Secreted proteins and surface markers on tubercle bacilli

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Table of contents

1. SUMMARY

Tuberculosis caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) is the major cause of mortality and morbidity of over two million people each year. Mapping of proteins exported by the pathogen to the extracellular environment is necessary to understand the biology of the pathogen and for identification of novel drug targets and vaccine candidates to eliminate the disease. On this background we investigated the secreted sub-proteome and membrane sub-proteome of *M. tuberculosis* and *M. bovis* in this study.

The secreted proteome of *M. tuberculosis* H37Rv culture filtrate made by Sadamu Nagai, was analysed using two different protein separation methods combined with MALDI-TOF and LC-MS/MS. We showed that this culture filtrate was particularly enriched in terms of secreted proteins compared to what has been reported previously. Furthermore, we searched with the generated mass spectra against a novel database including all predicted secreted proteins by the SignalP algorithms, after the removal of their predicted signal peptides, leading to the identification of the N-terminal peptides of over 40 proteins, confirming the bioinformatic prediction of the presence of a cleavable signal peptide in these proteins, with the AXA motif directly up-stream to the cleavage site as the most common recognized motif for signal peptidase I.

Further, due to the significance of proteins exported to the surface of the bacterium and their involvement in the pathogen-host interaction, the membrane and membrane-associated proteins were extracted using Triton X-114 phase separation from whole sonicated *M. bovis* BCG bacilli, a close relative to *M. tuberculosis*. Proteins from the lipid phase were precipitated by ethanol. To reduce the complexicity of the extracted protein mixture, two different separation strategies were used; both at protein and peptide level, combined with subsequent identification using LC-tandem mass spectrometry. As a result, over 100 membrane and membrane-associated proteins were identified including around 50% all predicted lipoproteins in the genome.

The culture filtrate protein mixture of *M. tuberculosis* used in our first work investigated to identify novel antigens. For this purpose, two different approaches were used to separate the proteins according to their hydrophobicity and pH followed by molecular mass based separation. A total of 20 serological reactive proteins were identified, including 4 novel

antigens. The immunogenicity of the identified antigens and their relative antibody quantities were measured using Image Master software. We show that antibodies against proteins belonging to the antigen 85 complex were the most abundant in the serum of *M. tuberculosis* infected patients, and that secreted lipoproteins were the most immunogenic proteins in term of high antibody to protein amount ratio were lipoprotein, illustrating the importance of this group of proteins for serodiagnostic development.

2. LIST OF PAPERS

This thesis is based on the following papers, hereafter cited by their Roman numerals:

I. **Hiwa Målen**, Frode S. Berven, Kari E. Fladmark, Harald G. Wiker (2007). Comprehensive analysis of exported proteins from *Mycobacterium tuberculosis* H37Rv. Proteomics 7: 1702 – 1718.

II. **Hiwa Målen**, Frode S. Berven, Tina Søfteland, Magnus Øverlie Arntzen, Clive S. D'Santos, Gustavo De Souza, and Harald G. Wiker. Membrane - and membrane associated proteins in Triton X-114 extracts of *Mycobacterium bovis* BCG identified using a combination of gel-based and gel-free fractionation strategies. (Revised version submitted for publication)

III. **Hiwa Målen**, Tina Søfteland, and Harald G. Wiker. Antigen analysis of *Mycobacterium tuberculosis* H37Rv culture filtrate proteins. (Submitted for publication)

3. ABBREVIATIONS

ACN	Acetonitril
BCG	Bacillus Calmette-Guérin
CD	Cluster of differentiation
dH_2O	Distilled water
DNA	Deoxy ribonucleic acid
DTT	Dithiothreitol
ESAT-6	Early secretory antigenic target-6 protein
ELISA	Enzyme-linked immunosorbent assay
HIV	Human immunodeficiency virus
IFN	Interferon
LPP	lipoprotein
MHC	Major histocompatibility complex
MtbC	*Mycobacterium tuberculosis* complex
NK cell	Natural killer cell
NO	Nitic oxide
PAMPs	Pathogen associated molecular patterns
PBS	Phosphate-buffered saline
PPD	Purified protein derivative
QFN	Quantiferon
SDS	Sodium dodecyl sulphate
GSP	General secretory pathway
TLR	Toll-like receptor
TM	Transmembrane
TNF-α	Tumor necrosis factor-α

4. INTRODUCTION

Tuberculosis is one of the most prevalent infections of human beings and contributes to the death of approximately two million people each year worldwide. Tuberculosis is a bacterial infection, and is caused by four species of Mycobacteria; *M. tuberculosis*, *M. bovis*, *M. microti* and *M. africanum*. At present the disease due to *M. tuberculosis* is the most common.

4.1 THE HISTORICAL BACKGROUND AND THE PRESENT BURDEN OF THE TUBERCULOSIS DISEASE

Tuberculosis is an ancient disease. It has been around throughout the known history and prehistory (Daniel 2006), and is one of the most prevalent infections of human beings that contributes considerably to illness and death around the world. The earliest evidence of tubercuolsis in man is provided by ca. 5400 year old Egyptian mummies, showing typical skeletal abnormalities of tuberculosis, including characteristic Pott's deformities, and recovery of DNA fragment sequences from bone samples, were consistent with *Mycobacterium* DNA (Zimmerman 1979; Morse *et al.* 1964; Crubezy *et al.* 1998).

In the first part of the nineteenth century, Rene´ Laennec described the pathology of the disease and the physical symptoms accompanied with pulmonary tuberculosis (Daniel 2006). Fifty years later, Jean-Antoine Villemin, a French military surgeon showed the infectious nature of the disease in experiments performed on rabbits infected with liquids from tuberculous cavities. However, the major breakthrough came in 1882, when Robert Koch managed to cultivate the causative agent of tuberculosis and reproduced the disease in animals by inoculating them with pure cultures. He also developed a staining method of the rod-shaped organism for microscopic examination. Further, he observed that *M. tuberculosis* infected persons could be identified by the appearance of a local reaction that followed the injection of a small amount of tuberculin, a cocktail of complex mycobacterial antigens also discovered by Koch, into the skin. The tuberculosis bacillus was re-named *M. tuberculosis* after L. Neumann in 1896 defined the genus Mycobacterium (meaning fungus-like-bacterium) as a group of bacteria that grew as mould-like pellicles on the surface of stationary liquid medium (Daniel 2005; Kaufmann 2003).

During the years 1908-1920, Calmette and Guérin developed an attenuated strain of *M. bovis*, a species closely related to *M. tuberculosis* that causes tuberculosis in cattle, by serially sub-culturing a virulent strain in a defined medium. After nearly 200 rounds of sub-culturing, the resulting strain called Bacillus Calmette-Guérin (BCG) had lost its virulence and was used as the first vaccine against tuberculosis (Barreto *et al.* 2006).

Current estimates show that nearly one third of the world population is already infected with *M. tuberculosis* and 8-10 million people develop active disease resulting in nearly two million deaths each year. Nearly 95% of active tuberculosis cases and 98% of deaths occur in the poor and developing countries of the world where pulmonary tuberculosis accounts for 85% of active tuberculosis cases (Frieden *et al.* 2003).

Although the incidence of tuberculosis had declined steadily from the 1950s to 1980s in the developed countries of the world due to BCG vaccination, rising standards of living and easy availability of an effective therapy, there has been a resurgence in the incidence of tuberculosis during the last two decades (Dye *et al.* 1999; Bloom and Murray 1992). Several factors have contributed to the resurgence of tuberculosis which varies from region to region and country to country around the globe. The rapid population growth in several countries, increased urbanization, civil wars and mass migration of populations, homeless individuals and the HIV epidemic have all contributed to this worsening of the situation (Frieden *et al.* 2003).

A more alarming trend is the emergence of multi-drug resistance tuberculosis (MDR-TB), which is currently defined as tuberculosis caused by strains resistant to at least isoniazid and rifampicin, the first line anti-tuberculosis drugs (Dye *et al.* 2002). In *M. tuberculosis*, resistance arises by spontaneous single step mutations, which occur at different frequencies for different medications, and is mostly seen as a consequence of previous anti-tuberculosis drug treatment (Frieden *et al.* 1993). Infection with multi-drug resistant *M. tuberculosis* strains has a large impact for the treatment of tuberculosis, in terms of prolonging the treatment period required and increasing the economical burden of the disease. Recently, even more disturbing reports have emerged, describing the appearance of extensively drug-resistant tuberculosis (XDR-TB) in different parts of the world. These strains of *M. tuberculosis* are resistant to isoniazid and rifampicin among the first line treatment drugs, and several main classes of the second line anti-tuberculosis drugs (Raviglione and Smith 2007). These reports reflect the urgent need to gather more knowledge about the pathogen and the mechanisms

underlying its pathogenicity, and also necessitate the search for new and more effective diagnostic and preventive tools to control the disease.

4.2 NATURAL COURSE OF INFECTION IN HUMANS

4.2.1 Primary infection

Tuberculosis is a transmissible disease and its mainly spread through airborne droplet nuclei, which are particles of 1-5 μm in diameter that contain *M. tuberculosis* bacilli. Due to their small size, the particles containing bacilli can remain airborne for minutes to hours after expectoration by people with pulmonary or laryngeal tuberculosis during coughing and sneezing (Wells 1955). After inhaling the droplet nuclei containing the *M. tuberculosis* bacilli, and uptake by alveolar macrophages, the bacilli start replicating slowly and grow shortly after infection (Morse *et al.* 1964). In most infected individuals, cell-mediated immunity develops 2-8 weeks after infection. The expression of acquired resistance to *M. tuberculosis* is associated with progressive growth of the bacilli, formation of granuloma structures characteristically being necrotic (caseating or cheese-like) at the site of infection. Activated T-lymphocytes and macrophages from granulomas limit further replication and spread of the organism (Schluger and Rom 1998). All the bacilli, however, might not be eliminated by the immune response and some persist in the body, and in a later stage, sometimes many years later, can begin to grow, resulting in clinical disease, known as reactivated tuberculosis.

4.2.2 Primary disease

Failure of the immune system to constrain the replication of *M. tuberculosis* bacilli leads to active disease. Tuberculous disease during the first five years following primary infection is classified as primary tuberculosis. Although an effective host immune response can initially constrain *M. tuberculosis* infection, several factors can trigger subsequent development of active disease from the primary infection. HIV is the greatest single risk factor for progression to active disease in adults. Other medical conditions that can also compromise the immune system and predispose to development of active disease include poorly controlled diabetes mellitus, renal failure, underlying malignant disease, chemotherapy, extensive corticosteroid therapy, malnutrition, and deficiency of vitamin D or A (Wilkinson *et al.* 2000; Karyadi *et al.* 2002). Defects in the production of interferon-γ (IFN-γ) (Sodhi *et al.* 1997; Hirsch *et al.* 1999)

or tumour necrosis factor-α (TNF-α) also contribute to the development of primary disease (Sterling *et al.* 2001; Keane *et al.* 2001).

4.2.3 Latent infection

Latent infection is defined as the presence of tubercle bacilli in the body without symptoms of the disease. Latency can be achieved either through the early restriction of *M. tuberculosis* growth, or after primary disease being "cured" either by itself or after chemotherapy. Most people exposed to *M. tuberculosis* mount a vigorous cell-mediated immune response that arrests progression of the infection, largely limiting it to the initial site of invasion in the lung parenchyma and the local draining lymph nodes (Cardona 2007). This results in the so-called "Ghon complex" as observed on X-ray, and comprises a calicified peripleural lymph node and calcified hilar lymph nodes, this clinical latency can persist throughout the person's lifetime. However, *M. tuberculosis* bacilli have been also observed in normal lung tissues in persons that have died due to other causes than tuberculosis. The bacilli reside in different cell types and remain latent in the absence of any histological reaction (Hernandez-Pando *et al.* 2000). However, in some circumstances the host immune response is perturbed, and reactivation of latent infection results. This process can occur, for example, through HIV infection, malnutrition, the use of steroids or other immunosuppressive medications (Flynn and Chan 2001). Although the estimated lifetime risk of developing reactivated tuberculosis is between 2% and 23%, the risk for individuals immunosuppressed by HIV infection is estimated to be as high as 10% per year.

4.2.4 Post-primary tuberculosis

Tuberculosis which occurs more than five years after a primary infection is classified as post-primary tuberculosis, and may develop as the result of reactivation of the endogenous primary infection or as a result of a recent exogenous re-infection occurring mostly in adults and elderly. Figure 1 summarizes the possible outcomes of *M. tuberculosis* exposure, showing that a small percentage of individuals, despite exposure to *M. tuberculosis*, remains uninfected, most likely due to the expression of high innate immunity. However, in the majority of individuals who are exposed to *M. tuberculosis*, the innate response cannot protect from infection, and effector T-helper-1 cell cytokines of the adaptive immune response are necessary to restrict bacterial growth and mediate protection. The adaptive immunity generated in these people, although protective, nonetheless does not induce sterilizing

immunity. These individuals therefore remain latently infected, and are vulnerable to disease reactivation when their immune surveillance weakens or when their immune response is compromised.

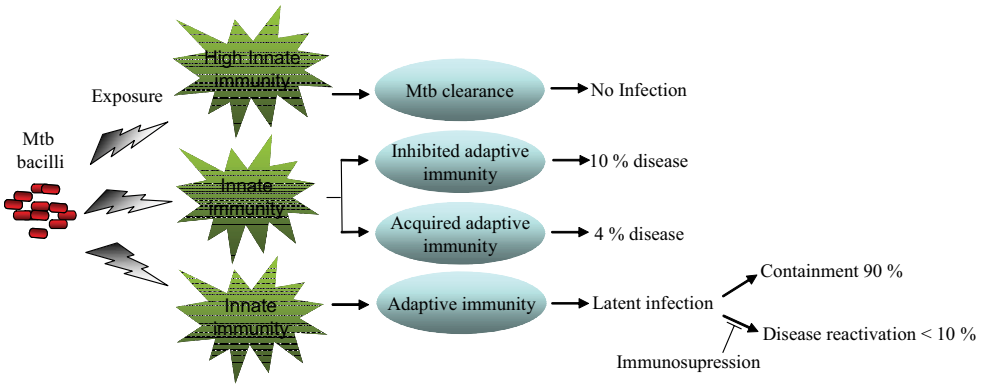

Figure 1. An overview over the possible outcomes of *M. tuberculosis* exposure.

4.3 THE HOST IMMUNE RESPONSE TO *M. tuberculosis*

4.3.1 The innate immune response

The innate immune response comprises several different cell types, has its own receptor system to recognize the presence of pathogens, and is a key to the initiation of an adaptive immune response in the host. The host response against *M. tuberculosis* is dominated by the fine-tuned interaction between innate and adaptive immune responses. Following the inhalation of *M. tuberculosis*-containing aerosols into the pulmonary alveoli, the bacteria bind to phagocytic receptors and enter resident alveolar macrophages, dendritic cells, and monocytes recruited from the bloodstream. Besides expressing phagocytic receptors, macrophages and dendritic cells also express Toll-like receptors (TLRs) that recognize conserved molecular patterns expressed on pathogens, so called pathogen associated molecular patterns (PAMPs) (Medzhitov and Janeway, Jr. 2000). Binding of TLRs to these molecules initiates a signal transduction pathway in the host cell that culminates in the activation of nuclear factor-kappa b (NFκb) and the induction of cytokines and chemokines that are crucial for eliciting the adaptive immune response against the pathogen (Aderem and Ulevitch 2000), composed of both humoral and cellular immune responses. In addition to macrophages and dendritic cells, γδ T-cells, natural killer (NK) cells, and natural killer T (NKT) cells also participate in the innate immune response to tuberculosis. Murine studies have indicated that the induction of γδ T-cells in the immune response against tuberculosis

precedes that of conventional CD4 and CD8 cells and hence plays an important role in modulating the effector response against tuberculosis. Following infection, the early recruitment of cells to the lung is mediated by the chemokines (C-X-C motif) ligand 2 (CXCL2) and CXCL10, and interleukin (IL)-12 and IL-17, released by macrophages and dendritic cells in the lungs. Once activated, the γδ T-cells secrete IFN-γ and TNF-α. The production of these cytokines strengthens the bactericidal capacity of macrophages (Ferrero *et al.* 2003; Lockhart *et al.* 2006).

4.3.2 The adaptive immune response

The humoral immune response

The humoral response which is the antibody mediated immunity, has been primarily used for diagnostic purposes in tuberculosis. Since *M. tuberculosis* is an intracellular pathogen, the serum components are thought not to get access to the pathogen and hence, may not play any protective role. However, this view has been challenged by recent studies, showing that the humoral immune response has shown effectiveness against other intracellular pathogens (Robbins *et al.* 1996; Glatman-Freedman 2003; Glatman-Freedman and Casadevall 1998; Glatman-Freedman 2006), suggesting that it may contribute to protective immunity to tuberculosis. In two different studies it was shown that monoclonal antibodies against surface antigens of *M. tuberculosis* give rise to protective immunity in mice, and prolong their survival after infection with lethal doses of *M. tuberculosis* or *M. bovis* through a more organized and compact granuloma formation where the bacilli were contained (Teitelbaum *et al.* 1998; Chambers *et al.* 2004). It is important to note that intracellular pathogens can also be found in the extracellular space during their life cycle, either before entering the host cells or after cell death, and then can easily be reached by antibodies, preventing their dissemination.

Cell mediated immune response

At the cellular level, an effective host immune response begins with alveolar macrophages infected with *M. tuberculosis,* that interact and activate T-lymphocytes via secretion of several cytokines, leading to activation of macrophages, dendritic cells, CD4 and CD8 αβ-T cells, CD1 restricted T cells, γδ-T cells and cytotoxic T-cells as well as the cytokines produced by these immune cells (Flynn and Chan 2001; Tufariello *et al.* 2003). In the mouse model, within 1 week of infection with virulent *M. tuberculosis*, the number of activated CD4 and CD8 T cells in the lung-draining lymph nodes increases (Serbina *et al.* 2000; Feng *et al.* 1999).

Between 2 and 4 weeks post-infection, both CD4 and CD8 T cells migrate to the lungs and demonstrate an effector/memory phenotype ($CD44^{hi}CD45^{lo}CD62L^{-}$); approximately 50% of these cells are CD69 (Serbina *et al.* 2000; Feng *et al.* 1999; Serbina and Flynn 1999). This indicates that activated T-cells migrate to the site of infection and are interacting with antigen presenting cells (APCs). The tuberculous granuloma contains both CD4 and CD8 T-cells (Flynn *et al.* 1992; Randhawa 1990) that participate in the continuous battle to contain the infection within the granuloma and prevent dissemination.

Apoptosis, the programmed cell death, has also been described as a part of the host immune response against the pathogen, where dying cells promote priming of CD8 T-cells by transferring antigens in apoptic vesicles to other cells to be presented (Winau *et al.* 2006), this process is also referred to as cross-presentation. Interestingly, Mustafa and co-workers (Mustafa *et al.* 1999a) showed in slowly progressive mouse models that *M. tuberculosis* inhibits apoptosis by modulating the expression of the apoptosis related proteins, Fas, Fas-ligand, Bcl-2 and Bax, and consequently manages to escape from the immune response by creating a safe heaven inside infected macrophages (Mustafa *et al.* 1999a; Mogga *et al.* 2002).

IFN-γ is the key cytokine for a protective immune response against *M. tuberculosis* (Flynn and Chan 2001). Within the granuloma, IFN-γ stimulates macrophages to kill mycobacteria through a variety of pathways (Saunders and Britton 2007). Consequently, humans and mice defective in IFN-γ or IFN-γ recptor genes are more susceptible to *M. tuberculosis* infection (Flynn *et al.* 1993; Ottenhoff *et al.* 1998). The IFN-γ also is important in antigen presentation process, leading to recruitment of CD4 T cells and/or cytotoxic CD8 T cells, which participates in mycobacterial killing. Furthermore, IFN-γ induces the transcription of more than 200 genes in macrophages including the up-regulation of major histocompatibility complex class-II (MHC-II) expression and the production of antimicrobial effectors such as oxygen radicals and nitric oxide (NO) and other reactive nitrogen intermediates (RNI) by macrophages (Scanga *et al.* 2001; Chan and Flynn 2004).

4.4 DIAGNOSIS OF THE DISEASE

4.4.1 Active tuberculosis

Tests for the diagnosis of tuberculosis vary in sensitivity, specificity, speed, and cost. The majority of TB cases in the world are diagnosed based on demonstration of acid fast bacilli in

sputum smear samples. This is not particularly sensitive, but the most contagious cases are found in this way. Nevertheless, culture is required for the definite diagnosis and is also essential for drug susceptibility testing. The sputum smear is an inexpensive test that can be carried out rapidly. Although the organism can take 6 weeks or longer to grow on solid culture media (eg, the egg-based Løwenstein-Jensen medium or the agar-based Middlebrook 7H10 or 7H11), growth generally occurs within 7-21 days with liquid culture media (Morgan *et al.* 1983). Ideally, when cultures are done, both solid and liquid culture media should be used, because the former allow examination of colony morphology and the identification of mixed cultures, and the later enable more rapid diagnosis.

Radiographic findings suggesting tuberculosis include upper-lobe infiltrates, cavitary lesions, and hilar or paratracheal adenopathy. In many patients with primary progressive disease and those with HIV infection, radiographic findings are more subtle and can include lower-lobe infiltrates or a miliary pattern. HIV-infected patients, particularly those in the late course of HIV infection, generally experience greater weight loss and fever but are less likely to have cavitary disease and positive smears for acid-fast bacilli (Perlman *et al.* 1997) than those not infected with HIV, and in one study, 8% of HIV-infected patients with pulmonary tuberculosis had normal chest radiographs (Burman and Reves 2000).

Immunological tests based on the presence of antibodies against various antigens of *M. tuberculosis* in sera of active tuberculosis patients are an attractive alternative to the more technically demanding nucleic acid-based methods. These tests are rapid, inexpensive and can easily be performed under the conditions commonly encountered in poor and developing countries (Chan *et al.* 2000). The ELISA-based serological tests have been used to detect antibodies to various purified or complex antigens of *M. tuberculosis* (Chan *et al.* 2000; Kanaujia *et al.* 2005; Gennaro 2000; Weldingh *et al.* 2005; Silva *et al.* 2003). However, these tests need to be further improved with respect to sensitivity and specificity, mainly due to large variations seen in the antibody response of tuberculosis patients to various mycobacterial antigens (Abebe *et al.* 2007; Lyashchenko *et al.* 1998).

Nucleic-acid amplification assays can be used directly on clinical specimens; they are most reliable in smear-positive respiratory samples from patients with previously untreated tuberculosis. In such samples, the sensitivity and specificity can be as high as 95% and 98%, respectively. The sensitivity is 48-53% in smear-negative respiratory samples, but the specificity is roughly 95%. In areas of high tuberculosis prevalence, there is no need to

confirm a heavily positive sputum smear, which will in most cases reflect *M. tuberculosis*. However, where concentrated smears are used and either the prevalence of HIV is high or the prevalence of tuberculosis is low, amplification techniques can be useful in distinguishing positive smears due to *M. tuberculosis* from positive smears with other mycobacteria.

Widespread implementation of nucleic-acid amplification assays has been limited by high costs and potential for poor performance under field conditions. Amplification tests do not replace the sputum smear which provides a gauge of infectiousness or culture which is necessary for drug-susceptibility testing. The assays can still give positive results after effective treatment because of detection of residual genetic material, so they may not be as useful in people with previous disease or in monitoring response to therapy.

Another approach to diagnose tuberculosis is by immunohistochemistry (IHC) using specific antibodies, which has the potential to reveal any mycobacterial antigen, and the presence of an intact bacillary cell wall is not a prerequisite. It has been generally considered to be more sensitive than acid-fast staining and has been investigated in order to improve the diagnosis of tuberculosis, mainly by the use of a commercially available anti-Bacillus Calmette Guérin (BCG) antiserum (Ulrichs *et al.* 2005; Mustafa *et al.* 1999b). Recently, Mustafa and co-workers showed that the diagnostic potential of immunohistochemistry can be improved by using an antibody to the secreted antigen MPT64, which is specific for the *M. tuberculosis* complex organisms and therefore can distinguish between the infection caused by pathogenic or atypical mycobacteria (Mustafa *et al.* 2006).

4.4.2 Latent tuberculosis

The persons infected with *M. tuberculosis* may be identified by a tuberculin skin test six to eight weeks after exposure to the bacilli. The test is based on a delayed-type hypersensitivity response to a complex cocktail of *M. tuberculosis* antigens, known as purified protein derivatives (PPD). The induration of more than 10 mm, recorded 48 to 72 hours after injection of PPD, are likely to develop tuberculosis in the future (Enarson 2004). Surveys conducted with PPD skin testing suggest that nearly a third of the world and half of Asia‘s population is infected with *M. tuberculosis* (Dye *et al.* 1999). Skin test reaction over 20 mm is usually due to active disease.

More sensitive and specific tests have been developed recently. One test measures IFN-γ production by T-lymphocytes in response to two *M. tuberculosis*-specific antigens [(early secretory antigenic target-6 (ESAT-6) and culture filtarte protein-10 (CFP-10)] (Doherty *et al.* 2002). This test performed better than PPD in discriminating latent tuberculosis infection from atypical mycobacterial reactivity or reactivity due to BCG vaccination (Brock *et al.* 2001). Two commercial tests have been developed based on ESAT-6 and CFP-10 [(QuantiFERON QFN-TB Gold (Cellestis Limited, Australia) and T SPOT-TB (Oxford immunotec, UK)] have been approved by FDA for detecting latent infection in Europe and USA. The tests are based on IFN-γ production by T-lymphocytes (QFN-TB Gold) or detection of *M. tuberculosis* single T-cells producing IFN-γ (T SPOT-TB) (Mazurek and Villarino 2003; Meier *et al.* 2005).

4.4.3 Prevention of tuberculosis by BCG

The control, prevention and eventual elimination of tuberculosis are highly dependent on the availability of a potent vaccine. More than 3 billion people worldwide have been vaccinated with BCG over the past five decades, however, the incidence of tuberculosis is still staggering (Frieden *et al.* 2003). The BCG vaccination prepare the immune response against primary infection and thus, protects children from the disease (particularly from tuberculous meningitis). However, the efficacy of BCG in preventing adult pulmonary tuberculosis varies from 0% to nearly 80% (Aronson *et al.* 2004; Flynn 2004; Fine 1995), and is most effective in developed countries and apparently more effective in countries far from the equator. The follow up study of a double blind vaccination trial in northern USA showed considerable protective effect of a single dose of BCG after 60 years which indicates that BCG may provide life long protection (Aronson 2004). Interestingly, several studies on the effect of repeated BCG vaccination have failed to demonstrate that revaccination by BCG provide additional protection. Unfortunately, due to the failure of BCG to protect against tuberculosis in many populations, it is clear that BCG is not sufficient to control the global epidemic of tuberculosis, therefore, more efficient vaccines are urgently needed.

4.4.4 New vaccine candidates

Despite the variable effectiveness of BCG vaccination, it will be difficult to totally replace it, due to its beneficial effects on preventing the dissemination of tuberculosis in childhood. The aim is rather to improve BCG vaccination in the future. A promising alternative is a recombinant BCG expressing listeriolysin from *Listeria monocytogenes* (Eddine and

Kaufmann 2005; Hess *et al.* 1998). Other vaccine candidates introduced are based on over-expression of antigen 85B in BCG (Horwitz *et al.* 2000) or recombinant BCG expressing cytokines (Murray *et al.* 1996). In addition, it may be possible to boost the effect of the BCG vaccine by introducing a second vaccine different from BCG at a later time point, and to use a heterologous prime–boost strategy for prevention of tuberculosis (McShane and Hill 2005). Booster vaccines are represented by Vaccinia virus expressing antigen 85A (McShane *et al.* 2004), DNA vaccines (Huygen 2005; Tollefsen *et al.* 2002) or subunit vaccines encoding fusion proteins (Andersen and Doherty 2005; Skeiky *et al.* 2004) of major antigens like antigen 85B and ESAT-6 (Olsen *et al.* 2004) or 85B and an ESAT-6 homolog termed TB10.4 (Dietrich *et al.* 2005). It should be noted that almost all the new vaccine candidates are based on secreted proteins; therefore, further exploration of the secreted sub-proteome of *M. tuberculosis* should provide more candidates for future vaccination strategies.

4.5 MOLECULAR EVOLUTION OF *M. tuberculosis*

Mycobacteria can be divided into two groups: fast-growing and slow-growing. Sequence data from various genes, including 16S rRNA, *rpo*B and *hsp*65 genes, have been used to construct phylogenetic relationships (Devulder *et al.* 2005; Adekambi and Drancourt 2004). One of the sequenced strains of *M. tuberculosis*, H37Rv, has been shown to contain 20 cytochrome P450-containing mono-oxygenases that catalyse mixed oxidation of hydrophobic compounds; this is an activity that is associated with free-living saprophytes in soil, which perhaps indicates that the ancestor of *M. tuberculosis* was a soil mycobacterium.

M. tuberculosis is part of the *M. tuberculosis* complex (MtbC), a group of closely-related slow-growing mycobacteria that includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti* and *M. canettii.* Although the members of the MtbC display different phenotypic characteristics and mammalian host ranges, they represent one of the most extreme examples of genetic homogeneity, with about 0.01%–0.03% synonymous nucleotide variation (Sreevatsan *et al.* 1997; Maniloff 1996; Saint-Ruf and Matic 2006; Zinser and Kolter 2004) and no considerable trace of genetic exchange among them (Maniloff 1996; Safi *et al.* 2004). Therefore, it is believed that the members of the MtbC are the clonal progeny of a single successful ancestor, resulting from a recent evolutionary bottleneck that occurred 20,000 to 35,000 years ago (Brosch *et al.* 2002; Gutierrez *et al.* 2005; Sreevatsan *et al.* 1997). However, it has been shown by differential hybridization arrays that there are 14 regions of difference

(RD1-14) ranging from 2 to 12.7 kb in size, with certain regions absent or present in certain strains between the members of the MtbC (Gordon *et al.* 1999; Behr *et al.* 1999) enabling them to adapt more successfully to different environments and hosts. On the other hand, the genes present in these regions represent interesting candidates for development of diagnostic tools that are able to distinguish between the different members of the MtbC, and also give an invaluable insight into the role of proteins present in these regions in the adaptation processes of the pathogen to the host environment.

4.5.1 The mycobacterial genome

One of the most striking advances in the history of molecular biology of mycobacteria are the genome projects, which have been established by Sanger, Pasteur and TIGR centers. Initially, construction of well characterized cosmid and bacterial artificial chromosome (BAC) libraries of *M. tuberculosis* (Brosch *et al.* 1998; Philipp *et al.* 1996) representing the complete genome opened wide venues for the study of genes involved in the physiological characteristics and pathogenesis of mycobacteria. In 1998, the entire genome of *M. tuberculosis* H37Rv was sequenced (Cole *et al.* 1998b), followed by the genome of the clinical isolate, *M. tuberculosis* CDC1551 (Fleischmann *et al.* 2002) and *M. bovis* AF2122/97 (Garnier *et al.* 2003). The complete genome sequence of the H37Rv strain of *M. tuberculosis* comprises 4,411,529 base pairs and contains around 4000 genes accounting for >91% of the potential coding capacity (Cole *et al.* 1998c; Brosch *et al.* 1998), with high guanine (G) and cytosine (C) content in the DNA (65.6%) (Cole 1998). Database comparisons searching for similarities between *M .tuberculosis* genes to other genes of known function, have led to tentative attribution of functions to roughly 40 % of the ca. 4000 predicted coding genes identified and these are predominantly involved in core metabolism. Some functional information or similarity to other gene products was found for a further 44 % of the protein coding genes, although over half of these belong to the class known as conserved hypothetical proteins found in a variety of bacteria. The remaining genes are probably typical for mycobacteria as they show no similarity to any other microbial sequences. About 51% of the genes have originated by gene duplication events, suggesting that there may be extensive functional redundancy or that *M. tuberculosis* is of recent evolutionary descent. More than 20% of the *M. tuberculosis* chromosome is devoted to genes encoding two different classes of proteins: enzymes involved in fatty acid metabolism and acidic, glycine-rich polypeptides of unknown function, the

proline-glutamic acid (PE) and proline-proline-glutamic acid (PPE) amino acid sequence motif containign proteins (Cole *et al.* 1998c).

The availability of the whole genome sequence has made it possible to identify genes and their regulatory units, and distinguish them from the repetitive or non-coding sequences by comparison with known sequences from other bacteria using bioinformatic programs, by which the role of proteins encoded by a gene can often be obtained.

4.5.2 The mycobacterial proteome

With the completion of the genome sequence of the H37Rv strain of *M. tuberculosis* (Cole *et al.* 1998c), a vast body of information about the bacterium's genes became available. This provided new insight into the biochemical and physiological processes that govern the life of the tubercle bacillus. Different bioinformatic tools have revealed the general properties of the proteome, and its subcellular locations. For example, a study by Tekaia and co-workers showed that the distribution of *M. tuberculosis* proteome throughout the p*I* range was similar to that seen with *Bacillus subtilis* (Tekaia *et al.* 1999). Interestingly, the proteome of *M. tuberculosis* contains about 4-fold more proteins with extremely basic p*I*. Many of these correspond to ribosomal, DNA-binding, cell envelope or transmembrane (TM) proteins. Proteins with acidic p*I* were mostly cytoplasmic enzymes involved in various metabolic functions. The sizes of the proteins range from 38 amino acids long, 50S ribosomal protein RpmJ, to the putative polyketide synthase, PKS12, comprising 4151 amino acids (Tekaia *et al.* 1999), with a mean length of 339 (+/- 284) amino acid residues, which is typical for other prokaryotes such as *Bacillus subtilis*.

Secreted proteins

Proteins secreted by *M. tuberculosis* have been the focus of interest for a number of years because they are highly relevant for vaccine development and for induction of protective immunity, and mapping this group of proteins is of great importance to elucidate their influence on the host immune response to the pathogen. Bioinformatic analysis of the NH_2-terminal sequences of proteins purified from the culture filtrate and their deduced amino acid sequences (Wiker *et al.* 1999; Young *et al.* 1992), indicates that many proteins of *M. tuberculosis* are secreted via the general secretory pathway (gsp). The gsp mediates protein translocation across the cytoplasmic membrane by means of an NH_2-terminal secretory signal peptide (Pugsley and Kornacker 1991). Following translocation, cleavage of the signal peptide

by signal peptidase I releases the mature protein, providing that there are no additional membrane-spanning segments or membrane-anchoring moieties. The conserved features of signal peptides such as length and amino acid composition plus the signal peptidase cleavage site (Watson 1984) make them amenable to identification by sequence analysis by using a bioinformatic approach like the Neural Network method or Hidden Markov Model that take advantage of the known nucleotide sequence of the *M. tuberculosis* genome (Emanuelsson *et al.* 2000; Nielsen *et al.* 1997; von Heijne 1986; Cole *et al.* 1998a).

However, not all secreted proteins are secreted through the general secretory pathway, and proteins lacking a classical signal peptide can still be secreted by other alternative secretion machineries, such ESAT-6 family member proteins. These proteins have been shown to be secreted by the secretion in mycobacteria (snm) pathway (Stanley *et al.* 2003), where a C-terminally located signal peptide mediates their translocation to the membrane and their subsequent secretion (Champion *et al.* 2006). Another alternative secretory pathway, twin-arginine translocase (TAT) secretion system, has also been described in *Mycobacterium smegmatis* (Posey *et al.* 2006), a close relative of *M. tuberculosis*, where proteins with two or more N-terminal tandem arginine residues can be recognised followed by their translocation. A large part of the secreted sub-proteome of *M. tuberculosis* is not designated any functions based on similarity to other known proteins, and therefore considered to be hypothetical. Therefore, mapping this group of proteins is of great importance to elucidate their influence on the host immune response to the pathogen.

In the first proteomic study of *M. tuberculosis*, that focused on secreted proteins by Nagai and co-workers, 12 culture filtrate proteins were chromatographically purified and partially characterized (Nagai *et al.* 1991). Following Nagai`s work, relatively few additional proteins with predicted N-terminal signal peptide, which can be recognized as secreted through the general secretory pathway, have been uncovered in subsequent studies by other groups (Rosenkrands *et al.* 2000a; Rosenkrands *et al.* 2000b; Mattow *et al.* 2003a; Sonnenberg and Belisle 1997; Beatty and Russell 2000a; Weldingh *et al.* 1998), probably due to considerable contamination of regular culture filtrates with intracellular proteins (Wiker 2001).

The major culture filtrate proteins secreted by *M. tuberculosis* are the antigen 85A, 85B and 85C proteins (collectively called antigen 85 complex), characterised Wiker and Harboe (Wiker and Harboe 1992). These proteins are closely related and share 68-80 % similarity at the amino acid level. *In vitro* assays performed with the three purified proteins showed that

they are involved in transferring mycolic acids to trehalose, leading to the formation of α,α´-trehalose monomycolate (TMM) and α,α´-trehalose dimycolate (TDM, cord factor) (Belisle *et al.* 1997), the dominating components of the *M. tuberculosis* cell wall which form a permeability barrier that shields the bacterium from environmental stress and enable it to resist many antibiotics. The antigen 85 complex proteins are also interacting with the host immune system and provoking both humoral and cell-mediated immune responses (Wiker and Harboe 1992).

Membrane proteins

Integral membrane proteins account for 20-30% of all genes in both prokaryotic and eukaryotic organisms (Krogh *et al.* 2001). Membrane and membrane associated proteins play an essential role in many vital processes such as cell-cell interactions, ion and nutrient transport and cell signalling, and participate in the key pathogenically relevant cellular mechanisms, they have also been shown to provoke both humoral (Laal *et al.* 1997) and cell-mediated (Sinha *et al.* 2005) immune responses. Membrane proteins have at least one and up to 16 transmembrane regions that traverse the cytoplasmic membrane. Many membrane associated proteins are actively exported through the cytoplasmic membrane via gsp (Pugsley and Kornacker 1991).

Distinct features of transmembrane regions have made it possible to identify them from the genome using different bioinformatic programs. The Hidden Markov Model-based prediction of transmembrane region algorithm (TMHMM) (Krogh *et al.* 2001; Sonnhammer *et al.* 1998) is considered to be among the programs with best performance (Moller *et al.* 2001) in terms of prediction of transmembrane regions. In *M. tuberculosis*, TMHMM predicts more than 780 proteins to be localized in the membrane, which constitutes around 20% of all predicted proteins. The essential role played by membrane proteins for the survival and adaptation of the pathogens has been the driving force behind many studies designed to map the membrane sub-proteome of *M. tuberculosis* (Gu *et al.* 2003; Mawuenyega *et al.* 2005; Sinha *et al.* 2005; Xiong *et al.* 2005).

Most integral membrane proteins are co-translationally inserted into the lipid bilayer of the membrane through a protein channel located in the membrane called the translocon complex. The translocon complex is composed of many different sub-units. Within the lipid bilayer, the protein sequence starts to acquire its free energy minimum and forms peptide α-helixes, which

are tightly packed transmembrane (TM) regions. Alpha-helices are formed by the consecutive joining of mostly non-polar amino acids, with typically 15-25 amino acids required to traverse the membrane bilayer. These amino acids exhibit positive hydropathy values, and hence are the major contributor to the hydrophobic characteristics commonly associated with membrane proteins (Scott and Barnett 2006). The topology of the inserted membrane proteins generally follows the positive-inside role, where lysine and arginine residues flank the transmembrane region in the cytosol (Nilsson *et al.* 2005).

Lipoproteins

Lipoproteins (LPP) are a subgroup of bacterial proteins characterized by the presence of a lipidated N-terminus. In *M. tuberculosis*, around 100 proteins have been predicted to be lipoproteins, which are thought to be involved in a variety of functions (Sutcliffe and Harrington 2004), including host-pathogen interactions and virulence (Sander *et al.* 2004; Moody *et al.* 2004). All bacteria localise specific proteins to their cell envelopes by post-translational lipid modification to produce membrane-anchored lipoproteins (Sutcliffe and Russell 1995). In mycobacteria lipid modification is likely to represent an important mechanism by which proteins are localised within the cell envelopes. LPPs of Gram-positive bacteria have been previously suggested to be functional equivalents of periplasmic proteins in Gram-negative bacteria (Sutcliffe and Russell 1995).

LPP biogenesis is dependent on the presence of specific type II signal peptide sequences (Klein *et al.* 1988; von 1989; Tjalsma *et al.* 2000; Sutcliffe and Harrington 2002), and the presence of a conserved consensus sequence motif called “lipobox” with a conserved cysteine residue at the +1 position. The lipobox directs the processing of the pre-lipoprotein to form the mature acylated LPP (Sutcliffe and Harrington 2004). The signal peptide directs lipoprotein export through the plasma membrane where a diacylglyceride unit is added by thioether linkage to the conserved cysteine residue in the +1 position. The enzyme that carries out this lipidation reaction, proLPP diacylglyceryl transferase (Lgt), is an essential enzyme in Gram-negative bacteria but is dispensable in the Gram-positive bacteria studied to date (Leskela *et al.* 1999). Following the lipidation by Lgt, the signal peptide is cleaved by a specific proLPP signal peptidase II enzyme (Lsp) at a cleavage site immediately preceding the lipidated cysteine, which consequently becomes the N-terminus of the mature LPP. However, analysis of several LPPs revealed that they might also be subjected to further cleavage by signal peptidase I since they have the recognition motif for this enzyme as well. This leads to

the conclusion that LPP can be alternatively or dually localized in both the membrane and outside the bacilli, probably as a consequence of different stimuli. It will be interesting to investigate whether a particular LPP can be localized differently in different strains as an adaptation tactic from the pathogen to a particular host environment.

5. AIM OF THE STUDY

The general aim of this study was to profile the culture filtrate and membrane fraction sub-proteome, and identify novel antigens to be used for both sero-diagnostic purposes and subunit vaccines against tuberculosis.

The specific aims:

1. Exploring the *M. tuberculosis* H37Rv culture filtrate proteins, and identification of novel secreted proteins.

2. Exploring the membrane fraction proteins of *M. bovis* BCG and identification of novel membrane and membrane-associated proteins.

3. Analysing the immunogenicity of *M. tuberculosis* H37Rv culture filtrate proteins, to identify novel candidates for serological diagnosis of tuberculosis.

6. MATERIALS AND METHODS

M. tuberculosis H37Rv culture filtrate

The culture filtrate proteins used in the first and third study belonged to *M. tuberculosis* H37Rv ATCC27294 from the National Institute of Health, Tokyo, Japan. Bacteria were cultured as surface pellicle on the wholly synthetic Sauton medium for 3 weeks without shaking. Then the bacteria were removed by filtration and the culture filtrate proteins were concentrated by 80% ammonium sulphate precipitation. Precipitated proteins were dissolved in phosphate-buffered saline (PBS) buffer (pH 7.4) and dialyzed against distilled water and lyophilised.

M. bovis BCG substrain Moreau

The membrane fraction proteins used in the second work belonged to *M. bovis* BCG substrain Moreau, which was cultured in 1 litre stationary glass bottles on the wholly synthetic Sauton medium as surface pellicle for 2-3 weeks at 37 °C. Bacteria were separated from the culture medium by paper filtration and washed with phosphate-buffered saline, pH 7.4. Collected bacterial cells were stored at -20 °C.

Human sera

A serum pool from sixteen patients with active tuberculosis, and a pool of ten healthy persons were used as control. The sera were a kind gift from M. E. Patarroyo, Bogota, Colombia.

One-dimensional gel electrophoresis

Fifty µg of *M. tuberculosis* H37Rv culture filtrate proteins were mixed with 25 µl sodium-dedocyl-sulphate (SDS) loading buffer and boiled for 5 minutes prior to separation on a 10 cm long, 1 mm thick 12% SDS-polyacrylamide (SDS-PAGE) gel. The protein migration was allowed to proceed until the blue dye had migrated to the bottom of the gel. The protein bands were visualized with Coomassie Brilliant Blue R-250 (CBB) (Bio-Rad, Hercules, CA, USA). The molecular mass standard, full-range-rainbow-RPN800 (Amersham Biosciences AB Uppsala, Sweden), was used to divide each lane into 10 segments. Each segment was cut into smaller pieces, destained, and in-gel digested with trypsin (Promega, Woods Hollow Road, Madison, U.S.A.).

Two-dimensional gel electrophoresis

Two mg of *M. tuberculosis* H37Rv culture filtrate proteins were mixed with 350 µl of rehydration buffer (7 M urea, 2 M thiourea, 4% 3-[(3-Cholamidopropyl)Dimethyl-Ammonio]-1-Propanesulfonate (CHAPS), 2% dithiothreitol (DTT), 2% Immobiline pH Gradient buffer (IPG buffer) pH 4-7). Isoelectric focusing was performed at 20°C on 18 cm immobiline dry strips (Amersham Biosciences AB, Uppsala, Sweden) with pH intervals (3-10; 4-5; 4.5-5.5; 5.3-6.5) using Multiphor II Electrophoresis System (Amersham Biosciences AB Uppsala, Sweden). Running conditions: current 2 mA; power 5 W using the EPS 3501 XL Power Supply in gradient mode and with check option for current turned off. Prior to the second dimension, the strips were incubated for 15 minutes in equilibration buffer (6 M urea, 2% SDS, 0.375 M Tris, pH 8.8, 20% glycerol) with 130 mM DTT first and then with 135 mM iodoacetoamide. The equilibrated strip was then placed on an ExcelGel XL SDS 12-14% (Amersham Biosciences). The second dimension was run at: current 40 mA and power 40 W for 4 hours. Proteins were visualized with CBB. Protein spots were excised from the gel and digested with trypsin.

Immunobloting

Proteins separated by SDS PAGE were electroblotted to nitrocellulose membranes (Amersham Biosceinces) and were blocked with 5% non-fat milk in PBS containing 0.5% Tween 20 (PBST) for 1 hour at room temperature (RT). Afterward, the membranes were washed with PBST for 10 min, this was repeated three times. After the last wash, the membranes were incubated overnight at 4°C with 1:500 diluted sera with 1% non-fat milk in PBS containing 0.1% Tween 20. A pool composed of sera from 16 tuberculosis patient, or sera from 10 healthy persons as negative control were used. The blots were washed thoroughly with PBST as described above, and probed with Horse Reddish Peroxidase (HRP) conjugated anti-human IgG (1:2000 dilution) (Amersham Biosciences) for 1 hour at RT. Antigen-antibody complexes were visualized by chemiluminescent (Pierce, Rockford, IL, U.S.A.) using Las-3000 (Fujifilm Life Science, Stamford, U.S.A).

In-gel digestion

Sliced gel spots or bands were washed twice with 50% acetonitrile (ACN) in 25 mM ammonium bicarbonate (NH_4HCO_3) for 15 minutes at room temperature (RT). The gel pieces

were dehydrated by incubating them with 50 µl 100% ACN for 20 minutes at RT. Proteins were reduced using 10 mM DTT and alkylated with 55 mM iodoacetamide (IAA); both in 100 mM NH_4HCO_3. The gel pieces were dehydrated with ACN as described above, and rehydrated in 25 mmol/l NH_4HCO_3 containing 0.01 µg/µl modified trypsin. Proteins were digested with trypsin for 16-20 h at 37°C. Then, the tryptic peptides were eluted by incubating the gel pieces with 50 µl 1% trifluoroacetic acid (TFA) for 20 minutes at RT. The supernatant containing tryptic peptides were collected by centrifugation at 15700 *g* for 10 minutes. Additional peptides were extracted from gel pieces by incubation with 50 µl 0.1% TFA in 50% ACN for 20 minutes at RT, followed by centrifugation at 15700 *g*. The supernatant was collected and added to the previous one. Finally, the gel pieces were dehydrated by incubating the gel pieces with 50 µl 100% ACN for 20 minutes at RT, and the supernatant was collected by centrifugation as described above and added to the pool.

In-solution digestion

Protein pellet was dissolved in aqueous 6 M urea (Ultragrade Fluka), 100 mM Tris (Merck) pH 8 and 10 mM DTT (Amersham Biosciences), and incubated for 1 hour at 37ºC. Iodoacetoamide (IAA) was added to a final concentration of 25 mM followed by one hour incubation in the dark at 37ºC. To avoid unwanted protease alkylation, DTT corresponding to 2.5 mM was added followed by 20 minute incubation at 37ºC. The sample volume was diluted 1:3 with MilliQ water (Sigma), and $CaCl_2$ was added to a final concentration of 1 mM. Trypsin was added to obtain a protein:trypsin ratio of 1:50, and a 16 hour incubation at 37ºC was conducted. The reaction was quenched by adding TFA to obtain a pH<3 in the digested solution.

Matrix-assisted laser desorption ionization time-off-flight mass spectrometry (MALDI-TOF MS)

The tryptic peptides extracted from gel slices were concentrated and desalted using $ZipTip_{C18}$ (Millipore, Billerica, MA, USA), and eluted with saturated alpha-cyano-4-hydoxy-cinnamic acid solution (CHCA) (Sigma) in 50% ACN and 0.1% TFA. One µl sample eluted from the matrix was applied to the steel target plate and analysed by MALDI-TOF MS (Autoflex & Ultraflex, Bruker Daltonics) and MALDI with tandem mass spectrometry (MS/MS) (Ultraflex, Bruker Daltonics). The obtained mass spectra were searched against the *M. tuberculosis* complex database using MASCOT (http://www.matrixscience.com). The search

parameters were: 100 ppm tolerance as maximum mass error, monoisotopic mass value, and fixed modification of cysteine by carboxymethyl. A protein was regarded identified if the matched peptide mass fingerprint covered 20% of the complete protein sequence. An assignment with sequence coverage below 20% was only accepted if one or more of the main peaks were identified by MALDI MS/MS with a significant MASCOT score (above 95% certainty).

Strong cation-exchange chromatography

Peptides were separated by salt-step cation-exchange chromatography using an LC-Packing *Ultimate* capillary-High Performance Liquid Chromatography (HPLC) system (Dionex, CA, USA) equipped with a strong cation-exchange column (LC-Packings Poros 10S 300 μm inner diameter and 150 mm long). The washed and solubilized digested membrane protein fractions were injected onto the SCX-column in 0.1% formic acid. An isocratic flow-rate of 20 μl/min of 0.05% (vol/vol) formic acid in 2% (vol/vol) ACN was delivered by the loading pump. After loading of the samples, the SCX-column was switched off-line and the flow-through (FT) and eluate were applied to a trap-column cartridge (LC Packings C18 PepMap 100 5 μm 100Å 1.0 mm in diameter and 15 mm long) and washed/desalted three minutes before isocratic elution with 0.1% (vol/vol) formic acid in 70% (vol/vol) ACN delivered by the capillary pump at 6 μl/min. Increasing concentrations of NaCl were injected as samples to elute stronger bound peptides. Salt-steps used for peptide elution were 40 mM, 100 mM and 250 mM of NaCl.

Liquid chromatography-ESI-MS/MS

On-line LC-MS/MS was performed on tryptic peptides using a Dionex *Ultimate 3000* nano-HPLC system coupled to an ESI-Q-Tof (Ultima Global, Waters, Massachusetts, USA). Reprosil-Pur 3.5 μm C18 resin (Dr.Maisch Gmbh, Ammerbuch, Germany) was packed in a 15 cm long and 75 μm inner diameter fused silica capillary column and used for analytical peptide separation. Solvent A was 0.1% (vol/vol) formic acid in 2% (vol/vol) ACN, and solvent B was 0.1% (vol/vol) formic acid in 90% ACN. The flow rate through the column was 0.3 μl/min. The gradient developed as follows: 5% to 10% B in 2 minutes, 10% to 30% B in 28 minutes, 30% to 50% B in 20 min, 50% to 95% B in 1 minute and ending with constant 95% B for 9 minutes before regeneration of the column. Proteolytic peptide profiles were acquired in data-dependent MS/MS mode from 0 to 65 minutes with a 1 second MS survey scan. The three most intense precursors were selected for fragmentation and then excluded for

90 seconds. Collision induced dissociation (CID) spectrum acquisition was allowed for 2 seconds on every selected precursor or stopped when the signal intensity fell below five counts per second. The electrospray voltage was set to 2.9 kV and fused silica capillaries with a 20 µm aperture (Waters) served as spray emittors. The obtained data was searched against the publicly available *M. tuberculosis complex* database using MASCOT software. Figure 2 illustrates protein identification by LC-MS/MS.

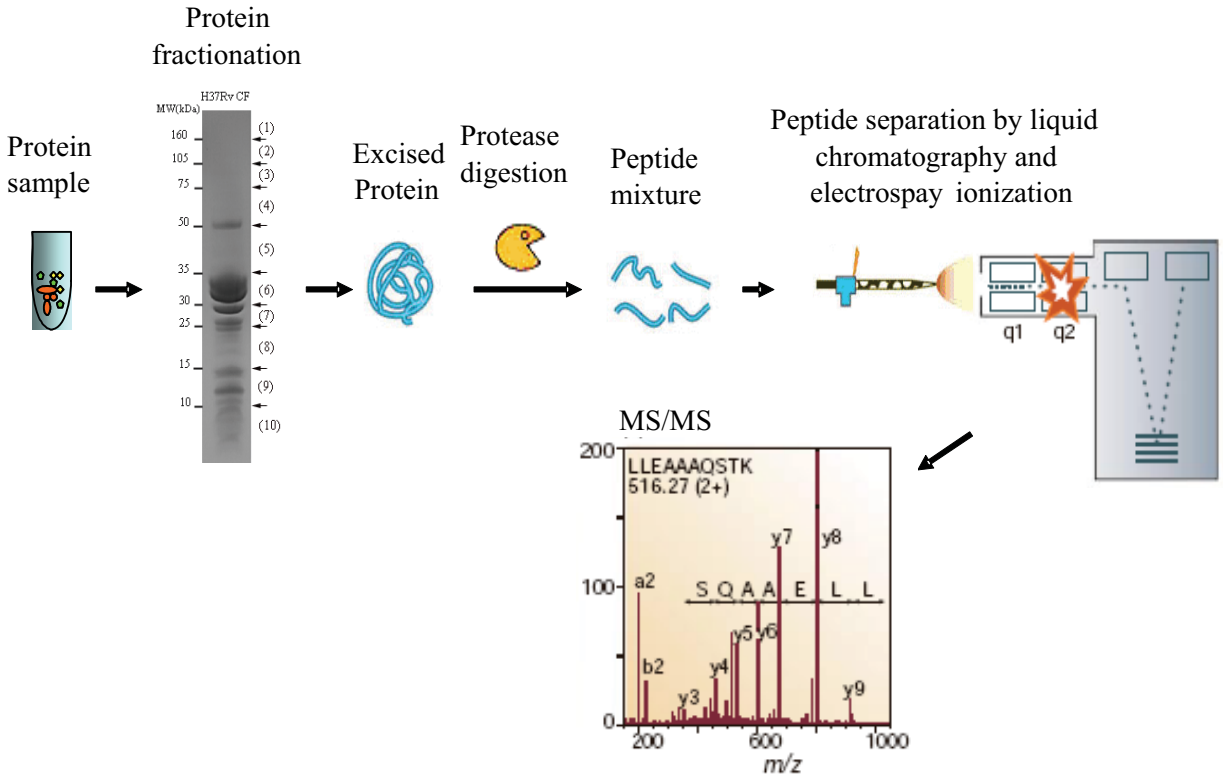

Figure 2. An illustration of protein mass spectrometry by LC-MS/MS. Proteins to be analysed are typically fractionated by gel electrophoresis, and digested enzymatically by a protease, usually trypsin, to yield peptide fragments. The peptides in their turn are further separated by one or more high pressure liquid chromatography and eluted into an electrospray ion source where they are nebulised in small, highly charged droplets containing peptides. After evaporation these droplets, multi-protonated peptides enter the mass spectrometer, where they undergo fragmentation by energetic collision with gas, which is referred to as tandem mass spectrometry (MS/MS) and the resulting mass spectra are then stored for search against protein sequence databases and identification of the parent protein. Adapted and modified from (Aebersold and Mann 2003)

Determination of N-terminal start sites in predicted secreted proteins

The Neural Network method (SignalPNN) and the Hidden Markov Model (SignalPHMM) for predicting signal peptides are publicly available at the SignalP server (http://www.cbs.dtu.dk/services/SignalP/). All proteins in the proteome of *M. tuberculosis*

H37Rv that were predicted to have a signal peptidase I cleavage site by either of the two SignalP methods (v 2.0) were compiled in a MASCOT searchable database after removal of the predicted signal peptide. This database is referred to as the Removed-Signal-Sequence database and is available at (http://www.bioinfo.no/publications/wiker2006/)

7. SUMMARY OF THE RESULTS

I. Profiling of *M. tuberculosis* H37Rv culture filtrate proteins

To profile the sub-proteome of *M. tuberculosis* H37Rv culture filtrate, three different batches were compared, that showed to be highly similar in 2D-PAGE pattern. A representative batch was selected for protein profiling. Two different strategies were applied to map the proteome content of the culture filtrate fraction: 1) 2D-PAGE coupled to MALDI-TOF MS, and 2) 1D-PAGE coupled to LC-MS/MS. In total 256 proteins were identified by both methods, out of which 159 of them had a predicted signal peptide.

Determination of signal peptidase I cleavage sites

To identify the cleavage site for signal peptidase I of secreted proteins of *M. tuberculosis* H37Rv and to verify a cleavable signal peptide, each mass finger print generated by both MALDI-TOF MS and LC-MS/MS were searched against the Removed-Signal-Sequence database using MASCOT. Out of the 159 identified proteins with a predicted signal peptide, 41 N-terminal peptides lying immediately C-terminally to a predicted cleavage site were identified, which confirms the existence of a cleavable signal sequence in many of these proteins.

Interestingly, the SignalP method predicted in total 188 cleavage sites in 127 of the proteins predicted to be processed by signal peptidase I, and 85 of these cleavage sites had an AXA motif at the N-terminal side. However, among the 127 proteins, 35 out of 41 experimentally verified cleavage sites had an AXA motif. The one-sided binomial test showed that this observation was highly signficant ($p<0.000003$). We have also detected the N-terminal cleavage site of signal peptidase I predicted by SignalP in 5 potential lipoproteins (Rv0526, Rv0999, Rv2911, Rv3668c and Rv3759c), with the predicted lipobox further N-terminally to the detected cleavage sites.

II. Identification of *M. bovis* membrane and membrane-associated proteins

The aim of this study was to perform a comprehensive proteomic analysis of exported and membrane proteins of *M. bovis* BCG substrain Moreau. For this purpose, membrane and membrane associated proteins were extracted using Triton X-114 phase separation from whole sonicated bacilli. Proteins from the lipid phase were precipitated by ethanol. To reduce the complexity of the protein mixture, they were subjected to two different separation strategies.

Firstly, proteins separated by 1D-PAGE coupled to nano-ESI-LCMS/MS, and secondly, in-gel digested peptides were separated by SCX coupled to nano-ESI-LCMS/MS. As a result, 351 different proteins were identified, 103 had one or more predicted TMH region, another 34 porteins were predicted as lipoproteins, in addition to 84 proteins which had a positive GRAVY value, suggesting that these proteins are membrane associated. Of the 351 proteins identified in this study, 54 proteins were not previously identified, including 24 predicted integral membrane proteins with at least one predicted TMH region.

III. Identification of novel antigens from *M. tuberculosis* H37Rv culture filtrate proteins

Two different approaches were used to separate the of *M. tuberculosis* H37Rv culture filtrate protein mixture analysed in paper I, using protein fractionation according to their hydrophobicity by a HPLC-C18 chromatography column and molecular mass. In parallel, proteins were separated by two-dimensional gel electrophoresis, based on their isoelectric point and their molecular mass. Twenty serologicaly reactive proteins were identified by both methods, including 4 novel antigens. Further, to estimate the immunogenicity of the identified culture filtrate proteins, the relative antibody quantities were measured using Image Master software. We observed that the antibodies against proteins belonging to the antigen 85 complex were the most abundant in the serum of patients with active tuberculosis. However, the most immunogenic proteins in terms of high antibody to protein ratio were Rv3881c and three lipoproteins, Rv0934 (the 38 kDa antigen), Rv0932 (pstS2), and Rv3006 (LppZ). Rv3881c is located in the region of difference 1 (RD1) which is deleted from *Mycobacterium bovis* BCG, and is therefore a particularly promising candidate for development of serodiagnostic assays to detect active tuberculosis.

8. GENERAL DISCUSSION

Secreted proteins

Secreted proteins by *M. tuberculosis* are believed to mediate important biological functions by interacting with host cells and are potentially important for virulence and pathogenesis of the bacterium (Beatty and Russell 2000b; Stewart *et al.* 2005; Malik *et al.* 2003; Fratti *et al.* 2003; Pieters and Gatfield 2002). Even though the *M. tuberculosis* culture filtrate proteins have been the focus of many previous studies (Sonnenberg and Belisle 1997; Beatty and Russell 2000c; Rosenkrands *et al.* 2000a; Rosenkrands *et al.* 2000b; Mattow *et al.* 2003b; Bahk *et al.* 2004), the number of identified proteins predicted to be secreted, as based on the presence of a signal peptide in the preprotein, has been relatively low. This is probably due the overrepresentation of proteins derived from the cytoplasm of the bacteria overshadowing the secreted ones.

In our first study (Paper I), we provided a comprehensive picture of the secreted protein repertoire of *M. tuberculosis* H37Rv. This was achieved by studying the culture filtrate of *M. tuberculosis* H37Rv after culturing on the wholly synthetic Sauton medium (Sadamu Nagai, Osaka, Japan). These culture filtrates have a very low content of intracellularly derived proteins. By applying two different protein fractionation strategies we managed to identify 159 predicted secreted proteins, in addition to several ESAT-6 family member proteins that lack a signal peptide in their primary sequences, but are shown to be secreted by an alternative pathway. This is the largest number of predicted secreted proteins of *M. tuberculosis* reported in one study, and certainly will contribute to shed light on the unexplored part of the pathogen's sub-proteome.

The N-terminal peptides of mature secreted proteins were identified in 41 of 159 exported proteins predicted by SignalP. These results verify the existence of a cleavable signal sequence in those proteins, and were in agreement with the SignalP predictions of the signal peptide and its cleavage site in a large fraction of the secreted proteins. We found that the majority of the identified N-terminal peptides of mature secreted proteins had an AXA motif N-terminally to their cleavage sites, showing that the mycobacterial signal peptidase I preferentially recognizes the AXA motif. This motif is underestimated by SignalP, possibly due to the presence of only a few mycobacterial proteins in the SignalPs training set. This might partly explain the failure to identify the N-terminal peptide of the other predicted secreted proteins

identified in this study. Our results show that there is a need for a separate algorithm for prediction of secreted proteins in acid-fast bacilli. This has not been possible before because few signal peptidase I cleavage sites in mycobacteria have been known.

Membrane proteins

Membrane proteins are close to the interaction surface between host cells and pathogens, therefore, a lot of effort has been spent to map the proteins on the membrane of *M. tuberculosis*, in order to better understand the molecular mechanisms that govern the host-pathogen interaction (Gu *et al.* 2003; Mawuenyega *et al.* 2005; Sinha *et al.* 2005; Xiong *et al.* 2005). Membrane proteins are embedded in the membrane by transmembrane peptides commonly folded as α-helixes (TMH). In this work we extracted hydrophobic proteins prior to their identification by mass spectrometry by applying Triton X-114 phase separation using whole sonicated bacilli directly instead of pre-separation of membranes, and application of two different fractionation strategies, both at protein and peptide level to reduce the complexity of the extracted proteins, we managed to identify 351 different proteins of which 103 (29.3%) proteins had at least one predicted TMH region, including 56 proteins with 2 or more TMH regions. We also identified proteins without any predicted TMH and with negative GRAVY values that might still be functionally associated to the membrane through interaction and formation of protein complexes with membrane-anchored proteins.

Further we observed that there is considerable overlap between TMH prediction and signal peptide prediction, making the categorization of a protein as secreted or membrane anchored more difficult for proteins with only one predicted TMH coinciding with their signal peptide prediction. The overlap in predictions of TMH regions and signal peptides is a general problem that inflict upon distinction between soluble secreted proteins and membrane proteins. More data are needed to resolve this problem, but our data suggest that the majority of proteins with multiple TMH regions and with an N-terminal TMH which is also predicted to be a signal peptide, are retained uncleaved. On the other hand, most of the proteins with predicted signal peptide and only 1 predicted TMH region positioned in the N-terminus, probably represent exported proteins with cleavable signal peptides. Therefore, it is important to separate proteins with dual prediction as membrane and as secreted proteins, and also report the SignalP prediction values, as well as their TMHMM prediction scores in future proteomic studies.

By comparing our data and a comparison to what has been published by others, we found that certain proteins have been identified in many different studies, indicating that these proteins are abundant. Abundant proteins in the membrane fraction of the bacilli are especially interesting both in terms of a better understanding of the dominant mechanisms occurring in the membrane and also in terms of selection of candidates for therapeutic purposes.

Lipoproteins

In our study we observed lipoproteins in both the culture filtrate (Paper I) and the lipid phase of the Triton X-114 extract (paper II). In total, we have identified over 50% of the all predicted lipoproteins in the genome. More detail investigation is needed to elucidate their role for the bacteria and its survival inside the host macrophages.

Exported lipoproteins have been shown to be exposed at the surface of *M. tuberculosis* and *M. bovis*. They are however vulnerable to proteolytic cleavage relatively close to the N-terminal cysteine and are subsequently released as soluble proteins in the culture filtrate. Similar observations have also been reported in *Bacillus subtilis* [55]. The lipoproteins we identified in the culture filtrate (Paper I) may represent such proteolytically processed proteins. Further, we found five potential N-terminal peptides among the 36 predicted lipoproteins. Closer inspection revealed that the cleavage site for signal peptidase I, predicted by SignalP and observed in our study, lies C-terminally from the predicted cleavage site for signal peptidase II. This finding suggests that some lipoproteins may be alternatively processed by signal peptidase I or II, and represent a mechanism for dual localization in A) the extracellular environment or B) as lipoprotein in the cell wall.

In the second paper we identified 43 lipoproteins, 21 of them were also identified in the culture filtrate, indicating a dual localization of these proteins. The lipoproteins identified only in the lipid phase are most likely modified by lipids, which anchor them to the membrane, and become more hydrophobic, otherwise most of the identified lipoproteins had a negative GRAVY value and only few of them had a transmembrane region in addition to their predicted lipobox motif.

Immunogenic proteins in the culture filtrate of *M. tuberculosis*

Due to the immunogenicity of secreted proteins, we analyzed the *M. tuberculosis* culture filtrate which is substantially enriched with secreted proteins with the aim of identifying novel antigens to be evaluated for future development of diagnostic methods. We used two different strategies to separate the proteins based on different physicochemical properties, to ensure that highly purified single proteins were identified as the target for antibody binding. We identified 20 antigens in the culture filtrate, including 4 novel antigens (Rv0063, Rv3587c, Rv2911 and Rv0932). All of the novel antigens have a predicted signal peptide, and the N-terminal peptide of Rv0063 and Rv2911 were identified in our previous study (Målen *et al.* 2007), confirming the existence of a cleavable signal peptide in these proteins.

Among the identified antigens, three of them were predicted to be lipoproteins, including Rv0934 (38-kDa antigen), which is known to be a surface exposed phosphate transport protein. This antigen is well known and has been extensively used for development of assays for the detection of tuberculosis (Harboe and Wiker 1992) and is also present in several commercially available assays for serodiagnosis of tuberculosis. Another lipoprotein observed in this study (LppZ, Rv3006) was recently reported to be seroreactive in both cavitary and non-cavitary tuberculosis patients (Sartain *et al.* 2006). A third lipoprotein that is closely related to the 38-kDa antigen, and also involved in phosphate transport (Braibant *et al.* 2000; Peirs *et al.* 2005), pstS2 (Rv0932), was also identified for the first time as a good serodiagnostic antigen in this study.

Four of the identified antigens, Rv0063, Rv0040 (MPT28), Rv3587c and Rv3881c were detected in more than one protein spot, with different molecular mass and isoelectric points. This is probably due to proteolytic digestion or post-translational modifications (PTMs) of these proteins such as amidation, glycosylation, phosphorylation or other modifications. However, all the isoforms of these proteins were serologically reactive, suggesting that the epitopes recognized by serum antibodies are on the peptide backbone of the protein and not on probable modification sites.

The immunogenicity of the identified antigens were evaluated by a relative quantification of the antigen spots displayed on the SYPRO-Ruby stained 2D-PAGE gels, and their corresponding spots on immunoblots probed with antibodies from tuberculosis serum pool. We found that the major antigens in the *M. tuberculosis* culture filtrate are the antigen 85 homologues 85A, 85B and 85C proteins (Wiker and Harboe 1992), with relative large amount of proteins and similarly relative large amount of antibodies in the tuberculosis patient sera. However, Rv3881c and

three other antigens predicted to be lipoproteins (Rv0934 (pstS1), Rv0932 (pstS2) and Rv3006 (LppZ)) were the most immunogenic considering the low amount of proteins present in the culture filtrate, compared to the relative amount of specific antibodies in the tuberculosis patient sera. This illustrates the significance of lipoproteins as potential diagnostic markers, and they should certainly be investigated further.

9. CONCLUSIONS

Data from this thesis shows that:

1. The culture filtrate produced by Sadamu Nagai was enriched with secreted proteins compared to what have reported by other investigators, leading to the identification of over 70 novel proteins predicted to be secreted to the extracellular environment by the pathogen.

2. AXA motif directly up-stream to signal peptides cleavage site is important for recognition and cleavage of signal peptides in *M. tuberculosis*.

3. Triton X-114 phase separation is a powerful method for extraction and enrichment of membrane and membrane associated proteins.

4. Several lipoproteins have dual localization, both in the culture filtrate as soluble proteins, and as hydrophobic porteins on the surface of the bacilli.

6. Estimation of the relative volume percentage values of antigens contained in the culture filtrate of *M. tuberculosis* show that lipoproteins are strongly immunogenic.

10. FUTURE PERSPECTIVES

Based on the findings of the present study, further studies on the identified novel proteins will help revealing their functions in terms of pathogen-host interaction and its essential for understanding the pathogenicity of the *M. tuberculosis*.

Membrane and membrane associated proteins extracted by Triton X-114 phase separation method, should be investigated in the aim of identification of novel antigens. Differentially expressed proteins from the membrane and membrane associated proteins between virulent strains and avirulent strains would be very interesting.

The specificity and sensitivity of the novel antigens identified in this study need to be evaluated by testing the immunogenecity of the purified antigens on a larger group of tuberculosis patients and controls.

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ERRATA

Typographical mistakes		**The word(s) to be corrected**	**The corrected word(s)**
The bacteria designation throughout (Introduction & paper III)		Mycobacterium tuberculosis	*Mycobacterium tuberculosis*
Protein annotation throughout (Introduction & paper III)		Rv0932	Rv0932c
Other corrections:			
Page	**Line**	**The word(s) to be corrected**	**The corrected word(s)**
4 (Introduction)	5	..amount ratio were lipoprotein, llustrating..	..amount ratio, illustrating
30 (Introduction)	10	..peptidase I of secreted..	..peptidase I in secreted..
31 (Introduction)	18	..protein ratio..	..protein amount ratio..

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Paper I

Comprehensive analysis of exported proteins from *Mycobacterium tuberculosis* H37Rv

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Comprehensive analysis of exported proteins from *Mycobacterium tuberculosis* H37Rv

Abstract:

Proteins secreted by *Mycobacterium tuberculosis* play an essential role in the pathogenesis of tuberculosis. The culture filtrates of *M. tuberculosis* H37Rv made by Sadamu Nagai (Japan), are considerably enriched for secreted proteins compared to other culture filtrates. Complementary approaches were used to identify the secreted proteins in these culture filtrates: (i) 2-DE combined with MALDI-TOF MS and (ii) LC coupled MS/MS. Peptides derived from a total of 257 proteins were identified of which 144 were identified by more than one peptide. Several members of the immunologically important early secretory antigenic target-6 (ESAT-6) family of proteins were found to be major components. The majority of the identified proteins, 159 (62%), were predicted to be exported through the general secretory pathway. We experimentally verified
that the signal peptides, which mediate translocation through the cell membrane, had been removed in 41 of the identified proteins, and in 35 of those, there was an AXA motif N-terminally to the cleavage site, showing that this motif is important for the recognition and cleavage of signal peptides in mycobacteria. A large fraction of the secreted proteins were unknown, suggesting that we have mapped an unexplored part of the exported proteome of *M. tuberculosis.*

Keywords:

Liquid chromatography-tandem mass spectrometry / Mass spectra / *Mycobacterium tuberculosis* / Secreted protein / Two-dimensional gel electrophoresis

Paper I
Supplementary table I

Supplementary table 1. A detailed list of *M. tuberculosis* H37Rv culture filtrate proteins identified by 2D-PAGE combined with MALDI-TOF MS and liquid chromatography coupled MS/MS

Gene number [a]	Theoretical molecular mass (kDa) [c]	Observed molecular mass (kDa) [d]	Theoretical isoelectric point (pI) [e]	Observed isoelectric point (pI) [f]	Identification method [g]	Sequence coverage [h]	Peptide sequence [i]	Fraction number [j]	Batch number [k]	Score	Minimum significance score ($p<0.05$)	Charge
Rv0002	42.1	50	4.76	4.79	MS	7/20%			A	52	51	
Rv0009	19.2	17	5.81	6.28	MS	7/57%			A	89	51	
Rv0012	23.22/23.22		6.57/6.57		LC-MS/MS	1	IDSVHGRSVDTALAAMQR	6	A	17	11	3
Rv0015c	43.34/-	31	10.33/-	4.88	MS	6/20%			A	54	51	
Rv0019c	10.33/-		8.38/-		LC-MS/MS	1	QGLVLQLTR	10	B	20	13	2
Rv0040c	28.92/27.29	30	8.68/8.91	5.8	MS	8/37%			A	105	43	
					LC-MS/MS	7	ALDITLPMPPR	10	A,C	20	12	2
					LC-MS/MS		WTQVPDPNVPDAFVVIADR	6	A,B,C	33	18	2
					LC-MS/MS		ENDMTLNTSR	6	A	37	21	2
					LC-MS/MS		HVIATSGADK	6	A	15	14	2
					LC-MS/MS		LGNSVYTSNAQLVVYR	6	A,B,C	97	23	2
					LC-MS/MS		LIGDFDPAEAITHGYIDSQK	6	A,B,C	51	21	2
					LC-MS/MS		LLAWQTTNASMANFDGFPSSIIEGTYR	8	A	80	19	3
Rv0054	17.35	20	5.12	5.2	MS	7/73%			A	143	51	
Rv0062	32.62/-	31.5	4.96/-	4.87	MS	9/64%			A	97	51	
Rv0063	46.21/46.21	35	6.81/6.81	6.83	MS	8/25%			A	54	51	
					LC-MS/MS	8	AMAFAAANNLK	7	A	67	22	2
					LC-MS/MS		AYSVGGYVNYLEVNQPPAR	4	A,C	41	24	2
					LC-MS/MS		DPAASGWEALSSALGGK	5	C	105	11	2
					LC-MS/MS		ILATCPAGSGGSVAAAIVSAVGTQPTG-TENHTFNYLDLVR	7	A	21	20	3
					LC-MS/MS		VLQPDDGPQFATAK	7	A,C	64	22	2
					LC-MS/MS		VTVTPATGLYAMHQVLAAAGR	3	A,C	15	12	3

					LC-MS/MS		YFGPNLSR	4	A,C	42	22	2
					LC-MS/MS		YLAVGNLNPSPLGYVGGSDVFTTITPA-TAQGIASAVDAFPR	7	A	70	19	3
Rv0064	103.44/102.95		6.26/6.38		LC-MS/MS	1	YELLSSGRK	5	A	21	13	1
Rv0066	82.54		5.7		LC-MS/MS	1	VPDNLAELGR	3	A	40	19	2
Rv0075	37.7		5.1		LC-MS/MS	1	ANRDHLAR	3	A	19	16	2
Rv0116c	23.88/22.13		7.36/7.36		LC-MS/MS	1	TVVMDSR	10	A	19	13	2
Rv0125	31.50/31.50	29	4.53/4.53	4.5	MS/MS	5	TQDVAVLQLR		A	64	51	1
					LC-MS/MS		TQDVAVLQLR	10	A,C	53	10	2
					LC-MS/MS		TGNVTLAEGPPA	8	A,C	18	11	2
					LC-MS/MS		GAGGLPSAAIGGGVAVGEPVVAMGNSGGQGGTPR	8	A,C	73	12	3
					LC-MS/MS		SGGGSPTVHIGPTAFLGLGVVDNNGNGAR	10	C	23	11	3
					LC-MS/MS		APPALSQDR	6	A	26	11	2
Rv0129c	32.02/32.02	32.5	4.99/4.99	5.6	MS	9/21%			A	80	51	
					LC-MS/MS	13	ADIQHVLNGATPPAAPAAPAA	8	A,C	18	10	2
					LC-MS/MS		DTYAADGGR	10	A	51	21	2
					LC-MS/MS		EMPAWLQANK	10	A,B,C	39	14	2
					LC-MS/MS		FLEGLTLR	10	A,B,C	36	13	2
					LC-MS/MS		FSRPGLPVEYLQVPSASMGR	8	A,B,C	43	16	3
					LC-MS/MS		IWVYCGNGTPSDLGGDNIPAK	6	A,B,C	10	9	2
					LC-MS/MS		LVANNTR	8	A	41	24	2
					LC-MS/MS		NDPMVQIPR	5	A,C	36	22	2
					LC-MS/MS		NGVFNFPPNGTHSWPYWNEQLVAMK	5	A,B,C	46	23	3
					LC-MS/MS		RNDPMVQIPR	5	A	38	22	2
					LC-MS/MS		TNQTFR	8	A	26	21	2
					LC-MS/MS		VQFQGGGPHAVYLLDGLR	1	A,B,C	97	10	3
					LC-MS/MS		WETFLTR	10	A,C	41	22	2
Rv0140	13.76		4.17		LC-MS/MS	1	MSNRIVLEPSADHPITIEPTNR	3	A	18	17	3
Rv0164	17.06/-	13	5.34/-	4.47	MS/MS	1	YPEWNEGVK		A	56	51	1
Rv0170	34.19/34.61		7.93/8.50		LC-MS/MS	1	ALHLVDGGRR	3	A	11	11	3

Rv0172	53.08/52.95		4.85/4.85		LC-MS/MS	1	TQVPTEWDELR	6	A	32	24	2
Rv0173	38.81[b]		6.01[b]		LC-MS/MS	1	LLAYVGGRSEVLNR	10	A,C	12	13	2
Rv0174	51.71/51.23		5.16/5.16		LC-MS/MS	2	GTVPSEIGPALDNSNR	8	A	28	13	2
					LC-MS/MS		VTAVEPTDQGAR	6	A	54	23	2
Rv0192	38.9		7.24		LC-MS/MS	1	LTVSDAVR	4	A	26	24	2
Rv0203	10.27/10.34		6.23/6.29		LC-MS/MS	1	AHFEANPK	10	A	31	23	2
Rv0211	67.25		4.68		LC-MS/MS	1	ALHSVGAPLEPGQK	3	A	33	17	3
Rv0219	16.27/16.27		10.96/10.96		LC-MS/MS	1	AGCSRVDAIDEE	10	C	15	12	3
Rv0237	37.67[b]	32	5.27[b]	5.2	MS	11/45%			A	76	51	
Rv0242c	46.83		6.4		LC-MS/MS	2	VVVVGGTPEAAASTNER	7	A	36	22	2
					LC-MS/MS		GQTNYATTK	5	A	39	22	2
Rv0244c	66.01	34	5.19	4.48	MS	6/21%			A	53	51	
Rv0265c	32.73[b]		5.43[b]		LC-MS/MS	1	AVLDAADVLIWMTESPEDEK	10	A	7	6	3
Rv0283	55.94		7.32		LC-MS/MS	1	SPIDLADHAVTSGLGLGADVPAPR	10	A	20	19	3
Rv0285	6.59/6.59		4.00/4.00		LC-MS/MS	2	AGVGVGESGASYLAGDAAAAATYGVVGG	8	A	67	23	3
					LC-MS/MS		APVITAVVPPAADPVSLQTAAGFSA-QGVEHAVVTAEGVEELGR	10	A	65	23	3
Rv0287	9.7	10	5.99	6	MS/MS	3	SLLDAHIPQLVASQSAFAAK		A	86	51	1
					LC-MS/MS		SLLDAHIPQLVASQSAFAAK	10	A	77	22	3
					LC-MS/MS		HTIGQAEQAAMSAQAFHQGESSAAFQAAHAR	10	A,C	78	19	4
					LC-MS/MS		VNTLLDVAQANLGEAAGTYVAADAAAASTYTGF	10	A	60	20	3
Rv0291	43.48/42.59	40	5.17/5.30	4.7	MS	7/21%			A	65	51	
					LC-MS/MS	2	GEGQLVAIIDTGVQPGPR	5	B	36	19	2
					LC-MS/MS		LVALSGTSYAAGYVSGVAALVR	5	B	35	19	3
Rv0309	19.09/18.62		8.48/8.49		LC-MS/MS	2	MDVYQR	10	A	31	22	2
					LC-MS/MS		HSVVMGVNK	10	A	27	23	2
Rv0315	28.81/27.11	22	4.79/4.77	4.75	MS	6/36%			A	66	51	
					LC-MS/MS	9	EWPFNDPGYK	6	C	43	24	2
					LC-MS/MS		FNCLAPGMWPAWWLSNDDPGR	9	C	13	9	3
					LC-MS/MS		GGIGTTWEAR	9	A,C	41	23	2

					LC-MS/MS		VFPVLNLAVGGSGGGDPATGSYPQEMLVDWVR	9	A,C	78	19	3
					LC-MS/MS		QNVFLDGNSNLVLR	9	C	65	22	2
					LC-MS/MS		SGEIDLIEWYGNGTWPSGTTVHANP-DGTAFETCPIGVDGGWHNWR	9	A	44	17	4
					LC-MS/MS		VTWNPSGMYFWLDYADGIEPYFSVPATGIEDLNEPIR	10	A	21	20	3
					LC-MS/MS		WQVSNHR	9	A	44	22	2
							TPIKNPVGFDRPQFFGQYR	9	C	20	14	2
					LC-MS/MS		YFGGLVHGLWR	8	C	42	23	2
Rv0340	18.35		4.53		LC-MS/MS	2	ANSLLDFVISLVR	7	A	38	22	2
					LC-MS/MS		SIAEAHLTDVTR	8	A	33	28	2
Rv0350	66.83	65	4.85	4.79	MS	6/36%			A	232	51	
					LC-MS/MS	7	NTTIPTKR	5	A	52	24	2
					LC-MS/MS		MPAVTDLVK	5	A	24	23	2
					LC-MS/MS		NQAVTNVDR	3	A	43	23	2
					LC-MS/MS		YTAPEISAR	3	A	41	22	2
					LC-MS/MS		NQAETLVYQTEK	3	A	54	21	2
					LC-MS/MS		AALGGSDISAIK	3	A	42	23	2
					LC-MS/MS		TTPSIVAFAR	3	A,C	34	23	2
Rv0363c	36.54	37	5.49	6	MS	10/44%			A	74	51	
Rv0398c	19.16/19.16	17	5.36/5.36	5.4	MS	10/48%			A	97	51	
					LC-MS/MS	5	DVASVFLPLQR	7	A,C	33	22	2
					LC-MS/MS		ISQQLFSFGDPTVQEVDGSDAAQFI-TAAAAVADRDVASVFLPLQR	10	A	32	18	4
					LC-MS/MS		VLGCQQNTAGSGAGFGAR	10	A,C	92	21	2
					LC-MS/MS		ISQQLFSFGDPTVQEVDGSDAAQFITAAAAVADR	10	A	35	19	3
					LC-MS/MS		RGEEGYFVLLAGTASDFCSAPNANYR	6	A	49	9	3
Rv0402c	100.65/99.77		6.31/6.21		LC-MS/MS	1	VADLSTLTDQLQRMIDITQR	7	A	12	12	4
Rv0408	72.94		5.2		LC-MS/MS	2	KIDTALELMDR	8	A	27	23	3
					LC-MS/MS		LRDSPVAGR	3	A	17	17	2
Rv0411c	32.87[b]	31	5.04[b]	5.25	MS	11/33%			A	91	51	

					LC-MS/MS	3	ILSAAER	3	A	16	15	2
					LC-MS/MS		TMSITCER	8	A	18	12	2
					LC-MS/MS		EIAPPPVIVSVVNWADCLVALQQR	1	A	28	11	3
Rv0436c	27.55/26.03		10.01/10.06		LC-MS/MS	1	ILDAQSR	8	A	18	14	2
Rv0446c	26.41/26.41		10.57/10.57		LC-MS/MS	1	GATPVQALRK	10	A	18	14	2
Rv0453	-/49.88		-/4.59		LC-MS/MS	1	MPMLPGAWDLGTWDR	4	C	13	11	3
Rv0455c	13.70/13.70	16	5.72/5.72	5.3	MS	5/41%			A	68	51	
					LC-MS/MS	2	DSTEDFPIPR	10	C	55	14	2
					LC-MS/MS		DTSPVYYQR	10	A	33	22	2
Rv0462	49.2	50	5.7	5.6	MS	11/30%			A	98	51	
					LC-MS/MS	1	ALPNEDADVSK	6	A	42	23	2
Rv0472c	26.38		7.72		LC-MS/MS	1	MLAVMLR	2	A,C	21	15	2
Rv0477	13.52/13.52	12.5	4.56/4.56	4.5	MSMS	4	LVDHTEWAQWGSLPSLR		A	75	51	1
					LC-MS/MS		DPEADPGAGEANYGGPPSSPR	10	A	44	13	2
					LC-MS/MS		VYPSQVGR	10	A	36	23	2
					LC-MS/MS		LVDHTEWAQWGSLPSLR	9	A	27	12	3
Rv0490	42.77/42.77		6.07/6.07		LC-MS/MS	1	SRATGGSGLGLAIVK	7	A	17	11	3
Rv0501	41.06	32	10.38	5.2	MS	8/22%			A	54	51	
Rv0506	13.44/12.52		6.99/6.31		LC-MS/MS	1	IAYLDPDAR	9	A	29	19	2
Rv0526	20.06[b] 19.66/19.30	18	5.17[b] 5.00/5.16	5.5	MS	11/60%			A	112	51	
					LC-MS/MS	3	GAGVSFLGIDVR	10	A	26	23	2
					LC-MS/MS		VAEEEPSGR	10	A	59	24	2
					LC-MS/MS		AEVSQLQR	4	A	45	24	2
Rv0559c	9.75/9.75		7.01/7.01		LC-MS/MS	5	GTTQGQAFQFLGAAIDHYCPEHVGVLQR	9	A	73	12	4
					LC-MS/MS		GVDGDAYK	10	A	30	21	2
					LC-MS/MS		SATFLQR	4	A	24	23	2
					LC-MS/MS		FGIYGPQDYNAWLAK	10	A	59	22	2
					LC-MS/MS		DDYDAPFNNTIHR	10	A,C	27	10	2
Rv0563	26.58/26.18		9.45/9.45		LC-MS/MS	1	IVRELATSAHQPMPR	6	C	14	10	3

Rv0583c	21.83[b]		4.39[b]		LC-MS/MS	1	LLPESSR	9	A	14	13	2
Rv0631c	119.5		6.39		LC-MS/MS	1	TRNHIAR	4	A	17	16	2
Rv0674	24.28/24.28		7.02/7.02		LC-MS/MS	1	SADGYRLSDR	5	A	13	9	2
Rv0677c	12.98/10.32	12	4.57/4.26	4.24	MS	5/61%			A	57	51	
					LC-MS/MS	2	ITVDGEVKDER	10	A	37	23	2
					LC-MS/MS		VFADDPEPFDPK	9	C	15	10	2
Rv0680c	10.33/10.33	9	4.65/4.65	4.6	MS/MS	1	NGDPFIWDR		A	56	51	1
Rv0732	43.93/41.85		9.23/9.32		LC-MS/MS	1	FEELR	6	A	12	11	1
Rv0774c	24.84/27.36		5.41/5.90		LC-MS/MS	1	ASGEDAGAMVLNELIPLLDTQR	6	A	24	10	2
Rv0787	31.70/-	23	4.78/-	5.18	MS	6/31%			A	58	51	
					LC-MS/MS	1	ACQLGAPLQSPSVTDDEPTR	10	A	98	21	2
Rv0835	20.83[b]	21	4.82[b]	4.83	MS	6/27%			A	52	51	
					LC-MS/MS	1	EPPEADTNVPGPCR	7	A	21	13	2
Rv0838	24.98[b]	24	5.97[b]	5.78	MS	6/33%			A	105	43	
					LC-MS/MS	3	AAGLVDVR	9	A	40	25	2
					LC-MS/MS		GVVPDAAIDLR	6	C	31	4	2
					LC-MS/MS		SVDVTFASAQR	7	C	18	9	3
Rv0843	35.72		5.87		LC-MS/MS	1	LIAAGTTR	8	A	33	24	2
Rv0851c	-/26.76		-/5.53		LC-MS/MS	1	GARVVLGDVDKPGLR	10	C	6	10	3
Rv0867c	36.75/36.31		3.64/3.64		LC-MS/MS	2	ATDGEWDQVAR	4	A	12	12	2
					LC-MS/MS		GLSNATPR	5	A	44	24	2
Rv0876c	57.93		11.35		LC-MS/MS	1	VMPPTIDLVR	3	A	21	20	2
Rv0884c	40.23	47.5	4.77	4.8	MS	8/37%			A	72	51	
Rv0928	35.84[b]	35	5.26[b]	4.8	MS	8/27%			A	56	51	
					LC-MS/MS	3	YPDSQVGTAVK	6	A	21	12	2
					LC-MS/MS		SDESGTTDNFQR	7	A	36	22	2
					LC-MS/MS		RPGSYPIVLATYEIVCSK	6	B	32	19	3
Rv0932c	35.73[b]	35	4.76[b]	5.9	MS	5/30%			A	66	58	
					LC-MS/MS	2	SGTSDNFQK	6	A	20	9	2
					LC-MS/MS		AFMQAAIGPGQEGLDQYGSIPLPK	6	B	20	17	3

Rv0934	35.90[b]	32	4.82[b]	4.75	MS	9/37%			A	65	51	
					LC-MS/MS	6	ASFLDQVHFQPLPPAVVK	5	B	45	18	3
					LC-MS/MS		DAATAQTLQAFLHWAITDGNK	5	B	29	21	2
					LC-MS/MS		GLMNIALAISAQQVNYNLPGVSEHLK	5	B	37	18	3
					LC-MS/MS		SDGSGDTFLFTQYLSK	6	B	24	17	2
					LC-MS/MS		SDGSGDTFLFTQYLSKQDPEGWGK	5	B	63	17	3
					LC-MS/MS		TWDDPQIAALNPGVNLPGTAVVPLHR	7	B	50	18	3
Rv0982	48.69/48.69		7.23/7.23		LC-MS/MS	1	MALNLMDNAAKWSPPGGHVGVR	9	C	10	9	3
Rv0996	35.97/36.16		4.62/4.60		LC-MS/MS	1	LGVENTR	9	A	15	14	2
Rv0999	20.88[b] 20.30/20.02		6.13[b] 6.07/6.08		LC-MS/MS	1	TTASTGDIAK	10	A	23	13	2
Rv1037c*	9.8	10	4.48	4.6	MS/MS	2	NFQVIYEQANAHGQK		A	110	51	1
					LC-MS/MS		TINYQFGDVDAHGAMIR	9	A	24	24	2
Rv1038c*	11	10	5.17	5.2	MS	4/33%			A	59	51	
					LC-MS/MS	4	FEVHAQTVEDEAR	10	A,C	96	23	2
					LC-MS/MS		FEVHAQTVEDEARR	9	A,C	53	18	4
					LC-MS/MS		NIVNMLHGVR	9	A	51	22	2
					LC-MS/MS		FMTDPHAMR	10	A	69	45	2
Rv1050	-/29.46		-/10.41		LC-MS/MS	1	SGRIMNMSSVVGR	10	A	14	11	3
Rv1074c	42.65	47	4.92	4.8	MS	11/45%			A	113	51	
Rv1075c	30.88/29.31		10.52/10.49		LC-MS/MS	1	ALAHTRGVR	7	A	11	9	2
Rv1077	48.63	50	5.17	5.2	MS	8/28%			A	74	51	
Rv1098c	50.14		5.18		LC-MS/MS	1	TAANSFEAQAAR	6	A	35	23	2
Rv1158c	18.54/15.12		4.23/4.56		LC-MS/MS	2	VDLPQLPYLPLQVPQQLSLPADLPALASGV-IPAAPIAPTPPAPGAPALPPGPPSLLAALP	4	A	9	6	4
					LC-MS/MS		DPAPAPAPAPNIPQQLISSAANAPQILQN-LATALGATPPLSAPK	10	A	23	10	3
Rv1161	136.92		6.67		LC-MS/MS	1	GVPVR	5	A	24	17	1
Rv1166	63.48[b]		5.14[b]		LC-MS/MS	1	VENIDPQR	5	A	13	11	2
Rv1174c	8.34/8.34		4.23/4.23		LC-MS/MS	3	NFLAAPPPQR	10	A,C	50	21	2

					LC-MS/MS		DPVDAVINTTCNYGQVVAALNATDPGA-AAQFNASPVAQSYLR	2	A	70	10	4
					LC-MS/MS		AAMAAQLQAVPGAAQYIGLVESVAGSCNNY	10	A	37	12	3
Rv1183	104.06/103.78		8.98/8.88		LC-MS/MS	2	GAQPNTSLADASISMSGYPVMLRDIR	9	A	14	9	3
					LC-MS/MS		DNGTLDKVVGLAR	7	C	17	12	3
Rv1186c	57.52		5.9		LC-MS/MS	1	HPSDSVVAGAVR	7	A	17	17	2
Rv1197*	10.96		5.02		LC-MS/MS	1	MWASAQNISGAGWSGMAEATSLDTMAQMNQAF	10	A	32	20	2
Rv1198	9.9	5	4.97	4.5	MS/MS	2	AQAGLLEAEHQAIIR		A	78	51	1
					LC-MS/MS		AQAGLLEAEHQAIIR	10	A	73	23	2
Rv1252c	17.71[b]		5.21[b]		LC-MS/MS	1	SEQPWNPEPLAGNYNECAQLSAVVIK	10	C	11	10	3
Rv1269c	9.08/9.08	8	6.26/6.26	5.7	MS	3/32%			A	45	43	
Rv1270c	22.60[b]		4.93[b]		LC-MS/MS	7	DASVAGSQQADGVATTK	9	A	21	13	2
					LC-MS/MS		GLANLLANLK	7	B	38	19	2
					LC-MS/MS		ITGNSSADDIATLAGSR	7	B	92	18	2
					LC-MS/MS		LAVTGDVPNLR	7	A,B	33	19	2
					LC-MS/MS		LEGDISNTPQTVATGSATLLVGNK	7	B	20	18	3
					LC-MS/MS		LEGDISNTPQTVATGSATLLVGNKSEDAK	7	B	22	17	3
					LC-MS/MS		TVPTTVWIASDGSSHLVQIQIAPTK	7	B	26	18	3
Rv1310	53		4.6		LC-MS/MS	1	NFGGTSVFAGVGER	3	B	19	18	3
Rv1323	40.08	40	4.91	5	MS	11/35%			A	71	51	
Rv1352	9.98/9.98	11	4.63/4.63	4.58	MS/MS	2	TEGPSNPLILVFGR		A	127	51	1
					LC-MS/MS		TEGPSNPLILVFGR	10	A,B,C	92	23	2
					LC-MS/MS		ETGEQFPGDGVFLVGTDIAPGTYR	10	A	44	9	3
Rv1371	55.12	10	9.44	4.33	MS	10/5%			A	55	51	
Rv1382	14.43/15.83		5.21/9.29		LC-MS/MS	1	AVLTRYPSGIMVER	8	A	18	15	2
Rv1386	6.39/7.03		4.63/4.63		LC-MS/MS	2	SGVGVAESGASYAAR	3	A	36	12	2
					LC-MS/MS		DALAAASYLSGGL	9	A	24	14	2
Rv1415	46.01	42.5	5.46	4.75	MS	9/33%			A	53	51	
Rv1419	13.65/13.65		4.18/4.18		LC-MS/MS	2	WNLTDDR	10	A	44	23	2
					LC-MS/MS		LQPCVNWISQHWTVQPDGLVK	9	C	12	11	3

Rv1435c	15.22/15.22		3.76/3.76		LC-MS/MS	1	DALTDPAPAGGPVPGQPVLPGPSASAPAGAR	10	A	33	13	3
Rv1436	35.95	35	5.19	5.25	MS	7/37%			A	52	51	
Rv1437	42.51	45	4.83	4.81	MS	9/36%			A	89	51	
Rv1438	27.4	30	5.54	5.66	MS	6/37%			A	53	51	
Rv1448c	40.72	50	4.87	4.88	MS	9/34%			A	101	51	
					LC-MS/MS	3	GQAGVANAR	5	A	29	23	2
					LC-MS/MS		ALSEGHTYDAQIAELAAR	5	B	32	19	3
					LC-MS/MS		TVTTDDVR	5	A	31	22	2
Rv1449c	75.55		4.78		LC-MS/MS	1	EDVLTHTR	4	A	24	22	2
Rv1454c	34.04	24	5.37	4.52	MS	12/72%			A	108	51	
Rv1475c	102.49	105	4.95	4.95	MS	32/44%			A	228	51	
					LC-MS/MS	6	IDTPGEADYYR	3	A	25	22	2
					LC-MS/MS		SNLIGMGVIPLQFPEGK	3	A	30	23	2
					LC-MS/MS		AVNDNDLSVTAVLSGNR	3	A	68	22	2
					LC-MS/MS		NGGILQYVLR	3	A	35	23	2
					LC-MS/MS		NEDGSNITK	2	A	27	23	2
					LC-MS/MS		AVIAESFER	2	A	26	22	2
Rv1477	45.72/45.72		6.07/6.07		LC-MS/MS	2	DANAAIAAAQHR	8	A	42	14	2
					LC-MS/MS		DPQTDTIAALIADVAK	7	C	22	11	2
Rv1488	37.88/37.88		6.15/6.15		LC-MS/MS	1	VARVELR	7	A	13	13	2
Rv1498A	7.62		6.23		LC-MS/MS	2	ALDWFEVQSIR	5	B,C	71	18	2
					LC-MS/MS		GHLVDGAVAHFQVTMK	5	B	26	18	3
Rv1541c	20.01[b]		6.20[b]		LC-MS/MS	1	DACAQDTDPR	3	A	11	11	3
Rv1729c	33.62		4.55		LC-MS/MS	1	TISNPFRCHGVDVDLASLVYTGPR	10	B	21	18	3
Rv1759c	-/71.30		-/5.07		LC-MS/MS	1	AGLYGNGGDGGAGGDGATSGKGGAG-GNAVVIGNGGNGGNAGK	10	A	13	10	3
Rv1784	101.47		5.46		LC-MS/MS	1	DVPVKPGR	4	A	17	16	2
Rv1793	10	9	4.76	4.45	MS/MS	1	AQAASLEAEHQAIVR		A	80	51	1
					LC-MS/MS		AQAASLEAEHQAIVR	7	A	68	19	3
Rv1804c	8.93/8.93	8.5	6.29/6.29	6.2	MS	3/54%			A	49	43	

Rv1810	8.43/8.43		4.50/4.50		LC-MS/MS	3	FAAIASGAYCPEHLEHHPS	10	A	39	12	2
					LC-MS/MS		DPTGDDAAFLAALDQAGITYADPGHAITAAK	10	A	97	9	3
					LC-MS/MS		DYNPGLTMDSAAK	10	A	31	13	2
Rv1812c	40.00/39.93		6.49/6.49		LC-MS/MS	1	TGVSVAAVSPGGVTLSSGERLAAATVVWCAGMR	8	A	12	6	4
Rv1815	20.18/20.18	24	5.33/5.33	5.4	MS/MS	5	QDNHVCTLGYVDPALK		A	121	51	1
					LC-MS/MS		QDNHVCTLGYVDPALK	9	A	43	16	3
					LC-MS/MS		STSEQVHADLGVTPLA	10	A	50	21	3
					LC-MS/MS		DNTPSGSTVATHELIADYEAIVLADDVTASNILPSGR	10	A	80	21	3
					LC-MS/MS		DPVLVFPGMEIR	10	A,C	54	13	2
					LC-MS/MS		GDSGGPVYLAPDGGPAQIVGIFNSVWGGFPAAVSWR	10	A	43	20	2
Rv1827	17.25	20	4.29	4.2	MS	4/39%			A	52	51	
Rv1833c	32.15		9.34		LC-MS/MS	1	TIIPR	6	A	19	19	1
Rv1837c	80.4	75	5.3	5.5	MS	23/37%			A	143	76	
Rv1845c	27.39/30.60		11.66/11.78		LC-MS/MS	1	RATWPLR	1	C	13	12	2
Rv1860	28.78/26.85	37	4.52/4.64	4.4	MS/MS	9	TTGDPPFPGQPPPVANDTR		A	65	51	1
					LC-MS/MS		TTGDPPFPGQPPPVANDTR	4	A,B,C	69	21	2
					LC-MS/MS		ALAESIRPLVAPPPAPAPAPAEPAPAP-APAGEVAPTPTTPTPQR	6	A,B	41	19	4
					LC-MS/MS		FSDPSKPNGQIWTGVIGSPAANAPDAGPPQR	7	A,B,C	45	12	3
					LC-MS/MS		IDNPVGGFSFALPAGWVESDAAHFDYGSALLSK	7	A	27	11	3
					LC-MS/MS		INQETVSLDANGVSGSASYYEVK	5	A,B	55	9	2
					LC-MS/MS		LYASAEATDSKAAAR	5	B	20	18	3
					LC-MS/MS		LGSDMGEFYMPYPGTR	4	A,B,C	51	10	2
					LC-MS/MS		LYASAEATDSK	5	A,B	65	23	2
					LC-MS/MS		WFVVWLGTANNPVDK	4	A,B,C	67	13	2
Rv1869c	41.61/42.11		4.92/4.93		LC-MS/MS	1	YDKLLLATGSAPR	4	C	11	12	2
Rv1876	18.34	18	4.5	4.48	MS	12/74%			A	145	51	
Rv1884c	11.21/-		8.01/-		LC-MS/MS	1	EQQIAVANR	5	A	32	23	2
Rv1886c	30.66/30.66	30	4.87/4.87	4.62	MS	8/41%			A	83	51	
					LC-MS/MS	10	AGCQTYKWETFLTSELPQWLSANR	8	A,B	57	20	3

					LC-MS/MS		FQDAYNAAGGHNAVFNFPPNGTHSWEYWGAQLNAMK	10	A	36	20	4
					LC-MS/MS		FQDAYNAAGGHNAVFNFPPNGTHSWEYWGAQLNAMKPDLQR	8	A	52	16	4
					LC-MS/MS		FSRPGLPVEYLQVPSPSMGR	8	A,B,C	54	22	3
					LC-MS/MS		LWVYCGNGTPNELGGANIPAEFLENFVR	8	A,B	41	13	3
					LC-MS/MS		NDPTQQIPK	4	A	30	13	2
					LC-MS/MS		VQFQSGGNNSPAVYLLDGLR	1	A,B,C	42	18	3
					LC-MS/MS		WETFLTSELPQWLSANR	8	A,B,C	32	19	2
					LC-MS/MS		AADMWGPSSDPAWER	6	A,B,C	50	12	2
					LC-MS/MS		AADMWGPSSDPAWERNDPTQQIPK	6	B	23	17	3
Rv1887	39.53		5.85		LC-MS/MS	1	VTSGEALTEPNPPEEQPNASAPQQDR	9	A	41	23	3
Rv1891	-/11.92		-/4.56		LC-MS/MS	1	GWQPGWFTGAGFFPPEP	10	A	16	12	2
Rv1899c	32.36[b]		9.79[b]		LC-MS/MS	1	HAGGVAAAIAR	1	C	12	13	2
Rv1906c	12.56/12.56		4.68/4.68		LC-MS/MS	2	KPQVVTIEPTDK	6	A	70	10	2
					LC-MS/MS		DPEPAPTPK	10	A	38	12	2
Rv1908c	80.7	45	5.13	5.5	MS	6/25%			A	61	51	
					LC-MS/MS	2	ALVEVYGADDAQPK	4	A	37	23	2
					LC-MS/MS		SSYGTGTGK	3	A	41	25	2
Rv1910c	17.57/16.49		5.71/5.38		LC-MS/MS	2	FTLYHLPAVPPLAGLAGTQAAR	10	A	28	22	3
					LC-MS/MS		APLAPKAAALGRSMPETPTGDVLTISSPAFADGAP	10	A	28	22	3
Rv1911c	17.57[b]		5.30[b]		LC-MS/MS	2	FTLYHLPVALQLPPGATGVQAAQAIAQAASGQAR	10	A	52	21	4
					LC-MS/MS		QGYFGPCPPAGTGTHHYR	6	A	43	10	3
Rv1926c	13.66/13.66	10.5	4.50/4.50	4.6	MS	5/52%			A	63	51	
					LC-MS/MS	7	GSVTPAVSQFNAR	10	A,B,C	50	10	2
					LC-MS/MS		IYFDVTGPSPTIVAMNNGMEDLLIWEP	10	A	50	23	2
					LC-MS/MS		LGSELTMTDTVGQVVLGWK	10	A,B,C	51	19	2
					LC-MS/MS		SSTAVIPGYPVAGQVWEATATVNAIR	10	A,B,C	63	17	3
					LC-MS/MS		SSTAVIPGYPVAGQVWEATATVNAIRGSVTPAVSQFNAR	10	A	35	21	3
					LC-MS/MS		TADGINYR	10	A	33	22	2
					LC-MS/MS		VLWQAAGPDTISGATIPQGEQSTGK	10	A,B,C	63	20	3
Rv1932	16.89	17	4.37	4.34	MS	7/61%			A	67	51	

Rv1980c	22.43/22.43	15	4.60/4.60	4.6	MS	5/26%			A	61	51		
					LC-MS/MS	10	AFDWDQAYR	6	A,B,C	26	16	2	
					LC-MS/MS		EAPYELNITSATYQSAIPPR	7	A,B,C	68	21	2	
					LC-MS/MS		FLSAATSSTPR	9	A	35	23	2	
					LC-MS/MS		GTDTGQACQIQMSDPAYNINISLPSYYPDQK	6	C	41	17	3	
					LC-MS/MS		GTQAVVLK	10	A	23	16	2	
					LC-MS/MS		KPITYDTLWQADTDPLPVVFPIVQGELSK	10	A	13	11	3	
					LC-MS/MS		QTGQQVSIAPNAGLDPVNYQNFAVTNDGVIF-FFNPGELLPEAAGPTQVLVPR	10	A	43	15	4	
					LC-MS/MS		SLENYIAQTR	6	A,B,C	42	22	2	
					LC-MS/MS		VYQNAGGTHPTTTYK	10	A	76	22	3	
					LC-MS/MS		DKFLSAATSSTPR	6	A,C	41	12	3	
Rv1984c	19.01/18.66		4.85/4.69		LC-MS/MS	4	ASASNGSDDASAHIQR	7	A	76	24	2	
					LC-MS/MS		GTHQASGLGDVGEAFVDSLTSQVGGR	8	C	36	21	3	
					LC-MS/MS		DPCSDIAVVFAR	10	A	35	22	2	
					LC-MS/MS		SIGVYAVNYPASDDYR	8	C	73	10	2	
Rv2006	14.57		6.25		LC-MS/MS	1	RLQVAGVR	4	A	30	23	2	
Rv2060	8.03/10.28		11.42/11.88		LC-MS/MS	1	GVPVR	5	A	24	17	1	
Rv2068c	30.02[b]	23	5.26[b]	5.1	MS	6/42%				74	51		
					LC-MS/MS	2	AGGGYDAEPR	9	A	32	23	2	
					LC-MS/MS		LITYTSDDIR	3	A	44	11	2	
Rv2074	15	12.5	9.5	4.47	MS	5/33%			A	55	51		
Rv2080	17.34[b]	15	5.19[b]	4.72	MS/MS	1	QIVAAADLQAVR		A	76	51	1	
Rv2110c	30.3	24	4.65	4.7	MS	8/40%			A	77	51		
Rv2140c	18.63	18	5.41	5.32	MS	5/76%			A	156	51		
					LC-MS/MS	1	TTSPDPYAALPK	10	A	34	22	2	
Rv2190c	35.71/35.71		5.74/5.74		LC-MS/MS	1	DPADDALAK	6	A	20	15	2	
Rv2192c	37.7		6.4		LC-MS/MS	1	HAAAVR	3	A	12	9	2	
Rv2200c	35.12/35.32		5.76/5.62		LC-MS/MS	1	GELAPQPVG	7	C	11	10	2	
Rv2201	70.21/70.21		6.45/6.45		LC-MS/MS	1	SFSGAQLR	2	C	10	9	2	

Rv2220	53.57	57	5.4	5	MS	5/45%			A	53	51	
					LC-MS/MS	1	IPITGSNPK	6	A	28	22	2
Rv2224c	52.65[b]		5.38[b]		LC-MS/MS	1	GVASSRPAIWCNSDADNDRLR	10	A	17	12	2
Rv2236c	33	25	10.85	5.3	MS	4/20%			A	55	51	
Rv2241	100.18		5.84		LC-MS/MS	1	IVPIIPDEAR	2	A	18	15	2
Rv2251	44.02/47.37		5.79/5.98		LC-MS/MS	1	ATLDPAGILNPGKLIP	10	A	20	13	2
Rv2253	15.21/15.14	14	5.59/5.59	6	MS	6/62%			A	47	43	
					LC-MS/MS	5	TGTSMAANRPEYPHK	10	A	13	11	3
					LC-MS/MS		ANYTFSSR	10	A	50	24	2
					LC-MS/MS		NEFIPRPIEYTWNGTQWVR	7	A,C	35	10	3
					LC-MS/MS		SITAYTPGQYGILTGVFHTDIASGTCK	7	A	29	13	3
					LC-MS/MS		GNVDMPVSAKPIVG	7	A	39	13	2
Rv2299	72.96		4.51		LC-MS/MS	1	LRIEALR	4	A	18	18	2
Rv2301	20.62/19.67	21	4.96/4.96	5.5	MS/MS	3	FEPPGIGTVGNAFVSALR		A	92	51	1
					LC-MS/MS		FEPPGIGTVGNAFVSALR	8	A,C	78	21	2
					LC-MS/MS		ACPDAEVVFAR	6	A	30	16	2
					LC-MS/MS		GRFEPPGIGTVGNAFVSALR	10	A,B	78	21	3
Rv2314c	48.71	50	4.93	4.96	MS	10/23%			A	61	51	
Rv2334	32.75	32.5	5.2	52	MS	11/53%			A	100	51	
Rv2346c	9.9	8	4.76	44.2	MS/MS	2	AQAGLLEAEHQAIVR		A	81	51	1
					LC-MS/MS		AQAGLLEAEHQAIVR	7	A	99	18	2
					LC-MS/MS		DVLAAGDFWGGAGSVACQEFITQLGR	9	A	103	20	3
Rv2376c	14.74/11.61	12	5.10/5.51	4.82	MS/MS	3	GSLVEGGIGGTEAR		A	71	51	1
					LC-MS/MS		GSLVEGGIGGTEAR	9	A,B,C	69	18	2
					LC-MS/MS		ASAMELLQAAGN	9	A,B	17	12	2
					LC-MS/MS		AAEHGDLPLSFSVTNIQPAAAGSATADVSVSGPK	10	A	32	22	3
Rv2401	11.1	15	5.17	4.28	MS	4/33%			A	54	51	
Rv2430c	21.98	27	4.77	4.98	MS/MS	2	APPPIAHSTVLVAPVSPSTASSR		A	91	51	1
					LC-MS/MS		APPPIAHSTVLVAPVSPSTASSR	9	A	40	18	3
					LC-MS/MS		SLDVEMTAVQR	9	A	44	22	2

Rv2431c	10.6	10.8	5.76	5.23	MS	4/69%			A	58	51	
					LC-MS/MS	1	YATAEADNIK	10	A	54	21	2
Rv2445c	14.5	17	5.34	5.26	MS	6/53%			A	65	51	
Rv2450c	14.78/14.78		4.12/4.12		LC-MS/MS	1	VAENVLR	10	A	17	14	2
Rv2465c	17.27	15.5	6.14	6.2	MS	6/40%			A	70	51	
Rv2469c	21.94/20.99		9.86/9.73		LC-MS/MS	1	ADTVDHVVPR	7	C	16	12	2
Rv2544	20.74[b]		5.25[b]		LC-MS/MS	1	FNDDSYGQDFYRNGSLCK	3	A	13	11	3
Rv2563	31.11/31.41		8.39/6.80		LC-MS/MS	1	TVDSMGVDAFVVK	1	C	16	14	2
Rv2575	30.8		4.93		LC-MS/MS	1	IQQQTTGR	9	A	33	24	2
Rv2576c	11.56/11.56		4.93/4.93		LC-MS/MS	3	YMITLHTPIAGGQPLVYTATLANPSQWAIVTASGGLR	10	A	44	19	3
					LC-MS/MS		DPVGHQVTYTVTTTSDLMANIR	10	A,C	46	10	3
					LC-MS/MS		YMSADPPSMAAFNADSSK	10	C	35	21	2
Rv2585c	55.43[b]		4.96[b]		LC-MS/MS	1	ALALCVPR	1	C	13	6	4
Rv2631	45.52	50	6.99	4.95	MS	11/21%			A	53	51	
Rv2668	16.22/15.31	25.5	4.90/4.87	4.67	MS	6/39%			A	66	51	
					LC-MS/MS	5	GDAPIGHIGDTLR	10	A	40	13	3
					LC-MS/MS		DPVSVVVLLDEK	10	A	63	25	2
					LC-MS/MS		GGVYWDAYRDPVSVVVLLDEK	10	A	40	22	3
					LC-MS/MS		VDTGTYVADVTVSSVVPVDPPPGFGYTR	10	A	67	20	3
					LC-MS/MS		SFPDSSVTR	10	A	41	23	2
Rv2672	51.15[b]	55	4.82[b]	4.81	MS	19/54%			A	169	51	
					LC-MS/MS		SGDMNLLSALINR	6	A	30	23	2
Rv2693c	17.10/20.10		9.62/10.01		LC-MS/MS	1	AGLLPAIGFALSMAGLILLWRLLR	10	A	13	12	3
Rv2721c	68.94/68.94	35	4.42/4.42	4.68	MS	15/28%			A	108	51	
					LC-MS/MS	2	AAGGAAGPLGAK	6	A	40	22	2
					LC-MS/MS		EFTTVPAVLAEQLK	6	A	21	13	2
Rv2766c	22.73/24.19		5.14/5.42		LC-MS/MS	1	VNAICPGVVRTR	2	A	17	14	2
Rv2799	19.89/17.11	23	5.24/5.22	5.4	MS	7/33%			A	67	51	
					LC-MS/MS	4	ANDLVPYYR	10	C	30	21	2
					LC-MS/MS		DIPFDVIQR	10	A,C	20	12	2

					LC-MS/MS		LGLAYTPPEAEEGLR	10	A,C	81	22	2
					LC-MS/MS		TYAQTLPPDAIETTIAGHR	10	A,C	14	12	3
					LC-MS/MS		AAQYWVR	10	A	22	13	2
Rv2823c	90.74		6.7		LC-MS/MS	1	WVYFLTR	4	A,C	33	18	2
Rv2831	26.63	30	5.82	5.75	MS	12/53%			A	157	51	
					LC-MS/MS		TSGDTIAANR	9	A	33	23	2
Rv2848c	44.05/45.55		5.96/6.11		LC-MS/MS	1	FVAHAACNTPRA	4	C	14	13	2
Rv2873	19.80[b]	17	4.67[b]	4.38	MS/MS	2	IDGTHQTLQGADLTVIGAR		A	98	51	1
					LC-MS/MS		LPAATIDQLK	10	A	25	23	2
Rv2874	71.16/71.16		9.64/9.64		LC-MS/MS	1	CGYHSHLTGGEFDVNR	6	A	5	3	4
Rv2875	16.31/16.31	16.7	4.31/4.31	4.36	MS/MS	5	QTLQGASVTVTGQGNSLK		A	57	51	
					LC-MS/MS		QTLQGASVTVTGQGNSLK	8	A	26	23	2
					LC-MS/MS		LPASTIDELKTNSSLLTSILTYHVVAGQTSPANVVGTR	10	A	22	20	4
					LC-MS/MS		LPASTIDELK	10	A	22	20	2
					LC-MS/MS		VGNADVVCGGVSTANATVYMIDSVLMPPA	10	A	24	20	3
					LC-MS/MS		TNSSLLTSILTYHVVAGQTSPANVVGTR	7	A	44	10	3
Rv2878c	14.62/14.62	12.5	4.57/4.57	4.2	MS	5/38%			A	55	51	
					LC-MS/MS	4	ADGTSTFVNNPTAAMSQDELSGR	10	A,B,C	48	22	3
					LC-MS/MS		ADVGAMQSFVSK	10	A,B,C	70	22	2
					LC-MS/MS		YNLNFTNLNDADGVIWAR	10	A,B,C	43	11	2
					LC-MS/MS		YNVPWQPAFVFYR	9	A,B,C	54	13	2
Rv2905	31.18[b]		4.96[b]		LC-MS/MS	1	YGLRSTAPPSDGR	7	C	14	11	3
Rv2911	28.38[b] 27.62/27.30	22	4.75[b] 4.75/4.75	4.7	MS	9/40%			A	54	51	
					LC-MS/MS	6	AATLGATSTHATTPSGLDGPGGSGASTAHDLVVIFR	7	B	20	17	4
					LC-MS/MS		DQNVAHPPASTIK	6	A	54	22	2
					LC-MS/MS		KTFVGAAAR	8	A	36	24	2
					LC-MS/MS		VLLALVALDELDLNSTVVADVADTQAECNCVGVKPGR	1	A	65	19	4
					LC-MS/MS		YPGAIGGK	8	A	51	23	2
					LC-MS/MS		QLLDGLLLVSGNDAANTLAHMLGGQDVTVAK	1	A	60	11	4

Rv2945c	22.36[b]	13	4.89[b]	5.3	MS	7/48%			A	62	51	
Rv2994	43.49/42.58		11.22/11.23		LC-MS/MS	1	CHAWPNGPR	7	A	18	12	2
Rv3004	7.60/8.70		10.61/11.32		LC-MS/MS	2	LRTLADER	9	A	14	14	2
					LC-MS/MS		WDDIDGLRFHR	8	A	15	12	2
Rv3006	36.41[b]	33	4.71[b]	4.61	MS	9/36%			A	75	51	
					LC-MS/MS	1	LAPSTGAVTGEPDVVR	7	A	60	23	2
Rv3016	20.23[b]	22	4.56[b]	4.38	MS/MS	1	TTFQDRPDGSLISEAAAAYR			61	51	1
Rv3031	57.79		7.2		LC-MS/MS	1	NRLLSESER	3	A	29	22	2
Rv3033	15.32/15.32		4.73/4.73		LC-MS/MS	1	VGARPDSVTCPDNLKGVEGAK	7	A	35	10	3
Rv3036c	22.08/20.94		4.91/4.91		LC-MS/MS	4	DGFVNVAQGSPLR	7	A,B,C	78	19	2
					LC-MS/MS		DQPYQMDATSEQHSSGQPPQATR	10	A,C	60	21	3
					LC-MS/MS		FFQDLGGAHPSTWYK	1	A,B	42	16	2
					LC-MS/MS		AFNYNLATSQPITFDTLFVPGTTPLDSIYPIVQR	10	A	36	19	3
Rv3044	35.20[b]	27	5.09[b]	4.97	MS	15/32%			A	130	51	
					LC-MS/MS	3	AAAAADPGPPTRPAHNAAGVSPEMVQVPAEAQR	3	A	33	6	4
					LC-MS/MS		IAAVDALITGFAEHATQVGTK	4	A,C	68	13	3
					LC-MS/MS		AYIEIGTTAADLAK	3	A	34	12	2
Rv3106	46.76/46.76		5.37/5.37		LC-MS/MS	1	AAGEPHGRPR	4	A	13	11	2
Rv3111	17.84	32	8.76	5.26	MS	6/29%			A	52	51	
Rv3158	48.68/52.08		7.97/9.25		LC-MS/MS	1	LLSQEAAMK	4	C	14	11	2
Rv3193c	103.24/103.24		8.53/8.53		LC-MS/MS	2	LDEAITK	2	A	17	12	2
					LC-MS/MS		AVVLARLR	4	A	17	12	2
Rv3194c	32.14/32.03		5.05/5.05		LC-MS/MS	1	VGQIGGITHK	3	A	13	6	2
Rv3201c	112.33/112.33		5.91/5.91		LC-MS/MS	2	LAWAALR	4	A	16	14	2
					LC-MS/MS		AEADGVKPPTAAVLVR	3	A	19	10	3
Rv3240c	105.98		5.09		LC-MS/MS	1	FLGLQVGVILATMTPDERR	1	A	21	16	3
Rv3244c	59.58[b]	57	5.12[b]	5.9	MS	11/24%			A	82	51	
					LC-MS/MS	3	IPVDSTAVASR	5	A	44	24	2
					LC-MS/MS		MPEQTAAAVSR	5	A	51	23	2
					LC-MS/MS		NTLYFADPTGK	9	A,C	17	13	2

Rv3248c	54.32		4.85		LC-MS/MS	1	IADLSLADFGR	6	A	32	23	2
Rv3267	48.06/47.79	50	4.87/4.87	4.82	MS	10/30%			A	59	51	
					LC-MS/MS	1	ADDLGAQQVAK	10	A	20	14	2
Rv3310	28.03/27.14	25	5.40/5.40	5.55	MS/MS	2	TNNSLLVVTWDEDDGSSR		A	90	51	1
					LC-MS/MS		SQAAIIGNK	9	A	27	24	2
Rv3354	9.70/9.70		3.72/3.72		LC-MS/MS	2	ALGQSVCPILAEPGGSFNTAVASVVAR	10	A	28	13	3
					LC-MS/MS		NPVDDAFIAALNNAGVNYGDPVDAK	10	A	76	13	3
Rv3356c	29.48	32	5.97	6.2	MS	8/32%			A	75	51	
Rv3369	15.7		5.69		LC-MS/MS	2	FGLTEAIAAYSTR	10	B	69	19	2
					LC-MS/MS		LTSDLYGWLTTVAR	9	B	47	18	2
Rv3397c	33.14		7.37		LC-MS/MS	1	MEIDWTGCRDFDELIVYCR	7	A	8	6	4
Rv3402c	-/42.90		-/6.45		LC-MS/MS	1	TGMADAGVR	10	A	19	10	2
Rv3413c	31.24		4.52		LC-MS/MS	2	QDLINEVNLLNTK	7	A	40	22	2
					LC-MS/MS		VEQMIAQGQWAEAQDELAEVSSTVQAVTDGSR	10	A	58	41	3
Rv3418c	10.7		4.62		LC-MS/MS	5	IPLDVAEGDTVIYSK	1	B	19	19	2
					LC-MS/MS		RIPLDVAEGDTVIYSK	9	B	32	19	3
					LC-MS/MS		VNIKPLEDKILVQANEAETTTASGLVIPDTAK	9	A	69	16	4
					LC-MS/MS		YGGTEIKYNGEEYLILSAR	9	A	36	20	3
					LC-MS/MS		YNGEEYLILSAR	10	A	48	19	2
Rv3428	45.46		9.19		LC-MS/MS	1	ERVTVPR	3	A	19	19	2
Rv3484	49.50/48.03	48	4.72/4.68	4.61	MS	9/23%			A	78	51	
Rv3485c	31/31		5.98/5.98		LC-MS/MS		VCTPLPR	8	C	20	19	2
Rv3491	17.40/17.40	17	5.47/5.47	5.5	MS/MS		LGPPPDGSYSFNQAGVSGVTWTITALCDQPSGTR		A	46	43	3
					LC-MS/MS	4	NMNDYSDPIVWAFNCALNVVSTTPQQITR	10	A	88	19	3
					LC-MS/MS		LQNFSGR	10	A	23	22	2
					LC-MS/MS		QPFSLQLIGPPPSPVQR	10	A,C	60	12	2
Rv3495c	39.14[b]		4.80[b]		LC-MS/MS	1	ALDTLPDAVR	10	A	12	11	2
Rv3509c	52.07	64	4.76	4.7	MS	16/40%			A	116	51	
Rv3572	16.29/16.22	13	4.75/4.75	4.55	MS	4/30%			A	55	51	
					LC-MS/MS	3	AGGSDVITTVYFGEGPPDK	10	A,C	9	7	2

					LC-MS/MS		LAYLDAHATSQFER	8	A,C	24	22	3
					LC-MS/MS		TPDGPTGFPPGLWAR	10	A,C	58	22	2
Rv3584	15.76[b]		8.11[b]		LC-MS/MS	4	IQAVQTSDFIQPGK	7	A	23	13	2
					LC-MS/MS		LTINNVLLR	7	A	67	7	2
					LC-MS/MS		AVDLVLVAVNQSPDVSDR	7	A	58	12	2
					LC-MS/MS		LPASGMLFVGTPDGQIVAPGPLPSNQAAK	7	A	24	10	3
Rv3587c	22.18/22.18	32	5.79/5.79	4.91	MS	7/32%			A	82	51	
					LC-MS/MS	5	DVGAAVLAAYVYSLDNK	6	A,B	21	13	2
					LC-MS/MS		DVGAAVLAAYVYSLDNKR	5	A,B,C	98	22	3
					LC-MS/MS		EGDDCPDSTLAVK	7	A	35	22	2
					LC-MS/MS		GLTNAPQYYVGDQPK	10	A,B,C	25	11	2
					LC-MS/MS		TFSPGEQVTTAVTWTGMGS	10	A	52	12	2
Rv3627c	43.89/41.33		5.71/4.96		LC-MS/MS	1	SRTPALDAGR	10	A	12	11	2
Rv3629c	34.21/36.57		9.41/8.80		LC-MS/MS	1	WIEVPFAR	7	A	12	11	2
Rv3668c	20.99[b] 20.18/20.18		4.63[b] 4.63/4.63		LC-MS/MS	4	VTPVAVFNGFAINGIGPDPSFGQIACK	10	A	18	10	3
					LC-MS/MS		DDKLPLGGGAGIVVNGDTMCTLTTIGHDK	10	A	22	11	4
					LC-MS/MS		YIPLHTPAVVMSINADLADINAK	6	A	72	10	3
					LC-MS/MS		NRPGAGFVPVPA	6	A	28	12	2
Rv3671c	36.45/-	25	6.38/-	4.45	MS	5/21%			A	52	51	
Rv3682	81.94/80.38	50	5.26/5.20	5.1	MS	12/20%			A	60	51	
					LC-MS/MS	1	LKDAGFQVADQTNSVNSSAK	10	A	25	21	3
Rv3693	42.92/42.92		11.30/11.30		LC-MS/MS	1	RVVIVLDTGR	9	C	14	10	2
Rv3705c	19.99/19.99		4.91/4.91		LC-MS/MS	3	QAVGVFASNDAADR	7	A,C	31	22	2
					LC-MS/MS		HPSEPGVVSYAVLGK	10	A,C	41	12	2
					LC-MS/MS		CFVQTR	10	A	27	21	2
Rv3710	70.11		5.2		LC-MS/MS	1	GGVAYIK		A	23	22	2
Rv3722c	47.34	40	5.63	5.65	MS	9/26%			A	69	51	
					LC-MS/MS		HQQILAPK	5	A	29	23	2
Rv3725	-/30.11		-/9.53		LC-MS/MS	1	CPIPR	7	A	31	23	2

Rv3759c	30.46[b] 30.10/30.10		5.20[b] 5.20/5.20		LC-MS/MS	1	DPLGSATGSVK	8	A	12	11	2
Rv3760	5.56/5.56		12.60/12.60		LC-MS/MS	1	KTHAAALR	4	A	15	11	2
Rv3803c	28.48/27.81	25	5.52/5.51	5.5	MS	9/41%			A	58	51	
					LC-MS/MS	9	DIPVAFLAGGPHAVYLLDAFNAGPDVSNW- VTAGNAMNTLAGK	9	A	48	6	4
					LC-MS/MS		GISVVAPAGGAYSMYTNWEQDGSK	10	A	43	23	2
					LC-MS/MS		GLAPGGHAAVGAAQGGYGAMALAAFHPDR	9	A,B,C	21	11	4
					LC-MS/MS		MFYNQYR	9	A,C	36	22	2
					LC-MS/MS		QWDTFLSAELPDWLAANR	6	A,B,C	58	21	2
					LC-MS/MS		SVGGHNGHFDFPASGDNGWGSWAPQLGAMSGDIVGAIR	9	A,C	39	19	4
					LC-MS/MS		VWVWSPTNPGASDPAAMIGQAAEAMGNSR	9	A,B,C	40	21	3
					LC-MS/MS		APYENLMVPSPSMGR	10	A,C	34	3	2
					LC-MS/MS		WHDPWVHASLLAQNNTR	6	A,C	38	10	3
Rv3804c	32.34/31.65	30	5.32/5.32	5.6	MS	7/21%			A	58	43	
					LC-MS/MS	11	ALGATPNTGPAPQGA	8	A,C	20	12	2
					LC-MS/MS		ASDMWGPK	8	A	48	24	2
					LC-MS/MS		EDPAWQR	4	A	26	23	2
					LC-MS/MS		FLEGFVR	6	A,B,C	25	12	2
					LC-MS/MS		LIANNTR	10	A	30	24	2
					LC-MS/MS		NDPLLNVGK	10	A,C	33	22	2
					LC-MS/MS		VQFQSGGANSPALYLLDGLR	6	A,B,C	36	23	3
					LC-MS/MS		VWVYCGNGKPSDLGGNNLPAK	5	A,B,C	30	21	3
					LC-MS/MS		WETFLTSELPGWLQANR	6	A,B,C	27	21	2
					LC-MS/MS		AGCQTYKWETFLTSELPGWLQANR	8	A,B,C	46	16	3
					LC-MS/MS		ASDMWGPKEDPAWQR	6	A,B	26	18	3
Rv3835	40.76/-	34	5.06/-	4.91	MS	10/23%			A	70	51	
Rv3841	20.44	20	4.73	4.81	MS/MS	1	AGANLFELENFVAR		A	88	51	1
Rv3846	23.3		5.96		LC-MS/MS	3	YAAATSQTK	10	A	59	22	2
					LC-MS/MS		AKEDHSAILLNEK	10	A	42	21	3

					LC-MS/MS		NLSPNGGDKPTGELAAAIADAFGSFDKFR	6	A	49	20	4
Rv3849	14.71	9	8.82	5.7	MS	5/45%			A	52	51	
Rv3872	9.27		3.95		LC-MS/MS	1	AGEAVQDVAR	7	A	50	45	2
Rv3874	10.79	10	4.59	4.5	MS	6/67%			A	55	51	
					LC-MS/MS		ADEEQQQALSSQMGF	10	A	56	22	2
					LC-MS/MS		AEMKTDAATLAQEAGNFER	9	A,C	47	25	2
					LC-MS/MS		FQEAANK	10	A	27	22	2
					LC-MS/MS		GAAGTAAQAAVVR	10	A	42	23	2
					LC-MS/MS		ISGDLK	10	A	24	24	2
					LC-MS/MS		QAGVQYSR	10	A	42	23	2
					LC-MS/MS		QELDEISTNIR	10	A	49	42	2
					LC-MS/MS		QKQELDEISTNIR	9	A,C	49	42	2
					LC-MS/MS		TDAATLAQEAGNFER	10	A,B,C	47	25	2
					LC-MS/MS		TQIDQVESTAGSLQGQWR	9	A,B	61	41	3
					MS/MS		TQIDQVESTAGSLQGQWR		A	114	51	1
Rv3875	9.9	9	4.48	4.25	MS/MS	2	WDATATELNNALQNLAR		A	75	51	1
					LC-MS/MS		WDATATELNNALQNLAR	10	A,B	38	26	2
					LC-MS/MS		LAAAWGGSGSEAYQGVQQK	10	A	67	41	2
Rv3881c	47.59	60	4.75	4.55	MS	10/39%			A	124	76	
					LC-MS/MS	10	ANEVEAPMADPPTDVPITPCELTAAK	5	A	22	20	3
					LC-MS/MS		DQILPVYAEYQQR	6	A,C	63	21	2
					LC-MS/MS		EHPTYEDIVGLER	5	A,C	26	22	2
					LC-MS/MS		EYLAAGAK	7	A	43	23	2
					LC-MS/MS		EAAALSGDVAVK	3	A	46	22	2
					LC-MS/MS		LYAENPSAR	6	A	39	23	2
					LC-MS/MS		TQSQTVTVDQQEILNR	5	A	97	22	2
					LC-MS/MS		VATAGEPNFMDLK	6	A,C	30	21	2
					LC-MS/MS		VLTEYNNK	6	A	32	22	2
					LC-MS/MS		AALEPVNPPKPPPAIK	4	A,C	25	23	3
Rv3899c	-/36.08		-/5.72		LC-MS/MS	1	RDSLPR	2	C	16	13	1

Rv3914	12.54	12	5.6	4.9	MS	4/39%			A	59	51		
					LC-MS/MS	5	ATDLTVAKLDVDTNPETAR	9	B	77	17	3	
					LC-MS/MS		MVAPVLEEIATER	9	A,B	61	18	2	
					LC-MS/MS		NFQVVSIPTLILFK	10	A,B,C	38	24	2	
					LC-MS/MS		ATDLTVAK	9	B	29	24	2	
					LC-MS/MS		NFQVVSIPTLILFKDGQPVK	10	B	48	19	3	
Rv3917c	34.63/34.46		5.96/6.12		LC-MS/MS	1	SLAGSQTGVRYQIVMGER	3	A	15	13	3	
MT0066,1 [a]	9.02/9.02	13.7	4.20/4.20	4	MS/MS	1	WGFGDLAVCDGEK		A	58	51	1	
MT2420 [a]	10.00	10	4.97	4.6	MS/MS	1	AQAAALEAEHQAIVR		A	131	51	1	
MT3437,1 [a]	17.35	9	11.07	5.18	MS	7/41%			A	52	51		

[a]Gene number as annotated for *M. tuberculosis* H37Rv (Rv) or *M. tuberculosis* CDC1551 (MT). Three proteins were only identified with the annoted genes for *M. tuberculosis* CDC1551. The nucleotide sequence of MT0066.1 is identical in *M. tuberculosis* H37Rv and found in positions 65012..65392 on the minus (-) strand overlapping with Rv0061 which is annotated as a conserved hypothetical protein (questionable ORF). The sequence of the peptide identified to belong to MT2420 is not encoded in the *M. tuberculosis* H37Rv genome. The peptide is highly homologous to a peptide found in Rv1793, the only difference being an A in MT2420 instead of an S. The peptide may therefore be derived from Rv1793 being modified posttranslationally by removal of a hydroxyl group to change S to A. The nucleotide sequence of MT3437.1 is identical in *M. tuberculosis* H37Rv and found in positions 3720757..3721236 on the plus (+) strand overlapping slightly with Rv3333c (position 3720782), extending in the gap between this gene and Rv3334 which starts at position 3721257.

[b]Theoretical molecular mass (kDa) or isoelectric point (p*I*) calculated after removing the predicted signal peptide as determined by prediction of lipoprotein consensus motif using Compute pI/MW tool, publicly available at (http://au,expasy,org/tools/pi_tool,html). See also supplementary table 2.

[c]Theoretical molecular mass (kDa) calculated after removing the predicted signal peptide by Neural Network/Hidden Markov model, using Compute pI/MW tool, publicly available at (http://au,expasy,org/tools/pi_tool,html). Only one figure is given when the predictions were concordant. If one of the methods did not predict a signal peptide it is designated with a "-". See also supplementary table 2.

[d]Observed molecular masses as determined by 2D-PAGE.

[e]Theoretical isoelectric point (p*I*) calculated after removing the predicted signal peptide by Neural Network/Hidden Markov model, Compute pI/MW tool, publicly available at (http://au,expasy,org/tools/pi_tool,html). If one of the methods did not predict a signal peptide it is designated with a "-". See also supplementary table 2.

[f]Observed p*I* as determined by 2D-PAGE.

[g]Peptides were identified by Matrix-assisted laser desorption ionization time-of-flight mass spectrometry of spots collected from 2D-PAGE (MS), Matrix-assisted laser desorption ionization time-off-flight mass spectrometry combined with tandem mass spectrometry (MS/MS) or Liquid chromatography Electrospray Ionisation with tandem mass spectrometry (LC- MS/MS)

[h]None of the identified peptides by MS or MS/MS were predicted signal peptides,

[i]Sequence coverage for individual proteins is given as number of peptides/percentage of sequence coverage for MS data, or as number of identified peptides for MS/MS and LC-MS/MS data.

[j] Fraction number of identified peptide after SDS-PAGE performed as shown in Figure 2. Explanation of the fraction numbers: (1) >160 kDa, (2) ranges from 105-160 kDa, (3) ranges from 75-105 kDa, (4)) ranges from 50-75 kDa, (5) ranges from 35-50 kDa, (6) ranges from 30-35 kDa, (7) ranges from 25-30 kDa, (8) ranges from 15-25 kDa, (9) ranges from 15-10 kDa, (10) <10 kDa. Many peptides were observed several times and also in more than one fraction. In such cases the fraction with most observations or most reliable observation is given.

[k] Batch number refers to the three different batches (A,B or C) of 3 - 4 week old *M. tuberculosis* H37Rv culture filtrates analysed.

* Two peptides identified by LC-MS/MS matched all of these proteins: EsxI (Rv1037c), EsxL (1198), EsxN (Rv1793), EsxO (Rv2346c) and EsxV (Rv3619c),

** One protein spot (Fig, 1, ¤) fingerprint matched all of these proteins: EsxJ (Rv1038c), EsxK (Rv1197), EsxP (Rv2347c) and EsxW (Rv3620c)

*** One peptide identified by LC-MS/MS matched both EsxK (Rv1197) and EsxP (Rv2347c).

Paper I
Supplementary table II

Supplementary table 2. List of predicted secreted and exported proteins of *M. tuberculosis* H37Rv culture filtrate proteins identified in this study, with their possible retention peptides.

Gene number	Signal peptide[a]	Signal peptidase	Retention signal	Predicted localization of protein
Rv0012	MRLTHPTPCPENGETMIDRRRSAWRFSVPLVCLLAGLLLAATHGVSG↓GTE	SPase I[c]	-	Secreted protein
Rv0019c	MQGLVLQLTRAGFLMLLWVFIWSVLRILKTDIYAPTGAVMMRRGLALRGTLLGARQRRHA**ARY	SPase I	-	Secreted protein
Rv0040c	MIQIARTWRVFAGGMATGFIGVVLVTAGKASA**DPLLPPPPIPAPVSAPA*TVP	SPase I	-	Secreted protein
Rv0063	MAREISRQTFLRGAAGALAAGAVFGSVRATA↓DPA[f]	SPase I	-	Secreted protein
Rv0116c	MRRVVRYLSVVVAITLMLTAESVSIATA**AVPPLQPIPGVASVSPANG*AVV	SPase I	-	Secreted protein
Rv0125	MSNSRRRSLRWSWLLSVLAAVGLGLATAPAQA↓APP	SPase I	-	Secreted protein
Rv0129c	MTFFEQVRRLRSAATTLPRRLAIAAMGAVLVYGLVGTFGGPATAGA↓FSR	SPase I	-	Secreted protein
Rv0164	MTAISCSPRPRYASRMPVLSKTVEVTADA**ASI	SPase I	-	Secreted protein
Rv0170	MKITGTVVKLGIVSVVLLFFTVMIIVIFG*QMR**FDR	SPase I	-	Secreted protein
Rv0172	MSTIFDIRNLRLPQLSRASVVIGSLVVVLALA**AG*IVG	SPase I	-	Secreted protein
Rv0174	MLTRFIRRQLILFAIVSVVA**IVVLG*WYY	SPase I	-	Secreted protein
Rv0203	MKTGTATTRRRLLAVLIALALPGAAVALLAEPSATG*A**SDP	SPase I	-	Secreted protein
Rv0285	MTLRVVPEGLAAASAAVEALTARLAAAHASA↓APV	SPase I	-	Secreted protein
Rv0309	MSRLLALLCAAVCTGCVAVVLAPVSLAVVNPWFA**NSVGN*ATQ	SPase I	-	Secreted protein
Rv0315	MLMPEMDRRRMMMMAGFGALAAALPAPTAWA**DPSRPAAPAGPTPAPAAPA*AAT	SPase I	-	Secreted protein
Rv0398c	MGVIARVVGVAACGLSLAVLAAAPTAGA↓EPT	SPase I	-	Secreted protein
Rv0453	MTSALIWMASPPEVHSALLSSGPGPGPVLA*AAT	SPase I	-	Secreted protein
Rv0455c	MSRLSSILRAGAAFLVLGIAAATFPQSAAA↓DST	SPase I	-	Secreted protein
Rv0477	MKALVAVSAVAVVALLGVSSAQA↓DPE	SPase I	-	Secreted protein
Rv0490	MTVFSALLLAGVLSALALAVGG↓AVG	SPase I	-	Secreted protein
Rv0506	MRMISVSGAVKRMWLLLAIVVVA**VVGGLGIYR*LHS	SPase I	-	Secreted protein
Rv0559c	MKGTKLAVVVGMTVAAVSLAAPAQA↓DDY	SPase I	-	Secreted protein
Rv0674	MPAMTARSVVLSVLLGAHPAWA↓TAS	SPase I	-	Secreted protein
Rv0677c	MIGTLKRAWIPLLILVVVAIA**GFTVQRIRTFFGSEGILVTPKVFA*DDP	SPase I	-	Secreted protein

Rv0774c	MPIRPNVHGMMARMPELSRRAVLGLGAGTVLGATSAYAIDM*LLQPRTSHAAPAAAIGTNVPLAPTPA**LDP	SPase I	-	Secreted protein
Rv0787	MHRPPWLAQLRRRLRIGVQL**GSR	SPase I	-	Secreted protein
Rv0851	MDGFPGRGAVITGGASGIGLATGTEFA*RRG	SPase I	-	Secreted protein
Rv0867c	MSGRHRKPTTSNVSVAKIAFTGAVLGGGGIAMA**AQATA*ATD	SPase I	-	Secreted protein
Rv0982	MWWFRRRDRAPLRATSSLSLRWRVMLLAMSMVAMVVVLMSFAVYAVISA↓ALY	SPase I	-	Secreted protein
Rv1050	MARQRFRDQVVLITGASSGIGEATAKAFA*REG	SPase I	-	Secreted protein
Rv1075c	MPRRSTIALATAGALASTGTA**YLGARNLLVGQATHA*RTV	SPase I	-	Secreted protein
Rv1158c	MPTIWTFVRAASSAALLTGGIAHA**DPAPAPAPAPNIPQQLISSAANAPQILQNLATALG*ATP	SPase I	-	Secreted protein
Rv1174c	MRLSLTALSAGVGAVAMSLTVGAGVASA↓DPV	SPase I	-	Secreted protein
Rv1269c	MTTMITLRRRFAVAVAGVATAAATTVTLAPAPANA↓ADV	SPase I	-	Secreted protein
Rv1352	MARTLALRASAGLVAGMAMAAITLAPGARA↓ETG	SPase I	-	Secreted protein
Rv1382	MNSGTLAGSLIFAAVLVMLIAVLA*RLMMRGWRRR**SER	SPase I	-	Secreted protein
Rv1386	MTLRVVPESLAGASAAIEAVTARLAAAHA*AAPFIA**AVI	SPase I	-	Secreted protein
Rv1419	MGELRLVGGVLRVLVVVGAVFDVAVLNAGAASA↓DGP	SPase I	-	Secreted protein
Rv1435c	MTLMAIVNRFNIKVIAGAGLFAAAIALSPDAAA↓DPL	SPase I	-	Secreted protein
Rv1477	MRRNRRGSPARPAARFVRPAIPSALSVALLVCTPGLATA↓DPQ	SPase I	-	Secreted protein
Rv1488	MQGAVAGLVFLAVLVIFAIIVVAKSVALIPQAEA↓AVI	SPase I	-	Secreted protein
Rv1759c	MSFVIAVPETIAAAATDLADLGSTIAGANAAAA*ANT	SPase I	-	Secreted protein
Rv1804c	MRVVSTLLSIPLMIGLAVPAHA↓GPS	SPase I	-	Secreted protein
Rv1810	MQLQRTMGQCRPMRMLVALLLSAATMIGLAAPGKA↓DPT	SPase I	-	Secreted protein
Rv1812c	MTRVVVIGSGFAGLWAALGA**A*RRL	SPase I	-	Secreted protein
Rv1815	MVRLVPRAFAATVALLAAGFSPATASA↓DPV	SPase I	-	Secreted protein
Rv1860	MHQVDPNLTRRKGRLAALAIAAMASASLVTVAVPATANA**DPEPAPPVPTTAASPPSTAAA*PPA	SPase I	-	Secreted protein
Rv1869c	MASSTTFVIVGGGLAGA*KAVEA**LRR	SPase I	-	Secreted protein
Rv1884c	MHPLPADHGRSRCNRHPISPLSLIGNASATSGDMSSMTRIAKPLIKSAMAAGLVTASMSLSTAVAHA**GPS	SPase I	-	Secreted protein
Rv1886c	MTDVSRKIRAWGRRLMIGTAAAVVLPGLVGLAGGAATAGA↓FSR	SPase I	-	Secreted protein
Rv1891	MIRELVTTAAITGAAIGGAPVAGA*DPQ	SPase I	-	Secreted protein
Rv1906c	MRLKPAPSPAAAFAVAGLILAGWAGSVGLAGA↓DPE	SPase I	-	Secreted protein
Rv1910c	MAHAFHRFALAILGLALPVALV**AYGGNGDSRKA*APL	SPase I	-	Secreted protein
Rv1926c	MKLTTMIKTAVAVVAMAAIATFAAPVALA↓AYP	SPase I	-	Secreted protein

Rv1980c	MRIKIFMLVTAVVLLCCSGVATA↓APK	SPase I	-	Secreted protein
Rv1984c	MTPRSLVRIVGVVVATTLALVSAPAGGRA**AHA*DPC	SPase I	-	Secreted protein
Rv2190c	MRLDQRWLIARVIMRSAIGFFASFTVSSGVLAANVLA↓DPA	SPase I	-	Secreted protein
Rv2201	MCGLLAFVAAPAGAAGPEGADA↓ASA	SPase I	-	Secreted protein
Rv2251	MRWRASSAPSISAPPIATGCCTPA*ASPPQTCCGAKTPVSRMRPTRCCCPAAPTGEDA**VAD	SPase I	-	Secreted protein
Rv2253	MSGHRKKAMLALAAASLAATLAPNAVA**A-*AEP	SPase I	-	Secreted protein
Rv2301	MNDLLTRRLLTMGAAAAMLAAVLLLTPITVPAGYPGAVAPATA**ACPDAEVVFAR*GRF	SPase I	-	Secreted protein
Rv2376c	MKMVKSIAAGLTAAAAIGAAAA**GVTSIMAGGPVVYQMQPVVFGAPLPLDPASA*PDV	SPase I	-	Secreted protein
Rv2450c	MKNARTTLIAAAIAGTLVTTSPAGIANA↓DDA	SPase I	-	Secreted protein
Rv2469c	MAHGKKRRGHRSSGVAAGVTGPASC**LHSVHSHR*LAS	SPase I	-	Secreted protein
Rv2576c	MPAGVGNASGSVLDMTSVRTVPSAVALVTFAGAALSGVIPAIARA↓DPV	SPase I	-	Secreted protein
Rv2668	MRHWLIVLATLLVAAAGVAAA**NDVPRAWA*GDA	SPase I	-	Secreted protein
Rv2766c	MTSLDLTGRTAIITGASRGIGLAIAQQLAA*AGAHVVLTARRQEA**ADE	SPase I	-	Secreted protein
Rv2799	MYTPGKGPPRAGGVVFTRVRLIGGLGALTA**AVVVVGTVGWQGIPPAPTGGDAVQLRSTA*APM	SPase I	-	Secreted protein
Rv2848c	MRVSAVAVAAPASGSG*KTTIATGLIGALRQA**GHT	SPase I	-	Secreted protein
Rv2875	MKVKNTIAATSFAAAGLAALAVAVSPPAAA↓GDL	SPase I	-	Secreted protein
Rv2878c	MSLRLVSPIKAFADGIVAVAIAVVLMFGLANTPRAVA↓ADE	SPase I	-	Secreted protein
Rv3004	MAHFAVGFLTLGLLVPVLTWPVSAPLLVIPVALS*ASIIRLRTLA**DER	SPase I	-	Secreted protein
Rv3033	MAHSIVRTLLASGAATALIAIPTACSFSIGTSHSHSVSKA↓EVA	SPase I	-	Secreted protein
Rv3036c	MRYLIATAVLVAVVLVGWPAAGA**PPSCAGLGGTVQA*GQI	SPase I	-	Secreted protein
Rv3106	MRPYYIAIVGSGPSAFFAAASLLKA↓ADT	SPase I	-	Secreted protein
Rv3158c	MILPAPHVEYFLLAPMLIVFSVAVAGVLAEA*FLPRRWRYGAQVTLALGGSAVALIAVIVVARS**IHG	SPase I	-	Secreted protein
Rv3194c	MNRRILTLMVALVPIVVFGVLLAVVTVPFVA**L*GPG	SPase I	-	Secreted protein
Rv3201	MTQTAAPARYSPAELACALGLFPPTAEQAAVIAAPPGPLVVIAGA↓GAG	SPase I	-	Secreted protein
Rv3267	MMSAQRVVRTVRTARAISTALAVAIVLGTGVA**WS*SVR	SPase I	-	Secreted protein
Rv3310	MLRGIQALSRPLTRVYRALAVIGVLAASLLASWVGA**VPQVGLAASA*LPT	SPase I	-	Secreted protein
Rv3354	MNLRRHQTLTLRLLAASAGILSAAAFAAPAQA↓NPV	SPase I	-	Secreted protein
Rv3402	MKIRTLSGSVLEPPSAVRA*TPG	SPase I	-	Secreted protein
Rv3484	MARSEGNRPRHRAVPQPSRIRKRLSRGVMTLVSVVALLMTGAGY**WVAHGALGGITISQA*LTP	SPase I	-	Secreted protein
Rv3485c	MNSRAPRNLAVSSPSAQVTGR**MVQ	SPase I	-	Secreted protein

Rv3491	MNIRCGLAAGAVICSAVALGIALHSGDPARA↓LGP	SPase I	-	Secreted protein
Rv3572	MTRLIPGCTLVGLMLTLLPAPTSA**A*GSN	SPase I	-	Secreted protein
Rv3587c	MLDLEPRGPLPTEIYWRRRGLALGIAVVVVGIAVAIVIAFVDSSAGA↓KPV	SPase I	-	Secreted protein
Rv3627c	MGPTRWRKSTHVVVGAAVLAFVAVVVAAA**ALVTTGGHRAGVRAPAPPPRPPTVKA*GVV	SPase I	-	Secreted protein
Rv3682	MPERLPAAITVLKLAGCCLLASVVATA**LTFPFAGGLGLMSNR*ASE	SPase I	-	Secreted protein
Rv3693	MILTGRTGLLALICVLPIALSPWPARAFVMLLVALAVAVTVDTLLA↓AST	SPase I	-	Secreted protein
Rv3705c	MRIAAAVVSIGLAVIAGFAVPVADA↓HPS	SPase I	-	Secreted protein
Rv3725	MQNATMRVLVTGGTGFVGGWTAKAIA*DAG	SPase I	-	Secreted protein
Rv3803	MKGRSALLRALWIAALSFGLGGVAVA**AEPTAKA*APY	SPase I	-	Secreted protein
Rv3804	MQLVDRVRGAVTGMSRRLVVGAVGAALVSGLVGA**VGGTATAGA*FSR	SPase I	-	Secreted protein
Rv3835	MLDAPEQDPVDPGDPASPPHGEAEQPLPGPRWPRALRASATRRALLLTALGGLLIAGLVTA**IPA	SPase I	-	Secreted protein
Rv3899c	MVTGQPAAAGAHSLSEGAMTAMQSGSVPPPQATPPITTPPVVSAPTMAAG*IEA	SPase I	-	Secreted protein
Rv3917c	MTQPSRRKGGLGRGLAALIPTGPA**DG*ESG	SPase I	-	Secreted protein
MT0066.1	MESAESIQRLTEFEMKLKFARLSTAILGCAAALVFPASVASA↓DPP	SPase I	-	Secreted protein
Rv0015c	MSPRVGVTLSGRYRLQRLIA**TGG	SPase I	1 TM[e]	Transmembrane protein
Rv0062	MTRRTGQRWRGTLPGRRPWTRPAPATCRRHLAFVELRHYFARVMSSAIGSVARWIVPLLGVAAVA**SIG	SPase I	1 TM	Transmembrane protein
Rv0064	METGSPGKRPVLPKRARLLVTAGMGMLALLLFGPRLV**DIYV*DWL	SPase I	6 TM	Transmembrane protein
Rv0219	MFDIATRFKNSYGSGPLHLLAMVSGFALLGYIVATA↓RPS	SPase I	4 TM	Transmembrane protein
Rv0291	MIRAAFACLAATVVVAGWWTPPAWA**IGPIGPPVVDAAA*QPP	SPase I	1 TM	Transmembrane protein
Rv0402c	MRSQRLAGHLSAAARTIHALSLPIILFWVALTIVVNVVA**PQLQSVAR*THS	SPase I	11 TM	Transmembrane protein
Rv0436c	MIGKPRGRRGVNLQILPSAMTVLSICAGLTAIKFA**LEHQPKAAMALIAAA*AIL	SPase I	4 TM	Transmembrane protein
Rv0446c	MVTSVSALAVAVVHSVAFA↓IGR	SPase I	4 TM	Transmembrane protein
Rv0563	MTWHPHANRLKTFLLLVGMSALIVAVGALFGRTALMLA**ALFA*VGM	SPase I	4TM	Transmembrane protein
Rv0680c	MKWNTVAASLAAGVITIAVALAAPPPAAHA↓KNG	SPase I	1 TM	Transmembrane protein
Rv0732	MLSAFISSLRTVDLRRKILFTLGIVILYRVGAA**LPSPGVNFPNVQQCIKEASA*GEA	SPase I	8 TM	Transmembrane protein
Rv0996	MPSIPQSLLWISLVVLWLFVLVPMLISKR*DA**VVR	SPase I	2 TM	Transmembrane protein
Rv1183	MVGCWVALALVLPMAVPSLAEMA**QR*HPV	SPase I	10 TM	Transmembrane protein
Rv1845c	MSALAFTILAVLLAGPTPALLA*RATWPLRAPRAAMVLWQAIALAAVLSSFSA**GIA	SPase I	4 TM	Transmembrane protein
Rv2060	MLTVVCLLVVTVLAICYRPLLFATVDPEVAAA*RGVPVRALGIVFAALMGVVAAQA**VQI	SPase I	3 TM	Transmembrane protein
Rv2200c	MTPRGPGRLQRLSQCRPQRGSGGPARGLRQLALAAMLGALAVTVSGCSWS*EA**LGI	SPase I	3TM	Transmembrane protein

Rv2563	MLFAALRDVQWRKRRLVIAIVSTGLVFAMTLVLTGLVNGFR*VEA**ERT	SPase I	4 TM	Transmembrane protein
Rv2693c	MNANRTSAQRLLAQAGGVSGLVYSSLPVVTFVVASSA*AGLLPAIGFALSMAGLILLWRLLRRESA**RPV	SPase I	5 TM	Transmembrane protein
Rv2721c	MNGQRGQLSTLIGRTLLGLAATAVTAVLLAPTVAA↓SPM	SPase I	1 TM	Transmembrane protein
Rv2874	MVESRRAAAAAASAYASRCGIAPATSQRSLA↓TPP	SPase I	4 TM	Transmembrane protein
Rv2994	MSRDPTGVGARWAIMIVSLGVTASSFLFINGVAF**LIPRLENA*RGT	SPase I	7 TM	Transmembrane protein
Rv3193c	MGMRSAARMPKLTRRSRILIMIALGVIVLLLAGPRLIDA↓YVD	SPase I	6 TM	Transmembrane protein
Rv3629c	MSTFRIFGFSLLMTVVALVTGYLHG*GPTALFLLAVLALLEVSLSFDNA**IIA	SPase I	9 TM	Transmembrane protein
Rv3671c	MTPSQWLDIAVLAVAFIAAISGWRAGALGSMLSFGGVLLGATA**GVL	SPase I	3 TM	Transmembrane protein
Rv3760	MPGSVPGKAPEEPPVKFTRAAAVWSALIVGFLILILLLIFIAQNTASA↓QFA	SPase I	3 TM	Transmembrane protein
Rv0526	MQSRATRRSGALTMRRLVIAAAVSALLLTG↓↓CSGRDAVA*QGG**DAV	SPase I/II	Lipid	Secreted protein / Surface lipoprotein
Rv0999	MRPPLAPQFAADLLVKTVSTLRSSGAAGRLTTMRKAVLAVGSVCWLVG↓↓CSSGASS**TTA*STG	SPase I/II	Lipid	Secreted protein / Surface lipoprotein
Rv2911	MRKLMTATAALCA↓↓CAVTVSAGA**AWA*DAD	SPase I/II	Lipid	Secreted protein / Surface lipoprotein
Rv3668c	MQTAHRRFAAAFAAVLLAVV↓↓CLPANTAAA↓DDK	SPase I/II	Lipid	Secreted protein / Surface lipoprotein
Rv3759c	MRMLRRLRRATVAAAVWLATVCLVAS↓↓CANA↓DPL	SPase I/II	Lipid	Secreted protein / Surface lipoprotein
Rv0173	MMSVLARMRVMRHRAWQGLVLLVLALLLSS↓↓CGW	SPase II[d]	Lipid	Surface lipoprotein
Rv0237	MAFPRTLAILAAAAALVVA↓↓CSH	SPase II	Lipid	Surface lipoprotein
Rv0265c	MRQGCSRRGFLQVAEAAAATGLFAG↓↓CSS	SPase II	Lipid	Surface lipoprotein
Rv0411c	MTRRALLARAAAPLAPLALAMVLAS↓↓CGH	SPase II	Lipid	Surface lipoprotein
Rv0583c	MKHFTAAVATVALSLALAG↓↓CSF	SPase II	Lipid	Surface lipoprotein
Rv0835	MCCSTAAKSAVIVCCAAIATTA↓↓CSF	SPase II	Lipid	Surface lipoprotein
Rv0838	MRLIGRLRLLMVGLVVICGACA↓↓CDR	SPase II	Lipid	Surface lipoprotein
Rv0928	MKLNRFGAAVGVLAAGALVLSA↓↓CGN	SPase II	Lipid	Surface lipoprotein
Rv0932c	MKFARSGAAVSLLAAGTLVLTA↓↓CGG	SPase II	Lipid	Surface lipoprotein
Rv0934	MKIRLHTLLAVLTAAPLLLAAAG↓↓CGS	SPase II	Lipid	Surface lipoprotein
Rv1166	MGVPSPVRRVCVTVGALVALACMVLAG↓↓CTV	SPase II	Lipid	Surface lipoprotein
Rv1252c	MPGVWSPPCPTTPRVGVVAALVAATLTG↓↓CGS	SPase II	Lipid	Surface lipoprotein
Rv1270c	MKHPPCSVVAAATAILAVVLAIGG↓↓CST	SPase II	Lipid	Surface lipoprotein

Rv1541c	MRWIGVLVTALVLSA↓↓CAA	SPase II	Lipid	Surface lipoprotein
Rv1899c	MSRAAGLPRLSWFAGLTWFAGGSTGAG↓↓CAA	SPase II	Lipid	Surface lipoprotein
Rv1911c	MTSTLHRTPLATAGLALVVALGG↓↓CGG	SPase II	Lipid	Surface lipoprotein
Rv2068c	MRNRGFGRRELLVAMAMLVSVTG↓↓CAR	SPase II	Lipid	Surface lipoprotein
Rv2080	MPHSTADRRLRLTRQALLAAAVVPLLAG↓↓CAL	SPase II	Lipid	Surface lipoprotein
Rv2224c	MGMRLSRRDKIARMLLIWAALAAVALVLVG↓↓CIR	SPase II	Lipid	Surface lipoprotein
Rv2544	MIAPQPIPRTLPRWQRIVALTMIGISTALIGG↓↓CTM	SPase II	Lipid	Surface lipoprotein
Rv2585c	MAPRRRRHTRIAGLRVVGTATLVAATTLTA↓↓CSG	SPase II	Lipid	Surface lipoprotein
Rv2672	MATVVGMSRPMTSTAMLVALTCSATVLAA↓↓CVP	SPase II	Lipid	Surface lipoprotein
Rv2873	MINVQAKPAAAASLAAIAIAFLAG↓↓CSS	SPase II	Lipid	Surface lipoprotein
Rv2905	MRARPLTLLTALAAVTLVVVAG↓↓CEA	SPase II	Lipid	Surface lipoprotein
Rv2945c	MNDGKRAVTSAVLVVLGA↓↓CLA	SPase II	Lipid	Surface lipoprotein
Rv3006	MWTTRLVRSGLAALCAAVLVSSG↓↓CAR	SPase II	Lipid	Surface lipoprotein
Rv3016	MVGLTRPLLLCGATLLIAA↓↓CTR	SPase II	Lipid	Surface lipoprotein
Rv3044	MRSTVAVAVAAAVIAASSG↓↓CGS	SPase II	Lipid	Surface lipoprotein
Rv3244c	MRLTILLFLGAVLAG↓↓CAS	SPase II	Lipid	Surface lipoprotein
Rv3495c	MNRIWLRAIILTASSALLAG↓↓CQF	SPase II	Lipid	Surface lipoprotein
Rv3584	MNRCNIRLRLAGMTTWVASIALLAAALSG↓↓CGA	SPase II	Lipid	Surface lipoprotein

[a] Sec-type signal peptides were identified by using SignalP, publicly available at (http://www.cbs.dtu.dk/services/SignalP-3.0/).
↓ Signal peptidase I cleavage site concordantly predicted by Neural Network method and Hidden Markov model.
* Signal peptidase I cleavage site predicted only by Hidden Markov Model.
** Signal peptidase I cleavage site predicted only by Neural Network.
↓↓ Predicted Signal peptidase II cleavage sites. Lipoprotein signal peptides were identified by Lipo-tool, which is publicly available at (http://www.bioinfo.no/tools/lipo) or by using the ProSite PS00013 lipoprotein concensus motif.
[b] Identified export signals are Sec-type signal peptides (Sec), and lipoprotein signal peptides (Lipo).
[c] Signal peptidase I
[d] Signal peptidase II
[e] Transmembrane (TM) domains present in the mature part of the protein after processing by the Signal peptidase.
[f] The first 3 amino acids of verified mature sequences are underlined. See also table 2.

Paper II

Membrane - and membrane associated proteins in Triton X-114 extracts of *Mycobacterium bovis* BCG identified using a combination of gel-based and gel-free fractionation strategies.

Membrane- and membrane associated proteins in Triton X-114 extracts of *Mycobacterium bovis* BCG identified using a combination of gel-based and gel-free fractionation strategies

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Abstract

Tuberculosis is an ancient disease that remains a significant global health problem. Because many membrane and membrane-associated proteins of this pathogen represent potential targets for drugs, diagnostic probes or vaccine components, we have analysed *M. bovis*, bacillus Calmette-Guérin (BCG) substrain Moreau, using Triton X-114 for extraction of lipophilic proteins, followed by identification with liquid chromatography coupled tandem mass spectrometry. We identified 351 different proteins in total, and 103 (29%) were predicted as integral membrane proteins with at least one predicted trans-membrane region and another 84 (23.9%) proteins had a positive grand average of hydropathicity (GRAVY) value, indicating increased probability for membrane association. All together 43 predicted lipoproteins were identified which is close to 50% of the total number of lipoproteins in the genome. Fifty-four proteins, including 24 (12.8%) predicted integral membrane proteins and 7 predicted lipoproteins are described for the first time. The proportion of hydrophobic membrane and membrane associated proteins shows that Triton X-114 is a highly efficient method for extraction of membrane proteins from bacteria, without the need for pre-isolation of membranes. ATP synthase, NAD(P) transhydrogenase, ubiquinone oxidoreductase and ubiquinol-cytochrome C reductase appear to represent major enzyme complexes in the membrane of *M. tuberculosis* complex organisms.

Keywords: *Mycobacterium bovis* /Strong cation-exchange chromatography /Triton X-114 / ATP synthase /NAD(P) transhydrogenase /ubiquinone oxidoreductase / ubiquinol-cytochrome C reductase /

1 Introduction

Tuberculosis is an airborne infection caused by *Mycobacterium tuberculosis*. It is estimated that one-third of the world's population is latently infected with *M. tuberculosis*, and more than 10 million new cases and 2-3 million deaths occur annually [1]. The emergence of drug-resistant stains of this pathogen is further worsening the threat (WHO, 2003). In 1998, the entire genome of *M. tuberculosis* H37Rv was sequenced [2], followed by the genome of the clinical isolate *M. tuberculosis* CDC1551 [3], and *Mycobacterium bovis* AF2122/97 [4]. The availability of whole genomic sequences of *M. tuberculosis* complex organisms has greatly facilitated the analysis of the global proteome of the bacterium.

Membrane and membrane associated proteins play an essential role in many vital processes such as cell-cell interactions, ion and nutrient transport and cell signalling, and participate in the key pathogenically relevant cellular mechanisms. Membrane proteins have at least 1 and up to 16 transmembrane regions that traverse the cytoplasmic membrane. Many membrane associated proteins are actively exported through the cytoplasmic membrane via the well-characterized general secretory (Sec) pathway that transports unfolded proteins across the cytoplasmic membrane to the bacterial envelope [5]. Proteins targeted to this system contain specific N-terminal signal sequences which consist of three distinct regions: A positively charged stretch of polar residues followed by a hydrophobic core which consists of 10 to 15 hydrophobic amino acid residues, and a stretch of short uncharged polar residues, usually carrying the consensus sequence AXA at position -1 to -3 relative to the cleavage site for Signal peptidase I [6-9]. Many proteins required for the pathogenicity of *Mycobacteria* are surface proteins that are involved in lipid metabolism and transport across the membrane [10, 11]. Surface proteins are exposed to the external environment. As a result, these proteins are ideally positioned to protect the bacterium or to modify the host immune response to the bacillus. More than half of all membrane proteins represent candidate pharmaceutical targets [12]. Identification of these proteins is crucial in order to fully understand the molecular mechanisms behind the virulence and pathogenicity of the bacteria, and should furthermore lead to the discovery of new therapeutic targets.

Proteomics, the global analysis of the proteins expressed in a cell or tissue, provides a powerful approach for large scale identification of proteins, their complexes, and their functions, which is required for the design of more effective and precise therapeutics. A

number of proteomic studies, mainly based on two-dimensional gel electrophoresis (2-DE), have been published identifying exported proteins in mycobacterial strains and their subcellular localization, including culture filtrate proteins, cell wall and cytosolic fractions [9, 13-17]. There have also been several studies reporting extraction of membrane and membrane associated proteins using centrifugation to obtain purified cell wall and cell membrane fractions, combined with 1D-PAGE and LC-MS/MS [18-21]. Common for these studies is pre-isolation of the membrane and cell wall of the bacteria, and application of different washing techniques before protein extraction by detergents. In this study, we separated hydrophobic membrane and membrane associated proteins directly from sonicated *M. bovis* bacilli by phase separation with Triton X-114 [22]. Proteins from the lipid phase were analyzed by two different strategies: 1) Extracted proteins were split into three equal portions and each separated by 1D-PAGE. Each lane was cut into 10 pieces each and the gel slices digested with either trypsin, GluC or a combination of both proteases. The resulting peptides were extracted and analyzed by nano-ESI-LCMS/MS. 2) A parallel sample of extracted proteins was similarly split into three equivalent portions and directly digested, in-solution, with either trypsin, GluC or a combination of both proteases. These peptides were separated by strong cation-exchange (SCX) chromatography and the different peptide fractions eluted at different salt concentrations were analyzed by nano-ESI-LCMS/MS. Our results demonstrated that Triton X-114 extraction of hydrophobic proteins in combination with two different fractionation strategies is a powerful method for enrichment of both membrane and membrane associated proteins, without the need for pre-isolation of membrane and cell walls.

2 Experimental procedures

2.1 Bacterial strains

M. bovis BCG substrain Moreau was cultured in 1 litre stationary glass bottles on the wholly synthetic Sauton medium as surface pellicle for 2-3 weeks at 37 °C. Bacteria were separated from the culture medium by paper filtration and washed with phosphate-buffered saline, pH 7.4. Collected bacterial cells were stored at -20 °C.

2.2 Triton X-114 extraction of BCG proteins from whole bacteria

Triton X-114 phase-partitioning was used to isolate lipophilic proteins following the method of Bordier [22]. In brief, 2-3 week old bacilli were probe-sonicated and centrifuged, initially at 2.300 *g* to remove unbroken cells and cell-wall debris. Triton X-114 was added to the supernatant (final detergent concentration 2 %, w/v) and the suspension was stirred at 4 ºC to obtain the protein extract in a single phase. Residual insoluble matter was removed by centrifugation at 15700 *g* for 10 min, and the phases were separated after 10 minutes incubation at 37 ºC. Upper (aqueous) and lower (detergent) phases were collected and washed four times. Proteins in the pooled aqueous and detergent phases were recovered by ethanol precipitation.

2.3 Gel electrophoresis and in-gel digestion of proteins

Proteins from three separate batches of *M. bovis* BCG Triton X-114 lipid phase (50 µg) were mixed with 25 µl sodium-dedocyl-sulphate (SDS) loading buffer and boiled for 5 minutes before separation on a 10 cm long 1 mm thick 12% SDS polyacrylamide gel. The protein migration was allowed to proceed until the bromophenol dye had migrated to the bottom of the gel. The protein bands were visualized with Coomassie Brilliant Blue R-250 staining (Bio-Rad, Hercules, CA, USA). Protein lanes were excised along the visible protein bands ranging from ~3 kDa to ~188 kDa and washed twice with 50% acetonitrile (ACN) in 25 mM ammonium bicarbonate (NH_4HCO_3) for 15 minutes at room temperature (RT). The gel pieces were dehydrated by incubating them with 50 µl 100% ACN for 20 minutes at RT. The Proteins were reduced using 10 mM dithiotreitol and alkylated with 55 mM iodoacetamide; both in 100 mM NH_4HCO_3. The gel pieces were dehydrated by 100% ACN as described above, and rehydrated in 25 mmol/l NH_4HCO_3. The proteins from the three different batches were digested by either trypsin (Promega), GluC (Roche Diagnostics, Mannheim, Germany) or a combination of both proteases for 16–20 h at 37°C. Then, the digested peptides were

eluted by incubating the gel pieces with 50 µl 1% formic acid (FA) for 20 minutes at RT. The supernatant containing the peptides were collected after centrifugation at 15700 *g* for 10 minutes. Then, the gel pieces were incubated with 50 µl 0.1% FA in 50% ACN for 20 minutes at RT, followed by centrifugation at 15700 *g*. The supernatant was collected and combined with the previous one. Finally, the gel pieces were dehydrated by incubating with 50 µl 100% ACN for 20 minutes at RT, and the supernatant was collected after centrifugation as described above and added to the pool.

2.4 Strong cation-exchange Chromatography

Peptides were separated by salt-step cation-exchange chromatography using an LC-Packing *Ultimate* capillary-High Performance Liquid Chromatography (HPLC) system (Dionex, CA, USA) equipped with a strong cation-exchange column (LC-Packings Poros 10S 300 µm inner diameter and 150 mm long). The washed and solubilized digested membrane protein fractions were injected onto the SCX-column in 0.1% formic acid. An isocratic flow-rate of 20 µl/min of 0.05% (vol/vol) formic acid in 2% (vol/vol) ACN was delivered by the loading pump. After loading of the samples, the SCX-column was switched off-line and the flow-through (FT) and eluate were applied to a trap-column cartridge (LC Packings C18 PepMap 100 5 µm 100Å 1.0 mm in diameter and 15 mm long) and washed/desalted three minutes before isocratic elution with 0.1% (vol/vol) formic acid in 70% (vol/vol) ACN delivered by the capillary pump at 6 µl/min. Increasing concentrations of NaCl were injected as samples to elute stronger bound peptides. Salt-steps used for peptide elution were 40 mM, 100 mM and 250 mM of NaCl.

2.5 Liquid chromatography-ESI-MS/MS

On-line LC-MS/MS was performed on tryptic peptides using a Dionex *Ultimate 3000* nano-HPLC system coupled to an ESI-Q-Tof (Ultima Global, Waters, Massachusetts, USA). Reprosil-Pur 3.5 µm C18 resin (Dr.Maisch Gmbh, Ammerbuch, Germany) was packed in a 15 cm long and 75 µm inner diameter fused silica capillary column and used for analytical peptide separation. Solvent A was 0.1% (vol/vol) formic acid in 2% (vol/vol) ACN, and solvent B was 0.1% (vol/vol) formic acid in 90% ACN. The flow rate through the column was 0.3 µl/min. The gradient developed as follows: 5% to 10% B in 2 minutes, 10% to 30% B in 28 minutes, 30% to 50% B in 20 min, 50% to 95% B in 1 minute and ending with constant 95% B for 9 minutes before regeneration of the column. Proteolytic peptide profiles were acquired in data-dependent MS/MS mode from 0 to 65 minutes with a 1 second MS survey

scan. The three most intense precursors were selected for fragmentation and then excluded for 90 seconds. CID spectrum acquisition was allowed for 2 seconds on every selected precursor or stopped when the signal intensity fell below five counts per second. The electrospray voltage was set to 2.9 kV and fused silica capillaries with a 20 μm aperture (Waters) served as spray emittors.

The obtained data was searched against a locally modified version of the publicly available *M. tuberculosis complex* database using MASCOT software, including a concatenated database with the reversed sequences of the original ORFs in order to determine false-positive thresholds of the Mascot identification engine [23]. Mascot scoring showed that our $p>0.01$ was equivalent to a score of 30. The criterium for a positive identification of proteins identified with at least 2 peptides was a minimal score of 30 for each peptide (which represents a 1:10000 protein false positive rate). Under such criteria we observed no false-positive identifications from the reversed database. However, for proteins identified with only one peptide, we considered the maximal score observed for a reversed sequence hit (Rv2073c, Score 34), as a threshold for false-positive identifications. Therefore, we eliminated all proteins identified in our study with only one peptide and a score lower or equal to 34. For visualization and validation of spectra, we used MSQuant version +1.4.2, an open source tool available at (http://msquant.sourceforge.net) and widely used for LC-MS/MS data analysis [24]. The obtained data was searched against the publicly available *M. tuberculosis complex* database using MASCOT software. The *M. bovis* AF2122/97 genome sequence is the most relevant annotation available to that of *M. bovis* BCG and this was used for primary identification of peptides. The orthologous proteins of *M. tuberculosis* H37Rv were also indicated.

2.6 Determination of N-terminal start sites in predicted exported proteins

The Neural Network method (SignalPNN) and the Hidden Markov Model (SignalPHMM) for predicting signal peptides are publicly available at the SignalP server (http://www.cbs.dtu.dk/services/SignalP/). All proteins in the proteome of *M. tuberculosis* that were predicted to have a signal peptidase I cleavage site by either of the two SignalP methods (version 2.0) were compiled in a MASCOT Deamon searchable database (http://www.matrixscience.com) after removal of the predicted signal peptide. This database is referred to as the Removed-Signal-Sequence database and is available at

(http://www.bioinfo.no/publications/wiker2006/). The generated protein spectra were searched against this database to find possible cleavage sites of the exported proteins.

3 Results

3.1 Identification of Triton X-114 extracted proteins

The aim of this study was to perform a comprehensive proteomic analysis of exported and membrane proteins of *M. bovis* BCG substrain Moreau. For this purpose, membrane and membrane associated proteins were extracted using Triton X-114 phase separation from whole sonicated bacilli. Proteins from the lipid phase were precipitated by ethanol. To reduce the complexity of the protein mixture, they were subjected to two different separation strategies. Firstly, proteins from three separate batches were fractionated by 1D-PAGE (Figure 1), the three gel lanes were cut into ten slices each, and subjected to in-gel digestion by two different proteases. Secondly, protein pellets from another three batches of the lipid-phase of Triton X-114 extracts were subjected to in-solution digestion. Peptides were then separated by SCX chromatography. The individual fractions of peptides from both methods were then analysed by nano-ESI-LCMS/MS. Figure 2 illustrate the sequence obtained for protein Rv0583c (M+2H 551.32, VLQFAPGELK) with a Mascot score of 35, which was the miniuml for a positive identification of proteins with only one peptide. This demonstrates the quality of data collection reported in this study. Such fragmentation data contains almost a full observation of the expected y-series daughter ions, plus the presence of other ions which indicates the correct assignment as a2, b2 and, most importantly, a highly abundant y-ion of a proline amino acid (y5), which is a very typical observation.

In total, 1616 peptides were identified from both methods, with 791 peptides from the 1D-PAGE in-gel digestion, and 825 from SCX peptide fractionation. As a result 351 different proteins were identified, 103 proteins by the 1D-PAGE method, 58 identifications by the SCX method, and 190 proteins were identified by both methods (Table 1 and Supplementary table 1). Of the 351 proteins identified in this study, 54 proteins were not previously identified; including 24 predicted integral membrane proteins with at least one predicted TMH region. Table 1 lists all of the identified predicted integral membrane proteins. The complete lists of all proteins with identified peptides are provided as supplementary information (Supplementary tables 1 and 2). Information about the criteria of protein identifications, such as number of peptides matching each protein, score and identification threshold are given in Supplementary table 2.

3.2 Topology and localization analysis of the *M. bovis* profiled proteins

Several of the computational methods used in membrane protein topology analysis have provided the basis for the characterization of the mycobacterial membrane proteins. Many tools are publicly available to predict the topology of membrane proteins [25]. TMHMM 2.0 [26, 27] is considered to be among the programs with best performance [25], and was chosen for the prediction of trans-membrane proteins among the proteins identified in this study. Of the 351 identified protein sequences submitted for characterization, TMHMM 2.0 predicted that 103 proteins had 1 or more TMH regions (table 1). Figure 3 shows the distribution of TMH numbers for the predicted trans-membrane proteins. Fifty-six of the trans-membrane proteins had from 2 to 14 TMHs. Among the proteins with only 1 TMH, 16 of them were lying in the first N-terminal 60 amino acids and coincident with a predicted signal peptide.

In order to identify membrane associated proteins, the 'grand mean of hydropathicity' (GRAVY) scores [28] were calculated for the identified proteins using the PROTPARAM tool (http://us.expasy.org/tools/protparam.html) (Figure 4) in which a score >-0·4 (mean score for the cytosolic proteins) suggests increased probability for membrane association; the higher the score, the greater the probability [28]. Eighty-four proteins among those that lacked a TMH region had a positive GRAVY score, suggesting that these proteins are membrane associated. In total, the predicted membrane proteins, lipoproteins as well as the 84 proteins with positive GRAVY score constituted 65.2% of all the identified proteins in this study (supplementary table 1).

3.3 Identification of the N-terminal cleavage site of exported proteins

Possible cleavage sites for signal peptidase I can be determined by various computational methods. The SignalP method for prediction of exported proteins based on the Neural Network method (NN) or the Hidden Markov Model (HMM) [29, 30] were used for this purpose. To identify the cleavage site for signal peptidase I of exported proteins of *M. bovis* and to verify a cleavable signal peptide, each mass fingerprint was searched against the Removed-Signal-Sequence database by using MASCOT. Out of the 119 identified proteins with a predicted signal peptide, only 5 N-terminal peptides lying immediately C-terminally to a predicted cleavage site were identified (Table 2). Interestingly, the N-terminal cleavage site of signal peptidase I in a potential lipoprotein (Mb2547c) was also detected; with the predicted lipobox and cleavage site for signal peptidase II further N-terminally to the detected cleavage site. Another observation from our data revealed the signal peptidase I cleavage of

Mb2217c, predicted to have two TMH regions by TMHMM; showing that the most N-terminal predicted TMH region is a signal peptide. Further, two peptides (GPDVISPDVIDR and GLAFAAAMVVLR) within the predicted signal sequences of two membrane proteins, Mb2220c and Mb2565, respectively, were also found, indicating that the predicted signal peptides were not cleaved.

3.4 Identification of lipoproteins

All bacteria including *Mycobacterium* retain specific proteins to their cell envelope by post-translational lipid modification to produce membrane-anchored lipoproteins (Lpp), and several of them are found to be surface accessible. These proteins are functionally diverse, and are suggested to be involved in host-pathogen interactions. In this study we report the identification of 43 lipoproteins, predicted to have lipid modification sites by the ScanProsite program (http://expasy.org/tools/scanprosite/). Seven of them had not been identified previously and represent novel identifications. This is the largest number of mycobacterial lipoproteins identified in one single report, and is close to 50% of all predicted lipoproteins in the genome. Thirty-seven of these had no predicted TMH region and are presented in supplementary table 1.

3.5 Functional distribution of the identified *M. bovis* strain proteins

The predicted *M. bovis* proteins have been classified into 12 distinct functional groups (http://genolist.pasteur.fr/bovilist). The 351 proteins identified here by 1D-PAGE and SCX chromatography coupled by LC-MS/MS are distributed across nine of those functional groups (Figure 5). The largest group in this study was cell wall and cell processes (functional group 3, 29%). The second major group belonged to intermediary metabolism and respiration (functional group 7, 27.6%) (Supplementary table 1). Notably, among identified proteins that lacked a TMH region, 31 proteins (8.8%) belonged to functional group 1 that includes proteins involved in fatty acid synthesis and transfer, and 60 proteins (17%) belonged to functional group 10 that includes conserved hypothetical proteins. These proteins have not been annotated with any biological function and needs to be further investigated.

3.6 p*I* and molecular weight distribution of the identified *M. bovis* proteins.

The protein p*I* values varied widely among the proteins identified in this study, and ranged from a p*I* value of 3.55 (Conserved hypothetical protein, Mb1501) to p*I* value 12.13 which belonged to the possible conserved trans-membrane protein, Mb2242. While the majority of

the identified proteins clustered in the area of p*I* values 4-8, 29 proteins had a p*I* value greater than 10, reflecting that many integral and strongly hydrophobic proteins have a very basic p*I* value. The protein with lowest molecular weight in this study was Hypothetical protein, Mb3519, identified in the SCX FT fraction 3 at 5.69 kDa. Possible multifunctional mycocerosic acid synthase, *mas* (Mb2965c) with a molecular weight of 224.39 kDa represented the largest identified protein in this study and was observed in 1D-PAGE fractions number 1 and 3 (Figure 1). The majority of the proteins were found in the range between 20 to 80 kDa (Figure 4).

4 Discussion

Due to the anticipated importance of membrane and membrane associated proteins of *M. tuberculosis* in host-pathogen interactions, it is essential to map these proteins. Therefore, the goal of this study was to improve the identification of membrane and membrane associated proteins. For this purpose, we used Triton X-114 phase separation for extraction of membrane proteins from whole sonicated *M. bovis* BCG bacilli, a close relative of *M. tuberculosis*, with 99.9% identity at the DNA level [31]. Two different strategies were applied to reduce the complexity of the extracted protein mixtures, and were subsequently identified by LC-MS/MS. Out of the 351 different proteins observed, 103 (29.3%) had at least one predicted TMH region, including 56 proteins with 2 or more TMH regions, and another 34 proteins lacking TMH but predicted as lipoproteins. This is the largest number of membrane and membrane associated proteins for mycobacteria reported in one study. The high proportion of membrane proteins to total number of proteins identified, is in line with previous reports [20, 32] , using Triton X-114 for protein extraction, and confirms its high efficacy for extraction of hydrophobic proteins.

Of the 351 proteins identified in this study, eighty-four proteins had a positive GRAVY value, indicating increased probability of membrane association, even though they lacked a defined TMH region. In general, proteins without typical membrane or membrane associated sequence characteristics might still be functionally membrane associated proteins through formation of protein complexes with membrane-anchored proteins. For example, the soluble protein encoded by the *pntAa* gene has been shown in other bacteria [33] to be associated with two other proteins encoded by *pntAb* and *pntB*, genes, forming a protein complex on the membrane. In *M. bovis* BCG these proteins, annotated as possible NAD(P) transhydrogenases,

are represented by one hydrophilic subunit, Mb0160 (*pntAa*), and two other hydrophobic subunits, Mb0161 (*pntAb*) and Mb0162 (*pntB*) that are integral membrane proteins with 3 and 9 TMH regions, respectively. All three proteins were observed in this study, and their adjacent genes are encoded in the same operon [2]. Another example of proteins functionally associated with the membrane is ATP synthase complex subunits. The protein complex consists of a membrane-embedded portion, F_0, containing a, and b subunits, and a soluble portion, F_1, composed of α, β, γ, δ, and ε subunits. We identified six subunits of the ATP synthase complex: a (Mb1336), b (Mb1338); α (Mb1340), β (Mb1342), γ (Mb1341), and δ (Mb1339). The subunits: a, b, and δ are integral membrane proteins, with 5 TMH regions in subunit a, and 1 TMH region in both subunit b and δ. In contrast, subunits α, β, and γ do not have TMH regions (Table 1). Similar observation on the ATP synthase complex were also reported previously [21]. Proteins belonging to ubiquinone oxidoreductase (complex I), involved in respiratory chain pathway and composed of 14 subunits, represent another example. The protein complex has a characteristic L-shaped structure, with one arm embedded in the membrane and the other one protrudes into the cytoplasm [34, 35]. Five subunits of the ubiquinone oxidoreductase complex were identified in this study: NuoA (Mb3169), NuoB (Mb3170), NuoJ (Mb3178), NuoM (Mb3181), and NuoN (Mb3182). Except for the NuoB subunit that lack a defined TMH, all the other subunits identified here, are integral membrane proteins with three or more predicted TMH regions (Table 1). These examples show that several hydrophilic proteins are retained in the lipophilic membrane fraction due to interaction with hydrophobic proteins.

The N-terminal peptides of five mature exported proteins were observed, verifying the presence of both predicted signal peptides and their cleavage sites in these proteins (Table 2). Among these, Mb2217, possible ubiquinol-cytochrome c reductase protein, had two predicted TMH regions. This protein is encoded by the *qcrC* gene which is a part of an operon composed of 3 conserved genes (*qcrC*, *qcrB*, and *qcrA*) [2]. The protein products of these genes form the cytochrome bc_1 complex in the membrane, and are central components in the energy transduction system [36]. Therefore, even though we show the presence of a cleavable signal peptide in the C subunit, the mature protein, with one TMH region left, is probably retained in the membrane and not secreted to the extracellular environment.

In our previous study [9], we observed 22 secreted proteins annotated as trans-membrane proteins due to the prediction of a TMH region in the signal peptide by TMHMM. However,

the detection of a cleavable signal sequence predicted by SignalP, showed that these proteins were secreted and not trans-membrane proteins. In total, 60 of the 159 predicted secreted proteins identified in our previous study [9], had also a predicted TMH lying in the first 60 to 70 N-terminal amino acid residues, and coinciding with the hydrophobic part of their signal sequence. Similarly, we now observed a number of proteins with more than one membrane-spanning region which had predicted N-terminal signal sequences coinciding with a TMH region. These are marked with an asterix in the column showing the number of TMH regions per protein in table 1. These observations show that there is considerable overlap between TMH prediction and signal peptide prediction. In our previous study on secreted proteins [9] we did not observe any peptides from within predicted signal peptides. In this study however, we identified a peptide within the predicted signal sequence for each of the proteins Mb2220c and Mb2565 (Supplementary table 2). Both these proteins were identified with one additional peptide further C-terminally and outside the N-terminal TMH region/predicted signal peptide, and both have 4 predicted TMH regions. These observations suggest that their predicted signal peptides are not cleaved, and are rather TMH regions than signal peptides. The overlap in predictions of TMH regions and signal peptides is a general problem that inflict upon distinction between soluble secreted proteins and membrane proteins. More data are needed to resolve this problem, but our data suggest that the majority of proteins with multiple TMH regions and with an N-terminal TMH which is also predicted to be a signal peptide, are retained uncleaved. On the other hand, most of the proteins with predicted signal peptide and only 1 predicted TMH region positioned in the N-terminus, probably represent exported proteins with cleavable signal peptides.

A closer look at our data revealed that 49 proteins identified here, had only one TMH region predicted by TMHMM. Twenty-one of these proteins had an N-terminal TMH and simultaneously also a predicted signal peptide in the same region (Table 1, marked with an asterix). In general, out of the 787 proteins predicted to be membrane proteins in the genome based on the TMHMM method, 207 of them have only one predicted TMH region lying in the first 60-70 N-terminal amino acid residues of the protein sequence, out of which 178 were also predicted as signal peptide by SignalP. Although it is difficult to categorize this group of proteins either as membrane proteins or as exported proteins, our observations are in favour of the conclusion that the majority of these proteins are secreted or membrane associated proteins. Taking into account the observations made in this study and in our previous one [9], it is important to separate proteins with dual prediction as membrane and as secreted proteins,

and also report the SignalP prediction values, as well as their TMHMM prediction scores in future proteomic studies.

Abundant proteins in the membrane fraction of the bacilli are interesting both in terms of better understanding of the dominant mechanisms occurring in the membrane and also in terms of selection of candidates for therapeutic purposes. We don't have direct quantitative data on the membrane proteins, but proteins observed in several studies or proteins that yield several different peptides are likely to be abundant. As an example, Mb2218, the probable Rieske iron-sulfur protein QcrA with 3 predicted TMH regions, was identified in this study by 12 different peptides (Supplementary table 3), and has been observed in several other studies [18-21, 32]. This shows that Mb2218 is likely to be an abundant protein, which is present in the membrane of both *M. tuberculosis* H37Rv, *M. bovis* BCG Copenhagen and *M. bovis* BCG Moreau. Another example is the possible conserved membrane protein, Mb0232c, with 2 predicted TMH regions, identified here by 16 different peptides, and has also been identified in the three mycobacterial strains mentioned above [18-21, 32]. Other examples of probable abundant proteins are: Mb0162, possible NAD(P) transdehydrogenase [18-21, 32], Mb1517, possible membrane protein [18, 21, 32, 37], and Mb2219, possible ubiquinol-cytochrome C reductase [18, 21, 32] (Supplementary table 3). The last two proteins, Mb1517 and Mb2219 have also been observed previously based on a surrogate expression system approach [37]. These are interesting proteins and should be further explored.

Among the identified proteins there were also 43 lipoproteins, including 7 lipoproteins not previously described. This is the largest number of lipoproteins identified in one single report, and is close to 50% of all predicted lipoproteins in the genome. Interestingly, most of the lipoproteins observed in this study had negative GRAVY values after removal of their predicted signal peptides that mediate their recognition and translocation to the membrane through the general secretory pathway. Identification of these lipoproteins in the Triton X-114 lipid phase indicates that these proteins were possibly modified by lipidation at their predicted lipobox motifs, which anchor them to the bacillary membrane and increase their hydrophobicity leading to their fractionation to the lipid phase of the Triton X-114 detergent.

In conclusion, we have obtained a more comprehensive picture of the *M. bovis* BCG membrane protein repertoire by Triton X-114 based protein extraction from whole sonicated bacilli. Three-hundred and fifty-one proteins were identified by a combination of both gel-based and gel-free protein and peptide fractionation methods. One hundred and two proteins

had one or more predicted trans-membrane regions. Further, we show that Triton X-114 based protein extraction is a powerful method for enrichment of lipoproteins from mycobacteria. The 54 novel proteins identified in this study provide further insight into the *M. bovis* membrane and membrane associated proteins, and reveals a large portion of previously unidentified proteins with unknown function, which might be involved in the pathogenesis of tuberculosis.

Acknowledgement

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Figure legends

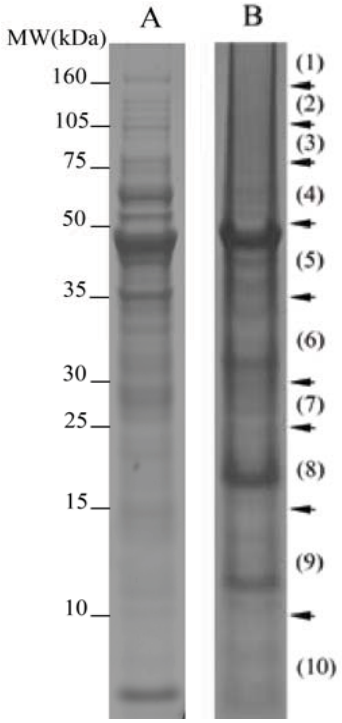
Figure 1. 1D-PAGE analysis of the extracted *M. bovis* BCG proteins. A) crude protein extract, and B) Triton X-114 lipid phase proteins. The molecular weight standard is shown on the left, and the fraction numbers on the right. Explanation of the fraction numbers: (1) >160 kDa, (2) 105-160 kDa, (3) 75-105 kDa, (4) 50-75 kDa, (5) 35-50 kDa, (6) 30-35 kDa, (7) 25-30 kDa, (8) 15-25 kDa, (9) 15-10 kDa, (10) <10 kDa.

Figure 2. CID fragmentation pattern of ion M+2H 551.32. The sequence identified by Mascot engine was VLQFAPGELK, from protein Rv0583c, with a Mascot Score of 35. On such spectra is possible to observe 9 of 10 expected y-series daughter ions, plus the a2 and b2 ions. We also indicate in the spectra the y ion that possibly contains the proline amino acid on the N-terminal (P y5), that is highly abundant compared to other y ions, which is expected due to the low fragmentation susceptibility of this amino acid.

Figure 3. Number of TMH regions in membrane proteins identified in the Triton X-114 lipid phase fraction of *M. bovis* BCG.

Figure 4. A) GRAVY score distribution, and B) Virtual 2D map of the proteins identified in this study. Calculation was done by JvirGel [38].

Figure 5. Functional categorization of the identified *M. bovis* BCG proteins. Explanation of functional category numbers: (0) virulence, detoxification, and adaptation, (1) lipid metabolism, (2) information pathways, (3) cell wall and cell processes, (6) PE/PPE family member proteins, (7) intermediary metabolism and respiration (9) regulatory proteins, (10) conserved hypothetical proteins, and (16) conserved hypothetical proteins with an orthologue in *M. tuberculosis.* Functional group codes are taken from the web server (http://genolist.pasteur.fr/BoviList/).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75
50
35
30
25
15
10
(1)
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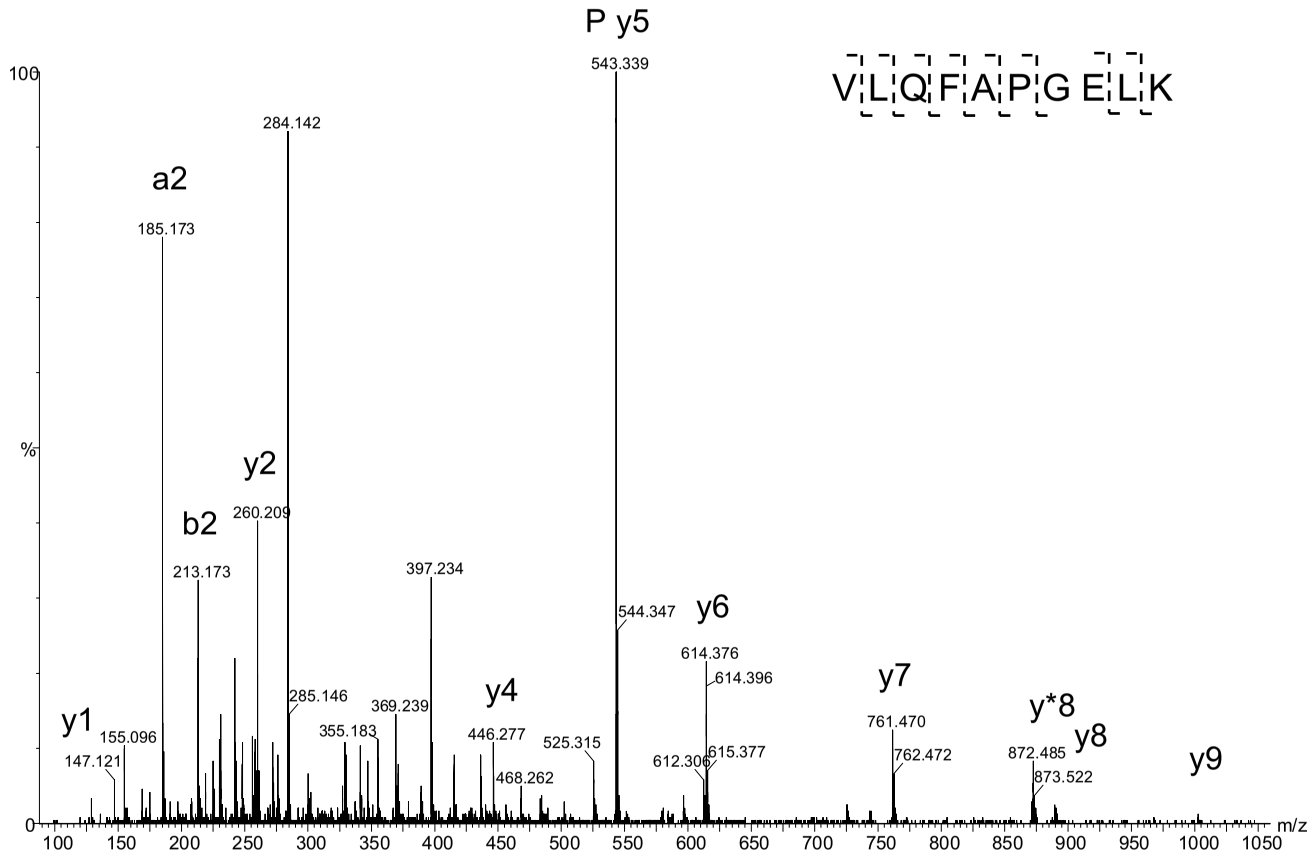
(5)
(6)
(7)
(8)
(9)
(10)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V L Q F A P G E L K
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%
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a2
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y2
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b2
213.173
397.234
544.347
y6
614.376
614.396
y7
761.470
762.472
y*8
872.485
y8
873.522
y9
y1
155.096
147.121
285.146
369.239
355.183
y4
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468.262
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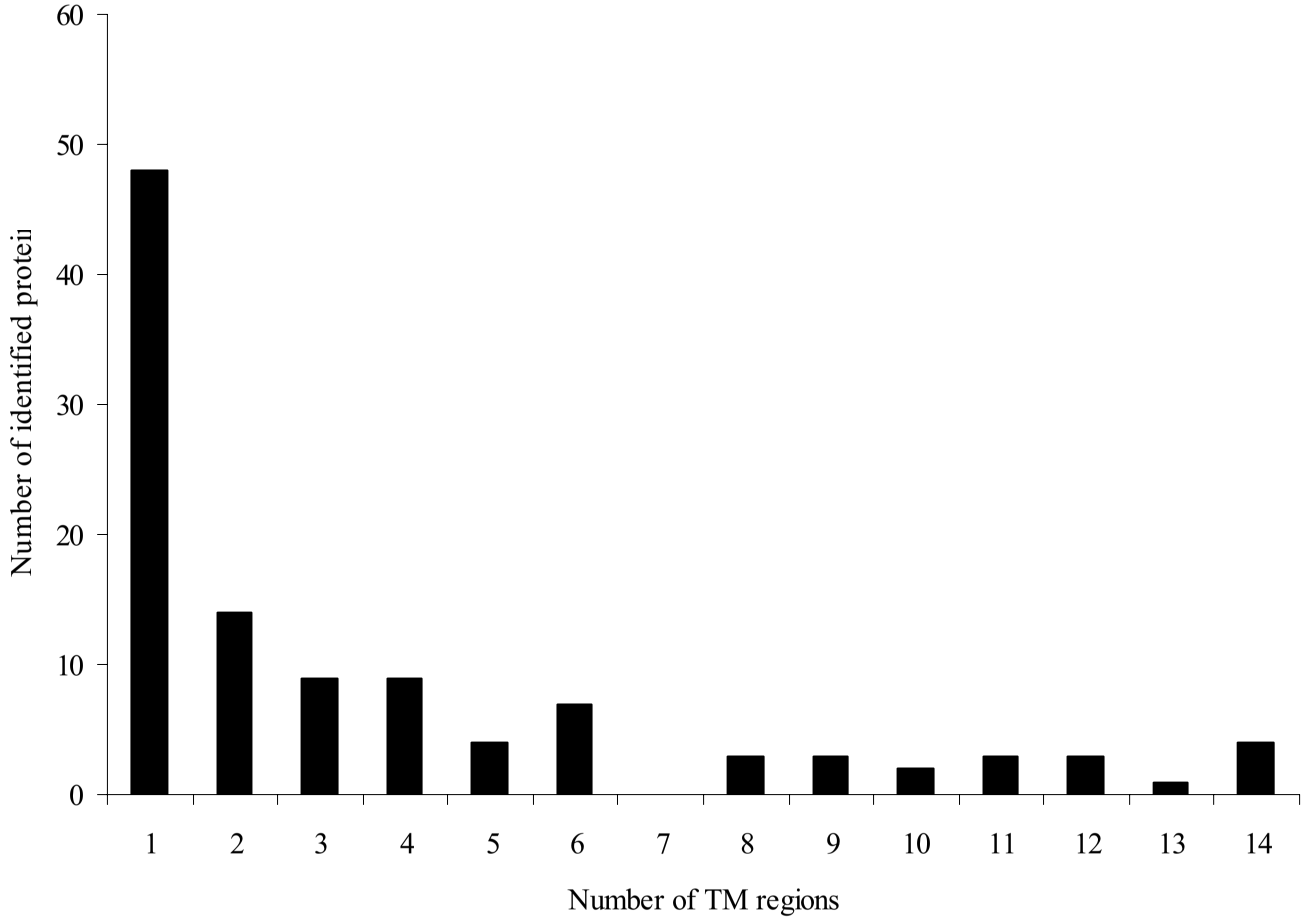
m/z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50
40
30
20
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Number of identified protei
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6
7
8
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10
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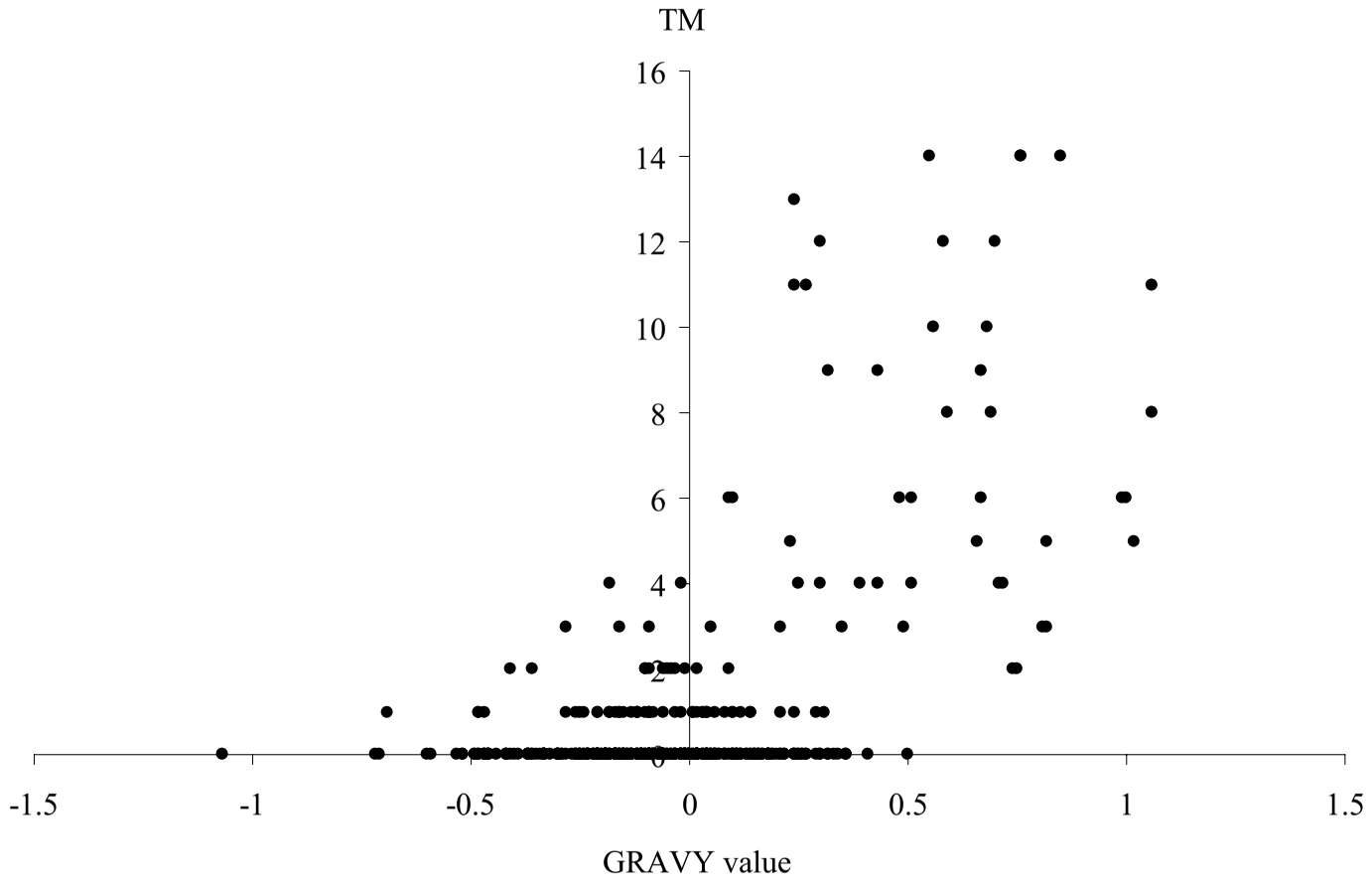
12
13
14
Number of TM regions

A)



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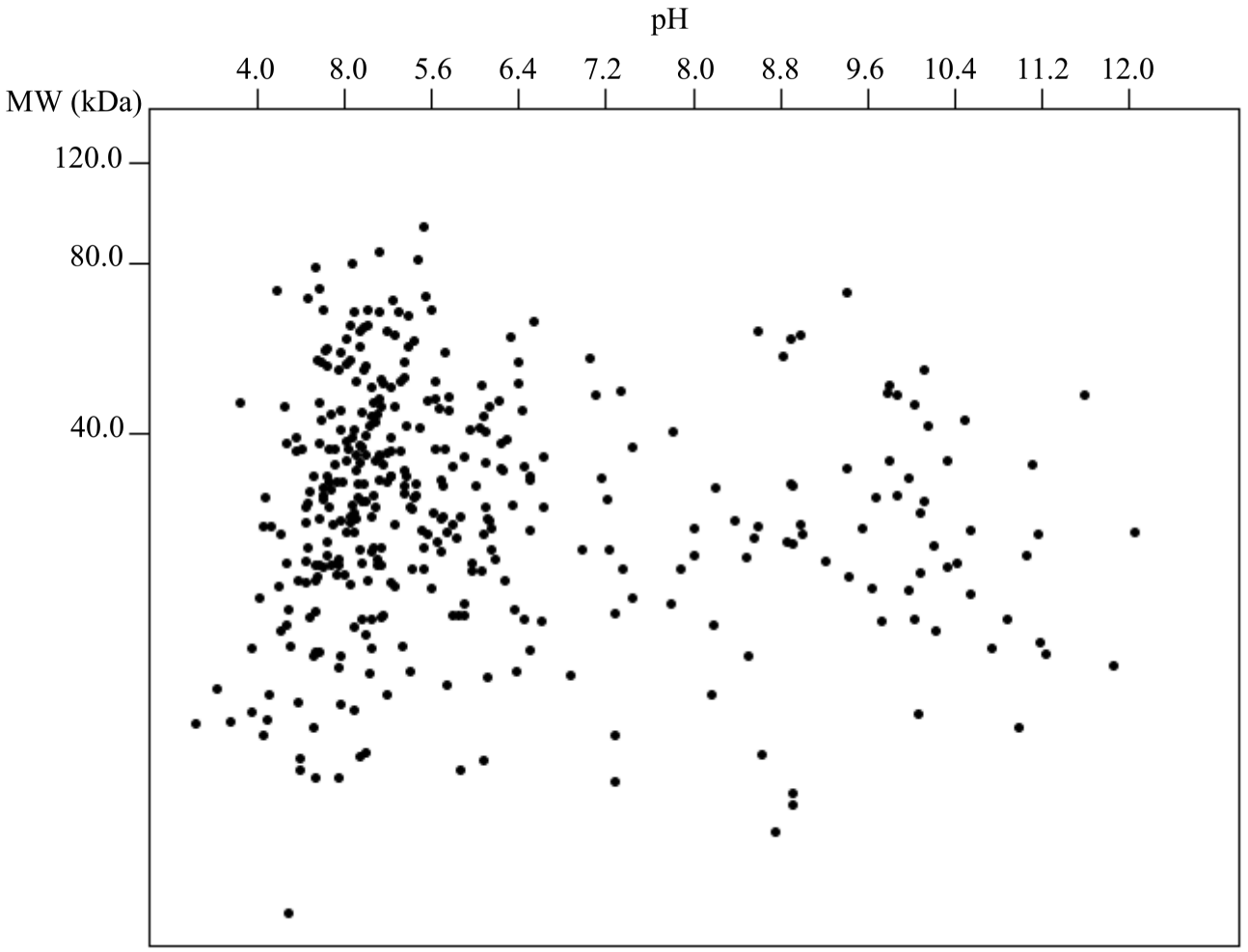

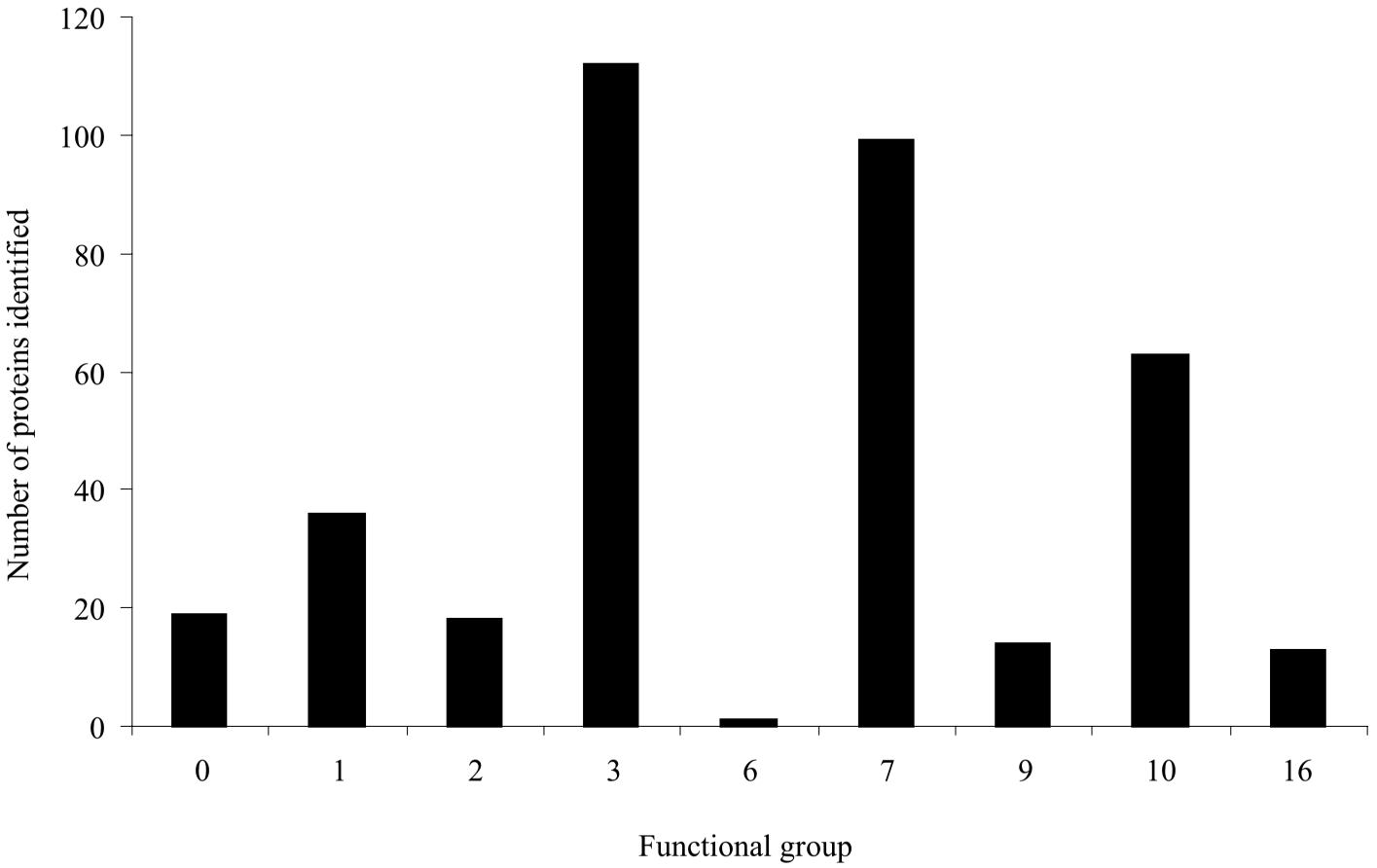
120
100
80
60
40
20
0
Number of proteins identified
0
1
2
3
6
7
9
10
16
Functional group

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Table 1: List of *M. bovis* integral membrane proteins identified in this study

M. bovis	H37Rv	Gene name	Protein identity	Fractionation method	NN	HMM	No. of TM[1]	GRAVY score	
Mb1445c	Rv1410c	-	Aminoglycosides/tetracycline transport integral membrane protein	Both	0.46	0.93	14*	0.76	[21]
Mb3181	Rv3157	*nuoM*	Possible NADH dehydrogenase I	Both	0.77	0.76	14*	0.76	
Mb3182	Rv3158	*nuoN*	Possible NADH DEhydrogenase	Both	0.52	0.56	14*	0.85	[18]
Mb3822	Rv3793	*embC*	Integral membrane indolylacetylinositol arabinosyl transferase	Both	0.56	0.07	13*	0.24	[19]
Mb2151	Rv2127	*ansP1*	Possible L-asparagine permease	SCX	0.32	0.00	12	0.70	
Mb3069c	Rv3043c	*ctaD*	Possible cytochrome c oxidasepolypeptide I	Both	0.38	0.00	12	0.58	[21]
Mb3824	Rv3795	*embB*	Integral membrane indolylacetylinositol arabinosylLtransferase	Both	0.79	0.80	12*	0.30	[19]
Mb0212c	Rv0206c	*mmpL3*	Possible conserved trans-membrane transport protein	SCX	0.63	0.70	11*	0.24	[19, 21]
Mb1633	Rv1607	*chaA*	Possible ionic transporter integral membrane protein	Both	0.75	1.00	11*	1.06	
Mb2203	Rv2181	-	Possible conserved integral membrane protein	SCX	0.63	0.07	10*	0.68	[18]
Mb2408	Rv2387	-	Conserved hypothetical protein	Both	0.46	0.00	10	0.56	
Mb0162	Rv0157	*pntB*	Possible NAD(P) transhydrogenase	Both	0.47	0.18	9	0.67	[18, 21, 32]
Mb1649c	Rv1623c	*cydA*	Possible integral membrane cytochrome D ubiquinol oxidase	Both	0.55	0.01	9*	0.43	
Mb2219	Rv2196	*qcrB*	Possible ubiquinol-cytochrome C reductase	Both	0.24	0.00	9	0.32	[18, 21, 32]
Mb0211	Rv0205	-	Possible conserved trans-membrane protein	SCX	0.63	0.00	8*	1.06	
Mb2692	Rv2673	-	Possible conserved integral membrane protein	1D-PAGE	0.58	0.01	8*	0.59	[18]
Mb0173	Rv0167	*yrbE1A*	Conserved hypothetical integral membrane protein	SCX	0.37	0.00	6	1.00	[18]
Mb0186c	Rv0180c	-	Possible conserved trans-membrane protein	Both	0.78	0.71	6*	0.48	[18, 21]
Mb2617c	Rv2586c	*secF*	Possible protein-export membrane protein	Both	0.30	0.00	6	0.10	[18, 19, 21]
Mb2618c	Rv2587c	*secD*	Possible protein-export membrane protein	1D-PAGE	0.60	0.04	6*	0.09	[18, 19, 21]
Mb3511c	Rv3481c	-	Possible integral membrane protein	SCX	0.69	0.95	6*	0.99	
Mb0255c	Rv0249c	-	Possible succinate dehydrogenase	SCX	0.39	0.00	5	0.23	
Mb1336	Rv1304	*atpB*	Possible ATP synthase A chain	Both	0.38	0.00	5	0.82	[18, 21]
Mb2144c	Rv2120c	-	Possible conserved integral membrane protein	Both	0.90	1.00	5*	1.02	[18]
Mb3178	Rv3154	*nuoJ*	Possible NADH dehydrogenase I	SCX	0.65	0.51	5*	0.66	[21]
Mb0578	Rv0563	*htpX*	Possible protease trans-membrane protein heat shock protein	Both	0.88	0.97	4*	0.30	[21]
Mb0979	Rv0954	-	Possible conserved trans-membrane protein	Both	0.29	0.00	4	-0.02	[21]

Mb1617	Rv1591	-	Possible trans-membrane protein	1D-PAGE	0.55	0.55	4*	0.43	[18, 21]
Mb1853	Rv1822	*pgsA2*	Possible CDP-diacylglycerol-3-phosphate 3-phosphatidyltrasferase	1D-PAGE	0.60	0.11	4*	0.72	
Mb2220c	Rv2197c	-	Possible conserved trans-membrane protein	Both	0.46	0.69	4*	0.51	[18]
Mb2565	Rv2536	-	Possible conserved trans-membrane protein	Both	0.75	0.73	4*	0.25	[21]
Mb2592	Rv2563	-	Possible glutamine-transport trans-membrane protein	Both	0.66	0.95	4*	0.71	[18, 21]
Mb3128c	Rv3101c	*ftsX*	Putative cell division protein	1D-PAGE	0.57	0.97	4*	0.39	[18, 21]
Mb3750	Rv3723	-	Possible conserved trans-membrane protein	SCX	0.42	0.00	4	-0.18	[18, 21]
Mb0161	Rv0156	*pntAb*	Possible NAD(P) transhydrogenase	SCX	0.35	0.25	3	0.82	[19]
Mb0182	Rv0176	-	Possible conserved mce associated trans-membrane protein	SCX	0.32	0.01	3	0.05	[18]
Mb0206	Rv0200	-	Possible conserved trans-membrane protein	SCX	0.70	0.82	3*	0.49	[18]
Mb0508	Rv0497	-	Possible conserved trans-membrane protein	Both	0.14	0.00	3	-0.28	
Mb1517	Rv1481	-	Possible membrane protein	Both	0.67	0.01	3	0.21	[18, 21, 32]
Mb2218	Rv2195	*qcrA*	Possible rieske iron-sulfur protein	Both	0.08	0.00	3	-0.16	[18, 21, 32]
Mb2223c	Rv2200c	*ctaC*	Possible trans-membrane cytochrome c oxidase	Both	0.53	0.84	3*	-0.09	[18, 21]
Mb3131c	Rv3104c	-	Possible conserved trans-membrane protein	1D-PAGE	0.45	0.00	3	0.35	
Mb3169	Rv3145	*nuoA*	Possible NADH dehydrogenase	Both	0.84	1.00	3*	0.81	[16]
Mb0232c	Rv0227c	-	Possible conserved membrane protein	Both	0.84	1.00	2*	-0.41	[18, 21, 32]
Mb1107	Rv1078	*pra*	Possible Proline-rich antigen homolog	Both	0.26	1.00	2*	0.09	[18]
Mb1271c	Rv1239c	*corA*	Possible magnesium and cobalt transport trans-membrane protein	SCX	0.14	0.00	2	-0.09	[21]
Mb1475	Rv1440	*secG*	Possible protein-export membrane protein	SCX	0.59	0.83	2*	0.74	[18]
Mb1523	Rv1487	-	Conserved membrane protein	Both	0.79	0.91	2*	0.75	
Mb1812	Rv1783	-	Possible conserved membrane protein	Both	0.33	0.51	2	-0.10	[18]
Mb1824	Rv1796	-	Conserved hypothetical pro-rich protease	1D-PAGE	0.77	1.00	2*	-0.03	
Mb1825	Rv1797	-	Conserved membrane protein	Both	0.68	0.64	2*	-0.06	[19]
Mb2217	Rv2194	*qcrC*	Possible ubiquinol-cytochrome c reductase	Both	0.72	1.00	2*	-0.05	[21]
Mb2242	Rv2219	-	Possible conserved trans-membrane protein	SCX	0.41	0.00	2	-0.04	[19, 21]
Mb2740c	Rv2721c	-	Possible conserved trans-membrane alanine and glycine rich protein	Both	0.76	1.00	2*	-0.10	[18, 21]
Mb3117	Rv3090	-	Hypothetical alanine and valine rich protein	Both	0.71	0.87	2*	-0.01	[16, 18, 21]
Mb3640c	Rv3610c	*ftsH*	Membrane-bound protease	Both	0.75	0.93	2*	-0.36	[18, 19]
Mb0008c	Rv0008c	-	Possible membrane protein	1D-PAGE	0.20	0.00	1	-0.28	[18, 21]
Mb0014c	Rv0014c	*pknB*	Trans-membrane serine/threonine-protein kinase B	1D-PAGE	0.11	0.00	1	-0.17	
Mb0049c	Rv0048c	-	Possible membrane protein	1D-PAGE	0.46	0.03	1	-0.13	[18, 21]
Mb0205	Rv0199	-	Possible conserved membrane protein	1D-PAGE	0.35	0.00	1	-0.21	[21]
Mb0368	Rv0361	-	Possible conserved membrane protein	Both	0.23	0.00	1	-0.48	[21]

Mb0400c	Rv0394c	-	Possible secreted protein	Both	0.45	0.83	1	-0.08	
Mb0923	Rv0899	*ompA*	Possible outer membrane protein	1D-PAGE	0.40	0.01	1	0.04	[21]
Mb1127c	Rv1097c	-	Possible membraneglycine and proline rich protein	SCX	0.24	0.00	1	-0.09	[16, 19, 21]
Mb1338	Rv1306	*atpF*	Possible ATP synthase B chain	Both	0.49	0.10	1	0.04	[18, 21]
Mb1512	Rv1476	-	Possible membrane protein	Both	0.21	0.00	1	0.02	[18]
Mb1867c	Rv1836c	-	Conserved hypothetical protein	Both	0.07	0.00	1	-0.09	[18]
Mb2118c	Rv2091c	-	Possible membrane protein	Both	0.19	0.00	1	-0.69	[16, 18, 21]
Mb2193	Rv2171	*lppM*	Possible conserved lipoprotein	1D-PAGE	0.92	1.00	1	0.06	
Mb2221c	Rv2198c	*mmpS3*	Possible conserved membrane protein	Both	0.19	0.00	1	-0.48	[18, 21]
Mb2226	Rv2203	-	Possible conserved membrane protein	Both	0.15	0.00	1	-0.18	
Mb2993c	Rv2969c	-	Possible conserved membrane or secreted protein	Both	0.41	0.10	1	0.10	[18, 21]
Mb3068c	Rv3042c	*serB2*	Possible phosphoserine phosphatase	1D-PAGE	0.25	0.01	1	0.36	
Mb3231c	Rv3206c	*moeB1*	Possible molybdenum cofactor biosynthesis protein	1D-PAGE	0.30	0.00	1	0.08	[18]
Mb0019c	Rv0019c	-	Conserved hypothetical protein	Both	0.59	0.41	1*	0.12	[18, 19]
Mb0134c	Rv0129c	*fbpC*	Secreted antigen 85C	1D-PAGE	0.67	1.00	1*	-0.25	[16, 19]
Mb0426	Rv0418	*lpqL*	Possible lipoprotein aminopeptidase	Both	0.79	1.00	1*	-0.06	[18, 19, 21]
Mb0427	Rv0419	*lpqM*	Possible lipoprotein peptidase	1D-PAGE	0.86	0.99	1*	-0.02	
Mb0439	Rv0431	-	Putative tuberculin related peptide	1D-PAGE	0.60	0.94	1*	0.29	[21]
Mb0463c	Rv0455c	-	Conserved hypothetical protein	SCX	0.90	1.00	1*	-0.18	[9]
Mb1241	Rv1209	-	Conserved hypothetical protein	1D-PAGE	0.91	1.00	1*	0.24	[18]
Mb1301c	Rv1270c	*lprA*	Possible lipoprotein	1D-PAGE	0.75	1.00	1*	0.01	[18, 19, 21]
Mb1339	Rv1307	*atpH*	Possible ATP synthase delta chain	Both	0.55	0.02	1*	0.03	[18, 19, 21]
Mb1403	Rv1368	*lprF*	Possible conserved lipoprotein	1D-PAGE	0.67	1.00	1*	-0.03	[18, 19, 21]
Mb1524	Rv1488	-	Possible exported conserved protein	Both	0.76	1.00	1*	-0.10	[18, 19, 21]
Mb1917c	Rv1885c	-	Conserved hypothetical protein	1D-PAGE	0.57	1.00	1*	-0.26	
Mb2121c	Rv2094c	*tatA*	Possible sec-independent protein traslocase membrane-bound protein	Both	0.58	0.29	1*	-0.24	
Mb2248c	Rv2224c	-	Possible exported protease	Both	0.74	1.00	1*	-0.21	[18, 19, 21]
Mb2311	Rv2289	*cdh*	Possible CDP-diacylglycerol pyrophosphatase	SCX	0.91	1.00	1*	-0.16	[16, 18, 19, 21]
Mb2323	Rv2301A	*cut2*	Possible cutinase	Both	0.83	1.00	1*	0.14	[13, 16]
Mb2547c	Rv2518c	*lppS*	Possible conserved lipoprotein	Both	0.83	1.00	1*	-0.12	
Mb2619c	Rv2588c	*yajC*	Possible conserved membrane protein secretion factor	SCX	0.57	0.10	1*	-0.15	[18]
Mb2628	Rv2597	-	Possible membrane protein	1D-PAGE	0.84	1.00	1*	0.03	[18, 21]
Mb2691	Rv2672	-	Possible secreted protease	Both	0.84	1.00	1*	0.04	[21]
Mb2900	Rv2875	*mpt70*	Major secreted immunogenic protein	Both	0.88	1.00	1*	0.31	[39]

Mb2945c	Rv2921c	*ftsY*	Possible cell division protein	Both	0.56	0.09	1*	0.10	[18, 21]
Mb2970c	Rv2945c	*lppX*	Possible conserved lipoprotein	Both	0.75	1.00	1*	0.01	[18, 19, 21]
Mb3422	Rv3390	*lpqD*	Possible conserved lipoprotein	Both	0.65	1.00	1*	-0.09	[18, 21]
Mb3615	Rv3584	*lpqE*	Possible conserved lipoprotein	Both	0.88	1.00	1*	0.21	[18, 21]
Mb3618c	Rv3587c	-	Possible conserved membrane protein	SCX	0.52	0.98	1*	-0.12	
Mb3707	Rv3682	*ponA2*	Possible bifunctional membrane-associated pencillin-binding protein	Both	0.76	1.00	1*	-0.12	[16, 18, 21]
Mb3834c	Rv3804c	*fbpA*	Secreted antigen 85A	1D-PAGE	0.68	1.00	1*	-0.16	[16, 19]
Mb3840	Rv3810	*pirG*	Exported repetitive protein	SCX	0.85	1.00	1*	0.14	

[1] Number of trans-membrane regions predicted by TMHMM (v 2.0), publicly available at (http://www.cbs.dtu.dk/services/TMHMM/).

[*] Signal peptide in the first 60 amino acid residues predicted by SignalP (v 2.0) based on either Neural Network and/or Hidden Markov Model of the protein sequence, publicly available at (http://www.cbs.dtu.dk/services/SignalP)

Table 2: Proteins with experimentally identified N-terminal peptide C-terminally to the predicted SignalP N-terminal signal sequence.

M. bovis	H37Rv	Gene Name	-1 to -3 position	N-terminal Sequence	Method of fractionation	No. of TM regions	Signal peptide Pred. method
Mb0463c	Rv0455c	-	AAA	↓DSTEDFPIPR	SCX	1	NNHMM[a]
Mb1917c	Rv1885c	-	ARA	↓DGTSQLAELVDAAAER	SCX	1	NNHMM
Mb2217	Rv2194	qcrC	AVA	↓DESSSALLR	1D-PAGE	2	NNHMM
Mb2323	Rv2301A	cut2	ATA	↓ACPDAEVVFAR	1D-PAGE	1	NNHMM
Mb2547c	Rv2518c	-	VIA	↓DKGTPFADLLVPK	SCX	1	NNHMM

↓ The experimentally identified N-terminal signal peptide cleavage site by signal peptidase I.

[a] N-terminal Signal peptide predicted by both Neural Network (NN) and Hidden Markov Model (HMM).

Paper II
Supplementary table I

Supplementary Table 1: List of all *M. tuberculosis bovis* Triton X-114 extracted proteins identified by 1D-PAGE and SCX combined with liquid chromatography coupled to MS/MS.

M. bovis	*M. tub.* H37Rv	Gene name	Protein identity	Fractionation Method	Functional group	NN method	HMM method	No. of TM[1]	GRAVY score	References
Mb0173	Rv0167	*yrbE1A*	Conserved hypothetical integral membrane protein	SCX	0	0.37	0.00	6	1.00	G
Mb0257c	Rv0251c	*hsp*	Heat shock protein	SCX	0	0.23	0.00	0	-0.47	G
Mb0358	Rv0350	*dnaK*	Possible chaperone protein	1D-PAGE	0	0.14	0.01	0	-0.37	Mt,G,R
Mb0440	Rv0432	*sodC*	Possible periplasmic superoxide dismutase	Both	0	0.69	1.00	0	-0.06	X,G
Mb0448	Rv0440	*groEL2*	60 KDA chaperonin	Both	0	0.09	0.00	0	-0.09	X,M,Sc
Mb0578	Rv0563	*htpX*	Possible protease trans-membrane protein heat shock protein	Both	0	0.88	0.97	4	0.30	X
Mb0651c	Rv0634c	-	Possible glyoxalse II	1D-PAGE	0	0.21	0.00	0	-0.17	Sc
Mb1111	Rv1082	*mca*	Mycothiol conjugate amidase	Both	0	0.14	0.00	0	-0.48	G
Mb1943c	Rv1908c	*katG*	Catalase-peroxidaseperoxynitirase	1D-PAGE	0	0.10	0.00	0	-0.33	X,M,G,R
Mb2057c	Rv2031c	*hspX*	Heat shock protein	Both	0	0.31	0.00	0	-0.52	X,M,Sc,Mt
Mb2262c	Rv2238c	*ahpE*	Peroxiredoxin	Both	0	0.31	0.00	0	-0.09	G
Mb2321c	Rv2299c	*htpG*	Possible chaperone protein	Both	0	0.09	0.00	0	-0.44	M,G
Mb2455	Rv2429	*ahpD*	Alkyl hydroperoxide reductase D protein	Both	0	0.18	0.00	0	0.17	G,R
Mb2612c	Rv2581c	-	Possible glycosylase II	Both	0	0.44	0.02	0	-0.02	G,R
Mb3297	Rv3269	-	Conserved hypothetical protein	Both	0	0.49	0.57	0	-0.04	Mt,G,R
Mb3451c	Rv3417c	*groEL1*	60 kDa chaperonin 1	Both	0	0.20	0.00	0	0.11	X,M,G,R
Mb3452c	Rv3418c	*groES*	10 kDa chaperonin	Both	0	0.16	0.00	0	-0.33	M,Sc,Mt
Mb3876	Rv3846	*sodA*	Superoxide dismutase	1D-PAGE	0	0.05	0.00	0	-0.21	X,M,Mt,R
Mb0134c	Rv0129c	*fbpC*	Secreted antigen 85C	1D-PAGE	1	0.67	1.00	1	-0.25	Sc,Mt
Mb0249	Rv0243	*fadA2*	Possible acetyl-CoA acetyltransferase	Both	1	0.47	0.37	0	-0.05	X,M,Mt,G
Mb0276	Rv0270	*fadD2*	Possible fatty-acid-CoA ligase	Both	1	0.14	0.00	0	-0.07	X,M,G,R
Mb0477	Rv0468	*fadB2*	Possible 3-hydorxybutyryl-CoA dehydrogenase	Both	1	0.15	0.04	0	0.17	M,Mt,G,R

Mb0478	Rv0469	*umaA*	Possible mycolic acid synthase	1D-PAGE	1	0.09	0.00	0	-0.30	Sc,G
Mb0515c	Rv0503c	*cmaA2*	Cyclopropane-fatty-acyl-phopholipid synthase 2	Both	1	0.16	0.00	0	-0.47	M,Sc,G
Mb0662c	Rv0643c	*mmaA3*	Methoxy mycolic acid synthase 3	1D-PAGE	1	0.23	0.00	0	-0.18	
Mb0663c	Rv0644c	*mmaA2*	Methoxy mycolic acid synthase 2	Both	1	0.07	0.00	0	-0.23	G
Mb0664c	Rv0645c	*mmaA1*	Methoxy mycolic acid synthase 1	Both	1	0.08	0.00	0	-0.33	G
Mb0847c	Rv0824c	*desA1*	Possible acyl-[acyl carrier- protein] desaturase	SCX	1	0.10	0.00	0	-0.46	X,M,Sc,Mt,G
Mb1124	Rv1094	*desA2*	Possible acyl-[acyl-carrier protein	Both	1	0.15	0.00	0	-0.19	X,M,Sc,G
Mb1502c	Rv1467c	*fadE15*	Possible acyl-CoA dehydrogenase	Both	1	0.07	0.00	0	-0.02	X,M,G
Mb1519	Rv1483	*fabG1*	Possible 3-oxoacyl-[acyl-carrier protein] reductase	1D-PAGE	1	0.31	0.00	0	0.04	Sc,G
Mb1520	Rv1484	*inhA*	NADH-dependent enoyl-[acyl-carrier-protein] reductase	Both	1	0.23	0.02	0	0.15	X,Sc,G,R
Mb1571	Rv1544	-	Possible ketoacyl reductase	SCX	1	0.24	0.17	0	0.18	X,Sc,G,R
Mb1706	Rv1679	*fadE16*	Possible acyl-CoA dehydrogenase	Both	1	0.18	0.00	0	0.32	Sc,G,R
Mb1853	Rv1822	*pgsA2*	Possible diacylglycerol-3-phosphate 3-phosphatidyltrasferase	1D-PAGE	1	0.60	0.11	4	0.72	
Mb2025	Rv2002	*fabG3*	Possible 20-beta-hydroxysteroid dehydrogenase	1D-PAGE	1	0.51	0.75	0	0.27	G
Mb2268	Rv2244	*acpM*	Meromycolate extension acyl carrier protein	Both	1	0.05	0.00	0	-0.25	X,M,G
Mb2270	Rv2246	*kasB*	3-oxoacyl-[acyl-carrier protein] synthase 2	Both	1	0.27	0.00	0	0.00	X,G,R
Mb2311	Rv2289	*cdh*	Possible CDP-diacylglycerol pyrophosphatase	SCX	1	0.91	1.00	1	-0.16	X,Sc,G
Mb2953	Rv2928	*tesA*	Possible thioesterase	SCX	1	0.47	0.00	0	-0.32	G
Mb2965c	Rv2940c	*mas*	Possible multifunctional mycocerosic acid synthase	Both	1	0.21	0.01	0	-0.05	M
Mb2966	Rv2941	*fadD28*	Fatty-acid-CoA ligase	Both	1	0.58	0.00	0	-0.13	G
Mb3163	Rv3139	*fadE24*	Possible acyl-CoA dehydrogenase	Both	1	0.62	0.02	0	-0.06	M,Sc,G
Mb3164	Rv3140	*fadE23*	Possible acyl-CoA dehydrogenase	Both	1	0.22	0.00	0	-0.15	G
Mb3165	Rv3141	*fadB4*	Possible NADPH quinone oxidoreductase	1D-PAGE	1	0.46	0.00	0	0.18	G
Mb3586c	Rv3556c	*fadA6*	Possible acetyl-CoA acetyltransferase	SCX	1	0.16	0.00	0	-0.06	Sc,G
Mb3787c	Rv3761c	*fadE36*	Possible acyl-CoA dehydrogenase	Both	1	0.08	0.00	0	-0.11	Sc,G
Mb3830c	Rv3800c	*pks13*	Polyketide synthase	SCX	1	0.10	0.00	0	-0.19	M,G
Mb3834c	Rv3804c	*fbpA*	Secreted antigen 85A	1D-PAGE	1	0.68	1.00	1	-0.16	M,Sc,R

Mb0009	Rv0009	*ppiA*	Possible iron-regulated peptidyl cis-trans isomearse	1D-PAGE	2	0.19	0.00	0	-0.30	M,Sc,Mt,G
Mb0054	Rv0053	*rpsF*	Possible 30S ribosomal protein S6	1D-PAGE	2	0.09	0.00	0	-0.25	Sc,Mt,G,R
Mb0658	Rv0639	*nusG*	Possible transcription anti termination protein	1D-PAGE	2	0.09	0.00	0	-0.18	Mt,G,R
Mb0670	Rv0651	*rplJ*	Possible 50S ribosomal protein L10	1D-PAGE	2	0.19	0.00	0	0.08	G
Mb0671	Rv0652	*rplL*	Possible 50S ribosomal protein L7/L12	Both	2	0.25	0.00	0	0.18	M,Sc,G,R
Mb0686	Rv0667	*rpoB*	DNA-directed RNA polymerase	Both	2	0.18	0.04	0	-0.33	X,M,G
Mb0687	Rv0668	*rpoC*	DNA-directed RNA polymerase	SCX	2	0.09	0.00	0	-0.36	X,M,G
Mb0704	Rv0685	*tuf*	Possible iron-regulated elongation factor	Both	2	0.83	0.00	0	-0.29	X,M
Mb0737	Rv0716	*rplE*	Possible 50S ribosomal protein L5	Both	2	0.21	0.00	0	-0.34	X,G,R
Mb1329	Rv1297	*rho*	Possible transcription terminationfactor RHO homolog	SCX	2	0.12	0.00	0	-0.71	M,Sc,G,R
Mb1425	Rv1390	*rpoZ*	Possible DNA-directed RNA polymerase	SCX	2	0.30	0.09	0	-0.21	
Mb1676	Rv1649	*pheS*	Possible phenylalanyl-tRNA synthetase, alpha chain	1D-PAGE	2	0.20	0.03	0	-0.13	G
Mb2154c	Rv2130c	*cysS2*	Possible cysteinyl-tRNA syntethase	SCX	2	0.17	0.00	0	-0.21	G
Mb2913c	Rv2889c	*tsf*	Possible elongation factor	1D-PAGE	2	0.26	0.01	0	-0.07	M,G,R
Mb3016c	Rv2992c	*gltS*	Possible glutamyl-tRNA synthase	Both	2	0.37	0.00	0	-0.17	M
Mb3486c	Rv3457c	*rpoA*	Possible DNA-directed RNA polymerase	Both	2	0.24	0.00	0	-0.20	M,Sc,R
Mb3487c	Rv3458c	*rpsD*	Possible 30S ribosomal protein s4	1D-PAGE	2	0.45	0.00	0	-0.72	X,M,G
Mb0008c	Rv0008c	-	Possible membrane protein	1D-PAGE	3	0.20	0.00	1	-0.28	X,G
Mb0049c	Rv0048c	-	Possible membrane protein	1D-PAGE	3	0.46	0.03	1	-0.13	X,G
Mb0111c	Rv0107c	*ctpI*	Possible cation-transporter ATPase I	1D-PAGE	3	0.43	0.97	0	0.10	X,M
Mb0182	Rv0176	-	Possible conserved mce associated trans-membrane protein	SCX	3	0.32	0.01	3	0.05	G
Mb0186c	Rv0180c	-	Possible conserved trans-membrane protein	Both	3	0.78	0.71	6	0.48	X,G
Mb0205	Rv0199	-	Possible conserved membrane protein	1D-PAGE	3	0.35	0.00	1	-0.21	X
Mb0206	Rv0200	-	Possible conserved trans-membrane protein	SCX	3	0.70	0.82	3	0.49	G
Mb0211	Rv0205	-	Possible conserved trans-membrane protein	SCX	3	0.63	0.00	8	1.06	
Mb0212c	Rv0206c	*mmpL3*	Possible conserved trans-membrane transport protein	SCX	3	0.63	0.70	11	0.24	X,M
Mb0232c	Rv0227c	-	Possible conserved membrane protein	Both	3	0.84	1.00	2	-0.41	X,M,G

Mb0243	Rv0237	*lpqI*	Possible conserved lipoprotein	Both	3	0.84	1.00	0	0.12	X,M
Mb0271c	Rv0265c	-	Possible periplasmic iron-transport lipoprotein	Both	3	0.53	1.00	0	0.03	G
Mb0351c	Rv0344c	*lpqJ*	Possible lipoprotein	1D-PAGE	3	0.95	1.00	0	-0.15	
Mb0368	Rv0361	-	Possible conserved membrane protein	Both	3	0.23	0.00	1	-0.48	X
Mb0386	Rv0379	*secE2*	Possible protein transport protein	1D-PAGE	3	0.17	0.00	0	-0.26	Sc,Mt
Mb0400c	Rv0394c	-	Possible secreted protein	Both	3	0.45	0.83	1	-0.08	
Mb0419c	Rv0411c	*glnH*	Possible glutamine-binding lipoprotein	Both	3	0.71	1.00	0	0.02	MA
Mb0426	Rv0418	*lpqL*	Possible lipoprotein aminopeptidase	Both	3	0.79	1.00	1	-0.06	X,M,G
Mb0427	Rv0419	*lpqM*	Possible lipoprotein peptidase	1D-PAGE	3	0.86	0.99	1	-0.02	
Mb0439	Rv0431	-	Putative tuberculin related peptide	1D-PAGE	3	0.60	0.94	1	0.29	X
Mb0485	Rv0475	*hbhA*	Iro-regulated heparin binding hemagglutinin	Both	3	0.25	0.00	0	-0.59	X,M,G,R
Mb0508	Rv0497	-	Possible conserved trans-membrane protein	Both	3	0.14	0.00	3	-0.28	
Mb0598c	Rv0583c	*lpqN*	Possible conserved lipoprotein	Both	3	0.64	1.00	0	-0.16	X,G
Mb0844c	Rv0821c	*phoY2*	Possible phosphate-transport system transcriptional protein	1D-PAGE	3	0.11	0.00	0	0.00	
Mb0923	Rv0899	*ompA*	Possible outer membrane protein	1D-PAGE	3	0.40	0.01	1	0.04	X
Mb0951	Rv0928	*pstS3*	Periplasmic phosphate-binding lipoprotein	Both	3	0.79	1.00	0	-0.15	X,Sc
Mb0956c	Rv0932c	*pstS2*	Periplasmic phosphate-binding lipoprotein	Both	3	0.91	1.00	0	-0.14	X,G
Mb0959	Rv0934	*pstS1*	Periplasmic phosphate-binding lipoprotein	Both	3	0.93	1.00	0	0.07	X,M,R
Mb0979	Rv0954	-	Possible conserved trans-membrane protein	Both	3	0.29	0.00	4	-0.02	X
Mb1067c	Rv1038c	*esxJ*	Putative esat-6 like protein	SCX	3	0.13	0.00	0	-0.60	M,G,R
Mb1127c	Rv1097c	-	Possible membrane glycine and proline rich protein	SCX	3	0.24	0.00	1	-0.09	X,M,Sc
Mb1198	Rv1166	*lpqW*	Putative lipoprotein	1D-PAGE	3	0.91	1.00	0	0.03	MA
Mb1230	Rv1198	*esxL*	Putative esat-6 like protein	SCX	3	0.11	0.00	0	-0.11	M,Mt,G
Mb1267	Rv1235	*lpqY*	Possible sugar-binding lipoprotein	1D-PAGE	3	0.95	1.00	0	0.00	
Mb1271c	Rv1239c	*corA*	Possible magnesium and cobalt transport membrane protein	SCX	3	0.14	0.00	2	-0.09	X
Mb1301c	Rv1270c	*lprA*	Possible lipoprotein	1D-PAGE	3	0.75	1.00	1	0.01	X,M,G
Mb1305	Rv1274	*lprB*	Putative lipoprotein	1D-PAGE	3	0.94	1.00	0	-0.19	Sc

Mb1306	Rv1275	*lprC*	Possible lipoprotein	Both	3	0.76	1.00	0	0.13	M,G
Mb1403	Rv1368	*lprF*	Possible conserved lipoprotein	1D-PAGE	3	0.67	1.00	1	-0.03	X,G
Mb1445c	Rv1410c	-	Aminoglycosides/tetracycline transport membrane protein	Both	3	0.46	0.93	14	0.76	X
Mb1446c	Rv1411c	*lprG*	Possible conserved lipoprotein	Both	3	0.88	1.00	0	-0.13	X,M,G
Mb1475	Rv1440	*secG*	Possible protein-export membrane protein	SCX	3	0.59	0.83	2	0.74	G
Mb1498	Rv1463	-	Possible conserved ATP-binding protein ABC transporter	Both	3	0.08	0.00	0	-0.17	Mt,G,R
Mb1512	Rv1476	-	Possible membrane protein	Both	3	0.21	0.00	1	0.02	G
Mb1517	Rv1481	-	Possible membrane protein	Both	3	0.67	0.01	3	0.21	X,G
Mb1523	Rv1487	-	Conserved membrane protein	Both	3	0.79	0.91	2	0.75	
Mb1524	Rv1488	-	Possible exported conserved protein	Both	3	0.76	1.00	1	-0.10	X,M,G
Mb1617	Rv1591	-	Possible trans-membrane protein	1D-PAGE	3	0.55	0.55	4	0.43	X,G
Mb1633	Rv1607	*chaA*	Possible ionic transporter integral membrane protein	Both	3	0.75	1.00	11	1.06	
Mb1704	Rv1677	*dsbF*	Possible conserved lipoprotein	SCX	3	0.63	1.00	0	0.04	
Mb1812	Rv1783	-	Possible conserved membrane protein	Both	3	0.33	0.51	2	-0.10	G,
Mb1821	Rv1793	*esxN*	Putative esat-6 like protein	Both	3	0.10	0.00	0	-0.11	Sc,Mt,G
Mb1956c	Rv1921c	*lppF*	Possible conserved lipoprotein	Both	3	0.80	1.00	0	-0.09	
Mb1957	Rv1922	-	Possible conserved lipoprotein	1D-PAGE	3	0.70	1.00	0	-0.06	
Mb2072	Rv2046	*lppI*	Possible lipoprotein	SCX	3	0.51	1.00	0	0.08	G
Mb2118c	Rv2091c	-	Possible membrane protein	Both	3	0.19	0.00	1	-0.69	X,Sc,G
Mb2121c	Rv2094c	*tatA*	Possible sec-independent protein translocase membrane protein	Both	3	0.58	0.29	1	-0.24	
Mb2144c	Rv2120c	-	Possible conserved integral membrane protein	Both	3	0.90	1.00	5	1.02	G
Mb2151	Rv2127	*ansP1*	Possible L-asparagine permease	SCX	3	0.32	0.00	12	0.70	
Mb2193	Rv2171	*lppM*	Possible conserved lipoprotein	1D-PAGE	3	0.92	1.00	1	0.06	
Mb2203	Rv2181	-	Possible conserved integral membrane protein	SCX	3	0.63	0.07	10	0.68	G
Mb2220c	Rv2197c	-	Possible conserved trans-membrane protein	Both	3	0.46	0.69	4	0.51	G
Mb2221c	Rv2198c	*mmpS3*	Possible conserved membrane protein	Both	3	0.19	0.00	1	-0.48	X,G
Mb2226	Rv2203	-	Possible conserved membrane protein	Both	3	0.15	0.00	1	-0.18	

Mb2242	Rv2219	-	Possible conserved trans-membrane protein	SCX	3	0.41	0.00	2	-0.04	X,M
Mb2248c	Rv2224c	-	Possible exported protease	Both	3	0.74	1.00	1	-0.21	X,M,G
Mb2323	Rv2301A	*cut2*	Possible cutinase	Both	3	0.83	1.00	1	0.14	Sc,Mt,R
Mb2504c	Rv2477c	-	Possible macrolide-transport ATP-binding protein	SCX	3	0.17	0.00	0	-0.41	X,M,G,R
Mb2547c	Rv2518c	*lppS*	Possible conserved lipoprotein	Both	3	0.83	1.00	1	-0.12	
Mb2565	Rv2536	-	Possible conserved trans-membrane protein	Both	3	0.75	0.73	4	0.25	X
Mb2592	Rv2563	-	Possible glutamine-transport trans-membrane protein	Both	3	0.66	0.95	4	0.71	X,G
Mb2616c	Rv2585c	-	Possible conserved lipoprotein	Both	3	0.88	1.00	0	-0.08	M
Mb2617c	Rv2586c	*secF*	Possible protein-export membrane protein	Both	3	0.30	0.00	6	0.10	X,M,G
Mb2618c	Rv2587c	*secD*	Possible protein-export membrane protein	1D-PAGE	3	0.60	0.04	6	0.09	X,M,G
Mb2619c	Rv2588c	*yajC*	Possible conserved membrane protein secretion factor	SCX	3	0.57	0.10	1	-0.15	G
Mb2628	Rv2597	-	Possible membrane protein	1D-PAGE	3	0.84	1.00	1	0.03	X,G
Mb2692	Rv2673	-	Possible conserved integral membrane protein	1D-PAGE	3	0.58	0.01	8	0.59	
Mb2740c	Rv2721c	-	Possible conserved membrane alanine and glycine rich protein	Both	3	0.76	1.00	2	-0.10	X,G
Mb2819c	Rv2796c	*lppV*	Possible conserved lipoprotein	1D-PAGE	3	0.64	0.99	0	-0.29	
Mb2898	Rv2873	*mpb83*	Cell surface lipoprotein	Both	3	0.89	1.00	0	0.19	X,G
Mb2900	Rv2875	*mpt70*	Major secreted immunogenic protein	Both	3	0.88	1.00	1	0.31	
Mb2945c	Rv2921c	*ftsY*	Possible cell division protein	Both	3	0.56	0.09	1	0.10	X,G
Mb2970c	Rv2945c	*lppX*	Possible conserved lipoprotein	Both	3	0.75	1.00	1	0.01	X,M,G
Mb2993c	Rv2969c	-	Possible conserved membrane or secreted protein	Both	3	0.41	0.10	1	0.10	X,G
Mb3031	Rv3006	*lppZ*	Possible conserved lipoprotein	1D-PAGE	3	0.87	1.00	0	-0.07	X,G
Mb3041	Rv3016	*lpqA*	Possible lipoprotein	1D-PAGE	3	0.78	1.00	0	0.21	MA
Mb3070	Rv3044	*fecB*	Possible FEIII-dicitrate-binding periplasmic lipoprotein	Both	3	0.65	1.00	0	0.04	X,Sc,G
Mb3128c	Rv3101c	*ftsX*	Putative cell division protein	1D-PAGE	3	0.57	0.97	4	0.39	X,M,G
Mb3131c	Rv3104c	-	Possible conserved trans-membrane protein	1D-PAGE	3	0.45	0.00	3	0.35	
Mb3272c	Rv3244c	*lpqB*	Possible conserved lipoprotein	Both	3	0.85	1.00	0	-0.03	X,M
Mb3329c	Rv3301c	*phoY1*	Possible phosphate-transport system transcriptional protein	Both	3	0.18	0.00	0	-0.07	X,M,G

Mb3422	Rv3390	*lpqD*	Possible conserved lipoprotein	Both	3	0.65	1.00	1	-0.09	X,G
Mb3511c	Rv3481c	-	Possible integral membrane protein	SCX	3	0.69	0.95	6	0.99	
Mb3615	Rv3584	*lpqE*	Possible conserved lipoprotein	Both	3	0.88	1.00	1	0.21	X,G
Mb3618c	Rv3587c	-	Possible conserved membrane protein	SCX	3	0.52	0.98	1	-0.12	MA
Mb3640c	Rv3610c	*ftsH*	Membrane-bound protease	Both	3	0.75	0.93	2	-0.36	M,G
Mb3707	Rv3682	*ponA2*	Possible membrane-associated pencillin-binding protein	Both	3	0.76	1.00	1	-0.12	Sc,G
Mb3750	Rv3723	-	Possible conserved trans-membrane protein	SCX	3	0.42	0.00	4	-0.18	X,G
Mb3789	Rv3763	*lpqH*	19 kDa lipoprotein antigen precursor	Both	3	0.82	1.00	0	0.11	X,G
Mb3822	Rv3793	*embC*	Integral membrane indolylacetylinositol arabinosyl transferase	Both	3	0.56	0.07	13	0.24	M
Mb3824	Rv3795	*embB*	Integral membrane indolylacetylinositol arabinosyl transferase	Both	3	0.79	0.80	12	0.30	M
Mb3840	Rv3810	*pirG*	Exported repetitive protein	SCX	3	0.85	1.00	1	0.14	
Mb1817	Rv1789	*PPE26*	PPE family protein	Both	6	0.35	0.07	0	0.34	
Mb0153	Rv0148	-	Possible short-chain type dehydrogenase/reductase	Both	7	0.10	0.00	0	0.11	X,Sc,G,R
Mb0160	Rv0155	*pntAa*	Possible NAD(P) transhydrogenase	1D-PAGE	7	0.28	0.00	0	0.14	G,R
Mb0161	Rv0156	*pntAb*	Possible NAD(P) transhydrogenase	SCX	7	0.35	0.25	3	0.82	M
Mb0162	Rv0157	*pntB*	Possible NAD(P) transhydrogenase	Both	7	0.47	0.18	9	0.67	X,G
Mb0189	Rv0183	-	Possible lysophospholipase	Both	7	0.10	0.00	0	-0.03	M,Sc,Mt
Mb0253c	Rv0247c	-	Possible succinate dehydrogenase	Both	7	0.13	0.00	0	-0.46	Sc,G,R
Mb0254c	Rv0248c	-	Possible succinate dehydrogenase	Both	7	0.19	0.00	0	-0.33	X,M,G,R
Mb0255c	Rv0249c	-	Possible succinate dehydrogenase	SCX	7	0.39	0.00	5	0.23	
Mb0323	Rv0315	-	Possible beat-1,3-glucanase	SCX	7	0.71	1.00	0	-0.35	Sc
Mb0415	Rv0407	*fgd1*	Possible f420-dependent glucose-6-phosphate dehydrogenase	Both	7	0.12	0.00	0	-0.25	X,G,R
Mb0447c	Rv0439c	-	Putative dehydrogenase	1D-PAGE	7	0.41	0.00	0	-0.23	G
Mb0539	Rv0526	-	Possible thioredoxin protein	Both	7	0.87	1.00	0	-0.14	X,G
Mb0568	Rv0553	*menC*	Possible muconate cycloisomerase	1D-PAGE	7	0.24	0.00	0	0.36	G
Mb0577	Rv0562	*grcC1*	Possible polyprenyl-diphosphate synthase	Both	7	0.22	0.30	0	0.01	G,
Mb0784c	Rv0761c	*adhB*	Possible alcohol dehydrogenase NAD dependent	Both	7	0.06	0.00	0	0.12	Sc,G

Mb0812	Rv0788	*purQ*	Possible phophoribosylformylglycinamidine synthase I	Both	7	0.20	0.03	0	0.22	G
Mb0826	Rv0803	*purL*	Phosphoribosylformylglycinamide synthase	1D-PAGE	7	0.07	0.00	0	0.04	Sc,G
Mb0838c	Rv0815c	*cysA2*	Possible thiosulfate sulfur tranferase	Both	7	0.11	0.00	0	-0.49	M,Sc
Mb0976	Rv0951	*sucC*	Possible succinyl-CoA synthase	Both	7	0.11	0.00	0	0.02	M,Sc
Mb0977	Rv0952	*sucD*	Possible succinyl-CoA syntethase	SCX	7	0.23	0.00	0	0.21	M,Sc,G,R
Mb1010	Rv0984	*moaB2*	Possible pterin-4-alpha-carbinolamine dehydratase	1D-PAGE	7	0.17	0.00	0	0.41	G,R
Mb1051	Rv1023	*eno*	Possible enolase	Both	7	0.16	0.00	0	0.02	G
Mb1123	Rv1093	*glyA1*	Possible Serine hydroxymethyltransferase 1	Both	7	0.10	0.00	0	0.05	M,G,R
Mb1128c	Rv1098c	*fum*	Possible fumarase	1D-PAGE	7	0.15	0.00	0	0.02	X,M,R
Mb1152	Rv1121	*zwf1*	Possible gulcose-6-phosphate 1-dehydrogenase	SCX	7	0.17	0.00	0	-0.19	G
Mb1211	Rv1178	-	Possible aminotrasferase	1D-PAGE	7	0.23	0.00	0	0.24	G
Mb1336	Rv1304	*atpB*	Possible ATP synthase A chain	Both	7	0.38	0.00	5	0.82	X,G
Mb1338	Rv1306	*atpF*	Possible ATP synthase B chain	Both	7	0.49	0.10	1	0.04	X,G
Mb1339	Rv1307	*atpH*	Possible ATP synthase delta chain	Both	7	0.55	0.02	1	0.03	X,M,G
Mb1340	Rv1308	*atpA*	Possible ATP synthase alpha chain	Both	7	0.06	0.00	0	-0.21	X,M,Sc,R
Mb1341	Rv1309	*atpG*	Possible ATP synthase gamma chain	SCX	7	0.26	0.00	0	-0.27	X,M,Sc,G
Mb1342	Rv1310	*atpD*	Possible ATP synthase beta chain	Both	7	0.11	0.00	0	-0.17	X,M,Sc,G
Mb1368	Rv1333	-	Possible hydrolase	SCX	7	0.15	0.17	0	0.50	
Mb1444	Rv1409	*ribG*	Possible bifunctional riboflavin biosynthesis protein	Both	7	0.09	0.00	0	0.09	M,G
Mb1471	Rv1436	*gap*	Possible glyceraldehyde 3-phosphate dehydrogenase	Both	7	0.16	0.00	0	0.02	X,M,Sc
Mb1472	Rv1437	*pgk*	Possible phophoglycerate kinase	Both	7	0.16	0.00	0	0.22	G
Mb1481c	Rv1446c	*opcA*	Putative OXPP cycle protein	Both	7	0.21	0.00	0	-0.02	G
Mb1482c	Rv1447c	*zwf2*	Possible glucose-6-phosphate 1-dehydrogenase	1D-PAGE	7	0.74	0.02	0	-0.30	M,G
Mb1499	Rv1464	*csd*	Possible cysteine desulfurase	Both	7	0.25	0.00	0	0.02	G,R
Mb1637	Rv1611	*trpC*	Possible indole-3-glycerol phosphate synthase	Both	7	0.23	0.00	0	0.25	M,G
Mb1639	Rv1613	*trpA*	Possible tryptophan synthase	Both	7	0.17	0.00	0	0.30	G
Mb1649c	Rv1623c	*cydA*	Possible integral membrane cytochrome D ubiquinol oxidase	Both	7	0.55	0.01	9	0.43	

Mb1687	Rv1659	*argH*	Possible Argininosuccinate lyase	Both	7	0.19	0.02	0	0.02	
Mb1729c	Rv1703c	-	Possible catechol-o-methyltransferase	SCX	7	0.13	0.00	0	-0.12	M,Sc,G,R
Mb1800	Rv1771	-	Possible oxidoreductase	SCX	7	0.14	0.00	0	-0.37	X,M,Sc,G
Mb1824	Rv1796	-	Conserved hypothetical pro-rich protease	1D-PAGE	7	0.77	1.00	2	-0.03	
Mb1857	Rv1826	*gcvH*	Possible glycine cleavage sysytem H protein	SCX	7	0.13	0.00	0	-0.07	
Mb1887c	Rv1856c	-	Possible oxidoreductase	1D-PAGE	7	0.06	0.00	0	0.10	G
Mb1903c	Rv1872c	*lldD2*	Possible L-lactate dehydrogenase	Both	7	0.35	0.00	0	0.06	X,M,Sc,G
Mb2094c	Rv2068c	*blaC*	Class beta-lactamase	Both	7	0.66	1.00	0	-0.09	X,M,Sc,G
Mb2146c	Rv2122c	*hisE*	Possible phosphoribosyl-AMP pyrophosphatase	Both	7	0.10	0.00	0	-0.13	
Mb2153c	Rv2129c	-	Possible oxidoreductase	Both	7	0.31	0.03	0	0.18	X,M,G
Mb2155c	Rv2131c	*cysQ*	Conserved hypothetical protein	Both	7	0.08	0.00	0	-0.07	G
Mb2200c	Rv2178c	*aroG*	Possible 3-deoxy-D-arabino-heptulosonatephosphate synthase	1D-PAGE	7	0.17	0.00	0	-0.21	M,Sc,G,R
Mb2217	Rv2194	*qcrC*	Possible ubiquinol-cytochrome c reductase	Both	7	0.72	1.00	2	-0.05	X
Mb2218	Rv2195	*qcrA*	Possible rieske iron-sulfur protein	Both	7	0.08	0.00	3	-0.16	X,M,G
Mb2219	Rv2196	*qcrB*	Possible ubiquinol-cytochrome C reductase	Both	7	0.24	0.00	9	0.32	X,M,G
Mb2223c	Rv2200c	*ctaC*	Possible trans-membrane cytochrome c oxidase	Both	7	0.53	0.84	3	-0.09	X,G
Mb2244	Rv2220	*glnA1*	Possible glutamine synthase	Both	7	0.10	0.00	0	-0.42	X,M,Sc,Mt
Mb2246c	Rv2222c	*glnA2*	Possible glutamine synthase	SCX	7	0.27	0.00	0	-0.26	X,M,G
Mb2316	Rv2294	-	Possible aminotransferase	Both	7	0.15	0.00	0	-0.08	Sc,G
Mb2318	Rv2296	-	Possible haloalkane dehalogenase	Both	7	0.09	0.00	0	-0.23	X,M,G,R
Mb2415	Rv2394	*ggtB*	Possible gamma-glutamyl transpeptidase precursor	1D-PAGE	7	0.76	1.00	0	-0.11	X,G
Mb2482c	Rv2455c	-	Possible oxidoreductase	SCX	7	0.08	0.00	0	-0.09	X,G
Mb2487c	Rv2460c	*clpP2*	Possible ATP-dependent clp protease proteolytic subunit 2	Both	7	0.22	0.00	0	-0.15	X,M,Sc,G
Mb2638c	Rv2606c	-	Conserved hypothetical protein	Both	7	0.16	0.00	0	0.15	M,G
Mb2691	Rv2672	-	Possible secreted protease	Both	7	0.84	1.00	1	0.04	X
Mb2697c	Rv2678c	*hemE*	Putative uroporhyrinogen decarboxylase	SCX	7	0.28	0.00	0	0.32	M
Mb2788c	Rv2766c	-	Possible short-chain type dehydrogenase	Both	7	0.46	0.78	0	0.26	G

Mb2805c	Rv2782c	*pepR*	Possible zinc protease	SCX	7	0.36	0.02	0	-0.08	G
Mb2917	Rv2893	-	Possible oxidoreductase	1D-PAGE	7	0.65	0.57	0	0.08	M
Mb2983c	Rv2959c	-	Possible methyltransferase	Both	7	0.60	0.77	0	-0.30	X,G
Mb2996	Rv2971	-	Possible oxidoreductase	1D-PAGE	7	0.24	0.00	0	-0.08	Sc,Sc,G,R
Mb3011c	Rv2987c	*leuD*	Possible 3-isopropylmalate dehydratase	Both	7	0.12	0.00	0	0.07	Sc,G
Mb3020c	Rv2996c	*serA1*	Possible d-3-phosphoglycerate dehydrogenase	Both	7	0.33	0.00	0	0.33	X,M,G
Mb3026c	Rv3001c	*ilvC*	Possible ketol-acid reductoisomerase	Both	7	0.08	0.00	0	-0.14	X,M,Sc,G
Mb3054c	Rv3028c	*fixB*	Possible electron transfer flavoprotein	Both	7	0.22	0.00	0	0.36	M,Sc,Mt
Mb3055c	Rv3029c	*fixA*	Possible electron transfer flavoprotein	Both	7	0.20	0.00	0	-0.11	Mt,G,R
Mb3069c	Rv3043c	*ctaD*	Possible cytochrome c oxidasepolypeptide I	Both	7	0.38	0.00	12	0.58	X
Mb3071	Rv3045	*adhC*	Possible NADP-dependent alcohol dehydrogenase	Both	7	0.29	0.00	0	0.04	M,Sc,R
Mb3143	Rv3116	*moeB2*	Possible molybdenum cofactor biosynthesis protein	SCX	7	0.21	0.00	0	0.06	
Mb3169	Rv3145	*nuoA*	Possible NADH dehydrogenase	Both	7	0.84	1.00	3	0.81	Sc
Mb3170	Rv3146	*nuoB*	Possible NADH dehydrogenase	Both	7	0.28	0.00	0	0.16	G
Mb3178	Rv3154	*nuoJ*	Possible NADH dehydrogenase I	SCX	7	0.65	0.51	5	0.66	X
Mb3181	Rv3157	*nuoM*	Possible NADH dehydrogenase I	Both	7	0.77	0.76	14	0.76	
Mb3182	Rv3158	*nuoN*	Possible NADH dehydrogenase	Both	7	0.52	0.56	14	0.85	X,G
Mb3231c	Rv3206c	*moeB1*	Possible molybdenum cofactor biosynthesis protein	1D-PAGE	7	0.30	0.00	1	0.08	G
Mb3276c	Rv3248c	*sahH*	Possible adeosylhomocysteinase	Both	7	0.16	0.00	0	-0.26	M,Sc,G
Mb3333c	Rv3305c	*amiA1*	possible n-acyl-l-amino acid amidohydrolase	Both	7	0.12	0.00	0	0.11	
Mb3440	Rv3406	-	Possible dioxygenase	Both	7	0.17	0.00	0	-0.17	M,R
Mb3493	Rv3464	*rmlB*	dTDP-glucose 4,6-dehydratase	Both	7	0.30	0.22	0	-0.52	M,Sc
Mb3613c	Rv3582c	*ispD*	Possible 4-diphosphocytidyl-2c-methyl-D-erythritol synthase	1D-PAGE	7	0.12	0.00	0	0.29	G
Mb3627c	Rv3596c	*clpC1*	Possible ATP-dependent protease ATP-binding subunit	1D-PAGE	7	0.34	0.00	0	-0.36	M,G,R
Mb3638c	Rv3608c	*folP1*	Dihydropterate synthase	Both	7	0.22	0.00	0	0.25	
Mb3648c	Rv3624c	*hpt*	Hypoxanthine-guaninephosphoribosyltransferase	1D-PAGE	7	0.37	0.03	0	0.06	G
Mb3736c	Rv3709c	*ask*	Aspartokinase	SCX	7	0.28	0.00	0	0.10	Sc,G

Mb3820	Rv3791	-	Putative short-chain type dehydrogenase	1D-PAGE	7	0.18	0.00	0	0.16	X,G
Mb0014c	Rv0014c	*pknB*	Trans-membrane serine/threonine-protein kinase B	1D-PAGE	9	0.11	0.00	1	-0.17	
Mb0591	Rv0576	-	Possible transcriptional regulatory protein	Both	9	0.26	0.00	0	-0.01	G
Mb1047	Rv1019	-	Possible transcriptional regulatory protein	1D-PAGE	9	0.19	0.00	0	-0.16	
Mb1367	Rv1332	-	Possible transcriptional regulatory protein	Both	9	0.22	0.00	0	-0.42	G
Mb1414	Rv1379	*pyrR*	Possible pyrimidine operon regulatory protein	1D-PAGE	9	0.15	0.00	0	-0.09	
Mb1515	Rv1479	*moxR1*	Possible transcriptional regulatory protein	Both	9	0.14	0.23	0	0.05	X,M,Sc
Mb1652	Rv1626	-	Possible two-component system transcriptional regulator	1D-PAGE	9	0.19	0.00	0	-0.16	M,Sc,G,R
Mb2282c	Rv2258c	-	Possible transcriptional regulatory protein		9	0.18	0.01	0	0.18	G
Mb2379	Rv2358	-	Possible transcriptional regulatory protein	1D-PAGE	9	0.26	0.03	0	0.02	
Mb2730	Rv2711	*ideR*	Iron-dependent repressor and activator	Both	9	0.07	0.00	0	-0.17	Sc,Mt,G
Mb3157c	Rv3133c	*devR*	Two component transcriptional regulatory protein	SCX	9	0.09	0.00	0	-0.02	X,Mt,G,R
Mb3234c	Rv3208	*TB9.4*	Conserved hypothetical protein	1D-PAGE	9	0.45	0.00	0	-0.23	Sc,Mt,G
Mb3274c	Rv3246c	*mtrA*	Two component sensory transduction transcriptional protein	SCX	9	0.14	0.00	0	-0.06	Sc,Mt,G,R
Mb3885	Rv3855	*ethR*	Transcriptional regulatory repressor protein	1D-PAGE	9	0.46	0.00	0	-0.19	M
Mb0019c	Rv0019c	-	Conserved hypothetical protein	Both	10	0.59	0.41	1	0.12	M,G
Mb0020c	Rv0020c	*TB39.8*	Conserved hypothetical protein	Both	10	0.15	0.00	0	-1.07	M,Mt,G
Mb0289	Rv0281	-	Conserved hypothetical protein	Both	10	0.37	0.13	0	-0.24	Sc,G
Mb0303c	Rv0295c	-	Conserved hypothetical protein	1D-PAGE	10	0.48	0.05	0	-0.40	M
Mb0321	Rv0313	-	Conserved hypothetical protein	SCX	10	0.09	0.00	0	-0.53	G
Mb0451	Rv0443	-	Conserved hypothetical protein	1D-PAGE	10	0.14	0.00	0	-0.17	G
Mb0463c	Rv0455c	-	Conserved hypothetical protein	SCX	10	0.90	1.00	1	-0.18	MA
Mb0473c	Rv0464c	-	Conserved hypothetical protein	Both	10	0.42	0.00	0	-0.15	X,M,G
Mb0538	Rv0525	-	Conserved hypothetical protein	1D-PAGE	10	0.26	0.00	0	-0.18	Sc,G,R
Mb0595c	Rv0580c	-	Conserved hypothetical protein	Both	10	0.36	0.00	0	-0.06	X,M,R
Mb0655	Rv0636	-	Conserved hypothetical protein	1D-PAGE	10	0.10	0.00	0	0.02	M,Sc,Mt,R
Mb0656	Rv0637	-	Conserved hypothetical protein	Both	10	0.14	0.00	0	-0.12	Sc,G

Mb0698c	Rv0679c	-	Conserved hypothetical threonine rich protein	Both	10	0.78	1.00	0	-0.06	G
Mb0759	Rv0738	-	Possible transcriptional regulatory protein	1D-PAGE	10	0.27	0.00	0	-0.10	Sc
Mb0854c	Rv0831c	-	Conserved hypothetical protein	1D-PAGE	10	0.12	0.00	0	-0.21	M,G
Mb0930	Rv0906	-	Conserved hypothetical protein	1D-PAGE	10	0.75	1.00	0	-0.03	X,G,R
Mb0993	Rv0968	-	Conserved hypothetical protein	SCX	10	0.53	0.05	0	0.13	X,G,R
Mb1107	Rv1078	*pra*	Possible Proline-rich antigen homolog	Both	10	0.26	1.00	2	0.09	G
Mb1241	Rv1209	-	Conserved hypothetical protein	1D-PAGE	10	0.91	1.00	1	0.24	G
Mb1333	Rv1301	-	Conserved hypothetical protein	1D-PAGE	10	0.21	0.00	0	0.19	
Mb1463c	Rv1428c	-	Conserved hypothetical protein	1D-PAGE	10	0.09	0.00	0	-0.04	G
Mb1501	Rv1466	-	Conserved hypothetical protein	Both	10	0.08	0.00	0	0.00	
Mb1573	Rv1546	-	Conserved hypothetical protein	1D-PAGE	10	0.11	0.00	0	-0.21	G
Mb1584	Rv1558	-	Conserved hypothetical protein	1D-PAGE	10	0.12	0.00	0	-0.48	Sc,Mt,G,R
Mb1812	Rv1784	-	Conserved hypothetical protein	Both	10	0.15	0.00	0	-0.12	M,Sc
Mb1822	Rv1794	-	Conserved hypothetical protein	SCX	10	0.10	0.00	0	-0.12	Sc,Sc,G,R
Mb1825	Rv1797	-	Conserved membrane protein	Both	10	0.68	0.64	2	-0.06	M
Mb1860	Rv1829	-	Conserved hypothetical protein	Both	10	0.12	0.00	0	-0.10	G
Mb1867c	Rv1836c	-	Conserved hypothetical protein	Both	10	0.07	0.00	1	-0.09	X,G
Mb1917c	Rv1885c	-	Conserved hypothetical protein	1D-PAGE	10	0.57	1.00	1	-0.26	
Mb1954c	Rv1919c	-	Conserved hypothetical protein	1D-PAGE	10	0.37	0.12	0	-0.28	X,G
Mb2016c	Rv1993c	-	Conserved hypothetical protein	Both	10	0.34	0.17	0	0.15	G
Mb2028c	Rv2005c	-	Conserved hypothetical protein	1D-PAGE	10	0.21	0.14	0	0.16	M,Sc,G,R
Mb2058	Rv2032	-	Conserved hypothetical protein	1D-PAGE	10	0.19	0.00	0	-0.19	M,Sc,G
Mb2207c	Rv2185c	*TB16.3*	Conserved hypothetical protein	1D-PAGE	10	0.07	0.00	0	-0.39	G,R
Mb2227c	Rv2204c	-	Conserved hypothetical protein	SCX	10	0.29	0.00	0	-0.20	Mt,G
Mb2235	Rv2212	-	Conserved hypothetical protein	Both	10	0.12	0.00	0	0.20	
Mb2320	Rv2298	-	Conserved hypothetical protein	1D-PAGE	10	0.14	0.00	0	-0.03	M,G
Mb2408	Rv2387	-	Conserved hypothetical protein	Both	10	0.46	0.00	10	0.56	

Mb2433c	Rv2410c	-	Conserved hypothetical protein	Both	10	0.18	0.00	0	-0.17	X,G
Mb2586c	Rv2556c	-	Conserved hypothetical protein	1D-PAGE	10	0.16	0.00	0	-0.01	
Mb2656	Rv2623	*TB31.7*	Conserved hypothetical protein	Both	10	0.29	0.07	0	0.10	X,M,Sc
Mb2659c	Rv2626c	-	Conserved hypothetical protein	Both	10	0.10	0.01	0	0.04	M,Sc
Mb2747c	Rv2728c	-	Conserved hypothetical alanine rich protein	1D-PAGE	10	0.52	0.92	0	0.24	
Mb2760	Rv2740	-	Conserved hypothetical protein	Both	10	0.19	0.00	0	0.05	M,G
Mb2765c	Rv2744c	*35kd-ag*	Conserved 35 kDa alanine rich protein	Both	10	0.38	0.24	0	-0.46	X,M,Sc,G
Mb3072c	Rv3046c	-	Conserved hypothetical protein	Both	10	0.34	0.00	0	-0.24	Mt
Mb3126c	Rv3099c	-	Conserved hypothetical protein	1D-PAGE	10	0.13	0.00	0	-0.07	M,G
Mb3236c	Rv3210c	-	Conserved hypothetical protein	1D-PAGE	10	0.18	0.00	0	-0.09	
Mb3339	Rv3311	-	Conserved hypothetical protein	Both	10	0.26	0.00	0	-0.22	G
Mb3645c	Rv3615c	-	Conserved hypothetical protein	Both	10	0.15	0.00	0	-0.01	
Mb3646c	Rv3616c	-	Conserved hypothetical alanine and glycine rich protein	SCX	10	0.16	0.00	0	0.24	X,G
Mb3657	Rv3633	-	Conserved hypothetical protein	1D-PAGE	10	0.10	0.00	0	-0.22	G
Mb3761c	Rv3734c	-	Conserved hypothetical protein	Both	10	0.10	0.00	0	-0.02	X,M,Sc,G
Mb3809	Rv3780	-	Conserved hypothetical protein	Both	10	0.11	0.08	0	-0.37	G
Mb3825	Rv3796	-	Conserved hypothetical protein	1D-PAGE	10	0.56	0.74	0	-0.01	
Mb3895	Rv3865	-	Conserved hypothetical protein	Both	10	0.43	0.00	0	0.18	
Mb3898	Rv3868	-	Conserved hypothetical protein	1D-PAGE	10	0.23	0.08	0	-0.19	G
Mb3910c	Rv3880c	-	Conserved hypothetical protein	Both	10	0.24	0.00	0	-0.22	G
Mb3951c	Rv3920c	-	Hypothetical protein similar to jag protein	1D-PAGE	10	0.08	0.00	0	-0.49	M,G
Mb0091	Rv0088	-	Hypothetical protein	Both	16	0.53	0.00	0	-0.37	M,Sc,G,R
Mb1026	Rv09992	-	Hypothetical protein	1D-PAGE	16	0.62	0.80	0	0.14	X,G
Mb1033	Rv1006	-	Hypothetical protein	Both	16	0.76	1.00	00	-0.30	X,M,Sc,G
Mb1320	Rv1289	-	Hypothetical protein	1D-PAGE	16	0.17	0.00	0	-0.09	M
Mb1454	Rv1419	-	Hypothetical protein	1D-PAGE	16	0.50	1.00	0	0.06	MA
Mb1623	Rv1597	-	Hypothetical protein	SCX	16	0.12	0.00	0	-0.15	M

Mb2327	Rv2305	-	Hypothetical protein	Both	16	0.36	0.00	0	0.12	X,G
Mb3059	Rv3033	-	Hypothetical protein	1D-PAGE	16	0.68	1.00	0	-0.10	Sc,G
Mb3117	Rv3090	-	Hypothetical alanine and valine rich protein	Both	16	0.71	0.87	2	-0.01	X,Sc,G
Mb3519	Rv3489	-	Hypothetical protein	SCX	16	0.11	0.00	0	-0.19	
Mb3848	Rv3818	-	Hypothetical protein	Both	16	0.21	0.00	0	-0.23	G

[1] Number of trans-membrane regions predicted by TMHMM v2.0, publicly available at (http://www.cbs.dtu.dk/services/TMHMM/).
[2] Abbreviation explanation of the references: [G] [1], [X] [2] , [M] [3, 4], [Sc] [5], [Ma] [6], [R] [7], [Mt] [8].

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Paper II
Supplementary table II

Supplementary table 2: A detailed list of *M.bovis* Triton X-114 lipid phase extracted proteins identified by SDS-PAGE and SCX combined with liquid chromatography coupled MS/MS.

M. bovis	*M. tub*. H37Rv	MW	p*I*	Peptide sequence	No. of identified peptides	Fractionation Method	Fraction no.	Score	Threshold	Charge
Mb0008c	Rv0008c	15.67	11.91	GLAYSAVGPVDVTR	1	SDS-PAGE	9	37	18	2
Mb0009	Rv0009	19.24	6.23	HTIFGEVIDAESQR	3	SDS-PAGE	8	85	19	3
				TVANFVGLAQGTK		SDS-PAGE	8	54	17	2
				VIQGFMIQGGDPTGTGR		SDS-PAGE	8	49	19	2
Mb0014c	Rv0014c	66.51	5.08	DIPDVAGQTVDVAQK	4	SDS-PAGE	4	26	18	2
				GQSSADAIATLQNR		SDS-PAGE	4	44	18	2
				TSLLSSAAGNLSGPR		SDS-PAGE	6	54	17	2
				VMDFGIAR		SDS-PAGE	4	30	19	2
Mb0019c	Rv0019c	17.15	11.29	ADDSTLVLTDDYASTR	3	SCX	FT3	72	20	2
				ITLSEQPVLIGR		SDS-PAGE	9	39	19	2
				YLVVTEGALTGAR		SDS-PAGE	9	26	18	2
Mb0020c	Rv0020c	56.07	4.73	GGQGQGRPDEYYDDR	4	SCX	7	23	20	3
				LPDTGVSR		SCX	FT3	51	20	2
				QDYGGGADYTR		SCX	FT2	53	17	2
				WQLADGDVIRLGHSE		SDS-PAGE	4	23	21	3
Mb0049c	Rv0048c	30.78	7.58	AVTLGDLQR	3	SDS-PAGE	7	37	21	2
				LVIYPEYASLDR		SDS-PAGE	7	38	17	2
				QLHSLGGLTGLLEQTR		SDS-PAGE	7	40	17	3
Mb0054	Rv0053	10.93	8.95	QLSLNESVLR	1	SDS-PAGE	10	36	20	2
Mb0091	Rv0088	24.62	11.23	LFGLPYR	1	SCX	FT1	70	20	2
Mb0111c	Rv0107c	167.82	5.40	TLAQLAVATR	1	SDS-PAGE	1	48	11	2

Mb0134c	Rv0129c	35.69	6.51	FLEGLTLR	3	SDS-PAGE	7	24	8	2
				VQFQGGGPHAVYLLDGLR		SDS-PAGE	7	30	11	3
				WETFLTR		SDS-PAGE	7	38	12	2
Mb0153	Rv0148	29.78	5.12	AVANYDSVATEDGAANIIK	17	SCX	FT1	77	19	2
				CADNASVYVVGGGK		SCX	FT2	31	15	2
				DGTGAGSAMADEVVAEIR		SCX	FT1	116	20	2
				DGAANIIKTALDE		SCX	FT2	37	16	2
				EYALTLAGEGASVVVNDLGGAR		SCX	4	27	15	2
				FVAPVVAYLCTEE		SCX	FT3	43	23	2
				ITDLSGAKIAGFKL		SCX	FT2	26	21	3
				LGLVGLINTLALE		SCX	1	73	20	2
				LGLVGLINTLALEGAK		SCX	FT1	79	20	2
				MSFENWDAVLK		SCX	2	54	20	2
				MTQDILPPEVLEK		SCX	FT1	66	21	2
				PGVQDRVIVVTGAGGGLGRE		SDS-PAGE	7	55	19	3
				TALDEFGAVHGVVSNAGILR		SDS-PAGE	7	79	16	2
				VALFGNDGANFDKPPSVQDVAAR		SDS-PAGE	7	43	18	3
				VHLYGGYHVLR		SDS-PAGE	7	73	18	3
				VIVVTGAGGGLGR		SDS-PAGE	7	46	20	2
				WAEITDLSGAK		SDS-PAGE	7	46	19	2
Mb0160	Rv0155	37.69	4.64	GGQTLIGFLAPR	1	SDS-PAGE	6	61	20	2
Mb0161	Rv0156	11.72	7.62	KPAVPAKPDR	1	SCX	1	55	19	3
Mb0162	Rv0157	48.86	6.51	DLATLLEDR	6	SCX	FT3	47	19	2
				NETSSPIYGMPILNVDK		SCX	FT3	49	22	2
				SVTEVSEELK		SDS-PAGE	4	56	18	2
				TSSPIYGMPILNVDK		SDS-PAGE	1	33	18	2
				YAIHPVAGR		SDS-PAGE	4	23	18	2

Mb0173	Rv0167	27.74	9.04	DQLQTPLTLVGGFFR	1	SCX	7	49	20	2
Mb0182	Rv0176	35.40	11.23	TFADLLLR	1	SCX	1	45	20	2
Mb0186c	Rv0180c	47.59	9.85	AGTLAASIAGQTLTR	2	SCX	FT3	68	16	2
				DAGPAGQQIVDGLVSGLDK		SDS-PAGE	3	33	9	2
Mb0189	Rv0183	30.26	6.18	ALLQVGETMPR	8	SCX	1	46	20	2
				APALTAPLLVLHGTDDR		SCX	FT2	38	14	3
				DPEVVQAYNTDPLVHHGR		SCX	1	58	20	3
				LDFTAISRDPE		SCX	FT3	73	23	2
				LGAAGLVTYALDHR		SDS-PAGE	8	43	13	3
				RAPALTAPLLVLHGTDDR		SDS-PAGE	7	22	20	3
				VVQAYNTDPLVHHGR		SDS-PAGE	7	53	18	3
				YTADFDTLVGIATR		SDS-PAGE	7	46	18	2
Mb0205	Rv0199	23.52	4.59	AVAIDTVHRE	2	SDS-PAGE	8	37	14	2
				LDVSDVDGK		SDS-PAGE	8	57	21	2
Mb0206	Rv0200	24.02	9.34	TVASFSPSAPAAAVDR	1	SCX	FT3	64	16	2
Mb0211	Rv0205	38.04	7.93	GEQIDNAGNAAIEALR	1	SCX	FT1	64	20	2
Mb0212c	Rv0206c	100.87	9.91	HVDALGVR	2	SCX	7	31	11	2
				LLGDDCWWAPR		SCX	8	51	13	2
Mb0232c	Rv0227c	45.53	4.58	ANHYFAR	16	SCX	1	28	10	2
				DSGLLLAIVDTVTLNR		SCX	FT1	40	12	2
				FTQNVGYTPEGK		SCX	1	17	14	2
				GGFEEPVPGAEAETEK		SCX	1	14	13	2
				GLNDENPPTAIPLR		SCX	1	16	13	2
				HDGLSYR		SCX	FT1	30	12	2
				KTAMAVSDDTHTGGAVQKPR		SCX	5	23	6	3
				LALWSR		SCX	7	33	14	2
				LGSAQPPPPPDAGHPDPGPPER		SCX	FT3	17	13	3

				LPTQRPDFPR		SDS-PAGE	5	19	12	3
				TAMAVSDDTHTGGAVQKPR		SDS-PAGE	4	32	8	4
				TEGALMAASGDR		SDS-PAGE	5	39	13	2
				TERANHYFARDPLKPE		SDS-PAGE	5	74	13	3
				TFWVDPVSGTIVK		SDS-PAGE	5	47	14	2
				TYPYFDPIAQK		SDS-PAGE	5	48	12	2
				YPSLYAGDEDGK		SDS-PAGE	5	32	10	2
Mb0243	Rv0237	39.53	5.38	AGELPMSAVDR	9	SCX	FT2	30	10	2
				DAGVLPVLK		SCX	7	13	11	2
				DKLAQLLVVGVR		SDS-PAGE	6	68	11	2
				FGVSEAVLR		SDS-PAGE	6	48	12	2
				IVAGGGPLPLAVSVDEE		SDS-PAGE	6	69	13	2
				LAQLLVVGVR		SDS-PAGE	6	33	14	2
				SCAEPAGIPALLSPR		SDS-PAGE	6	17	9	2
				SLIGGTGPSAR		SDS-PAGE	6	62	13	2
				TLVTQAPVGVMVGHLQVPGLTGSEPASLSK		SDS-PAGE	6	21	11	3
Mb0249	Rv0243	46.08	6.64	AHSLAPLAYLVDAE	4	SCX	7	49	15	2
				FGLAGER		SDS-PAGE	5	34	20	2
				LGLDAALGSIDR		SDS-PAGE	6	35	15	2
				LVGTLPASLGVEIPANSEPR		SDS-PAGE	5	62	19	2
Mb0253c	Rv0247c	28.63	6.15	CCTEVCPENIK	11	SCX	FT1	24	20	2
				CFLCQNVCHVVR		SCX	7	50	23	3
				CGSCSAEINGKPR		SCX	1	35	20	3
				DHEENKDAFAGPR		SCX	7	39	21	3
				DLVTDVSFNYQK		SCX	FT1	32	20	2
				ELQPSEYR		SCX	7	62	24	2
				HGLGYCNITK		SCX	7	45	19	2

				KYDPVVWLGSK		SCX	FT3	46	20	2
				LQQTQTPDLAVR		SCX	FT1	95	20	2
				MSTFDEDEIVTVTPMR		SCX	FT3	29	20	2
				YDPVVWLGSK		SDS-PAGE	7	25	20	2
Mb0254c	Rv0248c	70.68	5.81	AGLGAADYVR	8	SCX	FT2	38	20	2
				DQGAIAGAFGYWR		SCX	FT2	25	15	2
				FMFDYIPPVFK		SCX	7	90	19	2
				LEKYYTDEE		SCX	8	24	23	2
				LGGNSLSDLLVFGR		SDS-PAGE	4	26	22	2
				RLPSMYHQFKE		SDS-PAGE	5	19	18	3
				TGLELIR		SDS-PAGE	5	85	17	2
				VFAECTITELLK		SDS-PAGE	5	44	19	2
Mb0255c	Rv0249c	31.08	9.73	LTCYYYR	3	SCX	7	30	20	2
				SVWQSPTSCAVPEPR		SCX	FT3	56	20	2
				YWIWTQVSK		SCX	8	28	19	2
Mb0257c	Rv0251c	17.78	5.04	PVWDVEPWDR	1	SCX	8	42	23	2
Mb0271c	Rv0265c	35.25	6.14	ALLADPEIAASQATAQR	2	SCX	FT2	45	6	2
				QLPPQISQILGA		SDS-PAGE	6	72	10	2
Mb0276	Rv0270	59.89	6.76	DLQFNPSTVGPVVK	4	SCX	1	74	20	2
				EVIFLDELPR		SDS-PAGE	5	42	18	2
				IILLNSEFSGPQIK		SDS-PAGE	5	50	19	2
				LIAHSSTAPAPK		SDS-PAGE	5	67	20	2
Mb0289	Rv0281	32.96	4.63	AVGRPVPGPESE	4	SCX	FT1	29	20	2
				EVLASHGAQPR		SCX	FT2	15	14	2
				GLLIYLPATAQE		SCX	2	54	19	2
				YFDEYFR		SDS-PAGE	7	19	17	2
Mb0303c	Rv0295c	29.77	6.52	SGSTLLVESLR	1	SDS-PAGE	7	39	19	2

Mb0321	Rv0313	13.92	4.17	FLSSSGAGTGWSAIFEDLSR	2	SCX	FT2	55	19	2
				TAGEAGDGVWAIYTVDADGGAR		SCX	FT3	51	21	2
Mb0323	Rv0315	32.15	4.61	TCPIGVDGGWHNWR	1	SCX	8	38	16	3
Mb0351c	Rv0344c	19.27	6.24	DSVGCEAPFTNSPLR	1	SDS-PAGE	7	56	10	2
Mb0358	Rv0350	66.83	4.59	DAGQIAGLNVLR	5	SDS-PAGE	5	28	19	2
				DTLNKVDAAVAE		SDS-PAGE	4	35	19	2
				ITQDLLDR		SDS-PAGE	4	45	20	2
				SQALGQAIYE		SDS-PAGE	4	23	15	2
				TTPSIVAFAR		SDS-PAGE	5	29	15	2
Mb0368	Rv0361	29.98	4.64	RFTAPGFDAK	5	SCX	5	18	16	2
				RRFTAPGFDAKE		SCX	FT2	16	13	3
				SYPLVPHDAETE		SCX	FT3	71	23	2
				TQVIVTAHE		SDS-PAGE	8	28	19	2
				VFQTNQAPTTPPR		SDS-PAGE	8	24	13	2
Mb0386	Rv0379	7.97	9.08	VIEQDMAVDSAGK	1	SDS-PAGE	10	71	18	2
Mb0400c	Rv0394c	25.27	5.80	EICESVGGADTVLSR	6	SCX	FT1	82	12	2
				IDKNPELEPLLSQAIEAATR		SCX	8	62	14	3
				LLILPSGGSAPTGDHPTPHPSTSR		SCX	FT2	11	8	3
				SGHILIHDVSDFGHR		SDS-PAGE	7	79	9	3
				SVGGADTVLSRIDKNPE		SDS-PAGE	7	67	11	3
				AATRTSME		SDS-PAGE	3	10	3	2
Mb0415	Rv0407	36.96	5.13	FLELFQSDLAPR	4	SCX	1	51	20	2
				FWAPLSLTAEQK		SDS-PAGE	6	75	18	2
				ISYDPDPELALNNTR		SDS-PAGE	6	36	18	2
				LLLGTSVLTPTFR		SDS-PAGE	6	37	18	2
				VDFDGDYYR		SDS-PAGE	5	53	19	2
Mb0419c	Rv0411c	35.40	5.21	FVNGTLER	4	SCX	7	50	12	2

				LVNFSTVYLDANQR		SCX	FT3	48	13	2
				NDGTWNTLYR		SDS-PAGE	6	45	12	2
				WLTVLGPAPAPPTPR		SDS-PAGE	6	62	12	2
Mb0426	Rv0418	52.04	4.78	AVGTPGYQASVDYVVNTLR	13	SCX	FT3	92	14	2
				DAAAQRGAVALIIADNIDE		SDS-PAGE	5	25	11	2
				FGLIGSRNYVE		SDS-PAGE	5	32	12	2
				FSARVFKAE		SDS-PAGE	5	65	12	2
				GAVALIIADNIDEQAMGGTLGANTDVK		SDS-PAGE	5	40	8	3
				GVVTLGGNTVEAR		SDS-PAGE	5	64	6	2
				KGVVTLGGNTVE		SDS-PAGE	5	40	13	2
				LPVSGAVVLVDR		SDS-PAGE	5	77	12	2
				LQDIANANDGTR		SDS-PAGE	5	34	11	2
				LTASTQSFK		SDS-PAGE	5	45	12	2
				NSGFDVQTPEFSAR		SDS-PAGE	5	14	8	2
				NYVESLDIDALK		SDS-PAGE	5	50	12	2
				TAQDTSFDGR		SDS-PAGE	5	46	12	2
Mb0427	Rv0419	52.97	5.06	ALSILNDPFR	1	SDS-PAGE	5	64	12	2
Mb0439	Rv0431	16.83	4.29	TLGAAVELR	1	SDS-PAGE	8	61	14	2
Mb0440	Rv0432	23.84	6.43	GDGSAMLVTTTDAFTMDDLLSGAK	5	SCX	2	22	13	3
				LTPGFHGLHIHQVGK		SCX	FT3	68	10	3
				TAIIIHAGADNFANIPPER		SDS-PAGE	8	29	11	3
				YVQVNGTPGPDETTLTTGDAGK		SDS-PAGE	7	14	8	3
				YVQVNGTPGPDETTLTTGDAGKR		SDS-PAGE	7	79	11	3
Mb0447c	Rv0439c	33.65	10.10	VVQSSAQSHDKDLQR	1	SCX	FT3	49	20	3
Mb0448	Rv0440	55.88	4.74	AGAATEVELK	79	SCX	FT2	21	20	2
				AKTIAYDEE		SDS-PAGE	6	25	17	2
				ALSTLVVNK		SDS-PAGE	5	40	16	2

AMDKVGNEGVITVEE	SCX	FT1	62	19	2
APGFGDR	SCX	3	43	21	2
APLKQIAFNSGLEPGVVAE	SCX	4	23	15	2
ASVPGGGDMGGMDF	SCX	FT2	49	16	2
ATGANIVKVALE	SCX	4	27	16	2
AVLEDPYILLVSSK	SCX	4	54	15	2
AVVADKPEKE	SCX	FT2	16	16	2
DAVRNAKAAVEE	SCX	FT1	114	20	2
DETTIVEGAGDTDAIAGR	SCX	2	24	20	2
DLLPLLEK	SCX	FT1	54	19	2
DLLAAGVADPVK	SCX	4	29	16	2
EIELEDPYEK	SCX	FT3	41	24	2
EIELEDPYEKIGAELVK	SCX	FT2	32	16	3
EQIAATAAISAGDQSIGDLIAEAMDK	SCX	4	50	16	3
GAGDTDAIAGRVAQIRQE	SCX	7	19	15	3
GDEATGANIVKVALE	SCX	FT1	60	20	2
GEALSTLVVNK	SCX	FT3	71	23	2
GIVAGGGVTLLQAAPTLDE	SCX	1	49	19	2
GLNALADAVK	SCX	4	32	15	2
GLRNVAAGANPLGLKRGIE	SCX	FT2	49	17	3
GLRNVAAGANPLGLKRGIEKAVE	SCX	4	21	16	3
GLRNVAAGANPLGLKRGIEKAVEKVTE	SCX	FT2	61	15	4
GMRFDKGYISGYFVTDPERQE	SCX	7	87	20	3
GYISGYFVTDPER	SCX	4	73	15	2
GYISGYFVTDPERQE	SCX	FT2	34	16	2
HRIEDAVR	SCX	FT2	53	22	3
IENSDSDYDRE	SCX	FT2	45	14	2

IENSDSDYDREKLQE	SCX	3	28	20	3
KASVPGGGDMGGMDF	SCX	4	32	14	2
KIGAELVKE	SCX	FT1	95	19	2
KKWGAPTITNDGVSIAKE	SCX	1	71	19	3
KTDDVAGDGTTTATVLAQALVR	SCX	FT1	50	20	3
KVIGAGKPLLIIAE	SCX	FT3	31	16	2
KVIGAGKPLLIIAEDVE	SCX	2	23	20	2
KVIGAGKPLLIIAEDVEGE	SCX	FT2	43	12	3
KVRNLPAGHGLNAQTGVYE	SCX	FT2	51	13	3
KWGAPTITNDGVSIAKE	SCX	FT3	62	23	2
LAGGVAVIK	SCX	FT2	24	15	2
LEDPYEKIGAE	SCX	FT1	126	19	2
LEGDEATGANIVK	SCX	FT1	30	20	2
NADLSLLGK	SCX	4	36	17	2
NADLSLLGKARKVVVTKDE	SCX	FT2	36	16	3
NLPAGHGLNAQTGVYE	SCX	FT2	64	16	2
NLPAGHGLNAQTGVYEDLLAAGVADPVK	SCX	FT3	45	23	3
NVAAGANPLGLK	SCX	FT1	55	17	2
PTITNDGVSIAK	SCX	FT2	52	21	2
QEAVLEDPYILLVSSK	SCX	FT1	65	20	2
QEIENSDSDYDR	SDS-PAGE	5	71	18	2
QEIENSDSDYDREK	SDS-PAGE	5	43	19	2
QIAFNSGLEPGVVAEK	SDS-PAGE	9	21	18	2
QIAATAAISAGDQSIGDLIAE	SDS-PAGE	1	37	21	2
RGLNALADAVKVTLGPKGRNVVLE	SDS-PAGE	4	25	16	4
RLAKLAGGVAVIKAGAATE	SDS-PAGE	5	30	14	3
RLAKLAGGVAVIKAGAATEVE	SDS-PAGE	9	48	18	3

				SALQNAASIAGLFLTTE		SDS-PAGE	9	26	18	2
				SALQNAASIAGLFLTTEAVVADKPEK		SDS-PAGE	6	25	17	3
				SNTFGLQLE		SDS-PAGE	4	16	15	2
				TDDVAGDGTTTATVLAQALVR		SDS-PAGE	6	63	18	2
				TIAYDEEAR		SDS-PAGE	5	25	13	2
				TIAYDEEARR		SDS-PAGE	2	48	18	2
				TKEQIAATAAISAGDQSIGDLIAE		SDS-PAGE	5	20	15	2
				TLLKGAKE		SDS-PAGE	4	49	16	2
				TLLKGAKEVE		SDS-PAGE	5	73	14	2
				TTIVEGAGDTDAIAGR		SDS-PAGE	5	32	20	2
				TTIVEGAGDTDAIAGRVAQIRQE		SDS-PAGE	1	39	20	3
				VAKKTDDVAGDGTTTATVLAQALVRE		SDS-PAGE	3	28	15	4
				VALEAPLK		SDS-PAGE	3	40	19	2
				VIGAGKPLLIIAE		SDS-PAGE	5	61	16	2
				VIGAGKPLLIIAEDVE		SDS-PAGE	4	21	14	2
				VIGAGKPLLIIAEDVEGEALSTLVVNK		SDS-PAGE	1	20	18	3
				VRNLPAGHGLNAQTGVYE		SDS-PAGE	5	23	15	2
				VTLGPKGRNVVLE		SDS-PAGE	5	78	19	2
				VVVTKDETTIVEGAGDTDAIAGR		SDS-PAGE	5	44	12	3
				WGAPTITNDGVSIAK		SDS-PAGE	1	17	16	2
				WGAPTITNDGVSIAKE		SDS-PAGE	2	18	16	2
				AAVEEGIVAGGGVTLLQAAPTLDELK		SCX	FT2	70	15	3
Mb0451	Rv0443	18.96	5.20	APADLLSGYYHAVHK	1	SDS-PAGE	9	42	20	3
Mb0463c	Rv0455c	16.63	6.94	DSTEDFPIPR	1	SCX	1	36	14	2
Mb0473c	Rv0464c	21.30	10.07	QLGPVNWLVAK	2	SCX	1	52	19	2
				QQALLQATDELIK		SDS-PAGE	9	28	19	2
Mb0477	Rv0468	30.69	4.95	ALGLLTFTTDLNDLSDR	4	SCX	FT3	29	19	2

				SDAIQRVGVVGAGQMGSGIAE		SCX	FT2	45	16	2
				TLVTDEAAAAR		SCX	FT3	64	21	2
				VTVFEPAEALITAGR		SDS-PAGE	7	28	20	2
Mb0478	Rv0469	33.10	4.73	TLNIWAANLEANK		SDS-PAGE	7	46	19	2
Mb0485	Rv0475	21.53	9.84	AENSNIDDIK	10	SCX	FT1	75	20	2
				ALGTVASQTR		SCX	FT3	31	23	2
				ALGTVASQTRAVGE		SCX	FT3	57	22	2
				APLLAALGAADLALATVNE		SCX	FT3	24	23	2
				LQEDLPEQLTE		SCX	FT1	59	20	2
				LQEDLPEQLTELR		SCX	4	27	16	2
				SQQSFEEVSAR		SDS-PAGE	9	59	20	2
				SRARLTKLQEDLPEQLTE		SDS-PAGE	10	28	19	3
				YNELVER		SDS-PAGE	8	56	19	2
				AAEGYLEAATSR		SCX	FT3	32	20	2
Mb0508	Rv0497	33.09	5.23	ALDVVPTLWR	9	SCX	7	43	20	2
				DDHHHAGPDAHASQSPAANGR		SCX	FT3	55	21	4
				HAAAAAAGTDVEGDGAAEAR		SCX	1	37	20	2
				QISVAELLAR		SDS-PAGE	6	18	15	2
				RGDSDAITVAELTGEIPIIR		SDS-PAGE	2	29	16	3
				SSGNRQISVAE		SDS-PAGE	6	51	19	2
				TESSGNRQISVAE		SDS-PAGE	6	36	17	2
				TVYWSQPEPR		SDS-PAGE	6	30	20	2
				VQVGEAAPQSPAEPVAEQVAEEPTR		SDS-PAGE	6	33	19	3
Mb0515c	Rv0503c	34.66	4.91	DKYTDVCQFTLVK	18	SCX	1	53	20	2
				FDEPVDRIVSLGAFE		SCX	FT2	43	16	2
				FILTEIFPGGR		SCX	FT3	38	20	2
				FYNLTPDDGR		SCX	FT1	87	19	2

				HAVAEYDVNVIGLTLSENQYAHDK		SCX	7	49	23	3
				HFADGAGDAGFER		SCX	FT1	43	20	2
				IGANYVPTLNAWADALQAHK		SCX	7	44	20	3
				IGANYVPTLNAWADALQAHKDE		SCX	7	59	20	3
				IQGWEEFDEPVDR		SCX	7	86	20	2
				ISQVDYYSSNAGWK		SCX	1	38	20	2
				IVSLGAFEHFADGAGDAGFER		SCX	7	32	24	3
				LGLTSPMSLLR		SCX	8	31	23	2
				RYDTFFK		SCX	7	50	19	2
				TSQGDTTSGTQLKPPVEAVR		SCX	8	23	22	3
				TYDIYMHYLR		SCX	7	20	20	3
				YDTFFK		SCX	FT2	27	16	2
				YDVNVIGLTLSE		SCX	FT3	30	20	2
				YTDVCQFTLVK		SDS-PAGE	6	45	19	2
Mb0538	Rv0525	22.21	6.72	DIVAVIASPLQR	1	SDS-PAGE	8	53	19	2
Mb0539	Rv0526	23.21	8.05	ELLAADLQPVVER	2	SCX	7	50	12	2
				GAGVSFLGIDVR		SDS-PAGE	8	59	13	2
Mb0568	Rv0553	33.94	4.60	VAPLGGISALLDIAAR	1	SCX	FT1	58	19	2
Mb0577	Rv0562	35.53	4.72	ALLNGPVDDDAEVR	5	SCX	FT1	70	19	2
				ALAALVDYTVSR		SCX	FT3	26	20	2
				IIADTFAQLVTGQMR		SCX	7	30	23	2
				TPATVVAGVDLGDAVFAAAVR		SDS-PAGE	6	29	17	3
				VMSDSLLHLFNAGGK		SDS-PAGE	6	42	19	3
Mb0578	Rv0563	30.68	10.20	AMHAQPVSELQAPAMYR	5	SCX	FT3	69	14	3
				AVLGHELSHVYNR		SDS-PAGE	8	28	10	3
				LYISDTAAPNAFATGR		SDS-PAGE	8	73	11	2
				NAAVCCTTGILR		SDS-PAGE	8	72	10	2

				SGAVLTGDPLALASALR		SDS-PAGE	8	30	6	2
Mb0591	Rv0576	46.41	5.97	GWNHFLDR	2	SCX	2	38	20	2
				SLAIIGAAAGAQLAPR		SDS-PAGE	6	64	18	2
Mb0595c	Rv0580c	18.034	10.36	AVVSELFLR	8	SCX	1	41	20	2
				HNFSDGAAAQVVYDGK		SCX	FT1	52	19	2
				HNFSDGAAAQVVYDGK		SCX	1	55	20	2
				IPTLEEFAEAVDR		SDS-PAGE	9	33	18	2
				LVNPILR		SDS-PAGE	9	22	19	2
				MLGLSFR		SDS-PAGE	8	37	19	2
				SLLHTPLAGPLR		SDS-PAGE	8	31	19	3
				TQLMVVSFTGR		SDS-PAGE	8	28	18	2
Mb0598c	Rv0583c	23.682	4.39	APYGGIVYTQPADPNDPPTIVAILSK	5	SCX	FT3	58	11	3
				DNHIQETPVHHGDPGSPTIDLPVPDDWR		SCX	7	24	10	4
				LTGDIDPAK		SCX	FT3	15	7	2
				TMTLMDAANVIDE		SCX	FT3	38	12	2
				VLQFAPGELK		SDS-PAGE	7	33	12	2
Mb0651c	Rv0634c	25.89	4.71	VTGIPVGDLITHEHGDK	1	SDS-PAGE	7	49	18	3
Mb0655	Rv0636	14.93	6.53	FTAVVPVPNDGK	2	SDS-PAGE	10	23	21	2
				VGDQLPEK		SDS-PAGE	10	39	19	2
Mb0656	Rv0637	18.93	5.02	YPDYFIVGR	2	SCX	1	50	21	2
				YVQLDFFR		SDS-PAGE	9	55	20	2
Mb0658	Rv0639	25.41	4.42	ETPVELTFGQVSK	2	SDS-PAGE	7	31	20	2
				VLPGYILVR		SDS-PAGE	7	50	18	2
Mb0662c	Rv0643c	33.26	5.19	FIVTDIFPGGR	1	SDS-PAGE	7	38	19	2
Mb0663c	Rv0644c	32.68	5.53	EDMTLEEAQIAK	7	SCX	7	40	20	2
				ILPPDGVLLLHTITGLTR		SCX	2	27	20	3
				IVSIGAFEHFGHDR		SCX	8	40	20	3

				RQSLQPHYAR		SCX	FT1	66	19	3
				SEAIAIQSEEVYER		SCX	FT3	65	20	2
				VGYIDVNQFTLAK		SDS-PAGE	7	60	18	2
				VLLAGWEQFNEPVDR		SDS-PAGE	6	20	18	2
Mb0664c	Rv0645c	33.15	4.69	HVQLLQQHYAR	3	SCX	8	50	23	3
				SYDILPDDGR		SCX	2	27	21	2
				YLTFFER		SDS-PAGE	3	23	19	2
Mb0670	Rv0651	18.48	8.46	GLTVANLAELR	1	SDS-PAGE	9	49	19	2
Mb0671	Rv0652	13.44	4.30	AKDLVDGAPKPLLE	11	SCX	FT2	59	16	2
				DLVDGAPKPLLEK		SCX	FT2	36	15	3
				EIVSGLGLK		SCX	FT2	25	16	2
				EMTLLELSDFVK		SCX	FT1	57	20	2
				IVSGLGLKE		SDS-PAGE	9	87	19	2
				LEAAGATVTVK		SDS-PAGE	10	32	16	2
				LLDAFKE		SDS-PAGE	10	17	14	2
				LSDFVKKFEE		SDS-PAGE	10	45	14	3
				LSTDELLDAFK		SDS-PAGE	9	29	15	2
				VTAAAPVAVAAAGAAPAGAAVE		SDS-PAGE	10	22	19	2
				AAGDKKIGVIKVVRE		SDS-PAGE	9	52	20	4
Mb0686	Rv0667	129.22	4.67	FGFSEIMR	4	SCX	1	58	20	2
				RGDVNPVGGLEE		SDS-PAGE	3	29	14	2
				SEAPLVGTGMELR		SDS-PAGE	2	20	20	2
				VVVSQLVR		SDS-PAGE	1	25	19	2
Mb0687	Rv0668	146.73	6.08	LEDIWSTFTK	1	SCX	1	48	20	2
Mb0698c	Rv0679c	16.55	4.59	LVVVGLDNTVTYK	3	SCX	FT3	54	11	2
				NGDPTIDNLGAGNR		SCX	FT2	16	4	2
				TVTVGGANNKIAFDRIDE		SDS-PAGE	8	34	11	3

Mb0704	Rv0685	43.56	5.12	AFDQIDNAPEER	20	SCX	FT1	57	19	2
				EHVLLAR		SCX	FT1	75	20	2
				ELLAAQEFDEDAPVVR		SCX	FT2	38	15	2
				ETDKPFLMPVEDVFTITGR		SCX	FT3	87	19	3
				GQVYILSKDE		SCX	FT2	39	14	2
				HYAHVDAPGHADYIK		SCX	FT2	31	14	3
				LIQPVAMDEGLR		SCX	FT2	22	15	2
				LLDQGQAGDNVGLLLR		SCX	7	42	21	2
				NMITGAAQMDGAILVVAATDGPMPQTR		SCX	8	39	20	3
				QVGVPYILVALNK		SCX	FT1	66	21	2
				RGQVVTKPGTTTPHTE		SCX	1	24	20	3
				RGQVVTKPGTTTPHTEFE		SDS-PAGE	5	20	19	3
				RGVINVNEE		SDS-PAGE	5	74	17	2
				RGVINVNEEVE		SDS-PAGE	5	32	15	2
				SIPDPVRE		SDS-PAGE	5	32	18	2
				TDKPFLMPVEDVFTITGR		SDS-PAGE	5	79	17	3
				TKPHVNIGTIGHVDHGK		SDS-PAGE	6	27	15	4
				TTLTAAITK		SDS-PAGE	5	50	17	2
				TTVTGVEMFR		SDS-PAGE	6	39	18	2
				VLHDKFPDLNETK		SDS-PAGE	5	48	18	3
Mb0737	Rv0716	21.02	10.75	LTSIALPR	2	SCX	2	35	19	2
				MWEFLDR		SDS-PAGE	8	43	20	2
Mb0759	Rv0738	46.41	5.97	ALVGPQFR	3	SDS-PAGE	9	106	19	2
				AQDAFAALLANVR		SDS-PAGE	9	34	21	2
				ERPPADQLAAFLGR		SDS-PAGE	9	53	19	3
Mb0784c	Rv0761c	39.72	4.93	LNLDDMVTTAYK	3	SCX	FT3	77	20	2
				NLCDLGAGLLAGESVTDGSFR		SCX	FT1	82	20	2

				YVFAVEPVEWK		SDS-PAGE	6	31	19	2
Mb0812	Rv0788	23.60	4.57	FAPVMDEVVAAADR	4	SCX	FT1	22	20	2
				FEPDADLLVPLK		SDS-PAGE	8	27	18	2
				IGVVTFPGTLDDVDAAR		SDS-PAGE	9	20	19	2
				VASTSTAWTSR		SDS-PAGE	8	33	19	2
Mb0826	Rv0803	79.47	4.53	LLAAVLSSASR	1	SDS-PAGE	4	37	20	2
Mb0838c	Rv0815c	31.01	4.95	DEVLAAINVK	11	SCX	FT1	33	20	2
				DFVDAQQFSK		SCX	FT3	58	24	2
				ELLGHQNVK		SCX	FT1	76	23	2
				LYADAGLDNSK		SCX	4	32	17	2
				LYADAGLDNSKE		SCX	3	41	20	2
				PVTSYTASPPDNTIR		SCX	6	33	24	2
				SNLHAPKVVFVE		SCX	1	25	21	2
				SSHTWFVLR		SDS-PAGE	7	29	21	3
				SSHTWFVLRE		SDS-PAGE	7	34	18	2
				TDLQDPVKR		SDS-PAGE	7	44	19	2
				TIAYCRIGE		SDS-PAGE	7	21	15	2
Mb0844c	Rv0821c	23.51	5.14	AIVSAIQMVADIDR	1	SDS-PAGE	8	74	19	2
Mb0847c	Rv0824c	38.77	6.68	NLHMIFYR	1	SCX	9	44	23	2
Mb0854c	Rv0831c	30.18	4.85	HLLINDLPIER	2	SDS-PAGE	7	35	15	2
				IRHPTTDSLTE		SDS-PAGE	7	55	19	2
Mb0923	Rv0899	33.54	7.51	GASALSLSLLSISR	1	SDS-PAGE	6	49	18	2
Mb0930	Rv0906	40.64	6.56	APFLVPLGVGAHLR	1	SDS-PAGE	6	39	10	3
Mb0951	Rv0928	37.93	5.85	AFLQSTIGAGQSGLGDNGYIPIPDEFK	11	SCX	FT3	54	13	3
				DFTLPGER		SCX	7	48	14	2
				FNGNQTDFGGSDVPLSKDE		SCX	FT1	26	13	2
				IFNGSITQWNNPAIQALNR		SDS-PAGE	6	38	6	2

				IHVVFR		SDS-PAGE	6	35	10	2
				RPGSYPIVLATYE		SDS-PAGE	6	54	10	2
				RPGSYPIVLATYEIVCSK		SDS-PAGE	6	75	9	3
				SDESGTTDNFQR		SDS-PAGE	6	57	13	2
				SFQGGVGEGAR		SDS-PAGE	6	61	14	2
				YLQAASNGAWGK		SDS-PAGE	6	36	13	2
				YPDSQVGTAVK		SDS-PAGE	6	28	10	2
Mb0956c	Rv0932c	37.83	4.75	AFMQAAIGPGQEGLDQYGSIPLPK	11	SCX	FT3	19	12	3
				ELHSSGSTAQENAME		SCX	FT2	48	12	2
				ELHSSGSTAQENAMEQFVYAYVR		SCX	FT2	36	11	3
				GVSTLNLDGPTTAK		SCX	FT3	28	14	2
				IVCSKYPDATTGTAVRAFMQAAIGPGQE		SCX	FT1	80	12	3
				QFVYAYVR		SCX	FT3	102	15	2
				QLNMAQIITSAGPDPVAITTE		SCX	FT3	93	12	2
				QLNMAQIITSAGPDPVAITTESVGK		SDS-PAGE	6	74	4	3
				TDFAGSDVPLNPSTGQPDR		SDS-PAGE	6	65	10	3
				YLDGASNGAWGK		SDS-PAGE	6	51	8	2
				YPDATTGTAVR		SDS-PAGE	6	63	11	2
Mb0959	Rv0934	38.21	5.02	AQLGNSSGNFLLPDAQSIQAAAAGFASK	8	SCX	FT3	85	12	3
				GLGEAQLGNSSGNFLLPDAQSIQAAAAGFASK		SCX	FT3	82	12	3
				GLMNIALAISAQQVNYNLPGVSEHLK		SCX	FT3	28	15	3
				SDGSGDTFLFTQYLSK		SCX	FT1	36	10	2
				SPGFGTTVDFPAVPGALGE		SCX	FT3	34	16	2
				TPGCVAYIGISFLDQASQRGLGE		SDS-PAGE	6	11	10	3
				TWDDPQIAALNPGVNLPGTAVVPLHR		SDS-PAGE	6	56	10	3
				VLAAMYQGTIK		SDS-PAGE	6	107	9	2
				YAIVNNR		SDS-PAGE	6	32	8	2

Mb0976	Rv0951	40.93	4.74	ALGMLGDEANKPLVVR	11	SCX	1	48	20	3
				ANHPLVTLVATMDE		SCX	FT1	48	20	2
				GVDLDFAR		SCX	FT1	64	19	2
				HNVPSTPGR		SCX	1	22	20	2
				ILTEANHPLVTLVATMDEAADK		SCX	FT3	47	23	3
				ITLDGNADFR		SCX	1	38	19	2
				LDGNNVEEGRR		SDS-PAGE	6	39	19	3
				MDLFEYQAK		SDS-PAGE	6	69	18	2
				SIAEQGHLPAEVLDTAAVTIAK		SDS-PAGE	6	42	19	3
				VMAAGLDVVLGDQQVK		SDS-PAGE	6	37	20	2
				YAATPQDAYEHAK		SDS-PAGE	6	76	17	3
				AATDPLELK		SDS-PAGE	6	53	18	2
Mb0977	Rv0952	31.20	5.64	AIDAEIPLLVVITE	2	SCX	FT3	31	15	2
				VIVQGITGSEATVHTAR		SCX	1	47	19	3
Mb0979	Rv0954	30.20	6.79	FDPYGQYGR	1	SCX	7	41	20	2
Mb0993	Rv0968	10.26	6.25	LMFADVLAEASER	1	SCX	FT1	63	20	2
Mb1010	Rv0984	18.44	4.29	EILGIAEAIR		SDS-PAGE	9	33	19	2
Mb1026	Rv09992	25.94	8.95	AIDPGFFSAR	5	SDS-PAGE	8	77	12	2
				DLLVQAVAAVR		SDS-PAGE	8	43	12	2
				SGFGPEYTVTDVTPR		SDS-PAGE	8	40	13	2
				VLQAVVGGSAR		SDS-PAGE	8	37	14	2
				VTFSGTQLR		SDS-PAGE	8	57	13	2
Mb1033	Rv1006	61.26	5.32	DDSGFYPVATLDIPR	7	SCX	FT1	41	12	2
				ELFADEVTNK		SDS-PAGE	5	46	6	2
				KYDFSVIDNALTR		SDS-PAGE	5	74	10	2
				LVDSTGQVVR		SDS-PAGE	5	40	13	2
				SSDQPTPASVAETVR		SDS-PAGE	5	64	13	2

				VDLSGLPAGHYTLR		SDS-PAGE	5	19	11	3
				YSVEAQPGSQALAGK		SDS-PAGE	5	65	11	2
Mb1047	Rv1019	21.70	5.32	EGLYAVVVDR	2	SDS-PAGE	8	35	19	2
				VALALLTYVEER		SDS-PAGE	8	42	19	2
Mb1051	Rv1023	44.93	4.26	ILDSRGNPTVEVE	5	SCX	5	34	15	2
				RVAKYNQLLRIEE		SDS-PAGE	5	87	18	3
				VQIVGDDIFVTNPER		SDS-PAGE	5	116	19	2
				YAGDLAFPR		SDS-PAGE	5	56	19	2
				AAADSAELPLFR		SDS-PAGE	6	20	16	2
Mb1067c	Rv1038c	10.99	5.02	NIVNMLHGVR	2	SCX	7	42	19	2
				VHAQTVEDEAR		SCX	FT3	60	23	2
Mb1107	Rv1078	25.06	7.28	TMPTESYTPWITR	3	SCX	7	34	13	2
				TTGQPIGFGMSVVR		SDS-PAGE	2	35	12	2
				VVSETTGQPIGFGMSVVR		SDS-PAGE	7	23	9	3
Mb1111	Rv1082	32.70	4.93	GEILNPAMDLPDVHGR	5	SCX	2	22	20	3
				LYYVHGFLR		SCX	4	19	14	3
				MQMLQDEFAR		SCX	FT1	31	21	2
				RLWPTEEFE		SDS-PAGE	7	36	20	2
				VLVVTLTGGER		SDS-PAGE	7	37	18	2
Mb1123	Rv1093	45.03	6.58	DSPLDGQAAEDLLHE	3	SCX	FT3	24	22	2
				VIIAGWSAYPR		SCX	1	32	20	2
				VLDFAAFR		SDS-PAGE	4	28	18	2
Mb1124	Rv1094	31.35	4.58	AQKPVADALTLE	8	SCX	FT1	51	19	2
				EVDPVANEDVR		SCX	8	16	15	2
				HLHAIALRE		SCX	FT3	99	20	2
				ITAWGLAGEPSLK		SCX	2	23	20	2
				LQNVADAGIFGKPQLR		SDS-PAGE	7	38	15	3

				NLAAQIEEPILAGLIDR		SDS-PAGE	7	52	19	2
				WTAEEHLHAIALR		SDS-PAGE	7	60	18	3
				AADLDVLGADIEAYR		SDS-PAGE	7	66	19	2
Mb1127c	Rv1097c	29.87	4.56	VAITNFVTTMDVAAQASK	2	SCX	FT3	74	21	2
				VDADITIADSSR		SCX	FT3	86	20	2
Mb1128c	Rv1098c	50.14	5.18	AVDADSANYRIE	3	SDS-PAGE	5	35	16	2
				LSIEDLDR		SDS-PAGE	5	52	19	2
				TAANSFEAQAAR		SDS-PAGE	5	20	19	2
Mb1152	Rv1121	52.16	4.99	FANQALAELWDR	3	SCX	1	22	19	2
				GWQSPWLPR		SCX	8	39	20	2
				WAGVPIFVR		SCX	2	22	20	2
Mb1198	Rv1166	66.13	5.27	AGAPAALADSVR	5	SDS-PAGE	4	16	13	2
				ALPVTGGQFR		SDS-PAGE	4	21	12	2
				DIPGGFASGLAR		SDS-PAGE	4	20	12	2
				NINDVITAVEPR		SDS-PAGE	5	36	13	2
				QAGGNLATLLASR		SDS-PAGE	4	19	13	2
Mb1211	Rv1178	37.70	6.10	GILVAPGDFYGPGGAQHVR	2	SDS-PAGE	6	41	18	3
				LTEAAVLPVIGTK		SDS-PAGE	6	32	18	2
Mb1230	Rv1198	9.97	4.54	NFQVIYEQANAHGQK	1	SCX	FT1	83	20	2
Mb1241	Rv1209	13.08	4.84	ATTATTLPAFGVTR	1	SDS-PAGE	10	37	13	2
Mb1267	Rv1235	49.76	5.43	FTIAQVSLPR	3	SDS-PAGE	5	57	14	2
				GGVPFLPLNR		SDS-PAGE	5	21	13	2
				VTIGGLNLAVAK		SDS-PAGE	5	30	11	2
Mb1271c	Rv1239c	41.48	5.27	HGEHGGLSEVR	2	SCX	1	31	20	2
				KLDIEPIYLLK		SCX	7	46	20	2
Mb1301c	Rv1270c	24.87	5.21	DASVAGSQQADGVATTK	6	SDS-PAGE	8	52	13	2
				DTSVTLTMSDWGK		SDS-PAGE	8	13	11	2

				GLANLLANLK		SDS-PAGE	8	27	13	2
				ITGNSSADDIATLAGSR		SDS-PAGE	8	46	13	2
				LAVTGDVPNLR		SDS-PAGE	7	121	11	2
				LEGDISNTPQTVATGSATLLVGNK		SDS-PAGE	7	53	11	3
Mb1305	Rv1274	19.69	6.76	HGPFFPQCGGVSDQTVTELTR	3	SDS-PAGE	9	41	11	3
				LTRQSIANSK		SDS-PAGE	9	15	14	2
				VTGLVNTAK		SDS-PAGE	9	38	6	2
Mb1306	Rv1275	18.87	6.48	AIQGVDSIVMR	5	SCX	FT3	62	12	2
				ECEVLTTDILAK		SDS-PAGE	10	24	12	2
				FWFEQGSLSNER		SDS-PAGE	9	18	11	2
				LMELTLATNA		SDS-PAGE	9	21	13	2
				WQAANPAGLIDITR		SDS-PAGE	10	26	13	2
Mb1320	Rv1289	23.54	5.27	TRFPVGVR	2	SDS-PAGE	8	32	19	2
				YPTLGVGTR		SDS-PAGE	8	38	21	2
Mb1329	Rv1297	65.10	5.42	TTILQDIANAITR	1	SCX	7	70	19	2
Mb1333	Rv1301	22.58	4.57	MPLHPVAIELLR	1	SDS-PAGE	8	36	18	3
Mb1336	Rv1304	27.47	6.60	GHVTLLAPINLVEE	3	SCX	FT3	56	22	2
				NQVESAIGMR		SCX	FT3	41	22	2
				VTSTDVPGGVQLFFE		SDS-PAGE	3	20	19	2
Mb1338	Rv1306	18.32	4.86	AHVGTMSATLASR	3	SCX	FT1	44	20	2
				ILGVDLTASAATR		SDS-PAGE	9	39	19	2
				SDEQFAAAQADYDEAMTEAR		SDS-PAGE	9	64	19	2
Mb1339	Rv1307	48.81	5.26	ADSTVNPVVVALLSHTVE	14	SCX	FT3	37	22	2
				AGQVDEVEDQLFR		SCX	FT2	47	20	2
				GQAVEEAVLFLAEVAVAR		SCX	FT1	72	20	3
				LAILLGDCAVPAEGR		SCX	7	22	20	2
				LELGHESVR		SCX	FT1	64	20	2

				NHVADQAQQSATVDR		SCX	FT2	21	16	2
				QALLELAER		SCX	FT1	59	20	2
				QQLADAAAAADR		SCX	FT1	55	20	2
				RADSTVNPVVVALLSHTVE		SDS-PAGE	5	58	19	3
				RGEIVAQVGAAAELSDAQR		SDS-PAGE	4	35	18	3
				VEDQLFR		SDS-PAGE	3	29	19	2
				VGAPTLEVLR		SDS-PAGE	5	73	18	2
				WSANSDLIDAIEHVSR		SDS-PAGE	5	72	18	3
				YLTVPAEDATPR		SDS-PAGE	5	87	17	2
Mb1340	Rv1308	59.29	4.78	ASEEEILTEIR	6	SCX	2	43	20	2
				HVLIIFDDLTK		SCX	FT1	32	20	2
				LSDDLGGGSLTGLPIIETK		SCX	FT3	24	23	2
				TGEVLSVPVGDGFLGR		SDS-PAGE	5	32	18	2
				VLSVPVGDGFLGR		SDS-PAGE	3	23	21	2
				VVNPLGQPIDGR		SDS-PAGE	5	27	19	2
				YVSSFTADTSREE		SDS-PAGE	5	56	19	2
Mb1341	Rv1309	33.89	5.15	SEELFSLLR		SCX	1	45	20	2
Mb1342	Rv1310	53.09	4.60	DTALVFGQMDEPPGTR	33	SCX	FT1	66	20	2
				ETIEAFDR		SCX	FT1	113	18	2
				FEHWSIHR		SCX	1	26	20	3
				FLSQNMMAAEQFTGQPGSTVPVK		SCX	FT1	33	18	3
				FTQAGSEVSTLLGR		SCX	FT3	46	21	2
				GHVFNALGDCLDEPGYGEK		SCX	1	51	21	3
				GIFPAVDPLASSSTILDPSVVGDEHYR		SCX	FT3	69	23	3
				GVEVIDTGR		SCX	4	20	15	2
				GVKGHVFNALGDCLDEPGYGEKFE		SCX	FT3	41	15	3
				IALFGGAGVGK		SCX	8	22	21	2

				KPPAFEELEPR		SCX	FT3	41	20	2
				MPSAVGYQPTLADEMGELQER		SCX	FT1	55	19	3
				NFGGTSVFAGVGER		SCX	FT1	59	20	2
				QAFFLIGGLDDLAK		SCX	FT2	73	19	2
				QAFFLIGGLDDLAKKAE		SCX	FT1	22	21	3
				QFTGQPGSTVPVKE		SCX	7	35	23	2
				RFLSQNMMAAE		SCX	FT2	38	12	2
				SISVPVGEGVK		SCX	FT2	25	14	2
				SLAKTLTLE		SCX	FT3	85	20	2
				TEMLETGLK		SCX	1	34	20	2
				TISLQPTDGLVR		SDS-PAGE	3	19	17	2
				TLTLEVAQHLGDNLVR		SDS-PAGE	4	61	18	3
				TREGNDLWVE		SDS-PAGE	4	54	21	2
				TVLIQEMINR		SDS-PAGE	5	30	21	2
				VALSALTMAEWFR		SDS-PAGE	4	50	20	2
				VAQEVIR		SDS-PAGE	5	30	19	2
				VAQHLGDNLVR		SDS-PAGE	5	91	18	2
				VAQHLGDNLVRTISLQPTDGLVRGVE		SDS-PAGE	5	52	17	4
				VIDTGRSISVPVGE		SDS-PAGE	1	36	17	2
				VSTLLGRMPSAVGYQPTLADE		SDS-PAGE	5	41	15	2
				VTGPVVDVEFPR		SDS-PAGE	5	39	20	2
				VVDLLTPYVR		SDS-PAGE	2	38	18	2
Mb1367	Rv1332	24.05	5.14	DSSSPSDELEEITGIK	3	SCX	7	88	20	2
				NLAGAMIGLLDDR		SDS-PAGE	8	24	18	2
				SDANAWIAAVNDLR		SDS-PAGE	8	67	17	2
Mb1368	Rv1333	33.95	4.61	AVLAGVLNAQPVAGIPTYR	1	SCX	FT1	77	19	2
Mb1403	Rv1368	26.85	9.15	GLGAVVGQVQNPTIQGR	4	SDS-PAGE	7	48	8	2

				IYDPGIILDK		SDS-PAGE	7	35	9	2
				KPTTASSPSPGSPSPEAQQILQDSSK		SDS-PAGE	7	37	14	3
				MKPNTPVVATEFLVTNK		SDS-PAGE	7	76	12	3
Mb1414	Rv1379	20.63	7.94	AVQLAVLVDR	1	SDS-PAGE	8	47	19	2
Mb1425	Rv1390	11.81	4.08	IHADLLEHTEGE	1	SCX	FT2	37	16	2
Mb1444	Rv1409	35.37	6.89	AIADAILVGTGTVLADDPALTAR	7	SCX	FT1	44	20	2
				IVGAGGTEPAGGDHAE		SCX	FT3	57	19	2
				LADGSLAPQQPLR		SDS-PAGE	6	72	19	2
				SGVLAEQVAAGPLR		SDS-PAGE	6	96	18	2
				SAAADGSSQWISSEAAR		SDS-PAGE	6	28	17	2
				TDVLLEGGPTLAGAFLR		SDS-PAGE	6	48	19	2
				VGPDLLLSLVAR		SDS-PAGE	6	43	19	2
Mb1445c	Rv1410c	54.68	7.50	VQEQPTLAPQVEPL	1	SCX	FT3	46	9	2
Mb1446c	Rv1411c	24.55	8.23	DTINGQNTIR	8	SCX	FT3	37	14	2
				GSGNSVQMTLSK		SCX	FT1	58	12	2
				IPGLSLK		SDS-PAGE	8	25	12	2
				KVQVTKPPVS		SDS-PAGE	8	36	13	2
				SAHMVLTVNGK		SDS-PAGE	8	32	11	2
				TGDHQLAQAQLDR		SDS-PAGE	8	33	13	3
				TLSGDLTTNPTAATGNVK		SDS-PAGE	7	19	6	2
				VQVTKPPVS		SDS-PAGE	8	26	11	2
Mb1454	Rv1419	16.85	4.12	WNLTDDR	1	SDS-PAGE	10	45	14	2
Mb1463c	Rv1428c	29.89	7.09	IVMQVLDPINLTK	1	SDS-PAGE	7	40	20	2
Mb1471	Rv1436	35.92	5.05	ASVDEINAAFK	9	SCX	FT3	38	20	2
				GLMTTIHAYTQDQNLQDGPHK		SCX	7	50	20	3
				LPCDVGLEGDDTIVVGR		SCX	FT3	22	20	2
				LVDLVTLVGK		SCX	FT1	59	19	2

				VLDDEFGIVK		SCX	FT3	60	22	2
				VPIPTGSVTDLTVDLSTR		SCX	FT3	30	19	2
				VVAANDITDNSTLAHLLK		SDS-PAGE	6	31	18	3
				YYDAPIVSSDIVTDPHSSIFDSGLTK		SDS-PAGE	6	39	18	3
				AAALNIVPTSTGAAK		SDS-PAGE	6	45	19	2
Mb1472	Rv1437	42.51	4.59	GLTGGDILLLE	3	SCX	FT2	43	16	1
				LPVDLVVTEK		SDS-PAGE	6	48	19	2
				VLSREQPTGGVL		SDS-PAGE	6	23	15	2
Mb1475	Rv1440	8.16	10.62	GGGLSTLFGGGVQSSLSGSTVVEK	1	SCX	FT3	49	20	2
Mb1481c	Rv1446c	32.72	4.98	SIEAANDASHEHPSR	2	SCX	FT3	66	23	3
				VGADAGAGEFVVLR		SDS-PAGE	6	51	18	2
Mb1482c	Rv1447c	57.34	5.51	AVNAVFPEEAVFR	4	SDS-PAGE	5	33	18	2
				ETVQNILALR		SDS-PAGE	4	22	19	2
				LAEPLDQTTSR		SDS-PAGE	1	51	20	2
				VQPDEGVTLR		SDS-PAGE	1	30	19	2
Mb1498	Rv1463	28.82	6.08	HEILQLELLKPK	6	SCX	1	53	20	2
				IAILDETDSGLDVDALR		SCX	8	49	22	2
				RHEILQLELLKPK		SDS-PAGE	7	47	18	4
				SQHGGILLITHYTR		SDS-PAGE	10	54	18	3
				SVNEGFSGGEK		SDS-PAGE	9	48	18	2
				AAMAALDIDPAFAER		SDS-PAGE	7	49	18	2
Mb1499	Rv1464	44.60	6.05	ALNLVSYVLGDSR	2	SCX	7	53	23	2
				IDLDSLYLDDR		SDS-PAGE	6	55	19	2
Mb1501	Rv1466	12.38	3.55	SALVGSGLVDDIR	1	SCX	FT3	54	20	2
Mb1502c	Rv1467c	65.90	4.90	FLFDVETGEPGER	5	SCX	8	51	16	2
				NFLPLLTSTR		SDS-PAGE	4	23	16	2
				NIFHLVLARPE		SDS-PAGE	5	63	18	2

				VNDLMLPVVK		SDS-PAGE	5	26	20	2
				VSRLAEGPVAE		SDS-PAGE	5	36	19	2
Mb1512	Rv1476	19.60	4.35	AEVAALGVSAPPAATPGLLEVVQHAR	4	SCX	FT1	33	17	3
				IVLLDHNPPNDTPLR		SCX	FT2	20	15	3
				VTLEAGEDHSK		SCX	1	38	21	2
				VAALGVSAPPAATPGLLE		SDS-PAGE	9	43	20	2
Mb1515	Rv1479	40.76	6.36	ASLGIIAAAR	16	SCX	FT1	54	19	2
				DYVIPQDVIEVIPDVLR		SCX	1	38	20	2
				GHVLLEGVPGVAK		SCX	FT1	90	21	2
				IIVGQDQLVER		SCX	FT3	83	20	2
				INVGYPSPEEER		SCX	FT3	67	16	2
				IQFTPDLVPTDIIGTR		SCX	FT1	45	21	2
				IAANNFVHHALVDYVVR		SCX	8	24	20	3
				KPEQLGMNDVK		SCX	FT1	56	19	2
				LGPVVANFLLADE		SDS-PAGE	6	48	20	2
				LGPVVANFLLADEINR		SDS-PAGE	6	29	20	2
				MLVGLLSK		SDS-PAGE	6	60	18	2
				QILSTGDLLR		SDS-PAGE	5	31	19	2
				SWVAFGASPR		SDS-PAGE	6	58	19	2
				TFPMPSPFLVMATQNPIEHE		SDS-PAGE	6	52	20	3
				TLAVETFAR		SDS-PAGE	6	53	19	2
				VQSALLEVMQER		SDS-PAGE	6	56	20	2
Mb1517	Rv1481	36.02	9.93	AIFTALQAIATVGAVIGGGDTPPPAR	3	SDS-PAGE	6	55	19	3
				AVYSSLQQQIGYETIK		SDS-PAGE	6	55	18	2
				FANMELLESVAPK		SDS-PAGE	6	23	18	2
				VAQLSGGNSYNAATLAELR		SCX	FT3	74	19	2
Mb1519	Rv1483	25.66	9.10	IQQGALQFIPAK	1	SDS-PAGE	7	40	19	2

Mb1520	Rv1484	28.53	6.02	AMPAYNWMTVAK	4	SCX	1	37	21	2
				QGAQLVLTGFDR		SCX	FT3	45	19	2
				SNLVAAGPIR		SDS-PAGE	7	26	17	2
				VAQEQGAQLVLTGFDR		SDS-PAGE	7	46	18	2
Mb1523	Rv1487	15.16	5.07	GVQLGIEALEGK	2	SCX	FT1	33	16	2
				LDGQVWTAR		SDS-PAGE	9	55	12	2
Mb1524	Rv1488	41.25	6.17	AGRPTPEMLAYQYLQTLPEMAR	21	SDS-PAGE	6	38	18	3
				AIARKPVEGSLGTPPRLTQ		SCX	FT1	29	11	3
				AMILTAEGTR		SCX	FT1	38	15	2
				AVATAEAIAR		SCX	FT1	32	11	2
				DQINAQLR		SCX	FT3	52	15	2
				FEPSPVEDQPK		SCX	4	35	6	2
				GAKQAAILAAE		SCX	1	43	12	2
				GQKQAQILAAE		SCX	1	53	13	2
				GSLGTPPRLTQ		SCX	FT1	59	14	2
				ISNYIVGVEQLTTTTLR		SCX	FT1	39	15	2
				KPVEGSLGTPPR		SCX	FT1	40	12	2
				KRAMILTAEGTRE		SCX	FT3	29	15	3
				LLGKPGEDGVFR		SDS-PAGE	6	26	11	3
				NVVGGMTLEQTLTSR		SDS-PAGE	6	31	11	2
				QAQILAAEGAK		SDS-PAGE	6	32	9	2
				QAAILAAEADR		SDS-PAGE	6	54	15	2
				SIDPPPSIQASMEK		SDS-PAGE	6	14	7	2
				SVALIPQAEAAVIER		SDS-PAGE	6	28	11	2
				TVSGQLTLLVPFIDR		SDS-PAGE	6	33	13	2
				VAGWFSTDTDPSIAR		SDS-PAGE	5	22	7	2
				AAAYLQAQGQAK		SDS-PAGE	6	16	9	2

Mb1571	Rv1544	28.19	8.51	FWELPFAR	1	SCX	FT2	44	19	2
Mb1573	Rv1546	15.32	6.81	ALFGPLGSAAAR	1	SDS-PAGE	9	46	18	2
Mb1584	Rv1558	16.35	8.78	QIPVFVLTPVR	1	SDS-PAGE	9	48	18	2
Mb1617	Rv1591	23.15	7.84	YGALDFDTVPLSR	1	SDS-PAGE	8	40	11	2
Mb1623	Rv1597	27.98	5.90	WWTEIAR	1	SCX	2	35	21	2
Mb1633	Rv1607	36.77	7.09	DFFLPVAQK	3	SCX	FT3	34	14	2
				GAVEDDSHADPPSTR		SCX	2	26	13	2
				HRDFFLPVAQK		SDS-PAGE	4	18	14	3
Mb1637	Rv1611	28.02	4.86	ASVSIPVLR	8	SCX	FT2	36	16	2
				DLMTLDVDR		SDS-PAGE	7	55	19	2
				EASVSLSEIK		SDS-PAGE	7	22	17	2
				FQGSLDDLDAVR		SDS-PAGE	7	51	20	2
				GVRADVAARE		SDS-PAGE	7	63	19	2
				IAPGLPSSVIR		SDS-PAGE	7	51	18	2
				IVSVVTEQR		SDS-PAGE	7	47	19	2
				AAAAAAPPPLDVMAALR		SDS-PAGE	7	30	19	2
Mb1639	Rv1613	27.72	4.68	ALTGELAAGVR	4	SCX	FT1	26	17	2
				AQAAQIAQYADGVIVGSALVTALTEGLPR		SDS-PAGE	7	63	19	3
				DAVSQAAPELVGR		SDS-PAGE	7	48	20	2
				DTLAAVEAISIAGGR		SDS-PAGE	7	25	20	2
Mb1649c	Rv1623c	53.83	6.85	DLQQEYQQR	5	SCX	FT3	85	20	2
				ISGVTLQGIR		SCX	FT3	28	23	2
				QPWVVVPNPTGDQLVR		SCX	FT3	32	22	2
				SLCDTQTDPNFSVLTVGR		SDS-PAGE	2	40	19	2
				VIEVPYVLPFLAE		SDS-PAGE	3	22	18	2
Mb1652	Rv1626	22.66	4.74	EITALEGEVATLSER	3	SDS-PAGE	8	33	18	2
				IAPIVVLTAFSQR		SDS-PAGE	8	32	18	2

				VAEVVLETLGTPK		SDS-PAGE	8	46	19	2
Mb1676	Rv1649	37.37	5.25	ELPVYIISIGR	2	SDS-PAGE	6	52	19	2
				GEQDTFYIAPEDSR		SDS-PAGE	6	35	19	2
Mb1687	Rv1659	49.74	4.93	FAGGPSDALAALSK	4	SCX	7	54	20	2
				LAPAGYTLATDLAE		SCX	FT1	25	19	2
				LIGNLAGLLATLK		SDS-PAGE	5	20	17	2
				VATGVLDVVGALAEQAAAHPSAIMPGK		SDS-PAGE	7	22	12	3
Mb1704	Rv1677	19.20	6.34	VTFVGVAGLDQVPAMQE	1	SCX	FT3	45	8	2
Mb1706	Rv1679	37.88	4.86	AGSVMAPTADALYDFYGR	13	SCX	7	61	19	2
				ASVNDAALTITESAMR		SCX	FT1	57	19	2
				ATPGVVQEVVSVAAEHAER		SCX	FT1	45	21	3
				AYLEVAANSVSSPDDTTLTHVLGVK		SCX	8	45	19	3
				GNASAPMAVDIR		SCX	1	40	20	2
				KTGLLGLVLPR		SCX	FT1	78	19	2
				LEHLGGSLAELPTIR		SCX	FT3	48	21	2
				QLGTLAFSEPGSR		SCX	FT3	97	19	2
				SHFWAPVSTASADGDGIAVR		SDS-PAGE	6	59	17	3
				TGLLGLVLPR		SDS-PAGE	3	28	19	2
				VAGTFTGMGLR		SDS-PAGE	6	41	19	2
				VDTDCAFPAEAVDALR		SDS-PAGE	6	35	18	2
				VAANSVSSPDDTTLTHVLGVK		SDS-PAGE	6	47	15	2
Mb1729c	Rv1703c	21.53	5.94	AYLPDLQSILDR	1	SCX	7	58	20	2
Mb1800	Rv1771	48.04	7.64	SLPIMFPIEVR	1	SCX	7	41	20	2
Mb1812	Rv1783	48.59	5.07	DLNFGVVR	4	SCX	8	24	13	2
				LAEAYEEIGQR		SDS-PAGE	1	30	14	2
				WLPHFGDSR		SDS-PAGE	2	14	13	2
				YQSVVYNLPK		SDS-PAGE	1	37	11	2

Mb1812	Rv1784	101.47	5.36	FGVEQVR	5	SCX	FT3	60	20	2
				IMPTAVDVLDSIGR		SCX	1	37	20	2
				TTTLATIMSEIGR		SCX	1	46	20	2
				TVAELLALVR		SDS-PAGE	1	56	19	2
				VGTVIIDQLR		SDS-PAGE	1	28	20	2
Mb1817	Rv1789	38.57	4.18	AVAPYVAWMSAAAAQAE	6	SCX	FT1	71	20	2
				GWLGPASAAMAE		SCX	FT3	40	23	2
				MDFGALPPEVNSVR		SCX	FT3	28	16	2
				QAATQARAAAAAFE		SCX	FT2	25	17	2
				AAFAATVPPPLIAANR		SCX	FT3	45	20	2
				AAAAAFEAAFAATVPPPLIAANR		SDS-PAGE	10	41	16	3
Mb1821	Rv1793	9.94	4.56	AQAASLEAEHQAIVR	2	SCX	FT1	113	19	2
				DVLAAGDFWGGAGSVACQE		SDS-PAGE	10	121	18	2
Mb1822	Rv1794	32.39	4.64	HVAPELR	6	SCX	1	35	20	2
				RGQHWVSAVR		SCX	2	21	19	3
				SGFNVFSGGDLR		SCX	7	57	23	2
				SIHHADPAAINAVNVPMEE		SCX	7	57	16	3
				TVLDTLPYGEWK		SCX	FT3	36	19	2
				VLAAPDLEVVALLSR		SCX	FT1	89	20	2
Mb1824	Rv1796	60.02	5.44	FPELSAYQIINR	3	SDS-PAGE	6	40	9	2
				QLSAPLVVPQPPAPR		SDS-PAGE	6	61	9	2
				QNPIFDPLQPDDPR		SDS-PAGE	6	47	9	2
Mb1825	Rv1797	44.18	6.88	TWLVLR	1	SDS-PAGE	2	38	9	2
Mb1853	Rv1822	22.91	10.17	LGALLDPAVDR	1	SDS-PAGE	1	37	18	2
Mb1857	Rv1826	14.14	3.76	SALTTLLDAEAYR	1	SCX	7	67	22	2
Mb1860	Rv1829	18.11	4.20	FLDSVSPDDFKAT	3	SCX	FT2	20	17	2
				VEQPQNQPVLLLR		SDS-PAGE	9	55	18	2

				VRIVDLQE		SDS-PAGE	9	24	15	2
Mb1867c	Rv1836c	69.68	4.53	AVAQLSGGSYQNLE	11	SCX	FT1	77	19	2
				DTVAVIADPSIADQVK		SCX	FT2	20	16	2
				ESADSYNASAGPVGDR		SCX	FT1	29	21	2
				GGNSRLSNVVAALE		SCX	FT3	90	20	2
				GPGDWQAGHR		SCX	FT3	55	23	2
				LSNVVAALENR		SCX	FT3	41	23	2
				LTGAAGSQAISDSR		SDS-PAGE	5	28	18	2
				QTSAASAFAR		SDS-PAGE	4	58	16	2
				SLVISPVLLAVRPE		SDS-PAGE	4	86	19	2
				TEVPAGPLADPVNGQPR		SDS-PAGE	4	63	20	2
				VSDVKPPSSPVTSFPALPSTLSVGDDSMR		SDS-PAGE	4	18	15	3
Mb1887c	Rv1856c	23.23	5.86	AYSVSDTANAWR	2	SDS-PAGE	8	57	19	2
				LALFLTTPAAR		SDS-PAGE	8	56	19	2
Mb1903c	Rv1872c	45.34	10.15	AIEILQTGVIR	14	SCX	FT1	70	20	2
				APVPFHLLPHVAR		SCX	FT1	29	20	3
				AYLYGLMAGGEAGVNR		SCX	FT3	31	23	2
				DIEFHPTILR		SCX	FT3	42	25	2
				DLAPLLQFNRPQFDTSK		SCX	FT1	99	20	3
				DLVIAVPQGR		SCX	FT1	46	20	2
				GIQTLDDAR		SCX	FT1	40	19	2
				GVDGIVLSNHGGR		SDS-PAGE	4	21	19	2
				ILQTGVIR		SDS-PAGE	5	38	19	2
				LGAALTIQDLR		SDS-PAGE	6	34	19	2
				LLGVTCLEELSPR		SDS-PAGE	6	61	20	2
				LMHTEGEIAGAR		SDS-PAGE	6	35	19	2
				NGMSIPPALTLR		SDS-PAGE	6	55	18	2

				TVLDAMGHPR		SDS-PAGE	6	60	19	2
Mb1917c	Rv1885c	21.92	5.30	LEVADPVAAFK	3	SDS-PAGE	9	51	4	2
				DGTSQLAELVDAAAER		SCX	3	73	12	2
				LVDAAAERLE		SDS-PAGE	9	17	13	2
Mb1943c	Rv1908c	80.57	4.87	ALVEVYGADDAQPK	7	SDS-PAGE	3	21	17	2
				DGAGAGTIPDPFGGPGR		SDS-PAGE	4	31	20	2
				EATWLGDER		SDS-PAGE	4	41	20	2
				PEQHPPITE		SDS-PAGE	4	30	19	2
				SPAGAWQYTAK		SDS-PAGE	2	32	20	2
				SPTMLATDLSLR		SDS-PAGE	4	27	20	2
				VDPIYER		SDS-PAGE	4	53	19	2
Mb1954c	Rv1919c	16.80	10.93	TSSAPAATLFR	1	SDS-PAGE	9	39	19	2
Mb1956c	Rv1921c	45.00	5.43	EFLQSAIDANSR	5	SCX	7	36	14	2
				NDGTPIPDQVDSVF		SCX	7	64	14	2
				VAIFDWDNTVVK		SDS-PAGE	6	65	13	2
				VWAGEVGLDDQHVVGVR		SDS-PAGE	6	59	12	3
				YLTDAAANALSVACGK		SDS-PAGE	6	31	12	2
Mb1957	Rv1922	39.06	4.83	YGAGIVSR		SDS-PAGE	6	40	12	2
Mb2016c	Rv1993c	9.33	9.25	ASVTVMEWGLR	3	SCX	1	32	20	2
				EAPLPAGARVDE		SCX	FT1	41	19	2
				IGEEAPLPAGAR		SDS-PAGE	10	35	14	2
Mb2025	Rv2002	27.03	4.79	STALELGPSGIR	1	SDS-PAGE	7	35	11	2
Mb2028c	Rv2005c	30.99	5.62	GLLGSVSSSLVR	1	SDS-PAGE	7	47	20	2
Mb2057c	Rv2031c	16.23	4.75	AELPGVDPDK	11	SCX	FT1	71	19	2
				AELPGVDPDKDVDIMVR		SCX	9	45	24	3
				ATTLPVQR		SCX	5	17	15	2
				DGQLTIK		SCX	FT2	45	15	2

				FAYGSFVR		SCX	3	33	20	2
				GILTVSVAVSEGKPTEK		SCX	9	35	20	3
				GKPTEKHIQIRSTN		SDS-PAGE	9	39	21	3
				RTEQKDFDGRSE		SDS-PAGE	9	21	19	3
				SEFAYGSFVR		SDS-PAGE	10	36	15	2
				TVSLPVGADEDDIK		SDS-PAGE	9	25	18	2
				TVSLPVGADEDDIKATYDK		SDS-PAGE	10	17	14	3
Mb2058	Rv2032	36.57	5.23	VGLAPEMEEPPPATPR	1	SDS-PAGE	10	41	18	2
Mb2072	Rv2046	22.21	5.13	AGHPVDPAAYHVATR	1	SCX	7	54	15	3
Mb2094c	Rv2068c	32.56	6.04	ALLTDWMAR	4	SCX	1	29	13	2
				APLVAAVLHQNPLTHLDK		SCX	7	26	12	3
				EALLAEAATCVAGVLA		SCX	FT1	59	11	2
				LITYTSDDIR		SDS-PAGE	7	50	11	2
Mb2118c	Rv2091c	26.02	5.87	AQAGVQQVLTDE	7	SCX	FT3	39	23	2
				AQAGVQQVLTDETTGYGAK		SCX	FT1	49	20	2
				GATFECTVSIDGTSK		SCX	FT1	36	20	2
				GTYEVGRPQ		SCX	7	33	19	2
				QPWQPPGQGADHSSDPTVAAGYPWQQQPTQE		SCX	FT1	27	19	3
				RVTVTFQDNK		SDS-PAGE	6	62	17	2
				VTVTFQDNK		SDS-PAGE	6	37	18	2
Mb2121c	Rv2094c	8.91	9.32	ASIETPTPVQSQR	3	SCX	FT3	34	23	2
				VDPSAASGQDSTEAR		SCX	FT1	68	22	2
				VDPSAASGQDSTEARPA		SDS-PAGE	10	63	18	2
Mb2144c	Rv2120c	16.37	11.36	DSVAVLGGFAIVAAAAAL	3	SCX	FT1	16	6	2
				GGHDLPIALLE		SDS-PAGE	3	33	14	2
				TVTPVFAVR		SDS-PAGE	8	18	6	2
Mb2146c	Rv2122c	10.24	4.38	GLSLDDVYR	2	SCX	1	51	21	2

				TFEDLFAELGDR		SDS-PAGE	10	30	19	2
Mb2151	Rv2127	52.43	10.23	AGESPFVTFFSK	1	SCX	7	62	20	2
Mb2153c	Rv2129c	31.33	10.03	ADLPAFAELLAR	1	SCX	1	35	21	2
Mb2154c	Rv2130c	45.59	5.17	SDELDALLWR	1	SCX	1	64	21	2
Mb2155c	Rv2131c	28.41	4.65	VAPILLGAIADAWR	2	SCX	7	49	16	2
				VWIIDPLDGTR		SDS-PAGE	7	69	18	2
Mb2193	Rv2171	23.81	4.81	DTGPALDGDVPFSQK	3	SDS-PAGE	8	35	12	2
				GRADLTSVSDPDADVE		SDS-PAGE	8	29	9	2
				IEPEVVQWK		SDS-PAGE	8	36	10	2
Mb2200c	Rv2178c	50.64	5.56	AYANASAAMNLVR	6	SDS-PAGE	3	26	19	2
				DLLPPIVEK		SDS-PAGE	5	46	20	2
				GDMINGFAPDAAAR		SDS-PAGE	1	38	18	2
				LQEQLAQVAK		SDS-PAGE	1	21	19	2
				SADIDALGLR		SDS-PAGE	3	40	18	2
				YEALATEIDR		SDS-PAGE	4	21	19	2
Mb2203	Rv2181	47.14	11.66	ETTAVWWR	3	SCX	8	37	20	2
				IGAAALNTDQNIAGALAR		SCX	FT3	58	19	2
				TTAVWWR		SCX	8	36	24	2
Mb2207c	Rv2185c	16.29	4.48	DTLIMSYEWPEDR	4	SDS-PAGE	9	18	18	2
				EVEILEADDEGYPK		SDS-PAGE	9	53	18	2
				MLMDAAIFK		SDS-PAGE	9	30	21	2
				TTQTIYIDADPGEVMK		SDS-PAGE	9	26	17	2
Mb2217	Rv2194	29.12	10.23	AQIDAIGAYVQANGGGPTVVR	6	SCX	FT3	25	14	2
				DESSSALLR		SCX	1	42	13	2
				KDIIAYVK		SCX	3	46	10	2
				KDPIFDEAQIDAIGAYVQANGGGPTVVR		SCX	FT3	57	16	3
				NPDGSIATQSLR		SDS-PAGE	1	67	9	2

				QQILTAMLTGPQNMPK		SDS-PAGE	8	30	12	2
Mb2218	Rv2195	46.92	6.03	ATGTEDGPPFIK	12	SCX	FT3	51	20	2
				AVLWTSGWTPR		SCX	FT1	71	19	2
				EPDEAALAAMSNQE		SCX	7	62	20	2
				EPDEAALAAMSNQELLALGGK		SCX	1	26	22	2
				IAYKEPR		SCX	FT3	35	19	2
				IKPSDLGR		SCX	FT3	26	22	2
				IVPGPNPQDGAK		SCX	2	24	20	2
				TVVANLTDAFEGSTIR		SDS-PAGE	5	93	19	2
				VCSHLGCPSSLYE		SDS-PAGE	6	68	19	2
				VCSHLGCPSSLYEQQSYR		SDS-PAGE	3	43	19	3
				YQGETIYLAR		SDS-PAGE	6	32	21	2
Mb2219	Rv2196	61.01	8.75	EHQDSIMGSPDGEH	13	SCX	1	66	20	2
				FTGDYAHHNLLQR		SCX	8	27	19	3
				FTGDYAHHNLLQRPR		SCX	FT2	73	16	4
				HQDSIMGSPDGEH		SCX	FT1	78	20	2
				HTQFPGPGR		SCX	FT3	33	15	2
				IFFTGAFR		SCX	FT1	60	20	2
				LGSAGSPGSGSFLFADSAAEDAALR		SCX	7	22	18	2
				LHQPLGPVDE		SDS-PAGE	1	20	20	2
				LSPPNIGEVLAR		SDS-PAGE	2	43	18	2
				QRALAALRE		SDS-PAGE	1	51	18	2
				SVLEHGVETGIIK		SDS-PAGE	1	30	17	2
				TEHNVVGVR		SDS-PAGE	2	27	14	2
				VMPVFAFK		SDS-PAGE	1	37	15	2
Mb2220c	Rv2197c	22.48	9.48	GPDVISPDVIDR	2	SDS-PAGE	9	42	18	2
Mb2221c	Rv2198c	30.95	4.12	APGDIISVTYVDAAGR	5	SCX	FT3	82	21	2

				AYSAPESEHVTGGPYVPADLR		SCX	7	58	23	3
				QVTYSVTGTK		SDS-PAGE	5	38	14	2
				SEHVTGGPYVPADLR		SDS-PAGE	5	52	16	3
				TDAYSRAYSAPE		SDS-PAGE	5	56	20	2
Mb2223c	Rv2200c	40.45	7.98	AINQPPLAVTTHPFDTR	18	SCX	7	56	12	2
				DGTLTYDGADPER		SCX	FT1	30	13	2
				DKYGEELVGPVR		SCX	FT2	26	6	2
				DVMPNPVANNSVNVFQIEEITK		SCX	FT1	40	13	3
				IPVLVLPSGK		SCX	2	36	11	2
				ITKTGAFVGHCAE		SCX	FT3	40	15	3
				MCGTYHSMMNFE		SCX	FT3	73	14	2
				NTDTELPR		SCX	7	40	17	2
				RGELAPQPVG		SCX	FT1	18	15	2
				TGAFVGHCAE		SDS-PAGE	6	55	13	2
				TLGTSTEIPVLVLPSGK		SDS-PAGE	6	76	10	2
				TLGTSTEIPVLVLPSGKRIE		SDS-PAGE	6	56	10	3
				TYLNFDK		SDS-PAGE	3	26	12	2
				TYLNFDKVE		SDS-PAGE	6	15	13	2
				TYLNFDKVETLGTSTEIPVLVLPSGK		SDS-PAGE	6	31	10	3
				VETLGTSTEIPVLVLPSGK		SDS-PAGE	6	47	10	2
				VVTPNDFK		SDS-PAGE	6	49	12	2
				YGEELVGPVR		SDS-PAGE	6	29	11	2
Mb2226	Rv2203	24.37	8.63	GQVQGIAQLLFQR	2	SCX	7	52	20	2
				TAIQGYLNALENR		SDS-PAGE	7	53	19	2
Mb2227c	Rv2204c	12.54	4.19	YNLFFDDR	1	SCX	1	48	21	2
Mb2235	Rv2212	39.74	4.41	ALVGEDGAFGLLR	3	SCX	FT3	48	20	2
				GFDAPVMAFELHDNPR		SDS-PAGE	6	62	18	3

				LVAAAAPGQILAAAQLR		SDS-PAGE	6	54	17	2
Mb2242	Rv2219	26.82	12.13	AQLWQAFTLQR	1	SCX	7	50	20	2
Mb2244	Rv2220	53.53	4.84	GGYFPVAPNDQYVDLR	9	SCX	FT3	45	21	2
				IEPVNIRPHPYE		SCX	FT3	55	19	3
				IPITGSNPK		SCX	FT1	51	20	2
				LVPGYEAPINLVYSQR		SCX	FT3	80	24	2
				MLTNLINSGFILEK		SCX	5	22	16	2
				SVFDDGLAFDGSSIR		SDS-PAGE	5	38	18	2
				TGYAGLSDTAR		SDS-PAGE	5	47	18	2
				TWISFKRENE		SDS-PAGE	1	25	20	2
				VDAISGWWNTGAATE		SDS-PAGE	5	34	13	2
Mb2246c	Rv2222c	49.61	5.21	FADALSMADNVMTFR	4	SCX	FT1	41	20	2
				LVQGGEAPTAASWGAANR		SCX	FT3	46	20	2
				SHVTPYELR		SCX	1	25	22	2
				TEWANYR		SCX	1	25	19	2
Mb2248c	Rv2224c	55.89	6.07	FDLVGFDPR	6	SCX	FT3	34	13	2
				GDTLLALADMYMR		SCX	8	19	16	2
				IGSAYAEEFPQR		SDS-PAGE	5	24	13	2
				LAVPVDYDRPDGDVAALALIR		SDS-PAGE	5	26	10	3
				LGQPVEWTPCR		SDS-PAGE	5	42	13	2
				RFDLVGFDPR		SDS-PAGE	5	33	12	2
Mb2262c	Rv2238c	16.81	5.12	DQNQQLVTLR	3	SCX	FT3	33	20	2
				GTFVVDR		SDS-PAGE	9	42	18	2
				MLNVGATAPDFTLR		SDS-PAGE	9	41	20	2
Mb2268	Rv2244	12.49	3.74	AKIESENPDAVANVQAR	10	SCX	4	39	15	3
				IAVQTEDKYGVK		SCX	FT3	39	23	2
				IESENPDAVANVQAR		SCX	2	49	20	2

				IPDEDLAGLR		SCX	FT3	73	24	2
				LEEENPEAAQALR		SCX	7	55	19	2
				SENPDAVANVQAR		SDS-PAGE	10	30	15	2
				SENPDAVANVQARLE		SDS-PAGE	1	41	19	2
				TVGDVVAYIQK		SDS-PAGE	10	25	15	2
				VTGIEPSEITPE		SDS-PAGE	10	43	18	2
				AAQALRAKIE		SDS-PAGE	10	76	15	2
Mb2270	Rv2246	46.39	5.16	ADAAICGGVETR	5	SCX	FT3	54	23	2
				AINNALGGNRPAVYAPK		SCX	1	22	20	3
				IEAVPIAGFAQMR		SCX	7	41	24	2
				SILTVLALR		SCX	1	25	20	2
				TLDDPFVEEFDLPVR		SDS-PAGE	1	28	19	2
Mb2282c	Rv2258c	37.51	4.35	LGLANATFER	2	SDS-PAGE	6	42	19	2
				VGAYDVITVFDAIHDQAQPAR		SDS-PAGE	6	31	17	3
Mb2311	Rv2289	28.57	6.52	NYLYDAWAAR	1	SCX	8	35	13	2
Mb2316	Rv2294	44.25	6.15	IDLDALQEAFSSAR	3	SCX	2	33	21	2
				TGGNWLDALLR		SDS-PAGE	5	31	18	2
				WFLDHAR		SDS-PAGE	5	26	19	2
Mb2318	Rv2296	33.36	6.98	HIPGAAGQPHAR	2	SCX	FT3	39	20	3
				VLAPDLIGFGR		SDS-PAGE	6	46	19	2
Mb2320	Rv2298	34.98	9.51	ALNPLFGTENLR	4	SDS-PAGE	6	30	19	2
				IGLGTWQFGSR		SDS-PAGE	6	23	18	2
				IVIAYSPLAQGLLGGK		SDS-PAGE	6	58	18	2
				VFPVAPFPAVIK		SDS-PAGE	6	28	19	2
Mb2321c	Rv2299c	72.96	4.51	DGQQQIFYATGETR	20	SCX	FT1	48	20	2
				DVQSSRPEDYR		SCX	FT3	78	20	2
				EEVVDLIGTLAK		SCX	FT2	39	15	2

				EGLLSDIDNR		SCX	8	46	24	2
				ELISNASDALDK		SCX	FT2	45	16	2
				FAELLAER		SCX	FT3	51	19	2
				GEVDLSSEEDTSEAEREER		SCX	FT1	28	19	3
				GGALEDPARFAE		SCX	FT3	49	24	2
				GVVDAQDMSLNVSR		SCX	FT2	66	16	2
				HVAHAWDDPLE		SCX	FT2	43	20	2
				ILELNPSHPLVTGLR		SDS-PAGE	4	14	11	3
				LISNASDALDKLRIE		SDS-PAGE	4	71	18	3
				LTESPACLITDAFGMTPALAR		SDS-PAGE	2	26	20	2
				NAHVEQLE		SDS-PAGE	2	58	18	2
				SLAETAELLYGTALLAEGGALEDPAR		SDS-PAGE	4	29	18	3
				TFWTQFGR		SDS-PAGE	1	38	20	2
				VLLLTDPVDE		SDS-PAGE	4	74	18	2
				VVDLIGTLAK		SDS-PAGE	4	18	15	2
				VVDLIGTLAKSGTAE		SDS-PAGE	2	20	15	2
				YSDFIAWPIR		SDS-PAGE	4	48	19	2
Mb2323	Rv2301A	23.89	5.10	ACPDAEVVFAR	3	SCX	FT1	14	12	2
				FEPPGIGTVGNAFVSALR		SCX	FT2	10	7	2
				LCHGDDPVCHPADPNTWE		SDS-PAGE	9	33	13	3
Mb2327	Rv2305	46.12	5.76	DLFDVLIR	3	SCX	1	31	20	2
				QLQLLLPLVEDR		SCX	1	33	20	2
				TLYWEVPDR		SDS-PAGE	5	36	18	2
Mb2379	Rv2358	14.42	6.16	EILDAAGELLR	1	SDS-PAGE	9	44	18	2
Mb2408	Rv2387	45.01	5.35	DSGTNASIFSR	5	SCX	7	23	18	2
				EHPNWDEDGVKDSGTNASIFSR		SCX	FT1	53	20	3
				GIEEELELSLEK		SCX	FT3	24	20	2

				IVGQWFPATGASIG		SCX	FT3	60	24	2
				PAHSDSLTAQAER		SDS-PAGE	2	37	19	2
Mb2415	Rv2394	66.49	5.84	NLATNPEIATGYR	4	SDS-PAGE	6	43	11	2
				QSSGLSAITRSE		SDS-PAGE	8	45	13	2
				SSMAPTLVFDHSSAGR		SDS-PAGE	8	34	13	3
				VNLAEQSSGLSAITR		SDS-PAGE	9	17	9	2
Mb2433c	Rv2410c	36.02	5.11	NAEALYWIGR	2	SCX	1	51	19	2
				VGDSASSPAWVTLLR		SDS-PAGE	6	45	18	2
Mb2455	Rv2429	18.78	7.06	EAIFEALK	4	SCX	FT3	64	23	2
				MNIIANPGIPK		SCX	FT1	70	20	2
				AAAIVSGVAQALATIE		SDS-PAGE	9	35	20	2
				AAAIVSGVAQALATIEALSPS		SDS-PAGE	9	32	21	2
Mb2482c	Rv2455c	69.15	5.29	LNLGQLAQVLR	1	SCX	7	37	23	2
Mb2487c	Rv2460c	23.51	4.76	ADIQTVCLGQAASAAAVLLAAGTPGK	6	SCX	4	21	15	3
				AKDYGIIDTVLE		SCX	FT2	18	16	2
				DYGIIDTVLEYR		SDS-PAGE	8	28	17	2
				SNPYNKLFEE		SDS-PAGE	1	28	18	2
				TLMETTLAR		SDS-PAGE	5	25	15	2
				VLIHQPSLSGVIQGQFSDLE		SDS-PAGE	8	43	19	2
Mb2504c	Rv2477c	61.89	4.95	GPDQQKPAGVLSGGER	1	SCX	7	36	19	3
Mb2547c	Rv2518c	43.36	6.52	GAFYWLNNR	5	SCX	8	40	16	2
				GAFYWLNNRE		SCX	5	25	10	2
				GTPFADLLVPK		SDS-PAGE	6	42	13	2
				DKGTPFADLLVPK		SCX	3	15	9	2
				YTLNATALGLGGAATR		SDS-PAGE	6	27	9	2
Mb2565	Rv2536	24.63	4.64	ADDSPTGEMQVAQPEAQTAAVATVER	9	SDS-PAGE	7	58	19	3
				AQTAAVATVER		SCX	FT1	76	13	2

				GLAFAAAMVVLR		SCX	FT3	48	16	2
				REAPTEVIRTTE		SCX	FT2	22	6	2
				SDTPTEVIRTDTE		SCX	1	42	12	2
				TDTEADQTKPGDEPK		SCX	FT1	27	6	3
				TDTEADQTKPGDEPKKD		SCX	FT1	77	14	3
				TTESDTPTEVIR		SDS-PAGE	7	39	8	2
				AADTDVFSAVR		SDS-PAGE	2	34	9	2
Mb2586c	Rv2556c	13.89	5.46	IVDLTDAVR	1	SDS-PAGE	10	48	19	2
Mb2592	Rv2563	36.02	10.50	APGVLAAAPLATAPSTIR	7	SCX	FT3	56	12	2
				APSTPDEVAVSSTLGR		SCX	FT1	63	13	2
				IVGIVPESTALAK		SDS-PAGE	7	24	14	2
				NLGDDLQVGAR		SDS-PAGE	3	33	9	2
				QLPDGYQTVNR		SDS-PAGE	2	38	13	2
				TVDSMGVDAFVVK		SDS-PAGE	2	16	13	2
				VVAIDPALAFGGP		SDS-PAGE	7	29	13	2
Mb2612c	Rv2581c	24.17	6.63	FMLTDPIYGLGPR	3	SCX	1	38	21	2
				FNPFLEGLSR		SDS-PAGE	8	44	19	2
				IAQLVAGAFFR		SDS-PAGE	8	55	20	2
Mb2616c	Rv2585c	58.58	5.70	ALALCVPR	5	SCX	FT3	109	10	2
				LSPATDDALTDADGAAEAR		SCX	8	30	13	2
				LWNSTWDLKPGR		SCX	8	28	13	3
				TLTGFGYHGPDGQVVADR		SDS-PAGE	5	31	9	3
				WGAGWNMDR		SDS-PAGE	5	27	14	2
Mb2617c	Rv2586c	47.01	10.00	LGHHSFLSR	1	SDS-PAGE	2	45	19	2
Mb2618c	Rv2587c	60.23	9.06	EALAQAQQIISAR	2	SDS-PAGE	8	45	20	2
				LGIDLQGGTR		SDS-PAGE	8	74	19	2
Mb2619c	Rv2588c	12.96	4.06	AMQATIDLHDSLQPGER	4	SCX	FT1	72	20	3

				ATIVAIADDTIDLE		SCX	FT2	30	16	2
				IAPGVVTTWMK		SCX	FT3	57	24	2
				ILPDDDIDEELNEDLDKDVDDVAGER		SCX	FT1	19	19	3
Mb2628	Rv2597	21.91	4.43	IATGKSVLAGE	5	SDS-PAGE	8	34	13	2
				MDYVDCASAGQGADE		SDS-PAGE	8	29	13	2
				QLGPGAIVSHGGIDYVVR		SDS-PAGE	8	33	7	3
				RDPLAFDAMPQFGPR		SDS-PAGE	8	24	6	3
				RWAPDMGWE		SDS-PAGE	8	33	10	2
Mb2638c	Rv2606c	31.36	5.05	ATTFFDDPDVLAK	3	SCX	FT3	45	20	2
				ELQAPYELVAEVAR		SCX	FT2	16	15	2
				GVFVGSGIFKSGAPE		SDS-PAGE	7	56	19	2
Mb2656	Rv2623	31.65	5.56	AGPPTVHSEIVPAAAVPTLVDMSK	11	SCX	FT1	90	20	3
				DAVLMVVGCLGSGR		SCX	FT3	41	23	2
				GGYAGMLVGSVGETVAQLAR		SCX	FT3	52	22	2
				HLIDDALK		SCX	7	30	23	2
				IVPAAAVPTLVDMSK		SDS-PAGE	7	31	18	2
				LLGSVSSGLLR		SDS-PAGE	7	37	17	2
				SEEAQLVVVGSR		SDS-PAGE	7	65	17	2
				TPVIVAR		SDS-PAGE	1	25	21	2
				VATWLEVPLPPGVLR		SDS-PAGE	7	48	20	2
				VVEQASLR		SDS-PAGE	7	69	19	2
				YPNVAITR		SDS-PAGE	7	53	20	2
Mb2659c	Rv2626c	15.52	4.77	ADIARHLPE	13	SCX	4	25	15	2
				DIMNAGVTCVGEHETLTAAAQYMR		SCX	1	53	20	3
				EHDIGALPICGDDDR		SCX	1	23	20	3
				GLAAGLDPNTATAGELAR		SCX	FT1	85	20	2
				HLPEHAIVQFVK		SCX	FT2	63	16	2

				HQVRRVPVISE		SCX	4	37	15	3
				LHGMLTDR		SDS-PAGE	7	22	16	2
				LVGIVTEADIAR		SDS-PAGE	6	43	19	2
				RVPVISEHR		SDS-PAGE	7	37	20	3
				TLTAAAQYMRE		SDS-PAGE	10	51	19	2
				TTARDIMNAGVTCVGE		SDS-PAGE	7	72	20	2
				TTARDIMNAGVTCVGEHE		SDS-PAGE	7	26	15	3
				VPVISEHR		SDS-PAGE	7	27	18	2
Mb2691	Rv2672	53.99	4.60	GAVSALLSAAR	5	SCX	FT3	24	13	2
				GMGMSSPIDCR		SCX	1	72	11	2
				SGDGPGGASVAAVANAVATALGFPDSGR		SDS-PAGE	5	54	13	3
				SGDMNLLSALINR		SDS-PAGE	5	49	14	2
				VYSNAGIPAAPGVK		SDS-PAGE	5	78	11	2
Mb2692	Rv2673	48.88	9.83	TGAPSTATILR	1	SDS-PAGE	1	59	20	2
Mb2697c	Rv2678c	37.59	5.55	VPVWFMR	1	SCX	8	45	20	2
Mb2730	Rv2711	25.23	5.05	LPAGSPVAVVVRQLTE	4	SCX	FT2	45	15	2
				LTELPAGSPVAVVVR		SCX	5	29	16	2
				RDGLLRVAGDRHLE		SDS-PAGE	8	33	17	3
				TIYDLEEEGVTPLR		SDS-PAGE	8	41	19	2
Mb2740c	Rv2721c	72.33	4.22	AGGDTSTLGVR	9	SCX	FT3	41	15	2
				FSGGEVSWNR		SCX	FT1	43	13	2
				FTTVPAVLAEQLK		SCX	7	41	14	2
				GEILAKYE		SDS-PAGE	4	23	13	2
				LGAPVGDQTVDGDVVSQK		SDS-PAGE	4	13	10	2
				MFFTPATGAK		SDS-PAGE	4	17	6	2
				VFFSPATGANAVEGEILAK		SDS-PAGE	4	34	10	2
				VTAQKFSGGE		SDS-PAGE	4	16	6	2

				YLYGPLLDKYE		SDS-PAGE	4	78	11	2
Mb2747c	Rv2728c	23.46	4.69	ALDDALASGDLAVLTR	1	SDS-PAGE	8	56	10	2
Mb2760	Rv2740	16.59	4.57	DYFDVYDMFK	4	SCX	FT2	26	20	2
				GLVALVVPSLK		SCX	FT3	74	20	2
				IGADGAAVLTER		SDS-PAGE	9	75	19	2
				TDALIIGPLR		SDS-PAGE	9	60	17	2
Mb2765c	Rv2744c	29.26	5.77	AFAAQLVTAEQSVEDLK	17	SCX	FT3	73	22	2
				ALPAGGTTATPRPATE		SCX	4	60	14	2
				AQRTHQALTQQAAQVIGNQRQLE		SCX	FT3	70	20	3
				LLSQLEQAK		SCX	FT2	21	16	2
				MQEQVSASLR		SCX	FT2	19	15	2
				QALTLADQATAAGDAAK		SCX	1	73	19	2
				QVSASLRSMSE		SCX	FT1	85	20	2
				RNAMVLQQKIAE		SCX	FT2	31	16	2
				RRYANAIGSAE		SCX	4	40	15	2
				SMSELAAPGNTPSLDEVR		SCX	FT1	82	19	2
				THQALTQQAAQVIGNQR		SDS-PAGE	6	37	20	3
				TLHDQALSAAAQAK		SDS-PAGE	7	59	18	2
				TSGGAIAEQPYGQ		SDS-PAGE	7	37	20	2
				VEQAGIQMAGHSRLE		SDS-PAGE	7	32	17	3
				VQIQQAIEEAQR		SDS-PAGE	7	55	18	2
				YANAIGSAELAESSVQGR		SDS-PAGE	7	62	16	2
				YLMALFSSK		SDS-PAGE	7	49	15	2
Mb2788c	Rv2766c	27.13	5.82	CVDLTLER	5	SCX	FT1	42	18	2
				GIGLAIAQQLAAAGAHVVLTAR		SDS-PAGE	7	50	20	3
				QLALELSPR		SDS-PAGE	7	55	12	2
				TAIITGASR		SDS-PAGE	7	52	13	2

				VNAICPGVVR		SDS-PAGE	7	41	12	2
Mb2805c	Rv2782c	47.07	7.50	TTLPGGLR	1	SCX	FT3	41	21	2
Mb2819c	Rv2796c	20.20	6.25	AGEEGPLSPEK	3	SDS-PAGE	9	58	13	2
				DGVEQFGATGFGVMK		SDS-PAGE	9	38	12	2
				VAELENPLR		SDS-PAGE	9	16	10	2
Mb2898	Rv2873	22.07	4.73	DDLMVNNAGLVCGGVHTANATVYMIDTVLMPPAQ	10	SCX	FT2	12	10	4
				GCAQYAAQNPTGPGSVAGMAQDPVATAASNNPMLSTL						
				TSALSGK		SCX	FT1	32	10	4
				IDGTHQTLQGADLTVIGAR		SCX	7	58	15	3
				LLSSILTYHVIAGQASPSR		SCX	FT2	12	10	3
				LNPDVNLVDTLNGGE		SCX	3	50	14	2
				LNPDVNLVDTLNGGEYTVFAPTNAAFDK		SCX	7	62	13	3
				LNPDVNLVDTLNGGEYTVFAPTNAAFDKLPAATIDQLK		SDS-PAGE	7	27	3	4
				LPAATIDQLK		SDS-PAGE	7	44	13	2
				YTVFAPTNAAFDK		SDS-PAGE	7	47	12	2
				YTVFAPTNAAFDKLPAATIDQLK		SDS-PAGE	7	65	7	3
Mb2900	Rv2875	19.07	4.54	LPASTIDELK	2	SCX	FT1	30	11	2
				TNSSLLTSILTYHVVAGQTSPANVVGTR		SDS-PAGE	9	56	10	3
Mb2913c	Rv2889c	28.78	5.01	TVEQAIAELSAK	1	SDS-PAGE	7	47	19	2
Mb2917	Rv2893	34.61	6.71	DIAEVIALLR	1	SDS-PAGE	6	69	10	2
Mb2945c	Rv2921c	43.99	4.97	ADAIAPPEGRLE	1	SCX	FT1	38	20	2
Mb2953	Rv2928	29.15	4.99	EMLDLFTR	2	SCX	1	38	18	2
				FFVGALPTLR		SCX	7	62	23	2
Mb2965c	Rv2940c	224.39	4.84	HGVVPPLLHFNR	12	SCX	8	43	19	2
				QAQIVKPHDSGER		SCX	7	44	19	2
				LFMLSSTSSDALR		SDS-PAGE	1	48	19	2
				LFIDDDGQEQR		SDS-PAGE	1	32	19	2

				ELAEFEGELPR		SDS-PAGE	1	55	19	2
				ANLEQAGLR		SDS-PAGE	1	44	19	2
				FPIIDDR		SDS-PAGE	1	27	20	2
				SVEFAEQIR		SDS-PAGE	1	37	18	2
				SVAVTPEQAPLYR		SDS-PAGE	1	39	18	2
				LVSAATATGLPLR		SDS-PAGE	11	66	20	2
				TPMDLSVWPPE		SDS-PAGE	4	18	15	2
				LPVTTWDVR		SDS-PAGE	1	24	20	2
Mb2966	Rv2941	62.64	5.06	DLGNILTILSGSER	1	SCX	1	41	20	2
Mb2970c	Rv2945c	24.14	4.81	ASIDLGSGSIQLTQSK	6	SCX	FT3	79	15	2
				ITGTIPASSVK		SCX	7	68	16	2
				LFDDWSNLGSISE		SCX	FT1	24	12	2
				PATVWIAQDGSHHLVR		SDS-PAGE	7	87	11	3
				VDSLLGITSADVDVR		SDS-PAGE	8	40	13	2
				VLDPAAGVTQLLSGVTNLQAQGTEVIDGISTTK		SDS-PAGE	8	61	14	3
Mb2983c	Rv2959c	27.84	5.34	IFAILDSDHSMNHVLAE	6	SCX	FT3	25	13	2
				LLRPLLSAGDYLVVE		SCX	FT3	51	15	2
				NKFGWTSAPNGFLIRN		SCX	4	17	4	3
				REPDVLFVESSSTDPAIAEQIQR		SCX	FT2	17	13	3
				WYYGNLVWTK		SDS-PAGE	7	30	12	2
				YGGSAVYFANIMR		SDS-PAGE	7	53	11	2
Mb2993c	Rv2969c	26.83	5.77	DFLCPACGIFE	11	SCX	FT1	66	20	2
				DFLCPACGIFER		SCX	FT3	22	16	2
				DGVAGPGDAVR		SCX	FT3	50	20	2
				DIQPAELGK		SCX	1	23	21	2
				EIVGDVPGIDSAAATATS		SCX	FT2	27	16	2
				FHAALFSK		SCX	FT3	92	18	2

				IVGDVPGIDSAAATATS		SDS-PAGE	8	34	19	2
				LVTQPGTSNPK		SDS-PAGE	8	20	19	2
				VDGLAAAVNVHATPTVR		SDS-PAGE	8	25	21	2
				VNGTEYEWSTPAALVAK		SDS-PAGE	8	27	18	2
				VPDCINSGK		SDS-PAGE	8	51	17	2
				AAAAAYCVADESIEAFR		SDS-PAGE	7	29	18	2
Mb2996	Rv2971	30.35.	4.51	LIDTAYAYGNEAAVGR	2	SDS-PAGE	7	62	18	2
				LLDNPTVTSIASEYVK		SDS-PAGE	7	32	17	2
Mb3011c	Rv2987c	21.78	4.89	FGDIFR	9	SCX	8	25	23	2
				GACAYWKPR		SCX	FT3	64	24	2
				GLDDIALTLR		SCX	4	19	15	2
				GLDDIALTLRKLDE		SCX	FT3	31	20	3
				GSVLVAGPDFGTGSSR		SCX	FT3	54	19	2
				IITAATVVLPFK		SDS-PAGE	8	24	19	2
				SDPAFVLNLSPFDR		SDS-PAGE	8	49	18	2
				SNVDTDQIIPAVFLK		SDS-PAGE	8	27	19	2
				TGFEDGLFAGWR		SDS-PAGE	8	57	19	2
Mb3016c	Rv2992c	53.83	5.58	GEDLLPSTPR	8	SCX	FT3	52	20	2
				GPVTFAAGSVPDFALTR		SCX	7	38	20	2
				HTGGTFVFR		SCX	7	26	19	2
				KADALNAEHIR		SCX	8	53	23	3
				MLDVGDFTVR		SCX	2	49	20	2
				SYLALLDALR		SCX	FT1	20	19	2
				TALFNWAYAR		SDS-PAGE	1	20	19	2
				VAATGTTVSPPLFESLELLGR		SDS-PAGE	2	48	19	2
Mb3020c	Rv2996c	54.52	4.56	LLSLDDLLAR	3	SCX	FT3	62	24	2
				LLAAVPEADALLVR		SCX	7	52	23	2

				VAPWLDLVR		SDS-PAGE	5	53	19	2
Mb3026c	Rv3001c	36.09	4.80	DLMSWVDRPITE	9	SCX	7	60	23	2
				DLMSWVDRPITETA		SCX	FT3	55	20	2
				GDGLALALSYAK		SCX	FT1	48	19	2
				GVPCLVAVEQDPR		SCX	FT3	61	19	2
				LIVDLMYEGGLAR		SDS-PAGE	6	38	19	2
				LVADVEGGNK		SDS-PAGE	6	87	17	2
				MFYDDDADLSIIQGR		SDS-PAGE	3	34	19	2
				VEEQGLDVDTPAEVAK		SDS-PAGE	1	33	19	2
				VGVIGYGSQGHAHSLSLR		SDS-PAGE	6	43	18	3
Mb3031	Rv3006	38.81	4.57	ECPAPGVMQGCLE	6	SDS-PAGE	6	20	7	2
				ITGAVEEISISAEPK		SDS-PAGE	6	75	12	2
				LAPSTGAVTGEPDVVR		SDS-PAGE	6	29	7	2
				LMYAYISTPTDNR		SDS-PAGE	6	55	13	2
				MSPDGNVWGATVNK		SDS-PAGE	6	50	12	2
				TVIPVDPAGDGGLMDIVLSPTYSQDR		SDS-PAGE	6	63	14	3
Mb3041	Rv3016	22.14	4.45	QGMLDVATILLDQSR	1	SDS-PAGE	8	45	10	2
Mb3054c	Rv3028c	31.69	4.43	AEVLVLVEHAEGALK	10	SCX	FT2	20	14	3
				AEVLVLVEHAEGALKK		SCX	FT1	72	20	3
				ALGEPAAVVVGVPGTAAPLVDGLK		SCX	FT3	45	14	3
				EPAVAGDRPELTEATIVVAGGR		SCX	FT1	36	16	3
				GGVGVHSIFGGAFTVE		SDS-PAGE	7	23	17	2
				IGSGLLVDVVDVR		SDS-PAGE	7	26	17	2
				IYVAESDLVDK		SDS-PAGE	7	53	19	2
				SSAPAGVLIAATADGKE		SDS-PAGE	7	100	19	2
				YLITPAVDVLAGLAESSAPAGVLIAATADGK		SDS-PAGE	7	32	17	3
				AAVDSGYYPGQFQVGQTGK		SDS-PAGE	6	34	17	2

Mb3055c	Rv3029c	28.08	4.37	DDGMHGSDVIQTGWALAR	5	SCX	4	35	13	3
				EAADAVLDEINER		SCX	FT2	18	16	2
				GSVTVLTAGPERATE		SDS-PAGE	7	65	18	2
				RKLTDGDFTLDRE		SDS-PAGE	7	33	15	3
				TNIVVLIKQVPDTWSE		SDS-PAGE	7	50	17	2
				VTDEGEGGNQIVQYLVAQK		SDS-PAGE	7	46	18	3
Mb3059	Rv3033	19.27	5.26	FDMVETVDK	1	SDS-PAGE	9	45	15	2
Mb3069c	Rv3043c	63.65	6.99	AHVGRHHDEPAMVTSS	7	SCX	4	31	16	3
				APGMTMFR		SCX	FT1	26	19	2
				HHDEPAMVTSS		SCX	FT1	51	20	2
				HNFTELPR		SCX	2	21	20	2
				LHYPHMVER		SCX	FT1	26	19	2
				SERPAFELHYPHMVER		SDS-PAGE	1	41	19	4
				TAEAPPLGELEAIRPYPAR		SDS-PAGE	1	26	16	3
Mb3070	Rv3044	36.91	5.11	AYIEIGTTAADLAK	5	SCX	1	33	13	2
				IVVLSGDQLDALCALGLQSR		SCX	FT1	27	12	2
				IVAAALPNSSSSQPSYLGTTVHDLPGVGTR		SCX	FT1	92	12	3
				IAAVDALITGFAEHATQVGTK		SDS-PAGE	6	48	11	3
				AAVILDSDPWR		SDS-PAGE	6	69	12	2
Mb3071	Rv3045	37.07	4.93	FAGICHSDIHTVK	7	SCX	7	41	20	3
				HWNAGANTR		SCX	1	38	20	2
				IAGVVTAVGSEVTK		SCX	FT3	23	23	2
				LGAAMGADVTVLSQSLK		SCX	1	32	20	2
				RDPGPHDVAIDIK		SCX	7	51	20	3
				SYYATADPDTFR		SDS-PAGE	6	61	18	2
				WGQPNYPVVPGHE		SDS-PAGE	6	38	17	2
Mb3072c	Rv3046c	13.35	4.73	STPLTEEQIGEVVR	4	SCX	FT1	77	20	2

				TFSHPHFFR		SCX	7	44	20	3
				VALLSLLR		SCX	FT3	59	23	2
				VTHHDAGPENIQR		SDS-PAGE	10	23	19	3
Mb3117	Rv3090	32.27	8.32	GISPLGCWPGSSALPTIAVPGR	6	SCX	FT1	51	12	2
				LGNQSTALADVSIR		SCX	1	23	14	2
				NLDVSPLPSLAK		SCX	FT1	43	12	2
				QAAAPELFQQYK		SDS-PAGE	7	104	12	2
				SISDDPNVVVQNCITAAINK		SDS-PAGE	7	42	14	2
				VFAGFNPLDPR		SDS-PAGE	1	23	13	2
Mb3126c	Rv3099c	30.48	5.02	VLLELTGPLSR	2	SDS-PAGE	7	50	19	2
				VVDDFGGPAPTATIR		SDS-PAGE	7	49	19	2
Mb3128c	Rv3101c	32.79	9.00	QQAYDDAIR	1	SDS-PAGE	2	40	11	2
Mb3131c	Rv3104c	33.38	5.97	AVVDIPVPTSADLGR	3	SDS-PAGE	7	38	19	2
				LDLGFTESDALVR		SDS-PAGE	8	28	19	2
				SVNLSKDWAR		SDS-PAGE	7	26	19	2
Mb3143	Rv3116	41.66	5.08	PYVWGSIYR	1	SCX	8	38	24	2
Mb3157c	Rv3133c	23.26	5.45	TLLGLLSEGLTNK	1	SCX	1	60	20	2
Mb3163	Rv3139	49.65	5.92	AEQGLPFAR	16	SCX	FT1	33	19	2
				DYFDLTPDDDQK		SCX	FT1	86	20	2
				GMQIGLDGVQLLGGHGYTK		SCX	FT1	132	19	3
				GAALGQVELCGVSVPLNAR		SCX	1	23	21	2
				HRSSVTNVLVAE		SCX	FT1	75	23	2
				LGEDEASDNDYSEALALAR		SCX	FT1	73	20	2
				LGITAINIPEDFDGIAEHR		SCX	FT3	24	21	3
				LRDPLNR		SCX	FT1	30	19	2
				PALFIVESAASGLTVK		SCX	7	69	21	2
				QAFGEPIAHR		SCX	FT2	35	17	2

				SLIPAAADAELFIVGAQLGGK		SDS-PAGE	4	28	19	2
				TGIDVALALLTPIVGQE		SDS-PAGE	4	55	18	2
				TMFATAGAATR		SDS-PAGE	5	30	17	2
				VLRPAAHDADDAATYPSDLTAK		SDS-PAGE	5	48	13	3
				VLRPAAHDADDAATYPSDLTAKAAE		SDS-PAGE	5	109	15	4
				AAELGITAINIPEDFDGIAEHR		SDS-PAGE	5	60	18	2
Mb3164	Rv3140	43.34	5.01	AINLELPR	20	SCX	FT3	52	20	2
				EHPGVTVER		SCX	FT1	31	20	2
				GNLLGNPEIEVGK		SCX	FT3	81	23	2
				GTQQIQQLVVAR		SCX	FT2	22	13	2
				GTQQIQQLVVARRLLGLSSSE		SCX	FT1	106	19	3
				ILDIFEGTQQIQQLVVAR		SCX	FT2	17	16	2
				IRSVLTGAGVE		SCX	7	23	22	2
				ISYDKPSHTQSAAAAE		SCX	FT2	30	15	3
				ISYDKPSHTQSAAAAEFLR		SCX	FT3	40	13	3
				LDTLINLFE		SCX	4	42	14	2
				LDTLINLFEGAAE		SCX	FT2	21	19	2
				LLGLSSSELK		SCX	7	67	22	2
				LPRKLQAIIVKTHQGAAE		SCX	FT2	40	16	3
				MEADWEASYLLSLR		SCX	FT1	27	24	2
				TFDNTRPIVAAMAVGIGR		SCX	FT1	77	20	3
				TFDNTRPIVAAMAVGIGRAALEE		SCX	FT2	15	15	3
				THQGAAEMMR		SDS-PAGE	5	37	18	2
				TVELAGTTGYSEQSLLEK		SDS-PAGE	5	22	15	2
				VGKGFAGVME		SDS-PAGE	5	35	16	2
				AAWQADNNIPNSK		SDS-PAGE	5	23	19	2
Mb3165	Rv3141	33.85	5.22	LLVIGFTGGEIPTVK	1	SDS-PAGE	6	55	18	2

Mb3169	Rv3145	13.95	8.40	TSIGPGAASGQR	1	SCX	FT1	54	12	2
Mb3170	Rv3146	20.18	7.95	GLEEQLPGGILLSTVEK	3	SCX	FT1	26	20	2
				QADLMIVAGR		SDS-PAGE	9	36	20	2
				AALLARPTIE		SDS-PAGE	5	44	14	2
Mb3178	Rv3154	27.59	8.87	FRPGGHPTPLPNPGVYAR	3	SCX	7	61	12	3
				GLAALIFSR		SCX	7	53	16	2
				HNAVDVAALLPDGSYSE		SCX	FT3	53	15	2
Mb3181	Rv3157	59.17	8.98	APLWPFHR	6	SCX	8	15	11	2
				EIVAGVAAGR		SCX	7	41	16	2
				IGDLVGR		SCX	FT1	16	11	2
				STPATAVLMMAVMDK		SDS-PAGE	3	38	11	2
				VGTFGMLR		SDS-PAGE	1	23	13	2
				YPQRPAPPR		SDS-PAGE	4	34	15	2
Mb3182	Rv3158	55.34	8.92	GADGSAGSEDADLSHWAGLGQR	3	SCX	7	33	13	3
				LPGLDSFTPQASAVPGSDAER		SCX	FT3	66	13	2
				QNTLAVAR		SDS-PAGE	1	15	13	2
Mb3231c	Rv3206c	42.14	5.13	DSIVAINPLIR	1	SDS-PAGE	6	48	19	2
Mb3234c	Rv3208	9.40	4.77	GQLLVVASDVFVDR	3	SDS-PAGE	8	54	19	2
				HVENLVSGVHQALSTTTDNR		SDS-PAGE	8	23	18	3
				YWLDADKPISK		SDS-PAGE	8	24	19	3
Mb3236c	Rv3210c	25.12	4.96	ISMASMAAAEMGHYELLR	3	SDS-PAGE	8	23	16	3
				TGHSQFVVAE		SDS-PAGE	8	28	18	2
				AALSETGHSQFVVAEVR		SDS-PAGE	8	39	14	3
Mb3272c	Rv3244c	61.15	5.01	AIQDPASGQPAR	6	SCX	FT1	28	14	2
				GPYVINADGAPLEDR		SCX	FT3	31	14	2
				IPVDSTAVASR		SCX	1	36	11	2
				NTLYFADPTGK		SDS-PAGE	5	62	13	2

				TAEGQLPDPGPIELVK		SDS-PAGE	5	53	13	2
				TDAAHPVSYVNLDGVNSDAPSR		SDS-PAGE	5	62	13	3
Mb3274c	Rv3246c	25.27	5.18	DVLLEQVWGYR	1	SCX	1	35	20	2
Mb3276c	Rv3248c	54.32	4.85	DIIMLEHIK	12	SCX	1	23	20	2
				EQAEYLGVDVEGPYKPDHYR		SCX	7	27	18	3
				GPYKPDHYRY		SCX	FT3	28	17	3
				GVTEETTTGVLR		SCX	7	23	21	2
				HSLIDGINR		SDS-PAGE	2	42	19	2
				IADLSLADFGR		SDS-PAGE	4	38	20	2
				IDPINALQAMME		SDS-PAGE	5	44	17	2
				SIIVLSEGR		SDS-PAGE	5	26	18	2
				VFLNLLR		SDS-PAGE	5	19	19	2
				VLICGYGDVGK		SDS-PAGE	5	37	18	2
				VNVKPQVDLWTFGDTGR		SDS-PAGE	3	44	20	3
				YLGVDVEGPYKPDHYR		SDS-PAGE	5	25	15	3
Mb3297	Rv3269	9.75	7.80	ATTTVITGLAGVTAYE	6	SCX	FT3	35	22	2
				ATTTVITGLAGVTAYEILK		SCX	FT1	57	19	2
				IGEESPTPAISDLHDHDH		SCX	FT3	81	14	3
				QTAVSAAALGLR		SDS-PAGE	10	76	13	2
				SARLKVADVMAE		SDS-PAGE	10	14	3	2
				VADVMAEAR		SDS-PAGE	10	39	11	2
Mb3329c	Rv3301c	24.83	6.04	ADIGAAEQVIR	12	SCX	FT3	69	24	2
				ATQALLEADIGAAEQVIR		SCX	FT1	76	19	2
				DPQEAAQLHDRDDAMDDLHR		SCX	1	40	19	4
				EIFSAVQIIADTER		SCX	8	39	19	2
				EYPNQVLPEEVR		SCX	FT3	80	24	2
				FADHAVEVGR		SCX	4	20	15	2

				HLLSVLIDR		SCX	FT3	51	22	2
				IFSAVQIIADTER		SDS-PAGE	7	33	18	2
				TALLGRFFE		SDS-PAGE	8	27	19	2
				VAIALGDSAR		SDS-PAGE	8	18	18	2
				VGVETALLGR		SDS-PAGE	7	71	20	2
				VIFMVTGVLPTEDE		SDS-PAGE	8	51	19	2
Mb3333c	Rv3305c	41.23	5.83	ADMDALPMAER	8	SCX	FT3	84	22	2
				GVPPVVNEEISTR		SCX	7	28	19	2
				LADAGLNPK		SCX	7	60	19	2
				LMPGGAIDAIAAGALAGVSR		SCX	FT1	45	21	2
				QEYATTQFVAER		SDS-PAGE	6	79	19	2
				QTWVDLEELVR		SDS-PAGE	6	27	18	2
				VLPGGTGLTCDFGPQHQPR		SDS-PAGE	6	55	18	3
				VMVNIIEQAAAH		SDS-PAGE	6	59	19	2
Mb3339	Rv3311	45.73	3.90	CYLDDRPIFLGR	8	SCX	2	22	20	3
				DAGDEWEAFLGK		SCX	FT1	71	20	2
				DIGDYSEDSAVDK		SCX	FT3	34	23	2
				FFNGNPILGTVSGGLE		SCX	FT1	75	20	2
				FFNGNPILGTVSGGLEHFTGK		SCX	FT2	41	14	3
				ISVFGSER		SCX	1	36	21	2
				LLRDIGDYSEDSAVDKALE		SCX	1	25	20	3
				WNSIAEVIGR		SDS-PAGE	5	25	18	2
				YTLWAPR		SDS-PAGE	5	33	20	2
Mb3422	Rv3390	25.06	7.58	AEAQQVAHQVSR	6	SCX	1	19	19	2
				DSLLTTHPLPNIGR		SCX	FT1	76	9	3
				LVEWDGIR		SCX	FT1	81	13	2
				NAQSQANADGIIDTDMPGSGLSADGK		SDS-PAGE	8	44	8	3

				SITLTFIR		SDS-PAGE	8	59	12	2
				VVITGNPVTGWR		SDS-PAGE	8	42	13	2
Mb3440	Rv3406	32.58	6.44	AIDDYDDQHR	11	SCX	FT3	55	20	2
				CLTENLWALHTNR		SCX	FT1	20	19	3
				IGAQIDGVR		SCX	FT3	41	20	2
				LGGDLDPAAVNEIR		SCX	1	41	19	2
				QVFEKPDFR		SCX	1	24	20	2
				SFVGLDSHESR		SDS-PAGE	5	55	19	2
				TLLAGDFVR		SDS-PAGE	4	33	19	2
				VLFEVLQR		SDS-PAGE	10	56	19	2
				VTLMGDVPVDVYGQASR		SDS-PAGE	4	41	19	2
				YDYVTTKPLTAAQR		SDS-PAGE	6	44	18	3
Mb3451c	Rv3417c	55.88	4.74	AEIDKSDSDWDREK	34	SCX	FT1	40	20	3
				AFGGPTVTNDGVTVAR		SCX	7	55	20	2
				AFLEDLAVVTGGQVVNPDAGMVLR		SCX	FT1	81	20	3
				ALATLVVNAIR		SCX	FT3	33	24	2
				ALLASATPVSGKTGIAQVATVSSRDEQIGDLVGE		SCX	FT2	26	15	3
				AMSKVGHDGVVSVEE		SCX	7	38	23	2
				AVANRAKHLRAE		SCX	1	68	20	3
				DALILLHQDK		SCX	FT1	64	21	2
				DDTVIVDGGGTAEAVANR		SCX	FT1	76	20	2
				DEQIGDLVGEAMSK		SCX	FT3	72	24	2
				DLAVVTGGQVVNPDAGMVLRE		SCX	FT2	17	16	2
				EIELEDPFEDLGAQLVK		SCX	FT2	44	15	2
				ESVEDAVAAAK		SCX	FT1	51	20	2
				EVGLEVLGSAR		SCX	FT2	35	17	2
				GIVPGGGASLIHQAR		SCX	FT1	57	19	2

				GIVPGGGASLIHQARKALTE		SCX	FT3	49	20	3
				GPYFGDR		SCX	FT1	72	20	2
				IDKSDSDWDREKLGE		SCX	FT1	48	20	3
				ISSLPDLLPLLEK		SCX	4	18	15	2
				KESVEDAVAAAK		SCX	FT3	83	18	2
				LIEYDETAR		SDS-PAGE	5	28	19	2
				LRASLTGDE		SDS-PAGE	5	31	18	2
				LVAAGVNPIALGVGIGK		SDS-PAGE	6	58	19	2
				SAVLNASSVAR		SDS-PAGE	5	55	18	2
				SKLIEYDE		SDS-PAGE	5	43	16	2
				SVEDAVAAAKAAVEE		SDS-PAGE	6	30	18	2
				TGIAQVATVSSR		SDS-PAGE	5	25	16	2
				TNDVAGDGTTTATILAQALIK		SDS-PAGE	5	59	20	2
				TVVVDKPAKAE		SDS-PAGE	6	49	18	2
				VGAATETALK		SDS-PAGE	5	18	15	2
				VLGSARRVVVSKDDTVIVDGGGTAE		SDS-PAGE	5	54	19	3
				VSELPAGHGLNVNTLSYGDLAADGVIDPVK		SDS-PAGE	5	51	20	3
				AADAVSEALLASATPVSGK		SDS-PAGE	6	49	18	2
				AAVEEGIVPGGGASLIHQAR		SDS-PAGE	5	56	19	3
Mb3452c	Rv3418c	10.77	4.34	AKVNIKPLE	11	SCX	FT2	38	16	2
				AKVNIKPLEDKILVQANEAE		SCX	FT2	34	16	3
				DKILVQANEAE		SCX	5	21	15	2
				DVLAVVSK		SCX	FT1	77	20	2
				EKPQEGTVVAVGPGR		SCX	7	44	20	3
				GDTVIYSKYGGTE		SCX	1	58	20	2
				IPLDVAEGDTVIYSK		SDS-PAGE	10	17	16	2
				TTTASGLVIPDTAKEKPQE		SDS-PAGE	10	59	16	3

				VNIKPLEDK		SDS-PAGE	10	67	18	2
				YLILSARDVLAVVSK		SDS-PAGE	9	23	14	3
				YNGEEYLILSAR		SDS-PAGE	10	27	21	2
Mb3486c	Rv3457c	37.71	4.38	EDVTEIILNLK	4	SCX	1	42	20	2
				IPVDSIYSPVLK		SDS-PAGE	6	24	19	2
				TLLSSIPGAAVTSIR		SDS-PAGE	6	25	20	2
				TLVELFGLAR		SDS-PAGE	6	37	19	2
Mb3487c	Rv3458c	23.48	10.46	LDNVIYR	4	SDS-PAGE	8	30	19	2
				SLNTVPFQIAR		SDS-PAGE	8	22	19	2
				TDLVGGDQAFEK		SDS-PAGE	8	24	19	2
				VSQYDIVDVR		SDS-PAGE	8	33	20	2
Mb3493	Rv3464	37.56	5.39	DNESWWRPLK	4	SCX	2	23	20	2
				EHPDDAVTVLDALTYAGR		SCX	7	44	20	3
				ESLADVEDAIR		SDS-PAGE	6	52	17	2
				LMDRDPDDFDHVTDR		SDS-PAGE	6	64	20	3
Mb3511c	Rv3481c	23.79	10.54	HTEMPGWMR	1	SCX	8	39	13	2
Mb3519	Rv3489	5.69	4.33	IFLSMLGIGPAK	1	SCX	FT3	43	20	2
Mb3586c	Rv3556c	40.90	4.86	LFTTMLGELER	1	SCX	1	48	20	2
Mb3613c	Rv3582c	24.01	4.49	LDLLLAQAIVR	1	SDS-PAGE	8	62	18	2
Mb3615	Rv3584	18.79	9.92	AGQGSVMVPISAGLATPHE	9	SCX	FT1	86	12	3
				ATVNLTKPIANGLTYNFTFK		SCX	FT1	87	13	3
				AVDLVLVAVNQSPDVSDR		SCX	FT2	16	9	2
				IQAVQTSDFIQPGK		SCX	FT3	56	12	2
				KAGQGSVMVPISAGLATPHE		SCX	FT3	83	13	3
				LPASGMLFVGTPDGQIVAPGPLPSNQAAK		SDS-PAGE	10	32	12	3
				LTINNVLLR		SDS-PAGE	9	36	12	2
				LVGITSDIGSVTVAGDAR		SDS-PAGE	9	40	9	2

				PIANGLTYNFTFK		SDS-PAGE	9	36	13	2
Mb3618c	Rv3587c	27.06	5.92	PAIGPGTYNLVVQLGNLR	1	SCX	FT1	54	13	2
Mb3627c	Rv3596c	93.55	5.66	NNPVLIGEPGVGK	1	SDS-PAGE	4	49	19	2
Mb3638c	Rv3608c	28.81	4.94	ADLLASVADAVAAGVDPAR	2	SCX	FT3	74	20	2
				LVLDPGLGFAK		SDS-PAGE	7	55	20	2
Mb3640c	Rv3610c	81.99	5.61	DVLDTLAGELLEK	3	SCX	FT1	60	12	2
				GVLLYGPPGTGK		SDS-PAGE	4	17	13	2
				LTMFDDFGGR		SDS-PAGE	4	20	12	2
Mb3645c	Rv3615c	10.79	5.00	IYSEADEAWR	2	SCX	1	74	20	2
				TENLTVQPER		SDS-PAGE	10	33	20	2
Mb3646c	Rv3616c	39.89	5.01	LAAAFPGDGWLGSAADK	2	SCX	FT3	56	23	2
				QLISLIHDQANAVQTTR		SCX	7	63	20	3
Mb3648c	Rv3624c	23.60	4.72	DLSYIGTLDPR	2	SDS-PAGE	8	62	19	2
				GAVLFVTDLAR		SDS-PAGE	8	33	19	2
Mb3657	Rv3633	32.44	5.57	LLPVIEGVLGR	1	SDS-PAGE	7	48	18	2
Mb3707	Rv3682	84.64	5.13	EQVATGGYLIR	7	SCX	FT3	22	16	2
				GQVPAVSTMVDAK		SDS-PAGE	4	17	8	2
				GSMNVTDALATSPNTAFAK		SDS-PAGE	4	24	14	2
				LISQVGVGR		SDS-PAGE	4	21	10	2
				SAGFVGFTNR		SDS-PAGE	4	39	12	2
				TTLDPEVQAPVK		SDS-PAGE	4	50	12	2
				VPSVAGLDVDAAR		SDS-PAGE	4	40	12	2
Mb3736c	Rv3709c	44.43	4.77	VTIVGLPDIPGYAAK	1	SCX	FT3	41	20	2
Mb3750	Rv3723	27.37	9.71	EAPAAETATAEEPR	1	SCX	FT1	76	21	2
Mb3761c	Rv3734c	49.30	6.85	DLLELTSR	4	SCX	FT3	65	20	2
				MAGSVVGLAPSTLK		SCX	7	46	20	2
				TLSADPDDAEVR		SDS-PAGE	1	30	20	2

				TRPPSDGSSLLDALFK		SDS-PAGE	3	47	18	3
Mb3787c	Rv3761c	38.53	5.01	AVGLSDFGKPDGYLER	7	SCX	2	32	20	3
				DTAAESVPDVVAPLIAR		SCX	FT3	60	19	2
				LDGLDLGALDR		SCX	1	34	19	2
				RPPLHGLTPSAHDMAR		SCX	2	36	21	4
				VVAALGDTPVPVAR		SDS-PAGE	6	30	17	2
				VYDDASSWLVR		SDS-PAGE	6	20	18	2
				WGSQWELVR		SDS-PAGE	6	31	18	2
Mb3789	Rv3763	15.11	7.18	ITGTATGVDMANPMSPVNK	2	SCX	FT3	38	10	2
				SVGLGNVNGVTLGYTSGTGQGNASATK		SDS-PAGE	9	49	3	3
Mb3809	Rv3780	19.48	4.51	DIHATSIR	7	SCX	FT3	27	23	2
				ELEDGLAPELR		SCX	FT2	25	16	2
				IAQAQLVGWLE		SCX	FT2	31	16	2
				LDRLTLPFNE		SCX	FT3	76	20	2
				LDRLTLPFNEDAVPSDAE		SCX	7	27	21	2
				LTLPFNEDAVPSDAELR		SDS-PAGE	9	25	18	2
				SGQHGHGTGQYL		SDS-PAGE	9	66	19	2
Mb3820	Rv3791	27.46.	8.21	ELVWAPAAFR	1	SDS-PAGE	7	47	20	2
Mb3822	Rv3793	117.50	10.14	HAGYMANYYR	6	SCX	8	27	24	2
				LVADDQDLAPQHWIALTPPR		SCX	FT1	31	20	2
				WFDEHIR		SCX	2	37	21	2
				LPYNLDPAR		SDS-PAGE	2	34	20	2
				APLLVVTAAGR		SDS-PAGE	2	21	19	2
				APLSAIPSTATQVR		SDS-PAGE	3	27	18	2
Mb3824	Rv3795	117.93	9.91	NVVILSVPR	2	SCX	8	33	13	2
				VADHAGYMSNYFR		SDS-PAGE	1	42	9	3
Mb3825	Rv3796	39.45	6.74	DYGIDNVADLVK	1	SDS-PAGE	6	58	13	2

Mb3830c	Rv3800c	186.43.	4.58	ADVAESQENAPAER	4	SCX	FT1	55	20	2
				HSVYFTHGIR		SCX	8	23	20	3
				HVWEYAPR		SCX	2	28	21	2
				LADWMQSPE		SCX	FT2	32	16	2
Mb3834c	Rv3804c	35.69	6.51	ALGATPNTGPAPQGA	4	SDS-PAGE	7	19	9	2
				FLEGFVR		SDS-PAGE	7	34	12	2
				NDPLLNVGK		SDS-PAGE	7	26	11	2
				VQFQSGGANSPALYLLDGLR		SDS-PAGE	7	25	11	2
Mb3840	Rv3810	27.66	4.11	LGASQAIDLLK	1	SCX	FT3	75	17	2
Mb3848	Rv3818	57.62	5.03	DAVVLLPDFPVPDLR	3	SCX	FT1	29	20	2
				YGFAIAPELVR		SCX	8	34	23	2
				YLYTFFK		SDS-PAGE	5	42	19	2
Mb3876	Rv3846	22.98.	6.43	AKEDHSAILLNEK	1	SDS-PAGE	8	47	18	3
Mb3885	Rv3855	23.72	4.93	EAVLLTLLDR	1	SDS-PAGE	8	48	20	2
Mb3895	Rv3865	10.62	6.52	ADDGLAGVIDK	5	SCX	FT1	50	21	2
				ATDTVAGISGR		SCX	FT1	80	20	2
				FNDTLQEFETTR		SCX	FT1	64	20	2
				SSTGTGLQGVTSGLANNLLAAAGAYLK		SCX	7	36	20	3
				VQLTHGSFTSK		SDS-PAGE	10	74	20	3
Mb3898	Rv3868	62.43	4.82	TDPFGQEALDTLLAR	1	SDS-PAGE	5	52	19	2
Mb3910c	Rv3880c	12.17	4.48	ALHNAQAAASAYNDAAGE	12	SCX	FT3	28	18	2
				ALHNAQAAASAYNDAAGEQLTAALSAMSR		SCX	FT3	51	19	3
				ALHNAQAAASAYNDAAGEQLTAALSAMSRAMNE		SCX	FT2	21	16	3
				DGLLKKLGAE		SCX	4	39	16	2
				DGLLKKLGAEAVAQRVNE		SCX	FT3	133	20	3
				FQSALDGTLNQMNNGSFR		SCX	FT3	46	20	2
				IEDGLLK		SCX	FT1	54	21	2

				KLGAEAVAQR		SCX	7	58	23	2
				LGAEAVAQR		SDS-PAGE	10	24	21	2
				SMDELDPHVAR		SDS-PAGE	10	32	16	2
				VTINGHQWLTGLR		SDS-PAGE	10	53	20	3
				GSGVDVAISPTPR		SCX	1	18	14	2
Mb3951c	Rv3920c	20.56	4.06	EELAALADEVAR	2	SDS-PAGE	8	60	19	2
				GGEVLDALQELTR		SDS-PAGE	8	36	20	2

Paper II
Supplementary table III

Supplementary table 3: List of all identified *M. bovis* proteins, arranged according to number of identified peptides.

M. bovis	*M. tub.* H37Rv	No. of identified peptides	No. of TM
Mb0448	Rv0440	79	0
Mb3451c	Rv3417c	34	0
Mb1342	Rv1310	33	0
Mb1524	Rv1488	21	1
Mb2321c	Rv2299c	20	1
Mb0704	Rv0685	20	0
Mb3164	Rv3140	20	0
Mb2223c	Rv2200c	18	3
Mb0515c	Rv0503c	18	0
Mb0153	Rv0148	17	0
Mb2765c	Rv2744c	17	0
Mb0232c	Rv0227c	16	2
Mb1515	Rv1479	16	0
Mb3163	Rv3139	16	0
Mb1339	Rv1307	14	1
Mb1903c	Rv1872c	14	0
Mb2219	Rv2196	13	9
Mb0426	Rv0418	13	1
Mb1706	Rv1679	13	0
Mb2659c	Rv2626c	13	0
Mb2218	Rv2195	12	3
Mb2965c	Rv2940c	12	0
Mb3276c	Rv3248c	12	0
Mb3329c	Rv3301c	12	0
Mb3910c	Rv3880c	12	0
Mb0951	Rv0928	11	0
Mb0956c	Rv0932c	11	0
Mb1867c	Rv1836c	11	1
Mb2993c	Rv2969c	11	1
Mb0253c	Rv0247c	11	0
Mb0671	Rv0652	11	0
Mb0838c	Rv0815c	11	0
Mb0976	Rv0951	11	0
Mb2057c	Rv2031c	11	0
Mb2656	Rv2623	11	0
Mb3440	Rv3406	11	0
Mb3452c	Rv3418c	11	0
Mb2898	Rv2873	10	0
Mb0485	Rv0475	10	0
Mb2268	Rv2244	10	0
Mb3054c	Rv3028c	10	0
Mb2565	Rv2536	9	4

Mb0508	Rv0497	9	3
Mb2740c	Rv2721c	9	2
Mb0243	Rv0237	9	0
Mb3615	Rv3584	9	1
Mb1471	Rv1436	9	0
Mb2244	Rv2220	9	0
Mb3011c	Rv2987c	9	0
Mb3026c	Rv3001c	9	0
Mb0959	Rv0934	8	0
Mb1446c	Rv1411c	8	0
Mb0189	Rv0183	8	0
Mb0254c	Rv0248c	8	0
Mb0595c	Rv0580c	8	0
Mb1124	Rv1094	8	0
Mb1637	Rv1611	8	0
Mb3016c	Rv2992c	8	0
Mb3333c	Rv3305c	8	0
Mb3339	Rv3311	8	0
Mb3069c	Rv3043c	7	12
Mb2592	Rv2563	7	4
Mb1033	Rv1006	7	0
Mb2118c	Rv2091c	7	1
Mb3707	Rv3682	7	1
Mb0663c	Rv0644c	7	0
Mb1444	Rv1409	7	0
Mb1943c	Rv1908c	7	0
Mb3071	Rv3045	7	0
Mb3787c	Rv3761c	7	0
Mb3809	Rv3780	7	0
Mb3181	Rv3157	6	14
Mb3822	Rv3793	6	13
Mb0162	Rv0157	6	9
Mb2217	Rv2194	6	2
Mb3117	Rv3090	6	2
Mb0400c	Rv0394c	6	1
Mb1301c	Rv1270c	6	1
Mb2248c	Rv2224c	6	1
Mb2970c	Rv2945c	6	1
Mb3031	Rv3006	6	0
Mb3272c	Rv3244c	6	0
Mb3422	Rv3390	6	1
Mb1340	Rv1308	6	0
Mb1498	Rv1463	6	0
Mb1817	Rv1789	6	0
Mb1822	Rv1794	6	0
Mb2200c	Rv2178c	6	0

Mb2487c	Rv2460c	6	0
Mb2983c	Rv2959c	6	0
Mb3297	Rv3269	6	0
Mb1649c	Rv1623c	5	9
Mb0578	Rv0563	5	4
Mb0368	Rv0361	5	1
Mb0440	Rv0432	5	0
Mb0598c	Rv0583c	5	0
Mb1198	Rv1166	5	0
Mb1306	Rv1275	5	0
Mb1956c	Rv1921c	5	0
Mb2221c	Rv2198c	5	1
Mb2408	Rv2387	5	0
Mb2616c	Rv2585c	5	0
Mb2628	Rv2597	5	1
Mb2691	Rv2672	5	1
Mb3070	Rv3044	5	0
Mb0358	Rv0350	5	0
Mb0577	Rv0562	5	0
Mb1026	Rv09992	5	0
Mb1051	Rv1023	5	0
Mb1111	Rv1082	5	0
Mb1502c	Rv1467c	5	0
Mb1812	Rv1784	5	0
Mb2270	Rv2246	5	0
Mb2547c	Rv2518c	5	0
Mb2788c	Rv2766c	5	0
Mb3055c	Rv3029c	5	0
Mb3895	Rv3865	5	0
Mb1812	Rv1783	4	2
Mb2455	Rv2429	4	2
Mb0014c	Rv0014c	4	1
Mb0419c	Rv0411c	4	0
Mb1403	Rv1368	4	1
Mb1512	Rv1476	4	1
Mb2094c	Rv2068c	4	0
Mb2619c	Rv2588c	4	1
Mb3834c	Rv3804c	4	1
Mb0020c	Rv0020c	4	0
Mb0249	Rv0243	4	0
Mb0276	Rv0270	4	0
Mb0289	Rv0281	4	0
Mb0415	Rv0407	4	0
Mb0477	Rv0468	4	0
Mb0686	Rv0667	4	0
Mb0812	Rv0788	4	0

Mb1482c	Rv1447c	4	0
Mb1520	Rv1484	4	0
Mb1639	Rv1613	4	0
Mb1687	Rv1659	4	0
Mb2207c	Rv2185c	4	0
Mb2246c	Rv2222c	4	0
Mb2320	Rv2298	4	0
Mb2415	Rv2394	4	0
Mb2638c	Rv2606c	4	0
Mb2730	Rv2711	4	0
Mb2760	Rv2740	4	0
Mb3072c	Rv3046c	4	0
Mb3486c	Rv3457c	4	0
Mb3487c	Rv3458c	4	0
Mb3493	Rv3464	4	0
Mb3761c	Rv3734c	4	0
Mb3830c	Rv3800c	4	0
Mb3182	Rv3158	3	14
Mb1633	Rv1607	3	11
Mb2203	Rv2181	3	10
Mb0255c	Rv0249c	3	5
Mb1336	Rv1304	3	5
Mb2144c	Rv2120c	3	5
Mb3178	Rv3154	3	5
Mb1517	Rv1481	3	3
Mb3131c	Rv3104c	3	3
Mb1107	Rv1078	3	2
Mb1824	Rv1796	3	2
Mb3640c	Rv3610c	3	2
Mb0019c	Rv0019c	3	1
Mb0049c	Rv0048c	3	1
Mb0134c	Rv0129c	3	1
Mb0698c	Rv0679c	3	0
Mb1267	Rv1235	3	0
Mb1305	Rv1274	3	0
Mb1338	Rv1306	3	1
Mb1917c	Rv1885c	3	1
Mb2121c	Rv2094c	3	1
Mb2193	Rv2171	3	1
Mb2819c	Rv2796c	3	0
Mb0009	Rv0009	3	0
Mb0664c	Rv0645c	3	0
Mb0759	Rv0738	3	0
Mb0784c	Rv0761c	3	0
Mb1123	Rv1093	3	0
Mb1128c	Rv1098c	3	0

Mb1152	Rv1121	3	0
Mb1367	Rv1332	3	0
Mb1472	Rv1437	3	0
Mb1652	Rv1626	3	0
Mb1860	Rv1829	3	0
Mb2016c	Rv1993c	3	0
Mb2235	Rv2212	3	0
Mb2262c	Rv2238c	3	0
Mb2316	Rv2294	3	0
Mb2323	Rv2301A	3	0
Mb2327	Rv2305	3	0
Mb2612c	Rv2581c	3	0
Mb3020c	Rv2996c	3	0
Mb3170	Rv3146	3	0
Mb3234c	Rv3208	3	0
Mb3236c	Rv3210c	3	0
Mb3848	Rv3818	3	0
Mb3824	Rv3795	2	12
Mb0212c	Rv0206c	2	11
Mb0186c	Rv0180c	2	6
Mb2617c	Rv2586c	2	6
Mb2618c	Rv2587c	2	6
Mb2282c	Rv2258c	2	5
Mb2220c	Rv2197c	2	4
Mb3128c	Rv3101c	2	4
Mb0161	Rv0156	2	3
Mb1271c	Rv1239c	2	2
Mb1523	Rv1487	2	2
Mb1825	Rv1797	2	2
Mb0205	Rv0199	2	1
Mb0271c	Rv0265c	2	0
Mb0539	Rv0526	2	0
Mb1127c	Rv1097c	2	1
Mb1704	Rv1677	2	0
Mb1957	Rv1922	2	0
Mb2226	Rv2203	2	1
Mb2504c	Rv2477c	2	1
Mb2900	Rv2875	2	1
Mb2945c	Rv2921c	2	1
Mb3059	Rv3033	2	0
Mb3618c	Rv3587c	2	1
Mb3789	Rv3763	2	0
Mb0054	Rv0053	2	0
Mb0091	Rv0088	2	0
Mb0321	Rv0313	2	0
Mb0451	Rv0443	2	0

Mb0473c	Rv0464c	2	0
Mb0478	Rv0469	2	0
Mb0568	Rv0553	2	0
Mb0591	Rv0576	2	0
Mb0651c	Rv0634c	2	0
Mb0655	Rv0636	2	0
Mb0656	Rv0637	2	0
Mb0658	Rv0639	2	0
Mb0737	Rv0716	2	0
Mb0854c	Rv0831c	2	0
Mb0977	Rv0952	2	0
Mb1010	Rv0984	2	0
Mb1047	Rv1019	2	0
Mb1067c	Rv1038c	2	0
Mb1211	Rv1178	2	0
Mb1320	Rv1289	2	0
Mb1368	Rv1333	2	0
Mb1414	Rv1379	2	0
Mb1463c	Rv1428c	2	0
Mb1481c	Rv1446c	2	0
Mb1499	Rv1464	2	0
Mb1676	Rv1649	2	0
Mb1821	Rv1793	2	0
Mb1887c	Rv1856c	2	0
Mb2025	Rv2002	2	0
Mb2028c	Rv2005c	2	0
Mb2146c	Rv2122c	2	0
Mb2155c	Rv2131c	2	0
Mb2318	Rv2296	2	0
Mb2433c	Rv2410c	2	0
Mb2953	Rv2928	2	0
Mb2966	Rv2941	2	0
Mb2996	Rv2971	2	0
Mb3126c	Rv3099c	2	0
Mb3638c	Rv3608c	2	0
Mb3645c	Rv3615c	2	0
Mb3646c	Rv3616c	2	0
Mb3648c	Rv3624c	2	0
Mb3951c	Rv3920c	2	0
Mb1445c	Rv1410c	1	14
Mb2151	Rv2127	1	12
Mb0211	Rv0205	1	8
Mb2692	Rv2673	1	8
Mb0173	Rv0167	1	6
Mb3511c	Rv3481c	1	6
Mb0979	Rv0954	1	4

Mb1617	Rv1591	1	4
Mb1853	Rv1822	1	4
Mb3750	Rv3723	1	4
Mb0182	Rv0176	1	3
Mb0206	Rv0200	1	3
Mb3169	Rv3145	1	3
Mb1475	Rv1440	1	2
Mb2242	Rv2219	1	2
Mb0008c	Rv0008c	1	1
Mb0111c	Rv0107c	1	0
Mb0323	Rv0315	1	0
Mb0351c	Rv0344c	1	0
Mb0427	Rv0419	1	1
Mb0439	Rv0431	1	1
Mb0463c	Rv0455c	1	1
Mb0923	Rv0899	1	1
Mb0930	Rv0906	1	0
Mb1241	Rv1209	1	1
Mb1454	Rv1419	1	0
Mb2072	Rv2046	1	0
Mb2747c	Rv2728c	1	0
Mb3041	Rv3016	1	0
Mb3231c	Rv3206c	1	1
Mb3840	Rv3810	1	1
Mb0160	Rv0155	1	0
Mb0257c	Rv0251c	1	0
Mb0303c	Rv0295c	1	0
Mb0386	Rv0379	1	0
Mb0447c	Rv0439c	1	0
Mb0538	Rv0525	1	0
Mb0662c	Rv0643c	1	0
Mb0670	Rv0651	1	0
Mb0687	Rv0668	1	0
Mb0826	Rv0803	1	0
Mb0844c	Rv0821c	1	0
Mb0847c	Rv0824c	1	0
Mb0993	Rv0968	1	0
Mb1230	Rv1198	1	0
Mb1329	Rv1297	1	0
Mb1333	Rv1301	1	0
Mb1341	Rv1309	1	0
Mb1425	Rv1390	1	0
Mb1501	Rv1466	1	0
Mb1519	Rv1483	1	0
Mb1571	Rv1544	1	0
Mb1573	Rv1546	1	0

Mb1584	Rv1558	1	0
Mb1623	Rv1597	1	0
Mb1729c	Rv1703c	1	0
Mb1800	Rv1771	1	0
Mb1857	Rv1826	1	0
Mb1954c	Rv1919c	1	0
Mb2058	Rv2032	1	0
Mb2153c	Rv2129c	1	0
Mb2154c	Rv2130c	1	0
Mb2227c	Rv2204c	1	0
Mb2311	Rv2289	1	0
Mb2379	Rv2358	1	0
Mb2482c	Rv2455c	1	0
Mb2586c	Rv2556c	1	0
Mb2697c	Rv2678c	1	0
Mb2805c	Rv2782c	1	0
Mb2913c	Rv2889c	1	0
Mb2917	Rv2893	1	0
Mb3143	Rv3116	1	0
Mb3157c	Rv3133c	1	0
Mb3165	Rv3141	1	0
Mb3274c	Rv3246c	1	0
Mb3519	Rv3489	1	0
Mb3586c	Rv3556c	1	0
Mb3613c	Rv3582c	1	0
Mb3627c	Rv3596c	1	0
Mb3657	Rv3633	1	0
Mb3736c	Rv3709c	1	0
Mb3820	Rv3791	1	0
Mb3825	Rv3796	1	0
Mb3876	Rv3846	1	0
Mb3885	Rv3855	1	0
Mb3898	Rv3868	1	0

Paper III

Antigen analysis of *Mycobacterium tuberculosis* H37Rv culture filtrate proteins.

Antigen analysis of *Mycobacterium tuberculosis* H37Rv culture filtrate proteins

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Abstract

Recently, we showed that the culture filtrate of *Mycobacterium tuberculosis* H37Rv produced by Sadamu Nagai, Osaka, Japan, was enriched with secreted proteins and had a minimal content of intracellular proteins compared to what have been reported for *M. tuberculosis* culture filtrates by other investigators, including early culture filtrates. The goal of the present study was to identify antigens among these culture filtrate proteins recognized by a serum pool from tuberculosis patients. Two different approaches were used to separate the culture filtrate protein mixture: 1) Proteins were fractionated according to their hydrophobicity using a HPLC-C18 chromatography column followed by separation based on their molecular mass by SDS-PAGE and subsequent western blotting, or 2) Proteins were separated by two-dimensional gel electrophoresis, based on their isoelectric point and their molecular mass. Twenty serological reactive proteins were ultimately identified by both methods, including 4 novel antigens. Further, to estimate the immunogenecity of the identified culture filtrate proteins the relative antibody quantities were measured using Image Master software. Our results show that the antibodies against proteins belonging to the antigen 85 complex were the most abundant in the serum of patients with active tuberculosis. The most immunogenic proteins in terms of high antibody to protein ratio were Rv3881c and three lipoproteins Rv0934 (the 38 kDa antigen), Rv0932 (pstS2), and Rv3006 (LppZ). Rv3881c is located in the region of difference 1 (RD1) which is deleted from *Mycobacterium bovis* BCG, and is therefore a particularly promising candidate for development of serodiagnostic assays to detect active tuberculosis. The proteins from the *M. tuberculosis* H37Rv culture filtrate are strong candidates to be evaluated for improvement of the serodiagnostic tests of tuberculosis.

Keywords: *Mycobacterium tuberculosis* / Two-dimensional polyacrylamide gel electrophoresis / Mass spectrometry / Secreted proteins

Introduction

Tuberculosis (TB) is a major health problem throughout the world. According to the WHO, one-third of the world's population is latently infected and there are more than 10 million new cases around the globe causing 2 million deaths annually [1]. This contagious disease, though preventable, has had an increased incidence in recent years, mainly due to its association with human immunodeficiency virus disease [2] and also due to the occurrence of multi-drug resistance [3].

The immune response involved in combating the pathogen includes both humoral and cell mediated immunity. Currently, it is believed that the cellular immunity is the major component required for protection, while the role of humoral immunity remains controversial. Nevertheless, it has been shown that monoclonal antibodies against surface epitopes and proteins can modify the course of infection leading to better control and containment of the pathogen [4,5].

Most of our current knowledge on humoral immunity in TB derives from serodiagnostic studies [6]. The diagnosis of TB plays an important role in disease control. Therefore, the development of sensitive and specific serological tests for diagnosis of mycobacterial infections is critical to control the pathogen in early stages and preventing it from reactivation and dissemination. Proteins secreted to the extracellular environment by *M. tuberculosis* are recognized by the immune system in the infected host, constituting an important source of antigens that induce protective immunity and immune responses with diagnostic value [7,8].

A significant number of mycobacterial proteins inferred from the genome are predicted to be secreted via the well-characterized general secretory (Sec) pathway, which transports unfolded proteins across the cytoplasmic membrane to the bacterial envelope and the extracellular environment. Our previous work showed that the *M. tuberculosis* H37Rv culture filtrate produced by Sadamu Nagai [9] was considerably enriched with secreted proteins [10] compared to what have been reported by other investigators [11-15]. Therefore, the aim of the present study was to characterize the immunogenecity of this culture filtrate protein mixture to identify novel antigens as potential candidates for serological diagnosis of tuberculosis. Here, the culture filtrate protein mixture was separated by two different methods using both hydrophobicity based chromatography and

2D-PAGE electrophoresis, and proteins were probed with sera from *M. tuberculosis* infected patients. Subsequently, reactive protein spots with patient sera were identified by LC-MS/MS. Further, we estimated the relative abundance of antibodies as compared to protein staining of the identified antigens.

Materials and methods

Bacterial cultivation and sample preparation

M. tuberculosis H37Rv ATCC27294 from the National Institute of Health, Tokyo, Japan was cultured as surface pellicle on the wholly synthetic Sauton medium for 3-4 weeks without any shaking. Bacteria were removed by filtration and the culture filtrate was concentrated by 80% ammonium sulphate precipitation. Precipitated proteins were dissolved in buffer and dialyzed against distilled water and lyophilised [16]. Three separate culture filtrate batches were a generous gift from Sadamu Nagai, Osaka, Japan.

HPLC-C18 chromatography separation of *M. tuberculosis* H37Rv culture filtrate proteins

Two-hundred µg of culture filtrate proteins were resuspended in buffer containing 6M urea and 1% acetic acid and were loaded onto a macroporous reversed-phase C18 column (mRP-C18) (Agilent Technologies, Waldbronn, Germany) using an LC Packings *Ultimate 3000* capillary-HPLC system (Dionex, Sunnyvale, U.S.A.). The column was operated at an elevated temperature of 80˚C during the run. The compositions of the solvents used to develop the elution gradient were as follows: buffer A contained 0.1% (vol/vol) trifluoracetic acid/2% (vol/vol) acetonitrile, while buffer B contained 0.08% (vol/vol) trifluoracetic acid/90% (vol/vol) acetonitrile. The proteins were eluted with a gradient of acetonitrile in buffer B ranging from 15% to 100% with a flow rate of 300 µl/min over 35 min. Only fractions between 5 and 28 min were collected, fourteen fractions in total, with 100 sec collection time for each of them. The collected fractions were then dried out using a speed-vac centrifuge and resuspended in SDS sample buffer (Invitrogen, Carlsbad, CA, U.S.A.).

Two-dimensional gel electrophoresis

Two-hundred µg of *M. tuberculosis* H37Rv culture filtrate proteins were mixed with 155 µl of rehydration buffer (7 M urea, 2 M thiourea, 4% 3-[(3-Cholamidopropyl) Dimethyl-Ammonio]-1-Propanesulfonate (CHAPS), 2% dithiothreitol (DTT), 2% Immobiline pH Gradient buffer (IPG buffer) pH 4-7). Isoelectric focusing was performed at 20°C on 7 cm immobiline dry strips (Amersham Biosciences AB, Uppsala, Sweden) with pH intervals (3-10; 4-5; 4.5-5.5; 5.3-6.5) using Zoom IPG Runner system (Invitrogen). Running conditions: current ~50 µA; power 0.1 W and voltage 2000 V using the EPS 3501 XL Power Supply in

gradient mode and with check option for current turned off. Prior to the second dimension, the strips were incubated for 15 minutes in equilibration buffer (6 M urea, 2% SDS, 0.375 M Tris, pH 8.8, 20% glycerol) with 130 mM DTT first and then with 135 mM iodoacetoamide (IAA). The equilibrated strip was then placed on pre-cast SDS gels 12-14% (Invitrogen) and run as described below.

One dimensional electrophoresis

M. tuberculosis H37Rv culture filtrate protein fractions collected by mRP-C18 chromatography were mixed with 30 µl SDS loading buffer (Invitrogen) and boiled for 5 minutes at 95°C prior to separation on a 10 cm long, 1 mm thick 12% SDS-polyacrylamide (SDS-PAGE) gel (Invitrogen). Each protein samples collected by mRP-C18 fractionation, or IPG strips focused by Zoom IPG Runner system, were run in three parallels. The protein migration was allowed to proceed until the blue dye had migrated to the bottom of the gel. Proteins were visualized with SYPRO-Ruby (Bio-Rad, Hercules, CA, USA), in one of the gels, while proteins in the other two parallels were transferred to nitrocellulose membranes (Amersham Biosciences AB, Uppsala, Sweden) using electrobloting. The full-range-rainbow-RPN800 (Amersham Biosciences) was used as molecular mass standard.

Human Sera

A serum pool from sixteen patients with active tuberculosis, and a pool of ten healthy persons were used as control. The sera were a kind gift from M. E. Patarroyo, Bogota, Colombia.

Western blot

Proteins separated by SDS-PAGE were electroblotted to nitrocellulose membranes (Amersham Biosciences) and were blocked with 5% non-fat milk in PBS containing 0.5% Tween 20 (PBST) for 1 hour at room temperature (RT). The membranes were then washed with PBST for 10 min. This was repeated three times. After the last wash, the membranes were incubated overnight at 4°C with sera diluted 1:500 in PBS with 1% non-fat milk and 0.1% Tween 20. A pool composed of sera from 16 tuberculosis patient, or a pool of sera from 10 healthy persons was used. The blots were washed thoroughly with PBST as described above, and probed with Horse Reddish Peroxidase (HRP) conjugated anti-human IgG (1:2000 dilution) (Amersham Biosciences) for 1 hour at RT. Antigen-antibody

complexes were visualized by chemiluminescent reaction(Pierce, Rockford, IL, U.S.A.) using Las-3000 (Fujifilm Life Science, Stanford, U.S.A).

In-gel digestion

Sliced gel spots or bands were washed twice with 50% acetonitrile (ACN) in 25 mM ammonium bicarbonate (NH_4HCO_3) for 15 min at RT under shaking at 400 rpm. The gel pieces were dehydrated by incubating them with 50 µl 100% ACN for 2 min at RT. Proteins were then reduced using 10 mM DTT and alkylated with 55 mM IAA; both in 100 mM NH_4HCO_3. The gel pieces were dehydrated by 100% ACN as described above, and rehydrated in 25 mmol/l NH_4HCO_3 containing 0.01 µg/µl modified trypsin (Promega, Madison, U.S.A.) (ca 200 µg trypsin per protein spot). Proteins were digested by trypsin for 16-20 h at 37°C. Then, the tryptic peptides were eluted by incubating the gel pieces with 50 µl 1% trifluoroacetic acid (TFA) for 20 min at RT with shacking at 400 rpm. The supernatant containing the eluted peptides were collected by centrifugation at 15700 *g* for 10 min. Then, the gel pieces were incubated with 50 µl 0.1% TFA in 50% ACN for 20 min at RT with shaking at 400 rpm, followed by centrifugation. The supernatant collected and added to the previous one. Finally, the gel pieces were dehydrated by incubating them with 50 µl 100% ACN for 2 min at RT, and the supernatant was collected by centrifugation as described above and added to the pool. The volume of peptide pools were reduced to ca 10 µl and filtered through C8 columns (Agilent Technologies) before loading onto Q-TOF for protein identification.

Liquid chromatography-ESI-MS/MS

On-line LC-MS/MS was performed on tryptic peptides using a Dionex *Ultimate 3000* nano-HPLC system coupled to an ESI-Q-Tof (Ultima Global, Waters, Massachusetts, USA). Reprosil-Pur 3.5 µm C18 resin (Dr.Maisch Gmbh, Ammerbuch, Germany) was packed in a 15 cm long and 75 µm inner diameter fused silica capillary column and used for analytical peptide separation. Solvent A was 0.1% (vol/vol) formic acid in 2% (vol/vol) ACN, and solvent B was 0.1% (vol/vol) formic acid in 90% ACN. The flow rate through the column was 0.3 µl/min. The gradient was as follows: 5% to 10% B in 2 minutes, 10% to 30% B in 28 minutes, 30% to 50% B in 20 min, 50% to 95% B in 1 minute and ending with constant 95% B for 9 minutes before regeneration of the column. Proteolytic peptide profiles were acquired in data-dependent MS/MS mode from 0 to 65 minutes with a 1 second MS survey scan. The three most intense precursors were selected for fragmentation and then excluded for 90 seconds. CID spectrum acquisition was allowed for 2 seconds on every selected

precursor or stopped when the signal intensity fell below five counts per second. The electrospray voltage was set to 2.9 kV and fused silica capillaries with a 20 μm aperture (Waters, Milford, U.S.A.) served as spray emittors. The obtained data was searched against the publicly available *M. tuberculosis complex* database using MASCOT software. The criterium for a positive identification of proteins identified with at least 1 peptide was a minimal score of 30 for each peptide.

Documentation and image analysis

Digital images for the stained gels with SYPRO-Ruby were made using UVItec chemiluminescence and fluorescence documentation system (UVItec, Cambridge, U.K.), while the blotted membranes were documented by Laser 3000 (Fujifilm Life Science). Image Master version 5.0 (Amersham Biosciences) was used to identify spots on the gels; this software was also used for relative quantification of the spots, both on the gels stained with SYPRO-Ruby and the blot membranes.

Results

HPLC-C18 chromatography antigen analysis of *M. tuberculosis* H37Rv culture filtrate proteins

The goal of this study was to do antigen analysis of the culture filtrates of *M. tuberculosis* H37Rv mapped in our previous work in order to identify new potential candidates to be used in diagnosis of TB. To reduce the complexity of the protein mixture, proteins were fractionated according to their hydrophobicity (Fig. 1A) by using mRP-C18 chromatography with an elution gradient of ACN and TFA. Fractions of eluted proteins were collected and divided in three parallels and run on SDS-PAGE gels under the same conditions. One of the gels was stained for protein visualisation (Fig. 1B), and the other two parallels were transferred to a nitrocellulose membrane and incubated with the serum pool from TB patients or the serum pool from healthy controls (Fig. 1C&D). Protein bands in the stained gel corresponding to bands binding with serum antibodies from infected TB patients on the immunoblots, were cut out and identified by LC-MS/MS mass spectrometry. Out of 35 analysed protein bands, 20 different proteins were identified (Table 1). Only bands corresponding to one protein were considered. The control serum pool showed weak reactivity with bands corresponding to the antigen 85 complex proteins and MPT32 (Fig. 1D).

Using the mRP-C18 chromatography column we resolved the protein mixture into 14 different fractions, with 5-20 proteins in each lane. Our results show that closely related proteins such as the 85 antigen complex member proteins cluster in few fractions, due to their similar hydrophobicity, making it possible to identify antigens that might be hidden under these major proteins in 2D PAGE gels. Further, it was observed a correlation between molecular mass and elution time where proteins with low molecular mass were eluted at lower concentration of solution B. Apparently, larger proteins were more hydrophobic and therefore eluted at higher concentration of B solution (Fig. 1A&B). However, all the culture filtrate proteins were eluted between 37 to 49% of solution B, showing the soluble nature of these proteins.

Antigen analysis by 2D-PAGE

The *M. tuberculosis* H37Rv culture filtrate proteins were also analysed using 2D PAGE gel electrophoresis (Fig. 2). Three parallels of the culture filtrate proteins were separated by 2D

PAGE gels using IPG strips with narrow pH intervals. One of the parallel gels was stained for protein visualization (Fig. 2A) while the others were subjected to electroblotting and incubated with the serum pool from TB patients or the healthy control serum pool (Fig. 2B&C). Image Master software was used to identify the spots on the stained gels corresponding to reactive spots on the immunoblots. Subsequently these spots were identified by LC-MS/MS mass spectrometry. Thirty six spots were analysed, corresponding to 20 unique proteins (Fig. 2). Antigen spots selected for identifications were only accepted if they represented only one protein. The control sera showed weak reactivity with spots corresponding to the antigen 85 homologues proteins (85A, 85B, and 85C) and MPT32 (Fig. 2C).

Relative quantification of proteins and antigens in *M. tuberculosis* H37Rv culture filtrate

Using the Image Master software, the relative abundance of the *M. tuberculosis* H37Rv culture filtrate proteins were analysed (Fig. 3) in terms of the relative amount of the antigens compared to the relative amount of specific antibody present in sera recognizing that particular antigen. Antigen 85 complex proteins were the most immunogenic antigens present in the culture filtrate. However, four proteins Rv38831c, Rv0934 (pstS1), Rv0932 (pstS2) and Rv3006 (LppZ) were the most immunogenic in the sense that these proteins stained relatively stronger by western blotting as compared to staining intensity and spot size on the protein-stained-gel. For most of the other observed antigens there was a close correlation between the relative amount of protein and the relative amount of specific antibodies in the TB patient sera.

Table 1 List of *M. tuberculosis* H37Rv culture filtrate antigens identified in this study by LC-MS/MS.

Sanger ID	*Gene name*	*Protein identity*	*References*
Rv0040	*mtc28*	Secreted proline-rich protein	[17]
Rv0063	-	Possible oxidoreductase	
Rv0129c	*fbpC*	Secreted antigen 85C (Myocolyl transferase)	[14,18,19]
Rv0287	*esxG*	ESAT-6 like protein	[12]
Rv0932	*pstS2*	Periplasmic phosphate-binding lipoprotein	
Rv0934	*pstS1*	Periplasmic phosphate-binding lipoprotein	[12,14,15]
Rv1837	*glcB*	Possible malate synthase	[18,20]
Rv1860	*MPT32*	Alanine-, proline-rich secreted antigenic protein MPT32	[12-15,19]
Rv1886c	*fbpB*	Secreted antigen 85B (Myocolyl transferase)	[12-15,19]
Rv1926c	*mpt63*	Immunogenic protein MPT63	[12,13]
Rv1980	*mpt64*	Immunogenic protein MPT64	[12-15,19]
Rv2878c	*mpt53*	Soluble secreted antigen MPT53	[12-15,19]
Rv2911	*dacB2*	Possible alanyl-D-analine carboxypeptidase	
Rv3006	*lppZ*	Probable conserved lipoprotein	[21]
Rv3418c	*groES*	10 kDa Chaperonin	[12,13]
Rv3587c	-	Possible conserved membrane protein	
Rv3803c	*fbpD*	Secreted antigen MPT51	[12-15,19]
Rv3804c	*fbpA*	Secreted antigen 85A (Myocolyl transferase)	[12-15,19]
Rv3874	*esxB*	10 kDa culture filtrate antigen (CFP10)	[12,13,18]
Rv3881c	-	Conserved hypothetical alanine and glycine rich protein	[19]

Discussions

Due to the immunogenecity of secreted proteins, we analyzed the *M. tuberculosis* culture filtrate which is substantially enriched with secreted proteins with the aim of identifying novel antigens to be evaluated for future development of diagnostic methods. We used two different strategies to separate the proteins based on different physicochemical properties, to ensure that highly purified single proteins were identified as the target for antibody binding. In total, we managed to identify 20 antigens in the culture filtrate, including 4 novel antigens (Rv0063, Rv3587c, Rv2911 and Rv0932). All of the novel antigens have a predicted signal peptide, the N-terminal peptide of two of them have been identified in our previous study [10], confirming the existence of a cleavable signal peptide in these proteins.

Out of the 20 identified antigens identified in this study, three of them are predicted to be lipoproteins, including Rv0934 (38-kDa antigen), which is known to be a surface exposed phosphate transport protein. This antigen is extensively used for development of assays for the detection of TB [22] and is also present in several commercially available assays for serodiagnosis of TB. Another lipoprotein observed in this study (LppZ, Rv3006) was recently reported to be sero-reactive in both cavitary and non-cavitary TB patients [23]. A third lipoprotein that is closely related to the 38-kDa antigen, both involved in phosphate transport [24,25], pstS2 (Rv0932) was also identified as a good serodiagnostic antigen in this study.

Four of the identified antigens, Rv0063, Rv0040 (MPT28), Rv3587c and Rv3881c were detected in more than one protein spot, with different molecular mass and isoelectric points. This is probably due to proteolytic digestion or post-translational modifications (PTMs) of these proteins such as amidation, glycosylation, phosphorylation or other modifications. However, all the isoforms of these proteins were serologically reactive, suggesting that the epitopes recognized by serum antibodies are on the peptide backbone of the protein and not on probable modification sites.

Another interesting observation was with the Rv1860 protein. Apparently, only certain isoforms of the protein were strongly immunogenic, while the others showed little or no sign to reaction with the serum pool of TB patients. This is probably due to certain post-translational modifications (PTMs) or conformational changes of these isoforms providing

epitopes that have been recognized by the immune system of the host. Further investigation of these isoforms certainly will provide better insight to this phenomenon.

Visible bands and spots on immunoblots incubated with sera from healthy controls were also observed for Rv3881c and the antigen 85 complex proteins, which are probably due to previous exposure to mycobacteria or BCG vaccinations. However, the stronger reactivity of the TB sera with these antigens suggests that the mycobacterial proteins posses sero-dominant epitopes.

To evaluate the immunogenicity of the identified antigens, we performed a relative quantification of the antigen spots displayed on the SYPRO-Ruby stained 2D-PAGE gels, and their corresponding spots on immunoblots probed with antibodies from TB serum pool. We found that the major antigens in the *M. tuberculosis* culture filtrate are the antigen 85 homologues 85A, 85B and 85C proteins [26], with relative large amount of proteins and similarly relative large amount of antibodies in the TB patient sera. However, Rv3881c and three other antigens predicted to be lipoproteins (Rv0934 (pstS1), Rv0932 (pstS2) and Rv3006 (LppZ)) were the most immunogenic taken to consideration the low amount of proteins present in the culture filtrate, yet a significant relative amount of specific antibodies in the TB patient sera against them. This illustrates the significance of lipoproteins as potential diagnostic markers, and they should certainly be investigated further.

The identified *M. tuberculosis* proteins represent potential antigens for improved antigen-antibody-based diagnostic tests. Because of ubiquity of mycobacteria in the environment along with the conservation of their proteins, it may be necessary to use only partial proteins or peptides to obtain optimal sensitivity and specificity. Over-expression and purification of the identified proteins will help to evaluate their potential as diagnostic markers. Furthermore, the culture filtrate used in this study should be further investigated in terms of using sera from patients at different disease stages, since the different disease stages can change the profile of culture filtrate proteins recognized by serum antibodies from TB patients [27,28].

Acknowledgment

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Figure legends

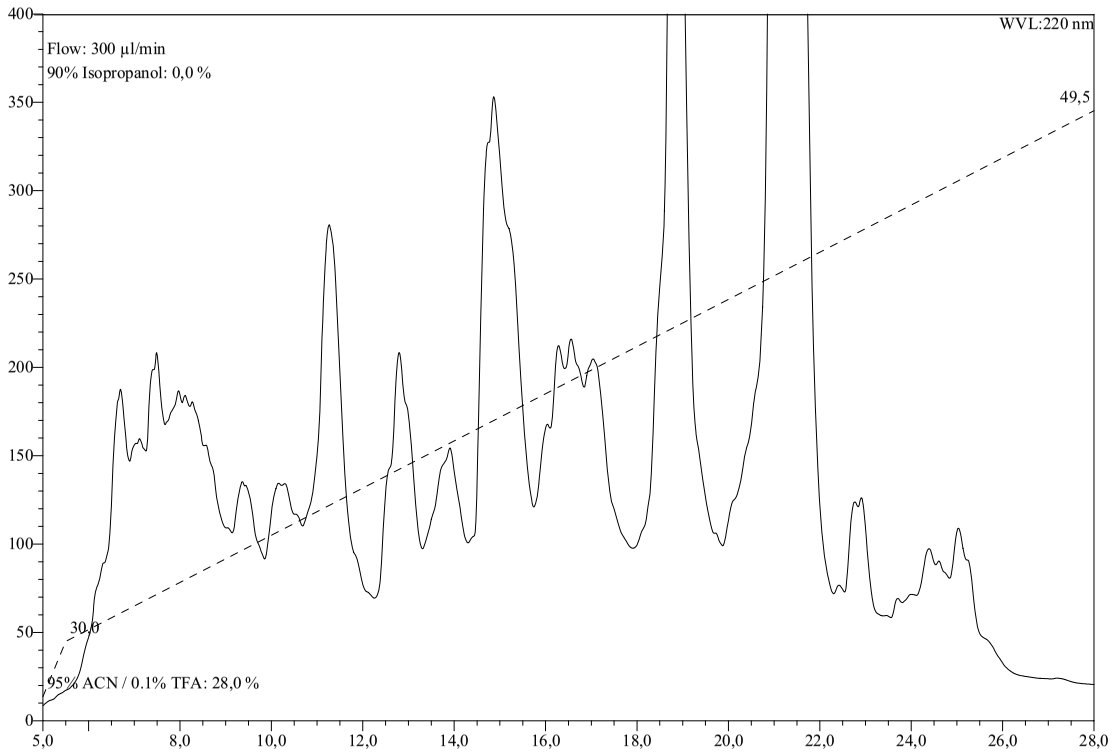
Figure 1. *M. tuberculosis* H37Rv culture filtrate protein fractionation by HPLC-chromatography. A) Protein separation chromatogram using mRP-C18 column, the gradient is indicated by pointed line, B) Proteins separated by SDS PAGE and stained with SYPRO-Ruby, C) Proteins separated by SDS PAGE and transferred to nitrocellulose membrane and probed with sera from *M. tuberculosis* infected patients, and D) Proteins separated by SDS PAGE and transferred to nitrocellulose membrane and probed with sera from healthy controls. The fraction numbers are indicated at the top and the molecular mass standard is indicated at the left.

Figure 2. *M. tuberculosis* H37Rv culture filtrate protein separated by 2D PAGE. A) Proteins separated by 2D PAGE and stained with SYPRO-Ruby, B) Proteins separated by 2D PAGE and transferred to nitrocellulose membrane and probed with sera from *M. tuberculosis* infected patients, and C) Proteins separated by SDS PAGE and transferred to nitrocellulose membrane and probed with sera from healthy controls. Circled spots mark the identified antigens. Molecular mass standard is indicated at the left.

Figure 3. Relative quantification of the *M. tuberculosis* H37Rv culture filtrate antigens using Image Master software. The black bars represent the relative volume percentage of antigen spots on the immunoblots, while the red ones represent the relative volume percentage of antigen spots on SYPRO-Ruby stained gels.

Figure 1

A)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WVL:220 nm
49,5
30,0
95% ACN / 0.1% TFA: 28,0 %
400
350
300
250
200
150
100
50
0
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12,0
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16,0
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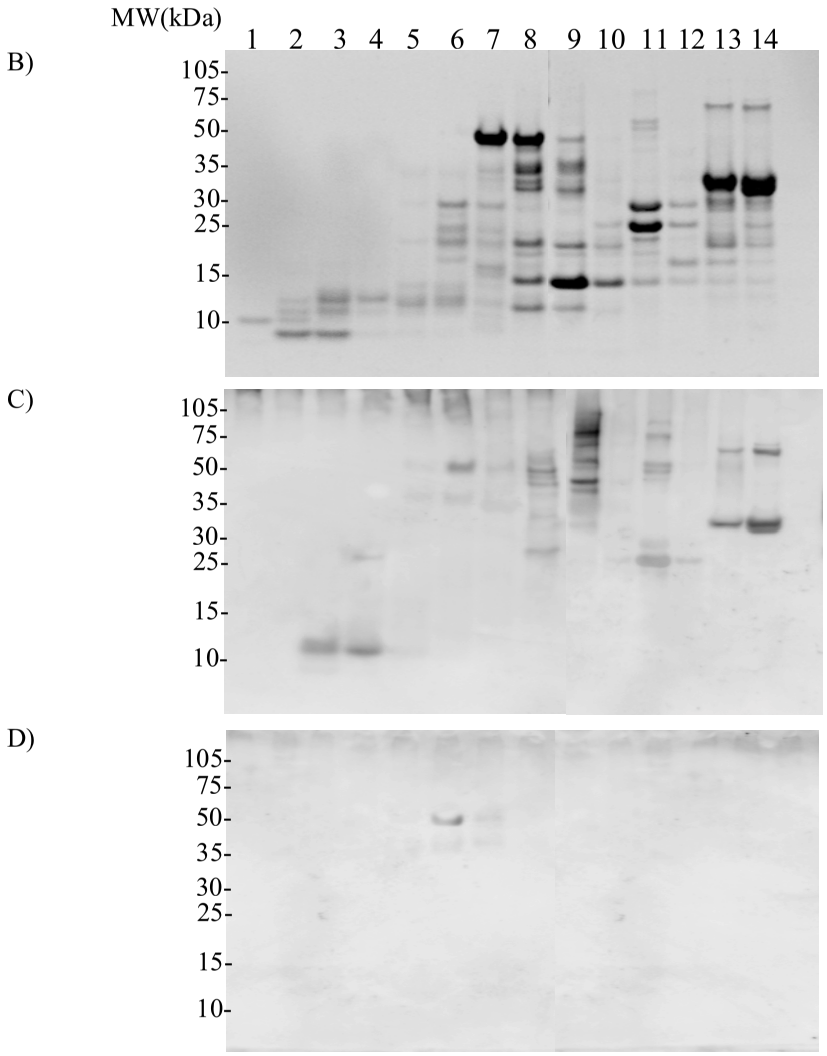
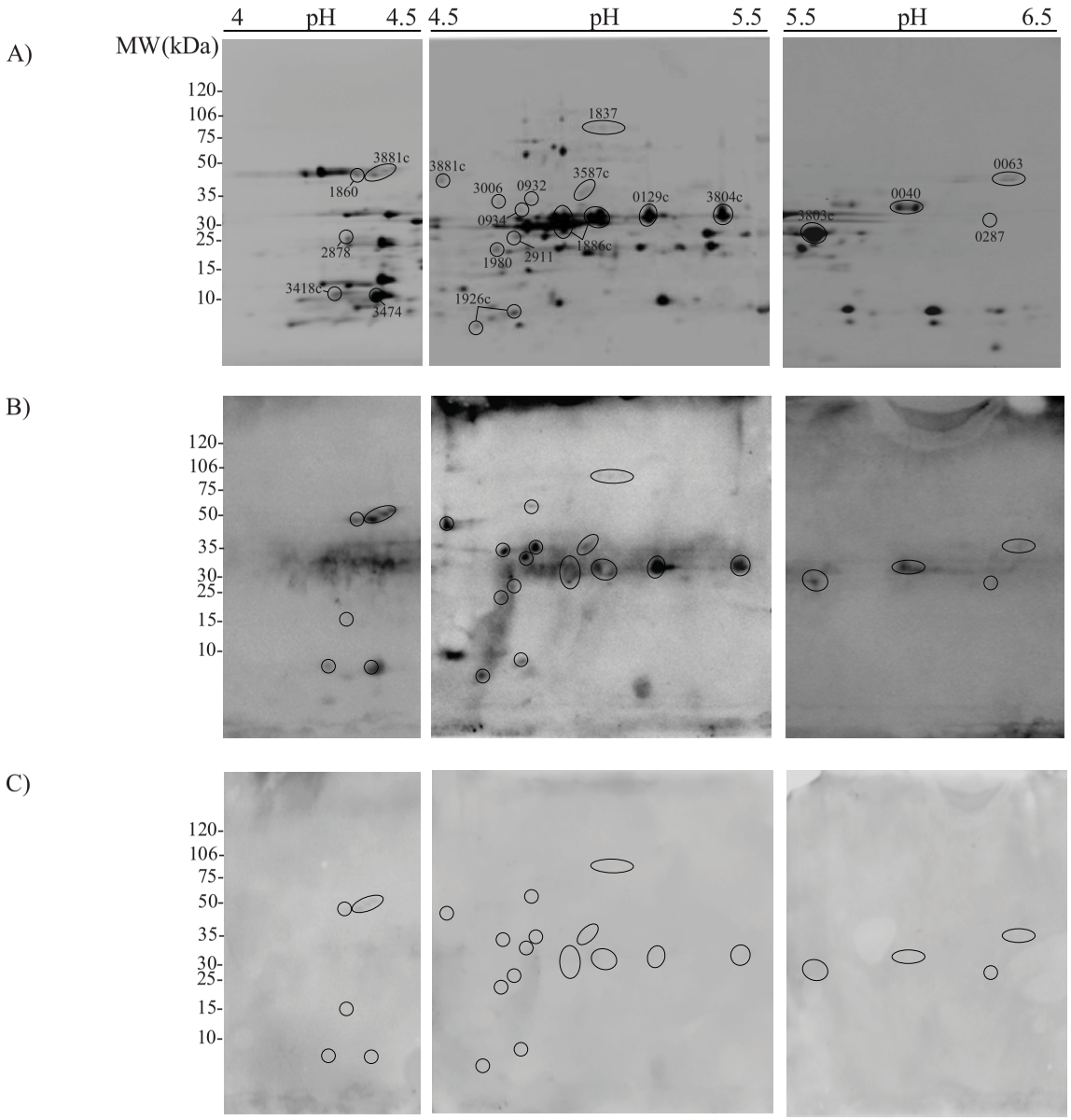

MW(kDa)
1 2 3 4 5 6 7 8 9 10 11 12 13 14
B)
105-
75-
50-
35-
30-
25-
15-
10-
C)
105-
75-
50-
35-
30-
25-
15-
10-
D)
105-
75-
50-
35-
30-
25-
15-
10-

Figure 2


A)
B)
C)
4
pH
4.5
4.5
pH
5.5
5.5
pH
6.5
MW(kDa)
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75-
50-
35-
30-
25-
15-
10-
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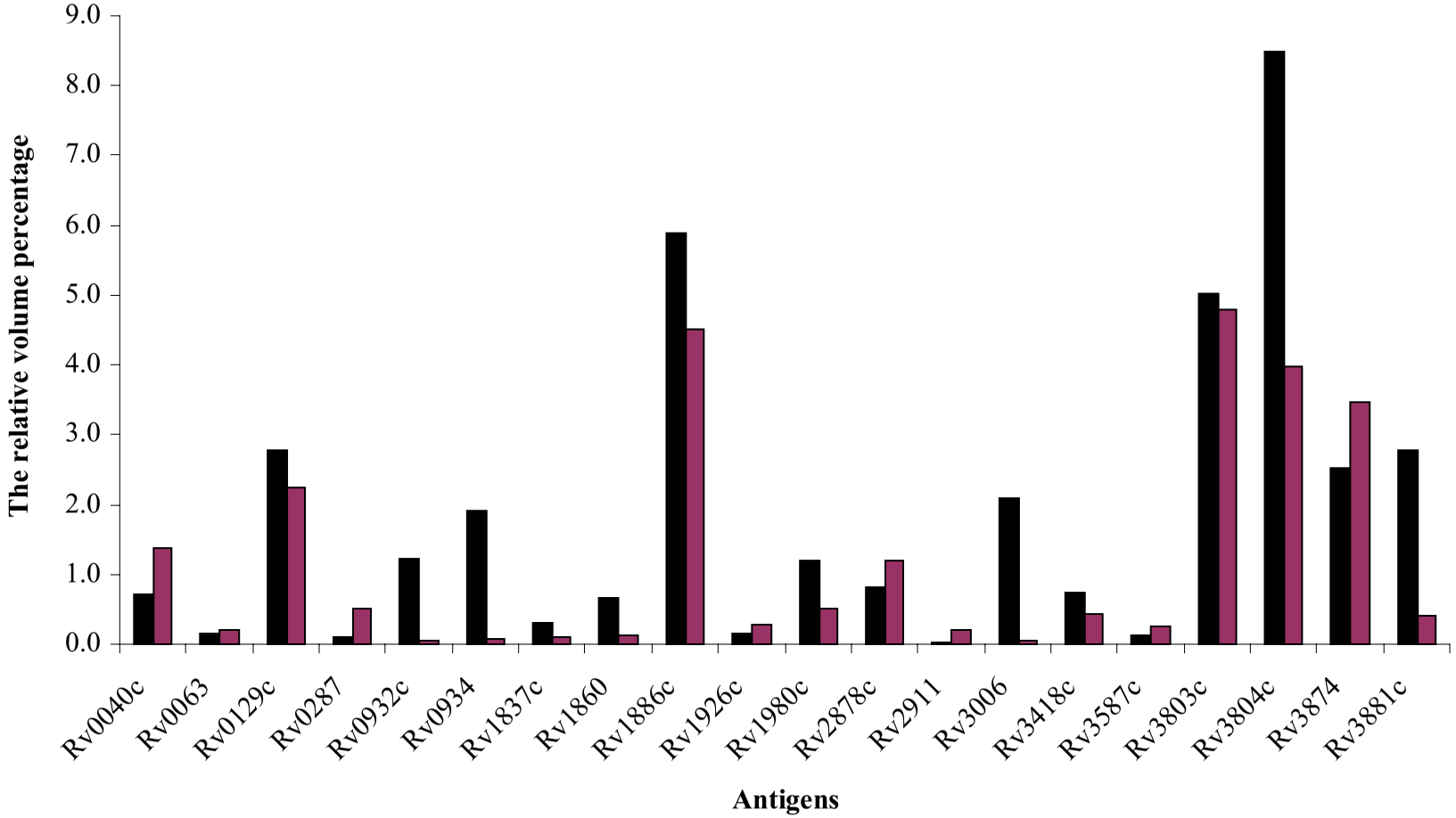
3803c
0287

Figure 3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8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
The relative volume percentage
Rv0040c
Rv0063
Rv0129c
Rv0287
Rv0932c
Rv0934
Rv1837c
Rv1860
Rv1886c
Rv1926c
Rv1980c
Rv2878c
Rv2911
Rv3006
Rv3418c
Rv3587c
Rv3803c
Rv3804c
Rv3874
Rv3881c
Antigens

Paper III
Supplementary table I

Supplementary table 1. A detailed list of *M. tuberculosis antigens* identified in this study by liquid chromatography coupled to MS/MS.

Sanger ID	Theoretical MW	Theoretical pI	Peptide sequence	No. of peptide matches	Score	Threshold	Charge
Rv0040c	28.9	8.68	RHVIATSGADK	8	66	20	2
			LEAQRPHGFK		56	20	2
			ALDITLPMPPR		28	20	2
			WTQVPDPNVPDAFVVIADR		20	19	2
			LIGDFDPAEAITHGYIDSQK		47	16	3
			HVIATSGADK		29	20	2
			LGNSVYTSNAQLVVYR		111	20	2
			ENDMTLNTSR		27	17	2
Rv0063	46.21	6.81	YFGPNLSR	2	50	19	2
			AMAFAAANNLK		60	20	2
Rv0129c	32.02	4.99	FLEGLTLR	2	46	19	2
			LVANNTR		38	22	2
Rv0287	9.7	5.99	FVAAAAK	1	36	19	2
Rv0932c	36	4.76	YPDATTGTAVR	2	51	20	2
			GVSTLNLDGPTTAK		23	20	2
Rv0934	36	4.82	VLAAMYQGTIK	2	39	19	2
			SDGSGDTFLFTQYLSK		42	19	2
Rv1837c	80.4	5.3	IGIMDEER	15	36	18	2
			RTTVNLK		38	21	2
			VPDIHDVALMEDR		31	21	2
			MAPLVDR		42	21	2
			DELQAQIDK		31	21	2
			ASDVNGPLINSR		76	20	2
			TGSIYIVKPK		29	21	3
			FALNAANAR		56	21	2

			VIEPIDMDAYR		39	20	2
			VEDVLGLPQNTMK		22	20	2
			VVADLTPQNQALLNAR		83	19	2
			NYTAPGGGQFTLPGR		75	20	2
			GDLAAAVDKDGTAFLR		51	20	2
			VVFINTGFLDR		60	21	2
			ATIEQLLTIPLAK		69	18	2
Rv1860	28.78	4.52	LYASAEATDSK	3	56	20	2
			TTGDPPFPGQPPPVANDTR		44	18	2
			LGSDMGEFYMPYPGTR		32	14	2
Rv1886c	30.66	4.87	VQFQSGGNNSPAVYLLDGLR	2	64	20	3
			NDPTQQIPK		41	19	2
Rv1926c	13.6	4.5	LGSELTMTDTVGQVVLGWK	5	32	20	3
			SSTAVIPGYPVAGQVWEATATVNAIR		35	18	3
			GSVTPAVSQFNAR		41	20	2
			VLWQAAGPDTISGATIPQGEQSTGK		40	18	3
			TADGINYR		52	19	2
Rv1980c	22.43	4.6	VYQNAGGTHPTTTYK	6	55	14	3
			GTQAVVLK		25	22	2
			FLSAATSSTPR		56	20	2
			AFDWDQAYR		39	20	2
			DKFLSAATSSTPR		38	19	2
			SLENYIAQTR		61	19	2
Rv2878c	14.61	4.75	YNLNFTNLNDADGVIWAR	3	90	19	2
			ADVGAMQSFVSK		36	20	2
			YNVPWQPAFVFYR		40	20	2
Rv2911	28.38	4.75	DQNVAHPPASTIK	4	59	20	2
			YPGAIGGK		52	20	2
			TFVGAAAR		40	19	2
			LVIAMMYGLVK		48	21	2

Rv3006	36	4.71	LAPSTGAVTGEPDVVR	2	60	23	2
			TALVAERITGAVEEISISAEPK		45	19	3
Rv3418c	10.8	4.34	EKPQEGTVVAVGPGR	4	51	19	2
			DVLAVVSK		36	22	2
			VNIKPLEDK		39	21	2
			IPLDVAEGDTVIYSK		50	20	2
Rv3587c	22.18	5.79	GLTNAPQYYVGDQPK	1	30	19	2
Rv3803c	28.47	5.52	GISVVAPAGGAYSMYTNWEQDGSK	3	43	23	2
			QWDTFLSAELPDWLAANR		58	21	2
			APYENLMVPSPSMGR		34	13	2
Rv3804c	32.33	5.32	ALGATPNTGPAPQGA	3	24	19	2
			LIANNTR		29	22	2
			FLEGFVR		35	18	2
			NDPLLNVGK		27	20	2
Rv3874	10.79	4.59	TQIDQVESTAGSLQGQWR	8	39	17	2
			QAGVQYSR		32	19	2
			QKQELDEISTNIR		60	19	2
			ADEEQQQALSSQMGF		49	14	2
			GAAGTAAQAAVVR		26	21	2
			AEMKTDAATLAQEAGNFER		24	17	3
			QELDEISTNIR		62	20	2
			TDAATLAQEAGNFER		88	19	2
Rv3881c	47.59	4.75	EYLAAGAK	13	46	19	2
			EHPTYEDIVGLER		44	20	2
			DQILPVYAEYQQR		49	19	2
			GSQQEDEALYTEDR		105	19	2
			LATSLR		33	22	2
			REHPTYEDIVGLER		69	21	3
			EYLAAGAKER		33	19	2
			TQSQTVTVDQQEILNR		111	19	2

	LYAENPSAR	64	20	2
	VLTEYNNK	54	18	2
	VATAGEPNFMDLK	42	20	2
	NAAQQLVLSADNMR	39	21	2
	EAAALSGDVAVK	32	19	2