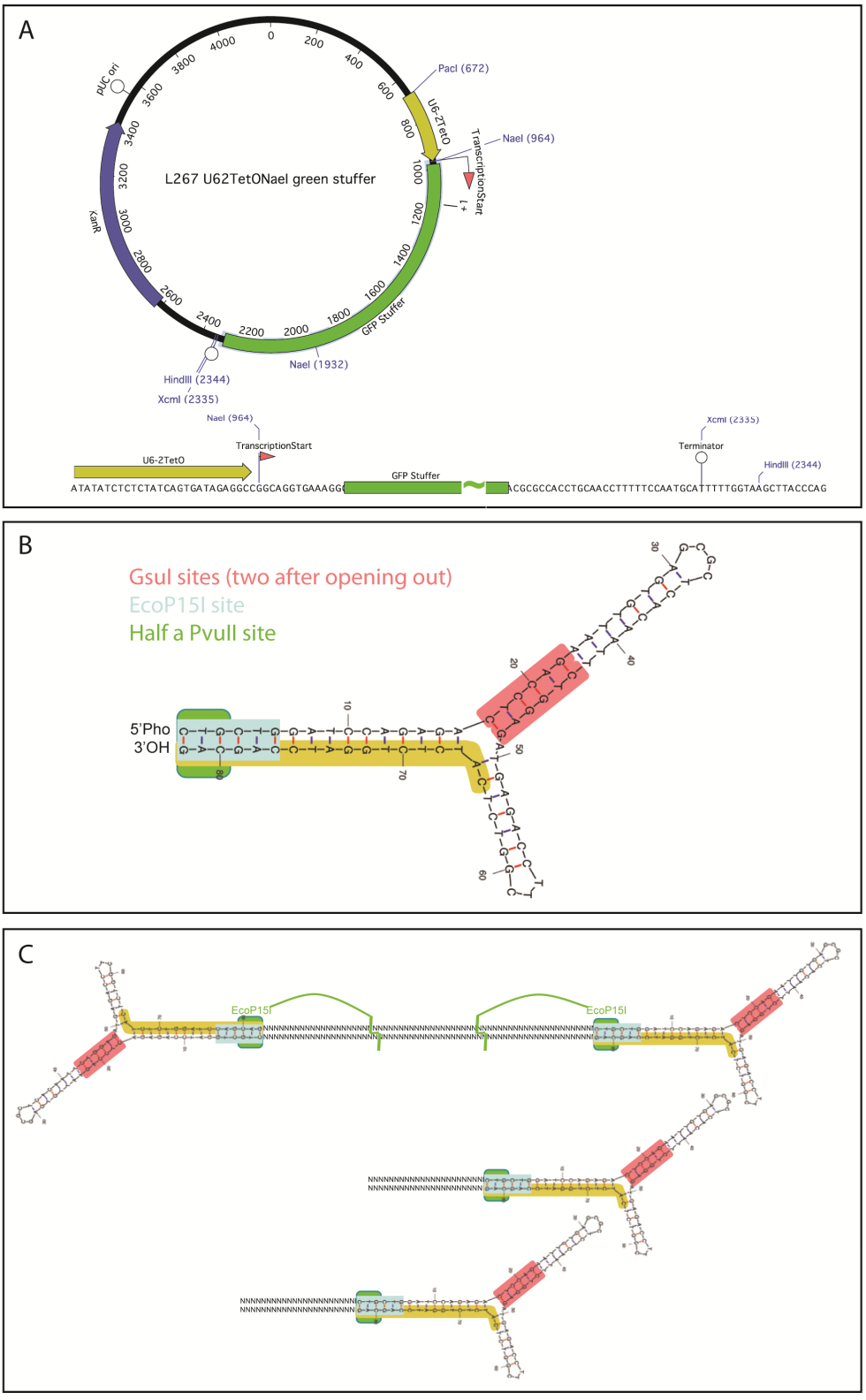
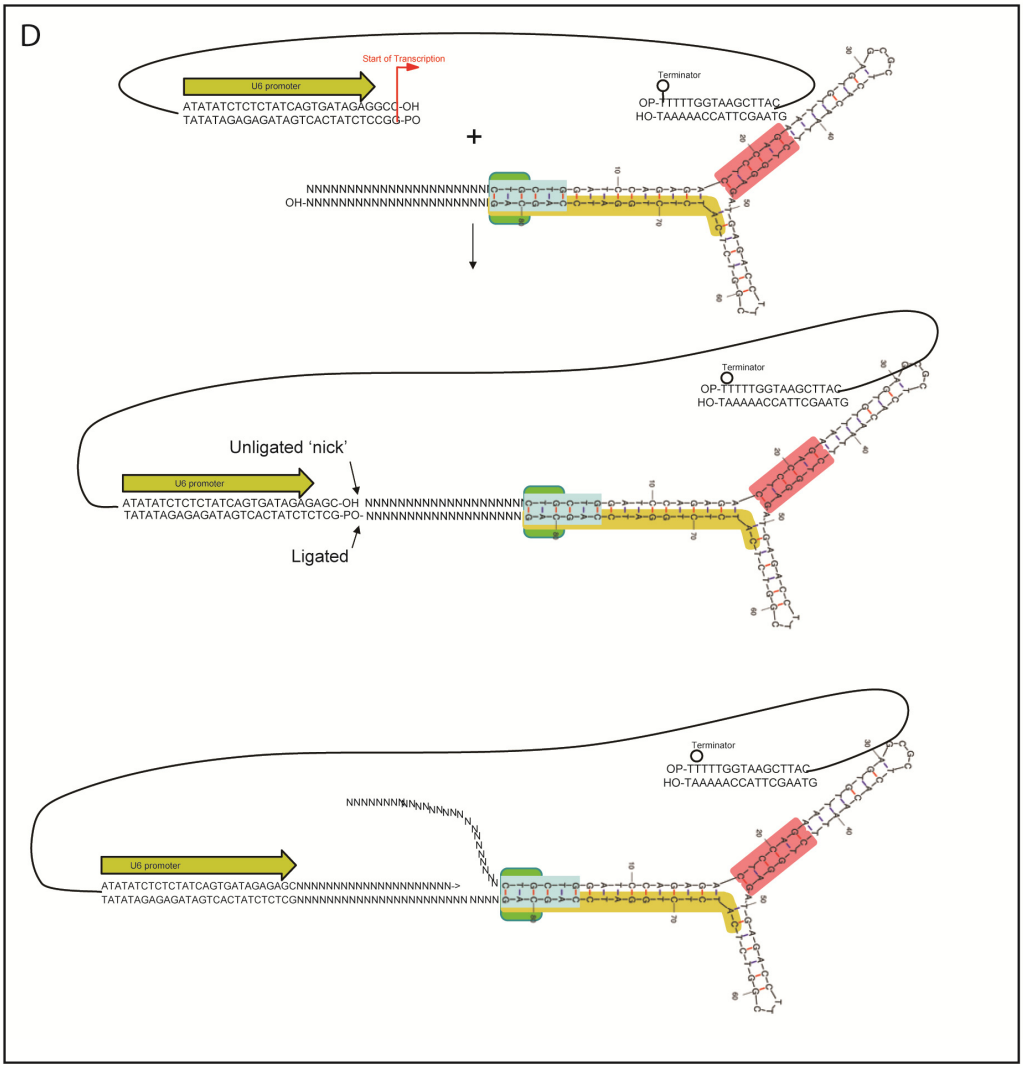
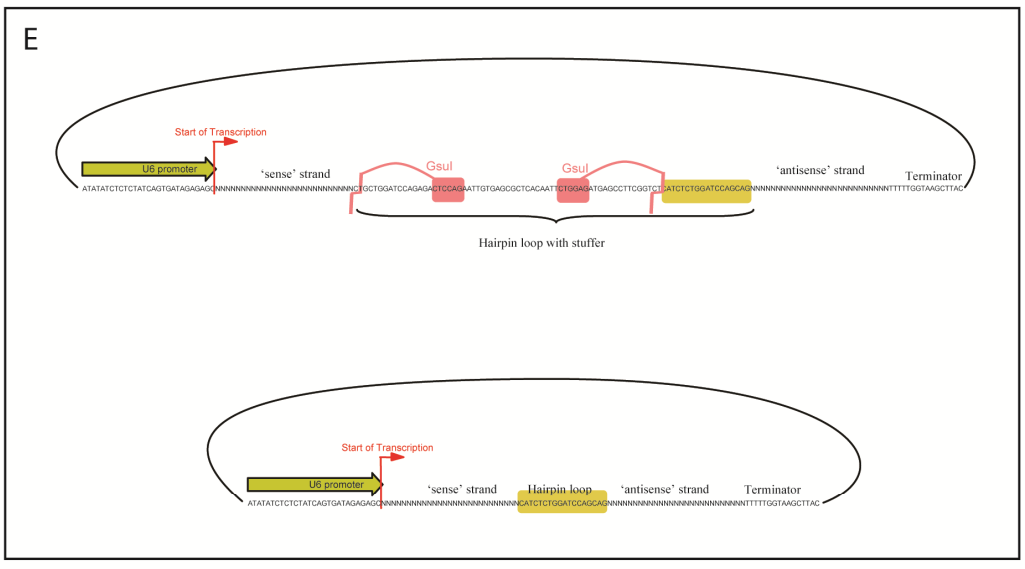
Supplementary figure 2




D
Start of Transcription
U6 promoter
ATATATCTCTCTATCAGTGATAGAGGCC-OH
TATATAGAGAGATAGTCACTATCTCCGG-PO
Terminator
OP-TTTTTGGTAAGCTTAC
HO-TAAAAACCATTCGAATG
NNNNNNNNNNNNNNNNNNNNNNNN
OH-NNNNNNNNNNNNNNNNNNNNNNNN
Unligated 'nick'
ATATATCTCTCTATCAGTGATAGAGAGC-OH
TATATAGAGAGATAGTCACTATCTCTCG-PO-
Ligated
ATATATCTCTCTATCAGTGATAGAGAGCNNNNNNNNNNNNNNNNNNNNNNNN->
TATATAGAGAGATAGTCACTATCTCTCGNNNNNNNNNNNNNNNNNNNNNNNN


E
Start of Transcription
U6 promoter
'sense' strand
GsuI
GsuI
'antisense' strand
Terminator
Hairpin loop with stuffer
Start of Transcription
U6 promoter
'sense' strand
Hairpin loop
'antisense' strand
Terminator

(A) L267 cloning vector with details of polylinker. Hairpins are expressed under the control of a modified U6 promoter, and cloned between the NaeI at the transcription start and the XcmI at the transcription terminator. PacI/HindIII restriction enzyme sites to facilitate cloning of the completed cassette into the final vector. (B) Structure of Hairpin Oligo DK540, including EcoP15I site (blue), GsuI sites (red) and a half-PvuII site (green). The loop sequence present in the completed hairpin cassette is in yellow.
(C) Hairpin Oligo DK540 ligated to each end of a short fragment of p53. Digestion with EcoP13I releases two hairpin-tagged sequences which are then blunt-ended with Klenow fragment and dephosphorylated. (D) NaeI/XcmI-digested vector is ligated to haripin-tagged sequences. Because the haripin tag lacks a 5' phosphate, the ligation product will have a 'nick' in the DNA phosphate backbone as indicated. DNA polymerisation from this nick using a strand-displacing polymerase opens out the hairpin into dsDNA. (E) Subsequent digestion with GsuI and recircularisation shortens the hairpin loop.