Supplementary figure 2

A

B

G sul sites (two after opening out)
EcoP15I site
Half a PvuII site

C

G sul
EcoP15I
(A) L267 cloning vector with details of polylinker. Hairpins are expressed under the control
of a modified U6 promoter, and cloned between the Nael at the transcription start and the
XcmI at the transcription terminator. Pacl/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) Nael/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.