CLONING SMALL HAIRPINS INTO LENTIVIRAL VECTORS

Annealing Step: in a single tube, mix the following:

23 µl ddH_2O
1 µl sense oligo (100µM)
1 µl antisense oligo (100µM)
25 µl 2X Annealing Buffer (recipe see right)
50 µl total reaction volume

2X Annealing Buffer:
200 mM potassium acetate
60 mM HEPES-KOH pH 7.4
4 mM Mg-acetate

Incubate the reaction for 5 minutes at 95°C and let the oligos gradually anneal to RT. These can then be placed on ice (or stored at –20°C) until used in ligation step. *This is the annealed oligo stock; prior to use, it will be necessary to prepare a 1:20 dilution.*

Ligation Step: in a single tube, mix in the following order

x µl ddH_2O
50-100 ng digested, gel purified vector
1 µl of 1:20 diluted annealed oligos
1 µl 10X T4 DNA ligase buffer
1 µl T4 DNA ligase
10 µl total reaction volume

Incubate the above reaction at room temperature for 1-4 hours and transform 2.5µl of the ligation reaction into DH5α cells.