CLONING SMALL HAIRPINS INTO LENTIVIRAL VECTORS

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

- 23 µl ddH₂O
- 1 µl sense oligo (100µM)
- 1 µl antisense oligo (100µM)
- 25 µl 2X Annealing Buffer (recipe see right)
- 50 µl total reaction volume

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 ddH₂O
- 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.