TRiP

Transgenic RNAi Project at Harvard Medical School

Cloning hairpins into VALIUM20 and VALIUM22

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1. Oligo design:

Select a 21nucleotide sequence based on the algorithm of Vert *et al*. (2006). This oligo design eliminates off target effects starting at 16 nucleotides.

Based on a miR1 scaffold, for the top strand oligo, add ctagcagt to the 5' end of the passenger strand DNA, add tagttatattcaagcata between the passenger strand DNA and the guide strand DNA, add gcg to the 3' end of guide strand DNA, so the resulting oligo will be:

ctagcagt**NNNNNNNNNNNNNNNNNNNN**tagttatattcaagcata**NNNNNNNNNNNNNNNNNNNN**gcg

For the bottom strand oligo, add aattcgc to the 5' end of the passenger strand DNA, add tatgcttgaatataacta between the passenger strand DNA and the guide strand DNA, add actg to the 3' end of the guide strand DNA, so the resulting oligo will be:

aattcgc**NNNNNNNNNNNNNNNNNNNN**tatgcttgaatataacta**NNNNNNNNNNNNNNNNNNNN**actg

2. Annealing the top and bottom strand oligos:

Add10ul top strand oligo (10-20uM) and 10ul bottom strand oligo (10-20uM) into 80ul annealing buffer (10mM Tris-HCl, pH 7.5, 0.1M NaCl, 1mM EDTA).

Mix, incubate at 95°C for 5 min, then slowly cool down to room temperature.

The resulting DNA fragment has overhangs for *NheI* and *EcoRI*.

3. Ligation:

Directly clone this DNA fragment into a VALIUM20 or VALIUM22 vector, which has been linearized by *NheI* and *EcoRI.*

6ul annealing product

2ul 10X ligation buffer

1ul T4 DNA ligase (1U/ul)

1ul 40ng/ul backbone (gel purified VALIUM20 or VALIUM22 cut with *NheI* and *EcoRI*)

Add ddH2O to 20ul total volume.

Mix, incubate at 16°C for 1 hour.

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4. **Transformation:**

   Add 10ul ligation product into 50ul TOP10 competent cells, following standard transformation protocol.

5. **Colony selection:**

   PCR select the correct clone by appearance of a 350bp PCR product using the following PCR primers:

   pVALIUM20: F: 5'-ACCAGCAACCAAGTAAATCAAC-3'
   R: 5'-TAATCGTGTGTGATGCCTACC-3'

   pVALIUM22: F: 5'-GGTGATAGAGCCTGAACCAG-3'
   R: 5'-TAATCGTGTGTGATGCCTACC-3'

6. **Sequencing:**

   Confirm the correct shRNA construct using the following sequencing primers:

   pVALIUM20: 5'-ACCAGCAACCAAGTAAATCAAC-3'

   pVALIUM22: 5'-GGTGATAGAGCCTGAACCAG-3'

7. **DNA miniprep and injection.**

Good Luck!

The TRiP Team at HMS

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