TRiP

Transgenic RNAi Project at Harvard Medical School

Cloning hairpins into VALIUM10 (or WALIUM10*)

Jian-Quan Ni, Matt Booker, Norbert Perrimon

1. Oligos design

The TRiP oligos were designed using SnapDragon (http://www.flyrnai.org/cgi-bin/RNAi_find_primers.pl) with the following criteria:

- Product size was set to 400 to 600 bp
- 19 bp predicted off-targets were avoided
- Only splice-form universal portions of each gene were selected for oligo design

2. PCR

Forward primer 20uM 0.5ul

Reverse primer 20uM 0.5ul

DNA Template (make sure to use genomic DNA or cDNA) 1ul

Taq polymerase mix (Promega, Cat No. M7122) 12.5ul

Add ddH_2O to .....25ul total volume

Once the PCR finishes (30 Cycles), load 10ul onto an agarose gel to verify the quality and quantity of the PCR product. If multiple bands are seen, cut out the relevant band and purify it.

3. TOPO Ligation

PCR product (there's no need for purification) 1ul

pENTR/D-TOPO entry vector (Invitrogen, Cat.No. K240020).....1ul

Salt Solution (1.2M NaCl, 0.06M $MgCl_2$) 1ul

Add ddH_2O to ..... 6ul total volume

Mix, incubate at room temperature for 10 min

The Transgenic RNAi Project at Harvard Medical School
NRB 356 Bay 7, 77 Avenue Louis Pasteur
Boston, MA 02115
Email: TRiP@flyrnai.org, Fax: 617-432-6238

4. Transformation

Transfer all ligation mix to 50ul TOP10 cells, place on ice for 30 min, heat shock, add SOC medium and incubate at 37°C for 50 min, then plate (Kan resistant).

5. Select clone, culture in LB medium (Kan resistant) and miniprep.

6. Sequence pENTR/D-TOPO entry vector to ensure the insertion and its orientation is correct; i.e., 3' to 5'.

Oligo used for sequencing, M13-F: 5'-TTGTAAAACGACGGCCAGTC-3'

7. Recombination

45-85ng/ul pENTR/D-TOPO entry vector (from step 5)..... 0.3ul

200ng/ul VALIUM10 (or WALIUM10)..... 0.3ul

LR clonase (Invitrogen, Cat No. 11791-100) 0.8ul

Add ddH_2O (or TE buffer, pH8.0) ..... 4ul total volume

Mix, incubate at 25°C for 1 hr, and then add 1ul proteinase K (From LR clonase kit), further incubate at 37°C for 10 min to remove the recombinase.

8. Transformation

Recombination product 5ul

TOP10 competent cells 50ul

Mix, incubate on ice 30 min, 42°C heat shock, add SOC medium, incubate at 37°C for 30 min, then plate (Amp resistant)

9. Select clone, culture in 5ml LB medium (Amp resistant) and miniprep

At end elute with 100ul ddH_2O.

10. Sequence hairpin construct to confirm the *ftz* inton is in the correct orientation (in our experience: 40-60% of the clones are correct).

Primer used for DNA sequencing, Hsp70F: 5'-CGCAGCTGAACAAGCTAAAC-3'

The Transgenic RNAi Project at Harvard Medical School
NRB 356 Bay 7, 77 Avenue Louis Pasteur
Boston, MA 02115
Email: TRiP@flyrnai.org, Fax: 617-432-6238

11. Precipitation (DNA from step 9)

DNA…….. 90ul

3M NaAc, pH5.2…….. 10ul

EtOH……. 500ul

Mix, -20°C for 1 hr

12. Centrifuge (15,000 rpm, 20 min), wash once with 70% EtOH, and dissolve in 40ul ddH_2O

13. Construct is ready for injection

∗ The only difference between VALIUM10 and WALIUM10 is their selectable eye color marker; VALIUM10 uses Vermilion, WALIUM10 uses White.

Questions? Contact Jian-Quan Ni at jni@genetics.med.harvard.edu.

Good Luck!

The TRiP Team at HMS

The Transgenic RNAi Project at Harvard Medical School
NRB 356 Bay 7, 77 Avenue Louis Pasteur
Boston, MA 02115
Email: TRiP@flyrnai.org, Fax: 617-432-6238