TRiP

Transgenic RNAi Project at Harvard Medical School

Cloning a cDNA into pVALIUM10-moe with a MCS

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1. Oligos design

- At the 5'end add a restriction enzyme site, followed by a Kozak DNA sequence (ANNATGGNN).
- At the 3' end, add a stop codon followed by a restriction enzyme site.

2. PCR

- Make sure to use genomic DNA or cDNA as the PCR template
- Total reaction volume should be 40ul

3. PCR purification,

- See Qiagen manual for details (Qiagen, Cat No. 28104):
- Elute with 40ul ddH_2O.

4. Enzyme digestion

Purification product …….. 40ul
Buffer X ……. 4.8ul
Enzyme A and/or Enzyme B …….. 2ul each enzyme
Mix, incubate at 37°C for 2 hours

5. Purification

- Same as step 3 (above)

6. Ligation

Purification product …….. 6ul
10X ligation buffer ……. 2ul
ddH_2O ……. 9ul
Backbone (linearized by enzyme digestion) …… 2ul
T4 DNA ligase (Roche, Cat No. 13580621) ……. 1ul
Mix, incubate at 16°C for 1 hour

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7. Transformation

Ligation mix …… 10ul
Top10 competent cells …… 50ul
Mix, incubate on ice 30min followed by a 42°C heat shock

Add SOC medium …… 150ul
Mix, incubate at 37°C for 30min, then plate (Amp resistant)

8. Select correct clone by PCR

9. Miniprep

- See Qiagen manual for details (Qiagen, Cat No. 27104)
- DNA sequence to confirm the correct DNA sequence

14. Prepare DNA for injection

- Ethanol precipitate
- Dissolve in injection buffer (100nM NaPO4, pH 7.2, 5mM KCl)

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

Good Luck!

The TRiP Team at HMS

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