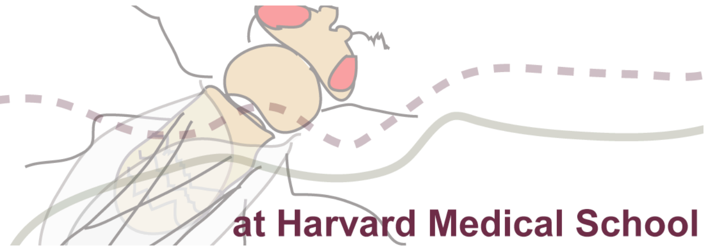
TRiP

Transgenic RNAi Project at Harvard Medical School



Clone DNA fragment into mENTRY

Jian-Quan Ni and Norbert Perrimon

1. Oligos design

- add restriction enzyme sites: at the 5' end add either *EcoRI* or *MfeI* and at the 3' end add either *XbaI* or *SpeI*

2. PCR

- Make sure to use genomic DNA or cDNA as the PCR template
- Total reaction volume should be 40ul
- Load 5ul onto an agarose gel to verify the quality and quantity of your PCR product. If multiple bands are seen, we cut out the relevant band and purify it.

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
Restriction enzymes (*EcoRI* or *MfeI* and *XbaI* or *SpeI*) … 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 *SpeI* and *EcoRI* digestion) .. 2ul
T4 DNA ligase (Roche, Cat No. 13580621) …….….... 1ul
Mix, incubate at 16°C for 1 hour

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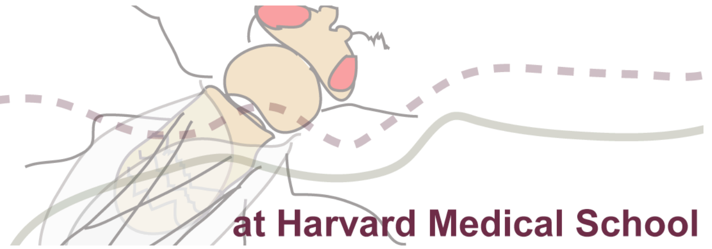

7. Transformation

Ligation mix 10ul
Top10 competent cells 50ul
Mix, place on ice for 30 min, heat shock, add SOC medium and incubate at 37°C for 50 min, then plate (Kan resistant).

8. Select clone by PCR

- Forward primer: 5'- CAAAAAAGCAGGCTCCGCGG -3'
- Reverse primer: 5'- GTACAAGAAAGCTGGGTCGG -3'

9. Miniprep

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

10. Recombination with destination vector

- See TRiP protocol for Cloning into VALIUM10-roe by recombination

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

Good Luck!

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