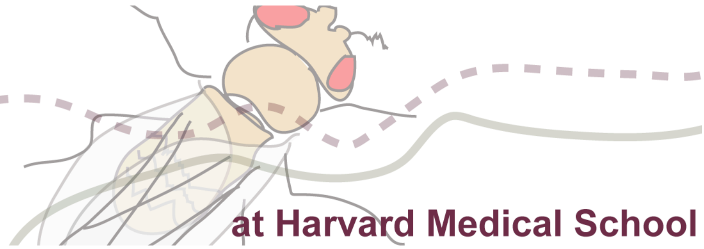
TRiP

Transgenic RNAi Project



at Harvard Medical School

# Cloning cDNAs into pVALIUM10-roe via Recombination

**Jian-Quan Ni and Norbert Perrimon**

## 1. Oligos design

- Make sure the 5’ primer has a Kozak DNA sequence (ANNATGGNN).
- There is no need to add a restriction enzyme site if using the PENTR/D TOPO vector (Invitrogen, K240020).
- If you are using the TRiP's modified mENTRY vector, you need add restriction enzyme sites to both at the 5’ end and 3’ end (from the mENTRY protocol: at the 5' end add either *EcoRI* or *MfeI* and at the 3' end add either *XbaI* or *SpeI*).

## 2. PCR

- For TOPO cloning the reaction volume is 20ul.
- For mENTRY vector cloning, the total reaction volume is 40ul.
- Make sure to use genomic DNA or cDNA as the PCR template.
- Load 5ul onto an agarose gel to verify the quality and quantity of your PCR product. If multiple bands are seen, we usually cut out the relevant band and purify it.

## 3. PCR purification

- See Qiagen manual for details (Qiagen, Cat No. 28104):
  Elute with 40ul dd$H_2O$.
- For TOPO cloning, there is no need to purify; i.e., directly use the PCR product for the ligation (For details see Invitrogen manual, Cat No. K240020).

## 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)

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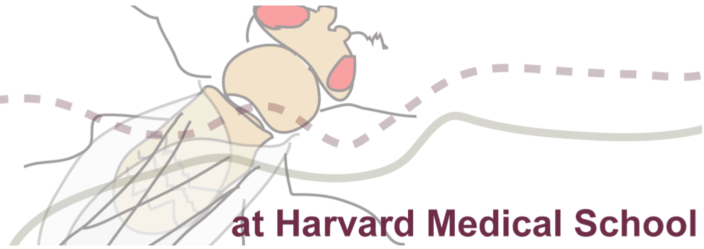

6. **Ligation**

Purification product ……………………………….. 6ul
10X ligation buffer ………………………………… 2ul
ddH$_2$O …………………………………………… 9ul
Backbone (linearized by enzyme digestion) …… 2ul
T4 DNA ligase (Roche, Cat No. 13580621) ……. 1ul
Mix, incubate at 16°C for 1 hour

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**

- make sure the direction of the insertion is correct if use single enzyme

9. **Miniprep**

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

10. **Recombination**

45-85ng/ul Entry vector ……………………………………………… 0.3ul
200ng/ul pVALIUM10-roe …………………………………………… 0.3ul
LR clonase (Invitrogen, Cat No. 11791-100) ……………………… 0.8ul
Add ddH$_2$O (or TE buffer, pH8.0) ………………………………….... 4ul total volume
Mix, incubate at 25°C for 1 hour

To stop the recombination, add 1ul proteinase K (from LR clonase kit), mix, incubate at 37°C for 10min.

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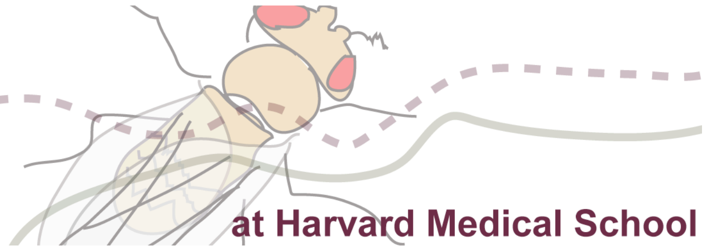


**11. Transformation**

Recombination product ........................................... 5ul
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)

**12. Select correct clone by PCR**

**13. Miniprep**

- See Qiagen manual for details (Qiagen, Cat No. 27104)

**14. Prepare DNA for injection**

- Ethanol precipitate
- Dissolve in injection buffer (100nM $NaPO_4$, pH 7.2, 5mM KCl)

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

**Good Luck!**

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