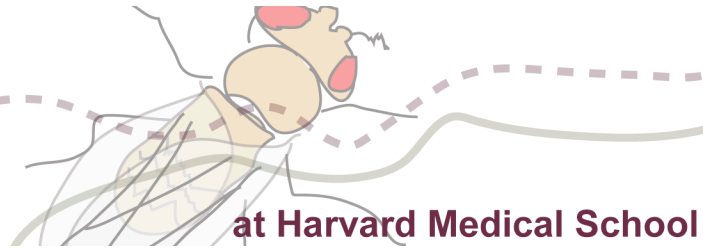


TRiP

Transgenic RNAi Project



at Harvard Medical School

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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