

# PCR fusion

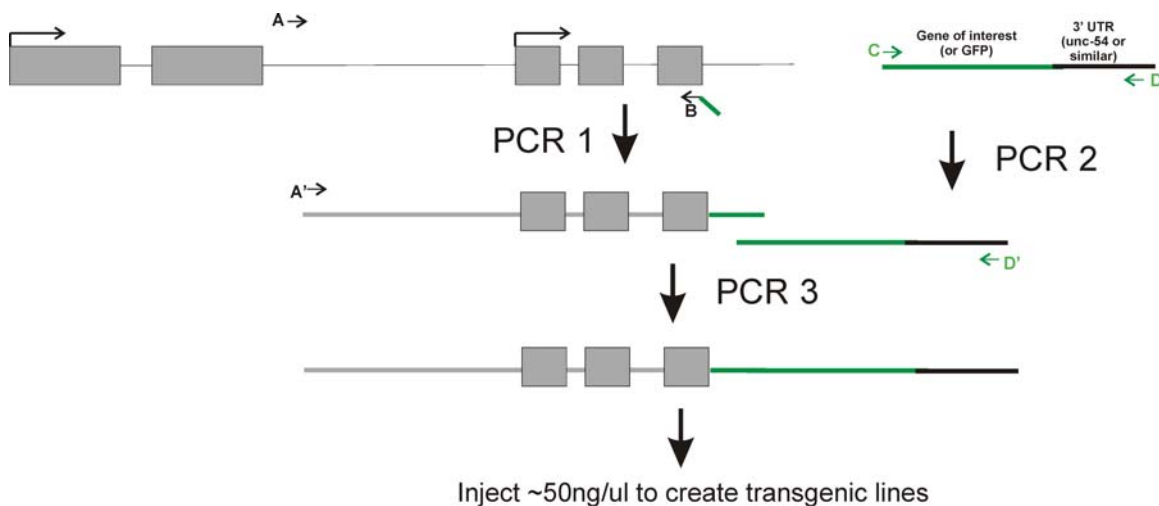
## Materials needed

- Primers

Primer A	Gene specific sense (21-24bp); ~2-3Kb from start ATG
Primer A'	Gene specific sense (21-24bp); 3-10bp downstream from primer A
Primer B	Chimeric primer; antisense; 1 <sup>st</sup> part of prime is homologous to fusion target (ie GFP or similar); 2 <sup>nd</sup> part of primer is homologous to promoter. For creating GFP fusions - 5' AGT CGA CCT GCA GGC ATG CAA GCT + XXX YYY ZZZ ... .. <ul style="list-style-type: none"> <li>- For transcriptional GFP fusions, XXX should be an antisense codon in exon 2</li> <li>- For translational GFP fusions, XXX should be the antisense codon just prior to the STOP codon</li> <li>- Region of homology should extend ~24bp</li> </ul>
Primer C	Fusion target sense primer. For GFP - 5' AGC TTG CAT GGC TGC AGG TCG ACT
Primer D	Fusion target antisense primer. For GFP - 5' AAG GGC CCG TAC GGC CGA CTA GTA GG
Primer D'	Fusion target antisense primer, should be 3-10bp upstream of Primer D; For GFP - 5' GGA AAC AGT TAT GTT TGG TAT ATT GGG

- Roche Expand Long PCR kit
- N2 genomic DNA, single worm DNA lysis solution, or other suitable DNA template

## Protocol



1. Set up PCR #1 - primer A + primer B + DNA template. Follow Expand Long protocol for PCR cycling conditions. Use Expand Long buffer B to start. If the reaction fails, try Buffers A and C.
2. Set up PCR #2 - primer C + primer D + DNA template (pPD95.75 for GFP)
3. Run 1 $\mu$ l of PCR #1 and #2 on a gel to ESTIMATE the concentration (Do NOT purify the fragments ; it will inhibit the fusion reaction)
4. Set up the fusion reaction (PCR #3) - Primer A', Primer D', 10-50ng of PCR product #1 and #2.
5. Run 1 $\mu$ l and 10 $\mu$ l of the fusion PCR to estimate concentration
6. Inject into worms without further purification at 10-50ng/ $\mu$ l along with the marker of your choice at 100ng/ $\mu$ l

### **Notes**

1. Purification of the PCR products often inhibits the fusion PCR for unknown reasons.
2. Even if the first PCR product is not visible on the gel, you may still be able to obtain a fusion product. Try using PCR #1 product undiluted in the fusion PCR reaction. If you still get nothing, try moving primer A around. Once PCR #1 works, the fusion reaction almost always works.
3. Most fusion PCR reactions will give a single band. However, another band is often visible, sometimes stronger than the fusion PCR product. Ignore it. It will act as 'carrier DNA' during the microinjection. Estimate the DNA concentration only from the correct sized fusion PCR product.

### **Reference**

Hobert, O., *PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic C. elegans*. *Biotechniques*, 2002. **32**(4): p. 728-30.