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This is a protocol for production of dsRNA to be injected in worms for RNAi.

Notes on primers

1. when designing primers, keep in mind that you should amplify a region which is 80% or more unique (at DNA level), in order to preserve specificity;
2. the forward and reverse primers should have the following extensions (19 bases each):
   direct primer: 5'-TAATACGACTCACTATAGG-your_seq-3'
   reverse primer: 5'-AATTAACCCTCACTAAAGG-your_seq-3'

Steps

1. **PCR** your fragment of interest. Make the following mix:
   10x PCR buffer 10µl
   Forward primer (25µM) 2µl
   Reverse primer (25µM) 2µl
   Template (e.g., 0.5ng genomic) 1µl
   10mM dNTPs 2µl
   DNA polymerase 1µl
   MQ water 82µl
2. PCR conditions
   92°C 1min
   92°C 20 sec
   anneal 40 sec
   extend XX sec
   72°C 7 min
   4°C hold
3. Annealing temp. depends on primer; extension temp. and time depend on the polymerase used.Run 2µl of each reaction on an agarose gel.Clean the PCR reactions using the Qiagen PCR cleanup kit; elute with 50µl.
4. **T3 and T7 transcription reactions**, using the Ambion kitMix in the order below, at room temperature:
   NTPs 10µl
   10X T3 OR T7 buffer 2.5µl
   DNA template 10µl
   T3 OR T7 enzyme mix 2.5µl
5. Incubate at 37 °C for 3-5 hours
6. **Remove template DNA**: add 1.3µl DNase to each reaction, incubate at 37°C for 15 minutes
7. Take 2µl samples of each reaction, mix with 2µl of sample buffer and 2µl or RNase-free H2O
8. **Clean the reactions** using the RNeasy kit. Elute each reaction with 30µl RNase-free water into the same tube. [alternatively, you could mix the two reactions and clean both in one round; this spares columns and, especially, pipetting work]
9. **Anneal the RNAs**: Add 30µl of 3X injection buffer to each tube. Incubate at 68°C for 10min and then at 37°C for 30min. Take 2µl of each sample for a gel and treat it as above.
10. **Check the RNAs:**Dilute 50x an aliquot of the final dsRNA solution and measure the OD260. (Assume that a solution of 50µg/ml gives an OD=1). Final yield should be around 1..2mg/ml.Run an agarose gel with the three samples per reaction. If everything is ok, proceed to injection.

Solutions

3X injection buffer

1. 120mM KPi pH 7.5
2. 18mM K citrate pH 7.5
   4% PEG 6K, filter 0.2µm