Single Worm PCR

Reagents:

Lysis Buffer*	1 X PCR buffer (see below for 10X PCR buffer)
Proteinase K:	20 mg/ml . Store in small aliquots at -80
10X PCR Buffer:	100 mM Tris, 500 mM KCl, 20 mM MgCl2 pH 8.3
dNTP mix:	25 mM/each
primers:	5-10 uM
Taq Polymerase:	approx 5U/ul

Procedure:

1. Add proteinase K to 1 X PCR buffer (95 µl 1 XPCR buffer + 5 µl 10mg/ml proteinase K) *Note: We do not use the "Lysis buffer" recipe anymore because 1 X PCR buffer seems to work fine.

2. Place 5-10 µl of 1 X PCR buffer + proteniase K in top of 200 µl PCR tube (if you use more than 1 worm use 10ul instead).

3. Pick single worm (or multiple worms) into buffer. Adding too many worms can inhibit the reaction.

4. Immediately (don't let sit too long in lysis buffer) spin down to bottom of tube by spinning in microfuge 15 seconds @ 14,000 rpm or just flick down.

5. Freeze tube in Liquid Nitrogen (or - 80 C freezer) at least 10min.Worms can be store ad infinitum at -80 C.

6. Lysis of worm and release of genomic DNA (You can use the PCR machine --"worm lysis" program)

   - heat tube to 65 degrees for 60-90 minutes
   - inactivate proteinase K by heating to 95 degrees for 15 minutes.
   - **If you do not use the worm DNA right away store at -80C.**

7. Perform PCR (50ul reaction)

   1. add 45-40 µl l of PCR "master mix" to each tube
   2. master mix: 1X PCR Buffer, 0.5 µM primers, 0.2 mM/each dNTPs
   3. Add 0.5µl of Taq to tubes at 95C (hot start) If you have many samples the master mix can include the Taq so you can skip this step.
   4. run PCR reaction for 30-35 cycles

Notes:

True Lysis buffer : 50 mM Kcl, 10 mM Tris pH 8.2, 2.5 mM MgCl2, 0.45% NP-40, 0.45% Tween-20, 0.01% DNA free gelatin. Mix 95 ul of Lysis buffer with 5 ul of 10 mg/ml Proteinase K.I find it doesn't make much of a difference compared to 1 X PCR buffer. We often leave out the gelatin and use Triton X-100 instead of NP-40.

Use oil on top during step 6 if you do not have a PCR machine with a heated lid.

Small changes in MgC2 concentration of PCR buffer can make a big difference in PCR efficiency. Can try at 1.5 or 2.5 mM MgCl final concentraion.

Refs:

A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. Williams BD; Schrank B; Huynh C; Shownkeen R; Waterston RH. Genetics, 1992 Jul, 131(3):609-24.

Cloning, sequencing, and mapping of an alpha-actinin gene from the nematode *Caenorhabditis elegans*. Barstead RJ; Kleiman L; Waterston RH. Cell Motility and the Cytoskeleton, 1991, 20(1):69-78.

Modified by M. Nonet from Aroian Lab protocol