

Single Worm PCR

Compiled by: Chad Rappleye, Aroian Lab Protocols, <http://aroianlab.ucsd.edu/protocols/worm-pcr.htm>

Reagents Needed:

single worm lysis buffer (common stock)
proteinase K

Procedure:

1. Add proteinase K to lysis buffer (90 μ L lysis buffer + 10 μ L 10mg/mL proteinase K).
2. Place 3-10 μ L of lysis buffer in top of 1.5mL Eppendorf tube.
3. Pick single worm into lysis buffer.
4. Spin worm to the bottom of tube by spinning in centrifuge for 15 seconds at 14,000 rpm.
5. Flash freeze the tube in dry ice and ethanol or in liquid nitrogen (poke a hole in the tube's lid if freezing in liquid nitrogen so the tube does not explode).
6. Freeze tube at -80°C for at least 1 hour.
7. Lyse the worm and release the genomic DNA by heating tube to 65°C for 60-90 minutes.
8. Inactivate the proteinase K by heating to 95°C for 15 minutes.
9. Perform PCR (common stock PCR reagents in -20°C stock freezer).
 - Run reaction for 30-35 cycles.

Recipes:

Worm PCR Lysis Buffer

* Common stock in -20°C stock freezer.

50mM KCl

10mM Tris (pH 8.3)

2.5mM MgCl₂

0.45% NP-40 (IGEPAL)

0.45% Tween-20

0.01% Gelatin

- Add 0.1mg/mL of proteinase K before use.

References:

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.

