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Genotyping *C. elegans*

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes a procedure to genotype *C. elegans* strains via genomic extraction and PCR amplification. You should always confirm strain information upon receipt of the strain.

Materials

Proteinase K, Molecular Biology Grade - 2 ml **New England Biolabs Catalog #**P8107S
Magnesium Chloride **Fisher Scientific Catalog #**AC223210010
Potassium chloride **P212121**
Tris HCl **P212121**
PCR tubes
Thermocycler
Platinum wire
PCR master mix
PCR primers
1% agarose DNA gels
New 6x Purple Loading Dye **New England Biolabs Catalog #**B7024S
Ultrapure water

Protocol materials

New 6x Purple Loading Dye **New England Biolabs Catalog #**B7024S
Proteinase K, Molecular Biology Grade - 2 ml **New England Biolabs Catalog #**P8107S
Magnesium Chloride **Fisher Scientific Catalog #**AC223210010
Potassium chloride **P212121**
Tris HCl **P212121**

Genomic extraction from single worms

15s

1 Prepare lysis buffer. You can prepare lysis buffer in advance and store it at Room temperature before adding proteinase K.

Lysis buffer (without proteinase K):
[M] 10 millimolar (mM) Tris HCl **P212121**
[M] 50 millimolar (mM) Potassium chloride **P212121**
[M] 2 millimolar (mM)
Magnesium Chloride **Fisher Scientific Catalog** #AC223210010
pH 8.3

2 Complete lysis buffer by adding
Proteinase K, Molecular Biology Grade - 2 ml **New England Biolabs Catalog** #P8107S
to a final concentration of [M] 0.5 mg/mL. Prepare ≥ 10 µL complete lysis buffer per sample.

3 Transfer 10 µL lysis buffer per tube to PCR tubes. Include an additional tube as a negative control.

4 **Pick a single worm from each strain and transfer to lysis buffer**
Flame-sterilize a platinum wire. Pick up one adult worm from plate, taking care to prevent puncturing the NGM. Transfer worm by gently submerging the platinum wire into the PCR tube under flame. Hold the wire in this position for several seconds to allow worms to detach. Flame-sterilize the wire before picking additional worms.

> Note
>
> Try to pick only a single worm per PCR tube. A couple of worms is fine, but too many can inhibit the reaction.

5 Immediately spin tubes in a benchtop microcentrifuge for 00:00:15. 15s

6 Freeze tubes in LN2 or -80 °C for ≥ 00:10:00. 10m

7 Lyse worms in a thermocycler according to the following protocol: 2h 45m
65 °C , 01:00:00 – 01:30:00 .
95 °C , 00:15:00 (proteinase K inactivation step).

You can store extracted worm DNA indefinitely at -80 °C .

PCR amplification

8 If using poison primer amplification, add 46 µL Q5 PCR master mix per tube to appropriate number of PCR tubes. To each, add 2 µL template (proteinase K extraction product) and 0.5 µL each primer.

If using standard amplification, add 47 µL Q5 PCR master mix per tube to appropriate number of PCR tubes. To each, add 2 µL template (proteinase K extraction product) and 0.5 µL each primer.

For negative control, add master mix and primers only.

9 Run PCR for 30–35 cycles according to Q5 manufacturer protocol. Determine annealing temperature based on primer sequences.

Analysis of PCR product via gel electrophoresis

10 Transfer 20 µL each PCR product to a new PCR tube and add 4 µL New 6x Purple Loading Dye **New England Biolabs Catalog #**B7024S .

11 Load onto 1% agarose DNA gel. Electrophorese at 120 V until dye front reaches the end of the gel.

12 Visualize under blue light.

Note

For large-scale deletion strains with validated poison primer sequences, gel analysis is usually sufficient to confirm genotype. In other cases, further analysis via Sanger sequencing is required.

Analysis of PCR product via sequencing

13 In a clean PCR tube, add 9 µL ultrapure water, 1 µL PCR product, and 1 µL sequencing primer.

14 Submit sample for Sanger sequencing.

Protocol references

Protocol modified from:
https://bpb-us-w2.wpmucdn.com/sites.wustl.edu/dist/0/1532/files/2018/07/Single-Worm-PCR-r8hwch.pdf