Single Worm PCR

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[Abstract] This protocol is used for genotyping single worms.

Materials and Reagents

1. Nonidet P-40 (Sigma-Aldrich, catalog number: 74385)
2. Gelatin (Sigma-Aldrich)
3. Proteinase K (Promega Corporation, catalog number: V3021)
4. KCl
5. Tris (pH 8.3)
6. $MgCl_2$
7. Nonidet P-40
8. Tween-20
9. Gelatinin
10. Lysis buffer (see Recipes)

Equipment

1. PCR tubes

Procedure

A. Transfer one adult worm directly from a plate to 5 µl of lysis buffer in each PCR tube.
   Note: To avoid transferring a large amount of bacteria, pick worm from region away from bacterial lawn. Also, try not to pick an old worm or starving worm.
B. Spin capped PCR tubes briefly to bring down worm to the bottom of the tube.
C. Freeze at -80 °C for 10 min or longer (up to a week).
D. Lyse the worms for release of genomic DNA.
E. Heat sample at 60 °C for 1 h, then inactivate protease K at 95 °C for 15 min. Store the worm DNA at -80 °C (or -20 °C for temporary storage) if needed.
F. Perform standard PCR reaction, use 5 µl worm DNA as template for 50 µl reaction.

Recipes

1. Lysis buffer
   50 mM KCl
   10 mM Tris (pH 8.3)
   2.5 mM $MgCl_2$
   0.45% Nonidet P-40
   0.045% Tween-20
   0.01% (w/v) gelatinin
   Autoclave and store at 4 °C (good for more than 6 months) or -20 °C for long-term storage. Right before use, add proteinase K stock to the lysis buffer with final concentration 60 μg ml^{-1}.

References

1. Williams, B. D., Schrank, B., Huynh, C., Shownkeen, R. and Waterston, R. H. (1992). A genetic mapping system in Caenorhabditis elegans based on polymorphic sequence-tagged sites. *Genetics* 131(3): 609-624.

