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genotyping_PCR

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ABSTRACT

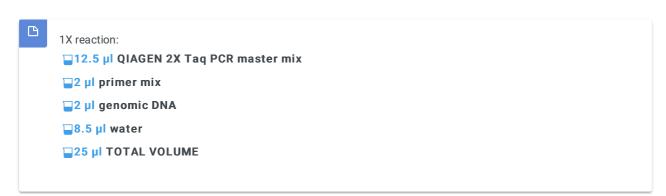
Protocol for doing PCR on single worm lysate. This is a basic protocol that can be adapted according to specific primers and amplicon size

MATERIALS

NAME >	CATALOG #	VENDOR ~
custom made primers		
Taq PCR Master Mix Kit	Cat No./ID: 201443	Qiagen

PCR program

1 Precalculate how many reactions are required for each pair of primers. Remember to include a minus-template control and add 1 to the final number to make sure you don't run out of reagents



Eg. for 6 reactions, need to prepare (6+1)X reactions

Prepare reagents

- 2 Get PCR master mix and gDNA out of the freezer. Thaw PCR mix on ice and spin down to collect at the bottom of the tube.
- 3 Get primers out of freezer or prepare new primers
- 3.1 If primers are ordered new, resuspend lypholised oligo to [M] 100 Micromolar (μ M) (== 100 μ mol/l). Volume of water (in μ l) to add is calculated as Volume = nmol*10 Eg. for primer provided as 28.5nmol, add 285 μ l of MQ water

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3.2 If diluting from stock primers, make a a primer mix of 10 μ M of each the forward and reverse primer.

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Eg. 30 μl water + 10 μl (100 μM) FW primer + 10 μl (100 μM) REV primer = TOTAL 100 μl primer mix
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- 4 Prepare and label PCR tubes
- 5 Prepare and label master mix eppendorfs

Assemble reagents

- 6 Assemble reagents in master mix tube in following order:
 - 1. Water
 - 2. Primers
 - 3. Tag PCR master mix
- 7 Dispense **■23** µl **PCR mix** into each PCR tube
- 8 Add $\mathbf{2}$ μ I genomic DNA to the PCR tube.
- 9 Close tubes, make sure the lids are firmly sealed, and flick to ensure all liquid is at the bottom of the tube

10 Put the PCR tubes in the thermocycler and run with the geno-PCR program



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Heat lid:
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8 105 °C

Initial denaturation:

©00:03:00 8 93 °C

35 cycles:

- 1. © 00:00:30 & 93 °C
- 2. © 00:00:30 § 55 °C adjust temperature according to primers
- 3. © 00:01:00 § 72 °C adjust time according to amplicon size. Rule: 1 min per 1kb

Final extension:

© 00:10:00 8 72 °C

Final Hold:

8 10 °C

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