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genotyping_PCR

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1 Works for me dx.doi.org/10.17504/protocols.io.7uphnvn

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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

1X reaction:

- **12.5 µl QIAGEN 2X Taq PCR master mix**
- **2 µl primer mix**
- **2 µl genomic DNA**
- **8.5 µl water**
- **25 µl TOTAL VOLUME**

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 (µM)** (== 100 µmol/l). Volume of water (in µl) to add is calculated as $Volume = nmol * 10$

Eg. for primer provided as 28.5nmol, add 285 µl of MQ water

3.2 If diluting from stock primers, make a a primer mix of 10 µM of each the forward and reverse primer.
Eg. **80 µl water** + **10 µl (100 µM) FW primer** + **10 µl (100 µM) REV primer** = TOTAL **100 µl primer mix**

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. Taq PCR master mix

7 Dispense **23 µl PCR mix** into each PCR tube

8 Add **2 µl 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

PCR program

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

Heat lid:
105 °C

Initial denaturation:
00:03:00 **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 **72 °C**

Final Hold:
10 °C