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🌐 genotyping_PCR

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Behavioural Genomics

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

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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

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custom made primers **Contributed by users**

Taq PCR Master Mix Kit **Qiagen Catalog #Cat No./ID: 201443**

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

Note

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 lyophilised oligo to **100 micromolar (µM)** (== 100 µmol/l). Volume of water (in µl) to add is calculated as $\text{Volume} = \text{nmol} \times 10$
Eg. for primer provided as 28.5nmol, add 285 µl of MQ water
 - 3.2 If diluting from stock primers, make 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

Note

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