Standard PCR protocol V.1

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ABSTRACT

Generic PCR protocol

PROTOCOL CITATION

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Master mix 10m

1 For a **25 µl** reaction volume use the following recipe:

- **14.9 µl** **dH_2O**
- **5 µl** **buffer**
- **1.5 µl** **$MgCl_2$**
- **1 µl** **FWD** primer (from a [M]**10 Micromolar (µM)** working solution)
- **1 µl** **REV** primer (from a [M]**10 Micromolar (µM)** working solution)
- **0.5 µl** **dNTPs** (from a [M]**10 Micromolar (µM)** mix)
- **0.1 µl** **Taq** polymerase

2 Prepare the master mix **On ice** allowing for 10% pipetting error (eg. if carrying out 10 reactions, calculate the master mix quantities needed for 11 reactions).

Remember to include a **negative control** with no DNA template and, if possible, a **positive control** using a known DNA template.

Preparing PCR reaction tubes 5m

3 Aliquot **24 µl** of master mix into each 0.2 mL PCR tube.

4 Add **1 µl** of either DNA template, dH_2O (negative control) or known DNA (positive control) to the PCR tubes and place in a thermocycler.

Thermocycling program

5 Standard PCR thermocycler program (30 cycles):

5.1 Initial denaturation at **94 °C** for **00:03:00** 3m

5.2 Denature at **94 °C** for **00:00:30** 30s

5.3 Annealing at ~ **55 °C** (depending on primers) for **00:01:00** 1m

5.4 Extension at **72 °C** for **00:02:00** 2m

x 29

5.5 Final extension at **72 °C** for **00:05:00** 5m

Storage

6 Store PCR products at **4 °C** , or at **-20 °C** for long term storage.

Analysis

7 Check if the PCR was successful by gel electrophoresis.

Gel electrophoresis
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PREVIEW RUN

7.1 For **200 mL** of a 1.2% agarose gel, add **2.4 g** of agarose to **200 mL** TAE or TBE in a conical flask and mix by swirling the flask.

7.2 Microwave on a high power for about **00:03:00** , or until boiling, swirling the flask approximately every **00:00:30** during heating to ensure all the agarose dissolves.

3m 30s

> Use heat proof gloves.

7.3 Allow the agarose to cool until you are able to comfortably touch the outside of the flask (approximately **00:05:00**).

5m

7.4 Add **10 µl** SafeView stain to the hot agarose and swirl to mix.

7.5 Pour the agarose into the casting tray. Pop any bubbles or move them to the edges of the mould using a pipette tip. Place the comb as desired.

7.6 Allow the gel to set for approximately **00:40:00** .

40m

7.7 Place the gel (still in the casting tray) gently into the tank, ensuring there is enough TAE or TBE running buffer to cover it.

7.8 If the buffer used for PCR already contains loading dye, skip to step 11.

7.9 On a piece of parafilm, dot **1 µl** of 6x loading dye using a pipette.

7.10 Pipette **5 µl** ladder or **6 µl** PCR product directly onto the loading dye and mix by pipetting up and down several times.

7.11 Load **6 µl** into each well.

7.12 Place the lid on the tank, making sure that the wells are at the negative electrode end of the tank. The samples should run from black to red (negative to positive).

7.13 Set the power supply to between **80-120V**, depending on the expected amplicon size and desired duration of electrophoresis.

7.14 Run the electrophoresis until the visible dye bands are about 75% of the way down the gel, approximately **1-1.5 hours.**

Once complete, ensure you disconnect the power supply from the gel tank, before removing the lid.