Gel electrophoresis

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1 Works for me Share This protocol is published without a DOI.

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ABSTRACT

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PROTOCOL CITATION

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In steps of
Standard PCR protocol

Making the gel 8m 30s

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.

2 3m 30s
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.

Use heat proof gloves.

3 5m
Allow the agarose to cool until you are able to comfortably touch the outside of the flask (approximately **00:05:00**).

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

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.

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

Loading the gel

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.

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

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

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

11 Load **6 µl** into each well.

Running the gel 1h

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).

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

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.