



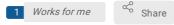
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## Standard PCR protocol

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ABSTRACT

Generic PCR protocol

PROTOCOL CITATION

Victoria Jackson 2021. Standard PCR protocol. **protocols.io** https://protocols.io/view/standard-pcr-protocol-bumenu3e

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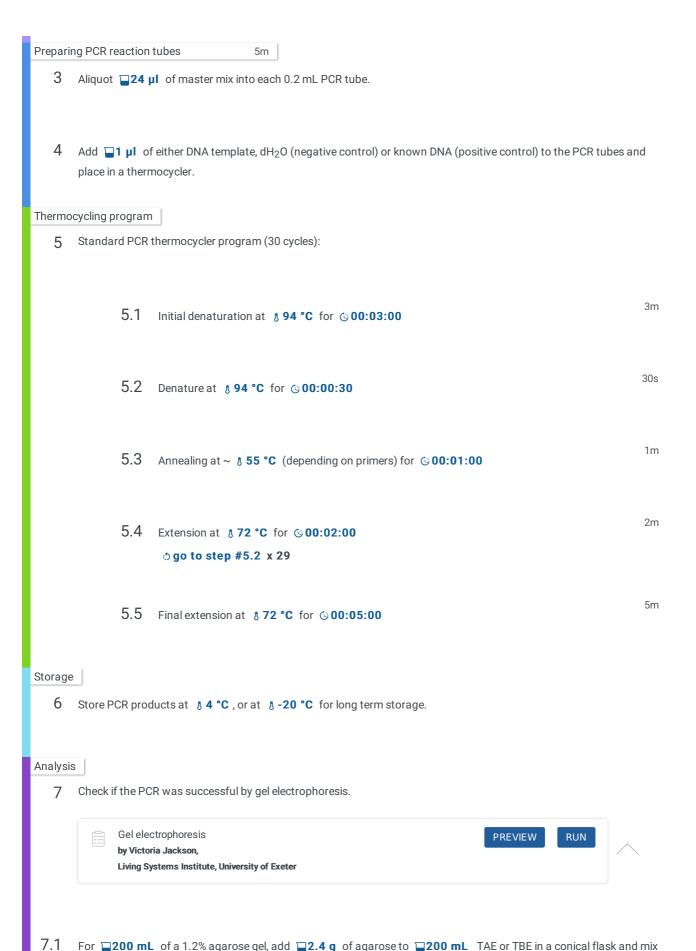
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Master mix 10m

- 1 For a **25 μl** reaction volume use the following recipe:
  - **■14.9 μl dH<sub>2</sub>0**
  - **■5** μl buffer
  - ■1.5 µl MgCl<sub>2</sub>
  - ■1 µl FWD primer (from a [M]10 Milimolar (mM) working solution)
  - □1 μl REV primer (from a [M]10 Milimolar (mM) working solution)
  - ■0.5 μl dNTPs (from a [M]10 Milimolar (mM) 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.



by swirling the flask.

40m

7.2	$\label{limiting} \mbox{Microwave on a high power for about } \textcircled{00:03:00} \mbox{ , or until boiling, swirling the flask approximately every}$	3m 30s
	© 00:00:30 during heating to ensure all the agarose dissolves.	

A	Use heat proof gloves.		

- Allow the agarose to cool until you are able to comfortably touch the outside of the flask (approximately © 00:05:00 7.3 ).
- 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**.
- Place the gel (still in the casting tray) gently into the tank, ensuring there is enough TAE or TBE running buffer to cover 7.7
- 7.8 If the buffer used for PCR already contains loading dye, skip to step 11.
- 7.9 On a piece of parafilm, dot  $\square 1 \mu I$  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 16 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.