Polymerase Chain Reaction for the Identification of a Plasmid

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

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ABSTRACT

Protocol for the identification of a plasmid by polymerase chain reaction followed by gel electrophoresis

MATERIALS

Target DNA
PCR master mix
Forward primer
Reverse primer
Sterile water

Materials available:
• Primers amp-F and amp-R, kan-F and kan-R.
• A commercially available PCR Master Mix at 2X concentration (containing Taq polymerase buffer, 1.5 mM MgCl2, 0.2 mM each of dATP, dCTP, dGTP and dTTP, Taq polymerase, and a red dye to allow direct loading of the completed reaction onto an agarose gel)
• Plasmids at 100 ng.µl-1 ; you will need to dilute this DNA to a working concentration of 0.1 ng.µl-1 before you use them

Making the PCR mix

1 Add 1 ng of the target DNA,

5 µL of each primer,
25 µL of 2X PCR master mix,
and sufficient sterile water to make the volume up to 50 µL.

Thermocycling

2 Thermocycle on the following settings:

	number of cycles	temperature, C	time, minutes
	1 cycle	94	1
	30 cycles	94	1
	30 cycles	55	1
	30 cycles	72	1
	1 cycle	72	5

cycle programme for PCR

Making the Gel

3 Weigh out the correct amount of agarose and place it in a 250 mL conical flask. Note: for a 1% gel you will need 1 g of agarose for every 100 mL of buffer.

4 Add 100 mL 1× TAE buffer and swirl gently to mix. Put an inverted small conical flask in the top of the flask.

5 Place in a microwave and heat for 1 minute, then gently swirl the contents.

Safety information

Remember to wear heatproof gloves and swirl the contents very carefully, as the liquid can become superheated.

6 Continue microwaving in 30-second blasts followed by gentle swirling until the agarose starts to boil.

Safety information

At this point you need to handle the flask very carefully.

7 Continue microwaving in 15-second blasts until all the agarose has dissolved - you should not be able to see any tiny pieces floating in the gel suspension.

8 Place the flask in a 50 °C water bath to cool. It is important to make sure the agarose has cooled to 50 °C before proceeding to the next stage. 30m

9 Whilst you are waiting for the gel to cool, prepare your gel tray by securely taping up the ends.

10 Once the agarose has cooled (5-10 minutes should be OK), add 4 µL of Midori Green direct **Contributed by users Catalog #MG06** to the cooled gel mixture and swirl to mix.

Safety information

Wear gloves to handle Midori Green and the resulting gel.

11 Pour the molten agarose into the gel tray and insert the comb (make sure it is straight and level) and leave to set. 30m

Loading and Running the Gel 1h

12 Add TAE buffer to the tank, then add the gel and ensure it is fully submerged in TAE.

13 Load the DNA samples into the gel, and add a DNA ladder to one of the wells

14 Run the gel at 100V for 01:00:00 1h

15 Observe the gel and locations of the DNA fragments