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Gel Electrophoresis

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We use this protocol and it's working

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Abstract

How to prepare and run gel electrophoresis using agarose gel for the verification of nucleic acid amplification products.

Guidelines

Gloves should be worn at all times.

Materials

- 50x TAE Buffer
- Agarose
- DNA ladder
- Loading dye (optional)
- Nucleic acid amplification solution (e.g. PCR or LAMP)
- Ethidium bromide
- Deionized water

Troubleshooting

Safety warnings

Gel bottle will be hot from microwave, use a hot mitt and be cautious to not get hot gel on your skin. Ethidium bromide may be a mutagen; skin contact should be avoided.

Make TAE buffer

1 In a large glass bottle, add 750 mL DI water.

2 Add 15 mL 50x TAE buffer to the deionized water.

3 Cap the bottle and mix by swirling

Make gel

4 Pour 300 mL TAE buffer into microwavable glass bottle.

5 Add the following amount of agarose powder to TAE buffer for different gel concentrations:

	Concentration (w/v)	Agarose (g)
	2.0%	6
	2.5%	7.5
	3%	9

Note

Swirl solution lightly to mix agarose into buffer. Swirling too vigorously will introduce bubbles that will make casting difficult.

6 Microwave in 00:00:40 intervals until completely dissolved, swirling bottle between heating intervals.

7 Pour into gel mold with well insert. Add to black line (~ 100 mL)

8 Allow gel to set for approximately 00:20:00 .

Load and run gel

9 Transfer gel boat with gel into electrophoresis chamber.

10 Add buffer to line marked on chamber, completely covering the gel.

11 Carefully load the wells with ladder (green) and amplification product solutions at volumes between 1 µL and 5 µL .

Note

Optionally, you can mix 1 µL loading dye with 4 µL amplification product solution in a separate microcentrifuge tube to make the loading process easier to visualize.

12 Put the lid on the chamber, turn on the power supply and press 'RUN'. Run for 00:50:00 .

Note

The power supply does not turn off automatically, so set a timer and shut off after 00:50:00 . This time may change based on factors such as gel concentration and amplicon length.

Stain the gel

13 Transfer the gel into the dying tray.

14 Check buffer level and add more if necessary.

15 Add 8 µL ethidium bromide to dying tray.

16 Turn on stage, and allow to stain for 00:20:00 .

Image gel

17 Place gel in imaging machine.

18 Turn on the machine by the switch on left side of machine. Press 'Trans UV'.

19 Take a picture with the camera.