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1% Agarose Gel Electrophoresis Prep

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ABSTRACT

Ararose (1%) gel electrophoresis for genomic DNA quality checking.

This protocol comes with no guarantees from the authors.

MATERIALS

NAME	CATALOG #	VENDOR
TAE Buffer (Tris-acetate-EDTA)	B49	
SYBR	S33102	Thermo Fisher Scientific
100g Agarose I (All Purpose, Electrophoresis Matrix (Low EEO))	RC-006	G-Biosciences
100bp DNA Ladder, 250ul (50 lanes)	G2101	Promega
6X Blue Loading Dye	L002	Gold Biotechnology

SAFETY WARNINGS

SyberSafe

Gel Prep

1 Weigh out 2.5g of powdered agarose.

2 Add 250mL of 1XTAE buffer to a 1.0L Erlenmeyer flask.

Note:
Be mindful to use best practices when making solutions. Make sure the glassware you are using has been properly cleaned and has been pre-rinsed with whatever solution you will be working with, in this case 1XTAE buffer.

3 Add the pre-weighed 2.5g of agarose to the 1XTAE in the 1.0L Erlenmeyer.

Note:
DO NOT OVERFILL THE FLASK! This solution will be heated and is subject to boiling when agitated. Ensure proper protective equipment.

4 Using a microwave, heat the agarose and buffer for 45 seconds.
Remove the flask and swirl. Use caution as the solution will be hot.

5 Continue to heat the solution in 30 second increments until it starts to boil, at which point the flask should be removed from the microwave. Cautiously swirl the solution to ensure all agarose has dissolved properly.

Solution has reached the correct temperature once all agarose crystals have dissolved and "crystal lenses" are no longer observed.

6 Allow solution to cool at room temperature.

7 While the agarose solution is cooling, ensure all equipment is clean and ready for use.

Note:
1) Pay special attention to the comb. Ensure all residue from previous gels are removed, otherwise well integrity may be compromised.
2) Fill the BioRad rig with 1XTAE Buffer. Add enough to fill both chambers and just cover bridge between them.
3) Clean the gel tray by wiping it down with distilled water, followed by 95% ethanol.
4) Set up the gel-casting tray; ensure a proper seal between the rubber gaskets and the open end of the gel tray.

8 Once the flask is warm to the touch, but does not induce pain with sustained gloved-hand contact, add 5uL of SybrSafe to the flask and swirl gently. Take care to avoid adding bubbles to the solution.

Note:
Do not add SybrSafe too early as it is denatured at high temperatures.

9 Carefully pour the agarose solution into the gel-casting tray. Again, try to avoid the creation of bubbles.

This step is time sensitive.

Notes
1) If bubbles are created either pop them with a clean pipette tip, or move them to the edge of the plate. Always remember that the gel polymerizes as it cools.
2) Place the comb into the second or third slot from the top of the gel tray and allow the gel to set. This placement may change depending on the number of samples or size of the gel.

Sample Prep

10 While gel polymerizes:

Transfer 10uL of sample into a clean strip-tube and add 2uL of 6X loading dye to each sample. Vortex to mix and spin down.

Loading Gel

11 Once the gel has solidified in the gel-casting tray, slowly and carefully remove the comb.

12 Transfer the gel and tray to the BioRad rig, orienting the wells closest to the black electrodes (or the chamber where the negative current will be applied).

13 Top up the buffer in the rig if needed. Approximately 2mm of 1XTAE should stand above the gel.

14 Load 12µL of sample and 3µL ladder to their respective wells.

15 Match up the black and red electrodes on the BioRad lid with that on the base.

Note:
Remember the chamber with the positive charge should be oriented at the bottom of the gel!

16 Plug the electrode lead into the machine and run at 130mV for 30 mins, checking the progress every 10-15 minutes.