Gel Electrophoresis Protocol

DNA Ladder Solution

Reagents

- 6X DNA Loading Dye
- TLE Buffer Solution
- 0.5 µg/µL DNA Ladder

Procedure

Prepare DNA ladder working stock containing a 4:1:1 ratio of TLE Buffer Solution, DNA Loading Dye, and DNA Ladder

1X SB Buffer Solution

Dilute one part 20X SB Buffer Solution in 19 parts deionized water.

Agarose Gel Casting

Note that GelGreen® is mutagenic so it is important to use gloves when making and handling agarose gels and to avoid contaminating surfaces and equipment in the lab with it. Wash off gloves if they have come into contact with solutions containing GelGreen® and thoroughly clean up any spills.

Reagents

- Agarose (CAS 9012-36-6)
- 1X SB Buffer Solution
- 10,000X GelGreen®

Procedure

- One small gel will require 50 mL of Agarose Gel
- One large gel will require 150 mL of Agarose Gel

1. Refer to the table below and add the appropriate number of grams of Agarose and milliliters of 1X SB Buffer to a flask.

DNA Size Resolution	Percent Agarose Gel (w/v)	Grams Per 100 mL 1X SB Buffer
1 kb – 30 kb	0.5%	0.5 grams
500 bp – 10 kb	1.0%	1.0 grams
50 bp – 2 kb	2.0%	2.0 grams

2. Place solution into the microwave until it begins boiling and then immediately turn off the microwave. Avoid allowing the solution to boil over!
3. Remove from the microwave and swirl solution to mix thoroughly. If solution has many bubbles suspended in it, place it back into the microwave very briefly until boiling again. Repeat until solution no longer has a large number of bubbles.
4. Add 0.5 µL GelGreen® per 100 mL and swirl solution in flask to mix.
5. Allow solution to cool to the point that you can hold your hand against the side for several seconds. This is to avoid warping the gel casting rig from high heat.
6. Pour solution into gel rig to a depth of 3-4 mm.
7. Put combs in place on the gel rig.
8. Allow gel to harden for at least 30 minutes.

Run DNA On Gel

1. Place gel into electrophoresis chamber.
2. Fill chamber with enough 1X SB buffer to cover the top of the gel.
3. Prepare samples by mixing 1µL of sample with 5µL
4. Load samples into gel. Samples should have at least 20 pg of DNA.
5. Add 1 µL of ladder per mm of well width.
6. Run gel at ~16V per cm of gel length until dye has reached the edge of the gel