Buffer and staining options for agarose gel electrophoresis

"One size fits all" protocol. Use 1% Agarose gel made in **TBE** (0.5x). Add 1µL of a dilute GelGreen stock per 10mL of gel while gel is molten (after cooling to pouring temperature ~50°C). Use **TBE** (0.5x) as the running buffer. Run at 150-180V (small tanks) or 200-250V (large tanks).

"Time is of the essence" protocol. Use 1% Agarose gel made in **LAB** (1x). Add 1µL of a dilute GelGreen stock per 10mL of gel while gel is molten (after cooling to ~50°C). Use **LAB** (1x) as the running buffer. Run at 250V. WARNING! this gel will be done in ~15 min.

"Higher-resolution required" protocol (DNA sizes **>**1.5 kb). Use 0.8% Agarose gel made in **TAE** (1x). Add 1µL of dilute GelGreen stock per 10mL of gel while gel is molten (after cooling to pouring temperature ~50°C). Use TAE (1x) as the running buffer. Run at <100V

"Higher-resolution required" protocol (DNA sizes **<**1.5 kb). Use 1.5-2% Agarose gel made in **TBE** (0.5x). Add 1µL of dilute GelGreen stock per 10mL of gel while gel is molten (after cooling to ~50 °C). Use TBE (0.5x) as the running buffer. Run at 150V (small tanks) or 200V (large tanks).

"Nice gel for publication" protocol. Use either of the above high-resolution protocols, but don't add GelGreen in the gel OR loading buffer. Instead, after the gel has run, add it to 100 ml of post-stain solution. Stain gel on rocking platform or orbital shaker (gentle shaking!) for 30-60 min. This staining solution can be reused a few times. Keep the post-stain solution covered in foil and keep in closed plastic box so it doesn't evaporate.

<u>Notes on recycling buffers:</u> Its OK to recycle TBE or LAB buffers quite a few times, but its important to use the same *batch* of buffer for both gel and tank buffer e.g. don't use fresh buffer for gel and recycled buffer for the tank. Don't recycle TAE – the acetate component is lost after the gel is run.

<u>Notes on loading buffers:</u> For all samples, use loading buffer that has GelGreen added to it (1 uL GelGreen per 1 ml loading buffer) – this pre-loading with dye helps to prevent the faster-running DNA bands from 'sweeping up' all the GelGreen in the agarose. For restriction digests, use loading buffer that has SDS added (purple lid for NEB buffer). For all other DNA samples, use loading buffer with no SDS (white lid for NEB buffer).

Reagents

TBE: 20X stock = 1.78 M Tris base, 1.78 M boric acid, 40 mM EDTA (adjust to pH 8). Use at 0.5X.

LAB: 25X stock = 250 mM lithium acetate, 250 mM boric acid. Use at 1X.

TAE: 20X stock = 800 mM TRIS base, 400 mM acetate, 20 mM EDTA (adjust to pH 8). Use at 1X.

<u>Pre stained No-SDS loading dye:</u> 1µL gel green stock concentrated stock per mL of loading dye (purple capped tube for NEB reagent)

<u>Pre stained SDS loading dye for digest samples:</u> 1µL gel green stock concentrated stock per mL of loading dye (white capped tube for NEB reagent)

<u>Dilute Gel Green stock:</u> 1µL Gel Green in 50µL RO water.

<u>Post Stain Solution</u>: 10 µL of conc. GelGreen dye in 100 mL RO water.