TopoGEN
Research, Diagnostic and Therapeutic
Biomedical Reagents, Kits and Services
www.topogen.com

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TIPS FOR OBTAINING HIGH QUALITY AGAROSE GEL RESULTS

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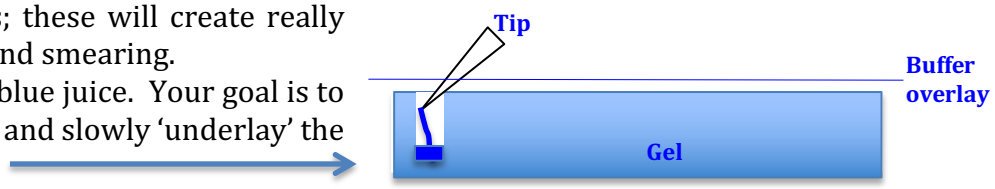
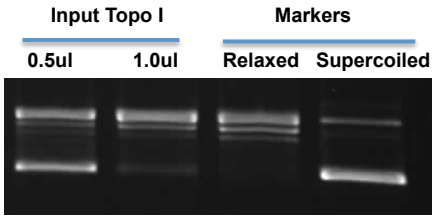
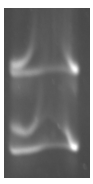
To obtain the most cosmetic, publication quality gels, here are some GUIDELINES.

1. Cast gels as required (EB or non-EB in gel, final ethidium bromide concentration 0.5ug/ml in gel for EB gels) using molten agarose that is gently mixed to avoid bubbles.
2. Cast gels at room temperature (optional: move to cold room to enhance solidifying gel). Be sure gel is well set up and solidified before removing comb.
3. GENTLY pull comb straight out with to and fro rocking motion to release from gel matrix. Pull straight up and do it slowly. The goal is to remove the comb without damaging the wells or leaving behind any debris that may contaminate band integrity.
4. Cover the gel with suitable running buffer (EB or non-EB). Don't use too much or too little overlay. Just enough to submerge the gel fully (about 0.5-1 cm overlay).
5. Use a p200 or similar pipettor to wash out each well. Inspect for debris or damage to well. Do not use damaged or debris-laden wells; these will create really ugly bands and cause a lot of band smearing.
6. Carefully load the sample in blue juice. Your goal is to place the tip just inside the well and slowly 'underlay' the sample. See image to right:
7. If the sample bleeds out or does not properly underlay in well, discard that well and try again.



8. Typically, we run our gels about 1-2 hr maximum run times for plasmid based assays, and 30' for kDNA gel separations (15-30' is usually adequate for kDNA). Set the voltage to get the dye front about ½ to ¾ down the gel length with these run times in mind.
PLASMID RUNS: typically 100 v.
kDNA RUNS: typically 100-200 v.
9. The reason we keep our run times short is to MINIMIZE band diffusion (which happens over time). For the same reason, keep your staining and de-staining times to a minimum. Usually 15' stain or 10' for de-stain (with water and several fluid changes). Take your pictures immediately. DO NOT let gels sit around more than necessary before photo-documenting. Take multiple images with different exposures to get the best picture possible.





Debris in well and poor loading leads to band distortion which are non-cosmetic.

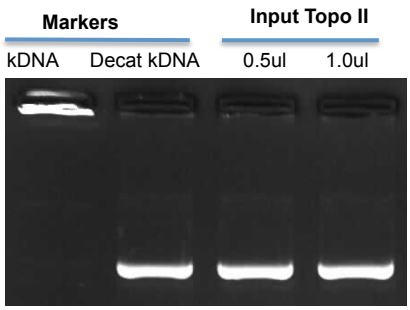


Fig 1. Topo I (Left) and Topo II Titration data (right). Your data should look similar to this. The topo I is plasmid based and topo II is kDNA based. In the case of topo I, the enzyme titration is obvious; in the case of topo II, there was excessive topo II so both lanes (0.5ul and 1 ul) displayed full activity. NOTE: Topo I gel has no EB; topo II gel has EB in gel/buffer.

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ADDITIONAL SUGGESTIONS:
EB gels are best for detecting cleavages (IFP) but not for relaxation (use EB gels to detect top1 and top2 cleavages).
NON-EB gels are best for relaxation or catalytic measurements of activity (used to confirm that enzyme is working properly.
Keep gel units clean and wash/rinse well before use.
AFTER electrophoresis, stain-destain appropriately and take pictures as soon as possible. This keeps bands tight and minimizes diffusion of bands.