

Casting 12 in. Gel for Quail Allele Scoring*

Polyacrylamide Gel Solution (4.8% Gel)

*Instructions are for preparing gel solution and casting a 12 in. well-to-read gel for the ABI 377 DNA Sequencer.

- 1) Make sure plates are clean and smudge-free. If necessary, wash again with lab soap and dH₂O and allow to air dry. Plates may be dried using larger Kimwipes.

Rinse spacers, comb and casting device with dH₂O as well and allow to air dry.

- 2) Prepare 10X TBE buffer, if necessary, and use within 7-10 days:

For 0.5 L: 54.0 g Tris, 27.5 g boric acid, 4.15 g EDTA in 0.5 L dH₂O

For 1.0 L: 108 g Tris, 55 g boric acid, 8.3 g EDTA

- 3) Add first 4 ingredients listed below together in a 150 ml or 250 ml beaker. Mix with a stir-bar on medium speed for 5 minutes.

A) Urea	5.4 g
B) 40% (19:1) Acryl/Bis	1.8 ml
C) dH ₂ O	9.0 ml
D) Amberlite Resin (AG501X8)	0.3 g
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E) 10X TBE	1.5 ml
F) APS (100 mg/ml)	120 μ l
G) TEMED	12 μ l
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Total Volume 15 ml

- 4) Set up filtering vacuum flask with 0.2 μ m filter and FIRST filter 10X TBE, then the acrylamide solution. Do not filter the TBE after the acrylamide solution--the Amberlite will remove the ions from the TBE (this is bad).
- 5) Degas filtered solution for 5 minutes.
- 6) While degassing solution, make sure plates and associated items are clean. Wipe face of plates off with Kimwipes if necessary.
- 7) Place cassette on top of Styrofoam blocks to raise it off working surface, Put some paper towels at bottom and top edge to soak up any leaky acrylamide. Place rear plate into the cassette (ETCHED SERIAL NUMBER DOWN), and slide forward until plate "locks" into place in cassette. Take spacers and moisten one

side of each (opposite sides of spacers as they should face each other with the same orientation: namely skinny part at top of rear plate and grooves pointed towards each other).

Stick spacers down onto rear plate and make sure there are no air-pockets between plate and spacers. Allow spacers to dry onto plate.

- 8) Place front plate on the top end of the rear plate, making sure that the space for the comb is oriented to the inside of the two plates (to allow comb to be placed in its home).
- 9) Get acrylamide solution. Get ready to add APS and TEMED to solution and then finish the rest of the process ASAP so gel does not polymerize while you are still futzing around with it.
- 10) Draw gel solution (after adding APS and TEMED) into syringe. Put the rear edge of the front plate in your other hand. Squirt (gently) gel solution under edge of front plate and gently move front plate so that it is resting on top of rear plate. Begin sliding front plate towards the bottom of the cassette while squirting acrylamide solution in front of plate edge. Back off if any bubbles form and resume when they are dislodged.

Slide front plate all the way down so that the bottom edges of the two plates are flush. Lock plates in place and place comb (sharks-tooth side pointing out –e.g. don't stick sharks tooth part into gel) into gel.

- 11) Place 3 “Bulldawg” clamps (funny name) across top of gel (equally spaced) and make sure that they do not contact the comb.
- 12) Allow 1.5 to 2 hours for gel to dry prior to use. If preparing for a morning run, wrap bottom end of plates in Saran Wrap so that gel does not crystallize.