SEQUENCING GELS

1. Standard 8% polyacrylamide/urea sequencing gel

   Wide plates for BRL or IBI box

   40% (w/v) acrylamide stock

   (acrylamide:bisacrylamide, 19:1) 12 ml

   Urea 28.8 g

   5 X TBE 12 ml

   H2O 13.5 ml
2. Dissolve urea with stirring (it gets cold) can warm to ÷30øC. NO FURTHER
3. De-gas solution under vacuum
4. ADD 10 ul TEMED 700 ul 10% ammonium persulphate (make fresh)
5. Pour gel immediately it cross links in about 10 minutes Can be used after ÷45 min
6. Pre-run 15 mins
7. Run at 60 watts constant power

_____ *** Denaturing Double Stranded Templates

- 2 ug DNA 2 ul
- H2O 16 ul
- 4 M NaOH 1 ul (.2 M final)
- 4 mM EDTA 1 ul (.2 mM final)
- Leave at room temperature 5 min.
- 2 M NH4OAc 2 ul (1/10th vol)
- 100% EtOH 55 ul (2.5 vol)
- Centrifuge 5 min, remove sup and resuspend in 7 ul H2O

PREPARING THE SEQUENCING GEL

1. Clean the glass plates with Kimwipes, first with diluted Liqinox, then with distilled water, then with ethanol until they are squeaky clean. (This is extremely important if you want to avoid air bubbles when pouring the gel.)
2. Spray a very small amount of Pam on one side of the larger plate and rub in very well with Kimwipes.
3. Assemble the plates with spacers on levelling plate on top of an empty blue tip box. Clamp with bulldog clamps, one on each side.
4. Pour the gel using a 60cc syringe to dispense the acrylamide; allowing it to run between

the plates by capillary action.

5. When the plates are filled with acrylamide, insert the comb upside down, approx. 1cm deep. Clamp the comb and the sides of the gel with bulldog clamps, but do not clamp the bottom unless you use a bottom spacer. Place plastic wrap around the bottom of the gel to prevent it from drying out.
6. Note: ACRYLAMIDE IS A NEUROTOXIN. WEAR GLOVES AND LAB COAT WHEN HANDLING. READ THE MSDS BEFORE USING THIS CHEMICAL.

A SIMPLE AND RAPID PROCEDURE FOR POURING SEQUENCING GELS

(BioFeedback)

The method is based on the physical phenomenom of surface tension and its corollary, capillary action. Because of capillary action, gravitaional force is not needed to draw the fluid into the gel assembly, so it can lie flat. Moreover, if the assembly lies flat, it is not neccesary to seal the edges for surface tension will prevent the solution from flowing out. The problem of air bubbles is almost non-existent.

Sequencing plates are detergent-cleaned, rinsed with distilled water and dried with ethanol. The glass surfaces that will be in contact with the gel are coated with Pam. The plates are carefully apposed with the side spacers in place. One clamp is used at the middle of each side. The assembly is placed on a flat level surface. Raising the assembly above the bench top (we use blue pipette tip boxes) makes it easier to fill. Gel solution at room temp. Containing 40ul N,N,N',N' - tetramethyethylenediamine (TEMED) and 500ul 10% ammonia persulfate per 100mL of solution is released slowly form 25 mL pipet into the middle of the well at what will be on top of the assembly. The solution is pulled into the space between the plates and fills it completely. Finally, the comb is inserted and the assembly fully clamped. It is particularly important to clamp the comb in place. Allow the filled and clamped assembly to remain in place until the gel has polymerized.

TIPS:

- --when leaving the gel overnight: wrap bottom with saran wrap to prevent drying of gel.
- --do not clamp bottom unless using bottom spacers.

References:

Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbour Press, Cold Spring Harbour, N.Y.

Taken from:

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