

PREPARATION OF A SEQUENCING GEL

- 1 IN A BEAKER : 33.6 g UREA
 8.0 mL 10 X TBE
 12.0 mL 40% ACRYLAMIDE (38:2)
 H₂O

 80.0 mL

 50.0 μ L TEMED
 240.0 μ L 10% APS

- 2 REACTION IS ENDOTHERMIC ----> HEAT & SWIRL IN A 37 C WATER BATH UNTIL UREA DISSOLVES

- 3 ADD 240 μ L 10% APS ----->SWIRL

- 4 TAKE 4 mL OF ACRYLAMIDE MIXTURE & ADD 7 μ L OF TEMED

- 5 RUN THE 4 mLs AROUND THE EDGE OF THE GEL (QUICK SEAL) ----> WAIT 5 MIN (WILL POLYMERIZE QUICKLY)

- 6 RINSE W/ WATER TO REMOVE XTRA TEMED

- 7 RINSE W/ ACRYLAMIDE W/O TEMED TO REMOVE THE XTRA WATER

- 8 ADD 50 μ L OF TEMED TO THE REMAINING MIXTURE AND POUR THE REST OF THE GEL

- 9 INSERT THE COMB (CAREFUL THERE ARE NO BUBBLES) AND CLAMP THE COMB IN PLACE & LAY FLAT

- 10 LEAVE FOR @ LEAST 2 HRS FOR GEL TO POLYMERIZE