Gradient Gels for titin

Granzier group protocol

1. Materials:

2% and 12% Acrylamide-bis-acrylamide (4C refrigerator in light proof bottles)
20% Ammonium persulfate
    0.2g APS in 1mL H2O
TEMED (4C refrigerator)

Small cooler and ice
6-flat glass gel plates, 3-curved glass gel plates, 6-plate spacers, 3-combs, and plate stand
Gel chamber, with cover and 4-clamps
Gel sealing grease
Gradient maker chamber, with stirrer, and variable height stand

2. Assemble Chamber

Wash plates, spacers and combs with 20% SDS, separate sponge, rinse with hot then deionized water, and dry on stand in 37C oven.
Stack flat plate, spacers, & curved plate:
    Place a flat plate on a clean paper towel
    Grease a "T" spacer along the sides where the glass plates will rest
    Place "T" spacer on one side of the flat plate, do the other side
    Place the curved glass plate on top
    Repeat placing the next two sets on top
Lay the chamber down and lightly grease the gasket
Change gloves
Slide the stack in from the top and place three flat plates on top
Put chamber cover into position, stand chamber on end, and press plates to the bottom.
Attach four 2 1/2" "Bulldog" clamps along the sides of the gel chamber

3. Seal Bottom of Chamber

Pipette 5mL 12% solution, 15uL APS, and 9uL TEMED into a test tube
Mix by covering with Parafilm and inverting several times
Pipette 5mL into chamber, running the solution down the back plate to prevent bubbles
Rock the chamber side to side to ensure a good coating and dislodge air bubbles
Cover chamber with plastic wrap.
Keep still and allow 5-10min for polymerization (rock to check.)

4. Rinse the Gradient Maker

Close the valves by moving each lever up
Fill the reservoirs with distilled H20
Turn on the mixer and open both valves
When empty, turn off mixer, cover with tissue and invert to remove the remaining water.

5. Pour Gels

Set height of mixer so that the bottom is level with the top of the chamber
Place filling tube so that it rests in the center of the first gel chamber
Pipette:
    12% solution into the 12% and Gradient test tubes
    2% solution into the Gradient test tube
    APS and TEMED into the test tubes
    mix each tube by inverting
Pipette an amount of gradient% equal to the amount of 12% made into the left gradient maker reservoir
Pour the full amount of 12% solution into the right reservoir (blot tube bottom first of any ice water)
Turn on mixer and open right valve, pushing top if necessary to start the siphon
Immediately after 12% solution flows, open the left valve
When the gel chamber is 1/2 full, very slowly lower the mixer two turns of the knob (1")
When the level of solutions in the reservoir is 2mm above the top of the exit drain, add the remaining gradient% solution (blot tube bottom)
When the fluid reaches to 5mm below (for 10 tooth comb) the lower part of the curved plate remove the filling tube and collect the remaining gradient% solution into its test tube to check for polymerization
Immediately insert the comb (very slowly so as not to disturb the gradient)
Immediately rinse the gradient maker reservoirs as described above
Repeat for the remaining two gel chambers

6. Finish

Let gels sit until polymerization is complete. Do not touch!!!
Disassemble chamber, store plates at 4C in a ziplock with a small amount of dH2O

Gel Recipes

Short Gels:

2.35% - 12% acrylamide-bis-acrylamide

	2.35%, 8 mL	12 %, 4 mL
2%	7.72 mL	none
12%	0.28 mL	4.0 mL
20% APS	44.0 uL	12.0 uL
TEMED	10.4 uL	7.2 uL

2.6% - 12% acrylamide-bis-acrylamide

	2.6%, 8 mL	12%, 4 mL
2%	7.52 mL	none
12%	0.48 mL	4.0 mL
20% APS	44.0 uL	12.0 uL
TEMED	10.4 uL	7.2 uL

Short Gel: Volume 12%, mL = 0.8 x desired% - 1.6

Volume 2%, mL = 8 - volume 12%

Tall Gels:

2.35% - 12% acrylamide-bis-acrylamide

	2.35%, 10.45 mL	12%, 6.45 mL
2%	10.08 mL	none
12%	0.365 mL	6.45 mL
20% APS	57.5 uL	19.35 uL
TEMED	13.59 uL	11.61 uL

2.45% - 12% acrylamide-bis-acrylamide

	2.45%, 10.45 mL	12%, 6.45 mL
2%	9.98 mL	none
12%	0.47 mL	6.45 mL
20% APS	57.5 uL	19.35 uL
TEMED	13.59 uL	11.61 uL

Tall Gel: Volume 12%, mL = 1.045 x desired% - 2.09

Volume 2%, mL = 10.45 - volume 12%

Stock Solutions

2% Acrylamide-Bis-Acrylamide solution

Acrylamide	5.0 g
Bis-Acrylamid	0.1 g
10X Fairbanks run. buff.	25 mL
20 % SDS	1.25 mL
100% Glycerol	12.46 mL
dH_2O	up to 250 mL

Store: 2-8° C, in dark container

12% Acrylamide-Bis-Acrylamide solution

Acrylamide	30.0 g
Bis-Acrylamide	0.6 g
10X Fairbanks run. buff.	25 mL
20 % SDS	1.25 mL
100 % Glycerol	37.52 mL
dH_2O	up to 250 mL

Store: 2-8° C, in dark container

10X Fairbanks running buffer, 1L, pH 7.5 with CH_3COOH

Trizma Base	40 mM (48.44 g)
Na-acetate	20 mM (16.4 g)
EDTA	2 mM (40 mL of 0.5 M stock sol.)
dH_2O	up to 1 L

Store: room temperature

1X Fairbanks running buffer, 500mL

10X Fairbanks sol.	50 mL
20 % SDS	2.5 mL
dH_2O	up to 500 mL
ß-Mercaptoethanol	400 uL (add just before using the solution)

Store: always make fresh