Laemmli Gels (Tris Glycine)
(>~20kDa)

Procedure:

1. Assemble gel apparatus

2. Prepare gel solution for the resolving portion of the gel first:

One 10% Acrylamide gel

Gel Volume:	15ml	30ml
Gel thickness:	(Thin)	(Thick)
(a) ddH_2O	6.2ml	12.4ml
(b) 4X LOWER Tris	3.75ml	7.5ml
(c) 30% acrylamide	5.0ml	10.0ml
(d) 10% APS	50µl	80µl
(e) Temed	5µL	8µL

3. Syringe in gel solution between glass plates (<u>be careful not to chip the glass plates with the metal</u>). Leave enough space above the resolving gel for the stacking gel (the rule of thumb is that the thickness of the stacking gel should be approximately the same as the comb's length.

(Hint: use 18g11/2 needle for 1.5mM spacer thickness gels; 22g11/2 for 0.75mM spacer thickness.)

If the gel polymerizes before you get it all out of the syringe try chilling the gel solution before adding the Temed; this will slow down the polymerization.

4. Gently overlay a thin layer of isopropanol over the gel with a Pasteur pipette; i.e., just enough to prevent oxygen from getting to the acrylamide. Let gel polymerize ~15-30 min (or more).

5. Drain or wick off isopropanol from resolving gel.

6. Insert comb into gel assembly apparatus.

7. Prepare gel solution for the stacking portion of the gel:

Gel Volume:	7ml	10ml
Gel thickness:	(Thin)	(Thick)
(a) dH_2O	3.9ml	5.6ml
(b) 4X UPPER Tris	1.9ml	2.7ml
(c) 30% acrylamide	1.3ml	1.8ml
(d) 10% APS	160µl	160µl
(e) Temed	8µL	9µL

8. Syringe gel solution in between plates.

9. Let polymerize ~40min +

10. Remove comb.

11. Add 1X gel running buffer to the wells. Wash the wells by pipetting the buffer up and down.

12. Add 2-ME to appropriate volume of 3X SAMPLE buffer.

13. Add appropriate volume of 3X sample buffer to sample.

14. Incubate samples 10-20min at ~ 68^{o}C.

15. Assemble upper chamber onto gel.

16. Add 1X gel running buffer to the lower and upper buffer chambers.

17. Run ~20mAmp for 15 – 20h (can run up to 60mAmp but don't let it run off)

<u>Solutions, buffers and stuff</u>:

1. 10X gel running buffer — 1X Concentration

(a) Tris base	60g	25mM
(b) Glycine	288.5g	192mM
(c) SDS	20g	0.1%
(d) ddH_2O to	2.0L	

(e) DO NOT pH. Leave as is; it should be ~8.2
(f) f, store RT

2. 4X Lower Reservoir Tris buffer — 1X Concentration

(a) Tris base	90.9g	375mM
(b) SDS	2g	0.1%
(c) pH w/12N HCl to	8.8	
(d) dH_2O to	500ml	

(e) f, store RT

3. 4X Upper Reservoir Tris Buffer — 1X Concentration

(a) Tris base	30.3g	125mM
(b) SDS	2g	0.1%
(c) pH w/12N HCl to	6.8g	
(d) dH_2O to	500ml	

(e) f, store RT

4. 3X SDS Sample buffer — 1X Concentration

(a) Glycerol	1.5ml	10%
(b) 20%SDS	1.5ml	2%
(c) 4X Upper buffer	1.875ml	64mM
(d) Bromophenol blue	~60mg	~0.1%

(e) Store RT
(f) Add 1uL 2-ME per 20μL immediately before adding to sample

5. 30% Acrylamide (100ml)

(a) Acrylamide	29g
(b) Bis-acrylamide	1g
(c) dH_2O to	100ml

(d) Filter through 0.45μM filter
(f) Add a small quantity of BIO-RAD AG 501-X8 (D) ion exchange resin; i.e., enough to cover bottle bottom.
(g) Store in brown bottle @ 4^{o}C

6. 10% Ammonium persulfate (APS)

   (a) APS 100mg
   (b) dH_2O 1ml
   (c) Store at -20^oC

   Note that APS does not last very long. Although it stores at -20^oC, it is best to make it up fresh if it is old.

7. Fixative (1.1L)

   (a) Glacial Acetic Acid 100ml
   (b) Methanol 500ml
   (c) dH_2O 500ml

8. Coomassie Blue Stain (1L)

   (a) Coomassie Brilliant Blue 1g
   (b) Methanol 500ml
   (c) Dissolve BB
   (d) Glacial Acetic Acid 70ml
   (e) dH_2O 430ml
   (f) (Sambrook/Maniatis recommends filtering through Whatman #1; I never did this)

9. Protein Destain (4L)

   (a) Glacial Acetic Acid 280ml
   (b) Methanol 800ml
   (c) Glycerol 120ml
   (d) dH_2O (to 4L) 2800ml

Notes:

Acrylamide is a neurotoxin. It is advisable to work in a fume hood when weighing the stuff out. Once polymerized acrylamide is supposedly benign