1. Set up plates using '2D' spacers (1.5mm thickness) or thin spacers (0.75mm thickness) and black clips.

2. Prepare gel solutions: In Erlenmeyer flasks mix running gel and stacking gel solutions. (Recipes are posted above bench top.) Wait before adding APS and Temed. Record % acrylamide of gel on gel sheet. Choose flasks that are appropriate size, i.e., too small will affect de-gassing. (For example, use 125ml flask for 1 running gel and 50ml flask for 1 stacking gel).

3. Degas, etc: 
   a) Check oil in de-gasser pump. Degas running gel for 7 min while you seal the plates.
   b) Thaw 10% APS (ammonium persulfate) in water bath.
   c) Mark 2.5cm from top center of eared plates.

4. Seal plates: with 1.3% agar (door of frig; microwave to melt with cap loosed or will explode; do not over-microwave) using Pasteur pipette (If you make a new solution of agar, you must microwave to almost boiling, swirl to dissolve, and microwave again and swirl several times to completely dissolve the agar). Do not use agarose for sealing. Extrude bubbles from the pipette before applying agar. Apply on one side, bottom, then other side. Especially at bottom junctions of spacers.

5. Pour plugs:
   a) Pull out 2ml (for thick gels; 1ml for thin gels) of running gel solution from degassing gel solution per gel. Put in Falcon tube or small flask. Continue to de-gas the rest of the running gel on ice if doing many gels so it won't polymerize too fast later when pour the gels.
   b) Add Temed (2\(\lambda\)/ml) and APS (8\(\lambda\)/ml).
   c) Quickly swirl and pour. If you're preparing more than one slab gel, add 2ml at a time with a pipette into a corner so it goes down the inside of a side spacer. Use a pipette.
   d) Squeeze bottom of the plates to release bubbles. Double check for slow streams; if so, give quick squeeze then leave alone. Don't try to get every bubble out. Move quickly because plug polymerizes fast.
   e) Let plug sit for 4min; check to make sure it polymerized.

6. Pour running gels:
   a) Add temed and APS (use chart for values).
   b) Quickly swirl gel solution and pour down middle of tipped back plate up to the 2.5cm mark. All the gels should be at the same level relative to the 2.5cm mark. This insures equal sized gels and thus allows comparison of proteins run on two different gels.
   c) Return flask to ice.
   d) Overlay gels with ddH2O. Use long Pasteur pipette with drawn out end (in flame) so ddH2O comes out slowly. Technique: Apply steady and constant stream of water, but not too much at one time or it will mix in with the gel mix.
   e) Let gel polymerize for at least 45min.

7. Pouring stacking gel:
a) De-gas stacking gel for 5-7 min.
b) Add Temed and 10% APS and swirl to mix.
c) Pour off the ddH20 from step 6.
d) Pour. Ok if stack overflows; let it brim over. (stack does not get overlaid with water.)
e) Insert thick comb (or thin one if you used thin spacers). Remove any bubbles. Avoiding having to take out comb while you're trying to get out bubbles, or else you will lose too much of the stack.
f) Let stack polymerize for 15 min to 45 min.

8. Prepare samples:
a) GSD should be thawed and mixed well.
b) Add GSD to make a 1:3 dilution with sample (or 1:4 or 1:5 or even 1:6 if volume is a consideration; a 1:3 dilution makes a final concentration of 2.2% SDS while a 1:6 makes a 1.1% which is still fine for coating proteins; however, there is less glycerol so the sample doesn't layer as well).
c) Boil samples for 3 min at 95-100°C (or longer if the volume is larger, ie, >100ul).
d) Marker lane: 5-7.5 markers + volume of buffer (same buffer as in adjacent lane sample, eg., lysis buffer) equivalent to the adjacent sample volume + GSD. Boil 3min.
e) Blank lanes: buffer (same volume and type as in adjacent sample or marker lane) + GSD.
   -If blank lane is between two lanes with different buffers, make it a transition lane that has 50% the level of each buffer. It is better to have two blank lanes if the transition is drastic, with each having identical constituents to the sample lane adjacent to it.
   -Don't have to boil because there are no proteins. The salts and glycerol contained in lysis buffer help keep the marker lane straight by preventing osmosis of proteins which could be caused by a salt gradient between the sample and marker lanes and the running buffer.

9. Fill out 1D Slab Gel Sheet. Record gel# and blot# (if necessary), % acrylamide, etc.

Example

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample volume</th>
<th>Extra (name) buffer</th>
<th>GSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>5</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Marker</td>
<td>5</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>A</td>
<td>50</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

10. Set up gel in apparatus:
a) Remove clips and bottom spacer and comb.
b) Put plates into apparatus.
c) Pour 1X running buffer into top well all the way to the top of the plate (Buffer should be well mixed after diluting from 10X stock). Use pasteur pipet to rinse out the lanes quickly before additional acrylamide polymerizes in the well (the additional acrylamide comes from the stacking gel solution at the top of the gel where the comb is inserted; oxygen from the air inhibits the polymerization of some of the acrylamide, but after the comb is pulled, the unpolymerized acrylamide flows to the bottom of the well; when the running buffer is added to the top chamber, no oxygen is there to inhibit the polymerization and you will get
variable amounts of additional polymerized acrylamide in the wells if you don’t rinse it out.

d) Then pour 1X running buffer into the bottom well until it just covers the entire bottom of the gel. Use curved pasteur pipet or syringe with curved needle to blow out bubbles from bottom of gel.

e) Load samples onto gel using long white round pipet tips that have 1cm cut off with razor blade.

f) Run gel overnight at 6-8 mAmps per gel. (at 10mA, ~14 hr to top of plug)

11. Further processing: Transfer gel onto nitrocellulose membrane (for Western blotting) or stain and/or dry gel.