Protein Gel

4% Stacking Gel	
40% acrylamide/bis 19:1	1 ml
Upper Tris	2.5 ml
dd H2O	6.3 ml
10% SDS	100 ul
10% APS	50 ul
TEMED	10 ul

Lower Gel	6%	9%	12.50%	15%
30% acrylamide/0.8% bis	6 ml	9 ml	12.5 ml	6.75 ml
Lower Tris	7.5 ml	7.5 ml	7.5 ml	7.5 ml
ddH2O	16.04 ml	13.04 ml	9.54 ml	7.04 ml
10% SDS	300 ul	300 ul	300 ul	300 ul
10% APS	150 ul	150 ul	150 ul	150 ul
TEMED	30 ul	30 ul	30 ul	30 ul

Mix the acrylamide, lower tris, and H_2O
Degas
Add the 10% SDS, 10% APS, and TEMED
Swirl the flask to mix
Pour the lower gel so that the are 2 inches between the top of the glass and the top of the lower gel
Add 1 mL 100% Isopropanol to even out the top of the gel
Pour off the Isopropanol and wash with 2 mL ddH_2O
Let gel polymerize for 10 minutes before adding the stacking gel
Make the stacking gel following the same steps as the lower gel (Mix, degas, add SDS, APS, and TEMED)
Pour the stacking gel and insert the comb

Prepare the samples:
Load a total of 10-20 uL of loading buffer and sample per lane
Make a dye mastermix of 20 uL dye:5 uL 1 M DTT (Bring to a final sample [] of 1x)
Aliquot the dye into clean eppendorf tubes
Add sample to each dye aliquot
Pipet up and down to mix
Incubate at 95 C for 2 minutes before loading on the gel

Setting up the gel:
Clamp the gel into the gel box
Add 500 mL 0.1% SDS-Tris/Glycine buffer to the top and bottom reservoirs of the gel box (100mL Tris, 10mL 10% SDS, 890 mL dH_2O)
Wash the wells of the gel by squirting buffer into them with a syringe
Remove air bubbles from the bottom of the gel by squirting buffer in between the glass plates
Load the samples using gel loading tips

Running the gel:
Run the gel at 30 milliamps for 5 hours