University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)		
	Version:	1		
	Description:	How to run a SDS-PAGE. Casting a Gel Video		
	Trigger:	Use this procedure to run gel electrophoresis on cell pellets.		
Last updated	Name:	Elizabeth Brookes	Date:	19.07.14
You will need	Time:	3 hours		
	PPE:	Gloves Lab coat		
	Equipment:	Vortex Heat Bath		
	Materials:	Stacking Gel Buffer: ○ 0.5M Tris-HCl pH 6.8 ○ 0.4% SDS Separating Gel Buffer: ○ 1.5M Tris-HCl pH 8.8 ○ 0.4% SDS Acrylamide Stock Solution: ○ 29% Acrylamide ○ 1% Bis-Acrylamide 10% Ammonium Persulfate TEMED Cell Pellet Milli-Q Water 2x Sample Buffer: ○ 60mM Tris-HCl pH 7.5 ○ 2% SDS ○ 10% Glycerol ○ 0.02% Bromophenol Blue ○ 1% β-ME Electrode Buffer: ○ 25mM Tris ○ 190mM Glycine ○ 0.1% SDS pH 8.4		

Making the SDS-PAGE Gel	
Step 1	Assemble the multi-casting chamber according to the manufacturer's instructions.
Step 2	Making the separating gel in a suitable beaker by following the formula below. Ammonium persulfate and TEMED should be added before pouring the gel. ***Note: Daisy recommends using the 15% formula for our protein.***

Separating Concentration	**8%**	**10%**	**12%**	**15%**
Separating Gel Buffer (mL)	16	16	16	16
Acrylamide Stock Solution (mL)	16	20	24	30
Water (mL)	27.4	23.4	19.4	13.4
10% Ammonium Persulfate (mL)	0.6	0.6	0.6	0.6

	TEMED (mL) 0.05 0.05 0.05 0.05 **NOTE: We are not doing this. We have purchased the Bio-Rad TGX Fast Cast acrylamide solution gel kit, which has it's own instructions in the box.**
Step 3	After adding the ammonium persulfate and TEMED immediately mix the gel solution gently and carefully introduce the solution into the gel casting chamber. Stop pouring the gel when the solution has reached about 6cm in height and layer about 0.5mL isobutanol on top of the separating gel solution to keep the gel surface flat. Allow the gel to polymerise for about 10-30 minutes. N.B. FOLLOW TGX FASTCAST DIRECTIONS INSTEAD
Step 4	When the gel has polymerized a distinct interface will appear between the separating gel and the isobutanol. Drain off the isobutanol and was the surface with distilled water.
Step 5	Make the stacking gel in a suitable beaker by following the formula below. Ammonium persulfate and TEMED should be added before pouring the gel. **Separating Concentration** | **8%** | **10%** | **12%** | **15%** **Stacking Gel Buffer (mL)** | 16 | 16 | 16 | 16 **Acrylamide Stock Solution (mL)** | 16 | 20 | 24 | 30 **Water (mL)** | 27.4 | 23.4 | 19.4 | 13.4 **10% Ammonium Persulfate (mL)** | 0.6 | 0.6 | 0.6 | 0.6 **TEMED (mL)** | 0.05 | 0.05 | 0.05 | 0.05 N.B. FOLLOW TGX FASTCAST DIRECTIONS INSTEAD
Step 6	After adding ammonium persulfate and TEMED immediately mix the gel solution gently and carefully introduce the solution onto the separating gel until the solution reaches the top of the front plate. Carefully insert the comb into the gel until the bottom of the teeth reach the top of the front plate. Allow the gel to polymerize within 10-30 minutes. N.B. FOLLOW TGX FASTCAST DIRECTIONS INSTEAD
Preparation of Samples for SDS-PAGE: Samples from Ni-NTA Purification	
Step 1	In separate microfuge tubes add 20μL of the various protein samples and 20μL of 2x sample buffer.
Step 2	Heat the microfuge tubes for 2-3 minutes at 100℃.
Step 3	Spin the microfuge tubes for 2 minutes.
Preparation of Samples for SDS-PAGE: Samples from Induction	
Step 1	Add 50μL of Milli-Q water to the cell pellet and vortex until the cells are completely resuspended.
Step 2	Then add 50μL SDS-PAGE 2x Sample Buffer and mix well by vortexing for 10 seconds.
Step 3	Heat for 2-3 minutes at 100℃ in a heat bath.
Step 4	Immediately vortex the material for 2 minutes to break up the viscous material.
Step 5	Spin in a microfuge for 2 minutes.
Step 6	Immediately remove the top 35μL to a fresh microfuge tube to avoid the viscous DNA-rich portion that has been pelleted. If there is a viscous sample clinging to the tip remove it with a tissue and continue to collect 35μL into a fresh tube. *Note: You may need to repeat the spin and collection to ensure that the sample can be removed.*
Preparing the Gel	

Step 1	After the stacking gel has polymerized remove the comb carefully and place the gel into an electrophoresis chamber. The gels should be facing inward (the short plate faces inward). There are two types of plastic holders sitting to the right of the casting apparatuses. Both work, but use the easy to use, pre-assembled one. Place the gels in the apparatus, then use the clear plastic clamps to secure the gels.
Step 2	Add electrode/running buffer to both the internal and external chambers. The buffer should fully cover the gel apparatus and wells. Note that the electrode/running buffer for the inside part of the well (touching the gels) can be reused once (inside being the buffer that touches the wells). The first time it runs, the mA on the power pack will start off at around 50-80 and then drop to below 50. The second time, it may start off above 100. (source: Dave; n.b. Cheng lab just throws out the inside buffer) The outside well buffer can be reused ad infinitum.
Loading Samples onto SDS-PAGE Gel	
Step 1	Load appropriate amount of molecular weight marker into both the first lane and the last lane: . For P7709V: Recommended load for SDS-PAGE Analysis is 15 µl, while 6 µl is recommended for Western blot analysis. George recommends 8 uL for WB. For P1172 there is no information online at https://www.neb.com/products/p7712-color-protein-standard-broad-range-11-245-kda#tabselect0, so maybe just use 8 uL. **This standard is supplied in gel loading buffer and is ready to use. Do not heat, dilute or add reducing agents before loading.**
Step 2	Load 20μL of each of the samples making a note of what each well contains. You should load the gels using a pipette with a drawn-out tip if available. Note the maximum capacity of a 15 well gel is about 36 uL.
Running SDS-PAGE	
Step 1	Using the electrode buffer run the electrophoresis at a constant voltage of 150V for 45 minutes-1.5 hr. Set the limiting current at 200 mA. The wires for the apparatus are under the bench in a bucket.
Step 2	When the dye front is about 1cm from the bottom of the gel, turn off the power supply and remove the gel from the apparatus.
Removing the Gel	
Step 1	Remove the gel sandwich from the apparatus and place it on a tray on the bench with the right side facing up i.e. well 1 to the left.
Step 2	For Coomassie staining, carefully insert a spatula between the plastic supports and gently prise the plates apart. For WB, keep the gel in the glass plates until you are ready to put it on the transfer sandwich.
Version history	*Describe the changes made in each new version of the protocol here.*