Cloning, expression, purification, and biophysical characterization of SSB from pathogenic bacteria

Week 5 – Purification of the SSB protein

- Resuspend the biomass (cells from the 1L growth) in 25 ml of cell lysis buffer (50 mM Tris-Cl, pH 8.3, 200 mM NaCl and 10% sucrose).

- Add 0.2 mg/ml lysozyme (final concentration) to the resuspended cells. Incubate at room temperature for 30 minutes.

- Sonicate the cells with two 1 minute pulses on ice and centrifuge the cells at 17000 rpm for 60 minutes (SS34 Oakridge tubes).

- Separate the supernatant into a clean 50 ml falcon tube.

- **PEI Precipitation**: Measure the volume of the supernatant and add PEI solution in a dropwise manner with stirring to a final concentration of 0.2%.

- Let the tube gently rock for an additional 20 minutes and spin the tube at 10000 rpm for 30 minutes.

- Transfer the supernatant to another tube and mark it at “PEI Supernatant”. Save a 40 ul sample in an Eppendorf tube and add 40 ul of the 2X SDS Gel loading dye. SAVE THIS SAMPLE FOR YOUR GEL “PEI SUP”

- To the pellet from the PEI step, add 35 ml of Buffer $T^{0.4}$ (50 mM Tris-Cl, pH8.3, 400 mM NaCl, 1 mM EDTA and 5 % glycerol). Resuspend with your pipette and break up the pellet. ~80% of the pellet will go into solution. Put your sample on the rocker for another 15 minutes at room temperature. Save a 40 ul sample in an Eppendorf tube and add 40 ul of the 2X SDS Gel loading dye. SAVE THIS SAMPLE FOR YOUR GEL “PEI PELLET”.

- Transfer the solution into a labeled SS34 Oakridge tube and spin at 13000 rpm for 15 minutes.

- Transfer the supernatant to a new 50 ml Falcon tube and proceed with Ammonium Sulfate Precipitation.

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- **Ammonium sulfate precipitation**

- Measure the volume of the supernatant fraction saved from the PEI step.

- Add ammonium sulfate to a final concentration of 30.8 %. To get this, add 170g/L of ammonium sulfate slowly to the tube and invert the tube to get it into solution.

- (170g/1000ml) * _____ ml = _____ g of ammonium sulfate.

- As a general rule of thumb, after weighing out the ammoniu sulfate, add one one fourths to the tube, get it resuspended, then add then next and so on…

- Incubate at room temberature (put the tube on the shaker) for an additional 20 minutes.

- Transfer the sample back into a new SS34 Oakridge tube and spin at 13000 rom for 30 minutes.

- Transfer the supernatant to a new 50 ml falcon tube and same the tube (label as AmS SUP). Save a 40 ul sample in an Eppendorf tube and add 40 ul of the 2X SDS Gel loading dye. SAVE THIS SAMPLE FOR YOUR GEL "AmS SUP".

- Resuspend the pellet in 2 ml of SSB Storage Buffer. Save a 40 ul sample in an Eppendorf tube and add 40 ul of the 2X SDS Gel loading dye. SAVE THIS SAMPLE FOR YOUR GEL "AmS PEL".

- This is the last step in your purification. The 2 ml resuspended sample is your SSB protein. Transfer the protein as 500 ul aliquots and save it at -20 DegC. Label it clearly!!

- Run the gel to make sure your purification was successful.

Checking protein expression using SDS-PAGE protocol

Prepare the SDS-gel using the following reagents:

12% Resolving gel layer (8 mL total):

DD water – 3.5 mL
30% acrylamide – 2.4 mL
1.5 M Tris pH 8.8 – 2.0 mL
20% SDS – 40 uL
10% APS – 80 uL
*TEMED – 7.5 uL
(mix everything and add Temed last –right before pouring the gel)

4% Stacking gel layer (7.5 mL total):

DD water – 5.5 mL
30% acrylamide – 1 mL
1 M Tris pH 6.8 – 940 uL
20% SDS – 40 uL
10% APS – 80 uL
*TEMED – 7.5 uL
(mix everything and add Temed last –right before pouring the stacking gel layer)

Gel preparation:

- Clean the 1.5 mm glass plate and spacer plate with DD water and ethanol and wipe clean.
- Assemble the gel plates using the Bio-Rad gel assembly cassette.
- Pour 7.5 ml of the resolving gel layer. Immediately top the gel with isopropanol to ensure an even gel-loading layer.
- Wait for 20-30 min to polymerize.
- Decant the isopropanol and wash with dd water. Wipe down the assembly.
- Pour the stacking gel till it reaches the top of the gel assembly. Slowly insert a clean 1.5 mm comb (inserting the comb at a slight angle-helps!). Also, make sue the lettering on the comb is facing you.
- Wait 20 more min

Loading samples and running the gel:

- Disassemble the gel assembly and keep the gel plates with the comb intact.
- Assemble the gel plates with the comb in the Bio-Rad gel loading cassette.
- Add 1L of 1X running buffer (100 mL of 10X running buffer + 900 mL ddwater). Load 10uL of sample in SDS-loading dye. Load protein size ladder.
- Run the gel at 120 milli amps for 1 hr.

Step 5. Coomassie Staining and destaining:

- Wash the gel cassette in ddwater to remove SDS buffer.
- Disassemble the gel cassette and add it to a plastic or glass dish.
- Add Coomassie stain-enough to cover the gel. Microwave on high for 20 sec and shake or rock at low speed for 15 min.
- Wash with dd water and add destaining solution enough to cover the gel.
- Crumple a Kim wipe and add it to the side of the dish (to soak up excess stain).
- Shake or rock gently for 1hr.
- Analyze gel bands using a light box.