Week 2. BIOL 4102

Protocol

SSB Protein Purification –Affinity Purification using Ni-NTA

Cell Lysis

1. Thaw the cells from the -80 freezer and resuspend the cells in 20 ml Resuspension buffer. Add lysozyme to the solution. Final concentration of lysozyme in the solution should be 0.4 mg/ml. The lysozyme stock solution provided is 10 mg/ml. [TA would have started the lysis before class to save time).

2. Lysozyme breaks the cell wall. Incubate the cells at 4 Deg C for 30 minutes. This step will break open the cells and release the contents (protein, chromosomal DNA, fatty acids, etc).

3. Sonicate the cells for 1 minute using the small sonicator tip. Sonication disrupts the chromosomal DNA and other cell debris. Sonicate in 10 sec gaps with 10 sec pulses; keep the sample on ice through the sonication procedure.

4. Transfer the sonicated sample to an Oakridge centrifuge tube. Balance the tubes and spin the sonicated sample for 45 minutes at 17000 rpm. This step will sediment the fatty acids, membranes, cell debris and most of the chromosomal DNA. The clear sample (supernatant) remaining after the spin is called the "clarified lysate". We assume your SSB sample to be soluble and will be in the clarified lysate. Save 40 ul of this sample in an Eppendorf tube and label it as 'CL'.

Fractionate over Ni^{2+}-NTA column

1. TA has packed a 4 ml Ni-NTA column and washed it first with water and then equilibrated it in binding buffer.

2. Pour the clarified lysate over the Ni-NTA column. Collect the sample that flows through in a clean tube and pour it again over the column. This step allows the SSB protein with the poly-His tag to bind to the Ni-NTA resin. Most off the other (non-specific) proteins from the cells will flow through. Save 40 ul of this sample in a Eppendorf tube and label it as "FT".

3. Wash the column with 100 ml of binding buffer. Save 40 ul of the wash sample in an Eppendorf tube and label it as 'W'.

4. Place a clean 15 ml falcon tube at the bottom of the column and add 10 ml of elution buffer to the column. The elution buffer contains 400 mM imidazole and should release the His-SSB from the column. Collect the 10 ml samples as 'eluate 1'. Repeat this elution step 2 more times and label the eluates as 'eluate 2' and 'eluate 3'. At the end of elution, you will have three tubes which should contain SSB protein.

5. Cap the column, don't let it run dry. Check to make sure that the protein is in the eluates using SDS-PAGE.

SDS-PAGE

1. Add 40 ul of 2X SDS Gel Loading dye to the Load, Wash and three eluate samples. Boil, spin and load the samples on a 12% SDS PAGE gel (like last week).

2. Run the gel, stain and destain the gel. Prepare the protein for dialysis.

DIALYSIS

1. Cut dialysis tubing enough to hold solution containing protein.

2. Wash the tubing with water and then with dialysis buffer.

3. Knot one open end and then pour the protein solution into the bag.

4. Tie the other end and place the tubing in the bucket containing buffer. Leave the solution at 4 Deg C until next week. The TA will change the dialysis buffer two times.