BME 128L Spring Quarter 2015
Professor DuBois

Lab Week #8: Test for expression of HAstV-SG capsid spike protein

Goal: To use SDS-PAGE and Western Blot to test for protein expression

Methods:

Prepare protein samples:

1. Resuspend/vortex your “pre-induction” and “post-induction” cell pellets each in 1ml H_2O.
2. In a new 1.5ml tube, mix 12 µl “pre-induction” sample and with 3 µl 5XSDS Loading Buffer. Label tube.
3. In a new 1.5ml tube, mix 6 µl “post-induction” sample with 6 µl H_2O and with 3 µl 5XSDS Loading Buffer. Label tube.
4. Make duplicates of both “pre-induction” and “post-induction” samples.
5. Boil all four samples 5-10 min at 100°C. Centrifuge tubes 30 sec on high speed.
6. Load samples, along with MW standards, on TWO different 15-well SDS-PAGE gels. Run gels 30-45 minutes at 200V until ladder is adequately resolved.
7. One gel will be stained with Coomassie gel stain. One gel will be transferred to a PVDF membrane and used for a Western Blot.

Run 4-12% Gradient SDS-PAGE

1. 5 µl Biorad Precision Plus Dual Color Standards
2. 15 µl “pre-induction” sample Construct 4
3. 15 µl “post-induction” sample Construct 4
4. 15 µl “pre-induction” sample Construct 5
5. 15 µl “post-induction” sample Construct 5
6. 15 µl “pre-induction” sample Construct 6
7. 15 µl “post-induction” sample Construct 6
8. 15 µl “pre-induction” sample Construct 8
9. 15 µl “post-induction” sample Construct 8
10. 15 µl “pre-induction” sample Construct 9
11. 15 µl “post-induction” sample Construct 9
12. 15 µl “pre-induction” sample Construct 10
13. 15 µl “post-induction” sample Construct 10

SDS-PAGE Coomassie Staining:

1. Carefully open gel cassette and transfer gel into container. Wash gel three times for 5 min each with ~200ml H_2O.
2. Add ~25ml Bio-safe coomassie stain and stain ~1 hour.
3. Rinse gel in ~200ml H_2O and take white-light image of gel.
Western Blot:

I. Semi-Dry Transfer:

1. Carefully open gel cassette and transfer gel into container Towbin transfer buffer and incubate for ~15 min.

   **Towbin Transfer Buffer (pH ~8.3)**
   - 25mM Tris
   - 192mM Glycine
   - 20% Methanol
   - 0.035% SDS

2. Put 2 thick filter papers and 1 membrane in transfer buffer. If membrane does not become wet, you may need to pre-wet quickly in 100% methanol before transferring to transfer buffer. Use a pencil to mark the spot on the membrane that the top of your molecular-weight markers will go.

3. Remove Trans-Blot Semi-Dry cover and stainless-steel cathode. Layer gel-membrane sandwich as follows. After placing each layer, roll out air bubbles with roller.

   4th/Top: Pre-wet filter paper
   3rd/Middle top: Equilibrated gel (align markers to spot on membrane)
   2nd/Middle bottom: Membrane (with pencil spot facing up)
   1st/Bottom: Pre-wet filter paper

4. Dry “sandwich” as much as possible without layers peeling back. Put a stack of paper towels over sandwich and roll out extensively to each edge. It should feel semi-dry (barely damp). If it is too damp, transfer is poor.

5. Secure stainless-steel cathode and cover. Run at 15V for 15 min using designated power source for Western Blots. Turn off power, take off cover and cathode, and gently peel up filter paper and gel at one corner – see if molecular-weight markers have transferred efficiently to the membrane. If not, run again at 15V for another 5-15min.

II. Buffers for Western Blot

- **TBS Buffer**
  - 25mM Tris pH 7.2
  - 150mM NaCl

- **Blocking Solution**
  - 25 mg/ml BSA in TBS Buffer

- **Primary Antibody Solution: HRP-conjugated Anti-His-tag antibody**
  - 5 ml Blocking Solution
  - 1.3 ul HRP-conjugated Anti-His-tag antibody (~3700X)

- **Wash Buffer**
  - TBS buffer with 0.05 % v/v Tween 20
III. Western Blot using SNAP i.d.: 


2. Add 30 ml Blocking Solution and turn on vacuum. When frame is empty, turn vacuum off.

3. Add 5ml Primary Antibody Solution and let incubate 10 minutes at room temperature.

1. Apply vacuum until dry. With vacuum continuously running, wash membrane with 4 x 30mL washes of Wash Buffer.

2. Remove membrane from blot-holder and lay protein side up on plastic wrap. Carefully pipet 2ml Detection Agent to top of membrane and incubate 5 minutes at room temperature. Pick up membrane with tweezers, let solution drip off, and place membrane on fresh plastic wrap. Place membrane on black tray and detect using imager with chemiluminescence setting.

Pre-lab Assignments for Week#9:

1. What does ultrasonication do to E. coli cells?

2. What will be the result of high-speed centrifugation of E. coli cell lysates?

3. What is the molecular mechanism of resin that allows easy purification of a 6His-tagged protein? How is the 6His-tagged protein eluted from the resin?