Saptasense Protocol

Eib Induction and Agglutination Assay

Background

In this experiment, we are retesting the effect of free Asn concentration on agglutination. Unlike previous experiments, we will be testing three different Asn concentrations and three replicates per experimental condition. Consistent results will allow us to make more conclusive statements than before. First, we will need to induce EibD expression using 0.001% rhamnose. Then, we will incubate the induced EibD cells with anti-Asn antibody, Asn-BSA beads, and Asn (30mM, 100mM, and 300mM).

Protocol

EibD Induction Protocol

- ☐ Add 49.5mL LB+CAM into two erlenmeyer flasks. One will be the experimental EibD culture and the other will be the negative control.
  - ☐ Make sure the LB+CAM is not cold.
- ☐ Add 0.5 mL ON culture into the respective flask.
- ☐ Grow up the cells at 37C with shaking until OD600 = 0.4-0.6
- ☐ Add 0.0005g 0.001% rhamnose to the experimental EibD culture.
- ☐ Incubate both flasks at 30C for 4 hours.
- ☐ Aliquot 1 mL uninduced cells (negative control) into Tube 1 - 0% rhamnose Eib pellet from -80C. Aliquot 1 mL of induced EibD cells (experimental) into Tubes 2-14. Make a few extra aliquots of induced just in case.
  1. EibD uninduced
  2. EibD induced (NO ANTIBODY, BEADS, OR ASN)
  3. Eib Induced + Antibody (NO BEADS OR ASN)
  4. Eib Induced + Antibody + Beads (NO ASN)
  5. Eib Induced + Antibody + 300 mM Asn (NO BEADS)
  6. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 1
  7. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 2
  8. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 3
  9. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 1
  10. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 2
  11. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 3
  12. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 1
  13. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 2

14. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 3

- ☐ Obtain pellets.
  - ☐ Centrifuge at 2500 rpm for 10 minutes.
  - ☐ Discard supernatant.
- ☐ Wash with 200 uL PBS three times.
  - ☐ Centrifuge at 2500rpm for 5 minutes and discard supernatant each time.
- ☐ Flash freeze and store final pellet at -80C unless using immediately.

Resuspension & Anti-Asparagine Antibody Incubation

- ☐ Resuspend cell pellets in 998 uL PBS.
- ☐ Take baseline OD600 measurements for each of the 14 samples, Tubes 1-14.
  - ☐ Collect measurements every 10s for the first 5 minutes, then at 6min, 7min, 10min, 20min, and 30min.
  - ☐ Take picture of cuvettes against black background (for qualitative data analysis).
- ☐ Add 2uL anti-asparagine antibody to Tubes 3-14. Add 2uL PBS to Tubes 1-2.
  1. EibD uninduced
  2. EibD induced (NO ANTIBODY, BEADS, OR ASN)
  3. Eib Induced + Antibody (NO BEADS OR ASN)
  4. Eib Induced + Antibody + Beads (NO ASN)
  5. Eib Induced + Antibody + 300 mM Asn (NO BEADS)
  6. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 1
  7. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 2
  8. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 3
  9. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 1
  10. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 2
  11. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 3
  12. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 1
  13. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 2
  14. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 3
- ☐ Incubate at room temperature and shake overnight.

Asn-BSA Bead Incubation

- ☐ Take 16h antibody incubation OD600 measurements for each of the 14 samples, Tubes 1-14.
  - ☐ Collect measurements every 10s for the first 5 minutes, then at 6min, 7min, 10min, 20min, and 30min.
  - ☐ Take picture of cuvettes against black background (for qualitative data analysis).
- ☐ Obtain pellets.
  - ☐ Centrifuge at 2500 rpm for 10 minutes.
  - ☐ Discard supernatant.
- ☐ Wash with 200 uL PBS two times.
  - ☐ Centrifuge at 2500rpm for 5 minutes and discard supernatant each time.
- ☐ Add 12.5 uL Asn-BSA beads + 987.5uL PBS into Tubes 4, 6-14. Add 1000uL PBS into Tubes 1-3, 5.
  1. EibD uninduced
  2. EibD induced (NO ANTIBODY, BEADS, OR ASN)
  3. Eib Induced + Antibody (NO BEADS OR ASN)
  4. Eib Induced + Antibody + Beads (NO ASN)
  5. Eib Induced + Antibody + 300 mM Asn (NO BEADS)
  6. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 1
  7. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 2
  8. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 3
  9. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 1
  10. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 2
  11. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 3
  12. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 1
  13. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 2
  14. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 3
- ☐ Incubate at room temperature and shake overnight.

30mM, 100mM, and 300mM Asn Incubation

- ☐ Take 12h bead incubation measurements for each of the 14 samples, Tubes 1-14.
  - ☐ Collect measurements every 10s for the first 5 minutes, then at 6min, 7min, 10min, 20min, 30min, 40 min, and 50 min.
  - ☐ Take picture of cuvettes against black background (for qualitative data analysis).
- ☐ Obtain pellets.
  - ☐ Centrifuge at 2500 rpm for 10 minutes.
  - ☐ Discard supernatant.
- ☐ Wash with 200 uL PBS two times.
  - ☐ Centrifuge at 2500rpm for 5 minutes and discard supernatant each time.

- ☐ Add 1000uL **30mM** Asn to Tubes 6-8.
  1. EibD uninduced
  2. EibD induced (NO ANTIBODY, BEADS, OR ASN)
  3. Eib Induced + Antibody (NO BEADS OR ASN)
  4. Eib Induced + Antibody + Beads (NO ASN)
  5. Eib Induced + Antibody + 300 mM Asn (NO BEADS)
  6. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 1
  7. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 2
  8. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 3
  9. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 1
  10. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 2
  11. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 3
  12. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 1
  13. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 2
  14. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 3

- ☐ Add 1000uL **100mM** Asn to Tubes 9-11.
  1. EibD uninduced
  2. EibD induced (NO ANTIBODY, BEADS, OR ASN)
  3. Eib Induced + Antibody (NO BEADS OR ASN)
  4. Eib Induced + Antibody + Beads (NO ASN)
  5. Eib Induced + Antibody + 300 mM Asn (NO BEADS)
  6. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 1
  7. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 2
  8. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 3
  9. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 1
  10. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 2
  11. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 3

12. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 1
13. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 2
14. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 3

- ☐ Add 1000uL **300mM** Asn to Tubes 5, 12-14.
    1. EibD uninduced
    2. EibD induced (NO ANTIBODY, BEADS, OR ASN)
    3. Eib Induced + Antibody (NO BEADS OR ASN)
    4. Eib Induced + Antibody + Beads (NO ASN)
    5. Eib Induced + Antibody + 300 mM Asn (NO BEADS)
    6. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 1
    7. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 2
    8. Eib Induced + Antibody + Beads + 30 mM Asn REPLICATE 3
    9. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 1
    10. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 2
    11. Eib Induced + Antibody + Beads + 100 mM Asn REPLICATE 3
    12. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 1
    13. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 2
    14. Eib Induced + Antibody + Beads + 300 mM Asn REPLICATE 3
- ☐ Add 1000uL PBS into Tubes 1-4.
- ☐ Incubate at room temperature and shake for 8 hours or overnight.

Final ODs

- ☐ Take 12h Asn incubation measurements for each of the 14 samples, Tubes 1-14.
    - ☐ Collect measurements at 10s, 20s, 30s, 40s, 50s, 1min, 2min, 3min, 4min, 5min, 10min, 15min, 20min, 30min.
    - ☐ Take picture of cuvettes against black background (for qualitative data analysis).