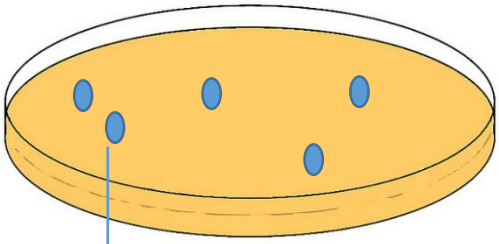
Cloning, expression, purification, and biophysical characterization of SSB from pathogenic bacteria

Week 3: Screen colonies of bacterial cells to determine if the SSB gene is inserted into the pCDF plasmid.

Overview:

- LB-spectinomycin plates from step V of week-2 class are screened for the presence or absence of colonies.

- If colonies are present, they are screened to determine if SSB gene is inserted into the pCDF plasmid.



LB-kanamycin plate

Step I-
Grow bacterial colony in LB-Kanamycin media overnight

Step II- Plasmid DNA isolation- Qiagen miniprep kit is used to break open the cells (lysis) and to release plasmid DNA followed by purification of the plasmid DNA

Step III- Restriction digestion - cut the extracted plasmid DNA with NdeI and BamHI to determine if SSB gene is inserted into the plasmid.

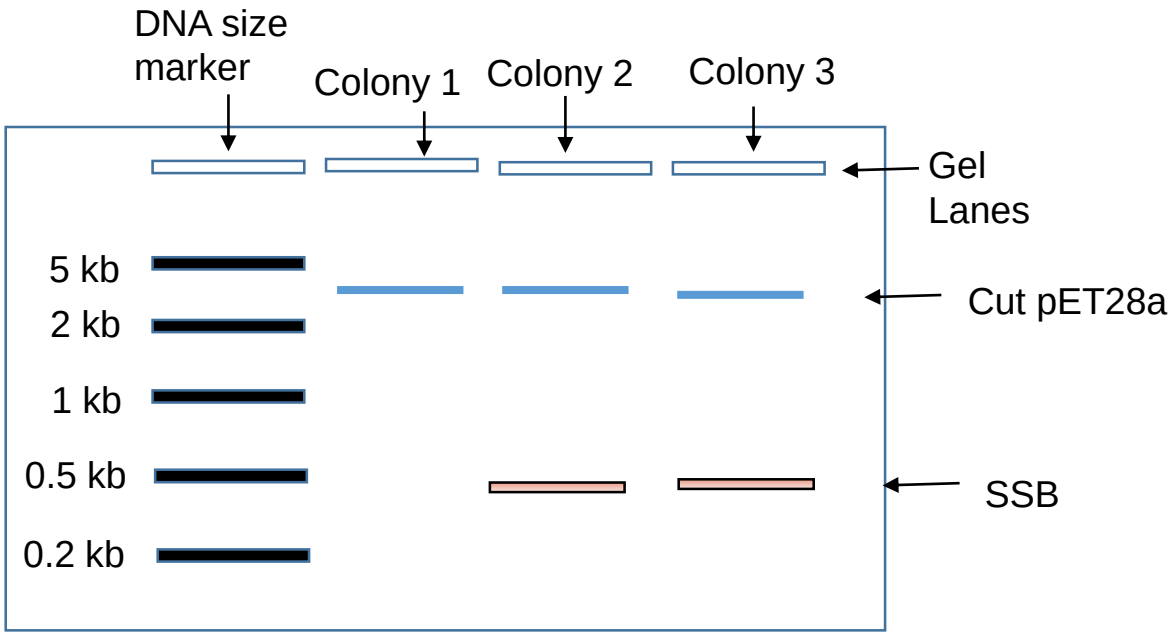
Step IV- Gel electrophoresis-Restriction digested DNA fragments are screened for size by agarose gel electrophoresis.

Week 3: Screen colonies of bacterial cells to determine the presence of SSB gene insert in the pET28a plasmid.

Overview- Cont'd.

Plasmid DNA isolated from bacterial colonies is screened for the presence of SSB DNA by restriction digestion with NdeI/BamHI. The digested products are analyzed by agarose gel electrophoresis.

Expected Sizes- **SSB~ 0.5kb**, **pCDF– 3.8kb**.



Example :
Colony 1 - Plasmid DNA isolated– no SSB found- cannot be used for expression.

Colony 2 - Plasmid DNA isolated – SSB found- can be used for expression

Colony 3 - Plasmid DNA isolated – SSB found- can be used for expression

Step I- Growing bacterial colony overnight- Protocol

1. Label two 15 mL sterile tubes as colony 1 and colony 2 and your group name.

2. Keep everything sterile. Next to a flame(careful!), add 5 mL LB +Spectinomycin (antibiotic) media to each 15mL tube.

3. Pick an individual colony using a sterile 10uL pipette tip and add the entire tip to the tube labeled as colony 1.

4. Pick another individual colony using a sterile 10uL pipette tip and add the entire tip to the tube labeled as colony 2.

5. Close cap loosely and tape it so it won't fall off . The loose cap helps with better aeration required for good bacterial growth.

6. Shake in an incubator at 220 rpm, overnight at 37°C.

Step II- Plasmid DNA isolation- Protocol

1. Follow Qiagen Miniprep kit protocol to isolate plasmid DNA-please see the attached-Qiagen company product sheet.

Step III- Restriction digestion of plasmid miniprep DNA

1. Label one 1.5mL microcentrifuge tube with your group SSB and as colony 1
2. Label another tube as SSB-colony 2
3. To colony1 tube- add 7uL of colony1 plasmid DNA
4. To colony2 tube- add 7ul of colony 2 plasmid DNA
5. To each tube add the following:

Master mix :
*NdeI---------- 0.5 uL
*BamHI---------- 0.5 uL
CutSmart buffer 10X --- 1 uL
DI water ---------- 1 uL

*Add enzymes- NdeI and BamHI last!

6. Gently mix and incubate at 37°C for 45 min
7. Continue to step IV

Step IV - Agarose gel electrophoresis to analyze restriction digestion products.

Prepare 1% agarose gel:

1. Add 0.5 g of agarose to 50 mL of 1X TAE buffer in a 200mL beaker.
2. Microwave solution till agarose is dissolved. (caution –hot!)
3. Once the beaker is cool to the touch – add 5 µL of SYBR Safety Stain.
4. Mix gently and pour into the casting tray.
5. Place the comb and wait for gel to polymerize (solidify).

Prepare sample to be loaded onto the gel:

1. Remove Colony1 and Colony2 tubes that were incubated at 37°C.
2. Add 2ul of 6X loading dye to colony 1 tube and gently mix.
3. Add 2 ul of 6X loading dye to colony 2 tube and gently mix.

Loading reactions onto the gel:

1. Load 10 uL of DNA marker in gel lane I
2. Leave lane 2 empty. Load the entire reaction (12 uL) of colony 1 tube in lane 3
3. Leave lane 4 empty. Load the entire reaction (12 uL) of colony 2 tube in lane 5
4. Run at 120V for 30 to 45 min.
5. Image gel using UV lamp.