**Protocol:** Purification via Heat-Soluble Precipitation

**Purpose:** To get purified IDPs from *E.coli* cell lysis from induction of protein expression via IPTG by heat shocking and verified the plasmid through SDS-PAGE

**Material:**

- Cell pellets and supernatants from induction of protein expression
- 25 mM Tris/HCl
- Water bath
- Centrifuge
- 2X SDS-PAGE sample buffer
- 1X SDS running buffer (see recipe)
- SDS stain solution (see recipe)
- SDS destain solution (see recipe)
- Mini protean TGX gels
- Power supply
- Electrophoresis Chambers

**Methods:**

1. Resuspend cell pellets in 500 µLof 25 mM Tris/HCl and take 500 µL aliquot from supernatants.
2. Heat shock in water bath at 80℃ for 15 minutes.
3. Centrifuge at 16,000 rcf for 40 minutes at room temperature.
4. Separate supernatant and pellets in different eppendorf tubes. (Votex them and make sure well dissolved)
5. Mix 6 µL of supernatants and pellets with 6 µL of 2X SDS-PAGE sample buffer (Votex and spin down)
6. Heat shock in water bath at 80℃ for 5 minutes.
7. Load 10 µL of the sample in gel and run the gel at 200V for around half an hour.
8. Take out the gel as soon as finish running. Gently open it and wash it with buffer in the wash box.
9. Stain the gel with 25 ml of stain solution for 30 to 60 minutes with shaking.
10. Collect the stain solution for reuse. Destain gel with 25ml of destain solution with shaking overnight.
11. Next morning, destain twice for one hour each time.

**Recipe:**

- SDS running buffer: Tris-Glycine Electrophoresis Buffer (10X  pH8.3)

| Reagents | Volume | Final Concentration |
|---|---|---|
| Tris Base | 30.3 g | 250mM |
| Glycine | 144 g | 1.9M |
| SDS | 10 g | 1% |
| Water | To 1 L | |

- SDS stain solution: “end of the day” stain (0.25% coomassie blue)

| Reagents | Final Concentration |
|---|---|
| Coomassie Blue | 0.25% |
| Acetic Acid | 10% |
| Methanol | 30% |

- SDS destain solution

| Reagents | Final Concentration |
|---|---|
| Acetic Acid | 10% |
| Methanol | 30% |