**Plan for activity screening:** Since β-Lactamase in pET vector is IPTG inducible, this is big hurdle right now so I am planning three methods in liquid culture. I will try plate method after this regardless if it is successful or not. Meantime I will have pure WT protein with some assay data which will be used to recalibrate our substrate concentrations (Amp and Cephalothin).

Overnight grown culture, regrow them in 150 µL (10 µL in to 150 µL) for 3 hrs so that it is active, log phase. After 3hrs, use 5 µL in assay. This optimization is for WT.

**Method 1**: Using culture inoculum without pre-induction with IPTG (No Pre-induction)

**Method 2**: Pre-induced with IPTG (0.5 mM for WT)

**Method 3**: Pre-induced with IPTG with large number of cells (50-100 µl).

**Method 1**: Using culture inoculum without pre-induction with IPTG (No Pre-induction)

**Ampicilin:**

Step 1: Make 2 ml of LB media with Kanamycin and 0.5 mM IPTG; add 4 µL of 166 mg/ml (so that final concentration is 332µg/ml)

Step 2: Make 10 ml LB with Kanamycin with 0.5 mM (final), use this for serial dilution so that highest concentration will be 333µg/ml and dilute up to 5.18 µg/ml. Leave last lane/well for “no Amp” control.

Step 3: Add 5 µL, 3 hrs grown culture. Grow 30OC for 30 min then switch to 37OC for overnight.

Follow same protocol for **Cephalothin** with concentration adjustment at step 1 e.g. add 12 µL of 200 µg/µl in 2 ml LB (so that final concentration will be 1200 µg/ml in LB).

Serial dilute in such a way that highest concentration is 1200 µg/ml and lowest will be 18.5µg/ml.

**Method 2**: Pre-induced with IPTG (0.5 mM for WT)

Everything remains same as in method 1 except step 3. In step 3, here, add 5 µl of three hour IPTG induced culture.

**Method 3** is still in thought process, evolving.. Basically it will be same as method 2 but 50-100 µl culture!!!