Test JDP Expression in ΔPatZ Strain

Goal: Test a variety of IPTG concentrations to optimize induction of expression of a JDP (A2) in ΔPatZ Strain

* Concentrations to test: 400uM, 600uM, 800uM, 1mM, 1.2mM

Saturday 072118

1. Autoclave 5 125mL E-flasks and 1 500mL E-flask with Liquid 20 cycle.
2. Pick three colonies and incubate in 8mL TB + 100ug/mL ampicillin overnight.

Sunday 072218

1. Add 4mL of overnight culture to 200mL TB + 100ug/mL ampicillin, grow at 37C, 180 RPM for 5 hours or until OD600 reaches about 0.8.
2. Cool cells down to 18C, 160 RPM for 1 hour.
3. Aliquot 20mL of culture into each of 5 250mL E-flasks. Induce each of the 5 flasks with respective volumes of IPTG stock to get the above concentrations (40uL, 60uL, 80uL, 100uL, 120uL of 200mM IPTG stock).
4. Collect 1mL of uninduced culture, spin down at max speed 30 sec, and store pellet in -30C freezer.
5. Incubate all 5 cultures at 18C, 160 RPM overnight.

Monday 072318

1. Collect 1mL of each of the 5 cultures, spin down at max speed 30 sec and remove supernatants.
2. Prepare 1X SDS sample buffer, and resuspend uninduced sample in 100uL and induced samples in 200uL of buffer.
3. Boil each sample for 5 min and then centrifuge at max speed for 5 min.
4. Load 8uL of each sample onto SDS-PAGE gel.