ScPho4 time-course: heat shock vs no heat shock

1. Grow KM2 overnight cultures in LB+amp-done

2. Transfer overnight culture to 2x 50ml LB+amp to a final OD of 0.05-0.1

3. Grow cultures to OD 0.5 at 37C

4. Transfer non-heat shock culture to 18C incubator until OD 0.6.

5. Heat shock the other culture at OD 0.5 42C 20 minutes, ICE for 15 minutes, then induce with 1mM IPTG and transfer to the 18C incubator-collect pre induction sample

6. When the non-heat shock culture reaches OD 0.6, induce it with 1mM IPTG final concentration-collect pre-induction sample

7. Track culture induction times, collecting each at their respective 10, 12, 14 and 16 hour post induction time points in 2x1ml aliquots (cultures will have to grow overnight)—measure OD at each timepoint—pellet culture, decant, and freeze pellets.

8. Collect the remaining culture at the end of the time course—pellet culture, decant, and freeze pellets.

SDS Page and Lysis—

1. Lyse pellets in 50ul 1x Bugbuster-20 min room temp incubation—collect 10ul aliquots of whole cell lysate for each pellet

2. Centrifuge remaining whole cell lysate, 25 min 14,000rpm at 4C

3. Collect 10ml aliquots of supernatant from each centrifuged sample

4. Fix samples 1:1 with 2x Laemmli+ 95C incubation 8min

5. Load SDS page 4ul fixed whole cell lysate, 10ul fixed supernatant 200V, 40min

6. Rinse gels in water 10min, switching to clean water after 5 min

7. Stain with Coomassie blue, 50ml per gel, at least 40 min.

8. Destain 30min, add fresh water at 15 min.