Aerobic, Anaerobic, LB and M9 experiment.

1)pucL, YgfU+pucL, LM4 Full, LM4 Optimum, eGFP were inoculated in 15mL LB medium in 15mL tube, 3ml LB medium in 12ml tube, 15ml M9 medium in 15mL tube, 3ml M9 medium in 12ml tube overnight (24h). Temperature 37 centi degree, 15ml tubes are placed in shaker with RPM=20; 12ml tubes are placed in shaker with RPM=22O.
2)All the medium with bacteria is measured in 96 well plate at OD600 at 24h.
3)All the samples are made to be ready for HPLC test.
4)All the tubes are cultivated in the same condition before till 48h.
5)All the medium with bacteria is measured in 96 well plate at OD600 at 48h.

Enzymes In Vitro Experiment

1)Bacteria is cultivated in 100ml LB in flask more than 500ml, 30 degrees, rpm=100, overnight
2)Bacteria is centrifugated 4000rpm 15min or 12000rpm 10min. Supernatant is discarded.
3)The protein extraction steps can be seen in eGFP purification part. Future purification steps are not performed in this experiment. BCA is performed to make sure all the samples with the same total protein mass. The crude extractions are prepared on the ice for reactions.
4)100ul crude protein extraction is added into 1.5ml tube with 900ul Urate PBS (PH=8.0, urate concentration depends on the specific experiment), reacted for specific time.
5)The 1.5ml tubes are placed in the 100 degrees metal heater for 10min.
6)HPLC test is performed then.