

### Characterization Pu Promoter

- 1 Seed cultures overnight with appropriate antibiotics in 37°C, 200 rpm shaking incubator.
  - 2 Morning: Dilute sample into linear OD range, read OD.
  - 3 Add fresh media, antibiotics to individual tubes with culture to achieve OD ~.1 in 10 mL (glass tube).
  - 4 Allow to grow to OD .6-.8 (typically), checking OD occasionally to find induction time (~3 hours in LB).
  - 5 When in induction OD range, take 150 µL sample with pipet. Induce with IPTG (.1 mM), aromatic (1 mM) where appropriate including wild type. Cap with rubber stopper and aluminum cap when induced to avoid losses to atmosphere.
  - 6 Take time points. Use syringe and needle to extract ~250 µL of culture, add to PCR tube. Transfer exactly 150 µL to microplate. Read OD 600 nm, excite 485/20, emission 528/20 nm. Time points typically consist of time 0, 1, 2, 3, 4, 6, 8, 10, 12, 24 hours.
- M9 induction OD 600 may vary as maximum growth is less than LB, typically >.4.

5 ml cultures of each strain in LB + CM\* media seeded from -80 frozen stock and grown overnight at 37° C and 200 RPM incubator. The following morning, cultures were reseeded to OD=.1 with fresh media<sup>x</sup> to 10 mL in 25 mL glass tubes. Cells were grown to OD=.6-.8 or OD=.35-.55 dependent on media<sup>y</sup> (2-3 hours) as measured by spectrofluorometer, then induced with .1 mM IPTG and 1 mM m-xylenes, when necessary. Cultures were capped with a rubber stopper and aluminum sealed to avoid atmospheric losses of volatile aromatics. Samples (~250 µL culture with syringe and needle) were taken, transferred to PCR tubes, and 150 µL were pipetted to a 96 well black, clear bottomed microplate for reading. This occurred just prior to adding IPTG (t=0) then 1, 2, 3, 4, 6, 8, 12, and 24 hours after induction. Plates were shaken for 5 seconds, OD 600 nm was measured, and GFP was measured via excitation under a 485/20 nm filter and emission measured under 528/20 nm filter.

\*CM = chloramphenicol plasmid retention strains only

media<sup>x</sup>= M9 media, M9 media with .1x NH<sub>4</sub>Cl, or LB dependent on test. Containing CM for chloramphenicol plasmid retention strains

media<sup>y</sup>= OD=.6-.8 for LB media, OD=.35-.55 for minimal media.