Protocols for Growing and Engineering *P. putida*

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Media and Cultivation

According to the ATCC, the recommended growth conditions of *P. putida* KT2440 are LB media at 37°C, whereas those of strain S12 are listed as TSB media at 30°C. Our experience has been that both rich media (as well as TB broth) work well for most applications, whereas we have given a preference to the use of 30°C for most cultivation work. Colony formation on solid media will be observed within 24 to 48 hours, in most cases.

For minimal media, we have found that M9 media works well, particularly when supplemented with trace minerals (we use ATCC MD-TMS). Both glucose and glycerol can be used as a carbon source. Stationary phase is reached within about 48 h at 30°C.

Antibiotics

P. putida is appropriately sensitive to both tetracycline (10 mg/L for pRK415 based plasmids) and gentamycin (20 mg/L for pMMB206G based plasmids) such that these antibiotics work well for plasmid maintenance, when appropriate. The use of plasmid with either ampicillin or chloramphenicol resistance markers, however, should be avoided.

Competent Cell Preparation

Begin with an overnight culture grown in 5 mL LB broth. Centrifuge culture for 5 min at 5000 rpm (~5500 x *g*) for 5 min. Pour off supernatant and resuspend

cells in 5 mL ice cold, sterile 10% v/v glycerol solution. Pellet as before. Repeat for a total of three glycerol washes. Whenever possible, keep cells chilled on ice between pelleting cycles. Following the final wash, pour off supernatant. Resuspend cells in ~100 uL 10% glycerol. Aliquot suspensions into sterile Eppendorf tubes, 20 uL per tube. Immediately store at -80°C until required. Cells should remain stable for several months.

Transformation and Selection

Remove frozen cells from cryogenic storage and thaw on ice. Place a 1 mm gap electroporation cuvette on ice to chill. Once thawed, add 1-2 uL of purified plasmid DNA (total if co-transformed) to cells and tap to mix. Apply cells to cuvette and place back on ice. Transform cells via electroporation (1.8 kV, 1 pulse), time constants should be in the range of 2-5 msec. Recover in 1 mL SOC media, for 2 hours at 30°C. Plate cells on LB agar with appropriate antibiotics and culture at 30°C. CFUs will appear in 24-48 hours.