Thursday 9/10/09 and Friday 9/11/09

Preparation of comp. cells and transformation of Pu GFP leu landing pad ligation

The preparation of comp. cells and transformation was done according to the protocol on 8/8/09 with the following changes:

- A replicate for *E. coli* DH5α and *P. putida* mt-2 (with TOL plasmid) was performed
    - The E. coli DH5α was grown in LB media
    - *P. putida* mt-2 (with TOL plasmid) was grown in ATTC sodium benzoate media prepared on 9/9/09
        - This strain must be grown with sodium benzoate otherwise it will lose the TOL plasmid
    - For the harvesting and washing steps the cultures were only centrifuged for 10 minutes instead of 15
    - Since the DNA was not purified from the salty ligation solution the voltage for transformation was lowered to 1.5 kV
    - No positive control or dilutions were plated because there were not enough sodium benzoate plates
- Transformations
    - 1:1 #1-3 Pu GFP ligation for both *E. coli* DH5α and *P. putida* mt-2
    - 1:3 #1-3 Pu GFP ligation for both *E. coli* DH5α and *P. putida* mt-2
    - 1:6 #1-3 Pu GFP ligation for both *E. coli* DH5α and *P. putida* mt-2
    - Negative *E. coli* DH5α control
    - Negitive *P. putida* mt-2 control

Results

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The comp. cell cultivation cultures were started at 11:50 AM. They were removed from the 30C incubator (*P. putida* mt-2 must grow at 30C) at 4:00 PM and had the following OD_{600} measured with the plate reader

Culture	Measurement 1	Measurement 2	Average Measurement	OD_{600}
DH5α	.735	.721	.728	.642
Blank LB	.092	.080	.086	-
Mt-2	.320	.357	.339	.260
Blank sodium benzoate	.079	.079	.079	-

The harvesting and washing steps were started at this point because the *E. coli* DH5α had grown out even though the *P. putida* mt-2 had not. During the final washing more care was taken to resuspend the *P. putida* comp. cells in less water to make them more concentrated.

For the transformations the time constant was between 3 and 4 and no popping occurred.

All of the plates were placed in the 30C shaker to grow out overnight