6-29-2010 and 6-30-2010

Pseudomonas putida KT2440 antibiotic tolerance

Goal

P. putida KT2440 has a high antibiotic tolerance due to the activity of efflux pumps it contains. We must find suitable concentrations of antibiotics that *P. putida* KT2440 cannot grow at to have a way of selecting cells with the desired plasmid when performing transformations. In literature the following antibiotic concentrations have been cited for transformations when working with *P. putida*:

- 1 mg/ml of carbenicillin[1]
- 50 mg/ml of tetracycline[1,2]
- 50 mg/mL of neomycin[1]
- 50 mg/mL of kanamycin[1,2]

From past experience, *P. putida* KT2440 was able to grow on chloramphenicol and ampicillin therefore these antibiotics should not be used to select for transformants.

With this information we can choose appropriate plasmid backbones for testing our parts constructed in *E. coli* in *P. putida* KT2440.

References

[1] Transformation of Pseudomonas putida and Escherichia coli with plasmid-linked drug-resistance factor DNA. Proceedings of the National Academy of Sciences of the United States of America [0027-8424] Chakrabarty yr:1975 vol:72 iss:9 pg:3647

[2] Improved transformation of *Pseudomonas putida* KT2440 by electroporation.

Media/Reagents

LB Media

Mix according to directions on bottle and autoclave
Previously prepared by Marc
Approximately 15 mL total

- 2 mL for overnight culture of *P. putida* KT2440
- 6 mL for testing kanamycin resistance
- 6 mL for testing tetracycline resistance

Kanamycin Stock Solution (50 mg/mL)*

Mix 250 mg of kanamycin in 5 mL of water and filter sterilize. Store in 1 mL aliquots in the -20C freezer.

TetracyclineStock Solution (50 mg/mL)*

Mix 250 mg of tetracycline in 2.5 mL of DI water and 2.5 mL of ethanol and filter sterilize. Store in 1 mL aliquos in the -20C freezer.

*Information about antibiotic was found on openwetware (http://openwetware.org)

Protocol

1. The night before start a culture of *P. putida* KT2440 in 2 mL of LB and grow in the 30C shaker overnight
2. Prepare media for antibiotic resistance experiment
    a. Kanamycin
        i. 25 mg/mL
            1. 50 uL of 50 mg/mL kan stock solution
            2. 1.930 mL of LB media
        ii. 50 mg/mL
            1. 100 uL of 50 mg/mL kan stock solution
            2. 1.880 mL of LB media
        iii. 75 mg/mL
            1. 150 uL of 50 mg/mL kan stock solution
            2. 1.830 mL of LB media
    b. Tetracycline
        i. 25 mg/mL
            1. 50 uL of 50 mg/mL tet stock solution
            2. 1.930 mL of LB media
        ii. 50 mg/mL
            1. 100 uL of 50 mg/mL tet stock solution
            2. 1.880 mL of LB media
        iii. 75 mg/mL
            1. 150 uL of 50 mg/mL tet stock solution
            2. 1.830 mL of LB media
    c. Control
        i. LB media
            1. 1.980 mL of LB media
3. Make an inoculation culture of *P. putida* KT2440
    a. Measure the OD_{600} using the spectrophotometer
    b. Spin down 1 mL of overnight culture at 13000 rpm for 1 minute
    c. Remove the supernatant
    d. Resuspend the cell pellet in designated about of LB media
        i. OD_{600} x 500 uL for a cell suspension with an OD of about 2
        ii. Must have at least 140 uL of cell suspension for this experiment

4. Inoculate seven cultures prepared in step 2 with 20 uL of cell suspension
5. Place in the 30 C shaker to grow out overnight
6. Check the next day to see which cultures have grown out overnight