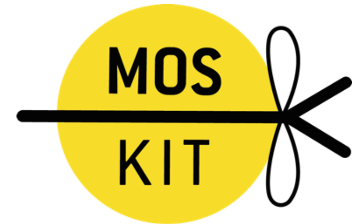
iGEM Pasteur Paris 2016
Protocols



TRANSFORMATION

Aim: To capture ligated plasmid-insert combination, and increase the amount of plasmids by transformation in competent cells.

The amount of plasmid supplied is insufficient to perform all our future experiments. Therefore, we need to amplify the amount of plasmids.

Materials:

- Microbiology equipment: 42 °C Water bath, 37 °C incubator (static, and shaking), Bunsen burner, sterile rake, petri dishes with appropriate antibiotic on agar, timer
- Antibiotics stock (carbenicillin 50 mg/ml, chloramphenicol 34 mg/ml)
- Competent cells DH5-α subcloning efficiency, BL21De3, or Top10
- pUC19 Control DNA (100 pg/µl)
- PSB1C3 plasmid (from shipped BioBrick-competent cells testing kit), chloramphenicol resistance
- pET43.1a (Novagen, Gift from D. Gopaul Lab, Institut Pasteur), ampicillin resistance (or carbenicillin)
- SOC (Super optimal Broth + glucose) media
- LB (Luria broth) Agar plates containing 50 µg/ml carbenicillin or 34 µg/ml chloramphenicol

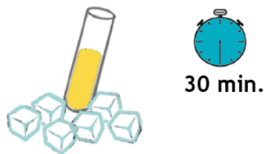
Protocol:

Positif control:

1. Thaw on ice one tube of DH5α^{TM} cells. Place 1.5 ml microcentrifuge tubes on ice.
2. Gently mix cells with the pipette tip and aliquot 50 µl of cells for each transformation into a 1.5 ml microcentrifuge tube.
3. Refreeze any unused cells in the dry ice/ ethanol bath for 5 minutes before returning to the -80°C freezer. Do not use liquid nitrogen.
4. Add 1 to 5 µl (1-10 ng) of DNA to the cells and mix gently by tapping the side of the tube. Do not mix by pipetting up and down. For the pUC19 control, add 2.5 µl (250 pg) of DNA to the cells and mix gently.
5. Incubate tubes on ice for 30 minutes
6. Transformation

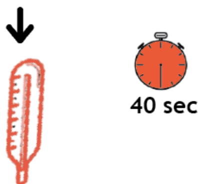
The transformation:

Note. Always keep cells in a sterile environment during each manipulation

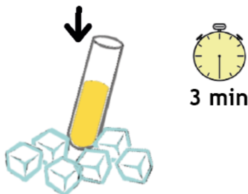


1.Thaw competent cells from -80°C on ice, thaw plasmids at 37°C, and place on ice, aliquot cells. Add plasmids to each competent cells vial and trap gently. Keep everything on ice.

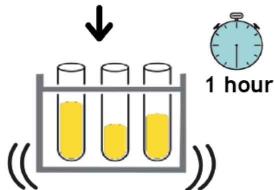
2. Warm LB agar plate at 37°C in incubator . Set Waterbath at 42°C



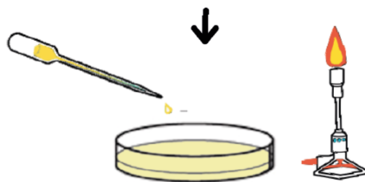
3. After 30 mins on ice place cells in 42°C (exactly 40 sec)



4. Then place immediately on ice (3 min)



5. Grow at 37°C in shaking incubator at 150 rpm (1 hour)



6.Take aliquot of culture and under the flame, streak with sterile rake on LB-agar plates containing the appropriate antibiotic

7. Place plates inverted in the static 37°C incubator overnight