Protocols

LB (Lysogeny Broth) – 1 L:

10 g Tryptone

10g NaCl

5g yeast extract

For agar plates add 15g agar

Standard digest

Digest:

10 µl DNA

5 µl Buffer

1 µl Enzyme 1

1 µl Enzyme 2 (optional)

Ad 50 µl H_2O

Buffer was chosen according to enzymes used.

NEBuffer 2.1 for EcoRI-HF/PstI

CutSmart for XbaI/SpeI

Reactions were scaled down if appropriate.

Standard Ligation

2 µl T4 DNA Ligase buffer

1 µl T4 DNA Ligase

X µl Vector

X µl Insert

Ad 20µl H_2O

Miniprep standard method from division of biochemistry:

To screen for successful ligation or transformation, the plasmid DNA was extracted via Miniprep. This method enables quick and efficient screening of many colonies for the desired plasmids.

Initially, a colony of *E. coli* is inoculated in 2 mL LB medium with antibiotic for selection. After incubation overnight at 37°C, 1.5ml of the culture is centrifuged and the pellet is resuspended in 100 µL TE buffer with RNAse. 200 µL of Solution 2 is then added, containing SDS (sodium dodecyl sulphate) and NaOH (sodium hydroxide), for alkaline lysis. This breaks the cell envelope and denatures proteins and DNA. Adding 150 µL of Solution 3, 3M KAc, the pH of the mixture is neutralized. Due to the neutralization, small plasmid DNA can renature, while the larger fragments of genomic DNA and most proteins remain denatured and can be pelleted by centrifugation. The supernatant, containing the plasmid DNA is then transferred to another tube and 1 mL Ethanol is added to precipitate the DNA. This step and the subsequent washing with 0.5 mL 70% ethanol remove some of the salts from the DNA. After washing, the DNA pellet was air-dried to remove all ethanol and solved in approx. 30µL MQ-H_2O. The resulting plasmid prep can then be analyzed via restriction digest and agarose gel electrophoresis.

Transformation of chemically competent E. coli

The cells, stored at -80°C, were thawed on ice. 5µl Ligation reaction was pipetted onto the cells and mixed gently. The cells were incubated on ice for 30 minutes. Heat shock at 42°C for 90 seconds followed. After cooling down on ice for 5 minutes, 1ml LB medium was added and the cells were regenerated by incubation at 37°C for approximately 60 minutes. After regeneration, the cells were pelleted using a microcentrifuge and resuspended in 50µl medium. This suspension was then plated on LB Agar containing the appropriate antibiotic for selection of the vector backbone. The plates were incubated overnight at 37°C and analyzed for colony growth the following day.

Growth of biofilms

The substrate (glass slides) were placed in a petri dish which was then filled with medium containing the appropriate selective antibiotic until the glass slides were barely submerged. Cells were then added as wel as IPTG for immediate induction of curli production. This culture was incubated on a rocking shaker for three days at room temperature. Medium was exchanged if the culture reached saturation.

Generation of competent cells

Source:
https://www.embl.de/pepcore/pepcore_services/cloning/cloning_methods/chemically_competent/

Scaled down to 10%

TB buffer

10 mM HEPES pH 6.7

15 mM $CaCl_2$

55 mM $MnCl_2$

250 mM KCl

Grow cells in 50ml LB to OD600 ~0.4, then chill on ice for 10 minutes, keep the cells cold from now on.

Centrifuge and resuspend pellet in 10ml cold TB

Centrifuge and resuspend pellet in 1.86ml cold TB

Add 140µl DMSO and incubate on ice for at least 10 minutes.

Aliquot 200µl each – should be enough for 10 transformations.

Freeze in liquid nitrogen and store at -80°C.