PRINTERIA

Notebook

Experiments with competent cell

EXPERIMENTS WITH COMPETENT CELLS

One of the problems which we are facing when we are creating Printeria is the way in which the competent cells will enter. The first way that we have tested is freeze drying competent cells.

Experiments

Friday 6/7/2018
We have made 10G electrocompetent cells

Tuesday 24/7/2018
We have made 250 ml of CCMB80 buffer

Wednesday 25/7/2018
We have made chemically competent and Mix and Go competent 10G cells.
We have done the transformation efficiency test for the chemically competent and the Mix and Go competent 10G cells using a control plasmid with ampicillin resistance and plates with ampicillin.

Thursday 26/7/2018
Results of the transformation efficiency test:

- Chemically competent cells: 1.64e5 CFUs/ µg
- Mix and Go competent cells: 5.5e4 CFUs/ µg

Lyophilization of 10 alliquotes of 10G chemically, electro and mix and go competent cells

Saturday 28/7/18
Rehydration of freeze dried cells: chemically, electro and mix and go competent cells

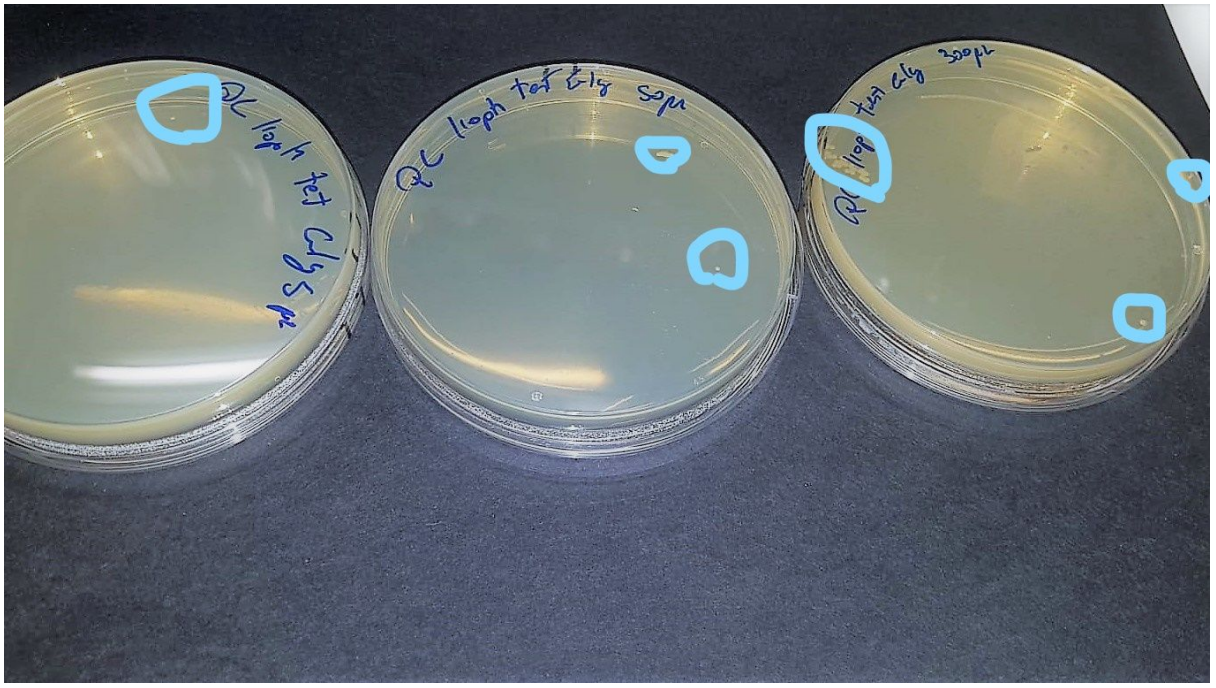
- 2 of each type with glycerol at 10%
- 2 of each type with MilliQ water

We have plated 1 aliquot of each type rehydrated with glycerol at 10% and 1 other rehydrated with MilliQ water on plates without antibiotic to check that the cells were viable.
We have done the transformation efficiency test for 1 aliquot of each type rehydrated with glycerol at 10% and 1 other rehydrated with MilliQ water using pGreen ***α***1 and plates with kanamycin. We have also done this test for 1 aliquot of the electrocompetent cells that we did on July 7.

Sunday 29/7/18
Results from saturday:

- All cells were viable rehydrated with glycerol at 10% and with MilliQ water.

- Only the chemically competent cells rehydrated with glycerol at 10% were competent (1e2). They have lost 3 orders of transformation efficiency but they remain competent. We must improve these results



Picture 1: Results of the transformation efficiency test for our 10G chemically competent cells

- Electrocompetent cells test: 1e9 CFUs/ µg

Another way that we have tried is using electrocompetent cells stored at -20°C:

Experiments

Thursday 2/8/2018
We have stored electrocompetent cells at -20°C

Thursday 9/8/2018
Transformation efficiency test for the electrocompetent cells stored at -20°C

Friday 10/8/2018
Results of the transformation efficiency test: 1.5e6 CFUs/ µg
They are still competent being a week at -20°C

Thursday 16/8/2018
Transformation efficiency test for the electrocompetent cells stored at -20°C.
Transformation efficiency test for one aliquot stored at room temperature (30.3°C) for 4 hours.

Friday 17/8/2018

Results of the transformation efficiency test:

- Stored at -20ºC: 5.88e5 CFUs/ µg
- Stored at RT: 1e4 CFUs/ µg

We can use this way.

Tuesday 4/09/18

We have made 10G electrocompetent cells.

Wednesday 5/09/18

Transformation efficiency test for the electrocompetent cells

Thursday 6/09/18

Results of the transformation efficiency test: 1e9 CFUs/ µg