Experiment 12.1 – Calculation of Competent Cell Efficiency Using iGEM Kit

Purpose:

We performed an experiment with the iGEM competent cell efficiency kit in order to calculate the actual efficiency of our competent cells. The 5 pg/ µL DNA stock from the iGEM transformation kit was used in each of the transformations.

Setup:

Trial	Cell Batch	Concentration of DNA	V (cells) : V (DNA)
1 (2x)	6/25	5 pg/µL	50 µL : 1 µL
2 (2x)	6/25	5 pg/µL	40 µL : 1 µL

Procedure:

Transformation protocol:

1. Thaw competent cells on ice for ~15 minutes
2. Aliquot 1 µL DNA into 1.5 mL Eppendorf tubes
3. Add 40/50 µL into DNA aliquots
4. Incubate on ice for ~30 minutes
5. Use water bath to heat shock sample for 1 min. @ 42° C
6. Add 200 µL SOC recovery medium to each sample
7. Allow cells to recover in shaker @ 37° C for ~2 hr.
8. Plate 100 µL of each sample and allow to dry
9. Store plates upside down in incubator @ 37° C overnight (~16 hr)

Calculating competent cell efficiency:

1. Count the colonies on each plate and take the average
2. Divide the average # of colonies by the mass (ng) of DNA (plated) x 1000 (ng/µg)
3. An efficiency of $1.5 \cdot 10^8$ or more is acceptable

Results:

Trial	Colony Count (1)	Colony Count (2)	Avg.	Efficiency
1	~568	~479	~524	$\sim 2.6 \cdot 10^8$
2	~372	~414	~393	$\sim 1.9 \cdot 10^8$

Discussion:

The results of this experiment show that the new batch of cells is indeed more efficient than the first batch made using Alex's protocol. This was suggested in a previous experiment, but has now been quantified using the transformation efficiency kit.