

Experiment 5.1 – 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 iGEM kit contains purified DNA of BBa_J04450, which is on a chloramphenicol backbone, at 5 different concentrations ranging from 50 pg/μL to 0.5 pg/μL. This experiment was performed using only 2 (5 & 10 pg/μL) concentrations, and was performed according to the transformation protocol recommended by iGEM to go with the kit.

Setup:

Trial	Cell Batch	Concentration of DNA	V (cells) : V (DNA)
1 (3x)	5/22	5 pg/μL	50 μL : 1 μL
2 (3x)	5/27	5 pg/μL	50 μL : 1 μL
3 (3x)	5/28	5 pg/μL	50 μL : 1 μL
4 (3x)	5/22	10 pg/μL	50 μL : 1 μL
5 (3x)	5/27	10 pg/μL	50 μL : 1 μL
6 (3x)	5/28	10 pg/μL	50 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 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 20 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)	Colony Count (3)	Efficiency
1	475+	575+	275+	$\sim 1.8 \cdot 10^8$
2	2	0	0	-
3	7	5	1	-
4	275+	375+	475+	$\sim 1.1 \cdot 10^8$
5	0	0	0	-
6	4	13	0	-

Discussion:

The results of this experiment show that only the competent cells made using Alex's protocol are reasonably acceptable. Both of the batches made using iGEM's protocol were thrown out immediately following this experiment. We will use the Alex's protocol if we end up needing more competent cells in the future.