Experiment 6.1 – Competent Cell Efficiency

Purpose:

This experiment was set up to test how efficient the new batch of competent cells is in comparison to the previous stock. All transformations were conducted with commercial pUC19 plasmids (5 pg/µL) with ampicillin resistance.

Setup:

	5/22 (1)	5/22 (2)	6/25 (1)	6/25 (2)
V pUC19 (5 pg/µL)	1 µL	1 µL	1 µL	1 µL
V pUC19 (5 pg/µL)	2 µL	2 µL	2 µL	2 µL

Procedure:

Preparation of competent cells: (5/22)

1. Grow overnight cultures of TOP10 cells in 5 mL SOB in shaker @ 37° C
2. Inoculate 250 mL SOB w/ 1.2 mL of overnight cultures (2x)
3. Grow up in shaker @ 37° C to an optical density of $A_{600} \approx 0.5$ ($A_{600} = 0.43, 0.40$)
4. Transfer to flat bottom centrifuge bottles and spin down @ 5000 rpm for 10 min. @ 4° C (JA-10 rotor)
5. Pour off supernatant and re-suspend each cell pellet with 50 mL 100 mM $MgCl_2$
6. Incubate on ice for ~30 min.
7. Spin down @ 4000 rpm for 10 min. @ 4° C
8. Pour off supernatant and re-suspend each cell pellet with 5 mL 100 mM $CaCl_2$ – 15% glycerol
9. Aliquot into 1.5 mL Eppendorf tubes and store @ -70° C

Preparation of competent cells: (6/25)

1. Grow overnight cultures of TOP10 cells in 5 mL SOB in shaker @ 37° C
2. Pre-chill rotor in cold-room overnight (0-4° C)
3. Inoculate 500 mL SOB w/ 5 mL of overnight cultures
4. Grow up in shaker @ 37° C to an optical density of $A_{600} \approx 0.5$ ($A_{600} = 0.485$)
5. Transfer to flat bottom centrifuge bottles and spin down @ 5000 rpm for 10 min. @ 4° C (JA-10 rotor)
6. Chill rotor and perform all subsequent steps in the cold room (0-4° C)
7. Pour off supernatant and re-suspend each cell pellet with 50 mL 100 mM $MgCl_2$
8. Incubate on ice for ~30 min.
9. Spin down @ 4000 rpm for 10 min. @ 4° C
10. Pour off supernatant and re-suspend each cell pellet with 5 mL 100 mM $CaCl_2$ – 15% glycerol
11. Aliquot into 1.5 mL Eppendorf tubes and store @ -70° C

Transformation protocol:

1. Thaw competent cells on ice for ~15 minutes
2. Aliquot 1-2 µL pUC19 into 1.5 mL Eppendorf tubes
3. Add 40 µL competent cells to DNA aliquots, aspirate w/ pipet to mix
4. Incubate on ice for ~30 minutes
5. Use water bath to heat shock @ 42° C for 60 s.
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

Results:

	(1)	(2)	Avg.	Efficiency
5/22 (5 pg)	8	5	6.5	$1.57 \cdot 10^7$
5/22 (10 pg)	6	1	3.5	$4.22 \cdot 10^6$
6/25 (5 pg)	8	10	9	$2.17 \cdot 10^7$
6/25 (10 pg)	19	18	18.5	$2.23 \cdot 10^7$

Discussion:

We saw from this experiment that the new batch of competent cells is more efficient that the old batch of cells. I pre-chilled the rotor overnight and between spins, as well as performed all of the steps after the first spin down in the cold room. Keeping the cells as close to 0° C as possible may have helped improve the efficiency of the cells. It is difficult to make an accurate calculation of efficiency, since there are many variables that are not specified by any method we have seen for performing this calculation.