

## Experiment 2.1 – Competent Cell Efficiency

### Purpose:

This experiment was designed to compare the efficiency of competent cells prepared using 2 variations of the iGEM protocol as well as the protocol suggested by our advisor, Alex. The effect of heat shock time and temperature at 2 sets of conditions were also re-evaluated due to inconclusive results from experiment 1.1, which was believed to be the result of poor technique. All transformations were performed using commercial pUC19 plasmids (5 pg/μL) with ampicillin resistance.

### Setup:

Trial	Cell Batch	V (cells) : V (pUC19)	Heat Shock
1 (2x)	5/22	50 μL : 1 μL	60 s. @ 42° C
2 (2x)	5/27	50 μL : 1 μL	60 s. @ 42° C
3 (2x)	5/28	50 μL : 1 μL	60 s. @ 42° C
4 (2x)	5/22	50 μL : 1 μL	10 min. @ 37° C
5 (2x)	5/27	50 μL : 1 μL	10 min. @ 37° C
6 (2x)	5/28	50 μL : 1 μL	10 min. @ 37° C
7 (3x)	1 of each	50 μL : 0 μL	60 s. @ 42° C

### 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: (5/27)

1. Grow overnight cultures of TOP10 cells in 5 mL SOB in shaker @ 37° C
2. Inoculate 250 mL SOB w/ 1.0 mL of overnight cultures
3. Grow up in shaker @ 37° C to an optical density of  $A_{600} \approx 0.3$  ( $A_{600} = 0.296$ )
4. Transfer to flat bottom centrifuge bottles and spin down @ 4200 rpm for 10 min. @ 4° C (JA-10 rotor)
5. Pour off supernatant and re-suspend cell pellet with 80 mL CCMB80 buffer
6. Incubate on ice for ~20 min.
7. Spin down @ 4200 rpm for 10 min. @ 4° C
8. Pour off supernatant and re-suspend cell pellet with 10 mL CCMB80 buffer
9. Test absorbance of 200 μL cells w/ 800 μL SOC ( $A_{600} = 1.36$ )
10. Incubate on ice for ~20 min.
11. Aliquot into 1.5 mL Eppendorf tubes and store @ -70° C

#### Preparation of competent cells: (5/27)

1. Grow overnight cultures of TOP10 cells in 5 mL SOB in shaker @ 37° C
2. Inoculate 250 mL SOB w/ 1.0 mL of overnight cultures
3. Grow overnight @ R.T. to an optical density of  $A_{600} \approx 0.3$  ( $A_{600} = 0.466$ )
4. Transfer to flat bottom centrifuge bottles and spin down @ 4200 rpm for 10 min. @ 4° C (JA-10 rotor)
5. Pour off supernatant and re-suspend cell pellet with 80 mL CCMB80 buffer
6. Incubate on ice for ~20 min.
7. Spin down @ 4200 rpm for 10 min. @ 4° C
8. Pour off supernatant and re-suspend cell pellet with 10 mL CCMB80 buffer
9. Test absorbance of 200  $\mu$ L cells w/ 800  $\mu$ L SOC ( $A_{600} = 1.44$ )
10. Incubate on ice for ~20 min.
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 50  $\mu$ L competent cells into 1.5 mL Eppendorf tubes
3. Add pUC19 into competent cell aliquots
4. Incubate on ice for ~30 minutes
5. Use water bath to heat shock samples at various conditions
6. Add 1 mL SOC recovery medium to each sample
7. Allow cells to recover in shaker @ 37° C for ~2 hr.
8. Plate 200  $\mu$ L of each sample and allow to dry
9. Store plates upside down in incubator @ 37° C overnight

#### Results:

Trial	Colony Count (1)	Colony Count (2)	Mean $\pm$ St. Deviation
1	22	26	23.0 $\pm$ 1.4
2	0 (*)	4	2.0 $\pm$ 2.8
3	6	6	6.0 $\pm$ 0
4	11	9	10.0 $\pm$ 1.4
5	3	1	2.0 $\pm$ 1.4
6	2	2	2 $\pm$ 0
7	all 3 negative controls were blank		N/A

(\*) – poor loading of DNA was observed during this trial

#### Discussion:

It was noted during the transformation procedure that the DNA was not being loaded directly into the cells on trial 2a. This vial was marked, and after transformation it was evident that proper loading technique is vital to the efficiency of transformation. We can also conclude that the competent cell protocol that was provided by Alex produces much higher efficiency cells than can be prepared using the protocol suggested by iGEM. Additional experiments will be conducted to test more parameters in the transformation protocol in an effort to further increase our transformation efficiency. We will use the competent cells prepared using Alex's protocol for all transformations moving forward.