Experiment 1.1 – Chemical Transformation Efficiency

Purpose:

This experiment was designed to investigate the effects of both competent cell : DNA ratio and heat shock temperature and duration on chemical transformation efficiency using commercial pUC19 plasmids (10 pg/ µL) with ampicillin resistance.

Setup:

Trial	V (µL) cells	V (µL) pUC19	Heat Shock
1 (2x)	50	0	-
2 (2x)	170	1	10 min @ 37° C
3 (2x)	50	2	10 min @ 37° C
4 (2x)	50	2	30 s @ 42° C
5 (2x)	50	2	60 s @ 42° C

Procedure:

Transformation protocol:

1. Thaw competent cells on ice for ~15 minutes
2. Aliquot 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 ~1 hr.
8. Plate 200 µL 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 ± St. Deviation
1	Film	Film	N/A
2	26	13	18.0 ± 9.2
3	20	16	18.0 ± 2.8
4	11	0	5.8 ± 7.8
5	22	0	11.0 ± 15.6

Discussion:

Trials 4.2 and 5.2 did not produce any colonies, which was most likely due to poor loading technique when adding the DNA to the competent cell aliquots. This did not allow us to draw any definite conclusions regarding the effect from varying the temperature and duration of heat shock. The most consistent results were produced using a 50 : 2 ratio of competent cells to DNA with a 10 minute heat shock at 37° C. We can conclude from this experiment that our original competent cells are viable, but may not be as efficient as desired. Additional experiments will be performed to test additional parameters and also to compare this batch of competent cells to those prepared using the protocol recommended by iGEM.