Experiment 2.3 – Choice of Recovery Medium and Volume

Purpose:

This experiment was designed to investigate the effect of using various types and volumes of growth media during the recovery phase of our transformation protocol. These experiments were conducted using commercial pUC19 (5 pg/µL) plasmids with ampicillin resistance.

Setup:

Trial	Type of Recovery Medium	Volume of Recovery Medium
1 (2x)	SOB	75 µL
2 (2x)	SOC	75 µL
3 (2x)	SOB	150 µL
4 (2x)	SOC	150 µL
5 (1x)	SOB	500 µL

Procedure:

Transformation protocol:

1. Thaw competent cells (5/22) on ice for ~15 minutes
2. Aliquot 50 µL competent cells into 1.5 mL Eppendorf tubes
3. Add 1 µL pUC19 into competent cell aliquots
4. Incubate on ice for ~5 minutes
5. Use water bath to heat shock samples for 10 min. @ 37° C
6. Add recovery medium to each sample as per experimental setup
7. Allow cells to recover in shaker for 1 hr. @ 37° C
8. Plate 150 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	12	29	20.5 ± 12.0
2	12	34	23.0 ± 15.6
3	13	28	20.5 ± 10.6
4	27	85	56.0 ± 41.0
5	23	N/A	N/A

Discussion:

This experiment showed that using a volume of 150 µL SOC during the recovery produced far and away the best results. The SOB recovery medium did not show much variation in colony count despite the difference in volume added. In theory, some minimum amount of growth medium must be added in order to allow the cells to properly recover. Adding more will allow the cells to recover but will dilute the amount of transformed cells that are plated at the end of the process, which would require a larger volume to be plated in order to balance out. We have decided to use 200 µL of SOC in all transformations moving forward.