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Goal: Transformations from the iGEM registry (Promoter and Coding Sequence)

Transformation Procedure for ONE SHOT

Materials:

Warm the vial of S.O.C. Medium made by Cara (6-6-17)

• Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates

for each transformation).• Place cuvettes on ice and set up your electroporator for bacterial transformation

as per the manufacturer's instructions.

• One 15 ml snap-cap tube per transformation

Transformation Procedure

Use this procedure to transform One Shot® TOP10 Electrocomp™ E. coli. We

recommend including the pUC19 control plasmid DNA supplied with the kit

(10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8) in your transformation

experiment to verify the efficiency of the competent cells. Do not use these cells for

chemically competent transformation.

1. Thaw, on ice, one vial of One Shot® TOP10 Electrocomp™ cells for each transformation.
2. Add 1 µl of the DNA (10 pg to 100 ng) into a vial of One Shot® cells and mix gently. For the pUC19 control, add 10 pg (1 µl) of DNA into a separate vial of One Shot® cells and mix gently.
3. Transfer the cells to the chilled electroporation cuvette on ice.
4. Electroporate the cells as per the manufacturer's recommended protocol.

5. Aseptically add 250 µl of pre-warmed S.O.C. Medium to each vial.

6. Transfer the solution to a 15 ml snap-cap tube and shake for at least 1 hour at 37°C to allow expression of the antibiotic resistance gene.

7. Spread 10 to 150 µl from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, dilute the transformation mix 1:50 into LB Medium (e.g. remove 20 µl of the transformation mix and add to 980 µl of LB Medium) and plate 20-100 µl.

8. Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.

9. Invert the selective plate(s) and incubate at 37°C overnight.

10. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

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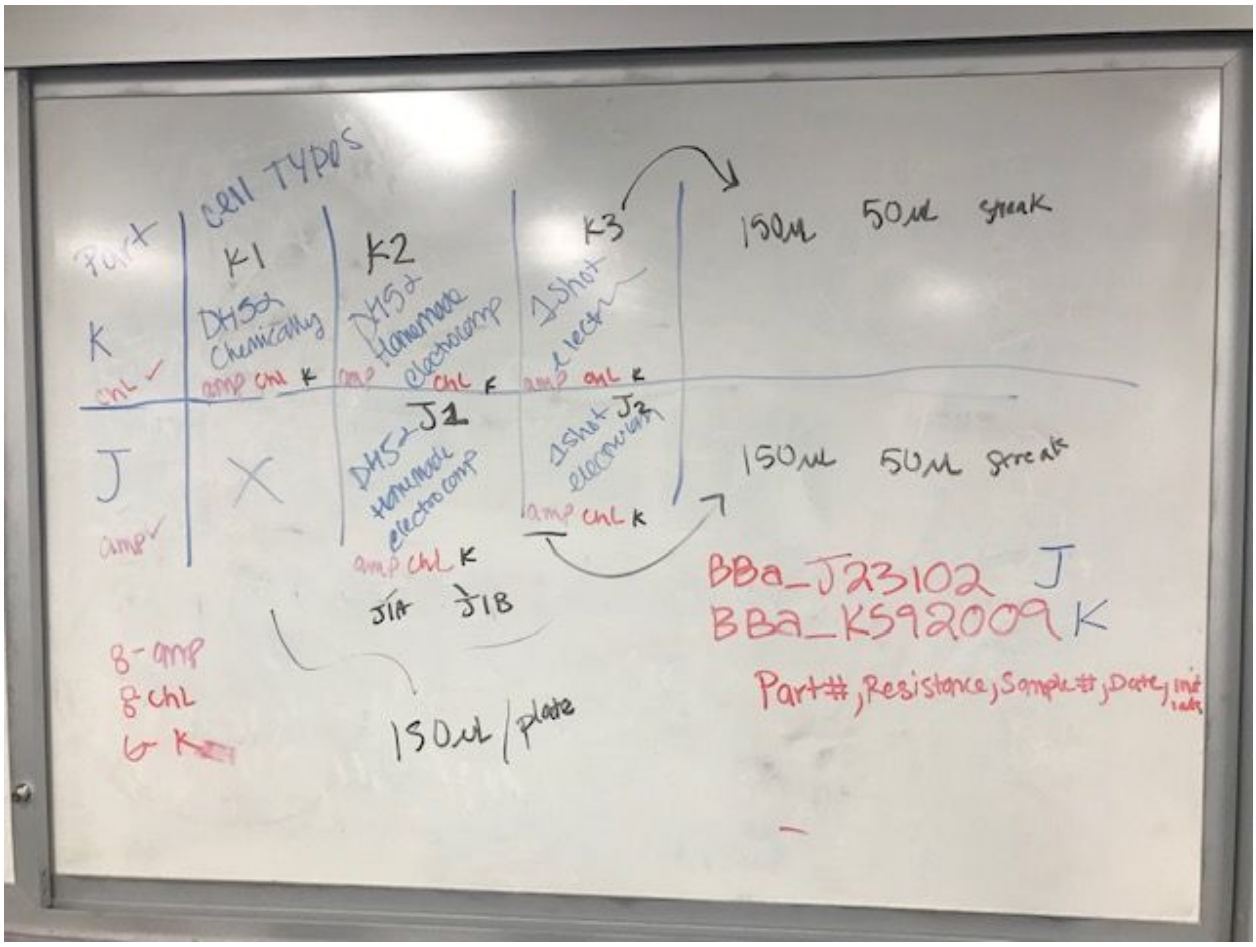
DH5 Transformations

Materials:

- DH5α chemically competent cells
- DH5α Homemade Cells(made in Spring Semester)
- SOC Medium made by Cara 6-6-17
- Eppendorf Tubes
- Cuvettes etc.

1. Combined 40 µL of electrically competent DH5α cells and 2 µL of ligated DNA to an Eppendorf tube.
2. Transferred the contents of the Eppendorf tube to a cuvette and lightly tapped the cuvette on the table to evenly distribute the contents and to get rid of air bubbles.
3. Placed the cuvette into the Bio-Rad MicroPulser and delivered an electric shock.
4. Immediately after, 900 µL of SOC medium was added to the cuvette and the solution was mixed via micropipette.
    a. DH5α A2 contained a newer SOC medium than DH5α A1 and DH5α B
5. Transferred the solution from the cuvette to a shaker tube and placed in the shaker at 37°C at 300 rpm for ~1 hour.

6. After shaking for ~1 hour, DH5α A1, A2, B, and Oneshot A and B were streaked and spread (100 µL) on kanamycin plates.
    a. DH5α A1 spread used 150 µl instead of 100 µ.
    b. 10 plates total
7. Plates left to incubate at 37 °C for 24 hours.
8. 

How to read the picture:

J is the promoter

Is supposed to be resistant to amp

2 plates were used to test if DH5alpha cells would frow (trials J1A and J1B)

3 plates were used to test 1 shot cells (J2) (150microL, 50microL and a streak plate)

K is the coding sequence

Is supposed to be resistant to chloramphenicol

1 plate test the DH5alpha chemically competent cells (K1)

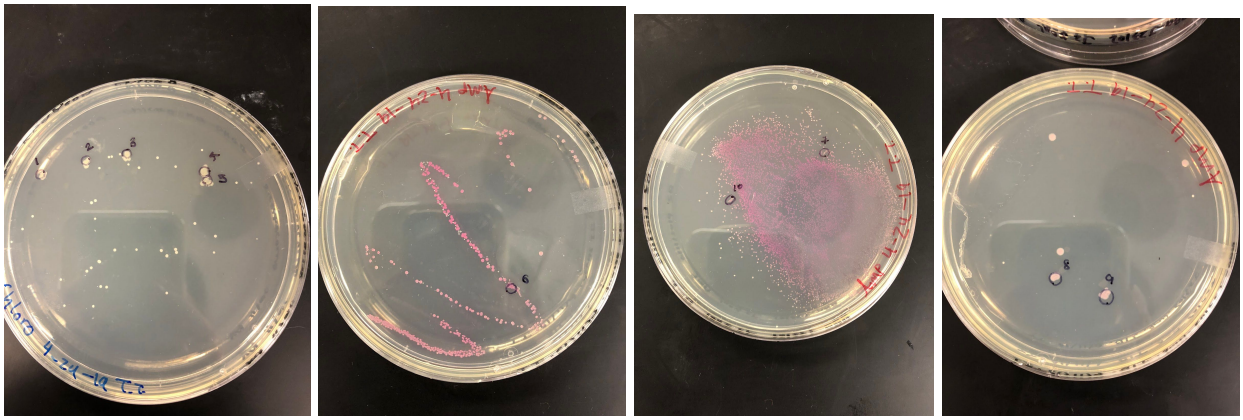
1 plate was used to test the DH5alpha electrocompetent(K2) (homemade cells)

3 plates were used to test 1 shot cells (K3) (150microL, 50microL and a streak plate)

Negative controls: The rest of the amp and chloramphenicol plates were used
6 plates of Kanamycin plates were used as a positive control.

Results

4 Plates looked promising:



Include pictures of your gel with a key of what is in each lane and a snapshot of what it should look like from SnapGene by simulating a gel.

Conclusion

Overall the goal was accomplished but has room for improvement. We were only able to get a little growth/very small number of colonies.

Remember: If more than one person worked on the same experiment only one entry is required. You do not have to be wordy, but be precise and informative. As long as someone can do this again following your protocol and knows what results to expect then you have succeeded! 😊