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Goal:

- Need to get plasmid, pCB302-gfp-MBD? , off of paper and transform using heat shock
    - Used protocol to elute DNA
- After transformation, perform PCR to amplify the DNA
- Pick colonies and start overnight cultures of transformed cells
- Characterize blue chromoprotein from the registry
    - How the pH affects the fluorescence of blue cp
    -

What do you want to accomplish, how will you do it (with PCR? Mini prep? Restriction digest?).

Materials

- TE Buffer ~300 µl
- Eppendorf Tube
- PCB302A
- PCB302B

Specific brand name and lot number

Protocol for removing DNA off of paper to transform:

1. Remove saran wrap from paper and locate the DNA via UV light
2. Use a razor blade to cut out the DNA and place into an Eppendorf tube.
3. Place 300µl of TE Buffer into the Eppendorf tube and let it sit for roughly 20 minutes.
4. Centrifuge at 13.3000 rpm for 5 minutes to sink the paper to the bottom of the tube and enable to draw out the liquid.
5. Transfer into new Eppendorf tubes
6. Use electroporation protocol to transform cells with the DNA

Transformation Protocols”

Transformation Procedure for ONE SHOT

Materials:

Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.

• Spread X-gal onto LB agar plates containing antibiotic, if desired.

• Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/ml ampicillin.

• 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 1-2 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.

Transformation with homemade cells from Spring 2019 (DH5a cell protocol)

Electroporation Transformation

1. 40 µL of the electrically competent cells and 1 µL of ligated DNA was added to an Eppendorf tube.
2. The contents of the Eppendorf tube was transferred to a cuvette and the cuvette was lightly tapped on the table to evenly distribute the contents.
3. The cuvette was placed into the Bio-Rad MicroPulser and an electric shock was delivered.
4. Immediately after, SOC medium was added to the cuvette and the solution was micropipette mixed.
5. The solution in the cuvette was added to a shaker tube and placed in a shaker at 37° at 200 rpm for 1 hour.
6. After shaking for 1 hour, 150 µL of the solution was streaked onto an agar plate with the respective antibiotics (ampicillin for pGEX).

Performed Transformations

Shakera and Christina did transformations on One-Shot Top Ten Cells

Laura and Chiara did transformations on Homemade Cells

Both groups did 4 transformation (2 pCB302 A and 2 pCB302 B)

pCB302 A and pCB302 B are the same plasmids but were on different sample paper

pCB302 A and pCB302 B are pCB302-gfp-MBD

Streaking Plates Protocol for all four transformations
There are 12 total Plates to streak (6 that are related to Oneshot Electrocompotent cell and 6 for homemade electrocompetent)

1. Add solutions to the center of the plate
2. Heat up streaker and make sure it is not hot to spread ethanol on a plate
3. Put the lid back on
4. Let it sit for about 10,15,20 minutes because of the increase of sample onto the plate.
5. Once you put the lid back on let it soak into the agar
6. Then flip the plate over with the lid so the part with agar is on the top
7. Put in the incubator at 37 ℃
8. Leave overnight

Pt 2:

Objective Transform Blue CP

Registry… to obtain needed samples

Transformation Procedure for ONE SHOTGUN

Materials:

Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.

• Spread X-gal onto LB agar plates containing antibiotic, if desired.

• Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/ml ampicillin.

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• *One 15 ml snap-cap tube per transformation*

Transformation Procedure

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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.

It was 50 ul of TopTen cells and 1ul of kit plate DNA in each vial. (2 vials)

After Recovering Cells

> Chiara, Asma, Christina, Jessica, Emilio and Kennex performed transformation on Blue Chromoprotein (coding seq) and J23102 (promotor). Afterwards plated both.

The promoter (J23102) which is ampicillin resistant recovered for about 25 minutes and does not need the full hour because it is slow reacting, and then was either streaked or spread. But the blue chromoprotein is chloramphenicol resistant and had to be incubated for the full hour before being plated.

3 Plates for J23102

- Streaked
- 100 microliters spread with hockey stick
- 150 microliters spread with hockey stick

3 Plates for Blue Chromoprotein

- Streaked
- 100 microliters spread with hockey stick
- 150 microliters spread with hockey stick

Results

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

Did you accomplish your goal? How has your work today helped with the overall project or in lab overall? Discuss gel results here, possible points of error or expected error. How will you proceed?

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! 😊

We want to perform transformation of the plasmid, pCB302, into symbiodinium microadriaticum to determine the best transformation protocol. Although, the Mexico lab has already transformed pCB302 into symbiodinium microadriaticum, we need to perform the transformation to amplify the DNA and make adjustments to their existing protocol.