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Goals:

1. Simulate an artificial ligation of blue chromoprotein part into J23102 (promoter and pcba1020-r0040 plasmid minus RFP) on snap gene
2. Ligate BP part with J23102 plasmid from Amirah's gel extraction
    a. BP was digested with NotI
    b. Promoter plasmid (J23102) was cut with SpeI and PstI
3. Ligate BP part with J23102 plasmid from Jessica and Christina's gel extraction
    a. BP (K592009) was digested with PSTI and BcuI
    b. Promoter plasmid (J23102) was cut with SpeI and PstI
4. Transform BP- promoter construct into DH5a chemically competent cells
5. Transform PCB302 (Mexico plasmid) into One Shot Top 10 chemically competent cells to eventually amplify that DNA
6. Restriction digest on pcb302 plasmid from previous mini-prepped samples on 6/14/19

Materials

- Extracted K DNA
- Extracted J DNA

Protocol

Kennex concocted the ligation mix. We ligated Jessica's and Christina's gel extractions. However, we had to throw the mix away because the ethanol precipitation hadn't been done yet. We then performed the correct ligation with Amirah's gel extractions. Later, we did a real ligation on Jessica and Christina's gel extractions (red tape). Did transformations (chemically) on both ligation reactions.

Purpose: To add gene into a plasmid

Ligation on Amirah's Gel Extraction

1. Add 6 µL of diH2O to a clean 1.5 mL Eppendorf tube, 1µL of T4 DNA Ligase Buffer, 1µL of plasmid DNA and 1µL of DNA part and mix.
2. Add 1µL of T4 DNA Ligase.
3. Pipet mix the tube and incubate at room temperature for 10 minutes.

Ligation on Jessica's Gel Extraction

1. Add 2 µL of diH2O to a clean 1.5 mL Eppendorf tube, 1µL of T4 DNA Ligase Buffer, 1µL of plasmid DNA and 5µL of DNA part and mix.
2. Add 1µL of T4 DNA Ligase.
3. Pipet mix the tube and incubate at room temperature for 10 minutes.

   *Adjusted the amount of DNA part based on the concentration of the DNA plasmid and DNA part.

Transformation

Transformation Protocol for one shot top 10 chemically competent

1. Centrifuge the vial(s) containing the ligation reaction(s) briefly and place on ice.

2. Thaw, on ice, one 50 µL vial of One Shot® cells for each ligation/transformation.

3. Pipet 1–5 µL of each ligation reaction directly into the vial of competent cells and mix by tapping gently. Do not mix by pipetting up and down. The remaining ligation mixture(s) can be stored at −20°C.

4. Incubate the vial(s) on ice for 30 minutes.

5. Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.

6. Remove vial(s) from the 42°C bath and place them on ice.

7. Add 300 µL of pre-warmed S.O.C medium to each vial. S.O.C is a rich medium; sterile technique must be practiced to avoid contamination.

8. Place the vial(s) in a microcentrifuge rack on its side and secure with tape to avoid loss of the vial(s). Shake the vial(s) at 37°C for exactly 1 hour at 225 rpm in a shaking incubator.

9. Spread 20–200 µL from each transformation vial on separate, labeled LB agar plates. The remaining transformation mix may be stored at 4°C and plated out the next day, if desired.

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

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

- 4 plates were made
  - 2 Ampicillin
    - One with puc19 positive control, one with pcb302 (should be negative for growth)
  - 2 Kanamycin
    - One with puc19 negative control, one with pcb302

Transformation done on Amirah and Jessica's gel extraction (2 ligation reactions) by Kennex. Asma plated the transformations.

One Shot™ MAX Efficiency™ DH5α-T1R Competent Cells

1. Briefly centrifuge the ligation reaction and place on ice.

2. Thaw, on ice, one 50 µl vial of One Shot® cells for each ligation/transformation.

3. Pipet 1 to 5 µl of each ligation reaction directly into the competent cells and mix by tapping gently. Do not mix by pipetting up and down. Store the remaining ligation reaction at -20°C.

* 1uL of ligation mix

4. Incubate the vial on ice for 30 minutes.

5. Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.

6. Remove vial from the 42°C bath and place on ice.

7. Add 250 µl of pre-warmed SOC medium to each vial. (SOC is a rich medium; sterile technique must be practiced to avoid contamination.)

8. Place the vial in a microcentrifuge rack on its side and secure with tape to avoid loss of the vial. Shake the vial at 37°C for exactly 1 hour at 225 rpm in a shaking incubator.

9. Spread 20 µl to 200 µl from each transformation vial on separate, labeled LB agar plates. We recommend that you plate two different volumes. Note: You may have to dilute cells 1:10 to obtain well-spaced colonies.

10. Store the remaining transformation reaction at +4°C and plate out the next day, if desired.

11. Invert the plates and incubate at 37°C overnight.

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

*Spread 2 plates for each ligation, so in total 4 plates. Each ligation reaction should have one 150uL spread and one 100uL spread.

- Ligation 1 = Amirah's gel extraction
- Ligation 2 = Jessica and Christina's gel extraction

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?

Notes

1. Transformation Procedure for ONE SHOT bl21 Chemically cells
    a. Only used for PROTEIN EXPRESSION. NOT CLONING.