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Date: 6/19/2019

Goal:

1. Run the gel for colony PCR on BCP K592009 + J23102 Promoter Ligation
2. Re-do overnight cultures
    a. Pcb302 in E. Coli from papers A & B
    b. Ligation 1
        i. 100 µL spread on plate
        ii. 150 µL spread on plate
    c. Ligation 2
        i. 100 µL spread on plate
        ii. 150 µL spread on plate

Date: 6/19/19

Goal:

1. Re-do overnight cultures
    a. pcb302
    b. Ligation 1 (K592009 + J23102)
        i. 100 µL spread on plate
        ii. 150 µL spread on plate
    c. Ligation 2 (K592009 + J23102)
        i. 100 µL spread on plate
        ii. 150 µL spread on plate

Protocol:

Overnight cultures

1. Add 6 mL of LB to a 15 mL Falcon tube along with 6 µL of antibiotics (Ampicillin for ligation reactions; Kanamycin for pcb302)
2. Dip a p10 tip into your selected colony and drop into the tube
3. Incubate in the water bath at 37° C at 220 rpm for 16-18 hours

Date: 6/19/19

Goal:

1. Run the gel for colony PCR on BCP + J Promoter Ligation
    a. K592009 & J23102

Protocol:

Preparing, Loading, and Running a 1% Agarose Gel

Preparing

1. Added 1 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask
2. Heated in the microwave until fully dissolved
3. Allowed the solution to cool until comfortable to touch
4. Added 10 µL GelRed Nucleic Acid Gel Stain and mixed
5. Inserted casting tray, made sure the rubber on the sides was not overlapping
6. Carefully poured the agarose into the tray and place the comb to create the wells
7. Allowed the gel to solidify
8. Once solidified, changed the orientation of casting tray where the rubber sides are not in contact with the sides of the system.
9. Poured in 1X TBE into the gel electrophoresis system to the fill line, being sure to submerge the gel, and removed the comb

Loading

1. Loaded ~5 µL of the ladder in the first well
2. Prepared samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and loaded

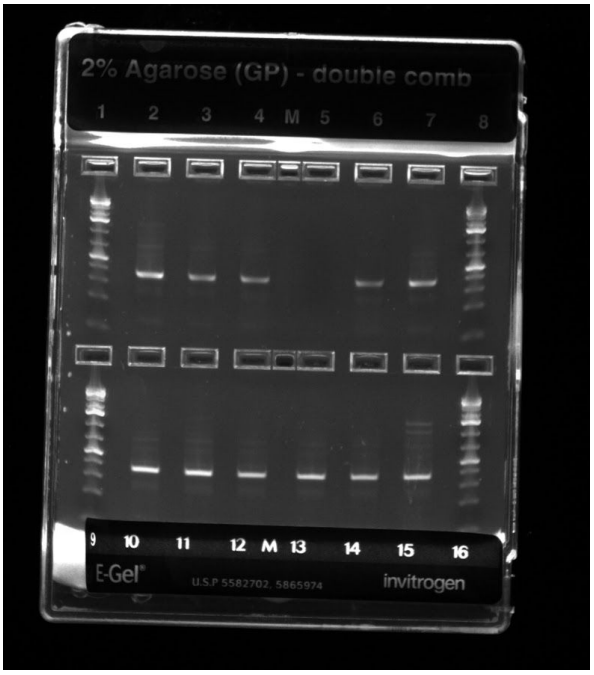
Running

1. Once the gel had been loaded, slid on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Ran for about 45 minutes to an hour

Results

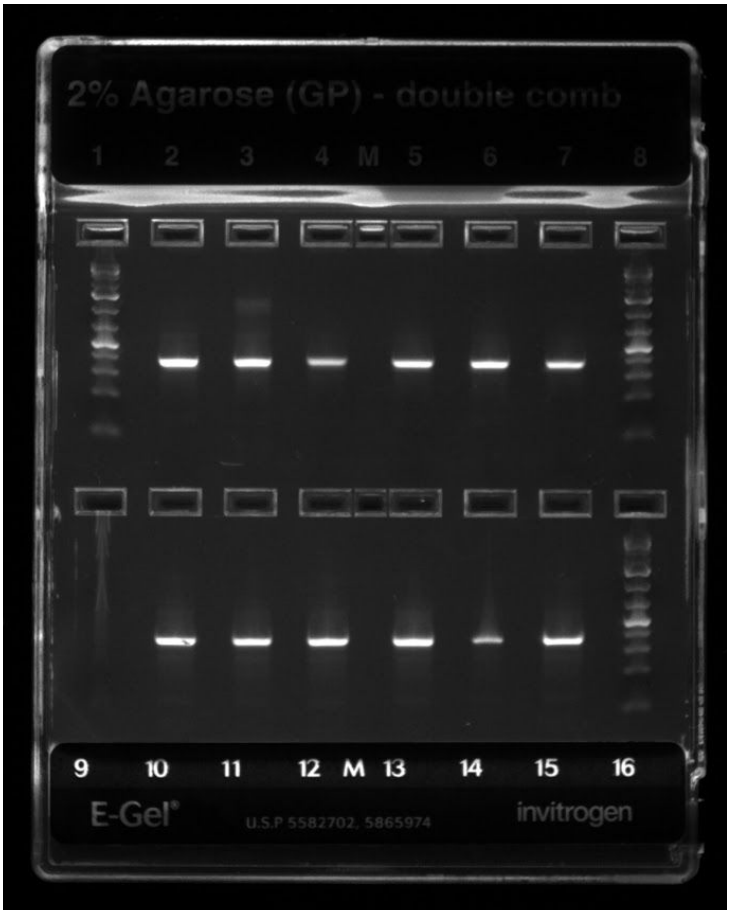
Gel1:

Lane 1	**2**	**3**	**4**	**M**	**5**	**6**	**7**	**8**
Gene Ruler 1 Kb plus	ligation 1 (100ul) colony 1	ligation 1 (100ul) colony 2	ligation 1 (100ul) colony 3	empty	ligation 1 (100ul) colony 4	ligation 1 (100ul) colony 5	ligation 1 (100ul) colony 6	Gene Ruler 1Kb plus
Gene Ruler 1 Kb plus	ligation 1 (150ul) colony 1	ligation 1 (150ul) colony 2	ligation 1 (150ul) colony 3	empty	ligation 1 (150ul) colony 4	ligation 1 (150ul) colony 5	ligation 1 (150ul) colony 6	Gene Ruler 1Kb plus

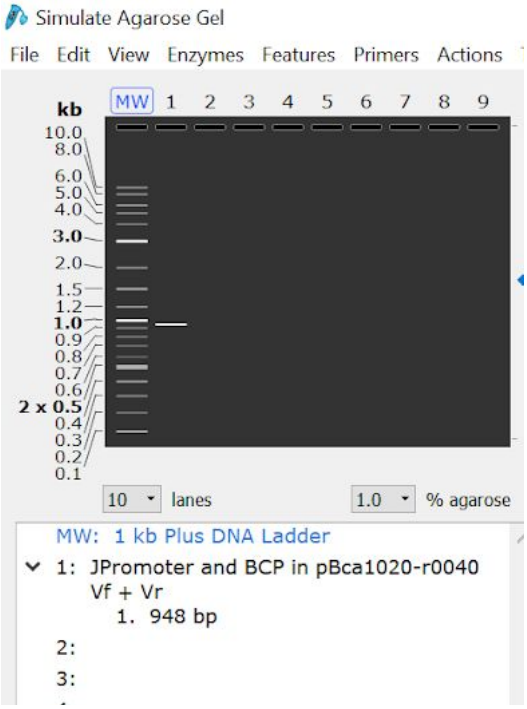


Gel 2:

Lane 1	**2**	**3**	**4**	**M**	**5**	**6**	**7**	**8**
Gene Ruler 1 Kb plus	ligation 2 (100ul) colony 1	ligation 2 (100ul) colony 2	ligation 2 (100ul) colony 3	empty	ligation 2 (100ul) colony 4	ligation 2 (100ul) colony 5	ligation 2 (100ul) colony 6	Gene Ruler 1 Kb plus
Gene Ruler 1 Kb plus	ligation 2 (150ul) colony 1	ligation 2 (150ul) colony 2	ligation 2 (150ul) colony 3	empty	ligation 2 (150ul) colony 4	ligation 2 (150ul) colony 5	ligation 2 (150ul) colony 6	Gene Ruler 1 Kb plus



Expected Results



Conclusions

It appears that each well contains at least one correct PCR product. They all have a band at around the 2000 base pair (bp) mark as expected. Therefore, we should set up a pH gradient to go ahead and characterize the blue chromoprotein part.