Online Notebook Template

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Date: 6/18/19

Goal:

1. Simulate gel for restriction digest on Snapgene
2. Run a gel on the Restriction Digest for pCB302 samples A and B from 6/17/19 and verify if we have the correct parts.
3. Colony PCR on ligations from previous day
4. Overnight culture on ligations as well as the pCB302 plasmid
5. Create Primers for pCB302 partial sequence on snapgene

Protocol

Colony PCR Protocol

Prepare 24 PCR tubes.

For 20 µL Reaction

1. Prepare a PCR concentration cocktail with the following proportions: 200µL of diH2O, 250 µL PCR Mastermix(2x), 25 µL of the forward primer, and 25 µL of the reverse primer. (Total 500uL PCR cocktail)
2. Add 20 µL of the concentration cocktail into each PCR tube.
3. Using a 10 µL micropipette, touch the tip onto the selected colony and swirl around in the PCR tube.
4. Place PCR tube in the thermocycler at the following generic settings:
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. 52° C for 1:00 minute *Annealing temperature varies depending on primer
    4. 72° C for 1:00 minute
    5. 30X (Go to Step 2)
    6. 72° C for 5:00 minutes

    Lid Temperature: 105° C

For the samples leaving in the machine, its label are as follows:
1 1:ligation 1 100 µL colony 1,1 2:ligation 1 100 µL colony 2,...,to 1 6
1’ 1:ligation 1 150 µL colony1,...to 1’6
2 1:ligation 2 100 µL colony 1, … to 2 6
2’1: ligation 2 150 µL colony 1, … to 2’6

Overnight Cultures

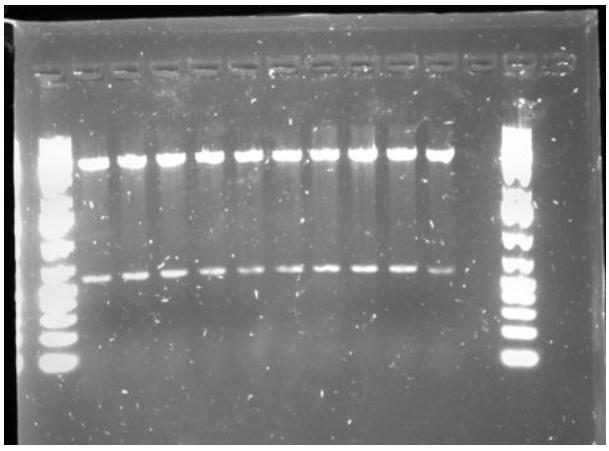
1. Add about 6 mL of LB(chloro added already) to a 15 mL Falcon tube
2. Dip a p10 tip into your selected colony and drop into the tube
3. Incubate in the water bath at 37° C at 220 rpm for 16-18 hours

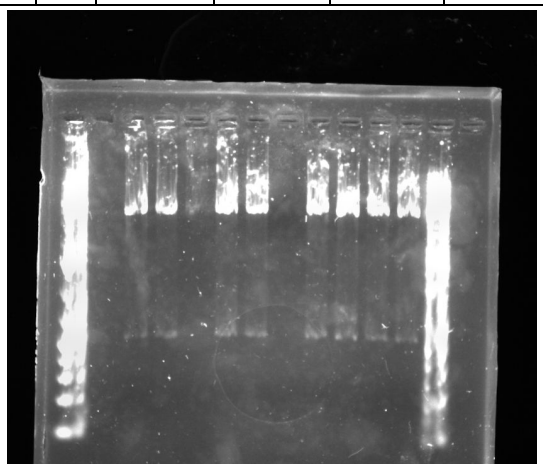
Results

S = sample

Gel 1: A samples

Lane 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gene Ruler 1 Kb plus	A1 s 1	A1s 2	A2 s 1	A2s 2	A3s 1	A3s 2	A4 s1	A 4s 2	A5s1	A5s2	empty	empty	Ladder

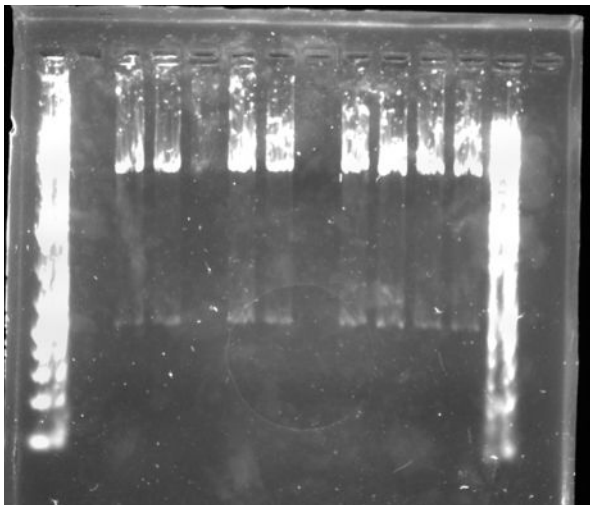


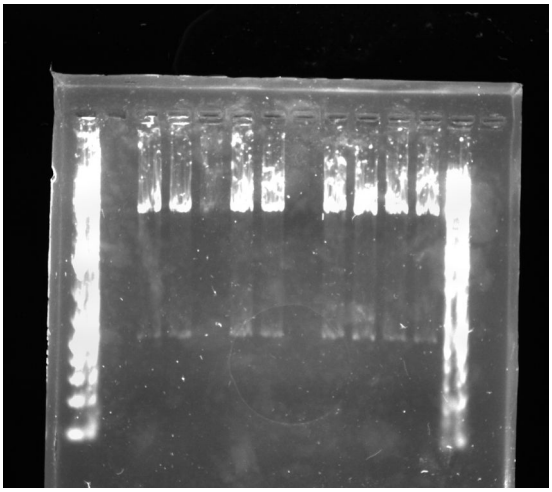


(run 20 min. longer)

Gel 2: B samples

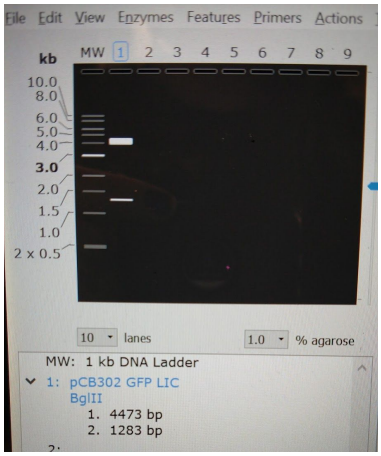
Lane 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gene Ruler 1 Kb plus	empty	B1 s 1	A1s 2	A2 s 1	A2 s2	A3s 1	A3s 2	A4 s1	A4 s2	B5 s1	B5 s2	Ladder	empty





(Run 20 min. longer)

Expected Results


File Edit View Enzymes Features Primers Actions
kb
MW 1 2 3 4 5 6 7 8 9
10.0
8.0
6.0
5.0
4.0
3.0
2.0
1.5
1.0
2 x 0.5
10 lanes
1.0 % agarose
MW: 1 kb DNA Ladder
1: pCB302 GFP LIC
BglII
1. 4473 bp
2. 1283 bp