

Name: Sijia, Jiazi, Kennex, Rehmat

Date: 7/31/19

Goals:

1. Mini-prep for pcb302 overnight cultures in E. Coli from papers 1 & 2 from 7/3/19 glycerol stocks
2. Restriction digest of pcb302 miniprep samples from today
3. Restriction digest on Dino III plasmid (XbaI and BglII)
4. Restriction digest on CO RFP (XbaI and BglII)

Name: Dr.Brewer, Jessica, Sijia, Jiazi

Date: 7/31/19

Goals:

1. Mini-prep of pcb 302 in E. Coli from overnight cultures 7/29/19

Protocol:

1. Added 1.5 ml overnight cultures to 6 tubes.
2. Centrifuged for 1 min at 8000 rpm .
3. Discarded the supernatant and resuspended pellet in one tube with 250 μ L Buffer P1 and combined 6 tubes.
4. Added 250 μ L of Buffer P2 and inverted 5 times.
5. Added 350 μ L of Buffer N3 and immediately mixed by inverting 5 times.
6. Repeated step 1-5 10 times for each sample, then got 10 tubes for each sample.
7. Centrifuged for 10 minutes at 13,000 rpm.
8. Divided 10 tubes into 2 groups for each sample.
9. Added 800 μ L of the clear supernatant into a spin column and centrifuged for 60 seconds and discarded the excess liquid.
10. Repeated step 9 five times for each group of each sample.
11. Added 500 μ L of PB and centrifuged the spin columns for 60 seconds. Discarded the flow through.
12. Added 750 μ L of PE to the spin columns, centrifuged for 60 seconds, and discarded the flow through.
13. Centrifuged the spin columns again for 60 seconds to remove residual wash buffer and discarded the flow through.
14. Transferred the spin columns to a clean eppendorf tube and added 50 μ L of EB to the center of the spin column to elute the DNA.
15. Allowed the spin column to stand for one minute and then centrifuged for one minute.
16. Recorded the concentrations for each sample.

Results:

Sample	Concentration(ng/ul)
pcb302 150ul colony 1,1	25
pcb302 150ul colony 1,2	25
pcb302 150ul colony 3,1	12.5
pcb302 150ul colony 3,2	15
pcb302 75ul colony 7,1	35

pcb302 75ul colony 7,2	30
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Conclusion:

The concentrations were higher than the original pcb302 samples from 7/3/19. We will do the restriction digest to check their bands, then send them to sequence.

Name: Sijia, Jiazi, Asma

Date: 7/31/19

Goals:

1. Restriction digest of pcb302
 - a. Enzyme: KpnI

Protocol:

Restriction Digest Protocol

30 μ L Fast Digest Restriction Digest

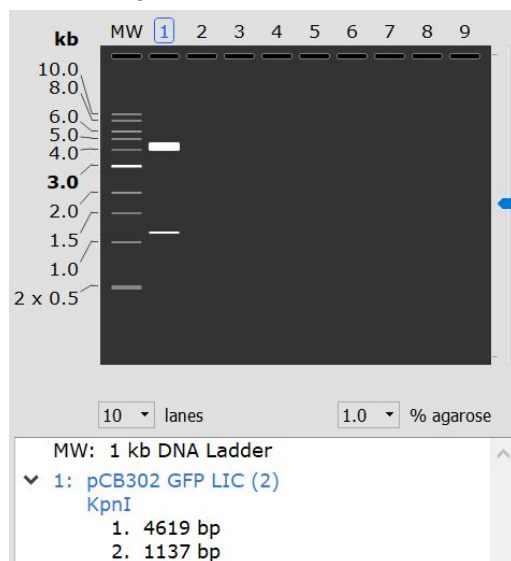
1. Prepared a Fast Digest concentration cocktail with the following proportions: 2 μ L Restriction Enzyme KpnI, 3 μ L of 10X Fast Digest Buffer, and 15 μ L of diH₂O.
2. Added 20 μ L of this cocktail to a clean 1.5 Eppendorf tube and then add 10 μ L of DNA
3. Incubated at 37° C for 30 minutes.

E-gel 2 % Agarose

1. Inserted E-gel into tray box
2. Loaded 5 μ L of MW ladder and DNA into each well
3. Ran for 25 minutes

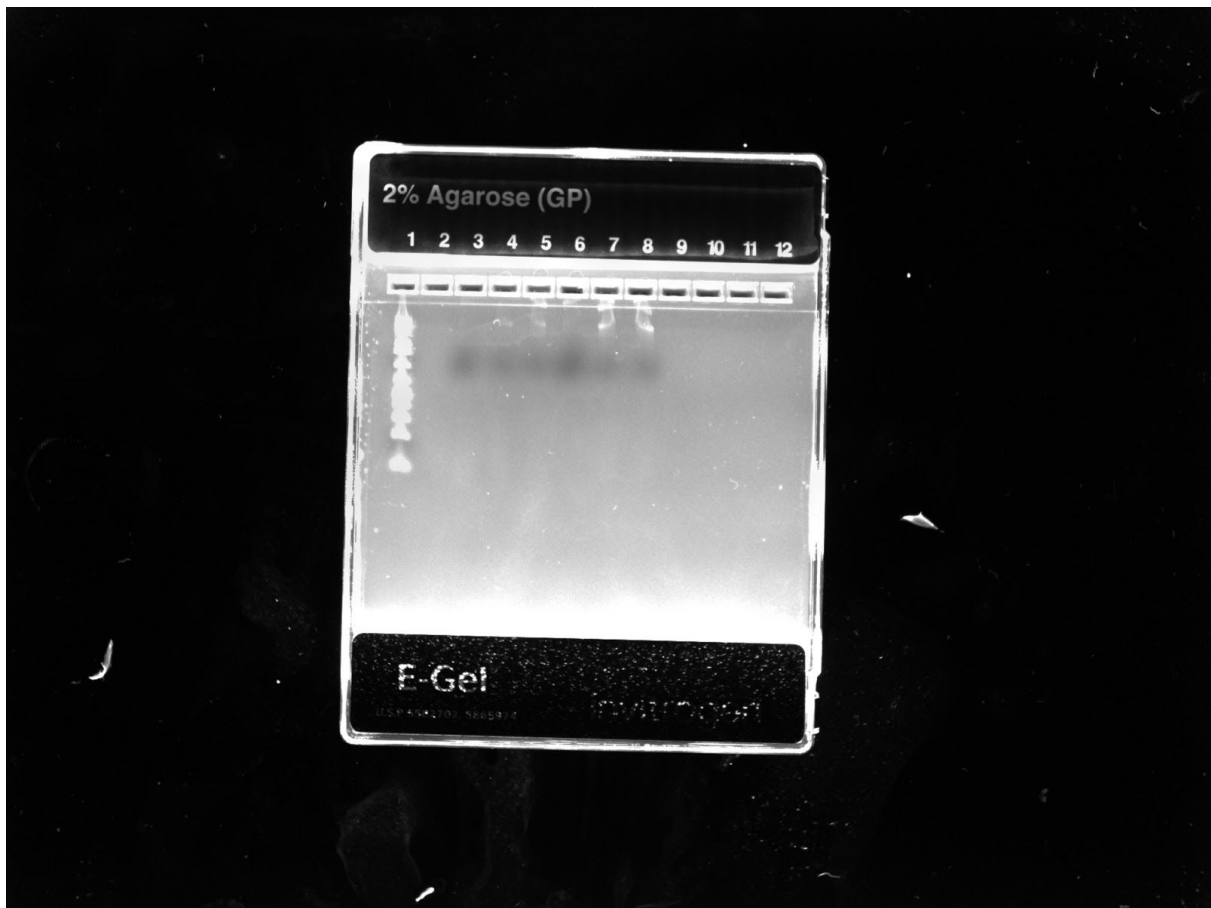
Results:

expected gel-



1	2	3	4	5	6	7	8	9	10	11	12
gene ruler 1kb plus	blank	7-1	7-2	3-1	3-2	1-1	1-2				

observed gel-



Conclusion:

The bands are not clearly visible. The samples may need to be incubated longer if the enzymes did not cut the samples.

Name: Kennex Lam

Date: 7/31/19

Goals:

1. Transform mCherry with One Shot™ BL21 Star™ (DE3) Chemically Competent E. coli

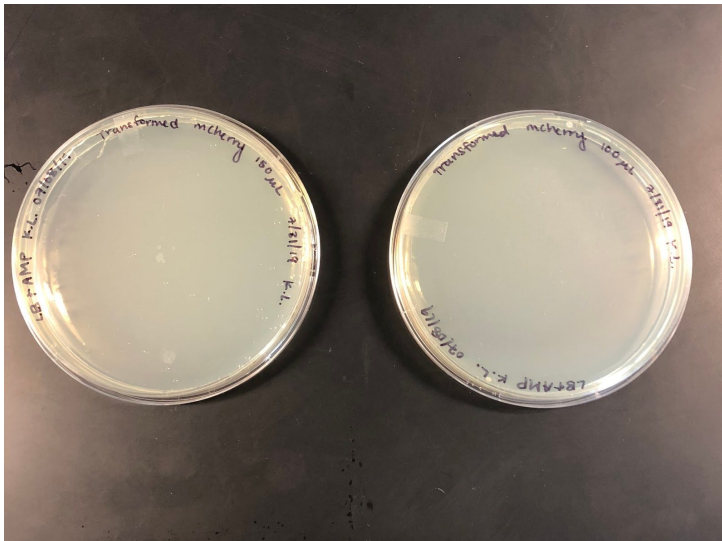
Protocol:

Transformation using One Shot BL21 Star (DE3) Cells

1. One vial of One Shot® cells was thawed on ice for one transformation.
2. Added 5–10 ng of DNA, in a volume of 1–5 µL to the cells and mixed by tapping gently. Did not mix cells by pipetting. **Deviation:** Diluted the initial concentration of 66 ng/uL of mCherry to 13.2 ng/uL in 5 uL. Added 1 uL of diluted mCHerry to 5 uL of competent cells.
3. Incubated the vial on ice for 30 minutes. **Deviation:** Incubated for 35 min.
4. Heat shocked the cells by incubating the vial(s) for exactly 30 seconds in the 42°C water bath. Did not mix or shake.
5. Removed the vial(s) from the 42°C bath and quickly placed on ice.
6. Added 250 µL of pre-warmed SOC medium to the vial(s). (SOC is a rich medium; used proper sterile technique to avoid contamination.)
7. Placed in a shaking incubator, and shook the vial(s) at 37°C for 1 hour at 225 rpm. **Deviation:** 300 rpm.
8. Plated two different volumes of the transformation reaction onto LB plates containing the appropriate antibiotic for plasmid selection. Included 34 µg/mL chloramphenicol if using BL21(DE3)pLysS or BL21(DE3)pLysE cells. Selected two volumes ranging from 20–200 µL to ensure well-spaced colonies on at least one plate. The remaining transformation reaction was stored at 4°C and plated out the next day, if needed.
9. Inverted the plates and incubated at 37°C overnight.
10. Selected transformants from the plates and culture as described on page 9.

Note: Clones may exhibit differences in expression of heterologous genes. We recommend choosing 3–4 transformants when characterizing clones for protein expression.

Results:



Conclusion:

The colonies did not grow. The protocol called for 5-10 ng, so I diluted to 13.2 ng/uL. However because it did not grow, we decided to transform the mCherry at its initial concentration of 66 ng/uL afterwards. One Shot BL21 Star competent cells are for protein expression while the NEB cells were for protein expression and cloning. There may be no RFP sequence within the mCherry DNA that we ordered.

Name: Kennex Lam, Asma Khimani

Date: 7/31/19

Goal:

1. Restriction Digest on
 - a. RFP Codon Opt. Midiprep #3 from 7/24
 - b. DinIII Miniprep #4 from 7/17

Protocol:

Restriction Digest Protocol

30 μ L Fast Digest Restriction Digest

1. Prepare a Fast Digest concentration cocktail with the following proportions: 1 μ L XbaI, 1 μ L BglII, 8 μ L of 10X Fast Digest Green Buffer, and 5 μ L of diH₂O.
2. Add 15 μ L of this cocktail to a clean 1.5 Eppendorf tube and then add 15 μ L of DNA.
3. Incubate at 37° C for 30 minutes.

* Used the same restriction enzymes for DinIII and Codon Optimized RFP.

Results:

Conclusion:

We digested them today but will run them on a gel tomorrow to proceed with gel extraction.