McMaster BioDesign Wet Lab Notebook

Laboratory Day #1

Overview

Date: 2025-08-27

Authors: Oviya S, Christina Z

Experiments: Lab organization, LB-agar plate preparation, LB broth preparation

Notes

Lab supplies moved into lab:

- Petri dishes + tubes
- 4 Eppendorf pipettes + tips
- Inoculation loops and cell spreader
- LB powder
- Agar powder
- Media bottles

LB-agar plates + LB broth preparation

- 20 x LB-kanamycin plates + 20x LB-no antibiotic plates made in BSC
- 500 mL LB-kanamycin and LB-no antibiotic broth autoclaved

Laboratory Day #2

Overview

Date: 2025-09-02

Authors: Christina Z, Eshana P, Varnigha M

Experiments: pUA66-PcspA-GFP streaking

Notes

- pUA66-PcspA-GFP agar stab shipment from addgene arrived and was immediately placed in the fridge

- LB-kanamycin plate was warmed in 37°C incubator before being streaked with the agar stab
- Streaked plate was inverted and placed upside down in incubator for overnight growth

Laboratory Day #3

Overview

Date: 2025-09-03

Authors: Christina Z, Eshana P, Varnigha M

Experiments: pUA66-PcspA-GFP Colony selection

Notes

- Around 100 colonies observed on the streaked LB-kanamycin plate
    - Some colonies not individual but several isolated colonies in the most streaked part of the plate
- Three isolated colonies were picked with an inoculation loop and put into 3 mL of LB-kanamycin broth in 15mL Falcon tubes
- Caps were loosely placed on the tube and taped shut before being placed in a 225 rpm shaking incubator at 37°C

Laboratory Day #4

Overview

Date: 2025-09-04

Authors: Christina Z, Eshana P, Irma L

Experiments: Miniprep

Notes

- Tubes were pulled from the incubator around 16 hours after inoculation
    - All 3 tubes had visible bacterial growth
- Each tube was miniprepped using the GeneJET plasmid miniprep following the Thermo recommended protocol
    - Samples stored in fridge overnight for quantification tomorrow

Laboratory Day #5

Overview

Date: 2025-09-05

Authors: Will PB, Oviya S

Experiments: Miniprep NanoQuant

Notes

- Minipreps were analyzed in duplicates using a NanoQuant plate with a Tecan M200 PRO for dsDNA
- GeneJET elution buffer used for individual blanking
- Results as follows in table below:

Replicate Number	**OD260/280 Ratio**	**Yield (ng/µL)**
1a	1.78	18.2
1b	1.78	17.8
Sample 1 Average:	**1.78**	**18.0**
2a	1.83	25.6
2b	1.81	25.1
Sample 2 Average:	**1.82**	**25.4**
3a	1.57	17.7
3b	1.64	17.9
Sample 3 Average:	**1.61**	**17.8**

- Minipreps stored in freezer after analysis

Laboratory Day #6

Info

Authors: Will PB, Oviya S

Date: 2025-09-09

Overview

- Prepared LB-agar and LB-agar w/ kanamycin
- Streaked pUA66-PcspA-GFP agar stab

Notes

Plate Preparation

- 500 mL of LB-agar and 500 mL of LB-agar with kanamycin prepared using 25 g of LB-Lennox powder and 12 g of agar before autoclaving
- Bottles placed in 60°C water bath for ~1 hour before pouring
- 500 µL of 50 mg/mL kanamycin stock added to the LB-agar with kanamycin bottle immediately before pouring
- 20 plates of each prepared and dried in BSC for ~1 hour before inverting and storing at 4°C in fridge

Colony Streaking

- 10 µL inoculation loops were briefly touched to the pUA66-PcspA-GFP agar stab, and streaked onto an LB-agar and LB-agar with kanamycin plate
- Plates were inverted and placed into a 37°C incubator

Laboratory Day #7

Info

Authors: Will PB, Oviya S

Date: 2025-09-10

Overview

- Checked on pUA66-PcspA-GFP plates
- Selected colonies for overnight growth

Notes

- Individual colonies were observed on both LB-agar and LB-agar with kanamycin plates
- A large well-separated colony from the LB-agar with kanamycin plate was used to inoculate 3 mL of LB-agar with kanamycin in a 15 mL Falcon tube
- The culture was loosly capped and sealed with parafilm before placing in a shaking 37°C incubator at 225 RPM

Laboratory Day #8

Info

Authors: Will PB, Oviya S

Date: 2025-09-11

Overview

- Miniprep of the overnight pUA66-PcspA-GFP colony
- NanoQuant of the miniprepped plasmid

Notes

Miniprep

- The overnight culture of pUA66-PcspA-GFP was removed from the incubator ~14 hours after inoculation, and was noticeably turbid
- The 3 mL culture was miniprepped using the GeneJET plasmid miniprep kit according to the manufacturer's instructions with the following modifications
    - Modifications were made based on consultation with Will PB
    - Elution buffer was placed into a 60°C water bath before use
    - The elution buffer incubated on the column before centrifugation for 5 minutes

NanoQuant

- The resulting miniprep was analyzed using the NanoQuant plate with a Tecan M200 PRO
- 3 wells were individually blanked with the GeneJET elution buffer before the 3 2 µL samples were analyzed
- Results shown in the table below:

Replicate Number	**OD260/280 Ratio**	**Yield (ng/µL)**
1	2.04	98.5
2	2.00	103.7
3	2.00	99
Average:	2.01	100.4 ng/µL

Laboratory Day #9

Info

Authors: Will PB, Oviya S

Date: 2025-09-12

Overview

- Screening of PCR conditions for PcspA extraction and linearization
- Gel analysis of PCR results
- Optimized PCR
- DpnI post-PCR treatment and cleanup

Notes

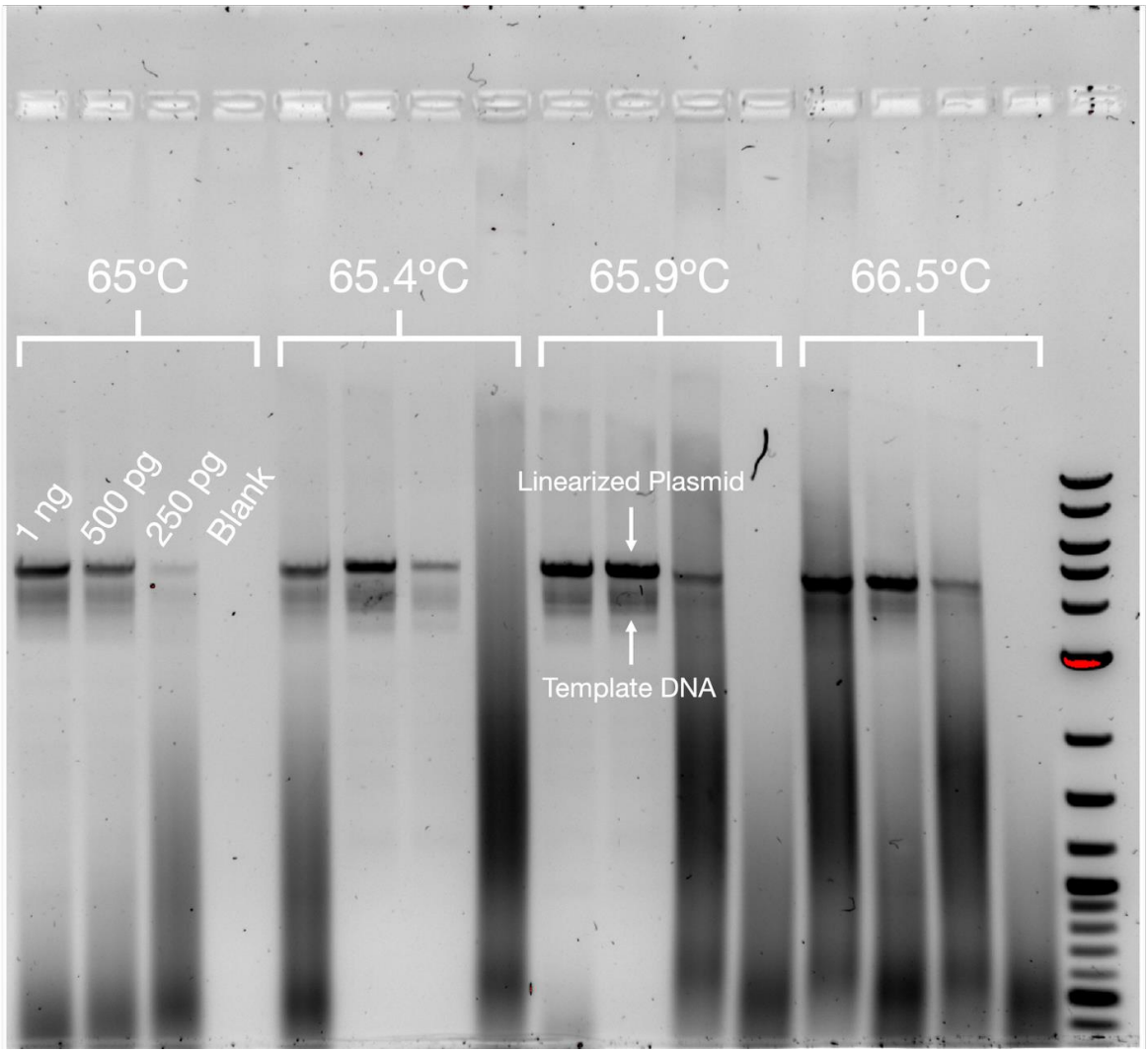
PCR Screening

- 20 µL reactions prepared with Q5 master mix using pUA66-PcspA-GFP miniprep from Day 8 at 0, 250 pg, 500 pg, and 1 ng starting concentration
- BioRad C1000 Touch thermocycler used
- Tested 4 annealing temperatures generated by gradient on the thermocycler around calculated temperature of 66ºC
    - 65, 65.4, 65.9, 66.5ºC
- Thermocycler conditions:

Step	**Temperature (ºC)**	**Time**
Initial Denaturation	98	30 seconds
20 Cycles	98	10 seconds
	Variable	20 seconds
	72	135 seconds
Final Extension	72	120 seconds
Hold	10	-

Gel Electrophoresis

- Each PCR reaction (16 total) was ran on a 1% agarose gel pre-stained with SYBR safe at 5V/cm in TAE buffer for 5 hours
    - Low and slow due to almost identical length of linearized vs vector plasmid
- Used NEB 1kb+ DNA ladder for size reference
- Imaged gel post-run on ChemiDoc XR, image below labeled with PowerPoint:



- 65.9ºC with 500 pg of plasmid vector was chosen as the optimal conditions due to strong amplification and minimal secondary product formation

PCR Product Generation

- PCR was reran using the identified conditions, then treated with NEB DpnI immediately after final extension of PCR
- Used Zymo cleanup kit to purify product using manufacturers protocol with following modifications used based on previous experience
    - Elution buffer was preheated to 60°C before use.
    - Elution buffer was incubated in the spin column for 5 minutes before centrifugation
    - Samples were eluted in 20 µL of elution buffer

- Resulting purified linearized DNA was analyzed using the NanoQuant plate blanked using the Zymo elution buffer
    - Only one sample taken to reduce the volume of sample measured
    - Yield: 20.1 ng/µL , OD260/280: 1.95

Laboratory Day #10

Overview

Date: 2025-09-15

Authors: Christina Z, Irma L, Varnigha M

Experiments: pUA66-PcspA-GFP transformation

Notes

- Thermo DH10B MAX cells and protocol used for transformation
- 1 ng of miniprep from Day #8 used
- Used plates from Day #1
- Serial diluted 10x and 100x in LB-without antibiotics broth before plating
- Each undiluted and diluted transformation plated on both LB-kanamycin and LB-without antibiotics plate
- Control LB-without antibiotics plate with untransformed DH10B prepare
- Placed in 37°C incubator inverted overnight

Laboratory Day #11

Overview

Date: 2025-09-16

Authors: Christina Z, Irma L, Varnigha M

Experiments: Transformation Plate Check

Notes

- Colonies only present on undiluted transformed plate without antibiotics
- No growth on control plate with untransformed DH10B

- Failure of negative control and growth on plates without antibiotics suggests error during the experimental procedure

- Competent cells potentially poorly viable?

Laboratory Day #12

Info

Date: 2025-09-17

Authors: Will PB, Oviya S

Overview

- Repeat transformation of pUA66-PcspA-GFP in DH10B MAX to confirm transformability of the plasmid

Notes

- Experimental procedure from Day #10 repeated to rule out issues with the competent cells and confirm protocol
- Used plates from Day #6

Laboratory Day #13

Info

Date: 2025-09-18

Authors: Will PB, Oviya S

Overview

- Checked the plates of previously transformed pUA66-PcspA-GFO

Notes

- Individual independent colonies were observed in all transformed plates with kanamycin
- Bacterial lawns were observed on the antibiotic free plates
- Transformation efficiency seems to be low (empirical observation) based on previous experience with plasmids like pUC19

Laboratory Day #14

Info

Date: 2025-09-22

Authors: Will PB, Oviya S

Overview

- Performed CPEC and NEBuilder-based assembly of linearized pUA66-PcspA-GFP for both J23100 and J23119 promoters
- Transformed DH10B with each of the new assemblies

Notes

- Both CPEC and NEBuilder prepared as 20 µL reactions with 50 ng of vector DNA and ~200 fmol of J23100 or J23119 oligos from IDT
- Transformed 2 µL of each assembly within half an hour of assembly completion
- Transformed with DH10B MAX according to mfg. protocol
- 10x and 100x serial dilution of each prepared
- Each reaction plated on both LB-agar with and without kanamycin
- 1 ng of pUA66-PcspA-GFP miniprep used as positive control

Laboratory Day #15

Info

Date: 2025-09-23

Authors: Will PB, Oviya S

Overview

- Checked overnight plates for assembly confirmation
- Repeated CPEC and NEBuilder DNA assemblies

Notes

- No colonies visible on any of the assembly plates with kanamycin, lawns on those without
- Positive control colony counts seemed low again, so suspected that the competent cell lot may not have great transformation efficiency
    - Received Stbl3 competent cells from Suky

- CPEC and NEBuilder repeated being extra careful that all protocol steps were followed to the minute to reduce any potential variability
- Assemblies stored at -20ºC in freezer to prevent degradation

Laboratory Day #16

Info

Date: 2025-09-24

Authors: Will PB, Oviya S

Overview

- Transformed previously prepared assemblies into Stbl3 E. coli

Notes

- Transformed 2 µL of each assembly into Stbl3 using the manufacturers protocol
- Serial diluted each to 10x and 100x before plating on LB-agar plates with and without kanamycin
- Transformed with 1 ng of pUA66-PcspA-GFP miniprep as positive control

Laboratory Day #17

Info

Date: 2025-09-25

Authors: Will PB, Oviya S

Overview

- Checked the plates for the second assembly round transformed into Stbl3

Notes

- No colonies observed on any of the assembly plates with kanamycin, lawns on plates without antibiotics
- Significantly more positive control colonies with Stbl3, suggesting failure is occurring during the assembly and not during transformation