Lab Notebook

Project: iGEM 2019
Authors: Grace Gu

MONDAY, 7/15/2019

Learned Basic Lab Techniques

- Pipetting
- Making agarose gel
- Making agar plates with LB + AMP

جامعة نيويورك أبوظبي
NYU ABU DHABI

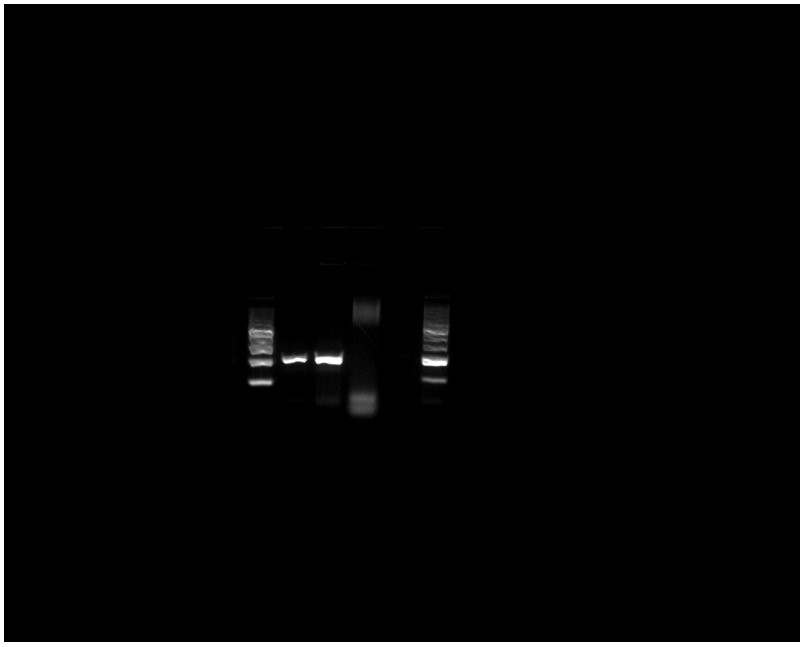
Agarose Gel Preparation (3%):

1. 1x TAE buffer was prepared by diluting a 50x TAE buffer
2. 1.5 g of Agarose were added to 50 ml of 1x TAE buffer (3%) in a conical flask
3. The solution was placed in a microwave and mixed until solution became clear
4. 3 microliters of Gel Red were added to the solution
5. A well comb was added to the gel cast, the solution was poured into the gel cast and allowed to solidify

Cas12 Cleavage with Cra gene:

1. Making 125nM gRNA: 0.2 µl of 1000 µM gRNA and 1.58 ml of water were mixed
2. Making 100nM CRISPR LbCas12a: 1 µl of 100 µM Cas12 and 999 µl of water
3. 1 µl of each solution were used to make 2 µl 62.5nM gRNA : 50nM Cas12a solution and added to approximately 18 µl of Cra DNA solution.
4. The same solution was made with 1 µl each of undiluted Cas12a and gRNA solutions (gRNA: 1000 µM; CAS12a: 100 µM).
5. The solutions were left to set for 1.5 hours in 37°C incubator.
6. A Gel electrophoresis was ran for the two samples and the control

CRISPR Cra PCR product_igem 2019_2_15 July.tif



Lane 1: ladder (100bp - 500bp), Lane 2: Control, Lane 3: sample with diluted CRISPR reagents, Lane 4: sample with undiluted reagents

Resuspended pcaA, HBcAg and IS481 gBlock gene fragments from IDT

1. For 500ng gBlocks, 50µL of molecular grade water was added to the tube. For 1000ng gBlocks, 100µL of molecular grade water was added to the tube to reach a final concentration of 10ng/µL
2. The tubes were vortexed briefly then incubated at 50°C for 15 mins
3. The tubes were briefly vortexed and centrifuged for 5 seconds
4. The concentration of each gBlock resuspension was taken with the NanoDrop

    pcaA: 8.7 ng/µL

    HBcAg: 11.2 ng/µL

    IS481: 8.2 ng/µL

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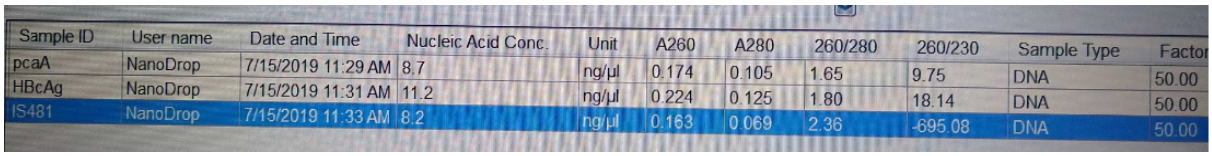

Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
pcaA	NanoDrop	7/15/2019 11:29 AM	8.7	ng/µl	0.174	0.105	1.65	9.75	DNA	50.00
HBcAg	NanoDrop	7/15/2019 11:31 AM	11.2	ng/µl	0.224	0.125	1.80	18.14	DNA	50.00
IS481	NanoDrop	7/15/2019 11:33 AM	8.2	ng/µl	0.163	0.069	2.36	-695.08	DNA	50.00

pJET Blunt End Ligation

5. Prepared Thermo Scientific CloneKet PCR Cloning Kit reagent mix in a PCR tube on ice
6. Added 10µL 2X Reaction Buffer
7. Added 1µL resuspended pcaA gBlock gene fragement
8. Added 1µL pJET 1.2/blunt cloning vector (50ng/µL)
9. Added 7µL nuclease-free water
10. Added 1µL T4 DNA Ligase
11. Incubated at room temperature for 5 minutes
12. Repeated for HBcAg and IS481 gBlocks

Transformation of E.coli via electroporation using ligated pJET vectors incubated for 5 minutes

1. Aliquoted 33µL of electrocompetent E.coli into 3 eppendorf tubes
2. Added either 10µL of pcaA, HBcAg or IS481 ligated pJET vectors into each of 3 eppendorf tubes
3. Pipette up and down to mix
4. Pipette contents of each eppendorf tube (cells with either pcaA, HBcAg or IS481) into electroporation cuvette
5. Place into electroporation machine and electroporate
6. Resuspend cells in 960µL of SOC by pipetting up and down in cuvette
7. Pour cuvette contents into 3 separate eppendorf tubes
8. Incubated on shaker at 220rpm and 37°C for one hour
9. Centrifuged for 1 minute at at 13000rpm, discarded 800µL supernatant from each eppendorf tube
10. Pellets were resuspended by pipetting up and down
11. Cells were spread on LB+AMP agar plates and placed in 37°C incubator overnight

Results of Ligation and Transformation using electroporation and 5 minute ligation incubation:
No growth on plates after overnight incubation.

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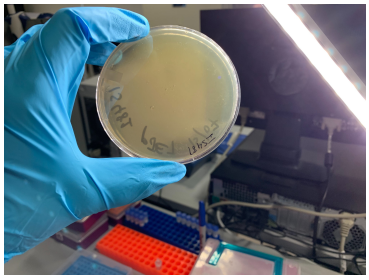
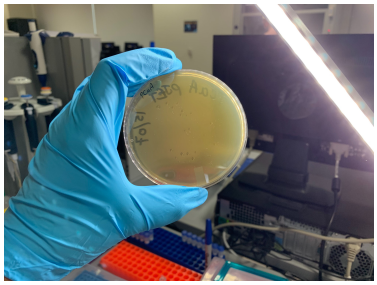


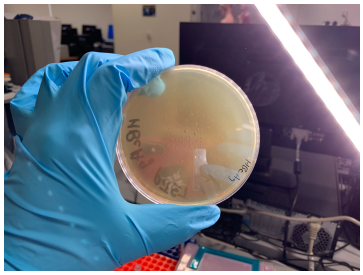
IS481 transformation result

image.png



pcaA transformation result

image.png



HbcAg transformation result

TUESDAY, 7/16/2019

Transformation with Heat Shock and 24 hour ligation incubation

pJET Blunt End Ligation

1. Prepared Thermo Scientific CloneKet PCR Cloning Kit reagent mix in a PCR tube on ice
2. Added 10µL 2X Reaction Buffer
3. Added 1µL resuspended pcaA gBlock gene fragement
4. Added 1µL pJET 1.2/blunt cloning vector (50ng/µL)
5. Added 7µL nuclease-free water
6. Added 1µL T4 DNA Ligase
7. Incubated at room temperature for 16 hours
8. Repeated for HBcAg and IS481 gBlocks

Transformation of E.coli via heat shock using ligated pJET vectors incubated for 16 hours

1. Aliquoted 33µL of electrocompetent E.coli into 3 eppendorf tubes
2. Added either 10µL of pcaA, HBcAg or IS481 ligated pJET vectors into each of 3 eppendorf tubes
3. Iced for 20 minutes,
4. 60 second heat shock in eppendorf heat block at 42°C

5. Returned tubes to ice for 2 minutes
6. Added 800µL of SOC
7. Incubated on shaker at 220 rpm and 37°C for one hour
8. Centrifuged for 1 minute at 13000rpm and discarded 700µL of supernatant
9. Carefully resuspended the pellet and plated on LB+AMP agar
10. Spreaded gently using plastic spreader
11. Incubated overnight (16 hours) at 37 °C

Results of Ligation and Transformation using heat shock and overnight ligation incubation:
Growth on plates.

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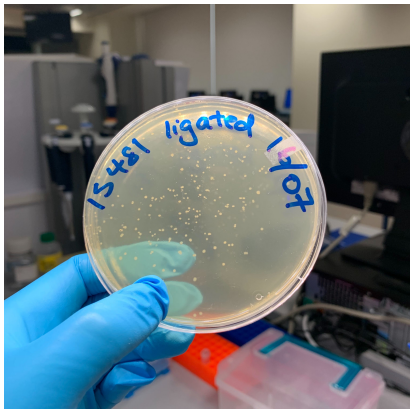

IS481 transformation result

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pcaA transformation result

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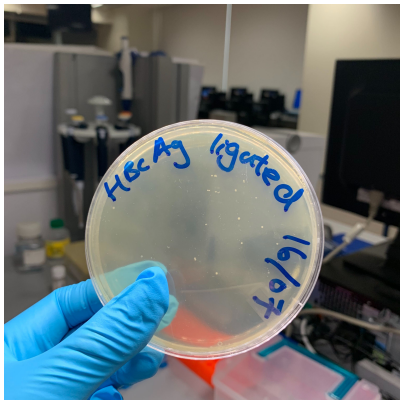

HbcAg transformation result

WEDNESDAY, 7/17/2019

Inoculation of pcaA, HBcAg and IS481 colonies from transformation

Inoculation steps:

1. 5mL LB broth was added to 3 15mL culture tubes
2. A plastic inoculation loop was used to select a colony from each plate and was swirled in the corresponding broth to dislodge the colony
3. The tubes were loosely capped and incubated on a shaker at 220rpm and 37°C overnight (16 hours)

THURSDAY, 7/18/2019

Mini-prepped inoculated pcaA, HBcAg and IS481 samples

DNA extraction:

1. Pellet 1–5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 x g) for 3 min at room temperature (15–25°C).
2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.
3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.
4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. If using LyseBlue reagent, the solution will turn colorless.
5. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.

October 2010
Quick-StartProtocol
Sample & Assay Technologies

6. Apply the supernatant from step 5 to the QIAprep spin column by decanting or pipetting. □ Centrifuge for 30–60 s and discard the flow-through, or □ apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source.
7. Recommended: Wash the QIAprep spin column by adding 0.5 ml Buffer PB.

 Centrifuge for 30–60 s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source.
Note: This step is only required when using endA+ strains or other bacteria strains with high nuclease activity or carbohydrate content.
8. Wash the QIAprep spin column by adding 0.75 ml Buffer PE.
 Centrifuge for 30–60 s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source. Transfer the QIAprep spin column to the collection tube.
9. Centrifuge for 1 min to remove residual wash buffer.
10. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

A nanodrop was used to measure the concentration of the plasmids.

Mini prep plasmid concentration

	A	B	C	D
1	Sample	Nucleic Acid Conc. (ng/µl)	260/280	260/230
2	pcaA 1	81.2	1.89	2.1
3	pcaA 2	52.9	1.94	2.21
4	pcaA 3	67.1	1.91	2.04
5	HBcAg 1	15.6	1.94	1.53
6	HBcAg 2	17.0	2.03	1.83
7	HBcAg 3	75.8	1.89	2.21
8	IS481 1	120.9	1.86	2.15
9	IS481 2	90.7	1.87	2.27
10	IS481 3	171.0	1.88	2.17
11	gbpa 1	37.1	1.84	1.88
12	gbpa 2	43.7	1.90	2.18
13	gbpa 3	78.6	1.78	1.66

*mini prep 2 and 3 of HBcAg gene are swapped

A gel electropheresis was run to confirm the ligation of the gblocks into the pJET vectors.

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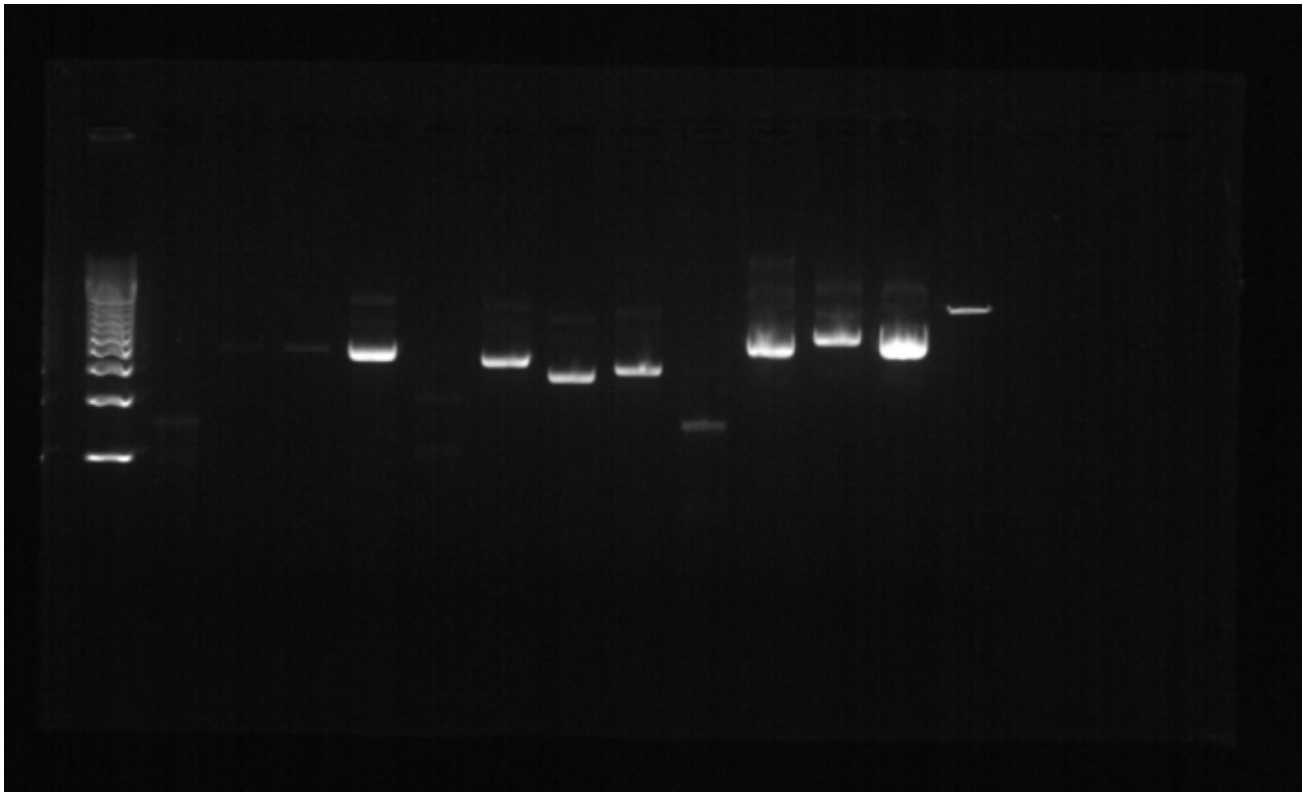
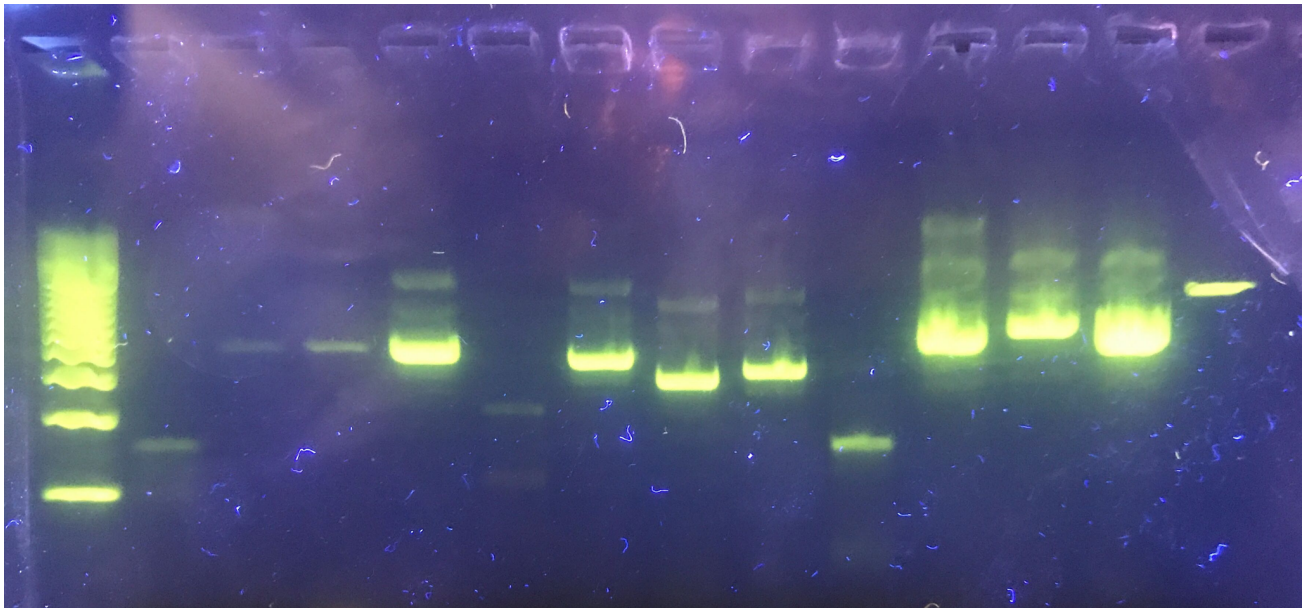


1. Ladder 2. HBcAg gblock 3. HBcAg MP1 4. HBcAg MP2 5. HBcAg MP3 6. pcaA gblock 7. pcaA MP1 8. pcaA MP2 9. pcaA MP3 10. IS481 gblock 11. IS481 MP1 12. IS481 MP2 13. IS481 MP3 14. pJET vector

image.png



1. Ladder 2. HBcAg gblock 3. HBcAg MP1 4. HBcAg MP2 5. HBcAg MP3 6. pcaA gblock 7. pcaA MP1 8. pcaA MP2 9. pcaA MP3 10. IS481 gblock 11. IS481 MP1 12. IS481 MP2 13. IS481 MP3 14. pJET vector

Resuspension, ligation and transformation of ypo2088 gblock

Resuspended ypo2088 gBlock gene fragment from IDT :

1. For 500ng gBlocks, 50µL of molecular grade water was added to the tube. For 1000ng gBlocks, 100µL of molecular grade water was added to the tube to reach a final concentration of 10ng/µL
2. The tubes were vortexed briefly then incubated at 50°C for 15 mins
3. The tubes were briefly vortexed and centrifuged for 5 seconds
4. The concentration of the gBlock resuspension was taken with the NanoDrop

Resupension Results:

ypo2088 gblock resuspension concentration

	A	B	C	D
1		Nucleic Acid Conc. (ng/µl)	260/280	260/230
2	YPO 2088 IDT Gblock	10.6	1.79	2.03

Transformation with Heat Shock and 2 hour ligation incubation

pJET Blunt End Ligation

1. Prepared Thermo Scientific CloneKet PCR Cloning Kit reagent mix in a PCR tube on ice
2. Added 10µL 2X Reaction Buffer
3. Added 1µL resuspended ypo2088 gBlock gene fragement
4. Added 1µL pJET 1.2/blunt cloning vector (50ng/µL)
5. Added 7µL nuclease-free water
6. Added 1µL T4 DNA Ligase
7. Incubated at room temperature for 16 hours

Incubate DNA at room temperature for two hours at room temperature.

Transformation of E.coli with ypo2088 via heat shock using ligated pJET vectors incubated for 16 hours

1. Added 50 µL of electrocompetent E.coli into eppendorf tube
2. Added 10µL of ypo2088 ligated pJET vectors into the eppendorf tube
3. Iced for 20 minutes, followed by a 60 second heat shock in eppendorf heat block at 42°C
4. Returned tibes to ice for 2 minutes
5. Added 800µL of SOC
6. Incubated on shaker at 220 rpm and 37°C for one hour
7. Centrifuged for 1 minute at 13000rpm and discarded 700µL of supernatant
8. Carefully resuspended the pellet and plated on LB+AMP agar. Less than or around 20 µL of the resuspended pellet were added to the plate.
9. Spreaded gently using plastic spreader
10. Incubated overnight (16 hours) at 37 °C

Results of Ligation and Transformation using heat shock and overnight ligation incubation:
Formation of very few and small colonies after overnight incubation.

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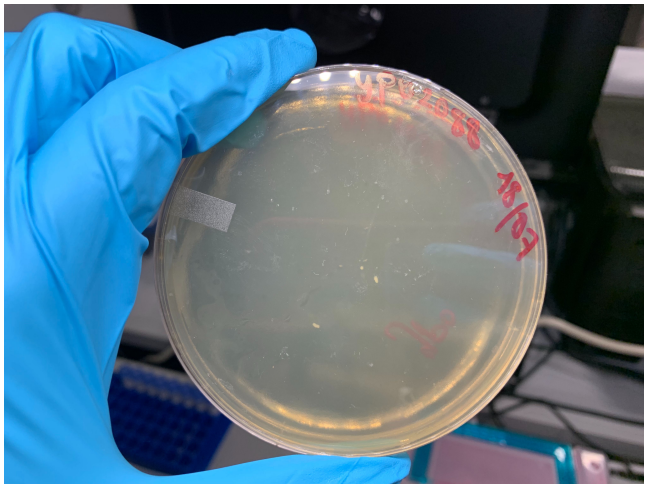


FRIDAY, 7/19/2019

Transformation with Heat Shock after 24 hour ligation incubation at room temperature of remaining 10µL of ypo2088 DNA and inoculation of ypo2088 transformed E.coli colonies from 7/18 transformation

Transformation of E.coli with ypo2088 via heat shock using ligated pJET vectors incubated for 16 hours

1. Added 50 µL of electrocompetent E.coli into eppendorf tube
2. Added 10µL of ypo2088 ligated pJET vectors into the eppendorf tube
3. Iced for 20 minutes, followed by a 60 second heat shock in eppendorf heat block at 42°C
4. Returned tibes to ice for 2 minutes
5. Added 800µL of SOC
6. Incubated on shaker at 220 rpm and 37°C for one hour
7. Centrifuged for 1 minute at 13000rpm and discarded 700µL of supernatant
8. Carefully resuspended the pellet and plated on LB+AMP agar. Less than or around 20 µL of the resuspended pellet were added to the plate.
9. Spreaded gently using plastic spreader
10. Incubated overnight (16 hours) at 37 °C

Inoculation of ypo2088 transformed E.coli colonies from transformation on Thurs 7/18:

1. 5mL LB broth was added to 2 15mL culture tubes
2. A plastic inoculation loop was used to select a colony from each plate and was swirled in the corresponding broth to dislodge the colony
3. The tubes were loosely capped and incubated on a shaker at 220rpm and 37°C overnight (16 hours)

SUNDAY, 7/21/2019

Inoculation of ypo2088 tranformed E.Coli colonies from tranformation on Friday 7/19 (one sample from 2 hours ligation incubation and three samples from overnight ligation incubation)

Mini-prepped two samples inoculated ypo2088 samples incubated for 2 hours and cultured for two nights

A nanodrop was used to measure the concentration of the plasmids.

Miniprep plasmid concentration

	A	B	C	D
1	Sample	Nucleic Acid Conc	260/280	260/230
2	ypo2088 1	49.0	2.03	2.42
3	ypo2088 2	56.8	2.07	2.07

MONDAY, 7/22/2019

LAMP

FIP, BIP B3, F3 primer stock solutions were diluted to 10µM concentrations:

Table 1

	A	B	C
1	Primers	Stock Conc. (µM)	
2	FIP	229.8	
3	BIP	272.6	
4	B3	353.8	
5	F3	366.8	

TUESDAY, 7/23/2019

RPA with IS481 and CRISPR

RPA reaction tubes : a. 1 microliter of miniprep 1 IS481
b. 1 microliter of miniprep 2 1S481
c. 1 microliter of miniprep 3 IS 481
d. 1 microliter of water - negative control
e. 3 microliter of miniprep 1 IS481 - (NB - check for higher template)

The adjusted RPA Protocol: 10µL final volume:

1. Reaction mix in 1.5 mL tube:
    a. Primer A (10µM) - 2.4µL
    b. Primer B (10µM) - 2.4µL
    c. Rehydration Buffer - 29.5µL
    d. dH2O - 8.2µL
2. Vortexed and spun the reaction mix briefly.
3. Added the reaction mix to freeze dried reaction. Pipetted up and down to mix.
4. Split the reaction into five volumes - 8.5µL to two separate PCR tubes.
5. Added 1µL of template in each tube.
6. Added 0.5µL of 280mM magnesium acetate and mixed well to start the reaction.
7. Incubate at 37-39°C for 20-40 min.

a. For low copy number: removed after 4 min, vortexed, spun briefly and put back into the heating device.

8. After 20-40 min cleaned the amplicons before running the gel.

CRA gene CRISPR using NEB and DETECTR protocals with DNase Substrate

3 Reaction tubes

Reagents in each tube

	A	B	C	D	E	F
1	Reagents	DNase Buffer	nuclease-free H2O	CRISPR reagents	Amplified Cra Samples	**Total Volume**
2	NEB protocol	5µL	5µL	27µL	3µL	40µL
3	DETECTR protocol	5µL	5µL	2µL	18µL	30µL
4	Control	5µL	45µL	None	None	

1. Mixing CRISPR reagents according to NEB protocal:
    a. Add into a 1.5 mL reaction tube the reagents in following order:
        I. 20µL nuclease-free water
        II. 3µL NEBuffer 2.1 Reaction Buffer (10X)
        III. 3µL 300nM gRNA (1µL 100mM + 99µL IDTE --> 6uL 1mM + 14µL IDTE)
        IV. 1µL 1µM LbaCas12a
    b. Pre-Incubated at room temperature for 15 minutes

2. Mixing CRISPR reagents according to DETECTR protocal:
    a. Add into a 1.5mL reaction tube:
        I. µL 125nM gRNA
        II. 1µL 100nM LbaCas12a

3. Added 5µL nuclease free water to 3 DNaseAlert substrate single-use tubes
4. Added 5µL of 10X DNAseAlert Buffer to each tube
5. Added the two CRISPR reaction mix to two tubes respectively, pipetted up and down to mix.
6. Added 40µL nuclease-free H2O in third tube
7. Incubated at 37℃ for 1 hour.
8. Viewed under 536nm LED light

IDTE Buffer (10mM Tris, 0.1mM EDTA, pH7.5) Preparation

1. Added into a 600mL beaker:
    a. 0.0146g of EDTA
    b. 0.605g of Tris
    c. 500mL of MilliQ water
2. Checked pH of the mixture.
3. 2M HCl was added until pH 7.5 was reached.
4. The mixture was autoclaved for 20 minutes.
5. After autoclave, small amount of the mixture was poured into a separate beaker and the pH was rechecked

Miniprep Plasmid concentration for ypo2088 after 24hour ligation incubation

A nanodrop was used to measure the concentration of the plasmids.

Table 2

	A	B	C	D
1	Sample	Nucleic acid conc	260/280	260/230
2	ypo2088 1 (24h ligation incubation)	86.5	1.89	2.19
3	ypo2088 2 (24h ligation incubation)	140.2	1.70	0.92
4	ypo2088 3 (24h ligation incubation)	14.1	2.04	1.02
5	ypo2088 4 (2h ligatio incubation)	6.4	2.83	1.42

CRISPR reaction following NEB protocol:

1. Assemble the reaction at room temperature in the following order*:
    a. 20µl Nuclease-free water
    b. 3µl NEBuffer 2.1 Reaction Buffer (10x)
    c. 3µl 300nM gRNA
    d. 1µl 1 µM EnGen Lba Cas12a (Cpf1)
2. Pre-incubate for 10 minutes at 25^{0}C.
3. Add 3 µl of 30 nM substrate DNA (30 µl final volume).
4. Vortex and pulse-spin in a microfuge.
5. Incubate at 37°C for 10 minutes.
6. Add DNase to each sample, Mix thoroughly and pulse-spin in a microfuge.
7. Incubate at room temperature for 10 minutes.
8. Add 5 µl of DNase alert buffer
9. Incubate for 60 minutes at 37°C.
10. Proceed with analysis.

ypo 2088 gel results:

gblock mini-prep 24 July 2019.tif

1. Ladder 2. ypo2088 gblock 3. ypo 2088 MP1 (21st July) 4. ypo2088 MP2 (July 21st) 5. ypo2088 MP3 (July 24th) 6.ypo2088 MP4 (July 24th) 7. ypo2088 MP5 (July 24th)

CRISPR Experiment Results from Cra Gene:

Cra_NEB_DETECTR_product.tif

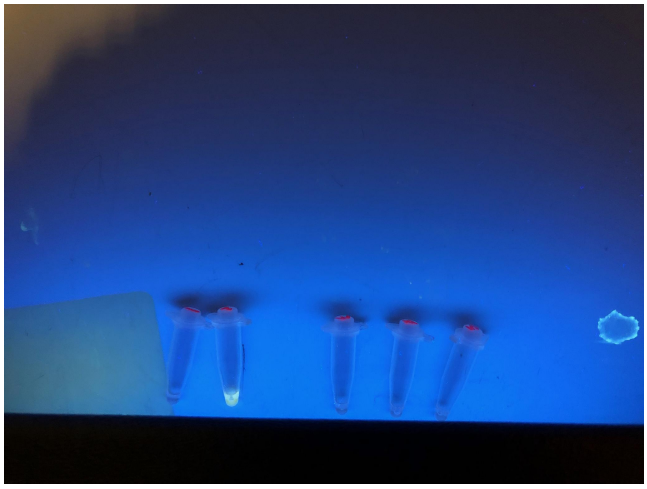
1. Ladder 2. Negative Control 3.DETECTR Protocol 4.NEB Protocol

RPA on IS481- MP1-MP2-MP3-NC-MP1 24 July 2019.tif

100 bp ladder- IS481 MP1-IS481 MP2- IS481 MP3- negative control-IS481 MP1

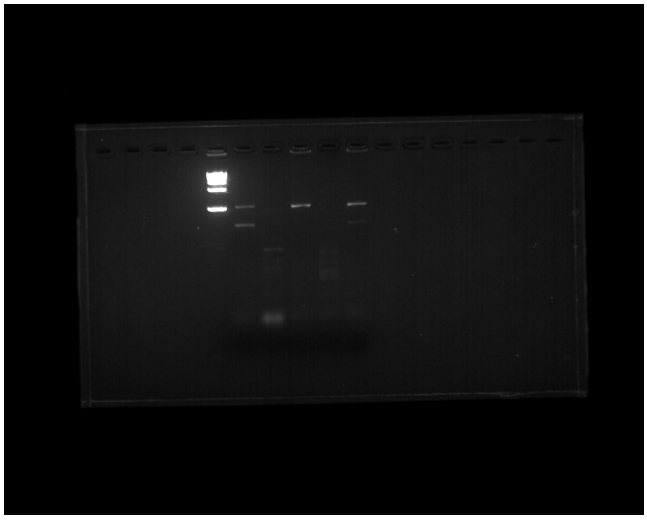
CRISPR on IS481:

CRISPR_ (NEB) 24 July 2019.jpeg



MP1-MP2-MP3-Negative control -concentrated MP1

RPA+CRISPR IS481 MP1-MP2-MP3-NC-MP1 24 July 2019.tif



1. ladder 2. MP1 3. MP2 4. MP3 5. -ve control 6. concentrated MP1

THURSDAY, 7/25/2019

IS481 RPA + CRISPR repeat

RPA reaction tubes : a. 1 microliter of miniprep 1 IS481
b. 1 microliter of miniprep 2 1S481
c. 1 microliter of miniprep 3 IS 481
d. 1 microliter of water - negative control
e. 0.5 microliter of miniprep 2 IS481

ypo2088 RPA + CRISPR

RPA reaction tubes: a. 1 microliter of miniprep 1 ypo2088
b. 1 microliter of miniprep 2 ypo2088
c. 1 microliter of miniprep 3 ypo2088
d. 1 microliter of water - negative control
e. 0.5 microliter of miniprep 1 ypo2088 + 0.5 microliter of water

- **A nanodrop was used to measure the concentration of the plasmids.**

Table 3

	A	B	C	D
1	Sample	Nucleic Acid Conc	260/280	260/230
2	ypo2088 1	49.0	1.90	2.40

TUESDAY, 7/30/2019

PCR of minipreped plasmids

Resuspension of Lyophilized Primers

a. Added volume of water indicated on order sheet (differed for each primer) to make 100 µM of each primer
b. Pipetted up and down to mix

Preparation of PCR mix

a. Prepared 10 µM working stock of each primer by extracting 5 µl of 100 µM stock and adding 45 µl of distilled water to a total of 50 µl.
b. Labelled each eppendorf tube with name of gene and sample number (1,2,3, postive control, negative control)
c. Added 20 µl PCR Mastermix to each tube
d. Added 1 µl of 10 µM forward primers to each appropriate tube. This was done for all genes.
e. Added 1 µl of 10 µM reverse primers to each appropriate tube. This was done for all genes.
f. Added 2ul DNA minipreps (1, 2, 3, and gblock) to each tube. None was added to negative control.
g. Water was added to reach a total volume ot 40 µl (16 µl for each tube with DNA and 18 µl for negative control)
h. PCR temperatures were set on machine according to BioRad protocol and 40 cycles were done.
i. PCR was left overnight.

WEDNESDAY, 7/31/2019

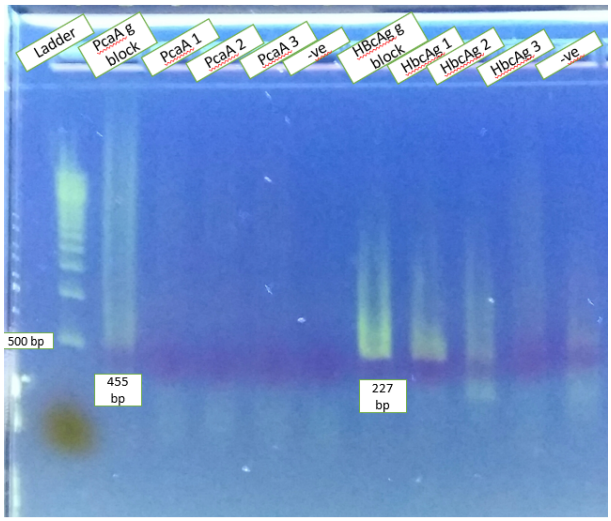
Visualition of PCR results using Gel Electrophoresis

E-Gel

a. 10 µl of ladder solution was added to 5 µl of H2O
b. 2.5 µl of dye was added to 12.5 µl of sample for all the genes and the replicates along with the negative control
c. The samples were loaded onto the ready-made E-gel
d. The gel was run for 20 minutes
e. The gel was observed under UV light for analysis

Results:
pcaA and HBcAg

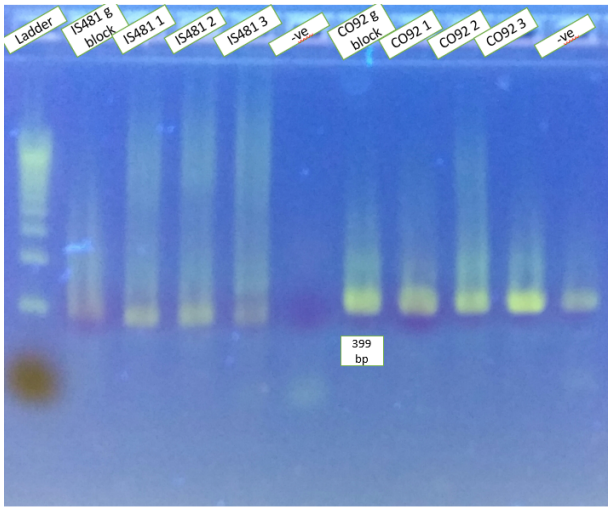
pcaA.PNG



No bands appeared for any of the pcaA samples, negative control contamination was observed for HBcAg

IS481 and CO92

IS481.PNG



Successful bands appeared for IS481, negative control contamination appeared for CO92

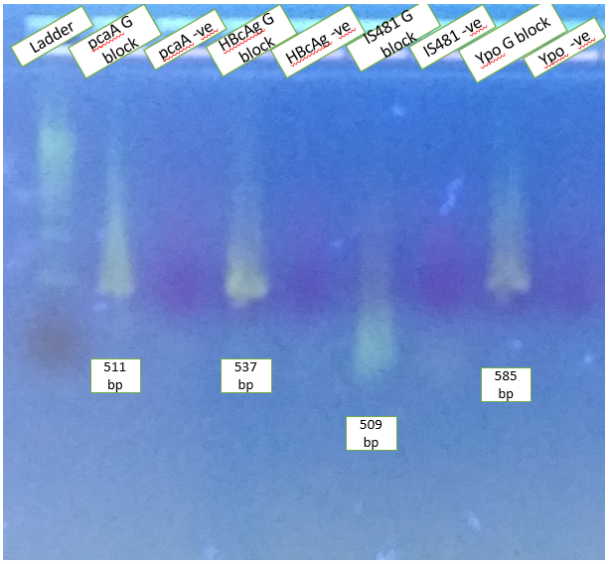
SUNDAY, 8/4/2019

Repeated PCR (as above) with same samples
Changed the annealing temperature for pcaA to 62
All other PCR settings remained similar
(IS481 miniprep 1 not PCRed)

TUESDAY, 8/6/2019

Visualization of PCR results (G-blocks and Negative Controls)

image.png

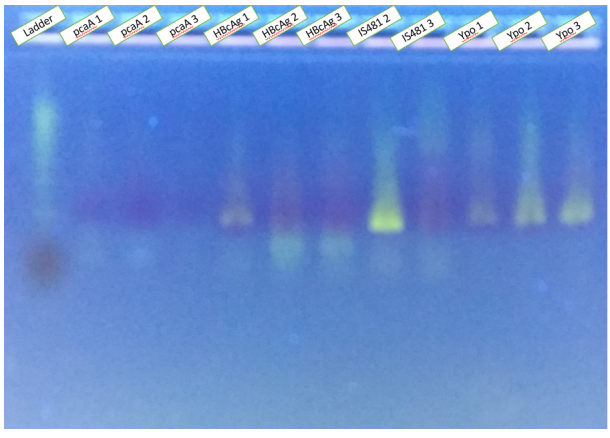


Note: IS481 G-block size appeared to be smaller than expected

WEDNESDAY, 8/7/2019

Visualization of PCR results for Miniprep samples

image.png



pcaA samples not visible

TUESDAY, 9/3/2019

Re-ligation of PJET vectors to PCR-amplified G-blocks

pJET Blunt End Ligation

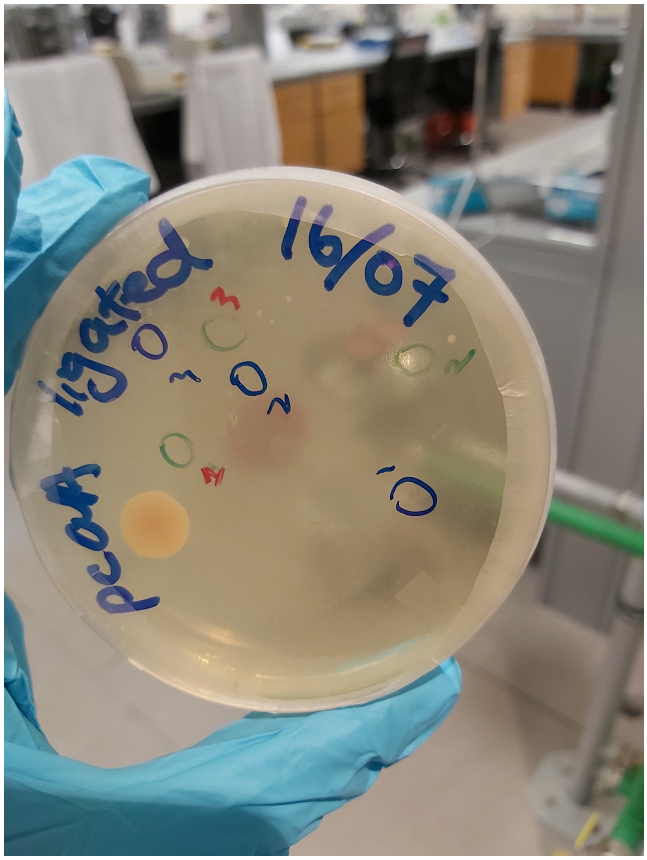
1. Prepared Thermo Scientific CloneKet PCR Cloning Kit reagent mix in a PCR tube on ice
2. Added 10µL 2X Reaction Buffer
3. Added 1µL resuspended ypo2088 gBlock gene fragement
4. Added 1µL pJET 1.2/blunt cloning vector (50ng/µL)
5. Added 7µL nuclease-free water
6. Added 1µL T4 DNA Ligase
7. Incubated at room temperature for 16 hours
8. Repeated for HBcAg and IS481 gBlocks

Transformation of E.coli via heat shock using ligated pJET vectors incubated for 16 hours for ypo2088, IS481, HbcAg, and IS481 MP 2

1. Aliquoted 200µL of electrocompetent E.coli into 4 eppendorf tubes
2. Added either 10µL of ypo, HBcAg or IS481 or MP2 ligated pJET vectors into each of 4 eppendorf tubes
3. Iced for 20 minutes, followed by a 60 second heat shock in eppendorf heat block at 42°C
4. Returned tubes to ice for 2 minutes
5. Added 800µL of SOC
6. Incubated on shaker at 220 rpm and 37°C for one hour
7. Centrifuged for 1 minute at 13000rpm and discarded 700µL of supernatant
8. Carefully resuspended the pellet and plated on LB+AMP agar
9. Spreaded gently using plastic spreader
10. Incubated overnight (16 hours) at 37 °C

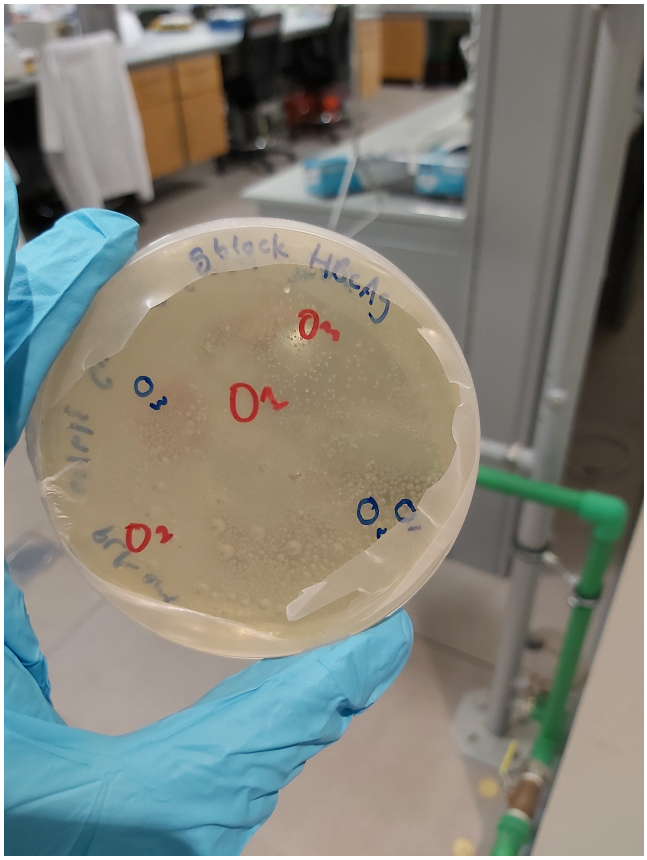
Results:

image.png



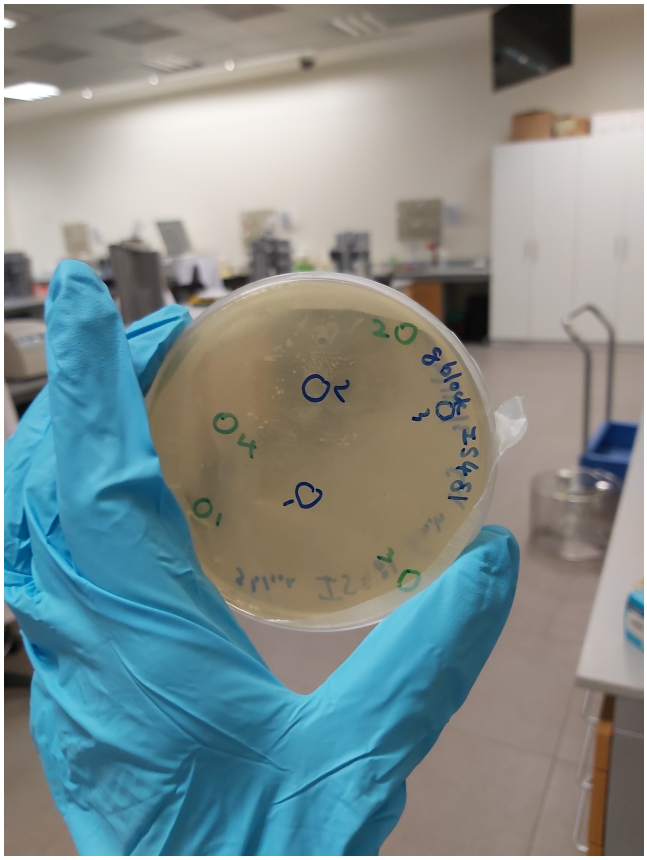
Colonies were extracted from the plates prepared on the 16th of July

image.png



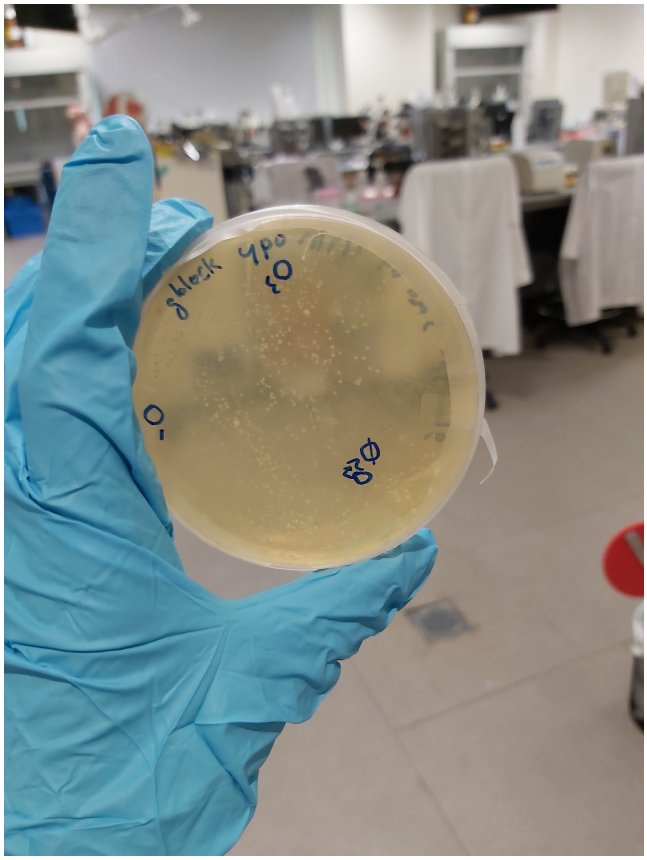
transformation results for HbcAg

image.png



transformation of IS481

image.png



transformation of ypo2088

WEDNESDAY, 9/4/2019

Innoculation of IS481 (from gblock and miniprep2), HBcAg, IS481, and YPO2088 from Transformations

Inoculation steps:

1. 10mL LB broth was added to 15mL culture tubes
2. A plastic inoculation loop was used to select a colony from each plate and was swirled in the corresponding broth to dislodge the colony
3. Step 2 was repeated for 3 colonies on each of the 5 plates
4. The tubes were loosely capped and incubated on a shaker at 220rpm and 37°C overnight (19 hours)

THURSDAY, 9/5/2019

Miniprepped the 15 innoculations from the day before

Miniprep procedures:

1. Pellet 5ml bacterial overnight culture by centrifugation at 7830 rpm (6800 x g) for 3 min at room temperature (15–25°C).
2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.
3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.
4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. If using LyseBlue reagent, the solution will turn colorless.
5. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.
6. Apply the supernatant from step 5 to the QIAprep spin column by decanting or pipetting. Centrifuge for 60 s and discard the flow-through,
7. Recommended: Wash the QIAprep spin column by adding 0.5 ml Buffer PB. Centrifuge for 60 s and discard the flow-through.

8. Wash the QIAprep spin column by adding 0.75 ml Buffer PE. Centrifuge for 60 s and discard the flow-through.
9. Centrifuge for 1 min to remove residual wash buffer.
10. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

Nanodrop with 1µl results (* indicates IS481 from previous miniprep2):

Table 4

	A	B	C	D
1	Data	Concentration	260/280	260/230
2	IS481* MP1	56.5	1.97	2.06
3	IS481* MP2	129.7	1.89	2.24
4	IS481* MP3	98.3	1.91	2.20
5	IS481 MP1	39.9	1.99	1.92
6	IS481 MP2	23.4	2.02	2.22
7	IS481 MP3	104.5	1.68	0.93
8	HBcAg MP1	75.9	1.68	0.68
9	HBcAg MP2	90.0	1.92	2.28
10	HBcAg MP3	62.1	1.94	2.07
11	ypo2088 MP1	57.4	1.94	2.15
12	ypo2088 MP2	13.6	2.15	1.84
13	ypo2088 MP3	77.5	1.93	2.32
14	pcaA MP1	34.6	2.01	2.34
15	pcaA MP2	63.1	1.72	0.99
16	pcaA MP3	59	1.76	1.02

Based on these and the gel results, we will be retransforming:

a. pcaA MP1
b. HbcAg MP2 and MP3
c. IS481* MP1, MP2, MP3
d. ypo MP1, MP2 MP3

PCR of minipreped plasmids

I. Prepared 10 µM working stock of each primer by extracting 5 µl of 100 µM stock and adding 45 µl of distilled water to a total of 50 µl.
II. Labelled each eppendorf tube with name of gene and sample number (1,2,3, postive control, negative control)
III. Added 20 µl PCR Mastermix to each tube
IV. Added 1 µl of 10 µM forward primers to each appropriate tube. This was done for all genes.
V. Added 1 µl of 10 µM reverse primers to each appropriate tube. This was done for all genes.
VI. Added 2ul DNA minipreps (1, 2, 3) to each tube. None was added to negative control.
VII. Water was added to reach a total volume ot 40 µl (16 µl for each tube with DNA and 18 µl for negative control)
VIII. PCR temperatures were set on machine according to BioRad protocol and 40 cycles were done.
IX. PCR was left overnight.

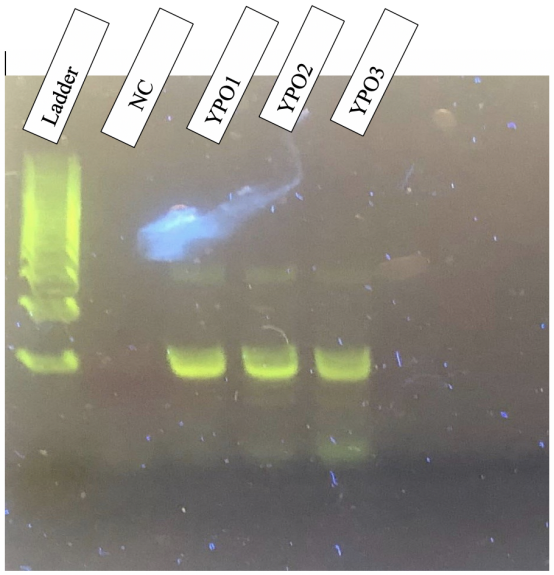
Running of PCR Samples on Gel to Confirm Proper Ligation

Agarose Gel Preparation and Running Samples:

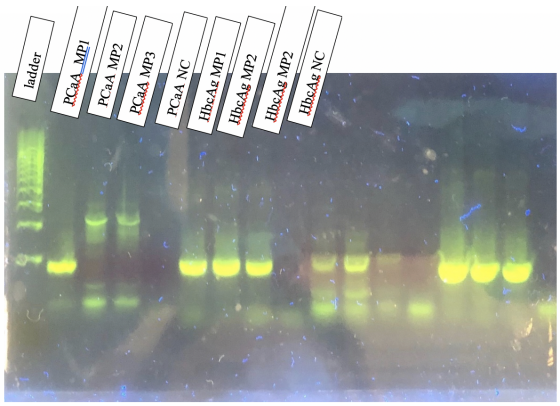
I. 1x TAE buffer was prepared by diluting a 50x TAE buffer
II. 1.5 g of Agarose were added to 50 ml of 1x TAE buffer (3%) in a conical flask
III. The solution was placed in a microwave and mixed until solution became clear
IV. 3 microliters of Gel Red were added to the solution
V. A well comb was added to the gel cast, the solution was poured into the gel cast and allowed to solidify
VI. After 30 min, 20 drops of 5 µl of loading dye was put on paraffin paper, after which 10 µl of samples from each PCRed miniprep was added.
VII. 5 µl of ladder was loaded first, followed by 15 µl of samples in the order: pcaA MP1, pcaA MP2, pcaA MP3, pcaA negative control, HbcAg MP1, HbcAg MP2, HbcAg MP3, HbcAg negative control, IS481 MP1, IS481 MP2, IS481 MP3, IS481 negative control, IS481 old MP1, IS481 old MP2, IS481 old MP3, IS481 old negative, ypo MP1, ypo MP2, ypo MP3, ypo negative control
VIII. The gels were left to run for 20 minutes and was later viewed under UV light

Result:

image.png



Screen Shot 2019-09-11 at 5.43.06 PM.png



MONDAY, 9/9/2019

Dilution of Minipreps (ypo MP3)

Table 5

	A	B	C	D	E
1	IS481				
2	**Serial Dilutions**	**Concentration (ng/µl)**	**From Previous Stock**	**dH2O**	**Remaining**
3		Highest Concentration (x ng/µl)	77.5		
4	1	60	19.35483871	5.64516129	14.58333333
5	2	25	10.41666667	14.58333333	15
6	3	10	10	15	12.5
7	4	5	12.5	12.5	20
8	5	1	5	20	22.5
9	6	0.1	2.5	22.5	22.5
10	7	0.01	2.5	22.5	22.5
11	8	0.001	2.5	22.5	
12	9	Negative Control (dH2O)	0	25	

TUESDAY, 9/10/2019

Dilution of Minipreps (other genes)

Serial Dilution on HbcAg MiniPrep 2:

Table 6

	A	B	C	D	E	F
1	**Serial Dilutions**	**Concentration (ng/µl)**	**From Previous Stock**	**dH2O**	**Remaining**	
2		Highest Concentration (x ng/µl)	90		30	
3	1	60	18	9	17	
4	2	25	10	14	14	
5	3	10	10	15	12.5	
6	4	5	12.5	12.5	20	
7	5	1	5	20	22.5	
8	6	0.1	2.5	22.5	22.5	
9	7	0.01	2.5	22.5	22.5	
10	8	0.001	2.5	22.5	25	
11	9	Negative Control (dH2O)	0	25		
12						

Serial Dilution on IS481* MiniPrep2.

Table 7

	A	B	C	D	E
1	**Serial Dilutions**	**Concentration (ng/µl)**	**From Previous Stock**	**dH2O**	**Remaining**
2		Highest Concentration (x ng/µl)	129.7		
3	1	60	11.56515035	13.43484965	14.58333333
4	2	25	10.41666667	14.58333333	15
5	3	10	10	15	12.5
6	4	5	12.5	12.5	20
7	5	1	5	20	22.5
8	6	0.1	2.5	22.5	22.5
9	7	0.01	2.5	22.5	22.5
10	8	0.001	2.5	22.5	
11	9	Negative Control (dH2O)	0	25	

PCR of serial dilutions for ypo and HbcAg:

1. Labelled each eppendorf tube with name of gene and concentration
2. Added 20 µl PCR Mastermix to each tube
3. Added 1 µl of 10 µM forward primers to each tube.
4. Added 1 µl of 10 µM reverse primers to each appropriate tube. This was done for all genes.
5. Added 2ul diluted sample to each tube. None was added to negative control.
6. Water was added to reach a total volume ot 40 µl (16 µl for each tube with DNA and 18 µl for negative control)
7. PCR temperatures were set on machine according to BioRad protocol and 35 cycles were done.
8. PCR was left overnight.

Transformation of pcaA Miniprep 1

1. Aliquoted 50µL of electrocompetent E.coli into an eppendorf tube
2. Added 10µL of pcaA MP 1 ligated pJET vector into eppendorf tube
3. Iced for 20 minutes, followed by a 60 second heat shock in eppendorf heat block at 42°C
4. Returned tubes to ice for 2 minutes
5. Added 800µL of SOC
6. Incubated on shaker at 220 rpm and 37°C for one hour
7. Centrifuged for 1 minute at 13000rpm and discarded 700µL of supernatant
8. Carefully resuspended the pellet and plated on LB+AMP agar
9. Spreaded gently using plastic spreader
10. Incubated overnight (16 hours) at 37 °C

WEDNESDAY, 9/11/2019

Inoculation of pcaA MP 1 transformation

Steps:

1. 5mL LB broth was added to 3 15mL culture tubes
2. A plastic inoculation loop was used to select a colony from each plate and was swirled in the corresponding broth to dislodge the colony, the colonies were marked
3. The tubes were loosely capped and incubated on a shaker at 220rpm and 37°C overnight (16 hours)

PCR of serial dilutions for IS481:

1. Labelled each eppendorf tube with name of gene and the concentration
2. Added 20 µl PCR Mastermix to each tube
3. Added 1 µl of 10 µM forward primers to each tube.
4. Added 1 µl of 10 µM reverse primers to each tube.
5. Added 2 µl of each dilution to each tube. None was added to negative control.
6. Water was added to reach a total volume ot 40 µl (16 µl for each tube with DNA and 18 µl for negative control)
7. PCR temperatures were set on machine according to BioRad protocol and 35 cycles were done.
8. PCR was left overnight.

LAMP reactions of serial dilutions

Resuspension of FIP, BIP, F3, B3 primers of ypo2088, HBcAg, and IS481

1. Added volume of water indicated on order sheet (differed for each primer) to make 100 µM of each primer.
2. Pipetted up and down to mix.

Preparation of LAMP Primer Mix ypo2088

1. Dilute 100μM FIP, BIP, F3, B3 primers to be 10μM following last year's LAMP optimization.
2. Mix them in one ependorff tube.
3. Pipetted up and down to mix

Preparation of LAMP Primer Mix stock (total volume 100μl) for HBcAg and IS481

1. Add 4μl of FIP (100μM), 4μl of BIP (100μM), 1μl of F3 (100μM),, 1μl of B3 (100μM), and 45μl of dH2O in the ependorff tube.
2. Pipetted up and down to mix.
3. Vortex the primer mix.

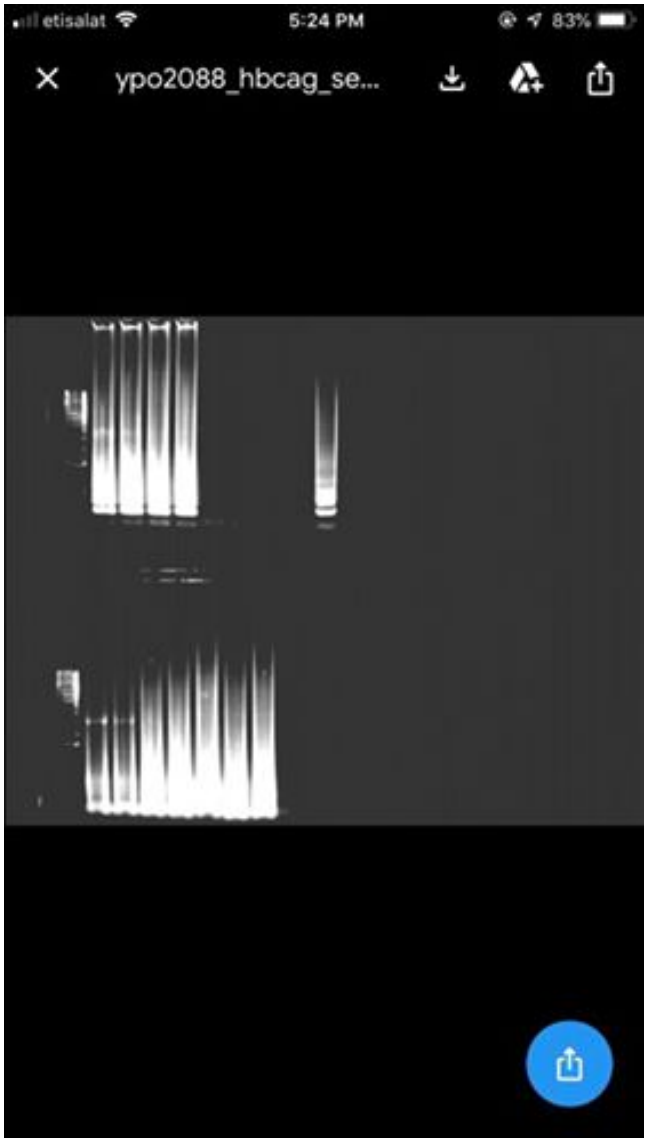
Run LAMP reaction for ypo2088 and HBcAg

1. Add 15μl of isothermal master mix to each tube (total 9 tubes).
2. Add 5μl of the primer mix to each tube.
3. Add 5μl of the dilution sample to each tube (8 dilution samples + 1 negative control).
4. Incubate at 65C for 20 minutes.

Visualition of LAMP results using Gel Electrophoresis

1. 2 μl of ladder solution was added to 5 μl of dye.
2. 1 μl of dye was added to 5 μl of sample for all the genes and the replicates along with the negative control
3. The samples were loaded onto the ready-made E-gel
4. The gel was run for 20 minutes
5. The gel was observed under UV light for analysis

image.png



Top: HBcAg: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control
Bottom: ypo2088: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

Redid the serial dilutions for is481* MP2 using the same volumes documented on the table from Tuesday 9/10

Gel Electrophoresis of PCR samples of diluted HbcAg and ypo2088 from day before:

Agarose Gel Preparation:

a. Prepared a 1x agarose gel by adding 0.5 grams of agarose to 50 ml of TAE buffer.
b. Heating in the solution up in 20 second interval (in the microwave) and stop after making sure that the solution is clear enough

Sample Loading:

1. 10 µl of samples from each PCRed diluted sample was added onto a piece of parafilm.
2. 5 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 5 µl of 500bp ladder was loaded first (for HbcAg, pre-dyed ladder was used, for ypo, 2 µl of ladder was mixed with 10 µl of purple dye), followed by 15 µl of samples in the order:

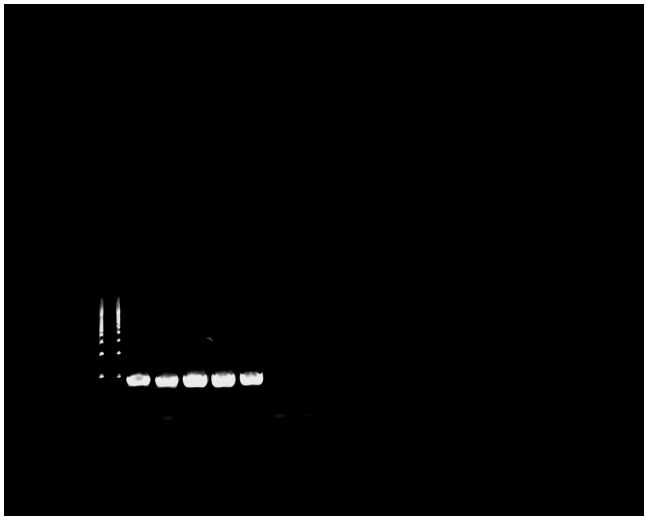
a. HbcAg: ladder, pcr sample 1 - 9 corresponding to the dilutions from high to low concentrations
b. ypo2088: ladder, pcr sample 1 - 9 corresponding to the dilutions from high to low concentrations

4. The gels were left to run for 20 minutes

Results:

HBcAg post serial dilution PCR results

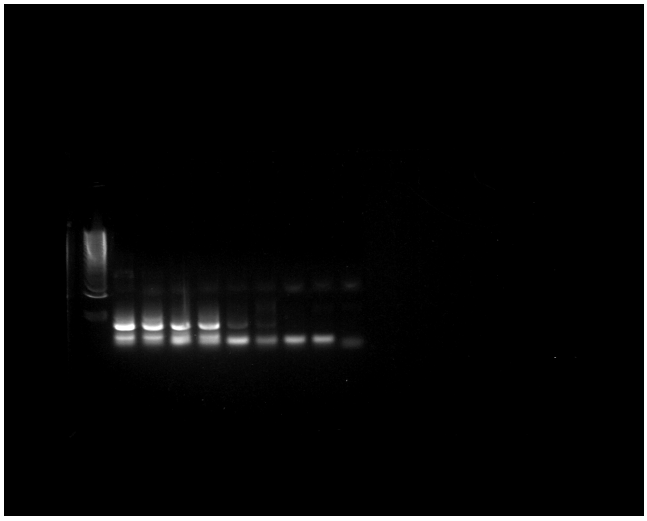
hbcag_serial_dilutions_PCR_Sept11.tif



Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

ypo2088 post serial dilution PCR results

ypo2088_serial_dilutions_PCR_Sept11.tif



Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

THURSDAY, 9/12/2019

Preparation of Quencher:

1. Added 380 µl of TE buffer to lyophilized quencher to get it to a working stock of 100 µM
2. Took 10 µl of 100 µM and added 10 µl of water to get a stock of 50 µM in 20 µl
3. Took 2 µl of 50 µM solution and added 98 µl of water to get a concentration of 1uM in 100 µl

LAMP primer mix for HBcAg (previous one is contaminated)

Preparation of LAMP Primer Mix ypo2088

1. Dilute 100µM FIP, BIP, F3, B3 primers to be 10µM following last year's LAMP optimization.
2. Mix them in one ependorff tube.
3. Pipetted up and down to mix

Preparation of LAMP Primer Mix stock (total volume 100µl) for HBcAg and IS481

1. Add 4µl of FIP (100µM), 4µl of BIP (100µM), 1µl of F3 (100µM),, 1µl of B3 (100µM), and 45µl of dH2O in the ependorff tube.
2. Pipetted up and down to mix.
3. Vortex the primer mix.

LAMP reaction for HBcAg and IS481* post serial dilution samples

1. Add 15µl of isothermal master mix to each tube (total 9 tubes for every gene).
2. Add 5µl of the primer mix to each tube.
3. Add 5µl of the dilution sample to each tube (8 dilution samples + 1 negative control each gene).
4. Incubate at 65C for 20 minutes.

Gel electrophoresis of LAMP HBcAg and IS481* post serial dilution samples

Agarose Gel Preparation:

a. Prepared a 1x agarose gel by adding 0.5 grams of agarose to 50 ml of TAE buffer.
b. Heating in the solution up in 20 second interval (in the microwave) and stop after making sure that the solution is clear enough

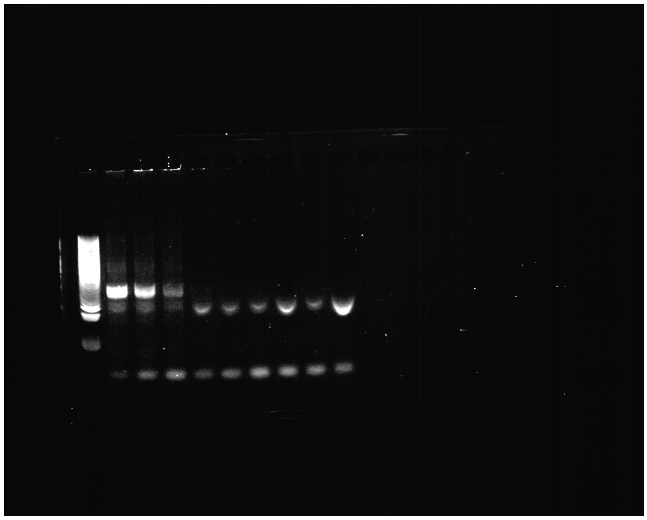
Sample Loading:

1. 10 µl of samples from each PCRed diluted sample was added onto a piece of parafilm.
2. 5 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 5 µl of 500bp ladder was loaded first (for HbcAg, pre-dyed ladder was used, for ypo, 2 µl of ladder was mixed with 10 µl of purple dye), followed by 15 µl of samples in the order:
    a. HbcAg: ladder, pcr sample 1 - 9 corresponding to the dilutions from high to low concentrations
    b. ypo2088: ladder, pcr sample 1 - 9 corresponding to the dilutions from high to low concentrations
4. The gels were left to run for 20 minutes

Results:

HBcAg post serial dilution LAMP results

HbcAg_serial_dilutions_LAMP_SEPT12_2019.tif



Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

IS481* post serial dilutions LAMP results

is481STAR_serial_dilutions_LAMP_SEPT12_2019.tif

Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

Miniprep PcaA (mini prep1 (from previous mp) - a, b, c)
Protocol on sheet

Nanodrop analysis for PcaA

Table 8

	A	B	C	D
1	Sample	Nucleic acid conc	260/280	260/230
2	pcaA mp1 (A)	83.4	1.95	2.36
3	pcaA mp1 (B)	59.7	1.99	2.40
4	pcaA mp1 (C)	111.9	1.93	2.26
5				

Serial dilution for PcaA (mini prep 1-C was chosen)

Table 9

	A	B	C	D
1	Pcaa			
2	**Serial Dilutions**	**Concentration (ng/µl)**	**From Previous Stock**	**dH2O**
3		Highest Concentration (x ng/µl)	111.9	
4	1	60	13.40482574	11.59517426
5	2	25	10.41666667	14.58333333
6	3	10	10	15
7	4	5	12.5	12.5
8	5	1	5	20
9	6	0.1	2.5	22.5
10	7	0.01	2.5	22.5
11	8	0.001	2.5	22.5
12	9	Negative Control (dH2O)	0	25

Overnight PCR for PcaA post serial dilution samples

1. Labelled each eppendorf tube with name of gene and the concentration
2. Added 20 µl PCR Mastermix to each tube
3. Added 1 µl of 10 µM forward primers to each tube.
4. Added 1 µl of 10 µM reverse primers to each tube.
5. Added 2ul of each dilution to each tube. None was added to negative control.
6. Water was added to reach a total volume ot 40 µl (16 µl for each tube with DNA and 18 µl for negative control)
7. PCR temperatures were set on machine according to BioRad protocol and 35 cycles were done.
8. PCR was left overnight.

TUESDAY, 9/17/2019

Serial dilutions for ypo2088 Miniprep 3:

Table 10

	A	B	C	D	E
1	ypo2088 (MP3)				
2	**Serial Dilutions**	**Concentration (ng/µl)**	**From Previous Stock**	**dH2O**	**Remaining**
3		Highest Concentratio n (x ng/µl)	77.5		
4	1	60	12.38709677	3.612903226	9.333333333
5	2	25	6.666666667	9.333333333	9.6
6	3	10	6.4	9.6	8
7	4	5	8	8	12.8
8	5	1	3.2	12.8	14.4
9	6	0.1	1.6	14.4	14.4
10	7	0.01	1.6	14.4	14.4
11	8	0.001	1.6	14.4	
12	9	Negative Control (dH2O)	0	16	

Overnight PCR for ypo2088 post serial dilution samples:

1. Added 20 µl PCR Mastermix to each PCR tube (not enough mastermix was left for the negative control/ just added what was left around 10ul)
2. Added 1 µl of 10 µM forward primers to each tube.
3. Added 1 µl of 10 µM reverse primers to each tube.
4. Added 2ul of each dilution to each tube. None was added to negative control.
5. Water was added to reach a total volume ot 40 µl (16 µl for each tube with DNA and 18 µl for negative control)
6. PCR temperatures were set on machine according to BioRad protocol and 35 cycles were done.
7. PCR was left overnight.

Serial dilution for IS481* mp2

Table 11

	A	B	C	D	E	F
1	IS481					
2	**Serial Dilutions**	**Concentration (ng/µl)**	**From Previous Stock**	**dH2O**	**Remaining**	
3		Highest Concentration (x ng/µl)	129.7			
4	1	60	11.6	13.4	14.58333333	<--This line is calculated assuming miniprep (highest concentration) is 75 ng/µl
5	2	25	10.4	14.6	15	
6	3	10	10	15	12.5	
7	4	5	12.5	12.5	20	
8	5	1	5	20	22.5	
9	6	0.1	2.5	22.5	22.5	
10	7	0.01	2.5	22.5	22.5	
11	8	0.001	2.5	22.5		
12	9	Negative Control (dH2O)	0	25		

Overnight PCR for IS481* mp2 post serial dilution samples:

1. Added 20 µl PCR Mastermix to each PCR tube (not enough mastermix was left for the negative control/ just added what was left around 10ul)
2. Added 1 µl of 10 µM forward primers to each tube.
3. Added 1 µl of 10 µM reverse primers to each tube.
4. Added 2ul of each dilution to each tube. None was added to negative control.
5. Water was added to reach a total volume ot 40 µl (16 µl for each tube with DNA and 18 µl for negative control)
6. PCR temperatures were set on machine according to BioRad protocol and 35 cycles were done.
7. PCR was left overnight.

10µl final volume RPA for HbcAg (done with 2 sets of RPA primers), IS481* (done with 2 sets of RPA primers), ypo2088 (done with first set of primers only) and pcaA (done with first set of primers only)

Protocal followed when doing HbcAg and IS481* RPAs:

1. Reaction mix in 1.5 mL tube:
    a. Primer A (10µM) - 4.8µL
    b. Primer B (10µM) - 4.8µL
    c. Rehydration Buffer - 59µL
    d. dH2O - 16.4µL
2. Pipetted up and down after addition of each component in step 1
3. Splitted the reaction mix in two (42.5µL) and added each half to 1 freeze dried reaction. Pipetted up and down to mix.

4. Splitted the reaction into 9 volumes - 8.5µL to 9 separate PCR tubes.
5. Added 1µL of template from each serial dilution in corresponding tube.
6. Added 0.5µL of 280mM magnesium acetate and mixed well to start the reaction.
7. Incubated at 38℃ for 40 min using thermocycler

Protocol followed when doing ypo2088

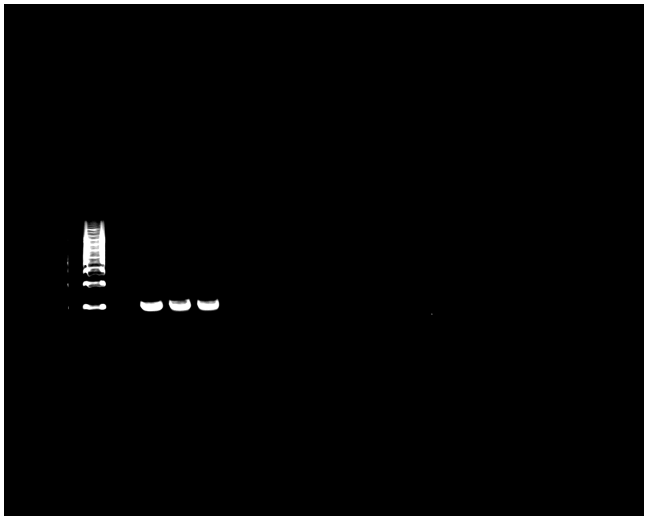
1. Reaction mix was done in 2 different 1.5mL eppendorf tube:
    a. Primer A (10µM) - 4.8µL
    b. Primer B (10µM) - 4.8µL
    c. Rehydration Buffer - 59µL
    d. dH2O - 16.4µL
2. Pipetted up and down after addition of each component in step 1
3. The 8.5reaction mix (for ypo (for fwd primer 1 and rev 1 primer) was added to the 9 freezed dried reaction (including Negative control) . Pipette up and down to mix
4. Added 1µL of template from each serial dilution to both set corresponding tubes.
5. Added 0.5µL of 280mM magnesium acetate and mixed well to start the reaction.
6. Incubated at 38℃ for 40 min using thermocycler

Sample Loading:

1. 10 µl of samples from each PCRed diluted sample was added onto a piece of parafilm.
2. 5 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 10 µl of 500bp ladder was mixed with 5 µl of green dye and loaded first followed by 15 µl of samples in the order: 1, 2, 3, 4, 5, 6, 7, 8, 9
4. The gels were left to run for 20 minutes

Results:

pcr_YPO2088_Sept 17 2019.tif



PCR of pcaA post serial dilutions

TE buffer preparation:

1. Added 20 ml of 50x TE buffer to 980 ml of water to make 1x buffer

Agarose Gel Preparation:

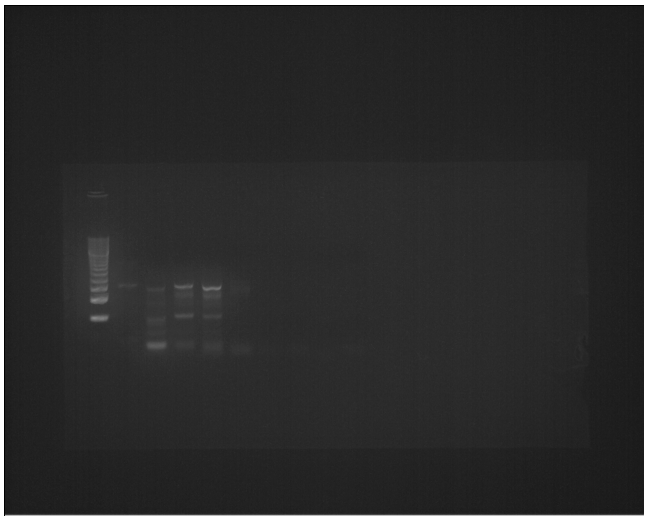
a. Prepared a 1x agarose gel by adding 0.5 grams of agarose to 50 ml of TAE buffer.
b. Heating in the solution up in 20 second interval (in the microwave) and stop after making sure that the solution is clear enough

Sample Loading:

1. 10 µl of samples from each PCRed diluted sample was added onto a piece of parafilm.
2. 5 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 10 µl of 500bp ladder was mixed with 5 µl of green dye and loaded first followed by 15 µl of samples in the order: 1, 2, 3, 4, 5, 6, 7, 8, 9
4. The gels were left to run for 20 minutes

Results:

PCR pcaA post dilution.tif



Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

WEDNESDAY, 9/18/2019

Gel Electrophoresis of RPA samples of diluted is481* and HbcAg from day before:

Agarose Gel Preparation:

a. Prepared a 1x agarose gel by adding 0.5 grams of agarose to 50 ml of TAE buffer.

b. Heating in the solution up in 20 second interval (in the microwave) and stop after making sure that the solution is clear enough Sample

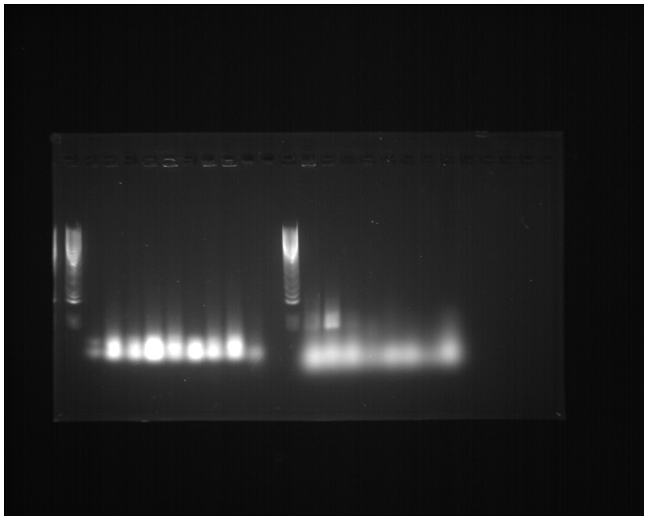
Loading:

1. 5 µl of samples from each RPA sample was added onto a piece of parafilm.
2. 1 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 1 µl of 500bp ladder (molecular ruler) was loaded first (for HbcAg, pre-dyed ladder was used, for ypo, 1 µl of ladder was mixed with 5 µl of purple dye), followed by 6 µl of samples in the order:
    a. IS481: ladder, pcr sample 1 - 9 corresponding to the dilutions from high to low concentrations
    b. HbcAg: ladder, pcr sample 1 - 9 corresponding to the dilutions from high to low concentrations
4. The gels were left to run for 20 minutes

Results:

IS481* RPA post serial dilution

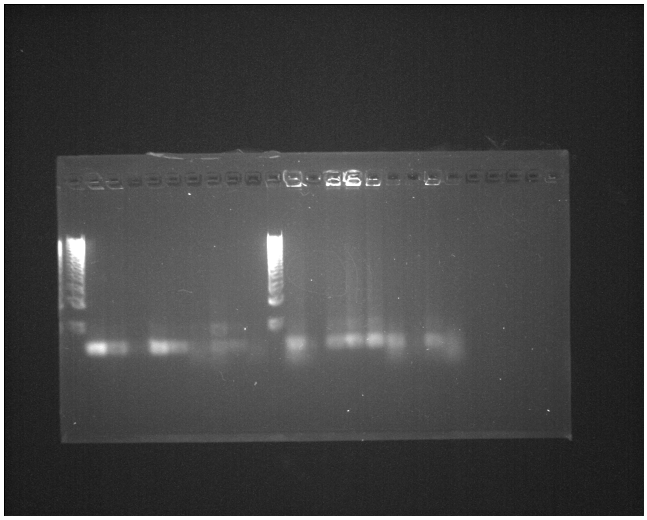
IS481STAR_serial_dilutions_RPA_primerSet2_1_SEPT 18.tif



Left: 2nd primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control
Right: 1st primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

HBcAg post serial dilutions RPA

HbcAg_serial_dilutions_RPA_primerSet1_2_SEPT18.tif



Left: 1st primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control
Right: 2nd primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

THURSDAY, 9/19/2019

Gel Electrophoresis of RPA samples of serial dilutions of ypo2088 from day (17/10/19)

Agarose Gel Preparation:

a. Prepared a 3% agarose gel by adding 1.5 grams of agarose to 50 ml of TAE buffer.

b. Heating in the solution up in 20-second interval (in the microwave) and stop after making sure that the solution is clear enough. In sum, it took 50seconds to obtain a clear solution.

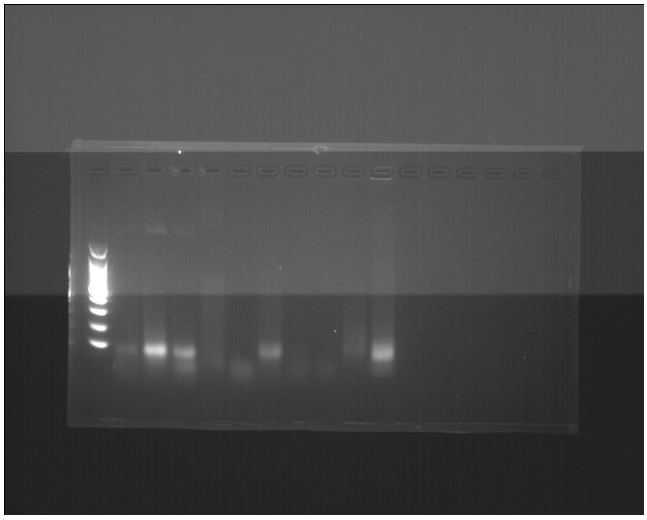
c. Pour the warm liquid agarose. Place the comb into the casting tray by placing the sides into the notches. . Wait until the gel polymerizes.

Sample loading

1. 5 µl of samples from each serial dilution of RPA samples of ypo2088 was added onto a piece of parafilm.
2. 1 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 1 µl of 500bp ladder (molecular ruler) was loaded first (pre-dyed ladder was used, for ypo, 1 µl of ladder was mixed with 5 µl of purple dye), followed by 6 µl of samples in the order:
    a. ypo: ladder, rpa serial dilutions sample 1 - 9 corresponding to the dilutions from high to low concentrations.
4. The gels were left to run for 20 minutes

Result:

ypo rpa serial dilutions with 2 PControl.tif



Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

Gel Electrophoresis of RPA samples of diluted is481* and HbcAg from Sept 17:

Agarose Gel Preparation:

a. Prepared a 1x agarose gel by adding 1.5 grams of agarose to 50 ml of TAE buffer.

b. Heating in the solution up in 20 second interval (in the microwave) and stop after making sure that the solution is clear enough Sample

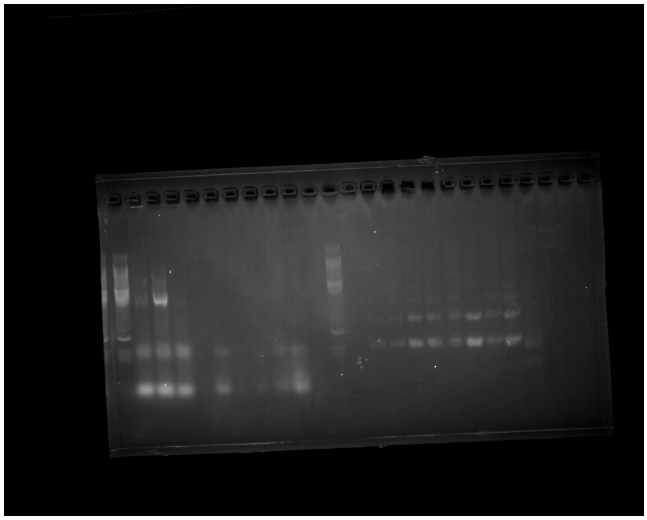
Loading:

1. 5 µl of samples from each RPA diluted sample was added onto a piece of parafilm.
2. 1 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 1 µl of 100bp ladder (molecular ruler) was loaded first (1 µl of ladder was mixed with 5 µl of purple dye), followed by 6 µl of samples in the order:
    a. IS481: ladder, rpa with primer set 1 sample 1 - 9 corresponding to the dilutions from high to low concentrations, empty space, ladder, rpa with primer set 2 sample 1-9 correspondinG to dilutiosn from high to low conc.
    b. HbcAg: ladder, rpa sample 1 - 9 corresponding to the dilutions from high to low concentrations, empty space, ladder, rpa with primer set 2 sample 1-9 correspondinG to dilutiosn from high to low conc.
4. The gels were left to run for 20 minutes

Results:

IS481* post serial dilution RPA

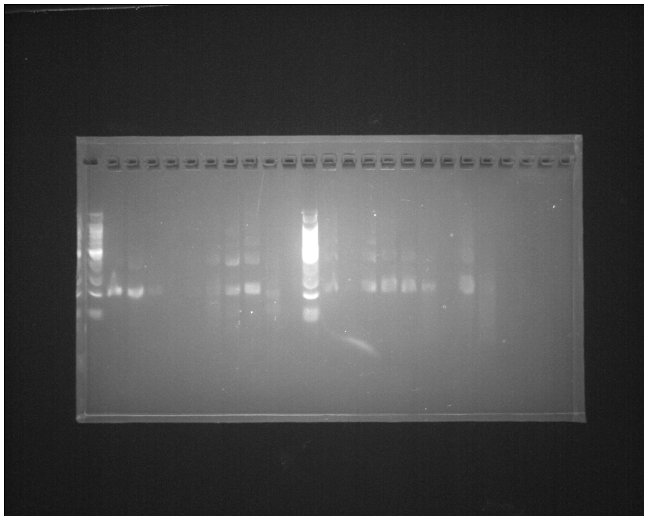
is481STAR_serial_dilutions_RPA_SEPT19_2019.tif



Left: 1st primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control
Right: 2nd primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

HBcAg post serial dilution RPA

HbcAg_serial_dilutions_RPA_SEPT19_2019.tif



Left: 1st primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control
Right: 2nd primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

Serial dilutions for HbcAg mp2:

Table 12

	A	B	C	D	E
1	**Serial Dilutions**	**Concentration (ng/µl)**	**From Previous Stock**	**dH2O**	**Remaining**
2		Highest Concentration (x ng/µl)	90		30
3	1	60	18	9	17
4	2	25	10	14	14
5	3	10	10	15	12.5
6	4	5	12.5	12.5	20
7	5	1	5	20	22.5
8	6	0.1	2.5	22.5	22.5
9	7	0.01	2.5	22.5	22.5
10	8	0.001	2.5	22.5	25
11	9	Negative Control (dH2O)	0	25	
12					

10µl final volume RPA for HbcAg (done with 2 sets of RPA primers), IS481* (done with 2 sets of RPA primers), pcaA (1st set of primers)

Protocal followed when doing HbcAg and IS481* RPAs:

1. Reaction mix in 1.5 mL tube:
    a. Primer A (10µM) - 4.8µL
    b. Primer B (10µM) - 4.8µL
    c. Rehydration Buffer - 59µL
    d. dH2O - 16.4µL
2. Pipetted up and down after addition of each component in step 1
3. Splitted the reaction mix in two (42.5µL) and added each half to 1 freeze dried reaction. Pipetted up and down to mix.
4. Splitted the reaction into 9 volumes - 8.5µL to 9 separate PCR tubes.
5. Added 1µL of template from each serial dilution in corresponding tube.
6. Added 0.5µL of 280mM magnesium acetate and mixed well to start the reaction.
7. Incubated at 38℃ for 40 min using thermocycler

Gel Electrophoresis of RPA samples of diluted is481* and HbcAg from above-said RPA run:

Agarose Gel Preparation:

a. Prepared a 1x agarose gel by adding 1.5 grams of agarose to 50 ml of TAE buffer.
b. Heating in the solution up in 20 second interval (in the microwave) and stop after making sure that the solution is clear enough

Sample Loading:

1. 5 µl of samples from each RPA diluted sample was added onto a piece of parafilm.
2. 1 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 1 µl of 100bp ladder (molecular ruler) was loaded first (1 µl of ladder was mixed with 5 µl of purple dye), followed by 6 µl of samples in the order:

a. IS481: ladder, rpa with primer set 1 sample 1 - 9 corresponding to the dilutions from high to low concentrations, empty space, ladder, rpa with primer set 2 sample 1-9 corresponding to dilutions from high to low conc.
b. HbcAg: ladder, rpa sample 1 - 9 corresponding to the dilutions from high to low concentrations, empty space, ladder, rpa with primer set 2 sample 1-9 corresponding to dilutions from high to low conc.

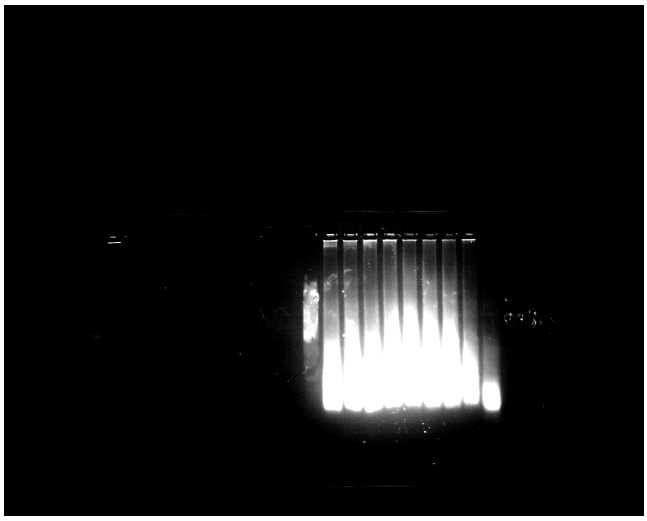
4. The gels were left to run for 20 minutes

Result:

IS481 post serial dilution

RPA:

is481STAR_serial_dilutions_RPA_SEPT19_2019_2.tif

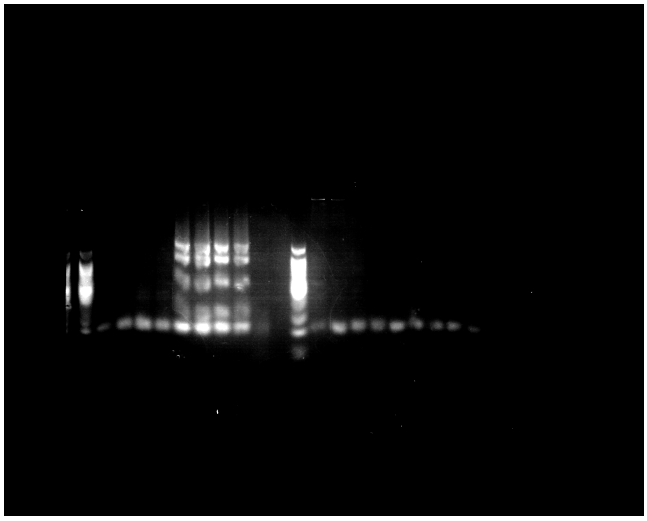


Left: 1st primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

Right: 2nd primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

HbcAg post serial dilution RPA:

HbcAg_serial_dilutions_RPA_SEPT19_2019_2.tif



Left: 1st primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

Right: 2nd primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

Overnight PCR for IS481* mp2 post serial dilution samples:

1. Added 20 µl PCR Mastermix to each PCR tube (not enough mastermix was left for the negative control/ just added what was left around 10ul)
2. Added 1 µl of 10 µM forward primers to each tube.
3. Added 1 µl of 10 µM reverse primers to each tube.
4. Added 2ul of each dilution to each tube. None was added to negative control.
5. Water was added to reach a total volume ot 40 µl (16 µl for each tube with DNA and 18 µl for negative control)
6. PCR temperatures were set on machine according to BioRad protocol and 35 cycles were done.
7. PCR was left overnight.

FRIDAY, 9/20/2019

Gel Electrophoresis of PCR samples of diluted is481* from Sept 19:

Agarose Gel Preparation:

a. Prepared a 1x agarose gel by adding 1.5 grams of agarose to 50 ml of TAE buffer.

b. Heating in the solution up in 20 second interval (in the microwave) and stop after making sure that the solution is clear enough Sample

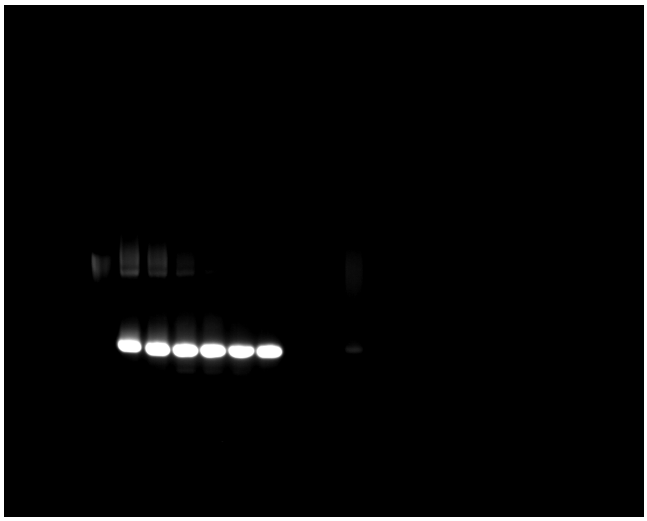
Loading:

1. 5 µl of samples from each PCR diluted sample was added onto a piece of parafilm.
2. 1 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 1 µl of 100bp ladder (molecular ruler) was loaded first (1 µl of ladder was mixed with 5 µl of purple dye), followed by 6 µl of samples in the order:
    a. IS481: ladder, rpa with primer set 1 sample 1 - 9 corresponding to the dilutions from high to low concentrations, empty space, ladder, rpa with primer set 2 sample 1-9 correspondinG to dilutiosn from high to low conc.
4. The gels were left to run for 20 minutes

Result:

IS481 post serial dilution PCR:

is481STAR_serial_dilutions_PCR_SEPT20_2019.tif



Left: 1st primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

Right: 2nd primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

RPA for post dilution IS481* (both primer 1 and 2 sets)

Protocal followed when doing IS481* RPAs:

1. Reaction mix in 1.5 mL tube:
    a. Primer A (10µM) - 4.8µL
    b. Primer B (10µM) - 4.8µL
    c. Rehydration Buffer - 59µL
    d. dH2O - 16.4µL
2. Pipetted up and down after addition of each component in step 1
3. Splitted the reaction mix in two (42.5µL) and added each half to 1 freeze dried reaction. Pipetted up and down to mix.
4. Splitted the reaction into 9 volumes - 8.5µL to 9 separate PCR tubes.
5. Added 1µL of template from each serial dilution in corresponding tube.
6. Added 0.5µL of 280mM magnesium acetate and mixed well to start the reaction.
7. Incubated at 38℃ for 40 min using thermocycler

Gel Electrophoresis of RPA samples of diluted is481* from above-said RPA run:

Agarose Gel Preparation:

a. Prepared a 1x agarose gel by adding 1.5 grams of agarose to 50 ml of TAE buffer.

b. Heating in the solution up in 20 second interval (in the microwave) and stop after making sure that the solution is clear enough Sample

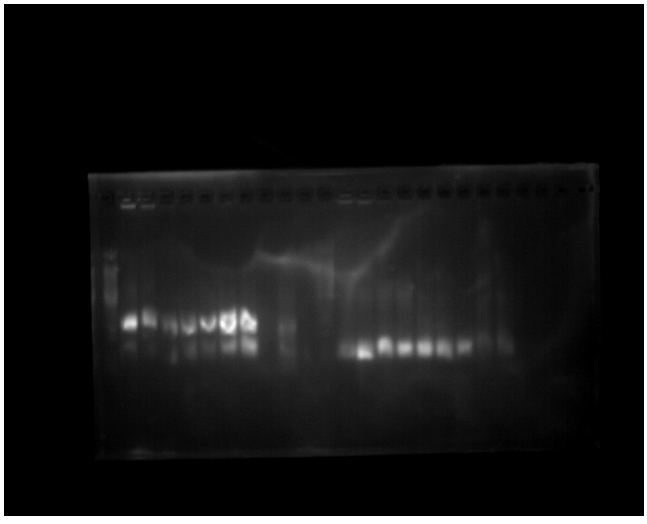
Loading:

1. 5 µl of samples from each RPA diluted sample was added onto a piece of parafilm.
2. 1 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 1 µl of 100bp ladder (molecular ruler) was loaded first (1 µl of ladder was mixed with 5 µl of purple dye), followed by 6 µl of samples in the order:
    a. IS481: ladder, rpa with primer set 1 sample 1 - 9 corresponding to the dilutions from high to low concentrations, empty space, ladder, rpa with primer set 2 sample 1-9 correspondinG to dilutiosn from high to low conc.
4. The gels were left to run for 20 minutes

Result:

IS481 post Serial dilution RPA:

is481STAR_serial_dilutions_RPA_SEP20_2019.tif



Left: 1st primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control
Right: 2nd primer set: Ladder-60 ng/µl dilution- 25ng/µl dilution- 10 ng/µl dilution- 5 ng/µl dilution- 1 ng/µl dilution- 0.1 ng/µl dilution- 0.01 ng/µl dilution- 0.01 ng/µl dilution- negative control

SATURDAY, 9/21/2019

Agarose Gel Preparation:

a. Prepared a 1x agarose gel by adding 1.5 grams of agarose to 50 ml of TAE buffer.
b. Heating in the solution up in 20 second interval (in the microwave) and stop after making sure that the solution is clear enough

Sample Loading: (PCR post serial dilutions for ypo2088 Sept 17th)

1. 5 µl of samples from each PCRed diluted sample was added onto a piece of parafilm.
2. 1 µl of purple loading dye was added to each sample drop and mixed by pipetting up and down.
3. 1 µl of 500bp ladder was mixed with 5 µl of dye and loaded first followed by 15 µl of samples in the order: 1, 2, 3, 4, 5, 6, 7, 8, 9
4. The gels were left to run for 20 minutes

10µl final volume RPA for pcaA (2nd set of primers)

Protocal followed when doing HbcAg and IS481* RPAs:

a. Reaction mix in 1.5 mL tube:
    I. Primer A (10µM) - 4.8µL
    II. Primer B (10µM) - 4.8µL
    III. Rehydration Buffer - 59µL
    IV. dH2O - 16.4µL
b. Pipetted up and down after addition of each component in step 1
c. Splitted the reaction mix in two (42.5µL) and added each half to 1 freeze dried reaction. Pipetted up and down to mix.
d. Splitted the reaction into 9 volumes - 8.5µL to 9 separate PCR tubes.
e. Added 1µL of template from each serial dilution in corresponding tube.
f. Added 0.5µL of 280mM magnesium acetate and mixed well to start the reaction.
g. Incubated at 38°C for 40 min using thermocycler

SUNDAY 9/22/2019

CRISPR of RPA samples of diluted is481* and HbcAg from above-said RPA run:

CRISPR reaction following NEB protocol:

1. Assemble the reaction at room temperature in the following order*:
    a. 20µl Nuclease-free water
    b. 3µl NEBuffer 2.1 Reaction Buffer (10x)
    c. 3µl 300nM gRNA
    d. 1µl 1 µM EnGen Lba Cas12a (Cpf1)
2. Pre-incubate for 10 minutes at 25^{0}C.
3. Add 3 µl of substrate DNA (30 µl final volume).
4. Vortex and pulse-spin in a microfuge.
5. Incubate at 37°C for 10 minutes.
6. Add 1.5 µl of 1uM FQ quencher
7. Incubate for 60 minutes at 37°C. Check the fluorescence every 10 minutes using fluorescent microscope.

Result: No fluorescence was seen.