Table of Contents

Introduction

Thursday, September 20, 2018

To Do:

1. ~~Explain lab notebook formatting~~
2. Media prep

Results and Data:

For each day, copy the previous entry headers and update the date. Save the same ongoing copy of the lab notebook to the lab google drive ONLY. Do not save separate physical copies. At the 1st of each new month, a read-only PDF file of the lab notebook must be saved and given to Dr. Ramsey electronically. **File contents converted to PDF MUST NOT BE EDITED after PDF conversion**. Continue to keep using the same word file until the end of the calendar year. New lab notebook files run in 6 month intervals.

For the To do list, update this each day with new tasks, as tasks are done, use the ~~strikethrough~~ font on the day they are completed and leave them out of the list on the next day.

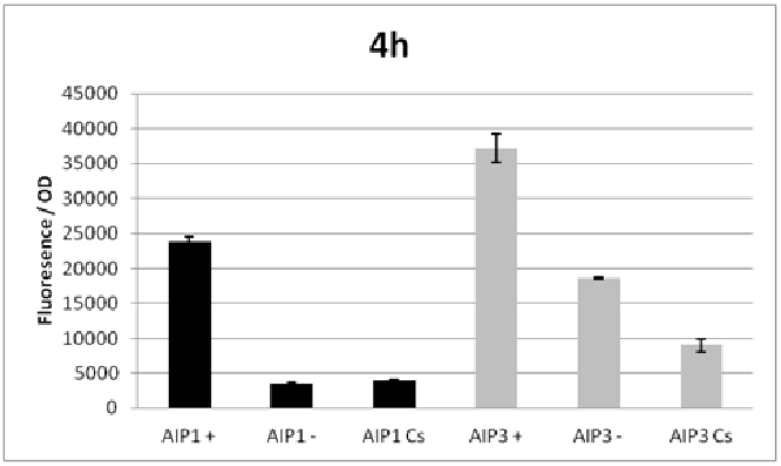


Figure 1

Figures are inserted as inline .png files when possible, .jpg, .gif, .tif otherwise are acceptable. Figure legends are always inserted in Word (right click – 'insert caption') and use "**Heading 2**" text to properly format in the TOC. Table legends are handled the same way. Figure images must be saved in a separate folder where the source data is saved.

Thoughts or questions. When you have a significant observation, question, confusing point or contradiction that you have identified in your data or protocol, use the "**Heading 3**" text heading on a descriptive brief title or single word heading so you can refer to it in your TOC later.

Protocol 1

1. This is the format for a protocol in your notebook.

2. The protocol title must be formatted in "**Heading 2.**"

3. Reagents which must be made for a specific protocol (buffers, solutions) should be listed in a "Reagents" Section, formatted in "**Heading 3**."

4. The protocol must be in numerical steps.

5. Use standard notation and carefully describe units for your protocol.

6. Use ½" indent for protocol text.

Reagents

Specific buffers

For initial lab notebooks, write as much detail as possible. As time goes by you will be able to refer to written protocols by their heading and/or page number within the document. If you make any modifications to a protocol you must state how and why.

File Formatting Protocol

1. Filenames begin with your initials, an underscore, and the date, formatted as the last 2 digits of the year, the month, then the day, ex: "KMR_180920_Sample_file1v1.xls"

2. This ensures all files will be sorted by their creator and by their date. You must use this file formatting system for all data files (including photos) that will be shared with the lab.

Bibliography data will be saved as (author/date) and using Mendeley at this time with the TOC inserted by order cited at the end of the lab notebook in the Bibliography section.

Example is a recent publication (Ramsey and Dove, 2016).

October 2018

Monday, October 22, 2018

To Do:

1. ~~Media prep (LB Agar)~~
2. ?

Results and Data:

LB Agar Preparation Protocol (500mL)

1. Weigh into 1L flask
    - 10g agar
    - 5g NaCl
    - 5g tryptone
    - 2.5g yeast extract
2. Add 500mL water
3. Stir with hot plate and stir bar until solute dissolves
4. Place tin foil and autoclave tape on top of flask with date written
5. Autoclave using LIQ 30 cycle
6. Pour near flame using sterile technique into sterile petri dishes, approximately 20mL per plate
7. Rinse agar flask and stir bar with water immediately once it is empty

Tuesday, October 23, 2018

To Do:

1. ~~Make LB Media~~
2. ~~Sterilize sticks and culture tubes~~

Results and Data:

LB Broth Protocol (for 1000mL LB)

1. Weigh into a 1L graduated cylinder (with stir bar)
    - 10g tryptone
    - 10g NaCl
    - 5g yeast extract
2. Add type 1 DEI H2O to 1L graduated cylinder
3. Mix until dissolved
4. Aliquot out
    - 3 x 200 mL in 1 L flasks
    - 4 x 75 mL in 100 mL bottled

E. coli Competent Cells Protocol

Reagents:

- Solution A
  - 10 mL of 1.0M $MnCl_2$
  - 50 mL 1.0M $CaCl_2$
  - 200mL MES, pH 6.3
  - 740mL of ddiH2O

- Solution A + 15% glycerol
  - 10 mL of 1.0M $MnCl_2$
  - 50 mL 1.0M $CaCl_2$
  - 200mL MES, pH 6.3
  - 590mL of ddiH2O
  - 150mL of glycerol

How to make 250 mL of 55mM of MES

FW of MES = 213.25 g/mol

Calculation:

$$\frac{50 mmol}{1\ L\ soln} \times \frac{1\ L}{1000\ mL} \times \frac{250\ mL}{1} \times \frac{1\ mol}{1000\ mmol} \times \frac{213.25\ g}{1\ mol} = \mathbf{2.67g}$$

Wednesday, October 24, 2018

To Do:

1. ~~Wash a load of glassware in dishwasher at MRamsey lab.~~
2. ~~Streak 4 plates of LB Agar (made 10/22/18) with XL1 Blue *E. coli* cells and incubate.~~

Results and Data:

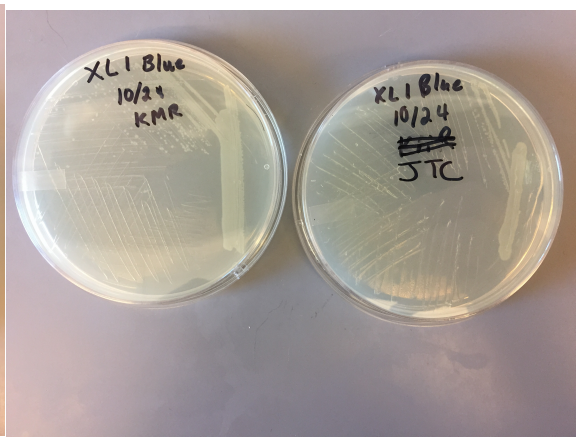
XL1 Blue plates were stored in incubator at MRamsey lab.

Thursday, October 25, 2018

To Do:

1. ~~Take pictures of incubated agar plates (streaked 10/24/18) with isolated colonies.~~
2. ~~Wash 200mL flask that the Nelson lab returned.~~

Results and Data:



Friday, October 26, 2018

To Do:

1. ~~Make Solution A + 15% Glycerol~~

Results and Data:

Solution A + 15% Glycerol is labeled “Solution A 15%”

Monday, October 29, 2018

To Do:

1. Make and test 3 PCR solutions
    - John’s primers: p824, 825 (always need 2 primers to amplify a region)
    - Control primers: p780 and p781
    - Should amplify 772 BPs
    - Steps
        - Add water to 1.5 mL tube
        - Add dNTPs
        - Add KOD buffer
        - Add enzyme KOD (this should be last)
        - Mix master-mix by pipetting up and down (vortexing would expose enzyme to oxygen)
        - Add MM to Tube 3
        - Add template to Tube 3 (3.4 uL)
        - Add MM to Tubes 1 and 2
        - Aliquot into PCR tubes
        - Pipette 1.5 uL of primers into Tube 1
        - Pipette 1.5 uL of controls into Tube 2 and Tube 3
        - Add 1 uL of H2O to Tube 3 (missing 1 uL template)
    - Notes
        - Label master-mix PCR tube “MM” (PCR tube = microphige tube/Ependorph tube)
        - Dispense H2O into PCR tube and label
        - Transfer 44 uL H2O into MM tube
    - Thermocycler – STD 1:
        - Heat at 94 degrees for 2 minutes,
        - 94 degrees C for 20 seconds
        - 50 degrees C for 30 seconds

- 68 degrees C for 1 minute (COD polymerase functions properly at 68 degrees C; TAC polymerase is different temp)
- Go back to step 2
- Repeat 32x
- 68 degrees C for 5 minutes
- 12 degrees C for infinity

Results and Data:

Total reaction volume	50
Total number of reactions	3

Component	Stock concentration	Final concentration	1 rxn volume	Factor 4.4
ddiH2O			10	44
KOD buffer	2x	1x	25	110
dNTPs	2 mM	0.4 mM	10	44
oligo F	10 uM	0.3 uM	1.5	-
oligo R	10 uM	0.3 uM	1.5	-
template	100 ng/ul	2 ng/ul	1	4.4
KOD	1 U/ul	0.02 U/ul	1	4.4
		Total volume	50	220

Thursday, September 20, 2018

To Do:

1. ~~Loading agarose gel (left to right)~~

Loading agarose gel (left to right)

Lane	Contents	Expected size
1	2-log NEB ladder	varies
2	CS #1 P820 P821	994 bp
3	CS #2 P780 P781	443 bp
4	CS #3 P780 P781	0 bp
5	JC #1 P825 P825	772 bp
6	JC #2 P780 P781	443 bp
7	JC #3 P780 P781	0 bp
8	KR #1 P820 P821	994 bp

9 KR #2 P780 P781 443 bp

10 KR #3 P780 P781 0 bp

11 – 15 Empty

November 2018

Thursday, November 1, 2018

To Do:

1. ~~Making chemically competent cells (step 3)~~

Results and Data:

Day 3

1. Add 3 mL of sterile $MgCl_2$ to 200 mL LB in 1 L flask. Add antibiotic if appropriate. We used 1 L flask that is not baffled.
2. Inoculate 200 mL LB with 0.5 mL of culture grown overnight.
3. Incubate 200 mL culture at 37°C, shaking until culture reaches an OD600 of 0.5.
4. Monitor culture growth by assessing OD600 using the spectrophotometer:
   - At 3, 4, and 5 hours or at appropriate times between, measure OD600
   - Use a cuvette with 1 mL LB as a blank
   - Add 1 mL culture from flask to a cuvette
   - Use MRamsey lab spectrophotometer set to a wavelength of 600nm
   - After ~6 hours, OD600 = 0.55 and we split culture into 4 50 mL conicals to spin down
5. When cultures approach correct OD, cool down centrifuge to 4°C (Dutta lab benchtop centrifuge)
6. When culture reaches an OD600 of approximately 0.5, transfer culture volume to sterile tubes (4x 50 mL conical) to pellet bacteria
7. Place tubes in cool centrifuge and pellet bacteria by spinning (in Dutta lab benchtop centrifuge, 15 minutes at 4000 rpm).
8. Remove tubes from centrifuge, decant the supernatant into a waste bottle, and keep cell pellets on ice
9. Add a total of 60 mL cold solution A to cell pellets from the original 200 mL culture. If you are using four 50 mL conical tubes, each tube should contain 15 mL cold solution A.
10. VERY gently resuspend cell pellet by pipetting up and down. Don't completely dispense liquid with each cycle, to prevent creating bubbles/froth. The cells should be completely homogeneous when done (no clumps or chunks)
11. Incubate resuspended cells on ice for at least 20 minutes (can stay on ice for up to 3 hours).
12. Place tubes in cool centrifuge and pellet bacteria by spinning (in Dutta lab benchtop centrifuge, 15 minutes at 4000 rpm).
13. While cells are spinning, prepare tubes for final competent cell aliquots: label and pre-chill on ice.
14. Remove tubes from centrifuge, decant the supernatant into a waste bottle, and keep cell pellets on ice
15. Add a total of 12 mL cold solution A + 15% glycerol to cell pellets from the original 200 mL culture. If you are using four 50 mL conical tubes, each tube should contain 3 mL cold solution A + 15% glycerol.
16. VERY gently resuspend cell pellet by pipetting up and down as previously.
17. Aliquot competent cells in 550 uL volumes into sterile pre-cooled 1.5 mL microcentrifuge tubes.

18. If available, freeze cells immediately upon aliquoting using using dry ice.
19. Store competent cells at -80°C.
20. At first use, test competency of cells by transforming with a known amount of supercoiled plasmid and record the transformation efficiency.

Monday, November 5, 2018

To Do:

1. ~~Make kanamycin stock solution~~
2. ~~Make kanamycin plates~~

Results and Data:

Kanamycin Protocol

1. Weigh out 1g of kanamycin into a nonsterile 50 mL conical
2. Add 15 mL of type 1 ddiH2O to the conical
3. Dissolve the kanamycin by vortexing
4. Pour into a graduated cylinder
5. Add type 1 ddiH2O until volume reaches 20 mL
6. Filter sterilize using a syringe filter into sterile 50 mL conical
    - DO NOT touch filter tip
    - Attach filter by screwing it onto the end of the syringe
    - Load kanamycin into syringe by removing stopper, pouring kanamycin, and placing stopper back in
7. Aliquot out 750 uL amounts of sterile kanamycin solution into 1.5 mL microfuge tubes

Wednesday, November 7, 2018

To Do:

1. ~~Count colonies from yesterdays transformations.~~
2. ~~Calculate transformation efficiency using pUC19 data (we know how much pUC19 DNA we added to the transformation, 50 pg). See below for formula and example.~~
3. ~~Start inoculate small (5mL) cultures with colonies containing the different plasmids (pKL02, pKL80, and pF). Cultures will grow overnight and we'll isolate the plasmid DNA tomorrow.~~

Results and Data:

From the New England Biolabs website (https://bit.ly/2nyifPU), here is how to calculate transformation efficiency:

Transformation efficiency (TE) equation:

TE = Colonies/µg/Dilution

Colonies = the number of colonies counted on the plate

µg = the amount of DNA transformed expressed in µg

Dilution = the total dilution of the DNA before plating

Plasmid Construct					
Volumes Aliquoted	pKL02	pKL80	pF	PUC19	None
20 uL	7	39	7	0	0
100 uL	80	290	122	8	0
Remaining ~800 uL	TMTC	TMTC	TMTC	17	0
Transformation Efficiency					

Dilution = 100/1100 = 0.0909

8colonies/0.05ug/0.0909dilution = 1.76x10^6

It would help to make a table

Wednesday, November 7, 2018

To Do:

1. ~~Mini prep plasmids using Miniprep kit~~

Results and Data:

QIAprep Spin Miniprep Kit Protocol

1. Pellet 5 mL bacterial overnight culture by centrifuging at 10000 x g for 1 minute at room temperature (15-25 deg C). Pipette 1.5 mL culture into the tube, spin, discard the supernatant, and repeat until all 5 mL are gone (3x).
2. Resuspend pelleted bacterial cells in 250 uL Buffer P1 (stored at 4 deg C) and transfer to a microcentrifuge tube
3. Add 250 uL 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 minutes. If using LyseBlue reagent, the solution will turn blue.
4. Add 350 uL Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times. If using LyseBlue, the solution will turn colorless.
5. Centrifuge for 10 minutes at 13,000 rpm (or max speed) in a tabletop microcentrifuge
6. Apply 800 uL supernatant from step 5 to the QIAprep 2.0 spin column by pipetting. For centrifuge processing, follow the instructions marked with a triangle. For vacuum manifold processing, follow the instructions marked with a circle. Centrifuge for 30-60 seconds and discard the flow-through.
7. Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB. Centrifuge for 30-60 seconds and discard the flow through. Transfer the QIAprep 2.0 spin column to the collection tube.
8. Wash with Buffer PE. Centrifuge for 30-60s.
9. Centrifuge for 3 minutes to remove residual wash buffer. Ethanol in DNA

10. Place the QIAprep 2.0 column in a clean 1.5 mL microcentrifuge tube. To elute DNA, add 0.1x 50 uL Buffer EB (10 mM TrisCl, pH 8.5) or water to the center of the QIAprep 2.0 spin column, let stand for 1 minute, and centrifuge for 1 minute.
11. Analyzing on a gel would show us how pure the chromosome is because supercoiled plasmid DNA moves faster.

Tuesday, November 13, 2018

To Do:

1. ~~PCR Round A on pKL114~~

Results and Data:

Number	Primers	Target	Expected Size	For plasmid
1	P818 and P695	PriM FR1	746 bp	pKL115
2	P696 and P819	PriM FR2	615 bp	pKL115
3	P818 and P819	positive control	1334 bp	-
4	P818 and P819	negative control	-	-

Total reaction volume	25
Total number of reactions	4

Component	Stock concentration	Final concentration	1 rxn volume	Factor 5.5
ddiH2O			5	27.5
KOD buffer	2x	1x	12.5	68.75
dNTPs	2 mM	0.4 mM	5	27.5
oligo F	10 uM	0.3 uM	0.75	4.125
oligo R	10 uM	0.3 uM	0.75	4.125
template	100 ng/ul	2 ng/ul	0.5	(2.25)
KOD	1 U/ul	0.02 U/ul	0.5	2.75
		Total volume	25	137.5

Template = pKL114 diluted 1:100

PCR Protocol by John Church

1. Acquire and label 4 PCR tubes with initials and designate as Tubes 1-4
    - The tubes comes in strips of 8 and they can be split into 4 tube pieces so that the first 3 tubes are used and the fourth is unused
2. Get a container of ice to keep the components on
3. Acquire the following components and put them on ice, labeling tubes if necessary:
    - ddi H2O in 1.5 mL microfuge tube
    - uL KOD buffer
    - dNTPs

- oligo F
- oligo R
- template
- Note: KOD enzyme should be kept in the freezer until it is used as it is expensive and should be added last

4. Centrifuge the microfuge tubes to get any solution out of the microfuge tube cover
5. If any of the solutions are frozen, be sure to vortex the microfuge tube in order to dissolve it (tubes with frozen components may not be homogenized)
    - DO NOT vortex the KOD enzyme itself or any solution with KOD enzyme because vortexing will expose it to oxygen and degrade it
6. Use PCR_worksheet.xlsx to make establish the specifics of what will be added
    - The file is located in the Protocols folder
    - For this protocol, a “Total reaction volume” of 100 uL and 4 “Total number of reactions” were used – the following volumes are based on these specifications
7. Add 0.75 uL of each experiment specific primer (forward and reverse) to PCR Tubes 1 and 2 (oligos forward and reverse)
    - The amount added should be calculated by taking the total volume for 1 reaction (in worksheet) and subtracting the volumes for 1 reaction that have not yet been added to the master-mix
8. Add 0.75 uL of each control primer (oligos forward and reverse) to PCR Tubes 3 and 4
9. Add 0.5 uL ddi H2O to PCR Tube 4 so that all 4 PCR Tubes have an even amount of solution to account for the lack of template in Tube 4
10. Prepare a master-mix in a 1.5 mL microfuge tube by adding the following according to the worksheet and using micropipettes:
    - Add 27.5 uL ddi H2O
    - Add 27.5 uL dNTPs
    - Add 68.75 uL KOD buffer
    - Add 2.75 uL KOD enzyme
11. Mix the master-mix solution by pipetting up and down
    - Do not vortex to mix
12. Add 23 uL of master-mix to PCR Tube 4
13. Add 2.25 uL template to Master Mix
14. Add 23.5 uL master mix to each PCR Tube 1-3 and pipette up and down to mix (conserves tips)
15. Close PCR Tubes 1-4 until the caps are tight (push until the caps do not squeak when you push on them)
16. Place the PCR Tubes in the thermocycler on STN 1 – the following settings should be in place:
    - Heat at 94 degrees for 2 minutes,
    - 94 degrees C for 20 seconds
    - 50 degrees C for 30 seconds

- 68 degrees C for 1 minute and 20 second [modified because product is 1334 bp which is over 1 kbp] (KOD polymerase functions properly at 68 degrees C; TAC polymerase is different temp)
- Go back to step 2
- Repeat 32x
- 68 degrees C for 5 minutes
- 12 degrees C for infinity

Wednesday, November 14, 2018

To Do:

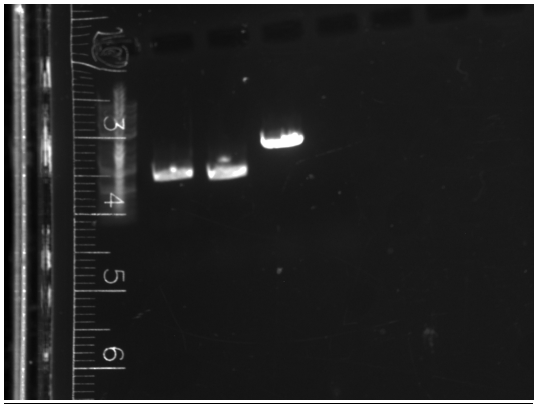
1. ~~Run gel for PCR~~

Results and Data:

Agarose Gel Protocol – written by Joe Paquette

(Note: all ddiH2O is type I)

1. Mix 25x TAE buffer with ddiH_2O to obtain 1x TAE solution.
   - To make 1 L of 1x TAE, add 40 mL 25x TAE into 1 L graduated cylinder and fill to 1 L with ddiH2O.
2. Add 1 g agarose powder to 100 mL 1x TAE buffer in 250 mL.
3. Add stirbar to container.
4. Heat to dissolve the agarose while stirring (don't let over boil, should look like clear liquid, no solids).
5. Once the sugar has dissolved, make a 1x concentration of SYBR safe in the 1% agarose solution.
   - To make, add 10µL of 10,000x SYBR safe dye to the 1% agarose solution
6. Let the 1% agarose solution cool to approximately 50-55°C.
7. Apply autoclave tape to the edges of the gel cast (ensure the tape is tightly bound).
8. Pour 1% agarose - 1x SYBR safe solution into the cast and insert a comb to mold wells in the gel.
9. Let sit until the 1% agarose – 1x SYBR safe solution has cooled and solidified into a gel.
10. Carefully remove the comb.
11. Pour the 100 ml of 1x TAE buffer solution into the gel tank (add just enough to slightly submerge the gel itself).
12. Obtain 5 µL of each PCR sample.
13. Make 1x loading dye in 6 µL of solution.
    - To make 6 µL of 1x loading dye, combine 5 µL PCR sample with 1 µL of 6x loading dye
14. Load 5 µL of the PCR-dye mixture into the wells in sequential order.
15. Insert the electrodes and run the gel at about 113 volts.



Thursday, November 15, 2018

To Do:

1. ~~PCR Round B on pKL114~~

Results and Data:

Number	Primers	Target	Expected Size	For plasmid
1	P818 and P819		1344	pKL115
2	P818 and P695	positive control	746	-
3	P818 and P695	negative control	-	-

Total reaction volume		100		
Total number of reactions		3		
				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	4.4
ddiH2O			20	88
KOD buffer	2x	1x	50	220
dNTPs	2 mM	0.4 mM	20	88
oligo F	10 uM	0.3 uM	3	13.2
oligo R	10 uM	0.3 uM	3	13.2
template	100 ng/ul	2 ng/ul	2	(6.8)
KOD	1 U/ul	0.02 U/ul	2	8.8
		Total volume	100	440

Reaction Set B Protocol

1. Acquire and label 3 PCR tubes with initials and designate as Tubes 1-3
    - The tubes comes in strips of 8 and they can be split into 4 tube pieces so that the first 3 tubes are used and the fourth is unused
2. Get a container of ice to keep the components on
3. Acquire the following components and put them on ice, labeling tubes if necessary:

- ddi H2O in 1.5 mL microfuge tube
- uL KOD buffer
- dNTPs
- oligo F
- oligo R
- template (for Reaction Set B, the template is a dilution from Reaction Set A)
  - To make template, dilute Reaction Set A PCR Tubes 1 and 2 using a 1:10 dilution, adding 1 uL of each together into a microfuge tube, and then adding 8 uL of ddi H2O to bring the volume to 10 uL
- Note: KOD enzyme should be kept in the freezer until it is used as it is expensive and should be added last

4. Centrifuge the microfuge tubes to get any solution out of the microfuge tube cover
5. If any of the solutions are frozen, be sure to vortex the microfuge tube in order to dissolve it (tubes with frozen components may not be homogenized)
   - DO NOT vortex the KOD enzyme itself or any solution with KOD enzyme because vortexing will expose it to oxygen and degrade it
6. Use PCR_worksheet.xlsx to make establish the specifics of what will be added
   - The file is located in the Protocols folder
   - For this protocol, a “Total reaction volume” of 100 uL and 3 “Total number of reactions” were used – the following volumes are based on these specifications
7. Add 3 uL of each experiment specific primer (forward and reverse) to PCR Tube 1 (oligos forward and reverse)
8. Add 3 uL of each control primer (oligos forward and reverse) to PCR Tubes 2 and 3
9. Add 2 uL ddi H2O to PCR Tube 3 so that all 3 PCR Tubes have an even amount of solution
   - This is equal to the template for 1 reaction volume
10. Prepare a master-mix in a 1.5 mL microfuge tube by adding the following according to the worksheet and using micropipettes:
    - Add 88 uL ddi H2O
    - Add 88 uL dNTPs
    - Add 220 uL KOD buffer
    - Add 8.8 uL KOD enzyme
11. Mix the master-mix solution by pipetting up and down
    - Do not vortex to mix
12. Add 92 uL of master-mix to PCR Tube 3
    - The amount added should be calculated by taking the total volume for 1 reaction (in worksheet) and subtracting the volumes for 1 reaction that have not yet been added to the master-mix
13. Add 6.8 uL template to Master Mix
14. Add 94 uL master mix to each PCR Tube 1-3 and pipette up and down to mix (conserves tips)
15. Close PCR Tubes 1-3 until the caps are tight (push until the caps do not squeak when you push on them)
16. Place the PCR Tubes in the thermocycler on STN 1 – the following settings should be in place:
    - Heat at 94 degrees for 2 minutes,

- 94 degrees C for 20 seconds
- 50 degrees C for 30 seconds
- 68 degrees C for 1 minute and 20 second [modified because product is 1334 bp which is over 1 kbp] (KOD polymerase functions properly at 68 degrees C; TAC polymerase is different temp)
- Go back to step 2
- Repeat 32x
- 68 degrees C for 5 minutes
- 12 degrees C for infinity

17. The PCR tubes are marked in orange and were stored in the freezer until after Thanksgiving break

December 2018

Monday, December 3, 2018

To Do:

1. ~~Run gel for PCR Round B tubes.~~

Results and Data:

Agarose Gel Protocol – written by Joe Paquette

(Note: all ddiH2O is type I)

1. Mix 25x TAE buffer with ddiH_2O to obtain 1x TAE solution.
   - To make 1 L of 1x TAE, add 40 mL 25x TAE into 1 L graduated cylinder and fill to 1 L with ddiH2O.
2. Add 1 g agarose powder to 100 mL 1x TAE buffer in 250 mL.
3. Add stirbar to container.
4. Heat to dissolve the agarose while stirring (don't let over boil, should look like clear liquid, no solids).
5. Once the sugar has dissolved, make a 1x concentration of SYBR safe in the 1% agarose solution.
   - To make, add 10µL of 10,000x SYBR safe dye to the 1% agarose solution
6. Let the 1% agarose solution cool to approximately 50-55°C.
7. Apply autoclave tape to the edges of the gel cast (ensure the tape is tightly bound).
8. Pour 1% agarose - 1x SYBR safe solution into the cast and insert a comb to mold wells in the gel.
9. Let sit until the 1% agarose – 1x SYBR safe solution has cooled and solidified into a gel.
10. Carefully remove the comb.
11. Pour the 100 ml of 1x TAE buffer solution into the gel tank (add just enough to slightly submerge the gel itself).
12. Obtain 5 µL of each PCR sample.
13. Make 1x loading dye in 8 µL of solution.
    - To make 8 µL of 1x loading dye, combine 5 µL PCR sample with 3 µL of 6x loading dye

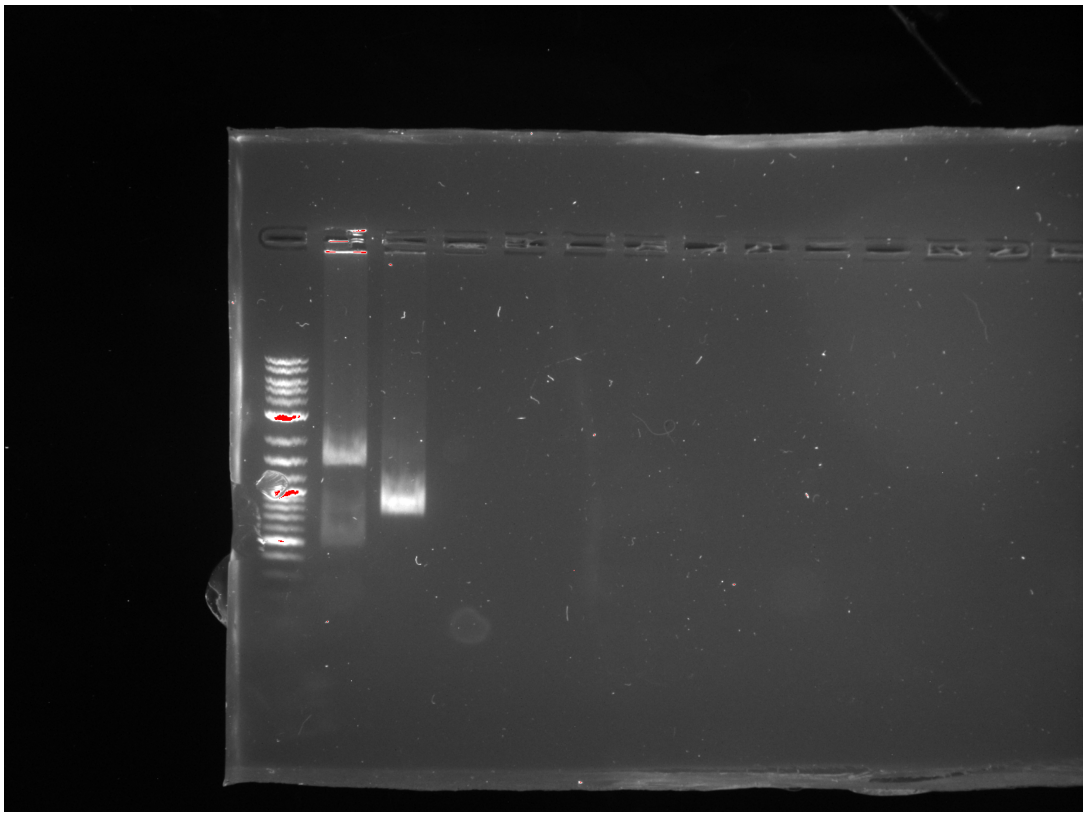
14. Load 10 µL of ladder into slot 1
15. Load 8 µL of the PCR-dye mixture into the wells in sequential order.
16. Insert the electrodes and run the gel at about 113 volts.

Materials needed:

- 1.0g of agarose powder
- SYBR safe dye
- 1 packet of 25x TAE buffer mix

PCR Imaging Machine

1. Insert flash drive into computer
2. Remove agarose gel from the plastic tray and place it onto the imaging tray
3. Select the option to view the gel and adjust it so that the wells that were run are in view
4. Select the option to image the gel
5. Deselect the box to show DNA
6. Save the picture of the gel to the computer under the KRamsey_lab folder
7. Save the picture of the gel from the computer to the flash drive



Above is the imaging of the gel for the PCR. The positive control DNA on the PCR matches with the expected size, however, the PCR from Tube 1 is slightly higher than expected, meaning that there may be more DNA in the PCR sample than expected.

Tuesday, December 4, 2018

To Do:

1. ~~Start PCR Purification Kit on PCR Round B~~
2. ~~Cut purified PCR DNA and plasmid DNA~~

Results and Data:

QIAquick PCR Purification Kit Protocol

1. Add 5 volumes Buffer PB to 1 volume of the PCR reaction and mix. (Have approximately 100 uL of PCR, so 500 uL Buffer PB will be used.)
2. Place a QIAquick column in the provided 2 mL tube.
3. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30-60s. Discard flow-through and place the QIAquick column back in the same tube.
4. To wash, add 750 uL Buffer PE to the QIAquick column and centrifuge for 30-60s. Discard flow-through and place the QIAquick column back in the same tube.
5. Centrifuge the QIAquick column once more in the provided 2 mL collection tube for 1 min to remove the residual wash buffer.
6. Place each QIAquick column in a clean 1.5 mL microcentrifuge tube.
7. To elute DNA, add 30 uL 0.1x Buffer EB (10mM Tris.Cl, pH 8.5) or water (pH 7.0-8.5) to the center of the QIAquick membrane and centrifuge the column for 1 min. For increased DNA concentration, add 30 uL elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.

Master Mix Solution	1x (uL)	3x (uL)
H2O	10.8	32.4
10x Cutsmart Buffer	3.0	9.0
DNA	(15.0)	-
BamH2 – HF	0.6	1.8
Kpn2 - HF	0.6	1.8
Total	30.0 (15.0 actual b/c of DNA)	90.0

Tube	DNA	DNA Volume (uL)	H2O Volume (uL)
1	Purified PCR	15	-
2	pKL80	5	10

DNA Cutting Protocol

1. To master mix tube (MM), add 32.4 uL H2O and 9.0 uL Cutsmart Buffer.
2. To individual DNA tubes, add 15 uL of each DNA type (Purified PCR and pKL80 plasmid).
3. Add 1.8 uL of each enzyme to the MM.
4. Mix it by pipetting up and down.
5. Add 15 uL of MM to individual tubes.
6. Incubate at 37^{o} C overnight.

Wednesday, December 5, 2018

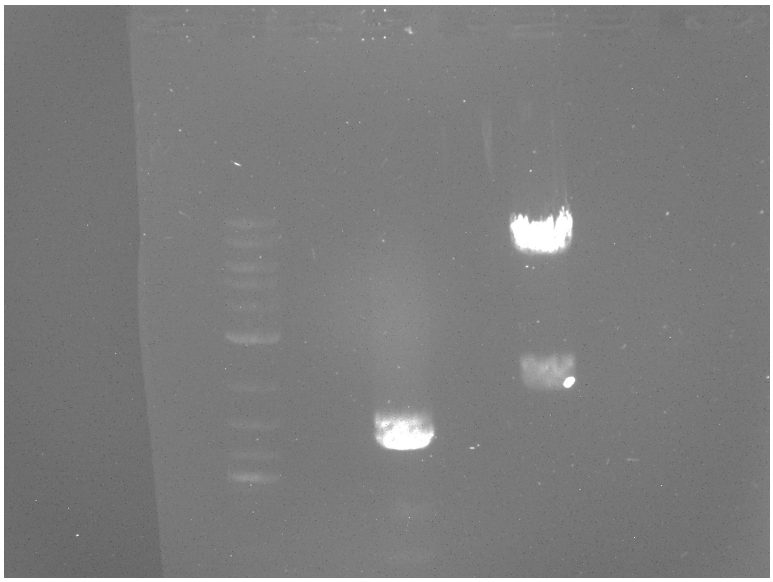
To Do:

1. ~~Remove phosphate ends from plasmid DNA.~~
2. ~~Run a gel (from existing gel) using plasmid and purified PCR DNA.~~

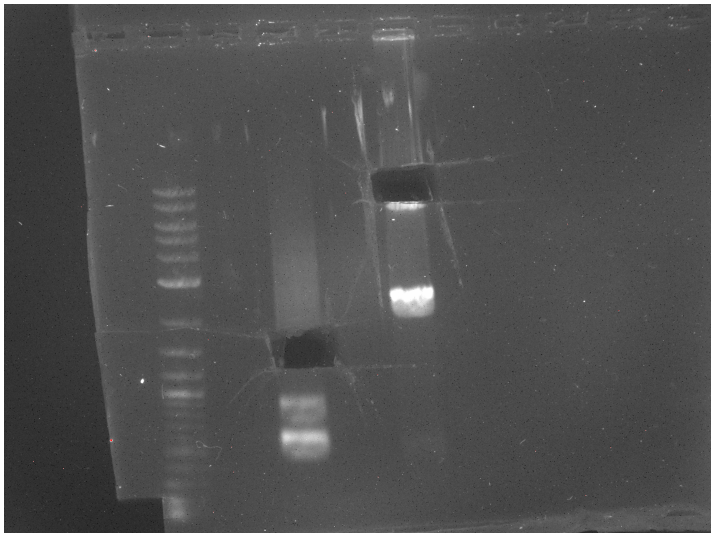
Results and Data:

Gel Protocol for Plasmid and PCR DNA

1. Take existing gel from fridge and add dye to the gel in solution so that it diffuses back into the gel.
2. Load 6 uL of dye into each of the DNA tubes.
3. Load 36 uL of each DNA into the gel and run.



Above is the gel of the PCR and Plasmid DNA. The PCR DNA is shown on the left and the plasmid DNA is shown on the right. This image was taken before cutting the gel.



Above is the image of the same gel, but the bands have been cut to remove the PCR and plasmid DNA.

Thursday, December 6, 2018

To Do:

1. ~~Purify gel from 12/5/18.~~
2. ~~Perform ligation reaction with digested, purified PCR and d.p. plasmid to connect insert and cut plasmid together.~~

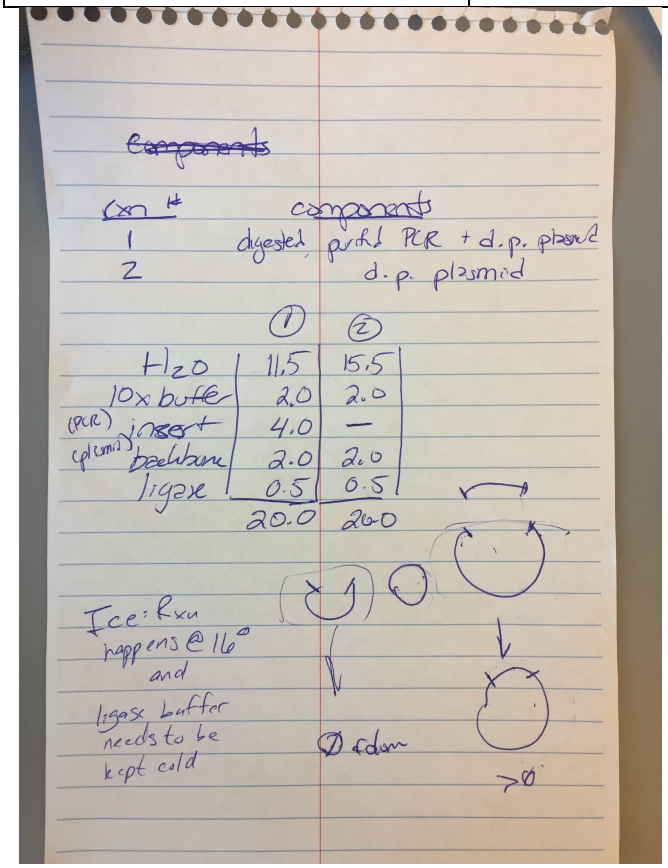
Results and Data:

QIAquick Gel Extraction kit Protocol

1. Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume gel (100 mg gel ~ 100 uL). The maximum amount of gel per spin column is 400 mg.
2. Incubate at 50° C for 10 minutes (or until the gel slice has completely dissolved). Vortex the tube every 2-3 minutes to help dissolve the gel.
3. Add 1 gel volume isopropanol to the sample and mix.
4. Place the QIAquick spin column in a provided 2 mL collection tube. To bind the DNA, apply the sample to the QIAquick column and centrifuge for 1 minute. Discard flow-through and place the QIAquick column back into the same tube. For sample volumes of >800 uL, load and spin again.
5. Add 500 uL Buffer QG to the QIAquick column and centrifuge for 1 minute. Discard flow-through and place QIAquick column back into the same tube.
6. To wash, add 750 uL Buffer PE to QIAquick column and centrifuge for 3 minutes. Discard the flow-through and place the QIAquick column back into the same tube.
7. Place QIAquick column into a clean 1.5 mL microcentrifuge tube.
8. To elute DNA, add 30 uL 0.1x Buffer EB (10mM Tris.Cl, pH 8.5) or water to the center of the concentration, add 30 uL Buffer EB to the center of the QIAquick membrane and centrifuge for 1 min.

Mass of Empty Microfuge Tube	1.0g	Volume of QB to Add
Total Mass of PCR Tube	1.7g	2100 uL
Total Mass of Plasmid Tube	1.7g	2100 uL

	Reaction 1 (uL)	Reaction 2 (uL)
H2O	11.5	15.5
10x buffer	2.0	2.0
Insert (PCR)	4.0	-
Backbone (plasmid)	2.0	2.0
Ligase	0.5	0.5
TOTAL	20.0	20.0



The purpose of this is to ligate the cut plasmid backbone to the strand of PCR DNA.

Ligation Reaction of Digested, Purified PCR and Plasmids Protocol

1. Get a container of ice to keep the reactants on. This is important, as the reaction happens at 16^{o} C and the ligase buffer needs to be kept cold in order to avoid breaking down.
2. Get and label two PCR tubes to use for the reactions. Be sure to include the date.
3. Add the volumes of the above chart to each of two tubes for H2O, 10x buffer, insert, and backbone, leaving ligase in the freezer until it is ready to be used. When using the ligase, be use to use the cooler from the freezer to keep it stored.
4. After all of the components have been added, mix each tube with a pipette set to 18 uL.
5. Place in the thermocycler overnight at 16^{o}C.

Thursday, December 6, 2018

To Do:

1. ~~Transform chemically competent *E. coli* cells.~~

Results and Data:

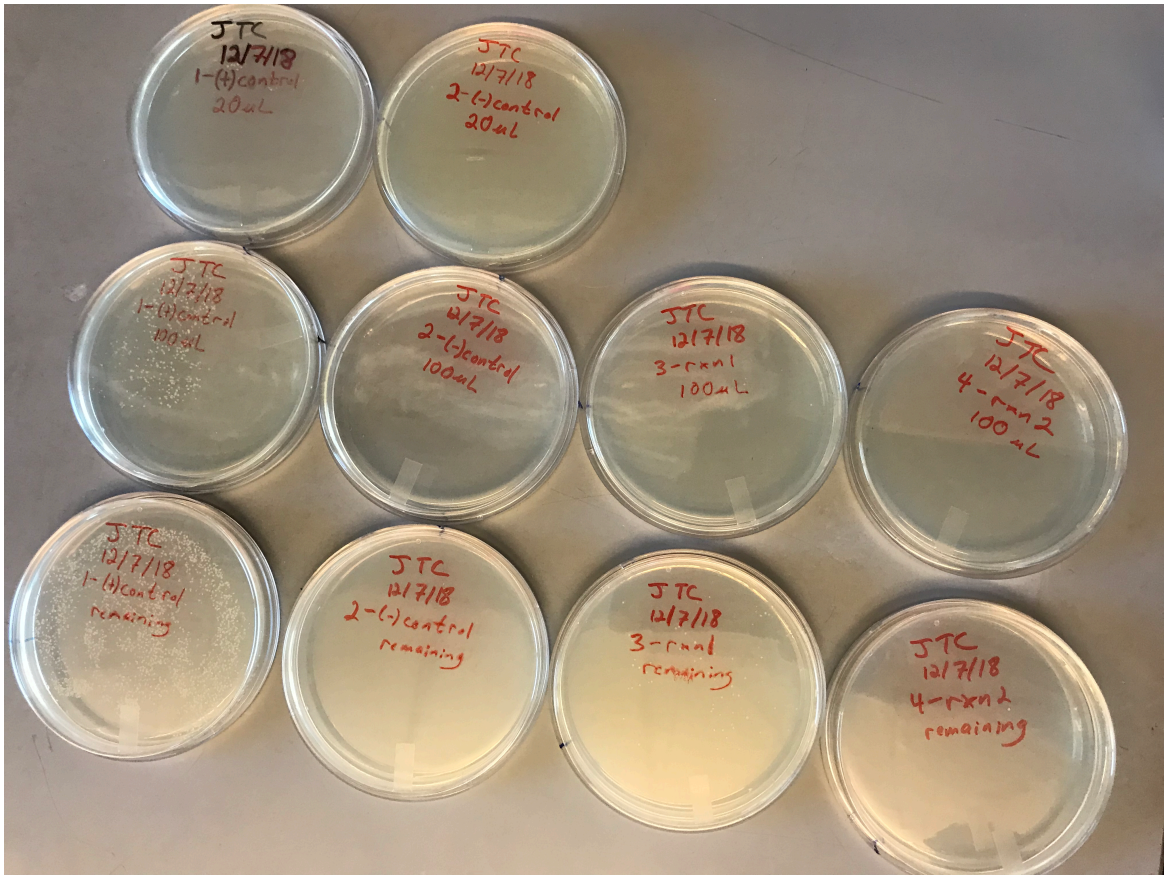
Transform Chemically Competent *E. coli* Cells Protocol

1. Set up reaction table. **Always include a positive and negative control for each antibiotic**. If transforming plasmids (from previous plasmid prep), use 0.5 – 1 uL of plasmid. If transforming ligations, use 8 uL per ligation. If transforming plasmid, plate 20 uL, 100 uL, and remaining culture. If transformation a ligation, plate 100 uL and remaining culture.

Reaction table

Tube number	Purpose	DNA	Volume of DNA	Final volume to plate	Number of kanamycin-containing plates	Number of carbenicillin-containing plates
1	(+) control	pKR1	1 uL	20 ul, 100 ul, remaining	3	
2	(-) control	None	0	20 ul, 100 ul, remaining	3	
3	Reaction 1	Ligation of PCR and Plasmid	8 uL	100 ul, remaining	2	
4	Reaction 2	Ligation of Plasmid	8 uL	100 ul, remaining	2	
				Total number of plates	10	0

2. Check to be sure you have enough plates with appropriate antibiotic. If plates were stored at 4°C, warm at 37°C until needed.
3. Obtain DNA and thaw on ice if necessary.
4. Thaw appropriate number of competent cell tubes on ice (5 reactions per tube of competent cells)
5. Label sterile tubes as indicated in reaction table. Add indicated volume of indicated DNA on ice.
6. When competent cells are thawed (check by probing for frozen cells using a sterile pipette tip), gently pipette 100 uL of cells into each reaction tube directly onto DNA using aseptic technique.
7. Incubate cells on ice for 20 minutes. During incubation, find or set heat block to 42°C.
8. Place tubes with cells and DNA onto 42°C heatblock for 30 seconds (heat shock step).
9. After heat shock, place tubes back on ice until next step (don't keep them here too long).
10. Using aseptic technique, add 1 mL LB (no antibiotic) to each microfuge tube.
11. Using autoclave tape, tape microfuge tubes down in shaking incubator set to 37°C.
12. Allow cells to recover for 1 hour at 37°C, shaking. Place in a rack after shaking (NOT back on ice).
13. Using aseptic technique, plate indicated amount of cells on appropriate antibiotic plates, spreading until plates look dry. For "remaining" volume, spin tubes at max speed in benchtop centrifuge for 30 seconds. Remove 800 uL of media. Using 200 uL pipette, resuspend cells at bottom of tube and plate all the remaining culture.
14. Incubate plates at 37°C.



The above picture was taken on 12/10/18 and was taken by Dr. Kathryn Ramsey. It shows the expected results. The plates were placed in the fridge.

Monday, December 17, 2018

To Do:

1. ~~Start cultures from plates from the transformed chemically competent *E. coli* colonies.~~

Results and Data:

Tubes	Purpose	Plate Taken From
1-6	Culture	3 – reaction 1 (remaining)
7	(+) control	1 – positive control (remaining)
8	(-) control	4 – reaction 2 (remaining)

1. Add 45 mL of LB broth to a 50 mL conical.
2. Add 45 uL of Kanamycin to the conical.
3. Label 8 test tubes (6 for reaction 1, 1 from the positive control, and one from reaction 2 to be used as a negative control).

4. Pipette out the quantity of the conical and aliquot out 5 mL of solution Kanamycin broth into each of the tubes.
5. Inoculate each of the tubes with the corresponding plate.
6. Place in the shaking incubator overnight.

Tuesday, December 18, 2018

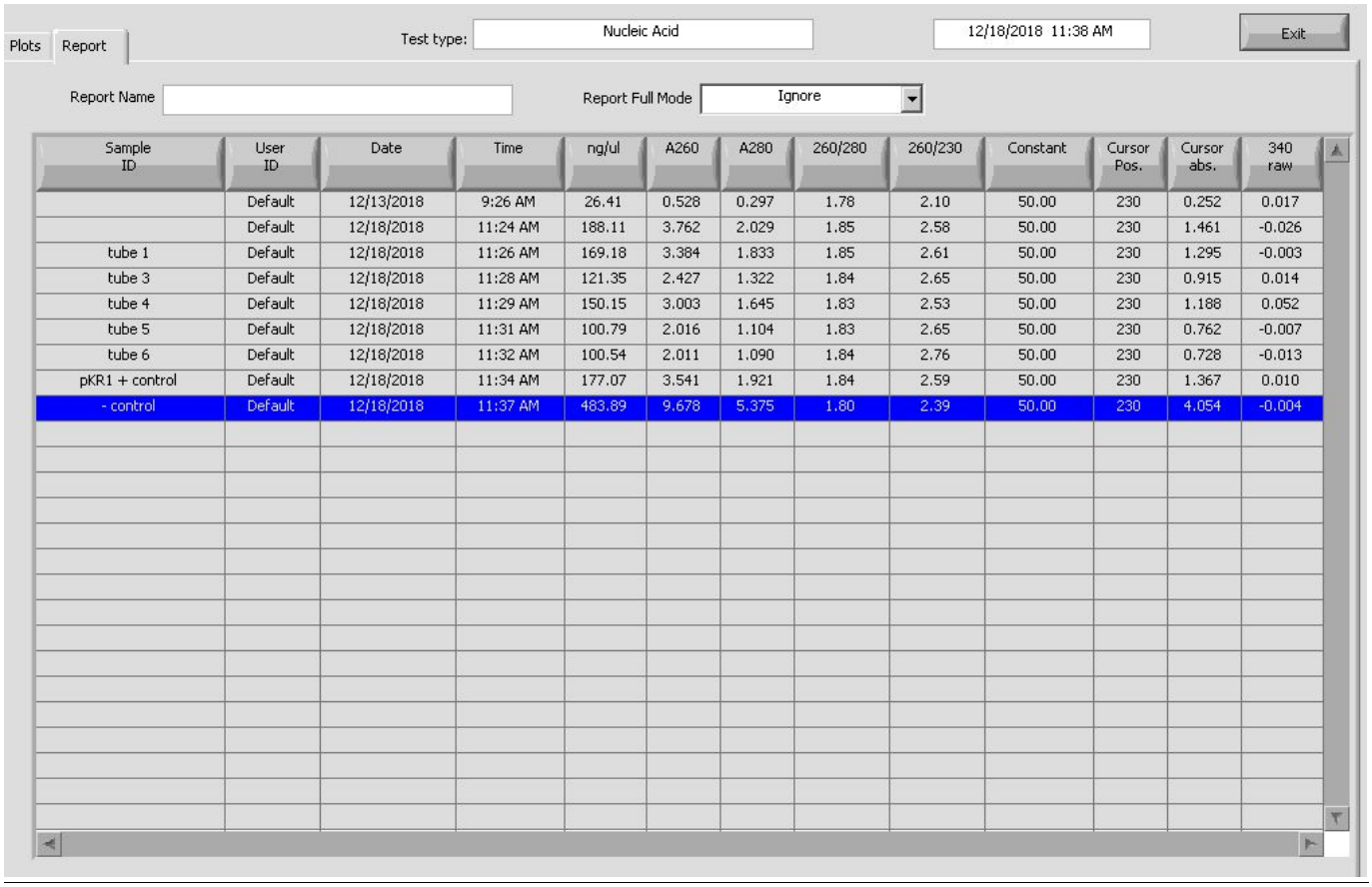
To Do:

1. ~~Miniprep the cultures of transformed chemically competent *E. coli* cells from 12/17/18.~~

Results and Data:

QIAprep Spin Miniprep Kit Protocol

1. Pellet 5 mL bacterial overnight culture by centrifuging at 10000 x g for 1 minute at room temperature (15-25 deg C). Pipette 1.5 mL culture into the tube, spin, discard the supernatant, and repeat until all 5 mL are gone (3x).
2. Resuspend pelleted bacterial cells in 250 uL Buffer P1 (stored at 4 deg C fridge) and transfer to a microcentrifuge tube
3. Add 250 uL 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 minutes. If using LyseBlue reagent, the solution will turn blue.
4. Add 350 uL Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times. If using LyseBlue, the solution will turn colorless.
5. Centrifuge for 10 minutes at 13,000 rpm (or max speed) in a tabletop microcentrifuge
6. Apply 800 uL supernatant from step 5 to the QIAprep 2.0 spin column by pipetting. Centrifuge for 30-60 seconds and discard the flow-through.
7. Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB. Centrifuge for 30-60 seconds and discard the flow through. Transfer the QIAprep 2.0 spin column to the collection tube.
8. Wash with 0.75 mL Buffer PE. Centrifuge for 30-60s.
9. Centrifuge for 3 minutes to remove residual wash buffer. Ethanol in DNA
10. Place the QIAprep 2.0 column in a clean 1.5 mL microcentrifuge tube. To elute DNA, add 0.1x 50 uL Buffer EB (10 mM TrisCl, pH 8.5) or water to the center of the QIAprep 2.0 spin column, let stand for 1 minute, and centrifuge for 1 minute.
11. Analyzing on a gel would show us how pure the chromosome is because supercoiled plasmid DNA moves faster.



Plots | Report

Test type: Nucleic Acid | 12/18/2018 11:38 AM | Exit

Report Name | Report Full Mode: Ignore

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
	Default	12/13/2018	9:26 AM	26.41	0.528	0.297	1.78	2.10	50.00	230	0.252	0.017
	Default	12/18/2018	11:24 AM	188.11	3.762	2.029	1.85	2.58	50.00	230	1.461	-0.026
tube 1	Default	12/18/2018	11:26 AM	169.18	3.384	1.833	1.85	2.61	50.00	230	1.295	-0.003
tube 3	Default	12/18/2018	11:28 AM	121.35	2.427	1.322	1.84	2.65	50.00	230	0.915	0.014
tube 4	Default	12/18/2018	11:29 AM	150.15	3.003	1.645	1.83	2.53	50.00	230	1.188	0.052
tube 5	Default	12/18/2018	11:31 AM	100.79	2.016	1.104	1.83	2.65	50.00	230	0.762	-0.007
tube 6	Default	12/18/2018	11:32 AM	100.54	2.011	1.090	1.84	2.76	50.00	230	0.728	-0.013
pKR1 + control	Default	12/18/2018	11:34 AM	177.07	3.541	1.921	1.84	2.59	50.00	230	1.367	0.010
- control	Default	12/18/2018	11:37 AM	483.89	9.678	5.375	1.80	2.39	50.00	230	4.054	-0.004

The above picture is from the Nanodrop Spectrophotometer. Note: the tubes go in descending order from blank to tubes 1 through 8. The sample labeled “tube 1” is actually mislabeled and should read tube 2. The first sample (top row) was a blank. Tubes 1-8 were placed in the Undergraduate Researchers box in the freezer.

Bibliography

Ramsey, K. M. and Dove, S. L. (2016) ‘ A response regulator promotes Francisella tularensis intramacrophage growth by repressing an anti-virulence factor ’, *Molecular Microbiology*. doi: 10.1111/mmi.13418.