# Table of Contents

# Introduction

## Thursday, September 20, 2018

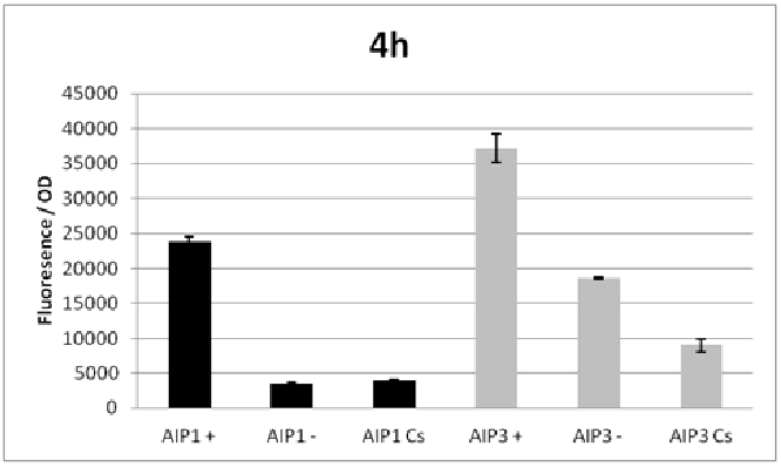
**To Do:**

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. Prep for LB Media

### LB Broth Protocol 500 mL

1. Weigh out and add into 1L Graduated cylinder/Flask

- 10g Agar
- 5g NaCl
- 5g Tryptone
- 2.5g Yeast extract

2. Add 500mL di$H_2O$ (Don't forget to add stir bar)

3. Stir solution in hot plate until solutes dissolve

4. Cover cylinder/flask with tinfoil and stick it with autoclave tape with date written out

5. Autoclave under Liq 30℃ cycle setting

6. Pour media onto plates using good microbiological technique

## Tuesday, October 23, 2018

**To Do:**

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

**Results and Data:**

### LB Broth Protocol 1000 mL

1. Weigh out 10g of tryptone into a 1L graduated cylinder/Flask
2. Add 10g NaCl and 5g yeast extract
3. Add ddiH2O (type 1) 1 liter
4. Mix until dissolved add stir bar
5. Aliquot out 3x200 mL 1L flasks and 4x75 mL in 100mL bottles
6. Autoclave on 30 C liquid cycle, with water in the bin

### Making Chemically Competent Cells Protocol

Inoculate overnight culture with a single colony in 3mL of LB plus appropriate antibiotic

Incubate culture in 37 °C overnight

Add 3mL of filter sterilized 1M MgCl2 to 200 mL of LB, also add appropriate antibiotic

Use 0.5 mL of overnight culture to inoculate the 200mL of LB

Incubate culture at 37 °C with aeration until OD600 of 0.5 is reacted, approximately 4 hrs for DH5$\alpha$ cells.

Transfer cells to a large sterile centrifuge bottle

Pellet cells by centrifugation at 4°C

Resuspend cell pellet in 60mL of cold solution A

Incubate suspension on ice for 20 min

Pellet cells by centrifugation again at 4 and resuspend in 12mL of cold solution A containing 15% glycerol

Aliquot competent cells in 0.5-1.0 mL volumes in sterile pre-cooled 1.5mL microcentrifuge tubes

Quick freeze cells using dry ice

Smaller scale:

Add 300µL of filter sterilized 1M MgCl2 to 20 mL of LB, also add appropriate antibiotic

Use 50µL of overnight culture to inoculate the 20mL of LB

Incubate culture at 37 °C with aeration until OD600 of 0.5 is reacted, approximately 3 hrs.

Transfer cells to falcon tube or 2mL eppi tubes

Pellet cells by centrifugation at 4°C

Resuspend cell pellet in 6mL of cold solution A

Incubate suspension on ice for 20 min

Pellet cells by centrifugation again at 4 and resuspend in 1.2mL of cold solution A containing 15% glycerol

Aliquot competent cells in 500-100 µL volumes in sterile pre-cooled 1.5mL microcentrifuge tubes

Store at -80(flash freeze optional)

Solution A:

Combine 10mL of 1$M$ $MnCl_2$, 50 mL 1$M$ $CaCl_2$, 200 mL 50 m$M$ 2-morpholino-ethanesulfonic acid (MES) pH 6.3, and 740 mL dd$H_2O$

Solution A %15 glycerol

10mL of 1.0 MnCl2

50mL 1.0M CaCl2

200mL MES pH 6.3

590 mL of ddiH2O

150mL of glycerol

Need 250mL of MES

MES= 213.25g/mol

**Wednesday, October 24, 2018**

**To Do:**

1. ~~Streak E. coli XL1 Blue on LB plates~~

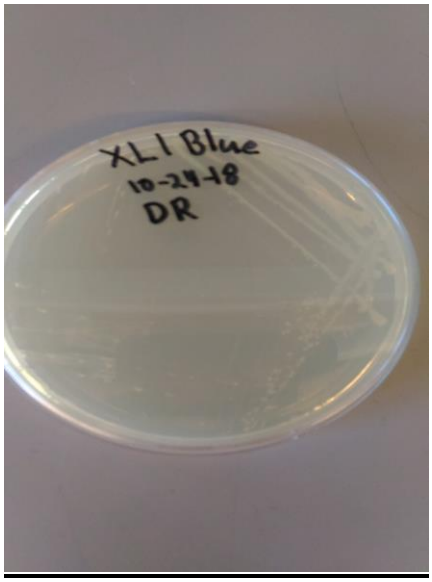
**Thursday, October 25, 2018**

**To Do:**

1. ~~Record results of E. coli XL1 Blue streak plate~~

**Results:**

Isolated colonies present




## Friday, October 26, 2018

**To Do:**

1. ~~Make 1L 50mM MES solution pH (6.3)~~
2. ~~Make 1L Solution A and 1L Solution A + 15% Glycerol~~

## Monday, October 29, 2018

**To Do:**

1. PCR of oligos positive, negative, and experimental controls (p822 For and p823 Rev) 304 bp region amplification region

   control primers: p780 and p781 (443 bp range)

### Standard PCR Protocol

1. Add dd$iH_2O$ to 1.5mL tube
2. Add KOD buffer
3. Add KOD
4. Mix by pipetting
5. Aliquot into PCR tubes tube#1 1.5µ of p822 and1.5µL of p823
6. Aliquot controls into tube 2 and tube 3

| | | | | |
|---|---|---|---|---|
| Total reaction volume | | 50 | | |
| Total number | | 3 | | |

| of reactions | | | | |
|---|---|---|---|---|
| | | | | **Factor** |
| **Component** | **Stock concentration** | **Final concentration** | **1 rxn volume** | 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 |

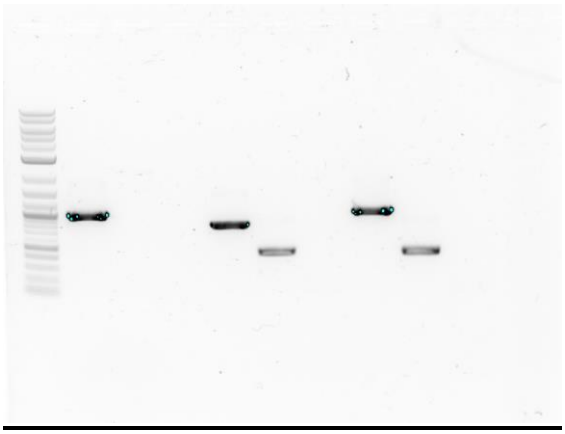
## Tuesday, October 30, 2018

**To Do:**

1. ~~**Run 1% Agarose gel with PCR products of 10/29/18**~~
2. ~~**Visualize gel**~~

| | | Expected Size |
|---|---|---|
| Lane 1 | Contents 2 Log NEB Ladder | Varies |
| Lane 2 | Contents CS#1 | 904 bp |
| Lane 3 | Contents CS#2 | 781bp |
| Lane 4 | Contents CS#3 | 0 bp |
| Lane 5 | Contents JC#1 | p824/p825 824bp |
| Lane 6 | Contents JC#2 | 443 bp |
| Lane 7 | Contents JC#3 | |
| Lane 8 | Contents KR#1 | |
| Lane 9 | Contents KR#2 | |
| Lane 10 | Contents KR#3 | |

## Results:



**Wednesday, October 31, 2018**

**To Do:**

1. ~~**Make O/N of XL1 Blue**~~
2. ~~**Restructure Lab Notebook**~~**.**

# November 2018

**Thursday, November 1, 2018**

## Making Chemically Competent E. Coli

1. Add 3 mL of sterile $MgCl_2$ to 200 mL LB in 1 L flask. Add antibiotic if appropriate.
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.~6 hours incubation time yielded a $OD_{600}$ of .55
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
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.

## Tuesday, November 6, 2018

**To Do:**

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

| Tube number | Purpose | DNA | Volume of DNA | Final volume to plate | Number of kanamycin-containing plates | Number of carbenicillin-containing plates |
|---|---|---|---|---|---|---|
| 1 | (+) control | pKL02 | 1 uL | 20 ul, 100 ul, remaining | 3 | 0 |
| 2 | (+) control | pKL80 | 1µL | 20 ul, 100 ul, remaining | 3 | 0 |
| 3 | (+) control | pF | 1 uL | 20 ul, 100 ul, remaining | 3 | 0 |
| 4 | (-) control | None | 0 | 20 ul, 100 ul, remaining | 3 | 0 |
| 5 | (+) control | pUC19 | 1 uL | 20 ul, 100 ul, remaining | 0 | 3 |
| 6 | (-) control | None | | 20 ul, 100 ul, remaining | 0 | 3 |
| | | | | **Total number of plates** | 12 | 6 |

**Reaction Table**

1. Check to be sure you have enough plates with appropriate antibiotic. If plates were stored at 4°C, warm at 37°C until needed. ( Blue Streak=Kanamycin Black Streak= Carbenicilin)
2. Obtain DNA and thaw on ice if necessary.
3. Thaw appropriate number of competent cell tubes on ice (5 reactions per tube of competent cells)
4. Label sterile tubes as indicated in reaction table. Add indicated volume of indicated DNA on ice.

5. 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.
6. Incubate cells on ice for 20 minutes. During incubation, find or set heat block to 42°C.
7. Place tubes with cells and DNA onto 42°C heat block for 30 seconds (heat shock step).
8. After heat shock, place tubes back on ice until next step (don't keep them here too long).
9. Using aseptic technique, add 1 mL LB (no antibiotic) to each microfuge tube.
10. Using autoclave tape, tape microfuge tubes down in shaking incubator set to 37°C.
11. Allow cells to recover for 1 hour at 37°C, shaking.
12. 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(Tube 4 & Tube 6 were not spun) . Remove 800 uL of media. Using 200 uL pipette, resuspend cells at bottom of tube and plate all the remaining culture. Then incubate @ 37 degrees O/N

## Wednesday, November 7, 2018

**To Do:**

1. ~~**Count colonies on spread plates**~~
2. ~~**Calculate Transformation Efficiencies**~~
3. ~~**Inoculate 5mL tube with colonies of the pKL02, pKL80, and pF constructs**~~

| Plasmid Construct | | | | | |
|---|---|---|---|---|---|
| **Volumes Aliquoted** | **pKL02** | **pKL80** | **pF** | **PUC19** | **None** |
| **20µL** | **7** | **39** | **7** | **0** | **0** |
| **100µL** | **80** | **290** | **122** | **8** | **0** |
| **Remaining ~800µL** | **TMTC** | **TMTC** | **TMTC** | **17** | **0** |
| **Transformation Efficiency** | **20µL=** | **20µL=** | **20µL=** | **20µL=0** | **20µL=0** |
| | **100µL=** | **100µL=** | **100µL=** | **100µL=1.76x10$^6$** | **100µL=0** |
| | **Remaining=** | **Remaining=** | **Remaining=** | **Remaining=6.12x10$^6$** | **Remaining=0** |

**Thursday, November 8, 2018**

**To Do:**

1. ~~**Miniprep XL1 Blue constructs**~~

**Wednesday, November 14, 2018**

## Guanine Quadruplexes

**To Do:**

1. ~~**Run LVS Genome Fasta file through G4 IPDB web tool**~~
2. ~~**Create Excel sheet of LVS G4 IPDB results**~~

**Results:**

Refer to 18.11.14 LVS Genome G4 Predictor v.2 (XLSX File) Date modified Dec.4.18 in Team Google Drive - KRamsey Lab – Dan Rosario

**https://drive.google.com/open?id=1ZMhzlF0yH9DvGdUipqSWmEm-K3xd5AyJ**

**Thursday, November 15, 2018**

**To Do:**

1. ~~**Create BED Files of LVS G4 IPDB files**~~

**Results:** Time in laboratory was used on creating Bed File for Antisense file.

Refer to 181115_LVSG4_Predictor_AntiSenseStrand.bed in Team Google Drive – Kramsey Lab – Dan Rosario

**https://drive.google.com/open?id=1ZkzjpjALFxR_CoJpauRn1YPJ1hSxQqn3**

**Week of November 18, 2018 - November 24, 2018**

**Thanksgiving Grace Period - NO LAB**

**Week of November 25, 2018 - December 1, 2018**

**LAB Cancellation**

**Reason: Dr. Ramsey's Sick Days**

# December 2018

## Tuesday, December 4, 2018

**To Do:**

1. **Practice and become familiar with unix/linux command terminus Task: Use BED tools on created LVS promoter, G4 Prediction antisense BED files**
2. **Create worklist/work document of command prompts**
3. **Enlist help to install linux on personal laptop**

### Results:

**18.12.04**

I tried to use the help command:

143:~ kathrynramsey$ bedtools --help

```
bedtools is a powerful toolset for genome arithmetic.


Version:   v2.27.1
About:     developed in the quinlanlab.org and by many contributors worldwide.
Docs:      http://bedtools.readthedocs.io/
Code:      https://github.com/arq5x/bedtools2
Mail:      https://groups.google.com/forum/#!forum/bedtools-discuss


Usage:     bedtools <subcommand> [options]


The bedtools sub-commands include:


[ Genome arithmetic ]
    intersect     Find overlapping intervals in various ways.
    window        Find overlapping intervals within a window around an interval.
    closest       Find the closest, potentially non-overlapping interval.
    coverage      Compute the coverage over defined intervals.
    map           Apply a function to a column for each overlapping interval.
    genomecov     Compute the coverage over an entire genome.
    merge         Combine overlapping/nearby intervals into a single interval.
    cluster       Cluster (but don't merge) overlapping/nearby intervals.
    complement    Extract intervals _not_ represented by an interval file.
    shift         Adjust the position of intervals.
    subtract      Remove intervals based on overlaps b/w two files.
    slop          Adjust the size of intervals.
    flank         Create new intervals from the flanks of existing intervals.
    sort          Order the intervals in a file.
    random        Generate random intervals in a genome.
    shuffle       Randomly redistrubute intervals in a genome.
    sample        Sample random records from file using reservoir sampling.
    spacing       Report the gap lengths between intervals in a file.
    annotate      Annotate coverage of features from multiple files.


[ Multi-way file comparisons ]
    multiinter    Identifies common intervals among multiple interval files.
    unionbedg     Combines coverage intervals from multiple BEDGRAPH files.
```

I tried to use the Bedtools intersect command

bedtools intersect -wb -a Promoters.bed -b 181115_LVSG4_Predictor_AntiSenseStrand.bed.txt > 181204_Promoter_Gpredict_antisense1.bed

bedtools intersect -wb -a Promoters.bed -b 181115_LVSG4_Predictor_AntiSenseStrand.bed.txt > 181204_Promoter_Gpredict_Sense1.bed

https://drive.google.com/open?id=1axV7-DX-Szct3t59DtHNF3kDf02Dqvc5xl3wQ0Md3Vs

## Wednesday, December 5, 2018

**To Do:**

1. **Compare Promoter Sense and Antisense G4 prediction files for sequence overlaps**

**Results:**

**18.12.05**

Cat 181204_Promoter_GPredict_Sense1.bed | head

```
NC_007880  10945  10964  NC_007880  10945  10964  100
NC_007880  19346  19371  NC_007880  19346  19371  90
NC_007880  35824  35852  NC_007880  35824  35852  100
NC_007880  39598  39619  NC_007880  39598  39619  100
NC_007880  39749  39775  NC_007880  39749  39775  90
NC_007880  59174  59197  NC_007880  59174  59197  90
NC_007880  61646  61674  NC_007880  61646  61674  110
NC_007880  67877  67904  NC_007880  67877  67904  110
NC_007880  79978  80004  NC_007880  79978  80004  100
NC_007880  81311  81339  NC_007880  81311  81339  80
```

cat 181204_Promoter_Gpredict_Sense1.bed | wc -l

169

cat 181204_Promoter_Gpredict_antiSense1.bed | wc -l

Dan Rosario kathrynramsey$ cat 181204_Promoter_Gpredict_antiSense1.bed | wc -l

169

The 181204_Promoter_Gpredict_antiSense1.bed file and the 181204_Promoter_Gpredict_Sense1.bed file yielded the same output of 169 overlaps. The identical results indicate that the bed intersect command prompts were mislabeled.

bedtools intersect -wb -a Promoters.bed -b 181115_LVSG4_Predictor_AntiSenseStrand.bed.txt > 181204_Promoter_Gpredict_antisense1.bed

bedtools intersect -wb -a Promoters.bed -b 181115_LVSG4_Predictor_AntiSenseStrand.bed.txt > 181204_Promoter_Gpredict_Sense1.bed

---

Yield of same output of 169 overlaps for both strands suggest that the bed intersect command prompts were mislabeled.

Correction

bedtools intersect -wb -a Promoters.bed -b 181204_LVSG4_Predictor_SenseStrand.bed > 181204_Promoter_Gpredict_Sense1.bed

**18/12/06**

Command line bedtools
Output: Bedtools feature data

daniel@daniel-Virtualbox:~$ ls
change directory (up one directory)

daniel@daniel-Virtualbox:~$ cd ..
daniel@daniel-Virtualbox:~$ ls

daniel@daniel-Virtualbox:~$ cd ..

daniel@daniel-Virtualbox:~$ ls

daniel@daniel-Virtualbox:~$ cd ..

daniel@daniel-Virtualbox:~$ cd home/daniel/
daniel@daniel-Virtualbox:~$ ls

Ideally it would be beneficial to copy, paste, and or cut terminal command line inputs from the terminal itself to the main computer outside Virtualbox(Ubuntu) for the purpose of documentation. After many attempts of trying to copy/paste between the host machine and the virtualbox machine I decided to try copy pasting inside of the virtualbox

1. I logged into my google drive to access this note file using the Firefox web browser within Ubuntu
2. Copied the command lines that allowed me to change directory
3. I then returned to this note and was able to ctrl v the commands and their output. Commands and their outputs are listed below

Out of ease and convenience I can perform all computational work within the Ubuntu machine in Virtualbox and document it.

```
daniel@daniel-VirtualBox:~$ ls
181204_Promoter_Gpredict_antisense1.bed  Downloads       Pictures  Videos
Desktop                                  examples.desktop  Public
Documents                                Music             Templates
daniel@daniel-VirtualBox:~$ cd ..
daniel@daniel-VirtualBox:/home$ ls
daniel
daniel@daniel-VirtualBox:/home$ cd ..
daniel@daniel-VirtualBox:/$ ls
bin   dev  initrd.img      lib64       mnt  root  snap  sys  var
boot  etc  initrd.img.old  lost+found  opt  run   srv   tmp  vmlinuz
```

```
cdrom  home  lib         media     proc  sbin  swapfile  usr  vmlinuz.old
daniel@daniel-VirtualBox:/$ cd ..
daniel@daniel-VirtualBox:/$ ls
bin   dev  initrd.img     lib64     mnt   root  snap     sys  var
boot  etc  initrd.img.old  lost+found  opt   run   srv      tmp  vmlinuz
cdrom  home  lib         media     proc  sbin  swapfile  usr  vmlinuz.old
daniel@daniel-VirtualBox:/$ cd ..
daniel@daniel-VirtualBox:/$ cd home/
daniel@daniel-VirtualBox:/home$ cd daniel/
```

**18.12.07**

Changing terminal directories to “Documents”. “Documents” contains the working bed files. The transcript from the terminal below shows my attempts at changing the directories. http://www.linuxandubuntu.com/home/10-basic-linux-commands-that-every-linux-newbies-should-remember
The web link above is an indispensable resources for teaching basic linux commands. My rationale for using for “mkdir” which stands for make directory was ultimately not necessary since “Documents” was already a directory with the bed files and the ramseylabwork folder within it.; therefore ignore this part of the transcript altogether. cd was utilized to chnge to documents but “documents” is the incorrect directory. “cd Documents” proved to be the correct command and was verified by “ls”

```
daniel@daniel-VirtualBox:~$ mkdir ramseylabwork
daniel@daniel-VirtualBox:~$ mkdir
mkdir: missing operand
Try 'mkdir --help' for more information.
daniel@daniel-VirtualBox:~$ mkdir help
daniel@daniel-VirtualBox:~$ mkdir --help
Usage: mkdir [OPTION]... DIRECTORY...
Create the DIRECTORY(ies), if they do not already exist.

Mandatory arguments to long options are mandatory for short options too.
  -m, --mode=MODE   set file mode (as in chmod), not a=rwx - umask
  -p, --parents     no error if existing, make parent directories as needed
  -v, --verbose     print a message for each created directory
  -Z                set SELinux security context of each created directory
                     to the default type
      --context[=CTX]  like -Z, or if CTX is specified then set the SELinux
                     or SMACK security context to CTX
      --help     display this help and exit
      --version  output version information and exit

GNU coreutils online help: <http://www.gnu.org/software/coreutils/>
Full documentation at: <http://www.gnu.org/software/coreutils/mkdir>
```

```
or available locally via: info '(coreutils) mkdir invocation'
daniel@daniel-VirtualBox:~$ mkdir documents
daniel@daniel-VirtualBox:~$ sudo mkdir
[sudo] password for daniel:
mkdir: missing operand
Try 'mkdir --help' for more information.
daniel@daniel-VirtualBox:~$ sudo mkdir
mkdir: missing operand
Try 'mkdir --help' for more information.
daniel@daniel-VirtualBox:~$ mkdir documents
mkdir: cannot create directory ‘documents’: File exists
daniel@daniel-VirtualBox:~$ mkdir ramseylabwork
mkdir: cannot create directory ‘ramseylabwork’: File exists
daniel@daniel-VirtualBox:~$ cd documents
daniel@daniel-VirtualBox:~/documents$ ls
daniel@daniel-VirtualBox:~/documents$ cd Documents
bash: cd: Documents: No such file or directory
daniel@daniel-VirtualBox:~/documents$ cd ..
daniel@daniel-VirtualBox:~$ ls
181204_Promoter_Gpredict_antisense1.bed  examples.desktop  ramseylabwork
Desktop                                  help             Templates
documents                                Music            Videos
Documents                                Pictures
Downloads                                Public
daniel@daniel-VirtualBox:~$ cd Documents/
daniel@daniel-VirtualBox:~/Documents$ ls
181115_LVSG4_Predictor_AntiSenseStrand.bed.txt  desktop.ini
181204_LVSG4_Predictor_SenseStrand.bed.txt      Promoters.bed
181204_Promoter_Gpredict_antisense1.bed         ramseylabwork
181204_Promoter_Gpredict_Sense1.bed
daniel@daniel-VirtualBox:~/Documents$
```

Correction from 18.12.05

bedtools intersect -wb -a Promoters.bed -b 181204_LVSG4_Predictor_SenseStrand.bed > 181204_Promoter_Gpredict_Sense1.bed

Preliminary Result: Could not open Exiting

I noticed the typo Strange instead of Strand for the file before the carrot. Success was obtained by correcting the typo and including the txt. file extension after the bed extension.

```
daniel@daniel-VirtualBox:~/Documents$ bedtools intersect -wb -a Promoters.bed -b
181204_LVSG4_Predictor_SenseStrange.bed> 181204_Promoter_Gpredict_Sense1.bed
Error: Unable to open file 181204_LVSG4_Predictor_SenseStrange.bed. Exiting.
daniel@daniel-VirtualBox:~/Documents$ bedtools intersect -wb -a Promoters.bed -b
181204_LVSG4_Predictor_SenseStrand.bed> 181204_Promoter_Gpredict_Sense1.bed
```

Error: Unable to open file 181204_LVSG4_Predictor_SenseStrand.bed. Exiting.
daniel@daniel-VirtualBox:~/Documents$ bedtools intersect -wb -a Promoters.bed -b 181204_LVSG4_Predictor_SenseStrand.bed.txt 181204_Promoter_Gpredict_Sense1.bed

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| NC_007880 | 8212 | 8239 | 1 | NC_007880 | 8212 | 8239 | 120 |
| NC_007880 | 8259 | 8269 | 1 | NC_007880 | 8259 | 8269 | 80 |
| NC_007880 | 25495 | 25520 | 1 | NC_007880 | 25495 | 25520 | 90 |
| NC_007880 | 66340 | 66368 | 1 | NC_007880 | 66340 | 66368 | 110 |
| NC_007880 | 78869 | 78888 | 1 | NC_007880 | 78869 | 78888 | 90 |
| NC_007880 | 81216 | 81244 | 1 | NC_007880 | 81216 | 81244 | 110 |
| NC_007880 | 124548 | 124574 | 1 | NC_007880 | 124548 | 124574 | 130 |
| NC_007880 | 148724 | 148745 | 1 | NC_007880 | 148724 | 148745 | 120 |
| NC_007880 | 158523 | 158551 | 1 | NC_007880 | 158523 | 158551 | 120 |
| NC_007880 | 178059 | 178082 | 1 | NC_007880 | 178059 | 178082 | 100 |
| NC_007880 | 181637 | 181658 | 1 | NC_007880 | 181637 | 181658 | 100 |
| NC_007880 | 190574 | 190600 | 1 | NC_007880 | 190574 | 190600 | 90 |
| NC_007880 | 192525 | 192545 | 1 | NC_007880 | 192525 | 192545 | 100 |
| NC_007880 | 213661 | 213686 | 1 | NC_007880 | 213661 | 213686 | 110 |
| NC_007880 | 213945 | 213967 | 1 | NC_007880 | 213945 | 213969 | 90 |
| NC_007880 | 222996 | 223019 | 1 | NC_007880 | 222996 | 223019 | 80 |
| NC_007880 | 223338 | 223365 | 1 | NC_007880 | 223338 | 223365 | 90 |
| NC_007880 | 225226 | 225254 | 1 | NC_007880 | 225226 | 225254 | 100 |
| NC_007880 | 268117 | 268146 | 1 | NC_007880 | 268117 | 268146 | 120 |
| NC_007880 | 327029 | 327042 | 1 | NC_007880 | 327029 | 327042 | 90 |
| NC_007880 | 327770 | 327794 | 1 | NC_007880 | 327770 | 327794 | 130 |
| NC_007880 | 336032 | 336060 | 1 | NC_007880 | 336032 | 336060 | 90 |
| NC_007880 | 338822 | 338849 | 1 | NC_007880 | 338822 | 338849 | 130 |
| NC_007880 | 344375 | 344391 | 1 | NC_007880 | 344375 | 344391 | 110 |
| NC_007880 | 344055 | 344082 | 1 | NC_007880 | 344055 | 344082 | 100 |
| NC_007880 | 352392 | 352411 | 1 | NC_007880 | 352392 | 352411 | 90 |
| NC_007880 | 373707 | 373736 | 1 | NC_007880 | 373707 | 373736 | 110 |
| NC_007880 | 404281 | 404307 | 1 | NC_007880 | 404281 | 404307 | 90 |
| NC_007880 | 405453 | 405475 | 1 | NC_007880 | 405453 | 405475 | 120 |
| NC_007880 | 406551 | 406564 | 1 | NC_007880 | 406551 | 406564 | 80 |
| NC_007880 | 414280 | 414297 | 1 | NC_007880 | 414280 | 414297 | 110 |
| NC_007880 | 415569 | 415595 | 1 | NC_007880 | 415569 | 415595 | 130 |
| NC_007880 | 422922 | 422951 | 1 | NC_007880 | 422922 | 422951 | 110 |
| NC_007880 | 435559 | 435580 | 1 | NC_007880 | 435559 | 435580 | 100 |
| NC_007880 | 502373 | 502402 | 1 | NC_007880 | 502373 | 502402 | 120 |
| NC_007880 | 521242 | 521270 | 1 | NC_007880 | 521242 | 521270 | 120 |
| NC_007880 | 537122 | 537137 | 1 | NC_007880 | 537114 | 537137 | 110 |
| NC_007880 | 555874 | 555901 | 1 | NC_007880 | 555874 | 555901 | 140 |
| NC_007880 | 582517 | 582530 | 1 | NC_007880 | 582517 | 582537 | 90 |
| NC_007880 | 584676 | 584704 | 1 | NC_007880 | 584676 | 584704 | 90 |
| NC_007880 | 606167 | 606187 | 1 | NC_007880 | 606167 | 606187 | 90 |
| NC_007880 | 636615 | 636640 | 1 | NC_007880 | 636615 | 636640 | 100 |
| NC_007880 | 801864 | 801876 | 1 | NC_007880 | 801864 | 801893 | 110 |
| NC_007880 | 805457 | 805479 | 1 | NC_007880 | 805457 | 805479 | 100 |
| NC_007880 | 824484 | 824499 | 1 | NC_007880 | 824484 | 824513 | 150 |
| NC_007880 | 826501 | 826520 | 1 | NC_007880 | 826501 | 826520 | 90 |
| NC_007880 | 831838 | 831864 | 1 | NC_007880 | 831838 | 831864 | 100 |
| NC_007880 | 845448 | 845464 | 1 | NC_007880 | 845442 | 845464 | 100 |
| NC_007880 | 873852 | 873877 | 1 | NC_007880 | 873852 | 873877 | 100 |
| NC_007880 | 874836 | 874861 | 1 | NC_007880 | 874836 | 874861 | 100 |
| NC_007880 | 880022 | 880049 | 1 | NC_007880 | 880022 | 880049 | 110 |
| NC_007880 | 918322 | 918350 | 1 | NC_007880 | 918322 | 918350 | 100 |
| NC_007880 | 935709 | 935721 | 1 | NC_007880 | 935709 | 935721 | 90 |
| NC_007880 | 971339 | 971356 | 1 | NC_007880 | 971339 | 971356 | 110 |
| NC_007880 | 975531 | 975560 | 1 | NC_007880 | 975531 | 975560 | 90 |

```
NC_007880  1015545  1015572  1  NC_007880  1015545  1015572  130
NC_007880  1028699  1028718  1  NC_007880  1028699  1028718  90
NC_007880  1029362  1029387  1  NC_007880  1029362  1029387  90
NC_007880  1055003  1055030  1  NC_007880  1055003  1055030  100
NC_007880  1086238  1086261  1  NC_007880  1086238  1086261  90
NC_007880  1119187  1119213  1  NC_007880  1119187  1119213  130
NC_007880  1130499  1130521  1  NC_007880  1130499  1130521  80
NC_007880  1144903  1144929  1  NC_007880  1144903  1144929  110
NC_007880  1172400  1172417  1  NC_007880  1172394  1172417  100
NC_007880  1184210  1184223  1  NC_007880  1184210  1184223  80
NC_007880  1184234  1184245  1  NC_007880  1184234  1184247  80
NC_007880  1199005  1199022  1  NC_007880  1199005  1199022  90
NC_007880  1201251  1201278  1  NC_007880  1201251  1201278  100
NC_007880  1229592  1229621  1  NC_007880  1229592  1229621  110
NC_007880  1232890  1232916  1  NC_007880  1232890  1232916  120
NC_007880  1232920  1232947  1  NC_007880  1232920  1232947  100
NC_007880  1282355  1282380  1  NC_007880  1282355  1282380  100
NC_007880  1293099  1293126  1  NC_007880  1293099  1293126  130
NC_007880  1323215  1323243  1  NC_007880  1323215  1323243  110
NC_007880  1338877  1338890  1  NC_007880  1338877  1338890  80
NC_007880  1338953  1338966  1  NC_007880  1338953  1338979  100
NC_007880  1340052  1340078  1  NC_007880  1340052  1340078  120
NC_007880  1349887  1349914  1  NC_007880  1349887  1349914  90
NC_007880  1420655  1420671  1  NC_007880  1420655  1420671  90
NC_007880  1437004  1437010  1  NC_007880  1437004  1437017  80
NC_007880  1463183  1463198  1  NC_007880  1463183  1463198  110
NC_007880  1490564  1490591  1  NC_007880  1490564  1490592  110
NC_007880  1522503  1522528  1  NC_007880  1522503  1522528  110
NC_007880  1572130  1572143  1  NC_007880  1572130  1572143  90
NC_007880  1648067  1648093  1  NC_007880  1648067  1648093  120
NC_007880  1648528  1648556  1  NC_007880  1648528  1648556  110
NC_007880  1683742  1683762  1  NC_007880  1683742  1683762  90
NC_007880  1685007  1685021  1  NC_007880  1685007  1685021  90
NC_007880  1686510  1686524  1  NC_007880  1686510  1686524  100
NC_007880  1701179  1701203  1  NC_007880  1701179  1701203  100
NC_007880  1746188  1746202  1  NC_007880  1746188  1746202  100
NC_007880  1847034  1847063  1  NC_007880  1847034  1847063  90
NC_007880  1861800  1861822  1  NC_007880  1861800  1861822  120
NC_007880  1881271  1881300  1  NC_007880  1881271  1881300  90
NC_007880  1881725  1881750  1  NC_007880  1881725  1881750  80
NC_007880  1884734  1884757  1  NC_007880  1884734  1884757  110
NC_007880  1894070  1894084  1  NC_007880  1894070  1894084  90
```

daniel@daniel-VirtualBox:~/Documents$ bedtools intersect -wb -a Promoters.bed -b 181204_LVSG4_Predictor_SenseStrand.bed.txt > 181204_Promoter_Gpredict_Sense1.bed

## Viewing the header of the Promoter Gpredict_Sense file (first 10 lines)

cat 181204_Promoter_Gpredict_Sense1.bed | head

```
NC_007880  8212  8239  NC_007880  8212  8239  120
NC_007880  8259  8269  NC_007880  8259  8269  80
NC_007880  25495  25520  NC_007880  25495  25520  90
NC_007880  66340  66368  NC_007880  66340  66368  110
NC_007880  78869  78888  NC_007880  78869  78888  90
```

```
NC_007880   81216   81244   NC_007880   81216   81244   110
NC_007880   124548   124574   NC_007880   124548   124574   130
NC_007880   148724   148745   NC_007880   148724   148745   120
NC_007880   158523   158551   NC_007880   158523   158551   120
NC_007880   178059   178082   NC_007880   178059   178082   100
```

This was done to prepare to use the wc -l command

daniel@daniel-VirtualBox:~/Documents$ cat 181204_Promoter_Gpredict_Sense1.bed | wc -l
97

The significance of 97 is that there are purported to be 97 overlaps between the predicted Sense1 bed file and the reference Promoter bed file that contains experimentally determined promoters.

## Original G4 predictions on LVS chromosome

daniel@daniel-VirtualBox:~/Documents$ cat 181204_LVSG4_Predictor_SenseStrand.bed.txt | wc -l
1293
daniel@daniel-VirtualBox:~/Documents$ cat 181115_LVSG4_Predictor_AntiSenseStrand.bed.txt | wc -l1103
1103

| | Sense | Antisense | Total of both Strands |
|---|---|---|---|
| Overlap with Promoter file Prediction | 97 | 169 | 266 |
| # of Putative G4s | 1,293 | 1,103 | 2,396 |
| Percentage | 0.07% | 0.15% | 0.11% |

# January 2019

## Arbitrary PCR and Transposon Mapping

### Monday, January 28, 2019

**To Do:**

1. ~~**Familiarize oneself with Arbitrary PCR**~~
2. **Create AR - PCR Protocol**

### Wednesday, January 30, 2019

**To Do:**

1. ~~**Create AR-PCR Protocol**~~
2. ~~**Create AR-PCR Worksheet**~~

**Results and Data:**

Delayed run of Arbitrary PCR since designed primers have yet to arrive.

| | | | | | | |
|---|---|---|---|---|---|---|
| **PCR #1** | | | | | | |
| **Component** | **Stock Concentration** | | **Final Concentration** | | **1 Rxn Volume (µL)** | Factor |
| **OneTaq Rxn Buffer** | 5X | | **1X** | | **5** | 25 |
| **Template** | 500ng | | **160ng/µL** | | **3.13** | indiv |
| **dNTPs** | **10 mM** | | **0.2mM** | | **0.5** | 2.5 |
| | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **ddiH2O** | **-** | | **-** | | **14.245** | 71.225 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **OneTaq Polymerase** | **0.25µL** | | **1.25 units/50µL** | | **0.125** | 0.625 |
| **KROL87 Arb1 or KROL 89 Arb 6** | **100µM** | | **4.0µM** | | **1** | 5 |
| **Internal Specific Primer: KROL90 Tn_Mar1** | **10µM** | | **0.4µM** | | **1** | 5 |
| **Total volume** | | | | | **25** | |

**Run PCR samples through thermocycler program for reaction 1.**

Initial Denaturation: 5 min. 95°C
5-6X:

- Denature 30 sec. 94°C
- Anneal 30 sec. 30°C
- Extend 60 sec. 72°C

30X:

- Denature 30 sec. 94° C
- Anneal

30 sec.
45-55° C

- Extend
  120 sec.
  72°C

Final Extension:

- Extend 5 min.
  72°C

| | | | | | | |
|---|---|---|---|---|---|---|
| **PCR #2** | | | | | | |
| **Component** | **Stock Concentration** | | **Final Concentration** | | **1 Rxn Volume (µL)** | |
| **PCR 1 Amplicon** | _ | | _ | | **0.75** | 3.75 |
| **dNTPs** | **10 mM** | | **0.2mM** | | **0.5** | 2.5 |
| | | | | | | |
| **OneTaq Rxn Buffer** | **5X** | | **1X** | | **5** | 25 |
| **KROL88 Arb2** | **100µM** | | **4.0µM** | | **1** | 5 |
| **External Specific Primer: KROL91 Tn_Mar2** | **10µM** | | **0.4µM** | | **1** | 5 |
| **OneTaq Polymerase** | | | **1.25 units/50µL** | | **0.125** | 0.625 |
| | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **ddiH2O** | _ | | _ | | **16.625** | 83.125 |
| **Total volume** | | | | | **25** | 125 |

Run ArbPCR reaction amplicons through Thermocycler Program for ARBPCR reaction 2.

PCR Rxn #2

30X:

- Denature: 30 sec. 94°C
- Anneal: 30 sec. 45-55°C
- Extend: 60 sec. 72°C

Final Extension:

- 5 min. 72°C

# February 2019

## Monday, February 4, 2019

**To Do:**

1. ~~**Run Arbitrary PCR of 19/1/30**~~

AP-PCR reactions 1 and 2 done with Arb1-Arb2 pairing subjected to 55°C annealing temperature during both reactions. No mixing of master mix components.

## Wednesday, February 6, 2019

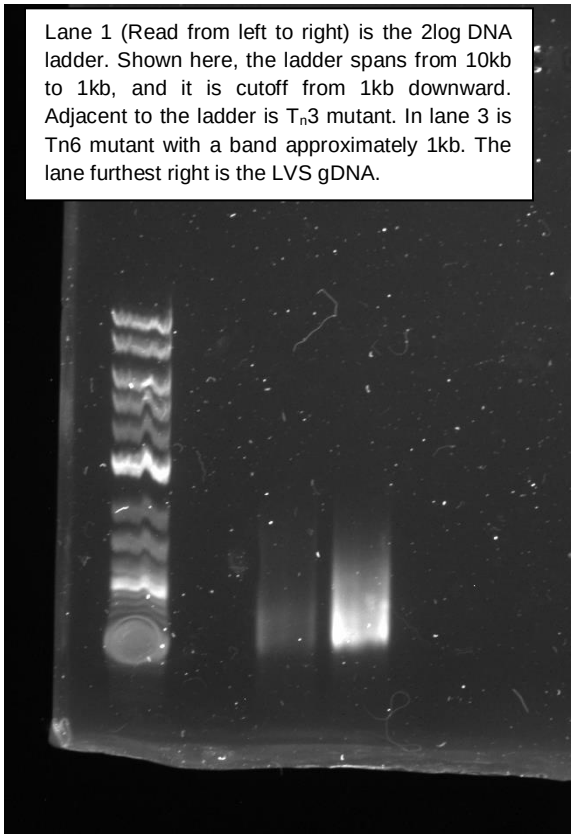
**To Do:**

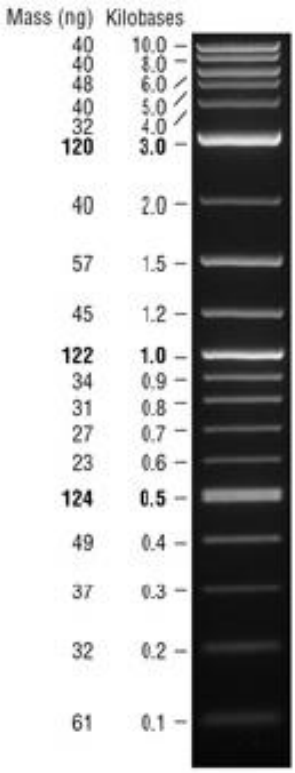
1. **Gel run Arbitrary Amplicons of 19.02.04**

**Ran a 1% agarose gel with 1X TAE buffer.**

| Well | Volume(uL) | Contents |
|---|---|---|
| 1 | 10 | $Log_2$ DNA Ladder |
| 2 | 30 | PCR2 Tn3 Mutant |
| 3 | 30 | PCR2 Tn6 Mutant |
| 4 | 30 | PCR2 LVS |

PCR samples volume samples were loaded as 25uL. 5uL of 6X orange G dye were added to each DNA template sample totaling a volume of 30uL. Not shown is the negative control




Results

Gel image does not depict any size bands less than 1kb, everything below 1kb has run off the gel. We are uncertain of the presence of any bands less than 1kb, and therefore have decided to retry PCR#2 from arbitrary PCR#1 stock. Currently the gel image shows bands present in the LVS lane as well as the PCR#2 Tn6 lane. LVS was our negative control of expected result of no bands. The presence of bands in the LVS lane indicates that amplification was arbitrary on the LVS gDNA. Retry scheduled for Friday February 8, 2019.

## Friday, February 8, 2019

**To Do:**

1. Retry PCR#2 of 2/8/19
2. Retry Gel run of PCR #2 amplicons

Data:

| Well | Volume(uL) | Contents |
|---|---|---|
| 1 | 10 | $Log_2$ DNA Ladder |
| 2 | 30 | PCR2 Tn3 Mutant |
| 3 | 30 | PCR2 Tn6 Mutant |

| 4 | 30 | PCR2 LVS |
|---|---|---|

**PCR #2**

| Component | Stock Concentration | Final Concentration | 1 Rxn Volume (µL) | |
|---|---|---|---|---|
| **PCR 1 Amplicon** | _ | _ | **0.75** | 3.75 |
| **dNTPs** | **10 mM** | **0.2mM** | **0.5** | 2.5 |
| **OneTaq Rxn Buffer** | **5X** | **1X** | **5** | 25 |
| **KROL88 Arb2** | **100µM** | **4.0µM** | **1** | 5 |
| **External Specific Primer: KROL91 Tn_Mar2** | **10µM** | **0.4µM** | **1** | 5 |
| **OneTaq Polymerase** | | **1.25 units/50µL** | **0.125** | 0.625 |
| **ddiH2O** | _ | _ | **16.625** | 83.125 |
| **Total volume** | | | **25** | 125 |

Run ArbPCR reaction amplicons through Thermocycler Program for ARBPCR reaction 2.

PCR Rxn #2

30X:

- Denature:
  30 sec.

94°C
- Anneal: 30 sec. 45-55°C
- Extend: 60 sec. 72°C

Final Extension:
- 5 min. 72°C

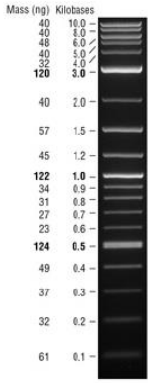
This 2°Rxn of Arb1-Arb2 pairing was done at 55°C annealing temperature with no mixing of master mix components.

110mL of agarose in 1X TAE was poured into the gel casting tray and 6µL of SYBR Safe was added to the casting tray. A comb was used to help diffuse the SYBER Safe in the gel. I waited 25 minutes for the gel to solidify before loading samples and applying current. 10µL of 2log DNA ladder was added to lane 1 of the gel and 5µL of Orange G loading dye was added to each 25µL sample. Applied current was set for 90 mV until the gel front reached the midpoint of the gel. Successive runs from halfway down the gel were conducted at 90mV and 100-103mV with varied times. The gel was visualized using the gel dock reader and image lab a total of five times. Several images were taken but only four were saved. The saved images are the presented images below and are representative of different time points of the run.

Thoughts:

In comparing the gel images of this rerun through the experiment with the image of February 6, 2019 there is a striking difference in appearance and band presentation. Immediately clear are the bands in the No Template lane which suggest contamination. The image of February 6, 2019 shows bands with a lighting bolt appearance whereas the bands in the rerun gel are actual bands but much closer together, making size differential difficult but not impossible. When I was visualizing the gel using the gel dock reader and image lab I noticed that the gel was gliding along the surface of the dock platform when I was trying to position the gel within UV light focus. Gels are

like sponges especially when saturated with TAE buffer. Using Kim wipes I tried to soak up as much of the residual




TAE I could, to then take the picture.

Results:

**Contamination in the No template lane voids all other results. Another rerun of this experiment is deemed appropriate.**

Lanes from left to right are: 2log ladder, Tn3 mutant, Tn6 Mutant, LVS gDNA, and No Template

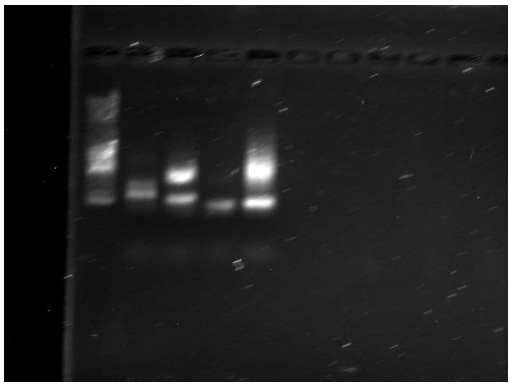


Image saved 08/02/19 1:22 PM

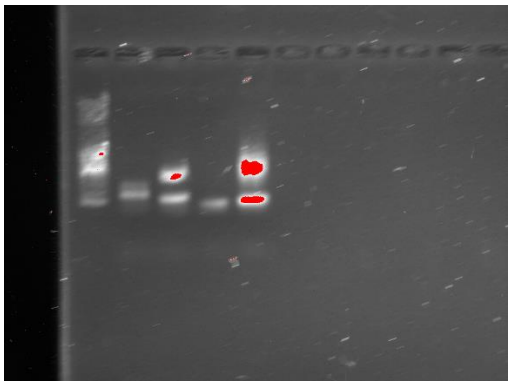
← The 2log DNA ladder in the image spans from 10kb to 0.5kb.

The Tn3 mutant lane shows two bands, one is of size 0.6kb and the other I think is 0.7kb.

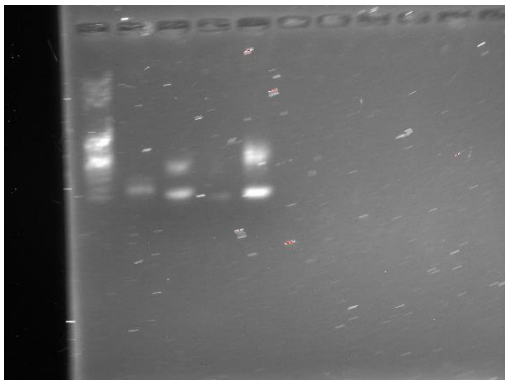
Looking at theTn6 mutant lane there is an intense band at the 0.8kb mark and a band matching up to 0.5 kb.

The LVS gDNA lane presents with one band that is less than 0.5kb. The 2log ladder in the image does not show any band less than 0.5kb but considering the proximity that the sole band in the LVS lane is to 0.5kb I consider it to be 0.4kb.

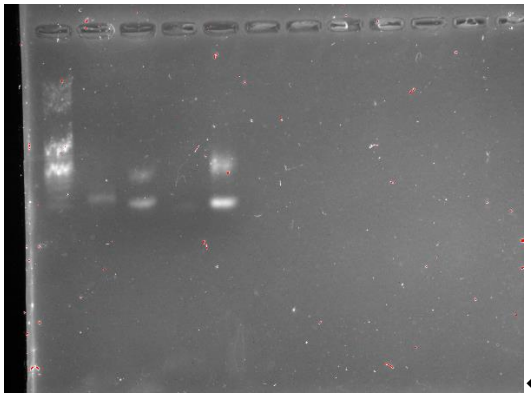
No template lane shows two intense bands. The bottom most lines up with the 0.4kb band found in the LVS lane. The intense band appears to be 0.8kb.



← This image is a duplicate of the image above with the brightness of the image raised and the fluorescent intensity of the bands highlighted through image lab. Image saved 08/02/19 1:24 PM



←This image is the result of running the gel further past the point of where the gel front reached the midpoint of the gel itself. Voltage current was set at 90V and time elapsed was 5 minutes. After 5 minutes I stopped the power source but did not immediately visualize the state of the gel. Image saved 08/02/19 1:38 PM



← The gel at this point was subjected to 100V of current for 7 minutes since the prior time it was visualized. Image saved 08/02/19 1:48 PM

**Monday, February 11, 2019**

**To do:**

1. **Lab maintenance (Autoclave pipette boxes)**
2. **Lab Maintenance (Make Agar Media)**

## Wednesday, February 13, 2019

**To do:**

1. **Retry PCR#2 Gel for Arbitrary PCR Transposon mutagenesis**

| **PCR #1** | | | | | | |
|---|---|---|---|---|---|---|
| **Component** | **Stock Concentration** | | **Final Concentration** | | **1 Rxn Volume (µL)** | Factor |
| **OneTaq Rxn Buffer** | 5X | | **1X** | | **5** | 25 |
| **Template** | 500ng | | **160ng/µL** | | **3.13** | indiv |
| **dNTPs** | **10 mM** | | **0.2mM** | | **0.5** | 2.5 |
| **ddiH2O** | **-** | | **-** | | **14.245** | 71.225 |
| **OneTaq Polymerase** | **0.25µL** | | **1.25 units/50µL** | | **0.125** | 0.625 |
| **KROL87 Arb1 or KROL 89 Arb 6** | **100µM** | | **4.0µM** | | **1** | 5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Internal Specific Primer: KROL90 Tn_Mar1** | **10µM** | | **0.4µM** | | **1** | 5 |
| **Total volume** | | | | | **25** | |

**Run PCR samples through thermocycler program for reaction 1.**

Initial Denaturation: 5 min. 95°C
5-6X:

- Denature 30 sec. 94°C
- Anneal 30 sec. 30°C
- Extend 60 sec. 72°C

30X:

- Denature 30 sec. 94° C
- Anneal 30 sec. 45-55° C
- Extend 120 sec. 72°C

Final Extension:

- Extend 5 min. 72°C

| | | | | | | |
|---|---|---|---|---|---|---|
| **PCR #2** | | | | | | |
| **Component** | **Stock Concentration** | | **Final Concentration** | | **1 Rxn Volume (µL)** | |
| **PCR 1 Amplicon** | _ | | _ | | **0.75** | 3.75 |
| **dNTPs** | **10 mM** | | **0.2mM** | | **0.5** | 2.5 |
| | | | | | | |
| **OneTaq Rxn Buffer** | **5X** | | **1X** | | **5** | 25 |
| **KROL88 Arb2** | **100µM** | | **4.0µM** | | **1** | 5 |
| **External Specific Primer: KROL91 Tn_Mar2** | **10µM** | | **0.4µM** | | **1** | 5 |
| **OneTaq Polymerase** | | | **1.25 units/50µL** | | **0.125** | 0.625 |
| **ddiH2O** | _ | | _ | | **16.625** | 83.125 |
| **Total volume** | | | | | **25** | 125 |

Run ArbPCR reaction amplicons through Thermocycler

Program for
ARBPCR reaction
2.

PCR Rxn #2

30X:

- Denature:
  30 sec.
  94°C
- Anneal:
  30 sec.
  45-
  55°C
- Extend:
  60 sec.
  72°C

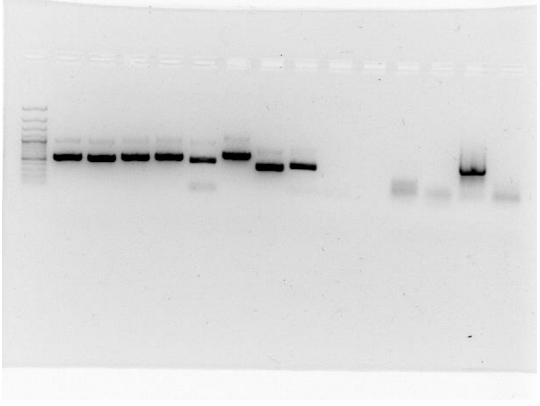
Final Extension:

- 5 min.
  72°C

AP-PCR done with Arb1 & Arb2 pairing at 55°C and with no mixing of master mix components to homogenize the solution.

**Results:**

**Using the DNA Gel ladder from the Dutta Laboratory Hannah (HT) and I used the entirety of a 1% gel. HT loaded lanes 2-10 for her experiment. A lane was intentionally left blank to separate my samples from HT's samples. I loaded my samples in lanes 12-15. Lane 12 is transposon Tn3 mutant, Lane 13 is transposon Tn6 mutant, lane 14 is LVS gDNA, and finally lane 15 is No template. The gel shows a fluorescently strong band in the LVS lane and faint bands in all other lanes of interest.**



## Friday, February 15, 2019

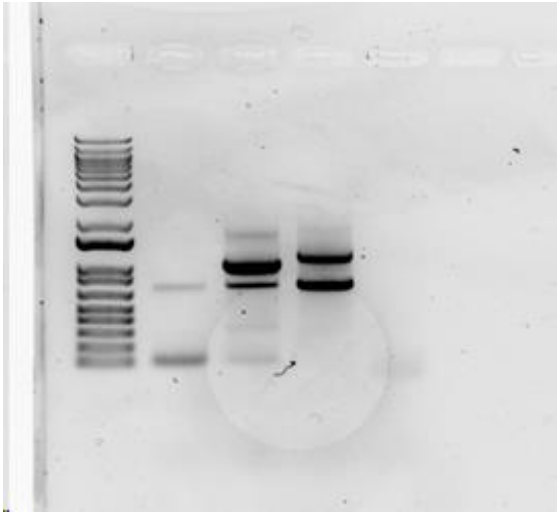
**To do:**

1. **Run Arbitrary PCR stage 1 with KROL 88 (Arb 6) primer**

**Results and Data:**

| PCR #1 | | | | |
|---|---|---|---|---|
| **Component** | **Stock Concentration** | **Final Concentration** | **1 Rxn Volume (µL)** | **Factor** |
| OneTaq Rxn Buffer | 5X | 1X | 5 | 25 |
| Template | 500ng | 160ng/µL | 3.13 | indiv |
| dNTPs | 10 mM | 0.2mM | 0.5 | 2.5 |
| ddiH2O | - | - | 14.245 | 71.225 |
| OneTaq Polymerase | 0.25µL | 1.25 units/50µL | 0.125 | 0.625 |
| KROL87 Arb1 (or KROL 88Arb 6) | 100µM | 4.0µM | 1 | 5 |
| Internal Specific Primer: KROL90 Tn_Mar1 | 10µM | 0.4µM | 1 | 5 |
| Total volume | | | 25 | |
| | | | | |
| PCR #2 | | | | |
| **Component** | **Stock Concentration** | **Final Concentration** | **1 Rxn Volume (µL)** | |

| | | | | |
|---|---|---|---|---|
| **PCR 1 Amplicon** | _ | _ | **0.75** | **3.75** |
| **dNTPs** | **10 mM** | **0.2mM** | **0.5** | **2.5** |
| **OneTaq Rxn Buffer** | **5X** | **1X** | **5** | **25** |
| **KROL88 Arb2** | **100µM** | **4.0µM** | **1** | **5** |
| **External Specific Primer: KROL91 Tn_Mar2** | **10µM** | **0.4µM** | **1** | **5** |
| **OneTaq Polymerase** | | **1.25 units/50µL** | **0.125** | **0.625** |
| **ddiH2O** | _ | _ | **16.625** | **83.125** |
| **Total volume** | | | **25** | **125** |



Bands in LVS lane and their intensity suggests many random primer hybridization events occurred. The Tn6 lane shows approximately similar size bands. These findings inspired the decision to image a PCR reaction 1 of samples 25.02.19. Lanes read left to right: DNA ladder, Tn3 mutant, Tn6 mutant, LVS gDNA, and No template.

**Reaction occurred under 55°C annealing temperature and master with a master mix solution that were not thoroughly mixed well.**

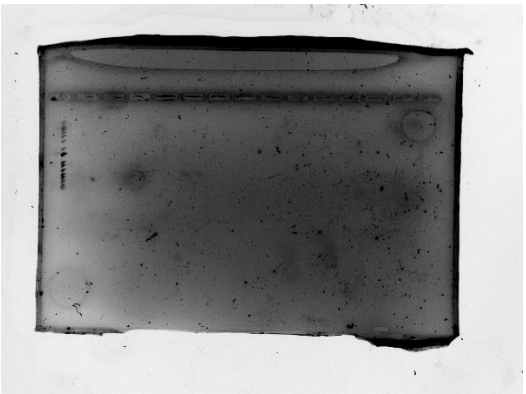
**Monday, February 25, 2019**

**To do:**

1. **Run a 1% agarose gel with Arb6PCR reaction 1 samples.**

**Thoughts and Results**

**Wasteful errors during gel loading on my part caused poor quality gel with no information to be gleam.**



**Friday, March 1, 2019**

**To do:**

1. Run Arb6 PCR reaction 1 and a 1% agarose gel from the generated products

## March 2019

**Monday, March 4, 2019**

**To do:**

**1.**Run Arb6 PCR reaction 1 and a 1% agarose gel from the generated PCR products of Monday, February 25, 2019

This AP-PCR trial was done with Arb6-Arb2 pairing at 55°C annealing temperature. No thorough mixing of the master mix components was done.

| **PCR #1** | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |

| **Component** | **Stock Concentration** | | **Final Concentration** | | **1 Rxn Volume (µL)** | Factor |
|---|---|---|---|---|---|---|
| **OneTaq Rxn Buffer** | 5X | | **1X** | | **5** | 25 |
| **Template** | 500ng | | **160ng/µL** | | **3.13** | indiv |
| **dNTPs** | **10 mM** | | **0.2mM** | | **0.5** | 2.5 |
| **ddiH2O** | **-** | | **-** | | **14.245** | 71.225 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **OneTaq Polymerase** | **0.25µL** | | **1.25 units/50µL** | | **0.125** | 0.625 |
| **KROL87 Arb1 or KROL 89 Arb 6** | **100µM** | | **4.0µM** | | **1** | 5 |
| **Internal Specific Primer: KROL90 Tn_Mar1** | **10µM** | | **0.4µM** | | **1** | 5 |
| **Total volume** | | | | | **25** | |

**Run PCR samples through thermocycler program for reaction 1.**

Initial Denaturation: 5 min. 95°C
5-6X:

- Denature 30 sec. 94°C
- Anneal 30 sec. 30°C
- Extend 60 sec. 72°C

30X:

- Denature 30 sec. 94° C
- Anneal 30 sec. 45-55° C
- Extend 120 sec. 72°C

Final Extension:

- Extend 5 min. 72°C

| | | | | | | |
|---|---|---|---|---|---|---|
| **PCR #2** | | | | | | |
| **Component** | **Stock Concentration** | | **Final Concentration** | | **1 Rxn Volume (µL)** | |
| **PCR 1 Amplicon** | _ | | _ | | **0.75** | 3.75 |
| **dNTPs** | **10 mM** | | **0.2mM** | | **0.5** | 2.5 |
| | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **OneTaq Rxn Buffer** | **5X** | | **1X** | | **5** | 25 |
| **KROL88 Arb2** | **100µM** | | **4.0µM** | | **1** | 5 |
| **External Specific Primer: KROL91 Tn_Mar2** | **10µM** | | **0.4µM** | | **1** | 5 |
| **OneTaq Polymerase** | | | **1.25 units/50µL** | | **0.125** | 0.625 |
| **ddiH2O** | _ | | _ | | **16.625** | 83.125 |
| **Total volume** | | | | | **25** | 125 |

Run ArbPCR reaction amplicons through Thermocycler Program for ARBPCR reaction 2.

PCR Rxn #2

30X:

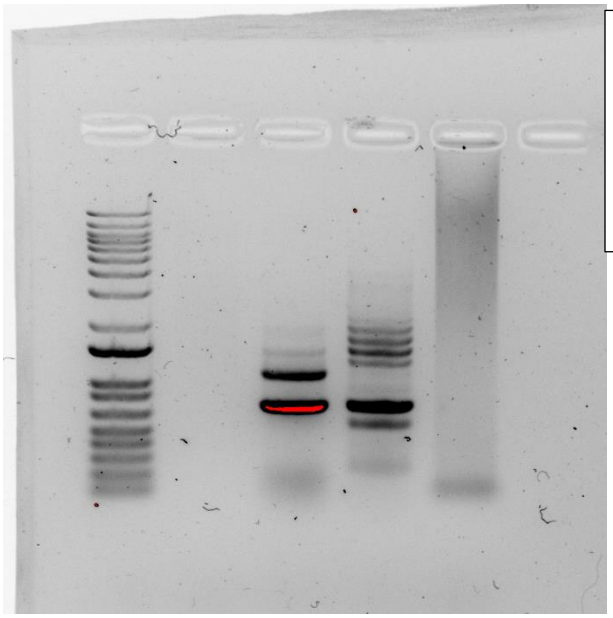
- Denature: 30 sec. 94°C
- Anneal: 30 sec. 45-55°C
- Extend: 60 sec.

72°C

Final Extension:

- 5 min.
  72°C

**Results & Thoughts**



Lanes Left to Right: DNA ladder, Tn3, Tn6, LVS gDNA, and No template. Seen here is AP-PCR primary reaction with Arb6-KROL90 pairing. Reaction done at 55°C and no mixing of master mix components.

Retry PCR reaction #1, this time describe every step of the preparation process and work process in as much detail as possible. Lack of band in the Tn3 mutant lane suggest that no PCR reaction occurred.

**Wednesday, March 6, 2019**

**To do:**

1. **Rerun Arbitrary PCR reaction 1 with pKROL 89 (Arb6) primer**

## Protocol

Arbitrary PCR of embellished Transposon elements in mutant genomic or clonal DNA is a powerful technique that allows the researchers to map out where transposon elements have inserted into bacterial chromosome and allows answers to questions related to promoters' positions and phenotype profiling of mutants.

Create a PCR master mix for the first reaction

Samples

| Sample | Tn3 | Tn6 | LVS gDNA | No Template |
|---|---|---|---|---|
| Concentration (ng/ul) | 160 | 160 | 160 | - |

Arbitrary PCR Master Mix Reaction One Recipe

| **PCR #1** | | | | |
|---|---|---|---|---|
| **Component** | **Stock Concentration** | **Final Concentration** | **1 Rxn Volume (µL)** | **Factor** |
| **OneTaq Rxn Buffer** | **5X** | **1X** | **5** | **25** |
| **Template** | **500ng** | **160ng/µL** | **3.13** | **indiv** |
| **dNTPs** | **10 mM** | **0.2mM** | **0.5** | **2.5** |
| **ddiH2O** | **-** | **-** | **14.245** | **71.225** |
| **OneTaq Polymerase** | **0.25µL** | **1.25 units/50µL** | **0.125** | **0.625** |
| **KROL87 Arb1 (or KROL 88Arb 6)** | **100µM** | **4.0µM** | **1** | **5** |
| **Internal Specific Primer: KROL90 Tn_Mar1** | **10µM** | **0.4µM** | **1** | **5** |

| | | | | |
|---|---|---|---|---|
| **Total volume** | | | **25** | |

Make up the master mix by aliquoting…

i. 71.225uL of dd$iH_2O$
ii. 25uL OneTaq Rxn Buffer
iii. 2.5uL dNTPs
iv. 5.0uL KROL 87 Arb1 or KROL 88 Arb6
v. 5.0uL KROL 90 Tn_Mar1
vi. 0.625uL OneTaq Polymerase

Once Master mix is prepared obtain four PCR strip tubes and label the four tubes as: Tn3, Tn6, LVS gDNA, and No Template.

Each PCR strip tube will contain 3.13uL of DNA sample and will total to 25uL. Load ~21.9uL of the master mix into each PCR strip tube and then aliquot 3.13uL of the DNA sample. Once the tubes contain master mix and DNA they are ready for the thermocycler.

Thermocycler Program

Initial Denaturation: 5 min. 95°C
5-6X:

- Denature 30 sec. 94°C
- Anneal 30 sec. 30°C
- Extend 60 sec. 72°C

30X:

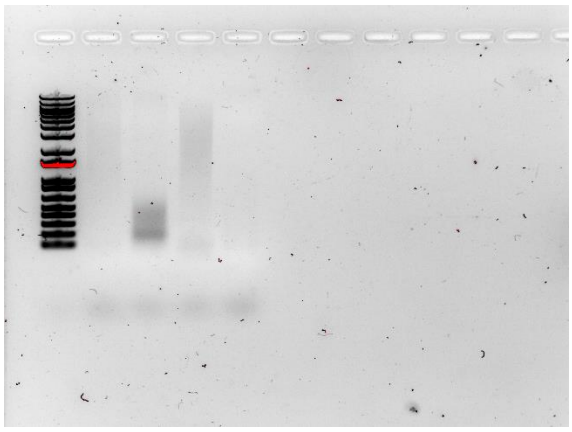
- Denature 30 sec. 94° C
- Anneal 30 sec. 45-55° C
- Extend 120 sec. 72°C

Final Extension:

- Extend 5 min. 72°C

Once samples have been amplified run a gel electrophoresis using only 20uL of the amplicons. Melt 1% agarose gel in 1X TAE if the lab supplied with a flask. If not, make up 500ml of 1% Agarose in 1X TAE in a 1,000mL flask. Obtain an empty clean 1,000mL flask and pour 20ml of 25X TAE into it and fill it to 500mL with 480mL of dd$iH_2O$, add 5g of pure agarose powder, and finally add a stir bar. Heat and stir the flask until all the agarose powder is dissolved in solution. Pour ~100mL of lukewarm Agarose solution into the casting tray and add ~5uL of SYBERSafe, mix well with comb. Place the comb near the top of the gel to form wells and let gel sit for 25 minutes to solidify. Once the gel solidifies fill the two chambers until the gel is submerged with 1X TAE. Add 5uL of OrangeG DNA stain to 25uL samples. Add 10uL of DNA 1kbplus Ladder to well #1. The subsequent wells will have the stained DNA samples loaded onto them.

Thoughts and Results:



This primary reaction of Arb 6-KROL 90 pairing was subjected to 55°C annealing temperature.

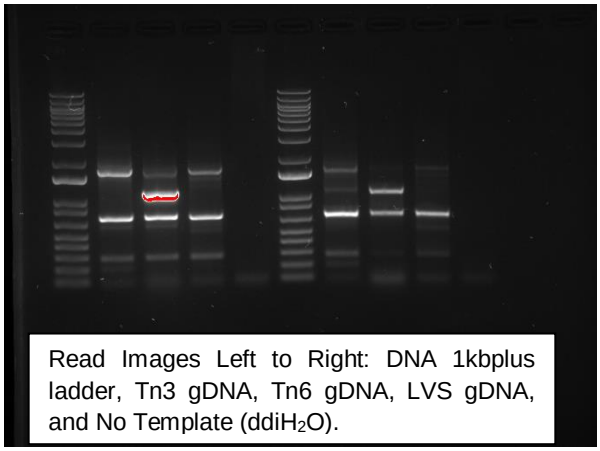
With every trial of arbitrary PCR there is variability in the resultant gels of first PCR reaction. Nothing except the procedure is constant, sometimes bands appear and disappear in lanes and their sizes vary from one image to the next. In the image above there are two band smears in the Tn6 mutant lane and primer dimers in all lanes. How to validate this result is contingent on what is causing the variability of the gel images.

**Friday, March 8, 2019**

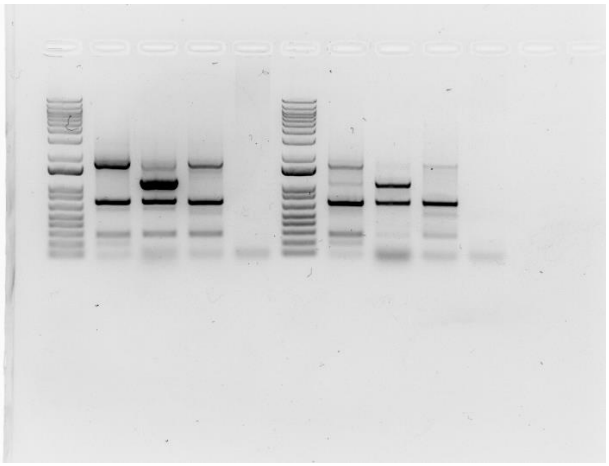
**To do:**

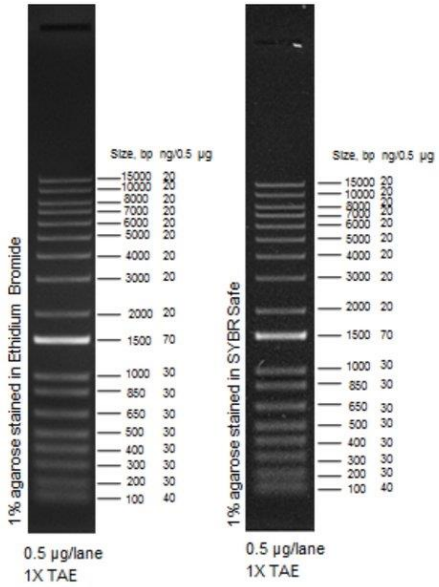
1. **Execute Arbitrary PCR with KROL88 Arb6 Primer and compare results to KMR's trail.**

In running this trial the samples present with consistency and symmetry. On reflection, since first undertaking this experiment endeavor I have not been spinning down the master mix or letting all components completely defrost. These results are based on careful prudent technique practices and the consistency between my samples and KMR is evident of no variability.



Read Images Left to Right: DNA 1kbplus ladder, Tn3 gDNA, Tn6 gDNA, LVS gDNA, and No Template (ddi$H_2O$).






**Results and Data:**

Reactions during this trial ran under 55°C annealing temperature and master mix components and reagents were allowed to thaw and were thoroughly mixed well by vortex and sonication

Shared bands between Tn3, Tn6, and LVS are annealing products of same size what is noteworthy are the bands in lanes Tn3 and Tn6 that are above

**Monday, March 18, 2019**

**To Do:**

1. **Execute ArbPCR1 with KROL87**

## Protocol

Arbitrary PCR of embellished Transposon elements in mutant genomic or clonal DNA is a powerful technique that allows the researcher to map out where transposon elements have inserted into bacterial chromosome via RNA sequencing or ChIP Sequencing and allows answers to questions related to promoters' positions and phenotype profiling of mutants.

Create a PCR master mix for the first reaction

Samples

| Sample | Tn3 | Tn6 | LVS gDNA | No Template |
|---|---|---|---|---|
| Concentration (ng/ul) | 160 | 160 | 160 | - |

Arbitrary PCR Master Mix Reaction One Recipe

| **PCR #1** | | | | |
|---|---|---|---|---|
| **Component** | **Stock Concentration** | **Final Concentration** | **1 Rxn Volume (µL)** | **Factor** |
| **OneTaq Rxn Buffer** | **5X** | **1X** | **5** | **25** |
| **Template** | **500ng** | **160ng/µL** | **3.13** | **indiv** |
| **dNTPs** | **10 mM** | **0.2mM** | **0.5** | **2.5** |
| **ddiH2O** | **-** | **-** | **14.245** | **71.225** |
| **OneTaq Polymerase** | **0.25µL** | **1.25 units/50µL** | **0.125** | **0.625** |
| **KROL87 Arb1** | **100µM** | **4.0µM** | **1** | **5** |

| | | | | |
|---|---|---|---|---|
| **Internal Specific Primer: KROL90 Tn_Mar1** | **10µM** | **0.4µM** | **1** | **5** |
| **Total volume** | | | **25** | |

Make up the master mix by aliquoting…

vii. 71.225uL of ddi$H_2O$
viii. 25uL OneTaq Rxn Buffer
ix. 2.5uL dNTPs
x. 5.0uL KROL 87 Arb1 or KROL 89 Arb6
xi. 5.0uL KROL 90 Tn_Mar1
xii. 0.625uL OneTaq Polymerase

Once Master mix is prepared obtain four PCR strip tubes and label the four tubes as: Tn3, Tn6, LVS gDNA, and No Template.

Each PCR strip tube will contain 3.13uL of DNA sample and will total to 25uL. Load ~21.9uL of the master mix into each PCR strip tube and then aliquot 3.13uL of the DNA sample. Once the tubes contain master mix and DNA they are ready for the thermocycler.

Thermocycler Program

Initial Denaturation: 5 min. 95°C
5-6X:

- Denature 30 sec. 94°C
- Anneal 30 sec. 30°C
- Extend 60 sec. 72°C

30X:

- Denature 30 sec. 94° C
- Anneal 30 sec. 45-55° C
- Extend 120 sec. 72°C

Final Extension:

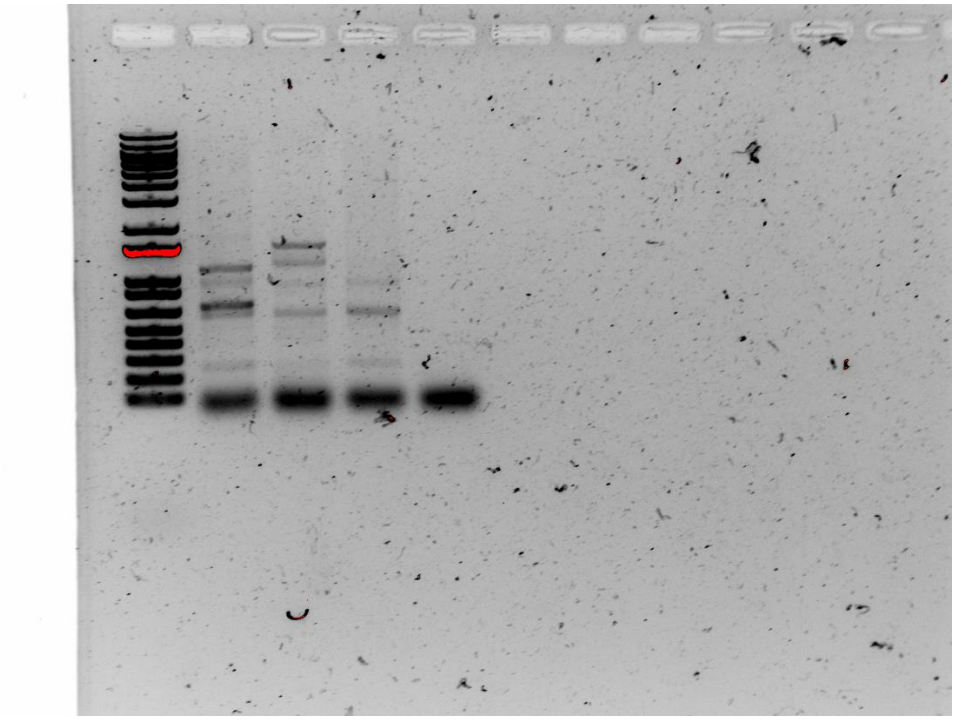
- Extend 5 min. 72°C

Once samples have been amplified run a gel electrophoresis using only 20uL of the amplicons (Samples were run under 55°C annealing temperature). Melt 1% agarose gel in 1X TAE if the lab supplied with a flask. If not, make up 500ml of 1% Agarose in 1X TAE in a 1,000mL flask. Obtain an empty clean 1,000mL flask and pour 20ml of 25X TAE into it and fill it to 500mL with 480mL of ddi$H_2O$, add 5g of pure agarose powder, and finally add a stir bar. Heat and stir the flask until all the agarose powder is dissolved in solution. Pour ~100mL of lukewarm Agarose solution into the casting tray and add ~5uL of SYBERSafe, mix well with comb. Place the comb near the top of the gel to form wells and let gel sit for 25 minutes to solidify. Once the gel solidifies fill the two chambers until the gel is submerged with 1X TAE. Add 5uL of OrangeG

DNA stain to 25uL samples. Add 10uL of DNA 1kbplus Ladder to well #1. The subsequent wells will have the stained DNA samples loaded onto them.

1° Reaction occurred with 55°C annealing temperature and well thorough mixing of the master mix reagents and components.

**Results:**



**Notes from KMR For 3/20/19**

Template is PCR rxn 1 diluted 1:100 and 2nd PCR rxn annealing temperature @ 65 Celsius.

**Wednesday, March 20, 2019**

**To Do: 1. Make a 1:100 of the Arbitrary PCR reaction 1 amplicons of 3/18/19 and run Arbitrary PCR reaction 2 with a melting temperature of 65°C**

**Thoughts/Reflection**

**1µL of Tn3, Tn6, LVS, and No template gDNA were used for a 1:100 to dilution leaving 9µL of each sample left over. Next, I loaded the diluted samples into the thermocycler and ran the following program…**

30x 94°C 30”

45-55°C 30”

72°C 1’

72°C 5’

**I did so under the assumption that once diluted the amplicons of reaction 1 that were made on 3/18/19, could at once be put through the thermocycler program for the second arbitrary PCR reaction since they are a solution of the PCR components: DNA polymerase, dNTPS, and primers. This is faulty thinking since Reaction 1 and Reaction 2 of the Arbitrary PCR protocol I have been following do not have the same set of annealing primers. Ultimately, my productivity on this day in laboratory amount to nothing more than a sham due to confusion and being swayed by an unsound assumption.**

## Friday, March 22, 2019

**To Do: 1. Re-dilute 1:100 dilutions of Arbitrary PCR sample amplicons of 3/18/19 and suspend them into Arbitrary PCR#2 master mix aliquots, to run thermo cycler program for Arbitrary PCR reaction #2**

**This AP-PCR trial run occurred with 55°C annealing temperature and a thoroughly mixed master mix.**

### Protocol

Arbitrary PCR of embellished Transposon elements in mutant genomic or clonal DNA is a powerful technique that allows the researcher to map out where transposon elements have inserted into bacterial chromosome via RNA sequencing or ChIP Sequencing and allows answers to questions related to promoters’ positions and phenotype profiling of mutants.

Samples

| Sample | Tn3 | Tn6 | LVS gDNA | No Template |
|---|---|---|---|---|
| Concentration (ng/ul) | 160 | 160 | 160 | - |

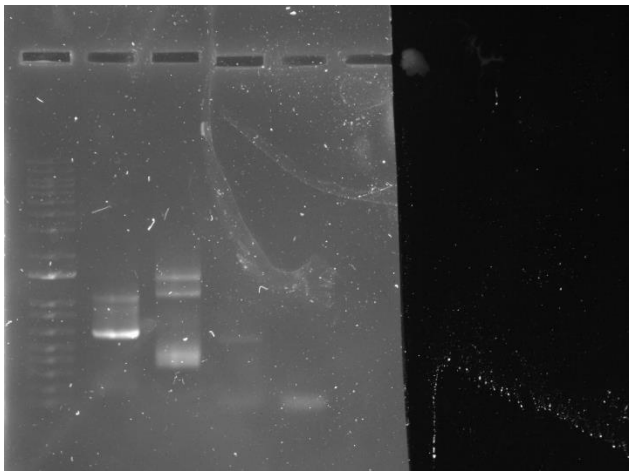
| **PCR #2** | | | | |
|---|---|---|---|---|
| **Component** | **Stock Concentration** | **Final Concentration** | **1 Rxn Volume (µL)** | |

| | | | | |
|---|---|---|---|---|
| **PCR 1 Amplicon** | _ | _ | **0.75** | **3.75** |
| **dNTPs** | **10 mM** | **0.2mM** | **0.5** | **2.5** |
| **OneTaq Rxn Buffer** | **5X** | **1X** | **5** | **25** |
| **KROL88 Arb2** | **100µM** | **4.0µM** | **1** | **5** |
| **External Specific Primer: KROL91 Tn_Mar2** | **10µM** | **0.4µM** | **1** | **5** |
| **OneTaq Polymerase** | | **1.25 units/50µL** | **0.125** | **0.625** |
| **ddiH2O** | _ | _ | **16.625** | **83.125** |
| **Total volume** | | | **25** | **125** |

Make up the master mix by aliquoting…

i. 83.125uL of ddi$H_2O$
ii. 25uL OneTaq Rxn Buffer
iii. 2.5uL dNTPs
iv. 5.0uL KROL 88 Arb2
v. 5.0uL KROL 91 Tn_Mar2
vi. 0.625uL OneTaq Polymerase

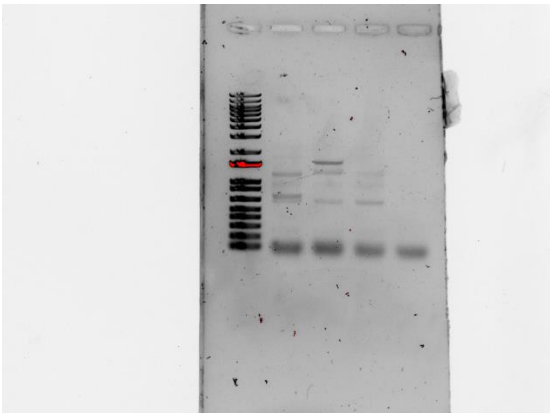
Results



Left to Right Lanes: DNA 1kb plus ladder, Tn3, Tn6, LVS gDNA, and Control(+). ArbPCR amplicon of KROL 89 Arb 6

**Monday, March 25, 2019**

**To Do: Run arbitrary PCR protocol 1 with arbitrary primer KROL87 at 60°C annealing temperature**



Lanes read Left to Right: DNA 1kb plus ladder, Tn3, Tn6, LVS gDNA, Control (+).

**Results and Data:**

The AP-PCR reaction master mix was thoroughly mixed well.

Arbitrary PCR Master Mix Reaction One Recipe

| **PCR #1** | | | | |
|---|---|---|---|---|
| **Component** | **Stock Concentration** | **Final Concentration** | **1 Rxn Volume (µL)** | **Factor** |
| **OneTaq Rxn Buffer** | **5X** | **1X** | **5** | **25** |
| **Template** | **500ng** | **160ng/µL** | **3.13** | **indiv** |

| | | | | |
|---|---|---|---|---|
| **dNTPs** | **10 mM** | **0.2mM** | **0.5** | **2.5** |
| **ddiH2O** | **-** | **-** | **14.245** | **71.225** |
| **OneTaq Polymerase** | **0.25µL** | **1.25 units/50µL** | **0.125** | **0.625** |
| **KROL87 Arb1** | **100µM** | **4.0µM** | **1** | **5** |
| **Internal Specific Primer: KROL90 Tn_Mar1** | **10µM** | **0.4µM** | **1** | **5** |
| **Total volume** | | | **25** | |

Make up the master mix by aliquoting…

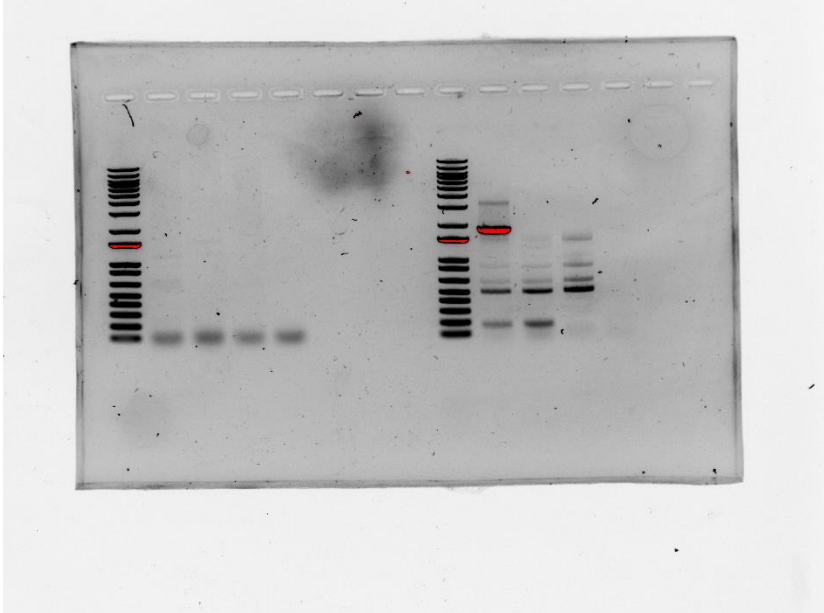
i. 71.225uL of ddi$H_2O$
ii. 25uL OneTaq Rxn Buffer
iii. 2.5uL dNTPs
iv. 5.0uL KROL 87 Arb1 or KROL 89 Arb6
v. 5.0uL KROL 90 Tn_Mar1
vi. 0.625uL OneTaq Polymerase

## Wednesday, April 3, 2019

**To Do: Perform arbitrary PCR using KROL 87 and KROL 89 and run at 65°C annealing temperature under arb PCR thermocycler program #1**

**Results & Data:**

**The left portion of the gel image below is where the KROL 87 sample set was loaded (Lane 1 DNA 1kb plus ladder, Lane 2 Tn3 mutant, Lane 3: Tn6 mutant, Lane 4: LVS gDNA, and Lane 5: - control ($H_2O$). The right side of the gel image is where KROL 89 sample set was loaded into with the same sample chronology as that of the sample set of lanes 1-5.**



**Monday, April 8, 2019**

**To Do: 1. Objective: Retry the ArbPCR 1° rxn of KROL 87 and KROL 89 at 65°C annealing temperature**

**Procedure:**

Samples

| Sample | Tn3 | Tn6 | LVS gDNA | No Template |
|---|---|---|---|---|
| Concentration (ng/ul) | 160 | 160 | 160 | - |

Arbitrary PCR Master Mix Reaction One Recipe

| **PCR #1** | | | | |
|---|---|---|---|---|
| **Component** | **Stock Concentration** | **Final Concentration** | **1 Rxn Volume (µL)** | **Factor** |
| **OneTaq Rxn Buffer** | **5X** | **1X** | **5** | **25** |

| | | | | |
|---|---|---|---|---|
| **Template** | **500ng** | **160ng/µL** | **3.13** | **indiv** |
| **dNTPs** | **10 mM** | **0.2mM** | **0.5** | **2.5** |
| **ddiH2O** | **-** | **-** | **14.245** | **71.225** |
| **OneTaq Polymerase** | **0.25µL** | **1.25 units/50µL** | **0.125** | **0.625** |
| **KROL87 Arb1** | **100µM** | **4.0µM** | **1** | **5** |
| **Internal Specific Primer: KROL90 Tn_Mar1** | **10µM** | **0.4µM** | **1** | **5** |
| **Total volume** | | | **25** | |

Make up the master mix by aliquoting…

vii. 71.225uL of ddi$H_2O$
viii. 25uL OneTaq Rxn Buffer
ix. 2.5uL dNTPs
x. 5.0uL KROL 87 Arb1 or KROL 89 Arb6
xi. 5.0uL KROL 90 Tn_Mar1
xii. 0.625uL OneTaq Polymerase

Once Master mix is prepared obtain four PCR strip tubes and label the four tubes as: Tn3, Tn6, LVS gDNA, and No Template.

Each PCR strip tube will contain 3.13uL of DNA sample and will total to 25uL. Load ~21.9uL of the master mix into each PCR strip tube and then aliquot 3.13uL of the DNA sample. Once the tubes contain master mix and DNA they are ready for the thermocycler.

Thermocycler Program

Initial Denaturation: 5 min. 95°C
5-6X:

- Denature 30 sec. 94°C
- Anneal 30 sec. 30°C
- Extend 60 sec. 72°C

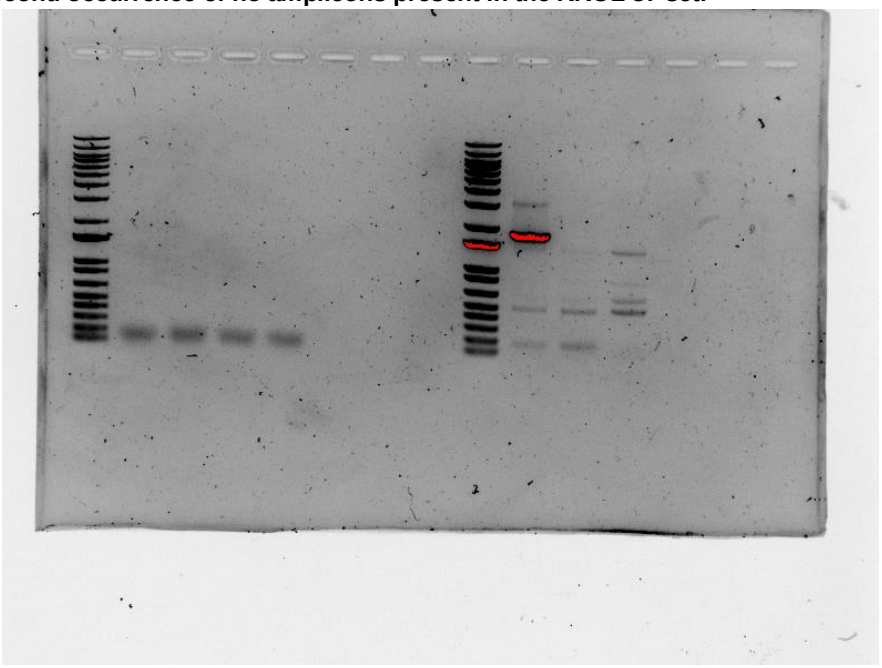
30X:

- Denature 30 sec. 94° C
- Anneal 30 sec. 45-55° C **[65° C for increasing annealing specificity]**
- Extend 120 sec. 72°C

Final Extension:

- Extend 5 min. 72°C

**Thoughts & Results: Unlike Wednesday April 3, 2019 in which I concluded that I forgot to add the genomic DNA of transposons mutants Tn3, Tn6, LVS gDNA to the KROL 87 set of PCR tubes, this time I made certain to add 3.13 µL of gDNA to each PCR reaction tube. In hindsight I most likely did add the KROL 87 sample to their wells on 19.04.03. This is the second occurrence of no amplicons present in the KROL 87 set.**



## Wednesday, April 10, 2019

**To Do: Perform Arb PCR 2° Reaction with 1:100 diluted amplicons of April 8, 2019 at 65° annealing temperature.**

**Procedure:**

| **PCR #2** | | | | |
|---|---|---|---|---|
| **Component** | **Stock Concentration** | **Final Concentration** | **1 Rxn Volume (µL)** | |
| **PCR 1 Amplicon** | _ | _ | **0.75** | **3.75** |
| **dNTPs** | **10 mM** | **0.2mM** | **0.5** | **2.5** |
| **OneTaq Rxn Buffer** | **5X** | **1X** | **5** | **25** |
| **KROL88 Arb2** | **100µM** | **4.0µM** | **1** | **5** |
| **External Specific Primer: KROL91 Tn_Mar2** | **10µM** | **0.4µM** | **1** | **5** |
| **OneTaq Polymerase** | | **1.25 units/50µL** | **0.125** | **0.625** |
| **ddiH2O** | _ | _ | **16.625** | **83.125** |
| **Total volume** | | | **25** | **125** |

Make up the master mix by aliquoting…

i. 83.125uL of ddi$H_2O$
ii. 25uL OneTaq Rxn Buffer
iii. 2.5uL dNTPs
iv. 5.0uL KROL 88 Arb2
v. 5.0uL KROL 91 Tn_Mar2
vi. 0.625uL OneTaq Polymerase

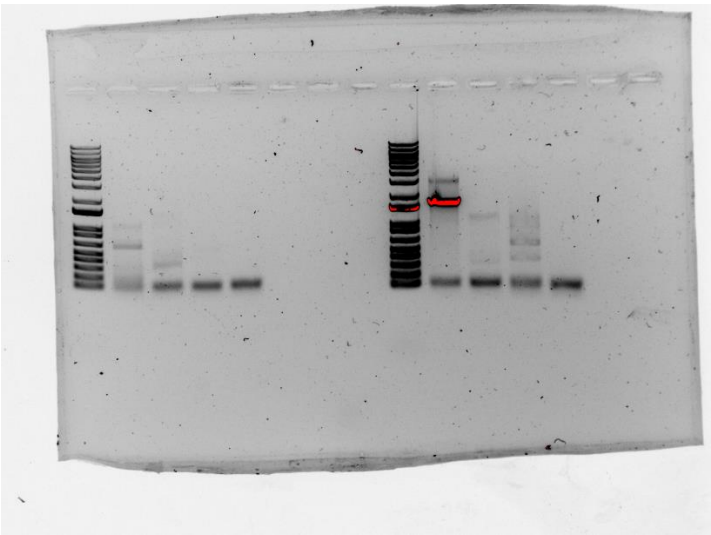
AP-PCR Thermo Cycler Program 2° Rxn

30x 94°C 30”

45-55°C 30” **[65° C for increasing primer annealing specificity]**

72°C 1’

72°C 5’



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**Friday, April 12, 2019**

**To Do: 1. Dilute AP-PCR from 4/8/19 1:10 and set up secondary PCR rxn upscaled to 50ul and run it under 65°C annealing temperature then PCR purify. Elute in 30ul of 0.1X EB buffer and run 5ul of each sample on gel.**

**Procedure:**

| **PCR #2** | | | | |
|---|---|---|---|---|
| **Component** | **Stock Concentration** | **Final Concentration** | **1 Rxn Volume (µL)** | |
| **PCR 1 Amplicon** | _ | _ | **0.75** | **3.75** |

| | | | | |
|---|---|---|---|---|
| **dNTPs** | **10 mM** | **0.2mM** | **0.5** | **2.5** |
| **OneTaq Rxn Buffer** | **5X** | **1X** | **5** | **25** |
| **KROL88 Arb2** | **100µM** | **4.0µM** | **1** | **5** |
| **External Specific Primer: KROL91 Tn_Mar2** | **10µM** | **0.4µM** | **1** | **5** |
| **OneTaq Polymerase** | | **1.25 units/50µL** | **0.125** | **0.625** |
| **ddiH2O** | _ | _ | **16.625** | **83.125** |
| **Total volume** | | | **25** | **125** |

Make up the master mix (50µL reaction) by aliquoting…

i. 166.25uL of ddi$H_2O$
ii. 50uL OneTaq Rxn Buffer
iii. 5uL dNTPs
iv. 10.0 uL KROL 88 Arb2
v. 10.0uL KROL 91 Tn_Mar2
vi. 1.25 uL OneTaq Polymerase

AP-PCR Thermo Cycler Program 2° Rxn

30x 94°C 30”

45-55°C 30” **[65° C for increasing primer annealing specificity]**

72°C 1’

72°C 5’

**QIAquick PCR Purification**

**Add 5 volumes buffer PB to 1 volume of the PCR reaction and mix. (50µL of PCR sample and 250 µL of PB buffer)**

**Place a QIAquick column into a 2ml collection/microcentrifuge tube.**

**Aliquot PB buffer-PCR sample into the spin column and centrifuge @ 13,000 rpm for 1 min. Discard flow-through.**

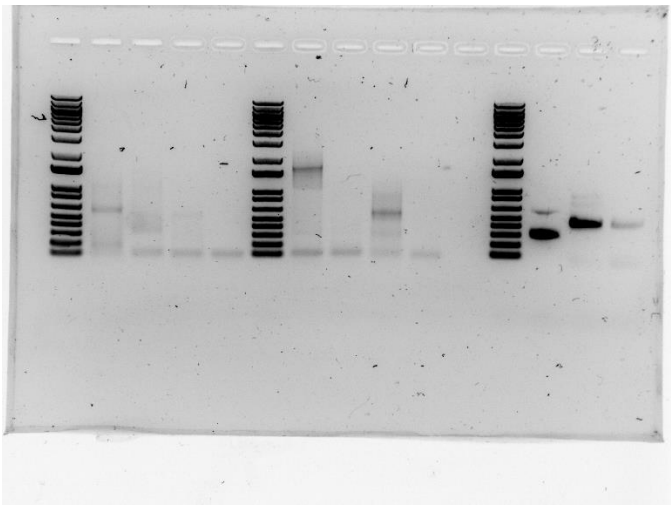
**Wash with 750µL of PE buffer to the QIAquick column and centrifuge @13,000 rpm for 1 min. Discard flow-through.**

**Centrifuge once more at 13,000 rpm for 1 min. to remove any residual wash buffer.**

**Elute DNA samples using 30µL of 0.1% EB buffer and let sit for 1 minute in the column. Centrifuge for 1 min. at 13,000 rpm.**

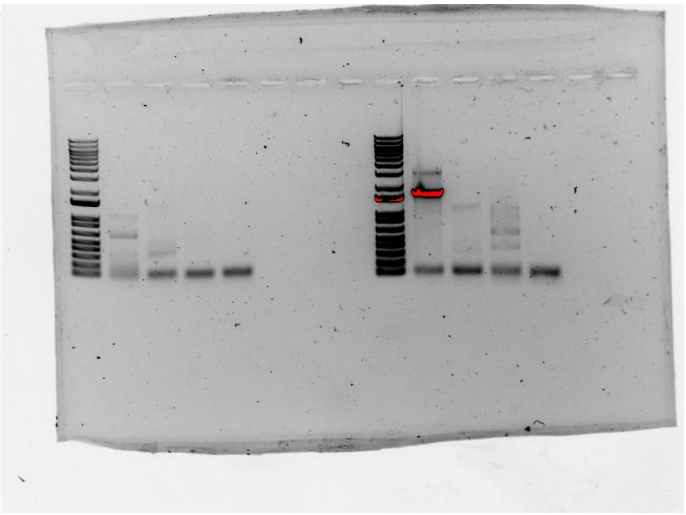
**Resolve the sample on 1% agarose gel and mix 1 µl Orange G dye to 5µL of sample.**

**Results:**



**Friday April 12, 2019**

**1:10 dilution of PCR secondary amplicons of 4/8/19**



**Wednesday April 10, 2019**

**1:100 dilution of PCR secondary amplicons 4/8/19**

## Tuesday, April 16, 2019

**To Do: Quantify purified PCR products of 4/12/19 using the nanodrop and set up DNA sequencing reactions for Tn3 and Tn6 samples.**

**Results:**

| Sample ID | User ID | Date | Time | ng/ul | A260 | A280 | 260/280 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Tn3 | Default | 4/16/2019 | 1:36 PM | 33.56 | 0.671 | 0.366 | 1.83 | 2.15 | 50.00 | 230 |
| | 0.312 | 0.008 | | | | | | | | |
| Tn6 | Default | 4/16/2019 | 1:37 PM | 24.12 | 0.482 | 0.268 | 1.80 | 1.89 | 50.00 | 230 |
| | 0.255 | 0.026 | | | | | | | | |

## Monday, April 22, 2019

**To Do:**

1. **Process sequencing results of 4/19/19 through BLAST and record genetic loci of Tn3 and Tn6 samples. Next figure out where transposon insertion and flanking occurred. Annotate the insertion and flanking on the LVS genome file of Snapgene.**
2. **Create KROL primers: For Tn3 create primers that anneal 100 bp upstream and downstream from Transposon. For Tn6 create primers that anneal 150 bp upstream and downstream from the transposon.**

**Results:**

Genbank information for Tn3 sample

Francisella tularensis subsp. holarctica LVS complete genome

Sequence ID: AM233362.1Length: 1895994Number of Matches: 1

Range 1: 698190 to 698244GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 102 bits(55) | 2e-23 | 55/55(100%) | 0/55(0%) | Plus/Plus |

```
Query  1       AAAGTACCACTAGTTTTAGATGTAATATATTTTCTATGTTCGGGATTATCTTCAT  55
               |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  698190  AAAGTACCACTAGTTTTAGATGTAATATATTTTCTATGTTCGGGATTATCTTCAT  698244
```

Genbank Information for Tn6 sample

Francisella tularensis subsp. holarctica LVS complete genome

Sequence ID: AM233362.1Length: 1895994Number of Matches: 1

Range 1: 721139 to 721198GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 111 bits(60) | 3e-26 | 60/60(100%) | 0/60(0%) | Plus/Plus |

```
Query  1       CACATCAAAAAGTTGAGATATCAGGTATACCATATTACCGATAATCACAGCTTTCAAAGC  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721139  CACATCAAAAAGTTGAGATATCAGGTATACCATATTACCGATAATCACAGCTTTCAAAGC  721198
```

## Wednesday, April 24, 2019

**To Do: 1. Using the newly created primers of 4/22/19 validate findings of 4/22/19 with PCR**

**Procedure**

| | |
|---|---|
| Total reaction volume | 20 |
| Total number of reactions | 4 |

| Component | Stock concentration | Final concentration | 1 rxn volume | Factor 5.5 |
|---|---|---|---|---|
| ddiH2O | | | 4 | 22 |
| KOD buffer | 2x | 1x | 10 | 55 |
| dNTPs | 2 mM | 0.4 mM | 4 | 22 |
| oligo F | 10 uM | 0.3 uM | 0.6 | 3.3 |
| oligo R | 10 uM | 0.3 uM | 0.6 | 3.3 |
| template | 100 ng/ul | 2 ng/ul | 0.4 | |
| KOD | 1 U/ul | 0.02 U/ul | 0.4 | 2.2 |
| | | Total volume | 20 | 110 |

**Make Two Master Mixes**

1. **MM#1 KROL 160 + KROL 161**
2. **MM#2 KROL 162 + KROL 163**

**KROL 160 + KROL 161**

**Tn size = 4616**

**WT KROL 160 and KROL 161 = 221 bp**

**WT product bands = (4616 +221) = 4837 bp**

**KROL 162 + KROL 163**

**Tn size = 4616 bp**

**WT KROL 162 and KROL 163 = 358 bp**

**WT product bands= (4616+ 358) = 4974 bp**

**PCR Program**

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 (KOD polymerase functions properly at 68 degrees C; Taq polymerase is different temp)

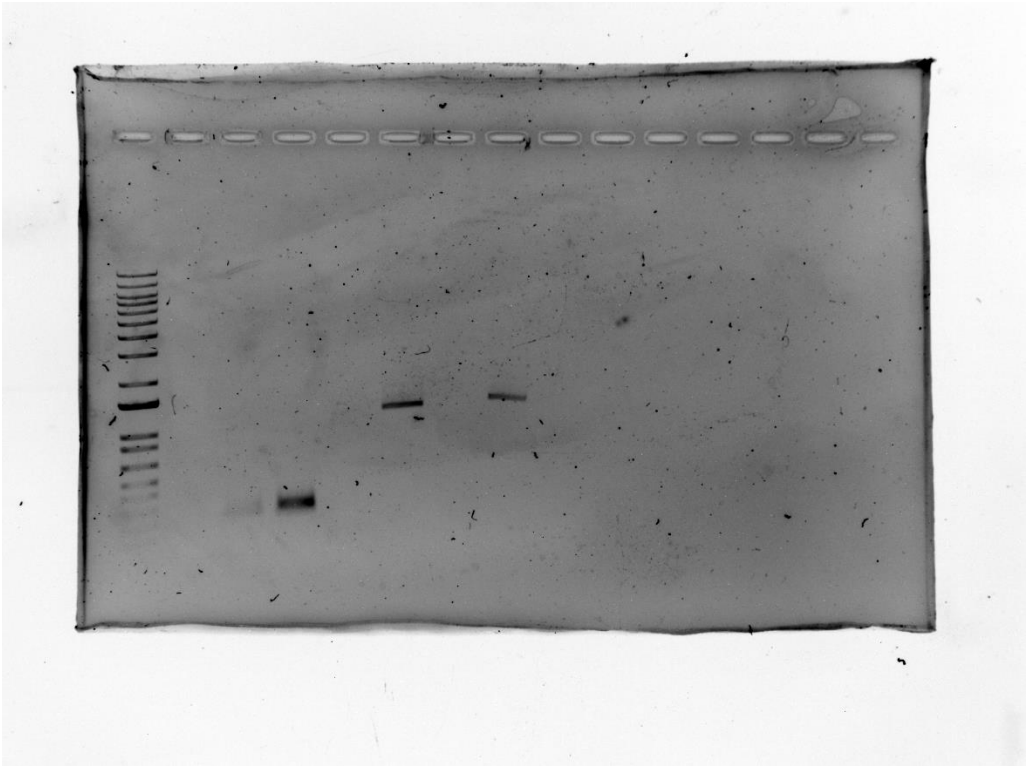
Go back to step 2

Repeat 32x

68 degrees C for 5 minutes

12 degrees C for infinity

**Results:** Failing to set the extension time for 5 minutes during the multiple cycles of PCR gave low abundance of amplicon products. KOD polymerase requires time for nucleotide polymerization. Running the gel for close to an hour allowed a portion of the gel front to run off the gel.



**Friday, April 24, 2019**

**To Do:**

**1. Run Tn3, Tn6, LVS gDNA, and $H_2O$ PCR reactions with KROL 160 and KROL 161 primers.**

**Procedure**

| | |
|---|---|
| Total reaction volume | 20 |
| Total number of reactions | 4 |

| | | | | **Factor** |
|---|---|---|---|---|
| **Component** | **Stock concentration** | **Final concentration** | **1 rxn volume** | 5.5 |
| ddiH2O | | | 4 | 22 |
| KOD buffer | 2x | 1x | 10 | 55 |
| dNTPs | 2 mM | 0.4 mM | 4 | 22 |
| oligo F | 10 uM | 0.3 uM | 0.6 | 3.3 |
| oligo R | 10 uM | 0.3 uM | 0.6 | 3.3 |
| template | 100 ng/ul | 2 ng/ul | 0.4 | |
| KOD | 1 U/ul | 0.02 U/ul | 0.4 | 2.2 |

| | | |
|---|---|---|
| Total volume | 20 | 110 |

**Make One Master Mix using KROL 160 and KROL161**

1. **MM#1 KROL 160 + KROL 161**
2. **MM#2 KROL 162 + KROL 163**

**KROL 160 + KROL 161**

**Tn size = 1441 bp**

**WT KROL 160 and KROL 161 = 221 bp**

**WT product bands = (1441 +221) = 1662 bp**

**KROL 162 + KROL 163**

**Tn size = 1441 bp**

**WT KROL 162 and KROL 163 = 358 bp**

**WT product bands= (1441+ 358) = 1799 bp**

**PCR Program**

Heat at 94 degrees for 2 minutes,

94 degrees C for 20 seconds

50 degrees C for 30 seconds

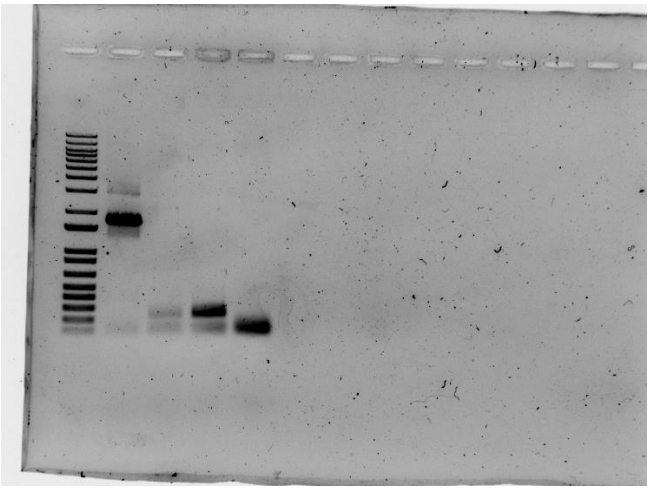
68 degrees C for 5 minutes (KOD polymerase functions properly at 68 degrees C extending elongation step to 5 minutes grants KOD polymerase a greater turnover number) Go back to step 2

Repeat 32x

68 degrees C for 5 minutes

12 degrees C for infinity

**Results:**



**Originally on April 24th, 2019 the estimated size value of the transposon inserts was 4616bp, it wasn't until KMR realized the mishap of using the wrong reference plasmid map for results verification and results were evaluated with another reference plasmid map estimating inserts to be around 1.7 kb.**

## 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.