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Monday, Dec 2nd , 2019

To ~~Do:~~

1. ~~Make sucrose plates~~
2. ~~Streak out pKL116 primary~~
3. ~~Streak out Tn 7 samples~~

Struck out 4 ΔpmrA Tn7 samples on CHA-Kan to test by colony PCR tomorrow

Struck out pKL116-3 primary integrin to do sucrose selection on

Wednesday, Dec 4th , 2019

To ~~Do:~~

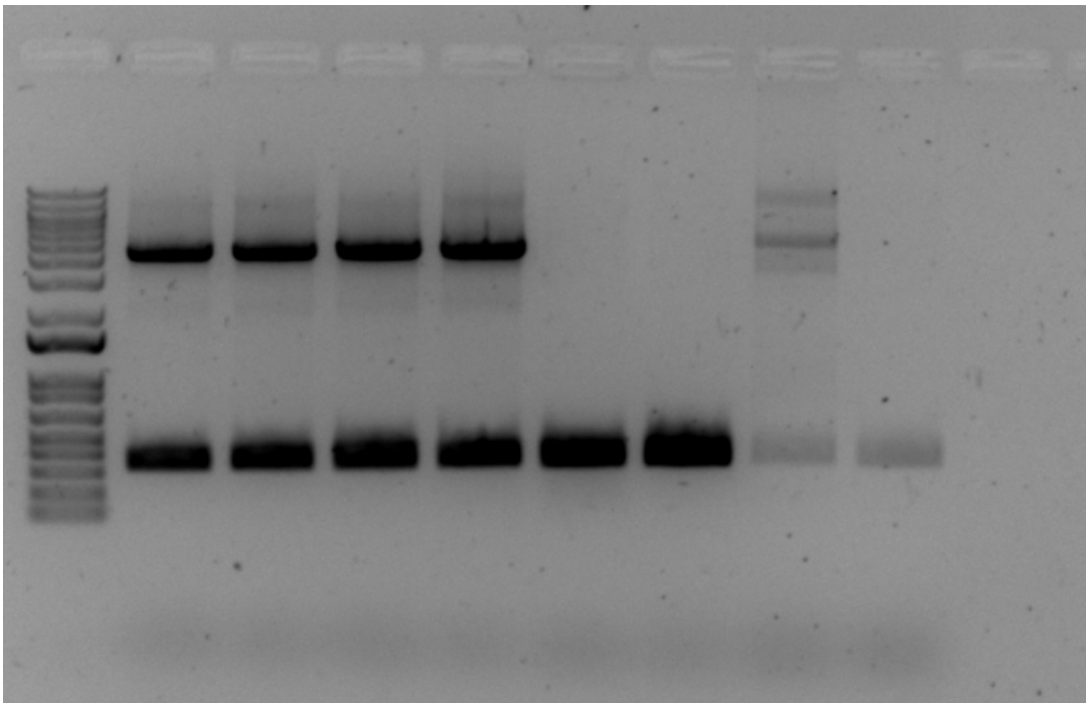
1. ~~Colony PCR of Tn7 samples~~
2. ~~Make electrocompetent cells~~
3. ~~Sucrose selection~~

Total reaction volume	20
Total number of reactions	8

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	9
ddiH2O			10.8	97.2
PrimeSTAR GXL Buffer	5x	1x	4	36
dNTPs	2.5 mM	0.2 mM	1.6	14.4
oligo F KROL252	10 uM	0.3 uM	0.6	5.4
oligo R KROL253	10 uM	0.3 uM	0.6	5.4
Template	100 ng/ul	2 ng/ul	2	

PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	3.6
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	ΔpmrA 1-1	2	KROL252, KROL253	2843
2	ΔpmrA 2-1	3	KROL252, KROL253	2843
3	ΔpmrA 2-2	4	KROL252, KROL253	2843
4	ΔpmrA 2-3	5	KROL252, KROL253	2843
5	LVS Colony	6	KROL252, KROL253	350
6	LVS gDNA	7	KROL252, KROL253	350
7	pKR57 plasmid	8	KROL252, KROL253	
8	- control (no template)	9	KROL252, KROL253	



Looks like there might be a mixed population. Faint band seen around 3kb could be desired insertion.

Performed sucrose selection on pKL116-3 primary integrin

Thursday, Dec 5th , 2019

To ~~Do~~:

1. ~~Electroporations with VSVG tag~~

Electroporated pKL75 into ΔpmrA mpk1B cells x3 and 1 control and in ΔpmrA no_C1 cells x2, the 2 in the no_C1 cells arced but still had a time constant of ~2.2

Friday, Dec 6th , 2019

To ~~Do~~:

1. ~~Pour plates for mac assay~~

Pour 1.5 flasks of square plates and 0.5 flasks of round plates for mac assay next week

Also poured 1 flask of hygromycin plates to follow up with the Tn7

Monday, Dec 9th , 2019

To ~~Do~~:

1. ~~Pour plates for mac assay~~

Cross patched 12 colonies from pKL116 primary integrin sucrose selection on CHA and CHA-Kan
Note: there were a mix of small and large colonies from the sucrose selection

Cross patched the single colonies from the ΔPmrA PriM+ onto CHA Hyg and CHA-Kan to see if they have lost the helper plasmid. Will do another colony PCR to try to find samples that are not a mixed population

Thawed 1 vial of J774A.1 P4 macrophage

Tuesday, Dec 10th , 2019

To ~~Do:~~

1. ~~Colony PCR of pKL116-3~~
2. ~~Seed macrophage~~
3. ~~Streak out mac assay strains~~
4. ~~Colony PCR of Tn7 samples~~

Confirm pKL116 with primers 703 and 704 and digest with SapI

Total reaction volume	20
Total number of reactions	15

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	16
ddiH2O			10.8	172.8
PrimeSTAR GXL Buffer	5x	1x	4	64
dNTPs	2.5 mM	0.2 mM	1.6	25.6
oligo F KROL252	10 uM	0.3 uM	0.6	9.6
oligo R KROL253	10 uM	0.3 uM	0.6	9.6
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	6.4
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	P703, P704	500
2	2	3	P703, P704	500
3	3	4	P703, P704	500
4	4	5	P703, P704	500
5	5	6	P703, P704	500
6	6	7	P703, P704	500
7	7	8	P703, P704	500
8	8	9	P703, P704	500

9	9	10	P703, P704	500
10	10	11	P703, P704	500
11	11	12	P703, P704	500
12	12	13	P703, P704	500
13	LVS gDNA	14	P703, P704	500
14	pKR57 plasmid	15	P703, P704	
15	- control (no template)	16	P703, P704	

Patched out 4 colonies from ΔPmrA mpk-1 electroporation with pKL75 to CHA-Kan

Diagnostic Digest:
Buffer: 2ul (per sample)
DNA: 15.0ul (per sample)
Enzyme: 0.5ul (per sample)
Don't digest or purify plasmid or LVS colony control

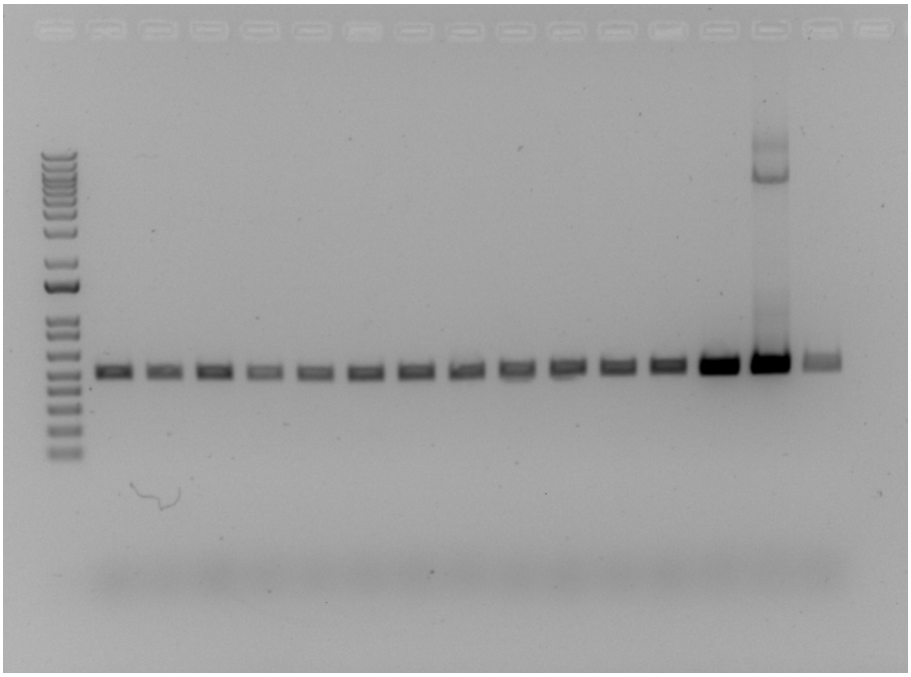
Components	**Volumes in 1 reaction (uL)**	**Volumes in Master Mix13 x (uL)**
H_2O	2.5	32.5
10x Buffer*	2.0	26.0
DNA	(15.0)	-
Enzyme (Sap I)	0.5	6.5
Total	20.0 (15.0 actual b/c of DNA)	

Total reaction volume	20
Total number of reactions	19

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	20
ddiH2O			10.8	216
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL252	10 uM	0.3 uM	0.6	12
oligo R KROL253	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	2	

PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	ΔpmrA 1-1 A	2	KROL252, KROL253	2843
2	ΔpmrA 1-1 B	2	KROL252, KROL253	2843
3	ΔpmrA 1-1 C	2	KROL252, KROL253	2843
4	ΔpmrA 1-1 D	2	KROL252, KROL253	2843
5	ΔpmrA 2-1 A	3	KROL252, KROL253	2843
6	ΔpmrA 2-1 B	4	KROL252, KROL253	2843
7	ΔpmrA 2-1 C	4	KROL252, KROL253	2843
8	ΔpmrA 2-1 D	4	KROL252, KROL253	2843
9	ΔpmrA 2-2 A	5	KROL252, KROL253	2843
10	ΔpmrA 2-2 B	5	KROL252, KROL253	2843
11	ΔpmrA 2-2 C	5	KROL252, KROL253	2843
12	ΔpmrA 2-2 D	5	KROL252, KROL253	2843
13	ΔpmrA 2-3 A	5	KROL252, KROL253	2843
14	ΔpmrA 2-3 B	5	KROL252, KROL253	2843
15	ΔpmrA 2-3 C	5	KROL252, KROL253	2843
16	ΔpmrA 2-3 D	5	KROL252, KROL253	2843
17	LVS gDNA	7	KROL252, KROL253	350
18	pKR57 plasmid	8	KROL252, KROL253	
19	- control (no template)	9	KROL252, KROL253	



Gel from colony PCR of pKL116 primary integrin 3. All samples seem to be wildtype as they were not digested by SapI, got contamination in the negative control again, I suspect its these primers

Wednesday, Dec 11th , 2019

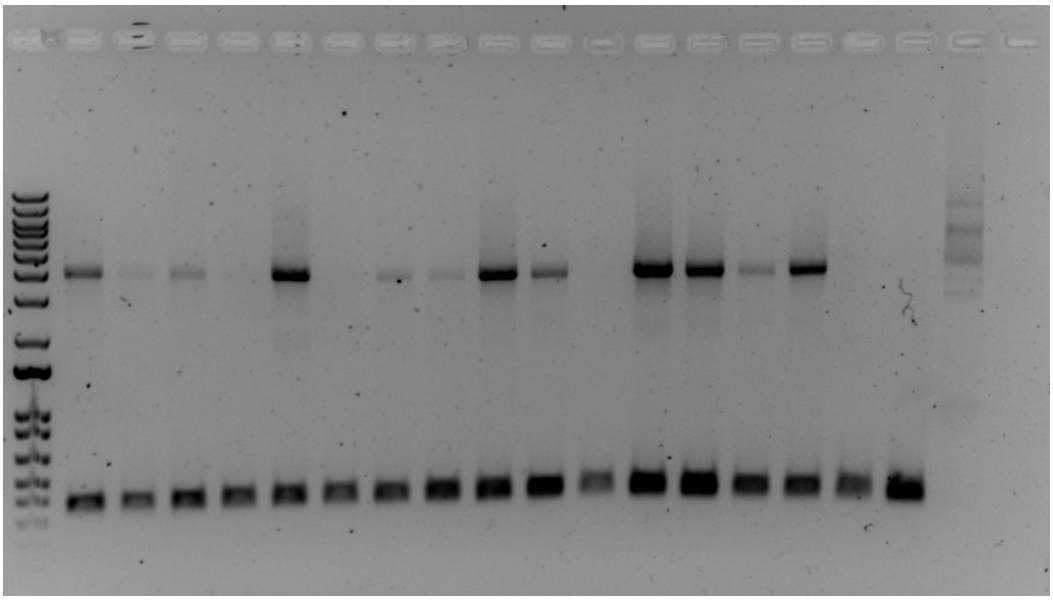
To ~~Do:~~

1. ~~Redo sucrose selection of pKL116-3~~
2. ~~Mac assay day 1~~
3. ~~Make LVS PriM+ EC~~
4. ~~Run gel of Tn7 colony PCR~~

Performed sucrose selection on ΔpmrA pKL116-3 again to try and sample more colonies

Made LVS PriM+ EC cells to try and delete PmrA in that background since the Tn7 hasn't been working

Started mac assay testing the PriM mtip2 mutants



Gel from ΔPmrA Tn7 colony PCR, single colonies were cross patched and about 75% had lost the Hyg resistance, however it still seems like there is a mixed population even after streaking to single colony, likely means the PriM integrated into the wrong region and may not be worth continuing on with

Thursday, Dec 12th , 2019

To Do:

1. ~~Mac assay day 2~~
2. ~~Electroporations~~
3. ~~Pour plates for next mac assay~~

Electroporated pKL37 into LVS PriM+ (3x with 1 control) and pKL75 into ΔpmrA no_c1 x2

Friday, Dec 13th , 2019

To Do:

1. ~~Electroporations~~
2. ~~Pour plates for next mac assay~~
3. ~~Thaw cells~~

Electroporated pKL75 into ΔpmrA mpk1 x3 with one control to try to tag PriM with the VSVG tag in this background

Poured 2 more flasks of round CHA plates for mac assay
Thawed 1 vial of P4 J774A.1 cells

Sunday, Dec 15th , 2019

To Do:

1. ~~Check electroporations~~
2. ~~Seed for mac assay~~
3. ~~Streak out strains for mac assay~~

Struck out 4 colonies from LVS PriM+ electroporation with pKL37 trying to delete PmrA onto CHA-Kan
Struck out 2 colonies from pKL75 into ΔPmrA no_C1 electroporation onto CHA-Kan

Struck out LVS, LVS no_C1, ΔPmrA no_C1, and ΔPmrA onto CHA plates for mac assay
Seeded macrophage (P4) for mac assay

Monday, Dec 16th , 2019

To Do:

1. ~~Check electroporations~~
2. ~~Mac assay day 1~~
3. ~~Freeze down primary integrins~~

Froze down one primary integrin from pKL37 PriM+ to try sucrose selection next week

Tuesday, Dec 17th , 2019

To Do:

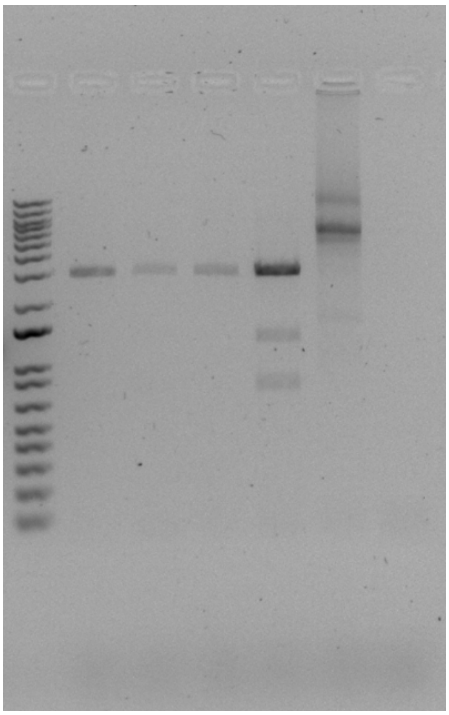
1. ~~Check electroporations~~
2. ~~Mac assay day 2~~
3. ~~Freeze down primary integrins~~

Going to check for VSVG tag integration using primers KROL150 and KROL240

Total reaction volume	20
Total number of reactions	6

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	7
ddiH2O			10.8	75.6
PrimeSTAR GXL Buffer	5x	1x	4	28
dNTPs	2.5 mM	0.2 mM	1.6	11.2
oligo F KROL	10 uM	0.3 uM	0.6	4.2
oligo R KROL	10 uM	0.3 uM	0.6	4.2
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	2.8
		Total volume	20	140

Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL150, KROL240	~1000
2	2	3	KROL150, KROL240	~1000
3	3	4	KROL150, KROL240	~1000
4	LVS gDNA	5	KROL150, KROL240	
5	pKR75 plasmid	6	KROL150, KROL240	
6	- control (no template)	7	KROL150, KROL240	



Non-specific bands coming up for all 3 potential integrins as well as in the gDNA with desired product coming up at around 1000 bp

Going to try again with primers KROL 150 and P035, and KROL150 and KROL276

Total reaction volume	20
Total number of reactions	6

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	7
ddiH2O			10.8	75.6
PrimeSTAR GXL Buffer	5x	1x	4	28
dNTPs	2.5 mM	0.2 mM	1.6	11.2

oligo F KROL	10 uM	0.3 uM	0.6	4.2
oligo R KROL	10 uM	0.3 uM	0.6	4.2
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	2.8
		Total volume	20	140

Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL150, KROL276	~1000
2	2	3	KROL150, KROL276	~1000
3	3	4	KROL150, KROL276	~1000
4	LVS gDNA	5	KROL150, KROL276	
5	pKR75 plasmid	6	KROL150, KROL276	
6	- control (no template)	7	KROL150, KROL276	

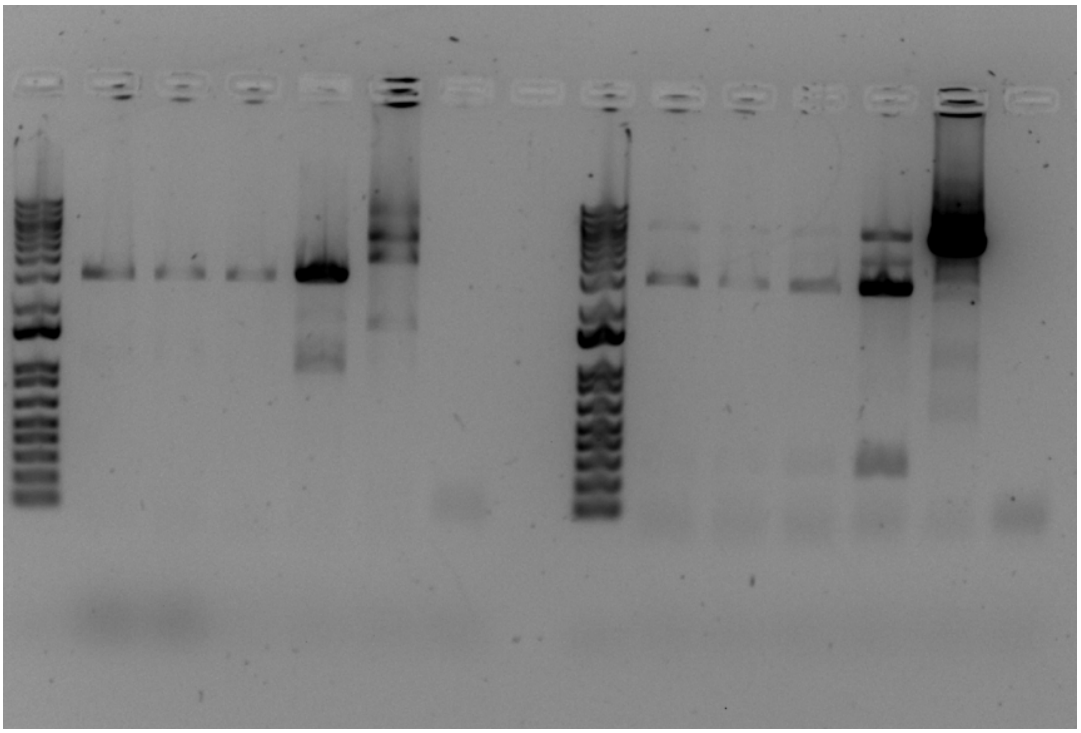
Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL150, P035	~1000
2	2	3	KROL150, P035	~1000
3	3	4	KROL150, P035	~1000
4	LVS gDNA	5	KROL150, P035	
5	pKR75 plasmid	6	KROL150, P035	
6	- control (no template)	7	KROL150, P035	

Struck out the 3 VSVG tagged primary integrins in ΔpmrA no_C1 for possible western tomorrow with Hannah

Wednesday, Dec 18th , 2019

To Do:

1. ~~Grow up cultures to test by western~~
2. ~~Make electrocompetent cells~~
3. ~~PCR gDNA from strains for sequencing~~



Total reaction volume	50
Total number of reactions	4

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	5
ddiH2O			31	155
PrimeSTAR GXL Buffer	5x	1x	10	50
dNTPs	2.5 mM	0.2 mM	4	20
oligo F	10 uM	0.3 uM	1.5	7.5
oligo R	10 uM	0.3 uM	1.5	7.5
template	100 ng/ul	2 ng/ul	1	5
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	1	5
		Total volume	50	250

Reaction Number	Sample	Lane on gel	Primers	Size

1	ΔpmrA no_C1	2	KROL151, P703	~1000
2	ΔpmrA mpk1	3	KROL151, P703	~1000
3	ΔpmrA mtip2	4	KROL151, P703	~1000
4	LVS PriM+	5	KROL252, KROL253	

PCR reactions were purified in preparation for sequencing

Thursday, Dec 19th , 2019

To Do:

1. ~~Colony PCR on pKL116-3~~
2. ~~Western for VSVG tagged strains~~

Confirm pKL116 with primers 703 and 704 and digest with SapI

Total reaction volume	20
Total number of reactions	19

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	20
ddiH2O			10.8	216
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL252	10 uM	0.3 uM	0.6	12
oligo R KROL253	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

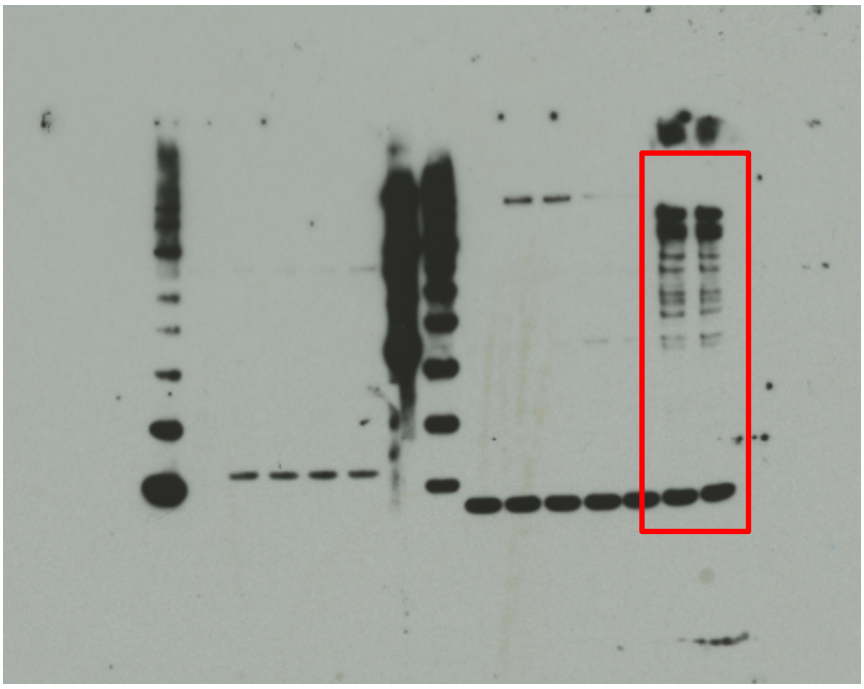
Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	P703, P704	500
2	2	3	P703, P704	500
3	3	4	P703, P704	500
4	4	5	P703, P704	500
5	5	6	P703, P704	500
6	6	7	P703, P704	500
7	7	8	P703, P704	500
8	8	9	P703, P704	500

9	9	10	P703, P704	500
10	10	11	P703, P704	500
11	11	12	P703, P704	500
12	12	13	P703, P704	500
13	13	14	P703, P704	500
14	14	15	P703, P704	500
15	15	16	P703, P704	500
16	16	17	P703, P704	500
17	LVS gDNA	18	P703, P704	500
18	pKL116 plasmid	19	P703, P704	500
19	- control (no template)	20	P703, P704	500

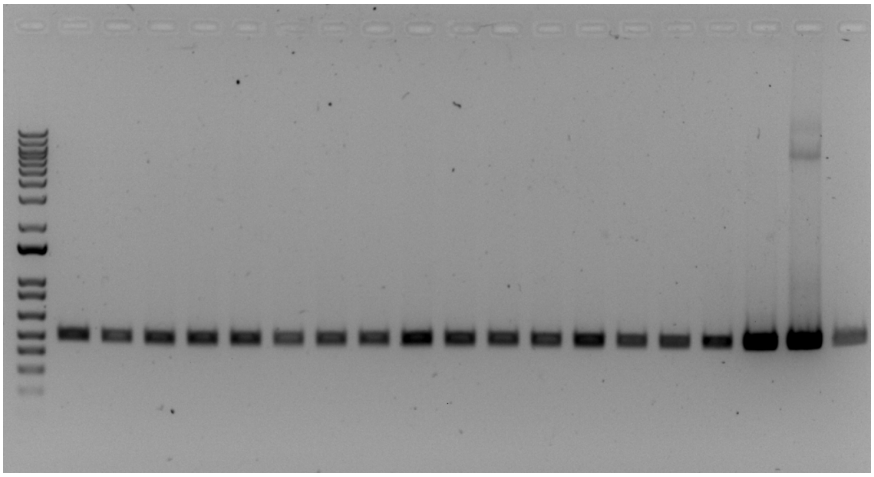
Samples were PCR purified in preparation for diagnostic digest

Diagnostic Digest:
Buffer: 2ul (per sample)
DNA: 15.0ul (per sample)
Enzyme: 0.5ul (per sample)
Don't digest or purify plasmid or LVS colony control

Components	**Volumes in 1 reaction (uL)**	**Volumes in Master Mix17 x (uL)**
H_2O	2.5	42.5
10x Buffer*	2.0	34.0
DNA	(15.0)	-
Enzyme (Sap I)	0.5	8 .5
Total	20.0 (15.0 actual b/c of DNA)	



Western blot testing the 2 potentially VSVG tagged ΔPmrA no_C1 strains samples are the last 2 on the right in red box. PriM should run at ~53kDa and the highest bands are running around 80-100kDa



None of the pKL116-3 colonies have the mutation. Best to try making a new primary integrin.
Either P703 or P704 is definitely contaminated, so next time going to check with primers KROL151 and KROL152 that will make a product of about 1021bp and SapI will cut around 700 and 300 for the mutation

Monday, Dec 30th , 2019

To Do:

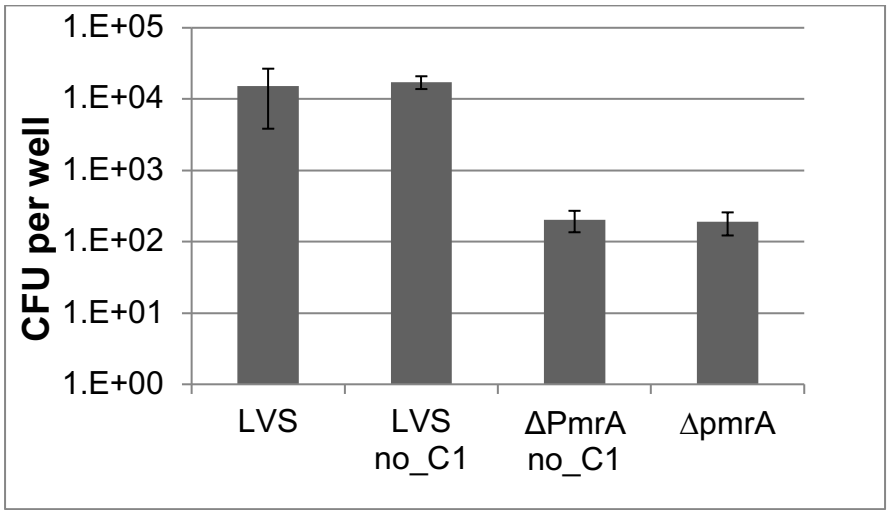
1. ~~Electroporations~~

2. ~~Streak out primary integrins for sucrose selection~~
3. ~~Streak out ΔpmrA to make new glycerol stocks~~

Electroporated pKL75 into ΔpmrA mpk1 cells (x3 and 1 control) and electroporated pKL116 into ΔpmrA (x2)

Going to streak out the ΔpmrA PriM+ primary integrin and LVS pKL116 primary integrins

- Struck out ΔpmrA PriM+ and LVS pKL116 1-1 and 1-2



Results from the mac assay from before break confirming the results of the cysteine mutant which showed again that removing the disulfide bond seems not to restore virulence

Tuesday, Dec 31th , 2019

To Do:

1. ~~Sucrose selection~~

Performed sucrose selection on LVS pKL116 (1-1) and on ΔpmrA PriM+ primary integrins

Thursday, Jan 2nd , 2020

To Do:

1. ~~Electroporations~~
2. ~~Check sucrose selection~~

Electroporated pKL75 into ΔpmrA mtip2 (x2 and 1 control) and electroporated pKL116 into ΔpmrA (x2)

Sucrose selection from Tuesday (LVS pKL116) already has some colonies so going to cross patch them for colony PCR tomorrow (patched out 16 colonies)

Friday, Jan 3rd , 2020

To Do:

1. ~~Electroporations~~
2. ~~Colony PCR~~

Either P703 or P704 is definitely contaminated, so next time going to check with primers KROL151 and KROL152 that will make a product of about 1021bp and SapI will cut around 700 and 300 for the mutation

Total reaction volume	20
Total number of reactions	14

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	15
ddiH2O			10.8	162
PrimeSTAR GXL Buffer	5x	1x	4	60
dNTPs	2.5 mM	0.2 mM	1.6	24
oligo F KROL252	10 uM	0.3 uM	0.6	9
oligo R KROL253	10 uM	0.3 uM	0.6	9
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	6
		Total volume	20	400

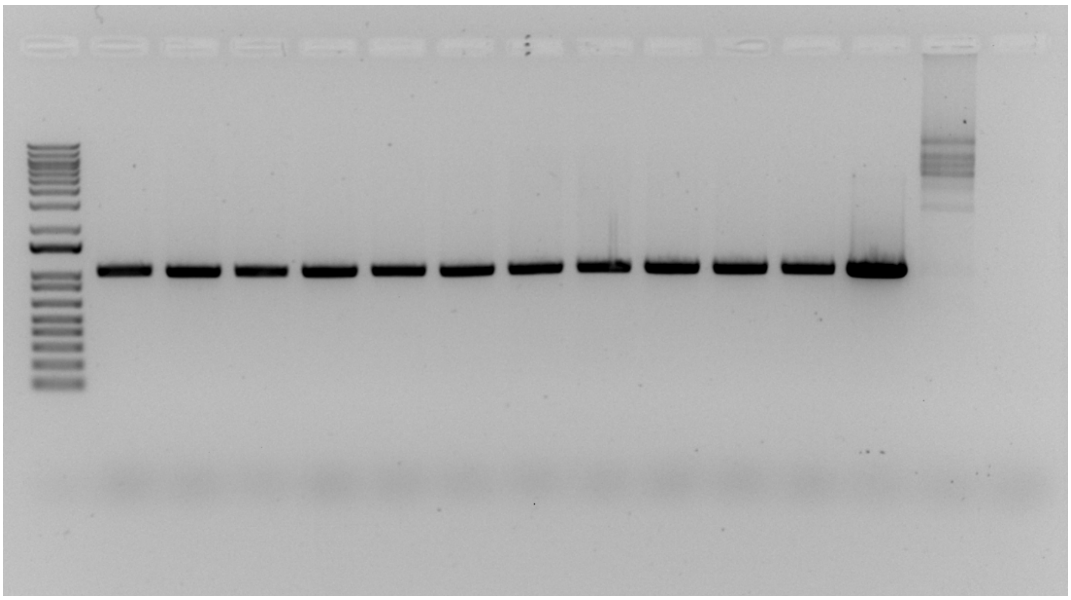
Reaction Number	Sample	Lane on gel	Primers	Size
1	2	2	KROL151, KROL152	1021
2	3	3	KROL151, KROL152	1021
3	4	4	KROL151, KROL152	1021
4	6	5	KROL151, KROL152	1021
5	9	6	KROL151, KROL152	1021
6	10	7	KROL151, KROL152	1021
7	11	8	KROL151, KROL152	1021
8	12	9	KROL151, KROL152	1021

9	13	10	KROL151, KROL152	1021
10	14	11	KROL151, KROL152	1021
11	15	12	KROL151, KROL152	1021
17	LVS gDNA	13	KROL151, KROL152	1021
18	pKL116 plasmid	14	KROL151, KROL152	1021
19	- control (no template)	15	KROL151, KROL152	1021

Samples were PCR purified in preparation for diagnostic digest

Diagnostic Digest:
Buffer: 2ul (per sample)
DNA: 15.0ul (per sample)
Enzyme: 0.5ul (per sample)
Don't digest or purify plasmid or LVS colony control

Components	**Volumes in 1 reaction (uL)**	**Volumes in Master Mix12 x (uL)**
H_2O	2.5	30.0
10x Buffer*	2.0	24.0
DNA	(15.0)	-
Enzyme (Sap I)	0.5	6
Total	20.0 (15.0 actual b/c of DNA)	



Seems as though none of the samples worked again, but looked up SapI enzyme and NEB website mentions that the enzyme may not be working because it needs to be mixed in the tube, so going to retry the digest after mixing the tube and also try to use a plasmid that is cut by SapI as a control.

Monday, Jan 6th , 2020

To Do:

1. ~~Electroporations~~
2. ~~Colony PCR~~
3. ~~Seed for mac assay~~

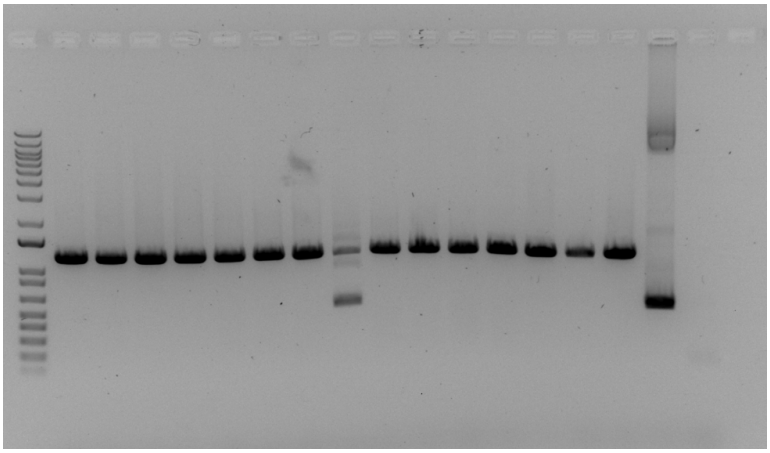
Colony PCR to check for deletion of pmrA in the LVS PriM+ background

Total reaction volume	20
Total number of reactions	17

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	18
ddiH2O			10.8	194.4
PrimeSTAR GXL Buffer	5x	1x	4	72
dNTPs	2.5 mM	0.2 mM	1.6	28.8
oligo F KROL252	10 uM	0.3 uM	0.6	10.8
oligo R KROL253	10 uM	0.3 uM	0.6	10.8
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	7.2
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL61, KROL62	1204
2	2	3	KROL61, KROL62	1204

3	3	4	KROL61, KROL62	1204
4	4	5	KROL61, KROL62	1204
5	5	6	KROL61, KROL62	1204
6	6	7	KROL61, KROL62	1204
7	7	8	KROL61, KROL62	1204
8	8	9	KROL61, KROL62	1204
9	10	10	KROL61, KROL62	1204
10	11	11	KROL61, KROL62	1204
11	12	12	KROL61, KROL62	1204
12	13	13	KROL61, KROL62	1204
13	14	14	KROL61, KROL62	1204
14	15	15	KROL61, KROL62	1204
15	LVS gDNA	16	KROL61, KROL62	1204
16	pKL37 plasmid	17	KROL61, KROL62	1204
17	- control (no template)	18	KROL61, KROL62	1204



All but 1 seem to be wildtype where pmrA was not deleted. Sample number 8 (patch #8) seems to be a mixed population where some of the cells actually have pmrA deleted. Going to streak this patch to single colony. Struck to single colony on 2 plates to increase the number of colonies.

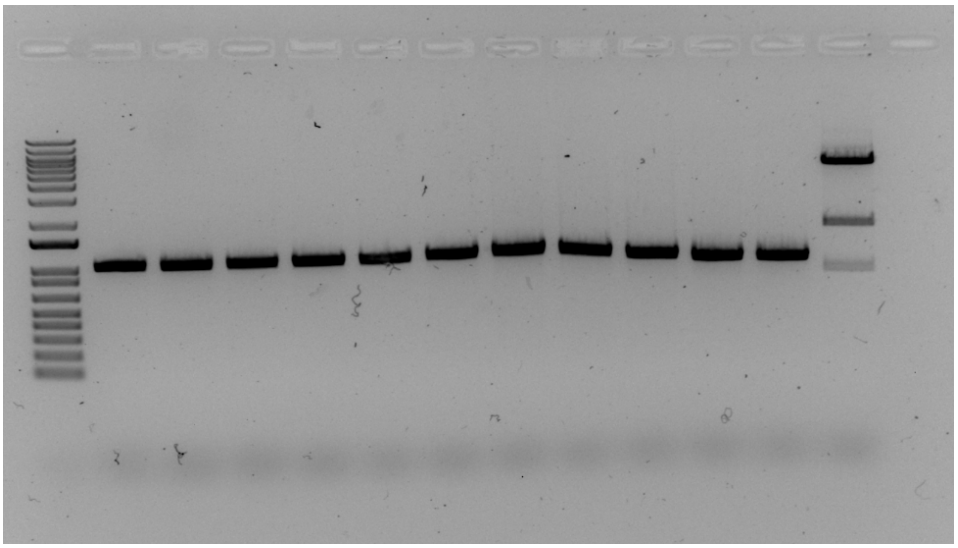
Electroporated pKL75 into ΔpmrA mpk1 (x3) and ΔpmrA mtip2 (x2) to try to tag these strains with the VSVG tag

Diagnostic Digest:
Buffer: 2ul (per sample)
DNA: 15.0ul (per sample)
Enzyme: 0.3ul (per sample)
Don't digest or purify plasmid or LVS colony control

Components	**Volumes in 1 reaction (uL)**	**Volumes in Master Mix12 x (uL)**
H_2O	2.7	32.4

10x Buffer*	2.0	24.0
DNA	(15.0)	-
Enzyme (Sap I)	0.3	3.6
Total	20.0 (15.0 actual b/c of DNA)	

pKL116 is being used as a positive control and is cut 3 times with SapI bands should be seen at 846bp, 1664bp, 2987bp, and 477bp.



SapI enzyme worked on positive control plasmid, so it wasn't the enzyme that was an issue.

Struck out strains for mac assay. Going to be testing the mtip2 mutants

Tuesday, Jan 7th , 2020

To Do:

1. ~~Mac assay~~
2. ~~Check electroporations~~

Got one colony on ΔpmrA mtip2 pKL75 electroporation that I patched onto CHA-Kan

Wednesday, Jan 8th , 2020

To Do:

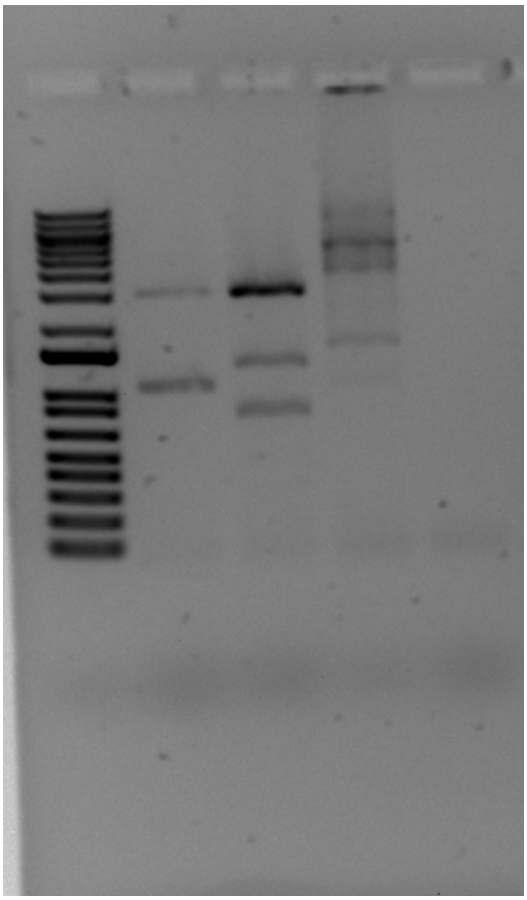
1. ~~Mac assay day 2~~
2. ~~Test primary integrin for VSVG tag~~

Going to try again with primers KROL 150 and P035, and KROL276, and KROL240

Total reaction volume	20
Total number of reactions	4

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	5
ddiH2O			10.8	54
PrimeSTAR GXL Buffer	5x	1x	4	20
dNTPs	2.5 mM	0.2 mM	1.6	8
oligo F KROL150	10 uM	0.3 uM	0.6	3
oligo R KROL240	10 uM	0.3 uM	0.6	3
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	2
		Total volume	20	140

Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL150, KROL240	~1000
2	LVS gDNA	3	KROL150, KROL240	
3	pKR75 plasmid	4	KROL150, KROL240	
4	- control (no template)	5	KROL150, KROL240	



Looks like sample has a band at the correct size indicating that the strain would have the VSVG but there is another band that shows up in the gDNA lane as well but may be just spillover

Struck out 2 primary integrins for pKL116, LVS 2-1 and 2-2 for sucrose selection

Thursday, Jan 9th , 2020

To Do:

1. ~~Sucrose selection~~
2. ~~Colony PCR~~
3. ~~Electroporations~~

Electroporated pKL116 into ΔpmrA (x4) and pKL75 into ΔpmrA mpk1 (x2)
Only one of the primary integrins grew that was struck out yesterday so performed sucrose selection on LVS pKL116 2-1
Colony PCR to check for deletion of pmrA in the LVS PriM+ background

Total reaction volume	20

Total number of reactions	17

Component	Stock concentration	Final concentration	1 rxn volume	Factor 18
ddiH2O			10.8	194.4
PrimeSTAR GXL Buffer	5x	1x	4	72
dNTPs	2.5 mM	0.2 mM	1.6	28.8
oligo F KROL252	10 uM	0.3 uM	0.6	10.8
oligo R KROL253	10 uM	0.3 uM	0.6	10.8
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	7.2
		Total volume	20	400

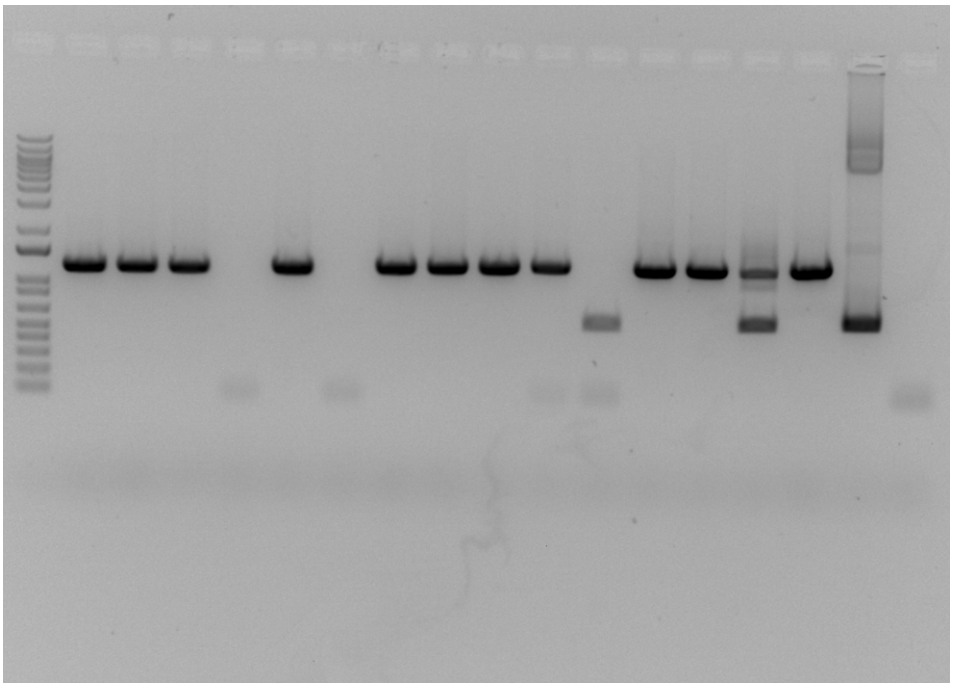
Reaction Number	Sample	Lane on gel	Primers	Size
1	17	2	KROL61, KROL62	1204
2	18	3	KROL61, KROL62	1204
3	19	4	KROL61, KROL62	1204
4	20	5	KROL61, KROL62	1204
5	21	6	KROL61, KROL62	1204
6	22	7	KROL61, KROL62	1204
7	23	8	KROL61, KROL62	1204
8	24	9	KROL61, KROL62	1204
9	25	10	KROL61, KROL62	1204
10	26	11	KROL61, KROL62	1204
11	27	12	KROL61, KROL62	1204
12	28	13	KROL61, KROL62	1204
13	8-3	14	KROL61, KROL62	1204
14	8-8	15	KROL61, KROL62	1204
15	LVS gDNA	16	KROL61, KROL62	1204
16	pKL37 plasmid	17	KROL61, KROL62	1204
17	- control (no template)	18	KROL61, KROL62	1204

Going to try sucrose selection again with the dPmrA PriM+ primary integrin and plate more of the lower dilutions to try and get good single colonies

Friday, Jan 10th , 2020

To Do:

1. ~~Sucrose selection~~
2. ~~Electroporations~~
3. ~~Run gel~~



Gel from yesterday's colony PCR, trying to delete pmrA from LVS PriM+ strain. It looks like patch number 27 has the deletion and so far does not show growth on CHA-Kan. Will streak to single colony and hopefully by Monday I will have the more diagnostic primers that will show the deletion but will not amplify the plasmid.

Made electrocompetent cells of ΔpmrA no_c1 and ΔpmrA mpk-1 and the used these cells for electroporations of pKL75 (2 for each strain and a control for each strain) with 1 control per strain

Started counting plates from the macrophage assay and contamination is seen throughout all the plates, but less on the 24 hour plates (probably bc the macrophage killed off the contaminant) and no contamination was seen in the LVS only well which means it may have been in the macrophage the whole time.
Re did the sucrose selection of pKL37 PriM+ primary integrant with 2 10^-5 and 2 10^-6 plates to have a better chance of getting more smaller single colonies

Monday, Jan 13th , 2020

To Do:

1. ~~Pour plates for mac assay~~
2. ~~Patch out colonies~~
3. ~~Check electroporations~~
4. ~~Thaw macrophage~~

Thawed one vial of P4 macrophage to re do last weeks mac assay
Cross patched 9 single colonies from the dpmrA PriM+ strain and going to try colony PCR tm with the new primers that wont amplify the plasmid

Tuesday, Jan 14th, 2020

To Do:

1. ~~Pour plates for mac assay~~
2. ~~Patch out colonies~~
3. ~~Check electroporations~~
4. ~~Colony PCR~~

Patched out some primary integrins for ΔpmrA mpk1 and ΔpmrA no_c1 pKL75 primary integrins

Cross patched 16 colonies from sucrose plates of LVS pKL116 2-1 onto CHA and CHA-kan

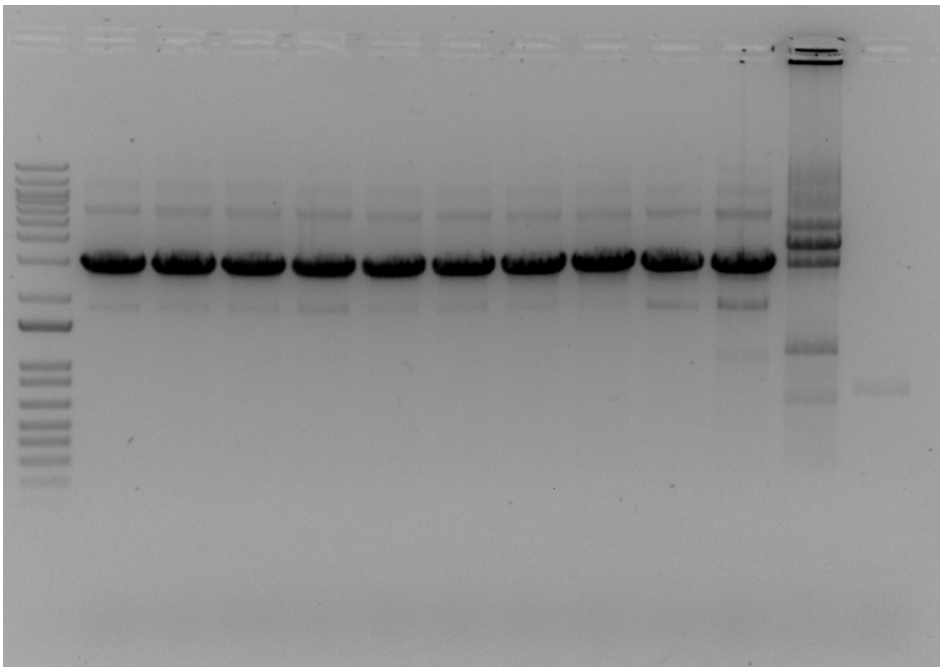
Colony PCR to check for deletion of pmrA in the LVS PriM+ background

Total reaction volume	20
Total number of reactions	12

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	13
ddiH2O			10.8	140.4
PrimeSTAR GXL Buffer	5x	1x	4	52
dNTPs	2.5 mM	0.2 mM	1.6	20.8
oligo F KROL252	10 uM	0.3 uM	0.6	7.8
oligo R KROL253	10 uM	0.3 uM	0.6	7.8
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	5.2
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL308, KROL309	512
2	2	3	KROL308, KROL309	512
3	3	4	KROL308, KROL309	512
4	4	5	KROL308, KROL309	512

5	5	6	KROL308, KROL309	512
6	6	7	KROL308, KROL309	512
7	7	8	KROL308, KROL309	512
8	8	9	KROL308, KROL309	512
9	10	10	KROL308, KROL309	512
10	11	11	KROL308, KROL309	512
11	12	12	KROL308, KROL309	512
15	LVS gDNA	16	KROL308, KROL309	2952
16	pKL37 plasmid	17	KROL308, KROL309	
17	- control (no template)	18	KROL308, KROL309	



So it looks like I might have struck the wrong patch to single colony so I'm going back to the original plate to do a test PCR to make sure I get the same phenotype and restreak the correct patch to single colony

Colony PCR to check for deletion of pmrA in the LVS PriM+ background

Total reaction volume	20

Total number of reactions	12

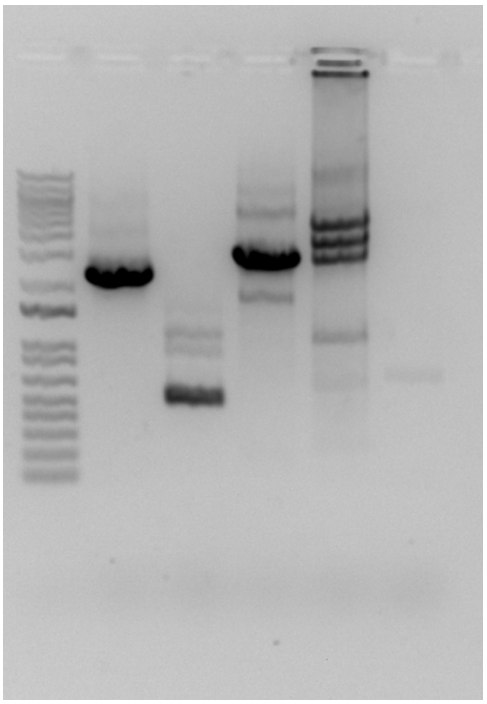
Component	Stock concentration	Final concentration	1 rxn volume	Factor 5
ddiH2O			10.8	54
PrimeSTAR GXL Buffer	5x	1x	4	20
dNTPs	2.5 mM	0.2 mM	1.6	8
oligo F KROL252	10 uM	0.3 uM	0.6	
oligo R KROL253	10 uM	0.3 uM	0.6	
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	2
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	dPmrA PriM+ 27	2	KROL308, KROL309	512
2	dPmrA PriM+ 27	3	KROL61, KROL62	512
15	LVS gDNA	16	KROL308, KROL309	2952
16	pKL37 plasmid	17	KROL308, KROL309	
17	- control (no template)	18	KROL308, KROL309	

Wednesday, Jan 15th , 2020

To Do:

1. ~~Check electroporations~~
2. ~~Colony PCR~~
3. ~~Seed macrophage~~
4. ~~Streak out strains for mac assay~~



Gel from test PCR of dpmrA PriM+ patch number 27. First lane shows the colony PCR from the patch using the new primers and the second lane shows the older primers. Deletion of pmrA should be around 2200bp using the new primers which looks correct

Total reaction volume	20
Total number of reactions	19

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	20
ddiH2O			10.8	216
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL151	10 uM	0.3 uM	0.6	12
oligo R KROL152	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

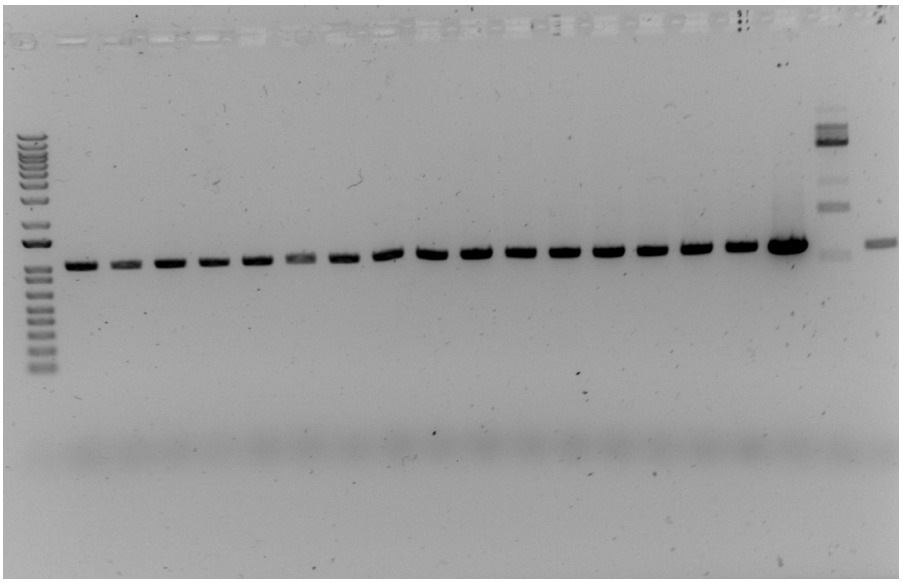
Reaction Number	Sample	Lane on gel	Primers	Size
1	2	2	KROL151, KROL152	1021
2	3	3	KROL151, KROL152	1021
3	4	4	KROL151, KROL152	1021

4	6	5	KROL151, KROL152	1021
5	9	6	KROL151, KROL152	1021
6	10	7	KROL151, KROL152	1021
7	11	8	KROL151, KROL152	1021
8	12	9	KROL151, KROL152	1021
9	13	10	KROL151, KROL152	1021
10	14	11	KROL151, KROL152	1021
11	15	12	KROL151, KROL152	1021
17	LVS gDNA	13	KROL151, KROL152	1021
18	pKL116 plasmid	14	KROL151, KROL152	1021
19	- control (no template)	15	KROL151, KROL152	1021

Samples were PCR purified in preparation for diagnostic digest

Diagnostic Digest:
Buffer: 2ul (per sample)
DNA: 15.0ul (per sample)
Enzyme: 0.3ul (per sample)
Don't digest or purify plasmid or LVS colony control

Components	**Volumes in 1 reaction (uL)**	**Volumes in Master Mix18 x (uL)**
H_2O	2.5	45.0
10x Buffer*	2.0	36.0
DNA	(15.0)	-
Enzyme (Sap I)	0.3	5.4
Total	20.0 (15.0 actual b/c of DNA)	



None of the samples cut again meaning that I don't have my mutation should try sequencing the plasmid just to make sure its correct

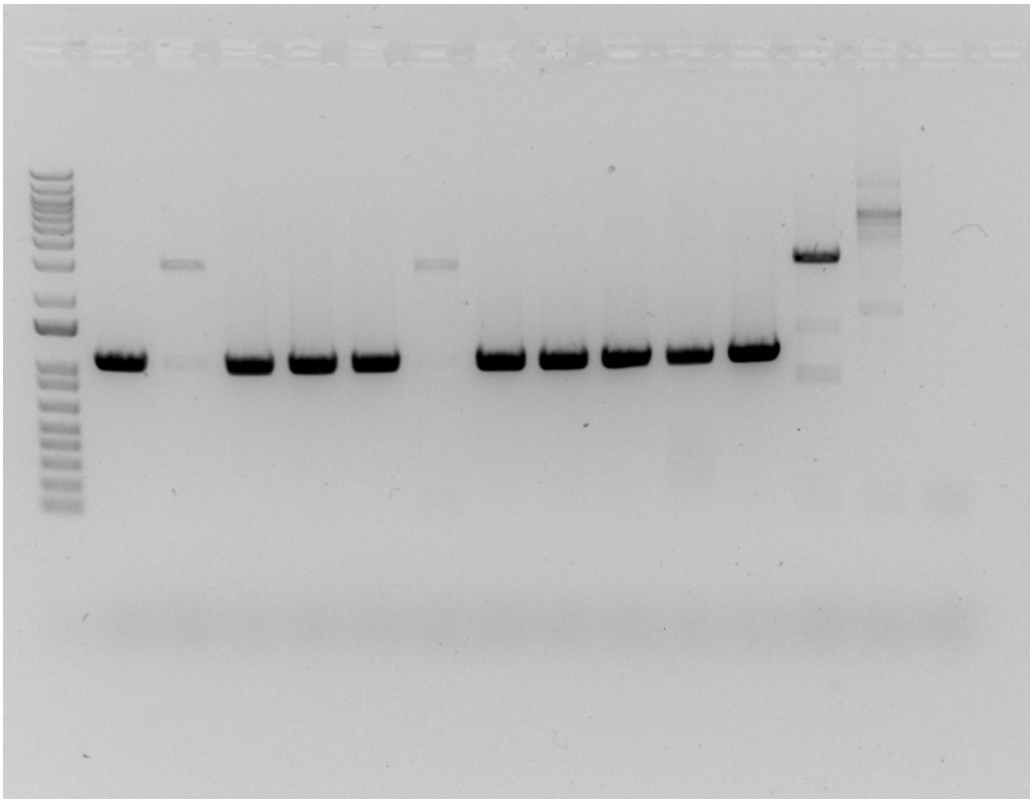
Colony PCR for checking integration of the VSVG tag from patches of electroporations

Total reaction volume	20
Total number of reactions	15

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	16
ddiH2O			10.8	172.8
PrimeSTAR GXL Buffer	5x	1x	4	64
dNTPs	2.5 mM	0.2 mM	1.6	25.6
oligo F KROL150	10 uM	0.3 uM	0.6	9.6
oligo R KROL240	10 uM	0.3 uM	0.6	9.6
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	6.4
		Total volume	20	140

Reaction Number	Sample	Lane on gel	Primers	Size
1	ΔpmrA no_C1-V-1	2	KROL150, KROL240	~1000
2	ΔpmrA no_C1-V-2	3	KROL150, KROL240	
3	ΔpmrA no_C1-V-3	4	KROL150, KROL240	

4	ΔpmrA no_C1-V-4	5	KROL150, KROL240	
5	ΔpmrA no_C1-V-5	6	KROL150, KROL240	
6	ΔpmrA no_C1-V-6	7	KROL150, KROL240	
7	ΔpmrA mpk1-V-1	8	KROL150, KROL240	
8	ΔpmrA mpk1-V-2	9	KROL150, KROL240	
9	ΔpmrA mpk1-V-3	10	KROL150, KROL240	
10	ΔpmrA mpk1-V-4	11	KROL150, KROL240	
11	ΔpmrA mpk1-V-5	12	KROL150, KROL240	
12	LVS gDNA	13	KROL150, KROL240	
13	pKR75 plasmid	14	KROL150, KROL240	
14	- control (no template)	15	KROL150, KROL240	



Going to freeze down 2 of each strain and will soon validate by western and need to run LVS, dpmrA PriM-V, and then start with 1 replicate per sample and then grow to single colony and do 3 biological replicates (patch out 3 single colonies)

For mac assay going to try streaking out strains later in the day and then picking the newest growth for the assay

Thursday, Jan 16th , 2020

To Do:

1. ~~Mac assay day 1~~
2. ~~Make cultures of the VSVG tagged strains for western~~
3. ~~Patch out single colonies of PriM+~~

Started 5mL cultures of ΔpmrA mtip2-V, ΔpmrA no_C1-V, and ΔpmrA mpk1-V with Kan at 0.1 OD going to take 1mL to spin down when OD is ~0.3-0.4 and resuspend with 1x SLB based on the OD

Froze down the strains at the following ODs:
ΔpmrA mtip2: 0.432
ΔpmrA no_C1: 0.445
ΔpmrA mpk1: 0.324

Friday, Jan 17th , 2020

To Do:

1. ~~Run gel and transfer for VSVG tagged strains~~
2. ~~Colony PCR~~

Lane	Volume	Contents
1	10ul	1x SLB
2	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
3	10ul	LVS
4	10ul	ΔpmrA PriM-V 1:2 (5ul sample, 5ul 1x SLB)
5	10ul	ΔpmrA mtip2-V 1:2 (5ul sample, 5ul 1x SLB)
6	10ul	ΔpmrA no_C1-V 1:2 (5ul sample, 5ul 1x SLB)
7	10ul	ΔpmrA mpk1-V 1:2 (5ul sample, 5ul 1x SLB)
8	10ul	1xSLB
9	10ul	1xSLB
10	10ul	Benchmark prestained ladder

Ran gel for about 1 hour at 150V and then did a wet transfer in the cold room at 20V for 1 hour and cut the top left corner (top of where the benchmark ladder is) and set in 10mL blocking buffer in the cold room shaking for over the weekend

Going to probe for VSVG tag and Tul4 (loading control) as the primary antibodies
In the past used Tul4 at 1:50,000 (anti mouse)
Use anti-VSVG at 1:2222 (anti rabbit), may do less because of how much PriM is produced

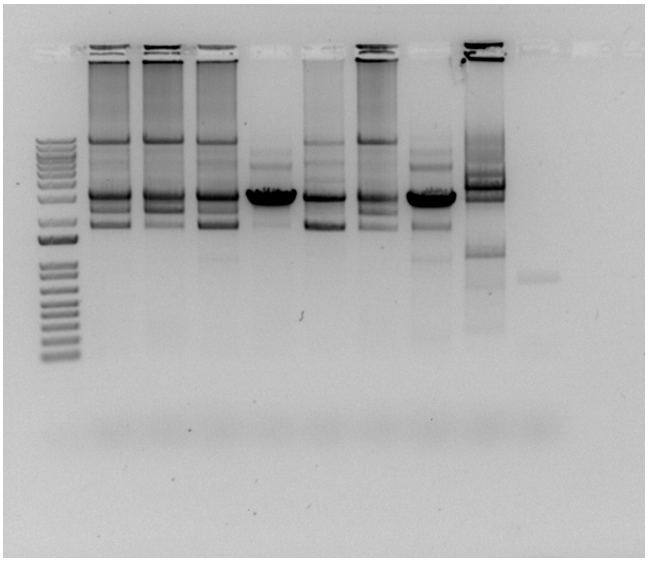
Colony PCR to check for deletion of pmrA in the LVS PriM+ background

Total reaction volume	20
Total number of reactions	9

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	10
ddiH2O			10.8	108
PrimeSTAR GXL Buffer	5x	1x	4	40
dNTPs	2.5 mM	0.2 mM	1.6	16
oligo F KROL252	10 uM	0.3 uM	0.6	6
oligo R KROL253	10 uM	0.3 uM	0.6	6
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	4
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	ΔpmrA PriM+ A	2	KROL308, KROL309	512
2	ΔpmrA PriM+ B	3	KROL308, KROL309	512
3	ΔpmrA PriM+ C	4	KROL308, KROL309	512
4	ΔpmrA PriM+ D	5	KROL308, KROL309	512
5	ΔpmrA PriM+ E	6	KROL308, KROL309	512
6	ΔpmrA PriM+ F	7	KROL308, KROL309	512
7	LVS gDNA	8	KROL308, KROL309	2952
8	pKL37 plasmid	9	KROL308, KROL309	

9	- control (no template)	10	KROL308, KROL309	



There is still a lot of primary integrant contamination so I struck out the patch again to single colony on 3 plates to get more spread out colonies

Monday, Jan 20th , 2020

To Do:

1. ~~Patch out single colonies~~
2. ~~Arbitrary PCR~~

Patched out 16 colonies of the ΔPmrA PriM+ single colonies to test tomorrow
Patched out 4 of each of the VSVG tagged strains on CHA-Kan to grow up in triplicate to test by western

Arbitrary PCR Protocol- Going to have 2 master mixes to test each of the arbitrary primers (KROL 87 + KROL89)
Primers with their sequences used for the arbitrary PCR

Primer	Purpose	Sequence
KROL87 Arb 1	Non-specific amplification	GGCCACGCGTCGACTAGTACNNNNNNNNNGATAT
KROL88 Arb 2	Amplifies the region from the first set of primers	GGCCACGCGTCGACTAGTAC
KROL89 Arb 6	Non-specific amplification	GGCCACGCGTCGACTAGTACNNNNNNNNNACGCC
KROL90 Mar1	Amplifies the transposon	tttgtcaaacaagctctcgattttaac
KROL92 Mar3	Amplifies the transposon	attaagtttatttttcaaagcttgaagg

Example:

Sample number	DNA
1	pSD26 Tn insertion 1 (Tn3) (KROL87)
2	pSD26 Tn insertion 2 (Tn6) (KROL87)
3	LVS gDNA (KROL87)
4	-DNA (KROL87)
5	pSD26 Tn insertion 1 (Tn3) (KROL89)
6	pSD26 Tn insertion 2 (Tn6) (KROL89)
7	LVS gDNA (KROL89)
8	-DNA (KROL89)

3) Set up reaction 1, using 500 ng of gDNA in a total reaction volume of 25 uL. See worksheet for details.

Reaction 1		**# of reactions here:**		4
		# of reactions allowing for error:		5.3
			Volume of each component (uL)	
Component	**Stock Concentration**	**Final Concentration**	**Per reaction**	**Master Mix**
ddiH_2O	-	-	14.245	75.5
OneTaq Rxn Buffer	5X	1X	5	26.5
dNTPs	10 mM	0.2 mM	0.5	2.7
KROL87 Arb1 (or KROL89 Arb6?)	100 µM	4.0 µM	1	5.3
Internal Specific Primer: KROL90 Tn_Mar1	10 µM	0.4 µM	1	5.3
Template	500 ng	160 ng/µL	3.13	-
OneTaq Polymerase	5 units/uL	6.25 units	0.125	0.7
Total volume			25	132.5

Thermocycler program:	**Step**	**Temp**	**Time**
	1	95°C	5'
	2	94°C	30"
	3	30°C	30"
	4	72°C	60"
	5	Go to step 2, repeat 5x	
	6	94°C	30"
	7	65°C	30"
	8	72°C	2'
	9	Go to step 6, repeat 29x	
	10	72°C	5'
	11	12°C	hold

For western take 5mL of 1:50,000 tul-4 and dilute 1:2 with blocking buffer Kathryn is going to check what concentration I should use the VSVG antibody at
Make standard VSVG aliquot and take 250ul of that dilution and add to 9.75mL of blocking buffer (~1:44000)

Tuesday, Jan 21st, 2020

To Do:

1. ~~Develop western~~
2. ~~Arbitrary PCR (second round)~~
3. ~~Patch out more single colonies~~

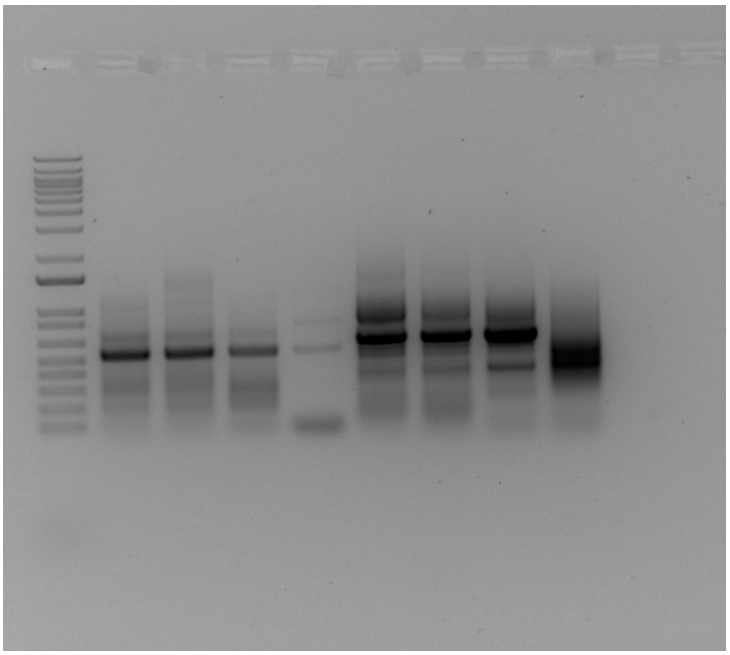
For VSVG western using a 1:44000 dilution of VSVG and 1:100,000 of tul4 for loading control saved the rest of the standard VSVG aliquot (1:2222) in the -20C

Arbitrary PCR round 2:

Reaction 2		**# of reactions here:**		8
		# of reactions allowing for error:		9.3
			Volume of each component (uL)	
Component	**Stock Concentration**	**Final Concentration**	**Per reaction**	**Master Mix**
ddiH_2O	_	_	16.625	154.6
OneTaq Rxn Buffer	5X	1X	5	46.5
dNTPs	10 mM	0.2 mM	0.5	4.65
KROL88 Arb2	100 µM	4.0 µM	1	9.3
External Specific Primer: KROL92 Tn_Mar3	10 µM	0.4 µM	1	9.3
PCR 1 Amplicon, diluted 1:10	-	-	0.75	-
OneTaq Polymerase	5 units/uL	6.25 units	0.125	1.16
Total volume			25	225.5

Thermocycler program:	**Step**	**Temp**	**Time**
	1	94°C	30"
	2	65°C	30"
	3	72°C	30"
	4	Go to step 1, repeat 29x	
	5	72°C	5'
	6	12°C	hold

Sample number	DNA	Lane on gel
1	pSD26 Tn insertion 1 (Tn3) (KROL87)	2
2	pSD26 Tn insertion 2 (Tn6) (KROL87)	3
3	LVS gDNA (KROL87)	4
4	-DNA (KROL87)	5
5	pSD26 Tn insertion 1 (Tn3) (KROL89)	6
6	pSD26 Tn insertion 2 (Tn6) (KROL89)	7
7	LVS gDNA (KROL89)	8
8	-DNA (KROL89)	9



Got product for both primer sets, but have negative control contamination and the bands that are showing up in the transposon samples are the same size as the LVS gDNA (which should ideally have no bands) Looked back at Daniel's notes and he tried doing 1:100 as well as 1:10 for the second round of arb PCR, so I am going to try that as well and include another negative control, but we may also have to play around with the annealing temps in the protocol.

Primers with their sequences used for the arbitrary PCR

Primer	Purpose	Sequence
KROL87 Arb 1	Non-specific amplification	GGCCACGCGTCGACTAGTACNNNNNNNNNGATAT
KROL88 Arb 2	Amplifies the region from the first set of primers	GGCCACGCGTCGACTAGTAC
KROL89 Arb 6	Non-specific amplification	GGCCACGCGTCGACTAGTACNNNNNNNNNNACGCC
KROL90 Mar1	Amplifies the transposon	tttgtcaaacaagctctcgattttaac
KROL92 Mar3	Amplifies the transposon	attaagtttatttttcaaagcttgaagg

Reaction 2	**# of reactions here:**	8
	# of reactions allowing for error:	9.3
	Volume of each component (uL)	

Component	Stock Concentration	Final Concentration	Per reaction	Master Mix
ddiH_2O	_	_	16.625	154.6
OneTaq Rxn Buffer	5X	1X	5	46.5
dNTPs	10 mM	0.2 mM	0.5	4.65
KROL88 Arb2	100 µM	4.0 µM	1	9.3
External Specific Primer: KROL92 Tn_Mar3	10 µM	0.4 µM	1	9.3
PCR 1 Amplicon, diluted 1:100	-	-	0.75	-
OneTaq Polymerase	5 units/uL	6.25 units	0.125	1.16
Total volume			25	225.5

Thermocycler program:	Step	Temp	Time
	1	94°C	30"
	2	65°C	30"
	3	72°C	30"
	4	Go to step 1, repeat 29x	
	5	72°C	5'
	6	12°C	hold

Of the 16 single colonies patched out yesterday, 14 still grew on CHA-Kan so going to be testing patches number 8 and 11 to see if they have the mutation

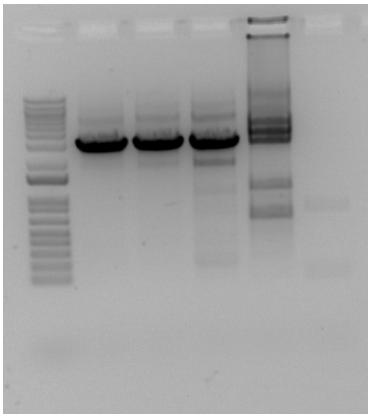
Colony PCR to check for deletion of pmrA in the LVS PriM+ background

Total reaction volume	20
Total number of reactions	5

Component	Stock concentration	Final concentration	1 rxn volume	Factor 6
ddiH2O			10.8	64.8
PrimeSTAR GXL Buffer	5x	1x	4	24
dNTPs	2.5 mM	0.2 mM	1.6	9.6
oligo F KROL252	10 uM	0.3 uM	0.6	3.6
oligo R KROL253	10 uM	0.3 uM	0.6	3.6
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	2.4
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	ΔpmrA PriM+ 8	2	KROL308, KROL309	512

2	ΔpmrA PriM+ 11	3	KROL308, KROL309	512
3	LVS gDNA	4	KROL308, KROL309	2952
4	pKL37 plasmid	5	KROL308, KROL309	
5	- control (no template)	6	KROL308, KROL309	



The two patches that did not grow on Kan turned out to actually be wildtype

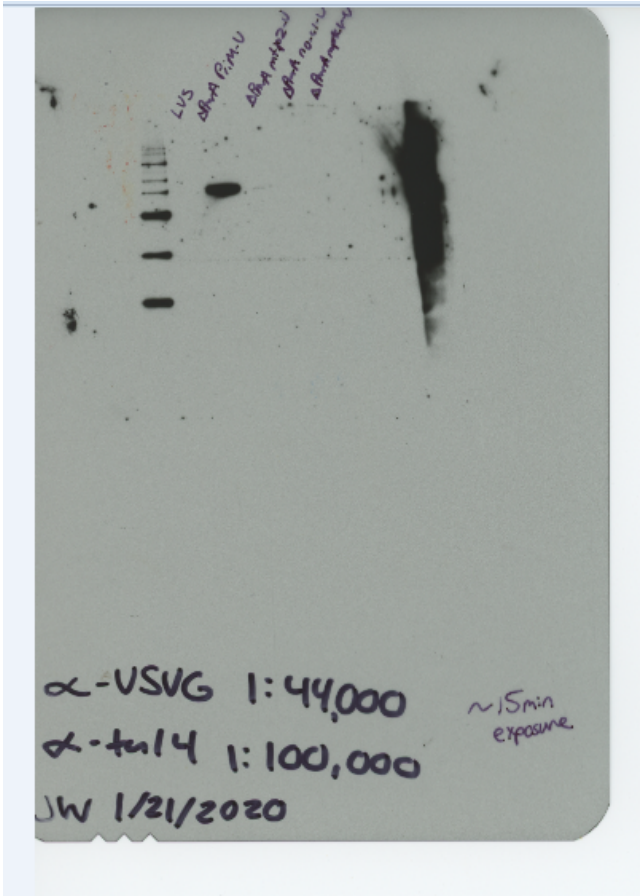
Cross patched 48 more colonies onto CHA and CHA-Kan to try to isolate the mutant

Wednesday, Jan 22nd , 2020

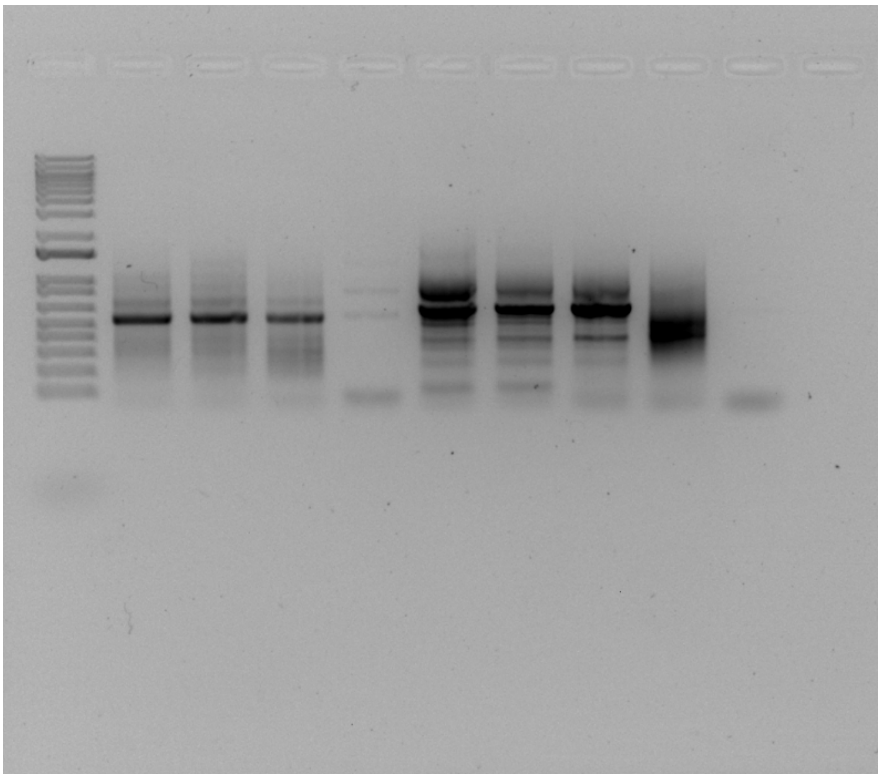
To Do:

1. ~~Run gel of Arbitrary PCR (second round)~~
2. ~~Streak out primary integrin for sucrose selection~~

All 48 single colonies that were cross-patched grew on Kan so now I have to re do the sucrose selection on that primary integrin



Film from western developed yesterday. Control showed up but none of my mutants did. Also tul4 loading control did not show up, but the aliquot I used for the primary may have been bad since Hannah's showed up fine so I'm going to use that one next time and also try loading 10ul of sample for the next gel instead of 5ul



KROL89 giving more robust results so going to continue with that set for now, and going to try to increase specificity by adjusting the annealing temp and can do that using the gradient function on the thermocycler

Arbitrary PCR Protocol

Primers with their sequences used for the arbitrary PCR

Primer	Purpose	Sequence
KROL87 Arb 1	Non-specific amplification	GGCCACGCGTCGACTAGTACNNNNNNNNNNGATAT
KROL88 Arb 2	Amplifies the region from the first set of primers	GGCCACGCGTCGACTAGTAC
KROL89 Arb 6	Non-specific amplification	GGCCACGCGTCGACTAGTACNNNNNNNNNNACGCC
KROL90 Mar1	Amplifies the transposon	tttgtcaaacaagctctcgattttaac
KROL92 Mar3	Amplifies the transposon	attaagtttatttttcaaagcttgaagg

Samples:

Sample number	**DNA**
1	pSD26 Tn insertion 1 (Tn3)
2	pSD26 Tn insertion 2 (Tn6)

3	LVS gDNA
4	-DNA

3) Set up reaction 1, using 500 ng of gDNA in a total reaction volume of 25 uL. See worksheet for details.

Reaction 1		**# of reactions here:**		4
		# of reactions allowing for error:		5.3
			Volume of each component (uL)	
Component	**Stock Concentration**	**Final Concentration**	**Per reaction**	**Master Mix**
ddiH_2O	-	-	14.245	75.5
OneTaq Rxn Buffer	5X	1X	5	26.5
dNTPs	10 mM	0.2 mM	0.5	2.7
KROL89 Arb6	100 µM	4.0 µM	1	5.3
Internal Specific Primer: KROL90 Tn_Mar1	10 µM	0.4 µM	1	5.3
Template	500 ng	160 ng/µL	3.13	-
OneTaq Polymerase	5 units/uL	6.25 units	0.125	0.7
Total volume			25	132.5

Thermocycler program:	**Step**	**Temp**	**Time**
	1	95°C	5'
	2	94°C	30"
	3	30°C	30"
	4	72°C	60"
	5	Go to step 2, repeat 5x	
	6	94°C	30"
	7	65°C	30"
	8	72°C	2'
	9	Go to step 6, repeat 29x	
	10	72°C	5'
	11	12°C	hold

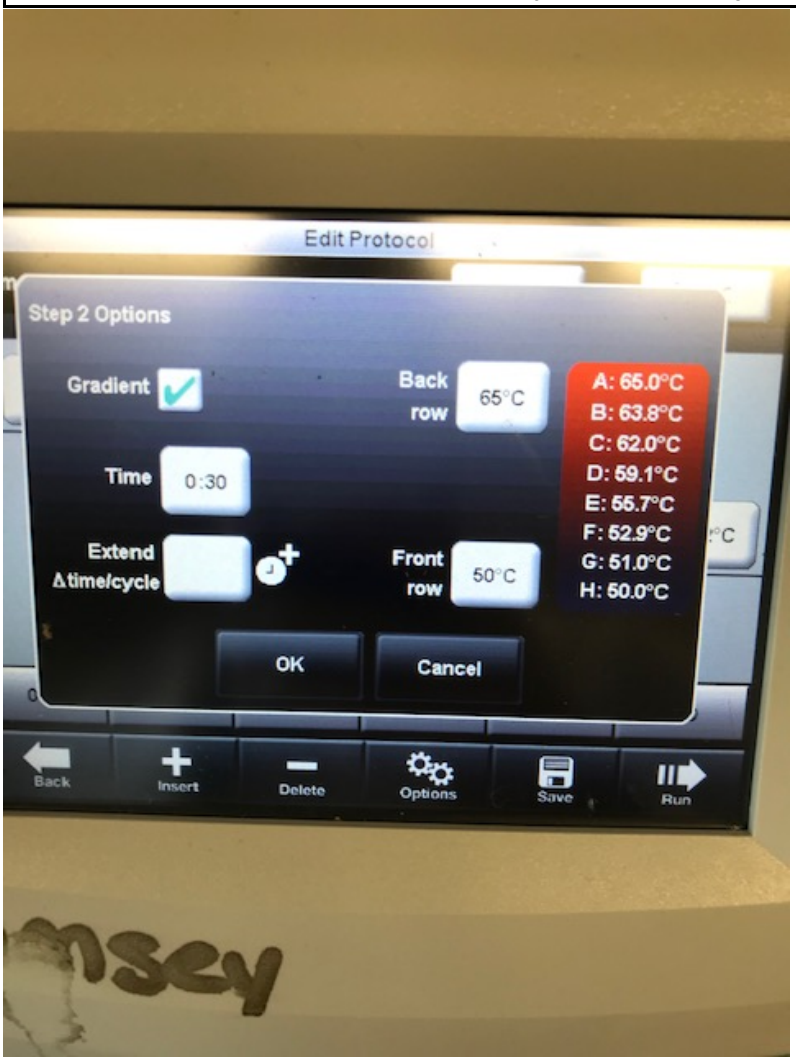
Diluting the first PCR rxn 1:100 and then running the second reaction with a gradient of annealing temps to see if one gives us a better result. Each sample tested will be tested with 8 temperatures (8 reactions per sample) from a range of 50-65C

Reaction 2		**# of reactions here:**		8
		# of reactions allowing for error:		9.3
			Volume of each component (uL)	
Component	**Stock Concentration**	**Final Concentration**	**Per reaction**	**Master Mix**
ddiH_2O	_	_	16.625	154.6
OneTaq Rxn Buffer	5X	1X	5	46.5

dNTPs	10 mM	0.2 mM	0.5	4.65
KROL88 Arb2	100 µM	4.0 µM	1	9.3
External Specific Primer: KROL92 Tn_Mar3	10 µM	0.4 µM	1	9.3
PCR 1 Amplicon, diluted 1:100	-	-	0.75	-
OneTaq Polymerase	5 units/uL	6.25 units	0.125	1.16
Total volume			25	225.5

Thermocycler program:

Step	Temp	Time
1	94°C	30"
2	65°C	30"
3	72°C	30"
4	Go to step 1, repeat 29x	
5	72°C	5'
6	12°C	hold



Range of annealing temps used for each set of reactions
Reactions are labeled 1-8 will sample 1 corresponding to the front (lowest temp) and sample 8 corresponding to the back (highest temp)

Thursday, Jan 23rd , 2020

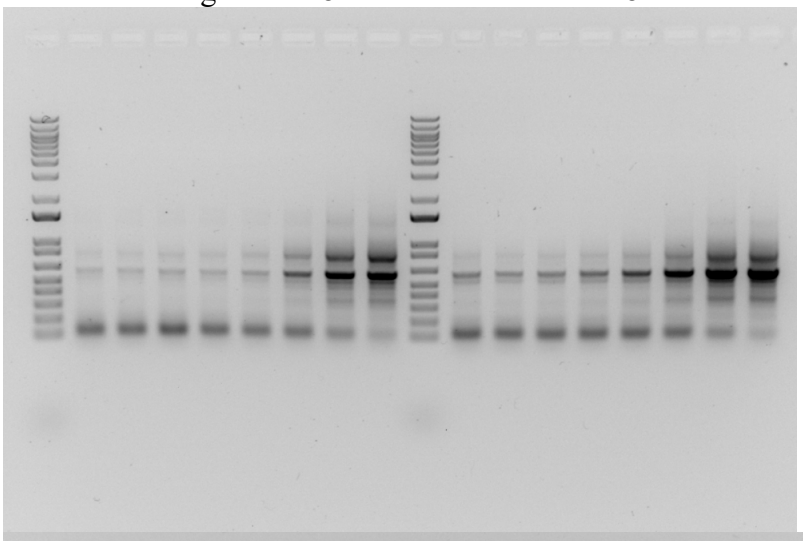
To Do:

1. ~~Run gel of Arbitrary PCR (second round)~~
2. ~~Sucrose selection of dpmrA PriM+~~
3. ~~Electroporations~~

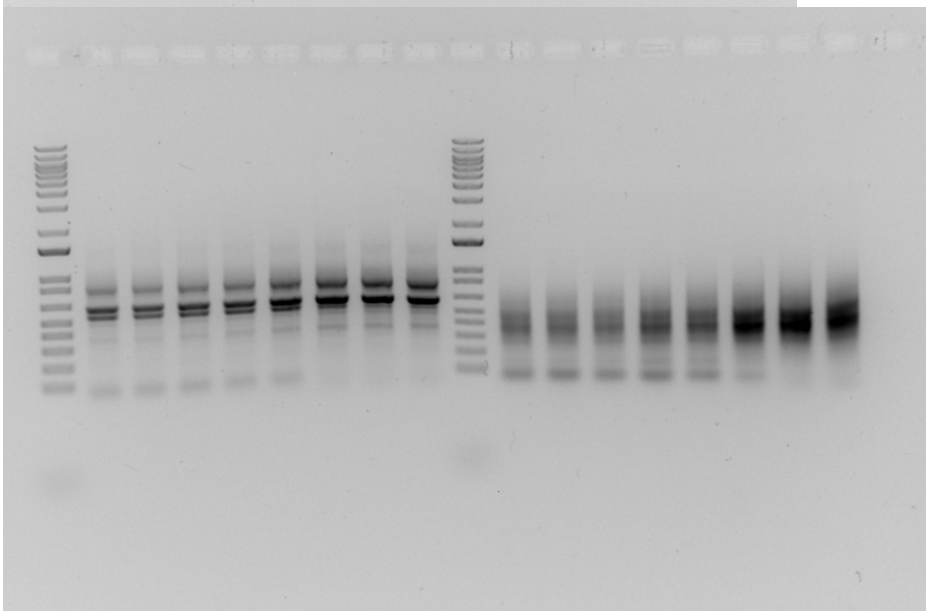
Made LVS electrocompetent cells and used in electroporations:

Electroporated pKR58 (to create the suppressor mutant) into LVS (2x)
Electroporated pKL116 into LVS (3x)

For the arb PCR needed 2 gels: sample 1 correlated to the lowest temp (50C) and sample 8 correlated to the highest temp (65C)
Gel 1 has Tn3 1-8 and Tn6 1-8
Gel 2 has LVS gDNA 1-8 and -DNA controls 1-8



Gel 1 of Arb PCR:
First set done with Tn3 gDNA
Second set done with Tn6 gDNA



Gel 2 of Arb PCR:
First set is LVS gDNA
Second set is -DNA controls

Picked 4 colonies from pKL116 transformation into 5mL LB cultures with Kan for minipreps

Friday, Jan 24th , 2020

To Do:

1. ~~Miniprep pKL116~~

Miniprepped 4 samples of pKL116 and eluted in 50ul so made an extra 200ul of plasmid

Monday, Jan 27th , 2020

To Do:

1. ~~Patch out electroporations~~
2. ~~Patch out single colonies for western~~
3. ~~Arb PCR~~
4. ~~Patch out colonies from sucrose selection~~

Patched out 4 colonies from the LVS pKR58 electroporation for John on CHA-Kan to do sucrose selection tomorrow
Patched out 5 colonies from the LVS pKL116 electroporation for sucrose selection tomorrow
Redoing the arbitrary PCR with the gradient with the new primers that came in to try and get rid of that contamination
KROL89 giving more robust results so going to continue with that set for now, and going to try to increase specificity by adjusting the annealing temp and can do that using the gradient function on the thermocycler
Arbitrary PCR Protocol
Example:

Sample number	DNA
1	pSD26 Tn insertion 1 (Tn3)
2	pSD26 Tn insertion 2 (Tn6)
3	LVS gDNA
4	-DNA

3) Set up reaction 1, using 500 ng of gDNA in a total reaction volume of 25 uL. See worksheet for details.

Reaction 1		# of reactions here:		4
		# of reactions allowing for error:		5.3
			Volume of each component (uL)	
Component	Stock Concentration	Final Concentration	Per reaction	Master Mix
ddiH_2O	-	-	12.245	64.9
OneTaq Rxn Buffer	5X	1X	5	26.5
dNTPs	2mM	0.2 mM	2.5	13.25
KROL89 Arb6	100 µM	4.0 µM	1	5.3
Internal Specific Primer: KROL90 Tn_Mar1	10 µM	0.4 µM	1	5.3
Template	500 ng	160 ng/µL	3.13	-
OneTaq Polymerase	5 units/uL	6.25 units	0.125	0.7
Total volume			25	132.5

Thermocycler program:	Step	Temp	Time
	1	95°C	5'
	2	94°C	30"
	3	30°C	30"
	4	72°C	60"
	5	Go to step 2, repeat 5x	
	6	94°C	30"
	7	65°C	30"
	8	72°C	2'
	9	Go to step 6, repeat 29x	
	10	72°C	5'
	11	12°C	hold

Tuesday, Jan 28th, 2020

To Do:

1. ~~Perform sucrose selection on pKL116 primary integrin~~
2. ~~Colony PCR~~
3. ~~Grow up cultures of VSVG tagged strains for western~~

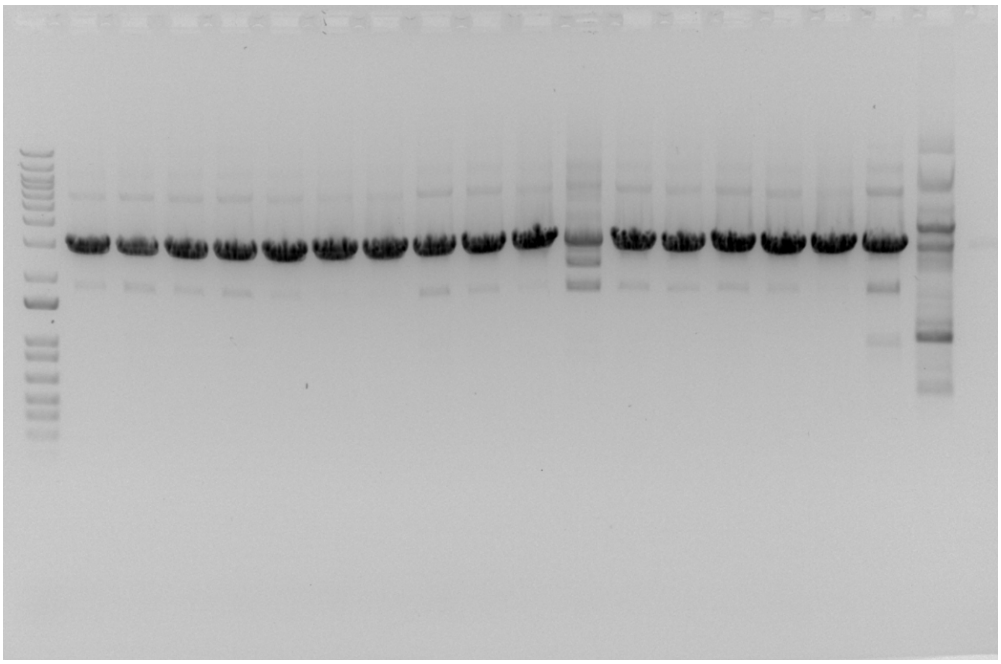
Colony PCR to check for deletion of pmrA in the LVS PriM+ background

Total reaction volume	20
Total number of reactions	19

Factor

Component	Stock concentration	Final concentration	1 rxn volume	20
ddiH2O			10.8	216.0
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL252	10 uM	0.3 uM	0.6	12
oligo R KROL253	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL308, KROL309	512
2	2	3	KROL308, KROL309	512
3	3	4	KROL308, KROL309	512
4	4	5	KROL308, KROL309	512
5	5	6	KROL308, KROL309	512
6	6	7	KROL308, KROL309	512
7	7	8	KROL308, KROL309	512
8	8	9	KROL308, KROL309	512
9	10	10	KROL308, KROL309	512
10	11	11	KROL308, KROL309	512
11	12	12	KROL308, KROL309	512
15	LVS gDNA	16	KROL308, KROL309	2952
16	pKL37 plasmid	17	KROL308, KROL309	
17	- control (no template)	18	KROL308, KROL309	



Grew up 5mL cultures for western of VSVG tagged strains all in triplicate and froze down with 1x SLB normalized to OD.
Goal was to freeze down at mid log (OD 0.3-0.4) but ΔpmrA mpk1 PriM-V and ΔpmrA mtip2 PriM-V did not grow well and were frozen down at a lower OD (cultures might not have been happy)
OD that each sample was frozen down at:

LVS 1: 0.357	ΔpmrA PriM-V 1: 0.337	ΔpmrA mpk1 PriM-V 1: 0.151	ΔpmrA no_C1 PriM-V 1: 0.327	ΔpmrA mtip2 PriM-V 1: 0.113
LVS 2: 0.353	ΔpmrA PriM-V 2: 0.326	ΔpmrA mpk1 PriM-V 2: 0.130	ΔpmrA no_C1 PriM-V 2: 0.327	ΔpmrA mtip2 PriM-V 2: 0.130
LVS 3: 0.319	ΔpmrA PriM-V 3: 0.327	ΔpmrA mpk1 PriM-V 3: 0.120	ΔpmrA no_C1 PriM-V 3: 0.337	ΔpmrA mtip2 PriM-V 3: 0.107

Wednesday, Jan 29th , 2020

To Do:

1. ~~Freeze down primary integrins~~
2. ~~Arb PCR~~
3. ~~Run gel for western~~
4. ~~Seed for mac assay~~
5. ~~Streak out strains for mac assay~~

Froze down LVS pKL116 primary integrins as 3-1 to 3-5 performed sucrose selection yesterday on LVS pKL116 3-1

Cross patched 32 colonies from the sucrose selection of ΔpmrA PriM+

Lane	Volume	Contents
1	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
2	10ul	LVS
3	10ul	ΔpmrA PriM-V-1
4	10ul	ΔpmrA PriM-V-2
5	10ul	ΔpmrA PriM-V-3
6	10ul	ΔpmrA mtip2-V-1
7	10ul	ΔpmrA mtip2-V-2
8	10ul	ΔpmrA mtip2-V-3
9	10ul	ΔpmrA no_C1-V-1
10	10ul	ΔpmrA no_C1-V-2
11	10ul	ΔpmrA no_C1-V-3
12	10ul	ΔpmrA mpk1-V-1
13	10ul	ΔpmrA mpk1-V-2
14	10ul	ΔpmrA mpk1-V-3
15	10ul	Benchmark prestained ladder

Ran gel for about 1 hour at 150V and then did a wet transfer in the cold room at 20V for 1 hour and cut the top left corner (top of where the benchmark ladder is) and set in 10mL blocking buffer in the cold room shaking until Friday

Going to probe for VSVG tag and Tul4 (loading control) as the primary antibodies
Going to use Tul4 at 1:100,000 (anti mouse)
Going to use anti-VSVG at 1:4444 (anti rabbit), half of the standard dilution

Diluting the first PCR rxn 1:100 and then running the second reaction with a gradient of annealing temps to see if one gives us a better result. Each sample tested will be tested with 8 temperatures (8 reactions per sample) from a range of 50-65C

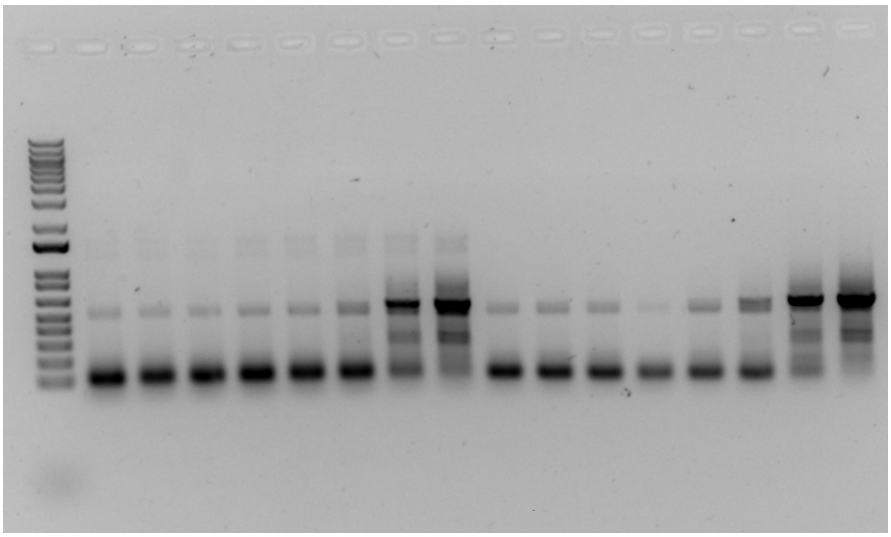
Reaction 2		**# of reactions here:**		8
		# of reactions allowing for error:		9.3
			Volume of each component (uL)	
Component	**Stock Concentration**	**Final Concentration**	**Per reaction**	**Master Mix**
ddiH_2O	_	_	16.625	154.6
OneTaq Rxn Buffer	5X	1X	5	46.5

dNTPs	2 mM	0.2 mM	2.5	23.25
KROL88 Arb2	100 µM	4.0 µM	1	9.3
External Specific Primer: KROL92 Tn_Mar3	10 µM	0.4 µM	1	9.3
PCR 1 Amplicon, diluted 1:100	-	-	0.75	6.98
OneTaq Polymerase	5 units/uL	6.25 units	0.125	1.16
Total volume			25	225.5

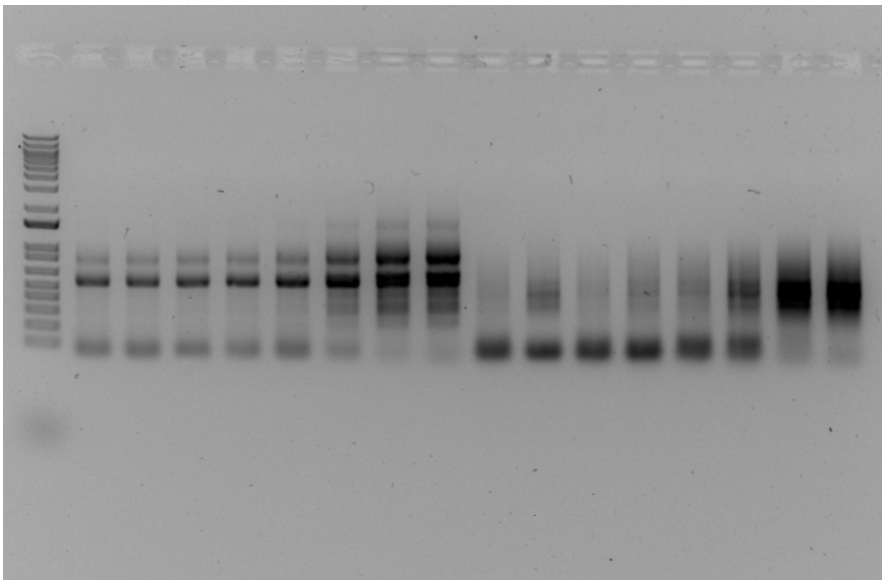
Thermocycler program:

Step	Temp	Time
1	94°C	30"
2	65°C	30"
3	72°C	30"
4	Go to step 1, repeat 29x	
5	72°C	5'
6	12°C	hold

Each set will have 8 samples tested at different annealing temps with number 1 being the lowest temp and number 8 being the highest temp



Gel 1 of Arb PCR reaction 2:
First set done with Tn3 gDNA
Second set done with Tn6 gDNA



Gel 2 of Arb PCR reaction 2:
First set is LVS gDNA
Second set is -DNA controls

Thursday, Jan 30th , 2020

To Do:

1. ~~Electroporations~~
2. ~~Mac assay~~
3. ~~Colony PCR~~

Performed 4 electroporations for deleting pmrA from LVS PriM+ strain using pKL37 and making the cells fresh right before electroporating the plasmid

Started mac assay testing the effect of adding cysteine:
Added 2ul of 1x PBS to strains 1 and 2 and added 2ul of 500mM cysteine to strains 3 and 4

Colony PCR to check for deletion of pmrA in the LVS PriM+ background

Total reaction volume	20
Total number of reactions	19

Factor

Component	Stock concentration	Final concentration	1 rxn volume	20
ddiH2O			10.8	216.0
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL252	10 uM	0.3 uM	0.6	12
oligo R KROL253	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

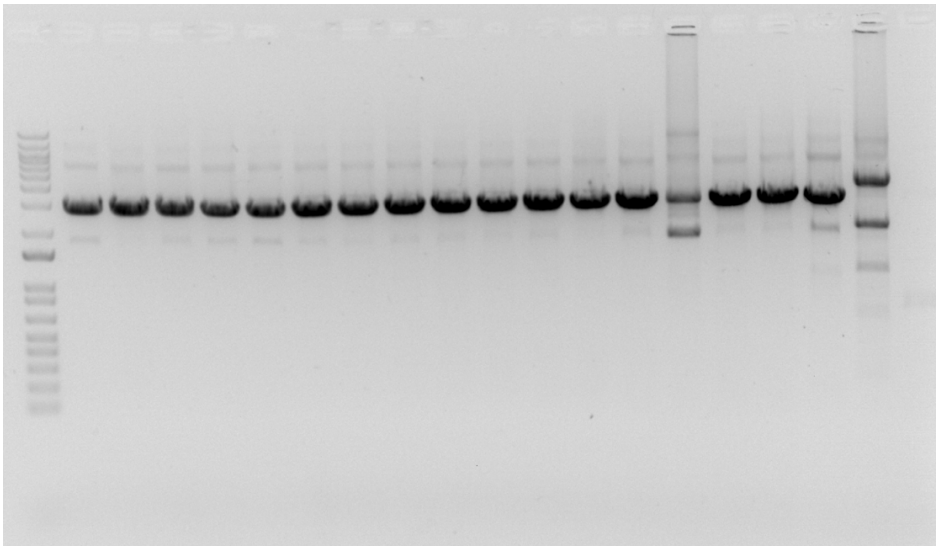
Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL308, KROL309	512
2	2	3	KROL308, KROL309	512
3	3	4	KROL308, KROL309	512
4	4	5	KROL308, KROL309	512
5	5	6	KROL308, KROL309	512
6	6	7	KROL308, KROL309	512
7	7	8	KROL308, KROL309	512
8	8	9	KROL308, KROL309	512
9	9	10	KROL308, KROL309	512
10	10	11	KROL308, KROL309	512
11	11	12	KROL308, KROL309	512
12	12	13	KROL308, KROL309	512
13	13	14	KROL308, KROL309	512
14	14	15	KROL308, KROL309	512
15	15	16	KROL308, KROL309	512

16	16	17	KROL308, KROL309	512
17	LVS gDNA	18	KROL308, KROL309	2952
18	pKL37 plasmid	19	KROL308, KROL309	
19	- control (no template)	20	KROL308, KROL309	

Friday, Jan 31st , 2020

To Do:

1. ~~Develop western~~
2. ~~Mac assay day 2~~
3. ~~Colony PCR~~
4. ~~Run gel from colony PCR~~



Gel from yesterday's colony PCR testing 16 patches from the dPmrA PriM+ sucrose plates

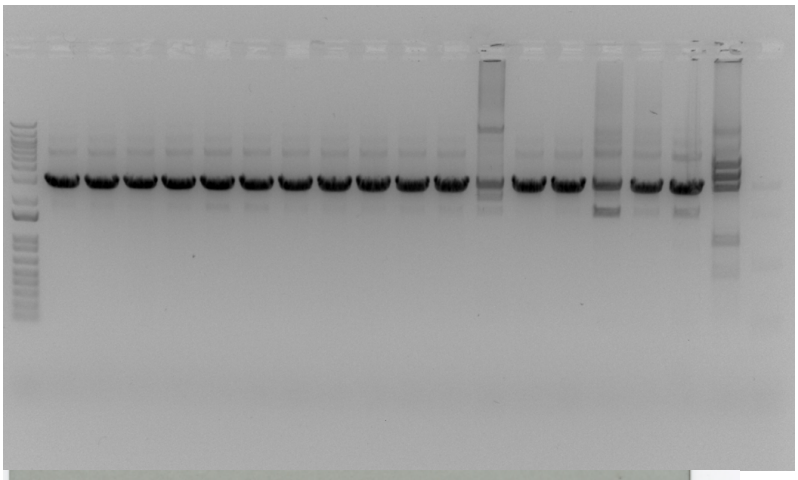
Colony PCR to check for deletion of pmrA in the LVS PriM+ background

Total reaction volume	20
Total number of reactions	19

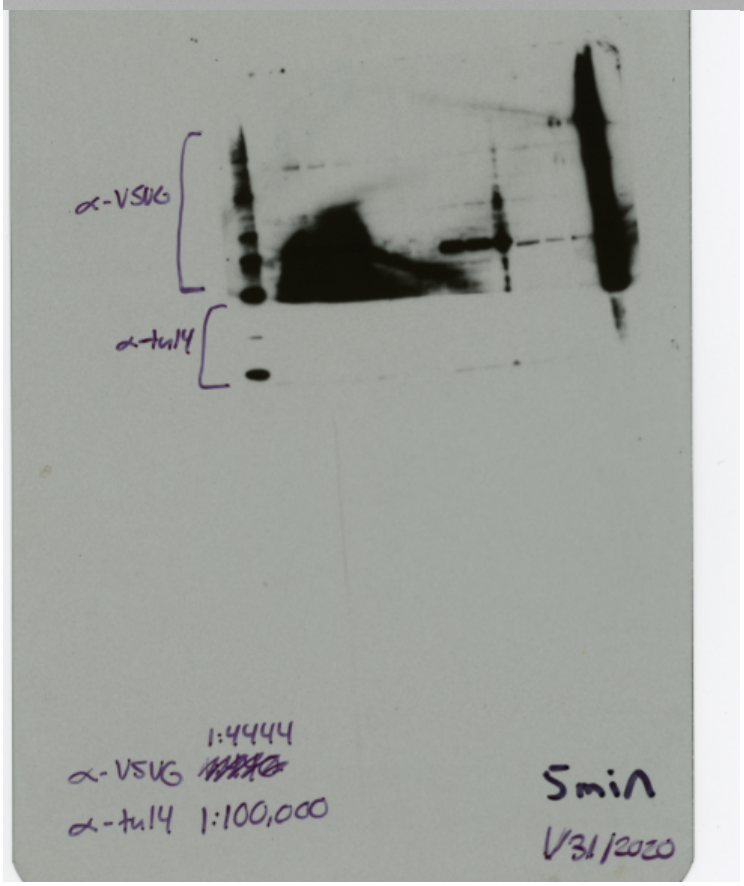
				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	20
ddiH2O			10.8	216.0
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL252	10 uM	0.3 uM	0.6	12

oligo R KROL253	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	17	2	KROL308, KROL309	512
2	18	3	KROL308, KROL309	512
3	19	4	KROL308, KROL309	512
4	20	5	KROL308, KROL309	512
5	21	6	KROL308, KROL309	512
6	22	7	KROL308, KROL309	512
7	23	8	KROL308, KROL309	512
8	24	9	KROL308, KROL309	512
9	25	10	KROL308, KROL309	512
10	26	11	KROL308, KROL309	512
11	27	12	KROL308, KROL309	512
12	28	13	KROL308, KROL309	512
13	29	14	KROL308, KROL309	512
14	30	15	KROL308, KROL309	512
15	31	16	KROL308, KROL309	512
16	32	17	KROL308, KROL309	512
17	LVS gDNA	18	KROL308, KROL309	2952
18	pKL37 plasmid	19	KROL308, KROL309	
19	- control (no template)	20	KROL308, KROL309	



Gel of colony PCR testing for deletion of PmrA in LVS PriM+ background. All samples seem to be wildtype besides a few that are primary integrins



Western came out nicely and shows a lot of PriM with the ΔpmrA PriM-V samples. The no_C1 mutants show less PriM production and the mtip2 mutants which are next to the PriM controls cannot be seen since the signal is blown out by the control, the mpk mutants show the least amount of PriM production in this blot

For western gel need to dilute the dPmrA PriM 1:10, and reorder no_C1, then mtip2, then mpk1

Monday, Feb 3rd , 2020

To Do:

1. ~~Run gel and transfer~~
2. ~~Count mac assay plates~~
3. ~~Check electroporations~~

Lane	Volume	Contents
1	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
2	10ul	LVS
3	10ul	ΔpmrA PriM-V-1 (1:10)
4	10ul	ΔpmrA PriM-V-2 (1:10)
5	10ul	ΔpmrA PriM-V-3 (1:10)
6	10ul	ΔpmrA no_C1-V-1
7	10ul	ΔpmrA no_C1-V-2
8	10ul	ΔpmrA no_C1-V-3
9	10ul	ΔpmrA mtip2-V-1
10	10ul	ΔpmrA mtip2-V-2
11	10ul	ΔpmrA mtip2-V-3
12	10ul	ΔpmrA mpk1-V-1
13	10ul	ΔpmrA mpk1-V-2
14	10ul	ΔpmrA mpk1-V-3
15	10ul	Benchmark prestained ladder

Ran gel using MES buffer for about 1 hour at 150V and then did a wet transfer in the cold room at 20V for 1 hour and cut the top left corner (top of where the benchmark ladder is) and set in 10mL blocking buffer in the cold room shaking until tomorrow

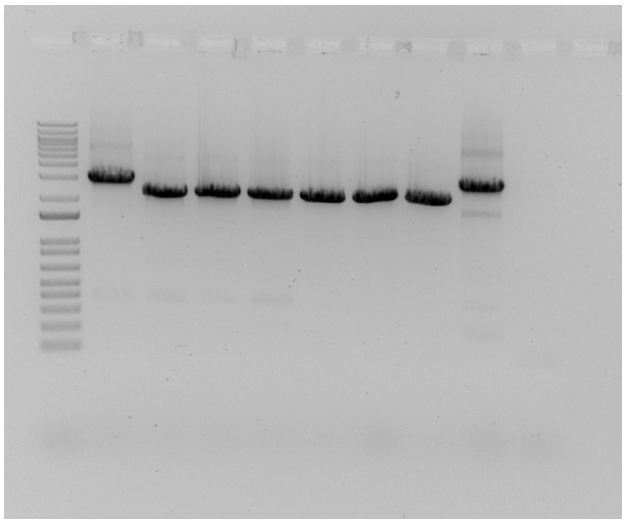
Results from last week's mac assay with adding cysteine seem to show that the cysteine does increase replication, but it showed this for both LVS and for ΔpmrA, however the ΔpmrA samples showed both large and small colonies and I'm worried that there might be LVS contamination in my stocks, so I am doing a colony PCR from the colonies on the mac assay plate to check for deletion of pmrA

Total reaction volume	20
Total number of reactions	9

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	10
ddiH2O			10.8	108
PrimeSTAR GXL Buffer	5x	1x	4	40
dNTPs	2.5 mM	0.2 mM	1.6	16
oligo F KROL252	10 uM	0.3 uM	0.6	6
oligo R KROL253	10 uM	0.3 uM	0.6	6

Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	4
		Total volume	20	400

Reaction Number	Sample	Lane on gel	Primers	Size
1	LVS colony	2	KROL308, KROL309	512
2	ΔpmrA large-1	3	KROL308, KROL309	512
3	ΔpmrA large-2	4	KROL308, KROL309	512
4	ΔpmrA large-3	5	KROL308, KROL309	512
5	ΔpmrA small-1	6	KROL308, KROL309	512
6	ΔpmrA small-2	7	KROL308, KROL309	512
7	ΔpmrA small-3	8	KROL308, KROL309	512
8	LVS gDNA	9	KROL308, KROL309	2952
9	- control (no template)	10	KROL308, KROL309	



Was able to confirm that both the large and small colonies are still lacking PmrA

Tuesday, Feb 4th, 2020

To Do:

1. ~~Colony PCR~~
2. ~~Develop western~~
3. ~~Sucrose selection~~
4. ~~Freeze down primary integrins~~

Total reaction volume	20
Total number of reactions	19

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	20
ddiH2O			10.8	216
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL	10 uM	0.3 uM	0.6	12
oligo R KROL	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	

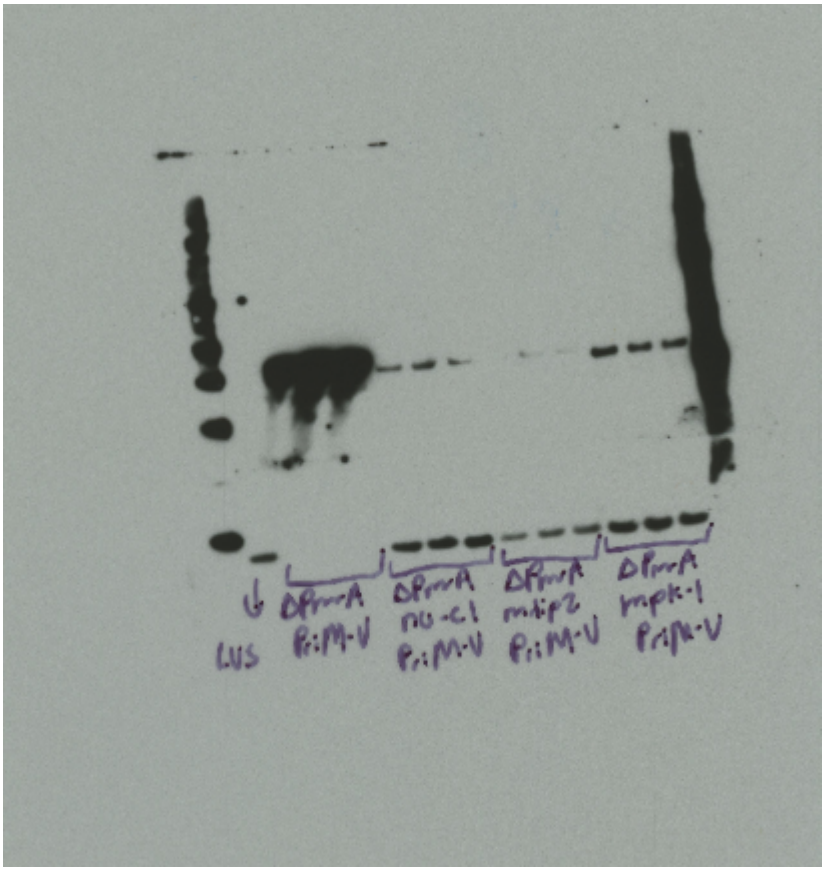
Reaction Number	Sample	Lane on gel	Primers
1	Colony 1	2	KROL151, KROL152
2	Colony 2	3	KROL151, KROL152
3	Colony 3	4	KROL151, KROL152
4	Colony 4	5	KROL151, KROL152

5	Colony 5	6	KROL151, KROL152
6	Colony 6	7	KROL151, KROL152
7	Colony 7	8	KROL151, KROL152
8	Colony 8	9	KROL151, KROL152
9	Colony 9	10	KROL151, KROL152
10	Colony 10	11	KROL151, KROL152
11	Colony 11	12	KROL151, KROL152
12	Colony 12	13	KROL151, KROL152
13	Colony 13	14	KROL151, KROL152
14	Colony 14	15	KROL151, KROL152
15	Colony 15	16	KROL151, KROL152
16	Colony 16	17	KROL151, KROL152
17	LVS gDNA	18	KROL151, KROL152
18	pKL116	19	KROL151, KROL152
19	- control (no template)	20	KROL151, KROL152

Samples were PCR purified in preparation for diagnostic digest

Diagnostic Digest:
Buffer: 2ul (per sample)
DNA: 15.0ul (per sample)
Enzyme: 0.3ul (per sample)
Don't digest or purify plasmid or LVS colony control

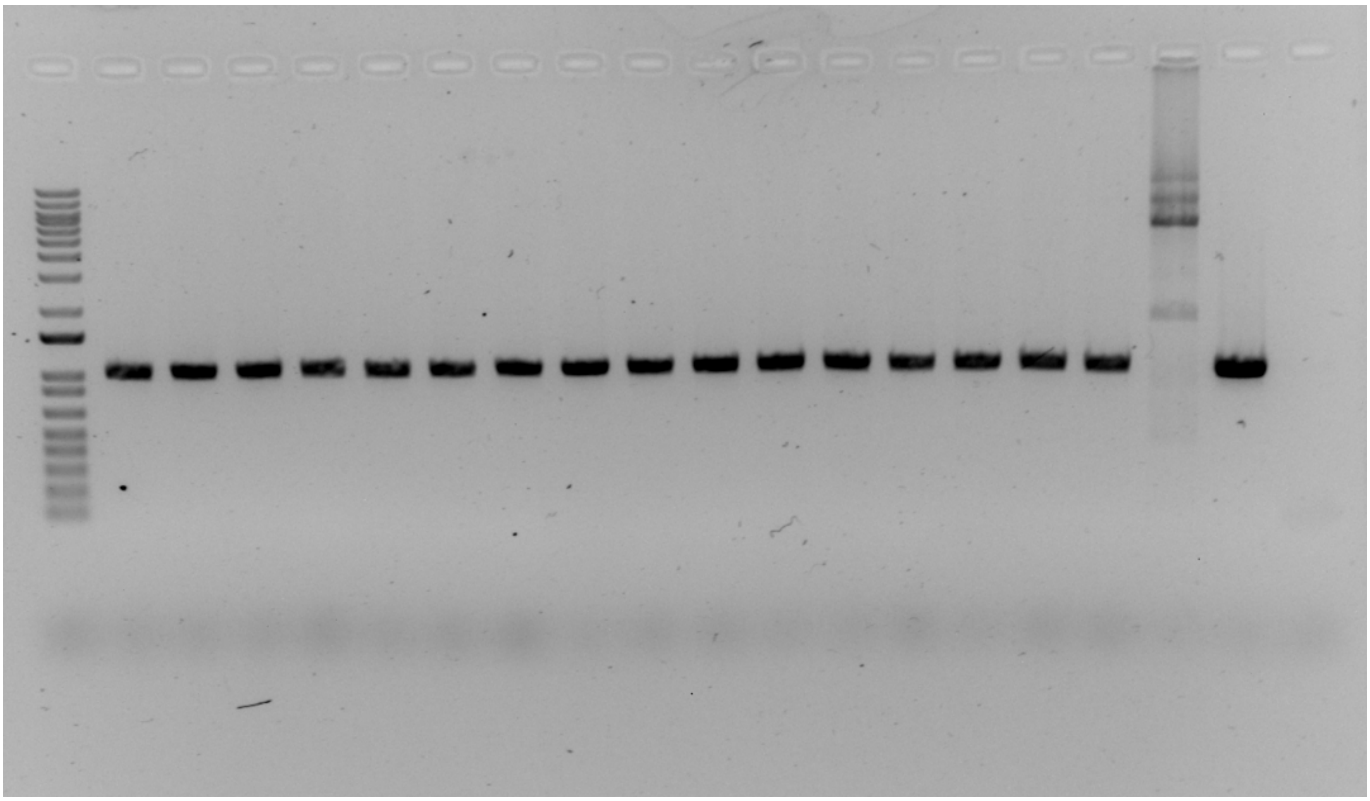
Components	**Volumes in 1 reaction (uL)**	**Volumes in Master Mix18 x (uL)**
H_2O	2.5	45.0
10x Buffer*	2.0	36.0
DNA	(15.0)	-
Enzyme (Sap I)	0.3	5.4
Total	20.0 (15.0 actual b/c of DNA)	



Wednesday, Feb 5th , 2020

To Do:

1. ~~Digest and gel from colony PCR~~



Still unable to obtain a colony with the mutation and all samples came out as wildtype so set up sequencing reactions to sequence the plasmid to make sure there aren't any errors

Monday, Feb 10th , 2020

To Do:

1. ~~Re run protein gel and transfer~~
2. ~~Colony PCR~~

Lane	Volume	Contents
1	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
2	10ul	LVS
3	10ul	ΔpmrA PriM-V-1 (1:15)

4	10ul	ΔpmrA PriM-V-2 (1:15)
5	10ul	ΔpmrA PriM-V-3 (1:15)
6	10ul	ΔpmrA no_C1-V-1
7	10ul	ΔpmrA no_C1-V-2
8	10ul	ΔpmrA no_C1-V-3
9	10ul	ΔpmrA mtip2-V-1
10	10ul	ΔpmrA mtip2-V-2
11	10ul	ΔpmrA mtip2-V-3
12	10ul	ΔpmrA mpk1-V-1
13	10ul	ΔpmrA mpk1-V-2
14	10ul	ΔpmrA mpk1-V-3
15	10ul	Benchmark prestained ladder

Ran gel using MES buffer for about 1 hour at 150V and then did a wet transfer in the cold room at 20V for 1 hour and cut the top left corner (top of where the benchmark ladder is) and set in 10mL blocking buffer in the cold room shaking until tomorrow

Colony PCR to check for deletion of pmrA in the LVS PriM+ background going to test 32 colonies worth

Total reaction volume	20
Total number of reactions	35

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	36
ddiH2O			7.8	280.8
PrimeSTAR GXL Buffer	5x	1x	4	144
dNTPs	2.5 mM	0.2 mM	1.6	57.6
oligo F KROL252	10 uM	0.3 uM	0.6	21.6
oligo R KROL253	10 uM	0.3 uM	0.6	21.6
Template	100 ng/ul	2 ng/ul	5	

PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	14.4
		Total volume	20	400

Sample table for colonies from primary 2-1

Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL308, KROL309	512
2	2	3	KROL308, KROL309	512
3	3	4	KROL308, KROL309	512
4	4	5	KROL308, KROL309	512
5	5	6	KROL308, KROL309	512
6	6	7	KROL308, KROL309	512
7	7	8	KROL308, KROL309	512
8	8	9	KROL308, KROL309	512
9	9	10	KROL308, KROL309	512
10	10	11	KROL308, KROL309	512
11	11	12	KROL308, KROL309	512
12	12	13	KROL308, KROL309	512
13	13	14	KROL308, KROL309	512
14	14	15	KROL308, KROL309	512
15	15	16	KROL308, KROL309	512
16	16	17	KROL308, KROL309	512
17	LVS gDNA	18	KROL308, KROL309	2952
18	pKL37 plasmid	19	KROL308, KROL309	
19	- control (no template)	20	KROL308, KROL309	

Sample table for primary integrin 2-2

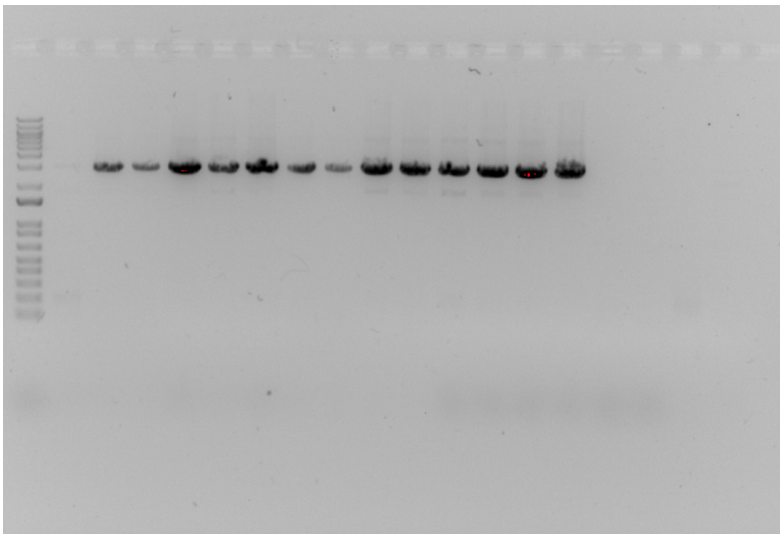
Reaction Number	Sample	Lane on gel	Primers	Size
1	1	2	KROL308, KROL309	512
2	2	3	KROL308, KROL309	512
3	3	4	KROL308, KROL309	512
4	4	5	KROL308, KROL309	512
5	5	6	KROL308, KROL309	512
6	6	7	KROL308, KROL309	512
7	7	8	KROL308, KROL309	512
8	8	9	KROL308, KROL309	512
9	9	10	KROL308, KROL309	512
10	10	11	KROL308, KROL309	512
11	11	12	KROL308, KROL309	512
12	12	13	KROL308, KROL309	512
13	13	14	KROL308, KROL309	512
14	14	15	KROL308, KROL309	512
15	15	16	KROL308, KROL309	512
16	16	17	KROL308, KROL309	512
17	LVS gDNA	18	KROL308, KROL309	2952
18	pKL37 plasmid	19	KROL308, KROL309	
19	- control (no template)	20	KROL308, KROL309	

Tuesday, Feb 11th , 2020

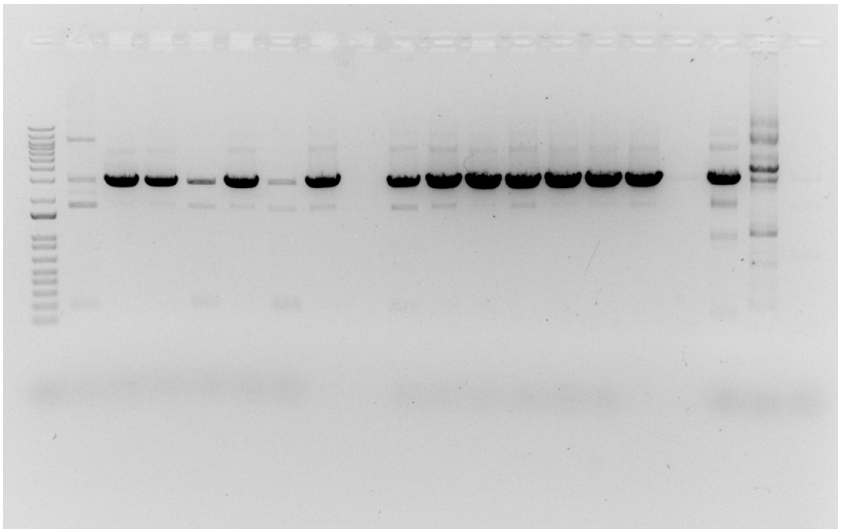
To Do:

1. ~~Develop western~~
2. ~~Run gel from colony PCR~~

3. ~~Patch out VSVG tagged strains to grow up tomorrow~~



Gel from colony PCR testing deletion of pmrA from PriM+ strain primary integrin 2-1 all samples coming out as wildtype except a few that did not grow well and the PCR didn't work



Gel from colony PCR testing deletion of pmrA from PriM+ strain primary integrin 2-2 all samples coming out as wildtype except the first sample that still have primary integrin contamination

Going to try streaking the first patch to single colony on sucrose plate to redo the selection

Making 1L of 10x TBS according to pouches concentration for 1x should be 25mM for Tris and 0.15M for NaCl and pH should be 7.2

Wednesday, Feb 12th , 2020

To Do:

1. ~~Run gel and transfer for western~~
2. ~~Colony PCR~~
3. ~~Sucrose selection~~

Colony PCR to check for deletion of pmrA in the LVS PriM+ background from primary integrin 2-2

Total reaction volume	20
Total number of reactions	19

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	20
ddiH2O			10.8	216
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL252	10 uM	0.3 uM	0.6	12
oligo R KROL253	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	5	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

Sample table for colonies from primary 2-1

Reaction Number	Sample	Lane on gel	Primers	Size
1	17	2	KROL308, KROL309	
2	18	3	KROL308, KROL309	
3	19	4	KROL308, KROL309	
4	20	5	KROL308, KROL309	
5	21	6	KROL308, KROL309	
6	22	7	KROL308, KROL309	
7	23	8	KROL308, KROL309	
8	24	9	KROL308, KROL309	
9	25	10	KROL308, KROL309	
10	26	11	KROL308, KROL309	
11	27	12	KROL308, KROL309	
12	28	13	KROL308, KROL309	
13	29	14	KROL308, KROL309	
14	30	15	KROL308, KROL309	

15	31	16	KROL308, KROL309	
16	32	17	KROL308, KROL309	
17	LVS gDNA	18	KROL308, KROL309	2952
18	pKL37 plasmid	19	KROL308, KROL309	
19	- control (no template)	20	KROL308, KROL309	

Performed sucrose selection on LVS pKL116 primary integrin 3-2

Gel for PriM VSVG tagged strains

Lane	Volume	Contents
1	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
2	10ul	LVS
3	10ul	ΔpmrA PriM-V-1 (1:15)
4	10ul	ΔpmrA PriM-V-2 (1:15)
5	10ul	ΔpmrA PriM-V-3 (1:15)
6	10ul	ΔpmrA no_C1-V-1
7	10ul	ΔpmrA no_C1-V-2
8	10ul	ΔpmrA no_C1-V-3
9	10ul	ΔpmrA mtip2-V-1
10	10ul	ΔpmrA mtip2-V-2
11	10ul	ΔpmrA mtip2-V-3
12	10ul	ΔpmrA mpk1-V-1
13	10ul	ΔpmrA mpk1-V-2
14	10ul	ΔpmrA mpk1-V-3
15	10ul	Benchmark prestained ladder

Ran gel using MES buffer for about 1 hour at 150V and then did a wet transfer in the cold room at 20V for 1 hour and set in 10mL blocking buffer in the cold room shaking

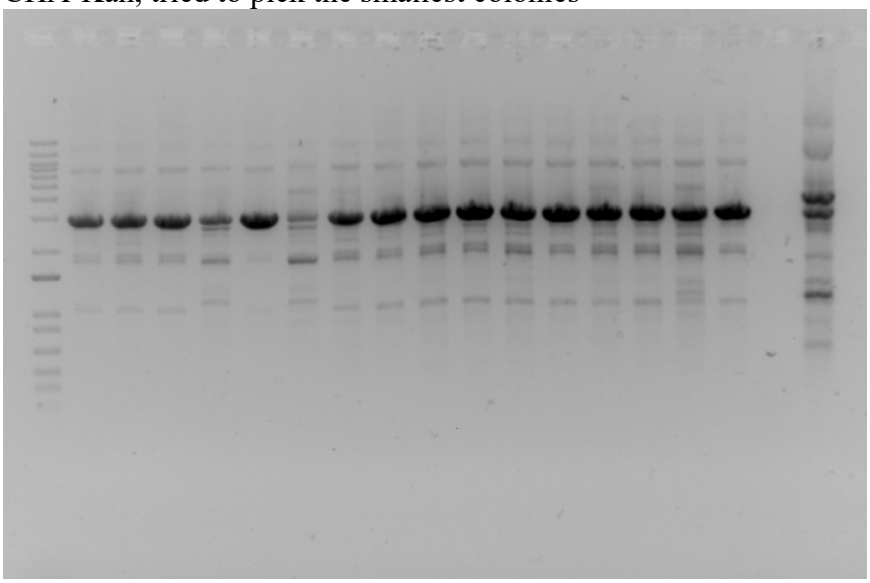
Patched out more colonies to start up cultures of VSVG tagged strains for western

Thursday, Feb 13th , 2020

To Do:

1. ~~Run gel from colony PCR~~

Picked additional colonies from sucrose plates for colony PCR of ΔpmrA PriM+ and cross patched to CHA-Kan, tried to pick the smallest colonies



Gel from colony PCR testing deletion of pmrA from PriM+ strain, looks like the gDNA didn't amplify, but struck out patches 20 and 22 to sngle colony since they look a bit different from the others

Friday, Feb 14th , 2020

To Do:

1. ~~Develop western~~
2. ~~Electroporate transposon~~
3. ~~Colony PCR~~
4. ~~Make cultures for VSVG tagged strains~~

Electroporated pKL97 (transposon plasmid) into ΔpmrA electrocompetent cells (x2) from 11-19 and will continue to use those cells from here on out. Goal is to determine the efficiency of this plasmid in this batch of cells. Need to recover at 37C for 3 hours and then dilute and spot plate on CHA-Kan plates to determine efficiency. Recovering in 4mL of MHB.

Set up new cultures for the PriM VSVG tagged strains to test by western. Did in 5mL MHB in triplicate starting at an OD of 0.1

Colony PCR to check for deletion of pmrA in the LVS PriM+ background from primary integrin 2-2

Total reaction volume	20
Total number of reactions	19

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	20
ddiH2O			10.8	216
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL252	10 uM	0.3 uM	0.6	12
oligo R KROL253	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	5	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

Sample table for colonies from primary 2-1

Reaction Number	Sample	Lane on gel	Primers	Size
1	17	2	KROL308, KROL309	
2	18	3	KROL308, KROL309	
3	19	4	KROL308, KROL309	
4	20	5	KROL308, KROL309	
5	21	6	KROL308, KROL309	
6	22	7	KROL308, KROL309	
7	23	8	KROL308, KROL309	
8	24	9	KROL308, KROL309	
9	25	10	KROL308, KROL309	
10	26	11	KROL308, KROL309	
11	27	12	KROL308, KROL309	
12	28	13	KROL308, KROL309	
13	29	14	KROL308, KROL309	

14	30	15	KROL308, KROL309	
15	31	16	KROL308, KROL309	
16	32	17	KROL308, KROL309	
17	LVS gDNA	18	KROL308, KROL309	2952
18	pKL37 plasmid	19	KROL308, KROL309	
19	- control (no template)	20	KROL308, KROL309	

Monday, Feb 17th , 2020

To Do:

1. ~~Calculate transposon efficiency~~
2. ~~Pour plates for mac assay~~
3. ~~Thaw macrophage~~

For transposon electroporation only observed 1-3 colonies in the undiluted spots of 5ul so for 1mL of culture should get ~400 colonies so should plate 1mL of electroporation culture per plate of CHA-Kan

Poured 2 flasks of CHA square plates for Kathryn's macrophage assay

Thawed one vial of P4 macrophage

For near infrared western going to do the tul4 at 1:50,000 (red mouse) and VSVG at 1:2222 (green rabbit)

For tul 4 dilute 1:10 in PBS (27ul) then use 3ul of that for western and aliquot the rest as 5ul aliquots of the 1:10

Tuesday, Feb 18th , 2020

To Do:

1. ~~Transposon electroporation~~
2. ~~Pour plates for mac assay~~
3. ~~Run gel and transfer~~

Performed 4 electroporations using pKL97 transposon plasmid. Plated 1mL of electroporation onto 1 CHA-kan plate for a total of 16 plates. Also used a CHA-Kan track plate to plate 10ul of the straight culture as well as 10ul of a 1:10 dilution (will be used to determine efficiency)

Gel for PriM VSVG tagged strains using samples grown on 2/15

Lane	Volume	Contents
1	3ul	Precision protein ladder
2	13ul	LVS
3	13ul	ΔpmrA PriM-V-1 (1:50)
4	13ul	ΔpmrA PriM-V-2 (1:50)
5	13ul	ΔpmrA PriM-V-3 (1:50)
6	13ul	ΔpmrA no_C1-V-1
7	13ul	ΔpmrA no_C1-V-2
8	13ul	ΔpmrA no_C1-V-3
9	13ul	ΔpmrA mtip2-V-1
10	13ul	ΔpmrA mtip2-V-2
11	13ul	ΔpmrA mtip2-V-3
12	13ul	ΔpmrA mpk1-V-1
13	13ul	ΔpmrA mpk1-V-2
14	13ul	ΔpmrA mpk1-V-3
15	10ul	1x SLB

Ran gel using MES buffer for about 1 hour at 150V and then did a wet transfer in the cold room at 20V for 1 hour and set in 25mL blocking buffer in the cold room shaking

Wednesday, Feb 19th , 2020

To Do:

1. ~~Develop western~~

\

Thursday, Feb 20th , 2020

To Do:

1. ~~Re-run gel and transfer~~

2. ~~Streak out more primary integrants~~

Gel for PriM VSVG tagged strains using samples grown on 2/15

Lane	Volume	Contents
1	3ul	Precision protein ladder
2	13ul	LVS
3	13ul	ΔpmrA PriM-V-1 (1:50)
4	13ul	ΔpmrA PriM-V-2 (1:50)
5	13ul	ΔpmrA PriM-V-3 (1:50)
6	13ul	ΔpmrA no_C1-V-1
7	13ul	ΔpmrA no_C1-V-2
8	13ul	ΔpmrA no_C1-V-3
9	13ul	ΔpmrA mtip2-V-1
10	13ul	ΔpmrA mtip2-V-2
11	13ul	ΔpmrA mtip2-V-3
12	13ul	ΔpmrA mpk1-V-1
13	13ul	ΔpmrA mpk1-V-2
14	13ul	ΔpmrA mpk1-V-3
15	10ul	1x SLB

Ran gel using MES buffer for about 1 hour at 150V and then did a wet transfer in the cold room at 7V for overnight hour and set in 25mL blocking buffer in the cold room shaking

Add 1.5ul of anti-tul4 to 15mL of blocking buffer and 6.8ul of anti-VSVG

Friday, Feb 21st , 2020

To Do:

1. ~~Develop western~~
2. ~~Perform sucrose selection~~
3. ~~Pour plates~~
4. ~~Scrape up electroporations~~
5. ~~Electroporate transposon plasmid~~

For western blot trying a higher dilution of tul4 (1:10,000) (1.5ul in 15mL)
Need a lambda pir e coli strain to transform that contains amp resistance

Scraped up plates from Tuesday's electroporation with the transposon plasmid. Scraped up all 16 plates into 4mL MHB and then add 1mL of 75% glycerol to make 5, 1mL glycerol stocks

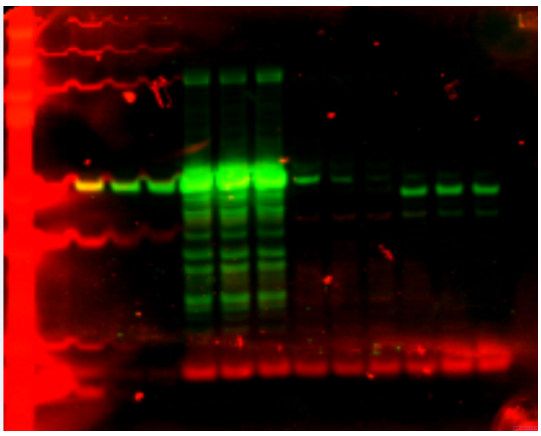
Electroporation Number	Number of Colonies
1-1	53
1-2	67

1-3	70
1-4	60
2-1	400-500
2-2	400-500
2-3	400-500
2-4	400-500

Electroporation Number	Number of Colonies
3-1	400-500
3-2	8
3-3	400-500
3-4	400-500
4-1	1
4-2	3
4-3	6
4-4	0

At this point, can assume that we have about 3000-3500 transposon mutants

Performed 3 more electroporations of pKL97 into ΔpmrA EC cells and plated 1mL (x4) for each reaction onto CHA-Kan for a total of 12 plates
Performed sucrose selection on ΔpmrA PriM+ primary integrins 2-4 and 2-5



When you increase the exposure on the red channel you can actually see the tul4! So next time going to reduce the amount of ladder to be able to detect the tul4

Monday, Feb 24th , 2020

To Do:

1. ~~Run gel and transfer~~
2. ~~Patch colonies from sucrose selection~~

Going forward with the project, going to try and target aim 2 to specifically look at the suppressor mutant

1. Growth curve with suppressor, LVS, new ΔpmrA
2. Does the suppressor have less PriM at the level of transcription (qRTPCR)

a. Isolate RNA in triplicate, streak to single colony, patch out cells and grow to mid-log (6mL total), want to have extra cDNA from ΔpmrA new, could try 30mL in flasks for growth curve as well

3. VSVG tag the PriM in the suppressor

Patched out 16 colonies from primary integrins 2-4 and 2-5 sucrose plates

Gel for PriM VSVG tagged strains using samples grown on 1/28

Lane	Volume	Contents
1	3ul	Precision protein ladder (1:10)
2	13ul	LVS
3	13ul	ΔpmrA PriM-V-1 (1:50)
4	13ul	ΔpmrA PriM-V-2 (1:50)
5	13ul	ΔpmrA PriM-V-3 (1:50)
6	13ul	ΔpmrA no_C1-V-1
7	13ul	ΔpmrA no_C1-V-2
8	13ul	ΔpmrA no_C1-V-3
9	13ul	ΔpmrA mtip2-V-1
10	13ul	ΔpmrA mtip2-V-2
11	13ul	ΔpmrA mtip2-V-3
12	13ul	ΔpmrA mpk1-V-1
13	13ul	ΔpmrA mpk1-V-2
14	13ul	ΔpmrA mpk1-V-3
15	10ul	1x SLB

Ran gel using MES buffer for about 1 hour at 150V and then did a wet transfer in the cold room at 20V for an hour and set in 25mL blocking buffer in the cold room shaking

Froze down 2 vials of p6 macrophage at an average count of 99 cells/mL

Tuesday, Feb 25th , 2020

To Do:

1. ~~Develop western~~
2. ~~Colony PCR~~

Colony PCR to check for deletion of pmrA in the LVS PriM+ background from primary integrin 2-4

Total reaction volume	20

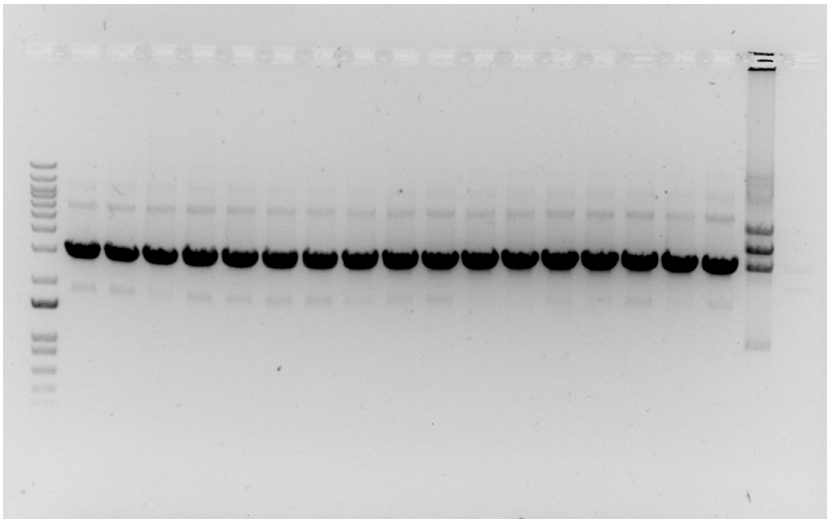
Total number of reactions	19

Component	Stock concentration	Final concentration	1 rxn volume	Factor 20
ddiH2O			10.8	216
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL308	10 uM	0.3 uM	0.6	12
oligo R KROL309	10 uM	0.3 uM	0.6	12
Template	100 ng/ul	2 ng/ul	5	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

Sample table for colonies from primary 2-1

Reaction Number	Sample	Lane on gel	Primers	Size
1	17	2	KROL308, KROL309	
2	18	3	KROL308, KROL309	
3	19	4	KROL308, KROL309	
4	20	5	KROL308, KROL309	
5	21	6	KROL308, KROL309	
6	22	7	KROL308, KROL309	
7	23	8	KROL308, KROL309	
8	24	9	KROL308, KROL309	
9	25	10	KROL308, KROL309	
10	26	11	KROL308, KROL309	
11	27	12	KROL308, KROL309	
12	28	13	KROL308, KROL309	
13	29	14	KROL308, KROL309	
14	30	15	KROL308, KROL309	

15	31	16	KROL308, KROL309	
16	32	17	KROL308, KROL309	
17	LVS gDNA	18	KROL308, KROL309	2952
18	pKL37 plasmid	19	KROL308, KROL309	
19	- control (no template)	20	KROL308, KROL309	



All samples came back as wildtype. Going to try a second set of samples from the other primary integrin

Wednesday, Feb 26th , 2020

To Do:

1. ~~Colony PCR~~

Colony PCR to check for deletion of pmrA in the LVS PriM+ background from primary integrin 2-5

Total reaction volume	20
Total number of reactions	19

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	20
ddiH2O			10.8	216
PrimeSTAR GXL Buffer	5x	1x	4	80
dNTPs	2.5 mM	0.2 mM	1.6	32
oligo F KROL308	10 uM	0.3 uM	0.6	12
oligo R KROL309	10 uM	0.3 uM	0.6	12

Template	100 ng/ul	2 ng/ul	5	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	8
		Total volume	20	400

Sample table for colonies from primary 2-1

Reaction Number	Sample	Lane on gel	Primers	Size
1	17	2	KROL308, KROL309	
2	18	3	KROL308, KROL309	
3	19	4	KROL308, KROL309	
4	20	5	KROL308, KROL309	
5	21	6	KROL308, KROL309	
6	22	7	KROL308, KROL309	
7	23	8	KROL308, KROL309	
8	24	9	KROL308, KROL309	
9	25	10	KROL308, KROL309	
10	26	11	KROL308, KROL309	
11	27	12	KROL308, KROL309	
12	28	13	KROL308, KROL309	
13	29	14	KROL308, KROL309	
14	30	15	KROL308, KROL309	
15	31	16	KROL308, KROL309	
16	32	17	KROL308, KROL309	
17	LVS gDNA	18	KROL308, KROL309	2952
18	pKL37 plasmid	19	KROL308, KROL309	
19	- control (no template)	20	KROL308, KROL309	

Colony PCR on strains KMLFT 72.1 and 72.2 (ΔpmrA PriM-V) to check for suppressor mutation

Total reaction volume	50
Total number of reactions	2

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	3
ddiH2O			30	90
PrimeSTAR GXL Buffer	5x	1x	10	30
dNTPs	2.5 mM	0.2 mM	4	12
oligo F KROL270	10 uM	0.3 uM	1.5	4.5
oligo R KROl271	10 uM	0.3 uM	1.5	4.5
template	100 ng/ul	2 ng/ul	2	2
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	1	3
		Total volume	50	100

Thursday, Feb 27th , 2020

To Do:

1. ~~Colony PCR~~
2. ~~Sequencing~~

PCR purified samples to be sequenced to check for the suppressor mutation and submitted for sequencing to see if I'll need to make a new strain with the VSVG tag on the PriM in the suppressor strain

Friday, Feb 28th , 2020

To Do:

1. ~~RNA isolation~~
2. ~~Pour plates for mac assay~~

Started cultures for RNA isolation. Used 4 of each strain (1-LVS, 2-ΔpmrA, and 3-ΔpmrA-supressor) Resuspended to 0.1OD in 6mL MHB and will let grow to mid-log, will only use 3 samples from each strain, but made an extra just in case one doesn't grow well

Took a time 0 OD for each sample before setting to shake at 37C

Sample	OD600 T=0	OD600 at harvest
1-1	0.105	0.335
1-2	0.097	0.328
1-3	0.098	0.327
1-3A	0.099	
2-1	0.096	0.304
2-2	0.101	0.293
2-3	0.104	0.319
2-3A	0.111	
3-1	0.092	0.341
3-2	0.108	0.335
3-3	0.110	0.300
3-3A	0.104	

Nanodrop results from RNA extraction: All values

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Constant	Cursor Pos.
1-1	Default	2/28/2020	3:43 PM	652.88	16.322	7.569	2.16	2.27	40.00	230	7.181	0.159
1-2	Default	2/28/2020	3:45 PM	719.10	17.978	8.454	2.13	2.38	40.00	230	7.538	0.297
1-3	Default	2/28/2020	3:46 PM	657.78	16.445	7.779	2.11	2.37	40.00	230	6.927	0.072
2-1	Default	2/28/2020	3:46 PM	677.75	16.944	7.921	2.14	2.40	40.00	230	7.052	0.092
2-2	Default	2/28/2020	3:48 PM	627.14	15.678	7.251	2.16	2.37	40.00	230	6.628	0.320
2-3	Default	2/28/2020	3:49 PM	651.49	16.287	7.618	2.14	2.42	40.00	230	6.728	0.087
3-1	Default	2/28/2020	3:50 PM	665.78	16.645	7.714	2.16	2.40	40.00	230	6.936	0.098
3-2	Default	2/28/2020	3:51 PM	690.39	17.260	8.006	2.16	2.41	40.00	230	7.151	0.295
3-3	Default	2/28/2020	3:51 PM	535.77	13.394	6.176	2.17	2.40	40.00	230	5.590	0.205

Monday, Mar 02nd , 2020

To Do:

1. ~~Streak out strains for mac assay~~
2. ~~Seed for mac assay~~
3. ~~Pour plates~~

Cross patched 32 single colonies from dpmrA PriM+ from patches that had grown on Kan

Tuesday, Mar 03rd , 2020

To Do:

1. ~~Mac assay day 1~~
2. ~~Check cross patches~~
3. ~~Patch out VSVG tagged strains~~

Patched out 5 single colonies from VSVG tagged strains onto CHA-Kan

Performed inoculation and 2 hour time point for macrophage assay testing suppressor mutant strains

Wednesday, Mar 04th , 2020

To Do:

1. ~~Mac assay day 2~~
2. ~~Make cultures for PriM western~~
3. ~~Colony PCR~~

Colony PCR to check for deletion of pmrA in the LVS PriM+ background from primary integrin 2-2

Total reaction volume	20
Total number of reactions	19

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	28
ddiH2O			10.8	302.4
PrimeSTAR GXL Buffer	5x	1x	4	112
dNTPs	2.5 mM	0.2 mM	1.6	44.8
oligo F KROL252	10 uM	0.3 uM	0.6	16.8
oligo R KROL253	10 uM	0.3 uM	0.6	16.8
Template	100 ng/ul	2 ng/ul	5	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	11.2
		Total volume	20	400

Sample table for colonies from primary 2-1

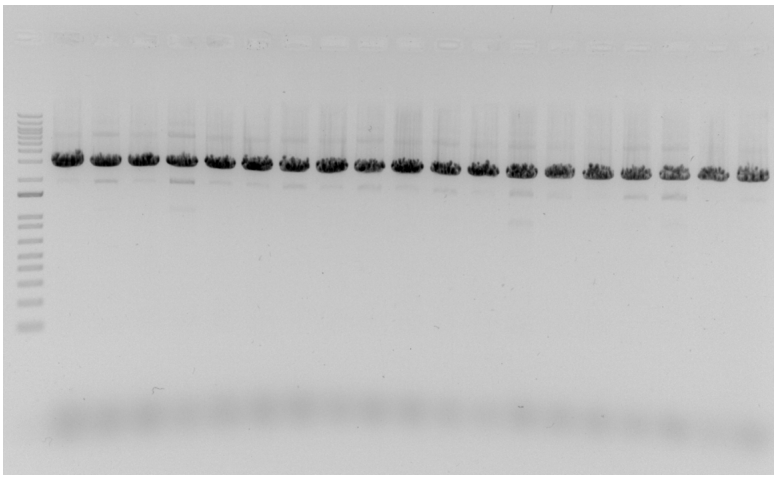
Reaction Number	Sample	Lane on gel	Primers	Size
1	17	2	KROL308, KROL309	
2	18	3	KROL308, KROL309	
3	19	4	KROL308, KROL309	
4	20	5	KROL308, KROL309	
5	21	6	KROL308, KROL309	
6	22	7	KROL308, KROL309	
7	23	8	KROL308, KROL309	
8	24	9	KROL308, KROL309	
9	25	10	KROL308, KROL309	

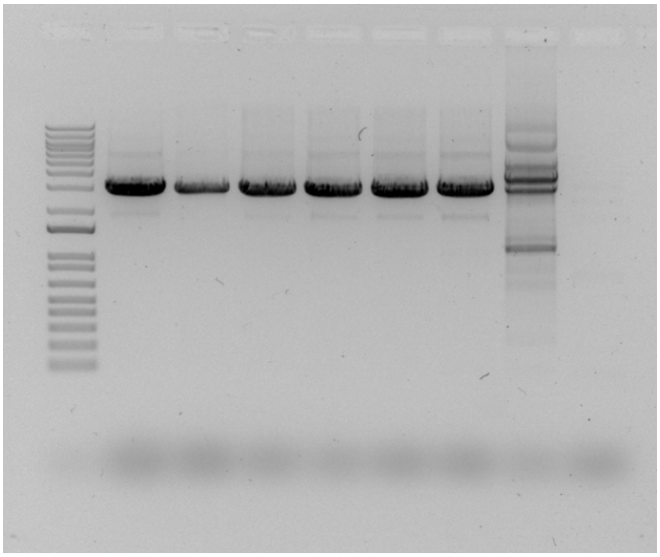
10	26	11	KROL308, KROL309	
11	27	12	KROL308, KROL309	
12	28	13	KROL308, KROL309	
13	29	14	KROL308, KROL309	
14	30	15	KROL308, KROL309	
15	31	16	KROL308, KROL309	
16	32	17	KROL308, KROL309	
17	LVS gDNA	18	KROL308, KROL309	2952
18	pKL37 plasmid	19	KROL308, KROL309	
19	- control (no template)	20	KROL308, KROL309	

Started cultures in triplicate for PriM VSVG tagged strains at 0.1 OD in 5mL and harvested at mid log (0.3-0.4OD)

Spun down 1mL of cultures and resuspended with normalized amount of 1xSLB at the following ODs:

Strain	OD600
ΔpmrA PriM-V-1	0.329
ΔpmrA PriM-V-2	0.328
ΔpmrA PriM-V-3	0.337
ΔpmrA no_C1 PriM-V-1	0.310
ΔpmrA no_C1 PriM-V-2	0.318
ΔpmrA no_C1 PriM-V-3	0.330
ΔpmrA mtip2 PriM-V-1	0.333
ΔpmrA mtip2 PriM-V-2	0.358
ΔpmrA mtip2 PriM-V-3	0.351
ΔpmrA mpk1 PriM-V-1	0.370
ΔpmrA mpk1 PriM-V-2	0.363
ΔpmrA mpk1 PriM-V-3	0.365



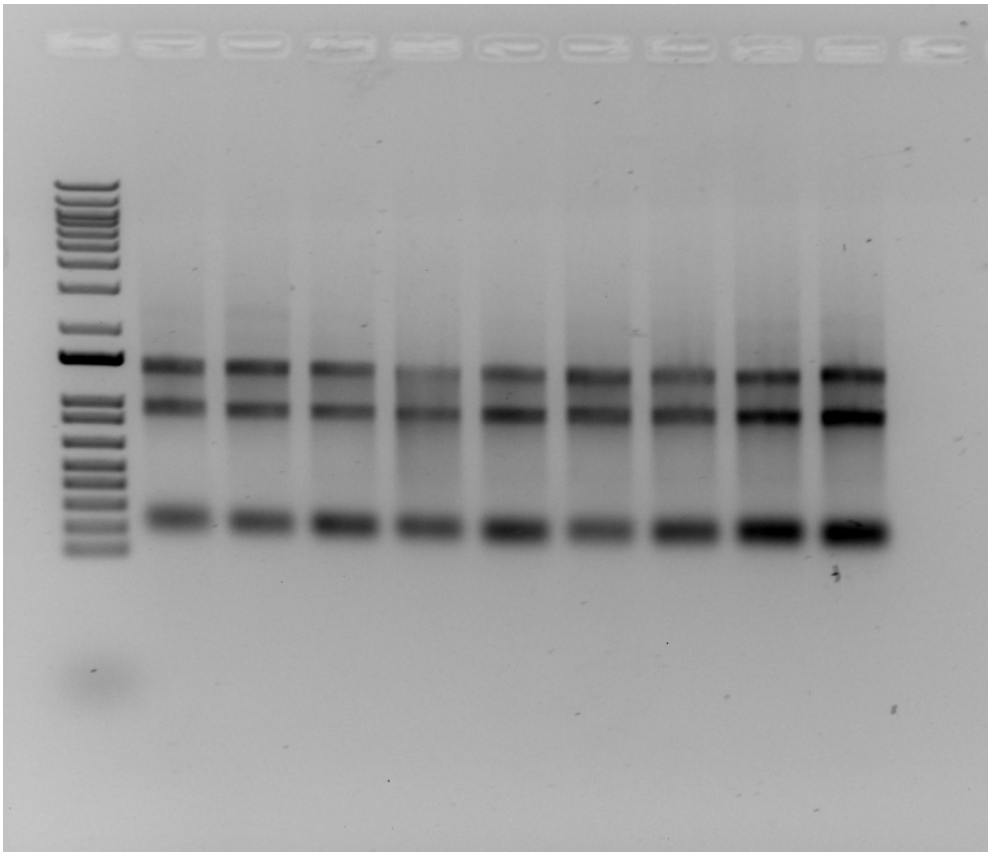


Tested all patches that did not grow on Kan and they all turned out to be wildtype again.

Thursday, Mar 05th , 2020

To Do:

1. ~~DNase treatment of RNA samples~~
2. ~~Pour plates for mac assay~~



I was able to successfully isolate RNA from each of my 9 samples as seen by the 3 distinct bands

Tuesday, Mar 10th , 2020

To Do:

1. ~~Thaw cells~~
2. ~~Run gel and transfer~~

Gel for PriM VSVG tagged strains using samples grown on 3/04

Lane	Volume	Contents
1	1ul	Precision protein ladder (1:5)
2	13ul	ΔpmrA PriM-V-1 (1:50)
3	13ul	ΔpmrA PriM-V-2 (1:50)
4	13ul	ΔpmrA PriM-V-3 (1:50)
5	13ul	ΔpmrA no_C1-V-1
6	13ul	ΔpmrA no_C1-V-2

7	13ul	ΔpmrA no_C1-V-3
8	13ul	ΔpmrA mtip2-V-1
9	13ul	ΔpmrA mtip2-V-2
10	13ul	ΔpmrA mtip2-V-3
11	13ul	ΔpmrA mpk1-V-1
12	13ul	ΔpmrA mpk1-V-2
13	13ul	ΔpmrA mpk1-V-3
14	13ul	LVS
15	10ul	1x SLB

Ran gel using MES buffer for about 1 hour at 150V and then did a wet transfer in the cold room at 20V for an hour and set in 25mL blocking buffer in the cold room shaking

Wednesday, Mar 11th , 2020

To Do:

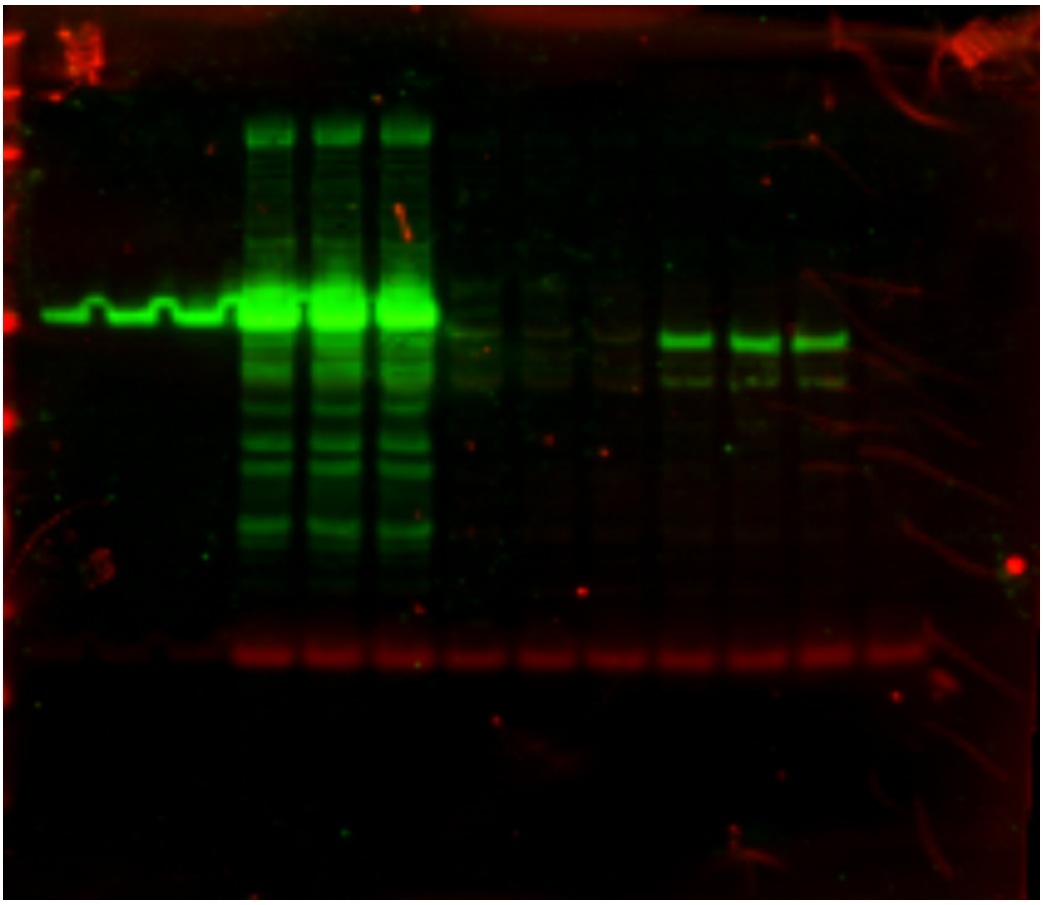
1. ~~Develop western~~
2. ~~Seed macrophage~~
3. ~~Streak out strains for mac assay~~
4. ~~Make cDNA~~

Component	Volume or Amount	Volume for Master Mix (11x)	Final Concentration
5X 1st strand buffer	12 ul	132	1x
RNase-free water	5.75 ul	63.25	
100 mM DTT	6.0 ul	66	10 mM
10 mM dNTPs	3.0 ul	33	0.5 mM
Superscript III (200 U/ul)	3.25	35.75	10.8 U/ul

Results from the cDNA nanodrop. Note: concentrations are a bit low because I forgot the do the long spin after the PE buffer wash so the volume was a bit higher than it should have been.

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	340 raw
1-1	Default	########	3:22 PM	17.9	0.542	0.317	1.71	2.09	33	0.005
1-2	Default	########	3:24 PM	21.03	0.637	0.382	1.67	1.72	33	0.015

1-3	Default	########	3:24 PM	17.68	0.536	0.309	1.73	2.13	33	0.012
2-1	Default	########	3:26 PM	20.2	0.612	0.365	1.68	2.03	33	0.012
2-2	Default	########	3:26 PM	19.09	0.579	0.343	1.69	1.86	33	0.026
2-3	Default	########	3:26 PM	16.73	0.507	0.286	1.77	1.91	33	0.019
3-1	Default	########	3:27 PM	12.84	0.389	0.246	1.58	1.65	33	0.019
3-2	Default	########	3:28 PM	17.76	0.538	0.317	1.7	1.99	33	0.022
3-3	Default	########	3:29 PM	14.43	0.437	0.27	1.62	1.46	33	0.011
3-1	Default	########	3:29 PM	16.98	0.515	0.305	1.68	2	33	0.008



Western testing the new samples of PriM-V mutants isolated on 3_04. Next time going to try loading 8ul to avoid spillover and make sure to position the blot so that the ladder doesn't get cut off.

Thursday, Mar 12th , 2020

To Do:

1. ~~Mac assay day 1~~
2. ~~Streak out cells for electroporations~~

Streaked out ΔpmrA suppressor strain and ΔpmrA FTL_0146SNP for electroporations tomorrow

Friday, Mar 13th , 2020

To Do:

1. ~~Mac assay day 2~~
2. ~~Electroporations~~
3. ~~qPCR primer efficiencies~~

Testing RT Primer Efficiencies Protocol

Note: Efficiencies should be run on same "type" of DNA as you plan to do the experiment on; i.e. ChIP= sonicated DNA, RNA expression = cDNA or RNA w/ 2-step kit

1) Determine concentration of DNA
2) Make a dilution that's final concentration is 1.5ng/ul.
3) Make 1:10 serial dilutions so you have 4 different DNA concentrations (1.5ng/ul, 0.15ng/ul, 0.015ng/ul, 0.0015ng/ul); named them A, B, C, and D respectively.
4) Put 17.5ul of each DNA sample into separate tubes (enough for 3.5rxns) – use strip tubes so you can use the electronic pipetter.
5) Set up primer master mixes in separate eppendorfs (make one for each primer pair)
   Since you have 4 DNA samples you have 14rxns/primer pair (add 3.5 to this for volume error)= 17.5

   example:

10ul of SYBR green	x 17.5 = 175
1ul of 5uM primer set	x 17.5 = 17.5
4ul ddH2O	x 17.5 = 70
	TOTAL = 262.5

5) Since 3.5 rxns x 20ul rxn volume = 70; and tubes already have 17.5ul of DNA in them you need to add 52.5ul (70-17.5) of primer master mix to each DNA tube
6) Pipette 20ul of each rxn into 3 separate wells on the 96 well plate as follows:

1A1	1A2	1A3	1B1	1B2	1B3	1C1	1C2	1C3	1D1	1D2	1D3
2A1	2A2	2A3	2B1	2B2	2B3	2C1	2C2	2C3	2D1	2D2	2D3

7) Spin plate down
8) Place in real-time machine and run rxn.

Parameters for old step-one program:

1. 95°C 10'
2. 95°C 15"
3. 60°C 60"
4. Go to step 2, 39x (total 40 cycles)
5. 95°C 10"
6. 65°C 60"
7. 97°C 60"

Calculations

ChIP enrichment

Primer Efficiency ^ (Ct_diff.)

Ct_diff. = (control_ChIP_Ct –test_ChIP_Ct) - (control_Input_Ct – test_Input_Ct)

Primer Efficiency = (10^(-1/slope))

Where the slope is the slope of the standard curve plot

Username and password are: K_Ramsey Lab, Roche480

1 – p437, p438 – priM

2 - Controls: KROL63 + 64 – tul4

After analyzing data have decided to stick with 1.5ng of DNA for qPCR

Monday, Mar 16th , 2020

To Do:

1. ~~Count mac assay plates~~
2. ~~qPCR~~

Then I diluted the cDNA to get a concentration of 1.5 ng/uL:

Since I'm doing 9 DNA samples today:
9 DNA samples per primer pair = 9 x 3.5 + 3.5 = 35
Set up primer master mixes in separate 1.5 mL tubes (make one for each primer pair, 2 total)

Example:

10 µL PowerUp SYBR Green Master Mix	x 35	= 350.0 µL
1 µL 5µM combined F and R primer	x 35	= 35 µL
8 µL ddiH_2O	x 35	= 280 µL
	TOTAL =	598.5 µL

Add primer master mix to tubes containing DNA.

3.5 reactions x 20 µL volume = 70 µL.
DNA tubes already have 3.5µL of DNA.
Add 66.5 µL of primer master mix to each tube

Primer Set #	Primers	Gene amplified
A	P437, p438	PriM
B	KROL63, 64	Tul4

DNA Tube	Sample
1	LVS 1
2	LVS 2
3	LVS 3
4	ΔpmrA sup-1
5	ΔpmrA sup-2
6	ΔpmrA sup-3
7	ΔpmrA-1
8	ΔpmrA-2
9	ΔpmrA-3

Plate 1:

1A	LVS-1	LVS-1	LVS-1	LVS-2	LVS-2	LVS-2	LVS-3	LVS-3	LVS-3		
1B	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-3	ΔpmrAS-3	ΔpmrAS-1		

1C	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2	ΔpmrA-3	ΔpmrA-3	ΔpmrA-3		
1D	LVS-1	LVS-1	LVS-1	LVS-2	LVS-2	LVS-2	LVS-3	LVS-3	LVS-3		
1E	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-3	ΔpmrAS-3	ΔpmrAS-1		
1F	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2	ΔpmrA-3	ΔpmrA-3	ΔpmrA-3		
1G			2G			3G			4G		
1H			2H			3H			4H		

Tuesday, Mar 17th , 2020

To Do:

1. ~~Streak out new patches for RNA Isolation~~

Patches had not sufficiently grown to allow for making cultures for the RNA extraction

Tuesday, Mar 17th , 2020

To Do:

1. ~~Growth curve~~
2. ~~RNA extraction~~

Colony PCR to check for suppressor mutation

Total reaction volume	50
Total number of reactions	4

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	5
ddiH2O			30	150
PrimeSTAR GXL Buffer	5x	1x	10	50
dNTPs	2.5 mM	0.2 mM	4	20
oligo F KROL270	10 uM	0.3 uM	1.5	7.5
oligo R KROl271	10 uM	0.3 uM	1.5	7.5
template	100 ng/ul	2 ng/ul	2	2
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	1	5
		Total volume	50	100

Friday, Jun 5th , 2020

To Do:

1. ~~Grow up cultures for western~~

Spun down 1mL of cultures and resuspended with normalized amount of 1xSLB at the following ODs:

Strain	OD600
ΔpmrA PriM-V-1	0.
ΔpmrA PriM-V-2	0.
ΔpmrA PriM-V-3	0.
ΔpmrA no_C1 PriM-V-1	0.
ΔpmrA no_C1 PriM-V-2	0.
ΔpmrA no_C1 PriM-V-3	0.
ΔpmrA mtip2 PriM-V-1	0.
ΔpmrA mtip2 PriM-V-2	0.
ΔpmrA mtip2 PriM-V-3	0.
ΔpmrA mpk1 PriM-V-1	0.
ΔpmrA mpk1 PriM-V-2	0.
ΔpmrA mpk1 PriM-V-3	0.

Monday, Jun 8th , 2020

To Do:

1. ~~Set up qPCR~~

Need to set up qPCR with iglA and pdpB with dpmrA and sup dpmrA to see what's going on in the FPI

qPCR primers:

pdpB: KROL298-KROL303 (3 sets of primers)
iglA: KROL306-307

Still use tul4 as a control?
There isn't enough room for LVS, dpmrA-sup, dpmrA for 3 genes

Remember to sign up with the GSC!
Username and password are: K_Ramsey Lab, Roche480

Since I'm doing 9 DNA samples today:
9 DNA samples per primer pair = 9 x 3.5 + 3.5 = 35
Set up primer master mixes in separate 1.5 mL tubes (make one for each primer pair, 2 total)

Example:

10 µL PowerUp SYBR Green Master Mix	x 35	= 350.0 µL
1 µL 5µM combined F and R primer	x 35	= 35 µL
8 µL ddiH_2O	x 35	= 280 µL
	TOTAL =	598.5 µL

Add primer master mix to tubes containing DNA.

3.5 reactions x 20 µL volume = 70 µL.
DNA tubes already have 3.5µL of DNA.
Add 66.5 µL of primer master mix to each tube

Primer Set #	Primers	Gene amplified
A	KROL306, KROL307	iglA
B	KROL300, KROL301	pdpB
C	KROL63, 64	Tul4

DNA Tube	Sample
1	LVS 1
2	LVS 2
3	LVS 3
4	ΔpmrA -1
5	ΔpmrA -2
6	ΔpmrA -3
7	ΔpmrA sup-1
8	ΔpmrA sup-2
9	ΔpmrA sup-3

Plate 1:

1A	LVS-1	LVS-1	LVS-1	LVS-2	LVS-2	LVS-2	LVS-3	LVS-3	LVS-3	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1
1B	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-3	ΔpmrAS-3	ΔpmrAS-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2
1C	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2	ΔpmrA-3	ΔpmrA-3	ΔpmrA-3	ΔpmrAS-3	ΔpmrAS-3	ΔpmrAS-3
1D	LVS-1	LVS-1	LVS-1	LVS-2	LVS-2	LVS-2	LVS-3	LVS-3	LVS-3			
1E	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-3	ΔpmrAS-3	ΔpmrAS-3			
1F	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2	ΔpmrA-3	ΔpmrA-3	ΔpmrA-3			
1G	LVS-1	LVS-1	LVS-1	LVS-2	LVS-2	LVS-2	LVS-3	LVS-3	LVS-3			
1H	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2	ΔpmrA-3	ΔpmrA-3	ΔpmrA-3			

Tuesday, Jun 9th, 2020

To Do:

1. ~~Make EC cells~~
2. ~~Collect qPCR data~~
3. ~~DNase treatment~~
4. ~~Run gel and transfer~~

Lane	Volume	Contents
1	1ul	Precision protein ladder (1:5)
2	10ul	ΔpmrA PriM-V-1 (1:50)
3	10ul	ΔpmrA PriM-V-2 (1:50)
4	10ul	ΔpmrA PriM-V-3 (1:50)
5	10ul	ΔpmrA no_C1-V-1
6	10ul	ΔpmrA no_C1-V-2
7	10ul	ΔpmrA no_C1-V-3
8	10ul	ΔpmrA mtip2-V-1
9	10ul	ΔpmrA mtip2-V-2
10	10ul	ΔpmrA mtip2-V-3
11	10ul	ΔpmrA mpk1-V-1
12	10ul	ΔpmrA mpk1-V-2
13	10ul	ΔpmrA mpk1-V-3
14	10ul	LVS
15	10ul	1x SLB

Ran gel using MES buffer for about 1 hour at 150V and then did a wet transfer in the cold room at 20V for an hour and set in 25mL blocking buffer in the cold room shaking

Wednesday, Jun 10th , 2020

To Do:

1. ~~Make cDNA~~
2. ~~Develop western~~
3. ~~Plate Eps~~

Generate cDNA

Adapted from Lory lab microarray protocol

Combine the first components for primer annealing:

Component	Volume or Amount	Final Concentration
RNA	5 - 10 ug	267 - 333 ng/ ul
$(NS)_5$ Primer (250 ng/ul)	3 ul	25 ng/ul
RNase-free water	up to 30 ul	

Incubate using program JSScDNA1:

Step	Temp	Time
1	70°C	10'
2	25°C	10'
3	4°C	hold

While waiting, prepare the cDNA synthesis reaction in master mix format:

Component	Volume or Amount	Volume for Master Mix (10x)	Final Concentration
5X 1st strand buffer	12 ul	120	1x
RNase-free water	5.75 ul	57.5	
100 mM DTT	6.0 ul	60	10 mM
10 mM dNTPs	3.0 ul	30	0.5 mM
Superscript III (200 U/ul)	3.25	32.5	10.8 U/ul

Aliquot 30 ul per reaction

Incubate using program JSScDNA2

Step	Temp	Time
1	25°C	10'
2	37°C	60'
4	42°C	60'
5	70°C	10'
6	4°C	hold

Remove RNA

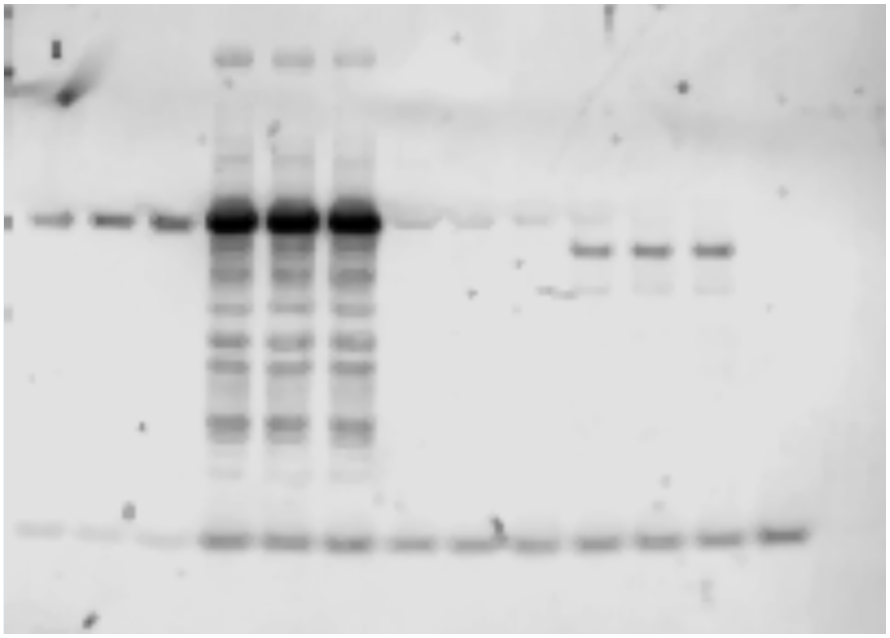
Transfer reactions to 1.5 mL tubes
Add 20 ul of 1N NaOH
Incubate 65°C for 30'
Neutralize with 20 ul of 1N HCl
Final volume is 100 ul
Purify cDNA using Qiagen PCR clean-up column
Elute in 60 ul of 0.1x EB

Still need to nanodrop cDNA samples

For western blot trying a higher dilution of tul4 (1:10,000) (1.5ul in 15mL)
6.8ul vsvg

Developed western blot

Hannah electroporated pKL75 into dpmrA Sup strain x2 and I plated them



Results from western, got a nice blot but the ladder got cut off, also was a lot of background noise so would like to reduce that.

Friday, Jun 12th , 2020

To Do:

1. ~~qPCR~~

Remember to sign up with the GSC!
Username and password are: K_Ramsey Lab, Roche480

Since I'm doing 9 DNA samples today:
9 DNA samples per primer pair = 9 x 3.5 + 3.5 = 35
Set up primer master mixes in separate 1.5 mL tubes (make one for each primer pair, 4 total)

Example:

10 µL PowerUp SYBR Green Master Mix	x 35	= 350.0 µL 700
1 µL 5µM combined F and R primer	x 35	= 35 µL 70
8 µL ddiH_2O	x 35	= 280 µL 560
	TOTAL =	598.5 µL

Add primer master mix to tubes containing DNA.

3.5 reactions x 20 µL volume = 70 µL.
DNA tubes already have 3.5µL of DNA.
Add 66.5 µL of primer master mix to each tube

Primer Set #	Primers	Gene amplified
A	KROL306, KROL307	iglA
B	KROL300, KROL301	pdpB
C	p437, p438	PriM
D	KROL63, 64	Tul4

DNA Tube	Sample
1	LVS 1
2	LVS 2
3	LVS 3
4	ΔpmrA-1
5	ΔpmrA-2
6	ΔpmrA-3
7	ΔpmrAS-1
8	ΔpmrAS-2
9	ΔpmrAS-3

1A	LVS-1	LVS-1	LVS-1	LVS-2	LVS-2	LVS-2	LVS-3	LVS-3	LVS-3			
1B	LVS-1	LVS-1	LVS-1	LVS-2	LVS-2	LVS-2	LVS-3	LVS-3	LVS-3			
1C	LVS-1	LVS-1	LVS-1	LVS-2	LVS-2	LVS-2	LVS-3	LVS-3	LVS-3			
1D	LVS-1	LVS-1	LVS-1	LVS-2	LVS-2	LVS-2	LVS-3	LVS-3	LVS-3			
1E	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2	ΔpmrA-3	ΔpmrA-3	ΔpmrA-3			
1F	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2	ΔpmrA-3	ΔpmrA-3	ΔpmrA-3			
1G	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2	ΔpmrA-3	ΔpmrA-3	ΔpmrA-3			
1H	ΔpmrA-1	ΔpmrA-1	ΔpmrA-1	ΔpmrA-2	ΔpmrA-2	ΔpmrA-2	ΔpmrA-3	ΔpmrA-3	ΔpmrA-3			

Monday, Jun 15th , 2020

To Do:

1. ~~qPCR~~

Remember to sign up with the GSC!
Username and password are: K_Ramsey Lab, Roche480

Since I'm doing 9 DNA samples today:
9 DNA samples per primer pair = 9 x 3.5 + 3.5 = 35
Set up primer master mixes in separate 1.5 mL tubes (make one for each primer pair, 4 total)

Example:

10 µL PowerUp SYBR Green Master Mix	x 35	= 350.0 µL 175
1 µL 5µM combined F and R primer	x 35	= 35 µL 17.5
8 µL ddiH_2O	x 35	= 280 µL 140
	TOTAL =	598.5 µL

Add primer master mix to tubes containing DNA.
3.5 reactions x 20 µL volume = 70 µL.

DNA tubes already have 3.5µL of DNA.
Add 66.5 µL of primer master mix to each tube

Primer Set #	Primers	Gene amplified
A	KROL306, KROL307	iglA
B	KROL300, KROL301	pdpB
C	p437, p438	PriM
D	KROL63, 64	Tul4

DNA Tube	Sample
1	LVS 1
2	LVS 2
3	LVS 3
4	ΔpmrA -1
5	ΔpmrA-2
6	ΔpmrA-3
7	ΔpmrAS -1
8	ΔpmrAS-2
9	ΔpmrAS-3

1A	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-3	ΔpmrAS-3	ΔpmrAS-3			
1B	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-3	ΔpmrAS-3	ΔpmrAS-3			
1C	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-3	ΔpmrAS-3	ΔpmrAS-3			
1D	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-1	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-2	ΔpmrAS-3	ΔpmrAS-3	ΔpmrAS-3			
1E												
1F												
1G												
1H												

Colony PCR for checking integration of the VSVG tag from patches of electroporations

Total reaction volume	20
Total number of reactions	15

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	16
ddiH2O			10.8	172.8
PrimeSTAR GXL Buffer	5x	1x	4	64
dNTPs	2.5 mM	0.2 mM	1.6	25.6
oligo F KROL150	10 uM	0.3 uM	0.6	9.6
oligo R KROL240	10 uM	0.3 uM	0.6	9.6
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	6.4
		Total volume	20	140

Reaction Number	Sample	Lane on gel	Primers	Size
1	ΔpmrA PriM-sup	2	KROL150, KROL240	~1000
2	ΔpmrA PriM-sup	3	KROL150, KROL240	
3	ΔpmrA PriM-sup	4	KROL150, KROL240	
4	ΔpmrA PriM-sup	5	KROL150, KROL240	
5	ΔpmrA PriM-sup	6	KROL150, KROL240	
6	ΔpmrA PriM-sup	7	KROL150, KROL240	
7	ΔpmrA PriM-sup	8	KROL150, KROL240	
8	ΔpmrA PriM-sup	9	KROL150, KROL240	
9	LVS gDNA	10	KROL150, KROL240	
10	pKR75 plasmid	11	KROL150, KROL240	
11	- control (no template)	12	KROL150, KROL240	

Tuesday, Jun 23rd , 2020

To Do:

1. make cultures for western
2. check electroporations by PCR

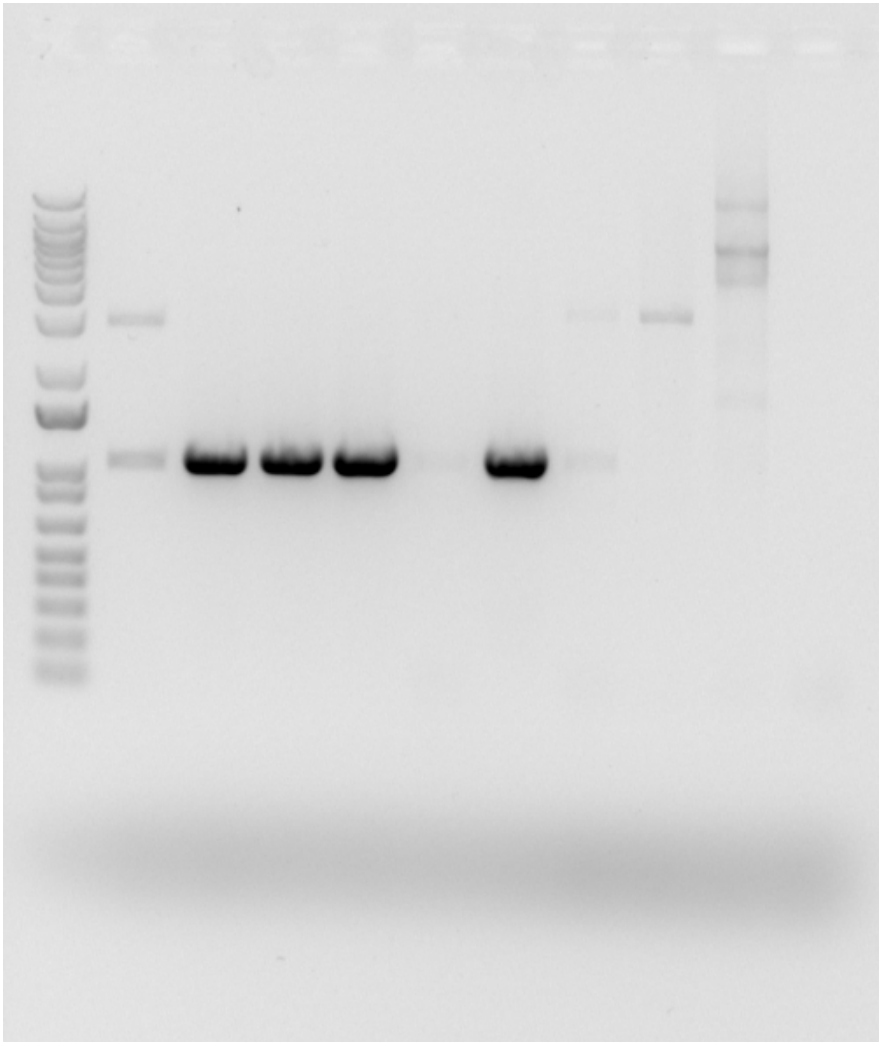
Colony PCR for checking integration of the VSVG tag from patches of electroporations

Total reaction volume	20
Total number of reactions	15

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	12
ddiH2O			10.8	129.6
PrimeSTAR GXL Buffer	5x	1x	4	48
dNTPs	2.5 mM	0.2 mM	1.6	19.2
oligo F KROL150	10 uM	0.3 uM	0.6	7.2
oligo R KROL240	10 uM	0.3 uM	0.6	7.2
Template	100 ng/ul	2 ng/ul	2	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	4.8
		Total volume	20	140

Reaction Number	Sample	Lane on gel	Primers	Size
1	ΔpmrA PriM-sup	2	KROL150, KROL240	~1000
2	ΔpmrA PriM-sup	3	KROL150, KROL240	
3	ΔpmrA PriM-sup	4	KROL150, KROL240	
4	ΔpmrA PriM-sup	5	KROL150, KROL240	
5	ΔpmrA PriM-sup	6	KROL150, KROL240	
6	ΔpmrA PriM-sup	7	KROL150, KROL240	
7	LVS gDNA	8	KROL150, KROL240	
8	VSVG gDNA	9	KROL150, KROL240	

9	pKR75 plasmid	10	KROL150, KROL240	
10	- control (no template)	11	KROL150, KROL240	



Lane	Volume	Contents
1	1ul	Precision protein ladder (1:5)
2	10ul	LVS
3	10ul	LVS
4	10ul	ΔpmrA PriM-V (1:50)
5	10ul	ΔpmrA PriM-V (1:50)
6	10ul	ΔpmrA(sup) PriM-V
7	10ul	ΔpmrA(sup) PriM-V
8	10ul	1x SLB
9	1ul	Precision protein ladder (1:5)
10	10ul	LVS
11	10ul	LVS
12	10ul	ΔpmrA PriM-V (1:50)
13	10ul	ΔpmrA PriM-V (1:50)
14	10ul	ΔpmrA(sup) PriM-V (1:10)
15	10ul	ΔpmrA(sup) PriM-V (1:10)

Ran gel using MES buffer for about 1 hour at 150V and then did a wet transfer in the cold room at 20V fo r an hour and set in 25mL blocking buffer in the cold room shaking

For western blot trying a higher dilution of tul4 (1:10,000) (1.5ul in 15mL)
6.8ul vsvg

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.