

## Table of Contents

December 2019 .....	2
Future To-Do .....	10
Bibliography .....	11

**December 2019****Monday, December 2, 2019****To Do:**

1. Digest gDNA for inverse PCR test

**Results and Data:****Digest**

LVS gDNA is 901 ng/uL

Reagent	
Water	21.0
10X CSB	3.0
gDNA	5.5
PstI-HF	0.5
<b>Total</b>	<b>30</b>

Leave at 37°C O/N

**Tuesday, Wednesday, December 3-4, 2019**

Sick ☹ Will need to re-do digest later.

**Friday, December 6, 2019****To Do:**

1. Digest gDNA for inverse PCR test

**Results and Data:****Digest**

LVS gDNA is 901 ng/uL. Digest 5 ug total.

Reagent	
Water	21.0
10X CSB	3.0
gDNA	5.5
PstI-HF	0.5
<b>Total</b>	<b>30</b>

Leave at 37°C O/N, Hannah will purify tomorrow and set up ligations.

**Friday, December 13, 2019****To Do:**

4.  $\Delta$ chaC cell viability expt

**Results and Data:****Test viability of  $\Delta$ chaC cells and suppressor mutant.**

Check viable CFU per OD600:

Number	Strain	Measured OD600	Actual OD600	Volume for 0.03 in 1.5 mL (uL)	Volume media (pipette 2x)	Final OD600
1	LVS	0.134	1.34	33.6	733.2	0.031
2	KMLFT114 LVS $\Delta$ chaC	0.200	2	22.5	738.6	0.031
3	KRLVS42.1 LVS $\Delta$ chaC	0.195	1.95	23.1	738.5	0.035
4	LVS $\Delta$ chaC_S 8-8	0.250	2.5	18.0	741	0.033

All cells resuspended in DMEMF, diluted serially to  $10^{-5}$  in 1X PBS, plated 10 uL undiluted plus all 5 dilutions on track plates 2x, put at 37°C.

**Monday, December 16, 2019****To Do:**

1. Count colonies from  $\Delta$ chaC cell viability expt

**Results and Data:****Assess viability of  $\Delta$ chaC cells and suppressor mutant.**

(pulled out plates from incubator yesterday and left at room temp)

#		Track Plate 1					Track Plate 2					Dilution factor counted	Average Cells	CFU per mL
		1	2	3	4	5	1	2	3	4	5*			
1	LVS	TMTC	TMTC	TMTC	TMTC	98	TMTC	TMTC	TMTC	TMTC	54	0.0001	76	7.60E+07
2	KMLFT114.1 $\Delta$ chaC	TMTC	TMTC	TMTC	TMTC	53	TMTC	TMTC	TMTC	TMTC	33	0.0001	43	4.30E+07
3	KRLVS42.1 $\Delta$ chaC	TMTC	TMTC	TMTC	TMTC	60	TMTC	TMTC	TMTC	TMTC	40	0.0001	50	5.00E+07
4	KRLVS42.1 $\Delta$ chaC S8-8	TMTC	TMTC	TMTC	TMTC	95	TMTC	TMTC	TMTC	TMTC	96	0.0001	96	9.55E+07
	Dilution Factor	1	0.1	0.01	0.001	0.0001	1	0.1	0.01	0.001	0.0001			

\* all strains except #4 had drops extending to bottom of plate, so counts are artificially low

? Okay. Apparently essentially no difference between the  $\Delta$ chaC strains and LVS with respect to viability. MAYBE a 2x difference.

On the original plate (used to prepare these inoculums) the  $\Delta$ chaC strains are very clearly mucoid-looking on the original plate and LVS and the suppressor look normal.

The colonies for the original (KMLFT)  $\Delta$ chaC strain look more "normal" and have some variability in size (accumulation of suppressor?).

The colonies from the second  $\Delta$ chaC strain (KRLVS42) look different- shinier/mucoid? No clear size differences.

**Prepare to perform a macrophage experiment on Thursday comparing  $\Delta$ chaC (KRLVS42) +/- cysteine. Use dptA(-) as a control (KRLVS1).**

**Tuesday, December 17, 2019****To Do:**

1. Autoclave media for mac expt
2. Thaw macrophage
3. Pour plates for mac expt

**Results and Data:****Mac expt preparation**

Use	Number of plates		Notes
	Round	Square	
Patch strains for infections	6		2x each
Plate inoculums	0	12	3 strains, 2 conditions in duplicate
Timepoint 2 hours	36	0	3 strains x 2 conditions x 3 wells x 2 plates
Timepoint 24 hours	6	30	Square: 2.5 strains x 2 conditions x 3 wells x 2 plates; round: 1 strain x 1 condition x 3 wells x 2 plates
Total plates	48	42	Round plates: 24 mL, 25 per flask; Square plates: 30 mL, 20 per flask
Flasks of 600 mL CHA	1.92	2.1	
Total number of CHA flasks	4.02		

Tala prepared 5 x 300 mL hemoglobin

I autoclaved 5 x 300 mL CHA.

Jamie had 9 square plates left from her expts

I poured 2.5 flasks of square plates (50 plates) and 2.5 flasks of round (62 plates).

Thawed 2 vials of J774A.1 P4s into one 100mm plate with 10mL DMEMF prepped by JW.

### Wednesday, December 18, 2019

#### To Do:

1. ~~Streak out strains for mac expt~~
  - a. ~~LVS~~
  - b. ~~KRLVS1 *dptA*(-)~~
  - c. ~~KRLVS42  $\Delta$ *chaC*~~
2. Seed macrophage

#### Results and Data:

Not enough macrophage! About  $\frac{1}{2}$  the amount necessary. Re-do later.

### Thursday, December 26, 2019

#### To Do:

1. ~~Thaw macrophage~~

#### Results and Data:

Thawed 2 vials of P4 J774A.1 cells into a single 100 mm dish.

### Friday, December 27, 2019

#### To Do:

1. ~~Feed macrophage~~

#### Results and Data:

Fed macrophage.

### Saturday, December 28, 2019

#### To Do:

1. ~~Streak out strains for mac expt~~
  - a. ~~LVS~~
  - b. ~~KRLVS1 *dptA*(-)~~
  - c. ~~KRLVS42  $\Delta$ *chaC*~~
2. ~~Seed macrophage~~
3. ~~Passage macrophage~~

#### Results and Data:

##### Streak out strains:

All from original stocks, no single-use aliquots

LVS, KRLVS1.1 (*dptA*(-)), KRLVS42.1 ( $\Delta$ *chaC*)

##### Seed macrophage:

Remove old media, replace with 10 mL fresh media

Scrape up all cells

Spin 4' 4000 rpm in Howlett lab centrifuge

Discard supernatant

Resuspend cells in 7 mL DMEMF

Check cell density using hemocytometer:

6	19	14	11	
9	10	10	6	
Average				10.625
Undiluted				21.25
Density				2.13E+05

Good density, but my original calculations called for 12 mL of cells at 1.67e5, because I intended to seed using a multichannel and there is quite a bit of waste with the reservoir- I only actually need 5.4 mL (9 wells per row x 4 rows x 0.15 mL) . There aren't enough cells for that waste, so simply add enough media to dilute to 1.67e5 and use a single channel to seed. Will continue propagating the remainder of the cells.

$$(7 \text{ mL})(21.25e4) / 16.7e4 = 8.9 \text{ mL}$$

Add 1.9 mL DMEM and transfer 150 uL to wells in 96-well plates, **2X**, as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	LVS	LVS	LVS		$\Delta$ chaC	$\Delta$ chaC	$\Delta$ chaC		dptA(-)	dptA(-)	dptA(-)	
<b>B</b>												
<b>C</b>												
<b>D</b>	LVS	LVS	LVS		$\Delta$ chaC	$\Delta$ chaC	$\Delta$ chaC		dptA(-)	dptA(-)	dptA(-)	
<b>E</b>												

Have about 3mL left, transfer with 7 mL DMEMF to new 100 mm TC plate.

Prepare cysteine (or mock) supplementation:

**Cysteine Supplementation Calculations**

Total final volume in well	0.2
Volume to plate (mL)	0.05
Total volume needed	4
Final conc cysteine in well (mM)	5
Stock cysteine (mM)	500
Conc cysteine to add (mM)	20
Volume stock cysteine (mL)	0.16
Volume media	3.84

Using multichannel, add 50uL mock to row A and 50uL cysteine to row D in both plates

Put 96-well plates and TC plate in Dutta lab CO2 incubator.

Check density of cells plated:

2nd measurement				
5	7	9	4	
5	16	7	3	
6	17	6	4	
8	9	11	8	
Average				7.8125
Undiluted				15.625
Density				1.56E+05

A tiny bit lower than I was aiming for, but certainly close enough. Final estimation of macrophage seeded per well:  $2.34 \times 10^4$

**Sunday, December 29, 2019**

**To Do:**

1. Prepare inoculum
2. Infect macs
3. Wash macs
4. T=2

**Results and Data:**

## Plan for inoculums:

Bacterial Calculations	Actual
MOI	5
Macrophage cells per well	2.34E+04
Volume bacteria to add (mL)	0.05
Bacterial density needed (cells/mL)	2.34E+06
Cells/mL per OD600	5.81E+09
OD needed for given density	0.00040
Resuspend to	0.040
Final MOI 5, dilute 1:100	0.00040

\*Concerned about  $\Delta$ chaC viability. This was recently tested (see results, 12/16/19) but I tested cells diluted to  $\sim$ 0.03. Maybe they are less viable at lower densities? Serially dilute and plate cells from the “dilution” tube, which should be at  $\sim$ 0.04, as well as the inoculum. Use the older plates Jamie had left over from her last macrophage assay for this- will only need 6 square plates, because only 3 strains at this point.

Prepare 96-well plates with 1x PBS for dilution of inoculums and additional dilution.

Note that in pipetting the PBS for the inoculum dilution, made minor error pipetting row “D.” Add extra row of PBS (to “F” instead of as normal, to “E”) and don’t use that row for dilutions.

Scrape up cells into 300  $\mu$ L DMEMF, resuspend, add 650  $\mu$ L more DMEMF, check OD600 with 1:10 dilution. Calculate the volume of resuspended cells to add to new tube of DMEMF to get 1.6 mL of 0.04. Use 1mL to check OD600. Calculate the volume of diluted cells to add to 5 mL DMEMF to get a final OD600 of 0.0004. Before actually performing dilution for the inoculum, serially dilute and plate the cells that are at  $\sim$ 0.04.

Number	Strain	Resuspend cells to (OD600)	For final vol 1.6 mL at 0.04			Volume to dilute in 1 mL (usually 1:100)	Volume PBS or cysteine	Volume media removed from 5mL
			Cells ( $\mu$ L)	Media ( $\times$ 2, $\mu$ L)	OD600			
1	LVS	1.2	53.3	773.3	0.044	45.45	50	95.5
2	$\Delta$ chaC	0.95	67.4	766.3	0.038	52.63	50	102.6
3	dptA(-)	1.58	40.5	779.7	0.037	54.05	50	104.1

Do 2x: once in media with mock, once in +cysteine media

Dilute cells for inoculum in 50 mL conicals- have 6 tubes, representing 3 strains +/- cysteine.

#		Cysteine?
1	LVS	-
2	$\Delta$ chaC	-
3	dptA(-)	-
4	LVS	+
5	$\Delta$ chaC	+
6	dptA(-)	+

Bring inoculum, 96-well plates, and other necessary items to hood upstairs.

Pour inoculums from 50 mL conical into 3-well reservoir, 3 at a time. Use multichannel (2-50) to add 50uL cells to each well as indicated in plate setup. Transfer 200uL each inoculum to 96-well plate for serial dilutions in PBS.

Put 96-well plate in 37°C CO<sub>2</sub> incubator in Camberg lab TC room (has water in bottom).

### Infection done at 11:30AM.

Serially dilute inoculums (1 -> 10<sup>-4</sup>) and plate on square CHA plates using drip method. Skipped row "D" in dilution plate (see above) but just plate all the wells anyway- good test to be sure I am not getting bacteria everywhere! Put square plates at 37°C.

### Wash at 1:30PM

Have prepared 7mL DMEMF + gentamycin +/- cysteine.

#### Cysteine

Total volume needed (uL)	7000
Final conc cysteine (mM)	5
Stock cysteine (mM)	500
Volume stock cysteine (uL)	70

#### Gentamycin

Gentamycin media (uL)	7000
Stock gentamycin (ug/mL)	50000
Desired concentration (ug/mL)	10
Volume gentamycin (uL)	1.4

Pipetted 7 mL DMEMF into 50mL conicals

Removed 70uL DMEMF

Added cysteine or PBS mock

Added 1.4uL gentamycin

Remove media from all wells

Add 280uL 1x PBS to all wells (wash 1)

Remove PBS

Add 280uL 1x PBS to all wells (wash 2)

Remove PBS

Add 200 uL DMEMF + gentamycin +/- cysteine to all wells as indicated in plate setup.

Put 96-well plate in 37°C CO<sub>2</sub> incubator in Camberg lab TC room (has water in bottom).

### T=2 at 3:30pm

Have labeled round CHA plates and added glass beads for spreading.

Remove 1 infected 96-well macrophage plate from incubator.

Remove media from all wells

Add 250uL 1x PBS to all wells (wash 1)

Remove PBS

Add 250uL 1x PBS to all wells (wash 2)

Remove PBS

Add 200 uL 1% saponin in 1x PBS to all wells

Let set at room temp for 30 mins

Plate 50uL each well on a round plate, 2x.

Put plates at 37°C.

Remove the last bit of lysates from wells 1A -> 3C, transfer to individual microfuge tubes and freeze at -20°C.

**Monday, December 30, 2019****To Do:**1. ~~T=24~~**Results and Data:****T=24 at 1:30pm**

Have labeled square and round CHA plates and added glass beads for spreading to round plates.

Have two 96 well plates prepped with 1X PBS for serial dilutions.

Remove last infected 96-well macrophage plate from incubator.

Remove media from all wells

Add 250uL 1x PBS to all wells (wash 1)

Remove PBS

Add 250uL 1x PBS to all wells (wash 2)

Remove PBS

Add 200 uL 1% saponin in 1x PBS to all wells

Let set at room temp for 30 mins

Transfer 180 uL of row A1-A7 to new plate with PBS for serial dilutions

Transfer 180 uL of row D (all) to new plate for serial dilutions.

Perform serial dilutions.

For row A8 – A10, plate 50uL each well on a round plate, 2x.

Plate serial dilutions 2x on square plates.

Put plates at 37°C.

Remove the last bit of lysates from wells 1A -> 3C, transfer to individual microfuge tubes and freeze at -20°C.

**Tuesday, December 31, 2019****To Do:**1. ~~Count inoculum plates~~2. ~~Move remainder of plates to RT over NYD holiday~~**Results and Data:****Plate counts**

Inoculum												
	Cysteine?	Rep.	2	3	4	Dilution factor counted	Cells / mL	Average Cells / mL	St dev	CFU/well	St dev	MOI
LVS	-	1A	TMTC	111	9	0.01	1.11E+06	1.15E+06	4.95E+04	5.73E+04	2.47E+03	2.4
	-	1B	TMTC	118	9	0.01	1.18E+06					
$\Delta$ chaC	-	2A	TMTC	132	12	0.01	1.32E+06	1.27E+06	7.07E+04	6.35E+04	3.54E+03	2.7
	-	2B	TMTC	122	9	0.01	1.22E+06					
dptA(-)	-	3A	TMTC	159	-	0.01	1.59E+06	1.70E+06	1.48E+05	8.48E+04	7.42E+03	3.6
	-	3B	TMTC	TMTC	18	0.001	1.80E+06					
LVS	+	7A	TMTC	126	-	0.01	1.26E+06	1.32E+06	8.49E+04	6.60E+04	4.24E+03	2.8
	+	7B	TMTC	138	-	0.01	1.38E+06					
$\Delta$ chaC	+	8A	TMTC	133	-	0.01	1.33E+06	1.33E+06	#VALUE!	6.65E+04	#VALUE!	2.8
	+	8B	6	-	-		#VALUE!					
dptA(-)	+	9A	TMTC	TMTC	19	0.001	1.90E+06	1.70E+06	2.83E+05	8.50E+04	1.41E+04	3.6
	+	9B	TMTC	TMTC	15	0.001	1.50E+06					
Dilution Factor			0.1	0.01	0.001							

MOI is a bit low, but inoculums are consistent.

There was one oddity, which was the  $\Delta$ chaC strain + cysteine. Note that the  $\Delta$ chaC colonies had a distinctly different appearance than the LVS or dptA(-) strains, smaller and a bit shinier, which makes me think I didn't accidentally plate the wrong wells. One of the plates looked normal for  $\Delta$ chaC, but the other plate (which would have been plated directly before or after the first plate) had a few colonies on the second dilution spot, none on any more dilute spots, and in the first dilution track there was a haze of cell with a few colonies of varying sizes. On all the other plates, there were clear colonies, even if they were a bit small- nothing like the variability in size on this plate. Very strange, but not worth worrying about too much at this point (unless the 2 hour timepoint looks odd).

## Thursday, January 2, 2020

### To Do:

1. Count T=2 plates
2. Move T=24 plates to 37°C incubator

## Results and Data:

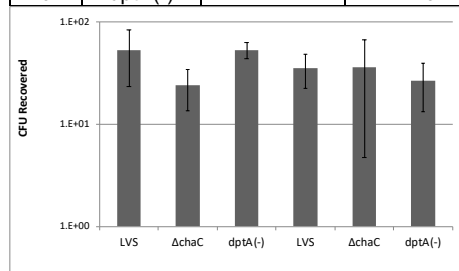
### Plate counts

	Cysteine?	Plate	Plate 1	Plate 2	Dilution factor counted	Average Cells	CFU per well	T-test
LVS	-	1A	8	11	1	10	3.80E+01	1.000
		1B	19	25	1	22	8.80E+01	
		1C	10	7	1	9	3.40E+01	
$\Delta$ chaC	-	2A	7	8	1	7.5	3.00E+01	0.186
		2B	10	5	1	7.5	3.00E+01	
		2C	6	0	1	3	1.20E+01	
dptA(-)	-	3A	12	9	1	10.5	4.20E+01	1.000
		3B	14	15	1	14.5	5.80E+01	
		3C	14	16	1	15	6.00E+01	
LVS	+	7A	11	3	1	7	2.80E+01	0.394
		30	10	15	1	12.5	5.00E+01	
		7C	9	5	1	7	2.80E+01	
$\Delta$ chaC	+	8A	14	12	1	13	5.20E+01	0.527
		8B	5	23	1	14	5.60E+01	
		8C	0	0	1	0	0.00E+00	
dptA(-)	+	9A	7	3	1	5	2.00E+01	1.000
		9B	8	13	1	11	4.20E+01	
		9C	4	5	1	5	1.80E+01	

50 ul cells plated

Great, all the macrophage were infected and to similar extents!

#		Cysteine?	Average CFU per well	St Dev	Original MOI	T-test	Fold Change
1	LVS	-	5.33E+01	3.01E+01	2.4		1.00
2	$\Delta$ chaC	-	2.40E+01	1.04E+01	2.7	0.186	-2.22
3	dptA(-)	-	5.33E+01	9.87E+00	3.6	1.000	1.00
4	LVS	+	3.53E+01	1.27E+01	2.8	0.394	-1.51
5	$\Delta$ chaC	+	3.60E+01	3.12E+01	2.8	0.527	-1.48
6	dptA(-)	+	2.67E+01	1.33E+01	3.6	0.233	-2.00



**Friday, January 3, 2020**

**To Do:**

- Count T=24 plates

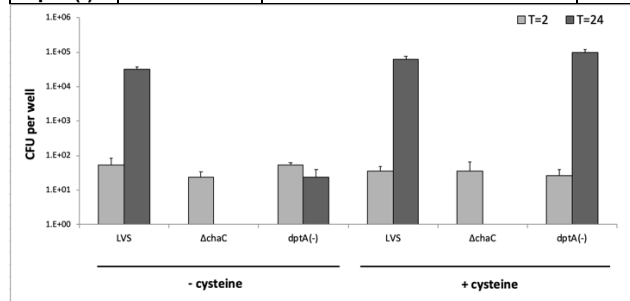
**Results and Data:**

**Plate counts**

	Plate	Track Plate 1				Track Plate 2				Dilution factor counted	Average Cells	CFU per well
		1	2	3	4	1	2	3	4			
LVS	1A	TMTC	149			TMTC	128			0.1	139	2.77E+04
	1B	TMTC	TMTC	14		TMTC	TMTC	16		0.01	15	3.00E+04
	1C	TMTC	TMTC	14		TMTC	TMTC	23		0.01	19	3.70E+04
ΔchaC	2A	0				0				1	0	0.00E+00
	2B	0				0				1	0	0.00E+00
	2C	0				0				1	0	0.00E+00
dptA(-)*	3A	8				2				1	5	2.00E+01
	3B	2				6				2	4	4.00E+01
	3C	0				3				3	1.5	1.00E+01
LVS	7A	TMTC	TMTC	31		TMTC	TMTC	43		0.01	37	7.40E+04
	7B	TMTC	TMTC	31		TMTC	TMTC	31		0.01	31	6.20E+04
	7C	TMTC	TMTC	23		TMTC	TMTC	25		0.01	24	4.80E+04
ΔchaC	8A	0				0				1	0	0.00E+00
	8B	0				0				1	0	0.00E+00
	8C	0				0				1	0	0.00E+00
dptA(-)	9A	TMTC	TMTC	47		TMTC	TMTC	41		0.01	44	8.80E+04
	9B	TMTC	TMTC	86		TMTC	TMTC	35		0.01	61	1.21E+05
	9C	TMTC	TMTC	32		TMTC	TMTC	46		0.01	39	7.80E+04
Dilution Factor		1	0.1	0.01	0.001	1	0.1	0.01	0.001			

\*Plated 50 ul on circular plate

T=24							
	Cysteine?	Average CFU per well	St Dev	Original MOI	T-test (vs LVS)	Fold Change	
LVS	-	3.16E+04	4.84E+03	2.4		1.00	
ΔchaC	-	0.00E+00	0.00E+00	2.7	0.000	#DIV/0!	
dptA(-)	-	2.33E+01	1.53E+01	3.6	0.000	-1352.86	
LVS	+	6.13E+04	1.30E+04	2.8	0.021	1.94	
ΔchaC	+	0.00E+00	0.00E+00	2.8	0.000	#DIV/0!	
dptA(-)	+	9.57E+04	2.25E+04	3.6	0.009	3.03	



**Future To-Do**

Move 1° LVS pKR10-1 into strain box

## **Bibliography**