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September 2019

Tuesday, September 3, 2019

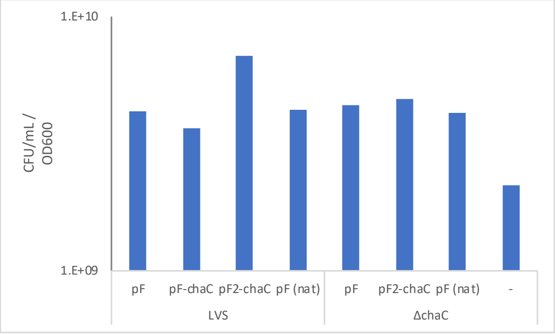
To Do:

1. ~~Count colonies from viable CFU expt~~
2. ~~Patch out cells for JW mac assay (KRLVS115, ΔnudH)~~
3. ~~Check for ΔchaC by PCR~~

Results and Data:

Check viable CFU per OD600:

Number		5	6	5	6	Dilution factor counted	Average Cells	CFU per mL	OD600	CFU per mL per OD600
1	**LVS pF**	TMTC	**18**	TMTC	**14**	0.00001	16	1.60E+08	0.038	4.21E+09
2	**LVS pF-chaC**	TMTC	**14**	TMTC	**12**	0.00001	13	1.30E+08	0.036	3.61E+09
3	**LVS pF2-chaC**	TMTC	**26**	TMTC	**23**	0.00001	25	2.45E+08	0.035	7.00E+09
6	**LVS pF (nat)**	TMTC	**16**	TMTC	**13**	0.00001	15	1.45E+08	0.034	4.26E+09
4	**ΔchaC pF**	TMTC	**16**	TMTC	**15**	0.00001	16	1.55E+08	0.035	4.43E+09
5	**ΔchaC pF2-chaC**	TMTC	**19**	TMTC	**13**	0.00001	16	1.60E+08	0.034	4.71E+09
7	**ΔchaC pF (nat)**	TMTC	**17**	TMTC	**13**	0.00001	15	1.50E+08	0.036	4.17E+09
8	**ΔchaC**	TMTC	**6**	TMTC	**9**	0.00001	8	7.50E+07	0.035	2.14E+09
	Dilution Factor	0.0001	0.00001	0.0001	0.00001					



NOTE the Y-axis! All essentially the same.

I'm still not seeing a strong phenotype for the ΔchaC viability loss, but I'm concerned about contamination. In the visible single colonies only on plates for ΔchaC, there are some that look normal (opaque and white) vs potentially more mucoid (pearly and generally slightly smaller). There are fewer of these pearly colonies. Patch out 6 pearly colonies and 3 white colonies and LVS and confirm ΔchaC genotype by PCR tomorrow. If there is lots of wild-type contamination in the ΔchaC strain, will need to repeat the experiment. If not, these are potentially suppressors?

Transposon mutagenesis of PchaC-lacZ

Most colonies have small dot of blue in center of colony. Picked some potentially larger blue dot colonies (2) and some potentially white colonies (a few, mostly small so the blue dot may just not be obvious yet). Patched to CHA-X-gal-Kan with a normal-looking colony on top as a control.

Re-making ΔchaC:

All patches grew on Kan except 1-4. Check the 1-4 patches by PCR.

Reaction numbers	Sample	Expected size
1	pKL133 1-4-A	1460
2	pKL133 1-4-B	1460
3	pKL133 1-4-C	1460
4	pKL133 1-4-D	1460
5	LVS colony	2243
6	LVS gDNA	2243
7	pKL133 (1:10)	no product
8	-DNA	no product
Total reaction volume		20
Total number of reactions		8

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	9.5
ddiH2O			12.4	117.8
PrimeSTAR GXL Buffer	5x	1x	4	38
dNTPs	2.5 mM	0.2 mM	1.6	15.2
P794	10 uM	0.3 uM	0.6	5.7
P795	10 uM	0.3 uM	0.6	5.7
template	100 ng/ul	2 ng/ul	0.4	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	3.8
		Total volume	20	186.2

Run standard program STN1 with 2:30 extension

Wednesday, September 5, 2019

To Do:

1. ~~PCR to check for ΔchaC in viable cells expt~~
2. ~~Run gel with PCRs (yesterday and today)~~
3. ~~Freeze down pSD26 Tn mutants (dark blue tiny)~~
4. ~~Patch pSD26 Tn mutants on X-gal (dark blue tiny?)~~

Results and Data:

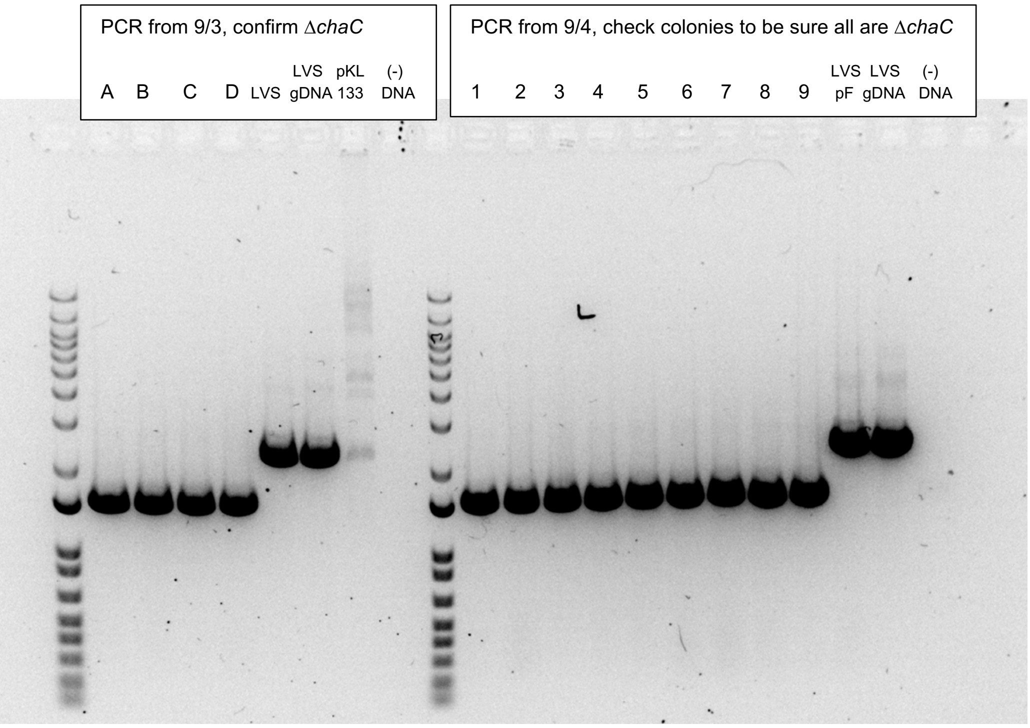
PCR from patches from ΔchaC viable cells experiment. Saw increased viability of ΔchaC strain but heterogeneous colony morphology (smaller and pearly vs larger and whiter). Concerned that I may have contaminated my ΔchaC only with wild-type cells.

Reaction numbers	Sample	Expected size
1	ΔchaC colony 1	1460
2	ΔchaC colony 2	1460
3	ΔchaC colony 3	1460
4	ΔchaC colony 4	1460
5	ΔchaC colony 5	1460
6	ΔchaC colony 6	1460
7	ΔchaC colony 7	1460
8	ΔchaC colony 8	1460
9	ΔchaC colony 9	1460
10	LVS pF colony 1	2243
11	LVS gDNA	2243
12	-DNA	no product
Total reaction volume		20

Total number of reactions	11

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	12.5
ddiH2O			12.4	155
PrimeSTAR GXL Buffer	5x	1x	4	50
dNTPs	2.5 mM	0.2 mM	1.6	20
P794	10 uM	0.3 uM	0.6	7.5
P795	10 uM	0.3 uM	0.6	7.5
template	100 ng/ul	2 ng/ul	0.4	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	5
		Total volume	20	245

Run standard program STN1 with 2:30 extension



Re-making ΔchaC: freeze down patches with the most cells, specifically clones D (KRLVS42.1) and C (KRLVS42.2). They both appear mucoid.

Confirming colonies- all 6 of the “pearly” looking colonies (1-6) and all 3 of the “white” looking colonies (7-9) are all ΔchaC. Patch to CHA and CHA-Kan to see if the “white” colonies are kanamycin-resistant (aka, have plasmid).

Thursday, September 6, 2019

To Do:

1. Check X-gal plates with rpsU1-lacZ Tn mutants (dark blue tiny)- are they still dark blue and slow-growing?
2. Check PchaC-lacZ patches
3. Check ΔchaC cells struck to CHA and CHA-Kan plates

Results and Data:

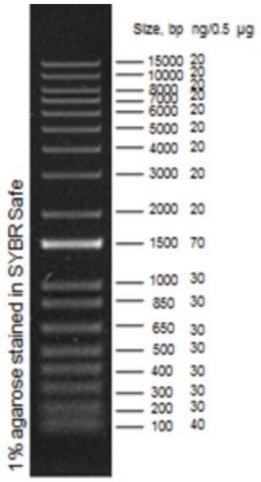
PCR f

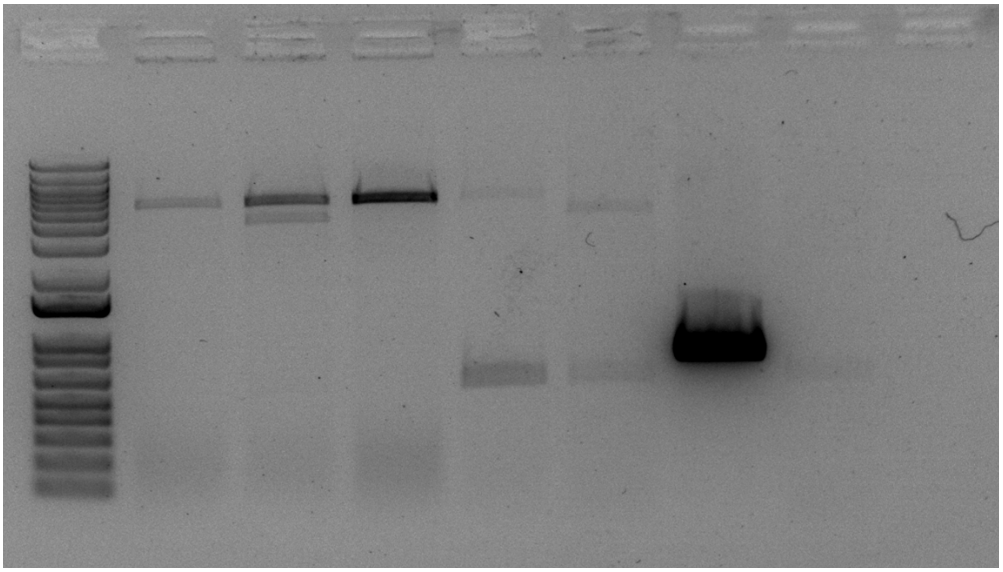
Results and Data:

Transposon mutagenesis of reporter strains:

Electroporation	Strain	EC cells	Plasmid	Volume	Notes
70	PchaC-lacZ	KMR 8.23.19	pSD26	3 uL	
71	PchaC-lacZ	KMR 8.23.19	pSD26	3 uL	

pSD26 was at 333 ng/uL so about 1 ug per electroporation.
Recovering at ~8:30am – 11:30. Plated 3x 100 ul, 1x 300 ul, 2x 500 ul on CHA-Kan-X-gal





Reaction numbers	Sample	Expected size
1	rpsU1-lacZ pSD26-1 (68-1)	3841 or 4282
2	rpsU1-lacZ pSD26-2 (68-2)	3841 or 4282
3	rpsU1-lacZ pSD26-3 (69-1)	3841 or 4282
4	rpsU1-lacZ pSD26-4 (69-2)	3841 or 4282
5	rpsU1-lacZ	3841
6	LVS gDNA	723
7	-DNA	no product

Future To-Do

Tuesday
chaC experiment:
Resuspend cells to same OD600, dilute and plate for CFUs
Reporter strains:
If significantly different, freeze down Tn insertions.
Plan on gDNA isolations.

Move 1° LVS pKR10-1 into strain box

Bibliography