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February 2021

Wednesday, February 3, 2021

To Do:
1. Finish WB on HT's T6SS samples

Results and Data:

Cut membrane and start incubating in 1° antibodies- reuse from last time.

Top	anti-sigma70	67.7 kDa	1:250	HT dilution from 200115	Third use?
Bottom	anti-IglC	22 kDa	1:750	HT dilution from 200115	Third use?

Incubate for ~1 hour

Plan for WB:
4-12% gel with MOPS buffer
Check IglC (22 kDa) and sigma70 (67.7 kDa) (tul4 similar to IglC in size?).

Friday, February 12, 2021

To Do:
1. Grow cells for T6SS experiment

Results and Data:

Yesterday, struck out LVS and ΔpigR cells. Today, scrape up cells, resuspend in sBHIc (all fresh components at the time, version "A") that Hannah made on 1/26/21.

Check ODs:

Number	Strain	Measured OD (50 uL in 450 uL)	Resuspend in BHIc to:	For 8 mL at 0.8
1	LVS	0.268	2.68	239
2	LVS ΔpigR	0.328	3.28	195

Dilute into either:
A: sBHIc
B: sBHIc + 5% KCl

Media	Number	12:15PM	3PM	3:55PM	4:30	1x SLB	2xSLB for supernatant samples
A: sBHIc	1A	0.089	0.202	0.297	0.392	392	86.2
	2A	0.087	0.131	0.178	0.226	226	49.7
B: sBHIc + 5% KCl	1B	0.118	0.103	0.112	0.123	123	27.1
	2B	0.0098	0.092	0.097	0.1	100	22.0

Pellet 1 mL (spin max speed 5'), transfer 900 ul of supernatant to be supernatant 1, unfiltered.
Pellet 2 x 1.8 mL tubes of each sample, filter sterilize with either:
Supernatant 2: New PES membranes
Supernatant 3: Old Pall membranes

Check volumes and add 1/10 volume 100% TCA -> 4°C O/N to precipitate
Sup1: 900 ul 90 ul TCA
Sup2: 1.5 mL 150 ul TCA
Sup3: 1.6 mL 160 ul TCA

Saturday, February 13, 2021

To Do:
1. Precipitate supernatant samples
2. Run gel for WB
3. Transfer gel for WB

Results and Data:

Finish prepping supernatant samples:
1. Pellet supernatant samples by centrifuging at 4°C for 30' at max speed
2. Remove supernatant (pellet will not be very visible)
3. Add 1 mL 100% ice-cold ethanol
4. Spin at 4°C for 30' at max speed
5. Remove ethanol, let pellets air-dry completely
6. Add 1x SLB, normalizing for cell density in original sample. Use the following formula:
   a. (35 uL)/(lowest density sample OD600) x (current sample OD600) = uL 1x SLB to add

Filter protocol	Media	Number	Volume precipitated	OD600	2xSLB for supernatant samples
Supernatant 1: unfiltered	A: sBHIc	1A	900	0.392	86.2
		2A	900	0.226	49.7
	B: sBHIc + 5% KCl	1B	900	0.123	27.1
		2B	900	0.1	22.0
Supernatant 2: PES filtered	A: sBHIc	1A	1500	0.392	143.7
		2A	1500	0.226	82.9
	B: sBHIc + 5% KCl	1B	1500	0.123	45.1
		2B	1500	0.1	36.7
Supernatant 2: old filters	A: sBHIc	1A	1600	0.392	153.3
		2A	1600	0.226	88.4
	B: sBHIc + 5% KCl	1B	1600	0.123	48.1
		2B	1600	0.1	39.1

7. Heat pellet samples for 5-10' at 95°C.

Run 4 -12% Bis-tris gel with MOPs buffer:
Gel 1

Lane	volume	Contents			
1	10	prestained ladder			
2	10	1x SLB			
3	10	1:10 Magic Mark			
4	10	1x SLB			
		Sample #	**Strain**	**Media**	**Component**
5	10	1A	LVS	sBHIc	pellet
6	10	2A	Δ*pigR*		
7	10	1A sup1	LVS	sBHIc	supernatant 1
8	10	2A sup1	Δ*pigR*		
9	10	**2A sup2**	LVS	sBHIc	supernatant 2
10	10	**1A sup2**	Δ*pigR*		
11	10	1A sup3	LVS	sBHIc	supernatant 3
12	10	2A sup3	Δ*pigR*		
13	10	1x SLB			
14	10	1x SLB			
15	10	1x SLB			

Note the accidental switch between samples 1A and 2A, supernatant 2.

Gel 2

Lane	volume	Contents			
1	10	prestained ladder			
2	10	1x SLB			
3	10	1:10 Magic Mark			
4	10	1x SLB			
		Sample #	**Strain**	**Media**	**Component**
5	10	1B	LVS	sBHIc + 5% KCl	pellet
6	10	2B	Δ*pigR*		
7	10	1B sup1	LVS	sBHIc + 5% KCl	supernatant 1
8	10	2B sup1	Δ*pigR*		
9	10	1B sup2	LVS	sBHIc + 5% KCl	supernatant 2
10	10	2B sup2	Δ*pigR*		
11	10	1B sup3	LVS	sBHIc + 5% KCl	supernatant 3
12	10	2B sup3	Δ*pigR*		
13	10	1x SLB			
14	10	1x SLB			
15	10	1x SLB			

Run at 150V for ~60 minutes
Transfer to PVDF 20 V for 60'
-Gel 1- diagonal slash at top right
-Gel 2- square cut-out at top right
Leave in blocking buffer at 4°C.

Tuesday, February 16, 2021

To Do:

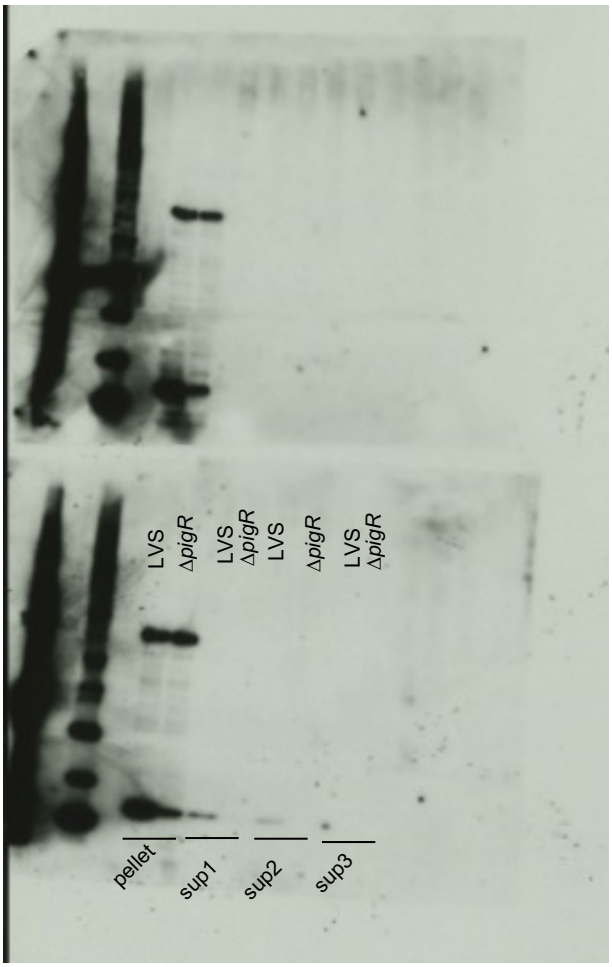
1. Finish T6SS WB

Results and Data:

Cut membrane and start incubating in 1° antibodies- reuse from last 2 times (make new dilutions for next blot).

Top	anti-sigma70	67.7 kDa	1:250	HT dilution from 200115	Fourth use?
Bottom	anti-IglC	22 kDa	1:750	HT dilution from 200115	Fourth use?

Incubate for ~1 hour
Wash 4 x ~ 10'
Re-block for ~40 minutes in 10 mL blocking buffer plus 50 uL 20% NP-40.
Add 2 uL goat anti-mouse to each tray with blocking buffer
Wash 4 x ~ 10'
Develop. Try pico kit first, then moved to atto kit. Needed atto kit to really see bands.



Anti-sigma^{70}

Blot 1: Samples grown in sBHIc

Anti-IglC

Anti-sigma^{70} 1:250

Blot 2: Samples grown in sBHIc + 5% KCl

Anti-IglC 1:750

Results:
I can see bands corresponding to IglC in both sup1 and sup2 samples from media with 5% KCl! That means unfiltered and filtered using the PES membrane!
*Note that I don't see the IglC band in the sup3 samples, the samples from the old filters.

I think this means that the issue was always with the supplements needing to be made fresh- and I would have seen secretion of IglC in the last blot if the sample hadn't mostly left the well.

Unclear why the old filters didn't work, but great that I don't have to worry about trying to source a discontinued item!

Finally, there is reasonable detection of IglC in the unfiltered supernatant and no apparent (detected) sigma70, suggesting that I may not need to filter the supernatants to get reasonable data.

But it is an important point that the detection is terrible. I think I could (1) load more, particularly if we get 10 well gels; (2) concentrate more supernatant. If I do this, will I need to filter-sterilize? Possibly-probably.

Wednesday, February 17, 2021

To Do:

1. Electroporation for starting to remake ΔpriM

Results and Data:

Make fresh EC LVS (not very dense! But move forward).
3 electroporations, 5 uL pKL37 (plasmid from common lab prep), time constants between 5-6-5.7
Recover in 5 mL MHB starting at 9:30 (Always forget to reduce the volume to 4 mL!!)

Future To-Do

Move 1° LVS pKR10-1 into strain box

Bibliography

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Hoang, K., Fitch, J., White, P., Mohapatra, N., Gunn, J. (2020). **The sensor kinase QseC regulates the unlinked PmrA response regulator and downstream gene expression in Francisella** Journal of Bacteriology https://dx.doi.org/10.1128/jb.00321-20