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**March 2020****Thursday, March 12, 2020****To Do:**

1. Start cultures for T6SS assay
2. Check culture OD600s
3. Collect samples for T6SS assay
  - a. cell pellets
  - b. supernatant; filter-sterilize, add 100% TCA, put at 4°C O/N

**Results and Data:**

Yesterday, made BHlc +/- 5% KCl (250 mL each) and struck out LVS,  $\Delta pigR$ ,  $\Delta rpsU2$  (HT's stock)

Today, supplement BHlc (6.25 mL 20% glucose, 2.5 mL  $\beta$ -NAD [1mg/mL], 2.5 mL heme-histidine)  
Resuspend cells in sBHlc (-KCl):

Number	Strain	Resuspended cells to	Desired volume	Desired OD600	Volume cells
1	LVS	6.65	7000	0.08	84.2
2	$\Delta pigR$	6.31	7000	0.08	88.7
3	$\Delta rpsU2$	5.11	7000	0.08	109.6

Inoculated cultures at ~11am, used 600 uL to check OD600 values

Number	Strain	Media	11:00am	1:45pm	3:20pm
1	LVS	sBHlc	0.081	0.231	0.522
2	$\Delta pigR$	sBHlc	0.081	0.255	0.578
3	$\Delta rpsU2$	sBHlc	0.079	0.159	0.271
4	LVS	sBHlc + 5% KCl	0.105	0.113	0.128
5	$\Delta pigR$	sBHlc + 5% KCl	0.113	0.15	0.218
6	$\Delta rpsU2$	sBHlc + 5% KCl	0.102	0.107	0.112

At 3:20pm, harvested 1 mL cell pellets (kept supernatant) and extra 1.8 mL supernatant.

Resuspended pellets in 1x SLB (OD in mL), boiled, froze.

Filter-sterilized supernatant, aliquoted 1.5 mL in one tube with 150 uL 100% TCA, 450 uL into another tube with 45uL 100% TCA. Put in cold room overnight.

**Friday, March 13, 2020****To Do:**

1. Pellet TCA-precipitated proteins
2. Add SLB

**Results and Data:**

Spin the tubes with more supernatant for 30' at 4°C max speed.

Discard supernatant.

Vortex the tubes with the remaining supernatant.

Transfer liquid to first set of tubes, spin 30' 4°C max speed.  
 Add 1 mL ice-cold 100% ethanol, spin 4°C 30' max speed.  
 Remove supernatant, let pellet dry.  
 Add appropriate amount of 1x SLB to normalize to OD600 (as indicated below).

Number	Strain	Media	Final OD600	1X SLB
1	LVS	sBH1c	0.522	163.1
2	$\Delta$ pigR	sBH1c	0.578	180.6
3	$\Delta$ rpsU2	sBH1c	0.271	84.7
4	LVS	sBH1c + 5% KCl	0.128	40.0
5	$\Delta$ pigR	sBH1c + 5% KCl	0.218	68.1
6	$\Delta$ rpsU2	sBH1c + 5% KCl	0.112	35.0

Boil samples 5', put at -20°C until ready to run gel.

After ~ 17 hrs of transfer (7V, 4°C), stopped transfer. Cut membranes to pieces with 3 well each (hopefully!) and incubate in blocking buffer in cold room, rocking, until Monday.

### Wednesday, January 15, 2019

#### To Do:

1. Perform WB

#### Results and Data:

Blot	Protein	kDa	Rec dilution	Planned dilution	Volume in 10 mL
1	Tul4	17	1:5000	1:20000	0.5
2	IglA	20.9	1:2000	1:2000	5
3	IglB	57.9	1:1000	1:1000	10
4	IglC	22.1	1:1000	1:1000	10
5	IglD	46.4	1:1000	1:1000	10
6	PdpC	155.9	1:2500	1:1000	10
7	PdpB	127.5	1:1000	1:1000	10
8	PdpA	95.3	1:1000	1:500	20
9	PdpB	127.5	1:1000	1:500	20
10	PdpA	95.3	1:1000	1:200	50

Don't bother with PdpD antibody- would be 140kDa, but is a pseudogene in LVS.

Incubate in 10 mL 1° for 1 hr

Wash 4 x 10' (approx.- sometimes a bit more)

Re-block in 5 mL blocking buffer for 20' (+ probably 10-15' more)

Add 2° antibody directly to 5 mL blocking

buffer- 1 uL of goat anti-mouse or goat anti-rabbit, as indicated below. Incubate 1 hr.

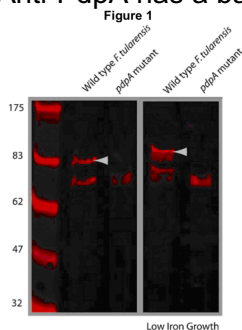
Blot	Protein	Antibody type
1	Tul4	mouse monoclonal
2	IglA	rabbit polyclonal
3	IglB	mouse monoclonal
4	IglC	mouse monoclonal
5	IglD	mouse monoclonal
6	PdpC	rabbit polyclonal
7	PdpB	mouse monoclonal
8	PdpA	mouse monoclonal
9	PdpB	mouse monoclonal
10	PdpA	mouse monoclonal

Wash 4 x 10'

Develop ~3' with 5 mL developer mix each

**Initial general impressions:**

Too much anti-rabbit secondary. Both blot 2 and 6 have LOTS of background  
Anti-PdpA has a background band (just as here, in the CoA):



Actually, I thought the background band on my blot was above the PdpA protein...

May need to look more carefully at the ladder. Should be at 93 kDa, the background on this blot is closer to 70 kDa.

Can't really see PdpC. Maybe if there was less background? The CoA blot isn't particularly convincing. IgB, IgC, IgD antibodies look great!

IgA looks pretty good with a VERY brief exposure. Will probably be great with less background.

Tul4 / LpnA continues to look excellent.

**WT vs  $\Delta$ rpsU2 impressions**

Much less PdpA!

Much less PdpB!

Can't tell about PdpC

May actually be slightly less IgB, C, D – although this may be a loading issue. No control, and LpnA/Tul4 blot is cut off, so can't tell relative amounts.

**Aliquot plan**

Tul4 / LpnA : 3 uL

IgA: 10 uL

IgB: 20 uL

IgC: 20 uL

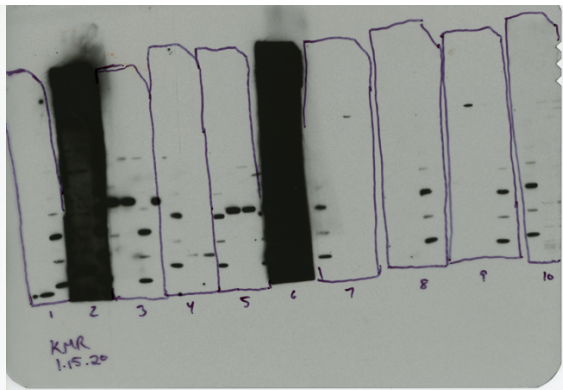
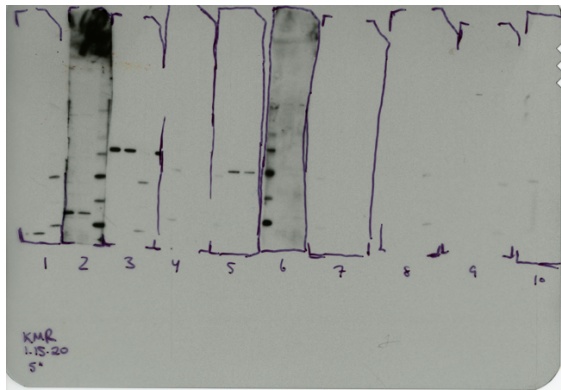
IgD: 20 uL

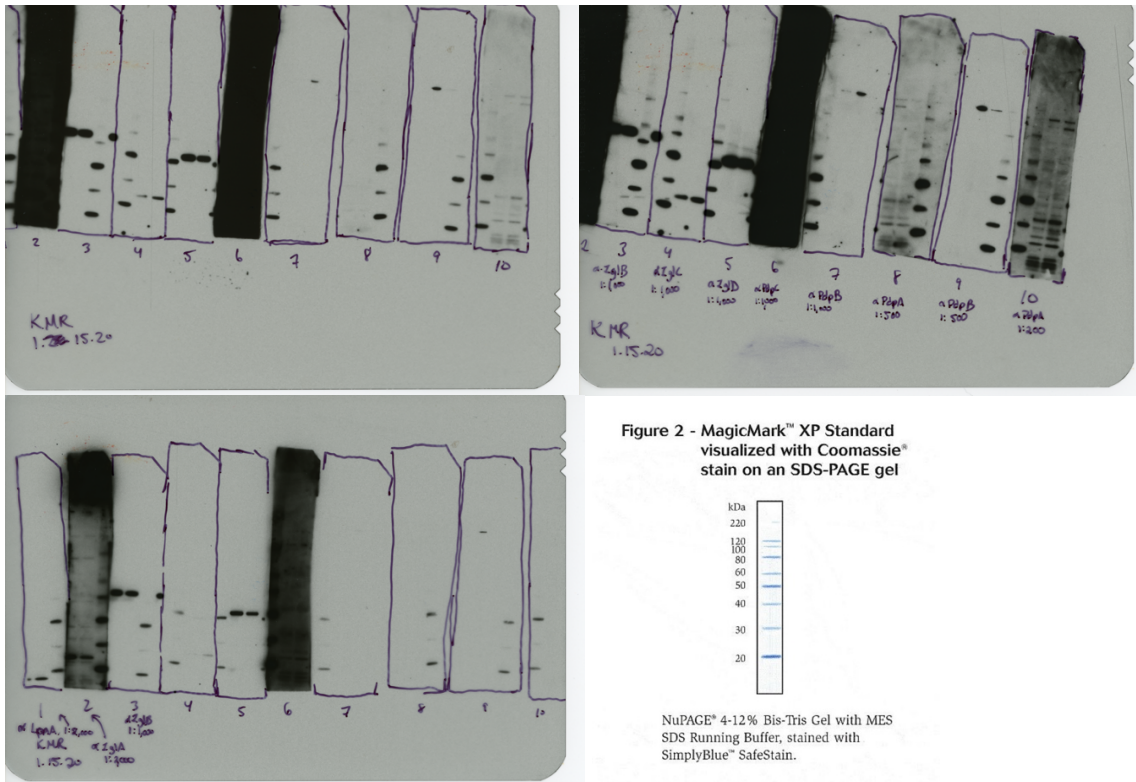
PdpC: 50 uL

PdpB: 20 uL

PdpA: 40 uL

Made 5 aliquots of each (only 4 of Tul4- there is only 100 uL total and I'm sending 15 uL to Mougous lab)

**Scans of the blots:**



Note that for PdpA, there is an extra band running around the size of PdpB, which is 127.5 kDa. It makes sense that the slightly lower band (closer to the 100 kDa band) would be PdpA, which is 95.3 kDa.

### Future To-Do

Move 1° LVS pKR10-1 into strain box

## **Bibliography**