Table of Contents

Introduction

Thursday, September 20, 2018

To Do:

1. ~~Explain lab notebook formatting~~
2. Media prep

Results and Data:

For each day, copy the previous entry headers and update the date. Save the same ongoing copy of the lab notebook to the lab google drive ONLY. Do not save separate physical copies. At the 1st of each new month, a read-only PDF file of the lab notebook must be saved and given to Dr. Ramsey electronically. **File contents converted to PDF MUST NOT BE EDITED after PDF conversion**. Continue to keep using the same word file until the end of the calendar year. New lab notebook files run in 6 month intervals.

For the To do list, update this each day with new tasks, as tasks are done, use the ~~strikethrough~~ font on the day they are completed and leave them out of the list on the next day.

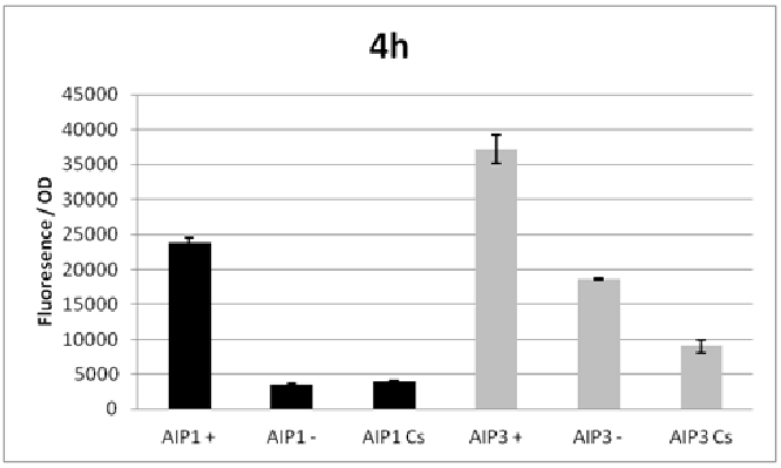


Figure 1

Figures are inserted as inline .png files when possible, .jpg, .gif, .tif otherwise are acceptable. Figure legends are always inserted in Word (right click – 'insert caption') and use "**Heading 2**" text to properly format in the TOC. Table legends are handled the same way. Figure images must be saved in a separate folder where the source data is saved.

Thoughts or questions. When you have a significant observation, question, confusing point or contradiction that you have identified in your data or protocol, use the "**Heading 3**" text heading on a descriptive brief title or single word heading so you can refer to it in your TOC later.

Protocol 1

1. This is the format for a protocol in your notebook.

2. The protocol title must be formatted in "**Heading 2.**"

3. Reagents which must be made for a specific protocol (buffers, solutions) should be listed in a "Reagents" Section, formatted in "**Heading 3**."

4. The protocol must be in numerical steps.

5. Use standard notation and carefully describe units for your protocol.

6. Use ½" indent for protocol text.

Reagents

Specific buffers

For initial lab notebooks, write as much detail as possible. As time goes by you will be able to refer to written protocols by their heading and/or page number within the document. If you make any modifications to a protocol you must state how and why.

File Formatting Protocol

1. Filenames begin with your initials, an underscore, and the date, formatted as the last 2 digits of the year, the month, then the day, ex: "KMR_180920_Sample_file1v1.xls"

2. This ensures all files will be sorted by their creator and by their date. You must use this file formatting system for all data files (including photos) that will be shared with the lab.

Bibliography data will be saved as (author/date) and using Mendeley at this time with the TOC inserted by order cited at the end of the lab notebook in the Bibliography section.

Example is a recent publication (Ramsey and Dove, 2016).

October 2018

Tuesday, October 23, 2018

To Do:

1. ~~Review recipe for cystine heart agar with hemoglobin (CHA)~~
2. ~~Review recipe for supplemented Mueller-Hinton broth~~

Cystine heart agar with hemoglobin plates protocol

For 600 mL of CHA

1. Weigh out 30.6g of cystine heart agar into 1L flask (non-baffled; 10.2g/100mL)

2. Add 300mL of ddiH_2O (type I)

3. Add stirbar to flask

4. Heat on low, stirring, for about 10 minutes (media should be totally dissolved)

5. Autoclave on 30' cycle, filling the water bin up to the height of the media

6. Cool down (ideally to ~55C)

7. Separately (before), prepare hemoglobin 2% solution

8. Add 6g freeze-dried hemoglobin to 300mL of ddiH_2O (type I)

9. Autoclave on 15' liquid cycle with water in the bin

10. Cool down (ideally to ~55C)

11. Using sterile technique, pour hemoglobin into CHA

12. Using a 50mL pipet, aliquot 24mL of CHAH mixture into each 100mm plate (should make approximately 25 plates) Try to avoid bubbles!

Reagents

Cystine heart agar

2% hemoglobin

100mm plates

Mueller-Hinton broth protocol

For 500 mL of MHB

1. Weigh out 10.5g of Mueller-Hinton broth into 1L square-bottle

2. Add 500mL of ddiH_2O (type I)

3. Autoclave on 30' liquid cycle, filling the water bin up

4. Cool down to 37C or cooler

5. Can keep this sterile media indefinitely without supplements

6. Add 5mL of 10% glucose

7. Add 5mL of 2.5% iron pyrophosphate

8. Add isovitalex (combine 10mL from liquid vial with solids, add entire volume to broth)

9. Can keep this supplemented media for 2 weeks, storing at 4C

Reagents

Mueller-Hinton Broth media dry

10% Glucose (filter-sterilized)

2.5% Iron pyrophosphate (1.25g iron pyrophosphate in 50mL of ddiH_2O (type 1), dissolved overnight, fresh solution every 2 weeks)

Isovitalex (commercially available)

Wednesday, October 24, 2018

To Do:

1. ~~Make cystine heart agar with hemoglobin (CHA) plates~~
2. Compare old CHA with new CHA

Cystine heart agar with hemoglobin plates protocol

For 600 mL of CHA

1. Separately (before), prepare hemoglobin 2% solution

2. Add 6g freeze-dried hemoglobin to 300mL of ddiH_2O (type I)

3. Add stirbar to flask

3. Autoclave on 15' liquid cycle with water in the bin

4. Cool down (ideally to ~55C)

5. Weigh out 13.3g of (old) cystine heart agar into 1L flask (non-baffled; 10.2g/100mL)

6. Weigh out 13.3g of (new) cystine heart agar into 1L flask (non-baffled; 10.2g/100mL)

7. Add 150mL of ddiH_2O (type I) to each flask

8. Add stirbar to flask

9. Heat on low, stirring, for about 10 minutes (media should be totally dissolved)

10. Autoclave on 30' liquid cycle, filling the water bin up to the height of the media

11. Cool down (ideally to ~55C)

12. Using sterile technique, pour hemoglobin into CHA

13. Using a 50mL pipet, aliquot 24mL of CHAH mixture into each 100mm plate (should make approximately 25 plates) Try to avoid bubbles!

Reagents

Cystine heart agar

2% hemoglobin

100mm plates

Thursday, October 25, 2018

To Do:

1. ~~Make hemoglobin for CHA plates (new)~~
2. Compare old CHA with new CHA

Making 2% Hemoglobin Solution

1. Add 3g freeze-dried hemoglobin to 150mL of ddiH_2O (type I)
2. Autoclave on 15' liquid cycle with water in the bin
3. Allow to cool, store in cold room at 4C

Friday, October 26, 2018

To Do:

1. ~~Compare old CHA with new CHA~~
2. ~~Pour CHA plates (new CHA)~~

Monday, October 29, 2018

To Do:

1. ~~Grow up LVS~~
2. ~~Make buffer 1 for TAP Purification~~

O.D. 600 Readings for LVS Strains

Strain	08:35	10:30	11:45
1: beta-prime-TAP	0.171	0.196	0.216
2: beta-prime-TAP	0.188	0.217	0.240
3: ΔpigR	0.095	0.104	0.111

Tuesday, October 30, 2018

To Do:

1. ~~Grow up LVS~~

Plan for growth experiment:

3mL cultures

Tube Number	Condition
1	MHB
2	MHB
3	MHB + 30ul 2.5% ironpyrophosphate
4	MHB + 30ul 2.5% ironpyrophosphate
5	MHB + 30ul 10% glucose
6	MHB + 30ul 10% glucose
7	MHB + 60ul isovitalex
8	MHB + 60ul isovitalex
9	MHB + all supplements
10	MHB + all supplements

MHB = Mueller Hinton broth supplemented by Dr. Ramsey on 10/28

Target OD600 of 0.08. We'd like to inoculate about 50ul into each tube.

Calculation:

~~(3000ul * 0.08)/50ul= 4.8 OD600~~

~~1st OD600= 0.068 * 50 = 3.4~~

~~2nd OD600= 0.122 * 50 = 6.1~~

~~If we add 50ul at OD600 6.1 to 3mL the OD600 should be 0.102~~

OD600 0.381 * 20 = 7.62

If we add 50ul at OD600 7.62 to 3mL the OD 600 should be 0.127

Mock tube OD600 0.082

To take OD600 of all 10 tubes 0.5ml into 0.5MHB

Results:

Growth experiment OD600

Tube Number	2hr 10' (OD600)	3hr 10' (OD600)	4hr (OD600)	5.5hr (OD600)
1	0.294	0.316	0.428	0.566
2	0.296	0.304	0.426	0.586
3	0.302	0.316	0.432	0.582
4	0.312	0.344	0.438	0.598
5	0.294	0.332	0.434	0.582
6	0.294	0.320	0.428	0.582
7	0.136	0.088	0.134	0.146
8	0.124	0.096	0.120	0.126
9	0.126	0.092	0.124	0.136
10	0.146	0.108	0.146	0.158

Wednesday, October 31, 2018

To Do:

1. ~~Perform last OD from growth experiment~~
2. ~~Streak out plates for cell harvesting~~

Tube Number	2hr 10' (OD600)	3hr 10' (OD600)	4hr (OD600)	5.5hr (OD600)	21.5hr (OD600)
1	0.294	0.316	0.428	0.566	2.19
2	0.296	0.304	0.426	0.586	1.86
3	0.302	0.316	0.432	0.582	1.94
4	0.312	0.344	0.438	0.598	1.92
5	0.294	0.332	0.434	0.582	1.85
6	0.294	0.320	0.428	0.582	1.95
7	0.136	0.088	0.134	0.146	0.530
8	0.124	0.096	0.120	0.126	0.350
9	0.126	0.092	0.124	0.136	0.400
10	0.146	0.108	0.146	0.158	0.480

2 plates struck out with LVS and beta-prime TAP glycerol stocks

Thursday, November 1, 2018

To Do:

1. ~~Prepare cultures of LVS and Beta prime TAP strains for cell harvesting~~

Cells were scraped off the plates prepared on October 31st 2018 into 3mL of S-MHB

1:20 starting OD600 calculations:

	C1	V1	C2	V2
Beta prime	3.46	2.9	0.05017	200
LVS	8	2.9	0.116	200
LVS	8	1.25425	0.05017	200

Added 2.9mL (whole culture) of Beta Prime TAP to 200mL S-MHB

Added 1.25mL of LVS culture to 200mL S-MHB

Cultures were put in shaking incubator at 37C at 09:45

2mL of 2.5% iron pyrophosphate added to both cultures at 11:30

OD600 taken 4hrs after inoculation:

Beta Prime TAP: 0.148

LVS: 0.170

OD600 taken ~6hrs after inoculation:

Beta Prime TAP: 0.233

LVS: 0.272

Cultures stopped and harvested for protein purification at 4:15

Tuesday, November 6, 2018

To Do:

1. ~~Perform first day of TAP purification~~

Sample	Date Prepared	Labeled	Volume of Cells	OD600 at harvest
1	11/01/2018	LVS	200mL	0.272
2	11/01/2018	β' TAP	200mL	0.233

Incubation in cold room started at 10:30

Incubation ended at 2:00

Wednesday, November 7, 2018

To Do:

1. ~~Perform second day of TAP purification~~
2. ~~Run protein gel~~

Use 10% NuPAGE Bis-Tris gels (don't forget to take sticker off the bottom)

Add 500mL (25mL 20x stock with 475mL type I DDih20,1.25mL antioxidant stored at 4C) MES buffer in gel apparatus

Remove combs

While samples are heating, gently wash wells with running buffer from inside the chamber using a 200ul pipet, wash each well twice

Combine 25ul of each protein sample with 25ul 2x sample loading buffer

(for 20ul sample add 22ul of 2x SLB)

Heat samples at ~95C for 5 minutes, quick vortex and spin once they come off heating block **ladder does not need to be heated**

Load as indicated in table

Run gel using Bloom power source ~115 volts for ~1hr 20min until the dye front is just running off the gel

Gel 1: Coomassie Stain

Lane	Volume	Contents
1	10ul	Benchmark unstained ladder (undiluted)
2	20ul	LVS-Lysate
3	20ul	LVS- FT
4	20ul	LVS- E1
5	20ul	LVS- FT2
6	20ul	LVS- elution final
7	20ul	Beta-prime elution final
8	20ul	Beta-prime FT2
9	20ul	Beta-prime E1
10	20ul	Beta-prime FT
11	20ul	Beta-prime Lysate
12	10ul	1x Sample Loading Buffer (SLB)

Gel 2: Silver Stain

Dilute ladder (1ul) into 9ul 1x sample loading buffer

Lane	Volume	Contents
1	10ul	Benchmark unstained ladder (1:10)
2	20ul	LVS-Lysate
3	20ul	LVS- FT
4	20ul	LVS- E1
5	20ul	LVS- FT2
6	20ul	LVS- elution final
7	20ul	Beta-prime elution final
8	20ul	Beta-prime FT2
9	20ul	Beta-prime E1
10	20ul	Beta-prime FT
11	20ul	Beta-prime Lysate
12	10ul	1x Sample Loading Buffer (SLB)

Both gels started at 3:35, gels ran for ~1hour

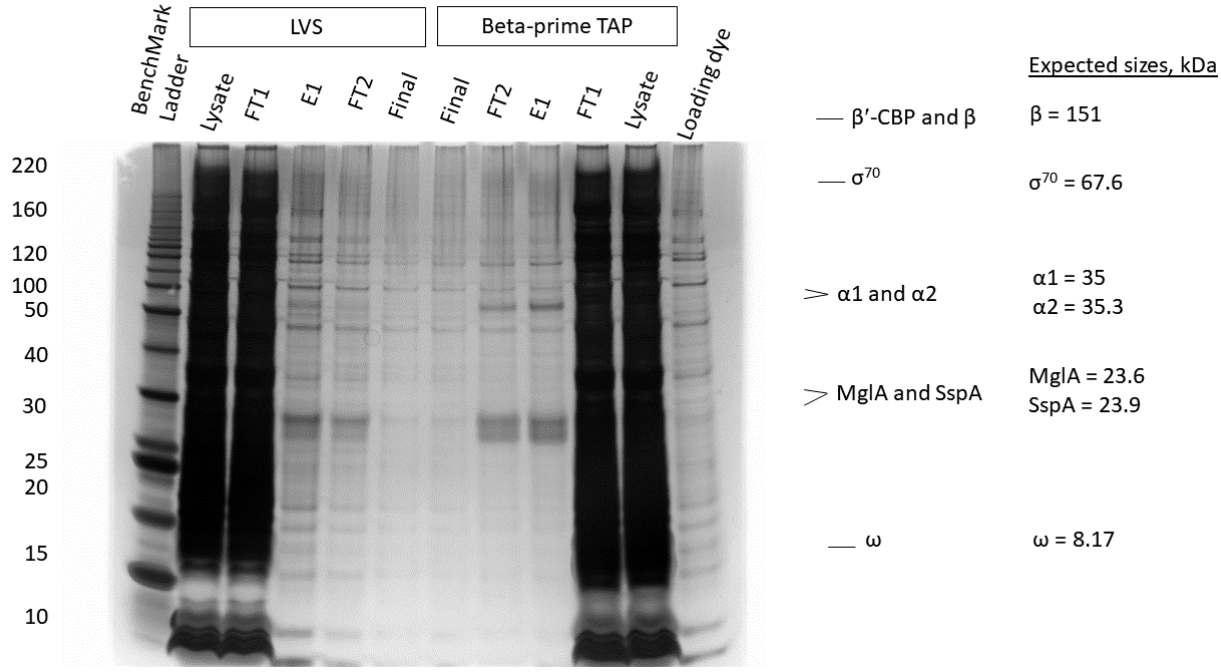
Gels started fixing at ~4:45

Thursday, November 8, 2018

To Do:

1. ~~Stain Coomassie and silver protein gels~~

Followed protocols for staining for silver and Coomassie blue gels.

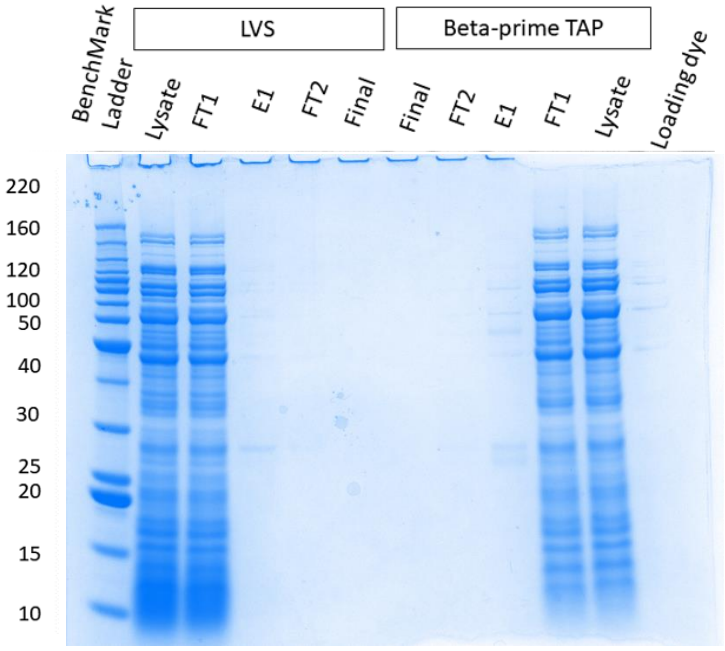


Friday, November 9, 2018

To Do:

1. ~~Make 4, 500mL bottles of media~~
2. ~~Make a plan to test growth conditions to induced production of the σ32 protein~~
3. ~~Take picture of Coomassie gel~~

Coomassie gel



Plan to induce production of σ32 protein:

Grow 2 flasks σ32 cells overnight at 37C (target OD of 0.3-0.4)

Determine OD and split into 6 flasks (duplicates)

Keep 1 flask at 37C, put one flask at 45C, put one flask at a temperature between 37C and 45C (41C)

Take samples for protein analysis at:

T=0,10,20,30,45,60mins, 2hrs

Take an OD at each timepoint to determine cell concentration (1mL)

Take samples at each time point for a western blot (1mL)

Determine at which temperature/timepoint the most σ32 is produced.

Tuesday, November 13, 2018

To Do:

1. ~~Supplement media flasks~~
2. ~~Inoculate at 0.005 for overnight culture~~
3. ~~Make glycerol stocks with extra cells~~

Want to inoculate at OD600 of 0.005

LVS: 300mL (2 flasks) OD600: 23.6

Beta-prime TAP: 300mL (2 flasks) OD600: 28.6

RpoH: 2 flasks of 200mL OD600: 25.4

Started shaking at 37C at 5:00PM inoculated at OD600: 0.005

Loading Buffer Recipe: (For 20mL)

5mL of 4x dye

2mL of DTT (10%)

Type I ddiH20 up to 20mL (13mL)

Wednesday, November 14, 2018

To Do:

1. ~~Do sigma 32 experiment~~
2. ~~Harvest LVS and Beta-prime TAP bacteria~~

Temperature Incubated	Flask Number
37 A	1
37 B	2
41 A	3
41 B	4
45 A	5
45 B	6

Flask Number	Timepoint	OD600
1	0	0.433
	10	0.443
	20	0.456
	30	0.470
	45	0.484
	60	0.504
	2 hrs	0.562
2	0	0.439
	10	0.448
	20	0.460
	30	0.472
	45	0.489
	60	0.507
	2 hrs	0.565
3	0	0.438
	10	0.444
	20	0.460
	30	0.473
	45	0.493
	60	0.516
	2 hrs	0.589
4	0	0.439
	10	0.449
	20	0.463
	30	0.477
	45	0.498
	60	0.520
	2 hrs	0.589
5	0	0.436
	10	0.454
	20	0.467
	30	0.473
	45	0.503
	60	0.525
	2 hrs	0.559
6	0	0.433
	10	0.443
	20	0.465
	30	0.485
	45	0.504
	60	0.520
	2 hrs	0.559

Plan to run western blot gel:

Well Number

1. Flask 1 T=0

2. Flask 3 T=0

3. Flask 5 T=0

4. 1, T=30

5. 3, T=30

6. 5, T=30

7. 1, T=60

8. 3, T=60

9. 5, T=60

10. 1, T=2hrs

11. 3, T=2hrs

12. 5, T=2hrs

Thursday, November 15, 2018

To Do:

1. ~~Run western blot gel~~
2. ~~Transfer western gel to membrane~~

Lane	Volume	Contents
1	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
2	12ul	1, T=0
3	12ul	3, T=0
4	12ul	5, T=0
5	12ul	1, T=30
6	12ul	3, T=30
7	12ul	5, T=30
8	12ul	1, T=60
9	12ul	3, T=60
10	12ul	5, T=60
11	12ul	1, T=2hr
12	12ul	3, T=2hr
13	12ul	5, T=2hr
14	10ul	Benchmark Unstained Ladder
15	12ul	Control

Monday, November 26, 2018

To Do:

1. ~~Run western blot gel again also run another gel of 0, 20, 30, 45 (flasks 1,3,5)~~
2. ~~Transfer to membrane, keep in blocking buffer~~
3. Tuesday- Day 1 of protein purification
4. Wednesday- Day 2 purification, run gels
5. Thursday, gel staining and western blot development

Run 2 gels (Nu-Page 4-12% MES)

Lane	Volume	Contents
1	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
2	12ul	1, T=0
3	12ul	3, T=0
4	12ul	5, T=0
5	12ul	1, T=30
6	12ul	3, T=30
7	12ul	5, T=30
8	12ul	1, T=60
9	12ul	3, T=60
10	12ul	5, T=60
11	12ul	1, T=2hr
12	12ul	3, T=2hr
13	12ul	5, T=2hr
14	10ul	Benchmark Prestained Ladder
15	12ul	Control (1:20)

Lane	Volume	Contents
1	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
2	12ul	1, T=0
3	12ul	3, T=0
4	12ul	5, T=0
5	12ul	1, T=20
6	12ul	3, T=20
7	12ul	5, T=20
8	12ul	1, T=30
9	12ul	3, T=30
10	12ul	5, T=30
11	12ul	1, T=45
12	12ul	3, T=45
13	12ul	5, T=45
14	10ul	Benchmark Prestained Ladder
15	12ul	Control (1:20)

Tuesday, November 27, 2018

To Do:

1. ~~Tuesday- Day 1 of protein purification~~
2. Wednesday- Day 2 purification, run gels
3. Thursday, gel staining and western blot development

JW purifying samples 1 and 2

KMR purifying samples 3 and 4

Sample	Date Prepared	Labeled	Volume of Cells	OD600 at harvest
1	11/14/2018	LVS	300mL	0.490
2	11/14/2018	β' TAP	300mL	0.427
3	11/14/2018	LVS	300mL	0.463
4	11/14/2018	β' TAP	300mL	0.441

Wednesday, November 28, 2018

To Do:

1. ~~Wednesday- Day 2 purification, run gels~~
2. Thursday, gel staining and western blot development

Use 10% NuPAGE Bis-Tris gels (don't forget to take sticker off the bottom)

Add 500mL (25mL 20x stock with 475mL type I DDih20,1.25mL antioxidant stored at 4C) MES buffer in gel apparatus

Remove combs

While samples are heating, gently wash wells with running buffer from inside the chamber using a 200ul pipet, wash each well twice

Combine 11ul of each protein sample with 11ul 2x sample loading buffer

Heat samples at ~95C for 5 minutes, quick vortex and spin once they come off heating block **ladder does not need to be heated**

Gel: Silver Stain

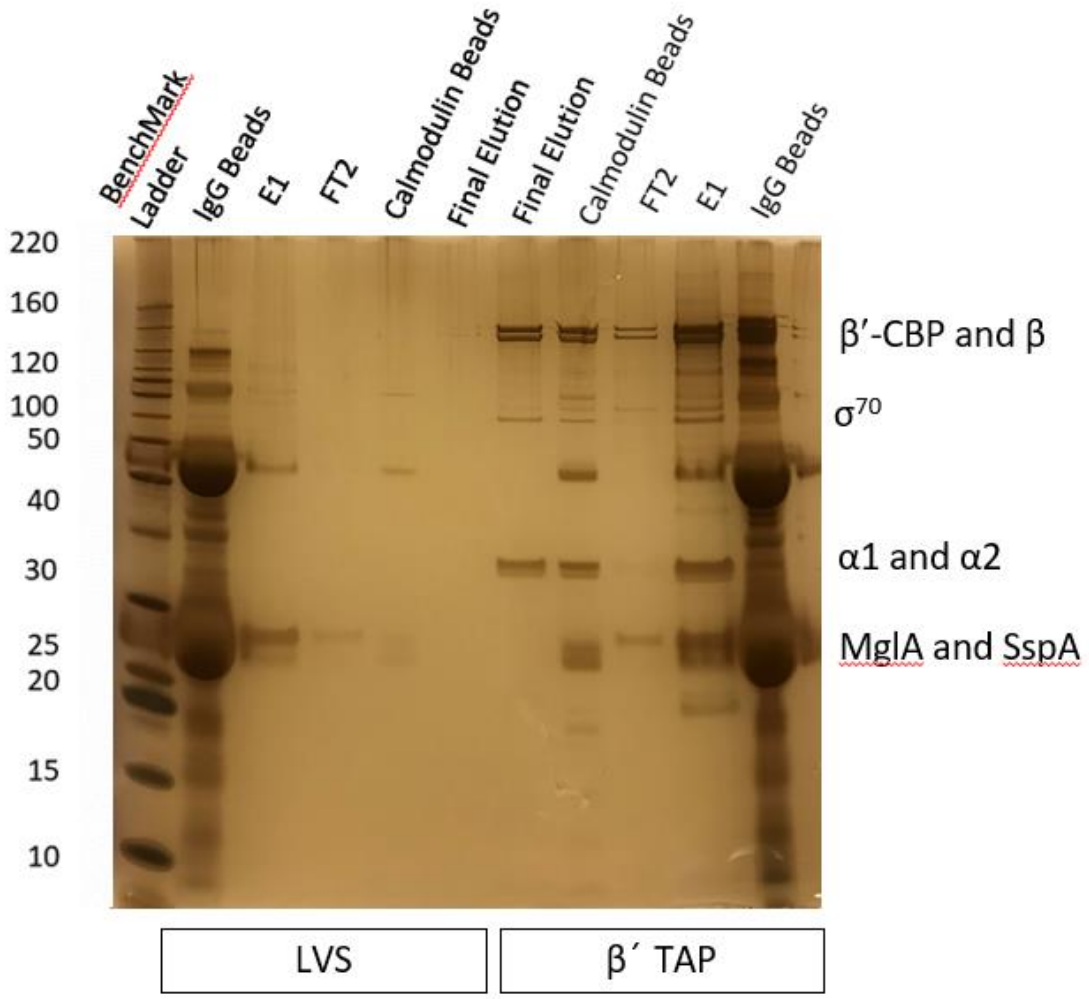
Dilute ladder (1ul) into 9ul 1x sample loading buffer

Lane	Volume	Contents
1	10ul	Benchmark unstained ladder (1:10)
2	20ul	LVS- IgG Beads
3	20ul	LVS- E1
4	20ul	LVS- FT2
5	20ul	LVS- Calmodulin Beads
6	20ul	LVS- elution final
7	20ul	Beta-prime elution final
8	20ul	Beta-prime Calmodulin Beads
9	20ul	Beta-prime FT2
10	20ul	Beta-prime E1
11	20ul	Beta-prime IgG Beads
12	10ul	1x Sample Loading Buffer (SLB)

Thursday, November 29, 2018

To Do:

1. ~~Silver stain gel staining~~
2. ~~Western blot development~~



Wednesday, December 12, 2018

To Do:

1. ~~Create plan for new heat shock experiment~~

Plan to induce production of σ32 protein:

Grow 2 flasks σ32 TAP tagged cells overnight at 37C (target OD of 0.3-0.4)

Also grow up 1 flask of LVS and subject it to the kickdown in temperature and take timepoint at time 0

Allow cells to grow at 30C for 1-2 hours for acclimation

Determine OD and split into 6 flasks (duplicates)

Keep 2 flasks at 30C, 2 flasks at 37C, 2 flasks at 45C

Take samples for protein analysis at:

T=0,15,30,45,60mins, 2hrs

Take an OD at each timepoint to determine cell concentration (1mL)

Take samples at each time point for a western blot (1mL)

Determine at which temperature/timepoint the most σ32 is produced.

Thursday, December 13, 2018

To Do:

1. ~~Learn to and start designing primers~~

Monday, December 17, 2018

To Do:

1. ~~Set up flasks for heat shock experiment~~

Target OD600 of 0.3-0.4

Inoculate flasks at about OD600 0.005

LVS: 100mL OD600: 1.29

RpoH: 2 flasks of 200mL OD600: 1.69

Flasks started shaking at 4:45

Temperature Incubated	Flask Number
30 A	1
30 B	2
37 A	3
37 B	4
45 A	5
45 B	6

Tuesday, December 18, 2018

To Do:

1. ~~Perform heat shock experiment~~

Shaker stopped in the middle of the night (it was on a 1h timer) was started again in the morning.

9:00am OD600

LVS: 0.158

RpoH A: 0.101

RpoH B: 0.115

12:00pm OD600

LVS: 0.316

RpoH A: 0.204

RpoH B: 0.224

Flask Number	Timepoint	OD600
1	0	0.341
	15	0.350
	30	0.361
	45	0.372
	60	0.382
	120	0.422
2	0	0.365
	15	0.376
	30	0.387
	45	0.399
	60	0.410
	120	0.451
3	0	0.341
	15	0.354
	30	0.368
	45	0.381
	60	0.396
	120	0.453
4	0	0.366
	15	0.380
	30	0.394
	45	0.409
	60	0.425
	120	0.482
5	0	0.341
	15	0.362
	30	0.381
	45	0.397
	60	0.407
	120	0.428
6	0	0.366
	15	0.389
	30	0.408
	45	0.423
	60	0.434
	120	0.457

Wednesday, December 19, 2018

To Do:

1. ~~Run western gel and transfer~~

Run western gel (Nu-Page 4-12% MES)

150V for ~1hour 10mins

Lane	Volume	Contents
1	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
2	12ul	1, T=0
3	12ul	3, T=0
4	12ul	5, T=0
5	12ul	1, T=30
6	12ul	3, T=30
7	12ul	5, T=30
8	12ul	1, T=60
9	12ul	3, T=60
10	12ul	5, T=60
11	12ul	1, T=2hr
12	12ul	3, T=2hr
13	12ul	5, T=2hr
14	10ul	Benchmark Prestained Ladder
15	12ul	LVS negative control

Thursday, December 20, 2018

To Do:

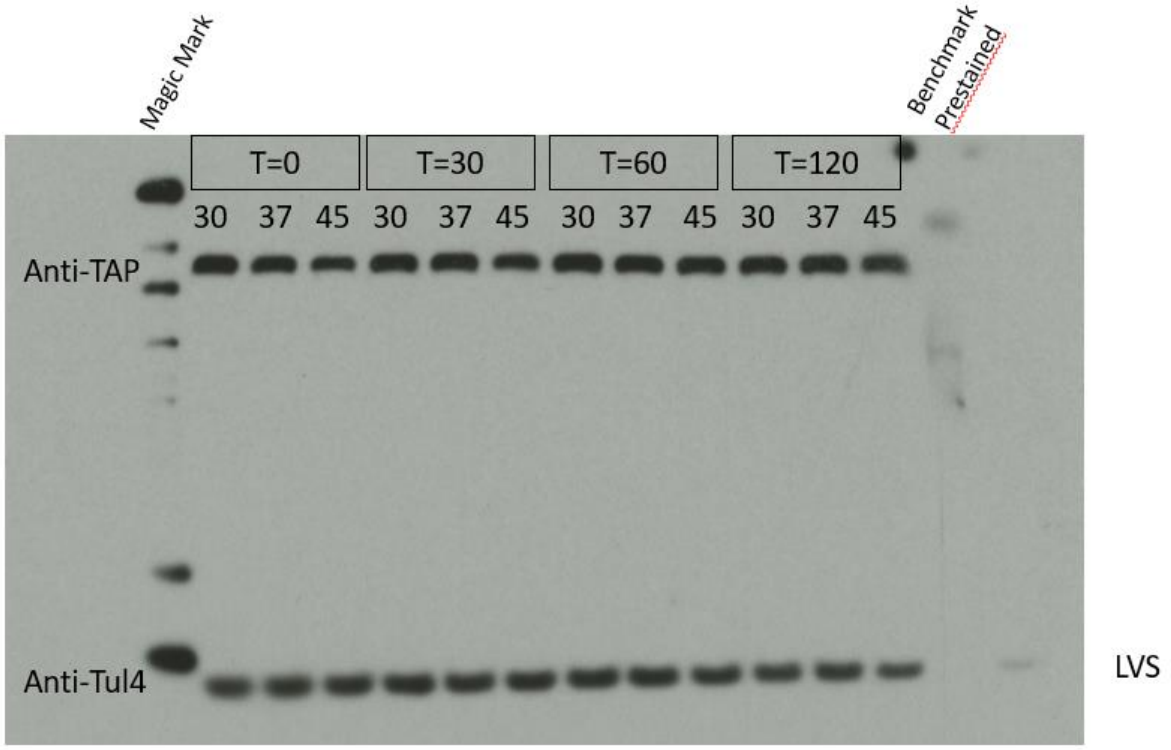
1. ~~Develop western~~

Primary Antibody

Anti-TAP (Rabbit IgG) 1:4000 (2.5ul in 10mL blocking buffer) (fisher catalog number CAB1001)

Anti-Tul4 (Mouse IgG) 1:50,000 (prefrozen) (NR-50326)

5 second exposure



Plan: Run gel again with only 3ul of sample

Friday, December 28, 2018

To Do:

1. ~~Run western gel and transfer~~

Run western gel (Nu-Page 4-12% MES)

150V for ~1hour 10mins

Lane	Volume	Contents
1	10ul	Magic Mark Ladder (2ul ladder, 8ul 1x sample loading dye)
2	3ul	1, T=0
3	3ul	3, T=0
4	3ul	5, T=0
5	3ul	1, T=30
6	3ul	3, T=30
7	3ul	5, T=30
8	3ul	1, T=60
9	3ul	3, T=60
10	3ul	5, T=60
11	3ul	1, T=2hr
12	3ul	3, T=2hr
13	3ul	5, T=2hr
14	10ul	Benchmark Prestained Ladder
15	3ul	LVS negative control

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.