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October 2022

Tuesday, October 18, 2022

To Do:

1. ~~Make and autoclave sucrose-containing CHA~~
2. ~~Pour CHA-sucrose plates~~
3. ~~Ask HT to streak 1°~~

Results and Data:

CHAH plates with 10% sucrose

In 500 mL flask, mix:
5 g Beef Heart Infusion
5 g Protease Peptone
5 g Glucose
0.5 g L-Cystine
7.5 g Agar
with 150 mL type I ddiH_2O. Stir on low heat until completely dissolved, about 10 minutes.
In 500 mL flask, dissolve 5 g hemoglobin in 250 mL ddiH2O. Stir on low heat until completely dissolved, about 10 minutes.

Put both flasks in 10 qt InstantPot with ~400 mL distilled water. Pressure cook for 30 minutes, keep warm setting on. While autoclaving, warm sterilized 50% sucrose in water bath at 55°C. After cycle finishes, have keep warm setting on and let sit for 20'. Release pressure valve manually if still pressurized.

After all components are sterilized and equilibrated to 55°C, pour hemoglobin into CHA flask. Add 100 mL 50% sucrose to CHA-hemoglobin flask. Mix media, use sterile pipette, pour ~24 mL media per plate.

**During first run, InstantPot had C6 H error. This error suggests that there is a problem with the pressure sensor. We might need to replace the Instantpot if that is the case, but there are reports that these errors can pop up and not be consistent. I ran another cycle and there was no error, so I re-made the media and ran the cycle without an error. The downside- I hurried a bit to get the media in, so the hemoglobin wasn't as well-dissolved as I thought. The media also sat in the InstantPot for 30 minutes (during group meeting) instead of 20, so the CHA was closer to solid that I'd like! Managed to pour the plates, but I put a plate without cells and some with LVS only at 37°C overnight to check growth.

Hannah struck out the 1° integrant (Δhfq with pKR11) for me around 3:15pm.

Wednesday, October 19, 2022

To Do:

1. ~~Plate cells on CHA and CHA sucrose~~

Results and Data:

Scrape up cells and serially dilute to 10^{-6}.

Plate 100ul of 10^{-2} to 10^{-6} dilutions on CHA-sucrose x2 and plate 10^{-6} on CHA only. Put in 37°C standing incubator.

Thursday, October 20, 2022

To Do:

1. ~~Out~~

Friday, October 21, 2022

To Do:

1. ~~Joint group meeting~~
2. ~~Check plates~~
3. ~~Prepare for bS21-2 IP~~
    a. ~~Make media~~

Results and Data:

Have 1 x 500 mL MHB in 1 L baffled flask. Prepare 3 more, can include with Aisling's autoclave cycle.

Sunday, October 23, 2022

To Do:

1. Patch
    a. ~~LVS pF (from KMR working stock box, froze on 8/16/22)~~
    b. ~~LVS pKR72 pF-rpsU2-TAP (from KMR working stock box, froze on 8/16/22)~~
    c. ~~LVS for EC cells.~~
2. ~~Transform XL1 blue cells with pKR147~~

Results and Data:

Looked at sucrose plates- all the colonies are still pretty small and we're looking for small colonies. Wait another day before patching.

Patch out LVS with pF and pKR72.

Only 2 plates of CHA left. Make more, then streak out 4 plates for EC cells. Made a half flask of plates, but worried that I lost some volume of CHA in the instantpot. Don't use 2 remaining CHA plates, but dry the ones I poured and plate 4 with LVS (test and potential EC cells).

Transform XL1Blue cells with pKR147. Also include pKR144. Use 0.5 uL plasmid from working stocks, 100 uL XL1blue cells from my own chem competent stocks, incubate on ice until heat block goes from 65°C to 42°C (assisted by removal), about 10 mins. Heat-shock for 30" at 42°C, add 1 mL LB, put at 37°C shaking for about 1 hr. Plate 20 ul, 100 ul, remaining for pKR144 and pKR147 and only remaining for negative control. Put plates at 30°C.

Monday, October 24, 2022

To Do:

1. ~~Help Aisling make EC LVS~~
2. ~~Patch potential ΔhfqΔrpsU2 colonies to CHA and CHA-Kan~~
3. ~~Start growing LVS pF and LVS pKR72 pF-rpsU2-TAP O/N~~

Results and Data:

Helped Aisling make EC LVS from cells plated yesterday. Made 6 tubes with 105 uL each, she will do a test electroporation tomorrow to determine electroporation efficiency. That will leave 10 electroporations to potentially do on Friday.

Patched out potential ΔhfqΔrpsU2 colonies to CHA and CHA-Kan. Patched 12 colonies, 1 – 11 were small and 12 was larger.

Start cells O/N for TAP purification. Supplement 2 x 500 mL of MH broth in baffled flasks.

Number	Cells	OD600	vol cells	vol media	dilution factor	Actual OD600	For 0.003 in 500 mL
1	LVS pKR72	0.350	50	950	20	7.00	214
2	LVS pF	0.235	25	975	40	9.40	160

Mixed up volumes, so started LVS pF at 0.004 and LVS pF72 at 0.00224. Add 128 uL more LVS pKR72 to get to approx. 0.004. Put in shaking incubator at approx. 4:20pm.

No colonies visible on carb plates from transformation yesterday. Check tomorrow.

Tuesday, October 25, 2022

To Do:

1. Check yesterday's patches by PCR
2. Patch potential ΔhfqΔrpsU2 colonies to CHA and CHA-Kan
3. Harvest LVS pF and LVS pKR72 pF-rpsU2-TAP, freeze

Results and Data:

Potential ΔhfqΔrpsU2 patches- not grown up well enough yet. Wait to check by PCR until tomorrow.

Harvest cells from cultures grown overnight at around 9:45am:

1 LVS pF 0.586
2 LVS pKR72 0.432

Spin down max speed in 250 mL bottles for 10' (while centrifuge cools to 4°C), resuspend in MHB, transfer to 50 mL conicals, spin down 5' max speed 4°C. Discard supernants and freeze pellets at -80°C.

Wednesday, October 26, 2022

To Do:

1. ~~Check potential ΔhfqΔrpsU2 patches by PCR~~
2. ~~Run PCR on gel~~
3. ~~TAP purification day 1~~

Results and Data:

Purify bS21-2-TAP using modified TAP protocol to IP, capturing Protein A moiety on IGG beads. Buffers:

Buffer A + PI +BB (modified Buffer 1)

Final composition	Stock solutions	For 25 mL
20 mM KHEPES pH 7.9	1 M KHEPES	500
10 mm $MgCl_2$	1 M $MgCl_2$	250
10 mM NH_4Cl	1 M NH_4Cl	250
0.5 mM DTT	0.5 M DTT	25
1X Bug Buster	10X Bug Buster	2.5
10% glycerol	75% glycerol	3.325
Water		18.15

+ 2 tablets protease inhibitor

1. Resuspend samples in **10 mL** Buffer A + PI + BB by pipetting with 5 mL pipette. Be sure no clumps remain (larger volume, hoping better/more efficient lysis).
2. Add 10 uL DNase I (Lucigen/Epiceter), mix by pipetting
3. Incubate at 37°C for 30'. Cool down centrifuge while waiting.
4. Spin 50 mL conical tubes at 4°C for 20' at max speed (14635xg).
5. Transfer lysates into new 50 mL conical tubes, leaving behind insoluble material at bottom (okay to leave some behind; better than taking too much)
6. Filter-sterilize lysates using 30mL syringe and syringe filter into new 50mL conical tube. Specifically, pull plunger out from 30mL syringe, thread tip of syringe onto syringe use filter (use MillexGP 0.22 micrometer filter, has 33mm diameter), pour/pipette lysate into syringe, insert plunger and recover filtered lysate into new (nonsterile) 50mL conical tube
7. Obtain a good estimate of sample volume using pipette.
8. Adjust buffer concentration to buffer B by adding to each sample:
   50 uL 1M NH_4Cl / mL sample
   10 uL 10% NP-40 /mL sample
   Both samples: ~9 mL
   450 uL for final 50 mM NH_4Cl
   90 uL for final 0.1% NP-40
9. Remove 50 uL into microfuge tube for control analysis (LYS).

   Have Buffer B made previously.

In cold room

10. Cut tip of 1mL pipette tip slightly
11. Resuspend IgG Sepharose beads completely
12. Pipette 300 uL IgG Sepharose beads into each column, placing column on top of Qiagen tip holder placed on 50 mL conical for flow-through
13. Wash beads with 10 mL of buffer B
14. When all the buffer B has passed through the column, cap the bottom of the column

15. Add 1 sample per polyprep column, add top cap to close column (make sure column is closed completely and is not dripping liquid)
16. Incubate samples with beads, rocking at 4°C, for 2 hours (actually about 2hrs 15mins)During incubation, prepare:
    a. TEV Cleavage Buffer MOD for this set of purifications, ~13 mL per purification

TEV Cleavage Buffer MOD

Composition	Stock solutions	For 40 mL
25 mM KHEPES pH 7.9	1 M KHEPES	1000 uL
10 mm $MgCl_2$	1 M $MgCl_2$	400 uL
50 mM NH_4Cl	1 M NH_4Cl	2000 uL
0.1% NP40	10.00%	400 uL
1 mM DTT	0.5 M	80 uL
	Water	36.12 mL

18. After 2 hour incubation, open columns and let lysate pass through column.
19. Save 50 uL aliquot of flow through in microfuge tube (FT1), discard remaining.
20. Wash columns 3x with 10 mL buffer B, discarding flow through
21. Wash columns with 10 mL TEV Cleavage buffer MOD, discarding flow through
22. Cap column bottoms, add 1 mL TEV Cleavage buffer MOD and 10 uL AcTEV Protease to each.
23. Incubate rocking at 4°C overnight- make sure columns are closed completely and are not dripping liquid. **Remainder of protocol FOR TOMORROW:** Recover TEV eluates by gravity flow (1 ml)Rinse each column with additional 200 ul of TEV Cleavage Buffer MOD

Buffer B (modified buffer IPP150)

Final composition	Stock solutions	For 150 mL
20 mM KHEPES pH 7.9	1 M KHEPES	3 mL
10 mm $MgCl_2$	1 M $MgCl_2$	1.5 mL
50 mM NH_4Cl	1 M NH_4Cl	7.5 mL
0.1% NP-40	10% NP-40	1.5 mL
Water		136.5 mL

pKR147

Check on transformations from Sunday 10/23 (plates have been at 30°C). Have nice colonies from pKR144 but no colonies from pKR147. Asked Hannah about the concentration of the plasmid, when she sent it to sequence it was at ~78 ng/uL. Surprised the transformation didn't work. Try again with DH5alpha cells. Need more LB-carb plates first, though!
Pour a 500mL flask of LB carb 100 ug/mL plates.

Check on transformation plates again at end of day (have been at room temp)- there are colonies on the pKR147 plates! Keep them at room temp overnight and plan to do a maxiprep soon.

PCR to check for ΔrpsU2

Use primers KROL148 and KROL148.
WT size: 1275 bp
ΔrpsU2 size: ~1095 bp

Sample Number	Sample
1	Δhfq ΔrpsU2 7-1-1
2	Δhfq ΔrpsU2 7-1-2
3	Δhfq ΔrpsU2 7-1-3
4	Δhfq ΔrpsU2 7-1-4

5	Δhfq ΔrpsU2 7-1-5
6	Δhfq ΔrpsU2 7-1-6
7	Δhfq ΔrpsU2 7-1-7
8	Δhfq ΔrpsU2 7-1-8
9	Δhfq ΔrpsU2 7-1-9
10	Δhfq ΔrpsU2 7-1-10

11	Δhfq ΔrpsU2 7-1-11
12	Δhfq ΔrpsU2 7-1-12
13	1° ΔrpsU2 Δhfq E7-1
14	ΔrpsU2 gDNA (KRLVS109.1)
15	LVS gDNA
16	-DNA

KRLVS109.1, LVS ΔrpsU2 Tn7::Ptul4-pdpA 5'UTR1-mut2v2-6aa-lacZ aphA

Total reaction volume	20
Total number of reactions	16
Number of reactions plus error	17.6

Component	Stock concentration	Final concentration	1 rxn volume	Master Mix
ddiH2O			11.8	207.68
PrimeSTAR GXL Buffer	5x	1x	4	70.4
dNTPs	2.5 mM	0.2 mM	1.6	28.16
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	7.04
KROL147	10 uM	0.3 uM	0.6	10.56
KROL148	10 uM	0.3 uM	0.6	10.56
template	100 ng/ul	2 ng/ul	1	indiv
		Total volume	20	303.68

Use STN1 with 1:30 extension time.
Run entire reaction on gel.

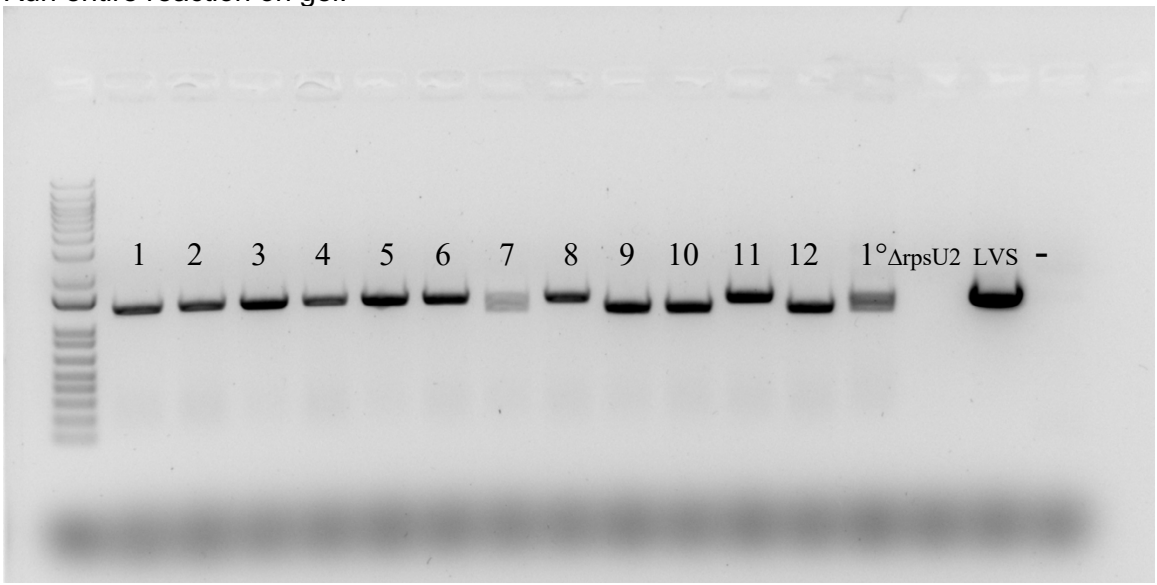


Figure 1. Gel of PCR to identify ΔrpsU2 cells – early view

Apparent deletion mutants:
9, 10, 12. Note that 7 is the only patch with decent growth on the Kan plate, so probably 1° or mixed- don't streak to single colony. Clearly forgot to add gDNA to the ΔrpsU2 control tube.

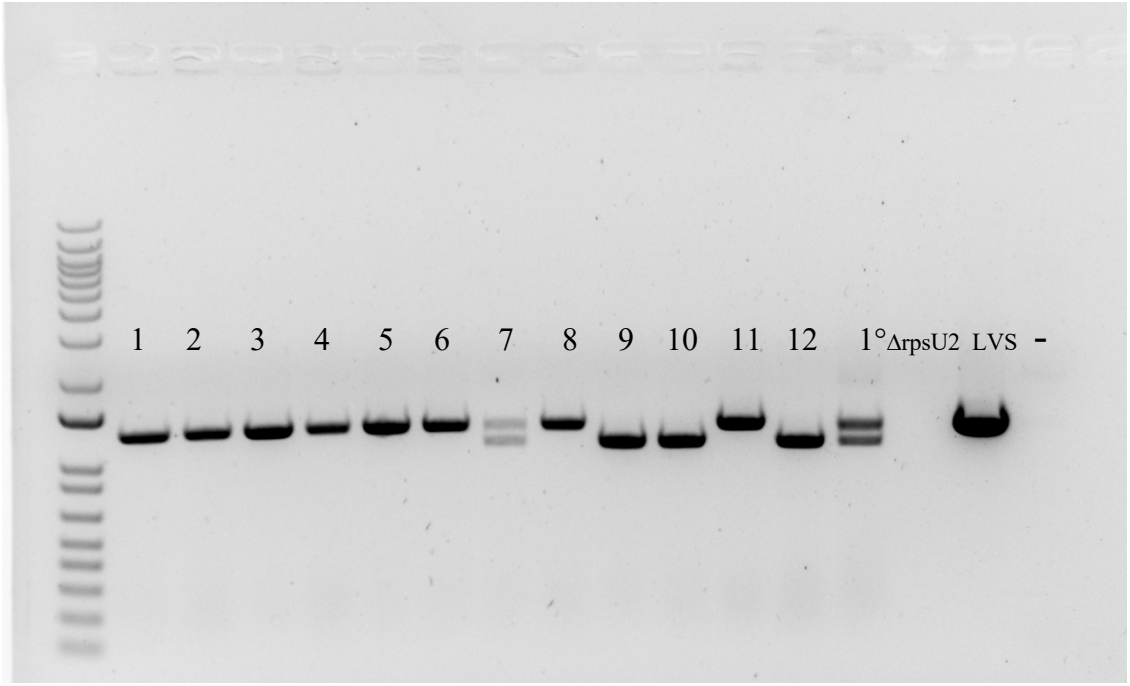


Figure 2. Gel of PCR to identify ΔrpsU2 cells – later view
Better resolution, very clear that 9, 10, and 12 seem to be deletion mutants while 7 is a mixed population (as is the 1° integrant sample).

Thursday, October 27, 2022

To Do:
1. ~~Finish TAP purification~~
2. Prepare for gradients tomorrow

Results and Data:

TAP purification continued: Recover TEV eluates by gravity flow (1 ml)Washed columns with 200 uL TEV Cleavage Buffer MOD to get beads to the bed.
3. Rinse each column with additional 200 ul of TEV Cleavage Buffer MOD.
4. Keep samples at 4°C.

Δhfq ΔrpsU2
Check on streak plates, there is growth on the first streak so things look promising!

Prepare sucrose solutions for gradients:

Component	Desired Concentration	Stock solutions	10%	50%
KHEPES pH7.9	25 mM	1 M KHEPES	750 uL	750 uL
MgCl2	10 mm	1 M $MgCl_2$	300 uL	300 uL
NH4Cl	50 mM	5 M NH_4Cl	300 uL	300 uL
sucrose			3 g	15 g
water			25.65	13.65
	Total		30 mL	30 mL

Friday, October 28, 2022

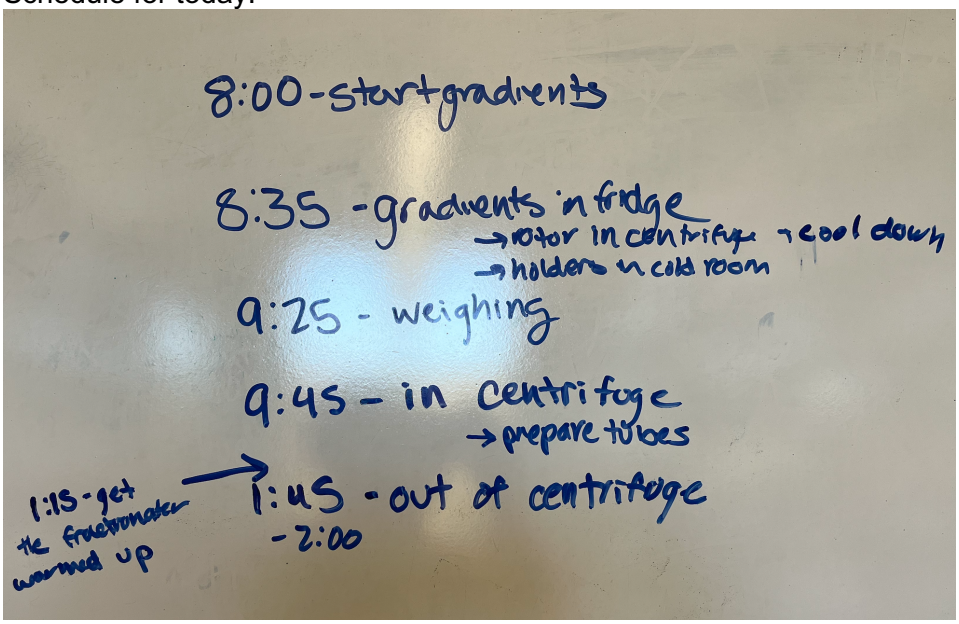
To Do:

1. ~~Electroporations with Aisling~~
2. ~~Gradient of TAP-purified proteins~~
3. ~~Check on ΔhfqΔrpsU2 cells~~

Results and Data:

Electroporations with Aisling. Performed 10 electroporations into EC LVS with 1 ug of pKR141 each (200 ng/ul, 5 uL). Recovered starting at 7:45 – 7:55am. Started plating around 11:15am, each electroporation onto one 150mm CHA-Kan plate.

Gradient to assess TAP purification of bS21-2-TAP (started on 10/26, finished 10/27).
Schedule for today:



Making a Sucrose Gradient

1. Have prepared a light solution (i.e. 10% sucrose) and a heavy solution (50% sucrose) that has been autoclaved and filter-sterilized. Sucrose solutions should be in the same buffer as your sample. Also prepare about 205 uL of your sample, diluted to 0.6 ug/ul.
   Prepared heavy and light solutions yesterday, will use TAP purification sample undiluted.
2. Put a tube in the marker block and draw a line at the top ledge
   Tubes are in drawer under Hannah's bench labeled "polysomes." Label tubes.
3. With a 30mL syringe, push into light solution and pump the air bubbles out. Draw out light solution (about 8 mL per gradient) and plunge needle to the very bottom of the tube. Lift the needle as solution pours out, be careful to keep the tip of the needle just under the surface of the solution. Stop at just above the line.
   Pumping out bubbles takes some time- be careful not to get any in the tube!
4. With a 10mL syringe, push into heavy solution and pump the air bubbles out. Draw out heavy solution, then wipe the needle and push a drop out, dabbing onto a kimwipe (making sure there's no air). Holding the tube with thumb and middle finger, use forefinger as a guide as you plunge needle to bottom of tube, resting the needle along the wall, and push out gently just enough to form a pool. Keep pushing out solution as you draw up the needle, keeping the tip about 1 cm

below the interface of the layers until there is about 2-3mm of space at the top. Make sure needle is resting against the wall of the tube as you quickly draw it out. There should be a visible line between the layers.
Easier to see interface when there is a colored background (not white). Used aluminum foil for second gradient, which was helpful. Aim to get the same amount of heavy solution in both tubes- this really drives differences in weight when balancing.

5. Use light solution to adjust the top layers, making sure all samples are at the same level
Hannah basically adds light solution until it's at the very top.
6. Cap the tubes, making sure the hole is the last part to seal. Some liquid should be visible inside the cap, if there is visible air or you can't see liquid in cap add some more light solution.
Hannah aims to have liquid spill-over – want to avoid bubbles that touch the interface with the liquid in the tube (i.e., bubble entirely enclosed by the cap seems to be fine).
7. Turn on gradient station in back, select "GMST"
8. Use the up and down keys on the gradient station and the level to level the plate. When level press "done".
9. Put tubes in tube holder (no need to balance); Go to exit -> gradient -> recent-> confirm rotor -> use (confirm 10:50) -> run (recent should be the 10-50% sucrose gradient because that's the one we have been using. If you need to find a different one go to recent and scroll through)
10. Put back in tube rack and refrigerate for 45 minutes. Now is a good time to cool down the ultracentrifuge.
Today there was a previous user, so the centrifuge was in use and cool up until we needed to use it. Move the rotor and sample holders to the cold room to cool.
11. Take off caps, remove 40uL of top layer and add your sample using P200.
Keep tubes on ice when not manipulating- use finger to make hole for tube, then secure ice around the tube. Also, this is slightly different in order- previously the protocol specified weighing first, but Hannah found it was necessary to weigh afterwards anyway, so just weigh afterwards.

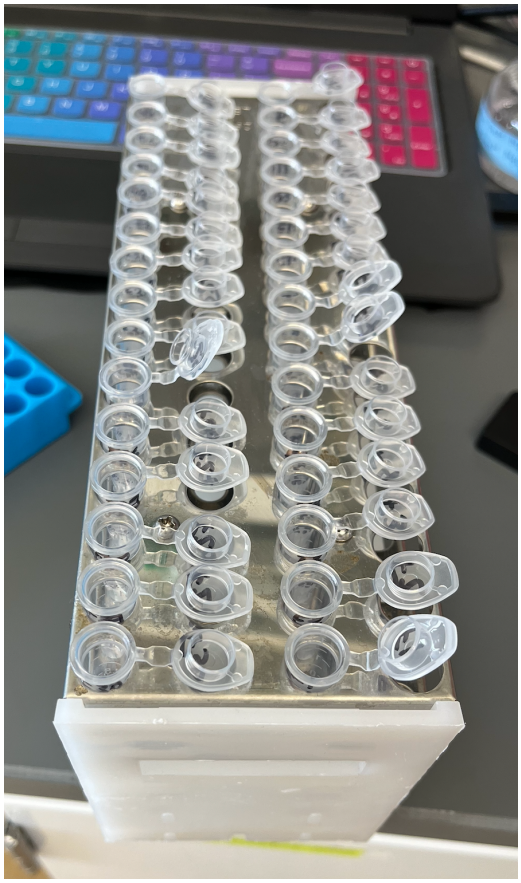
Sample	Contents
1	LVS pF TAP purification (mock)
2	LVS pKR72 TAP purification (IP of bS21-2-TAP)

12. Using sample buffer, adjust as necessary until there is no more than .002 difference in weight.
Today we had significant difficulty and the control gradient was ruined. Continued and kept using that ruined gradient as balance for actual sample. To re-balance we used a combo of heavy and light solution to minimize the new volume needed to re-adjust.
13. Put samples on ice and bring to INBRE to spin on ultracentrifuge with rotor SW40 Ti. 40,000 rpm for 4 hours at 4C, using 5 for acceleration and deceleration.
Put tubes in sample holders (need metal tweezers). Make sure tube holders are secure on the rotor. Today one fell off when we tried to spin (ruined gradient, lucky didn't ruin centrifuge). Stay with centrifuge until it reaches speed.
14. After spin, continue with fractionation protocol.

Fractionating

1. Before launching the Triax software, turn on both gradient station and fractionator on (buttons on side), then set gradient station to "scan"
2. Choose the username, click "single UV OD scan". Channel A Wavelength should be 260 nm
Labs have joint usernames. Also fill in experiment name.
3. On the bottom of the screen fill out Gradients necessary info (i.e. sample volume, gradient type, speed, etc)
4. Under the rotor settings (SW40Ti) select "number of fractions" as the mode to fractionate by
Didn't see this option today. Can specify the number of fractions one of the next screens.

5. On the LED Power screen, make sure Channel A is reading between 800,000 and 900,000. Push water through the cell if it needs to be adjusted
   Start by pushing water through cell, then check channel A readings. Hannah says she lets it stabilize a bit, but we didn't wait too long on that screen today.
6. Go to scan set up, make sure everything is filled out correctly
7. Go to graph. Pump water through the cell again to calibrate. Y axis should be close to 0, with at least two zeros following the decimal point (e.g. 0.002)
   Pump water through top cell and through side pump as well. Click Y autoscale if it's not selected. Wait for the reading to stabilize, approx. 20 minutes. Check on it occasionally.
8. Purge air through the system, then load the first sample. Cap the sample with the flow cell cap by turning and pushing down and put sample in the flow cell, using bottom lock. Slide it on the fractionator then rotate 90ºC to fix it in place (window should face front)
   Careful with the flow cell cap – be sure it's on properly but don't break the tube. When putting the flow cell on the station, have the window facing the right first, then twist 90° to have it face front.
9. Load microcentrifuge tubes in rack in the middle two rows, then put the rack on the fractionator. The 29 should face the back. The dispenser will make a U moving back then left then forwards, so load tubes accordingly.



Loaded tubes incorrectly! The tubes on the far left should be in the middle left row, not all the way to the left.

Tubes on the right are loaded correctly and the rack is facing the correct direction (29 in back).

10. On software, hit "start scan". When all of the tubes are filled, hit "end" to move the tubing to the back. Flush with water for 10 seconds then air for 10 seconds. Repeat for a total of 3 washes. Rinse screw-on cap with DI water.
    Typically don't need to actually hit "end", generally does this by itself. If you need to hit "end", the button is on the fraction collector itself. If no more samples (like today), go directly to step 13 when fractionating is finished.
11. Save run as csv when prompted and graph via file -> save graph image.

12. Repeat Step 9 for the remaining samples.
13. When all of the samples are complete, push water through the flow cell, then flush 5 times with water, 3 times with 70% isopropyl alcohol, and 5 times with air only using the dry syringe. Switch metal lever down to assist with air flushes.
    Dry syringe is 50 mL, should never get wet.
14. Flush the line your sample goes through with DI water.
    Do this **before** the air/dry steps, pre-13.
15. Save fractions at -20C if interested in proteins, -80C if interested in RNA.
    Stored fractions 1 – 19 at -20°C. Only recovered a portion of fractions 16 – 19 bc of error loading microfuge tubes.

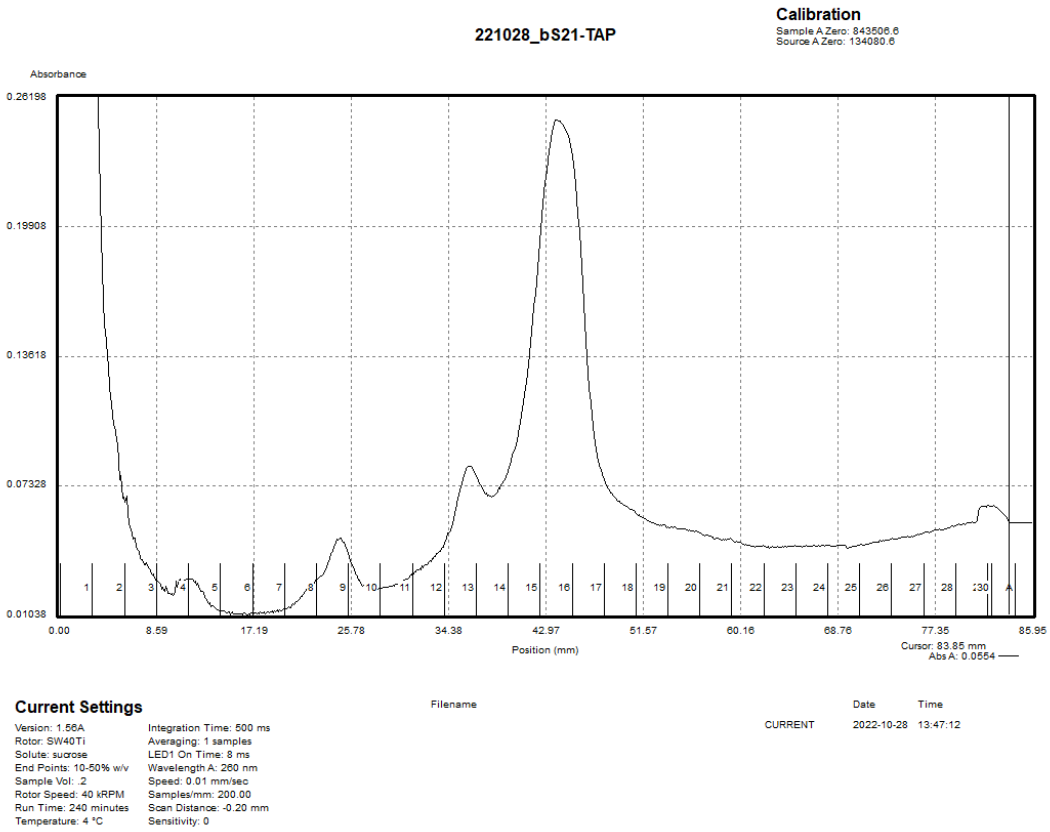


Figure 3. Gradient of bS21-TAP purification sample

Looks promising! There isn't a ton of material (note the absorbance ratio) but a good likely 70S peak. 50S peak is poorly resolved- did I immunoprecipitate 50S subunits or did some of the 70S dissociate? Should do a western to see.

Δhfq ΔrpsU2

Check on streak plates, continued growth. As expected, no single colonies yet. Maybe Sunday.

Monday, October 31, 2022 – Tuesday November 1, 2022

To Do:

1. Give seminar at University of Delaware

Tuesday, November 29, 2022

To Do:

1. Resuspend BioSamA primer for InSeq protocol

Results and Data:

Need a working concentration of 1 uM BioSamA.

Linear PCR ● TIMING 2 h

21| Assemble the linear PCR reactions on ice.

Component	Volume per sample
dH_2O	to 100 µl
Pfx buffer	10 µl
10 mM dNTPs	2 µl
50 mM $MgCl_2$	2 µl
BioSamA (1 pmol $µl^{-1}$)	5 µl
Clean DNA (from Step 20)	0.5–2 µg
Pfx polymerase	1 µl

Spec sheet for oligo:

17-Oct-2022

Order No. **18922250**

Ref. No. **421362344**

Sequence - KROL584 BioSamA_v2 — 100 nmole DNA Oligo, 27 bases

5'- /5BiotinTEG/AAG ACG GCA TAC GAG ATT ACG AAG ACC -3'

Properties

Tm (50mM NaCl):* 59.9 °C
GC Content: 48.1%
Molecular Weight: 8,817
nmoles/OD260: 3.6
ug/OD260: 31.4
Ext. Coefficient: 281,200 L/(mole·cm)

Amount Of Oligo

6.9 = OD_{260} 24.4 nmoles = 0.22 mg

For 100 µM: add 244 µL

Shipped To

KATHRYN RAMSEY
UNIVERSITY OF RHODE ISLAND
7 GREENHOUSE RD
KINGSTON, RI 02881-2018
USA
4018742932
Customer No. 503562 *PO No. Credit Card*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -1.92 at 25 °C
Strongest Folding Tm: 52.1 °C
Secondary structure should not affect yield or purity for this oligo.

Oligo Base Types	Quantity
DNA bases	27
Modifications and Services	**Quantity**
HPLC Purification	1
5' Biotin-TEG	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Received 24.4 nmoles, so add 244 ul to get a stock concentration of 100 uM.

Resuspended in 0.1x EB to get 100 uM stock.
To make 10 uM stock, dilute 20 uL of 100 uM stock in 180 uL 0.1x EB
To make 1 uM stock, dilute 20 uL of 10 uM stock in 180 uL 0.1x EB

Put in new InSeq box and store at -20°C

Future To-Do

Check on plasmids that might restore growth to ΔrpsU1ΔrpsU2 cells (Hannah's gDNA insert screen, Oli's Fall 2021 project).

Move 1° LVS pKR10-1 into strain box

Bibliography

Suh, Moo-Jin et al. “Extending ribosomal protein identifications to unsequenced bacterial strains using matrix-assisted laser desorption/ionization mass spectrometry.” Proteomics vol. 5,18 (2005): 4818-31. doi:10.1002/pmic.200402111