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December 2022

Wednesday, December 14, 2022

To Do:

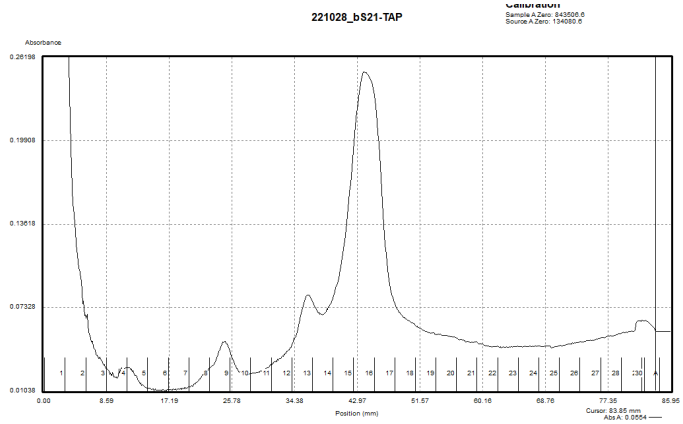
1. Run protein gel for WB of ribosome purifications

Results and Data:

Gel to assess modified TAP purification and subsequent sucrose gradient (see 10/28/22).

Would like to see the following:

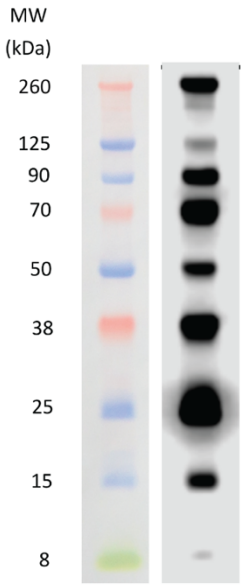
1. Where bS21-CBP is present in the gradient samples



2. Where most of the tagged bS21-2 is present
    a. Lysate
    b. flowthrough
    c. elution
    d. Samples 1 – 3 from sucrose gradient?
    e. beads

For goal 1:
4-12% gel, run in MES bufffer, 17 well.
Combine samples 1:1 with 2x SLB.

Lane	Volume	Contents
1	5 uL	WesternSure ladder
2	10 uL	Fraction 3
3	10 uL	Fraction 4
4	10 uL	Fraction 5
5	10 uL	Fraction 6
6	10 uL	Fraction 7
7	10 uL	Fraction 8
8	10 uL	Fraction 9
9	10 uL	Fraction 10
10	10 uL	Fraction 11
11	10 uL	Fraction 12
12	10 uL	Fraction 13
13	10 uL	Fraction 14
14	10 uL	Fraction 15
15	10 uL	Fraction 16
16	10 uL	Fraction 17
17	10 uL	Fraction 18



Using “new” Western blot ladder, Li-Cor 926-98000 WesternSure® Pre-stained Chemiluminescent Protein Ladder, 5 uL per western blot.

For goal 2:
4-12% MES, 15 well. Combine samples 1:1 with 2x SLB.

Lane	Volume	Contents
1	5 uL	WesternSure ladder
2	10 uL	Lysate 1 LVS pF
3	10 uL	Lysate 2 pF-bS21-2-TAP
4	10 uL	Flowthrough 1
5	10 uL	Flowthrough 2
6	10 uL	Elution 1
7	10 uL	Elution 2
8	10 uL	1X SLB
9	10 uL	Beads 1
10	10 uL	beads 2
11	10 uL	1x SLB
12	10 uL	Fraction 1
13	10 uL	Fraction 2
14	10 uL	Fraction 3
15	10 uL	Fraction 16 (control)

Make running buffer –800 mL for 2 gels
1x MES for <50 kD proteins
760 mL ddiH2O
40 mL 20x MES
2 mL NuPAGE antioxidant
Wash wells of gels with 200 uL tips
Load samples. Note that I had the pieces that push the gel against the rig backwards, so had to remove them and replace with samples in the well. Not ideal, but seemed to have worked. Added buffer over the tops of the gels and started running. Started at 100 V, went up to 120, then up to 150 when I saw the protocol (and realized I didn’t have much time!).

Prepare transfer buffer:
850 mL water
50 mL 20X transfer buffer
100 mL methanol

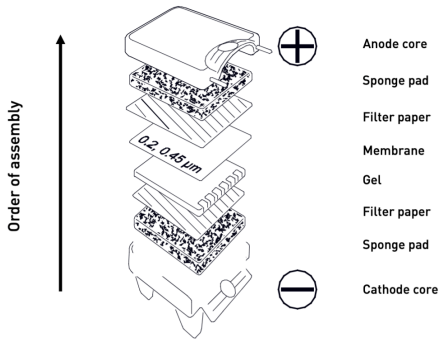
Store at -20C until ready for transfer. Still need to add 1 mL antioxidant.

Cut membrane (2) and filter paper (4) to size.
Have 3 sponges and one transfer apparatus per transfer.

Set up transfer:

Overview

IMPORTANT! When the blot sandwich is fully assembled, the **gel is closer to the cathode core (–)**, while the **membrane is closer to the anode core (+)**.



Only difference is that I have two sponges closer to the (-).
Used the power source on Hannah's bench, 20 V for 60 minutes.
Hannah transferred to blocking buffer (diluted 1:5 in PBS). Does not look great- the transfer was not efficient and all bands in the ladder (lower and higher MW) are still visible in the gel. Hannah thought it might have been due to insufficient buffer in the chamber. Consider if continuing with these blots is best or better to re-try.

Note that Hannah says the 2nd day takes 4-4.5 hours before imaging.

Wednesday, January 4, 2023

To Do:

1. ~~Run protein gels for WB of ribosome purifications~~
2. ~~Transfer gels~~

Results and Data:

Repeat running gel and transfer as above, but this time use more transfer buffer so the transfer is good.

Modifications:
Set up gel rig properly this time, but then loaded the 15 well gel with 17 well gel samples. Washed out samples and re-loaded all gels properly.
Run at 150 V for undocumented amount of time, until blue dye reaches the end but it still on the gel.
Set up transfer and fill apparatus with *all* 1L of the transfer buffer.
Run at 20V for 60 minutes.

Move blots to blocking buffer (diluted 1:5 in PBS) and leave at 4°C O/N.
Angled cut on top right corner of membrane from 17 well gel; square cut on top right corner

Thursday, January 5, 2023

To Do:

1. WB
    a. ~~Primary antibody~~
    b. ~~reblock~~
    c. ~~Secondary antibody~~
    d. ~~Detection~~
2. ~~Linear PCR~~
3. ~~Bind products to beads~~
4. Order from ~~NEB~~, Wilkem

Results and Data:

TAP Tag Polyclonal Antibody (anti-CBP), Fisher CAB1001
Using chemiluminescence, the Dove lab used 3 ul anti-TAP antibody / 25 ml 1x blocking buffer, a dilution of 1:8,333. No idea how much will be necessary using these samples and the near-IR detection. Try at 1:1,000 and see how it goes.

-Incubation in primary antibody: add 10 uL anti-CBP to 10 mL blocking buffer (diluted 1:5 in PBS), rock 1 hour at room temp
-While incubating, prepare wash buffers:

1x Wash Buffer (400 mL)
40 mL 10X PBS
360 mL dH20
Split into two separate bottles, 300 mL and 100 mL.
To the 300 mL bottle, add 1.5 mL Surfact-Amps(NP-40?). This is your primary wash buffer. The remaining 100 mL will be for the final two washes after the secondary antibody, to remove traces of detergent, which may show up as background on the Li-Cor.

-Wash 4x on rotator for 10 minutes each, using 25 mL wash buffer per wash.
xXxX
-Use 25 mL diluted blocking buffer and block again, for 30 min.

-Add 1 uL of 800CW [green channel] IRDye secondary antibody (anti-rabbit) to 10 mL wash buffer (the one with Surfact-Amps, aka PBS-T). Also add 0.01% SDS to the wash buffer (5 uL of 20% SDS). These secondary antibodies are stored at 4°C.
-Label for 1 hour on rocker at room temperature. Cover the box with foil or use a black box (the secondary antibodies are light sensitive).

-Wash 4x on rotator for 10 minutes each, using 25 mL wash buffer per wash.
XxXx

-Wash 2x on rotator for 10 minutes each, using 25 mL of wash buffer WITHOUT detergent.
Xx

(-If faint bands are expected, do a quick methanol rinse by dunking the blot in methanol for 2 seconds then letting air dry for 2 minutes prior to imaging.) – not doing this yet

Imaging

-Leave the membrane in the box containing the final wash buffer. Bring the box, gloves, forceps, and a timer to the imager in the INBRE facility.
-For imaging, use 84 uM resolution and “high” clarity.

Imaging Notes:

-The WesternSure ladder only shows up in 700 cW channel
-About ~40 mins to image 1 full blot using 84 uM resolution and “high” clarity.

Preview of 15-well gel blot looked like there were some strong bands, so didn’t bother with methanol drying step. Imaging the entire blot using the indicated settings took 40 minutes. On the plus side, the blot didn’t dry out bc I used the mat to cover it.

Preliminary notes:
There seem to be 2 sizes of eluted reactive bands. Antibody is rabbit IgG. According to ThermoFisher “The CAB1001 immunogen is a KLH conjugated peptide representing the C-terminus of the TAP

construct after TEV cleavage." – which should be the calmodulin binding peptide (CBP). The cleaved portion of the TAP tag is the Protein A, which interacts with IgG.
Based on the predicted protein sequence (looking at expression plasmid, pKR72), bS21-2-CBP should be 12.7 kD and there are two Protein A domains encoded after the TEV cleavage site. So bS21-2-TAP should be 28.3 kDa. Excellent- these are absolutely the right protein! I think the question is why so much uncleaved (probably?) protein is being eluted?

When I imaged the membrane from the 17-well gel, the signal looked poor. I did the methanol drying trick, but then imaged the wrong side of the blot! Try again with smaller area, so it's a bit faster.

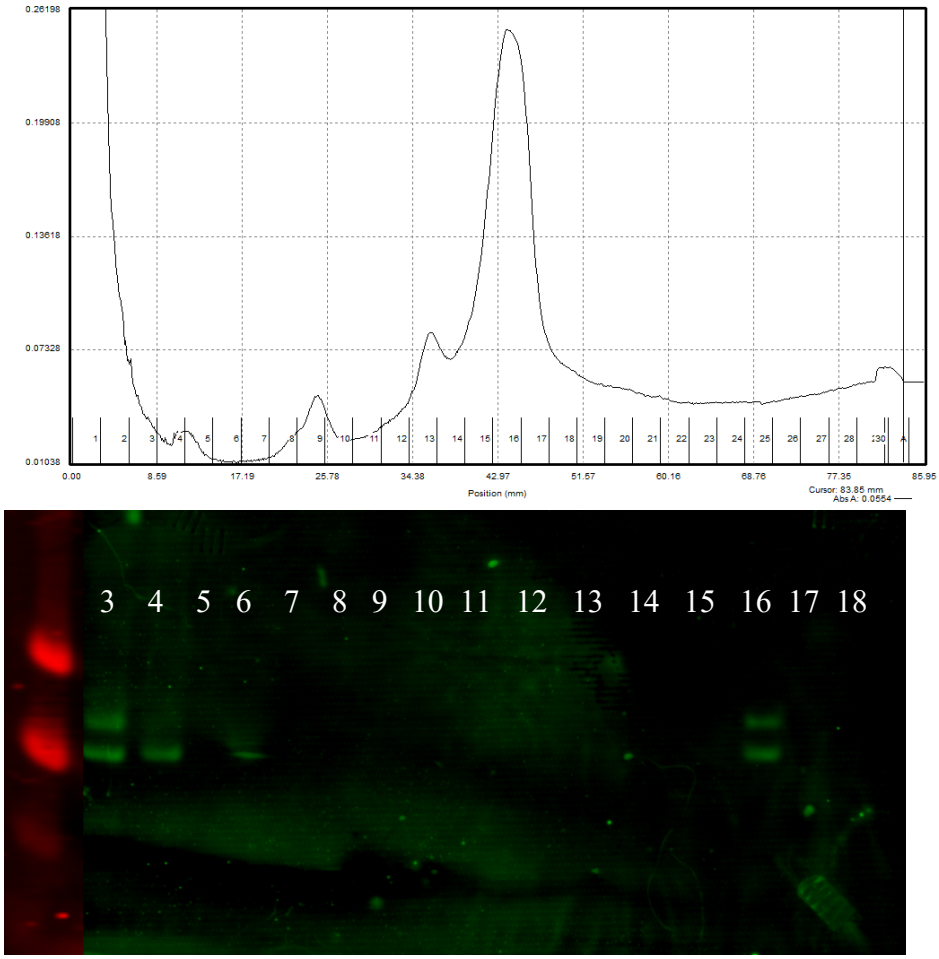


Figure 1. Western blots of samples from sucrose gradient

bS21-2-CBP should be 12.7 kD. Looks like we can see some bS21-2 in first low-density fractions, not associated with ribosomes. Unclear why there is a doublet of reactive bands? Not sure these are really running at the right size (is the ladder accurate?). Note that I think the green in sample 6 is not real / artifact – didn't look like a band, just some smudge.

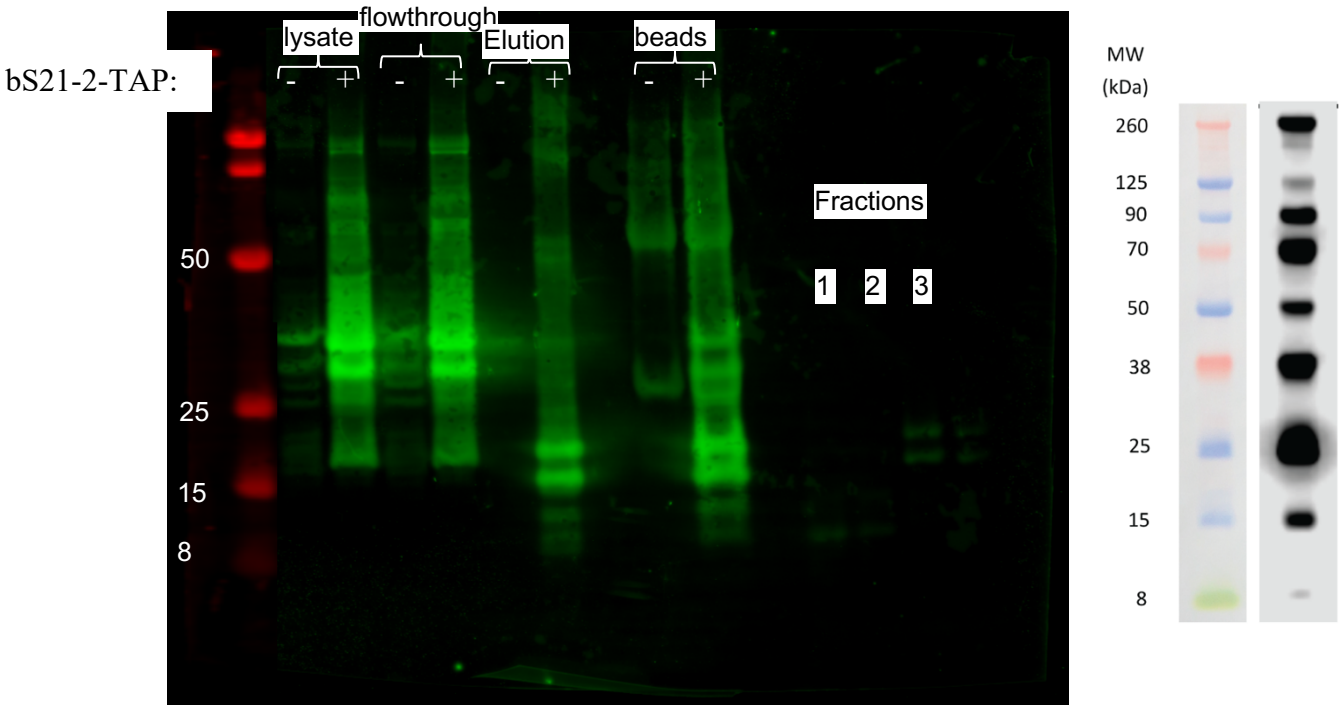


Figure 2. Western blots of samples from TAP purification (and some sucrose gradient)

Again, not sure why bS21-2-TAP and bS21-2-CPB are reacting as a doublet- but clearly they are. I think the reactivity in the "-" lane might be spill-over? There is a fair amount of bS21-2-TAP in the flow-though- failed to capture a fair amount. There is a reasonable amount in the elution, but also a bunch left on the beads. Should consider how to modify to (a) capture more of the tagged protein, (b) get more of it off the beads.

bS21-2-CBP should be 12.7 kD, bS21-2-TAP should be 28.3 kDa. (note TEV protease is 27 kDa)

Also, regarding the sucrose gradient fractions, looks like there is some amount of bS21-2-CPB in in fraction 3 (ran on both gels), but not actually in the first two fractions. I would have thought we'd see it in all three fractions.

Tn-Seq: Linear PCR (2 hours)

1. Assemble the linear PCR reactions on ice

Sample #	Template	Concentration (ng/uL)	Volume to add for 2 ug	0.1x EB to normalize concentration
1	day 0 - Input	1883.2	1.06	18.94
2	LVS gDNA	100	20.00	0.00
3	(-) DNA	-	0.00	20.00

Total Reaction Vol	100
Total # of reactions	3
Factor	3.5

Component	[Stock]	[Final]	1 rxn vol	Master mix	Added
ddiH20			52	182	X
Platinum SuperFi Buffer	5x	1 x	20	70	X
dNTPs	10 mM	0.2 mM	2	7	X
BioSamA	1 uM	0.05 uM	5	17.5	X
DNA		2 ug	20	add individually	X
Platinum SuperFi Polymerase	2 U/uL	0.02 U/uL	1	3.5	x
		Total Vol	**100**		

2. Prepare master mix without DNA, add 80 ul per 1 DNA sample in PCR strip tube, mix.
3. Portion 50 uL into second labeld PCR tube for each reaction, (splitting reactions into 2 x 50 uL)
4. Run on thermocycler using LIN_PCR program: 94°C for 2 min, followed by 50 cycles of 94°C for 15s and 68°C for 1 minute. 4°C hold (~1 hour and 40 minutes total)

While waiting, prep buffers:
"Bind and wash (B&W) buffer (2×) B&W buffer is prepared using 2 M NaCl, 10 mM Tris, and 1 mM EDTA; pH is adjusted to 7.5 with concentrated HCl."

2X B&W buffer

Reagent	[Stock] (M)	[Final] (M)	Volume (mL)
Water			17.64
NaCl	5	2	12
Tris-HCl pH 7.5	1	0.01	0.3
EDTA	0.5	0.001	0.06
Total Volume			30

"B&W buffer (1×) Dilute 2× B&W buffer to 1:1 with dH2O."
"LoTE buffer Combine 3 mM Tris and 0.2 mM EDTA; adjust pH to 7.5 with concentrated HCl"

LoTE buffer

Reagent	[Stock] (M)	[Final] (M)	Volume (mL)
Water			29.898
Tris-HCl pH 7.5	1	0.003	0.09
EDTA	0.5	0.0002	0.012
Total Volume			30

5. Pool the tubes containing the same DNA sample, run them over a QIAquick PCR cleanup column according to the instructions, and elute them in 50 uL of buffer EB.

Tn-Seq: Bind linear PCR products to beads (1 hour)

6. Resuspend beads (Dynabeads™ M-280 Streptavidin) by shaking.
7. Add beads to microfuge tube, 32 uL per sample: 3 samples = 96 uL beads.
8. Place tube on magnetic particle concentrator (MPC) for 1 – 2 min.

9. Remove supernant with pipette
10. Remove tube from MPC and add 1 mL 1x B&W buffer. Gently resuspend by pipetting.
11. Repeat steps 8 – 10 for a total of three washes.
12. Remove final wash and add 52 uL 2x B&W buffer per sample (3 x 52 = 156 uL). Aliquot into PCR strip tube, 50 uL per tube, 1 tube per sample.
13. Add entire volume of purified PCR to appropriate tube with beads
14. Mix at room temperature for 30'. Taped to vortex and kept at lowest setting (pretty vigorous!)
15. Place tubes on MPC for 2'
16. Remove all of supernatant with pipette
17. Remove tubes from MPC and add 100 uL 1x B&W buffer, mix by pipetting
18. Repeat steps 15 – 17 twice but use 100 uL of LoTE buffer.
19. Keep samples bound to beads in 100 uL LoTE buffer overnight, stored at 4°C.

Friday, January 6, 2023

To Do:

1. ~~Second strand synthesis~~
2. MmeI digestion (2.5 hrs)
3. Linker ligation (2.5 hrs)

Results and Data:

Tn-Seq: Second strand synthesis

1. Denature samples by heating in thermocycler: 95°C for 2 minutes, then 4°C.
2. Meanwhile, prepare second strand synthesis master mix:

	uL	x3.5
dH_2O	16	56
10x hexanucleotide mix	2	7
10 mM dNTPs	1	3.5
Klenow (exo-)	1	3.5

3. Collect beads on MPC, discard supernatant, remove tubes from MPC
4. Resuspend beads in 20 uL second strand mix
5. Incubate in a thermocycler at 37°C for 30 minutes. Mix by gently tapping every 10 minutes
6. Add 100 uL LoTE buffer to sample, collect beads on MPC, carefully discard supernatant
7. Repeat step 6.
8. Resuspend beads in 100 uL LoTE buffer.
9. Keep beads at 4°C until next step.

Never ordered the M12 oligos!!! Major limiting factor for next step! ☹ Will have to pause until they arrive.

Monday, January 16, 2023

To Do:

1. ~~Streak out cells for bS21-2-TAP purification~~
2. ~~MmeI digestion (2.5 hrs) (only took ~2 hours!)~~

Results and Data:

Streak out strains with Ben Moore:

KRLVS120 LVS pF
KRLVS249 LVS pF-bS21-2-TAP

Tn-Seq: MmeI digestion

1. Prepare 50 uM double stranded M12 oligos by combining in a PCR tube:

M12_top (100 uM in EB)	15 uL
M12_bottom (100 uM in EB)	15 uL
1 M NaCl	1.5 uL

2. Anneal by running program ANNEALOL: 95°C for 5', cool to 4°C with ramp speed of 0.1°C/second.
3. Store M12 dsDNA in 5 ul aliquots at -20°C.
4. Prepare MmeI buffer mix on ice:

		x 5
dH2O	15.8 uL	84 uL
10 rCutsmart	2 uL	10 uL
M12 dsDNA	0.2 uL	1 uL

*Note that MmeI now comes with rCutsmart buffer instead of Buffer 4 +SAM, so modify protocol accordingly. Also increased volume significantly (x5 instead of x3.5) for ease of pipetting.

5. Collect beads from second strand synthesis on MPC, discard supernatant, remove tubes from MPC
6. Resuspend each sample in 19 uL MmeI buffer mix
7. Add 1 uL MmeI to each sample (lot 10151401 exp 5/24, shipped from NEB on 1/5/23).
8. Incubate in thermocycler at 37°C for 1 hour, gently mixing samples every 10 mins.
9. Add 100 ul LoTE buffer to samples, collect beads on MPC, discard supernatant, remove tubes from MPC.
10. Repeat step 9.
11. Resuspend beads in 100 uL LoTE and store at 4°C.

Tuesday, January 17, 2023

To Do:

1. Seminar at WPI

Wednesday, January 18, 2023

To Do:

1. Harvest cultures with Ben

Results and Data:

Words go here.

Thursday, January 19, 2023

To Do:

1. Linker Ligation (~2.5 hours)

Results and Data:

Tn-Seq: Linker Ligation

1. Prepare stocks of barcoded, double-stranded sequencing adapters by combining in a PCR tube:

LIB_AdaptT_(X) (100 uM in EB)	15 uL
LIB_AdaptB_(X) (100 uM in EB)	15 uL
1 M NaCl	1.5 uL

   Resuspend oligos and prepare primers for indexes A, B, C, and D.
2. Anneal by running program ANNEALOL: 95°C for 5', cool to 4°C with ramp speed of 0.1°C/second.
3. Store adapters in 5 ul aliquots at -20°C.
4. Dilute adapters for use from 50 uM to 1 uM in 1x T4 DNA ligase buffer. For this experiment, only use adapter A. Don't want to pipette less than 1 uL, so combine on ice:

   44 uL of water
   5 uL of 10x T4 DNA ligase buffer
   1 uL of 50 uM adapter

5. Prepare ligation buffer master mix on ice:

Per sample:	x4
14.3 ul water	57.2 uL
1.7 uL of 10x T4 DNA ligase buffer	6.8 uL

6. Collect the beads from MmeI digestion on MPC, discard the supernatant, remove the tubes from the MPC
7. Resuspend the beads in 16 uL of ligation mix
8. Add 3 uL of 1 uM dsDNA sequencing adapter (from step 4) to sample (typically, a different adapter for each sample to be sequenced! Here there is only one sample and two controls, so only use one adapter). Record here which barcode is used for which sample- all barcode A.
9. Add 1 ul T4 DNA ligase (NEB M0202T, 2,000,000 U/mL) to each sample.
10. Incubate at 16°C in thermocycler for 1 hour. Gently mix every 10 minutes.
11. Add 100 ul LoTE buffer to samples, collect beads on MPC, discard supernatant, remove tubes from MPC.
12. Repeat step 11.
13. Resuspend beads in 100 uL LoTE and store at 4°C.

Tuesday, January 24, 2023

To Do:

1. PCR and final purification (4 hrs)

Results and Data:

Tn-Seq: PCR and final purification

1. Assemble PCR mix on ice

Total Reaction Vol	50
Total # of reactions	3
Factor	3.5

Component	[Stock]	[Final]	1 rxn vol	Master mix
ddiH20			23.5	82.25
Platinum SuperFi Buffer	5x	2 x	20	70
dNTPs	10 mM	0.2 mM	2	7
LIB-PCR5	5 uM	0.2 uM	2	7
LIB-PCR3	5 uM	0.2 uM	2	7
Platinum SuperFi Polymerase	2 U/uL	0.02 U/uL	0.5	1.75
		Total Vol	**50**	

2. Collect the beads from Linker ligation on MPC, discard the supernatant, remove the tubes from the MPC
3. Resuspend the beads in 50 uL of the PCR mix.
4. Place tubes in thermocycler and run program:
    1. 94°C, 2:00
    2. 94°C, 0:15
    3. 60°C, 1:00f
    4. 68°C, 2:00
    5. Goto step 2, 17 times
    6. 68°C, 4:00
5. While PCR is running, prepare 2% agarose gel (2.4 g agarose and 120 mL fresh TAE). Pour a large gel using the wide side of the widest combs available (12-well comb currently).
6. When PCR is done, collect the beads on the MPC and transfer PCR product to a new tube.
7. Add 10 uL of 6x orange-G dye to each 50 uL volume (final volume 60 uL).
8. Load gel:
    a. Ladder (plus some 5x blue dye from Qiagen kits [contains Bromophenol blue])
    b. Empty
    c. Sample 1
    d. Empty
    e. Sample 2

f. Empty
g. Sample 3
h. Empty

9. Run gel at 200 V for 30 minutes
10. Using the blue light screen, visualize bands and take image.
11. Using a clean blade for each band, excise bands at ~130 bp. Minimize the amount of agarose per band (Band today was 290 mg). Image gel post-excision.

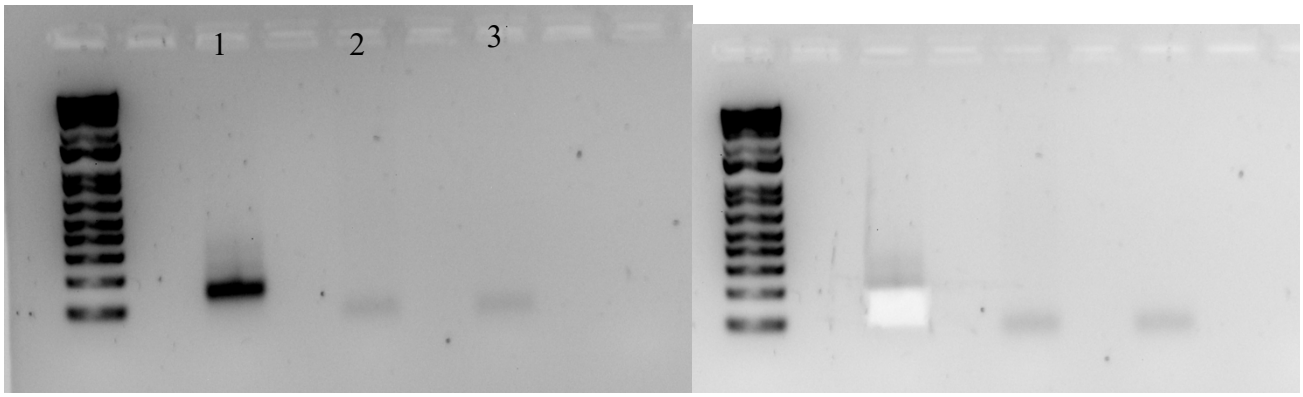


Figure 3. Gel of final Tn-Seq PCR, pre and post-band excision
Sample 1 is the Tn library input sample. Sample 2 is just LVS gDNA without a Tn, and sample 3 is no template. Looks great- we can see a clear band around 129 bp in sample 1, but basically primer dimers in the other lanes.

12. Purify bands using QIAquick gel purification column. Elute in 40 uL of 0.1x EB.

Run at 120 V for 50 min. Yellow dye and blue dye are visible at bottom of gel.
Use SilverQuest kit to stain. **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 supernatants 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.

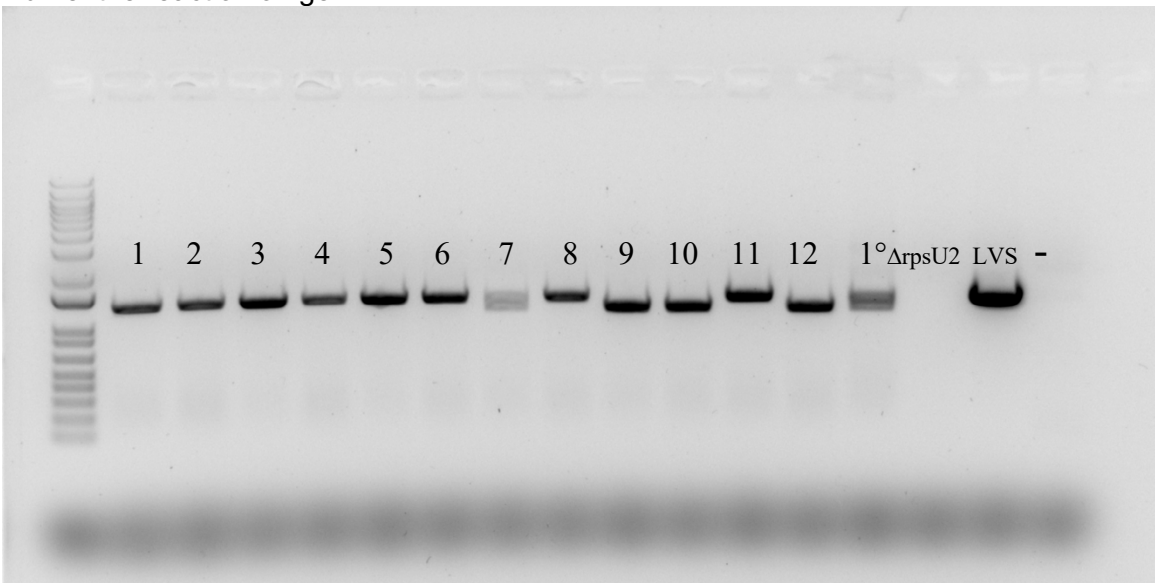


Figure 1. Gel of PCR to identify ΔrpsU2 cells – early view

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.

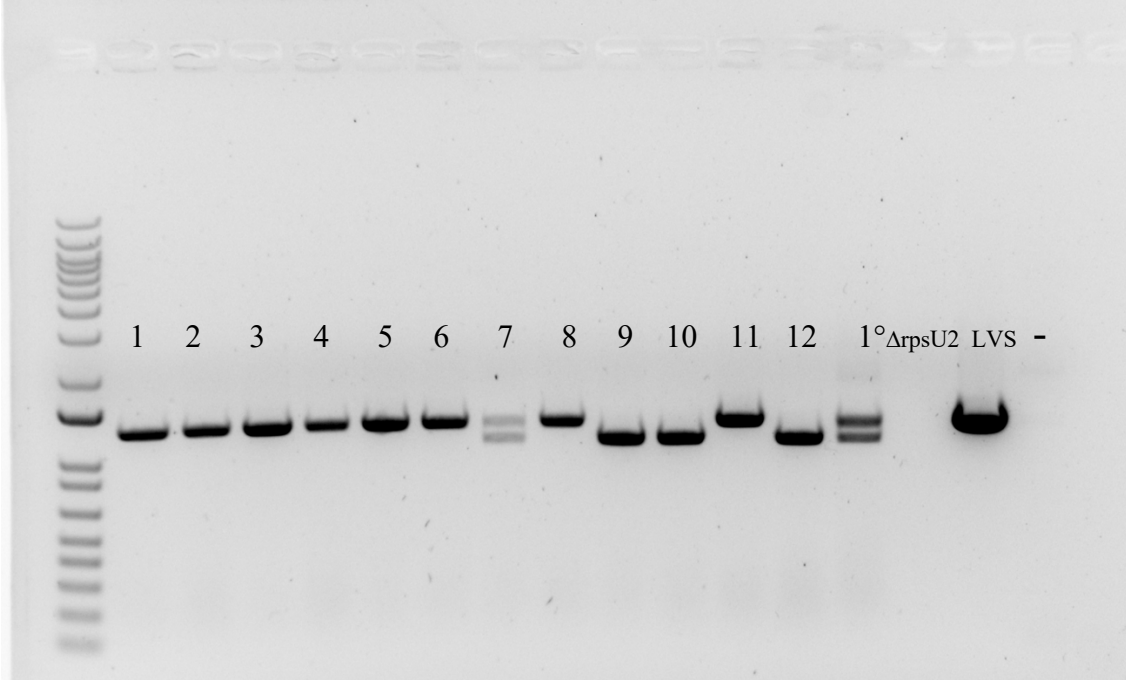


Figure 2. Gel of PCR to identify ΔrpsU2 cells – later view
Better resolution, very clear that 9, 10, and 12 seem to be deletion mutants while 7 is a mixed population (as is the 1° integrant sample).

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

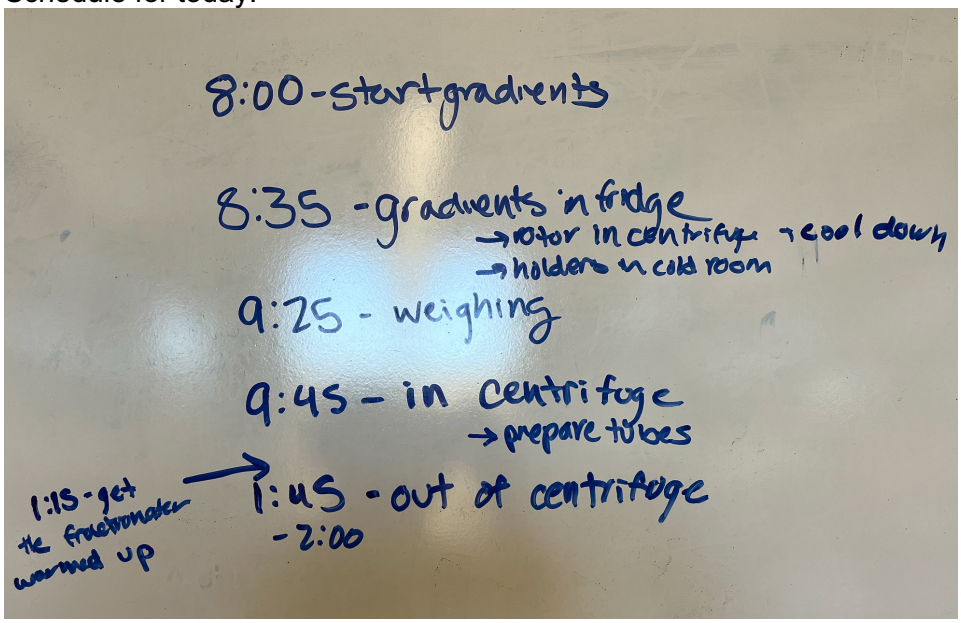
To Do:

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.

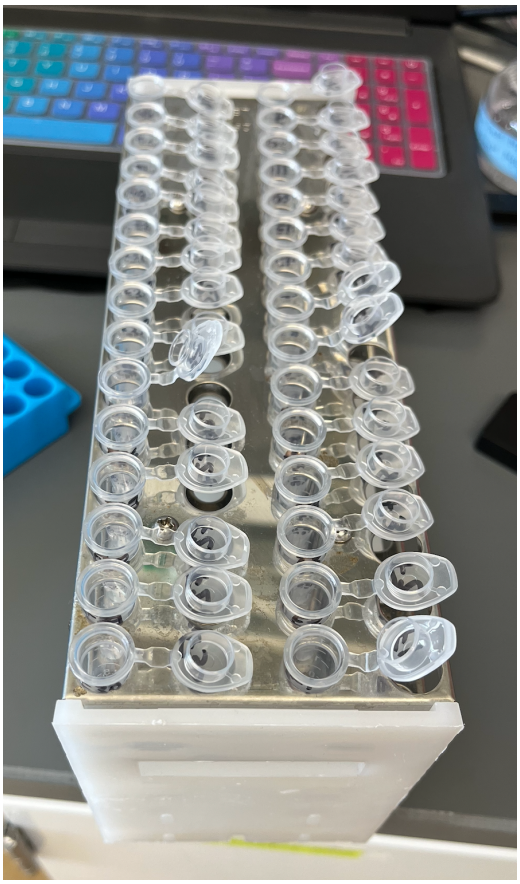
Sample	Contents
1	LVS pF TAP purification (mock)
2	LVS pKR72 TAP purification (IP of bS21-2-TAP)

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.

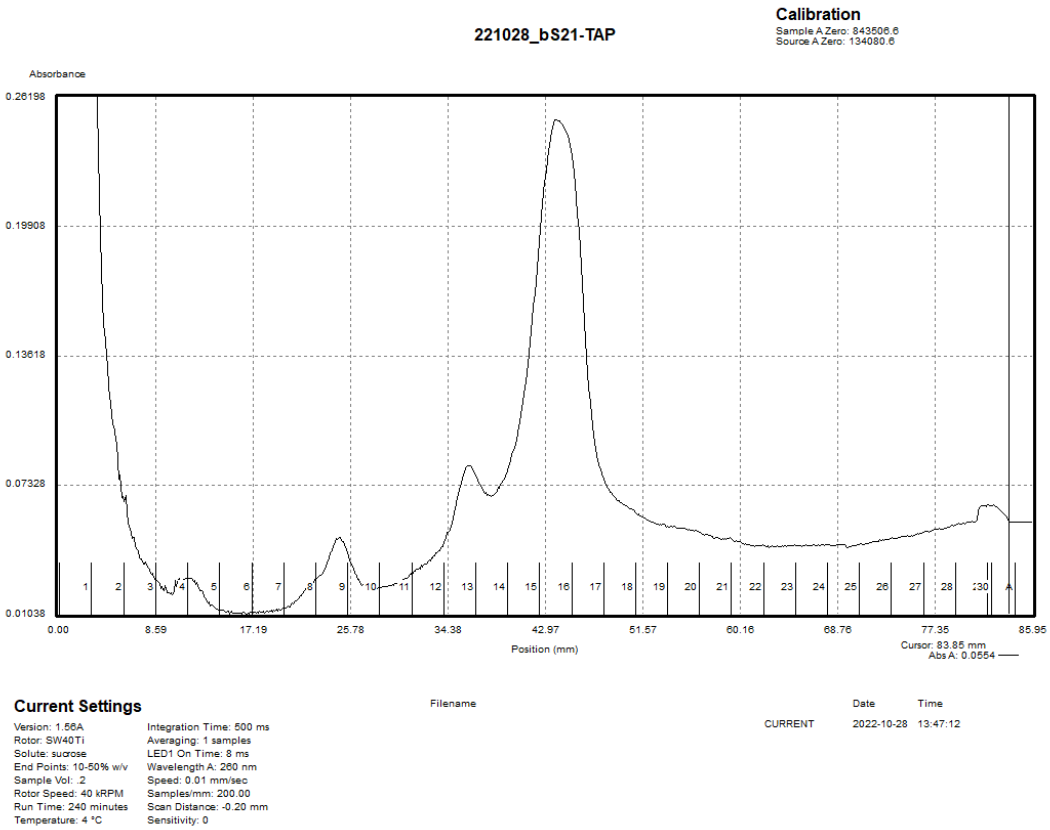


Figure 3. Gradient of bS21-TAP purification sample

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

Wednesday, November 1, 2022

To Do:

2. Run protein gel of ribosome purifications
3. Run protein gel for WB of ribosome purifications

Results and Data:

Words

Gel to assess modified TAP purification. Use the remainder of the E2A purification from 10/22/20. Combine elutions 1:1 with 2x SLB.

4-12% MES, 15 well

Lane	Volume	Contents
1	10 uL	Benchmark ladder 1:10
2	10 uL	E2- LVS pF
3	10 uL	E1- LVS pF-rpsU2-TAP
4	10 uL	Ribosomes diluted 1:10
5	10 uL	Benchmark ladder 1:10
6	5 uL	E2- LVS pF
7	5 uL	E1- LVS pF-rpsU2-TAP
8	10 uL	Ribosomes diluted 1:100
9	5 uL	E2A: LVS pF-rpsU-TAP (10/22/20 purification)
10	5 uL	Ribosomes diluted 1:10

Ribosome from 3/22/2019 (26.7 ug/ul): For today, dilute 2 ul into 23 ul 1xSLB (~2.1 ug/ul) and dilute further as indicated.

Run at 120 V for 50 min. Yellow dye and blue dye are visible at bottom of gel.
Use SilverQuest kit to stain.

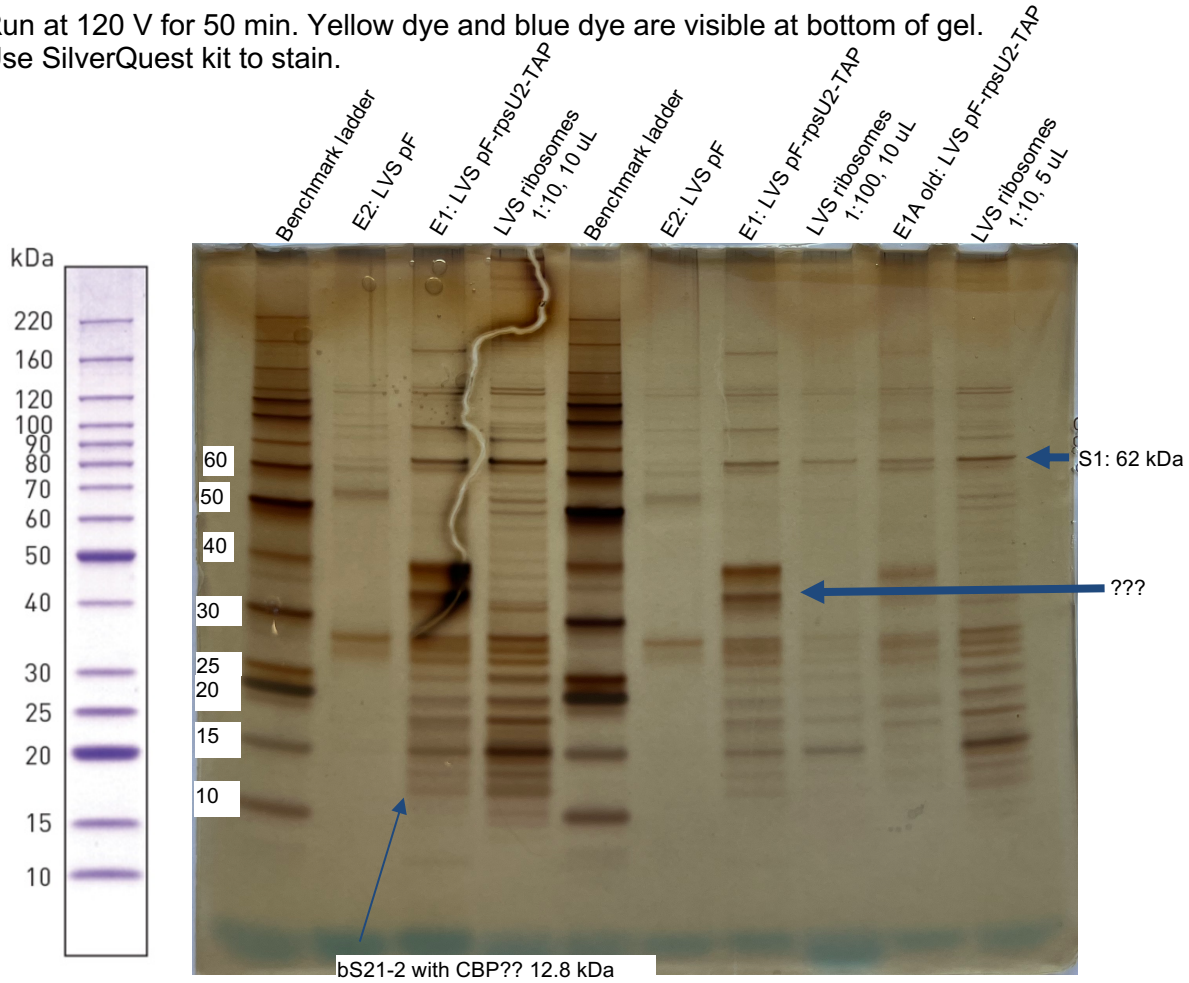


Figure 8. bS21-2-TAP purification from 9/2/22.

bS21-2 with the CBP moiety should be 12.76 kDa. Can see S1. There seem to be some lower bands missing- is this only the 30S and not the 50S? And what is between 30kDa and 40kDa? Not something from the purification, because it's not appearing in the LVS pF control.

Looks pretty good, but I should send to mass spec to see what is actually there. Where to send?

These are the ribosomal proteins purified from our previous mass spec, in ascending size order:

Molecular Weight (kDa)	Gene Locus	Gene Name	Protein
5	FTL_0175	*rpmH*	50S ribosomal protein L34
6	FTL_0521	*rpmG*	50S ribosomal protein L33
7	FTL_1143	*rpmF*	50S ribosomal protein L32
7	FTL_0254	*rpmD*	50S ribosomal protein L30
7	FTL_1405	*rpmI*	50S ribosomal protein L35
8	FTL_0244	-	50S ribosomal protein L29
8	FTL_1025	*rpsR*	30S ribosomal protein S18
8	FTL_1303	*rpmE*	50S ribosomal protein L31
8	FTL_1047	*rpsU2*	30S ribosomal protein S21-2
8	FTL_0456 / FTL_1360	*rpsU1 / rpsU3*	30S ribosomal protein S21
9	FTL_0522	*rpmB*	50S ribosomal protein L28
9	FTL_1738	*rpsP*	30S ribosomal protein S16
9	FTL_1452	*rpmA*	50S ribosomal protein L27
10	FTL_1538	*rpsO*	30S ribosomal protein S15
10	FTL_0240	*rpsS*	30S ribosomal protein S19
10	FTL_0245	*rpsQ*	30S ribosomal protein S17

10	FTL_0070	*rpsT*	30S ribosomal protein S20
11	FTL_0247	*rplX*	50S ribosomal protein L24
11	FTL_0950	-	50S ribosomal protein L25
11	FTL_0238	*rplW*	50S ribosomal protein L23
12	FTL_0249	*rpsN*	30S ribosomal protein S14
12	FTL_0235	*rpsJ*	30S ribosomal protein S10
12	FTL_0241	*rplV*	50S ribosomal protein L22
12	FTL_1453	*rplU*	50S ribosomal protein L21
13	FTL_1745	*rplL*	50S ribosomal protein L7/L12
13	FTL_0246	*rplN*	50S ribosomal protein L14
13	FTL_0252	*rplR*	50S ribosomal protein L18
13	FTL_1735	*rplS*	50S ribosomal protein L19
13	FTL_0258	*rpsM*	30S ribosomal protein S13
13	FTL_1404	*rplT*	50S ribosomal protein L20
13	FTL_1024	*rpsF*	30S ribosomal protein S6
14	FTL_0250	*rpsH*	30S ribosomal protein S8
14	FTL_0259	-	30S ribosomal protein S11
14	FTL_0232	*rpsL*	30S ribosomal protein S12
15	FTL_1186	*rpsI*	30S ribosomal protein S9
15	FTL_1748	*rplK*	50S ribosomal protein L11
15	FTL_0255	*rplO*	50S ribosomal protein L15
16	FTL_1026	*rplI*	50S ribosomal protein L9
16	FTL_1187	*rplM*	50S ribosomal protein L13
16	FTL_0243	*rplP*	50S ribosomal protein L16
17	FTL_0262	*rplQ*	50S ribosomal protein L17
18	FTL_0253	*rpsE*	30S ribosomal protein S5
18	FTL_0233	*rpsG*	30S ribosomal protein S7
19	FTL_1746	*rplJ*	50S ribosomal protein L10
19	FTL_0251	*rplF*	50S ribosomal protein L6
20	FTL_0248	*rplE*	50S ribosomal protein L5
22	FTL_0236	*rplC*	50S ribosomal protein L3
23	FTL_0260	*rpsD*	30S ribosomal protein S4
23	FTL_0237	*rplD*	50S ribosomal protein L4
25	FTL_0242	*rpsC*	30S ribosomal protein S3
25	FTL_1747	*rplA*	50S ribosomal protein L1
26	FTL_0224	*rpsB*	30S ribosomal protein S2
30	FTL_0239	*rplB*	50S ribosomal protein L2
62	FTL_1912	*rpsA*	30S ribosomal protein S1

Patch out LVS pF, LVS pKR153, LVS pKR154, four patches each (A-D) and put at 37°C O/N.
Electroporation plates look great:

EP	Cells	Plasmid	20 uL plate	200 ul plate	remaining plate
1	LVS	pKR153	22	>100	-
2	LVS	pKR154	17	>100	-
3	LVS	pF	~80	>100	-
4	LVS	-		~4 pinprick-sized	~50? Pinprick sized

Good reminder that Kan selection isn't perfect- LVS will start growing on kan plates if left long enough- not genetically resistant. Can see some pinprick-sized colonies on the transformations with plasmid DNA, probably background just like on negative control.

Patch out LVS pF, LVS pKR153, LVS pKR154, four patches each (A-D) and put at 37°C O/N.

Tueday, 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

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

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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