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September 2021

Lab goals for July and future

Paper: PmrA / PriM
Re-do macrophage assay with ∆pmrA strains
Isolate gDNA from ∆pmrA experiment in (April?) and check by qPCR

Paper: ∆rpsU2
Re-do macrophage assay with ∆rpsU2 and complements

Plan out future grants. New Investigator? Beckman? R21?

Wednesday, September 8, 2021

To Do:

1. Pour plates for sucrose selection for inducible triple mutant (working with Sierra)
2. Streak out cells for sucrose selection

Results and Data:

For 300 mL CHA:
- 6 g Beef Heart Infusion
- 6 g Protease Peptone
- 6 g Glucose
- 0.6 g L-Cystine
- 3 g salt
- 9 g Agar

CHAH plates with 10% sucrose
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.
Autoclave 30’, being EXTREMELY careful media does not boil over
While autoclaving, warm 250 mL of 2% hemoglobin and **sterilized 50% sucrose (**in 50°C oven or in water bath at 55°C)
Place CHA flask in 50°C oven or in water bath at 55°C, let temperature equilibrate
Wipe down flask and bottles with ethanol and using sterile technique, 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 (~20 plates for 500 mL media)

Using cells lacking ΔrpsU1ΔrpsU3 containing pKR113 (natR with ATc-inducible rpsU2-V), 1° integrants with pKR11

HT primary integrants
-80 freezer row 7C
5 most recent samples (back across)
ΔrpsU1ΔrpsU3 pKR113 pKR11 labeled E1-6, E4-1, E4-2, E4-3

Work with E1-6, E4-2
Streak out on plates with nat (5 ug/ml), ATc (100 ng/mL), Kan (5 ug/mL)

500 mL sucrose plates

Final conc	Stock	Volume to add
5 ug/mL nat	100 mg/mL	
100 ng/mL ATc	500 ug/mL	

Thursday, September 9, 2021

To Do:

1. Plate out cells for on sucrose plates for sucrose selection

Results and Data:

E4-2 didn't grow overnight, so move forward with E1-6 only on sucrose, ATc, nat. Sierra diluted and plated 10^{-2} – 10^{-7} (with 10^{-7} on ATc nat only).

Monday, September 13, 2021

To Do:

1. Check in with Steve about MALDI reagents

Results and Data:

Can't find calibration proteins, but do have the TFA, acetonitrile, matrix, and plate to attempt MALDI on ribosomes tomorrow!

Tuesday, September 14, 2021

To Do:

1. Talk to Al about MALDI sample prep
2. Prep samples
3. Instrument training

Results and Data:

From protocol Steve sent:

2.2. Mass spectrometry

1. Prepare 10 mg/ml sinapinic acid matrix solution by dissolving 3.0 mg of the solid in 200 ul of 0.1% TFA plus 100 ul of acetonitrile. (In order to save matrix and solvent)
2. Acidify ribosomes by adding 1 ul of 10% aqueous TFA to 9 ul of ribosome solution.
3. Prepare calibration protein solutions by dissolving separately 1 mg of each protein in 1 ml of distilled, deionized water.
4. Mix 1 ul of TFA-treated ribosome solution with 9 ul of the matrix solution.
5. Prepare the calibration protein-matrix solution by adding 1 ul of each calibration protein solution to 9 ul of matrix solution.
6. Apply 1 ul of the acidified ribosome-matrix solution to the sample probe and allow to air dry.
7. Apply 1 ul of the calibration protein-matrix solution to the sample probe near the ribosome samples and allow to air dry.
8. Acquire MALDI mass spectra, externally calibrate spectra, and measure masses of observed peaks.
9. (Optional) Improved mass calibration may be achieved by using several observed peaks and their known sequence masses to internally calibrate the mass spectrum.
10. Compare masses measured by mass spectrometry to the masses calculated from the amino acid sequences of known proteins.

Reference

Spedding, G. "Ribosomes and Protein Synthesis: A Practical Approach", *Oxford University Press*, pp.1-29 (1990).

Plan:

Make up 100 uL of **10% TFA:**
10 ul TFA added to 90 uL water (do what you oughter, add acid to water!).

Make 250 uL of **0.1% TFA**:
25 uL 10% TFA added to 225 uL water.

Weigh out ~~3~~ mg of ~~sinapinic~~ acid matrix into 1.5 mL tube.
We have sinapic acid (Sigma 85429-5G, lot BCBW5428)- use this. Difficult to weigh out accurately- managed to get 9 mg in a microfuge tube, so move forward with that amount.
~~Add by pipetting: 200 ul of 0.1% TFA, 100 ul of acetonitrile~~
Add by pipetting: 600 ul of 0.1% TFA, 300 ul of acetonitrile

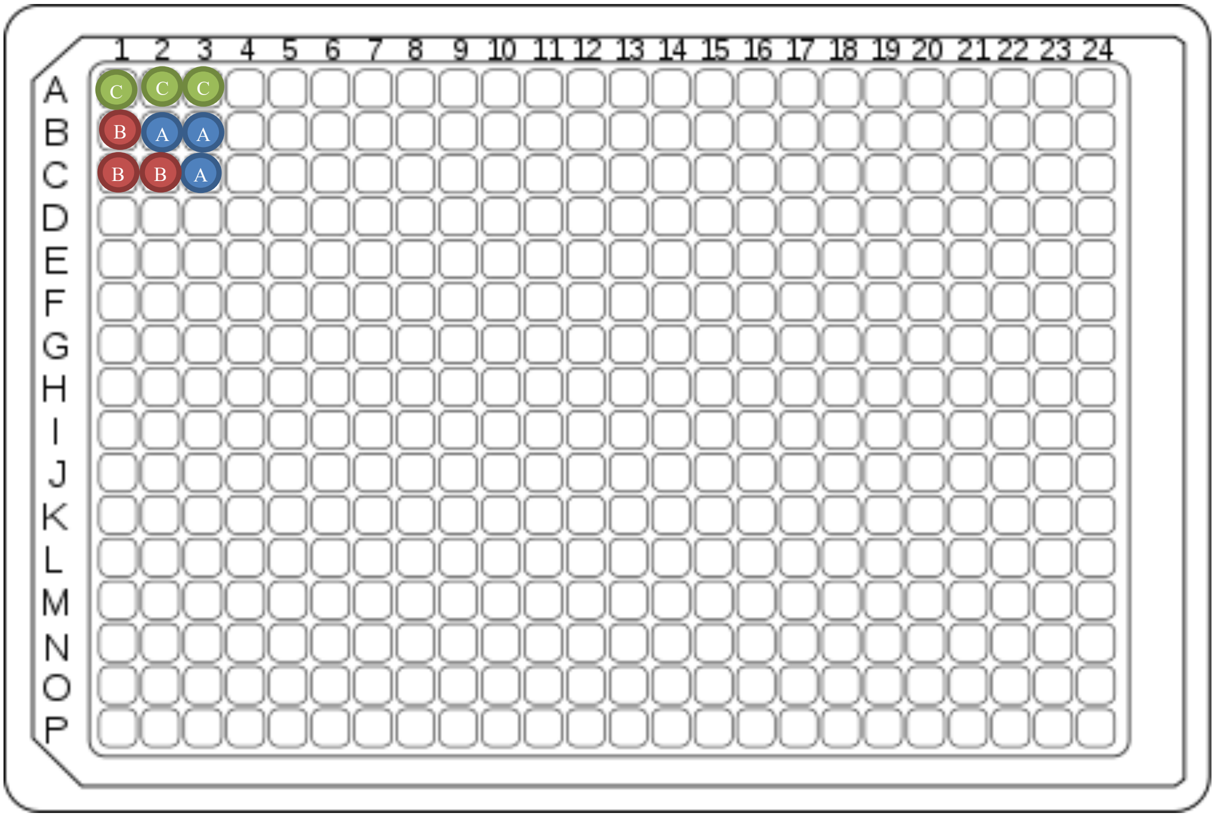
Mix by taping to vortex and leaving (5-10 mins?) while prepping ribosome samples.

In separate tube, prepare ribosomes.
Have WT ribosomes

Genotype	Date	Concentration (pmol/ul)	ug/ul	A260	OD at Harvest	High salt wash?	Brij?	BME in buffers?
WT	11/13/20	6.799	18.359	266.2	0.359	No	No	No

According to Suh et al., 2005, "One microliter of sample prepared by mixing 1 uL of acidified ribosomal solution (around 2pmol ribosome) with 9uL of matrix was loaded into a MALDI target and allowed to air dry."
So need a final concentration of 2 pmol/ul of acidified ribosomes.
(10 ul)(2 pmol)/(6.8 pmol) = 2.9 uL ribosomes (make up volume in buffer or water?)

Ribosomes	2.9 uL
Buffer (water?)	6.1 uL. $H^{10}M^{10}A^{50}$ (buffer) – did not use any buffer.
10% TFA	1.0 uL
Final	10 uL



Start with

A. 6.12 pmol/ul ribosomes added to matrix
B. 2.04 pmol/ul ribosomes added to matrix
C. No ribosomes added to matrix

A 9 ul ribosomes (6.8 pmol/ul) + 1 ul 10% TFA, add 1 ul to 9 ul matrix, make 3 spots
B Dilute ribosomes by 2/3- mix 3 ul (9 ul ribosomes + 1 ul 10% TFA) with 6 ul of 0.1% TFA, add 1 ul to matrix, make 3 spots
C Add 1 ul 0.1% TFA to 9 ul matrix, make 3 spots

Air-drying at 9:30am
Dry enough for MALDI at 10am!

Tried a variety of laser powers (50-90)- did not detect any ions.
Clean plate by flushing with methanol, wipe with Kimwipe, let air dry. Looks clean!

Future To-Do

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