Purification of Native $tRNA^{phe}$ from Overexpression (Byron Hetrick)

Day 1

- Transform DH5α cells with pBsPhe plasmid

  2x YT Plates (add 50mg L^{-1} Ampicillin):

2xYT Media	6.2g
Agar	3g
Water	200mL

  - Thaw competent cells on ice.
  - Add 1µL miniprepped (or megaprepped) DNA
    - pBsPhe is a low copy number plasmid so, if you do a miniprep or megaprep of this plasmid, follow the low copy number protocol.
  - Incubate on ice 30min.
  - Heat shock 42°C 90 seconds
  - Add 1mL 2xYT (no antibiotics)
  - Incubate 37°C 1hour (no shaking)
  - Plate 50 and 200µL on 2x YT-Amp (50mg L^{-1}) plates
  - Incubate 37°C overnight

Day 2

- Inoculate 4x 500mL 2x YT-Amp (50mg L^{-1} → 1.25mL of Amp stock) with a single colony from O/N plates)
- Incubate 37°C shaking 20hours

Day 3

- Centrifuge cultures 5,000xg 20min. 4°C
- Resuspend pellets in 8.5mL buffer A per 1L culture

  Lysis Buffer:

1mM Tris-Cl pH 7.5	(1M)	20µL
10mM MgoAc	(1M)	200 µL
Final Volume		20mL

- Transfer to 2 oak ridge tubes
- Add 10mL citrate buffered phenol to each (water saturated phenol also works)
- Vortex 30sec 2x
- Centrifuge 12,000RPM 20°C 1hour in Beckman JA17 rotor
- Transfer aqueous phase to new tubes
- Ethanol precipitate with 2.5 vol. ice cold ethanol 30 min. on ice
- Centrifuge 15,000RPM 4°C 30min. in Beckman JA17 rotor
- Air dry pellet
- Resuspend pellet in 4mL 1M NaCl each (will be a milky but lump-free suspension)
- Centrifuge 10,000RPM 30min. 4°C in Beckman JA17 rotor
- Collect supernatant
- Ethanol precipitate with 2.5 vol. ice cold ethanol 30min. on ice
- Centrifuge 15,000RPM 4°C 30min. in Beckman JA17 rotor
- Air dry pellet
- Resuspend in 4mL 1M Tris-Cl pH 8.0
- Incubate 37°C 2hours
- Ethanol precipitate with 2.5 vol. ice cold ethanol overnight at -20°C

Day 4

- Centrifuge ethanol precipitate 15,000RPM 30min. 4°C in Beckman JA17 rotor
- Air dry pellet
- Resuspend pellet in 8mL Buffer B ea.

Phenyl Column Loading Buffer:

20mM NaoAc pH 5.2	(3M)	53.3µL
10mM MgoAc	(1M)	80.0µL
2M $(NH_4)_2SO_4$	(3M)	5.3mL
Water		2.6mL
		8.0mL

- Filter sample with syringe filter
- Purify on linear FPLC gradient
  - Program: sean phe 725
  - Use 10mL loop
  - Change "empty loop volume" to 15mL
  - Hitrap 5mL phenyl column
  - Store column in 20% ethanol

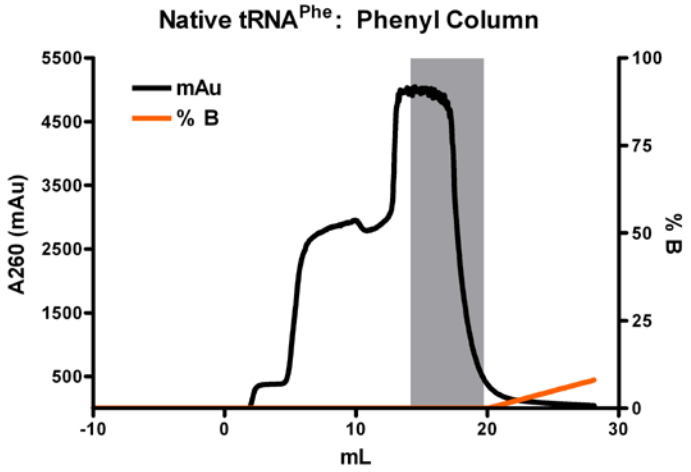
Phenyl Column Buffer A:

20mM NaoAc pH 5.2	(3M)	6.7mL
10mM $MgCl_2$	(1M)	10mL
1.5M $(NH_4)_2SO_4$	(3M)	500mL
Water		483.3mL
		1000mL

Phenyl Column Buffer B:

20mM NaoAc pH 5.2	(3M)	6.7mL
10mM $MgCl_2$	(1M)	10mL
Water		983.3mL
		1000mL

- Pool samples corresponding to shaded portion of profile below.
- Store at 4°C overnight



FPLC profile of tRNA^{phe} eluting from phenyl column. This profile is HIGHLY variable but you get the idea.

Day 5

- Add 2x vol. Q Column Buffer A to sample

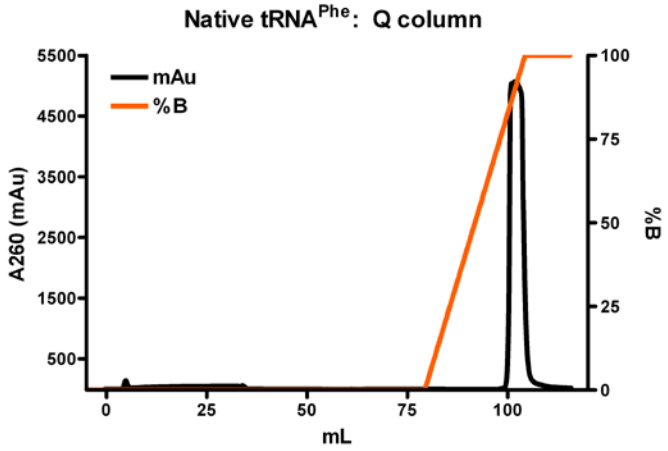
Q Column Buffer A:

50mM NaoAc pH 5.2	(3M)	16.7mL
Water		983.3mL
		1000mL

Q Column Buffer B:

50mM NaoAc pH 5.2	(3M)	16.7mL
1M NaCl	(5M)	200mL
Water		783.3mL
		1000mL

- Load column with super loop
  - Program: "Byron Phe Desalt"
  - Change "empty loop volume" to 1mL < sample volume
- Pool fractions and ethanol precipitate with 2.5 vol. -20°C overnight



Day 6

- Aliquiot overnight ethanol precipitation into 1.5mL eppendorf tubes
- Centrifuge 30min 4°C at max in microcentrifuge
- Resuspend in ~400µL water (total volume) (this can vary)
- Purify 100µL at a time on C18 µ-bonda pak HPLC column

C18 Column Buffer A:

20mM Tris-Ac pH 5.2	(2M)	10mL
400mM NaCl	(5M)	80mL
10mM MgoAc	(1M)	10mL
Water		900mL
		1000mL

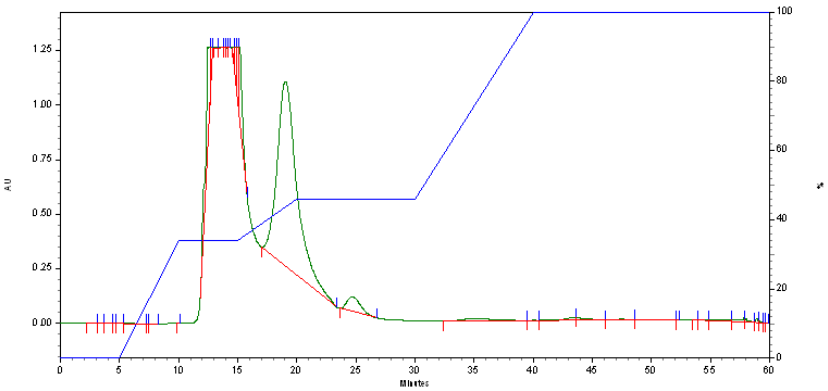
C18 Column Buffer B:

20mM Tris-Ac pH 5.2	(2M)	10mL
400mM NaCl	(5M)	80mL

10mM MgoAc	(1M)	10mL
60% Methanol		600mL
Water		300mL
		1000mL

HPLC Gradient:

% B	Time (min.)
0	5
0 →34	5
34	10
34 →46	5
46	10



- Collect fractions and ethanol precipitate with 2.5 vol ethanol (4^{o}C 30min or -20^{o}C overnight)

Day 7

- Aliquot into 1.5mL eppendorf tubes (there will be a ton of these, probably more than 100 total)
- Centrifuge 30min 4^{o}C at max in microcentrifuge
- Dry pellets
- Resuspend in ~200uL water