Protocol for preparing *E. coli* samples for Mass Spectrometry

Materials and Supplies

- OP50 media – ~1200ml (1L sol'n – 5g Tryptone, 2.5g Yeast Extract)
- LB plate (1)
- Autoclaved Test Tubes (2-4)
- OD_{600nm} Spectrometer
- Autoclaved 250ml Beckmann centrifuge bottles (6)
- Centrifuge with rotor that holds 250ml bottles
- 37°C incubator
- Roller or shaker that holds test tubes
- Shaker for 1L Erlenmeyer flasks
- Ice buckets w/ ice (2)
- French Press with refrigerated cell (cleaned before use)
- Autoclaved 1.5ml Eppies
- Sterile 15ml Falcon conical tubes
- Microcentrifuge
- Disposable 25ml pipets
- Bio-Rad RC DC Protein Assay Kit (Cat# 500-0122)
- 0.2 % *Rapi*Gest SF Powder in 50mM Ammonium Bicarbonate pH 7.8 (Waters Corporation, 5 Pack of 1 ml Vials, 186001860)
- 50mM Ammonium Bicarbonate buffer pH 7.8
- 500mM DTT
- 500mM IAA (Iodoacetamide – light sensitive)
- 500mM HCl
- 250 ng/µl Trypsin, modified, sequencing grade in 0.01% Acetic Acid (Roche, 4 x 25 µg, Cat # 1418033)
- 100 mM $CaCl_2$

Sample Preparation

DAY 1 – Plate growth

- Streak plate of TJ2 strain of OP50 E. coli located in freezer in J256.
- Use LB Agar Plate found in J180C 4°C room.
- Grow up colonies overnight at 37°C (K231).

DAY2 – Culture Growth part 1

- Inoculate 1-3 test tube(s) with 5 ml OP50 media with single colonies from plate. I grow up several cultures in case some don't grow.
- Grow overnight (8-12 hrs) at 37°C on roller or shaker (K231 or K310 hallway).

DAY3 – Culture Growth part 2

- Remove 10 ml from 1L of OP50 media to use as a zero for spectrophotometer and add 10ml overnight cultures to the liter of media for a 1:100 dilution.
- Grow bacteria at 37°C on shaker for ~5 hours or until OD_{600nm} reading on the spectrophotometer(in J180) is 0.5.
- Pour 200ml of bacteria into 5 x 250ml Beckmann centrifuge bottles and use the extra bottle as a balance with 200ml water.
- Chill on ice for 5 minutes (I usually chill the centrifuge bottles ahead of time as well).
- Spin down samples at 3000rpm for 5 minutes at 4°C using Beckman centrifuge in either J180 or J160.
- Remove supernatant form pellets.
- Add 2ml of cold 50mM Ammonium Bicarbonate pH 7.8 to each pellet. Making sure to fully resuspend the pellets in the buffer.
- Combine all resuspended pellets into a sterile 15ml Falcon conical tube.
- Freeze sample at -20°C until ready to lyse sample.

DAY4 – Lysis

- Lyse 10 ml sample with French Press (J432) using Klevit lab cell in K464 (see separate protocol for French Press lysis technique)
- Spin sample at 4000rpm for 30 minutes at 4°C using Waterston lab centrifuge in K313 hall.
- Remove supernatant from pelleted debris.
- Spin sample at 14K for 10 minutes to separate insoluble fraction from soluble.
- Use Bio-Rad Protein Assay Kit to quantitate protein (see separate protocol). Spec is in Fangman/Brewer lab J135.

Sample Digestion

- Make 0.2% *Rapi*Gest diluted in 50 mM Ammonium Bicarbonate pH 7.8 (1 mg *Rapi*Gest per 500 µl 50mM Ammonium Bicarbonate pH 7.8).
- Using a locking lid microcentrifuge tube, add 100 µl 0.2% *Rapi*Gest per 100 µl protein mixture(1:1) -[final concentration of *Rapi*Gest should be 0.1 % (w/v)]. If protein is in pellet form add 25-50 µl of 0.1% *Rapi*Gest. Vortex the sample.
- Boil sample at 99°C for ~2 minutes, then let cool for a couple minutes.
- Quick spin the sample and add DTT to a final concentration of 5mM.
- Incubate sample at 60°C for 30 minutes.
- Cool the sample to room temperature.
- Add IAA to a final concentration of 15mM.
- Place sample **IN DARK** at room temperature for 30 minutes.
- Add CaCl2 to a final concentration of 1mM.
- Then add Trypsin for a final concentration of 1:100 enzyme:protein.

- Incubate overnight with shaking at 37°C .
- Prior to mass spectrometry run, add HCl to a final concentration of 50mM.
- Incubate at 37°C for 45 minutes.
- Spin sample at 14K, 4°C for 10 minutes.
- A cloudy pellet should appear. Separate your supernatant form the pellet into a fresh Eppendorf tube.