Bioplatforms Australia Antibiotic Resistant Sepsis Pathogens Initiative
Bacterial prep for *Klebsiella pneumoniae* (Jonathan Wilksch)

Day 1

- Streak isolates from glycerol stock to Luria agar plates
- Incubate at 37°C overnight

Day 2

- Pick single colony from Luria agar plate and inoculate into a 500 ml flask containing 60 ml Luria broth.
- Grow shaking (200 rpm) at 37°C overnight

Day 3

- Thaw pooled human sera
- Pipet out 25 ml of overnight culture into falcon tube
- Pellet bacterial cells by spinning at 14,000 x g at 4°C for 15 min and remove supernatant with a serological pipette (do not pour off supernatant because the cell pellet is soft and will be lost).
    - Sera - Resuspend pellet in 25 ml and incubate at 37°C shaking (200 rpm) for 2 hrs
    - RPMI - Resuspend pellet in 25 ml and incubate at 37°C shaking (200 rpm) for 1 hr

- Transfer culture into tubes for the following protocols at their respective volume
    - RNA transcriptomics – 1 ml (10 ml tube)
    - Metabolomics – 10 ml (50 ml tube)
    - Proteomics – 10 ml (50 ml tube)

- Follow protocol
    - Isolation of RNA for transcriptomics
    - Bacterial harvest for metabolomics (see page 2)
    - Proteomic bacterial cell pellet washing protocol (see below)

Proteomic bacterial cell pellet washing protocol

1. Pellet cultures by centrifugation (14,000 *g* at 4°C for 15 m)
2. Remove culture supernatants and resuspend cell pellets by vortexing in 1mL ice-cold 1× PBS
3. Repeat centrifuge/wash steps twice more before removing the supernatant and storing the washed cell pellets at -80°C until it is ready for shipping to William Klare and Stuart Cordwell.

Bacterial harvest for metabolomics

Materials:

- 3:1 Methanol:Water (containing 2µM ^{13}C-sorbitol + 4µM ^{13}C,^{15}N-Valine,)
- Dry ice/ethanol bath
- Liquid nitrogen
- 1 x PBS
- Ice/Water slurry (in esky)
- 50ml Falcon tubes
- Eppendorf brand 1.5ml centrifuge tubes

Sample Harvest/Metabolic Arrest:

1. Set up 50ml falcon tubes containing 3 volumes of 1 x PBS (relevant to culture volume required).
2. Place in an ice/water slurry to chill
3. Infuse culture into tube containing PBS – allow to sit for at least 5 mins
4. Centrifuge at 3750 rpm in refrigerated centrifuge, 1°C, 10mins
5. Remove supernatant, and resuspend pellet into 1 ml PBS
6. Transfer to 1.5ml Eppendorf tube
7. Centrifuge at 14000 rpm, 30 seconds, 1°C
8. Remove supernatant
9. Wash once more with 1ml PBS
10. Repeat steps 7 and 8, being very careful to remove all PBS
11. Store pellets at -80°C until the pellets are sent to Metabolomics Australia, Bio21 Institute for extraction.