# SDS-PAGE
# Connor Trotter – 01/08/2019

## Materials

Protein or cell sample

1.5 mL Eppendorf tubes

5x SDS-loading buffer (63 μL 1M Tris-HCL pH 6.9; 240 μL 100% glycerol, 687 μL dd$H_2O$, 10 μL bromophenol blue, 500 μL 10% SDS solution)

SDS-PAGE gel

- Gel percentage differs between target protein size
- Buffer dam required if only 1 gel

SDS-PAGE gel tank

1x SDS-Running buffer

Coomassie Brilliant Blue

Destain buffer (20% v/v ethanol, 10% v/v acetic acid)

## Methods

1. Resuspend cell pellets in 50 μL 5x SDS-loading buffer or add 1:1 ratio for protein samples (eg from purification) in a 1.5 mL Eppendorf tube
2. Boil samples at 100 °C for 10 minutes, opening every 2/3 minutes and vortexing to avoid pressure build up
3. Load the SDS-PAGE gel into the SDS-PAGE tank
    a. Add fresh 1x SDS-running buffer to the inner gasket; recycled 1x SDS-running buffer can be used for the external basket if visibly free from contamination
4. Load 5-7 μL of protein ladder to the first well
    a. Volume depends on size of wells
5. Load 10-15 μL of sample to each well
6. Plug in the gel and run at 180 V for until blue dye front is at the bottom of the gel
    a. Typically >40 minutes
7. Drain the tank and obtain the gel
8. Stain gel in Coomassie Brilliant Blue for 2/3 hours

9. Destain the gel in destain buffer overnight
10. Image the gel using appropriate gel dock equipmen