

WHOLE CELL LYSATE PROTEIN PREPARATION

EXTRACTION OF WHOLE CELL LYSATE FOR WESTERNS

- Set up overnight culture of strains
- Use cultures from step 1 to set up cultures of 30-50mL to reach O.D. 0.8 tomorrow (or your required O.D.)
- Next day, spin down culture for 12 minutes at 6000g at room temperature in 50mL falcon
- Remove supernatant and resuspend culture in 2mL of 18% SW
- Spin down culture for 12 minutes at 6000 xg at room temperature
- Remove supernatant and resuspend in 1mL of 18% SW and place into 5 ml eppendorf
- Place samples on ice
- Sonicate for 8U, 5 times for 20 seconds each (or repeat until clear)
- Spin down sonicated culture at 12,000g for 30 minutes
- Remove supernatant to new tube/store at -80 if not being used straight away
- Prior to loading, mix equal volumes of protein sample and 2x lamelli buffer
- Heat at 95 for 10 minutes prior to loading onto SDS-PAGE gel