mCherry nanobody expression and purification

Transform mCherry nanobody plasmid (D33 LAM4) into BL21 Star (DE3) RIL

- Plate on LB/Amp/chloramphenicol/0.4% glucose plates, grow O/N 37°C

Protein Expression Conditions

Starter culture

- inoculate 100ml LB/Amp/chloramphenicol/0.4% glucose culture from single colony
- Grow O/N at 30°C

Large Cell Culture

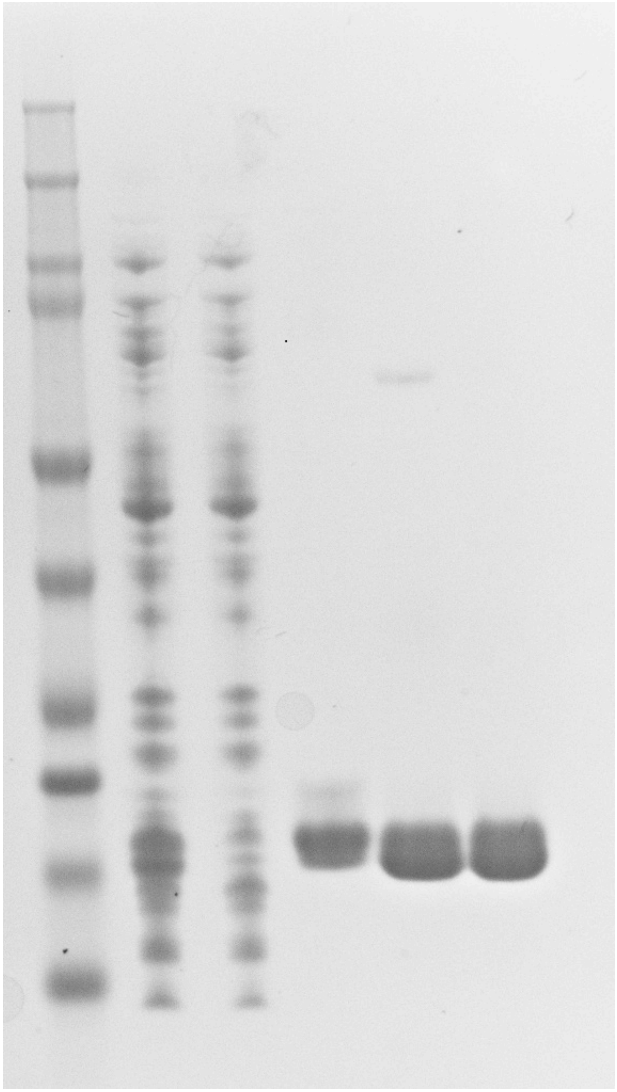
- Inoculate each 1L 2xYT/Amp/chloramphenicol culture with 5ml of the starter culture.
- Grow at 30°C until OD600=0.4
- Start cool down of shaker to 18°C
- Keep growing until OD600=0.6
- Add IPTG to final concentration of 1mM
- Cool shaker to 18°C
- Shake for 24hr

Harvest

- Centrifuge cells at 4000g, 15 min, 4°C
- Resuspend cells in 1XPBS buffer (20ml per L culture)
- Freeze resuspended cells at -80°C in 2L aliquots in 50ml conical tubes

Prep (will change this later)

- Break cells using high-pressure homogenizer
- Spin down at 80,000g, 45min, 4°C
- Equilibrate cobalt column with PBS + 5mM imidazole
- Add 5mM imidazole to supernatant
- Wash with 10 CV of PBS + 5mM imidazole
- Wash with 10 CV of buffer + 15mM imidazole
- Elute with PBS + 150mM imidazole
- Dialyze two times against: PBS
- Spin down or filter any precipitation
- Determine concentration based on A280
- Add glycerol to final concentration of 20%
- Freeze -20°C, aliquot 0.5mL fractions
- Expect 75mg mcherry nanobody for 12L prep

- 

1 MW std Precision Plus Dual Color
2 Load
3 Flowthrough
4 15mM imidazole wash
5 150mM imidazole elute
6 after dialysis