

## **GFP nanobody expression and purification**

### **Transform GFP nanobody plasmid (pSK003) into BL21 Star (DE3) RIL**

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

### **Protein Expression Conditions**

#### **Starter culture**

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

#### **Large Cell Culture**

- Inoculate each 1L 2xYT 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 20-24hr

#### **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

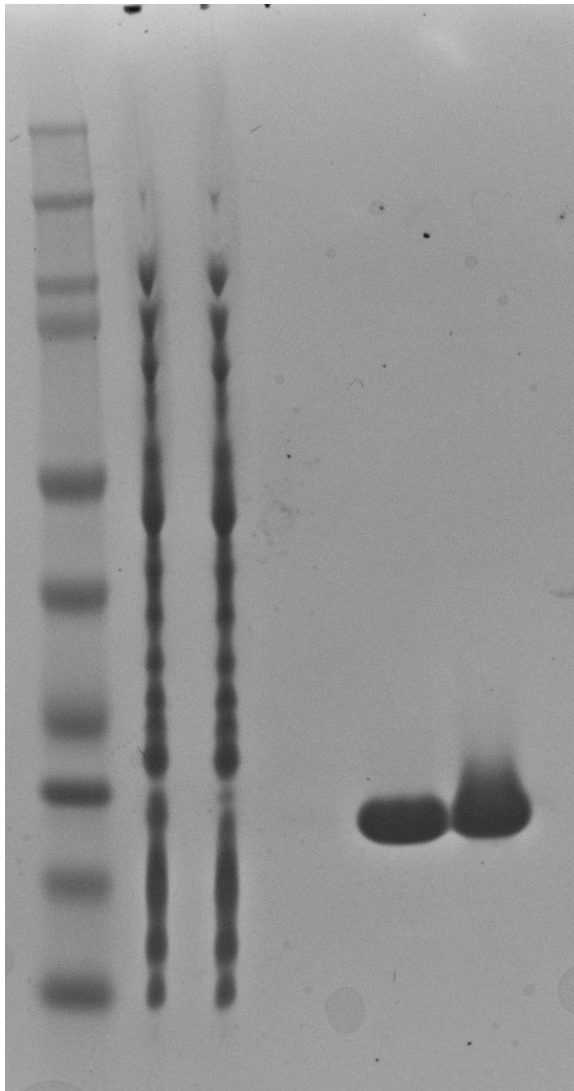
#### **Prep (will change this later)**

- Break cells using high-pressure homogenizer
- Spin down at 75,000g, 45min, 4°C
- Equilibrate 20ml cobalt column with 1x PBS + 5mM imidazole
- Add 5mM imidazole to supernatant
- Wash with 10 CV of 1x PBS + 5mM imidazole
- Wash with 10 CV of 1x PBS + 15mM imidazole
- Elute with 1x PBS + 150mM imidazole
- Dialyze overnight against 1x PBS
- Next day, dialyze against 1x PBS for 3-4 h
- Spin down or filter any precipitation
- Determine concentration based on A280 (extinction coefficient: 1.91)
- For freezing:
  - Add glycerol to final concentration of 20%
  - Freeze -20°C, aliquot 0.5mL fractions
  - Expect 55mg GFP nanobody for 12L prep

#### **Conjugation**

- Dilute GFPnb to 1mg/mL in PBS

- Add 1:1 v/v pre-treated NHS sepharose (pretreatment with 150mL cold 1mM HCl for 25mL resin, then 1x PBS until pH of flowthrough is 7.4)
- Incubate resin and protein with nutation overnight at 4°C
- Spin down resin and remove unbound protein fraction (can quantify by Pierce Protein Assay)
- Add 1CV 100mM Tris pH 8 and incubate for 30 min.
- Spin down and remove flowthrough.
- Repeat the above two steps 2x
- Add 1CV Tris pH 8 and incubate overnight.
- Wash resin with 10CV 20% EtOH and store in 20% EtOH



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

**GFPnb Construct Sequence:**

MAVQLVESGGALVQPGGSLRLSCAASGFPVNRYSMRWYRQAPGKEREWVA  
GMSSAGDRSSYEDSVKGRFTISRDDARNTVYLMNSLKPEDTAVYYCNVNVG  
FEYWGQGTQVTVSSKLEHHHHHH