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 1X PBS 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:
  o Add glycerol to final concentration of 20%
  o Freeze -20°C, aliquot 0.5mL fractions
  o 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
GFPnb Construct Sequence:
MAQVQLVESGGALVQPGGLRLSCAASGFVPNYRQAPGKEREWVA
GMSSAGDRSYEDSVEKGRFTISRDDARNTVYLQMNLKPEDTAVVYCNVNVG
FEYWGQGTQVTSSKLEHHHHHH