PreScission protease expression and purification

Transform GST-PreScission protease into BL21 Star (DE3)

- Use 1ul of GST-Precission Protease DNA for 1 aliquot of cells
    - Use heat shock method for chemically competent cells
- Plate on LB/Amp/0.4% glucose plates, grow O/N @ 37°C
    - Use filtered 50% glucose and add with Amp after LB agar is cooled to 60°C

Protein Expression Conditions

Starter culture

- Pick single colony and inoculate 50ml LB/Amp/0.4% glucose culture
- Grow O/N at 37°C

Large Cell Culture

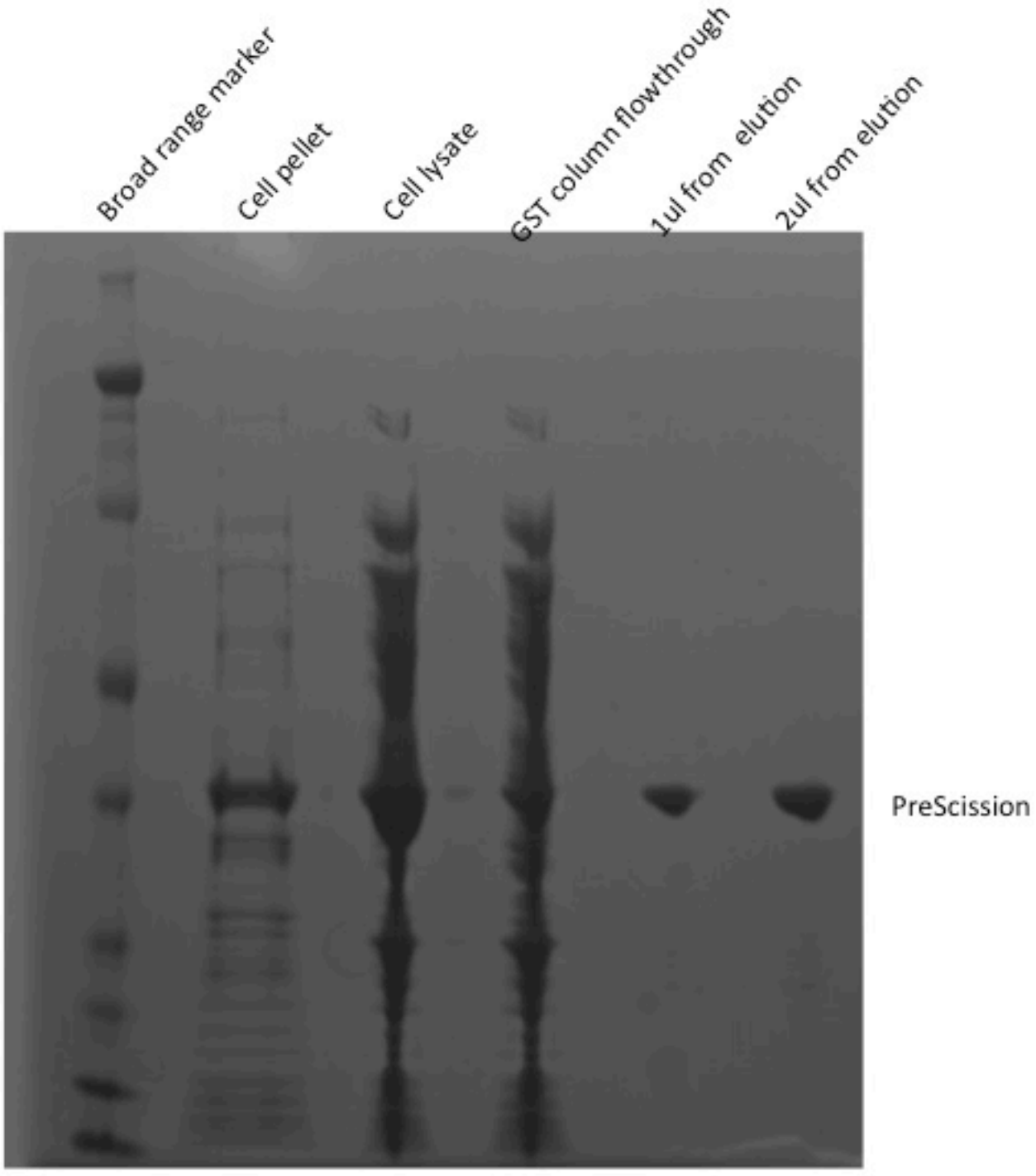
- Inoculate each 1L LB/Amp culture with 5ml of the starter culture.
- Grow at 37°C until OD600=0.5
- Start cooling shaker down to 18°C
- When OD600=0.7, add IPTG to final concentration of 0.2mM
- Grow for 24 hr

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

- Break cells using sonication (or high-pressure homogenizer):
    - For 4L prep: thaw cells, add BME to 5mM, use metal beaker on ice, sonicate at 35%, pulse total 5min, 1.5 sec on, 3 sec off each step.
- Spin down at 60-80,000g, 45min, 4°C
- Equilibrate GST sepharose column with wash buffer:
    - Wash buffer: 1X PBS, 5mM BME, 1mM DTT
    - 5mL GST beads per 1L cells broken
- Use gravity flow or batch method to add lysate to resin
    - Wash extensively (~300ml buffer)
    - Elute with elution buffer: 50mM Tris pH 8, 150mM NaCl, + 5mM BME + 10mM reduced Glutathione (pH should be about 7.5)
    - Elution volume around 100ml from 20ml of resin
- Dialyze: 50mM Tris pH 8, 150mM NaCl, 1mM DTT, 1mM EDTA, 4°C, O/N
- Check by SDS-PAGE
- Concentrate to ~4mg/ml using A280
    - Will not get a perfect peak without gel filtration but estimate of concentration is ok here, usually compare by gel to last prep to estimate
- Add glycerol to final concentration of 20%
- Freeze -20°C, aliquot 0.5mL fractions


Broad range marker
Cell pellet
Cell lysate
GST column flowthrough
1ul from elution
2ul from elution
PreScission