**Parallel rapid expression and purification of proteins for crystallography**

**PREPX large scale (1 L cultures)**

**Materials**

His Gravitrap Ni columns from GE healthcare, £ 145 for 10 columns; oracle supplier item 11564865

<http://www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences-uk/11003399>

Labmate reservoirs to increase the load volume to 40mL, £ 22.54 for 10 adapters; oracle supplier item 11787819

<http://www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences-uk/18321603>

PD-10 spin adapters on the columns to stop them wobbling in the racks £ 9 ea for 10 adapters; oracle supplier item 11717829

<http://www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences-uk/28923245>

PD10 columns £161 for 30 columns; oracle supplier item 11768488

<http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-uk/products/AlternativeProductStructure_17084/17085101>

Nalgene 24 place 30 mm racks (5970-0030) £ 10 ea; oracle supplier item 10644101

<http://www.thermoscientific.com/en/product/nalgene-unwire-test-tube-racks-resmer-manufacturing-technology.html>

Autoinduction TB from ForMedium (£50 for 1 Kg, around 20 L of medium, AIMTB0210)

<http://www.formedium.com/products/escherichia-coli-media/aim-auto-induction-medium/aim-terrific-broth-base-including-trace-elements.html>

2.5 L Ultrayield flasks from Thomson

<https://www.generon.co.uk/flasks-for-e-coli-and-microbial-3798/ultra-yield-flask-2-5l-sterile-269000001.html>

Optional but useful

Multitube vortexer

<http://www.benchmarkscientific.com/rpproducts/benchmixer-xl-vortex-mixer/>

**Method overview**

Standard workflow is expression via autoinduction followed by purification using IMAC/PD-10/revIMAC and serial gel filtration

**Expression**

**Materials (1 L cultures)**

Plates with LB-agar+antibiotics

1 L of autoclaved autoinduction TB + 20 g/L glycerol + antibiotics

1 mL of 10 % Antifoam 204 (Sigma) in ethanol

2.5 L Ultra Yield flasks (fitted with loose foil cover\*\*)

**Methods**

1. Either transform BL21[DE3}R3 with appropriate plasmid or streak from glycerol stock onto agar plate and incubate o/n 37 °C\*
2. Grow 10 mL o/n in 50 mL tube of each clone in superbroth + 1 % glucose + antibiotics
3. Use 10 mL to inoculate 1 L AIM-TB (+ Antibiotics + Antifoam 204) in baffled flask\*\*
4. Grow 4 h 37 °C 250 rpm shaking USE LOOSE FOIL COVER AS AERATION IS ESSENTIAL!
5. Grow 40-48 h 18 °C 250 rpm shaking
6. Harvest at 4000 g 20 minutes 4 °C
7. Scrape out pellet and place in plastic polygrip bag and freeze -80 °C

Final WCW typically 50 g

\* Freshly transformed or re-streaked cells always give better yields than growing overnights directly from frozen glycerol stocks

\*\*an upturned 500 mL plastic beaker with a 2 mL microcentrifuge tube taped to the side of the flask to act as a spacer can also be used

**Purification**

**Materials (1 L cultures)**

1 L of Base Buffer 10 mM HEPES, 5 % Glycerol, 500 mM NaCl, 0.5 mM TCEP, pH 7.5

100 mL of 3 M imidazole pH 7.5

100 mL of 10 % Triton X-100 in water

50 mg/mL Lysozyme solution (100 x)

1 mg/mL homemade benzonase (1000x)

2 x His GraviTrap column/L (GE healthcare) fitted with LabMate extender and PD-10 spin adapter in 24 place Nalgene rack

2 x PD10 column/L fitted with LabMate extender and PD-10 spin adapter in 24 place Nalgene rack

2 x 50 mL/L centrifuge tubes in in 24 place Nalgene rack

**Methods**

1. Place polygrip bag on flat surface and smash cell pellet into small pieces and pour into 500 mL beaker
2. Add 3 mL Base Buffer/g cell pellet (10 mM HEPES, 500 mM NaCl, 5 % Glycerol, 0.5 mM TCEP, pH 7.5) + 0.5 mg/mL Lysozyme, 1 µg/mL Benzonase, 1 % Triton X-100, 20 mM imidazole
3. Leave 30 minutes RT
4. Use stripette to dissolve pellet and put up to 45 mL in a 50 mL tube (4 tubes in total)
5. Freeze -80 °C 1-2 h
6. Thaw in RT water bath 1h and mix
7. Centrifuge 4,000 g 1 h 4 °C
8. Apply SN from 2 \* 50 mL tubes to 1 mL His GraviTrap column (GE healthcare) fitted with LabMate extender
9. Wash 10 mL Base Buffer + 20 mM Imidazole\*\*
10. Slot His GraviTrap column into PD10 column fitted with LabMate extender (pre-equilibrated in Base Buffer + 20 mM Imidazole)
11. Elute protein with 2.5 mL of Base Buffer + 500 mM Imidazole directly onto PD10 column
12. Remove His GraviTrap column
13. Place PD10 into 50 mL falcoln tube add 3.5 mL Base Buffer + 20 mM Imidazole and collect
14. Measure A280
15. Add protease 1 OD unit TEV for every 10 OD units target and incubate o/n 4 °C\*\*\*
16. Run back over His GraviTrap column equilibrated in Base Buffer + 20 mM Imidazole
17. Washed column 2.5 mL 20 mM Imidazole
18. Check purity of 6 mL pool
19. Concentrate to 1 mL ish
20. Transfer to 1.6 mL glass autosampler vial ensure at least 1.1 mL in vial!
21. Run through serial gel filtration system injecting 1 mL
22. Take peak fraction(s) only (1-2 mL) and concentrated to 10-20 mg/mL if possible

\*Imidazole concentration can be increase to 40 mM in most cases, but may affect yield, see below

\*\*10 mL of a 40 mM or 70 mM imidazole wash can also be done, but this is very target dependent and may lead to significant reduction in final yield BUT can also increase purity substantially, worth trying if your purity is poor.

\*\*\*Some targets exhibit significant affinity for IMAC columns even after TEV cleavage try increasing the imidazole concentration to 40 or 70 mM or use an MBP-TEV construct so that the protease can be removed using an amylose column rather than reverse IMAC.

**Column regeneration**

**PD-10**

1. Wash PD-10 columns with 50-100 mL of Milli-Q water

**His GraviTrap**

1. Wash IMAC colums 40 mL Milli-Q
2. Wash IMAC columns 10 mL 1 M NaOH
3. Wash IMAC colums 40 mL Milli-Q
4. Wash IMAC columns 10 mL 1 M Acetic Acid + 1 % Triton X-100
5. Wash IMAC colums 40 mL Milli-Q
6. Wash IMAC columns 10 mL 20 % Ethanol + 0.1 M EDTA\*
7. Wash IMAC colums 40 mL Milli-Q
8. Wash IMAC columns 0.5 mL 100 mM Nickel Sulfate + 20 mM Tris.HCl pH 8\*
9. Wash IMAC colums 40 mL Milli-Q

\*PUT NICKEL WASTE IN APPROPRIATE CONTAINER FOR DISPOSAL!

Store all columns in water at 4 °C