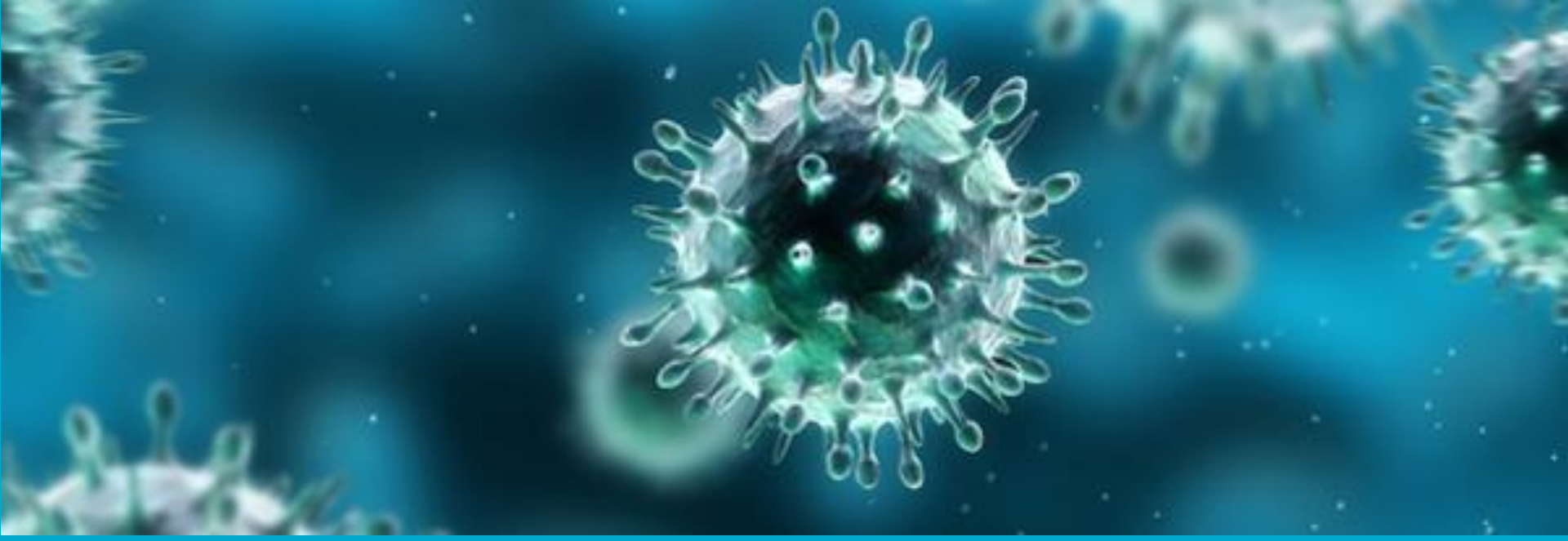
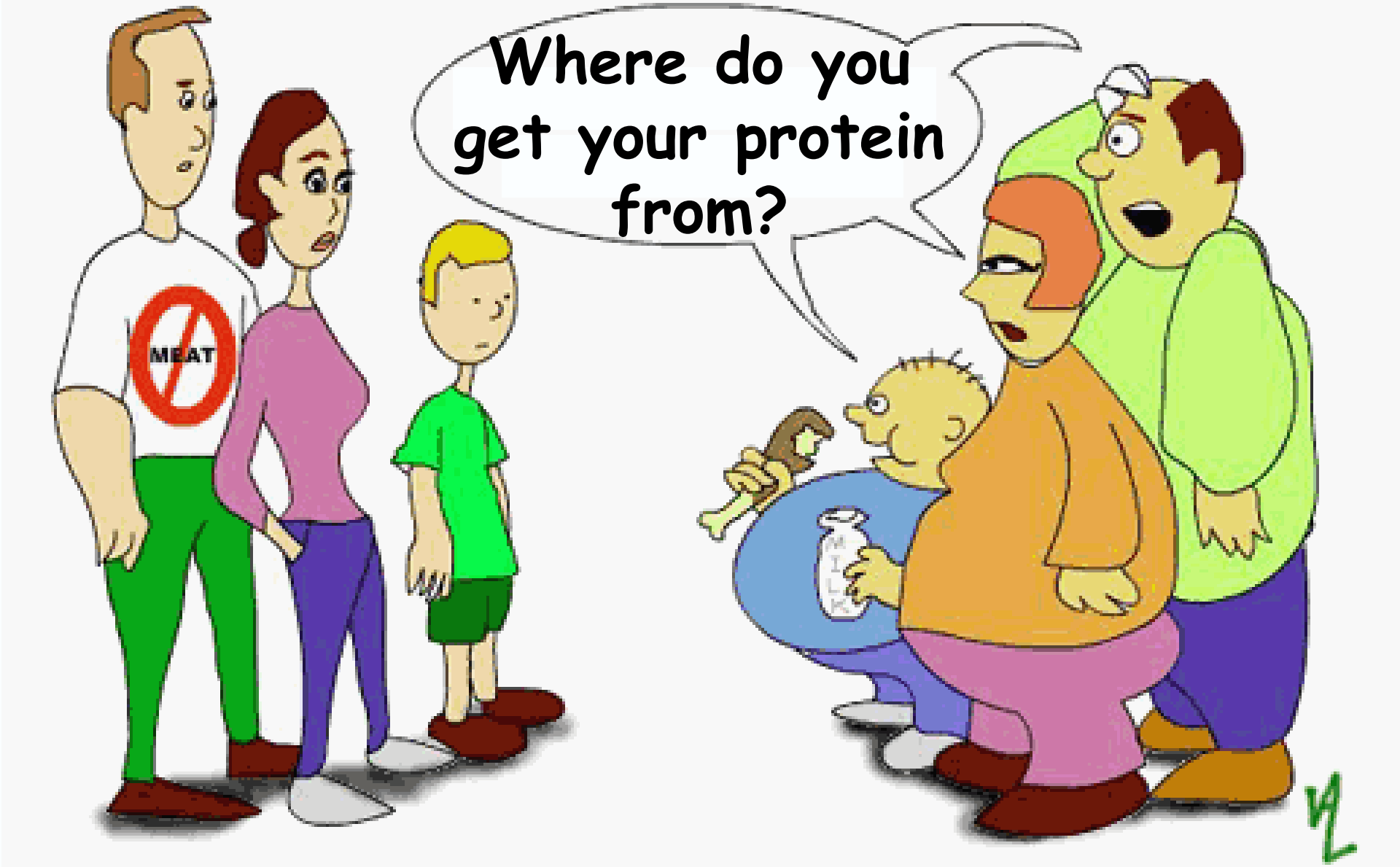


Strategies for expressing recombinant protein in bacteria and their purification

PEWS 2014

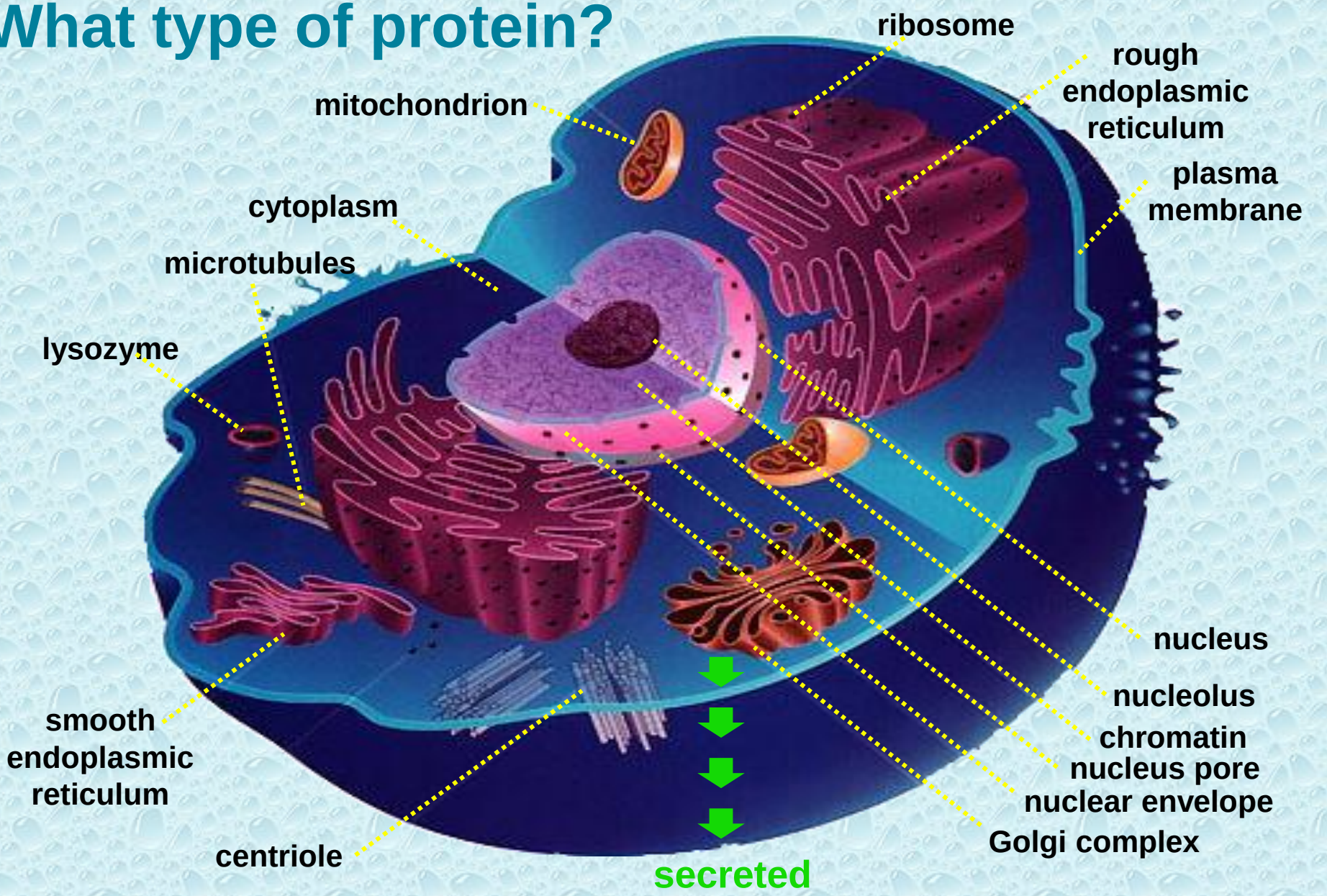
Bill McKinstry | Research Team Leader – Protein Purification

30 July 2014

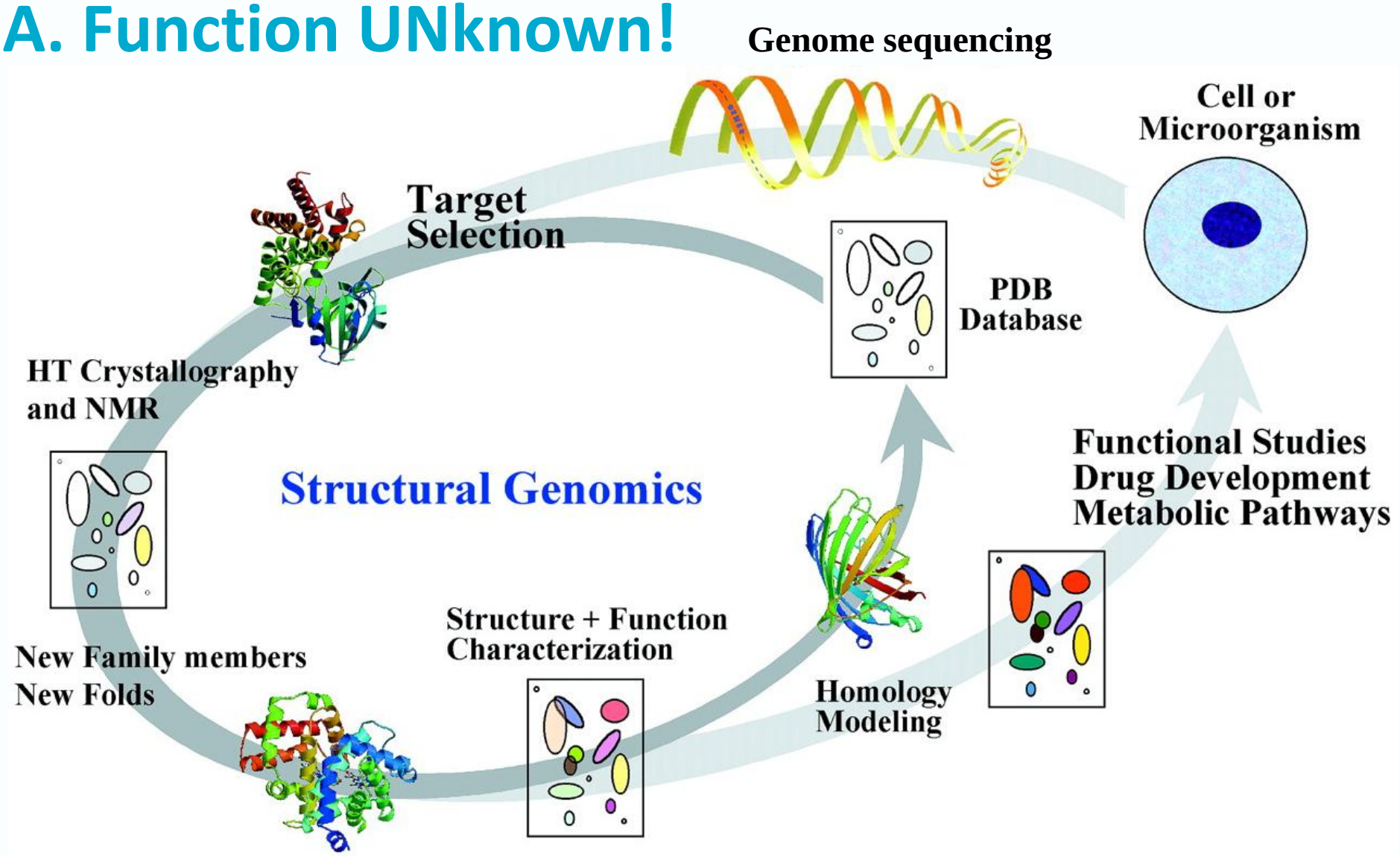

Where do you get your protein from?
MEAT


FIND YOUR PROTEIN HERE!
SHOP FOR YOUR FAVORITE PROTEIN FLAVORS AND
THEY'LL BE DELIVERED RIGHT TO YOUR DOOR.
BUY NOW!
PICK YOUR
PERFECT
PROTEIN

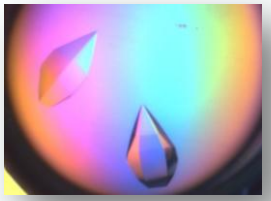
What type of protein?



Q. What are FUN proteins?
A. Function UNknown!

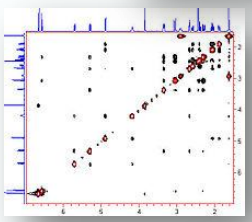


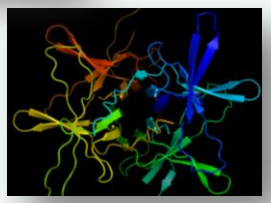
PURPOSE



Structural studies protein crystallography/NMR

- HT parallel cloning, expression, purification
- soluble, mono-disperse, high purity, >10 mg
- ± removal solubility/purification tags
- ^{13}C, ^{15}N, seleno-methionine labeling



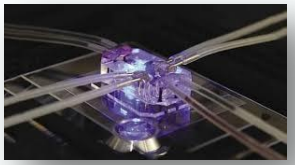


Antibodies – polyclonal, monoclonal

- soluble or denatured protein for immunisation and screening
- fusion protein OK, > 90% purity, 1 - 5 mg

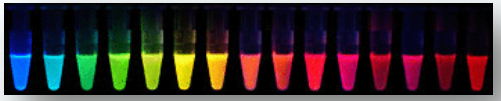
Lab-on-a-chip, high-throughput screening

- functionally active, fusion protein OK, conjugation chemistry
- soluble, high purity, stable, >10 mg



In vitro assays

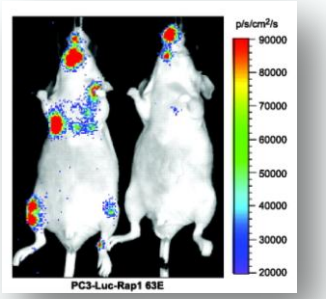
- functionally active, fusion protein OK, conjugation chemistry
- high purity, ± LET, stable, >10 mg



PURPOSE cont'd

In vivo assays

- soluble, functionally active, ± fusion protein
- suitable for bio-conjugation, high purity, LET, >100 mg



Biologicals

- soluble, functionally active, minimal COGS
- ± fusion protein, > 90% purity, >10 kg

Biotherapeutics

- soluble, functionally active, stable, tag removal
- suitable for bio-conjugation
- high purity, LET, low HCP, >1 kg

Subunit vaccines

- soluble, functionally active, stable, tag removal
- high purity, LET, low HCP, >1 kg, minimal COGS

Checklist

Name of protein

Function

Organism

Location: nuclear, cytosolic, membrane, secreted

Features: signal/leader sequence, trans-membrane domains, ligand sites, cofactor requirements, oligomeric status, binding proteins

PTMs: disulphide bonds, glycosylation, phosphorylation, etc

DNA sequence

Amino acid sequence

Literature review (www.ncbi.nlm.nih.gov/pubmed) (supplementary data)

Bioinformatic and sequence analysis (http://www.expasy.org)

Structure review (www.pdb.org) and orthologue search

Requirements

Research or commercial use

Expression vector

Purification Tag (eg. poly His, myc, FLAG, HA, GST, MBP, Halo, GFP, Fc)

Solubility Tag (GST, MBP, NusA, TRX, GFP)

Protease cleavage site (TEV, thrombin, Factor Xa, SUMO, enterokinase)

Template DNA or Codon optimization/synthetic genes

Host organism/protein expression strain

Amount of protein required

Protein purity

Ideal protein concentration

Preferred buffer

Suitable storage condition

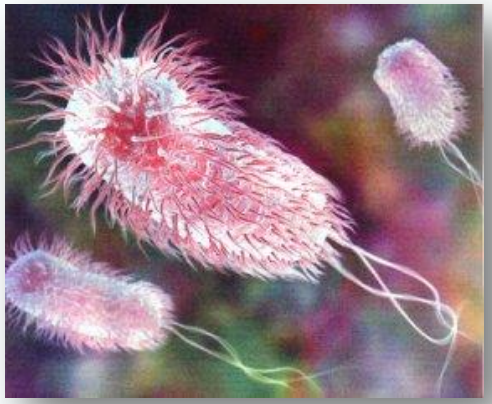
Endotoxin removal

Escherichia coli

pioneering host for heterologous recombinant protein production

well characterised genetics, physiology, culture requirements, leading to rapid growth

potential for high density cultivation



target expression to various intracellular compartments

dominated by BL21(DE3) strain and its derivatives

induction of T7 RNA promotor (DE3) mediated by IPTG

plasmids containing antibiotic resistance genes as selectable markers to introduce gene of interest

if transformation does not yield colonies due to toxicity try pLysS containing strains

E. coli protein expression strains

Strain	Description	Use	Antibiotic	Supplier
AD494 (DE3)	thioredoxin reductase (*trxB*) mutant	*trxB* expression host; disulfide bond formation in cytoplasm	Kan	Novagen
B834 (DE3)	methionine auxotroph protease deficient	general expression host; labeling of proteins with ^{35}S-methionine and selenomethionine	none	G. Stier
BL21 (DE3)	deficient in *lon* and*ompT* proteases	general purpose expression host	none	Novagen
BL21 AI	deficient in *lon* and*ompT* proteases	general purpose expression host; expression is induced by arabinose	none	Invitrogen
BL21 SI	deficient in *lon* and*ompT* proteases	general purpose expression host; expression is induced by salt	none	Invitrogen
BL21 (DE3) pLysS	deficient in *lon* and*ompT* proteases	high-stringency expression host	Cam	Novagen
B834 (DE3) pRARE	methionine auxotroph; protease deficient; contains plasmid encoding *argU, argW, glyT, IleX, leuW, metT, proL, thrT, thrU,* and *tyrU*	high-stringency expression host; labeling of proteins with ^{35}S-methionine and selenomethionine allows expression of genes encoding tRNAs for rare arg codons AGA, AGG, and CGA, glyc codon GGA, ilu codon AUA, leu codon CUA, and pro codon CCC	Cam	A. Geerlof
BL21(DE3)-R3-pRARE2		Lac-inducible, bacteriophage-resistant, rare-codon optimized	Cam	SGC
BL21 (DE3) CodonPlus-RIL	deficient in *lon* and*ompT* proteases; contains plasmid encoding *argU*,*ileY*, and *leuW*	expression host; allows expression of genes encoding tRNAs for rare argenine codons AGA and AGG, isoleucine codon AUA, and leucine codon CUA	Cam	Stratagene

E. coli protein expression strains

Strain	Description	Use	Antibiotic	Supplier
BL21 (DE3) CodonPlus-RP	deficient in *lon* and*ompT* proteases; contains plasmid encoding *argU* and*proL*	expression host; allows expression of genes encoding tRNAs for rare argenine codons AGA and AGG and proline codon CCC	Cam	Stratagene
Rosetta (DE3)	lactose permease (*lacY*) mutant, deficient in *lon* and*ompT* proteases; contains plasmid encoding *argU, argW, glyT, IleX, leuW, metT, proL, thrT, thrU,* and *tyrU*	expression host; allows expression of genes encoding tRNAs for rare codons AUA, AGG, AGA, CUA, CGA, CCC, and GGA	Cam	Novagen
Rosetta (DE3) 2	lactose permease (*lacY*) mutant, deficient in *lon* and*ompT* proteases; contains plasmid encoding *argU, argW, glyT, IleX, leuW, metT, proL, thrT, thrU,* and *tyrU*	expression host; allows expression of genes encoding tRNAs for rare codons AUA, AGG, AGA, CUA, CGA, CCC, GGA, and CGG	Cam	Novagen
Origami (DE3)	*trxB* and *gor* mutant	expression host; disulfide bond formation in cytoplasm	Kan, Tet, Str	Novagen
Origami (DE3) 2	*trxB* and *gor* mutant	expression host; disulfide bond formation in cytoplasm; kanamycin sensitive	Tet, Str	Novagen
Rosetta Gami (DE3)	contains plasmid encoding *argU, argW, glyT, IleX, leuW, metT, proL, thrT, thrU,* and *tyrU; trxB* and *gor* mutant	expression host; allows expression of genes encoding tRNAs for rare codons AUA, AGG, AGA, CUA, CGA, CCC, and GGA	Kan, Tet, Str , Cam	Novagen
Rosetta Gami (DE3)2	contains plasmid encoding *argU, argW, glyT, IleX, leuW, metT, proL, thrT, thrU,* and *tyrU; trxB* and *gor* mutant	expression host; allows expression of genes encoding tRNAs for rare codons AUA, AGG, AGA, CUA, CGA, CCC, GGA, and CGG	Tet, Str , Cam	Novagen

E. coli protein expression strains

Strain	Description	Use	Antibiotic	Supplier
C41(DE3)	F – *ompT hsdSB (rB- mB-) gal dcm* (DE3)	effective in expressing toxic and membrane proteins from all classes of organisms	none	Lucigen
C43(DE3)	F – *ompT hsdSB (rB- mB-) gal dcm* (DE3)	effective in expressing toxic and membrane proteins from all classes of organisms	none	Lucigen
Artic Express (DE3)	Cpn10 and Cpn60 chaperones	engineered for improved protein processing at low temperatures	none	Agilent
Clear Coli BL21(DE3)	*F– ompT hsdSB (rB- mB-) gal dcm lon λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5]) msbA148 ΔgutQΔkdsD ΔlpxLΔlpxMΔpagPΔlpxPΔeptA*	genetically modified LPS does not trigger endotoxic response in human cells	none	Lucigen
Various Chaperone co-expression	*DnaK/DnaJ/GrpE, GroEL/GroES, IbpA/IbpB, Skp, trigger factor and FkpA*	assist with protein refolding	various	Various
Engineering N-glycan biosynthetic pathways	*5 glycosyltransferases (galE, pglDEF); oligosaccharyl transferase (pglB), 4 enzymes involved in sugar biosynthesis (galE, pglDEF0, flippase (wlaB)*	Glycosylated proteins	?	Wacker

Peptide affinity purification tags

Tag	Description
poly His (5 - 10)	binds to Ni^{2+} or Co^{2+} IMAC, elution with imidazol, EDTA, low pH
poly Glu (EEEEEE)	binding to anion-exchange resin (Mono-Q), NaCl elution
poly Arg (RRRRR)	binding to cation-exchange resin (SP), NaCl elution
FLAG (DYKDDDDK)	binds to specific M2 mAb, elution with free peptide/low pH
c-myc (EQKLISEEDL)	binds to specific 9E10 mAb, elution with free peptide/low pH
HA (YPYDVPDYA)	binds to specific HA-7 mAb, elution with free peptide/low pH
S (KETAAAKFERQHMDS)	binds to specific S-protein, elution with free peptide/low pH
E (GAPVPYPDPLEPR)	binds to specific mAb, elution with free peptide/low pH
V5 (GKPIPNPLLGLDST)	binds to specific mAb, elution with free peptide/low pH
VSV (YTDIEMNRLGK)	binds to specific mAb, elution with free peptide/low pH
CBP (KRRWKKNFIAVSAANRFKKISSSGAL)	binds to immobilised Calmodulin, elution with EGTA
Xpress (DLYDDDDK)	binds to specific mAb, elution with free peptide/low pH
Strep-tag II (WSHPQFEK)	binds to streptavidin or streptactin, elution with d-desthiobiotin
Avi (GLNDIFEAQKIEWHE)	enzymatic biotinylation with BirA biotin ligase, binds to streptavidin elution with biotin

Protein solubility and purification tags

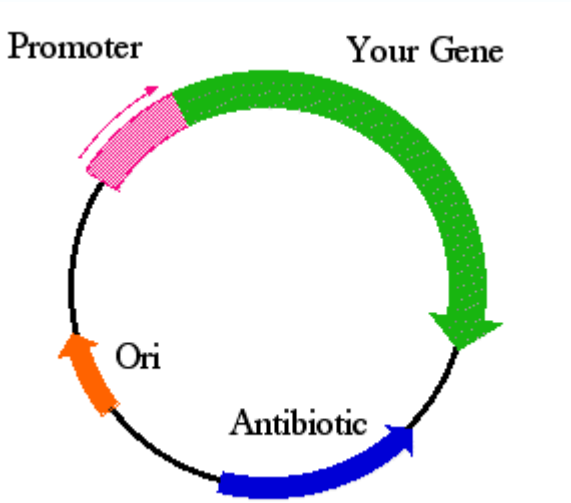
Tag	MW	Description
GST	26	assists solubility, dimerises, binds to immobilised glutathione, elute with 10 mM reduced GSH
GFP	27	auto-fluorescent provided fusion protein is correctly folded, binds to nano-bodies
MBP	41	enhances protein solubility, binds to immobilised amylose, elutes with 10 mM maltose
NusA	55	hydrophilic tag, enhances protein solubility, used in conjunction with various affinity tags
Trx	12	hydrophilic tag, enhances protein solubility, used in conjunction with various affinity tags
PDI	55	hydrophilic tag, enhances protein solubility, used in conjunction with various affinity tags
Fc	32	allows dimerisation, binds to immobilised Protein A/G, increases plasma half life
BCCP	16	protein is biotinylated in the presence of biotin ligase, with subsequent binding to streptavidin/streptactin
SUMO	12	enhances protein solubility, conformational cleavage sequence, used in conjunction with affinity tags
GB1	8	enhances protein solubility, IgG affinity purification
Halo	34	enhances protein solubility, binds covalently to synthetic ligands attached to beads/fluorophores, specifically cleaved by HaloTEV

Proteases used to remove fusion tags

Protease	Source	Class	Cleavage site
TEV	Tobacco etch virus protease	cysteine protease	ENLYFQ/X
3C	Human rhinovirus 3C protease	cysteine protease	EVLFQ/GP
Xa	Factor Xa	serine protease	IEGR/ or IDGR/
EK	Enterokinase	serine protease	DDDDK/
Thr	Thrombin	serine protease	LVPR/GS
Caspase	Caspase-3	cysteine-aspartic acid protease	DXXD/
PreScission	3C-GST fusion protein	cysteine protease	LEVLFQ/GP
WELQuT	His-tagged *Staphylococcus aureus* serine protease	serine protease	WELQ/X
SUMO	Ubl-specific protease 1 fragment	cysteine protease	Tertiary structure

Protein expression vectors

- contains all the elements necessary for protein expression
- origen of replication site
- strong inducible promotor usually based on lac operon or T7 promotor
- eg pGEX vector under control of tac promotor
- eg pET vector under control of T7 promotor
- translation initiation eg Shine-Dalgarno sequence ribosomal binding site
- start and termination codons
- transcription termination sequence
- +/-protein solubility tag(s), N and/or C terminal
- +/- affinity tag(s), N and/or C terminal
- multiple cloning site
- antibiotic selectable marker
- co-express 2 or more plasmids (different antibiotic selection)
- poly-cistronic vector containing 2 or more genes



Cloning strategy

Ligation Dependent Cloning (PCR amplification of template DNA + RE sites)

- N-terminal 6xHis tag

  NH_2-MGHHHHHHGTENLYFQGS **INSERT** -COOH

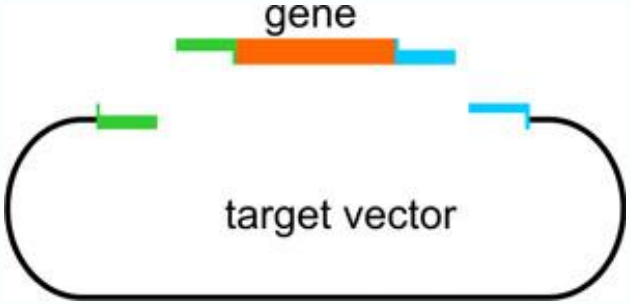
  TEV

- C-terminal 6xHis tag

  NH_2-MGS **INSERT** ASHHHHHH-COOH

Ligation Independent Cloning (PCR amplification of template DNA no RE sites or use of DNA ligase) (Infusion System – Clontech)

- PCR generated insert containing 15 bp ss over-hangs
- Vector cut with RE to generate complementary over-hang
- Annealing vector and insert with T4 DNA Pol



Growth media, feeding strategies, fermentation technologies

- rich media (LB, 2YT, TB)
- slow release glucose (Enpresso)
- auto-induction
- defined media
- feeding strategies
- carbon sources
- pH, temperature, and oxygenation control
- prevention of foaming

Parallel expression strategies

- **vectors containing different tags and/or fusion partners**

  His, myc, FLAG, GST, MBP, DsbA, NusA,
  location of tag: N- or C-terminal

- **different *E. coli* host strains**

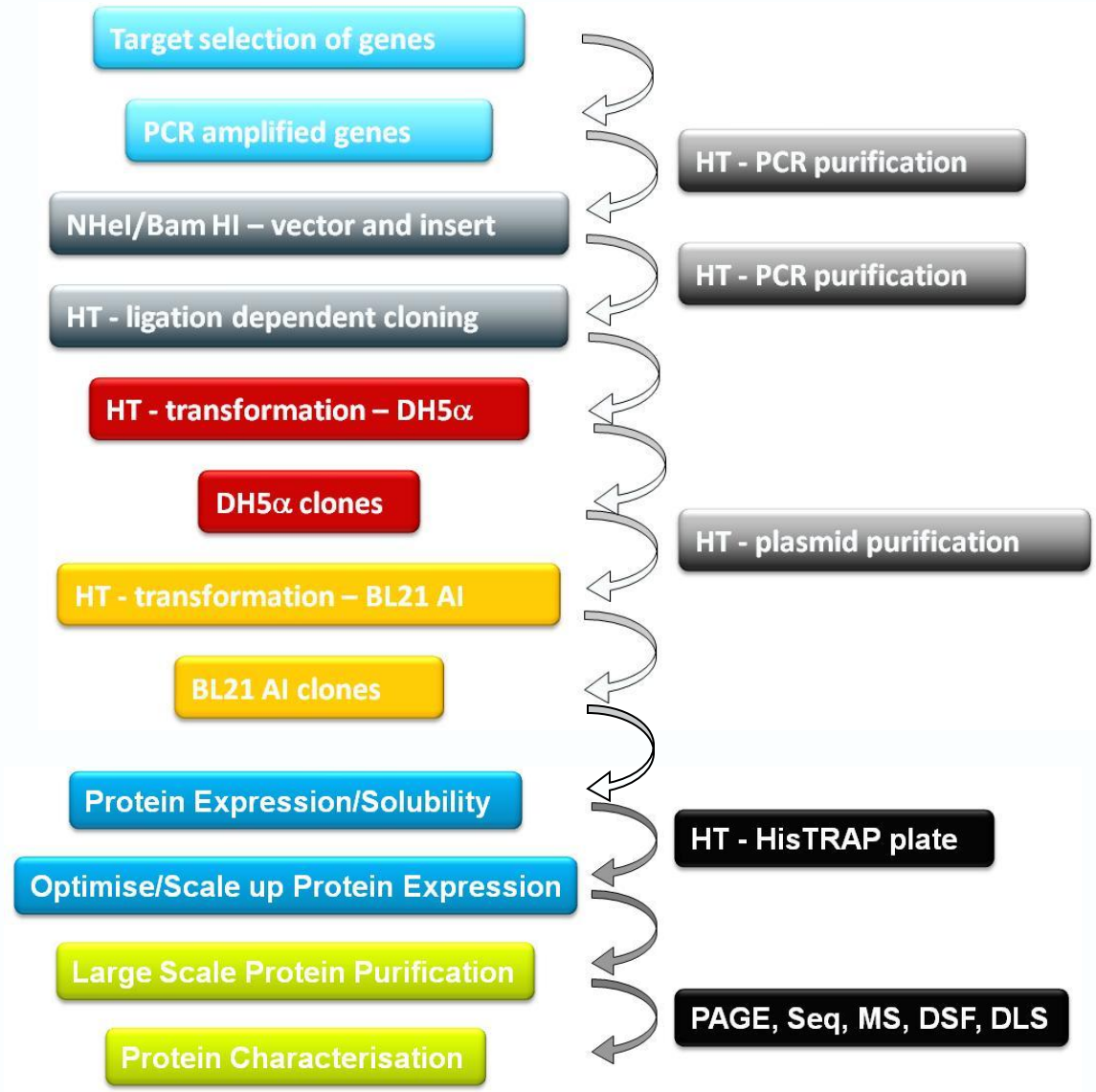
  rare E. coli codons
  BL21(DE3) CodonPlus RIL; BL21(DE3) CodonPlus RP; Rosetta (DE3) or Rosetta(DE3)2
  disulphide bonds (oxidising cytoplasmic environment)
  AD494 (thrioredoxin reductase (trxB mutation)
  Origami (trxB and glutathione reductase gor mutation)
  protein toxic to cell (tighten regulation of expression systems using T7 promotor
  pLysS or pLysE (lysozyme which binds to and inactivates T7 RNA pol)
  membrane bound proteins
  C41(DE3) and C43(DE3)

- **expression conditions (temperature, media, aeration, glucose)**

  18 ^{o}C, 30 ^{o}C or 37 ^{o}C
  carbenicillin instead of ampicillin

- **induction conditions (temperature, aeration, duration, IPTG, arabinose)**

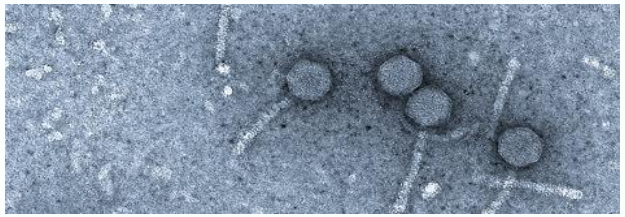
HT cloning, experssion and purification flow path



8-10 days

> 1 week

Bacteriophage structural genomics – HT techniques



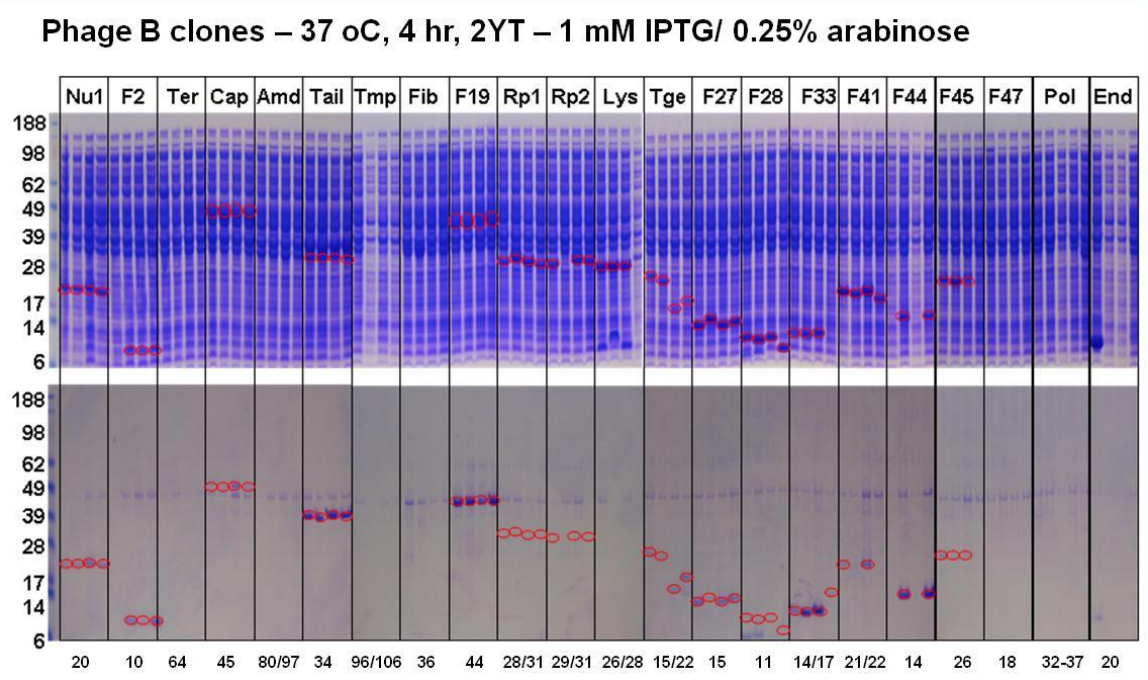
Lactococcus lactis bacteriophage

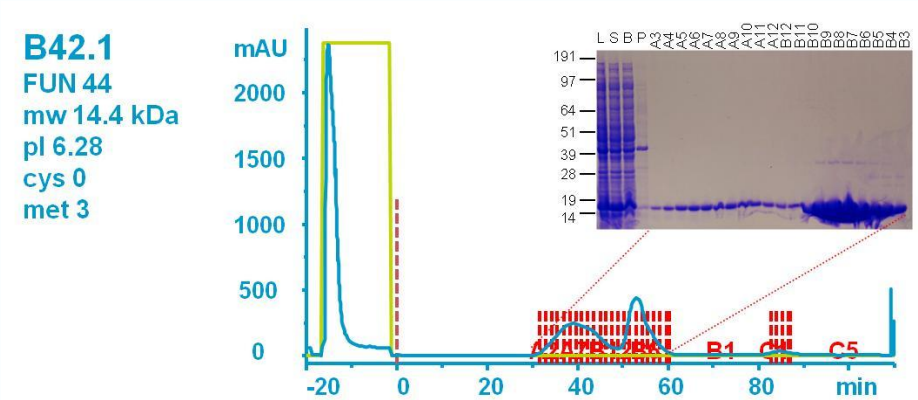
ds DNA viruses

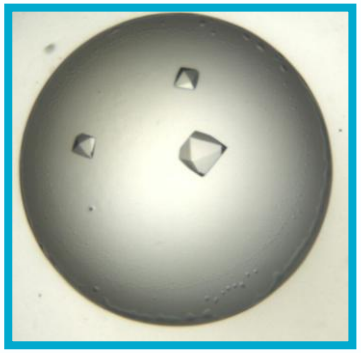
50 – 70 genes, pooly annotated

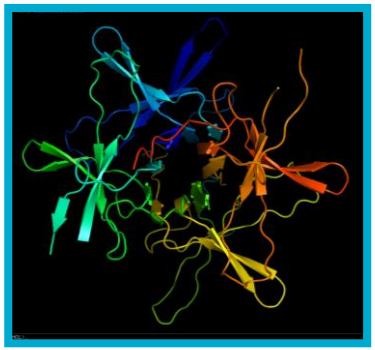
22 gene families; 4 orthologues

48 genes, 96 constructs







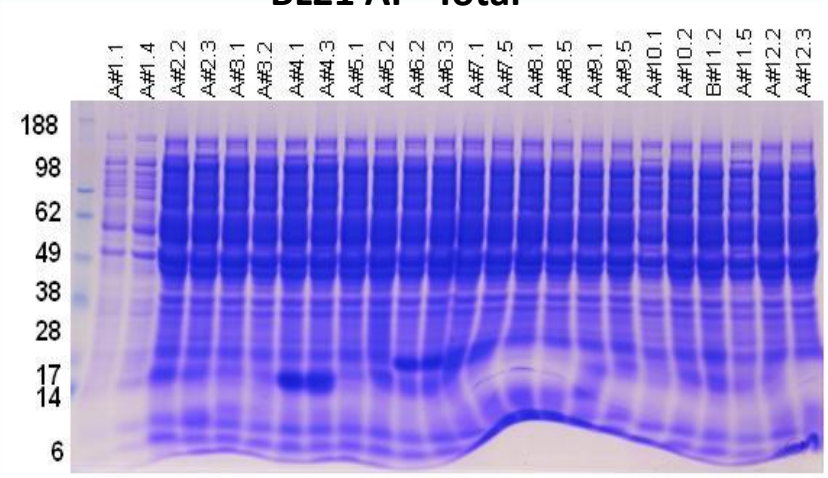


McKinstry et al, unpublished data

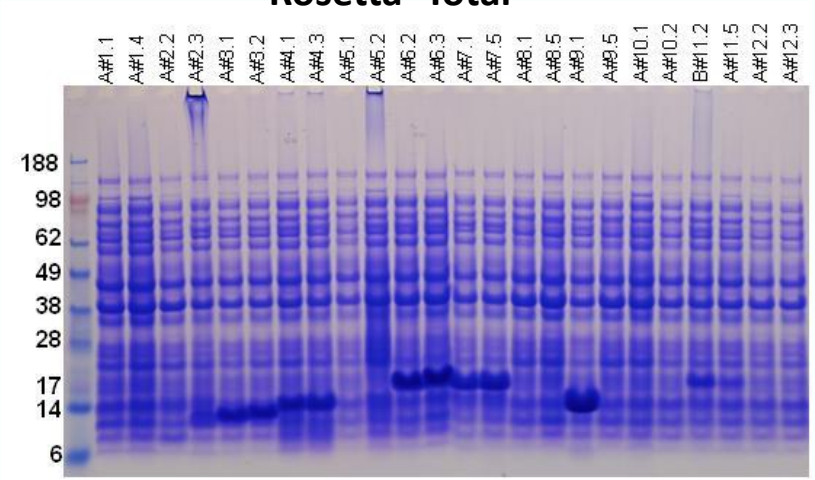
Arbovirus Structural Genomics –

Comparison of BL21 AI vs Rosetta for the expression of soluble protein

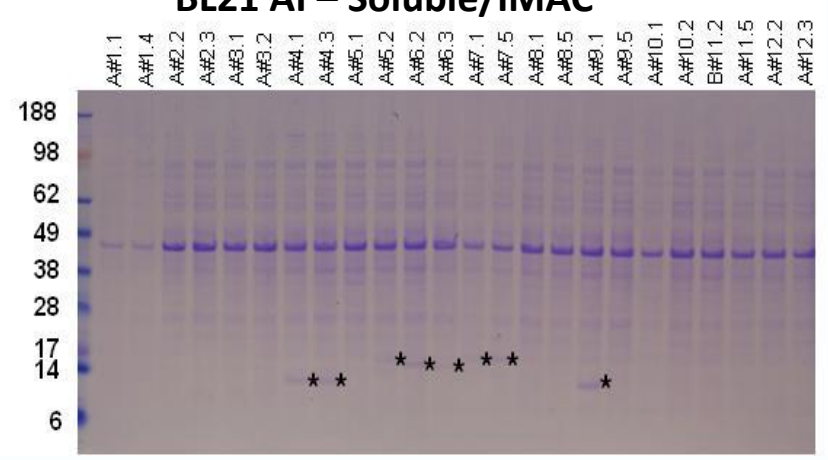
BL21 AI - Total



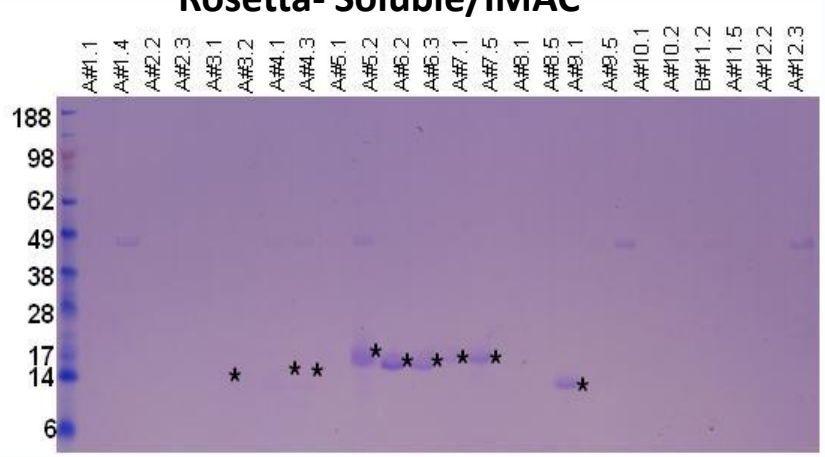
Rosetta- Total



BL21 AI – Soluble/IMAC



Rosetta- Soluble/IMAC



McKinstry et al, unpublished data

HT technologies allow us to explore various methods and strategies systematically and speed up protein production – which could benefit everyone, particularly those interested producing quality recombinant proteins

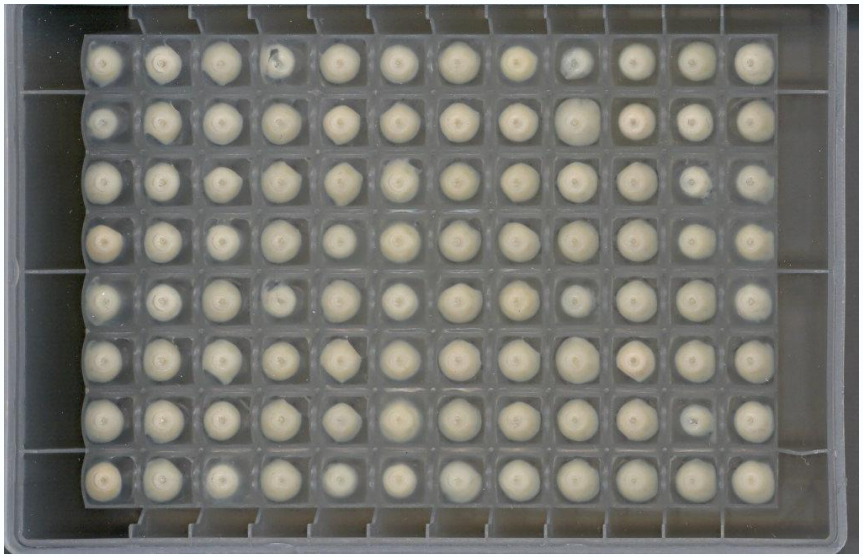
- BL21(DE3)-R3-pRARE2: Lac-inducible, bacteriophage-resistant, rare-codon optimized
- Some proteins work best from freshly transformed cells
- Generate multiple versions of protein (N- and C-terminal tags)
- Explore a range of solubility and/or purification tags
- Grow cells at 37 ^{o}C in TB, but induce at 18 ^{o}C overnight
- Increased solubility and expression levels with TB compared to 2YT, LB or MM
- Aeration is key – low yield often due to poor aeration
- Reusable polypropylene shake flasks Tunair® or Thomson UltraYield®
- 2.5L flasks can hold 1L of medium with excellent results
- Fermentation/bubbling is also good; eg LEX system (Harbinger Biotech)

Aled Edwards, SGC, personal experiences

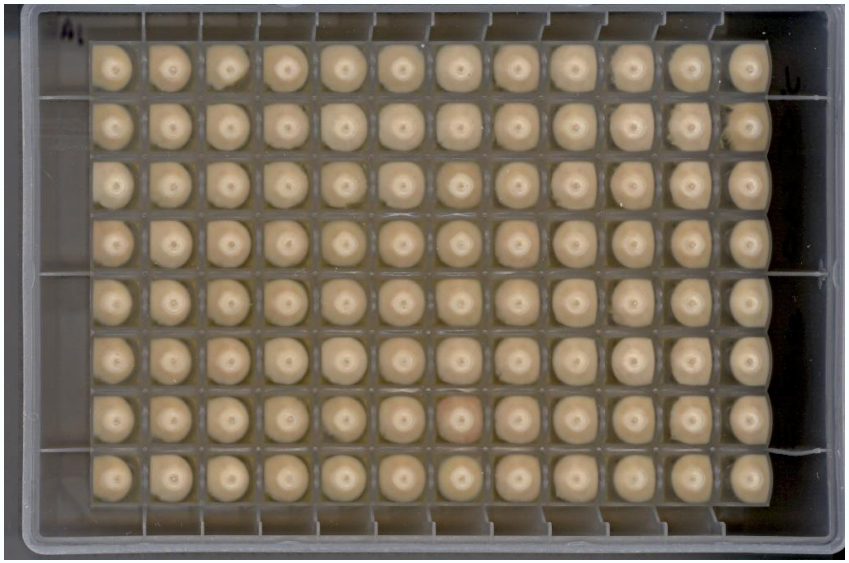
Comparison of expression levels and solubilities of FUN proteins

Host cell: E. coli BL21 AI

TB - 30 °C O/N



Enpresso - 30 °C O/N

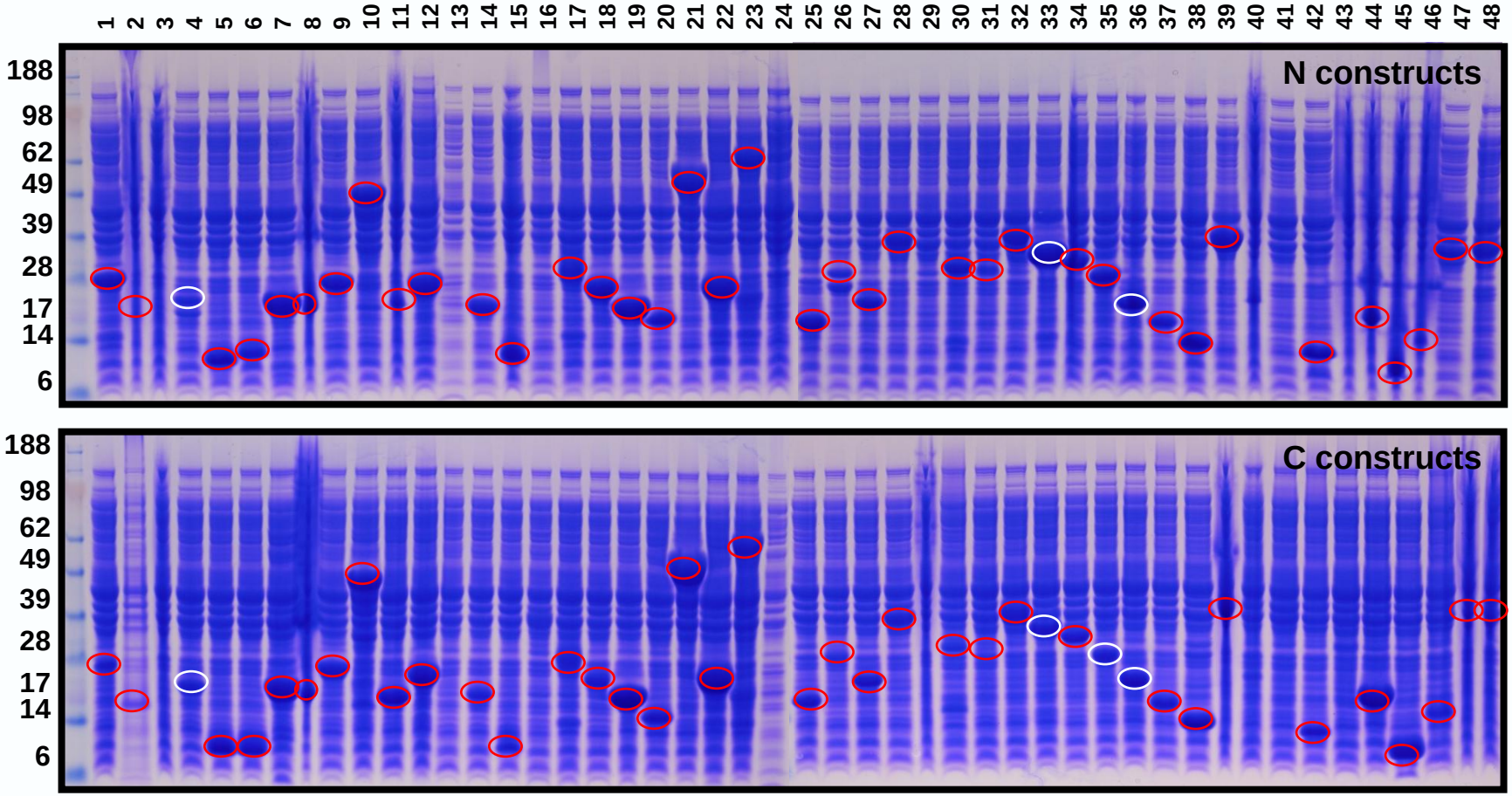


McKinstry et al, unpublished data

Comparison of expression levels and solubilities of Salmonella FUN proteins

Host cell: E. coli BL21 AI

Enpresso, 30 °C O/N, Total protein

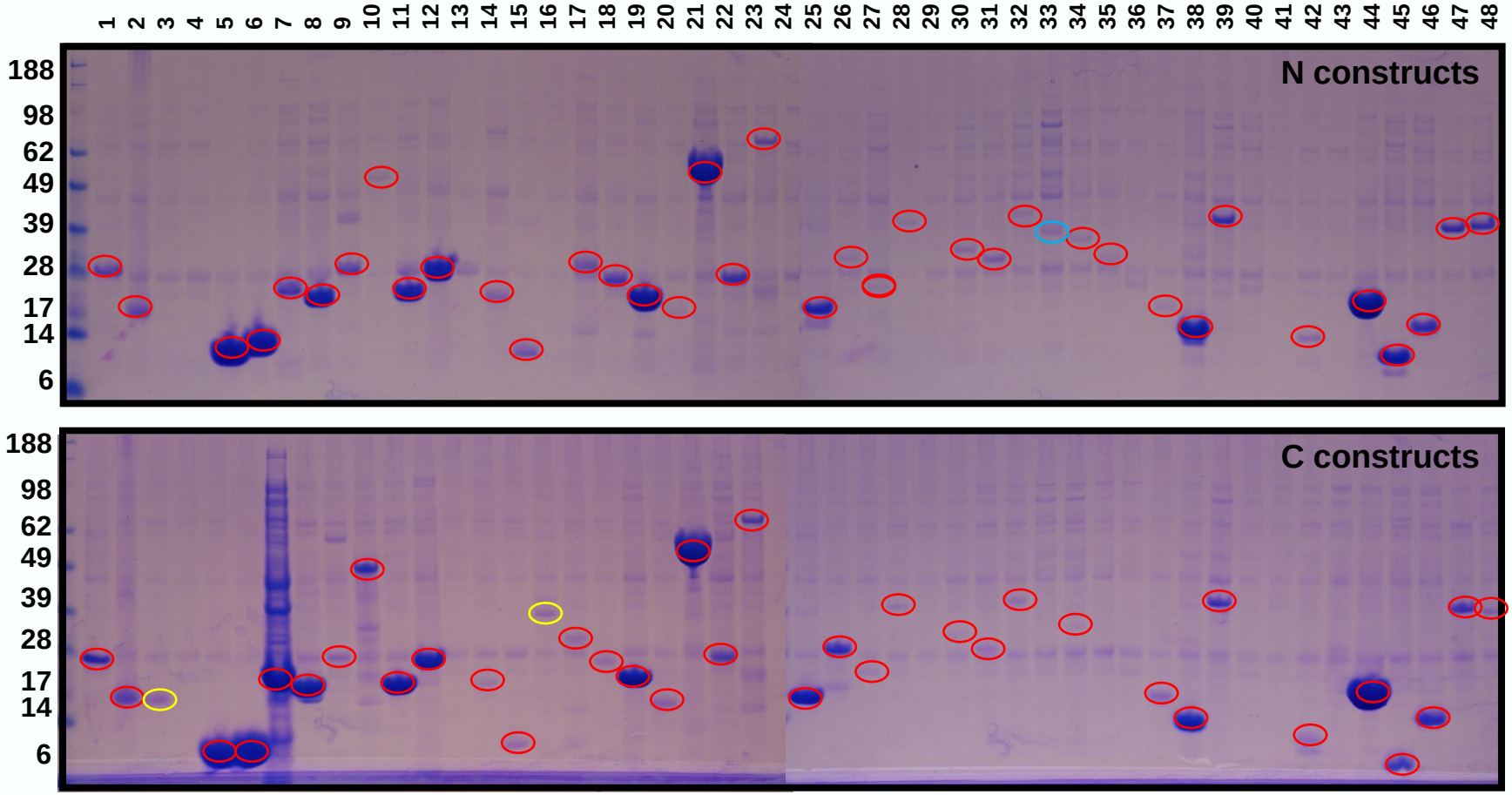


McKinstry et al, unpublished data

Comparison of expression levels and solubilities of Salmonella FUN proteins

Host cell: E. coli BL21 AI

Enpresso, 30 °C O/N, soluble protein – IMAC purified

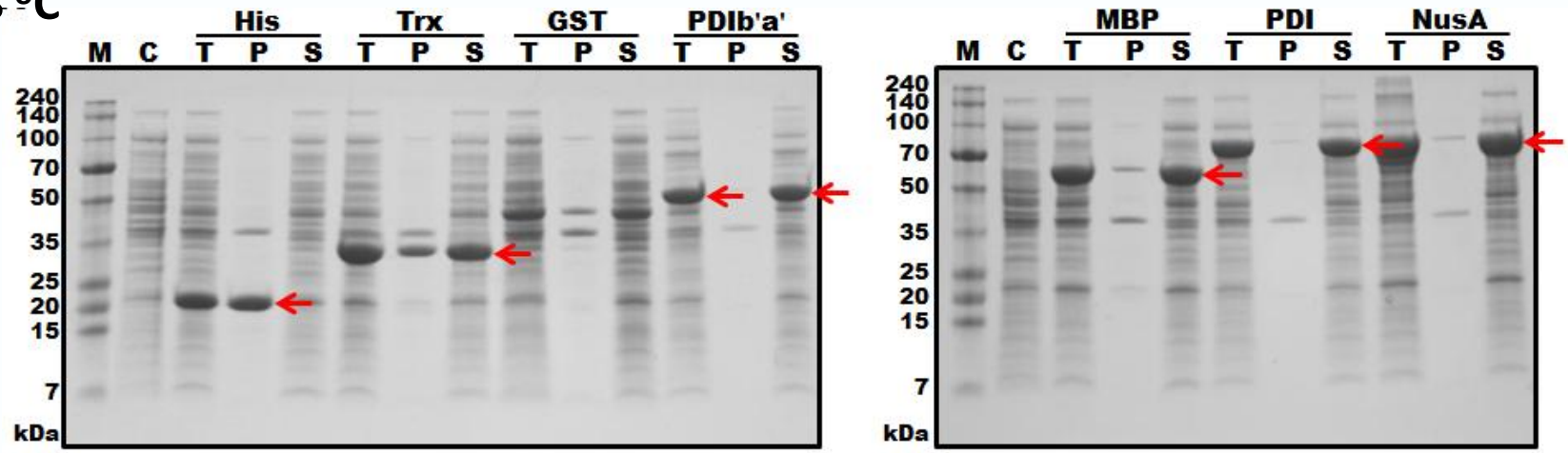


McKinstry et al, unpublished data

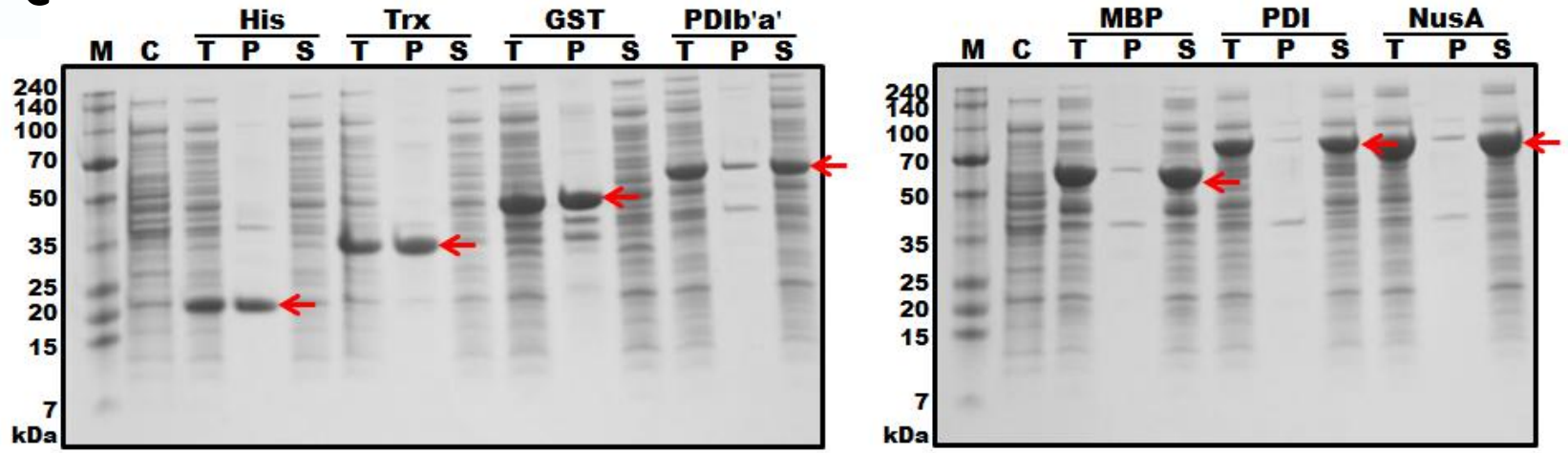
Expression levels and solubilities of hG-CSF fusion proteins

Host cell: E. coli Origami 2(DE3)

18 °C



30 °C

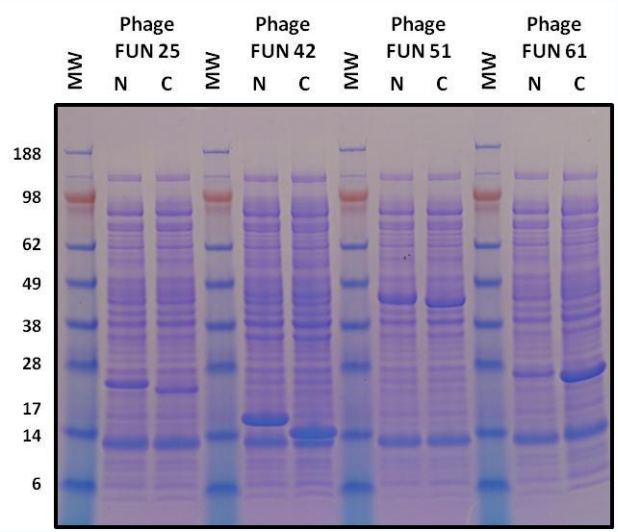


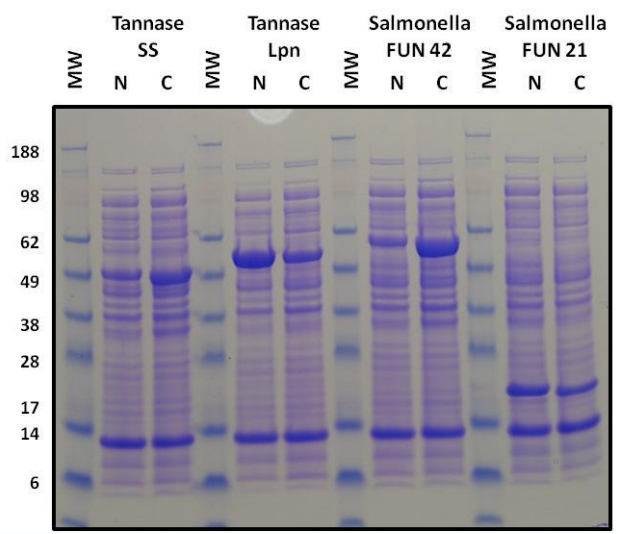
Do BH, Ryu HB, Hoang P, Koo BK, Choe H (2014). PLoS One, 9: e89906

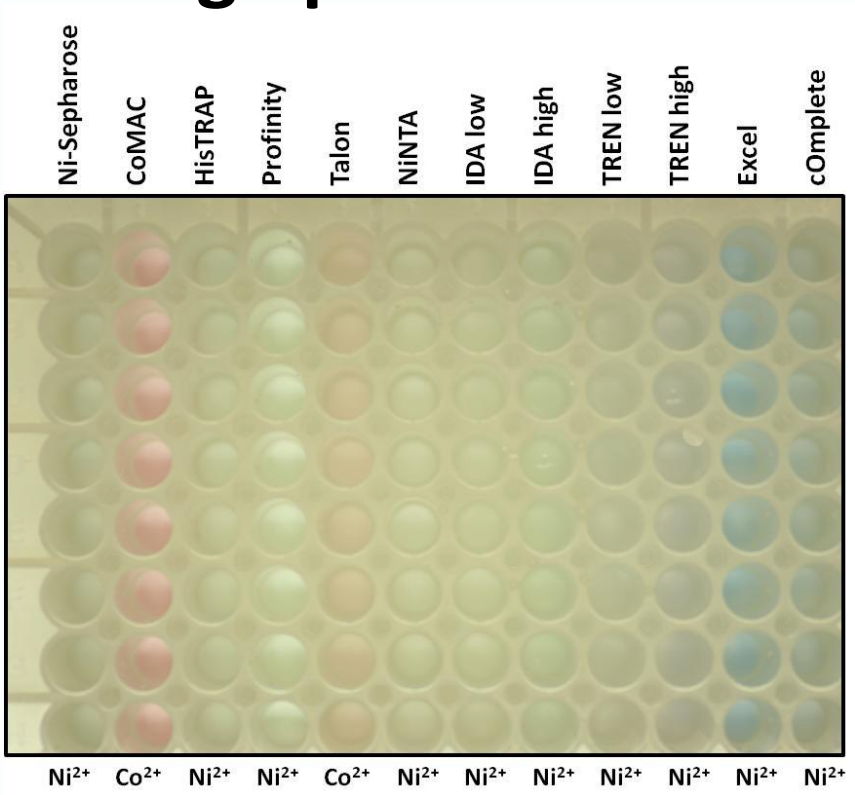
High-throughput IMAC screening - resins

Resin	Metal	Chemistry	Bead	Chemical compatibility
Chelating sepharose fast flow	Ni^{2+}	iminodiacetic acid	X-linked agarose,	20 mM βME, 500 mM imidazol, 6 M guanidine-HCl, 8 M urea; avoid EDTA
Co MAC His Bind Fractogel	Co^{2+}	iminodiacetic acid	Tentacle polymethylacrylate	
HisTRAP FF	Ni^{2+}	Propriety	Sepharose 6	5 mM DTT, 20 mM βME, 5 mM TCEP, 500 mM imidazol, up to 100 mM TRIS, HEPES, MOPS buffers, 6 M guanidine-HCl, 8 M urea; avoid EDTA
Profinity	Ni^{2+}	iminodiacetic acid	UNOsphere	< 0.1 mM EDTA/EGTA, <30 mM βME, 5 mM DTT, 10 mM TCEP, 500 mM imidazol, up to 50 mM TRIS, HEPES, MOPS buffers, 6 M guanidine-HCl, 8 M urea
HiTRAP Talon crude	Co^{2+}	tetra-dentate chelator	X-linked agarose	10 mM βME, 500 mM imidazol, up to 50 mM TRIS, HEPES, MOPS buffers, 6 M guanidine-HCl, 8 M urea; avoid DTT and EDTA
Ni-NTA Agarose	Ni^{2+}	nitrilotriacetic acid	Sepharose CL-6B	10 mM DTT, 20 mM βME, 500 mM imidazol, 6 M guanidine-HCl, 8 M urea; avoid EDTA
Workbeads IDA40 low	Ni^{2+}	iminodiacetic acid	X-linked agarose	6 M guanidine-HCl, 8 M urea; avoid EDTA
Workbeads IDA40 high	Ni^{2+}	iminodiacetic acid	X-linked agarose	6 M guanidine-HCl, 8 M urea; avoid EDTA
Workbeads TREN low	Ni^{2+}	Tris(2-ethylaminoethyl)-amine	X-linked agarose	6 M guanidine-HCl, 8 M urea; avoid EDTA
Workbeads TREN high	Ni^{2+}	Tris(2-ethylaminoethyl)-amine	X-linked agarose	6 M guanidine-HCl, 8 M urea; avoid EDTA
HisTRAP Excel	Ni^{2+}	Propriety	X-linked agarose	5 mM DTT, 5 mM TECP, 20 mM βME, 10 mM EDTA, 500 mM imidazol, 6 M guanidine-HCl
Complete IMAC	Ni^{2+}	Not stated	Sepharose CL-6B	10 mM DTT, 10 mM EDTA, 500 mM imidazol, 6 M guanidine-HCl, 8 M urea

High-throughput IMAC screening - proteins

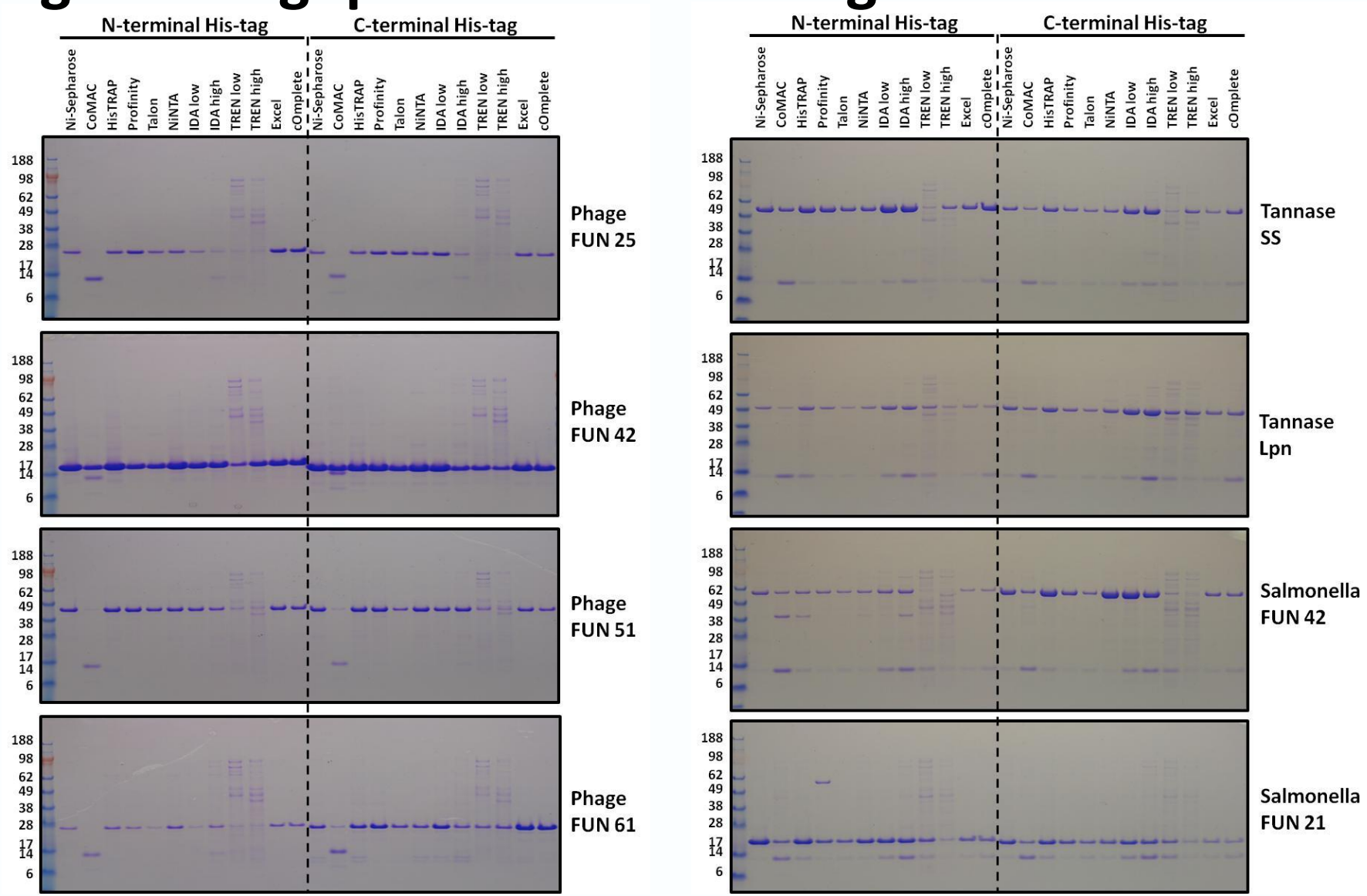






Binding	**TBS + 150 mM NaCl, 10 mM imidazole, 5 mM DTT**
Wash 1	**TBS + 150 mM NaCl, 10 mM imidazole, 5 mM DTT**
Wash 2	**TBS + 150 mM NaCl, 50 mM imidazole, 5 mM DTT**
Elution	**TBS + 150 mM NaCl, 250 mM imidazole, 5 mM DTT**

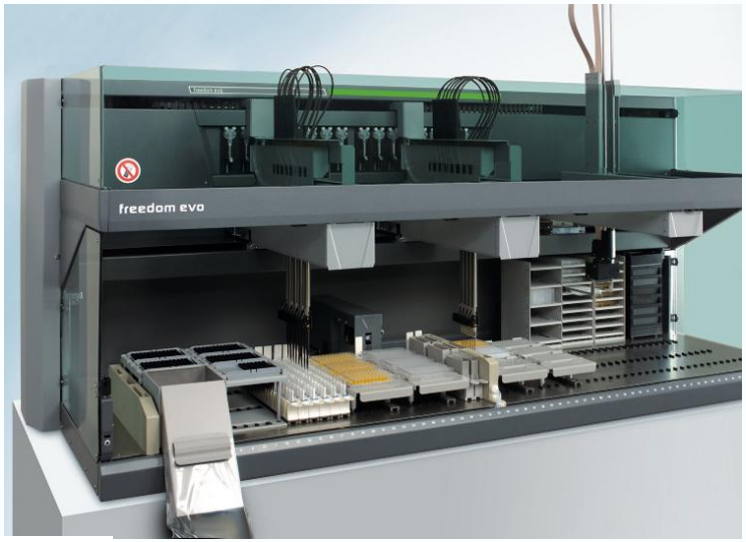
High-throughput IMAC screening - results

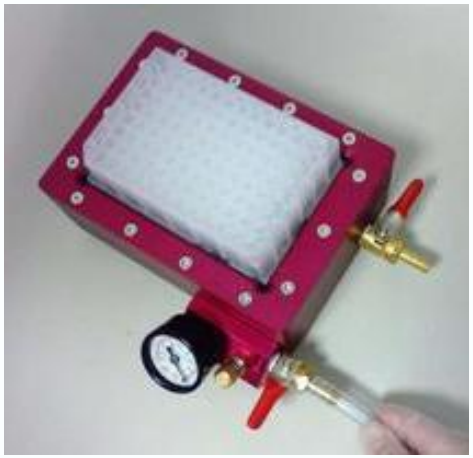


Protein purification – tips

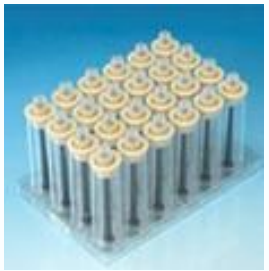
- DTT is good (intracellular proteins)
- low temperatures are good
- glycerol is good
- speed is good
- consider using 300 – 500 mM NaCl
- NaCl is not always the best, try ammonium sulphate, sodium phosphate, potassium acetate
- gel filtration is not a purification step
- refolding does not always work
- include cofactors, ligands, protein stabilisers

High-throughput technology and labware



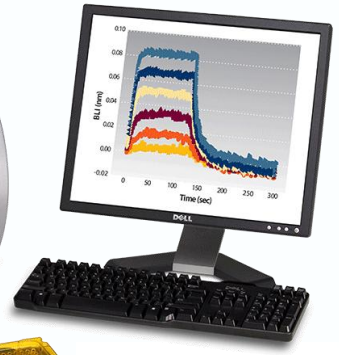


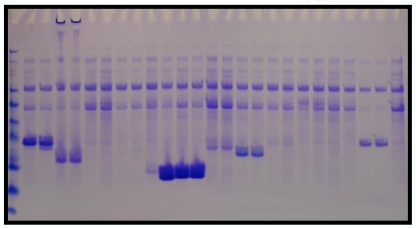
High-throughput expression systems

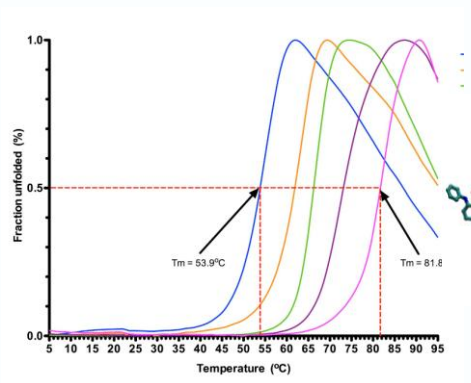


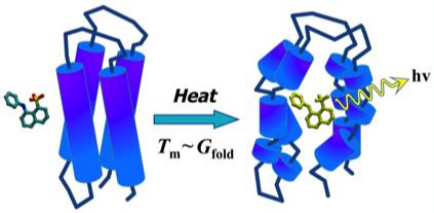
Protein extraction and purification- technology

High throughput protein analysis- technology









OTHER BACTERIAL RECOMBINANT PROTEIN EXPRESSION SYSTEMS

Host	Main Features	Case Protein
Caulobacter crescentus	ABC transporter secretion system, easy purification of secreted RSaA fusions	Haempoietic necrosis virus capsid
Rodhobacter sphaeroides	Membrane/LH2 fusion proteins	Membrane proteins
Cold adapted bacteria	Improved protein folding	Fab fragments, NGF, β-lactamase, peptidases, glucosidase
Pseudomonas sp.	Efficient secretion	G-CSF, SC Fv, Penicillin G acylase
Halophilic bacteria	Solubility favoured	β-lactamase, nucleotide diphosphate kinase
Streptomyces sp.	Efficient secretion	TB antigens, trypsin
Nocardia sp.	Efficient secretion	lysine 6 aminotransferase
Mycobacterium smegmatis	PTMs	Hsp-hIL-2 fusion, TB antigens
Coryneform sp.	High level production and secretion;GRAS	glutaminase, transglutaminase, cellulases
Bacillus sp.	High level production and secretion	β-galactosidase, disulphide isomerase, Abs, subtilisin, amylases
Lactococcus lactis	Single membrane envelope , secretion, GRAS	Fibronectin- binding protein , internalin A, GroEL
Lactobacillus sp.	Secretion, GRAS	β-galactosidase, chemokines, PA-1, pediocin, VP2/VP3 IPNV

IF ALL ELSE FAILS

CSIRO Recombinant Protein Production Facility

Contact: Bill McKinstry (bill.mckinstry@csiro.au)
Website: www.csiro.au/places/Recombinant-Protein-Facility

UQ Protein Expression Facility

Contact: Linda Lua; (l.lua@uq.edu.au; pef@uq.edu.au)
Websites: http://pef.aibn.uq.edu.au/
http://www.aibn.uq.edu.au/nbf

Monash University Protein Production Unit

Contact: Noelene Quinsey; (noelene.quinsey@monash.edu)
Websites: www.monash.edu.au/research/infrastructure/platforms/protein.html
www.med.monash.edu.au/biochem/protein-production-capability.html

Websites

Bioinformatics and tools

www.expasy.org
www.ccmb.med.umich.edu/bioinf-core/tools
www.ebi.ac.uk/services/proteins
www.bioinformatics.org/wiki

Plasmids

www.addgene.org
www.thesgc.org/reagents-resources

Synthetic genes

www.dna20.com
www.genscript.com
www.lifetechnologies.com/au/en/home/life-science/cloning/gene-synthesis.html

Recombinant proteins

www.embl.de/pepcore/pepcore_services/index.html
www.genscript.com/protein_news.html
www.oppf.rc-harwell.ac.uk/OPPF/
http://biosilta.com/recombinant-proteins/
http://en.wikipedia.org/wiki/List_of_recombinant_proteins
www.nigms.nih.gov/Research/SpecificAreas/PSI/Pages/default.aspx
www.gelifesciences.com/handbooks

Websites

http://wolfson.huji.ac.il/expression/bac-strains-prot-exp.html
http://wolfson.huji.ac.il/purification/index.html
www.piercenet.com/method/overview-affinity-purification

Protein structure

www.pdb.org
www.sbkb.org

Structural genomics

www.nigms.nih.gov/Research/SpecificAreas/PSI/Pages/default.aspx
www.thesgc.org
www.jcsg.org
www.nesg.org
www.nysgrc.org
www.csgid.org
www.ssgcid.org
www.uwstructuralgenomics.org
www.mcsg.anl.gov
www.strgen.org

Thank you

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Research Team Leader

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