Overcoming the difficulties in obtaining pure recombinant protein



Sandrine Thieffine, Rahul Yadav¹, Stephanie Duclos¹, Hara Black¹, James Errey¹, John Barker¹, Dirk Ullmann²

¹Evotec (UK) Ltd. Abingdon, United Kingdom, ²Evotec (München) GmbH, Munich, Germany

Summary

Evotec is a recognised leader in pharmaceutical research and offers a comprehensive range of capabilities to support drug design, discovery and development. High quality protein for biochemical assay and structural biology studies is essential for any drug discovery project. A challenging target may require multiple optimisation rounds to obtain soluble, pure and active protein. Here we present diverse methodologies to overcome those challenges through construct design, vector and host selection. We also present the rigorous control methods necessary to ensure the high quality of protein produced for a variety of applications.

From gene to protein (flexible entry points)

Proven track record

Proven track record in protein production on a wide range of drug targets including enzymes, cytokines, growth factors, hormones, receptors, transcription factors, antibodies, antibody fragments

Unique expertise in protein engineering multiple expression hosts





Unique expertise	2	high-throughput protein production, labelled proteins, secreted proteins, membrane proteins and protein refolding all enhanced with		Insect			Tagging	- SPR	
		structure guided construct design							
One-ston		One-Stop Service available: from gene design, protein expression	Construct			protein	Isotope labelling	NMR	NMR
shop	3	to purification and functional characterisation. Parallelisation of workflows to maximise efficiency and minimise costs	design	Mammalian			Endotoxin-free	- In vivo	
Flovible		Experienced protein production team, Lean 6σ process trained, flexible resource across three sites. Proactive project management		м	٢		Complexes		
resource	4	open communication with scientist to scientist dialogue, extension						Bio	
		of your lab		Yeast			Antibody antigen	Chem	
Customised solutions	5	 We offer customised solutions, bespoke and modular workflows, flexible production scale, capacities ranging from pilot optimisation to 450 L fermenter 							
							L	Antigen	

Construct design for improved solubility								
Secondary structure	Homology model	Sequence analysis	Panel of new constructs					
Domain identification, flexible loops, disordered area <i>etc</i> . using bioinformatics tools	Built using MOE based on known structures pinpoints active site architecture, hydrophobic patches, disulphide bridges <i>etc</i> .	Modifications identified for increased expression, solubility and/or crystallisability	New sequences with new boundaries, tags and mutations					

Expression scouting across multiple hosts

End Use	Target	Starting Point	Outcome	Resource	Timelines
Assays and crystallography	Cytosolic	Construct design	3 mg/L, >90% purity	1 FTE	2 months

• Aim: Identify expression conditions across 3

hosts

- Minimum input, maximum information output approach.
- Multiple vector selection for various expression strategies/host
- Established high-throughput downstream workflows for construct characterisation
- 1 FTE/month for analysis and design of up to 300 constructs through the above workflow

ATG CAG GGG ATC CTA....



- Expression optimisation carried out with different constructs design, Host, multiplicities of infection and timepoints
- Target showed low expression in mammalian cells and no expression in *E. coli*
- Baculovirus expression system gave high expression
- Protein expression led to successful purification with high yields
- High quality protein enabled multiple assays and shortened project timelines



Protein purification development

End Use	Target	Starting Point	Outcome	Resource	Timelines
NMR	Cytosolic	Construct design	>100 mg, >95% purity	1.2 FTE	3 months

• Aim: Produce homogeneous labelled and nucleotide loaded protein for NMR



Protein quality control....on top of the traditional





• Expression optimisation carried out with different tagging strategy and *E.coli* conditions

- Large-scale at 20 L scale using optimum conditions
- Purification refinement carried out with diverse affinity chromatography and size exclusion
- Nucleotide loading boosted to >95%
- QC by Mass Spec, HPLC and aSEC confirmed protein homogeneity and quality



QC extendable to comprehensive biophysics platform and complex assays