



Gene to Protein

<i>E. coli</i> , <i>Pichia pastoris</i> , Insect cell/baculovirus, Mammalian cell	• Clone the gene of interest into chosen expression vector • Small-scale expression screening • Large-scale (≥ 1 L) expression culture • Primary purification
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Molecular Engineering

Gene synthesis	Generate the DNA sequence of a gene of interest. Codon optimisation (optional) for the host expression system.
Expression construct	Clone the gene of interest into chosen expression vector. DNA sequence verification of cloned construct.
Mutagenesis	Single or multiple nucleotide base change e.g. amino acid substitution, insert/delete restriction enzyme site, stop codon and small tag. DNA sequence verification of cloned construct.
Plasmid production	Production and purification of plasmid DNA from mini- to mega-prep scale.

Expression Optimisation

<i>E. coli</i>	Expression screen in multiple conditions. Each construct tested in two different expression strains, two temperatures and two media. Analyse expression and Ni-NTA binding by SDS-PAGE. Resin-binding assay for 6xHis tagged constructs only. Determine optimal expression condition.
<i>Pichia pastoris</i>	Expression screen of 10 yeast clones per construct. Analyse expression and Ni-NTA binding by SDS-PAGE. Resin-binding assay for 6xHis tagged constructs only. Determine optimal expression condition.
Insect cell/baculovirus	Expression screen in multiple conditions. Each construct tested in two cell lines and at two temperatures. Analyse expression and Ni-NTA binding by SDS-PAGE and/or Western blot. Resin-binding assay for 6xHis tagged constructs only. Determine optimal expression condition.
Mammalian cell	Transient expression screen in two cell lines Analyse expression and Ni-NTA binding by SDS-PAGE and/or Western blot. Resin-binding assay for 6xHis tagged constructs only. Determine optimal expression condition

<i>In vitro</i> cell-free	Expression screen using <i>Leishmania tarentolae</i> lysate. Analyse expression by SDS-PAGE.
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Scale-up Expression

<i>E. coli</i> or <i>Pichia pastoris</i> expression in shake flask	Expression culture at ≥ 1 L in shake flasks. Cells and/or supernatant collected at optimal time of harvest. Analysis by SDS-PAGE.
Insect cell/baculovirus expression in shake flask	Scale-up of virus stock. Expression culture at ≥ 1 L in shake flasks. Cells and/or supernatant collected at optimal time of harvest. Analysis by SDS-PAGE and/or Western blot.
Mammalian cell transient expression in shake flask	Maxi-prep plasmid production. Expression culture at ≥ 1 L in shake flasks. Cells and/or supernatant collected at optimal time of harvest. Analysis by SDS-PAGE and/or Western blot.
<i>E. coli</i> or <i>Pichia pastoris</i> fermentation in bioreactor	Fermentation in a stirred tank bioreactor, from 2 L to 20 L. Analysis by SDS-PAGE.
Insect or mammalian cell expression in bioreactor	Expression in a WAVE bioreactor, from 1 L to 25 L. Analysis by SDS-PAGE and/or Western blot.

Protein Purification

Primary purification from cell pellet	Sample extraction followed by affinity chromatography (e.g. IMAC, GST, MBP, Strep(II), heparin). Analysis by SDS-PAGE.
Primary purification from supernatant	Tangential flow ultrafiltration for sample concentration and buffer exchange. Primary purification by affinity chromatography (e.g. IMAC, GST, MBP, Strep(II), heparin, Protein A). Analysis by SDS-PAGE.
Secondary or polishing purification	Secondary or polishing purification to improve product purity by chromatography techniques such as affinity, ion exchange, hydrophobic interaction or size exclusion. Analysis of by SDS PAGE.
Fusion tag removal	Scout optimal protease cleavage condition to remove fusion tag from protein. Proteases used are Enterokinase, Factor Xa, HRV-3C, SUMO, TEV or Thrombin. Chromatography to recover untagged protein. Analysis by SDS PAGE.
Endotoxin removal	Removal of endotoxin from purified protein. Analysis by SDS-PAGE and endotoxin determination by FDA-licensed LAL cartridges.

Protein Characterisation and Analysis	
Endotoxin measurement	Endotoxin level determination by FDA-licensed LAL cartridges (for samples prior to animal studies)
Mass spectrometry – intact protein analysis	Accurately determine native protein molecular weight of purified sample
Mass spectrometry – peptide mass fingerprint	Identification of purified protein or excised band from polyacrylamide gel
Analytical size exclusion chromatography	Estimate native protein molecular weight, determine multimeric structure and degree of aggregation
Dynamic light scattering	Determine size distribution profile of proteins and protein complexes. Suitable for high throughput detection of protein aggregation, thermal stability and storage conditions
Asymmetric flow-field flow-fractionation	Accurately determine size distribution profile of virus-like particles and protein complexes
Transmission electron microscopy	Imaging and morphological characterisation of virus-like particles and protein complexes

Please note the services listed here are indicative of typical processes. Individual processes can be tailored to suit the needs of the user.