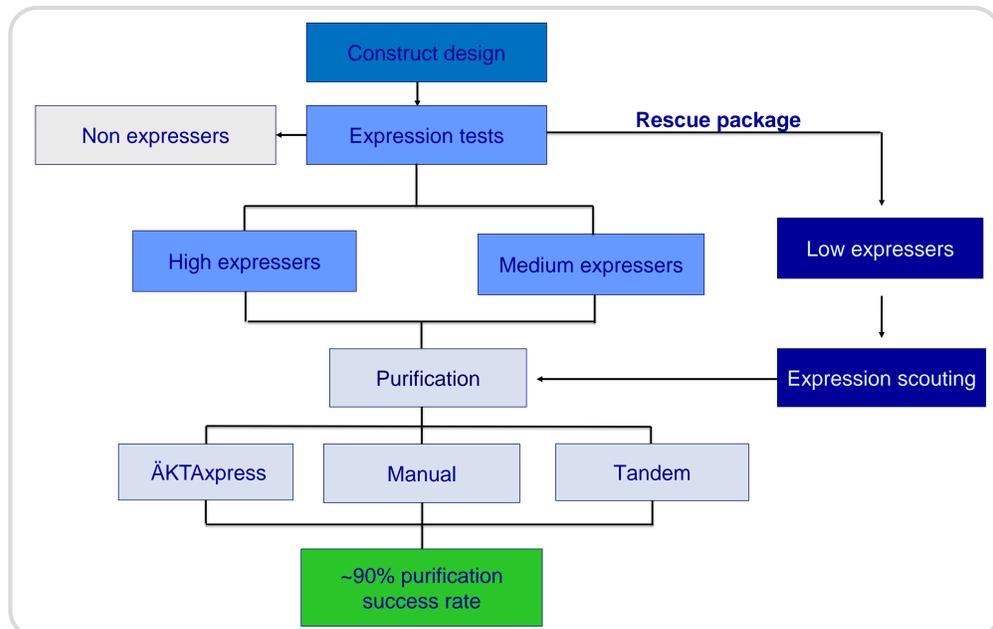


Towards a LEANer platform for parallel protein production



Summary

Evotec is a recognised leader in pharmaceutical research and offers a comprehensive range of capabilities to support drug design and discovery. Evotec's in-depth experience across multiple expression and purification systems can offer high quality proteins up to a hundred milligram scale for a variety of applications, including biophysics (e.g. X-ray crystallography) and bioassays (SPR, NMR, etc.). High-throughput processes have been developed and optimised based on LEAN 6 σ principles for rapid production of large numbers of protein targets. The workflow includes expression in bacteria, insect, yeast or mammalian cells, expression scouting and construct triage, and semi-automated parallel purification.



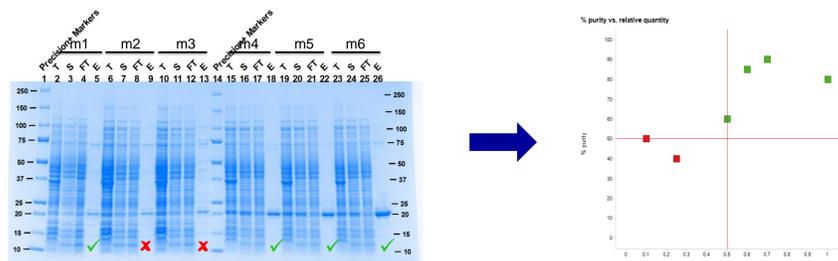
Small scale expression scouting and construct triage

Evotec routinely runs campaigns for expression and purification of multiple targets in bacteria, insect and mammalian cells. Total expression and protein yields are determined through pull-downs followed by SDS-PAGE and densitometry. Time and reagent requirements are significantly reduced by designing a workflow around semi-automated devices such as iBlot™ for protein transfer and SNAP ID™ for immunodetection. PhyNexus™ robot allows isolation of pure, concentrated sample from small-scale expression for multiple constructs in parallel.



Experimental set up for rapid parallel processing of multiple constructs

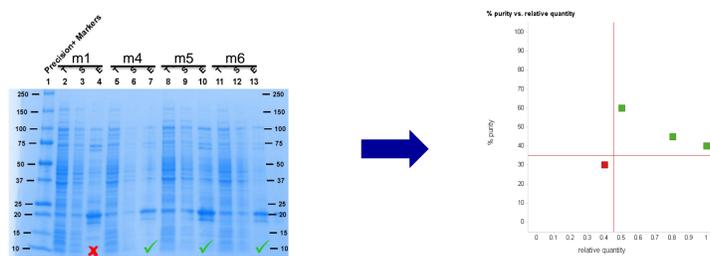
Expression data is used to triage the constructs by densitometry analysis of their purity and quantity. Based on a substantial amount of data sets, a model has been devised in order to predict the likelihood of success at the purification step. Constructs predicted to fail are rejected at this stage while constructs that go through are categorised according to expression level and the culture volume adjusted accordingly.



Early stage construct triage is the basis of this Lean workflow

Large scale expression

Successful constructs at small scale expression scouting are expressed in large scale and analysed. Their expression data are fitted to the model for a second round of triaging to account for culture scale-up. The expression profile will determine the purification method of choice in order to maximise final yield and purity.



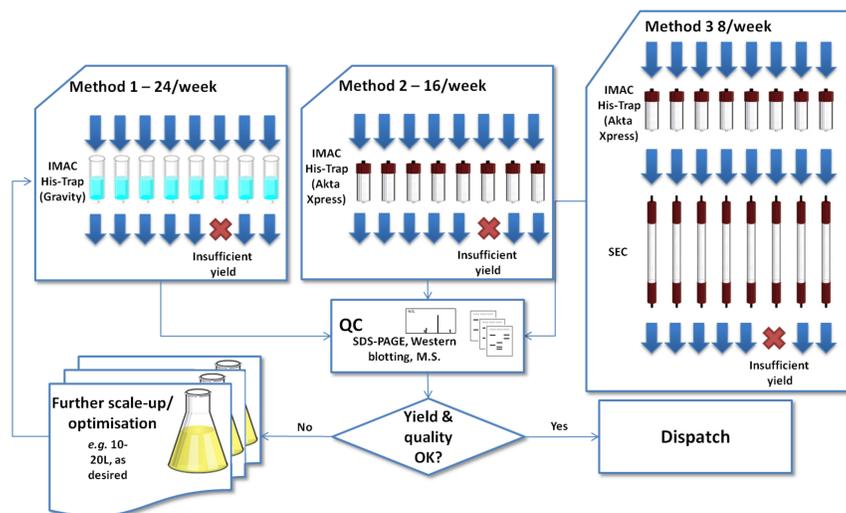
Second round of construct triage ensures high purification success rate and cost efficiency

Semi-automated parallel protein purification

Three different purification methods are offered depending on the expression profile and the desired downstream application.

- One-step IMAC on an ÄKTExpress
- Manual method by gravity flow
- IMAC in Tandem with SEC

This simplified and streamlined process provides the capacity to perform a high throughput experiment in a reproducible, parallel and cost efficient manner.



Experimental set up for rapid parallel processing of multiple constructs

Typical yields are in the range of 5 to 100 mg of pure protein allowing for a large number of experiments to be performed on each target in an economical fashion.



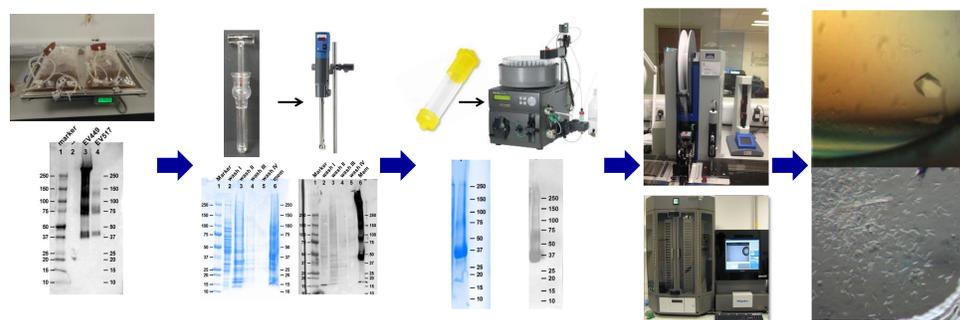
A case study using the Tandem method: from cell pellet to initial crystal hits in four days

Constructs that showed poor expression can follow an alternative path where expression in different cell lines and conditions is explored. Once expression levels have been improved, these constructs can be purified with the standard workflow.

Membrane proteins: from construct design to crystal structure

Evotec's established membrane protein capabilities have been expanded and adjusted to a parallel, LEANer workflow. Such a workflow includes:

- Construct design
- Expression screening
- Fluorescence based solubilisation condition screening
- Ligand binding assays
- Crystallisation trials



Membrane protein production and crystallisation workflow

Conclusion

Evotec is well-equipped to provide soluble and membrane protein production and structural biology activities as a service and has seen increased client demand for high throughput protein production for biophysics and crystallography. A robust and LEAN platform has been developed to screen for expression and triage constructs for parallel purification. In this way, we deliver high quality proteins for multiple applications in a rapid and cost efficient way. Evotec has expertise in screening of targets, reagent production and structural characterisation, offering integrated and stand-alone projects with disparate entry points to meet client requirements. A flexible and modular approach sets us apart from our competitors.