VIB Protein Core

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Activities

The PSF offers solutions for producing mg amounts of research grade proteins, or to optimize expression vectors and production conditions in different systems. When the pure protein is not the endpoint, also ***Conjugation*** of the purified protein is possible. The PSF services include scale up of microbial cultures up to 6 or 20 L, mammalian cell culture up to 12 L, and downstream processing and purification adapted to those scales. The deliverable is the end product of your process (usually a protein) and a documentation that each step is carried out to the state of the art. In order to rapidly optimize expression analysis, PSF uses a technology platform for parallel expression analysis with numerous possible combinations, i.e. ***FastScreen***. Expression optimization is offered in *Escherichia coli*, *Pichia pastoris* and mammalian cells (HEK293F). Matrix-approach based optimizations are also available for refolding of inclusion bodies, i.e. ***FastFold***, and for purification process optimization, i.e. ***FastClean***. Based on the ***FastScreen*** result, a ***Production*** can be performed. The ***FastClean*** can lead to a ***Purification*** strategy to make mg amounts of pure protein. Depending on the purpose of the pure protein; fluorescent labels, polymers, can be attached by ***Conjugation***. ***Protein Analytics*** can be performed to guarantee the quality of the delivered protein. Some ***Protein Analytics*** are done in house, others in collaboration with specialized groups or institutes. Not only the purity, concentration or LPS content is important but also the activity or affinity of a protein. Therefor PSF performs also different (cellular) ***Activity Assays*** and invests in technologies for ***Protein-Protein Interactions***. Beyond servicing, ***Research*** on new technologies and opportunities available on the market is done regularly by the PSF. By the enormous interest in single domain antibodies (also called nanobodies®), PSF has specialized in ***Nanobody® Production and Purification***.

Services

- **FastScreen**

FastScreen is a 3-4 week feasibility study service for Escherichia coli, Pichia pastoris and mammalian cell expression solutions that is routinely used at PSF. At the end, (± 1 month) FastScreen offers a go/no-go decision for expression strategies to our staff. The service presents comparison of several production strategies in a uniform vector frame, allowing true strategy comparison.

- **Production**

Scale up facilities for fermentation of microbial organisms up to 20 liter (fed batch fermentation) and mammalian cells up to 12 liter are available at PSF. Accordingly, standard operating procedures for collection and lysis of cells adapted to these scales are available.

- **FastFold**

Production in inclusion bodies is a well-established strategy in Escherichia coli. Inclusion bodies are usually more resistant to proteases and thus accumulate to high amounts. Also, after isolation of the inclusion bodies, the protein of interest is already reasonable pure. However, the process of refolding the denatured proteins can be a difficult task, with low yield and laborious methods. PSF offers a 2-3 week study using a matrix screening to find the optimal refolding buffer. This matrix screen is designed in-house. Also some methods of refolding can be compared (dilution, dialysis, on-column).The deliverable is a study and an outline for a refolding process, which can also be validated.

- **FastClean**

If no purification method is available, a screen can be set up to test a range of different (or combinations of) matrices to purify the protein of interest.

- **Conjugation**

Fluorescent molecules (antibodies) for microscopy are very expensive for VIB research groups. When the hybridoma cell line is available for the production of an antibody, PSF can produce easily mg amounts (1-100 mg) for labeling. A lot of labeling is done with AlexaFluor groups which are easy to use. PSF has also expression vectors available for fusion of proteins to fluorescent proteins (GFP, mCherry). Beside fluorescent labeling; small, very stable molecules like nanobodies®, alphabodies®, affibodies® are becoming more and more important for therapeutic use. A disadvantage of these molecules is their low serum half-life. This can be increased by covalent labeling with all kinds of molecules (like PEG). PSF has also in this field expertise.

- **Nanobody production and purification**

After the selection of your nanobodies® by the Nanobody Service Faility (NSF), PSF can help you with the production and purification. PSF has already produced and purified hundreds of nanobodies®: from Escherichia coli, from Pichia pastoris, with fusion tag, without fusion tag, small scale, 20 liter fermenter scale, monovalent, bivalent, fused to cytokines, ... Escherichia coli is still the most used system for the expression of monovalent nanobodies® and the easiest one. The yield varies from nanobody® to nanobody®. When you want to increase the yield, PSF can shift the production from Escherichia coli to Pichia pastoris. In Pichia pastoris is the yield much higher. Also the yield is going down from monovalent to bivalent, trivalent, ... nanobodies® in Escherichia coli. When you start with bivanlent nanobodies®, you have to shift to Pichia pastoris to be good. Also, when you want to express nanobodies® without any affinity tag, Pichia pastoris is the system to use. In this way, you can purify the nanobody® with conventional chromatography (ion-exchange, hydrophobic interaction, ...). When you start with nanobodies® fused to other proteins like cytokines, mammalian cells (HEK293F) are needed when post-translational modification is important.

- **Protein-Protein Interactions**

Folding of proteins and protein-protein interactions are becoming more and more important. A limited amount of technologies to analyze this are available in PSF and distributed over different VIB research groups (Biacore, SwitchSense, AlphaScreen). PSF wants to invest in the knowledge of these technologies and instruments when their opportunities are proven. Initial projects can be done on demo s and instruments available in the protein service facility consortium in Europe (P4EU). Important technologies:

FACS, CD spectrometry, DLS, SLS, MALS, isothermic titration calometry, microscale thermophoresis, ...

- Activity Assays

For some proteins, not only the purity, affinity, concentration, is important but also the activity. Most of these assays are cellular assays. PSF has the knowledge to perform some assays for cytokines (TNFα, IL2,) . For these cytokine assays, WHO standards are used to refer to.

Available Stock Proteins

Hamster anti-mCD11C (418)	Rat anti-mCD103	Caspase3 (murine)	LIF (murine)
Hamster anti-mCD11C (N418)AlexaFluor700 conjugate	Rat anti-mCD8α (53-6-7)	Cre	M-CSF (human)
Hamster anti-mCD28	Rat anti-mIFNγ (AN18)	FLT3 (human)	M-CSF (murine)
Hamster anti-mCD28 (37.51)	Rat anti-mIFNγ (DB1)	GM-CSF (human)	RANKL (murine) extracellular domain
Hamster anti-mCD3e (145-2c11)	Rat anti-mIFNγ (DB2)	GM-CSF (murine)	TAT-Cre
Mouse anti-hCD3 (OKT3)	Rat anti-mIL4 (11B11)	IFNγ (murine)	TNFα (human)
Rat anti-mCD103 (M290)	Rat anti-mIL5 (TRFK4)	IL1β (murine)	TNFα (human) FLAG fusion
Rat anti-mCD16/32 (2.4G2)	Rat anti-mIL5 (TRFK5)	IL2 (murine)	TNFα (murine)
Rat anti-mCD205 (NLDC145)	Rat anti-mTNFα (1F3F3D4)	IL22 (murine)	TNFα (murine) FITC conjugate
Rat anti-mCD24 (J11D)	Rat anti-mIL10 (2A5.1)	IL6 (human)	EGF165 (murine)

Selected publications

1. Generation and characterization of small single domain antibodies inhibiting human TNF receptor 1. Journal of Biological Chemistry 290, 4022-4037, 2015.
2. Phenylcoumaran benzylic ether reductase prevents accumulation of compounds formed under oxidative conditions in poplar xylem. Plant Cell 26, 3775-3791, 2014.
3. Caffeoyl Shikimate Esterase (CSE) Is an Enzyme in the Lignin Biosynthetic Pathway. Science 1241602, 2013.
4. Generation and in vivo characterization of a chimeric alphavbeta5-targeting antibody 14C5 and its derivatives. EJNMMI Research 3, 25, 2013.
5. Production of antibody derivatives in the methylotrophic yeast pichia pastoris. Methods in Molecular Biology 907, 325-340, 2012.
6. Fed-batch fermentation of GM-CSF-producing glycoengineered Pichia pastoris under controlled specific growth rate. Microbial Cell Factories 9, 93, 2010.
7. Further pharmacological and genetic evidence for the efficacy of PlGF inhibition in cancer and eye disease. Cell 141, 178-190, 2010.
8. Efficient production of human bivalent and trivalent anti-MUC1 Fab-scFv antibodies in Pichia pastoris. BMC Biotechnology 9, 2009.
9. New Strategies in Polypeptide and Antibody Synthesis: an overview. Cancer Biother Radiopharm 19, 97-107, 2004.
10. An advanced vector system for high-level recombinant gene expression in E. coli. Biotechnology International II: Latest developments in the Biotechnology industry and research. Universal Medical Press. 2, 165-172, 1999.
11. Increased stability of phage T7g10 mRNA is mediated by either a 5'- or a 3'-terminal stem-loop structure. Biol Chem 377, 811-7, 1996.
12. Tight transcriptional control mechanism ensures stable high-level expression from T7 promoter-based expression plasmids. Biotechnology 13, 175-9, 1995.
13. Versatile, multi-featured plasmids for high-level expression of heterologous genes in Escherichia coli: overproduction of human and murine cytokines. Gene 164, 9-15, 1995.

Contact

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