iba
Solutions
For Life Sciences

Protein Production &
Assays

Integrated Protein Services

Custom protein expression & purification

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Expression strategy

The first step in the recombinant protein generation process is to design an appropriate expression strategy. This needs the consideration of many factors which according to IBA's experience may be critical in relation to which is requested in the concrete case. IBA offers this to the customer as a service.

When cloning has been achieved, the next step is to perform a test expression to evaluate how efficient recombinant protein production in fact works and whether the desired purity grade is achieved by standard procedures.

Subsequent to the testing phase, the established procedure needs to be scaled up to generate the requested protein amounts.

All the services listed in this document and Figure 1 can be ordered independently from each other. E.g. if only purification is needed the service can be ordered alone.

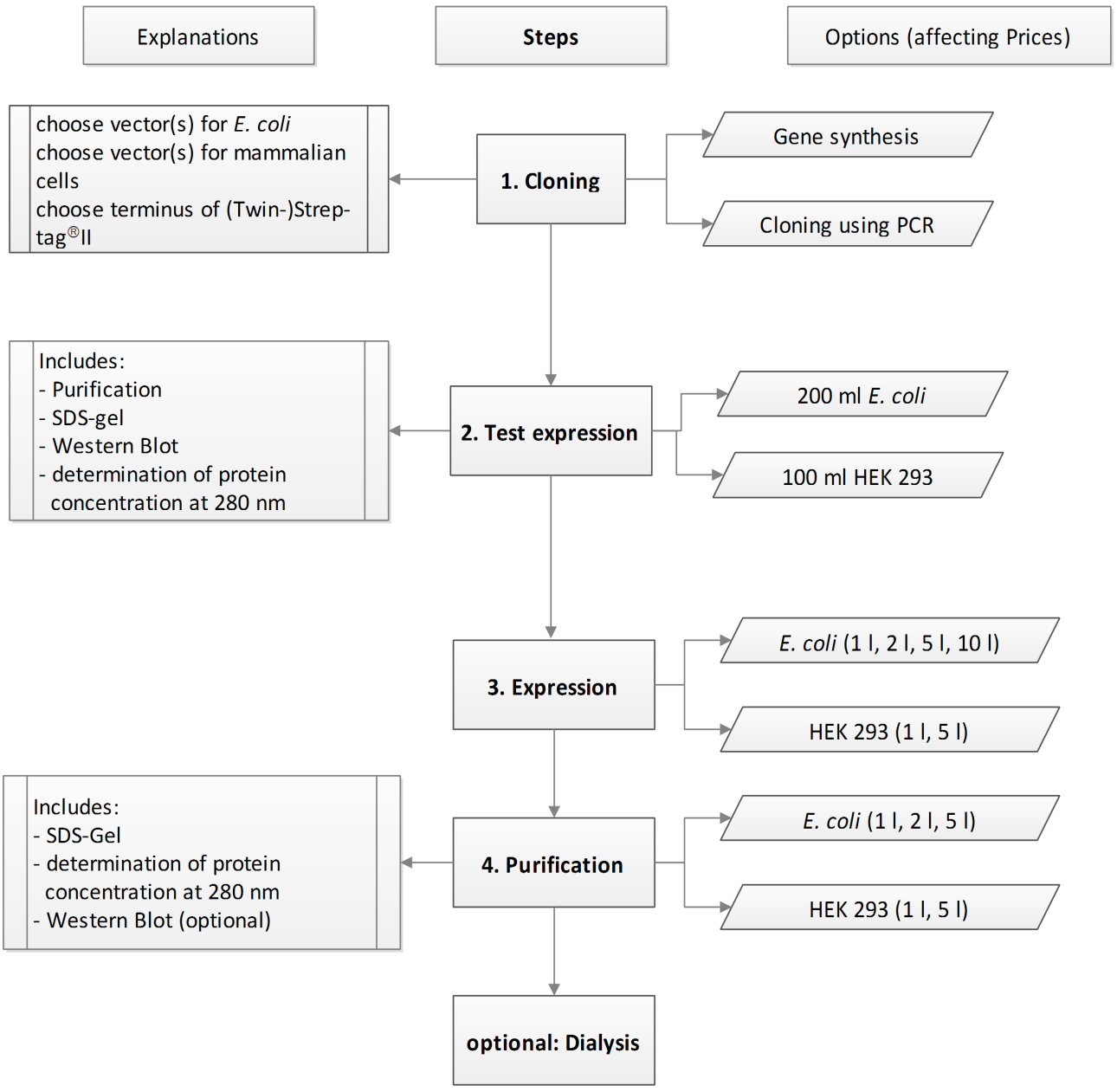

Figure 1: Custom protein production strategy from cloning to final formulation. Each service can be chosen independently from the others.

1.1 Protein expression and purification services involving the proprietary Strep-tag®II/Twin-Strep-tag® technology

Strep-tag is one of the most widely used technologies in the field of recombinant protein production and analysis offering unparalleled possibilities. IBA holds all intellectual property rights for Strep-tag®II/Twin-Strep-tag® and provides exclusive application services using this technology.

IBA's standard offer for a start-to-finish service from genes to recombinant Strep-tag fusion proteins comprises:

- Complete cloning service incl. control sequencing
- Expression in *E. coli* and mammalian HEK 293 cells
- Purification of the recombinant fusion protein

Further services including the development of downstream assays on the basis of Strep-tag®II/Twin-Strep-tag®, large-scale production of recombinant proteins (or nucleic acids) or monoclonal antibody production can be offered upon request.

Please note that IBA does not offer the refolding of insoluble proteins (e.g. proteins from inclusion bodies). If proteins are expressed in an insoluble form, IBA can denature the protein, purify it and send the purified protein in a denatured form to the customer.

1.2 Requirements for IBA's custom service

The complete gene sequence of interest in electronically readable form or its "Genbank" accession number is needed. In addition, any information concerning the biochemistry of the protein of interest would be helpful for planning a suitable expression strategy.

1.3 Integrity, secrecy and licensing

Our custom service requires the exchange of sensitive data. Therefore, all proprietary information and intellectual property transferred to IBA is kept absolutely confidential and is covered by a secrecy agreement if requested.

As a service provider we do not intend to claim any rights on proteins which we manufacture for our clients as long as the proteins are used for in-house research purposes only. A separate license for use of the Strep-tag®II/Twin-Strep-tag® technology is necessary, however, if direct commercialization of ordered proteins is intended. This is also the case if clients use the Strep-tag®II/Twin-Strep-tag® technology for the production of recombinant proteins in their own facilities with subsequent commercialization. IBA is authorized and ready to grant licenses for those purposes.

Please note that IBA is not able to provide pharma grade proteins. Therefore, all products from our service cannot be used for therapeutic issues and are only for research usage.

2 Cloning

This service comprises the precise cloning of the desired gene into IBA's StarGate expression vectors including our proprietary vectors with optimal control of bacterial expression (*tet* promoter) or mammalian expression. Due to a special cloning procedure using StarGate, we are able to insert the gene of interest into the appropriate vector yielding a protein with minimal additional amino acids which often remain from the multiple cloning sites. The resulting protein is modified only by appending purification tags to either the N- and/or the C-terminus.

General procedure:

- Consulting and planning of the cloning strategy
- *de novo* synthesis of the desired gene
- Subcloning into the appropriate expression vector and screening for positive clones
- Isolation of clonal plasmid DNA
- Control sequencing

2.1 Gene synthesis

The gene of interest (GOI) will be chemically synthesized (optimization of codon usage optional) based on the complete gene sequence of interest in electronically readable form or its "Genbank" accession number, provided by the customer.

Time required: 3-4 weeks

2.2 Cloning of the GOI into StarGate expression vectors

The gene will be cloned into the expression vector directly. The expression vector can be provided on request. The gene can be cloned into a StarGate Entry vector on request. This procedure allows you to use the StarGate system for further experiments.

For available expression vectors please refer to:

https://www.iba-lifesciences.com/cloning-expression-vectors.html

Time required: 1-2 weeks

IBA does not guarantee that the encoded gene will be expressed.

Product	Cat. No.
Gene synthesis, costs per bp	2-2002-006
Direct transfer-cloning into expression vector	2-2004-001
Each additional cloning	2-2004-011

Cloning of your gene of interest into StarGate compatible vectors is possible on request

3 Expression in *E. coli*

Strep-tag®II/Twin-Strep-tag® purification of the recombinant protein.

General procedure:

- *E. coli* test culture (200 ml) and induction of protein expression
- Strep-Tactin®/Strep-Tactin®XT affinity purification, SDS-PAGE
- Estimation of the necessary culture volume to reach the requested amount of protein
- *E. coli* culture in the desired volume, preparation and affinity purification via Strep-Tactin®/Strep-Tactin®XT
- Optional: Western Blot, Dialysis

3.1 *E. coli* test expression, purification and SDS-PAGE

A 200 ml test expression and purification via standard Strep-Tactin®/Strep-Tactin®XT affinity chromatography will be performed. At this stage the customer is able to evaluate the behavior of the recombinant protein and to estimate further culture volumes required to obtain the requested amount of protein.

3.2 Expression and purification (*E. coli*)

After test expression, the customer has a reasonable basis to choose between different *E. coli* culture scales (1 liter to 10 liter). Protein purification can be performed using Strep-Tactin®/Strep-Tactin®XT. Please refer to the order information below.

Time required: 1-2 weeks each

Product		**Cat. No.**
E. coli test expression, purification and SDS-PAGE		2-2100-100
Expression in *E. coli*	1 liter scale	2-2101-010
	2 liter scale	2-2101-020
	5 liter scale	2-2101-050
	10 liter scale	2-2101-100
Strep-tag®II/Twin-Strep-tag® purification from *E. coli* extract	1 liter scale	2-2102-010
	2 liter scale	2-2102-020
	5 liter scale	2-2102-050
	10 liter scale	2-2102-100

4 Expression in transient transfected mammalian cells (HEK 293)

Strep-tag®II/Twin-Strep-tag® purification of the recombinant protein.

General procedure:

- Transient transfection and test expression (100 ml) in HEK 293
- Strep-Tactin®/Strep-Tactin®XT affinity purification, SDS-PAGE
- Estimation of the necessary culture volume to reach the requested amount of protein
- Mammalian culture in the desired volume, preparation and affinity purification via Strep-Tactin®/Strep-Tactin®XT
- Optional: Western Blot, Dialysis

4.1 Mammalian test expression, purification, SDS-PAGE and Western Blot

A 100 ml test expression and purification via standard Strep-Tactin®/Strep-Tactin®XT chromatography will be performed. At this stage the customer is able to evaluate the behavior of the recombinant protein and to estimate further culture volumes required to obtain the requested amount of protein. Expressions can be done in HEK 293 cells.

Time required: 3-5 weeks

4.2 Expression and purification (mammalia)

After test expression, the customer has a reasonable basis to choose between different mammalian culture scales (1 liter to 5 liter). Protein purification can be performed using Strep-tag®II/Twin-Strep-tag®. Please refer to the order information below.

Time required: 6-8 weeks

Product		**Cat. No.**
Test expression in mammalian cells, purification, SDS-PAGE and Western Blot		2-2250-001
Expression in mammalian cells	1 liter scale	2-2251-010
	5 liter scale	2-2251-050
Strep-tag®II/Twin-Strep-tag® purification from mammalian extract/supernatant	1 liter scale	2-2252-010
	5 liter scale	2-2252-050

General remarks about IBA's protein expression and purification services

- The purified protein is supplied as eluted from the Strep-Tactin®/Strep-Tactin®XT affinity column in elution buffer containing desthiobiotin or biotin. Dialysis into a different buffer can be performed optionally.
- Protein concentrations are determined using the theoretical extinction coefficient at 280 nm of the recombinant protein, which is determined by the addition of the extinction coefficients of the tryptophane and tyrosine residues included in the recombinant protein or by a colorimetric assay according to Bradford with BSA as reference protein.
- IBA is not obliged to accept orders. Shipping costs are charged separately.

If you have any questions, please do not hesitate to contact us. We will be glad to assist.

Looking forward to working with you.

The IBA Team

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