

## **E. coli EXPRESSION SERVICE**

RELIATech offers a variety of custom services for gene expression (especially signal proteins from higher organisms) in *E. coli*. This “*E. coli Expression Service*” is divided into a series of steps which can be performed either individually or in combination so that you can select the steps you actually need. These services are designed to adequately cover most of your needs, from gene cloning into an *E. coli* expression vector, screening for the best vector and/or *E. coli* strain, production of biomass, as well as protein purification. Along with the regular project updates, you will receive progress reports at the end of each step of your program. You will then be asked to authorize us to proceed to the next step. Each step is described hereafter:

### **A1) Gene cloning within an *E. coli* expression vector**

The complexity of this step is highly dependent on the gene to be expressed. Conforming to customer's needs, RELIATech can perform all the experiments, including gene modification, prior to insertion into a expression vector. Alternatively, assistance is provided to the customer allowing him to choose the right expression vector and the right conditions to virtually ensure the optimal gene expression. Because *E. coli* are not able to cleave the signal peptide of secreted proteins the cDNA has to be modified in this way. Therefore the cDNAs are normally generated by PCR deleting the sequence for the signal peptide and introducing the appropriate restriction sites for cloning (in many *E. coli* expression vectors: NdeI and BamHI).

#### Material needed

- 10-30 µg purified and characterised plasmid cDNA bearing the gene to be expressed and containing the appropriate restriction sites for subcloning (e.g. NdeI/BamHI).

#### The service includes

- Subcloning of the cDNA into the *E. coli* expression vector e.g. pET-9a, pET-15bp or pCytexP3 or in an expression vector chosen by the customer.

**Note:** If there are no appropriate restrictions sites, the cDNA has to be generated by PCR introducing the appropriate restriction sites (see A2).

- Plasmid-preparation of 10 recombinant clones.
- Analysis by agarose gel electrophoresis.
- Mid-scale plasmid preparation of a positive clone.

#### Provided to the customer

- An *E. coli* expression vector containing the cDNA of interest.
- A detailed report sheet.

Expected time range:

2 - 4 weeks

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### **A2) Gene cloning within an *E. coli* expression vector by PCR**

#### Material needed

- 10-30 µg purified and characterised plasmid cDNA bearing the gene to be expressed.

### The service includes

- Generation of specific oligonucleotide primers containing the appropriate restriction sites for subcloning.

**Note:** For an optimal performance of a new protein it is recommended to test different *E. coli* strains and *E. coli* expression vectors either induced by a temperature shift or by IPTG.

**Note:** With respect to the purification procedure we would recommend introducing a “tag” at the N- or C-terminal end of the recombinant protein. There are *E. coli* expressions vectors available containing different “tags”. If the recombinant protein is used mainly in *in vitro* experiments (e.g. cell culture studies) we recommend for example a “His-tag” (6x Histidin) or a “Strep-tag II” (8 amino acids) [www.iba-go.de]. The “tag” is normally too small to interfere with the activity of the protein. For “Strep-tag II” the possibility exists to create an authentic protein by cleavage of the tag. If the recombinant protein is needed for animal studies (e.g. mouse, rat) we recommend the use of an “Fc-tag” (about 26 kDa) to increase the stability and half-life of the protein in the circulation. As an “Fc-tag” RELIA**Tech** can offer the human IgG1 and the mouse IgG 2b fragments.

- PCR with the customer’s cDNA as template.
- Characterisation by agarose gel electrophoresis.
- Subcloning of the PCR-fragment in the *E. coli* expression vector e.g. pET-9a, pET-15bp or pCytexP3 or in an expression vector chosen by the customer.
- Plasmid-preparation of 10 recombinant clones.
- Analysis by agarose gel electrophoresis.
- Mid-scale plasmid preparation of a positive clone.
- Verification by sequencing.

**Note:** All cDNA’s generated by PCR have to be completely sequenced due to possible mutations!

### Provided to the customer

- an *E. coli* expression vector containing the cDNA of interest.
- A detailed report sheet.

Expected time range:

4 - 6 weeks

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## **B) Generation of a recombinant *E. coli* strain**

### Material needed:

- 10-30 µg purified and characterised *E. coli* expression vector DNA bearing the gene to be expressed. The vector is either provided by the customer or prepared at RELIA**Tech** if the service A was also performed.

**Note:** For an optimal performance of a new protein it is recommended to test the bacterial host by using several different *E. coli* strains for the analytical production, e.g. BL21-CodonPlus<sup>TM</sup>(DE3)-RIL, BL21 Star<sup>TM</sup>(DE3), and TG-1.

### The service includes

- Generation of competent cells of the appropriate *E. coli* strain using standard protocols.
- Transformation with the *E. coli* expression vector.
- Isolation and cultivation of 6 single clones over night
- Analytic production (1ml scale); Induction by temperature shift or IPTG.
- SDS-PAGE and subsequent Coomassie stain of total lysate from induced/non-induced samples.

Provided to the customer

- A detailed report sheet.

Expected time range:

1.- 3 weeks

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**C) Optimisation of the conditions for production**

Material needed:

- Competent *E. coli* strain evaluated under point B.

The service includes

- Generation of pre-cultures and subsequent induction of protein production by different incubation periods and either different concentrations of IPTG or temperature shift.
- Preparation of inclusion bodies (IB) and cytosolic fraction (CF).
- SDS-PAGE and subsequent Coomassie stain of IBs and CFs from induced/non-induced samples.

Provided to the customer

- A detailed report sheet.

Expected time range:

2 - 3 weeks

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**D) Generation of biomass (2L scale)**

Material needed:

- Competent *E. coli* strain evaluated under point B.

The service includes

- Generation of the pre-culture and subsequent induction of protein production by the concentration of IPTG evaluated under point C or temperature shift.
- Time of incubation as determined under point C.
- Harvesting of the biomass.

Expected time range:

1 week

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**E) Establishing of the purification protocol**

Material needed:

- Biomass either provided by the customer or prepared at RELIA*Tech* if the service D was also performed.

The service includes

*1. Preparation of the „Inclusion Bodies“ (IB)*

- Disintegration of bacteria by lyses buffer and sonification.
- Accumulation of „IBs“ by several centrifugation steps.
- Check of „IB-preparation“ by SDS/PAGE and Coomassie stain.

## 2. Solubilisation of „Inclusion Bodies“

- Solubilisation of „IBs“.
- Check of „IBs“ by SDS/PAGE and Comassie stain.

**Note:** The proceeding depends on the protein to be purified whether (1) there already exist an established purification protocol, (2) the protein is fused to a “tag”, (3) the native protein is a dimer, or (4) there has to be established a new protocol. In the case of point 3 the next step would be the dimerization reaction followed by a gel filtration to separate monomers from dimers. In case of point 4 one would start with ion exchange chromatography (e.g. anion/cation).

### Provided to the customer

- All purified protein.
- A detailed report sheet.

Expected time range:

depends on the protocol!

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