

# Escherichia coli

## Expression vector

### pOPE101



<b>Vector Name</b>	pOPE101 (for details see page 3)
<b>Category</b>	Cassette vector for the expression of functional recombinant single-chain Fv antibody fragments in <i>Escherichia coli</i>
<b>Application</b>	Cloning of heavy and light chain variable domain genes of Fv antibody fragments selected from hybridomas, single B-lymphocytes or phage display libraries. Cloning sites: <i>NcoI</i> and <i>HindIII</i> for the heavy chain V <sub>H</sub> gene <i>MluI</i> and <i>NotI</i> for the light chain V <sub>L</sub> gene. Expression of single-chain Fv antibody fragments in <i>Escherichia coli</i> and secretion of functional antibody fragments into the periplasm.
<b>Vector Size</b>	3970 bp
<b>Resistance</b>	The vector contains an ampicillin resistance gene.
<b>Purification</b>	Qiagen Plasmid Purification Kit
<b>Volume/Format</b>	10 µL; concentration is 0.5 µg/µl in TE buffer
<b>Stability/Storage</b>	Stable for minimum 1 year when stored at -20 °C upon receipt.
<b>Quantity</b>	5 µg

**For research use only !**

#### Reference

- Schmiedl, A., Breitling, F., Winter, C. Queitsch, I. and Dübel, S. (2000) Effect of engineered Cysteines on yield, solubility and activity in various recombinant antibody formats expressed in *E. Coli*. *J. Immunol. Meth.* 242, 101-14.
- Schmiedl, A. and Dübel, S. (2001) Functional Minipreps. In: *Antibody Engineering*, ed: R. Kontermann and S. Dübel, Springer Verlag, Heidelberg.

Cat. No. PR3004

# Escherichia coli Expression Vector pOPE101



***Cassette vector for the expression of functional recombinant single-chain Fv antibody fragments in Escherichia coli***

<b>Cat. No.:</b>	<b>Vector Name</b>	<b>Quantity</b>
PR3004	pOPE101	5.0 µg

**Storage upon receipt:** -20 °C

**In vitro Test** FOR RESEARCH USE ONLY

## 1. Introduction

Complete immunoglobulin molecules cannot be produced in *Escherichia coli*, but within the past decade, recombinant antibody technologies have been widely used to express various recombinant single-chain Fv antibody fragments (scFv) of different specificity. ScFv antibody fragments have first been deposited as cytoplasmic inclusion bodies followed by refolding *in vitro*. Due to the general low folding efficiency, however, another strategy has been developed which better imitates the folding conditions of antibodies in eucaryotic cells. The secretion of the antibody fragments into the periplasmic space of *E. coli* permits their production as soluble and functional proteins with correctly formed intramolecular disulfide bonds. The total yields are usually lower than those of inclusion body refolding, however, it is the method of choice for a rapid assessment of antigen binding activity and specificity.

PROGEN now offers the cassette vector pOPE101 (Genebank accession no. Y14585) for the expression of functional recombinant single-chain Fv antibody fragments in *Escherichia coli*. The vector was specifically designed for cloning of heavy and light chain Fv gene fragments isolated from hybridomas, individual B cell clones as well as antibody libraries by using PROGEN's PCR primer sets F2000 or F2010.

## 2. Vector Design

The cassette vector pOPE101 was designed for the convenient insertion of heavy and light chain variable domain coding regions and for production of functional monomeric single-chain Fv antibody fragments in *E. coli* (Schmiedl *et al.*, 2000). The corresponding DNA fragments of human or mouse origin can be amplified by PCR using PROGEN's primer sets F2000 or F2010, respectively. The amplified gene fragments encoding the

variable heavy and the variable light chain domain are cloned in-frame between a pelB-leader sequence for the secretion of the fusion protein into the periplasmic space, and a short region encoding tags to facilitate detection and purification. The V<sub>H</sub> and V<sub>L</sub> genes were joined by a DNA-fragment coding for a flexible 18 amino acid residue linker containing the first six amino acids of the C<sub>H</sub>1 constant region domain and the hydrophilic pig brain alpha-tubulin peptide sequence EEGEFSEAR. At the 3'-end of the V<sub>L</sub> domain coding region, a short DNA fragment codes for a peptide tag of the proto-oncogene product *c-myc*. It contains the linear epitope EEKLISEEDL, which is recognised by the mouse monoclonal antibody mab Myc1-9E10 (Evan *et al.*, 1985). This is followed by six histidine residues, facilitating purification of the fusion protein by IMAC. The vector backbone further provides a strong synthetic promoter, the T7 terminator, the ColE1 origin of replication and an ampicillin resistance marker for selection.

In pOPE101 the recognition sites of the restriction endonucleases *Nco* I and *Hind* III allow the insertion of a V<sub>H</sub> gene fragment. For insertion of a V<sub>L</sub> gene fragment the sites of the restriction endonucleases *Mlu* I and *Not* I are recommended.

## 3. Material Required

Equipment for DNA cloning

## 4. Contents

5.0 µg purified plasmid DNA, 0.5 µg/µl.

## 5. Preparation of Reagents

The DNA should be diluted and transferred into *E. coli* cells as described in standard protocols. Use an overnight culture of a single clone to extract enough DNA for the following cloning procedures.

## 6. Cloning procedure

The immunoglobulin heavy and light chain Fv domain coding regions must be cloned in-frame into pOPE101 (see attached graphic). To clone a VH gene fragment the recognition sites of the restriction endonucleases *Nco* I and *Hind* III are recommended. The corresponding VL gene fragment should be introduced by using the recognition sites of the enzymes *Mlu* I and *Not* I. The cloning sites of the selected Fv gene fragments have to match the corresponding amino acid positions given by the vector. Gene fragments of genes derived from human or mouse origin by using PROGEN's PCR primer sets F2000 or F2010 can be directly cloned into pOPE101. After cloning, we recommend to check the sequence of the inserts to confirm that no mutation occurred in the open reading frames. The vector is now ready-to-use for the expression of functional recombinant single-chain Fv antibody fragments in *Escherichia coli*.

The expressed antibody fragments can be purified from periplasmic extracts as described in standard protocols (Schmiedl *et al.*, 2000; Schmiedl and Dübel, 2001).

## 7. References

1. Evan, G.I., Lewis, G.K., Ramsay, G. and Bishop, J.M. (1985) Isolation of Monoclonal Antibodies Specific for Human *c-myc* Proto-Oncogene Product. *Mol. Cell Biol.* 5, 3610-6.
2. Schmiedl, A., Breitling, F., Winter, C., Queitsch, I and Dübel, S. (2000) Effect of engineered Cysteines on yield, solubility and activity in various recombinant antibody formats expressed in *E. Coli*. *J. Immunol. Meth.* 242, 101-14.
3. Schmiedl, A. and Dübel, S. (2001) Functional Minipreps. In: *Antibody Engineering*, ed: R. Kontermann and S. Dübel, Springer Verlag, Heidelberg.
4. Zhou, H., Fisher, R.J. and Papas, T.S. (1994) Optimization of primer sequences for mouse scFv repertoire display library construction. *Nucleic Acids Research* 22, 888-9.

