E. coli Expression Vector pOPE101

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

Cat. No.:	**PR3004**
Vector Name:	**pOPE101**
Quantity:	**5 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 deposed 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 offers the cassette vector pOPE101 (Genebank accession no. Y14585) for the expression of functional recombinant single-chain Fv antibody fragments in *E. 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 and Application

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 VH and VL genes were joined by a DNA-fragment coding for a flexible 18 amino acid residue linker containing the first six amino acids of the CH1 constant region domain and the hydrophilic pig brain alpha-tubulin peptide sequence EEGEFSEAR. At the 3´-end of the VL 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 (IPTG inducible), 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 VH gene fragment. For insertion of a VL gene fragment the sites of the restriction endonucleases *Mlu*I and *Not*I are recommended.

3. Specification and Quality

Quantity	5.0 µg plasmid DNA (0.5 µg/µl in TE Buffer)
Purification	Plasmid Purification Column
Stability	Minimum 1 year when stored at -20°C
Vector	3970 bp, AmpR
Host Strains	*E. coli* lacIq genotype (e.g. XL1-Blue)
Cloning Sites	*Mlu*I and *Not*I for light chain VL genes *Nco*I and *Hind*III for heavy chain VH genes
Purity	UV-Scan (220-320) with peak at 258 nm

For plating we recommend to use LB Agar plates containing 100 mM Glucose and 100 µM Ampicillin.

4. 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.

5. 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 *E. coli.*

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

References

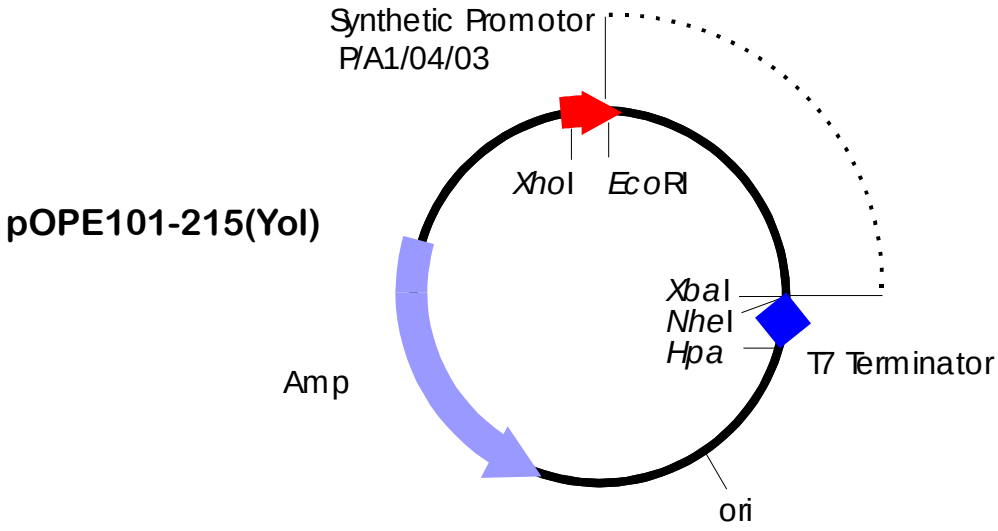
Evan, G.I., Lewis, G.K., Ramsay, G. and Bishop, J.M. (1985) Isolation of Monoclonal Antibodies Specific for Human c-*myc* Proto-Oncogene Product. Mol. Cell Biol. *5,* 3610-6.

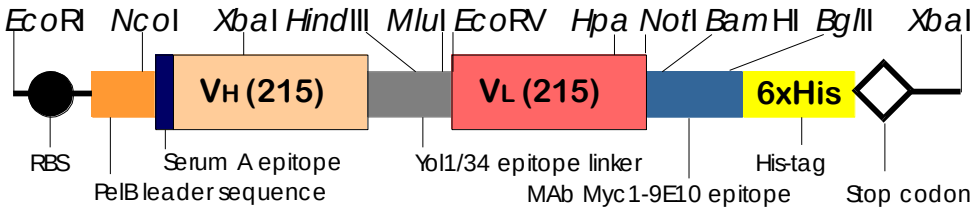
Schmiedl, A, Breitling, F, Winter, C, Queitsch, I and Dübel, S. (2000) Effect of engineered Cysteines on yield, solubility and activity in various recombinant antibody formats expressed in *E. coli.* J. Immunol. Meth. *242,* 101-14.

Schmiedl, A. and Dübel, S. (2001) Functional Minipreps. In: Antibody Engineering, ed: R. Kontermann and S. Dübel, Springer Verlag, Heidelberg.

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.

PROGEN Biotechnik GmbH
Maaßstrasse 30
69123 Heidelberg, Germany
T +49 (0) 6221 8278-0
F +49 (0) 6221 8278-24
info@progen.com
www.progen.com





VH insertion region of pOPE101

```
           NcoI                                        HindIII                      MluI
ATG..54bp..GCCATGGCGCAAGTTCAGCTGCAG..339bp..TCCGCCCCAAAGCTTGAAGAAGGTGAATTTTCAGAAGCACGCGT..
M..      ..A  M  A  Q  V  Q  L  Q..       ..S  A  P  K  L  E  E  G  E  F  S  E  A  R..
<----------------> <-----------------------------------> <----------------------->
  Signal Sequence                    VH                          YoI-tag
```

VL insertion region of pOPE101

```
                          MluI                        NotI    BamHI
..GAAGAAGGTGAATTTTCAGAAGCACGCGTAGATATC..330bp..AAAGGTGCGGCCGCTGGATCC
..E  E  G  E  F  S  E  A  R  V  D  I..       ..K  R  A  A  A  G  S..
  <----------------------> <----------------->
          YoI-tag                  VL
```