**E. coli** Expression Vector pOPE101

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

<table>
<thead>
<tr>
<th>Cat. No.:</th>
<th>PR3004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector Name:</td>
<td>pOPE101</td>
</tr>
<tr>
<td>Quantity:</td>
<td>5 µg</td>
</tr>
<tr>
<td>Storage Upon Receipt:</td>
<td>-20 °C</td>
</tr>
<tr>
<td><strong>In vitro Test</strong></td>
<td>For Research Use Only!</td>
</tr>
</tbody>
</table>

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 offers the cassette vector pOPE101 (Genebank accession no. Y14585) for the expression of functional recombinant single-chain Fv antibody fragments in *E. coli*. The corresponding DNA fragments of human or mouse origin can be amplified by PCR using PROGEN's primer sets F2000 or F2010 respectively. The vector was specifically designed for cloning of antibody heavy and light chains in-frame into pOPE101 (see attached graphic). To clone a VH gene fragment the coding regions must be cloned in-frame 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 expressed antibody fragments can be purified from periplasmic extracts as described in standard protocols (Schmiedl et al., 2000; Schmiedl and Dübel, 2001).

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 can be produced 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 EEGFSEAR. 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 recognized by the mouse monoclonal antibody mab Myc1-9E10 (Evans 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 *NcoI* and *HindIII* allow the insertion of a VH gene fragment. For insertion of a VL gene fragment the sites of the restriction endonucleases *MluI* and *NofI* are recommended.

3. Specification and Quality

<table>
<thead>
<tr>
<th>Quantity</th>
<th>5.0 µg plasmid DNA (0.5 µg/µl in TE Buffer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purification</td>
<td>Plasmid Purification Column</td>
</tr>
<tr>
<td>Stability</td>
<td>Minimum 1 year when stored at -20°C</td>
</tr>
<tr>
<td>Vector</td>
<td>3970 bp, AmpR</td>
</tr>
<tr>
<td>Host Strains</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Cloning Sites</td>
<td><em>MluI</em> and <em>NofI</em> for light chain VL genes</td>
</tr>
<tr>
<td></td>
<td><em>NcoI</em> and <em>HindIII</em> for heavy chain VH genes</td>
</tr>
<tr>
<td>Purity</td>
<td>UV-Scan (220-320) with peak at 258 nm</td>
</tr>
</tbody>
</table>

For plating we recommend to use LB Agar plates containing 100 µM 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 *NcoI* and *HindIII* are recommended. The corresponding VL gene fragment should be introduced by using the recognition sites of the enzymes *MluI* and *NofI*. 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


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Eco Nco Xba Hind Mlu Eco Hpa Not Bam Bgl Xba
RI I III I RV I HI II I

V (215)

H

L

6xHis

RBS

PelB leader sequence

T7 Terminator

Xho

I

Eco

RI

ori

Amp

Synthetic Promotor

P/A1/04/03

Xba

Hind

Hpa

NotI BamHI BglII Xba

NcoI Xba HindIII MluI EcoRV Hpa NotI BamHI BglII Xba

VL (215)

MAb Myc1-9E10 epitope

His-tag

Stop codon

Serum A epitope Yol1/34 epitope linker

6xHis

VL insertion region of pOPE101


<----------------------->     <---------------->

Yol-tag                    VL

VL insertion region of pOPE101

MluI ..GAAGAAGGGTGTTCTAGAAGGCGCGCCGCTGGATCC

<------------------------->

Yol-tag