Surface Expression Phagemid Vector pSEX81

Phagemid cassette vector for the expression of functional recombinant single-chain Fv antibody - pIII fusion protein libraries on the surface of M13 filamentous phage.

Cat. No.:	**PR3005**
Vector Name:	**pSEX81**
Quantity:	**5 µg**
Storage Upon Receipt:	**-20 °C**
***In vitro* Test**	**For Research Use Only!**

1. Introduction

Recombinant antibody technologies have been widely used to produce various single-chain Fv antibody fragments of different specificity. The randomized combination of PCR-amplified immunoglobulin variable heavy and light chain genes further led to the construction of large human or mouse scFv antibody libraries. The isolation of high affinity antibodies derived thereof was established by the expression of recombinant antibody fragments on the surface protein pIII of M13 bacteriophages, which can be rapidly screened against antigens bound to a solid phase.

The M13 bacteriophage protein pIII is located at one end of its tubular virion structure. It is a relative flexible and accessible molecule composed of two functional domains: an N-terminal domain that binds to the F pilus of bacteria during infection and a C-terminal domain buried within the virion. ScFv antibody fragments can be inserted near its N-terminus without destroying its function.

2. Vector Design and Application

PROGEN now offers the phagemid cassette vector pSEX81 for the expression of functional single-chain Fv antibody – pIII fusion proteins on the surface of M13 bacteriophages. The phagemid was specifically constructed for cloning of immunoglobulin heavy and light chain gene fragments isolated from human or mouse antibody libraries generated with PROGEN PCR primer sets F2000 or F2010.

The cassette vector pSEX81 was designed for the convenient insertion of heavy and light chain variable domain coding regions and for production of functional single-chain Fv antibody – pIII fusion proteins on the surface of M13 bacteriophages. The corresponding DNA fragments of human or mouse origin can be amplified by PCR using PROGEN primer sets F2000 or F2010, respectively. The amplified gene fragments encoding the variable heavy or light chain domain are cloned in-frame between a signal peptide sequence of bacterial pectate lyase (pelB) for the secretion of the fusion protein into the periplasmic space, and the pIII gene of M13 bacteriophage. 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. The vector backbone further provides a strong promoter (IPTG inducible), the T7 terminator, the ColE1 origin of replication, the intergenic region of phage F1 and an ampicillin resistance marker for selection.

In pSEX81 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	Qiagen Plasmid Purification Kit
Stability	Minimum 1 year when stored at -20°C
Vector	4883 bp, AmpR
Host Strains	*E. coli lacI*^{q} 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	OD 260/280 ratio: 1.8 – 2.0
Quality Check	Efficiency in standard transformation procedure with chemically competent *E. coli* cells (XL1-Blue) > 1 x 10^8 cfu/µg DNA

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

4. Preparation of Reagents

The DNA should be diluted and transferred into *E. coli* cells as described in standard protocols. To avoid possible mutations of the vector culture medium should include 100 mM Glucose. DNA preparation should be done by standard protocols.

5. Cloning procedure

The immunoglobulin heavy and light chain Fv domain coding regions must be cloned in-frame into pSEX81 (see attached graphic). To clone VH gene fragments the recognition sites of the restriction endonucleases *Nco*I and *Hind*III are recommended. VL gene fragments 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 derived from human or mouse origin with PROGEN PCR primer sets F2000 or F2010 can be directly cloned into pSEX81.

After cloning, the vector is ready-to-use for the expression of functional recombinant single-chain Fv antibody – pIII fusion proteins in *E. coli*. If the modified pSEX81 is co-transfected with M13 bacteriophage as described in standard protocols (Schmiedl *et al.*, 2000; Schmiedl and Dübel, 2001), the scFv antibody – pIII fusion protein is expressed on the surface of resulting phage particles. For expression of scFv antibody libraries on the surface of M13 bacteriophages, we recommend to use the M13 Hyperphage from PROGEN (Cat. No. PRHYPE).

References

Breitling, F., Dübel, S., Seehaus, T., Klewinghaus, I. and Little, M. (1991) A surface expression vector for antibody screening. Gene 104, 147-53.

Broders, O., Breitling, F., and Dübel, S. (2003). Hyperphage - Improving Antibody Presentation in Phage Display. Methods Mol. Biol. 205, 295-302.

Koch, J. and Dübel., S. (2001) Generation of antibody libraries from human donors. In: Antibody Engineering, ed: R. Kontermann and S. Dübel, Springer Verlag, Heidelberg.

Liang, M., Dübel, S., Li, D., Queitsch, I., Li, M. and Bautz, E.K.F. (2001) Baculovirus expression cassette vectors for rapid production of complete human IgG from phage display selected antibody fragments. J. immunol. Meth. *247*, 119-30.

Little, M., Breitling, F., Micheel, B. and Dübel, S. (1994) Surface display of antibodies. Biotechnology Advances *12*, 539-55.

Micheel, B., Heymann, S., Scharte, G., Böttger, V., Vogel, F., Dübel, S., Breitling, F., Little, M. and Behrsing, O. (1994) Production of monoclonal antibodies against epitopes of the main coat protein of filamentous fd phages. J. Imm. Methods *171*, 103-9.

Rondot, S., Koch, J., Breitling, F. and Dübel, S. (2001) A helper-phage to improve single chain antibody presentation in phage display. Nature Biotechnol., *19*, 75-8

Welschof, M., Terness, P., Kipriyanov, S., Stanescu, D., Breitling, F., Dorsam, H., Dübel, S., Little, M. and Opelz, G. (1997) The antigen binding domain of a human IgG-anti-F(ab')2 autoantibody. PNAS 94, 1902-7.

Zhou,H., Fisher,R.J. and Papas,T.S. (1994) Optimization of primer sequences for mouse scFv repertoire display library construction.

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 pSEX81

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            NcoI                                        HindIII                      MluI
ATG..54bp..GCCATGGCCCAGGTGCAGCTGGTG..366bp..TCCGCCCCAAAGCTTGAAGAAGGTGAATTTTCAGAAGCACGCGT..
M..       ..A  M  A  Q  V  Q  L  V..        ..S  A  P  K  L  E  E  G  E  F  S  E  A  R..
<---------------> <-----------------------------------> <----------------------->
  Signal Sequence                    VH                          Yol-tag
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VL insertion region of pSEX81

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                          MluI                      NotI     BamHI
..GAAGAAGGTGAATTTTCAGAAGCACGCGTACAGTCT..324bp..GGTGCGGCCGCTGGATCC
..E  E  G  E  F  S  E  A  R  V  Q  S..        ..G  A  A  A  G  S..
  <----------------------> <----------------->
          Yol-tag                   VL
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