Mo Bi Tec

pSKAN 8 Phagemid Vector

Product Information and Instructions
May 2000

Mo Bi Tec
MOLECULAR BIOLOGISCHE TECHNOLOGIE

Content

1. Introduction 3
2. Preparation of Your Own Library with pSKAN8 3
2.1. Amplification of DNA Oligos 3
2.1.1. Amplification Primers 3
2.1.2. Protocol 3
2.2. Modification of DNA 4
2.2.1. Restriction Reactions 4
2.2.2. Ligation of DNA Fragments 4
2.3. Preparation of Competent Cells 5
2.4. Transformation of *E. Coli* Cells by Electroporation 5
3. Vector Map 7
4. Primer and Restriction Site Positions in pSKAN8 8
5. Important literature 9
6. Order Information, Shipping & Storage 10

For research use only. Not for use in diagnostic or therapeutic procedures.

1. Introduction

pSKAN8 is a phagemid vector used to prepare your own phagemid display library.
Below you find the protocol we used for constructing the library HyC, which is available as part of our pSKAN Phagemid Display System with ready-to-use libraries. This protocol can be used for the construction of your own library as well.

Note: The system is designed for the presentation of a hypervariable amino acid loop of 6 - 8 amino acids. We have no experience with inserting larger loops. Thus, this will have to be determined empirically.

2. Preparation of Your Own DNA Library with pSKAN8

2.1. Amplification of DNA Oligos

2.1.1. Amplification Primers

primer F (# 4403) 5' GCTACAACGAGCTCAACGGTTGC 3'

primer B (# 4404) 5' GGGTAAGTGTCACCGTCGGTACCGCA 3'

Note: These primers are **not available** at **MoBiTec.**

2.1.2. Protocol

In a 0.5 ml reaction tube for PCR* (polymerase chain reaction) fill:

1 pmole of the hypermutated oligo

40 pmole of primer F (# 4403)

40 pmole of primer B (# 4404)

10 µl DNA-polymerase buffer (+ $MgCl_2$) (10x)

5 µl $MgCl_2$ (25 mM)

10 µl dNTP solution (2 mM, each)

0.25 µl of Taq-DNA polymerase (5 U/µl)

*PCR is a process covered by patents owned by Hoffmann-La Roche. Use of this process requires a license.

Add H_2O to a final volume of 100 µl. If necessary, cover the solution with 50 µl mineral oil to prevent evaporation and place the reaction tubes in a PCR thermocycler. Carry out 3 to 5 subsequent cycles of 1 minute at 94°C (DNA denaturation), 2 minutes at 50°C (primer annealing) and 3 minutes at 72°C (DNA synthesis). We highly recommend the Quick Step PCR Purification Kit from Edge Bio Systems (distributed by **MoBiTec** in Germany). Alternatively, purify the DNA by phenol/chloroform extraction and precipitate with ethanol. Dissolve in 20µl TE buffer.

Note: Also available at **MoBiTec** in Germany are thermostable polymerases and tubes for thermocyclers, which can be used without mineral oil (see **MoBiTec** catalog chapter 7).

2.2. Modification of DNA

2.2.1. Restriction Reactions

for *Sst* I (= *Sac* I) and *Kpn* I restrictions:

PCR product	16 µl
Kpn I, *Sac* I	20 units
restriction buffer (10x)	3 µl
H_2O ad	30 µl

Mix and incubate the tubes at 37°C for at least 2 hours and check the restriction products by polyacrylamide gel electrophoresis (ca.20%). Purify the DNA by phenol/chloroform extraction and ethanol precipitation. In order to be able to work with optimal buffer conditions, the two restriction reactions may also be performed in two steps. For a convenient buffer exchange inbetween the reactions, MobiSpin columns are available from **MoBiTec**.

2.2.2. Ligation of DNA Fragments

To ligate cleaved DNA fragments mix in an Eppendorf reaction tube:

vector and insert DNA fragments at a molar ratio of 1 : 4

1:10 of end volume of ligation buffer (10x)

0.5 units ligase per µg DNA

add H_2O to reach an end volume according to a DNA concentration of 0.01 µg/µl.

Mix and incubate overnight at 8°C or 3 - 4 hours at room temperature[1]. Add 1 volume of H_2O and inactivate the enzyme by heating to 65°C for 10 minutes. Purify the DNA by phenol/chloroform extraction and ethanol precipitation and redissolve it in 10 µl of TE buffer.

2.3. Preparation of Competent Cells

Inoculate 10 ml of LB/Tc medium with a single colony of *E. coli WK6λmutS*[2] and incubate at 37°C and 180 rpm overnight.

The next day, inoculate 1000 ml of LB/Tc medium (2 x 500 ml Erlenmeyer flasks) at 1% with the overnight grown culture and incubate again at same conditions until an optical density of OD_{600} = 0.6 has been reached.

Transfer 250 ml aliquots of the culture into centrifuge tubes (GS3), chill them on ice and centrifuge for 15 minutes at 6,000 rpm and 4°C (Sorvall RC5C centrifuge; GS3 rotor). Resuspend each pellet in 250 ml of ice-cold H_2O and repeat the centrifugation step. Resuspend each pellet in 125 ml of ice-cold H_2O, pour together two aliquots and centrifuge again. Resuspend each pellet in 10 ml of ice-cold glycerol (10%) collect both aliquots in an GSA centrifuge tube and centrifuge for 15 minutes at 8,000 rpm. Finally resuspend the bacterial pellet in 1 ml of glycerol (10%).

Fill 50 µl aliquots in precooled, sterile Eppendorf reaction tubes, freeze immediately in liquid nitrogen and store at -70°C until the transformation by electroporation.

[1] Ligation at 8°C is efficient, but slow.

[2] *E. coli WK6λmut*S
Genotype: *gal*E, *str*A, *nal*T, Δ(*lac-pro*AB), λ^+, *mut*S::Tn10, F´[*lac*I^q, *lac*ZΔM15, *pro*AB$^+$]

2.4. Transformation of *E. coli* Cells by Electroporation

Place frozen aliqouts of competent *E. coli WK6λmutS* cells on ice and let them thaw. To each aliquot add 1 µl of ligated DNA (0.01 - 0.1 µg) and incubate on ice for 1 minute. Fill the suspension in a precooled electroporation cuvette (use a Pasteur pipette), place the cuvette in the electroporation sled and give a pulse at a voltage of 2.5 kV, a capacity of 25 µF and a resistence of 200 Ω (Gene Pulser and Puls Controller, Bio-Rad). Immediately add 1 ml of LB medium, mix with a

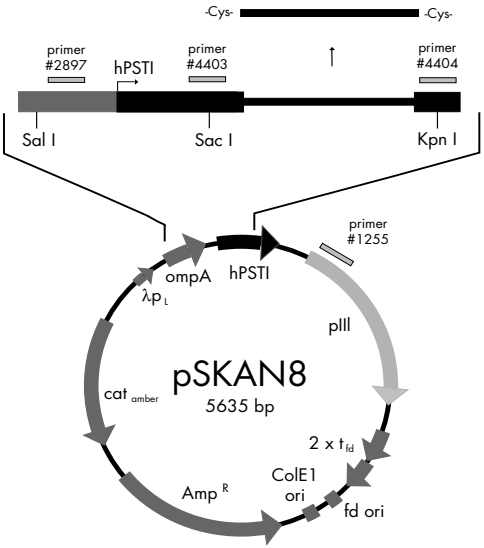
Pasteur pipette and transfer the suspension in an Eppendorf reaction tube. Incubate for 1 hour at 37°C and plate on LB agar (1 ml/135 mm plate) containing ampicillin (100 µg/ml) and tetracycline (20 µg/ml). Incubate overnight at 37°C. In the same way carry out a transformation with and without pSKAN-DNA as a control and plate out on LB/Tc and LB/Amp/Tc plates. Also diluted aliquots of transformed cells should be plated in order to calculate the size of the final library.
As a control, the individual clones of the library can be sequenced using the following primers:

primer # 1255 5´ GGGATTTTGCTAAACAAC 3´

primer # 2897 5´ GGAGGTCTAGATAACGAGG 3´

Double stranded sequencing is recommended. One primer may be sufficient.

3. Vector Map

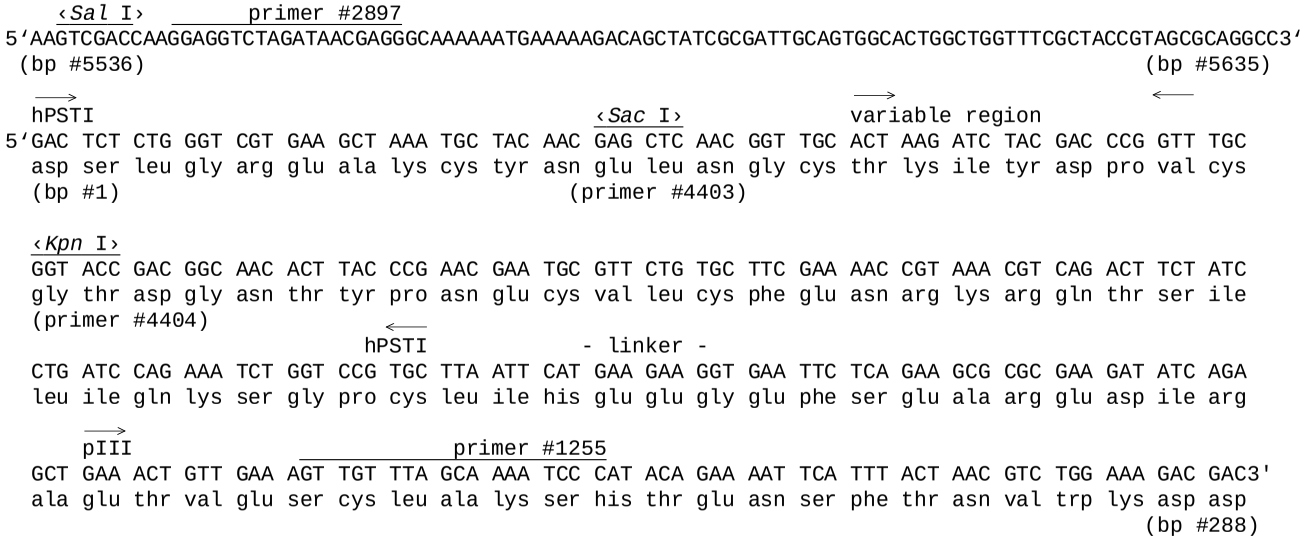


Phagemid vector construction.

pSKAN8 is based on the vector pMAMPF-3. Between the two Cys a hypervariable amino acid loop (with $(NNG/T)_{6-8}$) can be created using the protocol in chapter 2. (See also the ready-to-use pSKAN libraries.)
The phagemid contains the genetic elements:

Amp^R:	selection marker (ampicillin resistance)
ColE1 ori:	plasmid origin of replication
fd ori:	origin of replication for the fd phage
λp_L:	λ-promoter left
ompA:	signal sequence for secretion
hPSTI:	gene of the human pancreatic secretory trypsin inhibitor
pIII:	gene for the phage coat protein III
$2 \times t_{fd}$:	two phage fd transcription terminator sequences
cat_{amber}:	chloramphenicol resistance is inactive due to an amber mutation

4. Primer and Restriction Site Positions in pSKAN8



The sequence of pSKAN8 in this overview starts with the last bases of the linear sequence (bp # 5536 to # 5635). In the circular plasmid bp # 1 follows bp # 5635.

5. Literature

1. Maywald, F. *et al.*, Gene 68, 357-369 (1988)
2. Böldicke, T. *et al.*, Eur. J. Biochem. 175, 259-264 (1988)
3. Collins, J. *et al.*, Biol. Chem. Hoppe Seyler 371, 29-36 (1990)
4. Paulsen, J. *et al.*, Ber. Bunsenges. Phys. Chem. 93, 1015-1019 (1989)
5. Paulsen, J. *et al.*, DECHEMA Biotech. Conf., DECHEMA, Frankfurt, pp1031 (1989)
6. Hecht, H. J. *et al.*, J. Mol. Biol. 220, 711-722 (1991)
7. Collins, J. *et al.*, in Wooley *et al.* (ed.) D.F.I. Print, Aarhus, pp 34 (1990)
8. Klaus, W. and Schomburg, D., J. Mol.Biol. 229, 695-706 (1993)
9. Hecht, H. J. *et al.*, J. Mol. Biol. 225, 1095-1103 (1992)
10. Szardenings, M. *et al.*, Protein Engeneering 8, 45-52 (1995)
11. Röttgen, P. and Collins, J., Gene 164, 243-250 (1995)
12. Zell, R. and Fritz, H.-J., EMBO J. 6, 1809-1815 (1987)
13. Tesar, M. *et al.*, Immunotechnology 1, 53-64 (1995)
14. Sambrook, J. *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY. 1 (1989)

6. Order Information, Shipping & Storage

order #	description	amount
shipped at room temperature; store at +4°C:		
pSKAN8	pSKAN8, lyophilized vector DNA	5µg
PSKAN4	pMAMPF-3-PSTI4, lyophilized vector DNA	5µg
PS1255	sequencing primer # 1255 (18 mer) lyophilized	500 pmol
PS2897	sequencing primer # 2897 (19 mer) lyophilized	500 pmol
PSKAN2	anti-hPSTI antibody, monoclonal mouse lyophilized	40 µg
PSKAN3	anti-pIII (g3p) antibody, monoclonal mouse lyophilized	100 µl
shipped on dry ice; store at -70°C:		
PSKAN	pSKAN Phagemid Display System HyA, HyB, HyC pSKAN libraries inclusive *E. coli* host strain *WK6λmut*S	3 x 1ml 1ml