Mo Bi Tec

Cloning Vector p3T

Product Information and Instructions
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Mo Bi Tec
MOLECULAR BIOLOGISCHE TECHNOLOGIE

Content

The p3T Vector was developed by Dr. David Mitchell, Life Systems Design, Birsfelden, Switzerland.

The p3T vector

1. Summary

The p3T vector provides a flexible system for the direct cloning of PCR products. Based on pBluescript II SK + it allows the cloning of PCR products via both single and multiple T overhangs. Utilizing a unique series of restriction sites the same vector can be cleaved by different enzymes to produce 1, 2 or 3 T overhangs. This permits either the direct cloning of PCR products (via a single A extension) or the option of polyadenylating the PCR fragment and cloning via multiple A extensions. This procedure has the advantage that less material is required for cloning. The vector also contains a *Sma* I site to reduce vector background. *Msc* I sites (blunt) flank the insert allowing excision with minimal flanking regions. Blue/white selection by α-complementation can still be used with the p3T vector.

2. Introduction

A number of vectors exist for the direct cloning of PCR products (Ref. 1-4). Many of these are based on the addition of single T residues or tandem restriction sites that can be cleaved excising a small fragment to produce single T-overhangs. A major disadvantage is that due to the inefficient ligation of single base overhangs a large amount of PCR fragment is required and/or the use of extremely efficient competent cells. The p3T vector (Ref. 5) can be cut with *Pfl* MI to generate a 3-T overhang. If the PCR fragment is polyadenylated using terminal deoxynucleotidyl transferase (available at MoBiTec, see chapter 8), it can be cloned with high efficiency. The polyadenylation is a simple procedure requiring only a five minute reaction time.

The p3T vector was constructed using a pBluescript II SK+ backbone. The cassette was synthesized and cloned between the *Pst* I and *BamH* I sites in the polylinker. The insertion is 48 nucleotides long and preserves the β-galactosidase reading frame permitting blue/white selection. A naturally occuring *Bcg* I site in the ampicillin gene was removed by site directed mutagenesis. This vector is shown in Figure 1.

PCR products can be cloned directly using *Xcm* I cut vector or, more efficiently, cloned using *Pfl* MI cut vector and polyadenylating the PCR fragment. *Xcm* I leaves a single dT overhang, whereas *Pfi* MI produces a multiple dT overhang (cleavage sites see chapter 4.2.1.).

Advantages

- **single or multiple T-overhangs can be utilized**
- **direct cloning of PCR products (single dA extension)**
- **or polyadenylation and cloning via multiple dA extensions**
- **requires less amplified DNA**
- ***Sma* I site to reduce the vector background**
- ***Msc* I sites flank the insert for optimal excision**
- **cloning with high efficiency**
- **blue/white selection by α-complementation**

3. The p3T Vector

A schematic presentation of the p3T vector is shown in Figure 1. The complete sequence is deposited in the EMBL database (accession number Z46733) or can be obtained from MoBiTec by E-mail.

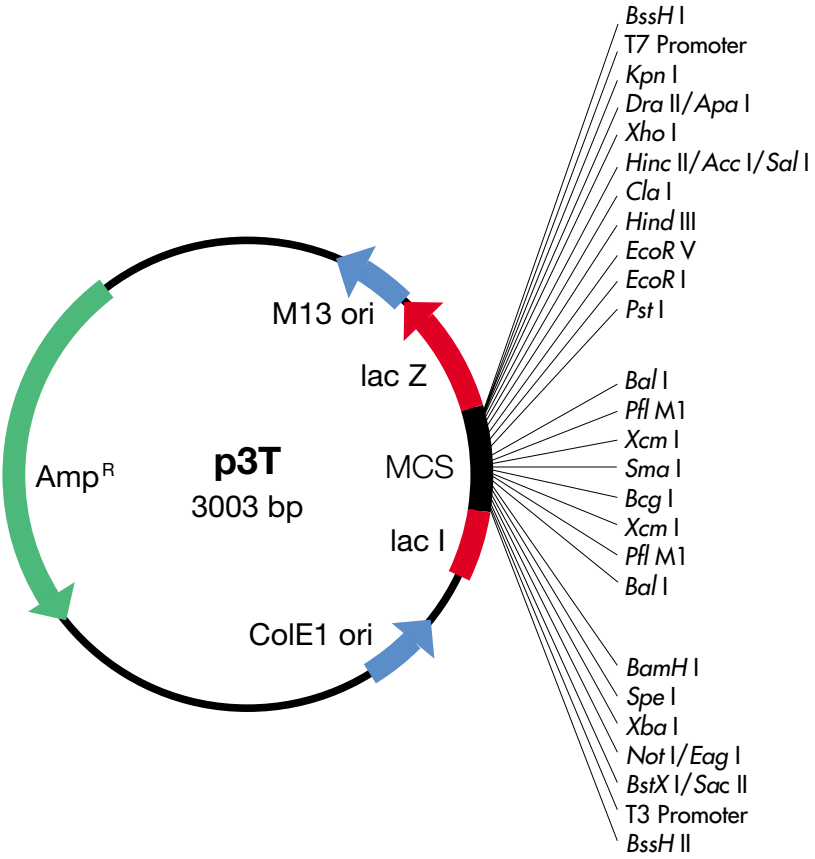
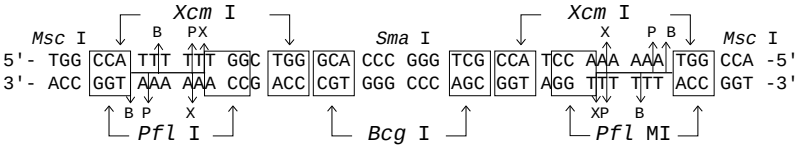


Figure 1.

The p3T vector. Amp^R: ampicillin resistance gene; ColE1 ori: pBR322 derived origin of replication; lac I: β-galactosidase promoter sequence; lacZ: the alpha peptide of β-galactosidase; fd: the f1 filamenteous phage derived origin of replication.



The insert sequence in p3T. The insert orientation in the plasmid is from Pst *I to* BamH *I (left to right). The restriction enzyme recognition sites are boxed and the sites of cleavage are indicated by arrows. B,* Bcg *I; P, Pfl M1; X,* Xcm *I.*

4. Protocols

For a protocol overview, please see page 6.

4.1. Amplification of DNA

Use standard reaction conditions.

- *Note*: MoBiTec tubes for thermocyclers do not require oil.

4.2. Cloning by polyadenylation

4.2.1. Restriction digest and elution of p3T

- For a single dT overhang, digest 5 µg of p3T with 20-30 U of restriction enzyme *Xcm* I for about 4 hours at 37 °C. The enzyme concentration and incubation time may be reduced.

Xcm I

5´ CCANNNNNNNNNTGG 3´

3´ GGTNNNNNNNNNACC 5´

Pfl MI

5´ CCANNNNNTGG 3´

3´ GGTNNNNNACC 5´

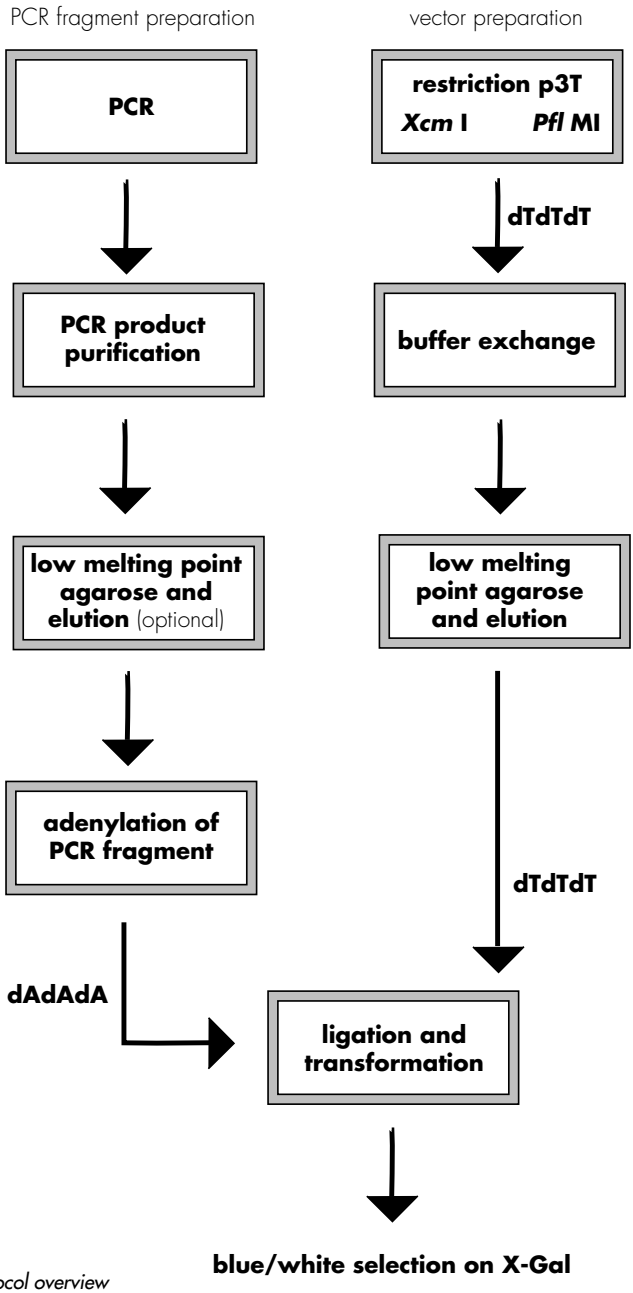


Fig. 3 Protocol overview

- For a multiple dT overhang digest 5 µg of p3T with 20-30 U of restriction enzyme *Pfl* M1 for about 4 hours at 37°C. The enzyme concentration and incubation time may be reduced.
- Confirm by analysis on a 0.8 % agarose gel, that the digestion is complete.
- Precipitate DNA with 1/10 vol 3 M sodium acetate pH 5.2 and 2 volumes of 100 % ethanol.
- Resuspend the pellet in an appropriate volume of 1X TE pH 7.5 and loading buffer, run on a 0.8 % low melting point agarose gel and elute, in order to separate the small excised fragment from the linearized vector.

Note: To simplify the elution procedure, the agarose with the appropriate band of DNA may be frozen and then filled into an empty Mobicol with filter (see chapter 8). After mixing the agarose with buffer, the Mobicol is centrifuged within an Eppendorf tube. The agarose remains on the filter and most of the DNA is eluted into the Eppendorf tube. An ethanol precipitation might be advisable after this procedure.

4.2.2. Elution and adenylation of PCR fragment

- Extract the PCR amplified DNA with an equal volume of chloroform isoamylalcohol (25:1) and precipitate with 1/10 vol 3 M NaOAc pH 5.2 and 2 volumes of 100 % ethanol.
- Resuspend the pellet in an appropriate volume of 1x TE pH 7.5 and loading buffer, run on a 1.2-1.5 % low melting point agarose gel and recover the fragment. If the produced PCR fragments are very uniform, the agarose gel and elution may be omitted.
- For the ligation to multiple dT overhangs, set up the 50 µl adenylation reaction described below.

10 µl 5x adenylation buffer (as in chapter 5)

0.1 mM (final concentration) DTT

1 µl 1 mM dATP

10 U terminal deoxynucleotidyltransferase (see chapter 8)

0.5-1.0 pmol 3'-OH termini of eluted PCR fragment

(add just before incubation!!)

H_2O to 50 µl

- incubate the reaction for 5 minutes. Stop the reaction by adding 2 µl 0.5 M EDTA pH 8.0. Add 50 µl 1x TE pH 7.5 and extract immediately, twice with phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (25:1). Precipitate with a 1/10 vol 3 M sodium acetate pH 7.0 and 2 volumes of 100 % ethanol. Resuspend in 1x TE pH 7.5 at the desired concentration.

4.2.3. Ligation and transformation

Ligation and transformation should be performed according to standard procedures (Ref. 6).

A *Sma* I digest after ligation and before transformation decreases the vector background, since it linearizes uncut vectors without insert. If there is the possibility of a *Sma* I site within the insert, this step should be omitted.

Transformants are selected on LB-agar plates containing ampicillin, X-Gal and IPTG (standard protocols).

5. Buffers

TE 10x

100 mM Tris
10 mM EDTA
pH 7.5

5x adenylation buffer (as supplied with MoBiTec´s Terminal Deoxynucleotidyl Transferase):

500 mM K Cacodylate buffer pH 7.2
10 mM $CoCl_2$

10 mM DTT (supplied in an extra vial)

6. Bacterial strains

E. coli standard strains such as XL-1 Blue may be used for propagation of p3T and the selection of transformants.

7. References

1. Holton, T.A. and Graham, M.W. (1991) *Nucleic Acids Res.* **19** 1156.
2. Kovalic, D., *et al.* (1991) *Nucleic Acids Res.* **19** 4560.
3. Marchuck, D. *et al.* (1991) *Nucleic Acids Res.* **19** 1154.
4. Mead, D. A. *et al.* (1991) *Bio/Technology* **9** 657-663.
5. Mitchell, D. B. *et al.* (1992) *PCR Meth. App.* **2** 81-82.
6. Sambrook *et al.* Molecular Cloning, (1989) Cold Spring Harbour
7. Sandhu, G. S. *et al.* (1989) *Biotechniques* **7** 689-690.

Note: In the references, the vector used to be called p123T.

8. Order Information

order #	**description**	**amount**
P123T	p3T vector DNA, lyophilized	5 µg
TDT200	Terminal deoxynucleotidyl transferase (including adenylation buffer)	200 units
TDT100	Terminal deoxynucleotidyl transferase (including adenylation buffer)	1000 units
M1002	Mobicols with two different screw caps (filters available separately! please request order information)	50