Cloning Vector p3T

Order #
P123T

Contents

1. Features ... 3

2. The p3T Vector ... 3

3. Vector Map ... 4

4. Cloning Procedure ... 5

5. Quality Warranty ... 6

6. References ... 6

7. Order Information, Shipping, and Storage ... 6

8. Contact and Support ... 6

1. Features

- Multiple Cloning Site with diverse cloning options:
    - Direct cloning of PCR products (single dA extension)
    - Cloning of polyadenylated fragments
- Enables efficient ligation and requires low amounts of insert DNA
- Linearization site (SmaI) to reduce background of “empty” vector clones
- BalI sites flank MCS for optimal excision of the insert
- High-efficiency-cloning
- Blue/white selection by α-complementation

2. The p3T Vector

The p3T vector provides a flexible system for direct cloning of PCR products. The vector is based on a pBluescript II SK+ backbone (Mitchell, D.B. *et al.*, 1992). It contains the lac operon of *E. coli* with CAP binding site, lac promoter (Plac), Lac repressor (LacR) binding site, and the 5’-terminal part of the lacZ gene encoding for the N-terminal part of β-galactosidase. This 5’-terminal part of the lacZ gene contains the multiple cloning site (MCS) and its expression is IPTG inducible. It is capable of intra-allelic α-complementation of a partial deleted chromosomal lacZ copy (*E. coli* host strain: lacZΔM15, e.g., DH5α, DH10B, JM101, JM109). In the presence of IPTG, transformants expressing both fragments of the ß-galactosidase (the vector encoded N-terminal part and the chromosomal encoded C-terminal part) will form a functional enzyme and can be detected as blue colonies on agar plates containing X-Gal. Cloning into the MCS will lead to a nonfunctional N-terminal fragment of the ß-galactosidase and to the abolishment of α-complementation. White colonies will grow on X-Gal/IPTG plates.

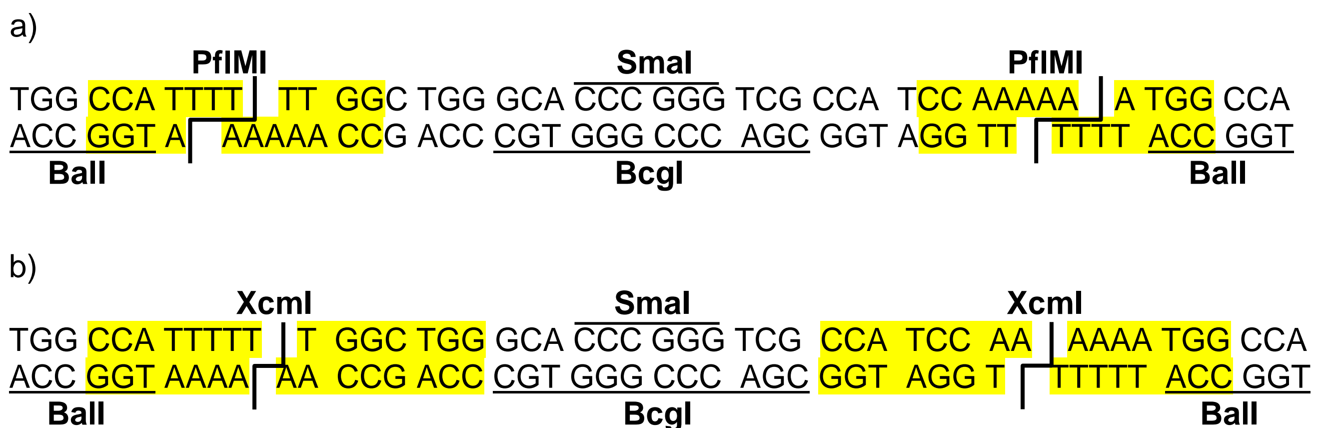
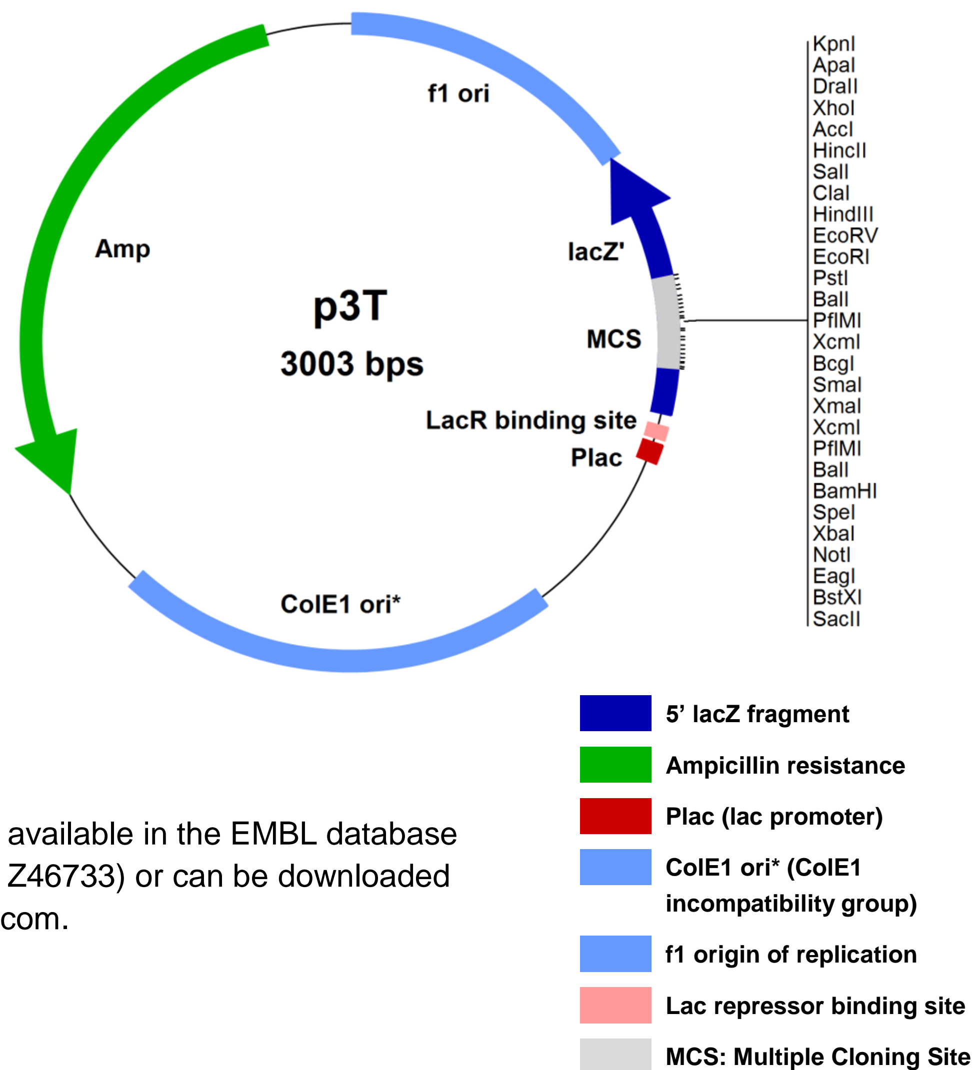


Fig1: Multiple Cloning Site of p3T
a) recognition sites of PflMI (yellow), BalI, BcgI and SamI (underlined)
b) recognition sites of XcmI (yellow), BalI, BcgI and SmaI (underlined)

Due to a unique series of restriction sites the p3T vector can be cleaved by different enzymes (XcmI, BcgI or PflMI) to produce 1, 2 or 3 T (thymin) overhangs. This permits either the direct cloning of PCR products via a single A (adenin) extension or polyadenylating the PCR fragment and cloning via multiple A extensions. If the PCR fragment is polyadenylated using terminal deoxynucleotidyl transferase, it can be cloned with high efficiency. The polyadenylation is a simple procedure requiring only a five minute reaction time.

3. Vector Map



Sequence data are available in the EMBL database (accession number Z46733) or can be downloaded from www.mobitec.com.

4. Cloning Procedure

Digest the p3T vector with restriction enzyme XcmI for single dT-overhang, with BcgI for 2T-overhang and with PflMI for 3T-overhang.

BcgI

5'.....$_{10}$(N)CGANNNNNNTGC(N)$_{12}$.....3'
3'.....$_{12}$(N)GCTNNNNNNACG(N)$_{10}$.....5'

XcmI

5' CCANNNNNNNNNTGG 3'
3' GGTNNNNNNNNNACC 5'

PflMI

5'CCANNNNNTGG3'
3'GGTNNNNNACC5'

PCR fragment preparation

PCR

Purification of PCR product

Adenylation of PCR fragment
(required for cloning with PflMI)

dAdAdA

vector preparation

Digestion of p3T with restriction enzyme

Purification of digested vector

dTdTdT

Ligation and transformation

blue/white screening

Fig. 2: Cloning procedure
To avoid clones containing vectors without an insert, a SmaI digestion after ligation and before transformation is recommended. This step linearizes vectors without insert. Do not perform SmaI digestion, if there is a SmaI site within the insert. Transformants are selected on LB-agar plates containing ampicillin, X-Gal, and IPTG.

MoBiTec GmbH, Germany ● Phone: +49 551 70722 0 ● Fax: +49 551 70722 22 ● E-Mail: info@mobitec.com ● www.mobitec.com

5. Quality Warranty

DNA concentration and purity were checked by UV spectrophotometry. All restriction sites specified in the vector map were checked by sequencing. Functionality of α-complementation was checked by transformation and plating the transformants on IPTG/X-Gal agar plates.

6. References

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

7. Order Information, Shipping, and Storage

Order#	Product	Quantity
P123T	p3T, *E. coli* PCR Product Cloning Vector	5 µg
shipped at RT; store at 4 °C		

8. Contact and Support

MoBiTec GmbH ◆ Lotzestrasse 22a ◆ D-37083 Goettingen ◆ Germany

Customer Service – General inquiries & orders
phone: +49 (0)551 707 22 0
fax: +49 (0)551 707 22 22
e-mail: order@mobitec.com

Technical Service – Product information
phone: +49 (0)551 707 22 70
fax: +49 (0)551 707 22 77
e-mail: info@mobitec.com

MoBiTec in your area: Find your local distributor at www.mobitec.com