Cloning Vector p3T

Order # P123T
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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
- Bal 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 β-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 β-galactosidase and to the abolishment of α-complementation. White colonies will grow on X-Gal/IPTG plates.

![Multiple Cloning Site of p3T](image)

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

\[
\text{BcgI} \\
5'\ldots…10(N)CGANNNNNTGG(N)_{12}\ldots3' \\
3'\ldots…12(N)GCTNNNNNACG(N)_{10}\ldots5' \\
\]

\[
\text{XcmI} \\
5'\text{CCANNNNNNTGG}3' \\
3'\text{GGTNNNNNACC}5' \\
\]

\[
\text{PflMI} \\
5'\text{CCANNNNTGG3'} \\
3'\text{GGTNNNACC5'} \\
\]

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.

**Fig. 2: Cloning procedure**

A diagram illustrating the cloning procedure, including steps such as PCR, purification of PCR product, adenylation of PCR fragment (required for cloning with PflMI), digestion of p3T with restriction enzyme, purification of digested vector, dTdT addition, ligation and transformation, and blue/white screening.
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


7. Order Information, Shipping, and Storage

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<td>p3T, <em>E. coli</em> PCR Product Cloning Vector</td>
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</table>

8. Contact and Support

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