

# DATA SHEET

Version: 2  
Revision date: 14/03/2023

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## 1. Identification

**Product name** pMBL-T Vector DNA Cloning Kit  
20 reactions

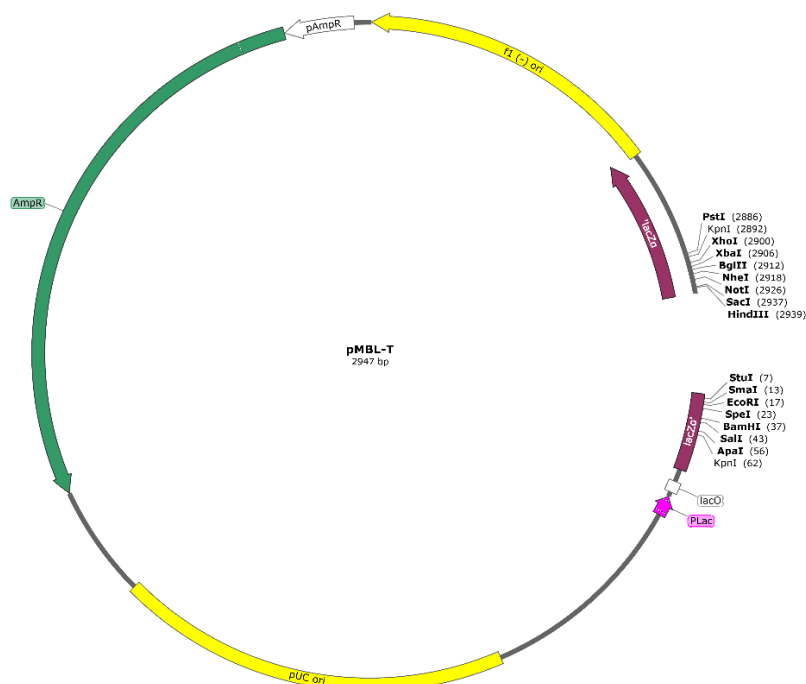
**Cat. No** C0030

## 2. Description

The pMBL-T Vector DNA Cloning Kit is a convenient system for the cloning of PCR products. The vector is prepared by cutting pMBL vector with EcoRV and adding a 3' terminal thymidine to both ends. These single 3'-T overhangs at the insertion site greatly improve the efficiency of ligation of a PCR product into the plasmids by preventing recircularization of the vector and providing a compatible overhang for PCR products generated by certain thermostable polymerases such as Taq DNA Polymerase. These polymerases often add a single deoxyadenosine, in a template-independent fashion, to the 3'-ends of the amplified fragments.

### Maps and features for pMBL-T vector:

The locations of genes are indicated by arrows. Selected unique restriction endonuclease sites are shown



## 3. Composition

Item	Volume
pMBL-T Vector (1µg, 50 ng/µL)	20 rxn
T4 DNA Ligase (100 U, 5 U/µL)	20 µl
10x T4 DNA Ligase Buffer : 400mM Tris-HCl, 100 mM MgCl <sub>2</sub> , 100 mM DTT, 5mM ATP, pH 7.8	100 µl
Control Insert (600 bp)	5 µl

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### 4. Quality specifications

More than 90% white colonies in a transformation with supplied control insert.

The total number of colonies in the supplied control insert should be higher than 1000, provided that *E. coli* cells have a competence of more than  $1 \times 10^7$  colonies/ $\mu\text{g}$  of circular pUC18.

### 5. Storage specifications

pMBL-T Vector DNA Cloning Kit MUST be stored at **-20°C** in a non-frost free freezer since temperature rises above 0°C daily in frost-free freezers. If properly stored, kits are guaranteed for 12 months from the date of purchase.

### 6. Applications

- Cloning of PCR fragments into DNA Cloning vector.
- Blue/white screening for recombinants.

### 7. Further information

**Product Use Limitations** This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

**Disclaimer** The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

**References** Zaccolo, M., Williams, D.M., Brown, D.M., and Gherardi, E. 1996. An approach to random mutagenesis of DNA using mixtures of triphosphate derivatives of nucleoside analogues. *J Mol Biol*, 255, 589–603.



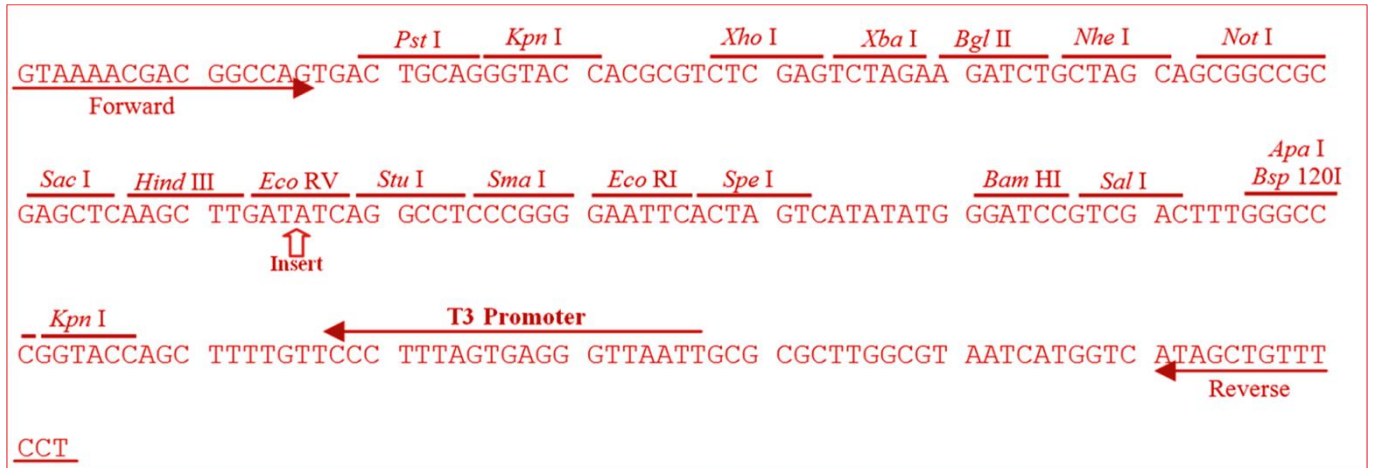
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## RECOMMENDED PROTOCOL

### 1. Amplify your insert of interest.

### 2. Ligate.

Mix the following:

	<u>Final Concentration</u>
1 µL T <sub>4</sub> DNA ligase 10X Buffer	1X
1 µL pMBL T-Vector (50 ng/µL)	5 ng/µL
x µL of DNA insert	
1 µL T <sub>4</sub> DNA ligase (5U)	0.5 U/µL
H <sub>2</sub> O up to 10 µL	

We recommend a 1:5 molar ratio of vector insert according to the following formula:

$$\text{ratio vector to insert} = \frac{\text{ng of vector} \times \text{insert length in bp}}{\text{vector length in bp} \times \text{ng of insert}}$$

Incubate the above mixture between 20°C to 22°C for up to 2 hours.

### 3. Transform

Transform 5 µL into 50µL of competent E. coli cells.

We recommend not to transform with a volume of ligation mix higher than 7.5µL

### 4. Grow and plate Incubate 37 °C overnight

