DESCRIPTION

EasyCloning Kit is designed for quick and effective PCR cloning. With our proprietary recombinase enzyme, this kit is especially powerful in directly cloning any single or multiple PCR products into designated vectors effectively without the tedious and limiting tasks of selecting proper restriction enzymes, phosphatases, or ligases.

KEY FEATURES

- **Fast:** One step cloning procedure completed within 30 minutes.
- **Easy:** PCR DNA and linearized vectors immediately used in cloning. No restriction enzymes, phosphatases or ligases are needed.
- **Effective:** Long PCR DNA up to 100 kb can be efficiently cloned vectors with a success rate >95%.

APPLICATIONS

- PCR cloning up to 100 kb
- Gene transfer from one vector to another
- High-throughput (HTP) PCR cloning
- *In vitro* joining of DNA fragments
- Gene insertion, knockout, mutagenesis et.al

CONTENTS for 10 or 20 Reactions

Kit Components	TBS4050-10	TBS4050-20
EasyCloning Enzyme (5 U/μL)	10μL	20μL
10X CloneEZ® Buffer	20μL	40μL
Manual	1	1

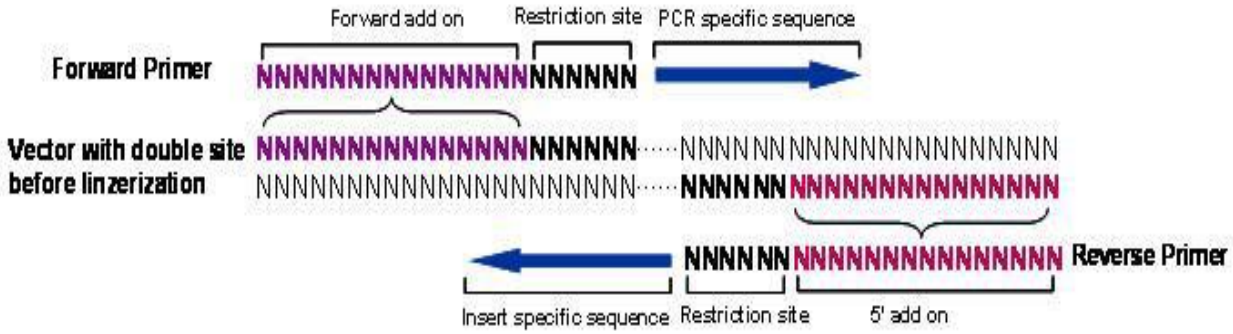
STORAGE

The kit should be stored at -20°C. It will remain stable for at least one year.

GENERAL PROTOCOL

A. PCR Amplification of Target DNA: To clone any DNA fragment into a linearized vector using this kit, the insert fragment should be obtained by PCR using primers with an add-on of 15-30 base sequences homologous to either side of the restriction site that is used to linearize the vector (>15 base increases efficacy. >30 base shows no further increase). Therefore, both primers should cover a 15-30 base sequence add-on at the 5'-end, an optional restriction site in the middle, and the insert-specific sequence at the 3'-end.

(**Note: The restriction site in the middle of the primer is optional and does not have to be the same site used to linearize the vector; you may also add any other sequence in the middle for frame adjustment or tag addition**.)



Note: PCR products may not need to be purified, however, as primers are inhibitory to the cloning reaction, purified PCR products would be a good choice for cloning.

B. Preparation of Linearized Vector:

To achieve a successful cloning reaction, complete linearization of the vector is critical. Incomplete linearization of the vector will result in high background. The linearized vector should be purified using a gel or PCR purification kit.

C. EasyCloning Recombination Procedure

Set up the following reaction in a 0.5 ml Eppendorf tube by mixing the following reagents gently and then spin down briefly to collect the reagents at the bottom of the tube.

Linearized vector (100-200 ng/μL)	6μL
Purified PCR products (100-200 ng/μL)	nμL
10X EasyCloning Buffer	2μL
EasyCloning Enzyme	2μL
Deionized water	up to 20μL

Incubate the reactions at 25°C for 5-30 minutes, and then transfer tubes to ice and incubate on ice for five minutes and proceed with transformation (Section D). The reaction can also be stored at -20°C for later transformation.

D. Transformation

MATERIALS NEEDED BUT NOT PROVIDED WITH KIT

Water bath or heating block (42°C)

SOC liquid medium

DH5α competent cells (>1×10$_8$ cfu/μg)

1. Thaw one vial of frozen 50μL competent cells on ice. Tap the tube gently to ensure that the cells are suspended.
2. Add 5–8μL of reaction mixture to the competent cells. Tap the tube gently and incubate the tube on ice for 30 minutes.
3. Heat shock the cells by placing them in 42°C water bath for 45–90 seconds and then place the tube on ice for 2–3 minutes.
4. Add 600μL of SOC medium to the cells and then incubate the cells on a shaker set at 250 rpm at 37°C for 60 minutes.
5. Centrifuge the cell down at 4000 rpm for five minutes and then remove and discard about 500μL of medium. Gently suspend the cells by tapping the tube.
6. Transfer 10μL and 100μL of the suspension to two different plates containing appropriate antibiotics, respectively. Spread the cells evenly on the plates.
7. Incubate the plates overnight at 37 °C.

TROUBLESHOOTING

Problem	Probable Cause	Solution
Few or no colonies are obtained from the transformation.	The competent cells have low transformation efficiency.	Check the transformation efficiency. Competent cells with >1×10^8 cfu/μg are recommended.
	Too much reaction mixture is used.	Do not add more than 10 μl of reaction mixture to 50 μl of competent cells. Too much reaction mixture inhibits the transformation.
	There are inhibitory contaminants from PCR DNA or from linearized vector.	Both of the PCR DNA and the linearized vector should be purified.
	The molar ratio of vector to insert is off.	Usually an insert/vector molar ratio of 2:1 is optimal. If the insert is as large as the linearized vector, a molar ratio of 1:1 can also be used.
Most of the colonies contain no insert.	The cloning vector is not completely linearized.	Gel-purify the linearized vector.
	The cloning reaction is contaminated with plasmids having the same antibiotic resistance.	Purified PCR DNA may contain the template plasmid, so gel-purify the PCR DNA.

Note: even there are only one or two colonies, it is likely to be positive clones.

This product is for Research Use Only.