CloneJET PCR Cloning Kit

The Thermo Scientific™ CloneJET™ PCR Cloning Kit can be used with PCR products generated by any DNA polymerase, therefore supporting both blunt- and sticky-end fragments from 6 bp to 10 kb long.

Recommendations

- Thoroughly mix every vial before use.
- Gel-analyze the PCR product for specificity and yield before cloning.
  - Specific PCR products of <1 kb appearing as one discrete band on the gel can be used for ligation directly from the PCR reaction mixture without any purification.
  - Gel purification is recommended for PCR products longer than 1 kb, PCR products contaminated with nonspecific sequences or primer-dimers, and PCR templates containing the β-lactamase (ampicillin resistance) gene.
  - For efficient cloning of gel-purified DNA fragments, it is important to avoid DNA damage by ethidium bromide and UV light.
  - The optimal insert:vector molar ratio is 3:1. Refer to Table 1 or use dedicated software (**thermoscientific.com/reviewer**) to calculate the optimal amount of PCR product to use.
  - For PCR products >3 kb, ligation can be prolonged to 30 min.

Note: All components of this kit should be stored at 20°C.

Table 1. Recommended amount of PCR product for the ligation reaction.

Length of PCR product	Optimal quantity of PCR product for ligation reaction (0.15 pmol ends)
100 bp	5 ng
300 bp	15 ng
500 bp	25 ng
1,000 bp	50 ng
2,000 bp	100 ng
3,000 bp	150 ng
4,000 bp	200 ng
5,000 bp	250 ng

Protocols

Blunt-end cloning

- For cloning blunt-end PCR products generated by proofreading DNA polymerases, if the supplier of a DNA polymerase does not specify the end structure of the PCR products it generates, follow the “Sticky-end cloning” protocol.
- For cloning blunt-end DNA fragments generated by restriction enzyme digestion, gel-purify the DNA fragment prior to ligation and use in a 3:1 molar ratio with pJET.2/blunt, the positive-selection vector.

Steps

1. Set up the ligation reaction on ice:

Component	Volume
2X reaction buffer	10 µL
Unpurified PCR product, purified PCR product, or other blunt-end DNA fragment	1 µL (0.15 pmol ends)
pJET1.2/blunt (50 ng/µL)	1 µL (0.05 pmol ends)
Water, nuclease-free	To 19 µL
T4 DNA ligase	1 µL
Total volume	**20 µL**

Vortex briefly and centrifuge for 3–5 sec.

2. Incubate the ligation mixture at room temperature (20°C) for 5 min. For PCR products >3 kb, ligation can be prolonged to 30 min.
3. Use the ligation mixture directly for transformation.

Sticky-end cloning

- For cloning PCR products with 3´ A overhangs generated by *Taq* DNA polymerase.
- For cloning PCR products when the supplier of a DNA polymerase does not specify the end structure of the PCR products it generates.
- For cloning DNA fragments with 5´ or 3´ overhangs generated by restriction enzyme digestion; gel-purify the DNA fragment prior to ligation and use in a 3:1 molar ratio with pJET.2/blunt, the positive-selection vector.

Steps

1. Set up the blunting reaction on ice:

Component	Volume
2X reaction buffer	10 µL
Unpurified PCR product, purified PCR product, or other sticky-end DNA fragment	1 µL (0.15 pmol ends)
Water, nuclease-free	To 17 µL
DNA blunting enzyme	1 µL
Total volume	**18 µL**

Vortex briefly and centrifuge for 3–5 sec.

2. Incubate the mixture at 70°C for 5 min, then chill on ice.
3. Set up the ligation reaction on ice. Add the following to the blunting reaction mixture:

Component	Volume
pJET1.2/blunt (50 ng/µL)	1 µL (0.05 pmol ends)
T4 DNA ligase	1 µL
Total volume	**20 µL**

Vortex briefly and centrifuge for 3–5 sec.

4. Incubate the ligation mixture at room temperature (20°C) for 5 min. For PCR products >3 kb, ligation can be prolonged to 30 min.
5. Use the ligation mixture directly for transformation.

Note: Keep the ligation mixture at –20°C if transformation is postponed. Thaw on ice and mix carefully before transformation.

Transformation

- The CloneJET kit is compatible with all common laboratory *E. coli* strains. Transformation of competent *E. coli* cells with the ligation mixture can be performed using different transformation methods (Table 2).
- For successful cloning, competent *E. coli* cells should have an efficiency of at least 1 x 10^6 CFU/µg supercoiled plasmid DNA. To check the efficiency, prepare a control transformation with 0.1 ng of a supercoiled plasmid vector.

Table 2. Guidelines for transformation of ligation mixture.

Transformation method	Treatment before transformation	Volume for transformation	Volume of competent cells for transformation
Thermo Scientific™ TransformAid™ Bacterial Transformation Kit (Cat. No. K2710)	Do not heat ligation mixture prior to use for transformation.	≤2.5 µL	50 µL
Calcium chloride transformation	Do not heat ligation mixture prior to use for transformation.	≤5 µL	50 µL
Electro-transformation	Spin column or chloroform extraction; do not heat ligation mixture prior to use for transformation.	0.5–1 µL of purified ligation mixture	40 µL

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