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Zero Blunt® PCR Cloning Kit

Catalog Number K2700-20, K2700-40, K2750-20, and K2750-40

Pub. No. MAN0007763 **Rev.** A.0

Produce blunt-end PCR products

1. Produce PCR products using an appropriate proofreading polymerase and your own protocol. Use no more than 30 cycles of amplification for optimal ligation efficiencies.

   IMPORTANT! If you used a *Taq* polymerase, you must blunt-end your PCR product.

2. Analyze 5–10 µL of each PCR sample by agarose gel electrophoresis.
3. Quantify the amount of DNA for each PCR product.

Clone into pCR®-Blunt

1. Add the following reagents to an autoclaved, 1.5-mL microcentrifuge tube:

pCR®-Blunt (25 ng)	1 µL
Blunt PCR product	1–5 µL
5X ExpressLink™ T4 DNA Ligase Buffer	2 µL
Sterile water to a total of	**9 µL**

2. Add 1 µL of ExpressLink™ T4 DNA Ligase (5 units) to the mixture.
3. Incubate the ligation reaction at room temperature for a minimum of 5 minutes. Longer ligation times increase the cloning efficiency.

One Shot® chemical transformation

1. Thaw One Shot® cells on ice.

   Note: S.O.C medium and positive control DNA are provided in kits with the One Shot® chemically competent cells.

2. Pipet 2 µL of each ligation reaction into the cells and gently stir the mixture with a pipette tip to mix.
3. Incubate the vials on ice for 30 minutes.
4. Heat shock the cells for 45 seconds at 42°C without shaking. Transfer the vials to ice.
5. Add 250 µL of S.O.C. medium to each vial.
6. Shake the vials at 37°C for 1 hour at 225 rpm.
7. Plate 10–100 µL from each transformation vial on an LB plate containing 50 µg/mL kanamycin or on a Low Salt LB plate containing 25 µg/mL Zeocin™ selective antibiotic.
8. Incubate plates overnight at 37°C.

Analyze positive clones

1. Pick at least 10 transformants for analysis.
2. Analyze the plasmid DNA by restriction analysis or sequencing to verify the correct insert and orientation. We recommend using the PureLink® HQ Mini Plasmid Purification Kit for purifying your plasmid DNA.
3. Purify the colony and make a glycerol stock for long-term storage. We recommend that you store a stock of plasmid DNA at –20°C.

Obtaining support

A detailed protocol, "Zero Blunt® PCR Cloning Kit User Guide" is available online, go to:

www.lifetechnologies.com/support

At the website, you can also :

- Access additional products used with the Zero Blunt® PCR Cloning Kit
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Obtain information about customer training
- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search for other user documents including SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Download detailed protocols and manuals

Limited product warranty

For Research Use Only. Not for use in diagnostic procedures.

The information in this guide is subject to change without notice.

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