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Fast-Link™ DNA Ligation and Screening Kits

Cat. Nos. LK08050 and LK08100

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1. Introduction

The Fast-Link™ DNA Ligation and Screening Kits provide rapid and efficient DNA ligation and subsequent screening of plasmid, cosmid, fosmid, and BAC clones without the need to grow cultures or perform minipreps. The DNA ligation components of the kit have been optimized for high efficiency ligation of cohesive-end DNAs in 5 minutes, blunt-end DNAs in 15 minutes at room temperature and ligation of PCR products containing A-overhangs to T-overhang cloning vectors in 1 hour at 16°C.

Following ligation and transformation, recombinant screening of insert-containing plasmid, cosmid, fosmid, and BAC clones can be accomplished in less than one day using the EpiBlue™ and EpiLyse™ Buffers provided in the kits. The screening reagents, EpiBlue Buffer and EpiLyse Buffer are also sold separately in the Colony Fast-Screen™ Kit.

2. Product Specifications

Storage: Store the Fast-Link DNA Ligation and Screening Kit at –20°C in a freezer without a defrost cycle until used. Once opened, the EpiBlue and EpiLyse Buffers can be stored at room temperature. Return the Fast-Link DNA Ligase, Fast-Link 10X Reaction Buffer and ATP to –20°C storage.

Storage Buffer: Fast-Link DNA Ligase is supplied at a concentration of 2 U/µl in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton® X-100, and 1 mM dithiothreitol (DTT).

Unit Definition: One unit of Fast-Link DNA Ligase converts 1 nmol of pyrophosphate into Norit-adsorbable material in 20 min. at 37°C.[1]

Quality Control: Fast-Link DNA Ligase activity is assayed in a reaction containing 33 mM Tris-acetate (pH 7.5), 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT, 1 mM ATP, 1 µg *Hin*d III-cut lambda DNA, and 0.001-1.0 unit of enzyme.

Contaminating Activity Assays: Fast-Link DNA Ligase is free of detectable exo- and endonuclease and RNase activities as judged by agarose gel electrophoresis following incubation of 1 µg of DNA and RNA substrates with 150 U of the enzyme for 16 hours at 37°C.

3. Kit Contents

Desc.	Concentration	Quantity
The Fast-Link DNA Ligation and Screening Kit is available in two sizes: 50 Ligations with 250 Screenings (Cat. # LK08050) and 100 Ligations with 500 Screenings (Cat. # LK08100) reaction sizes. The smaller kit contains the following reagents:		
Fast-Link DNA Ligase	@ 2 U/µl	50 µl
10X Fast-Link Buffer (330 mM Tris-acetate [pH 7.5], 660 mM potassium acetate, 100 mM magnesium acetate, 5 mM DTT)		100 µl
ATP (10 mM)		100 µl
EpiBlue™ Buffer		2.5 ml
EpiLyse™ Buffer		5 ml

4. Related Products

The following products are also available:

- Fast-Link™ DNA Ligation Kits
- Colony Fast-Screen™ Kits
- T4 DNA Ligase
- GELase™ Agarose Gel-Digesting Preparation
- APex™ Heat-Labile Alkaline Phosphatase
- T4 Polynucleotide Kinase
- Transformation and Storage Solution
- TransforMax™ EC100™ Electrocompetent *E. coli*
- TransforMax™ EC100D™ *pir*$^+$ and *pir*-116 Electrocompetent *E. coli*
- End-It™ DNA End-Repair Kit

5. General Considerations

1. **Preparation of Vector and Insert DNA molecules:** Prepare the vector and insert DNA molecules by restriction digestion, PCR amplification, or other physical/enzymatic methods. The ends of the DNA molecules must be similar for ligation to occur efficiently. If necessary, separate the vector and insert from other contaminating molecules by any one of several methods, including electrophoretic, physical (e.g., columns), or organic extraction followed by ethanol precipitation. During purification of DNA molecules (i.e., using agarose gel electrophoresis), minimize exposure of insert and vector DNA to UV irradiation, which can cause formation of pyrimidine dimers. Following purification, quantify the DNA by gel electrophoresis (comparing with a known amount of DNA) or fluorescence (i.e., using Hoechst dye 33258[2]).
2. **Dephosphorylation of Vector DNA Molecules:** Treatment of vector molecules to remove the 5′-phosphate groups will prevent recircularization, minimizing the recovery of nonrecombinant clones. This is especially important for vector molecules with compatible ends (nondirectional cloning).
3. **Cloning of PCR Amplification Products:** Phosphate groups must be added to either the 5′ end of primers used in the synthesis of PCR amplification products, or the amplification products themselves must be directly phosphorylated, if these products are cloned into a dephosphorylated vector. Alternatively, incorporate restriction endonuclease sites near the 5′ end of the PCR primers, and following amplification, digest the amplification products and the vector with the appropriate enzyme. If digestion at the restriction site is inefficient, the amplification products can be treated with a combination of T4 polynucleotide kinase to add phosphate groups; Klenow or T4 DNA polymerase to blunt the ends; and T4 DNA ligase to form concatamers.[3] The concatamer is then digested with the restriction endonuclease and the linear fragment cloned into an appropriate vector.
4. **Ratio of Vector DNA:Insert DNA:**[4,5] In general, for ligations of fragments with cohesive ends, one can achieve the greatest number of recombinants when the concentration of insert DNA is 2X that of the concentration of vector DNA (i.e., a molar ratio of 2:1, insert:vector). A lower ratio of insert-to-vector will favor

recircularization of the plasmid, whereas a higher ratio will favor formation of vectors containing multiple inserts. For a 3-kb vector, the optimum concentration of vector DNA is 10-20 ng/µl final volume; for a 10-kb vector, the optimum concentration of vector DNA is 5-10 ng/µl final volume. (A lower concentration of DNA is required for larger vectors as the likelihood that the ends of the same molecule will interact is less than for smaller vectors.) To achieve a 2:1 molar ratio of insert:vector DNA, the concentration of insert DNA will depend upon its size relative to the size of the vector. Ligations of DNA molecules with blunt ends require higher concentrations of insert DNA to achieve the greatest number of recombinants.[6] We recommend a molar ratio of 5:1, insert to plasmid, for most blunt end ligations. For ligations involving linkers, use a 100:1 molar ratio of linker to vector.[4]

5. **ATP Concentration:** High concentrations of ATP (i.e., greater than 0.5 mM) inhibit ligation of DNA molecules with blunt ends.[7]
6. **Enzymatic Treatment of Ligation Reactions:** Following ligation, the DNA molecules may be treated in the Fast-Link reaction buffer with most common restriction endonucleases or nucleases highly specific for linear double-stranded DNA (e.g., Plasmid-Safe™ DNase).
7. **Electroporation:** Aliquots (1-2 µl) of the ligation reaction may be used directly in electroporation without desalting.

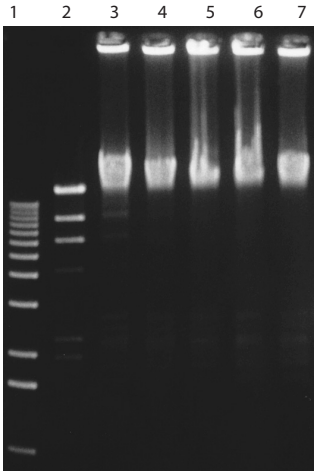

Figure 1. Time course for a cohesive-end ligation using the Fast-Link Kit. Lambda *Hin*d III markers were ligated in a standard Fast-Link reaction using 2 U of Fast-Link DNA Ligase (lanes 3-7).
Lane 1, 1 kb ladder; Lane 2, no enzyme; Lanes 3-7, 5 min, 15 min, 30 min, 1 hr, 2 hr, respectively.

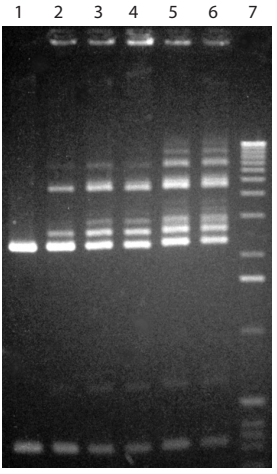

Figure 2. Time course for blunt-end ligation using the Fast-Link Kit. pUC19 digested with *Pvu* II was ligated in a standard Fast-Link reaction using 2 U of Fast-Link DNA Ligase (lanes 2-6). Lanes 1, no enzyme; Lanes 2-6, 5 min, 15 min, 30 min, 45 min, 1 hr, respectively; Lane 7, 1 kb ladder.

8. **Time of Ligation:** The ligation of DNA molecules with compatible cohesive ends occurs quickly at room temperature, being essentially complete within 5 minutes (Figure 1). The ligation of molecules with blunt ends proceeds more slowly, although a substantial amount of product is formed within 15 minutes (Figure 2). Note that the length of incubation for any of the ligation reactions may be extended overnight with no deleterious effects.

6. Protocol for Ligation of DNA Molecules with Cohesive or Blunt Ends

This protocol is suitable for the ligation of insert DNAs with cohesive or blunt ends into plasmid vectors with like ends.

1. Assemble the reaction in a microcentrifuge tube at room temperature as outlined, adding the ligase last (Numbers in parentheses indicate molar ratios.):

Ligations of Insert DNA with Cohesive Ends

1.5	µl	10X Fast-Link Ligation Buffer
1.5	µl	10 mM ATP
x	µl	vector DNA (1)
x	µl	insert DNA (2)
x	µl	sterile water to a volume of 14 µl
1	µl	Fast-Link DNA Ligase
15	µl	Total reaction volume

Ligations of Insert DNA with Blunt Ends

1.5	µl	10X Fast-Link Ligation Buffer
0.75	µl	10 mM ATP
x	µl	vector DNA (1)
x	µl	insert DNA (5)
x	µl	sterile water to a volume of 14 µl
1	µl	Fast-Link DNA Ligase
15	µl	Total reaction volume

2. Incubate the reaction 5 minutes at room temperature for cohesive ends and 15 minutes for blunt ends (See Figures 1 and 2, above).
3. Transfer the reaction to 70°C for 15 minutes to inactivate the Fast-Link DNA ligase; failure to inactivate the ligase may decrease transformation efficiencies.[6]
4. Spin briefly in a microcentrifuge.
5. Transform competent *E. coli* cells with 1/10 of the ligation reaction, keeping the volume of the ligation to no more than 5% of the volume of competent cells,[4] or follow the manufacturer's recommendations. If electroporating the ligated molecules, use no more than 2 µl of the ligation reaction with 50 µl of electrocompetent cells.
6. To determine the extent of ligation, inactivate the ligase and run 5 µl of the ligation reaction on an agarose gel and visualize.

7. Protocol for Ligation of PCR Products into T-Vectors

This protocol is suitable for ligation of PCR products containing a non-template, 3′ A-overhang[8] into plasmids containing a 3′ T-overhang[9] (T-vectors). Separate PCR products from reaction components (e.g., template DNA and buffer salts). Prepare[10] or purchase a T-vector suitable for cloning PCR products (e.g., TA Cloning® vector, Invitrogen).

1. Assemble the reaction in a microcentrifuge tube as outlined, adding the Fast-Link DNA Ligase last (Numbers in parentheses indicate molar ratio):

Ligations of PCR Products into T-Vectors	
1.5 µl	10X Fast-Link Ligation Buffer
0.75 µl	10 mM ATP
x µl	T-vector DNA (1)
x µl	PCR product (1)
x µl	sterile water to a volume of 14 µl
1 µl	Fast-Link DNA Ligase
15 µl	Total reaction volume

2. Incubate the reaction for 1 hour at 16°C.
3. Transfer the reaction to 70°C for 15 minutes to inactivate the Fast-Link DNA ligase; failure to inactivate the ligase may decrease transformation efficiencies.[6]
4. Spin briefly in a microcentrifuge.
5. Transform competent *E coli* cells with 1/10 of the ligation reaction, keeping the volume of the ligation to no more than 5% of the volume of competent cells,[4] or follow the manufacturer's recommendations. If electroporating the ligated molecules, use no more than 2 µl of the ligation reaction with 50 µl of electrocompetent cells.
6. To determine the extent of ligation, inactivate the ligase and run 5 µl of the ligation reaction on an agarose gel and visualize.

8. Protocol for Screening of Recombinants

After transformation and selection of recombinants, use the EpiBlue and EpiLyse Buffers to screen the clones. An overview of the colony screening process is shown in Figure 3.

1. Using a sterile toothpick gently touch the colony on the plate to collect a small amount (less than the size of a pin head) of cells from a chosen colony. Deposit the cells at the bottom of a 0.5-ml tube or the bottom of a microtiter plate well. Repeat the process using a fresh toothpick for each colony chosen and deposit the cells from each colony into its own tube or microtiter plate well.

   Note: *The Colony Fast-Screen process is extremely sensitive. The most common error is to collect too large a portion of cells from a colony.*
2. Resuspend the cells by adding 10 µl of EpiBlue Buffer and vortexing vigorously for 10 seconds.
3. Add 20 µl of EpiLyse Buffer into the resuspended cells.
4. Incubate the tubes or microtiter plate at 70°C for 15 minutes.

5. After the incubation, mix each tube by vigorous vortexing for 10 seconds. Load 10 µl from each independently, into the wells of an agarose gel. Use supercoiled DNA size markers.

   Notes:

   1) If a solution is too viscous and difficult to pipette, then too many cells were collected from the colony in step 1. Add an additional 20 µl of EpiLyse Buffer to the highly viscous solutions, mix thoroughly and load 10 µl onto the agarose gel.
   2) Cosmid, fosmid, and BAC clones may be difficult to detect because they often migrate with the chromosomal DNA fragments released from the host cell. To detect cosmid, fosmid, and BAC clones, run longer agarose gels (e.g. 14 cm gels) at lower voltage (e.g., 80 V) for longer times (e.g. 4 hours).
   3) Standard ethidium bromide staining may not be sensitive enough to detect low copy BAC clones. To detect BAC clones, stain the gels with ethidium bromide for longer times or use a highly sensitive stain (e.g., SYBR® Gold at 1:10,000 dilution in TE buffer or water).

6. Cover and store the solution remaining in each tube or microtiter plate well at room temperature for up to 1 week in the event additional gels need to be run.

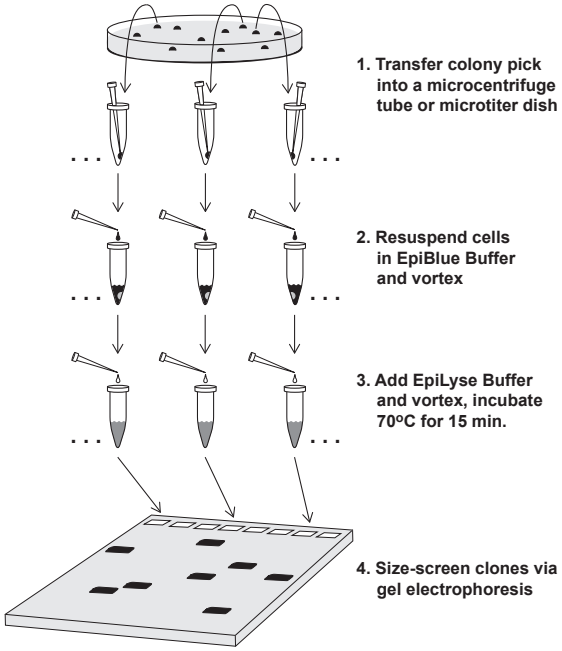

Figure 3. Screening Protocol.

9. References

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