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Simple and Fast
Techniques to Sequencing

Genomic Analysis Tools to accelerate sequencing projects

- GeneJumper™ Kit
- GeneJumper™ oriV Transposon Kit
- TOPO® Shotgun Subcloning Kit
- TOPO® Walker Kit

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Efficient Gene Sequencing—Faster Results

With Invitrogen's Genomic Analysis Tools, you get templates for total sequencing coverage quickly and efficiently. We offer complete kits for easy, uncomplicated analysis of cosmids, genomic DNA, cDNA, and artificial chromosomes (BACs, PACs, YACs). You'll avoid unnecessary steps when you're preparing templates for sequencing, as we'll as when constructing shotgun libraries, and filling in gap sequences. Functionally tested to ensure accurate results, these tools are designed for fast, efficient use. Discover which technology is right for you.

Get your entire sequence with convenient tools

Complete sequencing is easy and fast with our Genomic Analysis Tools. GeneJumper™, GeneJumper™ oriV Transposon, TOPO® Shotgun Subcloning, and TOPO® Walker Kits represent a significant advance over traditional methods by eliminating unnecessary steps for creating sequencing templates. Table 1 below shows the tools we offer to simplify your sequencing efforts.

Table 1 – Sequencing Tools from Invitrogen

Kit Name	Application	Template
GeneJumper™ Kit	Random insertion of primer-binding sites	BACs, PACs, cosmids, and cDNA
GeneJumper™ oriV Transposon Kit	Random insertion of primer binding sites and inducible origin of replication for generating increased yield of sequencing templates	BACs
TOPO® Shotgun Subcloning Kit	Construction of shotgun libraries for sequencing	BACs, YACs, cosmids, and genomic DNA
TOPO® Walker Kit	Determination of unknown gap sequence	Partially sequenced BACs, YACs, and PACs

Designed for efficient and random insertion

The GeneJumper™ Kits facilitate the random insertion of sequencing primer binding sites into just about any plasmid. These kits allow you to expedite lengthy protocols and eliminate the need to generate collections of sequencing primers. The GeneJumper™ Transposon (Figure 1) is designed to include primer-binding sites at each end that can be used for bi-directional sequencing or mapping by colony PCR. The transposon also contains several convenient restriction sites to facilitate downstream cloning procedures.

The GeneJumper™ Transposon is engineered to offer several conveniences and contains the following key features:

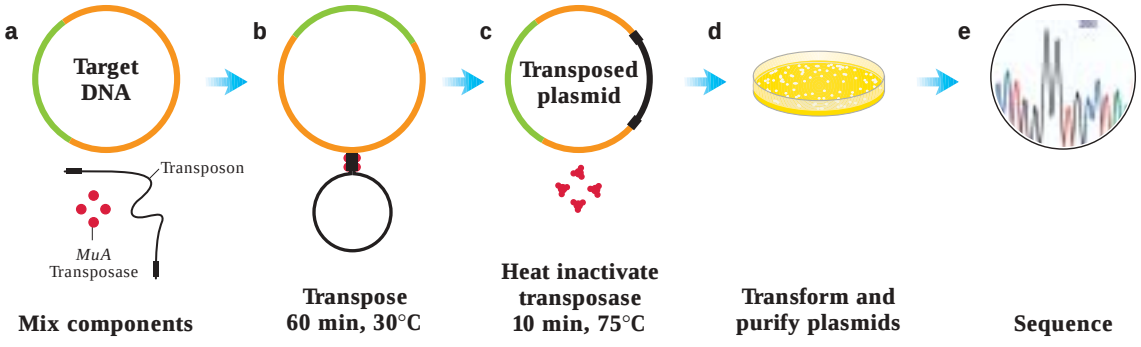
- Binding sites for the *MuA* Transposase (R1 and R2) to carry out the transposition reaction
- *Mu* end primer binding sites for performing PCR with a vector-specific primer to map inserts before sequencing
- Sequencing primer sites (Seq A and Seq B) for convenient bi-directional sequencing
- Antibiotic resistance for efficient selection of transposed plasmids
- Restriction sites for mapping

Figure 1 – Diagram of the GeneJumper™ Transposon

Get only the transposition activity that you want

Since transposases are common throughout many cell types, we specifically designed the GeneJumper™ transposon sequences to prevent unwanted transposition activity. The GeneJumper™ transposon contains sequences that are essential for binding only *MuA* transposase. A *MuA* transposase inactivation step ensures that transposition activity is limited to the *in vitro* reaction with your target DNA. Optimized reaction conditions reduce the frequency of multiple transposon insertions to well below 1%. This means you get a single, priming reaction that results in clean PCR and sequencing data. Figure 2 demonstrates the transposition reaction protocol and shows how quickly you can work on your sequencing results.

Figure 2 – The GeneJumper™ Kit *in vitro* transposition reaction



Generate stable BAC DNA template

The GeneJumper™ oriV Transposon Kit generates large quantities of stable BAC DNA for rapid, easy sequencing. The specialized transposon in the GeneJumper™ oriV Transponson Kit includes the oriV origin of replication[1], This origin allows easy induction of replication from "single copy" plasmid per cell to high copy number allowing you to generate high yields of DNA suitable for sequencing. Because the GeneJumper™ transposon already contains the sequencing primers, you're ready to sequence right away (Figure 3). With this inducible system, you no longer need to grow large BAC cultures to produce sufficient template, for complete sequencing coverage.

In the GeneJumper™ oriV Transposon Kit, transposed BAC plasmids are electroporated into GeneHogs® *trfA*-competent *E. coli*, which carry a mutant *trfA* gene under an arabinose-regulated promoter. After L-arabinose induction, the expressed TrfA protein interacts with oriV to initiate replication, increasing the copy number from one to more than 20 per cell. Induction produces a three- to six-fold higher yield of BAC DNA than an uninduced culture. Isolated plasmids can be sequenced using the primers provided. Make your BAC sequencing project easier with the GeneJumper™ oriV Transposon Kit.

Figure 3 – The GeneJumper™ oriV transposon

Accelerate shotgun subcloning and primer walking

We've paired up shotgun subcloning technology and primer walking with our patented TOPO® mediated technology to:

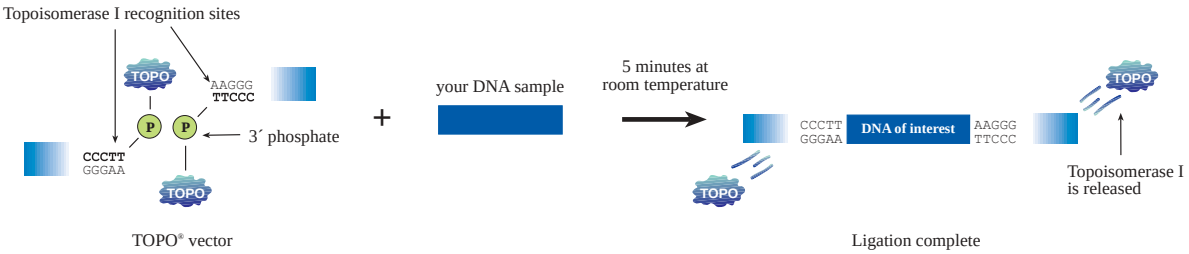
- Expedite DNA preparation
- Remove gel purification steps
- Decrease cloning time

TOPO® Cloning makes ligation faster and more successful. It enables 5-minute, bench-top ligation and yields ≥95% recombinants. This speed and efficiency saves you hours of time over ligase-mediated methods There's no overnight incubation, and you won't waste time repeating failed ligations.

TOPO® vectors are provided linearized with topoisomerase I covalently bonded to each 3´ phosphate, which enables the vectors to readily ligate DNA sequences with compatible ends (Figure 4). After a 5-minute incubation at room temperature, the ligation is complete and ready for transformation into *E. Coli*.

Read on to find out how to utilize our TOPO® cloning technology to expedite your sequencing experiments.

Figure 4 – How the TOPO® Cloning method works



Streamlined shotgun subcloning

The TOPO® Shotgun Subcloning Kit is specifically designed to expedite traditional shotgun subcloning procedures by saving both time and effort in each step of a traditional protocol. This technology was built upon examining each step of a traditional shotgun subcloning protocol and eliminating tedious steps and lengthy incubations (see Figure 5). This kit includes a specialized vector with numerous features to make shotgun subcloning easier than ever before. The TOPO® Shotgun Subcloning Kit includes pCR®4Blunt-TOPO® vector (Figure 6) that allows you to:

- **Easily Construct Shotgun Libraries**—Readily accepts blunt-ended DNA fragments. Cloning takes just 25 minutes.
- **Eliminate Multiple Inserts**—Only vectors containing single inserts will circularize and propagate.
- **Keep Background Low** —Expression of a lethal *ccd*B gene ensures only recombinant clones will grow.
- **Streamline Sequencing**—Increase efficiency by reading more insert and less vector.

Figure 5 – Cloning time using a traditional shotgun procedure versus the TOPO® Shotgun Subcloning Kit

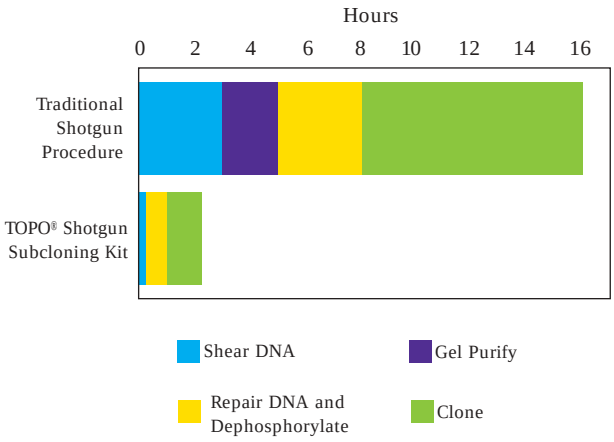
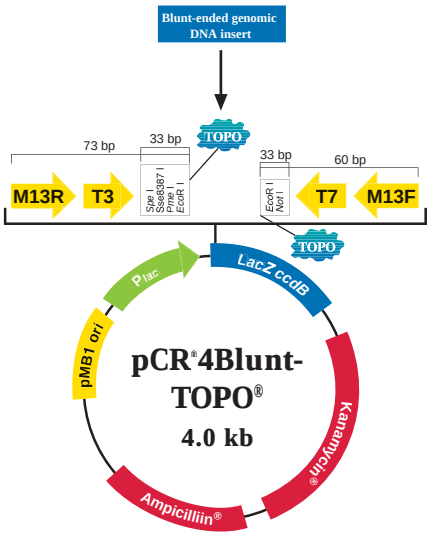


Figure 6 – pCR®4Blunt-TOPO® vector



Features of pCR®4Blunt-TOPO® include:
- Flanking *Eco*R I sites to simplify excision of cloned products
- Unique *Sse*8387 I site in the multiple cloning site to simplify performing nested deletions
- T7, T3, M13(-20) forward, and M13 reverse sequencing primer sites

The TOPO® Shotgun Subcloning Kit utilizes a nebulizer—a small plastic device used to atomize liquids—and compressed air to shear large DNA into 2kb fragments suitable for cloning.

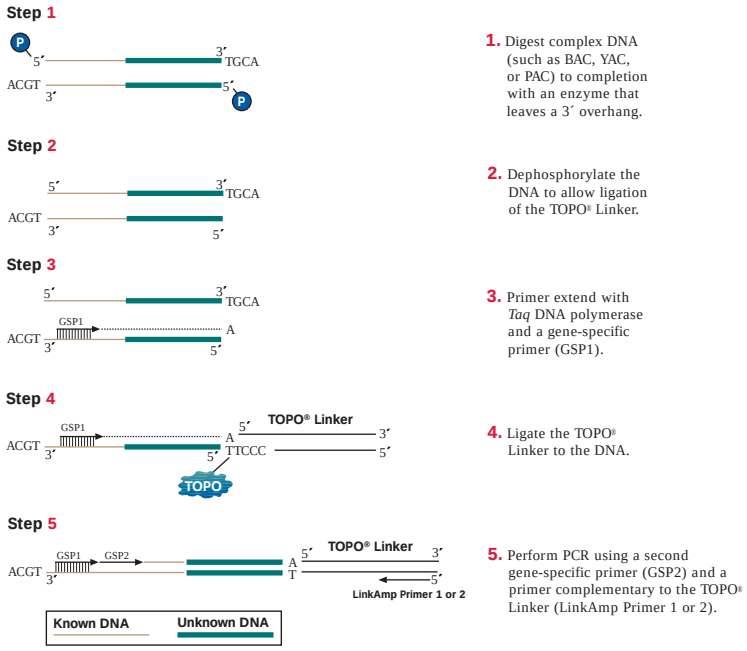
The fast way to fill in sequence gaps

The TOPO® Walker Kit eliminates the need for hybridization-based library screens when isolating unknown DNA sequences. Instead, the kit uses a simple 5-step protocol to save time in your chromosome walking experiments (Figure 7). Once the unknown DNA fragment is amplified, the PCR product can be sequenced directly. There's no need to carry out cloning experiments that can add additional days to your experiment.

TOPO® Walker Kit saves you time by:

- ligating a topoisomerase-activated linker to the unknown end of a DNA fragment in just 5 minutes.
- using PCR to rapidly amplify the unknown sequence
- sequencing the PCR product directly—no sub-cloning steps are required

Figure 7 – How TOPO® Walker works

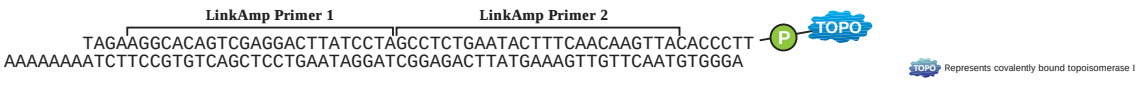


1. Digest complex DNA (such as BAC, YAC, or PAC) to completion with an enzyme that leaves a 3´ overhang.
2. Dephosphorylate the DNA to allow ligation of the TOPO® Linker.
3. Primer extend with *Taq* DNA polymerase and a gene-specific primer (GSP1).
4. Ligate the TOPO® Linker to the DNA.
5. Perform PCR using a second gene-specific primer (GSP2) and a primer complementary to the TOPO® Linker (LinkAmp Primer 1 or 2).

PCR makes it easy — TOPO® technology makes it fast

The key to the speed of the TOPO® Walker Kit is the TOPO® Linker (Figure 8). The TOPO® Linker is a 58 bp, double-stranded DNA sequence containing two PCR primer sites, and a 3´- T overhang in which topoisomerase I is covalently bound. In just 5 minutes you can ligate the TOPO® Linker to the 3´-A overhang of your DNA sequence. Subsequent PCR using a primer specific to the linker and a gene-specific primer from your known sequence amplifies the sequence gap.

Figure 8 – The topoisomerase-activated TOPO® Linker



Order today

Our Genomic Analysis tools offer many ways to easily get you sequencing templates. Choose from these kits to streamline and accelerate your sequencing projects.

Description	Quantity	Cat. no.
GeneJumper™ Kit for large DNA		
With chloramphenicol-resistant transposon	10 rxns	T1000-01
With kanamycin-resistant transposon	10 rxns	T1005-01
GeneJumper™ Kit for small plasmids		
With chloramphenicol-resistant transposon	10 rxns	T1050-01
With kanamycin-resistant transposon	10 rxns	T1055-01
GeneJumper™ oriV Transposon Kit	10 rxns	T1060-01
TOPO® Shotgun Subcloning Kit		
With One Shot®TOP10 Chemically Competent E .coli	5 libraries	K7000-01
With One Shot®TOP10 Electrocomp™ E. coli	5 libraries	K7050-01
TOPO® Shotgun Subcloning Kit-no nebulizers		
With One Shot®TOP10 Chemically Competent E. coli	5 libraries	K7010-01
With One Shot®TOP10 Electrocomp™ E. coli	5 libraries	K7060-01
Nebulizers	5	K7025-05
TOPO® Walker Kit	40 rxns	K8000-01

Reference.

1. Hradecna, Z. *et.al.* (1998) *Microbial and Comp Genomics* **3**: 58

US Patent No. 5,874,259. The oriV-trfA - mutant DNA amplification system was developed in the laboratory of W. Szybalski by Wild, Hradecna, and Szybalski at the University of Wisconsin-Madison.

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