

# Confidently conquer cloning of long DNA fragments

High-efficiency cloning with TOPO XL-2 kit

The Invitrogen™ TOPO™ XL-2 Complete PCR Cloning Kit provides all the necessary elements for efficient cloning of extra-long PCR products (up to 13 kb). The kit uses the linearized and topoisomerase I-activated Invitrogen™ pCR™-XL-2-TOPO™ vector, which is compatible with the cloning of blunt-end PCR fragments (Figure 1). Amplification of long PCR fragments is enabled by Invitrogen™ Platinum™ SuperFi™ Green PCR Master Mix, which is included in the kit. Topoisomerase I activation of the vector enables PCR products to be ligated in just 5 minutes on your benchtop, resulting in high cloning efficiency (up to 90% positive recombinants).

### The complete kit includes:

- **TOPO XL-2 PCR Cloning Kits** containing the pCR-XL-2-TOPO vector
- **Platinum SuperFi Green PCR Master Mix**—featuring a proofreading DNA polymerase (>300x fidelity compared to *Taq* polymerase) with high processivity and a density gradient to generate accurate, long PCR amplicons ready to load onto an agarose gel for gel extraction

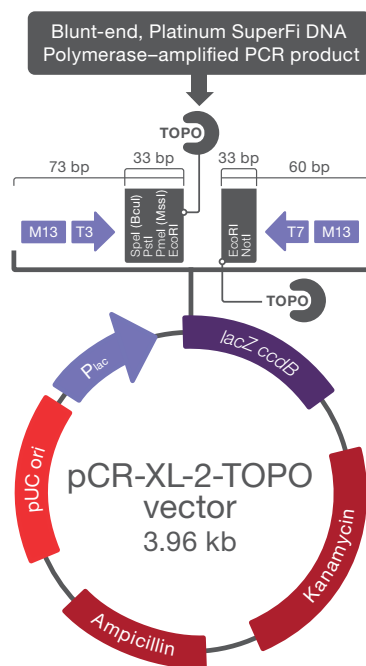


Figure 1. The pCR-XL-2-TOPO vector.

- **Invitrogen™ PureLink™ Quick Gel Extraction and PCR Purification Combo Kit**—designed to purify DNA fragments in less than 30 minutes from agarose gels or direct PCR purification using a silica-based spin cartridge
- **Invitrogen™ One Shot™ OmniMAX™ 2 T1<sup>R</sup> Chemically Competent *E. coli* Cells**—an improved, high-efficiency chemically competent cell line, perfect for use in all cloning applications



### High cloning efficiency

The pCR-XL-2-TOPO vector enables high cloning efficiency (Figure 2) and includes:

- *ccdB* gene for positive selection
- EcoRI site flanking the PCR product insertion site for easy excision of inserts
- Ampicillin- and kanamycin-resistance genes for your choice of antibiotic selection
- T7 promoter/priming site for *in vitro* transcription
- T7, T3, and M13 forward- and reverse-primer sites for sequencing

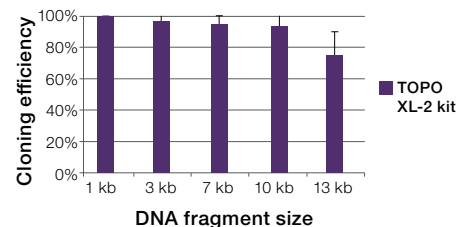
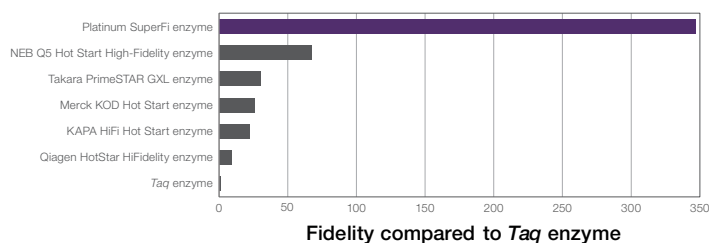


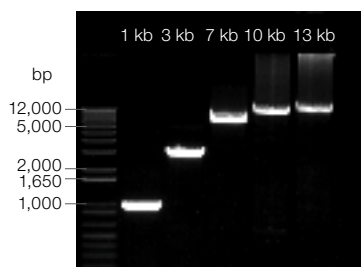
Figure 2. TOPO XL-2 PCR cloning kits show high cloning efficiencies. The TOPO XL-2 kit offers very high cloning efficiency for a broad range of different sized targets. Thermo Scientific™ Lambda genomic DNA targets ranging from 1 to 13 kb were cloned using the TOPO XL-2 cloning kit workflow.

## Exceptional accuracy of your clone

Platinum SuperFi Green PCR Master Mix contains a proofreading DNA polymerase, which combines exceptional fidelity with the trusted Invitrogen™ Platinum™ hot-start technology, designed for the highest success in PCR (Figure 3). Featuring >300x *Taq* fidelity, Platinum SuperFi Green PCR Master Mix is ideally suited for cloning, mutagenesis, and other applications benefiting from superior sequence accuracy, such as working with long DNA fragments (Figure 4).



**Figure 3. Platinum SuperFi DNA Polymerase exhibits superior fidelity.** Polymerase fidelity was measured by next-generation sequencing using unique molecular identifiers (UMIs), and reads from the same UMI family were aligned to call errors. The polymerase fidelities were normalized to *Taq* polymerase.



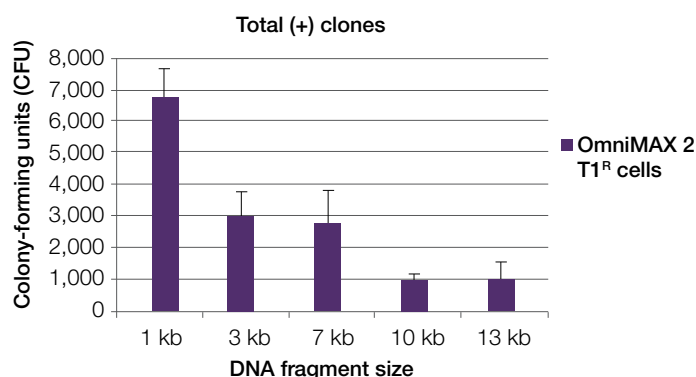
**Figure 4. Platinum SuperFi DNA Polymerase has high specificity with amplifying larger targets.** 1, 3, 7, 10, and 13 kb targets from Lambda genomic DNA were amplified following manufacturer's standards for PCR cycling parameters.

## Ordering information

Product	Size	Cat. No.
TOPO XL-2 Complete PCR Cloning Kit, with One Shot OmniMAX 2 T1 <sup>R</sup> Chemically Competent <i>E. coli</i> Cells	20 reactions	K8050-20
	10 reactions	K8050-10

## High transformation efficiency

The OmniMAX 2 T1<sup>R</sup> cells offer one of the highest transformation efficiencies (>5 x 10<sup>9</sup> transformants/μg pUC19) among chemically competent *E. coli* cells in the One Shot format. These highly versatile cells also provide efficient transformation of highly methylated DNA, since OmniMAX 2 T1<sup>R</sup> cells lack the *E. coli* K12 restriction systems (*mcrA* Δ(*mrr hsdRMS-mcrBC*)). In addition, the strain carries the *tonA* genotype, which confers resistance to T1 and T5 phage infection. This helps protect your samples and minimize the possibility of downtime in your lab due to phage contamination (Figure 5).



**Figure 5. The chemically competent OmniMAX 2 T1<sup>R</sup> cells yield high numbers of CFU.** Clones were selected on plates containing kanamycin and 1 mM IPTG. A total of 10–12 transformants were analyzed by colony PCR.

Request the TOPO XL-2 Complete PCR Cloning Kit for your Supply Center at [thermofisher.com/scproductrequests](http://thermofisher.com/scproductrequests)

Find out more at [thermofisher.com/topoxl2](http://thermofisher.com/topoxl2)

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