



IMPORTANT!
-20°C Storage Required
Immediately Upon Receipt

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DNATerminator[®] End Repair Kit

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DNATerminator[®] End Repair Kit

Components & Storage Conditions

The DNA Terminator End Repair Kit contains one tube each of End Repair Enzyme Mix and 5X End Repair Buffer in the following amounts:

DNATerminator Kit Components

Store at -20°C

Catalog #	Reactions	End Repair Enzyme	5X End Repair Buffer
40035-1	10	20 µL	100 µL
40035-3	20	40 µL	200 µL
40035-2	50	100 µL	500 µL

Description

The DNATerminator End Repair Kit supplies the buffer and enzymes needed to efficiently convert 3' and 5' overhangs into blunt ends for ligation into blunt cloning sites. It has been developed to maximize the efficiency of shotgun library construction, in which insert DNA is fragmented by shearing or restriction digestion. Physical methods of DNA fragmentation (e.g., sonication, hydrodynamic shearing, nebulization) are often preferred over enzymatic methods because they are more random (1, 2). However, shearing results in a heterogeneous mix of blunt and 3'- and 5'-overhanging ends that may or may not ligate efficiently. The DNATerminator End Repair Kit is a proprietary mix of DNA repair activities that generates blunt, phosphorylated ends that are efficiently ligated into blunt sites of cloning vectors.

Conventional end repair protocols are not optimal for generating blunt ends for library construction. Using the DNATerminator End Repair kit can result in a significant improvement in the number of colonies obtained in a shotgun library (Figure 1).

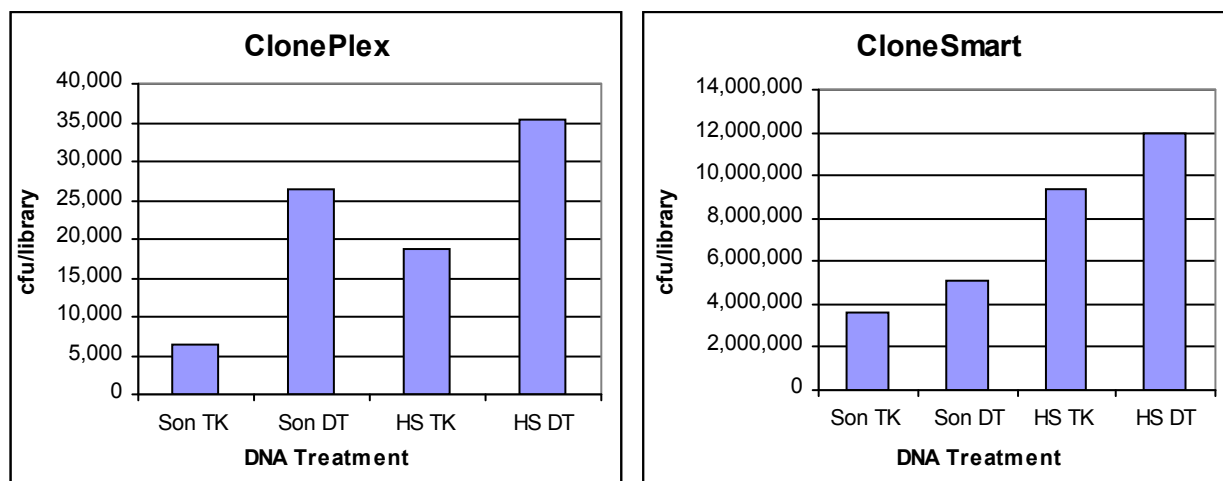


Figure 1. Effect of shearing and end repair methods on library construction efficiency. Shotgun libraries were constructed using 250 ng of lambda DNA sheared by sonication (Son) or by the GeneMachines HydroShear device (HS). Dual insert libraries were generated with Lucigen's ClonePlex[®] system (ClonePlex) and single insert libraries were generated with Lucigen's CloneSmart[®] system (CloneSmart). Sheared DNAs were repaired with T4 DNA polymerase and Klenow fragment (TK) or DNATerminator[®] End Repair Kit (DT). Values are colony forming units (cfu) per library.

DNATerminator[®] End Repair Kit

The DNATerminator is NOT recommended for end-repairing PCR fragments that contain a 3' single-base overhang, such as those produced by Taq or Tfl polymerases. Lucigen's PCR Terminator Kit was specifically developed for processing these ends.

Materials and Equipment Needed

The DNATerminator Kit requires the use of common molecular biology equipment, supplies, and reagents. In addition, the kit requires the purification of the DNA before and after performing the DNATerminator reaction. The DNA can be purified using your method of choice, such as commercial purification kits, gel electrophoresis, or ethanol precipitation.

Protocol

DNA Purification Before End Repair

The DNA needs to be relatively free of RNA before end repairing. Large amounts of contaminating RNA will severely impair the efficiency of the end repair reaction, resulting in DNA with poor cloning capabilities. We recommend the use of RNase I (Lucigen Cat. # 30104-1 or -2), which is an exonuclease that breaks RNA down into nucleosides, to remove most of the residual RNA often associated with DNA purification protocols. RNase A, which is a site specific endonuclease, will not degrade the RNA sufficiently and is not recommended.

Buffers used for fragmentation of the DNA also must be removed before beginning the DNATerminator reaction. After purification, the DNA should be eluted or dissolved in water.

DNATerminator End Repair Reaction

The DNATerminator End Repair Kit has been optimized for processing approximately 0.2-15 pmol of DNA fragments (equivalent to ~1-30 μg of DNA fragmented to 5 kb).

Mix the following components in a microfuge tube:

y μL purified, fragmented DNA
x μL H ₂ O
10 μL 5X DNATerminator [®] End Repair Buffer
2 μL DNATerminator [®] End Repair Enzymes
<hr/>
50 μL final volume

Incubate 30 minutes at room temperature.
Stop the reaction by incubation at 70°C for 15 minutes.
Purify the DNA.

Note: the heat denaturation step may be omitted only if the DNA is *immediately* purified using a protein denaturing reagent (e.g., addition of phenol or column binding buffer that contains guanidinium HCl).

Do not exceed the recommended enzymatic treatment of the fragment. Excessive enzyme or incubation time can lead to nucleolytic degradation of the fragments. For less than 0.2 pmol of DNA, the amount of enzyme may be scaled down and the time decreased to 15 minutes.

Purification and Size Fractionation of DNA After End Repair

DNATerminator[®] End Repair Kit

After stopping the DNATerminator End Repair reaction, the DNA must be purified from the enzymes. Stopping and reaction and purifying the DNA essential to achieve the maximum number of transformants.

Agarose gel electrophoresis, which is commonly used to isolate fragments of the appropriate size range (1), can be used for DNA purification as well as for size fractionation.

If the DNA is *not* size fractionated, it must be purified by other means. Failure to remove the end-repair enzymes may lead to inefficient ligation or high background of non-recombinant clones.

Tips on Maximizing Yield of Transformants

Avoid exposing the DNA to short wave UV radiation

It is important to note that using a typical short wavelength UV transilluminator (254 or 302 nm) to visualize DNA in the gel can reduce cloning efficiencies by several orders of magnitude (Figure 2).

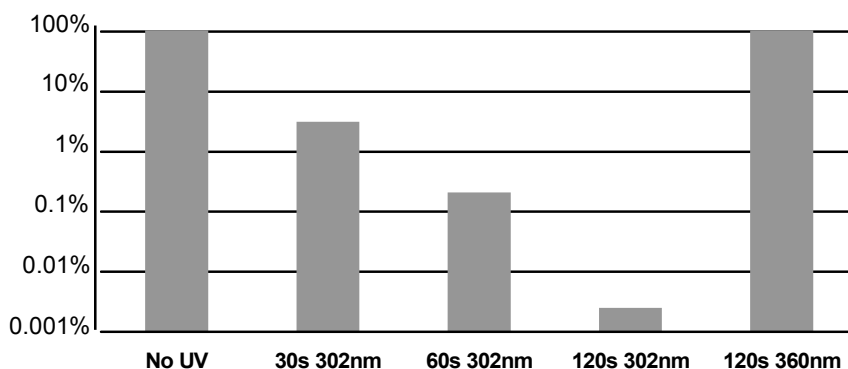


Figure 2. Relative cloning efficiency of pUC19 after exposure to short or long wavelength UV light. Intact pUC19 DNA was transformed after no UV exposure (“No UV”) or exposure to 302 nm UV light for 30, 60, or 90 seconds (“30s 302nm, 60s 302nm, 120s 302nm”) or to 360 nm UV light for 120 seconds (“120s 360nm”). Cloning efficiencies were calculated relative to un-irradiated pUC19 DNA.

IMPORTANT: Exposure to genotoxic short wavelength UV light (e.g., 254 or 302 nm) must be avoided when preparing samples for cloning. Use a long UV wavelength (e.g., 360 nm) low intensity lamp and short exposure times.

Do not exceed the recommended incubation time

Exceeding the 30-minute incubation time can lead to nucleolytic degradation of the fragments, which can reduce the yield of transformants.

Purify the fragments after the end repair step

Although the enzymes are denatured by heating at 70°C, purification of the DNA by gel electrophoresis, phenol/chloroform extraction, or column adsorption is required for maximum cloning efficiency.

Quantify the DNA before and after the end repair reaction

The DNATerminator kit protocol is designed to treat one to ten micrograms of fragments. Using an amount of input DNA outside this range can alter the kit’s effectiveness. In addition, care must be taken during the purification step. A common cause of variability in the number of transformants is variability in the amount of insert DNA. We recommend that a portion of the end-repaired fragments be accurately quantitated by gel electrophoresis next to mass standards.

DNATerminator[®] End Repair Kit

Avoid using ammonium ions prior to end repair

Ammonium ions interfere with the end repair reactions. Avoid introducing ammonium to the fragments prior to the end repair step (e.g., do not precipitate with ammonium acetate prior to the end repair step).

References

1. Sambrook, J. and Russell D. W. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, Cold Spring Harbor , New York) (2001).
2. Thorstenson YR, Hunicke-Smith SP, Oefner PJ, Davis RW. 1998. An automated hydrodynamic process for controlled, unbiased DNA shearing. *Genome Res* 8: 848-55.