CLONE DIRECT™
Rapid Ligation Kit

IMPORTANT!
-20°C Storage Required
Immediately Upon Receipt

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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user, especially the DNA targets to be cloned, are of the highest quality. Please follow the manual carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

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CloneDirect™ Kit Designations

Lucigen offers three versions of the CloneDirect Rapid Ligation Kit based on reaction size. The catalog numbers are listed below.

Catalog numbers of CloneDirect™ Kits

Product	Reactions	Catalog Number
CloneDirect Rapid Ligation Kit	24	40020-2
	48	40020-4
	96	40020-5

Components & Storage Conditions

The CloneDirect™ Kits require storage at **-20°C.**

CloneDirect™ Ligation Components **Store at -20°C**

	24 Reactions	48 Reactions	96 Reactions
CloneDirect™ 10X Ligation Buffer (Includes ATP)	24 ul	48 ul	96 ul
CloneSmart™ DNA Ligase (2U/ul)	24 ul	48 ul	96 ul

Quality Control and Stability

- Cohesive end ligation: Treatment of 50 ng of *Hin*dIII-digested pUC19 for 15 minutes with the CloneDirect Rapid Ligation Kit results in >95% re-ligated plasmid.
- Blunt-end ligation: Treatment of 50 ng of *Hin*cII-digested pUC19 for 30 minutes with the CloneDirect Rapid Ligation Kit results in >50% re-ligated plasmid.
- This kit is stable for one year from the date received if stored as recommended.

Applications and Description

The CloneDirect™ Rapid Ligation Kit provides the necessary components for convenient, reproducible ligation of DNA fragments. The Kit allows ligation of cohesive ends in as little as 5 minutes or blunt ends in as little as 30 minutes. However, for optimal results, a two-hour ligation is recommended. CloneDirect kits are ideally suited for all ligation needs, including:

- Shotgun library construction
- Cloning of blunt or cohesive end restriction fragments
- PCR cloning
- cDNA cloning
- Linker ligation

For projects requiring insertion of fragments into a cloning vector, we highly recommend Lucigen's CloneSmart™ Blunt Cloning Kits. The CloneSmart Kits combine the speed and efficiency of the CloneDirect Ligation Kit with the added benefits of cloning into a gap-free vector for unbiased results. Please contact Lucigen or visit our web site for more information.

Insert Preparation

The quality of insert DNA, particularly purity, quantity of insert, and compatibility of ends, is critical to the success of a ligation. In addition, the ends of at least one of the fragments must be 5' phosphorylated. All available restriction enzymes leave phosphorylated ends; however, fragments generated by physical shearing or PCR may have heterogeneous, non-phosphorylated ends. We recommend Lucigen's DNATerminator® Kit to end-repair physically sheared fragments to generate uniform blunt, phosphorylated ends. For cloning PCR products, we recommend repairing the ends with the PCRTerminator® Kit.

Ligation can be routinely achieved with DNA fragments obtained directly from a heat-killed restriction digest; however, highest yields are obtained from fragments that are first purified by gel electrophoresis, phenol/chloroform extraction, or use of an appropriate commercial DNA purification kit. After purification, the fragments should be dissolved or eluted in purified water. The components of common buffers such as TE can interfere with the ligation reaction. Before ligation, the concentration and integrity of the fragments should be verified by agarose gel electrophoresis with a mass standard. If the DNA is isolated by gel electrophoresis, only brief exposure to long wavelength UV (e.g. 360 nm) should be used to visualize the DNA (see below). Other physical characteristics of the DNA will affect cloning efficiency. For example,

fragments with high MW, skewed GC content, strong promoters, or toxic coding sequence will be cloned less efficiently.

After digestion, it is advisable to dephosphorylate the vector to decrease the background of non-recombinants that arise from self-ligation. Molecular biology-grade calf intestinal phosphatase should be used according to the manufacturer's instructions. Gel purification of the vector is often recommended to reduce the frequency of aberrant clones.

Insert: Vector Ratio

Typically, a 3:1 molar ratio of insert to vector results in the highest yield of transformants with single inserts, although a ratio as high as 5:1 is sometimes used to improve the efficiency of blunt end ligation. A lower amount of insert reduces the efficiency of ligation; a higher amount increases the probability of obtaining transformants with multiple inserts.

To maximize yield, it is important to accurately quantify both the cut vector and the insert, whether physically sheared and end repaired (as in a shotgun library), restriction digested, or amplified. Quantification is best performed by agarose gel electrophoresis with a mass standard or by fluorescence in the presence of a dye such as Hoechst 33258.

Best results are obtained using ~0.03 pmol of vector DNA (equivalent to ~50 ng of a 2 kb vector, or ~150 ng of a 6 kb vector). The amount of insert should be ~0.1 pmol (equivalent to ~150 ng of a 2 kb insert or ~450 ng of a 6 kb insert).

Sensitivity of DNA to Short Wavelength UV Light

DNA resolved on agarose gels is generally stained with ethidium bromide and visualized by illumination with ultraviolet light. Exposure to short wavelength ultraviolet light (e.g., 254, 302, or 312 nm) can reduce cloning efficiencies by several orders of magnitude (Figure 3). Note that the wavelength of most UV transilluminators, even those designated specifically for DNA visualization, is typically 302 nm or 312 nm. **IMPORTANT: Use a long UV wavelength (e.g., 360 nm) low intensity lamp and short exposure times.**

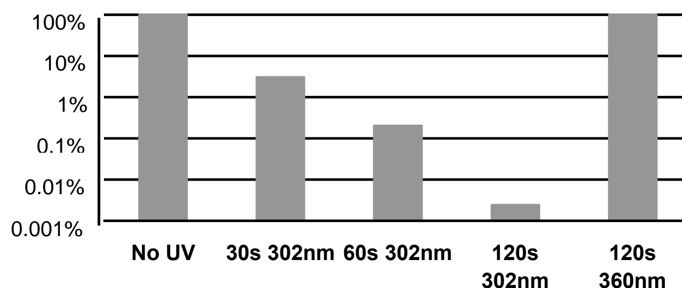

Figure 3. Relative cloning efficiency of pUC19 after exposure to short or long wavelength UV light. Intact pUC19 DNA was transformed after no UV exposure ("No UV") or exposure to 302 nm UV light for 30, 60, or 90 seconds ("30s 302nm, 60s 302nm,120s 302nm") or to 360 nm UV light for 120 seconds ("120s 360nm"). Cloning efficiencies were calculated relative to un-irradiated pUC19 DNA.

Protocol: CloneDirect™ Ligation Reaction

1. Quantify the cut vector and the insert.
2. Determine the appropriate amount of insert and vector (see Insert:Vector Ratio, above).
3. Prepare the following mix:

~0.03 pmol vector (50 ng of a 2-3 kb vector)
~0.1 pmol insert (150 ng of a 2-3 kb insert)
1.0 µl of CloneDirect™ 10X Ligation Buffer
1.0 µl CloneSmart™ DNA Ligase
Bring volume to 10 µl with water.

4. Mix the reaction by gently pipeting up and down.
5. Incubate the reaction at room temperature (22-25ºC) for 5 min (cohesive ends) or 30 min (blunt ends). Incubation times up to 2 hours may significantly improve ligation efficiency.
6. **ESSENTIAL:** Stop the reaction by incubating at 70ºC for 15 minutes.
7. Use 1-2 µl of the ligation for transformation of competent cells.

Optional Control Reaction:

Un-ligated control	Set up the above reaction without ligase. Run 5 µl of ligated and un-ligated reactions on an agarose gel. Ligation will be indicated by a clear reduction in the amount of the vector band and the presence of a higher molecular weight smear in the ligated reaction.

Appendix A: Troubleshooting Guide

Problem	Probable Cause	Solution
Very few or no transformants	Inefficient end repair.	Check the insert DNA for self-ligation by gel electrophoresis. Repeat end repair if necessary.
	Contaminating enzymes in ligation reaction, particularly alkaline phosphatase from vector preparation or restriction enzyme.	Heat-denature restriction digest 10 minutes at 70°C. Purify vector DNA by extraction or adsorption to matrix.
	No insert DNA, degraded DNA, or insufficient amount of DNA.	Check insert DNA by gel electrophoresis. Determine concentration of insert and add the correct amount.
	Incompatible or un-ligatable ends on insert or vector DNA.	Check the insert and vector DNA for self-ligation by gel electrophoresis. Repeat end repair if necessary. Be sure insert DNA is phosphorylated.
	Inadequate heat denaturation after ligation reaction.	DO heat denature for 15 min at 70°C. Skipping this step may lower the number of transformants by 2-3 orders of magnitude.
	Loss of DNA during precipitation.	DO NOT precipitate DNA after ligation reaction. It is not necessary with this protocol.
No inserts in vector.	Incompletely cut vector.	Recut the vector using sufficient enzyme and time to complete the digestion.
	Insert not compatible with vector ends.	Treat insert and vector with enzymes that leave compatible ends. Dephosphorylate vector after digestion or end repair.