

Please note: The concentration of the included pUC19 has been changed from 1ng/μl to 10pg/μl. Do not dilute the plasmid before performing the transformation positive control. Please contact Lucigen if you have any questions.

UltraClone[™]

DNA Ligation and Transformation Kit

IMPORTANT!
-80°C & -20°C Storage Required
Immediately Upon Receipt

Lucigen[®] Corporation
The Molecular Cloning Company[™]

2120 W. Greenview Drive
Middleton, WI 53562

Toll Free (888) 575-9695

Phone (608) 831-9011

FAX (608) 831-9012

www.lucigen.com

UltraCLONE™ DNA Ligation & Transformation Kit

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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UltraCLONE™ DNA Ligation & Transformation Kit

Table of Contents

Quality Control and Stability	3
UltraClone™ Kit Designations	4
Components and Storage	4
Applications and Description	5
Insert and Vector Preparation	5
Insert :Vector Ratio.....	6
Sensitivity of DNA to Short Wavelength UV Light	6
Protocol.....	7
<i>E. coli</i> ® Competent Cells.....	7
Transformation of Electrocompetent cells	8
Transformation of Chemically Competent cells	10
Appendix: Troubleshooting Guide	11

Quality Control and Stability

- Cohesive end ligation: Treatment of 50 ng of *Hind*III-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 *Hinc*II-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.

UltraCLONE™ DNA Ligation & Transformation Kit

UltraClone Kit Designations

Lucigen offers several versions of the UltraClone DNA Ligation and Transformation Kit. The kits differ in number of reactions and cells that are included. The catalog numbers are listed below.

Catalog numbers of UltraClone kits with cells

Product	Reactions	Catalog Number
UltraClone Kit with 10G Elite DUOs	12	40002-1
	24	40002-2
UltraClone Kit with 10G Elite SixPacks	24	40003-2
	48	40003-4
UltraClone Kit with 10GF' Elite DUOs	12	40004-1
	24	40004-2
UltraClone Kit with 10G Supreme DUOs	12	40008-1
	24	40008-2
UltraClone Kit with 10G Chemically Competent DUOs	12	40012-1
	24	40012-2

Components & Storage Conditions

The UltraClone Kits are packaged in two containers. The Ligation Components require storage at **-20°C**, and the Competent Cells require storage at **-80°C**.

Container 1: UltraClone Ligation Components

Store at -20°C

	12 Reactions	24 Reactions	48 Reactions
CloneDirect™ 10X Ligation Buffer (Includes ATP)	12 ul	24 ul	48 ul
CloneSmart® DNA Ligase (2U/ul)	12 ul	24 ul	48 ul

Container 2: *E. coli*® Competent Cells

Store at -80°C

	Catalog #	Reactions
<i>E. coli</i> 10G Elite Electrocompetent Cells or	60052-1	12 (6 x 50 µl)
	60052-2	24 (12 x 50 µl)
<i>E. coli</i> 10G Elite Electrocompetent Cells (SixPacks) or	60052-3	24 (4 x 150 µl)
	60052-4	48 (8 x 150 µl)
<i>E. coli</i> 10GF' Elite Electrocompetent Cells or	60061-1	12 (6 x 50 µl)
	60061-2	24 (12 x 50 µl)
<i>E. coli</i> 10G Supreme Electrocompetent Cells or	60080-1	12 (6 x 50 µl)
	60080-2	24 (12 x 50 µl)
<i>E. coli</i> 10G Chemically Competent Cells	60107-1	12 (6 x 50 µl)
	60107-2	24 (12 x 50 µl)
Control pUC19 DNA (10 pg/µl) - store at -20°C or -86°C	----	(1 x 20 µl)
Recovery Medium - store at -20°C or -86°C	----	12 (1 x 12 ml) 24 (2 x 12 ml) 48 (4 x 12 ml)

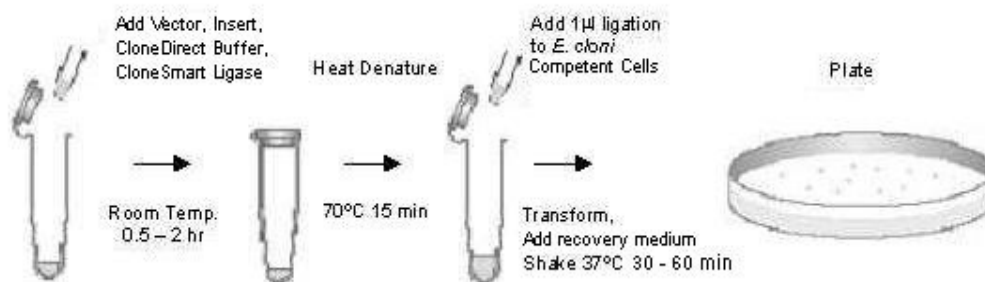
UltraCLONE™ DNA Ligation & Transformation Kit

Applications and Description

Lucigen's UltraClone™ DNA Ligation and Transformation Kit provides the necessary components for reproducible ligation of DNA fragments, as well as the competent cells needed for transformation. The Kit allows ligation of cohesive ends in as little as 5 minutes or blunt ends in as little as 30 minutes. UltraClone 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

UltraClone kits improve the fundamental cloning procedures of ligation and transformation. Featuring Lucigen's unique technology, UltraClone kits increase the number of recombinant colonies 3-10 fold, regardless of the cloning vector used. UltraClone kits also eliminate post-ligation cleanup steps, simplifying the process of transforming competent cells and reducing the chance of losing precious material. After the UltraClone ligation reaction, a 15 minute incubation at 70 °C is all that is required to prepare the ligated DNA for high efficiency transformation into *E. coli*® competent cells. *E. coli* cells allow blue/white screening, support high yield of intact plasmids, and upon infection with helper phages are suitable for production of ssDNA from plasmids containing the f1 origin of replication.



Insert and Vector Preparation

The quality of insert DNA, particularly purity, quantity, and compatibility of ends, is critical to the success of a ligation. In addition, the ends of the fragment or the vector 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,

UltraCLONE™ DNA Ligation & Transformation Kit

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.

Note: For cloning fragments into bacterial vector, we highly recommend Lucigen's CloneSmart® Blunt Cloning Kits. The CloneSmart Kits combine the speed and efficiency of the UltraClone 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: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 restriction digested, amplified, or physically sheared and end repaired (as in a shotgun library). 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). With a dephosphorylated vector preparation, lower insert/vector ratios are acceptable.

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. **Therefore, it is critical to use a low intensity, long UV wavelength lamp (e.g., hand-held, 360 nm) and short exposure times.**

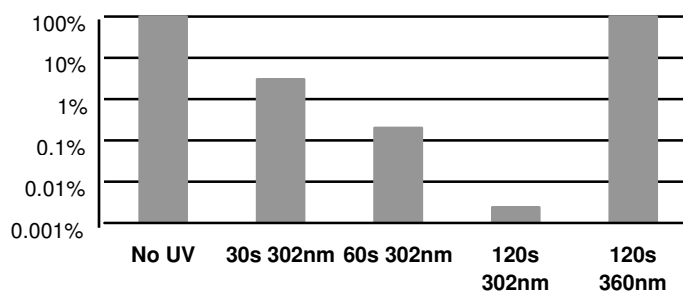


Figure 3. Relative cloning efficiency of pUC19 after exposure to short or long wavelength UV light. Intact pUC19 DNA was transformed after no UV exposure ("No UV") or exposure to 302 nm UV light for 30, 60, or 90 seconds ("30s 302nm, 60s 302nm, 120s 302nm") or to 360 nm UV light for 120 seconds ("120s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

UltraCLONE™ DNA Ligation & Transformation Kit

Protocol: UltraClone™ 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 (22-25°C) for 5 min (cohesive ends) or 30 min (blunt ends). Incubation times up to 2 hours may significantly improve ligation efficiency.
6. **ESSENTIAL: AFTER LIGATION, THE REACTION MUST BE INCUBATED AT 70°C 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 the CloneSmart Ligase. Run 5 µl of ligated and un-ligated reactions on an agarose gel. Ligation will be indicated by a reduction in the amount of the vector band and the presence of a higher molecular weight smear in the ligated reaction.
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E. cloni® Competent Cells

E. cloni 10G and 10GF' Competent Cells are *E. coli* strains optimized for high efficiency transformation. They are ideal for cloning and propagation of BAC, cosmid, or plasmid clones. They give high yield and high quality plasmid DNA due to the *endA1* mutation.

E. cloni 10G and 10GF' contain the inactive *mcr* and *mrr* mutations, allowing methylated genomic DNA that has been isolated directly from mammalian or plant cells to be cloned without deletions or rearrangements. The *rpsL* mutation confers resistance to streptomycin.

E. cloni 10GF' has the same chromosomal genotype as 10G, but it harbors the F' plasmid. This plasmid confers tetracycline resistance and allows the cells to be infected with M13 for ssDNA production. The F' plasmid also carries the *lacIq* repressor allele; therefore, when using a blue/white screening vector (such as Lucigen's pEZSeq or pUC19) addition of IPTG is necessary to induce expression of the *lacZα* peptide. In the absence of IPTG, blue/white screening will produce only faint color, and the associated transcription of insert DNA from the *lacZ* promoter will be minimal.

UltraCLONE™ DNA Ligation & Transformation Kit

Lucigen's UltraClone DNA Ligation and Transformation Kits are available with the following preparations of *E. cloni*® Competent Cells:

***E. cloni* 10G SUPREME Electrocompetent Cells** deliver $\geq 4 \times 10^{10}$ cfu/ μ g. Ideal for the most demanding applications that require the greatest number of transformants, such as construction of large, high complexity libraries or cloning difficult targets.

***E. cloni* 10G or 10GF' ELITE Electrocompetent Cells** deliver $> 2 \times 10^{10}$ cfu/ μ g. Provide large numbers of transformants from hard-to-clone fragments or limited DNA at a lower price than Supreme cells.

***E. cloni* 10G Chemically Competent Cells** deliver $\geq 1 \times 10^9$ cfu/ μ g. Unbeatable performance and value for routine applications.

Genotypes

***E. cloni* 10G:**

F- *mcrA* Δ (*mrr-hsdRMS-mcrBC*) ϕ 80*dlacZ* Δ M15 Δ *lacX74* *endA1* *recA1* *araD139* Δ (*ara, leu*)7697 *galU* *galK* *rpsL* *nupG* λ - *tonA*

***E. cloni* 10GF' Genotype:**

[F' *pro A+B+ lacIqZDM15::Tn10* (TetR)] / *mcrA* Δ (*mrr-hsdRMS-mcrBC*) ϕ 80*dlacZ* Δ M15 Δ *lacX74* *endA1* *recA1* *araD139* Δ (*ara, leu*)7697 *galU* *galK* *rpsL* *nupG* λ - *tonA*

- *E. cloni* Competent Cells are provided with supercoiled pUC19 DNA at a concentration of 10 pg/ μ l as a transformation control—use 1 μ l for transformation.
- For highest transformation efficiency, use the provided Recovery Medium to resuspend the cells after electroporation. Use of TB or other media may result in 10-20% lower transformation efficiencies.

Transformation of Electrocompetent cells

E. cloni 10G and 10GF'Elite and Supreme Electrocompetent Cells are provided in 50 μ l aliquots (DUOs), sufficient for two transformation reactions of 25 μ l each. *E. cloni* 10G Elites are also available in 150 μ l aliquots (SixPacks), sufficient for six transformation reactions of 25 μ l each.

Transformation is carried out in a 0.1 cm gap cuvette. Optimal settings for electroporation are listed in the table below. Typical time constants are 3.5 to 4.5 msec.

Optimal Setting	Alternate Settings (~ 20-50% lower efficiencies)
1.0 mm cuvette 10 μ F 600 Ohms 1800 Volts	1.0 mm cuvette 25 μ F 200 Ohms 1400 – 1600 Volts
Bio-Rad Micro Pulser #165-2100 Bio-Rad <i>E. coli</i> Pulser #165-2102	Bio-Rad Gene Pulser II #165-2105 BTX ECM630 Electroporation System

Optional transformation control reactions include electroporation with 1 μ l (10 pg) of supercoiled pUC19 DNA.

UltraCLONE™ DNA Ligation & Transformation Kit

To ensure successful transformation results, the following precautions must be taken:

- **ESSENTIAL: After ligation, the reaction must be heat killed at 70°C for 15 minutes!**
- Ligation reactions performed with Lucigen's UltraClone Kits can be used directly in electroporation, without purification of the ligation products.
- Microcentrifuge tubes and electroporation cuvettes must be thoroughly pre-chilled on ice before use. Successful results are obtained with cuvettes from BTX (Model 610) or BioRad (Cat. #165-2089). Users have reported difficulties using *E. coli* cells with Invitrogen cuvettes (Cat. # 65-0030).
- The cells must be completely thawed **on ice** before use.

Transformation Protocol (Electrocompetent cells)

1. Prepare nutrient agar with appropriate antibiotic.
2. Have Recovery Medium and 17 mm x 100 mm sterile culture tubes readily available at room temperature (one tube for each transformation reaction).
3. Place electroporation cuvettes (0.1 cm gap) and microcentrifuge tubes on ice (one cuvette and one tube for each transformation reaction).
4. Remove *E. coli* cells from the -80 °C freezer and place on wet ice until they thaw **completely** (10-20 minutes).
5. Add 25 µl of *E. coli* cells to the chilled microcentrifuge tube on ice.
6. Add 1 µl of the heat-denatured UltraClone Ligation reaction to the 25 µl of cells on ice. (Failure to heat-inactivate the ligation reaction will prevent transformation.) Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.
7. Carefully pipet 25 µl of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well. Electroporate according to the conditions recommended above.
8. Within 10 seconds of the pulse, add 975 µl of Recovery Medium to the cuvette and pipet up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.
9. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.
10. Spread up to 100 µl of transformed cells on nutrient agar plates containing the appropriate antibiotic.
11. Incubate the plates overnight at 37 °C.
12. Transformed clones can be further grown in any rich culture medium.

UltraCLONE™ DNA Ligation & Transformation Kit

Transformation of Chemically Competent cells

E. cloni® 10G Chemically Competent Cells are provided in 80 µl aliquots (DUOs), sufficient for two transformation reactions of 40 µl each.

Transformation is performed by heat shock at 42 °C, followed by incubation on ice.

To ensure successful transformation results, the following precautions must be taken:

- **ESSENTIAL: After ligation, the reaction must be heat killed at 70°C for 15 minutes!**
- Ligation reactions performed with Lucigen's UltraClone Kits can be used directly in electroporation, without purification of the ligation products.
- All microcentrifuge tubes must be thoroughly pre-chilled on ice before use.
- The cells must be completely thawed **on ice** before use.

Transformation Protocol (Chemically Competent cells)

1. Chill sterile culture tubes on ice (17 mm x 100 mm tubes, one tube for each transformation reaction).
2. Remove *E. cloni* cells from the -86 °C freezer and thaw completely on wet ice (10-20 minutes).
3. Add 40 µl of *E. cloni* cells to the chilled culture tube.
4. Add 1 µl of the heat-denatured UltraClone Ligation reaction to the 40 µl of cells on ice. (Failure to heat-inactivate the ligation reaction will prevent transformation.) Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.
5. Incubate on ice for 30 minutes.
6. Heat shock cells by placing them in a 42 °C water bath for 45 seconds.
7. Return the cells to ice for 2 minutes.
8. Add 960 µl of room temperature Recovery Medium to the cells in the culture tube.
9. Place the tubes in a shaking incubator at 250 rpm for 1 hour at 37 °C.
10. Plate up to 100 µl of transformed cells on nutrient agar plates containing the appropriate antibiotic.
11. Incubate the plates overnight at 37 °C.
12. Transformed clones can be further grown in any rich culture medium

UltraCLONE™ DNA Ligation & Transformation Kit

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 the restriction digest 10 minutes at 70°C. Purify vector DNA by extraction or spin column.
	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.	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.