Xi-Clone™ High Speed Cloning Kit

Instruction Manual

Catalog Number

XC005050

A Division of Gene Therapy Systems, Inc.
10190 Telesis Court
San Diego, CA 92121 USA
Telephone: 858-457-1919
US Toll-free: 888-428-0558
Fax: 858-623-9494
E-mail: tech1@genlantis.com
Website: http://www.genlantis.com

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The purchase price paid for the Xi-Clone™ High Speed Cloning Kit by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section). This kit is intended **for internal research only** by the purchaser. Such use is limited to the amplification, transfection, and expression of nucleic acids as described in the product manual. Furthermore, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Gene Therapy Systems, Inc ("GTS").

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Genlantis, a division of Gene Therapy Systems, Inc.
10190 Telesis Court, San Diego, CA 92121.
Telephone: 858-457-1919
Fax: 858-623-9494.
Email: **licensing@genlantis.com**

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OVERVIEW

Kit Contents

The Xi-Clone™ High Speed Cloning Kit contains sufficient materials for generating two Xi-Clone™ adapted vectors and performing 40 PCR cloning reactions.

Contents	Description	Quantity
Xi-Clone™ Enzyme Mix	Enzyme mix for treating two linear vectors so that they are ready to accept inserts in DH5α Chemically Competent *E. coli.*	4 µl
Xi-Clone™ Reaction Buffer	Reaction buffer for the Xi-Clone™ Enzyme Mix	12 µl
DNA Cleanup Spin Columns	Columns for cleaning linearized vectors and PCR products	45 reactions

Shipping and Storage

The Xi-Clone™ High Speed Cloning Kit is shipped frozen. For maximum stability and long-term use, store DNA Cleanup Spin Columns at room temperature and all other reagents at –20°C. All components are stable for six months when stored properly.

Accessory Products

Optimized DH5α Competent Cells for use in Xi-Clone transformation

Product Name	Cat. No.	Quantity
SmartCells™ Competent *E. coli*	C101020	20 x 50 µl
SmartCells™ F' Competent *E. coli*	C101020	20 x 50 µl

Xi-Clone™ ready vectors for high-level mammalian expression

Product Name	Cat. No.	Quantity
phCMV1 Xi-Clone™ Kit (Native protein)	XC003120	20 PCR Cloning Reactions
phCMV2 Xi-Clone™ Kit (N-term HA fusion)	XC003220	20 PCR Cloning Reactions
phCMV3 Xi-Clone™ Kit (C-term HA fusion)	XC003320	20 PCR Cloning Reactions

Xi-Clone™ ready vectors for high-level cell-free expression

Product Name	Cat. No.	Quantity
pIX Xi-Clone™ Kit (Native protein)	XC004120	20 PCR Cloning Reactions
pIX2 Xi-Clone™ Kit (N-term 6xHis fusion)	XC004220	20 PCR Cloning Reactions
pIX3 Xi-Clone™ Kit (C-term HA fusion)	XC004320	20 PCR Cloning Reactions

Accessory Products (continued)

Highly efficient transfection reagent for diverse cell lines

Product Name	Cat. No.	Quantity
GenePORTER™ 2 Transfection Reagent	T202007	75 reactions (0.75 ml)
GenePORTER™ 2 Transfection Reagent	T202015	150 reactions (1.5 ml)
GenePORTER™ 2 Transfection Reagent	T202075	750 reactions (5 x 1.5 ml)

Product Support

Telephone: 858-457-1919 OR 888-428-0558 (US toll free)	**Fax:** 858-623-9494
E-mail: tech1@genlantis.com	**Web:** http://www.genlantis.com

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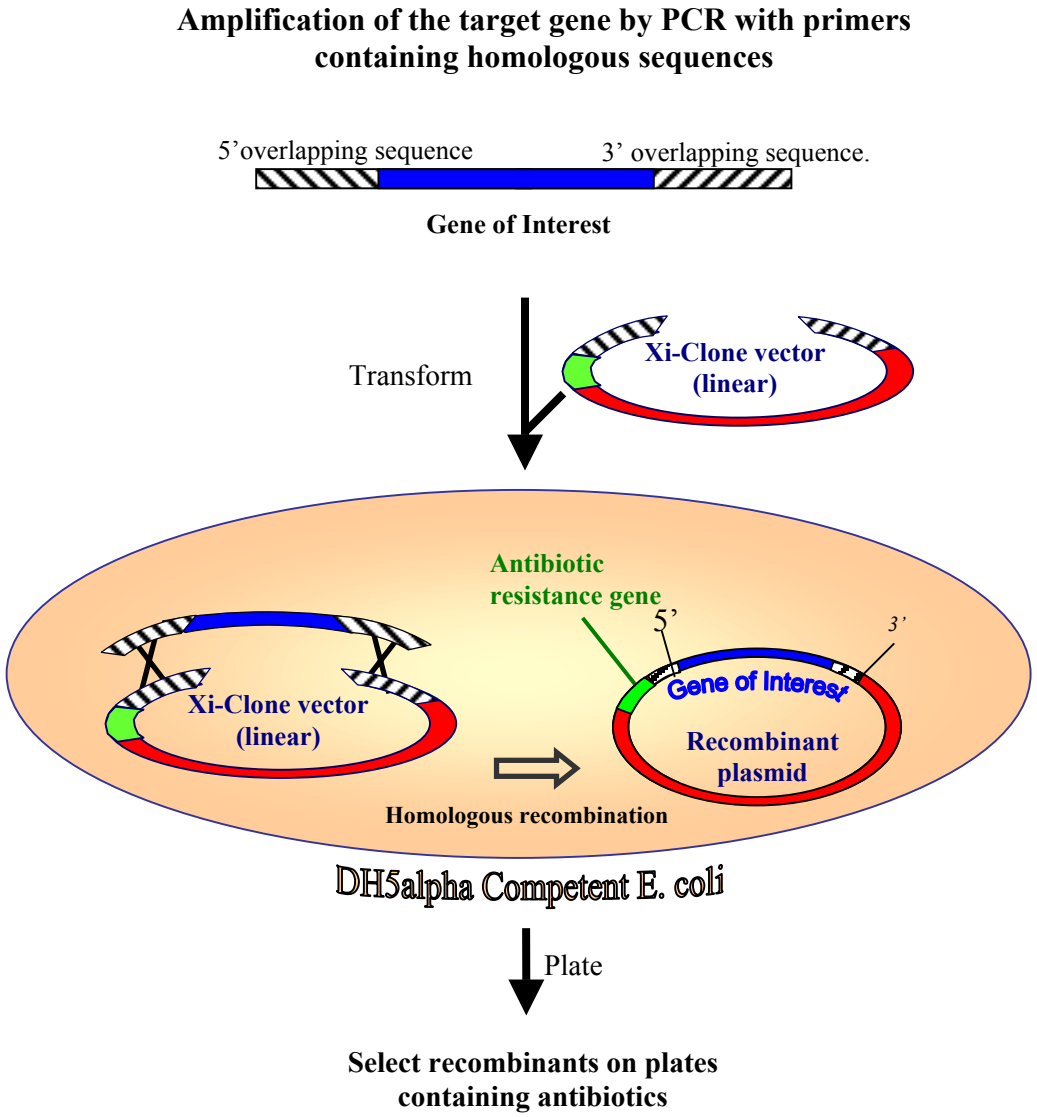
Introduction

Xi-Clone™ PCR Cloning Technology allows rapid, efficient, and directional cloning of PCR products. With a Xi-Clone™-ready vector, there is no need for ligase, overnight ligations, or restriction enzymes in the ensuing cloning experiments. The Xi-Clone™ High Speed Cloning Kit allows researchers to convert their favorite vector into a Xi-Clone™-ready vector so that multiple cloning experiments can be performed with great speed and ease.

Figure 1. Summary of Xi-Clone™ PCR Cloning Process



How Xi-Clone™ Works.

The Xi-Clone™ PCR Cloning Technology relies on the principle that certain *E. coli* cells are able to recombine homologous sequences with a high rate of specificity. With Xi-Clone™, linear DNA fragments are generated through the PCR process to include sequences on the 5' and 3' ends that are homologous with end sequences on a linear Xi-Clone™-adapted vector (Page 7, Fig. 1). When the PCR fragments and the linear Xi-Clone vector are mixed and transformed into competent *E. coli*, bacterial recombinase activity is able to join the two DNA fragments resulting in a circular plasmid. Most commercially available chemically competent DH5α *E. coli* strains facilitate efficient homologous recombination including those that the have *recA*$^-$ genotype which minimizes unwanted recombination in cloning.

If multiple cloning experiments need to be performed using the same vector, Xi-Clone™-adapted vectors can provide tremendous time savings. Because no enzymes are needed, there is no need for overnight ligation or restriction enzyme digests. In our laboratory, the Xi-Clone™ protocol consistently generates greater than 85% of the desired recombinant clones, virtually eliminating screening. In addition, because the Xi-Clone™ process does not utilize multiple cloning sites or enzyme recognition sites for the cloning step, you can accurately insert only the sequences you desire and still make sure that they are in-frame. This is especially important when you need to minimize interference caused by unwanted sequences.

Flexible and Simple Vector Conversion.

With other recombination PCR cloning technologies (*e.g.* TOPO® cloning, Gateway®, *etc.*), researchers are limited by the vendor on which vectors they can use. In contrast, researchers can take full advantage of the Xi-Clone™ PCR Cloning Technology with virtually any vector of their choice. Making a vector Xi-Clone™ ready is easy: simply cut the plasmid and treat it with the supplied Xi-Clone™ enzyme mix and buffer. The plasmid is now ready to accept properly designed PCR fragment containing the gene of interest. Once the vector is Xi-Clone™ adapted, it can remain stable for at least six months.

METHODS AND PROCEDURES

1. Convert Plasmid into a Xi-Clone™-ready Vector

Sufficient reagents are supplied for Xi-Clone treatment of two different vectors.

1.1. Linearize plasmid by restriction digestion

1.1.1 Identify a unique restriction site within the target region, for instance, the region between promoter and transcription terminator where you intend to insert the gene of interest for expression. Since complete digestion is crucial for making a good Xi-Clone™ vector, choose more efficient restriction enzymes that are available in high concentrations. We recommend using *BamHI, Eco RI, Eco RV, Nco I, Pst I, and Xho I*, and avoiding to use poor cutters such as *Sal I, Not I, Nsi I,* and *Sca I.*

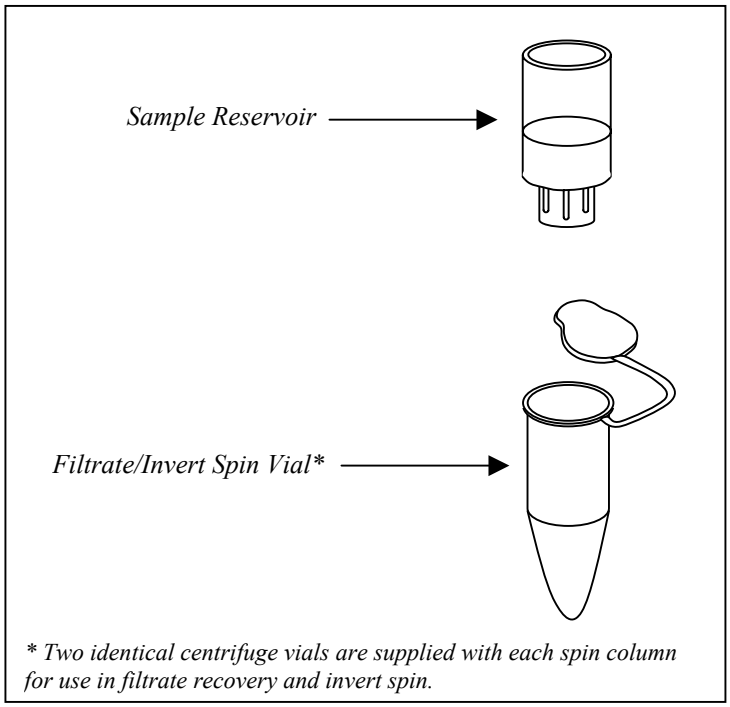
1.1.2 Set up a restriction digestion reaction using 1 µg of plasmid DNA in 40 µl total reaction volume. Add 1 µl restriction enzyme (~20 units) and incubate at 37°C for 3 hours.

1.1.3 Add additional 1 µl of the restriction enzyme and let it digest for another 3 hours**.**

1.1.4 Add additional 1 µl of the restriction enzyme followed by an overnight incubation at 37°C.

1.2 Treat linearized vector with Xi-Clone™ Enzyme Mix

1.2.1 Purify restricted plasmid with supplied DNA Cleanup Spin Column.



** Two identical centrifuge vials are supplied with each spin column for use in filtrate recovery and invert spin.*

1.2.1.1 Insert the sample reservoir into one of the vials provided.

1.2.1.2 Pipette 400 µl TE buffer into sample reservoir without touching the membrane with the pipette tip. Add entire restriction digestion reaction mix (from 1.1.4) to the reservoir. Seal with attached cap.

1.2.1.3 Place assembly in a micro-centrifuge and counterbalance with a similar device. Spin at 1000 g for 15 minutes (Note: do not centrifuge more than 15 minutes).

1.2.1.4 Remove assembly from centrifuge. Separate vial from sample reservoir, place the reservoir upright into a clean vial and add 50 µl dH_2O (Avoid touching the membrane surface).

1.2.1.5 Invert the reservoir into the same clean vial and spin at 1,000 g for 2 minutes.

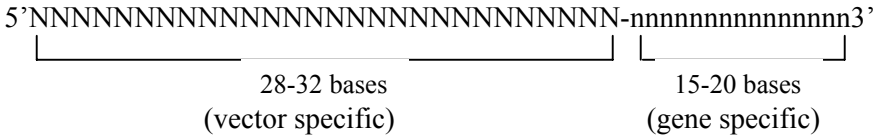
1.2.2 Mix 5.5 µl of Xi-clone Reaction Buffer with 50 µl recovered DNA from 1.2.1.5 and add 2 µl of Xi-clone Enzyme Mix followed by incubation at room temperature for 30 minutes.

1.2.3 Purify the treated linear vector using a DNA Cleanup Spin Column as in Step 1.2.1. Resuspend DNA in 50 µl of TE buffer with final concentration of about 20 ng/µl. Store the sample at -20°C.

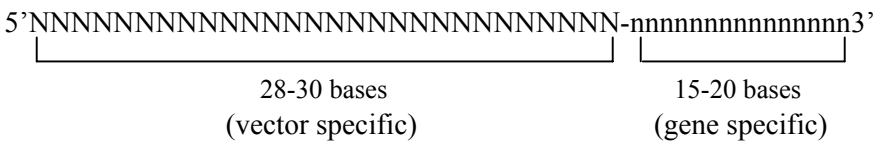
2. PCR Cloning

2.1 Design gene-specific PCR primers

2.1.1 The 5' PCR primer contains about 28 to 32 nucleotides overlapping with the 5' end of linear vector ('N') plus 15 to 20 nucleotides that are gene specific sequence ('n'). The 5' Xi-clone end is always the same for the same cloning vector and the gene specific sequence varies depending on the target gene.



2.1.2 The 3' custom primer contains about 28-30 nucleotides that are complementary to 3' end sequence of the linear vector ('N',) plus 15 to 20 nucleotides that are gene specific ('n').



2.2 PCR protocol

Following is a recommended PCR protocol for generating Xi-Clone inserts. You may modify this protocol based on the composition of your primer sequences and your DNA polymerase.

Prepare a 100 µl reaction mix as follows*:

86-x µl	ddH_2O
10 µl	10 x PCR buffer
1 µl	10 mM dNTP each
1 µl	DNA template (cDNA or linearized plasmid, ~100 pg)
1 µl	5' primer (1µg/µl)
1 µl	3' primer (1µg/µl)
x µl	DNA polymerase (Amount varies depending on the supplier)

PCR program:

94°C for 3 minutes

94°C for 30 seconds
58°C for 30 seconds
68°C for 1 minute/1kb
} 35 cycles

68°C for 5 minutes
4°C storage

2.3 Clean up PCR products

2.3.1 Purify your PCR fragments with PCR Cleanup Spin Column supplied using protocols described in Step 1.2.1

2.3.2 Recover the purified PCR fragment in 50 µl TE buffer.

IMPORTANT If your template is a plasmid, we recommend you linearize it before performing PCR in order to minimize background in the ensuing transformation step. In addition, we recommend that you clean up your PCR fragments by using the supplied PCR DNA purification columns before proceeding to the next step.

3. Transformation

While most commercially-available DH5α chemically competent *E. coli* should work for the Xi-Clone system, for maximal transformation and recombination efficiencies, we recommend that you use the SmartCells™ Competent *E. coli* from Genlantis:

Product Name	Cat. No.	Quantity
SmartCells™ Competent *E. coli*	C101020	20 x 50 µl
SmartCells™ F' Competent *E. coli*	C101020	20 x 50 µl

The following transformation protocol was developed using SmartCells™ Competent *E. coli*. For other DH5α chemically competent *E. coli*, follow the manufacturer's recommended protocol or your in-house protocol.

3.1 Thaw one tube of the SmartCells™ competent cells on ice (10-15 minutes).

3.2 Mix 10 µl of PCR fragment from Step 2.3 (1-2 µg) with 2 µl of linear Xi-Clone vector from step 1.2.3 in a pre-chilled tube.

3.3 Add one tube of SmartCells™ Competent *E. coli* to the above tube, mix gently and incubate on ice for 30 minutes. Immediately store any unused SmartCells™ *E. coli* at -70°C.

3.4 Heat the mix at 42°C for 1 minute.

3.5 Add 100 µl SOC medium and incubate at 37°C for 1 hour in an air incubator

3.6 Spread transformation mixture on LB/Agar plates containing the appropriate antibiotics.

3.7 Pick colonies and analyze positive recombinant plasmids by digesting miniprep DNA and electrophoresing on an agarose gel.

TIP The directional cloning by Xi-Clone™ allows quick identification of recombinant clones by running colony lysates directly on an agarose gel.. This method saves hours of time spent on growing up bacteria and eliminates the need for plasmid DNA minipreps.

1. Pipette 30 µl of TE buffer and 30 µl of phenol/chloroform (1:1 pre-saturated with TE) into a microfuge tube.

2. Pick up a full size bacterial colony (grown overnight) with a clean pipette tip and lyse the bacteria by pipetting up and down several times in the TE/phenol/chloroform mix

3. Use 20 µl of the aqueous phase (top phase) for gel analysis on a 0.8% agarose gel.

4. Run the agarose gel in 1x TBE or TAE. Load 0.5 µg of the supercoiled blank vector as a control. Recombinant plasmid with insert should run slower (higher) than the control vector. The actual difference in size varies depending on the size of your insert.

TIP In addition to facilitating Xi-Clone™ high speed cloning, SmartCells™ Chemically Competent *E. coli* offer superior transformation efficiency under diverse transformation conditions. They are especially useful when you are not sure how good your plasmid prep is, or when you wish to use more of the ligation mix in your transformation.

APPENDIX

Quality Control

To ensure robust performance of each lot of the Xi-Clone™ High Speed Cloning Kit, each kit component is rigorously tested to ensure quality and consistency. The complete kit is qualified for cloning PCR products in the correct orientation at greater than 85% efficiency using in-house control templates and vectors.

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
No or very few colonies on agar plates.	No vector	Make sure that the PCR fragments are mixed with the linear Xi-Clone™ vector before transformation
	Wrong antibiotic selection	Check that your agar plates contain the correct antibiotic and that the plates are freshly made.
	Competent cells are dead due to improper storage or shipping conditions	The efficiency of competent cells can be verified using a plasmid such as pUC19. Make sure you store your Competent *E. coli* at –70°C. Verify that the cells are not older than 6 months.
No insert or low number of recombinants	Not enough PCR fragment or vector.	Increase the amount of PCR fragments used by 1 µg increments. Alternatively, you can try increasing the amount of vector two-fold to help improve the efficiency of the cloning reaction.
	PCR fragments contain a high level of impurities or contaminants.	Clean and purify the PCR fragments by using ethanol precipitation or commercial PCR cleaning kits before cloning.
	Incomplete linearization of vector	Use filter pipette tips. More vigorous restriction enzyme digestion.

For additional troubleshooting assistance, please contact our Technical Support Department at:

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