

A Revolution in Cloning Technology

Xi-Clone™ Rapid PCR Cloning System: Questions & Answers

1. What is Xi-Clone™?

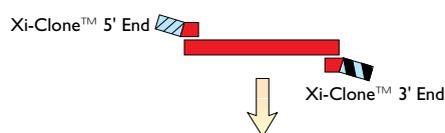
Xi-Clone™ is a powerful new PCR cloning system allowing researchers to save tremendous time and resources by cloning their genes-of-interest into high expression plasmid vectors without using any restriction or ligation enzymes. By simply mixing the PCR amplified gene with the linearized Xi-Clone vector, and then adding this mix to the SmartCells™ competent *E. coli* (included with the Xi-Clone kit), researchers can insert their gene of interest into a powerful mammalian or *in vitro* expression vector after only a few minutes of labor.

2. How does Xi-Clone work?

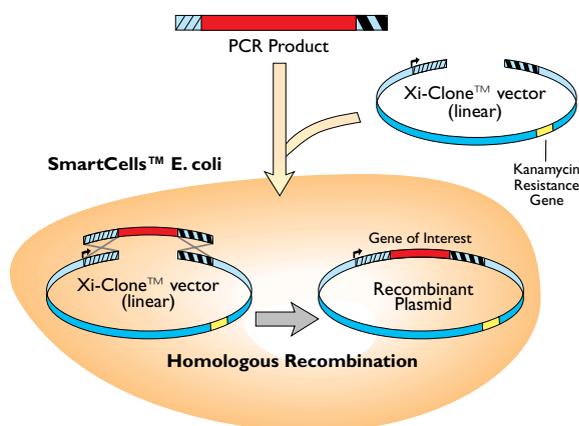
The Xi-Clone system uses homologous recombination in *E. coli* to insert the gene of interest into a linearized Xi-Clone expression vector (Figure 1). Initially, the gene is amplified by PCR using primers that contain both gene-specific and Xi-Clone end sequences. Next, the PCR product is mixed with the supplied linearized Xi-Clone vector, and the mixture is added to the SmartCells competent *E. coli*. After transformation, endogenous bacterial recombinases incorporate the gene of interest into the Xi-Clone vector, and > 85% of the transformants contain the insert in the correct orientation

Figure 1. How Xi-Clone Works

1. Amplify your gene of interest with PCR primers containing Xi-Clone ends.



2. Mix the PCR product with the supplied Xi-Clone vector and transform into SmartCells competent *E. coli*.



3. Plate and screen for recombinants containing kanamycin

3. What are the available Xi-Clone expression vectors?

At present, Xi-Clone expression vectors are available for CMV promoter based constitutive expression in mammalian cells (pCMV/Xi vectors) and in T7 promoter based *in vitro* expression systems (pIX/Xi vectors). For your convenience, each of these vectors is available for expression of recombinant proteins in native or HA epitope-tagged formats.

4. I use traditional restriction enzyme / ligase cloning. Why should I switch to Xi-Clone PCR Cloning?

The Xi-Clone system will save you days of labor compared to traditional restriction enzyme / ligase cloning. With the Xi-Clone system, there are no restriction digestions and no overnight ligations. In addition, screening will be significantly reduced because >85% of the transformants are the desired clones. More importantly, no extra sequences (i.e., restriction site nucleotides) are incorporated into your expression cassette. This means your expression product will not contain any unwanted amino acids.

5. I use TOPO/TA cloning, which is simple and takes 5 minutes. Why should I switch to Xi-Clone PCR Cloning?

If you are currently using TOPO/TA cloning system, you would benefit from using the Xi-Clone system because:

a. There are no topoisomerase recognition sequences necessary with Xi-Clone. So, no junk sequences are incorporated into your expression element, and no unwanted amino acids are incorporated into your expression product.

b. With Xi-Clone, your gene is always cloned in the correct orientation, whereas depending on which TOPO kit you use, you may get recombinants with your gene in both orientations.

c. The phCMV/Xi mammalian expression vectors utilize a highly optimized CMV promoter to give expression levels up to 5 times higher than those obtained with TOPO/TA counterparts.

d. For high-throughput users, no incubation steps are necessary.

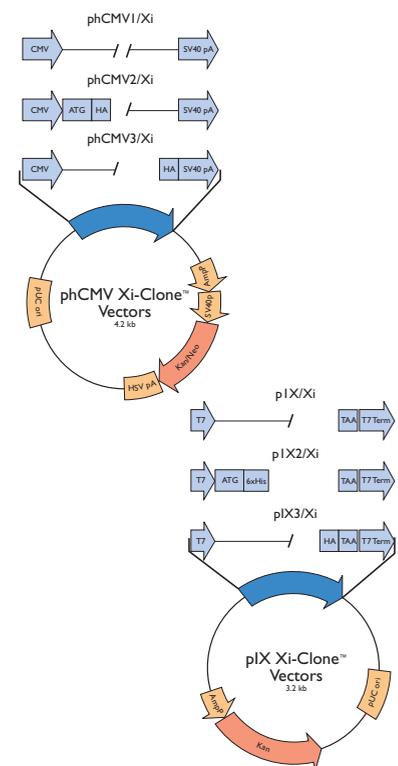
6. What is the largest gene I can insert into the Xi-Clone vector?

An upper size limit for the gene insert has not been determined. However, the largest gene we have successfully cloned into the Xi-Clone vector is 3.7 kb.

7. Can I use my own vector for the Xi-Clone process?

GTS provides a custom vector service in which we can convert your vector into a Xi-Clone compatible vector. Contact our Technical Support Department at 888-428-0558, Ext. 3 for details.

Figure 2. Xi-Clone phCMV and pIX Vectors



Product	Quantity	Catalog no.	Price
phCMV1 Xi-Clone Kit for Mammalian Expression	20 rxn.	XC003120	\$360
phCMV2 Xi-Clone Kit for Mammalian Expression	20 rxn.	XC003220	\$360
phCMV3 Xi-Clone Kit for Mammalian Expression	20 rxn.	XC003320	\$360
pIX Xi-Clone Kit for in vitro Translation	20 rxn.	XC004120	\$345
pIX2 Xi-Clone Kit for in vitro Translation	20 rxn.	XC004220	\$345
pIX3 Xi-Clone Kit for in vitro Translation	20 rxn.	XC004320	\$345

*Each kit also contains sufficient SmartCells™ Chemically Competent *E. coli* for 20 transformations.

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