

StrataClone™ PCR Cloning Systems

High-Performance PCR Cloning with Stratagene's DNA Topoisomerase I Technology

- + Clone both long and short amplicons with every kit
- + Kits for both UA and blunt-end cloning
- + Save time and money with just 5 minutes from PCR to transformation
- + High efficiency yields >95% clones with insert
- + More restriction enzyme sites in the multiple cloning site

OUR STRATACLONE™ PCR CLONING KIT MAKES CLONING YOUR PCR PRODUCTS EASIER, FASTER, AND MORE RELIABLE WITH DNA TOPOISOMERASE I TECHNOLOGY. GO FROM PCR TO TRANSFORMATION IN ONE SHORT, THREE-STEP PROTOCOL GUARANTEED TO GET YOUR CLONE THE FIRST TIME, EVERY TIME.

Fast, Easy, Reliable PCR Cloning

Our innovative cloning systems have facilitated the cloning and characterization of genes for over 20 years. With our StrataClone™ PCR Cloning Systems[®], cloning your PCR products is now easier, faster, and more reliable than conventional PCR cloning methods. Topoisomerase-based cloning saves time and money with simple primer design, no PCR clean-up, and an easy three-step process. Simply add your PCR product to the vector mix, incubate 5 minutes at room temperature, and transform ligated DNA into competent cells. With >95% efficiency, you are sure to get the right clone the first time every time (Figure 1).

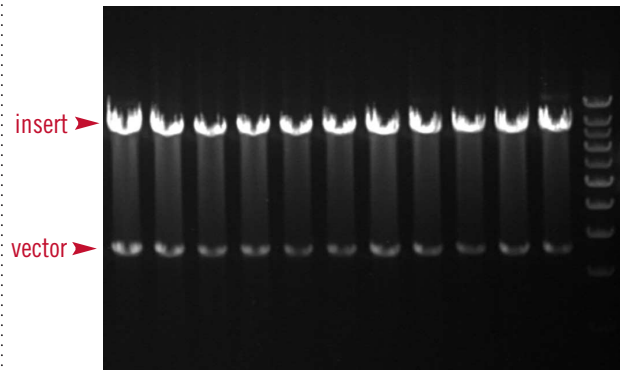


Figure 1
Get Your Clone the First Time, Every Time

We used the StrataClone Ultra™ PCR Cloning System to amplify and clone a blunt end 9.2-kb lambda fragment. The PCR product was amplified from lambda genomic DNA with our *PfuUltra*™ II Fusion HS DNA Polymerase and gel purified prior to cloning. Colonies were picked at random and DNA mini-preparations were digested with *EcoRI*.

Incubate PCR product with
Topoisomerase I-charged vector arms (5 minutes)

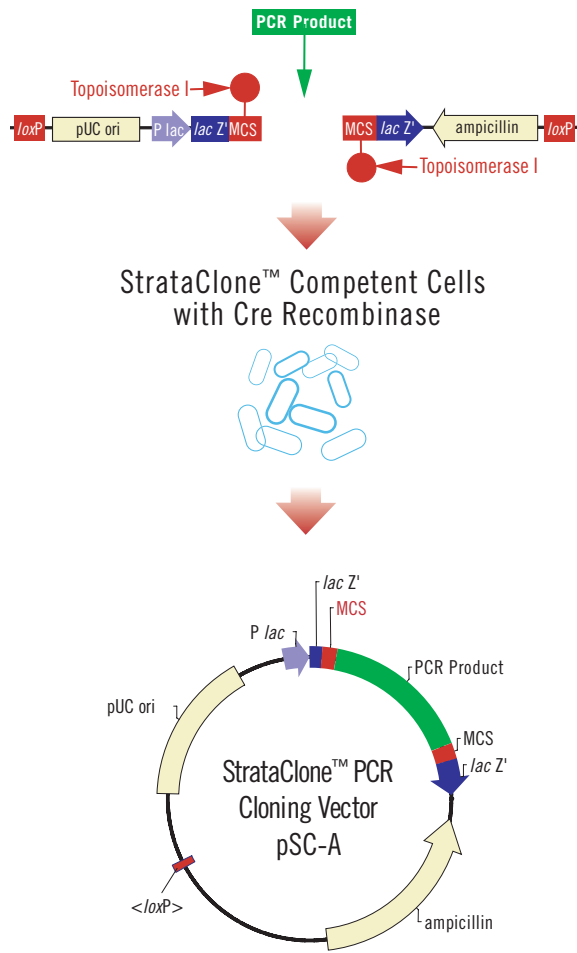


Figure 2
StrataClone™ PCR Cloning Technology

Our StrataClone™ PCR Cloning Systems exploit the combined efforts of two enzymes, *Vaccinia* topoisomerase I and bacteriophage P1 Cre recombinase.

Blunt-end and UA Cloning

We offer StrataClone topoisomerase I cloning kits for both blunt-end and UA cloning, and every kit can be used for long or short amplicons (Table 1). We have combined the highest accuracy in PCR amplification with easy, reliable, and affordably priced topoisomerase-based cloning in our StrataClone Ultra™ PCR Cloning Kit, by including our *PfuUltra* II Fusion HS DNA Polymerase[®]. Our StrataClone Blunt PCR Cloning Kits and StrataClone PCR Cloning Kits for UA cloning are available in both 10 and 20 reaction sizes. The StrataClone Combo PCR Cloning Kit contains 10 reactions of each.

StrataClone™ Technology

Our StrataClone PCR cloning systems exploit the combined efforts of two enzymes, DNA topoisomerase I from *Vaccinia* virus and Cre recombinase from bacteriophage P1 (Figure 2). In *Vaccinia*, DNA topoisomerase I assists in DNA replication by relaxing and rejoining DNA strands. The enzyme's site-specific endonuclease activity generates linear DNA with defined termini, and the DNA ligase activity closes the strand when replication is complete. When topoisomerase I cleaves a phosphodiester backbone of the DNA duplex, a covalent DNA-enzyme intermediate is formed, which conserves bond energy to be used for religating the cleaved DNA back to the original strand or to a heterologous DNA acceptor. The site-specific Cre recombinase enzyme catalyzes DNA recombination between asymmetric 8-bp core regions of two *loxP* recognition sequences. The StrataClone PCR cloning vector mix contains DNA arms charged with topoisomerase I on one end and *loxP* recombination sequences on the other. When your PCR product is added to the mix, the DNA topoisomerase I ligates the vector arms to the PCR product resulting in a linear molecule (vector arm - PCR product - vector arm). The ligated DNA is then transformed, with no clean-up steps required, into our high-efficiency competent cell line engineered to transiently express Cre recombinase. The linear DNA is circularized by the Cre recombinase at the *loxP* sites and the recombinant vector is amplified in an overnight incubation. Blue/white screening with *LacZ* allows quick identification of insert-positive clones.

PCR Insert	PCR Insert Size	Total Colonies	Blue	White	% White
1	664 bp	262	3	259	99%
2	1.8 kb	178	1	177	99%
3	3.5 kb	774	4	770	99%
4	6.0 kb (gel purified)	635	3	632	99%
5	9.2 kb (gel purified)	466	7	459	98.5%

Table 1
StrataClone™ PCR Cloning Results in >95% Clones with Insert

The StrataClone™ PCR Cloning Kit was used to clone PCR fragments from a common fluorescent protein (664 bp), γ -globin (1.8 kb), β -globin (3.5 kb), and two fragments from human α -1-antitrypsin (6 kb and 9.2 kb). We used undiluted PCR reaction mix (2 μ l) to clone PCR fragments 1-3. Larger PCR products 4 and 5 were gel purified prior to cloning. PCR fragment 1 was amplified from plasmid DNA, fragment 2 from first-strand cDNA, and fragments 3, 4, and 5 were amplified from human genomic DNA. PCR was performed using our *Taq2000*™ DNA Polymerase (664bp and 1.8kb), Easy-A™ High-Fidelity PCR Cloning enzyme (3.5kb), and *TaqPlus*™ Long PCR System (6 and 9.2 kb). Colonies were picked at random and screened by restriction digests to verify results.

For more information about StrataClone PCR cloning kits, please check our website at www.stratagene.com/strataclone.

Stratagene US and Canada
Order: 800-424-5444 x3
Technical Service: 800-894-1304 x2

Stratagene Europe
Order: 00800-7000-7000
Technical Service: 00800-7400-7400

Stratagene Japan K.K.
Order: 3-5821-8077
Technical Service: 3-5821-8076

For a list of worldwide distributors, please visit our website.

www.stratagene.com

StrataClone™ PCR Cloning Kits

StrataClone Ultra™ PCR Cloning Kit (includes StrataClone™ Blunt Vector Mix and <i>PfuUltra</i> ™ II Fusion HS DNA Polymerase)	20 reactions	240218
StrataClone™ Blunt PCR Cloning Kit	10 reactions	240208
	20 reactions	240207
StrataClone™ PCR Cloning Kit (UA cloning)	10 reactions	240206
	20 reactions	240205
StrataClone™ Combo PCR Cloning Kit (UA + blunt-end cloning)	20 reactions	240209

PfuUltra™, StrataClone™, StrataClone Ultra™, and *Taq2000*™ are trademarks of Stratagene in the United States. *TaqPlus*™ and Easy-A™ are registered trademarks of Stratagene in the United States.

a. Patents pending
b. U.S. Patent Nos. 6,734,293, 6,489,150, 6,444,428, 6,183,997, 5,948,663, 5,866,395, 5,545,552 and patents pending.

STRATAGENE
An Agilent Technologies Company

For Research Use Only. Not for use in diagnostic procedures.

© Stratagene, an Agilent Technologies company, 2008
Printed in USA, April 2008
5989-8266EN