Agilent StrataClone Cloning Systems

Data Sheet

Introduction

Our Agilent StrataClone kits make cloning your PCR products 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 required, and an easy 3-step process. Simply add your PCR product to the vector mix, incubate 5 minutes at room temperature, and transform ligated DNA into supplied competent cells.

- High-efficiency yields of >95 % clones with insert

- Clone both long and short amplicons with every kit

- Kits for both UA and blunt-end cloning

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

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 %

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 µl) 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 (664 bp and 1.8 kb), Easy-A High-Fidelity PCR Cloning enzyme (3.5 kb), and TaqPlus Long PCR System (6 and 9.2 kb). Colonies were picked at random and screened by restriction digests to verify results.

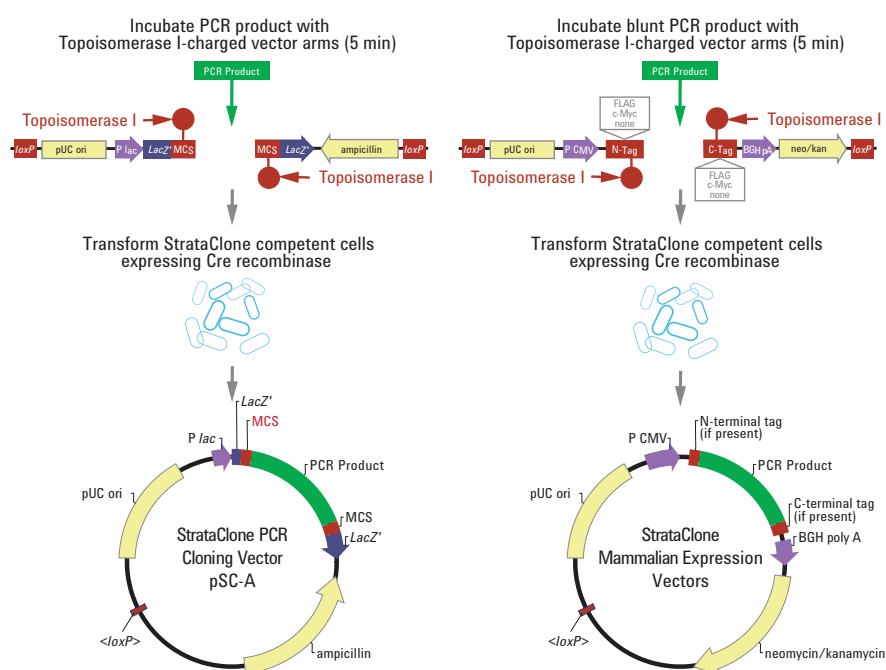**Agilent Technologies**

UA and Blunt-End Cloning

The StrataClone PCR Cloning Kit allows high efficiency cloning of Taq-amplified PCR products, while the StrataClone Blunt PCR Cloning Kit and the StrataClone Ultra Blunt PCR Cloning Kit are designed for blunt-ended PCR products. The latter kit, in addition to enabling easy, robust topoisomerase-based cloning, contains our highest fidelity enzyme for extremely accurate PCR amplification.

Mammalian Cloning and Expression

The Mammalian Expression Vector System offers five unique vectors, supplied as two linear topoisomerase-activated arms. The vectors are available with highly immunoreactive c-Myc or FLAG® epitope tags, which simplify detection of your protein by eliminating the need for protein-specific antibodies.

StrataClone Technology

Our StrataClone system exploits the combined efforts of two enzymes, Vaccinia virus topoisomerase and Cre recombinase from bacteriophage P1. The StrataClone 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, 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. *LacZ* allows quick identification of insert-positive clones via blue/white screening.

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

StrataClone Mammalian Expression Cloning Method
Five varieties of vector arms are available, with either an N-terminal tag (FLAG or c-Myc), a C-terminal tag (FLAG or c-Myc), or no tag.

Ordering information

Product Description	Part Number
StrataClone PCR Cloning Kit	240205
StrataClone Blunt PCR Cloning Kit	240207
StrataClone Ultra Blunt PCR Cloning Kit	240218
StrataClone Mammalian Expression Vector System, Untagged	240228
StrataClone Mammalian Expression Vector System, N-terminal FLAG	240229
StrataClone Mammalian Expression Vector System, C-terminal FLAG	240230
StrataClone Mammalian Expression Vector System, N-terminal c-Myc	240231
StrataClone Mammalian Expression Vector System, C-terminal c-Myc	240232

Each product contains enough material for 20 reactions.

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