

Ultra High Fidelity PCR Cloning

Cloning PCR products is a very powerful tool for genetic analysis. Current PCR cloning vectors offer a variety of methods to ligate PCR products into the vector, such as complimentary base overhangs (TA cloning[®]), use of topoisomerase instead of DNA ligase (TOPO cloning[®]), or adding restriction sites to the PCR fragment. However these cloning systems suffer from relatively low cloning efficiencies, necessitating the extra work and expense of colony screening. Further, they often completely fail to clone PCR products containing AT- or GC-rich regions, toxic genes, repeats, strong promoters, or other "difficult-to-clone" sequences, resulting in inaccurate results and lost data. Finally, current PCR cloning systems offer a limited choice of vectors that are often quite expensive.

Lucigen offers two advances for fast, easy, and highly accurate cloning of PCR products: 1) the PCR-SMART[™] Cloning Kits and 2) the PCRterminator[®] End Repair Kit.

PCR-SMART Cloning Kits

These Kits contain the new pcrSMART[™] vector, incorporating Lucigen's unique CloneSmart[®] transcription-free technology, to achieve unprecedented accuracy and efficiency in cloning PCR products.

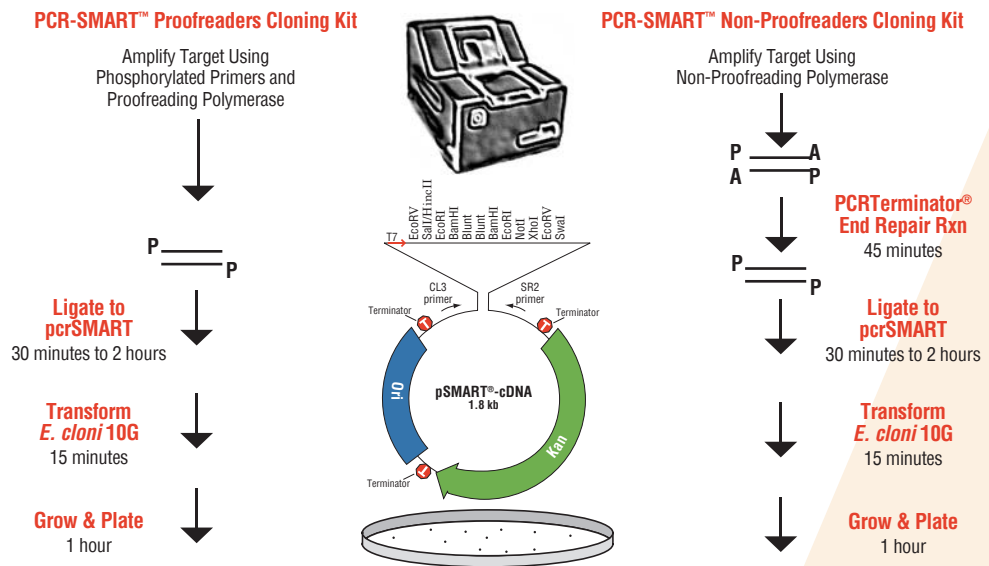
eLucidations[™]

1-888-575-9695

www.lucigen.com



*e*⁷



More accurate and reliable data. The pcrSMART vector contains transcription terminators and lacks an indicator gene to prevent transcription and translation of the insert, allowing high efficiency cloning of sequences that are unstable in other vectors. Consequently, cloning results accurately represent the population of input PCR products. Nothing is lost or changed in the cloning process.

High cloning efficiency from nanograms of insert. Blunt cloning with the pcrSMART vector is more efficient than other methods of PCR cloning. Yields of 10^4 - 10^5 recombinants are obtained from 50 ng of PCR product.

Saves time and effort. Greater than 99.5% of the clones have inserts...a much higher efficiency than is possible with any other PCR cloning system. Essentially all colonies contain a DNA insert, so no colony screening is needed.

Wide insert size range for standard or long PCR. PCR products from less than 100 bp to >10 kb can be cloned into the pcrSMART vector. The small vector size (1.8 kb) facilitates easier analyses, including sequencing and mutagenesis.

Clone PCR products from any PCR enzyme. PCR can be done with any DNA polymerase. Choose the PCR-SMART Kit that corresponds to the type of DNA polymerase (proofreader or non-proofreader) used in the PCR step:

PCR-SMART Proofreaders Cloning Kits are designed for highly efficient, error-free cloning of blunt PCR products produced by proofreading PCR enzymes (e.g., Vent® or Pfu DNA polymerase) or a mixture of proofreading and non-proofreading polymerases.

PCR-SMART Non-Proofreaders Cloning Kits are for high efficiency, error-free cloning of PCR products with terminal overhangs, produced by Taq, Tfl, Tth, and related enzymes.



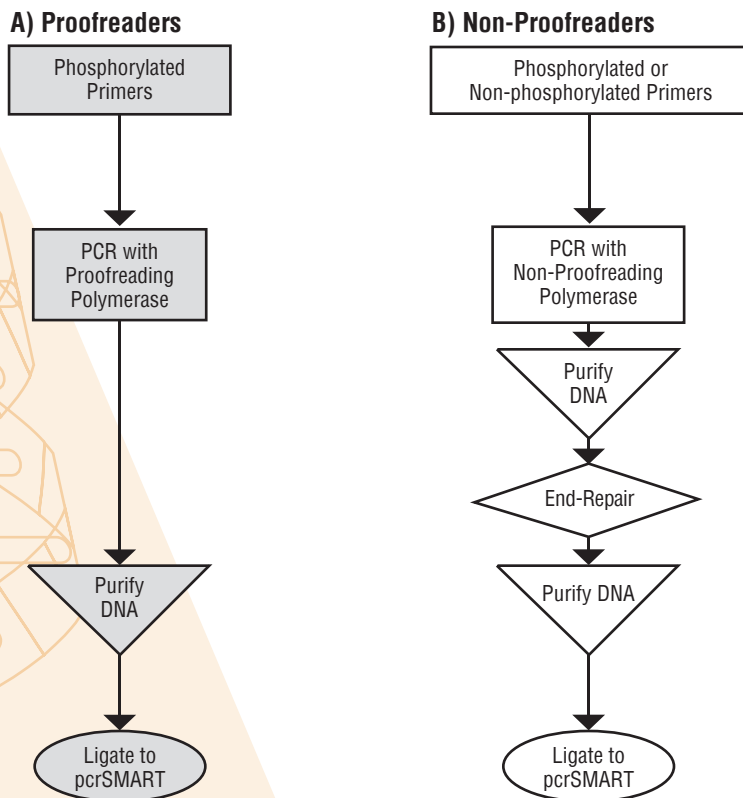


Figure 2. Alternative strategies for cloning with the PCR-SMART kit.

Strategies for cloning with the PCR-SMART Kit

The simplest protocol uses 5' phosphorylated primers and a proofreading polymerase for the PCR (Figure 1, page 8). However, the Kits also contain the necessary reagents for cloning PCR products made with non-phosphorylated primers and non-proofreading polymerases (Figure 2, above). The PCR-SMART Kits can be used with any combination of PCR amplification conditions.

A) Proofreaders. The most efficient way to clone PCR products is to use the PCR-SMART Proofreaders Kit, along with PCR primers that have been chemically synthesized with 5' phosphate groups (shaded pathway). Primers synthesized without 5' phosphate groups can be treated briefly with T4 polynucleotide kinase (included in the Proofreaders Kit) and used directly in PCR.

B) Non-Proofreaders. Products made with phosphorylated OR non-phosphorylated primers and a non-proofreading polymerase require an end-repair step to create blunt phosphorylated ends. The PCR product must be purified before end-repair, because the repair enzymes are not active in PCR buffer. The End Repair enzymes and buffer are included in the Non-Proofreaders Kit.

In all cases, we recommend gel purification as one of the purification steps to remove primers and to select for the correct-sized PCR product.

PCR-SMART Kits contain all needed reagents for phosphorylation, ligation, and transformation, including Lucigen's ultra high efficiency *E. cloni*[®] ELITE Electrocompetent Cells (>2 x 10¹⁰ cfu/μg). Kits are also available without cells, as the pcrSMART vector can be used with any chemically competent or electrocompetent *E. coli* cells.

PCRTerminator[®] End Repair Kits

Lucigen's PCRTerminator End Repair Kit allows high efficiency cloning of PCR fragments into any blunt cloning vector, greatly increasing your options for cloning PCR products. The Kit improves cloning results both for proofreading DNA polymerases (e.g., Vent[®] or Pfu) and non-proofreading DNA polymerases (e.g., Taq, Tfl, Tth).

For PCR with proofreaders or non-proofreaders. The PCRTerminator Kit effectively repairs ragged ends and phosphorylates the termini of any type of PCR product. The products can be cloned into any blunt vector. A separate treatment step with polynucleotide kinase is not needed.

The PCRTerminator Kit is easy to use. Simply add your PCR product to the PCRTerminator components. After a short incubation, purify the blunt PCR fragments and ligate directly into the vector of your choice.

Order Information

PCR-SMART Kit contents: pcrSMART Kan[®] High Copy Number Vector Premix, CloneSmart T4 DNA Ligase, two Sequencing Primers, Positive Control Insert DNA, Positive Control Transformation plasmid, T4 Polynucleotide Kinase and Buffer, and a complete protocol. The Non-Proofreaders Kit also includes PCR-SMART End Repair Enzyme Mix. Kits are available with or without *E. cloni* 10G ELITE Electrocompetent Cells (> 2 x 10¹⁰ cfu/μg DNA). ■

PCRTerminator Kit contents: End Repair Buffer, End Repair Enzyme, protocol.

Cat. No.	Product	Size
41103-1	PCR-SMART Proofreaders Cloning Kit	10 reactions
41103-2	PCR-SMART Proofreaders Cloning Kit	20 reactions
41101-2	PCR-SMART Proofreaders Cloning Kit (w/o cells)	20 reactions
41101-4	PCR-SMART Proofreaders Cloning Kit (w/o cells)	40 reactions
41124-1	PCR-SMART Non-Proofreaders Cloning Kit	10 reactions
41124-2	PCR-SMART Non-Proofreaders Cloning Kit	20 reactions
41120-2	PCR-SMART Non-Proofreaders Cloning Kit (w/o cells)	20 reactions
41120-4	PCR-SMART Non-Proofreaders Cloning Kit (w/o cells)	40 reactions
40037-1	PCRTerminator End Repair Kit	10 reactions
40037-2	PCRTerminator End Repair Kit	50 reactions

