

NEW CopyRight® v2.0 BAC Cloning Kits

- High efficiency BAC library construction
- General cloning of any DNA

Lucigen's new CopyRight v2.0 vectors and BAC-Optimized Replicator™ v2.0 Electrocompetent Cells combine the revolutionary, patented CloneSmart® transcription-free technology, inducible copy amplification, and a *lacZ-sacB* stuffer fragment that eliminates uncut vector from recombinants. The result is unprecedented accuracy, reliability, and efficiency in BAC cloning and library construction. Unlike other BAC cloning systems, Lucigen's CloneSmart technology assures that even "unclonable" inserts are stably maintained. Sequences are not lost or rearranged in the cloning process. A simple "on command" amplification system built into the CopyRight v2.0 vectors and BAC-Optimized Replicator v2.0 Electrocompetent Cells gives a 20-50 fold increase in copy number for high yields and easy purification (Wild, J., *et al.* (2002). *Genome Res.* 12,1434).

CopyRight pSMART® BAC vectors

The CopyRight pSMART BAC vector (Figure 1) incorporates transcription-free cloning for the highest stability BAC cloning. The vector is supplied pre-cut at a choice of BamHI, EcoRI, or HindIII sites, with dephosphorylated ends. Insert DNAs with 5'-phosphorylated ends are ligated to the CopyRight vector and transformed into Lucigen's BAC-Optimized Replicator v2.0 Electrocompetent Cells.

The new pSMART BAC vectors have a *lacZ-sacB* stuffer region, allowing uncut vector to be detected by blue/white screening and selected against by plating on sucrose. This feature completely eliminates uncut vector background, improving the efficiency of colony picking. However, unlike other cloning vectors, the promoter and the coding sequence of the stuffer fragment are completely removed during processing. This design prevents active expression of the insert DNA by the *lacZ* or *sacB* promoter, contributing greatly to plasmid stability, especially for inserts containing toxic coding sequences, strong secondary structure, or other deleterious features. It also prevents selection against clones that contain promoters, because the coding regions of the *lacZ* and *sacB* genes have been removed.

Advantages

Greater cloning fidelity. Transcription-free, single-copy cloning minimizes cloning bias and sequencing gaps. Clone even "unclonable" DNAs such as centromeric regions, toxic genes, strong promoters, repeats, hairpins, and AT-rich sequences with the pSMART BAC transcription-free vector (see Figure 1).

Maximum insert stability. CloneSmart transcription terminators in the pSMART BAC vector minimize deletions and rearrangements.

Higher throughput. Zero background eliminates wasted time in colony picking and analysis.

Inducible Copy Number. Increase DNA yields 20-50 fold with simple chemical induction.

Fast and easy cloning protocol. Simply add your DNA, incubate, and transform.

Higher cloning and sequencing efficiencies. Ultra-high efficiency BAC-Optimized Replicator v2.0 Electrocompetent Cells ($\geq 4 \times 10^{10}$ cfu/ μ g pUC 19) maximize recombinant yields.

Better Value. CopyRight Kits offer superior performance for BAC cloning (Figure 2) at a much better price than other available kits.

CopyRight Kits are also available with pEZ BAC vector for blue/white screening. Please see the Lucigen website (www.lucigen.com) for more information.

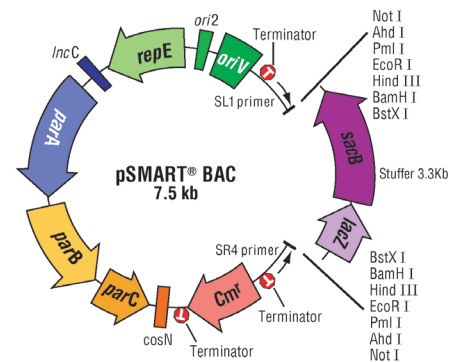


Figure 1. Diagram of CopyRight vector pSMART BAC. *ori2*, *repE*, *IncC* - origin of replication (single copy); *oriV* - inducible origin of replication; *parA,B,C* - partition genes; *Cm^r* - chloramphenicol-resistance gene; *cosN* - lambda packaging signal; T - CloneSmart transcription terminators; *sacB*, sucrose gene; *lacZ*, alpha peptide portion of the β -galactosidase gene. Approximate positions of sequencing primers and transcription terminators are indicated.

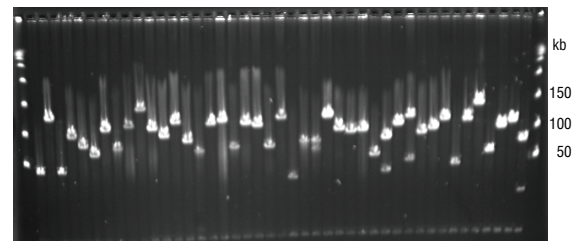


Figure 2. *Arabidopsis* Random Shear BAC Library in the pSMART BAC vector. 7,680 clones were obtained with an average insert size of 105 kb and ~5X genome coverage.

CopyRight® v2.0 BAC Cloning Kits *continued*

ORDER INFORMATION

Each CopyRight v2.0 BAC Cloning Kit contains: pre-cut vector (pSMART® BAC with a choice of BamHI or EcoRI or HindIII sites), CopyRight DNA Ligase, CopyRight 5X Ligation Buffer, Positive Control Insert DNA, Sequencing Primers, BAC-Optimized Replicator™ v2.0 Electrocompetent Cells in DUO packaging (2 transformations per tube), Positive Control Insert DNA, Recovery Medium, Arabinose Induction Solution, and complete protocols.

Product	Size	Cat. No.
CopyRight v2.0 BAC Cloning Kit, pSMART BAC BamHI w/BAC-Optimized Replicator v2.0 Electrocompetent Cells	10 rxns 20 rxns	42030-1 42030-2
CopyRight v2.0 BAC Cloning Kit, pSMART BAC EcoRI w/BAC-Optimized Replicator v2.0 Electrocompetent Cells	10 rxns 20 rxns	42031-1 42031-2
CopyRight v2.0 BAC Cloning Kit, pSMART BAC HindIII w/BAC-Optimized Replicator v2.0 Electrocompetent Cells	10 rxns 20 rxns	42032-1 42032-2

CloneSmart Technology is covered by U.S. Patent 6,709,861, and other patents issued or pending in the U.S. and other countries and assigned to Lucigen. Inducible Copy Amplification is covered by U.S. Patent 5,874,259, and other patents issued or pending in the U.S. and other countries and licensed to Lucigen.

\$500 Reward!

If you develop a novel application or would like to share technical insights related to our products, we would like to hear from you. Please email us (lucigen@lucigen.com) with comments or ideas for articles. We offer a \$500 cash honorarium for articles published in **eLucidations**.