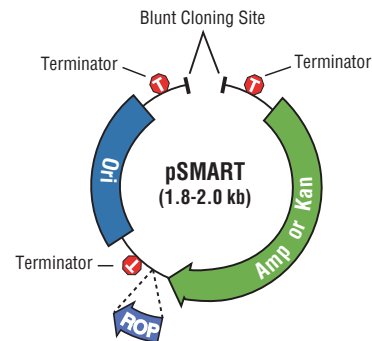


# Missing a Gene? Avoiding the Problem of Lost Sequences

## INTRODUCTION

Nearly all molecular biologists have encountered sequences that seem “impossible” to clone. Months of effort can be spent trying to obtain a particular clone, often with no success. In library construction projects, difficult sequences lead to numerous clone gaps that complicate the finishing process. This problem is especially serious when only nanograms of DNA are available for cloning.

To avoid the problem of lost inserts, Lucigen has developed the pSMART™ series of transcription-free cloning vectors (U.S. Pat. No. 6,709,861) that are designed specifically for unbiased, high-efficiency cloning. In contrast to conventional vectors, pSMART vectors eliminate indicator genes and the associated promoters that cause vector-driven transcription of the cloned insert. In addition, terminators flank the cloning site to prevent cloned promoters from transcribing into vector regions (Figure 1).



**Figure 1. Schematic diagram of the pSMART vectors.** Transcription terminators prevent transcription into or out of the insert. The pSMART vectors are available with either ampicillin or kanamycin selection, and the ROP gene is present in high or low copy versions.

The result is stable cloning of any DNA, including toxic genes, AT- or GC-rich sequences, strong promoters, sequence repeats, hairpins, large fragments, and trace amounts of target. Other important features of pSMART vectors include a choice of drug resistance markers and high or low copy number.

Lucigen's proprietary methods of vector processing ensure cloning efficiencies of  $\geq 99\%$ . Because nearly every recovered clone will be a recombinant, the blue/white indicator gene or direct selection is not needed, and error-prone colony screens are avoided completely.

## RESULTS

pSMART vectors provide accurate, reliable, and high efficiency cloning with any DNA. In particular, the unique benefits of transcription-free cloning are essential when working with many types of difficult or "unclonable" DNAs. Several examples are described below.

**Toxic genes.** Many regions encode polypeptides that are toxic to *E. coli*, so they are difficult to clone in conventional vectors. These sequences can be cloned intact, with high efficiency, and in either orientation with pSMART vectors. Examples include:

- **Toxic regions of the Mouse Hepatitis Virus genome.** These sequences are highly resistant to cloning in standard vectors (e.g., pGEM<sup>®</sup>, pTOPO<sup>®</sup>), but they were easily cloned and very stable in pSMART vectors.<sup>1</sup>
- **Novel enzymes.** A pSMART library contained multiple intact copies of a 3 kb gene encoding a saccharolytic enzyme. In pUC-based vectors, only deleted or rearranged versions of the gene were detected.
- **Lethal genes.** Many intact clones encoding a prokaryotic RNase, inserted in either orientation, were obtained in pSMART vectors. In a pUC19-based vector, the gene was only recovered in the "reverse" orientation.

**Strong promoters.** The strong P<sub>R</sub> promoter of bacteriophage Lambda was easily cloned and maintained in the pSMART high copy and low copy vectors, whereas intact clones were very rare in pUC19-based vectors (Table 1).

**Table 1.** The P<sub>R</sub> promoter (400 bp, 25 pg) was cloned into pSMART and pUC19-based vectors. The number of colonies per plate and the proportion of clones containing the intact promoter are shown.

Vector	Total cfu per plate	Intact $\lambda$ P <sub>R</sub> clones
pSMART-HC	170	75%
pSMART-LC	72	75%
pUC19	2000 Blue 20 White	-- 25%



1. Ralph Baric, University of North Carolina, *J. Virol.* 76: 11065 (2002).  
pGEM is a trademark of Promega Corp. TOPO is a trademark of Invitrogen Corp.

**AT- or GC-rich DNA.** Successfully cloning DNAs with skewed base composition – particularly AT-rich fragments – is problematic in standard vectors but highly reliable in pSMART™ vectors (Figure 2).

**Large fragments (>10 kb).** Inserts of >10 kb can be readily constructed with pSMART low copy number vectors (Figure 3).

For cloning inserts that are larger or particularly unstable, Lucigen offers the CopyRight™ BAC, Fosmid, and Large Insert Cloning Kits. These kits contain the pSMART VC vector for unprecedented cloning power (Figure 4).

**Trace amounts of target.** Nanogram quantities of DNA can be cloned with CloneSmart Kits, whereas other systems typically require micrograms. For this reason, the pSMART vectors are an essential part of Lucigen's Nanoclone™ technology, described in the accompanying article, *Lucigen's Custom Services...Doing It Better & Faster*.

**Many more recombinants from every cloning reaction**

The high efficiency, pre-processed pSMART vectors greatly accelerate all types of cloning projects. Tables 2 and 3 demonstrate the increased productivity made possible by using pSMART vectors.

**Table 2.** Results from sequencing a genomic shotgun library prepared using a pSMART vector.

Clones sequenced	Clones with inserts	Empty vector clones	Cloning Efficiency
32,000	31,960	40	99.9%

-- Data courtesy of Peter Wilson, Australian Genome Research Facility

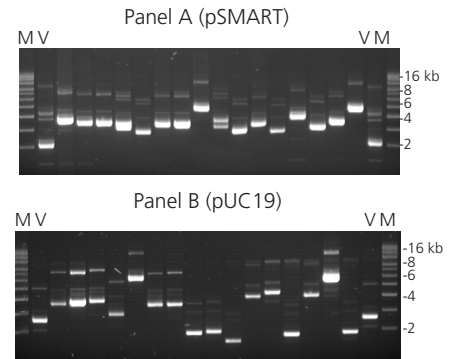
**Table 3.** Results from sequencing a BAC library. BACs containing mouse genomic DNA were sheared to 1.5-4 kb, end-repaired, and used for construction of libraries in the pSMART-HCAmp vector.

Number of libraries (#successful / #attempts)	Clones with inserts*	Clones/library	Read Length
33 / 33	~2400 / 2400	2-4 x 10 <sup>5</sup>	> 500 bp

\* 96 Clones from each of 24 libraries were sequenced to determine frequency of inserts. No empty vector was detected. Similar results were obtained with 22 additional libraries. Data courtesy of Kate Montgomery, Cecilia Shim, Jeremy Decker, Wendy Zencheck, Li Li, George Grills, and Raju Kucherlapati. Harvard Partners Genome Center

**CONCLUSION**

Lucigen's transcription-free, ultra high efficiency pSMART vectors provide much greater accuracy, reliability, and recombinant yields than any other cloning system and without the need for vector preparation or screening. Essentially all input sequences are recovered intact in the cloning process.

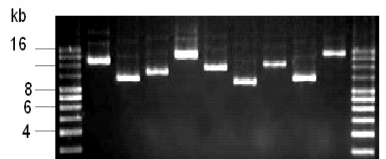


**Figure 2.** A cosmid containing genomic DNA from *Pneumocystis carinii* (70% AT) was subcloned into either the pSMART-HCKan vector or a pUC19-based vector. Plasmid DNA from transformants was analyzed by agarose gel electrophoresis.

**Panel A)** Plasmids from randomly picked pSMART transformants were all within the expected size range, demonstrating high stability.

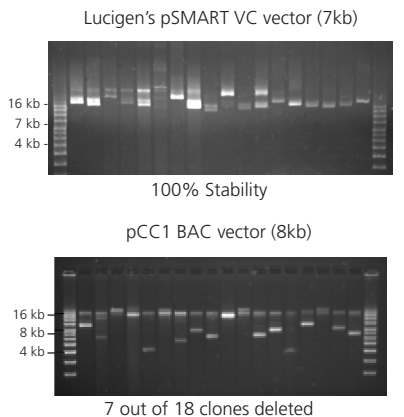
**Panel B)** Over 25% of the pUC19 vector transformants were unstable, yielding plasmids smaller than the parent vector. M, supercoiled plasmid ladder V, empty vector control

-- In association with James Stringer and Melanie Cushion, University of Cincinnati



**Figure 3.** Genomic DNA from *Shigella dysenteriae* was sheared, size-selected to 8-14 kb, end-repaired, and cloned into pSMART LCamp. Over 20,000 clones were obtained. Many new genes with no significant homology to known genes were recovered.

-- In association with Thomas Whittam, Michigan State University



**Figure 4.** Genomic DNA from *Tetrahymena thermophila* (75% AT) was sheared, size-selected to 10-20 kb, and cloned into pSMART VC or a leading BAC/large Insert vector.



These unique advantages are available in a series of Lucigen kits for every cloning application:

- **General cloning:** CloneSmart® Blunt Cloning Kits
- **cDNA cloning:** cSMART™ cDNA Cloning Kits
- **PCR cloning:** PCR-SMART™ Cloning Kits
- **BAC, Fosmid, & Large Insert cloning:** CopyRight™ Cloning Kits

For more information on each of these cloning kits, including ordering information, please see the Lucigen web site [www.lucigen.com](http://www.lucigen.com). ■

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