

The CloneSmart™ Advantage: Easy Cloning of “Difficult” DNA Targets

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Introduction

Molecular biologists often encounter certain genes, chromosomal regions, or entire genomes that are especially difficult to clone. The CloneSmart™ system was designed to eliminate many of the difficulties inherent to cloning, whether the target is an individual gene or a complete genome.

The CloneSmart™ Blunt Cloning Kits incorporate the pSMART™ vectors, which employ transcriptional terminators to minimize unintended transcription out of DNA inserts (Figure 1). The pSMART™ vectors do not use a promoter or indicator gene, so transcription through the insert is eliminated. This lack of transcription greatly decreases the bias against cloning strong promoter regions, sequences that encode toxic peptides, or other unstable elements.

In contrast, conventional vectors employing direct selection schemes or the blue/white screen (such as pUC19 or related vectors) have a high level of transcription and translation of the insert. Transcription initiated within cloned inserts can conflict with the indicator gene promoter or may interfere with maintenance of the plasmid.

The following examples illustrate the power and simplicity of using the CloneSmart™ system. DNA refractory to cloning in other plasmids was readily cloned in the pSMART™ vectors. High-throughput sequencing showed that over 99.5% of randomly picked clones contained insert DNA. The lack of empty vector background eliminated the effort, expense, and uncertainty of using XGal/IPTG for blue/white colony screening.

Cloning Difficult Targets

Cloning AT rich genomic DNA is notoriously difficult. While the reasons are not clear, one suggestion is that the cloned sequences behave as transcriptional promoters in *E. coli*. Transcription driven by the insert may then proceed into the vector and interfere with its replication or expression of drug resistance.

The genome of *Lactobacillus helveticus*, which is approximately 65% AT, provides a stringent test of cloning vectors. Attempts to generate genomic libraries of 2-4 kb fragments in pUC based vectors resulted in a small number of colonies with a substantial number of deleted clones (Figure 2). Assembly of 19,000 clones from the pUC libraries was expected to provide nearly 4X coverage of the genome, but instead only 70% of the genome was represented. Clone stacking was up to 40 deep in some single-copy loci (1).

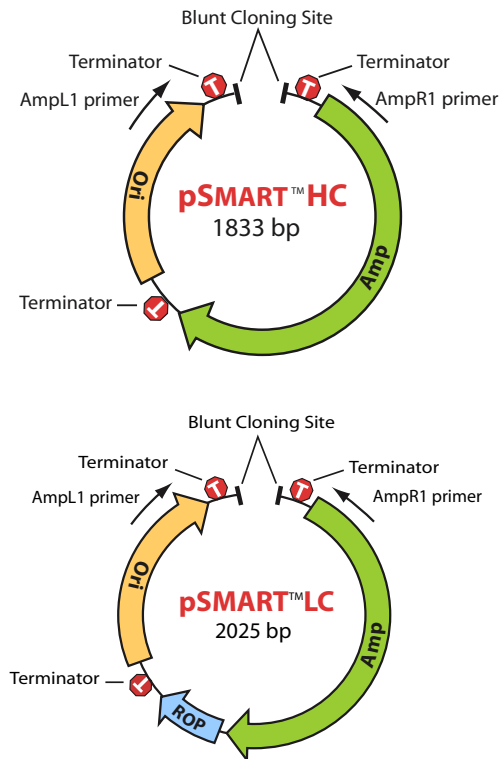


Figure 1. Diagram of the pSMART™ vectors. The high and low copy-number versions of the pSMART™ vectors are illustrated, showing positions of terminators (T), origin of replication (Ori), and ampicillin (Amp) gene. The repressor of primer (ROP) gene lowers the plasmid copy number to that of pBR322.

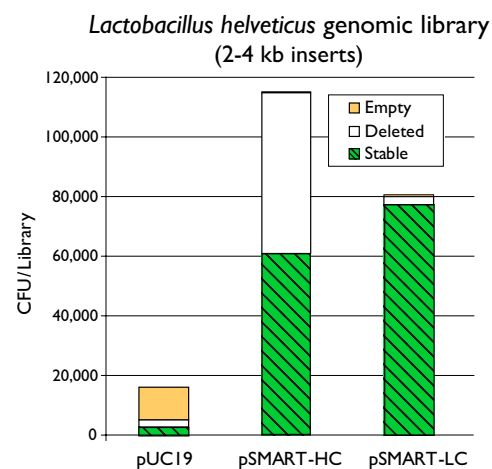


Figure 2. Efficiency of Cloning AT-rich DNA in CloneSmart™. Genomic DNA from *Lactobacillus helveticus* was sheared to 2-4 kb, gel isolated, and cloned into pUC19 or the pSMART™ vectors. DNA from transformants was analyzed by agarose gel to determine insert size. Clones having inserts of less than 0.2 kb were designated as “Deleted”.

In contrast, libraries of 2-3 kb genomic fragments created in pSMART™-HC and -LC contained 25-30 fold as many stable clones (Figure 2). Furthermore, 97% of the pSMART™-LC clones were intact. Sequencing 7300 clones from the pSMART™-LC library increased the genomic coverage to 97.5%. Minimal stacking was detected. These results demonstrated that the CloneSmart™ system produced broad and even coverage of this difficult-to-clone genome.

Target inserts that were problematic to clone in pUC type vectors were obtained readily in pSMART™.

The low-copy pSMART™-LC vector is also ideal for cloning large DNA fragments. Libraries containing inserts of 8-14 kb can be obtained routinely in this vector (Figure 3). Although the vector is maintained at a low copy number, minipreps from 1.5 ml cultures provided ample amounts of DNA for subsequent analyses.

Another significant problem in cloning can arise from vector-driven transcription of insert DNA. Blue/white screening or direct selection plasmids are based upon expression of an actively transcribed indicator gene. Because the multiple cloning site is imbedded within the indicator gene, any fragment inserted into the site is potentially transcribed and translated. The resulting expression can select against inserts that contain toxic coding sequences.

Numerous attempts were made to clone a gene encoding a specific saccharolytic enzyme into pUC based vectors. However, the resulting plasmids invariably suffered deletions within the insert, which precluded cloning the entire gene in a single plasmid. In contrast, full-length genomic clones of this gene were isolated from a pSMART™-LC library enriched for this sequence (data not shown).

Convenient and Reliable Library Construction

Because the CloneSmart™ vectors are provided pre-cut and dephosphorylated, researchers are freed from having to prepare their own vector. Further, the unique design of the CloneSmart™ system ensures virtually zero background of

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empty vector, so there is no need to screen for recombinants. Therefore, all viable colonies can be picked for analysis, eliminating the error-prone manual or robotic discrimination between white, light blue, and authentic blue colonies.

Several library construction projects have confirmed the ease of use and lack of background with the CloneSmart™ Kit

Size Distribution of Clones in *Shigella dysenteriae* genomic library

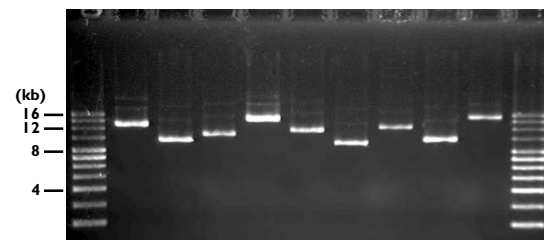
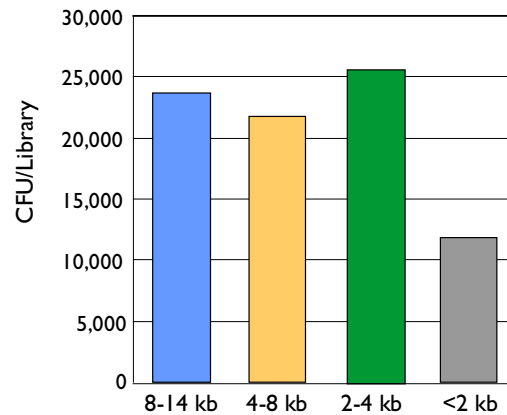


Figure 3. Cloning large inserts with CloneSmart™. Genomic DNA from *Shigella dysenteriae* was sheared to 8–14 kb, gel isolated, and cloned into pSMART™-LC. Upper image: size distribution of inserts; lower image: DNA minipreps of plasmids containing 8–14 kb inserts.

(Table 1). A university-associated genomics center generated successful libraries of 400,000 clones each from 50–200 ng of BAC DNA sheared to 1.5–4 kb (2). Sequence analysis of 100 clones from each of 24 of these libraries revealed inserts in 100% of the plasmids. Another academic researcher reported 100% inserts in over 1500 clones analyzed (3).

Table 1. High-throughput sequence analysis of CloneSmart™ libraries.

Number of libraries (# successful/# attempts)	Amount of insert (ng/ligation)	Clones with inserts	Clones/Library	Read length	Ref.
33/33	5-200 ng	~2400/2400	4 x 10 ⁵	>500 bp	2
7/7	100-300 ng	1584/1584	5-10 x 10 ⁵	>500 bp	3

These results demonstrate the ease and efficiency of using the CloneSmart™ system. Target DNA inserts that were problematic to clone in pUC type vectors were obtained readily in pSMART™. Genomic libraries of small and large inserts were generated in pSMART™ with minimal effort. Finally, the lack of empty vector background eliminated the need for colony screening, as well as the time and expense of sequencing non-recombinant clones.

References

1. Jim Steele (University of Wisconsin, Madison, WI) and Jeff Broadbent (Utah State University, Logan, UT).
2. Kate Montgomery, Cecilia Shim, Jeremy Decker, Wendy Zencheck, Li Li, George Grills, Raju Kucherlapati (Harvard Partners Genome Center, Cambridge, MA).
3. Forest Rohwer (San Diego State University, San Diego, CA).

Product Information

Product	Size	Prod. #
CloneSmart™-HCM Blunt Cloning Kit	10 libraries	40051-1
High copy number pSMART™-HC vector premix	20 libraries	40051-2
<i>E. cloni</i> ™ MCI2 electrocompetent cells	100 libraries	40051-3
Ligase, two sequencing primers, positive control insert DNA, positive control transformation plasmid.		
CloneSmart™-HCG Blunt Cloning Kit	10 libraries	40052-1
High copy number pSMART™-HC vector premix	20 libraries	40052-2
<i>E. cloni</i> ™ 10G electrocompetent cells	100 libraries	40052-3
Ligase, two sequencing primers, positive control insert DNA, positive control transformation plasmid.		
CloneSmart™-LCM Blunt Cloning Kit	10 libraries	40210-1
Low copy number pSMART™-LC vector premix	20 libraries	40210-2
<i>E. cloni</i> ™ MCI2 electrocompetent cells		
Ligase, two sequencing primers, positive control insert DNA, positive control transformation plasmid.		
CloneSmart™-LCG Blunt Cloning Kit	10 libraries	40311-1
Low copy number pSMART™-LC vector premix	20 libraries	40311-2
<i>E. cloni</i> ™ 10G electrocompetent cells		
Ligase, two sequencing primers, positive control insert DNA, positive control transformation plasmid.		

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