

## CloneSmart® Cloning Kits

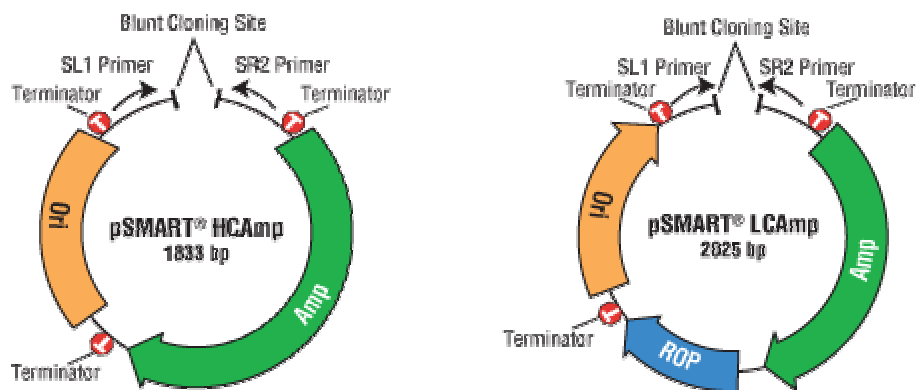
### Gap-Free Cloning

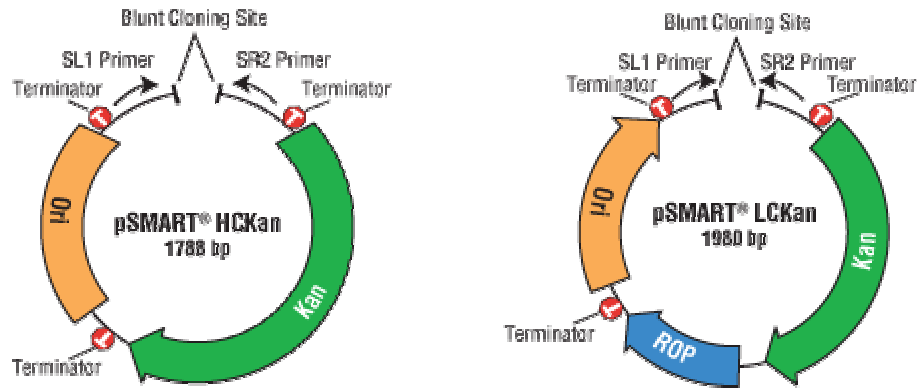
Lucigen's patented pSMART® vectors are a new generation of transcription- and translation-free cloning vectors designed to eliminate cloning gaps commonly found with many plasmids. Conventional cloning vectors contain promoters and associated signals that constitutively transcribe and translate the insert sequence. For example, the lac promoter that drives the expression of the b-galactosidase indicator gene in pUC type plasmids also transcribes and translates any DNA inserted into the multiple cloning site. This transcription and coupled translation can destabilize or select against recombinant plasmids, especially for inserts containing coding regions, strong promoters, short repeats, or incompatible secondary structures.

The pSMART vectors (Fig. 1) minimize many of the problems associated with cloning recalcitrant DNA in conventional plasmid vectors. Vector-driven transcription and translation of the insert DNA is eliminated, as these vectors do not contain a promoter or indicator gene that may initiate transcription through the cloning site. Likewise, insert-driven transcription into the vector is blocked by strong terminators flanking the cloning site. The low copy number versions of pSMART further increase the chances of cloning intact sequences that are difficult to maintain in typical vectors. For example, shotgun cloning of an AT-rich genome such as *Lactobacillus helveticus* in pSMART resulted in 30 times more stable clones than in pUC19. A number of additional difficult-to-clone genes and genomes have been obtained with this technology.

### No Background Problems

The pSMART vectors are supplied pre-digested, with blunt dephosphorylated ends, and are qualified to produce 99.5% recombinant clones in typical experiments. The ultra-low background of empty vector (less than 0.5%) eliminates the need to screen for recombinants and enables library construction from nanogram amounts of DNA. Because no screening is required, CloneSmart technology eliminates the need for XGAL/IPTG and removes the uncertainty of false negatives (light blue pUC colonies) and false positives (white colonies that lack inserts).





**Figure 1.** High copy (HC) and low copy (LC) versions of the pSMART transcription-free cloning vectors. Amp, ampicillin resistance gene; Kan, kanamycin resistance gene; Ori, origin of replication; ROP, "Repressor of Primer" copy number gene.

## Convenient Success

Plate your shotgun sequence library today - the CloneSmart Blunt Cloning Kits eliminate tedious vector and competent cell preparation, as well as time-consuming QC testing experiments. Kits include optimized reagents, ligation-ready vector (no post-ligation cleanup step required), highly efficient competent cells (up to  $>4 \times 10^{10}$  cfu/ $\mu$ g for the electrocompetent cells;  $>1 \times 10^9$  cfu/ $\mu$ g for the chemically competent cells), detailed instructions, and trouble-shooting guides to simplify cloning and sequencing.

## Advantages

- Gap-free cloning.
- $<1\%$  background.
- Cleanup-free transformation protocol - no post-ligation cleanup prior to transformation saves valuable time.
- Small vector sizes (1.7-2.0 kb) to facilitate subcloning, mutagenesis and transposition experiments.
- Choice of antibiotic resistance, use ampicillin or kanamycin selection.
- Pre-processed pSMART to eliminate vector digestion, gel purification, and dephosphorylation.
- High efficiency cloning with either electrocompetent or chemically competent cells.

## Specifications

- As many as 1,000,000 single insert clones can be obtained using the reagents and controls supplied in the electrocompetent cell kits. Yields are lower using the chemically competent cell kits. A background of  $<1\%$  is observed using the supplied reagents and controls.

	Copy No.	General Purpose	cDNAs	PCR Products	Short ORFs, Promoters	Toxic ORFs	Large inserts, Repeats, AT/GC-Rich	Expression	Insert Size
<b>Single Insert Cloning</b>									
<b>CloneSmart® LC Kits</b>									
Kan	low	+	+	+	+	++	++	-	$<15$ kb
Amp	low	+	+	+	+	+	+	-	$<15$ kb
<b>CloneSmart® HC Kits</b>									
Kan	High	++	+	+	++	+	+/-	-	$<6$ kb
Amp	High	+	+	+	+	+	+/-	-	$<6$ kb

++: Optimal vector

+: Suitable vector

+/-: Some clones may be difficult to obtain

## **Applications**

- General purpose cloning
- Shotgun and cDNA library construction
- Cloning phage and viral genomes with high densities of potentially toxic coding sequences
- Cloning A/T rich genomes and sequences
- Cloning problematic genomes and toxic genes
- Blunt PCR cloning

**[Click here for - CloneSmart Blunt HC LC Cloning Kit Manual](#)**