

## Advanced Vectors for Cloning BACs, Fosmids, & Large Insert Plasmids

### Introduction

BACs (Bacterial Artificial Chromosomes) and fosmids (single-copy plasmids containing the *cos* site for *in vitro* lambda packaging) have become important tools in genomic analysis, allowing easier isolation of genes, gene clusters, and expression control sequences. BAC and fosmid genomic libraries also have enabled physical mapping and efficient sequencing of entire genomes. Recently, thermal stable DNA polymerases with higher processivity have enabled “long” PCR amplifications of 10-20 kb. Cloning these large DNAs into BAC, fosmid, or standard plasmid vectors has been difficult, often resulting in biased results, poor yields, cloning and sequencing gaps, and incorrect data.

A recent improvement to BAC and fosmid vectors is conditional amplification, which allows cloning at low copy number to maintain insert stability, followed by induction to high copy number for increased yield and easier purification.<sup>1-4</sup> However, the improved vectors still have significant problems of cloning bias, i.e., rearrangement or loss of DNA segments containing AT- or GC-rich regions, strong promoters, repeats, hairpins, toxic genes, or other sequences. They also suffer from empty vector background and relatively few recombinants per reaction. The large size of current BAC vectors (> 8 kb) results in wasted time re-sequencing the vector rather than the insert.

These problems can be overcome with Lucigen’s CopyRight™ cloning vectors. By combining transcription-free CloneSmart® technology<sup>5</sup> with inducible copy number amplification, the CopyRight vectors greatly increase the stability of large inserts to allow cloning of large DNAs that were previously “unclonable”.

### Results

The difficulty of cloning AT-rich genomes and genes in conventional cloning vectors and hosts is well documented.<sup>6</sup> AT-rich DNA fragments as small as 2-4 kb are often deleted or rearranged when cloned into pUC-type plasmids. In contrast, these sequences can be accurately cloned with the high-stability pSMART® LCKan vector (Lucigen).

Some genomes appear to be particularly difficult to clone. One of the most difficult samples to clone is genomic DNA of the protozoan *Tetrahymena thermophila*, whose 160 Mb macronuclear genome is 75% AT. Lucigen scientists constructed several genomic libraries of various insert sizes in the pSMART-LCKan vector. The largest insert size tolerated with 95% efficiency of cloning was 4-5 kb, and a successful 20X coverage library was produced. An 8-12 kb insert library showed a significantly reduced cloning efficiency, with numerous deletions observed in the recombinant clones.

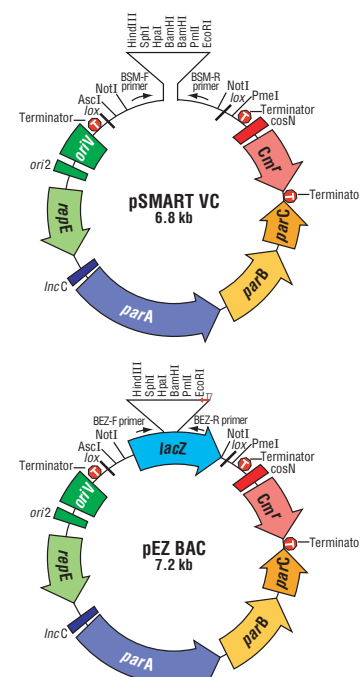
To enable large-insert library construction from such refractory DNAs, Lucigen developed a single-copy BAC vector incorporating the transcription-free CloneSmart technology. The *oriV* inducible replication origin was added to facilitate DNA preparation.

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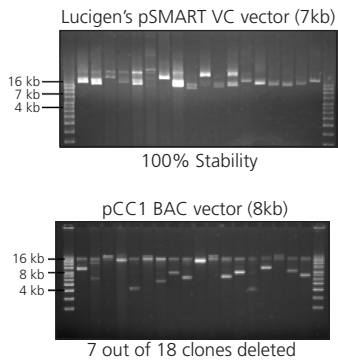
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**Figure 1. Diagram of the CopyRight™ Vectors.** The pSMART VC vector (transcription-free) and pEZ BAC vector (blue/white screening) are illustrated, showing positions of terminators (T), chloramphenicol-resistance gene (Cm<sup>r</sup>), *cos* site, partition genes (*parA*, *parB*, *parC*), single copy origin (*ori2*, *incC*, *repE*), inducible origin (*oriV*), cloning site, and primer binding sites.



**Figure 2. Stability of AT-rich DNA in pSMART VC.** Genomic DNA from *T. thermophila* was sheared to ~10-20 kb using the HydroShear™ device (Gene Machines, San Carlos, CA), end-repaired with the DNATerminator® kit (Lucigen), and size fractionated on an agarose gel. This material was cloned into either the pSMART VC vector or the pCC1BAC vector. DNA was isolated from randomly chosen pSMART VC colonies or white pCC1FOS colonies.

The resulting plasmid is the pSMART VC vector (Figure 1). An alternate vector, termed pEZ™ BAC, contains blue/white screening capability (Figure 1).

To demonstrate the efficacy of pSMART VC, a library of 10-20 kb fragments of *Tetrahymena thermophila* DNA was constructed. In contrast to libraries made in any other vector, the pSMART VC library was successful, as all 96 clones analyzed had inserts of the expected size (Figure 2, top panel). The same DNA preparation was cloned into pCC1BAC (8.2 kb, Epicentre), which has active transcription and translation of inserts from the *lacZ* gene. The pCC1BAC vector gave 50% fewer recombinants, and they spanned a wide range of sizes. In fact, 7 out of 18 clones showed an obvious deletion, as they were smaller than the size of the parental vector (Figure 2, lower panel). These results demonstrate that a transcription-free BAC vector can stabilize otherwise deleterious sequences.

**“On our first attempt, a library with 9-12 kb inserts worked brilliantly!”**

To confirm the activity of the oriV inducible origin of replication, genomic DNA from *Anopheles gambia* was packaged as a fosmid library into pSMART VC to generate inserts of ~40 kb. Miniprep DNA was isolated from several clones before and after copy number induction. The clones showed 20-50 fold amplification after 3 hours of induction (Figure 3).

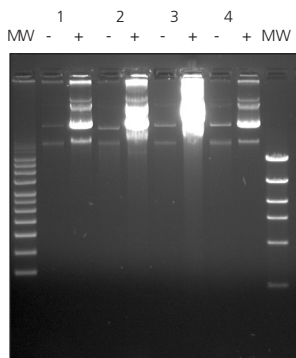
## Discussion

Large DNA inserts are generally difficult to clone; for certain genomes, obtaining stable inserts of 10 kb or greater has been impossible. Recent results show that the pSMART VC vector maintains such DNA with very high integrity. Three features of the vector are likely responsible for the high stability: the lack of vector-driven transcription into the insert, terminators at the cloning site to prevent insert-driven transcription into the vector, and cloning at single copy number. The advantage of transcription-free cloning is evident in the comparison of the pSMART VC and pCC1BAC vectors (Figure 2). Although these vectors have similar copy numbers, the pSMART VC vector exhibited dramatically increased stability.

The inducible replication origin greatly simplifies isolation of DNA from recombinant clones. The copy number of clones typically increases 20-50 fold following amplification (Figure 3). Reasons for variation in the final copy number clones have not been determined, but they may reflect sequence or structural characteristics of the inserts.

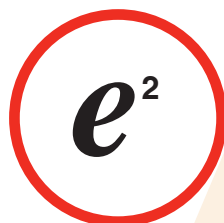
## References

1. Hradecna, Z., Wild, J., and Szybalski, W. (1998). Conditionally amplifiable inserts in pBAC vectors. *Microbial. & Comp. Genomics* 3, 58.
2. Wild, J., Hradecna, Z., and Szybalski, W. (2001). Single-copy/high-copy (SC/HC) pBAC/oriV novel vectors for genomics and gene expression. *Plasmid* 45, 142.
3. Wild, J., Hradecna, Z., and Szybalski, W. (2002). Conditionally amplifiable BACs: switching from single-copy to high-copy vectors and genomic clones. *Genome Res.* 12, 1434.



**Figure 3. Copy number amplification of CopyRight clones.** Clones were grown overnight without copy number induction. An aliquot of each culture was induced for 3 hours. Each lane contains 1/10th of the miniprep DNA from a 1-ml culture.

- no induction  
+ copy number induction



4. US Patent 5,874,259 and pending.
5. US Patent 6,709,861 and pending.
6. Gardner, MT, et al. (2002). Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 419, 498.

### CopyRight™ Kit Product Information

*"On our first attempt, a library with 9-12 kb inserts worked brilliantly!"*

-P. Wilson, Australia Genome Research Facility

The pSMART VC and pEZ BAC vectors are part of the CopyRight Cloning Kits. Applications, features, and benefits of the CopyRight vectors are described below:

**pSMART VC *Bam*HI-cut or blunt vector.** The pSMART VC vector does not include the *lacZ* gene, to provide maximum stability of inserts. Colony screening is not needed due to the ultra high cloning efficiency of this vector ( $\geq 99\%$ ). The pSMART VC vector provides absolutely the best method for cloning fosmid and 10-20 kb fragments, including long PCR products, as even "impossible" sequences are stably maintained. It is also very useful for BAC cloning, provided the insert DNA is very carefully isolated.

**pEZ BAC *Bam*HI-cut or blunt vector.** The pEZ BAC vector contains the *lacZ* gene for blue/white screening. It is ideal for either BAC or fosmid cloning and library construction, or >10 kb shotgun library construction. The pEZ BAC vector cloning efficiency is  $\geq 99\%$ .

Each vector is available in pre-processed, *Bam*H1-cut or blunt cloning versions. In addition to cloning large inserts (10-100+ kb), the pEZ BAC and pSMART VC vectors can be used for cloning any DNA that has blunt or *Bam*HI-cut ends.

The CopyRight vectors contain the following features:

- Single-copy replication origin and inducible medium copy replication origin
- Transcriptional terminators to stabilize recombinant clones
- Transcription/translation-free cloning for unstable DNAs (pSMART VC only)
- Bacteriophage lambda *cos* site for lambda packaging or terminase cleavage
- Phage T7 polymerase promoter for *in vitro* transcription (pEZ BAC only)
- *loxP* sites for Cre-recombinase cleavage
- Rare-cutting restriction sites on either side of the insert
- Chloramphenicol-resistance gene
- Minimal vector size

The CopyRight Kits offer these benefits in cloning large DNAs:

**Greater cloning fidelity.** Transcription-free, single copy cloning minimizes cloning bias and sequencing gaps. Clone even "unclonable" DNAs such as toxic genes, strong promoters, repeats, hairpins, and AT- or GC-rich sequences with the pSMART VC transcription-free vector. See Figure 2, previous page.

# CopyRight™ Cloning Kits

## Order Information

Each CopyRight Kit contains:

- Choice of: pEZ™ BAC blue/white *Bam*HI-cut vector, or pEZ BAC blue/white blunt cloning vector, or pSMART® VC transcription-free *Bam*HI-cut vector, or pSMART VC transcription-free blunt vector
- CloneSmart® DNA Ligase
- CloneDirect™ 10X Ligation Buffer (includes ATP)
- Sequencing Primers
- Control Insert DNA
- Replicator™ ultra high efficiency Electrocompetent Cells ( $>2 \times 10^{10}$  cfu/ $\mu$ g) with Replicator Induction Solution, Transformation Control DNA, and Recovery Media.

**Maximum insert stability.** CloneSmart transcription terminators in both pEZ BAC and pSMART VC minimize deletions and rearrangements.

**Inducible Copy Number.** Conditional amplification using a simple chemical induction step increases DNA yields 20-50 fold. See Figure 3, previous page.

**Fast and easy protocol.** Simply add the DNA, incubate, and transform.

**Higher cloning and sequencing efficiencies.** Ultra-high efficiency Replicator™ Electrocompetent Cells (>2 x 10<sup>10</sup> cfu/μg) maximize recombinant yields. Small vector sizes minimize wasted sequencing resources.

**Better Value.** CopyRight Kits offer superior performance at a much better price than any other available BAC, fosmid, or large DNA cloning kits. Replicator Cells contain the gene necessary for conditional amplification of vector copy number. If copy amplification is not needed, CopyRight Kits can be used with Lucigen's *E. cloni*® chemically competent or electrocompetent cells, or with any other competent *E. coli* cells.

**Replicator Cells are also available separately.** Cells include: Replicator Induction Solution, Transformation Control DNA, and Recovery Media. ■

Catalog No.	Product	Size
42001-1	CopyRight pEZ BAC <i>Bam</i> HI Cloning Kit	10 reactions
42001-2	CopyRight pEZ BAC <i>Bam</i> HI Cloning Kit	20 reactions
42012-1	CopyRight pEZ BAC <i>Bam</i> HI Cloning Kit w/o cells	10 reactions
42005-1	CopyRight pEZ BAC Blunt Cloning Kit	10 reactions
42005-2	CopyRight pEZ BAC Blunt Cloning Kit	20 reactions
42016-1	CopyRight pEZ BAC Blunt Cloning Kit w/o cells	10 reactions
42008-1	CopyRight pSMART VC <i>Bam</i> HI Cloning Kit	10 reactions
42008-2	CopyRight pSMART VC <i>Bam</i> HI Cloning Kit	20 reactions
42019-1	CopyRight pSMART VC <i>Bam</i> HI Cloning Kit w/o cells	10 reactions
42011-1	CopyRight pSMART VC Blunt Cloning Kit	10 reactions
42011-2	CopyRight pSMART VC Blunt Cloning Kit	20 reactions
42022-1	CopyRight pSMART VC Blunt Cloning Kit w/o cells	10 reactions
60201-1	Replicator Electrocompetent Cells	12 transformations
60201-2	Replicator Electrocompetent Cells	24 transformations

