

# High Stability Vectors for cDNA Cloning and Microarray Production

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## Introduction

DNA microarrays are a powerful tool for studying the complex genetic alterations that occur in cancer cells and other physiologically altered cells. The production of microarrays often depends on amplification of cDNA or other appropriate libraries. However, large sections of viral, microbial, and eukaryotic genomes are refractory to cloning or unstable when cloned into conventional host-vector systems. This cloning bias leads to inaccurate and incomplete analyses.

Insert instability is due to three factors common to most plasmid vectors: 1) vector-driven expression of deleterious genes, 2) insert-driven transcription interfering with vector stability, and 3) inappropriate antibiotic resistance. Most plasmid vectors induce strong transcription and translation of inserted fragments, which prevents cloning of numerous coding sequences. Standard vectors also allow transcription initiated by cloned promoters to interfere with plasmid replication or selection, causing additional bias. In addition, ampicillin resistance is mediated by a secreted enzyme,  $\beta$ -lactamase, which rapidly degrades ampicillin in the medium. The rapid loss of antibiotic selection strongly favors cells with deleted or non-recombinant plasmids.

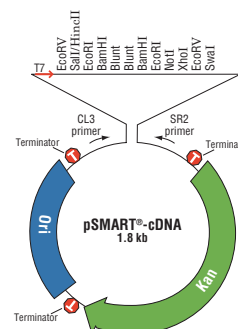
To circumvent these problems, we used the pSMART<sup>®</sup>-cDNA transcription-free cloning vectors for cDNA library analysis. These vectors lack an indicator gene and associated promoter, have termination signals on either side of the insertion site, and are kanamycin resistant (Figure 1).

## Results

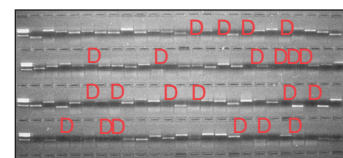
To examine gene expression in cardiac tissue of the teleost fish *Fundulus*, we constructed a microarray of relevant cDNAs. A cDNA library was constructed in a pUC based vector, and the inserts from approximately 5000 clones were amplified by PCR.

Sequence analysis was used to identify PCR products containing genes of interest, and bacterial stocks from 1152 clones (12 x 96-well plates) were re-grown to allow reamplification of the inserts. However, only 788 of the clones (68%) yielded a PCR product after re-growth of the stock cultures (Figs. 2 and 4).

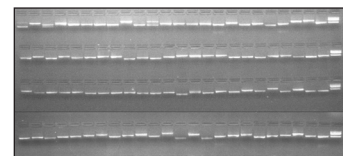
**“...identical groups of inserts confirmed the increased stability in the pSMART-cDNA vector.”**



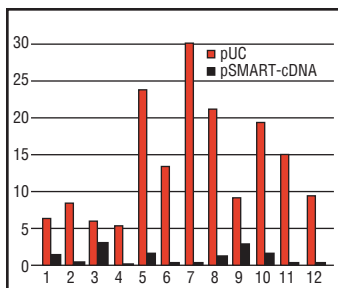
**Figure 1. Transcription-free pSMART-cDNA vector.** Transcriptional terminators prevent vector-driven transcription of insert DNA and terminate transcripts that initiate from cloned promoters. Kanamycin is used for selection. The background of non-recombinant vector is < 0.1%.



**Figure 2. Amplification of cDNA clones from a pUC library.** Only 68% of the clones from the pUC library were successfully re-amplified.



**Figure 3. Amplification of cDNA clones from a pSMART-cDNA library.** Over 99% of the cDNA clones in a pSMART-cDNA vector were stable upon re-growth and re-amplification, successfully producing PCR bands.



**Figure 4. Instability of cDNA clones in pUC vs pSMART-cDNA vectors.** Distribution of failed clones among twelve 96-well plates from cDNA libraries created in pUC and the pSMART-cDNA vector. All clones were initially positive by PCR screening.

A new library from the same tissue was constructed in a pSMART-cDNA vector. Inserts from this library were similarly amplified by PCR and sequenced. Strikingly, upon re-growth and re-amplification, 1144 of 1152 pSMART-ARRAY clones (>99%) resulted in successful PCR amplification (Figure 3, previous page, and Figure 4).

Sequence comparison of the original PCR products from the 1152 pUC and 1152 pSMART clones showed that these two groups had 157 cDNA clones in common. Successful re-growth and re-amplification was achieved for all 157 clones in the pSMART-cDNA vector, but only 133 of the pUC clones (85%) produced a PCR band. Thus, analysis of identical groups of inserts confirmed the increased stability in the pSMART-cDNA vector.

### Discussion

When used for production of a cDNA library, >99% of pSMART-cDNA clones were stable during re-growth and re-amplification. In contrast, only 68% of clones in a standard vector remained intact. For production of a 10,000-spot array, this difference represents a loss of 3200 genes. The increased stability of clones in the pSMART-cDNA vector was directly demonstrated by comparing the stability of the same 157 cDNA sequences in the two types of vectors. These clones, which were previously positive in PCR screening, were 100% stable in the pSMART vector, whereas 15% of the pUC clones failed to re-amplify.

Other specific examples of increased stability in transcription-free pSMART vectors have been noted. For example, toxic regions of the mouse hepatitis virus genome were readily cloned and stable in pSMART-cDNA, but they were deleted, rearranged, and slow-growing in TOPO® and pGEM® vectors. A lethal microbial nuclease gene was completely stable in a pSMART vector, but unclonable in conventional plasmids.

The efficiency, yield, and success rate of cloning in pSMART-cDNA libraries is a significant improvement over traditional vectors. These tools should improve the quality and quantity of data collected from microarrays, thereby increasing the likelihood of finding statistically significant differences in expression.

### Order Information

The pSMART-cDNA vector is available as a component of the cSMART cDNA Cloning Kit.

**The cSMART™cDNA Cloning Kit contains:** the pSMART-cDNA vector pre-cut at a choice of restriction enzyme sites (EcoR1+Not1, Not1+blunt, or blunt alone), CloneSmart® T4 DNA Ligase, sequencing primers, controls, and a choice of *E. coli*®10G ELITE (>2 x 10<sup>10</sup> cfu/μg) or SUPREME (≥4 x 10<sup>10</sup> cfu/μg) ultra high efficiency electrocompetent cells. Kits are also available without competent cells. The pSMART-cDNA vectors can be used with any chemically competent or electrocompetent *E. coli* type cells. ■



Cat. No.	Product	Size
<b>(RN = EcoRI+NotI insertion sites)</b>		
41012-2	cSMART cDNA HK-RN Cloning Kit w/o Cells	20 rxns
41012-4	cSMART cDNA HK-RN Cloning Kit w/o Cells	40 rxns
41015-1	cSMART cDNA HK-RN 10G ELITE Cloning Kit	10 rxns
41015-2	cSMART cDNA HK-RN 10G ELITE Cloning Kit	20 rxns
41018-1	cSMART cDNA HK-RN SUPREME Cloning Kit	10 rxns
41018-2	cSMART cDNA HK-RN SUPREME Cloning Kit	20 rxns
<b>(BluntN = Blunt+NotI insertion sites)</b>		
41034-2	cSMART cDNA BluntN Cloning Kit w/o Cells	20 rxns
41034-4	cSMART cDNA BluntN Cloning Kit w/o Cells	40 rxns
41036-1	cSMART cDNA BluntN 10G ELITE Cloning Kit	10 rxns
41036-2	cSMART cDNA BluntN 10G ELITE Cloning Kit	20 rxns
41038-1	cSMART cDNA BluntN SUPREME Cloning Kit	10 rxns
41038-2	cSMART cDNA BluntN SUPREME Cloning Kit	20 rxns
<b>(Blunt = Both insertion sites are blunt)</b>		
41045-2	cSMART cDNA HK-Blunt Cloning Kit w/o Cells	20 rxns
41045-4	cSMART cDNA HK-Blunt Cloning Kit w/o Cells	40 rxns
41047-1	cSMART cDNA HK-Blunt ELITE 10G Cloning Kit	10 rxns
41047-2	cSMART cDNA HK-Blunt ELITE 10G Cloning Kit	20 rxns
41049-1	cSMART cDNA HK-Blunt SUPREME Cloning Kit	10 rxns
41049-2	cSMART cDNA HK-Blunt SUPREME Cloning Kit	20 rxns