

## Construction of Large Unbiased cDNA Libraries with the CloneSmart® System

J. Matheson, D.Wilson, R. Drinkwater. Xenome, Ltd., Brisbane, Australia

Xenome focuses on the discovery of novel molecules, especially peptides from the venoms of Australian animals. We routinely make cDNA libraries from animal tissue that is normally only available in small quantities. As such, we have developed a protocol that generates end adapted double stranded cDNA from this tissue that is then used as a template in PCR. The DNA product from this reaction is cloned and sequenced. Two aspects of this method have often plagued the efficiency of the work. The first is the lack of consistency in the production of large, unbiased cDNA libraries, and the second is the frequency of clones in these libraries with truncated inserts. Our interest in Lucigen's CloneSmart Blunt Cloning Kit was primarily aimed at resolving these issues, that is, to generate large, unbiased libraries containing full length genes.

We have tested Lucigen's high copy number CloneSmart Blunt Cloning Kit (#40052) and the results are impressive. Each of the three cDNA libraries that have been generated with the CloneSmart Kit have produced between 600,000 and 950,000 cfu. With libraries of this size, we are very confident that we do not have sequence bias issues that previously existed. The insert size of the clones appears to mimic quite closely the molecular weight range of the insert template used in the cloning. We have sequenced approximately 500 clones from each of the CloneSmart libraries, and in all cases the inserts appear to be full length. In other vector types, there was a large proportion of quite obviously truncated sequences. Further, the insert orientation does not appear to be biased in the pSMART® vector, in contrast to results observed with blunt end cloning in other vectors.

A related result is that we are now seeing sequence types in a standard random cloning reaction that previously required the selection of DNA of a specific molecular weight. This again strengthens our view that the cDNA libraries generated with Lucigen's CloneSmart vector are less prone to the bias we have observed with other vector types.

---

*Another pleasing feature...  
was the low percentage  
of empty clones*

---

Sequencing appears to run equally well from either primer site on the CloneSmart vector. Most of the sequence data extends out to around 900 bp using standard sequencing chemistries analysed on a PE3700.

Interestingly, the sequencing reactions appear to run through the poly-A tails of the inserts more effectively than we would normally expect. This may be attributed to the quality of the sequencing primers provided with the vector or to the physical structure of the CloneSmart vector that facilitates high quality (low noise) sequence data.

Another pleasing feature of the CloneSmart system was the low percentage of empty clones. In two of the libraries the number of blank clones was effectively zero, whilst in the third about 10% of the clones were blank. This low background has now eliminated the need to screen clones for inserts prior to sequencing.

---

*We have tested Lucigen's  
CloneSmart Kit and the  
results are impressive.*

---

### Solve your cloning problems with the CloneSmart System and get a FREE kit!

Struggling with deleted inserts, small libraries, or DNA that seems impossible to clone into your present vector? Use the CloneSmart system to clone your difficult inserts, then send us a brief summary of your results. If your work is chosen for publication in *eLucidations*, you will receive a FREE 10-rxn CloneSmart kit of your choice.

For everyday cloning, choose the CloneSmart High Copy Number vector with 10G competent cells. For those difficult to clone regions, use the CloneSmart Low Copy Number vector with 10G competent cells.

See the back page for information on ordering your CloneSmart kit.

**CLONE**  
*SMART*®

#### IN THIS ISSUE

<b>Construction of Large Unbiased cDNA Libraries with the CloneSmart System.....</b>	<b>1</b>
<b>The Means To An End: Optimal Methods for DNA Fragmentation and End Repair.....</b>	<b>2</b>
<b>Product Information .....</b>	<b>4</b>
<b>How To Order .....</b>	<b>4</b>

**Lucigen**®  
The Molecular Cloning Company™

Continued on page 4

## Means To An End...Cont.

Use of this protocol typically results in libraries of >1,000,000 single insert clones or >10,000 dual insert clones with less than 0.1% empty vector background.

## References

1. Godiska R, Reuter M, Schoenfeld T, Sheets L, Derr A, and Mead D 2001. *eLucidations* 1: 1-3.
2. Bodenteich AS, Chissoe S, Wang Y-F, and Roe BA 1994. In *Automated DNA sequencing and analysis techniques* (ed. MD Adams, C Fields, and C Venter), pp.42-50. Academic Press, London, UK.
3. Deininger PL 1983. *Anal. Biochem.* 129: 216-223.
4. Thorstenson YR, Hunicke-Smith SP, Oefner PJ, Davis RW 1998. *Genome Res.* 8:848-55.

## How To Order

Order by telephone from 7:30 am - 5:30 pm (CST) or by fax, or e-mail anytime.

**Toll-free Phone:** 1-888-575-9695

**Fax:** 608-831-9012

**Phone:** 608-831-9011

**E-mail:** lucigen@lucigen.com

## Product Information

	Size	Electrocompetent Cells		Chemically Competent 10G Cells	w/o Cells	
		10G ELITE	10G SUPREME		Size	Cat.#
CloneSmart® HC Blunt Cloning Kits		Cat.#	Cat.#	Cat.#		
High copy number pSMART™ HCAmp Vector Premix	10 libraries	40052-1	40063-1	40074-1	20 rxns	40041-2
	20 libraries	40052-2	40063-2	40074-2	40 rxns	40041-4
High copy number pSMART HCKan Vector Premix	10 libraries	40706-1	40717-1	40728-1	20 rxns	40704-2
	20 libraries	40706-2	40717-2	40728-2	40 rxns	40704-4
CloneSmart LC Blunt Cloning Kits						
Low copy number pSMART LCAmp Vector Premix	10 libraries	40311-1	40322-1	40333-1	20 rxns	40300-2
	20 libraries	40311-2	40322-2	40333-2	40 rxns	40300-4
Low copy number pSMART LCKan Vector Premix	10 libraries	40832-1	40843-1	40854-1	20 rxns	40821-2
	20 libraries	40832-2	40843-2	40854-2	40 rxns	40821-4

All **CloneSmart** Kits include CloneSmart Ligase, two sequencing primers, positive control insert DNA, and positive control transformation plasmid.

### DNATerminator® End Repair Kit

	Size	Cat.#
End repair buffer, end repair enzyme mix	10 transformations	40035-1
	50 transformations	40035-2

## Construction of cDNA Libraries...Cont.

The CloneSmart Blunt Cloning Kit is certainly a very quick and easy system to use, requiring a much shorter time to generate libraries, which contain numerous previously elusive clones. With the CloneSmart Kit we now have large fully represented cDNA libraries – a simple remedy with a significant result! We will certainly continue with the CloneSmart system and recommend it highly.

**xenome**

Xenome Ltd.  
Brisbane Australia  
www.xenome.com

*eLucidations* is published to provide customers with useful information about Lucigen products. The articles are written by Lucigen staff and outside contributors.

If you have comments or suggestions about the current issue of *eLucidations*, please direct them to: Lucigen Corp., 2120 W. Greenview Drive, Middleton, WI 53562.

phone: 888-575-9695  
608-831-9011

fax: 608-831-9012

e-mail: lucigen@lucigen.com

web: www.lucigen.com

*pLEXX* and *eLucidations* are trademarks of Lucigen Corporation. *Lucigen*, *CloneSmart*, *ClonePlex*, *E. cloni*, *pSMART*, and *DNATerminator* are registered trademarks. The *CloneSmart* Kit is patented (U.S. Patent No. 6,709,861). © Lucigen Corporation. All rights reserved.