

Lucigen's Custom Services... Doing It Better & Faster

Lucigen's powerful cloning technologies and unparalleled cloning expertise are available to you as custom services. We specialize in delivering successful, cost-effective solutions to difficult cloning projects, with fast turnaround and complete confidentiality. Lucigen's custom services include:

- Custom cloning & library construction
- Custom construction of chemically competent or electrocompetent cells

GapFree™ & NanoClone™ Custom Cloning & Library Construction Services

Standard cloning procedures often require microgram quantities of input DNA. Unfortunately, only trace amounts of DNA are recovered from some samples, such as cells or viruses that are rare or difficult to culture. Conventional cloning methods also tend to select against DNA containing toxic genes, AT- or GC-rich regions, modified bases, promoters, repeats, or various other "unclonable" sequences.

Lucigen's unique GapFree and NanoClone custom cloning services solve this problem (Figure 1, next page).

GapFree libraries are constructed in Lucigen's transcription-free pSMART™ vectors to provide accurate, unbiased libraries, even with "unclonable" DNA. As a result, there are no cloning or sequencing gaps, and no sequences are lost or rearranged. In fact, GapFree libraries are not only more accurate, but they can produce up to 1,000-fold more recombinant colonies as compared to standard libraries (Figure 2, next page, upper right panel).

Libraries for protein analysis are constructed in Lucigen's pEZSeq™ or pSMART-cDNA vectors, which support transcription and translation.



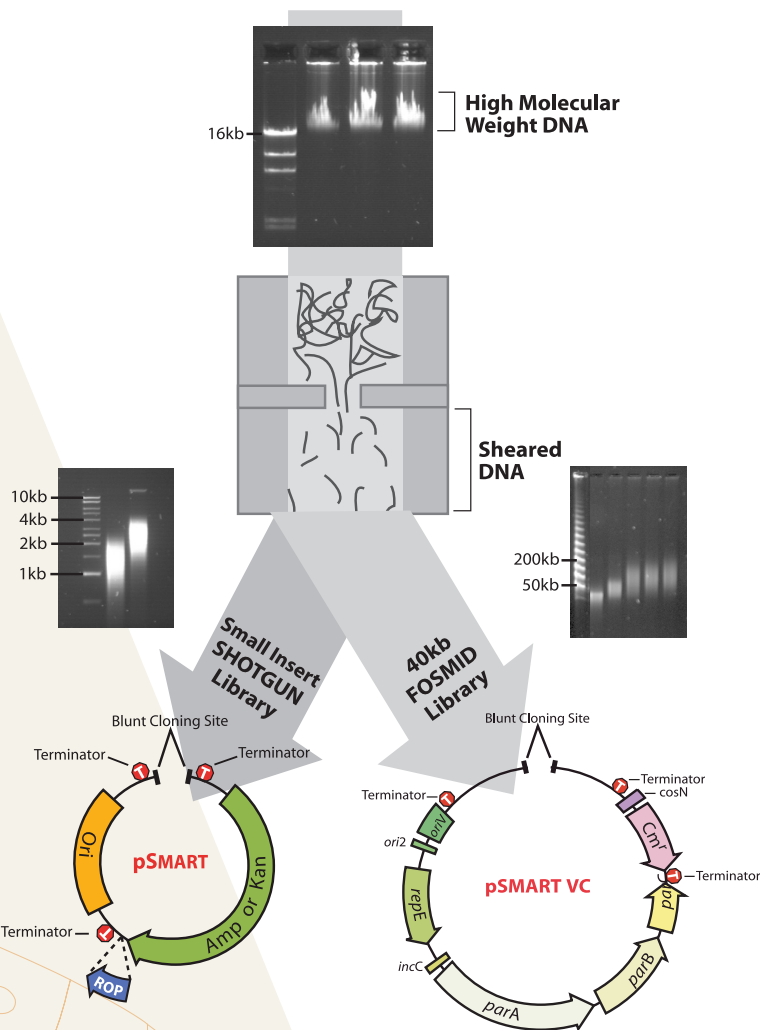


Figure 1. Construction of NanoClone™ and GapFree™ DNA libraries.

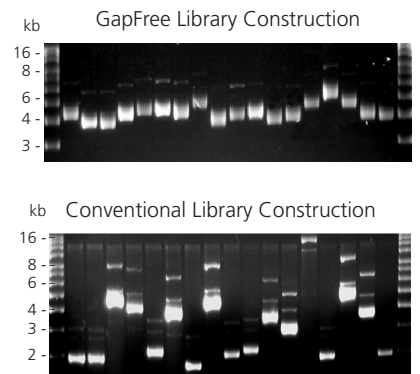


Figure 2. Increased stability of inserts in GapFree Libraries.

In Figure 2, *Streptococcus thermophilus* genomic DNA was Hydroshear™- fragmented to 2-3 kb and end-repaired using Lucigen's DNATerminator® Kit. The fragments were cloned into Lucigen's pSMART LCKan vector. Lucigen's GapFree library construction techniques resulted in approximately 1,000-fold more colonies with a dramatic increase in clone stability (upper panel), as compared to standard library construction methods (lower panel).

NanoClone technology likewise relies on the pSMART vectors, but it also employs a novel amplification process that allows successful cloning and library construction from as little as 1 nanogram of DNA. This technique has been used to clone trace amounts of DNA from

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numerous sources, including DNA that was unclonable by other techniques (Table 1; see also Breitbart, M. *et al.*, 2002, Genomic analysis of uncultured marine viral communities, Proc. Natl. Acad. Sci. USA **99**, 14250-14255).

Table 1. NanoClone libraries constructed from trace amounts of target DNA.

Sample	Amount of Starting DNA	CFUs per Library	Insert Sizes	Background
Inoue-Melnick virus	<1 ng	1 x 10 ⁵	1-2 kb	<1%
Cultured cyanophage	1 ng	1 x 10 ⁵	1-2 kb	<1%
Gel-isolated chromosomes				
Rat Y chromosome	<1 ng	1 x 10 ⁵	1-2 kb	<1%
<i>Pneumocystis carinii</i>	1 ng	1 x 10 ⁶	1-2 kb	<1%
<i>Pseudomonas</i> sp	50 ng	1 x 10 ⁶	1-2 kb	<1%
Uncultured freshwater phage	10 ng	1 x 10 ⁵	3-6 kb	~1%
Uncultured marine phage	100 ng	1 x 10 ⁶	1-2 kb	<1%
Cultured Roseophage	100 ng	1 x 10 ⁶	1-2 kb	<1%
Exiguobacterium	200 ng	5 x 10 ⁵	1-2 kb	<1%
Streptococcus	200 ng	5 x 10 ⁵	2-4 kb	<1%

GapFree and NanoClone cloning technologies, incorporating Lucigen's pSMART vectors and high efficiency *E. cloni*TM Electrocompetent Cells, provide the only reliable procedure to construct complex libraries from trace amounts of DNA or difficult sequences.

TransformanceTM Custom Competent Cell Service

For applications that require specialized competent cells, Lucigen's Transformance service produces electrocompetent or chemically competent cells from any strain of *E. coli*. Transformance processing incorporates the same proprietary methods used for manufacturing Lucigen's own *E. cloni* high efficiency competent cells, so optimal results are assured. Examples of competent cells produced by the Transformance procedure are shown in Table 2.

Table 2. Efficiencies of Transformance custom competent cells

Starting Sample	Resulting Transformation Efficiency (cfu/μg)
Strain "A"	2.5 x 10 ⁹
Strain "B"	1.8 x 10 ¹⁰
Strain "C"	2.4 x 10 ¹⁰
Strain "D"	3.0 x 10 ¹⁰

Transformance custom competent cells can be provided in small or large quantities. Each lot is quality controlled to assure high transformation efficiency and performance.

Contact Lucigen for a free quote on a custom service. ■

