

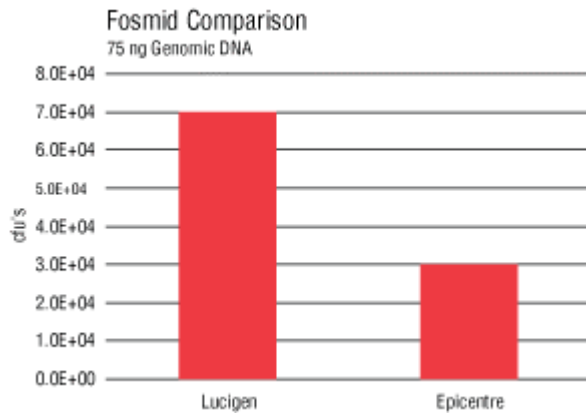
## CopyRight® v2.0 Fosmid Cloning Kit

**NEW! Better performance than any other fosmid cloning system**

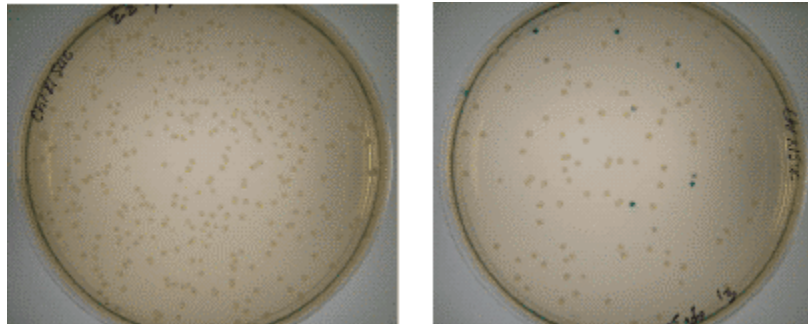
Lucigen's CopyRight v2.0 Fosmid Cloning Kit with the new pSMART® FOS vector and high efficiency Replicator™ FOS strain is the most advanced fosmid cloning system developed. The CopyRight v2.0 Fosmid Kit yields many more recombinants from less starting DNA, and the resulting fosmid libraries are complete, without missing clones. False-negatives and false-positives are dramatically reduced, and empty-vector background is essentially eliminated. Controllable genetic elements in the pSMART FOS vector and Replicator FOS cells allow amplification of vector copy number for high plasmid yields.

### CopyRight v2.0 Fosmid Kit Advantages

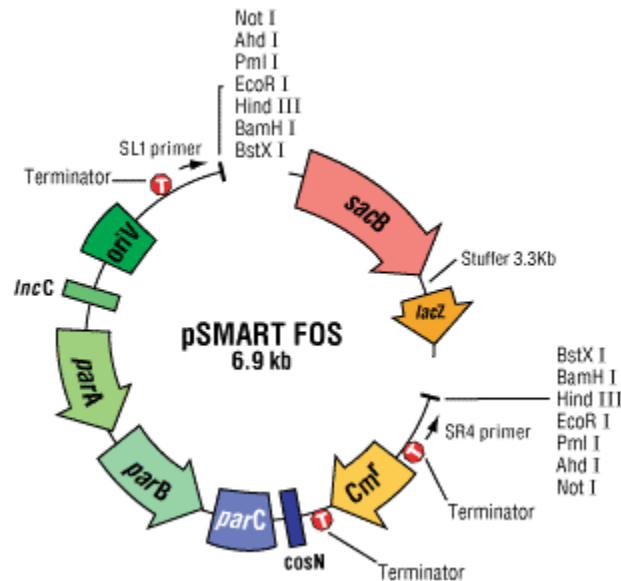
- **More recombinants from less DNA.** Conventional fosmid cloning systems have lower cloning efficiencies, requiring more target DNA, which can be difficult to obtain. The ultra-efficient CopyRight v2.0 Fosmid Kit gives high recombinant yields even when the amount of DNA is very limited (Figure 1).
- **Zero background.** A positive selection system and unique vector design eliminates empty-clone background for increased efficiency in robotic colony picking (Figure 2).
- Dramatically reduced false positives/negatives. The pSMART FOS vector contains a lacZ/sacB stuffer region that is removed during processing (Figure 3). The sacB gene is lethal to E. coli in the presence of 5% sucrose. Therefore, background of uncut vector can be detected or selected against without transcription of the insert sequence, for increased cloning efficiency.
- **No lost clones.** Conventional fosmid vectors are prone to instability due to transcriptional interference between the vector and the insert. The pSMART FOS vector incorporates Lucigen's patented\* CloneSmart® transcription terminators that prevent transcription both into and out of the insert DNA (Figure 3). Unlike other fosmid vectors, the marker and direct selection genes, as well as their promoters, are completely removed from the final vector preparation, eliminating the risk of clone loss. To further protect against unwanted transcription, the pSMART FOS vector has the chloramphenicol promoter facing away from the cloning site. This design effectively eliminates the cloning bias inherent in conventional vectors. As a result, fosmid libraries constructed in the pSMART FOS vector are complete, without missing clones or deleted sequences.
- **High DNA yields.** The CopyRight v2.0 Fosmid vector and Replicator FOS cells feature inducible amplification of copy number\*\*, increasing yields to as many as 50 copies per cell. CopyRight amplification is more robust than the similar CopyControl system (Figure 4) and permits easy isolation of plasmid DNA for sequencing, subcloning, or restriction mapping.
- **Better price and value!** Conventional fosmid kits often include unnecessary reagents and the Lambda packaging extract, which may not be optimal. CopyRight v2.0 Fosmid Kits are economically designed and priced to provide the best value available. The Kits can be used with Lambda packaging extracts from any vendor of your choice.



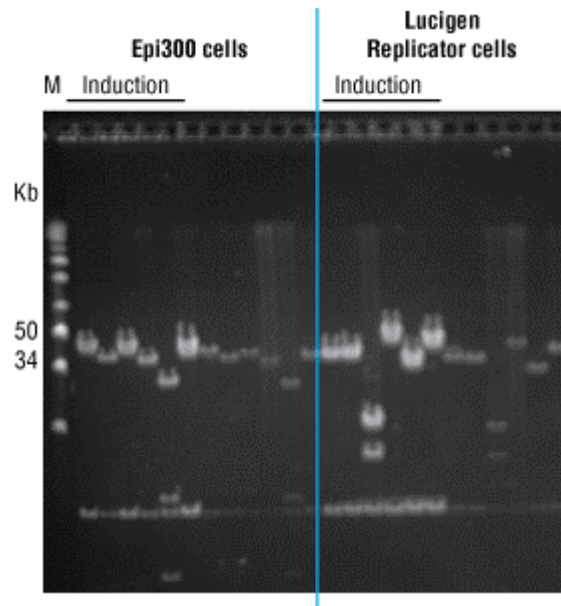
**Figure 1.** Higher recombinant yields. With a limiting amount of DNA, more than 2-fold more recombinants were obtained with the CopyRight v2.0 Fosmid Kit, as compared to a conventional fosmid cloning kit (CopyControl™ Fosmid Library Production Kit, Epicentre).



**Figure 2.** Zero background with the pSMART FOS vector. A fosmid library was constructed in either the pSMART FOS vector or the CopyControl™ pCC1FOS™ vector (Epicentre), packaged, infected into the same cell strain (EPI300™ cells, Epicentre), and plated. All colonies were recombinant with the pSMART FOS vector (left panel). However, the Epicentre vector (right panel) showed a significant background.



**Figure 3.** CopyRight v2.0 pSMART FOS vector. *sacB*, sucrase gene; *lacZ*, *lacZ* alpha peptide gene; *Cmr*- chloramphenicol resistance gene; *cosN* - lambda packaging signal; *par* A,B,C- partition genes; *ori2*, *repE* [not shown in drawing], *IncC* - origin of replication (single copy); *oriV* - inducible origin of replication. Approximate positions of sequencing primers and transcription terminators (T) are indicated. The *lacZ/sacB* stuffer region is completely removed during vector processing.



**Figure 4.** Copy number amplification. Copy amplification of fosmid clones from the CopyRight v2.0 Fosmid Kit (Replicator cells) was more consistent and robust than the similar amplification system in the Epicentre CopyControl Fosmid kit (EPI300 cells)

### CopyRight 2.0 pSMART FOS vector features:

- Single-copy replication origin and inducible medium-copy replication origin
- Transcription terminators to stabilize recombinant clones
- Transcription/translation-free cloning for unstable DNAs
- lacZ/sacB stuffer that is completely removed for minimal background and no bias
- Bacteriophage Lambda cos site for lambda packaging or terminase cleavage
- loxP site for Cre-recombinase recognition
- Rare-cutting restriction sites on either side of insert
- Chloramphenicol-resistance gene

### Replicator FOS Strain

Lucigen's Replicator FOS strain contains an inducible *trfA* gene, which is required for amplification of the pSMART FOS clones to high copy number. Replicator FOS cells are ideal for cloning and propagation of fosmid or plasmid clones. These cells contain the *endA1* and *recA1* mutations for high yield and high quality plasmid DNA. The *mcr* and *mrr* mutations allow cloning of methylated genomic DNA isolated directly from mammalian or plant cells to be cloned without deletions or rearrangements. They do not contain the F' plasmid.

**Replicator FOS genotype:**  $F^-$  *mcrA*  $\Delta(mrr-hsdRMS-mcrBC)$  *endA1* *recA1*  $\Phi 80dlacZ\Delta M15$   $\Delta lacX74$  *ara* $\Delta 139$   $\Delta ara, leu)7697$  *galU* *galK* *rpsL* *nupG* (*attL* *araC*-PBAD-*trfA250* *bla* *attR*)  $\lambda^-$ .

### DNATerminator® End Repair Kit

CopyRight v2.0 Fosmid Cloning Kits include Lucigen's high efficiency DNA Terminator Kit, which provides up to a 5-fold increase in cloning efficiency of fragments generated by physical shearing or restriction digestion.

[CopyRight v2.0 Fosmid Cloning Kit Manual](#)