

Ask Frank

by Fred and Hank



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Questions about CopyControl™ Cloning Products

Q: Why is maintaining clones at single copy so important?

A: Insert stability can be a significant problem when creating genomic libraries or cloning PCR products. When present at high-copy number inside the *E. coli* host, insert DNA containing repeats or regions with high AT or GC content are susceptible to recombination events. Also, some cloned genes express proteins that are toxic to *E. coli* (See page 20) but may be tolerated by the host at single copy. By maintaining clones at single copy, biases against specific DNA sequences and toxic gene products can be minimized or eliminated.

Q: How do CopyControl™ clones switch from single copy to high copy?

A: CopyControl™ Vectors have two origins of replication – a single-copy F-factor *ori* and an *oriV*. Multi-copy replication from *oriV* requires the *trfA* gene product, which is induced in TransforMax™ EPI300™ *E. coli* by adding CopyControl™ Induction Solution to the growth medium. Activation is very fast and replication continues from the *oriV* as long as the CopyControl inducer is present in the culture.

Q: Why is it important to control the length of time that clones are induced?

A: Clones should be induced for only a few hours before harvesting the cells and isolating the cloned DNA. If induction goes for too long, the stability problems that the CopyControl™ Cloning Systems are designed to eliminate can occur (deletions, rearrangements, sick/weak cells, etc.).

Q: What are the optimal insert sizes for the CopyControl™ Kits?

A: The optimal insert sizes vary for each of the three CopyControl Cloning Kits:

With the CopyControl™ PCR Cloning Kit, any PCR product or restriction fragment up to 15 kb can be reliably inserted. Success with clones up to 20 kb has been reported.

For the CopyControl™ Fosmid Library Production Kit the optimal insert size is between 30 and 45 kb. Inserts in this size range, ligated into the 8.1-kb pCC1FOS™ vector, give the appropriate amount of DNA that can be packaged into lambda phage heads. This kit includes the extremely efficient MaxPlax™ Lambda Packaging Extracts. Because lambda only packages DNA in a specific size range, this method produces virtually no “background” or recircularized, “vector-only” clones.

The CopyControl™ BAC Cloning Kits, with their highly purified, linearized, and dephosphorylated pCC1BAC™ vector, are used to produce very large clones for making genomic libraries with insert sizes up to 250 kb.

Q: Can I use the CopyControl PCR Cloning Kit with any PCR product?

A: Yes, or with any DNA fragment. The CopyControl PCR Cloning Kit contains end-repair reagents that will blunt-end any DNA fragment, including the 3'-A's on some PCR products. This allows “universal” cloning into the pCC1™ (Blunt Cloning-Ready) Vector. PCR products generated with proofreading enzymes, like *Pfu* DNA polymerase, have blunt ends and can be cloned directly into this pCC1 vector.

Q: What is the difference between a fosmid and a cosmid?

A: Fosmids and cosmids differ in their copy number. Cosmids are multi-copy vectors that generally have a high-copy origin of replication (such as the ColE1 replicon) and are normally present at

anywhere from 20-70 copies per cell. Fosmids contain the origin and partitioning genes from the F'-episome of *E. coli*, which keeps the clone at single copy. The CopyControl pCC1FOS™ Vector also contains the inducible multi-copy *oriV*.

Q: Why are libraries made with the CopyControl Fosmid Kit unbiased?

A: There are two reasons. First, the CopyControl Fosmid Library Production Kit uses sheared DNA, rather than restriction fragments, to generate DNA of the appropriate size. Shearing DNA is much more random than the distribution of restriction enzyme sites. Secondly, having all clones present at single copy increases the likelihood that ALL sequences will be represented. Some DNA sequences, if cloned into a high-copy vector like a cosmid, are unstable and, thus, are not represented in the library.

Q: How do the CopyControl BAC Cloning Kits reduce the time needed to construct a BAC library?

A: The CopyControl BAC Cloning kits reduce the time required to make BAC libraries by providing high-quality, “ready-to-go” reagents. The highly purified, linearized, and dephosphorylated cloning-ready BAC vectors save time in preparing cloning vectors. The optimized ligation reaction, using the proven Fast-Link™ DNA Ligase, takes only a fraction of the time usually required. A set of screening reagents facilitates rapid screening and sizing of BAC clones, and a simple “induction protocol” allows increased yields of BAC DNA (10-20 copies) for improved BAC DNA purification. The CopyControl BAC Cloning kits and vectors are available in three cloning formats: *Bam*HI Cloning-Ready, *Eco*RI Cloning-Ready, and *Hind*III Cloning-Ready.