FAQ's about CopyControl ™ products

(CopyControl PCR Cloning Kits, CopyControl BAC-cloning Kits, CopyControl Fosmid Library Production Kit)

CopyControl PCR Cloning Kits

Can I clone restriction fragments that I gel-purified using the CopyControl PCR Cloning Kit?

Can I use the CopyControl PCR Cloning Kits with ANY PCR product?

Can you explain to me how the induction works?

When I induce the cloned DNA to amplify it, what is the fidelity of the amplification?

How big a fragment can I clone with the CopyControl PCR Cloning Kit?

Will my cloning efficiency using the EPI300 chemically-competent cells be as high as when I use the electrocompetent cells?

What is the optimal size of DNA I can clone?

Can I induce the clone to high copy number right on the agar selection plates?

What is the inducer?

How well will the clone amplify if there are a lot of repeats in my cloned insert?

I got my CopyControl PCR clone just fine, but when I tried to induce it in a 1 ml culture, I got very little amplification (at most 71%) and the results from inducing 10 different colonies in 1 ml culture were very inconsistent. Why is the induction so bad and why so variable?

I can't get the induction to work. I tried using the Control Insert as you recommend and I get plenty of clones when I transform and screen on Chloramphenicol plates as you describe. When I try to induce the control

clone I get not increase in DNA yields at all. Are you sure that this technology works as you say?

CopyControl BAC-cloning Kits

How many clones must I screen to get a complete library for my organism?

What is the best way to prepare my DNA for cloning and making a BAC library?

Are there any published references out in the literature about using this kit?

CopyControl Fosmid Library Production Kit

How many clones do I need to have to get a true representation of by organisms's genome?

How much Chloramphenicol should I add to the medium for screening my primary library?

Do I need to add Maltose and magnesium to the medium to make the phage adsorb better to the cells?

Why is this kit better than the pWEB Cosmid Kit? What's the difference?

I already have a Fosmid Library made with your EpiFOS Kit. Is it possible to just transform my Fosmid clone I have into the EPI300 cells and induce it to higher copy number?

I gel-purified my DNA the way you said in the (old) product literature but I didn't get any clones at all. Why?

I only get a very limited number of colonies, and my control T7 reaction only gave me a few plaques, but my ligated lambda control gave me lots of good plaques. What is the problem?

I got almost no clones and my ligated lambda control and my T7 ligation control gave no plaques at all. What's wrong?

I can't get my clones to induce reproducibly. I tested ten different clones and did not get a consistent number of copies per cell in my clones. Why is this?

How would I check whether the fosmids are induced to multiple copy numbers? Is there any method to check the copy number?

Can I clone restriction fragments that I gel-purified using the CopyControl PCR Cloning Kit?

Yes you can, quite easily. Make sure that the fragments were not exposed to UV/Ethidium bromide for best results.

Can I use the CopyControl PCR Cloning Kits with ANY PCR product?

Yes, almost any. We've been successful with longer PCR products (15 kb) and those that are "difficult to clone".

Can you explain to me how the induction works?

By exposing the bacterial clone to a simple sugar, the cells take up the sugar which induces the trfA gene to start working. This in turn causes the oriV to function and start replicating the plasmid clone inside the cell. Depending on the cloned DNA and length of induction, you can get as much as 25-50x increase in the plasmid DNA yield from the cells. Aeration of the induced clones will improve the copy number induction. In general, the better the aeration, the more clone DNA you get.

When I induce the cloned DNA to amplify it, what is the fidelity of the amplification?

The "amplification" is not the same concept here as in PCR and thus "fidelity" questions are irrelevant. Each plasmid inside the cells is a carbon copy of the original, no mutations are introduced.

How big a fragment can I clone with the CopyControl PCR Cloning Kit? We say 15 kb in our marketing literature but we've been able to clone 20 kb on occasion.

How small a fragment can I clone with the CopyControl PCR Cloning Kit?
The limit is dependent on the size of DNA you can easily resolve by electrophoresis. Remember that the pCC1 vector is >8.1 kb, so you will need to be able to distinguish between recircularized vector and vector containing an insert.

Will my cloning efficiency using the EPI300 chemically-competent cells be as high as when I use the electrocompetent cells?

No, it will not. Expect at least two orders of magnitude less transformation efficiency in chemically competent cells vs. electrocompetent cells.

What is the optimal size of DNA I can clone?

There is no “optimum”; as said before you can clone reliably up to 15 kb. On the “low end” the smallest size will be determined how well you can differentiate between a re-ligated vector and the vector plus insert (PCR screening works well here).

Can I induce the clone to high copy number right on the agar selection plates?

Yes, and a protocol for this is provided in the tech literature. Just be aware that the cells will be induced almost immediately on the plates containing the inducer, and this may cause problems if the cloned DNA contains “toxic” sequences that would otherwise not be a problem if grown on plates without the inducer.

What is the inducer?

Sorry, can’t say specifically. Proprietary. We can say that it is a simple sugar.

How well will the clone amplify if there are a lot of repeats in my cloned insert?

We have not seen any major issues with deletions and other undesirable recombination events, but this does not rule out the possibility that this can occur. It will depend on the DNA being cloned.

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How many clones do I need to have to get a true representation of by organisms's genome?

The equation for this is provided in the product protocol.

How much Chloramphenicol should I add to the medium for screening my primary library?

We have now added this information to the product lit after getting many calls. We recommend 12.5 µg/ml on the screening plates.

Do I need to add Maltose and magnesium to the medium to make the phage adsorb better to the cells?

It's not necessary, but traditional lambda phage cloning kits indicate that it can help.

Why is this kit better than the pWEB Cosmid Kit? What's the difference?

The CopyControl Fosmid Library Kit combines the best of the pWEB Cosmid Kit (plenty of DNA to recover) with the EpiFOS Fosmid Kit (single copy cloning). You can get a unbiased Fosmid single-copy library and then induce the clones to higher copy so that you can get plenty of DNA for subcloning, sequencing, etc.

I already have a Fosmid Library made with your EpiFOS Kit. Is it possible to just transform my Fosmid clone I have into the EPI300 cells and induce it to higher copy number?

No, you'll need to have the oriV origin of replication in the fosmid in order to be able to induce to higher copy. You can retrofit your fosmid by using the EZ::TN <oriV/KAN-2> Insertion kit, which will then permit you to induce the fosmid to a higher copy number.

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CopyControl BAC-cloning Kits

How many clones must I screen to get a complete library for my organism?

It will depend on how big the genome of the organism is, as well as the average insert size. To determine the 99% confidence level that all sequences in the genome are represented, the following formula is used:
N = ln (1-P) / ln (1-f)
P is the desired probability (expressed as a fraction); f is the proportion of the genome contained in a single clone; and N is the required number of fosmid clones.
For example, the number of clones required to ensure a 99% probability of a given DNA sequence of Human DNA (genome = 3.3 Gb) being contained within a BAC library composed of 125 kb inserts is:
N = ln (1 - 0.99) / ln (1 - [1.25 x 10E5 bases / 3.3 x 10E9 bases]) = -4.61 / -0.000038
N = 121,702 clones

What is the best way to prepare my DNA for cloning and making a BAC library?

Embedding cells into agarose plugs, and following a very involved and time-sensitivie process. Contact Fred or Hank in tech Consulting for customer-friendly documents describing the protocols for creating DNA suitable for BAC Cloning.

Are there any published references out in the literature about using this kit?

Please contact technical support who will be happy to provide you with a list of publications. Also please see the references section on the product page.

CopyControl Fosmid Library Production Kit

How many clones do I need to have to get a true representation of by organisms's genome?

The equation for this is provided in the product protocol.

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I gel-purified my DNA the way you said in the (old) product literature but I didn't get any clones at all. Why?

When I run my T7 control DNA on a gel, it stays very close to the wells rather than migrating along properly. Why is this?
Contact technical support who will advise you

I only get a very limited number of colonies, and my control T7 reaction only gave me a few plaques, but my ligated lambda control gave me lots of good plaques. What is the problem?

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I can't get my clones to induce reproducibly. I tested ten different clones and did not get a consistent number of copies per cell in my clones. Why is this?

Contact technical support who will advise you

How would I check whether the fosmids are induced to multiple copy numbers? Is there any method to check the copy number?

The only real sure-fire way to look at whether or not a Fosmid has been induced is qPCR. In uninduced states, the Fosmids will always be at single copy.
But when induction occurs, the amplification of Fosmids will almost never be uniform and will vary based on the insert sequences and the ORFs contained therein.
There would also need to be a direct comparison with the uninduced Fosmids, too.