

Cloning Secrets Revealed - Tech Tips

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Q: What is the concentration of vector in the PreMix?

A: Vector:insert ratios are important when you're trying to find a trade-off between self-ligation of the vector and formation of concatemers of inserts. But the pSMART™ vectors have such a low background of self-ligation that the only real concern is using too much insert (e.g., >500 ng of 1kb fragments). There's usually no need to do any calculations - just add 50-500 ng of your insert to the CloneSmart ligation reaction. (And if you *must* know, it's 25 ng of vector per ligation.)

Q: Why do I have so many empty vector clones?

A: Most often, pSMART clones that appear to be empty usually have very small inserts (<50 bp). Sequencing a few of these clones will confirm whether they are truly empty or just contain micro-inserts.

So where do these little bits of DNA come from? No purification method is perfect. Even after size-selecting fragments on an agarose gel, oligonucleotides sometimes find their way into the sample. And those oligos are cloned far more efficiently than larger fragments. If you see small inserts, look for ways to reduce the source of the oligos.

Suppose you really do see empty vector clones? First, look at the number of colonies. The background of pSMART vectors in a typical ligation/transformation is less than 50 cfu per 100 µl of cells plated. If you're getting only 100 cfu from 100 µl of cells, your recombinant clones are not being created efficiently (usually due to bad ligation or a poorly tolerated clone). In this case, use the DNATerminator® Kit to maximize blunt ends on your inserts, or switch to a pSMART vector with lower copy number.

If you're getting hundreds of clones on your plate, and sequencing shows no insert at all, you've probably gotten some of those end-repair enzymes into your ligation reaction. And that means the vector is going to self-ligate!

Finding the source of the background will let you know what parts of your protocol to correct.

Q. What vector should I use for a 5 kb insert?

A. A quick guide:
Little insert (<5 kb) – use pSMART HC Kan.
Bigger insert (~4 -12 kb) – use pSMART LC Kan.
Nastiest insert (~8 - 150 kb) – use pSMART VC.

And don't even think about *lacZ* or ampicillin resistant plasmids unless you have a good reason.

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Here's the trouble: Blue/white screening misses a large fraction of clones that either grow as blue colonies or don't grow at all. Inserts in amp^R plasmids are often unstable, probably due to secretion of the beta-lactamase enzyme, which quickly degrades all the ampicillin in the medium. You've seen the satellite colonies on amp plates; these can also form in liquid medium and take over your culture. This doesn't happen with kanamycin or chloramphenicol selection.

Sometimes, though, you need to break these rules. If you'd like to express an insert, the lacZ α promoter in Lucigen's pEZSeq™ vector will work well. Or, if you'll be doing mutagenesis with a kan^R transposon, you'll need to use the amp^R version of the pSMART vector. These vectors retain all the other advantages of CloneSmart cloning, so they are still far better than using pUC-type vectors. ■

