Plasmid Handling

Clones, vectors and host strains are shipped as bacterial stabs or yeast slants in a small vial of agar media containing the appropriate selective agent. We recommend streaking out the stocks on an agar plate containing the appropriate selective agent as soon as possible after you receive your order.

If a stock does not grow when struck out from the stab or slant you received, check that you have used the correct selective agent at an appropriate concentration. We recommend using no more than 25 µg/ml kanamycin for full-length cDNA clones in pUNI and Gateway® vectors and for BAC clones from the P1 and TAC libraries. If you continue to see no growth, contact us.

Select several colonies from the agar plate on which you struck out the stock from a stab or slant culture, to inoculate cultures for verification of the stock you have received.

Use an appropriate method such as restriction analysis, PCR amplification, or sequencing of the insert to verify stocks.

Once you have obtained a verified culture, make a glycerol stock or isolate a sample of plasmid DNA for long-term storage. This is important as stabs received from ABRC may have spent several weeks in shipping and were not stored under ideal conditions during this time.

It is not possible for us to verify every stock before distribution to the community, so we rely on feedback from researchers to identify problems with stocks. If you verify or sequence a stock, please submit your results to ABRC.

If you are unable to verify a stock, check that you have used the correct selective agent at an appropriate concentration and that you have analyzed several colonies derived from the original culture. If possible, use more than one method of verification, such as PCR amplification and restriction analysis. Please report continuing problems to ABRC as soon as possible.

Some common problems with clones

1. A cDNA clone is not full length.

Not all clones in our collection represent full-length transcripts. Check the stock details on our web site to determine whether this stock represents a full-length clone.

Many clones in our collection were determined to represent full-length transcripts based on previous versions of the genome annotation. If sequence of a clone is available, compare it with the sequence of the current annotation of the gene.

If the sequence of the stock appears to represent a full-length transcript, but your verification suggests that the stock you received is not full-length, or is incorrect, please contact us. We will check our distribution stock and provide you with a replacement if possible.

2. A full-length pUNI clone appears to lack an insert.

We have documented some cases of these clones losing their inserts, but they appear to be relatively rare. Restriction analysis using SfiI is not always a reliable method of dropping out the insert, because the SfiI sites may be methylated. We recommend using HindIII and EcoRI. In cases where the insert really is lost, we find that we can maximize the chances of finding and insert by using a culture that is as fresh as possible and checking several colonies.

3. A restriction digest or sequencing indicates that you have received the wrong plasmid.

For clones such as BACs that were received and have been maintained in 384 well microtiter plates, or clones form high throughput collections, there is always some possibility that the stock we provided was selected from the wrong well, or that cross contamination between wells occurred. In some cases, checking several colonies will yield the correct clone. In others, we are able to supply a new stock derived from an archived stock that represents the correct clone.

4. Sequence of a BAC clone is similar, but does not correspond exactly, to the sequence submitted to GenBank, or the insert size is incorrect based on the sequence submitted to GenBank.

The sequence assigned to the BAC clones in our collection is actually the sequence of the annotation unit, rather than the clone. In some cases, sequence from am neighboring clones was added to the annotation unit, in others sequence was trimmed. The GenBank record for the sequence notes where this is the case.

If you need additional information about plasmids, including maps and protocols, please contact ABRC.