User Instructions: Glycerol Stock

Retrieve Vector Information

Your vector has a unique vector ID that is printed on the glycerol stock tube. You can use this ID to retrieve the vector map, vector sequence and vector component annotation through "Retrieve Vector Information" link on VectorBuilder's homepage (www.vectorbuilder.com).

Storage

The vector is shipped at ambient temperature as E. coli glycerol stock (15% glycerol). **We highly recommend that you inoculate LB liquid culture before freezing the vial.** Please do a flash spin before opening the tube to spin down residual liquid from the lid to avoid cross contamination.

The glycerol stock can be put in -80°C for long-term storage. We also recommend that you make a separate glycerol stock as a backup. The glycerol stock should be placed in 4°C for short-term storage (≤2 weeks) only.

Inoculation

The glycerol stock comes from a single clone. You can use it to directly inoculate LB liquid culture. However, in some cases, this approach could result in low DNA yield from the liquid culture. This problem typically goes away if you streak the E. coli on a plate first, and while the colonies are still fresh, use one colony to inoculate liquid culture. If the DNA yield is still low, please do a re-transformation, and pick a single colony to inoculate new LB liquid culture. If you do use a single colony derived from the original glycerol stock for subsequent work, there is a very small probability that this colony would acquire mutations relative to the parent stock. We therefore recommend that you validate the correctness of the colony (or a few colonies) by sequencing and restriction digest before proceeding with further experiments.

To inoculate from an unfrozen glycerol stock, simply aspirate one microliter of the stock into liquid LB medium supplemented with appropriate antibiotics. If inoculating from a frozen glycerol stock, scrape down a tiny chunk of the frozen stock using a sterile pipette tip, and carefully throw the tip into liquid LB medium. You can also streak an LB plate first and pick a single colony to inoculate LB liquid culture.

Some vectors, especially those with large sizes, repetitive sequences or unusual GC content, might have the tendency to undergo rearrangements such as deletions. One way to reduce this possibility is to grow the E. coli at 30°C on plate and in liquid culture instead of the standard 37°C.

Antibiotic Concentration

The antibiotic resistance of the vector is printed on the glycerol stock tube. Please use the antibiotic in LB plate or liquid culture at the recommended concentration below.

Ampicillin: 100 μg/ml
Kanamycin: 50 μg/ml
Chloramphenicol: 34 μg/ml
Tetracycline: 5 μg/ml
Streptomycin: 50 μg/ml
Gentamycin: 10 μg/ml

Host Strain

Please pay attention to the E. coli host strain information printed on the glycerol stock tube. Most vectors are constructed in Stbl3 host to ensure sequence stability. Some applications require the use of other hosts. For example, recombinant protein expression from pET vectors typically requires E. coli hosts carrying the T7 RNA polymerase gene, such as BL21(DE3). In such cases, vectors from the Stbl3 host need to be retransformed into the appropriate hosts.

Special Notice

VectorBuilder has 100% sequence guarantee for vectors constructed by us. If you decide to perform independent sequence validation, it is highly recommended to use Sanger sequencing which is the gold standard assay. In case of sequence error called by next-generation sequencing (NGS) or Nanopore sequencing, please double check the results by Sanger sequencing before contacting us for post-sales support. We have recently noticed high error rate associated with Nanopore sequencing from a number of cases.

Troubleshooting: Low Yields of Plasmid DNA

Possible Cause	Recommendation and Solution
Inoculum size is too small	Increase the inoculum size of glycerol stock usually could lead to a better plasmid yield.
Plasmid's copy number are varied	The origin of replication on the plasmid could inherent different types of copy number. Some plasmid vectors manufactured by VectorBuilder are medium- or low-copied type therefore their plasmid replicates less frequently in E. coli than the high-copy plasmid dose. To prepare low-copy plasmids, increase the amount of E. coli culture for the prep in order to obtain satisfying DNA yield.
Antibiotic issues	Correct use of antibiotics in E. coli culture is essential to obtain high plasmid yield. First of all, please read through the label on your glycerol stock tube and make sure that you are using the antibiotic corresponded to the antibiotic resistance gene on your plasmid. Misusage of antibiotics can inhibit bacterial propagation. Secondly, some antibiotics, such as ampicillin, degrade fast in liquid culture. As a result, bacteria that do not contain plasmids can propagate to a significant fraction of the culture, causing poor yield of the plasmid prep. To avoid this, please prepare ampicillin-containing growth medium freshly before use and make sure that enough ampicillin is supplied. Also, when culturing ampicillin-resistant bacteria, do not let the liquid culture saturate for too long before harvesting.
$EndA^+$ strain	$EndA^+$ strain retains the ability to express EndA endonuclease which causes degradation of plasmid DNA in your plasmid prep product. To remove endonuclease, apply extra wash on your plasmid prep column during the DNA clean-up step or preferably use an $EndA^-$ strain for cloning.
Microbial contamination	If you need to grow your E. coli in an antibiotic-free environment, please be very cautious that microbial contamination occurs. Microbials without transformed plasmids tend to propagate faster and become dominated in your bacteria culture, since plasmid replication consumes energy against cell division. If contamination already happen, please clean your bench and orbital shaker with 75% ethanol. Make sure your culture flask and pipet tips are well sterilized. You would also need to screen the correct strain colonies on agar plates with certain antibiotics.
Phage contamination	Phage contamination could cause cell lysis in bacteria culture. If phage contamination happens, please throw out the contaminated culture immediately. Discard all solutions that were used for preparing bacteria culture. Clean your bench surface and orbital shaker with 0.05% sodium hydroxide solution thoughtfully. All of your culture flask used for bacteria propagation should also be cleaned with sodium hydroxide solution by soaking and then autoclaved. Likewise, E. coli strains with fhuA mutation such as VB UltraStable™ chemically competent cells are preferred for cloning since they display resistance to T1 phage.

The liquid culture is directly inoculated from E. coli stock	Direct inoculation of a liquid culture from the E. coli liquid stock or stab culture you have received from VectorBuilder can very occasionally result in low yield. We recommend streaking the stock onto an LB agar plate containing the appropriate antibiotic first, and then inoculating a liquid culture with a fresh colony growing from that plate.
Inadequate bacteria culture used for plasmid extraction	Please check the binding capacity of your plasmid prep column and whether your plasmid is a high- or low-copy plasmid. For mini preps, we recommend using 1-5 ml of overnight bacterial culture. For maxi preps, if the plasmid is a high-copy type, we recommend using 100-150 ml of overnight bacterial culture; if the plasmid is a low-copy type, we recommend using 300-500 ml of overnight bacterial culture. Typically, for high-copy plasmids, ~5 ug of plasmid DNA can be extracted from every 1 ml of culture in mini prep and ~500 ug of plasmid DNA can be obtained from 150 ml of culture; for low-copy plasmids (e.g. pET), 1.5-2.5 ug of plasmid DNA can be harvested from every 1 ml of culture in mini prep and 150-200 ug of plasmid DNA can be obtained from 150 ml of culture.
You have not carefully followed the manual of the plasmid prep kit	If you use a plasmid prep kit, please carefully read the manual before use. Improper operations can often lead to poor performance of the kit.
Low-quality plasmid extraction kit	Some brands of plasmid DNA prep columns perform poorly or inconsistently for DNA preparation.