Lentiviral System FAQ

How should I store my lentivirus?

Aliquots should be made for the lentivirus and stored at -70 degrees Celsius.

How do I calculate the MOI?

MOI = Product Titer (IU/ml) x Virus Volume (ml) / Total Cell Number

Why is my lentiviral infection of lower efficiency than transduction?

There are many factors including insert size, insert sequence (specific sequence), and bacteria strain used for viral vector production, that can affect lentivirus packaging titer. No on can predict for sure which inserts will work better. Transduction can have higher transduction efficiency and higher level of gene expression as over 100 copies of DNA can get into cells. But only about 1% transduced cells integrated into host cells. Lentivirus efficiency can often be lower than transfection and expression level are also lower than transfection expression due to only 1-10 copies of viral particles getting into cells. In addition, the efficiency is also cell line dependent. We have documented well that lentiviruses can easily transduce 293 and MDA-MB-468 cells. Please test those two cell lines to be sure whether it is a titer issue or perhaps that this particular cell line is difficult for lentivirus to transduce.

What starting range of puromycin should I use for the antibiotic selection?

The concentration must be optimized case-by-case and is cell type dependent. On average, typical selection amounts are around 0.1 to 0.5 ug per ml.

What is the cell density that the customer should use for lentivirus infection?

20-30%

For the GIII third generation lentiviral vectors, are there recombination sites flanking the insert sequence?

Yes, there is an extra ~125bp to either side of the CDS insert. Thus, if EcoRV is used, the band will be the insert size plus 250bp.

Are they high copy or low copy plasmids? What is expected plasmid DNA yield?

These are high copy plasmids and should be propagated with an authentic DH5a strain. We typically see a yield of 300-500ug DNA from a 250mL culture.

What is your HA tag sequence?

5'-tacccatacgacgtcccagactacgct-3'

Do you have viral particles for this product?

We offer custom lentivirus packaging services for a range of viral titers. Please go to the following link for more details: http://www.abmgood.com/Custom-Lentivirus-Subcloning-Services.html

What is the difference between the pLenti-III and the pLenti-GIII vectors?

The difference is only in the availability of cloning sites for insertion of the gene.

I also need an empty vector as a control, how do I go about ordering this?

Please see our listings of available blank control vectors: http://www.abmgood.com/Lentiviral-Blank-Control-Vectors.html

We can also produce custom control vectors upon request, please email technical@abmgood.com with specific inquiries

Do your pLenti vectors include a chimeric RSV promoter upstream of the 5' LTR?

Yes, our pLenti vectors do include this RSV chimeric promoter sequence

How are your DNA pLenti vectors supplied?

DNA is supplied in 10mM Tris (unless otherwise requested) and intended for direct transformation in DH5alpha cells.

Which packaging system should I use, 2nd or 3rd generation?

All of abm's lentviral vectors are compatible with 2nd and 3rd generation packaging systems LV003 and LV053 available from abm. Please note, only packaging mixes produced by abm have been tested in house and therefore carry our guarantee for high titer virus production. If it is desirable to use other packaging plasmids obtained from a different source, the compatibility must be tested and determined by the end user.

Can you please advice on a protocol for vector extraction from filter paper?

1) Cut out the circle on the filter paper (this is where the DNA will be spotted), and then cut it into tiny pieces into a 1.5ml tube.

2) Put 30-50ul of PCR-grade water, 10mM Tris, or TE buffer to elute the DNA from the filter paper. The liquid may be completely soaked up by the filter, this is OK. Cap the tube and leave at room temperature for 5 minutes.
3) Centrifuge the tube at high speed for 1 minute to collect the liquid, or use a pipette and "compress" the filter paper at the bottom of the tube to squeeze out as much liquid as possible. Collect the liquid in a new tube, and then use DH5a competent cells to amplify the plasmid, use the suitable antibiotic to select for positive clones.

Where is the polyA signal in your vectors?

An SV40 poly A signal is located at the end of the 3'LTR. It is designed this way to ensure a high amount of transcriptional RNA is present so that a high viral titer is obtained. Having a polyA signal upstream of the 3'LTR can cause premature RNA transcription and reduce the amount of transductionally active RNA. Lentivirus is an RNA virus and having a polyA signal may affect viral packaging, thus it is removed from the expression cassette and placed at the end of the 3'LTR.

We have received positive feedback from previous customers in regards to the high and stable expression levels of our lentivectors.

What is the volume supplied?

Normally the concentration of our vectors are at 0.1ug/ul so there should be around 10ul in terms of volume.

Are design primers with a Kozak sequence required or is ATG enough?

Yes, a Kozak sequence immediately upstream of the ATG is required to ensure proper protein translation. This is true for all mammalian expression vectors that we offer.
Please note that blank vector/viruses do not express anything so the kozak sequence will be absent.

Which lentiviral genes were removed from the viral vector to render it replication deficient?

The gag, pol, rev, env, vif, vpr, tat, vpu, and nef elements involved in lentiviral replication are not present in our lentiviral systems.

Does your lentiviral system contain the WPRE gene? If so, is this the wildtype WPRE sequence?

The WPRE section is present in our lentiviral system and it is the standard wildtype version. You may align the wildtype sequence against our listed vector sequence.