Lentiviral Production FAQ

Where can I obtain lentiviral vector plasmids?

- www.addgene.com, RNAi knockdown plasmids can be obtained at openbiosystems. (eg. For MYC https://www.openbiosystems.com/Query/?i=0&q=MYC).

How should I prepare my vector plasmid DNA in preparation for submission?

- We recommend using a maxiprep kit to obtain better purity than miniprep kits. We have had good success using Invitrogen PureLink HiPure Plasmid Maxiprep (Invitrogen K2100-06, WSU Central stores 61485).

What E. coli strain should I use?

- Lentiviral vector plasmids have direct repeats (LTRs) and should be grown in an E. coli host suitable for maintaining direct repeats such as Invitrogen Stbl3™. We have had good success using electrocompetent "E. cloni" cells from lucigen (Cat# 60051-2)

How should we quantitate our vector plasmid?

- We use A260 but nanodrop or other methods are fine. However we recommend always confirming the concentration by agarose gel electrophoresis and EtBr stain of 500 ng of DNA. This will identify any inaccuracies resulting from salt or other impurities in the plasmid DNA prep.

What QC should be done before submission?

- We recommend cutting the vector plasmid with at least 1 restriction enzyme and preferably 2-3 enzymes that are good cutters and confirming the fragment sizes are as expected. It is best to use enzymes that cut no more than 2-3 sites in the plasmid. Each digest is with only one enzyme. This can identify any unexpected deletions that may have occurred during cloning or any recombination of LTRs during plasmid preparation. Any deletion in the lentiviral backbone can severely decrease or completely eliminate viral titers even if the transgene cassette is functional. Testing the transgene cassette (for example EGFP by transient transfection) is also a good idea if the vector has not been used before.

When can we drop off our plasmid?

- We ask that samples be dropped off before Thursday at noon for production the following week.

How are vector preparations made?

- We co-transfect 2nd generation lentiviral helper plasmids with your vector plasmid into HEK293 cells. The resulting VSV-G pseudotyped vector virions in cull culture medium are collected, 0.2um filtered and then concentrated by centrifugation. The pelleted virions are resuspended in IMDM medium and then frozen at -80C. We can resuspend in the medium or salt solution of your choice if you provide it with your vector plasmid.

How soon can we pick up our vector preparation after dropping off our vector plasmid?

- If we receive your plasmid before Thursday at noon we typically have the vector preparation ready in 13 days (Wed). Please bring over dry ice to keep your stocks frozen during transport.

How should vector preparations be stored? Can they be refrozen?

- Store at -80C until use. Freeze thawing is not recommended, it can decrease titers ~3-fold.

Why do we have to submit by Thurs and wait 13 days?

- Once we have your plasmid in hand we begin scaling up the HEK293 cells for the transfection to generate vector virions. The transfection occurs the following week. Vectors are concentrated then aliquoted and frozen on the following Friday. Vectors are titered either by transducing a test cell line and performing flow cytometry (or by p24 ELISA if there is no fluorophore the next week). We typically have the titering completed by the Wed, 13 days after submission.

Where can I get basic information about lentiviral vectors?

- http://tronolab.epfl.ch/lentivectors

Can we obtain assistance with lentiviral vector cloning?

- At this time we do not have the resources to help with this.

Can you make Adenoviral, AAV or gammaretroviral vectors?

- At this time we do not have the resources to help with this.