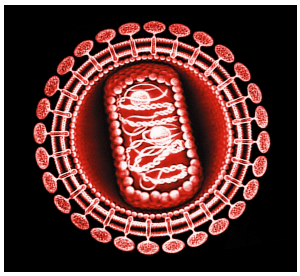
Care and Handling of Retroviruses



It can be useful to think of retroviruses as small cells. They have a membrane, a small amount of cytoplasm and the capsid containing the genome is somewhat like a nucleus. As such they are very unstable and should be treated gently like a cell would be. The half life is ~ 6-10 hours at 37°C.

Recombinant Retroviruses can have very different species and cell type specificities ("tropisms") with respect to their infection capabilities based on the envelope protein used for production.

	(Amphotropic)	(Ecotropic)	(Pantropic*)
Human	+++	N.S.	+++
Mouse	+++	+++	+++
Rat	+++	+++	+++
Monkey	+++	N.S.	+++
Cat	+++	N.S.	+++
Dog	+++	N.S.	+++
Hamster	+	N.S.	+++
Bird	N.S.	N.S.	+++
Fish	N.S.	N.S.	+++
Frog	N.S.	N.S.	+++
Insect	N.S.	N.S.	+++
Mollusk	N.S.	N.S.	+++

***Retrovirus must be packaged with a pantropic envelope protein such as VSVG**

N.S. = Not Suitable

Therefore, it is important to select a virus type that will infect your cells of interest. Retroviruses only infect dividing cells. One way to determine if a Retrovirus infects your cells is to do a literature search or ask other researchers working in your field which virus is best suited to a particular application. If you are not able to determine if a given virus will infect your cells of interest in this way, then you may have to test the infectivity of your cells. The virus core can provide a wide variety of **stock viruses encoding GFP, RFP, Neomycin Resistance or Puromycin Resistance as reporters** for this purpose.

After virus production, there are different methods for purification which may have certain advantages or disadvantages for your downstream applications. Also, it is essential to discuss buffer conditions that best meet your needs. **Virus production is not the same for every transfer plasmid.** Typically there may be a 10-fold range of virus productivities between various plasmid backbones and transgenes.

Quantification of viruses can be done by different methods – those methods are not comparable so they all need to refer to your own application. Concentrations of viral genomes per ml are not comparable with capsid numbers per ml determined by ELISA/Dot Blot or "infectious titers" determined after transduction of cells. The latter one is an absolutely cell-specific value. Please perform a dilution curve with your cells of interest, then you can always correlate those data with titers based on the same method.

Please discuss these issues with the Core Facility before virus production.

DON'T:

1. **Don't expose to environmental extremes** (such as pH, temperature, organic solvents, protein denaturants, strong detergents, or cation chelators such as EDTA). **ALL** liquids the virus is in (dilution buffers , cell culture media, etc.) should be pH= ~7.2. Add 10 mM HEPES if in doubt to buffer the pH.
2. In particular the VSV-G protein, which is on the surface of the virus and is required for infection, is extremely pH sensitive. It loses about 10-fold in infectious activity for each 0.3 unit drop in pH below ~pH 7.0. Although this effect is reversible to some degree the reversibility is time dependent. Because of this pH sensitivity **ALL** liquids the virus is in (dilution buffers , cell culture media, etc.) should be pH= ~7.2. Add 10 mM HEPES if in doubt to buffer the pH.
3. **Don't expose to any condition that disrupts membranes** (such as temperature, organic solvents, strong detergents). This will result in near complete inactivation because an intact viral membrane is required for viral infection.
4. **Don't introduce air into the virus** by vortexing, blowing bubbles and similar operations which result in protein denaturation. Denatured envelope proteins, which are required for infection, are inactive.
5. **Don't dry.** Drying will also result in membrane disruption and near complete inactivation of the virus.
6. **Don't freeze and thaw multiple times**. The titer may drop 2-3 fold (or more) with each freeze-thaw cycle. Therefore it is best to avoid.
7. **Don't expose to hydrophobic plastics** (especially polystyrene) for prolonged periods. Because retroviruses are surrounded by a mostly hydrophobic membrane they are very sticky and losses can occur if they are exposed to hydrophobic plastics while not frozen. It is best to store thawed retroviruses in siliconized or low protein binding tubes and pipette it with similar pipette tips.
8. **Don't filter.** Filtering can reduce titer because viruses can stick to the filter. If filtering is necessary it is essential that filters with 0.45 μm (or larger) pores are used. Since the diameter of a retroviruses is about 0.15-0.2 μm, if a filter with 0.2 μm (or smaller) pores is used substantial loss of infectious titer can occur.

DO:

1. **Do aliquot and freeze** at -80°C for long term storage if the virus is not used within ~1 week. Besides being intrinsically unstable it is possible for microorganisms to grow in residual cell culture media that is in the virus. Freezing helps prevent this.
2. **Thaw** on ice just before use.
3. **Use** virus within ~ 6 months of storage at -80°C unless the titer is much higher than needed. Even when stored at -80°C retroviruses will lose ~10x in titer every 6-12 months.