A guide to Retroviral production and plasmids

We produce retroviruses by transfecting multiple plasmids that between them provide all of the necessary components for vector particle production. However, the genome plasmid does not encode all, or even any, viral genes. Thus the infected cells make the transgene, eg GFP, but no viral genes. This makes vector production safe, with no risk of developing ongoing viral replication. This level of safety of vector production allows easy manipulation of the viral genes used to make the vector particles and use of the system for expressing gene products or RNAi etc.

For information on the contents and combinations of plasmids and safety considerations when generating retroviruses please read the guide on the Addgene website, followed by the information relevant to our lab below:
https://www.addgene.org/viral-vectors/lentivirus/lenti-guide/#components

<u>Additional Important Information</u>
Note that there are several classes of retrovirus. Lentiviruses, such as HIV-1, HIV-2, SIV, FIV, EIAV are a type of retrovirus. Gammaretroviruses, eg Moloney MLV, AKV, Gibbon ape leukaemia virus, are another type of retrovirus. Don't fall into the trap of calling gammaretroviruses retroviruses to distinguish from lentiviruses because we also work on other retroviruses that are not gammaretroviruses, eg. alpharetroviruses (RSV) and deltaretroviruses (HTLV).
We typically use first and second generation constructs. Third generation plasmids make lower titre vector preps. We use the vectors for two things, expressing proteins/RNAi/Crispr etc in cells ie as a tool, and we also use them for discovery research, ie making viral mutants and seeing what this does to infectivity. We use HIV-1 and MLV based vectors for expression and have a wider range of vector systems used for addressing research questions on that particular virus.
HIV-1 plasmids: The HIV-1 genome plasmids are based on the GFP-encoding genome called CSGW.
The HIV-1 packaging construct is called p8.91 (Gag-Pol, tat and rev). We also use p8.2 which encodes Gag-pol tat, rev, vpr, vpu, vif and nef.
The VSV-G envelope expression vector is pMDG.
MLV plasmids: There are lots of MLV genomes eg CNCG (CMV-Neo-CMV-GFP) or EXN: a Bieniasz lab version of LNCX (LTR-Neo-CMV-X), which can be used with the packaging vector CMVintron or CMVi.
A list of the vector plasmids we use is on our website. We're in the progress of making a webpage with all the maps and sequences attached.

<u>Cat3 plasmids encoding full length replication competent lentiviruses including HIV-1</u>
NL4.3 is the name of the first full length HIV-1 sequence that was cloned into a plasmid. So there are many plasmids called NL4.3. If a plasmid is just called "NL4.3" then it is probably the full-length replication competent molecular clone of HIV-1. If you transfect this then you get infectious virus so this has to be prepared in the Cat3 lab and must never be used in the Cat2 lab.
Other common clones in use are YU2, the first primary isolate to be cloned, which comes from a CSF sample. It is Vpu defective. R9 is a very early clone very closely related to NL4.3, ie the same virus stock but a slightly different sequence. Ba-L is another viral isolate, not a plasmid. Ba-L is very macrophage tropic so we have clones such as NL4.3 and R9 with a Ba-L Env cloned in. HIV is

either CCR5 tropic, CXCR4 tropic or dual tropic, infects both. This impacts whether a virus will preferentially infect macrophages or activated T cells although the relationship between tropism and co-receptor use is complex.

All full-length lentiviral plasmids should be stored separately from other plasmids in the specified freezer.

Know your plasmids before you transfect them

Lots of other plasmids contain bits of NL4.3, for example pSVIII NL4.3 is an Env expression vector derived from pNL4.3. p8.91, the HIV-1 packaging construct, is made from a chimeric virus called R9. This is part NL4.3 and part another chimeric virus. We have p8.91 versions with just NL4.3 sequence in called p8.91 NL4.3 as well as others. There are now many derivatives of p8.91 in use in the lab. The most common variant is p8.91Ex which has fewer sites in the backbone making more unique sites in Gag-Pol. Note that we also have an HIV-1 packaging plasmid called p8.2. This is the same as p8.91 but additionally encodes the accessory genes Vif, Vpr, Vpu, Nef.

The HIV-1 genomes are based on the original HIV-1 vector called HR'. This was modified to be a SIN (self-inactivating vector), see Addgene description, by Adrian Thrasher's lab and we call the GFP encoding version of this plasmid CSGW. There are many versions of this plasmid.
 There are also HIV-1 encoding plasmids called deltaEnv or ΔEnv. For example, pLAI ΔEnv, R9 ΔEnv and NL4.3 ΔEnv are in use in the lab. These are full length virus with a large section of the Env gene deleted. It is very important not to pseudotype these with the pSVIII Env constructs because they will recombine. Doing this will lead to infectious virus so this work must be carried out in the Cat3 lab.   You can pseudotype ΔEnv plasmids with VSV-G (pMDG plasmid) or a gammaretroviral Env eg Amphotropic Env in the Cat2. VSV-G is the envelope protein (G) from the vesicular stomatitis virus (VSV), which has a very broad tropism due to using a highly ubiquitous receptor. This envelope is most commonly used as the resulting pseudotyped virus can therefore infect most cell types.

Be aware that there are HIV-1 ΔEnv plasmids that should not be used in the Cat2. These only have a point mutation in Env and the virus can repair this by mutation. These constructs are therefore dangerous because they appear defective but can quickly produce infectious virus. We try to avoid these plasmids but they show up from time to time so it is very important that you know the origin and the structure of all of the plasmids you transfect.

Everyone is the lab is responsible for checking the map or sequence of all the plasmids that they are using, even if you have been given it from someone else in the lab- do not just take someone else's word for it as a lot of information is lost this way. Using the wrong combination of plasmids can result in the generation of infectious virus in the wrong level of containment and will put yourself and your labmates at risk, which is unacceptable.

Transfecting lentiviral genomes Addgene propose that lentiviral genomes cannot be used as expression plasmids. In our experience, using MLV genome plasmids such as EXN or HIV-1 genome plasmids such as CSGW works very effectively and if you can transfect the cells the transgenes express well. Of course, infecting with a vector prep always works better as it is more efficient, more cells are transduced, and integration ensures stable expression, and is the best option for cells that do not transfect efficiently.

Nomenclature

As is often the case with science if you want to talk about vectors you have to learn the language. Typically we switch the phrases around just to confuse everybody but broadly here is

the nomenclature we use in our lab **Vector** = either the genome plasmid or the product of the 3 plasmid transfection, i.e. the vector stock. Eg, Q. what vector plasmid are you using?
A. CSGW.

or, I infected the cells with HIV-1 vector

We don't typically call vector preps "virus" because we reserve the phrase "virus" for full length infectious virus
VLP (virus like particles) = we use this term to refer to viruses or vector preparations which carry no genome- meaning that no genome plasmid was transfected during preparation and so we consider them to be empty particles. We use VLPs to deliver proteins that are packaged into the viral particle, for example Vpx with SIV VLPs. It is important to remember that the VLP will contain not only the protein you want to deliver but all viral proteins encoded by the packaging plasmid that was transfected to produce them and this may include other viral accessory proteins, eg Vpr.
Bald vector. This is when no Env gene was transfected so vector particles are formed but they are not infectious because they have no Env. Bald particles are often used as a control for a vector that cannot enter the cells by receptor mediated means.
Packaging plasmid is the plasmid encoding the viral structural genes.   **Transfect** is the process of getting the DNA into the cells, **transform** is the process of getting DNA into bacteria, and **transduce** is the process of infecting cells with vector. Confusing I know.