Lentiviral Vector Safety Information

Your safety is our number one priority

The biosafety issues associated with using recombinant lentiviral vectors, i.e. the potential for producing oncogenic viruses or replication-competent lentivirus (RCL), can be greatly mitigated by carefully considering the nature of the transgene insert and by ensuring that viral replication is restricted to specific packaging cells that provide these essential functions, in trans. Lentiviral vectors can thus be used safely and are extremely useful tools for one-way transfers of exogenous genetic material into target cells.

Extra Safety with Lenti-X™ HTX

Most commercially available lentiviral packaging systems are 3rd generation versions that utilize split-genes to provide the viral packaging elements on individual plasmids that physically separate the viral envelope, env (usually VSV-G), sequence from the gag-pro-pol sequences. These split-gene packaging strategies reduce the risk of generating RCL because multiple recombination events are necessary to create a virus that harbors the sequences required for independent replication. Clontech’s Lenti-X HTX Packaging System also uses a split-gene packaging strategy, but adds another level of safety by further uncoupling pol (RT and IN) from gag-pro. The result is that gag, pol, and env reside on three physically distinct entities, rather than the standard two (Figure 1). This approach further reduces the possibility of creating RCL to a level below that of standard 3rd generation packaging systems, because extra recombination events are required to create such viruses. In fact, the emergence of RCL is unde-tectable from systems using this approach (1). These improvements significantly increase the safety profile of our Lenti-X HTX Packaging System.

Clontech’s Lenti-X HTX Packaging System consists of 5 separate components (Panel A), mixed in proprietary proportions for optimized packaging activity. The separation of the gag, pol, and env genes effectively reduces the incidence of RCL (1). Other 3rd generation systems (Panel B) which do not contain separate gag and pol sequences have higher RCL-generating potential. High levels of expression of essential viral components are driven by the Tet-Off® and Tat transactivators, resulting in high titers of virus. The pol gene is fused to vpr to ensure transport of the reverse transcriptase/integrase protein into the recombinant viral particle. Not all vector elements are shown.

Lenti-X Vector Safety

Clontech’s Lenti-X Vectors contain less than one-third of the wild-type HIV-1 genome. These wild-type sequences mainly consist of the viral LTRs and packaging signal (see vector maps for detailed sequence information). All essential replication genes have been completely removed and are instead supplied as separate DNA entities in the Lenti-X HTX Packaging Mix (described above).
Lentivirus Usage and Safety Guidelines

The protocols supplied with Clontech’s Lenti-X systems require the production, handling, and storage of infectious lentivirus. It is imperative to fully understand the potential hazards of, and necessary precautions for, the laboratory use of lentiviruses. The National Institutes of Health and Centers for Disease Control have designated recombinant lentiviruses as Level 2 organisms. Clontech® advises that you contact your health and safety facilities for local guidelines and regulations, and strongly recommends that new users of all viral technologies receive training from experienced personnel.

For more details, download the NIH’s “Biosafety Considerations for Research with Lentiviral Vectors.”

NIH guidelines require the maintenance of a Biosafety Level 2 (BL-2) facility for work involving lentivirus and others like it. The VSV-G pseudotyped lentiviruses packaged from the HIV-1-based vectors described here are capable of infecting human cells. The viral supernatants produced by these lentiviral systems could, depending on your insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to transfer and express genes in vivo.

For these reasons, due caution must be exercised in the production and handling of any recombinant lentivirus. The user is strongly advised not to create VSV-G pseudotyped lentiviruses capable of expressing known oncogenes.

For more information on Biosafety Level 2 agents and practices, download Biosafety in Microbiological and Biomedical Laboratories (BMBL), Fifth Edition (Revised December 2009), HHS Pub. No. (CDC) 21-1112, U.S. Department of Health and Human Services Centers for Disease Control and Prevention and NIH.

Summary of Biosafety Level 2 Practices

The following information is a brief description of Biosafety Level 2. It is neither detailed nor complete. Details of the practices, safety equipment, and facilities that combine to produce a Biosafety Level 2 are available in the above publication. If possible, observe and learn the practices described below from someone who has experience working with lentiviruses.

**Standard microbiological practices**
- Limited access to work area
- Biohazard warning signs posted
- Minimize production of aerosols
- Decontaminate potentially infectious wastes before disposal
- Use precautions with sharps (e.g., syringes, blades)
- Biosafety manual defining any needed waste decontamination or medical surveillance policies

**Safety equipment**
- Biological Safety Cabinet, preferably a Class II BSC/laminar flow hood (with a HEPA microfilter) used for all manipulations of agents that cause splashes or aerosols of infectious materials; exhaust air is unrecirculated
- PPE: protective laboratory coats, gloves, face protection as needed

**Facilities**
- Autoclave available for waste decontamination
- Chemical disinfectants available for spills

**Note:** The components that make up the Lenti-X family of products are designed to work together as a system. Substituting components from other manufacturers or that users have developed in-house, may affect performance and or safety. We recommend that you utilize our complete system, however, if you do decide to use components other than those developed by Clontech, please carefully consider performance and safety implications.

**Reference**

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