User Instructions: Lentivirus for in vivo Applications

Background

During the past decade, HIV-1 based defective lentivirus has been one of the most widely used gene transfer vectors. Lentivirus is an enveloped virus that contains a diploid positive-strand RNA genome. Upon infection of host cells, the virus uses reverse transcriptase to convert its RNA into double-stranded DNA, which is then permanently integrated into the chromosomes of the host cell.

Modified lentivirus, which has all viral structural genes removed leaving only the LTR and the packaging signal, and thus allowing for the introduction of therapeutic genes, is still able to infect cells. However, the viral genes required for producing new viral particles are no longer present. To improve safety, transfer vectors additionally contain a deletion in the 3’ LTR, rendering the virus “self-inactivating” (SIN) after integration.

The most advantageous feature of recombinant lentivirus is the ability to mediate efficient transduction, integration, and long-term expression of genomic material in both dividing and non-dividing cells. Its ability to infect non-dividing cells is facilitated by the pre-integration complex (PIC), which allows lentivirus to cross the nuclear envelope through the nuclear pore complex (NPC); most other retroviruses need the nuclear envelope to break down during mitosis to access host chromatin. As many cell types are considered to be largely quiescent in vivo, recombinant lentivirus has been the choice for many in vivo applications. Furthermore, unlike adenovirus, lentivirus is not immunogenic in vivo.

Since CD4 is the major receptor for binding to native HIV envelope glycoproteins, the tropism for lentiviral vectors is very restricted. CD4 receptors are only located on cells of immune lineage. In order to infect cells without CD4 expression, the vectors are pseudotyped with vesicular stomatitis virus glycoprotein (VSV-G), which is a rhabdovirus envelope protein that binds to ubiquitous phospholipid components of the plasma membrane rather than to specific cell surface receptors. Such viruses have an extremely broad host-cell range that includes cell types such as neurons, lymphocytes, and macrophages. Previously, retroviral vectors could not be used for these cell types. Moreover, lentiviral vectors have proven to be effective in transducing brain, liver, muscle, and retina in vivo.

A lentiviral particle binds to the cell membrane and enters the host cell. The viral RNA genome is released and reverse-transcribed to produce DNA. DNA is then stably integrated into the host genome at a random position by the viral integrase enzyme. A simplified illustration of the infection mechanism is shown in Figure 1.

Content

The following tables show the components associated with custom lentivirus. Virus titer is given in the certificate of analysis (COA) document.
Lentivirus for in vivo Applications User Instructions, V1.3, 2019-03-14

Storage and Handling

1. Upon receiving, lentivirus should be stored at -80°C for long term (stable for at least 6 months), or -20°C for use within one week. Polybrene can be stored at -20°C for long term, or 4°C for up to two weeks.

2. Thaw the vial of lentivirus on ice prior to use, and keep it on ice for the duration of the experiment. Virus titer drops rapidly at higher temperatures.

3. Depending on the amount used in your experiment, lentivirus can be dispensed in smaller aliquots, and refrozen, after it is thawed. However, this will typically result in a loss of ~20% in titer after each freeze-thaw cycle. Aliquots should be stored at -80°C.

   **CAUTION:** Repeated freeze-thaw cycles of lentivirus should be avoided, as this can cause a large titer drop.

Safety Precautions

All lentivirus from VectorBuilder is “self-inactivating”, meaning that they cannot replicate within target cells and infect other cells. This is because genes responsible for replication have been deleted from the viral genome. However, the virus can in theory pose a biohazard risk because it can transduce primary human cells. We recommend that the virus should be handled according to Biosafety Level 2 (BSL-2) criteria. All handling, storage and disposal of biohazard waste must be in accordance with published and institutional criteria.

Protocol for Stereotaxic Injection to the Rodent Brain

Intracerebral injections of tracers or engineered viruses in rodents have dramatically enhanced our understanding of the nervous system by targeting specific areas of the brain. These techniques are now commonly used in neuroscience.

An important aspect to consider in planning to use lentivirus for intracerebral injection is the extent of diffusion of the viral particles in the brain extracellular space (ECS). The ECS between cells in the vertebrate central nervous system has been estimated to be <40 nm. Unlike AAV particles, which have a diameter of approximately 20 nm, lentiviral particles are quite large (approximately 100 nm in diameter) and the particle size thus should strongly limit their free diffusion in the brain.

To determine the optimal injection for your study, you could conduct pilot testing in your animals by using reporter lentiviruses, such as EGFP-expressing lentivirus.
Materials:

1. Reagents
   - Ultra-purified lentivirus (a titer of $>1 \times 10^9$ infectious particles per ml is recommended)
   - Mice or rats
   - Disinfectant (ethanol 70%)
   - Anesthetics and analgesics (e.g., ketamine etc)
   - Lubricant eye ointment
   - Sterile phosphate-buffered saline (PBS)
   - Bone wax

2. Equipment
   - Electric hair shaver
   - Surgical tools including small surgical scalpel and scissors, fine forceps and surgical hooks
   - Small animal stereotaxic apparatus
   - Dissecting microscope
   - Cotton swabs
   - Hand-held drill with small dental drill bits
   - 10 ul injection syringe with rigid 33-gauge needle
   - 10 ml syringes with 27-gauge needles
   - Surgical sutures
   - Temperature-controlled cage
   - Heating pad

Procedures:

1. Anesthesia
   - Weigh the animal and calculate the dosage depending on the anesthetic used. For a ketamine-xylazine mixture, a dose of 80–100 mg ketamine and 10 mg xylazine per kilogram body weight is usually used for adult mice and rats.
   - Animals are commonly anesthetized via IP (intra-peritoneal) injection.
   - Place the animal on a heating pad to keep a steady body temperature.
   - The animal should reach deep anesthesia within ~10 min. Check the lack of response to nociceptive stimuli to confirm depth of anesthesia by pinching the tail.

2. Preparation of the animal
   - Shave the fur on the skull.
   - Clean the skin with 70% ethanol.
   - Apply lubricant eye ointment to both eyes to avoid keratitis during surgery.
   - Pull out the tongue using forceps to facilitate breathing.

3. Fixation of the animal in the stereotaxic apparatus
   - To place the animal in the stereotaxic apparatus, fix one ear bar in the apparatus, gently position the animal's head to lead its ear canal onto the ear bar, keep the animal's head in place and slowly position the second ear bar to complete the fixation.
   - Slowly move the incisor adapter into the animal's mouth until the animal's incisors 'fit' in the opening of the adapter, then gently pull back slightly and fix the adapter in place.
   - The last point of fixation, the nose clamp, should be used with very low pressure on the animal's nose.

Figure 2. Correctly positioned and fixed mouse
4. Craniotomy

- Make a midline incision with small surgical scissors or scalpel. Separate the subcutaneous and muscle tissue and use small surgical hooks to keep the area open.
- Clean the surface of the skull with a cotton swab until the bregma and lambda area are visible.
- Make sure that the top of the animal's skull is in the horizontal plane. For this, the head position may be adjusted using the screw at the incisor adapter.
- Point the tip of the syringe, held by the stereotact, to the bregma point, at all three axes. Write down the coordinates. This point would be considered as the zero point in all three axes.
- Lift the syringe in the vertical axis so that a planar movement would not scratch the skull and move the syringe head to the correct location.
- Lower the tip of the syringe until it touches the skull and mark the spot with a marker. Remove the syringe back to avoid stabbing.
- Thin the skull over the target area (about 1 mm x 1 mm for one injection site) using a hand-held drill by horizontal movement of the spinning drill bit while applying a slight pressure downward. We typically use x40 magnification of the dissecting microscope during drilling. Stop when the bone is very thin (blood vessels in the dura become clearly visible).
- Take a small needle (27-gauge) and carefully perforate the edges of the craniotomy. Next, with the tip of the needle, 'flip up' a piece of the thinned bone and then carefully remove it with fine forceps. The needle used for injection can easily penetrate the dura of mice and young rats. Opening of the dura is not necessary.
- Keep both the skull and exposed dura moist with PBS.

5. Injection of the lentivirus

- Withdraw desired volume (typically 4 ul) of lentivirus solution.
- Place the syringe above the hole and slowly lower it vertically until it reaches the surface of the skull. Continue to lower the syringe to penetrate the dura. After penetrating the dura, lower the syringe needle to the desired depth in the brain parenchyma.
- Set the digital pump to 0.02 ml/min (0.5 μl would be injected in 25 min) and start infusion. Slow infusion allows for effective spreading of the virus into the tissue and prevents back flow.
- After infusion is completed, wait for an additional 5 min to allow the material to spread into the brain instead of retreating back into the canal formed by the syringe.
- Remove the syringe very slowly.

6. Wound sealing and recovery

- Clean the injection site with moist cotton swabs. A small craniotomy (less than 1 mm x 1 mm) does not need to be covered with bone wax. For a larger craniotomy, apply a thin 'slip' of bone wax over the skull.
- Pull the edges of the skin together and suture the skin at 3 separate points.
- Place the animal in the temperature-controlled cage until full recovery (at least 2 days after surgery). Then return the animal to its home cage.