Lentiviral generation of stable animal cell lines

Lentiviral transduction enables stable integration of genetic material into dividing and non-dividing animal cells. This makes it a powerful tool for long-term expression of transgenes, genetic perturbation screens, and the generation of stable cell lines, especially in cell types that are difficult to transfect by conventional means.

The viral envelope protein determines host range ("tropism"). VSV-G is the most commonly used envelope protein, conferring broad tropism and high particle stability. Alternative envelopes such as RD114 or GALV may be preferred for hematopoietic or progenitor cells, or to reduce cytotoxicity.

Lentiviral vectors are derived from human immunodeficiency virus (HIV-1) and produced by co-transfection of a packaging cell line with a set of helper plasmids. This protocol uses a second-generation packaging system, in which the *gag*, *pol*, *rev*, and *tat* genes are encoded on a single plasmid. The recombinant envelope protein, typically VSV-G, is provided on a separate plasmid, and the gene of interest is cloned into a third, replication-deficient transfer vector.

Although second-generation vectors are widely used and considered safe when handled under BSL-2+ conditions, their simpler architecture—fewer plasmids and retention of *tat*—introduces a higher theoretical risk of recombination into replication-competent lentivirus compared to third-generation systems. Federal regulations and institutional biosafety policies require that researchers handling lentiviral vectors complete appropriate training, receive supervision, and follow strict decontamination and disposal protocols.

This is a bench card. Full protocol available online.


In the event of SPILL:

- ▷ **Evacuate the area and allow aerosols to settle for 30 min before re-entry**
- ▷ **Soak thoroughly with freshly prepared 1% sodium hypochlorite or equivalent virucidal disinfectant (Opti-Cide® 3).**

In the event of EXPOSURE:

- ▷ **Wash affected area for 15 min**
- ▷ **Seek medical attention within 1 hour**

Report to (all of):

- **Principal Investigator/Supervisor**
- **Biosafety Officer**

Reviewed: Jul 25, 2025

Procedures

›› *Production of virus particles*

- ☐ 6-well plate
- ☐ Flip-top filter, 0.45 µm, PES
- ☐ Lenti-X™ 293T
- ☐ DMEM + 10% FBS, without antibiotics
- ☐ Culture medium for the transduced cells
- ☐ Transfer plasmid (with transgene)
- ☐ Packaging plasmid
- ☐ Envelope plasmid

(1.) On the day of transfection, replace medium with antibiotic-free DMEM + 10% FBS.

> ***Critical:*** From this step forward, perform all steps in a certified class II biosafety cabinet. This includes transfection, medium changes, harvesting, and filtering virus-containing supernatant. Do not perform any viral manipulations on the open bench. Carry culture plates carefully using both hands. If moving between rooms or floors, place the cultures in a leak-proof secondary container lined with absorbent paper, and disinfect the exterior before transport.

(2.) Transfect Lenti-X™ 293T cells at 70% confluency with the following plasmids using chemical transfection with Lipofectamine® 2000 SOP 0012. By default, use one well of a 6-well plate per transgene:

	Format	Plates (per well)			
Plasmid		**6-well**		**12-well**	**24-well**
Packaging plasmid	psPAX2	0.22 pmol	1.46 µg	530 ng	300 ng
Envelope plasmid	pMD2.G (VSV-G)	0.12 pmol	0.43 µg	155 ng	90 ng
Transfer plasmid	pLJM1, pLKO.1, etc.	0.28 pmol	1.20 µg	430 ng	240 ng

> ***Critical:*** This SOP describes small-scale virus production (less than 100 mL total supernatant with titers less than 1×10^9 cfu/mL). Larger volumes or higher titers may require additional IBC review or enhanced containment measures.

(3.) *Optional:* After 18 h, replace the medium with 2 mL of the culture medium of the cells to transduce. 18 h

(4.) *Optional:* Supplement the culture medium with 10 mM sodium butyrate to restrict cell growth.

(5.) Harvest the virus-containing supernatant at 48 h, and optionally at 72 h and 96 h post transfection. Combine batches or store separately as needed.

> ***Critical:*** Handle supernatants with care; even replication-deficient vectors remain infectious until inactivated. Do not remove viral supernatant from the biosafety cabinet unless sealed and disinfected.

(6.) *Optional:* Pre-clear the viral supernatant at 500 × *g* for 5 min in a certified aerosol-tight centrifuge.

> ***Critical:*** Centrifugation must be performed in sealed buckets and followed by immediate disinfection. Skip this step for small-scale preparations or if a certified aerosol-tight centrifuge is not available near the biosafety cabinet.

(7.) Filter the virus-containing supernatant through a sterile 0.45 µm PES flip-top filter inside the biosafety cabinet to remove remaining packaging cells and debris. Do not use needles!

> ***Critical:*** Flip-top filters reduce the risk of aerosol generation and spills, increasing user safety. If using syringe filters, do not force the plunger or push air through the filter as this will generate aerosols, or can cause the filter to pop off.

(8.) Disinfect all tubes and external surfaces of containers with 1% sodium hypochlorite or equivalent virucidal disinfectant (Opti-Cide® 3) before removing them from the biosafety cabinet.

⊞ Optional: *PEG precipitation of viral supernatants*

- ☐ R0195 5 × PEG virus precipitation solution, 100 mL

(1.) Add 0.25 vol PEG virus precipitation solution per 1.0 vol of viral supernatant.

(2.) Mix well by shaking for 1 min. Incubate with constant rocking at 4 °C for at least 4 h. 6 h

(3.) Spin at 1 600 × *g* for 1 h at 4 °C in a certified aerosol-tight centrifuge. 1 h

> ***Critical:*** Centrifugation must be performed in sealed buckets and followed by immediate disinfection. Skip this step for small-scale preparations or if a certified aerosol-tight centrifuge is not available near the biosafety cabinet.

(4.) Carefully remove the supernatant without disturbing the pellet.

(5.) Thoroughly resuspend the viral pellet in 0.05–0.10 vol PBS or serum-free culture medium without antibiotics by pipetting up and down. Incubate for 10 min.

> ***Critical:*** Avoid generating bubbles which may inactivate the virus.

(6.) *Optional:* Transfer to a microcentrifuge tube and spin at full speed for 3 min to remove cellular debris.

⊞ Optional: *Concentration of viral supernatants by centrifugation*

(1.) If an ultracentrifuge is not available, place the viral supernatant on a 0.2 vol 50% sucrose cushion.

(2.) Concentrate depending on the available instrumentation,

- In a high-speed centrifuge at 10 000–20 000 × *g* for 240 min, or
- In an ultracentrifuge at 90 000 × *g* for 90 min.

(3.) Carefully remove the supernatant without disturbing the pellet. Resuspend as desired.

›› *Storage of lentiviral particles*

(1.) Store filtered viral supernatants at 4 °C for up to 24 h, or transfer in 1 mL aliquots to −80 °C storage.

> ***Critical:*** Use screw-cap polypropylene vials for storage. DO NOT submerge the vials into liquid nitrogen! Label the secondary container clearly with "***BIOHAZARD: Infectious Material (Lentivirus)***".

> ***Quality assurance:*** Freezing and thawing reduces viral infectivity by 20%. However, storing the supernatant at 4 °C for more than two days reduces infectivity by up to 40–60%. Avoid repeated freeze–thaw cycles.

›› *Transduction of host cells*

☐ Culture medium for the transduced cells	☐ R0134 10 g/L Hexadimethrine bromide, 100 mL

(1.) *Optional:* Determine the viral titer by ELISA, qPCR, or semi-quantitative lateral flow assays.

(2.) Seed cells to 25% confluency at the time of transduction.

(3.) Supplement 1 mL culture medium with 20 µL 10 g/L Polybrene® to a concentration of 0.2 g/L for a 20 × stock.

(4.) Prepare a dilution series of the viral supernatant in Polybrene®-supplemented medium. Apply to target cells to determine optimal transduction conditions. For a 6-well plate:

Dilution	**Neat**	**1:10**	**1:25**	**1:50**	**1:100**	**1:250**
Viral supernatant	1 500 µL	150 µL	60 µL	30 µL	15 µL	6 µL
Culture medium	0 µL	1 350 µL	1 440 µL	1 470 µL	1 485 µL	1 494 µL
Culture medium + 0.2 g/L Polybrene®	75 µL	75 µL	75 µL	75 µL	75 µL	75 µL

(5.) The next day, replace the medium with fresh culture medium.

> ***Safety:*** Do not aspirate viral medium using vacuum lines. Collect liquid waste into a flask containing bleach and incubate for at least 60 min before disposal!

(6.) The next day (or the day after), split the cells 1:3 to 1:5 depending on cell type, and proceed with selection or analysis as appropriate. For antibiotic selection, refer to SOP 0019.

> ***Critical:*** Maintain cells in a subconfluent state throughout antibiotic selection to outgrow successfully transduced clones.

> ***Critical:*** For biosafety reasons, all transduced cells must be cultured in a designated incubator for at least ***three to five additional passages,*** typically over two to three weeks, to allow clearance of any potentially infectious particles.

List of references

Recipe (available online) Troubleshooting (available online) Notes (available online)