

Lenti-based protocols

OriGene offers genome-wide [lenti-ORF](#) and [lenti-shRNA](#) in both plasmid and particle forms. The lenti vectors are 3rd generation; the safest vectors. Both 2nd generation and 3rd generation packaging plasmids can be used to package the 3rd generation vectors into pseudo lentiviral particles.

For lenti packaging, to produce high titer lentiviral particles, the optimized, high packaging efficiency [Lenti packaging kits](#) (Cat# TR30037 for regular lentivirus packaging, cat# TR30036 for integration-deficient lentivirus packaging) can be used.

NOTE: Performing Lentiviral experiments REQUIRES special laboratory conditions and/or permissions (BSL2). Follow the guidelines and regulations of your institution. Perform the experiments with due caution to avoid exposure to infectious materials.

Production of pseudo lentivirus (10 cm plate format, the production size can be scaled up or down accordingly):

1. Day 1, plate HEK293T cells in a 10 cm dish at approximately 40% confluency the day before transfection (antibiotic-free preferred). Cells should reach 65-70% confluency within 24 hours.
2. Day 2, Transfection
In a labeled Eppendorf tube, dilute the following DNA in 1.5 mL Opti-MEM, and pipet gently to mix completely.
 - a. 5 µg of pLenti-shRNA construct or
5 µg of pLenti-ORF expression construct
 - b. 6 µg of packaging plasmids
3. Add 33 µL of TurboFectin transfection reagent to the diluted DNA (not the reversed order), pipet gently to mix completely.
4. Incubate for 15 min at room temperature.
5. Add the transfection mixture prepared above dropwise to the cells. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly. Incubate at 37° in a CO2 incubator.

Note: With TurboFectin, no medium change is necessary, directly add the transfection mixture to cells.

6. Day 3, change the growth medium and continue to incubate the plate for 48 hours.
7. Day 5

- a. After 48-hour incubation, transfer the cell culture supernatant to a 15 mL centrifuge tube.
 - b. Centrifuge the tubes at 3K RPM for 5 mins and filter the supernatant through a syringe filter (0.45 micron) and collect the viral solution to a new sterile tube.
8. The viral particles are ready to be used. They can be stored at 4 °C for 2 weeks or aliquot and store at -80 °C for long-term.

Lentiviral transduction Protocols

The following protocol is based on a 24-well plate. If your experiments require a different size of culture plates, just scale up or down accordingly based on the relative surface area.

Tissue Culture Vessel	Growth area, cm ² /well
96-well plate	0.35
24-well plate	2
12-well plate	4
6-well plate	9.5
35 mm plate	8
60 mm plate	20
100 mm plate	60

Day 1, seed cells

Seed 0.5×10^5 HEK293T cells or your specific cells in each well of 24-well plate to 50% confluency upon transduction. Incubate 18–20 hours at 37°C in a humidified 5% CO₂ incubator.

Note: To determine how many cells to be seeded, please consider both the growth rates of your cells and the length of time the cells will be growing before transduction.

Day 2, transduction

1. Calculate the amount of viral particles to be added according to the desired multiplicity of infection (MOI).

Multiplicity of Infection (MOI):

Multiplicity of Infection (MOI) is the number of transducing lentiviral particles per cell

To calculate:

(Total number of cells per well) x (Desired MOI) = Total transducing units needed (TU)

$(\text{Total TU needed})/(\text{TU/mL}) = \text{Total mL of lentiviral particles to be added to each well}$

Note: When transducing a cell line for the first time, a range of volumes or MOI should be tested. The [transduction control particles](#) with a fluorescence marker can be used for MOI optimization. MOIs of 5, 10, 20, 50 and 100 or higher should be used to determine the optimal transduction efficiency for each cell line.

2. Thaw the lentiviral particles (if frozen) on ice. Gently spin down before opening. Keep them on ice. Mix gently before use.
3. Remove medium from wells and add appropriate amount of Lentiviral particles, culture medium, polybrene (final concentration is 8 $\mu\text{g/mL}$) to the total volume of 500 μL . Gently swirl the plate to mix.

Polybrene (Hexadimethrine bromide):

Polybrene is a small, positively charged molecule that binds to cell surfaces, neutralizes surface charge, increases binding between pseudoviral capsid and the cellular membrane. Polybrene has been proved to greatly enhance transduction efficiency. Some cells, like primary neurons, are sensitive to polybrene. Do not add polybrene to these types of cells. If working with a cell type for the first time, a polybrene control only well should be used to determine cell sensitivity.

4. Incubate 18–20 hours at 37°C in a humidified 5% CO₂ incubator. Overnight incubation may be avoided when toxicity of the lentiviral particles is a concern. Cells may be incubated for as short as 4 hours before changing the medium containing lentiviral particles.

Day 3, change media

Remove the medium containing lentiviral particles from wells and replace with 500 μL fresh pre-warmed complete culture medium.

Day4.

- a. For transient knockdown or gene overexpression: Do not disturb the cells until next day.
- b. For stable cell line generation if the vectors contain a mammalian selection marker: Split transduced cells (1:10 for puromycin, 1:20 for G418 selection) and apply complete medium containing the appropriate amount of antibiotics (puromycin or G418 or other antibiotics) (if you want to isolate single cell colonies, plate cells in a larger dish, such as 10 cm dish). Keep the cells cultured at 37°C in a humidified 5% CO₂ incubator.

Note: Perform a kill curve experiment for the right dose of puromycin in stable cell selection using the following guidelines:

- i. Plate 1.6×10^4 cells into wells of a 96-well plate with 120 μL fresh media.
- ii. The next day, add puromycin ranging from 0.5 to 10 $\mu\text{g/mL}$ to selected wells.

- iii. Examine cell viability daily.
- iv. Culture for 10-14 days. Replace the media containing puromycin every 3 days.
- v. The minimum concentration of puromycin that causes complete cell death after 4-7 days should be used for that cell type.

Day 5 and forward.

- a. For transient knockdown or gene overexpression: Harvest the cells and perform qRT-PCR or Western blot.

For stable cell line generation: replace medium with fresh, antibiotics-containing medium every 3-4 days until resistant colonies can be identified (generally, 10-12 days after drug selection). Pick a minimum of 5 colonies and expand each clone to assay for knockdown or overexpression of the target gene.

Note: This protocol is a guideline for your successful experiments. Conditions might be adapted to your cell line, the reagent, lentiviral vector and your lab's experiences.”