Lentiviral Production and Infection Protocol

Complete workflow from transfection to viral transduction

← All Protocols

Overview | Materials | Virus Production | Concentration | Infection

Overview

This protocol describes lentiviral production using HEK293T packaging cells and subsequent infection of target cells. Lentiviral vectors can transduce both dividing and non-dividing cells, making them ideal for stable gene transfer in a wide range of cell types.

Workflow Overview:

- **Day 0:** Plate 293T cells
- **Day 1:** Transfection
- **Day 2:** Media change
- **Day 3-4:** Virus collection
- **Day 4-5:** Virus concentration (optional)
- **Day 5+:** Target cell infection
- **Day 7-8:** Selection or sorting

Applications

- Stable gene overexpression
- shRNA/CRISPR knockdown
- Reporter gene expression
- Gene editing delivery
- Cell line engineering
- Primary cell transduction

Key Components

- **Transfer plasmid:** Contains your gene of interest
- **Packaging plasmid:** pSPAX2 or cDNL (gag-pol)
- **Envelope plasmid:** pMD2.G or VSVG
- **293T cells:** Virus-producing cells
- **Transfection reagent:** Delivers DNA to cells

Biosafety Considerations

- Lentiviral work typically requires BSL-2 containment
- Check institutional biosafety guidelines and obtain IBC approval
- VSV-G pseudotyped lentivirus has broad tropism - handle carefully
- Work in biosafety cabinet for all virus handling steps
- Decontaminate all materials with 10% bleach before disposal
- Never pipette by mouth - use mechanical pipettors
- Wear appropriate PPE: lab coat, gloves, eye protection