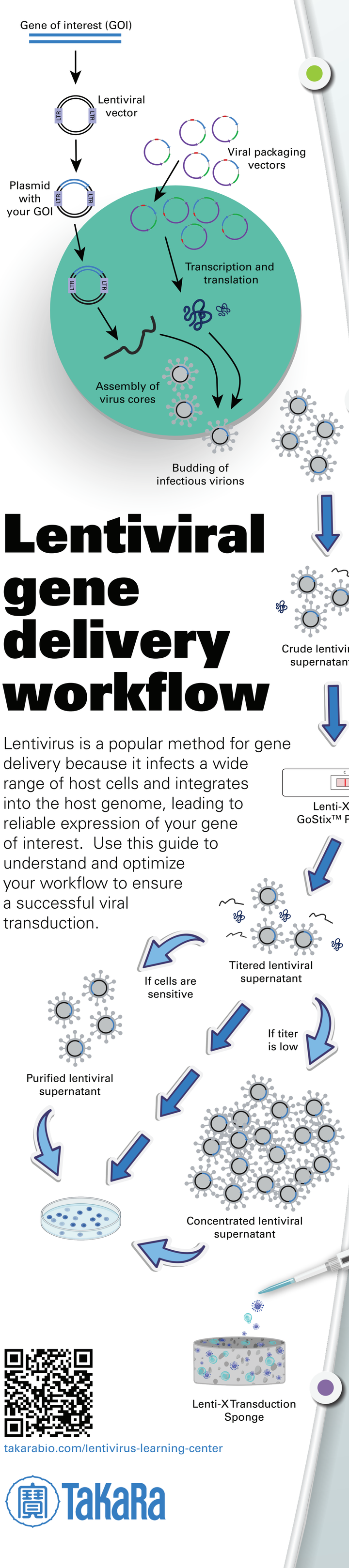
# Lentiviral gene delivery workflow

Lentivirus is a popular method for gene delivery because it infects a wide range of host cells and integrates into the host genome, leading to reliable expression of your gene of interest. Use this guide to understand and optimize your workflow to ensure a successful viral transduction.

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## 1 VECTOR SELECTION AND CLONING

Select the lentiviral vector backbone with the correct expression elements for your project for a reliable starting point. This will lead to a higher viral titer and improved transgene expression. Clone your gene of interest into your vector.

**Takara Bio tip:** Recycling old vectors found in your lab from previous projects may not yield optimal results because they weren't built for your current workflow. Use a vector backbone designed for specific applications, such as promoters that are appropriate for your desired expression level and target cell type, or genes for fluorescent protein expression or antibiotic selection markers. Get a reliable starting point with one of our Lenti-X™ or pLVX vector backbones.

**Takara Bio tip:** Lentiviral vectors may have limited restriction sites suitable for subcloning, which can make it difficult and time-consuming to perform PCR cloning using traditional ligation methods. Instead, save time with In-Fusion® cloning, which has a cloning accuracy of >95% and does not require restriction enzymes or ligase. Choose your cloning locus based on experimental preference, instead of being limited by available restriction sites.

## 2 VIRUS PACKAGING AND PRODUCTION

Co-transfect the lentiviral vector—containing your gene of interest—with packaging plasmids encoding essential viral genes into a packaging cell line. Choose a packaging cell line that is highly transfectable and will produce high lentiviral titers.

**Takara Bio tip:** Producing packaging vectors and optimizing the right ratios of the multiple plasmids and transfection reagent to achieve a high viral titer can be slow and laborious. Save time, increase expression, and improve safety with our 4th-generation Lenti-X Packaging Single Shots.

**Takara Bio tip:** Low quality cells don't produce enough lentivirus. If you aren't sure about the quality of the cells you found in the freezer, start fresh with Lenti-X 293T Cell Line, a subclone that produces six times more virus than its parental HEK 293 cell line.

## 3 HARVESTING AND TITRATION

Harvest your newly produced lentivirus—containing your gene of interest—from the supernatant. Titrate your virus now, before continuing with your workflow. There are two types of methods to titer your virus. Physical methods are fast and easy, and provide valuable data and reassurance that your viral titer is sufficient.

**Takara Bio tip:** Skipping titration will cost you time and effort later. Quantifying your virus is essential for successful transduction. Perform physical titration in just 10 minutes (not days) using the Lenti-X GoStix Plus and your smartphone.

### Functional titration methods

- ✓ Quantify functional lentiviral particles
- ✓ Provide accurate results
- ✗ Tedious and time consuming (days)
- ✓ Examples: flow cytometry, FACS, drug selection

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### Physical titration methods

- ✓ Quantify a component (protein or nucleic acid) of lentiviral particles
- ✓ Good correlation to the amount of functional viral particles
- ✓ Quick and easy to perform (minutes to hours)
- ✓ Examples: Lenti-X GoStix Plus, ELISA, qRT-PCR

## 4 CONCENTRATION AND/OR PURIFICATION

Your harvested lentiviral prep may require additional processing, depending on the titers or the multiplicity of infection (MOI) needed for your application.

**Takara Bio tip:** Low titer? Rescue your sample with Lenti-X Concentrator, which can achieve 100-fold concentration of your lentiviral supernatant.

### Concentrate when…

- Your viral titer is low
- You want to reduce sample volume
- You want to ensure consistent transduction downstream

### Purify when…

- Your target cells are sensitive to contaminants
- Your viral titer is low
- You want to reduce sample volume

## 5 TRANSDUCTION

Introduce your viral particles into the target cells when you have a high titer sample of your lentivirus—containing your gene of interest. High transduction efficiency is key to successful downstream applications.

**Takara Bio tip:** Spinoculation, a common method of transduction, is time consuming and requires polybrene, which is toxic to certain cells. Instead, implement the easy-to-use Lenti-X Transduction Sponge, a non-toxic macroporous alginate sponge that co-localizes your target cells and your lentivirus, leading to high transduction efficiency that is gentle on cells and easy to scale.