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RENDER Virus-like Particle Packaging

This protocol describes the preparation of packing virus-like particles (VLP) for delivery of genome and epigenome editing reagents, as described in Xu & Besselink et al. (2025).

Important notes and considerations before starting

• Maintain a culture of HEK293T or Lenti-X cells that does not exceed 90% confluency for several passages.

* Our RENDER platform (https://pmc.ncbi.nlm.nih.gov/articles/PMC12381050/) use an eVLP system (https://pmc.ncbi.nlm.nih.gov/articles/PMC8809250/) adapted from Moloney Murine Leukemia Virus (MMLV) for generating VLPs.

** In the RENDER paper, we used pBS-CMV-gagpol (https://www.addgene.org/35614/) for particle packaging. Recently work from David Liu's group (https://pmc.ncbi.nlm.nih.gov/articles/PMC12085157/) shows that an engineered Gag-pol variant improves eVLP production.

> • Therefore, I recommend using this new construct, v5 eVLP gag-pro-pol (https://www.addgene.org/228466/), which also performed better in our hands, for RENDER particles packaging.

*** We used this plasmid (https://www.addgene.org/181751/) as backbone template for eGag-editor plasmids cloning. For more eGag-editor constructs, please check the Nuñez lab's Addgene collection (https://www.addgene.org/browse/article/28259339/).

**** Mirus-LT1 Transfection Protocol:
https://tools.mirusbio.com/assets/protocols/ml001_transit_lt1_transfection_reagent.pdf

PROTOCOL

Day 0

1. Plate HEK293T or Lenti-X cells at 4×10^6 cells on a 10-cm plate.

Day 1

Perform the transfection 20-24 hours after seeding, or once cells are ~80% confluent, to ensure that the cells are actively dividing and are optimal for high transfection efficiency.

2. Transfect cells as follows:
   a. Prepare the plasmid mix as shown below. Click the plasmid for the Addgene entry.

Plasmid	**Amount (µg)**
eGag-pro-pol (v5)**	*x*
eGag-editor***	4.5-*x*
VSVG	0.5
sgRNA	5

Note: it is recommended to optimize the plasmids ratio.
For CRISPRoff (ZIM3), I typically use x=0.45. For a 10-cm dish, this corresponds to:

0.45 µg eGag-pro-pol (v5)
4.05 µg eGag-CRISPRoff (ZIM3)

b. While preparing the plasmid mix, take Opti-MEM and Mirus-LT1 out of the fridge and pre-warm them at room temperature. Gently vortex Mirus-LT1 before each use.
c. Mix 1.5 mL of Opti-MEM medium with the plasmid DNA mix, gently pipette few times, and add 45 µL Mirus-LT1. Pipette again and let it sit at room temperature for 15-30 minutes.****
d. Add mixture to cells dropwise. Mix by shaking the plates slightly (left to right, then front to back).

Day 4

(72 h after transfection)

3. (Optional, but recommended) Check the transfected cells under a fluorescent microscope. All our editor constructs in eGag-editor plasmids contain BFP fusion, and we integrate mCherry in the sgRNA plasmid backbone. Fluorescence can typically be observed as early as 24 hours post-transfection, and generally red/orange (mCherry) is much brighter than blue (BFP).

4. Harvest VLP-containing supernatant (optional: centrifuge for 5 min at 500 g to remove cell debris). Filter the clarified VLP-containing supernatant through a 0.45-µm PVDF filter.

5. Transfer the clarified supernatant to a sterile container and add Lenti-X Concentrator (Takara) at a 1:3 ratio (1 volume concentrator to 3 volumes supernatant). Mix by gentle inversion (for example, for 10 mL of media, add 3.33 mL of Lenti-X concentrator). Keep it in 4°C for at least 2 hours or overnight.

Day 5

6. Centrifuge the sample at 1,500 g for 45 minutes at 4°C (pre-chill the centrifuge and rotors). After centrifugation, an off-white pellet should be visible.

7. Carefully aspirate the supernatant without disturbing the pellet.

8. Briefly centrifuge again (1,500 g for 3 to 5 minutes at 4°C), then remove any residual supernatant using a P200 pipette tip.

9. Resuspend the pellet in PBS or Opti-MEM media (I typically do a 100-fold concentration, for example, use 100 µL PBS/Opti-MEM to resuspend the VLP pellet from 10 mL supernatant)

10. Freeze the VLP aliquots at a rate of -1°C/min and store at -80°C. Thaw VLPs on ice immediately prior to use.