GeticoFect™ 3000Plus Transfection Reagent for Lentivirus Production Instructions

Operation Steps

I. Experimental Preparation

(1) Reagents and Materials Preparation

Standard operating procedure (SOP) for lentivirus production using GeticoFect™ 3000Plus transfection reagent

(A) Material Preparation

Reagents:
DMEM (high glucose, with glutamine supplement, sodium pyruvate)
Fetal bovine serum (FBS)
Geneticin selective antibiotic (50 mg/mL)
Opti-MEM I reduced-serum medium (with glutamine supplement)
Opti-MEM I reduced-serum medium
Sodium pyruvate (100 mM)
Lentiviral packaging mixture
GeticoFect™ 3000Plus transfection reagent
pLenti6.3/V5-GW/EmGFP expression control vector
293, 293T, HT1080 cell lines
Polybrene™ reagent (10 mg/mL)

Reagents:
Crystal violet
Blasticidin S HCl

(B) Medium Preparation

1. **Complete medium**: For 293T and 293FT cell culture. In a sterile environment, mix 450 mL DMEM (high glucose, with glutamine supplement, sodium pyruvate) and 50 mL FBS. If using 293FT cells, additionally add 500 μg/mL geneticin selective antibiotic.
2. **Lentiviral packaging medium**: Mix 474 mL Opti-MEM I reduced-serum medium (with glutamine supplement), 25 mL FBS, and 1 mL sodium pyruvate (100 mM) under sterile conditions.

(C) Experimental Environment and Safety Precautions

- All virus preparation and related operations must strictly follow the Biosafety Level 2 (BL-2) protocol guidelines of the institution.
- After the experiment, all materials exposed to the virus must be treated with 10% bleach solution before disposal.

II. Lentivirus Production Process

(A) Forward Transfection (Taking 6-Well Plate as an Example, Adjust Dosages by Referring to the Table for Scaled-Up Cultivation)

Day 1 (Afternoon): Cell Seeding

- Resuspend appropriate 293T or 293FT cells in lentiviral packaging medium, seed into a 6-well culture plate at a density of 1.2×10^6 cells per well, adding 2 mL lentiviral packaging medium per well.
- Incubate the plate in a 37°C, 5% CO_2 incubator overnight.

Day 2 (Morning): Transfection Operation

- Cell density should reach 95–99% confluence for transfection. Equilibrate Opti-MEM I reduced-serum medium to room temperature, prepare Tube A and Tube B, and add reagents as per the table below:

Component	Volume in Tube A	Volume in Tube B
Opti-MEM I reduced-serum medium	250 µL	250 µL
GeticoFect™ 3000Plus transfection reagent	7 µL	-
T3000 enhancer reagent	-	6 µL
Lentiviral packaging mixture (1 µg/µL)	-	2.25 µL
pLenti expression vector	-	0.75 µg

- Slowly add the solution in Tube A to Tube B, pipette gently to mix, and incubate at room temperature for 10–20 minutes to form the lipid-DNA complex.
- During complex incubation, aspirate 1 mL medium from each culture well, leaving 1 mL medium per well.
- Slowly add 500 µL of the lipid-DNA complex to each culture well, dropping onto the well wall to avoid disturbing cells. Gently shake the plate after addition to distribute the complex evenly.
- Incubate the plate in a 37°C, 5% CO_2 incubator for 6 hours.
- After 6 hours, carefully aspirate the medium containing the lipid-DNA complex, treat with 10% bleach, and discard. Add 2 mL pre-warmed lentiviral packaging medium per well, handling gently to avoid cell damage.
- Return the plate to the incubator and continue incubation overnight at 37°C, 5% CO_2.

Day 3 (Morning): Harvest First Batch of Virus

- 24 hours after transfection, collect 2 mL cell supernatant from each culture well, transfer to a 15 mL centrifuge tube, and store at 4°C.
- Add 2 mL pre-warmed lentiviral packaging medium per well, return the plate to the 37°C, 5% CO_2 incubator, and continue incubation overnight.
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Day 4 (Afternoon): Harvest Second Batch of Virus

- Approximately 52 hours after transfection, collect 2 mL cell supernatant from each culture well, combine with the first batch, totaling 4 mL supernatant per well.
- Transfer 4 mL supernatant to a centrifuge tube, centrifuge at 2000 rpm for 10 minutes at room temperature to remove cell debris. After centrifugation, carefully aspirate the supernatant into a new centrifuge tube, discarding the pellet.
- Filter the clarified lentiviral supernatant through a 0.45 μm filter to further remove residual cell debris.
- Aliquot the filtered virus into cryotubes and store at -80°C. If virus concentration is needed, perform concentration before freezing, and minimize freeze-thaw cycles to maintain virus titer.

(B) Reverse Transfection (Taking 6-Well Plate as an Example, Adjust Dosages by Referring to the Table for Scaled-Up Cultivation)

Day 1 (Morning): Complex Formation and Cell Seeding

- Resuspend appropriate 293T or 293FT cells in pre-warmed lentiviral packaging medium, adjust cell concentration. For a 6-well plate, seed 3.6×10^6 cells per well.
- Equilibrate Opti-MEM I reduced-serum medium to room temperature, prepare Tube A and Tube B with the same reagent amounts as the forward transfection Day 2 operation.
- Add the solution in Tube A to Tube B, incubate at room temperature for 10–20 minutes.
- Add 500 μL of the lipid-DNA complex directly to each well of the 6-well plate.
- Immediately add 1 mL lentiviral packaging medium containing 3.6×10^6 cells to each well for seeding. Note: The cell seeding density in reverse transfection is 3 times higher than in forward transfection.
- Incubate the plate in a 37°C, 5% CO_2 incubator for 6 hours.

Day 1 (Afternoon): Medium Replacement

- 6 hours after transfection, carefully aspirate the medium containing the complex, treat with 10% bleach, and discard.
- Add 2 mL pre-warmed lentiviral packaging medium per well, then return the plate to the 37°C, 5% CO_2 incubator for overnight incubation.

Day 2 (Morning): Harvest First Batch of Virus

- Perform the same as the forward transfection Day 3 morning virus harvest steps.

Day 3 (Afternoon): Harvest Second Batch of Virus

- Perform the same as the forward transfection Day 4 afternoon virus harvest steps.

III. Lentivirus Titer Determination

(A) GFP-Based Titer Determination (Suitable for Lentiviruses Expressing GFP, Using 96-Well Plate for High-Throughput Flow Cytometry Analysis)

Day 1 (Morning)

- Seed HT1080 cells in a 96-well plate at 7000 cells per well with 100 µL culture medium.
- Incubate the 96-well plate in an incubator for 4–5 hours, waiting for virus transduction.
- Prepare fresh virus diluent before transduction: mix 15 mL fresh culture medium with 12 µL 10 mg/mL Polybrene reagent, vortex to mix, resulting in a final Polybrene concentration of 8 mg/mL.
- Take a 96-well round-bottom plate, add 135 µL of the above medium to each group of 4 wells.
- Add 15 µL lentiviral supernatant to the first row of wells in each group, making the total volume 150 µL for a 1:10 dilution, pipette gently to mix.
- Perform serial dilution from the first row: pipette 15 µL from the first row to the second row for a 1:100 dilution, and so on to achieve dilutions like 1:1000, 1:10000.
- Aspirate the original medium from HT1080 cell culture wells, add 100 µL of the prepared virus diluent at different concentrations to the corresponding HT1080 cell culture wells.
- Centrifuge the 96-well plate at 2000 rpm for 30 minutes at room temperature.
- After centrifugation, incubate the 96-well plate in an incubator overnight.

Day 2

- Aspirate the medium containing virus supernatant, replace with fresh HT1080 cell culture medium (without Polybrene reagent).
- Continue culturing cells for 3 days, then analyze the percentage of GFP-positive cells by flow cytometry.

Titer Calculation

- Determine the suitable dilution for titer calculation based on the percentage of GFP-positive cells, with an ideal transduction range of 1–20% GFP-positive cells.
- Calculate the titer using the formula.

(B) Blasticidin-Based Titer Determination (Taking 24-Well Plate as an Example)

Day 1 (Morning)

- Seed HT1080 cells in a 24-well plate at 42000 cells per well (30–50% confluence at seeding) with 500 µL culture medium.
- Incubate the 24-well plate in an incubator for 4–5 hours, waiting for virus transduction. Repeat titer determination is recommended.
- Prepare fresh virus diluent before transduction: same as the GFP-based method, mix 15 mL fresh culture medium with 12 µL 10 mg/mL Polybrene reagent, vortex to mix for a final Polybrene concentration of 8 mg/mL.
- Take 5 sterile centrifuge tubes, add 135 µL of the above medium to each, labeled as Tubes 1–5.
- Add 15 µL lentivirus to Tube 1, pipette to mix.
- Perform serial dilution for Tube 1: pipette 15 µL from Tube 1 to Tube 2 for a 1:100 dilution, and so on to Tubes 3–5 for dilutions of 1:1000, 1:10000, 1:100000.
- 4–5 hours after cell seeding, aspirate the medium from the 24-well plate, add 450 µL of the above Polybrene-containing medium to each well. Number the 24-well plate wells as 1–5 and a control well.
- Add 50 µL of the virus diluent from Tube 1 to Well 1, pipette to mix, resulting in a 1:100 virus dilution in Well 1 (total volume 500 µL, a 10-fold further dilution from the original 1:100).

- Add other diluted virus solutions to corresponding numbered wells similarly.
- Add 50 µL Polybrene-containing medium (without virus) to the control well.
- Gently shake the 24-well plate to mix the liquid evenly.
- Centrifuge the 24-well plate at 2000 rpm for 30 minutes at room temperature.
- After centrifugation, incubate the 24-well plate in an incubator overnight.

Day 2

- Aspirate the medium from each well, replace with 500 µL fresh culture medium (without Polybrene).
- Continue culturing cells for 24 hours.

Blasticidin Screening

- 48 hours after virus transduction, aspirate the medium, replace with selection medium containing blasticidin (final concentration 10 µg/mL).
- Replace the selection medium every 2 days for 10 consecutive days. Note: The control well should have no viable cells before staining.

Day 12

- Aspirate the medium, wash each well with 1 mL PBS.
- Prepare 1% crystal violet working solution (dissolve crystal violet in 10% ethanol aqueous solution).
- Add 250 µL diluted crystal violet solution to each well for staining, incubate at room temperature for 20 minutes.
- Aspirate the staining solution (reusable or dispose as specified), wash the plate multiple times with water to reduce background.

Titer Calculation.

IV. Transfection Method Calculation Tables

1. Scale-Up of Viral Production Using Forward Transfection (Table 1)

Cell Culture Vessel	Scale-Up Factor	Seeding Packaging Medium Volume (mL)	293T or 293FT Cells per Well	DNA Co-Transfection				GeticoFect 3000 (µL)	Replacement Lentiviral Packaging Medium Volume (mL)	Harvested Lentivirus Total Volume (mL)
				Opti-MEM I Medium Volume (mL)	Packaging Mixture (µg)	pLenti Vector (µg)	P3000 Reagent (µL)			
6-Well Plate	1	2	1.2×10^6	2×250 µL	2.25	0.75	6	7	2	2×2
60 mm	2.2	4	2.6×10^6	2×500 µL	5	1.7	13	15	4	2×4
10 cm	5.8	12	7.0×10^6	2×1.5 mL	13	4.3	35	41	12	2×12
T75	7.9	16	9.5×10^6	2×2 mL	17.8	5.9	47	55	16	2×16
T175	18.4	37	22.1×10^6	2×4.6 mL	41.4	13.8	111	129	37	2×37
Note: Scale-up factor calculated based on 6-well plate growth area										

2. Scale-Up of Viral Production Using Reverse Transfection (Table 2)

Cell Culture Vessel	Scale-Up Factor	Seeding Packaging Medium Volume (mL)	293T or 293FT Cells per Well	DNA Co-Transfection				Getico Fect 3000 (µL)	Replacement Lentiviral Packaging Medium Volume (mL)	Harvested Lentivirus Total Volume (mL)
				Opti-MEM I Medium Volume (mL)	Packaging Mixture (µg)	pLenti Vector (µg)	P3000 Reagent (µL)			
6-Well Plate	1	1	3.6×10^6	2×250 µL	2.25	0.75	6	7	2	2×2
60 mm	2.2	2	7.92×10^6	2×500 µL	5	1.7	13	15	4	2×4
10 cm	5.8	6	21×10^6	2×1.5 mL	13	4.3	35	41	12	2×12
T75	7.9	8	28.4×10^6	2×2 mL	17.8	5.9	47	55	16	2×16
T175	18.4	18	66.2×10^6	2×4.6mL	41.4	13.8	111	129	37	2X37