

Product Analysis Certificate

Ready-to-Use Packaging Plasmid Mix
Cat.# CPCP-K2A



Ready-to-Use Packaging Plasmid Mix

Product: Ready-to-Use Packaging Plasmid Mix

Catalog #: CPCP-K2A

Lot #: 12041101

Description:

The Ready-to-Use Packaging Plasmid Mix is a mixture of the human immunodeficiency virus (HIV) lentiviral packaging plasmid psPAX2 (Addgene, Cat.# 12260) and the pMD2.G plasmid (Addgene, Cat.# 12259) containing VSV-G. These plasmids provide all the necessary structural, regulatory, and replication proteins required to produce pseudotyped packaged lentiviral expression constructs when co-transfected with an HIV-based lentiviral expression construct (*e.g.* Collecta pRSI shRNA expression construct) into a producer 293T cell line (*e.g.* ATCC, Cat.# CRL-11268™). Pseudotyped lentiviral particles with VSV-G envelope protein can infect a variety of mammalian or non-mammalian, dividing or non-dividing cells. The kit contains 250 µg of lentiviral packaging plasmid mix that will be sufficient for 25 transfections in 10-cm culture plates.

The Ready-to-Use Packaging Plasmid Mix is compatible with most commercially-available second- and third-generation lentiviral vectors.

Biosafety Level: BSL-2

Storage: -20°C

Shelf Life: 12 months from date of receipt with proper storage

Shipping Conditions: Blue Ice or Dry Ice

Contents:

#	Catalog #	Description
1	CPCP-K2A	Ready-to-Use Packaging Plasmid Mix 250 µg, 0.5 µg/µl (500 µl × 1 tube)

Quality Control:

10 µg of Ready-to-Use Packaging Plasmid mix and 2 µg of pRSI12-U6-(sh)-UbiC-TagRFP-2A-Puro were co-transfected into ATCC 293T/17 (Addgene Cat.# CRL-11268™) producer cells with Lipofectamine® Reagent and Plus™ Reagent (Invitrogen) in a 10-cm culture plate as described in the included **Packaging of Individual Lentiviral Constructs** protocol. After transformation, the pRSI12 viral titer was $>1 \times 10^6$ TU/ml as determined by FACS analysis of 293T/17 cells infected by the pseudoviral supernatant.

APPROVED BY: _____

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PROTOCOLS

1. Packaging of Pooled Lentiviral Libraries
2. Packaging of Individual Lentiviral Constructs
3. Concentrating Pseudovirus (Optional)
4. Pseudoviral Titer Estimation

Required Materials:

- Cellecta Lentiviral shRNA Library or Construct (custom, from Cellecta; or generated by customer)
- 293T/17 Cell Line (ATCC, Cat.# CRL-11268™)
- Dulbecco's Modified Eagle Medium (D-MEM) (1X) (Mediatech CellGro, Cat.# 15-013-CV)
Note: ADD FRESH GLUTAMINE (1X) at the time a sealed bottle of D-MEM is opened, even if the label indicates glutamine has already been added. Glutamine in solution at +4°C has a half-life of 1 – 2 months, so glutamine(+) D-MEM purchased "off-the-shelf" from a supplier is to be regarded as glutamine(-). In our experience, the addition of glutamine increases titer approximately 2-fold. If GlutaMAX™ (Invitrogen) is used, addition of glutamine is not necessary.
- Glutamine (L-Alanyl-L-Glutamine, Dipeptide L-glutamine) (Mediatech, Cat.# 25-015-CI)
- Fetal Bovine Serum (recommended: Mediatech, Cat.# MT 35-010-CV)
- Puromycin
- D-PBS
- Trypsin-EDTA
- Polybrene® (hexadimethrine bromide) (Sigma-Aldrich, Cat.# 107689)
- 0.2-0.45 µm PES sterile syringe filters (Nalgene, Cat. # 194-2520)
- Tissue Culture Plates and Related Tissue Culture Supplies
- Lipofectamine™ Reagent (Invitrogen, Cat. # 18324-111)
- Plus™ Reagent (Invitrogen, Cat. # 11514-015)

Packaging of Pooled Lentiviral Libraries

The following protocol describes the generation of a packaged DECIPHER pooled lentiviral 27K shRNA library (27K shRNA complexity) using Invitrogen's Lipofectamine™ and Plus™ Reagent (see **Required Materials**). Other transfection reagents may be used, but the protocol should be adjusted to fit the manufacturer's protocol. The yield of recombinant lentiviral particles typically produced under these optimized conditions is 1-10 × 10⁶ TU/ml. In this protocol, using ten (10) 15-cm plates, at least 3 × 10⁸ TU of total pseudoviral particles can be made and then concentrated to up to 100-fold using several described methods.

1. Start growing 293T cells in D-MEM medium plus glutamine (see **Required Materials**), supplemented with 10% FBS without antibiotics, 2 to 3 days prior to transfection.

Day 1 – Plate Cells

2. Twenty four (24) hours prior to transfection, plate 12.5 × 10⁶ 293T cells in each of ten (10) untreated 15-cm plates (or 150 cm² flasks). Use 30 ml of media per plate. Disperse the cells and ensure even distribution. At the moment of transfection, the cells should have reached ~70%-80% confluency. Increase or decrease the number of 293T cells seeded if optimal confluency is not achieved in 24 hours. Incubate at 37°C in a CO₂ incubator for 24 hours.

Day 2 – Transfection

3. For each of the 10 plates, in sterile 14-ml polypropylene tubes, mix 60 µl (30 µg) of the Ready-to-use Packaging Plasmid Mix with 6 µg** of the plasmid library and add the plasmid mixture to 1,200 µl D-MEM medium without serum or antibiotics. Add 60 µl of Plus Reagent, mix, and incubate at room temperature for 15 min.

per plate	Component
60 µl	Ready-to-use Packaging Plasmid Mix (0.5 µg/µl)
6 µl	Plasmid shRNA Library (1 µg/µl) *
1,200 µl	D-MEM, no FBS, no antibiotics
60 µl	Plus Reagent
1,326 µl	Total volume

*** IMPORTANT: DO NOT use less than 60 µg (ten 15-cm plates) to package a batch of DECIPHER library. A smaller amount may cause shRNA insert representation to be adversely affected.**

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4. Add 990 μ l of Lipofectamine Reagent to 13.2 ml of D-MEM medium without serum or antibiotics in order to make a convenient master mix. Mix gently.

1 plate	10 plates (11X*)	Component
1,200 μ l	13,200 μ l	D-MEM, no FBS, no antibiotics
90 μ l	990 μ l	Lipofectamine
1,290 μ l	14,190 μ l	Total volume

* NOTE: Always add 1 or 2 extra reactions to avoid running out of master mix.

5. Add the diluted Lipofectamine Reagent (from step 4) to the DNA / Plus Reagent complex (from step 3), mix gently by flicking the tube or vortexing and incubate at room temperature for 15 min.
6. Add 2.616 ml of the DNA / Plus Reagent / Lipofectamine Reagent complex (from step 5) to each 15-cm plate from step 2, and mix complexes with medium by gentle rotation. Take care not to dislodge cells from the plate. Incubate at 37°C in the CO₂ incubator for 24 hours.

Day 3 – DNase Treatment (Optional)

7. At 24 hours post-transfection, replace the medium containing complexes with fresh D-MEM medium supplemented with 10% FBS, DNase I (1 U/ml), and MgCl₂ (4 mM). Continue incubation in the CO₂ incubator at 37°C for 24 hours. Overnight DNase I treatment before harvesting pseudovirus does not negatively affect viral titer or infectivity and helps prevent undesirable carryover of plasmid library into the pseudovirus prep.

Note: Failure to change the media the day after transfection results in large carryover of plasmid (free and/or Lipofectamine-bound) in your pseudoviral prep. This may cause problems with most downstream molecular biology applications, especially whenever there is a PCR step involved.

Day 4 – Collect Pseudoviral Supernatant

8. At 48 hours post-transfection, collect all 30 ml of the pseudovirus-containing medium from each plate and filter the supernatant (300 ml) through a Nalgene 0.2 – 0.45 μ m PES filter (a low protein binding filter) to remove debris and floating packaging cells. Failure to filter supernatant could result in carry-over of cells into your pseudoviral prep.

Note: Usually, the peak of pseudovirus production is achieved at 48 hours post-transfection. We recommend collecting the supernatant only once, at 48 hours post-transfection, in order to achieve higher titers. Supernatant can also be collected at 72 hours post-transfection—replace the collected 48-hour supernatant with 30 ml of fresh D-MEM medium supplemented with 10% FBS and continue incubation in the CO₂ incubator at 37°C for 24 hours.

Caution: You are working with infectious pseudoviral particles at this stage. Please follow the recommended guidelines for working with BSL-2 safety class materials (see **Safety Guidelines**).

9. Proceed to concentration step, or aliquot and store the non-concentrated supernatant at –80°C. Freezing and thawing may result in 10 – 20% loss of pseudoviral titer with each cycle.

Packaging of Individual Lentiviral Constructs

The following protocol describes the generation of ten (10) pseudoviral packaged lentiviral constructs using Invitrogen's Lipofectamine™ and Plus™ Reagent. Other transfection reagents may be used, but the protocol should be adjusted to fit the manufacturer's protocol. The yield of recombinant lentiviral particles typically produced under these optimized conditions is 1-10 \times 10⁶ TU/ml. In this protocol, at least 1 \times 10⁷ TU of total pseudoviral particles can be produced from each 10-cm plate and then concentrated up to 100-fold using several described methods.

1. Start growing 293T cells in D-MEM medium plus glutamine (see **Required Materials**), supplemented with 10% FBS without antibiotics, 2 to 3 days prior to transfection.

Day 1 – Plate Cells

2. Twenty four (24) hours prior to transfection, plate 4 \times 10⁶ 293T cells/plate in ten (10) untreated 10-cm plates. Use 10 ml of media per plate. Disperse the cells and ensure even distribution. At the moment of transfection, the cells should have reached ~70%-80% confluency. Increase or decrease the number of 293T cells seeded if optimal confluency is not achieved in 24 hours. Incubate at 37°C in a CO₂ incubator for 24 hours.

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Day 2 – Transfection

3. For each construct, mix 20 μl (10 μg) of the Ready-to-use Packaging Plasmid Mix (Cat.# CPCP-K2A) with 2 μg (2 μl – 20 μl depending on concentration) of the plasmid construct and then add the plasmid mixture to 1 ml D-MEM medium without serum or antibiotics. Add 20 μl of Plus Reagent, mix, and incubate at room temperature for 15 min. in a sterile 5 or 14-ml tube.

1 plate	Component
20 μl	Ready-to-use Packaging Plasmid Mix (0.5 $\mu\text{g}/\mu\text{l}$) *
4 μl	Plasmid shRNA Construct (0.5 $\mu\text{g}/\mu\text{l}$)
1,000 μl	D-MEM, no FBS, no antibiotics
20 μl	Plus Reagent
1,044 μl	Total volume

* For packaging mixes from other vendors, refer to manufacturer's protocol.

4. Make a master mix, using 30 μl Lipofectamine Reagent and 1 ml of D-MEM medium, without serum or antibiotics, per plate. Mix gently.

per plate	11X Master Mix	Component
1,000 μl	11,000 μl	D-MEM, no FBS, no antibiotics
30 μl	330 μl	Lipofectamine
1,030 μl	11,330 μl	Total volume

5. Add 1 ml of the diluted Lipofectamine Reagent (from step 4) to the DNA / Plus™ Reagent complex (from step 3), mix gently by flicking the tube or vortexing, and incubate at room temperature for 15 min.
6. Add 2 ml of the DNA / Plus Reagent / Lipofectamine Reagent complex (from step 5) to each 10-cm plate from step 2, and mix complexes with medium by gentle rotation. Take care not to dislodge cells from the plate. Incubate at 37°C in the CO₂ incubator for 24 hours.

Day 3 – DNase Treatment (Optional)

7. At 24 hours post-transfection, replace the medium containing complexes with fresh D-MEM medium supplemented with 10% FBS, DNase I (1 U/ml), and MgCl₂ (4 mM). Continue incubation in the CO₂ incubator at 37°C for 24 hours. Overnight DNase I treatment before harvesting pseudovirus does not negatively affect viral titer or infectivity and helps prevent undesirable carryover of plasmid construct into the pseudovirus prep.

Note: Failure to change the media the day after transfection results in large carryover of plasmid (free and/or Lipofectamine-bound) in your pseudoviral prep. This may cause problems with most downstream molecular biology applications, especially whenever there is a PCR step involved.

Day 4 – Collect Pseudoviral Supernatant

8. At 48 hours post-transfection, collect all 10 ml of the pseudovirus-containing medium from each plate and filter through a Nalgene 0.2 – 0.45 μm PES filter (a low protein binding filter) to remove debris and floating packaging cells. Failure to filter supernatant could result in carry-over of cells into your pseudoviral prep.

Note: Usually, the peak of pseudovirus production is achieved at 48 hours post-transfection. We recommend collecting the supernatant only once, at 48 hours post-transfection, in order to achieve higher titers. Supernatant can also be collected at 72 hours post-transfection—replace the collected 48-hour supernatant with 10 ml of fresh D-MEM medium supplemented with 10% FBS and continue incubation in the CO₂ incubator at 37°C for 24 hours.

Caution: You are working with infectious pseudoviral particles at this stage. Please follow the recommended guidelines for working with BSL-2 safety class materials (see Section H, Safety Guidelines).

9. Proceed to concentration step, or aliquot and store the non-concentrated supernatant at –80°C. Freezing and thawing may result in 10 – 20% loss of pseudoviral titer with each cycle.

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Concentrating Pseudovirus (Optional)

Although concentrating pseudovirus is optional, it is recommended if (1) very high titer pseudovirus stock may be needed to achieve desired MOI in hard-to-infect target cells or (2) pseudovirus should be suspended in another media (besides the standard PBS/10%FBS) which is optimal for sensitive target cells. However, because of the additional manipulation of samples, there is the added risk of contamination and loss of pseudovirus.

The following protocol was optimized to concentrate pseudovirus with high recovery. The protocol assumes that pseudoviral supernatant was harvested 48 hours after transfection and filtered as in step 8 above.

1. Aliquot pseudoviral supernatant in clear centrifuge tubes.
2. Add Polybrene to a final concentration of 5 µg/ml, and incubate for 1 hour at +4°C.
3. Centrifuge at 10,000 rpm for 1 hour at +4°C in a Beckman JA-14 (or JA-10) or equivalent rotor. Mark the tubes to identify the location where the pellet will be. At the end of centrifugation, you may or may not be able to see a pellet—assume it is at the location of the mark.
4. Immediately discard the supernatant by aspirating.
5. Place the tube on ice, resuspend the (in)visible pellet in PBS/10% FBS, make aliquots, and freeze at –80°C.

Alternatively, you may concentrate pseudovirus by the any of the methods below. However, the yield of pseudovirus is superior (~80% recovery) using Cellecta's protocol above.

- Ultracentrifugation at 50,000 x g for 90 min at +4°C
- Sucrose cushion ultracentrifugation
- PEG precipitation, followed by low speed centrifugation

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Pseudoviral Titer Estimation

Most Cellecta lentiviral vectors all express both fluorescent and puromycin resistance markers. Titers are calculated based on the percentage of either RFP- or GFP-positive or puromycin-resistant cells.

We recommend that you estimate the titer of the pseudovirus-containing supernatant before proceeding with transduction experiments for the following reasons:

- To ensure that pseudoviral stock is viable
- To determine the amount of pseudoviral particles (transduction units (TU)) necessary to achieve the desirable percentage of transduced target cells (multiplicity of infection (MOI))
- To control the number of copies of integrated pseudoviral constructs per target cell

To check pseudoviral titer, we recommend choosing a cell line appropriate for your experimental system. Most of the commonly used mammalian cell lines can be effectively transduced by lentiviral constructs. Relative titers can vary up to 50-fold depending on the chosen cell line.

Check Toxicity of Polybrene®

Polybrene is a polycation that neutralizes charge interactions to increase binding between the pseudoviral envelope and the cellular membrane. The optimal concentration of Polybrene depends on cell type and may need to be empirically determined. Excessive exposure to Polybrene can be toxic to some cells.

Before conducting the titer estimation experiment, we recommended performing a Polybrene toxicity titration in target cells. Grow cells in complete culture medium with a range of Polybrene concentrations (0 µg/ml, 2.5 µg/ml, 5 µg/ml) for 24 hours, and then replace old medium with Polybrene-free complete culture medium. Grow cells for an additional 48 hours, and then check toxicity by counting viable cells. For your experiments, use the highest concentration of Polybrene that results in less than 10% cell toxicity compared to no Polybrene (typically, 5 µg/ml is recommended). For some cell types, you cannot use Polybrene.

Transduction Protocol For Titering in HEK293 cells.

Please read the entire protocol before beginning your experiment. For other plate formats, the volumes should be adjusted depending on the growth area of the well or plate.

NOTE: We highly recommend also titering the library directly in your target cells prior to beginning your experiment. This ensures the greatest accuracy of MOI and reproducibility of transduction. Use of positive transduction controls (as is the common practice in the protocols of other vendors) is not recommended, because of batch-to-batch variation due to transduction conditions and quality of cells at that time.

Day 1.

1. Quickly thaw the pseudoviral particles in a water bath at 37°C. Transfer the thawed particles to a laminar flow hood, gently mix by rotation, inversion, or gentle vortexing, and keep on ice.

Caution: Only open the tube containing the pseudoviral particles in the laminar flow hood.

Note: Unused pseudoviral stock may be refrozen at –80°C, but it will result in a loss of about 10 – 20% in titer.

2. Trypsinize and resuspend cells to a density of 1×10^5 cells/ml* in D-MEM supplemented with 10% FBS and 5 µg/ml Polybrene. Aliquot 1 ml/well in a 12-well plate and add 0 µl, 1 µl, 3.3 µl, 10 µl, 33 µl, and 100 µl pseudoviral stock, prepared by serial dilution, to six different wells. Mix and return cells to CO₂ incubator. Grow cells under standard conditions for 24 hours.

***Note:** Be sure to record the **original** # of cells at Time of Infection, as this is critical in titer calculation.

Day 2.

3. At 24 hours post-transduction, replace media with fresh D-MEM supplemented with 10% FBS and without Polybrene. Return cells to CO₂ incubator, and grow under standard conditions for 24 hours.

Day 3. (48 hours after transduction)

Fluorescence-based titering:

By calculating the percentage of RFP- or GFP-positive cells using flow cytometry you can determine the pseudoviral titer (if the library vector contains a fluorescent marker). Detach cells from the plate by trypsin treatment, centrifuge, resuspend in 1X D-PBS at approximately 10×10^6 cells/ml, and determine the percentage of transduced (fluorescing) cells by flow cytometry. In order to set up a gate for counting RFP- or GFP-positive cells, first analyze the background (RFP or GFP) level of control non-transduced cells (0 µl of pseudoviral stock). Based on the percentage of transduced cells and the volume of pseudoviral stock used, calculate the original concentration of infective pseudoviral particles in the pseudoviral stock (transduction units) per ml (TU/ml).

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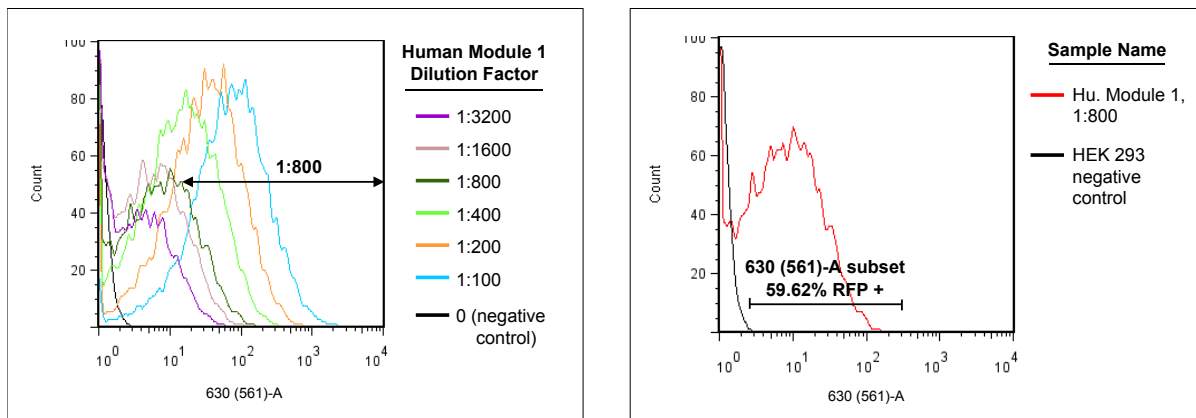
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Note: Depending on cell type, you may need to wait 72 hours after transduction before estimating titer by RFP or GFP fluorescence. For FACS Analysis of TagRFP, set excitation to 530 or 561 nm and emission between 580-600 nm.

Example of Titer Calculation:

FACS Analysis was performed on HEK 293 cells transduced with several dilutions of the DECIPHER Human Module 1 pseudoviral stock. Titer was calculated from a dilution that resulted in approximately 60% infected cells.



FACS Analysis of TagRFP for cells infected with varying dilutions of packaged DECIPHER library.

Formulas for determining titer and total TU:

$$(\text{Cell \# at Time of Infection}) \times (\text{Dilution factor}) \times (\% \text{ Positive cells}) / (\text{Volume of inoculum}) = \text{TU} / \text{Unit volume}$$

$$\text{TU} / \text{Unit volume} \times \text{Total Lentiviral Library volume} = \text{TOTAL TU}$$

Example Calculations:

$$(1.0 \times 10^5 \text{ cells}) \times (800) \times (.5962) / (0.3 \text{ ml}) = 3.18 \times 10^8 \text{ TU/ml for HEK 293 target cells}$$

$$3.18 \times 10^8 \text{ TU/ml} \times 1.5 \text{ ml Total Lentiviral Library volume} = 4.77 \times 10^8 \text{ TU}$$

Puromycin-based titering:

At 72 hours after infection, split cells into two samples. Grow one sample with antibiotic selection, and one sample without (**NOTE:** Before performing the following experiment, we recommend to first determine the optimal concentration of antibiotic using the **Puromycin Kill Curve** protocol below). After 72 hours of growth in puromycin, count the number of viable cells in the selected and unselected samples. The ratio of selected/unselected viable cells gives the percentage of infected cells. Then, calculate relative pseudoviral titer as previously described. This method of calculating virus titer does not take into account the effect of puromycin on the growth rate of puromycin-resistant cells. Please note that the titer determined by Puromycin selection may differ from the titer determined by counting RFP- or GFP-positive cells using flow cytometry, and it also depends on cell type and selection conditions.

Puromycin Kill Curve

In order to generate a purely transduced population of cells, it is important to determine the minimum amount of puromycin required to kill untransduced cells. This can be done empirically by generating a kill curve, as follows:

Trypsinize and resuspend cells to a density of 1×10^5 cells/ml in growth media, aliquot 1 ml per well in a 12-well plate, and add puromycin at 0 $\mu\text{g/ml}$, 0.5 $\mu\text{g/ml}$, 1 $\mu\text{g/ml}$, 2 $\mu\text{g/ml}$, 5 $\mu\text{g/ml}$, and 10 $\mu\text{g/ml}$ in six different wells. Mix and return cells to incubator. Grow cells under standard conditions for 48 hours.

For puromycin, the minimum antibiotic concentration to use is the lowest concentration that kills 99.9% of cells in 72 hours.

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Troubleshooting

Low Pseudoviral Titer (10^6 TU/ml in supernatant)

1. Poor transfection efficiency

Problem: 293T Cells have too high or too low density

Solution: Plate fewer or more cells in order to have about 70%-80% confluency at transfection stage.

Problem: Plasmid DNA/Lipofectamine/Plus Reagent ratios are incorrect

Solution: Optimize the ratios using the guidelines provided in the Lipofectamine protocol.

2. Inefficient production of the pseudovirus

Problem: 293T Cells are of poor quality

Solutions:

- Optimize growth conditions, check growth medium, and don't grow 293T cells for more than 20 passages.
- Check for mycoplasma contamination.
- Do not overgrow the cells (do not allow the cells to reach more than 90% confluency in order to keep the culture continuously in logarithmic growth phase).

Problem: Pseudoviral supernatant harvested too early or too late

Solution: Harvest supernatant 48 hours after transfection.

Inefficient Transduction of Packaged Library or Construct

1. Poor infection efficiency

Problem: Target cells have too high or too low density

Solution: Plate fewer or more cells in order to have about 50% confluency at infection stage.

Problem: Target cell line may be difficult to transduce

Solutions:

- Use a higher concentration of pseudoviral particles.
- Optimize the transduction protocol and use HEK293 cell line as positive control cells.
- Perform "Spinoculation" to improve transduction efficiency (email us at tech@cellecta.com for protocol).
- Check to see if Polybrene was added at 5 μ g/ml.

Problem: Wrong amount of Polybrene added during infection stage

Solution: If Polybrene is toxic to the target cells, optimize Polybrene concentration in the range of 0 – 5 μ g/ml.

Problem: Loss of pseudoviral titer during storage

Solution: Ensure storage of aliquoted packaged pseudovirus at -80°C . Each freeze-thaw cycle causes reduction of the titer by 10 – 20%. Use a fresh stock for transduction.

Problem: The assay is performed too early

Solution: Normally, the maximal expression of integrated provirus is expected to develop by 48-72 hours after infection. However, some cells exhibit delayed expression. Try the assay at a later time, such as 96 hours.

2. Transduction affects target cell viability

Problem: Polybrene is toxic for target cells

Solution: Optimize the concentration and exposure time to Polybrene during the transduction step. For some sensitive cells, Polybrene should not be used.

Problem: Pseudovirus-containing conditioned media is toxic to target cells.

Solution: Concentrate and resuspend the pseudovirus in target cell growth media.

Problem: Pseudovirus itself is toxic to target cells (Polybrene and/or conditioned media is not toxic).

Solutions:

- Decrease the incubation time of pseudovirus with target cells.
- Perform two sequential transductions, with short incubation times.

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3. No expression of fluorescent protein, selection marker, recombinant protein, shRNA, peptide, etc. in target cells

Problem: Promoter is not functional in target cells.

Solutions:

- Change the target cells.
- Replace the ineffective promoter(s) with EF1, CMV, PGK, UbiC for marker expression, and/or H1 or U6 (H1-Tet or U6-Tet) (for shRNA expression).
- Contact Cellecta at tech@cellecta.com to have the library or construct cloned in another vector with different promoter(s).

Technical Support

For additional information or technical assistance, please refer to the questions above and contact us by phone or email:

Phone: +1 (650) 938-3910
Toll-Free: +1 (877) 938-3910
Fax: +1 (650) 938-3911

E-mail:

Technical Support: tech@cellecta.com
General Information: info@cellecta.com
Sales: sales@cellecta.com
Orders: orders@cellecta.com

Blog: <http://www.cellecta.com/blog/>

Postal Mail: Cellecta, Inc.
320 Logue Ave.
Mountain View, CA 94043

For more information about Cellecta's products and services, please visit our web site at <http://www.cellecta.com>.

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Safety Guidelines

The HIV-based lentivector system is designed to maximize its biosafety features, which include:

- A deletion in the enhancer of the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- The RSV promoter upstream of 5'LTR in the lentivector allows efficient Tat-independent production of viral RNA, reducing the number of genes from HIV-1 that are used in this system.
- Number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev). The corresponding proteins are expressed from different plasmids lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector to prevent generation of recombinant replication-competent virus.
- None of the HIV-1 genes (gag, pol, rev) will be present in the packaged pseudoviral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.
- Pseudoviral particles will carry only a copy of your expression construct.

Despite the above safety features, use of HIV-based vectors falls within NIH Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus or the possibility of insertional mutagenesis. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at:

<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm>

It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviruses and follow standard microbiological practices, which include:

- Wear gloves and lab coat at all times when conducting the procedure.
- Always work with pseudoviral particles in a Class II laminar flow hood.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.

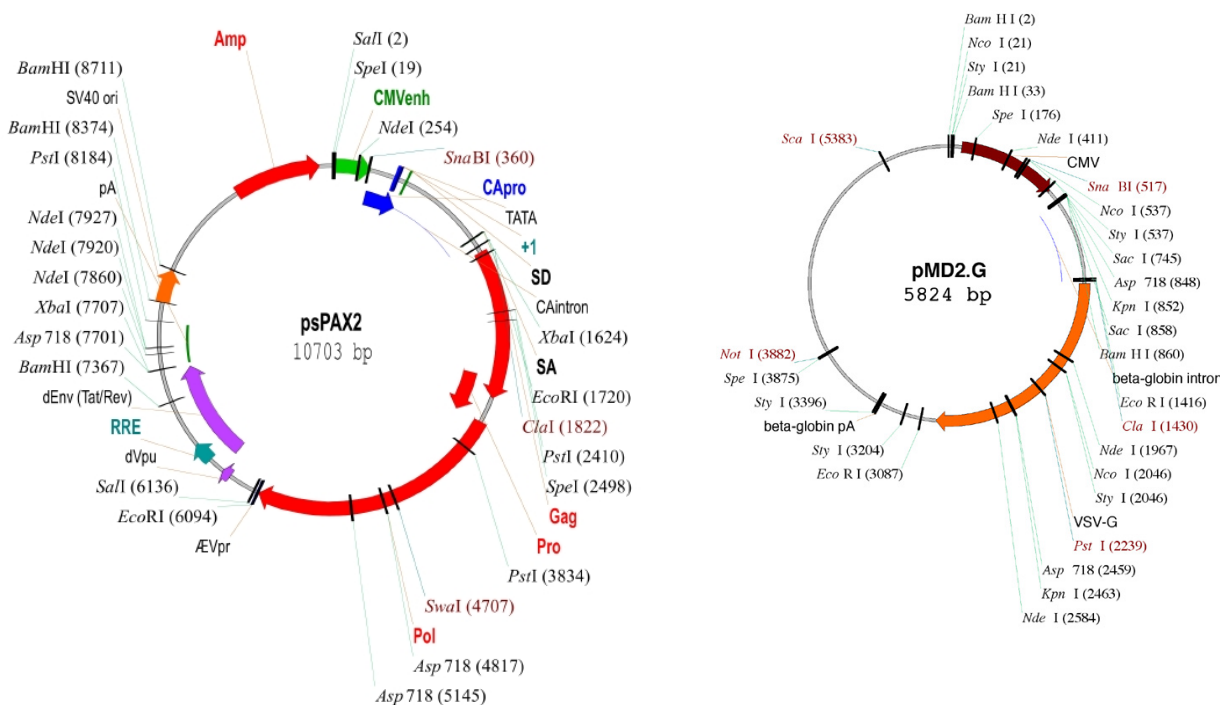
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Appendix

1. Packaging Plasmid Maps



For detailed maps, sequences, feature maps, and restriction maps for psPAX2 and pMD2.G, please see the Addgene website:

psPAX2: <http://www.addgene.org/12260/>

pMD2.G: <http://www.addgene.org/12259/>

For maps, sequences, cassette designs, feature maps, and restriction maps for the Lentiviral shRNA Expression Vectors **pRSI9-U6-(sh)-UbiC-TagRFP-2A-Puro** and **pRSI12-U6-(sh)-UbiC-TagRFP-2A-Puro**, please see the DECIPHER Project website:

<http://www.decipherproject.net/support/#vector-info>

For all other vectors, please contact Collecta at tech@collecta.com.

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Terms and Conditions

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