Product Manual

ViraBind™ Lentivirus Concentration and Purification Kit

Catalog Number

VPK-090 **2 preps**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

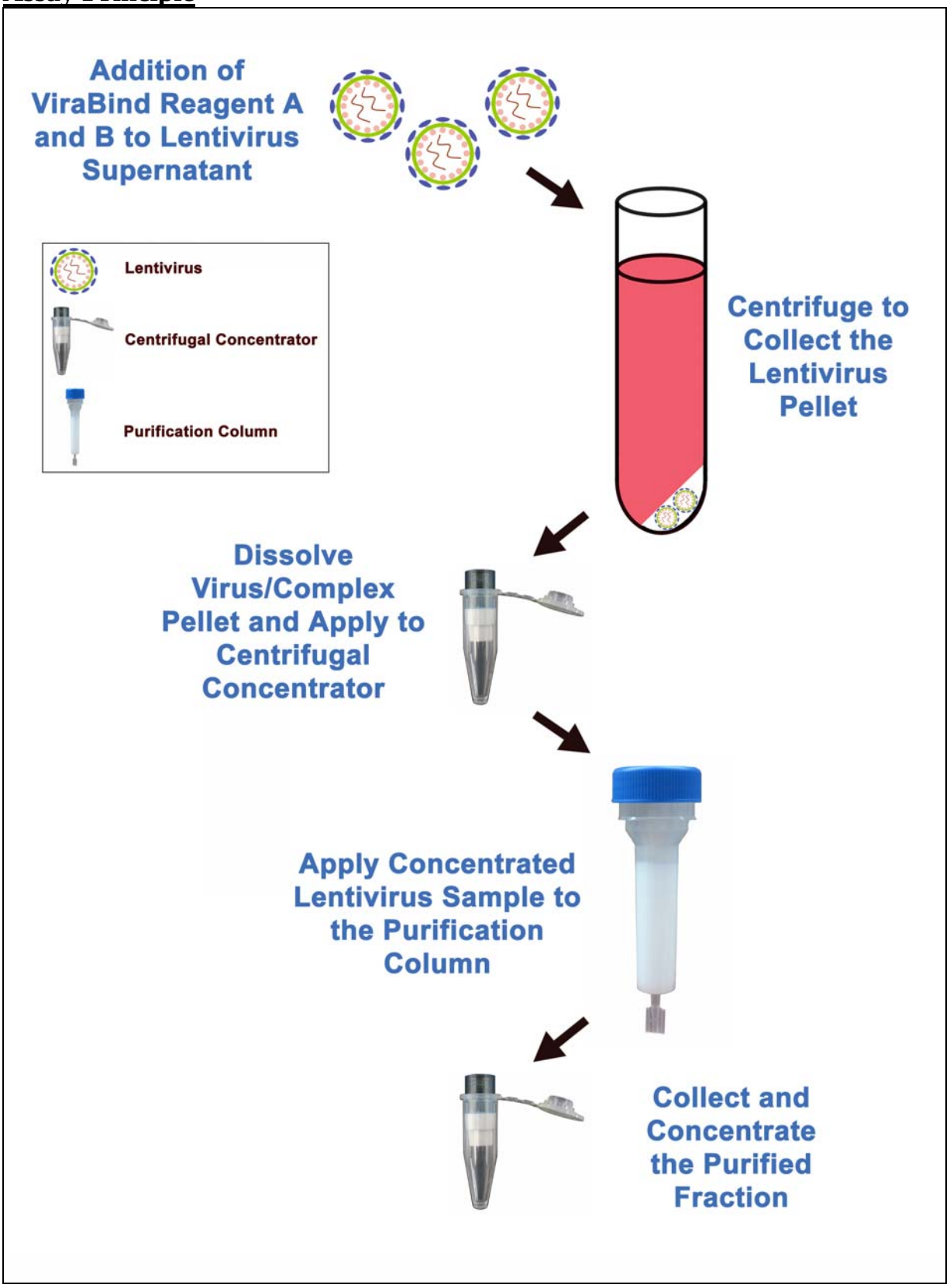
Lentivirus vectors based on the human immunodeficiency virus-1 (HIV-1) have become a promising vector for gene transfer studies. The advantageous feature of lentivirus vector is the ability for gene transfer and integration into dividing and non-dividing cells. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens the target cell range. Lentiviral vectors have been shown to deliver genes to neurons, lymphocytes and macrophages, cell types that previous retrovirus vectors could not be used. Lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses. Recently, the lentivirus system is widely used to integrate siRNA efficiently in a wide variety of cell lines and primary cells both *in vitro* and *in vivo*.

Lentivirus particles are produced from 293T cells through transient transfection of 3 or 4 plasmids that encodes for the components of the virion. Viral medium containing viral particles produced by packaging cells within 48-72 hr can be harvested and frozen. To obtain a higher titer, pseudovirus supernatant can be concentrated by ultracentrifuging. As a consequence, the ultracentrifugation step also concentrates cellular debris, membrane fragments, and denatured proteins derived from culture media of virus-producing cells. This unwanted material in the crude vector preparation is toxic to target cells, especially primary cells, and may cause immunogenic reactions in experimental animal models by *in vivo* vector administration. Therefore, to reduce undesirable effects and increase gene transfer efficiency, the purification of virus vector becomes essential.

ViraBind™ Lentivirus Concentration and Purification Kit does not involve ultracentrifugation. The lentiviruses are first pelleted from viral supernatant with ViraBind™ reagents (patented technology), then further purified and concentrated through a column and a concentrator, respectively (see Assay Principle below). The entire procedure takes about 3 hrs. Each preparation can handle up to 100 mL of lentiviral supernatant (10^{6-7} TU/mL) resulting in 100 µL of highly purified lentivirus (10^{9-10} TU/mL).

ViraBind™ Lentivirus Concentration and Purification Kit provides an efficient system for quick lentiviral purification with high recovery (>60%). The highly purified and concentrated viruses can be used in primary cell infections and *in vivo* applications. The system may be adapted to purification of other viral types, such as MMLV based retrovirus.

Assay Principle



Related Products

1. VPK-200: ViraSafe™ Universal Lentivirus Expression System
2. LTV-100: 293LTV Lentiviral Cell Line
3. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
4. VPK-108-HIV-p24: QuickTiter™ Lentivirus Quantitation Kit (HIV p24 ELISA)
5. VPK-112: QuickTiter™ Lentivirus Quantitation Kit
6. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
7. VPK-130: ViraBind™ Retrovirus Concentration and Purification Kit
8. VPK-100: ViraBind™ Adenovirus Purification Kit

Kit Components

1. ViraBind™ Lentivirus Reagent A (100X) (Part No. 309001): One sterile tube – 2.0 mL.
2. ViraBind™ Lentivirus Reagent B (100X) (Part No. 309002): One sterile tube – 2.0 mL.
3. Purification Columns (Part No. 309003): Two columns containing purification media.
4. Centrifugal Concentrators (Part No. 309004): Four units with 8 recovery tubes.
5. Purification Buffer (Part No. 309005): One bottle – 50.0 mL.

Materials Not Supplied

1. Lentivirus packaging plasmid mix and expression construct
2. Transfection Reagent
3. HEK 293T cells and cell culture growth medium
4. Centrifuge (capable of 10,000 x g)
5. Microfuge (capable of 10,000 x g)

Storage

Store all kit components at 4ºC until their expiration dates.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms.

Pseudovirus Production

The following procedure is suggested for a 10cm dish and may be optimized to suit individual needs. Please refer to the user manual when the lentivirus expression systems from Invitrogen or System Biosciences are used.

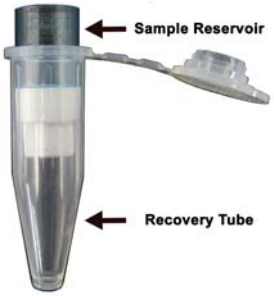
1. Use HEK 293T cells that have been passaged 2-3 times prior to transfection. Culture these cells until the monolayer is 70-80% confluent.
2. Replace the cell culture media with new growth media, 10 mL per 10 cm dish.
3. Transfect cells with the packaging plasmid mix and your expression construct. When using Lipofectamine™, please refer to Invitrogen's Lipofectamine™ reagent manual.
4. After 36-48 hrs, harvest all 10 mL medium in a 15 mL conical tube and centrifuge for 5 min at 3000 rpm to pellet the cell debris. Filter the supernatant through a 0.45 µm low protein binding filter.
5. The viral supernatant can be stored at -80ºC or immediately purified (see purification instructions below).

Protocol

I. Purification and Concentration

The following procedure is written for purification and concentration of 100 mL of lentiviral supernatant. For lentiviral samples that are less than 100 mL, the amount of ViraBind™ Lentivirus Reagent A, B (step 1) and Purification Buffer (step 5) needed should be calculated proportionally.

1. Add 1 mL of ViraBind™ Lentivirus Reagent A (100X) to 100 mL of viral supernatant, mix by inverting. Immediately add 1 mL of ViraBind™ Lentivirus Reagent B (100X) and mix by inverting.
2. Incubate for 60 minutes at 37ºC.
3. Centrifuge the complexed lentivirus for 15 minutes at 10,000 x g. A pellet should be visible.
4. Carefully aspirate the media and collect the pellet.
5. Resuspend and dissolve the complexed lentivirus pellet in 2 mL of Purification Buffer. Vortex the solution to dissolve the pellet.
   Note: The solution may appear hazy.
6. Centrifuge the solution in a microcentrifuge tube for 5 minutes at 10,000 x g to remove any insoluble material. Transfer the supernatant to another tube and centrifuge again for 5 minutes at 10,000 x g. Carefully recover the supernatant.
7. Assemble a Centrifugal Concentrator unit by inserting the blue sample reservoir into a recovery tube.



8. Apply 0.5 mL of the dissolved lentivirus solution to the sample reservoir of the Centrifugal Concentrator. Cap the concentrator and place into a tabletop centrifuge (Microfuge). Centrifuge for 10 minutes at 10,000 x g. As the sample is concentrated, top off the concentrator with additional lentivirus stock, centrifuging between. Flow through can be discarded.
9. Continue to concentrate the lentivirus solution until 200 µL remains in the sample reservoir.
10. Using a clean recovery tube, collect the lentivirus solution by inverting the sample reservoir into the tube and briefly centrifuging to collect all of the liquid. Adjust the final volume to 200 µL with Purification Buffer.

II. Purification Column

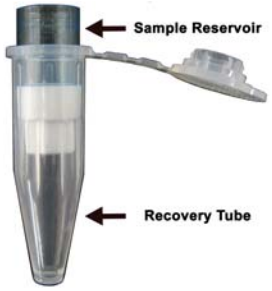
1. Completely resuspend the column packing material in the Purification Column by inverting and shaking (to create a slurry).
2. To pack the Purification Column, place in a 50-mL conical tube and centrifuge 3 minutes at 1000 rpm in a standard cell culture centrifuge (~200 x g).
3. Remove the break-off tip from the bottom of the Purification Column (by twisting) and immediately place into an empty 50-mL conical tube. Loosen the blue cap and allow the packing material liquid to drip through by gravity.

4. Once the liquid has completely come through, and the column stops dripping, add the 200 µL of concentrated lentivirus solution (step 10 above) to the top of the column. Slowly add the solution dropwise, distributing it evenly over the packing material.
   Note: To obtain maximum purification results, always add solutions dropwise to the column as not to disturb the column bed.

5. Allow the concentrated lentivirus solution to flow into the packing material. Once the flow has stopped, discard the flow through in the conical tube.
6. Slowly add 800 µL of Purification Buffer to the top of the column, dropwise, distributing it evenly over the packing material. Once the flow has stopped, carefully add another 3 mL of Purification Buffer to the column in the same manner.
   Note: Avoid disturbing the column bed.
7. Once the column flow has stopped, transfer the Purification Column to a clean, new 50-mL conical tube.
8. Finally, add 2.0 mL of Purification Buffer to the column and collect the flow through fraction (containing purified lentivirus).
   Note: A 2.0 mL fraction should be recovered.

III. Final Buffer Exchange and Concentration

1. Assemble a Centrifugal Concentrator unit by inserting the blue sample reservoir into a recovery tube.



2. Apply 0.5 mL of the recovered lentivirus fraction (step 8 above) to the sample reservoir of the Centrifugal Concentrator. Cap the concentrator and place into a tabletop centrifuge (Microfuge). Centrifuge for 5 minutes at 10,000 x g. As the fraction sample is concentrated, top off the concentrator with additional lentivirus solution, centrifuging between. Flow through can be discarded.
3. Continue to concentrate the lentivirus fraction until 100 µL remains in the sample reservoir.
4. Add 400 µL of PBS, or desired final buffer, to the Concentrator and continue to centrifuge until 100 µL remains. Repeat step 4 once more.
5. Finally, concentrate until the desired final volume.
6. Using a clean recovery tube, collect the concentrated lentivirus sample by inverting the sample reservoir into the tube and briefly centrifuging to collect all of the liquid.

Example of Results

The following figures demonstrate typical purification results. One should use the data below for reference only. This data should not be used to interpret actual results.

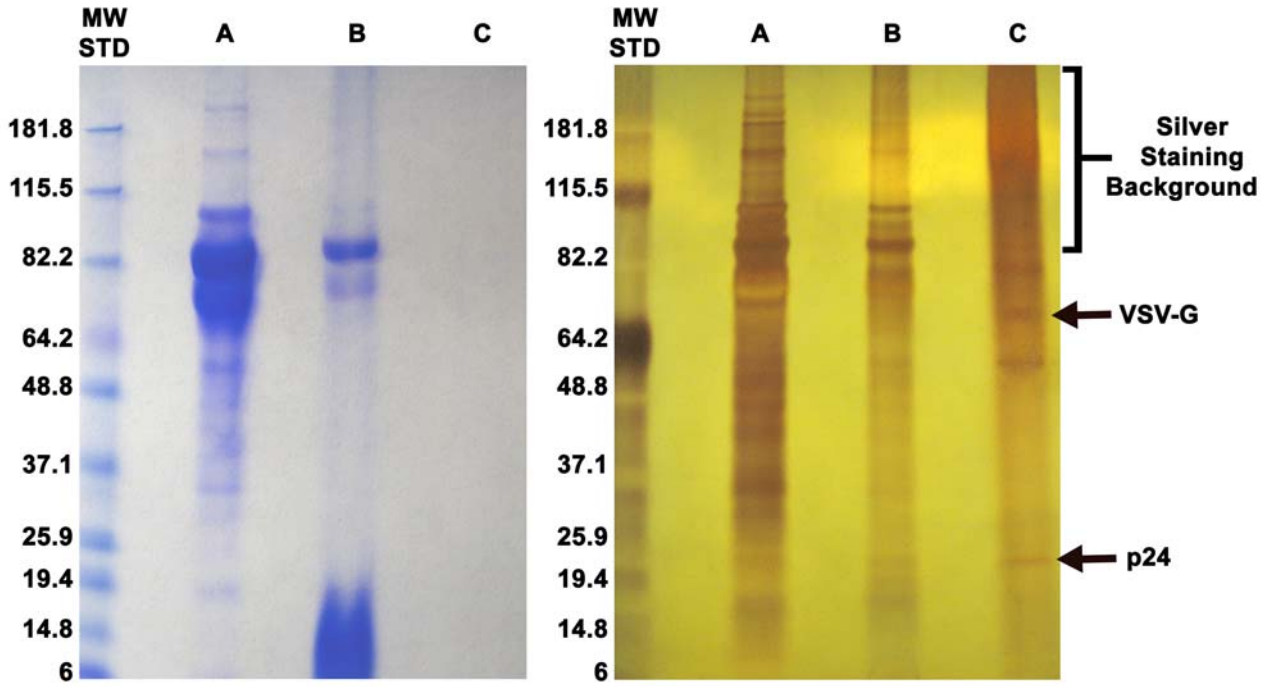


Figure 1: **Electrophoretic Profile of Purified GFP lentivirus.** 50 mL of GFP lentiviral supernatant was concentrated and purified according to the described Assay Instructions. Lentiviral Supernatant (A), Virus Pellet (B) and Purified Virus Fraction (C) were analyzed on SDS-PAGE. Proteins were visualized by Commassie blue stain (left) and silver stain (right). The VSV-G envelope protein and p24 coat protein (the most abundant proteins in the vector) are indicated.

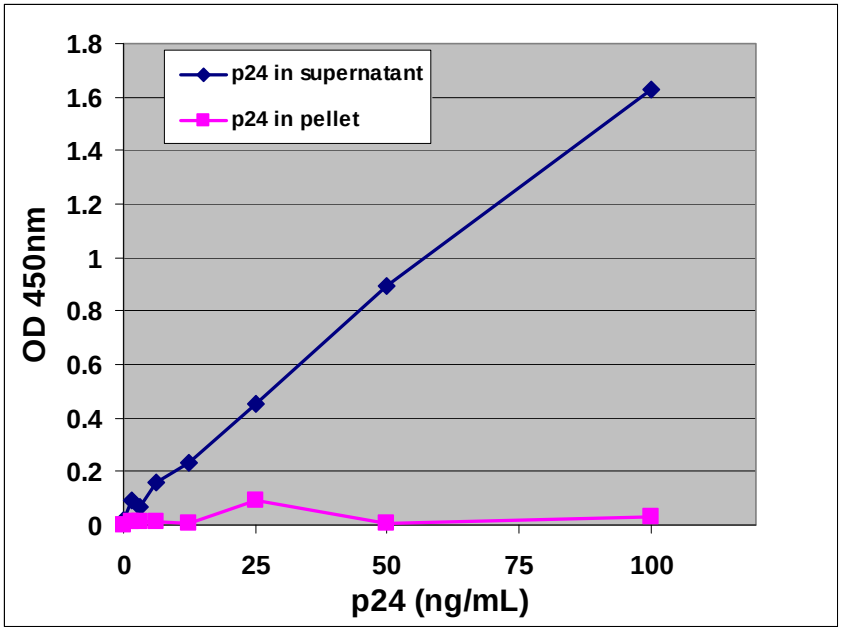


Figure 2: Free p24 does not complex with ViraBind™. Recombinant p24 diluted in culture medium was treated with ViraBind™ Lentivirus Reagents. The amount of p24 in supernatant and pellet was measured by p24 ELISA (Cat# VPK-107, Lentivirus Associated HIV p24 ELISA).

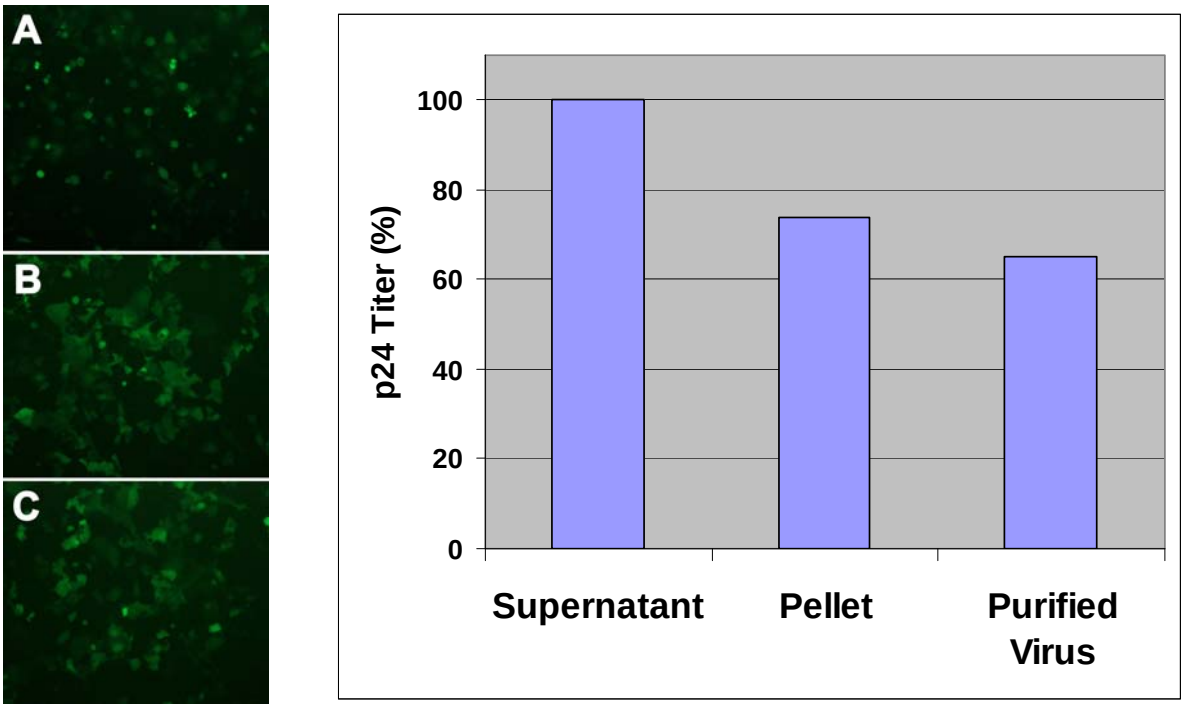


Figure 3: **Vector Yield Determined by p24 ELISA.** GFP lentiviral supernatant was concentrated and purified according to the Assay Instructions. Purification fractions of Lentiviral Supernatant (A), Virus Pellet (B) and Purified Virus Fraction (C) were used to infect 293 cells with GFP expression determined after 72 hr. The p24 titer of each fraction was determined by p24 ELISA (Cat.# VPK-107, Lentivirus Associated HIV p24 ELISA).

References

1. Naldini, L., U. Blomer, P. Gallay, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma, and D. Trono (1996) *Science* 272, 263-267.
2. Verma, I. M., and N. Somia **(**1997) *Nature* 389, 239-242
3. Kafri, T., U. Blomer, D. A. Peterson, F. H. Gage, and I. M. Verma (1997) *Nat. Genet.* 17, 314-317.
4. Beyer, W. R., M. Westphal, W. Ostertag, and D. von Laer (2002) *J. Virol.* 76, 1488-1495.

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