# 28

# Gene Delivery to Hematopoietic Stem Cells Using Lentiviral Vectors

**Hiroyuki Miyoshi**

## 1. Introduction

Hematopoietic stem cells (HSCs) are clonogenic cells capable of both self-renewal and multilineage differentiation. An efficient method for gene transfer into HSCs is required for exploring HSC biology as well as for gene therapy of hematopoietic disorders. Retroviral vectors have been the most widely used vectors for gene transfer to HSCs. However, retroviral vectors require cell division for integration, limiting their use for gene transfer into HSCs that are exclusively quiescent. Although prestimulation of HSCs with cytokines can enhance gene-transfer efficiency ***(1–7)***, exposure to cytokines also stimulates HSCs to differentiate, resulting in the reduction of long-term repopulating capacity ***(8–15)***. In contrast, lentiviral vectors based on the human immunodeficiency virus type 1 (HIV-1) can efficiently transduce human $CD34^+$ cells without cytokine prestimulation and long-term multilineage expression of the transgene is detected in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice after transplantation ***(16–22)***. Murine HSCs can also be easily transduced with lentiviral vectors without cytokine prestimulation ***(23–26)***.

In this chapter, the protocols for transduction of human $CD34^+$ cells that contain HSCs are described. The procedures include the isolation of human $CD34^+$ cells, preparation of lentiviral vectors, transduction of human $CD34^+$ cells, and in vivo analysis of transduced HSCs using NOD/SCID mice.

From: *Methods in Molecular Biology, vol. 246:*
*Gene Delivery to Mammalian Cells: Vol. 2: Viral Gene Transfer Techniques*
Edited by: W. C. Heiser © Humana Press Inc., Totowa, NJ

## 2. Materials

### 2.1. Reagents and Equipment

1. Ficoll-Paque PLUS (Amersham Biosciences, Uppsala, Sweden).
2. Magnetic cell separator MidiMACS, VarioMACS, or SuperMACS II (Miltenyi Biotec Inc., Auburn, CA).
3. MACS CD34 MicroBeads and FcR blocking reagent provided by Direct CD34 progenitor cell isolation kit (Miltenyi Biotec Inc.).
4. MACS separation column MS or LS (Miltenyi Biotec Inc.).
5. Ultracentrifuge with swinging-bucket rotors (Beckman SW28 and SW55 rotors or equivalent).

### 2.2. Solutions and Culture Medium

1. Phosphate-buffered saline (PBS) containing 2 m*M* EDTA.
2. MACS buffer: PBS containing 2 m*M* EDTA and 0.5% bovine serum albumin (BSA).
3. 2.5 *M* $CaCl_2$: Sterilize the solution by passing it through a pre-wetted 0.22-µm filter. Store the solution in aliquots at −20°C.
4. 2 × BBS: 50 m*M* BES, 280 m*M* NaCl, 1.5 m*M* $Na_2HPO_4$. Adjust the pH to 6.95 with NaOH. Sterilize the solution by passing it through a 0.22-µm filter. Store the solution in aliquots at −20°C.
5. Hank's balanced salt solution (HBSS) (Invitrogen/GIBCO, Carlsbad, CA).
6. Serum-free culture medium (SFM): Iscove's modified Dulbecco's medium (IMDM) (Invitrogen/GIBCO) supplemented with 10% StemSpan BIT 9500 Serum Substitute (Stem Cell Technologies Inc., Vancouver, BC).
7. Complete DMEM medium: Dulbecco's modified Eagle's medium (DMEM) (Invitrogen/GIBCO) supplemented with 10% fetal bovine serum (FBS), 2 m*M* L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate.

## 3. Methods

### 3.1. Isolation of Human $CD34^+$ Hematopoietic Progenitor Cells

Human HSCs are enriched in the $CD34^+$ fraction. Mononuclear cells from cord blood (CB), bone marrow (BM), or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (PB) are obtained by Ficoll-Paque density gradient centrifugation. $CD34^+$ cells are then magnetically labeled using MACS CD34 MicroBeads and enriched on positive selection columns in the magnetic field of the MACS separator.

1. Collect fresh human CB, BM, or G-CSF-mobilized PB samples and treat with an anticoagulant (e.g., heparin) (*see* **Note 1**).

2. Dilute the cells with 4 volumes of PBS containing 2 m*M* EDTA.
3. Carefully layer 35 mL of diluted cell suspension over 15 mL of Ficoll-Paque PLUS in a 50-mL conical centrifuge tube.
4. Centrifuge at 500*g* for 45 min at 20°C in a swinging-bucket rotor with no brake.
5. Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
6. Using a sterile pipet, carefully transfer the mononuclear cell (MNC) layer to a new 50-mL conical centrifuge tube.
7. Wash the cells by adding excess PBS containing 2 m*M* EDTA and centrifuge at 300*g* for 10 min at 20°C.
8. Resuspend the cell pellet in a final volume of 300 µL of MACS buffer per $10^8$ total cells. For less than $10^8$ total cells, use 300 µL.
9. Add 100 µL of FcR blocking reagent per $10^8$ total cells to the cell suspension and incubate for 10 min at 4°C.
10. Add 100 µL of MACS CD34 MicroBeads per $10^8$ total cells, mix well, and incubate for 30 min at 4°C.
11. Wash the cells by adding excess PBS containing 2 m*M* EDTA and centrifuge at 300*g* for 10 min at 4°C.
12. Resuspend the cell pellet in a final volume of 500 µL of MACS buffer per $10^8$ total cells.
13. Choose a positive selection MACS column type (MS or LS) according to the number of total unseparated cells and place it with column adaptor in the magnetic field of the MACS separator. Fill and rinse with buffer (MS: 500 µL; LS: 3 mL).
14. Pass the cells through 30-µm nylon mesh to remove clumps. Wet filter with buffer before use.
15. Apply the cells to the column, allow the cells to pass through the column and wash with buffer (MS: 2 mL; LS: 12 mL).
16. Remove column from separator, place column on a suitable tube, pipet MACS buffer on top of column (MS: 1 mL; LS: 5 mL) and elute retained cells using the plunger supplied with the column. Repeat this step twice for better cell yield.
17. For greater than 90% $CD34^+$ cell purity, repeat the magnetic separation (**steps 13**, **15**, and **16**). Apply the eluted cells to a fresh MS column, wash, and elute retained cells in MACS buffer.
18. Wash the cells by adding excess SFM and centrifuge at 300*g* for 10 min at 4°C.
19. Resuspend $CD34^+$ cells in SFM at a concentration of $5 \times 10^6$ cells/mL (*see* **Note 2**). $CD34^+$ cells can be stored in SFM with 10% dimethylsulfoxide (DMSO) in a liquid nitrogen freezer.

### 3.2. Preparation of Lentiviral Vectors

HIV-1-based lentiviral vectors pseudotyped with the vesicular stomatitis virus G glycoprotein (VSV-G) are generated by transient transfection of four plasmids, the packaging construct (pMDLg/pRRE) ***(27)***, the VSV-G-expressing construct (pMD.G) ***(28)***, the Rev-expressing construct (pRSV-Rev) ***(27)***, and the self-inactivating (SIN) lentiviral vector construct ***(29)***, into 293T cells. The packaging construct, pMDLg/pRRE, in which all accessory genes (*vif*, *vpr*, *vpu*, and *nef*) and regulatory genes (*tat* and *rev*) have been deleted, can be used to produce lentiviral vectors for transduction of $CD34^+$ hematopoietic progenitor cells ***(18,19,21,22)***.

1. Harvest exponentially growing 293T cells by trypsinization and seed $5 \times 10^6$ cells in poly-lysine coated 10-cm dish in 10 mL of complete DMEM medium (*see* **Note 3**).
2. Incubate the cells for 24 h at 37°C in a humidified incubator with an atmosphere of 10% $CO_2$. The cells should be about 75% confluent at the time of transfection.
3. Transfection: Prepare a total volume of 450 µL of plasmid DNA solution containing 17 µg of the SIN vector plasmid (*see* **Note 4**), 12 µg of the packaging plasmid (pMDLg/pRRE), 5 µg of the Rev-expressing plasmid (pRSV-Rev), and 5 µg of the VSV-G-expressing plasmid (pMD.G) in a 5-mL polystyrene tube (Falcon 2058) or a 1.5-mL microfuge tube. Add 50 µL of 2.5 *M* $CaCl_2$ and then 500 µL of 2 × BBS with gentle mixing. Incubate the mixture for 10–20 min at room temperature. Mix gently by pipetting and transfer the calcium phosphate-DNA solution dropwise to the dish. Rock the dish gently to mix the medium.
4. Incubate the cells for 12–16 h at 37°C in a humidified incubator with an atmosphere of 3% $CO_2$.
5. Remove the medium by aspiration and add 7.5 mL of prewarmed (37°C) complete DMEM medium.
6. Incubate for 48 h at 37°C in a humidified incubator with an atmosphere of 10% $CO_2$.
7. Remove the vector-containing medium and filter through a 0.45-µm filter (*see* **Note 5**).
8. Transfer the filtered medium to a conical ultracentrifuge tube that fits a Beckman SW28 rotor (or equivalent) and centrifuge at 50,000*g* (19,400 rpm in a Beckman SW28 rotor) for 2 h at 20°C.
9. Discard the supernatant and resuspend the pellet in an appropriate volume (up to 50 µL per dish) of HBSS (or IMDM) by pipetting. Try to avoid introducing bubbles. When dealing with small-scale preparations of vector, the following step can be omitted; store the vector suspension at −80°C.

10. Transfer the vector suspension to an ultracentrifuge tube that fits a Beckman SW55 rotor (or equivalent) and fill the tube with HBSS. Centrifuge at 50,000g (24,000 rpm in a Beckman SW55 rotor) for 2 h at 20°C.
11. Discard the supernatant and resuspend the pellet in an appropriate volume (up to 10 µL per dish) of HBSS (or IMDM) by pipetting. Try to avoid introducing bubbles.
12. Transfer the vector suspension to a tightly capped tube. Vortex the tube until no solid material is visible. If any debris is still visible, remove it by brief centrifugation and discard. Store the vector suspension in aliquots at −80°C (*see* **Note 6**).

### *3.3. Transduction of CD34$^+$ Cells by Lentiviral Vectors*

1. Incubate CD34$^+$ cells in SFM for 12–24 h before transduction at 37°C in a humidified incubator with an atmosphere of 5% $CO_2$. (If CD34$^+$ cells are frozen, thaw the cells, wash, and resuspend in SFM.)
2. Count the number of viable cells using the Trypan Blue exclusion method (*see* **Note 7**).
3. Wash the cells by adding excess SFM and centrifuging at 300*g* for 10 min at 4°C.
4. Resuspend the cells in SFM at a concentration of $5 \times 10^6$ cells/mL and transfer them to a 5-mL polypropylene round-bottom tube or a 96-well round-bottom cell culture dish (*see* **Note 8**).
5. Thaw the lentiviral vector stock and mix well by pipetting. Add the vector suspension to the cells at a multiplicity of infection (MOI) of 50–300 (*see* **Note 9**).
6. Incubate the vector-cell mixture for 5–12 h at 37°C in a humidified incubator with an atmosphere of 5% $CO_2$ (*see* **Note 10**).
7. Wash the cells with HBSS and centrifuge at 300*g* for 10 min at 4°C.
8. Resuspend the cells in an appropriate volume of HBSS. (The resuspension medium and volume should be determined based on subsequent assays.)

### *3.4. Analysis of Transduced Human CD34$^+$ Cells in NOD/SCID Mice*

In vitro assays for primitive hematopoietic cells, such as colony-forming cell (CFC) assays and long-term culture-initiating cells (LTC-IC) assays, can be used for analysis of transduced CD34$^+$ cells. However, HSCs can only be assayed by their ability to repopulate conditioned recipients. NOD/SCID mice are the most popular transplant recipients for human HSCs ***(30,31)***.

1. Irradiate NOD/SCID mice (8–10 wk old) at a sublethal dose (about 300 centigrays) 2–12 h before transplantation.

2. Intravenously inject 2–4 $\times$ $10^5$ transduced $CD34^+$ cells via the tail vein using 1-mL syringe with a 27 G needle.
3. At 6 wk or more after transplantation, collect blood samples from PB, spleen, and BM.
4. Analyze blood samples by flow cytometry with fluorescent antibodies against human specific cell-surface antigens or transgene products (**Fig. 1**).

## 4. Notes

1. HSCs are enriched in CB compared with BM or mobilized PB ***(32)***.
2. The purity of $CD34^+$ cells is usually greater than 95% as determined by flow cytometry.
3. For large-scale preparation, 15-cm dishes can be used. Scale up all materials in proportion to the area of the plates.
4. The latest SIN lentiviral vectors contain the central polypurine tract (cPPT) sequence, which improves the transduction efficiency ***(17,19,21)***, and the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), which enhances the expression of the transgene ***(33)***.
5. It is possible to add fresh medium and to collect vector-containing medium a second time from the cells. Add 7.5 mL of prewarmed (37°C) complete DMEM medium to the dish immediately after withdrawing the medium and continue incubation for another 48 h. Vector titers will be about 75% lower.
6. The titer of vectors is determined by measuring the amount of HIV-1 p24 gag antigen using an ELISA kit. (Commercial kits are available from PerkinElmer, Inc. (Boston, MA) and Advanced Biotechnologies, Inc., Columbia, MD). One ng of p24 would represent 5,000–10,000 infectious units (IU) on 293T or HeLa $CD4^+$ cells. In the case of vectors containing marker genes, the titer of vectors can be determined by infection of 293T or HeLa $CD4^+$ cells with serial dilutions of the vector stocks. Most vector stocks after two rounds of ultracentrifugation have titers ranging between 5 $\times$ $10^8$ and 2 $\times$ $10^9$ IU/mL. The titer of the vector stocks remains stable for at least 6 mo when they are stored in closed tubes at $-80$°C. In general, the vector stocks can be frozen and thawed several times with gradually loss of infectivity. If the yields of the vector are low, the number of infectious viral particles should be determined in samples taken at various stages during the purification to determine where losses are occurring.
7. To assay viable cells by Trypan Blue exclusion, mix 50 µL of cells with 50 µL of 0.4% Trypan Blue for 5 min. Transfer the cells to a hemocytometer and score the fraction of clear cells (alive) and dark blue cells (dead).
8. Using a smaller volume of SFM during transduction tends to increase the transduction efficiency ***(34)***.

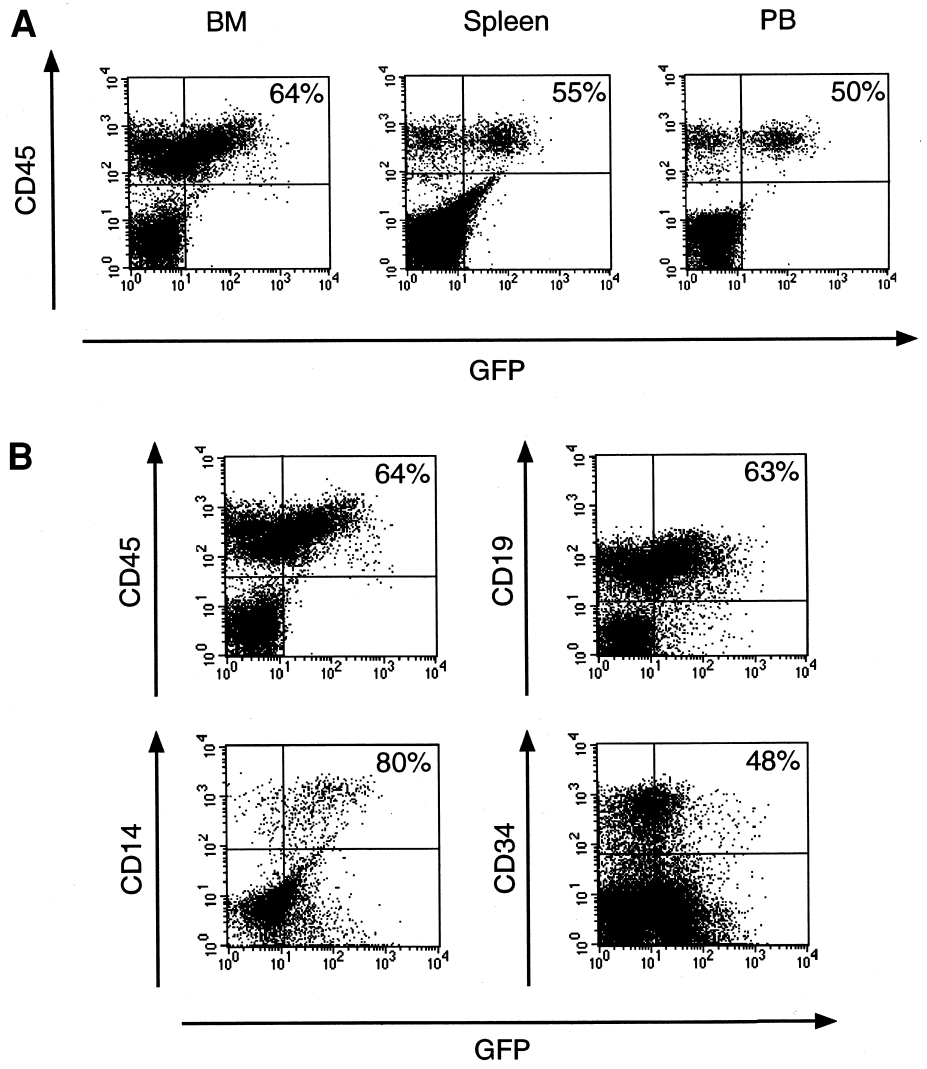


Fig. 1. Flow cytometry analysis of the transgene expression in NOD/SCID mice transplanted with lentiviral vector-transduced human CD34$^+$ cells. Human CB CD34$^+$ cells were transduced with an HIV-1-based lentiviral vector containing the green fluorescence protein (GFP) gene under the control of the cytomegalovirus (CMV) promoter and transplanted into sublethally irradiated NOD/SCID mice. At 15 wk posttransplantation, mononuclear cells were isolated from BM, spleen, and PB and analyzed by flow cytometry using FACSCalibur (Becton-Dickinson, Franklin Lakes, NJ) with CELLQuest software. **(A)** BM, spleen, and PB cells were stained with phycoerythrin (PE)-conjugated anti-human CD45. **(B)** BM cells were stained with phycoerythrin (PE)-conjugated anti-human CD14 (myeloid cells), CD19 (B cells), or CD34 (progenitor cells). Percentages of GFP$^+$ cells in engrafted human cell subpopulation are indicated.

9. Increases in MOI, addition of polybrene, and RetroNectin (recombinant human fibronectin fragment CH-296) (Takara Bio Inc., Otsu, Japan) have little effect on the transduction efficiency.
10. Incubation for 5 h is enough for transduction but longer incubation may improve the transduction efficiency.

## References


1. Cheng, L., Du, C., Lavau, C., Chen, S., Tong, J., Chen, B. P., et al. (1998) Sustained gene expression in retrovirally transduced, engrafting human hematopoietic stem cells and their lympho-myeloid progeny. *Blood* **92,** 83–92.
2. Conneally, E., Eaves, C. J., and Humphries, R. K. (1998) Efficient retroviral-mediated gene transfer to human cord blood stem cells with in vivo repopulating potential. *Blood* **91,** 3487–3493.
3. Marandin, A., Dubart, A., Pflumio, F., Cosset, F. L., Cordette, V., Chapel-Fernandes, S., et al. (1998) Retrovirus-mediated gene transfer into human CD34+38low primitive cells capable of reconstituting long-term cultures in vitro and nonobese diabetic-severe combined immunodeficiency mice in vivo. *Hum. Gene Ther.* **9,** 1497–1511.
4. Schilz, A. J., Brouns, G., Knoss, H., Ottmann, O. G., Hoelzer, D., Fauser, A. A., et al. (1998) High efficiency gene transfer to human hematopoietic SCID-repopulating cells under serum-free conditions. *Blood* **92,** 3163–3171.
5. van Hennik, P. B., Verstegen, M. M., Bierhuizen, M. F., Limon, A., Wognum, A. W., Cancelas, J. A., et al. (1998) Highly efficient transduction of the green fluorescent protein gene in human umbilical cord blood stem cells capable of cobblestone formation in long-term cultures and multilineage engraftment of immunodeficient mice. *Blood* **92,** 4013–4022.
6. Cavazzana-Calvo, M., Hacein-Bey, S., de Saint Basile, G., Gross, F., Yvon, E., Nusbaum, P., et al. (2000) Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* **288,** 669–672.
7. Dorrell, C., Gan, O. I., Pereira, D. S., Hawley, R. G., and Dick, J. E. (2000) Expansion of human cord blood CD34(+) CD38(−) cells in ex vivo culture during retroviral transduction without a corresponding increase in SCID repopulating cell (SRC) frequency: dissociation of SRC phenotype and function. *Blood* **95,** 102–110.
8. van Beusechem, V. W., Bart-Baumeister, J. A., Hoogerbrugge, P. M., and Valerio, D. (1995) Influence of interleukin-3, interleukin-6, and stem cell factor on retroviral transduction of rhesus monkey CD34+ hematopoietic progenitor cells measured in vitro and in vivo. *Gene Ther.* **2,** 245–255.
9. Peters, S. O., Kittler, E. L., Ramshaw, H. S., and Quesenberry, P. J. (1996) Ex vivo expansion of murine marrow cells with interleukin-3 (IL-3), IL-6, IL-11, and stem cell factor leads to impaired engraftment in irradiated hosts. *Blood* **87,** 30–37.
10. Yonemura, Y., Ku, H., Hirayama, F., Souza, L. M., and Ogawa, M. (1996) Interleukin 3 or interleukin 1 abrogates the reconstituting ability of hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA* **93,** 4040–4044.

11. Yonemura, Y., Ku, H., Lyman, S. D., and Ogawa, M. (1997) In vitro expansion of hematopoietic progenitors and maintenance of stem cells: comparison between FLT3/FLK-2 ligand and KIT ligand. *Blood* **89,** 1915–1921.
12. Bhatia, M., Bonnet, D., Kapp, U., Wang, J. C., Murdoch, B., and Dick, J. E. (1997) Quantitative analysis reveals expansion of human hematopoietic repopulating cells after short-term ex vivo culture. *J. Exp. Med.* **186,** 619–624.
13. Gothot, A., van der Loo, J. C., Clapp, D. W., and Srour, E. F. (1998) Cell cycle-related changes in repopulating capacity of human mobilized peripheral blood CD34(+) cells in non-obese diabetic/severe combined immune-deficient mice. *Blood* **92,** 2641–2649.
14. Rebel, V. I., Tanaka, M., Lee, J. S., Hartnett, S., Pulsipher, M., Nathan, D. G., et al. (1999) One-day ex vivo culture allows effective gene transfer into human nonobese diabetic/severe combined immune-deficient repopulating cells using high-titer vesicular stomatitis virus G protein pseudotyped retrovirus. *Blood* **93,** 2217–2224.
15. Szilvassy, S. J., Meyerrose, T. E., and Grimes, B. (2000) Effects of cell cycle activation on the short-term engraftment properties of ex vivo expanded murine hematopoietic cells. *Blood* **95,** 2829–2837.
16. Miyoshi, H., Smith, K. A., Mosier, D. E., Verma, I. M., and Torbett, B. E. (1999) Transduction of human CD34+ cells that mediate long-term engraftment of NOD/SCID mice by HIV vectors. *Science* **283,** 682–686.
17. Follenzi, A., Ailles, L. E., Bakovic, S., Geuna, M., and Naldini, L. (2000) Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. *Nat. Genet.* **25,** 217–222.
18. Guenechea, G., Gan, O. I., Inamitsu, T., Dorrell, C., Pereira, D. S., Kelly, M., et al. (2000) Transduction of human CD34+ CD38-bone marrow and cord blood-derived SCID-repopulating cells with third-generation lentiviral vectors. *Mol. Ther.* **1,** 566–573.
19. Sirven, A., Pflumio, F., Zennou, V., Titeux, M., Vainchenker, W., Coulombel, L., et al. (2000) The human immunodeficiency virus type-1 central DNA flap is a crucial determinant for lentiviral vector nuclear import and gene transduction of human hematopoietic stem cells. *Blood* **96,** 4103–4110.
20. Woods, N. B., Fahlman, C., Mikkola, H., Hamaguchi, I., Olsson, K., Zufferey, R., et al. (2000) Lentiviral gene transfer into primary and secondary NOD/SCID repopulating cells. *Blood* **96,** 3725–3733.
21. Sirven, A., Ravet, E., Charneau, P., Zennou, V., Coulombel, L., Guetard, D., et al. (2001) Enhanced transgene expression in cord blood CD34(+)-derived hematopoietic cells, including developing T cells and NOD/SCID mouse repopulating cells, following transduction with modified trip lentiviral vectors. *Mol. Ther.* **3,** 438–448.
22. Gao, Z., Golob, J., Tanavde, V. M., Civin, C. I., Hawley, R. G., and Cheng, L. (2001) High levels of transgene expression following transduction of long-term nod/scid-repopulating human cells with a modified lentiviral vector. *Stem Cells* **19,** 247–259.
23. Barrette, S., Douglas, J. L., Seidel, N. E., and Bodine, D. M. (2000) Lentivirus-based vectors transduce mouse hematopoietic stem cells with similar efficiency to moloney murine leukemia virus-based vectors. *Blood* **96,** 3385–3391.

24. Chen, W., Wu, X., Levasseur, D. N., Liu, H., Lai, L., Kappes, J. C., and Townes, T. M. (2000) Lentiviral vector transduction of hematopoietic stem cells that mediate long-term reconstitution of lethally irradiated mice. *Stem Cells* **18,** 352–359.
25. Mikkola, H., Woods, N. B., Sjogren, M., Helgadottir, H., Hamaguchi, I., Jacobsen, S. E., et al. (2000) Lentivirus gene transfer in murine hematopoietic progenitor cells is compromised by a delay in proviral integration and results in transduction mosaicism and heterogeneous gene expression in progeny cells. *J. Virol.* **74,** 11911–11918.
26. Tahara-Hanaoka, S., Sudo, K., Ema, H., Miyoshi, H., and Nakauchi, H. (2002) Lentiviral vector-mediated transduction of murine CD34(−) hematopoietic stem cells. *Exp. Hematol.* **30,** 11–17.
27. Dull, T., Zufferey, R., Kelly, M., Mandel, R. J., Nguyen, M., Trono, D., and Naldini, L. (1998) A third-generation lentivirus vector with a conditional packaging system. *J. Virol.* **72,** 8463–8471.
28. Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F. H., et al. (1996) In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* **272,** 263–267.
29. Miyoshi, H., Blomer, U., Takahashi, M., Gage, F. H., and Verma, I. M. (1998) Development of a self-inactivating lentivirus vector. *J. Virol.* **72,** 8150–8157.
30. Shultz, L. D., Schweitzer, P. A., Christianson, S. W., Gott, B., Schweitzer, I. B., Tennent, B., et al. (1995) Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *J. Immunol.* **154,** 180–191.
31. Dick, J. E. (1996) Normal and leukemic human stem cells assayed in SCID mice. *Semin. Immunol.* **8,** 197–206.
32. Wang, J. C., Doedens, M., and Dick, J. E. (1997) Primitive human hematopoietic cells are enriched in cord blood compared with adult bone marrow or mobilized peripheral blood as measured by the quantitative in vivo SCID-repopulating cell assay. *Blood* **89,** 3919–3924.
33. Zufferey, R., Donello, J. E., Trono, D., and Hope, T. J. (1999) Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. *J. Virol.* **73,** 2886–2892.
34. Haas, D. L., Case, S. S., Crooks, G. M., and Kohn, D. B. (2000) Critical factors influencing stable transduction of human CD34(+) cells with HIV-1-derived lentiviral vectors. *Mol. Ther.* **2,** 71–80.