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# Efficient Generation of Transgenic Rats Through the Male Germline Using Lentiviral Transduction and Transplantation of Spermatogonial Stem Cells

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## Abstract

Spermatozoa produced from spermatogonial stem cells (SSCs) are the vehicle by which genes of a male are passed to the next generation. A single SSC has the ability to self-renew and produce thousands of spermatozoa; therefore, it is an ideal target for genetic modification to efficiently generate transgenic animals in mammalian species. Rats are an important model organism for biological research; however, gene function studies have been difficult because of a limited ability to generate transgenic animals. Transgenic rat production through SSCs offers a means to overcome this obstacle. Because SSCs divide slowly both in vivo and in vitro, lentiviral vectors may be an ideal method for introducing stable genetic modification. Using a lentiviral vector, an enhanced green fluorescent protein (eGFP) transgene was introduced into the genome of cultured rat SSCs, which were microinjected into testes of immunodeficient mice to assess transduction efficiency. Approximately 40% of rat SSCs exposed to the lentiviral vector overnight carried the eGFP transgene and generated colonies of spermatogenesis. When transduced SSCs were transplanted into recipient rat testes, in which endogenous germ cells had been decreased but not eliminated by busulfan treatment, approximately 6% of offspring were transgenic. The transgene was stably integrated into the donor SSC genome and transmitted to and

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expressed by progeny in subsequent generations. Thus, lentiviral transduction of SSCs followed by transplantation is an effective means for generating transgenic rats through the male germline, and this approach may be applicable to other species in which existing methods are inadequate or not applicable.

Key words: lentivirus, spermatogenesis, testis

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Spermatogenesis is the complex process by which millions of sperm are produced daily in the testis. Continued functioning of this system is dependent on spermatogonial stem cells (SSCs), male germline stem cells capable of self-renewal and production of progeny committed to differentiation, thereby giving rise to the entire spermatogenic lineage. These abilities account for the persistent reproductive capacity of a male from puberty to death. Spermatozoa produced from SSCs are the vehicles by which genes of a male are passed to the next generation. Theoretically, a single rat SSC can give rise to 4096 spermatozoa each time it produces a committed progenitor type A spermatogonium ([Russell et al, 1990](#)). Thus, genetic modification of SSCs has the potential for efficiently generating transgenic animals.

Spermatogonial stem cell transplantation is an essential technique for achieving SSC-mediated transgenesis ([Brinster and Avarbock, 1994](#); [Brinster and Zimmermann, 1994](#)). Donor testis cells containing SSCs can be collected and genetically modified in vitro and then transplanted into infertile recipient testes, where donor cell-derived spermatogenesis will occur and produce spermatozoa that contain the genetic modification. These recipient animals can be mated to produce offspring carrying the modified donor genes. This approach has the potential to be more efficient and effective in generating transgenic rats and perhaps other animals than current methods. Insertion of a transgene into SSCs by lentiviral transduction assures germline transmission, and each recipient animal potentially can generate founder offspring, each with unique chromosomal integration sites.

The principle of generating transgenic animals through genetic modification of SSCs followed by transplantation has been applied in both mice ([Nagano et al, 2000, 2001, 2002](#)) and rats ([Hamra et al, 2002](#); [Orwig et al, 2002](#)). We have previously demonstrated the insertion of a LacZ marker transgene into the genome of SSCs using retroviral vectors with both species ([Nagano et al, 2000, 2001](#); [Orwig et al, 2002](#)). The Moloney murine leukemia virus (MMLV) has been one of the most widely used vectors for genetic modification because of its ability to stably integrate proviral DNA into the genome ([Miller, 1992](#); [Verma and Somia, 1997](#)). In mice, 2%–20% of the SSCs could be transduced by an MMLV retroviral vector, resulting in production of transgenic offspring following transplantation at a frequency of 4.5% ([Nagano et al, 2001](#)). In rats, only 0.5% of SSCs exposed to the MMLV retroviral vector were transduced and produced transgenic colonies of spermatogenesis in recipient testes ([Orwig et al, 2002](#)). Because retroviruses require active cell division for insertion of proviral DNA into the host cell genome, these vectors pose a limitation in application because SSCs divide slowly ([Meistrich et al, 1993](#); [Kubota et al, 2004](#); [Hamra et al, 2005](#); [Ryu et al, 2005](#)).

Lentiviruses, a subclass of retroviruses, are capable of transducing nondividing cells ([Bukrinsky et al, 1993](#); [Verma and Somia, 1997](#)), and thus have greater potential as a vector for efficiently altering the genome of slowly dividing cells such as SSCs. The ability of lentiviral vectors to transduce mouse SSCs has been demonstrated by insertion of the LacZ marker transgene ([Nagano et al, 2002](#)). Similarly, rat SSCs have been transduced with an eGFP transgene using a lentiviral vector, in which transgenic offspring were produced after transplantation ([Hamra et al, 2002](#)). In these studies

the efficiency of lentiviral transduction of SSCs was not fully examined. Thus, the usefulness of lentiviral vector transduction of SSCs compared to other means of generating transgenic animals remains unresolved, especially in the case of rats.

Laboratory rats are one of the most widely used animal models in biological sciences. With the abundant physiological and genomic information available, rats are an excellent model for studying gene function, and use of transgenesis to alter and control expression of specific genes would greatly expand their usefulness. However, development of methods for transgenesis, and eventually gene targeting, has been hindered because of specific characteristics of the rat model that differ in some respects from the well-established mouse system. Two significant problems are the difficulty in producing transgenic rats by egg pronuclear injection and the absence of embryonic stem cells to generate mutant rats. Thus, an impetus for attempting to use rat SSCs to produce transgenic animals is the possibility that the system would be more efficient than pronuclear injection and that it could be extended to generate targeted gene mutations. As a first step, this study was designed to evaluate the efficiency of using lentiviral vectors to stably transduce rat SSCs and efficiently generate transgenic progeny after transplantation. Because spermatogenesis is a well-conserved process in mammals ([Fritz, 1986](#)), techniques developed in rats for generation of transgenic animals through the male germline may be applicable to other species.

## ▶ **Materials and Methods**

### **Donor Rat Testis Cell Collection and SSC Enrichment**

Testis cells were collected from 8- to 14-day-old Sprague-Dawley (S/D) rat pups carrying a fusion transgene consisting of the *Escherichia coli* LacZ gene driven by the mouse metallothionein I (MT) promoter ([Rhim et al., 1994](#)). The LacZ gene is expressed by germ cells, which allows identification of donor-derived spermatogenesis in recipient testes following transplantation. Upon incubation of recipient testes with 5-bromo-4-chloro-indolyl B-D-galactosidase (X-gal), donor-derived spermatogenesis can be detected by blue staining. Donor testes cells were collected by a 2-step enzymatic digestion as previously described ([Ogawa et al., 1997](#)).

Cell suspensions were enriched for SSCs using flow cytometric cell sorting (FACS) based on Thy1<sup>lo</sup>β3-integrin<sup>-</sup> staining as previously described ([Ryu et al., 2004](#)). Sorting of cell populations was conducted using a dual-laser FACStar Plus (BD Biosciences, San Jose, Calif) equipped with 488-nm argon and 633-nm helium neon laser made available through the Cancer Center Flow Cytometry and Cell Sorting Shared Resource at the University of Pennsylvania. Cells were sorted into polypropylene tubes containing phosphate-buffered saline (PBS)-S (PBS with 1% fetal bovine serum, 1 mM pyruvate, 5.6 mM glucose, and 10 mM HEPES), pelleted by centrifugation at 600 x *g* for 7 minutes and resuspended in culture media (MEM $\alpha$ ; Invitrogen, Carlsbad, Calif).

### **Lentiviral Transduction**

The lentiviral vector used (EF1-eGFP) consisted of the enhanced GFP gene (eGFP) driven by the elongation factor 1 (EF1) promoter ([Chang et al., 1999](#)). The vector was produced by the University of Pennsylvania Vector Core. FACS-separated Thy1<sup>lo</sup>β3-integrin<sup>-</sup> cells (7.5– 35 x 10<sup>4</sup> cells/cm<sup>2</sup>) were cultured in MEM $\alpha$  medium with 4 μg/mL polybrene on mitotically inactivated SIM mouse embryo-derived thioguanine and ouabain resistant cell feeders seeded at a concentration of 5 x 10<sup>4</sup> cells/cm<sup>2</sup>. Cells were exposed to EF1-eGFP lentivirus for 15 hours at a multiplicity of infection (MOI) of either 20 or 80 at 37° C in an atmosphere of 5% CO<sub>2</sub> in air. On the next day, the infected cells were recovered

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by trypsin-EDTA digestion and washed twice in MEM $\alpha$  by centrifugation (600 x g for 7 minutes). Cells were then diluted in MEM $\alpha$  for transplantation into recipient testes.

### ***Recipient Animals and Transplantation***

Two different types of recipient animals were used for transplantation experiments. In order to assess transfection efficiency of donor MT-LacZ rat SSCs by the EF1-eGFP lentivirus following overnight exposure, approximately 10  $\mu$ L of cell suspension ( $4.5\text{--}9.2 \times 10^5$  cells/mL) was transplanted into NCr nude mice (nu/nu; Taconic, Germantown, NY) testes in which endogenous spermatogenesis had been abolished by busulfan treatment (44 mg/kg) 6–8 weeks prior ([Orwig et al., 2002](#)).

To generate transgenic offspring from rat SSCs transduced by the EF1-eGFP lentivirus, approximately 20  $\mu$ L of donor MT-lacZ or S/D FACS separated germ cell suspensions ( $5.2\text{--}15.2 \times 10^6$  cells/mL) exposed to the lentivirus overnight were injected into the seminiferous tubules of recipient S/D rats (18–22 days of age) via the efferent duct ([Ryu et al., 2003](#)). Endogenous spermatogenesis had been reduced in recipient S/D rat testes by busulfan treatment (10 mg/kg) at 8–10 days postpartum (DPP) ([Ogawa et al., 1999](#)). The Animal Care and Use Committee of the University of Pennsylvania approved all experimental procedures in accordance with The Guide for Care and Use of Laboratory Animals (National Academy of Sciences, Assurance no. A3079-0).

### ***Analysis of Recipient Testes and Assessment of Transfection Efficiency***

Assessment of transfection efficiency and toxicity of the EF1-eGFP lentivirus was conducted 3 months after transplantation of donor rat cells into recipient nude mouse testes. Recipient testes were collected and viewed using an epi-fluorescent dissecting microscope to detect eGFP-positive colonies of spermatogenesis. Testes were subsequently stained with X-gal to visualize colonies of spermatogenesis derived from the MT-lacZ donor cells. The number of eGFP-positive and LacZ-stained colonies of spermatogenesis were counted for each recipient testis and digital images of both colony types were captured. Colonies expressing eGFP were considered those derived from donor rat SSCs transduced by the EF1-eGFP lentivirus and thus containing integration of the eGFP transgene. Blue X-gal-stained colonies represented total colonization by the donor MT-lacZ SSCs irrespective of lentiviral transduction. Lentiviral transduction efficiency was determined by dividing the number of transduced eGFP colonies by the number of total lacZ-stained colonies of spermatogenesis in each recipient testis.

### ***Production and Analysis of Transgenic Offspring***

To produce transgenic offspring from transduced rat SSCs, recipient S/D rats transplanted with donor MT-lacZ or S/D germ cells exposed to the EF1-eGFP lentivirus were mated with S/D females beginning 1.5 months after transplantation. Breeding was allowed to continue for 11–21 months, after which recipient males were sacrificed and the testes weighed. Pups positive for eGFP expression were identified from litters by ultraviolet (UV) light examination. Using this method, both male and female founders were identified and used for generating eGFP transgenic lines. Offspring from these founder animals were confirmed eGFP-positive by UV light examination, and some were sacrificed and the organs examined for eGFP expression.

### ***Southern Blotting***

Southern blotting was used to confirm the eGFP phenotype of the transgenic animals and determine the integration pattern of founders and their F1 progeny. Genomic DNA (8  $\mu$ g) was extracted from tail samples of transgenic and control offspring, digested with *Bam*H1, separated on a 0.8% agarose gel, and transferred to a nylon membrane (Nytran SuPerCharge; Schleicher and Schuell, Keene, NH). The

membrane was hybridized overnight at 68° C with a <sup>32</sup>P-labeled PCR-amplified fragment of eGFP cDNA (445 bp; Prime-It DNA Labeling kit; Stratagene, Cedar Creek, Tex).

## Statistics

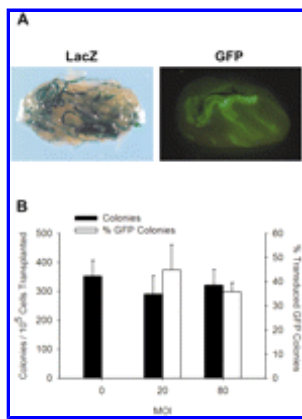
Significant differences in transduction efficiency between the 2 MOIs and toxicity of the lentiviral vector were evaluated using a Student's *t* test and 1-way analysis of variance, respectively. All statistical analyses were conducted using SPSS 13.0 software (SPSS Inc, Chicago, Ill).

## Results

### Lentiviral Transduction Efficiency of Rat SSCs

Transduction efficiency of SSCs is a key aspect of generating transgenic animals through the male germline. Because the SSC slowly divides, transduction using retroviral vectors, which require active cell division, is challenging. Lentiviral vectors have greater potential for more efficient transduction because active cell division is not essential. By using MT-lacZ donor rats, we were able to specifically investigate SSC transduction efficiency by comparing total SSC colonization, based on blue lacZ colonies, with eGFP colonies that were transduced with the lentiviral vector ([Figure 1](#)). The SSC transplantation technique is better developed in mice, and rat spermatogenesis can occur in the mouse testis ([Clouthier et al, 1996](#)). Therefore, we used xenogeneic transplant of rat germ cells into nude mouse testes as an assay for assessing transduction efficiency and toxicity of the lentiviral vector. Selection of Thy1<sup>lo</sup>β3-integrin<sup>-</sup> cells from donor rat pup testes resulted in SSC enrichment of the cell population, in which approximately 1 in 15 cells is an SSC ([Ryu et al, 2004](#)). SSC-enriched cell populations using this selection strategy were exposed to the EF1-eGFP lentivirus at 2 different MOIs to test its effectiveness at transducing rat SSCs, and the cells were subsequently transplanted into seminiferous tubules of germ cell-depleted nude mouse recipient testes. Three months after transplantation, both eGFP-stained and lacZ-stained donor colonies of spermatogenesis were counted, and the lentiviral transduction efficiency was found to be 44.8 ± 10.5% ( $\bar{x} \pm \text{SEM}$ , n = 8 testes) and 35.8 ± 3.7% (n = 5 testes) at an MOI of 20 or 80, respectively. There was no significant difference between the 2 MOIs. Also, a toxic effect of the lentiviral vector on SSCs from increasing the MOI was not observed; this was evaluated based on comparison between the number of lacZ colonies generated by cells exposed to the lentivirus at an MOI of 20 (290.3 ± 63.6,  $\bar{x} \pm \text{SEM}$ , n = 8 testes) or an MOI of 80 (321.9 ± 53.5, n = 5 testes) and nonexposed control cells (352.5 ± 53.4, n = 12 testes). Starting with an enriched cell population, rat SSCs can be efficiently transduced with lentiviral vectors up to at least an MOI of 80 without significant toxic effects.

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Figure 1. Assessment of transduction efficiency and toxicity by the lentiviral vector EF1-eGFP on rat spermatogonial stem cells (SSCs) using xenogeneic transplantation into nude mouse testes. **(A)** Colonization of recipient nude mouse testis by metallothionein I (MT)-LacZ donor SSCs following overnight exposure to the EF1-eGFP lentiviral vector. Both eGFP-expressing (green) and LacZ-stained (blue) colonies of donor-derived spermatogenesis could be detected 3 months after transplantation. Each donor colony of spermatogenesis represents a single biologically active SSC in the transplanted cell suspension ([Nagano et al., 1999](#)). **(B)** Transduction efficiency and toxicity of the EF1-eGFP lentiviral vector on donor rat SSCs. SSC-enriched donor germ cell populations were exposed to the lentiviral vector overnight at multiplicities of infection (MOIs) of 20 and 80 followed by transplantation into recipient nude mouse testes. Transduction efficiency, determined by comparing the number of LacZ-stained (representing total donor SSC number) and eGFP-expressing (representing donor SSCs transduced with the lentiviral vector) colonies of spermatogenesis, was  $44.8 \pm 10.5\%$  ( $\bar{x} \pm \text{SEM}$ ,  $n = 8$  testes) and  $35.8\% \pm 3.7\%$  ( $n = 5$  testes) at an MOI of 20 or 80, respectively. The number of LacZ colonies generated from  $10^5$  cells transplanted was  $352.5 \pm 53.4$  ( $\bar{x} \pm \text{SEM}$ ,  $n = 12$  testes),  $290.3 \pm 63.6$  ( $n = 8$  testes), and  $321.9 \pm 53.5$  ( $n = 5$  testes) at an MOI of 0 (control, nonexposed cells), 20, or 80, respectively. Also, based on total LacZ-stained colonies, overnight exposure to the EF1-eGFP lentiviral vector did not have a significant toxic effect on the SSCs. There were no significant differences between the MOIs for transduction efficiency ( $P = .118$ ) or lentiviral toxicity ( $P = .735$ ).

### *Production of Transgenic Rats from Lentiviral Transduced SSCs*

Generation of transgenic animals using SSCs requires both effective transduction and successful transplantation in which spermatogenesis is restored in recipient males to a level at which fertility is reestablished ([Ogawa et al., 1999](#); [Ryu et al., 2003](#)). At 18–22 days of age, rats that had been treated with busulfan as pups (8–10 dpp) were used as recipients for transplantation. A total of 10 busulfan-treated recipient rats were transplanted with donor rat germ cells that had been exposed to the EF1-eGFP lentivirus (Table). Eight of the 10 recipients regained fertility between 104 and 256 days after transplantation. All 8 recipients sired at least 1 eGFP-positive pup, demonstrating that normal spermatozoa capable of fertilization and generation of transgenic offspring could be derived from lentiviral transduced SSCs. The actual success of transgenic progeny production was variable between recipient animals, ranging from 1.3% to 13.6% of the total progeny produced. Overall, 5.8% of the total progeny from all recipient males were eGFP positive and thus derived from donor SSCs transduced with the EF1-eGFP lentiviral vector in vitro.

Transgenic eGFP founder animals were produced by mating recipient males to wild-type S/D females. Two EF1-eGFP founders (1 male and 1 female) were generated by breeding recipient male A2107 to a wild-type female ([Figure 2](#)). The eGFP transgene was inherited and expressed by at least 3 generations of progeny from this recipient male, demonstrating its stable integration beginning with the original donor SSC genome (ie, the transgene was not silenced during development). The pedigree for progeny produced from this recipient demonstrates that the transgene was transmitted and expressed by both sexes, and inheritance followed a Mendelian pattern ([Figure 2](#)). Progeny generated from mating the founders with wild-type females expressed the eGFP transgene in all tissues and organs ([Figure 3](#)). Examination of a founder male, produced from the mating of recipient rat A2107, and his progeny using Southern blotting revealed a single and identical integration site of the eGFP transgene ([Figure 4](#)). Thus, stable integration and expression of a transgene can be achieved through the male germline using a lentiviral vector to modify the SSC genome. [✚](#)

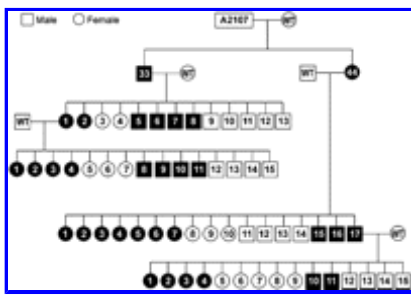


Figure 2. Pedigree of recipient animal A2107 that had been transplanted with Sprague-Dawley donor spermatogonial stem cells exposed to the EF1-eGFP lentiviral vector overnight. This pedigree demonstrates transmission of the transgene for 3 generations. Solid symbols indicate progeny expressing the transgene. Numbers in circles (females) and boxes (males) are identifiers for each progeny.

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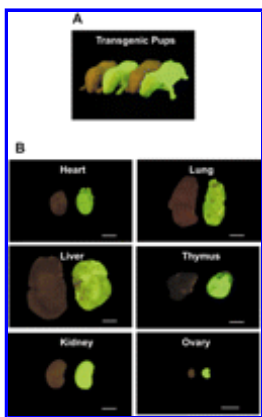


Figure 3. Production of transgenic rats by lentiviral transduction of rat SSCs followed by their transplantation into recipient testes and natural mating. **(A)** The enhanced GFP (eGFP) transgene was expressed in the skin of newborn pups, which allowed for identification of transgenic pups and ultimately the establishment of founders. **(B)** Expression of the eGFP transgene in various organs of adult rats generated from founder animals. The transgene was expressed in all organs of both males and females.

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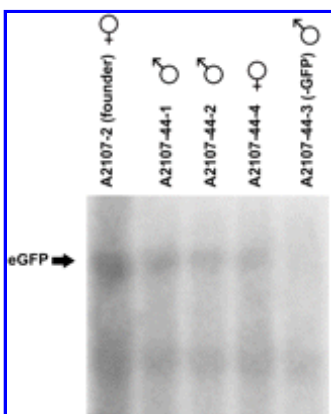


Figure 4. Southern blot analysis showing the integration pattern of the enhanced GFP (eGFP) transgene (arrow) in progeny of recipient rat A2107. The founder animal from this recipient (A2107-2) had a single integration site, characteristic of a lentiviral vector. Animal A2107-44-3 was not fluorescent and did not contain the inserted eGFP gene. An identical integration pattern was seen in progeny from these 2 founder animal demonstrating stable integration of the eGFP transgene. The lower band is not related to interpretation because it was also present in the negative control (A2107-44-3).

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*Production of transgenic rats from lentiviral transduction of spermatogonial stem cells\**

## Discussion and Conclusions

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In this study, SSCs from donor rat testes were efficiently transduced (cells expressing viral vector compared to total cells surviving viral exposure) with an eGFP transgene using a lentiviral vector, which resulted in the generation of transgenic offspring following transplantation of the transduced cells and natural mating of the recipient male. We have previously shown that lentiviral vectors can be used to modify the SSC genome of mice ([Nagano et al, 2002](#)) and retroviral vectors can transduce both mouse and rat SSCs ([Nagano et al, 2000, 2001](#); [Orwig et al, 2002](#)). Transgenic rats have also previously been produced by lentiviral transduction of SSCs followed by transplantation ([Hamra et al, 2002](#)). Although multiple studies regarding genetic modification of the SSC genome have been conducted, a direct measure of transduction efficiency and toxicity of viral vectors have not been examined. A unique aspect of the present study was the use of lacZ-expressing donor rat SSCs for genetic modification by a lentiviral vector. Thus, lacZ-stained colonies of spermatogenesis represented total functional donor SSCs and eGFP-fluorescing colonies represented donor SSCs within the same cell population that were transduced by the EF1-eGFP lentiviral vector. By comparing lacZ-donor– derived and eGFP-donor– derived colonies of spermatogenesis, we made a direct assessment of transduction efficiency and toxicity. In this study, approximately 45% of the SSCs in the original cell population were transduced by the lentiviral vector at an MOI of 20. Also, this transduction was accomplished without a significant toxic effect from overnight exposure to the lentivirus. In our previous studies using retroviral and lentiviral vectors with both rats and mice, transduction efficiency was estimated to be in a range of 0.5%– 20% ([Nagano et al, 2000, 2001, 2002](#); [Orwig et al, 2002](#)). Thus, the techniques used in this study resulted in significant enhancement in the efficiency of transducing SSCs of rats and may be applicable to SSCs of other species.

In the present study, 80% of the recipients transplanted with lentiviral -exposed SSCs sired offspring containing the genetic modification. Overall, 5.8% of the pups sired by the recipient rats contained the genetic modification and were thus derived from SSCs transduced in vitro by the lentiviral EF1-eGFP vector. Transgenic offspring production by pronuclear injection is approximately 5% efficient (transgenic progeny produced compared to eggs injected) in mice ([Brinster et al, 1985](#)), and no greater than 5% efficient in rats ([Hi rabayashi et al, 2001](#)); however, the typical success rate is often lower, particularly in rats. Thus, SSC-mediated transgenic rat production in this study was at least as efficient as that of the best reported cases using pronuclear injection. In a previous study, 4 of 6 recipient rats transplanted with SSCs exposed to an eGFP lentiviral vector regained fertility and sired offspring, but only 1 animal (25% of the recipients) sired progeny containing the genetic modification in which 30% of the offspring were transgenic ([Hamra et al, 2002](#)). As can be observed from these 2 similar studies, the success of SSC transplantation is variable in rats, and enhancing the efficacy of the procedure can have a dramatic effect on the efficiency of generating transgenic animals through the male germline.



Multiple factors contribute to the overall efficacy of generating transgenic animals by transducing SSCs followed by transplantation and natural breeding, including SSC transduction efficiency and the success of transplantation. Use of an SSC-enriched testis cell population in this study was likely a contributing factor to increasing gene integration efficiency into stem cells and provides the basis for future experiments of generating transgenic animals through the male germline. In addition, the high efficiency of transduction using a lentiviral vector contributed to the high rate of gene integration into stem cells. Enhancing transplantation success for SSCs, defined by the degree of donor SSC colonization and amount of donor-derived sperm production relative to endogenous production in the recipient host testis, is a greater challenge, particularly in the rat. Transplantation success is greatly influenced by the removal of endogenous germ cells in recipient testes; however, a return of endogenous spermatogenesis is also essential for reestablishing fertility after transplantation of donor SSCs in the rat ([Ogawa et al., 1999](#)). We have previously shown that busulfan treatment of rat pups at 8–10 days of age is an effective means for preparing recipients for transplantation ([Ryu et al., 2003](#)). In this study, the effectiveness of this strategy was variable in inhibiting endogenous spermatogenesis of the recipient rat as an adult, as evidenced by the differences in testis sizes, which could have a major effect on the recipient's capability to sire progeny containing donor genetics. Preparation of recipient animals for transplantation represents an aspect of the procedure for generating transgenic rats using SSCs that requires further development. Indeed, generation of transgenic animals using transplantation of SSCs may be challenging in other species with the current methods for creating recipient animals, because maintenance of endogenous spermatogenesis may be essential for development of recipient fertility, or, in some species, it may be impossible to completely eliminate endogenous spermatogenesis.

Natural mating of recipient rats transplanted with donor SSCs that had been transduced by the EF1-eGFP lentiviral vector generated founder animals that were used to create homozygous transgenic rat lines. Similarly to other transgenic animal studies, the transgene was stably inserted into the donor SSC genome and inherited by both male and female progeny following a Mendelian pattern. Also, expression of the transgene was present in all tissues and remained constant for several generations. Thus, lentiviral vectors are clearly capable of efficiently transducing SSCs, and when this is combined with transplantation, transgenic animals can be effectively produced. Recently, long-term culture of rat SSCs has been achieved ([Hamra et al., 2005](#); [Ryu et al., 2005](#)), providing a key tool for realizing the full potential for generating transgenic rats through the male germline. Maintaining SSCs in culture after transfection could allow for stably transduced cells to be selected and purified and for the numbers to be expanded. Using the principles reported in this investigation and previous ones, transgenesis is a technique that can potentially be utilized in future experimentation to assess gene function in rat models using gene knockout strategies developed for embryonic stem (ES) cells and recently reported for mouse SSCs ([Kanatsu-Shinohara et al., 2006](#)).

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## ► **Footnotes**

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