Low titer lentiviral transgenesis in rodents with simian immunodeficiency virus vector

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BioTechniques 55:137-140 (September 2013) doi 10.2144/000114078
Keywords: Lentiviral transgenesis; low titer; SIV; transgenic mice; transgenic rat

Efficient production of transgenic animals using low-titer lentiviral constructs remains challenging. Here we demonstrate that microinjection of simian immunodeficiency virus-derived lentiviral constructs can produce transgenic mice and rats with high efficiency even when using low-titer virus preparations.

Transgenic animals are vital in vivo models for functional genetics and biomedical research. Lentivirus-mediated transgenesis is a new and powerful tool for mammalian genome manipulation (1). The most commonly used lentiviral vectors are derived from human immunodeficiency virus (HIV), but other lentiviral vectors are also capable of producing transgenic mammals. For example, transgenic pigs have been generated using an equine infectious anemia virus-derived vector (2). Transgenic rhesus monkeys (3) and transgenic rabbits (4) have been also been produced using simian immunodeficiency virus (SIV)-derived vectors.

Lentiviral transgenesis using an SIV vector is similar to the HIV-based system, although less well-characterized. Transgene delivery can be performed using perivitelline space injection with the lentiviral construct. Alternative delivery methods, such as co-culture with denuded zygotes or microdrilled zona pellucida zygotes, are technically demanding. The main limitation of lentivirus transduction by microinjection into oocytes or early embryos is the need for high titer lentivirus preparations.

Transgenesis using low-titer lentiviral vectors can be carried out by repeated injection into the perivitelline space (5) or by using a piezo impact micromanipulator (6). However, these methods are time-consuming or require special laboratory equipment.

Here we report a simple and efficient adaptation of SIV-based transgenesis in rodent models that allows the use of low-titer lentiviral vectors. We produced transgenic mice and a transgenic rat by perivitelline space injections using a second generation packaging system and an SIV-based vector (7) encoding EGFP as a fluorescent marker driven by a ubiquitous CAG promoter. The CAG promoter is an artificial composite regulatory element harboring the cytomegalovirus (CMV) early enhancer element and chicken β-actin early enhancer.

Previous studies of SIV vectors indicated that they are similar to those derived from HIV viruses with respect to the insertion of transgenes in nonproliferating cells (8). In some cell lines, SIV-based transformation performed better than HIV-based transformation (9). The SIV vector system contains a central polypurine track and the posttranscriptional regulatory element of woodchuck hepatitis virus to increase transformation efficiency and expression level. The VSVg envelope protein was supplied on a separate plasmid.

Our virus vector system and the packaging construct were cotransfected into 293T cells. Viral particles were purified (10) and later titrated with Jurkat cells by measuring GFP-positive cells using FACS and adjusted to 1×106 TU/mL. The physical titer was quantified by qRT-PCR with primers specific for the LTR region (forward: SivQF 5’-TGTTCCCTGCTAGACTCTCA-3’; reverse: SivQR 5’-GGAAACACACACTGGCTTACT-3’) and found to be 2.6×108 RNA copies/mL (11). For transgenesis, female FVB/N mice and Sprague Dawley rats were superovulated. Following the 3R principles for animal research, only two donor rats and two donor mice were used for the experiments. Fertilized embryos were infected by injecting 70–130 pl of virus suspension into the perivitelline space under biosafety level 2 precautions. After a short culturing time,

Table 1. Transgenesis using SIV-derived EGFP vector

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Rat</th>
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</thead>
<tbody>
<tr>
<td>Injected zygotes</td>
<td>20</td>
</tr>
<tr>
<td>Transferred zygotes</td>
<td>19</td>
</tr>
<tr>
<td>Fosters</td>
<td>2</td>
</tr>
<tr>
<td>Newborns</td>
<td>8</td>
</tr>
<tr>
<td>Positive by PCR</td>
<td>3</td>
</tr>
<tr>
<td>Positive by fluorescence</td>
<td>3</td>
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Method summary:
Here we report the efficient production of transgenic mice and rats by a low-titer simian immunodeficiency virus vector. 1×106 TU/mL lentivirus stocks were generated in 293T cells and microinjected into the perivitelline space of rodent zygotes.
zygotes were transferred to pseudo-pregnant female CD1 mice or Wistar rat recipients. GFP fluorescence was detected in the offspring using a GFPS-5 headset (Biological Laboratory Equipment, Maintenance and Service Ltd., Budapest, Hungary) with blue light illumination and a barrier filter cut-off below 500 nm. Offspring were also screened for the presence of the transgene by genomic PCR using primers (forward: 5´- CTCGTGACCATGACCTAC -3´; reverse: 5´- CATGATATAGACGTTGCTGTT- 3´) (Figure 1F).

After single subzonal injections, approximately 10% of embryos died due to mechanical damage after micromanipulation (Table 1). Micromanipulation was carried out without the usage of automatic or semiautomatic micromanipulators. Nineteen mouse zygotes survived and were transferred to two foster mice. The 8 rat zygotes that survived were transferred to a foster rat. Altogether, eight mice and four rats were born and tested (Table 1).

GFP expression in the F0 generation was evaluated by in vivo fluorescence imaging and PCR. Three mice and one rat (Figure 1C and 1D) showed strong and ubiquitous expression of the transgene. Two of the three founder mice are shown in Figure 1A and 1B. Rates of transgenesis were 37.5% of live founder mice and 15.7% of transferred mouse zygotes. These rates were 25% and 12.5% in rats, respectively. Similar or higher transgenic rates have been reported previously (12,13,14). However, our results were achieved using a low-titer virus preparation. The transgenic status of the animals was confirmed by genomic PCR (Figure 1F). All PCR-positive animals expressed the transgene.

To demonstrate efficient germline transmission of the transgene one founder mouse and the founder rat were mated. Both founders transmitted the transgene to the next generation. For rats, the germline transmission rate was 5/10, the expected Mendelian value for single-copy integration. Single-copy integration was confirmed in the founder rat by Southern blot analysis (Figure 1E). For mice, the 5/9 transmission rate was close to the expected Mendelian value, assuming single- or low-copy integration. Low- or single-copy integrations are beneficial in establishing a well-characterized transgenic animal line. We presume that low- or single-copy integrations occurred due to the low titer of the lentiviral vector.
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constructs. The transgenic mouse line was kept for two generations and none of the transgenic offspring showed any transgenic silencing. The transgenic rat line was kept for four generations.

Interestingly, homozygous transgenic rats had a greater fluorescence intensity (Figure 2). Fluorescence intensity was determined by iTEM 5.0 (Olympus Soft Imaging Solution GmbH, Germany) and was found to be 1.62 times stronger in homozygous than in hemizygous rats. Homozygous status was confirmed by quantitative real time PCR using GFP-specific primer pairs. Amplification was analyzed using the Power SYBR Green PCR Master Mix (Life Technologies, Grand Island, NY) run on Rotorgene RG-3000 (Corbett Research, Sidney, Australia). Real time PCR confirmed the fluorescence observations. As homo- and hemizygous animals could be identified based on their fluorescence intensity, this transgenic rat line offers a special opportunity for cell-based therapeutic experiments and cellular transplantation.

In summary, we have shown for the first time that SIV-derived vectors have the capability to produce transgenic rodents with high efficiency, even when low titer viral preparations are used. Founder animals harbor single- or low-copy integrations, which promotes the establishment of transgenic lines. This cost-effective method results in well-characterized transgenic mouse and rat lines in a short period of time.

Acknowledgments

This work was supported by the following grants: OTKA-K68126, IGRULOMB (OM-00118/2008), Regulomix OMFB-00312,00313/2010. The authors thanks for F.L. Cosset for guidance and advice in the conduct of this research.

Competing interests

The authors declare no competing interests.

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Received 25 September 2012; accepted 05 August 2013.

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