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Lentiviral transgenesis in mice *via* a simple method of viral concentration

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ABSTRACT

Transgenic animals are important *in vivo* models for biological research. However, low transgenic rates are commonly reported in the literature. Lentiviral transgenesis is a promising method that has greater efficiency with regard to generating transgenic animals, although the transgenic rate of this approach is highly dependent on different transgenes and concentrated lentiviruses. In this study, we modified a method to concentrate lentiviruses using a table centrifuge, commonly available in most laboratories, and carried out analysis of the transgenic efficiency in mice. Based on 26 individual constructs and 627 live pups, we found that the overall transgenic rate was more than 30%, which is higher than obtained with pronuclear microinjection. In addition, we did not find any significant differences in transgenic efficiency when the size of inserts was less than 5000 bp. These results not only show that our modified method can successfully generate transgenic mice but also suggest that this approach could be generally applied to different constructs when the size of inserts is less than 5000 bp. It is anticipated that the results of this study can help encourage the wider laboratory use of lentiviral transgenesis in mice.

1. Introduction

Transgenic animals have been widely applied in the fields of agriculture, biology, and biomedicine. Scientists have focused on *in vivo* studies using transgenic mouse models in biomedical research because of the high similarities that the physiological and genomic conditions of these animals have with humans. The first transgenic mouse model was generated *via* pronuclear microinjection in 1982, and since then, there have been many studies of transgenic animals in different fields [1]. However, one critical disadvantage of pronuclear microinjection is its low transgenic efficiency. In mice, the transgenic rate of this approach is less than 10%, and much lower efficiency, even less than 1%, has been observed in larger animals [2–4]. This suggests that it is necessary to increase the transgenic rate to facilitate the further application of transgenic animals.

Several methods for animal transgenesis have been developed to increase this efficiency, such as the use of electroporation, sperm vectors, nuclear transfer, and transposons [5,6]. However, most of these approaches only increase transgenic efficiency to a limited extent. In contrast, viral transgenesis has shown to greatly improve the transgenic rate by taking advantages of viral properties. This technique was first developed in 1976 [7] and has since been modified using different viral backbones [8–11]. One of these viral backbones, a lentiviral vector, is the most widely used and efficient one because of several unique

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characteristics, such as its infection ability and the ability to carry out expression profiling of transgenes [12,13]. On the basis of previous studies, lentiviral transgenesis has been applied in mice, rats, and monkeys, showing high transgenic efficiency in different species [11,14–16]. These results suggest lentiviral transgenesis could be a powerful tool for generating transgenic animals.

Several factors affect the transgenic efficiency of lentiviral transgenesis, including the size of transgenes, titer of lentiviruses, and the researchers' micromanipulation skills [12,17]. The size of transgenes is a critical factor influencing the packaging of lentiviruses. In general, the size of a whole lentiviral vector containing genes of interest is suggested to be less than 13,000 bp. In addition, the longer the lentiviral vectors, the lower the efficiency of the lentiviruses [18]. Therefore, one disadvantage of the lentiviral vector approach is that it is size limited. Furthermore, the generation of high-titer lentiviruses is a critical step that is needed to achieve a high transgenic rate in transgenic animals. At least 1×10^8-10^9 CFU/mL of lentiviral titer is usually suggested, and an ultracentrifuge is needed to obtain this [17]. Because an ultracentrifuge is not available in most laboratories, in the present study, we developed a method using a table microcentrifuge to concentrate lentiviruses and examined its transgenic efficiency with an in-depth investigation of mice.

2. Materials and methods

2.1. Preparation of lentiviral constructs

Lentiviral backbone was a gift from David Baltimore (Addgene plasmid # 14883). The map is shown in Supporting Information 1. The lentiviral vector containing 5′ long terminal repeat, human immunodeficiency virus 1 (HIV-1 flap), woodchuck hepatitis virus posttranscriptional regulatory element, and 3′ self-inactivated long terminal repeat was used in this study (Fig. 1) [11]. Twenty-six different transgenes carrying various promoters and functional genes were inserted into lentiviral vectors. The detailed information is listed in Supporting Information 2. Here, we define the "insert" as including the promoters and functional genes, and the size of inserts ranges from 1183 to 4925 bp. As a result, the total size of these lentiviral vectors ranges from approximately 9000 to 13,000 bp.

2.2. Production of lentiviruses

To produce the lentiviruses, lentiviral vectors coding for different genes, pΔ8.9, and vesicular stomatitis virus glycoprotein vector (pVSV-G; Invitrogen) were cotransfected into 293FT human embryonic kidney packaging cells (Invitrogen) *via* the $CaCl_2$ method as described previously [19]. Supernatant with lentiviruses was collected from one 10-cm dish 3 days after transfection and then subjected to centrifugation using a table microcentrifuge (Eppendorf 5430R) with 30,130 *g* at 4 °C for 8 hours. Supernatant was removed, and lentiviral pellet was resuspended using 20–30 μL of cold phosphate-buffered saline buffer. The concentrated lentiviruses were aliquoted and kept at −80 °C for further experiments.

2.3. Generation of transgenic mice through lentiviral transgenesis

All animal procedures performed in this study were approved by the Institutional Animal Care and Use Committee at National Cheng Kung University, Taiwan. Approval numbers include 98223, 99056, 98218, 102121, 103009, 103097, 103097, 103098, and 104265. All mice were housed under a 13:11 hours light:dark cycle with food and water *ad libitum* at normal room temperature (22°C–25°C)

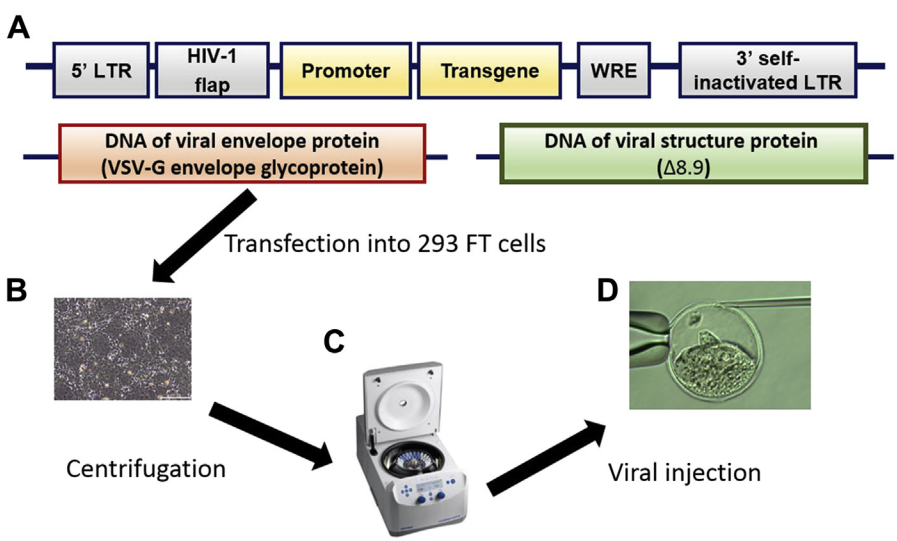

Fig. 1. The flow chart of lentiviral transgenesis in mice. Different transgenes with different promoters were inserted into lentiviral vectors (A) and then subjected to cotransfection with DNA of viral envelope and structure proteins in 293FT cells (B). Viral supernatant was concentrated using a table centrifuge (C), and concentrated viruses were used for viral injection in mice (D). LTR, long terminal repeat; HIV-1 flap, human immunodeficiency virus 1; WRE, woodchuck hepatitis virus posttranscriptional regulatory element.