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Highly efficient transgenesis in *Xenopus tropicalis* using *I-SceI* meganuclease

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Abstract

In this study, we report a highly efficient transgenesis technique for *Xenopus tropicalis* based on a method described first for Medaka. This simple procedure entails co-injection of meganuclease *I-SceI* and a transgene construct flanked by two *I-SceI* sites into fertilized eggs. Approximately 30% of injected embryos express transgenes in a promoter-dependent manner. About 1/3 of such embryos show incorporation of the transgene at the one-cell stage and the remainder are 'half-transgenics' suggesting incorporation at the two-cell stage. Transgenes from both classes of embryos are shown to be transmitted and expressed in offspring. The procedure also works efficiently in *Xenopus laevis*. Because the needle injection procedure does not significantly damage embryos, a high fraction develop normally and can, as well, be injected with a second reagent, for example an mRNA or antisense morpholino oligonucleotide, thus allowing one to perform several genetic manipulations on embryos at one time. This simple and efficient technique will be a powerful tool for high-throughput transgenesis assays in founder animals, and for facilitating genetic studies in the fast-breeding diploid frog, *X. tropicalis*.

Keywords: *Xenopus tropicalis*; *Xenopus laevis*; Transgenesis; *I-SceI*

1. Introduction

Xenopus has been widely used in studies of developmental biology, because of the ease with which one can perform embryological manipulations and inject molecules (e.g. mRNAs) into embryos. In addition, the transgenesis technique by restriction endonuclease mediated integration (REMI) that was developed about 10 years ago provided a powerful new tool for the *Xenopus* system, because it was more efficient than transgenic procedures in other vertebrates (Kroll and Amaya, 1996; Amaya and Kroll, 1999). In this method, decondensed sperm nuclei are incubated with a linearized transgene construct, restriction enzyme, and *Xenopus* egg extract, and subsequently transplanted into unfertilized eggs. After the transplantation the transgene is immediately integrated into the partially digested host genome. Hence, the transgene shows non-mosaic, correct spatio-temporal expression pattern in founder transgenic animals.

The REMI method was originally developed in *Xenopus laevis*, the most favored species in the *Xenopus* genus for developmental biology studies. The REMI method made it possible to manipulate the *Xenopus* genome, including procedures which could be used for insertional mutagenesis (Bronchain et al., 1999). However, because of its pseudotetraploid genome and long generation time (1–2 years under normal conditions (Duellman and Trueb, 1986)), genetic studies in *X. laevis* remain problematic.

To overcome this drawback, we have been involved in the development of *X. tropicalis*, a close relative of *X. laevis*, as a new experimental system for developmental genetics (Amaya et al., 1998; Offield et al., 2000; Hirsch et al., 2002a). *X. tropicalis* is the only diploid species in the genus (de Sa and Hillis, 1990) with a genome size nearly half that of mouse (1.7×10^9 bp/haploid nucleus (Tymowska, 1973)), and a significantly shorter generation time (3–5 months) than *X. laevis*. Their adult size is approximately one quarter that of *X. laevis*, allowing more animals to be housed in equivalent space for multi-generation experiments. Their embryos are smaller than those of *X. laevis* (0.7–0.8 versus 1.0–1.3 mm, respectively), but are sufficiently large for embryological manipulations and injection of molecules like mRNA. The REMI method was also adapted to *X. tropicalis* (Offield et al., 2000),

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and further modified to improve the transgenesis efficiency (Hirsch et al., 2002b).

Up to now, however, transgenic *X. tropicalis* have not been as widely generated by many researchers as one might expect. The major reason is the challenges associated with the *Xenopus* REMI transgenesis procedure. The sperm nuclear transplantation, which uses a relatively large needle, is a burden on the recipient eggs, so its success strongly depends on egg quality. In *X. laevis*, the rate of transplanted eggs that develop normally to tadpoles fluctuates between 1 and 16% (20–40% of

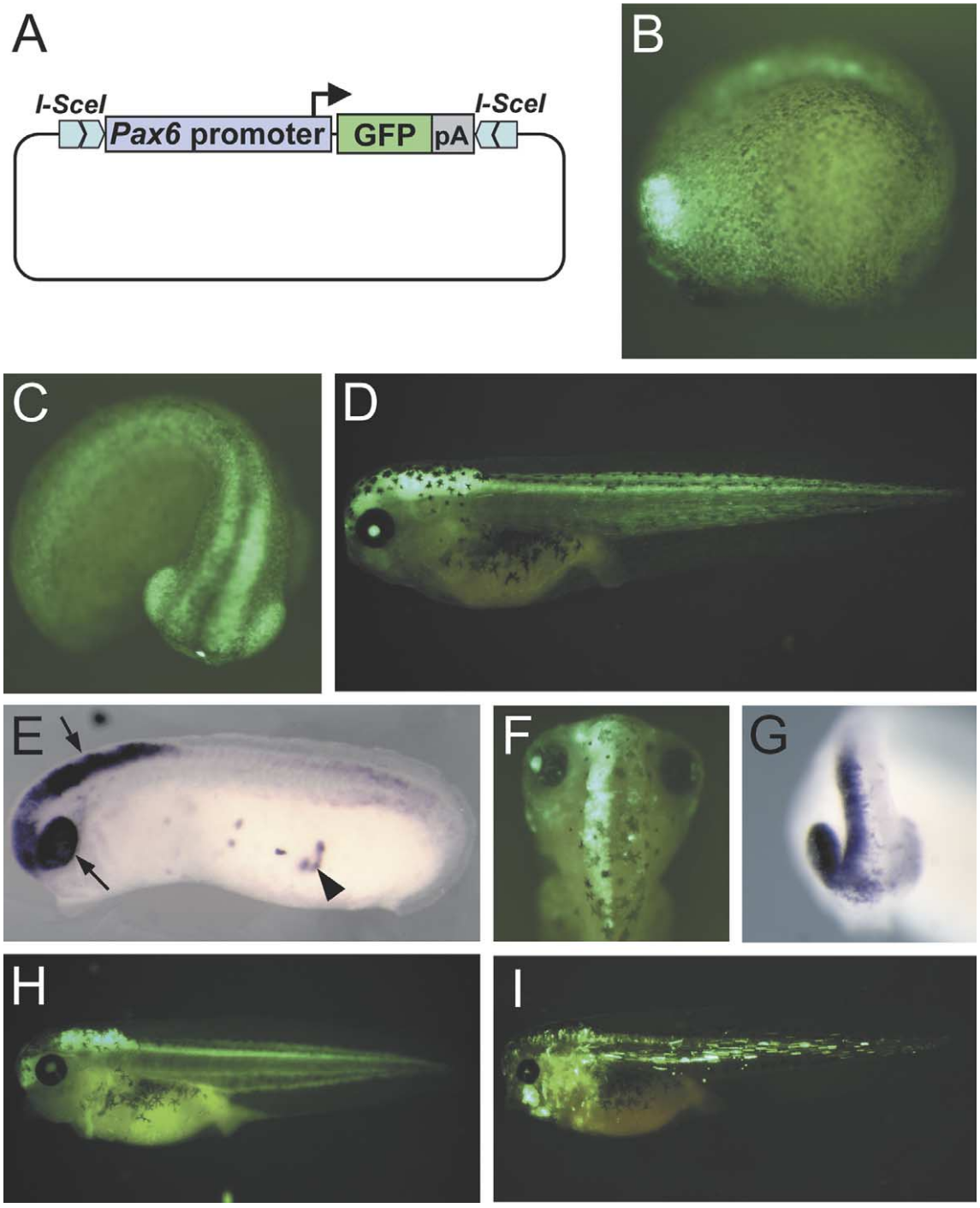

Fig. 1. Expression of the *Pax6* reporter co-injected with *I-SceI* meganuclease into *X. tropicalis* embryos. (A) A reporter construct (IS-Pax6GFP) introduced into embryos shown in panels B–G, and I. The *Pax6* promoter-GFP cassette is flanked at both ends by two *I-Sce1* recognition sites. (B–D) GFP fluorescence observed in embryos co-injected with IS-Pax6GFP and *I-SceI* meganuclease (B, stage 20; C, stage 25; D, stage 41). (E) In situ hybridization analysis of GFP expression in a stage 26 embryo co-injected with IS-Pax6GFP and *I-SceI* meganuclease. The smooth expression in the eye and brain tissues and the spotty ectopic expression in ventral cells are indicated by arrows and arrowhead, respectively. (F,G) Putative half transgenic embryos showing GFP expression only in one side (F, GFP fluorescence at stage 41; G, in situ hybridization with GFP probe at stage 26). (H) GFP expression in stage 41 transgenic embryo generated by the REMI method using the same *Pax6* promoter-GFP cassette. (I) Mosaic GFP expression in stage 41 embryo injected without *I-SceI*.