GENERATION OF TRANSGENIC-CLONED GUANGXI HUANJIANG XIANG PIGS SYSTEMICALLY EXPRESSING GREEN FLUORESCENT PROTEIN*

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Introduction

Guangxi Huanjiang Xiang pig is a unique miniature pig strain that is originally from Huanjiang Maonan Autonomous County of Guangxi, China, and it has great potential in agricultural and biomedical research. Although cloning and genetic modification of pigs would enhance their application value,cloning of Guangxi Huanjiang Xiang pig has not yet been reported. The present study aimed to generate transgenic-cloned Huanjiang Xiang pigs, laying the foundation for the conservation and utilization of this valuable swine resource.

Materials and Methods

Fibroblasts were isolated from tails of Huanjiang Xiang pig, and genetically modified using Xfect transfection. Positive transgenic fibroblasts were selected by G418 resistance screening, and positive transgenic fibroblasts expressing green fluorescent protein (GFP) were used as donor cells for reconstructing transgenic embryos by somatic cell nuclear transfer (SCNT). Embryos transfer was performed at the day of SCNT. Pregnancy was monitored, and the surrogates were delivered by natural parturition on Day 114 to 120 of gestation (SCNT was performed on Day 0). Genomic DNA was extracted from tail biopsies of newborn cloned piglets and PCR reactions were used for the detection of GFP gene integration. One transgenic-cloned offspring was autopsied to collect organs and tissues for histological analysis of GFP expression.

Results

Positive transgenic fibroblasts expressing GFP were selected by G418 resistance screening and then were used as donor cells for reconstructing transgenic embryos by SCNT. After the transfer of 504 transgenic SCNT embryos to two surrogate recipients, one of which was pregnant and gave birth to three live piglets. Overall cloning efficiency was 0.6%. One piglet died at Day 7 after birth because of the feeding problem. Another one was autopsied for histological analysis, and the 3rd transgenic-cloned Huanjiang Xiang pig is still alive with clinically healthy. All of three piglets were positive transgenic identified by PCR reaction, and the histological analysis showed that GFP transgene systemically expressed in the transgenic cloned Huanjiang Xiang pig.

Conclusion

The successful generation of transgenic-cloned Huanjiang Xiang pigs lays the foundation for the conservation and utilization of this valuable swine resource.

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