Utility and Efficiency of Homologous Recombination for Introducing Targeted Modifications to the Pig Genome

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Summary and Implications

The production and utilization of genetically modified animals greatly improves their utility in agriculture, as biomedical research models of human disease, for the production of recombinant pharmaceutical proteins, and for production of organs with greater potential for xenotransplantation. While numerous strategies have been utilized in the production of transgenic large animals, cell-based transgenesis followed by somatic cell nuclear transfer (SCNT) is currently the most widely applied method. We constructed a targeting vector with 2774 and 1890 bp arms of homology flanking a neomycin resistant gene. Following delivery of the DNA targeting vector into the porcine fetal fibroblasts by electroporation and selection with G418, PCR and sequencing confirmed one of 547 transgenic clonal colonies contained the targeted introduction of the neomycin resistance gene. Genetically modified fibroblast donor cells can be utilized for SCNT for the development of genetically modified pigs to study biological mechanisms important for animal agriculture and to create biomedical models.

Introduction

Transgenic technology has potential for rapidly improving quantity and quality of agricultural products, compared to traditional selection and breeding methods in domestic animals that are time-consuming when attempting to alter the desired allele frequency for specific traits. Additionally, transgenic animals can be used as biomedical research models or directly for human health, by producing recombinant pharmaceutical proteins and/or organs for xenotransplantation. Due to the advantage of bypassing the need of embryonic stem (ES) cells that are difficult to isolate in domestic animal species, cell-based method of transgenesis followed by somatic cell nuclear transfer (SCNT) is currently widely applied. In this study, we aimed to introduce a targeted genetic modification to the X chromosome of the swine genome to determine the efficiency by which targeted genetic modifications can be made using traditional targeting techniques.

Materials and Methods

Two arms of homology (2774 and 1890 bp in length) were PCR amplified form the X chromosome and introduced into a targeting vector to flank a neomycin resistance gene. The entire vector was sub-cloned into the PUC19 vector, transfected into DH5α E.coli and propagated. A linearized DNA targeting vector (12.5 µg /mL) was delivered into porcine fetal fibroblasts using electroporation (three 1 ms pulses of 300 V to 200 µL containing $1x10^6$ cells/mL). Electroporated cells were selected by adding G418 (Geneticin) into culture medium.

Results and Discussion

Following G418 marker screening after electroporation of DNA vectors into the fibroblasts, 547 clones were obtained. PCR and sequencing confirmed one (No. 495) of these 547 clones was genetically modified through homologous recombination (Figure 1).

While producing genetically modified pigs via SCNT is currently the only method of producing pigs with targeted genetic modifications, the process of introducing targeted genetic modification to somatic cells remains a very inefficient process. The combined application of new technologies, including Zinc-finger nucleases (ZFNs) or transcription activator-like effector nucleases (TALNs) to enhance targeted genome modification and the emerging ability to create induced pluripotent stem (iPS) cells in domestic animals represent potential pathways for improving the success rate of targeted genome manipulation strategies to create genetically modified pigs.

Acknowledgements

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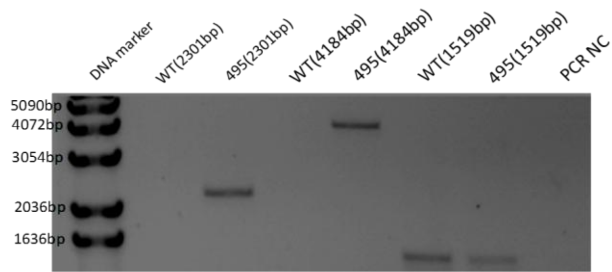

Figure 1. PCR genotyping of clone 495. Primer pairs to amplify 2301 and 4184 bp length DNA fragments are specific to targeting the targeted modification. Primer pair to amplify 1519 bp fragment is specific to pig genome, showing bands in both clone 495 and wildtype DNA. WT: wildtype pig DNA. PCR NC: PCR negative control.