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Establishment of cell-based transposon-mediated transgenesis in cattle

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ABSTRACT

Transposon-mediated transgenesis is a well-established tool for genome modification in small animal models. However, translation of this active transgenic method to large animals warrants further investigations. Here, the piggyBac (PB) and sleeping beauty (SB) transposon systems were assessed for stable gene transfer into the cattle genome. Bovine fibroblasts were transfected either with a helper-independent PB system or a binary SB system. Both transposons were highly active in bovine cells increasing the efficiency of DNA integration up to 88 times over basal nonfacilitated integrations in a colony formation assay. SB transposase catalyzed multiplex transgene integrations in fibroblast cells transfected with the helper vector and two donor vectors carrying different transgenes (fluorophore and neomycin resistance). Stably transfected fibroblasts were used for SCNT and on *in vitro* embryo culture, morphologically normal blastocysts that expressed the fluorophore were obtained with both transposon systems. The data indicate that transposition is a feasible approach for genetic engineering in the cattle genome.

1. Introduction

The advent of high throughput DNA sequencing methods and comprehensive annotated genome maps concomitantly with advanced active transgenic techniques promise to revolutionize the field of animal biotechnology. In particular, areas like disease modeling, biopharming, and basic research will benefit enormously by introducing precise genetic engineering tools to manipulate livestock genomes. Initial transgenic methods relayed on passive (nonfacilitated) genomic integration of transgenes at sites of spontaneously arising double-strand breaks of chromosomes after direct injection of naked DNA into zygotes (pronuclear injection) or transfection of cultured cells followed by SCNT. Homologous recombination in somatic cells of livestock is an extremely rare event, and only a few

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genes were targeted in recent years [1–7]. These inefficient and unreproducible approaches were gradually superseded by a new generation of active methods in which genomic insertion of heterologous DNA molecules is catalyzed by exogenously provided enzymes (reviewed in [8,9]).

For gain-of-function approaches in farm animals, the engineered transposon systems Sleeping Beauty (SB) and piggyBac (PB) gained increasing interests in recent years [10,11]. Transposon-based transgenic methods, derived from naturally occurring DNA transposable elements, are nonviral gene delivery systems capable of efficient enzyme-mediated genomic insertion of DNA segments into the genome. During transposition, a single copy of the sequence of interest framed by inverted terminal repeats (ITRs) is integrated into the genome through a precise, transposase-catalyzed mechanism, providing long-term expression of the gene of interest in cells [12]. Bicomponent transposon-based transgenic systems comprise a donor vector containing the transgene flanked by transposase-specific ITRs and the transposase enzyme provided as protein, mRNA, or most commonly as a helper DNA vector. In addition, systems that combine both components in a single vector, known as helper-independent transposons, have been developed and validated in cells and animals [13–16]. Transposase catalyzes both the excision of the transgene from the donor vector and its integration into a genomic target site. Integration occurs at short consensus sequences, for example Tc1/mariner transposases, such as SB, recognize TA dinucleotides [17], and PB transposase recognizes TTAA tetranucleotides [18]. Through this mechanism one monomeric copy of a transposon is integrated in the genome, leaving the empty backbone of the donor plasmid, which is eventually degraded [12,19,20] or, rarely, randomly integrated [14,21]. Expression units introduced by transposition are less prone to epigenetic silencing and show long-term expression of the transgene [12], suggesting that transposons have a tendency to land in genomic regions that are transcriptionally permissive [22].

PiggyBac and SB transposons have been extensively studied for transgenesis in mice, rats, and rabbits [13,23–26]. Both *in vivo* (intracytoplasmic injection of zygotes) and *in vitro* (SCNT) approaches have been exploited to generate transposon–transgenic pig models [12,20,27–31]. Zygote microinjection with SB transposon components has resulted in single-copy integration units into the pig preimplantation embryo genome [20], into born F0 animals and successful transmission to F1 generation [12]. Garrels et al. [12] demonstrated segregation of individual transposons in the F1 offspring, copy number–dependent expression of reporter protein over a prolonged time with no evidence of gene silencing. Similarly, transgenic pigs generated by microinjection of a helper-independent, self-inactivating PB transposon had monogenic and often single transgene genomic integration and the absence of concatemers or variegated transgene expression [28].

Alternatively, genetic modification of somatic cells by PB or SB transposition followed by SCNT is an avenue to generate transgenic livestock. Here, we assessed the suitability of the PB and SB transposon systems for the genetic modification of bovine fibroblasts, which were subsequently used in SCNT.

To this end, cultured fibroblast cells were transfected or electroporated with both transposon systems, respectively, and on selection or enrichment of transgenic cells, they were used as nuclear donor in SCNT. The use of transposons is associated with an enhanced proportion of stably transfected cells, as it has been documented for established immortalized and primary porcine cells [32] transfected with SB, PB, Tol2, or passport transposon systems [20,32] as well as for primary bovine fibroblasts transfected with PB [33]. These promising results warrant more research that extends and adapts transposon-based methods to functional transgene products and to economically important livestock species such as cattle.

2. Materials and methods

2.1. Experimental design

The experimental design is summarized in the Figure 1. Fibroblast cultures were established from fetuses collected at a local abattoir. Fibroblasts were transfected with either SB transposon components or PB helper-independent plasmids followed by a 3-week antibiotic selection period.

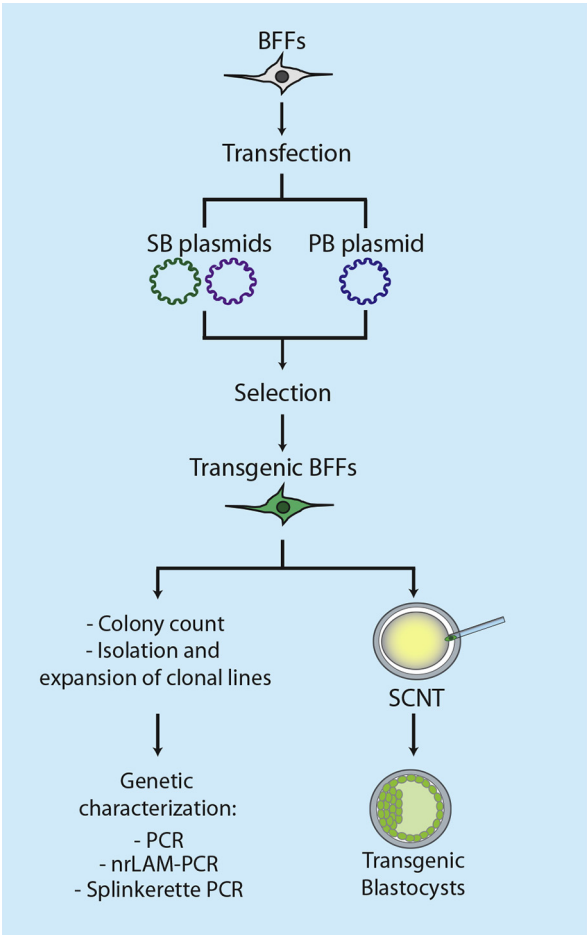

Fig. 1. Schematic representation of the experimental design.