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Review

Transposon-based reprogramming to induced pluripotency

Dharmendra Kumar[1], Thirumala R. Talluri[2], Taruna Anand[3] and Wilfried A. Kues[4]

[1]Animal Physiology and Reproduction Division, ICAR-Central Institute for Research on Buffaloes, Hisar, Haryana, India, [2]Equine Production Campus, ICAR National Research Centre on Equines, Bikaner, Rajasthan, India, [3]VTCC, ICAR-National Research Centre on Equines, Hisar, Haryana, India and [4]Friedrich-Loeffler-Institute, Mariensee, Germany

Summary. Induced pluripotent stem (iPS) cells represent a recent innovation in the field of stem cells. Commonly, iPS cells are generated by viral transduction of core reprogramming genes, such as Oct4, Sox2, Klf4, c-Myc, Nanog and Lin28. However, integrating viruses, like retro- and lentiviral vectors, may cause insertional mutagenesis and may increase the risk of tumor formation. Therefore, alternative methods which avoid these safety concerns are intensively investigated. Here, we review the current status of transposon-based methods to induce pluripotency. DNA transposons are non-viral elements, which can be effectively integrated into a genome by their corresponding transposase enzyme. The advantages of transposon-based gene transfer are their increased safety, their large cargo capacity, their relatively simple design, and the availability of hyper-active and mutated transposase enzymes. For example, integration-deficient, excision-competent transposase variants allow the complete removal of the reprogramming transposon after successful reprogramming to obtain transposon-free reprogrammed cells. Transposon-based reprogramming broaden the toolbox for iPS cell production and will advance the establishment of safe, non-viral methods.

Key words: Induced pluripotent stem cells, Reprogramming, Transposition, Sleeping Beauty, PiggyBac, Stemness, Ontogenesis, Synthetic biology

Offprint requests to: Wilfried A Kues, Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics, Dept. Biotechnology, Höltystr. 10, 31535 Mariensee, Germany. e-mail: wilfried.kues@fli.bund.de

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Introduction

Induced pluripotent stem (iPS) cells represent a recent innovation in stem cell research and developmental biology. Commonly, iPS cells are generated by viral transduction of core reprogramming genes, such as Oct4, Sox2, Klf4, c-Myc, Nanog and Lin28 (Takahashi and Yamanaka, 2006; Okita et al., 2007; Takahashi et al., 2007; Yu et al., 2007). The viral gene delivery of the reprogramming factors is associated with considerable risks of insertional mutagenesis and genotoxicity (Wu and Dunbar, 2011). To overcome these risks, alternative methods, such as non-integrating adenoviral vectors (Stadtfeld et al., 2008), plasmids (Yu et al., 2009), recombinant proteins (Zhou et al., 2009), modified mRNAs (Warren et al., 2010), and small molecules (Shi et al., 2008) were assessed for iPS cell derivation. However, the efficiency of reprogramming using these methods is significantly lower than that of retro- or lentiviral vectors and the alternative methods may require repetitive treatments to maintain pluripotency (Kumar et al., 2015).

Non-autonomous DNA transposon systems represent a promising alternative to these approaches. Recently generated hyperactive transposon elements have improved gene delivery to levels similar to that obtained with viral vectors (Mates et al., 2009; Yusa et al., 2011; Li et al., 2013). DNA transposons, specifically Sleeping Beauty (SB) and piggyBac (PB), have emerged as useful alternatives to virus-mediated reprogramming of somatic cells in different species (Table 1), including human (Woltjen et al., 2009; Davis et al., 2013), mouse (Woltjen et al., 2009; Muenthaisong et al., 2012; Grabundzija et

al., 2013; Talluri et al., 2014; Tsukiyama et al., 2014), pig (Kues et al., 2013), horse (Nagy et al., 2011), bat (Mo et al., 2014), monkey (Debowski et al., 2015), rat (Ye et al., 2015) and cattle (Talluri et al., 2015). The different transposon systems have a number of advantages over the viral vectors, such as: (i) no or minimal bias for integration in expressed genes or promoter regions, (ii) a DNA cargo capacity of up to 100 kb (Rostovskaya et al., 2012; Skipper et al., 2013), (iii) the possibility of seamless removal of the transposon after reprogramming (Woltjen et al., 2009), (iv) a cost-effective production of the basic plasmids, (v) less innate immunogenicity, (vi) the co-delivery of multiple genes, and (vii) no requirement for a specialized biohazard containment facility during production or handling.

Transposons or mobile genetic elements were first described by Barbara McClintock as "jumping genes" responsible for color mosaicism of kernels in maize cobs (McClintock, 1950). Transposons are discrete pieces of DNA that can move from one site to another within a genome, and sometime between genomes (Ivancevic et al., 2013; Walsh et al. 2013). Species-specific transposons are found in the genomes of all pro- and eukaryotes, and in humans about 45% of the genome is derived from retro- (RNA) and DNA transposons (Lander et al., 2001), however the gross majority of the naturally occurring transposons accumulated mutations and lost the ability to jump. Due to their wide distribution and diversity, they are a considerable source of genomic variation and as such, they constitute powerful drivers of genome evolution (Ivancevic et al. 2013; Ayarpadikannan and Kim, 2014; Erwin et al., 2014; Chenais, 2015; Ayarpadikannan et al., 2015).

To obtain a clinically relevant transposon that could be used for cellular reprogramming, it was important to identify transposable elements, which are capable of efficient transposition in mammalian cells. In this direction, SB and PB DNA transposon systems have been developed as efficient cellular reprogramming vectors. SB originates from salmonid fish species, where it existed as inactive element, which was reawakened by an *in vitro* mutagenesis approach (Ivics et al., 1997). PB was identified as an active element in the moth *Trichoplusia ni* (Fraser et al., 1996). Importantly, no orthologous elements to SB and PB are known in mammalian species, thus the re-mobilisation by potential endogenous transposases is unlikely. In transgenic mice and pigs this has been experimentally verified (Dupuy et al., 2002; Garrels et al., 2012a).

Importantly, the hyperactive SB (SB100X) and PB (hypPB) transposases have similar activity levels in mammalian cells, and are independent of cellular co-factors (Mátés et al., 2009; Doherty et al., 2012). Both transposon systems have been shown to be suitable for the derivation of iPS cells (Woltjen et al., 2009; Nagy et al., 2011; Grabundzija et al., 2013; Salewski et al., 2013; Kues et al., 2013; Talluri et al., 2014, 2015). Here, we review the current status and perspectives of transposon-based methods to induce pluripotency.

Transposon systems and reprogramming by engineered transposons

Several DNA transposon systems with active transpositional competence in mammalian cells and organisms have been developed in recent years. The best characterized transposon systems, such as SB, PB, Tol2, Frog Prince and Passport originate from non-mammalian species (Kawakami et al., 2000; Miskey et al., 2003; Ivics et al., 2009; Clark et al., 2009), but active mammalian DNA transposons, like Hsmar1, also have been reconstructed (Miskey, 2007).

For genetic engineering the SB and PB transposon are commonly designed as non-autonomous systems, splitting the functional elements into two bacterial plasmids, which can be amplified in *E. coli* under standard laboratory conditions. One plasmid (helper plasmid) carries the transposase gene, and a second plasmid carries the inverted terminal repeat (ITR) flanked gene of interest (transposon) (Fig.1). Here, the transposon plasmid carries the reprogramming factors on a polycistronic construct driven by a single promoter (Fig. 1). Co-delivery of both plasmids into mammalian cells, results in transcription of the episomal helper plasmid and expression of the transposase, which subsequently transposes the ITR-flanked reprogramming factor cassette into the genome. Transposition includes two basic basepair-accurate enzymatic reactions, a precise cutting at the ends of the ITRs, and an introduction of the transposon into a genomic site (cut-and-paste mechanism) as shown in Fig. 1. Both steps are performed by the transposase protein. In the first step of transposition, the transposase binds to the ITRs followed by formation of a synaptic complex. The minimal consensus sequence for SB-catalyzed integrations is a TA-dinucleotide (Ivics et al., 1997), and for PB transposition a TTAA-tetranucleotide sequence is required (Ding et al., 2005; Collier and Largaespada, 2007; Cadinanos and Bradley, 2007). Tol2 does not seem to use a specific recognition site (Grabundzija et al., 2010; Meir et al., 2011), but all transposases seem to require additional, less well explored topological conformations of the target DNA. In particular, chromatin condensation and CpG-methylation patters seem to affect the transposition rate (Jursch et al., 2013; Claeys-Bouuaert and Chalmers, 2013). Genomic regions with higher AT content organized in a palindromic core unit were found to be the preferential insertion sites for SB (Vigdal et al., 2002; Yant et al., 2005), while a recent study showed that the first 100 nucleotides surrounding the TTAA integration site are important for PB transposase target selection (Meir et al., 2011).

Nevertheless, at a genomic scale, SB transposon integrations appear to be randomly distributed (Vigdal et al., 2002; Yant et al., 2005; Berry et al., 2006; Huang et al., 2010), whereas PB and Tol2 show a slight bias for promoter and exonic regions (Skipper et al., 2013). Saturation analyses indicated a minor correlation between the integration profile of transposons and the

transcriptional status of targeted genes (Yant et al., 2005). Consequently, the integration pattern of the transposon systems seem to be safer as compared to retroviral vectors, which show a strong bias for promoter and exonic regions of transcribed genes (Wu et al., 2003; Berry et al., 2006; Hackett et al., 2007). Importantly, both SB and PB seem to favour transcriptional permissive loci in the genome, ensuring robust expression of the reprogramming factors (Talluri et al., 2014).

For iPS cell reprogramming, the transposon is used as a bi-component vector system for delivering of the reprogramming factors. The transposon is excised from the donor plasmid and is integrated at a chromosomal site by the transposase through the cut-and-paste mechanism. The mobilisation of the transposon requires that the transposase gene is transcribed; the matured messenger transcript is exported into the cytoplasm, and then becomes translated. The transposase protein is then folded, and enters the nucleus, where transposition occurs (Fig. 1) (Beall et al., 2002).

The transposon could accommodate individual cDNAs or polycistronic constructs (Fig. 2). The employment of 2A self-cleaving peptide sequences allow the stochiometric co-expression of several protein products from a single transcript (Kues et al., 2013; Grabundzija et al., 2013; Davis et al., 2013; Mo et al., 2014; Tsukiyama et al., 2014; Talluri et al., 2014, Talluri et al., 2015). The design of 2A constructs requires the removal of the stop codons and the in-frame integration of 2A sequences. The 2A sequences encode short oligopeptides (18-22 amino acids) that auto-catalytically cleave the nascent protein sequence inside the 2A oligopeptide before the last proline (de Felipe, 2004; Szymczak et al., 2004; Yusa et al., 2009; Kaji et al., 2009; Szymczak-Workman et al., 2012). Thus, the resulting individual reprogramming factors will carry short 2A peptides on the carboxy terminal ends. Recently, 2A peptide mediated co-expression of transgenes has gained much popularity due to its small size, efficacy, stoichiometry and availability of various functional variants. Its small size is an invaluable feature in terms of designing reprogramming vectors assembling several protein-coding cDNA on a single construct. The use of 2A variants dispenses with the need for several promoter elements and thereby reduces potential intermolecular homologous recombination events and promoter crosstalk (Henikoff, 1998).

Transposon removal and maintenance of pluripotency

Maintaining genomic integrity is critical for therapeutic applications of iPS cells. Transposon-based

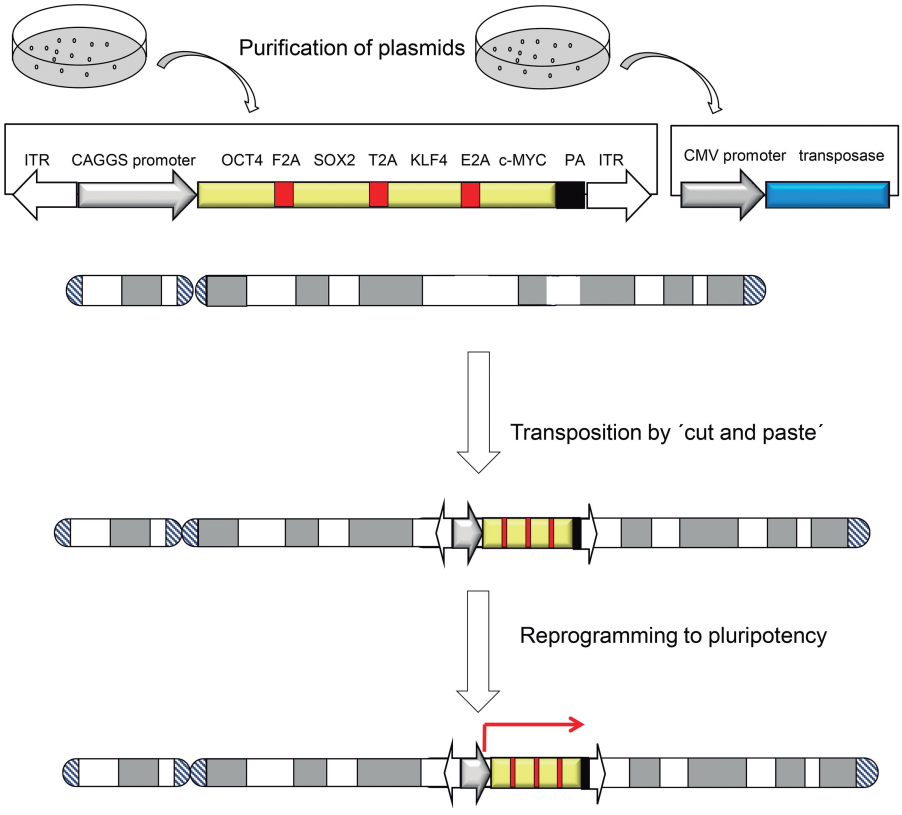

Fig. 1. Transposition of a multi-gene reprogramming transposon. A non-autonomous transposon system consists of two plasmids, which are separately amplified in bacteria. The transposon plasmid carries an ITR-flanked reprogramming construct of a strong promoter driving a multi-gene arrangement of reprogramming factors. The transposase (helper) plasmid carries the corresponding transposase gene driven by a strong promoter. Upon co-transduction into mammalian cells, the transposase gene is expressed, and catalyzes precise integration of the ITR-flanked transposon into the genome.

delivery systems offer the possibility of seamless removal of the reprogramming cassette. This was demonstrated as a proof-of-principle in murine iPS cells (Woltjen et al., 2009; Yusa et al., 2009), and then in human iPS cells (Woltjen et al., 2011; Igawa et al., 2014). The integrated PB transposon can be re-mobilized by supplying the transposase in *trans* (Fig. 3) (VandenDriessche et al., 2009; Woltjen et al., 2011) and permits the isolation of transgene-free iPS cells, which makes the system more attractive and relevant in producing safe and clean iPS cells (Yusa et al., 2009; Ivics et al., 2009; Woltjen et al., 2011, Mo et al., 2014). The integrated PB transposon could be excised from iPS cells at a rate of higher than 90% by transient expression of PB transposase (Hotta et al., 2009). During PB transposon excision the TTAA integration sites used by PB transposase are repaired to the original sequence (Fraser et al., 1996), resulting in a seamless removal of transposons.

In contrast, Davis et al. (2013) were unable to generate a transgene-free iPS cell line following re-expression of the SB transposase in the reprogrammed cells. SB mediated iPS cells containing a single copy of the transposon might improve the likelihood of generating transgene-free iPS cells. However, these excision approaches are complex and time consuming, since they require the identification of iPS cells with minimal copy insertions, mapping of integration sites, excision of the reprogramming cassette, and validation of factor-free clones (Park et al., 2008).

Alternatively, recombinase-specific sites such as lox P could be incorporated into the transposon vector to enable recombinase mediated excision (Kaji et al., 2009). In this case, the short terminal repeat sequences would remain in the genome (Cadinanos and Bradley, 2007; Walisko et al., 2008).

Recently, an inducible PB transposon-based system to regulate protein stability of the reprogramming factors by the small molecule, trimethoprim (TMP), was established for murine and porcine iPS cell derivation (Sui et al., 2014). Here, the half-life of one or more reprogramming factors is reduced by engineering fusion proteins with the destabilizing domain of *E. coli* dihydrofolate reductase, which targets the fusion protein to proteasomal degradation (Iwamoto et al. 2010; Sui et al, 2014). The media supplementation of TMP prevents degradation in a reversible and dose-dependent manner (Sui et al., 2014), allowing a fine-tuning of the levels of domain-tagged exogenous reprogramming factors.

Transposon-mediated induced pluripotent stem cells

The most commonly applied methods for the generation of iPS cells rely on the introduction of different combinations of reprogramming factors in the form of DNA, mRNA or protein into somatic cells. The early studies applied multiple retro- and lentiviruses integrations into the host genome for high expression of

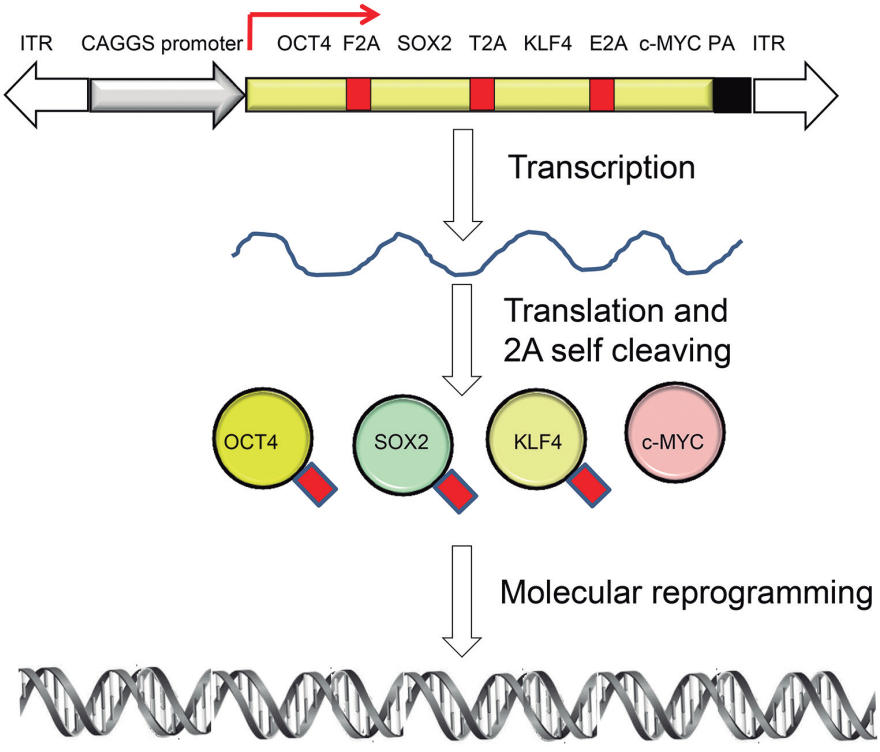

Fig. 2. Translation of reprogramming factors from a transposon 2A construct . A single transcript is formed from the multigenic construct. During translation, the self-cleaving 2A peptide sequences catalyze the production of individual reprogramming factors.

the encoded cargo genes (Takahashi and Yamanaka, 2006; Sommer et al., 2009). But, oncogenicity of the factors used in cell reprogramming and the potential for insertional mutagenesis caused by integrating viral gene transfer vectors limited the value of the resulting iPS cells for clinical applications (Okita et al., 2007; Nakagawa et al., 2008; Aoi et al., 2008), and it is believed that avoiding viral insertions will be a strict requirement for clinical translation of iPS cells. Therefore, transposon- mediated generation of iPS cells is now an alternative choice due to their high gene delivery efficiency along with the ability to be excised from the cells after reprogramming. The introduction of the reprogramming factors into somatic cells using transposon systems is relatively straightforward, requiring only one transfection. The overall efficiency of transposons-mediated cellular reprogramming is ~ 0.02% (Grabundzija et al., 2013, Davis et al., 2013; Talluri et al., 2014), which comes close to the initially obtained reprogramming efficiencies by viral vectors. This is higher than that reported for non-integrative delivery strategies using either replicating episomal vectors or minicircles (Yu et al., 2009; Jia et al., 2010), although lower than Sendai viral vectors or synthetic mRNA (Fusaki et al., 2009; Warren et al., 2010). Transposons-mediated delivery of core reprogramming factors have been successfully used to derive pluripotent cells in various species, including human (Woltjen et al., 2009; Davis et al., 2013), mouse (Muenthaisong et al., 2012; Grabundzija et al., 2013; Talluri et al., 2014; Tsukiyama et al., 2014), pig (Kues et al., 2013), horse (Nagy et al., 2011), bat (Mo et al., 2014), monkey

Table 1. Transposon-based reprogramming approaches.

Species	Cell type	Transposon system	Reprogramming factors	Differentiation		Chimera	Germline contribution	Reference
				in vitro	*in vivo*			
Bat	Fetal fibroblasts	PB	OSKMNL + Nr5a2, and miR302/367	EBs	Teratoma	NA	NA	Mo et al., 2014
Cattle	Fetal fibroblasts	PB/SB	SOKMNL	EBs	Teratoma	NA	NA	Talluri et al., 2015
Horse	Fetal fibroblasts	PB	OSKM	EBs	Teratoma	NA	NA	Nagy et al., 2011
Human	Skin fibroblasts	PB	OSKML	EBs	Teratoma	Ethically not allowed	Ethically not allowed	Igawa et al., 2014
	Fetal fibroblasts	SB	OSKM	EBs	NA	Ethically not allowed	Ethically not allowed	Davis et al., 2013
Monkey	Skin fibroblasts	PB	OSKMNL	EBs	Teratoma	NA	NA	Debowski et al., 2015
Mouse	Fetal fibroblasts	PB	OSKM	EBs	Teratoma	Yes	Yes	Yusa et al., 2009
	Fetal fibroblasts	PB	$O^{dd}K^{dd}S^{dd}$/ $O^{dd}KS$	NA	NA	NA	NA	Sui et al 2014
	Fetal fibroblasts	PB	SOKMNL/SOKM/SOK	EB	Teratoma	NA	NA	Talluri et al. 2014
	Fetal fibroblasts	SB	OKSM	EB	Teratoma	NA	NA	Talluri et al., 2014
Pig	Fetal fibroblasts	SB	OSKM	Neuronal lineage	Teratoma	NA	NA	Kues et al., 2013
Rat	Fetal fibroblasts	PB	OSKM	chondrocyte	NA	NA	NA	Ye et al., 2015

O, Oct4, S, Sox2; K, Klf4; M, c-Myc; N, Nanog; L, Lin28; EBs, Embryoid bodies; NA, Not application; PB, piggyBac; SB, Sleeping Beauty; dd, Destabilizing domain

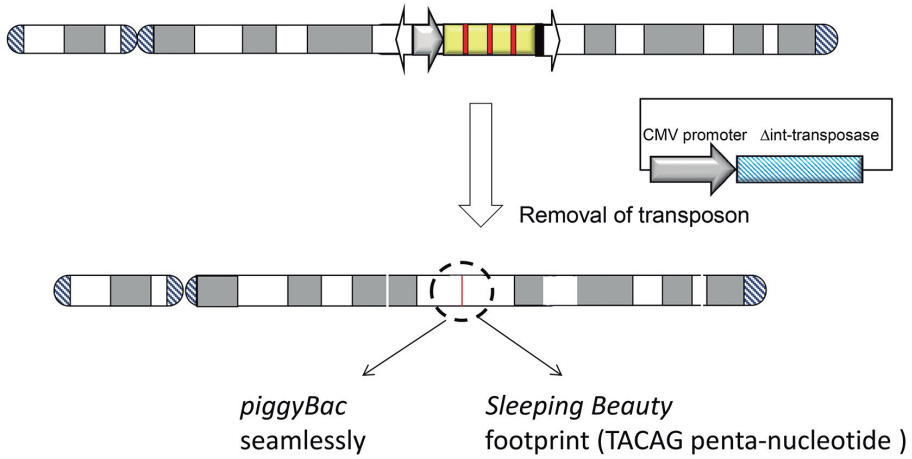

Fig. 3. Removal of a reprogramming transposon from iPS cells. By supplying an excision competent/integration-deficient transposase variant, the integrated transposon can be effectively removed from the genome. In the case of PB transposon this happens seamlessly, in the case of SB a pentameric footprint remains in the integration site.

(Debowski et al., 2015), rat (Ye et al., 2015) and cattle (Talluri et al., 2015), which showed typical hallmarks of pluripotency, such as differentiation *in vivo* and teratoma formation (Table 1). Importantly, multiple previous attempts with embryonic stem cells (ESC), primordial germ cells (PGC) and other stem cells from non-rodent and non-human species failed to achieve teratoma-competent pluripotent stem cells over the last 20 years (Gandolfi et al., 2012; Nowak-Imialek and Niemann, 2012; Kumar et al., 2015). In contrast to these ESC and PGC approaches, iPS cells can be conveniently reprogrammed from different somatic cells. Thus, the iPS methodology advanced the stem cell field with a huge leap over previous attempts, and allowed to identify discrete, critical reprogramming factors.

The progress in iPS cell development in farm animals lags behind those in rodents, but large mammalian models will be instrumental for preclinical tests of novel cell therapies, enhanced pharmaceutical studies and regenerative studies, including the restoration of fertility (Kumar et al., 2015). The pig is an attractive large animal model for preclinical testing of safety and efficacy of cell based therapies (Gün and Kues, 2014). Porcine organs are largely similar in size and physiology to their human counterparts rendering the domestic pig a suitable model for cardiovascular disease (Turk and Laughlin, 2004), muscular dystrophies (Emery, 2002), atherosclerosis (Ishii et al., 2006), wound repair (Graham et al., 2000), diabetes (Dyson et al., 2006), ophthalmological diseases (Petters et al., 1997) and xenotransplantation. Recently, we reported the first SB reprogrammed porcine iPS cells (Kues et al., 2013) (Fig. 4). The porcine iPS cells maintained long-term proliferation *in vitro* (>40 passages), expressed transcription factors typical of embryonic stem cells, such as OCT4, NANOG, SOX2, REX1, ESRRB, DPPA5, and UTF1 and surface markers of pluripotency, including SSEA-1 and TRA-1-60. *In vitro* differentiation resulted in derivatives of ecto-, meso- and endodermal cell types. Upon injection of putative iPS cells under the skin of immunodeficient mice, we observed mature teratomas (Kues et al., 2013). In addition, we successfully applied the PB transposon system for induction of pluripotency in porcine somatic cells (unpublished data), demonstrating the suitability of both transposon systems for reprogramming of porcine fibroblasts.

Most of the iPS cells derived through transposon-mediated reprogramming showed the *in vitro* differentiation to embryoid bodies (EBs) and readily formed teratomas *in vivo* (Table 1). Teratoma formation demonstrated that the reprogrammed iPS cells had the developmental potential to differentiate into tissues of all three primary germ layers, i.e. ectoderm, mesoderm and endoderm (Fig. 5). Perhaps the most stringent test for pluripotency of iPS cells is their ability to form germline-competent chimeras. The formation of murine chimeras from transposon reprogrammed iPS cells has been demonstrated (Woltjen et al., 2009). However, most of the current transposon iPS lines carry constructs, in which a strong promoter is constitutively driving the reprogramming factors (Nagy et al., 2011; Kues et al.,

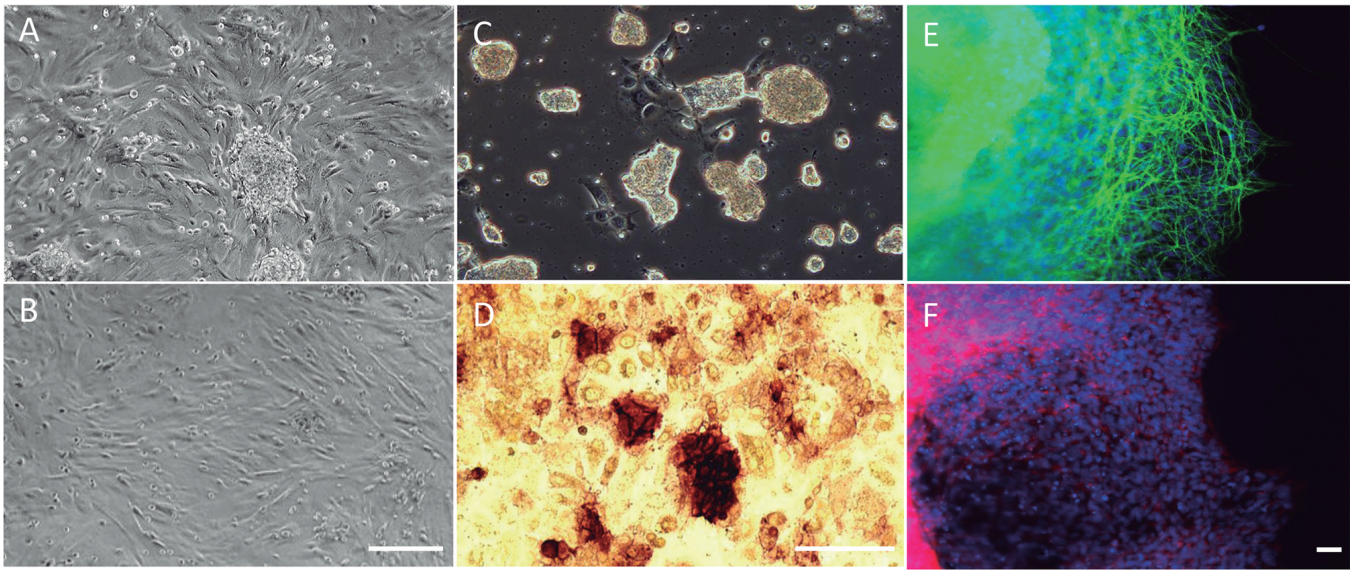

Fig. 4. SB transposon reprogramming of porcine fibroblasts. **A)** Initial cell colonies formed 9-13 days after electroporation of the SB transposon system. **B)** Control culture. Bar = 20 µm. **C)** Stable porcine iPS line. **D)** Histochemical determination of alkaline phosphatase expression (red) in iPS colonies. Bar = 50 µm. **E)** Immunodetection of neuronal markers in *in vitro*-differentiated porcine iPS cells. Tuj1 (green) axonal marker, nuclei (blue (Hoechst 33342)). **F)** Immunodetection of neuronal marker in *in vitro*-differentiated porcine iPS cells. Nestin (red) neuronal progenitor, nuclei (blue (Hoechst 33342)). Bar = 10 µm. (modified from Kues et al., 2013).

2013; Talluri et al., 2015), which will prevent their contribution to a regular ontogenesis. Thus the transposon iPS lines from several species have not yet been tested for their capability to contribute to chimera formation and germline transmission. The recent development of integration-deficient, but excision-competent transposase variants (Yusa et al., 2011) will simplify the transposon removal after complete reprogramming and the achievement of autonomous stemness.

Application of transposons in gene therapy, transgenesis and genetic engineering

A highly efficient genetic modification system holds remarkable potential for biomedical research, but also for facilitating the advancement of molecular medicine, including gene and cell therapies. Transposon systems have been applied for genetic modification of human ES cells (Wilber et al., 2007). More recently, transposons have also been used to insert bacterial artificial chromosomes (BACs) in human ES cells (Rostovskaya et al., 2012). Both SB and PB have been used to genetically modify hematopoietic stem cells (Grabundzija et al., 2010). Recently, a hyperactive transposase variant of SB showed efficient transgenesis in mouse, rabbit and pig (Garrels et al., 2011, 2012b; Ivics et al., 20014a-c), with a preferential integration into transcriptionally permissive genomic loci (Garrels et al., 2012c; Bosch et al., 2015). This has been verified for

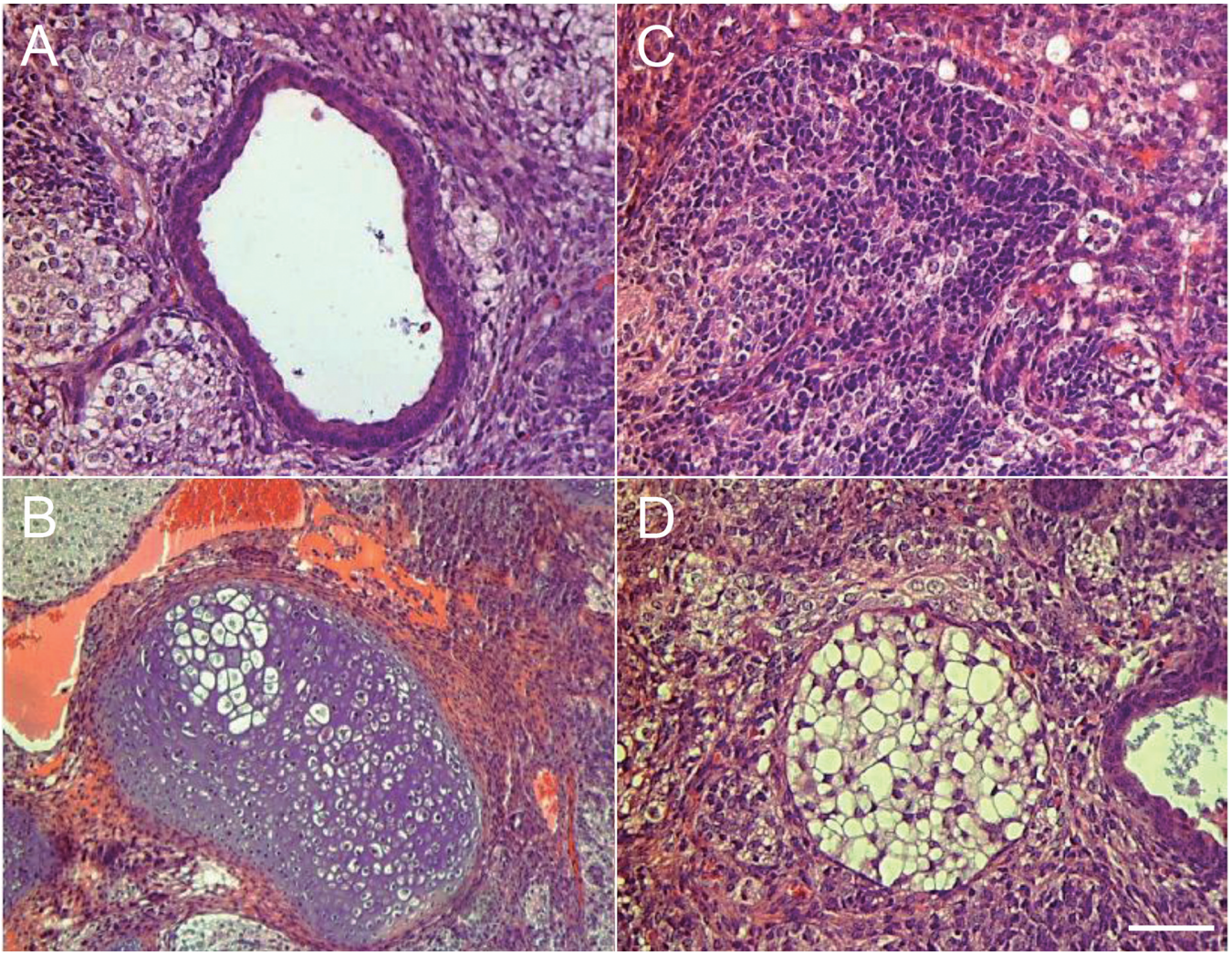

Fig. 5. Teratoma of transposon-derived bovine iPS cells. **A-D)** Representative images of a mature teratoma depicting fully differentiated cells of the three germ layers. About 1 x 106 bovine iPS cells were injected under the skin of an immuno-deficient nude mouse. Four weeks later the tumor was removed and formaldehyde fixed. Paraffin-embedded tissue was sectioned in 10 µm slides, and stained with hematoxylin and eosin (modified from Talluri et al., 2015, Supplementary data). Bar: 40 µm.

PB-mediated transgenesis in the pig (Li et al., 2014). In contrast, random transgenesis by non-facilitated methods frequently resulted in silencing of transgenes (Henikoff, 1998; Kues et al., 2006; Bosch et al., 2015). Importantly, the SB transposase has a close-to-random insertion profile in mammalian genomes (Mátés et al., 2009; Skipper et al., 2013). The majority of SB insertions occur in intergenic regions, unlike retro- and lentiviral integrations, which favour promoter and exonic regions (Nakagawa et al., 2008; Stadtfeld et al., 2008; Yu et al., 2009). Thus, the SB catalysed DNA integration reduces the risk of insertional mutagenesis and represents a rather safe method of gene delivery (Mátés et al., 2009; Garrels et al., 2012a). Thus transposon-mediated gene transfer provides an effective mechanism for permanent or reversible genetic modification of several cell types, which may be developed to novel cell therapies.

For the first time transposon vectors were used as tools for gene therapy in liver of mice by Yant et al., (2000). Since then, there have been many successful preclinical gene therapy studies benefiting from the use of transposon vectors. For example, the SB system has been used for delivering blood clot factors VIII (Kren et al., 2009), IX (Yant et al., 2000; Yant et al., 2002), and lysosomal enzyme (Aronovich et al., 2007). Furthermore, transposon mediated *in vivo* gene delivery had been achieved by poly ethylene-imine (PEI) into lung (Liu et al., 2006a) and brain tumors (Ohlfest et al., 2005). PEI-based systemic administration of SB transposon encoding endothelial nitric oxide synthase resulted in endothelial nitric oxide synthase expression in pulmonary endothelial cells, leading to inhibition of induced pulmonary hypertension in rats (Liu et al., 2006b). Further, transposon systems have been used for the treatment of epidermolysis bullosa (Ortiz-Urda et al., 2003), tyrosinemia type I (Montini et al., 2002), Fanconi anemia type c (Smith and Wagner, 2012), Huntington's disease (Chen et al., 2005), sickle cell anaemia (Belcher et al., 2010), T cell malignancy (Nakazawa et al., 2011), liver disease (Burnight et al., 2012) and hepatic gene deficiency disorders (Anderson et al., 2013).

The first clinical trials using SB vector for patients with B lineage malignancies are in progress at the MD Anderson Cancer Centre, in which T cells with engineered CD19-specific chimeric antigen receptors (CAR) have been developed using a binary transposon system (Switzer et al., 2012; Singh et al., 2014). This approach is a combination of immunotherapy with gene therapy techniques (Singh et al., 2014). Previously, CAR-positive T cells were generated by several groups using viral vectors, which was time-consuming and costly (Singh et al., 2014). The SB system has also been approved for a human clinical trial involving immunotherapy for CD19-positive malignancies (Hackett et al., 2010). Further, the successful introduction of a SB therapeutic transgene into CD34-positive cells from sickle cell anaemic patients and *in vitro* evidence of its ability to improve the disease characteristics are important milestones in progressing toward transposon-mediated gene therapies (Sjeklocha et al. 2011). Recently, Nakazawa, et al. (2013) reported that the long-term transgene expression in PB-modified human T lymphocytes is encouraging for therapies that are dependent on prolonged gene expression.

Perspectives

The development of transposon-mediated reprogramming of somatic cells has considerable potential in speeding up patient-specific cell based therapies. The advancement in cellular reprogramming is likely to develop faster than gene therapy due to the available improved methodologies. Recent promising results highlight the potential of transposon-mediated somatic cells reprogramming, bringing the hope of cell-based therapies closer to reality (Filareto et al., 2013). The continuing efforts in transposase engineering have recently resulted in the emergence of robust transposon platform technologies that can now be explored to genetically modify clinically relevant stem cells. This should pave the way toward the validation of these emerging technologies for cell-based gene therapies in preclinical disease models that mimic the cognate human diseases (Di Matteo et al., 2012). Currently, transposon-based gene delivery in primary stem/progenitor cell such as hematopoietic stem cells, mesenchymal stem cells, muscle stem/progenitor cells, and iPS cells resulted in prolonged and relatively robust expression of the gene of interest (Di Matteo et al., 2012). However, it will be crucial to conduct additional preclinical studies in both small and large animal models to further evaluate the efficacy and safety of emerging transposon technologies. In addition, the functional consequences of transposition on tumorigenic risk need to be assessed in the appropriate cellular models or by *in vivo* analysis in tumor-prone mouse models (Di Matteo et al., 2012; Vand Rajabpour et al., 2014).

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References

Anderson C.D., Urschitz J., Khemmani M., Owens J.B., Moisyadi S., Shohet R.V. and Walton C.B. (2013). Ultrasound directs a transposase system for durable hepatic gene delivery in mice. Ultrasound Med. Biol. 39, 2351- 2361.

Aoi T., Yae K., Nakagawa M., Ichisaka T., Okita K., Takahashi K., Chiba T. and Yamanaka S. (20080. Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science 321, 699-702.

Aronovich E.L., Bell J.B., Belur L.R., Gunther R., Koniar B., Erickson D.C., Schachern P.A., Matise I., McIvor R.S., Whitley C.B. and

Hackett P.B. (2007). Prolonged expression of a lysosomal enzyme in mouse liver after Sleeping Beauty transposon-mediated gene delivery: implications for non-viral gene therapy of mucopolysaccharidoses. J. Gene Med. 9, 403-415.

Ayarpadikannan S. and Kim H.S. (2014). The impact of transposable elements in genome evolution and genetic instability and their implications in various diseases. Genomics Inform. 12(3), 98-104.

Ayarpadikannan S., Lee H.E., Han K. and Kim H.S. (2015). Transposable element-driven transcript diversification and its relevance to genetic disorders. Gene 558(2), 187-194.

Beall E.L., Mahoney M.B. and Rio D.C. (2002). Identification and analysis of a hyperactive mutant form of Drosophila P-element transposase. Genetics 162, 217-227.

Belcher J.D., Vineyard J.V., Bruzzone C.M., Chen C., Beckman J.D., Nguyen J., Steer C.J and Vercellotti G.M. (2010). Heme oxygenase-1 gene delivery by Sleeping Beauty inhibits vascular stasis in a murine model of sickle cell disease. J Mol Med. 88, 665- 675.

Berry C., Hannenhalli S., Leipzig J. and Bushman F.D. (2006). Selection of target sites for mobile DNA integration in the human genome. PLoS Comput. Biol. 2: e157.

Bosch P., Forcato D.O., Alustiza F.E., Alessio A.P., Fili A.E., Olmos Nicotra M.F., Liaudat A.C, Rodríguez N., Talluri T.R. and Kues W.A. (2015). Exogenous enzymes upgrade transgenesis and genetic engineering of farm animals. Cell Mol. Life Sci. 72(10), 1907-1929.

Burnight E.R., Staber J.M., Korsakov P., Li X., Brett B.T., Scheetz T.E., Craig N.L. and McCray P.B. Jr. (2012). A hyperactive transposase promotes persistent gene transfer of a piggyBac DNA Transposon. Mol. Ther. Nucleic Acids 1: e50.

Cadinanos J. and Bradley A. (2007). Generation of an inducible and optimized piggyBac transposon system. Nucleic Acids Res. 35(12), e87.

Chen Z.J., Kren B.T., Wong P.Y., Low W.C. and Steer C.J. (2005). Sleeping Beauty mediated down-regulation of huntingtin expression by RNA interference. Biochem. Biophys. Res. Commun. 329, 646-652.

Chénais B. (2015). Transposable elements in cancer and other human diseases. Curr. Cancer Drug Targets 17 (Epub ahead of print).

Claeys-Bouuaert C. and Chalmers R. (2013). Hsmar1 transposition is sensitive to the topology of the transposon donor and the target. PLoS One 8(1), e53690.

Clark K.J., Carlson D.F., Leaver M.J., Foster L.K. and Fahrenkrug S.C. (2009). Passport, a native Tc1 transposon from flatfish, is functionally active in vertebrate cells. Nucleic Acids Res. 37, 1239-1247.

Collier L.S. and Largaespada D.A. (2007). Transposons for cancer gene discovery: Sleeping Beauty and beyond. Genome Biol. 8(Suppl 1), S15.

Davis R.P., Nemes C., Varga E., Freund C., Kosmidis G., Gkatzis K., de Jong D., Szuhai K., Dinnyés A. and Mummery C.L. (2013). Generation of induced pluripotent stem cells from human foetal fibroblasts using the Sleeping Beauty transposon gene delivery system. Differentiation 86(1-2), 30-37.

de Felipe P. (2004). Skipping the co-expression problem: the new 2A "CHYSEL" technology Genet. Vaccines Ther. 2(1), 13.

Debowski K., Warthemann R., Lentes J., Salinas-Riester G., Dressel R., Langenstroth D., Gromoll J., Sasaki E. and Behr R. (2015). Non-viral generation of Marmoset monkey iPS cells by a six-factor-in-one-vector approach. PLoS One 10(3), e0118424.

Di Matteo M., Belay E., Chuah M.K. and Vandendriessche T. (2012). Recent developments in transposon-mediated gene therapy. Expert Opin. Biol. Ther. 12(7), 841-858.

Ding S., Wu X., Li G., Han M., Zhuang Y. and Xu, T. (2005). Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice. Cell 122, 473-483.

Doherty J.E., Huye L.E., Yusa K., Zhou L., Craig N.L. and Wilson M.H. (2012). Hyperactive piggyBac gene transfer in human cells and *in vivo*. Hum. Gene Ther. 23(3), 311-320.

Dupuy A.J., Clark K., Carlson C.M., Fritz S., Davidson A.E., Markley K.M., Finley K., Fletcher C.F., Ekker S.C., Hackett P.B., Horn S. and Largaespada D.A. (2002). Mammalian germ-line transgenesis by transposition. Proc. Natl. Acad. Sci. USA. 99(7): 4495-4499.

Dyson M., Allososh M., Vuichetich J.P., Mokeleke E.A., Sturek M. (2006). Components of metabolic syndrome and coronary artery disease in female Ossabaw swine fed excess atheriogenic dieat. Transplant Rev. 21, 54-63.

Emery A.E. (2002).The muscular dystrophies. Lancet 359, 687-695.

Erwin J.A., Marchetto M.C. and Gage F.H. (2014). Mobile DNA elements in the generation of diversity and complexity in the brain. Nat. Rev. Neurosci. 15(8), 497-506.

Filareto A., Parker S., Darabi R., Borges L., Iacovino M., Schaaf T., Mayerhofer T., Chamberlain J.S., Ervasti J.M., McIvor R.S., Kyba M. and Perlingeiro R.C. (2013). An ex vivo gene therapy approach to treat muscular dystrophy using inducible pluripotent stem cells. Nat. Commun. 4, 1549.

Fraser M.J., Ciszczon T., Elick T. and Bauser C. (1996). Precise excision of TTAA-specific lepidopteran transposons piggyBac (IFP2) and tagalong (TFP3) from the baculovirus genome in cell lines from two species of Lepidoptera, Insect Mol. Biol. 5, 141-151.

Fusaki N., Ban H., Nishiyama A., Saeki K. and Hasegawa M. (2009). Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc. Jpn. Acad. Ser. B. Phys. Biol. Sci. 85, 348-362.

Gandolfi F, Pennarossa G, Maffei S, Brevini T. (2012) Why is it so difficult to derive pluripotent stem cells in domestic ungulates? Reprod Domest Anim. 47 Suppl 5,11-17.

Garrels W., Mates L., Holler S., Taylor U., Petersen B., Niemann H., Izsvak Z., Ivics Z. and Kues W.A. (2011). Germline transgenesis by Sleeping Beauty transposition in porcine zygotes and targeted insertion in the pig genome. PLoS One 6, e23573.

Garrels W., Holler S., Cleve N., Niemann H., Ivics Z. and Kues W.A. (2012a). Assessment of fecundity and germ line transmission in two transgenic pig lines produced by sleeping beauty transposition. Genes (Basel). 3(4), 615-633.

Garrels W., Ivics Z. and Kues W.A. (2012b). Precision genetic engineering in large animals. Trends Biotechnol. 7, 387-393.

Garrels W., Cleve N., Niemann H. and Kues W.A. (2012c). Rapid non-invasive genotyping of reporter transgenic mammals. BioTechniques Rapid Dispatches 1, 1-4; doi 10.2144/000113874

Grabundzija I., Irgang M., Mátés L., Belay E., Matrai J., Gogol-Döring A., Kawakami K., Chen W., Ruiz P., Chuah M.K., VandenDriessche T., Izsvák Z. and Ivics Z. (2010). Comparative analysis of transposable element vector systems in human cells. Mol. Therapy18(6), 1200-1209.

Grabundzija I., Wang J., Sebe A., Erdei Z., Kajdi R., Devaraj A., Steinemann D., Szuhai K., Stein U., Cantz T., Schambach A., Baum C., Izsvák Z., Sarkadi B. and Ivics Z. (2013). Sleeping Beauty transposon-based system for cellular reprogramming and targeted

gene insertion in induced pluripotent stem cells. Nucleic Acids Res. 41(3),1829-1847.

Graham J.S., Reid F.M., Smith J.R., Stotts R.R., Tucker E.S., Shumaker S.M, Niemuth N.A. and Janny S.J. (2000). A cutaneous full-thickness liquid sulfur mustard burn model in weanling swine: Clinical pathology and urinary excretion of thiodiglycol. J. App. Tox. 20, S161-S172.

Gün G., Kues W.A. (2014) Current progress of genetically engineered pig models for biomedical research. Biores. Open Access. 3, 255-264.

Hackett C.S., Geurts A.M. and Hackett P.B. (2007). Predicting preferential DNA vector insertion sites: implications for functional genomics and gene therapy. Genome Biol. 8, S12.

Hackett P.B., Largaespada D.A. and Cooper L.J. (2010). A transposon and transposase system for human application. Mol. Ther. 18, 674-683.

Henikoff S. (1998). Conspiracy of silence among repeated transgenes. Bioessays 20(7): 532-535.

Hotta A., Cheung A.Y., Farra N., Vijayaragavan K., Seguin C.A., Draper J.S., Pasceri P., Maksakova I.A., Mager D.L., Rossant J., Bhatia M. and Ellis J. (2009). Isolation of human iPS cells using EOS lentiviral vectors to select for pluripotency. Nat. Methods 6, 370-376.

Huang X., Guo H., Tammana S., Jung Y.C., Mellgren E., Bassi P., Cao Q., Tu Z.J., Kim Y.C., Ekker S.C., Wu X., Wang S.M. and Zhou X. (2010). Gene transfer efficiency and genome-wide integration profiling of Sleeping Beauty, Tol2, and piggyBac transposons in human primary T cells. Mol. Ther. 18,1803-1813.

Igawa K., Kokubu C., Yusa K., Horie K., Yoshimura Y., Yamauchi K., Suemori H., Yokozeki H., Toyoda M., Kiyokawa N., Okita H., Miyagawa Y., Akutsu H., Umezawa A., Katayama I. and Takeda J. (2014). Removal of reprogramming transgenes improves the tissue reconstitution potential of keratinocytes generated from human induced pluripotent stem cells. Stem Cells Transl. Med. 3(9), 992-1001.

Ishii A., Vinuela F., Murayama Y., Yuki I., Nien Y.L., Yeh D.T. and Vinters H.V. (2006). Swine model of carotid artery atherosclerosis: Experimental induction by surgical partial ligation and dietary hyperchloestrolemia. Am. J. Neurorad. 27, 1893-1899.

Ivancevic A.M., Walsh A.M., Kortschak R.D. and Adelson D.L. (2013). Jumping the fine LINE between species: horizontal transfer of transposable elements in animals catalyses genome evolution. Bioessays 35(12),1071-1082.

Ivics Z., Hackett P.B., Plasterk R.H. and Izsvak Z. (1997). Molecular reconstruction of Sleeping Beauty, a Tc1- like transposon from fish, and its transposition in human cells. Cell 91, 501-510.

Ivics Z., Li M.A., Mátés L., Boeke J.D., Nagy A., Bradley A. and Izsvák Z. (2009). Transposon-mediated genome manipulation in vertebrates. Nat. Methods 6(6), 415-422.

Ivics Z., Garrels W., Mátés L., Yau T.Y., Bashir S., Zidek V., Landa V., Geurts A., Pravenec M., Rülicke T., Kues W.A. and Izsvák Z. (2014a). Germline transgenesis in pigs by cytoplasmic microinjection of Sleeping Beauty transposons. Nat. Protoc. 9(4), 810-827.

Ivics Z., Hiripi L., Hoffmann O.I., Mátés L., Yau T.Y., Bashir S., Zidek V., Landa V., Geurts A., Pravenec M., Rülicke T., Bösze Z. and Izsvák Z. (2014b). Germline transgenesis in rabbits by pronuclear microinjection of Sleeping Beauty transposons. Nat. Protoc. 9(4), 794-809.

Ivics Z., Mátés L., Yau T.Y., Landa V., Zidek V., Bashir S., Hoffmann O.I., Hiripi L., Garrels W., Kues W.A., Bösze Z., Geurts A., Pravenec M., Rülicke T. and Izsvák Z. (2014c). Germline transgenesis in rodents by pronuclear microinjection of Sleeping Beauty transposons. Nat. Protoc. 9(4), 773-793.

Iwamoto M., Björklund T., Lundberg C., Kirik D. and Wandless T.J. (2010). A general chemical method to regulate protein stability in the mammalian central nervous system. Chem. Biol. 17(9), 981-988.

Jia F., Wilson K.D., Sun N., Gupta D.M., Huang M., Li Z., Panetta N.J., Chen Z.Y., Robbins R.C., Kay M.A., Longaker M.T. and Wu J.C. (2010). A nonviral minicircle vector for deriving human iPS cells. Nat. Methods 7, 197-199.

Jursch T., Miskey C., Izsvák Z. and Ivics Z. (2013). Regulation of DNA transposition by CpG methylation and chromatin structure in human cells. Mob. DNA. 4(1), 15.

Kaji K., Norrby K., Paca A., Mileikovsky M., Mohseni P. and Woltjen K. (2009). Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature 458, 771-775.

Kawakami K., Shima A. and Kawakami N. (2000). Identification of a functional transposase of the Tol2 element, an Ac-like element from the Japanese medaka fish, and its transposition in the zebrafish germ lineage. Proc. Natl. Acad. Sci. USA. 97, 11403-11408.

Kren B.T., Unger G.M., Sjeklocha L., Trossen A.A., Korman V., Diethelm- Okita B.M., Reding M.T and Steer C.J. (2009). Nanocapsule-delivered Sleeping Beauty mediates therapeutic Factor VIII expression in liver sinusoidal endothelial cells of hemophilia A mice. J. Clin. Invest. 119, 2086-2099.

Kues W.A., Schwinzer R., Verhoeyen E., Lemme E., Herrmann D., Barg-Kues B., Hauser H., Wonigeit K. and Niemann H. (2006). Epigenetic silencing and tissue-independent expression of a novel tetracycline inducible system in doubletransgenic pigs. FASEB J. 20, E1-E10.

Kues W.A., Herrmann D., Barg-Kues B., Haridoss S., Nowak-Imialek M., Buchholz T., Streeck M., Grebe A., Grabundzija I., Merkert S., Martin U., Hall V.J., Rasmussen M.A., Ivics Z., Hyttel P. and Niemann H. (2013). Derivation and characterization of sleeping beauty transposon-mediated porcine induced pluripotent stem cells. Stem Cells Dev. 22(1), 124-135.

Kumar D., Talluri T.R., Anand T. and Kues W.A. (2015). Induced pluripotent stem cells: Mechanisms, achievements and perspectives in farm animals. World J. Stem Cells 7(2), 315-328.

Lander E.S., Linton L.M., Birren B., Nusbaum C., Zody M.C., Baldwin J., Lander E.S., Linton L.M., Birren B., Nusbaum C., Zody M.C., Baldwin J., Devon K., Dewar K., Doyle M., FitzHugh W., Funke R., Gage D., Harris K., Heaford A., Howland J., Kann L., Lehoczky J., LeVine R., McEwan P., McKernan K., Meldrim J., Mesirov J.P., Miranda C., Morris W., Naylor J., Raymond C., Rosetti M., Santos R., Sheridan A., Sougnez C., Stange-Thomann N., Stojanovic N., Subramanian A., Wyman D., Rogers J., Sulston J., Ainscough R., Beck S., Bentley D., Burton J., Clee C., Carter N., Coulson A., Deadman R., Deloukas P., Dunham A., Dunham I., Durbin R., French L., Grafham D., Gregory S., Hubbard T., Humphray S., Hunt A., Jones M., Lloyd C., McMurray A., Matthews L., Mercer S., Milne S., Mullikin J.C., Mungall A., Plumb R., Ross M., Shownkeen R., Sims S., Waterston R.H., Wilson R.K., Hillier L.W., McPherson J.D., Marra M.A., Mardis E.R., Fulton L.A., Chinwalla A.T., Pepin K.H., Gish W.R., Chissoe S.L., Wendl M.C., Delehaunty K.D., Miner T.L., Delehaunty A., Kramer J.B., Cook L.L., Fulton R.S., Johnson D.L., Minx P.J., Clifton S.W., Hawkins T., Branscomb E., Predki P., Richardson P., Wenning S., Slezak T., Doggett N., Cheng J.F,.Olsen A, Lucas S, Elkin C, Uberbacher E., Frazier M., Gibbs

R.A, Muzny DM, Scherer SE, Bouck JB, Sodergren E.J, Worley K.C, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson D.L., Weinstock G.M., Sakaki Y., Fujiyama A., Hattori M., Yada T., Toyoda A., Itoh T., Kawagoe C., Watanabe H., Totoki Y., Taylor T., Weissenbach J., Heilig R., Saurin W., Artiguenave F., Brottier P., Bruls T., Pelletier E., Robert C., Wincker P., Smith D.R., Doucette-Stamm L., Rubenfield M., Weinstock K., Lee H.M., Dubois J., Rosenthal A., Platzer M., Nyakatura G., Taudien S., Rump A., Yang H., Yu J., Wang J., Huang G., Gu J., Hood L., Rowen L., Madan A., Qin S., Davis R.W., Federspiel N.A., Abola A.P., Proctor M.J., Myers R.M., Schmutz J., Dickson M., Grimwood J., Cox D.R., Olson M.V., Kaul R., Raymond C., Shimizu N., Kawasaki K., Minoshima S., Evans G.A., Athanasiou M., Schultz R., Roe B.A., Chen F., Pan H., Ramser J., Lehrach H., Reinhardt R., McCombie W.R., de la Bastide M., Dedhia N., Blöcker H., Hornischer K., Nordsiek G., Agarwala R., Aravind L., Bailey J.A., Bateman A., Batzoglou S., Birney E., Bork P., Brown D.G., Burge C.B., Cerutti L., Chen H.C., Church D., Clamp M., Copley R.R., Doerks T., Eddy S.R., Eichler E.E., Furey T.S., Galagan J., Gilbert J.G., Harmon C., Hayashizaki Y., Haussler D., Hermjakob H., Hokamp K., Jang W., Johnson L.S., Jones T.A., Kasif S., Kaspryzk A., Kennedy S., Kent W.J., Kitts P., Koonin E.V., Korf I., Kulp D., Lancet D., Lowe T.M., McLysaght A., Mikkelsen T., Moran J.V., Mulder N., Pollara V.J., Ponting C.P., Schuler G., Schultz J., Slater G., Smit A.F., Stupka E., Szustakowski J., Thierry-Mieg D., Thierry-Mieg J., Wagner L., Wallis J., Wheeler R., Williams A., Wolf Y.I., Wolfe K.H., Yang S.P., Yeh R.F., Collins F., Guyer M.S., Peterson J., Felsenfeld A., Wetterstrand K.A., Patrinos A., Morgan M.J., de Jong P., Catanese J.J., Osoegawa K., Shizuya H., Choi S. and Chen Y.J. (2001). Initial sequencing and analysis of the human genome. Nature 409(6822), 860-921.

Li X., E.R. Burnight, A.L. Cooney, Malani N., Brady T., Sander J.D., Staber J., Wheelan S.J., Joung J.K., McCray P.B. Jr, Bushman F.D., Sinn P.L. and Craig N.L. (2013). PiggyBac transposase tools for genome engineering. Proc. Natl. Acad. Sci. USA 110, E2279-E2287.

Li Z., Zeng F., Meng F., Xu Z., Zhang X., Huang X., Tang F., Gao W., Shi J., He X., Liu D., Wang C., Urschitz J., Moisyadi S. and Wu Z. (2014). Generation of transgenic pigs by cytoplasmic injection of piggyBac transposase-based pmGENIE-3 plasmids. Biol. Reprod. 90(5), 93.

Liu H., Liu L., Fletcher B.S. and Visner G.A. (2006a). Sleeping Beauty-based gene therapy with indoleamine 2,3-dioxygenase inhibits lung allograft fibrosis. FASEB J. 20, 2384-2386.

Liu L., Liu H., Visner G. and Fletcher B.S. (2006b). Sleeping Beauty-mediated eNOS gene therapy attenuates monocrotaline-induced pulmonary hypertension in rats. FASEB J. 20, 2594-2596.

Mates L., Chuah M.K., Belay E., Jerchow B., Manoj N., Acosta-Sanchez A., Grzela D.P., Schmitt A., Becker K., Matrai J., Ma L., Samara-Kuko E., Gysemans C., Pryputniewicz D., Miskey C., Fletcher B., VandenDriessche T., Ivics Z. and Izsvák Z. (2009). Molecular evolution of a novel hyperactive Sleeping Beauty transposase enables robust stable gene transfer in vertebrates. Nat. Genet. 41, 753-761.

McClintock B. (1950). The Origin and behavior of mutable loci in maize. Proc. Natl. Acad. Sci. USA. 36(6), 344-355.

Meir Y.J., Weirauch M.T., Yang H.S., Chung P.C., Yu R.K. and Wu S.C. (2011). Genome-wide target profiling of piggyBac and Tol2 in HEK 293: pros and cons for gene discovery and gene therapy. BMC Biotechnol. 11, 28.

Miskey C., Izsvak Z., Plasterk R.H. and Ivics Z. (2003). The Frog Prince: a reconstructed transposon from Rana pipiens with high transpositional activity in vertebrate cells. Nucleic Acids Res. 31, 6873-6881.

Miskey C., Papp B., Mátés L., Sinzelle L., Keller H., Izsvák Z. and Ivics Z. (2007). The ancient mariner sails again: transposition of the human Hsmar1 element by a reconstructed transposase and activities of the SETMAR protein on transposon ends. Mol. Cell Biol. 27, 4589-4600.

Mo X., Li N. and Wu S. (2014). Generation and characterization of bat-induced pluripotent stem cells. Theriogenology 82(2), 283-293.

Montini E., Held P.K., Noll M., Morcinek N., Al-Dhalimy M., Finegold M., Yant S.R., Kay M.A. and Grompe M. (2002). *In vivo* correction of murine tyrosinemia type I by DNA mediated transposition. Mol. Ther. 6, 759-769.

Muenthaisong S., Ujhelly O., Polgar Z., Varga E., Ivics Z., Pirity M.K. and Dinnyes A. (2012). Generation of mouse induced pluripotent stem cells from different genetic backgrounds using Sleeping beauty transposon mediated gene transfer. Exp. Cell Res. 318(19), 2482-2489.

Nagy K., Sung H.K., Zhang P., Laflamme S., Vincent P., Agha-Mohammadi S., Woltjen K., Monetti C., Michael I.P., Smith L.C. and Nagy A. (2011). Induced pluripotent stem cell lines derived from equine fibroblasts. Stem Cell Rev. 7, 693-702.

Nakagawa M., Koyanagi M., Tanabe K., Takahashi K., Ichisaka T., Aoi T., Okita K., Mochiduki Y., Takizawa N. and Yamanaka S. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat. Biotechnol. 26, 101-106.

Nakazawa Y., Huye L.E., Salsman V.S., Leen A.M., Ahmed N., Rollins L., Dotti G., Gottschalk S.M., Wilson M.H. and Rooney C.M. 2011. PiggyBac mediated cancer immunotherapy using EBV specific cytotoxic T-cells expressing HER2-specific chimeric antigens receptor. Mol. Ther. 19, 2133-2143.

Nakazawa Y., Saha S., Galvan D.L., Huye L., Rollins L., Rooney C.M. and Wilson M.H. (2013). Evaluation of long-term transgene expression in piggyBac modified human T lymphocytes. J. Immunother. 36, 3-10.

Nowak-Imialek M. and Niemann H. (2012). Pluripotent cells in farm animals: state of the art and future perspectives. Reprod. Fertil. Dev. 25(1), 103-128.

Ohlfest J.R., Demorest Z.L., Motooka Y., Vengco I., Oh.S, Chen E., Scappaticci F.A., Saplis R.J., Ekker S.C., Low W.C., Freese A.B. and Largaespada D.A. (2005). Combinatorial anti-angiogenic gene therapy by non-viral gene transfer using the sleeping beauty transposon causes tumor regression and improves survival in mice bearing intracranial human glioblastoma. Mol. Ther. 12, 778-788.

Okita K., Ichiaka T. and Yamanaka S. (2007). Generation of germline-competent induced pluripotent stem cells. Nature 448, 313-317.

Ortiz-Urda S., Lin Q., Yant S.R., Keene D., Kay M.A. and Khavari P.A. (2003). Sustainable correction of junctional epidermolysis bullosa via transposon-mediated non-viral gene transfer. Gene Ther. 10, 1099-1104.

Park I.H., Arora N., Huo H., Maherali N., Ahfeldt T., Shimamura A., Lensch M.W., Cowan C., Hochedlinger K. and Daley G.Q. (2008). Disease-specific induced pluripotent stem cells. Cell 134, 877-886.

Petters R.M., Alexander C.A., Wells K.D., Collins E.B., Sommer J.R., Blanton M.R., Rojas G., Hao Y., Flowers W..L, Banin E., Cideciyan A.V., Jacobson S.G. and Wong F. (1997). Genetically engineered large animal model for studying cone photoreceptor survival and

degeneration in retinitis pigmentosa. Nat. Biotechnol. 15, 965-970.

Rostovskaya M., Fu J., Obst M., Baer I., Weidlich S., Wang H., Smith A.J., Anastassiadis K. and Stewart A.F. (2012). Transposon-mediated BAC transgenesis in human ES cells. Nucleic Acids Res. 40(19): e150.

Salewski R.P., Buttigieg J., Mitchell R.A., van der Kooy D., Nagy A., Fehlings M.G. (2013). The generation of definitive neural stem cells from piggyBac transposon-induced pluripotent stem cells can be enhanced by induction of the NOTCH signaling pathway. Stem Cells Dev. 22, 383-396.

Shi Y., Desponts C., Do J.T., Hahm H.S., Scholer H.R. and Ding S. (2008). Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small molecule compounds. Cell Stem Cell 3, 568-574.

Singh H., Huls H., Kebriaei P. and Cooper L.J. (2014). A new approach to gene therapy using Sleeping Beauty to genetically modify clinical-grade T cells to target CD19. Immunol. Rev. 257, 181-190.

Sjeklocha L.M., Park C.W., Wong P.Y., Roney M.J., Belcher J.D., Kaufman D.S., Vercellotti G.M., Hebbel R.P., Steer C.J. (2011). Erythroid-specific expression of β-globin from Sleeping Beauty-transduced human hematopoietic progenitor cells. PLOS ONE. 6, e29110.

Skipper K.A., Andersen P.R., Sharma N and Mikkelsen J.G. (2013). DNA transposon-based gene vehicles - scenes from an evolutionary drive. J. Biomed. Sci. 20: 92.

Smith A.R. and Wagner J.E. (2012). Current clinical management of Fanconi anemia. Expert Rev. Hematol. 5, 513-522.

Sommer C.A., Stadtfeld M., Murphy G.J., Hochedlinger K., Kotton D.N. and Mostoslavsky G. (2009). Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. Stem Cells 27, 543-549.

Stadtfeld M., Nagaya M., Utikal J., Weir G. and Hochedlinger K. (2008). Induced pluripotent stem cells generated without viral integration. Science 322, 945-949.

Sui D., Sun Z., Xu C., Wu Y., Cappecci M.R., Wu S. and Li N. (2014). Fine-tuning of iPSC derivation by an inducible reprogramming system at the protein level. Stem Cell Reports 2(5), 721-733.

Switzer K., Rabinovich B. and Cooper J.N.L. (2012). Transposon-based engineering of clinical-grade T cells for cancer therapy. Curr Drug Ther. 7, 36-41.

Szymczak A.L., Workman C.J., Wang Y., Vignali K.M., Dilioglou S., Vanin E.F. and Vignali D.A. (2004). Correction of multi-gene deficiency *in vivo* using a single 'self-cleaving' 2A peptide-based retroviral vector. Nat. Biotechnol. 22, 589-594.

Szymczak-Workman A.L., Vignali K.M. and Vignali D.A. (2012). Verification of 2A peptide cleavage. Cold Spring Harb Protoc. 2012(2), 255-257.

Takahashi K. and Yamanaka S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-676.

Takahashi K., Tanabe K., Ohnuki M., Narita M., Ichisaka T., Tomoda K. and Yamanaka S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861-872.

Talluri T.R., Kumar D., Glage S., Garrels W., Ivics Z., Debowski K., Behr R. and Kues W.A. (2014). Non-viral reprogramming of fibroblasts into induced pluripotent stem cells by Sleeping Beauty and piggyBac transposons. Biochem. Biophys. Res. Commun. 450(1), 581-587.

Talluri T.R., Kumar D., Glage S., Garrels W., Ivics Z., Debowski K., Behr R., Niemann H. and Kues W.A. (2015). Derivation and characterization of bovine induced pluripotent stem cells by transposon-mediated reprogramming. Cell Reprogram. 17(2), 131-140.

Tsukiyama T., Kato-Itoh M., Nakauchi H. and Ohinata Y. (2014). A comprehensive system for generation and evaluation of induced pluripotent stem cells using piggyBac transposition. PloS One 9, e92973.

Turk J.R. and Laughlin M.H. (2004). Physical activity and atherosclerosis: which animal model? Can. J. Appl. Physiol. 29, 6547-683.

Vand Rajabpour F., Raoofian R., Habibi L., Akrami S.M. and Tabrizi M. (2014). Novel trends in genetics: transposable elements and their application in medicine. Arch. Iran Med. 17(10), 702-712.

VandenDriessche T., Ivics Z., Izsvák Z. and Chuah M.K. (2009). Emerging potential of transposons for gene therapy and generation of induced pluripotent stem cells. Blood 114(8), 1461-1468.

Vigdal T.J., Kaufman C.D., Izsvak Z., Voytas D.F. and Ivics Z. (2002). Common physical properties of DNA affecting target site selection of sleeping beauty and other Tc1/mariner transposable elements. J. Mol. Biol. 323, 441-452.

Walisko O., Schorn A., Rolfs F., Devaraj A., Miskey C., Izsvak Z. and Ivics Z. (2008). Transcriptional activities of the Sleeping Beauty transposon and shielding its genetic cargo with insulators. Mol. Ther. 16, 359-369.

Walsh A.M., Kortschak R.D., Gardner M.G., Bertozzi T. and Adelson D.L. (2013). Widespread horizontal transfer of retrotransposons. Proc. Natl. Acad. Sci. USA. 110(3), 1012-1016.

Warren L., Manos P.D., Ahfeldt T., Loh Y.H., Li H., Lau F., Ebina W., Mandal P.K., Smith Z.D., Meissner A., Daley G.Q., Brack A.S., Collins J.J., Cowan C., Schlaeger T.M. and Ross, D.J. (2010). Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7, 618-630.

Wilber A., Linehan J.L., Tian X., Woll P.S., Morris J.K., Belur L.R., McIvor R.S. and Kaufman D.S. (2007). Efficient and stable transgene expression in human embryonic stem cells using transposon-mediated gene transfer. Stem Cells 25(11), 2919-2927.

Woltjen K., Michael I.P., Mohseni P., Desai R., Mileikovsky M., Hamalainen R., Cowling R., Wang W., Liu P., Gertsenstein M. Kaji K., Sung H.K. and Nagy A. (2009). piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458, 766-770.

Woltjen K., Hämäläinen R., Kibschull M., Mileikovsky M. and Nagy A. (2011). Transgene-free production of pluripotent stem cells using piggyBac transposons. Methods Mol. Biol.767, 87-103.

Wu X., Li Y., Crise B., Burgess S.M. (2003). Transcription start regions in the human genome are favored targets for MLV integration. Science 300, 1749-1751.

Wu C. and Dunbar C.E. (2011). Stem cell gene therapy: The risks of insertional mutagenesis and approaches to minimize genotoxicity. Front. Med. 5, 356-371.

Yant S.R., Ehrhardt A., Mikkelsen J.G., Meuse L., Pham T. and Kay M.A. (2002). Transposition from a gutless adeno-transposon vector stabilizes transgene expression *in vivo*. Nat. Biotechnol. 20, 999-1005.

Yant S.R., Meuse L., Chiu W., Ivics Z., Izsvak Z. and Kay M.A. (2000). Somatic integration and long-term transgene expression in normal and haemophilic mice using a DNA transposon system. Nat. genet. 25, 35-41.

Yant S.R., Wu X., Huang Y., Garrison B., Burgess S.M. and Kay M.A. (2005). High-resolution genome-wide mapping of transposon integration in mammals. Mol. Cell Biol. 25, 2085-2094

Ye J., Hong J. and Ye F. (2015). Reprogramming rat embryonic fibroblasts into induced pluripotent stem cells using transposon vectors and their chondrogenic differentiation *in vitro*. Mol. Med. Rep. 11(2), 989-994.

Yu J., Vodyanik M.A., Smuga-Otto K., Antosiewicz-Bourget J., Frane J.L., Tian S., Nie J., Jonsdottir G.A., Ruotti V., Stewart R., Slukvin II. and Thomson J.A. (2007). Induced pluripotent stem cell lines derived from human somatic tissue. Science 318, 1917-1920.

Yu J., Hu K., Smuga-Otto K, Tian S., Stewart R., Slukvin II. and Thomson J.A. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. Science 324, 797-780.

Yusa K., Rad R., Takeda J. and Bradley A. (2009). Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. Nat. Methods 6, 363-369.

Yusa K., Zhou L., Li M.A., Bradley A. and Craig N.L. (2011). A hyperactive piggyBac transposase for mammalian applications. Proc. Natl. Acad. Sci. USA. 108, 1531-1536.

Zhou H., Wu S., Joo J.Y., Zhu S., Han D.W., Lin T., Trauger S., Bien G., Yao S., Zhu Y., Siuzdak G., Scholer H.R., Duan L. and Ding S. (2009). Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 4, 381-384.

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