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Genetic Modification of Domestic Animals for Agriculture and Biomedical Applications

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1. Introduction

Transgenic technology has been applied mainly in the study of gene structure and function in model organisms and gene therapy for human diseases. 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. However, due to the limitations in making genetic modifications and SCNT, producing genetically modified animals is still inefficient. Fortunately, the current advancement of new techniques and methods in both gene targeting (Urnov et al., 2010) and abilities to produce pluripotent stem cells (Voigt and Serikawa, 2009) holds great promises for this field.

In this chapter, we will review the recent progress and technical route of the cell-based method of transgenesis by SCNT and discuss the newly emerging methods to enrich the gene targeting frequency of somatic cells. We will also discuss factors to improve the efficiency of SCNT and our future perspectives on the promises of this field.

2. Recent progress and applications of transgenic domestic animals

2.1 Methods of creating genetically modified animals

2.1.1 Pronuclear microinjection /viral-mediated /sperm-mediated /ICSI (Intractyoplasmic sperm injection)- mediated gene transfer

Numerous methods have been successfully used to introduce genetic modifications and produce transgenic animals, including pronuclear microinjection of foreign DNA into zygotes (Hammer et al., 1985; Pursel and Rexroad, 1993), viral-mediated gene transfer (Chan et al., 1998; Cabot et al., 2001; Whitelaw et al., 2008), sperm-mediated gene transfer (Castro et al., 1991; Chang et al., 2002; Lavitrano et al., 2002 and 2006) and intracytoplasmic injection (ICSI) of a sperm head carrying foreign DNA (Perry et al., 1999; Osada et al., 2005; Moisvadi et al., 2009; García-Vázquez et al., 2010). Despite the proven successful application of these techniques, some problems, such as inefficiency and mosaicism (transgene not going into the germline) (Table 1) remain to be solved and limit the practical application of these methods.

Table 1. The Advantages and disadvantages of different transgenic methods.

Method	Advantages	Disadvantages
Pronuclear microinjection	The first method successfully used for different animal species.	Low embryo survival, low and random integration, multiple copies, high cost in domestic animals.
Viral-mediated DNA transfer	Infect both dividing and non-dividing cells, less damage by co-culture with zona-free zygotes or injection into the perivitelline space compared with pronuclear microinjection, high integration.	Limited DNA capacity, random integration.
Sperm-mediated DNA transfer	Relatively high efficiency as compared to pronuclear injection, low cost, ease of use.	No control of integration sit
Intracytoplasmic sperm injection -mediated DNA transfer	Allow introduction of very large DNA transgenes, relatively high efficiency as compared to pronuclear injection.	No control of integration sit

Gene targeting by homologous recombination often offers more precise and site-specific integration, sometimes at single nucleotide level. This is particularly important since single nucleotide changes can be a common culprit for some of the human diseases, which require more precise manipulation to build biomedical models using transgenic animals.

2.1.2 Cell-based transgenesis via SCNT

The first live animal by SCNT was produced in 1997, "Dolly" (Wilmut et al., 1997), demonstrating the ability of a differentiated somatic cell to produce live offspring following nuclear remodeling and reprogramming by an oocyte. Then, the birth of "Polly" in the same year (Schnieke et al., 1997), the first transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts, demonstrated a route to create transgenic cloned animals. This cell-based method of transgenesis by SCNT can bypass the absence of ES cells, offers the reliability of germline transmission by avoiding mosaic transgene integration, and provides the only currently used strategy to knock out a gene in domestic animals (reviewed by Ross et al., 2009a). Currently, SCNT using transgenic cells cultured *in vitro* as a source of donor nuclei is becoming the most utilized technique to produce the transgenic domestic animals. However, while the advantages and success of this strategy are well documented (Table 2), the procedure is still labor-intensive and inefficient. Recently, some new techniques have been reported that may have the potential to improve the production efficiency of cloned transgenic domestic animals by increasing the efficiency of gene targeting or nuclear remodeling and reprogramming following SCNT.

2.2 Utilization of transgenic models

The potential for transgenic domestic animals to benefit humans is not only in agricultural production by providing more and better agricultural products for human consumption but also in biomedicine, such as for producing recombinant pharmaceutical proteins, making organs suitable for xenotransplantation and establishing human disease models. An overview list of the transgenic domestic animals produced via SCNT is given in Table 2 to demonstrate their applications.

2.2.1 Improved animal agriculture production

Increased utilization of domestic animals and their products requires breeding and selection strategies for specific traits. However, classical breeding and genetic selection have some disadvantages, such as the inability to control gene frequency of desired genotypes coupled with long generation intervals. The application of transgenic technology offers a powerful tool to rapidly improve agriculture production by developing domestic animals that express desired traits via genome manipulation strategies. Previous studies have demonstrated the practical application of transgenesis to improve numerous agricultural traits of domestic animals, including increased growth rate (Pursel et al., 1999), increased meat quality (Saeki et al., 2004), enhanced disease resistance (Lo et al., 1991; Clements et al., 1994), and better milk production and composition (Wheeler et al., 2001; Reh et al., 2004). Nevertheless, utilization of the cell-based method of transgenesis via SCNT has also been successfully used to alter characteristics of pork quality (Lai et al., 2006), enhance disease resistance (Denning et al., 2001a; Wall et al., 2005; Richt et al., 2007) and improve milk composition (Brophy et al., 2003).

Species	Application	Donor cells	Key Molecule	Construct	DNA delivery method	Reference
Sheep	Bioreactor	serum starved fetal fibroblasts	human clotting factor IX (FIX)	pMIXI	Lipofection	Schnieke et al., 1997
	Disease model	serum starved fetal fibroblasts	alpha1(I) procollagen (COL1A1)	COLT-1/COLT-2	Lipofection	McCreath et al., 2000
	Xenotransplantation; disease resistance	serum starved fetal fibroblasts	alpha(1,3)galactosyl transferase (GGTA1), prion protein (PrP)	GGTA1; PrP	Electroporation	Denning et al., 2001a
Cattle	Marker gene	actively dividing fetal fibroblasts	β-galactosidase–neomycin, cytomegalovirus (CMV)	pCMV/ βGEO	Not described	Cibelli et al., 1998
	Bioreactor	serum starved fetal/ear fibroblasts	bovine prochymosin coding gene, αS1-casein promoter	TFF1/TFF2	Lipofection	Zakhartchenko et al., 2001
	GFP-reporter	dividing fetal fibroblast	enhanced, humanized version of the GFP-reporter	CEEGFP	Lipofection	Bordignon et al., 2003
	Bioreactor	serum starved fetal fibroblasts and G1 fibroblasts	β-casein and Kκ-casein	$CSN2^{A5}$ / $CSN2/3^{B}$	Lipofection	Brophy et al., 2003
	Bioreactor	fetal fibroblasts	bispecific single-chain variable fragment (biscFV) molecule with anti-human CD28 × anti-human melanoma specificity	Bi-scFV r28M	Lipofection	Grosse-Hovest et al., 2004
	Bioreactor	fetal fibroblasts	growth hormone (GH)	hGH vector	Not described	Salamone et al., 2006
	Bioreactor	fetal oviduct epithelial cells	human α-lactalbumin	phLa4-EGFP-NEO	Electroporation	Wang et al., 2008
	Bioreactor	fetal fibroblasts	human lactoferrin	hLF BAC	Microinjection/ Electroporation/ Lipofection	Yang et al., 2008
Goat	eGFP	fetal fibroblasts	green fluorescent protein reporter gene	CEeGFP	Lipofection	Keefer et al., 2001
Pig	Xenotransplantation	fetal fibroblasts	alpha -1,3-galactosyltransferase locus	pGalGT	Electroporation	Lai et al., 2002a
	Xenotransplantation	fetal fibroblasts	alpha -1,3-galactosyltransferase locus	pPL654 and pPL657	Electroporation	Dai et al., 2002
	Disease model	fetal fibroblasts	cystic fibrosis (CF); CF transmembrane conductance receptor (CFTR)	CFTR-*null and* CFTR-ΔF508	Adeno-associated virus (AAV)	Rogers et al., 2008
	Disease model	ear fibroblasts	Alzheimer's disease; neuronal variant of the human amyloid precursor protein gene with the Swedish mutation	pPDGFbEGFP	Lipofection	Kragh et al., 2009
	Xenotransplantation	fetal fibroblasts	human A20 gene	pCAGGSEhA20-IRESNEO	Electroporation	Oropeza et al., 2009
	Disease model	fetal fibroblasts	Huntington's disease	pCAG-HTT-2A-ECFP	Electroporation	Yang et al., 2010
Rabbit	eGFP	serum starved adult fibroblasts	green fluorescent protein reporter gene	pEGFP-C1	Lipofection	Li et al., 2009

Table 2. Overview on successful transgenic domestic animals via SCNT.

2.2.2 Xenotransplantation

Due to the potential application of human organ transplantation and the growing gap between the demand and availability of organs for transplantation, the pig has long been considered as an alternative source to provide organs for humans. In contrast to organs from other animal species, domestic pig organs share many similarities to those of humans including size, anatomy and physiology. However, despite these similarities, significant immunological barriers exist impeding the success of pig to human xenotransplantation (Cooper et al., 2008), in addition to concerns regarding the transmission of pig specific viruses to the human genome (Magre et al., 2003). The immunological obstacles of xenotransplantation include rapid hyperacute rejection (HAR), delayed acute vascular rejection (AVR), and the cellular immune response that occurs within weeks (Auchincloss and Sachs, 1998). Two transgenic strategies have been successfully applied to overcome HAR. One is to express human proteins that inhibit the complement cascade in transgenic pigs (Fodor et al., 1994; Cozzi and White, 1995; Diamond et al., 2001). For example, transgenic expression of human complement inhibitor CD59, CD46 and DAF (decay-accelerating factor, also named as CD55) in pigs prolongs survival rates from minutes to days and months following heart and kidney transplantation into baboons or monkeys by blocking the damage from HAR (Diamond et al., 1996 and 2001; Byrne et al., 1997; Zaidi et al., 1998; Bhatti et al., 1998; Chen et al., 1999). The second strategy to avoid HAR is to knockout the genes that induce the production of antigenic structures (α-gal-epitopes) on the surface of pig organs (Lai et al., 2002a; Dai et al., 2002; Phelps et al., 2003; Yamada et al., 2005). α-gal-epitopes on endothelial cells of porcine transplanted organs can be recognized by human xenoreactive natural antibodies (XNA) and activate the HAR cascade (Galili, 1993). Genetically engineering of pigs to lower or inhibit the expression level of XNA targets is thought to be a promise way to eliminate the HAR. Following the successful production of α-1,3-galactosyltransferase knockout pigs (Lai et al., 2002a; Dai et al., 2002; Phelps et al., 2003; Yamada et al., 2005), van Poll et al. (2010) recently showed that exposure of isolated xenogeneic pig liver sinusoidal endothelial cells (LSECs) from α-1,3-galactosyltransferase-deficient pigs to human and baboon serum reduces IgM binding and complement activation levels as compared to wild-type pig LSECs. However, Diswall et al. (2010) found a different reactivity pattern of baboon and human serum to pig glycolipid antigens isolated from α-1,3-galactosyltransferase knockout and wild-type pig hearts and kidneys, suggesting that non-human primates may not be an ideal model for modeling pig to human xenotransplantation. If HAR is controlled, the next obstacle to xenotransplantation is AVR which is due to the loss of porcine thrombomodulin in xenograft rejection or the inability of porcine thrombomodulin to activate human protein C. One of genetic engineering strategies to overcome AVR is to express human thrombomodulin in pigs. Petersen et al. (2006) showed the production of transgenic cloned pigs using CD59/DAF and human thrombomodulin triple transgenic adult donor cells.

With regard to risks associated with xenotransplantation, previous studies have shown that the risk of cross-transmission of pig endogenous retrovirus (PERV) to human patients or nonhuman primate recipients is low (Paradis et al., 1999; Switzer et al., 2001), although it has been found PERV can infect human cells in culture (Patience et al., 1997). Never-the-less, some investigators have been working to further reduce the possibility by creating

pigs with suppressed expression of endogenous retroviruses (Ramsoondar et al., 2009). In addition to PERV, herpesvirus is another concern regarding biosafety in xenotransplantation (Mueller et al., 2011). Overall, dramatically increased knowledge will facilitate the clinical application of transgenic strategies of pig to human xenotransplantation.

2.2.3 Production of recombinant proteins

The mammary gland and blood of transgenic domestic animals, including sheep, goats, cows, pigs and rabbits, have been successfully used as bioreactor to produce numerous recombinant proteins, such as antibodies (Grosse-Hovest et al., 2004), growth factors (Schnieke et al., 1997) and pharmaceuticals (reviewed by Melo et al., 2007). Using various mammary gland-specific or blood-specific promoters to drive the expression of specific protein-coding genes, transgenic domestic animals can continuously produce the recombinant proteins in large quantities in their milk or blood. Recombinant proteins, including human von Willebrand factor (Lee et al., 2009), human erythropoietin (Park et al., 2006), human insulin-like growth factor-I (Monaco et al., 2005), human factor VIII (Paleyanda et al., 1997) and bovine alpha-lactalbumin (Bleck et al., 1998) have been produced in the milk of transgenic pigs. Transgenic goats, capable of synthesizing human butyrylcholinesterase (Huang et al., 2007) and human longer acting tissue plasminogen activator (Ebert et al., 1991) in their milk have also been created. Human salmon calcitonin in milk of transgenic rabbits (McKee et al., 1998); human factor IX (Schnieke et al., 1997) and alpha-1-antitrypsin (Wright et al., 1991) in milk of transgenic sheep; and human lactoferrin (van Berkel et al., 2002; Yang et al., 2008), human growth hormone (Salamone et al., 2006) and human α-lactalbumin (Wang et al., 2008) in milk of transgenic cows are all additional examples of using transgenic domestic animals and the mammary gland as a bioreactor for production of recombinant proteins. Table 2 summarizes some recombinant proteins expressed in milk or blood of cloned transgenic domestic animals. One of the major advantages of using domestic animals for this purpose is that the produced protein is thought to undergo more accurate posttranslational processing to ensure their biological activity. While this application of transgenic technology to produce recombinant protein products is rapidly developing, research efforts exploring the efficacy of these products are still needed.

2.2.4 Biomedical models of human diseases

Another important application of genetically modified domestic animals is to create better and novel biomedical models of human diseases. Pig models of different human diseases, including retinitis pigmentosa, cardiovascular disease, diabetes, Huntington's disease, cystic fibrosis and Alzheimer's disease have been well discussed by Prather et al. (2008). Many of these biomedical models created by SCNT are listed in Table 2.

3. Technical aspects of cell-based transgenesis by SCNT

The general procedure of cell-based transgenesis via SCNT is to construct a DNA vector, deliver the vector into cultured somatic cells, select transgenic cell lines, utilize SCNT and transfer cloned embryo into surrogates (Figure 1).

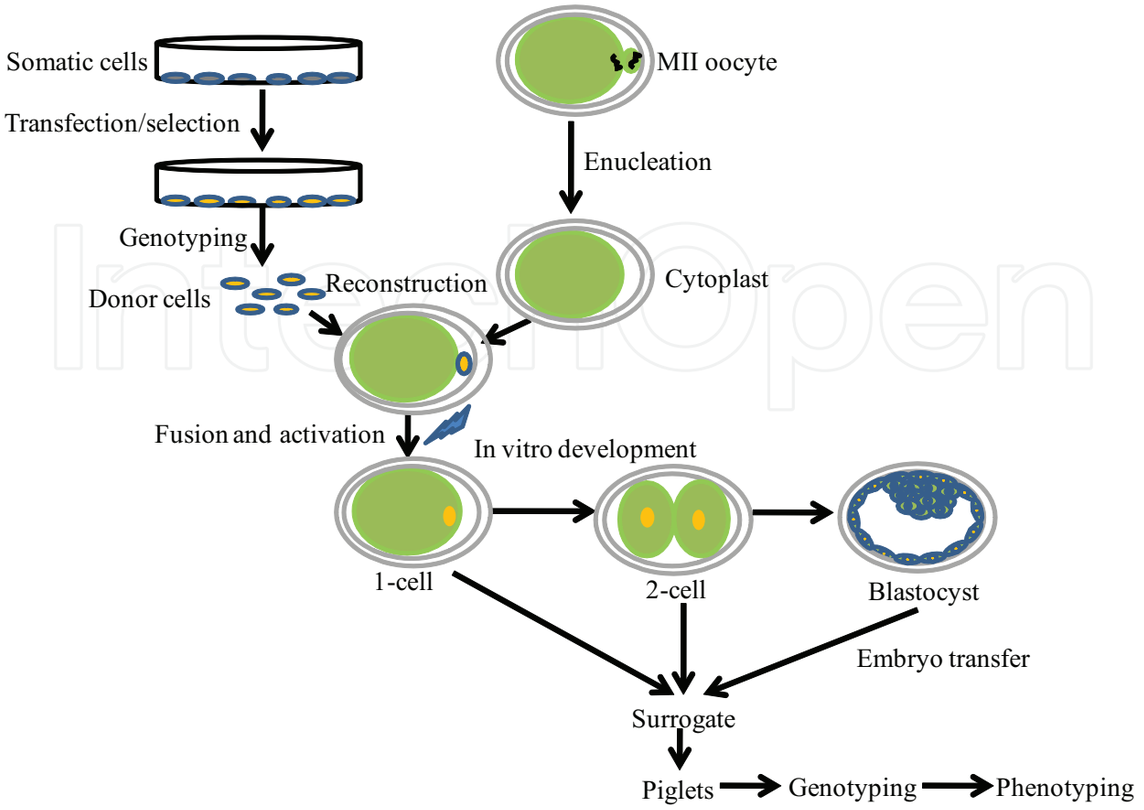


Fig. 1. Technical diagram of cell-based transgenesis followed by SCNT in pigs.

3.1 Vector construction

Currently, the whole genome sequence of several domestic animals, including cattle, poultry, cats, dogs, horses, pigs and rabbits is available via the ensembl database (www.ensembl.org), providing researchers useful sequence information for a large number of genes useful in designing DNA constructs for transgenic genome modification. Precise design and construction of DNA constructs is critical efficiently creating transgenic domestic animals. Transgenesis involves adding a gene to a host genome (transgenic), physically deleting a specific region of the host genome making a non-functional gene (knock-out), replacing an active gene by another active gene (knock-in), or introducing a point mutation (point mutation knock-in). Depending on the objective of the transgenic modification, different strategies of vector design need to be carefully considered to ensure success.

3.1.1 Transgenic vs. Knock-out vs. Knock-in

The design strategy for the transgenic vector, which is based on random integration, essentially includes a gene ORF (open reading frame), a promoter element and the appropriate RNA processing component(s). The promoter is a major transcriptional regulatory element that normally includes regulatory elements and the transcriptional start site typically located in 5′ sequence of the gene. Utilization of specific promoter with a transgene enables tissue or cell specific expression and can significantly impact the expression efficiency of the transgene. Carefully choosing a well-characterized promoter

will enable the precise control of transgene expression. The gene ORF is usually derived from the cDNA for the protein of interest, which includes translational start (ATG) and stop codons. Examination of extra sequence, if any, existing between the transcriptional start site and the translational start codon should be performed to ensure the absent of potential regulatory elements. Furthermore, consideration should be given to remove non-coding sequence of genes to avoid the introduction of the regulatory elements although this may also have consequences impacting mRNA stability. Reliable transgene expression is not only regulated by the sequence in the expression vector, but also by intrinsic factors in the host genome following transgene integration. Several additional points should be considered in transgenic vector construction. (i) How GC rich regulatory sequence are, especially CpG islands in the promoters can have significant implications for the expression of the transgene as methylation of CpG islands can inactivate the promoter and silence the transgene expression. (ii) Inclusion of specific elements that favor mRNA maturation and transfer to the cytoplasm, as it has been demonstrated that the inclusion of an intron in the transgenic vector can increase the transgene expression level (Choi et al., 1991; Duncker et al., 1997). These genomic regions may have critical sequence motifs affecting mRNA splicing and accumulation. (iii) Removal of unnecessary plasmid DNA sequence used in recombinant DNA cloning. In mice, it was found that plasmid sequence existing in the transgenic vector can decrease transgene expression (Kjer-Nielsen et al., 1992). Furthermore, the local chromatin status of the transgenic locus can also affect expression meaning that the integration of the same transgene in different genomic locations can have profound effects on the expression level of the transgene.

The primary strategy for targeted genome modifications, including knock-out and knock-in applications, is to use homologous recombination to introduce precise, site-specific genome alterations. In knock-out targeting vector systems, the primary approach is to delete DNA fragments (entire gene or partial deletion) important for the gene function by homologous recombination. Compared with the knock-out targeting vector, in addition to the targeting arms and positive selection cassette, the knock-in targeting vector includes the extra replacement cassette that will replace the target gene with a new gene (a set of genes or a point mutation). Targeting vectors relying on homologous recombination contain 5′ and 3′ homologous arms flanking a positive selection cassette (Rogers et al., 2008; Sun et al., 2008). Several principles should be considered when designing a successful targeting vector. (i) Avoid excessive repetitive DNA. In mouse ES cells it has been demonstrated that the excessive repetitive DNA within the targeting vector can significantly reduce targeting frequency (Wu et al., 2008). (ii) Use isogenic DNA as the source for producing exogenous homologous arms. While gene targeting in domestic species by using non-isogenic DNA as a source for targeting arms is possible (McCreath et al., 2000; Denning et al., 2001a and b; Kuroiwa et al., 2004; Marques et al., 2006; Richt et al., 2007), the use of isogenic DNA can largely improve the efficiency of gene targeting (te Riele et al., 1992). (iii) Increase the length of continuous exogenous homologous arms. The efficiency of gene targeting was generally found to be increased with the length of targeting arms in mouse ES cells (Hasty et al., 1991a). (iv) Use multiple cell lines; when targeting the CFTR (cystic fibrosis transmembrane conductance receptor) gene in pig fetal fibroblast cell lines, Rogers et al. (2008) demonstrated drastic differences in the targeting efficiencies between cell lines derived from littermate pig fetuses.

3.1.2 Tissue specificity and inducible promoters

As mentioned, the promoter used in a transgenic DNA construct determines when, where and to what extent the transgene is expressed. Hundreds of promoters can be isolated for the expression of transgenes, and are generally classified as constitutive promoters, tissue/cell-specific or Developmental stage-specific promoters and inducible promoters. Constitutive promoters, such as commonly used SV40 (simian virus 40 promoter), CMV (cytomegalovirus immediate-early promoter), PGK (mouse phosphoglycerate kinase 1 promoter) and CAGG (chicken β-Actin promoter coupled with CMV early enhancer) promoters for mammalian systems (Qin et al., 2010), can continuously drive transgene expression in all tissues and species. Tissue/cell-specific or developmental stage-specific promoters can restrict transgene expression to specific tissue(s) or only during certain developmental stages. For example, the 5.5-Kb osteopontin (OPN) promoter has been used to drive GFP expression in transgenic mice in the same cell-specific and developmental stage-specific manner as endogenous OPN expression (Higashibata et al., 2004). Inducible promoters, as their name suggests, may be activated by the presence of endogenous or exogenous factors. Exogenous factors include chemical compounds such as antibiotics or physical factors such as heat and light. For example, TRE promoter (tetracycline-responsive element promoter) can be activated by the rtTA (reverse tetracycline-controlled transcriptional activator) in a doxycycline-inducible manner (Qin et al., 2010). Antibiotic-induced promoters are the most commonly used in animal genetic modification because of easy manipulation. Inducible promoters provide a very useful tool in animal genetic engineering to turn on or off transgene expression in a particular tissue or at certain developmental stages.

3.1.3 Positive/negative selection strategies

Considering the rarity of a homologous recombination event relative to random integration of the targeting vector, an efficient targeting vector design should incorporate a good selection strategy, providing a powerful tool to improve the frequency of targeted colonies and reduce screening cell lines that result from random integration. Promoter-less gene targeting vector, also referred to the promoter-trapping method, has been used to enrich the gene targeting events in somatic cells in pigs and sheep (McCreath et al., 2000; Denning et al., 2001a and b). In a promoter-trapping vector, the selectable gene lacks its own promoter but it becomes activated from the target gene promoter after correctly integrating into the genome. In a fibroblast cell line, promoter-less vectors can enrich targeting frequency 5,000- to 10,000-fold (Hanson and Sedivy, 1995). Despite this potential improvement, the major limitation of the promoter-trapping method is that it requires active transcription of the targeted gene to drive expression of the selectable marker used in the targeting vector. Thus, if the target gene is only active in cell types that are difficult to culture, it is nearly impossible to target the gene locus using this method. Compared with promoter-trapping method, a more widely used strategy includes utilization of both positive and negative selection (PNS) (Jin et al., 2003; Kuroiwa et al., 2004; Richt et al., 2007). A positive selection gene (antibiotic-resistant gene such as neo) in the targeting vector is needed to select cells with an integrated construct a negative selection gene (cytotoxic genes such as the thymidine kinase (TK) gene or the diphtheria toxin A-chain (DT-A) gene) to further select against random integration event. The negative selection marker in the targeting vector usually is placed downstream of homologous arms and recombined away during the process of homologous recombination. The enrichment of the PNS selection is the ratio of

clones recovered with the positive selection only (PS) versus the positive and negative dual selections. The PNS strategy can be used to target both active and inactive gene loci. It has been shown that the targeting efficiency at the COL1A1 locus in fibroblasts by the promoter-trapping strategy is 15.7-fold higher than by PS only (Marques et al., 2006).

3.2 Delivery of DNA vector into cultured somatic cells

Following vector construction and preparation of the DNA construct for delivery into a somatic cell, it is important to identify an efficient method of DNA delivery into somatic cells as efficiency between delivery methods varies greatly. There are numerous methods to introduce an exogenous DNA construct into somatic cells, which can be categorized as liposome-mediated DNA transfer (Hyun et al., 2003a; Lee et al., 2005), electroporation (Dai et al., 2002; Ramsoondar et al., 2003; Watanabe et al., 2005) and viral-mediated delivery (Lai et al., 2002b; Rogers et al., 2008).

3.2.1 Liposome-mediated DNA delivery

Liposome-mediated DNA transfer can easily transfect a large number of somatic cells without the need of specialized equipment and expertise, compared with other methods. Lee et al. (2005) demonstrated the efficiency of gene transfection with a plasmid containing the enhanced green fluorescence protein gene into fetal-derived bovine fibroblast cells by lipids was significantly higher than that obtained by electroporation. They also validated that transfection efficiency in fetal-derived bovine fibroblast cells, regardless of the delivery methods, was significantly higher than delivering DNA into cumulus-derived fibroblast cells and adult ear skin-derived fibroblast cells, establishing that both delivery method and cell line origin affect the efficiency of gene transfection. Using liposome-mediated DNA delivery followed by SCNT, genetically modified pigs (Hyun et al., 2003a) and sheep (McCreath et al., 2000) have successfully been created.

3.2.2 Electroporation

In contrast to liposome-mediated DNA delivery, electroporation has been widely used for the delivery of exogenous DNA into the cytoplasm of somatic cells to generate genetically modified cell lines for nuclear transfer. Electroporation has been utilized to successfully provide genetically modified donor cells for SCNT to create transgenic cloned domestic animals, including cattle (Kuroiwa et al., 2004), goat (Yu et al., 2006; Zhu et al., 2009), pig (Dai et al., 2002; Lai et al., 2002a; Ramsoondar et al., 2003; Watanabe et al., 2005) and sheep (Denning et al., 2001a). Ross et al. (2010a) indentified optimal electroporation conditions (three 1 ms pulses of 300 V to 200 μL of $1x10^6$ cells/mL in the presence of 12.5μg DNA/mL), which can consistently deliver DNA vector into the 65-80% surviving porcine fetal fibroblasts and have been used to produce healthy, viable transgenic piglets (Ross et al., 2009b). In adult rhesus macaque fibroblasts, it has been demonstrated that electroporation can generate more transfected cells than liposome-mediated methods (Meehan et al., 2008), which is consistent with other similar comparisons (Yáñez and Porter, 1999). Of the numerous delivery methods, electroporation was demonstrated to have the greatest efficiency in generating targeted cell lines via homologous recombination (Vasquez et al., 2001). Targeting the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus, Mir and Piedrahita (2004) demonstrated that the electroporation of a DNA construct with a nuclear localization signal into s-phase synchronized cells can increase targeting efficiency

sevenfold and decrease random integration events 54-fold in primary fetal bovine fibroblasts. Later, Meehan et al. (2008) confirmed this method by successful gene targeting of the HPRT locus in adult rhesus macaque fibroblasts achieved by electroporation of S-phase synchronized cells with a construct containing a SV40 enhancer.

3.2.3 Viral-mediated delivery

Because viruses have the natural ability to stably transfect somatic cells with high efficiency, utilizing viral particles to delivery exogenous DNA into somatic cells has been widely successful. In contrast to liposome-mediated delivery and electroporation, which deliver linear, double-strand DNA into the cytoplasm of somatic cells, viral delivery of exogenous DNA delivers a high number of the linear, intact single-strand DNA molecules into the nucleus of cells. One report has demonstrated the efficient targeting of the PRNP gene encoding the prion protein PrP in bovine fetal fibroblasts by adeno-associated virus (AAV) vectors (Hirata et al., 2004). Also, the same transfection method followed by SCNT was used to successfully produce the CFTR-null and CFTR-DeltaF508 heterozygous pigs and CFTR-deficient ferrets (Rogers et al., 2008; Sun et al., 2008). Despite high transfection efficiency and production of transgenic cloned animals using viral-mediated delivery method, the need to produce high concentrations of virus particles in addition to limitations of the size of DNA capable of being delivered via virus limits the application of this method.

3.3 Selection and characterization of transgenic cell lines

3.3.1 Selection by marker

Following DNA construct delivery into somatic cells, transfected cells are cultured 24-48h in the absence of selection, followed by selection. Selection agents are chosen according to the DNA construct and added in cell-type specific concentrations into the cell culture medium. Typically, G418 (Geneticin) is used when the neomycin resistance gene is the positive selection marker and gangciclovir when using the TK gene for negative selection. Due to the limited lifespan of many of the somatic cell lines that are typically used for nuclear transfer and the significant amount of time to produce clonal colonies, it is important to perform the genetic screening by PCR or southern blotting as early as possible, prior to cell senescence.

3.3.2 Genotyping by PCR or southern blotting

Screening selected colonies by PCR for a gene targeting event typically involves using one PCR primer specific to the host genome sequence and the other primer specific to the selectable marker between the arms of homology in the targeting vector. This approach provides a simple, rapid and highly sensitive method to identify the gene targeting event in the transfected cells (Gómez-Rodríguez et al., 2008). Furthermore, PCR can be performed using the lysate from the small amount of cells, and also can be facilitated by pooled analysis of multiple cell lines. These types of PCR analysis sometimes require optimization as the amplicon is typically several thousand bp depending on the length of the targeting arms. Following the initial screening via PCR, the targeted colonies can be further expanded for analysis by southern blotting. A strategy for identifying targeted cells by southern blotting should be incorporated in the vector design. Two DNA probes on each side of the wild type gene, but outside the targeting vector, are designed to detect a change in fragment size resulting from either introduction or elimination. It is necessary to verify that the probes for southern blotting work well on wild-type genomic DNA from the somatic cells before

the targeting experiment. Also, it is important to perform southern blotting analysis using both probes to confirm the double-crossover event since homologous recombination can occur at only one end of the targeting vector (Hasty et al., 1991b; Moens et al., 1992). Of the methods of screening for gene targeting in somatic cells, southern blotting is considered to be the golden approach to identify correctly targeted colonies despite being time-consuming and relatively expensive compared to PCR approaches.

3.3.3 Fluorescence In situ hybridization

Fluorescence in situ hybridization (FISH) is most commonly used to identify the number of integration sites following random integration of a transgene. FISH has been successfully used to detect the number of bacterial artificial chromosome (BAC) sequences integrated and chromosomal location(s) in mouse ES cells (Yang and Seed, 2003). Although the chromosomal location can be shown directly by FISH, Gómez-Rodríguez et al. (2008) found that screening of BAC-based constructs by FISH can be prone to false positives because of small pieces of integrated DNA that are below the limits of detection by fluorescent hybridization.

3.4 SCNT using genetically modified somatic cells

Once the cell line containing the appropriate genetic modification has been identified, those cells can then be used for SCNT as described in Figure 1. SCNT is a process in which the nucleus of a somatic cell is transferred into the cytoplasm of an enucleated oocyte. The hereby reconstructed SCNT embryos can be activated to initiate development and then are transferred into a synchronized surrogate mother immediately or after short-term *in vitro* culture. In addition to the technical factors involved in the SCNT process, the status of the donor cells and the quality of unfertilized oocytes are considered to largely affect the overall efficiency of the SCNT. This point will be discussed more below.

4. Improvement of the efficiency of cell-based transgenesis via SCNT

Since cell-based transgenesis via SCNT relies extensively on both transgenic and SCNT techniques, the efficiency could be improved by increasing SCNT efficiency and/or gene transfer efficiency.

4.1 Factors affecting SCNT efficiency

SCNT is a technique that requires precise skills in micromanipulation. In addition to technical skills, numerous other factors also impact SCNT efficiency, including donor cell types, quality of the recipient oocytes, cell cycle synchronization of both donor cell and recipient cytoplasm, the epigenetic status of the donor cell, method of reconstructed zygote activation, epigenetic reprogramming following activation, in vitro culture conditions of reconstructed embryos and embryo transfer into surrogate mothers. Here, we will focus on the cell cycle synchronization and methods being used to improve nuclear remodeling and reprogramming during SCNT.

4.1.1 Type of cell and synchronization of cell and recipient oocyte

Different types of donor cells and the different origins of recipient oocyte have been successfully used to produce the cloned animals in domestic species (Table 2). Generally, nuclei from less differentiated donor cells demonstrate greater SCNT efficiency than those

from differentiated donor cells (Hill et al., 2000; Lee et al., 2003). SCNT embryos created from *in vivo*-derived MII oocytes have better developmental potential than *in vitro*-maturated MII oocytes; and *in vitro*-maturated oocytes from sexually mature animals have been demonstrated to more efficiently produce cloned embryos than oocytes *in vitro*-matured from prepubertal animals (Lai et al., 2002a; Lee et al., 2003; Hyun et al., 2003b).
Since the first SCNT animal was produced by transferring the nuclei from quiescent cells (G0), synchronization of the donor nuclei into G0/G1 is thought to be crucial to the success of SCNT (Wilmut et al., 1997). The G0 cells, with lower transcription activity and different chromatin configuration in contrast to the cells at other stages of the cell cycle, may be more responsive to factors inside the recipient oocyte cytoplasm that impact the nuclear remodeling and reprogramming process following SCNT. The cell cycle of cultured somatic cells can be synchronized by serum starvation, contact inhibition and chemical treatments (Cho et al., 2005; Gibbons et al., 2002). Serum starvation to induce the donor cells into G0 is widely used for the production of cloned transgenic animals (Table 2). However, recent studies have demonstrated that synchronizing the cell cycle of donor cells by serum starvation can cause apoptosis (Dalman et al., 2010) and reduce blastocyst production in cattle (Miranda Mdos et al., 2009). The production of the cloned transgenic calves from non-quiescent fetal fibroblasts demonstrated that the synchronization of the donor cells is not an absolute requirement for SCNT success (Cibelli et al., 1998). However, the relative cell cycle combination of both donor cells and recipient oocyte, not just donor cells, is thought to be important for the maintenance of correct ploidy and the subsequent development of reconstructed embryos (reviewed by Campbell, 1999).

4.1.2 Methods to improve nuclear remodeling and reprogramming

Following transfer of a donor nucleus into a recipient oocyte cytoplasm and subsequent activation, nuclear remodeling events of the chromatin structure, such as changes of DNA methylation patterns and histone modifications, result in the reprogramming of gene expression to recapitulate developmental patterns observed in a normal fertilized embryo (Whitworth and Prather, 2010). In contrast to less differentiated donor nuclei, the relatively high level of DNA methylation and low histone acetylation exist in the chromatin of the highly differentiated nuclei. These epigenetic modifications are used to maintain the temporal and spatial patterns of gene expression specific to the cell type or developmental stage. When differentiated nuclei are transferred into the enucleated oocyte cytoplasm, correctly establishing normal patterns of zygotic gene expression is crucial to the full term development of SCNT animals. However, numerous studies have demonstrated improper reprogramming of genes in embryos and tissues of domestic animals following SCNT (Wrenzycki et al., 2001; Pfister-Genskow et al., 2005; Aston et al., 2010; Ross et al., 2010b). Thus, various strategies are under development to facilitate and promote appropriate nuclear reprogramming of the transferred nucleus following nuclear transfer or to pre-program the genome of the donor nucleus prior to SCNT. Of these strategies, one widely used is to treat reconstructed SCNT embryos, but not the donor cells, with histone deacetylase inhibitors (HDACi), such as trichostatin A (TSA), 6-(1,3-dioxo-1H, 3H-benzo [de] isoquinolin-2-yl)-hexanoic acid hydroxyamide (Scriptaid), sodium butyrate and valproic acid. It has been demonstrated that histone deacetylase inhibitor treatment after SCNT can improve both *in vitro* development of SCNT embryos to the blastocyst stage and *in vivo* development to term following embryo transfer (Li et al., 2008; Cervera et al., 2009; Zhao et al., 2009a, 2010; Das et al., 2010; Himaki et al., 2010a; Miyoshi et al., 2010). However,

these treatments may be with some level of toxicity, as one group has reported that offspring from TSA-treated rabbit embryos did not survive to adulthood (Meng et al., 2009). The exact mechanism by which the HDACi treatment significantly improves the cloning efficiency remains largely unknown, although it has been shown to increase levels of global histone acetylation after HDACi treatment which may subsequently change the structure of chromatin and improve nuclear reprogramming (Shi et al., 2008; Iager et al., 2008; Zhao et al., 2010; Das et al., 2010).

Accruing investigations have demonstrated that abnormal DNA methylation patterns contribute to the lower developmental competency of SCNT derived embryos (Kang et al., 2001, 2002; Bourc'his et al., 2001; Santos et al., 2003; Wrenzycki et al., 2006), suggesting the inability of oocyte to fully restore the DNA methylation pattern of differentiated donor nuclei to that of normal totipotent 1-cell stage embryos. Thus, the second method used to assist epigenetic reprogramming is to reduce the DNA methylation level in donor cells or reconstructed embryos by treating them with the DNA methyl-transferase inhibitor such as 5-aza-2′-deoxycytidine (5-aza-dC). Unfortunately, previous studies have demonstrated that 5-aza-dC treatment of donor cells or cloned embryos does not improve the *in vitro* and *in vivo* development of SCNT derived embryos (Enright et al., 2005; Tsuji et al., 2009). However, combining the treatment of donor cells and embryos with both TSA and 5-aza-dC resulted in improved blastocyst development (Ding et al., 2008). Furthermore, an additional study has demonstrated enhanced gene targeting frequency in ES cells with low genomic methylation levels, suggesting the epigenetic status of targeted loci may influence the efficiency of gene targeting by affecting the accessibility for the homologous recombination machinery (Domínguez-Bendala and McWhir, 2004).

An additional strategy to promote developmental reprogramming of cloned embryos is to treat them with latrunculin A (LatA), an actin polymerization inhibitor. One group has reported that post-activation treatment with LatA is effective to improve *in vitro* developmental capacity of gene-modified cloned miniature pig embryos and embryos treated with LatA have the ability to develop into fetuses (Himaki et al., 2010b). Pre-reprogramming donor nuclei prior to SCNT has also been attempted. Rathbone et al (2010) reported that pretreatment of permeabilized ovine fetal fibroblasts with a cytoplasmic extract produced from germinal vesicle (GV) stage *Xenopus laevis* oocytes improves the live birth rate, but not development to blastocyst stage or pregnancy rate following embryo transfer.

4.2 Methods to improve gene transfer efficiency

Production of transgenic domestic animals has been widely accomplished, however, several limitations remain. Typically, plasmid based DNA constructs are limiting in the size of exogenous DNA to transfer and creating animals with targeted genetic modifications has been significantly more challenging. Thus, it is important to continue development of new strategies that broaden application and increase the efficiency of creating targeted genetic modifications in domestic animals.

4.2.1 Artificial chromosomes as DNA transfer vector

In contrast to plasmid based vector, artificial chromosomes have the capacity to carry Megabase-sized pieces of DNA that are maintained as autonomous, replicating chromosomes. Artificial chromosome vectors include a centromere, two telomeres and origins of replication (Robl et al., 2007). A 10 Mb human artificial chromosome (HAC) vector

containing the entire unarranged sequences of the human immunoglobulin heavy and light chain loci (1-1.5 Megabase for each locus) have been transferred into bovine fibroblasts using a microcell-mediated chromosome transfer approach. Following SCNT using selected cells, trans-chromosomal cloned bovine offspring were produced that expressed human immunoglobulin proteins in the blood. HAC was retained as an independent chromosome with the proportion of cells ranged from 78 to 100% in most animals and HAC retention rate has not changed over several years. This system provides a useful tool to produce human therapeutic polyclonal antibodies using trans-chromosomal cloned domestic animals (Kuroiwa et al., 2002; Robl et al., 2007). In domestic animals, the swine artificial chromosome (SAC) (about 310 kb) containing pig centromeric DNA and the neomycin resistance gene was constructed and introduced into pig cell lines, and one positive clone was characterized, showing the possibility for producing transgenic pigs for xenotransplantation and other purposes (Poggiali et al., 2002).

4.2.2 Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALNs)

The low frequency of homologous recombination hampers rapid progress and wide application of gene targeting in domestic animals. The generation of a site-specific double-stranded DNA break (DSB) within the desired locus can facilitate gene targeting by resulting in additions and deletions causing inactivation of gene function. Naturally occurring DNA-binding proteins, including zinc finger proteins (ZFPs) and meganucleases, have been engineered to bind site-specific DNA sequence. Zinc finger nucleases (ZFNs) combine the specific DNA-binding domain (ZFP) with the non-specific cleavage domain of the restriction endonuclease *FokI* and offer powerful tools to create a site-specific DSB to facilitate local homologous recombination. The ZFN induced DSB can lead to incorporating exogenous DNA in a site specific manner by utilization of a homologous recombination targeting vector that overlaps with the DSB region. Additionally, DSBs repaired by non-homologous end-joining (NHEJ) can result in loss of a single nucleotide, multiple nucleotides, or small regions; all capable of rendering a targeted gene dysfunctional. For increasing the specificity of DNA binding, multiple zinc fingers, each recognizing and binding to a 3-bp sequence of DNA nucleotides can be linked in tandem to recognize a unique genomic locus. In human somatic cells, custom-designed ZFNs yielded more than 18% targeting efficiency at the X-linked interleukin-2 receptor gamma gene locus and about 7% of the cells possessed a bi-allelic gene modification (Urnov et al., 2005). ZFNs can also promote the addition of novel DNA sequence into a targeted endogenous locus of human cells at a frequency ranging from 5% to 15% depending on the size of extra-chromosomal DNA (Moehle et al., 2007). Thus, the efficiency of gene targeting using ZFNs offers the possibility of gene therapy for human genetic disease and an approach for improvement of genetic engineering in domestic animals. Recently, targeted gene disruption of exogenous EGFP gene was achieved in porcine somatic cells using ZFNs (Watanabe et al., 2010) and transgenic EGFP knockout pigs were produced using ZFNs followed by SCNT (Whyte et al., 2011).

The appropriate design of a site-specific ZFN is critical to successfully introduce ZFN-mediated genetic modifications. Of the available zinc-finger engineering methods, modular assembly is the most easily performed method, but is also associated with high failure rates to yield a functional three–zinc finger array for the majority of potentially targetable sites (Ramirez et al, 2008). The second method is to combine selection-based methods with ZFNs

made using modular assembly. While effective, this method is labor intensive and requires additional expertise. The third method, referred to as OPEN (Oligomerized Pool ENgineering) is based on bacterial 2-hybrid (B2H) selection and has been proven to be a rapid platform for plant and human cells with high targeting efficiency (ranging from 1 to 50% at different loci) and less toxicity compared to modular assembly system. However, utilization of OPEN requires an archive of pre-selected zinc-finger pools and *E. coli* selection (Maeder et al., 2008). Furthermore, due to the challenge of engineering the endonucleases, orthophenanthroline (OP, a DNA cleaving molecule) was conjugated with triplex-forming oligonucleotides (TFOs, sequence-specific binding capacity) to induce targeted DSBs and stimulate mutations at the target site in approximately 10% of treated human cells (Cannata et al., 2008). TFO conjugating to OP or other DNA cleaving molecules may provide a useful tool to induce targeted gene modification because triplex-forming sequences are frequent in mammalian genes. While ZFN-driven gene targeting can be much more efficient than homologous recombination-based methods, the design and development of highly specific ZFNs remain difficult because of the lack of a simple correspondence between amino acid sequence and DNA recognition sequence.

Recently, several groups have shown that transcription activator–like effectors (TALEs) from the bacterial genus *Xanthomonas* contain a central domain of tandem repeats that can be readily engineered to bind virtually any DNA sequence (Boch et al., 2009; Christian et al., 2010; Morbitzer et al., 2010). The structure of the central protein domain, highly conserved in all the known TALEs, includes 17.5 tandem repeats with 34 amino acids per repeat. In each repeat monomer of a TALE, only amino acid positions 12 and 13 are hypervariable (repeat variable diresidues) (Boch et al., 2011), which can specifically recognize a single nucleotide in the target site (Boch et al., 2009; Moscou et al., 2009). Thus, the correspondence between each repeat variable diresidues and the binding nucleotide in DNA sequence opens the possibility to create novel sequence-specific DNA binding proteins by rearrangement of TALE repeats. The engineered hybrid TALE nucleases (TALNs), produced by fusion of the *FokI* endonuclease domain with the high-specificity DNA-binding domains of TALEs, can bind and create targeted DSBs in tobacco and yeast (Mahfouz et al., 2011; Li et al., 2011), showing the feasibility of engineering TALE-based hybrid nucleases capable of generating site-specific genome modification. Recently, Miller et al. (2011) reported the generation of discrete edits or small deletions within endogenous human NTF3 and CCR5 genes and the insertion of 46-bp sequence at CCR5 locus into the genome of human K562 cells using designed TALNs, demonstrating the effective application of TALNs to modify endogenous genes. While the simple DNA-binding code of TALEs enables easier design strategies as compared to ZFPs, the repetitive nature of TALE DNA-binding domains results in difficulty to efficiently synthesize new TALEs by currently used vector construction methods. To overcome this problem, Zhang et al. (2011) recently developed a new strategy to construct repeat domains of TALEs by hierarchical ligation.

4.3 Induced pluripotent stem (iPS) cells

Owing to the lack of ES cells in domestic animals, it is difficult to replicate strategies routinely used to create genetically modified mice. As an alternative, cell-based transgenesis via SCNT is currently used to produce genetically modified domestic animals. Recent advancements in the ability to generate induced pluripotent stem (iPS) cells may open another potential strategy to improve the efficiency of SCNT in domestic animals. Induced pluripotent stem cells in mice and human were successfully generated by reprogramming

somatic cells with viral delivery of a combination of four defined transcription factors including Sox2, Oct4, Klf4, and c-Myc or Sox2, Oct4, Nanog, and Lin28 (Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Yu et al., 2007). The iPS cells are similar to ES cells in morphology, biochemistry, gene expression and the ability to differentiate into many cell types and self renew (Wernig et al., 2007; Lowry et al., 2008). Furthermore, subsequent studies have optimized existing procedures and discovered novel reprogramming protocols to generate the iPS cells, including non-integrating viruses (Stadtfeld et al., 2008), non-viral vectors (Okita et al., 2008), non-integration episomal vectors (Yu et al., 2009) and RNA-induced reprogramming (Warren et al., 2010), which greatly decrease the biosafety concerns associated with the application of iPS cells. The use of iPS cells can benefit animal transgenesis in several aspects. (i) Stable genetic modification in iPS cells may be more efficient compared to somatic cells as is the case with ES cells. (ii) Genetically modified iPS cells can be used to produce chimeric animals since it has been reported that iPS cells produce viable, live-born and fertile mice offspring through tetraploid complementation (Zhao et al., 2009b; Boland et al., 2009). (iii) The use of genetically modified iPS cells as donors may increase the efficiency of cell-based transgenesis via SCNT owing to the pluripotent status of iPS cells. (iv) In contrast to the limited lifespan of somatic cells, true iPS cells are immortalized. These advantages, coupled to successful derivation of iPS cells from domestic animals (Esteban et al., 2009; Ezashi et al., 2009; Wu et al., 2009) presents a new opportunity to produce transgenic animals using iPS cells.

5. Summary

While the potential opportunities of transgenic domestic animals in biomedicine and agriculture are significant, current procedures, including cell-based transgenesis via SCNT, to produce genetically modified domestic animals are not without limitations. The combination of new technologies, including ZFNs/TALNs to enhance targeted genome modification and iPS cells and other strategies to improve epigenetic remodeling of SCNT embryos, represent pathways for improving the success rates of current genome manipulation strategies resulting in transgenic domestic animals. These modern approaches may have limitations of their own, such as the difficulty and high cost to design, produce and validate the target-specific ZFPs, constructing custom-designed TALEs, and maintenance of iPS cells. However, despite these limitations, we expect to see these strategies become widely utilized as a result of the potential opportunities that utilization of these strategies offers to the field of targeted genome manipulation in domestic animals.

6. References

Aston, K.I., Li, G.P., Hicks, B.A., Sessions, B.R., Davis, A.P., Rickords, L.F., Stevens, J.R., White, K.L. 2010. Abnormal levels of transcript abundance of developmentally important genes in various stages of preimplantation bovine somatic cell nuclear transfer embryos. Cell Reprogram. 12(1): 23-32.

Auchincloss, H. Jr., Sachs, D.H. 1998. Xenogeneic transplantation. Annu Rev Immunol 16: 433-470.

Bhatti, F.N., Zaidi, A., Schmoeckel, M., Cozzi, E., Chavez, G., Wallwork, J., White, D.J., Friend, P.J. 1998. Survival of life-supporting HDAF transgenic kidneys in primates is enhanced by splenectomy. Transplant Proc 30(5): 2467.

Bleck, G.T., White, B.R., Miller, D.J., Wheeler, M.B. 1998. Production of bovine alpha-lactalbumin in the milk of transgenic pigs. J Anim Sci 76(12):3072-3078.

Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A., Bonas, U. 2009. Breaking the code of DNA binding specificity of TAL-type III effectors. Science 326(5959): 1509-1512.

Boch, J. 2011. TALEs of genome targeting. Nat Biotechnol 29(2): 135-136.

Boland, M. J., Hazen, J. L., Nazor, K. L., Rodriguez, A.R., Gifford, W., Martin, G., Kupriyanov, S., Baldwin, K.K. 2009. Adult mice generated from induced pluripotent stem cells. Nature 461 (7260): 91-94.

Bordignon, V., Keyston, R., Lazaris, A., Bilodeau, A.S., Pontes, J.H., Arnold, D., Fecteau, G., Keefer, C., Smith, L.C. 2003. Transgene expression of green fluorescent protein and germ line transmission in cloned calves derived from in vitro-transfected somatic cells. Biol Reprod 68: 2013-2023.

Bourc'his, D., Le Bourhis, D., Patin, D., Niveleau, A., Comizzoli, P., Renard, J.P., Viegas-Pequignot, E. 2001. Delayed and incomplete reprogramming of chromosome methylation patterns in bovine cloned embryos. Curr Biol 11: 1542-1546.

Brophy, B., Smolenski, G., Wheeler, T., Wells, D., L'Huillier, P., Laible, G. 2003. Cloned transgenic cattle produce milk with higher levels of beta-casein and kappa-casein. Nat Biotechnol 21(2):157-162.

Byrne, G.W., McCurry, K.R., Martin, M.J., McClellan, S.M., Platt, J.L., Logan, J.S. 1997. Transgenic pigs expressing human CD59 and decay-accelerating factor produce an intrinsic barrier to complement mediated damage. Transplantation 63: 149.

Cabot, R.A., Kühholzer, B., Chan, A.W., Lai, L., Park, K.W., Chong, K.Y., Schatten, G., Murphy, C.N., Abeydeera, L.R., Day, B.N., Prather, R.S. 2001. Transgenic pigs produced using in vitro matured oocytes infected with a retroviral vector. Anim Biotechnol 12(2):205-214.

Campbell, K.H. 1999. Nuclear transfer in farm animal species. Semin Cell Dev Biol. 10(3):245-252.

Cannata, F., Brunet, E., Perrouault, L., Roig, V., Ait-Si-Ali, S., Asseline, U., Concordet, J.P., Giovannangeli, C. 2008. Triplex-forming oligonucleotide-orthophenanthroline conjugates for efficient targeted genome modification. Proc Natl Acad Sci USA 105(28): 9576-9581.

Castro, F.O., Hernandez, O., Uliver, C., Solano, R., Milanes, C., Aguilar, A., Perez, R., De Armas, R., Herrera, N., Fuente, J.D.L. 1991. Introduction of foreign DNA into the spermatozoa of farm animals. Theriogenology 34: 1099-1110.

Cervera, R.P., Martí-Gutiérrez, N., Escorihuela, E., Moreno, R., Stojkovic, M. 2009. Trichostatin A affects histone acetylation and gene expression in porcine somatic cell nucleus transfer embryos. Theriogenology 72(8): 1097-1110.

Chan, A.W., Homan, E.J., Ballou, L.U., Burns, J.C., Bremel, R.D. 1998. Transgenic cattle produced by reverse-transcribed gene transfer in oocytes. Proc Natl Acad Sci USA 95(24): 14028-14033.

Chang, K., Qian, J., Jiang, M., Liu, Y.H., Wu, M.C., Chen, C.D., Lai, C.K., Lo, H.L., Hsiao, C.T., Brown, L., Bolen, J. Jr., Huang, H.I., Ho, P.Y., Shih, P.Y., Yao, C.W., Lin, W.J., Chen, C.H., Wu, F.Y., Lin, Y.J., Xu, J., Wang, K. 2002. Effective generation of transgenic pigs and mice by linker based sperm-mediated gene transfer. BMC Biotechnol 2: 5.

Chen, R.H., Naficy, S., Logan, J.S., Diamond, L.E., Adams, D.H. 1999. Hearts from transgenic pigs constructed with CD59/DAF genomic clones demonstrate improved survival in primates. Xenotransplantation 6: 194.

Cho, S.R., Ock, S.A., Yoo, J.G., Mohana kumar, B., Choe, S.Y., Rho, G.J. 2005. Effect of confluent, Roscovitine treatment and serum starvation on the cell-cycle synchronization of bovine foetal fibroblasts. Reprod Domest Anim 40: 171-176.

Choi, T., Huang, M., Gorman, C., Jaenisch, R. 1991. A generic intron increases gene expression in transgenic mice. Molecular Cellular Biology 11: 3070-3074.

Christian, M., Cermak, T., Doyle, E.L., Schmidt, C., Zhang, F., Hummel, A., Bogdanove, A.J., Voytas, D.F. 2010. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 186(2): 757-761.

Cibelli, J.B., Stice, S.L., Golueke, P.J., Kane, J.J., Jerry, J., Blackwell, C., Ponce de Leon, F. A., Robl. J.M. 1998. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 280: 1256-1258.

Clements, J.E., Wall, R.J., Narayan, O., Hauer, D., Schoborg, R., Sheffer, D., Powell, A., Carruth, L.M., Zink, M.C., Rexroad, C.E. 1994. Development of transgenic sheep that express the visna virus envelope gene. Virology 200: 370-380.

Cooper, D.K., Ezzelarab, M., Hara, H., Ayares, D. 2008. Recent advances in pig-to-human organ and cell transplantation. Expert Opin Biol Ther 8(1): 1-4.

Cozzi, E., White, D.J.G. 1995. The generation of transgenic pigs as potential organ donors for humans. Nat Med 1: 964-966.

Dai, Y., Vaught, T.D., Boone, J., Chen, S.H., Phelps, C.J., Ball, S., Monahan, J.A., Jobst, P.M., McCreath, K.J., Lamborn, A.E., Cowell-Lucero, J.L., Wells, K.D., Colman, A., Polejaeva, I.A., Ayares, D.L. 2002. Targeted disruption of the alpha1,3-galactosyltransferase gene in cloned pigs. Nat Biotechnol 20: 251-255.

Dalman, A., Eftekhari-Yazdi, P., Valojerdi, M., Shahverdi, A., Gourabi, H., Janzamin, E., Fakheri, R., Sadeghian, F., Hasani, F. 2010. Synchronizing Cell Cycle of Goat Fibroblasts by Serum Starvation Causes Apoptosis. Reprod Domest Anim. 45: e46–e53.

Das, Z.C., Gupta, M.K., Uhm, S.J., Lee, H.T. 2010. Increasing histone acetylation of cloned embryos, but not donor cells, by sodium butyrate improves their in vitro development in pigs. Cell Reprogram 12(1): 95-104.

Denning, C., Burl, S., Ainslie, A., Bracken, J., Dinnyes, A., Fletcher, J., King, T., Ritchie, M., Ritchie, W.A., Rollo, M., de Sousa, P., Travers, A., Wilmut, I., Clark, A.J. 2001a. Deletion of the lpha(1,3)galactosyl transferase (GGTA1) gene and the prion protein (PrP) gene in sheep. Nat Biotechnol 19: 559-562.

Denning, C., Dickinson, P., Burl, S., Wylie, D., Fletcher, J. Clark, A.J. 2001b. Gene targeting in primary fetal fibroblasts from sheep and pig. Cloning Stem Cells 3(4): 221-231.

Diamond LE, McCurry KR, Martin MJ, McClellan SB, Oldham ER, Platt JL, Logan JS. 1996. Characterization of transgenic pigs expressing functionally active human CD59 on cardiac endothelium. Transplantation 61(8): 1241-1249.

Diamond, L.E., Quinn, C.M., Martin, M.J., Lawson, J., Platt, J.L., Logan, J.S. 2001. A human CD46 transgenic pig model system for the study of discordant xenotransplantation. Transplantation 71(1): 132-142.

Ding X, Wang Y, Zhang D, Wang Y, Guo Z, Zhang Y. 2008. Increased pre-implantation development of cloned bovine embryos treated with 5-aza-2'-deoxycytidine and trichostatin A. Theriogenology 70(4): 622-630.

Diswall, M., Angström, J., Karlsson, H., Phelps, C.J., Ayares, D., Teneberg, S., Breimer, M.E. 2010. Structural characterization of alpha1,3-galactosyltransferase knockout pig heart and kidney glycolipids and their reactivity with human and baboon antibodies. Xenotransplantation 17(1): 48-60.

Domínguez-Bendala, J., McWhir, J. 2004. Enhanced gene targeting frequency in ES cells with low genomic methylation levels. Transgenic Res 13(1): 69-74.

Duncker, B.P., Davies, P.L., Walker, V.K. 1997. Introns boost transgene expression in Drosophila melanogaster. Mol Gen Genet 254: 291-296.

Ebert, K.M., Selgrath, J.P., DiTullio, P., Denman, J., Smith, T.E., Memon, M.A., Schindler, J.E., Monastersky, G.M., Vitale, J.A., Gordon, K. 1991. Transgenic production of a variant of human tissue-type plasminogen activator in goat milk: generation of transgenic goats and analysis of expression. Biotechnology (N Y) 9(9): 835-838.

Enright, B.P., Sung, L.Y., Chang, C.C., Yang, X., Tian, X.C. 2005. Methylation and acetylation characteristics of cloned bovine embryos from donor cells treated with 5-aza-2'-deoxycytidine. Biol Reprod 72(4): 944-948.

Esteban, M. A., Xu, J., Yang, J., Peng, M., Qin, D., Li, W., Jiang, Z., Chen, J., Deng, K., Zhong, M., Cai, J., Lai, L., Pei, D. 2009. Generation of induced pluripotent stem cell lines from Tibetan miniature pig. J Biol Chem 284(26): 17634-17640.

Ezashi, T., Telugu, B. P., Alexenko, A. P., Sachdev, S., Sinha, S., Roberts, R. M. 2009. Derivation of induced pluripotent stem cells from pig somatic cells. Proc Natl Acad Sci USA 106(27): 10993-10998.

Fodor, W.L., Williams, B.L., Matis, L.A., Madri, J.A., Rollins, S.A., Knight, J.W., Velander, W., Squinto, S.P. 1994. Expression of a functional human complement inhibitor in a transgenic pig as a model for the prevention of xenogeneic hyperacute organ rejection. Proc Natl Acad Sci USA 91(23): 11153-11157.

Galili, U. 1993. Interaction of the natural anti-Gal antibody with alpha-galactosyl epitopes: a major obstacle for xenotransplantation in humans (see comments). Immunol Today 14: 480-482.

García-Vázquez, F.A., Ruiz, S., Matás, C., Izquierdo-Rico, M.J., Grullón, L.A., De Ondiz, A., Vieira, L., Avilés-López, K., Gutiérrez-Adán, A., Gadea, J. 2010. Production of transgenic piglets using ICSI-sperm-mediated gene transfer in combination with recombinase RecA. Reproduction 140(2): 259-272.

Gibbons, J., Arat, S., Rzucidlo, J., Miyoshi, K., Waltenburg, R., Respess, D., Venable, A., Stice, S. 2002. Enhanced survivability of cloned calves derived from roscovitine-treated adult somatic cells. Biol Reprod 66: 895–900.

Gómez-Rodríguez, J., Washington, V., Cheng, J., Dutra, A., Pak, E., Liu, P., McVicar, D.W., Schwartzberg, P.L. 2008. Advantages of q-PCR as a method of screening for gene targeting in mammalian cells using conventional and whole BAC-based constructs. Nucleic Acids Res. 36(18): e117.

Grosse-Hovest, L., Müller, S., Minoia, R., Wolf, E., Zakhartchenko, V., Wenigerkind, H., Lassnig, C., Besenfelder, U., Müller, M., Lytton, S.D., Jung, G., Brem, G. 2004. Cloned transgenic farm animals produce a bispecific antibody for T cell-mediated tumor cell killing. Proc Natl Acad Sci USA 101(18): 6858-6863.

Hammer, R.E., Pursel, V.G., Rexroad, C.E. Jr, Wall, R.J., Bolt, D.J., Ebert, K.M., Palmiter, R.D., Brinster, R.L. 1985. Production of transgenic rabbits, sheep and pigs by microinjection. Nature 315(6021): 680-683.

Hanson, K.D., Sedivy, J.M. 1995. Analysis of biological selections for high-efficiency gene targeting. Mol Cell Biol. 15(1): 45-51.

Hasty, P., Rivera-Pérez, J., Bradley, A. 1991a. The length of homology required for gene targeting in embryonic stem cells. Mol Cell Biol. 11(11): 5586-5591.

Hasty, P., Rivera-Perez, J., Chang, C., Bradley, A. 1991b. Target frequency and integration pattern for insertion and replacement vectors in embryonic stem cells. Mol Cell Biol 11: 4509-4517.

Higashibata, Y., Sakuma, T., Kawahata, H., Fujihara, S., Moriyama, K., Okada, A., Yasui, T., Kohri, K., Kitamura, Y., Nomura, S. 2004. Identification of promoter regions involved in cell- and developmental stage-specific osteopontin expression in bone, kidney, placenta, and mammary gland: an analysis of transgenic mice. J Bone Miner Res 19(1): 78-88.

Hill, J.R., Winger, Q.A., Long, C.R., Looney, C.R., Thompson, J.A., Westhusin, M.E. 2000. Development rates of male bovine nuclear transfer embryos derived from adult and fetal cells. Biol Reprod 62(5): 1135-1140.

Himaki, T., Yokomine, T.A., Sato, M., Takao, S., Miyoshi, K., Yoshida, M. 2010a. Effects of trichostatin A on in vitro development and transgene function in somatic cell nuclear transfer embryos derived from transgenic Clawn miniature pig cells. Anim Sci J 81(5): 558-563.

Himaki, T., Mori, H., Mizobe, Y., Miyoshi, K., Sato, M., Takao, S., Yoshida, M. 2010b. Latrunculin A dramatically improves the developmental capacity of nuclear transfer embryos derived from gene-modified clawn miniature pig cells. Cell Reprogram 12(2): 127-131.

Hirata, R.K., Xu, C., Dong, R., Miller, D.G., Ferguson, S., Russell, D.W. 2004. Efficient PRNP gene targeting in bovine fibroblasts by adeno-associated virus vectors. Cloning Stem Cells 6(1): 31-36.

Huang, Y.J., Huang, Y., Baldassarre, H., Wang, B., Lazaris, A., Leduc, M., Bilodeau, A.S., Bellemare, A., Côté, M., Herskovits, P., Touati, M., Turcotte, C., Valeanu, L., Lemée, N., Wilgus, H., Bégin, I., Bhatia, B., Rao, K., Neveu, N., Brochu, E., Pierson, J., Hockley, D.K., Cerasoli, D.M., Lenz, D.E., Karatzas, C.N., Langermann, S. 2007. Recombinant human butyrylcholinesterase from milk of transgenic animals to protect against organophosphate poisoning. Proc Natl Acad Sci USA 104(34): 13603-13608.

Hyun, S., Lee, G., Kim, D., Kim, H., Lee, S., Nam, D., Jeong, Y., Kim, S., Yeom, S., Kang, S., Han, J., Lee, B., Hwang, W. 2003a. Production of nuclear transfer-derived piglets using porcine fetal fibroblasts transfected with the enhanced green fluorescent protein. Biol Reprod 69(3): 1060–1068.

Hyun, S.H., Lee, G.S., Kim, D.Y., Kim, H.S., Lee, S.H., Kim, S., Lee, E.S., Lim, J.M., Kang, S.K., Lee, B.C., Hwang, W.S. 2003b. Effect of maturation media and oocytes derived from sows or gilts on the development of cloned pig embryos. Theriogenology 59(7): 1641-1649.

Iager, A.E., Ragina, N.P., Ross, P.J., Beyhan, Z., Cunniff, K., Rodriguez, R.M., Cibelli, J.B. 2008. Trichostatin A improves histone acetylation in bovine somatic cell nuclear transfer early embryos. Cloning Stem Cells 10(3): 371-379.

Jin, D.I., Lee, S.H., Choi, J.H., Lee, J.S., Lee, J.E., Park, K.W., Seo, J.S. 2003. Targeting efficiency of a-1,3-galactosyl transferase gene in pig fetal fibroblast cells. Exp Mol Med. 35(6): 572-577.

Kang, Y.K., Koo, D.B., Park, J.S., Choi, Y.H., Chung, A.S., Lee, K.K., Han, Y.M. 2001. Aberrant methylation of donor genome in cloned bovine embryos. Nat Genet 28: 173–177.

Kang, Y.K., Park, J.S., Koo, D.B., Choi, Y.H., Kim, S.U., Lee, K.K., Han, Y.M. 2002. Limited demethylation leaves mosaic-type methylation states in cloned bovine pre-implantation embryos. EMBO J 21: 1092–1100.

Keefer, C. L., Baldassarre, H., Keyston, R., Wang, B., Bhatia, B., Bilodeau, A.S., Zhou, J.F., Leduc, M., Downey, B.R., Lazaris, A., Karatzas, C.N. 2001. Generation of dwarf goat (Capra hircus) clones following nuclear transfer with transfected and nontransfected fetal fibroblasts and in vitro-matured oocytes. Biol Reprod 64: 849-856.

Kjer-Nielsen, L., Holmberg, K., Perera, J.D., McCluskey, J. 1992. Impaired expression of chimaeric major histocompatibility complex transgenes associated with plasmid sequences. Transgenic Research 1: 182-187.

Kragh, P.M., Nielsen, A.L., Li, J., Du, Y., Lin, L., Schmidt, M., Bogh, I.B., Holm, I.E., Jakobsen, J.E., Johansen, M.G., Purup, S., Bolund, L., Vajta, G., Jorgensen, A.L. 2009. Hemizygous minipigs produced by random gene insertion and handmade cloning express the Alzheimer's disease-causing dominant mutation APPsw. Transgenic Res 18: 545-558.

Kuroiwa, Y., Kasinathan, P., Choi, Y.J., Naeem, R., Tomizuka, K., Sullivan, E.J., Knott, J.G., Duteau, A., Goldsby, R.A., Osborne, B.A., Ishida, I., Robl, J.M. 2002. Cloned transchromosomic calves producing human immunoglobulin. Nat Biotechnol 20: 889-894.

Kuroiwa, Y., Kasinathan, P., Matsushita, H., Sathiyaselan, J., Sullivan, E.J., Kakitani, M., Tomizuka, K., Ishida, I., Robl, J.M. 2004. Sequential targeting of the genes encoding immunoglobulin-mu and prion protein in cattle. Nat Genet 36(7): 775-780.

Lai, L., Kolber-Simonds, D., Park, K.W., Cheong, H.T., Greenstein, J.L., Im, G.S., Samuel, M., Bonk, A., Rieke, A., Day, B.N., Murphy, C.N., Carter, D.B., Hawley, R.J., Prather, R.S. 2002a. Production of a-1, 3-galactosyltransferase knockout pigs by nuclear transfer cloning. Science 295: 1089-1092.

Lai, L., Park, K.W., Cheong, H.T., Kuhholzer, B., Samuel, M., Bonk, A., Im, G.S., Rieke, A., Day, B.N., Murphy, C.N., Carter, D.B., Prather, R.S. 2002b. Transgenic pig expressing the enhanced green fluorescent protein produced by nuclear transfer using colchicine-treated fibroblasts as donor cells. Mol Reprod Dev 62(3): 300-306.

Lai, L., Kang, J.X., Li, R., Wang, J., Witt, W.T., Yong, H.Y., Hao, Y., Wax, D.M., Murphy, C.N., Rieke, A., Samuel, M., Linville, M.L., Korte, S.W., Evans, R.W., Starzl, T.E., Prather, R.S., Dai, Y. 2006. Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nat. Biotechnol 24: 435-436.

Lavitrano, M., Bacci, M.L., Forni, M., Lazzereschi, D., Di Stefano, C., Fioretti, D., Giancotti, P., Marfé, G., Pucci, L., Renzi, L., Wang, H., Stoppacciaro, A., Stassi, G.,

Sargiacomo, M., Sinibaldi, P., Turchi, V., Giovannoni, R., Della Casa, G., Seren, E., Rossi, G. 2002. Efficient production by sperm-mediated gene transfer of human decay accelerating factor (hDAF) transgenic pigs for xenotransplantation. Proc Natl Acad Sci USA 99(22): 14230-14235.

Lavitrano, M., Busnelli, M., Cerrito, M.G., Giovannoni, R., Manzini, S., Vargiolu, A. 2006. Sperm-mediated gene transfer. Reprod Fertil Dev 18(1-2): 19-23.

Lee, G.S., Hyun, S.H., Kim, H.S., Kim, D.Y., Lee, S.H., Lim, J.M., Lee, E.S., Kang, S.K., Lee, B.C, Hwang, W.S. 2003. Improvement of a porcine somatic cell nuclear transfer technique by optimizing donor cell and recipient oocyte preparations. Theriogenology 59(9): 1949-1957.

Lee, H.G., Lee, H.C., Kim, S.W., Lee, P., Chung, H.J., Lee, Y.K., Han, J.H., Hwang, I.S., Yoo, J.I., Kim, Y.K., Kim, H.T., Lee, H.T., Chang, W.K., Park, J.K. 2009. Production of recombinant human von Willebrand factor in the milk of transgenic pigs. J Reprod Dev 55(5): 484-490.

Lee, S.L., Ock, S.A., Yoo, J.G., Kumar, B.M., Choe, S.Y., Rho, G.J. 2005. Efficiency of gene transfection into donor cells for nuclear transfer of bovine embryos. Mol Reprod Dev 72(2): 191-200.

Li, J., Svarcova, O., Villemoes, K., Kragh, P.M., Schmidt, M., Bøgh, I.B., Zhang, Y., Du, Y., Lin, L., Purup, S., Xue, Q., Bolund, L., Yang, H., Maddox-Hyttel, P., Vajta, G. 2008. High in vitro development after somatic cell nuclear transfer and trichostatin A treatment of reconstructed porcine embryos. Theriogenology 70(5): 800-808.

Li ,S., Guo, Y., Shi, J., Yin, C., Xing, F., Xu, L., Zhang, C., Liu, T., Li, Y., Li, H., Du, L., Chen, X. 2009. Transgene expression of enhanced green fluorescent protein in cloned rabbits generated from in vitro-transfected adult fibroblasts. Transgenic Res 18: 227-235.

Li. T., Huang, S., Jiang, W.Z., Wright, D., Spalding, M.H., Weeks, D.P., Yang, B. 2011. TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain. Nucleic Acids Res 39(1): 359-372.

Lo, D., Pursel, V., Linton, P.J., Sandgren, E., Behringer, R., Rexroad, C., Palmiter, R.D., Brinster, R.L. 1991. Expression of mouse IgA by transgenic mice, pigs and sheep. Eur. J. Immunol 21: 1001–1006.

Lowry, W.E., Richter, L., Yachechko, R., Pyle, A.D., Tchieu, J., Sridharan, R., Clark, A.T., Plath, K. 2008. Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci USA 105(8): 2883-2888.

Maeder, M.L., Thibodeau-Beganny, S., Osiak, A., Wright, D.A., Anthony, R.M., Eichtinger, M., Jiang, T., Foley, J.E., Winfrey, R.J., Townsend, J.A., Unger-Wallace, E., Sander, J.D., Müller-Lerch, F., Fu, F., Pearlberg, J., Göbel, C., Dassie, J.P., Pruett-Miller, S.M., Porteus, M.H., Sgroi, D.C., Iafrate, A.J., Dobbs, D., McCray, P.B. Jr., Cathomen, T., Voytas, D.F., Joung, J.K. 2008. Rapid "open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification. Mol Cell 31(2): 294-301.

Magre, S., Takeuchi, Y., Bartosch, B. 2003. Xenotransplantation and pig endogenous retroviruses. Rev Med Virol 13(5): 311-329.

Mahfouz, M.M., Li, L., Shamimuzzaman, M., Wibowo, A., Fang, X., Zhu, J.K. 2011. De novo-engineered transcription activator-like effector (TALE) hybrid nuclease with novel DNA binding specificity creates double-strand breaks. Proc Natl Acad Sci USA 108(6): 2623-2628.

Marques, M.M., Thomson, A.J., McCreath, K.J., McWhir, J. 2006. Conventional gene targeting protocols lead to loss of targeted cells when applied to a silent gene locus in primary fibroblasts. J Biotechnol 125(2): 185-193.

McCreath, K. J., Howcroft, J., Campbell, K. H., Colman, A., Schnieke, A. E., Kind, A. J. 2000. Production of gene-targeted sheep by nuclear transfer from cultured somatic cells. Nature 405: 1066-1069.

McKee, C., Gibson, A., Dalrymple, M., Emslie, L., Garner, I., Cottingham, I. 1998. Production of biologically active salmon calcitonin in the milk of transgenic rabbits. Nat Biotechnol16(7): 647-651.

Meehan, D.T., Zink, M.A., Mahlen, M., Nelson, M., Sanger, W.G., Mitalipov, S.M., Wolf, D.P., Ouellette, M.M., Norgren Jr, R.B. 2008. Gene targeting in adult rhesus macaque fibroblasts. BMC Biotechnol 8: 31.

Melo, E.O., Canavessi, A.M., Franco, M.M., Rumpf, R. 2007. Animal transgenesis: state of the art and applications. J Appl Genet 48(1): 47-61.

Meng, Q., Polgar, Z., Liu, J., Dinnyes, A. 2009. Live birth of somatic cell-cloned rabbits following trichostatin A treatment and cotransfer of parthenogenetic embryos. Cloning Stem Cells 11(1): 203-208.

Miller, J.C., Tan, S., Qiao, G., Barlow, K.A., Wang, J., Xia, D.F., Meng, X., Paschon, D.E., Leung, E., Hinkley, S.J., Dulay, G.P., Hua, K.L., Ankoudinova, I., Cost, G.J., Urnov, F.D., Zhang, H.S., Holmes, M.C., Zhang, L., Gregory, P.D., Rebar, E.J. 2011. A TALE nuclease architecture for efficient genome editing. Nat Biotechnol 29(2): 143-148.

Mir, B., Piedrahita, J.A. 2004. Nuclear localization signal and cell synchrony enhance gene targeting efficiency in primary fetal fibroblasts. Nucleic Acids Res 32(3): e25.

Miranda Mdos, S., Bressan, F.F., Zecchin, K.G., Vercesi, A.E., Mesquita, L.G., Merighe, G.K., King, W.A., Ohashi, O.M., Pimentel, J.R., Perecin, F., Meirelles, F.V. 2009. Serum-starved apoptotic fibroblasts reduce blastocyst production but enable development to term after SCNT in cattle. Cloning Stem Cells 11(4): 565-573.

Miyoshi, K., Mori, H., Mizobe, Y., Asasaka, E., Ozawa, A., Yoshida, M., Sato, M. 2010. Valproic acid enhances in vitro development and oct-3/4 expression of miniature pig somatic cell nuclear transfer embryos. Cell Reprog 12: 67-74.

Moehle, E.A., Rock, J.M., Lee, Y.L., Jouvenot, Y., DeKelver, R.C., Gregory, P.D., Urnov, F.D., Holmes, M.C. 2007. Targeted gene addition into a specified location in the human genome using designed zinc finger nucleases. Proc Natl Acad Sci USA 104(9): 3055-3060.

Moens, C. B., Auerbach, A. B., Conlon, R. A., Joyner, A. L., Rossant, J. 1992. A targeted mutation reveals a role for N-myc in branching morphogenesis in the embryonic mouse lung. Genes Dev 6: 691-704.

Moisvadi, S., Kaminski, J.M., Yanagimachi, R. 2009. Use of intracytoplasmic sperm injection (ICSI) to generate transgenic animals. Comp Immunol Microbiol Infect Dis 32(2): 47-60.

Monaco, M.H., Gronlund, D.E., Bleck, G.T., Hurley, W.L., Wheeler, M.B., Donovan, S.M. 2005. Mammary specific transgenic over-expression of insulin-like growth factor-I (IGF-I) increases pig milk IGF-I and IGF binding proteins, with no effect on milk composition or yield. Transgenic Res 14(5): 761-773.

Morbitzer, R., Römer, P., Boch, J., Lahaye, T. 2010. Regulation of selected genome loci using de novo-engineered transcription activator-like effector (TALE)-type transcription factors. Proc Natl Acad Sci USA 107(50): 21617-21622.

Moscou, M.J., Bogdanove, A.J. 2009. A simple cipher governs DNA recognition by TAL effectors. Science 326(5959): 1501.

Mueller, N.J., Takeuchi, Y., Mattiuzzo, G., Scobie, L. 2011. Microbial safety in xenotransplantation. Curr Opin Organ Transplant 16(2): 201-206.

Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T., Yamanaka, S. 2008. Generation of mouse induced pluripotent stem cells without viral vectors. Science 322(5903): 949-953.

Oropeza, M., Petersen, B., Carnwath, J.W., Lucas-Hahn, A., Lemme, E., Hassel, P., Herrmann, D., Barg-Kues, B., Holler, S., Queisser, A-L., Schwinzer, R., Hinkel, R., Kupatt, C., Niemann, H. 2009. Transgenic expression of the human A20 gene in cloned pigs provides protection against apoptotic and inflammatory stimuli. Xenotransplantation 16: 522-534.

Osada, T., Toyoda, A., Moisyadi, S., Akutsu, H., Hattori, M., Sakaki, Y., Yanagimachi, R. 2005. Production of inbred and hybrid transgenic mice carrying large (>200 kb) foreign DNA fragments by intracytoplasmic sperm injection, Mol Reprod Dev 72: 329-335.

Paleyanda, R.K., Velander, W.H., Lee, T.K., Scandella, D.H., Gwazdauskas, F.C., Knight, J.W., Hoyer, L.W., Drohan, W.N., Lubon, H. 1997. Transgenic pigs produce functional human factor VIII in milk. Nat Biotechnol 15(10): 971-975.

Paradis, K., Langford, G., Long, Z., Heneine, W., Sandstrom, P., Switzer, W.M., Chapman, L.E., Lockey, C., Onions, D., Otto, E. 1999. Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. Science 285(5431): 1236-1241.

Park, J.K., Lee, Y.K., Lee, P., Chung, H.J., Kim, S., Lee, H.G., Seo, M.K., Han, J.H., Park, C.G., Kim, H.T., Kim, Y.K., Min, K.S., Kim, J.H., Lee, H.T., Chang, W.K. 2006. Recombinant human erythropoietin produced in milk of transgenic pigs. J Biotechnol 122(3): 362-371.

Patience, C., Takeuchi, Y., Weiss, R.A. 1997. Infection of human cells by an endogenous retrovirus of pigs. Nat Med 3(3): 282-286.

Perry, A.C.F., Wakayama, T., Kishikawa, H., Kasai, T., Okabe, M., Toyoda, Y., Yanagimachi, R. 1999. Mammalian transgenesis by intracytoplasmic sperm injection. Science 284:1180-1183.

Petersen, B., Kues, W., Lucas-Hahn, A., Queisser, A.L., Lemme, E., Hoelker, M., Carnwath J.W., Niemann, H. 2006. Generation of pigs transgenic for hCD59/DAF and human thrombomodulin by somatic nuclear transfer. Reprod Fertil Dev 18: 142.

Pfister-Genskow, M., Myers, C., Childs, L.A., Lacson, J.C., Patterson, T., Betthauser, J.M., Goueleke, P.J., Koppang, R.W., Lange, G., Fisher, P., Watt, S.R., Forsberg, E.J., Zheng, Y., Leno, G.H., Schultz, R.M., Liu, B., Chetia, C., Yang, X., Hoeschele, I., Eilertsen, K.J. 2005. Identification of differentially expressed genes in individual bovine preimplantation embryos produced by nuclear transfer: improper reprogramming of genes required for development. Biol Reprod 72(3): 546-555.

Phelps, C.J., Koike, C., Vaught, T.D., Boone, J., Wells, K.D., Chen, S.H., Ball, S., Specht, S.M., Polejaeva, I.A., Monahan, J.A., Jobst, P.M., Sharma, S.B., Lamborn, A.E., Garst, A.S.,

Moore, M., Demetris, A.J., Rudert, W.A., Bottino, R., Bertera, S., Trucco, M., Starzl, T.E., Dai, Y., Ayares, D.L. 2003. Production of alpha 1,3-galactosyltransferase-deficient pigs. Science 299(5605): 411-414.

Poggiali, P., Scoarughi, G.L., Lavitrano, M., Donini, P., Cimmino, C. 2002. Construction of a swine artificial chromosome: a novel vector for transgenesis in the pig. Biochimie 84(11): 1143-1150.

Prather, R.S., Shen, M., Dai, Y. 2008. Genetically modified pigs for medicine and agriculture. Biotechnology and Genetic Engineering Reviews 25: 245-266.

Pursel, V.G., Rexroad, C.E. Jr. 1993. Recent progress in the transgenic modification of swine and sheep. Mol Reprod Dev 36(2): 251-254.

Pursel, V.G., Wall, R.J., Mitchell, A.D., Elsasser, T.H., Solomon, M.B., Coleman, M.E., Mayo, F., Schwartz, R. J. 1999. Expression of insulin-like growth factor-1 in skeletal muscle of transgenic pigs. In 'Transgenic Animals in Agriculture'. (Eds J. D. Murray, G. B. Anderson, A. M. Oberbauer and M. M. McGloughlin.) pp. 131-144. (CABI Publishing: New York.)

Qin, J.Y., Zhang, L., Clift, K.L., Hulur, I., Xiang, A.P., Ren, B.Z., Lahn, B.T. 2010. Systematic Comparison of Constitutive Promoters and the Doxycycline-Inducible Promoter. PLoS ONE 5(5): e10611.

Ramirez, C.L., Foley, J.E., Wright, D.A., Müller-Lerch, F., Rahman, S.H., Cornu, T.I., Winfrey, R.J., Sander, J.D., Fu, F., Townsend, J.A., Cathomen, T., Voytas, D.F., Joung, J.K. 2008. Unexpected failure rates for modular assembly of engineered zinc fingers. Nat Methods 5(5): 374-375.

Ramsoondar, J.J., Machaty, Z., Costa, C., Williams, B.L., Fodor, W.L., Bondioli, K.R. 2003. Production of alpha 1, 3-galactosyltransferase-knockout cloned pigs expressing human alpha 1, 2-fucosylosyltransferase. Biol Reprod 69(2): 437-445.

Ramsoondar, J., Vaught, T., Ball, S., Mendicino, M., Monahan, J., Jobst, P., Vance, A., Duncan, J., Wells, K., Ayares, D. 2009. Production of transgenic pigs that express porcine endogenous retrovirus small interfering RNAs. Xenotransplantation 16(3): 164-180.

Rathbone, A.J., Fisher, P.A., Lee, J.H., Craigon, J., Campbell, K.H. 2010. Reprogramming of ovine somatic cells with Xenopus laevis oocyte extract prior to SCNT improves live birth rate. Cell Reprogram. 12(5): 609-616.

Reh, W.A., Maga, E.A., Collette, N.M., Moyer, A., Conrad-Brink, J.S., Taylor, S.J., DePeters, E.J., Oppenheim, S., Rowe, J.D., BonDurant, R.H., Anderson, G.B., Murray, J.D. 2004. Hot topic: using a stearoyl-CoA desaturase transgene to alter milk fatty acid composition. J Dairy Sci 87: 3510-3514.

Richt, J.A., Kasinathan, P., Hamir, A.N., Castilla, J., Sathiyaseelan, T., Vargas, F., Sathiyaseelan, J., Wu, H., Matsushita, H., Koster, J., Kato, S., Ishida, I., Soto, C., Robl, J.M., Kuroiwa, Y. 2007. Production of cattle lacking prion protein. Nat Biotechnol 25(1): 132-138.

Robl, J.M., Wang, Z., Kasinathan, P., Kuroiwa, Y. 2007. Transgenic animal production and animal biotechnology. Theriogenology 67(1): 127-133.

Rogers, C.S., Hao, Y., Rokhlina, T., Samuel, M., Stoltz, D.A., Li, Y., Petroff, E., Vermeer, D.W., Kabel, A.C., Yan, Z., Spate, L., Wax, D., Murphy, C.N., Rieke, A., Whitworth, K., Linville, M.L., Korte, S.W., Schnieke, A.E., Kind, A.J., Ritchie, W.A., Mycock, K., Scott, A.R., Ritchie, M., Wilmut, I., Colman, A., Campbell, K.H. 1997. Human factor

IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. Science 278: 2130-2133.

Rogers, C.S., Hao, Y., Roklina, T., Samuel, M., Stoltz, D.A., Li, Y., Petroff, E., Vermeer, D.W., Kabel, A.C., Yan, Z., Spate, L., Wax, D., Murphy, C.N., Rieke, A., Whitworth, K., Linville, M.L., Korte, S.W., Engelhardt, J.F., Welsh, M.J., Prather, R.S. 2008. Production of CFTR-null and CFTR-deltaF508 heterozygous pigs by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. J Clin Invest 118(4): 1571-1577.

Ross, J.W., Prather, R.S., Whyte, J.J., Zhao, J. 2009a. Cloning and transgenics: progress and new approaches in domestic animals. CAB reviews: perspectives in agriculture, veterinary science. Nutr Nat Resour 4(38): 13.

Ross, J.W., Zhao, J., Walters, E.M., Samuel, M., Narfstrom, K., Jeong, M., DeMarco, M.A., McCall, M.A., Kaplan, H.J., Prather, R.S. 2009b. Somatic cell nuclear transfer to create a miniature swine model of retinitis pigmentosa, April 18-22, New Orleans, LA

Ross, J.W., Whyte, J.J., Zhao, J., Samuel, M., Wells, K.D., Prather, R.S. 2010a. Optimization of square-wave electroporation for transfection of porcine fetal fibroblasts. Transgenic Res 19(4): 611-620.

Ross, P.J., Wang, K., Kocabas, A., Cibelli, J.B. 2010b. Housekeeping gene transcript abundance in bovine fertilized and cloned embryos. Cell Reprogram 12(6): 709-717.

Saeki, K., Matsumoto, K., Kinoshita, M., Suzuki, I., Tasaka, Y., Kano, K., Tagachui, Y., Mikami, K., Hirabayashi, M., Kashiwazaki, N., Hosoi, Y., Murata, N., Iritani, A. 2004. Functional expression of a Delta12 fatty acid desaturase gene from spinach in transgenic pigs. Proc. Natl. Acad. Sci. USA 101: 6361-6366.

Salamone, D., Barañao, L., Santos, C., Bussmann, L., Artuso, J., Werning, C., Prync, A., Carbonetto, C., Dabsys, S., Munar, C., Salaberry, R., Berra, G., Berra, I., Fernández, N., Papouchado, M., Foti, M., Judewicz, N., Mujica, I., Muñoz, L., Alvarez, S.F., González, E., Zimmermann, J., Criscuolo, M., Melo, C. 2006. High level expression of bioactive recombinant human growth hormone in the milk of a cloned transgenic cow. J Biotechnol. 124(2): 469-472.

Santos, F., Zakhartchenko, V., Stojkovic, M., Peters, A., Jenuwein, T., Wolf, E., Reik, W., Dean, W. 2003. Epigenetic marking correlates with developmental potential in cloned bovine preimplantation embryos. Curr Biol 13: 1116-1121.

Schnieke, A.E., Kind, A.J., Ritchie, W.A., Mycock, K., Scott, A.R., Ritchie, M., Wilmut, I., Colman, A., Campbell, K.H. 1997. Human factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. Science 278(5346): 2130-2133.

Shi, L.H., Ai, J.S., Ouyang, Y.C., Huang, J.C., Lei, Z.L., Wang, Q., Yin, S., Han, Z.M., Sun, Q.Y., Chen, D.Y. 2008. Trichostatin A and nuclear reprogramming of cloned rabbit embryos. J Anim Sci 86(5): 1106-1113.

Sun, X., Yan, Z., Yi, Y., Li, Z., Lei, D., Rogers, C.S., Chen, J., Zhang, Y., Welsh, M.J., Leno, G.H., Engelhardt, J.F. 2008. Adeno-associated virus-targeted disruption of the CFTR gene in cloned ferrets. J Clin Invest 118(4): 1578-1583.

Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G., Hochedlinger, K. 2008. Induced pluripotent stem cells generated without viral integration. Science 322(5903): 945-949.

Switzer, W.M., Michler, R.E., Shangmugam, V., Matthews, A., Hussain, A.I., Wright A, Sandstrom, P., Chapman, L.E., Weber, C., Safley, S., Denny, R.R., Navarro, A.,

Evans, V., Norin, A.J., Kwiatkowski, P., Heneine, W. 2001. Lack of cross-species transmission of porcine endogenous retrovirus infection to nonhuman primate recipients of porcine cells, tissues and organs. Transplantation 71: 959-965.

Takahashi, K., Yamanaka, S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126 (4): 663-676.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5): 861-872.

te Riele, H., Maandag, E.R., Berns, A. 1992. Highly efficient gene targeting in embryonic stem cells through homologous recombination with isogenic DNA constructs. Proc Natl Acad Sci USA. 89(11): 5128-5132.

Tsuji, Y., Kato, Y., Tsunoda, Y. 2009. The developmental potential of mouse somatic cell nuclear-transferred oocytes treated with trichostatin A and 5-aza-2'-deoxycytidine. Zygote 17(2): 109-115.

Urnov, F.D., Miller, J.C., Lee, Y.L., Beausejour, C.M., Rock, J.M., Augustus, S., Jamieson, A.C., Porteus, M.H., Gregory, P.D., Holmes, M.C. 2005. Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature 435(7042): 646-651.

Urnov, F.D., Rebar, E.J., Holmes, M.C., Zhang, H.S., Gregory, P.D. 2010. Genome editing with engineered zinc finger nucleases. Nat Rev Genet 11(9): 636-646.

van Berkel, P.H., Welling, M.M., Geerts, M., van Veen, H.A., Ravensbergen, B., Salaheddine, M., Pauwels, E.K., Pieper, F., Nuijens, J.H., Nibbering, P.H. 2002. Large scale production of recombinant human lactoferrin in the milk of transgenic cows. Nat Biotechnol 20(5): 484-487.

van Poll, D., Nahmias, Y., Soto-Gutierrez, A., Ghasemi, M., Yagi, H., Kobayashi, N., Yarmush, M.L., Hertl, M. 2010. Human immune reactivity against liver sinusoidal endothelial cells from GalTα(1,3)GalT-deficient pigs. Cell Transplant 19(6): 783-789.

Vasquez, K.M., Marburger, K., Intody, Z., Wilson, J.H. 2001. Manipulating the mammalian genome by homologous recombination. Proc Natl Acad Sci USA. 98(15): 8403-8410.

Voigt, B., Serikawa, T. 2009. Pluripotent stem cells and other technologies will eventually open the door for straightforward gene targeting in the rat. Dis Model Mech 2(7-8): 341-343.

Wall, R.J., Powell, A., Paape, M.J., Kerr, D.E., Bannermann, D.D., Pursel, V.G., Wells, K.D., Talbot, N., Hawk, H. 2005. Genetically enhanced cows resist intramammary *Staphylococcus aureus* infection. Nat Biotechnol 23: 445-451.

Wang, J., Yang, P., Tang, B., Sun, X., Zhang, R., Guo, C., Gong, G., Liu, Y., Li, R., Zhang, L., Dai, Y., Li, N. 2008. Expression and characterization of bioactive recombinant human α-lactalbumin in the milk of transgenic cloned cows. J Dairy Sci 91(12): 4466-4476.

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., Rossi, D.J. 2010. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7(5): 618-630.

Watanabe, S., Iwamoto, M., Suzuki, S., Fuchimoto, D., Honma, D., Nagai, T., Hashimoto, M., Yazaki, S., Sato, M., Onishi, A. 2005. A novel method for the production of

transgenic cloned pigs: electroporation-mediated gene transfer to non-cultured cells and subsequent selection with puromycin. Biol Reprod 72(2): 309-315.

Watanabe, M., Umeyama, K, Matsunari, H., Takayanagi, S., Haruyama, E., Nakano, K., Fujiwara, T., Ikezawa, Y., Nakauchi, H., Nagashima, H. 2010. Knockout of exogenous EGFP gene in porcine somatic cells using zinc-finger nucleases. Bio chem Biophys Res Commun 402(1): 14-18.

Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., Bernstein, B.E., Jaenisch, R. 2007. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 448(7151): 318-324.

Wheeler, M.B., Bleck, G.T., Donovan, S.M. 2001. Transgenic alteration of sow milk to improve piglet growth and health. Reproduction 58 (Suppl.): 313-324.

Whitelaw, C.B., Lillico, S.G., King, T. 2008. Production of transgenic farm animals by viral vector-mediated gene transfer. Reprod Domest Anim 43 Suppl 2: 355-358.

Whitworth, K.M., Prather, R.S. 2010. Somatic cell nuclear transfer efficiency: how can it be improved through nuclear remodeling and reprogramming? Mol Reprod Dev 77(12): 1001-1015.

Whyte, J.J., Zhao, J., Wells, K.D., Samuel, M.S., Whitworth, K.M., Walters, E.M., Laughlin, M.H., Prather, R.S. 2011. Gene targeting with zinc finger nucleases to produce cloned eGFP knockout pigs. Mol Reprod Dev 78(1): 2.

Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J., Campbell, K.H. 1997. Viable offspring derived from fetal and adult mammalian cells. Nature 385(6619): 810-813.

Wrenzycki, C., Wells, D., Herrmann, D., Miller, A., Oliver, J., Tervit, R., Niemann, H. 2001. Nuclear transfer protocol affects messenger RNA expression patterns in cloned bovine blastocysts. Biol Reprod 65(1): 309-317.

Wrenzycki, C., Herrmann, D., Gebert, C., Carnwath, J.W., Niemann, H. 2006. Gene expression and methylation patterns in cloned embryos. Methods Mol Biol 348: 285-304.

Wright, G., Carver, A., Cottom, D., Reeves, D., Scott, A., Simons, P., Wilmut, I., Garner, I., Colman, A. 1991. High level expression of active human alpha-1-antitrypsin in the milk of transgenic sheep. Biotechnology (N Y) 9(9): 830-834.

Wu, S., Ying, G.X., Wu, Q., Capecchi, M.R. 2008. A protocol for constructing gene targeting vectors: generating knockout mice for the cadherin family and beyond. Nature Protocols 3: 1056-1076.

Wu, Z., Chen, J., Ren, J., Bao, L., Liao, J., Cui, C., Rao, L., Li, H., Gu, Y., Dai, H., Zhu, H., Teng, X., Cheng, L., Xiao, L. 2009. Generation of pig induced pluripotent stem cells with a drug inducible system. J Mol Cell Biol 1(1): 46-54.

Yamada, K., Yazawa, K., Shimizu, A., Iwanaga, T., Hisashi, Y., Nuhn, M., O'Malley, P., Nobori, S., Vagefi, P.A., Patience, C., Fishman, J., Cooper, D.K., Hawley, R.J., Greenstein, J., Schuurman, H.J., Awwad, M., Sykes, M., Sachs, D.H. 2005. Marked prolongation of porcine renal xenograft survival in baboons through the use of α1,3-galactosyltransferase gene-knockout donors and the cotransplantation of vascularized thymic tissue. Nat Med 11: 32-34.

Yáñez, R.J., Porter, A.C. 1999. Influence of DNA delivery method on gene targeting frequencies in human cells. Somat Cell Mol Genet 25(1): 27-31.

Yang, Y., Seed, B. 2003. Site-specific gene targeting in mouse embryonic stem cells with intact bacterial artificial chromosomes. Nat Biotechnol 21(4): 447-451.

Yang, P., Wang, J., Gong, G., Sun, X., Zhang, R., Du, Z., Liu, Y., Li, R., Ding, F., Tang, B., Dai, Y., Li, N. 2008. Cattle mammary bioreactor generated by a novel procedure of transgenic cloning for large-scale production of functional human lactoferrin. PloS One 3(10): e3453.

Yang, D., Wang, C.E., Zhao, B., Li, W., Ouyang, Z., Liu, Z., Yang, H., Fan, P., O'Neill, A., Gu, W., Yi, H., Li, S., Lai, L., Li, X.J. 2010. Expression of Huntington's disease protein results in apoptotic neurons in the brains of cloned transgenic pigs. Hum Mol Genet 19(20): 3983-3994.

Yu, G., Chen, J., Yu, H., Liu, S., Chen, J., Xu, X., Sha, H., Zhang, X., Wu, G., Xu, S., Cheng, G. 2006. Functional disruption of the prion protein gene in cloned goats. J Gen Virol 87: 1019-1027.

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, I.I., Thomson, J.A. 2007. Induced pluripotent stem cell lines derived from human somatic cells. Science 318(5858): 1917-1920.

Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, I.I., Thomson, J.A. 2009. Human induced pluripotent stem cells free of vector and transgene sequences. Science 324(5928): 797-801.

Zaidi, A., Schmoeckel, M., Bhatti, F., Waterworth, P., Tolan, M., Cozzi, E., Chavez, G., Langford, G., Thiru, S., Wallwork, J., White, D., Friend, P. 1998. Life-supporting pig-to primate renal xenotransplantation using genetically modified donors. Transplantation 65: 1584.

Zakhartchenko, V., Mueller, S., Alberio, R., Schernthaner, W., Stojkovic, M., Wenigerkind, H., Wanke, R., Lassnig, C., Mueller, M., Wolf, E., Brem, G. 2001. Nuclear transfer in cattle with non-transfected and transfected fetal or cloned transgenic fetal and postnatal fibroblasts. Mol Reprod Dev 60: 362-369.

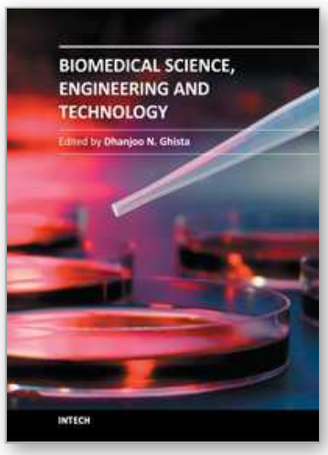
Zhang, F., Cong, L., Lodato, S., Kosuri, S., Church, G.M., Arlotta, P. 2011. Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. Nat Biotechnol 29(2): 149-153.

Zhao, J., Ross, J.W., Hao, Y., Spate, L.D., Walters, E.M., Samuel, M.S., Rieke, A., Murphy, C.N., Prather, R.S. 2009a. Significant improvement in cloning efficiency of an inbred miniature pig by histone deacetylase inhibitor treatment after somatic cell nuclear transfer. Biol Reprod 81(3): 525-530.

Zhao, J., Hao, Y., Ross, J.W., Spate, L.D., Walters, E.M., Samuel, M.S., Rieke, A., Murphy, C.N., Prather, R.S. 2010. Histone deacetylase inhibitors improve in vitro and in vivo developmental competence of somatic cell nuclear transfer porcine embryos. Cell Reprogram 12(1): 75-83.

Zhao, X. Y., Li, W., Lv, Z., Liu, L., Tong, M., Hai, T., Hao, J., Guo, C.L., Ma, Q.W., Wang, L., Zeng, F., Zhou, Q. 2009b. iPS cells produce viable mice through tetraploid complementation. Nature 461(7260): 86-90.

Zhu, C., Li, B., Yu, G., Chen, J., Yu, H., Chen, J., Xu, X., Wu, Y., Zhang, A., Cheng, G. 2009. Production of Prnp -/- goats by gene targeting in adult fibroblasts. Transgenic Res 18(2):163-171.



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