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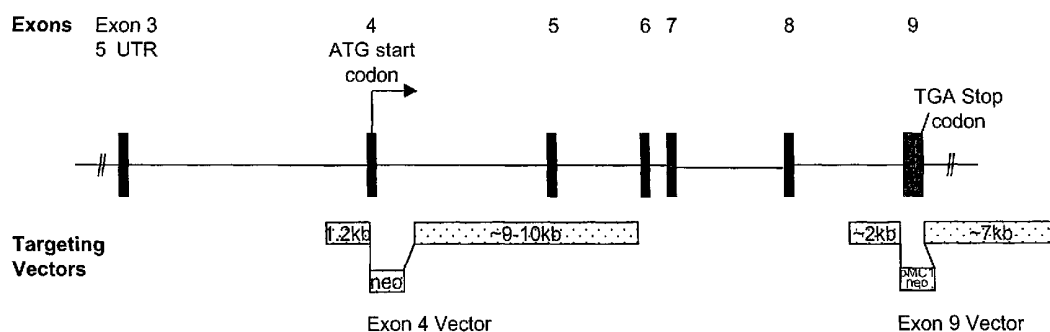
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(54) Title: ANIMAL TISSUE FOR XENOTRANSPLANTATION



(57) **Abstract:** This invention provides immunologically compatible animal tissue, suitable for xenotransplantation into human patients. Sequence of the cDNA for the sheep $\alpha(1,3)$ galactosyltransferase has been determined, along with information about the flanking untranscribed regions and introns of the $\alpha(1,3)$ GT gene. This in turn has led to the design and implementation of targeting vectors capable of inactivating the $\alpha(1,3)$ GT gene by deleting or interrupting the protein coding region. Successfully targeted fibroblasts are used as nuclear donors for the cloning of animals in which the $\alpha(1,3)$ GT gene is inactivated. The Gal $\alpha(1,3)$ Gal determinant does not form on endothelial and other cells of animals having homozygously inactivated $\alpha(1,3)$ GT. The tissues can be transplanted into human patients, without being subject to hyperacute rejection that would otherwise occur due to naturally occurring antibodies to the Gal $\alpha(1,3)$ Gal determinant present in human serum. Because of the limited availability of human organs for transplantation, immunologically compatible animal tissue is an important alternative for human organs that are in such short supply for transplantation therapy.

ANIMAL TISSUE FOR XENOTRANSPLANTATIONTECHNICAL FIELD

5 This invention relates generally to the fields of carbohydrate chemistry and animals engineered with a genetic knockout. More particularly, it relates to non-human mammals that are engineered to be deficient in the enzyme $\alpha(1,3)$ galactosyltransferase, and consequently do not express the Gal $\alpha(1,3)$ Gal xenoantigen.

REFERENCE TO RELATED APPLICATIONS

10 This application claims the priority benefit of the following pending U.S. patent applications: USSN 60/204,148, filed May 15, 2000; and USSN 09/593,316, filed June 13, 2000. For the purpose of prosecution in the U.S., the priority applications are hereby incorporated herein by reference in their entirety.

BACKGROUND

15 The acute shortage of human organs for transplantation provides a compelling need for the development of new sources of suitable tissue. An idea of considerable promise is to transplant patents with organs from non-human animals. The main challenge to overcome is rendering foreign tissue immunologically compatible with the patient being treated.

20 Tissue from most mammalian species would undergo hyperacute rejection when transplanted into humans. This is because human plasma contains natural antibodies against carbohydrate determinants of the animal tissue, thought to originate through prior immune stimulation by dietary antigen or mucosal microflora. Since the antibodies are pre-formed, rejection occurs within days of the transplant.

25 The main target for the natural antibodies mediating rejection is cell-surface oligosaccharides expressing the determinant Gal $\alpha(1,3)$ Gal (reviewed by Joziassse et al., Biochim. Biophys. Acta 1455:403, 1999). Humans, apes and Old World monkeys differ from other mammals in that they lack α -galactosyl epitopes in complex oligosaccharides. Other mammals express the Gal $\alpha(1,3)$ Gal epitope prominently on the surface of nucleated cells, including hepatic cells, renal cells, and vascular endothelium — which is especially problematic for xenotransplantation of whole organs.

30 The Gal $\alpha(1,3)$ Gal epitope is made by a specific enzyme, $\alpha(1,3)$ galactosyltransferase, abbreviated in this disclosure as $\alpha 1,3$ GT. The transferase uses UDP-galactose as a source of galactose, which it transfers specifically to an acceptor oligosaccharide, usually Gal $\beta(1,4)$ GlcNAc (N-acetyl lactosamine). In mammals that don't express the Gal $\alpha(1,3)$ Gal product, the $\alpha 1,3$ GT locus is inactivated (Gaillili et al., Proc. Natl. Acad. Sci. USA 15:7401, 1991). There are frameshift and nonsense mutations within the locus, turning it into a non-functional, processed pseudogene (Laarsen et al., J. Biol. Chem. 265:7055, 1990; Joziassse et al., J. Biol. Chem. 266:6991, 1991).

35 In humans, N-acetyl lactosamine acceptor oligosaccharides are processed differently. The enzyme $\alpha(1,2)$ fucosyltransferase builds the N-acetyl lactosamine into Fuca $\alpha(1,2)$ Gal $\beta(1,4)$ GlcNAc, which is blood group H substance. This in turn serves as an acceptor substance for blood group A GlcNAc-transferase, or blood group B Gal-transferase, forming A-substance or B-substance, respectively, depending on the blood type of

the individual. Naturally occurring antibodies circulating in the blood are reactive against the alternative carbohydrate determinants that are not self-antigens.

Larsen et al. (Proc. Natl. Acad. Sci. USA 86:8227, 1989) isolated and characterized a cDNA encoding murine α 1,3GT. Joziase et al. (J. Biol. Chem. 267:5534, 1992) detected four distinct mRNA transcripts, which predict four different isoforms of the α 1,3GT. The full-length mouse mRNA (including 5' untranslated mRNA) was reported to span at least 35-kb of genomic DNA, distributed over nine exons ranging from 36 base pairs to ~2600 base pairs in length. Numbering in the 5' to 3' direction, the coding region is distributed over Exons 4 to 9. The four transcripts are formed by alternative splicing of the pre-mRNA.

Joziase et al. (J. Biol. Chem. 264:14290, 1989) isolated and characterized a cDNA encoding bovine cDNA. The coding sequence was predicted to be a membrane-bound protein with a large glycosylated COOH-terminal domain, a transmembrane domain, and a short NH₂ terminal domain.

The porcine α 1,3GT cDNA sequence has been reported from several different laboratories: Strahan et al. (Immunogenetics 41:101, 1995); U.S. Patent 5,821,117; U.S. Patent 5,849,991; and International Patent Application WO 95/28412. The genomic organization of porcine α 1,3GT was reported by Katayama et al. (Glycoconjugate J. 15:83, 1998). Again, the coding region spans six exons, conserving the arrangement present in the mouse genome, and extending over nearly 24-kb.

It has been reported that about 95% of the naturally occurring xenospecific antibody in humans recognize the Gal α (1,3)Gal epitope (McKensie et al., Transpl. Immunol. 2:81, 1994). Antibody in human serum binds specifically to pig endothelial cells in a manner that is inhibitable by Gal α (1,3)Gal, or by Gal α (1,6)Glc (melibiose). New age monkeys have the same naturally occurring antibody, and demonstrate hyperacute rejection of pig organ xenotransplants. The rejection reaction can be obviated in experimental animals by infusing the recipient with the free carbohydrate (Ye et al., Transplantation 58:330, 1994), or by adsorbing antibody from the circulation on a column of Gal α (1,3)Gal or melibiose (Cooper et al., Xenotransplantation 3:102, 1996).

It has been suggested that xenotransplants of pig tissue could provide a source of various tissue components — heart valves, pancreatic islet cells, and perhaps large organs such as livers and kidneys (Cowley, Newsweek, Jan 1/2000). If xenotransplants from non-primates into humans is ever to become viable, then techniques need to be developed to prevent Gal α (1,3)Gal mediated rejection. Possible genetic manipulation strategies are reviewed by Gustafsson et al. (Immunol. Rev. 141:59, 1994), Sandrin et al. (Frontiers Biosci. 2:e1-11, 1997), and Lavitrano et al. (Forum Genova 9:74, 1999).

One approach is to prevent the formation of Gal α (1,3)Gal by providing another transferase that competes with α 1,3GT for the N-acetyl lactosamine acceptor. International Patent Application WO 97/12035 (Nextran-Baxter) relates to transgenic animals that express at least one enzyme that masks or reduces the level of the xenoreactive antigens by competing with α 1,3GT. The enzymes proposed are α (1,2)fucosyltransferase (that makes H antigen in humans), α (2,6)sialyltransferase, and β (1,3)N-acetylglucosaminyltransferase. It is thought that once N-acetyl lactosamine has been converted by one of these transferases, it can no longer act as an acceptor for α 1,3GT. The xenotransplantation cells of Application WO 97/12035 have at least one enzyme that reduces Gal α (1,3)Gal expression, and also express a complement inhibitor such as CD59, decay accelerating factor (DAF), or membrane cofactor protein (MCP). Expression of human CD59 in transgenic pig organs enhances organ survival in an ex vivo xenogeneic perfusion model (Kroshus et al., Transplantation 61:1513, 1996).

Another approach is to disassemble Gal α (1,3)Gal after it is formed. International Patent Application WO 95/33828 (Diacrin) suggests modifying cells for xenogeneic transplants by treating tissue with an α -glycosidase. Osman et al. (Proc. Natl. Acad. Sci. USA 23:4677, 1997) reported that combined transgenic

expression of both α -glycosidase and $\alpha(1,2)$ fucosyltransferase leads to optimal reduction in Gal $\alpha(1,3)$ Gal epitope. Splenocytes from mice overexpressing human α -glycosidase showed only a 15-25% reduction in binding of natural human anti-Gal $\alpha(1,3)$ Gal antibodies. Mice overexpressing human $\alpha(1,2)$ fucosyltransferase as a transgene showed a reduction of Gal $\alpha(1,3)$ Gal epitopes by ~90%. Doubly transfected COS cells
5 expressing both the glycosidase and the transferase showed negligible cell surface staining with anti-Gal $\alpha(1,3)$ Gal, and were not susceptible to lysis by human serum containing antibody and complement.

A further alternative is to prevent Gal $\alpha(1,3)$ Gal expression in the first place. Strahan et al. (Xenotransplantation 2:143, 1995) reported the use of antisense oligonucleotides for inhibiting pig $\alpha 1,3$ GT, leading to a partial reduction in expression of the major target for human natural antibodies on pig vascular
10 endothelial cells. Hayashi et al. (Transplant Proc. 29:2213, 1997) reported adenovirus-mediated gene transfer of antisense ribozyme for $\alpha 1,3$ GT and $\alpha(1,2)$ fucosyltransferase genes in xenotransplantation.

U.S. Patent 5,849,991 (Bresatch) describes DNA constructs based on the mouse $\alpha 1,3$ GT sequence. They are designed to disrupt expression of functional $\alpha 1,3$ GT by undergoing homologous recombination across Exon 4, 7, 8, or 9. The constructs contain a selectable marker such as *neo^R*, *hyg^R* or thymidine kinase.
15 It is proposed that such constructs be introduced into mouse embryonic stem (ES) cells, and recovering cells in which at least one $\alpha 1,3$ GT gene is inactivated. Experiments are reported which produced mice that are homozygous for inactivated $\alpha 1,3$ GT, resulting in lack of expression of Gal $\alpha(1,3)$ Gal epitope, as determined by specific antibody.

U.S. Patent 5,821,117 (Austin Research Inst.) report cDNA sequence data for porcine $\alpha 1,3$ GT. This
20 was used to probe a pig genomic DNA library, and two lambda phage clones were obtained that contain different regions of the porcine transferase gene. International Patent Application WO 95/28412 (Biotransplant) also reports cDNA sequence data for porcine $\alpha 1,3$ GT. It is proposed that genomic DNA fragments be isolated from an isogenic DNA library, and used to develop a gene-targeting cassette including a positive or negative selectable marker.

International Patent Application WO 99/21415 (Stem Cell Sciences, Biotransplant) reports construction of a DNA library from miniature swine. A vector is obtained comprising a *pgk-neo* cassette, and fragments of the $\alpha 1,3$ GT gene. This is used for homologous recombination to eliminate $\alpha 1,3$ GT activity in porcine embryonic fibroblasts. Costa et al., Alexion Pharmaceuticals (Xenotransplantation 6:6, 1999) report experiments with transgenic mice expressing the human complement inhibitor CD59. In $\alpha 1,3$ GT knockout
25 mice, the CD59 gene helped prevent human serum-mediated cytolysis. It had a similar effect in mice expressing $\alpha(1,2)$ fucosyltransferase. Combination of all three modifications provided no additional protective effect.

There have been no reports of the use of $\alpha 1,3$ GT inactivated tissue suitable for xenotransplantation into humans. In view of the paucity of available organs for human transplantation, there is a pressing need to
30 develop further options.

SUMMARY

Immunologically compatible animal tissue for xenotransplantation is described in this disclosure.
40 Sequence data for the sheep $\alpha(1,3)$ galactosyltransferase ($\alpha 1,3$ GT) gene is provided, which enables construction of targeting vectors for inactivating the $\alpha 1,3$ GT gene. Successfully targeted cells can be used as nuclear donors for obtaining animals lacking the Gal $\alpha(1,3)$ Gal determinant. The tissues can be transplanted into human patients, without being subject to hyperacute rejection by antibodies to the Gal $\alpha(1,3)$ Gal determinant normally present in human serum.

One embodiment of the invention is animal tissue devoid of Gal α (1,3)Gal determinants, such as can be detected by antibody binding according to standard techniques. Exemplary is tissue from ruminants of the subfamily caprinae, especially sheep. Tissue of this nature suitable for transplantation to humans includes but is not limited to lung tissue, kidney tissue, liver tissue, cardiac tissue, pancreatic tissue, and ocular tissue.

5 This invention also embodies a polynucleotide construct effective for inactivating an α 1,3GT gene. Exemplary constructs comprise at least two polynucleotide sequences from an ovine α 1,3GT gene in a non-natural arrangement, for inactivating the α 1,3GT gene by homologous recombination, leading to deletion or interruption of the α 1,3GT encoding sequence, or replacement with an alternative sequence. Optionally, the vector comprises an intron sequence of at least 30 consecutive nucleotides homologous to any of recombinant
10 phage plasmids illustrated below, or deposited in support of this disclosure. In certain applications, α 1,3GT gene is inactivated by contacting the cell with the polynucleotide construct of this invention under conditions suitable for homologous recombination of the construct into the genome of the cell.

Another embodiment of the invention is a cell that expresses glycosyl transferase enzymes but does not detectably express α (1,3)galactosyltransferase (α 1,3GT). The cell may be heterozygous or homozygous
15 for inactivation of an α 1,3GT gene.

Such cells and tissues can be obtained from a whole animal such as a sheep that is homozygous for inactivation of an α 1,3GT gene. Such animals can be produced by engrafting into a female of the same species embryo having at least one inactivated α 1,3GT allele. Embryos can be generated, for example, by transfer of a nucleus from a donor cell with an inactivated α 1,3GT gene, to an enucleated recipient cell. If the
20 birthed animal is heterozygous for α 1,3GT inactivation, homozygous inactivation can be achieved by selective breeding, or by a second round of gene targeting to inactivate the second allele.

A further embodiment of this invention is an isolated polynucleotide comprising a sequence of 30 or more consecutive nucleotides contained in (or capable of hybridization with) SEQ. ID NO:1 or 14 to 25, or which is contained in phage deposited in support of this application, but which does not appear in other known
25 sequences, such as SEQ. ID NOs: 3, 5, 7, 9, 11, and 13. Included are polynucleotide constructs effective for inactivating a α (1,3)galactosyltransferase (α 1,3GT) gene. Such polynucleotides can also be used in an assay for determining α 1,3GT expression, in which the polynucleotide is combined with analyte mRNA or cDNA, and the formation of a duplex between the two is correlated with expression of α 1,3GT by the cell.

Another embodiment of the invention is an isolated polypeptide comprising a sequence of 10 or more
30 consecutive amino acids contained in or homologous to SEQ. ID NO:2, or encoded in a polynucleotide of this invention, which is not fully contained in any other known sequence, such as SEQ. ID NOs: 4, 6, 8, 10, and 12. Such polypeptides may have glycosyltransferase activity, or be immunologically cross-reactive with α 1,3GT. In certain contexts, the polypeptides are used to prepare antibody. In other contexts, the polypeptides are used to prepare a Gal α (1,3)Gal determinant by combining with a galactose acceptor saccharide in the presence of
35 UDP-galactose.

Further embodiments of the invention are polyclonal and monoclonal antibodies (including immunoglobulin derivatives and equivalents) that bind specifically to a polypeptide of this invention, but not with other polypeptides. Some such antibodies react with α 1,3GT of other mammalian species, while others are specific for ovine α 1,3GT. They can be used, for example, in an assay to determine α 1,3GT in a sample,
40 in which the sample is combined with the antibody, and any complex formed is correlated with the presence of α 1,3GT.

Cells and tissues of this invention can be used to prepare tissue devoid of antibody-detectable Gal α (1,3)Gal determinants for treatment of the human body by surgery or therapy. This invention provides a

method of xenotransplantation, comprising transplanting tissue devoid of antibody-detectable Gal α (1,3)Gal determinants into a mammal that may have circulating antibody against Gal α (1,3)Gal determinants, such as a human.

These and other embodiments of the invention will be apparent from the description that follows.

5

DRAWINGS

Figure 1 is a chart providing certain sequence data of this invention, which is α (1,3)Galactosyltransferase cDNA in the sheep (α 1,3GT) (SEQ. ID NO:1). The predicted protein sequence is shown below (SEQ. ID NO:2). Numbers 4 to 9 indicate the projected 5' boundaries of Exons 4 to 9 in the genomic sequence.

10

Figure 2 is a chart comparing the cDNA nucleotide sequence of sheep α 1,3GT (SEQ. ID NO:1) with the bovine homolog (SEQ. ID NO:3). Identical residues are marked with an asterisk (*). The putative start codon is indicated with an arrow (\downarrow).

15

Figure 3 is a chart comparing the protein sequence of sheep α 1,3GT (SEQ. ID NO:2) with the bovine homolog (SEQ. ID NO:4). Identical residues are marked with an asterisk (*).

Figure 4 is a half-tone reproduction, showing Southern blot analysis of sheep genomic DNA. Probes for two different α 1,3GT exons yield one band, demonstrating that there is one copy of α 1,3GT in the sheep genome.

20

Figure 5 is a half-tone reproduction, showing Northern blot analysis of mRNA from sheep fetal fibroblasts. There is a single expressed transcription product for α 1,3GT ~3-kb in size.

Figure 6 is a restriction map for the sheep α 1,3GT gene. Phage clones were obtained for the regions shown, and used to determine intron sequences.

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Figure 7 is a map showing sequenced intron regions of α 1,3GT. The areas numbered 1 to 9 in the figure correspond to SEQ. ID NOs:15, 16, 19, 20, 21, 22, 23, 24, and 25, respectively.

Figure 8 is a map showing the design of two targeting vectors for inactivating the α 1,3GT gene by eliminating one of the exons through homologous recombination. Each targeting vector comprises a selectable marker (*neo*), flanked on one side by an intron sequence of 1-2-kb, and on the other side by an intron sequence of 7-10-kb. A number of vectors have been obtained, including some that target Exon 4, Exon 8, and Exon 9.

30

Figures 9 to 11 are drawings that compare the details of the targeting vectors p0054, p0079, and p0063, respectively, with the region of the α 1,3GT gene sequence targeted for homologous recombination (shown below). These vectors are designed to replace the coding region in Exon 4 with the selectable marker *neo* or *pac*.

35

Figure 12 is a drawing showing how vectors p0054 and p0079 can be truncated for use in an adeno-associated virus (AAV) vector.

Figure 13 is a drawing that compares targeting vector p0078 (above), with the targeted Exon 8 region of α 1,3GT (below), to be substituted with selectable marker *neo*.

40

Figures 14 and 15 are drawings that compare targeting vector p0047 and p0046, respectively, with the targeted Exon 9 region of α 1,3GT (shown below), to be substituted with selectable marker *neo*.

Figure 16 is a half-tone reproduction, showing PCR analysis of sheep fetal fibroblasts targeted with the p0054 vector. Using two primers for intron sequences that flank Exon 4 (upper panel), the expected product is 2.8-kb for native α 1,3GT, and 2.2-kb after homologous recombination. Using a primer for the

selectable marker (lower panel), amplification product is expected only after recombination. The results show that one of the samples is from a fibroblast that has successfully been targeted — replacing Exon 4 with the selectable marker. Since Exon 4 contains the translation initiation site, this would inactivate the $\alpha 1,3\text{GT}$ gene.

Figure 17 is a half-tone reproduction, showing PCR analysis of umbilical chord taken from a sheep fetus, generated by transfer of a $\alpha 1,3\text{GT}$ targeted nucleus to a quiescent oocyte, which was implanted into a surrogate female at estrus. Two bands were observed: 2.8 kb, corresponding to the wild type $\alpha 1,3\text{GT}$ gene, and 2.2 kb, appropriate for a targeted $\alpha 1,3\text{GT}$ gene. These results are consistent with inactivation of the $\alpha 1,3\text{GT}$ gene on one haplotype.

DETAILED DESCRIPTION

This invention provides new sequence data for the $\alpha(1,3)\text{galactosyltransferase}$ ($\alpha 1,3\text{GT}$) gene in the Black Welsh Mountain sheep. The data includes the cDNA sequence, encompassing the entire $\alpha 1,3\text{GT}$ encoding region, and certain portions of the genome sequence, from upstream of the coding region, from introns, and from downstream of the coding region.

The discovery of this sequence and nucleic acids containing them have made it possible to construct targeting vectors designed to disrupt expression of a $\alpha 1,3\text{GT}$ gene in eukaryotic cells, particularly sheep cells. Exemplary vectors targeting Exons 4 and 9 of the $\alpha 1,3\text{GT}$ gene are shown in **Figure 8**. These exemplary vectors have a knockout sequence flanked by two regions homologous to the genomic sequence. After recombination, the knockout sequence replaces a portion of the gene so as to disrupt transcription of a functioning $\alpha 1,3\text{GT}$ — by elimination of Exon 4 or Exon 9, which encompass the translation start and stop codons, respectively. The knockout sequences in these exemplars comprise a selectable marker that facilitates the separation of cells that have been genetically altered after targeting with the vector.

For certain aspects of this invention, mammalian cells that have had an $\alpha 1,3\text{GT}$ gene inactivated artificially in at least one haplotype are used to produce animals that do not express the $\text{Gal}\alpha(1,3)\text{Gal}$ epitope. Nuclear transfer permits a nucleus having an inactivated $\alpha 1,3\text{GT}$ gene to be transferred from a donor cell or cell line to an embryonic cell or oocyte. An embryo is then formed, engrafted into the uterus of a surrogate host, and used to birth a live neonate. Where the $\alpha 1,3\text{GT}$ gene is homozygously inactivated, the animal or its progeny are already able to supply cells and tissue lacking the $\text{Gal}\alpha(1,3)\text{Gal}$ epitope. Where the $\alpha 1,3\text{GT}$ gene is inactivated in only one haplotype, homozygous inactivation can be achieved by standard cross-breeding techniques. Tissue lacking the $\alpha 1,3\text{GT}$ epitope can then be used for transplantation into humans without risk of antigenicity for the natural antibody that is normally the linchpin for hyperacute rejection of xenografts.

Definitions

For purposes of this disclosure, the term $\text{Gal}\alpha(1,3)\text{Gal}$ (abbreviated GAL) refers to an oligosaccharide determinant present on endothelial cells and other cells of most non-primate mammals, for which humans have a naturally occurring antibody. The usual structure is $\text{Gal}\alpha(1,3)\text{Gal}\beta(1,4)\text{GlcNAc}$, although other forms of $\text{Gal}\alpha(1,3)\text{Gal}$ specifically detectable by the naturally occurring anti $\text{Gal}\alpha(1,3)\text{Gal}$ in human serum of B blood type are included. $\text{Gal}\alpha(1,3)\text{Gal}$ is distinct from the $\text{Gal}\alpha(1,3)[\text{Fuc}\alpha(1,2)]\text{Gal}\beta(1,4)\text{GlcNAc}$ determinant characteristic of the human B blood type antigen.

An “antibody detectable” determinant refers to a determinant that is present in an amount and is sufficiently accessible so that it can be detected by an antibody specific for the determinant in an appropriate

immunoassay — such as an agglutination reaction, optionally developed with an antiglobulin reagent, or by immunohistochemistry.

The term “ $\alpha(1,3)$ galactosyltransferase” and the abbreviation “ $\alpha 1,3GT$ ” refer to the enzyme present in non-primate mammals that catalyzes the formation of the $Gal\alpha(1,3)Gal$ determinant by attaching Gal in the $\alpha(1,3)$ position to the $Gal\beta(1,4)GlcNAc$ acceptor. $\alpha 1,3GT$ has the Enzyme Commission designation EC 2.4.1.124. $\alpha 1,3GT$ is not naturally expressed in humans, and the term does not include the galactosyltransferase that forms the human B blood group antigen.

An “acceptor” substance for $\alpha 1,3GT$ is a carbohydrate structure that can act as a substrate and become galactosylated by a transferase enzyme. Acceptors for $\alpha 1,3GT$ include both $Gal\beta(1,3)GlcNAc$ and $Gal\beta(1,4)GlcNAc$ (Basu et al., J. Biol. Chem. 248:1700, 1973; Blake et al., J. Biol. Chem. 256:5387, 1981).

A transferase is said to be “detectably expressed” by a cell at the mRNA level when mRNA encoding the transferase can be measured in the cell by some suitable technique, such as Northern analysis or PCR-reverse transcriptase. It may also be expressed at the protein level, as detected by a specific antibody or demonstration of the characteristic enzymatic activity. Scientists skilled in the art will recognize that some cells (such as mature red blood cells) do not express any glycosyltransferases, even though they display certain oligosaccharide determinants. Inhibition of $\alpha 1,3GT$ expression is only meaningful in cells capable of expressing other glycosyltransferase enzymes.

A gene is said to be “inactivated” when it is rendered incapable of transcribing a functional protein. For example, an inactivated gene may be missing necessary transcription or translation control elements, it may be lacking an essential part of the protein encoding region, or the encoding region may be placed out of phase. In another example, the gene may be interrupted by an inserted sequence, or mutated in such a way as to interfere with transcription or translation of the gene product. In a third example, the inactivated gene may produce a translation product that has been altered in such a way that it lacks important enzymatic activity of the native gene product.

The term “tissue” refers to a heterogeneous collection of cells responsible for maintaining one or more physiological functions. Of interest for certain embodiments of this invention are organs suitable for transplantation, such as a whole kidney; however, the term also includes organ fragments and other embodiments, such as a piece of connective tissue, or a collection of cells in a medical support device.

The term “embryo” as it is used in this disclosure refers to an organism developing in the uterus of a species of interest at any time after fertilization or intrauterine transfer, not limited to a particular developmental period. The terms “engrafting” or “transplanting”, in reference to embryo manipulation, refer to any process known in the art for artificially introducing one or more embryos into the uterus of a female animal.

An “individual” or “subject” refers to any vertebrate animal, usually a member of a mammalian species, including but not limited to domestic animals, non-human primates, and humans.

It is understood that a clinical or biological “sample” encompasses a variety of sample types obtained from a subject and useful in an in vitro procedure, such as a diagnostic test. The definition encompasses solid tissue samples obtained during surgery or autopsy, and liquid samples such as blood, spinal fluid, and fluid obtained by amniocentesis, and various subfractions, enrichments, or solubilized extracts derived from such collections.

A “host cell” denotes a prokaryotic or eukaryotic cell has been genetically altered, or is capable of being genetically altered by administration of an exogenous polynucleotide, such as a bacterial plasmid or recombinant vector. When referring to genetically altered cells, the term refers both to the originally altered cell and to the progeny thereof.

A cell is said to be "genetically altered" when it contains an artificially introduced polynucleotide, or is the progeny of a cell so altered that has inherited an introduced polynucleotide. The polynucleotide may contain a sequence that is heterologous to the cell, it may contain native sequences in an artificial arrangement (e.g., an encoding region linked to a different promoter), or it may provide additional copies of a native encoding sequence. The polynucleotide can be introduced by transfection using electroporation or liposome-mediated transfer, homologous recombination, transduction using a viral vector, any combination thereof, or any other technique known in the art. The polynucleotide will often comprise a transcribable sequence encoding a protein of interest, which enables the cell to express the protein at an elevated level. In the context of the present application, a "genetically altered cell" has a genetic alteration that is inheritable by progeny of the cell. For example, an embryo having genetically altered DNA, if carried to term, will give rise to a neonate that has cells containing the genetic alteration.

The terms "polynucleotide" and "oligonucleotide" are used interchangeably to refer to a polymeric form of nucleotides of any length. Included are genes and gene fragments, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA and RNA, nucleic acid probes, and primers. Also included are nucleotide analogs, including but not limited to thiol-derivatized nucleosides (U.S. Patent 5,578,718), oligonucleotides with modified backbones (U.S. Patent Nos. 5,541,307 and 5,378,825), and peptide nucleic acids (U.S. Patent No. 5,786,461). The term polynucleotide, as used in this disclosure, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of the invention that is a polynucleotide encompasses both a double-stranded form, and each of the two complementary single-stranded forms known or predicted to make up the double-stranded form.

Polynucleotide sequences are said to be in a "non-natural arrangement" when they are joined together or interposed with another sequence in an arrangement not found in nature.

When comparison is made between polynucleotides for degree of identity, it is implicitly understood that complementary strands are easily generated, and the sense or antisense strand is selected or predicted that maximizes the degree of identity between the polynucleotides being compared. A computer program that can be used for finding homologous sequences is the BLAST algorithm (Example 1). For purposes of the present disclosure, percentage of sequence identity is calculated by first aligning the polynucleotide being examined with the reference counterpart, and then counting the number of residues shared between the sequences being compared as a percentage of the region under examination. No penalty is imposed for the presence of insertions or deletions, but insertions or deletions are permitted only where clearly required to readjust the alignment. The percentage is given in terms of residues in the sequence being examined that are identical to residues in the comparison or reference sequence. Particularly desirable polynucleotide sequences preserve the function of the prototype: depending on context, an ability to hybridize with a target sequence, the function of a polypeptide it may encode, or (for certain gene targeting vectors) the ability to facilitate homologous recombination or gene inactivation.

"Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding can occur by Watson-Crick base pairing, Hoogsteen binding, triplex formation, or complexing in any other sequence-specific manner. A hybridization reaction will, on occasion, be a step in a more extensive process, such as part of PCR amplification. Hybridization reactions can be performed under conditions of different "stringency". Conditions that increase the stringency of a hybridization reaction are widely known (see e.g., Sambrook et al., *infra*). Examples of conditions in order of increasing stringency: incubation temperatures of 25°C, 37°C, 50°C, and 68°C; buffer concentrations of 10 x SSC, 6 x SSC, 1 x SSC, 0.1 x SSC (where SSC is

0.15 M NaCl and 15 mM citrate buffer, pH 7.2) and their equivalent using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 min to 24 h; 1, 2, or more washing steps; wash incubation times of 1, 5, or 15 min; and wash solutions of 6 x SSC, 1 x SSC, 0.1 x SSC, or deionized water. Typical conditions of high stringency for the binding of a probe of about 100 base pairs and above is a
5 hybridization reaction at 65°C in 2 x SSC, followed by repeat washes at 0.1 x SSC — or the equivalent combination of solvent and temperature conditions for the particular nucleic acids being studied.

A “hybrid” of polynucleotides, or a “complex” formed between any two or more components in a biochemical reaction (such as antibody and antigen), refers to a duplex or higher-order complex that is sufficiently long-lasting to persist between its formation and subsequent detection.

10 A “control element” or “control sequence” is a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide.

“Operatively linked” refers to an operative relationship between genetic elements, in which the function of one element influences the function of another element. For example, an expressible encoding
15 sequence may be operatively linked to control elements such as promoters and enhancers that permit transcription, and control elements for translation such as initiation sequences, stop codons, and signals for polyadenylation.

“Heterologous” means derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared. For example, a polynucleotide introduced by genetic engineering techniques into
20 an animal of a different species is said to be a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked is said to be a heterologous promoter.

The terms “polypeptide”, “peptide” and “protein” are used interchangeably in this disclosure to refer to polymers of amino acids of any length. The polymer may comprise modified amino acids, it may be linear or
25 branched, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, and/or phosphorylation.

Percentage of sequence identity is calculated for polypeptides by first aligning the polypeptide being examined with the reference counterpart or prototype, and then counting the number of residues shared
30 between the sequences being compared as a percentage of the region under examination. No penalty is imposed for the presence of insertions or deletions, but insertions or deletions are permitted only where clearly required to readjust the alignment. The percentage is given in terms of residues in the sequence being examined that are identical to residues in the comparison or reference sequence. Where substitutions are made, conservative substitutions (in which one amino acid is substituted by another with similar charge, size,
35 hydrophobicity, or aromaticity) are typically better tolerated. Desirable sequences preserve the function of the prototype: for example, the enzymatic activity, the binding of specific substrates, and the binding of specific antibody as detectable in a standard competition inhibition immunoassay.

The term “antibody” as used in this disclosure refers to both polyclonal and monoclonal antibody. The ambit of the term deliberately encompasses not only intact immunoglobulin molecules, but also such
40 fragments and genetically engineered derivatives of immunoglobulin molecules as may be prepared by techniques known in the art, and which retains the binding specificity of the antigen binding site.

An “immunogenic” compound or composition is capable of stimulating production of a specific immunological response when administered to a suitable host, usually a mammal.

An "isolated" polynucleotide, polypeptide, protein, antibody, or other substance refers to a preparation of the substance which is enriched in relation to some of the other components that may also be present in the environment where the substance or a similar substance is initially obtained or occurs naturally. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Enrichments by 2, 10, 100, and 1000 fold achieve improved degrees of purification. A substance can also be provided in an isolated state by a process of artificial assembly, such as by chemical synthesis or recombinant expression. An "isolated" cell is a cell that has been separated from the organism in which it was grown.

A polynucleotide used in a reaction, such as a probe used in a hybridization reaction or a vector used in gene targeting is referred to as "specific" or "selective" if it hybridizes or reacts with the intended target more frequently, more rapidly, or with greater duration than it does with alternative substances. Similarly, a polypeptide is referred to as "specific" or "selective" if it binds an intended target, such as a ligand, hapten, substrate, antibody, or other polypeptide more frequently, more rapidly, or with greater duration than it does to alternative substances. An antibody is referred to as "specific" or "selective" if it binds via at least one antigen recognition site to the intended target more frequently, more rapidly, or with greater duration than it does to alternative substances.

General Techniques

In general, the practice of this invention can be carried out using standard techniques of genetic engineering, protein manipulation, and cell culture. Textbooks that describe standard laboratory techniques include the current editions of *"Molecular Cloning: A Laboratory Manual"* (Sambrook et al.); *"Animal Cell Culture"* (R.I. Freshney, ed.); the series *"Methods in Enzymology"* (Academic Press, Inc.); *"Current Protocols in Molecular Biology"* (F.M. Ausubel et al., eds.); and *"Recombinant DNA Methodology II"* (R. Wu ed.). Techniques used in raising, purifying and modifying antibodies, and the design and execution of immunoassays, are described in *Handbook of Experimental Immunology* (D.M. Weir & C.C. Blackwell, eds.); *The Immunoassay Handbook* (Stockton Press NY); and *Methods of Immunological Analysis* (Masseyeff et al. eds., Weinheim: VCH Verlags GmbH).

Texts that describe reproductive techniques and embryo transfer in animals include *Manual of the International Embryo Transfer Society: A procedural guide and general information for the use of embryo transfer technology emphasizing sanitary procedures*, 3rd ed. (Stringfellow et al., Savoy, IL: International Embryo Transfer Society, Savoy IL); and *Embryo transfer in farm animals: A review of techniques and applications* (K.J. Betteridge, ed., Agriculture Canada Monographs No. 16, Ottawa, 1977).

Polynucleotides

The polynucleotides of this invention include those containing nucleotide sequences which are found within the $\alpha 1,3\text{GT}$ cDNA sequence, shown in SEQ. ID NO:1, or the genomic sequence and its 5' and 3' flanking regions, part of which is shown in SEQ. ID NOs:15 to 25. Further sequence of the $\alpha 1,3\text{GT}$ gene can be obtained by employing standard sequencing techniques known in the art to the phage plasmids deposited in support of this application.

Also included in this invention are polynucleotides containing $\alpha 1,3\text{GT}$ -like sequence that is from naturally occurring allelic variants, synthetic variants, and homologs of $\alpha 1,3\text{GT}$ with a percentage of residues identical to the $\alpha 1,3\text{GT}$ cDNA or gene sequence, determined as described above. It is understood that

substitutions, insertions, and deletions can be accommodated within a polynucleotide sequence without departing from the spirit of this invention. In certain embodiments, the polynucleotide sequences are at least 80% , 90%, 95%, 98%, or 100% identical to one of the sequences exemplified in this disclosure; in order of increasing preference. The length of consecutive residues in the identical or homologous sequence compared with the exemplary sequence can be at least about 15, 30, 50, 75, 100, 200 or 500 residues in order of increasing preference, up to the length of the entire clone, gene, or sequence.

Certain polynucleotides of this invention are distinct from polynucleotides already in the hands of the public, including previously known α 1,3GT cDNA or genomic sequences from other species (e.g., SEQ. ID NO:3, 5, 7, 9 and 11), other glycosyl transferase cDNA and gene sequences, and matching fragments from other published sequence data that are fortuitously identical over the length of the fragment (for example, certain regions of SEQ. ID NO:13). A polynucleotide of this invention can be "distinct" from other polynucleotides because of an internal sequence difference (a substitution, deletion, or insertion), or because it is defined to encompass additional sequence at either end. Also included in the invention are recombinant or synthetic polynucleotides in which an α 1,3GT-like sequence is linked to a heterologous sequence to form: for example, a heterologous promoter in an expression vector, or a selectable marker such as *neo* in a targeting vector.

Particular polynucleotides of this invention are useful for producing polypeptides of interest, as nucleotide probes and primers, and as targeting vectors for genetic knock-outs. Further description of the characteristics of such constructs is provided in a later section.

Preparation:

The polynucleotides of this invention can be prepared by any suitable technique in the art. Using the data provided in this disclosure or deduced from the deposited plasmids, sequences of less than ~50 base pairs are conveniently prepared by chemical synthesis, either through a commercial service or by a known synthetic method, such as the triester method or the phosphite method. A suitable method is solid phase synthesis using mononucleoside phosphoramidite coupling units (Hirose et al., *Tetra. Lett.* 19:2449-2452, 1978; U.S. Patent No. 4,415,732).

For use in antisense therapy, polynucleotides can be prepared by chemistry that produces compounds suitable stable for the pharmaceutical preparation for which they are intended. Non-limiting examples include thiol-derivatized nucleosides (U.S. Patent 5,578,718), oligonucleotides with modified backbones (U.S. Patent Nos. 5,541,307 and 5,378,825). Also of interest in the context of antisense constructs are peptide nucleic acids. Prototype PNAs have an achiral polyamide backbone consisting of N-(2-aminoethyl)glycine units, to which purine and pyrimidine bases are linked, for example, by way of a methylene carbonyl linker. PNAs are nuclease and protease resistant, and the uncharged nature of the PNA oligomers enhances the stability of PNA-nucleotide duplexes, thereby blocking transcription or translation. Uptake into cells can be enhanced by conjugating to lipophilic groups incorporating into liposomes, and introducing an amino acid side chain into the PNA backbone. See Soomets et al., *Front. Biosci.* 4:D782, 1999; U.S. Patents 5,539,082, 5,766,855, 5,786,461, and International Patent Application WO 8/53801.

Polynucleotides of this invention can also be obtained by PCR amplification of a template with the desired sequence. Oligonucleotide primers spanning the desired sequence are annealed to the template, elongated by a DNA polymerase, and then melted at higher temperature so that the template and elongated oligonucleotides dissociate. The cycle is repeated until the desired amount of amplified polynucleotide is obtained (U.S. Patent Nos. 4,683,195 and 4,683,202). Suitable templates depend on the nucleotide it is desired to obtain. Certain polynucleotides of this invention can be obtained from the deposited plasmids.

Polynucleotides transcribed into mRNA can be obtained from the cDNA of a suitable cell in the desired species or a close relative. Cells that express $\alpha 1,3$ GT include fibroblasts and endothelial cells. Intron sequences for $\alpha 1,3$ GT can be obtained from a genomic DNA library of the species of interest.

Production scale amounts of large polynucleotides are most conveniently obtained by inserting the desired sequence into a suitable cloning vector and reproducing the clone. Techniques for nucleotide cloning are given in Sambrook, Fritsch & Maniatis (supra) and in U.S. Patent No. 5,552,524. Exemplary cloning and expression methods are illustrated in Examples 1 and 2, below. Polynucleotides can be purified by standard techniques in nucleic acid chemistry, such as phenol-chloroform extraction, agarose gel electrophoresis, and other techniques known in the art, adapted according to the source of the polynucleotide.

Use of the polynucleotides:

Polynucleotides of this invention can be used to identify $\alpha 1,3$ GT nucleotide sequences in a sample of interest for research, diagnostic evaluation, or any other purpose. Generally, this will involve preparing a reaction mixture in which a sample suspected of containing an $\alpha 1,3$ GT-related sequence is contacted with a polynucleotide of this invention under conditions that permit the polynucleotide to hybridize specifically with the compound being tested for, detecting any stable hybrids that form, and correlating the hybrids with the presence of a $\alpha 1,3$ GT related sequence in the sample. The formation of stable hybrids can be detected by any suitable method known in the art. For example, the probe sequence with a detectable label such as a radioisotope, a chromophore, or a hapten such as avidin to which an signaling reagent can be attached. Alternatively, the reagent polynucleotide can be a primer for an amplification reaction in which the amount of product produced correlates with the formation of specific hybrids.

The specificity of the primer and stringency of hybridization conditions are both chosen with a view to facilitating detection of sequences of interest, while diminishing false positive reactions. Thus, when it is important to distinguish between $\alpha 1,3$ GT sequences of different species, then stringency conditions should be high, and the reagent polynucleotide should be nearly identical to the sequence being tested for. Conditions can be determined empirically so that the reagent polynucleotide will hybridize with the $\alpha 1,3$ GT sequence being tested for but not with $\alpha 1,3$ GT sequences from other species. In a more usual application, the question to be addressed is whether sample taken from an animal of a particular species expresses autologous $\alpha 1,3$ GT, or not.

For example, the polynucleotides of this invention can be used to determine whether a cell or tissue treated to inactivate the endogenous $\alpha 1,3$ GT gene expresses $\alpha 1,3$ GT at the mRNA level. Thus, polynucleotides of this invention are used as probes in mRNA blots or as primers in reverse PCR to detect endogenous $\alpha 1,3$ GT species. False positives will not arise due to expression of $\alpha 1,3$ GT from another species, but could arise from cross-reactivity from another glycosyl transferase sharing homology with $\alpha 1,3$ GT, or with another expressed sequence with a fortuitous sequence match. Again, one of skill in the art will know how to select the reagent sequence and hybridization conditions based on sequence information and empirical testing. Since the degree of sequence identity with other galactosyl transferases is typically less than 60%, the practitioner has more latitude in modifying the reagent sequence to enhance stability, sensitivity, or for any other purpose.

Polynucleotides of this invention can also be used to inhibit the transcription or translation of $\alpha 1,3$ GT in target cells, particularly those of the sheep. Such polynucleotides can be in the form of antisense constructs, which in some embodiments bind to $\alpha 1,3$ GT mRNA and prevent translation. Other polynucleotides of this invention are ribozymes having a substrate ($\alpha 1,3$ GT mRNA) binding portion, and an enzymatic portion

with endonuclease activity that cleaves the substrate. The design and use of ribozymes is described in U.S. Patent Nos. 4,987,071, 5,766,942, 5,998,193, and 6,025,167. Particular use of α 1,3GT sequences for the inhibition of porcine α 1,3GT synthesis is outlined in Strahan et al. (Xenotransplantation 2:143, 1995). The effectiveness of transcription inhibitors can be determined by transfecting a cell expressing α 1,3GT with a test compound, and measuring any alteration of expression by the cell — either at the level of mRNA expression (e.g., by quantitative mRNA blotting or reverse PCR), at the level of protein expression (e.g., by immunoassay using anti- α 1,3GT), or at the level of Gal α (1,3)Gal expression (e.g., by binding of a specific antibody or a specific lectin).

Of particular interest are polynucleotide constructs comprising α 1,3GT sequence of this invention that can be used for altering an endogenous α 1,3GT gene. In general, such constructs have a region of at least about 200 base pairs and more typically at least about 1-kb that are at least about 95% identical to the genomic sequence of interest, to permit specific targeting. The construct will also incorporate a substitution, deletion, or insertion designed to modify the targeted gene.

Also included in this invention are polynucleotides that encode polypeptides of interest. Characteristics of the polypeptides of this invention are described in the section that follows. For polypeptides that are fragments of naturally occurring α 1,3GT, there will be a corresponding naturally occurring polynucleotide encoding sequence. Those skilled in the art will recognize that because of redundancies in the amino acid code, any polynucleotide that encodes a peptide of interest can be used in a translation system to produce the peptide. Except where otherwise required, all possible codon combinations that translate into the peptide sequence of interest are included in the scope of the invention.

The polynucleotides of this invention can be in the form of an expression vector, in which the encoding sequence is operatively linked to control elements for transcription and translation in a prokaryotic or eukaryotic host cell of interest. Further details of expression systems for in vitro peptide production are provided below.

Also contemplated in this invention are constructs for introducing a transgene into a eukaryotic cell, for purposes of expressing α 1,3GT, and potentially forming the Gal α (1,3)Gal determinant on cells that would not otherwise present it. Suitable promoters include the endogenous α 1,3GT promoter, predicted to be present in the α 1,3GT genome sequence upstream from the translation start site. Other suitable promoters include constitutive promoters such as those for SV40 and CMV. J.H. White (Adv. Pharmacol. 40:339, 1997) describes modified steroid receptors and steroid-inducible promoters as genetic switches for gene therapy. Walther et al. (J Mol Med. 74:379, 1996) describe cell-type specific and inducible promoters for vectors in gene therapy as an approach for cell targeting.

In particular embodiments, the transgene is inserted into the genome of an embryonic cell (or a nucleus subsequently transferred to an embryonic cell). U.S. Patent No. 4,873,191 describes a process in which genetic material is introduced into a zygote so that it is genetically transformed, then transplanting the embryo into a female so that the embryo develops to term. International Patent Application WO 99/58703 reports a method of preparing transgenic sheep by microinjecting an expression system into a fertilized oosperm, identifying embryonic cells using a nested PCR technique, and then transferring a multicellular embryo into a suitable host. U.S. Patent No. 5,700,671 reports a method for producing mammals with a transgene that encodes a heterologous glycosyltransferase. Other techniques useful in preparing transgenic animals can be found, for example, in U.S. Patent Nos. 4,736,666 5,741,957, and 5,942,435. Individuals with an expressible α 1,3GT transgene, will not have naturally occurring antibodies against Gal α (1,3)Gal, and should accommodate a xenotransplant from other animals expressing α 1,3GT High α 1,3GT activity in a

transgenic cell will also decrease the availability of N-acetyl lactosamine acceptor carbohydrate for other glycosyltransferases using the same acceptor, shifting the balance of synthesized oligosaccharide structures away from the products of other transferases.

5 Polypeptides

The polypeptides of this invention include those that comprise amino acid sequences encoded within any of the polynucleotides of this invention, exemplified by SEQ. ID NO:2 and fragments thereof. Also included in this invention are polypeptides containing α 1,3GT-like sequence that is from naturally occurring allelic variants, synthetic variants, and homologs of α 1,3GT with a percentage of residues identical to the

10 α 1,3GT protein, calculated as described earlier.

It is understood that substitutions, insertions, and deletions can be accommodated within a protein sequence without departing from the spirit of this invention. Conservative substitutions are typically more tolerable, such as the substitution of charged amino acids with amino acids having the same charge, or substituting aromatic or lipophilic amino acids with others having similar features. Certain peptides of this

15 invention are at least 60%, 80%, 90%, 95%, or 100% identical to one of the sequences exemplified in this disclosure; in order of increasing preference. The length of the identical or homologous sequence compared with the prototype polypeptide can be about 7, 10, 15, 20, 30, 50 or 100 residues in order of increasing preference, up to the length of the entire protein.

Certain peptides of this invention are distinct from peptides already in the hands of the public, including previously known α 1,3GT proteins and non-functional homologs from other species (e.g., SEQ. ID

20 NOs:4, 6, 8, 10, and 12), and other glycosyl transferases, such as the human A and B blood group transferases. A polypeptide of this invention can be "distinct" from other polypeptides because of an internal sequence difference (a substitution, deletion, or insertion), or because it is defined to encompass additional sequence at either end. Also included in the invention are artificially engineered fusion proteins in which a

25 α 1,3GT-like sequence is linked to a heterologous sequence which modulates α 1,3GT activity, provides a complementary function, acts as a tag for purposes of labeling or affinity purification, or has any other desirable purpose.

Particular peptides of this invention are useful for their galactose transferase activity, for drug screening, and for raising specific antibody, as described below. mRNA splice variants of mouse α 1,3GT have

30 been observed that omit Exons 5 and 6, and may still produce functional enzyme. If the ovine α 1,3GT is analogous, then the catalytic activity probably resides somewhere else in the molecule. It is predicted that the catalytic activity of α 1,3GT resides further towards the -COOH terminus, probably at least partly in Exon 9.

Preparation:

Short polypeptides of this invention can be prepared by solid-phase chemical synthesis. The principles of solid phase chemical synthesis can be found in Dugas & Penney, Bioorganic Chemistry, Springer-Verlag NY pp 54-92 (1981), and U.S. Patent No. 4,493,795. Automated solid-phase peptide synthesis can be performed using devices such as a PE-Applied Biosystems 430A peptide synthesizer (commercially available from Applied Biosystems, Foster City CA).

Longer polypeptides are conveniently obtained by translation in an in vitro translation system, or by expression in a suitable host cell. To produce an expression vector, a polynucleotide encoding the desired polypeptide is operably linked to control elements for transcription and translation, and then transfected into a suitable host cell. Expression may be effected in prokaryotes such as E. coli (ATCC Accession No. 31446 or

27325), eukaryotic microorganisms such as the yeast *Saccharomyces cerevisiae*, or higher eukaryotes, such as insect or mammalian cells. Control elements such as the promoter are chosen to permit translation at an acceptable rate under desired conditions. A number of expression systems suitable for producing the peptides of this invention are described in U.S. Patent No. 5,552,524. Expression cloning is available from such commercial services as Lark Technologies, Houston TX.

Following production, the protein is typically purified from the producing host cell by standard methods in protein chemistry in an appropriate combination, which may include ion exchange chromatography, affinity chromatography, and HPLC. Expression products are optionally produced with a sequence tag to facilitate affinity purification, which can subsequently be removed by proteolytic cleavage.

Use of the polypeptides:

Polypeptides of this invention can be used for a number of worthwhile purposes, including but not limited to the production of Gal α (1,3)Gal-containing synthetic oligosaccharides, for drug screening, and for production of antibody.

At least three activities are present in the native α 1,3GT: binding of the substrate UDP-gal, binding of the acceptor oligosaccharide N-acetyl lactosamine, and catalytic activity whereby the acceptor oligosaccharide is galactosylated. The binding site for each acceptor can be determined by contacting a test fragment or homolog with the substrate, or with a competitive inhibitor that would be expected to bind with higher affinity, and determining whether a complex forms by a suitable separation technique, such as HPLC, or by an equilibrium quantitation technique, such as microchip analysis, which will better detect low-affinity reactions.

The site of catalytic activity can be determined by contacting the test peptide with both UDP-galactose and acceptor oligosaccharide, and measuring any Gal α (1,3)Gal formed, for example, by immunoassay. Optionally, a systematic approach can be used to determine functional regions and homologs of α 1,3GT according to any of these assays. For example, the assay system is confirmed on the native α 1,3GT structure; then a series of nested fragments is tested to determine the minimum fragment that provides the same activity. Similarly, substitutions can be introduced into the sequence until the activity is ablated, thereby determining what residues are critical. Henion et al. (Glycobiology 4:193, 1994) characterized the minimal size for catalytically active α 1,3GT in New World monkeys, finding that ~68 amino acids could be removed from the N-terminal end before losing activity, while removal of only ~3 amino acids from the C-terminal end resulted in loss of activity.

Native α 1,3GT, and fragments and homologs that preserve substrate binding or catalytic activity, can be used to screen for activity inhibitors and enhancers. For example, a reaction mixture can be prepared containing UDP-galactose, a carbohydrate acceptor, a peptide having α 1,3GT activity, and a test compound. The rate of formation of Gal α (1,3)Gal is then measured. When compared to a reaction mixture not containing the test compound, a decrease in activity correlates with inhibitory capacity of the test compound, while an increase in activity correlates with augmentation capacity.

Native α 1,3GT, and fragments and homologs with α 1,3GT activity are also of interest for the artificial production of complex oligosaccharides containing a Gal α (1,3)Gal determinant, or which build upon the Gal α (1,3)Gal linkage. Such structures may be employed for any worthwhile purpose. For example, oligosaccharides that terminate in Gal α (1,3)Gal are of interest for use in the context of minimizing a rejection reaction in an individual xenotransplanted with a Gal α (1,3)Gal-expressing antigen. Substances containing a high density of the determinant can be used as an adsorbant to remove naturally occurring anti-Gal α (1,3)Gal antibodies from plasma by ex vivo recirculation, or by adsorption in situ (see WO 98/42750). Certain

Gal α (1,3)Gal polymers, built into structures that have T-cell inhibitory epitopes, are proposed as immunosuppressants for the natural antibody against Gal α (1,3)Gal. Catalytic α 1,3GT peptides can optionally be adapted with amino acid additions, deletions, or substitutions for any worthwhile purpose, such as to improve stability or modify the specificity of the transferase in any way that is desirable.

Peptides of this invention are also of interest for the preparation and testing of antibodies against α 1,3GT. A polyclonal antiserum raised against intact α 1,3GT can be screened to map immunogenic portions of the primary sequence. To do this, sequential peptides about 12 residues long are synthesized that cover the entire protein (SEQ. ID NO:2), and overlapping by about 8 residues. The peptides can be prepared on a nylon membrane support by standard F-Moc chemistry, using a SPOTST[™] kit from Genosys according to manufacturer's directions. Prepared membranes are overlaid with the antiserum, washed, and overlaid with β -galactosidase conjugated anti-immunoglobulin. Positive staining identifies antigenic regions, which, in an appropriate context, may themselves be immunogenic. There will also be antibodies that span different parts of the primary structure, or which rely on a conformational component not displayed in smaller peptides.

Suitable methods for raising and testing α 1,3GT antibodies are described in the following section.

Antibodies

Antibody molecules of this invention include those that are specific for any novel peptide encompassed in this disclosure. These antibodies are useful for a number of purposes, including assaying for the expression of α 1,3GT, and purification of α 1,3GT peptides by affinity purification.

Polyclonal antibodies can be prepared by injecting a vertebrate with a polypeptide of this invention in an immunogenic form. If needed, immunogenicity of a polypeptide can be enhanced by linking to a carrier such as KLH, or combining with an adjuvant, such as Freund's adjuvant. Typically, a priming injection is followed by a booster injection is after about 4 weeks, and antiserum is harvested a week later. If desired, the specific antibody activity can be further purified by a combination of techniques, which may include Protein-A chromatography, ammonium sulfate precipitation, ion exchange chromatography, HPLC, and immunoaffinity chromatography using the immunizing polypeptide coupled to a solid support. Antibody fragments and other derivatives can be prepared by standard immunochemical methods, such as subjecting the antibody to cleavage with enzymes such as papain, pepsin, or trypsin.

Any unwanted cross-reactivity can be removed by treating the polyclonal antibody mixture with adsorbants made of those antigens attached to a solid phase, and collecting the unbound fraction. Contaminating activity against other transferases of the same species, or against α 1,3GT from other species, can all be removed by this technique if such cross-reactivity would interfere with the intended use of the antibody. Specificity of the original antisera can be improved to start with, by immunizing with peptide fragments of α 1,3GT that are substantially distinct from the equivalent region of the homologous protein.

Production of monoclonal antibodies is described in such standard references as Harrow & Lane (1988), U.S. Patent Nos. 4,491,632, 4,472,500 and 4,444,887, and *Methods in Enzymology* 73B:3 (1981). Briefly, a mammal is immunized as described above, and antibody-producing cells (usually splenocytes) are harvested. Cells are immortalized, for example, by fusion with a non-producing myeloma, transfecting with Epstein Barr Virus, or transforming with oncogenic DNA. The treated cells are cloned and cultured, and the clones are selected that produce antibody of the desired specificity.

Other methods of obtaining specific antibody molecules (optimally in the form of single-chain variable regions) involve contacting a library of immunocompetent cells or viral particles with the target antigen, and growing out positively selected clones. Immunocompetent phage can be constructed to express

immunoglobulin variable region segments on their surface. See Marks et al., *New Eng. J. Med.* 335:730, 1996, International Patent Applications WO 94/13804, WO 92/01047, WO 90/02809, and McGuinness et al., *Nature Biotechnol.* 14:1449, 1996.

The antibodies of this invention can be used in immunoassays to detect or quantitate any of the polypeptides of this invention, including $\alpha 1,3\text{GT}$. For example, it may be desirable to measure $\alpha 1,3\text{GT}$ in a biological sample to determine whether an individual expresses $\alpha 1,3\text{GT}$ (and hence the $\text{Gal}\alpha 1,3\text{Gal}$ epitope), or whether a cell has been successfully treated to inactivate the $\alpha 1,3\text{GT}$ gene. It may also be desirable to measure $\alpha 1,3\text{GT}$ in a biological sample taken from an individual suspected of having a disease that is correlated with altered expression of $\alpha 1,3\text{GT}$,

General techniques of immunoassay can be found in "The Immunoassay Handbook", Stockton Press NY, 1994; and "Methods of Immunological Analysis", Weinheim: VCH Verlags gesellschaft mbH, 1993). The antibody is combined with a test sample under conditions where the antibody will bind specifically to any modulator that might be present, but not any other proteins liable to be in the sample. The complex formed can be measured in situ (U.S. Patent Nos. 4,208,479 and 4,708,929), or by physically separating it from unreacted reagents (U.S. Patent No. 3,646,346). Separation assays typically involve labeled $\alpha 1,3\text{GT}$ reagent (competition assay), or labeled antibody (sandwich assay) to facilitate detection and quantitation of the complex. Suitable labels are radioisotopes such as ^{125}I , enzymes such as β -galactosidase, and fluorescent labels such as fluorescein. Antibodies of this invention can also be used to detect $\alpha 1,3\text{GT}$ in tissue sections by such techniques as immunohistology or flow cytometry. The antibody is contacted with the tissue, unreacted antibody is washed away, and then bound antibody is detected — either directly, or by using a labeled anti-immunoglobulin reagent.

Assays of this nature can also be used in a competitive format to identify antibodies that bind to the same epitope on a target compound. In one such format, the reference antibody is labeled, and tested for binding to $\alpha 1,3\text{GT}$ or a related peptide under conditions that permit specific antibody-peptide complexes to form. In parallel, the $\alpha 1,3\text{GT}$ peptide is first reacted with the test antibody, and the labeled reference antibody is added subsequently. The assay is read by separating free antibody from peptide-antibody complexes (e.g., by gel filtration or precipitation), and then determining the proportion of label associated with the complexes. If the bound proportion decreases as a result of preincubating with the test antibody, then the test antibody and the reference antibody share overlapping or conformationally related epitopes. Epitope sharing can also be evaluated by mapping the binding site of each antibody using peptide fragments, described earlier. Antibodies can also be screened to identify those with catalytic inhibitory capacity, according to the inhibition screening assay described in the preceding section.

Inactivation of the $\alpha(1,3)\text{Galactosyltransferase}$ gene

This invention provides cells and cell nuclei, in which a preexisting gene for $\alpha 1,3\text{GT}$ has been inactivated. This means that synthesis of the $\text{Gal}\alpha(1,3)\text{Gal}$ epitope by at least one $\alpha 1,3\text{GT}$ allele has been suppressed.

A gene for $\alpha 1,3\text{GT}$ can be inactivated by any one of a number of transformations. For example, the gene may be adapted so that no mRNA transcript is produced; for example, by deleting or altering a transcription control element, such as a promoter or a transcription start sequence. Alternatively, the gene may be adapted so that any mRNA that is produced is not transcribable into the protein product. This can be effected, for example, by deleting or altering a translation control element, such as a ribosomal binding site or a translation initiation codon. Alternatively, the gene may be adapted so that any protein that is produced

lacks the essential features of a glycosyl transferase. For example, the encoding region can be interrupted with stop codons, the encoding region can be placed out-of-phase, or critical portions of the protein may be missing, such as a structural component or a signal peptide for secretion. In a related alternative, the gene may be adapted so that the protein product lacks the specificity of $\alpha 1,3\text{GT}$ — either because the catalytic site is removed, or because substrate binding specificity has been sufficiently altered so that the enzyme is incapable of synthesizing the $\text{Gal}\alpha(1,3)\text{Gal}$ linkage.

A principle method for inactivating the $\alpha 1,3\text{GT}$ gene is to actually change the genetic structure, making the inactivation inheritable by progeny of the cell. Changes of this nature can be effected by disrupting the genome with an integrating vector or mutating the genome by chemical or biological treatment. Treated cells are then cloned, and tested to determine if $\alpha 1,3\text{GT}$ expression has been affected. Suitable means of testing include determination of mRNA expression by probe or amplification based assays, or determination of $\alpha 1,3\text{GT}$ protein expression by immunoassay of cell lysates or immunohistochemical staining of fixed cells.

Efficient targeting of the $\alpha 1,3\text{GT}$ gene generally requires a targeting vector, comprising nucleotide sequence identical or nearly identical to a portion of the gene of interest, linked to another structure capable of introducing the alteration. One such method uses homologous recombination, in which a DNA vector comprising homologous regions recombines at the targeted site, substituting its DNA sequence for that of the target. Cloned cells that have been selectively targeted can be identified by PCR amplification of a sequence exclusive to the targeting vector, restriction analysis of the recombination site, or expression phenotype.

Generally it is more convenient to include a selectable marker in the targeting construct, so that targeted cells can rapidly be separated from untargeted cells. U.S. Patent 5,614,396 describes a method for obtaining a cell containing a desired sequence in the cell's genome, by using a targeting vector having two regions homologous to the targeting sequence, flanking a sequence that is to be inserted, and having a selectable marker. The DNA undergoes homologous recombination at the target site, and recombined cells are recovered under selective culture conditions.

Positive selection markers include the *neo* gene, selectable using G418 or kanamycin; the *hyg* gene, selectable using hygromycin; the *pac* gene, selectable using puromycin; the *gpt* gene, selectable using xanthine; and hypoxanthine-phosphoribosyltransferase (HPRT), selectable using hypoxanthine. Negative selection markers include thymidine kinase (*tk*), selectable using acyclovir or ganciclovir; HPRT, selectable using 6-thioguanine; and cytosine deaminase, selectable using 5-fluoro-cytosine. Markers can also have an intrinsic label, like green fluorescent protein or β -galactosidase, which permit clones of targeted cells to be identified and selected.

Further methodology for homologous recombination is described in the published literature. U.S. Patents 5,464,764 and 5,631,153 provide a double-selection strategy, in which two sequences homologous to the gene target flank a positive selection marker, and a negative selection marker is attached to the 3' terminal of the second flanking region. Homologous integration retains the positive selection marker, but eliminates the negative selection marker, whereas random integration usually retains both markers. Thus, by screening for both markers sequentially or together, cells that have been correctly targeted will be positively selected, and those that have been incorrectly targeted are selected out. U.S. Patent 5,789,215 reports the use of homologous recombinant targeting vectors for modifying the cell genome of mouse embryonic stem cells. See also U.S. Patents 5,589,369 and 5,776,774.

Example 3 describes illustrative targeting vectors according to this invention. They are capable of inactivating the sheep $\alpha 1,3\text{GT}$ gene by eliminating part of the protein coding region via homologous recombination. Vectors p0054, p0079, and p0063 (Example 1, Figure 2) are targeted to eliminate Exon 4, which contains the $\alpha 1,3\text{GT}$ translation start codon. Other vectors have been obtained that target Exon 8 or

Exon 9, which is thought to encode at least part of the α 1,3GT catalytic site. The α 1,3GT gene in other species can be targeted in a similar fashion, using probes having flanking sequence for the α 1,3GT of that species. The bovine and porcine α 1,3GT cDNA sequences are provided in SEQ. ID NOs:5-8.

The vectors comprise flanking regions identical to the targeted α 1,3GT sequence, one side being about 1 kb, the other being at least 1 or 2 kb, in either order. In between the flanking regions is a selectable marker such as *neo*, designed to replace one of the Exons in the α 1,3GT coding sequence. The selectable marker genes are not provided with their own promoter, and require continued translation through the upstream α 1,3GT sequence in order to be expressed. This helps the marker select for properly integrated vector, because vector inserted at a random site will probably not link the marker gene to a suitable promoter, and resistance to the selector drug will not be conferred. In cells that normally express a high level of α 1,3GT and the Gal α (1,3)Gal epitope, the α 1,3GT promoter will be highly active and the drug resistance marker will be strongly expressed. Thus, a higher concentration of selector drug can be used to select out cells that have the vector inserted elsewhere.

Vectors p0054, p0079, and p0063 (Figures 9, 10, and 11) are targeted to eliminate Exon 4, which contains the α 1,3GT translation start codon. Vector p0078 (Figure 12) is targeted to eliminate Exon 8, and contains an additional feature of interest. The sequence replacing Exon 8 has an encoding region for a polypeptide linker, followed by the *neo* gene. Since *neo* is translated within the sequence of the rest of the α 1,3GT protein, the linker should improve the flexibility or accessibility of the *neo* gene product and enhance the neomycin resistance conferred. Vector p0047 and p0046 are designed to replace Exon 9, which is thought to contain at least part of the α 1,3GT catalytic site.

All these vectors, and other designs that would be effective in inactivating ovine α 1,3GT, can be constructed using the sequence data provided in this disclosure and optionally the deposited phage plasmids, according to standard protocols of genetic engineering. Also suitable are insertion vectors (the general structure of which is reported, for example, by Hasty et al., Mol. Cell Biol. 11: 4509, 1991). In this case, homologous recombination results in insertion of the linearized vector sequence, thereby disrupting the gene. Also suitable are shorter oligonucleotides, such as chimeroplasts (A. Cole-Strauss et al., Science 273:1386, 1996), which can direct single base pair changes to a specific gene sequence.

The targeting vectors can be used to inactivate α 1,3GT in sheep cells, or the cells of other phylogenetically related species that have sufficient identity through the targeted area that homologous recombination will take place. Of particular interest are ruminants of the subfamily caprinae, especially ovids and pseudois, exemplified by the sheep of any domestic breed.

Example 4 provides a non-limiting illustration of the use of such vectors. A suitable cell line is combined with the vectors in a culture medium, and the vectors are introduced into the cell. In the illustration, the vectors are introduced by optimized conditions of electroporation. The cells are then cultured for a time in an appropriate medium for maintenance of the cells, during which time the recombination event should occur. The cells are then subjected to culture conditions that permit outgrowth of cells bearing the selectable marker as a result of successful recombination.

Those skilled in the art will recognize that other means of introducing the targeting vector into the cell are also acceptable. For example, International Patent Application WO 29/48005 reports a strategy for modifying a gene in a cell by introducing a parvoviral vector comprising a sequence substantially identical to the target except for the modification being introduced, and permitting the viral vector to effect homologous recombination, thereby modifying the gene sequence. Figure 12 shows targeting constructs based on vectors p0054 and p0079, modified to ~4-kb, so that they will fit within the genome of adeno-associated virus. U.S.

Patent No. 5,932,241 describes cationic lipid DNA complexes for gene targeting. U.S. Patent No. 5,804,413 describes herpes simplex virus strains for gene transfer.

After genetic manipulation has been completed and altered cells have been selected, inactivation of the $\alpha 1,3$ GT gene can be confirmed by suitable testing. Assays for expression of $\alpha 1,3$ GT at the mRNA level and at the protein level have already been described. The nature of the genetic alteration can be determined by PCR amplification using primers bracketing the targeted recombination site, and characterizing the amplification product, or by Southern analysis. If the targeting vector contains a unique sequence, then correct integration can be confirmed using a primer specific for the inserted sequence and a primer specific for neighboring $\alpha 1,3$ GT sequence. Sequence information disclosed in this application relating to $\alpha 1,3$ GT encoding and intron regions (SEQ. ID NOs:15-25) can be used to develop such primers. As illustrated in Example 4, production of amplification product of the predicted size confirms correct integration.

Animals and cells that are homozygous for inactivated $\alpha(1,3)$ Galactosyltransferase

Certain aspects of this invention relate to animals and tissues in which the $\alpha 1,3$ GT gene of at least one haplotype is inactivated. To produce such animals, a cell with this characteristic is provided that has the potential to develop into a fertile embryo. In mice, it is possible to inactivate $\alpha 1,3$ GT in embryonic stem cells, inject the cells into a blastocyst in culture, and then engraft the blastocyst into a female that acts as a surrogate to bring the genetically altered embryo to term (U.S. Patent No. 5,849,991).

In ungulates, a convenient manner for producing genetically modified animals is by the use of nuclear transfer techniques. First, an $\alpha 1,3$ GT gene is inactivated in cultured cells. The nucleus is then transferred into an enucleated recipient cell (such as an oocyte or other pluripotent cell) that is capable of developing into a fertile embryo.

Nuclear donor cells are typically nucleated cells of the desired species that can be maintained in culture. Primary fibroblast lines are exemplary, although other types of cells (such as a stable endothelial cell culture) may also be suitable. For the production of $\alpha 1,3$ GT inactivated sheep, suitable donor cells can be taken from fetal tissue of the Black Welsh Mountain or Finn Dorset line. The selected breed need not be isogenic for the particular vector constructs illustrated in this disclosure, because there will be sufficient identity within the $\alpha 1,3$ GT gene for successful targeting. Primary fibroblast cultures can readily be produced from fetuses collected at about 35 days of gestation, and subjected to a mild trypsin/EDTA solution. Cells are then introduced with the targeting vector, cultured long enough to permit genetic alteration to take place, and then selected for proper recombination, as described in the previous section.

The process of producing a genetically altered animal can be facilitated by increasing the replicative capacity of the nuclear donor cell — in particular, by increasing the level of telomerase activity in the cell. This is conveniently accomplished by introducing the cells with a vector comprising an encoding region for telomerase reverse transcriptase (TERT) under control of a heterologous promoter. See Bodnar et al., Science 279:349, 1998. The human TERT gene sequence is provided in U.S. Patent 6,166,178, which also describes the use of TERT to increase replicative capacity of various cell types. Efficacy of transfection can be determined, for example, by TRAP assay (Kim et al., Science 266:2011, 1997), by RT-PCR for TERT mRNA, or by determining the replicative capacity by passaging cells and monitoring the number of cell doublings.

Nuclear transfer to produce animals from the targeted cells is conducted according to established methods. International Patent Application WO 97/07669 (Roslin Institute) describes quiescent cell populations for nuclear transfer. International Patent Application WO 97/07668 (Roslin Institute) describes inactivated oocytes as cytoplasm recipients for nuclear transfer. International Patent Application WO 99/01164 (University

of Massachusetts) relates to cloning pigs using donor nuclei from differentiated cells. U.S. Patent No. 5,994,619 (University of Massachusetts) reports production of chimeric bovine or porcine animals using cultured inner cell mass cells. U.S. Patent No. 6,011,197 (Infigen) relates to a method for cloning bovines, by reprogramming non-embryonic bovine cells using leukocyte inhibitory factor (LIF) and fibroblast growth factor (FGF), then transferring the nucleus into an enucleated oocyte. International Patent Application WO 99/21415 (Stem Cell Sciences) reports nuclear transfer for production of transgenic animal embryos. Loi et al. (Reprod. Nutr. Dev. 38:615, 1998) discuss embryo transfer and related technologies in sheep reproduction. Wells et al. (Biol. Reprod. 57:385, 1997) report production of cloned lambs from an established embryonic cell line. Liu et al. (Mol. Reprod. Dev. 47:255, 1997) discuss the effect of cell cycle coordination between nucleus and cytoplasm and the use of in vitro matured oocytes in nuclear transfer in sheep embryos. Campbell et al. (Nature 380:65, 1996) report sheep cloned by nuclear transfer from an established cell line.

Nuclear transfer methods are particularly effective if the nucleus of the donor cell is quiescent, which can be achieved by culturing the donor cell in a serum-free medium (WO 97/07669). In an exemplary method, the nucleus of a donor cell is transferred into an oocyte that is arrested in the metaphase of the second meiotic division, and subsequently activating the reconstituted cell. Briefly, unfertilized metaphase II oocytes are collected as follows: Female animals are synchronized using progestagen sponges for ~14 days, and induced to superovulate with single injections of follicle-stimulating hormone on two successive days. Ovulation is induced or synchronized with a suitable dose of gonadotrophin-releasing hormone or an analog thereof (e.g., ~8 mg GnRH Receptal™, Hoechst, UK) on the following day. The oocytes are recovered by flushing from the oviduct one day later, washed, and enucleated by treating with cytochalasin B and aspirating the nucleus using a glass pipette. Enucleated oocytes are then placed into contact with a single cell that acts as the nucleus donor.

Fusion of the donor nucleus into the enucleated recipient cell is effected by placing the couplet in a fusion chamber and aligning it between the electrodes. Electrical pulses are then applied to induce fusion, typically a low-voltage AC pulse for several seconds, followed by a plurality of very short high-voltage DC pulses. Following an incubation period, activation is induced by application of an additional electrical pulse. The reconstructed zygote is then cultured for a time before engrafting into a surrogate female. Further details and alternative procedures are described in the patent publications cited above.

Estrus in the surrogate female is typically synchronized artificially using a suitable combination of inducing agents. Cameron et al. (Aust. Vet. J. 66:314, 1989) discuss synchronization methods and other practical aspects for commercial embryo transfer in pigs. Blum-Reckow et al. (J. Anim. Sci. 69:3335, 1991) report experiments relating to transfer of pig embryos after long-term in vitro culture. Replacing medium every 12 h during culture improved survival, and pregnancy rate improved if the sexual cycle of recipients was 24 h behind that of the donor.

The embryos are introduced into the uterus of the recipient female using any suitable technique, including devices adapted for the purpose, or appropriate methods. For example, U.S. Patent 4,326,505 describes surgical procedures for embryo transplants in animals, in which the uterine horn is positioned in the peritoneal cavity proximate to the vaginal wall, a cannula is inserted through the vaginal wall and into the uterine horn, and the embryo is introduced through the cannula. Non-surgical methods include using a suitable device to manipulate the injection port through the folds of the cervix to the bifurcation of the uterus. For example, devices and techniques for porcine non-surgical embryo transfer are reported by Li et al. (J. Anim. Sci. 74:2263, 1996). Wallenhorst et al. (J. Anim. Sci. 77:2327, 1999) describe the effect of transferring pig embryos to different uterine sites.

The expression of the Gal α (1,3)Gal epitope is autosomal dominant, and prevention of Gal α (1,3)Gal mediated xenograft rejection is improved with homozygous inactivation of α 1,3GT. One option is to select cells for production of embryos that are already homozygously inactivated. For example, the α 1,3GT gene can be targeted for homologous recombination with a construct that incorporates a drug resistance gene, such as *neo*. Targeted cells are first cultured with neomycin at a level that will select for recombination of at least one haplotype. Selected cells are then cultured at a higher level of neomycin to select double recombinants (U.S. Patent No. 5,589,369). In another example, the α 1,3GT gene is targeted simultaneously or sequentially with two different constructs for the same region, each having a different drug resistance gene. The Exon 4 targeting vectors p0054 (*neo*) (Figure 9) and p0063 (*pac*) (Figure 11) can be used in this capacity. Cells are then selected by culturing with the two selection drugs simultaneously or sequentially to select double recombinants. In yet another example, targeting vectors are used that delete part of the α 1,3GT gene sequence, targeted cells are cloned, and their genomic DNA is subjected to Southern or PCR analysis to determine whether the sequence has been deleted from both haplotypes. In a further example, the vectors are used to target cells that actively synthesize and present on the plasma membrane a high density of Gal α (1,3)Gal (such as endothelial cells). After targeting, the cells are subject to multiple rounds of proliferation and selection using specific antibody to the determinant, for example, by flow cytometry or complement-mediated lysis. The cell population surviving repeated selection will be incapable of synthesizing the xenogeneic oligosaccharide, and will be enriched for cells having homozygous inactivation of α 1,3GT.

Animals homozygous for inactivated α 1,3GT can also be obtained by first producing animals heterozygous for inactivated α 1,3GT according to this disclosure — and then mating the heterozygous knockouts once they reach sexual maturity, to obtain progeny that have inherited inactivated α 1,3GT from both parents. Alternatively, cells from a heterozygous knockout can be targeted with an inactivation vector according to this invention to inactivate α 1,3GT on the other haplotype, and used to produce an embryo with homozygously inactivated α 1,3GT.

Tissue devoid of Gal α (1,3)Gal determinants

Tissue samples can be tested to determine whether α 1,3GT has been successfully inactivated. As described elsewhere in this disclosure, changes to the α 1,3GT gene can be determined by Southern analysis or PCR amplification of genomic DNA; mRNA expression can be determined by Northern analysis or reverse PCR, and protein expression can be determined by immunoassay or immunohistochemistry. Elimination of mRNA or protein expression correlates with homozygous inactivation of α 1,3GT, whereas quantitative reduction in mRNA or protein expression correlates with inactivation of α 1,3GT on one haplotype. If inactivation of α 1,3GT has been effected at the level of protein catalytic activity, then mRNA and protein levels may appear normal.

A useful test for successful α 1,3GT inactivation is expression of the Gal α (1,3)Gal epitope. This can be determined using an antibody or lectin specific for Gal α (1,3)Gal. Since it is a self-antigen in most mammals, specific antibodies cannot easily be obtained by immunizing the usual source animals. However, purified antibody can be obtained from pooled human serum by adsorbing on an affinity column of Synsorb 115 (Chembiomed, Alberta, Canada) or D(+) melibiose (Sigma). An alternative is the "IB4" lectin from *Bandeiraea (Griffonia) simplicifolia* (Sigma Cat. L 3019) which is specific for α -D-galactosyl residues (Hayes et al., J. Biol. Chem. 25:1904, 1976), and binds both the Gal α (1,3)Gal epitope, and B blood group substance. In most mammals, Gal α (1,3)Gal is expressed on red blood cells, and can be detected in a simple agglutination

reaction. The antibody or lectin can be used to stain for Gal α (1,3)Gal in fixed tissue or in flow cytometry analysis.

Density of Gal α (1,3)Gal in a particular tissue of interest can be quantitated using a complement lysis assay. Briefly, tissue cells are suspended (by treating with trypsin or collagenase, if necessary), washed, incubated in a medium containing ^{51}Cr for a few hours to incorporate the label, and then washed again. The labeled targets are then combined with diluted human serum as a source of both antibody and complement, and then incubated for several hours at 37°C. Release of the label correlates with density of Gal α (1,3)Gal on the surface of the target cells. For further details of assays for α 1,3GT inactivation and Gal α (1,3)Gal determination, the reader can consult U.S. Patent No. 5,849,991.

Once an animal has been produced with homozygously inactivated α 1,3GT, there should be no synthesis of Gal α (1,3)Gal by the α 1,3GT gene, and the phenotype should breed true. The animal or any progeny bearing the same phenotype can be used as a source of any tissue type that is desired for xenotransplantation. Possible harvest tissue includes but is not limited to any of the following: whole organs, such as kidney, liver, heart, lung, eyes, and pancreas; solid tissue, such as skin, cartilage, pancreatic islets, and vasculature of various types; and cell suspensions, such as progenitor cells for regeneration of neural tissue, hepatocytes, or other cell types.

The following examples provided as further non-limiting illustrations of particular embodiments of the invention.

EXAMPLES

Example 1: Cloning and characterization of the ovine α (1,3)-galactosyl transferase cDNA

The cDNA encoding ovine α (1,3)-galactosyl transferase (GT) was cloned using the reverse transcriptase polymerase chain reaction (PCR) amplification technique.

Two oligonucleotide primers were prepared. The sense oligonucleotide, 698007 (5'-GAGAAAATAA TGAATGTCAA AGGA-3'; SEQ. ID NO:26), included 5' untranslated sequence and the first five codons that encode mature α 1,3GT. The antisense oligonucleotide, 698006 (5'-TGATAATCCC AGCAGTATTC-3'; SEQ. ID NO:27), encoded about amino acids 336 to 342. These primers were used in combination with a Titan™ RT-PCR kit (Boehringer Mannheim) and total RNA (TriPure™ RNA) extraction kit, (Boehringer Mannheim). The mRNA was extracted from Black Welsh Mountain (BW) fetal fibroblasts.

The 1035bp PCR product was cloned into pCR-BluntII-TOPO vector (Invitrogen) and the resulting plasmid propagated by transforming TOP10 competent cells. One colony with plasmid containing the correct fragment was selected. Plasmid p0010 was isolated and the partial cDNA insert sequenced. The final 75 nucleotides of the coding sequence were obtained from phage G (see Example 3), giving a complete sequence of 1110 base pairs.

The 5' untranslated region (UTR) of the ovine α 1,3GT gene was cloned by rapid amplification of cDNA ends (RACE) PCR. A 5' race kit (Boehringer Mannheim) was used in accordance with the manufacturer's instructions. Total RNA was isolated from Black Welsh Mountain fetal fibroblasts and a reverse transcriptase reaction carried out. An anchor of known sequence was linked to the 5' termini of the cDNA molecules produced. First round PCR was performed with sense (supplied) and antisense 798006 (5'-CTTGATGGGT TTATCCAGAA CA-3'; SEQ. ID NO:28) primers. Second round PCR product, produced with the same sense primer but a nested antisense primer (798005, 5'-CTGTGGATAT ATTCCCAAAA CAC-3'; SEQ. ID NO:29), was cloned into pCR-BluntII-TOPO vector. Colonies that contained plasmid with insert were

selected. One clone was sequenced and extended the ovine sequence by 193bp into the 5' UTR (SEQ. ID NO:14).

Figure 1 shows the nucleotide sequence of the cDNA (SEQ. ID NO:1). There are 193bp at the 5' end that are untranslated. Also shown is the 369 amino acid sequence of the predicted protein product (SEQ. ID NO:2). The encoding region is residues 194 to 1300 of SEQ. ID NO:1.

Figures 2 and 3 compare the polynucleotide and amino acid sequences with those of the bovine α 1,3GT (SEQ. ID NOs:3 and 4). Identical residues are marked below with an asterisk.

The sheep α 1,3GT encoding sequence and translation product were compared with SEQ. ID NOs 3-11 using version 2.0 of the BLASTN, BLASTP, and TBLASTN algorithms (National Center for Biotechnology Information; Altschul et al., Nucleic Acids Res. 25:3389, 1997). Comparison of ovine α 1,3GT protein encoding sequence across the whole length of the murine sequence (Joziasse et al, 1992. J. Biol. Chem. 267:5534-5541) and bovine sequence (Joziasse et al, 1989. J Biol. Chem. 264:14290-14297) showed homologies of 78% or 95% respectively. A more detailed comparison is shown in Table 1.

TABLE 1: Comparison of Sheep α 1,3GT with other α 1,3GT sequences

Comparison with:		Sheep α 1,3GT residues matched	Percent identity in matched sequence
NUCLEOTIDES			
bovine α 1,3GT	SEQ. ID NO:3	194-1303	95 %
marmoset α 1,3GT	SEQ. ID NO:5	448-1303	87 %
pig α 1,3GT	SEQ. ID NO:7	503-1303	90 %
mouse α 1,3GT	SEQ. ID NO:9	657-1303	83 %
human α 1,3GT pseudogene	SEQ. ID NO:11	620-1303	87 %
AMINO ACIDS			
bovine α 1,3GT	SEQ. ID NO:4	1-369	94 %
marmoset α 1,3GT	SEQ. ID NO:6	1-369	82 %
pig α 1,3GT	SEQ. ID NO:8	1-369	82 %
mouse α 1,3GT	SEQ. ID NO:10	1-369	72 %
human α 1,3GT pseudogene	SEQ. ID NO:12	139-249	81 %

Example 2: Expression and Southern blot analysis of the Galactosyl Transferase gene

Based on the comparison of the ovine cDNA sequence with the genome and mouse sequence, predictions were made about the organization of the ovine α 1,3GT gene. Hybridization probes for the α 1,3GT gene outside the coding region were designed as follows: A partial cDNA probe, produced by excising a 1035bp fragment from plasmid p0010 (Example 1); an Exon 4 probe, produced by concatamerizing 95bp synthetic oligonucleotides (sense 998003, antisense 998004; SEQ. ID NOs:30 and 31); a 700bp fragment spanning from Exon 6 to 7, derived by PCR from phage G (Example 3) using primers 898003 (5'-GGTGGTTTCC GAGATGGTTT AACAA-3'; SEQ. ID NO:32) and 898005 (5'-GGGTTGAACC AGTCCGATAG

CTTA-3'; SEQ. ID NO:33); a 513bp fragment from Exon 9, produced by PCR using primers 698001 (5'-TCCAGGATGC CTTTGATAGA G-3';SEQ. ID NO:34) and 698006 (5'-TGATAATCCC AGCAGTATTC-3'; SEQ. ID NO:27). These probes were used for the analysis in this example, and for library screening (Example 3).

Figure 4 shows Southern blot analysis to determine the copy number of $\alpha 1,3GT$ in sheep. Ovine genomic DNA was digested with Apal, AseI, BamHI, BsmI, EcoRI or EcoRV, then transferred to nitrocellulose membrane, using standard techniques (Sambrook et al, 1989). Probing with Exon 4 or Exon 6-7 probes revealed a single band in each lane. The likelihood of achieving disruption of the ovine $\alpha 1,3GT$ gene using the targeting strategies in Example 3 is greater if the gene is transcribed and exists as a single copy in the genome.

Figure 5 shows Northern blot analysis for gene expression. Northern analysis was performed using 8 μg of Black Welsh Mountain (BW) fetal fibroblast-derived poly-A RNA, which was isolated using standard techniques (Sambrook et al, 1989). Probing with the $\alpha 1,3GT$ partial cDNA fragment revealed a single band of approximately 3-kb, similar to that previously reported in the mouse (Jozaisse et al, 1992. J. Biol. Chem. 267:5534-5541).

Together, these data provide evidence that the $\alpha 1,3GT$ gene is single copy and is expressed in Black Welsh Mountain fetal fibroblasts.

Example 3: Construction of targeting vectors from genomic Galactosyl Transferase DNA

Construction of vectors to disrupt the $\alpha 1,3GT$ gene by homologous recombination requires isolation of genomic sequences, isogenic (or at least sufficiently identical) to sequences in the target cells. To facilitate this, genomic DNA was isolated from Black Welsh Mountain fetal fibroblasts and a λ DASHII phage library constructed. Briefly, Sau3A partially digested genomic DNA was dephosphorylated and ligated to compatible BamHI vector arms (Stratagene). The ligation products were packaged to produce recombinant phage, which were propagated on *spi* selective XL1-Blue-MRA(P2) bacterial cells. The resulting library had a complexity of 1.4×10^6 recombinants and was subsequently amplified once.

Using the $\alpha 1,3GT$ Exon-4, Exon-6-7 and Exon-9 probes described in Example 2, phage clones spanning these regions were isolated according to standard methods (Sambrook et al, 1989). Six useful clones were isolated.

Figure 6 shows the restriction map of the $\alpha 1,3GT$ gene, and the regions included in Phage $\alpha 1,3GT$ clones. The clones designated **B** and **D** contain the 5' UTR of the $\alpha 1,3GT$ gene and Exon 4, which contains the start codon. The clones designated **C** and **N** contain Exons 4, 5, 6 and 7. The clone **G** spans Exon 6, 7, 8 and 9. Clone **Q** contains part of Exon 9 and the 3' UTR. These phage DNAs reflect the endogenous $\alpha 1,3GT$ gene. They were characterized by inter-exon PCR, sequencing and restriction analysis combined with exon-specific probing.

Recombinant phage **B**, **C** and **G** have been deposited as a pooled sample, which can be separated using the oligonucleotide probes 1198001 (5'-GGGAGGAAGC GAAGGTGCA-3'; SEQ. ID NO:35), 798006 (5'-CTTGATGGGT TTATCCAGAA CA-3'; SEQ. ID NO:28) and 698006 (5'-TGATAATCCC AGCAGTATTC-3'; SEQ. ID NO:27), that recognize 5'UTR, Exon 5 and Exon 9 sequence, respectively.

Figure 7 maps the sequenced intron regions of $\alpha 1,3GT$ to their positions in the gene. The areas numbered 1 to 9 in the figure correspond to SEQ. ID NOs:15, 16, 19, 20, 21, 22, 23, 24, and 25, respectively.

Figure 8 shows the position of exemplary targeting vectors designed for use in homologous recombination to disrupt gene expression by excising Exons 4 and 9. These and a number of other vectors are described below.

Figure 9 shows in more detail the promoterless targeting vector designated plasmid p0054, directed towards Exon 4. The vector comprises two regions that are complementary to genomic sequence; a 1.2-kb 5' arm, which includes sequence from Intron 3 leading up to and including the start codon of the $\alpha 1,3$ GT gene in Exon 4, and a ~9-kb 3' arm that initiates 1.5-kb into Intron 4, continuing to Intron 5. Separating these regions is *neo^R*-polyA sequence. After homologous recombination the vector confers neomycin phosphotransferase resistance to the cells and deletes 1.5-kb of genomic sequence, including the first coding exon of $\alpha 1,3$ GT gene. The entire cassette was cloned into pBlueScript™ for propagation in DH5 α bacterial cells.

The p0054 vector was constructed by amplifying a truncated left arm (300bp, includes EcoRI site) (using primers 199001, 5'-ACGTGGCTCC AAGAATTCTC CAGGCAAGAG TACTGG-3', SEQ. ID NO:36; and 199006, 5'-CATCTTGTTT AATGGCCGAT CCCATTATTT TCTCCTGGGA AAAGAAAAG-3', with tail complementary to the start of *neo* coding sequence, SEQ. ID NO:37), and a *neo*-polyA sequence obtained from Stratagene (using primers 199005, 5'-CTTTTCTTTT CCCAGGAGAA AATAATGGGA TCGGCCATTG AACAAAGATG-3', SEQ. ID NO:38, with tail complementary to left arm; and 199004, 5'-CAGGTCGACG GATCCGAACA AAC-3', SEQ. ID NO:39). These fragments were used to prime from each other to give a 1.2-kb fusion product. This was ligated to Intron 3 sequence, to extend the left arm, and to ~9-kb of 3' sequence to create the right arm, which initiates 1.5-kb into Intron 4, continuing to Intron 5.

Figure 10 shows the promoterless *neo*-polyA insertion vector designated plasmid p0079, also directed towards Exon 4. This vector contains the same left arm-*neo*-polyA fusion as in vector p0054, but with a modified right arm of 3.9-kb. The 3' region comprises a 1.5-kb fragment, generated by PCR (using primers 200011, 5'-CAGATCTAAC GAGGATTCAA TGTCAAAGGA AAAGTGATTG TGTCAAT-3', SEQ. ID NO:40; and 499006, 5'-CTGAAGTGAA TGTTTATCCA GGCCATC-3', SEQ. ID NO:41), which extends from the second codon in Exon 4 into Intron 4, replacing the sequence deleted in p0054. The 3' arm was extended by ligation to a 2.4-kb EcoRV downstream fragment.

Figure 11 shows the promoterless *pac*-polyA replacement vector designated plasmid p0063, also directed towards Exon 4. Construction of this vector was similar as for p0054, except that it contains the *pac* gene, which codes for puromycin N-acetyltransferase, rather than the *neo* gene. The *pac* sequence is available in plasmid pPUR from ClonTech™. The oligonucleotide primers used to generate the 5'-*pac*-polyA fusion were, for the 5' region, 199001 (SEQ. ID NO:36) and 699002 (5'-GCGCACCGTG GGCTTGACT CGGTCATTAT TTTCTCCTGG GAAAAGAAAA G-3', SEQ. ID NO:42), with tail complementary to the start of *pac* coding sequence; and, for *pac*-polyA, 699004 (5'-GAGAAAATAA TGACCGAGTA CAAGCCCACG GTGC-3' SEQ. ID NO:43), with tail complementary to left arm, and 699005 (5'-CTGGGGATCC AGACATGATA AGATAC-3' SEQ. ID NO:44).

Figure 12 shows restriction fragments of the Exon 4 directed targeting vectors (3.9-kb NsiI / BglII, from p0054; 3.6-kb NsiI / ClaI, from p0079; Figure 10). These fragments are of appropriate size for insertion into recombinant adeno-associated virus (AAV) for gene targeting according to the general approach outlined in International Patent Application WO 98/48005.

Figure 13 shows the promoterless *neo*-polyA replacement vector designated plasmid p0078, directed towards Exon 8. The vector comprises a 1.3-kb left arm, which is complementary to sequence from Intron 7 up to and including the first nucleotide of Exon 8, fused in frame to 5 \times GGA (glycine) repeats, then to *neo*-polyA sequence (obtained from Stratagene). The glycine linker is designed to permit more independent rotation of each functional domain (the remaining fragment of $\alpha 1,3$ GT, and *neo*), allowing *neo* to perform its function. The

vector was constructed by amplifying the left arm (using primers 200-005, 5'-CTGGTTGGTT CTAGAACAGG AGGA-3', SEQ. ID NO:45; and 200-007, 5'-CATCTTGTTT AATGGCCGAT CCCATTCCTC CTCCTCCTCC ACTGGTGACA AACACAGAGTC CATGAG -3' SEQ. ID NO:46, with tail complementary to the start of *neo* coding sequence) and *neo*-polyA sequence (using primers 200-006, 5'-CTCATGGACT CTGTTTGTG ACCAGTGGAG GAGGAGGAGG AATGGGATCG GCCATTGAAC AAGATG -3', SEQ. ID NO:47, with tail complementary to left arm; and 200-008, 5'-CAGGTCGACG GATCCGAACA AAC -3', SEQ. ID NO:48). These fragments were then used to prime from each other, giving a 2.2-kb fusion product. To complete the targeting vector, this product was ligated to a ~9-kb right arm, which initiates 0.7-kb into Intron 8, continuing to the 3'UTR.

Figure 14 shows the targeting vector designated plasmid p0047, designed to disrupt Exon 9 of the $\alpha 1,3$ GT gene. The pMC1-*neo*-polyA replacement vector comprises (from left to right) 1.8-kb of Intron 8 sequence, located 0.4-kb prior to Exon 9; pMC1-*neo*-polyA sequence (Stratagene); and a ~7-kb right arm (HindIII restriction sites blunt ended using T4 DNA polymerase obtained from Promega), initiating 60bp upstream of the stop codon of Exon 9, and continuing into the 3'UTR.

Figure 15 shows the targeting vector designated plasmid p0046, also directed towards Exon 9. The pIRES- β geo-polyA vector (obtained from Austin Smith, Centre for Genome Research, Edinburgh UK) contains the same right arm as p0047 extended to include the remainder of Intron 8 and the first ~120bp of Exon 9, which allows expression of the inserted selectable cassette: the internal ribosome entry site- β -galactosidase-*neo^R*-polyA.

After homologous recombination, the selectable marker in both p0047 and p0046 are designed to replace >80% of Exon 9, which is hypothesized to comprise at least part of the catalytic domain of $\alpha 1,3$ GT.

Example 4: Disruption of the galactosyl transferase gene by homologous recombination

Black Welsh Mountain fetal fibroblasts have previously been used in nuclear transfer experiments, creating live offspring (GB Patents 2318578 and 2331751). For the purposes of the present experiment, electroporation conditions were optimized using the β -galactosidase marker plasmid, pCMV-Sport- β gal (obtained from Gibco). Using a 0.4cm cuvette with 3×10^5 cells (0.8ml, 6 μ g plasmid DNA) and a setting of 250 μ F:400Volts (Gene Pulser, BioRad), 10-30% of the surviving fibroblasts stained positive for β -gal expression.

For targeting electroporations 10, 25 or 100 μ g of NotI linearized p0054 vector was mixed with 1×10^7 early passage Black Welsh Mountain fetal fibroblasts and pulsed as described above. Cells were grown on tissue culture plastic for 24 h before G418 (300 μ g/mL) was applied. After 10-14 days, colonies were isolated. Half the colony cell population was expanded for analysis, while the remainder was cryopreserved for later recovery.

Figure 16 shows the results of site specific recombination detected by PCR amplification. Wild type and targeted $\alpha 1,3$ GT alleles were detected using sense (399010, 5'-CAGCTGTGTG GGTATGGGAG GG-3'; SEQ. ID NO:49) and antisense (499006, 5'-CTGAAGTGA TGTATATCCA GGCCATC-3'; SEQ. ID NO:50) PCR primers, yielding products of 2.8-kb and 2.2-kb, respectively. A second PCR screen with primers 399010 (SEQ. ID NO:49) and 399005 (5'-AGCCGATTGT CTGTTGTGCC CAGTCAT-3'; SEQ. ID NO:51) produced a fragment of 1.5-kb only in clones that were correctly targeted. The frequency of site-specific recombination was 1 in 52 (6 in 312) clones in the 10 μ g experiment or 1 in 88 (10 in 877) from all electroporations.

The *neo^R* gene was tested in one sample by Southern blot analysis. DNA was digested with the restriction enzyme BsmI, and the blot was probed with *neo* coding sequence, producing a diagnostic band at

5.5-kb. However, all cryopreserved samples of targeted Black Welsh Mountain cells failed to grow after resuscitation. Analysis other than PCR was not possible due to inadequate amounts of DNA. The PCR data demonstrate successful gene disruption by homologous recombination in sheep somatic cells.

Additional vectors were constructed for targeting Exon 8 or 9, using the *pac* gene as a resistance marker. Transfection of Black Welsh Mountain fetal fibroblasts with the pIRES- β geo-polyA replacement vector failed to produce successful targeting in the 250 clones examined. Apparently, the efficiency of this vector was lower than the promoterless neo-polyA vector.

Example 5: Targeting of the α 1,3GT gene in other breeds

In this example, targeting vectors were used to affect the α 1,3GT gene in cells of a different breed.

Cell lines were created as follows. Finn Dorset (FD) day 35 fetuses were decapitated and eviscerated. The carcasses were dissociated manually, and then treated with trypsin/EDTA solution (Sigma). Primary cultures of fibroblasts were grown in GMEM media (Sigma), supplemented with 10% FCS (Globe Farm), on tissue culture plastic coated with 0.1% gelatin. Cells were exposed to gentamycin (Gibco) for the first 24 hours to prevent bacterial contamination. Once confluent, cells were cryopreserved in aliquots of $\sim 1 \times 10^6$ per vial. Euploid lines of male genotype were used for gene targeting.

Cell suspensions were targeted with 10 μ g of linearized promoterless neo-polyA replacement vector p0054. Electroporation was conducted by applying an electrical pulse of 125 μ F, 350 Volts. A total of 568 clones were derived from one electroporated primary culture, designated 7G65F4. Colonies were screened for successful targeting as described in Example 4. Eighteen colonies (3.2%) showed patterns consistent with homogeneous cell populations in which one α 1,3GT allele was deleted. A further 17 colonies containing mixed populations of targeted and non-targeted cells were detected. Surprisingly, even though the DNA of the targeting vectors is not isogenic with Finn Dorset gene sequences the targeting frequency was higher than in the experiments with Black Welsh Mountain cells (Example 4).

Homogeneous colonies of targeted FD cells stored in liquid N₂ were thawed rapidly and collected by centrifugation. Three clones (3C6, 5E1 and 4H2) from culture 7G65F4 retained a normal karyotype and fibroblast morphology, and grew successfully. Cells were grown for up to five days in normal medium containing 10% FBS, replacing the medium every 48 hours. The cells were then stressed for up to five days, in medium containing only 0.5% FBS, replacing the medium every 48 hours. Thereafter, culturing continued in 10% FBS and karyotyped. The 4H2 clone was unstable in longer periods of culture, but clones 3C6 and 5E1 grew in a slow, stable fashion, and thus suitable for nuclear donors.

The frequency of site-specific recombination observed in these experiments is shown in Table 2:

TABLE 2: Gene Targeting Efficiency in Primary Sheep Fibroblast Cultures

Parental culture	Target locus	Drug resistant colonies	Targeting events detected	Colonies suitable for nuclear transfer
Black Welsh	α 1,3GT	877	10 (1.1%)	0 (0%)
Finn Dorset	α 1,3GT	568	35 (6.2%)	2 (0.4%)

Example 6: Targeting of the $\alpha 1,3$ GT gene in telomerized fibroblasts

A vector containing an expression cassette for telomerase reverse transcriptase was found to increase functional telomerase activity and replicative capacity in sheep fibroblasts suitable for nuclear transfer. It is a retroviral vector containing sequences encoding telomerase reverse transcriptase (abbreviated here as hTERT) with a consensus Kozak sequence downstream of the myeloproliferative sarcoma virus (MPSV) promoter. It also contains puromycin and hygromycin resistant gene sequences and allows drug selection of the transfected clones.

Primary sheep fibroblast cell line designated BW6F2 (passage 6, obtained from a Black Welsh sheep) was transfected with linearized pGRN145. The cells were plated in 96 well plates, and selected using puromycin at 1 μ g/mL. PCR screening with puromycin primers showed that all but one of the selected clones contained the vector sequence.

Fourteen of the clones were developed into cell lines. hTERT expression was measured in the cloned sheep fibroblasts by Western blot and by immunocytology. Functional telomerase activity was measured by TRAP assay, and was found to be positive in 10 of these clones, compared with the original BW6F2 line.

In order to determine the replicative capacity of the cloned fibroblast cell lines, the cells were passaged continuously using standard culture conditions. The clones expressing hTERT were grown through over 260 population doublings (PDs) like young cells. Cells transfected with a control plasmid without hTERT cDNA or the transfected cells not expressing hTERT grew less than 83 PDs. The parental cells only replicate through 127 PDs, when they become senescent.

The hTERT expressing sheep fibroblasts were also analyzed to determine whether or not the hTERT expressing cells showed signs of transformation to a malignant phenotype by karyotype analysis, response to serum starvation (0.1% serum for 7 days, followed by resynchronization for 24 h in 10% serum). Telomere length was assessed by extracting DNA from cloned cells using a blotting assay. The DNA was digested with *Rsa*I and *Hinf*I, separated on 0.7% agarose, blotted onto a nylon membrane, and probed with 32 P-labeled (TTAGGG) $_3$ oligonucleotide.

It was found that by passage ~150, some clones have telomere shortening (GRN 1-1, 2-7 and 2-8), while others show no change (GRN 2-1, 2-5 and 2-10), or show elongated telomeres (GRN 2-2). Clones with higher hTERT expression levels (detected by Western blot and immunostaining with 1A4 antibody) maintained their telomere length, while clones with lower hTERT expression levels were typically the ones showing shortened telomeres.

GRN1-1 cells at passage 5 or 25 were resuscitated into T175 flasks and grown to subconfluency. Cells (2.8×10^6 , passage 5; 8.3×10^6 , passage 25) were electroporated with 10 μ g of *Not*I linearized p0054 $\alpha 1,3$ GT targeting vector, using a setting of 125 μ F: 350 V in Flowgen™ 0.4 cm / 800 μ l cuvettes. Diluted cells were plated to 20 \times 96 well plates. The next day, G418 (600 μ g /mL) was added to the medium to begin the selection process. Cell death appeared after 8-10 days in G418, much longer than when using parental BW6F2 cells. Colonies were observed after ~2 weeks and replica plated (41 colonies from passage 5 cells; 2 colonies from passage 25 cells) on day 20 of selection.

PCR analysis was conducted on DNA isolated from selected colonies. One correct targeting event (clone B9) was detected from the passage 5 electroporation. This clone and eight non-targeted clones were resuscitated in 24 well plates. All clones grew to confluency. The B9 (correctly targeted) cell line, and the C9 cell line (one of the eight containing randomly integrated $\alpha 1,3$ GT) grew fastest. Clones B9 and C9 have been karyotyped, and both are 54XY.

Thus, telomerized sheep fibroblasts were successfully targeted with the promoterless neo $\alpha 1,3GT$ targeting vector, p0054. The targeted clone (B9) has been expanded, and retains a stable karyotype. This clone exists as a pure population of targeted cells and continues to grow at passage 17 (~80 doublings).

5 Example 7: Animals in which an $\alpha 1,3GT$ gene is inactivated

Successfully targeted fibroblast cell lines 3C6 and 5E1 are used as donors for nuclear transfer. The cells are grown for up to five days in 10% FBS, and then starved for up to five days in 0.5% FBS, as described in Example 5. Prepared cells are trypsinized for ~5 min, and collected by low-speed centrifugation (800 rpm for 3 min). They are then resuspended in < 100 μL 10% FCS medium, and used immediately for nuclear transfer.

Six weeks before the transfer, adult female breeding sheep are selected, and brought in to the facility to acclimatize to the surroundings. For each nuclear transfer experiment, about 8 animals are used as oocyte donors, 2 as temporary recipients, and 10 as final recipients.

Oocytes are collected as follows. Time of estrus is controlled in donor animals by treatment with an intravaginal progestagen sponge for 11 to 16 days, replacing once if necessary. Ovine follicular growth is promoted by injection of gonadotrophin, with or without subcutaneous or intramuscular injection of 0.6 mg (2 mL) follicle stimulating hormone (FSH) twice daily over 4 consecutive days, followed by a single injection of 500 i.u. (2.5 mL) pregnant mare's serum gonadotropin (PMSG) on the day of sponge removal. Time of ovulation is controlled by subcutaneous or intramuscular injection of an analogue of gonadotrophin releasing hormone (BuserelinTM, given 24 h after sponge removal). Between one and twelve days after the onset of estrus, general anesthesia is induced by an intravenous injection of a barbiturate, followed by intubation and maintenance with gaseous anesthetics. The reproductive tract is exposed by mid-ventral laparotomy, and the oviduct or uterus is temporarily cannulated as a passageway for sterile medium. Three small puncture incisions made at sites anterior to the udder, through which a laparoscope, manipulating forceps and a needle are used to manipulate the uterus. After oocytes are collected, the incision is sutured closed, and the donor animal is allowed to recover from anesthetic.

Nuclear transfer is conducted as follows. First, the oocytes are stripped of cumulus cells by triturating with a pipette and incubating with hyaluronidase. They are then enucleated by removing the first polar body and metaphase plate. A single 3C6 or 5E1 targeted nuclear donor cell is introduced under the zona of each oocyte. The cell combination is subject to simultaneous electrofusion and activation (0.25 kV cm^{-1} AC for 5 sec. to align oocyte and donor cell, followed by 3 pulses of 1.25 kV cm^{-1} DC for 80 μsec to fuse and activate the reconstructed embryo). The activated cell is maintained in culture overnight at 39°C. Next day, the cells are embedded in agar chips to protect from macrophages, and then transferred to the ligated oviduct of a temporary recipient.

Estrous is controlled in the temporary recipient by treatment with intravaginal progestagen sponge for 11 to 16 days, with or without subcutaneous or intramuscular injection of 500 i.u. of PMSG. The timing brings the temporary recipients to estrus ~3 days before the oocyte donors. Cells are collected under general anesthesia using barbiturate followed by gaseous anesthetics. The reproductive tract is exposed by midventral laparotomy; placing ligatures of silk at each uterotubal junction, and embryos are transferred through the fimbriated end of the oviduct. The laparotomy is then closed, and a long-acting antibiotic is administered. The embryos are flushed from the temporary recipient after 6 days, and developing embryos are removed from the agar chip.

Blastocysts and morula are then transferred into the recipients that will carry the embryo to term. Estrus is controlled by treatment with an intravaginal progestagen sponge for 11 to 16 days, bringing the final

recipients to estrus simultaneously with the oocyte donor. The permanent recipients are anesthetized by intravenous barbiturate and gaseous anesthetics, the reproductive tract is exposed by mid-ventral laparotomy, and the oviduct or uterus is temporarily cannulated for transfer of the embryos. Alternatively, three small puncture incisions are made anterior to the udder, and a laparoscope, manipulating forceps and needle are inserted for manipulation of the uterus. The oviduct or uterus is temporarily cannulated for transfer of the embryos, and the incision is sutured closed.

Recipients of oocytes with a targeted nucleus, engrafted in the manner outlined, were monitored for the status of their pregnancy by subcutaneous ultrasonic scanning on a weekly basis. Ten recipients were determined to be pregnant 35 days after engraftment. At the time these observations were recorded, insufficient time had passed for any engrafted pregnancies to reach term. Some of the pregnancies were stable, and some started to regress. A fetus and umbilical cord were recovered from one of the regressing pregnancies for analysis.

Figure 17 shows the results of PCR analysis of the tissue from the umbilical cord. Using primers for the 5' untranslated region and the neo gene, a band corresponding to 1.5 kb was detected, which should only be present in progeny from a cell successfully targeted with a neo-containing deletion vector. Using primers for the 5' and 3' untranslated regions, two bands were observed: 2.8 kb, corresponding to the wild type $\alpha 1,3$ GT gene, and 2.2 kb, appropriate for a targeted $\alpha 1,3$ GT gene. These results are consistent with inactivation of the $\alpha 1,3$ GT gene on one haplotype.

For animals maintaining their pregnancy, the progress of the fetus is monitored regularly by ultrasound, and brought to term. Blood cells are collected after birth, to verify that at least one $\alpha 1,3$ GT allele has been inactivated.

BIOLOGICAL DEPOSIT

The recombinant phage designated **B**, **C** and **G**, have been deposited with the National Collections of Industrial and Marine Bacteria Limited (NCIMB), 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland, United Kingdom (Tel: +44 (0)1224 273332; Fax: +44 (0)1224 272461).

A deposit comprising equal concentrations of each phage was received on April 25, 2000, and is catalogued under Accession No. NCIMB 41056. The concentration is 0.8×10^8 pfu/mL for each of the three phage, for a total of 2.4×10^8 pfu/mL. Each phage can be separated from the mixture using the following probes:

- Phage **B**: probe GGGAGGAAGCGAAGGTGCA (SEQ. ID NO:35), 5'UTR
- Phage **C**: probe CTTGATGGGTTTATCCAGAACA (SEQ. ID NO:28), Exon 5
- Phage **G**: probe TGATAATCCCAGCAGTATTC (SEQ. ID NO:27), Exon 9

Each recombinant phage was deposited with the NCIMB separately on May 30, 2000. Accession numbers are: Clone **B**, No. 41059; Clone **C**, No. 41060; and Clone **G**, No. 41061. Each deposit has a concentration of 2×10^8 pfu/mL.

The phage are useful for obtaining sequence information about the sheep $\alpha 1,3$ Galactosyltransferase gene (described in Example 3) and as an amplification template for preparing certain polynucleotides of this invention.

The deposits are made under terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. For purposes of prosecution of this and any related application in the United States, the deposit is made under terms and with the undertakings of 37 CFR §§ 1.801-1.808. Subject to 37 CFR § 1.808(b), all restrictions imposed on the availability to the public

5 of the deposited material will be irrevocably removed upon the granting of a U.S. patent thereto.

SEQUENCE DATA

TABLE 3: Sequences listed in this Disclosure

SEQ. ID NO:	Designation	Reference
1	Sheep α 1,3-GT cDNA sequence	This Invention.
2	Sheep α 1,3-GT amino acid sequence	This Invention.
3	Bovine α 1,3-GT cDNA sequence	GenBank Accession J04989 Joziassse et al. "Bovine α 1- \rightarrow 3-galactosyltransferase" J. Biol. Chem. 264, 14290 (1989)
4	Bovine α 1,3-GT amino acid sequence	GenBank Accession P14769 Joziassse et al. (1989), supra.
5	Marmoset α 1,3-GT cDNA sequence	GenBank Accession S71333 lymphoid cell line B95.8, mRNA Partial Macher et al. "Defining the minimal size of catalytically active primate α 1,3 galactosyltransferase" Glycobiology 4,193 (1994)
6	Marmoset α 1,3-GT amino acid sequence	GenBank Accession S71333 Macher et al., supra.
7	Pig α 1,3-GT cDNA sequence	GenBank Accession L36152 Sus scrofa alpha-1,3-galactosyltransferase mRNA. Strahan et al. "cDNA sequence and chromosome localization of pig α 1,3 galactosyltransferase" Immunogenetics 41, 101 (1995) See also GenBank Accession L36535 Sandrin et al. "Characterization of cDNA clones for porcine α (1,3)galactosyl transferase" Xenotransplantation (1994)
8	Pig α 1,3-GT amino acid sequence	GenBank Accession L36152 Strahan et al., supra.
9	Mouse α 1,3-GT cDNA sequence	GenBank Accession M26925 Larsen et al. "Isolation of a cDNA encoding a murine UDPgalactose: β -D-galactosyl-1,4-N-acetyl-D-glucosaminide alpha-1,3-galactosyltransferase" Proc. Natl. Acad. Sci. USA 86, 8227 (1989) See also GenBank Accession IM85153 Joziassse et al. "Murine alpha-1,3-galactosyltransferase: A single gene locus specifies four isoforms of the enzyme by alternative splicing" J. Biol. Chem. 267, 5534 (1992)
10	Mouse α 1,3-GT amino acid sequence	GenBank Accession M26925 Larsen et al., supra.
11	Human α 1,3-GT pseudogene DNA sequence	GenBank Accession M60263 Joziassse et al. "Characterization of an alpha-1- \rightarrow 3-galactosyltransferase homologue on human chromosome 12 that is organized as a processed pseudogene" J. Biol. Chem. 266, 6991 (1991)

TABLE 3: Sequences listed in this Disclosure

SEQ. ID NO:	Designation	Reference
12	Human $\alpha 1,3$ -GT pseudogene open reading frame amino acid sequence	calculated from GenBank Accession M60263 Joziassse et al., supra.
13	Bovine γ globin gene and globin pseudogene	GenBank Accession M63452 C.H. Duncan (direct submission)
14	Exon 4 of the sheep $\alpha 1,3$ -GT gene	This Invention.
15	Pre-Exon 4 sequence (Phage B and C)	This Invention. Region 1 of Figure 7
16	Post-Exon 4 sequence Exon 4 / intron 4 boundary	This Invention. Region 2 of Figure 7
17	Post-Exon 4 sequence	This Invention.
18	Post-Exon 4 sequence	This Invention.
19	Post-Exon 4 sequence	This Invention. Region 3 of Figure 7
20	Pre-Exon 8 sequence	This Invention. Region 4 of Figure 7
21	Intron sequence approx -4kb to -2kb from Exon 9	This Invention. Region 5 of Figure 7
22	Intron sequence approx -2kb to -1kb from Exon 9	This Invention. Region 6 of Figure 7
23	Intron sequence 5' to Exon 9	This Invention. Region 7 of Figure 7
24	Intron sequence 3' to Exon 9	This Invention. Region 8 of Figure 7
25	Intron sequence approx -2kb to -1kb from Exon 9	This Invention. Region 9 of Figure 7
26 to 51	Probes and PCR primers	This Invention.

It is understood that certain adaptations of the description and illustrations provided in this disclosure are a matter of routine optimization for those skilled in the art, and can be implemented without departing from the spirit of the invention, or the scope of the appended claims.

5

CLAIMS

What is claimed as the invention is:

1. Ovine tissue devoid of antibody-detectable Gal α (1,3)Gal determinants.
2. The tissue of claim 1, which is selected from the group consisting of lung tissue, kidney tissue, liver tissue, cardiac tissue, pancreatic tissue, and ocular tissue.
3. Isolated ovine cell or tissue that expresses glycosyl transferase enzymes but does not detectably express α (1,3)galactosyltransferase (α 1,3GT).
4. An ovine cell that is heterozygous or homozygous for inactivation of an α 1,3GT gene.
5. The cell of claim 4, produced by transfer of a nucleus from a donor cell heterozygous or homozygous for inactivation of an α 1,3GT gene, to an enucleated recipient cell.
6. An ovine animal that is homozygous for inactivation of an α 1,3GT gene.
7. A polynucleotide construct effective for inactivating an α 1,3GT gene in an ovine cell.
8. The polynucleotide construct of claim 7, comprising at least two polynucleotide sequences from an ovine α 1,3GT gene in a non-natural arrangement, for inactivating the α 1,3GT gene by homologous recombination.
9. The polynucleotide construct of claims 7-8, comprising an intron sequence of at least 30 consecutive nucleotides found in any of recombinant phage **B**, **C** and **G**, deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061.
10. The polynucleotide construct of claims 7-9, comprising a selectable marker.
11. The polynucleotide construct of claim 10, wherein the selectable marker is flanked on either side by polynucleotide sequences from an ovine α 1,3GT gene.
12. A method for inactivating an α 1,3GT gene in an ovine cell, comprising contacting the cell with the polynucleotide construct of any of claims 7-11 under conditions suitable for homologous recombination of the construct into the genome of the cell.

13. A method for producing an ovine that is homozygous for inactivation of an $\alpha 1,3$ GT gene, comprising providing an ovine embryo of cells according to claim 4, engrafting the embryo into the uterus of a female, birthing an ovine with an inactivated $\alpha 1,3$ GT gene from the engrafted female, and if the birthed ovine has the $\alpha 1,3$ GT gene inactivated on only one allele, then mating it with another ovine with an inactivated $\alpha 1,3$ GT gene, thereby producing an ovine that is homozygous for inactivation of the $\alpha 1,3$ GT gene.
14. A method for producing an isolated ovine cell that expresses glycosyl transferase enzymes but does not detectably express $\alpha 1,3$ GT, comprising isolating the cell from an ovine homozygous for inactivation of an $\alpha 1,3$ GT gene according to claim 6.
15. A method for producing ovine tissue devoid of antibody-detectable Gal $\alpha(1,3)$ Gal determinants, comprising harvesting the tissue from an ovine homozygous for inactivation of an $\alpha 1,3$ GT gene according to claim 6.
16. Use of ovine tissue devoid of antibody-detectable Gal $\alpha(1,3)$ Gal determinants according to claim 1 or claim 2, for treatment of the human body by surgery or therapy.
17. A method of xenotransplantation, comprising transplanting tissue devoid of antibody-detectable Gal $\alpha(1,3)$ Gal determinants according to claim 1 or claim 2 into a mammal having circulating antibody against Gal $\alpha(1,3)$ Gal determinants.
18. An isolated polynucleotide that comprises a sequence of at least 30 consecutive nucleotides with at least one of the following properties:
 - a) it is contained in SEQ. ID NO:1 or any of SEQ. ID NOS:14 to 25, but not in any of SEQ. ID NOS: 3, 5, 7, 9, 11, and 13;
 - b) it is contained in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061; but not in λ -phage or any of SEQ. ID NOS: 3, 5, 7, 9, 11, and 13; or
 - c) it hybridizes under stringent conditions to a polynucleotide with the sequence in SEQ. ID NO:1 or any of SEQ. ID NOS:14 to 25, but not to a polynucleotide with the sequence in any of SEQ. ID NOS: 3, 5, 7, 9, 11, and 13
19. The isolated polynucleotide of claim 18, wherein said sequence is contained in SEQ. ID NO:1 or any of SEQ. ID NOS:14 to 25; or hybridizes under stringent conditions to a polynucleotide consisting of SEQ. ID NO:1 or any of SEQ. ID NOS:14 to 25 under stringent conditions; but wherein said sequence is not contained in any of SEQ. ID NOS: 3, 5, 7, 9, 11, and 13.
20. The isolated polynucleotide of claim 18, wherein said sequence is contained in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061; or hybridizes under stringent conditions to phage **B**, **C** and **G**; but wherein said sequence is not contained in λ -phage or any of SEQ. ID NOS: 3, 5, 7, 9, 11, and 13.

20. The isolated polynucleotide of claim 18, wherein said sequence hybridizes under stringent conditions to a polynucleotide with the sequence in SEQ. ID NO:1 or any of SEQ. ID NOs:14 to 25, but not to a polynucleotide with the sequence in any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13.
21. The polynucleotide of claims 18-20, which is a construct effective for inactivating a $\alpha(1,3)$ galactosyltransferase ($\alpha1,3GT$) gene in an ovine cell by homologous recombination.
22. An isolated polypeptide that comprises a sequence of at least 10 consecutive amino acids with at least one of the following properties:
 - a) it is contained in SEQ. ID NO:2 but not in any of SEQ. ID NOs: 4, 6, 8, 10, and 12;
 - b) it is encoded in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061., but not encoded in λ -phage or present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12; or
 - c) it is at least 80% identical to 15 consecutive amino acids contained in SEQ. ID NO:2, wherein said sequence is not present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12
23. The isolated polypeptide of claim 22, wherein said sequence is contained in SEQ. ID NO:2 but not in any of SEQ. ID NOs: 4, 6, 8, 10, and 12.
24. The isolated polypeptide of claim 22, wherein said sequence is encoded in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061., but not encoded in λ -phage or present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12.
25. The isolated polypeptide of claim 22, wherein said sequence is at least 80% identical to 15 consecutive amino acids contained in SEQ. ID NO:2, wherein said sequence is not present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12.
26. The polypeptide of claim 22, which has glycosyltransferase activity.
27. An isolated polynucleotide comprising a sequence encoding a polypeptide according to any of claims 22-26.
28. An isolated polyclonal antibody or a monoclonal antibody that binds specifically to a polypeptide with the sequence SEQ. ID NO:2 but not to a peptide with the sequence present in any of SEQ. ID NOs: 4, 6, 8, or 10.
29. An assay method for determining $\alpha1,3GT$ expression by a cell, comprising contacting a polynucleotide according to any of claims 18-21 with the cell or with mRNA or cDNA obtained from the cell, detecting any hybrids that form as a result, and correlating presence of the hybrids with expression of $\alpha1,3GT$ by the cell.
30. A method for producing the antibody specific for sheep $\alpha1,3GT$, comprising immunizing an animal or contacting an immunocompetent particle with a polypeptide according to any of claims 22-26.

31. A method for preparing a Gal α (1,3)Gal determinant, comprising contacting a galactose acceptor saccharide with the polypeptide of claim 25 or claim 26 in the presence of UDP-galactose.
32. An assay method for determining α 1,3GT in a sample, comprising preparing a reaction mixture comprising the sample and an antibody according to claim 28 under conditions that permit the antibody to complex with α 1,3GT, and correlating any complex formed with the presence or amount of α 1,3GT in the sample.

Figure 1(A)

5' -AGCCGAGGACGCCGCCGGGGAGCCGAGGCTCCGGCCAGCCCCAGCGCGCCAGCT
TCTGCAGATCAGGAGTCAGAACGCTGCACCTTCGCTTCCTCCCAGCCCTGCCTCCTTCTG
CAAAACGGAGCTCAATAGAACTTGGTACTTTTGCCTTTTACTCTGGGAGGAGAGAAGCAG
ACGATGAG | 4 | GAGAAAATA ATGAATGTCAAAGGAAAAGTGATTCTGTCAATGCTGGTTG
M N V K G K V I L S M L V V
TCTCAACTGTCATTGTTGTGTTTGGGAATATATCCACAG | 5 | CCCAGAAGGCTCTTTGT
S T V I V V F W E Y I H S P E G S L F
TCTGGATAAACCCATCAAG | 6 | AAACCCAGAAGTCAGTGGCGGCAGCAGCATTCAAGAGG
W I N P S R N P E V S G G S S I Q K G
GCTGGTGGTTTCCGAGATGGTTTAAACAATGG | 7 | TTACCAAGAAGAAGATGAAGACGTAG
W W F P R W F N N G Y Q E E D E D V D
ACGAAGAAAAGGAACAAAGAAAGGAAGACAAAAGCAAGCTTAAGCTATCGGACTGGTTCA
E E K E Q R K E D K S K L K L S D W F N
ACCCATT | 8 | TAAACGCCCTGAGGTTGTGACTATGACAGATTGGAAGGCACCCGTGGTGT
P F K R P E V V T M T D W K A P V V W
GGGAAGGCACCTTACAACAGAGCCGTCTTAGACGATTACTACGCCAAGCAGAAAATTACCG
E G T Y N R A V L D D Y Y A K Q K I T V
TCGGCCTGACGGTTTTCGCCGTCGGAA | 9 | GATACATTGAGCATTACTTGGAGGAGTTCT
G L T V F A V G R Y I E H Y L E E F L
TAACGTCTGCTAATAAGCAC'TTCATGGTTGGCCACCGAGTCATCTTTTACGTCATGGTGG
T S A N K H F M V G H R V I F Y V M V D
ACGACGTCTCCAGGATGCCTTTGATAGAGCTGGGCCCTCTGCGCTCCTTCAAAGTGTTTG
D V S R M P L I E L G P L R S F K V F E
AGGTCAAGCCTGAGAGGAGGTGGCAGGACGTCAGCATGGTGCATGAAGACCATCGGGG
V K P E R R W Q D V S M V R M K T I G E
AGCACATCGTGGCCACATCCAGCGTGAGGTTGACTTCCTCTTCTGCATGGACGTGGACC
H I V A H I Q R E V D F L F C M D V D Q

Figure 1(B)

AGGTCTTCCAAGACGAGTTTCGGGGTGGAGACCCTGGGTGAGTCGGTGGCCCAGCTACAGG
V F Q D E F G V E T L G E S V A Q L Q A

CCTGGTGGTACAAGGCAGATCCCGATGAGTTTACCTACGAGAGGCGCAAGGAGTCTGCAG
W W Y K A D P D E F T Y E R R K E S A A

CATACATTCCCTTCGGCGAAGGGGATTTTTATTACCACGCAGCCATTTTTGGGGGAACAC
Y I P F G E G D F Y Y H A A I F G G T P

CCACTCAGGTCCTTAACATCACCCAGGAATGCTTCAAAGGAATCCTCAAGGACAAGAAAA
T Q V L N I T Q E C F K G I L K D K K N

ATGACATAGAAGCCCAATGGCATGATGAGAGCCATCTAAACAAGTATTTCTTCTCAACA
D I E A Q W H D E S H L N K Y F L L N K

AACCCACTAAAATCTTATCCCCGGAATACTGCTGGGATTATCATATAGGCCTACCTGCGG
P T K I L S P E Y C W D Y H I G L P A D

ATATTAAAGCTTGTCAGATGTCTTGGCAGACAAAAGAGTATAATGTGGTTAGAAATAACG
I K L V K M S W Q T K E Y N V V R N N V

TCTGAAGCCGAGGACGCCCGGGGAGCCGAGGCTCCGCCAGCCCCAGCGCGCCAGC

TTCTGCAGATCAGGAGTCAGAACGCTGCACCTTCGCTTCCTCCCAGCCCTGCCTCCTTCT

GCAAAACGGAGCTCAATAGAACTTGGTACTTTTGCCTTTTACTCTGGGAGGAGAGAAGCA

GACGATGAGGAGAAAATA

Figure 2(A)

```
sheep -----
bovine CCGGGGGCCGGGCCGAGCTGGGAGCGTCGAGCCCGCTGCCCAGCGCCCGCCGGCTCCCTC

sheep -----
bovine GCGCCCCCTGCCCGCCGCCCCGGAGGAGCGCCCGGCGGCCGGCCGACGGGAGCGCAGCGGC

sheep -----
bovine ACACCCCGCCCCGGCAGCCCCGCGGGGCTCGGGAGGAGGCAGCGCGCCGACTGTTCGGC

sheep AGCCGAGGACGCCCGCGGGGAGCCGAGGCTCCGGCCAGCCCCAGCGCGCCAGCTTCTG
bovine AGCCGAGGACGCCCGCGGGGAGCCGAGGCGCCGCGCCAGCCCCAGCGCGCCAGCTTCTG
*****

sheep CAGATCAGG-----
bovine CGGATCAGGAAACACGTGTCCTCAAGTGGCCAGCCAGCTGTCCCCAAGAGGAACTTGC
* *****

sheep -----AGTCAGAACGCTGCAC
bovine CTGGCATTTCACGGAAGACGAGACACTTCACAAAATCAACGGAGTCAGAAGGCTGCAC
*****

sheep CTTCGCTTCCTCCCAGCCCTGCCTCCTTCTGCAAAACGGAGCTCAATAGAACTTGGTACT
bovine CTTCGCTTCCTCCCAGCCCTGCCTCCTTCTGCAAAACGGAGCTCAGTAGAACTTGGTACT
*****
↓
sheep TTTGCCCTTTTACTCTGGGAGGAGAGAAGCAGACGATGAGGAGAAAATAATGAATGTCAAA
bovine TTTGCCCTTTTACTCTAGGAGGAGAGAAGCAGACGATGAGGAGAAAATAATGAATGTCAAA
*****

sheep GGAAAAGTGATTCTGTCAATGCTGGTTGTCTCAACTGTCATTGTTGTGTTTGGGAATAT
bovine GGAAAAGTGATTCTGTCAATGCTGGTTGTCTCAACTGTCATTGTTGTGTTTGGGAATAT
*****

sheep ATCCACAGCCCAGAAGGCTCTTTGTTCTGGATAAACCCATCAAGAAACCCAGAAGTCAGT
bovine ATCCACAGCCCAGAAGGCTCTTTGTTCTGGATAAACCCATCAAGAAACCCAGAAGT---T
*****

sheep GCGGGCAGCAGCATTCAGAAGGGCTGGTGGTTTCCGAGATGGTTTAACAATGGTTACCAA
bovine GGTGGCAGCAGCATTCAGAAGGGCTGGTGGCTTCCGAGATGGTTTAACAATGGTTACCAT
** *****
```

Figure 2(B)

```
sheep      GAAGAAGATGAAGACGTAGACGAAGAAAAGGAACAAAGAAAGGAAGACAAAAGCAAGCTT
bovine     GAAGAAGATGGAGACATAAACGAAGAAAAGGAACAAAGAAACGAAGACGAAAGCAAGCTT
          ***** ** *****

sheep      AAGCTATCGGACTGGTTCAACCCATTTAAACGCCCTGAGGTTGTGACTATGACAGATTGG
bovine     AAGCTATCGGACTGGTTCAACCCATTTAAACGCCCGAGGTTGTGACCATGACGAAGTGG
          ***** ***** *

sheep      AAGGCACCCGTGGTGTGGGAAGGCACCTTACAACAGAGCCGTCTTAGACGATTACTACGCC
bovine     AAGGCTCCAGTGGTGTGGGAAGGCACCTTACAACAGAGCCGTCTTAGACAATTATTATGCC
          ***** ** *****

sheep      AAGCAGAAAATTACCGTCGGCCTGACGGTTTTCGCCGTCGGAAGATACATTGAGCATTAC
bovine     AAGCAGAAAATTACCGTCGGCCTGACGGTTTTCGCCGTCGGAAGATACATTGAGCATTAC
          *****

sheep      TTGGAGGAGTTCTTAACGTCCTGCTAATAAGCACTTCATGGTTGGCCACCGAGTCATCTTT
bovine     TTGGAGGAGTTCTTAACGTCCTGCTAATAAGCACTTCATGGTTGGCCACCGAGTCATCTTT
          *****

sheep      TACGTCATGGTGGACGACGTCCTCCAGGATGCCTTTGATAGAGCTGGGCCCTCTGCGCTCC
bovine     TATATCATGGTAGATGATGCTCCAGGATGCCTTTGATAGAGTTGGGTCCCTCTGCGCTCC
          ** *****

sheep      TTCAAAGTGTTTGAGGTCAAGCCTGAGAGGAGGTGGCAGGACGTCAGCATGGTGCGCATG
bovine     TTCAAAGTGTTTAAGATCAAGCCTGAGAAGAGGTGGCAGGACATCAGCATGATGCGCATG
          *****

sheep      AAGACCATCGGGGAGCACATCGTGGCCACATCCAGCGTGAGGTTGACTTCCTCTTCTGC
bovine     AAGACTATCGGGGAGCACATGTGGCCACATCCAGCATGAGGTTGACTTCCTTTTCTGC
          *****

sheep      ATGGACGTGGACCAGGTCTTCCAAGACGAGTTCGGGGTGGAGACCTGGGTGAGTCGGTG
bovine     ATGGATGTGGACCAGGTCTTCCAAGACAAGTTTGGGGTGGAGACCTGGGCGAGTCGGTG
          *****

sheep      GCCCAGCTACAGGCCCTGGTGGTACAAGGCAGATCCCAGATGAGTTTACCTACGAGAGGCGC
bovine     GCCCAGCTACAAGCCTGGTGGTACAAGGCAGATCCCAATGACTTCACCTACGAGAGGCGC
          *****

sheep      AAGGAGTCTGCAGCATACATTCCCTTCGGCGAAGGGGATTTTATTACCACGCAGCCATT
bovine     AAGGAGTCTGCAGCATACATTCCCTTCGGCGAAGGGGATTTTATTACCATGCAGCCATT
          *****
```

Figure 2(C)

sheep	TTTGGGGGAACACCCACTCAGGTCCTTAACATCACCCAGGAATGCTTCAAAGGAATCCTC
bovine	TTTGGGGGAACACCCACTCAGGTCCTTAACATCACCCAGGAATGCTTCAAAGGAATCCTC

sheep	AAGGACAAGAAAAATGACATAGAAGCCCAATGGCATGATGAGAGCCATCTAAACAAGTAT
bovine	AAGGACAAGAAAAATGACATAGAAGCCCAATGGCATGATGAAAGCCATCTAAACAAGTAT

sheep	TTCCTTCTCAACAAACCCACTAAAATCTTATCCCCGGAATACTGCTGGGATTATCATATA
bovine	TTCCTTCTCAACAAACCTACTAAAATCTTATCCCCGGAATACTGCTGGGATTATCACATA

sheep	GGCCTACCTGCGGATATTAAGCTTGTCAGATGTCCTTGGCAGACAAAAGAGTATAATGTG
bovine	GGCCTACCTGCGGATATTAAGCTTGTCAGATGTCCTTGGCAGACAAAAGAGTATAATGTG

sheep	GTTAGAAATAACGTCTGA-----
bovine	GTTAGAAATAATGTCTGACTTTGTGCCAGTACATTTCTGAATTGAGAGAGTATTATTCT

Figure 3

	1				50
bovine	MNVKGVILS	MLVVSTVIVV	FWEYIHSPEG	SLFWINPSRN	PEV.GGSSIQ
sheep	MNVKGVILS	MLVVSTVIVV	FWEYIHSPEG	SLFWINPSRN	PEVSGGSSIQ
	*****	*****	*****	*****	*** *****
	51				100
bovine	KGWWLPRWFN	NGYHEEDGDI	NEEKEQRNED	ESKLKLSDF	NPFKRPEVVT
sheep	KGWWFPRWFN	NGYQEEDEDV	DEEKEQRKED	KSKLKLSDF	NPFKRPEVVT
	****	*****	*****	*****	*****
	101				150
bovine	MTKWKAPVVW	EGTYNRAVL	NYAKQKITV	GLTVFAVGRY	IEHYLEEFIT
sheep	MTDWKAPVVW	EGTYNRAVL	DYAKQKITV	GLTVFAVGRY	IEHYLEEFIT
	**	*****	*****	*****	*****
	151				200
bovine	SANKHFMVGH	PVIFYIMVDD	VSRMPLIELG	PLRSFKVFKI	KPEKRWDIS
sheep	SANKHFMVGH	RVIFYVMVDD	VSRMPLIELG	PLRSFKVFEV	KPERRWQDVS
	*****	****	*****	*****	*** **** *
	201				250
bovine	MMRMKTIGEH	IVAHIQHEVD	FLFCMDVDQV	FQDKFGVETL	GESVAQLQAW
sheep	MVRMKTIGEH	IVAHIQREVD	FLFCMDVDQV	FQDEFGVETL	GESVAQLQAW
	*****	*****	*****	*****	*****
	251				300
bovine	WYKADPNDFI	YERRKESAAY	IPFGEQDFYY	HAAIFGGTPT	QVLNITQECF
sheep	WYKADPDEFT	YERRKESAAY	IPFGEQDFYY	HAAIFGGTPT	QVLNITQECF
	*****	*****	*****	*****	*****
	301				350
bovine	KGILKDKKND	IEAQWHDESH	LNKYFLLNKP	TKILSPEYCW	DYHIGLPADI
sheep	KGILKDKKND	IEAQWHDESH	LNKYFLLNKP	TKILSPEYCW	DYHIGLPADI
	*****	*****	*****	*****	*****
	351		370		
bovine	KLVKMSWQTK	EYNVVRNNV*			
sheep	KLVKMSWQTK	EYNVVRNNV*			
	*****	*****			

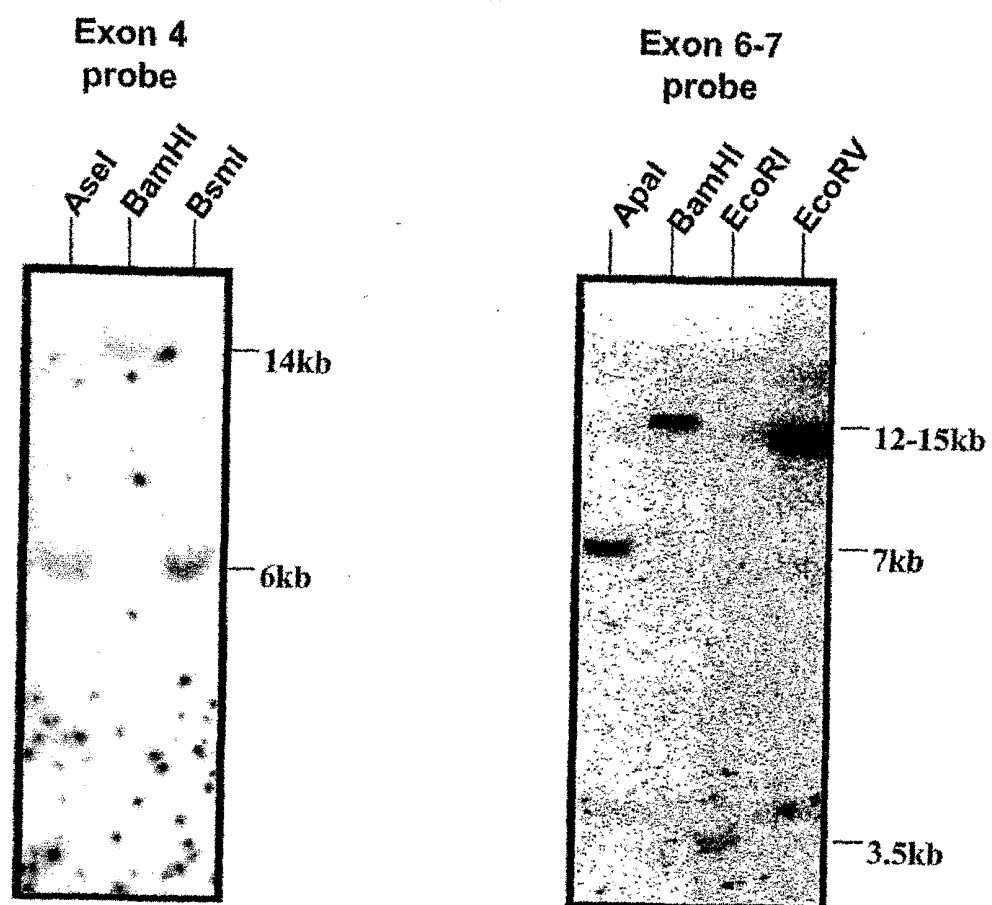
Figure 4

Figure 5

$\alpha(1,3)$ -GT



GapDH

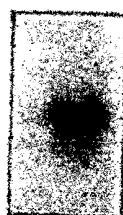


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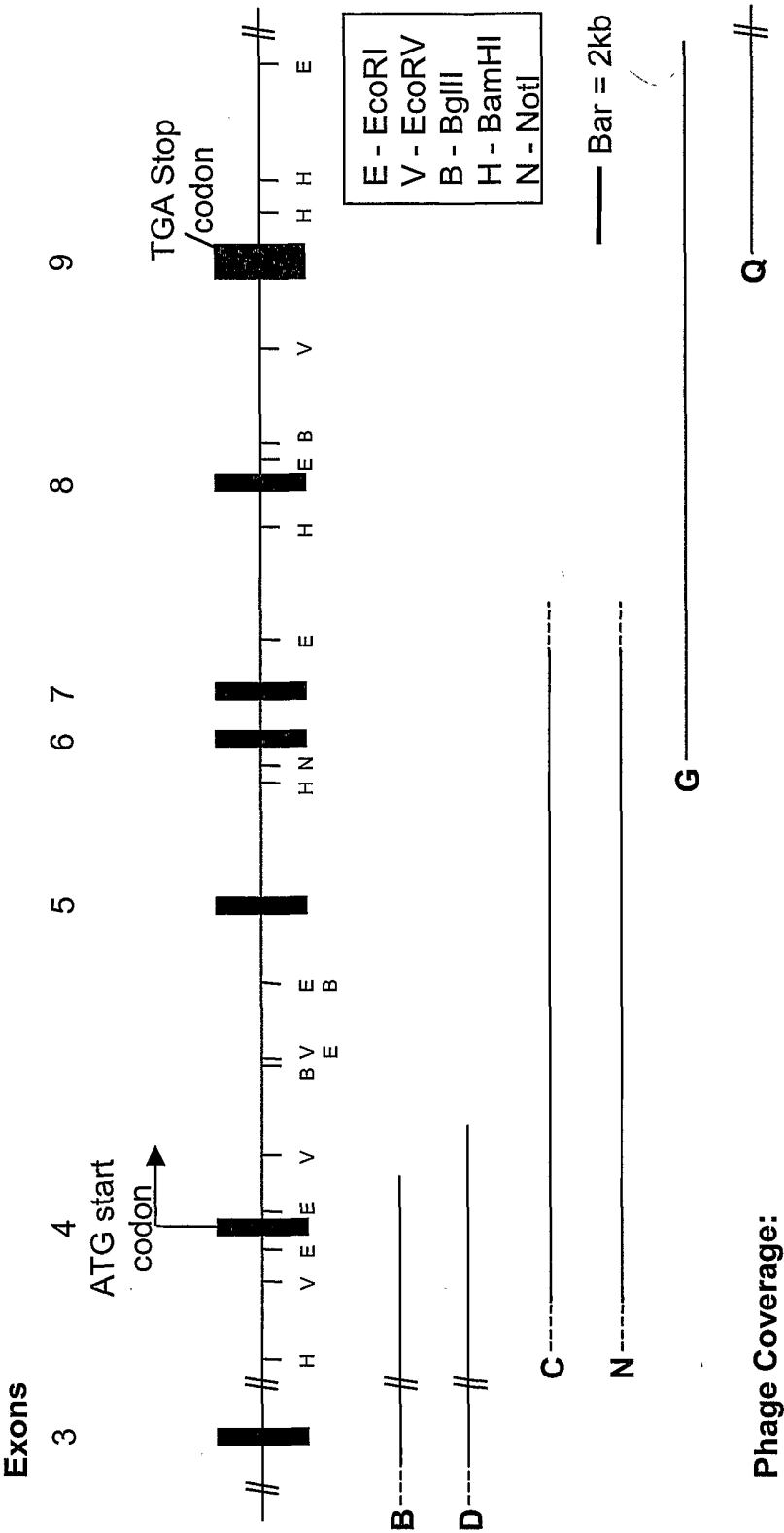


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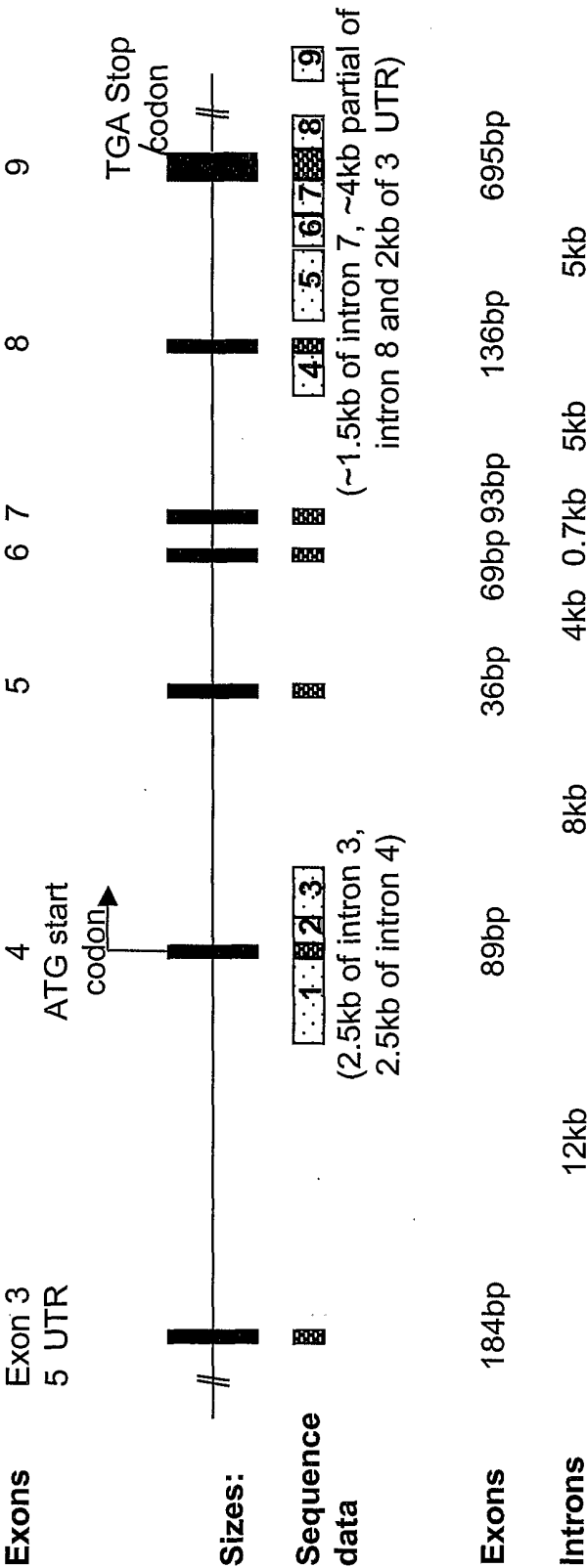


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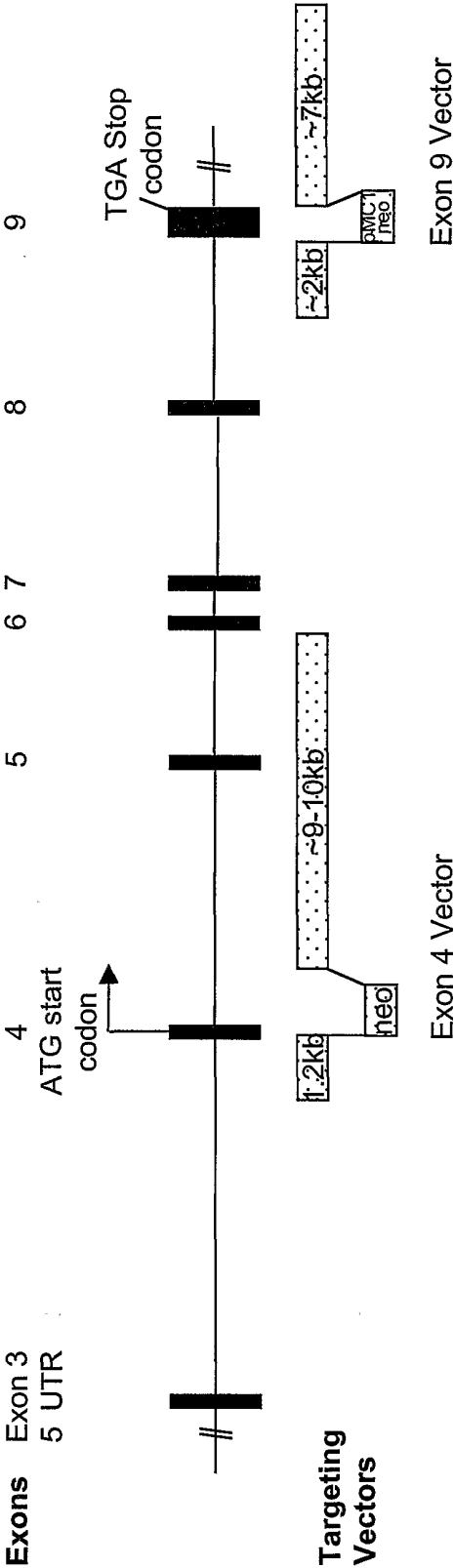


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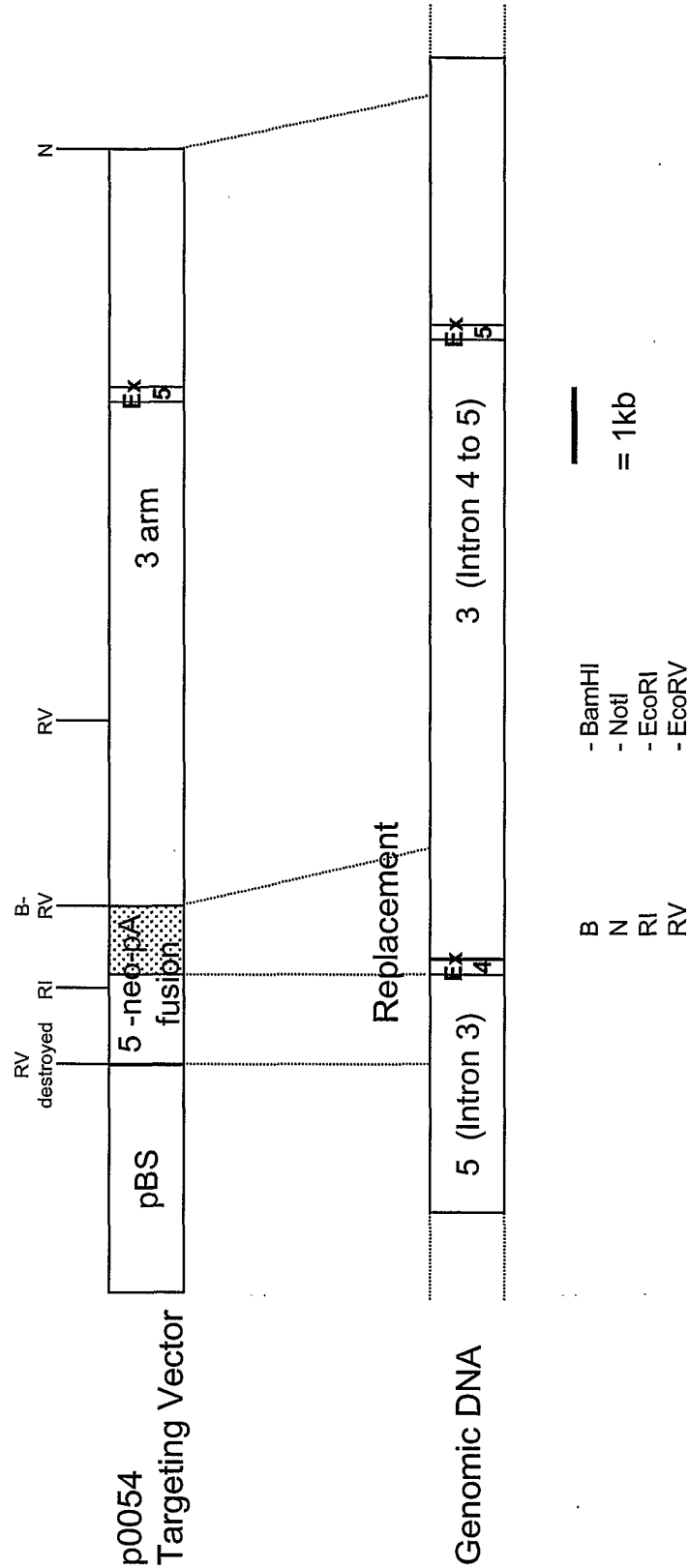


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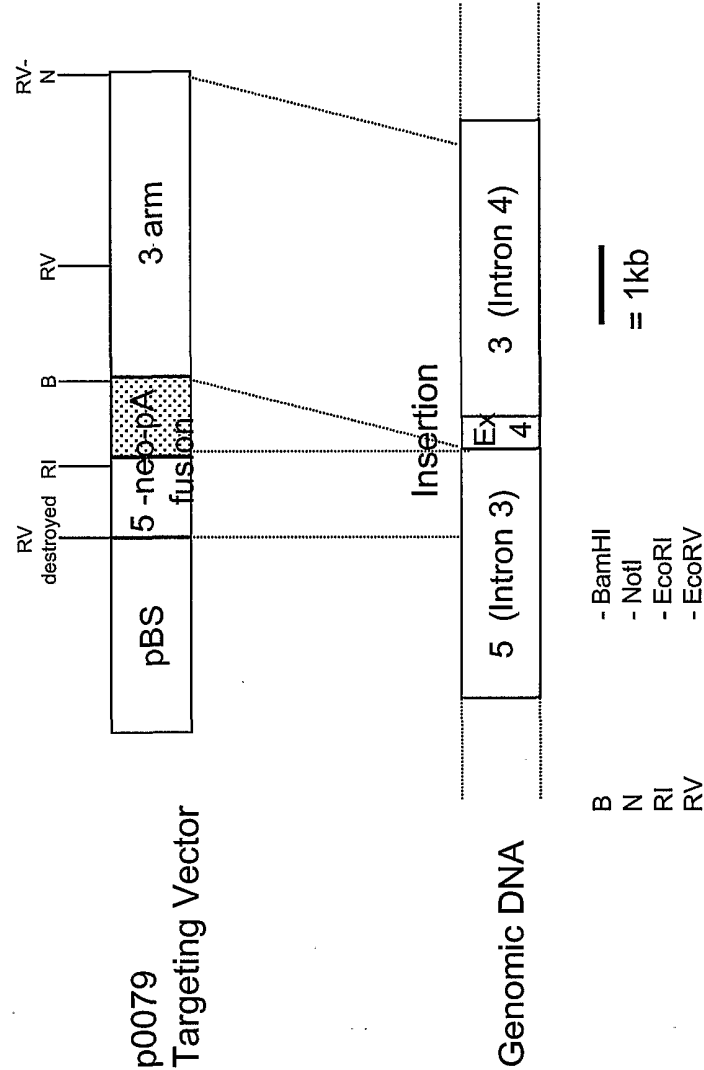


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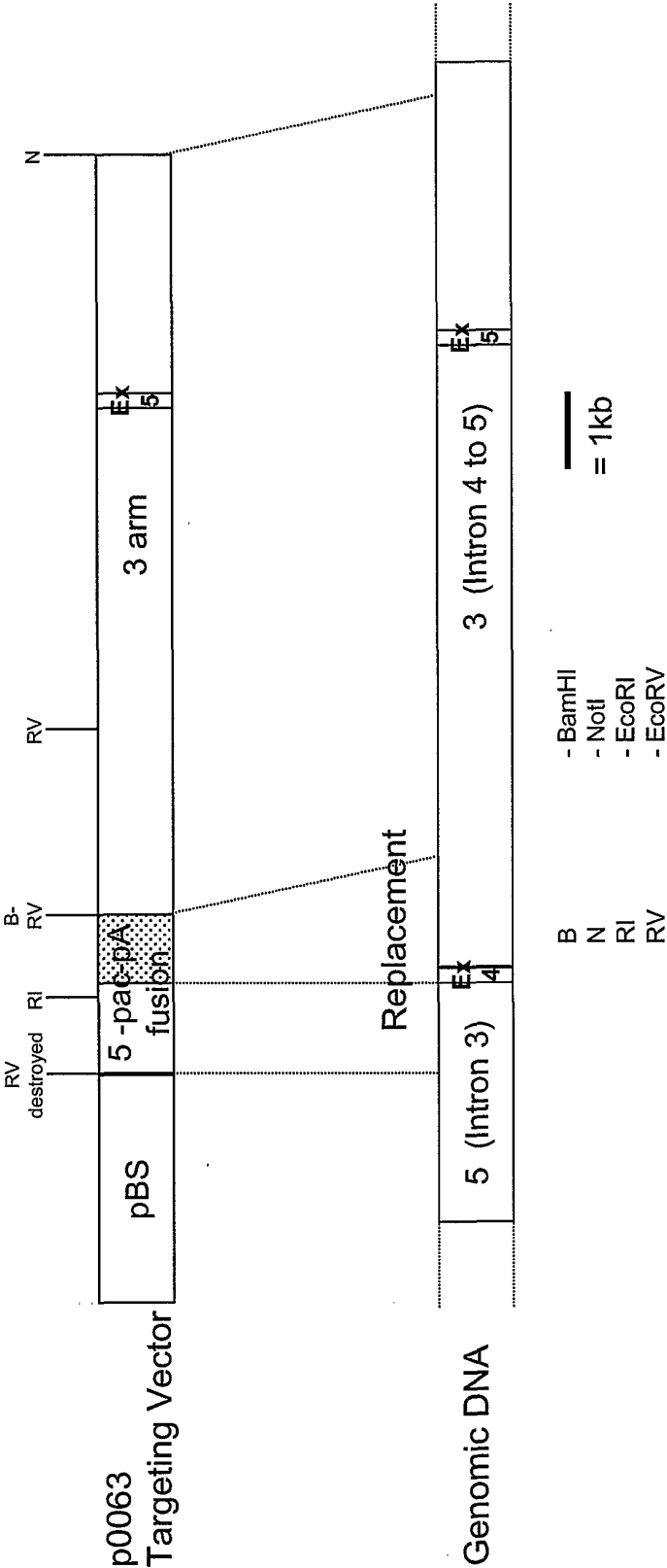


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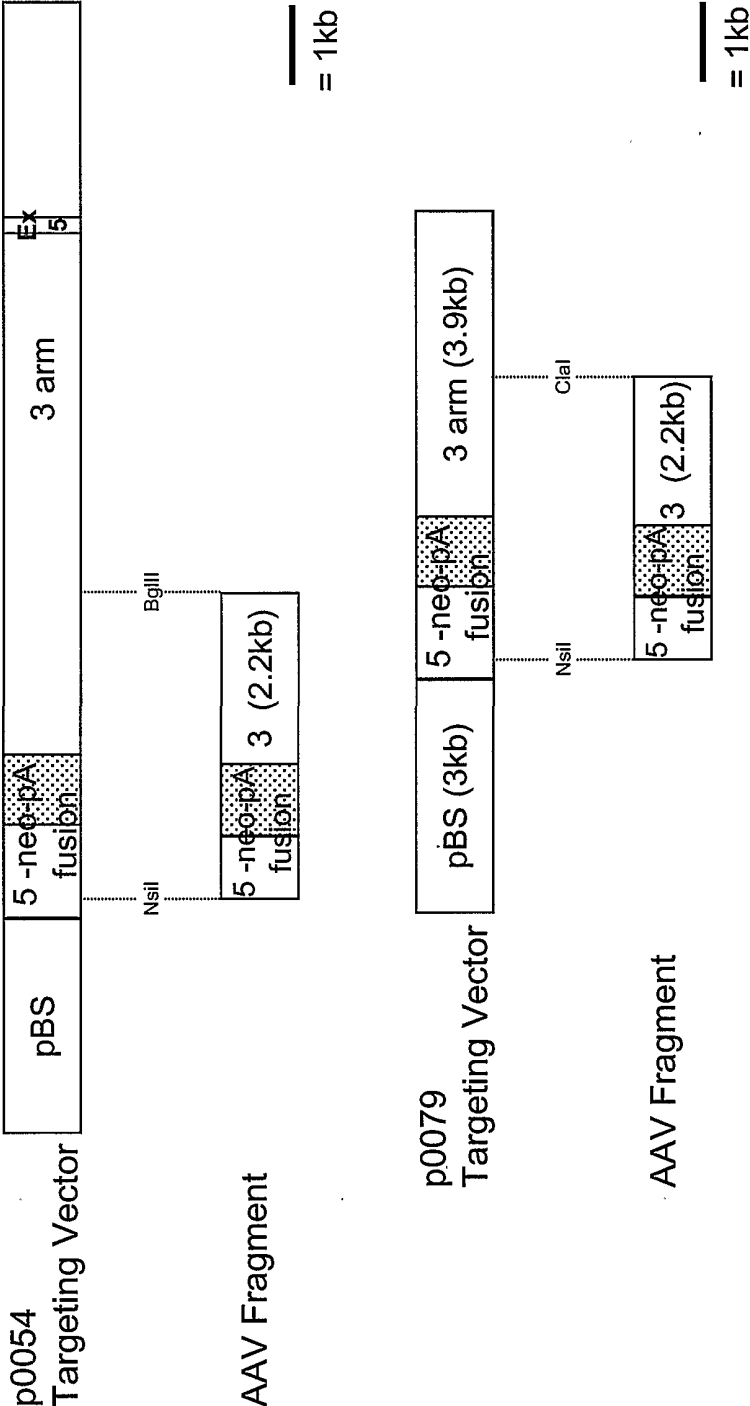


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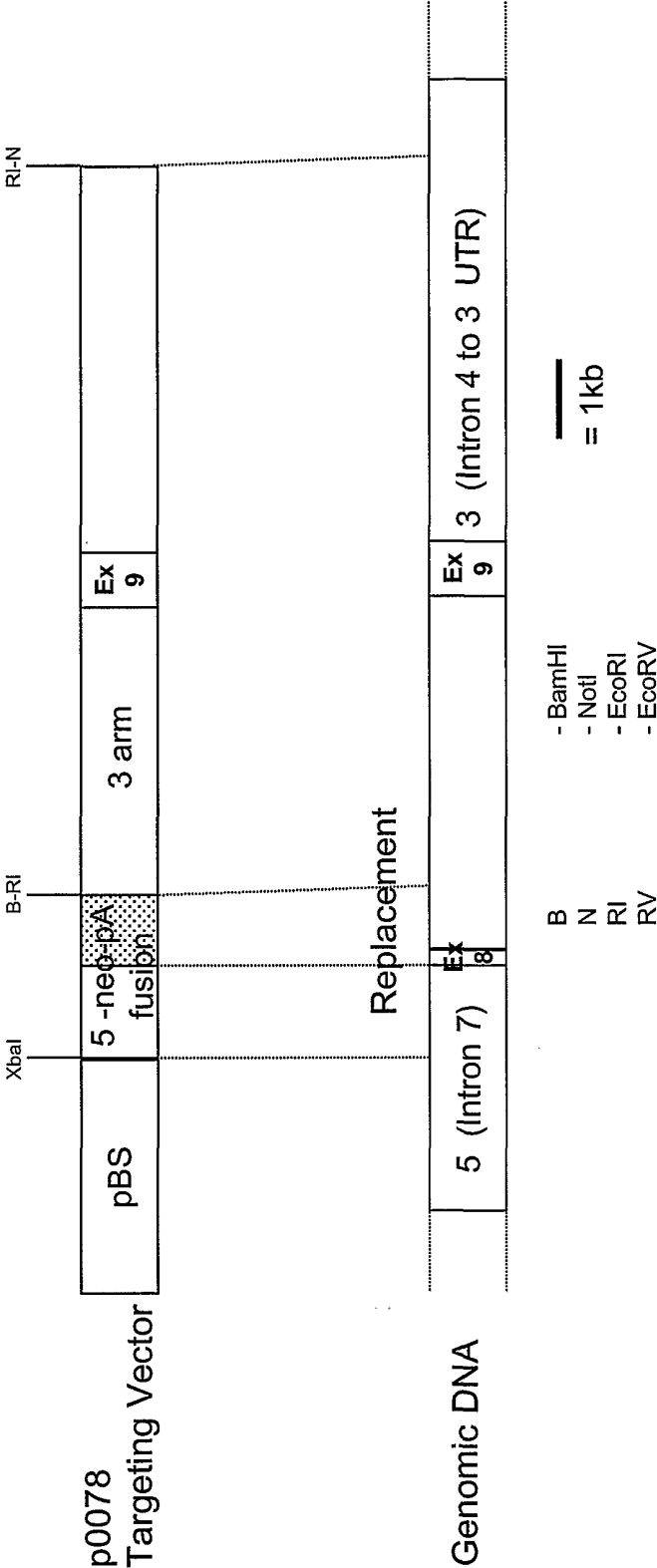


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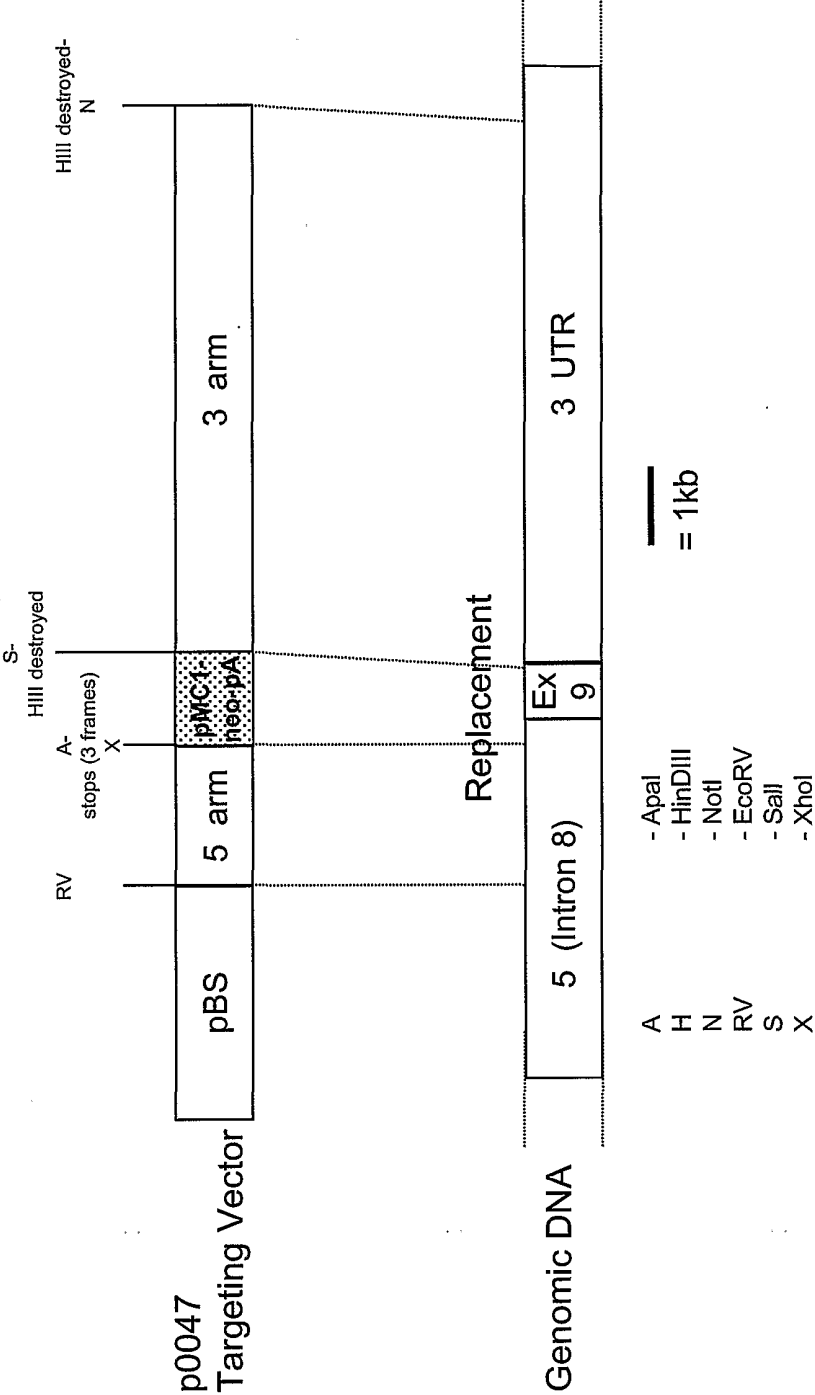


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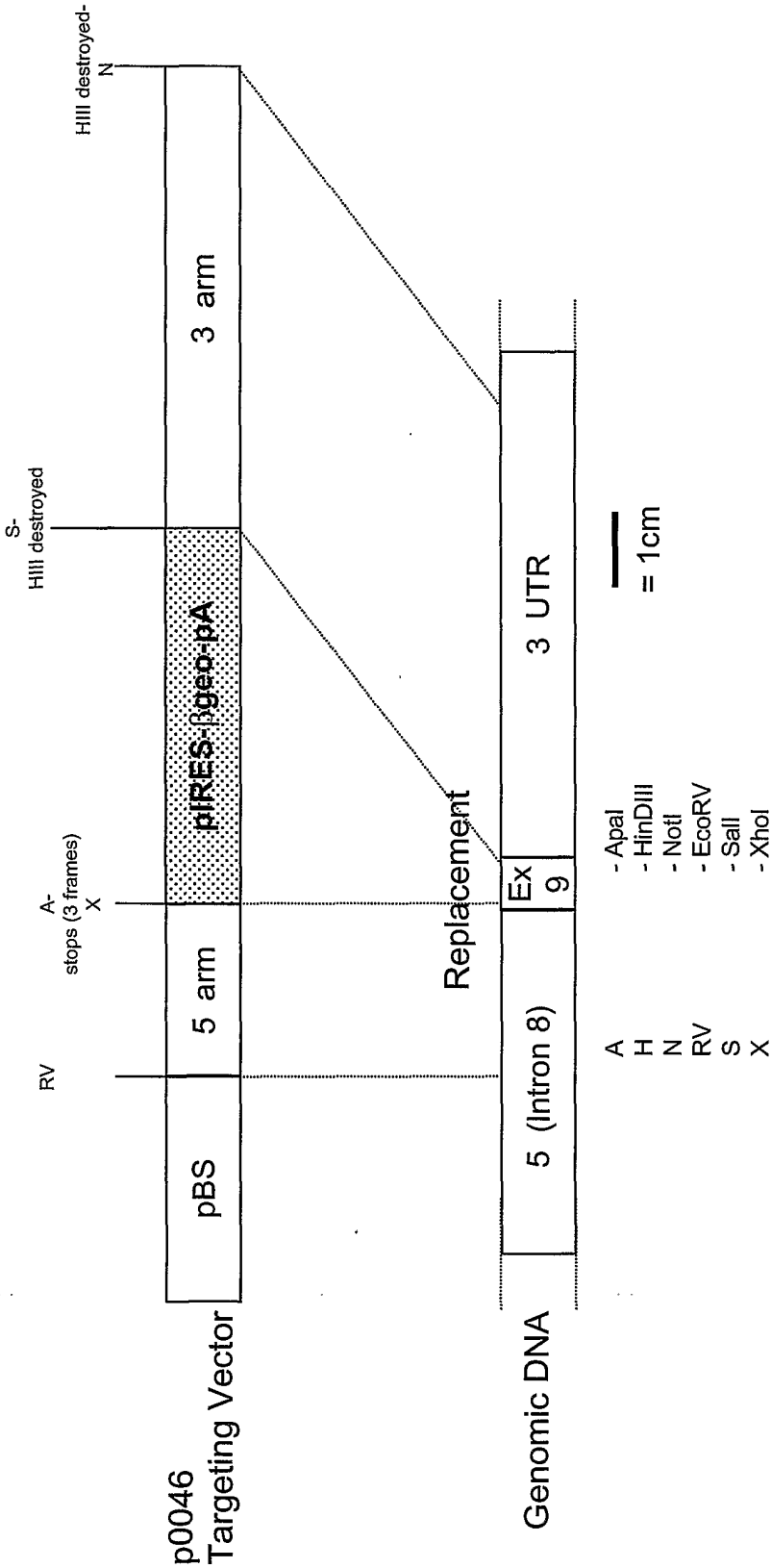


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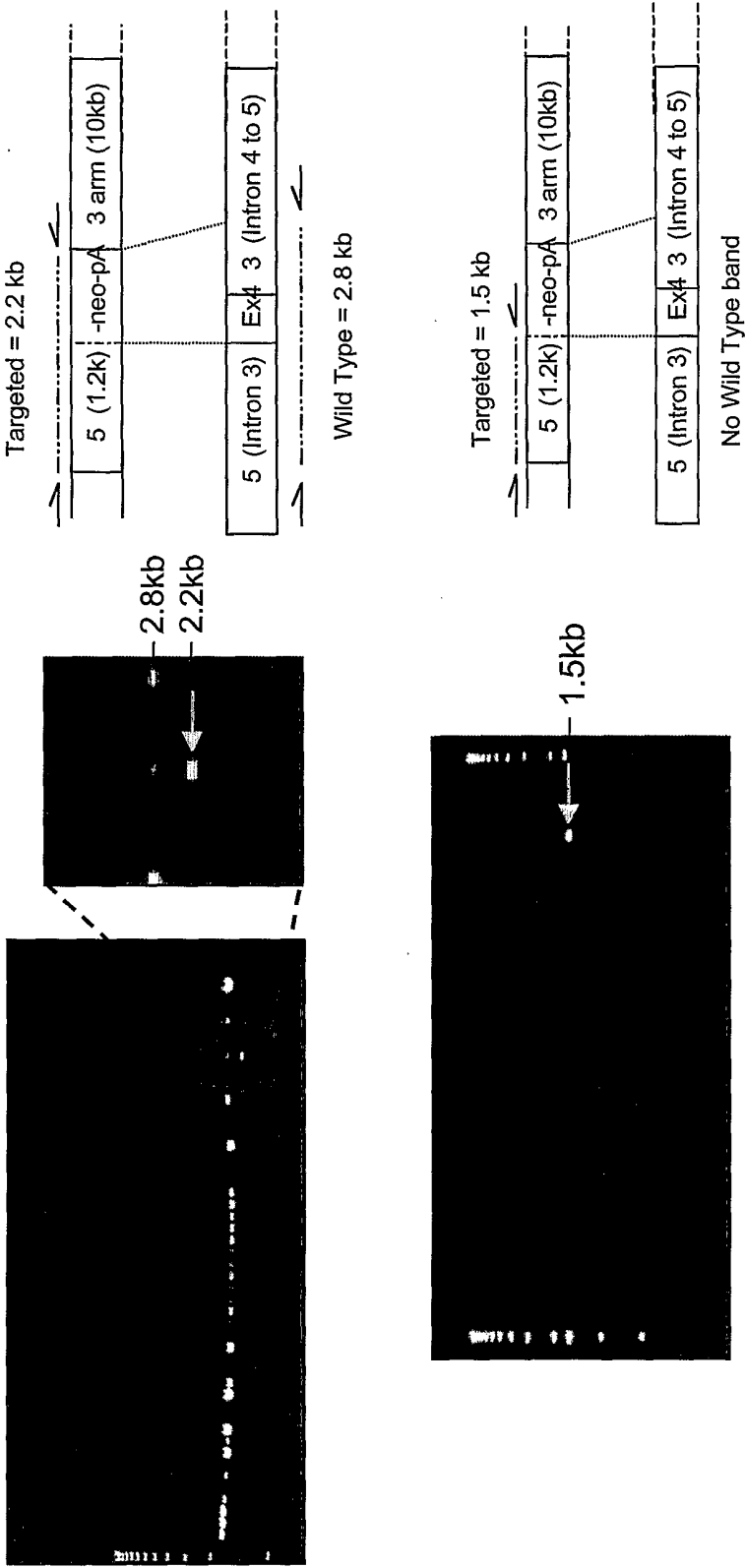
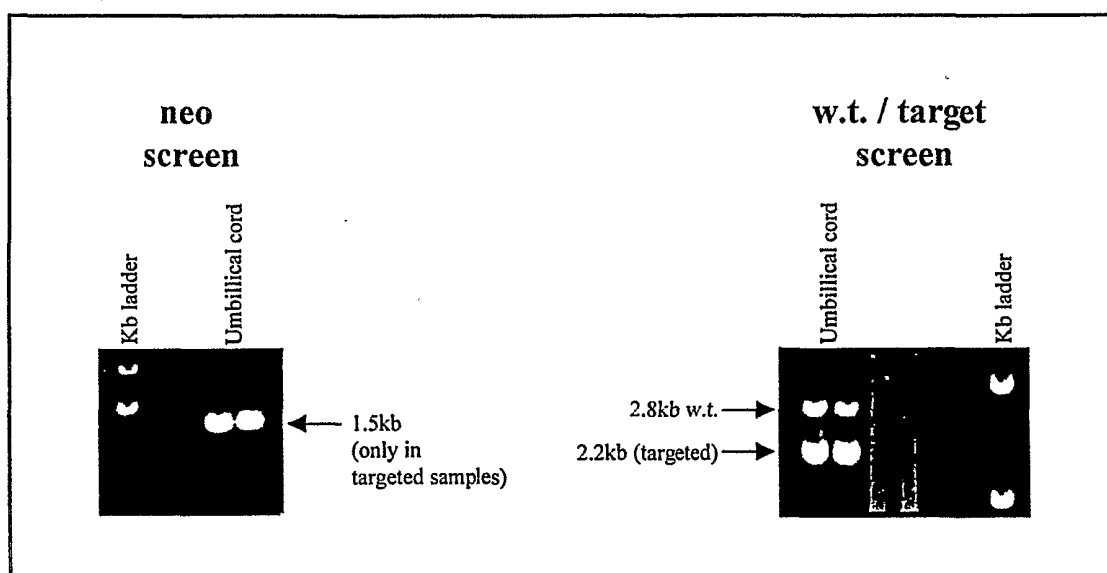


Figure 17

SEQUENCE LISTING

<110> Denning, Chris
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               95           100           105
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Val Trp Glu Gly Thr Tyr Asn Arg Ala Val Leu Asp Asp Tyr Tyr Ala
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Lys Gln Lys Ile Thr Val Gly Leu Thr Val Phe Ala Val Gly Arg Tyr
               125           130           135           140
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               175           180           185
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Phe Leu Phe Cys Met Asp Val Asp Gln Val Phe Gln Asp Glu Phe Gly
          225          230          235
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Val Glu Thr Leu Gly Glu Ser Val Ala Gln Leu Gln Ala Trp Trp Tyr
          240          245          250
aag gca gat ccc gat gag ttt acc tac gag agg cgc aag gag tct gca 997
Lys Ala Asp Pro Asp Glu Phe Thr Tyr Glu Arg Arg Lys Glu Ser Ala
          255          260          265
gca tac att ccc ttc ggc gaa ggg gat ttt tat tac cac gca gcc att 1045
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          270          275          280
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Phe Gly Gly Thr Pro Thr Gln Val Leu Asn Ile Thr Gln Glu Cys Phe
285          290          295          300
aaa gga atc ctc aag gac aag aaa aat gac ata gaa gcc caa tgg cat 1141
Lys Gly Ile Leu Lys Asp Lys Lys Asn Asp Ile Glu Ala Gln Trp His
          305          310          315
gat gag agc cat cta aac aag tat ttc ctt ctc aac aaa ccc act aaa 1189
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          320          325          330
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Phe Trp Ile Asn Pro Ser Arg Asn Pro Glu Val Ser Gly Gly Ser Ser
          35          40          45
Ile Gln Lys Gly Trp Trp Phe Pro Arg Trp Phe Asn Asn Gly Tyr Gln
          50          55          60
Glu Glu Asp Glu Asp Val Asp Glu Glu Lys Glu Gln Arg Lys Glu Asp
          65          70          75          80
Lys Ser Lys Leu Lys Leu Ser Asp Trp Phe Asn Pro Phe Lys Arg Pro
          85          90          95
Glu Val Val Thr Met Thr Asp Trp Lys Ala Pro Val Val Trp Glu Gly
          100          105          110
Thr Tyr Asn Arg Ala Val Leu Asp Asp Tyr Tyr Ala Lys Gln Lys Ile
          115          120          125
Thr Val Gly Leu Thr Val Phe Ala Val Gly Arg Tyr Ile Glu His Tyr
          130          135          140
Leu Glu Glu Phe Leu Thr Ser Ala Asn Lys His Phe Met Val Gly His
          145          150          155          160
Arg Val Ile Phe Tyr Val Met Val Asp Asp Val Ser Arg Met Pro Leu
          165          170          175
Ile Glu Leu Gly Pro Leu Arg Ser Phe Lys Val Phe Glu Val Lys Pro
          180          185          190
Glu Arg Arg Trp Gln Asp Val Ser Met Val Arg Met Lys Thr Ile Gly
          195          200          205
Glu His Ile Val Ala His Ile Gln Arg Glu Val Asp Phe Leu Phe Cys
          210          215          220
Met Asp Val Asp Gln Val Phe Gln Asp Glu Phe Gly Val Glu Thr Leu
          225          230          235          240
Gly Glu Ser Val Ala Gln Leu Gln Ala Trp Trp Tyr Lys Ala Asp Pro

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245 250 255
 Asp Glu Phe Thr Tyr Glu Arg Arg Lys Glu Ser Ala Ala Tyr Ile Pro
 260 265 270
 Phe Gly Glu Gly Asp Phe Tyr Tyr His Ala Ala Ile Phe Gly Gly Thr
 275 280 285
 Pro Thr Gln Val Leu Asn Ile Thr Gln Glu Cys Phe Lys Gly Ile Leu
 290 295 300
 Lys Asp Lys Lys Asn Asp Ile Glu Ala Gln Trp His Asp Glu Ser His
 305 310 315 320
 Leu Asn Lys Tyr Phe Leu Leu Asn Lys Pro Thr Lys Ile Leu Ser Pro
 325 330 335
 Glu Tyr Cys Trp Asp Tyr His Ile Gly Leu Pro Ala Asp Ile Lys Leu
 340 345 350
 Val Lys Met Ser Trp Gln Thr Lys Glu Tyr Asn Val Val Arg Asn Asn
 355 360 365
 Val

<210> 3
 <211> 1617
 <212> DNA
 <213> BOVINE
 <220>
 <221> CDS
 <222> (469)..(1575)
 <400> 3

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 gcgcccctgc ccgcccgcggc ggaggagcgc ccggcgggcg gccgacggga gcgcagcggc 120
 acaccccgcc ccggcacgcc cgcggggctc gggaggaggc agcgcgccga ctgttccggc 180
 agccgaggac gccgcccggg agccgaggcg ccggccagcc ccagcgcgcc ccagcttctg 240
 cggatcaggg aaaccacgtg tcctcaagtg gccagccagc tgtccccaag aggaacttgc 300
 ctggcatttg cacggaaaaga cgagacactt cacaaaatca acggagtcag aaggctgcac 360
 cttcgcttcc tcccagccct gcctccttct gcagaacgga gctcagtaga acttgggtact 420
 tttgcctttt actctaggag gagagaagca gacgatgagg agaaaata atg aat gtc 477
 Met Asn Val
 1
 aaa gga aaa gtg att ctg tca atg ctg gtt gtc tca act gtc att gtt 525
 Lys Gly Lys Val Ile Leu Ser Met Leu Val Val Ser Thr Val Ile Val
 5 10 15
 gtg ttt tgg gaa tat atc cac agc cca gaa ggc tct ttg ttc tgg ata 573
 Val Phe Trp Glu Tyr Ile His Ser Pro Glu Gly Ser Leu Phe Trp Ile
 20 25 30 35
 aac cca tca aga aac cca gaa gtt ggt ggc agc agc att cag aag ggc 621
 Asn Pro Ser Arg Asn Pro Glu Val Gly Gly Ser Ser Ile Gln Lys Gly
 40 45 50
 tgg tgg ctt ccg aga tgg ttt aac aat ggt tac cat gaa gaa gat gga 669
 Trp Trp Leu Pro Arg Trp Phe Asn Asn Gly Tyr His Glu Glu Asp Gly
 55 60 65
 gac ata aac gaa gaa aag gaa caa aga aac gaa gac gaa agc aag ctt 717
 Asp Ile Asn Glu Glu Lys Glu Gln Arg Asn Glu Asp Glu Ser Lys Leu
 70 75 80
 aag cta tcg gac tgg ttc aac cca ttt aaa cgc ccc gag gtt gtg acc 765
 Lys Leu Ser Asp Trp Phe Asn Pro Phe Lys Arg Pro Glu Val Val Thr
 85 90 95
 atg acg aag tgg aag gct cca gtg gtg tgg gaa ggc act tac aac aga 813
 Met Thr Lys Trp Lys Ala Pro Val Val Trp Glu Gly Thr Tyr Asn Arg
 100 105 110 115
 gcc gtc tta gac aat tat tat gcc aag cag aaa att acc gtc gcc ctg 861
 Ala Val Leu Asp Asn Tyr Tyr Ala Lys Gln Lys Ile Thr Val Gly Leu
 120 125 130
 acg gtt ttc gcc gtc gga aga tac att gag cat tac ttg gag gag ttc 909
 Thr Val Phe Ala Val Gly Arg Tyr Ile Glu His Tyr Leu Glu Glu Phe
 135 140 145
 tta acg tct gct aat aag cac ttc atg gtg ggc cac cca gtc atc ttt 957
 Leu Thr Ser Ala Asn Lys His Phe Met Val Gly His Pro Val Ile Phe
 150 155 160
 tat atc atg gta gat gat gtc tcc agg atg cct ttg ata gag ttg ggt 1005
 Tyr Ile Met Val Asp Asp Val Ser Arg Met Pro Leu Ile Glu Leu Gly
 165 170 175

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cct ctg cgc tcc ttc aaa gtg ttt aag atc aag cct gag aag agg tgg 1053
Pro Leu Arg Ser Phe Lys Val Phe Lys Ile Lys Pro Glu Lys Arg Trp
180 185 190 195
cag gac atc agc atg atg cgc atg aag act atc ggg gag cac att gtg 1101
Gln Asp Ile Ser Met Met Arg Met Lys Thr Ile Gly Glu His Ile Val
200 205 210
gcc cac atc cag cat gag gtt gac ttc ctt ttc tgc atg gat gtg gac 1149
Ala His Ile Gln His Glu Val Asp Phe Leu Phe Cys Met Asp Val Asp
215 220 225
cag gtc ttc caa gac aag ttt ggg gtg gag acc ctg ggc gag tcg gtg 1197
Gln Val Phe Gln Asp Lys Phe Gly Val Glu Thr Leu Gly Glu Ser Val
230 235 240
gcc cag cta caa gcc tgg tgg tac aag gca gat ccc aat gac ttc acc 1245
Ala Gln Leu Gln Ala Trp Trp Tyr Lys Ala Asp Pro Asn Asp Phe Thr
245 250 255
tac gag agg cgg aag gag tct gca gca tac att ccc ttc ggc gaa ggg 1293
Tyr Glu Arg Arg Lys Ser Ala Ala Tyr Ile Pro Phe Gly Glu Gly
260 265 270 275
gat ttt tat tac cat gca gcc att ttt ggg gga aca ccc act cag gtc 1341
Asp Phe Tyr Tyr His Ala Ala Ile Phe Gly Gly Thr Pro Thr Gln Val
280 285 290
ctt aac atc acc cag gaa tgc ttc aaa gga atc ctc aag gac aag aaa 1389
Leu Asn Ile Thr Gln Glu Cys Phe Lys Gly Ile Leu Lys Asp Lys Lys
295 300 305
aat gac ata gaa gcc caa tgg cat gat gaa agc cat cta aac aag tat 1437
Asn Asp Ile Glu Ala Gln Trp His Asp Glu Ser His Leu Asn Lys Tyr
310 315 320
ttc ctt ctc aac aaa cct act aaa atc tta tcc ccg gaa tac tgc tgg 1485
Phe Leu Leu Asn Lys Pro Thr Lys Ile Leu Ser Pro Glu Tyr Cys Trp
325 330 335
gat tat cac ata ggc cta cct gcg gat att aag ctt gtc aag atg tct 1533
Asp Tyr His Ile Gly Leu Pro Ala Asp Ile Lys Leu Val Lys Met Ser
340 345 350 355
tgg cag aca aaa gag tat aat gtg gtt aga aat aat gtc tga 1575
Trp Gln Thr Lys Glu Tyr Asn Val Val Arg Asn Asn Val
360 365
ctttgtgccg gtacatttct gaatttgaga gagtattatt ct 1617

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<210> 4
<211> 368
<212> PRT
<213> BOVINE
<400> 4

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Met Asn Val Lys Gly Lys Val Ile Leu Ser Met Leu Val Val Ser Thr
1 5 10 15
Val Ile Val Val Phe Trp Glu Tyr Ile His Ser Pro Glu Gly Ser Leu
20 25 30
Phe Trp Ile Asn Pro Ser Arg Asn Pro Glu Val Gly Gly Ser Ser Ile
35 40 45
Gln Lys Gly Trp Trp Leu Pro Arg Trp Phe Asn Asn Gly Tyr His Glu
50 55 60
Glu Asp Gly Asp Ile Asn Glu Glu Lys Glu Gln Arg Asn Glu Asp Glu
65 70 75 80
Ser Lys Leu Lys Leu Ser Asp Trp Phe Asn Pro Phe Lys Arg Pro Glu
85 90 95
Val Val Thr Met Thr Lys Trp Lys Ala Pro Val Val Trp Glu Gly Thr
100 105 110
Tyr Asn Arg Ala Val Leu Asp Asn Tyr Tyr Ala Lys Gln Lys Ile Thr
115 120 125
Val Gly Leu Thr Val Phe Ala Val Gly Arg Tyr Ile Glu His Tyr Leu
130 135 140
Glu Glu Phe Leu Thr Ser Ala Asn Lys His Phe Met Val Gly His Pro
145 150 155 160
Val Ile Phe Tyr Ile Met Val Asp Asp Val Ser Arg Met Pro Leu Ile
165 170 175
Glu Leu Gly Pro Leu Arg Ser Phe Lys Val Phe Lys Ile Lys Pro Glu
180 185 190
Lys Arg Trp Gln Asp Ile Ser Met Met Arg Met Lys Thr Ile Gly Glu

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      195              200              205
His Ile Val Ala His Ile Gln His Glu Val Asp Phe Leu Phe Cys Met
      210              215              220
Asp Val Asp Gln Val Phe Gln Asp Lys Phe Gly Val Glu Thr Leu Gly
225      230      235
Glu Ser Val Ala Gln Leu Gln Ala Trp Trp Tyr Lys Ala Asp Pro Asn
      245      250      255
Asp Phe Thr Tyr Glu Arg Arg Lys Glu Ser Ala Ala Tyr Ile Pro Phe
      260      265      270
Gly Glu Gly Asp Phe Tyr Tyr His Ala Ala Ile Phe Gly Thr Pro
      275      280      285
Thr Gln Val Leu Asn Ile Thr Gln Glu Cys Phe Lys Gly Ile Leu Lys
      290      295      300
Asp Lys Lys Asn Asp Ile Glu Ala Gln Trp His Asp Glu Ser His Leu
305      310      315
Asn Lys Tyr Phe Leu Asn Lys Pro Thr Lys Ile Leu Ser Pro Glu
      325      330      335
Tyr Cys Trp Asp Tyr His Ile Gly Leu Pro Ala Asp Ile Lys Leu Val
      340      345      350
Lys Met Ser Trp Gln Thr Lys Glu Tyr Asn Val Val Arg Asn Asn Val
      355      360      365

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<210> 5
<211> 1131
<212> DNA
<213> Mermoset alpha 1,3-GT
<220>
<221> CDS
<222> (1)..(1131)
<400> 5

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atg aat gtc aaa gga aaa gta att ctg tcg atg ctg gtt gtc tca act 48
Met Asn Val Lys Gly Lys Val Ile Leu Ser Met Leu Val Val Ser Thr
      5      10      15
gtg att gtt gtg ttt tgg gaa tat atc aac agc cca gaa ggc tct ttc 96
Val Ile Val Val Phe Trp Glu Tyr Ile Asn Ser Pro Glu Gly Ser Phe
      20      25      30
ttg tgg ata tat cac tca aag aac cca gaa gtt gat gac agc agt gct 144
Leu Trp Ile Tyr His Ser Lys Asn Pro Glu Val Asp Asp Ser Ser Ala
      35      40      45
cag aag gac tgg tgg ttt cct ggc tgg ttt aac aat ggg atc cac aat 192
Gln Lys Asp Trp Trp Phe Pro Gly Trp Phe Asn Asn Gly Ile His Asn
      50      55      60
tat caa caa gag gaa gaa gac aca gac aaa gaa aaa gga aga gag gag 240
Tyr Gln Gln Glu Glu Glu Asp Thr Asp Lys Glu Lys Gly Arg Glu Glu
      65      70      75      80
gaa caa aaa aag gaa gat gac aca aca gag ctt cgg cta tgg gac tgg 288
Glu Gln Lys Lys Glu Asp Asp Thr Thr Glu Leu Arg Leu Trp Asp Trp
      85      90      95
ttt aat cca aag aaa cgc cca gag gtt atg aca gtg acc caa tgg aag 336
Phe Asn Pro Lys Lys Arg Pro Glu Val Met Thr Val Thr Gln Trp Lys
      100      105      110
gcg ccg gtt gtg tgg gaa ggc act tac aac aaa gcc atc cta gaa aat 384
Ala Pro Val Val Trp Glu Gly Thr Tyr Asn Lys Ala Ile Leu Glu Asn
      115      120      125
tat tat gcc aaa cag aaa att acc gtg ggg ttg acg gtt ttt gct att 432
Tyr Tyr Ala Lys Gln Lys Ile Thr Val Gly Leu Thr Val Phe Ala Ile
      130      135      140
gga aga tat att gag cat tac ttg gag gag ttc gta aca tct gct aat 480
Gly Arg Tyr Ile Glu His Tyr Leu Glu Glu Phe Val Thr Ser Ala Asn
      145      150      155      160
agg tac ttc atg gtc ggc cac aaa gtc ata ttt tat gtc atg gtg gat 528
Arg Tyr Phe Met Val Gly His Lys Val Ile Phe Tyr Val Met Val Asp
      165      170      175
gat gtc tcc aag gcg ccg ttt ata gag ctg ggt cct ctg cgt tcc ttc 576
Asp Val Ser Lys Ala Pro Phe Ile Glu Leu Gly Pro Leu Arg Ser Phe
      180      185      190
aaa gtg ttt gag gtc aag cca gag aag agg tgg caa gac atc agc atg 624
Lys Val Phe Glu Val Lys Pro Glu Lys Arg Trp Gln Asp Ile Ser Met

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      195              200              205
atg  cgt  atg  aag  acc  atc  ggg  gag  cac  atc  ttg  gcc  cac  atc  caa  cac    672
Met  Arg  Met  Lys  Thr  Ile  Gly  Glu  His  Ile  Leu  Ala  His  Ile  Gln  His
      210              215              220
gag  gtt  gac  ttc  ctc  ttc  tgc  atg  gat  gtg  gac  cag  gtc  ttc  caa  gac    720
Glu  Val  Asp  Phe  Leu  Phe  Cys  Met  Asp  Val  Asp  Gln  Val  Phe  Gln  Asp
      225              230              235              240
cat  ttt  ggg  gta  gag  acc  ctg  ggc  cag  tcg  gtg  gct  cag  cta  cag  gcc    768
His  Phe  Gly  Val  Glu  Thr  Leu  Gly  Gln  Ser  Val  Ala  Gln  Leu  Gln  Ala
      245              250              255
tgg  tgg  tac  aag  gca  gat  cct  gat  gac  ttt  acc  tat  gag  agg  cgg  aaa    816
Trp  Trp  Tyr  Lys  Ala  Asp  Pro  Asp  Asp  Phe  Thr  Tyr  Glu  Arg  Arg  Lys
      260              265              270
gag  tcg  gca  gca  tat  att  cca  ttt  ggc  cag  ggg  gat  ttt  tat  tac  cat    864
Glu  Ser  Ala  Ala  Tyr  Ile  Pro  Phe  Gly  Gln  Gly  Asp  Phe  Tyr  Tyr  His
      275              280              285
gca  gcc  att  ttt  gga  gga  aca  ccg  att  cag  gtt  ctc  aac  atc  acc  cag    912
Ala  Ala  Ile  Phe  Gly  Gly  Thr  Pro  Ile  Gln  Val  Leu  Asn  Ile  Thr  Gln
      290              295              300
gag  tgc  ttt  aag  gga  atc  ctc  ctg  gac  aag  aaa  aat  gac  ata  gaa  gcc    960
Glu  Cys  Phe  Lys  Gly  Ile  Leu  Leu  Asp  Lys  Lys  Asn  Asp  Ile  Glu  Ala
      305              310              315              320
gag  tgg  cat  gat  gaa  agc  cac  cta  aac  aag  tat  ttc  ctt  ctc  aac  aaa    1008
Glu  Trp  His  Asp  Glu  Ser  His  Leu  Asn  Lys  Tyr  Phe  Leu  Leu  Asn  Lys
      325              330              335
ccc  tct  aaa  atc  tta  tct  cca  gaa  tac  tgc  tgg  gat  tat  cat  ata  ggc    1056
Pro  Ser  Lys  Ile  Leu  Ser  Pro  Glu  Tyr  Cys  Trp  Asp  Tyr  His  Ile  Gly
      340              345              350
ctg  cct  tca  gat  att  aaa  act  gtc  aag  cta  tca  tgg  caa  aca  aaa  gag    1104
Leu  Pro  Ser  Asp  Ile  Lys  Thr  Val  Lys  Leu  Ser  Trp  Gln  Thr  Lys  Glu
      355              360              365
tat  aat  ttg  gtt  aga  aag  aat  gtc  tga
Tyr  Asn  Leu  Val  Arg  Lys  Asn  Val
      370              375

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<210> 6

<211> 376

<212> PRT

<213> MerMoset alpha 1,3-GT

<400> 6

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Met  Asn  Val  Lys  Gly  Lys  Val  Ile  Leu  Ser  Met  Leu  Val  Val  Ser  Thr
  1              5              10              15
Val  Ile  Val  Val  Phe  Trp  Glu  Tyr  Ile  Asn  Ser  Pro  Glu  Gly  Ser  Phe
              20              25              30
Leu  Trp  Ile  Tyr  His  Ser  Lys  Asn  Pro  Glu  Val  Asp  Asp  Ser  Ser  Ala
      35              40              45
Gln  Lys  Asp  Trp  Trp  Phe  Pro  Gly  Trp  Phe  Asn  Asn  Gly  Ile  His  Asn
      50              55              60
Tyr  Gln  Gln  Glu  Glu  Asp  Thr  Asp  Lys  Glu  Lys  Gly  Arg  Glu  Glu
      65              70              75              80
Glu  Gln  Lys  Lys  Glu  Asp  Asp  Thr  Thr  Glu  Leu  Arg  Leu  Trp  Asp  Trp
      85              90              95
Phe  Asn  Pro  Lys  Lys  Arg  Pro  Glu  Val  Met  Thr  Val  Thr  Gln  Trp  Lys
      100              105              110
Ala  Pro  Val  Val  Trp  Glu  Gly  Thr  Tyr  Asn  Lys  Ala  Ile  Leu  Glu  Asn
      115              120              125
Tyr  Tyr  Ala  Lys  Gln  Lys  Ile  Thr  Val  Gly  Leu  Thr  Val  Phe  Ala  Ile
      130              135              140
Gly  Arg  Tyr  Ile  Glu  His  Tyr  Leu  Glu  Glu  Phe  Val  Thr  Ser  Ala  Asn
      145              150              155              160
Arg  Tyr  Phe  Met  Val  Gly  His  Lys  Val  Ile  Phe  Tyr  Val  Met  Val  Asp
      165              170              175
Asp  Val  Ser  Lys  Ala  Pro  Phe  Ile  Glu  Leu  Gly  Pro  Leu  Arg  Ser  Phe
      180              185              190
Lys  Val  Phe  Glu  Val  Lys  Pro  Glu  Lys  Arg  Trp  Gln  Asp  Ile  Ser  Met
      195              200              205
Met  Arg  Met  Lys  Thr  Ile  Gly  Glu  His  Ile  Leu  Ala  His  Ile  Gln  His
      210              215              220

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Glu Val Asp Phe Leu Phe Cys Met Asp Val Asp Gln Val Phe Gln Asp
 225 230 235 240
 His Phe Gly Val Glu Thr Leu Gly Gln Ser Val Ala Gln Leu Gln Ala
 245 250 255
 Trp Trp Tyr Lys Ala Asp Pro Asp Asp Phe Thr Tyr Glu Arg Arg Lys
 260 265 270
 Glu Ser Ala Ala Tyr Ile Pro Phe Gly Gln Gly Asp Phe Tyr Tyr His
 275 280 285
 Ala Ala Ile Phe Gly Gly Thr Pro Ile Gln Val Leu Asn Ile Thr Gln
 290 295 300
 Glu Cys Phe Lys Gly Ile Leu Leu Asp Lys Lys Asn Asp Ile Glu Ala
 305 310 315 320
 Glu Trp His Asp Glu Ser His Leu Asn Lys Tyr Phe Leu Leu Asn Lys
 325 330 335
 Pro Ser Lys Ile Leu Ser Pro Glu Tyr Cys Trp Asp Tyr His Ile Gly
 340 345 350
 Leu Pro Ser Asp Ile Lys Thr Val Lys Leu Ser Trp Gln Thr Lys Glu
 355 360 365
 Tyr Asn Leu Val Arg Lys Asn Val
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 <211> 1269
 <212> DNA
 <213> Sus scrofa
 <220>
 <221> CDS
 <222> (16)..(1131)
 <400> 7

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 1 5 10
 ctt gtc tca act gta atg gtt gtg ttt tgg gaa tac atc aac agc cca 99
 Leu Val Ser Thr Val Met Val Val Phe Trp Glu Tyr Ile Asn Ser Pro
 15 20 25
 gaa ggt tct ttg ttc tgg ata tac cag tca aaa aac cca gaa gtt ggc 147
 Glu Gly Ser Leu Phe Trp Ile Tyr Gln Ser Lys Asn Pro Glu Val Gly
 30 35 40
 agc agt gct cag agg ggc tgg tgg ttt ccg agc tgg ttt aac aat ggg 195
 Ser Ser Ala Gln Arg Gln Trp Trp Phe Pro Ser Trp Phe Asn Asn Gly
 45 50 55 60
 act cac agt tac cac gaa gaa gaa gac gct ata ggc aac gaa aag gaa 243
 Thr His Ser Tyr His Glu Glu Glu Asp Ala Ile Gly Asn Glu Lys Glu
 65 70 75
 caa aga aaa gaa gac aac aga gga gag ctt ccg cta gtg gac tgg ttt 291
 Gln Arg Lys Glu Asp Asn Arg Gly Glu Leu Pro Leu Val Asp Trp Phe
 80 85 90
 aat cct gag aaa cgc cca gag gtc gtg acc ata acc aga tgg aag gct 339
 Asn Pro Glu Lys Arg Pro Glu Val Val Thr Ile Thr Arg Trp Lys Ala
 95 100 105
 cca gtg gta tgg gaa ggc act tac aac aga gcc gtc tta gat aat tat 387
 Pro Val Val Trp Glu Gly Thr Tyr Asn Arg Ala Val Leu Asp Asn Tyr
 110 115 120
 tat gcc aaa cag aaa att acc gtg ggc ttg acg gtt ttt gct gtc gga 435
 Tyr Ala Lys Gln Lys Ile Thr Val Gly Leu Thr Val Phe Ala Val Gly
 125 130 135 140
 aga tac att gag cat tac ttg gag gag ttc tta ata tct gca aat aca 483
 Arg Tyr Ile Glu His Tyr Leu Glu Glu Phe Leu Ile Ser Ala Asn Thr
 145 150 155
 tac ttc atg gtt ggc cac aaa gtc atc ttt tac atc atg gtg gat gat 531
 Tyr Phe Met Val Gly His Lys Val Ile Phe Tyr Ile Met Val Asp Asp
 160 165 170
 atc tcc agg atg cct ttg ata gag ctg ggt cct ctg cgt tcc ttt aaa 579
 Ile Ser Arg Met Pro Leu Ile Glu Leu Gly Pro Leu Arg Ser Phe Lys
 175 180 185
 gtg ttt gag atc aag tcc gag aag agg tgg caa gac atc agc atg atg 627
 Val Phe Glu Ile Lys Ser Glu Lys Arg Trp Gln Asp Ile Ser Met Met
 190 195 200

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cgc atg aag acc atc ggg gag cac atc ctg gcc cac atc cag cac gag 675
Arg Met Lys Thr Ile Gly Glu His Ile Leu Ala His Ile Gln His Glu
205 210 215 220
gtg gac ttc ctc ttc tgc atg gac gtg gat cag gtc ttc caa aac aac 723
Val Asp Phe Leu Phe Cys Met Asp Val Asp Gln Val Phe Gln Asn Asn
225 230 235
ttt ggg gtg gag acc ctg ggc cag tcg gtg gct cag cta cag gcc tgg 771
Phe Gly Val Glu Thr Leu Gly Gln Ser Val Ala Gln Leu Gln Ala Trp
240 245 250
tgg tac aag gca cat cct gac gag ttc acc tac gag agg cgg aag gag 819
Trp Tyr Lys Ala His Pro Asp Glu Phe Thr Tyr Glu Arg Arg Lys Glu
255 260 265
tcc gca gcc tac att ccg ttt ggc cag ggg gat ttt tat tac cac gca 867
Ser Ala Ala Tyr Ile Pro Phe Gly Gln Gly Asp Phe Tyr Tyr His Ala
270 275 280
gcc att ttt ggg gga aca ccc act cag gtt cta aac atc act cag gag 915
Ala Ile Phe Gly Gly Thr Pro Thr Gln Val Leu Asn Ile Thr Gln Glu
285 290 295 300
tgc ttc aag gga atc ctc cag gac aag gaa aat gac ata gaa gcc gag 963
Cys Phe Lys Gly Ile Leu Gln Asp Lys Glu Asn Asp Ile Glu Ala Glu
305 310 315
tgg cat gat gaa agc cat cta aac aag tat ttc ctt ctc aac aaa ccc 1011
Trp His Asp Glu Ser His Leu Asn Lys Tyr Phe Leu Leu Asn Lys Pro
320 325 330
act aaa atc tta tcc cca gaa tac tgc tgg gat tat cat ata ggc atg 1059
Thr Lys Ile Leu Ser Pro Glu Tyr Cys Trp Asp Tyr His Ile Gly Met
335 340 345
tct gtg gat att agg att gtc aag ata gct tgg cag aaa aaa gag tat 1107
Ser Val Asp Ile Arg Ile Val Lys Ile Ala Trp Gln Lys Lys Glu Tyr
350 355 360
aat ttg gtt aga aat aac atc tga ctttaaattg tgccagcagt tttctgaatt 1161
Asn Leu Val Arg Asn Asn Ile
365 370
tgaaagagta ttactctggc tacttctcca gagaagtagc acttaatttt aacttttaaa 1221
aaaataactaa caaaatacca acacagtaag tacatattat tcttcctt 1269

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<210> 8

<211> 371

<212> PRT

<213> Sus scrofa

<400> 8

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Met Asn Val Lys Gly Arg Val Val Leu Ser Met Leu Leu Val Ser Thr
1 5 10 15
Val Met Val Val Phe Trp Glu Tyr Ile Asn Ser Pro Glu Gly Ser Leu
20 25 30
Phe Trp Ile Tyr Gln Ser Lys Asn Pro Glu Val Gly Ser Ser Ala Gln
35 40 45
Arg Gly Trp Trp Phe Pro Ser Trp Phe Asn Asn Gly Thr His Ser Tyr
50 55 60
His Glu Glu Glu Asp Ala Ile Gly Asn Glu Lys Glu Gln Arg Lys Glu
65 70 75 80
Asp Asn Arg Gly Glu Leu Pro Leu Val Asp Trp Phe Asn Pro Glu Lys
85 90 95
Arg Pro Glu Val Val Thr Ile Thr Arg Trp Lys Ala Pro Val Val Trp
100 105 110
Glu Gly Thr Tyr Asn Arg Ala Val Leu Asp Asn Tyr Tyr Ala Lys Gln
115 120 125
Lys Ile Thr Val Gly Leu Thr Val Phe Ala Val Gly Arg Tyr Ile Glu
130 135 140
His Tyr Leu Glu Glu Phe Leu Ile Ser Ala Asn Thr Tyr Phe Met Val
145 150 155 160
Gly His Lys Val Ile Phe Tyr Ile Met Val Asp Asp Ile Ser Arg Met
165 170 175
Pro Leu Ile Glu Leu Gly Pro Leu Arg Ser Phe Lys Val Phe Glu Ile
180 185 190
Lys Ser Glu Lys Arg Trp Gln Asp Ile Ser Met Met Arg Met Lys Thr
195 200 205
Ile Gly Glu His Ile Leu Ala His Ile Gln His Glu Val Asp Phe Leu

```

210 Phe Cys Met Asp Val Asp Gln Val Phe Gln Asn Asn Phe Gly Val Glu
 225 Thr Leu Gly Gln Ser Val Ala Gln Leu Gln Ala Trp Trp Tyr Lys Ala
 245 His Pro Asp Glu Phe Thr Tyr Glu Arg Arg Lys Glu Ser Ala Ala Tyr
 260 Ile Pro Phe Gly Gln Gly Asp Phe Tyr Tyr His Ala Ala Ile Phe Gly
 275 Gly Thr Pro Thr Gln Val Leu Asn Ile Thr Gln Glu Cys Phe Lys Gly
 290 Ile Leu Gln Asp Lys Glu Asn Asp Ile Glu Ala Glu Trp His Asp Glu
 305 Ser His Leu Asn Lys Tyr Phe Leu Leu Asn Lys Pro Thr Lys Ile Leu
 325 Ser Pro Glu Tyr Cys Trp Asp Tyr His Ile Gly Met Ser Val Asp Ile
 340 Arg Ile Val Lys Ile Ala Trp Gln Lys Lys Glu Tyr Asn Leu Val Arg
 355 Asn Asn Ile
 370

<210> 9
 <211> 1500
 <212> DNA
 <213> Musca sp.
 <220>
 <221> CDS
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tggtttccat ggcaactaat gccatagttg.ggccaaagtga cccagactaa gctaatacaac 10920
attttccctc cccccgcccc caggggggca gatgatctaa actagtccaa tcaagttgaa 10980
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<210> 19
<211> 1813
<212> DNA
<213> Ovis sp.
<400> 19

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tgcagactca aagaacaggc ttgtggacac agtgagggaa ggagagggtg caatgaactg 60
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gctgggtgct gcaggagatg caaatccaat gctctgtgac aatttagagg ggtgtgatgg 180
gatgggtggt ggaggaggagg ttcaagaggg akgggacata tgtatacgta tgactgattc 240
atgtttatgt atggcagaaa ccaacacaaat attgttatta tcctccaatt agaaataaat 300
tcaaatataat aaaaaaggcc accgtacaac aactctgggc caggagcact gtcttgtcac 360
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ttcagttcag ttcagtcggt cagtcgtgtc ckactctttg cgaccccatg taatcgcagc 480
acgccaggcc tccctgtcta tcaccaactc ctagagttca ctcaaactcg tgtccattga 540
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ccacagttca aaagcatcga ttcttcgggg ctacagcctt tcatagttcc aactctcaca 840
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taatgtctct gcttttgaat atgtataaaa cattagaatg ttgctttcag ttcttaaat 960
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aaccagttct ggagtattat tgtttcattc tgaaaaaactt gttttaaaag aaacaggaca 1140
agctagagag tgaccatggt aaaggggtct acaaacaggg tctttcaaat tgaaggaaac 1200
catgggtact tagtctgaag aaaaggtgac tcagtgttgg atgcagggac cctggggctc 1260
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ctgggctccc ata 1813

```

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<210> 20
<211> 1379
<212> DNA
<213> Ovis sp.
<400> 20

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actggcacac ccgggggtgc aggtagagct gggctgtggg tttgagacga gacccggggc 60
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gcggaaggac aggagggtgt gacgagtgct tcttgggggc tggggtgagg aggagggtgac 180
ctggccttgc ccagccccc tggaaaaaact tagcttcacc cctggcagag gggcagtggt 240
gtgaaaaccc actctccacc catgtgtgag ggagagagga agttctcagt gggctgatct 300
atttgccaat atctcttga tagcctcacc aaggccactt gcacacagca ggggcccgat 360
ggggccacca agactttgcc ccagtcactg ggcctctccc cgcagtggct gaagtgcagg 420
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gaggataggg gaggaaggaa agggggacag agggcatgag gtataaagg aagaccccn 1140
tagggccccc gctaaagcag cccccaggca cctggggcac cgtctcctgt tctcaccca 1200
gaagatgccg cagatgtctc actgccagct gactgttggg gttaaactcc cattaggatt 1260

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caaggatggt taccaccagc cacggctgag ccgttttcca tcagatgtct ctgtcccacc 1320
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<210> 21
 <211> 1973
 <212> DNA
 <213> Ovis sp.
 <400> 21

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 agatagcccc aacgtgtgtc attgtttctc ccacacactc aagaccccaa ctgagaacta 120
 aggaccgaag tgactaagcg cggagcttcc ccacgtcagc ctggctgacc actctccccg 180
 ggcttccgca gctgcctctg cccttctect cctagaggct ttggcccccg gagatctggc 240
 caaccagAAC ttcttcttcc agaggttgga tgggttccctg ttctgtcttc catttctctg 300
 tttagaatga cttttctccc caaactgcaa tcattcttac ttaaaagtcg gagctgtgat 360
 aatagttttaa aatataaacc attttgttcc tttgggtgatt ttttttttcc tttatcaaaag 420
 cgaagacaaa ggtttacttt tcctattagc ctctccctcg caacccccct ccacgggcct 480
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 ccagcccaga gccagaaagc catcgccctc accctactgg tcaagtcctg atgtcaccac 660
 ctgtgctgat ccagggggca tctgtgatgg agaagggtga cctctggaatt ttgacatttt 720
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 cggatgaat aagtaaatag ctaaaatacc acatctatta gaaggacct agtccaaaac 1560
 actgacaaca caaactgctg gcaaaaatat ggagcaagca gaactctgat tcaactgttg 1620
 cgagaaggca agatggttca gctgctttgg aagaaagctg aacataattct tatcatcacag 1680
 tctagcaatt gcatccttgg tattcaccaa aaaaagctga aactttacag ccgcacagaa 1740
 acttacatac agatgtttaa acgcagcttt agtcacagtt tctaaaactt ggacataact 1800
 aagatgtcct ttagtaggtg aatggattaa taaactgttg tggcacgtca ggtggtggaa 1860
 tatattatca gtgcaaaaac acaaatctgt caagccatgg aagagataga ggaacttaat 1920
 gcatattatt aagtaaaaga accatctgaa agattgcctg ctgtcagatt cca 1973

<210> 22
 <211> 920
 <212> DNA
 <213> Ovis sp.
 <400> 22

agggaaacaaa agctgggtac gatatcatat atagtgaaaa atacttgtca ttacatattt 60
 gtcgaacccc atagaatata cacaaccaag agtgaacttt ttttagaaaa ggaaatgaga 120
 tagttaaaaat aagttttaa atcagaggtgt ttgctctggt atttgatgg gtcttataat 180
 ggtggtagct ggataatgga gtgggttttg gggtaaccac tagaaaattc ttaaaggga 240
 tgtataatgt ggaaggatat tcatgtcccc cataaccat tctctgggtc ccagagaaga 300
 agagagtggt gatgcttagt cttaaggcat ccccaaggga cctaaggagc ttgtcaccct 360
 ttcatctgat agggcaggat aacattggag cggatgttgg ggtttctgag agatgcctga 420
 gaactagcgc cccctgatgt tgatcacggg gtacaacctt ccagttataa aatgagcaag 480
 ttctggggat ctgatgtaaa gcattgtggt tattgttaat aatattgttt tctatactca 540
 taaagtattt cagataataa atctcaaatc ttctcccaa aacagaaatg atgattatgt 600
 gatgcaatga aggtgttaac taatgctact gctgctgctg agtcgcttca gtcgtgtgag 660
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 gcaagaacac tggagtggtg tgccatttcc ttctccaatg catgaagggtg aaaagagaaa 780
 gtgaagtcac tcagtcgtgt ctgactctta gcgaccccat ggactgcagc ctaccagcct 840
 tctccgtcca tgggattttc caggcaagag tactggagtg ggggtgccatt gccttctccg 900
 aactaatgct acagtgtggt 920

<210> 23
 <211> 650
 <212> DNA

<213> Ovis sp.
<400> 23

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cctcctccag gggctcttcc tgaccccagg cgtcaaaccg gcctctcctg cgtctcttgc 60
atcacaggca gattctctac ccactgagcc atctgggaag cccttttata atatatgaat 120
ataccaaaac aacacttggc acattttaaa cttaaaactat gctatatgtc aattatatct 180
caataaaaaca ggacaaaaaa aggactagca cactttcacc taccgggagt ctgaaccaac 240
tgataaataa gacaaaagga taaataaggt agatggtagt gatgatcatc ttactgtgtg 300
taaaagctga tattgaagaa tatgggttat aaaactgaag gccctgggcc tcctgatgtt 360
gaaccacaga attagaaaga cgtaggcacc cttcatccat agcatgagaa actgcagggtg 420
tctggatgtt ggagaagggc ctgtatcatt tagctacatt gcagggccct agatagatag 480
agatctgttt aaacctcgag ggccggaggg tcgagcagtg tggttttcaa gaggaagcaa 540
aaagcctctc caccagggcc tggaatgttt ccaccaatg tcgagcagtg tggttttgca 600
agaggaagca aaaagcctct ccaccaggc ctggaatgtt tccaccaat 650
```

<210> 24
<211> 705
<212> DNA
<213> Ovis sp.
<400> 24

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tataatgtgg ttagaaataa cgtctgactt tgtgccagta catttttgaa tttgagagag 60
tattattctg gctacttccct cagaaaagta acacttaatt ttaacttaaa aaaaaaaaaa 120
aactaacaaa agaccaacac agcaagtaca cattatttct ccttgtaaca ttgagccttg 180
taatacggga gaatgaacct gtggtaatcg gatgtaaatt cccagtgtat tcttacctat 240
tctcagttgc gggggagggg aatggataca ctatcagttg aacaaaaaaa gagaaaaaat 300
tatcatagac aaaagccaga aacattctac atgtgccaat taacttactg gagagaagta 360
cgcaaaggga cgtgtttggc aacaagacaa tgattgtgag gggctgtcct cttgatattca 420
gtgtcttctc gtctctgctg ggtctgaagc aacacagagt tgcttttgca gcaggagaaac 480
tcttagtagg acatagtcta ccttgccgat cctcaaatgg tttagttagt gtcctttcct 540
tatcaggata ttggcttctg tgccaagact agtcaagatt cccagtacat atggtgaaga 600
tggtggggaa tcacaggcaa tctgagtcag tgggtgctca gcacaaaatg aaaacaaagc 660
ctccaagtaa gttggccatg aaataagcaa agagagagta gaaag 705
```

<210> 25
<211> 900
<212> DNA
<213> Ovis sp.
<400> 25

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actcacaag tgtgaaacac catagaaggc atgaatacat aataaatgat gcataccttt 60
taaagagaga aagctagggg aagtggggat ctactgttca agtctcaggg attcaaacc 120
agcactgtct gactctaaat cctttgttct ttccacagct caagtcttct tctttcaaac 180
acacacacac acacacacac acacacacac ttttaaccatt ttgttttga gaccgagggc 240
atcaaggaat caccaccaag aactctcaat tcttgtaata gcattaaaga aagagaactt 300
agttgactca aacttcacag atggatggta ggtctaaact catcctggag cctggctgga 360
aagcagtcac acagaacctg agcgatacga ggctcaggga agttcaaggg ctgagagggt 420
acagaggaga gaaggtctgg gaggtggaaa cagtctcaga ggggctgctt ctgaattaag 480
cactgagaga agagaaagg atcactaaga agtggaaatg gaatgaggtt tcggagagag 540
gaacagcagg tacgaagcat ggaggactat tctgaggctt ttagggagac cctggatgaa 600
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ggagctgcac atttgcccag catctgtgtc tttctttctc gctttatctc cttcatagag 720
tctatgtggt ttccatggca actaatgcc aagttgggcc aagtgaccca gactaagcta 780
atcaacattt tccctcccc cgccccagg ggggcagatg atctaaacta gtccaatcaa 840
gttgaatctc aggcctttta ttaagcgcc gccaccgcgg tggagctcca ttcgcctat 900
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<210> 26
<211> 24
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Probes and PCR
Primers
<400> 26

gagaaaataa tgaatgtcaa agga

24

<210> 27
<211> 20

<212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 27

 tgataatccc agcagtattc 20

 <210> 28
 <211> 22
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 28

 cttgatgggt ttatccagaa ca 22

 <210> 29
 <211> 23
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 29

 ctgtggatat attccccaaa cac 23

 <210> 30
 <211> 95
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 30

 aattcgagaa aataatgaat gtcaaaggaa aagtgattct gtcaatgctg gttgtctcaa 60
 ctgtcattgt tgtgttttgg gaatatatcc acagg 95

 <210> 31
 <211> 95
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 31

 aattcctgtg gatataattcc caaaacacaa caatgacagt tgagacaacc agcattgaca 60
 gaatcatttt tcctttgaca ttcattatatt tctcg 95

 <210> 32
 <211> 24
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 32

 ggtggtttcc gagatggttt aaca 24

 <210> 33
 <211> 24
 <212> DNA

<213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR Primers
 <400> 33
 gggttgaacc agtccgatag ctta 24
 <210> 34
 <211> 21
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR Primers
 <400> 34
 tccaggatgc cttgataga g 21
 <210> 35
 <211> 19
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR Primers
 <400> 35
 gggaggaagc gaaggtgca 19
 <210> 36
 <211> 36
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR Primers
 <400> 36
 acgtggctcc aagaattctc caggcaagag tactgg 36
 <210> 37
 <211> 49
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR Primers
 <400> 37
 catcttgctic aatggccgat cccattattt tctcctggga aaagaaaag 49
 <210> 38
 <211> 49
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR Primers
 <400> 38
 cttttctttt cccaggagaa aataatggga tcggccattg aacaagatg 49
 <210> 39
 <211> 23
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR

Primers
 <400> 39
 caggctcgacg gatccgaaca aac 23
 <210> 40
 <211> 47
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 40
 cagatctaac gaggattcaa tgtcaaagga aaagtgattc tgtcaat 47
 <210> 41
 <211> 27
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 41
 ctgaactgaa tgtttatcca ggccatc 27
 <210> 42
 <211> 51
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 42
 gcgcaccgtg ggcttgact cggtcattat tttctcctgg gaaaagaaaa g 51
 <210> 43
 <211> 34
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 43
 gagaaaataa tgaccgagta caagcccacg gtgc 34
 <210> 44
 <211> 26
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 44
 ctggggatcc agacatgata agatac 26
 <210> 45
 <211> 24
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 45

ctggttggtt ctagaacagg agga 24

<210> 46
 <211> 66
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 46

catcttggtc aatggccgat cccattcctc ctctcctcc actggtgaca aaacagagtc 60
 catgag 66

<210> 47
 <211> 66
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 47

ctcatggact ctgttttgtc accagtggag gaggaggagg aatgggatcg gccattgaac 60
 aagatg 66

<210> 48
 <211> 23
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 48

caggtcgacg gatccgaaca aac 23

<210> 49
 <211> 22
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 49

cagctgtgtg ggtatgggag gg 22

<210> 50
 <211> 27
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 50

ctgaactgaa tgtttatcca ggccatc 27

<210> 51
 <211> 27
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Probes and PCR
 Primers
 <400> 51


agccgattgt ctgttggtgcc cagtcac 27

Applicant's or agent's file reference number	730/200PCT	International application No.
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page 31, line 25-41	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution National Collections of Industrial and Marine Bacteria Limited	
Address of depositary institution (including postal code and country) 23 St. Machar Drive Aberdeen AB2 1RY Scotland UK	
Date of deposit Apr 25, 2000 and May 30, 2000	Accession Number 41056, 41059, 41060, 41061
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
[all states]	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	
<input checked="" type="checkbox"/>	This sheet was received with the international application
Authorized officer 	

For International Bureau use only	
<input type="checkbox"/>	This sheet was received by the International Bureau on:
Authorized officer	