COMPOSITIONS AND METHODS FOR TREATMENT OF TYPE VII COLLAGEN DEFICIENCIES

SEQUENCE LISTING

[001] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 9, 2017, is named 0100-0016PR1_SL.txt and is 92,924 bytes in size.

TECHNICAL FIELD OF THE INVENTION

[002] The present invention relates to compositions and methods for the treatment of Type VII collagen deficiencies such as dystrophic epidermolysis bullosa comprising the administration of autologous genetically modified cells comprising a nucleotide sequence encoding Type VII collagen (C7) or a functional variant thereof.

BACKGROUND OF THE INVENTION

[003] Type VII collagen (C7) is a protein important for anchoring fibril formation at the dermal-epidermal junction (DEJ), which holds together the layers of skin (Burgeson 1993; Leigh *et al.* 1988). Dystrophic epidermolysis bullosa (DEB) is an inherited disease characterized by a deficiency in C7 resulting in an impairment of anchoring fibrils to adhere between the epidermis and the underlying dermis affecting the skin and organs. Mutations of the COL7A1 gene encoding C7 causes the C7 deficiency. Patients suffering from DEB are highly susceptible to severe blistering. Dominant dystrophic epidermolysis bullosa (DDEB) is characterized by generalized blistering. Transient bullous dermatosis is a form of DDEB, which corrects itself during infancy. Recessive dystrophic epidermolysis bullosa (RDEB) is a debilitating skin-blistering disorder. RDEB inversa is another form of RDEB, where blisters form on areas where skin rubs on skin. Without these fibrils, skin layers separate causing severe blistering, open wounds and scarring in response to any kind of friction, including normal daily activities like rubbing or scratching.

[004] There are currently no curative treatments and patients largely rely on palliative wound care. Preclinical studies employing a number of strategies show encouraging results suggesting that the effects of the disease can be corrected by restoring C7 function in RDEB skin. Initial approaches relied on grafting fragile engineered epidermal tissue and require removal of the skin tissue to be replaced, resulting in scarring (Mavilio *et al.* 2006; Chen, Nat. Genet. 2002; Siprashvili 2010). Other methods have included delivery of the corrective C7 by direct virus-based expression and systemic delivery of recombinant protein (Remington 2009; Woodley *et al.* 2003). All of these potential treatments have a number of drawbacks including cost and biosafety concerns.

[005] The use of replication-defective, self-inactivating (SIN) lentiviral vectors (LV) offers several advantages for gene therapy: 1) ability to transduce both dividing and non-dividing cells, 2) high transduction efficiency, 3) long-term sustained transgene expression, 4) the ability to dispense sequences encoding viral proteins that might trigger an immune response, 5) an increased safety profile due to transcriptionally inactive SIN long terminal repeat (LTR), and 6) an ability to package large transgenes. Safety studies from clinical trials using LV vectors for gene therapy have showed no preferential integration in or near proto-oncogenes or tumor suppressor genes. Patients who have been dosed at 10 billion LV vector-transduced T cells per subject and followed for a median of 4 years, showed no leukemia or other adverse events. Trials with the indications of metachromatic leukodystrophy, Wiskott-Aldrich Syndrome (WAS) and X- adrenoleukodystrophy (ALD), revealed no aberrant clonal expansion seen up to 21 months, 32 months, and 36 months of follow up, respectively (Biffi *et al.* 2013; Aiuti *et al.* 2013).

[006] Treatment with autologous genetically-modified human dermal fibroblasts (GM-HDF) offers a promising alternative based on correcting the hereditary defect ex vivo in the patients' own fibroblasts (Ortiz-Urda *et al.* 2003; Woodley *et al.* 2003). It has been reported that hCOL7A1 gene-corrected fibroblasts are better than keratinocytes at supplying C7 to the basement membrane zone (BMZ) in mice with RDEB skin grafts (Goto *et al.* 2006). In addition, injection of fibroblasts can be more easily completed than grafting of keratinocytes.

SUMMARY OF THE INVENTION

[007] The present invention is directed to a method of treating a patient suffering from a Type VII collagen (C7) deficiency comprising obtaining cells from the dermis or epidermis, preferably fibroblasts or keratinocytes, of a C7-deficient patient, contacting the cells with a transducing lentiviral vector particle comprising the *COL7A1* gene or a functional variant thereof to form an autologous genetically modified cell comprising the *COL7A1* gene or functional variant thereof having a vector copy number wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell, culturing said autologous genetically modified cell, and administering the genetically modified cells to the C7-deficient patient. The administration may be done by injection, preferably intradermal injection. In one aspect of the invention, the C7 deficiency is dystrophic epidermolysis bullosa (DEB), such as recessive dystrophic epidermolysis bullosa (RDEB) or dominant dystrophic epidermolysis bullosa (DDEB). Subtypes of RDEB are also envisioned to be treated according to the present invention including Hallopeau-Siemens, non-Hallopeau-Siemens RDEB, RDEB inversa, pretibial RDEB, acral RDEB, and RDEB centripetalis.

[008] In one embodiment of the invention, the transducing lentiviral vector particle is constructed from a transfer lentiviral vector comprising (a) a modified 5' long terminal repeat in LTR, wherein the promoter of the modified 5' LTR is a cytomegalovirus promoter, (b) the *COL7A1* gene or a functional variant thereof, (c) at least one lentiviral central polypurine tract, (d) a hepatitis B virus post- transcriptional regulatory element (PRE), and (e) a modified 3' LTR, wherein the modified 3' LTR comprises a deletion relative to the wild-type 3' LTR, wherein the *COL7A1* gene or functional variant is incorporated into the cells to form genetically modified cells having a functional *COL7A1* gene or functional variant thereof.

[009] In an embodiment, there are at least two lentiviral central polypurine tract elements. In another embodiment, the PRE is woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). Preferably, the transfer lentiviral vector used to construct the transducing vector is selected from the group consisting of pSMPUW and pFUGW. Preferably, the transducing lentiviral vector particle is INXN-2004 or INXN-2002.

[010] Administration of the genetically modified fibroblasts autologous to the C7-deficient patient may be done in any suitable manner, including by injection, topically, orally, or embedded in a biocompatible matrix.

[011] Another aspect of the invention is directed to the autologous genetically modified fibroblasts from a C7-deficient patient, such as RDEB or DDEB patient, transduced with a lentiviral vector particle comprising a functional *COL7A1* gene and expressing type VII collagen, wherein the lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

[012] Another embodiment is directed to a self-inactivating lentiviral vector formed from a transfer vector comprising (a) a modified 5' long terminal in LTR, wherein the promoter of the modified 5' LTR is a cytomegalovirus promoter, (b) the *COL7A1* gene or a functional variant thereof, (c) at least one lentiviral central polypurine tract element, (d) a hepatitis B virus post-transcriptional regulatory element (PRE), and (e) a modified 3' LTR, wherein the modified 3' LTR comprises a deletion relative to the wild-type 3' LTR. In one embodiment, the vector comprises at least two lentiviral central polypurine tract elements and the PRE is woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). The vector of the invention includes the vector designated INXN-2004 and comprising the sequence of IGE308 plasmid; or the vector designated INXN-2002.

[013] Another embodiment of the invention is directed to pharmaceutical compositions comprising a fibroblast obtained from a patient suffering from RDEB or DDEB transduced with a lentiviral vector designated INXN-2004 comprising the sequence of IGE308 plasmid; or transduced with a lentiviral vector designated INXN-2002.

[014] In one embodiment, the present invention is directed to cells transduced in vitro or ex vivo using the vectors, such as INXN-2002 and INXN-2004.

[015] In one embodiment, the present invention is directed to transduced autologous fibroblasts using vectors INXN-2002 or INXN-2004, which have been obtained and propagated according to methods described in U.S. Patent No. 8,728,819 and International Patent Application.

[016] WO2008/027984 entitled "Methods for culturing minimally-passaged fibroblasts and uses thereof", each of which is hereby incorporated by reference herein.

[017] In one embodiment, the present invention is directed to use of concentrated lentivirus for transduction of human autologous dermal cells, such as fibroblasts and/or keratinocytes.

[018] In another embodiment, the present invention is directed to transduction of human autologous dermal cells, such as fibroblasts and/or keratinocytes via centrifugation with lentivirus.

[019] In one embodiment, the present invention is directed to use of both concentrated lentivirus and centrifugation for transduction of human autologous dermal cells, such as fibroblasts and/or keratinocytes.

[020] In an embodiment, the present invention is directed to use of human autologous dermal cells, such as fibroblasts and/or keratinocytes, which have been contacted with lentivirus in two or more separate transduction processes.

[021] In one embodiment, the present invention is directed to use human autologous dermal cells, such as fibroblasts and/or keratinocytes, which have been contacted with lentivirus in two or more separate transduction processes, wherein the cells are cultured and passaged one, two, or three times, before the second or any subsequent transduction process.

[022] The present invention also relates to the treatment of patients suffering from pseudosyndactyly comprising administering to said patient an autologous population of cells obtained from said patient transduced with a lentiviral vector particle comprising a functional *COL7A1* gene or its functional variant thereof, and expressing type VII collagen.

[023] The invention further relates to a method of treating, inhibiting or reducing the blistering of a dystrophic epidermolysis bullosa (DEB) or methods of treating lesions in patients suffering from RDEB comprising administering the autologous genetically modified cells of the present invention.

[024] Another aspect of the invention relates to isolated population of genetically modified fibroblasts autologous to a C7-deficient patient, such as DDEB or RDEB patient, transduced

with a vector particle comprising a functional *COL7A1* gene or functional variant thereof and expressing type VII collagen, as well as methods of making these isolated populations.

[025] The invention also relates to a method of increasing the integrated transgene copy number per cell in genetically modified human dermal fibroblasts or keratinocytes comprising contacting a transducing lentiviral vector comprising a nucleotide sequence encoding a *COL7A1* gene or a functional variant thereof with a human dermal fibroblast or keratinocyte obtained from a C7-deficient patient to form a transduction composition. The transduction composition is subjected to spinoculation to form transduced human dermal fibroblast or keratinocyte, wherein the integrated transgene copy number of the transduced human dermal fibroblasts or keratinocytes is higher relative to a transduction composition not subjected to spinoculation. The invention further includes a super-transduction step, wherein the transduced cells are further contacted with a second transducing lentiviral vector to form a second tranducsction composition, which is optionally subjected to spinoculation. In one aspect, the transducing lentiviral vector is INXN-2002 or INXN-2004, preferably INXN-2004. It has been found that the the transduced human dermal fibroblast or keratinocytes have at least or about 1, 2, 5, 10, 20, 25, 27, 28, 29, 30, 35, 40, 45, or 50-fold greater integrated transgene copy number per cell relative to a transduction composition not subjected to spinoculation or super-transduction. In another embodiment, the invention relates to having an integrated transgene copy number per cell of at least 0.05, 0.09, 0.41, or 0.74 or a range of between about 0.1 to about 5, 0.1 to about 1, 0.4 to about 1, or 0.4 to about 0.75. Expression has been found to be increased using the transduction methods described herein. For example, it has been found that expression of C7 has increased by 10, 25, 50, 100, 150, or 200-fold relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction.

BRIEF DESCRIPTION OF THE DRAWINGS

[026] A more complete understanding of the present invention may be obtained by reference to the accompanying drawings, when considered in conjunction with the subsequent detailed description. The embodiments illustrated in the drawings are intended only to exemplify the invention and should not be construed as limiting the invention to the illustrated embodiments.

[027] Figure 1A depicts type VII collagen (C7) trimers, which form anchoring fibrils. The *COL7A1* gene encodes a 290-kDa alpha chain and three of the chains form a triple helix (trimer). Image from Bruckner- Tuderman L. *Molecular Therapy* (2008) 17:6–7.

[028] Figure 1B depicts the general structure of normal and RDEB skin. C7 anchoring fibrils bind to other collagens, extracellular matrix proteins, and Lam332 to mediate attachment of the dermis to the epidermis. Absence of anchoring fibrils can lead to blister formation.

[029] Figure 2 describes a cGMP-scale GM-HDF production process according to one aspect of the present invention. A C7 expression cassette was cloned into a self-inactivating lentivirus backbone. A pilot-scale production of lentiviral vector (INXN-2002) comprising *COL7A1* gene (LV-COL7) with a titer of ~9 x 10^6 IU/mL was generated for use in the cGMP-scale production process. Fibroblasts were isolated from RDEB patient biopsies, grown, then split into three arms for mock, high-, and low-dose LV-COL7 transduction. Each arm of fibroblasts was grown to 2 x CS10 scale then cryopreserved (Drug Substance). For patient treatment, Drug Substance vials are thawed and formulated (Drug Product), then shipped back to the clinic for wound-site injection of the originating patient.

[030] Figure 3A provides the LV-COL7 (for INXN-2002) copy number per cell. Drug Substance vials were thawed and assayed for LV-COL7 DNA copies per cell using qPCR. Primers were specific for the LV shuttle vector. Results demonstrate dose-dependent levels of copies per cell with an average of 0.38 and 0.18 copies from the High and Low Dose arms, respectively.

[031] Figure 3B provides the C7 expression levels produced by RDEB patient fibroblasts transduced with LV-COL7: Drug Substance vials were thawed and cultured for 3 days. Conditioned cell culture supernatants were collected and assayed for C7 levels by ELISA. Results show virus dose-dependent protein expression that ranges from 60 to 120 ng/mL C7 in LV-COL7-transduced cells.

[032] Figure 3C displays the trimeric form of C7 produced by RDEB patient fibroblasts transduced with LV- COL7: Conditioned cell culture supernatants were also used in an immunoprecipitation assay. Immunoprecipitated C7 was separated on non-denaturing SDS-PAGE and visualized by western blot. The C7 produced by RDEB fibroblasts was predominantly trimeric with LV-COL7-transduced cells expressing more COL7 than mock-transduced cells (Arm C). Some lower molecular weight species showing immunoreactivity, were also observed.

[033] Figure 4A depicts binding of purified COL7 to Lam332. COL7 was expressed in CHO-DG44 cells, was purified by size exclusion chromatography, and was assayed for preferential binding to Lam332 as compared to BSA to establish the assay (increase in OD450 corresponds to increase in COL7 binding to Laminin332).

[034] Figure 4B depicts Lam332 binding of COL7 in LV-COL7-transduced fibroblasts (as transduced by the INXN-2002 vector) culture supernatants. Drug Substance vials were thawed and cultured for 3 days. Conditioned cell culture supernatants were collected and assayed for binding to Lam332 compared with BSA control. Results show virus dose-dependent binding to Lam332.

[035] Figure 5 depicts composite RDEB skin grafts on the dorsum of SCID mice that were injected intradermally with 1 x 10^6 GM-HDF (as transduced by the INXN-2002 vector) and analyzed by immunofluorescent staining with human COL7 specific antibodies. Representative images are shown. Localization of COL7 was observed in composite grafts (n=4) generated with RDEB keratinocytes at Day 10-post intradermal injection of 1 x 10^6 GM-HDF. Positive control grafts generated from normal keratinocytes and fibroblasts showed intense COL7 staining and negative control grafts did not show COL7 staining at baseline measurements (arrows at DEJ for negative baseline comparison).

[036] Figure 6 provides a schematic of the pSMPUW lentiviral expression plasmid vector.

[037] Figure 7 compares the features of the pSMPUW lentiviral expression vector relative to 3rd generation lentivirus expression vectors.

[038] Figure 8 provides a schematic of the pFUGW lentiviral expression plasmid vector.

[039] Figure 9 depicts an electron micrograph of HIV-1 virus particles.

[040] Figure 10 provides the immunoprecipitation results for TR8 GM-HDFs detecting the formation of C7 trimers.

[041] Figure 11A provides the immunoprecipitation results for TR10 and ER1 GM-HDFs detecting the formation of C7 trimers.

[042] Figure 11B provides the immunoprecipitation results for TR9 detecting the formation of C7 trimers.

[043] Figure 12 shows the results of Lam332 binding results by C7 as expressed by GM-HDFs.

[044] Figure 13 provides a schematic of the lentiviral vector plasmid construct, which encodes a *COL7A1* gene.

[045] Figure 14 presents a schematic of the proviral RNA genome structure of the INXN-2002 lentiviral vector.

[046] Figure 15 shows a schematic depicting the details of the mature INXN-2002 virus particle.

[047] Figure 16 provides a schematic of the *in vitro* immortalization assay to assess the potential for insertional genotoxicity of INXN-2002.

[048] Figure 17 represents typical cell morphology and structures of FXC-007 in culture.

[049] Figure 18 provides a graph of the C7 expression levels as determined by ELISA for particular dosages of the TR8 GM-HDFs.

[050] Figure 19 provides the western blot showing the immunoprecipitation of C7 trimers against NC1-specific antibodies as produced by FCX-007 GM-HDFs.

[051] Figure 20 shows the assay readout (optical density at 450 nm (OD450)) for a binding assay to detect the interaction of C7 with laminin 332 or bovine serum albumin (BSA)-coated

wells. Bound C7 was detecting using a C7 NC1-specific antibody and an HRP-conjugated secondary antibody.

[052] Figure 21A graphically represents the amount of cell migration of normal and RDEB patient fibroblasts transduced with the LV-COL7 over time.

[053] Figure 21B provides images of the cell migration of normal and RDEB patient fibroblasts transduced with the LV-COL7 over time.

[054] Figure 22 provides the schematic for the cloning of human *COL7A1* gene.

[055] Figure 23 provides the schematic of the cloning of the *COL7A1* gene into the pSMPUW expression vector to produce the INXN-2002 lentiviral vector transfer plasmid, IGE230.

[056] Figure 24 provides the schematic representation of the construction of the INXN-2004 lentiviral vector transfer plasmid (IGE308).

[057] Figure 25A schematically depict the gene elements of the IGE308 plasmid.

[058] Figure 25B schematically depict the gene elements of the IGE308 plasmid.

[059] Figure 26 provides the immunofluorescence analysis of GM-HDF injected composite grafts.

[060] Figure 27 provides a graph representing the LV-COL7 transcript levels as measured by its fold change value over control cells.

[061] Figure 28 provides representative images of C7 staining for INXN-2002 and LV-HA-COL7-transduced RDEB fibroblasts.

[062] Figure 29 provides a graphical representation of the C7 protein levels as measured in the cell supernatants of GM-HDFs transduced by TR8, TR9, T10 and ER1.

[063] Figure 30 graphically represents the correction of hypermotility of GM-HDFs over time for TR8, TR9, TR10 and ER1.

[064] Figure 31 depicts representative indirect immunofluorescence (IF) images to examine C7 protein expression by GM-HDF cells using an antibody specific for C7 at two magnifications, 20x and 5x. (Only Arm A was tested for TR12.1 and TR13. Only Arm A of TR8 is shown for comparison of previous processes.)

[065] Figure 32 provides the percentage of C7-positive (C7+) cells of GM-HDFs for each training run arm of TR11, TR12.1 and TR13.

[066] Figure 33 provides a graph representation of the amount of C7 protein levels expressed for TR8, TR11, TR12.1 and 13. Error bars represent standard deviation; n=3. Only Arm A results are shown for TR8, TR12.1, and TR13.

[067] Figure 34 provides the SDS-PAGE/immunoblots for the detection of C7 trimers (a) TR11, (b) TR12.1 and TR13 GM-HDFs.

[068] Figure 35 depicts a graph of C7 expressed by GM-HDF that bind to Laminin 332 (Lam332) for TR11, TR12.1 and TR13.

[069] Figure 36 provides the results of cell migration assays depicting the correction of hypermotility (% migration) over time for GM-HDFs for TR11, TR12.1 and TR13. The hypermotility of normal human dermal fibroblasts (NHDF) and a control fibroblasts from recessive dystrophic epidermolysis bullosa cells are provided.

DETAILED DESCRIPTION OF THE INVENTION

[070] Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of this invention. Although any compositions, methods, kits, and means for communicating information similar or equivalent to those described herein can be used to practice this invention, the preferred compositions, methods, kits, and means for communicating information are described herein.

[071] All references cited herein are incorporated herein by reference to the full extent allowed by law. The discussion of those references is intended merely to summarize the assertions made

by their authors. No admission is made that any reference (or a portion of any reference) is relevant prior art. Applicants reserve the right to challenge the accuracy and pertinence of any cited reference.

[072] In order that the present invention may be more readily understood, certain terms are herein defined. Additional definitions are set forth throughout the detailed description.

[073] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[074] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects. Typically, the term is meant to encompass approximately or less than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% variability depending on the situation.

[075] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

[076] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

[077] Nucleic acids and/or nucleic acid sequences are "homologous" when they are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. Proteins and/or protein sequences are homologous when their encoding DNAs are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. The homologous

molecules can be termed homologs. For example, any naturally occurring proteins, as described herein, can be modified by any available mutagenesis method. When expressed, this mutagenized nucleic acid encodes a polypeptide that is homologous to the protein encoded by the original nucleic acid. Homology is generally inferred from sequence identity between two or more nucleic acids or proteins (or sequences thereof). The precise percentage of identity between sequences that is useful in establishing homology varies with the nucleic acid and protein at issue, but as little as 25% sequence identity is routinely used to establish homology. Higher levels of sequence identity, e.g., 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% or more can also be used to establish homology. Methods for determining sequence identity percentages (e.g., BLASTP and BLASTN using default parameters) are described herein and are generally available.

[078] The terms "identical" or "sequence identity" in the context of two nucleic acid sequences or amino acid sequences of polypeptides refers to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. A "comparison window", as used herein, refers to a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are aligned optimally. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482; by the alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443; by the search for similarity method of Pearson and Lipman (1988) Proc. Nat. Acad. Sci U.S.A. 85:2444; by computerized implementations of these algorithms (including, but not limited to CLUSTAL in the PC/Gene program by Intelligentics, Mountain View Calif., GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis., U.S.A.); the CLUSTAL program is well described by Higgins and Sharp (1988) Gene 73:237-244 and Higgins and Sharp (1989) CABIOS 5:151-153; Corpet et al. (1988) Nucleic Acids Res. 16:10881-10890; Huang et al (1992) Computer Applications in the Biosciences 8:155-165; and Pearson et al. (1994) Methods in Molecular Biology 24:307-331. Alignment is also often performed by inspection and manual alignment.

[079] In one class of embodiments, the polypeptides herein are at least 70%, generally at least 75%, optionally at least 80%, 85%, 90%, 98% or 99% or more identical to a reference polypeptide, or a fragment thereof, e.g., as measured by BLASTP (or CLUSTAL, or any other available alignment software) using default parameters. Similarly, nucleic acids can also be described with reference to a starting nucleic acid, e.g., they can be 50%, 60%, 70%, 75%, 80%, 85%, 90%, 98%, 99% or more identical to a reference nucleic acid or a fragment thereof, e.g., as measured by BLASTN (or CLUSTAL, or any other available alignment software) using default parameters. When one molecule is said to have certain percentage of sequence identity with a larger molecule, it means that when the two molecules are optimally aligned, said percentage of residues in the smaller molecule finds a match residue in the larger molecule in accordance with the order by which the two molecules are optimally aligned.

[080] The term "substantially identical" as applied to nucleic acid or amino acid sequences means that a nucleic acid or amino acid sequence comprises a sequence that has at least 90% sequence identity or more, preferably at least 95%, more preferably at least 98% and most preferably at least 99%, compared to a reference sequence using the programs described above (preferably BLAST) using standard parameters. For example, the BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1992)). Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Preferably, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, more preferably over a region of at least about 100 residues, and most preferably the

sequences are substantially identical over at least about 150 residues. In a most preferred embodiment, the sequences are substantially identical over the entire length of the coding regions.

[081] A "functional variant" of a protein disclosed herein can, for example, comprise the amino acid sequence of the reference protein with at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 conservative amino acid substitutions. The phrase "conservative amino acid substitution" or "conservative mutation" refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and Schirmer, R. H., Principles of Protein Structure, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids may be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and Schirmer, R. H., supra). Examples of conservative mutations include amino acid substitutions of amino acids within the sub-groups above, for example, lysine for arginine and vice versa such that a positive charge may be maintained; glutamic acid for aspartic acid and vice versa such that a negative charge may be maintained; serine for threonine such that a free --OH can be maintained; and glutamine for asparagine such that a free --NH_2 can be maintained. In one embodiment of the present invention, a *COL7A1* gene encodes for the C7 protein or a functional variant thereof, provided that the variant is capable of anchoring the fibrils between the epidermis and dermis.

[082] Alternatively or additionally, the functional variants can comprise the amino acid sequence of the reference protein with at least one non-conservative amino acid substitution. "Non-conservative mutations" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with, or inhibit the biological activity of, the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the reference sequence.

[083] Proteins disclosed herein (including functional portions and functional variants thereof) may comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

[084] The invention includes the vectors INXN-2002; and IGE308 also referred to herein as INXN-2004, as well as vectors substantially identical and/or homologous thereto as well as functional variants thereof.

[085] The term "patient" or "subject" refers to mammals, including humans and animals.

[086] The term "treating" refers to reducing or alleviating the symptoms, and/or preventing relapses and/or the progression of DEB, including RDEB and/or DDEB.

[087] The term "autologous cells" refers to cells obtained and then returned to the same individual. In particular, cells may be removed from the body of a DEB patient, which may subsequently be genetically modified, cultivated to proliferate, and then returned to the body of the patient from which they were originally removed.

[088] The term "transduction" refers to the delivery of a gene(s) using a viral or retroviral vector by means of viral infection rather than by transfection.

[089] The term "transducing lentiviral vector" or "transducing lentiviral vector particle" refers to the infectious lentiviral vector particles formed from the co-transfection of a packaging cell line with the lentiviral expression/transfer plasmid vector comprising the *COL7A1* gene or functional variant thereof, a packaging vector(s), and an envelope vector. The transducing

lentiviral vector is harvested from the supernatant of the producer cell culture after transfection. Suitable packaging cell lines are known in the art and include, for example, the 293T cell line.

[090] The term "lentiviral vector" refers to a vector containing structural and functional genetic elements outside the LTRs that are primarily derived from a lentivirus.

[091] The term "self-inactivating vector" (SIN) refers to vectors in which the 3' LTR enhancer-promoter region, known as the U3 region, has been modified (e.g., by deletion or substitution) to prevent viral transcription beyond the first round of viral replication. Consequently, the vectors are capable of infecting and then integrating into the host genome only once, and cannot be passed further. SIN vectors greatly reduce risk of creating unwanted replication-competent virus since the 3' LTR U3 region has been modified to prevent viral transcription beyond the first round of replication, eliminating the ability of the virus to be passed.

[092] The term "pharmaceutically acceptable carrier" is employed herein to refer to liquid solutions which are, within the scope of sound medical judgment, suitable for use in contact with the autologous, genetically-modified cells without affecting their activity, and without being toxic to the tissues of human beings and animals or causing irritation, allergic response, or other complications, commensurate with a reasonable benefit/risk ratio. A useful pharmaceutically acceptable carrier may be an injectable solution which is biocompatible with the autologous, genetically-modified cells and does not reduce their activity or cause their death.

[093] The present invention relates to an autologous, genetically-modified cell therapy for C7-deficient patients. "C7-deficient patients" either lack or have substantially reduced production of type VII collagen (C7), which is important for anchoring fibril formation at the dermal-epidermal junction (DEJ), resulting in dystrophic epidermolysis bullosa (DEB). The present invention relates to harvesting DEB patient cells, such as fibroblasts or keratinocytes, genetically modifying the harvested cells to insert a functional *COL7A1* gene or functional variant thereof, expanding the genetically modified cells, and administering a population of the genetically-modified cells back into the DEB patient.

[094] Cells are harvested from a patient suffering from DEB. Fibroblasts or keratinocytes are preferred. The cells may be obtained through known methods, including from patient skin

samples, scrapings, and biopsies, for example. In one embodiment, the cells are obtained from the non-blistering skin of a DEB patient. In another embodiment, the cells are obtained from the blistering skin of a DEB patient. The cells harvested from a DEB patient have either a mutated, non-functional or missing *COL7A1* gene. These cells are cultured using standard cell culture techniques. The harvested patient's cells are subsequently treated *ex vivo* with genetic material encoding a functional type VII collagen gene (C7) or functional variant thereof.

[095] The *COL7A1* gene has the nucleic acid, SEQ ID NO:1, which encodes for a 290 kDa alpha chain, wherein three chains form a triple helix (trimer), as depicted in Figure 1A. C7 has the amino acid sequence of SEQ ID NO: 2 and is the protein important for anchoring fibril formation at the dermal-epidermal junction (DEJ). Mutations in the C7 gene cause an absence or reduction of C7, which make up anchoring fibrils that maintain binding of the epidermis to the dermis. C7 anchoring fibrils bind to other collagens, extracellular matrix proteins, and Lam332 that mediate the attachment of the dermis to the epidermis, as shown in Figure 1B. The absence of anchoring fibrils leads to blistering of the skin. The invention also includes using vectors comprising nucleic acids and polypeptides substantially identical to *COL7A1* and C7 respectively. In another embodiment, the present invention includes vectors that comprise nucleic acids that encode a functional variant of the COL7A1 or C7 protein, provided that the variant is capable of anchoring the fibrils between the epidermis and dermis. Another aspect of the invention includes the use of only the open reading frame of the *COL7A1* gene, which encodes the C7 protein or a functional variant thereof. In some embodiments, the *COL7A1* nucleic acid is codon-optimized for increased expression of C7 in the transduced cell, as is known in the art.

[096] In accordance with the present invention, the *COL7A1* can be transduced into the harvested DEB cells using a transducing lentiviral vector, which is replication defective and self-inactivating. Self-inactivating lentiviral vectors are derived from the human immunodeficiency virus (HIV-1), which are pseudotyped with a heterologous VSV-G envelope protein instead of the HIV-1 envelope protein. As previously reported, a 400 bp deletion is introduced to the U3 region of the LTR resulting in a self-inactivating (SIN) vector (Zuffery 1998). The vectors of the present invention are distinguished from 2nd and 3rd generation SIN vectors. In particular, the

vectors of the present invention have been modified to enhance the safety features and increase the cloning capacity as further described herein.

[097] Vectors used to construct the transducing lentiviral vectors of the present invention are introduced via transfection or infection into a packaging cell line. The packaging cell line produces transducing lentiviral vector particles that contain the vector genome. After cotransfection of the packaging vectors, transfer vector, and an envelope vector to the packaging cell line, the recombinant virus is recovered from the culture media and titered by standard methods used by those of skill in the art. Thus, the packaging constructs can be introduced into human cell lines by calcium phosphate transfection, lipofection or electroporation, generally together with a dominant selectable marker, such as kanamycin, neomycin, DHFR, or Glutamine synthetase, followed by selection in the presence of the appropriate drug and isolation of clones. The selectable marker gene can be linked physically to the packaging genes in the construct.

[098] Stable cell lines wherein the packaging functions are configured to be expressed by a suitable packaging cell are known. For example, see U.S. Pat. No. 5,686,279; and Ory et al., (1996), which describe packaging cells. The packaging cells with a lentiviral vector incorporated in them form producer cells. Producer cells are thus cells or cell-lines that can produce or release packaged infectious viral particles carrying the therapeutic gene of interest. An example of a suitable lentiviral vector packaging cell lines includes 293 cells.

[099] In one embodiment of the invention, the transducing lentiviral vector is constructed from a lentiviral expression plasmid vector comprising (a) a modified 5' long terminal repeat (LTR), wherein the promoter of the modified 5' LTR is a cytomegalovirus promoter, (b) the *COL7A1* gene, (c) at least one lentiviral central polypurine tract element, and (d) a modified 3' LTR, wherein the modified 3' LTR comprises a deletion relative to the wild-type 3' LTR and comprises a deletion of a hepatitis B virus post- transcriptional regulatory element (PRE).

[0100] In another aspect of the invention, the deleted hepatitis B virus post- transcriptional regulatory element (PRE) is a woodchuck post-transcriptional regulatory element PRE (WPRE).

[0101] In another aspect of the invention, the lentiviral expression plasmid vector comprises two or more lentiviral central polypurine tract (cPPT) elements. Incorporation of cPPT element has been found to increase gene expression levels.

[0102] In another aspect of the invention, the 3' LTR may be modified to delete a 400 bp fragment (as disclosed by Zuffery 1998), instead of the commonly used 133 bp deletion of standard 3rd generation SIN LV vectors. The 400 bp deletion is believed to increase safety by preventing read-through transcription as well increase viral titer because the vector transcript is more stable in packaging cells.

[0103] In yet another aspect of the invention, the lentiviral expression plasmid may incorporate a hepatitis B virus post-transcriptional regulatory element (PRE), which is preferably woodchuck post-transcriptional regulatory element PRE (WPRE). WPRE has been found to increase the viral titer of the vector.

[0104] The addition of the cPPT, and modified 3'LTR deletion has been shown to improve the function of the lentiviral vector comprising the *COL7A1* gene of the present invention to increase gene expression levels, viral titer, and safety.

[0105] The present invention further includes the use of lentiviral transfer vectors in which certain elements common for 3rd generation lentiviral expression vectors are deleted. In particular, safety concerns for use of lentiviral vectors in humans hinge on the fear of generating a replication-competent lentivirus, which may arise from recombination between split genomes of 3rd generation lentiviral vectors. (Tareen *et al.*, 2013.). The gag sequence and/or the rev-responsive elements (RRE) may be deleted from the viral vector, in order to reduce sequence homology with other helper plasmids and thereby increase safety to humans. Accordingly, in one embodiment of the invention, the gag sequence is absent in the vector. In another embodiment of the invention, both the gag sequence and RRE is absent in the vector.

[0106] The starting materials to generate the lentiviral vectors of the present invention is preferably a lentiviral expression plasmid vector that comprises a cPPT and PRE that can accommodate the insertion of the large *COL7A1* gene (8.89 kbp) or a functional variant thereof. Preferably, the starting material to construct the transducing lentiviral vector of the present

invention is selected from the lentiviral expression plasmid vectors, pSMPUW (Cell Biolabs, Inc., San Diego, CA) and pFUGW (Addgene, Cambridge, MA).

[0107] In one aspect of the invention, the pSMPUW lentiviral expression plasmid vector is selected for construction of the transducing lentiviral vector. Figure 6 shows a schematic of the genetic elements in the pSMPUW lentiviral expression plasmid vector. Features of the pSMPUW lentiviral expression vector have been modified from 3rd generation lentivirus expression vectors in order to enhance gene expression levels and safety features, as depicted in Figure 7. In particular, the pSMPUW lentiviral expression vector encodes for a multicloning site (MCS) followed by the Woodchuck Hepatitis Virus Post-transcriptional regulatory element (WPRE). The residual gag (Δgag) and the RRE element were removed from the pSMPUW vector construct. Additionally, the pSMPUW vector construct utilized a larger 400 bp deletion in the 3'LTR U3 region instead of the commonly used 133 bp deletion in a standard 3rd generation SIN LV vector. Wild type *COL7A1* gene was incorporated into the pSMPUW vector.

[0108] In another aspect of the invention, the pFUGW lentiviral expression plasmid is selected for construction of the transducing lentiviral vector. Figure 8 shows a schematic of the genetic elements in this plasmid vector. Features of the pFUGW vector include a RRE, two cPPT elements, and a WPRE element, which may improve lentiviral production and transgene expression.

[0109] In another aspect of the invention, the WPRE element has been deleted from the lentiviral vector.

[0110] In an embodiment of the present invention, transducing lentiviral vector particles of the present invention are designated INXN-2002 (vector transfer plasmid - IGE230) or INXN-2004 (vector transfer plasmid - IGE308), or a substantially identical vector comprising functional variants thereof.

[0111] The cells harvested from the DEB patient are transformed with a functional *COL7A1* gene. In one aspect of the invention, the cells are transduced with a lentiviral vector having the *COL7A1* gene. In another aspect of the invention, the lentiviral vector is a self-inactivating vector. The copy number of the integrated transgene can be assessed using any known methods.

For example, copy number may be determined through quantitative PCR, multiplex ligation-dependent probe amplification, fluorescent in situ hybridization (FISH), microarray-based copy number screening, and conventional karyotyping. The number of copies of the transgene integrated into each cell may be modulated by the virus dose given to the cells during production. The integrated transgene copy number per cell in the RDEB harvested cells transduced with a *COL7A1*-containing vector is dose dependent.

[0112] Cells harvested from the DEB patient and transformed with the functional *COL7A1* gene will have a functional *COL7A1* gene and exhibit normal cell morphologies. These cells may be caused to proliferate or expanded in culture using standard cell culture techniques.

[0113] Normal fibroblast morphological characteristics are observed among harvested fibroblasts transduced with *COL7A1*-transducing vectors within the scope of the present invention. For example, normal fibroblast morphologies include cells displaying elongated, fusiform or spindle appearance with slender extensions. Normal morphologies further include cells appearing larger, flattened stellate cells which may have cytoplasmic leading edges. Figure 9 displays an example of normal cell morphology for fibroblasts transduced with a *COL7A1*-transducing vector.

[0114] The production of C7 produced by harvested DEB patient cells transduced with a *COL7A1*-transducing vector has also been observed. In particular, the formation of C7 trimers is important in the assembly of anchoring fibrils. It has been found that DEB patient cells transduced with a *COL7A1*-transducing vector are capable of forming the C7 trimers with the correct structure, size, and function.

[0115] In one aspect of the invention, it is possible to verify that the C7 expressed by fibroblasts transduced with a *COL7A1*-transducing vector will be capable of forming anchoring fibrils using immunoprecipitation with an anti-C7 specific antibody. For example, the anti-C7 specific antibody, fNC1, may be used for selective capture and the concentration of C7 from culture supernatants for detection by SDS-PAGE/immunoblot. For example, Figures 9 and 10 demonstrate that C7 produced by fibroblasts transduced with a *COL7A1*-transducing vector were predominantly trimeric.

[0116] In another aspect of the invention, the function of C7 may be assessed using known methods, such as using a laminin binding assay or cell migration assay. C7 has been shown to bind immobilized extracellular matrix (ECM) components, including fibronectin, Laminin 332 (Lam332), COL1, and COL4 (Chen, et al., 2002a). The interaction between C7 and Lam332 occurs through the NH2-terminal NC1 domain of C7 and is dependent upon the native conformation of both Lam332 and C7 NC1 (Rousselle, et al., 1997). The association between C7 and Lam332 is important for establishing correct Lam332 architecture at the dermal-epidermal junction. Such organization is important for interactions with extracellular ligands and cell surface receptors, and for cell signaling (Waterman, et al., 2007). In one aspect of the invention, an ELISA using an antibody against the C7 NC1 domain to detect binding of C7 to purified Lam332 has been developed. Although a C7/Lam332 binding ELISA has already been described in the literature (Chen, et al., 2002a), to our knowledge it has never been used to test C7 present in the supernatants of transduced cells. Results in Figure 12 show dose-dependent binding to Lam332 by C7 expressed by GM-HDFs from the Training and Engineering runs.

[0117] In addition, a cell migration assay can be used to assess functional C7 activity. Previous studies have shown that RDEB fibroblasts and keratinocytes show an increase in motility relative to their normal counterparts, and that normal motility can be restored by expression of C7 (Chen, et al., 2000; Chen, et al., 2002b; Cogan, et al. 2014; Baldeschi et al., 2003). Suitable assay includes the colloidal gold salt migration assay to measure the migration of fibroblasts and keratinocytes, or a wound healing assay to measure the migration of keratinocytes. Cells having functional C7 activity will exhibit a reduced motility relative to RDEB cells in such assays.

[0118] The present invention is directed, in one aspect, to pharmaceutical formulations comprising the autologous, genetically-modified cells from DEB patients. These cells may be present in any amount suitable for the delivery to the patient in which the cells were originally harvested. For example, that cells may be present in a cell concentration of $1.0 - 5.0 \times 10^7$ cells/mL, $1.0 - 4.0 \times 10^7$ cells/mL, $1.0 - 3.0 \times 10^7$ cells/ mL, or $1.0 - 2.0 \times 10^7$ cells/mL. The cells are present in present in a suspension suitable to sustain the viability of the cells, such as in Dulbecco's Modified Eagle's Medium (DMEM). In particular, the viability of the cells in the formulation are present in an amount of $\geq$ 60%, 70%, 75% or 80%. Suitable excipients may also be present in the formulation, such as phosphate buffered saline which may be used to wash

the cells from thawed vials containing the autologous, genetically-modified. Preferably, no phenol red is present in the final formulation.

[0119] The autologous genetically modified cells of the present invention have a transducing vector copy number of at least or about 0.05, 0.09, 0.41, or 0.74 or in the range of about 0.1 to about 6.0, about 0.1 to about 5.5, about 0.1 to about 5.0, about 0.1 to about 4.5, about 0.1 to about 4.0, about 0.1 to about 3.5, about 0.1 to about 3.0, about 0.1 to about 1, 0.4 to about 1, or 0.4 to about 0.75. Preferably, the transducing vector copy number is in the range of about 0.1 about 5.0. It has been found that the the transduced human dermal fibroblast or keratinocytes have at least or about 1, 2, 5, 10, 20, 25, 27, 28, 29, 30, 35, 40, 45, or 50-fold greater integrated transgene copy number per cell relative to a transduction composition not subjected to spinoculation or super-transduction. Moreover, the C7 protein expression values of the FCX-007 cells of the present invention are $\geq$ 300, 350, 400, 450, 500, 550, or 600 ng/day/E6 cell. Preferably, the C7 protein expression values of the autologous genetically-modified cells of the present invention are $\geq$ 500 ng/day/E6 cell. The present invention has also found that expression of C7 has increased by 10, 25, 50, 100, 150, or 200-fold relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction.

[0120] The multiplicity of infection (MOI) refers to the number of vector particles per cell used in transduction. In accordance with the present invention, the desired transducing vector copy number has been achieved even as the MOIs decreases, as shown in the examples below. For example, the invention relates to MOIs of $\leq$ 15, $\leq$ 14, $\leq$ 13, $\leq$ 12, $\leq$ 11, or $\leq$ 10. Preferably, the MOI is between about 1 to 10.

[0121] The formulations of the present invention may be used to treat various maladies of patients suffering from Type VII deficiency. In particular, the formulations of the present invention are suitable to treat DEB, including DDEB or RDEB. In an embodiment of the invention, subtype of RDEB may also be treated, including but not limited to Hallopeau-Seiemens, non-Hallopeau-Siemens RDEB, RDEB inversa, pretibial RDEB, acral RDEB, and RDEB centripetalis.

[0122] The invention further relates to the treatment, reduction, prevention, and/or inhibition of various symptoms attributed to C7 deficiencies. For example, DEB is known to cause scarring

after blisters, which may cause contracture deformities, swallowing difficulty if the mouth and esophagus are affected, fusion of fingers and toes, and limited mobility. Therefore, in one aspect of the invention envisions the treatment, reduction, inhibition, and/or prevention of pseudosyndactyly, also known as mitten hand syndrome. In another aspect, the invention relates to the treatment, reduction, inhibition, and/or prevention of deep fibrosis and/or scarring associated with RDEB and/or DDEB, which may result in milia, joint contractures, generalized soft tissue fibrosis, organ fibrosis, corneal lesions, scarring plaques, scarring alopecia, nail dystrophy, ankyloglosia, and increased frequency of dental caries, for example. In another aspect, the invention relates to the treatment, reduction, prevention, and/or inhibition of oral mucosa lesions and gastrointestinal lesions associated with RDEB. Another embodiment relates to the treatment, prevention, reduction and/or inhibition of blisters associated with DEB patients.

[0123] Administration of the autologous genetically modified cells may be done at any appropriate time as one skilled in the art would be capable of determining based on the needs of the patient. For example, administration may be done once, once a day, once a month, once a quarter, or 1-2 times per year.

[0124] The formulation of the present invention containing the autologous genetically modified cells may be administered to the patient by any known method, including but not limited to injection, topically, orally, or embedded in a biocompatible matrix. The injection may be, e.g., parenteral, intradermal, subcutaneous, intramuscular, intravenous, intraosseous, intraarterial,ocular and intraperitoneal or direct injection into a specific organ/tissue, e.g. prostate or liver. Topically, the formulation may be administered directly to an affected site, such as at the site of a lesion. Debridement of the affected tissue can precede the direct application of the formulation to the site. Alternatively, the formulation may be encapsulated in a suitable delivery system, such as in a polymer capsule, or embedded in a biocompatible matrix or graft, e.g., collagen matrix, in a hydrogel, skin graft, or a mesh.

[0125] The autologous genetically modified cells of the present invention may be administered solely or in combination with other treatments for a patient suffering from DEB. Examples of therapeutic agents used for treating DEB include topical care, such as Zorblisa, skin grafts, anti-inflammatories, antibodies, other potential gene or cell-based therapies. In another embodiment,

the modified cells of the present invention may be administered for for the skin or muoscal areas where bone marrow transplant therapies (or other system cellular therapies such as mesenchymal stem cells) did not provide sufficient therapy. The C7-expressing ability of the autologous genetically modified cells is preferably not impaired by a combination with other therapeutic agents.

[0126] The present invention further relates to a method of enhancing or increasing the integrated transgene copy number per cell in the genetically modified cells obtained from C7-deficient patients and transduced according to the present invention. In particular, it has been found that certain steps during transduction substantially enhanced the copy number of the transgene in these cells. In one aspect of the invention, the cells obtained from the C7-deficient patient are contacted with the lentiviral vector of the present invention and that composition is subjected to spinoculation, also known as spin transduction. In this manner, the lentiviral vectors are spun onto the targeted cells. The addition of spinoculation has unexpectedly increased the copy number per cells in the genetically modified human dermal fibroblasts by a level of $\geq$ 2--fold relative to a transduction without spinoculation or transduction. However, in order to identify additional methods to increase copy number, it has unexpectedly been found that a second transduction step (or super-transduction) in which the targeted cells are passaged through the first transduction with spinoculation, and then subsequently passaged through a second transduction optionally with spinoculation. In this manner, it has been found that this super-transduction with spinoculation increases the copy number by an additional 2-fold increase relative to the original traditional transduction method that did not include spinoculation or super-transduction.

[0127] In another embodiment, it has been found that changing the lentiviral vector from INXN-2002 to INXN-2004 increased the transgene copy number in the genetically modified human dermal fibroblasts by 4-fold relative to traditional transduction without spinoculation or super-transduction.

[0128] Through extensively evaluating integrated transgene copy numbers, it has been found the cumulative change of adding spinoculation, adding super-transduction with spinoculation, and changing from INXN-2002 to INXN-2004, relative to the original transduction process that did

not include spinoculation or super-transduction, unexpectedly increased the copy number > 27-fold (*see* Example 9: comparing TR12.1 and TR3 to TR8 in original studies (which did not include spinoculation or super-transduction). Accordingly, the present invention relates to increased the copy number of the transduced cells from the C7-deficient patient.

[0129] To further illustrate the invention, the following non-limiting examples are provided.

EXAMPLES

[0130] FCX-007 is a suspension of live, autologous human dermal fibroblast cells genetically modified using either a lentiviral vector (INXN-2002) (as shown in Example 1) or a lentiviral vector (INXN-2004) (as shown in Example 2) to express the human collagen type 7 protein.

[0131] EXAMPLE 1

A. Elucidation of Structure and Characteristics of FCX-007 transduced with Lentiviral Vector INXN-2002

[0132] The FCX-007 cells derived from the lentiviral vector INXN-2002 cells are suspended in a cryopreservation medium consisting of Iscove's Modified Dulbecco's Medium (IMDM) without fetal bovine serum (50.0%), Profreeze-CDMTM (42.5%) and dimethyl sulfoxide (DMSO) (7.5%). The structural features for FCX-007 Drug Substance (DS) include the primary structure of the autologous human dermal fibroblast (HDF) cells and the structure of the lentiviral vector used to transduce and gene modify the HDF cells.

B. Lentiviral Vector (INXN-2002)

[0133] INXN-2002 Lentiviral Vector (LV), which is used to transduce and to introduce the human collagen 7A1 gene into the HDF cells, is a recombinant lentiviral vector encoding the human collagen 7A1 gene. INXN-2002 LV is a self-inactivating (SIN) lentiviral vector that is constructed based on the human immunodeficiency virus type 1 (HIV-1) pseudotyped with a heterologous VSV-G envelope protein. The virus particle for INXN-2002 lentiviral vector is approximately 120 nm in diameter and is comprised of numerous proteins with two copies of a single-stranded RNA genome. A specific molecular formula, molecular weight, or stereochemistry is not available.

[0134] Structural features for INXN-2002 LV include the primary structure of the RNA viral genome and the structure of the viral particle. The primary structure of the RNA genome of INXN-2002 is determined by the full nucleotide sequencing of the viral genome. The viral particles structure is deduced from the particle structure of HIV-1 with added VSV-G protein pseudotyping. An overview of the nucleic acid structure and the structure of the viral particle are provided below.

C. INXN-2002 RNA Viral Genome Structure

[0135] INXN-2002 LV is a self-inactivating (SIN) lentiviral vector that is constructed based on the human immunodeficiency virus type 1 (HIV-1) pseudotyped with a heterologous VSV-G envelope protein instead of the HIV-1 envelope protein. A 400 bp deletion is introduced to the U3 region of the LTR resulting in a self-inactivating (SIN) vector (Zuffery 1998). The pSMPUW lentiviral expression plasmid vector (Cell Biolabs, Inc., San Diego, CA) was selected for construction of the INXN-2002 LV. Figure 6 shows a schematic of the genetic elements in the pSMPUW lentiviral expression plasmid vector.

[0136] The coding elements between the 5' and 3' LTRs of the HIV-1 virus are fully gutted. The vector encodes for a multicloning site followed by the Woodchuck Hepatitis Virus Posttransscriptional Regulatory Element (WPRE). In order to maximize the cloning capacity of the vector, these elements were removed by digesting the vector with BamHI from the multicloning site and KpnI at the 5' end of the 3' left terminal repeat. The COL7A1 gene expression cassette with a CMV promoter is cloned into the digested vector by single strand annealing to generate the lentiviral vector plasmid construct encoding the COL7A1 gene as shown in Figure 13.

[0137] The lentiviral vector plasmid construct is co-transfected into HEK293T cells with three helper plasmids (pCMV-G, pCMV-Rev2 and pCgp) to produce the INXN-2002 lentiviral vector particles. pCMV-G plasmid provides the VSV-G pseudotyping surface protein, pCMV-Rev2 provides the HIV-1 Rev protein for efficient RNA transport and packaging, and pCgp provides the structural and viral enzyme proteins for production of the INXN-2002 lentiviral vector particles. Figure 14 shows a schematic of the proviral RNA genome structure of the INXN-2002 lentiviral vector.

[0138] D. INXN-2002 Lentiviral Vector Particle Structure

[0139] INXN-2002 lentiviral vector is constructed based on a HIV-1 derived vector backbone and has a similar viral particle structure to the HIV-1 virus. Figure 9 shows an electron micrograph of HIV-1 particles.

[0140] The HIV-1 particle has a spherical shape of approximately 120 nm in diameter, with an estimated molecular weight of 277MDa (Carlson 2008). The particle has a lipid bilayer membrane stubbed with envelope protein. The envelope protein interacts with the receptors on the target cells for infection and delivery of the RNA viral genome to the target cells. A cone shaped nucleo-core, where two strands of viral RNA genome is housed, can be observed inside the virus particle. The cone shaped nucleo-core is formed by the viral capsid protein.

[0141] For INXN-2002, VSV-G (glycoprotein of the vesicular stomatitis virus (VSV-G)) is used as a substitute for the HIV-1 envelope proteins resulting in improved vector stability, target cell tropism, and transduction efficiency (Cronin 2005).

[0142] During production, INXN-2002 viral particles assemble and bud out from the surface of transfected HEK293T cells. The VSV-G protein is provided by the pCMV-G helper plasmid, the vector core and enzyme proteins are provided by the pCgp helper plasmid, and the Rev protein, which is needed for efficient RNA genome transport and packaging into the viral particle, is provided by the pCMV-Rev2 plasmid. It is noted that all the other HIV-1 accessory proteins including Vpu, Vif, Vpr, Nef, and Tat, are deleted from the INXN-2002 vector.

[0143] After budding from the producer cell surface, the protease enzyme packaged inside the virus particle cleaves the Gag precursor protein into its constituent proteins (MA, CA, NC), to convert the immature virion into a mature infectious INXN-2002 vector particle. Figure 15 shows a schematic depicting the details of the mature INXN-2002 virus particle.

[0144] Two strands of the INXN-2002 RNA genome are packaged inside the cone shaped core formed by the Capsid protein (CA). The nucleocapsid (NC) protein forms a stable complex with the RNA genome inside the capsid core. The matrix protein forms a coat on the inner surface of the membrane. The virus buds through the cell plasma membrane spiked with the VSV-G envelope protein and forms the lipid envelope. Based on the recent three dimensional analysis of

HIV-1 virus particle structure (Carlson 2008), Table 1 shows the component proteins that make up the INXN-2002 lentiviral vector and a brief description of the function of each of the components.

Table 1: INXN-2002 Lentiviral Vector Major Component Proteins

Component Proteins	**Protein MW (kD)**	**Protein functions**
Capsid (CA)	24	Forms the nucleo-core
Matrix (MA)	17	Forms the protein coat on the inner surface of the lipid membrane
Nucleocapsid (NC)	7	Forms a stable complex with the RNA genome
VSV-G	69	Pseudotyped envelope protein interacts and binds to receptors on target cells for transduction to deliver the vector RNA genome to the cells
Protease	11	Plays an important role in the maturation of the INXN-2002 vector by cleaving the Gag proteins to its functional constituents, CA, MA, and NC
Reverse transcriptase	66/51	Builds a DNA copy of the viral RNA genome
Integrase	31	Inserts the DNA copy of the viral RNA genome into the infected cell genome

E. INXN-2002 Characterization

[0145] Table 2 provides a list of the characterization assays and specifications for INXN-2002 manufactured by City of Hope. Characterization assay results are provided in the attached CoA for INXN-2002 lot number 0786-240-0002-1, the lot intended for use in manufacture of the FCX-007 clinical product.

Table 2: Characterization of INXN-2002

Category	Assay	Test Method	Lab	SOP	Target Specification
Identity	Vector identity	RT-PCR	CoH	QC-SOP-0843	Band of correct size detected
	Vector insert stability	Southern blot analysis	Indiana University	VP-10-17.16	Report result
Potency	Viral antigen Physical Titer (p24)	ELISA	Indiana University	VP-10-05.06	Report result
	TU titer	H1299 transduction with qPCR readout	BioReliance	016135.BSV	Report result
	C7 expression (Potency)	ELISA	BioReliance	016136.BSV	Report result
Appearance and pH	Appearance	Visual inspection	CoH	QC-SOP-0734	Opaque solution
	pH	pH meter	CoH	QC-SOP-0675	6.9 - 7.8

F. Characterization of INXN-2002 by an in vitro Immortalization Assay

[0146] The potential for insertional genotoxicity of INXN-2002 was evaluated using an in vitro immortalization (IVIM) assay. The test was conducted at Cincinnati Children's Hospital Medical Center, Division of Experimental Hematology & Cancer Biology (CCHMC).

[0147] The principle of the IVIM test is based on the understanding that normal Lineage negative (Lin-) bone marrow (BM) cells will stop proliferating after 3-4 weeks in vitro, but in the presence of certain vector integrations which cause upregulation of proto-oncogenes, some clones will continue to proliferate after 5 or more weeks. The number of such immortalized clones is representative of the oncogenic potential of the vector. The immortalized clones are expanded and further analyzed for stem cell markers by FACS, vector copy number by qPCR or the site of integration by LAM-PCR. Insertion in common integration sites (cis) such as Evi1 or Prdm 16 is frequently observed in immortalized clones generated in IVIM assays (Calmels et al., 2005; Modlich et al., 2008).

[0148] Lineage-negative (Lin-) bone marrow (BM) cells from C57BL/6 mice were isolated from complete BM by magnetic sorting using lineage-specific antibodies. The Lin- BM cells were then cultured and stimulated in complete growth medium for 2 days. On Day 4, cells were transduced with INXN-2002 vector in a 48-well plate coated with RetroNectin (10μg/cm^2, Takara). Figure 16 shows a schematic setup of the IVIM assay.

[0149] To enhance the INXN-2002 transduction efficiency on Lin- BM cells, INXN-2002 vector was concentrated approximately 14-fold using a spin concentrator. For transduction, 80 μL of the concentrated INXN-2002 was added to each well containing 1.5 x 10^5 cells with 150 μL of transduction medium. The plate was centrifuged for 1000g at 32°C for 70 minutes (spinocculation) to further enhance the transduction efficiency. Cells were incubated at 37°C, 5% CO_2 incubator O/N.

[0150] In order to achieve a high INXN-2002 vector copy number in the transduced Lin- BM cells, INXN-2002 transduction was repeated on Day 5, Day 6, and Day 7 for a total of 4 consecutive transductions.

[0151] The transduced Lin- BM cells were expanded for 10 days and cell concentration was adjusted to 2 – 4 x 10^5 cells/ml every two days, and fresh medium was provided as needed. On Day 10 post transduction, cells were harvested and counted. A portion of the cells was then submitted for DNA isolation and qPCR to determine the vector copy number (VCN). The remaining cells were placed back into culture for potential plating of the IVIM assay between days 18 – 21.

[0152] Table 3 shows the pilot test run result of INXN-2002 transduced Lin- BM cells harvested on Day 10.

Table 3: Day 10 Post-Transduction Lin- BM Cells Analysis

Test Article	Total Cells	Cell Viability	Vector Copy per Cell
INXN-2002	1.5×10^6	89%	0.09
Control vector-1	2.7×10^6	95%	0.81
Control vector-2	2.1×10^6	91%	3.58
Mock	3.0×10^6	88%	0.0

[0153] Despite the use of concentrated INXN-2002 vector and 4x consecutive spinocculation transduction, the vector copy number reached in the Lin- BM cells was significantly lower than the targeted copy number of 1-3 expected for the IVIM test. The pilot test was terminated.

[0154] Because of the low vector copy numbers experienced in the IVIM assays as well as the low vector copy numbers achieved in all the RDEB fibroblast cell transduction runs, it is believed that this lot of INXN-2002 poses minimal risk of insertional genotoxicity when used to transduce RDEB fibroblast cells. No further analysis of this lot of INXN-2002 by the IVIM assay was pursued.

G. FCS-007 Transduced with INXN-2002

[0155] The human dermal fibroblast cells used for the manufacture of FCX-007 are derived from a live skin biopsy. The biopsy is digested using Liberase® (Roche) to release the dermal fibroblast cells and then the cells are expanded in culture using standard cell culture techniques. At the completion of culture expansion after INXN-2002 transduction, the cells are harvested and washed, then formulated to contain $1.0 - 3.0 \times 10^7$ cells/mL. The DS is tested for purity and confirmed to contain $\geq 98\%$ fibroblasts by CD90 staining with cell viability of $\geq 85\%$.

[0156] FCX-007 cells transduced with the INXN-2002 LV in the DS formulation display typical fibroblast morphologies when cultured on tissue culture surfaces. Specifically, cells may display an elongated, fusiform or spindle appearance with slender extensions, or cells may appear as larger, flattened stellate cells which may have cytoplasmic leading edges. A mixture of these morphologies may also be observed. Figure 17 shows a typical cell morphology and structure of FCX-007 DS in culture.

[0157] The cells express proteins characteristic of normal fibroblasts including the fibroblast specific marker, CD90 (Thy-1), a 35 kDa cell-surface glycoprotein, and the extracellular matrix proteins, such as various types of collagen.

H. FCX-007 Derived from Lentiviral Vector INXN-2002: Drug Substance Release Characterization

[0158] FCX-007 Drug Substance is characterized for release at three stages during manufacturing: in- process, bulk harvest, and after cryopreservation (DS). Table 4 provides a list of the characterization assays and specifications for release of FCX-007 DS manufactured by PCT. Characterization assay results for the finished Drug Substance are provided in the attached CoTs for Training Run 8 Arm A, Arm B and Arm C (Non-Transduced Control).

Table 4: FCX-007 Drug Substance Release Characterization

Stage	Assay	Test Method	SOP	Specification
In-Process	Morphology	Microscopic evaluation	PCT SOP-0334	Pass
	Cell confluence	Microscopic evaluation	PCT SOP-0334	Pass
Bulk Harvest	Cell count	Hemacytometer	PCT SOP-0329	≥1E9
	Cell viability	Hemacytometer	PCT SOP-0329	≥ 85%
Drug Substance-Cryovial	Cell Count	Hemacytometer	PCT SOP-0329	1.0-2.7E7 cells/mL
	Cell Viability	Hemacytometer	PCT SOP-0329	>/= 85%
	Purity	FACS	PCT SOP WI-1067	>/= 98% CD-90+
	COL7A1 gene copy #	qPCR	BioReliance109011.BSV	Report result
	C7 expression	ELISA	PCT SOP-0338	Report result

I. Additional FCX-007 DS Derived from INXN-2002 Characterization

[0159] Additional characterization of FCX-007 has been performed on the training production runs to confirm expression of functional C7. The representative assessments shown below are from Training Run 8 (TR8) where cells were transduced with either a high dose (3.4 IU/cell) or

low dose (1.7 IU/cell) of LV-COL7, or were mock-transduced. These transduction arms are also referred to as Arm A, B, and C, respectively.

J. FCX-007 DS Transduced with INXN-2002: C7 Expression Level

[0160] An enzyme-linked immunofluorescence assay (ELISA) was developed for the purpose of quantifying C7 expression by FCX-007. For this assay, TR8 Drug Substance vials (high dose, low dose, and mock-transduced) were thawed and cultured for 3 days and conditioned cell culture supernatants were collected and assayed for C7. Results in Figure 18 show virus dose-dependent protein expression that ranges from 60 to 120 ng/mL C7 in LV-COL7-transduced cells.

K. FCX-007 DS Transduced with INXN-2002: C7 Trimer Formation

[0161] Anchoring fibrils are formed from the assembly of C7 trimers. The proper expression and formation of C7 trimers by FCX-007 can be detected by immunoprecipitation of C7 followed by non-denaturing SDS-PAGE/immunoblot analyses. For Figure 19, C7 was immunoprecipitated from FCX-007 cell culture supernatants at passage 1 and passage 2 post- thaw using a NC1-specific antibody and was separated on non-denaturing SDS-PAGE then visualized by western blot. The C7 produced by RDEB fibroblasts was predominantly trimeric (red arrow; ~870 kDa) with LV-COL7-transduced cells (Transduction Arms A and B) expressing more C7 than mock-transduced cells (Arm C, starred). Monomeric (290 kDa) and dimeric (580 kDa) forms were also observed. Assay controls included immunopreciptation of purified C7 (Pur COL7) and immunoprecipitation without antibody or test sample (IP Controls).

L. FCX-007 Transduced with INXN-2002: C7 Binding to Lam332

[0162] C7 interacts with Laminin332 at the dermal/epidermal junction (DEJ). The interaction between C7 and Lammin332 is important for anchoring fibril functionality (Chen 2002, Rousselle 1997, Waterman 2007). A binding assay for detection of this interaction was developed at Intrexon. Drug Substance vials (high dose, low dose, and mock-transduced) were thawed and cultured for 2 days. Conditioned cell culture supernatants were collected and were incubated with Lam332 or bovine serum albumin (BSA)-coated wells and bound C7 was detected using a C7 NC1- specific antibody and an HRP-conjugated secondary antibody. The

assay readout is optical density at 450 nm (OD450). Results in Figure 20 show virus dose-dependent binding to Lam332 compared with a BSA control.

M. FCX-007 Derived from INXN-2002 Migration Assessment

[0163] A migration assay was developed to assess function of C7. Chen 2000 has demonstrated that RDEB patient skin cells migrate faster than normal skin cells into an artificial wound margin created on tissue culture vessels, and that application of C7 can restore the migration rate. Normal human dermal fibroblasts (NHDFs; Lonza) and cells from FCX-007 Drug Substance vials were thawed and cultured. Cells were seeded into culture dishes with an insert to prevent cell adherence in a small strip of the culture dish. The strip was then removed and the rate at which the cells migrated into the open area was monitored by microscopy and quantified using the irregular shape delineation plugin for ImageJ software. Figures 21A-21B show both the percent migration (A) and the images of migration (B) for this assay. The results show that the mock-transduced RDEB patient fibroblasts migrate into the open area faster than NHDFs, and transduction with LV-COL7 reverts the patient cells to a rate of migration similar to NHDFs. These results are consistent with those described by Chen 2000.

Example 2: FCX-007 Transduced with INXN-2004 Lentiviral Vector

[0164] FCX-007 is an autologous fibroblast cell product genetically modified by INXN-2004 lentiviral vector (LV-COL7) to express the human collagen 7 protein (C7). The materials used to manufacture FCX-007 DS/INXN-2004 are described below are similar to that set forth above in Example 1, except that the IGE-230 LV-COL7 vector plasmid was used for the production of INXN-2002. IGE-308 LV-COL7 vector plasmid was used to make the INXN-2004 vector. The same helper plasmids, pCMV-G, pCMV-Rev2, and pCgp were used to co-transfect a 293 WCB cell line.

[0165] INXN-2004 Lentiviral Vector

[0166] IGE308 LV-COL7 Vector Transfer Plasmid

[0167] The IGE308 plasmid was constructed using standard molecular cloning methods. The construction process involved cloning of the human *COL7A1* gene and introduction of the cloned

COL7A1 gene into a lentiviral vector (SIN) backbone, pFUGW, to produce the IGE308 LV-COL7 vector transfer plasmid.

Cloning of the Human *COL7A1* Gene

[0168] The *COL7A1* gene cloned into the IGE308 LV-COL7 vector transfer plasmid is the same *COL7A1* gene cloned into INXN-2002 (IGE230 vector transfer plasmid).

[0169] Primers were designed and produced with the Takara PrimeScript reverse transcriptase to amplify the *COL7A1* gene from human genomic cDNA. Four primer pairs, as shown in Table 5 were designed, each to amplify approximately 2 kb of the *COL7A1* gene.

Table 5: Primer Sets used to Amplify the Human *COL7A1* Gene

Forward Primer	Primer Sequence	Reverse Primer	Primer Sequence	Expected Amplicon Size (bp)
CollF1 (SEQ ID NO: 3)	CGACTTGTGTTGGGACTGG CTAGCGCCACCATGACGCT GCGGCTTCTGGTGGC	CollR3 (SEQ ID NO: 7)	CCCGCACAGTGTAGCTAA GCCC	1753
CollF3 (SEQ ID NO: 4)	GGGCTTAGCTACACTGTGC GGG	CollR2 (SEQ ID NO: 8)	CCTTTGGACAATACACTG GGCAGG	2059
CollF2 (SEQ ID NO: 5)	CCTGCCCAGTGTATTGTCC AAAGG	CollR4 (SEQ ID NO: 9)	CAGATGCCTTGATGCCAG CAG	2127
CollF4 (SEQ ID NO: 6)	CTGCTGGCATCAAGGCATC TG	CollR1 (SEQ ID NO: 10)	CGTGATTTCATTTGCTAC ACGTAATCGATTCAGTCC TGGGCAGTACCTGTCCC	2993

[0170] Products were amplified with two primer pairs (CollF2/CollR4 and CollF4/CollR1) designed to amplify the 5' end 3790 base pairs (bp) of *COL7A1* from human genomic cDNA along with a 5' overhang for cloning into the expression vector. The two 5' PCR products (2127 bp and 2993 bp) were joined by overlap extension PCR to generate a 3790 bp final product.

[0171] In order to clone the remainder of the gene, a fragment of DNA (341bp, Col1A1-3, Table 6 was synthesized encompassing the final 322 bp of the *COL7A1* gene with overlaps to the PCR amplified 5' 3790bp gene fragment and the cloning vector.

Table 6: Col1A1-3 Fragment Sequence

Fragment	Sequence
Col1A1-3 (SEQ ID NO: 11)	CGCTCCCAGAACATCACCTACCACTGCAAGAACAGCGTGGCCTACATG GACCAGCAGACTGGCAACCTCAAGAAGGCCCTGCTCCTCCAGGGCTCC AACGAGATCGAGATCCGCGCCGAGGGCAACAGCCGCTTCACCTACAGC GTCACTGTCGATGGCTGCACGAGTCACACCGGAGCCTGGGGCAAGACA GTGATTGAATACAAAACCACCAAGACCTCCCGCCTGCGCATCATCGAT GTGGCCCCCTTGGACGTTGGTGCCCCAGACCAGGAATTCGGCTTCGAC GTTGGCCATGTCTGCTTCCTGTAAATCGATTACGTGTAGCAAATGAAAT CACG

[0172] The two *COL7A1* gene fragments (3790 bp and Col1A1-3) were assembled into an inducible expression vector (VVN-257673) that had previously been digested with NheI and ClaI using the In-Fusion HD Cloning Kit. Bacterial clones were screened by PCR with primers specific to the backbone vector and *COL7A1* sequence to identify candidates for sequencing. Positive clones were confirmed by digestion and *COL7A1* fragment DNA sequencing. The resulting plasmid was named VVN-4311835.

[0173] VVN-4311835 and a *COL7A1* expression plasmid (SC300011) purchased from Origene were digested with BstZ17I and SapI. A 6276 bp fragment of the *COL7A1* gene was cloned from SC300011 into VVN-4311835. The resulting plasmid, VVN-4311835 (used the same plasmid number), was completely sequenced and the *COL7A1* sequence was confirmed to be complete and without mutations.

[0174] VVN-4311835 plasmid was digested with NheI and ClaI, two restriction enzymes with sites just outside of the *COL7A1* coding sequence. The excised *COL7A1* gene was cloned into a constitutive expression vector, VVN-257231 which was also digested with NheI and ClaI, to produce VVN-4319958. VVN-4319958 expresses the full length human *COL7A1* under the control of the constitutive CMV promoter.

[0175] Figure 22 provides a schematic for the cloning of the human *COL7A1* gene.

[0176] Introduction of the Cloned *COL7A1* Gene into a Lentiviral Vector (SIN)

[0177] The pSMPUW lentiviral expression vector (VPK-211, Cell Biolabs, Inc., San Diego, CA) was initially selected for construction of INXN-2002 lentiviral vector encoding the *COL7A1* gene based on its enhanced safety features and large cloning capacity.

[0178] Figure 7 compares the pSMPUW vector to a standard 3rd generation SIN LV vector. The pSMPUW vector encodes for a multicloning site (MCS) followed by the Woodchuck Hepatitis Virus Post-transcriptional regulatory element (WPRE). The residual gag (Δgag) and the RRE element were removed from the pSMPUW vector construct. Additionally, the pSMPUW vector construct utilized a larger 400 bp deletion in the 3'LTR U3 region instead of the commonly used 133 bp deletion in a standard 3rd generation SIN LV vector.

[0179] Details of the cloning of the *COL7A1* gene into the pSMPUW lentiviral expression vector for construction of INXN-2002 lentiviral vector were described above in Example 1, and are further described below.

[0180] An insert was generated containing a CMV promoter followed by a Kozak sequence and a truncated version of the *COL7A1* gene with BclI and SapI restriction sites to use for introduction of the entire coding sequence. This insert was synthesized in two fragments, CColG and ColG2 (IDT) (Table 8). These two fragments were assembled with the digested pSMPUW vector using the In-Fusion HD Cloning Kit. Positive clones were identified using colony PCR with a primer specific to the plasmid backbone (63968-3R), and a primer specific to the *COL7A1* gene (ColS14). Plasmids from positive clones were purified and sequence confirmed to generate IGE228. IGE228 was digested with FspI and BamHI.

[0181] Initial attempts to clone the *COL7A1* gene using the BclI and SapI sites were unsuccessful. An alternate strategy was devised using a PCR product as a linker to bypass the BclI restriction site. Primers EPF5 and CollR3 were used to generate a PCR product from the plasmid template VVN-4319958. This product was digested with BamHI, which cuts 48 bp upstream of the 5' end of *COL7A1*, and FspI, which cuts 1630 bp into the 5' end of *COL7A1* to generate the 5' linker.

[0182] The wild type *COL7A1* gene was cut with FspI and SapI from VVN-4319958. This fragment was ligated to the digested IGE228 and the 5' linker to generate a lentivirus construct for the expression of *COL7A1*, VVN-4580853 (also named IGE230). Positive clones were identified using the primers ColS13, specific to *COL7A1* and 63968-3R, specific to the lentiviral backbone. The plasmid was sequenced from the CMV promoter to the 3' end of *COL7A1*.

[0183] Table 7 below shows the synthetic gene sequences and primers used for introduction of the *COL7A1* gene into the pSMPUW lentiviral expression vector.

Table 7: Synthetic Gene Elements and Primers

Gene Element / Primer Name	Sequence
CColG (SEQ ID NO: 12)	TTCAAATTTTCGGGGGATCGCATTAGTTATTAATAGTAATCAATTACGGGG TCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAA ATGGCCCGCCTGGTTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAA TGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAAT GGGTGGAGTATTTACGGTAAACTGCCCACTTGGTAGTACATCAAGTGTATC ATACGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCT GGTATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACAT CTGCGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATC AATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCC ATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTA CGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGC TAGCATTGTGTGCTTTTCGGGCCACCATGACGCTGCGGCTTCTGGTGGCCGC GCTCTGCGCCGGGATCCTGGCAGAGGCGCCCCGAGTGCGAGCCCAGCACA GGGAGAGAGTGAC
ColG2 (SEQ ID NO: 13)	AGTGCGAGCCCAGCACAGGGAGAGAGTGACCTGCACGCGCCTTTACGCCG CTGACATTGTGTTCTTACTGGATGGCTCCTCATCCATTGGCCGCAGCAATTT CCGCGAGGTCCGCAGCTTTCTCGAAGGGCTGGTGCTGCCTTTCTCTGGAGC AGCCAGTGCACAGGGTGTGCGCTTTGCCACAGTGCAGTACAGCGATGACCC ACGGACAGAGTTCGGCCTGGATGCACTTGGCTCTGGGGGTGATGTGATCCG CGCCATCCGTGAGCTTAGCTACAAGGGGGGCAACACTCGCACAGGGGCTG CAATTCTCCATGTGGCTGACCATGTCTTCCTGCCCCAGCTGGCCCGACCTGG TGTCCCCAAGGTCTGCATCCTGATCACGTCTCTCATGCAGAGGAGGAAGAG CGGGTACCCCCTGAGGATGATGAGTACTCTGAATACTCCGAGTATTCTGTG GAGGAGTACCAGGACCCTGAAGCTCCTTGGGATAGTGATGACCCCTGTTCC CTGCCACTGGATGAGGGCTCCTGCACTGCCTACACCCTGCGCTGGTACCAT CGGGCTGTGACAGGCAGCACAGAGGCCTGTCACCCTTTTGTCTATGGTGGC TGTGGAGGGAATGCCAACCGTTTTGGGACCCGTGAGGCCTGCGAGCGCCGC TGCCCACCCCGGGTGGTCCAGAGCCAGGGGACAGGTACTGCCCAGGACTG AGTACCTTTAAGACCAATGACTTACAA

Gene Element / Primer Name	Sequence
63968-3R (SEQ ID NO: 14)	ATGGAAAAACGCCAGCAACG
ColS14 (SEQ ID NO: 15)	CTGTCACCCTTTTGTCTATG
EPF5 (SEQ ID NO: 16)	GTTGCCGGACACTTCTTGTCCTCT
CollR3 (SEQ ID NO: 17)	CCCGCACAGTGTAGCTAAGCCC
ColS13 (SEQ ID NO: 18)	AGAGGCCCCGAAGGACTTCA

[0184] Figure 23 provides a schematic of the cloning of the *COL7A1* gene into the pSMPUW expression vector to produce the INXN-2002 lentiviral vector transfer plasmid, IGE230.

[0185] Results from continuing development indicated that deletion of the RRE element and the use of a large 400 bp deletion in the 3'LTR U3 region in the pSMPUW vector construct, combined with the requirement to package a large *COL7A1* gene (8.8 kbp), resulted in a negative impact on LV-COL7 vector production (infectious titer) and subsequent transduction and C7 protein expression in RDEB fibroblast cells.

[0186] A 3rd GEN SIN vector, pFUGW (FUGW, plasmid #14883, www.addgene.org) was selected to construct a second generation LV-COL7 vector, INXN-2004. INXN-2004 was constructed to improve the vector copy number. Figure 8 provides a schematic of the genetic elements in the pFUGW lentiviral expression plasmid vector.

[0187] The pFUGW vector contains a RRE, two cPPT elements, as well as a WPRE (woodchuck hepatitis virus posttranscriptional regulatory element) element for achieving improved lentiviral vector production and transgene expression. The IGE308 lentiviral vector transfer plasmid was constructed to maximize the transgene cloning capacity to accommodate the insertion of the large *COL7A1* gene (8.8 kbp).

[0188] The WPRE element, the hUBC promoter and the GFP reporter gene were removed through digestion of the vector with PacI and XhoI. An insert was generated containing the 5' end of the CMV promoter followed by a small fragment of the 3' end of *COL7A1* gene. This insert (ColCN) was synthesized at IDT as a G-Block fragment (sequence in Table 8). The fragment was assembled with the digested pFUGW vector using a Gibson cloning derivative method. Positive clones were identified using colony PCR with primers specific to the plasmid backbone (FugwQCF and FugwQCR). Plasmids from positive clones were purified and sequence confirmed to generate IGE301.

[0189] IGE301 was then digested with BaeI. IGE230 was digested with AseI and SapI to create a fragment containing the 3' end of the CMV promoter, the 5' UTR, and the majority of the *COL7A1* gene. The fragment was transferred to the BaeI-digested IGE301 vector using a Gibson cloning derivative method. Positive clones were identified using the primers ColS13, specific to *COL7A1* and FugwQCR, specific to the lentiviral backbone. Plasmids from positive clones were purified and sequence-confirmed to generate IGE308.

[0190] Table 8 below provides the synthetic gene sequences and primers used for introduction of the *COL7A1* gene into the pFUGW lentiviral expression vector.

[0191] Plasmids were Maxi-prepped with the Qiagen MaxiPrep Kit per the manufacturer's protocol.

Table 8: Synthetic Gene Elements and Primers used in the Final Steps of IGE308 Creation

Gene Element / Primer Name	Sequence
ColCN (g-block) (SEQ ID NO: 19)	Acagcagagatccagtttggttaattaagcattagttattaatagtaatcaattacggggtcattaagactcggcctta gaaccccagtatcagcagaaccgcgtctctcatgcagaggaggaagagcgggtacccccctgaggatgatgagt actctgaatactccgagtattctgtggaggagtaccaggaccctgaagctccttgggatagtgatgacccctgttcc ctgccactggatgagggctcctgcactgcctacaccctgcgctggtaccatcgggctgtgacaggcagcacaga ggcctgtcacccttttgtctatggtggctgtggagggaatgccaaccgttttgggacccgtgaggcctgcgagcgc cgctgcccacccccgggtggtccagagccaggggacaggtactgcccaggactgatcgataccgtcgacctcga gacctagaaaaacat
FugwQCF (SEQ ID NO: 20)	GTAGACATAATAGCAACAGAC

Gene Element / Primer Name	Sequence
FugwQCR (SEQ ID NO: 21)	TATTGCTACTTGTGATTGCTC
ColS13 (SEQ ID NO: 22)	AGAGGCCCCGAAGGACTTCA

[0192] Figure 24 provides a schematic representation of the construction of the INXN-2004 lentiviral vector transfer plasmid (IGE308).

[0193] Production of IGE-308 Plasmid

[0194] The IGE308 plasmid was produced using a process consisting of five steps: transformation, glycerol stock production, scale up, capture, diafiltration and formulation.

[0195] Transformation: a seed stock of the IGE308 plasmid was provided to Aldevron for transformation into the competent *E. coli* (DH10B). Transformation plates were stored at 34°C which is the standard for lentiviral constructs.

[0196] Glycerol stock production - Seed cultures were created by picking isolated colonies from the transformation plate. Each culture was mini prepped and the best seed culture, as determined via agarose gel, was used to make the working glycerol stocks. Scale Up - One 500 mL culture was grown in each of the following media types: Rapid Growth media and Maximum Yield media (for a total of two 500 mL cultures) in order to compare media. The cultures were inoculated using the glycerol stocks created in step two and a full panel of QC assays was run on each prep. This also allowed Aldevron to test the stability of the plasmid in each media type.

[0197] Based on the initial QC results Rapid Growth media was chosen for growth of the transformed *E. coli* in shaker flasks at 34°C. A total of 8L of culture was prepared.

[0198] Capture - The 8 L of *E. coli* culture was lysed and initially purified using DMAE anion exchange chromatography which produced a total of 833.8 mg plasmid. An additional purification step was run using HIC (Hydrophobic Interaction Chromatography) chromatography over OS resin. This step yielded 663 mg of purified DNA.

[0199] Concentration and Formulation - The purified plasmid was then adjusted to the final buffer and concentration via serial diafiltration. The final buffer used was TE.

[0200] A total of 200 mg of IGE308 plasmid was shipped at a concentration of 1.4 mg/mL. The IGE308 plasmid was analyzed prior to further manufacture of the INXN-2004.

[0201] Analysis of IGE308 Plasmid Sequence

[0202] The IGE308 plasmid used for INXN-2004 manufacture was fully sequenced at SeqWright under GLP conditions using primer walking and oligonucleotide synthesis to yield 4-fold, bi-directional sequence coverage. The IGE308 plasmid has a size of 16,777 bp. The sequence showed 100% match to the IGE308 construction reference sequence provided to SeqWright. Gene elements in the IGE308 plasmid are illustrated in Figures 25A-25B. Table 9 provides a list of gene elements with their corresponding functions.

Table 9: Size and Functions of Gene Elements in IGE308 Plasmid

Location	Size (bp)	Element	Function
1-238	238	pFUGW backbone	Backbone element
239-815	577	CMV promoter	Promoter to drive expression of the proviral genome
239-1015	777	5' LTR	Proviral genome packaging and integration
835-894	60	R region of 5' LTR	Repeat region in 5' and 3' LTRs, transcription initiation site
895-1015	121	U5 region of 5' LTR	Unique 5' sequence contains the Tat binding site and packaging sequences of HIV
1016-1125	110	pFUGW backbone	Backbone element
1126-1170	45	Psi packaging signal	RNA target site for packaging the viral RNA genome into viral capsid during replication
1171-1679	509	pFUGW backbone	Backbone element
1680-1913	234	RRE	Facilitates mRNA transcript nucleus export when bound to Rev protein
1914-2443	529	pFUGW backbone	Backbone element

Location	Size (bp)	Element	Function
2444-2459	16	cPPT	Recognition site for proviral DNA synthesis, increases transduction efficiency and transgene expression
2460-2601	142	pFUGW backbone	Backbone element
2602-3199	598	CMV promoter	Promoter to drive the expression of *COL7A1* gene inside the LV-COL7 vector
3200-3217	18	5UTR	Synthetic 5'UTR fragment to improve the translation of the *COL7A1 (derived from* Rahnella aquatilis, ATCC 33071)
3218-3223	6	Kozak	Translation enhancement
3224-12058	8835	Collagen 7A1 ORF with TGA stop codon	Collagen 7A1 ORF encoding the human wild type collagen 7 protein
12059-12070	12	Stuffer	Remnant sequence resulted from DNA cloning
12071-12762	692	3'LTR	Polyadenylation signal and proviral genome packaging and integration
12071-12581	511	U3 region of 3' LTR	SIN deletion (133 bp) located in this region
12245-12260	16	cPPT	Recognition site for proviral DNA synthesis, increases transduction efficiency and transgene expression
12582-12641	60	R region of 3' LTR	Repeat region in 5' and 3' LTRs, transcription initiation site
12642-12762	121	U5 region of 3' LTR	Unique 5' sequence contains the Tat binding site and packaging sequences of HIV
12763-12790	28	pFUGW backbone	Backbone element
12791-13018	228	bGHpA terminator	Sequence to stop transcription of DNA

Location	Size (bp)	Element	Function
13019-13080	62	pFUGW backbone	Backbone element
13081-13387	307	F1 origin	F1 phage origin of replication
13388-13948	561	pFUGW backbone	Backbone element
13949-14323	375	Zeocin resistance gene	Mammalian selection marker
14324-14455	132	pFUGW backbone	Backbone element
14456-14575	120	SV40 pA terminator	Sequence to stop transcription of DNA
14576-15009	434	pFUGW backbone	Backbone element
15010-15629	620	pUC origin	Bacterial DNA replication start
15630-15783	154	pFUGW backbone	Backbone element
15784-16644	861	Ampicillin resistance gene	Plasmid selection marker
16645-16685	41	pFUGW backbone	Backbone element
16686-16714	29	Ampicillin resistance promoter	Promoter to drive expression of the ampicillin resistance gene
16715-16777	63	pFUGW backbone	Backbone element

[0203] The *COL7A1* gene sequence in the IGE308 plasmid (without the stop codon TGA) was compared to the GenBank Homo sapiens collagen, type VII, alpha 1 (*COL7A1*) sequence (NM_000094.3). Sequence alignment is provided in Appendix 4. Three silent point mutations were identified as listed in Table 10 below. No sequence gap was identified. The three silent point mutations have no impact on the encoded C7 protein.

Table 10: Sequence Comparison of *COL7A1* in IGE308 and GenBank Consensus

Nucleotide Position	*COL7A1* in IGE308	GenBank Consensus (NM_000094.3)	Impact
6040	G	A	Silent point mutation
8674	A	G	Silent point mutation
8731	G	C	Silent point mutation

[0204] Helper Plasmids, pCMV-G, pCMV-Rev2 and pCgp

[0205] The three helper plasmids used for INXN-2004 LV vector production, pCMV-G, pCMV-Rev2 and pCgp, were provided by CoH.

[0206] 293T Working Cell Bank (WCB)

[0207] The 293T WCB used for INXN-2004 LV vector production was provided by CoH.

[0208] Other Starting Materials for INXN-2004 Manufacture

[0209] Other starting materials used for INXN-2004 LV vector production were also provided by CoH.

[0210] Biopsy Tissue

[0211] Three 3-4 mm punch skin biopsies (dermis and epidermis layers) are collected from an un-blistered area of the RDEB subject's body using standard aseptic practices. The biopsies are collected by the treating physician, placed into a vial containing cold, sterile phosphate buffered saline (PBS), and shipped via next day delivery in a refrigerated Infectious Shipper container, which is appropriate for shipping potentially biohazardous tissue and is designed to maintain a temperature of 2-8°C.

a. Reagents, Solvents, and Auxiliary Materials

i. Reagents and Solvents

[0212] Two animal-sourced reagents are used in the FCX-007 Drug Substance manufacturing process: Fetal Bovine Serum (FBS) and Trypsin-EDTA solution. Complete Growth Medium

[0213] Complete Growth Medium (CGM) is used in FCX-007 manufacturing during all the cell culture expansion steps. CGM is prepared by mixing 18 L of IMDM in a 20 L bag with 2 L of FBS through aseptic transfer. The formulated CGM bag is stored in a 5±3°C refrigerator.

a. Initiation Growth Medium

[0214] Initiation Growth Medium (IGM) is used in FCX-007 manufacturing for the initial seeding of fibroblasts after biopsy digestion into a T-75 cell culture flask in the presence of

antibiotics. Initiation Growth Medium is prepared fresh by adding 0.6 mL of the100-fold concentrated antibiotic solution (GA) aseptically inside an ISO5 BSC to 58.2 mL of CGM in a 500 mL square media bottle. Final concentrations are 0.4 mg/mL gentamicin, 0.295 µg/mL amphotericin B. GSH solution (1.2 mL of 50X solution) is also added to the 500 mL square media bottle. The bottles are closed and inverted 3-5 times to mix. The IGM is warmed inside a 37°C incubator for at least 60 minutes before immediate use.

[0215] The Initiation Growth Medium is prepared fresh immediately prior to use and therefore no expiration date is applied and no Quality Control release tests are performed.

b. Transduction Medium

[0216] Transduction Medium (TM) is used during the INXN-2004 transduction of fibroblast cells. Transduction Medium is prepared fresh inside an ISO5 BSC by adding 40 mL IMDM to a 250 mL centrifuge tube containing 10 mL CGM to dilute the FBS concentration to 2% in the medium. The 250 mL tube is mixed and placed inside a 37.0°C Incubator to warm until needed for INXN-2004 transduction.

[0217] The Transduction Medium is prepared fresh immediately prior to use and therefore no expiration date is applied and no Quality Control release tests are performed.

c. GSH Solution

[0218] GSH is supplemented to the Initiation Growth Medium (IGM) and CGM used in the early stage T-75 and T-175 fibroblast cell cultures to enhance cell growth. A 50X GSH stock solution is prepared inside an ISO5 BSC by dissolving 51.1 g of GSH into 1 L of IMDM. The solution is 0.22 µm filtered. The filtered solution is aliquoted into 50 mL centrifuge tubes at 20 mL per tube and stored frozen at -80°C for further use.

d. RetroNectin™ Solution

[0219] RetroNectin™ is used in the FCX-007 production process to enhance the INXN-2004 transduction efficiency on fibroblast cells. Inside an ISO5 BSC, using a 5 mL syringe and a 18g needle, 2.5 mL WFI is aseptically transferred into a vial of clinical grade RetroNectin® to reconstitute RetroNectin® at 1.0 mg/mL. RetroNectin® is dissolved thoroughly by swirling

gently. The entire contents of the RetroNectin® vial are withdrawn using the attached 5 mL syringe. The 18g needle is aseptically replaced with a 0.22 μm syringe filter onto the syringe, and the reconstituted RetroNectin® is sterile filtered into a 250 mL centrifuge tube. Using an appropriately sized pipette, 122.5 mL PBS is transferred to the reconstituted RetroNectin® in the 250 mL centrifuge tube to dilute the RetroNectin® to 20μg/mL, which is then mixed well using a pipette. Using an appropriately sized pipette, 6.3 mL of the diluted RetroNectin® solution is added to each of T-25 flasks to coat the surface with RetroNectin® at 5 $\mu g/cm^2$. For the first round of INXN-2004 transduction, 5x to 10x T-25 flasks are prepared. For INXN-2004 super transduction, 18x T-25 flasks are prepared.

[0220] The RetroNectin™ solution is prepared fresh immediately prior to use and therefore no expiration date is applied and no Quality Control release tests are performed.

[0221] Cryopreservation Medium

[0222] Cryopreservation Medium is a two-fold concentrated solution that is added to harvested and washed fibroblasts during the FCX-007 manufacturing process to formulate the cell seed stock and FCX-007 Drug Substance for storage in liquid nitrogen. Cryopreservation Medium is prepared by mixing 0.85 volume ProFreeze™-CDM (2X) with 0.15 volume DMSO to obtain one volume Cryopreservation Medium.

[0223] The Cryopreservation Medium is prepared fresh immediately prior to use and therefore no expiration date is applied and no Quality Control release tests are performed.

[0224] Example 3: Training Run 8, 9 and 10 and Enhanced Biopsy Enzymatic Digestion

[0225] Training Run 8 and Enhanced Biopsy Enzymatic Digestion

[0226] In the above TRs, the biopsy digestion method was suspected to not be able to achieve effective digestion of the 3-4mm sized biopsy tissue. In TR8, the application of additional shear force during the digestion process was incorporated into the process to improve the overall digestion efficiency. The digestion process was modified to the following: pulse vortex the centrifuge tube at maximum setting for 5 seconds, every 15 ± 2 minutes during the digestion,

returning the centrifuge tube to the orbital shaker after each vortex; at the end of the 60 minutes of incubation, pulse vortex the centrifuge tube at maximum setting for 10 seconds.

[0227] A biopsy from an RDEB donor was processed and digested using the enhanced digestion method. Cells from the digestion were seeded into a T-75 flask using culture medium supplemented with GSH. Cells showed good growth and reached 90% confluence on day 14 for passaging. To evaluate the INXN-2002 transduction conditions, cells harvested from the T-75 flask were seeded into 3x T-175 flasks, as Control, Arm A, and Arm B. Cells in all three arms were expanded, transduced with the 10 L pilot LV-COL7 vector in 1-CS (the control arm was mock transduced), and then further expanded into 2x 10-CS before being harvested for cryopreservation.

Table 11: Cell Growth and Cell Yields in Training Run 8

Activity	Days in Culture	Viable Cells	Cell Viability	Note
Biopsy digestion, seed into one T-75 flask, with 35 mL culture medium, 37°C and 5% CO_2	0	3.4×10^5	99%	Good cell growth was observed in the T-75 flask; approximately 90% confluence on Day 14.
Passage 1: to three T-175 flasks with 50 mL culture medium, 37°C and 10% CO_2	14	6.5×10^6	97%	Flask 1: mock transduction control Flask 2: LV transduction (Arm A) Flask 3: LV transduction (Arm B)
Passage 2: to 1-CS and LV transduction Control Arm A Arm B	 20 20 20	 1.4×10^7 1.0×10^7 1.0×10^7	 98% 97% 98%	Cells were harvested from each of the T-175 flask and expanded into corresponding 1-CS for LV-COL7 transduction (CoH Pilot LV-COL7).
Passage 3: to 10-CS Control Arm A Arm B	 26 27 26	 6.1×10^7 6.6×10^7 6.4×10^7	 99% 99% 99%	Cells from each arm were harvested and expanded to one corresponding 10-CS.
Passage 4: to 2x 10-CS Control Arm A Arm B	 35 39 38	 4.0×10^8 3.3×10^8 3.8×10^8	 99% 98% 98%	Cells from each arm were harvested and expanded to two corresponding 10-CS.

Activity	Days in Culture	Viable Cells	Cell Viability	Note
Harvest: 2x10-CS				Cells were harvested from each of the 2x10-CS for cryopreservation and testing.
Control	45	6.9×10^8	95%	
Arm A	47	4.0×10^8	98%	
Arm B	46	5.3×10^8	97%	

[0228] The total number of cells harvested from the 10-CS was considered adequate for further production of FCX-007 drug product for injection. The harvested cells were tested for COL7A1 gene copy number (transduction efficiency), C7 protein expression, cell viability, and cell purity. Table 12 provides the analysis results for the harvested cells from all three arms.

Table 12: Analysis of Cells Harvested from Training Run 8

Condition	INXN-2002 Transduction MOI (IU/cell)[1]	COL7 A1gene copy/cell[2]	C7 protein expression[2] (ng/mL)	Purity[2] (% $CD90^+$)	Cell Viability (%)	Endotoxin
Arm A	3.4	0.021	59.4	100%	95%	Pass
Arm B	1.7	0.017	46.6	100%	93%	Pass
Control	Mock	BLQ[3]	BLQ[3]	100%	96%	Pass

[1] INXN-2002 IU titer: 9.2×10^6 IU/mL which was determined at Intrexon using an early development stage H1299 infectious titer assay.
[2] Details of the assays are provided in Section 3.2.S.4.
[3] BLQ: below limit of quantitation

[0229] A Multiplicity of Infection (MOI) dose dependent increase in COL7A1 gene copy number and C7 protein expression was observed. Details of the INXN-2002 transduction development and optimization are described below. Although low INXN-2002 IU titer limited the transduction MOI, and the subsequent COL7A1 gene copy number, C7 protein expression was observed to be biologically relevant in the preclinical setting as evidenced by the in vitro and in vivo results. Overall, the results show that RDEB fibroblast cells can be transduced with INXN-2002 and express functional C7 protein.

[0230] The Arm A cell product from TR8 was used for Proof of Concept studies and toxicology and biodistribution studies.

[0231] Training Run 9

[0232] A biopsy from an RDEB donor was processed and digested using the enhanced digestion method. Cells from the digestion were seeded into a T-75 flask using culture medium supplemented with GSH. Similar to TR8, cells showed good growth and reached 90% confluence on Day 19 for passaging. To evaluate the LV-COL7 transduction conditions, cells harvested from the T-75 flask were seeded into 3x T-175 flasks, as Control, Arm A, and Arm B. Cells in all three arms were expanded, transduced with LV-HA-COL7 vector (containing an HA tag in the construct) in 1-CS (the control arm was mock transduced), then further expanded into 2x 10-CS before being harvested. The harvested cells were tested for COL7A1 gene copy number (transduction efficiency), C7 protein expression, and cell purity. Table 13 provides the cell growth in TR9.

[0233] Table 13: Cell Growth and Cell Yields in Training Run 9

Activity	**Days in Culture**	**Viable Cells**	**Cell Viability**	**Note**
Biopsy digestion, seed into one T-75 flask, with 35 mL culture medium, 37°C and 5% CO_2	0	NA	NA	Good cell growth was observed in the T-75 flask; approximately 90% confluence on Day 19.

Activity	Days in Culture	Viable Cells	Cell Viability	Note
Passage 1: to three T-175 flasks with 50 mL culture medium, 37°C and 10% CO_2	19	4.5×10^6	99%	Flask 1: mock transduction control Flask 2: LV transduction (Arm A) Flask 3: LV transduction (Arm B)
Passage 2: to 1-CS and LV transduction				Cells were harvested from each of the T-175 flask and expanded into corresponding 1-CS for LV-COL7 transduction (CoH Pilot LV-HA-COL7, a research vector) [1]
Control	26	1.0×10^7	99%	
Arm A	25	7.1×10^6	97%	
Arm B	25	7.8×10^6	98%	
Passage 3: to 10-CS				Cells from each arm were harvested and expanded to one corresponding 10-CS.
Control	33	3.8×10^7	99%	
Arm A	32	5.3×10^7	98%	
Arm B	31	2.5×10^7	97%	
Passage 4: to 2x 10-CS				Cells from each arm were harvested and expanded to two corresponding 10-CS. [2]
Control	46	2.6×10^8	97%	
Arm A	42	3.6×10^8	98%	
Arm B	45	2.8×10^8	97%	
Harvest: 2x10-CS				Cells were harvested from each of the 2x10-CS for cryopreservation and testing.
Control	61	7.6×10^8	100%	
Arm A	53	5.3×10^8	97%	
Arm B	60	5.5×10^8	98%	

[1] LV-HA-COL7 vector is a research vector which incorporated an HA tag to the COL7A1 sequence in the INXN-2002 LV vector.

[2] The total number of cells harvested from the 10-CS is considered adequate for further production of FCX-007 drug product for injection.

[0234] Table 14 provides the analysis results for the harvested cells from all three arms.

Table 14: Analysis of Cells Harvested from Training Run 9

Condition	LV-HA-Col7 Transduction MOI (IU/cell)[1]	Col7 Gene Copy/Cell[2]	Col7 Protein Expression (ng/mL)	Purity[2] (% $CD90^+$)	Cell Viability (%)	Endotoxin
Arm A	1.0	0.009	71.4	100%	92%	Pass
Arm B	1.9	0.021	91.6	99%	95%	Pass
Control	Mock	BLQ[3]	BLQ[3]	100%	92%	Pass

[1] LV-HA-Col7 vector titer: 2.0 x 10^6 IU/mL which was determined at Intrexon using an early development stage H1299 infectious titer assay.
[2] Details of the assays are provided in Section 3.2.S.4.
[3] BLQ: below limit of quantitation

[0235] An MOI dose dependent increase in COL7A1 gene copy number and C7 protein expression was again observed. Again, the results show that RDEB fibroblast cells can be successfully grown, transduced with LV-HA-COL7 vector, and express C7 protein.

[0236] Training Run 10 and Scale-Up to Six 10-CS

[0237] According to the proposed clinical protocol (See Module 5), 4 x 10^8 FCX-007 drug product cells are needed for a single treatment dose, with a possibility of repeat dosing. To meet the projected FCX-007 product needs, it is necessary to scale-up the final cell expansion step to six 10-CSs based on the cell yields attained in TR8 and TR9.

[0238] A biopsy from an RDEB donor was processed and digested using the enhanced digestion method. Cells from the digestion were seeded into a T-75 flask using culture medium supplemented with GSH. Cells showed good growth and reached 90% confluence on Day 19 for passaging. Cells harvested from the T-75 flask were seeded into 2x T-175 flasks, one for INXN-2002 transduction, and one for cell substrate control. Cells from one flask were expanded, transduced with INXN-2002 in 1-CS, and then further expanded into 6x 10-CS before being harvested. Table 15 provides the cell growth in TR10.

Table 15: Cell Growth and Cell Yields in Training Run 10

Activity	Days in Culture	Viable Cells	Cell Viability	Note
Biopsy digestion, seed into one T-75 flask, with 35mL culture medium, 37°C and 5% CO_2	0	NA	NA	Good cell growth was observed in the T-75 flask; approximately 90% confluence on Day 19.
Passage 1: to two T-175 flasks with 50mL culture medium, 37°C and 10% CO_2	19	3.5×10^6	98%	Flask 1: LV transduction Flask 2: control cells for analysis
Passage 2: to 1-CS and LV transduction	30	4.6×10^6	96%	Cells from Flask 1 were harvested and expanded into 1-CS for LV-COL7 transduction (GMP grade INXN-2002).
Passage 3: to 10-CS	38	2.1×10^7	97%	Cells were harvested and expanded to one 10-CS.
Passage 4: to 6x 10-CS	60	1.9×10^8	95%	Cells were harvested and expanded to six 10-CS.
Harvest: 6x 10-CS	75	5.8×10^8	99%	Cells were harvested from the 6x10-CS for cryopreservation and testing.

[0239] Relative to cells in TR8 and TR9, cells in TR10 grew slower and yielded fewer cells at each passage step, indicating potential variability among different RDEB donors.

[0240] The total number of cells harvested from the 6x 10-CS is considered adequate for production of one dosage of FCX-007 drug product for injection.

[0241] Table 16 provides the analysis results for the harvested cells.

Table 16: Analysis of Cells Harvested from TR10

INXN-2002 Transduction MOI (IU/cell)[1]	COL7 Gene Copy/Cell[2]	C7 Protein Expression[2] (ng/mL)	Purity[2] (% $CD90^+$)	Cell Viability (%)	Endotoxin
2.0	0.013	60.09	100%	92%	Pass

[1] INXN-2002 IU titer: 9.2×10^6 IU/mL which was determined at Intrexon using an early development stage H1299 infectious titer assay.

[2] Details of the assays are provided in Section 3.2.S.4.

Example 4

INXN-2002 Transduction Development and Optimization

[0242] Lentiviral transduction of dermal fibroblast cells was initially developed and optimized using normal human dermal fibroblast cells (NHDF; Lonza, CC-2511) cultured in 96-well plates with a model lentiviral GFP (GeneCopoeia, LP-EGFP-LV105-0205).

[0243] Using GeneCopoeia's LV transduction protocol as a starting point, initial optimization evaluated conditions including cell density at time of transduction, transduction culture volume, use of RetroNectin™, super-infection (re-transducing cells with virus on two consecutive days), time of cell plating (at time of transduction versus one day prior to transduction), and serum content in transduction media. The optimal transduction procedure was then implemented in a GMP production setting. Details of the studies are described below.

[0244] A. Effect of Cell Seeding Conditions and RetroNectin™ Coating for LV Transduction

[0245] NHDFs in exponential growth phase were harvested and seeded into 96-well plates on two different dates at different seeding densities. One set of non tissue culture treated 96-well plates were coated with RetroNectin™ per the manufacturer's recommendation (the RetroNectin™ coating density was 20 $\mu g/cm^2$). For cells seeded one day before LV transduction (Day -1), the spent medium is removed and fresh medium (100 µL) with the LV vector is added to the wells on Day 0 for transduction. For the day of transduction condition (Day 0), cells and LV vector in 100 µL of fresh medium is added simultaneously to the wells. The MOI used was 2000 vp/cell for all conditions. After an overnight incubation, the medium was removed and the cells were fed with 100 µL fresh medium for further culture. At ninety-six hours post-transduction, the cells were harvested for transduction efficiency analysis by FACS analysis of GFP signal on a BD LSRII and analyzed using FlowJo software (v.10). Table 17 provides the LV transduction efficiencies under the different conditions.

Table 17: Effect of Cell Seeding Condition and RetroNectin™ Coating on LV Transduction of Fibroblast Cells (% GFP Positive Cells)

Cell Seeding Density (Cells/Well; Cells/Cm^2)[1]	Without RetroNectin™ Coating		With RetroNectin™ Coating	
	Day -1 Cell Seeding	Day 0 Cell Seeding	Day -1 Cell Seeding	Day 0 Cell Seeding
3000; 9375	6.8%	11.2%	NC	22.6%
1500; 4688	3.3%	7.2%	NC	18.2%

[1] Surface area for a 96-well plate well: 0.32 cm^2
NC = Not conducted

[0246] The results demonstrate that it is not necessary to pre-seed the cells on the day before LV transduction. Cells and LV vector can be added simultaneously at the time of transduction, which is convenient for GMP manufacturing operations. Coating the culture surface with RetroNectin™ prior to LV transduction significantly increased the LV transduction efficiency. The higher cell seeding density of approximately 1 x 10^4 cells/cm^2 is desired during LV transduction, as the lower cell seeding density resulted in lower LV transduction efficiency.

[0247] B. Effect of MOI and Super-Transduction on LV Transduction Efficiency

[0248] In this study, the effects of MOI and super-transduction on LV transduction of fibroblast cells were examined.

[0249] A set of non tissue culture treated 96-well plates was pre-coated with RetroNectin™ (20 μg/cm^2) before being used for transduction. At the time of transduction, 3000 cells with LV vector in a volume of 100 μL were added to a well. After an overnight incubation, the medium was removed and the cells were fed with 100 μL fresh medium for further culture. For the super-transduction, the culture medium was removed from the wells one day after the initial transduction (approximately 24 hours), and the same amount of LV vector in 100 μL fresh medium was added to the wells. After 3 hours of transduction, the medium was removed and the cells were fed with 100 μL fresh medium for further culture. Ninety-six hours post-transduction, the cells were harvested for transduction efficiency analysis by FACS analysis of GFP signal on a BD LSRII and analyzed using FlowJo software (v.10). Table 18 provides the LV transduction efficiencies under the different conditions.

Table 18: Effect of MOI and Super-Transduction on LV Transduction of Fibroblast Cells (% GFP Positive Cells)

MOI (vp/cell)	Without Super-Transduction	With Super-Transduction
0	1.6%	2.0%
200	4.3%	5.8%
500	7.7%	10.0%
1000	15.3%	16.3%
2000	22.6%	27.5%

[0250] An increasingly higher MOI resulted in higher transduction efficiencies with and without super-transduction. Super-transduction resulted in an incremental increase in LV transduction efficiency. However, the increase was not considered significant, and was not implemented in the GMP production setting.

[0251] C. LV Transduction Culture Volume and FBS Concentration

[0252] Lentiviral vector particles first need to make contact with fibroblast cells in culture in order to achieve transduction. Like other viral particles, LV particles in solution follow Brownian motion, and a productive transduction is generally a random event. Reduction of culture medium depth to a minimum volume or the use of spino-transduction has been shown to improve viral vector transduction on target cells (Nyberg-Hoffman 1997). The effect of transduction volume/culture depth and medium FBS concentration on LV transduction of fibroblast cells were evaluated.

[0253] A set of non tissue culture treated 96-well plates was pre-coated with RetroNectin™ (20μg/cm^2) before being used for transduction. At the time of transduction, 3000 cells with LV vector in a volume of 100 μL or 50 μL of medium with 10% FBS were added to the wells. The same transduction conditions were repeated in medium with 2% FBS. After an overnight incubation, the medium was removed and the cells were all fed with 100 μL fresh medium with 10% FBS for further culture. The MOI used was 2000 vp/cell for all conditions. Ninety-six hours post-transduction, the cells were harvested for transduction efficiency analysis by FACS analysis of GFP signal on a BD LSRII and analyzed using FlowJo software. Table 19 provides the LV transduction efficiencies under the different conditions.

Table 19: Effect of Culture Volume (Depth) and FBS on LV Transduction (% GFP Positive Cells)

Transduction Medium Volume in 96-well plate (µL)	Transduction Medium Depth (mm)	Transduction Medium FBS %	
		2%	10%
50	1.6	46.7%	46.2%
100	3.1	31.8%	27%

[0254] As expected, reducing the transduction medium volume (depth) resulted in a noticeable increase in LV transduction efficiency. Additionally, results indicate that using a 2% FBS transduction medium is beneficial for LV transduction. Based on these results, a 2% FBS transduction medium was incorporated into the GMP manufacturing process, while keeping the transduction medium volume to a minimum, approximately 60 mL in 1-CS. In the GMP manufacturing process for FCX-007, a 1-layer CellSTACK® with a surface area of 636 cm^2 will be used for LV transduction of fibroblast cells. It was determined that it is feasible to use a transduction volume of 60 mL (depth 0.9mm) without causing detrimental effect (e.g. drying) on the cells during the 3 hours transduction period.

[0255] D. Evaluations of RetroNectin™ Coating and the Type of Culture Surface

[0256] RetroNectin™ coating of the culture surface significantly increased the LV transduction of fibroblast cells. The RetroNectin™ manufacturer recommends a RetroNectin™ coating amount in the range of 4 µg/cm^2 to 20 µg/cm^2. To minimize the amount of RetroNectin™ used without negatively impacting the LV transduction of fibroblast cells, the amount of RetroNectin™ used for coating was evaluated.

[0257] Furthermore, the RetroNectin™ manufacturer recommends use of a non tissue culture treated plastic surface for RetroNectin™ coating. However, culture flasks and CellSTACKs® used for fibroblast cell culture are all tissue culture-treated. Both tissue culture treated and non tissue culture treated 96-well plates were coated with different amounts of RetroNectin™ and evaluated for LV transduction of fibroblast cells.

[0258] Both non tissue culture treated and tissue culture treated 96-well plates were pre-coated with different amounts of RetroNectin™ (20 µg/cm^2, 10 µg/cm^2, and 5 µg/cm^2) before being

used for transduction. At the time of transduction, 3000 cells with LV vector in a volume of 50 µL of transduction medium with 2% FBS were added to each well. After 3 hours transduction, the medium was removed and the cells were all fed with 100 µL fresh medium with 10% FBS for further culture. The MOI used was 2000 vp/cell for all conditions. Ninety-six hours post-transduction, the cells were harvested for transduction efficiency analysis by FACS analysis of GFP signal on a BD LSRII and analyzed using FlowJo software. Table 20 provides the LV transduction efficiencies under the different conditions.

Table 20: Effect of RetroNectin™ Amount and Culture Surface Type on LV Transduction of Fibroblast Cells (% GFP Positive Cells)

	Amount of RetroNectin™ Used for Coating (µg/cm^2)		
Type of Culture Surface	**5**	**10**	**20**
Tissue culture treated	27.8%	25.8%	28.1%
Non tissue culture treated	38.2%	38.8%	38.5%

[0259] Comparable efficiencies of LV transduction of fibroblast were observed for all three doses of RetroNectin™ used for coating. As a result, a RetroNectin™ coating density of 5 µg/cm^2 was selected for use in the FCX-007 production process. Although lower LV transduction efficiency was observed in the tissue culture treated surface, the difference is not considered significant enough to prevent the use of tissue culture treated flasks in the INXN-2002 transduction step of the FCX-007 manufacturing process.

[0260] Summary of the Development and Optimization of Fibroblast LV Transduction

[0261] Based on the study results described above using the LV-GFP model vector for fibroblast cell transduction, the following LV transduction protocol was selected for INXN-2002 transduction of fibroblast cells derived from RDEB donor:

[0262] Pre-coat the culture surface with RetroNectin™ at 5 µg/cm^2. Tissue culture treated flasks can be used for RetroNectin™ coating.

[0263] Add cells and LV vector simultaneously at the time of transduction, with a cell seeding density of approximately1x10^4 cells/cm^2.

[0264] Use a high MOI which does not cause toxic effect on cells to achieve high transduction efficiency (10 mL of INXN-2002 LV-Col7 vector per transduction).

[0265] Transduce for 3 hours at 37°C in a transduction medium with 2% FBS, and then change or feed cells with a medium which contains 10% FBS.

[0266] Use a low transduction medium volume, 50 µL/well for a 96-well plate or 60 mL for a 1-layer CellSTACK®, for high transduction efficiency.

[0267] Super-transduction is not required.

[0268] F. INXN-2002 LV Transduction of Fibroblast Cells

[0269] Based on the LV transduction protocol developed above, INXN-2002 was used to transduce normal human fibroblast cells (NHDF, Lonza, CC-2511) in a 96-well plate. Considering the relative low titer of the INXN-2002, three doses of INXN-2002 were used for transduction, 12.5 µL (1:4 dilution), 3.1 µL (1:16 dilution), and 0.8 µL (1:64 dilution). The transduced cells were passaged three times to ensure stable integration of the LV-COL7 vector into the transduced cell genome. At passage 3, genomic DNA was extracted from the cells and analyzed by qPCR using a primer specific to the LV-COL7 vector sequence. The transduction efficiency was quantified as gene copy numbers per cell. Table 21 provides the transduction efficiency as measured by gene copy number per cell.

Table 21: INXN-2002 Transduction of Fibroblast Cells

INXN-2002 Vector Dosing[1]			
Dilution factor	**Vector volume per well of 96-well plate (µL)**	**Calculated MOI (IU/cell)**	**Gene Copy Number per Cell[2]**
1/4	12.5	40	0.08
1/16	3.1	10	0.86
1/64	0.8	2.5	0.11

[1] INXN-2002 IU titer: 9.2 x 10^6 IU/mL which was determined at Intrexon using an early development stage H1299 infectious titer assay.

[2] Gene copy number per cell was determined at Intrexon using an early development stage qPCR assay, which resulted in an approximately 10-fold higher gene copy number compared to the fully developed assay that was transferred to BioReliance for FCX-007 product analysis. The difference was due to the use of a different qPCR primer set and circular plasmid standards in the early development stage assay.

[0270] At the highest dose of INXN-2002 vector, ¼ dilution (MOI= 40), a significant toxic effect on cells were observed: most of the cells did not recover from the transduction step, resulting in a minimal gene copy number per cell at the end of the cell expansion. The optimal INXN-2002 MOI was approximately 10 IU/cell, resulting in a gene copy number of 0.86.

[0271] INXN-2002 LV Transduction in Training Runs

[0272] Following the LV transduction protocol (Section [0260]) and the INXN-2002 transduction vector dosing/MOI results from the 96-well studies, INXN-2002 transduction of RDEB fibroblast cells was conducted at scale in TR8, TR9, and TR10. The 1-layer CellSTACK® used for INXN-2002 transduction was pre-coated with RetroNectin™ at 5 µg/cm^2. At the time of transduction, cells and INXN-2002 were added simultaneously in 60 mL of transduction medium with 2% FBS to the 1-layer CellSTACK®. After 3 hours in the incubator at 37°C, the cells were fed with 130 mL of complete growth medium with 10% FBS. The transduced cells were passaged twice, first to one 10-layer CellSTACK®, and then to two or six 10-layer CellSTACKs® for cell harvest. Gene copy numbers in the harvested cells were analyzed by qPCR. Table 22 provides the INXN-2002 transduction results from the executed Training Runs.

Table 22: Summary of LV-COL7 Transduction in Training Runs

Training Run	**LV-COL7 Vector**	**LV-COL7 Vector Titer (IU/mL)**	**Arm**	**Amount of Vector Used for Transduction (mL) (dilution factor)**	**Number of Cells for Transduction**	**MOI[1] (IU/cell)**	**Gene Copy per Cell[2]**
8	INXN-2002 LV-COL7 (Pilot)	$9.2x10^6$	A	3.75 (1/16)	1.0×10^7	3.4	0.021
			B	1.88 (1/32)	1.0×10^7	1.7	0.017
			Control	0	1.4×10^7	0	BLQ[1]
9	LV-HA-COL7 (Pilot)	$2.0x10^6$	A	3.75 (1/16)	7.1×10^6	1.0	0.009
			B	7.5 (1/8)	7.4×10^6	1.9	0.021
			Control	0	1.0×10^7	0	BLQ[3]
10	INXN-2002 LV-COL7 (GMP)	$2.3x10^6$	TR10	4 (1/15)	4.6×10^6	2.0	0.013

[1] MOI was determined based on the INXN-2002 LV-Col7 vector IU titer determined at Intrexon using an early development stage H1299 infectious titer assay, which resulted in approximately 10-fold higher titer value compared to the fully developed assay that was transferred to BioReliance for FCX-007 product analysis. The

difference was due to the use of a different qPCR primer set and circular plasmid standards in the early development stage assay.
[2] Gene copy per cell was quantified using the fully developed qPCR assay, which was transferred to BioReliance for FCX-007 product analysis
[3] BLQ: Below the limit of quantitation

[0273] Following the LV transduction protocol developed using the small scale 96-well plates, successful INXN-2002 transduction of RDEB fibroblast cells was achieved at scale for FCX-007 manufacture. Gene copy numbers in the transduced cells increased with an increased MOI of transduction. Because of the low titer of INXN-2002, gene copy numbers in the transduced, harvested cell product were relatively low. In order to produce FCX-007 cell product with a feasibly high gene copy number, 10 mL of INXN-2002 was selected for GMP production transduction, as it caused minimal toxic effects on cells during transduction.

[0274] Engineering Run

[0275] In preparation for GMP manufacturing of FCX-007, and finalization of the production Master Batch Records (MBR), an engineering run was executed using a biopsy from an RDEB donor with approved production batch records. The GMP grade INXN-2002 LV vector was used for transduction. Table 23 provides cell growth figures from the Engineering Run.

Table 23: Cell Growth and Cell Yields in Engineering Run

Activity	Days in Culture	Viable Cells	Cell Viability	Note
Biopsy digestion, seed into one T-75 flask, with 35mL culture medium, 37°C and 5% CO_2	0	9.6×10^5	92%	Good cell growth in the T-75 flask, reached approximately 90% confluence on Day 19
Passage 1: to one T-175 flask and one T-25 flask, 37°C and 10% CO_2	20	3.5×10^6	99%	T-175 Flask: LV transduction T-25 Flask: control cells for analysis
Passage 2: to 1-CS and LV transduction	29	9.9×10^6	98%	Cells from T-175 Flask were harvested and expanded into 1-CS for LV-COL7 transduction (GMP grade INXN-2002)
Passage 3: to 10-CS	38	3.3×10^7	98%	Cells were harvested and expanded to one 10-CS.
Passage 4: to 6x 10-CS	49	3.1×10^8	96%	Cells were harvested and expanded to six 10-CS.

Activity	Days in Culture	Viable Cells	Cell Viability	Note
Harvest: 6x 10-CS	60	9.6 x 10^8	97%	Cells were harvested from the 6x10-CS for cryopreservation and testing

[0276] Cell growth and yields from the ER are comparable to those in TR8 and TR9, and are noticeably faster and higher relative to TR10, indicating cell growth variability among different RDEB donors. The harvested cells were filled and cryopreserved as shown in Table 24.

Table 24: FCX-007 Drug Substance Vials Filled in Engineering Run

Vials	Fill Volume (mL)	Number of Vials Filled	Purpose
2 mL cryovial	1.2	10	Bulk Drug Substance
5 mL cryovial	4.5	6	Bulk Drug Substance
2 mL cryovial	1.2	2	Sterility test
2 mL cryovial	0.6	4	QC test
2 mL cryovial	0.6	6	Bulk Drug Substance Stability Test

[0277] The total number of cells harvested from the 6x 10-CS is considered adequate for production of two doses of FCX-007 drug product for injection.

[0278] The harvested cells were tested according to the proposed FCX-007 Drug Substance Specifications. Table 25 provides the analysis results for the harvested cells.

Table 25: FCX-007 Drug Substance Engineering Run Analysis

Test[1]	Test Method	Lab	SOP	Specifications	Test Result
Mycoplasma	USP<63>	BioReliance	102063GMP.BSV	Negative	Negative
Replication Competent Lentivirus	C8166 cell	BioReliance	009130GMP.BUK	No RCL detected	ND[4]
Sterility	USP<71>	BioReliance	510120GMP.BSV	No Growth	No growth
Endotoxin	Endosafe	PCT	SOP-0249	≤ 5.0 EU/mL	< 2.00 EU/mL
Cell Count	Hemacytometer	PCT	SOP-0329	1.0 – 3.0x10^7 cells/mL	1.6x10^7 cells/mL
Cell Viability	Hemacytometer	PCT	SOP-0329	≥ 85% viability	94%
Purity	FACS Calibur	PCT	SOP-1067	≥ 98% CD 90^+	100%

Test[1]	Test Method	Lab	SOP	Specifications	Test Result
Residual VSV-G Protein	VSV-G ELISA	Intrexon[2]	VSV-G ELISA	Report Result	BLQ
COL7A Gene Copy Number	qPCR	Intrexon[2]	COL7A Gene Copy Number	Report Result	0.025 copies/cell
COL7A Gene Expression	Col 7 ELISA	Intrexon[3]	COL7A Gene Expression	Report Results	66.4 ng/mL

[1] Details of the assays are provided in Section 3.2.S.4.2.
[2] Intrexon in house assay. Assays are being transferred to BioReliance for GMP release test
[3] Intrexon in house assay. Assays are being transferred to PCT for GMP release test
[4] RCL testing was not performed on the cell product material as the material was not used for further studies

Example 5 - Description of Manufacturing Process Development to Improve LV-Col7 Transduction Efficiency

[0279] FCX-007 cell products manufactured using the process described above had low levels of gene modification efficiency as indicated by the low gene copy number and C7 expression levels when INXN-2002 vector was used for cell transduction. Two approaches were pursued to increase the LV-Col7 transduction efficiency on RDEB fibroblast cells: 1) development of a new LV-Col7 lentiviral vector (INXN-2004) which offers better vector titer and improved stable transgene (collagen VII) expression in the transduced fibroblast cells, and 2) further optimization of the LV-Col7 transduction process on fibroblast cells. Details of the development of the new INXN-2004 vector are described in Section 3.2.S.2.3. Studies in further optimization of the LV-Col7 transduction process, as well as cell expansion procedures are described below.

[0280] Spin Transduction (Spinoculation)

[0281] Spin transduction involves centrifugation of viral vectors onto the target cells under a g force environment. Spin transduction (spinoculation) has been reported in the literature to increase retroviral vector transduction efficiency on target cells (J Virol Methods. 1995 Aug;54(2-3):131-43. Centrifugal enhancement of retroviral mediated gene transfer. Bahnson AB1, Dunigan JT, Baysal BE, Mohney T, Atchison RW, Nimgaonkar MT, Ball ED, Barranger JA. and Hum Gene Ther. 1994 Jan;5(1):19-28. Improved methods of retroviral vector transduction and production for gene therapy. Kotani H1, Newton PB 3rd, Zhang S, Chiang YL, Otto E, Weaver L, Blaese RM, Anderson WF, McGarrity GJ.).

[0282] RDEB fibroblast cells from the previous TR8 were cultured. Cells in exponential growth phase were harvested and used for this study. The previous INXN-2002 vector lots were used for transduction. For spin transduction, $1x10^3$ cells with different MOIs of INXN-2002 in 50μL of transduction media (IMDM + 2%FBS) were added into each well of a 96-well tissue culture coated plates which were pre-coated with RetroNectin (5 $\mu g/cm^2$). The plates containing cells plus INXN-2002 vector were centrifuged at 1300g, 4°C for 1.5 hours. After centrifugation, the plates were placed into a 37°C incubator to allow the cells to recover and grow for 3 hours. After which, 100 μl of complete culture media (IMDM +10FBS+GSH) was added to each well. The plates were returned to the incubator for further culture. The standard non-spin transduction described above was used as a control. After transduction, cells were passaged three times, 3-5 days culture between each cell passage. At the last passage culture supernatants were harvested to evaluate C7 protein expression levels, while the cells were harvested for gene copy number per cell analysis. Table 26 shows the Col7 expression levels for the different transduction conditions.

Table 26: C7 Expression Level

INXN-2002	MOI (IU/cell)	Vector Dilution used for Transduction	Transduction Method	
			Spin Transduction	Non-spin transduction
GMP lot	6.31		257.4±62.17	311.75±70.89
	1.60		539.50±70.49	251.27±22.41
	0.4		243.94±3.40	207.51±25.26
Pilot lot	30		327.76±21.69	238.56±27.93
	15		391.59±42.82	208.39±16.80
	7.5		269.07±25.51	214.23±16.83

[000119] Table 27 provides the vector copy number per cells of the harvested cells.

Table 27: Vector Copy Number per Cell

INXN-2002	MOI (IU/cell)	Vector dilution used for transduction	Transduction Method	
			Spin Transduction	Non-spin transduction
GMP lot	6.31		0.11±0.02	0.10±0.02
	1.60		0.11±0.01	0.06±0.00
	0.4		0.10±0.03	0.04±0.01

INXN-2002	MOI (IU/cell)	Vector dilution used for transduction	Transduction Method	
			Spin Transduction	Non-spin transduction
Pilot lot	30		0.09±0.01	0.06±0.02
	15		0.09±0.02	0.05±0.01
	7.5		0.05±0.03	0.07±0.02

INXN-2002, IU titer: 2.36 x 10^6 IU/mL (determined at Intrexon)
INXN-2002, IU titer: 2.36 x 10^6 IU/mL (determined at Intrexon)

[0283] The results show a modest increase in both Col7 production levels and vector copy number per cell using the spin transduction method, especially at the lower MOIs and at the INXN-2002 vector dilutions tested.

[0284] Optimization of Spin Transduction Parameters

[0285] The spin transduction parameters, including centrifugation time, speed and temperature, were evaluated with an aim to further increase the INXN-2002 transduction efficiency. The same cell culture condition as described above was used in this study. In the initial experiment, centrifugation temperature was examined. 96-well plates containing cells plus INXN-2002 vector were centrifuged at either 4°C or 25°C (RT) for 1.5 hours at 1300g. This was followed by another experiment where 96-well plates containing cells plus INXN-2002 vector were centrifuged at 4°C or 25°C (RT) for 1 or 2 hours, at either a high speed of 1300g, or a low speed of 300g, to examine the effect of centrifugation speed on INXN-2002 transduction efficiency. In both experiments, cells were returned to a 37°C incubator for incubation post centrifugation. Three hours later, 100 µl of complete culture media was added to each well. Cells were passaged three times before being harvested for Col7 expression analysis in the culture supernatant and vector copy number per cell analysis in the harvested cells.

[0286] Table 28 provides the effect of spin transduction temperature on Col7 expression levels and vector copy number per cell. The standard non-spin transduction was included as a control.

[0287] Again, increased INXN-2002 transduction, as indicated by higher C7 protein expression and vector copy number per cell, was demonstrated using spin transduction compared to the standard no spin transduction. Spin transduction temperature did not demonstrate impact on the overall transduction efficiency.

[0288] Table 29 below provides the effect of centrifugation speed as well as temperature and time on INXN-2002 transduction efficiency.

Table 28: The Effect of Spin Transduction Temperature on INXN-2002 Transduction Efficiency

INXN-2002 MOI	C7 Expression (ng C7/day/E6 cells)			Vector Copy Number per Cell		
		Spin Transduction		No Spin	Spin Transduction	
	No spin	4°C	RT		4°C	RT
18	105.84±11.69	177.36±24.98	313.59±39.13	0.07±0.04	0.07±0.02	0.08±0.03
6	117.82±14.79	196.26±23.75	143.61±19.01	0.02±0.01	0.08±0.03	0.05±0.00
2	50.05±13.60	136.55±6.82	105.57±17.90	0.01±0.00	0.02±0.00	0.03±0.02

INXN-2002 IU titer: 2.36 x 10^6 IU/mL (determined at Intrexon)

Table 29: Effect of Spin Transduction Temperature, Time, and Speed on INXN-2002 Transduction Efficiency

INXN-2002 MOI	C7 Expression (ng C7/day/E6 cells)								Vector Copy Number per Cell							
Temp	4°C				RT				4°C				RT			
Speed (xg)	1300		300		1300		300		1300		300		1300		300	
Time (hr)	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
3.8	208.88 ±13.61	247.03±10.09	221.49±4.08	310.93±0.41	359.47±3.85	201.06±5.37	168.98±3.34	201.82±9.41	0.08 ±0.01	0.07 ±0.01	0.06 ±0.01	0.09 ±0.03	0.11 ±0.01	0.06 ±0.01	0.07 ±0.01	0.07 ±0.02
1.9	180.34 ±12.33	265.44±1.33	213.83±0.58	301.82±11.54	240.61±2.68	180.36±3.10	212.76±3.19	177.16±5.92	0.06 ±0.02	0.10 ±0.02	0.05 ±0.00	0.06 ±0.01	0.10 ±0.02	0.07 ±0.02	0.06 ±0.01	0.05 ±0.01

INXN-2002 IU titer: 2.36 x 10^6 IU/mL (determined at Intrexon)

[0289] The results indicate a modest improvement in INXN-2002 transduction efficiency, as indicated by C7 protein expression and vector copy number, when spin transduction was conduced at RT, 1300g, for 1 hour. The significance of the improvement is not known and the initially developed spin transduction parameters (centrifugation at 1300g for 1.5 hours at 4°C) were selected for future study use.

[0290] Super Transduction to Further Increase INXN-2002 Transduction Efficiency

[0291] Super transduction (a second transduction) was evaluated in the early development of INXN-2002 transduction, which is described above. Super transduction was evaluated again in combination with spin transduction to further increase INXN-2002 transduction efficiency on fibroblast cells. Unlike the previous method, in the current study's super transduction was performed on cells one passage after the initial spin transduction. The inclusion of the one cell passage is expected to allow the cells to recover from the initial spin transduction and become more receptive to the 2nd super transduction.

[0292] Similarly, RDEB fibroblast cells from the previous TR8 in exponential growth phase were harvested and used for this study. The optimized spin transduction method described above, centrifugation at 1300g for 1.5 hours at 4°C, was used for INXN-2002 transduction. The transduced cells were passaged once at 72-96 hours post the initial spin

transduction. After 72-96 hours of culture in the second passage, cells were harvested and re-transduced using the same spin transduction conditions. After the super spin transduction, cells were passaged an additional three times before being harvested for Col7 expression analysis in the culture supernatant, and vector copy number per cell analysis in the harvested cells. The INXN-2002 vector was used for the study. Table 30 provides the effect of super spin transduction on INXN-2002 transduction efficiency.

Table 30: Effect of Super Spin Transduction

INXN-2002 MOI	C7 Protein Expression		Vector Copy Number per Cell	
	One Round of Spin Transduction	Super Spin Transduction	One Round of Spin Transduction	Super Spin Transduction
1.9	257.8±3.37	431.9±95.5	0.07±0.03	0.22±0.07

INXN-2002 IU titer: 2.36 x 10^6 IU/mL (determined at Intrexon)

[0293] Super spin transduction resulted in a significant increase in INXN-2002 transduction efficiency on RDEB fibroblast cells. C7 protein expression increased approximately 1.7-fold and the vector copy number per cell increased approximately 3-fold.

[0294] The data demonstrate a 40% increase in Col7 expression and a 60% increase in copy number per cell with the additional transduction. Due to this increase, super-infection was added to the production protocol.

[0295] Transduction with the INXN-2004 Vector

[0296] Concurrent with the development of the improved transduction method, a new LV-Col7 vector (referenced as "INXN-2004") based on a different lentiviral vector backbone (that is, pFUGW) was also developed.

[0297] INXN-2004 Single Spin Transduction

[0298] RDEB fibroblast cells from the previous TR8 in exponential growth phase were harvested and used for this study. In the first study, single round of spin transduction as described above was carried out using a pilot lot of INXN-2004 vector. The standard no-spin transduction was included in the study as a control. Cells were passaged three times

before being harvested for Col7 expression analysis in the culture supernatant, and vector copy number per cell analysis in the harvested cells. Table 31 provides the Col7 expression levels and vector copy number per cell using the INXN-2004 vector transduction.

Table 31: C7 Protein Expression and Vector Copy Number per Cell using INXN-2004 Transduction

INXN-2004 MOI	C7 Protein Expression		Vector Copy Number per Cell	
	No Spin Transduction	Spin Transduction	No Spin Transduction	Spin Transduction
17	2312.46±0.03	3960.58±0.02	0.16±0.03	0.05±0.02
4.25	1668.46±0.06	3602.10±0.06	0.21±0.06	0.27±0.06
1.06	724.12±0.03	2566.88±0.10	0.06±0.03	0.21±0.10
0.27	168.61±0.00	1936.72±0.00	0.02±0.00	0.13±0.00

INXN-2004 IU titer: 2.36 x 10^6 IU/mL (determined at Intrexon)

[0299] Relative to the INXN-2002 vector, a significant improvement in transduction efficiency was achieved with use of INXN-2004 in combination with the spin transduction method. Additionally, the INXN-2004 vector appears to be significantly more active in transducing RDEB fibroblast cells even under a no-spin transduction condition, as indicated by the approximately 10-fold higher C7 protein expression and vector copy number per cell. The results support the use of the pFUGW base for construction of the new INXN-2004 vector.

[0300] Consistent to what is reported above, spin transduction appears to exacerbate the INXN-2004 vector toxic effect on fibroblast cells at higher MOI conditions, as indicated by the lower C7 expression levels and vector copy numbers at MOI17. This does not impact FCX-007 manufacturing as the use of a lower MOI is demonstrated to be as effective.

[0301] INXN-2004 Super Spin Transduction

[0302] In the second study, super spin transduction (as described above) was used for INXN-2004 transduction. The scale of transduction was increased from a 96-well plate to a T-25 flask. The transduction parameters remained unchanged, including the same

RetroNectin™ coating density of 5μg/cm2, and the same cell density at transduction of $1x10^4$ cells/cm^2. Transduction volume in the T-25 flask was 4.2 mL. For transduction, the T-25 flask containing the cells and virus was centrifuged at 4°C, for 1.5 hours at 1300g. Cells were returned to a 37°C incubator and incubated for three hours. After the initial incubation, 4.2 mL of complete culture media was added to the flask for further incubation. Cells were passaged three times before being harvested for Col7 expression analysis in the culture supernatant, and vector copy number per cell analysis in the harvested cells. Table 32 provides the effect of super spin transduction on INXN-2004 vector transduction efficiency. A pilot lot of INXN-2004 vector was used for the study.

Table 32: Effect of Super Spin Transduction for the INXN-2004 Vector

INXN-2004 MOI	C7 Protein Expression		Vector Copy Number per Cell	
	Single Spin Transduction	Super Spin Transduction	Single Spin Transduction	Super Spin Transduction
0.11	3178±20.53	4029±59.67	0.12±0.007	0.57±0.134

INXN-2004 IU titer: 2.36 x 10^6 IU/mL (determined at Intrexon)

[0303] As observed for the previous INXN-2002 vector, super spin transduction resulted in a significant increase, approximately 5-fold, in vector copy number per cell in the transduction cells, despite a relatively modest increase in Col7 protein expression level, under a low MOI transduction condition.

[0304] Summary of Transduction Development and Optimization

[0305] Significant improvements in LV-Col7 transduction efficiency on RDEB fibroblast cells was achieved in continued process development, both with the previous INXN-2002 and the new INXN-2004 vector. The improvements are to enable the manufacturing of a biologically potent FCX-007 cell product. Based on the manufacturing process development study results, the following transduction procedure was chosen for manufacturing of FCX-007 using INXN-2004 vector for transduction.

[0306] Pre-coat culture surface with RetroNectin at 5μg/cm^2. Tissue culture-treated flasks are used for RetroNectin™ coating.

[0307] Add cells and INXN-2004 vector simultaneously at the time of transduction. Cell seeding density is approximately 1 x 10^4 cells/cm^2.

[0308] Centrifuge cells and INXN-2004 vector transduction mixture in the transduction vessel at 4°C, for 1.5 hours at 1300g.

[0309] Incubate cells for 1.5- 2.0 hours at 37°C post transduction in transduction medium with 2%FBS, and then change or feed cells with complete culture medium with 10%FBS.

[0310] Use a low transduction medium volume (50µL/well for a 96-well plate, or 0.35 mL/cm^2).

[0311] Perform a super-transduction on cells after one passage post the initial transduction.

Example 6 - Analysis of RDEB Genetically Modified Human Dermal Fibroblasts Transduced by INXN-2002

[0312] RDEB Genetically Modified Human Dermal Fibroblasts (GM-HDF) derived from INXN-2002 lentiviral vector was analyzed in order to:

[0313] Evaluate localization of COL7 expression in established composite human skin grafts (prepared on porcine dermis) comprised of RDEB keratinocytes by ID injection.

[0314] Assess potential carcinogenicity/tumorgenicity in composite RDEB human skin grafted (prepared on porcine dermis) on SCID mice treated with RDEB GM-HDF

[0315] An initial attempt to evaluate GM-HDF (derived from INXN-2002) function by seeding fibroblasts on the reticular side of composite graft cultures was unsuccessful, possibly due to inadequate migration of GM-HDF into the devitalized porcine dermis or insufficient time for adequate diffusion and deposition of rC7 at Day 19. An alternative approach was used to evaluate function by direct ID injection of existing grafts in six back-up animals on study. RDEB composite grafts prepared on porcine dermis appear to be more robust than anticipated, making direct injection into established RDEB skin

grafts possible. This approach more closely mimics the disease and clinical route of administration.

[0316] This study report presents data from an initial evaluation of ID injection of GM-HDF into established RDEB grafts. Additional studies to achieve long term objectives for in vivo pharmacology are planned.

A. STUDY MATERIALS

A.1 Test Article(s)

[0317] Test article was prepared and tested for Drug Substance release specifications prior to shipment. The test article for skin graft injection was prepared one day prior to each scheduled day of treatment. GM-HDF was thawed, washed, resuspended in DMEM, and evaluated against release specifications. The GM-HDF was shipped for overnight delivery and administered within 48 hours. Non-corrected RDEB HDF (mock transduced) was isolated and expanded from the identical patient as the GM-HDF by the same methods.

[0318] The test article and cells to be used for preparation of composite skin grafts was shipped frozen. Cells used for skin graft preparation will be thawed and cultured for testing.

[0319] Lot number TR8 (Non-Transduced Control) was used as the RDEB-HDF negative control. Lot number TR8 Arm A (GM-HDF-LV-COL7) was used as the GM-HDF test article.

[0320] Test article analysis was performed at the manufacturing facility prior to shipment. Remaining test article will be stored at -70°C for 1 year. The test article will be stored in Dulbecco's Modified Eagle Medium (DMEM).

A.2. Other Chemicals and Materials

A list of other materials is provided below in Table 33.

Table 33: Other Study Materials

Description	Source
Normal Porcine Skin	Porcine cadaver
RDEB keratinocytes	Human skin biopsy
RDEB fibroblasts	Human skin biopsy
Normal keratinocytes	Human skin biopsy
Normal fibroblasts	Human skin biopsy

[0321] Group 1 (Negative Control) Cells: Untransduced RDEB cells from the donor from training run 8 were thawed, and passaged once prior to transfection. Two days after passaging the cells, the cells were collected and 1.2 x 10^6 cells were transfected in a 50ml conical tube in low serum growth (ATCC PCS-201-030 and PCS-201-041) media using the lipid based transfection agent Transfex (ATCC) with COL7 plasmid VVN 4317513. After a 10 minutes incubation with complexed Transfex, DNA and Optimem (LifeTech), 1 x 10^6 transfected cells were transferred to a T150 flask, and 2 x 10^5 cells were placed in a T25 and placed in a 37°C 5% CO2 incubator for 16 hours, at which time the media was removed and replace with full growth media (IMDM (Sigma), 920mg/L of L-Glutathione Reduced (Sigma) 10% heat inactivated FBS (Atlantic Biologicals) and 1XGlutamax (LifeTech)). Cells were then returned to the 37°C 5%CO2 incubator until time of shipment. At time of shipment all cell flask was filled to capacity, such that all parts of the flask were in contact with media regardless of the flask orientation.

B. STUDY DESIGN

B.1. Randomization

[0322] Due to high failure rates of RDEB human skin grafts, mice in these groups of animals are not randomized.

[0323] B.2. Justification for Species and Number on Study

[0324] The NOD. CB.17-PRKDC scid/scid mouse is the standard species for use in proof of concept preclinical efficacy/toxicology studies and is a rodent species of choice for such evaluations using the human xenograft models. This study was designed to

minimize the number of animals on study that would provide sufficient data to evaluate the test articles in.

[0325] B.3. Route of Administration

[0326] Fibroblasts will be administered by intradermal injection for normal skin grafts. Composite grafts will be administered cells by seeding prior to grafting.

[0327] B.4. Dose Administration

[0328] Intradermal cell injections of lentivirus transduced (COL7A1-corrected) RDEB GM-HDF (using INXN-2002) and negative controls (vehicle or RDEB-HDF) will be done with a 30-gauge needle. The injection will be performed by first piercing the skin, then directing the needle as superficially as possible back upward toward the surface.

[0329] Composite grafts will be administered cells by seeding prior to grafting according to protocol outlined in the in vitro study with the distinction that the composite culture system will not be lifted to air-fluid interface prior to grafting.

[0330] B.4.1. Preparation of Fibroblasts for Injection (Composite Grafts)

[0331] Fibroblasts were removed from the liquid nitrogen storage at one week prior to injection, thawed in 37°C water bath until a small ice crystal remains, then disinfected and transferred to BSC. Vials were resuspended in 10ml of PBS wash volume and spun for 10 minutes at 1000 RPM. The supernatant was discarded and the cells were washed in DMEM+10% FBS 1x Antibiotic media and re-spun for 10minutes at 1000RPM. The pellet was resuspended in appropriate volume and plated on 15cm TC dishes. Media was changed every other day and passaged at 80% confluence until grafts are ready to inject. On the day of injection, fibroblasts were trypsinized, neutralized in DMEM+10% FBS media and spun 10min at 10000 RPM, the pellet of cells was resuspended and an aliquot of cells was taken, cells were counted with trypan blue stain to determine viability. The cells were resuspended such that 1.0×10^6 cells in 50uL volume is drawn up into syringe. A 30g needle was attached and placed at 4°C until mouse was ready tobe injected.

Table 34: Composite Skin Grafts (Fibroblast Seeding)

Grp	N	Sex	Composite Skin Graft		Number of Cells		Collection of Specimens
			Keratinocytes	Fibroblasts	Keratinocytes	Fibroblasts	
1	3	M	WT	WT	1×10^6	1×10^6	Day 15
2	3	M	RDEB Keratinocytes	RDEB HDF	1×10^6	1×10^6	Day 15
3	3	M	RDEB Keratinocytes	RDEB-GM-HDF	1×10^6	1×10^6	Day 15
4	3	M	RDEB Keratinocytes	RDEB - GM-HDF	1×10^6	5×10^5	Day 15
5	3	M	RDEB Keratinocytes	RDEB - GM-HDF	1×10^6	2.5×10^5	Day 15
6	3	M	RDEB Keratinocytes	RDEB - GM-HDF	1×10^6	1.25×10^5	Day 15

Table 35: Composite Skin Grafts (Fibroblast Intradermal Injections)

Grp	N	Sex	Test Article	Dose	Injection	Graft Description
1	2	M	RDEB-HDF	1×10^6	Composite Graft	1 million RDEB GM-HDF, 1 million RDEB KC
2	4	M	RDEB-GM-HDF	1×10^6	Composite Graft	0.125 million RDEB GM-HDF, 1 million RDEB KC

Table 36: Study Groups for the Evaluation of ID-Injected Xenografts

Condition	Graft Pre-Seeding Prep Conditions		Injected Fibroblasts	Date of Harvest
Control Groups	**Keratinocytes**	**Fibroblasts**		
Normal	Normal KC	Normal FB	N/A	Day 19 post-grafting
FDEB	RDEB	RDEB	N/A	Day 19 post-grafting
Experimental Groups				
1A	RDEB	TR8/A 1 million	TR8 nontransduced	10 days post-injection
1B	RDEB	TR8/A 1 million	TR8 nontransduced	10 days post-injection
2A	RDEB	TR8/A 0.125 mil	TR8/ArmA	10 days post-injection
2B	RDEB	TR8/A 0.125 mil	TR8/ArmA	10 days post-injection
2C	RDEB	TR8/A 0.125 mil	TR8/ArmA	10 days post-injection
2D	RDEB	TR8/A 0.125 mil	TR8/ArmA	10 days post-injection

C. Skin Grafts

[0332] C.1 RDEB Human Skin Grafts

[0333] C.1.1 Fibroblasts

[0334] Genetically engineered human fibroblasts (RDEB GM-HDF) produced by lentiviral gene transfer, including the INXN-2002 lentiviral vector, along with normal fibroblasts and non-corrected RDEB fibroblasts as controls, are the cell types used for initially infusing dermis skin. Cells are cultured onto devitalized dermis to form skin equivalents approximately 2 cm square. Regenerated skin composites consist of human keratinocytes cultured atop fibroblast-infused human devitalized dermis.

[0335] Isolation and culture of primary keratinocytes

[0336] Wild-type keratinocyte cells are isolated from neonatal foreskin, whereas primary RDEB keratinocytes are isolated from patient skin samples. The latter is obtained as a 6mm punch biopsy submerged in 15 mL of keratinocyte growth medium 50/50V in a 15 mL centrifuge tube shipped on wet ice. No bubbles can be present in shipped samples. Medium 50/50V contains 50% medium 154 (Invitrogen) supplemented with HKGS (0.2% bovine pituitary extract [BPE], bovine insulin [5 mg/mL], hydrocortisone [0.18 mg/mL], bovine transferrin [5 mg/mL], human epidermal growth factor [0.2 ng/mL]) and 50% keratinocyte SFM (KSFM; Invitrogen) supplemented with recombinant human EGF1–53 BPE. Antibiotics AV100 (amikacin/vancomycin) are added to reduce bacterial contamination.

[0337] Separate dermis and epidermis by treating with 25 U/mL dispase (Becton Dickinson) overnight at 4°C. Epidermis is then peeled from dermis and placed in a 15 mL centrifuge tube with 5 mL TrypLE 10x. Epidermal "peel" is incubated in a 37°C water bath for 15-30 min with gentle agitation every 5 min. TrypLE is neutralized with equal volume DTI (defined trypsin inhibitor) or DMEM+10%FBS. Cells are pelleted at 1200 rpm and plated in 12 mL 50/50V onto T75 Corning® Purecoat™collagen 1 mimetic flasks. In the event of PureCoat™ flask unavailability from manufacturer, 1ml of collagen 1 coating solution per 10cm plastic dish for 15 minutes at 37°C can be used instead. Collagen 1 coating is a 0.2 uM filtered solution of Vitrogen 100 Collagen (1ml), HEPES (2ml), BSA (100uL of 100mg/ml) and 1x HBSS (100ml). The excess collagen coating is aspirated prior to cell seeding. Flasks are swirled to ensure an even distribution of cells and placed in a 37°C 5% CO_2 humidified incubator for at least one night before any manipulation or observation. Cells are allowed to adapt to tissue culture conditions for 3 days. After adaptation, medium is changed once every 2 days or as needed. Keratinocytes are passaged onto normal tissue culture plastic when they are approximately 70% confluent.

[0338] In the 50/50V media keratinocytes grow well for five passages. Keratinocytes are utilized within the first four passages.

[0339] Preparation of porcine devitalized dermis

[0340] Obtain sheets of split thickness porcine skin. This may be prepared in similar fashion to devitalized human dermis (as above) from porcine skin harvested by dermatome. Wash the porcine dermis 3x in sterile PBS containing 1x penicillin/streptomycin/Amphotericin/gentamicin and incubate in PBS with 1M NaCl and 2x antibiotics at 37°C for 72 hours. Separate epidermis and discard, wash 3x with 1XPBS containing antibiotics to remove excess salt and gentamicin in solution.

[0341] Confirm sterility by culturing a piece of porcine dermis in 50/50V or KGM for 4 days. Dermis can be stored at 4°C in antibiotic solution, changing the PBS weekly. Dermis should not be stored for more than a few months before use.

[0342] Organotypic Culture Seeding Fibroblasts

[0343] Cut devitalized dermis into 2 cm square pieces. Place dermis basement membrane side down into 6 well culture plates and allow dermis to dry slightly.

[0344] Seed fibroblasts into each well, centrifuge 1200 rpm for 5 minutes and return to 37°C with 5% humidified CO_2 for 3–4 days, changing media every day. During this time, fibroblasts attach to the dermis and begin to migrate into the tissue.

[0345] Seeding Keratinocytes

[0346] After the fibroblasts have migrated through the dermis, carefully detach dermis and transfer to annular dermal support (ADS). Add a thin layer of Matrigel to seal and secure dermis in device. Add 5 mL KGM to stromal compartment of ADS. KGM is a 3:1 mixture of DMEM:Ham's F12 supplemented with FBS (10%), adenine (1.8 x 10-4 M), hydrocortisone (0.4 μg/mL), insulin (5 μg/mL), cholera toxin (1 x 10-10 M), EGF (10 ng/mL), transferrin (5 μg/mL), and triiodo-L-thyronine (1.36 ng/mL). Trypsinize, count, and seed keratinocytes in a total volume of 100 μL of KGM per ADS. Return ADS assembly to incubator. Do not disturb ADS for at least 24hrs. The keratinocytes at the top of the dermis must remain at the air liquid interface. Change the KGM in the lower chamber every day. Organotypic culture will continue for 1-2 weeks.

[0347] Grafting Organotypic Culture

[0348] Organotypic cultures will be ready to graft after 7-10 days. Grafting will be conducted as described above for normal human skin grafts. Bandages and sutures will be removed after 7-10 days and animal will be rewrapped with fresh bandaging for another week. Thereafter graft will be exposed to air to mature.

[0349] Harvesting Grafts and Sectioning

[0350] Humanely sacrifice mice and carefully harvest graft. Bisect graft and cut an additional 1 mm slice. Trim slice down to a 1 mm square and send for immunoelectron microscopy (IEM). Freeze remaining halves in OCT on dry ice while noting the central edge. Sectioning will start at this edge. One half will be sectioned for immediate analysis and remaining half will be stored for later analysis. Cut ten 8 μm sections starting from the central edge and sequentially label. Discard the next forty sections. Repeat until end of graft is reached. Air dry and fix with ice cold 50/50 acetone/methanol.

[0351] Injection of Established RDEB Composite Grafts

[0352] Fibroblasts will be intradermally injected directly into the skin following of the established graft. Injections will be no greater than 50 μL in volume.

[0353] Injections were performed on mice previously grafted with composite grafts as described herein: Devitalized porcine dermis was seeded by centrifugation with variable dosage of RDEB GM- HDF and grown in tissue culture for 1 week. Subsequently, the dermis was flipped over and 1 million RDEB keratinocytes were seeded and allowed to grow in culture for one more week. After this two week period in tissue culture as described above, the grafts were placed on SCID mice and sutured in place, bandaged for 13 days. The dressings were removed at that time, and at 38-41 days post grafting, grafts were injected with 50uL volume of fibroblasts.

D. EXPERIMENTAL PROCEDURES

[0354] D.1. Localization of COL7

[0355] Immunofluorescent microscopy was used to evaluate expression of type VII collagen. For immunofluorescence (IF) analyses of skin cultures with anti-human C7 specific antibody NP185.

[0356] D.1.2. Methods for NP 185 Antibody Immunofluorescence Microscopy

[0357] Composite grafts were harvested from SCID mice immediately after CO_2 euthanasia was performed. The grafts were bisected and placed in OCT blocks. Frozen blocks of tissue were sectioned on a Leica CM1850 cryostat at 8μM thickness at -21°C and fixed in ice cold 50% methanol/50% acetone. Slides were rehydrated and washed in 1XPBS three times, incubated in primary antibody (NP185 10ug/ml; mouse anti-Human Collagen VII) for 1 hour at room temperature. Slides were washed and incubated in Alexa 488 tagged goat anti-mouse IgG antibody (1:400, 1hour, Room Temperature, life technologies) and a nuclear Hoescht 33342 counterstain (Life technologies). Samples were washed three times in 1xPBS, mounted using fluoromount prior to imaging. Images were taken on a Zeiss Observer.Z1 fluorescence microscope at 20X magnification.

[0358] Grafts harvested at Day 19 were included as positive and negative control arms. Experimental arms were injected Day 38, 39, or 41 and harvested Day 48, 49, or 51 (Table 37).

Table 37: Graft, Injection, and Harvest Days

Study Arm	N	Day of Graft	Day of Injection	Day of Harvest
Control Arms				
Positive (Normal keratinocytes, normal fibroblasts)	1	0	N/A	19
Negative (RDEB keratinocytes, RDEB fibroblasts)	1	0	N/A	19
Experiment Arms				
Non-transduced RDEB fibroblasts	2	0	41	51
GM-HDF	4	0	38 (n=3) or 39 (n=1)	48 (n=3) or 49 (n=1)

E. DATA ANALYSIS

[0359] IF images were prepared and provided to the PI in a blinded fashion. Data was analyzed and interpreted by the PI prior to unblinding.

F. RESULTS

[0360] Representative results from the IF analysis of the harvested graft tissue are presented in Figure 26.

[0361] C7 staining at DEJ was visualized in all NP185 staining conditions excluding the negative control (image "RDEB uncorrected" - see arrows at DEJ for negative baseline comparison). With regard to the positive controls, there was intense DEJ staining seen in the DEJ of normal keratinocytes/fibroblasts seeded grafts. There was a small focus of positive staining seen in the group 1 injected with uncorrected fibroblasts (Image 1b) in a small section that could represent previously seeded corrected fibroblasts, however the remainder of the uncorrected fibroblast injected grafts in both mice appear to be less intense (as represented in image 1a). There was C7 staining seen at the DEJ in the

representative images of four mouse grafts injected with corrected fibroblasts (4a-d) were observed even after only 10-days post-injection.

[0362] No tumors were observed in any grafts during the course of the study.

G. CONCLUSIONS

[0363] RDEB GM-HDF TR8 intradermally injected at the approximate proposed clinical dose produced C7 that localized to the DEJ in vivo in composite grafts of RDEB keratinocytes on devitalized pig dermis. Additionally results from seeding of GM-HDF in composite grafts prior to grafting indicate that GM-HDF could persist in expression C7 that localizes to the DEJ. This model can be used to characterize the localization, durability, persistence and phenotype correction approximating the effect of GM-HDF predictive of the clinical dose.

[0364] Results of these studies show intradermal injections of autologous GM-HDF as an effective treatment of RDEB in patients.

Example 7 - Non-GLP In Vitro GM-HDF Cell Characterization and Proof-of-Concept Assessments

[0365] The objectives of this study are the following: (a) characterize the GM-HDFs produced using the anticipated at-scale GMP production method to be used for the treatment of RDEB patients; (b) assess copy number of integrated LV and expression levels of C7; and (c) confirm the functionality of C7 expressed by the GM-HDFs

A. STUDY MATERIALS

A.1. Test Article(s)

[0366] GM-HDFs from Training runs 8, 9, and 10, and the Engineering run (as described above) were characterized.

Table 38: General production information for TR8, TR9, TR10, and ER1

Production Run	Arm	MOI (IU/cell)	LV-COL7 and TU titer	Production Scale
TR8	A	3.4	INXN-2002 (Pilot) $9.2x10^6$ IU/mL	2 x 10-layer CellSTACKs®
	B	1.7		
	Control[1]	0		
TR9	A	1.0	LV-HA-COL7 (Pilot) $2.0x10^6$ IU/mL	2 x 10-layer CellSTACKs®
	B	1.9		
	Control[1]	0		
TR10[2]	A	2.0	INXN-2002 (GMP) $2.3x10^6$ IU/mL	6 x 10-layer CellSTACKs®
	Control[1]	0		
ER1[2]	A	2.6	INXN-2002 (GMP) $2.3x10^6$ IU/mL	6 x 10-layer CellSTACKs®

[1]Control arm cells were mock-transduced

[2]Only one LV-COL7 transduction arm was generated for TR10 and for ER1

B. Primer and Probe sequences used in qPCR analyses

Table 39: Primer and Probe Sequences used for qPCR Assays

Name	Sequence[1]	Assay
PR13843 forward[2]	ACCTGAAAGCGAAAGGGAAAC (SEQ ID NO:25)	LV-COL7 copy number
PR13843 reverse[2]	CACCCATCTCTCTCCTTCTAGCC (SEQ ID NO: 26)	LV-COL7 copy number
PR13843 probe[2]	6-FAM-AGCTCTCTCGACGCAGGACTCGGC-3'IB FQ (SEQ ID NO: 27)	LV-COL7 copy number
PR13653 forward	CACTCCCAACGAAGACAAGAT (SEQ ID NO: 28)	COL7A1 mRNA expression
PR13653 reverse	GTCTAACCAGAGAGACCCAGTA (SEQ ID NO: 29)	COL7A1 mRNA expression
PR13653 probe	6-FAM TTTGTAAACCGGTGCAGCTGCTTT 3'IB FQ (SEQ ID NO: 30)	COL7A1 mRNA expression

[1]Primers/probes purchased from IDT.

[2]LV-specific primer/probe sequences derived from Greenberg et al. (2006).

C. EXPERIMENTAL PROCEDURES

C.1 LV-COL7 Copy Number

[0367] Nucleic acid isolation was performed using Qiagen's AllPrep kit according to the manufacturer's instructions. gDNA isolated from $3x10^5$ GM-HDF cells was normalized to 12.5 ng/µl. 8 µl of the normalized gDNA was used in a 20 µL assay (10 µL Taqman® Gene Express, 1.8 µL nuclease-free water, 0.06 µL of 100 µM forward primer, 0.06 µL of 100 µM reverse primer, 0.04 µL of 100 µM Taqman® probe). A standard curve of serially diluted linearized C7 lentiviral shuttle vector (1e6 copies/reaction to 5 copies/reaction), plus 4 µL of human gDNA (1.5e4 cells/reaction) were also assayed in the 20 µL assay mentioned above. The Taqman® assay used was PR13843. 8 µL of an

additional standard curve of commercial human gDNA (Clontech) (1.5e4 cells/reaction to 2e2 cells/reaction) in a 20 µL assay (10 µL Taqman® Gene Express, 1.0 L nuclease-free water, 1 µL of 20X ACTB primer/probe set) was also performed. All samples were run on an ABI7900 using the following cycling parameters: 2 minutes at 50°C, 10 minutes at 95°C, and 40 cycles of 15 sec at 95°C and 1 minute at 60°C.

C.2 COL7A1 RT-qPCR

[0368] Vials of GM-HDF cells were thawed and gDNA and RNA were isolated using Qiagen's AllPrep™ kit. RNA (800 µg) was used to generate cDNA using Quanta's qScript™ per manufacturer's instructions. The cDNA and gDNA were then tested using Taqman® assay PR13653 as described in above. Data were normalized to housekeeping gene ACTB (dCT), and then compared to Control data (ddCT). The resulting ddCT is converted to fold change using the formula 2^-(ddCT).

C.3 C7 Immunofluorescence

[0369] For immunofluorescence analyses, 1.2 x 10^4 GM-HDFs were allowed to attach to PDL/Lamin- coated coverslips in 24-well plates overnight and then fixed and permeabilized with a 50%/50% mix of methanol/acetone. The coverslips were washed 3 times with 1X PBS and then blocked with 10% goat serum in PBS for 30 minutes at room temperature. After three additional washes with PBS, the coverslips were incubated with 1.25 µg/mL fNC1 antibody in 1% goat serum/PBS, followed by 3 additional washes with PBS and incubation with 5 µg/mL Alexa Fluor® 555- conjugated goat anti-rabbit IgG in 1% goat serum/PBS for 1 hour at room temperature. Coverslips were stained with NucBlue® Live Cell Stain Ready Probes Reagent before being mounted onto slides. Images were acquired on a Zeiss Axio Observer microscope at 20X magnification using an exposure time of 290 ms. NHDFs or GM-HDFs were fixed, permeabilized, and stained with NucBlueLive® Cell Stain to visualize nuclei (blue) or with the fNC1 antibody and Alexa Fluor® 555-congugated goat anti-rabbit IgG (5 µg/mL) to visualize C7 expression (red). Images were acquired at 20X magnification using an exposure time of 290 ms.

C.4 C7 Protein ELISA

[0370] Briefly, a standard curve of purified His-NC1 fragment (9.8 – 625 ng/mL) or collected supernatants containing C7 protein (from GM-HDF in culture for three to five days) were immobilized to a Nunc MaxiSorp® 96-well plate overnight at 4°C. Standards and samples were tested in the same sample matrix (20% RDEB fibroblast conditioned media). Coated wells were washed with PBST and blocked with 3% BSA/PBS for 1 hour at 37°C. Detection was accomplished using a polyclonal anti-NC1 Ab (fNC1, 0.5 μg/mL) followed by incubation with secondary antibody donkey anti-rabbit IgG HRP (Jackson ImmunoResearch, 0.08 μg/mL). Bound antibodies were detected via colorimetric development with TMB substrate solution. Following quenching of the reaction, absorbance was measured at 450 nm on the SpectraMax® Plus 384 (Molecular Devices).

C.5 Immunoprecipitation of C7 trimers

[0371] First, magnetic Protein G beads were washed with 1 X PBS-T. Beads were bound to the magnet for a minimum of 2 minutes prior to removal of supernatant and in all subsequent steps. Following the washes, the beads were coated with 5 μg of anti-C7 fNC1 and incubated for 10 minutes with rotation (Glas-Col, setting 30 ~ 14 rpm at room temperature). Beads were bound to the magnet to remove the supernatant and washed with Ab binding/Wash buffer. Supernatants were collected from GM-HDFs in culture for three to five days. C7 containing supernatant was added to the bead/C7 supernatant mix and was incubated overnight at 4° C with rotation (setting 30 ~ 14 rpm). The next day the beads were bound to the magnet and washed three times using Wash buffer. Target antigen was eluted in 20 μL of elution buffer (50 mM glycine, pH 2.8, and 10 μl of 4X Loading Dye). The samples were denatured at 70°C for 10 minutes. 12 μl of a total of 30 μl (remaining sample was stored at 4°C) were then loaded (per well) into a 12 well 3-8% Tris Acetate gel and run for 4 hours at 150 volts. The gel was removed from the cassette and soaked in Transfer Buffer containing 10% methanol for 20 minutes. Overnight wet transfer of the gel to a nitrocellulose membrane was performed at 15 volts at 4°C. The next day, the voltage was increased to 50 volts for 30 min on ice. Upon completion of transfer, the blot was blocked using 5% milk in TBS-T with agitation (Labnet ProBlot™

Rocker 25, setting 60 rpm) for 2 hours at room temperature and incubated with commercially available anti-C7 LH7.2 (0.25 μg/mL) with agitation for 2 hours at room temperature. The blot was washed 3 times with 1X TBS-T (5 min each) with agitation before being probed with HRP-conjugated goat anti-mouse IgG (0.1 μg/mL) with agitation for 1 hour at room temperature. The blot was developed using Lumiglo Ultra™Chemiluminescent substrate and Fujifilm LAS 3000 Imaging System.

C.6 Lam332 Binding

[0372] 96-well MaxiSorp™ plates were coated with 1 μg Lam332 or BSA in 100 mM carbonate buffer, pH 9.3 overnight at 4°C. The plates were rinsed five times with PBS-T, and then blocked with 1% BSA in PBS-T at room temperature for 1 hour. Coated wells were rinsed five times with PBS-T and incubated with supernatants from transduced cells overnight at 4°C. Following three more washes with PBS-T, bound C7 was incubated with the fNC1 antibody (0.5 μg/mL, PBS-T) at room temperature for 3 hours, followed by incubation with HRP-conjugated donkey anti- rabbit IgG (0.8 μg/mL final concentration in PBS-T) at room temperature for 2 hrs. Detection of the C7-bound antibodies was via a colorimetric reaction using TMB and measured by reading the absorbance of the product at 450nm on a SpectraMax® Plus 384 plate reader (Molecular Devices). All incubations were carried out on an orbital shaker (GeneMate) at a speed setting of 2.

C.7. Cell Migration Assay

[0373] CytoSelect™ 24-well wound-healing assay kit from Cell Biolabs, Inc. was used for the cell migration assay. GM-HDFs (0.8×10^5) were seeded in the presence of a wound insert in 24-well plates and grown for 48 hours. The wound insert was then removed and the cells were further cultured in reduced (1%) serum media containing 15 μg/mL type I collagen (COL1) for 8 hours. Brightfield images of the wounds were acquired 0, 2, 4, 6, and 8 hours after removal of the wound insert using the Celigo® Imaging Cytometry System (Cyntellect). The surface area of the wound at each time

point was measured using ImageJ (National Institutes of Health). The data is reported as the percentage of migration (% Migration) covering the wound area.

C.7. DATA ANALYSIS

[0374] qPCR was performed using an ABI 7900 instrument utilizing SDS2.3 software. The experimental data were viewed and exported in SDS 2.4 software. ImagJ software was used to quantitate percent cell migration for the assay described above. ImageJ is an open source image processing program developed by the Nation Institute of Health and designed for scientific multidimensional images.

D. RESULTS

D.1. Characterization of C7 Expression

D.1.1. LV-COL7 Copy Number and Transcript Levels

[0375] Taqman® assays were designed against various regions of the viral shuttle vector. Of the Taqman® assays tested, designs targeting the C7 coding sequence demonstrated unacceptable levels of background amplification. The Taqman® assay selected, PR13843, was targeted to a sequence found at the 5' end of the LV backbone as described in Greenberg et al. (2006).

[0376] GM-HDF training run cells were obtained, washed with dPBS, and resuspended in RLT buffer. Qiagen's AllPrep kit was used to isolate the DNA (for copy number) and RNA (for mRNA transcript level) from the drug substance vials from each arm of each training run. The isolated DNA and RNA were then subjected to qPCR and RT-qPCR assays as described above in Section C.1 and C.2, respectively. Table 40 shows the copy number/cell from Training Runs 8, 9, and 10 (TR8, TR9 and TR10) and the Engineering Run (ER1). The data demonstrate that the number of copies of transgene integrated into each cell can be modulated by the virus dose given to the cells during production, and is less than 1 copy/cell. Similarly, the LV-COL7 transcript levels, shown in Figure 40, also correlate with the virus dose and copy number, with Arms A and B from the training runs

expressing higher levels (3 to 5 LOG) of the lentiviral C7 sequence compared to Control cells.

Table 40: LV-COL7 Copy Number in GM-HDF Production Run Cells

Production Run	LV-COL7 Used	Arm	MOI (IU/cell)	Gene Copy per Cell
TR8	INXN-2002 (Pilot)	A	3.4	0.021±0.007
		B	1.7	0.017±0.007
		Control[1]	0	BLQ[2]
TR9	LV-HA-COL7 (Pilot)	A	1.0	0.009±0.002
		B	1.9	0.021±0.002
		Control[1]	0	BLQ[2]
TR10[3]	INXN-2002 (GMP)	A (3)	2.0	0.013±0.002
		Control[1]	0	BLQ[2]
ER1[3]	INXN-2002 (GMP)	A (3)	2.6	0.025±0.005
		Control[1]	0	BLQ[2]

[1] Control arms were mock-transduced and were not exposed to LV-COL7

[2] BLQ: below limit of quantitation

[3] Only a single transduction arm was generated for TR10 and for ER1

D.1.2. C7 Protein Expression

[0377] Indirect immunofluorescence (IF) was used to examine C7 protein expression by GM-HDF cells using an antibody specific for the C7. Normal human dermal fibroblasts (NHDFs) were used as a positive control for this assay and the control arms of the training runs were used as negative controls. In the representative images shown in Figure 28 below, a small percentage (<10%) of INXN-2002 and LV-HA-COL7-transduced RDEB fibroblasts show C7 staining, which is consistent with the copy

number of integrated LV (Table 18). The signal from the GM-HDFs that are C7-positive is brighter than the signal from NHDFs, indicating greater amounts of C7 produced by GM-HDFs.

[0378] A direct ELISA was developed by Intrexon to measure C7 secreted by GM-HDFs into the cell culture media. This assay, as described above in Section C.4, utilized an NC1 region-specific polyclonal antibody. ELISA assay results of the GM-HDFs from the three training runs show that C7 expression is LV dose dependent with expression levels ranging from 45-90 ng/mL of cell supernatants (Figure 29).

D.2. Functional Assessment of C7 Expressed by GM-HDFs 9.2.1. Formation of C7 Trimers

[0379] The formation of C7 trimers is important in the assembly of anchoring fibrils. To verify that the C7 expressed by GM-HDFs will be capable of forming anchoring fibrils, we assessed whether or not the expressed C7 formed trimers using immunoprecipitation (IP) with an anti-C7 specific antibody (fNC1) for selective capture, then concentration of C7 from culture supernatants for detection by SDS-PAGE/immunoblot.

[0380] The optimal conditions for immunoprecipitation were established by testing a commercial C7 antibody and the fNC1 C7 antibody. The coating of protein G beads with fNC1 resulted in higher levels of C7 binding compared to coating with the commercially available antibody. Controls used in this assay include a no antibody control to rule out non-specific binding of C7 to the beads, as well as antibody coated beads incubated with and without purified C7 spiked into conditioned media as controls for specificity of C7 isolated from GM-HDF culture supernatants.

[0381] Immunoprecipitation results for TR8 and for TR10 and ER1 showed that the C7 produced by GM-HDF was predominantly trimeric (Figure 10, and Figure 11, respectively). Some lower molecular weight species showing immunoreactivity were also observed, and included the dimeric and 290 kDa monomeric forms. IP of C7 from GM-HDFs of TR9 was detected on the immunoblot at low levels by both an anti-C7 antibody

(left panel, Figure 11B) as well as an antibody specific for the HA tag incorporated into the C7 protein (right panel, Figure 11B).

D.2.2. Lam332 Binding by C7 Expressed from GM-HDFs

[0382] C7 has been shown to bind immobilized extracellular matrix (ECM) components, including fibronectin, Laminin 332 (Lam332), COL1, and COL4 (Chen, et al., 2002a). The interaction between C7 and Lam332 occurs through the NH2-terminal NC1 domain of C7 and is dependent upon the native conformation of both Lam332 and C7 NC1 (Rousselle, et al., 1997). The association between C7 and Lam332 is important for establishing correct Lam332 architecture at the dermal-epidermal junction. Such organization is important for interactions with extracellular ligands and cell surface receptors, and for cell signaling (Waterman, et al., 2007). Intrexon developed an ELISA using an antibody against the C7 NC1 domain to detect binding of C7 to purified Lam332. Although a C7/Lam332 binding ELISA has already been described in the literature (Chen, et al., 2002a), to our knowledge it has never been used to test C7 present in the supernatants of transduced cells. Results in Figure 12 show dose-dependent binding to Lam332 by C7 expressed by GM-HDFs from the Training and Engineering runs.

D.2.3. Cell Migration Assay

[0383] Previous studies have shown that RDEB fibroblasts and keratinocytes show an increase in motility relative to their normal counterparts, and that normal motility can be restored by expression of C7 (Chen, et al., 2000; Chen, et al., 2002b; Cogan, et al. 2014; Baldeschi et al., 2003). Most of these studies employed either the colloidal gold salt migration assay to measure the migration of fibroblasts and keratinocytes, or a wound-healing assay to measure the migration of keratinocytes. Here we used the CytoSelect™ 24-well wound-healing assay kit (Cell Biolabs, Inc.) to measure the motility of NHDFs and GM-HDFs.

[0384] Immunofluorescent staining for C7 revealed that only a small percentage (<10%) of transduced cells expressed high levels of the protein, raising the question as to whether a small number of C7-producing cells could have a global effect on cell migration. To

determine if the presence of C7 in the culture media is sufficient to reverse the RDEB hyper-motility phenotype, the wound healing assay was carried out on GM-HDFs from the three training runs, with NHDFs as a positive control and the mock-transduced Control (RDEB) arms as the negative control.

[0385] Control fibroblasts from TR8, TR10, and ER1 exhibit a hyper-motility phenotype that is reversed by the expression of C7 (Figure 30). However, the Control fibroblasts from TR9 do not exhibit a significant hyper-motility phenotype, making it difficult to investigate the functionality of HA- tagged C7 in this assay.

[0386] CONCLUSIONS

[0387] The integrated transgene copy number per cell in GM-HDFs was dependent on the virus dose ranging from 0.009 to 0.03 transgene copies per cell.

[0388] Dose-dependent C7 expression from the GM-HDF cells was confirmed by qRT-PCR, immunofluorescence staining, and ELISA.

[0389] The structure of the C7 expressed by the GM-HDF cells was confirmed to be predominantly trimeric by immunoprecipitation/SDS-PAGE/immunoblot analysis for TR8, TR10, and ER1. TR9 showed minimal trimer formation.

[0390] The C7 produced from the TR8 GM-HDF cells was demonstrated to be functional by binding to Laminin332 in an in vitro binding assay and by correction of the hypermotility phenotype of Control RDEB cells in an in vitro migration assay. TR9 showed binding to Laminin332, however, migration assay results were equivocal. TR9 HA-GM-HDFs generated from transduction with LV-HA-COL7 showed low levels of trimer, less binding to Lam332, and lack of clear hypermotility correction.

[0391] Due to lack of confirmation of in vitro functionality of HA-C7 expressed by TR9 HA- GM-HDFs, TR8 cells were chosen for in vitro and in vivo hybrid pharmacology/toxicology evaluations. This eliminated the ability to assess expression, localization and persistence in normal human skin or RDEB composite grafts prepared

with devitalized human skin. Ultimately, composite grafts prepared with devitalized porcine dermis were used for these studies. Immunostaining with human specific anti-C7 antibody was used to distinguish rC7 form native C7 in this approach.

EXAMPLE 8 – DRUG PRODUCT

[0392] FCX-007 Drug Product consists of a sterile suspension of each patient's own living autologous fibroblast cells, which are gene modified using INXN-2004 LV-COL7 to encode the human wild type *COL7A1* gene, formulated in Dulbecco's Modified Eagle's Medium (DMEM) without phenol red.

[0393] Formulation Development

[0394] Development of FCX-007 formulation and cell dosage was based on Fibrocell's manufacturing experience with azficel-T (LAVIV®); a live, autologous fibroblast cell product for injection. Fibroblast cells have been successfully formulated in DMEM at a cell concentration range of 0.5 – 3.0 x 10^7 cells/mL without viscosity concerns (azficel-T BLA #125348). Data suggest viscosity becomes a concern for product injection when cell concentration reaches 5.8 x 10^7 cells/mL. As a result, DMEM at a cell concentration of 1.0-3.0 x 10^7 cells/mL was selected as the formulation for FCX-007 DP.

[0395] Excess

[0396] Up to ten FCX-007 DP vials are shipped to the clinic as one dose for treatment injection. At the time of administration, the DP vial is gently inverted three times, and then 1 mL from each vial is drawn aseptically into a sterile 1 mL syringe with a 21 gauge needle. The 21 gauge needle is then replaced with a 30 gauge needle for intradermal administration. The process is repeated for the remaining 9 product vials.

[0397] FCX-007 DP vials are filled with 1.2 mL of the fibroblast cell suspension into a 2.0 mL capacity vial for a recoverable volume of 1.0 mL. The 0.2 mL excess is intended to ensure that 1.0 mL can be obtained from the vial at the time of administration in the clinic.

a. Physicochemical and Biological Properties

i. Physicochemical Properties

[0398] FCX-007 DP is presented as a gene modified autologous fibroblast cell suspension in DMEM without phenol red with a 1.2 mL fill in a 2 mL cryovial. The cells are demonstrated by assays to be a living culture. Table 41 shows the physicochemical properties of FCX-007 DP.

Table 41: Physicochemical Properties of FCX-007 DP

Physicochemical Property	Value
Cell Count	1.0-3.0 x 10^7 cells
Cell Viability	≥ 70%

[0399] Biological Properties

[0400] FCX-007 is a sterile autologous fibroblast cell product that is genetically modified to express the human collagen 7 protein (C7). INXN-2004 vector copy number per cell and expression of the C7 protein are unique biological properties of FCX-007 DP. FCX-007 DP is a live cell suspension product that is produced from FCX-007 DS by washing the thawed FCX-007 DS cells in PBS and DMEM buffers. INXN-2004 vector copy number per cell and C7 protein expression are analyzed on the FCX-007 DS prior to release to manufacture FCX-007 DP Table 42 shows the biological properties of FCX-007 DP.

Table 42: Biological Properties of FCX-007 DP

Biological Property	Value	Stage Measured
Cell Type Purity / Identity (fibroblast)	≥ 98% CD 90+	Drug Substance
INXN-2002 Vector Copy Number	0.1 – 5.0	Drug Substance
C7 Protein Expression	≥ 500ng/day/E6 cells	Drug Substance

Biological Property	Value	Stage Measured
Sterility	No Growth (Sterile)	Drug Product

BATCH FORMULA

[0401] To prepare the FCX-007 Drug Product, FCX-007 Drug Substance vials are removed from the vapor phase of liquid nitrogen frozen storage, thawed, and pooled inside an ISO 5 BSC. The cells are washed with phosphate buffered saline (PBS), followed by a wash with Dulbecco's Modified Eagle's Medium (DMEM) without phenol red before being suspended in DMEM without phenol red to a target concentration of 1.0 – 3.0 x 10^7 cells/mL. The formulated cells are then filled into 2 mL cryovials at 1.2 mL/vial. Drug Product material prepared for each injection treatment is defined as a batch. The proposed treatment batch size is up to ten (10) 2 mL vials containing 1.2 mL of cell suspension.

[0402] Table 43 provides a list of all components used in the Drug Product manufacturing process.

Table 43: Composition and Quantity of FCX-007 Drug Product Batch

Component	Function	Quality Standard	Quantity per DP Vial[1]	Quantity per Batch (10 vials per batch)
FCX-007 DS	Drug Substance	Drug Product Certificate of Analysis	1.2-3.6 x 10^7 cells	1.2 – 3.6 x 10^8 cells
PBS	Diluent used for washing Drug Substance cells	Manufacturer 's Certificate of Analysis	Minimal residual trace[2]	Minimal residual trace[2]
DMEM without phenol red	Buffer for cell suspension	Manufacturer 's Certificate of Analysis	1.2 mL	12 mL

[1] Vial contains 1.2 mL of FCX-007 cell suspension
[2] PBS wash residual is diluted with DMEM without phenol red such that trace amounts of PBS is possible but not quantifiable

DESCRIPTION OF MANUFACTURINIG PROCESS AND PROCESS CONTROLS

b. Manufacturing Process Flow Diagram

[0403] Manufacturing of the FCX-007 Drug Product commences when FCX-007 Drug Substance vials are removed from liquid nitrogen storage and thawed using a ThermoMixer®. In an ISO5 BSC the thawed FCX-007 DS cells are pooled into a 250 mL centrifuge tube containing >5x bulk volume of phosphate buffered saline (PBS) wash buffer for re-suspension. The cells are centrifuged at 1000 rpm for 10 minutes at 5 ± 3°C and the PBS wash buffer is removed. This is followed by a wash with > 5x bulk volume of Dulbecco's Modified Eagle Medium (DMEM) by re-suspension and centrifugation at 1000 rpm for 10 minutes at 5 ± 3°C. The DMEM wash buffer is removed. The washed cells are re-suspended in DMEM without phenol red to a target concentration of 2.0 x 10^7 cells/mL. The cell suspension is manually filled into sterile 2.0 mL cryovials in an ISO5 BSC to a volume of 1.2 mL per vial to produce the FCX-007 DP vials. The Drug Product vials are stored at 2-8°C until released by QA for shipment in a Credo Cube™ shipper to the clinical site.

[0404] Manufacturing Process Description

[0405] FCX-007 Drug Product is manufactured as indicated above. INXN-2002 is a synonymous name for FCX-007.

[0406] Step 1: Removal of FCX-007 Drug Substance Vials from Liquid Nitrogen Storage and Lot Verification

[0407] The FCX-007 Drug Substance, which is stored in a liquid nitrogen freezer, is requested from Materials Management. FCX-007 Drug Substance vials are then pulled for this batch of FCX-007 Drug Product production before being transferred to the cleanroom suite.

[0408] Step 2: Thaw Cryovials

[0409] A Line Clearance of the cleanroom suite is performed by QA per SOP-0099 GMP Production Line Clearance/GTP Line Cleaning in order to release the room for processing use. Operators initiate room, personnel, and BSC environmental monitoring (EM) per SOP-0129 (In Process Environmental and Personnel Monitoring) prior to processing and non-viable EM sampling in the BSC during processing.

[0410] The FCX-007 DS cyrovials are disinfected and thawed in a ThermoMixer® equilibrated to 37°C until cell suspension is almost completely thawed with a sliver of ice crystals remaining. The thawed vials are transferred to a BSC.

[0411] Step 3: PBS Wash

[0412] Inside the BSC, using a pipette, transfer approximately 150 mL of PBS (> 5 volumes of the FCX-007 DS cell suspension) into a 250 mL centrifuge tube. The contents of the thawed cryovials are transferred into the 250 mL centrifuge tube. Use the diluted cell suspension to rinse the pipette. Use a pipette to transfer 3.0 mL of fresh PBS to each cryovial as a rinse. Transfer the rinse to a 250 mL centrifuge tube. Swirl the tube gently to mix the cell suspension. Transfer the centrifuge tube to a centrifuge and centrifuge the cells at 1000 RPM for 10 minutes at 5 ± 3°C. After centrifugation, remove the PBS wash supernatant from the tube.

i. Step 4: DMEM Wash

[0413] Using a pipette, add approximately 200 mL of DMEM without phenol red (> 5 volumes of the FCX-007 DS cell suspension) to the tube to re-suspend the cell pellet. Swirl the tube gently to mix the cell suspension. Transfer the centrifuge tube to a centrifuge and centrifuge the cells at 1000 RPM for 10 minutes at 5 ± 3°C. After centrifugation, using a pipette, transfer 0.9 mL of the supernatant from the centrifuge bottle into each of 2 separate 2 mL cryovials (1.8 mL total) labeled "Lot, QC-Sterility". Set the cryovials labeled for sterility aside in the BSC. Aspirate the remaining DMEM wash supernatant from the centrifuge tube.

[0414] Step 5: Cell Pellet Re-suspension in DMEM

[0415] The volume of DMEM used to re-suspend the cell pellet is calculated as:

[0416] Volume of thawed FCX-007 DS cell suspension divided by 1.5

[0417] The 1/1.5 volume factor compensates for possible cell loss during the wash steps, in order to result in a cell concentration in the specified range of 1.0-3.0 x 10^7 cells/mL after re-suspension.

[0418] Use a pipette to transfer the calculated amount of DMEM to re-suspend the cell pellet in the centrifuge tube. Use a pipette to transfer 0.1 mL of the cell suspension into a 2 mL cryovial labeled "Lot, QC- Count and Viability" and submit the cryovial to QC for cell counting. Use a pipette to measure the remaining total cell suspension volume and place the cell suspension into a 2-8°C refrigerator for later final fill use.

[0419] Step 5a: Re-dilution (if needed)

[0420] If the QC cell concentration is above 3.0 x 10^7cells/mL, perform an additional dilution using fresh DMEM to target a cell concentration of 2.0 x 10^7 cells/mL. Re-submit a cell suspension sample to QC for cell count to confirm final cell concentration.

[0421] Step 6: Final Fill

[0422] Retrieve the cells from the refrigerator and transfer the cells to the BSC for final fill. Use a pipette to fill the well-mixed cell suspension manually into 2 mL cryovials in the following order:

[0423] Fill 0.1 mL of cell suspension into the first sterility sample vial (added to retained sample from Step 4)

[0424] Fill the product vials at 1.2 mL per vial

[0425] Fill 0.1 mL of cell suspension into the second sterility sample vial (added to retained sample from Step 4)

[0426] Fill the QC vial at ≤1.8 mL per vial

[0427] Submit the sterility and QC vials for testing.

[0428] Step 7: Store DP Vials for Release and Shipment

[0429] Hold the DP vials at 5 ± 3°C for release and shipment to the clinical site.

[0430] Description of the In-Process Controls

[0431] Environmental Controls

[0432] All cell culture manipulations are carried out in a certified ISO 5 Biological Safety Cabinets (BSCs) within an ISO7 environmental background using aseptic techniques. The final Drug Product 2 mL cryovial containers are purchased pre-sterilized from Corning. The operators performing the final fill are qualified for aseptic fill operations.

[0433] In-Process Controls and Testing

[0434] The process controls and testing performed for release of FCX-007 Drug Product are described below.

[0435] Step 1

[0436] The correct FCX-007 Drug Substance lot is verified by QA before use in FCX-007 DP manufacture.

[0437] Step 2

[0438] The temperature of the ThermoMixer® used to thaw FCX-007 Drug Substance vials is controlled at 37°C. The content of each vial is thawed until a sliver of ice still remains in the vial to prevent overheating.

[0439] Step 3

[0440] The PBS wash volume is controlled at ≥ 5 volumes of the FCX-007 DS cell pool to ensure adequate washing of the cells. The centrifugation conditions are controlled at 1000 rpm, 10 minutes and 5±3°C to ensure sufficient cell pelleting.

[0441] Step 4

[0442] Similarly to the PBS wash step, the DMEM wash volume is controlled at ≥ 5 volumes of the FCX-007 DS cell pool to ensure adequate washing of the cells. The centrifugation conditions are controlled at 1000 rpm, 10 minutes and 5±3°C to ensure sufficient cell pelleting. Two 900 µL aliquots of DMEM wash supernatant are taken for use in sterility testing of the final product.

[0443] Step 5 and 5(a) (if needed)

[0444] The re-suspended cell concentration is controlled at 1.0 -3.0 x 10^7 cells/mL, and viability ≥ 70%.

[0445] Step 6

[0446] Fill volume of the FCX-007 DP vials is controlled at 1.2 mL fill per vial. A final cell count and viability measurement, as well as Gram stain is performed on the filled QC vial. The two sterility vials are submitted for sterility testing. The results of this sterility test are not received until after Drug Product release, so release of DP is done on the basis of a negative Gram Stain. The use of a rapid microbial test in this situation is indicated in

Guidance for FDA Reviewers and Sponsors "Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigation New Drug Applications (INDs)" of April 2008.

ii. Control of Materials

[0447] The FCX-007 Drug Product manufacturing process utilizes single use, disposable products that will come in contact with raw materials (e.g. pipets, centrifuge tubes, and cryovials) to remove cleaning and carryover issues from consideration. Raw materials selected for use in the manufacturing process are acceptable for pharmaceutical processing as being USP VI compliant. All disposable items are purchased pre-sterilized by gamma irradiation. The PBS and DMEM wash buffers are purchased sterile from commercial suppliers. Steps in the process have the appropriate process parameters identified, such as time and temperature.

EXAMPLE 9 - In Vitro Characterization of Optimized GM-HDF

[0448] This example describes characterization of GM-HDFs generated using an enhanced LV transduction procedure the LV-COL7 construct INXN-2004. Three training runs (TR11, TR12.1, and TR13) were executed to generate cells for the analyses at GMP-scale. TR11 was transduced with the LV-COL7 vector INXN-2002 and was used to evaluate a centrifugation-enhanced (spinoculation) LV transduction procedure with and without a second transduction step (super-transduction). TR12.1 and TR13 were transduced with the LV-COL7 vector INXN-2004 utilizing enhanced transduction procedures as determined through evaluation of TR11.

[0449] GM-HDFs from each training run were characterized using assays to assess results such as copy number of integrated LV-COL7, protein expression of C7, and functionality of the C7 expressed by GM-HDFs.

[0450] LV integration and C7 expression levels increased first with the addition of the super-transduction step and increased further with use of the LV-COL7 INXN-2004. C7 expressed from GM-HDFs from all three training runs assembled into the trimeric form and bound to Laminin332, which is important in the formation of anchoring fibrils.

Expression of C7 by the GM-HDFs was able to reverse hypermotility observed in RDEB fibroblasts. GM-HDFs from TR12.1 and 13 were selected to be used in *in vivo* GLP toxicology studies.

[0451] Objectives in this study were to (a) demonstrate improvement in copy number of integrated LV and expression of C7 resulting from 1) an enhanced transduction procedure incorporated into the at-scale GMP production method and 2) adoption of the INXN-2004 LV-COL7 construct and (b) to confirm the functionality of C7 expressed by the GM-HDFs

1. STUDY MATERIALS

1.1. Test Article(s)

[0452] GM-HDFs from Training Runs (TR) 11, 12.1, and 13 were characterized.

Table 44: General production information for TR11, TR12.1, and TR13

Production Run	LV-COL7 and TU titer	Arm	MOI (IU/cell)	Super-transduction	Production Scale
TR11	INXN-2002 (GMP) $1.2x10^5$ IU/mL	A	0.77	Yes	2 x 10-layer CellSTACKs®
		B	0.77	No	
		Control[1]	0	n/a	
TR12.1	INXN-2004 (GMP) $1.4x10^5$ IU/mL	A	0.12	Yes	2 x 10-layer CellSTACKs®
		B	0.12	No	T-175[2]
TR13	INXN-2004 (GMP) $1.4x10^5$ IU/mL	A	0.12	Yes	2 x 10-layer CellSTACKs®
		B	0.12	No	T-175[2]

[1] Control arm cells were mock-transduced

[2] Arm B of TR12.1 and TR13 were for research only. Passaging of these arms terminated early and, thus, did not complete the full-scale production process.

1.2. Primer and Probe Sequences

Table 45: Primer and Probe Sequences used for qPCR Assays

Name	Sequence[1]	Assay
PR13843 forward[2]	ACCTGAAAGCGAAAGGGAAAC (SEQ ID NO: 31)	LV-COL7 copy number
PR13843 reverse[2]	CACCCATCTCTCTCCTTCTAGCC (SEQ ID NO: 32)	LV-COL7 copy number
PR13843 probe[2]	6-FAM-AGCTCTCTCGACGCAGGACTCGGC-3'IBFQ (SEQ ID NO: 33)	LV-COL7 copy number

[1] Primers/probes purchased from IDT.
[2] LV-specific primer/probe sequences derived from Greenberg *et al.* (2006).

2. EXPERIMENTAL PROCEDURES

2.1. LV-COL7 Copy Number

[0453] Nucleic acid isolation was performed using Qiagen's AllPrep kit according to the manufacturer's instructions. gDNA was isolated from $6.7x10^5$ GM-HDF cells, and 8 µL of gDNA was used in a 20 µL assay (10 µL Taqman® Gene Express, 1.8 µL nuclease-free water, 0.06 µL of 100 µM forward primer, 0.06 µL of 100 µM reverse primer, 0.04 µL of 100 µM Taqman® probe). A standard curve of serially diluted linearized LV-COL7 lentiviral shuttle vector (1e6 copies/reaction to 5 copies/reaction), plus 4 µL of human gDNA (1.5e4 cells/reaction) were also assayed in the 20 µL assay mentioned above. The Taqman® assay used was PR13843. 8 µL of an additional standard curve of commercial human gDNA (Clontech) (1.5e4 cells/reaction to 2e2 cells/reaction) in a 20 µL assay (10 µL Taqman® Gene Express, 1.0 L nuclease-free water, 1 µL of 20X ACTB primer/probe set) was also performed. All samples were run in triplicate on an ABI7900HT using the following cycling parameters: 2 minutes at 50°C, 10 minutes at 95°C, and 40 cycles of 15 sec at 95°C and 1 minute at 60°C.

2.2. C7 Immunofluorescence

[0454] For immunofluorescence analyses, 1.2×10^4 GM-HDFs were allowed to attach to PDL/Lamin-coated coverslips in 24-well plates overnight and then fixed and permeabilized with a 50%/50% mix of methanol/acetone. The coverslips were washed 3

times with 1X PBS and then blocked with 10% goat serum in PBS for 30 minutes at room temperature. After three additional washes with PBS, the coverslips were incubated with 1.25 μg/mL of a polyclonal fNC1 antibody (α-fNC1) in 1% goat serum/PBS, followed by 3 additional washes with PBS and incubation with 5 μg/mL Alexa Fluor® 555-conjugated goat anti-rabbit IgG in 1% goat serum/PBS for 1 hour at room temperature. Coverslips were stained with NucBlue® Live Cell Stain Ready Probes Reagent before being mounted onto slides. Images were acquired on a Zeiss Axio Observer microscope at 20X magnification using an exposure time of 290 ms. NHDFs or GM-HDFs were fixed, permeabilized, and stained with NucBlue® Live Cell Stain to visualize nuclei (blue) or with the fNC1 antibody and Alexa Fluor® 555-congugated goat anti-rabbit IgG (5 μg/mL) to visualize C7 expression (red). Images were acquired at 20X and at 5X magnification using an exposure time of 290 ms.

2.3. C7 Flow Cytometry

[0455] GM-HDFs (~500,000 cells) were collected into each well of a 96-well plate V-bottom plate, concentrated by centrifugation (1500 rpm, 5 min, room temp.), and the culture media supernatants removed. The cells were then resuspended in 200 μL of CytoFix, transferred to a 96-well V-bottom plate, and incubated for 30 min at 4°C. Following fixation, the fixative is then removed by centrifugation (1500 rpm, 5 min, 4°C) and the cells permeabilized with 200 μL ice-cold 100% methanol for 30 min on ice. Cells were then held at -20°C prior to staining procedure. For staining, permeabilized cells were washed twice with 200 μL incubation buffer (0.5 g BSA in 100 mL PBS), with centrifugation (300 rpm, 5 min, room temp.) in between washes to remove the buffer. Washed cells were resuspended in 100 μL of incubation buffer containing α-fNC1 diluted 1:400 and incubated for 1hour at room temp. Cells were then washed twice as described above and resuspended in 100 μL of incubation buffer containing AlexaFluor 555-Rabbit IgG secondary antibody diluted 1:2000 and incubated for 30 min at room temp. Cells were then washed twice as described above and resuspended in 100 μL of PBS. C7-expressing cells were enumerated by flow cytometry using BD LSRII and BD FACSDiva Software (V6.2) with the following parameters: FSC Voltage = 484, SSC Voltage = 209, PE = 500.

2.4. C7 Protein ELISA

[0456] Briefly, a standard curve of purified His-NC1 fragment (9.8 – 625 ng/mL) or collected supernatants containing C7 protein (from GM-HDF in culture for three to five days) were immobilized to a Nunc MaxiSorp® 96-well plate overnight at 4°C. Standards and samples were tested in the same sample matrix (10% RDEB fibroblast conditioned media). Coated wells were washed with PBST and blocked with 3% BSA/PBS for 1 hour at 37°C. Detection was accomplished using α-fNC1 Ab (0.5 µg/mL) followed by incubation with secondary antibody donkey anti-rabbit IgG HRP (Jackson ImmunoResearch, 0.08 µg/mL). Bound antibodies were detected via colorimetric development with TMB substrate solution. Following quenching of the reaction, absorbance was measured at 450 nm on the SpectraMax® Plus 384 (Molecular Devices).

2.5. Immunoprecipitation of C7 trimers

[0457] First, magnetic Protein G beads were washed with 1 X PBS-T. Beads were bound to the magnet for a minimum of 2 minutes prior to removal of supernatant and in all subsequent steps. Following the washes, the beads were coated with 5 µg of α-fNC1 and incubated for 10 minutes with rotation (Glas-Col, setting 30 ~ 14 rpm at room temperature). Beads were bound to the magnet to remove the supernatant and washed with Ab binding/Wash buffer. Supernatants were collected from GM-HDFs in culture for three to five days. C7 containing supernatant was added to the bead/C7 supernatant mix and was incubated overnight at 4° C with rotation (setting 30 ~ 14 rpm). The next day the beads were bound to the magnet and washed three times using Wash buffer. Target antigen was eluted in 20 µL of elution buffer (50 mM glycine, pH 2.8, and 10 µL of 4X Loading Dye). The samples were denatured at 70°C for 10 minutes. 12 µL of a total of 30 µL (remaining sample was stored at 4°C) were then loaded (per well) into a 12 well 3-8% Tris Acetate gel and run for 4 hours at 150 volts. The gel was removed from the cassette and soaked in Transfer Buffer containing 10% methanol for 20 minutes. Overnight wet transfer of the gel to a nitrocellulose membrane was performed at 15 volts at 4°C. The next day, the voltage was increased to 50 volts for 30 min on ice. Upon completion of transfer, the blot was blocked using 5% milk in TBS-T with agitation (Labnet ProBlot™ Rocker 25, setting 60 rpm) for 2 hours at room temperature and

incubated with commercially available anti-C7 LH7.2 (0.25 µg/mL) with agitation for 2 hours at room temperature. The blot was washed 3 times with 1X TBS-T (5 min each) with agitation before being probed with HRP-conjugated goat anti-mouse IgG (0.1 µg/mL) with agitation for 1 hour at room temperature. The blot was developed using Lumiglo Ultra™ Chemiluminescent substrate and Fujifilm LAS 3000 Imaging System.

2.6. Lam332 Binding

[0458] 96-well MaxiSorp™ plates were coated with 1 µg Lam332 or BSA in 100 mM carbonate buffer, pH 9.3 overnight at 4°C. The plates were rinsed five times with PBS-T, and then blocked with 1% BSA in PBS-T at room temperature for 1 hour. Coated wells were rinsed five times with PBS-T and incubated with supernatants from transduced cells overnight at 4°C. Following three more washes with PBS-T, bound C7 was incubated with the α-fNC1 (0.5 µg/mL, PBS-T) at room temperature for 3 hours, followed by incubation with HRP-conjugated donkey anti-rabbit IgG (0.8 µg/mL final concentration in PBS-T) at room temperature for 2 hrs. Detection of the C7-bound antibodies was via a colorimetric reaction using TMB and measured by reading the absorbance of the product at 450nm on a SpectraMax® Plus 384 plate reader (Molecular Devices). All incubations were carried out on an orbital shaker (GeneMate) at a speed setting of 2.

2.7. Cell Migration Assay

[0459] CytoSelect™ 24-well wound-healing assay kit from Cell Biolabs, Inc. was used for the cell migration assay. GM-HDFs (0.8×10^5) were seeded in the presence of a wound insert in 24-well plates and grown for 48 hours. The wound insert was then removed and the cells were further cultured in reduced (1%) serum media containing 15 µg/mL type I collagen (COL1) for 8 hours. Brightfield images of the wounds were acquired 0, 2, 4, 6, and 8 hours after removal of the wound insert using the Celigo® Imaging Cytometry System (Cyntellect). The surface area of the wound at each time point was measured using ImageJ (National Institutes of Health). The data is reported as the percentage of migration (% Migration) covering the wound area.

3. DATA ANALYSIS

[0460] qPCR was performed using an ABI 7900 instrument utilizing SDS2.3 software. The experimental data were viewed and exported in SDS 2.4 software. FlowJo V10 software was used to analyze Flow Cytometry data. ImagJ software was used to quantitate percent cell migration for the assay described in Section 2.7. ImageJ is an open source image processing program developed by the Nation Institute of Health and designed for scientific multidimensional images.

4. RESULTS

4.1. Characterization of C7 Expression

[0461] The overall objectives of this evaluation were to increase copy number and protein expression through a comparison of transduction methods and LV vectors. Specific objectives were to:

1. Evaluate the effect of super-transduction on integrated copy number
2. Evaluate the effect of spinoculation on integrated copy number
3. Compare copy number values between INXN-2002 and INXN-2004 under super-transduced and spinoculated conditions.

[0462] Two GMP lots of LV-COL7 constructs were used for these studies: the INXN-2002 and the construct INXN-2004 as described in this Example.

4.1.1. LV-COL7 Copy Number

[0463] GM-HDF training run cells were obtained and copy number was determined as described above in Section 2.1. Table 46 below provides the copy number/cell from Training Runs utilizing the new transduction methods. Initial optimization implemented the addition of centrifugation during the transduction step ("spinoculation"). Standard transduction had been used previously with INXN-2002 and resulted in integrated copy numbers ranging from 0.013 to 0.025. Addition of only the spinoculation step for transduction with INXN-2002 resulted in a ≥2-fold increase in the integrated copy

number to 0.05 per cell (TR11 Arm B, Table 46) compared to TR8 results from previous studies.

[0464] To further enhance the integrated copy number, a second transduction ("super-transduction") was added to the GM-HDF production process. Addition of super-transduction, also utilizing spinoculation, further increased integrated copy numbers of INXN-2002 by approximately 2-fold (TR11 Arm A, Table 46).

[0465] Small-scale research studies (not shown) demonstrated a second-generation INXN-2004 LV-COL7 construct, which utilizes an alternative 3rd-generation self-inactivating LV backbone, had the potential to achieve higher integrated copy numbers, resulting in higher expression levels, while utilizing a lower multiplicity of infection (MOI) than used for INXN-2002. When used at GMP-scale with the optimized transduction procedure (spinoculations with super-transduction), INXN-2004 had further improved integrated LV copy numbers of approximately 4- to 8-fold over the INXN-2002 construct to achieve average copy numbers of 0.41 to 0.74 (TR12.1 and TR13, respectively, Table 46).

[0466] Of note, Arms B of TR12.1 and TR13 were not tested for copy numbers as they were not passaged through the entire production process (Table 44). Accurate copy number assessments require cells that have been passaged several times following transduction to ensure that episomal copies of LV, which can artificially inflate measured copy numbers, no longer remain.

Table 46: LV-COL7 Copy Number in GM-HDF Production Run Cells

Production Run	LV-COL7 Used	Arm	MOI (IU/cell)	Super-transduction[1]	Gene Copy per Cell
TR8[2]	INXN-2002 (pilot)	A	3.4	No	0.021 ±0.005
TR11	INXN-2002 (GMP)	A	0.77	Yes	0.09±0.03
		B	0.77	No	0.05±0.01
		Control[3]	n/a	No	BLQ[4]
TR12.1[5]	INXN-2004 (GMP)	A	0.12	Yes	0.41±0.19

Production Run	LV-COL7 Used	Arm	MOI (IU/cell)	Super-transduction[1]	Gene Copy per Cell
TR13[5]	INXN-2004 (GMP)	A	0.12	Yes	0.74±0.10

[1] Only indicated arms were super-transduced; all TR11, TR12.1, and TR13 arms were spinoculated
[2] TR8 Arm A data is presented as a comparator for previous results presented in RDEB-ADSO-2
[3] Control arm was mock-transduced and was not exposed to LV-COL7
[4] BLQ: below lower limit of quantitation
[5] Arm B for both TR12.1 and TR13 was not carried through the full production process and not tested for LV-COL7 copy numbers

4.1.2. C7 Protein Expression

[0467] Indirect immunofluorescence (IF) was used to examine C7 protein expression by GM-HDF cells using an antibody specific for the C7. TR11 Arms A & B, and only Arm A of TR12.1 and TR13 were analyzed by IF. Normal human dermal fibroblasts (NHDFs) were used as a positive control and the control arm of TR11 was used as a negative control. Representative images at two magnifications, 20x and 5x are shown below in Figure 31. Also included as a comparator for the original process are IF images of cells from TR8 Arm A which had integrated LV-COL7 copy numbers of 0.015 per cell. Consistent with the copy number of integrated LV presented in Table 46 above, only a small number of TR11 GM-HDFs were visualized with C7 antibodies, though still greater than the number of C7-positive cells from TR8. Furthermore, the higher copy numbers measured from TR12.1 and TR13 GM-HDFs correlate to higher numbers of cells positive for C7 detection. Of note, the signal from the GM-HDFs that are C7-positive is brighter than the signal from NHDFs, suggesting greater amounts of C7 produced by GM-HDFs per cell.

[0468] The specific number of cells expressing C7 was then quantified using a flow cytometry assay. As described in Section 2.3, this assay utilized an NC1 region-specific polyclonal antibody. For this experiment, Arm B cells from TR12.1 and TR13 were included to compare the single transduction procedure (Arm B) with the super-transduction procedure (Arm A) using second-generation LV-COL7 construct (INXN-2004). As shown in Figure 32 below, the percentage of C7-positive (C7+) cells for each training run arm is consistent with the qualitative number of cells expressing C7 as

detected by IF (Figure 32), and correlates to the measured copy number (Table 46). Comparison of C7+ cells between Arm A and Arm B for TR12.1 and TR13 confirms the near doubling of expression levels that would be predicted by the addition of a second transduction.

[0469] A direct ELISA was used to measure C7 secreted by GM-HDFs into the cell culture media. This assay, as described in Section 2.4, also utilized the NC1 region-specific polyclonal antibody. ELISA assay results of the GM-HDFs from the three training runs show that C7 expression is dependent on the level of integrated LV copy numbers with expression levels reaching approximately 2300 ng/day/1e6 cells (Figure 33). Compared to TR8 Arm A as a representative result from previous studies (RDEB-ADSO-2), this represents a >200-fold increase in C7 expression (Figure 33).

4.2. Functional Assessment of C7 Expressed by GM-HDFs

4.2.1. Formation of C7 Trimers

[0470] The formation of C7 trimers is essential in the assembly of anchoring fibrils (Bruckner-Tuderman, 1999). To verify that the C7 expressed by GM-HDFs will be capable of forming anchoring fibrils, GM-HDFs were assessed for the formation of C7 trimers using immunoprecipitation (IP) with an anti-C7 specific antibody (fNC1) for selective capture, then concentration of C7 from culture supernatants for detection by SDS-PAGE/immunoblot. Purified recombinant C7 was used as a positive control and the capture step without C7-specific antibody was used as a negative control. IP results for the three training runs showed that the C7 produced by the GM-HDFs was predominantly trimeric (Figure 34) with some lower molecular weight species showing immunoreactivity present, likely the dimeric and monomeric forms of C7.

4.2.2. Lam332 Binding by C7 Expressed from GM-HDFs

[0471] C7 has been shown to bind immobilized extracellular matrix (ECM) components, including fibronectin, Laminin 332 (Lam332), COL1, and COL4 (Chen, et al., 2002a). The interaction between C7 and Lam332 occurs through the NH2-terminal NC1 domain

of C7 and is dependent upon the native conformation of both Lam332 and C7 NC1 (Rousselle, et al., 1997). The association between C7 and Lam332 is important for establishing correct Lam332 architecture at the dermal-epidermal junction. Such organization is critical for interactions with extracellular ligands and cell surface receptors, and for cell signaling (Waterman, et al., 2007). Intrexon developed an ELISA using an antibody against the C7 NC1 domain to detect binding of C7 to purified Lam332. Results from two experiments, presented in Figure 35, show copy number-dependent binding to Lam332 by C7 expressed by GM-HDFs from the three Training runs. TR11 Control cells were used as a negative control in both experiments.

4.2.3. Cell Migration Assay

[0472] Previous studies have shown that RDEB fibroblasts and keratinocytes show an increase in motility relative to their normal counterparts, and that normal motility can be restored by expression of C7 (Chen, et al., 2000; Chen, et al., 2002b; Cogan, et al. 2014; Baldeschi et al., 2003). Most of these studies employed either the colloidal gold salt migration assay to measure the migration of fibroblasts and keratinocytes, or a wound-healing assay to measure the migration of keratinocytes. In this Example a CytoSelect™ 24-well wound-healing assay kit (Cell Biolabs, Inc.) was used to measure the motility of NHDFs and GM-HDFs.

[0473] To determine whether the presence of C7 in the culture media is sufficient to reverse the RDEB hyper-motility phenotype, the wound healing assay was carried out on GM-HDFs from the three training runs, with NHDFs as a positive control and the mock-transduced Control (RDEB) arms as the negative control. Control fibroblasts from TR11 Arm C (RDEB) exhibited a hyper-motility phenotype that is reversed by the expression of C7 (Figure 36). Interestingly, the levels of phenotype reversal was not dependent upon the integrated copy number/C7 expression levels, suggesting that even low levels of C7 expression are enough to reverse the hyper-motility phenotype.

5. CONCLUSIONS

[0474] The integrated transgene copy number per cell in GM-HDFs was improved by both the addition of enhanced transduction methods (spinoculation and super-transduction) and a change to the LV-COL7 construct INXN-2004, with levels as high as 0.74 copies per cell achieved. The fold-improvements achieved with each process change are as follows:

- The addition of spinoculation: ≥2-fold
- The addition of super-transduction (with spinoculation): ~2-fold
- The change from INXN-2002 to INXN-2004: >4-fold
- The total cumulative change from original process: average of >27-fold (TR12.1 and TR13 compared to TR8 used in original studies)

[0475] Integrated copy number-dependent C7 expression from the GM-HDF cells was confirmed by immunofluorescence staining, flow cytometry, and ELISA. A >200-fold improvement in C7 expression was achieved relative to TR8 results presented in IND submission 16582 Serial 0000.

[0476] The structure of the C7 expressed by the GM-HDF cells was confirmed to be predominantly trimeric by immunoprecipitation/SDS-PAGE/immunoblot analysis.

[0477] In addition, the C7 produced from the GM-HDF cells was confirmed to be functional with binding to Laminin332 *in vitro* and by correction of the hypermotility phenotype of control RDEB cells in an *in vitro* migration assay.

APPENDIX 4

```
COL7A1          ATGACGCTGCGGCTTCTGGTGGCCGCGCTCTGCGCCGGGATCCTGGCAGAGGCGCCCCGA 60
Genbank         ATGACGCTGCGGCTTCTGGTGGCCGCGCTCTGCGCCGGGATCCTGGCAGAGGCGCCCCGA 60
                ************************************************************

COL7A1          GTGCGAGCCCAGCACAGGGAGAGAGTGACCTGCACGCGCCTTTACGCCGCTGACATTGTG 120
Genbank         GTGCGAGCCCAGCACAGGGAGAGAGTGACCTGCACGCGCCTTTACGCCGCTGACATTGTG 120
                ************************************************************

COL7A1          TTCTTACTGGATGGCTCCTCATCCATTGGCCGCAGCAATTTCCGCGAGGTCCGCAGCTTT 180
Genbank         TTCTTACTGGATGGCTCCTCATCCATTGGCCGCAGCAATTTCCGCGAGGTCCGCAGCTTT 180
                ************************************************************

COL7A1          CTCGAAGGGCTGGTGCTGCCTTTCTCTGGAGCAGCCAGTGCACAGGGTGTGCGCTTTGCC 240
Genbank         CTCGAAGGGCTGGTGCTGCCTTTCTCTGGAGCAGCCAGTGCACAGGGTGTGCGCTTTGCC 240
                ************************************************************

COL7A1          ACAGTGCAGTACAGCGATGACCCACGGACAGAGTTCGGCCTGGATGCACTTGGCTCTGGG 300
Genbank         ACAGTGCAGTACAGCGATGACCCACGGACAGAGTTCGGCCTGGATGCACTTGGCTCTGGG 300
                ************************************************************

COL7A1          GGTGATGTGATCCGCGCCATCCGTGAGCTTAGCTACAAGGGGGGCAACACTCGCACAGGG 360
Genbank         GGTGATGTGATCCGCGCCATCCGTGAGCTTAGCTACAAGGGGGGCAACACTCGCACAGGG 360
                ************************************************************

COL7A1          GCTGCAATTCTCCATGTGGCTGACCATGTCTTCCTGCCCCAGCTGGCCCGACCTGGTGTC 420
Genbank         GCTGCAATTCTCCATGTGGCTGACCATGTCTTCCTGCCCCAGCTGGCCCGACCTGGTGTC 420
                ************************************************************

COL7A1          CCCAAGGTCTGCATCCTGATCACAGACGGGAAGTCCCAGGACCTGGTGGACACAGCTGCC 480
Genbank         CCCAAGGTCTGCATCCTGATCACAGACGGGAAGTCCCAGGACCTGGTGGACACAGCTGCC 480
                ************************************************************

COL7A1          CAAAGGCTGAAGGGGCAGGGGGTCAAGCTATTTGCTGTGGGGATCAAGAATGCTGACCCT 540
Genbank         CAAAGGCTGAAGGGGCAGGGGGTCAAGCTATTTGCTGTGGGGATCAAGAATGCTGACCCT 540
                ************************************************************

COL7A1          GAGGAGCTGAAGCGAGTTGCCTCACAGCCCACCAGTGACTTCTTCTTCTTCGTCAATGAC 600
Genbank         GAGGAGCTGAAGCGAGTTGCCTCACAGCCCACCAGTGACTTCTTCTTCTTCGTCAATGAC 600
                ************************************************************

COL7A1          TTCAGCATCTTGAGGACACTACTGCCCCTCGTTTCCCGGAGAGTGTGCACGACTGCTGGT 660
Genbank         TTCAGCATCTTGAGGACACTACTGCCCCTCGTTTCCCGGAGAGTGTGCACGACTGCTGGT 660
                ************************************************************

COL7A1          GGCGTGCCTGTGACCCGACCTCCGGATGACTCGACCTCTGCTCCACGAGACCTGGTGCTG 720
Genbank         GGCGTGCCTGTGACCCGACCTCCGGATGACTCGACCTCTGCTCCACGAGACCTGGTGCTG 720
                ************************************************************

COL7A1          TCTGAGCCAAGCAGCCAATCCTTGAGAGTACAGTGGACAGCGGCCAGTGGCCCTGTGACT 780
Genbank         TCTGAGCCAAGCAGCCAATCCTTGAGAGTACAGTGGACAGCGGCCAGTGGCCCTGTGACT 780
                ************************************************************

COL7A1          GGCTACAAGGTCCAGTACACTCCTCTGACGGGGCTGGGACAGCCACTGCCGAGTGAGCGG 840
Genbank         GGCTACAAGGTCCAGTACACTCCTCTGACGGGGCTGGGACAGCCACTGCCGAGTGAGCGG 840
                ************************************************************

COL7A1          CAGGAGGTGAACGTCCCAGCTGGTGAGACCAGTGTGCGGCTGCGGGGTCTCCGGCCACTG 900
Genbank         CAGGAGGTGAACGTCCCAGCTGGTGAGACCAGTGTGCGGCTGCGGGGTCTCCGGCCACTG 900
                ************************************************************
```

```
COL7A1          ACCGAGTACCAAGTGACTGTGATTGCCCTCTACGCCAACAGCATCGGGGAGGCTGTGAGC 960
Genbank         ACCGAGTACCAAGTGACTGTGATTGCCCTCTACGCCAACAGCATCGGGGAGGCTGTGAGC 960
                ************************************************************

COL7A1          GGGACAGCTCGGACCACTGCCCTAGAAGGGCCGGAACTGACCATCCAGAATACCACAGCC 1020
Genbank         GGGACAGCTCGGACCACTGCCCTAGAAGGGCCGGAACTGACCATCCAGAATACCACAGCC 1020
                ************************************************************

COL7A1          CACAGCCTCCTGGTGGCCTGGCGGAGTGTGCCAGGTGCCACTGGCTACCGTGTGACATGG 1080
Genbank         CACAGCCTCCTGGTGGCCTGGCGGAGTGTGCCAGGTGCCACTGGCTACCGTGTGACATGG 1080
                ************************************************************

COL7A1          CGGGTCCTCAGTGGTGGGCCCACACAGCAGCAGGAGCTGGGCCCTGGGCAGGGTTCAGTG 1140
Genbank         CGGGTCCTCAGTGGTGGGCCCACACAGCAGCAGGAGCTGGGCCCTGGGCAGGGTTCAGTG 1140
                ************************************************************

COL7A1          TTGCTGCGTGACTTGGAGCCTGGCACGGACTATGAGGTGACCGTGAGCACCCTATTTGGC 1200
Genbank         TTGCTGCGTGACTTGGAGCCTGGCACGGACTATGAGGTGACCGTGAGCACCCTATTTGGC 1200
                ************************************************************

COL7A1          CGCAGTGTGGGGCCCGCCACTTCCCTGATGGCTCGCACTGACGCTTCTGTTGAGCAGACC 1260
Genbank         CGCAGTGTGGGGCCCGCCACTTCCCTGATGGCTCGCACTGACGCTTCTGTTGAGCAGACC 1260
                ************************************************************

COL7A1          CTGCGCCCGGTCATCCTGGGCCCCACATCCATCCTCCTTTCCTGGAACTTGGTGCCTGAG 1320
Genbank         CTGCGCCCGGTCATCCTGGGCCCCACATCCATCCTCCTTTCCTGGAACTTGGTGCCTGAG 1320
                ************************************************************

COL7A1          GCCCGTGGCTACCGGTTGGAATGGCGGCGTGAGACTGGCTTGGAGCCACCGCAGAAGGTG 1380
Genbank         GCCCGTGGCTACCGGTTGGAATGGCGGCGTGAGACTGGCTTGGAGCCACCGCAGAAGGTG 1380
                ************************************************************

COL7A1          GTACTGCCCTCTGATGTGACCCGCTACCAGTTGGATGGGCTGCAGCCGGGCACTGAGTAC 1440
Genbank         GTACTGCCCTCTGATGTGACCCGCTACCAGTTGGATGGGCTGCAGCCGGGCACTGAGTAC 1440
                ************************************************************

COL7A1          CGCCTCACACTCTACACTCTGCTGGAGGGCCACGAGGTGGCCACCCCTGCAACCGTGGTT 1500
Genbank         CGCCTCACACTCTACACTCTGCTGGAGGGCCACGAGGTGGCCACCCCTGCAACCGTGGTT 1500
                ************************************************************

COL7A1          CCCACTGGACCAGAGCTGCCTGTGAGCCCTGTAACAGACCTGCAAGCCACCGAGCTGCCC 1560
Genbank         CCCACTGGACCAGAGCTGCCTGTGAGCCCTGTAACAGACCTGCAAGCCACCGAGCTGCCC 1560
                ************************************************************

COL7A1          GGGCAGCGGGTGCGAGTGTCCTGGAGCCCAGTCCCTGGTGCCACCCAGTACCGCATCATT 1620
Genbank         GGGCAGCGGGTGCGAGTGTCCTGGAGCCCAGTCCCTGGTGCCACCCAGTACCGCATCATT 1620
                ************************************************************

COL7A1          GTGCGCAGCACCCAGGGGGTTGAGCGGACCCTGGTGCTTCCTGGGAGTCAGACAGCATTC 1680
Genbank         GTGCGCAGCACCCAGGGGGTTGAGCGGACCCTGGTGCTTCCTGGGAGTCAGACAGCATTC 1680
                ************************************************************

COL7A1          GACTTGGATGACGTTCAGGCTGGGCTTAGCTACACTGTGCGGGGTGTCTGCTCGAGTGGGT 1740
Genbank         GACTTGGATGACGTTCAGGCTGGGCTTAGCTACACTGTGCGGGGTGTCTGCTCGAGTGGGT 1740
                ************************************************************

COL7A1          CCCCGTGAGGGCAGTGCCAGTGTCCTCACTGTCCGCCGGGAGCCGGAAACTCCACTTGCT 1800
Genbank         CCCCGTGAGGGCAGTGCCAGTGTCCTCACTGTCCGCCGGGAGCCGGAAACTCCACTTGCT 1800
                ************************************************************

COL7A1          GTTCCAGGGCTGCGGGTTGTGGTGTCAGATGCAACGCGAGTGAGGGTGGCCTGGGGACCC 1860
Genbank         GTTCCAGGGCTGCGGGTTGTGGTGTCAGATGCAACGCGAGTGAGGGTGGCCTGGGGACCC 1860
                ************************************************************
```

```
COL7A1          GTCCCTGGAGCCAGTGGATTTCGGATTAGCTGGAGCACAGGCAGTGGTCCGGAGTCCAGC 1920
Genbank         GTCCCTGGAGCCAGTGGATTTCGGATTAGCTGGAGCACAGGCAGTGGTCCGGAGTCCAGC 1920
                ************************************************************

COL7A1          CAGACACTGCCCCCAGACTCTACTGCCACAGACATCACAGGGCTGCAGCCTGGAACCACC 1980
Genbank         CAGACACTGCCCCCAGACTCTACTGCCACAGACATCACAGGGCTGCAGCCTGGAACCACC 1980
                ************************************************************

COL7A1          TACCAGGTGGCTGTGTCGGTACTGCGAGGCAGAGAGGAGGGCCCTGCTGCAGTCATCGTG 2040
Genbank         TACCAGGTGGCTGTGTCGGTACTGCGAGGCAGAGAGGAGGGCCCTGCTGCAGTCATCGTG 2040
                ************************************************************

COL7A1          GCTCGAACGGACCCACTGGGCCCAGTGAGGACGGTCCATGTGACTCAGGCCAGCAGCTCA 2100
Genbank         GCTCGAACGGACCCACTGGGCCCAGTGAGGACGGTCCATGTGACTCAGGCCAGCAGCTCA 2100
                ************************************************************

COL7A1          TCTGTCACCATTACCTGGACCAGGGTTCCTGGCGCCACAGGATACAGGGTTTCCTGGCAC 2160
Genbank         TCTGTCACCATTACCTGGACCAGGGTTCCTGGCGCCACAGGATACAGGGTTTCCTGGCAC 2160
                ************************************************************

COL7A1          TCAGCCCACGGCCCAGAGAAATCCCAGTTGGTTTCTGGGGAGGCCACGGTGGCTGAGCTG 2220
Genbank         TCAGCCCACGGCCCAGAGAAATCCCAGTTGGTTTCTGGGGAGGCCACGGTGGCTGAGCTG 2220
                ************************************************************

COL7A1          GATGGACTGGAGCCAGATACTGAGTATACGGTGCATGTGAGGGCCCATGTGGCTGGCGTG 2280
Genbank         GATGGACTGGAGCCAGATACTGAGTATACGGTGCATGTGAGGGCCCATGTGGCTGGCGTG 2280
                ************************************************************

COL7A1          GATGGGCCCCCTGCCTCTGTGGTTGTGAGGACTGCCCCTGAGCCTGTGGGTCGTGTGTCG 2340
Genbank         GATGGGCCCCCTGCCTCTGTGGTTGTGAGGACTGCCCCTGAGCCTGTGGGTCGTGTGTCG 2340
                ************************************************************

COL7A1          AGGCTGCAGATCCTCAATGCTTCCAGCGACGTTCTACGGATCACCTGGGTAGGGGTCACT 2400
Genbank         AGGCTGCAGATCCTCAATGCTTCCAGCGACGTTCTACGGATCACCTGGGTAGGGGTCACT 2400
                ************************************************************

COL7A1          GGAGCCACAGCTTACAGACTGGCCTGGGGCCGGAGTGAAGGCGGCCCCATGAGGCACCAG 2460
Genbank         GGAGCCACAGCTTACAGACTGGCCTGGGGCCGGAGTGAAGGCGGCCCCATGAGGCACCAG 2460
                ************************************************************

COL7A1          ATACTCCCAGGAAACACAGACTCTGCAGAGATCCGGGGTCTCGAAGGTGGAGTCAGCTAC 2520
Genbank         ATACTCCCAGGAAACACAGACTCTGCAGAGATCCGGGGTCTCGAAGGTGGAGTCAGCTAC 2520
                ************************************************************

COL7A1          TCAGTGCGAGTGACTGCACTTGTCGGGGACCGCGAGGGCACACCTGTCTCCATTGTTGTC 2580
Genbank         TCAGTGCGAGTGACTGCACTTGTCGGGGACCGCGAGGGCACACCTGTCTCCATTGTTGTC 2580
                ************************************************************

COL7A1          ACTACGCCGCCTGAGGCTCCGCCAGCCCTGGGGACGCTTCACGTGGTGCAGCGCGGGGAG 2640
Genbank         ACTACGCCGCCTGAGGCTCCGCCAGCCCTGGGGACGCTTCACGTGGTGCAGCGCGGGGAG 2640
                ************************************************************

COL7A1          CACTCGCTGAGGCTGCGCTGGGAGCCGGTGCCCAGAGCGCAGGGCTTCCTTCTGCACTGG 2700
Genbank         CACTCGCTGAGGCTGCGCTGGGAGCCGGTGCCCAGAGCGCAGGGCTTCCTTCTGCACTGG 2700
                ************************************************************

COL7A1          CAACCTGAGGGTGGCCAGGAACAGTCCCGGGTCCTGGGGCCCGAGCTCAGCAGCTATCAC 2760
Genbank         CAACCTGAGGGTGGCCAGGAACAGTCCCGGGTCCTGGGGCCCGAGCTCAGCAGCTATCAC 2760
                ************************************************************

COL7A1          CTGGACGGGCTGGAGCCAGCGACACAGTACCGCGTGAGGCTGAGTGTCCTAGGGCCNGCT 2820
Genbank         CTGGACGGGCTGGAGCCAGCGACACAGTACCGCGTGAGGCTGAGTGTCCTAGGGCCNGCT 2820
```

```
                ********************************************************.***

COL7A1          GGAGAAGGGCCCTCTGCAGAGGTGACTGCGCGCACTGAGTCACCTCGTGTTCCAAGCATT 2880
Genbank         GGAGAAGGGCCCTCTGCAGAGGTGACTGCGCGCACTGAGTCACCTCGTGTTCCAAGCATT 2880
                ************************************************************

COL7A1          GAACTACGTGTGGTGGACACCTCGATCGACTCGGTGACTTTGGCCTGGACTCCAGTGTCC 2940
Genbank         GAACTACGTGTGGTGGACACCTCGATCGACTCGGTGACTTTGGCCTGGACTCCAGTGTCC 2940
                ************************************************************

COL7A1          AGGGCATCCAGCTACATCCTATCCTGGCGGCCACTCAGAGGCCCTGGCCAGGAAGTGCCT 3000
Genbank         AGGGCATCCAGCTACATCCTATCCTGGCGGCCACTCAGAGGCCCTGGCCAGGAAGTGCCT 3000
                ************************************************************

COL7A1          GGGTCCCCGCAGACACTTCCAGGGATCTCAAGCTCCCAGCGGGTGACAGGGCTAGAGCCT 3060
Genbank         GGGTCCCCGCAGACACTTCCAGGGATCTCAAGCTCCCAGCGGGTGACAGGGCTAGAGCCT 3060
                ************************************************************

COL7A1          GGCGTCTCTTACATCTTCTCCCTGACGCCTGTCCTGGATGGTGTGCGGGGTCCTGAGGCA 3120
Genbank         GGCGTCTCTTACATCTTCTCCCTGACGCCTGTCCTGGATGGTGTGCGGGGTCCTGAGGCA 3120
                ************************************************************

COL7A1          TCTGTCACACAGACGCCAGTGTGCCCCCGTGGCCTGGCGGATGTGGTGTTCCTACCACAT 3180
Genbank         TCTGTCACACAGACGCCAGTGTGCCCCCGTGGCCTGGCGGATGTGGTGTTCCTACCACAT 3180
                ************************************************************

COL7A1          GCCACTCAAGACAATGCTCACCGTGCGGAGGCTACGAGGAGGGTCCTGGAGCGTCTGGTG 3240
Genbank         GCCACTCAAGACAATGCTCACCGTGCGGAGGCTACGAGGAGGGTCCTGGAGCGTCTGGTG 3240
                ************************************************************

COL7A1          TTGGCACTTGGGCCTCTTGGGCCACAGGCAGTTCAGGTTGGCCTGCTGTCTTACAGTCAT 3300
Genbank         TTGGCACTTGGGCCTCTTGGGCCACAGGCAGTTCAGGTTGGCCTGCTGTCTTACAGTCAT 3300
                ************************************************************

COL7A1          CGGCCCTCCCCACTGTTCCCACTGAATGGCTCCCATGACCTTGGCATTATCTTGCAAAGG 3360
Genbank         CGGCCCTCCCCACTGTTCCCACTGAATGGCTCCCATGACCTTGGCATTATCTTGCAAAGG 3360
                ************************************************************

COL7A1          ATCCGTGACATGCCCTACATGGACCCAAGTGGGAACAACCTGGGCACAGCCGTGGTCACA 3420
Genbank         ATCCGTGACATGCCCTACATGGACCCAAGTGGGAACAACCTGGGCACAGCCGTGGTCACA 3420
                ************************************************************

COL7A1          GCTCACAGATACATGTTGGCACCAGATGCTCCTGGGCGCCGCCAGCACGTACCAGGGGTG 3480
Genbank         GCTCACAGATACATGTTGGCACCAGATGCTCCTGGGCGCCGCCAGCACGTACCAGGGGTG 3480
                ************************************************************

COL7A1          ATGGTTCTGCTAGTGGATGAACCCTTGAGAGGTGACATATTCAGCCCCATCCGTGAGGCC 3540
Genbank         ATGGTTCTGCTAGTGGATGAACCCTTGAGAGGTGACATATTCAGCCCCATCCGTGAGGCC 3540
                ************************************************************

COL7A1          CAGGCTTCTGGGCTTAATGTGGTGATGTTGGGAATGGCTGGAGCGGACCCAGAGCAGCTG 3600
Genbank         CAGGCTTCTGGGCTTAATGTGGTGATGTTGGGAATGGCTGGAGCGGACCCAGAGCAGCTG 3600
                ************************************************************

COL7A1          CGTCGCTTGGCGCCGGGTATGGACTCTGTCCAGACCTTCTTCGCCGTGGATGATGGGCCA 3660
Genbank         CGTCGCTTGGCGCCGGGTATGGACTCTGTCCAGACCTTCTTCGCCGTGGATGATGGGCCA 3660
                ************************************************************

COL7A1          AGCCTGGACCAGGCAGTCAGTGGTCTGGCCACAGCCCTGTGTCAGGCATCCTTCACTACT 3720
Genbank         AGCCTGGACCAGGCAGTCAGTGGTCTGGCCACAGCCCTGTGTCAGGCATCCTTCACTACT 3720
                ************************************************************
```

```
COL7A1          CAGCCCCGGCCAGAGCCCTGCCCAGTGTATTGTCCAAAGGGCCAGAAGGGGGAACCTGGA 3780
Genbank         CAGCCCCGGCCAGAGCCCTGCCCAGTGTATTGTCCAAAGGGCCAGAAGGGGGAACCTGGA 3780
                ************************************************************

COL7A1          GAGATGGGCCTGAGAGGACAAGTTGGGCCTCCTGGCGACCCTGGCCTCCCGGGCAGGACC 3840
Genbank         GAGATGGGCCTGAGAGGACAAGTTGGGCCTCCTGGCGACCCTGGCCTCCCGGGCAGGACC 3840
                ************************************************************

COL7A1          GGTGCTCCCGGCCCCCAGGGGCCCCCTGGAAGTGCCACTGCCAAGGGCGAGAGGGGCTTC 3900
Genbank         GGTGCTCCCGGCCCCCAGGGGCCCCCTGGAAGTGCCACTGCCAAGGGCGAGAGGGGCTTC 3900
                ************************************************************

COL7A1          CCTGGAGCAGATGGGCGTCCAGGCAGCCCTGGCCGCGCCGGGAATCCTGGGACCCCTGGA 3960
Genbank         CCTGGAGCAGATGGGCGTCCAGGCAGCCCTGGCCGCGCCGGGAATCCTGGGACCCCTGGA 3960
                ************************************************************

COL7A1          GCCCCTGGCCTAAAGGGCTCTCCAGGGTTGCCTGGCCCTCGTGGGGACCCGGGAGAGCGA 4020
Genbank         GCCCCTGGCCTAAAGGGCTCTCCAGGGTTGCCTGGCCCTCGTGGGGACCCGGGAGAGCGA 4020
                ************************************************************

COL7A1          GGACCTCGAGGCCCAAAGGGGGAGCCGGGGGCTCCCGGACAAGTCATCGGAGGTGAAGGA 4080
Genbank         GGACCTCGAGGCCCAAAGGGGGAGCCGGGGGCTCCCGGACAAGTCATCGGAGGTGAAGGA 4080
                ************************************************************

COL7A1          CCTGGGCTTCCTGGGCGGAAAGGGGACCCTGGACCATCGGGCCCCCCTGGACCTCGTGGA 4140
Genbank         CCTGGGCTTCCTGGGCGGAAAGGGGACCCTGGACCATCGGGCCCCCCTGGACCTCGTGGA 4140
                ************************************************************

COL7A1          CCACTGGGGGACCCAGGACCCCGTGGCCCCCCAGGGCTTCCTGGAACAGCCATGAAGGGT 4200
Genbank         CCACTGGGGGACCCAGGACCCCGTGGCCCCCCAGGGCTTCCTGGAACAGCCATGAAGGGT 4200
                ************************************************************

COL7A1          GACAAAGGCGATCGTGGGGAGCGGGGTCCCCCTGGACCAGGTGAAGGTGGCATTGCTCCT 4260
Genbank         GACAAAGGCGATCGTGGGGAGCGGGGTCCCCCTGGACCAGGTGAAGGTGGCATTGCTCCT 4260
                ************************************************************

COL7A1          GGGGAGCCTGGGCTGCCGGGTCTTCCCGGAAGCCCTGGACCCCAAGGCCCCGTTGGCCCC 4320
Genbank         GGGGAGCCTGGGCTGCCGGGTCTTCCCGGAAGCCCTGGACCCCAAGGCCCCGTTGGCCCC 4320
                ************************************************************

COL7A1          CCTGGAAAGAAAGGAGAAAAAGGTGACTCTGAGGATGGAGCTCCAGGCCTCCCAGGACAA 4380
Genbank         CCTGGAAAGAAAGGAGAAAAAGGTGACTCTGAGGATGGAGCTCCAGGCCTCCCAGGACAA 4380
                ************************************************************

COL7A1          CCTGGGTCTCCGGGTGAGCAGGGCCCACGGGGACCTCCTGGAGCTATTGGCCCCAAAGGT 4440
Genbank         CCTGGGTCTCCGGGTGAGCAGGGCCCACGGGGACCTCCTGGAGCTATTGGCCCCAAAGGT 4440
                ************************************************************

COL7A1          GACCGGGGCTTTCCAGGGCCCCTGGGTGAGGCTGGAGAGAAGGGCGAACGTGGACCCCCA 4500
Genbank         GACCGGGGCTTTCCAGGGCCCCTGGGTGAGGCTGGAGAGAAGGGCGAACGTGGACCCCCA 4500
                ************************************************************

COL7A1          GGCCCAGCGGGATCCCGGGGGCTGCCAGGGGTTGCTGGACGTCCTGGAGCCAAGGGTCCT 4560
Genbank         GGCCCAGCGGGATCCCGGGGGCTGCCAGGGGTTGCTGGACGTCCTGGAGCCAAGGGTCCT 4560
                ************************************************************

COL7A1          GAAGGGCCACCAGGACCCACTGGCCGCCAAGGAGAGAAGGGGGAGCCTGGTCGCCCTGGG 4620
Genbank         GAAGGGCCACCAGGACCCACTGGCCGCCAAGGAGAGAAGGGGGAGCCTGGTCGCCCTGGG 4620
                ************************************************************

COL7A1          GACCCTGCAGTGGTGGGACCTGCTGTTGCTGGACCCAAAGGAGAAAAGGGAGATGTGGGG 4680
Genbank         GACCCTGCAGTGGTGGGACCTGCTGTTGCTGGACCCAAAGGAGAAAAGGGAGATGTGGGG 4680
                ************************************************************
```

```
COL7A1          CCCGCTGGGCCCAGAGGAGCTACCGGAGTCCAAGGGGAACGGGGCCCACCCGGCTTGGTT 4740
Genbank         CCCGCTGGGCCCAGAGGAGCTACCGGAGTCCAAGGGGAACGGGGCCCACCCGGCTTGGTT 4740
                ************************************************************

COL7A1          CTTCCTGGAGACCCTGGCCCCAAGGGAGACCCTGGAGACCGGGGTCCCATTGGCCTTACT 4800
Genbank         CTTCCTGGAGACCCTGGCCCCAAGGGAGACCCTGGAGACCGGGGTCCCATTGGCCTTACT 4800
                ************************************************************

COL7A1          GGCAGAGCAGGACCCCCAGGTGACTCAGGGCCTCCTGGAGAGAAGGGAGACCCTGGGCGG 4860
Genbank         GGCAGAGCAGGACCCCCAGGTGACTCAGGGCCTCCTGGAGAGAAGGGAGACCCTGGGCGG 4860
                ************************************************************

COL7A1          CCTGGCCCCCCCAGGACCTGTTGGCCCCCGAGGACGAGATGGTGAAGTTGGAGAGAAAGGT 4920
Genbank         CCTGGCCCCCCCAGGACCTGTTGGCCCCCGAGGACGAGATGGTGAAGTTGGAGAGAAAGGT 4920
                ************************************************************

COL7A1          GACGAGGGTCCTCCGGGTGACCCGGGTTTGCCTGGAAAAGCAGGCGAGCGTGGCCTTCGG 4980
Genbank         GACGAGGGTCCTCCGGGTGACCCGGGTTTGCCTGGAAAAGCAGGCGAGCGTGGCCTTCGG 4980
                ************************************************************

COL7A1          GGGGCACCTGGAGTTCGGGGGCCTGTGGGTGAAAAGGGAGACCAGGGAGATCCTGGAGAG 5040
Genbank         GGGGCACCTGGAGTTCGGGGGCCTGTGGGTGAAAAGGGAGACCAGGGAGATCCTGGAGAG 5040
                ************************************************************

COL7A1          GATGGACGAAATGGCAGCCCTGGATCATCTGGACCCAAGGGTGACCGTGGGGAGCCGGGT 5100
Genbank         GATGGACGAAATGGCAGCCCTGGATCATCTGGACCCAAGGGTGACCGTGGGGAGCCGGGT 5100
                ************************************************************

COL7A1          CCCCCAGGACCCCCGGGACGGCTGGTAGACACAGGACCTGGAGCCAGAGAGAAGGGAGAG 5160
Genbank         CCCCCAGGACCCCCGGGACGGCTGGTAGACACAGGACCTGGAGCCAGAGAGAAGGGAGAG 5160
                ************************************************************

COL7A1          CCTGGGGACCGCGGACAAGAGGGTCCTCGAGGGCCCAAGGGTGATCCTGGCCTCCCTGGA 5220
Genbank         CCTGGGGACCGCGGACAAGAGGGTCCTCGAGGGCCCAAGGGTGATCCTGGCCTCCCTGGA 5220
                ************************************************************

COL7A1          GCCCCTGGGGAAAGGGGCATTGAAGGGTTTCGGGGACCCCCAGGCCCACAGGGGGACCCA 5280
Genbank         GCCCCTGGGGAAAGGGGCATTGAAGGGTTTCGGGGACCCCCAGGCCCACAGGGGGACCCA 5280
                ************************************************************

COL7A1          GGTGTCCGAGGCCCAGCAGGAGAAAAGGGTGACCGGGGTCCCCCTGGGCTGGATGGCCGG 5340
Genbank         GGTGTCCGAGGCCCAGCAGGAGAAAAGGGTGACCGGGGTCCCCCTGGGCTGGATGGCCGG 5340
                ************************************************************

COL7A1          AGCGGACTGGATGGGAAACCAGGAGCCGCTGGGCCCTCTGGGCCGAATGGTGCTGCAGGC 5400
Genbank         AGCGGACTGGATGGGAAACCAGGAGCCGCTGGGCCCTCTGGGCCGAATGGTGCTGCAGGC 5400
                ************************************************************

COL7A1          AAAGCTGGGGACCCAGGGAGAGACGGGCTTCCAGGCCTCCGTGGAGAACA▒GGCCTCCCT 5460
Genbank         AAAGCTGGGGACCCAGGGAGAGACGGGCTTCCAGGCCTCCGTGGAGAACA▒GGCCTCCCT 5460
                ************************************************.**********

COL7A1          GGCCCCTCTGGTCCCCCTGGATTACCGGGAAAGCCAGGCGAGGATGG▒AAACCTGGCCTG 5520
Genbank         GGCCCCTCTGGTCCCCCTGGATTACCGGGAAAGCCAGGCGAGGATGG▒AAACCTGGCCTG 5520
                ************************************************ ************

COL7A1          AATGGAAAAAACGGAGAACCTGGGGACCCTGGAGAAGACGGGAGGAAGGGAGAGAAAGGA 5580
Genbank         AATGGAAAAAACGGAGAACCTGGGGACCCTGGAGAAGACGGGAGGAAGGGAGAGAAAGGA 5580
                ************************************************************
```

```
COL7A1          GATTCAGGCGCCTCTGGGAGAGAAGGTCGTGATGGCCCCAAGGGTGAGCGTGGAGCTCCT 5640
Genbank         GATTCAGGCGCCTCTGGGAGAGAAGGTCGTGATGGCCCCAAGGGTGAGCGTGGAGCTCCT 5640
                ************************************************************

COL7A1          GGTATCCTTGGACCCCAGGGGCCTCCAGGCCTCCCAGGGCCAGTGGGCCCTCCTGGCCAG 5700
Genbank         GGTATCCTTGGACCCCAGGGGCCTCCAGGCCTCCCAGGGCCAGTGGGCCCTCCTGGCCAG 5700
                ************************************************************

COL7A1          GGTTTTCCTGGTGTCCCAGGAGGCACGGGCCCCAAGGGTGACCGTGGGGAGACTGGATCC 5760
Genbank         GGTTTTCCTGGTGTCCCAGGAGGCACGGGCCCCAAGGGTGACCGTGGGGAGACTGGATCC 5760
                ************************************************************

COL7A1          AAAGGGGAGCAGGGCCTCCCTGGAGAGCGTGGCCTGCGAGGAGAGCCTGGAAGTGTGCCG 5820
Genbank         AAAGGGGAGCAGGGCCTCCCTGGAGAGCGTGGCCTGCGAGGAGAGCCTGGAAGTGTGCCG 5820
                ************************************************************

COL7A1          AATGTGGATCGGTTGCTGGAAACTGCTGGCATCAAGGCATCTGCCCTGCGGGAGATCGTG 5880
Genbank         AATGTGGATCGGTTGCTGGAAACTGCTGGCATCAAGGCATCTGCCCTGCGGGAGATCGTG 5880
                ************************************************************

COL7A1          GAGACCTGGGATGAGAGCTCTGGTAGCTTCCTGCCTGTGCCCGAACGGCGTCGAGGCCCC 5940
Genbank         GAGACCTGGGATGAGAGCTCTGGTAGCTTCCTGCCTGTGCCCGAACGGCGTCGAGGCCCC 5940
                ************************************************************

COL7A1          AAGGGGGACTCAGGCGAACAGGGCCCCCCAGGCAAGGAGGGCCCCATCGGCTTTCCTGGA 6000
Genbank         AAGGGGGACTCAGGCGAACAGGGCCCCCCAGGCAAGGAGGGCCCCATCGGCTTTCCTGGA 6000
                ************************************************************

COL7A1          GAACGCGGGCTGAAGGGCGACCGTGGAGACCCTGGCCCTCAGGGGCCACCTGGTCTGGCC 6060
Genbank         GAACGCGGGCTGAAGGGCGACCGTGGAGACCCTGGCCCTCAGGGGCCACCTGGTCTGGCC 6060
                ************************************************************

COL7A1          CTTGGGGAGAGGGGCCCCCCCGGGCCTTCCGGCCTTGCCGGGGAGCCTGGAAAGCCTGGT 6120
Genbank         CTTGGGGAGAGGGGCCCCCCCGGGCCTTCCGGCCTTGCCGGGGAGCCTGGAAAGCCTGGT 6120
                ************************************************************

COL7A1          ATTCCCGGGCTCCCAGGCAGGGCTGGGGGTGTGGGAGAGGCAGGAAGGCCAGGAGAGAGG 6180
Genbank         ATTCCCGGGCTCCCAGGCAGGGCTGGGGGTGTGGGAGAGGCAGGAAGGCCAGGAGAGAGG 6180
                ************************************************************

COL7A1          GGAGAACGGGGAGAGAAAGGAGAACGTGGAGAACAGGGCAGAGATGGCCCTCCTGGACTC 6240
Genbank         GGAGAACGGGGAGAGAAAGGAGAACGTGGAGAACAGGGCAGAGATGGCCCTCCTGGACTC 6240
                ************************************************************

COL7A1          CCTGGAACCCCTGGGCCCCCCGGACCCCCTGGCCCCAAGGTGTCTGTGGATGAGCCAGGT 6300
Genbank         CCTGGAACCCCTGGGCCCCCCGGACCCCCTGGCCCCAAGGTGTCTGTGGATGAGCCAGGT 6300
                ************************************************************

COL7A1          CCTGGACTCTCTGGAGAACAGGGACCCCCTGGACTCAAGGGTGCTAAGGGGGAGCCGGGC 6360
Genbank         CCTGGACTCTCTGGAGAACAGGGACCCCCTGGACTCAAGGGTGCTAAGGGGGAGCCGGGC 6360
                ************************************************************

COL7A1          AGCAATGGTGACCAAGGTCCCAAAGGAGACAGGGGTGTGCCAGGCATCAAAGGAGACCGG 6420
Genbank         AGCAATGGTGACCAAGGTCCCAAAGGAGACAGGGGTGTGCCAGGCATCAAAGGAGACCGG 6420
                ************************************************************

COL7A1          GGAGAGCCTGGACCGAGGGGTCAGGACGGCAACCCGGGTCTACCAGGAGAGCGTGGTATG 6480
Genbank         GGAGAGCCTGGACCGAGGGGTCAGGACGGCAACCCGGGTCTACCAGGAGAGCGTGGTATG 6480
                ************************************************************

COL7A1          GCTGGGCCTGAAGGGAAGCCGGGTCTGCAGGGTCCAAGAGGCCCCCCTGGCCCAGTGGGT 6540
Genbank         GCTGGGCCTGAAGGGAAGCCGGGTCTGCAGGGTCCAAGAGGCCCCCCTGGCCCAGTGGGT 6540
                ************************************************************
```

```
COL7A1          GGTCATGGAGACCCTGGACCACCTGGTGCCCCGGGTCTTGCTGGCCCTGCAGGACCCCAA 6600
Genbank         GGTCATGGAGACCCTGGACCACCTGGTGCCCCGGGTCTTGCTGGCCCTGCAGGACCCCAA 6600
                ************************************************************

COL7A1          GGACCTTCTGGCCTGAAGGGGGAGCCTGGAGAGACAGGACCTCCAGGACGGGGCCTGACT 6660
Genbank         GGACCTTCTGGCCTGAAGGGGGAGCCTGGAGAGACAGGACCTCCAGGACGGGGCCTGACT 6660
                ************************************************************

COL7A1          GGACCTACTGGAGCTGTGGGACTTCCTGGACCCCCCGGCCCTTCAGGCCTTGTGGGTCCA 6720
Genbank         GGACCTACTGGAGCTGTGGGACTTCCTGGACCCCCCGGCCCTTCAGGCCTTGTGGGTCCA 6720
                ************************************************************

COL7A1          CAGGGGTCTCCAGGTTTGCCTGGACAAGTGGGGGAGACAGGGAAGCCGGGAGCCCCAGGT 6780
Genbank         CAGGGGTCTCCAGGTTTGCCTGGACAAGTGGGGGAGACAGGGAAGCCGGGAGCCCCAGGT 6780
                ************************************************************

COL7A1          CGAGATGGTGCCAGTGGAAAAGATGGAGACAGAGGGAGCCCTGGTGTGCCAGGGTCACCA 6840
Genbank         CGAGATGGTGCCAGTGGAAAAGATGGAGACAGAGGGAGCCCTGGTGTGCCAGGGTCACCA 6840
                ************************************************************

COL7A1          GGTCTGCCTGGCCCTGTCGGACCTAAAGGAGAACCTGGCCCCACGGGGGCCCCTGGACAG 6900
Genbank         GGTCTGCCTGGCCCTGTCGGACCTAAAGGAGAACCTGGCCCCACGGGGGCCCCTGGACAG 6900
                ************************************************************

COL7A1          GCTGTGGTCGGGCTCCCTGGAGCAAAGGGAGAGAAGGGAGCCCCTGGAGGCCTTGCTGGA 6960
Genbank         GCTGTGGTCGGGCTCCCTGGAGCAAAGGGAGAGAAGGGAGCCCCTGGAGGCCTTGCTGGA 6960
                ************************************************************

COL7A1          GACCTGGTGGGTGAGCCGGGAGCCAAAGGTGACCGAGGACTGCCAGGGCCGCGAGGCGAG 7020
Genbank         GACCTGGTGGGTGAGCCGGGAGCCAAAGGTGACCGAGGACTGCCAGGGCCGCGAGGCGAG 7020
                ************************************************************

COL7A1          AAGGGTGAAGCTGGCCGTGCAGGGGAGCCCGGAGACCCTGGGGAAGATGGTCAGAAAGGG 7080
Genbank         AAGGGTGAAGCTGGCCGTGCAGGGGAGCCCGGAGACCCTGGGGAAGATGGTCAGAAAGGG 7080
                ************************************************************

COL7A1          GCTCCAGGACCCAAAGGTTTCAAGGGTGACCCAGGAGTCGGGGGTCCCGGGCTCCCCTGGG 7140
Genbank         GCTCCAGGACCCAAAGGTTTCAAGGGTGACCCAGGAGTCGGGGGTCCCGGGCTCCCCTGGG 7140
                ************************************************************

COL7A1          CCTCCTGGCCCTCCAGGTGTGAAGGGAGATCTGGGCCTCCCTGGCCTGCCCGGTGCTCCT 7200
Genbank         CCTCCTGGCCCTCCAGGTGTGAAGGGAGATCTGGGCCTCCCTGGCCTGCCCGGTGCTCCT 7200
                ************************************************************

COL7A1          GGTGTTGTTGGGTTCCCGGGTCAGACAGGCCCTCGAGGAGAGATGGGTCAGCCAGGCCCT 7260
Genbank         GGTGTTGTTGGGTTCCCGGGTCAGACAGGCCCTCGAGGAGAGATGGGTCAGCCAGGCCCT 7260
                ************************************************************

COL7A1          AGTGGAGAGCGGGGTCTGGCAGGCCCCCCAGGGAGAGAAGGAATCCCAGGACCCCTGGGG 7320
Genbank         AGTGGAGAGCGGGGTCTGGCAGGCCCCCCAGGGAGAGAAGGAATCCCAGGACCCCTGGGG 7320
                ************************************************************

COL7A1          CCACCTGGACCACCGGGGTCAGTGGGACCACCTGGGGCCTCTGGACTCAAAGGAGACAAG 7380
Genbank         CCACCTGGACCACCGGGGTCAGTGGGACCACCTGGGGCCTCTGGACTCAAAGGAGACAAG 7380
                ************************************************************

COL7A1          GGAGACCCTGGAGTAGGGCTGCCTGGGCCCCGAGGCGAGCGTGGGGAGCCAGGCATCCGG 7440
Genbank         GGAGACCCTGGAGTAGGGCTGCCTGGGCCCCGAGGCGAGCGTGGGGAGCCAGGCATCCGG 7440
                ************************************************************

COL7A1          GGTGAAGATGGCCGCCCCGGCCAGGAGGGACCCCGAGGACTCACGGGGCCCCCTGGCAGC 7500
Genbank         GGTGAAGATGGCCGCCCCGGCCAGGAGGGACCCCGAGGACTCACGGGGCCCCCTGGCAGC 7500
```

```
                ************************************************************

COL7A1          AGGGGAGAGCGTGGGGAGAAGGGTGATGTTGGGAGTGCAGGACTAAAGGGTGACAAGGGA 7560
Genbank         AGGGGAGAGCGTGGGGAGAAGGGTGATGTTGGGAGTGCAGGACTAAAGGGTGACAAGGGA 7560
                ************************************************************

COL7A1          GACTCAGCTGTGATCCTGGGGCCTCCAGGCCCACGGGGTGCCAAGGGGGACATGGGTGAA 7620
Genbank         GACTCAGCTGTGATCCTGGGGCCTCCAGGCCCACGGGGTGCCAAGGGGGACATGGGTGAA 7620
                ************************************************************

COL7A1          CGAGGGCCTCGGGGCTTGGATGGTGACAAAGGACCTCGGGGAGACAATGGGGACCCTGGT 7680
Genbank         CGAGGGCCTCGGGGCTTGGATGGTGACAAAGGACCTCGGGGAGACAATGGGGACCCTGGT 7680
                ************************************************************

COL7A1          GACAAGGGCAGCAAGGGAGAGCCTGGTGACAAGGGCTCAGCCGGGTTGCCAGGACTGCGT 7740
Genbank         GACAAGGGCAGCAAGGGAGAGCCTGGTGACAAGGGCTCAGCCGGGTTGCCAGGACTGCGT 7740
                ************************************************************

COL7A1          GGACTCCTGGGACCCCAGGGTCAACCTGGTGCAGCAGGGATCCCTGGTGACCCGGGATCC 7800
Genbank         GGACTCCTGGGACCCCAGGGTCAACCTGGTGCAGCAGGGATCCCTGGTGACCCGGGATCC 7800
                ************************************************************

COL7A1          CCAGGAAAGGATGGAGTGCCTGGTATCCGAGGAGAAAAAGGAGATGTTGGCTTCATGGGT 7860
Genbank         CCAGGAAAGGATGGAGTGCCTGGTATCCGAGGAGAAAAAGGAGATGTTGGCTTCATGGGT 7860
                ************************************************************

COL7A1          CCCCGGGGCCTCAAGGGTGAACGGGGAGTGAAGGGAGCCTGTGGCCTTGATGGAGAGAAG 7920
Genbank         CCCCGGGGCCTCAAGGGTGAACGGGGAGTGAAGGGAGCCTGTGGCCTTGATGGAGAGAAG 7920
                ************************************************************

COL7A1          GGAGACAAGGGAGAAGCTGGTCCCCCAGGCCGCCCCGGGCTGGCAGGACACAAAGGAGAG 7980
Genbank         GGAGACAAGGGAGAAGCTGGTCCCCCAGGCCGCCCCGGGCTGGCAGGACACAAAGGAGAG 7980
                ************************************************************

COL7A1          ATGGGGGAGCCTGGTGTGCCGGGCCAGTCGGGGGCCCCTGGCAAGGAGGGCCTGATCGGT 8040
Genbank         ATGGGGGAGCCTGGTGTGCCGGGCCAGTCGGGGGCCCCTGGCAAGGAGGGCCTGATCGGT 8040
                ************************************************************

COL7A1          CCCAAGGGTGACCGAGGCTTTGACGGGCAGCCAGGCCCCAAGGGTGACCAGGGCGAGAAA 8100
Genbank         CCCAAGGGTGACCGAGGCTTTGACGGGCAGCCAGGCCCCAAGGGTGACCAGGGCGAGAAA 8100
                ************************************************************

COL7A1          GGGGAGCGGGGAACCCCAGGAATTGGGGGCTTCCCAGGCCCCAGTGGAAATGATGGCTCT 8160
Genbank         GGGGAGCGGGGAACCCCAGGAATTGGGGGCTTCCCAGGCCCCAGTGGAAATGATGGCTCT 8160
                ************************************************************

COL7A1          GCTGGTCCCCCAGGGCCACCTGGCAGTGTTGGTCCCAGAGGCCCCGAAGGACTTCAGGGC 8220
Genbank         GCTGGTCCCCCAGGGCCACCTGGCAGTGTTGGTCCCAGAGGCCCCGAAGGACTTCAGGGC 8220
                ************************************************************

COL7A1          CAGAAGGGTGAGCGAGGTCCCCCCGGAGAGAGAGTGGTGGGGGCTCCTGGGGTCCCTGGA 8280
Genbank         CAGAAGGGTGAGCGAGGTCCCCCCGGAGAGAGAGTGGTGGGGGCTCCTGGGGTCCCTGGA 8280
                ************************************************************

COL7A1          GCTCCTGGCGAGAGAGGGGAGCAGGGGCGGCCAGGGCCTGCCGGTCCTCGAGGCGAGAAG 8340
Genbank         GCTCCTGGCGAGAGAGGGGAGCAGGGGCGGCCAGGGCCTGCCGGTCCTCGAGGCGAGAAG 8340
                ************************************************************

COL7A1          GGAGAAGCTGCACTGACGGAGGATGACATCCGGGGCTTTGTGCGCCAAGAGATGAGTCAG 8400
Genbank         GGAGAAGCTGCACTGACGGAGGATGACATCCGGGGCTTTGTGCGCCAAGAGATGAGTCAG 8400
                ************************************************************

COL7A1          CACTGTGCCTGCCAGGGCCAGTTCATCGCATCTGGATCACGACCCCTCCCTAGTTATGCT 8460
```

```
Genbank         CACTGTGCCTGCCAGGGCCAGTTCATCGCATCTGGATCACGACCCCTCCCTAGTTATGCT 8460
                ************************************************************

COL7A1          GCAGACACTGCCGGCTCCCAGCTCCATGCTGTGCCTGTGCTCCGCGTCTCTCATGCAGAG 8520
Genbank         GCAGACACTGCCGGCTCCCAGCTCCATGCTGTGCCTGTGCTCCGCGTCTCTCATGCAGAG 8520
                ************************************************************

COL7A1          GAGGAAGAGCGGGTACCCCCTGAGGATGATGAGTACTCTGAATACTCCGAGTATTCTGTG 8580
Genbank         GAGGAAGAGCGGGTACCCCCTGAGGATGATGAGTACTCTGAATACTCCGAGTATTCTGTG 8580
                ************************************************************

COL7A1          GAGGAGTACCAGGACCCTGAAGCTCCTTGGGATAGTGATGACCCCTGTTCCCTGCCACTG 8640
Genbank         GAGGAGTACCAGGACCCTGAAGCTCCTTGGGATAGTGATGACCCCTGTTCCCTGCCACTG 8640
                ************************************************************

COL7A1          GATGAGGGCTCCTGCACTGCCTACACCCTGCGCTGGTACCATCGGGCTGTGACAGGCAGC 8700
Genbank         GATGAGGGCTCCTGCACTGCCTACACCCTGCGCTGGTACCATCGGGCTGTGACAGGCAGC 8700
                ************************************************************

COL7A1          ACAGAGGCCTGTCACCCTTTTGTCTATGGTGGCTGTGGAGGGAATGCCAACCGTTTTGGG 8760
Genbank         ACAGAGGCCTGTCACCCTTTTGTCTATGGTGGCTGTGGAGGGAATGCCAACCGTTTTGGG 8760
                ************************************************************

COL7A1          ACCCGTGAGGCCTGCGAGCGCCGCTGCCCACCCCGGGTGGTCCAGAGCCAGGGGACAGGT 8820
Genbank         ACCCGTGAGGCCTGCGAGCGCCGCTGCCCACCCCGGGTGGTCCAGAGCCAGGGGACAGGT 8820
                ************************************************************

COL7A1          ACTGCCCAGGAC 8832 (SEQ ID NO: 23)
Genbank         ACTGCCCAGGAC 8832 (SEQ ID NO: 24)
                ************
```

The following is a comparative analysis of 60010743-ITX-00001_CONTIG SEQUENCE (SEQ ID NO:34) relative to IGE308_REFSEQUENCE (SEQ ID NO: 34).

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

```
IGE308_RefSequence          #1   GTCGACGGAT CGGGAGATCT CCCGATCCCC TATGGTGCAC TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG
60010743-ITX-00001_Contig   #1   GTCGACGGAT CGGGAGATCT CCCGATCCCC TATGGTGCAC TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG

                            #1   GTCGACGGAT CGGGAGATCT CCCGATCCCC TATGGTGCAC TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG

IGE308_RefSequence          #81  TATCTGCTCC CTGCTTGTGT GTTGGAGGTC GCTGAGTAGT GCGCGAGCAA AATTTAAGCT ACAACAAGGC AAGGCTTGAC
60010743-ITX-00001_Contig   #81  TATCTGCTCC CTGCTTGTGT GTTGGAGGTC GCTGAGTAGT GCGCGAGCAA AATTTAAGCT ACAACAAGGC AAGGCTTGAC

                            #81  TATCTGCTCC CTGCTTGTGT GTTGGAGGTC GCTGAGTAGT GCGCGAGCAA AATTTAAGCT ACAACAAGGC AAGGCTTGAC

IGE308_RefSequence          #161 CGACAATTGC ATGAAGAATC TGCTTAGGGT TAGGCGTTTT GCGCTGCTTC GCGATGTACG GGCCAGATAT ACGCGTTGAC
60010743-ITX-00001_Contig   #161 CGACAATTGC ATGAAGAATC TGCTTAGGGT TAGGCGTTTT GCGCTGCTTC GCGATGTACG GGCCAGATAT ACGCGTTGAC

                            #161 CGACAATTGC ATGAAGAATC TGCTTAGGGT TAGGCGTTTT GCGCTGCTTC GCGATGTACG GGCCAGATAT ACGCGTTGAC

IGE308_RefSequence          #241 ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT CATAGCCCAT ATATGGAGTT CCGCGTTACA
60010743-ITX-00001_Contig   #241 ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT CATAGCCCAT ATATGGAGTT CCGCGTTACA

                            #241 ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT CATAGCCCAT ATATGGAGTT CCGCGTTACA

IGE308_RefSequence          #321 TAACTTACGG TAAATGGCCC GCCTGGCTGA CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT
60010743-ITX-00001_Contig   #321 TAACTTACGG TAAATGGCCC GCCTGGCTGA CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT

                            #321 TAACTTACGG TAAATGGCCC GCCTGGCTGA CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT

IGE308_RefSequence          #401 AGTAACGCCA ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA GTACATCAAG
60010743-ITX-00001_Contig   #401 AGTAACGCCA ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA GTACATCAAG

                            #401 AGTAACGCCA ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA GTACATCAAG

IGE308_RefSequence          #481 TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG CCCGCCTGGC ATTATGCCCA GTACATGACC
60010743-ITX-00001_Contig   #481 TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG CCCGCCTGGC ATTATGCCCA GTACATGACC

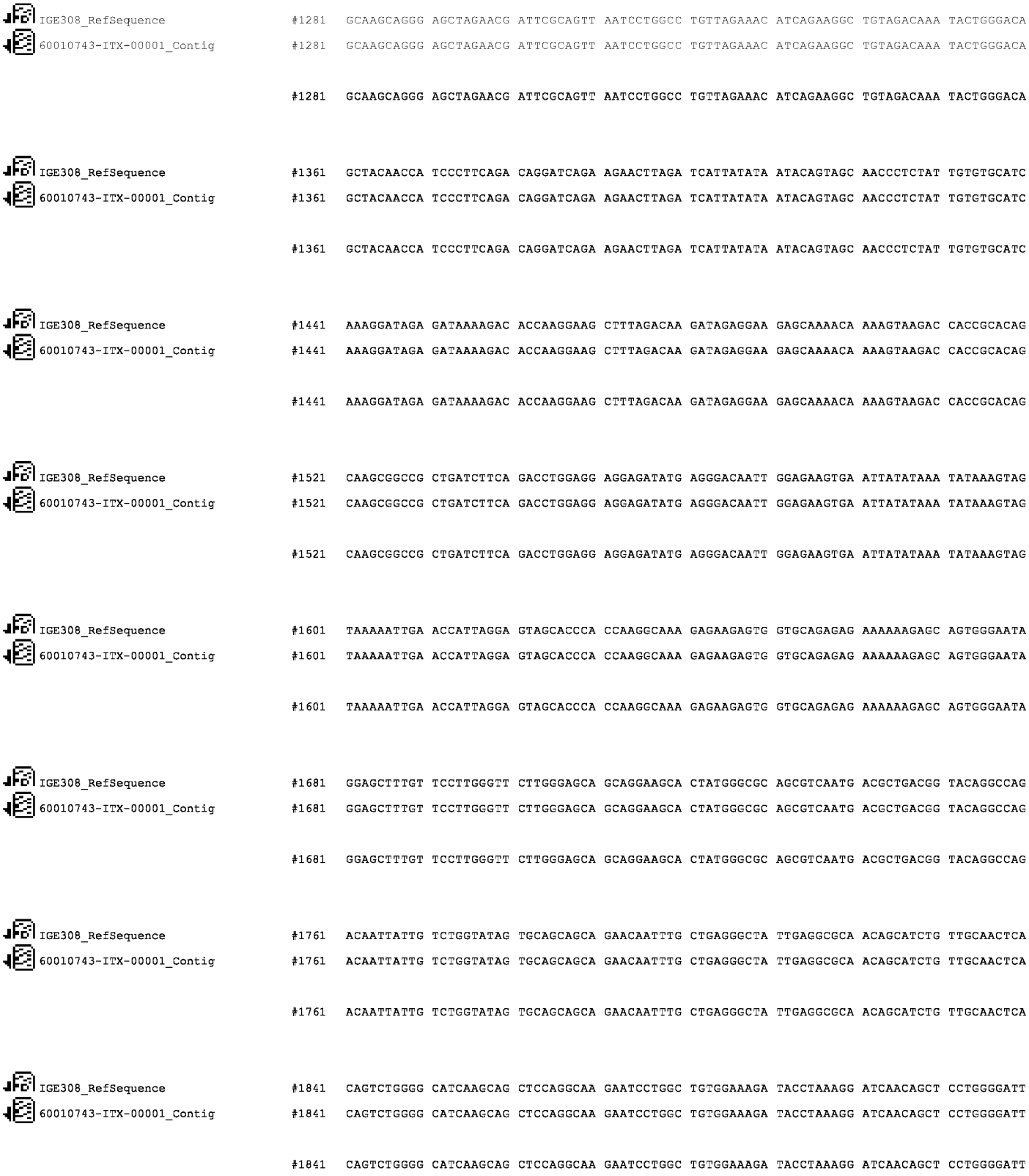
                            #481 TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG CCCGCCTGGC ATTATGCCCA GTACATGACC

IGE308_RefSequence          #561 TTATGGGACT TTCCTACTTG GCAGTACATC TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT
60010743-ITX-00001_Contig   #561 TTATGGGACT TTCCTACTTG GCAGTACATC TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT

                            #561 TTATGGGACT TTCCTACTTG GCAGTACATC TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT
```

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence	#641	CAATGGGCGT GGATAGCGGT TGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT TTGTTTTGGC
60010743-ITX-00001_Contig	#641	CAATGGGCGT GGATAGCGGT TGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT TTGTTTTGGC
	#641	CAATGGGCGT GGATAGCGGT TGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT TTGTTTTGGC
IGE308_RefSequence	#721	ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG
60010743-ITX-00001_Contig	#721	ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG
	#721	ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG
IGE308_RefSequence	#801	GAGGTCTATA TAAGCAGCGC GTTTTGCCTG TACTGGGTCT CTCTGGTTAG ACCAGATCTG AGCCTGGGAG CTCTCTGGCT
60010743-ITX-00001_Contig	#801	GAGGTCTATA TAAGCAGCGC GTTTTGCCTG TACTGGGTCT CTCTGGTTAG ACCAGATCTG AGCCTGGGAG CTCTCTGGCT
	#801	GAGGTCTATA TAAGCAGCGC GTTTTGCCTG TACTGGGTCT CTCTGGTTAG ACCAGATCTG AGCCTGGGAG CTCTCTGGCT
IGE308_RefSequence	#881	AACTAGGGAA CCCACTGCTT AAGCCTCAAT AAAGCTTGCC TTGAGTGCTT CAAGTAGTGT GTGCCCGTCT GTTGTGTGAC
60010743-ITX-00001_Contig	#881	AACTAGGGAA CCCACTGCTT AAGCCTCAAT AAAGCTTGCC TTGAGTGCTT CAAGTAGTGT GTGCCCGTCT GTTGTGTGAC
	#881	AACTAGGGAA CCCACTGCTT AAGCCTCAAT AAAGCTTGCC TTGAGTGCTT CAAGTAGTGT GTGCCCGTCT GTTGTGTGAC
IGE308_RefSequence	#961	TCTGGTAACT AGAGATCCCT CAGACCCTTT TAGTCAGTGT GGAAAATCTC TAGCAGTGGC GCCCGAACAG GGACTTGAAA
60010743-ITX-00001_Contig	#961	TCTGGTAACT AGAGATCCCT CAGACCCTTT TAGTCAGTGT GGAAAATCTC TAGCAGTGGC GCCCGAACAG GGACTTGAAA
	#961	TCTGGTAACT AGAGATCCCT CAGACCCTTT TAGTCAGTGT GGAAAATCTC TAGCAGTGGC GCCCGAACAG GGACTTGAAA
IGE308_RefSequence	#1041	GCGAAAGGGA AACCAGAGGA GCTCTCTCGA CGCAGGACTC GGCTTGCTGA AGCGCGCACG GCAAGAGGCG AGGGGCGGCG
60010743-ITX-00001_Contig	#1041	GCGAAAGGGA AACCAGAGGA GCTCTCTCGA CGCAGGACTC GGCTTGCTGA AGCGCGCACG GCAAGAGGCG AGGGGCGGCG
	#1041	GCGAAAGGGA AACCAGAGGA GCTCTCTCGA CGCAGGACTC GGCTTGCTGA AGCGCGCACG GCAAGAGGCG AGGGGCGGCG
IGE308_RefSequence	#1121	ACTGGTGAGT ACGCCAAAAA TTTTGACTAG CGGAGGCTAG AAGGAGAGAG ATGGGTGCGA GAGCGTCAGT ATTAAGCGGG
60010743-ITX-00001_Contig	#1121	ACTGGTGAGT ACGCCAAAAA TTTTGACTAG CGGAGGCTAG AAGGAGAGAG ATGGGTGCGA GAGCGTCAGT ATTAAGCGGG
	#1121	ACTGGTGAGT ACGCCAAAAA TTTTGACTAG CGGAGGCTAG AAGGAGAGAG ATGGGTGCGA GAGCGTCAGT ATTAAGCGGG
IGE308_RefSequence	#1201	GGAGAATTAG ATCGCGATGG GAAAAAATTC GGTTAAGGCC AGGGGGAAAG AAAAAATATA AATTAAAACA TATAGTATGG
60010743-ITX-00001_Contig	#1201	GGAGAATTAG ATCGCGATGG GAAAAAATTC GGTTAAGGCC AGGGGGAAAG AAAAAATATA AATTAAAACA TATAGTATGG
	#1201	GGAGAATTAG ATCGCGATGG GAAAAAATTC GGTTAAGGCC AGGGGGAAAG AAAAAATATA AATTAAAACA TATAGTATGG


IGE308_RefSequence #1281 GCAAGCAGGG AGCTAGAACG ATTCGCAGTT AATCCTGGCC TGTTAGAAAC ATCAGAAGGC TGTAGACAAA TACTGGGACA
60010743-ITX-00001_Contig #1281 GCAAGCAGGG AGCTAGAACG ATTCGCAGTT AATCCTGGCC TGTTAGAAAC ATCAGAAGGC TGTAGACAAA TACTGGGACA
#1281 GCAAGCAGGG AGCTAGAACG ATTCGCAGTT AATCCTGGCC TGTTAGAAAC ATCAGAAGGC TGTAGACAAA TACTGGGACA
IGE308_RefSequence #1361 GCTACAACCA TCCCTTCAGA CAGGATCAGA AGAACTTAGA TCATTATATA ATACAGTAGC AACCCTCTAT TGTGTGCATC
60010743-ITX-00001_Contig #1361 GCTACAACCA TCCCTTCAGA CAGGATCAGA AGAACTTAGA TCATTATATA ATACAGTAGC AACCCTCTAT TGTGTGCATC
#1361 GCTACAACCA TCCCTTCAGA CAGGATCAGA AGAACTTAGA TCATTATATA ATACAGTAGC AACCCTCTAT TGTGTGCATC
IGE308_RefSequence #1441 AAAGGATAGA GATAAAAGAC ACCAAGGAAG CTTTAGACAA GATAGAGGAA GAGCAAAACA AAAGTAAGAC CACCGCACAG
60010743-ITX-00001_Contig #1441 AAAGGATAGA GATAAAAGAC ACCAAGGAAG CTTTAGACAA GATAGAGGAA GAGCAAAACA AAAGTAAGAC CACCGCACAG
#1441 AAAGGATAGA GATAAAAGAC ACCAAGGAAG CTTTAGACAA GATAGAGGAA GAGCAAAACA AAAGTAAGAC CACCGCACAG
IGE308_RefSequence #1521 CAAGCGGCCG CTGATCTTCA GACCTGGAGG AGGAGATATG AGGGACAATT GGAGAAGTGA ATTATATAAA TATAAAGTAG
60010743-ITX-00001_Contig #1521 CAAGCGGCCG CTGATCTTCA GACCTGGAGG AGGAGATATG AGGGACAATT GGAGAAGTGA ATTATATAAA TATAAAGTAG
#1521 CAAGCGGCCG CTGATCTTCA GACCTGGAGG AGGAGATATG AGGGACAATT GGAGAAGTGA ATTATATAAA TATAAAGTAG
IGE308_RefSequence #1601 TAAAAATTGA ACCATTAGGA GTAGCACCCA CCAAGGCAAA GAGAAGAGTG GTGCAGAGAG AAAAAAGAGC AGTGGGAATA
60010743-ITX-00001_Contig #1601 TAAAAATTGA ACCATTAGGA GTAGCACCCA CCAAGGCAAA GAGAAGAGTG GTGCAGAGAG AAAAAAGAGC AGTGGGAATA
#1601 TAAAAATTGA ACCATTAGGA GTAGCACCCA CCAAGGCAAA GAGAAGAGTG GTGCAGAGAG AAAAAAGAGC AGTGGGAATA
IGE308_RefSequence #1681 GGAGCTTTGT TCCTTGGGTT CTTGGGAGCA GCAGGAAGCA CTATGGGCGC AGCGTCAATG ACGCTGACGG TACAGGCCAG
60010743-ITX-00001_Contig #1681 GGAGCTTTGT TCCTTGGGTT CTTGGGAGCA GCAGGAAGCA CTATGGGCGC AGCGTCAATG ACGCTGACGG TACAGGCCAG
#1681 GGAGCTTTGT TCCTTGGGTT CTTGGGAGCA GCAGGAAGCA CTATGGGCGC AGCGTCAATG ACGCTGACGG TACAGGCCAG
IGE308_RefSequence #1761 ACAATTATTG TCTGGTATAG TGCAGCAGCA GAACAATTTG CTGAGGGCTA TTGAGGCGCA ACAGCATCTG TTGCAACTCA
60010743-ITX-00001_Contig #1761 ACAATTATTG TCTGGTATAG TGCAGCAGCA GAACAATTTG CTGAGGGCTA TTGAGGCGCA ACAGCATCTG TTGCAACTCA
#1761 ACAATTATTG TCTGGTATAG TGCAGCAGCA GAACAATTTG CTGAGGGCTA TTGAGGCGCA ACAGCATCTG TTGCAACTCA
IGE308_RefSequence #1841 CAGTCTGGGG CATCAAGCAG CTCCAGGCAA GAATCCTGGC TGTGGAAAGA TACCTAAAGG ATCAACAGCT CCTGGGGATT
60010743-ITX-00001_Contig #1841 CAGTCTGGGG CATCAAGCAG CTCCAGGCAA GAATCCTGGC TGTGGAAAGA TACCTAAAGG ATCAACAGCT CCTGGGGATT
#1841 CAGTCTGGGG CATCAAGCAG CTCCAGGCAA GAATCCTGGC TGTGGAAAGA TACCTAAAGG ATCAACAGCT CCTGGGGATT

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #1921 TGGGGTTGCT CTGGAAAACT CATTTGCACC ACTGCTGTGC CTTGGAATGC TAGTTGGAGT AATAAATCTC TGGAACAGAT
60010743-ITX-00001_Contig #1921 TGGGGTTGCT CTGGAAAACT CATTTGCACC ACTGCTGTGC CTTGGAATGC TAGTTGGAGT AATAAATCTC TGGAACAGAT

#1921 TGGGGTTGCT CTGGAAAACT CATTTGCACC ACTGCTGTGC CTTGGAATGC TAGTTGGAGT AATAAATCTC TGGAACAGAT

IGE308_RefSequence #2001 TTGGAATCAC ACGACCTGGA TGGAGTGGGA CAGAGAAATT AACAATTACA CAAGCTTAAT ACACTCCTTA ATTGAAGAAT
60010743-ITX-00001_Contig #2001 TTGGAATCAC ACGACCTGGA TGGAGTGGGA CAGAGAAATT AACAATTACA CAAGCTTAAT ACACTCCTTA ATTGAAGAAT

#2001 TTGGAATCAC ACGACCTGGA TGGAGTGGGA CAGAGAAATT AACAATTACA CAAGCTTAAT ACACTCCTTA ATTGAAGAAT

IGE308_RefSequence #2081 CGCAAAACCA GCAAGAAAAG AATGAACAAG AATTATTGGA ATTAGATAAA TGGGCAAGTT TGTGGAATTG GTTTAACATA
60010743-ITX-00001_Contig #2081 CGCAAAACCA GCAAGAAAAG AATGAACAAG AATTATTGGA ATTAGATAAA TGGGCAAGTT TGTGGAATTG GTTTAACATA

#2081 CGCAAAACCA GCAAGAAAAG AATGAACAAG AATTATTGGA ATTAGATAAA TGGGCAAGTT TGTGGAATTG GTTTAACATA

IGE308_RefSequence #2161 ACAAATTGGC TGTGGTATAT AAAATTATTC ATAATGATAG TAGGAGGCTT GGTAGGTTTA AGAATAGTTT TTGCTGTACT
60010743-ITX-00001_Contig #2161 ACAAATTGGC TGTGGTATAT AAAATTATTC ATAATGATAG TAGGAGGCTT GGTAGGTTTA AGAATAGTTT TTGCTGTACT

#2161 ACAAATTGGC TGTGGTATAT AAAATTATTC ATAATGATAG TAGGAGGCTT GGTAGGTTTA AGAATAGTTT TTGCTGTACT

IGE308_RefSequence #2241 TTCTATAGTG AATAGAGTTA GGCAGGGATA TTCACCATTA TCGTTTCAGA CCCACCTCCC AACCCCGAGG GGACCCGACA
60010743-ITX-00001_Contig #2241 TTCTATAGTG AATAGAGTTA GGCAGGGATA TTCACCATTA TCGTTTCAGA CCCACCTCCC AACCCCGAGG GGACCCGACA

#2241 TTCTATAGTG AATAGAGTTA GGCAGGGATA TTCACCATTA TCGTTTCAGA CCCACCTCCC AACCCCGAGG GGACCCGACA

IGE308_RefSequence #2321 GGCCCGAAGG AATAGAAGAA GAAGGTGGAG AGAGAGACAG AGACAGATCC ATTCGATTAG TGAACGGATC GGCACTGCGT
60010743-ITX-00001_Contig #2321 GGCCCGAAGG AATAGAAGAA GAAGGTGGAG AGAGAGACAG AGACAGATCC ATTCGATTAG TGAACGGATC GGCACTGCGT

#2321 GGCCCGAAGG AATAGAAGAA GAAGGTGGAG AGAGAGACAG AGACAGATCC ATTCGATTAG TGAACGGATC GGCACTGCGT

IGE308_RefSequence #2401 GCGCCAATTC TGCAGACAAA TGGCAGTATT CATCCACAAT TTTAAAAGAA AAGGGGGGAT TGGGGGGTAC AGTGCAGGGG
60010743-ITX-00001_Contig #2401 GCGCCAATTC TGCAGACAAA TGGCAGTATT CATCCACAAT TTTAAAAGAA AAGGGGGGAT TGGGGGGTAC AGTGCAGGGG

#2401 GCGCCAATTC TGCAGACAAA TGGCAGTATT CATCCACAAT TTTAAAAGAA AAGGGGGGAT TGGGGGGTAC AGTGCAGGGG

IGE308_RefSequence #2481 AAAGAATAGT AGACATAATA GCAACAGACA TACAAACTAA AGAATTACAA AAACAAATTA CAAAAATTCA AAATTTTCGG
60010743-ITX-00001_Contig #2481 AAAGAATAGT AGACATAATA GCAACAGACA TACAAACTAA AGAATTACAA AAACAAATTA CAAAAATTCA AAATTTTCGG

#2481 AAAGAATAGT AGACATAATA GCAACAGACA TACAAACTAA AGAATTACAA AAACAAATTA CAAAAATTCA AAATTTTCGG

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #2561 GTTTATTACA GGGACAGCAG AGATCCAGTT TGGTTAATTA AGCATTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG
60010743-ITX-00001_Contig #2561 GTTTATTACA GGGACAGCAG AGATCCAGTT TGGTTAATTA AGCATTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG

#2561 GTTTATTACA GGGACAGCAG AGATCCAGTT TGGTTAATTA AGCATTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG

IGE308_RefSequence #2641 TTCATAGCCC ATATATGGAG TTCCGCGTTA CATAACTTAC GGTAAATGGC CCGCCTGGTT GACCGCCCAA CGACCCCCGC
60010743-ITX-00001_Contig #2641 TTCATAGCCC ATATATGGAG TTCCGCGTTA CATAACTTAC GGTAAATGGC CCGCCTGGTT GACCGCCCAA CGACCCCCGC

#2641 TTCATAGCCC ATATATGGAG TTCCGCGTTA CATAACTTAC GGTAAATGGC CCGCCTGGTT GACCGCCCAA CGACCCCCGC

IGE308_RefSequence #2721 CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGC CAATAGGGAC TTTCCATTGA CGTCAATGGG TGGAGTATTT
60010743-ITX-00001_Contig #2721 CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGC CAATAGGGAC TTTCCATTGA CGTCAATGGG TGGAGTATTT

#2721 CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGC CAATAGGGAC TTTCCATTGA CGTCAATGGG TGGAGTATTT

IGE308_RefSequence #2801 ACGGTAAACT GCCCACTTGG TAGTACATCA AGTGTATCAT ACGCCAAGTA CGCCCCCTAT TGACGTCAAT GACGGTAAAT
60010743-ITX-00001_Contig #2801 ACGGTAAACT GCCCACTTGG TAGTACATCA AGTGTATCAT ACGCCAAGTA CGCCCCCTAT TGACGTCAAT GACGGTAAAT

#2801 ACGGTAAACT GCCCACTTGG TAGTACATCA AGTGTATCAT ACGCCAAGTA CGCCCCCTAT TGACGTCAAT GACGGTAAAT

IGE308_RefSequence #2881 GGCCCGCCTG GTATTATGCC CAGTACATGA CCTTATGGGA CTTTCCTACT TGGCAGTACA TCTGCGTATT AGTCATCGCT
60010743-ITX-00001_Contig #2881 GGCCCGCCTG GTATTATGCC CAGTACATGA CCTTATGGGA CTTTCCTACT TGGCAGTACA TCTGCGTATT AGTCATCGCT

#2881 GGCCCGCCTG GTATTATGCC CAGTACATGA CCTTATGGGA CTTTCCTACT TGGCAGTACA TCTGCGTATT AGTCATCGCT

IGE308_RefSequence #2961 ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC GTGGATAGCG GTTTGACTCA CGGGGATTTC CAAGTCTCCA
60010743-ITX-00001_Contig #2961 ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC GTGGATAGCG GTTTGACTCA CGGGGATTTC CAAGTCTCCA

#2961 ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC GTGGATAGCG GTTTGACTCA CGGGGATTTC CAAGTCTCCA

IGE308_RefSequence #3041 CCCCATTGAC GTCAATGGGA GTTTGTTTTG GCACCAAAAT CAACGGGACT TTCCAAAATG TCGTAACAAC TCCGCCCCAT
60010743-ITX-00001_Contig #3041 CCCCATTGAC GTCAATGGGA GTTTGTTTTG GCACCAAAAT CAACGGGACT TTCCAAAATG TCGTAACAAC TCCGCCCCAT

#3041 CCCCATTGAC GTCAATGGGA GTTTGTTTTG GCACCAAAAT CAACGGGACT TTCCAAAATG TCGTAACAAC TCCGCCCCAT

IGE308_RefSequence #3121 TGACGCAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTGGTTTAG TGAACCGTCA GATCCGCTAG
60010743-ITX-00001_Contig #3121 TGACGCAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTGGTTTAG TGAACCGTCA GATCCGCTAG

#3121 TGACGCAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTGGTTTAG TGAACCGTCA GATCCGCTAG

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #3201 CATTGTGTGC TTTTCGGGCC ACCATGACGC TGCGGCTTCT GGTGGCCGCG CTCTGCGCCG GGATCCTGGC AGAGGCGCCC
60010743-ITX-00001_Contig #3201 CATTGTGTGC TTTTCGGGCC ACCATGACGC TGCGGCTTCT GGTGGCCGCG CTCTGCGCCG GGATCCTGGC AGAGGCGCCC

#3201 CATTGTGTGC TTTTCGGGCC ACCATGACGC TGCGGCTTCT GGTGGCCGCG CTCTGCGCCG GGATCCTGGC AGAGGCGCCC

IGE308_RefSequence #3281 CGAGTGCGAG CCCAGCACAG GGAGAGAGTG ACCTGCACGC GCCTTTACGC CGCTGACATT GTGTTCTTAC TGGATGGCTC
60010743-ITX-00001_Contig #3281 CGAGTGCGAG CCCAGCACAG GGAGAGAGTG ACCTGCACGC GCCTTTACGC CGCTGACATT GTGTTCTTAC TGGATGGCTC

#3281 CGAGTGCGAG CCCAGCACAG GGAGAGAGTG ACCTGCACGC GCCTTTACGC CGCTGACATT GTGTTCTTAC TGGATGGCTC

IGE308_RefSequence #3361 CTCATCCATT GGCCGCAGCA ATTTCCGCGA GGTCCGCAGC TTTCTCGAAG GGCTGGTGCT GCCTTTCTCT GGAGCAGCCA
60010743-ITX-00001_Contig #3361 CTCATCCATT GGCCGCAGCA ATTTCCGCGA GGTCCGCAGC TTTCTCGAAG GGCTGGTGCT GCCTTTCTCT GGAGCAGCCA

#3361 CTCATCCATT GGCCGCAGCA ATTTCCGCGA GGTCCGCAGC TTTCTCGAAG GGCTGGTGCT GCCTTTCTCT GGAGCAGCCA

IGE308_RefSequence #3441 GTGCACAGGG TGTGCGCTTT GCCACAGTGC AGTACAGCGA TGACCCACGG ACAGAGTTCG GCCTGGATGC ACTTGGCTCT
60010743-ITX-00001_Contig #3441 GTGCACAGGG TGTGCGCTTT GCCACAGTGC AGTACAGCGA TGACCCACGG ACAGAGTTCG GCCTGGATGC ACTTGGCTCT

#3441 GTGCACAGGG TGTGCGCTTT GCCACAGTGC AGTACAGCGA TGACCCACGG ACAGAGTTCG GCCTGGATGC ACTTGGCTCT

IGE308_RefSequence #3521 GGGGGTGATG TGATCCGCGC CATCCGTGAG CTTAGCTACA AGGGGGGCAA CACTCGCACA GGGGCTGCAA TTCTCCATGT
60010743-ITX-00001_Contig #3521 GGGGGTGATG TGATCCGCGC CATCCGTGAG CTTAGCTACA AGGGGGGCAA CACTCGCACA GGGGCTGCAA TTCTCCATGT

#3521 GGGGGTGATG TGATCCGCGC CATCCGTGAG CTTAGCTACA AGGGGGGCAA CACTCGCACA GGGGCTGCAA TTCTCCATGT

IGE308_RefSequence #3601 GGCTGACCAT GTCTTCCTGC CCCAGCTGGC CCGACCTGGT GTCCCCAAGG TCTGCATCCT GATCACAGAC GGGAAGTCCC
60010743-ITX-00001_Contig #3601 GGCTGACCAT GTCTTCCTGC CCCAGCTGGC CCGACCTGGT GTCCCCAAGG TCTGCATCCT GATCACAGAC GGGAAGTCCC

#3601 GGCTGACCAT GTCTTCCTGC CCCAGCTGGC CCGACCTGGT GTCCCCAAGG TCTGCATCCT GATCACAGAC GGGAAGTCCC

IGE308_RefSequence #3681 AGGACCTGGT GGACACAGCT GCCCAAAGGC TGAAGGGGCA GGGGGTCAAG CTATTTGCTG TGGGGATCAA GAATGCTGAC
60010743-ITX-00001_Contig #3681 AGGACCTGGT GGACACAGCT GCCCAAAGGC TGAAGGGGCA GGGGGTCAAG CTATTTGCTG TGGGGATCAA GAATGCTGAC

#3681 AGGACCTGGT GGACACAGCT GCCCAAAGGC TGAAGGGGCA GGGGGTCAAG CTATTTGCTG TGGGGATCAA GAATGCTGAC

IGE308_RefSequence #3761 CCTGAGGAGC TGAAGCGAGT TGCCTCACAG CCCACCAGTG ACTTCTTCTT CTTCGTCAAT GACTTCAGCA TCTTGAGGAC
60010743-ITX-00001_Contig #3761 CCTGAGGAGC TGAAGCGAGT TGCCTCACAG CCCACCAGTG ACTTCTTCTT CTTCGTCAAT GACTTCAGCA TCTTGAGGAC

#3761 CCTGAGGAGC TGAAGCGAGT TGCCTCACAG CCCACCAGTG ACTTCTTCTT CTTCGTCAAT GACTTCAGCA TCTTGAGGAC

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #3841 ACTACTGCCC CTCGTTTCCC GGAGAGTGTG CACGACTGCT GGTGGCGTGC CTGTGACCCG ACCTCCGGAT GACTCGACCT
60010743-ITX-00001_Contig #3841 ACTACTGCCC CTCGTTTCCC GGAGAGTGTG CACGACTGCT GGTGGCGTGC CTGTGACCCG ACCTCCGGAT GACTCGACCT

#3841 ACTACTGCCC CTCGTTTCCC GGAGAGTGTG CACGACTGCT GGTGGCGTGC CTGTGACCCG ACCTCCGGAT GACTCGACCT

IGE308_RefSequence #3921 CTGCTCCACG AGACCTGGTG CTGTCTGAGC CAAGCAGCCA ATCCTTGAGA GTACAGTGGA CAGCGGCCAG TGGCCCTGTG
60010743-ITX-00001_Contig #3921 CTGCTCCACG AGACCTGGTG CTGTCTGAGC CAAGCAGCCA ATCCTTGAGA GTACAGTGGA CAGCGGCCAG TGGCCCTGTG

#3921 CTGCTCCACG AGACCTGGTG CTGTCTGAGC CAAGCAGCCA ATCCTTGAGA GTACAGTGGA CAGCGGCCAG TGGCCCTGTG

IGE308_RefSequence #4001 ACTGGCTACA AGGTCCAGTA CACTCCTCTG ACGGGGCTGG GACAGCCACT GCCGAGTGAG CGGCAGGAGG TGAACGTCCC
60010743-ITX-00001_Contig #4001 ACTGGCTACA AGGTCCAGTA CACTCCTCTG ACGGGGCTGG GACAGCCACT GCCGAGTGAG CGGCAGGAGG TGAACGTCCC

#4001 ACTGGCTACA AGGTCCAGTA CACTCCTCTG ACGGGGCTGG GACAGCCACT GCCGAGTGAG CGGCAGGAGG TGAACGTCCC

IGE308_RefSequence #4081 AGCTGGTGAG ACCAGTGTGC GGCTGCGGGG TCTCCGGCCA CTGACCGAGT ACCAAGTGAC TGTGATTGCC CTCTACGCCA
60010743-ITX-00001_Contig #4081 AGCTGGTGAG ACCAGTGTGC GGCTGCGGGG TCTCCGGCCA CTGACCGAGT ACCAAGTGAC TGTGATTGCC CTCTACGCCA

#4081 AGCTGGTGAG ACCAGTGTGC GGCTGCGGGG TCTCCGGCCA CTGACCGAGT ACCAAGTGAC TGTGATTGCC CTCTACGCCA

IGE308_RefSequence #4161 ACAGCATCGG GGAGGCTGTG AGCGGGACAG CTCGGACCAC TGCCCTAGAA GGGCCGGAAC TGACCATCCA GAATACCACA
60010743-ITX-00001_Contig #4161 ACAGCATCGG GGAGGCTGTG AGCGGGACAG CTCGGACCAC TGCCCTAGAA GGGCCGGAAC TGACCATCCA GAATACCACA

#4161 ACAGCATCGG GGAGGCTGTG AGCGGGACAG CTCGGACCAC TGCCCTAGAA GGGCCGGAAC TGACCATCCA GAATACCACA

IGE308_RefSequence #4241 GCCCACAGCC TCCTGGTGGC CTGGCGGAGT GTGCCAGGTG CCACTGGCTA CCGTGTGACA TGGCGGGTCC TCAGTGGTGG
60010743-ITX-00001_Contig #4241 GCCCACAGCC TCCTGGTGGC CTGGCGGAGT GTGCCAGGTG CCACTGGCTA CCGTGTGACA TGGCGGGTCC TCAGTGGTGG

#4241 GCCCACAGCC TCCTGGTGGC CTGGCGGAGT GTGCCAGGTG CCACTGGCTA CCGTGTGACA TGGCGGGTCC TCAGTGGTGG

IGE308_RefSequence #4321 GCCCACACAG CAGCAGGAGC TGGGCCCTGG GCAGGGTTCA GTGTTGCTGC GTGACTTGGA GCCTGGCACG GACTATGAGG
60010743-ITX-00001_Contig #4321 GCCCACACAG CAGCAGGAGC TGGGCCCTGG GCAGGGTTCA GTGTTGCTGC GTGACTTGGA GCCTGGCACG GACTATGAGG

#4321 GCCCACACAG CAGCAGGAGC TGGGCCCTGG GCAGGGTTCA GTGTTGCTGC GTGACTTGGA GCCTGGCACG GACTATGAGG

IGE308_RefSequence #4401 TGACCGTGAG CACCCTATTT GGCCGCAGTG TGGGGCCCGC CACTTCCCTG ATGGCTCGCA CTGACGCTTC TGTTGAGCAG
60010743-ITX-00001_Contig #4401 TGACCGTGAG CACCCTATTT GGCCGCAGTG TGGGGCCCGC CACTTCCCTG ATGGCTCGCA CTGACGCTTC TGTTGAGCAG

#4401 TGACCGTGAG CACCCTATTT GGCCGCAGTG TGGGGCCCGC CACTTCCCTG ATGGCTCGCA CTGACGCTTC TGTTGAGCAG

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #4481 ACCCTGCGCC CGGTCATCCT GGGCCCCACA TCCATCCTCC TTTCCTGGAA CTTGGTGCCT GAGGCCCGTG GCTACCGGTT
60010743-ITX-00001_Contig #4481 ACCCTGCGCC CGGTCATCCT GGGCCCCACA TCCATCCTCC TTTCCTGGAA CTTGGTGCCT GAGGCCCGTG GCTACCGGTT

#4481 ACCCTGCGCC CGGTCATCCT GGGCCCCACA TCCATCCTCC TTTCCTGGAA CTTGGTGCCT GAGGCCCGTG GCTACCGGTT

IGE308_RefSequence #4561 GGAATGGCGG CGTGAGACTG GCTTGGAGCC ACCGCAGAAG GTGGTACTGC CCTCTGATGT GACCCGCTAC CAGTTGGATG
60010743-ITX-00001_Contig #4561 GGAATGGCGG CGTGAGACTG GCTTGGAGCC ACCGCAGAAG GTGGTACTGC CCTCTGATGT GACCCGCTAC CAGTTGGATG

#4561 GGAATGGCGG CGTGAGACTG GCTTGGAGCC ACCGCAGAAG GTGGTACTGC CCTCTGATGT GACCCGCTAC CAGTTGGATG

IGE308_RefSequence #4641 GGCTGCAGCC GGGCACTGAG TACCGCCTCA CACTCTACAC TCTGCTGGAG GGCCACGAGG TGGCCACCCC TGCAACCGTG
60010743-ITX-00001_Contig #4641 GGCTGCAGCC GGGCACTGAG TACCGCCTCA CACTCTACAC TCTGCTGGAG GGCCACGAGG TGGCCACCCC TGCAACCGTG

#4641 GGCTGCAGCC GGGCACTGAG TACCGCCTCA CACTCTACAC TCTGCTGGAG GGCCACGAGG TGGCCACCCC TGCAACCGTG

IGE308_RefSequence #4721 GTTCCCACTG GACCAGAGCT GCCTGTGAGC CCTGTAACAG ACCTGCAAGC CACCGAGCTG CCCGGGCAGC GGGTGCGAGT
60010743-ITX-00001_Contig #4721 GTTCCCACTG GACCAGAGCT GCCTGTGAGC CCTGTAACAG ACCTGCAAGC CACCGAGCTG CCCGGGCAGC GGGTGCGAGT

#4721 GTTCCCACTG GACCAGAGCT GCCTGTGAGC CCTGTAACAG ACCTGCAAGC CACCGAGCTG CCCGGGCAGC GGGTGCGAGT

IGE308_RefSequence #4801 GTCCTGGAGC CCAGTCCCTG GTGCCACCCA GTACCGCATC ATTGTGCGCA GCACCCAGGG GGTTGAGCGG ACCCTGGTGC
60010743-ITX-00001_Contig #4801 GTCCTGGAGC CCAGTCCCTG GTGCCACCCA GTACCGCATC ATTGTGCGCA GCACCCAGGG GGTTGAGCGG ACCCTGGTGC

#4801 GTCCTGGAGC CCAGTCCCTG GTGCCACCCA GTACCGCATC ATTGTGCGCA GCACCCAGGG GGTTGAGCGG ACCCTGGTGC

IGE308_RefSequence #4881 TTCCTGGGAG TCAGACAGCA TTCGACTTGG ATGACGTTCA GGCTGGGCTT AGCTACACTG TGCGGGTGTC TGCTCGAGTG
60010743-ITX-00001_Contig #4881 TTCCTGGGAG TCAGACAGCA TTCGACTTGG ATGACGTTCA GGCTGGGCTT AGCTACACTG TGCGGGTGTC TGCTCGAGTG

#4881 TTCCTGGGAG TCAGACAGCA TTCGACTTGG ATGACGTTCA GGCTGGGCTT AGCTACACTG TGCGGGTGTC TGCTCGAGTG

IGE308_RefSequence #4961 GGTCCCCGTG AGGGCAGTGC CAGTGTCCTC ACTGTCCGCC GGGAGCCGGA AACTCCACTT GCTGTTCCAG GGCTGCGGGT
60010743-ITX-00001_Contig #4961 GGTCCCCGTG AGGGCAGTGC CAGTGTCCTC ACTGTCCGCC GGGAGCCGGA AACTCCACTT GCTGTTCCAG GGCTGCGGGT

#4961 GGTCCCCGTG AGGGCAGTGC CAGTGTCCTC ACTGTCCGCC GGGAGCCGGA AACTCCACTT GCTGTTCCAG GGCTGCGGGT

IGE308_RefSequence #5041 TGTGGTGTCA GATGCAACGC GAGTGAGGGT GGCCTGGGGA CCCGTCCCTG GAGCCAGTGG ATTTCGGATT AGCTGGAGCA
60010743-ITX-00001_Contig #5041 TGTGGTGTCA GATGCAACGC GAGTGAGGGT GGCCTGGGGA CCCGTCCCTG GAGCCAGTGG ATTTCGGATT AGCTGGAGCA

#5041 TGTGGTGTCA GATGCAACGC GAGTGAGGGT GGCCTGGGGA CCCGTCCCTG GAGCCAGTGG ATTTCGGATT AGCTGGAGCA

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #5121 CAGGCAGTGG TCCGGAGTCC AGCCAGACAC TGCCCCCAGA CTCTACTGCC ACAGACATCA CAGGGCTGCA GCCTGGAACC
60010743-ITX-00001_Contig #5121 CAGGCAGTGG TCCGGAGTCC AGCCAGACAC TGCCCCCAGA CTCTACTGCC ACAGACATCA CAGGGCTGCA GCCTGGAACC

#5121 CAGGCAGTGG TCCGGAGTCC AGCCAGACAC TGCCCCCAGA CTCTACTGCC ACAGACATCA CAGGGCTGCA GCCTGGAACC

IGE308_RefSequence #5201 ACCTACCAGG TGGCTGTGTC GGTACTGCGA GGCAGAGAGG AGGGCCCTGC TGCAGTCATC GTGGCTCGAA CGGACCCACT
60010743-ITX-00001_Contig #5201 ACCTACCAGG TGGCTGTGTC GGTACTGCGA GGCAGAGAGG AGGGCCCTGC TGCAGTCATC GTGGCTCGAA CGGACCCACT

#5201 ACCTACCAGG TGGCTGTGTC GGTACTGCGA GGCAGAGAGG AGGGCCCTGC TGCAGTCATC GTGGCTCGAA CGGACCCACT

IGE308_RefSequence #5281 GGGCCCAGTG AGGACGGTCC ATGTGACTCA GGCCAGCAGC TCATCTGTCA CCATTACCTG GACCAGGGTT CCTGGCGCCA
60010743-ITX-00001_Contig #5281 GGGCCCAGTG AGGACGGTCC ATGTGACTCA GGCCAGCAGC TCATCTGTCA CCATTACCTG GACCAGGGTT CCTGGCGCCA

#5281 GGGCCCAGTG AGGACGGTCC ATGTGACTCA GGCCAGCAGC TCATCTGTCA CCATTACCTG GACCAGGGTT CCTGGCGCCA

IGE308_RefSequence #5361 CAGGATACAG GGTTTCCTGG CACTCAGCCC ACGGCCCAGA GAAATCCCAG TTGGTTTCTG GGGAGGCCAC GGTGGCTGAG
60010743-ITX-00001_Contig #5361 CAGGATACAG GGTTTCCTGG CACTCAGCCC ACGGCCCAGA GAAATCCCAG TTGGTTTCTG GGGAGGCCAC GGTGGCTGAG

#5361 CAGGATACAG GGTTTCCTGG CACTCAGCCC ACGGCCCAGA GAAATCCCAG TTGGTTTCTG GGGAGGCCAC GGTGGCTGAG

IGE308_RefSequence #5441 CTGGATGGAC TGGAGCCAGA TACTGAGTAT ACGGTGCATG TGAGGGCCCA TGTGGCTGGC GTGGATGGGC CCCCTGCCTC
60010743-ITX-00001_Contig #5441 CTGGATGGAC TGGAGCCAGA TACTGAGTAT ACGGTGCATG TGAGGGCCCA TGTGGCTGGC GTGGATGGGC CCCCTGCCTC

#5441 CTGGATGGAC TGGAGCCAGA TACTGAGTAT ACGGTGCATG TGAGGGCCCA TGTGGCTGGC GTGGATGGGC CCCCTGCCTC

IGE308_RefSequence #5521 TGTGGTTGTG AGGACTGCCC CTGAGCCTGT GGGTCGTGTG TCGAGGCTGC AGATCCTCAA TGCTTCCAGC GACGTTCTAC
60010743-ITX-00001_Contig #5521 TGTGGTTGTG AGGACTGCCC CTGAGCCTGT GGGTCGTGTG TCGAGGCTGC AGATCCTCAA TGCTTCCAGC GACGTTCTAC

#5521 TGTGGTTGTG AGGACTGCCC CTGAGCCTGT GGGTCGTGTG TCGAGGCTGC AGATCCTCAA TGCTTCCAGC GACGTTCTAC

IGE308_RefSequence #5601 GGATCACCTG GGTAGGGGTC ACTGGAGCCA CAGCTTACAG ACTGGCCTGG GGCCGGAGTG AAGGCGGCCC CATGAGGCAC
60010743-ITX-00001_Contig #5601 GGATCACCTG GGTAGGGGTC ACTGGAGCCA CAGCTTACAG ACTGGCCTGG GGCCGGAGTG AAGGCGGCCC CATGAGGCAC

#5601 GGATCACCTG GGTAGGGGTC ACTGGAGCCA CAGCTTACAG ACTGGCCTGG GGCCGGAGTG AAGGCGGCCC CATGAGGCAC

IGE308_RefSequence #5681 CAGATACTCC CAGGAAACAC AGACTCTGCA GAGATCCGGG GTCTCGAAGG TGGAGTCAGC TACTCAGTGC GAGTGACTGC
60010743-ITX-00001_Contig #5681 CAGATACTCC CAGGAAACAC AGACTCTGCA GAGATCCGGG GTCTCGAAGG TGGAGTCAGC TACTCAGTGC GAGTGACTGC

#5681 CAGATACTCC CAGGAAACAC AGACTCTGCA GAGATCCGGG GTCTCGAAGG TGGAGTCAGC TACTCAGTGC GAGTGACTGC

IGE308_RefSequence #5761 ACTTGTCGGG GACCGCGAGG GCACACCTGT CTCCATTGTT GTCACTACGC CGCCTGAGGC TCCGCCAGCC CTGGGGACGC
60010743-ITX-00001_Contig #5761 ACTTGTCGGG GACCGCGAGG GCACACCTGT CTCCATTGTT GTCACTACGC CGCCTGAGGC TCCGCCAGCC CTGGGGACGC

#5761 ACTTGTCGGG GACCGCGAGG GCACACCTGT CTCCATTGTT GTCACTACGC CGCCTGAGGC TCCGCCAGCC CTGGGGACGC

IGE308_RefSequence #5841 TTCACGTGGT GCAGCGCGGG GAGCACTCGC TGAGGCTGCG CTGGGAGCCG GTGCCCAGAG CGCAGGGCTT CCTTCTGCAC
60010743-ITX-00001_Contig #5841 TTCACGTGGT GCAGCGCGGG GAGCACTCGC TGAGGCTGCG CTGGGAGCCG GTGCCCAGAG CGCAGGGCTT CCTTCTGCAC

#5841 TTCACGTGGT GCAGCGCGGG GAGCACTCGC TGAGGCTGCG CTGGGAGCCG GTGCCCAGAG CGCAGGGCTT CCTTCTGCAC

IGE308_RefSequence #5921 TGGCAACCTG AGGGTGGCCA GGAACAGTCC CGGGTCCTGG GGCCCGAGCT CAGCAGCTAT CACCTGGACG GGCTGGAGCC
60010743-ITX-00001_Contig #5921 TGGCAACCTG AGGGTGGCCA GGAACAGTCC CGGGTCCTGG GGCCCGAGCT CAGCAGCTAT CACCTGGACG GGCTGGAGCC

#5921 TGGCAACCTG AGGGTGGCCA GGAACAGTCC CGGGTCCTGG GGCCCGAGCT CAGCAGCTAT CACCTGGACG GGCTGGAGCC

IGE308_RefSequence #6001 AGCGACACAG TACCGCGTGA GGCTGAGTGT CCTAGGGCCG GCTGGAGAAG GGCCCTCTGC AGAGGTGACT GCGCGCACTG
60010743-ITX-00001_Contig #6001 AGCGACACAG TACCGCGTGA GGCTGAGTGT CCTAGGGCCG GCTGGAGAAG GGCCCTCTGC AGAGGTGACT GCGCGCACTG

#6001 AGCGACACAG TACCGCGTGA GGCTGAGTGT CCTAGGGCCG GCTGGAGAAG GGCCCTCTGC AGAGGTGACT GCGCGCACTG

IGE308_RefSequence #6081 AGTCACCTCG TGTTCCAAGC ATTGAACTAC GTGTGGTGGA CACCTCGATC GACTCGGTGA CTTTGGCCTG GACTCCAGTG
60010743-ITX-00001_Contig #6081 AGTCACCTCG TGTTCCAAGC ATTGAACTAC GTGTGGTGGA CACCTCGATC GACTCGGTGA CTTTGGCCTG GACTCCAGTG

#6081 AGTCACCTCG TGTTCCAAGC ATTGAACTAC GTGTGGTGGA CACCTCGATC GACTCGGTGA CTTTGGCCTG GACTCCAGTG

IGE308_RefSequence #6161 TCCAGGGCAT CCAGCTACAT CCTATCCTGG CGGCCACTCA GAGGCCCTGG CCAGGAAGTG CCTGGGTCCC CGCAGACACT
60010743-ITX-00001_Contig #6161 TCCAGGGCAT CCAGCTACAT CCTATCCTGG CGGCCACTCA GAGGCCCTGG CCAGGAAGTG CCTGGGTCCC CGCAGACACT

#6161 TCCAGGGCAT CCAGCTACAT CCTATCCTGG CGGCCACTCA GAGGCCCTGG CCAGGAAGTG CCTGGGTCCC CGCAGACACT

IGE308_RefSequence #6241 TCCAGGGATC TCAAGCTCCC AGCGGGTGAC AGGGCTAGAG CCTGGCGTCT CTTACATCTT CTCCCTGACG CCTGTCCTGG
60010743-ITX-00001_Contig #6241 TCCAGGGATC TCAAGCTCCC AGCGGGTGAC AGGGCTAGAG CCTGGCGTCT CTTACATCTT CTCCCTGACG CCTGTCCTGG

#6241 TCCAGGGATC TCAAGCTCCC AGCGGGTGAC AGGGCTAGAG CCTGGCGTCT CTTACATCTT CTCCCTGACG CCTGTCCTGG

IGE308_RefSequence #6321 ATGGTGTGCG GGGTCCTGAG GCATCTGTCA CACAGACGCC AGTGTGCCCC CGTGGCCTGG CGGATGTGGT GTTCCTACCA
60010743-ITX-00001_Contig #6321 ATGGTGTGCG GGGTCCTGAG GCATCTGTCA CACAGACGCC AGTGTGCCCC CGTGGCCTGG CGGATGTGGT GTTCCTACCA

#6321 ATGGTGTGCG GGGTCCTGAG GCATCTGTCA CACAGACGCC AGTGTGCCCC CGTGGCCTGG CGGATGTGGT GTTCCTACCA

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #6401 CATGCCACTC AAGACAATGC TCACCGTGCG GAGGCTACGA GGAGGGTCCT GGAGCGTCTG GTGTTGGCAC TTGGGCCTCT
60010743-ITX-00001_Contig #6401 CATGCCACTC AAGACAATGC TCACCGTGCG GAGGCTACGA GGAGGGTCCT GGAGCGTCTG GTGTTGGCAC TTGGGCCTCT

#6401 CATGCCACTC AAGACAATGC TCACCGTGCG GAGGCTACGA GGAGGGTCCT GGAGCGTCTG GTGTTGGCAC TTGGGCCTCT

IGE308_RefSequence #6481 TGGGCCACAG GCAGTTCAGG TTGGCCTGCT GTCTTACAGT CATCGGCCCT CCCCACTGTT CCCACTGAAT GGCTCCCATG
60010743-ITX-00001_Contig #6481 TGGGCCACAG GCAGTTCAGG TTGGCCTGCT GTCTTACAGT CATCGGCCCT CCCCACTGTT CCCACTGAAT GGCTCCCATG

#6481 TGGGCCACAG GCAGTTCAGG TTGGCCTGCT GTCTTACAGT CATCGGCCCT CCCCACTGTT CCCACTGAAT GGCTCCCATG

IGE308_RefSequence #6561 ACCTTGGCAT TATCTTGCAA AGGATCCGTG ACATGCCCTA CATGGACCCA AGTGGGAACA ACCTGGGCAC AGCCGTGGTC
60010743-ITX-00001_Contig #6561 ACCTTGGCAT TATCTTGCAA AGGATCCGTG ACATGCCCTA CATGGACCCA AGTGGGAACA ACCTGGGCAC AGCCGTGGTC

#6561 ACCTTGGCAT TATCTTGCAA AGGATCCGTG ACATGCCCTA CATGGACCCA AGTGGGAACA ACCTGGGCAC AGCCGTGGTC

IGE308_RefSequence #6641 ACAGCTCACA GATACATGTT GGCACCAGAT GCTCCTGGGC GCCGCCAGCA CGTACCAGGG GTGATGGTTC TGCTAGTGGA
60010743-ITX-00001_Contig #6641 ACAGCTCACA GATACATGTT GGCACCAGAT GCTCCTGGGC GCCGCCAGCA CGTACCAGGG GTGATGGTTC TGCTAGTGGA

#6641 ACAGCTCACA GATACATGTT GGCACCAGAT GCTCCTGGGC GCCGCCAGCA CGTACCAGGG GTGATGGTTC TGCTAGTGGA

IGE308_RefSequence #6721 TGAACCCTTG AGAGGTGACA TATTCAGCCC CATCCGTGAG GCCCAGGCTT CTGGGCTTAA TGTGGTGATG TTGGGAATGG
60010743-ITX-00001_Contig #6721 TGAACCCTTG AGAGGTGACA TATTCAGCCC CATCCGTGAG GCCCAGGCTT CTGGGCTTAA TGTGGTGATG TTGGGAATGG

#6721 TGAACCCTTG AGAGGTGACA TATTCAGCCC CATCCGTGAG GCCCAGGCTT CTGGGCTTAA TGTGGTGATG TTGGGAATGG

IGE308_RefSequence #6801 CTGGAGCGGA CCCAGAGCAG CTGCGTCGCT TGGCGCCGGG TATGGACTCT GTCCAGACCT TCTTCGCCGT GGATGATGGG
60010743-ITX-00001_Contig #6801 CTGGAGCGGA CCCAGAGCAG CTGCGTCGCT TGGCGCCGGG TATGGACTCT GTCCAGACCT TCTTCGCCGT GGATGATGGG

#6801 CTGGAGCGGA CCCAGAGCAG CTGCGTCGCT TGGCGCCGGG TATGGACTCT GTCCAGACCT TCTTCGCCGT GGATGATGGG

IGE308_RefSequence #6881 CCAAGCCTGG ACCAGGCAGT CAGTGGTCTG GCCACAGCCC TGTGTCAGGC ATCCTTCACT ACTCAGCCCC GGCCAGAGCC
60010743-ITX-00001_Contig #6881 CCAAGCCTGG ACCAGGCAGT CAGTGGTCTG GCCACAGCCC TGTGTCAGGC ATCCTTCACT ACTCAGCCCC GGCCAGAGCC

#6881 CCAAGCCTGG ACCAGGCAGT CAGTGGTCTG GCCACAGCCC TGTGTCAGGC ATCCTTCACT ACTCAGCCCC GGCCAGAGCC

IGE308_RefSequence #6961 CTGCCCAGTG TATTGTCCAA AGGGCCAGAA GGGGGAACCT GGAGAGATGG GCCTGAGAGG ACAAGTTGGG CCTCCTGGCG
60010743-ITX-00001_Contig #6961 CTGCCCAGTG TATTGTCCAA AGGGCCAGAA GGGGGAACCT GGAGAGATGG GCCTGAGAGG ACAAGTTGGG CCTCCTGGCG

#6961 CTGCCCAGTG TATTGTCCAA AGGGCCAGAA GGGGGAACCT GGAGAGATGG GCCTGAGAGG ACAAGTTGGG CCTCCTGGCG

IGE308_RefSequence #7041 ACCCTGGCCT CCCGGGCAGG ACCGGTGCTC CCGGCCCCCA GGGGCCCCCT GGAAGTGCCA CTGCCAAGGG CGAGAGGGGC
60010743-ITX-00001_Contig #7041 ACCCTGGCCT CCCGGGCAGG ACCGGTGCTC CCGGCCCCCA GGGGCCCCCT GGAAGTGCCA CTGCCAAGGG CGAGAGGGGC

#7041 ACCCTGGCCT CCCGGGCAGG ACCGGTGCTC CCGGCCCCCA GGGGCCCCCT GGAAGTGCCA CTGCCAAGGG CGAGAGGGGC

IGE308_RefSequence #7121 TTCCCTGGAG CAGATGGGCG TCCAGGCAGC CCTGGCCGCG CCGGGAATCC TGGGACCCCT GGAGCCCCTG GCCTAAAGGG
60010743-ITX-00001_Contig #7121 TTCCCTGGAG CAGATGGGCG TCCAGGCAGC CCTGGCCGCG CCGGGAATCC TGGGACCCCT GGAGCCCCTG GCCTAAAGGG

#7121 TTCCCTGGAG CAGATGGGCG TCCAGGCAGC CCTGGCCGCG CCGGGAATCC TGGGACCCCT GGAGCCCCTG GCCTAAAGGG

IGE308_RefSequence #7201 CTCTCCAGGG TTGCCTGGCC CTCGTGGGGA CCCGGGAGAG CGAGGACCTC GAGGCCCAAA GGGGGAGCCG GGGGCTCCCG
60010743-ITX-00001_Contig #7201 CTCTCCAGGG TTGCCTGGCC CTCGTGGGGA CCCGGGAGAG CGAGGACCTC GAGGCCCAAA GGGGGAGCCG GGGGCTCCCG

#7201 CTCTCCAGGG TTGCCTGGCC CTCGTGGGGA CCCGGGAGAG CGAGGACCTC GAGGCCCAAA GGGGGAGCCG GGGGCTCCCG

IGE308_RefSequence #7281 GACAAGTCAT CGGAGGTGAA GGACCTGGGC TTCCTGGGCG GAAAGGGGAC CCTGGACCAT CGGGCCCCCC TGGACCTCGT
60010743-ITX-00001_Contig #7281 GACAAGTCAT CGGAGGTGAA GGACCTGGGC TTCCTGGGCG GAAAGGGGAC CCTGGACCAT CGGGCCCCCC TGGACCTCGT

#7281 GACAAGTCAT CGGAGGTGAA GGACCTGGGC TTCCTGGGCG GAAAGGGGAC CCTGGACCAT CGGGCCCCCC TGGACCTCGT

IGE308_RefSequence #7361 GGACCACTGG GGGACCCAGG ACCCCGTGGC CCCCCAGGGC TTCCTGGAAC AGCCATGAAG GGTGACAAAG GCGATCGTGG
60010743-ITX-00001_Contig #7361 GGACCACTGG GGGACCCAGG ACCCCGTGGC CCCCCAGGGC TTCCTGGAAC AGCCATGAAG GGTGACAAAG GCGATCGTGG

#7361 GGACCACTGG GGGACCCAGG ACCCCGTGGC CCCCCAGGGC TTCCTGGAAC AGCCATGAAG GGTGACAAAG GCGATCGTGG

IGE308_RefSequence #7441 GGAGCGGGGT CCCCCTGGAC CAGGTGAAGG TGGCATTGCT CCTGGGGAGC CTGGGCTGCC GGGTCTTCCC GGAAGCCCTG
60010743-ITX-00001_Contig #7441 GGAGCGGGGT CCCCCTGGAC CAGGTGAAGG TGGCATTGCT CCTGGGGAGC CTGGGCTGCC GGGTCTTCCC GGAAGCCCTG

#7441 GGAGCGGGGT CCCCCTGGAC CAGGTGAAGG TGGCATTGCT CCTGGGGAGC CTGGGCTGCC GGGTCTTCCC GGAAGCCCTG

IGE308_RefSequence #7521 GACCCCAAGG CCCCGTTGGC CCCCCTGGAA AGAAAGGAGA AAAAGGTGAC TCTGAGGATG GAGCTCCAGG CCTCCCAGGA
60010743-ITX-00001_Contig #7521 GACCCCAAGG CCCCGTTGGC CCCCCTGGAA AGAAAGGAGA AAAAGGTGAC TCTGAGGATG GAGCTCCAGG CCTCCCAGGA

#7521 GACCCCAAGG CCCCGTTGGC CCCCCTGGAA AGAAAGGAGA AAAAGGTGAC TCTGAGGATG GAGCTCCAGG CCTCCCAGGA

IGE308_RefSequence #7601 CAACCTGGGT CTCCGGGTGA GCAGGGCCCA CGGGGACCTC CTGGAGCTAT TGGCCCCAAA GGTGACCGGG GCTTTCCAGG
60010743-ITX-00001_Contig #7601 CAACCTGGGT CTCCGGGTGA GCAGGGCCCA CGGGGACCTC CTGGAGCTAT TGGCCCCAAA GGTGACCGGG GCTTTCCAGG

#7601 CAACCTGGGT CTCCGGGTGA GCAGGGCCCA CGGGGACCTC CTGGAGCTAT TGGCCCCAAA GGTGACCGGG GCTTTCCAGG

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #7681 GCCCCTGGGT GAGGCTGGAG AGAAGGGCGA ACGTGGACCC CCAGGCCCAG CGGGATCCCG GGGGCTGCCA GGGGTTGCTG
60010743-ITX-00001_Contig #7681 GCCCCTGGGT GAGGCTGGAG AGAAGGGCGA ACGTGGACCC CCAGGCCCAG CGGGATCCCG GGGGCTGCCA GGGGTTGCTG

#7681 GCCCCTGGGT GAGGCTGGAG AGAAGGGCGA ACGTGGACCC CCAGGCCCAG CGGGATCCCG GGGGCTGCCA GGGGTTGCTG

IGE308_RefSequence #7761 GACGTCCTGG AGCCAAGGGT CCTGAAGGGC CACCAGGACC CACTGGCCGC CAAGGAGAGA AGGGGGAGCC TGGTCGCCCT
60010743-ITX-00001_Contig #7761 GACGTCCTGG AGCCAAGGGT CCTGAAGGGC CACCAGGACC CACTGGCCGC CAAGGAGAGA AGGGGGAGCC TGGTCGCCCT

#7761 GACGTCCTGG AGCCAAGGGT CCTGAAGGGC CACCAGGACC CACTGGCCGC CAAGGAGAGA AGGGGGAGCC TGGTCGCCCT

IGE308_RefSequence #7841 GGGGACCCTG CAGTGGTGGG ACCTGCTGTT GCTGGACCCA AAGGAGAAAA GGGAGATGTG GGGCCCGCTG GGCCCAGAGG
60010743-ITX-00001_Contig #7841 GGGGACCCTG CAGTGGTGGG ACCTGCTGTT GCTGGACCCA AAGGAGAAAA GGGAGATGTG GGGCCCGCTG GGCCCAGAGG

#7841 GGGGACCCTG CAGTGGTGGG ACCTGCTGTT GCTGGACCCA AAGGAGAAAA GGGAGATGTG GGGCCCGCTG GGCCCAGAGG

IGE308_RefSequence #7921 AGCTACCGGA GTCCAAGGGG AACGGGGCCC ACCCGGCTTG GTTCTTCCTG GAGACCCTGG CCCCAAGGGA GACCCTGGAG
60010743-ITX-00001_Contig #7921 AGCTACCGGA GTCCAAGGGG AACGGGGCCC ACCCGGCTTG GTTCTTCCTG GAGACCCTGG CCCCAAGGGA GACCCTGGAG

#7921 AGCTACCGGA GTCCAAGGGG AACGGGGCCC ACCCGGCTTG GTTCTTCCTG GAGACCCTGG CCCCAAGGGA GACCCTGGAG

IGE308_RefSequence #8001 ACCGGGGTCC CATTGGCCTT ACTGGCAGAG CAGGACCCCC AGGTGACTCA GGGCCTCCTG GAGAGAAGGG AGACCCTGGG
60010743-ITX-00001_Contig #8001 ACCGGGGTCC CATTGGCCTT ACTGGCAGAG CAGGACCCCC AGGTGACTCA GGGCCTCCTG GAGAGAAGGG AGACCCTGGG

#8001 ACCGGGGTCC CATTGGCCTT ACTGGCAGAG CAGGACCCCC AGGTGACTCA GGGCCTCCTG GAGAGAAGGG AGACCCTGGG

IGE308_RefSequence #8081 CGGCCTGGCC CCCCAGGACC TGTTGGCCCC CGAGGACGAG ATGGTGAAGT TGGAGAGAAA GGTGACGAGG GTCCTCCGGG
60010743-ITX-00001_Contig #8081 CGGCCTGGCC CCCCAGGACC TGTTGGCCCC CGAGGACGAG ATGGTGAAGT TGGAGAGAAA GGTGACGAGG GTCCTCCGGG

#8081 CGGCCTGGCC CCCCAGGACC TGTTGGCCCC CGAGGACGAG ATGGTGAAGT TGGAGAGAAA GGTGACGAGG GTCCTCCGGG

IGE308_RefSequence #8161 TGACCCGGGT TTGCCTGGAA AAGCAGGCGA GCGTGGCCTT CGGGGGGCAC CTGGAGTTCG GGGGCCTGTG GGTGAAAAGG
60010743-ITX-00001_Contig #8161 TGACCCGGGT TTGCCTGGAA AAGCAGGCGA GCGTGGCCTT CGGGGGGCAC CTGGAGTTCG GGGGCCTGTG GGTGAAAAGG

#8161 TGACCCGGGT TTGCCTGGAA AAGCAGGCGA GCGTGGCCTT CGGGGGGCAC CTGGAGTTCG GGGGCCTGTG GGTGAAAAGG

IGE308_RefSequence #8241 GAGACCAGGG AGATCCTGGA GAGGATGGAC GAAATGGCAG CCCTGGATCA TCTGGACCCA AGGGTGACCG TGGGGAGCCG
60010743-ITX-00001_Contig #8241 GAGACCAGGG AGATCCTGGA GAGGATGGAC GAAATGGCAG CCCTGGATCA TCTGGACCCA AGGGTGACCG TGGGGAGCCG

#8241 GAGACCAGGG AGATCCTGGA GAGGATGGAC GAAATGGCAG CCCTGGATCA TCTGGACCCA AGGGTGACCG TGGGGAGCCG

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #8321 GGTCCCCCAG GACCCCCGGG ACGGCTGGTA GACACAGGAC CTGGAGCCAG AGAGAAGGGA GAGCCTGGGG ACCGCGGACA
60010743-ITX-00001_Contig #8321 GGTCCCCCAG GACCCCCGGG ACGGCTGGTA GACACAGGAC CTGGAGCCAG AGAGAAGGGA GAGCCTGGGG ACCGCGGACA

#8321 GGTCCCCCAG GACCCCCGGG ACGGCTGGTA GACACAGGAC CTGGAGCCAG AGAGAAGGGA GAGCCTGGGG ACCGCGGACA

IGE308_RefSequence #8401 AGAGGGTCCT CGAGGGCCCA AGGGTGATCC TGGCCTCCCT GGAGCCCCTG GGGAAAGGGG CATTGAAGGG TTTCGGGGAC
60010743-ITX-00001_Contig #8401 AGAGGGTCCT CGAGGGCCCA AGGGTGATCC TGGCCTCCCT GGAGCCCCTG GGGAAAGGGG CATTGAAGGG TTTCGGGGAC

#8401 AGAGGGTCCT CGAGGGCCCA AGGGTGATCC TGGCCTCCCT GGAGCCCCTG GGGAAAGGGG CATTGAAGGG TTTCGGGGAC

IGE308_RefSequence #8481 CCCCAGGCCC ACAGGGGGAC CCAGGTGTCC GAGGCCCAGC AGGAGAAAAG GGTGACCGGG GTCCCCCTGG GCTGGATGGC
60010743-ITX-00001_Contig #8481 CCCCAGGCCC ACAGGGGGAC CCAGGTGTCC GAGGCCCAGC AGGAGAAAAG GGTGACCGGG GTCCCCCTGG GCTGGATGGC

#8481 CCCCAGGCCC ACAGGGGGAC CCAGGTGTCC GAGGCCCAGC AGGAGAAAAG GGTGACCGGG GTCCCCCTGG GCTGGATGGC

IGE308_RefSequence #8561 CGGAGCGGAC TGGATGGGAA ACCAGGAGCC GCTGGGCCCT CTGGGCCGAA TGGTGCTGCA GGCAAAGCTG GGGACCCAGG
60010743-ITX-00001_Contig #8561 CGGAGCGGAC TGGATGGGAA ACCAGGAGCC GCTGGGCCCT CTGGGCCGAA TGGTGCTGCA GGCAAAGCTG GGGACCCAGG

#8561 CGGAGCGGAC TGGATGGGAA ACCAGGAGCC GCTGGGCCCT CTGGGCCGAA TGGTGCTGCA GGCAAAGCTG GGGACCCAGG

IGE308_RefSequence #8641 GAGAGACGGG CTTCCAGGCC TCCGTGGAGA ACAAGGCCTC CCTGGCCCCT CTGGTCCCCC TGGATTACCG GGAAAGCCAG
60010743-ITX-00001_Contig #8641 GAGAGACGGG CTTCCAGGCC TCCGTGGAGA ACAAGGCCTC CCTGGCCCCT CTGGTCCCCC TGGATTACCG GGAAAGCCAG

#8641 GAGAGACGGG CTTCCAGGCC TCCGTGGAGA ACAAGGCCTC CCTGGCCCCT CTGGTCCCCC TGGATTACCG GGAAAGCCAG

IGE308_RefSequence #8721 GCGAGGATGG GAAACCTGGC CTGAATGGAA AAAACGGAGA ACCTGGGGAC CCTGGAGAAG ACGGGAGGAA GGGAGAGAAA
60010743-ITX-00001_Contig #8721 GCGAGGATGG GAAACCTGGC CTGAATGGAA AAAACGGAGA ACCTGGGGAC CCTGGAGAAG ACGGGAGGAA GGGAGAGAAA

#8721 GCGAGGATGG GAAACCTGGC CTGAATGGAA AAAACGGAGA ACCTGGGGAC CCTGGAGAAG ACGGGAGGAA GGGAGAGAAA

IGE308_RefSequence #8801 GGAGATTCAG GCGCCTCTGG GAGAGAAGGT CGTGATGGCC CCAAGGGTGA GCGTGGAGCT CCTGGTATCC TTGGACCCCA
60010743-ITX-00001_Contig #8801 GGAGATTCAG GCGCCTCTGG GAGAGAAGGT CGTGATGGCC CCAAGGGTGA GCGTGGAGCT CCTGGTATCC TTGGACCCCA

#8801 GGAGATTCAG GCGCCTCTGG GAGAGAAGGT CGTGATGGCC CCAAGGGTGA GCGTGGAGCT CCTGGTATCC TTGGACCCCA

IGE308_RefSequence #8881 GGGGCCTCCA GGCCTCCCAG GGCCAGTGGG CCCTCCTGGC CAGGGTTTTC CTGGTGTCCC AGGAGGCACG GGCCCCAAGG
60010743-ITX-00001_Contig #8881 GGGGCCTCCA GGCCTCCCAG GGCCAGTGGG CCCTCCTGGC CAGGGTTTTC CTGGTGTCCC AGGAGGCACG GGCCCCAAGG

#8881 GGGGCCTCCA GGCCTCCCAG GGCCAGTGGG CCCTCCTGGC CAGGGTTTTC CTGGTGTCCC AGGAGGCACG GGCCCCAAGG


IGE308_RefSequence #8961 GTGACCGTGG GGAGACTGGA TCCAAAGGGG AGCAGGGCCT CCCTGGAGAG CGTGGCCTGC GAGGAGAGCC TGGAAGTGTG
60010743-ITX-00001_Contig #8961 GTGACCGTGG GGAGACTGGA TCCAAAGGGG AGCAGGGCCT CCCTGGAGAG CGTGGCCTGC GAGGAGAGCC TGGAAGTGTG
#8961 GTGACCGTGG GGAGACTGGA TCCAAAGGGG AGCAGGGCCT CCCTGGAGAG CGTGGCCTGC GAGGAGAGCC TGGAAGTGTG
IGE308_RefSequence #9041 CCGAATGTGG ATCGGTTGCT GGAAACTGCT GGCATCAAGG CATCTGCCCT GCGGGAGATC GTGGAGACCT GGGATGAGAG
60010743-ITX-00001_Contig #9041 CCGAATGTGG ATCGGTTGCT GGAAACTGCT GGCATCAAGG CATCTGCCCT GCGGGAGATC GTGGAGACCT GGGATGAGAG
#9041 CCGAATGTGG ATCGGTTGCT GGAAACTGCT GGCATCAAGG CATCTGCCCT GCGGGAGATC GTGGAGACCT GGGATGAGAG
IGE308_RefSequence #9121 CTCTGGTAGC TTCCTGCCTG TGCCCGAACG GCGTCGAGGC CCCAAGGGGG ACTCAGGCGA ACAGGGCCCC CCAGGCAAGG
60010743-ITX-00001_Contig #9121 CTCTGGTAGC TTCCTGCCTG TGCCCGAACG GCGTCGAGGC CCCAAGGGGG ACTCAGGCGA ACAGGGCCCC CCAGGCAAGG
#9121 CTCTGGTAGC TTCCTGCCTG TGCCCGAACG GCGTCGAGGC CCCAAGGGGG ACTCAGGCGA ACAGGGCCCC CCAGGCAAGG
IGE308_RefSequence #9201 AGGGCCCCAT CGGCTTTCCT GGAGAACGCG GGCTGAAGGG CGACCGTGGA GACCCTGGCC CTCAGGGGCC ACCTGGTCTG
60010743-ITX-00001_Contig #9201 AGGGCCCCAT CGGCTTTCCT GGAGAACGCG GGCTGAAGGG CGACCGTGGA GACCCTGGCC CTCAGGGGCC ACCTGGTCTG
#9201 AGGGCCCCAT CGGCTTTCCT GGAGAACGCG GGCTGAAGGG CGACCGTGGA GACCCTGGCC CTCAGGGGCC ACCTGGTCTG
IGE308_RefSequence #9281 GCCCTTGGGG AGAGGGGCCC CCCCGGGCCT TCCGGCCTTG CCGGGGAGCC TGGAAAGCCT GGTATTCCCG GGCTCCCAGG
60010743-ITX-00001_Contig #9281 GCCCTTGGGG AGAGGGGCCC CCCCGGGCCT TCCGGCCTTG CCGGGGAGCC TGGAAAGCCT GGTATTCCCG GGCTCCCAGG
#9281 GCCCTTGGGG AGAGGGGCCC CCCCGGGCCT TCCGGCCTTG CCGGGGAGCC TGGAAAGCCT GGTATTCCCG GGCTCCCAGG
IGE308_RefSequence #9361 CAGGGCTGGG GGTGTGGGAG AGGCAGGAAG GCCAGGAGAG AGGGGAGAAC GGGGAGAGAA AGGAGAACGT GGAGAACAGG
60010743-ITX-00001_Contig #9361 CAGGGCTGGG GGTGTGGGAG AGGCAGGAAG GCCAGGAGAG AGGGGAGAAC GGGGAGAGAA AGGAGAACGT GGAGAACAGG
#9361 CAGGGCTGGG GGTGTGGGAG AGGCAGGAAG GCCAGGAGAG AGGGGAGAAC GGGGAGAGAA AGGAGAACGT GGAGAACAGG
IGE308_RefSequence #9441 GCAGAGATGG CCCTCCTGGA CTCCCTGGAA CCCCTGGGCC CCCCGGACCC CCTGGCCCCA AGGTGTCTGT GGATGAGCCA
60010743-ITX-00001_Contig #9441 GCAGAGATGG CCCTCCTGGA CTCCCTGGAA CCCCTGGGCC CCCCGGACCC CCTGGCCCCA AGGTGTCTGT GGATGAGCCA
#9441 GCAGAGATGG CCCTCCTGGA CTCCCTGGAA CCCCTGGGCC CCCCGGACCC CCTGGCCCCA AGGTGTCTGT GGATGAGCCA
IGE308_RefSequence #9521 GGTCCTGGAC TCTCTGGAGA ACAGGGACCC CCTGGACTCA AGGGTGCTAA GGGGGAGCCG GGCAGCAATG GTGACCAAGG
60010743-ITX-00001_Contig #9521 GGTCCTGGAC TCTCTGGAGA ACAGGGACCC CCTGGACTCA AGGGTGCTAA GGGGGAGCCG GGCAGCAATG GTGACCAAGG
#9521 GGTCCTGGAC TCTCTGGAGA ACAGGGACCC CCTGGACTCA AGGGTGCTAA GGGGGAGCCG GGCAGCAATG GTGACCAAGG

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #9601 TCCCAAAGGA GACAGGGGTG TGCCAGGCAT CAAAGGAGAC CGGGGAGAGC CTGGACCGAG GGGTCAGGAC GGCAACCCGG
60010743-ITX-00001_Contig #9601 TCCCAAAGGA GACAGGGGTG TGCCAGGCAT CAAAGGAGAC CGGGGAGAGC CTGGACCGAG GGGTCAGGAC GGCAACCCGG

#9601 TCCCAAAGGA GACAGGGGTG TGCCAGGCAT CAAAGGAGAC CGGGGAGAGC CTGGACCGAG GGGTCAGGAC GGCAACCCGG

IGE308_RefSequence #9681 GTCTACCAGG AGAGCGTGGT ATGGCTGGGC CTGAAGGGAA GCCGGGTCTG CAGGGTCCAA GAGGCCCCCC TGGCCCAGTG
60010743-ITX-00001_Contig #9681 GTCTACCAGG AGAGCGTGGT ATGGCTGGGC CTGAAGGGAA GCCGGGTCTG CAGGGTCCAA GAGGCCCCCC TGGCCCAGTG

#9681 GTCTACCAGG AGAGCGTGGT ATGGCTGGGC CTGAAGGGAA GCCGGGTCTG CAGGGTCCAA GAGGCCCCCC TGGCCCAGTG

IGE308_RefSequence #9761 GGTGGTCATG GAGACCCTGG ACCACCTGGT GCCCCGGGTC TTGCTGGCCC TGCAGGACCC CAAGGACCTT CTGGCCTGAA
60010743-ITX-00001_Contig #9761 GGTGGTCATG GAGACCCTGG ACCACCTGGT GCCCCGGGTC TTGCTGGCCC TGCAGGACCC CAAGGACCTT CTGGCCTGAA

#9761 GGTGGTCATG GAGACCCTGG ACCACCTGGT GCCCCGGGTC TTGCTGGCCC TGCAGGACCC CAAGGACCTT CTGGCCTGAA

IGE308_RefSequence #9841 GGGGGAGCCT GGAGAGACAG GACCTCCAGG ACGGGGCCTG ACTGGACCTA CTGGAGCTGT GGGACTTCCT GGACCCCCCG
60010743-ITX-00001_Contig #9841 GGGGGAGCCT GGAGAGACAG GACCTCCAGG ACGGGGCCTG ACTGGACCTA CTGGAGCTGT GGGACTTCCT GGACCCCCCG

#9841 GGGGGAGCCT GGAGAGACAG GACCTCCAGG ACGGGGCCTG ACTGGACCTA CTGGAGCTGT GGGACTTCCT GGACCCCCCG

IGE308_RefSequence #9921 GCCCTTCAGG CCTTGTGGGT CCACAGGGGT CTCCAGGTTT GCCTGGACAA GTGGGGGAGA CAGGGAAGCC GGGAGCCCCA
60010743-ITX-00001_Contig #9921 GCCCTTCAGG CCTTGTGGGT CCACAGGGGT CTCCAGGTTT GCCTGGACAA GTGGGGGAGA CAGGGAAGCC GGGAGCCCCA

#9921 GCCCTTCAGG CCTTGTGGGT CCACAGGGGT CTCCAGGTTT GCCTGGACAA GTGGGGGAGA CAGGGAAGCC GGGAGCCCCA

IGE308_RefSequence #10001 GGTCGAGATG GTGCCAGTGG AAAAGATGGA GACAGAGGGA GCCCTGGTGT GCCAGGGTCA CCAGGTCTGC CTGGCCCTGT
60010743-ITX-00001_Contig #10001 GGTCGAGATG GTGCCAGTGG AAAAGATGGA GACAGAGGGA GCCCTGGTGT GCCAGGGTCA CCAGGTCTGC CTGGCCCTGT

#10001 GGTCGAGATG GTGCCAGTGG AAAAGATGGA GACAGAGGGA GCCCTGGTGT GCCAGGGTCA CCAGGTCTGC CTGGCCCTGT

IGE308_RefSequence #10081 CGGACCTAAA GGAGAACCTG GCCCCACGGG GGCCCCTGGA CAGGCTGTGG TCGGGCTCCC TGGAGCAAAG GGAGAGAAGG
60010743-ITX-00001_Contig #10081 CGGACCTAAA GGAGAACCTG GCCCCACGGG GGCCCCTGGA CAGGCTGTGG TCGGGCTCCC TGGAGCAAAG GGAGAGAAGG

#10081 CGGACCTAAA GGAGAACCTG GCCCCACGGG GGCCCCTGGA CAGGCTGTGG TCGGGCTCCC TGGAGCAAAG GGAGAGAAGG

IGE308_RefSequence #10161 GAGCCCCTGG AGGCCTTGCT GGAGACCTGG TGGGTGAGCC GGGAGCCAAA GGTGACCGAG GACTGCCAGG GCCGCGAGGC
60010743-ITX-00001_Contig #10161 GAGCCCCTGG AGGCCTTGCT GGAGACCTGG TGGGTGAGCC GGGAGCCAAA GGTGACCGAG GACTGCCAGG GCCGCGAGGC

#10161 GAGCCCCTGG AGGCCTTGCT GGAGACCTGG TGGGTGAGCC GGGAGCCAAA GGTGACCGAG GACTGCCAGG GCCGCGAGGC

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #10241 GAGAAGGGTG AAGCTGGCCG TGCAGGGGAG CCCGGAGACC CTGGGGAAGA TGGTCAGAAA GGGGCTCCAG GACCCAAAGG
60010743-ITX-00001_Contig #10241 GAGAAGGGTG AAGCTGGCCG TGCAGGGGAG CCCGGAGACC CTGGGGAAGA TGGTCAGAAA GGGGCTCCAG GACCCAAAGG

#10241 GAGAAGGGTG AAGCTGGCCG TGCAGGGGAG CCCGGAGACC CTGGGGAAGA TGGTCAGAAA GGGGCTCCAG GACCCAAAGG

IGE308_RefSequence #10321 TTTCAAGGGT GACCCAGGAG TCGGGGTCCC GGGCTCCCCT GGGCCTCCTG GCCCTCCAGG TGTGAAGGGA GATCTGGGCC
60010743-ITX-00001_Contig #10321 TTTCAAGGGT GACCCAGGAG TCGGGGTCCC GGGCTCCCCT GGGCCTCCTG GCCCTCCAGG TGTGAAGGGA GATCTGGGCC

#10321 TTTCAAGGGT GACCCAGGAG TCGGGGTCCC GGGCTCCCCT GGGCCTCCTG GCCCTCCAGG TGTGAAGGGA GATCTGGGCC

IGE308_RefSequence #10401 TCCCTGGCCT GCCCGGTGCT CCTGGTGTTG TTGGGTTCCC GGGTCAGACA GGCCCTCGAG GAGAGATGGG TCAGCCAGGC
60010743-ITX-00001_Contig #10401 TCCCTGGCCT GCCCGGTGCT CCTGGTGTTG TTGGGTTCCC GGGTCAGACA GGCCCTCGAG GAGAGATGGG TCAGCCAGGC

#10401 TCCCTGGCCT GCCCGGTGCT CCTGGTGTTG TTGGGTTCCC GGGTCAGACA GGCCCTCGAG GAGAGATGGG TCAGCCAGGC

IGE308_RefSequence #10481 CCTAGTGGAG AGCGGGGTCT GGCAGGCCCC CCAGGGAGAG AAGGAATCCC AGGACCCCTG GGGCCACCTG GACCACCGGG
60010743-ITX-00001_Contig #10481 CCTAGTGGAG AGCGGGGTCT GGCAGGCCCC CCAGGGAGAG AAGGAATCCC AGGACCCCTG GGGCCACCTG GACCACCGGG

#10481 CCTAGTGGAG AGCGGGGTCT GGCAGGCCCC CCAGGGAGAG AAGGAATCCC AGGACCCCTG GGGCCACCTG GACCACCGGG

IGE308_RefSequence #10561 GTCAGTGGGA CCACCTGGGG CCTCTGGACT CAAAGGAGAC AAGGGAGACC CTGGAGTAGG GCTGCCTGGG CCCCGAGGCG
60010743-ITX-00001_Contig #10561 GTCAGTGGGA CCACCTGGGG CCTCTGGACT CAAAGGAGAC AAGGGAGACC CTGGAGTAGG GCTGCCTGGG CCCCGAGGCG

#10561 GTCAGTGGGA CCACCTGGGG CCTCTGGACT CAAAGGAGAC AAGGGAGACC CTGGAGTAGG GCTGCCTGGG CCCCGAGGCG

IGE308_RefSequence #10641 AGCGTGGGGA GCCAGGCATC CGGGGTGAAG ATGGCCGCCC CGGCCAGGAG GGACCCCGAG GACTCACGGG GCCCCCTGGC
60010743-ITX-00001_Contig #10641 AGCGTGGGGA GCCAGGCATC CGGGGTGAAG ATGGCCGCCC CGGCCAGGAG GGACCCCGAG GACTCACGGG GCCCCCTGGC

#10641 AGCGTGGGGA GCCAGGCATC CGGGGTGAAG ATGGCCGCCC CGGCCAGGAG GGACCCCGAG GACTCACGGG GCCCCCTGGC

IGE308_RefSequence #10721 AGCAGGGGAG AGCGTGGGGA GAAGGGTGAT GTTGGGAGTG CAGGACTAAA GGGTGACAAG GGAGACTCAG CTGTGATCCT
60010743-ITX-00001_Contig #10721 AGCAGGGGAG AGCGTGGGGA GAAGGGTGAT GTTGGGAGTG CAGGACTAAA GGGTGACAAG GGAGACTCAG CTGTGATCCT

#10721 AGCAGGGGAG AGCGTGGGGA GAAGGGTGAT GTTGGGAGTG CAGGACTAAA GGGTGACAAG GGAGACTCAG CTGTGATCCT

IGE308_RefSequence #10801 GGGGCCTCCA GGCCCACGGG GTGCCAAGGG GGACATGGGT GAACGAGGGC CTCGGGGCTT GGATGGTGAC AAAGGACCTC
60010743-ITX-00001_Contig #10801 GGGGCCTCCA GGCCCACGGG GTGCCAAGGG GGACATGGGT GAACGAGGGC CTCGGGGCTT GGATGGTGAC AAAGGACCTC

#10801 GGGGCCTCCA GGCCCACGGG GTGCCAAGGG GGACATGGGT GAACGAGGGC CTCGGGGCTT GGATGGTGAC AAAGGACCTC

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #10881 GGGGAGACAA TGGGGACCCT GGTGACAAGG GCAGCAAGGG AGAGCCTGGT GACAAGGGCT CAGCCGGGTT GCCAGGACTG
60010743-ITX-00001_Contig #10881 GGGGAGACAA TGGGGACCCT GGTGACAAGG GCAGCAAGGG AGAGCCTGGT GACAAGGGCT CAGCCGGGTT GCCAGGACTG

#10881 GGGGAGACAA TGGGGACCCT GGTGACAAGG GCAGCAAGGG AGAGCCTGGT GACAAGGGCT CAGCCGGGTT GCCAGGACTG

IGE308_RefSequence #10961 CGTGGACTCC TGGGACCCCA GGGTCAACCT GGTGCAGCAG GGATCCCTGG TGACCCGGGA TCCCCAGGAA AGGATGGAGT
60010743-ITX-00001_Contig #10961 CGTGGACTCC TGGGACCCCA GGGTCAACCT GGTGCAGCAG GGATCCCTGG TGACCCGGGA TCCCCAGGAA AGGATGGAGT

#10961 CGTGGACTCC TGGGACCCCA GGGTCAACCT GGTGCAGCAG GGATCCCTGG TGACCCGGGA TCCCCAGGAA AGGATGGAGT

IGE308_RefSequence #11041 GCCTGGTATC CGAGGAGAAA AAGGAGATGT TGGCTTCATG GGTCCCCGGG GCCTCAAGGG TGAACGGGGA GTGAAGGGAG
60010743-ITX-00001_Contig #11041 GCCTGGTATC CGAGGAGAAA AAGGAGATGT TGGCTTCATG GGTCCCCGGG GCCTCAAGGG TGAACGGGGA GTGAAGGGAG

#11041 GCCTGGTATC CGAGGAGAAA AAGGAGATGT TGGCTTCATG GGTCCCCGGG GCCTCAAGGG TGAACGGGGA GTGAAGGGAG

IGE308_RefSequence #11121 CCTGTGGCCT TGATGGAGAG AAGGGAGACA AGGGAGAAGC TGGTCCCCCA GGCCGCCCCG GGCTGGCAGG ACACAAAGGA
60010743-ITX-00001_Contig #11121 CCTGTGGCCT TGATGGAGAG AAGGGAGACA AGGGAGAAGC TGGTCCCCCA GGCCGCCCCG GGCTGGCAGG ACACAAAGGA

#11121 CCTGTGGCCT TGATGGAGAG AAGGGAGACA AGGGAGAAGC TGGTCCCCCA GGCCGCCCCG GGCTGGCAGG ACACAAAGGA

IGE308_RefSequence #11201 GAGATGGGGG AGCCTGGTGT GCCGGGCCAG TCGGGGGCCC CTGGCAAGGA GGGCCTGATC GGTCCCAAGG GTGACCGAGG
60010743-ITX-00001_Contig #11201 GAGATGGGGG AGCCTGGTGT GCCGGGCCAG TCGGGGGCCC CTGGCAAGGA GGGCCTGATC GGTCCCAAGG GTGACCGAGG

#11201 GAGATGGGGG AGCCTGGTGT GCCGGGCCAG TCGGGGGCCC CTGGCAAGGA GGGCCTGATC GGTCCCAAGG GTGACCGAGG

IGE308_RefSequence #11281 CTTTGACGGG CAGCCAGGCC CCAAGGGTGA CCAGGGCGAG AAAGGGGAGC GGGGAACCCC AGGAATTGGG GGCTTCCCAG
60010743-ITX-00001_Contig #11281 CTTTGACGGG CAGCCAGGCC CCAAGGGTGA CCAGGGCGAG AAAGGGGAGC GGGGAACCCC AGGAATTGGG GGCTTCCCAG

#11281 CTTTGACGGG CAGCCAGGCC CCAAGGGTGA CCAGGGCGAG AAAGGGGAGC GGGGAACCCC AGGAATTGGG GGCTTCCCAG

IGE308_RefSequence #11361 GCCCCAGTGG AAATGATGGC TCTGCTGGTC CCCCAGGGCC ACCTGGCAGT GTTGGTCCCA GAGGCCCCGA AGGACTTCAG
60010743-ITX-00001_Contig #11361 GCCCCAGTGG AAATGATGGC TCTGCTGGTC CCCCAGGGCC ACCTGGCAGT GTTGGTCCCA GAGGCCCCGA AGGACTTCAG

#11361 GCCCCAGTGG AAATGATGGC TCTGCTGGTC CCCCAGGGCC ACCTGGCAGT GTTGGTCCCA GAGGCCCCGA AGGACTTCAG

IGE308_RefSequence #11441 GGCCAGAAGG GTGAGCGAGG TCCCCCCGGA GAGAGAGTGG TGGGGGCTCC TGGGGTCCCT GGAGCTCCTG GCGAGAGAGG
60010743-ITX-00001_Contig #11441 GGCCAGAAGG GTGAGCGAGG TCCCCCCGGA GAGAGAGTGG TGGGGGCTCC TGGGGTCCCT GGAGCTCCTG GCGAGAGAGG

#11441 GGCCAGAAGG GTGAGCGAGG TCCCCCCGGA GAGAGAGTGG TGGGGGCTCC TGGGGTCCCT GGAGCTCCTG GCGAGAGAGG

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #11521 GGAGCAGGGG CGGCCAGGGC CTGCCGGTCC TCGAGGCGAG AAGGGAGAAG CTGCACTGAC GGAGGATGAC ATCCGGGGCT
60010743-ITX-00001_Contig #11521 GGAGCAGGGG CGGCCAGGGC CTGCCGGTCC TCGAGGCGAG AAGGGAGAAG CTGCACTGAC GGAGGATGAC ATCCGGGGCT

#11521 GGAGCAGGGG CGGCCAGGGC CTGCCGGTCC TCGAGGCGAG AAGGGAGAAG CTGCACTGAC GGAGGATGAC ATCCGGGGCT

IGE308_RefSequence #11601 TTGTGCGCCA AGAGATGAGT CAGCACTGTG CCTGCCAGGG CCAGTTCATC GCATCTGGAT CACGACCCCT CCCTAGTTAT
60010743-ITX-00001_Contig #11601 TTGTGCGCCA AGAGATGAGT CAGCACTGTG CCTGCCAGGG CCAGTTCATC GCATCTGGAT CACGACCCCT CCCTAGTTAT

#11601 TTGTGCGCCA AGAGATGAGT CAGCACTGTG CCTGCCAGGG CCAGTTCATC GCATCTGGAT CACGACCCCT CCCTAGTTAT

IGE308_RefSequence #11681 GCTGCAGACA CTGCCGGCTC CCAGCTCCAT GCTGTGCCTG TGCTCCGCGT CTCTCATGCA GAGGAGGAAG AGCGGGTACC
60010743-ITX-00001_Contig #11681 GCTGCAGACA CTGCCGGCTC CCAGCTCCAT GCTGTGCCTG TGCTCCGCGT CTCTCATGCA GAGGAGGAAG AGCGGGTACC

#11681 GCTGCAGACA CTGCCGGCTC CCAGCTCCAT GCTGTGCCTG TGCTCCGCGT CTCTCATGCA GAGGAGGAAG AGCGGGTACC

IGE308_RefSequence #11761 CCCTGAGGAT GATGAGTACT CTGAATACTC CGAGTATTCT GTGGAGGAGT ACCAGGACCC TGAAGCTCCT TGGGATAGTG
60010743-ITX-00001_Contig #11761 CCCTGAGGAT GATGAGTACT CTGAATACTC CGAGTATTCT GTGGAGGAGT ACCAGGACCC TGAAGCTCCT TGGGATAGTG

#11761 CCCTGAGGAT GATGAGTACT CTGAATACTC CGAGTATTCT GTGGAGGAGT ACCAGGACCC TGAAGCTCCT TGGGATAGTG

IGE308_RefSequence #11841 ATGACCCCTG TTCCCTGCCA CTGGATGAGG GCTCCTGCAC TGCCTACACC CTGCGCTGGT ACCATCGGGC TGTGACAGGC
60010743-ITX-00001_Contig #11841 ATGACCCCTG TTCCCTGCCA CTGGATGAGG GCTCCTGCAC TGCCTACACC CTGCGCTGGT ACCATCGGGC TGTGACAGGC

#11841 ATGACCCCTG TTCCCTGCCA CTGGATGAGG GCTCCTGCAC TGCCTACACC CTGCGCTGGT ACCATCGGGC TGTGACAGGC

IGE308_RefSequence #11921 AGCACAGAGG CCTGTCACCC TTTTGTCTAT GGTGGCTGTG GAGGGAATGC CAACCGTTTT GGGACCCGTG AGGCCTGCGA
60010743-ITX-00001_Contig #11921 AGCACAGAGG CCTGTCACCC TTTTGTCTAT GGTGGCTGTG GAGGGAATGC CAACCGTTTT GGGACCCGTG AGGCCTGCGA

#11921 AGCACAGAGG CCTGTCACCC TTTTGTCTAT GGTGGCTGTG GAGGGAATGC CAACCGTTTT GGGACCCGTG AGGCCTGCGA

IGE308_RefSequence #12001 GCGCCGCTGC CCACCCCGGG TGGTCCAGAG CCAGGGGACA GGTACTGCCC AGGACTGATC GATACCGTCG ACCTCGAGAC
60010743-ITX-00001_Contig #12001 GCGCCGCTGC CCACCCCGGG TGGTCCAGAG CCAGGGGACA GGTACTGCCC AGGACTGATC GATACCGTCG ACCTCGAGAC

#12001 GCGCCGCTGC CCACCCCGGG TGGTCCAGAG CCAGGGGACA GGTACTGCCC AGGACTGATC GATACCGTCG ACCTCGAGAC

IGE308_RefSequence #12081 CTAGAAAAAC ATGGAGCAAT CACAAGTAGC AATACAGCAG CTACCAATGC TGATTGTGCC TGGCTAGAAG CACAAGAGGA
60010743-ITX-00001_Contig #12081 CTAGAAAAAC ATGGAGCAAT CACAAGTAGC AATACAGCAG CTACCAATGC TGATTGTGCC TGGCTAGAAG CACAAGAGGA

#12081 CTAGAAAAAC ATGGAGCAAT CACAAGTAGC AATACAGCAG CTACCAATGC TGATTGTGCC TGGCTAGAAG CACAAGAGGA

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #12161 GGAGGAGGTG GGTTTTCCAG TCACACCTCA GGTACCTTTA AGACCAATGA CTTACAAGGC AGCTGTAGAT CTTAGCCACT
60010743-ITX-00001_Contig #12161 GGAGGAGGTG GGTTTTCCAG TCACACCTCA GGTACCTTTA AGACCAATGA CTTACAAGGC AGCTGTAGAT CTTAGCCACT

#12161 GGAGGAGGTG GGTTTTCCAG TCACACCTCA GGTACCTTTA AGACCAATGA CTTACAAGGC AGCTGTAGAT CTTAGCCACT

IGE308_RefSequence #12241 TTTTAAAAGA AAAGGGGGGA CTGGAAGGGC TAATTCACTC CCAACGAAGA CAAGATATCC TTGATCTGTG GATCTACCAC
60010743-ITX-00001_Contig #12241 TTTTAAAAGA AAAGGGGGGA CTGGAAGGGC TAATTCACTC CCAACGAAGA CAAGATATCC TTGATCTGTG GATCTACCAC

#12241 TTTTAAAAGA AAAGGGGGGA CTGGAAGGGC TAATTCACTC CCAACGAAGA CAAGATATCC TTGATCTGTG GATCTACCAC

IGE308_RefSequence #12321 ACACAAGGCT ACTTCCCTGA TTGGCAGAAC TACACACCAG GGCCAGGGAT CAGATATCCA CTGACCTTTG GATGGTGCTA
60010743-ITX-00001_Contig #12321 ACACAAGGCT ACTTCCCTGA TTGGCAGAAC TACACACCAG GGCCAGGGAT CAGATATCCA CTGACCTTTG GATGGTGCTA

#12321 ACACAAGGCT ACTTCCCTGA TTGGCAGAAC TACACACCAG GGCCAGGGAT CAGATATCCA CTGACCTTTG GATGGTGCTA

IGE308_RefSequence #12401 CAAGCTAGTA CCAGTTGAGC AAGAGAAGGT AGAAGAAGCC AATGAAGGAG AGAACACCCG CTTGTTACAC CCTGTGAGCC
60010743-ITX-00001_Contig #12401 CAAGCTAGTA CCAGTTGAGC AAGAGAAGGT AGAAGAAGCC AATGAAGGAG AGAACACCCG CTTGTTACAC CCTGTGAGCC

#12401 CAAGCTAGTA CCAGTTGAGC AAGAGAAGGT AGAAGAAGCC AATGAAGGAG AGAACACCCG CTTGTTACAC CCTGTGAGCC

IGE308_RefSequence #12481 TGCATGGGAT GGATGACCCG GAGAGAGAAG TATTAGAGTG GAGGTTTGAC AGCCGCCTAG CATTTCATCA CATGGCCCGA
60010743-ITX-00001_Contig #12481 TGCATGGGAT GGATGACCCG GAGAGAGAAG TATTAGAGTG GAGGTTTGAC AGCCGCCTAG CATTTCATCA CATGGCCCGA

#12481 TGCATGGGAT GGATGACCCG GAGAGAGAAG TATTAGAGTG GAGGTTTGAC AGCCGCCTAG CATTTCATCA CATGGCCCGA

IGE308_RefSequence #12561 GAGCTGCATC CGGACTGTAC TGGGTCTCTC TGGTTAGACC AGATCTGAGC CTGGGAGCTC TCTGGCTAAC TAGGGAACCC
60010743-ITX-00001_Contig #12561 GAGCTGCATC CGGACTGTAC TGGGTCTCTC TGGTTAGACC AGATCTGAGC CTGGGAGCTC TCTGGCTAAC TAGGGAACCC

#12561 GAGCTGCATC CGGACTGTAC TGGGTCTCTC TGGTTAGACC AGATCTGAGC CTGGGAGCTC TCTGGCTAAC TAGGGAACCC

IGE308_RefSequence #12641 ACTGCTTAAG CCTCAATAAA GCTTGCCTTG AGTGCTTCAA GTAGTGTGTG CCCGTCTGTT GTGTGACTCT GGTAACTAGA
60010743-ITX-00001_Contig #12641 ACTGCTTAAG CCTCAATAAA GCTTGCCTTG AGTGCTTCAA GTAGTGTGTG CCCGTCTGTT GTGTGACTCT GGTAACTAGA

#12641 ACTGCTTAAG CCTCAATAAA GCTTGCCTTG AGTGCTTCAA GTAGTGTGTG CCCGTCTGTT GTGTGACTCT GGTAACTAGA

IGE308_RefSequence #12721 GATCCCTCAG ACCCTTTTAG TCAGTGTGGA AAATCTCTAG CAGGGCCCGT TTAAACCCGC TGATCAGCCT CGACTGTGCC
60010743-ITX-00001_Contig #12721 GATCCCTCAG ACCCTTTTAG TCAGTGTGGA AAATCTCTAG CAGGGCCCGT TTAAACCCGC TGATCAGCCT CGACTGTGCC

#12721 GATCCCTCAG ACCCTTTTAG TCAGTGTGGA AAATCTCTAG CAGGGCCCGT TTAAACCCGC TGATCAGCCT CGACTGTGCC

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

```
IGE308_RefSequence          #12801   TTCTAGTTGC CAGCCATCTG TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT
60010743-ITX-00001_Contig   #12801   TTCTAGTTGC CAGCCATCTG TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT

                            #12801   TTCTAGTTGC CAGCCATCTG TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT

IGE308_RefSequence          #12881   CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGGACAGC
60010743-ITX-00001_Contig   #12881   CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGGACAGC

                            #12881   CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGGACAGC

IGE308_RefSequence          #12961   AAGGGGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG ATGCGGTGGG CTCTATGGCT TCTGAGGCGG AAAGAACCAG
60010743-ITX-00001_Contig   #12961   AAGGGGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG ATGCGGTGGG CTCTATGGCT TCTGAGGCGG AAAGAACCAG

                            #12961   AAGGGGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG ATGCGGTGGG CTCTATGGCT TCTGAGGCGG AAAGAACCAG

IGE308_RefSequence          #13041   CTGGGGCTCT AGGGGGTATC CCCACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG CGCAGCGTGA
60010743-ITX-00001_Contig   #13041   CTGGGGCTCT AGGGGGTATC CCCACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG CGCAGCGTGA

                            #13041   CTGGGGCTCT AGGGGGTATC CCCACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG CGCAGCGTGA

IGE308_RefSequence          #13121   CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC
60010743-ITX-00001_Contig   #13121   CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC

                            #13121   CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC

IGE308_RefSequence          #13201   CGTCAAGCTC TAAATCGGGG GCTCCCTTTA GGGTTCCGAT TTAGTGCTTT ACGGCACCTC GACCCCAAAA AACTTGATTA
60010743-ITX-00001_Contig   #13201   CGTCAAGCTC TAAATCGGGG GCTCCCTTTA GGGTTCCGAT TTAGTGCTTT ACGGCACCTC GACCCCAAAA AACTTGATTA

                            #13201   CGTCAAGCTC TAAATCGGGG GCTCCCTTTA GGGTTCCGAT TTAGTGCTTT ACGGCACCTC GACCCCAAAA AACTTGATTA

IGE308_RefSequence          #13281   GGGTGATGGT TCACGTAGTG GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA
60010743-ITX-00001_Contig   #13281   GGGTGATGGT TCACGTAGTG GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA

                            #13281   GGGTGATGGT TCACGTAGTG GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA

IGE308_RefSequence          #13361   GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT TCTTTTGATT TATAAGGGAT TTTGCCGATT
60010743-ITX-00001_Contig   #13361   GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT TCTTTTGATT TATAAGGGAT TTTGCCGATT

                            #13361   GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT TCTTTTGATT TATAAGGGAT TTTGCCGATT
```


IGE308_RefSequence #13441 TCGGCCTATT GGTTAAAAAA TGAGCTGATT TAACAAAAAT TTAACGCGAA TTAATTCTGT GGAATGTGTG TCAGTTAGGG
60010743-ITX-00001_Contig #13441 TCGGCCTATT GGTTAAAAAA TGAGCTGATT TAACAAAAAT TTAACGCGAA TTAATTCTGT GGAATGTGTG TCAGTTAGGG
#13441 TCGGCCTATT GGTTAAAAAA TGAGCTGATT TAACAAAAAT TTAACGCGAA TTAATTCTGT GGAATGTGTG TCAGTTAGGG
IGE308_RefSequence #13521 TGTGGAAAGT CCCCAGGCTC CCCAGCAGGC AGAAGTATGC AAAGCATGCA TCTCAATTAG TCAGCAACCA GGTGTGGAAA
60010743-ITX-00001_Contig #13521 TGTGGAAAGT CCCCAGGCTC CCCAGCAGGC AGAAGTATGC AAAGCATGCA TCTCAATTAG TCAGCAACCA GGTGTGGAAA
#13521 TGTGGAAAGT CCCCAGGCTC CCCAGCAGGC AGAAGTATGC AAAGCATGCA TCTCAATTAG TCAGCAACCA GGTGTGGAAA
IGE308_RefSequence #13601 GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CATAGTCCCG CCCCTAACTC
60010743-ITX-00001_Contig #13601 GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CATAGTCCCG CCCCTAACTC
#13601 GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CATAGTCCCG CCCCTAACTC
IGE308_RefSequence #13681 CGCCCATCCC GCCCCTAACT CCGCCCAGTT CCGCCCATTC TCCGCCCCAT GGCTGACTAA TTTTTTTTAT TTATGCAGAG
60010743-ITX-00001_Contig #13681 CGCCCATCCC GCCCCTAACT CCGCCCAGTT CCGCCCATTC TCCGCCCCAT GGCTGACTAA TTTTTTTTAT TTATGCAGAG
#13681 CGCCCATCCC GCCCCTAACT CCGCCCAGTT CCGCCCATTC TCCGCCCCAT GGCTGACTAA TTTTTTTTAT TTATGCAGAG
IGE308_RefSequence #13761 GCCGAGGCCG CCTCTGCCTC TGAGCTATTC CAGAAGTAGT GAGGAGGCTT TTTTGGAGGC CTAGGCTTTT GCAAAAAGCT
60010743-ITX-00001_Contig #13761 GCCGAGGCCG CCTCTGCCTC TGAGCTATTC CAGAAGTAGT GAGGAGGCTT TTTTGGAGGC CTAGGCTTTT GCAAAAAGCT
#13761 GCCGAGGCCG CCTCTGCCTC TGAGCTATTC CAGAAGTAGT GAGGAGGCTT TTTTGGAGGC CTAGGCTTTT GCAAAAAGCT
IGE308_RefSequence #13841 CCCGGGAGCT TGTATATCCA TTTTCGGATC TGATCAGCAC GTGTTGACAA TTAATCATCG GCATAGTATA TCGGCATAGT
60010743-ITX-00001_Contig #13841 CCCGGGAGCT TGTATATCCA TTTTCGGATC TGATCAGCAC GTGTTGACAA TTAATCATCG GCATAGTATA TCGGCATAGT
#13841 CCCGGGAGCT TGTATATCCA TTTTCGGATC TGATCAGCAC GTGTTGACAA TTAATCATCG GCATAGTATA TCGGCATAGT
IGE308_RefSequence #13921 ATAATACGAC AAGGTGAGGA ACTAAACCAT GGCCAAGTTG ACCAGTGCCG TTCCGGTGCT CACCGCGCGC GACGTCGCCG
60010743-ITX-00001_Contig #13921 ATAATACGAC AAGGTGAGGA ACTAAACCAT GGCCAAGTTG ACCAGTGCCG TTCCGGTGCT CACCGCGCGC GACGTCGCCG
#13921 ATAATACGAC AAGGTGAGGA ACTAAACCAT GGCCAAGTTG ACCAGTGCCG TTCCGGTGCT CACCGCGCGC GACGTCGCCG
IGE308_RefSequence #14001 GAGCGGTCGA GTTCTGGACC GACCGGCTCG GGTTCTCCCG GGACTTCGTG GAGGACGACT TCGCCGGTGT GGTCCGGGAC
60010743-ITX-00001_Contig #14001 GAGCGGTCGA GTTCTGGACC GACCGGCTCG GGTTCTCCCG GGACTTCGTG GAGGACGACT TCGCCGGTGT GGTCCGGGAC
#14001 GAGCGGTCGA GTTCTGGACC GACCGGCTCG GGTTCTCCCG GGACTTCGTG GAGGACGACT TCGCCGGTGT GGTCCGGGAC

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #14081 GACGTGACCC TGTTCATCAG CGCGGTCCAG GACCAGGTGG TGCCGGACAA CACCCTGGCC TGGGTGTGGG TGCGCGGCCT
60010743-ITX-00001_Contig #14081 GACGTGACCC TGTTCATCAG CGCGGTCCAG GACCAGGTGG TGCCGGACAA CACCCTGGCC TGGGTGTGGG TGCGCGGCCT

#14081 GACGTGACCC TGTTCATCAG CGCGGTCCAG GACCAGGTGG TGCCGGACAA CACCCTGGCC TGGGTGTGGG TGCGCGGCCT

IGE308_RefSequence #14161 GGACGAGCTG TACGCCGAGT GGTCGGAGGT CGTGTCCACG AACTTCCGGG ACGCCTCCGG GCCGGCCATG ACCGAGATCG
60010743-ITX-00001_Contig #14161 GGACGAGCTG TACGCCGAGT GGTCGGAGGT CGTGTCCACG AACTTCCGGG ACGCCTCCGG GCCGGCCATG ACCGAGATCG

#14161 GGACGAGCTG TACGCCGAGT GGTCGGAGGT CGTGTCCACG AACTTCCGGG ACGCCTCCGG GCCGGCCATG ACCGAGATCG

IGE308_RefSequence #14241 GCGAGCAGCC GTGGGGGCGG GAGTTCGCCC TGCGCGACCC GGCCGGCAAC TGCGTGCACT TCGTGGCCGA GGAGCAGGAC
60010743-ITX-00001_Contig #14241 GCGAGCAGCC GTGGGGGCGG GAGTTCGCCC TGCGCGACCC GGCCGGCAAC TGCGTGCACT TCGTGGCCGA GGAGCAGGAC

#14241 GCGAGCAGCC GTGGGGGCGG GAGTTCGCCC TGCGCGACCC GGCCGGCAAC TGCGTGCACT TCGTGGCCGA GGAGCAGGAC

IGE308_RefSequence #14321 TGACACGTGC TACGAGATTT CGATTCCACC GCCGCCTTCT ATGAAAGGTT GGGCTTCGGA ATCGTTTTCC GGGACGCCGG
60010743-ITX-00001_Contig #14321 TGACACGTGC TACGAGATTT CGATTCCACC GCCGCCTTCT ATGAAAGGTT GGGCTTCGGA ATCGTTTTCC GGGACGCCGG

#14321 TGACACGTGC TACGAGATTT CGATTCCACC GCCGCCTTCT ATGAAAGGTT GGGCTTCGGA ATCGTTTTCC GGGACGCCGG

IGE308_RefSequence #14401 CTGGATGATC CTCCAGCGCG GGGATCTCAT GCTGGAGTTC TTCGCCCACC CCAACTTGTT TATTGCAGCT TATAATGGTT
60010743-ITX-00001_Contig #14401 CTGGATGATC CTCCAGCGCG GGGATCTCAT GCTGGAGTTC TTCGCCCACC CCAACTTGTT TATTGCAGCT TATAATGGTT

#14401 CTGGATGATC CTCCAGCGCG GGGATCTCAT GCTGGAGTTC TTCGCCCACC CCAACTTGTT TATTGCAGCT TATAATGGTT

IGE308_RefSequence #14481 ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC
60010743-ITX-00001_Contig #14481 ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC

#14481 ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC

IGE308_RefSequence #14561 ATCAATGTAT CTTATCATGT CTGTATACCG TCGACCTCTA GCTAGAGCTT GGCGTAATCA TGGTCATAGC TGTTTCCTGT
60010743-ITX-00001_Contig #14561 ATCAATGTAT CTTATCATGT CTGTATACCG TCGACCTCTA GCTAGAGCTT GGCGTAATCA TGGTCATAGC TGTTTCCTGT

#14561 ATCAATGTAT CTTATCATGT CTGTATACCG TCGACCTCTA GCTAGAGCTT GGCGTAATCA TGGTCATAGC TGTTTCCTGT

IGE308_RefSequence #14641 GTGAAATTGT TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG
60010743-ITX-00001_Contig #14641 GTGAAATTGT TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG

#14641 GTGAAATTGT TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

```
IGE308_RefSequence          #14721   TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG GGAAACCTGT CGTGCCAGCT GCATTAATGA
60010743-ITX-00001_Contig   #14721   TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG GGAAACCTGT CGTGCCAGCT GCATTAATGA

                            #14721   TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG GGAAACCTGT CGTGCCAGCT GCATTAATGA

IGE308_RefSequence          #14801   ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT
60010743-ITX-00001_Contig   #14801   ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT

                            #14801   ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT

IGE308_RefSequence          #14881   CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT AACGCAGGAA
60010743-ITX-00001_Contig   #14881   CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT AACGCAGGAA

                            #14881   CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT AACGCAGGAA

IGE308_RefSequence          #14961   AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC
60010743-ITX-00001_Contig   #14961   AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC

                            #14961   AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC

IGE308_RefSequence          #15041   CCCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT
60010743-ITX-00001_Contig   #15041   CCCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT

                            #15041   CCCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT

IGE308_RefSequence          #15121   TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT CTCCCTTCGG
60010743-ITX-00001_Contig   #15121   TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT CTCCCTTCGG

                            #15121   TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT CTCCCTTCGG

IGE308_RefSequence          #15201   GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG
60010743-ITX-00001_Contig   #15201   GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG

                            #15201   GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG

IGE308_RefSequence          #15281   CACGAACCCC CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT
60010743-ITX-00001_Contig   #15281   CACGAACCCC CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT

                            #15281   CACGAACCCC CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT
```

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #15361 ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT
60010743-ITX-00001_Contig #15361 ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT

#15361 ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT

IGE308_RefSequence #15441 GGCCTAACTA CGGCTACACT AGAAGAACAG TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT
60010743-ITX-00001_Contig #15441 GGCCTAACTA CGGCTACACT AGAAGAACAG TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT

#15441 GGCCTAACTA CGGCTACACT AGAAGAACAG TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT

IGE308_RefSequence #15521 GGTAGCTCTT GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA
60010743-ITX-00001_Contig #15521 GGTAGCTCTT GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA

#15521 GGTAGCTCTT GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA

IGE308_RefSequence #15601 AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT TAAGGGATTT
60010743-ITX-00001_Contig #15601 AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT TAAGGGATTT

#15601 AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT TAAGGGATTT

IGE308_RefSequence #15681 TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT CTAAAGTATA
60010743-ITX-00001_Contig #15681 TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT CTAAAGTATA

#15681 TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT CTAAAGTATA

IGE308_RefSequence #15761 TATGAGTAAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC
60010743-ITX-00001_Contig #15761 TATGAGTAAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC

#15761 TATGAGTAAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC

IGE308_RefSequence #15841 CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT GCAATGATAC
60010743-ITX-00001_Contig #15841 CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT GCAATGATAC

#15841 CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT GCAATGATAC

IGE308_RefSequence #15921 CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT
60010743-ITX-00001_Contig #15921 CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT

#15921 CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT

60010743-ITX-00001_Comparative_Analysis
60010743-ITX_Final.SPF

IGE308_RefSequence #16001 GCAACTTTAT CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG
60010743-ITX-00001_Contig #16001 GCAACTTTAT CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG

#16001 GCAACTTTAT CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG

IGE308_RefSequence #16081 CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC GGTTCCCAAC
60010743-ITX-00001_Contig #16081 CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC GGTTCCCAAC

#16081 CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC GGTTCCCAAC

IGE308_RefSequence #16161 GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT
60010743-ITX-00001_Contig #16161 GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT

#16161 GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT

IGE308_RefSequence #16241 AAGTTGGCCG CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT
60010743-ITX-00001_Contig #16241 AAGTTGGCCG CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT

#16241 AAGTTGGCCG CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT

IGE308_RefSequence #16321 TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GGCGACCGAG TTGCTCTTGC CCGGCGTCAA
60010743-ITX-00001_Contig #16321 TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GGCGACCGAG TTGCTCTTGC CCGGCGTCAA

#16321 TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GGCGACCGAG TTGCTCTTGC CCGGCGTCAA

IGE308_RefSequence #16401 TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG AAAACTCTCA
60010743-ITX-00001_Contig #16401 TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG AAAACTCTCA

#16401 TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG AAAACTCTCA

IGE308_RefSequence #16481 AGGATCTTAC CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC
60010743-ITX-00001_Contig #16481 AGGATCTTAC CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC

#16481 AGGATCTTAC CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC

IGE308_RefSequence #16561 CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA TGTTGAATAC
60010743-ITX-00001_Contig #16561 CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA TGTTGAATAC

#16561 CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA TGTTGAATAC

REFERENCES

A. Aiuti et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science, 2013. August; 341:6148.

Baldeschi C, Gache Y, Rattenholl A, Bouillé P, Danos O, Ortonne JP, Bruckner-Tuderman L, Meneguzzi G. 2003. Genetic correction of canine dystrophic epidermolysis bullosa mediated by retroviral vectors. Human Molecular Genetics 12: 1897-905.

A. Biffi et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science, 2013. August; 341:6148.

Bruckner-Tuderman L, Hopfner B, Hammami-Hauasli N. 1999. Biology of anchoring fibrils: lessons from dystrophic epidermolysis bullosa. Matirx Biology 18: 43-54.

Burgeson R.E. Type VII collagen, anchoring fibrils, and epidermolysis bullosa. J. Invest. Dermatol. 1993;101:252–255.

Chen M, O'Toole EA, Muellenhoff M, Medina E, Kasahara N, Woodley DT. 2000. Development and characterization of a recombinant truncated type VII collagen "minigene". Implication for gene therapy of dystrophic epidermolysis bullosa. J Biol Chem 275: 24429-35.

Chen M, Costa FK, Lindvay CR, Han YP, Woodley DT. 2002a. The recombinant expression of full-length type VII collagen and characterization of molecular mechanisms underlying dystrophic epidermolysis bullosa. J Biol Chem 277: 2118-24.

Chen M, Kasahara N, Keene DR, Chan L, Hoeffler WK, Finlay D, Barcova M, Cannon PM, Mazurek C, Woodley DT. 2002b. Restoration of type VII collagen expression and function in dystrophic epidermolysis bullosa. Nature Genetics 32: 670-5.

Cogan J, Weinstein J, Wang X, Hou Y, Martin S, South AP, Woodley DT, Chen M. 2014. Aminoglycosides restore full-length type VII collagen by overcoming premature termination codons: therapeutic implications for dystrophic epidermolysis bullosa. Molecular Therapy 22: 1741-52.

Deshpande P, Ralston DR, and S McNeil. The use of allodermis prepared from Euro skin bank to prepare autologous tissue engineered skin for clinical use. Burns. 2013. 39(6): 1170-1177.

Goto, M., et al., Fibroblasts show more potential as target cells than keratinocytes in COL7A1 gene therapy of dystrophic epidermolysis bullosa. J Invest Dermatol, 2006. 126(4): p. 766-72.

Greenberg KP, Edwin SL, Schaffer DV, Flannery JG. 2006. Gene Delivery to the Retina Using Lentiviral Vectors. Advances in Experimental Medicine and Biology 572: 255-66

Kim YH, Woodley DT, Wynn KC, Giomi W, Bauer EA. Recessive Dystrophic Epidermylosis Bullosa Phenotype is Preserved in Xenografts Using SCID Mice: Development of an Experimental In Vivo Model. J Invest Dermatol. 1992 Feb;98(2):191-7.

Keene DR, Sakai LY, Lunstrum GP, Morris NP, Burgeson RE. Type VII Collagen Forms an Extended Network of Anchoring Fibrils. J Cell Biol. 1987 Mar;104(3):611-21.

Leigh I.M. Eady R.A. Heagerty A.H. Purkis P.E. Whitehead P.A. Burgeson R.E. Type VII collagen is a normal component of epidermal basement membrane, which shows altered expression in recessive dystrophic epidermolysis bullosa. J. Invest. Dermatol. 1988;90:639–642.

Mavilio F. Pellegrini G. Ferrari S. Di Nunzio F. Di Iorio E. Recchia A. Maruggi G. Ferrari G. Provasi E. Bonini C. Capurro S. Conti A. Magnoni C. Giannetti A. De Luca M. Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. Nat. Med. 2006;12:1397–1402.

Ortiz-Urda S. Lin Q. Green C.L. Keene D.R. Marinkovich M.P. Khavari P.A. Injection of genetically engineered fibroblasts corrects regenerated human epidermolysis bullosa skin tissue. J. Clin. Invest. 2003;111:251–255.

Remington J. Wang X. Hou Y. Zhou H. Burnett J. Muirhead T. Uitto J. Keene D.R. Woodley D.T. Chen M. Injection of recombinant human type VII collagen corrects the

disease phenotype in a murine model of dystrophic epidermolysis bullosa. Mol. Ther. 2009;17:26–33.

Rousselle P, Keene DR, Ruggiero F, Champliaud MF, Rest M, Burgeson RE. 1997. Laminin 5 binds the NC-1 domain of type VII collagen. J Cell Bio, 138: 719-28.

Siprashvili Z, Ngon T. Nguyen, Maria Y. Bezchinsky, M. Peter Marinkovich, Alfred T. Lane, and Paul A. Khavari. Long-term type VII collagen restoration to human epidermylosis bullosa skin tissue. Hum Gene Ther. Oct 2010; 21(10): 1299–1310.

Tareen S, Nicolai C, Campbell D, Flynn P, Slough M, Vin C, Kelly-Clarke B, Odegard J, Robbins S. 2013. A Rev-Independent gag/pol Eliminates Detectable psi-gag Recombination in Lentiviral Vectors. Biores Open Access. 2013 Dec 1; 2(6): 421-430.

Waterman EA, Sakai N, Nguyen NT, Horst BA, Veitch DP, Dey CN, Ortiz-Urda S, Khavari PA, Marinkovich MP. 2007. A laminin-collagen complex drives human epidermal carcinogenesis through phosphoinositol-3-kinase activation. Cancer Research 67: 4264-70.

Woodley D.T. Keene D.R. Atha T. Huang Y. Ram R. Kasahara N. Chen M. Intradermal injection of lentiviral vectors corrects regenerated human dystrophic epidermolysis bullosa skin tissue in vivo. Mol. Ther. 2004;10:318–326.

Woodley D.T. Krueger G.G. Jorgensen C.M. Fairley J.A. Atha T. Huang Y. Clian L. Keene D.R. Chen M. Normal and gene-corrected dystrophic epidermolysis bullosa fibroblasts alone can produce type VII collagen at the basement membrane zone. J. Invest. Dermatol. 2003;121:1021–1028.

Woodley D.T. Keene D.R. Atha T. Huang Y. Lipman K. Li W. Chen M. Injection of recombinant human type VII collagen restores collagen function in dystrophic epidermolysis bullosa. Nat. Med. 2004;10:693–695.

CLAIMS

1. A method of treating a patient suffering from a type VII collagen (C7) deficiency comprising

obtaining cells from the dermis or epidermis of the C7-deficient patient,

contacting said cells with a transducing lentiviral vector comprising a nucleotide sequence encoding COL7A1 or a functional variant thereof to form an autologous genetically modified cell having a vector copy number wherein said lentiviral vector has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell,

culturing said autologous genetically modified cell,

and administering an effective amount of the genetically modified cells to the C7-deficient patient.

2. The method of claim 1, wherein the cells are selected from fibroblasts and keratinocytes.

3. The method of claim 2, wherein the cells are fibroblasts.

4. The method of claim 1, wherein the C7-deficiency is dystrophic epidermolysis bullosa.

5. The method of claim 4, wherein the dystrophic epidermolysis bullosa is selected from recessive dystrophic epidermolysis bullosa (RDEB) and dominant dystrophic epidermolysis bullosa (DDEB).

6. The method of claim 5, wherein the C7-deficiency is RDEB.

7. The method of claim 6, wherein the RDEB is a subtype selected from the group consisting of Hallopeau-Siemens, non-Hallopeau-Siemens RDEB, RDEB inversa, pretibial RDEB, acral RDEV, and RDEB centripetalis.

8. The method of claim 5, wherein the C7-deficiency is DDEB.

9. The method of claim 1, wherein said transducing lentiviral vector is in the form of a lentiviral vector particle.

10. The method of claim 9, wherein said transducing lentiviral vector particle is constructed from a transfer lentiviral vector comprising (a) a modified 5' long terminal repeat in LTR, wherein the promoter of the modified 5' LTR is a cytomegalovirus promoter, (b) a functional *COL7A1* gene, (c) at least one lentiviral central polypurine tract, and (d) a modified 3' LTR, wherein the modified 3' LTR comprises a deletion

relative to the wild-type 3' LTR, wherein a hepatitis virus post-transcriptional regulatory element (PRE) has been deleted, wherein the *COL7A1* gene or the functional variant thereof is incorporated into the cells to form genetically modified cells having a functional *COL7A1* gene.

11. The method of claim 10, wherein there are at least two lentiviral central polypurine tract elements.

12. The method of claim 10, wherein the deleted PRE is a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

13. The method of claim 9, wherein transducing lentiviral vector particle is constructed from a lentiviral expression plasmid vector selected from the group consisting of pSMPUW and pFUGW.

14. The method of claim 13, wherein said lentiviral expression plasmid vector is pFUGW.

15. The method of claim 9, wherein said transducing lentiviral vector particle is INXN-2004.

16. The method of claim 9, wherein said transducing lentiviral vector particle is INXN-2002.

17. The method of claim 1, wherein the genetically modified fibroblasts are administered to the patient by injection, topically, orally, or embedded in a biocompatible matrix.

18. The method of claim 17, wherein the genetically modified fibroblasts are administered by injection.

19. The method of claim 18, wherein the genetically modified cells are intradermally injected into the patient.

20. The method of claim 17, wherein the genetically modified fibroblasts are encapsulated in a polymer capsule.

21. The method of claim 17, wherein the genetically modified fibroblasts are embedded in a collagen matrix.

22. The method of claim 17, wherein the genetically modified fibroblasts are embedded in a hydrogel or mesh.

23. An autologous genetically modified fibroblast from a patient suffering from a Type VII collagen deficiency transduced with a lentiviral vector particle comprising a functional *COL7A1* gene or a functional variant thereof, and expresses type VII collagen, wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

24. An autologous genetically modified fibroblast from a patient suffering from a recessive dystrophic epidermolysis bullosa transduced with a lentiviral vector particle comprising a functional *COL7A1* gene or a functional variant thereof, and expresses type VII collagen, wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

25. An autologous genetically modified fibroblast from a patient suffering from a dominant dystrophic epidermolysis bullosa transduced with a lentiviral vector particle comprising a functional *COL7A1* gene or a functional variant thereof, and expresses type VII collagen, wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

26. A self-inactivating lentiviral vector formed from a transfer vector comprising (a) a modified 5' long terminal in LTR, wherein the promoter of the modified 5' LTR is a cytomegalovirus promoter, (b) the *COL7A1* gene or a functional variant thereof, (c) at least one lentiviral central polypurine tract element, and (d) a modified 3' LTR, wherein the modified 3' LTR comprises a deletion relative to the wild-type 3' LTR, and, wherein a hepatitis virus post-transcriptional regulatory element (PRE) has been deleted.

27. The vector of claim 26, wherein the vector comprises at least two lentiviral central polypurine tract elements.

28. The vector of claim 26, wherein the deleted PRE is a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

29. The vector of claim 26, wherein the vector comprises (a) a modified 5' long terminal in LTR, wherein the promoter of the modified 5' LTR is a cytomegalovirus promoter, (b) the *COL7A1* gene, (c) two lentiviral central polypurine tract elements, (d) further comprising a WPRE, and (e) a modified 3' LTR, wherein the modified 3' LTR comprises a deletion relative to the wild-type 3' LTR.

30. The vector of claim 26, wherein the vector is designated INXN-2004.

31. The vector of claim 26, wherein the vector is designated INXN-2002.

32. A transfer vector designated IGE-308.

33. A lentiviral vector particle designated INXN-2004.

34. A lentiviral vector particle designated INXN-2002.

35. A stable virus packaging cell line producing the INXN-2002 lentiviral vector particle.

36. A stable virus packaging cell line producing the INXN-2004 lentiviral vector particle.

37. A pharmaceutical composition comprising a fibroblast obtained from a patient suffering from RDEB transduced with a lentiviral vector designated INXN-2004 having the sequence of IGE-308.

38. A pharmaceutical composition comprising a fibroblast obtained from a patient suffering from RDEB transduced with a lentiviral vector designated INXN-2002.

39. A pharmaceutical composition comprising a fibroblast obtained from a patient suffering from DDEB transduced with a lentiviral vector designated INXN-2004 having the sequence of IGE-308.

40. A pharmaceutical composition comprising a fibroblast obtained from a patient suffering from DDEB transduced with a lentiviral vector designated INXN-2002.

41. A cell transduced in vitro or ex vivo with the vector of claim 33.

42. A cell transduced in vitro or ex vivo with the vector of claim 34.

43. A method of treating a patient suffering from pseudosyndactyly comprising administering to said patient an autologous population of cells obtained from said patient transduced with a lentiviral vector particle comprising a functional *COL7A1* gene or a functional variant thereof, and expressing type VII collagen, wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

44. The method of claim 43, wherein the autologous population of cells is selected from keratinocytes and fibroblasts.

45. The method of claim 43, wherein the autologous population of cells is fibroblasts.

46. The method of claim 43, wherein administration of said cells to the patient is by injection, topically, orally, or embedded in a biocompatible matrix.

47. A method of treating, inhibiting or reducing the blistering of a dystrophic epidermolysis bullosa (DEB) patient comprising administering to said patient an autologous population of cells obtained from said patient transduced with a lentiviral vector particle comprising a functional *COL7A1* gene or a functional variant thereof, and expressing type VII collagen, wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

48. The method of claim 47, wherein the autologous population of cells is selected from keratinocytes and fibroblasts.

49. The method of claim 48, wherein the autologous population of cells is fibroblasts.

50. The method of claim 47, wherein administration of said cells to the patient is by injection, topically, orally, or embedded in a biocompatible matrix.

51. The method of claim 47, wherein the patient suffers from recessive dystrophic epidermolysis bullosa.

52. The method of claim 47, wherein the patient suffers from dominant dystrophic epidermolysis bullosa.

53. A method of treating lesions in patients suffering from recessive dystrophic epidermolysis bullosa comprising administering to said patient an autologous population of cells obtained from said patient transduced with a lentiviral vector particle comprising a functional *COL7A1* gene or a functional variant thereof, and expressing type VII collagen, wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

54. The method of claim 53, wherein the autologous population of cells is selected from keratinocytes and fibroblasts.

55. The method of claim 53, wherein the autologous population of cells is fibroblasts.

56. The method of claim 53, wherein administration of said cells to the patient is by injection, topically, orally, or embedded in a biocompatible matrix.

57. The method of claim 53, wherein the lesions are present in oral mucosa.

58. The method of claim 53, wherein the lesions are present in the gastrointestinal tract.

59. An isolated population of genetically modified fibroblasts autologous to a patient suffering from a Type VII collagen deficiency transduced with a lentiviral vector particle

comprising a functional *COL7A1* gene or a functional variant thereof, and expresses type VII collagen, wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

60. The population of claim 59, wherein the lentiviral vector particle is INXN-2002.

61. The population of claim 59, wherein the lentiviral vector particle is INXN-2004.

62. An isolated population of genetically modified fibroblast autologous to a patient suffering from a recessive dystrophic epidermolysis bullosa transduced with a lentiviral vector particle comprising a functional *COL7A1* gene or a functional variant thereof, and expresses type VII collagen, wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

63. The population of claim 62, wherein the lentiviral vector particle is INXN-2002.

64. The population of claim 62, wherein the lentiviral vector particle is INXN-2004.

65. An isolated population of genetically modified fibroblasts autologous to a patient suffering from a dominant dystrophic epidermolysis bullase transduced with a lentiviral vector particle comprising a functional *COL7A1* gene or a functional variant thereof, and expresses type VII collagen, wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell.

66. The population of claim 65, wherein the lentiviral vector particle is INXN-2002.

67. The population of claim 65, wherein the lentiviral vector particle is INXN-2004.

68. A method of making an isolated population of Type VII collagen (C7) expressing genetically modified cells autologous to a patient suffering from C7 deficiency comprising harvesting cells from the dermis or epidermis of the C7-deficient patient, contacting said cells with a transducing lentiviral vector particle comprising the *COL7A1* gene or a functional variant thereof to form an autologous genetically modified cell comprising the *COL7A1* gene having a vector copy number of wherein said lentiviral vector particle has a transducing vector copy number in the range of 0.1 to 5.0 copies per cell, and culturing said autologous genetically modified cell to obtain an isolated population of C7 expressing genetically modified cells.

69. The method of claim 68, wherein the cells are selected from fibroblasts and keratinocytes.

70. The method of claim 69, wherein the cells are fibroblasts.

71. The method of claim 68, wherein the C7-deficiency is dystrophic epidermolysis bullosa.

72. The method of claim 71, wherein the dystrophic epidermolysis bullosa is selected from recessive dystrophic epidermolysis bullosa (RDEB) and dominant dystrophic epidermolysis bullosa (DDEB).

73. The method of claim 71, wherein the C7-deficiency is RDEB.

74. The method of claim 73, wherein the RDEB is a subtype selected from the group consisting of Hallopeau-Siemens, non-Hallopeau-Siemens RDEB, RDEB inversa, pretibial RDEB, acral RDEV, and RDEB centripetalis.

75. The method of claim 71, wherein the C7-deficiency is DDEB.

76. The method of claim 68, wherein the transducing lentiviral vector particle is INXN-2002.

77. The method of claim 68, wherein the transducing lentiviral vector particle is INXN-2004.

78. An isolated population of Type VII collagen (C7)-expressing genetically modified cells produced by the method of claim 68.

79. An isolated population of Type VII collagen (C7)-expressing genetically modified cells produced by the method of claim 76.

80. An isolated population of Type VII collagen (C7)-expressing genetically modified cells produced by the method of claim 77.

81. A pharmaceutical formulation comprising the isolated population of claim 78.

82. A pharmaceutical formulation comprising the isolated population of claim 79.

83. A pharmaceutical formulation comprising the isolated population of claim 80.

84. A method of increasing the integrated transgene copy number per cell in genetically modified human dermal fibroblasts or keratinocytes comprising contacting a transducing lentiviral vector comprising a nucleotide sequence encoding a *COL7A1* gene or a functional variant thereof with a human dermal fibroblast or keratinocyte obtained from a C7-deficient patient to form a transduction composition, and subjecting said transduction composition to spinoculation to form transduced human dermal fibroblast or keratinocyte, wherein the integrated transgene copy number of the transduced human

dermal fibroblasts or keratinocytes is higher relative to a transduction composition not subjected to spinoculation.

85. The method of claim 84, wherein the transduced human dermal fibroblasts are contacted to a second transducing lentiviral vector to form a second tranduscrion composition.

86. The method of claim 85, wherein the second transduction composition is subjected to spinoculation.

87. The method of claim 84, wherein the transduced human dermal fibroblasts have at least a 1-fold greater integrated transgene copy number per cell relative to a transduction composition not subjected to spinoculation.

88. The method of claim 84, wherein the transduced human dermal fibroblasts have at least a 2-fold greater integrated transgene copy number per cell relative to a transduction composition not subjected to spinoculation.

88. The method of claim 86, wherein the transduced human dermal fibroblasts have at least a 5-fold greater integrated transgene copy number per cell relative to a transduction composition not subjected to spinoculation or a second transduction.

88. The method of claim 86, wherein the transduced human dermal fibroblasts have at least a 10-fold greater integrated transgene copy number per cell relative to a transduction composition not subjected to spinoculation or a second transduction.

88. The method of claim 86, wherein the transduced human dermal fibroblasts have at least a 20-fold greater integrated transgene copy number per cell relative to a transduction composition not subjected to spinoculation or a second transduction.

88. The method of claim 86, wherein the transduced human dermal fibroblasts have at least a 27-fold greater integrated transgene copy number per cell relative to a transduction composition not subjected to spinoculation or a second transduction.

89. The method of claim 84, wherein the transduced human dermal fibroblasts have an integrated transgene copy number per cell of at least 0.05.

90. The method of claim 84, wherein the transduced human dermal fibroblasts have an integrated transgene copy number per cell of at least 0.09.

91. The method of claim 85, wherein the transduced human dermal fibroblasts have an integrated transgene copy number per cell of at least 0.41.

92. The method of claim 86, wherein the transduced human dermal fibroblasts have an integrated transgene copy number per cell of at least 0.74.

93. The method of claim 84, wherein the transduced human dermal fibroblasts have an integrated transgene copy number per cell of at between about 0.1 to about 5.

94. The method of claim 84, wherein the transduced human dermal fibroblasts have an integrated transgene copy number per cell of between about 0.1 to about 1.

95. The method of claim 84, wherein the transduced human dermal fibroblasts have an integrated transgene copy number per cell of between about 0.4 to about 1.

96. The method of claim 84, wherein the transduced human dermal fibroblasts have an integrated transgene copy number per cell of between about 0.4 to about 0.75 .

100. The method of claim 84, wherein the transducing lentiviral vector is in the form of a lentiviral vector particle.

101. The methd of claim 100, wherein the transducing lentiviral vector particle is INXN-2002.

102. The method of claim 100, wherein the transducing lentiviral vector particle is INXN-2004.

103. The method of claim 86, wherein the transduced human dermal fibroblasts or keratinocytes have an increased expression of C7 relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction.

104. The method of claim 86, wherein the transduced human dermal fibroblasts or keratinocytes have at least a 100-fold increased expression of C7 relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction.

105. The method of claim 86, wherein the transduced human dermal fibroblasts or keratinocytes have at least a 200-fold increased expression relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction.

106. A method of increasing the integrated transgene copy number per cell in genetically modified human dermal fibroblasts or keratinocytes comprising

(a) contacting a transducing lentiviral vector comprising a nucleotide sequence encoding a *COL7A1* gene or a functional variant thereof with a human dermal fibroblast

or keratinocyte obtained from a C7-deficient patient to form a first transduction composition,

(b) subjecting said first transduction composition to spinoculation to form transduced human dermal fibroblast or keratinocyte,

(c) contacting the transduced human dermal fibroblasts or keratinocytes of step (b) with a second transducing lentiviral vector to form a second transduction composition;

(d) subjecting said first transduction composition to spinoculation to form transduced human dermal fibroblast or keratinocyte,

wherein the integrated transgene copy number of the transduced human dermal fibroblasts or keratinocytes is at least 5, 10, 15, 20, 25, 27, 28, 29, 30, 35, 40, 45, or 50-fold higher relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction step.

107. The method of claim 106, wherein the first and second tranducing lentiviral vector are INXN-2004.

108. The method of claim 106, wherein the cells are human dermal fibroblasts.

109. The method of claim 106, wherein the integrated transgene copy number of the transduced human dermal fibroblasts or keratinocytes is at least 5-fold higher relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction step.

110. The method of claim 106, wherein the integrated transgene copy number of the transduced human dermal fibroblasts or keratinocytes is at least 10-fold higher relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction step.

111. The method of claim 106, wherein the integrated transgene copy number of the transduced human dermal fibroblasts or keratinocytes is at least 27-fold higher relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction step.

112. The method of claim 106, wherein the integrated transgene copy number of the transduced human dermal fibroblasts or keratinocytes is about 5-fold higher relative to

transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction step.

113. The method of claim 106, wherein the integrated transgene copy number of the transduced human dermal fibroblasts or keratinocytes is about 10-fold higher relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction step.

114. The method of claim 106, wherein the integrated transgene copy number of the transduced human dermal fibroblasts or keratinocytes is about 27-fold higher relative to transduced human dermal fibroblasts or keratinocytes not subjected to spinoculation or a second transduction step.