(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 January 2022 (27.01.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/020601 A1

(51) International Patent Classification:
A01K 67/027 (2006.01) *C12N 15/00* (2006.01)
A01K 67/02 (2006.01) *C12N 15/877* (2010.01)
A61D 19/04 (2006.01) *C12N 5/02* (2006.01)
A01K 67/00 (2006.01)

(21) International Application Number:
PCT/US2021/042790

(22) International Filing Date:
22 July 2021 (22.07.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/056,386 24 July 2020 (24.07.2020) US

(71) Applicant: **INGURAN, LLC** [US/US]; 22575 State Highway 6 South, Navasota, Texas 77868 (US).

(72) Inventors: **KENDALL, David**; 22575 State Highway 6 South, Navasota, Texas 77868 (US). **MORENO, Diego**; 22575 State Highway 6 South, Navasota, Texas 77868 (US).

(74) Agent: **RAHMAN, Hashim** et al.; 22575 State Highway 6 South, Navasota, Texas 77868 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) **Title:** METHODS FOR GENERATING, EVALUATING, GENE EDITING AND CLONING PLURIPOTENT STEM CELLS COMPRISING A LETHAL HAPLOTYPE

	Haplotype	Hap Annotation	Seq
SEQ ID NO 1	HH2	Unknown causal	AAATTTTCAAAGGTAAGAAAAGTGGGGAGTTTGTG[A/G]CTGCATGATACCAAATGAATACTGCAGGCATTTTA
SEQ ID NO 2	JH1	Causal	TTAGACAGACCACTCAGGATGCCCCTGAAGAGGTT[C/T]GAAACCGTGACTTCAGGAGAGAGTTGGAGGAGAGA
SEQ ID NO 3	HH2	Unknown causal	ATTCTAAATCACTGGACCACCAGGGAACTCCTTAT[C/T]CAACACTTTTCTTTGTAATAAAATGTTAGATAGTG
SEQ ID NO 4	JH1	Flanking	TCATTAGAGGGCTGTATCAATAATCCTCATATCCT[A/G]TTCCGAGATTCTTGCATGCTAGGAGTTGAATTTGA
SEQ ID NO 5	HH2	Unknown causal	CCCCAACAATCATGAGCTTCCCATCTCAATGTGAC[A/G]TCAGTGAAGGCCACATGAGGAGTAGTGATGAGGTA
SEQ ID NO 6	BH1	Unknown causal	TTCTAAGTATATGCTGGCCATTGTCCTCTTGATAA[A/G]AGGTGTCACTCATGTTGTGGTGACCAAAGCCTGCA
SEQ ID NO 7	HH3	Flanking	CACAGTTCTGTGCACCCAGGTGTCAACAACTCCAT[A/C]ACACTCGGCCTAAAGCATCGCTTTGCTGGTGTCTG
SEQ ID NO 8	HH2	Unknown causal	GTCTATTCCTGGAACTTTCCCCACTTCAGGGAACC[C/T]AGGAGCAGGATAGCAAACCCACCCAATCATTCCAC
SEQ ID NO 9	BH1	Unknown causal	AGCACGAGCTCACAGAAACTGCGAGAAGGACACCA[C/T]GAACACACGGAGCAAAGTCCAGTACCTCTTGACTC
SEQ ID NO 10	BH1	Unknown causal	AGACAAATGGAGGCAACAGAGCATAGATGTGAGAC[A/G]AGTTCCAGGCCTGACGCAGCACGAAGGAGAAACTG
SEQ ID NO 11	HH2	Unknown causal	AGGAAGAATGGAATATTAAAGCAGCAGATTAGATT[A/G]CAGGTAAAACCACCTTGGCCAGGGGGACTACAGCA
SEQ ID NO 12	JH2	Unknown causal	ATATAGTAGAGAAATATACATGTCTCAGACCACCC[C/T]TCCTCCTCTGATGGGCCCAGGTACATGAATTTTTT
SEQ ID NO 13	HH2	Unknown causal	ACTGATCTAATCTGACCCATTGGCCTTTTTTTTTT[A/T]AATTGAGCTTGCATGAGCTGTTAATGTATTTTGAA
SEQ ID NO 14	HH2	Unknown causal	ACTCCTACTGAGTCAACTGTAAATACTGTGTATCT[C/T]AGAAGTTAATTGTCAGTAAAAAACACATGAGCACA
SEQ ID NO 15	HH3	Flanking	TAGGACATGATACATGTTGGGTAAAGCAAAAGGCC[A/G]TGCCAAAATTCATATTGTTAAAAAGAAACTTGAGG
SEQ ID NO 16	BH1	Unknown causal	GTGCTCATCCAAGGGTGTTCTGAGCAGGAAAGGCC[A/G]CTGGTCAGGCAGCCCAGAAGGTGAGTTTGCCCATC
SEQ ID NO 17	BH2	Flanking	CTGACAGAATACTTGAATACAGGCAGAGCTTGCAG[A/G]TTTTGCATGCTTGGTTCCAACCACCACAATAAAGT
SEQ ID NO 18	BH1	Unknown causal	TGCTGCTCAATGTTCCCAAATTGTGTGCCACCAGA[A/G]GGCCTGATTTTGCAGGGAAAAGAGAAGTGGGTCCC
SEQ ID NO 19	BH1	Unknown causal	AGTGCTGGCCACCGCTGGACAATCAGCCTGAGGCC[A/G]GAGCTTGGAGGAACAAAGTCCAAGGACCGCAAACA
SEQ ID NO 20	BH1	Unknown causal	CTCCTGGCTGGGGCTGGGTGGCTGCAGGGAGGTGA[A/G]TCTGGGGCCAAGGTTAAAGCACCTGTAGACATTTC
SEQ ID NO 21	HH2	Unknown causal	CTTCCGGTGATTGAGGGTTATAAAAATGCCATTTA[C/T]CCAGCAAGGATGGTCATGTGAATTGGAGACTGATC
SEQ ID NO 22	JH2	Unknown causal	AGTAAATCTCTCAAACAAGTTTATAACCGTGGCCT[C/G]CACAAAAGGCATCAAATCTGGGTCTCGCATGAAAA
SEQ ID NO 23	HH2	Unknown causal	TTCATTCTGGTCTCTGCTTAAGTGTCATCTCCCCA[A/G]AGAGGCCTTTTTTGGCCACCCTATGTAAGGAGAGT
SEQ ID NO 24	BH1	Unknown causal	AGGCATGGCTCCCTCCACATAAACACAGAGCCTCA[C/T]AGCAAGGCCAGAACCTATAGGCAAAGGGAGTTCAA
SEQ ID NO 25	BH1	Unknown causal	CACCCCCTGCCAGCCTAAGTGGCTCCTAACCCCTC[A/G]GTGCATCACCTATAAGGCCTGCACACAGGCACAAA
SEQ ID NO 26	HH4	Flanking	ACAGGAGAAGGGAGAGAACACTGGCAGGCACTAAC[A/G]TATCCACTTTATCTACAGTGCTGTAGCTTTTAGTT

Figure 1A

(57) **Abstract:** The invention includes a method of evaluating a bovine embryo by fertilizing an egg obtained from a first bovine heterozygote of a recessive lethal haplotype with sperm cells obtained from a second bovine heterozygote of the recessive lethal haplotype; producing the embryo from the fertilized egg, wherein the embryo is homozygous for the lethal haplotype; establishing a cell culture from the embryo; collecting a plurality of cultured cells; and obtaining omics data, comprising one or more features, from the plurality of cultured cells.

WO 2022/020601 A1

[Continued on next page]

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

METHODS FOR GENERATING, EVALUATING, GENE EDITING AND CLONING PLURIPOTENT STEM CELLS COMPRISING A LETHAL HAPLOTYPE

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of United States Provisional Patent Application No 63/056,386 filed July 24, 2020. The entire disclosure of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

In livestock, recessive lethal haplotypes pose a significant problem for breeders, since a relatively large emphasis is placed on fertility, among other economic traits. Additionally, efforts by breeders to avoid producing offspring that are homozygous for these lethal haplotypes often result in suboptimal breeding values and slower genetic progress. Moreover, because certain lethal recessive haplotypes result in early embryonic death, studying and evaluating homozygous individuals has proven to be difficult if not impossible, thereby impairing the livestock industry's ability to develop more efficient breeding strategies and treatments using biotechnology.

SUMMARY OF THE INVENTION

One embodiment of the invention comprises a method of evaluating a bovine embryo comprising fertilizing an egg obtained from a first bovine heterozygote of a recessive lethal haplotype with sperm cells obtained from a second bovine heterozygote of the recessive lethal haplotype; producing the embryo from the fertilized egg, wherein the embryo is homozygous for the lethal haplotype; establishing a cell culture from the embryo; collecting a plurality of cultured cells; and obtaining omics data, comprising one or more features, from the plurality of cultured cells. A further embodiment comprises the steps of calculating feature weights for the one or more

features; and calculating a production value, a genotypic value or a breeding value based on the calculated feature weights. In a particular embodiment, the recessive lethal haplotype is selected from the group consisting of: AH1, HH1, HH2, HH3, HH4, HH5, HH6, JH1, JH2, BH1 and BH2. In an even more particular embodiment, the step of establishing a cell culture from the embryo comprises culturing the embryo at the blastocyst stage. In a further aspect of this embodiment, the omics data is comprised of genotypic, proteomic or transcriptomic data. In a more specific embodiment, the step of establishing a cell culture comprises placing the embryo, or a cell sample from the embryo, on a first substrate in a first culture media comprising a base media (e.g. TeSR-E6 (Stem Cell Technologies, Canada)), a low free-fatty acid BSA (bovine serum albumin), Fibroblast Growth Factor 2 and an inhibitor of Wnt signaling for 11 to 14 days and thereafter dissociating cell outgrowths on the first substrate and placing the cell outgrowths on a second substrate in a second culture media comprising a ROCK (Rho-associated coiled-coil containing kinase) inhibitor. More specifically, in a further embodiment, the first substrate and the second substrate are comprised of irradiated mouse embryonic fibroblasts. In a yet further embodiment, the cell sample from the embryo comprises cells from the inner cell mass of the embryo.

Another embodiment of the invention encompasses a method of evaluating a plurality of bovine embryos comprising fertilizing a plurality of eggs obtained from one or more female bovine heterozygotes of a recessive lethal haplotype with sperm cells obtained from one or more male bovine heterozygotes of the recessive lethal haplotype; producing the plurality of embryos from the plurality of fertilized eggs, wherein the embryos are homozygous for the lethal haplotype; identifying a first group of one or more embryos from the plurality of embryos that are underdeveloped or dead after a first period of time; obtaining omics data from the identified first group. In a particular embodiment, the omics data comprises one or more features, and in a further

embodiment comprises the steps of calculating feature weights for the one or more features; and calculating a production value, a genotypic value or a breeding value based on the calculated feature weights. An additional embodiment may also comprise the steps of identifying a second group of one or more embryos from the plurality of embryos that are underdeveloped or dead after a second period of time; and obtaining omics data from the identified second group. In a particular embodiment, the omics data is comprised of genotypic, proteomic or transcriptomic data. In an even more particular embodiment, the recessive lethal haplotype is selected from the group consisting of: AH1, HH1, HH2, HH3, HH4, HH5, HH6, JH1, JH2, BH1 and BH2.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A to 1I list SNP markers of lethal haplotypes.

DETAILED DESRIPTION OF THE INVENTION

One aspect of the invention is encompassed by a method of generating pluripotent stem cells from an individual that is homozygous for a lethal haplotype. An additional aspect of the invention encompasses the use of gene editing to correct a causative mutation (including one or more base substitutions, deletions or insertions) of a lethal haplotype. A further aspect of the invention encompasses differentiating pluripotent stem cells comprising a homozygous lethal haplotype into other cell types in order to study the effects of the lethal haplotype. Finally, the invention also encompasses a method for determining the developmental stage or age of an individual when the effects of a lethal haplotype phenotypically manifest themselves.

Generating Zygotes/Embryos that are Homozygous for a Lethal Haplotype

In one embodiment of the invention, in order to generate or produce an individual, which includes a zygote, an embryo or a fetus, that is homozygous for a lethal haplotype, one first identifies a male carrier and a female carrier of the lethal haplotype. Carriers of lethal haplotypes

can be identified by omics data, including but not limited to transcriptomic and genomic data, which may include DNA sequence data such as obtained by nucleotide sequencing and genotype data such as obtained using a single nucleotide polymorphism (SNP) microarray or nucleotide sequencing.

Examples of relevant lethal haplotypes in the bovine livestock industry that result in early embryonic death include but are not limited to Ayrshire Haplotype 1 (AH1), Brown Swiss Haplotype 1 (BH1), Brown Swiss Haplotype 2 (BH2), Holstein Haplotype 1 (HH1), Holstein Haplotype 2 (HH2), Holstein Haplotype 3 (HH3), Holstein Haplotype 4 (HH4), Holstein Haplotype 5 (HH5), Holstein Haplotype 6 (HH6), Jersey Haplotype 1 (JH1) and Jersey Haplotype 2 (JH2). Table 1 below lists the gene believed to be affected by each of these haplotypes.

Table 1.

Haplotype	Result of homozygous haplotype	Affected Gene	Location of Mutation (chromosome no.: base pair region)
BH1	Early embryonic or fetal death	unknown	7: 42,811,272 – 47,002,161
BH2	Early embryonic or fetal death	tubulin delta 1 (TUBD1)	19: 11,063,520
HH1	Early embryonic or fetal death	apoptotic peptidase activating factor 1 (APAF1)	5: 63,150,400
HH2	Early embryonic or fetal death	unknown	1: 94,860,836 – 96,553,339
HH3	Early embryonic or fetal death	structural maintenance of chromosomes 2 (SMC2)	8: 95,410,507

HH4	Early embryonic or fetal death	Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase (GART)	1: 1,277,227
HH5	Early embryonic or fetal death	transcription factor B1, mitochondrial (TFB1M)	9: 93,223,651 – 93,370,998
HH6	Early embryonic death	SDE2 telomere maintenance homolog (SDE2)	16: 27,833,776 – 29,661,958 or 16: 31,162,715 – 32,019,139
JH1	Early embryonic or fetal death	CWC15 spliceosome-associated protein (CWC15)	15: 15,707,169
JH2	Early embryonic or fetal death	unknown	26: 8,812,759 – 9,414,082

Additionally, Figure 1 lists SNP markers for certain lethal haplotypes (SEQ ID NO 1 to SEQ ID NO 221). In Figure 1, the SNP within each nucleotide sequence is denoted by brackets.

Each haplotype is identifiable in individuals through the use one or more genetic markers. These haplotypes are believed to be recessive, so only individuals with two copies of the haplotype (i.e., homozygous) will exhibit abnormalities. Individuals with only one copy, or no copies, of the haplotype will be normal. In the context of the invention, a haplotype may comprise one or more alleles.

One embodiment of the invention encompasses a method of producing a homozygous individual for a lethal haplotype in which gametes are obtained from a male carrier and a female carrier of the haplotype, and an obtained egg is fertilized with an obtained sperm cell in vitro to

produce a homozygous zygote or embryo. Specifically, in certain aspects of the invention, in vitro produced zygote and embryos are produced in the laboratory by non-typical harvest of cattle oocytes, in vitro fertilization and embryo culture methodologies. In peripubertal heifers, prophase I immature cumulus oocyte complexes (COCs) are recovered from live standing females by using ultrasound guided transvaginal oocyte recovery (TVOR) system, also referred to as ovum pickup (OPU). In prepubertal heifers, ultrasound guided laparoscopic OPU is employed for COC recovery. When immature COCs are brought into the laboratory, they are placed into typical in vitro maturation (IVM) culture system where the most developmentally capable oocytes undergo spontaneous and programmed meiosis. After an overnight culture period, those oocytes that progress through meiosis I and accordingly shed their second polar body progressing to metaphase of the second meiotic division, and remain a plasma membrane intact and morphologically normal, those mature oocytes are placed into in vitro fertilization. Mature oocytes from individual females are placed into traditional IVF drops and mated to specific sires, using highly screened and accurate sperm capacitation treatments and sperm concentration per oocyte fertilized. Zygotes (day 1) are placed into traditional co-culture system and may be cultured to uterine stages of development by day 7-8 of culture.

In other embodiments of the invention, homozygous zygotes or embryos for a lethal haplotype are produced in vivo by traditional methods for synchronized supernumerary follicle production, artificial insemination and scheduled non-surgical transvaginal catheterized intrauterine embryo recovery (i.e., flushing).

As noted above, one aspect of the invention encompasses in vitro production of a homozygous individual for a lethal haplotype. By way of example only, the following oocyte

maturation procedure, IVF procedure, in vitro culture procedure and co-culture procedure may be used with the invention.

Oocyte Collection. Collect slaughterhouse oocytes and wash 1X with about 3mL Hepes washing media and with 1X with TCM-199 (Invitrogen, Carlsbad, CA) + 10% Fetal Bovine Serum (FBS). Culture in maturation media for 22 hrs in a CO_2 incubator at 38.5°C. In one embodiment, the maturation media contains TCM-199, FBS, pyruvate, chorionic gonadotropin (e.g., Chorulon (Intervet, Summit NJ)), follicle stimulating hormone (FSH) (e.g., Folltropin (Bioniche, Belleville, Canada)), estradiol, and at least one antibiotic. In a further embodiment, Amikacin (Sigma-Aldrich, St. Louis, MO) can be used as the antibiotic. In another embodiment, the maturation media may also comprise luteinizing hormone.

In one embodiment, the maturation media may comprise 5-20 ml of TCM-199 Earl's; 0.5-2ml of FBS (Thermo Fisher Scientific, Waltham, MA); 10-30 µl of pyruvate (prepared by adding 0.05-0.20 g of sodium pyruvate (Sigma-Aldrich, St. Louis, MO) to 5-20 ml of saline solution); 50-200 µl of chorionic gonadotropin (prepared by adding 5-20 UI of Chorulon (Intervet, Summit NJ) to 5-20 ml of TCM-199 Earl's); 5-20 µl of FSH (prepared by adding 0.001-0.01 g of Folltropin (Bioniche, Belleville, Canada) to 5-20 ml of TCM-199 Earl's); 5-20 µl of estradiol (prepared by adding 0.001-0.05 g of estradiol (Sigma-Aldrich, St. Louis, MO) to 5-20 ml of Etanol (Sigma-Aldrich, St. Louis, MO)); and 10-30 µl Amikacin (prepared by adding 0.1-1 g Amikacin sulfate salt (Sigma-Aldrich) to 20-40 ml of saline solution). In alternative embodiments, the maturation media may comprise the aforementioned components using different volumes but in the same proportion to each other, e.g., in one embodiment, the maturation media may comprise 10-40 ml of TCM-199; 1-4 ml of FBS; 20-60 µl of sodium pyruvate, etc. In a further embodiment, the maturation media comprises the above preparations of TCM-199 Earl's, FBS, pyruvate, chorionic

gonadotropin, FSH, estradiol and an antibiotic in the approximate ratio of 9 : 1 : 0.02 : 0.1 : 0.01: 0.01 : 0.02 by volume, respectively.

In Vitro Fertilization. Trim away cumulus cells from matured oocytes. Transfer them to a fertilization dish and return to the CO_2 incubator. Thaw frozen semen straws using standard procedures, centrifuge in 800μL of Pure Sperm gradient (Nidacon, Molndal, Sweden), or a percoll or similar gradient at 2500 RPM for 10 minutes to remove egg components, glycerol and other debris. Remove supernatant, leaving a loose pellet of live sperm. Combine pellets using a small amount of fertilization media and repellet at 1500 RPM for 3 minutes. Carefully remove supernatant. Then gently mix the pellet. After determining the desired insemination dose, inseminate the oocytes by adding sperm to the pellet, then culture in a dish and return to the CO_2 incubator for about 18-22 hours.

In Vitro Culture. Remove presumptive zygotes from the fertilization dish and transfer into a sterile 1.5 mL eppendorf tube. Allow zygotes to form a loose pellet and remove excess media to form a 1:1 ratio of pellet and solution. Rinse the eppendorf tube with TCM-199, place contents into a dish and wash with BSA media. Then culture presumptive zygotes (discard disfigured oocytes, as well as oocytes with yellow colored cytoplasm or vacuolated cytoplasm) in a dual gas incubator (5% CO_2, 5% O2) at 38.5°C for about 48 hours.

Co-culture. Transfer cleaved zygotes to co-culture dishes comprising the cumulus cells from the mature oocytes and FBS media topped with mineral oil, and incubate in a CO_2 incubator at 38.5°C until needed.

Generating Pluripotent Stem Cells from Embryos Homozygous for a Lethal Haplotype

One embodiment of the invention encompasses a method for generating pluripotent stem cells that are homozygous for a lethal haplotype. One step of the method comprises producing an

embryo that is homozygous for a lethal haplotype using gametes obtained from known carriers of the lethal haplotype.

In a particular embodiment of the invention, a hatched embryo is used to establish a pluripotent stem cell culture. Alternatively, the zona pellucida of an embryo (e.g., an expanded blastocyst) that is not hatched can be mechanically or chemically removed. Mechanical removal of the zona pellucida from an embryo can be accomplished in any suitable embryo splitting media using a needle or a laser, for example. Alternatively, the zona pellucida can be chemically removed, using pronase for example. In a particular embodiment of the invention, a 21G needle is used for mechanical separation of the zona pellucida from an embryo. In one embodiment, once denuded, the embryo is placed into a culture dish or well that is coated with mitotically inactivated feeder cells. In a particular embodiment of the invention, the inactivated feeder cells are comprised of irradiated mouse fibroblasts, and in an even more particular embodiment, are comprised of CF1 mouse embryonic fibroblasts (MEFs). In an alternative embodiment, the embryo is placed into a culture dish free of feeder cells. In certain embodiments of the invention, a culture dish free of feeder cells can be coated with a cell support material, for example, fibronectin, vitronectin or Matrigel (Corning Life Sciences, Tewksbury, MA).

The embryos are then cultured in a serum-free media comprising an inhibitor or antagonist of the Wnt/β-catenin pathway and either 1) a fibroblast growth factor (FGF) or 2) a member of the transforming growth factor beta (TGF-β) family or both. Examples of inhibitors of the Wnt/β-catenin pathway that may be used in the invention include, but are not limited to, inhibitors of Wnt response (IWR) including IWR1, IWR2, IWR3, IWR4 and IWR5 and inhibitors of Wnt production (IWP) including IWP1, IWP2, IWP3 and IWP4 and may be used a concentration of 0.5-5 μM, 1-4 μM, 2-3 μM or 2.5 μM. Examples of fibroblast growth factors that may be used in the invention

include, but are not limited to, FGF1, FGF2, FGF3 and FGF4 and may be used at a concentration of 10-30 ng/ml, 15-25 ng/ml, 17-23 ng/ml or 20 ng/ml. Examples of members of the TGF-β family that may be used, include but are not limited to TGF-β1, Activin A and Activin B and may be used at a concentration of 10-30 ng/ml, 15-25 ng/ml, 17-23 ng/ml or 20 ng/ml. In a particular embodiment of the invention, the serum free media is comprised of an IWR and either 1) FGF2 or 2) Activin-A or both. In a more particular embodiment of the invention, the serum free media is comprised of IWR1 and FGF2. In another embodiment of the invention, the serum free media is comprised of IWR1, FGF2 and Activin A. In one embodiment of the invention, the serum-free media is comprised of TeSR-E6 (Stem Cell Technologies, Canada), although any serum-free media suitable for use in stem cell culturing may be used as a base media in the invention. In a particular embodiment of the invention, the serum-free media is supplemented with a serum protein, for example bovine serum albumin (BSA) at a concentration of 10-20 mg/ml, 12-17 mg/ml, 13-15 mg/ml or 13.4 mg/ml. In particular, embodiment of the invention, the BSA is comprised of a low free fatty acid BSA. The media in which the pluripotent stem cells are cultured may also comprise a suitable antibiotic, including but not limited to penicillin or streptomycin. In a particular embodiment of the invention, the serum free culture media comprises IWR1, FGF2, Activin A and BSA. In another embodiment, the serum free culture media comprises IWR1, FGF2 and Activin A. In a more particular embodiment, the serum fee culture media comprises TeSR-E6, IWR1 and FGF2; TeSR-E6, IWR1, FGF2 and BSA; TeSR-E6, IWR1, FGF2 and Activin A; or TeSR-E6, IWR1, FGF2, Activin A and BSA. In a particular embodiment of the invention, the serum-free media may be comprised of Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12), examples of which include TeSR1 media disclosed by Ludwig et al. (Nature Methods, vol. 3, no. 8, August 2006, pp. 637-646); modified mTeSR1 media as disclosed by Wu

et al. (Nature, 2015 May 21; 521(7552): 316-321), which lacked FGF2 and TGFβ1 but was supplemented with 20 ng/ml FGF2 and 2.5 μM IWR1; and N2B27 medium as disclosed by Tong et al. ("Generating gene knockout rats by homologous recombination in embryonic stem cells," Nat Protoc. 2011 June; 6(6)). In a particular embodiment of the invention, pluripotent stem cells can be cultured using a culture dish free of feeder cells and a serum-free media comprising FGF2, IWIR1 and Activin A. In an even more particular embodiment, pluripotent stem cells can be cultured using a culture dish free of feeder cells and a serum-free media comprising FGF2, IWIR1, Activin A and BSA.

Once the embryo is placed in a suitable culture media as above, the embryo is cultured at approximately 30-40°C, 35-39°C, 36-38°C, or 37°C and with approximately 5% CO_2. After approximately 50-100 hours, 60-90 hours, 70-80 hours, or 72 hours, the culture media is replaced. If an embryo has failed to attach to the layer of feeder cells in the culture at the time the culture media is replaced, the embryo can be mechanically pressed against the bottom of the culture dish, using for example, a needle. After approximately 11 to 14 days in culture, any outgrowths are dissociated using trypsin or TrypLE (Gibco, Thermo Fisher Scientific, Waltham, MA) and passed to a new culture dish or well coated with inactivated feeder cells, and the composition of the media used to establish the initial cell culture may be used again in the replacement media. In a particular embodiment, this media is used to replace the initial cell culture media is supplemented with an inhibitor of Rho-associated, coiled-coil containing protein kinase (ROCK) at a concentration of 1-20 μM, 3-17 μM, 5-15 μM or 10 μM. In a particular embodiment, inhibitors of ROCK with which the media can be supplemented, include but are not limited to, Y-27632 and fasudil. In an even more particular embodiment, the media used to replace the initial culture media is supplemented with Y-27632. In a particular embodiment, ROCK inhibitor (for example Y-27632) is used in the

culture media for 24 hours, and after 24 hours, the culture media is replaced with a culture media that does not comprise a ROCK inhibitor (for example Y-27632). On the day of the first cell passage (e.g., day 11) or at any passage thereafter, omics data may be obtained or extracted from for the cultured cells using a portion of the dissociated (i.e., unbound) cells or the culture media. In a more particular embodiment, dissociated cells may be genomically evaluated, by for example, genotying with a SNP microarray or by DNA sequencing. In an even further embodiment, the genotype or DNA sequence may be analyzed for the presence of a lethal haplotype using one or more genetic markers for the lethal haplotype.

Omics evaluation

In the context of the invention, "omics data" may include, but is not limited to, genomic, proteomic, transcriptomic, epigenomic, microbiomic or metabolomic data. In one embodiment of the invention, omics data is derived or obtained from molecules (small or large) or any other substances (ions, elements, etc.) obtained or extracted from a cell or tissue sample or detected in the cell or tissue sample. Additionally, omics data may be derived or obtained from molecules (small or large) or any other substances (ions, elements, etc.) obtained from fluid or media surrounding a cell or tissue sample. Both the presence and the quantity of such molecules or substances within a sample may be determined. Any known method in the art for detecting, measuring, quantifying or assaying molecules or other substances may be used with the invention, including but not limited to molecular hybridization, immunohistochemistry, real time quantitative PCR, quantitative reverse transcription PCR, blotting, nucleotide sequencing, protein sequencing, nuclear magnetic resonance spectroscopy, mass spectroscopy, liquid chromatography, gas chromatography and electrophoresis. In a specific embodiment, a transcriptome may be profiled using a microarray.

In a particular embodiment, transcriptomic, proteomic or metabolomic data can be derived from RNA, proteins or metabolites, respectively, found within a cell or tissue sample. Such a cell or tissue sample may be cryopreserved and then subsequently thawed for extraction of DNA or RNA or to obtain proteins or metabolites for profiling or any molecules providing omics data.

In one embodiment of the invention, omics data comprises features. For example, for metabolomic data, each assayed or measured metabolite can constitute a feature. In one embodiment, a feature may simply comprise the presence or absence of a particular molecule or substance, e.g., the presence of a particular metabolite or transcript, or alternatively a feature may comprise the quantity of a particular molecule or substance, e.g., the quantity of a particular metabolite or transcript. For example, the quantity of glucose in a tissue or blood sample can comprise a feature.

With respect to genomic data, in various embodiments of the invention, genomic data may comprise DNA or RNA-related data obtained from oligonucleotide arrays or other hybridization assays, DNA sequence data or RNA sequence data. In a specific embodiment of the invention, genomic data may be obtained from whole or partial genome sequencing using any technique known in the art. In addition to obtaining genomic DNA sequences, in other embodiments of the invention, RNA may also be sequenced, including messenger RNA (mRNA), precursor mRNA (pre-mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), non-coding RNA (ncRNA), long RNA, including long non-coding RNA (lncRNA) and small RNA, including micro RNA (miRNA), small interfering RNA (siRNA), small nucleolar RNA (snoRNAs), Piwi-interacting RNA (piRNA), tRNA-derived small RNA (tsRNA) and small rDNA-derived RNA (srRNA). In addition to sequencing such molecules, it is also contemplated that real time quantitative PCR or quantitative reverse transcription PCR may be used to quantify DNA or RNA in a sample.

DNA Extraction and Amplification

Another aspect of the invention encompasses sequencing or genotyping pluripotent stem cells homozygous for a lethal haplotype. In a specific embodiment, the DNA of cultured pluripotent stem cells can be used for sequencing or genotyping. Pluripotent stem cell DNA may first be extracted and then amplified (via PCR) so that there is a sufficient amount of DNA for sequencing or genotyping.

For genomic analysis, approximately 200 ng of double stranded DNA should be extracted per sample DNA at concentration per sample of 50 ng/ul. In certain embodiments of the invention, the DNA is used to confirm the presence of a lethal haplotype and/or confirm that the lethal haplotype has been corrected or suppressed via genetic modification. The remaining cells in culture remain in cell culture for passage and eventual harvest and cryopreservation for later diagnostic, cytogenetic and biological productive use such as gene editing and cloning.

By way of example, the following DNA extraction and amplification procedure may be used in certain embodiments of the invention. One skilled in the art will know that variations on this method exist and that this method should not be construed to limit the functionality or scope of the current invention. This method is illustrative only.

1.5 ml tubes containing a cell suspension of pluripotent stem cells are spun at ≥10000 x g in a microcentrifuge for 45 seconds to pellet the cells. The suspension solution is pipetted off carefully so as to not remove the pelleted cells. Approximately 50 µl of suspension solution is left in each tube. The tubes are then vortexed for 10 seconds to resuspend the cell pellets. 300 µl of Tissue and Cell Lysis Solution (Epicentre; Madison Wisconsin; Catalog # MTC096H) containing 1 µl of Proteinase K (Epicentre; Madison Wisconsin; at 50 ug/µl; Catalog #MPRK092) is then added to each tube and mixed. The tubes are incubated at 65°C for 30 minutes and vortexed at 15

minutes. The samples are cooled to 37°C. Afterwards 1 µl of 5 mg/µl RNase A (Epicentre; Madison Wisconsin; at 5 mg/ml; Catalog # MPRK092) is added to each sample and then mixed. The samples are then incubated at 37°C for 30 minutes. The samples are then placed in a 4°C cooler for 5 minutes. 175 µl of MPC Protein Precipitation Reagent (Epicentre; Madison Wisconsin; Catalog # MMP095H) is added to each sample, and the samples vortexed vigorously for 10-15 seconds. The samples are centrifuged in order to pellet debris for 8 minutes at ≥10000 x g. The supernatant is transferred to a clean microcentrifuge tube. 600 µl of cold (-20°C) isopropanol is added to the supernatant. Each tube is then inverted 30-40 times. The DNA is pelleted by centrifugation for 8 minutes in a microcentrifuge at ≥10000 x g. The isopropanol is poured off without dislodging the DNA pellet. The pellet is rinsed once with 70% ethanol and then the ethanol is carefully poured off so as not to disturb the DNA pellet. The residual ethanol is removed with a pipet, and the DNA pellet is allowed to air dry in the microcentrifuge tube. Once dried, the DNA pellet is resuspended in 20 µl Tris-EDTA.

In certain embodiments of the invention, DNA from pluripotent stem cells can be extracted using the Purelink Genomic Kit Cat # K1820-00 (Invitrogen). In further embodiments, once the DNA is extracted, it can be put through a whole genome amplification protocol using the Illustra Genomiphi V2 DNA amplification kit (GE Lifesciences), which uses the phi29 DNA polymerase to amplify the genome.

Genotyping DNA

In one aspect of the invention, extracted and/or amplified DNA from stem cells may be genotyped using genomic single nucleotide polymorphism (SNP) arrays or chips, which are readily available for various species of animals from companies such as Illumina and Affymetrix. Alternatively, the entire genome can be sequenced using methods well-known in the art. Low

density and high density chips are contemplated for use with the invention, including SNP arrays comprising from 3,000 to 800,000 SNPs. By way of example, a "50K" SNP chip measures approximately 50,000 SNPs and is commonly used in the livestock industry to detect lethal haplotypes and to establish genetic merit or genomic estimated breeding values (GEBVs).

Nucleotide Sequencing

One aspect of the invention comprises nucleotide sequencing extracted DNA or RNA. In certain embodiments of the invention, nucleic acid is extracted from pluripotent stem cells homozygous for a lethal haplotype, or that have been genetically modified, using any known method known in the art, including but not limited to Sanger sequencing and high throughput sequencing, which includes next generation (short read) sequencing and third generation (long read) sequencing. In one embodiment of the invention, one read with short read sequencing comprises approximately 100 to 300 base pairs, and one read with long read sequencing comprises approximately 10,000 or more base pairs. Nonlimiting examples of sequencing methods for use in the invention include single-molecule real time sequencing, ion semiconductor sequencing, pyrosequencing, sequencing by synthesis, combinatorial probe anchor synthesis, sequencing by ligation, nanopore sequencing, massively parallel signature sequencing, polony sequencing, DNA nanoball sequencing, heliscope single molecule sequencing and sequencing using droplet based microfluidics or digital microfluidics.

Cryopreservation of Cells

The following method of cryopreserving cells may be used in the invention and is presented by way of example only. Any method known in the art for cryopreserving stem cells may be used in the invention.

Cells are washed twice with phosphate-buffered saline (PBS) and then incubated with prewarmed TrypLE Select (Gibco) at 37°C for 8 to 10 minutes. Then, the cells are detached by gentle pipetting and the cell suspension is transferred to a conical 15mL tube containing 5 to 10 ml of culture media and centrifuged at 300 x g for 10 minutes. After centrifugation, the supernatant is removed carefully to avoid disturbing the pellet and cold (2-8°C) CryoStor CS10 (StemCell Technologies) is added. After mixing thoroughly, the suspension is transferred to a cryovial. The cells are cryopreserved using a standard slow-rate cooling procedure; cryovials are placed at -80°C in a styrofoam box for 24 hours and then stored in liquid nitrogen.

Genetic Modification

One aspect of the invention encompasses methods of genetically modifying stem cells of the invention. In particular, one aspect of the invention encompasses correcting or suppressing one or more mutations responsible for a lethal haplotype in a pluripotent stem cell and then 1) using the stem cell as a donor to create a clone or 2) using the stem cell to derive/generate gametes and then producing progeny from the gametes. In a particular embodiment, gene editing can be used to make targeted changes to gene sequences that remain after cell division. Clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR associated endonuclease (Cas9) may be used to repair a mutated gene such as in lethal haplotype by providing a DNA template of the normal gene that can be incorporated through a process known as homology directed repair (HDR). Supharattanasitthi et al. demonstrated CRISPR/Cas9-mediated one step bi-allelic change of genomic DNA in induced pluripotent stem cells in vitro using dual antibiotic selection (Scientific Reports (2019) 9:174). In one embodiment, the method outlined in Supharattanasitthi et al. can be used to alter the genomic DNA of the pluripotent stem cells to correct or eliminate a lethal haplotype.

Cloning

One aspect of the invention encompasses cloning using a pluripotent stem cell of the invention as a donor. Mammals have been cloned using embryonic stem cells. See, e.g., Wakayama et al., Proc Natl Acad Sci USA, 1999 Dec 21; 96(26):14984-14989. In a particular embodiment, a lethal haplotype is corrected/suppressed via gene editing in a pluripotent stem cell and then the cell is used as a donor cell in cloning. The following cloning procedure is presented by way of nonlimiting example only.

Oocyte Enucleation. *In vivo* matured oocytes are collected from donor females. Oocytes with attached cumulus cells or devoid of polar bodies are discarded. Cumulus-free oocytes are divided into two groups: oocytes with only one polar body evident (metaphase II stage) and the activated telophase II protocol (oocytes with one polar body and evidence of an extruding second polar body). Oocytes in telophase II are cultured in M199+10% FBS for 3 to 4 hours. Oocytes that are activated during this period, as evidenced by a first polar body and a partially extruded second polar body, are grouped as culture induced, calcium activated telophase II oocytes (Telophase II-Ca+2) and enucleated. Oocytes that have not activated are incubated for 5 minutes in PBS containing 7% ethanol prior to enucleation. Metaphase II stage oocytes (one polar body) are enucleated with a 25-30 micron glass pipette by aspirating the first polar body and adjacent cytoplasm surrounding the polar body (approximately 30% of the cytoplasm) presumably containing metaphase plate.

Telophase stage oocytes are prepared by two procedures. Oocytes are initially incubated in phosphate buffered saline (PBS, Ca^{+2}/Mg^{+2} free) supplemented with 5% FBS for 15 minutes and Cultured in M 199+10% FBS at 38° C. for approximately three hours until the telophase spindle configuration or the extrusion of the second polar body is reached. All the oocytes that

respond to the sequential culture under differential extracellular calcium concentration treatment are separated and grouped as Telophase II-Ca^{2+}. The other oocytes that do not respond are further incubated in 7% ethanol in M199+10% FBS for 5-7 minutes (Telophase II-ETOH) and cultured in M199+10% FBS for 2 to 4 hours. Oocytes are then cultured in M199+10%/ FBS containing 5 μg/ml of cytochalasin-B for 10-15 minutes at 38° C. Oocytes are enucleated with a 30 micron (OD) glass pipette by aspirating the first polar body and approximately 30% of the adjacent cytoplasm containing the metaphase II or about 10% of the cytoplasm containing the telophase II spindle. After enucleation the oocytes are immediately reconstructed.

Embryo Reconstruction. Pluripotent stem cells are harvested by trypsinizing (0.025% trypsin/0.5 mM EDTA) (Sigma) for 7 minutes. Single cells are resuspended in equilibrated M199+10% FBS supplemented with 2 mM L-glutamine, penicillin/streptomycin. The donor cell injection is carried out in the same medium as for enucleation. Donor cells are graded into small, medium and large before selection for injection to enucleated cytoplasts. Small single cells (10-15 micron) are selected with a 20-30 micron diameter glass pipette. The pipette is introduced through the same slit of the zona made during enucleation and donor cells are injected between the zone pellucida and the ooplasmic membrane. The reconstructed embryos are incubated in M199 30-60 minutes before fusion and activation.

Fusion and Activation. All reconstructed embryos (ethanol pretreatment or not) are washed in fusion buffer (0.3 M mannitol, 0.05 mM $CaCl_2$, 0.1 mM $MgSO_4$-, 9 mM K_2HPO_4, 0.1 mM glutathione, 0.1 mg/ml BSA in distilled water) for 3 minutes before electrofusion. Fusion and activation are carried out at room temperature, in a chamber with two stainless steel electrodes 200 microns apart (BTX® 200 Embryomanipulation System, BTX®-Genetronics, San Diego, Calif.) filled with fusion buffer. Reconstructed embryos are placed with a pipette in groups of 3-4 and

manually aligned so the cytoplasmic membrane of the recipient oocytes and donor CFF155-92-6 cells are parallel to the electrodes. Cell fusion and activation are simultaneously induced 32-42 hours post GnRH injection with an initial alignment/holding pulse of 5-10 V AC for 7 seconds, followed by a fusion pulse of 1.4 to 1.8 KV/cm DC for 70 microseconds using an Electrocell Manipulator and Enhancer 400 (BTX®-Genetronics). Embryos are washed in fusion medium for 3 minutes, then they are transferred to M199 containing 5 μg/ml cytochalasin-B (Sigma) and 10% FBS and incubated for 1 hour. Embryos are removed from M199/cytochalasin-B medium and co-cultured in 50 microliter drops of M199 plus 10% FBS with goat oviductal epithelial cells overlaid with paraffin oil. Embryo cultures are maintained in a humidified 39° C incubator with 5% CO_2 for 48 hours before transfer of the embryos to recipient females.

The following alternative cloning procedure is presented by way of nonlimiting example only.

Remove COC's from maturation medium and rinse through one dish of warm TL Hepes (MOFA GLOBAL, Bovi Pro Oocyte Washing Medium with BSA at 3 mg/ml, filtered). Transfer the COC's into a hyaluronidase drop within the dish of TL Hepes. The cumulus cells of the COC's are stripped by hand, first by using a 200 μl gel loader pipet tip to remove the outer layers of cumulus, then by using a denudation pipet to remove the remaining cumulus cells. Those oocytes that are clean (no cumulus cells) are transferred to a second dish of warm TL Hepes. If there are eggs that still have cumulus cells attached, transfer those to another drop of hyaluronidase and finish hand stripping them, then transfer to the TL Hepes dish. The maturation rate (MO, mature oocyte) is determined by checking each viable oocyte for an extruded polar body (PB). Those eggs that are not mature need to be enucleated immediately.

To a nunc well, add 0.50 ml of Cyto B medium (2 ml TL Hepes and 1µl Cytochalasin B (Sigma C-6762)) and 10 µl of Hoescht stain. Remove the oocytes from the maturation medium and rinse through warm TL Hepes, and then place the oocytes in the nunc well with the Hoescht stain for 15 minutes. At the end of the 15 minutes, rinse the oocytes through warm Cytochalasin B. Transfer to the lower drop of Cytochalasin B in a manipulation plate. Using the tip of a glass enucleation tip (25 µm inner diameter), pierce through the zona of an oocyte, carefully, bring your tip near to the chromosomes and slowly aspirate them out, taking as little cytoplasm as possible. When that oocyte is successfully enucleated move to a separate area of the dish and enucleate the rest of the oocytes. When you are ready to "reconstruct" (putting a cell into the enucleated oocyte), turn the UV light off, turn up the light to a comfortable level. Get an enucleated oocyte on the holder, using your tip, turn the oocyte until the "slit" made when enucleating the oocyte is in focus and in the same plane as your tip. Using the slit deposit one cell into the space between the zona and the cytoplasmic membrane of the oocyte to create a reconstructed embryo. Rinse all reconstructed embryos in TLHepes and then place into a maturation caffeine media (2 ml in vitro maturation media with 3.9 mg caffeine) until ready to fuse, at approximately 24 hours post maturation.

When ready to fuse (approximately at 23.5 – 24 hours post maturation), turn on the BTX machine (ECM Square Wave Electroporation System 830), making sure the settings are: mode LV, voltage 100, pulse length 40 µsec. Put the fusion chamber into the 100 mm dish, attach the red lead to the top wire, the black lead to the lower wire. Transfer reconstructed embryos in a dish comprising TLHepes. Pick up 8-10 reconstructed embryos and transfer to a dish containing a caffeine media (29.1 mg of caffeine in 15 ml of TLHepes) and let them sink. Transfer the 8-10 reconstructed embryos to another dish containing 2 ml of a SOR-based media (77.7 mg caffeine

in 50 ml of SOR media) and let the sink. Finally, transfer the 8-10 reconstructed embryos to the fusion chamber, which is filled with the SOR-based media, and once the reconstructed embryos are lined up, hit "pulse" on BTX machine. Once fused, transfer reconstructed embryos back to the dish containing the caffeine media for rinsing and then to a dish containing TLHepes, until all reconstructed embryos are fused. When fusions are completed, transfer all fused embryos to a dish containing 500 µl of a CR1aa/CR2 media (comprising 9.7 mg caffeine/5 ml CR2) for 1 hour.

To activate, (at 25 hours, 1 hour post-CR2 + caffeine), place reconstructed embryos in a nunc well along with 500 µl of ionomycin media (3 µl ionomycin and 3 ml TL Hepes) for 4 minutes. Remove and rinse three times in a dish of warm TL Hepes. Transfer the activated embryos to a nunc well containing 500 µl of cycloheximide media (at a concentration of 10 µg/ml of cycloheximide) and return to the incubator for 5 hours. After the 5 hours, remove the lysed oocytes, and rinse the remaining oocytes 6 times through the center area of the nunc well and return to the incubator. This is Day 0. On Day 5, move all embryos that are less than 8 cells to another nunc well, (count the number of 1 cells, cleaved, 8 cells and morula), leave the 8 cells and morula, add 25 µl of warm fetal bovine serum (FBS 5%) to the well and return to the incubator. On Day 6 and Day 7, check for any embryos that can be transferred. If there are, using a 2 ml Sartstedt tube, put 1 ml of Minitube holding media (BoviHold, Minitub International) into the tube and warm. Once the media is warm, transfer embryos to the tube and send to the farm for embryo transfer.

EXAMPLE 1

Embryonic stem cell line establishment

Five HH5 tested positive cows were submitted to artificial insemination (AI) by using frozen conventional sperm from a HH5 tested positive bull. 7 days post AI, the embryos were

recovered by uterine flushing and then were sent to the cell culture laboratory. To establish the cell lines, hatched embryos were used, and the expanded blastocyst that were not hatched were submitted to mechanical removal of the zona pellucida with a 21G needle into a drop of splitting media (ABT 360, USA). Ninety four denuded embryos were used, each embryo was placed into an individual well of a 12 well tissue culture-treated dish (Falcon) coated with irradiated CF1 Mouse Embryonic Fibroblasts (MEFs) (Gibco) and cultured with TeSR-E6 (Stem Cell Technologies, Canada) as base media, supplemented with (13.4 mg/mL) Low Free Fatty Acid BSA (MP Biomedicals NZ), 20 ng/mL bovine FGF2 (bFGF2) (Peprotech, USA), 2.5 μM IWR1 (Sigma, USA) and 1X penicillin-streptomycin (Gibco), at 37°C and 5% CO_2. After 72 hours, the culture media was changed for the first time, and the embryos that did not attach to the feeder layer, were mechanically pressed against the bottom of the well with a 21G needle, then the media was changed daily. After 11 days of culture, the outgrowths were dissociated using TrypLE (Gibco) and passed to a new well, previously coated with MEFs, and cultured in the same media supplemented with 10μM Y-27632 (Enzo life science). The day of the first passage, a sample of cells was collected to be sent for qPCR analysis for HH5 (Genetic Visions, Wisconsin, USA). A total of 25 cell lines were established and sent for qPCR analysis, 5 of them, were confirmed homozygous. Two samples of the confirmed homozygous HH5 gene were genotyped using (BovineHD BeadChip assay 777k, Illumina Inc., San Diego, CA). These 2 embryos – cell lines, received a homozygous evaluation for HH5 by the Council on Dairy Cattle Breeding (CDCB). These are the first global published results showing this event.

What we claim is

1. A method of evaluating a bovine embryo comprising:

fertilizing an egg obtained from a first bovine heterozygote of a recessive lethal haplotype with sperm cells obtained from a second bovine heterozygote of the recessive lethal haplotype;

producing the embryo from the fertilized egg, wherein the embryo is homozygous for the lethal haplotype;

establishing a cell culture from the embryo;

collecting a plurality of cultured cells; and

obtaining omics data, comprising one or more features, from the plurality of cultured cells.

2. The method of claim 1, further comprising the steps of:

calculating feature weights for the one or more features; and

calculating a production value, a genotypic value or a breeding value based on the calculated feature weights.

3. The method of claim 1, wherein the recessive lethal haplotype is selected from the group consisting of: AH1, HH1, HH2, HH3, HH4, HH5, HH6, JH1, JH2, BH1 and BH2.

4. The method of claim 1, wherein the step of establishing a cell culture from the embryo comprises culturing the embryo at the blastocyst stage.

5. The method of claim 1, wherein the omics data is comprised of genotypic, proteomic or transcriptomic data.

6. The method of claim 1, wherein the step of establishing a cell culture comprises placing the embryo, or a cell sample from the embryo, on a first substrate in a first culture media comprising a low free-fatty acid BSA (bovine serum albumin), Fibroblast Growth Factor 2 and an inhibitor of Wnt signaling for 5-15 days and thereafter dissociating cell outgrowths on the first substrate and placing the cell outgrowths on a second substrate in a second culture media comprising a ROCK (Rho-associated coiled-coil containing kinase) inhibitor.

7. The method of claim 6, wherein the first substrate and the second substrate are comprised of irradiated mouse embryonic fibroblasts.

8. The method of claim 6, wherein the cell sample from the embryo comprises cells from the inner cell mass of the embryo.

9. A method of evaluating a plurality of bovine embryos comprising:

fertilizing a plurality of eggs obtained from one or more female bovine heterozygotes of a recessive lethal haplotype with sperm cells obtained from one or more male bovine heterozygotes of the recessive lethal haplotype;

producing the plurality of embryos from the plurality of fertilized eggs, wherein the embryos are homozygous for the lethal haplotype;

identifying a first group of one or more embryos from the plurality of embryos that are underdeveloped or dead after a first period of time;

obtaining omics data from the identified first group.

10. The method of claim 9, further comprising the step of:

establishing cell cultures from one or more embryos in the identified first group.

11. The method of claim 9, wherein the omics data comprises one or more features.

12. The method of claim 11, further comprising the steps of

calculating feature weights for the one or more features; and

calculating a production value, a genotypic value or a breeding value based on the calculated feature weights.

13. The method of claim 9, further comprising the steps of:

identifying a second group of one or more embryos from the plurality of embryos that are underdeveloped or dead after a second period of time; and

obtaining omics data from the identified second group.

14. The method of claim 13, wherein the omics data is comprised of genotypic, proteomic or transcriptomic data.

15. The method of claim 9, wherein the recessive lethal haplotype is selected from the group consisting of: AH1, HH1, HH2, HH3, HH4, HH5, HH6, JH1, JH2, BH1 and BH2.

	Haplotype	Hap Annotation	Seq
SEQ ID NO 1	HH2	Unknown causal	AAATTTTCAAAGGTAAGAAAAGTGGGGAGTTTGTG[A/G]CTGCATGATACCAAATGAATACTGCAGGCATTTTA
SEQ ID NO 2	JH1	Causal	TTAGACAGACCACTCAGGATGCCCCTGAAGAGGTT[C/T]GAAACCGTGACTTCAGGAGAGAGTTGGAGGAGAGA
SEQ ID NO 3	HH2	Unknown causal	ATTCTAAATCACTGGACCACCAGGGAACTCCTTAT[C/T]CAACACTTTTCTTTGTAATAAAATGTTAGATAGTG
SEQ ID NO 4	JH1	Flanking	TCATTAGAGGGCTGTATCAATAATCCTCATATCCT[A/G]TTCCGAGATTCTTGCATGCTAGGAGTTGAATTTGA
SEQ ID NO 5	HH2	Unknown causal	CCCCAACAATCATGAGCTTCCCATCTCAATGTGAC[A/G]TCAGTGAAGGCCACATGAGGAGTAGTGATGAGGTA
SEQ ID NO 6	BH1	Unknown causal	TTCTAAGTATATGCTGGCCATTGTCCTCTTGATAA[A/G]AGGTGTCACTCATGTTGTGGTGACCAAAGCCTGCA
SEQ ID NO 7	HH3	Flanking	CACAGTTCTGTGCACCCAGGTGTCAACAACTCCAT[A/C]ACACTCGGCCTAAAGCATCGCTTTGCTGGTGTCTG
SEQ ID NO 8	HH2	Unknown causal	GTCTATTCCTGGAACTTTCCCCACTTCAGGGAACC[C/T]AGGAGCAGGATAGCAAACCCACCCAATCATTCCAC
SEQ ID NO 9	BH1	Unknown causal	AGCACGAGCTCACAGAAACTGCGAGAAGGACACCA[C/T]GAACACACGGAGCAAAGTCCAGTACCTCTTGACTC
SEQ ID NO 10	BH1	Unknown causal	AGACAAATGGAGGCAACAGAGCATAGATGTGAGAC[A/G]AGTTCCAGGCCTGACGCAGCACGAAGGAGAAACTG
SEQ ID NO 11	HH2	Unknown causal	AGGAAGAATGGAATATTAAAGCAGCAGATTAGATT[A/G]CAGGTAAAACCACCTTGGCCAGGGGGACTACAGCA
SEQ ID NO 12	JH2	Unknown causal	ATATAGTAGAGAAATATACATGTCTCAGACCACCC[C/T]TCCTCCTCTGATGGGCCCAGGTACATGAATTTTTT
SEQ ID NO 13	HH2	Unknown causal	ACTGATCTAATCTGACCCATTGGCCTTTTTTTTTT[A/T]AATTGAGCTTGCATGAGCTGTTAATGTATTTTGAA
SEQ ID NO 14	HH2	Unknown causal	ACTCCTACTGAGTCAACTGTAAATACTGTGTATCT[C/T]AGAAGTTAATTGTCAGTAAAAAACACATGAGCACA
SEQ ID NO 15	HH3	Flanking	TAGGACATGATACATGTTGGGTAAAGCAAAAGGCC[A/G]TGCCAAAATTCATATTGTTAAAAAGAAACTTGAGG
SEQ ID NO 16	BH1	Unknown causal	GTGCTCATCCAAGGGTGTTCTGAGCAGGAAAGGCC[A/G]CTGGTCAGGCAGCCCAGAAGGTGAGTTTGCCCATC
SEQ ID NO 17	BH2	Flanking	CTGACAGAATACTTGAATACAGGCAGAGCTTGCAG[A/G]TTTTGCATGCTTGGTTCCAACCACCACAATAAAGT
SEQ ID NO 18	BH1	Unknown causal	TGCTGCTCAATGTTCCCAAATTGTGTGCCACCAGA[A/G]GGCCTGATTTTGCAGGGAAAAGAGAAGTGGGTCCC
SEQ ID NO 19	BH1	Unknown causal	AGTGCTGGCCACCGCTGGACAATCAGCCTGAGGCC[A/G]GAGCTTGGAGGAACAAAGTCCAAGGACCGCAAACA
SEQ ID NO 20	BH1	Unknown causal	CTCCTGGCTGGGGCTGGGTGGCTGCAGGGAGGTGA[A/G]TCTGGGGCCAAGGTTAAAGCACCTGTAGACATTTC
SEQ ID NO 21	HH2	Unknown causal	CTTCCGGTGATTGAGGGTTATAAAAATGCCATTTA[C/T]CCAGCAAGGATGGTCATGTGAATTGGAGACTGATC
SEQ ID NO 22	JH2	Unknown causal	AGTAAATCTCTCAAACAAGTTTATAACCGTGGCCT[C/G]CACAAAAGGCATCAAATCTGGGTCTCGCATGAAAA
SEQ ID NO 23	HH2	Unknown causal	TTCATTCTGGTCTCTGCTTAAGTGTCATCTCCCCA[A/G]AGAGGCCTTTTTTGGCCACCCTATGTAAGGAGAGT
SEQ ID NO 24	BH1	Unknown causal	AGGCATGGCTCCCTCCACATAAACACAGAGCCTCA[C/T]AGCAAGGCCAGAACCTATAGGCAAAGGGAGTTCAA
SEQ ID NO 25	BH1	Unknown causal	CACCCCCTGCCAGCCTAAGTGGCTCCTAACCCCTC[A/G]GTGCATCACCTATAAGGCCTGCACACAGGCACAAA
SEQ ID NO 26	HH4	Flanking	ACAGGAGAAGGGAGAGAACACTGGCAGGCACTAAC[A/G]TATCCACTTTATCTACAGTGCTGTAGCTTTTAGTT

Figure 1A

SEQ ID NO 27	HH3	Flanking	TCATTTTCTTTGAAGGTCAATAGGGCCACTATCAC[A/G]TAAAATAAAAGTTACTTGGAAATAAGTTCTGTAAT
SEQ ID NO 28	BH2	Flanking	ATTAATAAACAGATACTTTCTTTTCCATTTCACCA[G/T]AAGTCTTTTGTACTGCGTTTTAAAACTAGCTTTGT
SEQ ID NO 29	HH2	Unknown causal	GAGTGGAGGCAATAGTGGTGGCCTCGCTGGGAAAT[G/T]AGCCTGCTTGCTGGGTCTATAGCAGGATGCCATTG
SEQ ID NO 30	BH1	Unknown causal	CAGCCACTCAGGCCTAGCCCTCCCTGCAAGTGGGT[G/T]GAGCAGATGAGAGCCCCGAAGGAAAGACTGAGCAT
SEQ ID NO 31	BH1	Unknown causal	CGGCCACGAGGATGCGGCCGTGCTCTTGGTAGTCC[A/G]CTTGGGTGCGCAGGTGCACGTGCGCGACCACAGCG
SEQ ID NO 32	BH1	Unknown causal	TACAGTGAGGCACCTTCCTCCACTGTCCGCGCCAG[C/T]ACAGGGGCCCAGGACAGCAAGCTGACCGGCTCAGG
SEQ ID NO 33	BH1	Unknown causal	CAGCCAGGTGGACACAGATCTATATTCAGACCTTG[C/T]CCACTGAGGCCTCTTGATACTTAGCAATATTTCAT
SEQ ID NO 34	HH2	Unknown causal	CTGGCTGATCTCCCACAAGTGAGGGGGCTTCACAG[G/T]GTACAGGAACCTTTCTCCTCTCACAGCTTCCTTCC
SEQ ID NO 35	HH4	Flanking	ACAATGTGAACAGTACCGCAGTAGGCCTTTGTCCC[A/G]TGGAAAGCTCCCTGTAAGGGTGTGAGGCCATCAGT
SEQ ID NO 36	JH1	Flanking	GTATTTAGCTACATTTATCTTTCCCAGGGAAAATT[C/T]CTGCTCCAAAGATTGGATAAGTGCCATTTTTATGG
SEQ ID NO 37	HH5	Not sure	CAATTTATGAAGTGTGGGCCATATCACTACTTGTC[A/C]AAAGGAACCCTATGTTTTAAAACTCAAAGGGGTTA
SEQ ID NO 38	HH2	Unknown causal	TCTTGGCTCTATCATAGAGCTCTATTTACTTCTTA[A/G]CCATCACTCTATTCCAAGCCACCATCATCTCCACC
SEQ ID NO 39	HHP	Flanking	CCAGAGCAGTCAGTTCACCCCAGGCCAGAGGCCAC[C/T]GGGAGACCCTGCTGCGTTGACTCATTACAGCGAAA
SEQ ID NO 40	HH2	Unknown causal	AGGGAGATGGAGGATAGAGGAAAGTGTTTTTGGCC[A/G]AAGGACTAGAGTGAGGCAAGACCCCAATTTAAGAC
SEQ ID NO 41	BH1	Unknown causal	AGACCCAGAAAGAATAGAATGGCTCTTGGGGATAT[A/G]AGGTGGCCATCAAATTGAACTCATGAGCTCTAAAC
SEQ ID NO 42	HH2	Unknown causal	TGAGCCTTAGCCCAGGTCCCACACACAGGCCTGAA[A/G]GTCTACCACAGTCAACTCTGACACAACTGTTAGGA
SEQ ID NO 43	HH3	Flanking	TACAGGTTGGTCTGGATACAAGAGTTGGCCCACAA[C/T]AGGAATTGAAGAAGCAGAGAATAGAAGAAGGCAGA
SEQ ID NO 44	BH1	Unknown causal	TGGCGGCGGAGGGACTGGAGGGGCACACGGTGGCC[A/G]CGTTACCGCCGTGTCTGCTCCCGGAGGATTAACTC
SEQ ID NO 45	BH1	Unknown causal	GGTGCGCGCACGCGACACGGCGTCGCCCATTGGCG[C/T]CTTCATGTGGCCGCTGCACTGGTCCATGTGGCTGG
SEQ ID NO 46	BH1	Unknown causal	CACAGAGCTCACACAGCACGGCCTGTTGGCACGGA[A/G]GCACTGCTCGGTTCTGCTCCTGACACTTAAGGCAT
SEQ ID NO 47	HH2	Unknown causal	CACATTTATTTGGGTCTGCATGATATGTCTCAAAT[A/G]TCTCCAGACTTGGCCTTTATTAAGCAAGTTCTACA
SEQ ID NO 48	JH2	Unknown causal	AGGAAATGACTCTCCCAGGCCTCCCTGAAGCTTCT[A/G]ATCTGGCAAGGAAGACCCTTTTCTGTCACTTCCAA
SEQ ID NO 49	BH1	Unknown causal	TTCCTAGAGACCCTGCTGTGGGTGGCCCAGGAAAC[G/T]TCCAGAGAAGCAGGAATAACTCTAAGTGTAAGTCA
SEQ ID NO 50	BH1	Unknown causal	TGGTGAGTGTGGGGTGGGAATCTAGCTGGTGGGGG[C/T]AGCTCCCAGATTCGGGGGGCCAGTTGTGTCTGCTG
SEQ ID NO 51	BH1	Unknown causal	ATTTCTTAATAAACCACATGTACTTTCGCCAACCA[G/T]TGAAATGGTCCCCTTGTTCCCTTAGTTCAGGCTAT
SEQ ID NO 52	BH1	Unknown causal	TTCTCCTACCCACATCCAAATCTCTGGCCTGCTCC[A/G]GGTCCCCCAGCAGACACATCCTTACTGTGGAGACA

Figure 1B

SEQ ID NO 53	BH1	Unknown causal	AGCCTACTACTACCAATTCGAGGCCCAAAGCAAAC[A/G]GAGTCCCAGTATCTTAAGTTTGGAAAATGTGTCAC
SEQ ID NO 54	BH1	Unknown causal	CACCTGCCCCCCACCCCTCGACCCCGGCCTTTCCT[A/C]TTCCTTCTGATGATGATTCCAGTGACATTTCCAGC
SEQ ID NO 55	HH2	Unknown causal	TTGGGCTCAGGAGCCCTACTGGTGGGCCCAGGGAC[G/T]CTTAATTCAGCAGAAGCTATTCAACCCTTGTCAAT
SEQ ID NO 56	HH2	Unknown causal	ATGGGCTCATGTATAGATGAGAGGTCTCATTTGTT[C/T]CTAAATCCTCAGGAAGGCCTGCCTGCTGAGAACTT
SEQ ID NO 57	HH2	Unknown causal	CCAGATATGAGAGCCAGATGGAGGCTCTCGCCTGG[A/G]AGCTTGGCCATGTTCAGGGGATGCTCCAGCATCGT
SEQ ID NO 58	HH4	Flanking	CCTTCACCTGTGTTTTTTTGCCTTTTTTCAGGATC[A/G]AACCTCCAAGCTCTCATAGACAGCACTAGGGAGCC
SEQ ID NO 59	JH2	Unknown causal	ACAGTTGAGCATTCATGAGTTACAAGTTCCCAAAG[C/T]CCCACAAGCTACGGGCCTGTTCTCTAGACGTACTA
SEQ ID NO 60	HH3	Flanking	TGCCATTAACAGTCATGTTTTTAACATTTTCTTTC[A/C]TGAGAACACACGCTCAGAGATTTTCAGTTCTAGTT
SEQ ID NO 61	HH2	Unknown causal	AATCCAATTTTCTGTTGATGGGCTGGGCTGAGTTC[A/G]CGCCCTGTTGTTTGGATGAGGCCAAACTGTTGACC
SEQ ID NO 62	JH2	Unknown causal	TAAAATCTCTGCAAGCAAAAAAGAGAAGAAAGGAT[G/T]GGGGAGAGAGCAGTTCAGGCAGAAGAAGTTGAAAT
SEQ ID NO 63	BH1	Unknown causal	GGGACCCCATCCTGCCCTGACCTGAGAGCAGCCTC[A/G]GGTGGAGCCCTCAGTGGCCAAACAGAACCCTGCTC
SEQ ID NO 64	HH2	Unknown causal	TATTAGATTTTAATTAATTGGTTTCTATCCTACCT[G/T]ATTTTGATTGGTGTTTGATTTGTTTTACAAGTTAA
SEQ ID NO 65	BH1	Unknown causal	TCCTTGGGGCTGGCCCTTGCCAGGGTCAATTCACC[A/G]GTCCTTTCTCATCTCGTACCTACTTTCCACACCGG
SEQ ID NO 66	JH1	Flanking	ATTCTTCCATTTTTATTTCTAGCATGTGTAAACTA[G/T]ATCCTAAATTTCCTGAGGCTACAGTTCCCATACTA
SEQ ID NO 67	HH2	Unknown causal	CCTTCTGGTAAAGGTGAAACCTGAGCCACCTGGCC[A/G]GAGTATCCAGGCACTGCTAGGTGAGGCTACCAAGG
SEQ ID NO 68	HHP	Flanking	TGGGATTTTTGTTGCTTAGAAAGAGGCCTAAATCC[A/G]GGGAATGCTCTCTCCCTCTAGCCCTTCTCTCCCTC
SEQ ID NO 69	BH1	Unknown causal	AAGGGAGGGGCAGGTGCCACCTCCTAAGGCCTCAC[C/T]GCTGAGATCACTCCAAGGGGGTGTTCAGGTGTGGT
SEQ ID NO 70	HH2	Unknown causal	TTTCTAATATCTCTTCATTGCAGGAGTCCCTGGTC[A/G]AGGGCTGGTTTTGATTGCCAATGTCATAATCTAAA
SEQ ID NO 71	BH1	Unknown causal	ATGGCTTAGGCCAAATTAGGTTCTCTTATGTCAAA[C/T]TTGCTGGCATATTAGGTTCTCAATATGCCAGAAAA
SEQ ID NO 72	HH4	Flanking	CCTGTCCTTTGCTAGTTCATCAACCATGACAAACA[G/T]TGTATATTGATTTGGGGCTTCGTAGATAACTGAAG
SEQ ID NO 73	BH1	Unknown causal	GGCAGCAAGTGGCCAGGCTCAAGTCTTGGAAATCC[C/T]ACCACCATGCTCTGCTGTGTGTGCTAAGTCACTTC
SEQ ID NO 74	HH2	Unknown causal	CAAGTCAGGGGCCCCCAGCCGCTGGGATCTAATGC[A/G]TGATGACCTGACGTGAAGCTGAGGTAACAATAACA
SEQ ID NO 75	BH1	Unknown causal	TGCAGCCCAGTCCCTAACAGGCAAGGACCAGTACC[A/G]GTCTATGGCCCGGGGTTTGGGGAGACCCCTGCACT
SEQ ID NO 76	HH2	Unknown causal	TCTCAGTCAACCAGAAAGAACATCCAACAGCTATA[C/T]GGTAAGGATTAGTTAGGAGACCTTAGAAATCAAAA
SEQ ID NO 77	BH1	Unknown causal	CATAGTAAAATGTCACTACAGTTAAATGTGTTGAA[A/C]AGGTGGTGAAGGCCTTTTTTCTTCCATCTTTTGAA

Figure 1C

SEQ ID NO 78	BH1	Unknown causal	CTGCCATGTTCATATATATGCGGCCTGTGTCTGAC[A/C]GATCCCCTACCCAGGACAAGATGGTATCAGCCTTC
SEQ ID NO 79	BH2	Flanking	AGTGGATTTCTCCTGTAGTGAAATAAGCCTTCCCC[G/T]GAGTAAGTAGGCTGGAACACACTTCCCAGCTGATG
SEQ ID NO 80	HH2	Unknown causal	ATGAGTGCAGAAACATCTGAATCTTGTTCTGTAAG[C/T]GGGCCAGTCTCTCTGTTCATGTTTTAACCAAATTG
SEQ ID NO 81	HH2	Unknown causal	ATGAATAATAAAATTTTAGTAAACGCGGTGCTTTC[C/T]CTATTTCATATATTGGGCAATTGCATATAACCATT
SEQ ID NO 82	HH2	Unknown causal	ATTCAATGTATTTCGCAAATCTCCTGACTTTTCTG[C/T]ATTTTCAAATTTAATGATTACAACTGTGGTTGAAA
SEQ ID NO 83	HH2	Unknown causal	GCAAGAGTACTGGAGTGGGGTGCCATTGTCTTCTC[C/T]TGTACTAGGCCAGGGTTCTTGAAAAATAATGCAGA
SEQ ID NO 84	HH2	Unknown causal	AAGCCCCGAAGAGGAACATTAAGTTCAGTGCAGTC[A/G]CTCAGTCATACCCGACTCTTTGCGAACCCATGAGC
SEQ ID NO 85	HH2	Unknown causal	GCAGACTCCTTGGTGCCTGCCAGATCTTGGAGGCG[A/G]CATGGTAATCCTTTGTTTTTGAGTTTGTTCAACAT
SEQ ID NO 86	JH2	Unknown causal	TGTGTAGGCTGTACAAATTGATTTCTGTCCAAAGA[A/G]CAGAGGATGGAAAGGTGGGCGGAAAGTAACTTTAC
SEQ ID NO 87	HH2	Unknown causal	AACCCATTACACTTGAATATCTGATTCTTGCCAAT[A/G]CATAATTTAAAGTCTTCACTTCAAAATAATTTGGT
SEQ ID NO 88	HH5	Not sure	CTAACTTTATTGACTTTAAAGGATTTCCATCATAG[C/T]GTGTAATTTTGCAAAATTTGTTGCATATTTCTGTT
SEQ ID NO 89	HH2	Unknown causal	CCAGTTCCATGGAGCTCTTGATCTTCAGCCTGTCC[A/G]ATGCCTCCTTTTGTGGCACACCACCCAGGTCTTCT
SEQ ID NO 90	HH2	Unknown causal	CACTTGTAAAATGCCATTTTTTTCTATAGAGATGT[A/C]TGATTTTTCAGACTTTATATATTCTGGATATGAGT
SEQ ID NO 91	BH1	Unknown causal	CCTAGGTCGATGTCCGTGAAGCCCTCAGCTGTGAT[A/G]GCATCAGCCGTGTTCTTGTCAATGTTCTCCAGACA
SEQ ID NO 92	HH2	Unknown causal	CAAGAGACAGGATAGTAAATAAAGGGCAGACTGTG[A/G]CATTTAAATCTTTTCAAAAAAGATGTCATCAGCTC
SEQ ID NO 93	HH2	Unknown causal	AGCCTCTCCTTCCTGGACCTCTGTTTCACCACCAC[A/G]ACTGTACCCCAAATGCTGTTCAACTTAGGCGGACC
SEQ ID NO 94	BH1	Unknown causal	AGGCTGAGGGGTGCCCAGATGGAGAACTGAATGAA[A/G]GCAGTCAAGTGGTATAAAGTTCCAGTTACAAGATA
SEQ ID NO 95	BH1	Unknown causal	ACTTTCATTTATTGCAGAATTTTTATTATTGCTGT[A/G]TCCCATGAAAGTGAATTGAGCAAGGAGTAATGAGA
SEQ ID NO 96	BH1	Unknown causal	TGCCCTGGGATGATGGGCAACCACTGGCCACTTAC[A/G]AGGCCCTCACAGACTGTCCTTATCAGCAGCAGTAC
SEQ ID NO 97	JH1	Flanking	TTTTTTTAAGATGTCTCTGCTTTTGTAGTTGTTAG[C/T]CTGTTCTGCTGTGTGAGGTTTAGATGAAATGAGAG
SEQ ID NO 98	HH1	Flanking	ACCAGGGAAGTCCTGTACATTCTTAAATAGCTTTG[C/G]AAGATGGGGATTCTGTGATGCTTGGGGTATGAAAT
SEQ ID NO 99	HH1	Flanking	CACAATGAAGCTTGACTACCCAGGCCAGTAAGTTC[G/T]TGTGTTATCAGTTGGCACTCATTATCCCTGGAATG
SEQ ID NO 100	HH3	Flanking	TTAACTCTAGATCTGAGATTCTGTCTCTTGGTTTC[A/G]CCATGATTCCTGGATTTAATTCGAGTACAAATTTA
SEQ ID NO 101	BH1	Unknown causal	TGCTATGGGCATAAGCCAGGAACGAGCAGCACAGA[A/G]TTAGGGAGGGGGCCTCTGGGGTAGTGGTCAGAGGT
SEQ ID NO 102	HH1	Flanking	TAAATGCTTTACTTACTGTAATACATTGTAGTAGC[A/G]AAGAGCACTGTTTTTGAAGCTACAAACCTAGGTTT
SEQ ID NO 103	HH2	Unknown causal	CTGGATAGTGACAGACTAGTATCAGGCATTCTTAC[A/G]TATGTCTAGCCAGGAGGGGGATTTTTTTTTTTTTT

Figure 1D

SEQ ID NO 104	HH2	Unknown causal	AACATGGGGTGAATTAATAAATTTGCTTCTGAAAA[C/T]GCCGAGTTTAACATAATGCAAGGACATTGCTATAG
SEQ ID NO 105	HH2	Unknown causal	GCCAGTGAGCTGCACTCTTGCTCTTAGATTTCTGC[C/T]GAGTCCTATTATCACTCTGAAGGAACTGGCTGCTT
SEQ ID NO 106	HH2	Unknown causal	GATGTCTCAAAGCATGGGTGATGATAACTTTAGTA[G/T]TTTAGATGCTAATGAAGCAGAAATCGAACCAGAAA
SEQ ID NO 107	HH2	Unknown causal	CATTATGGTGAAGTATTAGGGGATAAATAAATAGA[A/C]AAGATAAGTAGGCTGCCCAGACTGGGTGAGTTAGA
SEQ ID NO 108	JH2	Unknown causal	CAGCACACCAAATTCAGGTGATGACAGCTGTGCCC[A/G]GTCATCAGCTGTCCTTCTTGGAGGGACCATGAGAA
SEQ ID NO 109	HH3	Flanking	GTGTTGCCTCTATAGCAACTTGAAAAGGTTATAAT[C/T]GTATTACCAAGAAAAAGAAATCAGACACATTTAGG
SEQ ID NO 110	HH3	Flanking	TATAAACATATGTACTTCATTTATAAAGAATTGCA[C/T]TGGTGAAGGAAAAAATCTCTGACTAGCCTTGATAT
SEQ ID NO 111	BH2	Flanking	CTCATTATGATGAAAAAGCCACTCAAAGATTCGCA[C/T]TTCTCTACTTCCTTCCGGATTAGGTTCATTATAGA
SEQ ID NO 112	BH2	Flanking	ACTGTTTTTAAGGGAAAAACAATGCATGCCCTCGG[G/T]TATGGAGATGTTGAGTTTGAGGTACACATAAGAGA
SEQ ID NO 113	HH3	Flanking	CTAATATTTTATTAGTATTGATTTCCCATTTGCCT[A/G]GTAGCCTAGTATTAGTGAATTCACTGAACTATTAC
SEQ ID NO 114	BH2	Causal	CCTGCTTGATATTCATCAGCTTCACACAGATCTTA[C/T]GAACAGCATCGTTCTCATGAACAAGGAGGGCGTCT
SEQ ID NO 115	HH1	Flanking	GTGTGTATAGGTATTCAAAGCTGAAACAGGAGAGA[A/T]ACTTCTAGAAATCAAGGCTCATGAGGATGAAGTGC
SEQ ID NO 116	HH4	Flanking	TGGCTGTAGGAATTGTCATCTCGTTTCCACATACC[A/G]TGGGTGAAGGTTGGGCATGTGCTTTTTCAGTTTTA
SEQ ID NO 117	HH3	Flanking	GACTTTGGATCTATTTTTTCTACTCTTTTGCCTGG[C/T]GCTAATGCTATGCTTGCACCACCAGAAGGGCAGAC
SEQ ID NO 118	HH3	Flanking	TCAGAATTTGAGAATGCCTTTACTTCCAAGGTTCA[C/T]GATCTTTCAACTACACAACTGTTTCTAAAATATTA
SEQ ID NO 119	JH2	Unknown causal	TTAACCTAGGATATGAGGGTACAGGCTTGGATATA[A/G]GATGCATATTTCTAAGTCAAGACTAACAGATAGGG
SEQ ID NO 120	JH2	Unknown causal	AGATACTGAGAATAAATATTTAATGGAAATTAAAG[A/G]AACTCAAAACTGTGAGAATTCTCTATTAACAACTT
SEQ ID NO 121	HH2	Unknown causal	GTCTTATCTGCAAATTACTAAAAATAATCAGCTCT[A/G]AGAAGTGACCTTGGATGAAGCAGTATTAAATTGGC
SEQ ID NO 122	HH2	Unknown causal	CCTTACCTGAAAATTAGGAGTAAAATATTTAATAA[G/T]ATATCATCAATAAAAGACAATATCGAGTGCAATAC
SEQ ID NO 123	HH2	Unknown causal	ACCAATGGCCAGTCTGTGATGCTGAACTCAGTAAG[C/T]AGAATACATGAGCCTGGGAAACAAGATGTAAAAAT
SEQ ID NO 124	HH3	Flanking	TATGTTTATTGTTTCTCTCTAAGCAGTCCATGTTA[C/T]CTCCCCAAAGCGATTTTCCTTTCATTGCAGATATG
SEQ ID NO 125	HH2	Unknown causal	TCTTCTCCTCAACTCCCTATCAAGCTTTTGGCTGG[A/G]GTTTAGCTCCCAGATGTTAGCCTCCACTAACTGGC
SEQ ID NO 126	HH2	Unknown causal	ACAGTCATTCCAGACAATGCATTCTTCAGTGATGA[C/T]GCAGTCTTGATATTATCAAAAGAAATGTTTATTTC
SEQ ID NO 127	HH2	Unknown causal	ACTTTTACATAACATGCCTGCAGAATAGAAGCAGG[A/G]ATTTTTTTTTTTCTGGGAGATTATGAATTCTCATA
SEQ ID NO 128	BH1	Unknown causal	GCAGAGAGAAAGTCGGGCCTCAGCAGACAGCATGT[A/G]GAGTCTGGTTCCCGGGAGCAGCCAAGACCCAGAAG
SEQ ID NO 129	HH3	Flanking	AGTCAGCTCTTTGCATCAGGTGGCCAAAGTATAAT[C/T]CATATTAATTAATGGTATATAAGGGCATGTGAGTT

Figure 1E

SEQ ID NO 130	HH2	Unknown causal	GAATAGAATCAGGAGATTTTGAAGGAGGAGTGCAT[C/T]ACAGTATTAAGTGTGGCTAAAAAGATAAGAAGGAT
SEQ ID NO 131	HH2	Unknown causal	CATTTGTGTATATTACCAGTGCATATATTAGCAGC[A/G]TTTTCTAGGCTTCAGGAAATGCTTCTAAAATTACT
SEQ ID NO 132	HH2	Unknown causal	TACATTGCGTGTGTGTGTGTGCATTTTAGACACAC[A/G]TTTAGAAGACAAAATGTCAAAGAAGAGCCTCGAGA
SEQ ID NO 133	HH2	Unknown causal	TAAAGTTTTAGTAGAAGAACATTAGGATGTGTATA[A/G]AAAAGGAAGAATGGGCAGTCATATCAAGAAAGTAT
SEQ ID NO 134	HH3	Flanking	AGGTGGGAAGGAGGCACTGCAAATAGAATTCCTGG[A/G]GCTTTCAATCAGGCCAGAGAGAGGACATTGTGGGG
SEQ ID NO 135	BH1	Unknown causal	GACTAGAGGACATGGGCAGCGTTCTGGACATCAGG[C/T]AGTGACCATGGGCTCCCTGCCCTGATGGCCGGCAC
SEQ ID NO 136	JH2	Unknown causal	TCTAACTTGAAACTTATAACGGCATGTTTTTCTTT[G/T]GGGGGAAGAAAAAAGCCTAAAATCATGACTGAATC
SEQ ID NO 137	BH1	Unknown causal	GAAATCCACAAATCCACAGTGAGTTTTAAAAACTG[A/G]GCAAATAAATGGGGTAAAGTAGAAAAGTCTTCCTT
SEQ ID NO 138	BH1	Unknown causal	GGATGTACGAAAGTCAAGGATGATGTTAGTGCTTA[C/T]GATGAGTGTGTCTAACTAGAGGATTTATTTCTCAC
SEQ ID NO 139	BH1	Unknown causal	AGGAAGAAACCCCAGACAAGTAAACAGAGGAAGGT[A/G]TCAGAAAACAGGATGATGTCTAATCATGAATCGCA
SEQ ID NO 140	BH1	Unknown causal	CCAGCAGTATGAAATCAGAATTAAAGGTCTGAAAT[C/T]CCCATGCCATGATGAATGTGTTCTTTAACTATTAA
SEQ ID NO 141	HH3	Flanking	GGACCCTACCAACCGGTAAATACCTCCAGCCCCTC[A/G]CCTTTGTGGTGGCAGACTTGAGATTTGTTGTCCCG
SEQ ID NO 142	BH1	Unknown causal	AGACTTGACCTTTATATATCTGGTTAATGTCTAGA[C/T]TGTTGATTATTCCCTGGGGCTAGGAAGAACTGGAA
SEQ ID NO 143	HH2	Unknown causal	GGACATAGGGACAGGGGAGGAGGACCAGACACCAA[C/T]AGGTAATAGTAACTACCCTCACTGTTTCATCAACC
SEQ ID NO 144	HH2	Unknown causal	GGGCTTTCACACATTGCAAAGAAAATCAGTCACAA[C/T]AAAAACTGCCTGATGAGAGACTGTTGCACACTTTG
SEQ ID NO 145	BH1	Unknown causal	GAGCCAGGGCTCCAGGAGAGATAGAAACTGCACCC[C/T]GCCCCCCAACTCCTGACTGAAGGCTGGATCGCCCT
SEQ ID NO 146	HH2	Unknown causal	TTCTTTCAAATGAACATTGTAAAAATATGAACTAG[G/T]TTTCAACTTAAATTGAAAACAAAATTTAGGAACAT
SEQ ID NO 147	HH2	Unknown causal	TTATCTGTTTTTGCTCATCTTAATATAAAAATCTT[A/G]ACAGTTATGGTTCGAGTTCACCTGGATGAGATTTC
SEQ ID NO 148	HH2	Unknown causal	TTCCATAACTTAAAAGGACCATATGTTCATTGAGA[A/G]GGAATTACTAGAAATGATGAACGTAATTTTTCTTC
SEQ ID NO 149	HH2	Unknown causal	GATATTAGCTGGTTTTAAATGTATTTTTAACCTTT[A/G]GTGGTCGTTATTAAATTTTTAACATAAAATTCAAA
SEQ ID NO 150	HH2	Unknown causal	GGAGTGTATTATAATGATTTTTCTATACTATTTGT[A/G]TTCTTAGTCACTCAGTCATGTCCGACTCTTTGCAA
SEQ ID NO 151	BH1	Unknown causal	CCCCTCTGACCTTCTCACTCTCCAGTCAAACCAGA[C/T]GTCTTCTCCAGCACGGGTCCCTTCACTTATATCAG
SEQ ID NO 152	BH1	Unknown causal	ACCTCAGGGAAATAAAAGGTGATTGTTATGGCCCT[G/T]CCTGATTCCAGCAGCCCTGGGTCAGATGCTTCCCA
SEQ ID NO 153	HH3	Flanking	ATAAAGAGTTGGACAGGACTGAAGTGACTGAGCAT[A/G]CACACACTCAATCAGTATATATTTTGAATTATTTA
SEQ ID NO 154	HH2	Unknown causal	TTACCAGCGTTGTTAAACGCTGCCTGTGTTTCATT[C/T]TGCTTTGATGCTATTTGAGGTAGCATGCCAGCAGA
SEQ ID NO 155	HH2	Unknown causal	TAACGTCGCTTTTCCTCAGTTTCTGTAGCTCTGAA[C/T]AGGGGATAATAACAATATCTTCCTCAACACTTGTA

Figure 1F

SEQ ID NO 156	HH2	Unknown causal	CCTACAAACAGGCATGCTTGCCATCAATTGTAAAA[C/T]GTCTATATCCCCCAAGCCCAGGATTCCTCTCCTAT
SEQ ID NO 157	HH2	Unknown causal	CTTTGATGCCATTATTACCATTTGGTGACTCTGTG[A/G]TCAGCAAATGGTTTGTCAATTTAAAGTCTGGAATC
SEQ ID NO 158	HH2	Unknown causal	ATATTTTTGACATTAGCAAAAACTGCTTTAGAGGT[A/G]GATTGCTACTAATATCATATATTTTAATACTGTCA
SEQ ID NO 159	HH2	Unknown causal	GGAAGAAAAGTTTTTTTTTTTCTCTTAATGGAAAC[A/G]ATATAGCCTGGACCCTGTAAAGGTGCACACAAATA
SEQ ID NO 160	HH2	Unknown causal	CTGCACTGTAAGCCAGCTGTCATGAACATACTAAA[C/T]TATGTCATTGCCTATCGCTTTTATAGCATCTCCTA
SEQ ID NO 161	HH2	Unknown causal	AGACAGTAAATTAATTATAAGGTCATGTCAACAGA[A/G]AAGTAATGCACAAGACAAACAGCTCTTTATGTAAA
SEQ ID NO 162	HH3	Flanking	GCAACATGCCACAGAATGTGTTCATGGAGCTCACA[A/G]ATGGGCAGGAAAAAAAGATAGACTAGAAATGACCA
SEQ ID NO 163	BH1	Unknown causal	TAAAAGTGCTAAAATTTGTAACTACTTTCCAATTA[C/T]ATTTAACCCCAGGTATGTACTATTTTCATATTTTA
SEQ ID NO 164	BH1	Unknown causal	TAGTTAACATTGACTAAGGGTTGGAGGCGGCCAAT[A/G]GACCTCGAGAACAAGTATAAGCTTGTACTTCTCTG
SEQ ID NO 165	BH1	Unknown causal	CACTTTGCTCATGGGAGTATATGTGTATGTGAGTA[C/T]GTC
SEQ ID NO 166	JH2	Unknown causal	CATGTTTAGGTAAGAAAACTGTTAAAATGTATTCA[C/T]GATGCCACTTCCCTTTACTTGGTGCAGTTTGCCCA
SEQ ID NO 167	JH2	Unknown causal	GAAGCAGAGGTGGGTGAGGGGTCTAAACCAGGAGA[G/T]GAACTAGACCTTCAGACCTGAAGGTTATCTGATTC
SEQ ID NO 168	BH1	Unknown causal	TTTGGAGAATTCTGTGCCTTTTCATACTCTCCTCT[A/G]CTCTTCTTTTCCTCACACTCGACTTTGTTGCTTCT
SEQ ID NO 169	BH1	Unknown causal	AGACCAGTGGACCCTAACACAAGGACAGAGTTCAT[C/T]GGATATGGCCACAGGGTCCAGCCCATGAGGCTGCC
SEQ ID NO 170	HH3	Flanking	CAAGGGAAGGAGAGCACTTTTATGGAAGGAAGAGG[A/G]AATTGTGAGGGCTGCAGTCCACAAAGAGCCCTTGG
SEQ ID NO 171	BH1	Unknown causal	CCCCCTGGGTGCCTAGGGCTGATGGGTCTGACCCA[C/T]GGATGGAACGCCTTGAATCCAGAGCCAGTTGCCAG
SEQ ID NO 172	JH2	Unknown causal	AACTTTGTATCTACAGAAAGTACTCCTTGATCCCC[C/T]TCATCTCTTTGGGACTCACTCCAGTGATGCTGACT
SEQ ID NO 173	JH2	Unknown causal	GATGTTCTTTGCAACTGTTGTACCTGGTTGGTAGG[A/G]AATCATATAAAGGTGTGCTCCTGCAATCACTTCTC
SEQ ID NO 174	JH2	Unknown causal	CTATTTGACACAGTGGCATGTAGACTAGGCAGTCA[A/G]ACAATGTTGTCTGTCCCTCTGAGTTGGGACTCAAA
SEQ ID NO 175	BH1	Unknown causal	GATGCAGACGTTTCAGGAAGGAGGCACTGCACAAA[A/G]TGAGATAGAGGTACAGGGCCCCAGGCCCCCAGAGT
SEQ ID NO 176	BH1	Unknown causal	AATGAGGGTAATTGCCTTTCATGTTCCTAATATTT[G/T]TGATTTAGAAAAAAAAATCACCCCATTTATCATTC
SEQ ID NO 177	HH2	Unknown causal	GGTACTTTGGGTTAACAGACTATTACATTGATGAC[A/G]TGCATTATTTGCCTCTGTTGCACTGATTAAAGGGT
SEQ ID NO 178	BH1	Unknown causal	GGCCATTTGTCTTGGGCCTAATCTTGTTCACGCAG[C/T]GTGGGAGGCTCCAACTGATCCCAAATTTCCCCAAA
SEQ ID NO 179	BH1	Unknown causal	AAGAGTAGGGTACAAGTTTGGAAAGGGGGCTTCTG[A/G]GGCCCCTATGGGGTGAGAAATACGAGTATGAGAGT
SEQ ID NO 180	BH1	Unknown causal	AAACATTTTCAGAATTGAATGTGGTCTATGTTCAC[C/T]TTTTCCTCTCATTTGGGGAACTTTCCCACTTCCCA
SEQ ID NO 181	BH1	Unknown causal	TTCAGGGGAGTTCATTTGCAGGACCTGGAGGAAGA[C/T]GAGAGTATAGGAAGTCAGAATGAGGCTTGTGGGGA

Figure 1G

SEQ ID NO 182	BH1	Unknown causal	GTAGAAACTTGATGTTTGCAACTTGATGTTTTGGT[A/G]AAAAGTATATACCCATGAAATCATCATCACTATCA
SEQ ID NO 183	JH2	Unknown causal	AAAAGACTGTGTACACTCACTTGAATTTGCTTTCC[A/G]TTCTGTAAAAGGAGAGAATACGTTCTATTGTTTGT
SEQ ID NO 184	HH3	Flanking	AACCATTCTCTCATCAACGTAAGATAGATTAATAG[A/C]TTGCTAAATGCAACAGGATTTAATGTGAGTAGGCA
SEQ ID NO 185	HH2	Unknown causal	TCCACCACCTGGAGGCCCCTCCAGCTGCTAGTTTA[A/G]TCTTGGAGAGCATGATCTTAAACCCACTAGCCTAT
SEQ ID NO 186	HH3	Flanking	AATGAAATGTTATTATGGAGAAGAAGTTGAAACAG[A/C]AGCCAGAAACTTTAAGCCTGGCCAATACCTTAATA
SEQ ID NO 187	BH1	Unknown causal	TTCCAGTACTACAGTTTAAAAGAACGAATTCTTCC[A/G]CGTTCAGCCTTCTTTAGGGGCCAATACTCACGTCA
SEQ ID NO 188	BH1	Unknown causal	CCCACTTTCCGAATCCCATGTTCCAGCATCCAGCC[C/T]CCTTCACACCAGCAGAAACAGATTCAGGCTGGGTG
SEQ ID NO 189	BH1	Unknown causal	TGAGTGTCTCCTTCTGACCTTCATGTCGTATGACC[A/G]ATTTGTGGCTATTTGTAATCCCCTGCAATACACAG
SEQ ID NO 190	BH1	Unknown causal	GATCTTTTGCTTATTTATTCATTATATTCTTTATG[A/C]AATAGTTATCTGAACTCACTGTCATTAATCTGAAC
SEQ ID NO 191	BH1	Unknown causal	TTGTATTGCTATCTCTCCATAATAATTCAACTTTA[C/T]ACATCACAAAGGAGGAAACAGAGGGAATTTTCTCA
SEQ ID NO 192	BH1	Unknown causal	GGATGCCCAGAGGCCCATCCAACTCTGATATTTTT[A/G]GATCCTGCAGTCATCACTTTTCCTGCTGTGTATCA
SEQ ID NO 193	BH1	Unknown causal	GTCTCCTGCTGGGCCTGGGAGGGGGCGTGGGGAAG[A/G]ACCATCAGGAGACTGAGCTGCAAAAGACCCCTGGC
SEQ ID NO 194	BH1	Unknown causal	AATAAGGCAGAAGCCCTAATAATTTTGAAACAACA[A/G]CAGCAATCACTGTTGCTTCCTCTGTGGCAAGCTAT
SEQ ID NO 195	BH1	Unknown causal	AAATCCAAAAAAAAAGGGGGGAGGATTGTAAAAGTA[C/T]TAGTTAAAAATAAAGATGAATTATTTCATCTGCTC
SEQ ID NO 196	BH1	Unknown causal	GTTGAGTCGGACATAGCTGCCCTTGGATCCTAGCC[C/T]GGCCCCTGGGCACATGGAACCGTGGTGTCCTGGTC
SEQ ID NO 197	BH1	Unknown causal	CTCACTGGCAGTTGGCTGCATTCAGTTCCTGGCCG[C/T]GTGGGTCTTCCTGAATTTTCACCTTGGCTTCTAGC
SEQ ID NO 198	BH1	Unknown causal	GTTACTCTAACATGGGTGGGATTACAGGTGATGCT[C/T]GGCCTCTTCCTCCCCCCAGGTATTTTTAGTGGTCT
SEQ ID NO 199	BH1	Unknown causal	CTGAGGCCCAGAGGTGATCTATGGGCCCTAATGGA[C/T]GATGCAGTCCTTGGGGAAGGGGATGGACGAGTGGT
SEQ ID NO 200	BH1	Unknown causal	TTGCCGTCACAGGCTGCGTGGCCTCATCTGAGGCC[C/T]CTGTCTCACCGTTTCAGTTTCCCATTTCTTTAACA
SEQ ID NO 201	BH1	Unknown causal	ACAGCATCTGGAATTCCTTGAGTGGCAGTGCAGCC[A/G]AGCTCGGACTCTGAGGCCAGACTCCGGGGTTCAAG
SEQ ID NO 202	BH1	Unknown causal	TCTCTGGATTTCCAGCACCCTAGACTGTTGCCAAA[A/C]TTGGCTCCCATGTGCCTGGGAGGAGGTGCGGGCTG
SEQ ID NO 203	BH1	Unknown causal	ACAGAAAGATTTTAAAAGGCCAGCAATACTGTTCT[G/T]TAATTCAACATCAGCTCACGAGCTTTTCTCATGTG
SEQ ID NO 204	BH1	Unknown causal	CACTGATCCCAGGAACCAGCAGCCAGACACCAAGA[G/T]GTGACACACTCTATGGCTCATGAGAATAGAATAAC
SEQ ID NO 205	BH1	Unknown causal	ATCCCACTGAACAGATGTTTGTTTCCTGCCATGGT[C/T]AGTCATGGCAAAAGAAATGCTAATCTTCTGCTATT
SEQ ID NO 206	BH1	Unknown causal	TCCCCCAGGAACACTTATATCATGAGTCCCCAAGC[A/C]AAGAGGGCCTTCCCAGCTCCTCTAACATGGATCAC
SEQ ID NO 207	BH1	Unknown causal	AGGACTCATCAGCCCTGACCCTTACCTACTGTCAT[C/T]CTCAATCTTAGGCCCTTCTCATATCCATTTGTCCT

Figure 1H

SEQ ID NO 208	BH1	Unknown causal	TTTTAAAGGGATAGCCTAAGGAACCCCACTTGAAT[C/T]CTTGGAGGAGGGCATGGTTTCTGCATTGAGCCTGG
SEQ ID NO 209	BH1	Unknown causal	ACTTTCAGAAACCCATGTAAAAAACGATTATAAGC[C/T]CTGAACACAATGAATGTATCTGGGACTCCTGAGGG
SEQ ID NO 210	BH1	Unknown causal	GTGCATCACCAGGCCAGGATCCCTGGTCCAAAGCT[A/T]TAAAACAAGCTGGACACTCTTCCTCTTCTGAGCAC
SEQ ID NO 211	HH1	Flanking	TTTTCTTTTTAAGGTCTTTATTGATCTTGGATGAT[A/G]TTTGGGATCCTTGGGTGTTAAAAGCTTTTGACAAT
SEQ ID NO 212	HH3	Flanking	AGACATTTTTTCTAAGAAAGATATAGTAAGGCCCAC[A/G]GGCATGGGATTAGATACTTAACAACAGTAGTCATT
SEQ ID NO 213	JH1	Flanking	GCGGAGAGTCATGATGGCGTCTGTATGATCTCCGG[A/G]GGTAGCGGCGTCGGAGTTCGGCCAAAGTGAGCGGC
SEQ ID NO 214	HH1	Causal	GCCTCTGTGAACTGGAAACTTCAGAGGTTTATCGG[C/T]AAGCTAAGCTGCAGGCCAAGCAGGAGGTCGATAAC
SEQ ID NO 215	BH2	Flanking	GAGACGTATAGATTCCTGGGTCCTTTGCATCTGGA[C/T]GGTTTGAATGGATAAAGCACCACTTGACAAATAAG
SEQ ID NO 216	HBR	Flanking	AAGACGCTCCAAGAGGTGCTGCAGTGCTCCTGGTG[A/T]GGGTGGCAGTGCCGTCGTGTGCCCCAGGCCTGTGA
SEQ ID NO 217	HH3	Causal	TACTCAGAATATTGGACATATGCTACGTACTCATT[C/T]CACACATTCTCAGGTAAGAACCAAAAAGAGCCTCA
SEQ ID NO 218	HH4	Causal	GCTGACCAAGAACGGCCCCAAAGTTCTGGAATTTA[A/C]TTGCCGTTTCGGTGATCCAGAGTGCCAAGTGAGTA
SEQ ID NO 219	JH1	Flanking	TTATTAGCGTCCTTTGAGGGTGGGCTCTTGTGTTT[A/C]CAGCATTTCTTAAATTGTAACTTGGAAAAGATCTG
SEQ ID NO 220	BH2	Flanking	CGAGTGTGAATCTATAATATTACGGTACAACATAA[C/T]CTTCAATATTCATAACAGTACTCCTAAAGCAGGAA

Figure 1I

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US21/42790

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A01K 67/027; A01K 67/02; A61D 19/04; A01K 67/00; C12N 15/00; C12N 15/877; C12N 5/02 (2021.01)

CPC - A01K 2227/101; C12Q 2600/172; A01K 2267/02; C12N 15/8771; A01K 67/02; A01K 67/027; A01K 67/0273; A61D 19/04; C12N 5/0604; C12N 5/0608; G16B 20/00; C12Q 2600/124; C12N 5/0607

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2019/100018 A1 (INGURAN LLC) 23 May 2019; Summary of the invention, Detailed description of the invention, Page 30	1-5, 9-15 --- 6-8
Y	W0 2019/140260 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, ET AL.) July 18, 2019; paragraph [0144]	6-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 October 2021 (12.10.2021)	DEC 21 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US21/42790

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

An "Invitation to Furnish Nucleotide and/or Amino Acid Sequence Listing and to Pay, Where Applicable, Late Furnishing Fee" (Form PCT/ISA/225), was mailed on 03 September 2021 (03.09.2021). The sequence listing provided to the ISA/US in response to the Form PCT/ISA/225 cannot be used for search as the applicant failed to provide the late furnishing fee indicated under Rule 13ter.1(c). Therefore, under Rule 13ter.1(d), the international search will be carried out only to the extent possible .