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(54) ***BOS TAURUS* VARIETY 'HO840003150607238' AND METHODS OF USE THEREOF**

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(58) **Field of Classification Search**

CPC C12N 5/061; C12N 5/0611; C12N 5/0609; C12N 5/06; A01K 67/027; A01K 2227/101

See application file for complete search history.

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(57) **ABSTRACT**

The disclosure relates to Bovine germplasm of Bos taurus variety HO840M003150607238. Included in the present disclosure are cells comprising the genome of Bovine variety HO840M003150607238 characterized by the presence of homozygous loci and spermatozoa obtained from said cells. Also provided by the present disclosure are tissue cultures of cells, animals obtained from said cells, and parts thereof, including F1 spermatozoa. The disclosure further provides for methods of breeding, selecting, and using the germplasm to improve existing commercial cattle herds generated from in vitro fertilization methods and progeny cattle obtained from in vitro fertilization and implantation and artificial insemination methods.

22 Claims, No Drawings

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BOS TAURUS VARIETY 'HO840003150607238' AND METHODS OF USE THEREOF

INCORPORATION OF SEQUENCE LISTING

The Sequence Listing is hereby incorporated by reference in its entirety, including the file named P34675_ST25.txt, which is 47,645,133 bytes in size and was created on Mar. 26, 2019, which is likewise herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present disclosure relates to the field of Bos taurus breeding. In particular, the present disclosure related to Bos taurus variety HO840003150607238 having high multi-trait selection indices and high trait transmissibility.

BACKGROUND OF THE INVENTION

There are numerous steps in the development of any new, desirable Bos taurus germplasm. Bos taurus breeding begins with the analysis and definition of problems and weaknesses of the current germplasm, the establishment of program goals, and the definition of specific breeding objectives. The next step is selection of germplasm that possess the traits to meet the program goals. A goal is to combine in a single variety an improved combination of desirable traits from the parental germplasm. See Schefers et al., "Genomic selection in dairy cattle: Integration of DNA testing into breeding programs" Animal Frontiers 2(1):1-9 (2012).

During breeding, cattle breeders have a variety of sources when making breeding decisions. In addition to genomic data, a number of agencies and organizations collect and release analysis of population data and indexes. Every three months, the Animal Improvement Programs Laboratory (AIPL) of the United States Department of Agriculture releases the newest USDA-DHIA (Dairy Herd Improvement Association) genetic evaluations for dairy bulls and cows. The AIPL calculates genetic evaluations for type for various breeds, and many breed associations provide their own indexes or other strategies for evaluating certain breed-relevant traits. U.S. dairy genetic evaluations are computed every four months by the Council on Dairy Cattle Breeding (CDCB) and Holstein Association USA (HAU). Both CDCB and HAU traits provide the breeder within important comparative data to evaluate the complex genetic and phenotypic traits to develop improved and desirable Bos taurus germplasm. For Holstein and Jersey sires, for example, evaluations are genomically enhanced and represent a blending of genomic data, pedigree information, and results from progeny. These genetic evaluations provide the breeder important information for the selection of desirable germplasm and the development of new and valuable inseminates.

There is a continuous need to develop improved Bos taurus germplasm for use in improving production herds as well as for the continued improvement of elite animals. The present germplasm is the result of crosses between superior elite females ranked by performance as among the top 1% of the population of domesticated dams and elite bulls that are among the top 5% of the domestic population.

SUMMARY OF THE INVENTION

having at least 90% of the nucleic acid sequences selected from the group consisting of SEQ ID NOs:1 to 29048.

The present disclosure provides for, and includes, a plurality of Bos taurus gamete cells comprising at least 90% of the loci comprising the nucleic acid sequences selected from the group consisting of SEQ ID NOs: 1 to 29048.

In an aspect, the present disclosure includes, and provides for, an F1 Bos taurus animal, or part thereof, said F1 Bos taurus animal comprising a genome comprising at least 90% of the loci comprising the nucleic acid sequences selected from the group consisting of SEQ ID NOs: 1 to 29048.

In another aspect, the present disclosure provides for, and includes, an F1 Bos taurus animal, or part thereof, comprising a genome comprising at least 90% of the loci comprising the nucleic acid sequences selected from the group consisting of SEQ ID NOs: 1 to 29048.

In a further aspect, the present disclosure provides for, and includes a Bos taurus animal, or part thereof, comprising one or more cells having at least 25% of the loci comprising nucleic acid sequences selected from the group consisting of SEQ ID NOs:1 to 29048.

An even further aspect of the present disclosure is a plurality of Bos taurus cells, each comprising a diploid or haploid genome each diploid genome comprising homozygous loci comprising at least 90% of the nucleic acid sequences selected from the group consisting of SEQ ID NOs: 1 to 29048; and each haploid genome comprising at least 90% of the loci comprising the nucleic acid sequences selected from the group consisting of SEQ ID NOs: 1 to 29048.

DETAILED DESCRIPTION

A goal of a Bos taurus breeding program is to combine in a single Bos taurus variety an improved combination of desirable traits from the parental germplasm that provides for desirable progeny when used in artificial insemination and in vitro fertilization programs. Improved Bos taurus inseminate varieties are useful for various artificial breeding techniques, including artificial insemination ("AI") and embryo transfer ("ET"). Improved Bos taurus germplasm, varieties, and inseminates prepared therefrom, are desirable.

The present disclosure provides for, and includes, an improved elite SM germplasm obtained from a multigenerational breeding program. The germplasm is unique and readily distinguishable from germplasm present in non-selected cattle. Indeed, in the absence of continued selection, the germplasm reverts to heterogeneity and diversity. As provided herein, the germplasm of the present disclosure is identifiable using standard methods and the germplasm can be readily identified in progeny generations. Indeed, as few as 800 SNP markers are sufficient to identify parentage with greater than 99% accuracy. See McClure et al., "SNP Data Quality Control in a National Beef and Dairy Cattle System and Highly Accurate SNP Based Parentage Verification and Identification," Frontiers in Genetics 9(84):1-14 (2018). As provided here, the tens of thousands of sequences provide for tracking and selecting animals through multiple generations. Breeding with the germplasm provided herein, combined with the selection of suitable mates will maintain the desirable germplasm in subsequent generations. Moreover, genetic testing allows for the removal of progeny having germplasm that lacks that set of desired loci for the improvement of cattle herds.

The present disclosure provides for, and includes, cells,

germplasm characterized by SEQ ID NOs:1 to 41648 and homozygous loci comprising the nucleic acid sequences selected from the group consisting of SEQ ID NOs:1 to 29048, and listed in Table 1. Animal differs from the reference genome at 11832 homozygous loci, or about 28% of the total loci.

TABLE 1

Genotype of *Bos taurus* animal HO840003150607238 ("Animal")

	SEQ ID Range SEQ ID NOs: 1 to 29048		No. of Alternate
Homozygous	Reference Allele	Alternate Allele	Alleles
Chromosome 1	1-1110	17188-17963	775
Chr. 2	1111-01954	17964-18580	616
Chr. 3	1955-2883	18581-19199	618
Chr. 4	2884-3684	19200-19689	489
Chr. 5	3685-4439	19690-20169	479
Chr. 6	4440-5272	20170-20823	653
Chr. 7	5273-5962	20824-21347	523
Chr. 8	5963-6797	21348-21843	495
Chr. 9	6798-7455	21844-22309	465
Chr. 10	7456-8125	22310-22775	465
Chr. 11	8126-8809	22776-23290	514
Chr. 12	8810-9358	23291-23663	372
Chr. 13	9359-9951	23664-24058	394
Chr. 14	9952-10592	24059-24647	588
Chr. 15	10593-11148	24648-24987	339
Chr. 16	11149-11703	24988-25348	360
Chr. 17	11704-12314	25349-25729	380
Chr. 18	12315-12797	25730-26027	297
Chr. 19	12798-13309	26028-26360	332
Chr. 20	13310-13823	26361-26736	375
Chr. 21	13824-14279	26737-27082	345
Chr. 22	14280-14702	27083-27360	277
Chr. 23	14703-15016	27361-27615	254
Chr. 24	15017-15414	27616-27882	266
Chr. 25	15415-15770	27883-28107	224
Chr. 26	15771-16151	28108-28362	254
Chr. 27	16152-16444	28363-28574	211
Chr. 28	16445-16812	28575-28812	237
Chr. 29	16813-17187	28813-29048	235
		No. Alternate alleles	11832

Heterozygous	SEQ ID Range
Chromosome 1	29049-29832
Chr. 2	29833-30576
Chr. 3	30577-31069
Chr. 4	31070-31789
Chr. 5	31790-32326
Chr. 6	32327-32899
Chr. 7	32900-33505
Chr. 8	33506-34086
Chr. 9	34087-34609
Chr. 10	34610-35231
Chr. 11	35232-35844
Chr. 12	35845-36305
Chr. 13	36306-36749
Chr. 14	36750-36970
Chr. 15	36971-37461
Chr. 16	37462-37865
Chr. 17	37866-38181
Chr. 18	38182-38494
Chr. 19	38495-38763
Chr. 20	38764-39143
Chr. 21	39144-39454
Chr. 22	39455-39787
Chr. 23	39788-40099
Chr. 24	40100-40457
Chr. 25	40458-40689
Chr. 26	40690-40949
Chr. 27	40950-41236
Chr. 28	41237-41401
Chr. 29	41402-41648

The present disclosure provides for, and includes, a diploid Bos taurus cell or a plurality of diploid Bos taurus cells comprising improved germplasm characterized by a genome having homozygous loci comprising 90% to 100% of the nucleic acid sequences selected from the group consisting of SEQ ID NOs:1 to 29048.

Animal is the progeny of a cross between Sire HO840003135669665 ("Sire") and Dam HO840003128557405 ("Dam"). The genotype of Sire is represented by the SEQ ID NOs: 41649 to 82185 and homozygous loci comprising the nucleic acid sequences selected from the group consisting of SEQ ID NOs: 41649 to 68810 and listed in Table 2 ("Sire Genotype"). Sire differs from the reference genome at 10935 homozygous loci, or about 27% of the total loci.

TABLE 2

Genotype of Sire HO840003135669665 (Sire Genotype)

	SEQ ID Range SEQ ID NOs: 41649-68810		No. of Alternate
Homozygous	Reference Allele	Alternate Allele	Alleles
Chromosome 1	41649-42719	57847-58601	754
Chr. 2	42720-43499	58602-59159	557
Chr. 3	43500-44453	59160-59783	623
Chr. 4	44454-45260	59784-60266	482
Chr. 5	45261-45934	60267-60700	433
Chr. 6	459345-46671	60701-61265	564
Chr. 7	46672-47422	61266-61885	619
Chr. 8	47423-48224	61886-62406	520
Chr. 9	48225-48890	62407-62852	445
Chr. 10	48891-49556	62853-63309	456
Chr. 11	49557-50233	63310-63817	507
Chr. 12	50234-50737	63818-64185	367
Chr. 13	50738-51301	64186-64534	348
Chr. 14	51302-51833	64535-65004	469
Chr. 15	51834-52378	65005-65353	348
Chr. 16	52379-52865	65354-65668	314
Chr. 17	52866-53404	65669-65967	298
Chr. 18	53405-53798	65968-66197	229
Chr. 19	53799-54206	66198-66464	266
Chr. 20	54207-54696	66465-66813	348
Chr. 21	54697-55109	66814-67072	258
Chr. 22	55110-55539	67073-67329	256
Chr. 23	55540-55826	67330-67565	235
Chr. 24	55827-56213	67566-67821	255
Chr. 25	56214-56566	67822-68028	206
Chr. 26	56567-56940	68029-68274	245
Chr. 27	56941-57230	68275-68477	202
Chr. 28	57231-57518	68478-68648	170
Chr. 29	57519-57846	68649-68810	161
		No. Alternate alleles	10935

	SEQ ID Range
Heterozygous	68811-82185
Chromosome 1	68811-69568
Chr. 2	69569-70363
Chr. 3	70364-70760
Chr. 4	70761-71423
Chr. 5	71424-72041
Chr. 6	72042-72719
Chr. 7	72720-73111
Chr. 8	73112-73656
Chr. 9	73657-74158
Chr. 10	74159-74759
Chr. 11	74760-75333
Chr. 12	75334-75787
Chr. 13	75788-76276
Chr. 14	76277-76695
Chr. 15	76696-77157
Chr. 16	77158-77633
Chr. 17	77634-78084

TABLE 2-continued

Genotype of Sire HO840003135669665 (Sire Genotype)	
Chr. 19	78535-78953
Chr. 20	78954-79354
Chr. 21	79355-79764
Chr. 22	79765-80082
Chr. 23	80083-80419
Chr. 24	80420-80769
Chr. 25	80770-81008
Chr. 26	81009-81269
Chr. 27	81270-81548
Chr. 28	81549-81839
Chr. 29	81840-82185

The genotype of Dam is represented by the SEQ ID NOs: 82186-122589 and homozygous loci comprising the nucleic acid sequences selected from the group consisting of SEQ ID NOs: 82186 to 98686 and listed in Table 3 ("Dam Genotype"). Dam differs from the reference genome at 11237 homozygous loci, or about 28% of the total loci.

TABLE 3

Genotype of Dam HO840003128557405 ("Dam Genotype")

	SEQ ID Range SEQ ID NOs: 82186-98686		No. of Alternate
Homozygous	Reference Allele	Alternate Allele	Alleles
Chromosome 1	82186-83192	98687-99371	754
Chr. 2	83193-84062	99372-99977	557
Chr. 3	84063-84881	99978-100492	623
Chr. 4	84882-85725	100493-101051	482
Chr. 5	85726-86405	101052-101490	433
Chr. 6	86406-87219	101491-102080	564
Chr. 7	87220-87869	102081-102587	619
Chr. 8	87870-88653	102588-103065	520
Chr. 9	88654-89284	103066-103516	445
Chr. 10	89285-89899	103517-103940	456
Chr. 11	89900-90666	103941-104564	507
Chr. 12	90667-91210	104565-104932	367
Chr. 13	91211-91734	104933-105256	348
Chr. 14	91735-92282	105257-105760	469
Chr. 15	92283-92836	105761-106060	348
Chr. 16	92837-93376	106061-106423	314
Chr. 17	93377-93940	106424-106775	298
Chr. 18	93941-94380	106776-107030	229
Chr. 19	94381-94873	107031-107351	266
Chr. 20	94874-95367	107352-107726	348
Chr. 21	95368-95770	107727-108041	258
Chr. 22	95771-96219	108042-108305	256
Chr. 23	96220-96562	108306-108588	235
Chr. 24	96563-96993	108589-108894	255
Chr. 25	96994-97303	108895-109070	206
Chr. 26	97304-97640	109071-109263	245
Chr. 27	97641-97962	109264-109489	202
Chr. 28	97963-98283	109490-109703	170
Chr. 29	98284-98686	109704-109952	161
		No. Alternate alleles	11237

	SEQ ID Range
Heterozygous	109953-122589
Chromosome 1	109953-110857
Chr. 2	110858-111511
Chr. 3	111512-112144
Chr. 4	112145-112680
Chr. 5	112681-113278
Chr. 6	113279-113859
Chr. 7	113860-114455
Chr. 8	114456-115061
Chr. 9	115062-115588
Chr. 10	115589-116273
Chr. 11	116274-116642

TABLE 3-continued

Genotype of Dam HO840003128557405 ("Dam Genotype")	
Chr. 13	117066-117610
Chr. 14	117611-117973
Chr. 15	117974-118467
Chr. 16	118468-118832
Chr. 17	118833-119198
Chr. 18	119199-119559
Chr. 19	119560-119828
Chr. 20	119829-120190
Chr. 21	120191-120554
Chr. 22	120555-120841
Chr. 23	120842-121068
Chr. 24	121069-121323
Chr. 25	121324-121625
Chr. 26	121626-121970
Chr. 27	121971-122194
Chr. 28	122195-122406
Chr. 29	122407-122589

Notably, and as expected for a select cross between Sire and Dam, the number of homozygous alleles in Animal increases compared to both parents. As show in Table 4, Animal retains 70 to 80% of the homozygous alleles found in Sire and Dam and more than 15% of the heterozygous alleles in Sire and Dam are fixed as homozygous in Animal. These results demonstrate that improvements by selective are breeding are reflected in the germplasm and that such improvements do not require, but may be informed by, a priori knowledge of the genotype of the germplasm.

TABLE 4

Select cross increases homozygosity

				Homozygous Matches from parent		Just Parent type	
Alleles	Sire	Dam	Admiral	S %	D %	S %	D %
Homo ref	12944	13040	17187	32%	32%	80%	79%
Homo alt	8663	8313	11832	21%	21%	79%	74%
Homo-homo	21607	21353	29048	54%	53%	80%	77%
Het to homo	6724	6340		17%	16%	50%	50%

It is to be understood that the disclosure is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The disclosure is capable of other aspects or of being practiced or carried out in various ways.

As used herein the term "about" refers to ±10%.

As used herein, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof

Throughout this application, various embodiments of this disclosure may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as "from 1 to 6" should be considered to have specifically disclosed subranges such

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