ELICA

Chasing deleterious variants in Holstein dairy cattle

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XIX EAAP/Interbull meeting
Tallin, Estonia - August 28, 2017

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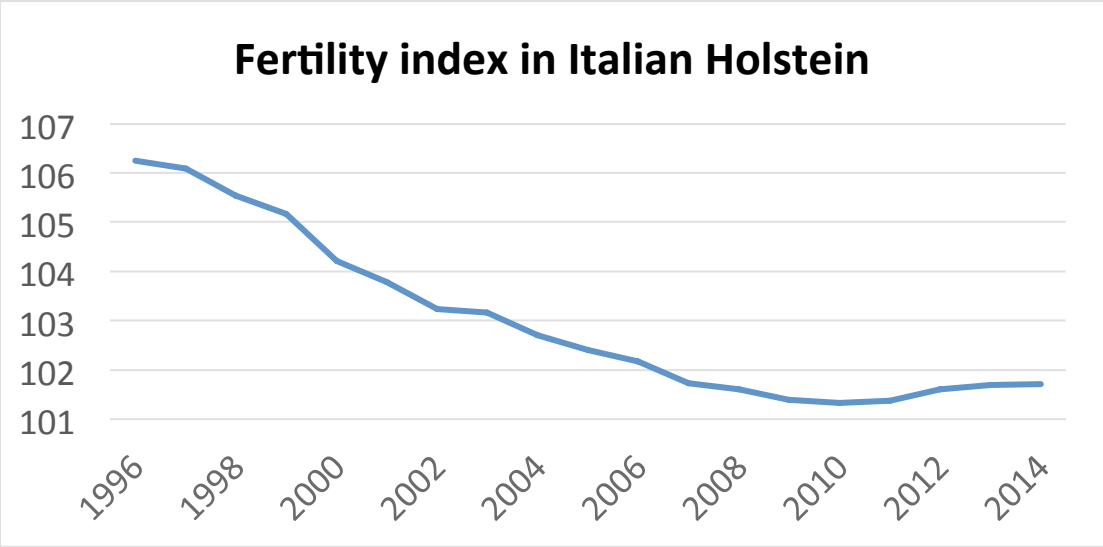
GENETIC TREND



–Negative energy balance

–Increased inbreeding (0.05 per year – Mrode *et al.*, 2005

–Deleterious recessives

DEFINITION

Deleterious variant:

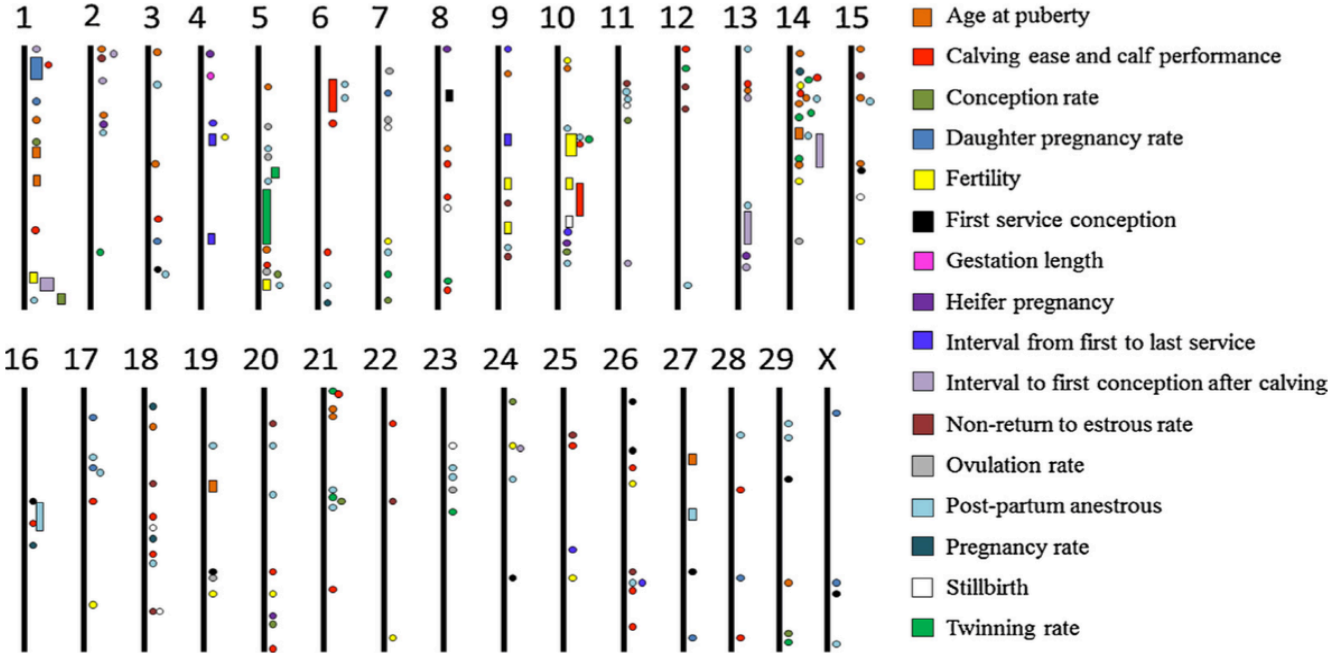
reduce the reproductive fitness of carriers, and would thus be targeted by purifying natural selection

MacArthur *et al.*, 2014

Unexpected high frequency

- Demographic events (e.g. bottlenecks)
- Breeding practice
  - In linkage with favourable alleles (hich-hiking)
  - Spread by important AI sires
- Variants in balancing selection (e.g. beta-globin - malaria)

QTL MAPPING



Fortes et al, 2013

HAPLOTYPES LACKING ONE OF THE HOMOZYGOUS CLASSES

Harmful recessive effects on fertility detected by absence of homozygous haplotypes

P. M. VanRaden,*[1] K. M. Olson,† D. J. Null,* and J. L. Hutchison*
*Animal Improvement Programs Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705-2350
†National Association of Animal Breeders, Columbia, MO 66205-1033

- No affected population
- No phenotype is used

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SEEKING CAUSAL VARIANTS

OPEN ACCESS Freely available online PLOS ONE

Identification of a Nonsense Mutation in CWC15 Associated with Decreased Reproductive Efficiency in Jersey Cattle

Tad S. Sonstegard[1], John B. Cole[2]*, Paul M. VanRaden[2], Curtis P. Van Tassell[1], Daniel J. Null[2], Steven G. Schroeder[1], Derek Bickhart[1], Matthew C. McClure[1]

1 Bovine Functional Genomics, United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, United States of America, **2** Animal Improvement Programs Laboratories, United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, United States of America

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Detection of Haplotypes Associated with Prenatal Death in Dairy Cattle and Identification of Deleterious Mutations in GART, SHBG and SLC37A2

Sébastien Fritz[1,2], Aurelien Capitan[1,2], Anis Djari[3], Sabrina C. Rodriguez[2,3], Anne Barbat[2], Aurélia Baur[1,2], Cécile Grohs[2], Bernard Weiss[2], Mekki Boussaha[2], Diane Esquerré[4], Christophe Klopp[3], Dominique Rocha[2], Didier Boichard[2]*

1 UNCEIA, Genetics Team, Paris, France, **2** INRA, UMR1313 Animal Genetics and Integrative Biology, Jouy-en-Josas, France, **3** INRA, Sigenae, UR875 Biométrie et Intelligence Artificielle, Castanet-Tolosan, France, **4** INRA, GeT Genomics Facility, UMR444 Laboratoire de Génétique Cellulaire, Castanet-Tolosan, France

OPEN ACCESS Freely available online PLOS ONE

Bovine Exome Sequence Analysis and Targeted SNP Genotyping of Recessive Fertility Defects BH1, HH2, and HH3 Reveal a Putative Causative Mutation in *SMC2* for HH3

Matthew C. McClure[1], Derek Bickhart[2], Dan Null[2], Paul VanRaden[2], Lingyang Xu[1], George Wiggans[2], George Liu[1], Steve Schroeder[1], Jarret Glasscock[3], Jon Armstrong[3], John B. Cole[2], Curtis P. Van Tassell[1], Tad S. Sonstegard[1]*

1 United States Department of Agriculture, Agriculture Research Service, Bovine Functional Genomics Laboratory, Beltsville, Maryland, United States of America, **2** United States Department of Agriculture, Agriculture Research Service, Animal Improvement Programs Laboratory, Beltsville, Maryland, United States of America, **3** Cofactor Genomics, St. Louis, Missouri, United States of America

Pausch *et al. BMC Genomics* (2015) 16:312
DOI 10.1186/s12864-015-1483-7

BMC Genomics

RESEARCH ARTICLE Open Access

Homozygous haplotype deficiency reveals deleterious mutations compromising reproductive and rearing success in cattle

Hubert Pausch[1*†], Hermann Schwarzenbacher[2†], Johann Burgstaller[3], Krzysztof Flisikowski[4], Christine Wurmser[1], Sandra Jansen[1], Simone Jung[1], Angelika Schnieke[4], Thomas Wittek[3] and Ruedi Fries[1]

USE OF SEQUENCE

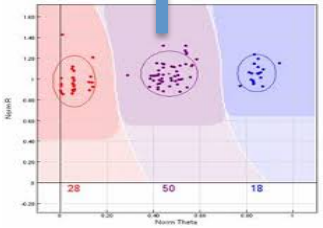
Proceedings, 10th World Congress of genetics Applied to Livestock Production

NGS-based Reverse Genetic Screen Reveals Loss-of-function Variants Compromising Fertility in Cattle.

C. Charlier[1], W. Li[1], C. Harland[1,2], M. Littlejohn[2], F. Creagh[2], M. Keehan[2], T. Druet[1], W. Coppieters[1], R. Spelman[2] & M. Georges[1]
[1]Unit of Animal Genomics, GIGA-R & Faculty of Veterinary Medicine, University of Liège, Belgium,
[2]Livestock Improvement Corporation, New Zealand.

LoF variants

No observed homozygotes



Ref/Ref	Alt/Ref	Alt/Alt
3	8	0

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Workflow

Data production

Bulls HD-SNP genotyping

1009 AI Italian Holstein bulls; 800K SNPs

Software used: PLINK 1.9; GHap

HD-SNP phasing and haplotyping

62,215 haploblocks; 445,042 haplotypes

VEP and SIFT analyses

147,209 variants

Exome sequencing

18 bulls; 54Mbp

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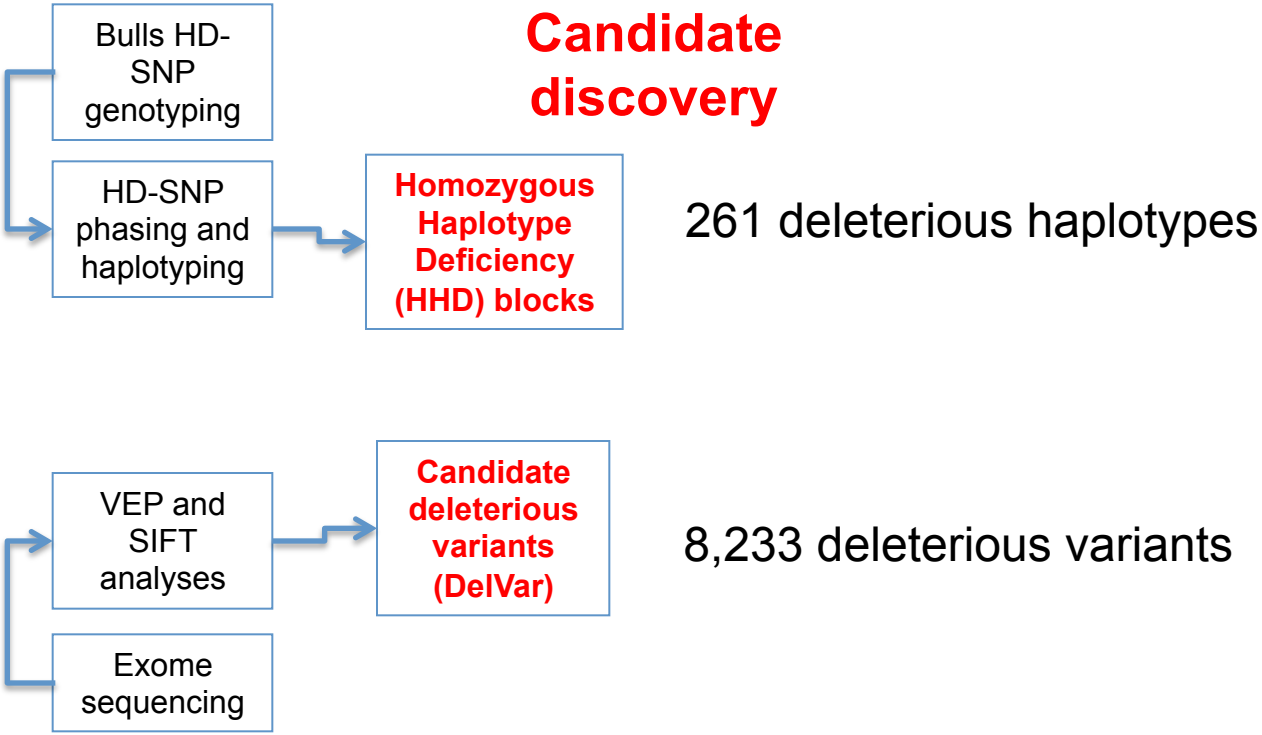
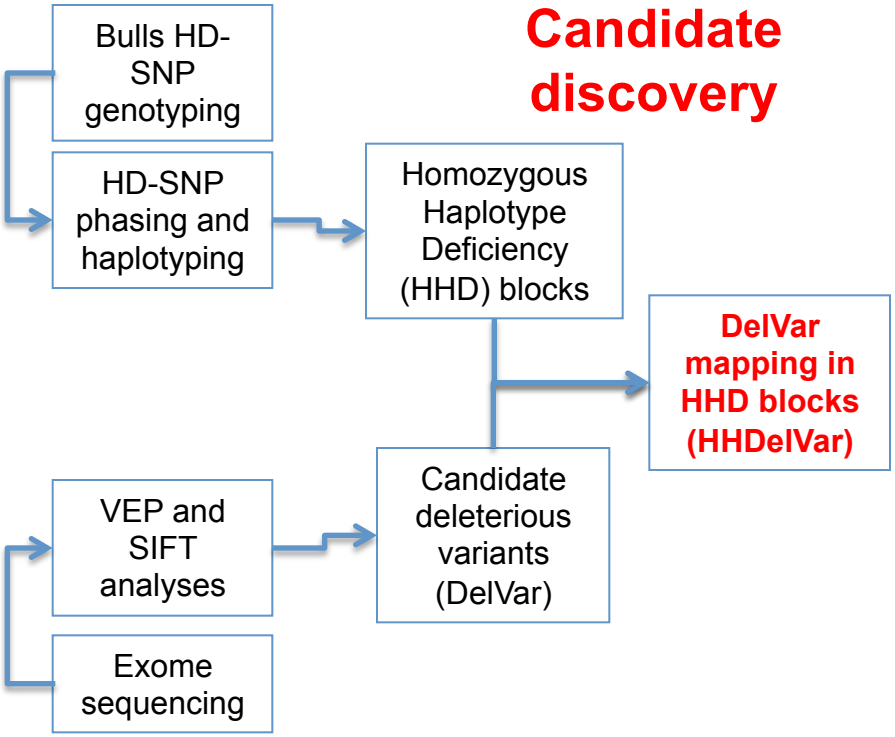
Workflow

Data production



Workflow

Data production



73 deleterious variants in 56 genes mapping in 51 deleterious haploblocks

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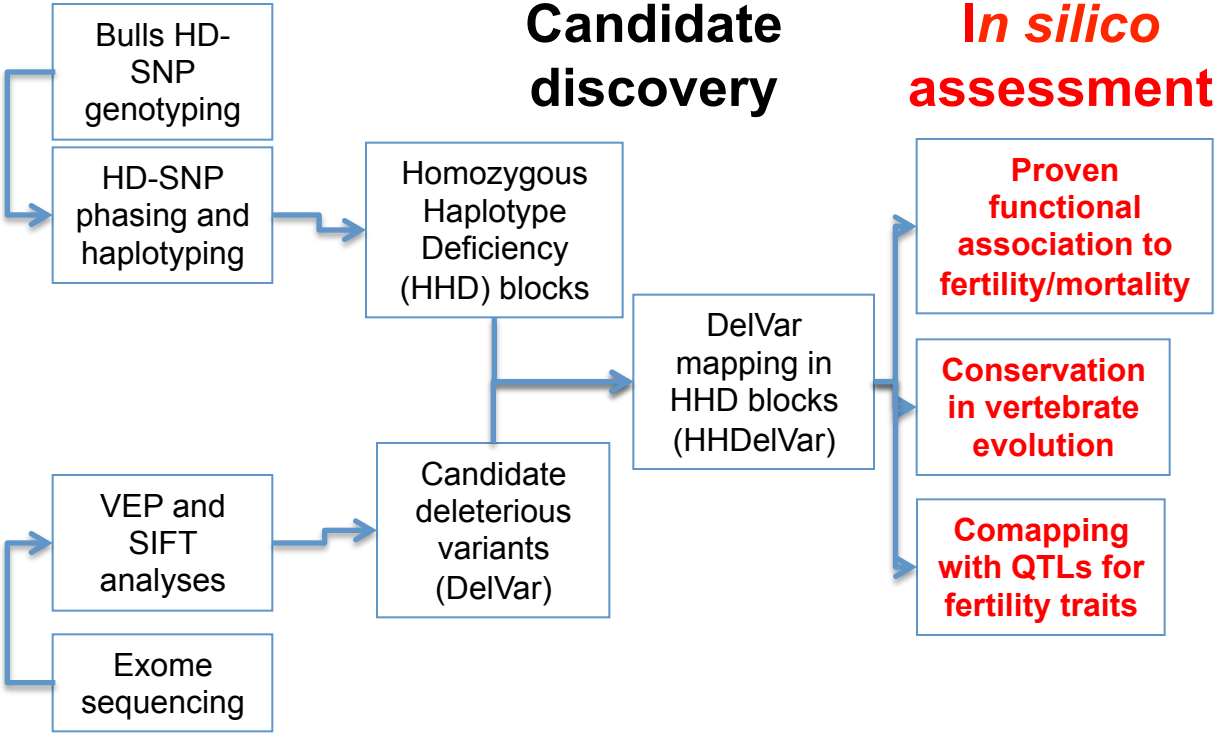
Workflow

Data production



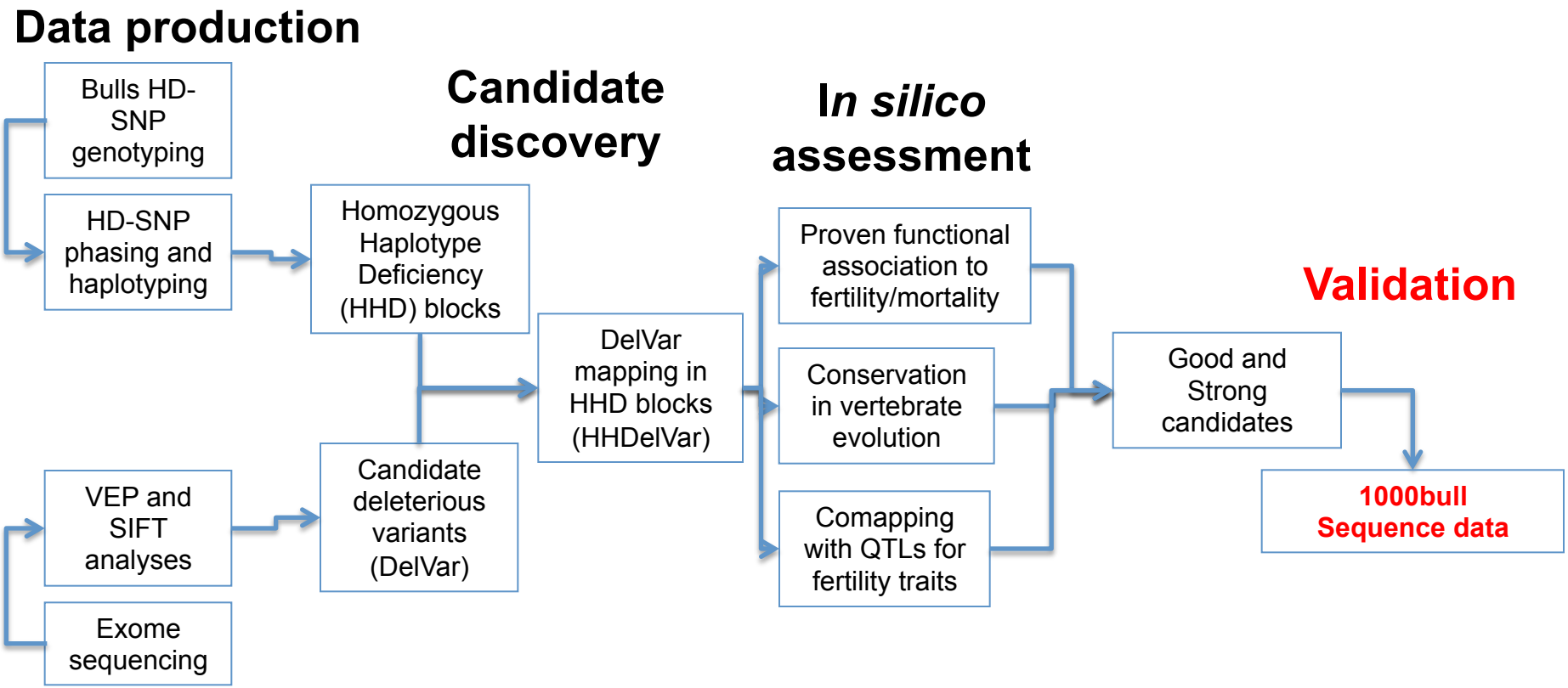
Software: PhyloP; FastCons
Database: OMIM; MGI

KRT8; DLAT1

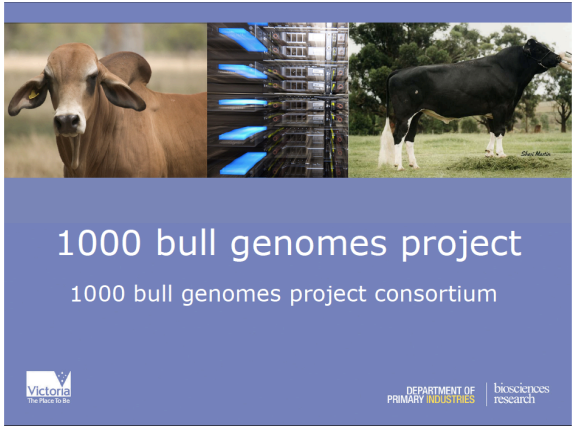
2 good candidates
7 strong candidates

ESPL1; RPTOR; TECRL; SETB2; SPATS2; UBR1; EPB42

Workflow


Data production
Bulls HD-SNP genotyping
HD-SNP phasing and haplotyping
VEP and SIFT analyses
Exome sequencing
Candidate discovery
Homozygous Haplotype Deficiency (HHD) blocks
Candidate deleterious variants (DelVar)
DelVar mapping in HHD blocks (HHDelVar)
In silico assessment
Proven functional association to fertility/mortality
Conservation in vertebrate evolution
Comapping with QTLs for fertility traits
Validation
Good and Strong candidates
1000bull Sequence data

VALIDATION



RUN5 (*Bos taurus* + *B. indicus*):

- 1682 animals
- 70 breed

Breed	N.
Holstein	450
Fleckvieh	145
Angus	141
Brown Swiss	105

GENOTYPE FREQUENCY IN 1000BULL: 450 HOLSTEIN

Gene	Position	Mutation	HOM. 1	HET.	HOM. 2	H-W
KR8	5_27217392	T/C Splice variant	10	127	313	N.S.
BUB1	11_1568943	G/A Splice variant	333	110	7	N.S
TECRL	6_81580936	T/C Missense	1	22	427	N.S.
UBR1	10_38335826	G/A Missense	3	55	392	N.S
……..						
…….						

8 out of nine candidate mutations not confirmed!

Genotype Frequency in 1000bull: Holstein and entire dataset

K→Q at position 641

Gene	Position	Mutation		HOM. 1	HET.	HOM. 2	H-W
DLAT	15_22697929	C/A Missense	Holstein	365	85	0	0.02*
DLAT	15_22697929	C/A Missense	Entire dataset	1594	88	0	

Allele frequency of derived allele in Holstein: 9.4%

Derived allele very rare in non Holstein breeds

HAPLOTYPE ALIGNMENT IN HETEROZYGOTES

= reference allele
= alternative allele

Chr15_22679557-Chr15_22711644

Missens variant

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EVIDENCE

- *DLAT* (dihydrolipoamide S-acetyltransferase) gene catalyses the conversion of pyruvate to acetyl coenzyme A.
- Up-regulated in the endometrium of pregnant cows
- Expressed in mouse testis and associated with epididymal sperm maturation and with sperm motility
- *DLAT* co-maps with two male fertility QTLs, one associated with semen volume in Holstein and the second with scrotal circumference in Angus.
- The deleterious missense variant changes the 641th amino acid (over 647) in domain associated to energy metabolism.
- The mutation site is highly conserved across vertebrates

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CONCLUSIONS

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FINAL CONSIDERATIONS

Have we identified a recessive deleterious causal mutation in *DLAT* gene?

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FINAL CONSIDERATIONS

May be……..

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ON THE TABLE.....

Gene	Position	Mutation	Dataset	HOM. 1	HET.	HOM.2	H-W
UBR1	10_38335826	G/A Missense	Holstein	3	55	392	N.S
UBR1	10_38396251	G/A Intron	Holstein	0	167	283	8,307E-09***
UBR1	10_38396251	G/A Intron	Entire dataset	0	513	1169	

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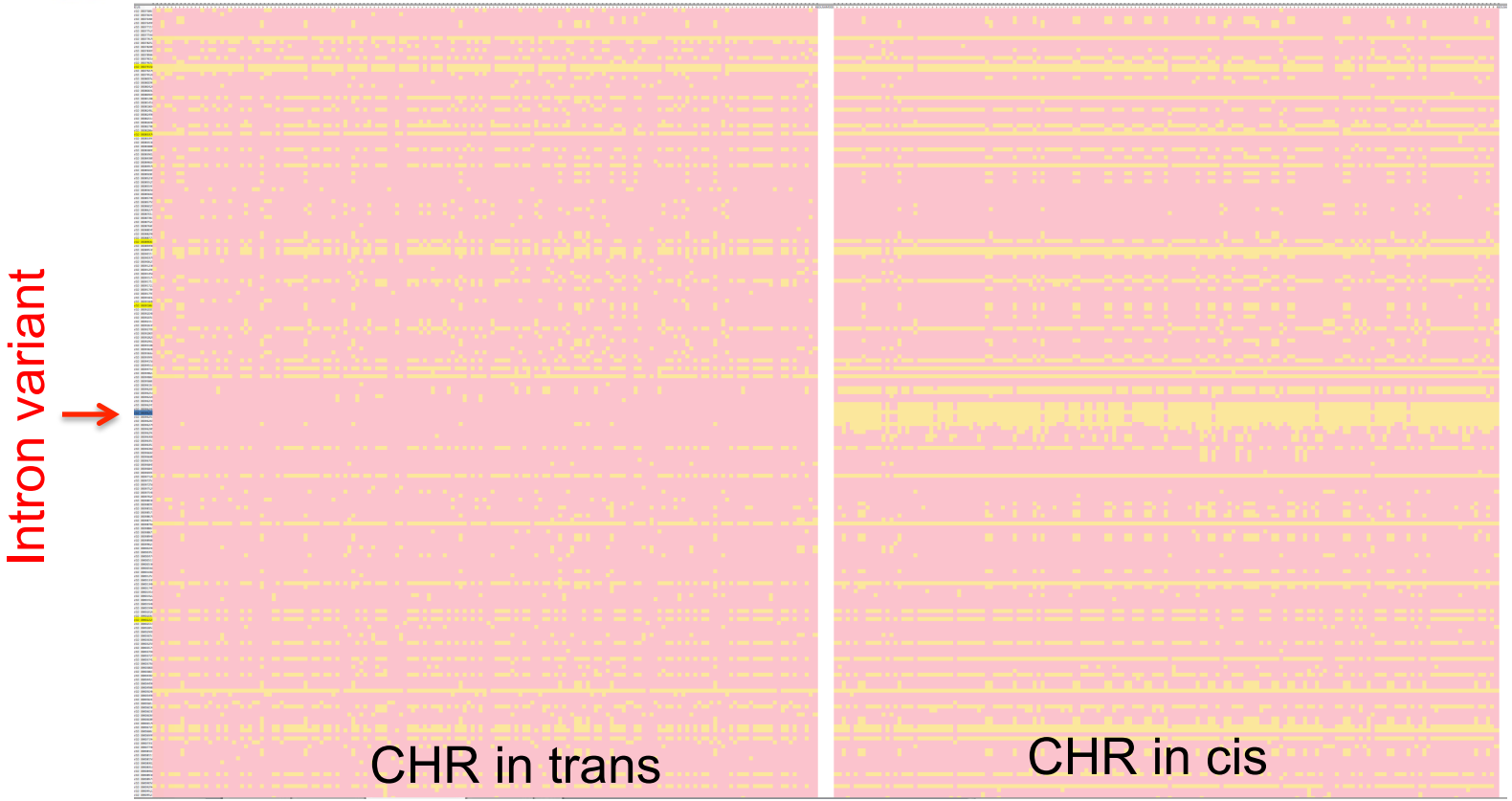
ON THE TABLE.....



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