A SNP Bovine Panel For High-Throughput Milk Production, Genetic Diseases, Traceability And Paternity Typing

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Introduction

Genomic data have the potential to contribute valuable information for animal selection and are being increasingly used in the genetic evaluation of animals and design of genetic improvement programs. Single Nucleotide Polymorphisms (SNPs) are the most frequent type of sequence variation of DNA. Thanks to the availability of very high-throughput genotyping technologies, SNPs have become the genetic markers of choice for high resolution genetics and genome-wide association studies.

The aim of this work was to create a SNP panel useful for the development of a high-throughput and easy-to-use DNA chip technology for SNP bovine genotyping. Therefore we increased a previous panel of 22 SNPs developed for typing the main mutations of bovine milk protein genes (Chessa et al., 2007), based on the ligation detection reaction (LDR) and universal array (UA).

The set up technology will be transferred to end-users with the aim to substitute their routine analyses for SNP genotyping performed with several technologies, such as RFLP-PCR, Real-Time PCR, SNaPshot, etc.

Material and methods

Starting with data obtained from NCBI, we identified different genes whose mutations were significantly associated with milk quality and production. The selected genes included the ones responsible for milk proteins synthesis*: CSN1S1*, *CSN2*, *CSN1S2*, *CSN3* (α_{S1}-casein, β-casein, α_{S2}-casein, and κ-casein, respectively), *LGB* (β-lactoglobulin), *LALBA* (α-lactalbumin), and *LTF* (lactoferrin). Genes involved at various levels in milk fat biosynthesis were also considered: *SCD* (Stearoil Co-A desaturase), *DGAT1* (acylCoA:diacylglicerol acyltransferase 1), *LEP* (leptin), *FASN* (fatty acid synthase). Finally genes having DNA-binding and transcriptional activation ability, and hormonal, receptor or transport functions were included: *PROP1* (PROP paired-like homeobox 1), *POU1F1* (POU class 1 homeobox 1), *STAT5* (signal transducer and activator of transcription 5A), *CXCR1* (interleukin 8 receptor, alpha), *ABCG2* (ATP-binding cassette, sub-family G member 2), and *PRL*

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(prolactin). For each gene, using the NCBI dbSNP collection (http://www.ncbi.nlm.nih.gov/SNP, Sherri et al. 2001) and the Bovine SNP Retriever (http://www.itb.cnr.it/ptp/bovine_snp_retriever/, Panzitta et al., 2008), a user-friendly tool for bovine SNP retrieval, annotations indicating the gene position on the chromosome, the type of mutation (missense or silent), the SNP location within the gene (intron, exon, 5'- or 3'-flanking regions), the association with a specific QTL trait, the SNP validation state, allelic frequencies data, and the correlated breed were collected.

Results and discussion

The first step of this study was to choose among all the available polymorphisms of interest for the Italian Friesian a 50 SNP panel, including a set of 15 SNP to be used for animal identification (Table 1) and other bovine polymorphisms located in genes involved in milk protein and fat biosynthesis (48 SNP) (Table 2) and genes responsible for genetic disease susceptibility (5 SNP) (Table 3). Among the SNPs localized in the genes described above, 68 SNPs were considered the most informative because some of them were found in the exon regions of the gene correlated to causative mutations and some others had high frequencies in Italian Holstein breed.

To validate the SNP panel we started with the DNA extraction from milk and blood of 500 Italian Friesian animals in collaboration with LGS (Genetics and Services Laboratory, Cremona, Italy). Different multiplex-PCR assays were developed to amplify all the DNA fragment containing the selected SNPs. Allele-specific probes were designed using ORMA software (Severgnini et al., 2009) to type the SNPs exploiting a microarray technology based on the ligation detection reaction (LDR) associated with the hybridization onto a universal array (UA).

Table 1: SNP used as marker for animal identification (15)

LOC.	ACC. Nr.	SNP	POSITION	EFFECT
BTA3250	AF458963	A>G	28.6	MAI
BTA6343	AF458965	C>G	80.7	MAI
BTA5597	AF458968	G>T	129.2	MAI
BTA4083	AF465157	C>T	73.6	MAI
BTA5277	AF465158	C>T	33.5	MAI
BTA3254	AF465160	C>T	130.5	MAI
BTA5033	AF465162	C>T	94.6	MAI
BTA5089	AF465163	G>T	78.9	MAI
BTA2121	AF465164	G>T	78.0	MAI
BTA3144	AF465167	G>T	62.0	MAI
BTA2889	AF465169	C>T	45.3	MAI
BTA6899	AF465171	C>G	38.7	MAI
BTA3463	AF465172	C>T	8.0	MAI
BTA5915	AF465177	C>T	56.8	MAI
BTACAPN1	AF465178	C>T	56.0	MAI

MAI = Marker for animal identification.

Table 2: SNP affecting milk production traits (48)

GENE	ACC. Nr.	SNP	POSITION	EFFECT
CSN1S1	X59856	g.10175 A>C	5' RR	MPB
		g.10283 TTTTT>Δ	5' RR	
		g.10331 A>G	5' RR	
		g.10371 G>C	5' RR	
	X59856	g.26181 A>G	Exon 17	MPB
CSN2	X14711	g.6690 G>A	Exon 6	MPB
		g.8101 C>A	Exon 7	
		g.8178 A>C	Exon 7	
		g.8219 C>A	Exon 7	
		g.8267 C>G	Exon 7	
CSN1S2	M94327	g.8879 G>T	Exon 7	MPB
CSN3	AY380228	g.8371 A>G	Intron 2	MPB
		g.13065 C>T	Exon 4	
		g.13068 C>T	Exon 4	
		g.13104 A>C	Exon 4	
		g.13124 A>G	Exon 4	
		g.13165 A>G	Exon 4	
LGB	X14710	g.3984 A>G	Exon 3	MPB
		g.5174 C>T	Exon 4	
		g.5263 T>C	Exon 4	
LALBA	X06366	g.753 G>A	5' RR	MPB
		g.851 G>A	Exon 1	
LTF	L19985	g.-915 T>C	5' RR	MPB
		g.-926 G>A	5' RR	
		g.-610 G>T	5' RR	
		g.-585 C>T	5' RR	
	AY036584	g.156 A>C	5' RR	
		g.216 C>G	5' RR	
	Z93399	g.5805 C>T	Exon 11	
SCD	AY241932	g.10329 C>T	Exon 5	MFB
DGAT1	BC118146	g.10433-4 AA>GC	Exon 8	MFB
LEP	U50365	g.1180C>T	Exon 2	MFB
		g.3100C>T	Exon 3	
FASN	AF285607	g.763 G>C	Exon 1	MFB
		g.17924 A/G	Exon 34	
	NM_174579	c.577 C>A	Exon 3	MFB
PROP1		c.545 G>A	Exon 2	
		c.606 T>C	Exon 3	
		c.647 A>G	Exon 3	
PIT1	EF090615	g.207G>A	Exon 6	L & MS
		g.230C>T	Exon 6	
STAT5	AJ237937	g.9501A>G	Intron 9	MGD & L
CXCR1	U19947	c. -1768 T/A	5' RR	CID
ABCG2	NW_931635	A>C	Exon 14	MYC
PRL	X16641	g. –1043A>G	Exon 4	L & MPB
	AF426315	g.8398A>G	Exon 4	
MC1R	U39469	g.296C>T	Exon 1	Coat color
		g.310G>del	Exon 1	

RR = Regulatory region; MPB = Milk proteins biosynthesis; MFB = Milk fat biosynthesis; L = Lactogenesis; MS = Milk secretion; MGD = Mammary gland development; CID = Chemokine gene expression in response to infection disease MYC = Milk yield and composition

Table 3: SNP responsible for genetic disease (5)

GENE	ACC. Nr.	SNP	POSITION	EFFECT
CD18	Y12672	g.383G>A	Exon 2	Bovine Leukocyte adhesion deficiency
SLC35A3	AY160683	g.559G>T	Exon 4	Complex Vertebral Malformation
LRP4	DQ462703	c.4863-4CG>AT	Exon 33	Syndactylysm
DUMPS	BC112872	C>T		Deficiency of uridine monophosphate synthase
ASS	BC102474	C>T	Exon 5	Citrullinaemia

Conclusion

In the present study, a SNP Panel useful for the development of a easy-to-use DNA chip for bovine genotyping in Italian Holsteins was created.
In particular, SNPs located in genes involved in milk protein and fat biosynthesis, genes responsible for genetic disease susceptibility and SNPs that together with the first ones could be used also as marker for animal identification were identified.
Further validation studies of our SNP panel on Italian Holsteins DNA samples are required.

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