



**DISCOVERY, ANALYSIS, AND UTILITY OF BOVINE SINGLE  
NUCLEOTIDE POLYMORPHISMS**

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## ABSTRACT

DNA sequence variation provides the fundamental material for improving livestock through selection. To date, few studies in cattle have concentrated on the isolation of single nucleotide polymorphisms (SNPs) to describe rates of nucleotide diversity across the bovine genome. In an attempt to determine the future value of SNP markers in livestock genomics, the screening of 97 bovine gene fragments amplified from a diverse panel of individuals was undertaken. Over 200 bovine SNPs were isolated from 70 known genes (1 SNP every 231bp), allowing the first large-scale analysis of SNP occurrence and comparison of the rates of bovine nucleotide polymorphism with other mammalian species. To further investigate the properties of bovine SNPs, pooled DNA sequencing analysis of 48 bovine gene fragments containing 187 SNPs were analyzed for minor allele frequency in four samples constructed from 10 common *Bos taurus* and *Bos indicus* cattle breeds. Analysis showed 28% of SNPs were common ( $\geq 20\%$ ) in all four pools providing vital evidence for the existence of informative bovine SNPs shared across many diverse cattle populations.

To clarify the level of assay flexibility, ease of implementation, and overall rates of genotyping success, two distinct SNP genotyping technologies based on either primer extension or primer hybridization were assessed. Analysis of different SNP base variants and in/dels coupled with the genotyping of over 1000 individuals per SNP clearly showed that primer extension-based SNP genotyping was a robust technology suitable for generating confident SNP genotypes. Furthermore, resultant SNP genotypes were used to highlight the successful application of SNPs in the genetic linkage mapping of type I markers for enhanced comparative map resolution and the development of candidate gene SNP haplotypes for linkage (QTL) and linkage disequilibrium mapping of 11 complex traits in cattle. Compelling evidence for a QTL on BTA2 affecting economically important carcass traits was found in the region harbouring the *MSTN* gene. Linkage disequilibrium analysis confirmed the association of *MSTN* haplotypes with total meat and fat percentage traits in Limousin cattle. In total, almost 290 bovine SNPs located within 70 known genes were described, resulting in a resource which will continue to provide key DNA markers for future comparative mapping strategies and association studies of candidate genes with complex traits in cattle.



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**ABBREVIATIONS**

<b>A</b>	adenine
<b>Abs</b>	absorbance
<b>AP</b>	alkaline phosphatase
<b>ASA</b>	allele specific amplification
<b>ASH</b>	allele specific hybridization
<b>BAC</b>	bacterial artificial chromosome
<b>bp</b>	base pair
<b>BSA</b>	bovine serum albumin
<b>BTA</b>	<i>Bos taurus</i>
<b>C</b>	cytosine
<b>°C</b>	degrees Celcius
<b>cm</b>	centimetre
<b>cM</b>	centiMorgan
<b>CpG</b>	C and G dinucleotide pair (running 5' to 3')
<b>cSNP</b>	coding single nucleotide polymorphism
<b>dATP</b>	2'-deoxyadenosine 5'-triphosphate
<b>dCTP</b>	2'-deoxycytosine 5'-triphosphate
<b>ddF</b>	dideoxy fingerprinting
<b>dGTP</b>	2'-deoxyguanosine 5'-triphosphate
<b>dH<sub>2</sub>O</b>	distilled water
<b>DNA</b>	deoxyribonucleic acid
<b>dNTP</b>	deoxyribonucleotide triphosphate
<b>dsDNA</b>	double stranded deoxyribonucleic acid
<b>dTTP</b>	2'-deoxythymidine 5'-triphosphate
<b>EDC</b>	1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride
<b>EDTA</b>	ethylenediamine tetra acetic acid
<b>EST</b>	expressed sequence tag
<b>EtBr</b>	ethidium bromide
<b>FISH</b>	fluorescence <i>in situ</i> hybridisation
<b>FITC</b>	fluorescein isothiocyanate
<b>fmol</b>	fentamole
<b>g</b>	gram
<b>G</b>	guanine
<b>hr</b>	hour

<b>HRP</b>	horse radish peroxidase
<b>HSA</b>	<i>Homo sapiens</i>
<b>in/del</b>	insertion/deletion
<b>kb</b>	kilobase pair
<b>L</b>	litre
<b>LB</b>	Luria broth
<b>LD</b>	linkage disequilibrium
<b>LOD</b>	Logarithm of odds score
<b>M</b>	molar
<b>MAS</b>	marker assisted selection
<b>mA</b>	milliampere
<b>Mb</b>	million base pairs
<b>MFI</b>	mean fluorescence intensity
<b>min</b>	minute
<b>mM</b>	millimolar
<b>mRNA</b>	messenger ribonucleic acid
<b>ng</b>	nanograms
<b>nm</b>	nanometre
<b>OAR</b>	<i>Ovis aries</i>
<b>OD</b>	optical density
<b>ODA</b>	<i>N,N</i> -dimethyloctylamine
<b>PBS</b>	phosphate buffer saline
<b>PCR</b>	polymerase chain reaction
<b>PE</b>	phycoerythrin
<b><math>P_E</math></b>	probability of exclusion
<b>pers. comm.</b>	personal communication
<b>pg</b>	picograms
<b>pmol</b>	picomole
<b>pNPPD</b>	p-Nitrophenyl phosphate solution
<b>PPD11</b>	p-phenylenediamine dihydrochloride, pH 11
<b>QTL</b>	quantitative trait loci
<b>RFLP</b>	restriction fragment length polymorphism
<b>RNA</b>	ribonucleic acid
<b>rpm</b>	revolutions per minute
<b>SA-HRP</b>	streptavidin-conjugated horse radish peroxidase

<b>SA-PE</b>	streptavidin-R-phycoerythrin
<b>SDS</b>	sodium dodecyl sulphate
<b>sec</b>	second
<b>SNP</b>	single nucleotide polymorphism
<b>SSC</b>	sodium chloride sodium citrate
<b>SSCP</b>	single stranded conformational polymorphism
<b>ssDNA</b>	single stranded deoxyribonucleic acid
<b>STR</b>	short tandem repeat
<b>STS</b>	sequence tagged site
<b>T</b>	thymine
<b>TAE</b>	tris acetate ethylenediaminetetra-acetic acid
<b>Taq</b>	<i>Thermus aquaticus</i>
<b>TBE</b>	tris borate ethylenediaminetetra-acetic acid
<b>TE</b>	tris ethylenediaminetetra-acetic acid
<b>TEMED</b>	<i>N,N,N',N'</i> -tetramethylethylenediamine
<b>T<sub>m</sub></b>	melting temperature
<b>TMAC</b>	tetramethylammonium-chloride
<b>TSA</b>	tyramide signal amplification
<b>µg</b>	microgram
<b>µl</b>	microlitre
<b>µM</b>	micromolar
<b>U</b>	restriction endonuclease unit
<b>UTR</b>	untranslated region
<b>UV</b>	ultraviolet light
<b>V</b>	volts
<b>VDA</b>	variation detection array
<b>v/v</b>	volume for volume
<b>w/v</b>	weight for volume

**DECLARATION**

I certify that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

G. S. Sellick  
23/7/02



## DEDICATION

For my father, who always wanted to see me wear a “black cap”.

Dad, I know you will always be there close, somewhere.

For my mother, whose enduring love, strength and support

has been forever inspirational.

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