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Detection of genetic diversity in cattle by microsatellite and SNP markers - a review

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Environmental challenges and preference of high-yielding breeds have resulted in extinction of various local cattle populations as well as the loss of genetic diversity in modern cattle breeds. Genetic diversity, however, plays a vital role both in cattle industry to meet current and future demand for milk and beef and in adaptation to different environmental challenges for animals. Thanks to developing molecular genetics and bioinformatics tools, genetic data including microsatellites and Single Nucleotide Polymorphisms (SNPs) can be detected across the genome and can be analysed to reveal genetic diversity within and between cattle populations. Until recently, microsatellite markers were commonly used to estimate genetic diversity in both local and exotic cattle breeds. Today, however, SNP arrays are the most preferred technology for genetic diversity analysis, since they are time-efficient and easy to access and apply. Moreover, developments in sequencing technology with affordable costs have made it possible to obtain SNP data across the genome via whole genome resequencing. It is foreseen that whole genome resequencing will be routinely used to estimate genetic diversity periodically not only in cattle but also in the other livestock species as well in the future. In this study, the most commonly preferred molecular methods to reveal genetic diversity in cattle were discussed and some bioinformatics tools to analyse genetic data were summarised.

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Introduction

As of domestication, cattle have mainly been a part of both diet and daily life of humankind [Abbink 2003, Caroli *et al.* 2009]. Today, cattle are reared in almost all parts of the world to obtain animal-derived products such as milk and beef providing essential amino acids, minerals and vitamins for human nutrition [Brito *et al.* 2021, Dawood *et al*. 2021]. Globally, 81% of milk and 21% of meat production are met by cattle [Kayikci *et al.* 2019, FAO 2022].

Cattle genome have been profoundly shaped by two events known as domestication and migration throughout history [Larson and Burger 2013, Senczuk *et al.* 2021]. Both archaeological and genomic data indicate that sharing the same ancestor (*Bos primigenius*), taurine (*Bos taurus*) and indicine (*Bos indicus*) cattle were first domesticated approximately 10.000 and 8.000 years ago in Fertile Crescent and Indus Valley, respectively [Pitt *et al.* 2019, Senczuk *et al.* 2021]. Domestication enabled early farmers to conduct non-systematic selection practises in which it is more likely that behavioural characteristics were taken into consideration rather than milk and meat yield [Larson and Burger 2013]. Additionally, cattle were introduced to new environmental conditions by human migration in which they developed adaptation to different environments via natural selection. These phenomena increased genetic diversity in locally adapted cattle populations [Groeneveld *et al.* 2010].

Genetic diversity defines the total of alleles and genotypes which shape the genome in terms of morphology, physiology and behaviour of an animal in a certain species [Frankham *et al.* 2002]. In livestock including cattle, maintaining genetic diversity at optimum level is of great importance to meet current and future production systems as well as demand for animal-derived products [Karsli *et al.* 2020a]. Additionally, genetic diversity is required for adaptation to diverse environmental stressors such as diseases and climate change [Demir *et al.* 2021a]. However, genetic diversity tends to decrease in both local and exotic cattle breeds due to many reasons [FAO 2019]. Today, several facts such as increasing human population, water and land scarcity for agriculture together with climate change have been forcing farmers to rear high-yielding cattle breeds [Srivastava *et al.* 2019]. Compared to local cattle breeds, exotic ones were developed based on genotype combinations of small part of genome including regions related to milk and meat traits. Today, these exotic breeds holding low genetic diversity are distributed across the world and are preferred by farmers for their high production capacity [FAO 2019]. On the other hand, local cattle populations not only conserve high genetic diversity but also they may carry unique genetic combinations related to environmental adaptation [Srivastava *et al.* 2019]. Unfortunately, effective population size of locally adapted native cattle breeds tends to reduce which directly decreases

genetic diversity as well. Besides, uncontrolled crossbreeding practices with exotic breeds results in genetic erosion in local cattle populations [Rahman *et al.* 2013].

Reduction in genetic diversity brings about conservation genetics in which genetic diversity can be detected via different molecular techniques. Today, numerous molecular techniques such as Polymerase Chain Reaction (PCR) based molecular markers, PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) [Karsli *et al.* 2022], microsatellites [Demir and Balcioglu 2019], mitochondrial DNA (mtDNA) [Di Lorenzo *et al.* 2018], Y chromosome [Yalta-Macedo *et al.* 2021], SNP array [Bhuiyan *et al.* 2021] and even whole genome resequencing [Xia *et al.* 2021] are available to obtained genomic data in cattle. These data including microsatellites and SNPs can be further analysed by bioinformatics tools in order to estimate genetic diversity in cattle breeds. In this regard, this paper aims (i) to review commonly used molecular techniques to obtain genomic data and (ii) to summarize bioinformatics tools to reveal genetic diversity in cattle breeds.

Molecular detection of genetic diversity in cattle

Invention of the PCR by Kary Mullis in 1984 has fundamentally revolutionized molecular genotyping techniques. While molecular genotyping of livestock species including cattle was mainly based on RFLP, numerous PCR-dependent genotyping techniques such as Random Amplification of Polymorphic DNA (RAPD), PCR-RFLP, Amplified Fragment Length Polymorphism (AFLP), microsatellites, mtDNA, Y chromosome etc., were discovered to reveal genetic variability in single gene or multiple loci [Shrivastava *et al.* 2018, Xia *et al.* 2019, Karsli *et al.* 2020b, Demir *et al.* 2020]. Additionally, based on selected breeds, numerous SNP arrays with variable densities are available to calculate genetic diversity within and between cattle populations. Recently, Olschewsky and Hinrichs [2021] have reviewed a total of 133 scientific papers published between 2005 and 2020 in terms of molecular genotyping methods including AFLP, RFLP, Whole Genome Sequencing (WGS), Y chromosome, mtDNA, microsatellites and SNPs) in some major farm animals (cattle, sheep, goat, chicken and pig). Authors highlighted that the most preferred molecular techniques to reveal genetic diversity were microsatellites (48%) and SNPs (29%), whereas WGS is becoming popular (6%) since 2010 [Olschewsky and Hinrichs 2021]. Hence, in this study priority was given to microsatellites, SNP arrays and WGS for genetic characterization of cattle populations.

Microsatellite markers in detection of genetic diversity in cattle

Microsatellites, also known as Short Tandem Repeats (STRs) or Simple Sequence Repeats (SSRs), refer to small DNA fragments generally less than 5 nucleotides which are distributed in both coding and noncoding regions across the eukaryotic genome [Bruford and Wayne 1993]. While microsatellite motifs are generally conserved in

livestock species, their repeat numbers show variability among different cattle breeds as well as among animals within a certain breed [Demir *et al.* 2021b]. Differences in repeat numbers of microsatellite markers result in presence of various alleles within cattle populations (Fig. 1). Based on these microsatellite alleles each individual could be easily genotyped. In microsatellite studies, loci are amplified with specific oligonucleotides also known as primers and band size are detected to obtain genotypes (Fig. 1). Since fragment size of PCR products may be close, they are visualised by fragment analyser devices rather than traditional agarose gel electrophoresis [Pashnick and Thum 2020]. Via bioinformatics tools, obtained genetic data could be further used to estimate genetic diversity parameters within and between populations.

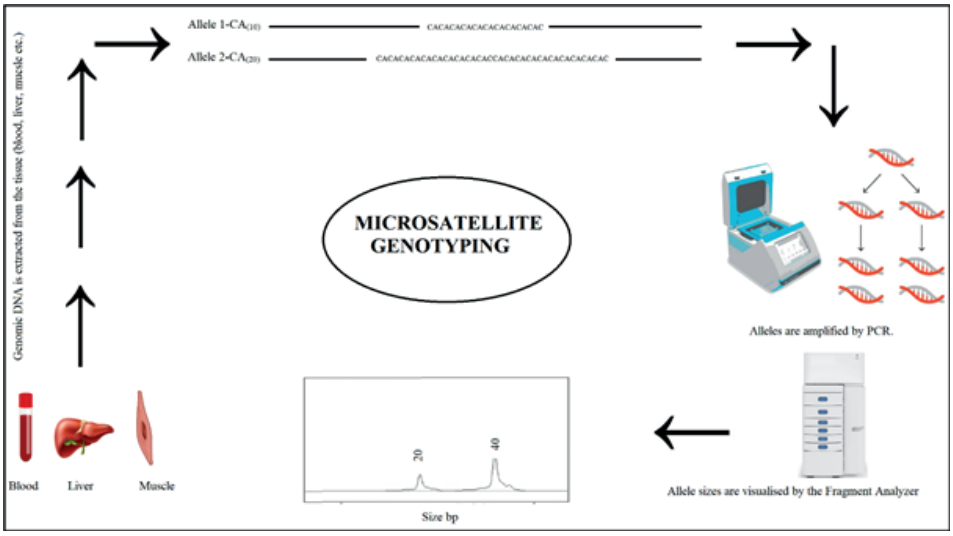

Fig. 1. An overview of microsatellite marker technique.

Microsatellites are of several advantages in revealing genetic diversity in cattle populations, since they are highly polymorphic, showing co-dominant inheritance, distributed across the genome, available in introns and exons, and easy to apply [Jaayid and Dragh 2013, Demir and Balcioglu 2019]. Microsatellite technique is also encouraged by Food and Agriculture Organization of the United Nations (FAO) in genetic characterization of livestock species [Demir and Balcioglu 2019]. Indeed, a list of 30 microsatellite loci has been published by FAO for 9 major livestock species including cattle not only for genetic characterization studies but also to make conducted studies comparable (Tab. 1) [FAO 2011]. Indeed, this panel of microsatellite loci allows for assessing genetic diversity from regional and/or national to international level meaning that genetic diversity in local cattle breeds could be assessed by the comparison of the results obtained from cosmopolitan breeds (dairy, beef and dual purpose) as well as the other local cattle breeds around the globe.

Table 1. Summary of microsatellite loci recommended by FAO

Locus	Chr	PS (5'-3')	AT (°C)	GAN	AR (bp)
BM1824	1	F: GAGCAAGGTGTTTTTCCAATC R: CATTCTCCAACTGCTTCCTTG	55-60	G18394	176-197
INRA023	3	F: GAGTAGAGCTACAAGATAAACTTC R: TAACTACAGGGTGTTAGATGAACTC	55	X67830	195-225
ETH152	5	F: TACTCGTAGGGCAGGCTGCCTG R: GAGACCTCAGGGTTGGTGATCAG	55-60	Z14040 G18414	181-211
ETH10	5	F: GTTCAGGACTGGCCCTGCTAACA R: CCTCCAGCCCACTTTCTCTTCTC	55-65	Z22739	207-231
ILSTS006	7	F: TGTCTGTATTTCTGCTGTGG R: ACACGGAAGCGATCTAAACG	55	L23482	277-309
HEL9	8	F: CCCATTCAGTCTTCAGAGGT R: CACATCCATGTTCTCACCAC	52-57	X65214	141-173
MM12	9	F: CAAGACAGGTGTTTCAATCT R: ATCGACTCTGGGGATGATGT	50-55	Z30343	101-145
ETH225	9	F: GATCACCTTGCCACTATTTCCT R: ACATGACAGCCAGCTGCTACT	55-65	Z14043	131-159
INRA037	10	F: GATCCTGCTTATATTTAACCAC R: AAAATTCCATGGAGAGAGAAAC	57-58	X71551	112-148
CSRM60	10	F: AAGATGTGATCCAAGAGAGAGGCA R: AGGACCAGATCGTGAAAGGCATAG	55-65	…	79-115
ILSTS005	10	F: GGAAGCAATGAAATCTATAGCC R: TGTTCTGTGAGTTTGTAAGC	54-58	L23481	176-194
INRA032	11	F: AAACTGTATTCTCTAATAGCTAC R: GCAAGACATATCTCCATTCCTTT	55-58	X67823	160-204
HEL13	11	F: TAAGGACTTGAGATAAGGAG R: CCATCTACCTCCATCTTAAC	52-57	X65207	178-200
INRA005	12	F: CAATCTGCATGAAGTATAAATAT R: CTTCAGGCATACCCTACACC	55	X63793	135-149
CSSM66	14	F: ACACAAATCCTTTCTGCCAGCTGA R: AATTTAATGCACTGAGGAGCTTGG	55-65	…	171-209
SPS115	15	F: AAAGTGACACAACAGCTTCTCCAG R: AACGAGTGTCCTAGTTTGGCTGTG	55-60	FJ828564	234-258
HEL1	15	F: CAACAGCTATTTAACAAGGA R: AGGCTACAGTCCATGGGATT	54-57	X65202	99-119
INRA035	16	F: TTGTGCTTTATGACACTATCCG R: ATCCTTTGCAGCCTCCACATTG	55-60	X68049	100-124
TGLA53	16	F: GCTTTCAGAAATAGTTTGCATTCA R: ATCTTCACATGATATTACAGCAGA	55	…	143-191
ETH185	17	F: TGCATGGACAGAGCAGCCTGGC R: GCACCCCAACGAAAGCTCCCAG	58-67	Z14042	214-246
TGLA227	18	F: CGAATTCCAAATCTGTTAATTTGCT R: ACAGACAGAAACTCAATGAAAGCA	55-56	…	75-105
INRA063	18	F: ATTTGCACAAGCTAAATCTAACC R: AAACCACAGAAATGCTTGGAAG	55-58	X71507	167-189
ETH3	19	F: GAACCTGCCTCTCCTGCATTGG R: ACTCTGCCTGTGGCCAAGTAGG	55-65	Z22744	103-133
TGLA126	20	F: CTAATTTAGAATGAGAGAGGCTTCT R: TTGGTCTCTATTCTCTGAATATTCC	55-58	…	115-131
TGLA122	21	F: CCCTCCTCCAGGTAAATCAGC R: AATCACATGGCAAATAAGTACATAC	55-58	…	136-184
HEL5	21	F: GCAGGATCACTTGTTAGGGA R: AGACGTTAGTGTACATTAAC	52-57	X65204	145-171
HAUT24	22	F: CTCTCTGCCTTTGTCCCTGT R: AATACACTTTAGGAGAAAAATA	52-55	X89250	104-158
BM1818	23	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	56-60	G18391	248-278
HAUT27	26	F: AACTGCTGAAATCTCCATCTTA R: TTTTATGTTCATTTTTTGACTGG	57	X89252	120-158

Chr – chromosome; PS – primer sequence; AT – annealing temperature; GAN – GeneBank Accession Number; AR – allele range.

As given in Table 1, FAO-recommended microsatellite loci are located at 20 different chromosomes making them useful to reveal genetic diversity across different genomic regions. Due to their advantages, microsatellites not only have been preferred for molecular genotyping in cattle [Demir and Balcioglu 2019] but also they were used to reveal genetic diversity in sheep [Ben Sassi-Zaidy *et al.* 2022], goats [Karsli *et al.* 2020a], chickens [Sabry *et al.* 2021] and pigs [Snegin *et al.* 2021] as well. Apart from genetic diversity, microsatellites have been applied for genome mapping [Ihara *et al.* 2004], parentage testing [Brenig and Schütz 2016], breed assignment [Jaiswal *et al.* 2016], conservation priority [González-Cano et al. 2022] in cattle populations.

SNP arrays in detection of genetic diversity of cattle

SNP is defined as the simplest form of DNA variation such as transition or transversion occurring at a frequency of about one per 1.000 base pairs throughout the genome [Brookes 1999]. Several studies have shown that these simple mutations are significantly related to production [Ali *et al.* 2020], reproduction [Lu *et al.* 2021] and diseases [Soares *et al.* 2021] in cattle. Similar to microsatellites, they are randomly distributed in both coding and noncoding regions of the genome, while they can be detected via numerous molecular tools such as Allele Specific (AS) PCR, PCR-RFLP, sequencing etc. in which SNP arrays are commonly preferred due to higher accuracy and feasibility [Kleinman-Ruiz *et al.* 2016]. SNP arrays are also called microarray,

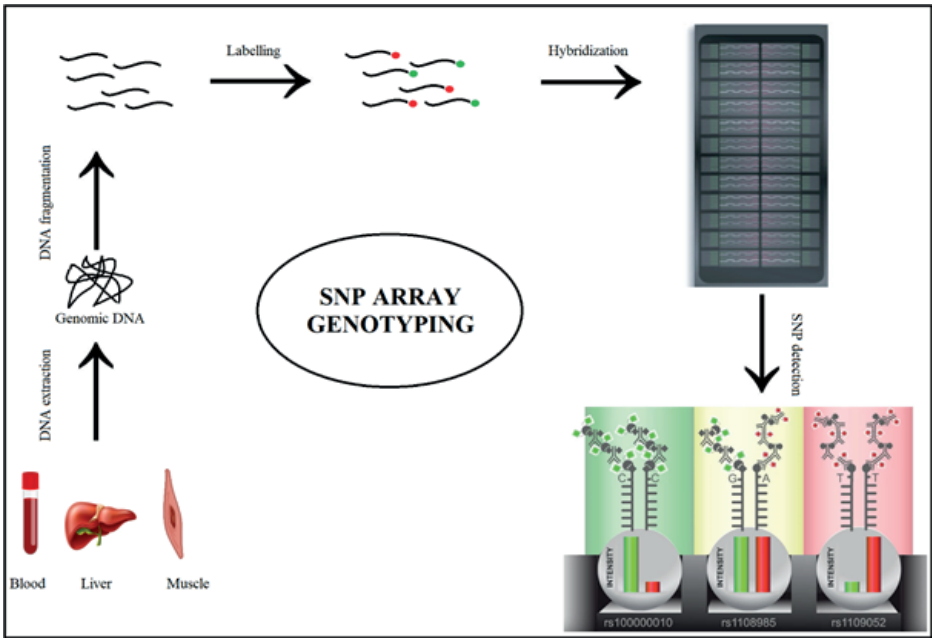

Fig. 2. Concept of genotyping based on SNP array.

SNP chip and BeadChip which are used to genotype animals in terms of thousands of SNPs based on spots on the microarray corresponding to different alleles [Flanagan and Jones 2019]. Each spot creates different colour patterns based on the density of hybridized nucleotides which enables to record individuals as homozygous or heterozygous (Fig. 2). Today, several commercially produced SNP arrays with different densities are available for cattle genotyping in which develop by Illumina Inc., LD, SNP50 v.2 and HD are able to detect approximately 6.909, 54.609 and 777.962 SNPs in cattle, respectively [Nicolazzi *et al.* 2015]. Due to their abundance and randomly distribution in intron and exon regions across the genome, SNP arrays have been utilized to reveal genetic diversity within and between cattle breeds [Saravanan *et al.* 2020] together with parentage analysis [Hu *et al.* 2021], genes associated with economic traits [Raza *et al.* 2020], heat tolerance [Jia *et al.* 2019], selection signatures [Moravčíková *et al.* 2019], susceptibility/resistance to diseases [Zeb *et al.* 2020, Chai *et al.* 2021].

WGS in detection of genetic diversity of cattle

Sanger sequencing, also known as chain-termination method, is referred to the first generation sequencing by which nucleotide order of a given DNA fragment could be detected by chemical reaction process [Sanger *et al.* 1977]. Sanger sequencing has been mainly utilized in cattle to reveal genetic diversity in single locus [Bayıl Oğuzkan and Bozkurt 2019] as well as partial mtDNA region such as displacement loop [Granado *et al.* 2021]. Although, this method provides nucleotide discovery with high accuracy (99.9%) [Shendure and Ji 2008], it is not cost-effective and limited to sequence small part of the genome (approximately 1000 bp). However, second generation sequencing such as Next Generation Sequencing (NGS) based on parallel sequencing of massive DNA fragments are available to detect SNPs by screening large part of the genome (from exome to entire genome sequencing) with cost-effective manner in cattle breeds. In NGS studies, numerous library preparation methods such as Restriction-Site-Associated DNA Sequencing (RAD-seq) and Double Digest Restriction-Site-Associated DNA Sequencing (ddRAD-seq) together with variable sequencing platforms such as Illumina, Roche 454 and AB SOLiD enable scientist to genotype local cattle breeds [Vineeth *et al.* 2020, Mao *et al.* 2021]. Library preparation process and sequence platforms were comprehensively reviewed elsewhere by Hess *et al.* [2020] and Gatew and Tarekegn [2018], respectively. Briefly, genomic DNA libraries are created by DNA extraction, digestion, barcoding, indexing and cleaning steps followed by PCR amplification (Fig. 3). These libraries covering different individuals are sequenced simultaneously at single run by a suitable sequence platform.

WGS tends to become popular due to advantages of NGS technologies. Breed-specific SNPs and variants with low frequencies could be detected by assembling genomic data obtained by WGS with reference cattle genome [Zhang *et al.* 2019].

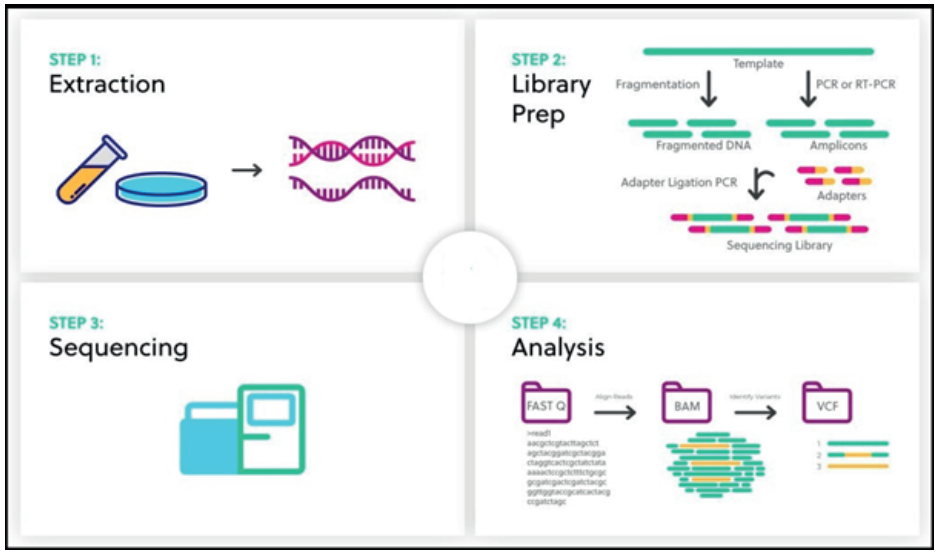

Fig. 3. Main steps of WGS (https://irepertoire.com/ngs-overview-from-sample-to-sequencer-to-results/).

Besides, indexing and barcoding process of NGS technologies allow to sequence whole genome of different individuals simultaneously at the same reaction which directly decreases economic burden of genotyping. Moreover, genomic data obtained from the entire genome not only increases accuracy of genetic diversity analysis but also enables scientists to conduct new statistical approaches such as copy number variations [Zhang 2020]. However, including more labour in laboratory practises as well as complex process of library preparation are one of the disadvantages of WGS in pre-sequencing steps [Zhao *et al.* 2020]. Additionally, raw data obtained from sequencer should be assembled to reference genome to detect SNPs and both SNPs and samples should be filtered by quality control process before downstream analyses via bioinformatic tools.

Comparison of microsatellite markers, SNP arrays and WGS in genetic diversity studies

Several criteria such as feasibility (time and labour efficiency) and accuracy may be considered to compare microsatellite and SNP data for genetic diversity analysis. In particular, obtaining SNP data is much easier than microsatellite data which requires PCR amplification and fragment analysis for each locus that are not feasible in terms of time and labour efficiency [Fernández *et al.* 2013].

On the other hand, SNPs are bi-allelic meaning that in theory, maximum 2 alleles and 3 genotypes could be detected at each SNP, whereas microsatellite loci are multi-

allelic indicating that multi alleles and genotype combinations could be observed per each locus [Vignal *et al.* 2002]. For example, a total of 37 alleles were reported at *ILSTS34* microsatellite locus in native Indian cattle breeds [Sharma *et al.* 2015]. Accordingly, Schopen *et al.* [2008] reported that for single microsatellite locus, three SNPs are required to achieve the same genetic information in cattle. However, microsatellite loci are less in number compared to SNPs across the cattle genome. In conservation genetics, evaluation of genetic diversity covering in as much of large part of the genome as possible is of vital importance to prioritize cattle populations [Karimi *et al*. 2016]. In this context, SNP data has advantages over microsatellite data, since genetic diversity may be estimated via millions of SNPs distributed across larger part of the genome. Indeed, screening larger part of genome for SNPs gives better results with higher accuracy to estimate genetic diversity in cattle populations [Bradbury *et al.* 2015].

Although, today, SNP arrays are commonly preferred to reveal genetic diversity in cattle populations due to their feasibility, they are produced to detect specific SNPs based on reference cattle breeds [Hou *et al.* 2012, De Donato *et al.* 2013]. This bias to reference cattle breeds hinders to detect unique SNPs in local cattle populations [Malik *et al.* 2018, Geibel *et al.* 2021]. It is known that local cattle populations contain beneficial mutations for specific environmental conditions. These mutations could not be detected by commercially available SNP arrays. On the other hand, developments in sequencing technologies enable scientists to re-sequence the whole genome of local cattle via NGS techniques such as Genotyping by Sequencing (GBS) and RADSeq [Malik *et al.* 2018, Wang *et al.* 2018]. These techniques are mainly based on enzymatic digestion, amplifying and partly sequencing cattle genome. These partially amplified sequences could be easily re-combined together according to update reference cattle genome to obtain SNPs across the genome. Whole genome resequencing makes it possible to observe unique SNPs which cannot be detected by SNP arrays.

Statistical analysis of genetic diversity via microsatellites and SNPs

In livestock including cattle, genetic diversity may be evaluated within and between populations. Statistical parameters such as number of alleles (N_a), allele frequency, number of effective alleles (*Ne*), observed (H_O) and expected heterozygosity (H_E) are commonly used to reveal genetic diversity within cattle populations. *Na* indicates the total number of detected alleles per breed, whereas Ne refers to the number of alleles contributing to genetic variation in a given locus [Kimura and Crow 1964]. H_O is the statistics of the frequency of total number of heterozygote individuals in terms of a certain loci, while H_E, also known as gene diversity, defines the proportion of heterozygous genotypes in the context of Hardy-Weinberg equilibrium [Nei 1973]. Being Wright's F statistics based on expected level of heterozygosity, F_{ST} and F_{IS} are referred to genetic differentiation between subpopulations and level of inbreeding in subpopulations, respectively. Among them, N_a and *Ne* are estimated by microsatellite

data, whereas H_O, H_E and F_{IS} are calculated via both microsatellite and SNP data to reveal genetic diversity [Herráez *et al.* 2005, Uzzaman *et al.* 2014]. Besides, there are numerous clustering-based statistical approaches such as Principal Component Analyses (PCA), Neighbour-Joining (NJ) tree analysis, STRUCTURE analysis and Analysis of Molecular Variance (AMOVA) to reveal genetic structure of cattle populations via microsatellite and SNP data. By applying orthogonal transformation to reduce correlated variables into uncorrelated variables, PCA assigns the highest percentage of total variance to the first (PC1) and second (PC2) components [Fraga *et al.* 2016]. NJ method enables to create tree diagram via binary distance matrix at

Table 2. Summary of some statistical softwares programs in genetic diversity

Software	Data Type	Na	Ne	F_{IS}	H_O	H_E	F_{ST}	PCA	NJ-Tree	Structure	AMOVA	Genetic distance	Reference
GenAlEx	SSR and SNP	+	+	+	+	+	+	+			+	+	Peakall and Smouse [2006]
GDA	SSR and SNP	+		+	+	+	+		+			+	Lewis [2001]
Popgene	SSR	+	+	+	+	+	+		+			+	Yeh *et al.* [1999]
Power Marker	SSR and SNP	+							+	+	+	+	Liu *et al.* [2005]
Cervus	SSR and SNP	+			+	+							Kalinowski *et al.* [2007]
Arlequin	SSR and SNP	+	+	+	+	+	+				+	+	Excoffier *et al.* [2005]
STRUCTURE	SSR and SNP									+			Pritchard *et al.* [2000]
Tassel	SNP	+			+	+		+	+	+		+	Bradbury *et al.* [2007]
DARwin	SSR and SNP	+			+	+		+	+				Perrier and Jacquemoud-Collet [2006]
PLINK	SNP	+			+	+				+		+	Purcell *et al.* [2007]
TFPGA	SSR	+		+	+	+	+					+	Miller [2008]
Genepop	SSR and SNP	+		+	+	+	+					+	Rousset [2008]
NTSYSPC	SSR and SNP							+	+			+	Rohlf [2002]
DnaSP	SNP	+			+	+	+					+	Rozas *et al.* [2017]
MacClade	SNP								+				Maddison and Maddison [2000]
PHYLIP	SNP				+	+			+			+	Felsenstein [1989]
FSTAT	SSR	+	+	+	+	+	+						Goudet [1995]
ADMIXTURE	SNP							+	+				Alexander and Lange [2011]
MEGA	SNP								+				Kumar *et al.* [2004]
GENETIX	SSR and SNP	+	+	+	+	+	+	+				+	Bonhomme *et al.* [1993]

Na – number of alleles; Ne – number of effective alleles; F_{IS} – inbreeding coefficient; H_O – observed heterozygosity; H_E – expected heterozygosity; F_{ST} – genetic differentiation coefficient, PCA – Principal Component Analysis; NJ-Tree – Neighbour Joining-Tree Analysis; AMOVA – Analysis of Molecular Variance.

individual and/or breed level. STRUCTURE analysis detects differences between populations by placing each of the samples in groups sharing similar patterns of variation using a Bayes iterative algorithm [Porras-Hurtado 2013]. Additionally, AMOVA developed by Excoffier *et. al* [1992] which is frequently used in many molecular studies, is a hierarchical analysis of variance method which divides genetic diversity in terms of its components, allowing it to be determined between populations, between individuals within the population, and within individuals.

In general, populations with higher heterozygosity and lower inbreeding possess higher genetic diversity allowing them for developing adaptation to different production systems and environmental conditions. Moreover, by revealing genetic diversity, farmers can increase heterozygosity and decrease inbreeding values via suitable management practices [Demir and Balcioglu 2019].

Rapid advances in bioinformatics have given rise to numerous statistical software which enables to handle genetic diversity in cattle populations (Tab. 2). Possessing different user interfaces, data types of input and output, and operating platforms [Saravanan *et al.* 2019], these programs have facilitated to both manipulate and analyse different molecular data including microsatellites and SNPs. Several studies have focused on explaining the methodologies and statistical approaches of several analyses [Excoffier *et al.* 1992, Pritchard et al. 2000, Excoffier 2004, Ringnér 2008, Dogan and Dogan 2016]. Although, statistical softwares are many in numbers, some of them are summarised in Table 2. As seen, most of them allow for conducting multiple analyses, whereas some of them enable to conduct specific analysis such as STRUCTURE. Therefore, multiple platforms should be adopted to reveal genetic diversity in cattle populations. However, several programs support specific input and output files such as phylip, genepop, mega etc., which requires additional programs to convert data types for specific analysis. PGD Spider [Lischer and Excoffier 2012] and FORMATOMATIC [Manoukis 2007] are one of the useful tools to convert input data for different programs.

Conclusion and perspectives

In this review, molecular identification of genetic diversity in cattle based on microsatellite and SNP markers as well as some statistical approaches and programs to handle these data were summarised. Today, trend in decreased genetic diversity threatens both farmers for maintaining agricultural production and local cattle populations for developing adaptation against environmental conditions which will likely change in the future due to global warming. Particularly, conservation of variations in the genomic regions related to environmental adaptation will be of great importance in the future due to ongoing climate change. The first step of conservation programs in local cattle populations is to reveal current genetic diversity. So far, microsatellite and SNP arrays have been used to reveal genetic diversity within and between populations. As mentioned above, they also have some disadvantages

and are unbiased for local cattle populations. Today, however, NGS technologies are promising to detect genetic diversity across the genome with higher accuracy compared to microsatellite and SNP array. It is believed that, NGS technologies will be cost effective and routinely be used for genetic diversity analysis in the close future.

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