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Journal of Veterinary Physiology and Pathology. 2025; 4(3): 20-30.

DOI: 10.58803/jvpp.v4i3.60

http://jvpp.rovedar.com/

Review Article

Advancing Goat Genomics Verification and Applying GBTS Liquid Chip for Precision Breeding

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ARTICLE INFO

Article History:
Received: 28/06/2025
Revised: 30/07/2025
Accepted: 18/08/2025
Published: 01/09/2025

check for updates

Keywords:
Breeding
Genetic marker
Genomic selection
Genotyping by target sequencing
Liquid chip
Milk goat genomic
Single-nucleotide polymorphism

ABSTRACT

Genotyping by target sequencing (GBTS) liquid chip is a cutting-edge genomic tool that enables the efficient detection of genetic markers for economically important traits, including milk yield, fat content, and disease resistance, in milk goats. The present study aimed to review the development, validation, and application of the GBTS liquid chip in goat genomics, emphasizing its role in precision breeding. The methodology involved extracting DNA from different goat breeds, designing probes for specific gene markers, performing genotyping using the GBTS liquid chip, verifying detected single-nucleotide polymorphisms (SNPs) through whole-genome resequencing, and assessing chip repeatability across batches. Sequence alignment, variant calling, and genome-wide association studies were conducted using bioinformatics tools such as BWA, PLINK, and GATK to ensure accurate identification of SNP loci. Advanced statistical methods, including principal component analysis and phylogenetic tree construction, are employed to demonstrate the chip's effectiveness in distinguishing genetic diversity and relationships among breeds. Functional annotation through databases such as Ensembl and KEGG helped interpret the biological roles of identified markers, while genomic prediction models, including genomic best linear unbiased prediction and BayesC, estimate breeding values for targeted selection. This integrated strategy, combining high-throughput genomic technologies, microfluidic platforms, and computational analysis, demonstrated the potential of GBTS liquid chip technology to enhance goat breeding programs by improving productivity, conserving genetic diversity, and ensuring sustainability.

1. Introduction

The discovery of gene markers in goats to enhance milk production, quality, and disease resistance is a significant breakthrough for dairy goat breeding programs. The genetic markers in goats are DNA sequences that indicate specific traits, revealing which traits are selected for breeding[1-3]. Targeted breeding is enabled for desirable traits, such as milk yield, fat content, and protein levels, as well as health-related traits such as disease resistance and longevity[2]. Genetic marker technology, particularly through different methods such as genomic liquid chips, enables breeders to use non-invasive genomic analysis, significantly reducing time and cost compared to conventional methods such as blood sampling and genomic sequencing[3]. The present study highlighted genetic markers in milk goats, emphasizing their application in different breeding and management strategies. This study covered 25 well-researched markers associated with milk yield, composition, disease resistance, and other important characteristics of health[1,2].

Genetic markers in milk goats are powerful tools for improving breeding programs to enhance production, quality, and health characteristics of milk. By being linked to valuable traits through DNA sequences, these markers serve as a precise tool for selective breeding. Especially valuable

Cite this paper as: Aziz U, Rehman A, Xu X, Zhu J, He Y, Wang Z, Fu L, Ur Rehman F, Li J, wang X, Yan H, and An X. Advancing Goat Genomics Verification and Applying GBTS Liquid Chip for Precision Breeding. 2025; 4(3): 20-30. DOI: 10.58803/jvpp.v4i3.60

for traits such as milk yield, fat content, protein composition, and disease resistance[4]. Breeders can do this non-invasively using genomic liquid chips and other advanced technologies, not only speeding up the process and saving time and money, but also including genome sequencing and blood collection, as suggested by conventional techniques[2]. Genomic liquid chip and related advanced technologies are integrated to offer a comprehensive view of goat farming improvement. Examples of the most studied markers include *diacylglycerol o-acyltransferase 1* (*DGAT1*), which affects milk fat metabolism, and *alpha S1-casein* (*CSN1S1*), which affects milk yield and protein content. The affected gene, *Beta-Casein* (*CSN2*), has become the focus of attention due to its variants A1 and A2, which impact milk composition, digestibility, and health benefits[5]. Another important marker involved in the production of unsaturated fatty acids and therefore healthier milk fat profiles includes *stearoyl-CoA desaturase* (*SCD*). The *KCNJ11* gene, which is related to energy metabolism, is associated with increased milk yield. The prolactin receptor gene (*PRLR*) regulates milk synthesis by controlling the important lactation hormone prolactin[6,7]. Moreover, genes such as the melanocortin 4 receptor (*MC4R*) and fatty acid synthase (*FASN*), which are associated with energy balance and fatty acid synthesis, can influence milk yield and fat content[7]. However, to strengthen immunity against diseases, especially mastitis (a common disease in all goats, especially dairy goats), markers such as the *major histocompatibility complex* (*MHC*) and *toll-like receptor 4* (*TLR4*) are important[8].

2. Genetic markers for milk yield and composition

The markers allow breeders to choose goats that are resistant to infection and more adaptable and productive. Important immune-regulating and anti-inflammatory genes, such as interleukin 10 (*IL-10*) and transforming growth factor beta (*TGF-β*), play a crucial role in maintaining overall goat health and reducing the likelihood of developing diseases, including mastitis[7]. For instance, genes producing milk, including *growth hormone 1* (*GH1*) and *alpha-lactalbumin* (*LALBA*), which affect milk yield and nutritional quality, can be selected for these markers. One of the markers is *superoxide dismutase 1* (*SOD1*), which contributes to stress tolerance and disease resistance in highly stressful environments[8]. In addition, *prolactin-releasing peptide 1* (*PrRP*)*, lactoglobulin beta* (*LGB*)*,* and *C-X-C chemokine receptor type 2* (*CXCR2*) genes appear to contribute to mastitis resistance and immunity. Furthermore, adiponectin *(ADIPOQ)* and *fucosyltransferase 2 (FUT2)* affect milk fat synthesis and milk oligosaccharide composition, and milk oligosaccharide composition[4,5]. Genetic markers in milk goats can be utilized to develop a comprehensive breeding strategy that enhances milk production efficiency, sustainability, and quality. Widespread use of this molecular approach will revolutionize the dairy industry, allowing producers and consumers alike to pick precisely for desirable traits[6].

The *SCD* gene has a significant impact on milk fat composition, as it not only enhances the quality of milk fat through the production of unsaturated fatty acids but also improves its nutritional profile, which is becoming increasingly important to consumers. In addition, it is essential that genetic markers related to energy balance, such as those associated with the *MC4R* genes, are crucial for optimal feed intake, which supports higher milk production and overall health[7]. Goats that utilize energy more efficiently are especially valuable for breeding programs aimed at maximizing production while minimizing resource use[7,8].

As many diseases become more prevalent and global challenges such as climate change threaten the agricultural industry, genetic selection is another key consideration for selecting breeding stock, with disease resistance being particularly critical[9]. Goats' resistant to infections are known to have markers, such as *TLR4*, that enhance their immune response. Therefore, it is essential to select goats that can withstand stress and environmental conditions, such as mastitis. Among the molecular markers, *MHC* contributes to disease resistance, and ongoing studies are revealing how these genes interact together to prevent many diseases in goats[9,10].

Though mastitis poses a significant challenge to milk production, it affects both the quantity and quality of milk. Breeders can select goats with more genetic markers associated with immune response and inflammation regulation, such as *IL-10* and *TGF-β*, that are less susceptible to infection and are more likely to produce uncontaminated milk[10,11]. Within the context of dairy goat farming development, milk quality-related genetic markers, especially *CSN1S1* and *CSN2*, can be exploited to supplement other dairy product production, including cheese[9-11]. However, as the world's demand for high-quality, nutritious milk rises, it is vital to gain an understanding of the genetic background of milk production to ensure breeders can meet this demand[11].

The *DGAT1* and *LALBA* are new-generation genetic markers that could help improve milk quality and transform the dairy industry[8-11]. Genomic liquid chip technology can be used to move forward, identify and analyze the key genes in milk goat genomics (Table 1) more rapidly and precisely than ever before, and apply more targeted and efficacious breeding programs. These genomic liquid chips, which are gaining widespread adoption, could usher in a new era for livestock genetics, utilizing genomic tools to produce healthier, more productive animals that meet an ever-changing market for dairy[12,13]. Furthermore, gene editing technologies such as CRISPR are starting to open new pathways for enhancing goat genetic materials. These tools could enable more precise changes, resulting in significant improvements in milk yield, milk composition, and disease resistance[13,14].

Table 1. Key genes and their role in milk goat genomics

Gene name	Description	Reference
Diacylglycerol o-acyltransferase 1(DGAT1)	Plays a central role in milk fat metabolism; variants linked to higher milk fat content are useful for breeding goats with superior fat yield.	Elsik et al. (2016)
Alpha S1-CASEIN (CSN1S1)	Major milk protein affecting yield and protein content; higher levels are associated with increased milk yield and improved milk quality, which is vital for cheese production.	Bokhari and Sauer (2005)
Beta-casein (CSN2)	Another key milk protein influencing yield and composition, variants such as A1 and A2, are linked to milk digestibility and health benefits.	Elsik et al. (2016)
Stearoyl-CoA desaturase (SCD)	Involved in converting saturated fats to unsaturated fats in milk; variations affect fat composition and nutritional quality.	Loukovitis et al. (2016)
Potassium channel, inwardly rectifying subfamily J member 11 (KCNJ11)	Associated with energy metabolism and milk yield, variants increase milk production efficiency in dairy goats.	Tamura et al. (2021)
Prolactin receptor (PRLR)	Regulates milk synthesis by interacting with the prolactin hormone; variants are linked to higher lactation potential and milk yield.	Singh et al. (2024)
Melanocortin 4 receptor (MC4R)	Regulates food intake and energy balance; variants linked to better feed intake and improved milk yield, enhancing metabolic efficiency.	Elsik et al. (2016)
Fatty acid synthase (FASN)	Involved in fatty acid production, impacting milk fat composition; specific variants are linked to higher fat yield and improved milk quality.	Clark and Elgar (2000)
Thyroglobulin (TG)	Regulates thyroid hormones, which influence metabolism and milk production; variants impact milk yield and metabolic efficiency.	Ye et al. (2025)
Growth hormone 1 (GH1)	Regulates growth and metabolism, influencing milk yield; specific alleles are linked to improved lactation performance and herd productivity.	Ye et al. (2025)
Major histocompatibility complex (MHC)	Central to immune function and disease resistance, variants protect goats from diseases such as mastitis, thereby improving overall herd health.	Rasheed et al. (2017)
Toll-like receptor 4 (TLR4)	Plays a role in immune response and detecting bacterial infections; specific variants reduce susceptibility to mastitis.	Tamura et al. (2021)
Lactoglobulin beta (LGB)	Protein involved in immune function; linked to mastitis resistance and overall milk quality.	Nystrom and McKay (2021)
Heat shock protein 70 (HSP70)	Protects cells from stress; variants linked to improved disease resistance and stress tolerance in challenging environments.	Xu et al. (2014)
Interleukin 10 (IL-10)	An anti-inflammatory cytokine regulating immune response; linked to better mastitis resistance, improving goat health and milk quality.	Zhang et al. (2022)
Transforming growth factor beta (TGF-β)	Regulates immune response and inflammation; variants are associated with mastitis and other inflammatory diseases.	Yang et al. (2010)
C-X-C chemokine receptor type 2 (CXCR2)	Involved in immune regulation and inflammation; variants linked to improved resistance to mastitis and other infections.	Elsik et al. (2016)
Follistatin (FST)	Involved in immune modulation and inflammatory response regulation; linked to better disease resistance, including mastitis.	Elsik et al. (2016)
Superoxide dismutase 1 (SOD1)	Antioxidant enzyme protecting cells from oxidative damage; variants linked to improved disease resistance and stress tolerance.	Singh et al. (2024)
Prolactin-releasing peptide 1 (PRM1)	Regulates prolactin release, which is essential for milk production; variants are linked to differences in lactation performance and milk yield.	Tamura et al. (2021)
Alpha-lactalbumin (LALBA)	Crucial for lactose synthesis in milk; variants influence milk yield and composition, particularly lactose content, improving milk quality.	Yáñez et al. (2023)
Carbonic anhydrase (CA)	Plays a role in regulating milk acidity; variants influence milk quality, including flavor and nutritional content.	Singh et al. (2024)
Sphingosine-1-phosphate receptor 1 (S1PR1)	Regulates milk lipid composition; variants affect milk fat content and overall milk quality.	Yang et al. (2010)
Adiponectin (ADIPOQ)	Involved in metabolic regulation and milk fat content; favorable variants improve milk fat synthesis and quality.	Yang et al. (2010)
Fucosyltransferase 2 (FUT2)	Plays a role in synthesizing oligosaccharides in milk; variants linked to milk composition, enhancing nutritional value for offspring.	Ma et al. (2012)

The use of genetic markers in goat breeding has the potential to revolutionize this industry, enabling it to adapt to changing customer demands and safeguard goat populations worldwide[13].

3. Marker selection

The first step was to select markers that have a strong influence on milk composition, such as *DGAT1, CSN1S1, CSN2,* and *FASN*, or on disease resistance, including *MHC, TLR4, IL10*, and *SOD1*. Each marker is a short section of DNA (15 to 30 base pairs) surrounding the polymorphism or genetic variant, which will enable probes to detect the presence of both the reference and alternative alleles, particularly in cases of single-nucleotide polymorphism (SNP)[14]. To verify the specificity of these probes, bioinformatic tools such as BLAST were used to determine that these probes only bind to the intended genetic regions and do not cross-react with other regions of the genome[13]. The probes' design represented factors such as GC content, which was maintained within a stable range of 40-60%, and melting temperature (Tm), which was optimized within 55-65°C for optimal hybridization. Oligo Analyser was used as a tool to screen hairpins, which are potential obstacles to binding. When the probes were designed and synthesised using commercial high-purity services, with possible modifications for detection such as fluorescent labels or biotin[10-13]. These probes were synthesized and tested in genomic assays, including real-time polymerase chain reaction (PCR) and genotyping microarrays. The genes *DGAT1*, *CSN1S1*, *CSN2*, and *FASN*, along with those related to disease resistance such as *MHC*, *TLR4*, *IL-10*, and *SOD*, were evaluated for their capacity to pinpoint the specific genes responsible for traits including illness resistance and milk quality. Probes for *MHC* and *TLR4* activity detect genetic variants linked to mastitis resistance and immune function, while probes for *LALBA* and *ADIPOQ* identify variants related to milk fat and lactose content[14]. The outcome of these tests would further refine breeding strategies by revealing the genetic factors that control milk production and quality. The chip fabrication process, which is essential for high-throughput genetic analysis, was included[15,16]. Initial fabrication begins by selecting and cleaning silicon wafers, followed by oxidation to create a thin silicon dioxide layer. Afterwards, a photoresist material was patterned on the wafer with photolithography and exposed to UV light using a mask. This pattern was then transferred onto the wafer's surface in order to create the correct circuit design. There were several stages involved in building integrated circuits, which were achieved through doping, etching, and layering processes, as well as further polishing to achieve a smooth surface. When the layers were complete, the wafer was cut into individual chips, and these were tested to ensure they met performance requirements. This method yielded a precise and controlled process for producing high-quality chips for genetic analysis[15].

4. Probe evaluation

Once the probe was designed, its binding efficiency and specificity to the target DNA sequence were thoroughly evaluated. This assessment ensures that the probe correctly recognizes and attaches to the intended genetic region without cross-reacting with unrelated sequences[17]. Prob evaluation included checking for potential secondary structures, such as hairpins, that may interfere with probe function, and verifying that the Tm falls within an optimal range (Typically 55-65°C) to promote stable hybridization. Additionally, *in silico* tools were used to confirm that the probe did not show significant non-specific binding to other genome regions. After these validations, the DNA probe was chemically synthesized in the laboratory for downstream applications[17].

4.1. Analysis of loci distribution and function

The next stage was to evaluate the distribution of small SNPs in the genome and their potential biological functions. The first step was to count the number of SNP loci by chromosome, which helped in understanding the distribution of SNPs and determining whether any chromosomes were related to specific traits or diseases. The SNP density was calculated across chromosomes using a sliding window of 1 megabase (1 Mb) to guarantee a homogenous distribution of SNP[17,18].

4.2. Gap distribution analysis

The regions of the chromosome containing SNPs were completed in the gaps that were missing. Areas lacking SNPs between two adjacent segments are known as gaps[17-18]. In studies analyzing SNP distribution in goat genomics, different methods were employed to identify gaps in genomic coverage, which are critical for ensuring comprehensive marker detection in breeding programs. A commonly used approach involves a threshold-based formula to detect regions with sparse SNP coverage, often referred to as gaps, which may indicate under-sampled genomic areas requiring further investigation.

Gap threshold formula, (D > T), where D means the distance in base pairs between two consecutive SNP loci on a chromosome, and T means the threshold distance, typically set at 1 megabase (1 Mb), based on sliding window analyses used to assess SNP density. When the distance (D) between adjacent SNP loci exceeds the threshold (T = 1 Mb), studies flag these regions as potential gaps, suggesting insufficient marker coverage that could affect the accuracy of genomic selection for traits such as milk yield or disease resistance in goats[18-20].

4.3. Single-nucleotide polymorphism functional annotations

After mapping the SNP, gene ontology (GO) analysis was performed to evaluate the potential functional relevance of the SNP by grouping genes based on biological processes, molecular functions, and cellular components. Kyoto encyclopedia of genes and genomes (KEGG) analysis was performed to identify the enriched pathways of the SNP loci. Functional annotation was done using tools such as DAVID

(Database for annotation, visualization, and integrated discovery), which provided information on potential biological functions, pathways, and protein interactions associated with the SNP. This method of functional analysis enables the understanding of how SNPs are linked to traits or diseases, such as immune functions or metabolic disorders, based on their gene associations[19].

5. Development of a microfluidic chip

The development of a microfluidic chip is a complex process that combines expertise from materials science, biology, chemistry, and engineering. These chips, which consist of microscale fluid channels etched onto a solid surface, are commonly used in diagnostics, genetic analysis, and other types of biochemical assays. A microfluidic chip is a time-efficient, economical, and high-throughput platform for utilizing genetic tests in livestock genomics, such as genetic trait identification in milk goats[18,19].

Chip design and fabrication are the initial steps wherein fluid dynamics, material properties, and assay requirements are considered. The chip is usually fabricated via soft lithography. The master mold is created using photolithography and is subsequently utilized to form the microfluidic channels on a polymer substrate, such as polydimethylsiloxane (PDMS). Functionalization comes after the chip fabrication. In this step, other biomolecules, including DNA probes or antibodies, are incorporated into the surface of the chip to detect the desired genetic markers or proteins[19]. An example of functionalization for milk goat genomics could be the immobilization of oligonucleotides complementary to genetic markers associated with specific traits such as milk yield, fat content, or disease resistance. A prominent example of such genes is the *DGAT1* gene, a major gene known to affect milk fat metabolism, and *CSN1S1*, which is associated with milk protein content (Table 1)[20].

After genes' functionalization, microfluidic chips are employed in genetic assays, such as PCR and hybridization-based techniques, to detect specific genetic variants in milk goat genomics. These platforms offered advantages, including faster PCR amplification and reduced reagent consumption, which lowers costs and shortens assay times. Moreover, microfluidic chips enable the integration of multiple assays on a single platform, facilitating simultaneous analysis of different genetic markers, such as those associated with milk yield, fat content, and disease resistance. This multi-target capability is particularly valuable for complex breeding programs that rely on multiparent analyses, where genetic contributions from multiple parental lines are evaluated to identify superior traits across diverse goat breeds, as noted in studies on genomic selection[21].

In the manufacture of microfluidic chips for results-based detection of genetic markers, detection systems are critical for accurate and sensitive analysis. Optical detection methods, such as fluorescence or absorbance, are commonly used due to their high sensitivity. For instance, genetic probes labeled with fluorophores (FAM or Cy5) emit fluorescence upon binding to target DNA sequences, which can be visualized using a fluorescence microscope or specialized equipment, including a microarray scanner or real-time PCR thermal cycler. Additionally, electrochemical detection methods, which measure changes in current or potential upon probe-target binding, are emerging as cost-effective and portable alternatives, enhancing the versatility of microfluidic platforms[21,22]. Electrochemical detection techniques are emerging as they are economical, portable, and allow fast and in situ analysis. These techniques detect changes in current or potential as the target molecules interact with the functionalized surface of the chip[22].

Milk goat genomics benefits from the application of microfluidic chips as a robust candidate marker discovery tool. This technology enables the automation of genetic assays, reduces costs, and allows for the simultaneous assessment of multiple traits[23]. In a microfluidic chip, a genetic marker diffuses toward the surface. When a microfluidic chip binds, it results in a measurable alteration in electrochemical characteristics, which is recognized by electrodes integrated into the chip (Figure 1). Electrochemical detection, integrated into the microfluidic chip, enables label-free detection in real-time by measuring changes in electrical signals upon the binding of genetic markers to probes, making it highly appealing for high-throughput genetic analysis due to its simplicity, portability, and reduced need for costly labeling reagents[23].

The final step in creating a microfluidic chip for genetic analysis is to incorporate the chip into a broader platform for data collection and analysis after the detection system is set up. Advances in software and hardware integration have reached a point where these chips can be operated with greater precision. These systems often have simple computer interfaces that enable researchers to monitor the progress of an assay, analyze the results, and create extensive reports. Furthermore, microfluidic chips can be improved by coupling with complementary technologies, such as microarray platforms or next-generation sequencing (NGS), to broaden their potential and applications[23,24].

Accordingly, designing a microfluidic chip for the identification of genetic markers in milk goats or other livestock requires multiple essential steps, including chip design and fabrication, surface functionalization, assay development, detection system integration, and platform integration. Therefore, these platforms have many advantages, especially high sensitivity, cost efficiency, and high-throughput large-scale genetic analyses. Microfluidic chips can help reduce the efficiency of livestock median breeding programs by offering a more accurate way of tracking desirable genes[25].

The development of genomic tools for the genetic enhancement of dairy goats, such as a genomic liquid chip, necessitates the integration of meticulous laboratory techniques, cutting-edge sequencing technologies, and bioinformatics. Through the combination of these methodologies, genetic markers associated with traits including milk yield, fat content, and disease resistance can be identified, thereby facilitating the creation of robust tools for genomic selection and breeding programs[26].

6. DNA extraction for milk goat genomic study

The first step in developing a genomic liquid chip involves collecting high-quality biological samples and extracting DNA. Accurate and pure DNA is essential for downstream applications, such as PCR, NGS, and marker analysis (Table 2)[27].

High-quality DNA is the foundation for accurate genomic analysis, ensuring reliability in genetic marker detection[27].

6.1. Molecular genetic marker analysis

To detect and analyze genetic markers, several methods are integrated into the genomic liquid chip, including PCR-based methods, NGS, and hybridization assays. Each technique serves a specific purpose and provides complementary advantages. When adapted to microfluidic platforms, these methods can be miniaturized, reducing reagent use and costs while enabling high-throughput genetic analysis (Table 3). Multiplex PCR can analyze multiple markers in a single microfluidic channel, increasing efficiency. Similarly, NGS can be integrated into advanced setups, though it demands more computational resources for data storage and analysis[28].

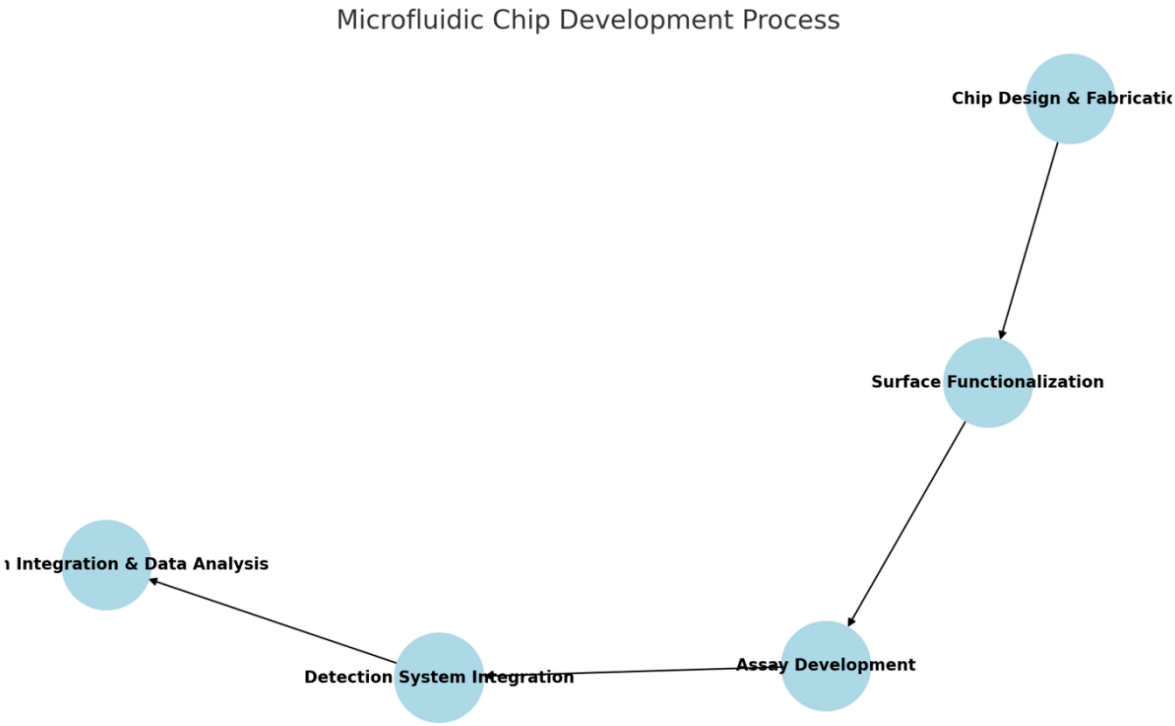

Figure 1. Microfluidic chip development process

Table 2. Steps and descriptions for DNA extraction in goat genomic research

Step	Description
Sample Collection	Common sources include blood, ear tissue, hair follicles, or saliva. Blood is preferred for its high yield and purity. Tissue samples must be stored in RNAlater or frozen immediately to avoid degradation.
Sample preparation	Blood samples are centrifuged at 3000 rpm for 10 minutes to isolate white blood cells. Tissue samples are homogenised, and the resulting material is transferred into a lysis buffer.
Cell lysis	A lysis buffer containing sodium dodecyl sulfate (SDS) is added to break open the cells. Proteinase K is used to degrade proteins, releasing DNA into solution.
DNA precipitation	DNA is precipitated using isopropanol or ethanol, centrifuged at 12,000 rpm, and washed with 70% ethanol to remove contaminants.
DNA resuspension	The DNA pellet is air-dried for 5-10 minutes to remove residual ethanol, and then resuspended in Tris-EDTA (TE) buffer (10 mM Tris-HCl, one mM EDTA, pH 8.0), which stabilizes the DNA by maintaining the pH and preventing nuclease activity. The resuspended DNA is stored at -20°C for short-term use (up to 3 months) or at -80°C for long-term preservation (Over 3 months) to ensure stability for downstream genomic applications such as PCR or sequencing.
Quality assessment	DNA concentration is measured using NanoDrop or Qubit, and quality is confirmed via agarose gel electrophoresis. Pure DNA should show intact bands and a 260/280 absorbance ratio of 1.8-2.0.

Table 3. PCR, next-generation sequencing, and hybridisation in genetic marker analysis

Method	Description	Advantages	Reference
PCR	Amplifies specific regions of DNA to detect genetic markers. Techniques include allele-specific PCR, Quantitative PCR, and multiplex PCR.	High sensitivity, low cost, and rapid detection. Ideal for analysing known markers, including milk fat content (*DGAT1*) or milk protein (*CSN1S1*).	Mandal et al. (2014)
NGS (Next-generation sequencing)	Allows for sequencing of entire genomes or large portions of the genome, providing comprehensive genetic data.	Identifies novel markers and complex genetic variations. Essential for understanding traits such as milk quality or disease resistance.	Mandal et al. (2014)
Hybridization-based assays	Fluorescence in situ hybridization (FISH): A cytogenetic technique that uses fluorescently labeled DNA probes to bind specific genomic regions, enabling visualization of genetic markers under a fluorescence microscope. In the context of milk goat genomics, FISH can detect chromosomal variations associated with traits like milk yield or disease resistance, complementing high-throughput array-based hybridization methods.	Enables simultaneous detection of multiple markers. Offers versatility in detecting Single-nucleotide polymorphism or other variations	Xiao et al. (2024), Mandal et al. (2014)

6.2. Bioinformatics in marker detection and genomic selection

Bioinformatics is essential for processing, analyzing, and validating genetic data in milk goat genomics, enabling the identification of genetic markers that influence traits such as milk yield, fat content, and disease resistance[27,28]. For instance, sequence alignment tools such as BWA align DNA sequences from goat samples to a reference genome, identifying SNPs associated with genes such as *DGAT1*, which regulates milk fat content. Variant calling tools, such as GATK, detect specific SNPs linked to traits, such as those in the *CSN1S1* gene for milk protein content or *TLR4* for mastitis resistance. Genome-wide association studies (GWAS) using PLINK correlate these SNPs with phenotypic data, revealing markers that significantly impact milk yield or disease resistance (Table 4). Functional annotation databases, including Ensembl and KEGG, map these SNPs to biological pathways, such as lipid metabolism for fat content or immune response for disease resistance. Additionally, genomic prediction models such as GBLUP estimate breeding values by integrating SNP data across the genome, guiding the selection of goats with superior traits for milk production and health, thereby enhancing breeding efficiency[29].

The GWAS plays a crucial role in connecting genetic markers to specific traits of interest, such as milk production and resistance to mastitis. Studies identified SNPs linked to these traits and used functional annotation tools such as Ensembl and KEGG to interpret their biological significance. Ensembl maps SNPs to genes, such as *CSN1S1*, while KEGG highlights relevant pathways, including lipid metabolism and immune response. By integrating multi-omics data, encompassing transcriptomics (Gene expression profiles of lactation-related genes), proteomics (Protein levels in milk fat synthesis), and metabolomics (Lipid profiles influencing milk quality), comprehensive insights into the molecular mechanisms that drive milk yield, fat content, and disease resistance can be gained. This comprehensive approach enhances the precision of genomic selection by enabling breeders to select goats with optimal genetic profiles for these traits, as supported by tools such as GBLUP for breeding value estimation[30].

6.3. Integrating methods into microfluidic chips

The genomic liquid chip combines DNA extraction, PCR, NGS, and bioinformatics into a single platform. These chips are designed to analyse multiple genetic markers simultaneously, offering significant advantages in livestock breeding programs (Table 5)[31].

By combining these features, the milk goat genomic liquid chip revolutionises the selection process, enabling breeders to identify desirable traits with greater efficiency, precision, and cost-effectiveness[32].

Table 4. Bioinformatics in marker detection and genomic selection

Bioinformatics tool/method	Purpose	Examples	Reference
Sequence alignment	Aligns DNA sequences to a reference genome to identify polymorphisms such as SNP, insertions, and deletions.	Tools: BWA, Bowtie2, HISAT2	Hajirasouliha et al. (2010)
Variant calling	Identifies specific genetic variations associated with desirable traits.	Tools: GATK, SAMtools, FreeBayes	Elsik et al. (2016)
Genome-wide association studies (GWAS)	Links genetic variations to phenotypic traits, such as milk yield or disease resistance.	Tools: PLINK, TASSEL, GEMMA	Hajirasouliha et al. (2010), Elsik et al. (2016)
Functional annotation	Predicts the biological impact of genetic variants by linking them to genes and pathways.	Databases: Ensembl, NCBI Gene, KEGG, GeneCards	Clark and Elgar (2000)
Genomic prediction models	Predicts the genetic potential of animals for breeding purposes.	Tools: GBLUP (Genomic Best Linear Unbiased Prediction), BayesC	Clark and Elgar (2000), Hajirasouliha et al. (2010)

Table 5. Integrating methods into microfluidic chips

Feature	Benefit
Miniaturization	Reduces reagent consumption, cost, and assay time.
High-throughput capacity	Simultaneously analyses multiple markers, ideal for complex breeding programs.
Integration of multiple methods	Combines PCR, hybridisation, and NGS into a single chip for comprehensive genetic analysis.
Real-time detection	Incorporates optical techniques such as fluorescence or electrochemical systems for rapid, real-time marker detection.
Portable systems	Can be integrated into portable platforms for on-site genomic analysis and data collection.

NGS: Next-generation sequencing.

7. Verification of genotyping by target sequencing liquid chip for goat milk

Scientists currently verify genotyping by target sequencing (GBTS) liquid chip technique because it detects SNP loci that characterise goat milk genetics[32]. The chip emerges as an effective coordination instrument for genomic selection programs, which combine DNA collection with validated SNP assays and repeatability testing alongside relevant bioinformatics treatments. The process of collecting DNA samples from goats' milk drawn from different breeds, namely Alpine, Saanen, and Boer breeds[33]. A range of genetic backgrounds is crucial for understanding milk trait phenotypes. To achieve reliable outcomes from tests, a minimum of 50 samples from dairy goats is required. When the DNA interacts with liquid chip probes, hybridization enables the measurement of the detection rate, and SNP coverage measurement calculates the number of SNP loci from the potential typing set that the chip accurately detects[31-33]. The effectiveness of genomic sampling with the GBTS liquid chip was determined by its high detection rate of targeted genetic sequences, such as SNPs linked to milk yield and disease resistance. Whole-genome resequencing (WGR) of 14 DNA samples from diverse goat breeds confirmed the accuracy of the SNPs identified by the GBTS chip. Following identification, researchers utilize either BLAST or BWA software programs to align SNPs to the goat reference genome for accuracy validation. In the verification study using 14 DNA samples from diverse goat breeds, SNP evaluations from the GBTS liquid chip's microarray aligned closely with outcomes from comprehensive whole-genome resequencing. This confirmed the chip's accuracy in detecting SNPs for traits such as milk yield and disease resistance[33]. The scientific community uses repeatability assessments to determine the reliability of GBTS liquid chips in delivering consistent performance over time, as demonstrated by Tsai et al.[22]. Intragroup replication verifies result consistency by repeatedly testing individual DNA samples within a single chip batch, while intergroup replication assesses cross-run consistency by analyzing identical samples across different chip batches. These assessments, encompassing both intragroup and intergroup replication, confirmed the chip's ability to reproducibly detect genetic markers, such as SNP linked to milk yield and disease resistance, with high accuracy and minimal variation in detection rates across runs. Consistent SNP detection rates above 95% and low genotyping error rates (<1%) across batches demonstrate robust chip performance, ensuring reliability for genomic selection in milk goat breeding programs[33,34].

Pooled SNP data, which comes from liquid chip systems or WGR technology analysis, is used to check chip performance by identifying breeds among non-dairy goats[32-34]. The SNP loci associated with *CSN1S1* and *TLR4* were processed through PLINK to validate genotype data and assess missingness. This ensured accurate identification of genetic markers across diverse goat breeds for the GBTS liquid chip. Data visualization forms the central component throughout this scientific process. The scientific study of gene relationship structures depends heavily on methods to visualize species classification processes[34] . Three R packages, named ggtree, ggplot2, and scatterplot3d, help visualize phylogenetic trees together with PCA plots. Through PCA visualization, examine clustering and divergence patterns between goat breeds based on SNP information, revealing insights into genetic structure. ggplot2 and scatterplot3d in the R packages are used for visualizing PCA and phylogenetic trees. PCA plots can illustrate how well individual goat breeds cluster together or diverge from each other in terms of their SNP profiles, facilitating insights into the population's genetic structure[35].

7.1. Bioinformatics tools

The process of marker refinement relies on bioinformatics tools after chip identification, along with consistency measurements and statistical analysis[34,35]. The BWA and Bowtie2 align reads against a reference genome to ensure precise analysis of genetic variants. The tools GATK, along with SAMtools and FreeBayes, enable the detection of SNP, as well as additional variants[36]. The GWAS research investigated the relationship between SNPs and phenotypic traits, such as milk production or fat composition. Ensembl, NCBI gene, and KEGG functional annotation tools help to understand the biological significance of identified markers. The present study identified SNPs in genes such as *DGAT1* and *CSN1S1*, which regulate milk fat metabolism and protein synthesis, as well as *TLR4*, which enhances mastitis resistance, using tools like Ensembl and KEGG. These findings elucidated functional attributes for milk production and disease protection in goat breeding[36,37]

Scientists have linked multi-omics data from transcriptomics combined with proteomics and metabolomics datasets to gain a deeper understanding of the molecular elements responsible for milk production qualities, according to Zhou et al.[37]. Breeders estimated the genetic potential of animals by analyzing entire genomes to improve their selection decisions. The GBTS liquid chip optimizes goat breeding results by combining genomic information with bioinformatics techniques to achieve

better milk production outcomes and disease resistance, while also improving the herd's overall traits[38]. The GBTS liquid chip verification serves as the benchmark for subsequent GBTS liquid chip operations, ensuring the accurate detection of genetic markers in goat milk with a high standard of efficiency[38,39]. The scientists at Vellore Institute of Technology (VIT), India, have established the groundwork for genomic selection through rigorous quality DNA procedures, alongside validated SNP detection, optimal repeat trials, and advanced bioinformatics methods[39]. Recent advances at the intersection of genetics and technology have the potential to transform goat breeding practices by enhancing productivity and promoting sustainable herd management, including the alignment of reads to a reference genome to accurately map genetic variants. Innovative approaches combine data from transcriptomics, proteomics, and metabolomics, which offer a more comprehensive insight into the molecular features that drive milk-related traits[38].

Genomic prediction models, including GBLUP and BayesC, are used to estimate the breeding values of individual goats. These models utilize whole-genome data to predict the genetic potential of animals, enabling breeders to make more informed selection decisions[39]. By incorporating bioinformatics with genomic data, the GBTS liquid chip enabled precision breeding that increases milk production, enhances disease resistance, and refines traits in goat herds[38,39].

The GBTS liquid chip verification provided a reference basis for GBTS liquid chips, making this chip a reliable, high-efficiency, and accurate tool for detecting genetic markers in goat milk. With proper DNA collection, SNP detection validation, repeatability testing, and bioinformatics processing, the researchers at Vellore may have a solid platform for genomic selection. The intersection of genetics and technology has the potential to revolutionize goat breeding programs, impacting productivity and sustainability in herds[40].

8. Challenges of genotyping by target sequencing

The development and application of the GBTS liquid chip for milk goat genomics face several challenges, particularly in achieving reliable genetic marker detection for breeding programs. Key challenges include obtaining sufficient DNA yield and quality from non-invasive samples, such as saliva or hair follicles, which often produce lower-quality DNA compared to blood samples, as noted in prior studies[7-9]. Variability in DNA extraction methods can further introduce inconsistencies, leading to degraded or contaminated samples that compromise SNP detection accuracy and result in biased phenotypic and genetic analyses. Additionally, the high costs associated with NGS technologies and the need for specialized bioinformatics expertise to analyze SNP data pose significant hurdles, particularly for small-scale breeding operations in resource-limited settings[40]. Additionally, observed in the processes of sample collection, DNA extraction, and data analysis, highlight the need for established protocols and accessible tools to enhance the GBTS chip's utility in genomic selection for different traits in goats[41].

Significant challenges arise when attempting to acquire DNA samples that accurately represent all genetic variations within a population, particularly when studying small or geographically isolated groups. Analysis becomes more difficult and less conclusive because different DNA extraction methods, along with other sampling preservation techniques, introduce additional data variability[40,41]. The combination of advanced genomic technologies with economical and large-scale production platforms presented a unique challenge to address[41]. The NGS and multiplex PCR require not only specialized infrastructure facilities but also high-performance computing hardware[42], along with a dedicated team to handle and effectively evaluate large datasets. In developing regions, small farms and breeding stations often face requirements that exceed financial feasibility[42]. The crossing of different heterologous lines remains a primary concern because small result variations can greatly influence breeding choices, including consistency tests across multiple chip batches and experiments. Genomic discoveries should be integrated into breeding approaches to enable farmers to utilize tools such as the GBTS liquid chip alongside traditional practices, despite challenges related to cost and expertise. Suggestions include developing user-friendly mobile apps for SNP data interpretation, providing farmer training programs, and subsidizing chip costs to enhance adoption. A full benefit from genomic technologies to boost goat milk production and disease resistance will only become possible by solving current limitations[42-44].

9. Conclusion

The GBTS liquid chip represented a transformative advancement in milk goat genomics, offering a high-throughput, cost-effective platform for detecting SNP associated with key traits such as milk yield, fat content, and disease resistance. By integrating advanced microfluidic technology, precise DNA extraction, and robust bioinformatics tools such as BWA, GATK, and PLINK, the chip enables accurate SNP identification and validation, as demonstrated through whole-genome resequencing and repeatability testing across diverse goat breeds. Functional annotation using Ensembl and KEGG elucidated the biological roles of markers, while genomic prediction models such as GBLUP enhance breeding precision. Despite challenges in DNA quality, cost, and bioinformatics expertise, solutions including user-friendly apps, farmer training, and cost subsidies can facilitate adoption. This technology promises to revolutionize dairy goat breeding by enhancing productivity, preserving genetic diversity, and promoting sustainable farming practices.

Declarations

Funding

No specific funding was received for the preparation of the present review article. The authors' contributions were supported by their respective institutions.

Ethical considerations

All cited studies were assumed to comply with ethical standards as per their original publications. The author has reviewed all ethical problems, including plagiarism, consent to publish, data fabrication, and falsification.

Authors' contributions

Umar Aziz, Abdul Rehman, Xiaolong Xu, Junru Zhu, Yonglong He, Zhanhang Wang, Li Fu, Jiayuan Li, Xugan Wang, and Hanbing Yan contributed to the literature review, data synthesis, and manuscript drafting. Xiaopeng An conceptualized the review, supervised the writing process, and finalized the manuscript as the corresponding author. All authors have read and approved the final edition of the manuscript for publication.

Acknowledgments

The authors would like to express their gratitude to the research teams and institutions that studied goat genomics and microfluidic technologies, which contributed to the knowledge synthesized in this review article. Additionally, acknowledgment is given to bioinformatics experts for providing insights into the tools.

Availability of data and materials

As a review article, the present study synthesized data from published literature cited in the references. No new primary data were generated, and all referenced materials are publicly available through their respective sources.

Conflict of interests

The authors declare no competing interests related to the content of this review article. No financial or personal relationships influenced the interpretation of the literature discussed.

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