Research Square

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Development and Application Validation of a 20K-Liquid Chip for the Qinchuan Cattle

Kaiyan Zhang
Northwest A&F University
Jun Ma
Northwest A&F University
Luyang Sun
Northwest A&F University
Dawei Wei
Ningxia University
Xue Gao
Chinese Academy of Agricultural Sciences
Weidong Ma
Shaanxi Agricultural and Animal Husbandry Seed Farm, Shaanxi Fufeng
Ningbo Chen
Northwest A&F University
Xiaoting Xia
Northwest A&F University
Yun Ma
Ningxia University
Chuzhao Lei
Northwest A&F University
Yu Jiang
Northwest A&F University
Yongzhen Huang
hyzsci@nwafu.edu.cn

Northwest A&F University

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Abstract

As a core indigenous beef cattle breed in China, Qinchuan cattle face bottlenecks in genetic improvement due to inadequate adaptability of generic SNP chips and the absence of dedicated molecular detection tools. This study developed and validated a Qinchuan-specific 20K low-density liquid-phase SNP chip based on targeted sequencing genotyping technology. Whole-genome resequencing data from 193 Qinchuan cattle were integrated with data from 274 external reference populations sourced from the European Gene Database. Core loci were selected through genome-wide homozygous fragment analysis and selection signal detection. Following quality control and association studies, 20k core target loci were finalized for chip synthesis. Chip validation demonstrated an average detection rate of 98.48% for core loci, with 99.01% genotypic consistency across technical replicates. Principal component analysis and phylogenetic tree analysis confirmed the chip's ability to accurately distinguish Qinchuan cattle from other breeds. This chip balances low cost and high specificity, providing a dedicated tool for genetic diversity assessment, breed identification, and genomic selection in Qinchuan cattle. It also establishes a technical foundation for innovative utilization of local beef cattle germplasm resources.

1. Introduction

Domesticated animals serve as excellent biological models for studying phenotypic development and evolution. Through prolonged human selection and domestication, distinct breeds have developed significant variations in morphological traits, physiological functions, and environmental adaptability [1–3]. Cattle (Bos taurus) represent a vital livestock resource for humanity, exhibiting rich phenotypic diversity. Their complex population evolutionary history and trait inheritance mechanisms have attracted considerable scholarly attention[4]. Qinchuan cattle, a highly representative local beef breed in China, are renowned for their imposing stature, docile temperament, rapid growth rate, tender meat quality, and strong stress resistance. Their body conformation traits are not only directly linked to economic characteristics such as dressing percentage and meat yield but also embody unique genetic codes shaped by long-term artificial selection and environmental adaptation. Decoding the genetic mechanisms underlying its body conformation holds significant practical value for targeted breeding and germplasm resource innovation. Current research on genetic mechanisms underlying animal body conformation has yielded substantial progress: studies in cattle have identified genes such as IGF1 and GHR as closely linked to body development[5]; comparative genomics has elucidated key genes regulating equine body conformation[6]; and population genomics analysis has uncovered novel loci potentially influencing poultry body variation[7]. The marked differentiation in body size between domesticated animals and their wild ancestors renders them ideal subjects for studying genetic variation in traits. As a core trait in both breeding practice and fundamental research, the genetic mechanisms underlying body size have been extensively explored and characterised[8]. With the rapid advancement of genomic sequencing technologies, it has become feasible to decipher the gene regulatory networks underlying complex traits such as body size in domesticated animals. For instance current research has successfully elucidated key genetic mechanisms, including cold tolerance

adaptationin high-latitude Chinese pigs[9]and canine adaptation to high-starch diets[10]. However, compared to these species, genetic research on Qinchuan cattle body conformation remains underdeveloped. Existing universal SNP chips lack coverage of its breed-specific loci, hindering accurate capture of genetic signals for body conformation traits—this limits identification of key functional genes and genomic selection efficiency, constraining breed improvement precision.

Single nucleotide polymorphisms (SNPs), as the most prevalent and stable form of genetic variation in genomes, constitute indispensable core molecular markers in genetic research. They exert profound influences on crucial biological processes including organismal growth and development, metabolic regulation, and environmental adaptability[11]. SNP chips, as the core technology enabling efficient SNP detection, have become indispensable due to their unique advantages of high throughput, high accuracy, and standardisation. They are employed for analysing population structure and admixture proportions, estimating effective population size[12–13], localising quantitative trait loci (QTL)[14–15], conducting genome-wide association studies (GWAS)[16–17], and implementing genomic selection (GS)[18–20]. The advent of targeted sequencing genotyping (GBTS) liquid chips has effectively addressed the limitations of traditional techniques, emerging as a more cost-effective alternative to whole-genome sequencing. Dueto its unique technical design, this approach has now been extensively applied in genetic breeding and germplasm resource innovation studies across various livestock and poultry species, including pigs, cattle, sheep, and chickens[21–22]. The GBTS liquid chip substantially lowers the technical barriers and economic costs of large-scale population genotyping, providinggrassroots breeding units and research institutions with a more accessible technological option[23]. GenoBaits technology employs liquid-phase probe hybridisation for selective DNA capture, making it more suitable for precise detection at medium-to-high numbers of loci[24–25]. These chip advantages provide efficient and reliable technical tools for genetic breeding in relevant livestock breeds, while also injecting new impetus into the conservation and innovative utilization of germplasm resources from local superior breeds[26–27].

Within Qinchuan cattle research, limited studies have employed generic chips such as the Illumina BovineSNP50 for genetic diversity analysis, however, existing tools exhibit significant limitations.The design for generic chips is based on imported breed genomes, resulting in inadequate coverage of Qinchuan cattle-specific loci and consequently low detection efficiency for loci associated with core economic traits[28]. Moreover, existing research has not sufficiently elucidated the genetic mechanisms underlying Qinchuan cattle's superior traits, and there is a lack of dedicated molecular detection tools tailored to their unique genetic background. This constrains the precision of genetic diversity assessment and molecular breeding for Qinchuan cattle [29]. Therefore, this study employed methods including homozygous fragment analysis, selection signal detection, and genome-wide association studies to identify candidate loci.

We developed a custom 10K functional locus gene chip. Utilising this chip technology, we conducted efficient detection and analysis of genetic variation in Qinchuan cattle and selected breeding lines. This aims to precisely identify key genes and molecular markers associated with Qinchuan cattle growth,

providing scientific tools and valuable data support for elucidating the genetic basis of their superior traits and optimising breeding strategies.

2. Materials and Methods

2.1 Sample Collection and Data Processing

In this study, jugular vein blood samples from 95 Qinchuan cattle (Bos taurus) collected by the laboratory in advance at the Shaanxi Qinchuan Cattle Breeding Station were used. Blood DNA was extracted using the standard phenol-chloroform method. Sequencing was performed by BGI using the DNBSEQ-T7 sequencer. Following quality control, gene libraries were constructed with an average fragment size of 300 bp per sample[30]. Descriptive statistics for nine trait data sets were performed using SPSS. For each trait, sample size, mean, standard deviation, minimum value, maximum value, and coefficient of variation (Coefficient of Variation = Standard Deviation / Mean × 100%). Data normality was verified via Shapiro-Wilk tests ($P > 0.05$ indicating normal distribution). Outliers (exceeding mean ± 3 standard deviations) were excluded, ultimately retaining complete phenotypic data from 189 Qinchuan cattle for subsequent analysis.

To conduct an in-depth analysis of Qinchuan cattle genetic information, we expanded our sample collection scope. We obtained 374 samples from the European genetic database for further analysis, which included Qinchuan cattle that exhibited larger body size and superior traits following selective breeding[31].Detailed data and grouping are presented in the supplementary table1. The raw sequencing data (FASTQ format) underwent quality control using Trimmomatic (version 0.38) with parameters set as LEADING:20, TRAILING:20, SLIDINGWINDOW:3:15, AVGQUAL:20, MINLEN:35 to filter low-quality bases ($Q < 20$) and adapter contamination sequences [32].

First, raw sequencing data underwent quality control using Trimmomatic (version 0.38). Parameters LEADING:20, TRAILING:20, SLIDINGWINDOW:3:15, AVGQUAL:20, and MINLEN:35 were used to filter out low-quality sequences and adapter contamination[33]. Subsequently, the processed sequences were aligned to the reference genome using BWA (Burrows-Wheeler Aligner). The aligned files were converted to BAM format via Samtools and sorted according to chromosomal coordinates. Picard Tools were then employed for deduplication, with parameters REMOVE_DUPLICATES=true, CREATE_INDEX=true, and VALIDATION_STRINGENCY=LENIENT set to remove PCR duplicates and generate an index file, preparing for subsequent variant detection analysis. During the variant detection phase, GATK (version 3.8) modules—Haplotype Caller, Genotype GVCFs, and Select Variants—were employed to detect SNPs. Subsequently, the Variant Filtration module was used to filter SNPs based on the following criteria: DP < 585 || DP > 5272 || QD < 2.0 || FS > 60.0 || MQ < 40.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0 || SOR > 3.0 [34]. Finally, additional quality control of SNPs was performed using BCFtool software[35], with quality control criteria including genotyping dropout rate < 0.01 and minor allele frequency > 0.05, ultimately retaining 21,183,737 SNPs.

2.2 Core Region Screening

PLINK 1.9 software was employed to conduct ROH analysis on sequenced populations of Qinchuan cattle and other subpopulations. Quality control was first performed, excluding SNPs with detection rates < 95%, samples with individual detection rates < 95%, and SNPs with minor allele frequencies < 0.05 or Hardy - Weinberg equilibrium test P-value < 10^{-6}, and excluded non-chromosomal and duplicate SNPs. ROH detection followed the methodology described in previous studies[36], employing a sliding window threshold of 0.05, window size of 50 SNPs, permitting 2 missing SNPs and 1 heterozygous SNP per window, with a maximum SNP spacing of 100 kb and minimum density of 1 SNP per 50 kb. The genomic inbreeding coefficient (FROH) was calculated as FROH = ∑LROH / Lgenome, where ∑LROH denotes the sum of lengths of all ROH segments on autosomes, and Lgenome represents the total physical length of autosomes[37]. Finally, the --homozyg group parameter was employed to merge adjacent ROH segments into larger ROH islands based on predefined ROH parameters.

The study employed VCFtools to calculate genetic differentiation indices (Fst) and nucleotide diversity (π) values between Qinchuan cattle populations and other sequenced breeds, retaining only loci unique to Qinchuan cattle. Fst analysis was conducted by calculating weighted FST values across all SNPs within the step size, followed by arithmetic averaging to obtain the mean FST value for all SNPs within the window. π analysis calculated nucleotide diversity values within the window [38]. Combining Fst and π analysis provides a more comprehensive reflection of population genetic structure and the influence of natural selection. In this study, the top 5% of values were used as a threshold to identify regions under selection, which were subsequently annotated and visualised. To systematically analyse the biological functions of the identified candidate genes, gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed using the online platform DAVID 6.8, with a threshold of $p < 0.05$.

Based on a genome-wide SNP dataset from 193 Qinchuan cattle individuals with somatic trait data, GWAS analyses was conducted for nine quantitative traits. The primary software employed for GWAS was GEMMA [39], utilising a mixed linear model for analysis. The first principal component and sex matrix were employed as covariates, with significance and suggestive site thresholds set at 5.5 $\times 10^{-8}$(1/17,937,451) respectively. Manhattan and QQ plots were generated using the CMplot package within the R software[40]. To further analyse SNP functionality, we employed a Perl script to identify significant SNPs within 100 kb upstream and downstream regions as candidate gene-targeting areas based on Ensembl database screening.

Deduplication of previously screened sites was performed, excluding duplicate sites, low-polymorphism sites (MAF < 0.05), and sites with high dropout rates (dropout rate > 0.01); sites were integrated according to "functional priority" and "genomic uniformity" principles: prioritise retaining SNPs within exonic regions and known functional genes, while ensuring uniform SNP distribution across the genome. This process ultimately identifies core SNPs for chip construction.

2.3 Microarray Synthesis

Based on the reference genome Bos taurus (ARS-UCD1.2_Btau5.0.1Y), the genome was partitioned into 26,204 sites using 95,000 bp windows. A sliding window scoring strategy was employed to select key candidate target sites, with specific DNA probes designed for each qualified site. Prioritising core functional sites identified through multidimensional screening, probes were designed with dual-probe coverage for critical sites to enhance capture stability. Probes measured 100–120 bp with 35–65% GC content, optimised via kmer alignment and deep learning models to ensure fewer than five homologous regions (≥ 95% sequence similarity). Optimised probes underwent in vitro transcription with biotin labelling. Labelled probes are mixed at equimolar concentrations to form a homogenised probe pool, supplemented with three exogenous quality control reference probes. The pool concentration is adjusted to 100 ng/μL and stored sealed at -20°C.

The chip assembly employed a liquid-phase capture scheme utilising targeted sequencing genotyping (GBTS) technology. The core components comprised: biotin-labelled probe pool, PCR amplification primers, hybridisation buffer, and magnetic bead enrichment reagent. All components were encapsulated according to standardised procedures. Each batch of chips included one positive control (Qinchuan cattle DNA sample with known genotype) and one negative control (no template DNA) for experimental quality control.

2.4 Chip Validation

To comprehensively evaluate chip performance, genotyping was performed on 135 samples (see supplementary table for details): Genomic DNA libraries were constructed using the GenoBaits DNA-seq library preparation kit. The library was hybridised with chip probes at 65°C for 16 hours; Dynabeads MyOne Streptavidin C1 were added to capture hybridised fragments. Following PCR amplification and magnetic bead purification, PE150 sequencing was performed on the MGISEQ-2000 platform. Sequencing data quality control was conducted using FastQC, with reference genome alignment via BWA and SNP genotyping performed using GATK. The SNP detection rate for each sample was calculated for each sample (number of detected SNPs / total core SNPs × 100%). For Qinchuan cattle samples undergoing whole-genome resequencing, compare chip-detected SNP genotypes with resequencing data genotypes to calculate concordance rate (number of identical genotype sites / total detected sites × 100%). Statistically analyse the MAF distribution and proportion of polymorphic sites among core SNPs in the sample (number of SNPs with MAF > 0.01 / total core SNPs × 100%), investigating the proportion of target polymorphic sites.

Principal component analysis was performed using PLINK software with the --pca 10 parameter to extract the top 10 principal components. The calculation was based on allele frequencies at core target loci, generating principal component score files and variance explained by principal components files. The ggplot2 package in R software was employed to read the principal component score files and plot scatter plots for the top principal components[41]. Additionally, VCF2Dis was employed to compute genetic differences between samples, generating a distance matrix for constructing a neighbour-neighbour tree. SNP data were processed to account for minor missing sites, preventing sample

exclusion due to localised data gaps. The ITOLsoftware package was used to read the phylogenetic tree file and refine the tree[42].

3. Results

3.1 Core Site Screening Results

In the statistics of ROH segment across populations(Fig. 1A and 1B), distinct characteristics emerged in the proportion and quantity of short The proportion of short ROH segments (0.5–1 Mb) was 76.61% (44,475 segments) in the sampled Qinchuan cattle, 79.40% (18,116 segments), and 14.97% (6,832 segments) in the medium-length ROH segments (1–2 Mb) in the selected Qinchuan cattle, significantly the proportion of 1-2Mb medium-length ROH segments was 14.97% (6,832), significantly higher than the 12.02% (2,178) observed in the selected Qinchuan cattle population. Figure 1C indicates more recent inbreeding events in the sampled Qinchuan cattle population, medium, and long segments among the Qinchuan cattle, the Qinchuan cattle population, and the selected Qinchuan cattle population. higher than the 1202% (18,116 segments) observed in the selected Qinchuan cattle (Fig. 1D). In terms of proportion, both populations were primarily composed of short ROHs of 0.5–1 Mb. The sampled Qinchuan cattle exhibited an average inbreeding coefficient of 0.055957, slightly higher than that of the selected Qinchuan cattle. This indicates that the overall inbreeding level of the sampled Qinchuan cattlemarginally higher than that of the selected Qinchuan cattle. Subsequently, the study analysed the distribution of regions of shared haplotypes (ROH) within the population and visualised them based on SNP positions on chromosomes (Fig. 1E). The majority of the 95 loci annotated within ROH islands were located within growth-related genes, providing a prioritisation basis for functional locus screening on the chip.

To identify candidate genomic regions under selection during growth and development in Qinchuan cattle compared to other populations, this study employed the Fst-π ratio method to detect selection signals, using the top 5% threshold as the screening criterion. Regions significantly detected by both methods were deemed genuinely selected. Figure 2 displays Manhattan plots of different Qinchuan cattle populations generated using the Fst-π ratio method with a 5% threshold. The results reveal distinct selection regions across populations identified by the Fst-π ratio approach,with a total of 291 overlapping selected sites detected. Functional enrichment analysis of these overlapping genes was conducted using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO). The most significantly enriched KEGG pathway was Metabolic pathways. GO terms with significant association (P-value < 0.05) among these overlapping genes included: nucleoplasm, protein binding, and cytoplasm.

Genome-wide association studies were conducted on eight body-size traits inQinchuan cattle(Fig. 3). The QQ plot indicated satisfactory model fit. A total of 137 significant SNPs (P < 5×10 − 8 were identified in 193 individuals) identified, with 14 genes located within exonic regions across all loci. PubMed literature review revealed these genes are associated with signal transduction, key pathways, and cell

proliferation, and are closely linked to growth and development in Qinchuan cattle. DHRS3, as a member of the short-chain dehydrogenase/reductase family, catalyses the reduction of all-trans retinaldehyde to retinol, maintaining its dynamic equilibrium and promoting skeletal muscle regeneration during myoblast differentiation[43]. CENPS functions primarily in chromosome segregation and the maintenance of genomic stability. CENPS maintains centromere integrity during the G1 phase via homologous recombination repair mechanisms, preventing chromosomal translocations[44]. SIK2 AMP-activated protein, a member of thekinase family, functions as a serine/threonine kinase. It primarily participates in multiple biological processes—including energy metabolism regulation and cell cycle progression—by phosphorylating downstream target proteins [45]. ANKRD11 is a multifunctional protein containing ankyrin repeat domains, playing a pivotal role in development and disease through involvement in transcriptional regulation, chromatin remodelling, and cellular signalling pathways[46]. UNC5B, a member of the UNC5 family, initiates pro-apoptotic signals in the absence of key factors. By activating downstream pathways, it induces apoptosis, participating in the clearance of surplus cells during tissue development to maintain cellular homeostasis. UNC5B regulates endothelial cell migration, proliferation, and vascular branching, thereby sustaining the normal structure of vascular networks[47].

The reference genome (ARS-UCD1.2_Btau5.0.1Y) was divided into 26,204 sites using 95,000-base window lengths across autosomes, yielding 23,183 successfully designed probes. Combining with the previously mentioned loci and excluding loci with a missing rate exceeding 50% in the test population, 20,566 loci were ultimately retained.

3.2 Microarray Synthesis Results

The study retained 20,566 valid probes, corresponding to 20,566 core target sites. The core target sites were evenly distributed across 29 autosomes, with no obvious clusters or gaps. Sites on chromosomes 1–6 exhibited higher density (8–10 sites per Mb on average), while chromosomes 25–29 showed relatively lower density (3–5 sites per Mb on average). The overall distribution met the uniformity requirements for chip design (Fig. 4A).

Functional sites (trait-associated + selected genes + immune-related) accounted for 62.3%, while Qinchuan cattle-specific sites constituted 28.7%, ensuring sites balanced functional targeting with genomic coverage. Exon regions contained 1,913 sites (9.3%), of which 832 (43.5%) were missense mutation sites; 11,973 sites (58.7%) were located in intron regions, including 621 sites associated with selected genes; 1,234 functional sites (6.0%) were in non-coding regions, comprising 412 sites in UTR regions and 387 sites associated with lncRNAs, ensuring coverage of key gene expression regulatory regions (Fig. 4B).

3.3 Chip Result Detection

The core SNP detection rate for test samples demonstrated excellent performance, with a population-average detection rate exceeding 98.48%. This indicates the chip's high efficacy in SNP genotyping for Qinchuan cattle samples, enabling stable capture of genotype information for the vast majority of core target loci. This meets the data integrity requirements for subsequent genomic analysis. The genotypic

concordance rate for duplicate sample pairs reached 99.01%, demonstrating the chip's excellent repeatability and reliability in Qinchuan cattle SNP genotyping. Genotyping results exhibit minimal susceptibility to experimental variation, with data credibility meeting genomic research standards. A total of 14,320 polymorphic sites (69.66%) exhibited MAF > 0.1, with 7,842 (54.7%) located within functional genes. This indicates that the chip sites balance genetic polymorphism with functional relevance (Fig. 4C). The proportion of polymorphic sites remained consistently high, indicating that the core SNPs selected for the chip effectively cover the genetic polymorphism of the Qinchuan cattle population and meet the requirements for polymorphism sites in genetic diversity assessment studies.

The NJ tree construction results in Fig. 4D show that all Qinchuan cattle samples clustered into a single branch with bootstrap values ≥ 92 (high node reliability), clearly distinguished from branches of reference breeds such as Angus and Jinnan cattle, with no cross-breed clustering observed. Consistent results from both analyses indicate that the core target sites selected via chip screening effectively capture breed-specific genetic characteristics of Qinchuan cattle, enabling precise analysis of their population genetic structure and breed identification ; Whether based on the 20K chip loci (Fig. 5A)or the whole-genome loci (Fig. 5B), Qinchuan cattle and non-Qinchuan control samples formed distinct population boundaries in the PC1-PC2 scatter plot. Qinchuan cattle samples clustered into an independent group, significantly separated from other breeds. PC1 explained 35.17% of genetic variation and PC2 explained 12.36%. This indicates that the 20K chip loci and genome-wide loci exhibithigh consistency in reflecting population genetic structure, demonstrating that these chip loci can effectively capture genetic differentiation information between populations and serve as a reliable alternative for genome-wide analysis.

4. Discussion

The size of domesticated animals is not only a commercial standard in livestock production but also a central focus in advancing research on poultry evolution and development [48–49]. As a prime example of a trait influenced by numerous complex factors, body size is affected by a large number of genes, which are often distributed across biologically similar loci[50]. The Research findings indicate genetic divergence between wild-type Qinchuan cattle and selectively bred Qinchuan cattle. The selectively bred population exhibits lower inbreeding levels due to more extensive hybrid gene exchange, consistent with their distinct breeding histories. This insight aids understanding of their genetic differentiation and evolutionary adaptation. This study, targeting the Qinchuan cattle's breed characteristics of rapid growth, tender meat quality, and strong adaptability successfully customised a 20K functional locus gene chip targeting. This addresses the current lack of dedicated tools in Qinchuan cattle genetic research and the low efficiency of screening markers for core economic traits.

Selection signal analysis (Fst versus π ratio method) identified 291 overlapping regions of recent common ancestry (ROH), which encompass multiple genes associated with bovine growth. Notably, a ROH island overlapping with selection signals on chromosome 4 contained 24 genes involved in metabolic regulation,which collectively support cellular proliferation and growth development. GWAS

analysis screened 137 significant SNPs, with genes in three overlapping regions exhibiting critical functions. MAN1A2, as a member of the mannosidase family, regulates glycoprotein modification by catalysing N-glycan trimming in the Golgi apparatus. This process directly influencing protein folding, transport, and stability while extensively participating in organ development. Duringoocyte development, its loss-of-function leads to ovarian dysfunction in cattle, disrupting oocyte maturation and subsequent embryonic development capacity. Additionally, animal models reveal its regulation of ciliary function impacts the transport of oocytes by fallopian tube cilia[51]. FAM83F promotes cell progression from G1 to S phase by interacting with cyclin or CDK4/6, accelerating the cell cycle and activating proliferation-related signalling pathways, thereby enhancing the expression of bovine proliferation-associated genes[52]. KIFC2 participates in intracellular material transport and division-related processes, and stabilizes regulatory factors governing the G1-to-S phase transition. The stability accelerates cell cycle progression and promotes cell proliferation. Concurrently, KIFC2 amplification correlates with pyrimidine metabolism activation. Pyrimidine metabolism supplies nucleotides as raw materials for DNA synthesis, providing the material foundation for cellular growth and division, thereby further driving bovine cell proliferation[53].

The core value of gene chips lies in their high compatibility with the genetic background of target breeds, with detection rates and genotypic concordance serving as key indicators of suitability. In this study, the average detection rate for core SNPs in samples exceeded 98.48%, while genotypic concordance in technical replicates reached 99.01%. Both metrics significantly surpassed conventional standards for genomic chip applications and outperformed most universal beef cattle chips when applied to local breeds[54]. By employing methods such as selection signals and genome-wide association studies to identify Qinchuan cattle-specific loci, thisstudy effectively circumvented the inadequate genomic matching issues inherent in generic chips when applied to local breeds. The high detection rate indicates the chip's stable capture of core genetic information in Qinchuan cattle, minimising interference from data loss in subsequent analyses. The high concordance demonstrates reliable genotyping results, providing high-quality data support for genetic diversity assessment and molecular marker screening, which fully reflects the chip's excellent compatibility with the Qinchuan cattle genetic background[55].

As a locally adapted breed with a long history of selective breeding, the genetic diversity and distribution of polymorphic sites in Qinchuan cattle directly constitute the genetic basis of breed characteristics. In the samples of this study polymorphic sites with MAF > 0.1 accounted for 69.66% of the total, which was significantly higher thanthe conventional level of low-polymorphism chips. This indicates that the core SNPs selected for the chip effectively cover key genetic variations within the Qinchuan cattle population. Regarding breed characteristics, Qinchuan cattle exhibit polygenic regulation across economic traits including growth development, meat quality, and stress resistance. The functional loci integrated into the chip precisely encompass regions associated with these traits[56]. This research provides a basis for constructing germplasm resource fingerprinting and breed identification, while also laying the groundwork for subsequent genome-wide association studies. The functional loci on this chip facilitate the localisation of key genes associated with target traits, accelerating the elucidation of the genetic mechanisms underlying Qinchuan cattle's economic traits.

Compared to traditional methods, the chip exhibitsthree core advantages: strong breed specificity, precise functional orientation, and cost-effectiveness compatible withindustrial applications. The customised Qinchuan cattle 10K functional locus gene chip developed in this study, with its high detection rate, high consistency, and strong specificity, provides an efficient tool for genetic analysis and molecular breeding of local cattle breeds. This chip not only supports fundamental research such as genetic diversity assessment and germplasm resource conservation in Qinchuan cattle but also directly serves industrial demands for breed improvement, facilitating the transition of Qinchuan cattle breeding from "traditional phenotypic selection" to "molecular design breeding".

5. Conclusions

To address the conservation and molecular breeding requirements of Qinchuan cattle, this study employed targeted sequencing-based genotyping technology. Through a multidimensional screening strategy incorporating GWAS, ROH analysis, selection signal analysis, and supplementary literature databases, a synthetic chip comprising 20,566 core target loci was finalised. Validation results demonstrated the chip's outstanding core performance. Functional validation further confirmed the chip's capability for Qinchuan cattle breed identification, providing a reliable tool for genetic analysis of key traits such as body measurements and body weight. Compared to generic commercial chips, the Qinchuan cattle chip offers significant advantages including high specificity and low cost. The successful development of this chip not only fills the gap in dedicated Qinchuan cattle chips but also provides efficient technical support for the precise conservation of Qinchuan cattle genetic resources, the identification of core breeding value genes, and the establishment of a genomic selection breeding system.

Abbreviations

SNP
Single Nucleotide Polymorphisms
GBTS
Genotyping by Targeted Sequencing
QTL
Quantitative Trait Loci
GWAS
Genome-Wide Association Studies
GS
Genomic Selection
ROH
Runs of Homozygosity
Fst
Fixation Index
π

Nucleotide Diversity
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
MAF
Minor Allele Frequency
PCA
Principal Component Analysis
NJ Tree
Neighbor-Joining Tree
HPC
High-Performance Computing
BWA
Burrows-Wheeler Aligner
GATK
Genome Analysis Toolkit
VCF
Variant Call Format
BCFtools
Binary Call Format tools
PLINK
Whole-Genome Association Analysis Toolset
Picard Tools
Picard Sequence Processing Tools
Trimmomatic
Illumina Sequence Data Trimmer
FASTQ
Fast Quality Format
BAM
Binary Alignment Map
DP
Depth of Coverage
QD
Quality by Depth
FS
Fisher Strand
MQ
Mapping Quality
MQRankSum

Mapping Quality Rank Sum Test
ReadPosRankSum
Read Position Rank Sum Test
SOR
Strand Odds Ratio
FROH
Genomic Inbreeding Coefficient Based on ROH
UTR
Untranslated Region
lncRNA
Long Non-Coding RNA
DAVID
Database for Annotation, Visualization and Integrated Discovery
GEMMA
Genome-wide Efficient Mixed-Model Association
CMplot
Circular Manhattan Plot
ggplot2
Grammar of Graphics Plot 2
VCF2Dis
VCF to Genetic Distance
ITOL
Interactive Tree Of Life
MGISEQ-2000
MGI Sequencing Platform 2000
DNBSEQ-T7
DNA Nanoball Sequencing Platform T7
Dynabeads MyOne Streptavidin C1
Dynabeads MyOne Streptavidin C1 Magnetic Beads
PCR
Polymerase Chain Reaction
PE150
Paired-End 150bp
ARS-UCD1.2_Btau5.0.1Y
Bos taurus Reference Genome Version ARS-UCD1.2_Btau5.0.1Y

Declarations

Ethics declarations

All procedures were conducted in accordance with the Chinese laws on animal experimentation and were approved by the Northwest A&F University's Experimental Animal Management Committee (Approval No. DK2022065), and conducted under the authority of the Project License. Animal use and care were in accordance with the ARRIVE guidelines (https://arriveguidelines.org/).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Author Contribution

K.Z., J.M., and L.S.were responsible for participating in sample collection, data collation, and experimental operations related to chip validation.D.W.² provided relevant research data support.X.G., W.M., N.C., X.X., Y.M., C.L., and Y.J.guided the experimental design and data analysis ideas, offered key technical support, and participated in the formulation of research schemes and discussion of results.Y.H.* (corresponding author) oversaw the entire research project, being responsible for experimental design, result interpretation, and manuscript writing and revision.All authors have read and approved the final manuscript.

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Not applicable.

Data Availability

The datasets generated and/or analyzed during the current study are available in the China National Center for Bioinformation (CNCB) repository, with the accession number PRJCA054984.

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Figures

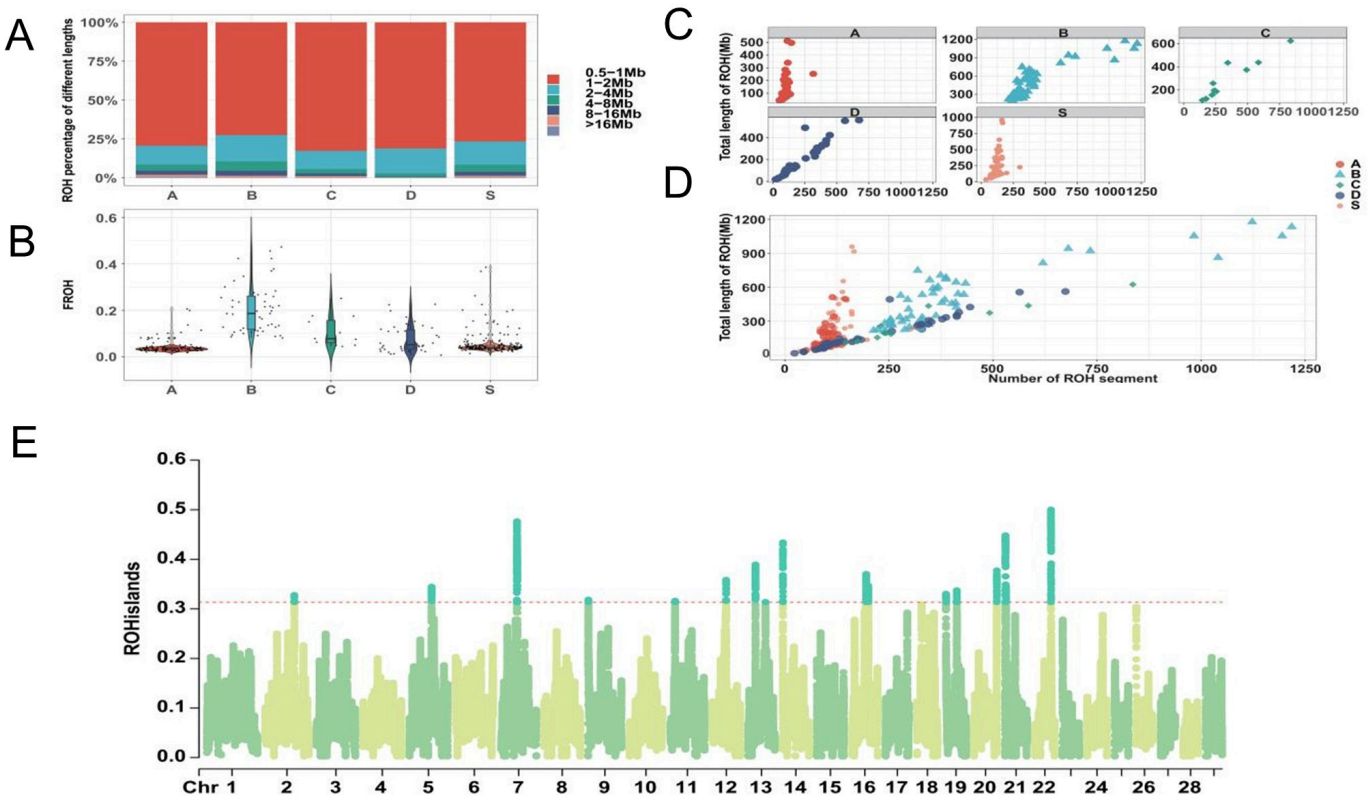


Figure 1

Characterisation and distribution of runs of homozygosity (ROH). (A) Genome coverage proportion by ROH length categories per group. Different colors represent distinct ROH length ranges: 0.5–1 Mb, 1–2 Mb, 2–4 Mb, 4–8 Mb, 8–16 Mb, and >16 Mb. The y-axis indicates the proportion of genome covered by ROHs of each length category. (B) ROH frequency versus length distribution. The x-axis represents ROH length, and the y-axis represents the number of ROH segments (frequency) corresponding to each length. (C) Scatter plots of ROH segment count versus largest segment size. Each point denotes an individual, with the x-axis showing the total number of ROH segments per individual and the y-axis showing the length of the largest ROH segment in that individual. (D) Correlation between total ROH length and segment count. The x-axis represents the total number of ROH segments, and the y-axis represents the total length of all ROH segments per individual. (E) Distribution of the linkage disequilibrium coefficient of ROH across the whole genome. The x-axis lists autosomes (Chr1–Chr29), and the y-axis represents the linkage disequilibrium coefficient of ROH regions on each chromosome.

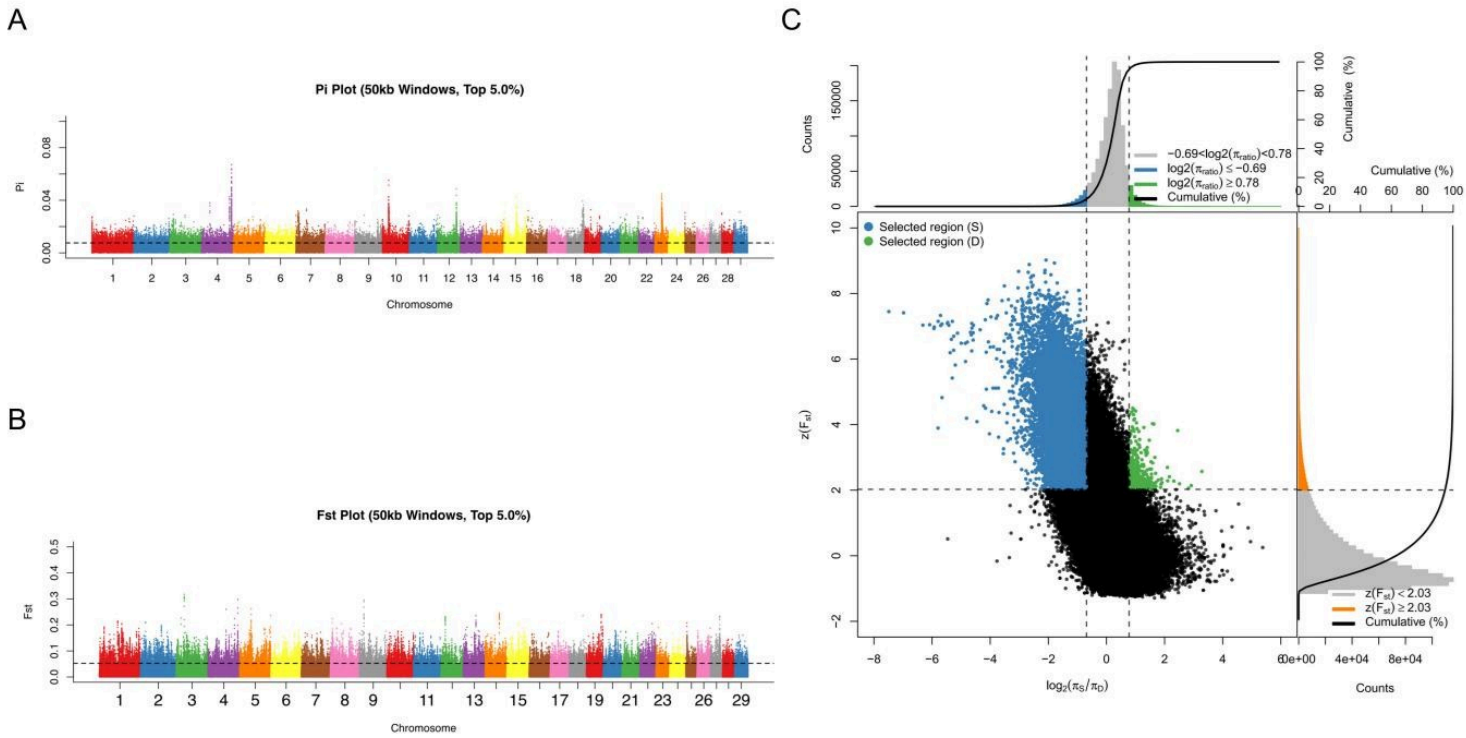


Figure 2

Selection signature results of Qinchuan cattle. (A) SvsA Fst Plot (50 kb windows, top 5% Fst). The x-axis shows chromosomes (Chr1–Chr29), and colored bars represent Fst values (genetic differentiation coefficient) of each 50 kb window. Bars exceeding the top 5% threshold indicate genomic regions with high genetic differentiation between populations. (B) SvsA Pi Plot (50 kb windows, top 5% Pi). The x-axis lists chromosomes, and colored bars represent Pi values (nucleotide diversity) of each 50 kb window. Bars exceeding the top 5% threshold highlight regions with high nucleotide diversity. (C) Relationships between genetic statistics (Fst and Pi) and their frequency distributions. The x-axis represents the value range of genetic statistics, and the y-axis represents the frequency of each value.

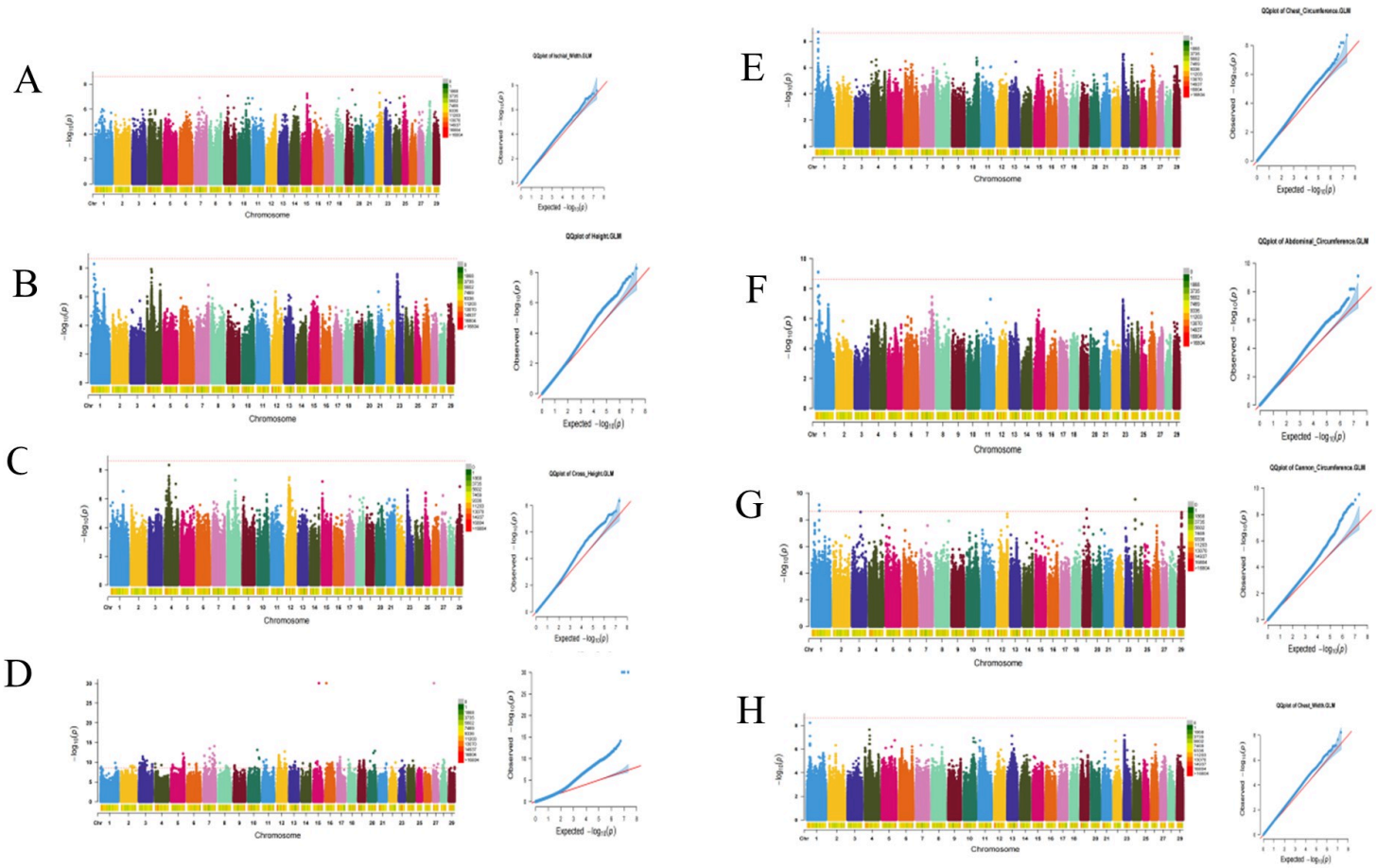


Figure 3

Genome-wide association study (GWAS) results for Qinchuan cattle. Manhattan plots (left) and QQ plots (right) for eight body-size traits: (A) ischial end width, (B) body height, (C) cross-shaped part height, (D) body length, (E) chest girth, (F) abdominal girth, (G) cannon circumference, and (H) chest width. For Manhattan plots: The x-axis shows chromosomes (Chr1–Chr29), and the y-axis represents $-\log_{10}$(P-values) of Single Nucleotide Polymorphisms (SNPs). Higher bars indicate stronger associations between the corresponding SNP and the trait. The horizontal dashed line denotes the significance threshold ($P < 5\times10^{-8}$). For QQ plots: The x-axis shows expected $-\log_{10}$(P-values) under the null hypothesis, and the y-axis shows observed $-\log_{10}$(P-values). Points close to the diagonal indicate a good fit of the GWAS model.

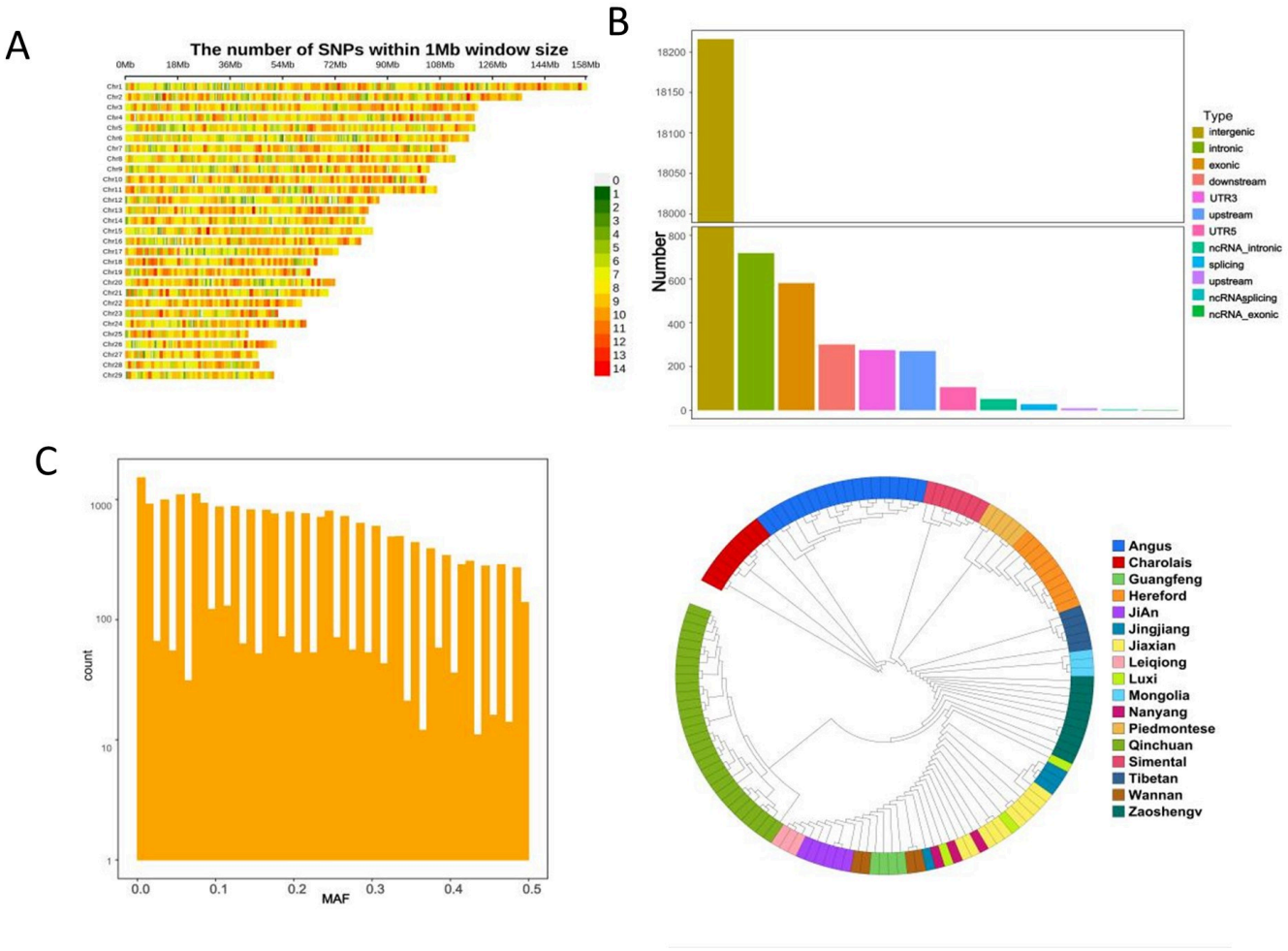


Figure 4

Chip SNP Locus Distribution and Characteristics Analysis Chart.(A) Heatmap of SNP distribution across chromosomes in 1 Mb windows. The x-axis lists autosomes (Chr1–Chr29) and their physical positions (in Mb), and the y-axis represents the number of SNPs per 1 Mb window (range: 0–14). Color intensity corresponds to SNP density, with darker colors indicating higher density. (B) Bar chart of SNP quantity statistics by functional type. The x-axis lists SNP functional categories: exon regions (including missense mutation sites), intron regions (including selected gene-associated sites), non-coding regions (including UTR regions and lncRNA-associated sites), and other regions. The y-axis represents the number of SNPs in each category. Functional sites (trait-associated + selected genes + immune-related) and Qinchuan cattle-specific sites are indicated in the chart annotation.(C) Minor Allele Frequency (MAF) distribution map of core loci in samples. The x-axis shows MAF ranges (0–0.5), and the y-axis represents the number of core SNPs corresponding to each MAF range. A total of 14,320 polymorphic sites (69.66%) had MAF > 0.1, with 7,842 (54.7%) located within functional genes. (D) Circular phylogenetic tree of Qinchuan cattle and reference breeds. Branches are labeled with breed names (e.g., Qinchuan, Angus, Jinnan, Hereford), and bootstrap values (≥92) are marked at key nodes, indicating high reliability of the clustering result. All Qinchuan cattle samples cluster into a single independent branch, clearly distinguished from other breeds.

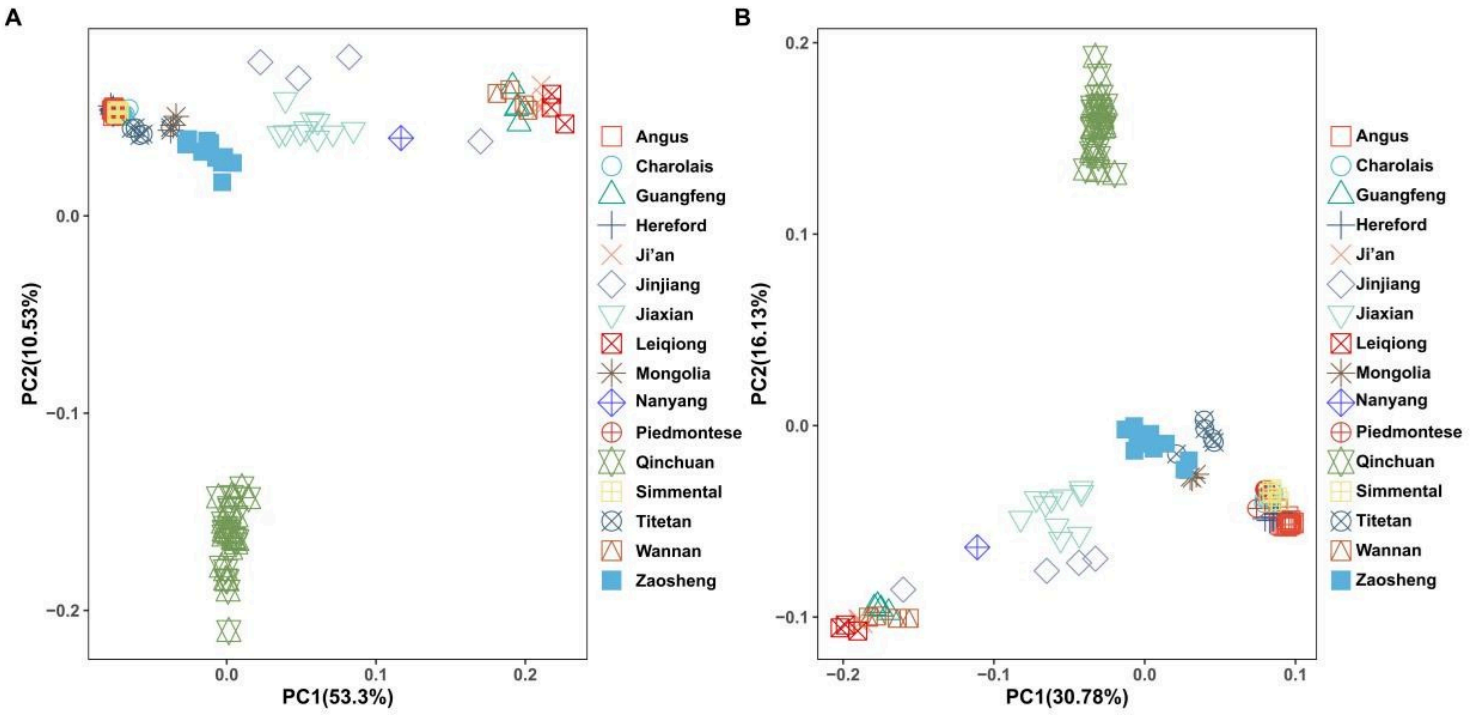


Figure 5

Principal Component Analysis (PCA) results. (A) PCA plot of cattle population genetic structure based on 20K chip-derived SNPs. The x-axis (PC1) explains 30.78% of genetic variation, and the y-axis (PC2) explains 12.36%. Different symbols and colors represent different breeds (e.g., Qinchuan [QIC], Angus [Aan], Charolais [Chals], Jinnan [Jinj]). Qinchuan cattle samples form an independent cluster, significantly separated from other breeds. (B) PCA plot of cattle population genetic structure based on whole-genome SNPs. The x-axis (PC1) explains 53.3% of genetic variation, and the y-axis (PC2) explains the remaining major variation. Symbols and colors correspond to those in (A). Qinchuan cattle and non-Qinchuan control samples form distinct population boundaries, consistent with the clustering result in (A), indicating high consistency between 20K chip loci and whole-genome loci in reflecting population genetic structure.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- file.xlsx