Systematic Profiling of Short Tandem Repeats in the Cattle Genome

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Abstract

Short tandem repeats (STRs), or microsatellites, are genetic variants with repetitive 2–6 base pair motifs in many mammalian genomes. Using high-throughput sequencing and experimental validations, we systematically profiled STRs in five Holsteins. We identified a total of 60,106 microsatellites and generated the first high-resolution STR map, representing a substantial pool of polymorphism in dairy cattle. We observed significant STRs overlap with functional genes and quantitative trait loci (QTL). We performed evolutionary and population genetic analyses using over 20,000 common dinucleotide STRs. Besides corroborating the well-established positive correlation between allele size and variance in allele size, these analyses also identified dozens of outlier STRs based on two anomalous relationships that counter expected characteristics of neutral evolution. And one STR locus overlaps with a significant region of a summary statistic designed to detect STR-related selection. Additionally, our results showed that only 57.1% of STRs located within SNP-based linkage disequilibrium (LD) blocks whereas the other 42.9% were out of blocks. Therefore, a substantial number of STRs are not tagged by SNPs in the cattle genome, likely due to STR’s distinct mutation mechanism and elevated polymorphism. This study provides the foundation for future STR-based studies of cattle genome evolution and selection.

Key words: cattle genome, short tandem repeat, whole genome sequencing.

Introduction

Short tandem repeats (STR) are highly variable genetic elements widely dispersed in mammalian genomes. Here, we focus on STRs with repetitive motifs of 2–6 base pairs, which are commonly referred to as microsatellites. The elevated level of polymorphism and mutability of STRs, due to the high incidence of replication slippage, has resulted in their application to the analysis of population differentiation, genetic diversity, and forensic identification (MacHugh et al. 1997; Chikhi et al. 2004; Li et al. 2007; Chambers et al. 2014).

An appreciable but unknown fraction of STRs contribute to gene regulation and have functional effects (Gemayel et al. 2010). Changes to the repeat length of STRs have been associated with gene function (Borel et al. 2012), transcriptional plasticity (Vinces et al. 2009), complex traits (Hammock and Young 2005; Queitsch et al. 2012; Gymrek et al. 2016), and morphological evolution (Wren et al. 2000; Fondon and Garner 2004). The potential for adaptive and deleterious STR mutation is considerable. For example, in human ~17% of genes contain STRs in their open reading frames. Several
Detecting Bovine STRs Using Sequencing Data

A comprehensive inventory of STRs in cattle genomes is significant for livestock breed management, livestock-based genome deep sequencing and genotyping, and provides a novel resource for the current research community. Furthermore, we perform an initial investigation of the population genetics and functional impacts of bovine STRs, and provide some new insights for further exploration of cattle genome.

Materials and Methods

Samples and Sequencing

The whole genome sequencing data was generated as previously described (Larkin et al. 2012). Five Holstein bulls (Bos taurus) were sequenced using the Illumina V3 PE 100 chemistry on a HiSeq 2000 platform to 30–50× coverage. The raw reads were first filtered using NGS QC toolkit to remove low-quality bases with quality score of 20 (–s 20) and the percentage of read length less than 75% of given quality (–I 75) (Patel and Jain, 2012). A summary of short read statistics was presented in supplementary table S1, Supplementary Material online.

Identification of STR in Cattle

We conducted a comprehensive survey of STR variants using high coverage NGS data in dairy cattle. The coverage of sequence data for each animal was around 30–50× (supplementary table S1, Supplementary Material online), allowing sufficient power to detect STRs using lobSTR (version 2.0) (Gymrek et al. 2012). lobSTR was applied with default parameters for alignment and STR discovery as previously described (Gymrek et al. 2012). Briefly, we first created a lobSTR reference index based on Bovine UMD 3.1’s STR data (downloaded from the UCSC genome browser) using lobstr_index.py script. Then we carried out lobSTR alignment to create the STR alignment bam files, and the final STR variants allelotypes were identified throughout all samples based on the merged alignment file. lobSTR employs an explicit model to enhance accuracy by avoiding stutter noise caused by PCR amplification of a STR locus. After filtering by quality of STR calling (QUAL > 30), we finally obtained 60,106 unique STRs across all analyzed animals.

STR-Overlapping Genes Annotation Using PANTHER and DAVID

The 60,106 STRs loci were intersected with RefSeq genes downloaded from the UCSC Genome Browser to obtain a total of 11,676 overlapped STRs. Based on 4,213 unique STR-containing genes, we tested the hypothesis that the PANTHER molecular function, biological process and pathway terms were under- or over-represented in genes regions after Bonferroni corrections (Mi et al. 2013). We also performed...
Validating lobSTR Accuracy Using Sanger Sequencing
To confirm lobSTR prediction, we randomly selected 18 STRs of different motif sizes (including di-, tri-, and tetranucleotides) and used Sanger sequencing to confirm whether the correct genotypes were derived after PCR and/or TA cloning. Primer information can be found in Table S9. DNA fragments obtained from five animals (Elevation, Blackstar, Starbuck, Chairman, and Ivanhoe) were sequenced by Sanger chemistry at Genewiz, Inc. according to standard procedures.

STRs Overlap with QTLs Associated with Important Traits
We downloaded QTL information from cattle QTLdb from http://www.animalgenome.org/cgi-bin/QTLdb/BT/index (last access November 2, 2016). Because previous QTL mapping studies have utilized both STR and SNP markers, and employed different design populations and mapping methods, we merged all QTLs into a set of unique non-redundant regions.

SNP-Based LD Block Estimation around STR
LD (R² proxy for LD) was calculated and LD blocks were detected using Bovine HD SNPs datasets in Holstein population using PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/; last access November 2, 2016) (Purcell et al. 2007). The SNPs within each block were overlapped with STRs. The STR overlapping with block region was considered as the potential candidate STRs which could be further tagged by proximate SNPs.

Selection and Population Genetic Analysis of STRs
A total of genotypes of 22,067 STRs were recovered in all five cattle, among which 20,059 (90.9%) were dinucleotides. Given their abundance, we restricted our analysis to this set of 20,059 dinucleotide STRs. We identified outlier loci based on two anomalous relationships that counter expected trends in microsatellite variation: (1) 100 invariant microsatellites with allele size > 20 (supplementary table S10, Supplementary Material online) and (2) 44 dinucleotide loci that are diallelic with a maximum allele size at least two time greater than the size of the alternate allele (supplementary table S11, Supplementary Material online).

Principal Component Analysis of Genetic Relatedness
We used microsatellite calls for all dinucleotide loci where data were available for all five cattle and maximum allele size was ≤ 30 (n = 19,338). We carried out principal components analysis (PCA) using EIGENSOFT (Patterson et al. 2006) and each microsatellite allele was coded as a binary string of all zeroes except for the position corresponding to the size of allele, which was set to one.

STRs Overlap with Regions under Selection Predicted Based on SNPs
To identify STRs linked to potential regions under positive selection, we overlapped STRs with the 49 positive selection regions identified by the same Holstein sequencing samples as previously reported (Larkin et al. 2012). In addition, to further study the potential genome signals involved in selection for STRs in Holstein population, we utilized the integrated haplotype score (iHS) using Bovine HD SNP array in 44 unrelated Holstein, iHS was estimated using selscan program with default settings (Szpiech and Hernandez 2014). In this study, we considered 100-kb nonoverlapping windows; the density of signal in each region was measured by the proportion of SNPs with |iHS| > 2. Then the empirical cutoffs for the top 1% of signals were considered as candidate selection regions (Regions with SNP number <10 were dropped here) (Voight et al. 2006). To assess significance for the analyses of regions, we performed 10,000 times permutation tests to get empirical P-values for these overlaps using an R/Bioconductor package regionR (Gel et al. 2016). To further explore the selection characteristics of STRs, we employed the recently developed ksk method; ksk values were estimated with window size 100,000 and step size 5000 as described previously (Haasl et al. 2014).

Results and Discussion
Identification of STR Using Whole Genomics Sequencing
We used five sequenced Holstein bulls with coverage depth between 30 and 50× for STR identification (supplementary table S1, Supplementary Material online). Using lobSTR, we mapped 218,078 aligned reads and identified 37,997 STRs covered with low number of reads (Blackstar), whereas we mapped over 1 million aligned reads and identified 62,378 STRs with high number of reads (Starbuck with an average of 10.4× coverage). In total, we detected 72,615 STRs based on NGS data derived from the five genomes. On average, we obtained 52,186 STRs for a 7.99× genome coverage (supplementary table S2, Supplementary Material online). After filtering with lobSTR calling quality (QUAL = 30), we obtained a
STRs, with counts of 35,402 and 14,697, respectively (supplementary fig. S1, Supplementary Material online). STRs with unique annotated cattle RefSeq genes (fig. 1). Among these STRs, 5624, 7517, 10,039, 14,859, and 22,067 STRs were identified in one, two, three, four, and five individual(s), respectively.

We observed a relatively uneven distribution of STRs with a maximum interval of 1,087,826 bp. The distribution of STRs shows large differences across chromosomes. For instance, we found 3683, 2926, and 2757 STRs on chromosome 1, 2, and 6, whereas only 931, 1071, and 1079 STRs on chromosome 25, 28, and 23. After normalizing by chromosome length, we observed that STR densities per Mb ranged from 20.54 to 23.26. Among 22,067 STRs found in all five sampled individuals, 20,059 (90.9%) were dinucleotide microsatellites, whereas 931, 1071, and 1079 STRs on chromosomes 1, 2, and 6, respectively. Among 20,059 STRs, 90.1% were dinucleotide microsatellites, which were by far the most common, confirming the earlier result based on a small dataset (Stone et al. 1995). Two motifs, (A)n and (AT)n, occupy a large proportion of our identified STRs, with counts of 35,402 and 14,697, respectively (supplementary fig. S1, Supplementary Material online). STRs with motifs longer than two nucleotides were rare in the cattle genome.

Because sequence and annotation of chrX and chrUn within the cattle genome are less satisfactory, we mainly focused on the high-confidence STRs on autosomes. Because lobSTR only has sufficient power to detect STRs with a motif size from 2 to 6 base pairs, we limited our analyses to these types of STRs on bovine autosomes. STRs with a motif size more than 6 bp were not considered in this study, which may require other detection programs such as VNTRseek (Gelfand et al. 2014).

STRs Overlap with Genes

In this study, we constructed the first STR map for five Holstein genomes. We observed 11,676 STRs overlapped with 4,213 unique annotated cattle RefSeq genes (fig. 1). Among these genes, we observed 188, 84, 45, 12 genes containing at least 10, 15, 20, 30 STRs, corresponding to total STR lengths of 11,7549, 72,981, 49,499, and 19,581 bp, respectively. Genes with over 30 STRs included RBFOX1, MACROD2, GALNT6, CTNNA2, CA10, NRXN1, PRKG1, DPYD, NEGR1, CDH18, CTNNA3, and NRG3 (supplementary fig. S2, Supplementary Material online).

Using the PANTHER classification system (Mi et al. 2010), STR-containing genes were enriched for the GO terms of synaptic vesicle exocytosis, heart development, visual perception, cellular amino acid metabolic process, and cell–cell adhesion (supplementary table S3, Supplementary Material online). We further divided the total 4,213 genes into four groups: group 1 with more than 10 STRs, group 2 with 5–10 STRs, group 3 with 2–4 STRs, and group 4 with one STR.

For group 1 genes with higher STR count, we observed that most of genes were enriched for heart development, nervous system development, ion transport and cell communication (with Enrichment Score > 1.3, i.e., P-values < 0.05 after the Benjamini and Hochberg correction for the multiple testing in supplementary table S4, Supplementary Material online). Similarly, group 2 genes were involved in phosphate-containing compound metabolic process, regulation of catalytic activity, and voltage-gated calcium channel activity (supplementary table S5, Supplementary Material online). Group 3 genes were enriched for visual perception, blood coagulation, catabolic process and cell communication (supplementary table S6, Supplementary Material online), whereas group 4 genes were enriched for vesicle-mediated transport, protein transport, and primary metabolic process (supplementary table S7, Supplementary Material online). To evaluate STR’s functional impacts, we further pinpointed 204 STRs located within the exons of 194 genes. DAVID results indicated that these genes are most enriched for neuron differentiation, lipid binding, and membrane-bounded vesicle (supplementary table S8, Supplementary Material online).

As an example, SLC11A1 overlaps with two STRs. This is a highly conserved gene across mammals and is associated with resistance and susceptibility to various intracellular pathogens in humans as well as in livestock species (Blackwell et al. 2001; Thomas and Joseph 2012). Previous studies have shown that microsatellite alleles localized in the 3’ UTR of the SLC11A1 are involved in macrophage function and resistance to Brucella abortus infections in both cattle and buffalo (Kumar et al. 2005; Borriello et al. 2006; Capparelli et al. 2007; Ganguly et al. 2008; Martinez et al. 2008; Kumar et al. 2011). Another gene ATP8B2 contains STR in its exon. This gene is involved in magnesium ion binding and cation-transporting ATPase activity. Notably, ATP8B2 has been specifically identified as a target of positive selection for the aquatic adaptation of dolphins by constructing whole-genome ortholog gene sets among five mammalian species, including dolphin, cow, dog, panda, and human (Sun et al. 2015).

Validating STR Predictions with Sanger Sequencing

To verify the STR detected using lobSTR in our cattle data, we performed PCR and Sanger sequencing. After sequencing these regions, we observed good concordance between lobSTR and the capillary sequencing results (table 1). We found that 16 (45.71%) regions were correctly genotyped by lobSTR and 14 (40%) regions were partly correctly genotyped (table 1). In only five instances, lobSTR called incorrect STR genotypes. We further performed two filters based on coverage and Q score: (1) After applying “DP ≥ 5” and “–LOG(1 – Q) ≥ 0.8” for each locus, 21 left validations showed similar results (42.86% completely correction rate); (2) After applying “DP ≥ 5” for each allele and “–LOG(1 – Q) ≥ 0.8” for each locus, 12 left validations showed a higher (58.33%) completely correction rate. It is noted that our lower validation rates might be related to the draft status of the
cattle genome assembly, which is 95% complete and contains many gaps and unplaced contigs.

We observed lobbSTR correctly called the homozygous STRs that were covered by low read coverage of \( \leq 2 \times \). For heterozygous STRs, lobbSTR may correctly call one allele and miss the other allele due to insufficient sequencing coverage. We observed for homozygous loci 9/10 (90.00%) were correctly called whereas other heterozygous showed lower correct rates (7/25, 28.00%). Our results also revealed the allelotyping algorithm in lobbSTR was not able to identify noisy reads and incorrectly assigned heterozygous genotypes to these loci. We also observed lobbSTR made more correct callings for STRs of

![Genomic landscape of STRs on autosomes in five Holsteins.](image)

**Fig. 1.**—Genomic landscape of STRs on autosomes in five Holsteins. Tracks from outside to inside are: STR frequencies across five Holsteins; chromosomes in different colors; frequencies of 11,676 STRs overlapped with genes; selected polymorphic genes; STR counts in each of 4,213 genes; Allele size plot for 100 invariant microsatellites with allele size \( \geq 20 \); Variance plot in allele size for 44 dinucleotide loci that are diallelic with a maximum allele size at least two time greater than the size of the alternate allele.
motif sizes of three and four (tri- and tetra-nucleotides) than dinucleotide STRs, although the dinucleotide STR is the most abundant STR type in cattle genome.

STRs Overlap with QTLs Associated with Important Traits

We found that 47.76% (28,705) of the STRs overlapped with the merged QTL regions (empirical $P$-value = 0.224). Most of overlapped QTLs were related to important milk and production traits. On the other hand, we also observed 52.24% of STRs did not overlap with any existing QTLs. These may represent some novel STRs, which may be used as new candidate markers to refine cattle QTLs after validation.

Selection and Population Genetic Analyses of Microsatellites

Of the 56,559 autosomal microsatellites, the genotypes of 22,067 microsatellites were recovered in all five cattle. Dinucleotide microsatellites were by far the most common (20,059 of 22,067 or 90.9%). Therefore, we restricted our analyses to diallelic STR loci with maximum allele size/C20, to avoid spurious allele calls due to short read lengths. This results in a set of 19,338 dinucleotide STRs. We observed the plot of mean variance in allele size versus maximum allele size corroborates the well-established positive correlation between allele size and variance in allele size (fig. 2). Because sample size was limited ($n = 5$ diploid individuals, 10 chromosomes),

| Table 1 |
|-----------------|-----------------|-----------------|
| No. | STR | Chr | Begin | End | Motif (bp) | Animal | A1 | A2 | Call | Coverage | Q score |
| 1 | BM1818 | 23 | 39,294,224 | 39,294,249 | GT 37 | Blackstar | 33 | 37 | 33 | 33 | P | 4 | 0.69 |
| 2 | BM1824 | 1 | 132,498,006 | 132,498,034 | CA 29 | Blackstar | 29 | 35 | 33 | 35 | P | 4 | 2.35 |
| 3 | BM2113 | 2 | 127,591,877 | 127,591,917 | AC 42 | Elevation | 26 | 48 | 28 | 38 | N | 14 | 5.52 |
| 4 | ETH10 | 5 | 56,657,954 | 56,657,996 | CA 41 | Elevation | 39 | 43 | 37 | 43 | P | 6 | 4.09 |
| 5 | ETH152 | 5 | 114,885,382 | 114,885,416 | TG 37 | Blackstar | 33 | 35 | 35 | 35 | P | 14 | 4.17 |
| 6 | ETH225 | 9 | 10,858,165 | 10,858,199 | CA 34 | Elevation | 32 | 34 | 30 | 32 | N | 8 | 0.69 |
| 7 | ETH3 | 19 | 56648417 | 56648461 | AC 46 | Blackstar | 42 | 52 | 44 | 52 | P | 6 | 6.00 |
| 8 | ILST5006 | 7 | 96,709,240 | 96,709,279 | TG 43 | Elevation | 39 | 43 | 35 | 41 | N | 6 | 4.12 |
| 9 | INRA023 | 3 | 33,011,005 | 33,011,044 | CA 43 | Blackstar | 31 | 37 | 31 | 31 | P | 28 | 3.62 |
| 10 | INRA037 | 10 | 76,365,534 | 76,365,555 | TG 32 | Blackstar | 31 | 38 | 31 | 31 | P | 8 | 1.94 |
| 11 | INRA063 | 18 | 40,699,867 | 40,699,892 | GT 35 | Blackstar | 27 | 27 | 27 | 27 | Y | 4 | 0.80 |
| 12 | STR_chr8 | 8 | 38,693,251 | 38,693,297 | ACT 46 | Blackstar | 39 | 39 | 39 | 39 | Y | 4 | 3.11 |
| 13 | STR_chr7 | 7 | 2,965,462 | 2,965,506 | ATCC 44 | Blackstar | 44 | 44 | 44 | 44 | Y | 4 | 1.23 |
| 14 | STR_chr10 | 10 | 37,414,396 | 37,414,434 | ATCC 38 | Blackstar | 38 | 38 | 38 | 38 | Y | 10 | 5.70 |
| 15 | STR_chr16 | 16 | 81,503,345 | 81,503,411 | ATCC 66 | Blackstar | 30 | 30 | 30 | 30 | Y | 4 | 3.01 |
| 16 | STR_chr19 | 19 | 46,403,668 | 46,403,701 | ATCC 33 | Blackstar | 33 | 37 | 33 | 37 | Y | 12 | 4.21 |
| 17 | STR_chr24 | 34 | 34,465,896 | 34,465,926 | AGAT 30 | Blackstar | 30 | 30 | 30 | 30 | Y | 2 | 0.70 |
| 18 | STR_chr25 | 24 | 34,465,896 | 34,465,926 | AGAT 30 | Blackstar | 26 | 26 | 30 | 30 | N | 8 | 2.41 |

Note.—Y: Both platforms agree. P: lobSTR reported only one allele out of two. N: lobSTR reported an allele that does not exist.
we were unable to use the approximate Bayesian computation method for inferring natural selection on microsatellites (Haasl and Payseur 2013). However, we did identify outlier loci based on two anomalous relationships that countered expected trends in microsatellite variation. First, we identified 100 invariant microsatellites with allele size \( \geq 20 \) (fig. 3A and B; supplementary table S10, Supplementary Material online; empirical \( P \)-value = 0.017). These outlier loci are unusual because they demonstrate no variance despite allele size \( \geq 20 \). Mutational studies (Marriage et al. 2009; Sun et al. 2012) as well as analyses of polymorphism data (Legendre et al. 2007; Brandstrom and Ellegren 2008; Kelkar et al. 2008; Payseur et al. 2011) have demonstrated that mutation rate of STRs increases with size. Thus, long STRs should be highly variable due to frequent mutation. The lack of variance at these loci therefore suggests that artificial selection (and perhaps genetic drift due to breed formation bottlenecks) has eliminated individuals that possessed mutated alleles at these loci. Second, we identified 44 diallelic microsatellites where the large allele was at least two times greater in size than the small allele (fig. 3C; supplementary table S11, Supplementary Material online; empirical \( P \)-value = 0.008). The second set of outlier loci is unusual for similar reasons. In each case, the longer allele is invariant, which defies the expectation that long alleles should be highly mutable. Furthermore, these loci are bimodal. Maintenance of two distinct allele sizes over time suggests elimination of mutated alleles by artificial and/or natural selection. A bimodal fitness surface, leading to the selection of two distinct alleles, might be especially common in regulatory regions, where specific STR sizes lead to the critical spacing patterns of promoter or enhancer sequence elements. Indeed, Elmore et al. (2012) identified promoter microsatellites in* Aspergillus flavus* where gene expression peaked at two distinct allele sizes, whereas intermediate allele sizes were associated with decreased expression (Elmore et al. 2012). An alternative possibility for the origin of both sets of outlier STRs is that the identified outlier loci are positioned in areas of reduced mutation. However, in the case of the bimodal outliers (supplementary table S11, Supplementary Material online) a low mutation rate begs the question of how the two alleles originated in the first place.

**Fig. 2.—** Mean variance in allele size versus maximum allele size for 19,338 dinucleotide STRs with maximum allele size \( \leq 30 \). Error bars are standard errors on the estimate of the mean variance in allele size.
In addition, we calculated \( ksk^2(20) \) values using 10-kb windows with a 5-kb step size as described previously (Haasl et al. 2014). \( ksk^2(20) \) was developed to estimate selection on STRs by comparing the number of haplotypes (K) and segregating sites (S), representing a moving average for each 200 kb region. We obtained a total of 503,607 \( ksk^2(20) \) values across the cattle genome, then chose the top 1% and top 5% highly negative values of \( ksk^2(20) \). Our analysis revealed 25,182 top 5% positions and 5,035 top 1% positions. We overlapped the 25,182 top 5% positions with STR regions (60,106 regions), and found 25 STRs overlapping with top 5% positions.

Within these 25 STRs, we indeed found seven genes which may involve in selection signature: \( FAM171B \), \( INHBB \), \( TFCP2L1 \), \( RPRD2 \), \( KLHL1 \), \( SP2 \), and \( WRN \). Three of these loci are of particular interest: \( INHBB \) has an important role in inhibin in reproduction (Chu et al. 2011; Lee et al. 2013); \( KLHL1 \) is involved in poor sperm motility in Holstein–Friesian bulls (Shin et al. 2014; Hering et al. 2014); and \( WRN \) gene is related to Werner syndrome (WS), also known as “adult progeria”, a rare and autosomal recessive progeroid syndrome (PS), which was characterized by the appearance of premature aging (Doan et al. 2012).

To investigate the potential selection involved in STRs, we next searched for all STRs that overlap with 1kb windows on either side of the midpoints of the top 5% of \( ksk^2(20) \) values. We obtained 512 STRs in 97 genes which overlap with the identified \( ksk^2(20) \) selection signature regions. Moreover, we found one STR located at 19,597 Mb on BTA21 overlapping with the detected outlier loci, indicating a strong selection signature with highly divergent alleles.

In order to estimate the genetic relatedness using identified STRs across five individual cattle, we used STR calls for all dinucleotide loci where data were available for all animals and maximum allele size was <30 (n = 19,338). Principal components analysis (PCA) was performed using EIGENSOFT (Patterson et al. 2006). The biplot of the first two principal components, which explained 63.5% of the variance, clearly showed that Starbuck and Elevation show little genetic differentiation (fig. 4). The other three individuals were divergent from each other as well as Starbuck and Elevation (fig. 4).

**STRs and SNP-Based LD Blocks**

STRs have been proposed as a major explanatory factor in explaining the heritability of complex traits in humans and model organisms (Press et al. 2014). Indeed, STRs are widely used for population genetics studies and linkage mapping complex traits due to their high variability. Although earlier studies based on a limited number of then known STRs suggested they are effectively tagged by SNPs (McClure et al. 2012, 2013), to fully explore the potential tagging relationship between STRs and SNPs, we investigated linkage disequilibrium (LD) pattern using SNPs around identified STRs, which is a simple method to understand their potential relationship without phasing STRs. We performed LD block estimation for each autosome using the 44 Holstein Bovine HD SNP array data retrieved from the Bovine HapMap panel.

We identified a total of 58,816 discrete LD blocks distributed across each chromosome with the maximum block size around 200 kb. Because genome regions characterized as LD blocks could imply the co-transmission of phenotype from parent to offspring, we overlapped the 60,106 STRs with the identified LD blocks. We found 57.1% of them (34,312 STRs) overlapped with 14,754 LD blocks, indicating the potential linkage characteristics between STRs and SNPs. The remaining 42.9% of STRs did not overlap with any SNP-base LD block, which suggests these STRs could serve as additional variants. Although SNP arrays and NGS have facilitated efficient SNP genotyping and QTL mapping, additional non-tagged STRs are likely to explain part of the heritability of complex trait missed by SNPs. Thus, a large proportion of the
novel STRs reported here could contribute to STR-based fine mapping of hereditary traits of interest and characterization of meioses not tagged by SNPs. Given the LD pattern of STRs across the genome remains largely unknown, the exploration of LD between STRs and functional genes may help us identify novel candidate STR markers related to complex traits.

STRs Overlap with Regions under Selection Predicted Based on SNPs

Similar to SNPs, STRs are one type of the common variants in the cattle genome which may be under selection. Given their abundance in the genome, STRs represent a notable gap in our knowledge to interrogate genomes for selection signatures. The selective regime of a multi-allelic STR is potentially more complex than that of a di-allelic SNP. In conjunction with its complicated mutational properties, STRs therefore represent a substantially different selective target than SNPs (Putman and Carbone 2014).

We divided STRs into two types to explore their selection signatures. Type 1 included STRs that can be tagged by SNPs; these STRs can be simply explored by estimating the extent of homozygosity of haplotypes. Type 2 included STRs that cannot be tagged effectively by SNPs. Thus, they cannot be handled by currently existing methods directly but require additional research (Putman and Carbone 2014). Because our results were limited by a small sample size (five resequenced genomes), we focused on type 1 STRs. In the future, additional genome-wide STR will facilitate full genome scans for selection based on microsatellite. Here, we present our initial evidence for STR selection as a first step towards this future direction.

A previous study investigated selection signatures based on BovineSNP50 array and NGS data of Chief and Mark and a total of 49 regions were reported to be under recent selection (Larkin et al. 2012). To investigate the selection signature involved in STRs, we first checked if any STRs were embedded in those 49 regions. We observed 5243 STRs overlapping with 45 identified regions, where each of these regions contained variable numbers of STRs (empirical \( P \)-value = 0.014). The maximum and minimum counts of STRs contained in these regions were 1062 and 6, respectively, with an average of 118 per region.

To explore the recent selection of genome regions involved in STRs, we further utilized the Bovine HapMap HD SNP data, and then produced phased haplotypes for each Holstein individual. We then estimated the EHH and iHS in 44 resultant Holstein samples. In total, we found 249 identified regions at the top 1% level and 1194 regions at the top 5% level.
Among these candidate regions, we observed 598 STRs within 54 genes overlapping with 220 regions at the top 1% level (empirical P-value = 0.037) and found 2700 STRs within 246 genes overlapping with 1025 regions at the top 5% level (empirical P-value = 0.003).

Notably, we identified some genes that may be as the potential targets of recent artificial selection for genetic improvement of milk production. For instance, gene SLC20A1 encodes a prostaglandin transporter which is involved in maternal recognition of pregnancy (Bauersachs and Wolf 2015) and mammary development (Gao et al. 2013). We also identified genes related to milk production, fertility and milk fatty acid traits. For example, ATP1A2 was recently reported to be subject to artificial selection for milk production and fertility traits in multiple Holstein populations (Larkin et al. 2012; Lee et al. 2014). PRKG1 was identified as one of 20 genes associated with milk fatty acid traits in Holstein (Li et al. 2014), and its functions include calcium channel regulator activity and cGMP-dependent protein kinase activity. This study also identified ACSL1 associated with the milk fatty acid traits (Li et al. 2014) and supported by other studies (Widmann et al. 2011; Gao et al. 2013; Weber et al. 2013). Other identified genes in our current study are involved in somatic cell score and meat quality traits, such as GRIN2B (Wu et al. 2014) and PLXNA4 (Strillacci et al. 2014).

**Conclusion**

Short tandem repeats are highly mutable genetic elements that often reside in functional genomic regions and are involved in genome evolution. The advances of whole genome sequencing and STR genotype calling algorithms have made it possible to readily identify STR variants across the cattle genome. We concluded that STRs represent a significant source of polymorphism in the cattle genome. We proposed some novel candidate STRs which may be involved in important dairy traits. Our findings suggest that future studies of STRs using NGS could lead to many novel insights into important dairy traits. For instance, gene SLCO2A1 encodes a prostaglandin transporter which is involved in maternal recognition of pregnancy (Bauersachs and Wolf 2015) and mammary development (Gao et al. 2013). We also identified genes related to milk production, fertility and milk fatty acid traits. For example, ATP1A2 was recently reported to be subject to artificial selection for milk production and fertility traits in multiple Holstein populations (Larkin et al. 2012; Lee et al. 2014). PRKG1 was identified as one of 20 genes associated with milk fatty acid traits in Holstein (Li et al. 2014), and its functions include calcium channel regulator activity and cGMP-dependent protein kinase activity. This study also identified ACSL1 associated with the milk fatty acid traits (Li et al. 2014) and supported by other studies (Widmann et al. 2011; Gao et al. 2013; Weber et al. 2013). Other identified genes in our current study are involved in somatic cell score and meat quality traits, such as GRIN2B (Wu et al. 2014) and PLXNA4 (Strillacci et al. 2014).

**Data Accessibility**

Cattle 60,106 STR genotypes predicted by lobSTR in the VCF format was uploaded under doi:10.5061/dryad.34v0d via Dryad. Raw sequencing data are available upon request (after a signed Material Transfer Agreement for exclusive research purpose).

**Supplementary Material**

Supplementary data are available at Genome Biology and Evolution online.

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