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Identification of simple sequence repeat markers in the dromedary (*Camelus dromedarius*) genome by next-generation sequencing

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Abstract: The availability of molecular markers in camels is limited. The aim of this study was to develop new simple sequence repeat (SSR) markers. Four breeds of pooled dromedary genome were sequenced at low coverage utilizing Roche and Illumina platforms. A total of 65,746 contigs, covering approximately 52 Mb (2316 contigs > 2 kb), were assembled. The partial genome revealed 613 SSR loci with a minimum number of 5 repeat units. Comparative chromosomal location for 60 camel loci was predicted against bovine genome assembly Baylor Btau_4.6.1/bosTau7. Ten markers (16.7%) returned matches with a >100 score and >80% identity. SSR abundance was 1 in every 84.3 kb of contigs. The SSR loci mainly comprised di- (80.8%), tri- (10.8%), tetra- (7.6%), and pentamer (0.8%) motifs. (TA)_n and (AC)_n were the most abundant (58.6%) dimers. Thirty SSR loci were experimentally characterized for both dromedary (16 animals) and Bactrian camels. The number of alleles ranged from 1 to 3, and the average number of fragments scored per animal ranged from 0.81 to 2. Polymorphic information content ranged from 0 to 0.66 with a mean value of 0.38. These SSR markers will be a valuable resource for further genetic studies of camels and related species.

Key words: Camel, dromedary, genome, microsatellites, next-generation sequencing

1. Introduction

The family Camelidae comprises 4 domesticated species belonging to 3 genera (1). These species are the Bactrian (*Camelus bactrianus*), the dromedary (*Camelus dromedarius*), the llama (*Lama glama*), and the alpaca (*Vicugna pacos*). In desert countries, camels provide resources that are integral for society such as milk, meat, and other products. Camels are heat stress-resistant animals (2), possessing the ability to apply remarkable adaptive thermoregulatory mechanisms to survive in arid and semiarid environments. Acquiring thermotolerance is a worldwide goal for animal producers (3,4).

An evaluation of genetic diversity based on morphological traits does not usually provide accurate estimates of genetic differences, as they are highly influenced by environmental factors. Several molecular markers have been developed and utilized in genotyping, breeding, and conservation of animals (5). Among the large variety of marker systems available, microsatellites or simple sequence repeats (SSRs) are the most abundant codominant and multiallelic markers (6,7). They are invaluable genetic tools for animal breeding and

quantitative trait locus (QTL) analysis (7,8). The SSR marker system has been widely used for camel genetic diversity (9–15).

Several studies developed SSR markers for different camelids, and each publication reported from 8 to 23 new loci (16–18). However, they were limited in number and not adequate for genetic mapping or QTL analysis. This is because the development of SSR markers is labor-intensive and requires library construction and screening (17). Most recently, high throughput of next-generation sequencing (NGS) enabled the development of genome-wide SSR markers such as alpaca transcriptome (19) and bovine genome (20). The goal of the present study was to identify SSR markers from the dromedary (*Camelus dromedarius*) genome and investigate their polymorphic nature for genetic applications by using camel breeds bred in Saudi Arabia.

2. Materials and methods

2.1. NGS and sequence analysis

Whole-genomic DNA was isolated from 4 female Arabian camels (dromedary) using the Wizard Genomic Kit

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(Promega, USA). DNA samples were pooled and used for NGS utilizing 2 sequencing platforms. The first run required the generation of a sequencing library followed by emulsion PCR. The data were generated from a half-plate 454 pyrosequencing reaction using a GS FLX titanium platform (Roche, USA). The second run was performed utilizing the Genome Analyzer (Illumina, USA). The data were generated from 1 lane with 101 paired-end cycles with a gap of approximately 450 bp. Combined reads were assembled in SeqMan NGen (DNASTAR, USA). SSRs were retrieved from assembled contigs using the Simple Sequence Repeat Identification Tool (SSRIT) (21) as a web interface. There was no sequence masking for any repetitive element or those with a minimum number of 5 repeat units. A total of 60 SSRs representing di-, tri-, tetra-, and pentamers were randomly selected, and their original contig sequences were retrieved from the assembly. Forward and reverse primers flanking each SSR locus were designed in Vector NTI (Invitrogen, USA). The marker sequences were compared to the bovine whole genome sequence (Baylor Btau_4.6.1/bosTau7) to identify potentially homologous sequences utilizing BLAT genome search. Default search parameters were used for this comparison (<https://genome.ucsc.edu/cgi-bin/hgBlat>).

2.2. SSR characterization and data analysis

A total of 16 Saudi camels (*C. dromedarius*), representing 4 breeds (ZU: Zurg, MJ: Majaheem, MG: Maghateer, SO: Sofr), were investigated to assess the applicability of the developed SSR markers. In addition, SSR markers were screened for one Bactrian camel (*C. bactrianus*). DNA was isolated using Wizard Genomic DNA purification kit (Promega, USA) from blood samples (dromedary) or hair samples (Bactrian). DNA samples were resuspended in TE buffer overnight at 4 °C and stored at -20 °C. The quality and quantity of genomic DNA were determined with a NanoDrop spectrophotometer.

Isolated DNA samples were first assessed for PCR by amplifying a repetitive sequence, which partially covered the 12S ribosomal gene developed in this study from the GenBank database using forward (5'-ACTCAAAGGACTTGGCGGTGC-3') and reverse (5'-GTGTGCGTGCTCCATGGC-3') primers. If the 12S is successfully amplified, then the DNA sample is ready for SSR analysis; otherwise, it may contain PCR inhibitors that preclude SSR amplification. PCR amplifications (both for 12S and SSR markers) were performed in 20- μ L reactions containing 20 ng of genomic DNA template (pooled from all 16 animals), 1X GoTaq Green Master Mix (Promega, USA), 0.1 μ M each forward and reverse primer, and nuclease-free water. Thermal cycling profile consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles (94 °C for 45 s, 50 °C for 45 s, and 72 °C for 1 min) and a final extension at 72 °C for 20 min. PCR products

were separated in 3% MetaPhore agarose (Lonza, USA) in 0.5X TBE buffer. HyperLadder IV (Biolone, UK) was used as the DNA marker. Gels were run under 60 V for 2 h. DNA was visualized with acridine orange (Sigma, USA) under UV light.

The expected heterozygosity (H_e) was calculated according to the Nei equation (22), and the observed heterozygosity (H_o) was calculated by dividing the number of heterozygotes at the locus by the number of individuals typed. Polymorphic information content (PIC) values were calculated for each SSR to estimate its allelic variation according to the formula described by Anderson et al. (23).

3. Results

The NGS with 454 GS FLX System yielded more than 700,000 reads with an average length of 375 bp, while the NGS with Genome Analyzer platform yielded more than 30×10^6 paired reads with approximately 100 bp. The reads were trimmed, and a draft dromedary genome was assembled into 65,746 contigs (2316 contigs longer than 2 kb) with N50 of 973 bp and an average of 786 bp, where N50 is the length of the longest contig of the lower half of all contigs (with a descending order from the longest to the shortest contig).

In total, 613 SSR loci with perfect repeats were detected in the assembly (Table 1). Singletons were not used to extract SSR motifs. The search was limited to motifs with 5 or more repeats. All 4 possible combinations of dimer motif groupings were found in 495 loci, of which 156 were AT/TA motifs. The trimer, tetramer, and pentamer combinations were detected in 66, 47, and 5 loci, respectively.

One-tenth of detected loci were randomly selected to be tested for SSR characterization utilizing local camel breeds. The designed PCR primers are listed in Table 2. The repeat number ranged from 5 to 22. These loci were numbered consecutively (*Cd00801* to *Cd00860*), and their sequences were deposited in GenBank (<http://www.ncbi.nlm.nih.gov>) with sequential accession numbers (JX093499–JX092558).

Comparative chromosomal location for the selected camel markers was predicted in the bovine genome by BLAT searches against bovine genome. All sequences returned a BLAT match (Table 3). Some markers returned multiple matches; however, 16.7% (10 markers) returned BLAT matches with >100 score and >80% identity. Putative camel homologs were found on each chromosome of bovine genome, except for BTA 25, 28, and Y. One camel SSR locus was placed on BTA 6, 7, 8, 12, 16, 19, 24, and 26, while BTA 11 and 14 reached 5 SSRs each with an average of 2 loci per chromosome. Conversely, 3 markers showed matches to unassigned contigs (UN).

The selected SSR primers were evaluated for their ability to prime PCR amplification of one pooled DNA

Table 1. SSR repeats detected in dromedary camel genome.

| Repeat motif grouping | Times repeated | Occurrence |
|-------------------------|----------------|------------|
| Dimers | | |
| AC/CA/TG/GT | 5–149 | 83 |
| AG/GA/CT/TC | 5–61 | 248 |
| AT/TA | 5–19 | 156 |
| GC/CG | 5–9 | 8 |
| Trimers | | |
| AAC/ACA/CAA/GTT/TTG/TGT | 5–17 | 16 |
| AAG/AGA/GAA/CTT/TTC/TCT | 0 | 0 |
| AAT/ATA/TAA/ATT/TTA/TAT | 5–18 | 8 |
| ACC/CCA/CAC/GGT/GTG/TGG | 5–8 | 5 |
| ACG/CGA/GAC/CGT/GTC/TCG | 5 | 1 |
| AGC/GCA/CAG/GTC/TCG/CGT | 5 | 3 |
| AGG/GGA/GAG/CCT/CTC/TCC | 5–14 | 10 |
| AGT/GTA/TAG/ACT/CTA/TAC | 5 | 2 |
| ATG/TGA/GAT/CAT/ATC/TCA | 5–17 | 4 |
| GGC/GCG/CGG/GCC/CCG/CGC | 5–17 | 17 |
| Tetramers* | | |
| AATT | 5 | 2 |
| ACCC | 5 | 1 |
| ACGC | 7 | 1 |
| AGAC | 5–11 | 6 |
| AGAT | 6–17 | 5 |
| ATGT | 6–12 | 3 |
| CAGG | 13–10 | 2 |
| CCCT | 7–19 | 2 |
| CCGC | 5–6 | 3 |
| CCTT | 5 | 1 |
| GGCT | 5 | 1 |
| GTAA | 8 | 1 |
| TAGT | 6 | 1 |
| TGAA | 6 | 2 |
| TTTA | 5–15 | 3 |
| TTTC | 6–20 | 6 |
| TTTG | 5–10 | 7 |
| Pentamers* | | |
| AAACA | 8 | 1 |
| AATAA | 7 | 1 |
| ACCAC | 8 | 1 |
| CCGCT | 5 | 1 |
| CGTGC | 6 | 1 |

*: Equivalent motifs in different reading frames or on a complementary strand were not listed to save space. Tetramers have equivalent motifs ACGC, CGCA, GCAC, CACG, GCGT, CGTG, and GTGC, while pentamers have 10 equivalent motifs.

sample (Figure 1). Among the 60 primer pairs, 56 (93%) primers showed clear amplified fragments and 4 (7%) did not amplify detectable products. After 3 independent PCRs, 30 primers showing consistent and reproducible amplification were selected to analyze 16 camels. In addition, they were all positive when tested for the Bactrian camel genome with similar allele amplifications (data not shown).

The 30 SSR primers revealed 61 amplified DNA fragments (alleles) that ranged from 1 to 3 alleles with an average of 2.03 alleles per primer combination across all 16 animals (Table 4). All primers showed an average of 62.8% polymorphism ranging from 0% (no polymorphism) to 100%. Results showed that more than 76% of primers produced more than 1 allele across all 16 animals. The number of SSR alleles scored per animal ranged from 1 to 3, and the average number of fragments ranged from 0.81 to 2. In total, applied markers generated 592 fragments across the tested animals; 14–32 fragments were generated per SSR marker with an average of 19.7. The PIC for all primers ranged from 0.0 to 0.66 with an average value of 0.38. The H_o and H_e values of each locus are presented in Table 4. The H_o ranged from 0 to 1 with an average of 0.26, whereas the H_e ranged from 0 to 0.69 with an average of 0.38.

4. Discussion

The present investigation was carried out to enrich the content of available camel molecular markers. The generated trace genome sequence served as the basis to achieve this goal. We assembled the reads into genomic contigs to extract SSR sequences. The utilization of NGS technology delivers more coverage than the conventional whole-genome sequencing approach (24). This coverage includes more SSR markers, as recorded in this study. The Illumina platform is very important for delivering good sequence depth and confidence, as shown in the SSR markers identified in alpaca (19). However, the Roche GS FLX platform is equally important in extending contig length, thus capturing long repeats flanked by unique signature sequences. Therefore, a mixed sequence would cover both good sequence depth and contig length.

The assembly generated contigs that were useful for primer design. The total SSR genome coverage varies between mammals. It can extend to 4.16% in mice, but decreases to a mere 0.78% in humans (20). The calculated SSR coverage in the analyzed partial camel genome was 0.021%, which represents a minor portion. However, this does not include motifs repeated twice, thrice, or 4 times. In fact, we observed many mononucleotide repeats within camel contigs. Mononucleotides are highly abundant in humans with an average appearance of 2.9 kb, thus exceeding all other nucleotide SSRs (25).

Table 2. Developed dromedary camel SSR markers with their repeats and PCR primers.

| Locus | Accession number | Repeat motif | Primer sequence (5'-3') | T _m (°C) | Size (bp) |
|----------------|------------------|----------------------|---|---------------------|-----------|
| <i>Cd00801</i> | JX093499 | (AAAT) ₁₅ | F: GATGCAACGGAGAAACGATC R: CCAAGATCATAAAGCTTAAGCC | 52.0 52.0 | 254 |
| <i>Cd00802</i> | JX093500 | (TA) ₁₂ | F: GTCTGAATTCCCAATGTAACCC R: CAGGATGCTCTGCAATGTCAC | 51.7 53.0 | 203 |
| <i>Cd00803</i> | JX093501 | (TTG) ₆ | F: TGTTCCTTGGGCTTACTTCC R: TGAGTCTTGCTACATAACCAGGC | 51.0 51.3 | 204 |
| <i>Cd00804</i> | JX093502 | (CA) ₈ | F: ATTCAAACCCAGGTCTCTGG R: GCAGAAGATCCATATGGAGCC | 50.4 52.8 | 239 |
| <i>Cd00805</i> | JX093503 | (GTAA) ₈ | F: GTTCGATCTTCAGGACTTCCG R: CTTGCTGTCGTGATTCCAGG | 52.9 53.0 | 322 |
| <i>Cd00806</i> | JX093504 | (GCG) ₁₂ | F: GTTCGTTGCTCGTGTGACG R: GCTGAGACTAAACACTGACGGC | 52.2 53.2 | 331 |
| <i>Cd00807</i> | JX093505 | (GA) ₁₅ | F: TCAAGCCGGCTTTACAAGG R: AGCCTGCTTGACCCATGG | 53.0 53.1 | 232 |
| <i>Cd00808</i> | JX093506 | (AT) ₉ | F: AGTGCAGGCACTTTATTGGG R: CGAGTTGGATGTTGTGTCTCC | 51.9 51.8 | 238 |
| <i>Cd00809</i> | JX093507 | (AGAT) ₁₀ | F: GCACACACGCACACACACAC R: TATCTAACGGAGGAGGAGGCC | 53.7 54.0 | 308 |
| <i>Cd00810</i> | JX093508 | (AAC) ₉ | F: TGGACTTGGGGAGTATTATGC R: TCCCTATCCCAGTCTTGCC | 51.3 51.3 | 217 |
| <i>Cd00811</i> | JX093509 | (GA) ₈ | F: ACGCCCTAGGCTTCAAGG R: CTAGCCCTGAAAATGGATGG | 51.3 51.8 | 283 |
| <i>Cd00812</i> | JX093510 | (AAC) ₁₀ | F: CCATGAGGTTCTCTGAAACCC R: GAGTAATTCCTGAAATGGCC | 52.5 52.0 | 292 |
| <i>Cd00813</i> | JX093511 | (GTTT) ₅ | F: AAAGCGTGCTGAACGATCC R: GACGTCAAAATCCTTAGGATGG | 52.7 52.1 | 261 |
| <i>Cd00814</i> | JX093512 | (TG) ₁₄ | F: GCATAATGCCATCCAAGTCC R: GCCAAGGTATGGAAGCAACC | 51.9 53.6 | 236 |
| <i>Cd00815</i> | JX093513 | (AAC) ₁₁ | F: CCATGAGGTTCTCTGAAACCC R: TGGCCCATCACTTGAAATACC | 52.5 53.8 | 262 |
| <i>Cd00816</i> | JX093514 | (CA) ₂₃ | F: GCAGGGTCATTTTAGCAGG R: ATGGTGAGCACAAGTGAGGG | 51.6 52.2 | 317 |
| <i>Cd00817</i> | JX093515 | (AT) ₉ | F: ATCACCTGTGCTTCCTGCC R: GAAGGAAGGGTGCTGAAGG | 52.2 51.1 | 285 |
| <i>Cd00818</i> | JX093516 | (TG) ₁₂ | F: AGTTATCCTTGAGGGCCTGC R: ACAGTGTTCCTGTTCCC | 52.5 52.6 | 320 |
| <i>Cd00819</i> | JX093517 | (AT) ₁₉ | F: AATCAGAAGCAGAACCCAAGC R: AAGGAGGTAAAGGAGGTGTGG | 52.7 51.5 | 287 |
| <i>Cd00820</i> | JX093518 | (CA) ₂₀ | F: CTGTACACGTCCCACGACATG R: AACCATGCAAGAAGCCAGG | 53.6 52.5 | 207 |
| <i>Cd00821</i> | JX093519 | (CA) ₂₀ | F: AGCTCATTCTCCCCAACCC R: AGTCCTCAGCTTGTAATTGC | 52.8 51.1 | 258 |
| <i>Cd00822</i> | JX093520 | (AATAA) ₇ | F: ACTCTCCGATCTAGGGCCC R: GGTTTAGTGGTTCAAAGCCG | 51.5 51.5 | 277 |
| <i>Cd00823</i> | JX093521 | (GCGG) ₆ | F: ATCCCTTTCACGCCAACCC R: TCGTAACAAGGTTCCGTAGG | 52.0 51.3 | 298 |

Table 2. (Continued).

| | | | | | |
|----------------|----------|----------------------|---|--------------|-----|
| <i>Cd00824</i> | JX093522 | (TTTG) ₅ | F: TCTTGTGATGCCTTTGTCTGG R: CATCCCACGAGGAAATGC | 52.6 52.7 | 210 |
| <i>Cd00825</i> | JX093523 | (TG) ₅ | F: AACACCATGCACTAAGCAAGG R: ATGTCTTGCCCTTCCCTTGC | 52.0 53.3 | 352 |
| <i>Cd00826</i> | JX093524 | (AC) ₁₁ | F: TGAATGGTCTTCTAGTGGCCC R: AATGAGCCTGGAGGTAAGTGG | 53.2 52.4 | 269 |
| <i>Cd00827</i> | JX093525 | (TTTG) ₅ | F: AATCCCAGTCTATCCCTTCCC R: TGCACCCCAATGTCATAGC | 52.7 53.2 | 368 |
| <i>Cd00828</i> | JX093526 | (GT) ₂₀ | F: AAGTGGTCCTTCTCCTTCAGC R: ACGTCTTGCCCTTCCCTAGC | 51.7 52.7 | 278 |
| <i>Cd00829</i> | JX093527 | (CA) ₁₀ | F: CAGTGTGGCTATGACCAAGC R: GGGGAATACTGACACAGAGGG | 52.3 52.4 | 342 |
| <i>Cd00830</i> | JX093528 | (TTA) ₁₈ | F: GCTCAGCAAATACAGCAGCC R: TTCATAGCTGTCTGGCGTGC | 52.7 53.8 | 352 |
| <i>Cd00831</i> | JX093529 | (AATT) ₅ | F: TGCTTAGCATGCACAAGGC R: GTGGGGAGGGCTATGTGG | 52.3 52.2 | 215 |
| <i>Cd00832</i> | JX093530 | (CATA) ₁₀ | F: TGTGGGTTCATTTTCAGGGC R: CTCCTATAAGCCCCTTTGG | 52.9 52.3 | 326 |
| <i>Cd00833</i> | JX093531 | (AC) ₂₂ | F: AATATGGGCTCAATTTGGCC R: CCTCTTGTTCATCTGGACTGG | 53.1 51.1 | 302 |
| <i>Cd00834</i> | JX093532 | (TTG) ₁₅ | F: TCTCACTCTGCCTCCAGGG R: CTGAGCTTGACACTGATTGCC | 52.3 52.3 | 237 |
| <i>Cd00835</i> | JX093533 | (AGAC) ₆ | F: AGGGAGACAGACAGACACGC R: CGGTGGCAGAAGGACTCC | 51.4 52.6 | 242 |
| <i>Cd00836</i> | JX093534 | (AC) ₁₀ | F: ACGTCCCTCTCCCACTGG R: GGGTGGGGCTAGAACTCTACC | 51.7 53.4 | 204 |
| <i>Cd00837</i> | JX093535 | (AC) ₁₆ | F: AACTGAGCTGATTCCAGCCC R: GGGAACAGGGAGTAGGTGG | 53.2 50.6 | 236 |
| <i>Cd00838</i> | JX093536 | (TG) ₁₇ | F: GAGCCTGGAGGCAAGTGG R: TCTAATGACCCTCCCAGTTGG | 52.7 53.0 | 257 |
| <i>Cd00839</i> | JX093537 | (CA) ₁₆ | F: CCAGTTGATTGGGAAATCCC R: TTCCAGATTGTGTGTGTGTC | 53.1 51.4 | 214 |
| <i>Cd00840</i> | JX093538 | (TG) ₁₅ | F: AAAGGTTTGAGCGCCACC R: CTGTCTTCCAAGTGTCTGC | 52.5 51.3 | 284 |
| <i>Cd00841</i> | JX093539 | (CA) ₅ | F: GCGTTCCCAACAAGCTAGG R: TGTGGAGGTGTACCAGCTCC | 52.3 52.2 | 210 |
| <i>Cd00842</i> | JX093540 | (AG) ₅ | F: CATACTCTTTGGCACTGTGG R: TCCTGCTATTGATTAGACACAGG | 52.2 50.6 | 303 |
| <i>Cd00843</i> | JX093541 | (AT) ₇ | F: TGCTGTTTCAAATTCCTGC R: GGAAGGGAAAGTAAATTTCCG | 52.7 53.0 | 609 |
| <i>Cd00844</i> | JX093542 | (AT) ₆ | F: CTTTGTGCTAGATGAACGAACG R: AATGGAACGGTTGCAGG | 52.0 53.0 | 255 |
| <i>Cd00845</i> | JX093543 | (CA) ₅ | F: GACTGGAAAACAGATTTGGAGC R: TCCTGTTTTGCTCGATGTACG | 52.2 52.9 | 127 |
| <i>Cd00846</i> | JX093544 | (TC) ₆ | F: TGGTCTTGACAAATCTTACGACC R: TAAGGCATGATCTTCACTCACC | 52.6 52.7 | 431 |
| <i>Cd00847</i> | JX093545 | (CA) ₅ | F: TAAGATGAAAAGAAAAGAGAGCC R: TCTTGCCAATATGAGAAATTGC | 51.4 50.9 | 242 |

Table 2. (Continued).

| | | | | | |
|----------------|----------|----------------------|--|--------------|-----|
| <i>Cd00848</i> | JX093546 | (TTG) ₅ | F: TGCACATGTTTCCTCAGGG R: AGGTGACTGCTTTCATAAATGC | 51.4 50.6 | 264 |
| <i>Cd00849</i> | JX093547 | (TATT) ₅ | F: CCATGCTGTACAGGAGGACC R: GCATTCTGAGTCCCAGAGAGG | 51.7 52.8 | 435 |
| <i>Cd00850</i> | JX093548 | (GT) ₇ | F: CCCAAATTTCCCTCTCAACC R: GGTAATTAGCGGAGTTCCCC | 52.5 52.0 | 211 |
| <i>Cd00851</i> | JX093549 | (ATA) ₅ | F: TCTTAGGGGTAGGATCAATTCC R: GTCAGTGCATCAGGCATCC | 50.9 50.7 | 310 |
| <i>Cd00852</i> | JX093550 | (TC) ₆ | F: TATACGAGGTTCCGGTGTAGC R: CGTGGATGATTGGCTTAAGG | 51.5 52.2 | 224 |
| <i>Cd00853</i> | JX093551 | (CTAT) ₁₁ | F: GGCAGCCCAGATCTATCTCC R: GCTCAGTGGTAGAGTGCATGC | 52.7 52.3 | 463 |
| <i>Cd00854</i> | JX093552 | (AC) ₁₀ | F: GTGGGAACGAGAGCTCTGC R: TGGAGGACAATTGAGAGATAAGG | 52.1 51.8 | 286 |
| <i>Cd00855</i> | JX093553 | (CA) ₁₃ | F: CTAGCCTCTTCCTCCATTTAGC R: CCTACAGGAGGCATACCTGC | 51.2 51.3 | 250 |
| <i>Cd00856</i> | JX093554 | (TC) ₇ | F: CAACTGGGTGTTTGCTTGC R: TCCTCAGCCCAAACCTCTCC | 51.4 51.4 | 445 |
| <i>Cd00857</i> | JX093555 | (GA) ₅ | F: GGGACTATGGTTGCAGATGC R: CCTCCTAGGGTCTTGAATGC | 51.9 52.1 | 322 |
| <i>Cd00858</i> | JX093556 | (GCC) ₇ | F: ATGGGAGCTAATCCTCAAGC R: CGAACTGATGGAATAGCTGC | 50.2 50.0 | 481 |
| <i>Cd00859</i> | JX093557 | (CG) ₅ | F: ACAGCCAGACAGACATACTAGCC R: GCTATCTATCTATGTGGGGAGGC | 52.0 52.9 | 288 |
| <i>Cd00860</i> | JX093558 | (TG) ₁₅ | F: ACAATGTCAGGAGACCCAGG R: CCTTTGCTTCATTTACCTCTCC | 51.0 51.7 | 513 |

T_m : Melting temperature.

SSR locus length can be calculated by multiplying the motif length with its repetition frequency (Table 1). Dimer motifs were found to be repeated up to 149 times (298 bp long). Dinucleotide repeat motifs tend to be longer than other repeats in several eukaryotic genomes (26). Long SSR motifs are expected to give a large number of alleles per locus due to greater potential for slippage (27). Few loci with many alleles will give an estimated genetic distance that is equivalent to that of many loci with few alleles (28). On the other hand, many loci with few alleles constitute crucial input for mapping purposes.

The abundance of specific SSR repeat motifs was investigated in several animals such as chicken (29) and alpaca (19). When studying the abundance of certain SSR motifs in any genome, all equivalent motifs in a grouping in different reading frames or on a complementary strand should be considered (26). Dimer SSRs have 4 groupings or classes, while trimers have 10 groupings (Table 1). Camel genome showed high frequency of dimer motif repeats (80.8%). This was likewise observed in several

other eukaryotes (26). Camel SSRs with dimer and trimer motifs were compared with those of the related alpaca (19). The most abundant dimer in camel was AG/GA/CT/TC, with 50.1% compared to 30% in alpaca. The lowest dimer occurrence was recorded for GC/CG, and the comparable figures were 1.6% (camel) and 1.4% (alpaca). The motif AT/TA represented 31.5% (camel) and 31.6% (alpaca) of all dimers. As a percentage of all repeats, AT/TA occurrence was 25.4% in camel compared to 13.1% in alpaca (Figure 2). Considering the source of SSR sequences (genomic in camel and ESTs in alpaca) and the presumed synteny between them, it is probable that AT/TA repeats are almost equally dispersed between genic and intergenic sequences in camels. In sheep, the most abundant dimer repeat was found to be AC/CA/TG/GT (67%) (30). However, the SSR sequences were extracted from skin EST sequences and thus do not reflect the whole genome.

The camel genome showed 2 abundant trimer motif groupings, namely GGC/GCG/CGG/GCC/CCG/CGC (25.8%) and AAC/ACA/CAA/GTT/TTG/TGT (24.2%).

Table 3. BLAT search results with bovine. Only the top hit is indicated for each locus (the used query-database type was nucleotide-nucleotide).

| Locus | BLAT Score | Start | End | Q size | Identity | Chromosome | Start | End | Span |
|----------------|------------|-------|-----|--------|----------|-----------------|-----------|-----------|------|
| <i>Cd00801</i> | 49 | 18 | 172 | 254 | 71.5% | 2 | 73873046 | 73873150 | 105 |
| <i>Cd00802</i> | 65 | 123 | 199 | 199 | 95.9% | 4 | 118771549 | 118771949 | 401 |
| <i>Cd00803</i> | 61 | 37 | 135 | 204 | 82.7% | 1 | 150016532 | 150016618 | 87 |
| <i>Cd00804</i> | 33 | 17 | 156 | 240 | 55.6% | 14 | 32951266 | 32951306 | 41 |
| <i>Cd00805</i> | 36 | 184 | 219 | 322 | 100% | 14 | 14237251 | 14237286 | 36 |
| <i>Cd00806</i> | 51 | 159 | 244 | 331 | 78.7% | 4 | 97209810 | 97209881 | 72 |
| <i>Cd00807</i> | 75 | 95 | 204 | 229 | 94.4% | 7 | 14999684 | 14999816 | 133 |
| <i>Cd00808</i> | 23 | 184 | 207 | 238 | 100% | 10 | 39983556 | 39983584 | 29 |
| <i>Cd00809</i> | 78 | 52 | 173 | 282 | 83.5% | 17 | 70719484 | 70719604 | 121 |
| <i>Cd00810</i> | 96 | 1 | 165 | 216 | 83.0% | 15 | 13203076 | 13203224 | 149 |
| <i>Cd00811</i> | 160 | 2 | 282 | 282 | 89.1% | Un_AAFC02248261 | 792 | 1021 | 230 |
| <i>Cd00812</i> | 88 | 10 | 281 | 291 | 90.9% | Un_JH126266 | 1826 | 2255 | 430 |
| <i>Cd00813</i> | 30 | 99 | 132 | 260 | 97.0% | 11 | 48406748 | 48406782 | 35 |
| <i>Cd00814</i> | 130 | 1 | 234 | 234 | 86.3% | X | 67270088 | 67270289 | 202 |
| <i>Cd00815</i> | 47 | 49 | 231 | 260 | 92.8% | 10 | 5700521 | 5700865 | 345 |
| <i>Cd00816</i> | 44 | 90 | 144 | 316 | 83.4% | 19 | 63666336 | 63666384 | 49 |
| <i>Cd00817</i> | 100 | 118 | 284 | 284 | 83.1% | 3 | 56808973 | 56809137 | 165 |
| <i>Cd00818</i> | 85 | 178 | 296 | 320 | 91.4% | 17 | 321136 | 321268 | 133 |
| <i>Cd00819</i> | 108 | 86 | 225 | 285 | 88.6% | 3 | 46283442 | 46283581 | 140 |
| <i>Cd00820</i> | 56 | 19 | 90 | 207 | 96.8% | 23 | 42335175 | 42335346 | 172 |
| <i>Cd00821</i> | 150 | 19 | 258 | 258 | 85.9% | 14 | 2508875 | 2509098 | 224 |
| <i>Cd00822</i> | 77 | 19 | 205 | 277 | 74.0% | 11 | 14487391 | 14487537 | 147 |
| <i>Cd00823</i> | 124 | 79 | 297 | 297 | 85.3% | 27 | 7281250 | 7281432 | 183 |
| <i>Cd00824</i> | 83 | 1 | 173 | 210 | 78.2% | 8 | 54427216 | 54427364 | 149 |
| <i>Cd00825</i> | 35 | 117 | 302 | 353 | 71.8% | 13 | 28557411 | 28557575 | 165 |
| <i>Cd00826</i> | 48 | 56 | 206 | 270 | 96.2% | 18 | 28948221 | 28948421 | 201 |
| <i>Cd00826</i> | 20 | 216 | 235 | 270 | 100% | 18 | 45252141 | 45252160 | 20 |
| <i>Cd00827</i> | 208 | 30 | 368 | 368 | 86.9% | X | 80748743 | 80749108 | 366 |
| <i>Cd00828</i> | 56 | 26 | 268 | 278 | 98.3% | 10 | 94786630 | 94787091 | 462 |
| <i>Cd00829</i> | 34 | 176 | 244 | 342 | 94.6% | 29 | 30233605 | 30234055 | 451 |
| <i>Cd00830</i> | 159 | 11 | 323 | 351 | 82.0% | 14 | 2477863 | 2478107 | 245 |
| <i>Cd00831</i> | 45 | 130 | 195 | 208 | 94.3% | 23 | 8829852 | 8829918 | 67 |
| <i>Cd00832</i> | 99 | 1 | 228 | 324 | 80.8% | 15 | 35395766 | 35395955 | 190 |
| <i>Cd00833</i> | 33 | 115 | 149 | 302 | 97.2% | 15 | 32937537 | 32937571 | 35 |
| <i>Cd00834</i> | 32 | 36 | 221 | 237 | 58.9% | 20 | 59854501 | 59854599 | 99 |
| <i>Cd00835</i> | 53 | 49 | 122 | 242 | 96.7% | 12 | 83018654 | 83018764 | 111 |
| <i>Cd00836</i> | 23 | 21 | 44 | 203 | 100% | 21 | 22051621 | 22051651 | 31 |
| <i>Cd00837</i> | 49 | 103 | 171 | 237 | 88.9% | 5 | 99220633 | 99220699 | 67 |
| <i>Cd00838</i> | 32 | 169 | 202 | 256 | 97.1% | 20 | 60265921 | 60265954 | 34 |
| <i>Cd00839</i> | 41 | 161 | 203 | 214 | 97.7% | 26 | 15543536 | 15543578 | 43 |

Table 3. (Continued).

| | | | | | | | | | |
|----------------|-----|-----|-----|-----|-------|-------------|-----------|-----------|--------|
| <i>Cd00840</i> | 40 | 67 | 108 | 282 | 100% | Un_JH126349 | 9255 | 9462 | 208 |
| <i>Cd00841</i> | 28 | 105 | 135 | 210 | 86.3% | 13 | 72622131 | 72622159 | 29 |
| <i>Cd00842</i> | 31 | 117 | 157 | 302 | 76.5% | 14 | 12022068 | 12022101 | 34 |
| <i>Cd00843</i> | 28 | 456 | 490 | 608 | 94.0% | 11 | 75030580 | 75030616 | 37 |
| <i>Cd00844</i> | 22 | 165 | 187 | 252 | 100% | Un_JH121384 | 233613 | 233637 | 25 |
| <i>Cd00845</i> | 22 | 62 | 83 | 173 | 100% | 9 | 93324886 | 93324907 | 22 |
| <i>Cd00846</i> | 40 | 134 | 290 | 431 | 79.6% | 1 | 23236292 | 23236439 | 148 |
| <i>Cd00847</i> | 29 | 38 | 78 | 239 | 94.0% | 11 | 11109619 | 11109665 | 47 |
| <i>Cd00848</i> | 45 | 49 | 116 | 264 | 83.6% | 15 | 5181726 | 5181796 | 71 |
| <i>Cd00849</i> | 130 | 54 | 258 | 435 | 86.6% | 4 | 118753014 | 118753221 | 208 |
| <i>Cd00850</i> | 32 | 105 | 137 | 211 | 100% | 27 | 45997276 | 46331741 | 334466 |
| <i>Cd00851</i> | 34 | 55 | 136 | 306 | 97.3% | 13 | 66004976 | 66014684 | 9709 |
| <i>Cd00852</i> | 53 | 21 | 86 | 225 | 90.8% | 1 | 142303731 | 142303798 | 68 |
| <i>Cd00853</i> | 100 | 109 | 277 | 461 | 81.9% | 6 | 50881280 | 50881447 | 168 |
| <i>Cd00854</i> | 88 | 1 | 187 | 282 | 78.9% | 21 | 1599943 | 1600124 | 182 |
| <i>Cd00855</i> | 44 | 53 | 134 | 250 | 81.3% | 22 | 55348889 | 55348961 | 73 |
| <i>Cd00856</i> | 23 | 266 | 294 | 445 | 89.7% | X | 42605701 | 42605729 | 29 |
| <i>Cd00856</i> | 23 | 366 | 388 | 445 | 100% | 2 | 15598394 | 15598416 | 23 |
| <i>Cd00856</i> | 23 | 334 | 358 | 445 | 96.0% | 27 | 34934426 | 34934450 | 25 |
| <i>Cd00856</i> | 20 | 373 | 392 | 445 | 100% | 1 | 3406491 | 3406510 | 20 |
| <i>Cd00857</i> | 62 | 57 | 281 | 322 | 82.9% | 11 | 68700495 | 68700712 | 218 |
| <i>Cd00858</i> | 28 | 208 | 238 | 499 | 96.7% | 5 | 121089630 | 121089663 | 34 |
| <i>Cd00859</i> | 55 | 121 | 181 | 287 | 98.4% | 24 | 29259853 | 29260218 | 366 |
| <i>Cd00860</i> | 24 | 471 | 499 | 513 | 92.9% | 16 | 40407501 | 40407531 | 31 |

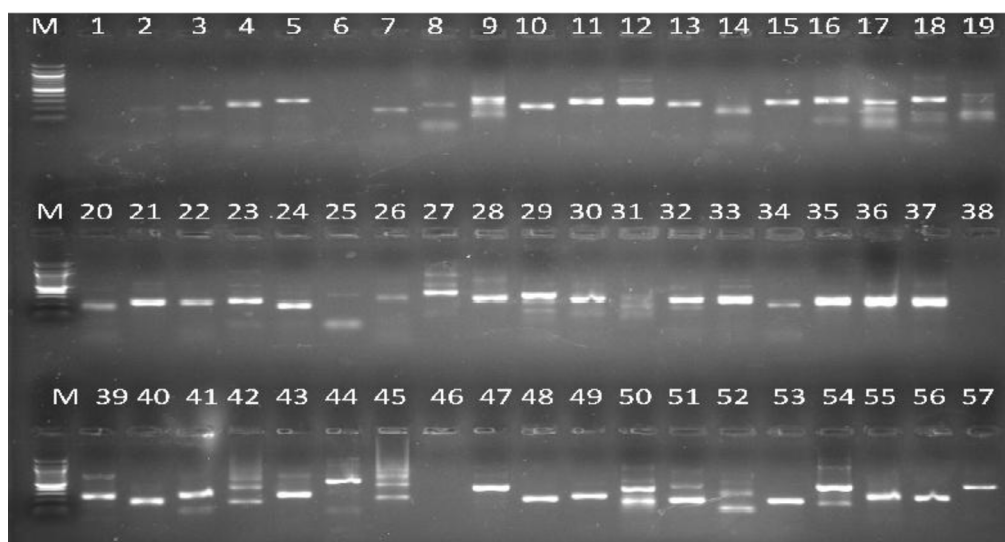


Figure 1. Screening of selected SSRs primers on pooled camel genomic DNA. M = 100-bp DNA ladder. Numbers 1–57 correspond to loci *Cd00801* and *Cd00857*, respectively.

Table 4. Characteristics of selected SSRs for genetic diversity in Saudi camels.

| Locus | Total alleles | Average number of fragments* | Total number of fragments | Polymorphism %** | H_o | H_e | PIC |
|----------------|---------------|------------------------------|---------------------------|------------------|-------|-------|-------|
| <i>Cd00811</i> | 2 | 2.00 | 32 | 0 | 1.00 | 0.52 | 0.50 |
| <i>Cd00812</i> | 2 | 2.00 | 32 | 0 | 1.00 | 0.52 | 0.50 |
| <i>Cd00815</i> | 3 | 1.56 | 25 | 100 | 0.56 | 0.67 | 0.66 |
| <i>Cd00816</i> | 3 | 2.00 | 32 | 67 | 1.00 | 0.55 | 0.53 |
| <i>Cd00818</i> | 2 | 1.94 | 31 | 50 | 0.94 | 0.51 | 0.50 |
| <i>Cd00824</i> | 2 | 1.00 | 16 | 100 | 0.00 | 0.44 | 0.43 |
| <i>Cd00827</i> | 3 | 1.00 | 16 | 100 | 0.00 | 0.56 | 0.54 |
| <i>Cd00828</i> | 2 | 1.00 | 16 | 100 | 0.00 | 0.52 | 0.50 |
| <i>Cd00829</i> | 3 | 1.19 | 19 | 100 | 0.19 | 0.28 | 0.35 |
| <i>Cd00832</i> | 2 | 1.00 | 16 | 100 | 0.00 | 0.51 | 0.49 |
| <i>Cd00833</i> | 3 | 2.00 | 32 | 67 | 1.00 | 0.59 | 0.58 |
| <i>Cd00835</i> | 2 | 1.44 | 23 | 100 | 0.64 | 0.52 | 0.50 |
| <i>Cd00836</i> | 2 | 2.00 | 32 | 0 | 1.00 | 0.52 | 0.50 |
| <i>Cd00837</i> | 2 | 0.88 | 14 | 100 | 0.00 | 0.51 | 0.49 |
| <i>Cd00839</i> | 1 | 1.00 | 16 | 0 | 0.00 | 0.00 | 0.00 |
| <i>Cd00840</i> | 1 | 1.00 | 16 | 0 | 0.00 | 0.00 | 0.00 |
| <i>Cd00841</i> | 1 | 1.00 | 16 | 0 | 0.00 | 0.00 | 0.00 |
| <i>Cd00843</i> | 3 | 0.81 | 13 | 100 | 0.00 | 0.49 | 0.47 |
| <i>Cd00844</i> | 3 | 1.00 | 16 | 100 | 0.00 | 0.57 | 0.55 |
| <i>Cd00847</i> | 2 | 1.00 | 16 | 100 | 0.00 | 0.44 | 0.43 |
| <i>Cd00848</i> | 2 | 1.00 | 16 | 100 | 0.00 | 0.51 | 0.49 |
| <i>Cd00849</i> | 1 | 1.00 | 16 | 0 | 0.00 | 0.00 | 0.00 |
| <i>Cd00850</i> | 1 | 0.88 | 14 | 0 | 0.00 | 0.00 | 0.00 |
| <i>Cd00851</i> | 1 | 1.00 | 16 | 0 | 0.00 | 0.00 | 0.00 |
| <i>Cd00852</i> | 2 | 1.00 | 16 | 100 | 0.00 | 0.23 | 0.22 |
| <i>Cd00853</i> | 2 | 1.00 | 16 | 100 | 0.00 | 0.51 | 0.49 |
| <i>Cd00854</i> | 3 | 1.00 | 16 | 100 | 0.00 | 0.69 | 0.66 |
| <i>Cd00855</i> | 2 | 1.31 | 21 | 100 | 0.50 | 0.39 | 0.44 |
| <i>Cd00856</i> | 1 | 1.00 | 16 | 0 | 0.00 | 0.00 | 0.00 |
| <i>Cd00860</i> | 2 | 1.00 | 16 | 100 | 0.00 | 0.44 | 0.43 |
| Total | 61 | ----- | 592 | ----- | ----- | ----- | ----- |
| Mean | 2.03 | 1.23 | 19.7 | 62.80 | 0.26 | 0.38 | 0.38 |

*: Average number of fragments scored per animal.

** : Polymorphism % equals number of polymorphic alleles divided by total alleles.

The latter was also the most abundant trimer in alpaca (21.8%), whereas the former was very rare (1.7%) (19).

Molecular markers have provided new opportunities to assess animal genetic variability at the DNA level. Microsatellite markers have been widely used, since they are polymorphic and randomly distributed in the genome. In this study, 30 microsatellite loci were characterized

using 16 Saudi camels that represented 4 morphologically diverse breeds. Twenty SSRs produced polymorphic information for the animals under study. They revealed 61 amplified DNA fragments (alleles) that ranged from 1 to 3 alleles with an average of 2.03. This range is comparable with that observed by Mehta et al. (11) in 3 Indian camel populations, where the range was 2–6 alleles using 16 SSR

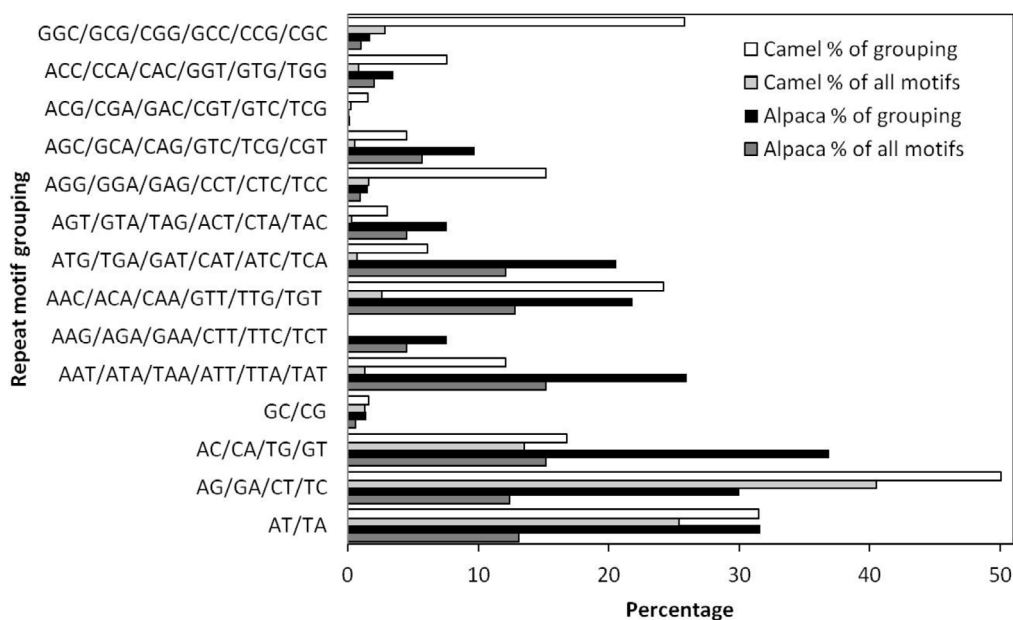


Figure 2. Abundance of SSR dimer and trimer motif groupings in camel (this study) and alpaca (24).

primers, and by Al-Swailem et al. (12) in 3 Saudi camel populations, where the range was 1–7 alleles. However, this number of alleles is considered low compared to earlier studies (14,15). Generally, the number of alleles is highly associated with sample size and the number of unique alleles in the population. As the sample size increases, the total number of expected alleles also increases. In a study on Saudi camels, Al-Swailem et al. (12) showed that 61 alleles were generated with an average of 3.81 alleles per locus, using 99 Saudi camels. Mburu et al. (9) found that a total of 115 alleles were observed at 14 loci in 332 camels from a study of 7 dromedary populations. Spencer and Woolnough (14) generated 185 alleles from 28 loci using 484 Australian camels belonging to 6 sampling locations.

PIC value is another important measure of polymorphism. The calculated PIC value in this study indicates relatively low polymorphism in the investigated population. The average PIC value was 0.38, which is close to the reported values of related studies using microsatellite markers in camel genetic diversity. The reported values were 0.48 (11), 0.51 (14), and 0.58 (15). Considerable polymorphism was detected among the investigated Saudi camels, which reflects their potential for future breeding purposes. In this study, H_o averaged 0.26, while H_e averaged 0.38. These values are considered low compared to reported data for Saudi camels, where H_e was 0.633, while H_o was 0.665, 0.605, and 0.662 for Majaheem, Maghateer, and Sofr breeds, respectively (15). Schulz et

al. (13) recorded a value of 0.633 for Arabian camels from different regions. Conversely, Mburu et al. (9) recorded a value of 0.51 for camels from the United Arab Emirates, which could indicate narrow genetic selection for many generations. The low heterozygosity values in our study could be attributed to the small population size, which was used for characterization purposes.

The developed camel SSRs had a high score of BLAT matches, reflecting good synteny between bovine and camel genomes. Such synteny is helpful in comparative analyses of genetic maps. In conclusion, the present study developed insights into camel genomic SSR abundance and polymorphism. Thirty SSR markers were experimentally characterized and can be potentially utilized in genetic diversity analyses for both dromedary and Bactrian camels. The developed camel SSRs are expected to expand the available molecular marker toolbox and be further utilized for genetic mapping, identification of important QTLs, and breeding.

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