Identification of Short Interspersed Elements (SINEs) in Iranian River Buffalo

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ABSTRACT

The aim of this research was to identify repeat sequences in Iranian river buffalo, which may be a basis for future genetic studies. Short Interspersed Element (SINE) is one of repeat sequences that mainly have used for evolutionary and phylogenetic studies. SINEs are non-viral retro transposable repetitive sequences with a length of 70–500bp that are widespread among eukaryotic genomes. While some SINEs are derived from 7SL RNA or 5S rRNA, most SINEs are derived from tRNA. Hence, the tRNA-like secondary structure as well as the conserved RNA polymerase III–specific internal promoter sequences (designated A and B boxes) allows new SINE elements to be distinguished from other repetitive elements in the genome. In this study 3 Short Interspersed Elements (SINEs) -like sequences referred to as BOVTA, CHR-I and CHR-II were identified. The isolation of SINEs began with RAPD-PCR enrichment. Several RAPD primers were used to amplify fragments in separate reactions. Many obtained intense bands were gel-extracted and ligated into PTZ57R/T vector and the plasmids transformed into DH5a cells. Plasmid DNA from each cloned fragment was purified and then DNA inserts were sequenced. Sequences masked with repeat masker program, and result showed the presence of three short interspersed like elements including BOVTA2, CHR-2_BT and CHR-2B. RNA polymerase III internal promoter and tRNA related sequence commonly found in short interspersed elements. BLAST search reveals significant homology with other previously described SINEs and tRNAs.

Keywords: *Iranian river buffalo, RAPD-PCR, Short interspersed element (SINE), BOVTA2, CHR-2_BT, CHR-2B*

INTRODUCTION

Short interspersed nucleotide elements (SINEs) are small nucleotide sequences with 10^4-10^5 copies per genome. Individual species may have more than one SINE family present in their genome. SINEs are defined by the presence of a region homologous to 7SL RNA and tRNA, together with the promoter sequences designated the A and B boxes (Okada, 1991), SINEs[1] represent nonautonomous transposable elements and exploit the enzymatic retrotransposition machinery of LINEs (Long INterspersed Element) (Kajikawa and Okada, 2002). Ray (2007) suggested that SINEs have applications in population structure, conservation genetics, the genetics of speciation, phylogeny reconstruction, inbreeding, estimates of ancestral population size, hetrozigosity and agreement with the expectation of Hardy-Weinberg equilibrium. Although several families of SINEs have been characterized in some members of the Bovidae, namely cattle and goat, none had been identified in buffalo (Jobse et al. 1995). In this research we identified three SINE like sequences in Iranian river buffalo (Bubalus bubalis).

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MATERIAL AND METHODS

SINEs like elements of Iranian river buffalo were identified trough cloning and sequencing of RAPD (Random Amplified Polymorphic DNA) primers used to isolation of microsatellite loci. Blood samples were taken from jugular vein of Iranian river buffalo and following DNA was extracted via salting out procedure (Miller et al., 1988). PCR was done in different reactions and programs depending of TM of primers and optimized conditions. Several RAPD primers were used to amplify fragments in separate reactions. Many obtained intense band were gel-extracted and ligated into PTZ57R/T vector and the plasmids transformed into DH5α cells. Plasmid DNA from each cloned fragment was purified and then DNA inserts were sequenced using ABI PRISM sequencer set. SINE sequence homology was made with a online software (Repeat masker). Sequence analysis and alignment were carried out using NCBI-BLASTN 2.2.14 version (Altschul et al., 1997) and CLUSTAL W 1.83 version for multiple sequence analysis (Gasteiger et al., 2003)

RESULT AND DISCUSSION

During screening of repetitive sequences from the genome of the Iranian river buffaloes, we identified three SINE like sequences that have significant homology with 3 reported SINEs (BovTA2, CHR-2B and CHR-2_BT) (Figure 1).

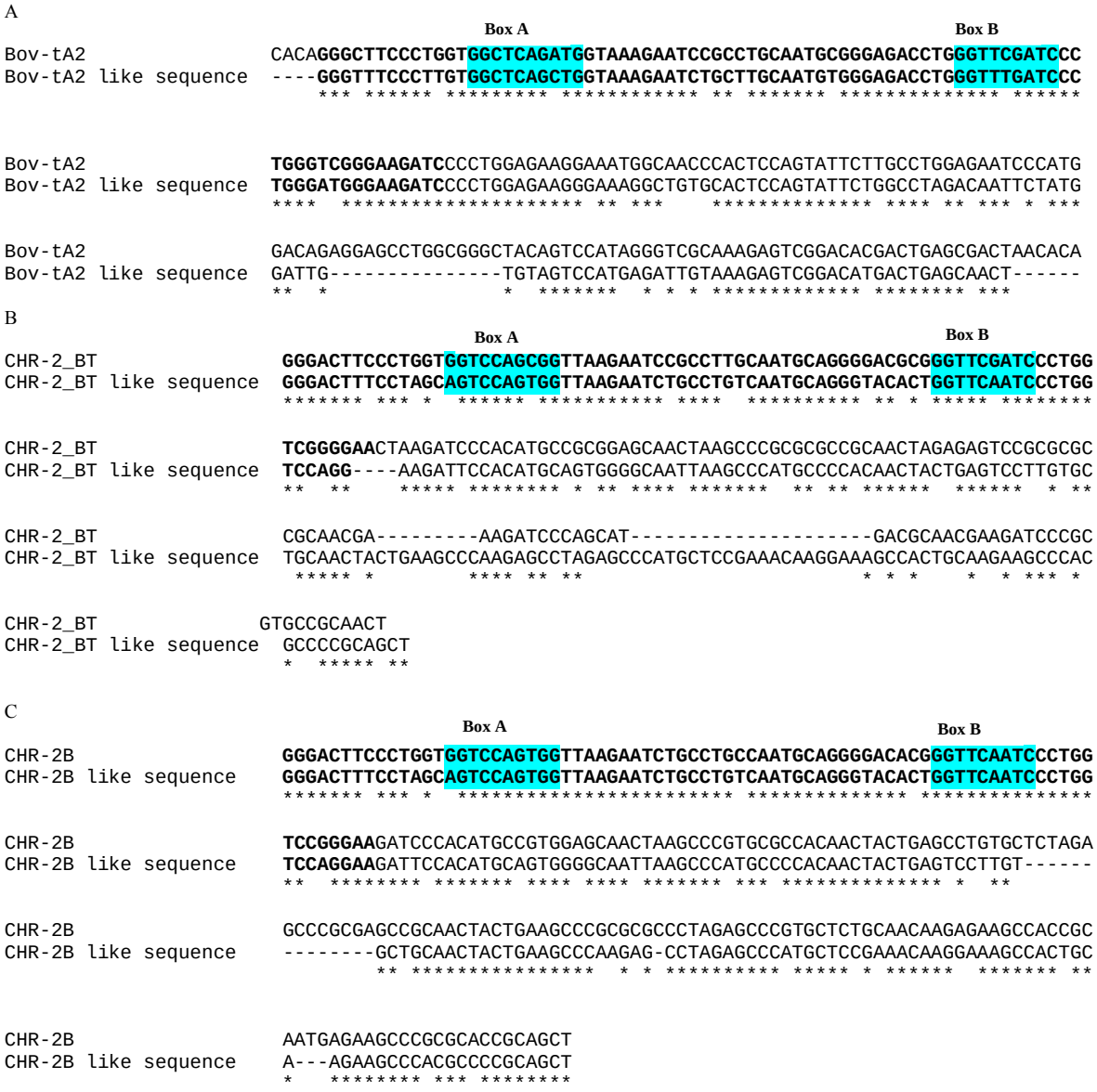

Fig 1: Alignment of the Bov-tA2, CHR-2_BT and CHR-2B like sequences of Iranian river buffalo with the Bov-tA2, CHR-2_BT and CHR-2B sequences. Shaded boxes shows RNA polymerase III 2nd promoter, bold letters show partial tRNA derived structure of SINEs, the identical nucleotide are shown by stars and deletions are shown by bars.

GenBank accession numbers of Bov-tA2, CHR-2_BT and CHR-2B like sequences are GQ463459, GQ463461 and GQ463462, respectively. Generally, identification of partial sequences of SINE elements in river buffalo can be useful for studies of genome evolution and other population genetics. Nucleotide deletion or insertion in these elements can create polymorphism in specific locus, shed the light on new features of genome arrangement, which needs wide genetic researches.

The results of this study allow the assignment of these elements to *bubalus bubalis* for the first time based on alignment of SINE-like element with reported SINE elements thus extending the river buffalo physical map.

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