***In-silico* amplification of randomly selected male - specific region – Y (MSY) genes as candidate genes for the selection of fertile indigenous bulls for artificial insemination**

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Abstract

Molecular markers-based selection is a novel tool for unbundling complexities associated with the selection of animals for breeding improvement programmes because these markers could contribute to full exploitation of animal potential prior to enrollment for improvement programmes. This study examined in-silico polymerase chain reaction amplification of some selected Male Specific region Y (MSY) genes in genome assemblies including Bos_taurus_UMD_3.1.1/bosTau8 and ARS-UCD 1.2/bosTau9; BaylorBtau_4.6.1/bosTau7, Bos_taurus_UMD_3.1/bosTau6, and Baylor4.0/bosTau4. The results of the amplification showed that 12 out of the selected 30 genes were expressed at an average primer melting temperature of 61.78 °C; while salt and annealing oligo concentrations were 50 mM and 50 nM per reaction, respectively. Out of expressed genes; 6, 5 and 1 genes were expressed in genome assemblies Bos_taurus_UMD_3.1.1/bosTau8, ARS-UCD1.2/bosTau9, and BaylorBtau_4.6.1/bosTau7, respectively. The study concluded that the expressed genes are potential markers for selection of fertile bulls and the primers used are suitable for in-vivo annotation of the genes in the prospective bull selection process for breeding programmes.

Keywords: Male-specific region-Y genes (MSY); molecular markers; bulls and *in-silico*

Introduction

The best reproductive technology with excellent potential for increasing herd level productivity in cattle is artificial insemination because semen from a single bull can sire multiple calves far and wide within and outside a given geographical location; but selection and recruitment of highly fertile bull is key to successful implementation of artificial insemination programmes especially in the tropical resources poor countries such as Nigeria. Challenges including inadequate record-keeping and poor implementation of deliberate improvement and breeding programme are limiting factors against

identification of highly fertile indigenous bulls in the tropics such as Nigeria. Meanwhile, identifying these bulls in highly desirable; therefore, the need for field-level molecular fertility markers is a worthy application of molecular biology for increasing livestock productivity as a way of fighting food insecurity in a country like Nigeria where population is currently growing at an alarming rate. Expression profiling of MSY genes are potential molecular markers of bull fertility but their mechanisms of action are relatively unexplored; hence, the need to investigate them becomes highly imperative for the purpose of establishing relationships

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between expression of these genes and fertility as well as tolerance of indigenous bulls to stress as a way of establishing these genes as field-level fertility markers in bulls selection and or recruitment for artificial insemination programmes for improved productivity (Hamilton *et al.*, 2012).

Bull fertility is a major cause of economic loss in cattle production because healthy bulls are at many times confused with fertile bulls; meanwhile, lots of bulls even when they are healthy, may either be infertile or sub-fertile (David *et al.*, 2014). Apart from fertility, in the tropical countries where increasing temperature is a growing source of stress affecting livestock productivity, mobilization of stress tolerant bulls for breeding programme is also a livestock production objective. Several factors including nutrition, genetics, environment, infectious diseases, and management may be the responsible cause of infertility or sub-fertility of bulls under different production systems; but what matters, is identification of infertile or sub-fertile bulls in order to prevent their use in breeding programmes. Annotation of genes which have been shown to regulate fertility in cattle holds significant promise for understanding the regulation of fertility in bulls. This is because, these genes when expressed in cattle reproductive tracts, will further be expressed in the semen and hence, will ensure successful conception. Therefore, the exploration of the genetics behind bull fertility is a powerful tool that can aid farmers' profits; in addition, identifying superior animals as the parents of the next generation cattle can contribute positively to yield of both milk and beef production enterprises (Noirin *et al.*, 2014). Therefore, this study carried out a computational amplification of randomly selected MSY genes for the purpose of providing useful insights that can speed up rates of their *in-*

vivo annotations and provision of complementary and comparative models for their expression analysis in bulls' recruitment for improvement and breeding programmes.

Methodology

Male-specific region-Y (MSY) genes were randomly selected and their oligo primers were designed using the nucleotides database of the National Centre for Biotechnology Information (NCBI) of the United State of America (USA). Then the primers as presented in Table 1 were used in computational polymerase chain reaction amplification. The cattle genome assemblies including ARS-UCD1.2/bosTau9, and Bos_taurus_UMD_3.1.1/bosTau8, BaylorBtau_4.6.1/bosTau7, Bos_taurus_UMD_3.1/bosTau6, and Baylor4.0/bosTau4 were used for the amplification of these genes (UCSC Genomics Institute).

Results

The results of the amplification showed that 12 out of the selected 30 genes were expressed at an average primer melting temperature of 61.78 °C; while salt and annealing oligo concentrations were 50 nM per reaction. Furthermore, out of the 12 genes expressed using the three genome assemblies in this study; there are 6 among the genes expressed in the genome assembly Bos_taurus_UMD_3.1.1/bosTau8; and there are 5 out of the genes that were expressed in the genome assembly Bos_taurus_UMD_3.1.1/bosTau9, while only 1 out of the genes was expressed in the genome assembly BaylorBtau_4.6.1/bosTau7; the details of the expressed genes and their amplification information are presented in Table 2.

Table 1: The oligo primer details of the genes amplified in the *in-silico* platform

S/N	Gene Name	Primers type	Primer Sequence (5' to 3')	Primers Length (bp)	Product length (bp)	Accession No
1	<i>Bos taurus</i> bovid-specific transcript Y-linked 1 (BTY1) mRNA	F	CCAGAAAGTCTGACGTTGGCT	20	201	JX899361.1
		R	CCTTTGGGCTCTGTAGCCAGG	20		
2	<i>Bos taurus</i> bovid-specific transcript Y-linked 10 (BTY10) mRNA	F	CTTCCACTCTGAACCTGCCT	20	243	JX899370.1
		R	TGTTTTTGCAGCATGACCTCAG	22		
3	<i>Bos taurus</i> heat shock transcription factor, Y-linked 2 (HSFY2), mRNA	F	TTTCCAGTCAGGCATAGCGA	20	240	NM_001077006.1
		R	TGTTGACATCTCCCAAAGTAACG	23		
4	<i>Bos taurus</i> testis-specific protein, Y-linked 1 (TSPY), mRNA	F	TGGGACGATCCCCTGAAGTA	20	206	NM_001244608.1
		R	GGATACGGTTCATGCGGAGAG	20		
5	<i>Bos taurus</i> TU29 transcribed RNA sequence, transcriptome shotgun assembly project	F	ATGGCCACCAGTCTTACTGC	20	375	GAQO01000001.1
		R	CAGTTGAATGTGCCTTGGGC	20		
6	<i>Bos taurus</i> TU35 transcribed RNA sequence, transcriptome shotgun assembly project	F	AAGTGTGAGCAGTCACCCAGG	20	464	GAQO01000002.1
		R	ACCATTCTGGCCTGGTCTG	20		
7	<i>Bos taurus</i> TU35 transcribed RNA sequence, transcriptome shotgun assembly project	F	AGCATCCAACTGGCATGAA	20	306	GAQO01000002.1
		R	GGTGACTGCTCACACTTGGA	20		

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8	<i>Bos taurus</i> PROP paired-like homeobox 1 (PROP1), mRNA	F	GCACGAGAAAGTCTCGCTCA	20	250	NM_174678.3
		R	TGGAGGGCTGAGATGGAAGA	20		
9	<i>Bos taurus</i> transmembrane protein 95 (TMEM95), mRNA	F	CACATCCACGTAGGCAGGAG	20	165	NM_001367291.1
		R	AGAAGACACAGGCCCTCAACG	20		
10	<i>Bos taurus</i> prostaglandin D2 synthase (PTGDS), mRNA	F	CGAGCTGTTCTCTGGAGAAG	20	214	NM_174791.4
		R	GTCTCTGCCACTGACACCTC	20		
11	<i>Bos taurus</i> estrogen receptor 2 (ESR2), mRNA	F	AACCTGCTGATGCTCCTGTC	20	150	XM_010809436.3
		R	CAAAGACTTGTGCCCCGAA	20		
12	<i>Bos taurus</i> chaperonin containing TCP1 subunit 8 (CCT8), mRNA	F	TCAGTACTGCATGGCATGGTT	21	248	XM_027541905.1
		R	GCCACTCTGCCACCTGTTAC	20		
13	<i>Bos taurus</i> testis expressed 261 (TEX261), mRNA	F	GCCCTGTTCTTCCCCCTAAAGC	21	200	NM_001035300.1
		R	ACCTCTGGCCCATATAAAGGA	21		

Table 2: The genes and their *in-silico* polymerase chain reaction amplification details

	Gene Name	Primer melting temperatures °C	Salt concentration	Oligo concentration	Genome/Assembly
1	<i>Bos taurus</i> bovid-specific transcript Y-linked 1 (BTY1) mRNA	60.4	50 mM	50 nM	Bos_taurus_UMD_3.1.1/bosTau8
2	<i>Bos taurus</i> bovid-specific transcript Y-linked 10 (BTY10) mRNA	59.4	50 mM	50 nM	BaylorBtau_4.6.1/bosTau7
3	<i>Bos taurus</i> heat shock transcription factor, Y-linked 2 (HSFY2), mRNA	61.8	50 mM	50 nM	Bos_taurus_UMD_3.1.1/bosTau8
4	<i>Bos taurus</i> testis specific protein, Y-linked 1 (TSPY), mRNA	62.7	50 mM	50 nM	Bos_taurus_UMD_3.1.1/bosTau8
5	<i>Bos taurus</i> TU29 transcribed RNA sequence	60.1	50 mM	50 nM	Bos_taurus_UMD_3.1.1/bosTau8
6	<i>Bos taurus</i> TU35 transcribed RNA sequence	58.9	50 mM	50 nM	Bos_taurus_UMD_3.1.1/bosTau8
7	<i>Bos taurus</i> PROP paired-like homeobox 1 (PROP1), mRNA	61.8	50 mM	50 nM	Bos_taurus_UMD_3.1.1/bosTau8
8	<i>Bos taurus</i> transmembrane protein 95 (TMEM95), mRNA	61.7	50 mM	50 nM	ARS-UCD1.2/bosTau9
9	<i>Bos taurus</i> prostaglandin D2 synthase (PTGDS), mRNA	61.7	50 mM	50 nM	ARS-UCD1.2/bosTau9
10	<i>Bos taurus</i> estrogen receptor 2 (ESR2), mRNA	60.4	50 mM	50 nM	ARS-UCD1.2/bosTau9
11	<i>Bos taurus</i> chaperonin containing TCP1 subunit 8 (CCT8), mRNA	61.5	50 mM	50 nM	ARS-UCD1.2/bosTau9
12	<i>Bos taurus</i> testis 261 (TEX261)	63	50 mM	50 nM	ARS-UCD1.2/bosTau9

Discussion

In the herd level productivity and improvement programmes, identification and recruitment of good animals into breeding is ultimate for a successful breeding programme because the discovery

and selection of bulls with required genes influencing traits of economic interest for the livestock industry is germane to profit making, it is a complex process and it is a continuous commercial application (Fortes *et al.*, 2013). Identification of these genes

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responsible for desirable traits unlike in traditional selection can improve accuracy of introducing desirable genes into the animal's next generation hence increasing productivity. This selection and breeding approach is a paradigm shift in animal production science with potential unmatched accuracy because it accounts for both phenotypic and genotypic traits in selection of an animal into breeding programme for improving productivity compared with traditional selection which is primarily based on phenotypic traits and their correlation with production performances. In addition, selection based on candidate genes using molecular evaluations will allow the application of modern reproductive technologies such as artificial insemination for improvement of indigenous low productive breeds (Meuwissen *et al.*, 2016). Since some of these breeds are adapted to local environment, molecular assessment of their genetic makeup can contribute to identification novel genes such as tolerance to stress as complementation to productivity improvement. The male - specific region of the mammalian Y chromosome (MSY) contains clusters of these genes which are described to be essential for superior male reproductive capacities; this region have highest gene density in the genome of bovine, and the genes are predominantly expressed as well as differentially regulated during testicular development, furthermore, it is a region of genomic niche regulating male reproduction successes (Chang *et al.*, 2013). Apart from their predominance during the testicular development, the genes in the MSY region are highly expressed in the testis of fertile mammals which supports their potentials as molecular markers of fertility because it can be infer that an animal with excellent expression profile of these genes could be

fertile since testis is a primary organ associated with reproductive excellence especially semen functional qualities (Paria *et al.*, 2011). Again some of the genes within the region in bovine since they are associated with testicular development, there are indications that they are important for determining reproductive performance capabilities of bulls. Corresponding positive diversity of these genes in bull can serve as ultimate signal for fertility because meiotic recombination that is an essential biological process which generates genetic diversity were reported to be more for bulls compared with cow in a large genome-wide association study involving up to a million cattle; and this diversity primarily occurs near the subtelomeric regions of all bovine autosomes which is an indication that most genetic richness of cattle is closely associated with bulls; identifying such bulls using MSY genes hold potential for herd-level productive improvement strategy while considering application of artificial insemination (Ma *et al.*, 2015).

Considering the growing population and increasing demand for cattle products in a country such as Nigeria, application of reproductive technologies such as artificial insemination is highly desirable. However, in order to eliminate bull infertility factor from breeding programmes; molecular identification using MSY region genes can be exploited to speed up selection process in breeding programmes. The successful amplification of some of the randomly selected MSY region genes in this study indicated these genes are potential explorable candidate genes for the recruitment of bulls for breeding programmes.

Conclusion

The study concluded that the expressed genes are potential markers for selection of fertile bulls and the primers used are

suitable for *in-vivo* annotation of the genes in the prospective bull selection process for breeding programmes.

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