*Original Research*

# Screening of Sahiwal Bulls for Fertility-Related PROP1 Gene Isoforms

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**Abstract**

*The Prophet of Pit1 (PROP1) gene isoforms are reported to be associated with production, growth and fertility trait in cattle. The present study was carried out to screen the Sahiwal bulls for the PROP1 gene isoforms resulting from H173R mutations in third exon of the gene. The exon 3 was characterized by sequencing the amplicons obtained after PCR amplification using custom designed primers. Phylogram of exon 3 of PROP1 identified that Sahiwal is closely related to Bos taurus and Karan Fries in evolutionary tree whereas it is distantly placed from rodents. The multiple alignment of the target region sequence with Bos taurus reference sequence revealed that the bulls under the study were free of mutations causing the PROP1-173R isoforms. No other variations were observed in the third exon thus giving the targeted region a highly conserved one.*

**Key words:** Bull Fertility, H173R, PROP1 Gene, Sahiwal

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## Introduction

The Prophet of Pit1 (PROP1) gene, a member of homeobox gene family and is located on chromosome 7 at location 41205895 to 41209913 bases, conferring a total length of 4019 bp. PROP1 comprises of 4 exons and 3 introns and encodes a paired class homeodomain transcription factor with a polypeptide length of 226 amino acids representing the prophet of Pit-1 transcription factor (also known as POU1F1 transcription factor) as it lies upstream of POU1F1 in pituitary development (Davis *et al*., 2010; Sornson *et al*., 1996). Thus, POU1F1 gene is a direct downstream target for the regulation of the PROP1 gene. Previous studies have found that PROP1 mutations affects the expression level of the POU1F1 gene, and thus the GH, PRL, and TSH-β expression levels (Carvalho *et al*., 2006; Davis *et al*., 2010). There are reports of associations of the POU1F1 pathway genes like POU1F1, GH, PRL, GHR, PRLR, STAT5A, OPN and UTMP with early embryonic survival, fertilization rate (Khatib *et al*., 2009) and male fertility (Khatib *et al*., 2010).





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PROP1 gene takes part in the Wnt/b-catenin Signaling pathway (Olson *et al*., 2006). It includes nuclear recruitment of b-catenin and activation of Wnt-dependent transcription factors leading to development and differentiation of the diverse reproductive tissues and affects implantation, decidualisation and placental differentiation (Sonderegger *et al*., 2010). WNT signaling and b-catenin in the testis has been associated with proliferation and selfrenewal of spermatogonia (Golestaneh *et al*., 2009) and also in male infertility (Boyer *et al*., 2008). In addition, the bovine PROP1 gene is located at a QTL on chromosome 7 which affects ovulation rate in cattle (Kappes *et al*., 2000; Kirkpatrick *et al*., 2000).

Recent studies found a missense single nucleotide polymorphism (rs136195618 A>G) that replaces a histidine amino acid with an arginine (H173R) in exon 3 of bovine PROP1 gene. The bovine isoform PROP1-173H, representing the "A" allele strongly activates transcription of the reporter gene and the PROP1-173R isoform was reported to be associated with a 5-fold reduction in binding capacity as well as a reduced activation capacity, when compared to the bPROP1-173H carboxyl domain (Showalter *et al*., 2002). The PROP1-173R isoform was found to be associated with a decrease in sire conception rate and an increase in productive life and protein yield in Holstein bulls (Lan *et al*., 2013). The PROP1-173R isoforms were found to have positive effect on growth traits in cattle (Pan *et al*., 2013).

Till date, it remains unknown whether there exist PROP1 gene isoforms in indigenous cattle breeds of India. Therefore, the present study was carried out with the objective to characterise the exon 3 of PROP1 gene and to screen H173R (rs136195618 A>G) polymorphism so as to identify the PROP1 gene isoform related to bull fertility.

## Materials and Methods

### *Sample Collection and DNA Isolation*

Frozen semen straws of sixty-two Sahiwal bulls were collected from Artificial Breeding Research Centre (ABRC) of National Dairy Research Institute, Karnal and were utilized for isolation of genomic DNA. The DNA isolation was performed using a protocol that involves two steps: Lysis and extraction. Lysis of spermatozoa was done as per Hossain *et al*. (1997), while the extraction of DNA was done by standard Phenol Chloroform extraction procedure (Sambrook and Russell, 2001). The concentrations of DNA were measured using Biospec-nano-spectrophotometer (Schimadzu Cooperation, Japan).

### *Primer Designing*

In silico primer designing for exon3 of bovine PROP1 gene was carried out using Primer3 software (http://www.primer3.ut.ee) (Untergrasser *et al*., 2012) using Bos taurus sequence (GenBank Ref Seq: AC_000164.1) as reference.





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***PCR Amplification***

PCR amplification was carried out in a total volume of 25 ml with 100 ng DNA template, 1x PCR buffer, 1.5 mM MgCl2, 200 mM of each dNTPs, 20 pmol of each primer and 1 unit of Taq DNA polymerase. The PCR programme was initial denaturation at 95°C for 5 min. The cycling protocol was denaturation at 95°C for 30 sec, then annealing temperature of 59°C for 30 sec then extension at 72°C for 30 sec followed by final extension at 72°C for 5 min. The number of cycles was 35. Quality and sizes of PCR products were checked on 2% agarose gel electrophoresis (Fig. 1).

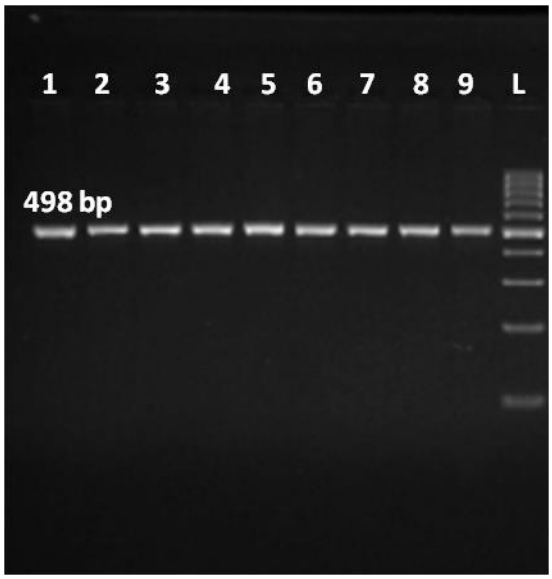


**Fig. 1:** Resolution of PCR product of exon3; L=100bp ladder

***Sequencing and SNP Screening***

PCR products were stored at -20ºC and sent to first BASE Sequencing INT, Singapore, for direct sequencing from both 5' and 3' ends. The raw sequences obtained after sequencing were edited using BioEdit software. Sequencing results were screened for (rs136195618 A>G) polymorphism and other reported/novel SNPs by ClustalW alignment analysis with Bos taurus reference sequence: AC_000164.1 (NCBI), and confirmed by visual inspection of chromatograms.

***BLAST Analysis***

Analysis of the sequence results was done to find homology with other species using BLASTN (http://blast.ncbi.nlm.nih.gov/). Phylogram was also constructed using online tool phylogeny.fr [(http://www.phylogeny.fr/) (Dereeper *et al*., 2008)].

**Results and Discussion**

The third exon of PROP1 gene was characterised in Sahiwal by DNA sequencing and the nucleotide sequence was submitted to GenBank and accession number 'KT198685.1' was obtained. BLAST analysis





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of the Sahiwal exon 3 nucleotide sequence showed 100% sequence identity with Karan Fries and Bos taurus. Phylogram of exon 3 of PROP1 (Fig. 2) shows that the Sahiwal is closely related to Bos taurus and Karan Fries in evolutionary tree whereas it is distantly placed from rodents.

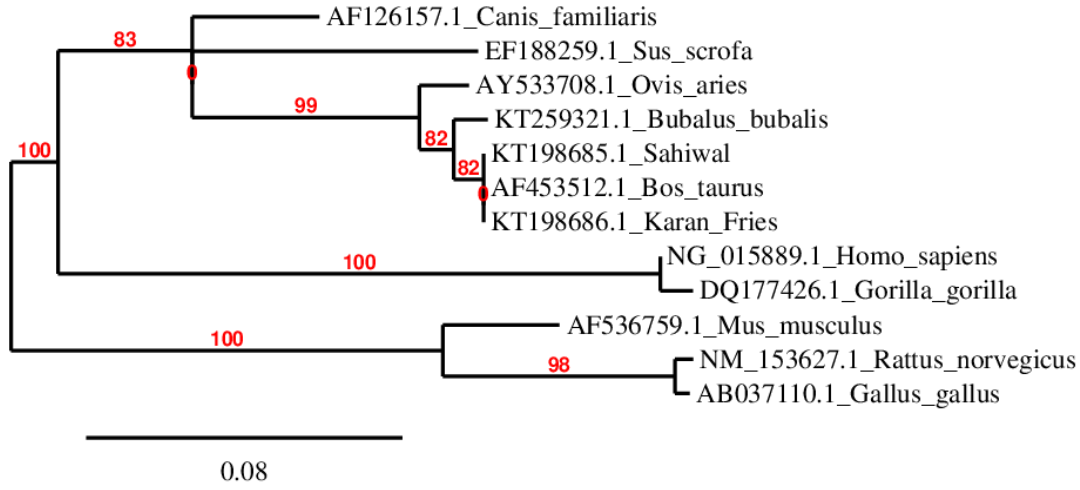


**Fig. 2:** Phylogram of Exon 3 of PROP1gene

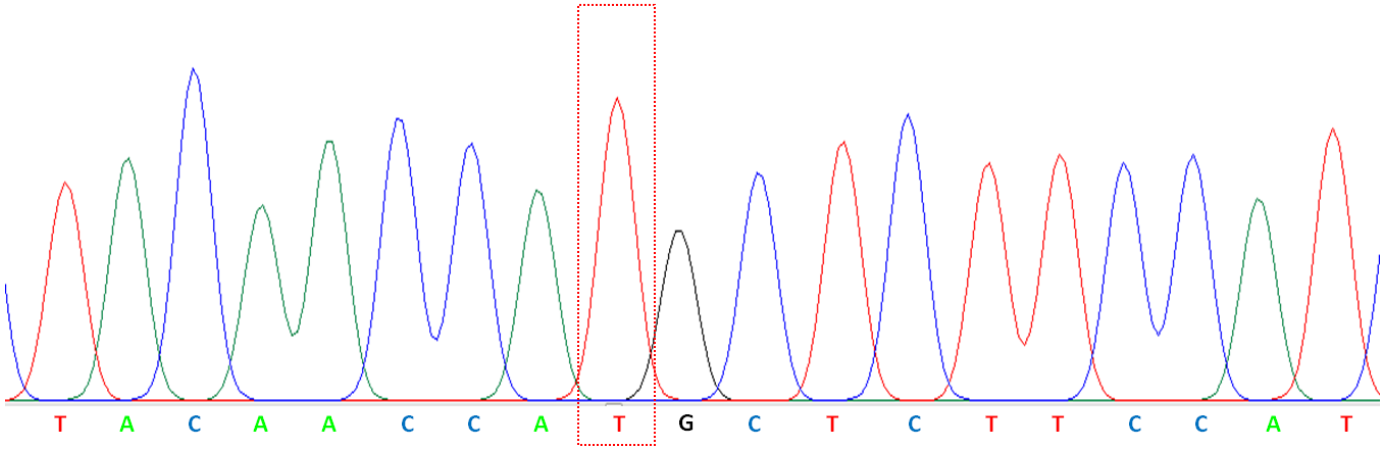


**Fig. 3:** Chromatogram showing A allele at rs136195618 locus (PROP1-173H isoform) in Sahiwal bulls

ClustalW multiple alignment analysis with Bos taurus (NCBI Ref Seq: AC_000164.1) did not found any nucleotide variation in the targeted region. Screening of SNP locus (rs136195618) showed that the reference allele A was present in all the animals included in the present study (Fig. 3). Thus, the studied bulls possessed the bovine PROP1-173H isoform. The SNP (rs136195618 A>G) was observed in different cattle breeds like German Holstein Friesian, US Holstein Friesian, German Flekvieh and indigenous cattle breeds of China. (Lan *et al*., 2013; Pan *et al*., 2013; Pausch *et al*., 2015). Despite the fact that Karan Fries is having Holstein Friesian blood the H173R polymorphism was not detected in the Karan Fries bulls (Vineeth *et al*.,





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2017). No reports are available about the H173R polymorphism status in other indigenous cattle breeds of India.

## Conclusion

The third exon of bovine PROP1 gene has been characterized for the first time in Sahiwal bulls for which NCBI GenBank accession number KT198685.1 was obtained. The screening for fertility related PROP1 isoforms revealed that the indigenous bull population under the present study was having PROP1-173H isoform, the one which is favorable for bull fertility. The results obtained in the present study emphasize the importance of prior screening of candidate functional SNPs in population before using them in selection programmes.

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