Section A: Project Details - Consolidated Annual Progress Report

FINAL REPORT OF DBT-TWINNING SCHEME
(01.04.2011 - 16.03.2015)

A1. GENETIC AND BIODIVERSITY STUDIES ON MITHUN (*Bos frontalis*): C2-40


Department of Animal Genetics and Breeding

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Jharnapani, Medziphema, Nagaland – 797 106, INDIA
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&

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   Dept of National facility for transgenic and Knockout Mice
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   E-mail: satishk@ccmb.res.in

A6. Total Cost of the Project: Rs. 52.93 lakhs (NRCM, Nagaland - Rs 30.48 lakhs and CCMB, Hyderabad - Rs. 22.45 lakhs)

A8. **Approved Objectives of Project**

- Characterisation of physical traits of four strains (Nagaland, Arunachali, Manipur, Mizoram) of Mithun
- To understand breeding and management practices of Mithun rearing in Nagaland.
- To understand domestication of Mithun and its relationship with cattle and other bovines.

A9. **Specific Recommendations made by the Task Force (if any)**

The project was reviewed by an Expert Committee in the mid-term monitoring and mentoring meeting of the on-going Twinning (Aqua and Animal Biotechnology) projects held on 12 April, 2013 and recommended as below -

- **Progress report- Good**
  - Sequencing gel with accessories not allowed. Facility for the same at CCMB may be used.
  - Capillary based methodology to be employed for diversity studies.
  - 1 contractual labour permitted for 3rd year.
**Section B: Scientific and Technical Progress**

**GENETIC AND BIODIVERSITY STUDIES ON MITHUN (Bos frontalis)**

Annual PROGRESS REPORT OF DBT-TWINING SCHEME

(01.04.2011 - 16.03.2015)

**B1. Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period** (1000-1500 words for interim reports; 2500-3500 words for final report; data must be included in the form of up to 3 figures and/or tables for interim reports; up to 7 figures and/or tables for final reports):

**Introduction**

Mithun (Bos frontalis), a bovine species, is indigenous to the eastern Himalayas. Several myths of domestication and origin were elaborated by Simoons et al. (1968) in their anthropological study of the mithun keeping tribes. Simoons and Simoons (1968) describes the unique use of mithun such as, for sacrificing in special occasions to enhance the owners’ prestige, matrimonial alliances, land and other essential goods. However, there are no indications of keeping for milk, meat, pack or draught animal and planned breeding. At higher elevations mithun territories are shared by Yak (Poephagus grunniens), while at lower altitudes domestic cattle and mithun co-habit. Hence, mithuns play an important role in the lives of the tribal population of the North Eastern Hilly region of this country.

The current consensus plan is to conserve this unique animal along with genetic improvement of the mithun population with regards to selection for meat as well as milk traits. Hence, there is a urgent need to characterize the strains of mithun to know their genetic constitution and diversity available in the populations. There are a large number of works reported in cattle and other livestock species. However, no report is available in mithun so far. This urgency of research leads to the formulation of the present project proposal.

Mithuns are reared under age-old traditional system in the forests without much human interventions except providing salt to these animals occasionally. These animals are bred naturally in the forests with the herd bulls and having no records keeping practices. We plan to gather quality information on three aspects of mithun production, and to undertake population genetics studies using genomic tools. Firstly, information on their breeding and management practices; Secondly, information on their phenotypic characteristics and differential body characters, if any between strains/breeds of mithun; and thirdly, information of bulls’ usage in the mithun herds. Finally, modern genomic tools will be used to study
genetic variation in mithun population and to address questions pertaining to domestication of this species.

**NRC-M (ICAR), Nagaland (NER Centre)**

**Work Plan for entire period (0-48 months)**

<table>
<thead>
<tr>
<th>Period of study</th>
<th>Achievable targets</th>
</tr>
</thead>
</table>
| **6 Months**    | • Sample survey and gathering of scientific information  
                    • Recording of phenotypic data  
                    • Random selection of Mithuns  
                    • Collection of samples from animals  
                    • Procurement of essential chemicals, equipments etc. |
| **12 Months**   | • Sample survey and gathering of scientific information to continue  
                    • Recording of phenotypic data to continue  
                    • Collection of samples and DNA preparation  
                    • Validation of microsatellite markers on a panel of unrelated animals. Selection of markers.  
                    • Testing of cattle microsatellite markers and finalization of a panel of markers. |
| **18 Months**   | • Collection of samples and DNA preparation  
                    • PCR amplification of Cytochrome B and D-loop region of mithun  
                    • Difficulty in amplification of D-loop region of mithuns  
                    • Sequencing of mitochondrial DNA based on Cytochrome B gene (cytb) and D-loop region |
| **24 Months**   | • Collection of samples and DNA preparation  
                    • Microsatellite analysis of mithun for breeding and management to continue  
                    • Sequencing of mitochondrial DNA to continue |
| **30-36 Months**| • Development of microsatellite library - mithun specific microsatellites  
                    • Genotyping with mithun specific microsatellites  
                    • Analysis of data  
                    • Statistical analysis and inferences. |
| **37-48 Months**| • The project was given an extension by DBT  
                    • Genotyping with mithun specific microsatellites  
                    • Genotyping/sequencing of more samples based on Cyt b gene  
                    • Analysis of various sequence data  
                    • Report writing |
Work Done during the entire period - NER Institute (NRCM, Nagaland)

- Recruitment of the Junior Research Fellow (JRF) again on 04.12.2012 after first recruitment on 20.05.2011.
- The phenotypic characters of all the Mithuns belonging to four strains (Nagaland, Arunachal Pradesh, Manipur, Mizoram) present in the Institute herd were recorded and analyzed statistically.
- Procurement of consumables from time to time as per requirements.
- Trips to Mithun habitats in the interior of Nagaland, Manipur, Mizoram and Arunachal Pradesh were made a number of times for collection of information of Mithun population, habitat, bull usage patterns and mithun blood samples.
- Isolation of genomic DNA from the collected samples.
- Most of these field trips were very challenging. However, trips to Manipur having the natural habitat of Mithuns for collection of samples and information on Mithun breeding and animal husbandry practices were more daunting due to prevailing social tension in the State.
- Collection of 14 samples of wild gaur (*Bos gaurus*) from Mysore zoo in three trips.
- 30 Micro-satellite markers which were reported in cattle (FAO, 1996) were tried in mithun and out of 30 such markers, 19 were found successful in mithun and wild gaur.
- Working DNA of all unrelated mithun was sent for genotyping (outsourcing).
- Blood samples collected from 10 Dam-Calf pair of Nagaland, Arunachal and Mizoram Strain and DNA isolated from these samples.
- The genomic DNA samples (178 - mithun, 14 gaurs and 8 - thro tho cattle) were PCR amplified using the 19 sets of micro satellite markers.
- The forward markers were fluorescently labeled, whereas the reverse primers were unlabeled.
- Four different fluorescent dyes were used (FAM, NED, PET & VIC).
- PCR products were multiplexed after all individual products were checked on agarose gel.
- The PCR product-multiplex mix was analyzed on an ABI Genetic Analyzer 3500xL, against Liz 500R size standard.
- POPGENE (Version 1.32) was used for Population Genetic Analysis.
- During the course of the present study, a library of 50 new micro/mini-satellites were generated. Out of these 50, 10 mithun specific microsatellites were identified and validated which are under the process of filing a patent.
- A total of 81 samples PCR amplified for Cyt b (1140bp) and further sent for sequencing.
- 9 samples PCR amplified for D loop F1/R1 (1kb) and further sent for sequencing. The forward and reverse primers are as follows- F1-5' CTG CAG TCT CAC CAT CAA CC -3' and R1-5' CTA GAG GGC ATT CTC ACT GGG-3'.
- 19 samples PCR amplified for D loop F5/R5 (750bp) and further sent for sequencing. The forward and reverse primers are as follows- F5-5' CAG GGA TCC CTC TCG CTG CT-3' and R5-5' GTC GTG AGC TAC AGG GTC TG-3'.
• A total of 4 D loop primers were tried in mithun of which only 2 primers worked and amplified successfully and sent further for sequencing.

• The D loop primers which did not work are as follows- 1) F4-5’ CTG CAG TCT CAC CAT CAA CC-3’ and R4-5’ GAT TAT AGA ACA GGC TCC TCC TC-3’ (around 500bp) and 2) F-5’ AAA TGT AAA ACG ACG GCC AGT AAT CCC AAC TCA ACA C-3’ and R-5’ AAC AGG AAA CAG CTA TGA CCA CTC ATC TAG GCA TTC TC-3’ (323bp).

• The D-loop region was found to have repeat sequences and the slippages were observed which was, even-if normal, disrupted the sequencing efforts.

• In situations whereby repeat sequences disrupt sequencing efforts, there are 2 options: first is to keep trying to sequence using internal primers and the other option is to amplify the region and go for fragment analysis. The fragment size will indicate the number of repeats present.

• Due to these sequencing difficulties, complete D-loop region of mithun could not be sequenced, and accordingly, only Cytochrome B gene was used as mitochondrial genotyping work.
Detailed Achievements against the Targets/Objectives

Objective - 1

- *Characterization of physical traits of four strains (Nagaland, Arunachal, Manipur and Mizoram) of mithun*

Achievements - Phenotypic characterization of Mithuns

Various phenotypic characters of Mithuns belonging to different strains were recorded in the proforma developed for this purpose.

The study consisted of a random sample of total 134 Mithuns belonging to four different strains (Nagaland, Arunachal, Manipur and Mizoram) under three age groups (young stock I, YS-I: up to 1 year of age, young stock II, YS-II: 1-3 year of age and adult stock, Adult: 3 year and above) to study various morphological traits and 149 Mithuns for study of physical coat colour patterns stationed at research farms situated at Medziphema and Porba, Nagaland. These included height at wither (HW), body length (BL), heart girth (HG), face length (FL), tail length (TL), neck circumference (NC), neck length (NL), ear length (EL), horn length (HL), horn circumference (HC), and point of shoulder to point of pin bone (PS). The visual observations of physical coat colour pattern of mithun consisted of colour of the coat, muzzle, hoof, tail switch, fore limbs and hind limbs, respectively. The recorded data tabulated and analyzed statistically using SAS package.

The physical coat colour was also analyzed. Nagaland and Mizoram mithuns are predominantly jet black. One of the most distinguishing features of mithun is white stocking of fore legs and hind legs irrespective of their sex. The present study will be helpful to generate mithun specific species descriptor to aid in genetic improvement and conservation programme of this unique animal.
Table 1a: Least squares means with standard error (± SE) of various morphometric characters of mithuns

<table>
<thead>
<tr>
<th>Factor</th>
<th>BW (kg)</th>
<th>SW (kg)</th>
<th>HW (cm)</th>
<th>BL (cm)</th>
<th>HG (cm)</th>
<th>FL (cm)</th>
<th>TL (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Overall (µ)</td>
<td>134</td>
<td>21.58 ± 0.24</td>
<td>305.67 ± 5.89</td>
<td>114.21 ± 1.18</td>
<td>157.91 ± 1.91</td>
<td>154.48 ± 1.75</td>
<td>35.79 ± 0.46</td>
</tr>
<tr>
<td>2 Strain</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Nagaland</td>
<td>56</td>
<td>21.66 ± 0.33</td>
<td>261.30 ± 10.91</td>
<td>105.48 ± 2.19</td>
<td>142.19 ± 3.54</td>
<td>138.45 ± 3.83</td>
<td>32.44 ± 0.86</td>
</tr>
<tr>
<td>Arunachal</td>
<td>29</td>
<td>21.77 ± 0.43</td>
<td>268.90 ± 12.89</td>
<td>110.17 ± 2.12</td>
<td>150.20 ± 3.44</td>
<td>146.25 ± 3.15</td>
<td>32.70 ± 0.83</td>
</tr>
<tr>
<td>Manipur</td>
<td>21</td>
<td>21.74 ± 0.52</td>
<td>248.29 ± 10.60</td>
<td>103.67 ± 2.58</td>
<td>140.01 ± 4.19</td>
<td>135.51 ± 3.24</td>
<td>31.65 ± 1.01</td>
</tr>
<tr>
<td>Mizoram</td>
<td>28</td>
<td>21.61 ± 0.44</td>
<td>238.01 ± 8.27</td>
<td>103.20 ± 1.66</td>
<td>134.10 ± 2.69</td>
<td>132.17 ± 2.46</td>
<td>30.62 ± 0.65</td>
</tr>
<tr>
<td>3 Sex</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Male</td>
<td>62</td>
<td>21.83 ± 0.31</td>
<td>273.73 ± 7.63</td>
<td>107.37 ± 1.53</td>
<td>143.54 ± 2.48</td>
<td>140.66 ± 2.27</td>
<td>32.85 ± 0.60</td>
</tr>
<tr>
<td>Female</td>
<td>72</td>
<td>21.56 ± 0.32</td>
<td>234.52 ± 7.82</td>
<td>103.89 ± 1.57</td>
<td>139.70 ± 2.54</td>
<td>135.53 ± 2.33</td>
<td>30.86 ± 0.61</td>
</tr>
<tr>
<td>4 Age group</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>YS-I</td>
<td>21</td>
<td>-</td>
<td>99.88 ± 12.51</td>
<td>90.56 ± 2.51</td>
<td>109.78 ± 4.06</td>
<td>109.90 ± 3.72</td>
<td>24.49 ± 0.98</td>
</tr>
<tr>
<td>YS-II</td>
<td>33</td>
<td>-</td>
<td>284.71 ± 9.87</td>
<td>98.64 ± 1.98</td>
<td>130.67 ± 3.21</td>
<td>122.81 ± 2.94</td>
<td>28.99 ± 0.77</td>
</tr>
<tr>
<td>Adult</td>
<td>80</td>
<td>-</td>
<td>377.79 ± 6.72</td>
<td>127.69 ± 1.34</td>
<td>184.41 ± 2.18</td>
<td>181.58 ± 2.00</td>
<td>42.08 ± 0.53</td>
</tr>
</tbody>
</table>

**P < 0.01 NS = Non-significant
Table 1b: Least squares means with standard error (± SE) of various morphometric characters of mithuns

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>NC (cm)</th>
<th>NL (cm)</th>
<th>EL (cm)</th>
<th>PS (cm)</th>
<th>HL (cm)</th>
<th>HC (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Overall (µ)</td>
<td>134</td>
<td>81.78 ± 1.78</td>
<td>37.69 ± 0.72</td>
<td>19.53 ± 0.70</td>
<td>70.90 ± 0.89</td>
<td>18.66 ± 0.54</td>
<td>23.84 ± 0.55</td>
</tr>
<tr>
<td>2 Strain</td>
<td></td>
<td>**</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>Nagaland</td>
<td>56</td>
<td>73.92 ± 3.31</td>
<td>33.87 ± 1.34</td>
<td>18.78 ± 0.98</td>
<td>84.32 ± 1.66</td>
<td>14.15 ± 0.76</td>
<td>19.12 ± 0.78</td>
</tr>
<tr>
<td>Arunachal</td>
<td>29</td>
<td>80.10 ± 3.21</td>
<td>36.76 ± 1.30</td>
<td>18.78 ± 1.26</td>
<td>84.03 ± 1.61</td>
<td>13.50 ± 0.97</td>
<td>19.01 ± 1.00</td>
</tr>
<tr>
<td>Manipur</td>
<td>21</td>
<td>72.56 ± 3.91</td>
<td>34.58 ± 1.58</td>
<td>16.73 ± 1.53</td>
<td>81.54 ± 1.96</td>
<td>11.84 ± 1.19</td>
<td>18.61 ± 1.21</td>
</tr>
<tr>
<td>Mizoram</td>
<td>28</td>
<td>68.43 ± 2.51</td>
<td>31.77 ± 1.01</td>
<td>17.15 ± 1.30</td>
<td>78.76 ± 1.26</td>
<td>13.01 ± 1.00</td>
<td>19.40 ± 1.03</td>
</tr>
<tr>
<td>3 Sex</td>
<td></td>
<td>**</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Male</td>
<td>62</td>
<td>84.13 ± 2.31</td>
<td>33.28 ± 0.93</td>
<td>18.36 ± 0.90</td>
<td>83.46 ± 1.16</td>
<td>16.16 ± 0.70</td>
<td>23.06 ± 0.72</td>
</tr>
<tr>
<td>Female</td>
<td>72</td>
<td>63.38 ± 2.37</td>
<td>35.21 ± 0.96</td>
<td>17.35 ± 0.93</td>
<td>80.86 ± 1.19</td>
<td>10.09 ± 0.72</td>
<td>15.02 ± 0.74</td>
</tr>
<tr>
<td>4 Age group</td>
<td></td>
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<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>YS-I</td>
<td>21</td>
<td>55.91 ± 3.79</td>
<td>27.70 ± 1.53</td>
<td>15.07 ± 1.49</td>
<td>52.01 ± 1.02</td>
<td>3.22 ± 1.15</td>
<td>9.47 ± 1.18</td>
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<tr>
<td>YS-II</td>
<td>33</td>
<td>68.20 ± 2.99</td>
<td>31.39 ± 1.21</td>
<td>17.22 ± 1.17</td>
<td>81.31 ± 1.90</td>
<td>9.93 ± 0.91</td>
<td>16.60 ± 0.93</td>
</tr>
<tr>
<td>Adult</td>
<td>80</td>
<td>97.15 ± 2.04</td>
<td>43.65 ± 0.82</td>
<td>21.29 ± 0.80</td>
<td>113.17 ± 1.50</td>
<td>26.23 ± 0.62</td>
<td>31.04 ± 0.63</td>
</tr>
</tbody>
</table>

**P < 0.01 NS = Non-significant
Objective - 2

- To understand breeding and management practices of Mithun rearing in Nagaland.

Achievements - Microsatellite characterization of Mithuns

Isolation of genomic DNA of Mithun

A total of 185 random blood samples from genetically unrelated mithuns from their native breeding tracts in four Indian states comprising Arunachal Pradesh (40), Nagaland (41), Manipur (16) and Mizoram (66) and 10 blood samples from wild Indian gaur (Bos gaurus) from Mysore zoo, Karnataka State, India were collected for diversity analysis. 12 samples of a local cattle breed, Tho-tho, were also collected for use as positive control. Genomic DNA from each sample was isolated from white blood cells by standard proteinase K digestion followed by phenol–chloroform extraction method (Sambrook et al., 1989) and stored at -20°C.

Blood samples were collected from Mithun (Bos frontalis) in heparinized vacuutainer tubes and genomic DNA was isolated from this whole blood using Promega Wizard DNA isolation kit as per manufacturer protocol. Quality and quantity was checked for each DNA samples and nanodrop rating (260/280) between 1.7-1.8 for each DNA was considered to be of good quality for further work.

PCR amplification standardization

30 Microsatellite primers for characterization of cattle as per FAO list were procured and these primers were tested one by one for possible amplification in Mithuns. Out of these 30 bovine microsatellite primers, 19 were found to be positive in Mithuns.
PCR amplification agarose gel images for 19 markers

![Agarose gel image](image_url)

Figure: PCR amplified products of 19 sets of primers with one genomic DNA were loaded on 2% agarose gel (Expected PCR product size: 100-200 bp)

1, 21, 30, 2, 3, 10, 13, 14, 16, 17, 18, 22, 23, 24, 25, 26, 27, 28, 29: 19 different markers
L: 100 bp DNA ladder

Analysis of microsatellite data

- Nineteen (63%) out of 30 cattle microsatellite markers revealed successful amplification patterns in 120 mithuns and 10 gaur samples (Table 1), while the remaining 11 cattle specific microsatellite markers failed to yield any amplification. Based on prior information on PCR products' sizes, 19 microsatellite loci were grouped into four sets, containing five (ETH225, HAUT27, BM1818, LSTS030, ETH185), four (HEL1, ETH3, BM1824, ILSTS034), five (MM12, ETH10, HEL13, ILSTS033, ETH152) and five loci (CSSM66, BM2113, ILSTS006, ILSTS011), respectively (Table 2). Fifteen of the positive clones which were sequenced and analyzed for presence of mithun-specific microsatellite repeats were submitted in Genbank (Accession KF564956-KF564970, Table 2). Genotype data collected from the 19 amplified microsatellites were used for genetic diversity studies of Indian mithun and wild gaur populations.
Fifteen (79%) of the 19 microsatellite loci were highly polymorphic with higher PIC value (> 0.50) in mithun and gaur, respectively. A total of 274 and 89 alleles were detected in mithun and gaur with allele numbers ranging from eight to 25 in mithuns and two to seven in gaur, respectively (Table 2). Hence, each of the panel of 15 microsatellites - Panel 1 (ETH225, BM1818, ILSTS030, ETH185, HEL1, ETH3, ILSTS034, MM12, HEL13, ILSTS033, ETH152, CSSM66, BM2113, ILSTS006, and ILSTS011), and Panel 2 (HAUT27, BM1818, ILSTS030, ETH185, HEL1, ETH3, BM1824, ILSTS034, MM12, HEL13, ETH152, BM2113, ILSTS006, ILSTS011 and
ILSTS054) from FAO standard panel for cattle diversity studies appear to be most suitable for diversity studies in Indian mithun and gaur, respectively due to their high informativeness.

- BM2113 loci revealed an allele of 121 bp in mithun and gaur populations only, but not in Thotho cattle. The locus ILSTS005 could not be amplified in both Indian mithuns and wild gaur.
- Observed heterozygosity \( (H_o) \) ranged from 0.15 to 0.94 in mithun and 0.01 to 0.99 in gaur, while Nei's unbiased expected heterozygosity values \( (H_e) \) ranged from 0.31 to 0.89 in mithun and 0.36 to 0.83 in gaur, respectively. PIC value ranged from 0.29 to 0.88 in mithun and 0.32 to 0.82 in gaur, respectively. The average estimates of \( H_o \), \( H_e \) and PIC across all the 19 microsatellite loci were 0.48, 0.66 and 0.63 in mithun, and 0.62, 0.71 and 0.61 in gaur, respectively.

**Hardy-Weinberg test**

- All 19 microsatellite loci showed significant deviation from Hardy-Weinberg equilibrium \( (P < 0.05) \) in Indian mithuns and gaur. Estimates of within population inbreeding coefficient \( (F_{IS}) \) as per Wright (1978) were 30.00% and 6.50% in mithun and gaur population, respectively which was high and positive \( (P < 0.05) \).

**Bottleneck effect**

- A normal L-shaped distribution of allelic frequencies was found in the bottleneck analysis in Indian mithun population by the qualitative graphical test (Fig. 4). There was no mode-shift found in the distribution of allelic frequencies showing the absence of any genetic bottleneck in the Indian mithun population, similar to Yunnan mithun (Qu et al., 2012). However, gaur population was found to have shifted mode indicating presence of bottleneck in recent history as the distribution of allelic frequency didn’t form exactly L-shaped distribution (Figure).
### Table: Characterization of 30 cattle microsatellite markers tested in Indian mithuns and wild gaur

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Marker(s)</th>
<th>Chromosome No. in cattle</th>
<th>Primer sequences (5’ - 3’)</th>
<th>Annealing Temperature (°C)</th>
<th>Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ETH225</td>
<td>9</td>
<td>GATCACCTTGCCACTTTTCCT, ACATGACAGGCAGCTGCTACT</td>
<td>58</td>
<td>FAM</td>
</tr>
<tr>
<td>2</td>
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DISCUSSION

- This is the first research investigation on genetic diversity analysis in Indian mithuns and wild gaurds employing cattle microsatellite markers following secondary guidelines of FAO.

- **Genetic diversity in Indian mithun and wild gaur**

  - The 15 microsatellite markers identified from FAO panel for cattle diversity studies appeared to be suitable for diversity studies in Indian mithun and gaur.
  
  - The 121 bp allele at BM2113 locus was in mithun and gaur populations only and not in Tho tho cattle suggested it possibly be the mithun and gaur specific allele.
  
  - Non-amplification of ILSTS005 locus in Indian mithuns and wild gaur indicated absence of this sequence in both these species which was in agreement with the observations of Ritz et al. (2000) and Nguyen et al. (2008), who also reported that this cattle microsatellite marker failed to amplify both in Mithan (mithun) and wild gaur.
  
  - The average observed heterozygosity values in mithun ($H_o = 0.48$) and gaur ($H_o = 0.62$) were significantly ($P<0.05$) lower than the average expected heterozygosity (Nei's unbiased mean heterozygosity) in both mithuns ($H_e = 0.66$) and gaur ($H_e = 0.66$), respectively. This indicated presence of overall low heterozygosity and low to medium genetic diversity in Indian mithun and gaur populations, which was similar to Yunan mithuns (Qu et al., 2012) with the corresponding estimates of observed heterozygosity, expected heterozygosity and PIC as 0.53, 0.63 and 0.60, respectively. The low heterozygosity might be due to non-random mating structure in mithuns with few bigger bulls getting more chances to mate with females. However, Pandey et al. (2008) reported higher observed heterozygosity (0.72) in Kherigarh cattle, which might be due to availability of larger population in cattle than mithuns.

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**Figure. Phylogenetic tree showing genetic relationship between mithun, gaur and tho tho cattle**
**Hardy-Weinberg test**

- Significant deviation from Hardy-Weinberg equilibrium (P < 0.05) in Indian mithuns and gaur population at all the 19 loci might be due to heterozygote deficiency under small population size.

- High degree of within population inbreeding estimate in Indian mithun population was possibly due to the fact that the mating system was non-random, probably the bigger bulls getting more chances for mating within the herd and less chance of gene flow into the herd through introduction of external germplasm, resulting in deviation of the population from Hardy-Weinberg equilibrium. However, lower level of inbreeding observed in wild gaur population was probably due to the zoo policy of introduction of gaur bulls from outside into the zoo herd from time to time. The study also further validates the fact that the normal practice of tribal mithun owners of a particular clan shun any exchange of their mithuns with other clans or tribal society and possibly assortative mating practised within the mithun groups giving rise to highly inbred mithun population or heterozygotic deficiency.

**Bottleneck effect**

- The normal L-shaped distribution of allelic frequencies in the bottleneck analysis without any mode-shift by the qualitative graphical test showed absence of genetic bottleneck in Indian mithun population. This was similar to the reports in Yunan mithuns (Qu et al., 2012), Kherigarh cattle (Pandey et al., 2006), Banni buffalo (Mishra et al., 2009) and Nagpuri buffalo (Kataria et al., 2009). Bottleneck analysis indicated that in spite of lower heterozygosity and small population size, there is not such evidence of genetic bottleneck in the Indian mithun population so far, which is an encouraging fact. However, genetic bottleneck was observed in wild gaur population, which was expected and may be attributed to small population size of wild gaur under zoo condition. This type of genetic bottleneck was also reported in other wild species viz. European bison (Luense et al., 2005) and giant panda (Zhang et al., 2002). However, this genetic bottleneck may not be indicative of any low reproductive capacity of wild gaur as no such evidence is present. This is the first study of genetic bottleneck in Indian mithun and wild gaur population.

- The study demonstrated that the cattle microsatellite markers can be used effectively on mithun and wild gaur for further genetic analysis of Indian mithuns and wild gaur populations and provided valuable information regarding the present genetic status of these bovine species. The present level of heterozygosity in Indian mithuns and gaur population with significant within population inbreeding level necessitates the development and introduction of a rational breeding policy particularly for mithuns in their native tracts for future genetic improvement of these unique bovines and their conservation which are under threat of extinction.
**Generation of a microsatellite library of Mithuns**

- Genomic DNA is digested with a 4 base cutter and purified.
- Around 20bp adapters were ligated to the purified digested DNA fragments.
- The DNA is then denatured to produce single stranded fragments, which is hybridized to biotinylated microsatellite probe molecules.
- Streptavidin coated Magnetic beads are added to the above mixture for enrichment. Since the microsatellite probes are attached to the beads, and any DNA fragments containing microsatellites are hybridized to these probes, the microsatellite DNA is also pulled out of solution.
- This 5 minute procedure separates the DNA of interest from the rest of the DNA which does not contain the selected microsatellites.
- The selected DNA is then amplified using primers designed to the linkers, cloned into a vector, and sequenced.
- Sequencing of these clones enables primers to be designed for each microsatellite locus.
- A total of 50 micro/minisatellites were generated for mithun in this procedure and presented below.

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</table>
• Out of these 50 micro/mini satellites, 10 mithun specific microsatellites were identified and validated. These are presented in the following table -

Table. Details of Validated Microsatellite markers used for genotyping -

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Marker No.</th>
<th>Clone No.</th>
<th>Amplicon Size</th>
<th>Repeat Seq.</th>
<th>No. of Repeats</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mithun</td>
<td>Tho tho</td>
<td>Gaur</td>
<td>Buffalo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Marker 1</td>
<td>1-29</td>
<td>216bp</td>
<td>CA</td>
<td>19</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Marker17</td>
<td>2-67</td>
<td>155bp</td>
<td>ACAC</td>
<td>21</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Marker29</td>
<td>4-28</td>
<td>124bp</td>
<td>CA</td>
<td>10</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>Marker13</td>
<td>2-25</td>
<td>68bp</td>
<td>GTCT</td>
<td>5</td>
<td>+ve</td>
</tr>
<tr>
<td>5</td>
<td>Marker63</td>
<td>V-17</td>
<td>97bp</td>
<td>GTT</td>
<td>4</td>
<td>+ve</td>
</tr>
<tr>
<td>6</td>
<td>Marker35</td>
<td>2-29</td>
<td>105bp</td>
<td>TTCTA</td>
<td>3</td>
<td>+ve</td>
</tr>
<tr>
<td>7</td>
<td>Marker20</td>
<td>2-65</td>
<td>74bp</td>
<td>TAGCAG</td>
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<td>+ve</td>
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<tr>
<td>8</td>
<td>Marker70</td>
<td>V-9</td>
<td>107bp</td>
<td>TGC</td>
<td>3</td>
<td>+ve</td>
</tr>
</tbody>
</table>

-ve indicates absence,
+ve indicates presence.
Objective - 3

- To understand domestication of mithun and its relationship with cattle and other bovines.

Achievements - Mitochondrial genotyping of Mithuns and phylogenetic study

- Blood samples were collected from the native breeding tract of mithuns falling under four North Eastern States (Nagaland, Manipur, Mizoram and Arunachal Pradesh).
- These animals were taken up randomly, hence unrelated animals were belonging to each of the four strains in a way to maximize the phenotypic/ geographical representation.
- Blood samples were also collected from few individuals of wild Bos gaurus from Mysore zoo of India and the local Tho tho cattle.
- These animals were genotyped for cytochrome b and D-loop regions of mitochondria (through DNA sequencing) to understand their maternal inheritance and origin of species.
- For the purpose of mitochondrial genotyping, we have targeted Cytochrome B (cytb) gene and D-loop region of mithuns.
- The complete cytb gene (1246 bp) of mithun was PCR amplified, cloned, sequenced and submitted in Genbank (Acc. No. )
- A total of 81 samples PCR amplified for Cyt b (1140 bp) and got them sequenced.
- The raw sequence data were aligned to construct consensus sequences (DNASTAR software) which were used to construct phylogenetic tree using Muscle online program.
- For D-loop PCR amplification, a number of primers were tried.
- A total of 4 D loop primers were tried in mithun of which only 2 primers worked and amplified successfully and sent further for sequencing.
- The D loop primers which did not work are as follows- 1) F4-5’ CTG CAG TCT CAC CAT CAA CC-3’ and R4-5’ GAT TAT AGA ACA GGC TCC TC-3’ (around 500bp) and 2) F-5’ AAA TGT AAA ACG ACG ACG GCC AGT AAT CCC AAT
AAC TCA ACA C-3' and R-5' AAC AGG AAA CAG CTA GCA TCA CTC ATC TAG GCA TTC TC-3' (323bp).

- 9 samples PCR amplified for D loop F1/R1 (1kb) and further sent for sequencing. The forward and reverse primers are as follows- F1-5' CTG CAG TCT CAC CAT CAA CC -3' and R1-5' CTA GAG GGC ATT CTC ACT GGG-3'.
- 19 samples PCR amplified for D loop F5/R5 (750bp) and further sent for sequencing. The forward and reverse primers are as follows- F5-5' CAG GGA TCC CTC TTC TCG CT-3' and R5-5' GTC GTG AGC TAC AGG GTC TG-3'.
- However, even after sequencing was done, consensus sequences could not be constructed due to internal repeat problems within the D-loop sequences.
- Consequently, cytb gene sequences were used for the study of maternal genetic analysis and origin of mithun in relation with other bovine species.
- The D-loop region was found to have repeat sequences and the slippages were observed which was, even-if normal, disrupted the sequencing efforts.
- Due to these sequencing difficulties, complete D-loop region of mithun could not be sequenced, and accordingly, only Cytochrome B gene was used for mitochondrial genotyping work.

Table: Details of primers used for Cytochrome B (cytb) Amplification

<table>
<thead>
<tr>
<th>Primer code</th>
<th>Primer sequence (5’ -------- 3’ )</th>
<th>Amplicon size approx (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>CGAAGCTTGATATGAAAAACCATCGTTG</td>
<td>1246 bp</td>
<td>50.0</td>
</tr>
<tr>
<td>R1</td>
<td>GGAATTCATCTCTCCGGTTTACAAGAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>CGAAGCTTGATATGAAAAACCATCGTTG</td>
<td>1140 bp</td>
<td>54.0</td>
</tr>
<tr>
<td>R2</td>
<td>GGAATTCATCTCTCCGGTTTACAAGAC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table:** Details of primers used for D-loop Amplification

<table>
<thead>
<tr>
<th>Primer code</th>
<th>Primer sequence (5’ ------ 3’)</th>
<th>Amplicon size expected (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>AAATGTTAAACGCAGGCAGCTCAATCCCAATACTCAACAC</td>
<td>323 bp</td>
<td>No amplification observed</td>
</tr>
<tr>
<td>R1</td>
<td>AACAGGAAACAGCTATGCCACTCATCTAGGGCTTCTC</td>
<td>323 bp</td>
<td>No amplification observed</td>
</tr>
<tr>
<td>R2</td>
<td>CTGCGAGTCTCACCATCAACC</td>
<td>1kb</td>
<td>58.0</td>
</tr>
<tr>
<td>R3</td>
<td>GTGATTAGAAGGCGCTTCTC</td>
<td>500 bp</td>
<td>57.4</td>
</tr>
<tr>
<td>R4</td>
<td>GGCGAGTCAGCTCACGGGTTG</td>
<td>750 bp</td>
<td>Multiple bands observed</td>
</tr>
</tbody>
</table>

**Figure:** Evolutionary relationships of mithun in relation with other bovines
• Cytochrome b gene of 81 mithuns, 8 Tho tho cattle and 10 gaus (*Bos gaurus*) was sequenced. The Cytb sequences of other related species like *Bos taurus, Bos indicus, Bubalus bubalis, Bos javonicus, Bos gruniens* (Yak) etc were downloaded from Genbank database and phylogenetic tree was constructed.

• The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in Muscle.

**Discussion**

• From the patterns of phylogenetic tree, it was observed that mithun showed the smallest distance estimate with gaur but was greater than the other bovine species.

• The topology of mt DNA phylogenetic trees consistently depicted a gaur-mithun cluster.

• The proximity between mithun and gaur was supported by bootstrap values.

• Mithuns residing in Manipur, Arunachal, Mizoram and Nagaland are more closely related to gaur, but are distantly related to cattle and yaks.

• Mithuns from the various regions are more closely related with one another and are coming under the same clade.

• Mithun is different from *Bos taurus* and *Bos indicus* but must have interbred at some point of time with its ancestor *Bos gaurus* during the historic times.

• However, it was separated from gaur into a distinct clade to be genetically distinct as found in present times.

• Hence, it is concluded that mithun was originated from wild gaur rather than cattle.
CONCLUSION

- The study demonstrated that the cattle microsatellite markers can be used effectively on mithun and wild gaur for further genetic analysis of Indian mithuns and wild gaur populations and provided valuable information regarding the present genetic status of these bovine species.

- The present level of heterozygosity in Indian mithuns and gaur population with significant within population inbreeding level necessitates the development and introduction of a rational breeding policy particularly for mithuns in their native tracts for future genetic improvement of these unique bovines and their conservation which are under threat of extinction.

- A set of new microsatellite markers were also generated under this study which were mithun specific and were found to be robust novel markers with high polymorphism and were suitable for multiplexing and easy for further application in any other laboratories. These microsatellite markers could be valuable for future population and genetic mapping studies in mithuns.

- It was also the first study which demonstrated through mitochondrial inheritance that Indian mithun was more closely related with gaurs and having descended from gaurs rather than cattle.

- The project was completed with the generation of a number of valuable information which has already been utilized for developing the suitable breeding strategy for Mithuns in their native habitat.
## Section C: Details of Grant Utilization

C1. Equipment acquired or placed order with actual cost (entire period)

<table>
<thead>
<tr>
<th>SI No.</th>
<th>Name of equipment procured</th>
<th>Cost</th>
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<tbody>
<tr>
<td>1</td>
<td>Nanodrop spectrophotometer (Eppendorf, Germany)</td>
<td>479380</td>
</tr>
<tr>
<td>2</td>
<td>Heating block (Genaxy, USA)</td>
<td>60337</td>
</tr>
<tr>
<td>3</td>
<td>Vortex mixer (Labnet, USA)</td>
<td>21849</td>
</tr>
<tr>
<td>4</td>
<td>Micropipettes (Capp, Denmark)</td>
<td>35707</td>
</tr>
<tr>
<td>5</td>
<td>Microwave oven (Samsung)</td>
<td>42500</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>639773</strong></td>
</tr>
</tbody>
</table>

Details of Expenditure – Please see the consolidated table below
C2. Details of Expenditure – Please see the table below

### Consolidated statement of Expenditure

DBT project entitled “Genetic and Biodiversity Studies on Mithun” (*Bos frontalis*)

<table>
<thead>
<tr>
<th>Name of Head</th>
<th>Receipt (Rs)</th>
<th>Expenditure (Rs)</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Year</td>
<td>2nd Year</td>
<td>3rd Year</td>
</tr>
<tr>
<td><strong>Non Recurring</strong></td>
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<tr>
<td>Equipment</td>
<td>640000</td>
<td>0</td>
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<tr>
<td><strong>Recurring</strong></td>
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<td>Manpower</td>
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<tr>
<td>Consumables</td>
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<td>400000</td>
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<td>Travel</td>
<td>100000</td>
<td>99000</td>
<td>0</td>
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<tr>
<td>Contingency</td>
<td>30000</td>
<td>30000</td>
<td>0</td>
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<tr>
<td>Overhead Charges</td>
<td>100000</td>
<td>75000</td>
<td>0</td>
</tr>
<tr>
<td><strong>Non Recurring + Recurring</strong></td>
<td>1617000</td>
<td>765000</td>
<td>0</td>
</tr>
</tbody>
</table>

*Refund amount sent back to DBT vide Demand Draft No 277189 dated 23.03.2016*
**Work done by Collaborating Centre (CCMB, Hyderabad)**

**Scientific Progress (upto-March 2013)**

I. **Sequencing of the Cytochrome b gene of the mitochondrial genome.** To amplify the entire Cytochrome b gene we designed a set of primer pair with respect to the cattle mitochondrial genome - forward 5’–CGAAGCTTGTATGAAAACCATCGTTG-3’ and reverse 5’-GGAATTCATCTCTCCGGTTTACAAGAC-3’. PCR reaction was performed using 1x reaction buffer, 1.5 mM MgCl₂, 5 pmol forward and reverse primer with following reaction condition 95 ºC for 5 min. 30 cycles of 94ºC for 45 sec, 57ºC for 45 sec, 72ºC for 1 min and final extension at 72ºC for 10 minute. Amplified product was treated with ExoSAP-IT, as per manufacturer’s instructions. Sequencing of the PCR product was performed by (BioServe). Finally, we obtained 838 bp amplicons of the 10 individuals (Annexure I).

![Fig. 1. Representative Cytochrome b sequence from *Bos frontalis*](image)

To investigate the relationship of the *Bos frontalis* with other closely related *Bos* species we performed neighbor – joining analysis of 10 sequenced Cytochrome b gene of the *Bos frontalis* with known sequences of closely related *Bos* species. Values over the node is percentage of the re samplings of 1000 bootstrap values. Analysis revealed *Bos frontalis* does have not close relationship with any other known *Bos* species (Fig. 2).
II. Sequencing of the mitochondrial D-loop of the mitochondrial genome. To amplify the entire displacement region (D-loop) of the mitochondrial genome, four set of overlapping primers were designed [Fig. 3, Table1]. *Bos indicus* (16339 bp) whole mitochondrial genome sequence (JN817305.1) was used as the reference sequence for primer designing (Fig. 3). PCR reaction was performed using 1X reaction buffer, 1.5 mM MgCl$_2$, 5 pmol forward and reverse primers, with following reaction condition 95 °C for 5 min; 35 cycles of 94 °C for 45 sec, 72 °C for 1 min and final extension at 72 °C for 10 minute.

Fig.2. Neighbor joining analysis of Cytochrome b gene from *Bos* genus

![Schematic representation showing the alignment of primers used for D-loop amplification](image)

**Fig.3.** Schematic representation showing the alignment of primers used for D-Loop amplification
Table 1: Details of primers used for D-loop Amplification

<table>
<thead>
<tr>
<th>Primer code</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplicon size expected (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD-F1</td>
<td>ACCCCCAAGCTGAAGTTCT</td>
<td>542 bp</td>
<td>57.4</td>
</tr>
<tr>
<td>MD-R1</td>
<td>AGATGAGATGGCCCTGAAGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD-F2</td>
<td>TAATTACCATGCGCGGTGA</td>
<td>516 bp</td>
<td>50.0</td>
</tr>
<tr>
<td>MD-R2</td>
<td>TTGACTTTTTGGAGTGCTAAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD-F3</td>
<td>CTAATCAGCCCATGCTCACA</td>
<td>670 bp</td>
<td>57.4</td>
</tr>
<tr>
<td>MD-R3</td>
<td>GTGGCTGGCAGAGATTTAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD-F4</td>
<td>TCCCAACTCCATAAACACATAGG</td>
<td>603 bp</td>
<td>57.4</td>
</tr>
<tr>
<td>MD-R4</td>
<td>GGTGAGGTTTATCGGGGTTTA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Amplicons were obtained from 2 Mithun samples using the F1-R1, F3-R3 and F4-R4 primer sets (Fig. 2).

![PCR amplified products from mitochondrial D-Loop of Bos frontalis](image)

**Fig.4.** PCR amplified products from mitochondrial D-Loop of *Bos frontalis*
ANNEXURE I

Cytochrome b SEQUENCE FROM various Bos frontalis sequence

>Cyto1
CCAGCTCCATCAACATCTCCTCTAGATGAATTTCTGGCTCTCCTCTGGGAGTAGTGTATTCATTACCTACA
AATCTCTACAGGCTCTATTCTCATGAGTAGACTACAGACTACACATCCGACATACAACAAGCGAGGCTTCAATG
TTTTTTATATGCTTATATGTCAGCTAGACGCCATATTACGCTTACACCTCTCTAGAAAC
ATGAAACATTGGAGTAAATCCCTCTACTACAGTAGTCATAGAGGACTTAGCTAATGCTATACCAT
GAGGGCAATGTCTATTTTGAGGAGCAACATGTATACACATACCAACCTCCTATCAGCAATCCCTTACATCGGC
ACAAATTTATGGCAATACTCGAGGTTGAGATCCATGAGTAAAGCGACCTTTACCAACGGAGAC
CTCCCTCGGAGAC

>Cyto2
CCAGCTCCATCAACATCTCCTCTAGATGAATTTCTGGCTCTCCTCTGGGAGTAGTGTATTCATTACCTACA
AATCTCTACAGGCTCTATTCTCATGAGTAGACTACAGACTACACATCCGACATACAACAAGCGAGGCTTCAATG
TTTTTTATATGCTTATATGTCAGCTAGACGCCATATTACGCTTACACCTCTCTAGAAAC
ATGAAACATTGGAGTAAATCCCTCTACTACAGTAGTCATAGAGGACTTAGCTAATGCTATACCAT
GAGGGCAATGTCTATTTTGAGGAGCAACATGTATACACATACCAACCTCCTATCAGCAATCCCTTACATCGGC
ACAAATTTATGGCAATACTCGAGGTTGAGATCCATGAGTAAAGCGACCTTTACCAACGGAGAC
CTCCCTCGGAGAC

>Cyto4
CCAGCTCCATCAACATCTCCTCTAGATGAATTTCTGGCTCTCCTCTGGGAGTAGTGTATTCATTACCTACA
AATCTCTACAGGCTCTATTCTCATGAGTAGACTACAGACTACACATCCGACATACAACAAGCGAGGCTTCAATG
TTTTTTATATGCTTATATGTCAGCTAGACGCCATATTACGCTTACACCTCTCTAGAAAC
ATGAAACATTGGAGTAAATCCCTCTACTACAGTAGTCATAGAGGACTTAGCTAATGCTATACCAT
GAGGGCAATGTCTATTTTGAGGAGCAACATGTATACACATACCAACCTCCTATCAGCAATCCCTTACATCGGC
ACAAATTTATGGCAATACTCGAGGTTGAGATCCATGAGTAAAGCGACCTTTACCAACGGAGAC
CTCCCTCGGAGAC

>cyto5
CCAGCTCCATCAACATCTCCTCTAGATGAATTTCTGGCTCTCCTCTGGGAGTAGTGTATTCATTACCTACA
AATCTCTACAGGCTCTATTCTCATGAGTAGACTACAGACTACACATCCGACATACAACAAGCGAGGCTTCAATG
TTTTTTATATGCTTATATGTCAGCTAGACGCCATATTACGCTTACACCTCTCTAGAAAC

CTCCCTCGGAGAC
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>cyto6
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>CYTO7
CCAGCTCCCATCAAACATCTCTCCTCATGATGAAATTTCGGCTCCCTCCTCTGGGAGTATGCTTAATCCTACAATATCTCCACAGGCTATTTCTCTAGCAATACACTACACATCCGATACAACAAGCATTCTCCTCCGTTACCCGAGCTTCTACATCAAGGCTCCAACAATCCAACAGGAATCTCCTCAGACGCAGACAAAATTCCATTCCACCCCTACTACACCATTAAAGACATCATTTAGGAACCTTGGTACTATCTTCTAGCCCTTTATATACTACTAGTGCTATTTCGACCCGACCTCCCCTCGGAGAC

>cyto8
CCAGCTCCCATCAAACATCTCTCCTCATGATGAAATTTCGGCTCCCTCCTCTGGGAGTATGCTTAATCCTACAATATCTCCACAGGCTATTTCTCTAGCAATACACTACACATCCGATACAACAAGCATTCTCCTCCGTTACCCGAGCTTCTACATCAAGGCTCCAACAATCCAACAGGAATCTCCTCAGACGCAGACAAAATTCCATTCCACCCCTACTACACCATTAAAGACATCATTTAGGAACCTTGGTACTATCTTCTAGCCCTTTATATACTACTAGTGCTATTTCGACCCGACCTCCCCTCGGAGAC
References


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Annexure-V

Publications

**DBT Twinning Programme**

"Genetic and Biodiversity Studies on Mithun..."

Research papers