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BIS-CATTLE: A Web Server for Breed Identification using Microsatellite DNA Markers

Sarika Jaiswal, Sandeep Kumar Dhanda, M.A. Iquebal, Vasu Arora, Tejas M. Shah, U.B. Angadi, Chaitanya G. Joshi, Gajendra P.S. Raghava, Anil Rai and Dinesh Kumar

Centre for Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, Library Avenue, PUSA, New Delhi 110012, India

Bioinformatics Centre, CSIR-Institute of Microbial Technology, Chandigarh 160036, India

Department of Animal Biotechnology, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand 388 001, Gujarat, India

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Abstract: Domestic cow, *Bos taurus* is one of the important species selected by humans for various traits, viz. milk yield, meat quality, draft ability, resistance to disease and pests and social and religious reasons. Cattle domestication initiated sometime in the Neolithic (8,000-10,000 years ago) with subsequent spread of cattle throughout the world is intertwined with human migrations and trade (Willham, 1986). At present, more than 1.5 billion cattle are reported which is liable to expand to 2.6 billion by 2050, as per FAO (2013). This high magnitude of numbers and breed

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Introduction

The domestic cow (*Bos taurus*) is economically and culturally important species of the globe facilitating nutrition to the entire human population. 800 different cattle breeds in the world have been selected by humans for various traits, viz. milk yield, meat quality, draft ability, resistance to disease and pests and social and religious reasons. Cattle domestication initiated sometime in the Neolithic (8,000-10,000 years ago) with subsequent spread of cattle throughout the world is intertwined with human migrations and trade (Willham, 1986). At present, more than 1.5 billion cattle are reported which is liable to expand to 2.6 billion by 2050, as per FAO (2013). This high magnitude of numbers and breed...
management needs accurate identification tool to identify the breeds at molecular level.

Every breed is a unique combination of genes evolved in response to a geo-climate along with adaptation of gene pool in a given ecological niches. In livestock animals, correct individual identification is essential for breeding purposes since their abilities are directly passed down to the next generation. The previous methods applied in cattle were use of tattoos and ear tags which contained individual identification numbers, followed by more informative electronic chips (Seo et al., 2000). With time, blood typing results based on the protein polymorphisms for parentage testing was also followed but due to experimental complexity of blood typing system, it was replaced with DNA testing as applicable in forensic sciences on human (Yoon, 2002).

Today, well defined breed descriptors declared by Breed Societies or Statutory Bodies are used to categorise the breed. These phenotypic descriptors have limitations as they cannot be used to identify semen, ova, embryo or a breed product. Moreover these phenotypic descriptors cannot predict breed in admixture or so called non-descriptive population. STR markers have been used to identify domestic animal breed in large number of studies.

MacHugh et al. (1998) used 20 STR for assessing genetic structure of seven European cattle breeds along with the locus minimization up to 10 where the correct breed designation can be inferred with accuracies approaching 100%. But the major limitations of this work are the standalone mode of analysis and lack of reference data. Genetic variability and relationships among six native French cattle breeds and one foreign breed were investigated using 23 microsatellite markers by Maudet et al. (2002) where French alpine breeds with smaller population sizes showed higher genetic variability than the larger Holstein breed. They used two different assignment tests for determining the breed of origin of individuals. The exclusion-simulation significance test correctly assigned fewer individuals than the direct approach but provided a confidence level (p<0.01) for each individual being assigned. The accuracy of assignment greatly decreased as the threshold level of confidence of assignment increased as well as when the level of population differentiation decreased below the level often found between related breeds (FST<0.1). Accurate assignment with high statistical confidence is required for animal traceability.

Breed signature using microsatellite DNA marker has been reported in various domestic animals for example, cattle (Blott et al., 1999; Maudet et al., 2002), sheep (Arranz et al., 2001; Niu et al., 2011), goat (Serrano et al., 2009; Hoda et al., 2011), pig (Fan et al., 2005), horse (Bjornstad and Roed, 2002), dog (Toskinen and Bredbadka, 1999), poultry and rabbit (Gotz and Thaller, 1998).

Present work aims at resolving challenging limitations in all earlier reported methods viz. reference data availability, relatively larger number of locus requirement and complexity of computation. Lack of reference data availability adds cost addition as each time one has to genotype all potential/suspected breeds in question. We present here a novel model approach for breed identification of using test data of 8 cattle breeds. This methodology can be used for more number of breeds/countries to have the advantage of less genotyping cost as reference data obviates the cost of genotyping each time. Web server based computation makes this approach much user friendly.

Materials and Methods

Data Generation/Availability

For reference allelic data generation, a total of 500 samples were collected using random stratified sampling in the respective native breeding tract. Blood samples were collected in vacutainer containing EDTA as anticoagulant. Due care was taken to collect genetically unrelated samples of each breed. The panel of 500 samples constituted Dangi (67) Khillar (66), Nimari (65), Malvi (68), Kankrej (56), Gir (33) Gaolao (75), Kenkatha (70) animals. These samples were considered as representative of gene pool for further DNA signature investigation. Eighteen microsatellite loci were chosen from the microsatellite loci panel recommended by the Food and Agricultural Organization (FAO) and the International Society for Animal Genetics and previous studies (Chaudhary et al., 2009; Kale et al., 2010). The 18 loci are CSRM60, ILSTS005, ILSTS011, ILSTS006, MM12, ILSTS030, BM1824, HAUT27, BM1818, ETH152, INRA035, ETH10, INRA005, ILSTS034, CSSM663, ETH3, INRA063 and ILSTS033. As microsatellite markers are co-dominant thus a combination of 18 co-dominant loci and 500 samples are expected to generate 18000 allelic data for the populations under study to develop reference data of breed signature. Allelic richness (R) from all over samples for each locus using the rarefaction method (El Mousadik and Petit, 1996) and pairwise genetic distance between breeds were estimated using Weir and Cockerham’s estimate of Wright’s FST (θ) (Weir and Cockerham, 1984) through FSTAT v2.9.3 (Goudet, 2002).

Statistical Approaches

Various statistical classifiers like Bayesian network, Memory-Based Learning with nearest neighbour (IB1) and Support Vector Machine (SVM) was applied to build accurate model for classification of cattle breeds.
After developing the model, the prediction quality of the model was examined through evaluation measures like accuracy, Mathew’s Correlation Coefficient (MCC), sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV). The brief description of classifiers used for model development is as follows.

**Bayesian Networks as Classifiers**

A Bayesian network \( B \) may be induced and encodes a probability distribution \( P_B(A_1, A_2, ..., A_n, C) \) from a given training set. The resulting model can be used so that given a set of attributes \( a_1, a_2, ..., a_n \), the classifier based on \( B \) returns the label \( c \) which maximizes the posterior probability, i.e.:

\[
P_B(c | a_1, a_2, ..., a_n)
\]

Let \( D = \{u_1, u_2, ..., u_N\} \) denotes the training data set. Here, each \( u_i \) is a tuple of the form \( \{a_i', a_2', ..., a_n'\} \) which assigns values to the attributes \( A_1, A_2, ..., A_n \) and to the class variable \( C \). The log likelihood function, which measures the quality of learned model can be written as:

\[
LL(B | D) = \sum_{i=1}^{N} \log P_B(c' | a_1', a_2', ..., a_n')
\]

\[
+ \sum_{i=1}^{N} \log P_B(a_1', a_2', ..., a_n')
\]

The first term in this equation measures efficiency of \( B \) to estimates the probability of a class given set of attribute values. The second term measures how well \( B \) estimates the joint distribution of the attributes. Since the classification is determined based on \( P_B(c | A_1, A_2, ..., A_n) \), only the first term is related to the score of the network as a classifier i.e., its predictive accuracy. This term is dominated by the second term, when there are many observations. As \( n \) grows larger, the probability of each particular assignment to \( A_1, A_2, ..., A_n \) becomes smaller, since the number of possible assignments grows exponentially in \( n \).

**Memory-Based Learning (MBL) Algorithm**

Memory-Based Learning (MBL) is based on the hypothesis that performance in cognitive tasks depends on reasoning on the basis of similarity of new situations to stored representations of earlier experiences, rather than on the application of mental rules abstracted from earlier experiences. This approach has surfaced in different contexts using a variety of alternative names such as similarity-based, example-based, exemplar-based, analogical, case-based, instance-based and lazy learning (Cost and Salzberg, 1993; Aha et al., 1991). Historically, MBL algorithms are descendants of the k-Nearest Neighbour (henceforth k-NN) algorithm (Aha et al., 1991).

An MBL system has two components, viz. a learning component which is memory-based since it involves adding training instances to memory and a performance component which is similarity-based. In the performance component, the product of the learning component is used as a basis for mapping input to output; this usually takes the form of performing classification.

The most basic metric that works for patterns with symbolic features is the Overlap metric (Hamming distance or Manhattan metric or city-block distance or L1 metric) given in following equations; where \( \Delta (X, Y) \) is the distance between instances \( X \) and \( Y \), represented by \( n \) features and \( \delta \) is the distance per feature. The k-NN algorithm with this metric is called IB1 (Aha et al., 1991):

\[
\Delta(X, Y) = \sum_{i=1}^{n} \delta(x_i, y_i)
\]

Where:

\[
\delta(x_i, y_i) = \begin{cases} 
abc & \text{if number, else} \\
0 & \text{if } x_i = y_i \\
1 & \text{if } x_i \neq y_i 
\end{cases}
\]

The major difference of k-NN algorithm with IB1 algorithm, implemented in Tilburg Memory-Based Learner (TiMBL) software originally proposed by (Aha et al., 1991), is that in TiMBL version, the value of \( k \) refers to \( k \)-nearest distances rather than \( k \)-nearest examples. With \( k = 1 \), for instance, TiMBL’s nearest neighbour set can contain several instances that are equally distant to the test instance. TiMBL, which is used in the study is an open source software package implementing several Memory-Based Learning (MBL) algorithms.

**Support Vector Machines**

Support vector machines are relatively new type of supervised machine-learning techniques, proven to be particularly attractive to biological analysis due to their ability to handle noise and large input spaces (Vapnik, 1999). The choice of the proper kernel function is an important issue for SVM training because the power of SVM comes from the kernel representation that allows the nonlinear mapping of input space to a higher dimensional feature space. Linear, polynomial, RBF and sigmoid are some typical choices of kernel function (Cristianini and Shawe-Taylor, 2000).
SVM can handle large feature spaces, effectively avoid over fitting by controlling the margin and automatically identify a small subset made up of informative points, i.e., support vectors, etc. The use of appropriate decision function can give better classification.

Cross Validation

In this study, five-fold cross validation technique was implemented to obtain estimate of prediction error, where the data sets were randomly divided into five equal sets. Among these, four sets were used for training and remaining one set for testing. The process was repeated five times such that each set has been used for testing. Average prediction on error estimation of five sets is calculated to estimate the prediction error.

Assessment of the Prediction Accuracy

The prediction quality of the model was examined through statistical estimation of the accuracy, Mathew’s Correlation Coefficient (MCC), sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV). The evaluation measures are defined as follows:

\[
\text{Sensitivity} = \frac{TP}{TP + FN}
\]

\[
\text{Specificity} = \frac{TN}{FP + TN}
\]

\[
\text{PPV} = \frac{TP}{TP + FP}
\]

\[
\text{NPV} = \frac{TN}{TN + FN}
\]

\[
\text{Accuracy} = \frac{(TP + TN)}{(TP + FP + TN + FN)}
\]

\[
\text{MCC} = \frac{(TP*TN - FP*FN)}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}}
\]

Implementation of Server

The web server is developed using Hyper Text Markup Language and Java. It is a user-friendly web server launched using web server software Apache. The user has to submit microsatellite allelic data in base. This can also be uploaded using .csv format, .txt format or directly in the submission form. A number of flexibilities have been added in this server. The user may opt for breed identification with five loci, 10 loci and 18 loci. It has seven tabs viz. home, submission, algorithm, tutorial, team, links and FAQs. The server has tutorial for the users for easy understanding with a sample data.

Results and Discussion

The microsatellite DNA marker data was used in this study to develop a model web server minimizing the computational complexity and reduction in number of loci for breed identification. In order to achieve this, three classifiers viz. Bayesian network, Memory-based learning (IB1) algorithm and Support Vector Machine were applied over the 18000 allelic/microsatellite data of cattle breeds. It was observed that models developed were with accuracies 95.45, 97 and 89.65%, respectively with five-fold cross validation. Hence, the model developed using Memory-based learning (IB1) algorithm was ultimately chosen to implement on the server using TiMBL: Tilburg Memory-Based Learner version 6.1 software (Daelemans et al., 2010). The sensitivity, specificity and MCC were found to be 88, 98.29 and 0.86, respectively (Table 1). The breed wise accuracy and MCC of the implemented algorithm were ranges from 91 to 100% and from 0.63 to 1.00, respectively (Table 2).

Though few indicine and taurine locus specific alleles are reported in very limited studies ARO23 (Metta et al., 2004), OCAM (MacHugh et al., 1967) and INRA124 (Giovambattista et al., 2000) which shows STR alleles are rarely private. Thus they cannot be used as DNA signature directly. Instead of STR allele per say, the frequency and genetic distance can be used as signature. This was also observed in our studies. We did not find private or breed specific allele in any population.

Breed assignment tests can be performed by means of a number of statistical tools based on genetic distances and differences in allelic frequencies (Cornuet et al., 1999). The later approach is widely reported in literature as it is usually obtained using a Bayesian approach (Ajmone-Marsan et al., 2007; Talle et al., 2005). Ranking of loci were performed on the basis of contribution of each loci in the average prediction using best (TiMBL) algorithm. From the rank, here we applied an incremental feature addition approach and on each step performance was noted.

Similarly our results support the identification of eight breeds of cattle with 18 different loci with accuracy of 97%. In our investigation, the role of number of loci in breed identification is depicted in Fig. 1. It shows that the accuracy and MCC increases from 88.50 to 97.00% and 0.47 to 0.86 respectively with increase in the number of locus (Table 3). We can achieve accuracy upto 95.55% with MCC 0.80 by selecting just five loci (CSRM60, ILSTS005, BM1824, ILSTS034, ETH3). While minimising the number of loci, we found 95.55% accuracy using five STR locus and 96.1% accuracy with ten loci. Our finding shows that beyond ten loci, there is no need of
locus genotyping saving cost of breed identification upto 50%. Addition of further eight more loci will increase accuracy by just 0.9% unnecessarily making the genotyping cost in magnitude of almost double which is not desirable. We found interesting values of top five loci which are highly differentiated ($F_{ST}>0.15$ and $R_t>8.0$). In case of top 10 loci, we found similar range except two loci viz. HAUT27 and INRA035 (Table 4). We also found in these loci where genetic differentiation ($F_{ST}$ value) is relatively less, the allelic richness ($R_t$ value) was high enough to compensate the informativeness of locus for potential breed identification. This finding is supported from literature too. There are cases of domestic animal breed predictions with as low as three loci in horse (Bjornstad and Roed, 2002). Minimum number of locus with high accuracy is always desirable and such success comes when loci are highly differentiable i.e. high $F_{ST}$ values for example in case of horse, $F_{ST}$ is 0.2-0.25. The maximum individual assignment success with $F_{ST}$ of 0.18 across 10 loci has been reported in dog (Koskinen, 2003). The results of genetic differentiation and analysis supported differentiation of the Murciana and Granadina populations with 25 microsatellites loci even with a low $F_{ST}$ value (0.0432) and with assignment of individuals to their populations with a success rate of more than 80% (Martinez et al., 2010).

Table 1. Performance of different classifiers

| Classifiers            | Sensitivity | Specificity | Accuracy | MCC  |
|------------------------|-------------|-------------|----------|------|
| Bayesian network       | 81.80       | 97.40       | 95.45    | 0.79 |
| Support vector machine | 58.60       | 94.09       | 89.65    | 0.53 |
| TiMBL-IB1 algorithm    | 88.00       | 98.29       | 97.00    | 0.86 |

Table 2. Prediction accuracies obtained for eight breeds of cattle

| Breeds   | Sensitivity | Specificity | Accuracy | MCC |
|----------|-------------|-------------|----------|-----|
| DAG      | 100.00      | 100.00      | 100.00   | 1.00|
| GAO      | 100.00      | 100.00      | 100.00   | 1.00|
| GIR      | 60.61       | 99.57       | 97.00    | 0.73|
| KAN      | 69.64       | 96.62       | 93.60    | 0.67|
| KEN      | 100.00      | 100.00      | 100.00   | 1.00|
| KHL      | 100.00      | 100.00      | 100.00   | 1.00|
| MAL      | 80.88       | 96.53       | 94.40    | 0.76|
| NIM      | 73.85       | 93.56       | 91.00    | 0.63|
| Wt. Avg  | 88.00       | 98.29       | 97.00    | 0.86|

DAG-Dangi; GAO- Gaolao; GIR- Gir; KAN- Kankrej; KEN- Kenkatha; KHL-Khillar; MAL- Malvi; NIM- Nimari

Table 3. Trend for average of sensitivity, specificity, accuracy and MCC with incrementing locus according to their rank

| No. of Loci | Sensitivity | Specificity | Accuracy | MCC  |
|-------------|-------------|-------------|----------|------|
| 1           | 54.00       | 93.43       | 88.50    | 0.47 |
| 2           | 70.00       | 95.71       | 92.50    | 0.66 |
| 3           | 73.80       | 96.26       | 93.45    | 0.70 |
| 4           | 77.60       | 96.80       | 94.40    | 0.74 |
| 5           | 82.20       | 97.46       | 95.55    | 0.80 |
| 6           | 81.80       | 97.40       | 95.45    | 0.79 |
| 7           | 83.00       | 97.57       | 95.75    | 0.81 |
| 8           | 83.00       | 97.57       | 95.75    | 0.81 |
| 9           | 83.00       | 97.57       | 95.75    | 0.81 |
| 10          | 83.00       | 97.57       | 95.75    | 0.81 |
| 11          | 84.40       | 97.77       | 96.10    | 0.82 |
| 12          | 85.40       | 97.91       | 96.35    | 0.83 |
| 13          | 85.00       | 97.86       | 96.25    | 0.83 |
| 14          | 85.20       | 97.89       | 96.30    | 0.83 |
| 15          | 86.00       | 98.00       | 96.50    | 0.84 |
| 16          | 86.40       | 98.06       | 96.60    | 0.84 |
| 17          | 87.20       | 98.11       | 96.95    | 0.85 |
| 18          | 87.80       | 98.26       | 96.95    | 0.86 |
| 19          | 88.00       | 98.29       | 97.00    | 0.86 |
Table 4. \(R_t\) and \(F_{ST}\) of top ten loci

| Locus     | \(R_t\) (Allelic richness) | \(F_{ST}\) (Genetic differentiation) |
|-----------|-----------------------------|---------------------------------------|
| CSRM60    | 13.69                       | 0.150                                 |
| ILSTS005  | 8.20                        | 0.270                                 |
| BM1824    | 8.17                        | 0.272                                 |
| ILSTS034  | 14.83                       | 0.151                                 |
| ETH3      | 8.09                        | 0.359                                 |
| ILSTS030  | 5.36                        | 0.150                                 |
| HAUT27    | 8.32                        | 0.088                                 |
| ETH152    | 9.33                        | 0.321                                 |
| INRA035   | 11.66                       | 0.093                                 |
| INRA005   | 8.23                        | 0.113                                 |

Fig. 1. Trend of Accuracy and MCC with locus

Although available bovine High-Density (HD) SNP Chip (778K) (Bai et al., 2012) and Low-Density (LD) SNP Chip (54 K) (Kuehn et al., 2010) can also be used for breed identification but at the moment they are not cost effective for most part of the globe. Moreover, for breed differentiation, using SNP 50 K data can be done by limited number of software for example Mendel (Lange et al., 2013) which is again not in server mode further compounding the issue of user-friendliness.

Conclusion

The present study reports world’s first model web server for domestic animal breed prediction. We report accuracy of 95.5, 96.10 and 97% of 8 cattle breeds with 5, 10 and 18 loci respectively. Selecting less number of loci will not only reduce the cost drastically but also provide greater computational ease to identify the breed at molecular level with degree of admixture too. This can be an indispensable tool for existing breed and new synthetic commercial breeds with their IP protection in case of sovereignty and bio-piracy dispute. This web server can be used as a model for other domestic species as well as all flora and fauna across globe in germplasm management. Though we develop this model on microsatellite DNA markers but similar server based approach with reference data is going to be warranted for high thorough put SNP chip based data to reap the benefit of genomics and computational tools.

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Author contributions

Sarika Jaiswal: Created the work-flow, web application and performed data analyses. Drafted the manuscript, read and approved the manuscript.
Sandeep Kumar Dhanda, Vasu Arora and U.B. Angadi: Created the work-flow, web application and performed data analyses, read and approved the manuscript.
M.A. Iquebal: Created the work-flow, web application and performed data analyses, drafted the manuscript, read and approved the manuscript.
Tejas M. Shah: Participated in sample collection and data generation, read and proved the manuscript.
Chaitanya G. Joshi: Drafted the manuscript, read and approved the manuscript.
Dinesh Kumar: Created the work-flow, web application and performed data analyses, drafted the manuscript, read and approved the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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