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Bovine EpiMap explorer: an interactive web application for genome-wide DNA methylation analysis in dairy cattle

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Abstract

Background DNA methylation in agriculture, particularly in cattle, is receiving increasing attention, not only for understanding underlying biological mechanisms, but also for the use of epigenetic markers in the selection of phenotypes of interest. Large datasets have been generated under predefined physiological contexts; however, no overview of bovine DNA methylation currently exists. Achieving such a view would require the integration of data from numerous studies.

Results *Bovine Epimap Explorer* is a user-friendly web application designed for the interactive exploration and visualization of processed DNA methylation data from bovine blood samples. It enables researchers to filter, visualize, and compare key methylation features, such as differentially methylated cytosines (DMCs), across various biological conditions including Age_Correlation, SubfertileVsFertile, MastitisVsControls, Interindividual_Variability, YoungVsOld_Mitochondria. The platform supports multiple visualization modes, including smoothed genome browser tracks, group-wise boxplots, volcano plots, Venn diagram and methylation density distributions. It integrates gene annotations and statistical metrics such as q-values, methylation differences, standard deviation, and correlation, while providing interactive filters and data download options.

Conclusion *Bovine EpiMap Explorer* aims to facilitate access to high-throughput DNA methylation datasets and accelerate research in animal epigenetics.

Keywords DNA methylation, Epigenetics, Bovine, Web application, Epimap: biomarker discovery, Blood, Epigenomic browser, EpiBov

Introduction

Livestock farming, particularly dairy cattle production, has become an area of growing interest in scientific research due to its economic importance and the transformative potential of biotechnologies to enhance key traits. Both genetic and more recently, epigenetic selection strategies aim to improve animal productivity and health while addressing the specific requirements of diverse agricultural systems [1, 2].

Among epigenetic mechanisms, DNA methylation has emerged as a critical layer of gene regulation that complements classical genetics. It plays a central role in gene expression, developmental processes, and disease response in mammals, including dairy cattle [3]. Notably, DNA methylation is highly influenced by environmental factors such as nutrition, stress, and age [4, 5], making it a promising target for studying environment genome interactions and for identifying robust biomarkers associated with animal welfare, disease resilience, and epigenomic selection [6–8].

Recent advances in high-throughput sequencing technologies, such as enzymatic methyl-seq (EM-seq) [9], now allow accurate and comprehensive profiling of genome-wide DNA methylation. Using EM-seq on blood-derived DNA from Holstein cows, thousands of differentially methylated cytosines (DMCs) have been identified, for example, between fertile and subfertile individuals [7]. Similar approaches have revealed methylation changes associated with mastitis resistance, including both DMCs and differentially methylated regions (DMRs) [6].

These types of analyses allow for the creation of categories of methylation marks based on their variability within a tissue. In blood, using several datasets and hundreds of animals, it has been estimated that about 5% of CpGs are variable across time or between individuals [10]. Therefore, by relying on a smaller dataset, the analysis can be accelerated while still providing robust q-values for phenotypic associations and for the selection of markers that are likely to persist throughout an individual's life once established.

However, the rapid accumulation of large-scale methylation datasets presents both opportunities and challenges. While these data are rich in biological information, their analysis often requires advanced bioinformatics expertise and high-performance computing resources tools that are not always accessible to all researchers.

Significant efforts have been made to characterize the human and murine epigenomes through large-scale initiatives such as ENCODE [11] and Roadmap Epigenomics [12]. In contrast, equivalent resources for livestock species remain limited, particularly for cattle. While genome browsers like IGV or the UCSC Genome Browser offer access to publicly available datasets (e.g., via GEO), they often lack the specificity and user-friendliness required for interpreting DNA methylation data from agricultural species.

To address this gap, we developed Bovine EpiMap Explorer, an interactive web application that enables intuitive and dynamic exploration of DNA methylation processed data derived from bovine blood samples. Blood, being minimally invasive and accessible, reflects both systemic and immune-related epigenetic signals, making it a valuable matrix for biomarker discovery in livestock.

The application supports hypothesis generation, biomarker identification, and functional exploration by integrating processed datasets, statistical filters, and interactive visualizations tailored to key biological conditions such as interindividual variability, age, fertility issues, mastitis, and age-associated mitochondrial methylation. By providing an accessible platform for non-specialists and advanced users alike, Bovine EpiMap Explorer contributes to democratizing access to epigenomic data in animal research.

Implementation

Data acquisition

Publicly available methylation datasets from previously published studies were integrated into the platform (Table 1). We included processed data obtained from

Table 1 The studies included in EpiMap Bovine explorer

Reference	Dataset	Title of article
Bouzeraa et al. [6]	MastitisVsControls	Decoding epigenetic markers: implications of traits and genes through DNA methylation in resilience and susceptibility to mastitis in dairy cows
Bouzeraa et al. [7]	SubfertileVsFertile	Epigenetic insights into fertility: involvement of immune cell methylation in dairy cows reproduction
Bouzeraa et al. [10]	Interindividual_Variability	Building a Bovine Blood Genomic DNA Methylation EpiMap Related to Disease Phenotypes
Bouzeraa et al. [13]	Age_Correlation + YoungVsOld_Mitochondria	Changes in Nuclear and Mitochondrial DNA Methylation in Cow Blood Associated with Age and Disease

whole-genome enzymatic methyl-seq (EM-seq) performed on blood samples from Holstein cows. These datasets were downloaded from their respective sources and filtered using the quality thresholds described in each original publication. For consistency, all data were reformatted to meet the input structure and requirements of the Bovine EpiMap Explorer platform.

The current implementation relies on EM-seq–based datasets, which provide improved library complexity and quantitative accuracy by avoiding bisulfite-induced DNA degradation [9]; however, the platform architecture is intentionally designed to support future integration of whole-genome bisulfite sequencing (WGBS) data and additional tissues.

The integrated dataset covers the following biological conditions:

- *Age_Correlation*: 48 healthy cows.
- *Subfertile vs Fertile*: 12 cows (6 fertile vs. 6 subfertile).
- *Mastitis vs Controls*: 12 cows (6 affected vs. 6 controls).
- *Interindividual_Variability*: 60 samples, including 30 healthy cows and 30 diseased cows (affected by mastitis, fertility issues, lameness, or metabolic disorders).
- *YoungVsOld_Mitochondria*: 40 healthy cows, with analyses conducted across CpG, CHG, and CHH methylation contexts in mitochondria.

For the *Age_Correlation* and *YoungVsOld_Mitochondria*, only CpG methylation sites derived from healthy animals [13] were included to avoid confounding effects associated with disease.

The *Cow Methylation Explorer* application was developed in R using the Shiny framework, enabling seamless and interactive exploration of genome-wide DNA methylation data from dairy cattle blood.

The application interface is organized into four main tabs:

1. *Home*—Presents an overview of the app and graphical abstract [7].
2. *Data Exploration*—Allows users to:

- Select a dataset (Age_Correlation, SubfertileVsFertile, MastitisVsControls, Interindividual_Variability, YoungVsOld_Mitochondria).
- Apply dynamic filters (chromosome, gene symbol, annotation type, q-value, methylation difference, correlation (Only for Age_Correlation dataset) or standard deviation (Only for Interindividual_Variability dataset).
- View interactive visualizations:

Data Table: Tabular output of filtered CpG sites with customizable columns.
Density Plot: Distribution of methylation values.
Genome Browser: A smoothed line and area plot showing average methylation levels along genomic coordinates. Annotated gene tracks are overlaid to identify nearby genes. Mouseover gene tracks to display gene names and coordinates.
Volcano Plot: Displays methylation difference versus $-\log_{10}$(q-value); available for MastitisVsControls, SubfertileVsFertile, Interindividual_Variability and YoungVsOld_Mitochondria datasets only.
Boxplots: Methylation by annotation regions or by group, displayed when a specific annotation type is selected.
Pie Chart: Genomic context distribution.

- Download filtered datasets (CSV) with embedded copyright notice.

3. *Data Intersection*—Implements an interactive Venn diagram interface, available for MastitisVsControls, SubfertileVsFertile, and Age_Correlation datasets only, allowing users to:

- Compare CpG overlap between datasets (by geneID or CpG position).
- Select intersection mode: *Intersection*, *Unique to each set*.
- Download subset-specific tables.

4. *Custom Upload*—Enables users to upload their own methylation datasets to perform interactive exploration within the platform. This module allows users to:

- Upload files (.csv or .rds format) containing methylation data.
- Apply dynamic filters.
- Generate interactive visualizations.
- Perform overlap analysis with existing reference datasets.
- Download filtered or processed results.

5. *Methods* and *About*—Document the experimental design, sequencing methodology (EM-seq), and user guidance, including ethical approval and resources.

All plots are interactive and responsive to user filters and can be downloaded for publication or further analysis. Annotations, tooltips and zooming enhance usability and data exploration.

Chromosome–gene selection logic

In the Data Exploration module, chromosome and gene-based filtering are implemented with adaptive logic to ensure biological consistency while preserving exploratory flexibility. When the chromosome filter is set to "All", users may freely query any gene symbol, and genome-wide matching is performed automatically, with the corresponding chromosomal location resolved internally. When a specific chromosome is selected, the gene selection is dynamically restricted to genes located on that chromosome, preventing biologically inconsistent queries. This design allows flexible genome-wide exploration while reducing user error and misinterpretation.

Backend design and performance

- Processed methylation datasets are stored as .rds objects for efficient memory loading.
- All operations (filtering, rendering) are reactive, ensuring smooth updates without full-page reloads.
- A smoothing algorithm enables dynamic genome browser visualization.
- Built-in downsampling optimizes performance for large datasets.

Construction of Customized Profiles

The application allows users to download filtered data in CSV format, including columns listed in Table 2. Filtering can be applied based on dataset type, chromosome, start and end positions, annotation type, gene symbol, q-value, methylation difference,

Table 2 Description of the columns available in the downloadable CSV files from the Bovine EpiMap Explorer application

Column Name	Description
Chromosome	Chromosome number where the CpG site is located
Start	Start position of the CpG methylation site on the chromosome
End	End position of the CpG methylation site on the chromosome
Correlation	Spearman correlation coefficient between methylation level and age; *available only for Age_Correlation dataset*
Qvalue	Adjusted p-value (q-value) for the statistical significance of the correlation or methylation difference
Sd_meth	Standard deviation of methylation levels across all individuals; *available only for Interindividual_Variability dataset*
Meth.diff	Methylation difference between compared groups; *Not available for Age_Correlation dataset*
GeneId	Ensembl gene identifier associated with the CpG site
Annot_large	Genomic context of the CpG site (e.g., TSS shores, exon, intron, intergenic); *Not available for YoungVsOld_Mitochondria dataset*
Genename	Full gene name associated with the CpG site
Symbol	Gene symbol (abbreviated gene name)
GeneChr	Chromosome number of the associated gene
GeneStart	Start position of the gene on the chromosome
GeneEnd	End position of the gene on the chromosome
Context	Sequence context of the methylation site (e.g., CpG, CHG, CHH); *available only for YoungVsOld_Mitochondria dataset*
MeanMeth_Fertile	*Average methylation level in the Fertile group; available only for SubfertileVsFertile dataset*
MeanMeth_SubFertile	Average methylation level in the Subfertile group; *available only for SubfertileVsFertile dataset*
MeanMeth_Control	Average methylation level in the Control group; *available only for MastitisVsControls and Interindividual_Variability dataset*
MeanMeth_Mastitis	Average methylation level in the Mastitis group; *available only for MastitisVsControls dataset*
MeanMeth_Diseased	Average methylation level in the overall Diseased group, *available only for Interindividual_Variability dataset*
MeanMeth_Young	Average methylation level in the Young age group; *available only for mitochondria dataset*
MeanMeth_Old	Average methylation level in the Old age group; *available only for YoungVsOld_Mitochondria datasets*
MeanMethGD	Average methylation level in the Control group; *available only for Age_Correlation dataset*

methylation standard deviation (for Interindividual_Variability dataset), and correlation values (for Age_Correlation dataset).

Visualizations are dynamically generated according to the applied filters and can also be downloaded in high-quality format. The Genome Browser plot supports zooming and navigation across genomic regions for detailed inspection.

The Data Intersection tab enables users to compare datasets using an interactive Venn diagram. Users can explore shared or unique features (by geneID or CpG position) and download the corresponding subset as a CSV table for further analysis.

Custom dataset upload and user-defined analyses

Bovine EpiMap Explorer supports custom dataset upload through a constrained input format requiring genomic coordinates, gene identifiers, and at least one statistical metric. Uploaded datasets are automatically validated and integrated into the same exploration, visualization, and intersection workflows as the curated reference datasets. This feature enables direct, standardized comparison between user-generated and reference epigenomic data.

Results

We can demonstrate the utility of the Bovine EpiMap Explorer by investigating DNA methylation patterns associated with fertility in dairy cows. The application enabled the rapid identification of the gene *KIAA0825*, previously highlighted by Bouzeraa et al. [7]. The genome browser within the app allowed visualization of methylation levels across the *KIAA0825* locus (Fig. 1).

Additionally, the group-wise boxplots provided by the application reveal clear methylation differences between fertile and subfertile groups in specific genomic regions associated with this gene (Fig. 2).

The Bovine EpiMap Explorer further allows users to reproduce the volcano plot presented by Bouzeraa et al. [7] by visualizing genes of interest, such as *RAP2A*. This facilitates rapid assessment of their differential methylation profiles and statistical significance within the same plot (Fig. 3).

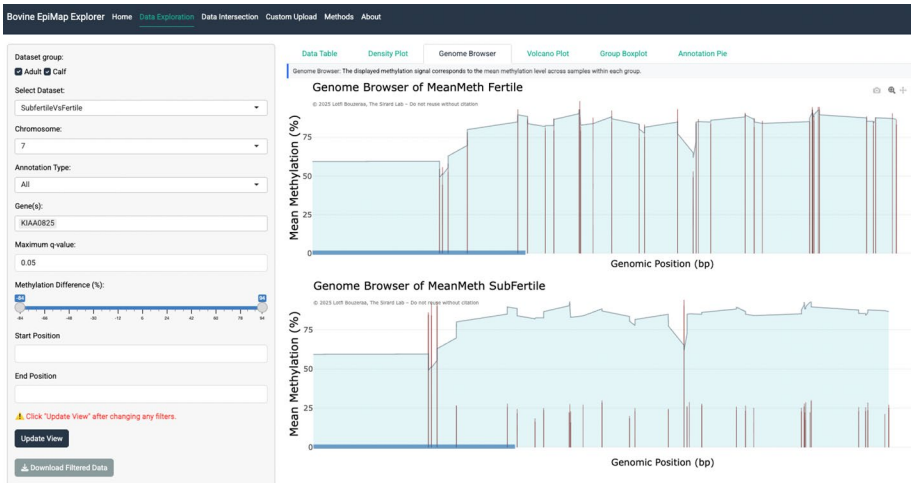

Fig. 1 Screenshot of genome browser view of DNA methylation across the *KIAA0825* locus in the SubfertileVsFertile dataset. Methylation levels are shown as smoothed line plots per group, with gene annotations and genomic coordinates displayed

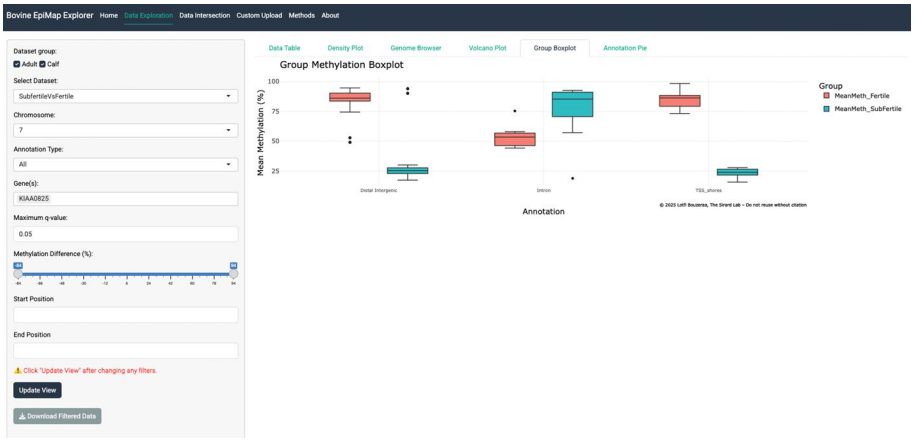

Fig. 2 Screenshot of Group-wise DNA methylation levels across annotated regions of the *KIAA0825* gene in fertile and subfertile cows. Boxplots display the distribution of mean methylation (%) by annotation category, comparing fertile (red) and subfertile (blue) groups within the SubfertileVsFertile dataset

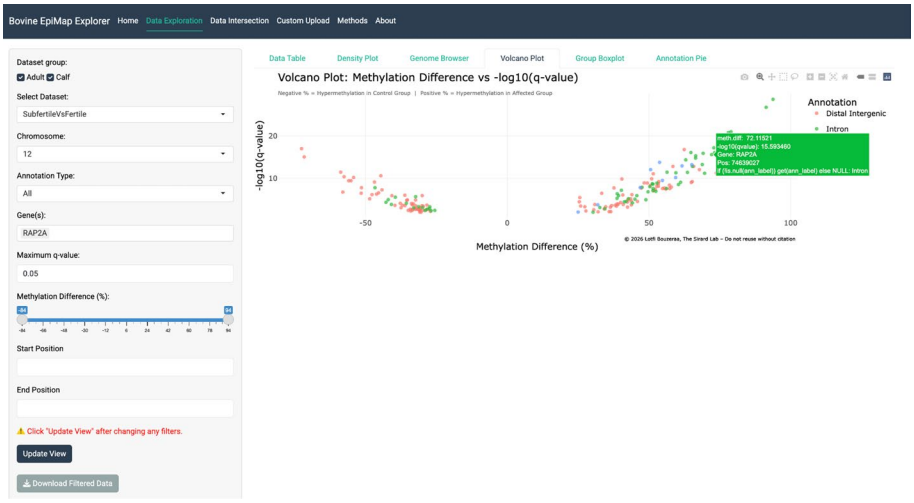

Fig. 3 Screenshot of volcano plot of differentially methylated cytosines (DMCs) in the SubfertileVsFertile dataset highlighting *RAP2A*. Each point represents a CpG site, plotted by methylation difference (%) on the x-axis and $-\log_{10}$(q-value) on the y-axis. Gene-specific selection enables the identification of significantly differentially methylated sites across key fertility-related genes

The Venn diagrams allow users to intersect datasets such as MastitisVsControls, SubferileVsFertile, and Age_Correlation to identify shared genes or genomic positions across conditions. Each portion of the diagram can be selected, and the corresponding subset of data can be downloaded as a CSV file for further analysis.

When the Venn filter mode is selected, the intersection table includes all information from the selected datasets (two or three, depending on the intersection). For example, in the central portion representing 9834 elements (Fig. 4), the resulting data table will contain 9834 × 3 entries, combining information from Age_Correlation, Subfertile versus Fertile, and Mastitis versus Controls.

Functional positioning of Bovine EpiMap Explorer relative to existing genome browsers :

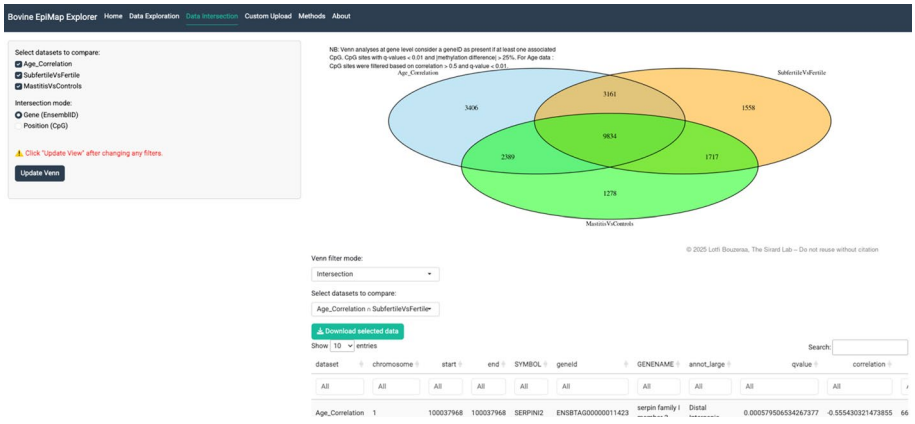

Fig. 4 Screenshot of venn diagram showing the overlap of genes across Age_Correlation, SubfertileVsFertile, and MastitisVsControls datasets. The intersection mode is set to "Gene", allowing identification of genes shared or unique to each condition. A total of 9834 genes are common to all three datasets, while others are specific to one or two biological contexts. This feature supports the extraction and download of the corresponding gene lists for downstream analysis

Table 3 Functional comparison between Bovine EpiMap Explorer and generic genome browsers (IGV and UCSC)

Feature	Bovine EpiMap explorer	IGV	UCSC genome browser
Main purpose	Integrated epigenomic analysis platform for bovine blood	Genome track visualization	Genome annotation & track visualization
Species focus	Bovine-specific (curated cattle datasets)	Multi-species	Multi-species (human/mouse-centric)
Built-in biological comparisons	Yes (age, mastitis, fertility, variability)	No	No
Statistical filtering	Yes (q-value, methylation difference, correlation, SD)	No	No
Group-level methylation summaries	Yes (mean methylation per group)	Precomputed tracks required	Custom tracks required
Interactive analysis plots	Yes (density, volcano, boxplots, pie charts)	No	No
Cross-dataset gene intersection	Yes (Venn diagrams + tables)	No	No
Downloadable analysis-ready tables	Yes	Limited	Limited
Mitochondrial methylation focus	Yes (CpG, CHG, CHH contexts)	No	No
Ease of use for non-specialists	High	Moderate	Moderate

To contextualize the scope and intended use of Bovine EpiMap Explorer, we compared its core functionalities with those of widely used generic genome browsers, including IGV and the UCSC Genome Browser (Table 3).

While generic genome browsers primarily focus on the visualization of user-provided genomic tracks, Bovine EpiMap Explorer is designed as a curated, analysis-oriented epigenomic resource. The platform integrates standardized EM-seq datasets with built-in biological contrasts, statistical filtering (e.g., q-values, methylation differences, correlation and variability metrics), and multiple interactive exploratory modules (including volcano plots, density plots, boxplots, annotation summaries, and cross-dataset intersections). This design enables rapid hypothesis generation and the direct export of

analysis-ready results, without requiring prior data preprocessing or advanced bioinformatics expertise.

Conclusion

Bovine EpiMap Explorer addresses a critical need for accessible and user-friendly tools in livestock epigenomics. By enabling non-specialists to visualize, compare, and interpret complex DNA methylation data, it bridges the gap between data generation and biological insight.

The platform complements traditional genome browsers by specifically focusing on blood-derived DNA methylation and by offering hypothesis-driven exploration tailored to research in animal health and reproduction. The application supports rapid filtering and rendering of large datasets.

Bovine EpiMap Explorer provides a foundational framework for expanding DNA methylation data resources in cattle. By centralizing high-quality, processed methylation datasets and offering interactive tools for their exploration, the platform serves as a starting point for future integration of additional tissues, developmental stages, and environmental contexts. It paves the way for building a comprehensive epigenetic reference map in bovines, facilitating large-scale comparative analyses and the identification of biomarkers relevant to animal health, productivity, and resilience.

Availability and requirements

Project name: Epibov. Project home page: https://www.epibov.ca. Operating system(s): Platform independent. Programming language: R. Other requirements: Basic Internet connection. License: CC-BY 4.0. Any restrictions to use by non-academics: Free to use. Users must cite the Epibov application, and cite the original publications associated with the datasets used.

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Author contributions
MAS secured the funding. LB designed and developed the web application, implemented data, and drafted the manuscript. LB and MAS contributed to application testing. All authors reviewed and approved the final version of the manuscript.

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Data availability
This study is based entirely on publicly available DNA methylation datasets. All raw sequencing data and processed files used in this analysis were retrieved from previously published studies and are accessible through the Gene Expression Omnibus (GEO) under the following accession numbers: GSE276263, GSE256195, GSE290131, and GSE296484.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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