Machine Learning Pipeline for Detecting PCR-Induced Chimeric Reads

MitoChime: Organellar Chimera Detection from Per-Read Features

Duran, Lin, Pailden

University of the Philippines Visayas
Philippine Genome Center Visayas

December 8, 2025

Outline

General Objective

- Develop and evaluate a machine-learning pipeline (MitoChime) to detect PCR-induced chimeric reads in *S. lemuru* mitochondrial sequencing data to improve downstream assembly quality.

Specific Objectives

1. Construct simulated *Sardinella lemuru* Illumina paired-end datasets contain ing both clean and PCR-induced chimeric reads.
2. Extract alignment-based and sequence-based features such as k-mer composition, junction complexity, and split-alignment counts from both clean and chimeric reads
3. Train, validate, and compare supervised machine learning models for classifying reads as clean or chimeric.
4. Determine feature importance and identify indicators of PCR-induced chimerism.
5. Integrate the optimized classifier into a modular and interpretable pipeline deployable on standard computing environments at PGC Visayas.

Scope of the Study

- Focuses on PCR-induced chimeric reads in *Sardinella lemuru* mitochondrial sequencing data to:
  - to limit interspecific variation in mitochondrial genome size, GC content, and repetitive regions so that differences in read patterns can be attributed more directly to PCR-induced chimerism
  - to align the analysis with relevant *S. lemuru* sequencing projects at PGC Visayas
  - to take advantage of the availability of *S. lemuru* mitochondrial assemblies and raw datasets in public repositories such as the National Center for Biotechnology Information (NCBI), which facilitates reference selection and benchmarking
  - to develop a tool that directly supports local studies on *S. lemuru* population structure and fisheries management produce tools applicable to local population and fisheries studies

Scope of the Study

- Uses wgsim-based simulations and selected empirical mitochondrial datasets
- Analysis targets low-dimensional alignment and sequence features (k-mers, GC content, clipping, split alignments) to maintain interpretability and computational accessibility
- Long-read platforms (Nanopore, PacBio) and other taxa are not included

Key Exclusions

- Naturally occurring chimeras
- NUMTs
- Large-scale nuclear genome rearrangements
- High-dimensional deep learning embeddings

Other Limitations

- No simulations with variable sequencing error rates
- No testing of alternative parameter settings (k-mer length, microhomology windows)
- Reliance on supervised machine learning may limit detection of novel/unknown chimeric patterns

Methodology

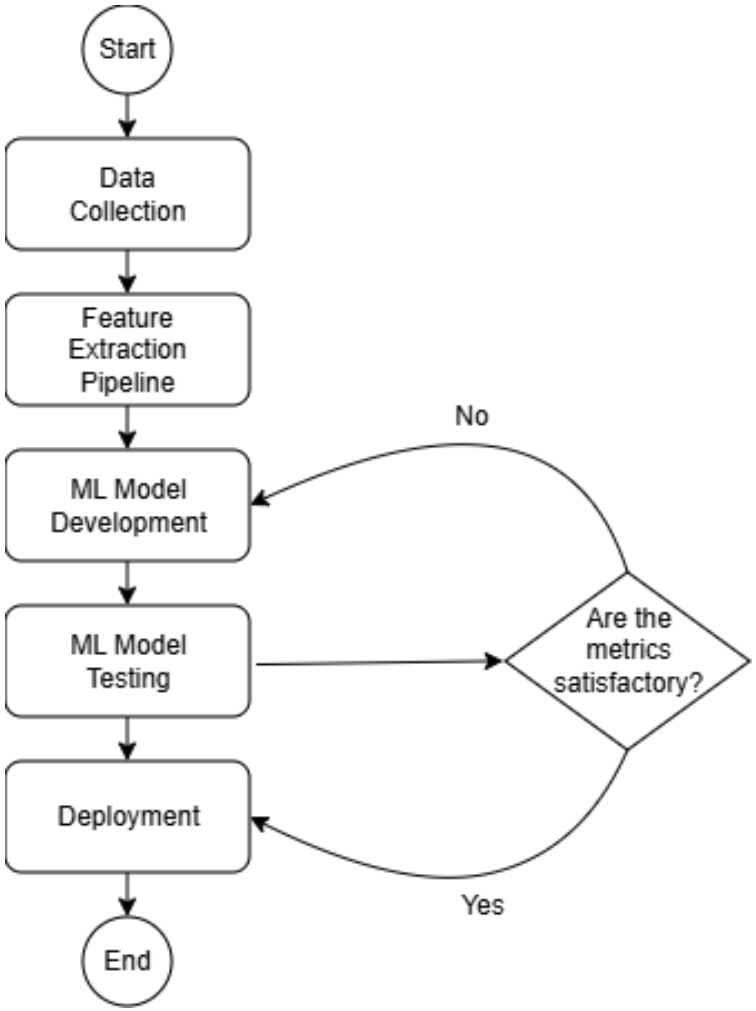


Figure: Process Diagram of the Special Project

Data Collection

The *S. lemuru* mitochondrial reference genome (NCBI: NC_039553.1) was downloaded in FASTA format and used as the basis for generating simulated reads.

Data Preprocessing

- A Python script was used to generate the reads.
- Clean reads were produced with wgsim from the reference genome.
- A chimeric reference was created by creating a custom script to combine non-adjacent segments with microhomology
- Chimeric reads were simulated with wgsim.
- All reads were mapped with minimap2 to extract alignment information.
- SAM/BAM files were converted, sorted, and indexed with samtools.

Data Preprocessing

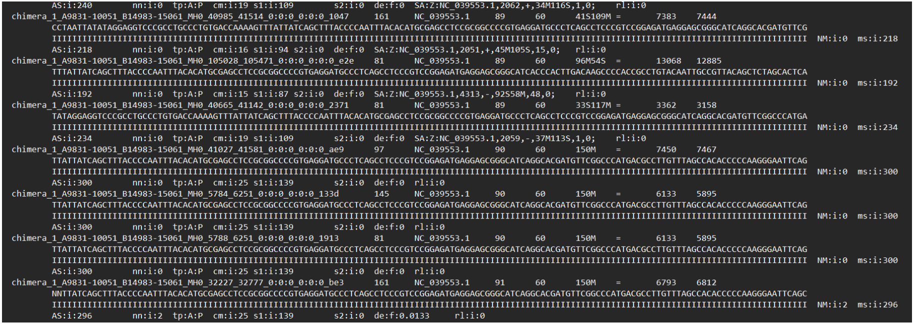
- Final dataset: 40k reads, roughly balanced between clean and chimeric (19,984 clean reads and 20,000 chimeric).
- Some of the clean reads failed to align due to the set error rate.

Data Preprocessing

```
NC_039553.1_3_540_8:0:0_6:0:0_ef2       163     NC_039553.1     3       60      150M    =       391     538
        TGGTGTAGCTTAAACAAAGCATAACACTGAAGATGTTACGATGGGCCGTGATAAGCCCCACACGCACTGAAAGGTTTGGTCCTGGCTTTATTATCAGCTTTACCGGAATTTACACCAGCGAGCCTCCGCGGCGCCGTGAGGATGGCCTCA
        ........................................................................................................................................................ NM:i:8  ms:i:220
        AS:i:220        nn:i:0  tp:A:P  cm:i:8  s1:i:164        s2:i:0  de:f:0.0533     rl:i:0
NC_039553.1_4_430_13:0:0_11:0:0_243d    163     NC_039553.1     4       60      150M    =       281     427
        GGTGTAGCTTAAACAAAGCATAACACTGCAGATGATCCGCTGGGCCGTGATAAGCCGCAGCAGGAGTGAAAGTTTTGGTCCAGGCTTTATTATCAGCTTTACCCCAAATTACACATGCGAGCCTCCGCGGCCCCGTGAGGATGCCCTCAG
        ........................................................................................................................................................ NM:i:13 ms:i:170
        AS:i:170        nn:i:0  tp:A:P  cm:i:9  s1:i:135        s2:i:0  de:f:0.0867     rl:i:0
NC_039553.1_5_495_6:0:0_11:0:0_1d49     163     NC_039553.1     5       60      150M    =       346     491
        GTGTAGCTTACACAAAGCATAACACTGAAGATGTTAAGATGGGCCGTGATCAGCCCCACAAGCACTGAAAGGTTAGGTCCTGGCTTTATTATCAGGTTTCCCCCAATTTACACATGCGAGCCTCCGCGGCCCCGTGAGGATGCGCTCAGC
        ........................................................................................................................................................ NM:i:6  ms:i:240
        AS:i:240        nn:i:0  tp:A:P  cm:i:12 s1:i:148        s2:i:0  de:f:0.04       rl:i:0
NC_039553.1_6_523_6:0:0_9:0:0_82c       163     NC_039553.1     6       60      150M    =       374     518
        TGTAGCTTAAACAAAGCATAACACTGAAGATGTTAAGATGGGCCGTGATAAGCCCCCCAAGCACTGCAAGGTTTGGTCGTGGCTATATTAACAGCTTTACCCCAATTTACACATGCGCGCCTCCGCGGCCCCGTGAGGATGCCCTCAGCC
        ........................................................................................................................................................ NM:i:6  ms:i:240
        AS:i:240        nn:i:0  tp:A:P  cm:i:10 s1:i:157        s2:i:0  de:f:0.04       rl:i:0
NC_039553.1_9_574_7:0:0_7:0:0_181b      163     NC_039553.1     9       60      150M    =       425     566
        AGCTTAAACAAAGCATAACACTGCAGATGTTAAGCTGGGCCGTGATAAGCCCCACAAGCACTGAAAGGTTTGGTCGAGGCTTTATTATCAGCTTTACCGCAATTTACACATGCGAGCCTCCGCGGCCCCGTGAGGCTGCCCTCCGCCTCC
        ........................................................................................................................................................ NM:i:7  ms:i:230
        AS:i:230        nn:i:0  tp:A:P  cm:i:12 s1:i:176        s2:i:0  de:f:0.0467     rl:i:0
NC_039553.1_10_391_9:0:0_8:0:0_256b     99      NC_039553.1     10      60      150M    =       242     382
        GCTTAAACAAAGCCTAACACTGAAGATGTAAAGATGGGCCGTGATAAGCCCCACAAGCACAGAAAGGTTTGTTCCTGGCTTTATTAACAGCTTTACCCCAATTTAGACATGCGAGCCTCCGCGGCCCCGTGATGCTGGCCTCAGCCTCCC
        ........................................................................................................................................................ NM:i:9  ms:i:210
        AS:i:210        nn:i:0  tp:A:P  cm:i:5  s1:i:156        s2:i:0  de:f:0.06       rl:i:0
NC_039553.1_11_509_6:0:0_11:0:0_a19     99      NC_039553.1     11      60      150M    =       360     499
        CTTCAACAAAGCATAACACTGAAGATGTTAAGATGGGCCGTTATAAGCCCGACAAGCACTGAAAGGTTAGGTCCTGGCTTTATTATGAGCTTTACCCCAATTTACACATGCGATCCTCCGCGGCCCCGTGAGGATGCCCTCAGCCTCCCG
        ........................................................................................................................................................ NM:i:6  ms:i:242
        AS:i:242        nn:i:0  tp:A:P  cm:i:10 s1:i:150        s2:i:0  de:f:0.04       rl:i:0
NC_039553.1_12_427_9:0:0_9:0:0_157      163     NC_039553.1     12      60      150M    =       278     416
        TTAAACAAAGCATAACACTGAAGAATTTCAGATGGGCCGTGCTAAGGCCCACAAGCACTGAAAGTTTTGGTCCTGGCTTTATTATCAGCTTTACCCCAATTTACACATGCGAGCCTCGGGGGCCCCGTGAGGATGCCCTCCGCCTCCCGT
        ........................................................................................................................................................ NM:i:9  ms:i:210
        AS:i:210        nn:i:0  tp:A:P  cm:i:8  s1:i:158        s2:i:0  de:f:0.06       rl:i:0
```

Figure: SAM File of Clean Reads

Data Preprocessing



```
        AS:i:240        nn:i:0  tp:A:P  cm:i:19 s1:i:109        s2:i:0  de:f:0  SA:Z:NC_039553.1,2062,+,34M116S,1,0;    rl:i:0
chimera_1_A9831-10051_B14983-15061_MH0_40985_41514_0:0:0_0:0:0_1047     161     NC_039553.1     89      60      41S109M =       7383    7444
        CCTAATTATATAGGAGGTCCCGCCTGCCCTGTGACCAAAAGTTTATTATCAGCTTTACCCCAATTTACACATGCGAGCCTCCGCGGCCCCGTGAGGATGCCCTCAGCCTCCCGTCCGGAGATGAGGAGCGGGCATCAGGCACGATGTTCG
        IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII  NM:i:0  ms:i:218
        AS:i:218        nn:i:0  tp:A:P  cm:i:16 s1:i:94 s2:i:0  de:f:0  SA:Z:NC_039553.1,2051,+,45M105S,15,0;   rl:i:0
chimera_1_A9831-10051_B14983-15061_MH0_105028_105471_0:0:0_0:0:0_e2e    81      NC_039553.1     89      60      96M54S  =       13068   12885
        TTTATTATCAGCTTTACCCCAATTTACACATGCGAGCCTCCGCGGCCCCGTGAGGATGCCCTCAGCCTCCCGTCCGGAGATGAGGAGCGGGCATCACCCACTTGACAAGCCCCACCGCCTGTACAATTGCCGTTACAGCTCTAGCACTCA
        IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII  NM:i:0  ms:i:192
        AS:i:192        nn:i:0  tp:A:P  cm:i:15 s1:i:87 s2:i:0  de:f:0  SA:Z:NC_039553.1,4313,-,92S58M,48,0;    rl:i:0
chimera_1_A9831-10051_B14983-15061_MH0_40665_41142_0:0:0_0:0:0_2371     81      NC_039553.1     89      60      33S117M =       3362    3158
        TATAGGAGGTCCCGCCTGCCCTGTGACCAAAAGTTTATTATCAGCTTTACCCCAATTTACACATGCGAGCCTCCGCGGCCCCGTGAGGATGCCCTCAGCCTCCCGTCCGGAGATGAGGAGCGGGCATCAGGCACGATGTTCGGCCCATGA
        IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII  NM:i:0  ms:i:234
        AS:i:234        nn:i:0  tp:A:P  cm:i:19 s1:i:109        s2:i:0  de:f:0  SA:Z:NC_039553.1,2059,-,37M113S,1,0;    rl:i:0
chimera_1_A9831-10051_B14983-15061_MH0_41027_41581_0:0:0_0:0:0_ae9      97      NC_039553.1     90      60      150M    =       7450    7467
        TTATTATCAGCTTTACCCCAATTTACACATGCGAGCCTCCGCGGCCCCGTGAGGATGCCCTCAGCCTCCCGTCCGGAGATGAGGAGCGGGCATCAGGCACGATGTTCGGCCCATGACGCCTTGTTTAGCCACACCCCCAAGGGAATTCAG
        IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII  NM:i:0  ms:i:300
        AS:i:300        nn:i:0  tp:A:P  cm:i:25 s1:i:139        s2:i:0  de:f:0  rl:i:0
chimera_1_A9831-10051_B14983-15061_MH0_5784_6251_0:0:0_0:0:0_133d       145     NC_039553.1     90      60      150M    =       6133    5895
        TTATTATCAGCTTTACCCCAATTTACACATGCGAGCCTCCGCGGCCCCGTGAGGATGCCCTCAGCCTCCCGTCCGGAGATGAGGAGCGGGCATCAGGCACGATGTTCGGCCCATGACGCCTTGTTTAGCCACACCCCCAAGGGAATTCAG
        IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII  NM:i:0  ms:i:300
        AS:i:300        nn:i:0  tp:A:P  cm:i:25 s1:i:139        s2:i:0  de:f:0  rl:i:0
chimera_1_A9831-10051_B14983-15061_MH0_5788_6251_0:0:0_0:0:0_1913       81      NC_039553.1     90      60      150M    =       6133    5895
        TTATTATCAGCTTTACCCCAATTTACACATGCGAGCCTCCGCGGCCCCGTGAGGATGCCCTCAGCCTCCCGTCCGGAGATGAGGAGCGGGCATCAGGCACGATGTTCGGCCCATGACGCCTTGTTTAGCCACACCCCCAAGGGAATTCAG
        IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII  NM:i:0  ms:i:300
        AS:i:300        nn:i:0  tp:A:P  cm:i:25 s1:i:139        s2:i:0  de:f:0  rl:i:0
chimera_1_A9831-10051_B14983-15061_MH0_32227_32777_0:0:0_0:0:0_be3      161     NC_039553.1     91      60      150M    =       6793    6812
        NNTTATCAGCTTTACCCCAATTTACACATGCGAGCCTCCGCGGCCCCGTGAGGATGCCCTCAGCCTCCCGTCCGGAGATGAGGAGCGGGCATCAGGCACGATGTTCGGCCCATGACGCCTTGTTTAGCCACACCCCCAAGGGAATTCAGC
        IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII  NM:i:2  ms:i:296
        AS:i:296        nn:i:2  tp:A:P  cm:i:25 s1:i:139        s2:i:0  de:f:0.0133     rl:i:0
```

Figure: SAM File of Chimeric Reads

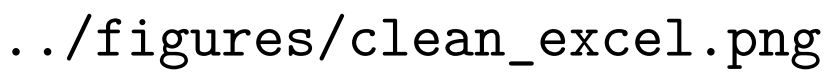
Feature Extraction Pipeline

- BAM files were processed with a Python script to build a TSV feature matrix.
- Used Pysam for parsing alignments and NumPy for computation.

Feature Extraction Pipeline

- Focused on three features linked to PCR-induced chimeras:
  1. **Supplementary Alignment (SA)**: Detects split alignments; counts and metrics extracted from SA tags
  2. **K-mer Composition Difference**: Breakpoints inferred; left/right segments compared using cosine and JS metrics.
  3. **Microhomology**: Overlap at junction quantified (length + GC content) within a defined window.
- Pipeline design and outputs to be validated by experts.

Feature Extraction Pipeline



Feature Extraction Pipeline

Dataset construction and split

- Simulated feature tables:
  - Clean reads (label 0)
  - PCR-induced chimeras (label 1)
- `build_datasets.py`:
  - Concatenate tables
  - Shuffle rows (avoid file-order artefacts)
- 80/20 **stratified** train–test split
- Test set held out and used **only once** at the end

Validation strategy

- Layer 1: 80/20 stratified train–test split
- Layer 2: 5-fold stratified cross-validation on training set
  - Train on 4 folds, validate on 1
  - Rotate so each fold is validation once
- Layer 3: Final evaluation on held-out test set
- Hyperparameter tuning:
  - `RandomizedSearchCV` inside CV for top models
- Goal: stable estimates and **unbiased** test performance

Model zoo and preprocessing pipeline

- **Baseline:** dummy majority-class classifier
- **Linear models:** logistic regression, calibrated linear SVM
- **Tree ensembles:**
  - Random Forest, Extra Trees
  - Gradient Boosting, XGBoost, LightGBM, CatBoost
- **Others:** bagging trees, k-NN, Gaussian NB, shallow MLP
- Common scikit-learn pipeline:
  - Median imputation (numeric missing values)
  - Standardisation (zero mean, unit variance)
- Ensures a **fair comparison** across models

Test-set performance (F1, tuned models)

Figure: Test F1 for tuned models (chimera class).

- Positive class: **chimeric reads**
- Dummy baseline:
  - F1 ≈ 0.67
- Linear models:
  - F1 ≈ 0.74 (logreg, linear SVM)
- Best ensembles:
  - CatBoost: F1 ≈ 0.769
  - Gradient Boosting: F1 ≈ 0.767
  - LightGBM: F1 ≈ 0.766
- k-NN, MLP: good but slightly below ensembles

Effect of hyperparameter tuning

Figure: Test F1: baseline vs tuned.

Figure: Test ROC–AUC: baseline vs tuned.

- Tuning done with `RandomizedSearchCV` on training set
- Small but consistent gains (F1, AUC ≈ 0.001–0.01)
- Top-ranked models remain the same (CatBoost, Gradient Boosting, LightGBM)

ROC and precision–recall curves

Figure: ROC (left) and PR (right) curves for CatBoost, Gradient Boosting, Random Forest, and logistic regression.

- Ensembles: ROC–AUC ≈ 0.84; logreg: ≈ 0.82
- Average precision ≈ 0.88 for ensembles
- Precision > 0.9 up to recall ≈ 0.5–0.6
- Good trade-off across thresholds

Confusion matrix: CatBoost (test set)

Figure: Confusion matrix heatmap for CatBoost.

- Clean reads:
  - Recall ≈ 0.95 (3782 / 3997)
- Chimeric reads:
  - Precision ≈ 0.92
  - Recall ≈ 0.66 (2631 / 4000)
- Behaviour at default threshold:
  - **Conservative chimera filter**
  - Protects clean reads, misses some subtle chimeras

Top features for CatBoost

Figure: Permutation importance (F1) for CatBoost.

- Strongest signals:
  - `kmer_js_divergence`
  - `total_clipped_bases`
  - `kmer_cosine_diff`
- Also important:
  - Left/right soft-clipping
  - Mapping quality (MAPQ)
  - SA count (supplementary alignments)
- Consistent with PCR chimera junctions

Feature family importance

Figure: Aggregated feature families for CatBoost.

- Aggregated permutation importance:
  - **Clipping** features dominate
  - **K-mer jump** features also strong
- Smaller contributions:
  - SA structure
  - Micro-homology
  - Other alignment context
- Same pattern for Gradient Boosting and Random Forest

ML component: summary and implications

- Per-read classifier for clean vs PCR chimeric reads
- Evaluation:
  - Stratified 80/20 split, 5-fold CV, held-out test set
- Best models: tree-based ensembles
  - CatBoost, Gradient Boosting, LightGBM
  - Test F1 ≈ 0.77, ROC–AUC ≈ 0.84
- Default threshold:
  - Conservative chimera filter (high clean recall, high chimera precision)
  - Removes $\sim 2/3$ of chimeras
- Features match PCR chimera biology
- Practical, interpretable pre-filter before mitochondrial assembly