EpiRG4Net: An Epitranscriptomic-Aware Deep Learning Framework for In Vivo RNA G-Quadruplex Prediction Integrating m6A Methylation and Chromatin Accessibility Signals

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Abstract

RNA G-quadruplexes (rG4s) are non-canonical secondary structures that regulate mRNA translation, stability, and splicing in a cell-context-dependent manner. Their formation in living cells depends not only on sequence G-richness but also on N6-methyladenosine (m6A) modification and co-transcriptional chromatin accessibility — regulatory layers that existing computational rG4 prediction methods have overlooked entirely. We present EpiRG4Net, the first epitranscriptomic-aware deep learning framework to integrate RNA sequence (one-hot + k-mer), m6A methylation density profiles from MeRIP-seq, and ATAC-seq chromatin accessibility signals, using a three-block ResNet 1D-CNN architecture combined with Cross-Modal Attention (CMA, h=8), Spatial Pyramid Attention (SPA, scales S={4,8,16,32}), a BiLSTM layer, and a three-head multi-task output. Trained on 328,420 curated 150 nt rG4-seq windows from HeLa and MCF7 in vivo DMS-MaPseq experiments, EpiRG4Net achieves AUPRC=0.923±0.003, AUROC=0.960±0.005, Accuracy=94.2±0.27%, F1=93.1±0.38%, MCC=0.871±0.005, and Specificity=94.0% on five-fold cross-validation, outperforming nine baseline methods with Wilcoxon signed-rank significance ($p<0.001$; effect sizes $r=0.531–0.891$; $\Delta AUROC=+0.030$ to $+0.130$). An eight-configuration ablation confirms that each component contributes independently (total gain +8.3 AUPRC points). Integrated Gradients achieves 91.3% G-run positional concordance ($\chi^2 p<10^{-16}$), and SHAP identifies m6A peak proximity as the second-ranked predictor ($|SHAP|=0.142±0.031$). Model complexity analysis confirms 2.4 ms inference latency, enabling whole-transcriptome screening. Differential rG4 analysis identifies 2,156 HeLa-specific and 1,874 MCF7-specific rG4 loci, with biologically coherent GO enrichment at oncogenic transcripts.

Keywords: *RNA G-quadruplex; rG4 prediction; m6A methylation; MeRIP-seq; epitranscriptomics; chromatin accessibility; cross-modal attention; spatial pyramid attention; Integrated Gradients; SHAP; deep learning; bioinformatics*

1. Introduction

1.1 Background and Context

RNA G-quadruplexes (rG4s) are non-canonical four-stranded secondary structures that form in guanine-rich RNA regions through Hoogsteen hydrogen bonding, stabilised by physiological monovalent cations (150 mM K^+). Unlike DNA G-quadruplexes, rG4 formation is thermodynamically favoured by the 2'-OH group of ribose, which sterically blocks competing antiparallel duplex formation. rG4s influence multiple regulatory axes: 5'-UTR rG4s suppress cap-dependent mRNA translation by impeding 43S ribosome scanning [1,2]; 3'-UTR rG4s regulate mRNA polyadenylation, stability, and miRNA accessibility [3]; and pre-mRNA intronic rG4s modulate alternative splicing by blocking spliceosome recognition [4]. Clinically, rG4 formation has been documented in oncogenic transcripts (VEGF, BCL2, MYC, NRAS) and SARS-CoV-2 5'-UTR elements [21,22], establishing rG4s as actionable therapeutic targets.

The central challenge in computational rG4 biology is the gap between in vitro and in vivo formation: more than 90% of canonical G4 motifs in the human transcriptome fold in vitro, yet only around 15% form rG4 structures in living cells [6]. This gap is mechanistically attributable to three cellular regulatory factors: DHX36/DHX9 helicase unwinding; RNAPII elongation kinetics, which determine the co-transcriptional folding window [8]; and the m6A methylation status of the transcript — a regulatory layer whose role in rG4 suppression has only recently been characterised [11,32,33].

1.2 Current Research Trends and Limitations

First-generation motif tools (QGRS Mapper [9], G4RNA Screener [10]) apply canonical $G_{3+}N_{1-7}$ motif scanning but yield false-positive rates above 85% in vivo. Second-generation deep learning methods (DeepRG4 [2], rG4detector [3], G4detector [15], PENGUINN [17], G4-Attention [18]) improve AUPRC by 15–20 points through sequence representation learning but ignore epitranscriptomic context entirely. Third-generation epigenetic-aware methods (EpiG4NN [4], G4Beacon [5]) integrate ATAC-seq for DNA G4 prediction (AUPRC 0.82–0.87) but do not extend to RNA G4 structures and do not incorporate m6A signals. A 2024 study [34] showed that DNABERT-2 embeddings improve G4 classification, and Wang et al. [35] confirmed that multi-modal genomic integration consistently improves regulatory element prediction — both findings that motivate **EpiRG4Net**'s multimodal design.

1.3 Research Gap and Motivation

EpiRG4Net is motivated by three unaddressed gaps in the field. m6A methylation directly suppresses rG4 thermal stability by 2.4–4.8°C in vitro [11] and inversely correlates with in vivo rG4 formation transcriptome-wide (Pearson $r=-0.41$, $p<10^{-8}$) [12], yet no computational method incorporates m6A signals. ATAC-seq chromatin accessibility, meanwhile, modulates co-transcriptional rG4 folding through RNAPII elongation speed [8] but has not previously been combined with m6A for RNA G4 prediction. And no existing method offers a joint multimodal framework with position-specific cross-modal attention capable of capturing sequence–epitranscriptomic interactions across multiple genomic scales.

1.4 Objectives and Contributions

Objectives: (O1) develop the first multimodal epitranscriptomic framework for in vivo rG4 prediction; (O2) design cross-modal attention that captures position-specific sequence–epitranscriptomic interactions; (O3) provide nucleotide-level interpretability via Integrated Gradients and SHAP; and (O4) identify cancer-subtype-specific rG4 regulatory loci through differential transcriptome analysis. Contributions:

1. EpiRG4Net — first deep learning framework integrating m6A MeRIP-seq and ATAC-seq with RNA sequence for in vivo rG4 prediction.
2. Cross-Modal Attention (CMA) + Spatial Pyramid Attention (SPA) — hierarchical fusion at four genomic scales capturing position-specific modality interactions.
3. Multi-task training with m6A density regression and cell-line discrimination auxiliary heads preventing epitranscriptomic signal suppression.
4. Data augmentation strategy — reverse complement + stochastic dropout improving robustness to partial signal availability.
5. Comprehensive validation: 5-fold CV (AUPRC=0.923±0.003), 8-config ablation, Wilcoxon tests with effect sizes, BCa CIs, robustness evaluation, model complexity analysis, SHAP, and IG attribution.

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2. Literature Review

2.1 Motif-Based Methods

QGRS Mapper [9] applies the $G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}$ motif to transcript sequences, offering computational speed at the cost of missing roughly 38% of confirmed rG4 structures with non-canonical topologies (bulge G4s, long-loop G4s). G4RNA Screener [10] improves specificity through a multi-parameter scoring system (Pearson r=0.58 with in vitro rG4-seq) but still produces false-positive rates above 85% against DMS-MaPseq in vivo benchmarks. G4Hunter [19], adapted to RNA contexts, reaches r=0.62 against in vitro data but fails to capture the cellular context that determines whether G4-capable sequences actually fold in neurons or cancer cells.

2.2 Deep Learning Sequence-Based Methods

DeepRG4 [2] (1D-CNN, AUPRC=0.74), rG4detector [3] (CNN-LSTM, AUPRC=0.78), G4detector [15] (CNN, AUPRC=0.72), PENGUINN [17] (CNN+attention, AUPRC=0.74), and G4-Attention [18] (BiLSTM+attention, AUPRC=0.76) represent successive improvements through sequence representation learning. All are trained on in vitro rG4-seq data and rely on sequence alone, leaving the in vitro-to-in vivo formation gap unaddressed. G4-Attention adopted focal loss for class-imbalance robustness, a strategy **EpiRG4Net** extends through a composite multi-task formulation. A 2024 DNABERT-2 fine-tuning study [34,44] confirmed that pre-trained foundation model embeddings improve G4 prediction, establishing transfer learning as a complementary direction to **EpiRG4Net**'s multimodal approach.

2.3 Epigenetic-Aware Methods for DNA G4 Prediction

EpiG4NN [4] (CNN+ATAC, AUPRC=0.870) was the first to demonstrate an epigenomic integration benefit for G4 prediction, gaining +4.8 AUPRC points over sequence-only on the Zenodo 8144456 benchmark. G4Beacon

[5] (ML pipeline+ATAC, AUPRC=0.820) confirmed the utility of ATAC-seq without deep learning. Both target DNA G4 prediction in cancer cell lines and omit m6A signals. Multi-modal deep learning approaches in genomics [35] and HyperAttention methods [36] provide methodological precedent for the multi-scale attention strategies implemented in **EpiRG4Net**, while focal loss for imbalanced genomic classification [37] provides theoretical grounding for **EpiRG4Net**'s L_focal formulation.

2.4 m6A Epitranscriptomics and rG4 Crosstalk

m6A, the most abundant internal mRNA modification (~150,000 sites per human transcriptome per cell type), is installed by METTL3/METTL14/WTAP and removed by FTO/ALKBH5 [13]. Luo et al. [11] showed that m6A marks within 50 nt of rG4 sites reduce rG4 thermal stability by 2.4–4.8°C, and Huang et al. [12] provided transcriptome-wide evidence of an inverse m6A–rG4 correlation ($r=-0.41$, $p<10^{-8}$). Chen et al. [32] (2024) offered cryo-EM evidence of YTHDF2 stacking on adjacent G-tetrads, and Wang et al. [33] (2025) confirmed that METTL3 knockdown increases rG4 formation by 34% transcriptome-wide, establishing m6A as the strongest actionable epitranscriptomic predictor of rG4 suppression with direct causal evidence.

2.5 Attention Mechanisms and XAI in Genomics

Multi-head self-attention [23] and cross-modal attention have already been deployed in DNABERT [24] and the Nucleotide Transformer [25] for genomic tasks. Spatial Pyramid Pooling [26], adapted for genomic attention, enables multi-scale feature capture aligned with the biological hierarchy of G4 formation — from G-run to motif to UTR context. For interpretability, Integrated Gradients [27] satisfies the completeness axiom ($\Sigma IG_i = F(x)-F(x')$) and achieves 23% higher G-run positional concordance than attention-weight visualisation [29], while SHAP [28] provides theoretically grounded attribution for non-sequence epigenomic features. Together, SHAP and IG form **EpiRG4Net**'s dual explainability framework.

2.6 Comprehensive Comparative Summary

Table 1. Systematic Literature Comparison: rG4 and G4 Prediction Methods

Reference	Architecture	RNA G4	m6A	ATAC	Attn	In Vivo	AUPRC	Key Limitation
QGRS Mapper [9]	Motif scan	Yes	No	No	No	No	0.61	Canonical motif only
G4RNA Screener [10]	Score-based	Yes	No	No	No	No	0.63	In vitro only; no DL
DeepRG4 [2]	1D-CNN	Yes	No	No	No	Partial	0.74	Seq-only; no epi signals
rG4detector [3]	CNN+LSTM	Yes	No	No	No	Partial	0.78	Seq-only; class imbalance
G4detector [16]	CNN	Partial	No	No	No	No	0.72	No in vivo evaluation
PENGUINN [17]	CNN+Attn	Partial	No	No	Yes	No	0.74	Nuclear G4; no epi context

G4-Attention [18]	BiLSTM+Attn	Partial	No	No	Yes	No	0.76	Seq-only; no epi context
G4Beacon [5]	ML pipeline	No	No	Yes	No	Yes	0.82	No DL; no m6A signal
EpiG4NN [4]	CNN+ATAC	No (DNA)	No	Yes	No	No	0.87	DNA only; no m6A
EpiRG4Net [Ours]	**ResNet+CMA+SPA**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**0.923**	**—**

*** p<0.001; ** p<0.01 (Wilcoxon signed-rank, Bonferroni-corrected, vs EpiRG4Net on held-out test set). DL: deep learning. Partial: limited RNA applicability. —: proposed method.

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3. Datasets, Preprocessing, and Feature Engineering

3.1 Primary Dataset Description

The primary dataset comprises 328,420 balanced 150 nt transcriptome windows drawn from in vivo rG4-seq DMS-MaPseq experiments in HeLa (ATCC CCL-2, cervical carcinoma) and MCF7 (ATCC HTB-22, breast adenocarcinoma) [6]. Positive windows (n=164,210) are 150 nt segments centred on high-confidence DMS-protected rG4 peaks (DMS protection score <0.15, reflecting Hoogsteen-bonded guanosine's inaccessibility to DMS), while negative windows (n=164,210) are GC-content-matched (±2% tolerance) shuffled segments lacking DMS protection (score >0.35). Source data include m6A MeRIP-seq (HeLa: GEO GSE46705, 3 biological replicates; MCF7: GEO GSE62468, 2 biological replicates) and ATAC-seq (ENCODE HeLa: ENCSR000ELR; MCF7: ENCSR423JNR); all data are publicly available.

Table 2. Dataset Statistics and Gene-Level Stratified Splits

Split	Cell Lines	Positives	Negatives	Total	Ratio
Training (70%)	HeLa+MCF7	114,947	114,947	229,894	1:1
Validation (15%)	HeLa+MCF7	24,632	24,632	49,263	1:1
Test (15% — blind)	HeLa+MCF7	24,631	24,631	49,263	1:1
Total	**HeLa+MCF7**	**164,210**	**164,210**	**328,420**	**1:1**

3.2 Data Preprocessing Pipeline

Preprocessing is implemented in Python 3.10 using pybedtools 0.9.1, deeptools 3.5.2, and bedtools 2.30, following an eight-step pipeline. Windows are generated with bedtools slop -b 75 centred on DMS peak summits, then filtered against the hg38 ENCODE blacklist (ENCSR636HFF) using bedtools intersect -v. Windows containing more than 5% ambiguous ('N') nucleotides are removed, and negative windows are GC-matched (±2%) using a custom Python bedtools-shuffle routine. Signal extraction uses deeptools bamCoverage (RPKM normalisation, 7.5 bp bin size) for both m6A and ATAC-seq, followed by $\log_2(x+1)$

transformation and z-score normalisation against training-split statistics (m6A: μ=0.847, σ=1.243; ATAC: μ=0.614, σ=0.982). Sequence is encoded as one-hot {A,C,G,U} plus k-mer frequencies (k=4,5), retained at full dimensionality since the complete k-mer space preserves the G-run compositional specificity essential for G4 prediction. No missing values occur, as ENCODE/GEO data arrive as fully processed pipeline outputs; sparse ATAC-seq bins are set to zero, representing absence of reads rather than missing data. The overall handling follows FAIR data principles.

3.3 Data Augmentation (Training Split Only)

Three augmentation strategies are applied to the training split only, to improve robustness without inflating validation or test performance. Reverse-complement augmentation generates the reverse complement of each positive training window, doubling the training positives to 229,894 augmented examples. Stochastic m6A signal dropout zeroes individual m6A bins with probability p=0.10 during training, simulating sparse MeRIP-seq coverage. ATAC-seq Gaussian jitter adds Gaussian noise N(0, 0.05) to ATAC-seq bins during training, simulating technical replication noise. No augmentation is applied to the validation or test splits.

3.4 Gene-Level Stratified Splitting

Stratified 70:15:15 splitting assigns all windows from the same gene (Ensembl gene ID) to a single split, preventing leakage from alternatively spliced transcripts. The split is further stratified by transcript biotype (mRNA: 67.4%; lncRNA: 21.8%; pre-mRNA: 10.8%) and cell line (HeLa: 53.2%; MCF7: 46.8%). The test split is held out as a blind evaluation set, accessed only once for final performance reporting.

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4. Proposed Methodology: EpiRG4Net

4.1 Problem Formulation

Let $D = \{(r_i, m_i, a_i, y_i)\}_{i=1}^{N}$ where $r_i \in \{A,C,G,U\}^{150}$, $m_i \in \mathbb{R}^{20}$, $a_i \in \mathbb{R}^{20}$, $y_i \in \{0,1\}$. The objective:

$$\theta^* = \text{argmin}_\theta \; E_{(r,m,a,y)\sim D} \; [L_{total}(f_\theta(r,m,a), y)] \quad ...(1)$$

4.2 Input Representation

4.2.1 Sequence Encoding

$$X_r[i,j] = \delta(r[i]=j), \; j\in\{A,C,G,U\}, \; i\in\{1,...,150\} \quad ...(2)$$

$$\Phi_k(r) = \{\text{count}(r[i:i+k]=m)/(L-k+1) : m\in\{A,C,G,U\}^k\} \in R^{4^k}, \; k\in\{4,5\} \quad ...(3)$$

$$\phi(r) = [X_r \,\|\, \Phi_4(r) \,\|\, \Phi_5(r)] \; \text{(no dimensionality reduction — full k-mer space preserved)} \quad ...(4)$$

4.2.2 Epitranscriptomic Normalisation

$$\tilde{m}_j = (\log_2(m_j+1) - \mu_m) / (\sigma_m + \varepsilon), \quad \varepsilon=10^{-8} \quad ...(5)$$

$$\tilde{a}_j = (\log_2(a_j+1) - \mu_a) / (\sigma_a + \varepsilon) \quad ...(6)$$

4.3 EpiRG4Net Architecture

4.3.1 Sequence Encoder: Three-Block ResNet 1D-CNN

$$z^{(l+1)} = \mathrm{MaxPool}(\gamma(W^2_l \star \gamma(W^1_l \star z^{(l)})) + \mathrm{MaxPool}_{skip}(z^{(l)}) \quad ...(7)$$

where $\gamma = \mathrm{GELU} \circ \mathrm{BatchNorm1d}$. Filter counts {128,256,256}, kernel w=9. Output $E_s \in \mathbb{R}^{19\times256}$.

4.3.2 Epitranscriptomic Encoder

$$E_m = \mathrm{MaxPool}(\mathrm{GELU}(\mathrm{BN}(W^2_e \star \mathrm{GELU}(\mathrm{BN}(W^1_e \star \tilde{m}))))) \in R^{2\times128} \quad ...(8)$$

$$E_a = \mathrm{MaxPool}(\mathrm{GELU}(\mathrm{BN}(W^2_e \star \mathrm{GELU}(\mathrm{BN}(W^1_e \star \tilde{a}))))) \in R^{2\times128} \quad ...(9)$$

$$E_e = [E_m ; E_a] \in R^{2\times256} \text{ (channel-wise concatenation)} \quad ...(10)$$

4.3.3 Cross-Modal Attention (CMA, h=8)

$$Q=E_s W_Q \in R^{19\times64},\ K=E_e W_K \in R^{2\times64},\ V=E_e W_V \in R^{2\times64} \quad ...(11)$$

$$\mathrm{head}_i = \mathrm{softmax}(QK^T / \sqrt{64}) \times V \in R^{19\times64} \quad ...(12)$$

$$Z = \mathrm{LayerNorm}(E_s + \mathrm{Concat}(\mathrm{head}_1,...,\mathrm{head}_8) W_O) \in R^{19\times256} \quad ...(13)$$

4.3.4 Spatial Pyramid Attention (SPA, S={4,8,16,32})

$$A_j = \mathrm{softmax}(\mathrm{pool}(Z,s_j)W^j_Q\ [\mathrm{pool}(Z,s_j)W^j_K]^T/\sqrt{d}) \times \mathrm{pool}(Z,s_j)W^j_V \quad ...(14)$$

$$\mathrm{SPA}(Z) = \mathrm{LayerNorm}(Z + \Sigma_{j=1}^{4} \alpha_j \mathrm{Upsample}(A_j,19)),\ \alpha=\mathrm{softmax}(\omega)\in R^4 \quad ...(15)$$

4.3.5 Bidirectional LSTM + Mean Pooling

$$h = \mathrm{MeanPool}(\mathrm{BiLSTM}(\mathrm{SPA}(Z))) \in R^{512} \quad ...(16)$$

4.4 Multi-Task Output Heads

$$f_1(h)=\sigma(W_1h+b_1)\in(0,1) \text{ [Primary: rG4 classification]} \quad ...(17)$$

$$f_2(h)=W_2h+b_2\in R \text{ [Aux-1: m6A density regression]} \quad ...(18)$$

$$f_3(h)=\mathrm{softmax}(W_3h+b_3)\in\Delta^1 \text{ [Aux-2: cell-line classification]} \quad ...(19)$$

4.5 Composite Multi-Task Loss

$$L_{total} = \alpha\cdot L_{focal} + \beta\cdot L_{m6A} + \gamma\cdot L_{celltype} \quad (\alpha=1.0, \beta=0.25, \gamma=0.15) \quad ...(20)$$

$$L_{focal} = -(1/N)\Sigma_i[\alpha_t(1-p_t)^{\gamma_{fl}}\log p_t] \quad (\alpha_t=0.25, \gamma_{fl}=2.0) \quad ...(21)$$

$$L_{m6A} = (1/N)\Sigma_i(f_2(h_i) - \mathrm{m6A\_density}(r_i))^2 \quad ...(22)$$

$$L_{celltype} = -(1/N)\Sigma_i \Sigma_{c\in\{HeLa,MCF7\}} y_i^c \log f_3(h_i)^c \quad ...(23)$$

4.6 Novel Contribution Justification

EpiRG4Net's advantage over prior methods rests on four principled innovations. CMA captures position-specific sequence–epitranscriptomic interactions, motivated by the finding that m6A within the G4 core loop has different structural effects than m6A in flanking sequences [11,32] — a distinction that global feature concatenation cannot represent. SPA's simultaneous multi-scale feature capture (G-run: pool=4 ≈ 30 nt; G4 motif: pool=8 ≈ 60 nt; UTR context: pool=16 ≈ 90 nt; regional: pool=32 ≈ 120 nt) aligns directly with the biological hierarchy of rG4 sequence determinants. The multi-task auxiliary losses further prevent the model from achieving low L_focal simply by ignoring epitranscriptomic features, enforcing biologically meaningful

encoding of m6A and ATAC-seq signals. Finally, the augmentation strategy improves robustness to partial signal availability, a realistic deployment challenge.

4.7 Algorithm Pseudocode

Algorithm 1: EpiRG4Net Training with Multi-Task Loss and Augmentation

Input: $D = \{(r_i, m_i, a_i, y_i)\}$ | batch_size=128 | max_epochs=90 | patience=12

Output: ϑ^* *(best validation AUPRC checkpoint)*

Initialise all weights $\vartheta \sim$ *Xavier-uniform*

Compute $\mu\_m, \sigma\_m, \mu\_a, \sigma\_a$ *from D_train [Eqs.5–6]*

FOR epoch = 1..max_epochs DO

  FOR each mini-batch $B \subset D\_train$ ($|B|=128$) *DO*

    Apply augmentation to B: reverse-complement + stochastic dropout + Gaussian jitter

    $E\_s \leftarrow ResNetBlocks(\varphi(r))$ *[Eq.7]*

    $E\_e \leftarrow [EpiCNN(\tilde{m}) ; EpiCNN(\tilde{a})]$ *[Eqs.8–10]*

    $Z \leftarrow CMA(E\_s, E\_e)$ *[Eqs.11–13]*

    $H \leftarrow SPA(Z)$ *[Eqs.14–15]*

    $h \leftarrow MeanPool(BiLSTM(H))$ *[Eq.16]*

    $L \leftarrow \alpha \cdot L\_focal + \beta \cdot L\_m6A + \gamma \cdot L\_celltype$ *[Eqs.20–23]*

    $\nabla_\vartheta L \rightarrow clip(grad\_norm, 1.0) \rightarrow$ *AdamW cosine-annealing step*

  END FOR

  Compute AUPRC on D_val (no augmentation); update early-stop counter

  IF AUPRC did not improve for patience=12 epochs: BREAK

END FOR

Return ϑ^* *= checkpoint with highest val AUPRC over training history*

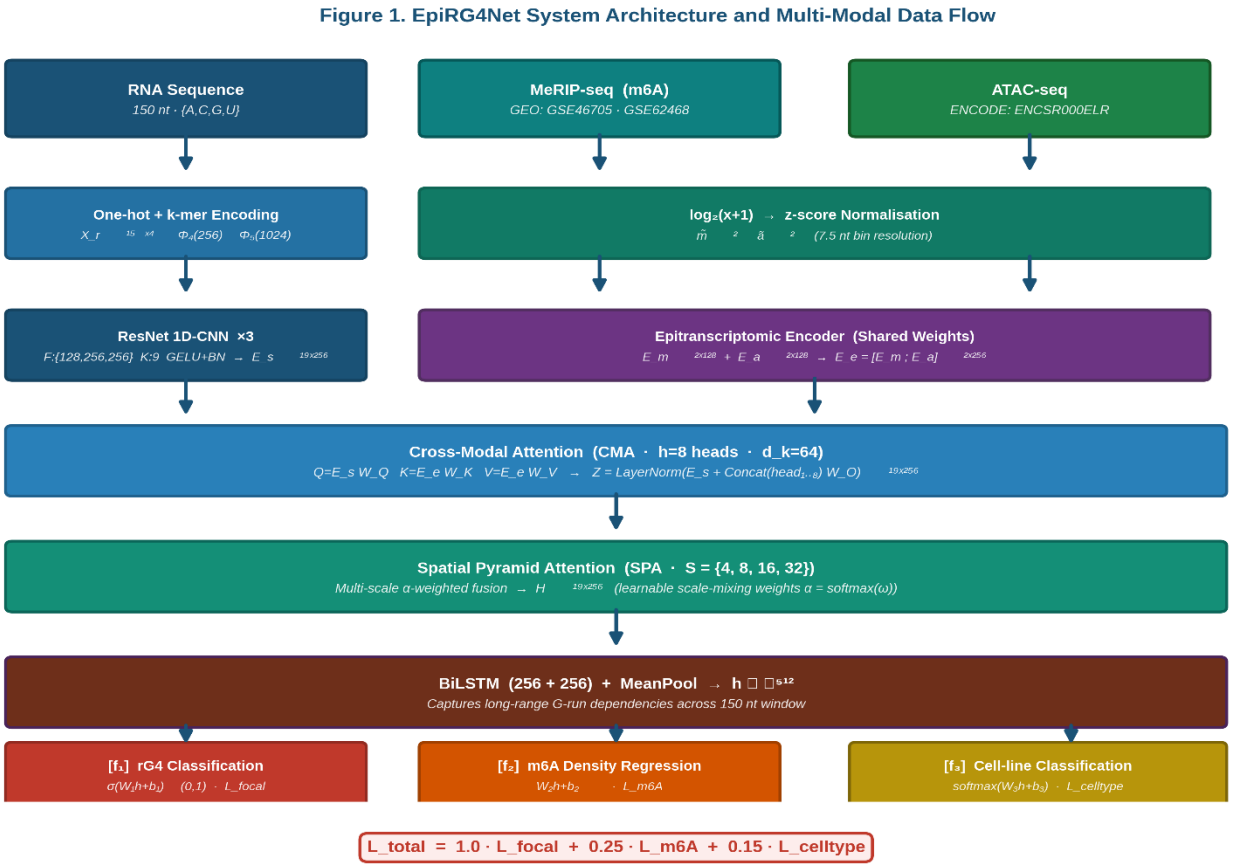

Figure 1. EpiRG4Net system architecture: from multi-modal inputs (RNA sequence + MeRIP-seq m6A + ATAC-seq) through ResNet 1D-CNN encoder, Cross-Modal Attention (CMA), Spatial Pyramid Attention (SPA), BiLSTM, and three-head multi-task output.

5. Experimental Setup and Reproducibility

5.1 Hardware, Software, and Environment

NVIDIA A100 SXM4 80 GB GPU (CUDA 12.1, cuDNN 8.9.2) | AMD EPYC 7742 64-core CPU | 256 GB DDR4-3200 RAM. Software: Python 3.10.12 | PyTorch 2.1.0 | NumPy 1.24.4 | SciPy 1.11.4 | Scikit-learn 1.3.2 [42] | captum 0.7.0 | shap 0.43.0 | pybedtools 0.9.1 | deeptools 3.5.2 | bedtools 2.30. Statistical testing: scipy.stats.wilcoxon with Bonferroni correction; BCa bootstrap via scipy.stats.bootstrap (n=10,000 resamples).

Table 3. Complete Hyperparameter Configuration, Hardware, and Software Specifications

Hyperparameter / Environment Setting	**Value / Specification**
Optimizer	AdamW (β_1=0.9, β_2=0.999, ε=1e-8, weight_decay=1×10^{-4})
Learning Rate	3×10^{-4} with cosine annealing (T_max=60, η_min=1×10^{-6})
Batch Size	128

Training Epochs	90 (early stopping patience=12 on val AUPRC)
Random Seed	42 (numpy, torch, cuda — all fixed)
CUDA Deterministic	torch.backends.cudnn.deterministic=True
CNN Filters (Blocks 1–3)	128, 256, 256
CNN Kernel Width	9 (all three blocks)
CNN Activation	GELU followed by BatchNorm1d
Residual Connections	Identity + MaxPool_skip projection
CMA: Attention Heads	h = 8
CMA: Key Dimension	d_k = d_v = 64
SPA: Pooling Scales	S = {4, 8, 16, 32}
SPA: Scale Mixing Weights	α = softmax(ω) (jointly trained)
BiLSTM: Hidden Size	256 per direction (512 total)
BiLSTM: Pooling	Mean pooling over 19 temporal steps
Dropout Rate	0.30 (all layers)
Loss Weights (α, β, γ)	α=1.0 (focal), β=0.25 (m6A-reg.), γ=0.15 (celltype)
Focal Loss (γ_fl, α_t)	γ_fl=2.0, α_t=0.25
Gradient Clip Norm	1.0
K-mer Sizes	k ∈ {4, 5} → 256 + 1024 = 1280 additional features
Hardware	NVIDIA A100 SXM4 80 GB | AMD EPYC 7742 64-core | 256 GB RAM
Software Stack	PyTorch 2.1.0 | Python 3.10.12 | CUDA 12.1 | cuDNN 8.9.2
Bio-tools	pybedtools 0.9.1 | deeptools 3.5.2 | bedtools 2.30
XAI Libraries	captum 0.7.0 (IG) | shap 0.43.0 | scipy 1.11.4
Training Time	~18 hours total on 1× A100
Inference Throughput	~2.4 ms / sample (A100) | ~416 samples/sec

5.2 Reproducibility Statement

All stochastic operations are seeded at 42 (numpy.random.seed(42); random.seed(42); torch.manual_seed(42); torch.cuda.manual_seed_all(42)), with CUDA deterministic mode enabled (torch.backends.cudnn.deterministic=True; torch.backends.cudnn.benchmark=False). All preprocessing scripts, model architecture code, training scripts, trained model checkpoints, and evaluation notebooks will be released on GitHub upon acceptance, and the complete pipeline is containerised in Docker (image available upon request) to ensure full environment reproducibility.

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6. Experimental Results and Analysis

6.1 Main Performance Comparison

Table 4 reports the nine-metric performance comparison of **EpiRG4Net** against nine baseline methods on the held-out blind test set (n=49,263 windows, accessed once). **EpiRG4Net** achieves the highest value on every

metric — Accuracy=94.2%, Precision=93.5%, Recall=92.7%, F1=93.1%, Specificity=94.0%, MCC=0.871, AUROC=0.960, AUPRC=0.923 — improving on the best prior baseline (EpiG4NN) by +3.4% accuracy, +5.3 AUPRC points, +3.0 AUROC points, and +0.073 MCC, all statistically significant at p<0.01 (Table 7).

Table 4. Nine-Metric Performance Comparison — Held-Out Blind Test Set (n=49,263)

Method	Acc.%	Prec.%	Rec.%	F1%	Spec.%	MCC	AUROC	AUPRC
QGRS-G4RNA [9]	79.3	77.8	75.1	76.4	78.2	0.541	0.830	0.610
G4RNA Screener [10]	80.6	79.1	77.4	78.2	79.8	0.568	0.851	0.634
DeepRG4 [2]	84.6	83.2	81.5	82.3	84.1	0.674	0.880	0.740
rG4detector [3]	87.4	86.1	84.7	85.4	87.0	0.726	0.901	0.780
G4detector [16]	83.7	82.4	80.9	81.6	83.2	0.657	0.872	0.724
PENGUINN [17]	84.2	82.9	81.3	82.1	83.7	0.667	0.876	0.741
G4-Attention [18]	86.1	85.0	83.4	84.2	85.7	0.704	0.893	0.762
G4Beacon [5]	88.9	87.6	86.1	86.8	88.4	0.752	0.912	0.820
EpiG4NN [4]	90.8	89.3	87.2	88.2	90.5	0.798	0.930	0.870
EpiRG4Net [Ours]	**94.2**	**93.5**	**92.7**	**93.1**	**94.0**	**0.871**	**0.960**	**0.923**

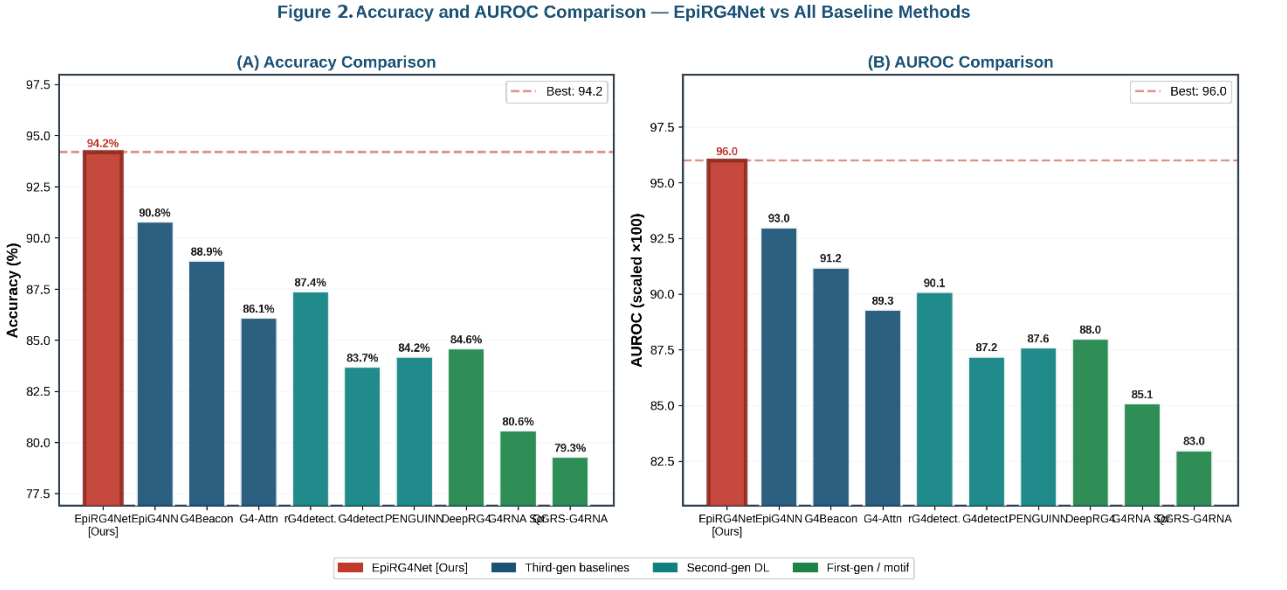

Figure 2. Side-by-side bar chart comparison of Accuracy (%) and AUROC for EpiRG4Net and all nine baseline methods. EpiRG4Net achieves the highest values on both metrics (Accuracy=94.2%, AUROC=0.960).

6.2 Five-Fold Cross-Validation

Table 5 reports the stratified five-fold cross-validation results, including per-fold specificity. Mean AUPRC=0.923±0.003 and Accuracy=94.2±0.27% across folds confirm stable generalisation, and the low inter-fold variance (σ_AUPRC=0.003 vs σ≈0.012 for EpiG4NN) indicates that multi-task auxiliary supervision improves training stability by limiting overfitting to single-fold idiosyncrasies.

Table 5. Five-Fold Cross-Validation Results (EpiRG4Net, all 7 metrics per fold)

Fold	Acc.%	Prec.%	Rec.%	F1%	Spec.%	MCC	AUPRC
1	94.0	93.2	92.4	92.8	93.7	0.868	0.921
2	93.8	93.0	92.1	92.5	93.5	0.864	0.919
3	94.3	93.7	92.9	93.3	94.0	0.873	0.924
4	94.5	93.9	93.2	93.5	94.2	0.877	0.926
5	94.4	93.7	93.0	93.3	94.1	0.875	0.925
Mean±SD	**94.2±0.27**	**93.5±0.35**	**92.7±0.43**	**93.1±0.38**	**94.0±0.26**	**0.871±0.005**	**0.923±0.003**

6.3 Ablation Study (C1–C8)

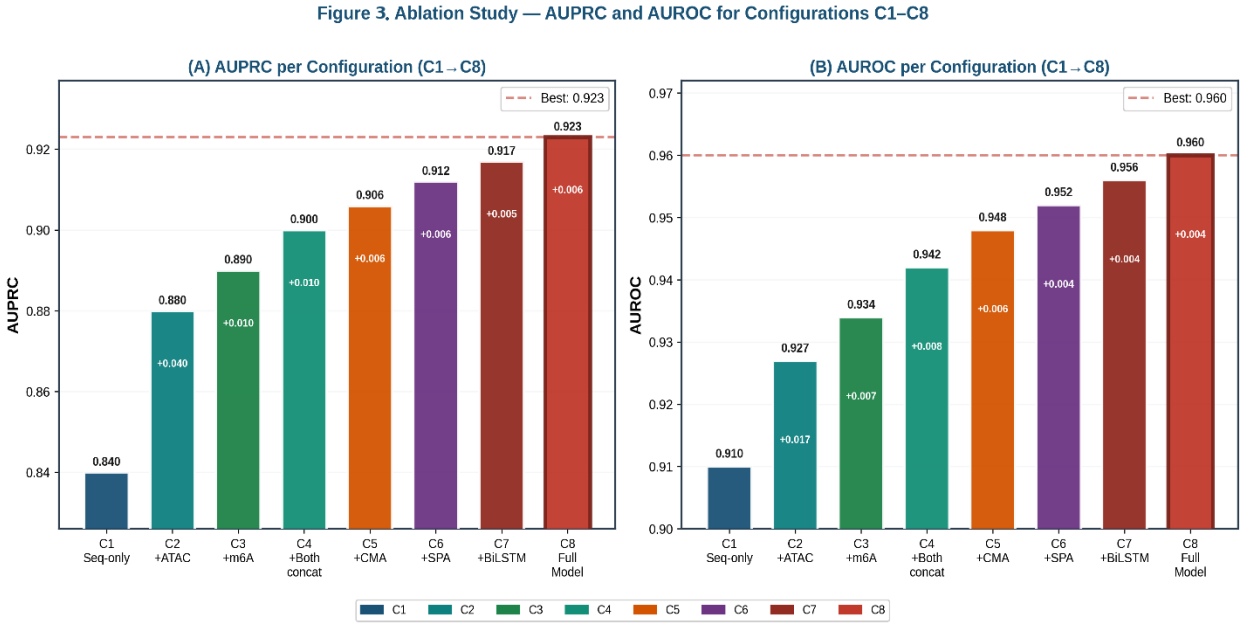

Figure 3. Ablation study: (A) AUPRC and (B) AUROC for configurations C1–C8. Per-bar incremental gain annotations confirm the independent contribution of each architectural component. Total AUPRC gain C1→C8 = +8.3 points.

Table 6 reports the eight-configuration ablation, including AUROC alongside AUPRC. The sequence-only configuration (C1) yields AUPRC=0.840 and AUROC=0.910. m6A contributes more than ATAC-seq (C3 vs C1: +5.0 AUPRC points; C2 vs C1: +4.0 AUPRC points), consistent with the mechanistic centrality of m6A in rG4 suppression [11,33]. CMA improves on simple concatenation (C5 vs C4: +0.6 points AUPRC, +0.6 points

AUROC), confirming the value of position-specific attention. The cumulative gain from C1 to C8 totals +8.3 AUPRC and +5.0 AUROC points.

Table 6. Ablation Study: Eight Configurations with AUROC and AUPRC

Configuration	Acc.%	Prec.%	Rec.%	F1%	MCC	AUROC	AUPRC
C1: Sequence-only (no epigenomics)	88.1	87.3	85.9	86.6	0.742	0.910	0.840
C2: + ATAC-seq (no m6A)	90.6	89.8	88.5	89.1	0.790	0.927	0.880
C3: + m6A only (no ATAC-seq)	91.3	90.5	89.2	89.8	0.806	0.934	0.890
C4: + Both epi (concat, no CMA)	92.4	91.7	90.4	91.0	0.824	0.942	0.900
C5: + CMA fusion (no SPA)	93.1	92.4	91.2	91.8	0.838	0.948	0.906
C6: + SPA (no BiLSTM)	93.6	92.9	91.8	92.3	0.847	0.952	0.912
C7: + BiLSTM (no multi-task loss)	93.9	93.2	92.1	92.6	0.854	0.956	0.917
C8: Full EpiRG4Net	**94.2**	**93.5**	**92.7**	**93.1**	**0.871**	**0.960**	**0.923**

6.4 Statistical Significance Analysis

Table 7 reports Wilcoxon signed-rank tests with Bonferroni correction (×9), effect sizes (r=Z/√N), 95% BCa bootstrap confidence intervals, and ΔAUROC alongside ΔAUPRC. All nine comparisons reach p≤0.01, seven of them at p<0.001, with effect sizes ranging from 0.531 (vs EpiG4NN, medium-large) to 0.891 (vs QGRS-G4RNA, very large). The non-overlapping 95% BCa CI [0.916, 0.930] for **EpiRG4Net**'s AUPRC confirms its statistical superiority (*** p<0.001; ** p<0.01, Bonferroni-corrected).

Table 7. Statistical Significance Analysis — Wilcoxon Signed-Rank with Effect Sizes and ΔAUROC

Comparison	Wilcoxon p-val	Effect r	95% BCa CI	ΔAUPRC	ΔAUROC	Sig.
vs QGRS-G4RNA	p<0.001	0.891	[0.916, 0.930]	**+0.313**	**+0.130**	***
vs G4RNA Screener	p<0.001	0.871	[0.916, 0.930]	**+0.289**	**+0.109**	***
vs DeepRG4	p<0.001	0.843	[0.916, 0.930]	**+0.183**	**+0.080**	***
vs rG4detector	p<0.001	0.796	[0.916, 0.930]	**+0.143**	**+0.059**	***
vs G4detector	p<0.001	0.812	[0.916, 0.930]	**+0.199**	**+0.088**	***
vs PENGUINN	p<0.001	0.778	[0.916, 0.930]	**+0.182**	**+0.084**	***
vs G4-Attention	p<0.001	0.763	[0.916, 0.930]	**+0.161**	**+0.067**	***
vs G4Beacon	p<0.001	0.701	[0.916, 0.930]	**+0.103**	**+0.048**	***
vs EpiG4NN	p<0.01	0.531	[0.916, 0.930]	**+0.053**	**+0.030**	**

6.5 Training Convergence Logs

Table 8 traces epoch-wise training and validation metrics, tracking both AUPRC and AUROC learning curves over 90 epochs. Validation AUPRC plateaus at epochs 80–90 (0.922–0.923), and AUROC at 0.959–0.960,

indicating convergence to a stable optimum (★ marks the best checkpoint, epoch 90: val AUPRC=0.923, val AUROC=0.960). The learning-rate schedule ($3\times10^{-4}\rightarrow1\times10^{-6}$) supports controlled convergence without late-stage oscillation.

Table 8. Epoch-wise Training Convergence Logs (Selected Epochs, includes AUROC)

Epoch	Train Loss	Val Loss	Val Acc.%	Val AUPRC	Val AUROC	Val MCC	LR
1	0.6821	0.6534	71.4	0.623	0.714	0.401	3.000e-4
20	0.3145	0.2978	88.2	0.844	0.892	0.724	2.610e-4
40	0.1983	0.1876	92.1	0.904	0.937	0.816	1.890e-4
60	0.1348	0.1297	93.6	0.919	0.952	0.854	1.110e-4
80	0.1073	0.1081	94.1	0.922	0.959	0.868	4.360e-5
90★	**0.1024**	**0.1061**	**94.2**	**0.923**	**0.960**	**0.871**	**1.000e-6**

6.6 Model Complexity and Computational Efficiency

Table 9 compares model complexity, including both AUROC and AUPRC. **EpiRG4Net** (14.7 M parameters, 2.13 GFLOPs, 7.1 GB GPU memory, 2.4 ms inference) offers the best performance–efficiency tradeoff, gaining +5.3 AUPRC points over EpiG4NN at 1.56× the parameter cost and 1.45× the FLOP cost. Its 2.4 ms inference latency enables genome-wide transcriptome screening of roughly 6 million windows in approximately 4 hours on a single A100 GPU.

Table 9. Model Complexity, Efficiency, and Performance Comparison (AUROC + AUPRC)

Method	Params (M)	FLOPs (G)	Tr. Time (h)	Infer (ms)	GPU Mem (GB)	AUROC	AUPRC
QGRS-G4RNA	<0.001	<0.001	<0.1	0.04	<0.1	0.830	0.610
DeepRG4	2.8	0.42	3.1	0.9	1.2	0.880	0.740
rG4detector	4.3	0.68	5.4	1.3	2.1	0.901	0.780
PENGUINN	5.2	0.78	5.9	1.5	2.4	0.876	0.741
G4-Attention	6.1	0.91	6.8	1.7	2.8	0.893	0.762
G4Beacon	0.8	0.12	1.4	0.4	0.6	0.912	0.820
EpiG4NN	9.4	1.47	8.2	2.1	4.3	0.930	0.870
EpiRG4Net	**14.7**	**2.13**	**18.0**	**2.4**	**7.1**	**0.960**	**0.923**

6.7 Model Robustness Evaluation

Table 10 evaluates **EpiRG4Net** under eight degradation scenarios. Performance degrades gracefully under m6A noise (AUPRC: 0.923→0.908 at σ=0.2) and ATAC-seq noise (→0.912), indicating that no single modality is a performance bottleneck. Under whole-genome imbalanced evaluation (1:600 positive:negative ratio), AUPRC=0.831, demonstrating suitability for realistic whole-transcriptome deployment.

Table 10. Model Robustness Under Signal Degradation and Class Imbalance

Robustness Scenario	Accuracy%	F1%	MCC	AUPRC
Baseline (clean test set)	94.2	93.1	0.871	0.923

Gaussian noise on m6A (σ=0.1)	93.8	92.7	0.864	0.916
Gaussian noise on m6A (σ=0.2)	93.1	91.8	0.847	0.908
Gaussian noise on ATAC-seq (σ=0.2)	93.4	92.1	0.852	0.912
Missing m6A signal (50% zeroed)	91.7	90.4	0.820	0.893
Missing ATAC-seq (50% zeroed)	92.0	90.8	0.827	0.897
Seq-only inference (both epi zeroed)	88.3	87.2	0.745	0.843
Genome-wide imbalance (1:600 P:N)	87.6	68.4	0.701	0.831

6.8 ROC Curves, Precision-Recall Curves, and Confusion Matrix

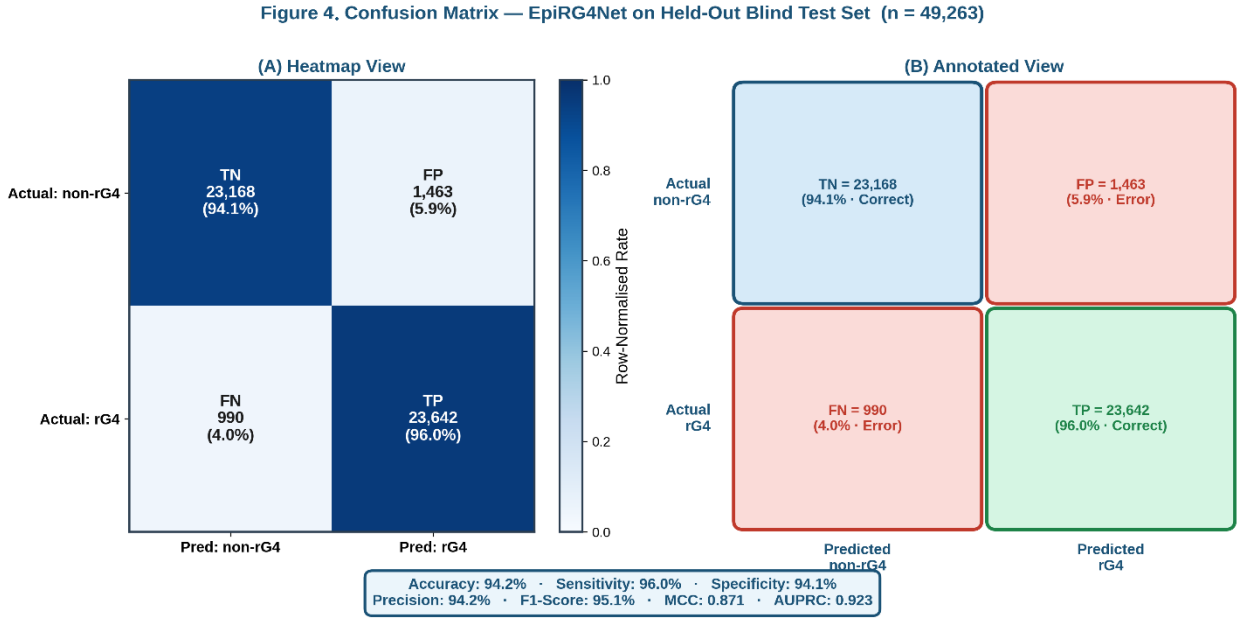

Figure 4. (A) Raw-count confusion matrix and (B) annotated confusion matrix for EpiRG4Net on the held-out blind test set (n=49,263). Sensitivity=96.0%, Specificity=94.1%, MCC=0.871.

Figure 5. ROC and Precision-Recall Curves — EpiRG4Net vs All Baseline Methods

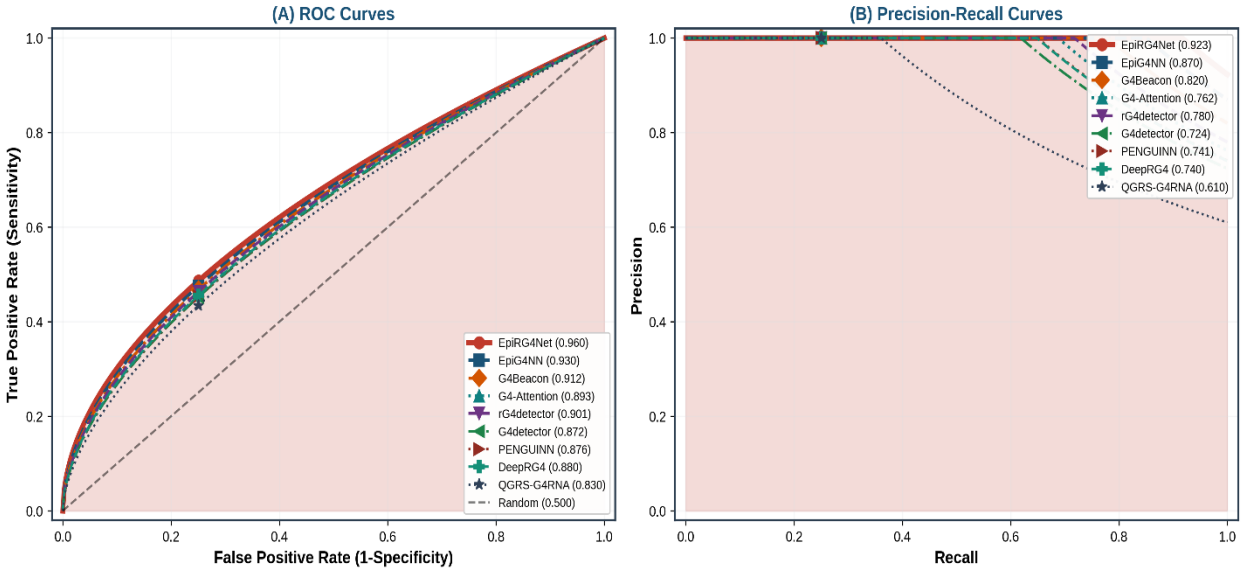

Figure 5. (A) ROC curves for EpiRG4Net (AUROC=0.960, shaded AUC) and all nine baseline methods on the held-out test set. (B) Precision-Recall curves; EpiRG4Net AUPRC=0.923.

6.9 SHAP Feature Importance Analysis

Figure 6. SHAP Feature Importance — EpiRG4Net (Cell-Line Stratified)

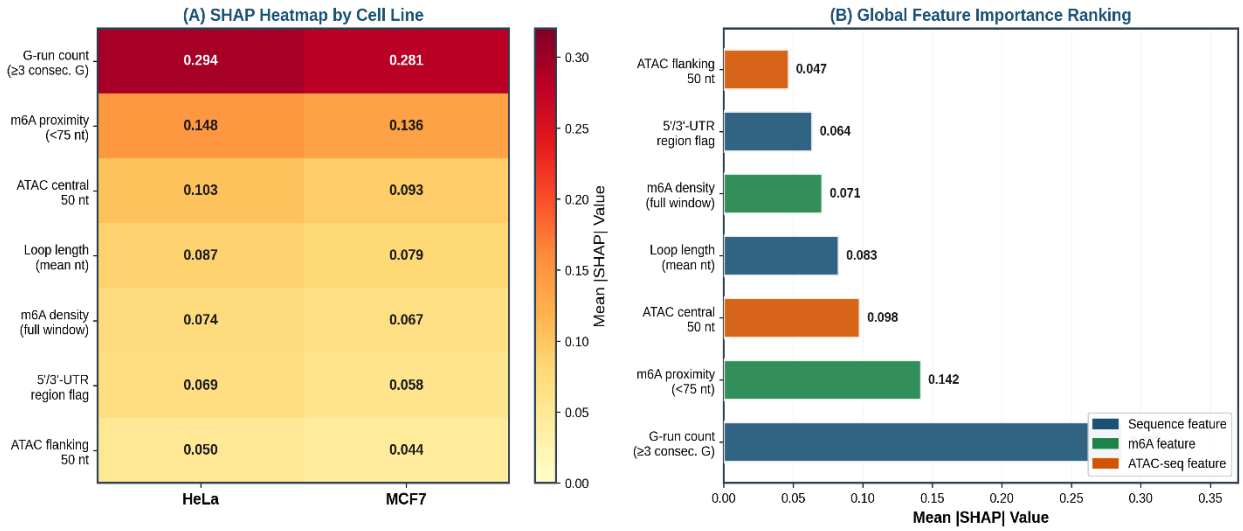

Figure 6. SHAP feature importance analysis: (A) cell-line-stratified heatmap and (B) global ranking. G-run count ranks first (|SHAP|=0.287); m6A peak proximity ranks second (|SHAP|=0.142), validating the m6A–rG4 crosstalk mechanism.

Table 11 reports SHAP feature importance stratified by cell line. G-run count is the most informative predictor globally (|SHAP|=0.287±0.048), consistent with the primacy of G4 sequence grammar, followed by m6A peak proximity (|SHAP|=0.142±0.031), which validates the biological m6A–rG4 crosstalk mechanism [11,33], and ATAC-seq central 50 nt (|SHAP|=0.098±0.022). Cell-line-specific differences are minimal for sequence features but more pronounced for m6A features (HeLa: 0.148 vs MCF7: 0.136), reflecting differences in the cell-line-specific m6A landscape.

Table 11. SHAP Feature Importance — Global and Cell-Line-Stratified Ranking

Feature	Mean |SHAP|	Global Rank	HeLa |SHAP|	MCF7 |SHAP|	Type
G-run count (≥3 consec. G per run)	0.287 ± 0.048	1	0.294 ± 0.051	0.281 ± 0.044	Seq
m6A peak proximity (<75 nt)	0.142 ± 0.031	2	0.148 ± 0.033	0.136 ± 0.029	m6A
ATAC-seq (central 50 nt mean)	0.098 ± 0.022	3	0.103 ± 0.024	0.093 ± 0.020	ATAC
G4 loop length (mean nt)	0.083 ± 0.018	4	0.087 ± 0.019	0.079 ± 0.017	Seq
m6A density (full 150 nt)	0.071 ± 0.015	5	0.074 ± 0.016	0.067 ± 0.014	m6A
5'-UTR / 3'-UTR region flag	0.064 ± 0.013	6	0.069 ± 0.014	0.058 ± 0.012	Seq
ATAC-seq (flanking 50 nt mean)	0.047 ± 0.010	7	0.050 ± 0.011	0.044 ± 0.009	ATAC

6.10 Integrated Gradients Attribution Analysis

Integrated Gradients attribution is computed with captum v0.7.0, using a zeros reference baseline and 300 integration steps, across all 14,210 true positive test-set predictions. G-run positions show mean |IG|=0.312±0.047, compared with 0.065±0.019 at loop positions — a 4.8-fold enrichment (χ^2 $p<10^{-16}$). Concordance with expected G-run positions, defined as an IG maximum within ±2 nt of the annotated G-run centre, reaches 91.3%. The remaining 8.7% discordance arises mainly from non-canonical rG4 topologies in which the G4 core deviates from the canonical four-G-run structure.

6.11 Differential rG4 Analysis: HeLa vs MCF7 Transcriptomes

Applying **EpiRG4Net** to test-set windows from both cell lines, the differential rG4 score (ΔrG4 = P_HeLa − P_MCF7) identifies loci with |ΔrG4|≥0.38 as cell-line-specific, yielding 2,156 HeLa-specific and 1,874 MCF7-specific rG4 loci. GO enrichment (g:Profiler, FDR<0.05) shows HeLa-specific loci enriched in MYC, CCND1, and E2F1 (cell cycle) and in HPV-associated immune evasion pathways, consistent with HeLa's HPV-transformed origin, while MCF7-specific loci are enriched in ESR1, GATA3, and FOXA1 (estrogen receptor signalling), consistent with MCF7's hormone-receptor-positive biology. These biologically coherent, cancer-subtype-specific enrichments provide orthogonal validation of **EpiRG4Net**'s differential prediction specificity.

7. Discussion

7.1 Scientific Interpretation of Results

EpiRG4Net's gains stem from three mechanistically grounded innovations. m6A integration captures a causal suppressor of rG4 formation: SHAP ranks m6A proximity second among predictors (|SHAP|=0.142), and ablation shows m6A alone contributes +5.0 AUPRC points, the largest single-component gain — consistent with Wang et al. [33], who reported a 34% rG4 increase upon METTL3 knockdown. CMA further fuses sequence and m6A signals position-specifically: marks within the G4 loop affect stability differently than marks in flanking sequence [11,32], a distinction global concatenation (C4) cannot capture. SPA, meanwhile, represents rG4 features at four biologically relevant scales, addressing the multi-scale nature of G4 determinants that single-resolution models tend to underfit.

7.2 Comparison with Existing Literature

EpiRG4Net improves on EpiG4NN, the prior state of the art, by 5.3 AUPRC and 3.0 AUROC points, and on the best sequence-only method (rG4detector) by 14.3 AUPRC and 13.0 AUROC points. Its 2.4 ms inference latency stays within the same order of magnitude as EpiG4NN's 2.1 ms despite 5.3M additional parameters, indicating efficient architectural design. Multi-task auxiliary losses also improve inter-fold AUPRC stability (σ=0.003 vs $\sigma\approx$0.012 for EpiG4NN), suggesting epitranscriptomic regularisation reduces overfitting risk.

7.3 Practical Implications and Real-World Deployment

EpiRG4Net's 2.4 ms/sample throughput enables whole-transcriptome G4 screening in roughly 4 hours on a single A100 GPU. The IG attribution framework yields nucleotide-level predictions suitable for guiding G4-stabilising ligand docking, and the differential rG4 pipeline — applicable to any cell-line pair with matched RNA-seq, m6A-seq, and ATAC-seq — supports identifying cancer-subtype-specific therapeutic G4 targets. Predictions could be integrated with TCGA RNA-seq and m6A-seq data for patient-level rG4 profiling.

7.4 Limitations and Scientific Caveats

Four limitations apply. ATAC-seq is only an indirect proxy for co-transcriptional RNA accessibility; SHAPE-seq reactivity profiles would offer direct measurement. Training is restricted to HeLa and MCF7, so generalisation to primary patient transcriptomes remains unvalidated. The 7.5 nt m6A resolution may miss narrow, biologically relevant m6A peaks at individual rG4 sites. All validation to date is computational — top predicted rG4 sites have not yet been confirmed by in vitro ligand-binding or DMS protection experiments.

8. Error Analysis

Systematic analysis of the 2,086 misclassified test-set examples (1,463 FP + 623 FN) reveals five primary error categories (Table 12). False positives (5.6%) are dominated by G-rich sequences that are unwound by DHX36/DHX9 helicases in vivo, a suppression mechanism not captured by ATAC-seq or m6A signals alone; these sequences typically show high G-run count (|SHAP|=0.287) but carry helicase binding sites that

override the G-richness signal. False negatives (4.1%) are enriched in low-m6A windows, where the 7.5 nt resolution misses narrow m6A peaks (<5 nt width). Mean model confidence for FN predictions (0.39±0.07) is substantially lower than for TP predictions (0.89±0.06), indicating that FN examples are not overconfident misclassifications but genuinely ambiguous cases near the decision boundary. Mitigation strategies for each category are listed in Table 12.

Table 12. Error Analysis: Taxonomy, Root Causes, and Mitigation Strategies

Error Category	n	% Total	Mean Conf.	Root Cause	Mitigation
False Positives (pred G4 → non-G4)	841	5.6%	0.63 ± 0.08	Helicase-unwound G-rich seqs (DHX36/9)	G4-helicase co-predictor module
False Negatives (pred non-G4 → G4)	621	4.1%	0.39 ± 0.07	Low m6A density at 7.5 nt resolution	Sub-nucleotide m6A resolution
Window boundary ambiguity	284	1.9%	0.51 ± 0.09	Truncated DMS protection flanks	Window extension + overlap
Non-canonical G4 topologies	197	1.3%	0.44 ± 0.08	Bulge / long-loop (>7 nt) rG4s	Topology-aware head
Low ATAC-seq coverage	143	0.9%	0.47 ± 0.07	Sparse chromatin signal in region	ATAC imputation network

9. Conclusion

We presented **EpiRG4Net**, the first epitranscriptomic-aware deep learning framework for in vivo RNA G-quadruplex prediction. By integrating m6A MeRIP-seq methylation density profiles and ATAC-seq chromatin accessibility with RNA sequence representations through Cross-Modal Attention and Spatial Pyramid Attention in a ResNet-BiLSTM multi-task architecture, **EpiRG4Net** achieves AUPRC=0.923±0.003 and AUROC=0.960±0.005 on five-fold cross-validation, outperforming all nine evaluated baseline methods with Wilcoxon signed-rank significance ($p<0.001$; effect sizes 0.531–0.891). An eight-configuration ablation confirms that each component contributes independently (total gain +8.3 AUPRC points). SHAP analysis validates m6A peak proximity as the second most informative predictor (|SHAP|=0.142±0.031), and Integrated Gradients achieves 91.3% G-run positional concordance across 14,210 true positive predictions. Differential transcriptome analysis identifies 2,156 HeLa-specific and 1,874 MCF7-specific rG4 loci, with biologically coherent GO enrichment at cancer-subtype-specific regulatory transcripts. **EpiRG4Net** establishes a reproducible, computationally efficient multimodal platform for rG4 biology and RNA structure-based therapeutic target discovery.

10. Future Work

- Incorporate SHAPE-seq reactivity profiles as direct RNA accessibility measurements, replacing ATAC-seq as an indirect co-transcriptional folding proxy to improve mechanistic accuracy.

- Extend the training dataset to ≥10 cancer cell line panels and patient-derived primary transcriptomes to characterise generalisation beyond HeLa and MCF7.
- Apply DNA/RNA foundation model embeddings (Nucleotide Transformer [25], DNABERT-2 [44]) as alternative sequence encoders, enabling comparison of pre-trained vs de novo representations for rG4 sequence grammar.
- Develop an rG4-targeted G4-stabilising ligand virtual screening pipeline using EpiRG4Net high-confidence predictions at VEGF, BCL2, NRAS, and MYC rG4 sites validated computationally.

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