

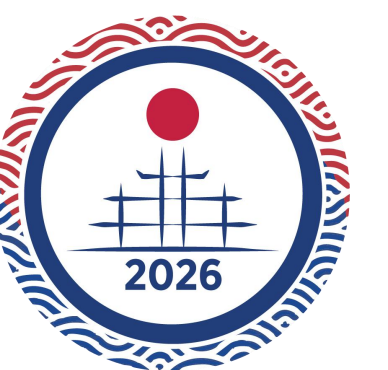


Rapid Diagnosis of Malignant Pleural Effusion Based on a Cell-Free DNA Multi-Omics Model Using Ultra-Low-Pass Nanopore Sequencing

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INTRODUCTION

Malignant pleural effusion (MPE) is a common complication of malignancies, affecting approximately 15% to 20% of cancer patients, and represents the second leading cause of exudative pleural effusions. Despite its high prevalence and severe prognostic implications, diagnosis remains challenging due to the low sensitivity (~60%) of routine cytological examination.

Cell-free DNA (cfDNA)-based liquid biopsy has emerged as a promising complementary diagnostic tool. Unlike conventional next-generation sequencing (NGS), which requires separate biochemical workflows to capture methylation alongside genomic and fragmentomic signals, Nanopore sequencing natively resolves CpG methylation states during base-calling without additional library modifications, enabling true single-run multi-omics profiling at ultra-low cost. In this study, we developed and independently validated a multi-omics cfDNA fusion model leveraging this capability to discriminate MPE from benign pleural effusion (BPE).

METHODS

In the discovery cohort, pleural effusion cfDNA from 111 patients (62 MPE, 49 BPE) was profiled by Nanopore sequencing at $\sim 1.2 \times$ mean depth. Three omics profiles were extracted: (i) methylation (5-kb bin-level hyper- and hypo-DMRs, filtered by age- and sex-adjusted GLM); (ii) copy number variations (ichorCNA-derived metrics); and (iii) fragmentomics (differential end-motifs and fragment size abundance). Three single-omics logistic regression classifiers were trained via 5-fold cross-validation ($n=77$) and evaluated on a test set ($n=34$).

An early-fusion model integrating all three feature domains was subsequently trained, optimizing feature selection via recursive feature elimination with cross-validation (RFECV). Depth robustness was assessed by *in silico* downsampling to $0.2 \times$, $0.1 \times$ (three replicates per depth). Finally, the model was evaluated in an independent validation cohort of 147 patients (72 MPE, 75 BPE) at a mean sequencing depth of $\sim 0.19 \times$.

RESULTS

- Single-omics classifiers yielded test-set AUCs ranging from 0.775 (fragmentomics) to 0.940 (methylation)(Figure 1). While the CNV classifier achieved highest sensitivity of 84.2%, no single-omics model simultaneously achieved both high sensitivity and specificity ($\leq 86.7\%$).
- RFECV identified five cross-domain features for the fusion model: global hyper and hypo-methylation DMRs means from methylation, tumor fraction; chr9p loss burden from CNV and AACT end-motif. Crucially, the multi-omics fusion model achieved a superior test-set AUC of 0.944; it successfully maintained the peak baseline sensitivity of 84.2% while substantially boosting specificity to 93.3%. In *in silico* downsampling confirmed the fusion model's stable performance down to $0.1 \times$, maintaining sensitivity $\geq 78.9\%$, specificity $\geq 86.7\%$, precision $\geq 88.2\%$ across all replicates (Figure 2).
- In the independent validation cohort, the multi-omics model demonstrated sustained diagnostic efficacy: AUC 0.924, sensitivity 80.6%, specificity 94.7%, and PPV 93.5% (Figure 3).

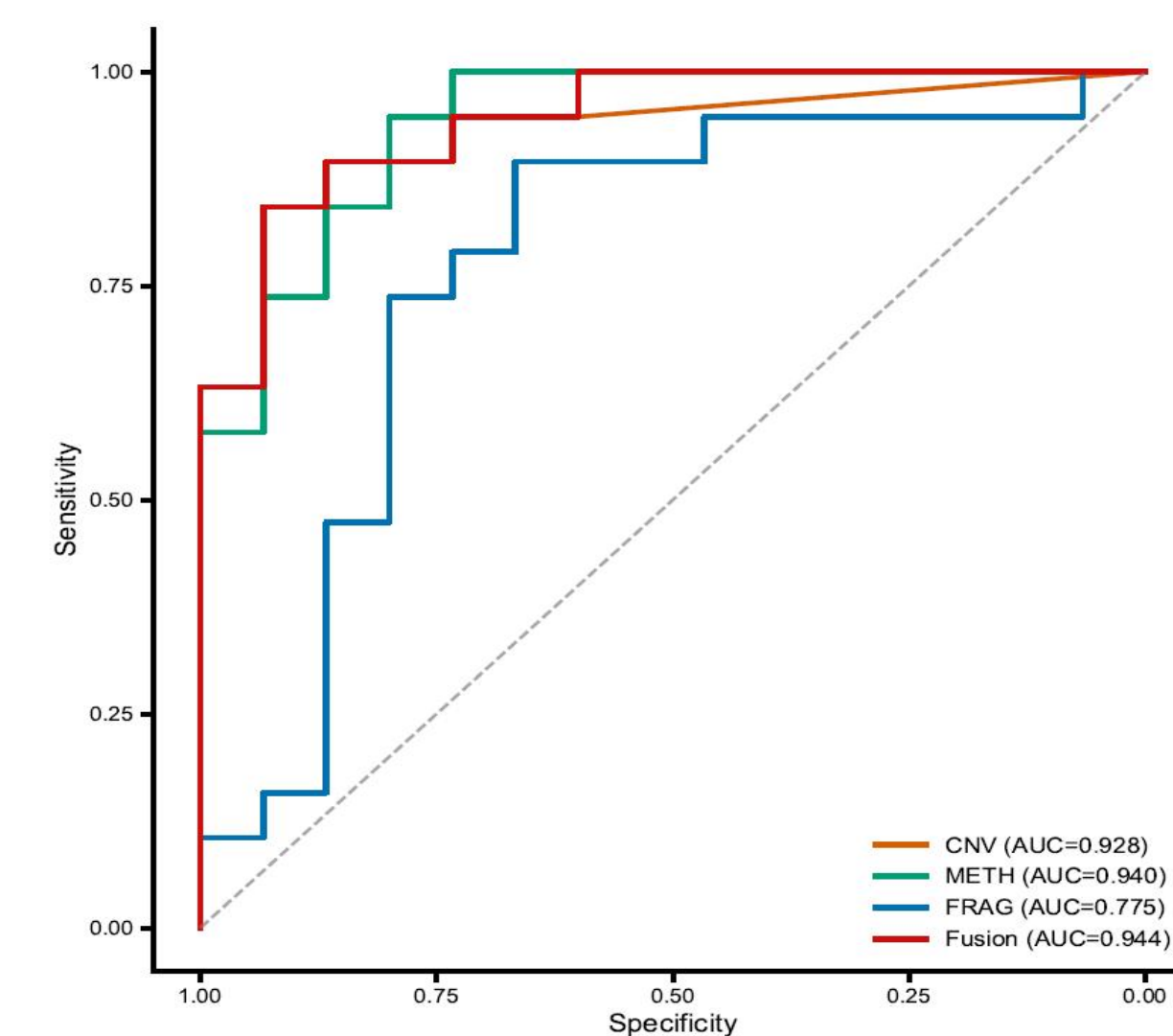


Figure 1. ROC curves of single-omics and multi-omics models in the test set.

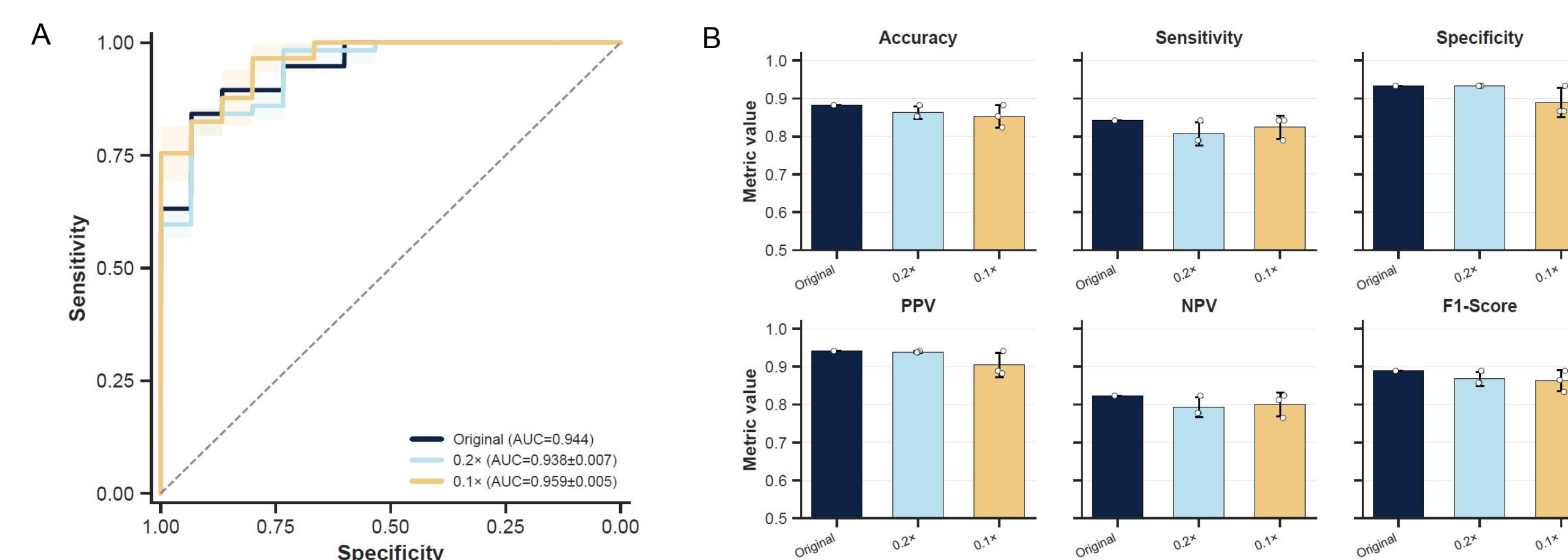


Figure 2. Performance of the multi-omics fusion model across sequencing depths: ROC curves (A) and diagnostic metrics (B).

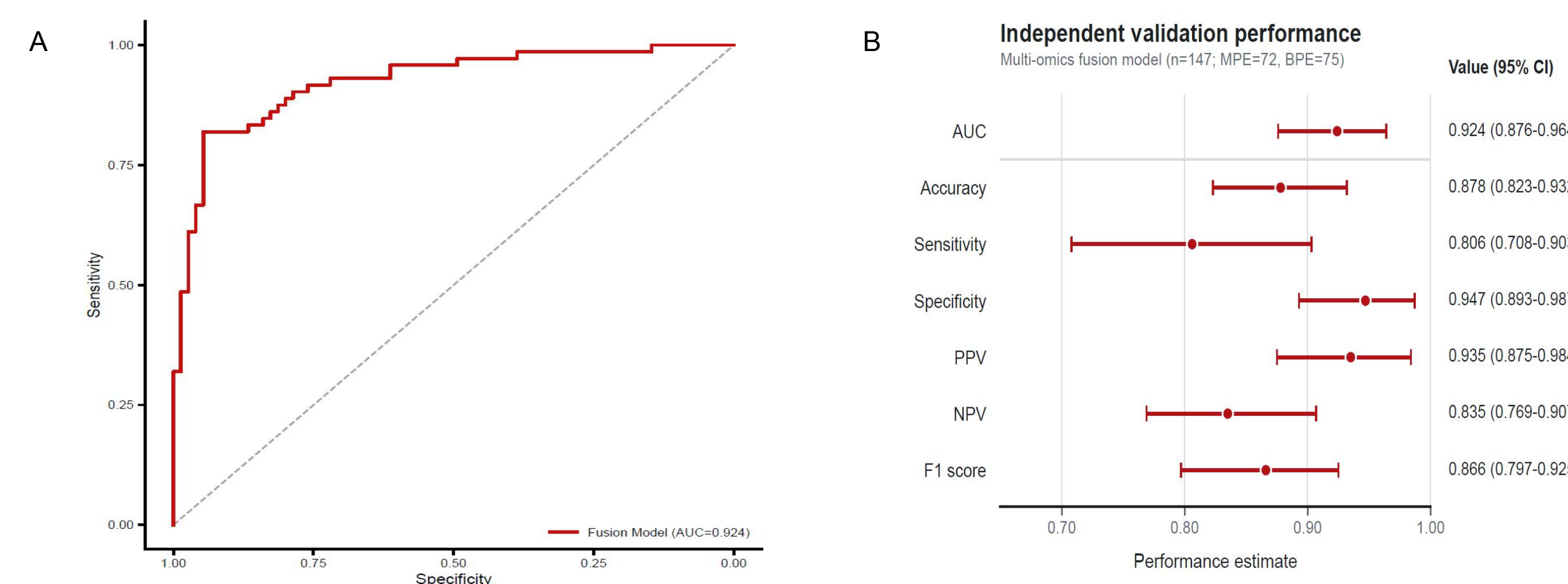


Figure 3. Diagnostic efficacy of the multi-omics fusion model in the independent validation cohort: ROC curve (A) and diagnostic performance metrics with 95% confidence intervals (B).

DISCUSSION AND CONCLUSION

- Ultra-low-pass Nanopore sequencing enables simultaneous multi-omics cfDNA profiling at depths as low as $0.2 \times$. The multi-omics fusion model achieved high diagnostic sensitivity across both discovery and independent validation cohorts, positioning it as a reliable adjunct in the diagnostic workup of pleural effusion.
- Benefiting from streamlined library preparation, rapid sequencing turnaround at ultra-low depths, and minimal sequencing costs of Nanopore sequencing, this platform offers a highly scalable and cost-effective complement to cytology, demonstrating strong translational potential for clinical settings.

REFERENCES & ACKNOWLEDGEMENTS

References:

- Katsman E, et al. Detecting cell-of-origin and cancer-specific methylation features of cell-free DNA from Nanopore sequencing. *Genome Biology*. 2022;23:158. Scan QR-code for the most critical references.

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Artificial Intelligence (AI) Use Disclaimer

No artificial-intelligence tools were used in the preparation of this e-poster.