

Developing a Novel Genomic Biomarker for the Early Detection of Neurodegeneration

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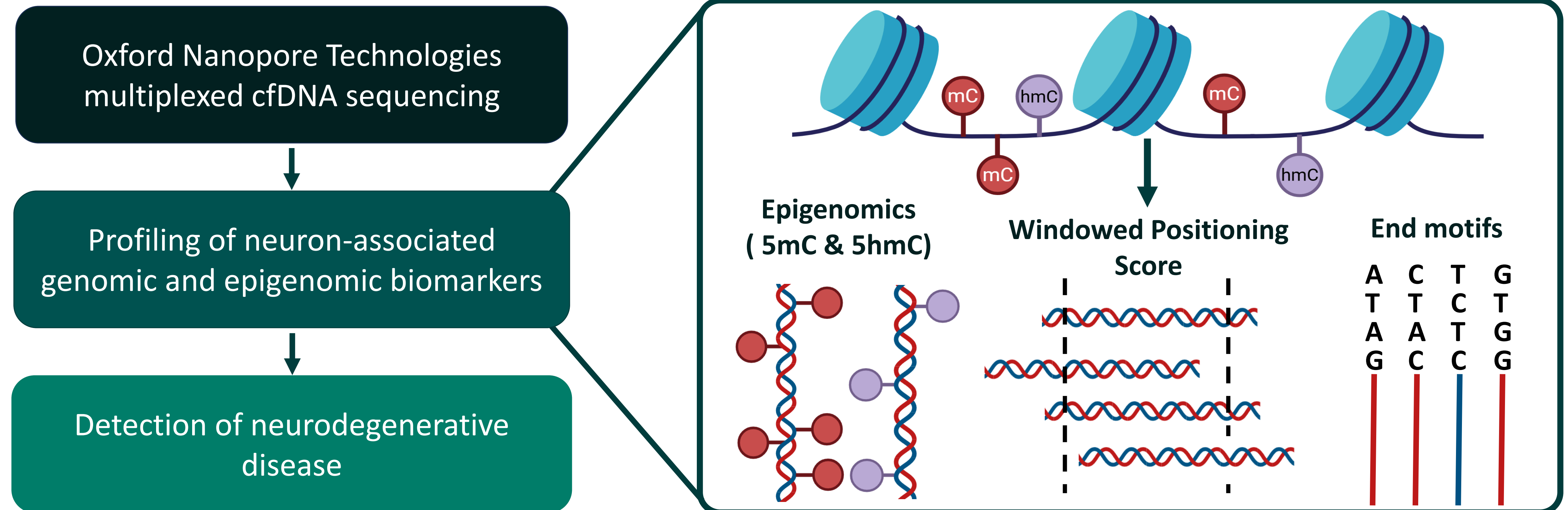
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Introduction

Current diagnostic techniques cannot reliably detect neurodegeneration before at the earliest stages of Alzheimer's disease (AD).¹ We aimed to develop a sequencing-based assay that utilises multiple features of **cell-free DNA (cfDNA)** in plasma as biomarkers of AD. The software developed, named **NEON** (NEuron-derived cfDNA biomarker of neurodegeneratiON), leverages **DNA methylation (5mC)**, **DNA hydroxymethylation (5hmC)**, **nucleosome positioning (Windowed Positioning Score; WPS)**, and **fragment end motif** derived signals from a single sequencing experiment to detect the cfDNA-associated signatures of neuronal damage.^{2,3}

Methods



Results

Figure 1: Deconvolution of cell type proportions in plasma samples from Alzheimer's disease patients & age-matched controls. Using NEONs cfDNA-optimised deconvolution algorithm, oligodendrocyte-derived cfDNA was found significantly be enriched in AD plasma ($\log_2FC = 1.55$, $p = 0.026$), whereas granulocyte-derived cfDNA was found to be significantly depleted in AD plasma ($\log_2FC = -0.34$, $p = 0.036$). Borderline significant enrichments were also observed in alveolar epithelium-derived cfDNA ($\log_2FC = 1.46$, $p = 0.055$) and neuron-derived cfDNA ($\log_2FC = 0.62$, $p = 0.087$) in AD plasma.

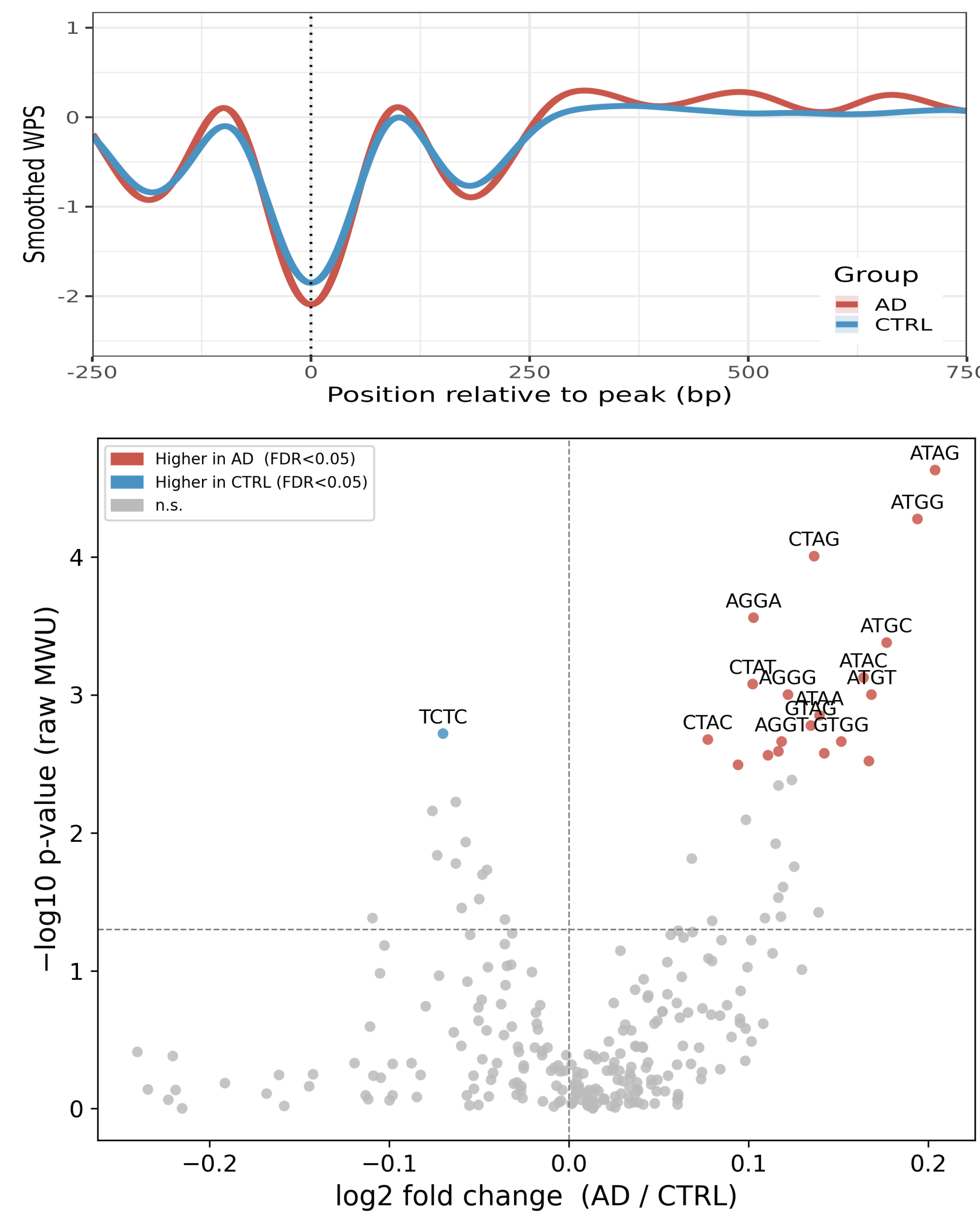
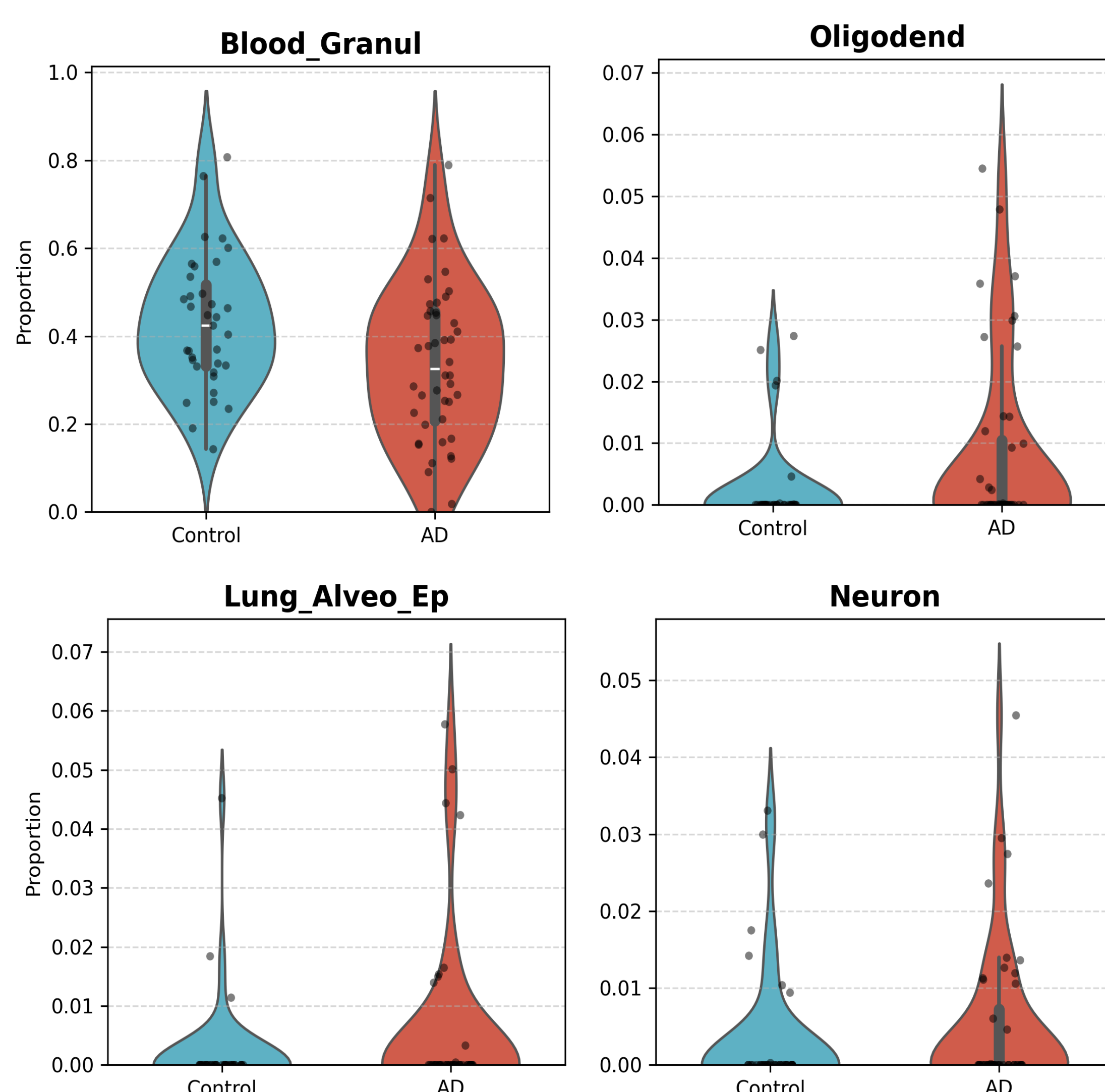
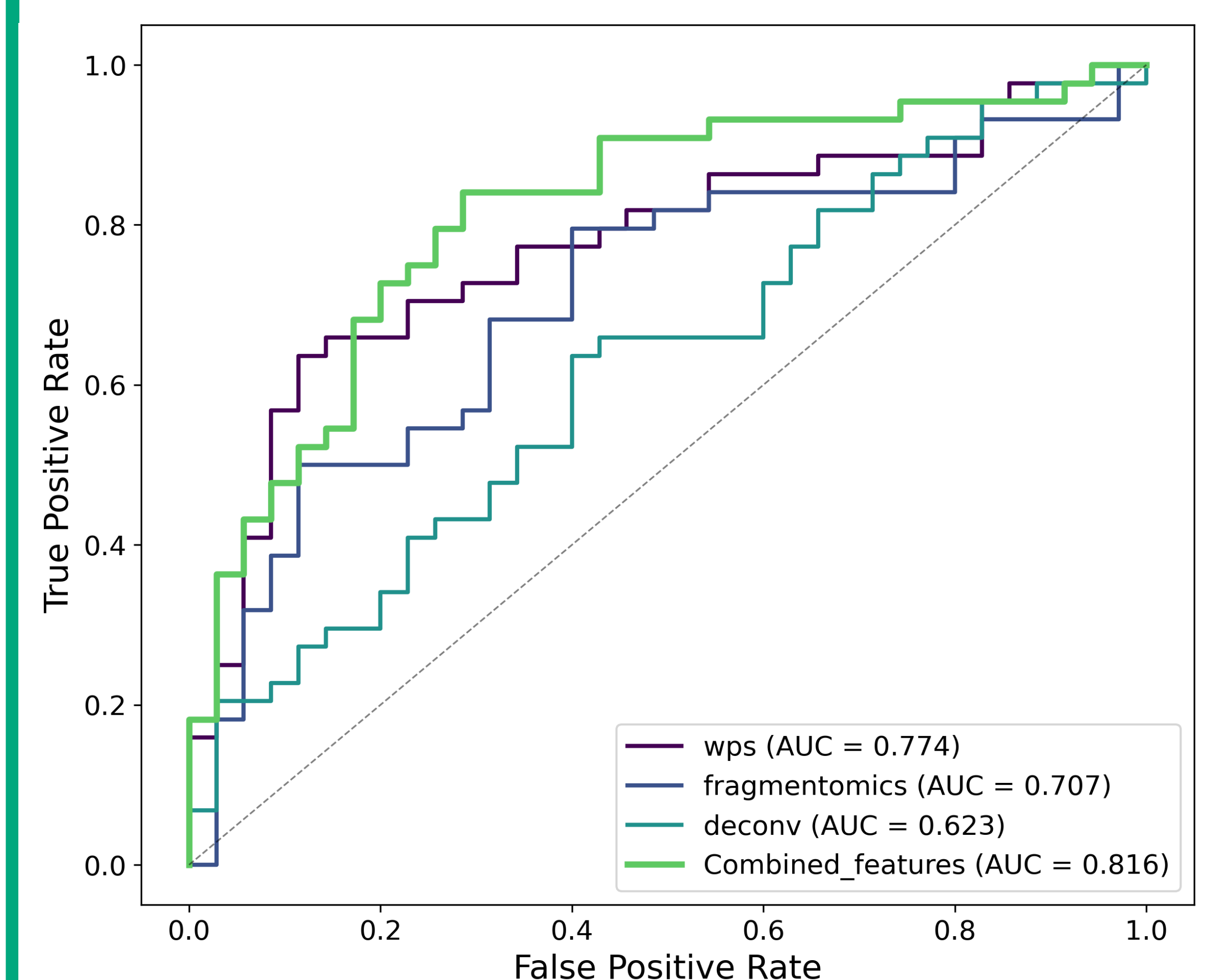


Figure 2: WPS and end motif analysis reveal characteristic fragmentomic differences in AD plasma. WPS analysis detects AD-associated open chromatin signatures at via plasma cfDNA, indicated by increased trace phasing. Fragment end motif analysis (bottom) suggests AD cfDNA is enriched for A-starting end motifs, suggesting decreased DNASE1L3-driven apoptotic fragmentation toward alternative nuclease activity.

Figure 3: Leave-One-Out cross validation using a deconvolution, window positioning score, fragment end motif, and a combined genomic biomarker feature panel. ROC analysis demonstrates that a ridge regression model trained on a combined panel of the most discriminative genomic biomarker features achieves superior classification ($AUC = 0.816$) compared to deconvolution ($AUC = 0.623$), fragment end motif ($AUC = 0.707$), and windows positioning core ($AUC = 0.774$) features alone.



Discussion

Preliminary results for using NEON to detect neuron-derived cfDNA as a biomarker neurodegeneration are promising, however testing of diagnostic capability in full-scale patient populations as well as integration with further undiscussed genomic biomarkers are required to refine this approach before it is fit for use in aiding Alzheimer's disease diagnosis.

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References

- Kimmineni KM, Cash DM, Poole T, Frost C, Benzing TL, Ahsan RL, et al. Presymptomatic atrophy in autosomal dominant Alzheimer's disease: A serial magnetic resonance imaging study. *Alzheimer's and Dementia*. 2018 Jan 1;14(1):43–53.
- Galetzki H, Franklin RM, Reich DS. Cell-free DNA-based liquid biopsies in neurology. *Vol. 146, Brain*. Oxford University Press; 2023. p. 1758–74.
- Titus AJ, Gallimore RM, Salas LA, Christensen BC. Cell-type deconvolution from DNA methylation: A review of recent applications. *Vol. 26, Human Molecular Genetics*. Oxford University Press; 2017. p. R216–24.
- Song J, Xuan PF. A systematic assessment of cell type deconvolution algorithms for DNA methylation data. *Vol. 23, Briefings in Bioinformatics*. Oxford University Press; 2022.
- De Ridder K, Che H, Leroy K, et al. Benchmarking of methods for DNA methylome deconvolution. *Not Commun* 15, 4134 (2024). <https://doi.org/10.1038/s41467-024-48466-z>
- Loyfer N, Vagstadheim J, Peretz A, Cann G, Bredino J, Klochender A, et al. A DNA methylation atlas of normal human cell types. *Nature*. 2023 Jan 12;613(7943):355–64.