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Ultra-mild bisulphite sequencing for DNA methylation analysis from low-input clinical specimens

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Abstract

In a recent paper published in Nature Communications, Dai and colleagues offer a practical re-engineering of a workhorse: ultra-mild bisulphite sequencing (UMBS-seq), which re-tunes classical bisulphite conversion so that fragmented, low-input clinical DNA can yield methylomes that are not merely usable, but technically reliable. In this commentary, we set the advance in its proper frame—less a revolution than a highly optimized re-engineering—and trace the practical consequences of minimising chemical damage to input DNA: reduced DNA damage, higher library complexity, and more uniform recovery across GC content, with performance that, on key metrics, performs better than conventional bisulphite workflows and is competitive with enzymatic alternatives. In the benchmarking presented by Dai et al., these gains are reflected in higher library yield and complexity at inputs spanning nanograms to picograms, profiles shifted toward longer fragments, improved CpG/GC coverage uniformity, and more consistent C-to-U conversion with minimal background at the lowest inputs—features that matter most when clinical DNA is both scarce and fragmented. The point isn't optimisation for its own sake; it's what steadier methylomes make possible. In pregnancy cohorts—where cord-blood methylation patterns have been associated with later islet autoimmunity risk and later metabolic risk—UMBS-seq becomes an enabling instrument: it turns limited, fragmented clinical material into data robust enough to support methylation risk scores—turning scarcity from a limitation into a design criterion.

A recent study published in *Nature Communications* [1] introduces ultra-mild bisulphite sequencing (UMBS-seq), a compelling method for low-input and fragmented clinical methylome profiling. Scientific progress often unfolds as a dialogue between what we aspire to measure and what our tools can reveal. In epigenomics, that dialogue has long been mediated by bisulphite conversion, a transformative workhorse that delivered the first genome-wide maps of 5-methylcytosine (5mC), yet imposes harsh chemical penalties. High-temperature denaturation and acidic bisulphite treatment fragment DNA, reduce library yield, introduce GC-dependent dropout and conversion bias, and can artefactually inflate methylation estimates—liabilities that become prohibitive for low-input, frag-

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mented clinical specimens such as cell-free DNA from plasma, single cells and formalin-fixed paraffin-embedded (FFPE) tissues [2]. The question is whether bisulphite interpretability can be retained while minimizing DNA damage and biases at low input. For non-specialists, recent methodological overviews chart the trade-offs that govern platform choice—arrays versus sequencing, bisulphite versus enzymatic conversion, short reads versus single-molecule long reads—and make plain the recurring failure points (DNA damage, loss of complexity, coverage bias, and cost), especially when starting material is scarce or fragmented [3, 4]. In liquid-biopsy settings, where fragmentation and low mass turn those trade-offs into primary constraints, the broader technology landscape and its failure modes are reviewed in detail [5].

The study by Dai et al. [1] introduces UMBS-seq, a re-engineered bisulphite chemistry that seeks to preserve the strengths of the original method while eliminating its major drawbacks. Unlike alternative bisulphite-free enzymatic approaches such as TET-assisted pyridine borane sequencing (TAPS) or Enzymatic Methyl-seq (EM-seq), UMBS-seq does not replace bisulphite; it refines it. This distinction matters because bisulphite chemistry is simple, robust, automatable and clinically scalable. The limitations are practical rather than conceptual; conventional bisulphite chemistry is harsh.

UMBS-seq addresses this by introducing a highly optimised conversion strategy. The method achieves high conversion efficiency without the collateral damage characteristic of traditional bisulphite sequencing. In their head-to-head benchmarking against conventional bisulphite (CBS-seq) and an enzymatic alternative (EM-seq), Dai et al. show that UMBS-seq produces higher library yield across a wide input range (down to picogram quantities), with lower duplication (often used as a practical proxy for library complexity), insert-size profiles comparable to EM-seq and shifted toward longer fragments than CBS-seq, and improved CpG/GC coverage uniformity in GC-rich regulatory elements; importantly, UMBS-seq also maintains highly efficient and consistent C-to-U conversion at low-input, with low background 'unconverted C' signals that rise more noticeably in enzymatic workflows at the lowest inputs.

The result is a method that retains the interpretive clarity of bisulphite while offering superior performance across key metrics relevant to translational epigenomics. Critically, UMBS-seq demonstrates markedly improved performance in limited -input contexts, where every fragment is indispensable. Nowhere is this clearer than in their low-input cell-free DNA benchmarking: compared head-to-head with EM-seq and conventional bisulphite, UMBS-seq produces higher library concentrations, lower duplication rates (greater usable complexity), and insert-size distributions that preserve cfDNA integrity; it also improves CpG site recovery and GC coverage uniformity, and—when paired with targeted methylation capture—delivers enhanced CpG coverage from the same 5 ng of clinical input. Consistent with this, UMBS-seq shows very low background unconverted cytosines (~0.1%) across inputs, whereas background rises substantially for EM-seq at the lowest inputs (exceeding 1% in their comparison).

The study's methodological design demonstrates a precise understanding of how incremental chemical adjustments can produce disproportionately large effects on data quality. By systematically optimising temperature, pH adjustment , reaction duration, and bisulphite concentration, the authors identify conditions that maintain high conversion efficiency while markedly reducing depurination and fragmentation—a balance validated across a wide range of DNA inputs, including sub -picogram samples. Their benchmarking strategy, evaluating UMBS-seq against both conventional bisulphite and EM-seq under identical analytical settings, further strengthens the work. The inclusion of synthetic standards alongside biologically challenging materials such as cell-free DNA, FFPE samples and GC-dense genomic regions provides a realistic assessment of performance in a translational context. The workflow preserves classical bisulphite principles without the complexity of enzymatic alternatives, enhances compatibility with existing automation and fits clinical sequencing infrastructure.

Placed in the wider landscape of methylation technologies, UMBS-seq offers a compelling alternative to enzymatic approaches whose advantages diminish as DNA input decreases. The authors' comparisons with EM-seq are particularly instructive. Although enzymatic methods are widely viewed as the next step in low-damage methylation profiling, Nuttall et al. [2] report that the present results suggest their advantages may not fully translate to low-input, fragmented clinical contexts, as reflected by reduced complexity, lower conversion efficiency, and increased noise in clinically relevant sample types.

EM-seq also introduces its own liabilities: enzyme instability, lengthy protocols and high reagent costs. UMBS-seq, by contrast, delivers bisulphite-level interpretability with substantially less damage, in a faster, simpler workflow that is friendly to automation and large-scale processing.

This advance represents a logical extension of the authors' previous Ultrafast Bisulphite Sequencing (UBS-seq), which accelerated traditional bisulphite reactions and improved conversion kinetics but retained high-temperature conditions that limited performance on fragile clinical DNA [6]. UMBS-seq advances this further, offering a more protective conversion environment that improves on UBS-seq in preserving molecular integrity, particularly for cell-free DNA and other fragmented

substrates. In this sense, UMBS-seq is less a departure than a culmination: a technique that addresses long-standing methodological contention.

Beyond its technical refinement, UMBS-seq signals a broader shift in the field: recognising that epigenetics is moving from discovery science to clinical translation. Early-detection biomarkers, ageing signatures, cell-free DNA methylation panels, and minimal residual disease assays all depend on reliable methylation profiling from small amounts of input DNA. A method that enables accurate 5mC mapping from low-input DNA is not merely an incremental upgrade; it is an enabling technology.

Important limitations remain. UMBS-seq, like other bisulphite methods, does not discriminate 5mC from oxidised derivatives such as 5hmC, and its performance across diverse real-world clinical pipelines will require larger multicentre validation. Nonetheless, by sharply reducing DNA damage while preserving bisulphite interpretability, UMBS-seq lowers a key barrier to clinical-grade methylome assays.

This translational need is already evident in pregnancy and paediatric cohorts, where low-input perinatal DNA is often the only feasible window into long-term metabolic risk. It is becoming increasingly clear that methylation risk scores can do more than predict disease; they can illuminate underlying biology. This is particularly evident in diabetes. A recent *Nature Metabolism* study reported that children born to mothers with type 1 diabetes carry methylation signatures associated with reduced risk of islet autoimmunity—an unexpected yet compelling insight made possible through conventional bisulphite sequencing [7]. In parallel, a study published in *Diabetes* used methylation risk scores to identify biomarkers predicting a child's future metabolic risk at 7, 11 and 18 years of age [8]. Together, these findings underscore how methylation profiling can reshape diagnostics, risk prediction and even therapeutic thinking. Yet their translation ultimately hinges on assay sensitivity and specificity—areas where UMBS-seq offers a substantial advantage. By reducing technical noise and enhancing true signal, UMBS-seq creates a clear opportunity to revisit and refine these biomarkers, moving them closer to clinically actionable epigenetic tools by assay-ready performance at low input.

The same technical bottlenecks extend beyond diabetes. In oncology, clinical translation of methylation biomarkers remains limited. UMBS-seq provides a route to revisit such biomarkers with higher-fidelity methylome profiling, potentially rescuing candidates previously constrained by technical artefacts rather than biological limitation. Notably, most clinical studies to date have relied on classical bisulphite platforms (qMS-PCR, MethyLight, pyrosequencing, targeted NGS panels, and microfluidic assays) rather than gentler low-damage workflows, reflecting cost and implementation barriers.

This is exactly where UMBS-seq could help shift the field: by enhancing sensitivity and specificity in low-input clinical samples, it offers a practical route to strengthen trial-ready methylation assays. More broadly, UMBS-seq does not just refine current workflows; it lowers a key barrier to clinical translation. By preserving DNA integrity while retaining bisulphite interpretability, UMBS-seq makes clinic-grade methylome profiling more scalable and accelerates the path from discovery to validation—and from validation to routine care.

Author contributions

RAZ, IK and AEO contributed equally to the writing of the manuscript. All the authors have read and approved the article.

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Data availability

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Declarations

Ethics approval and consent to participate

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Consent to publish

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Competing interests

The authors declare no competing interests.

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