Advancing Agilent Avida Methyl and Duo Workflows with Enzymatic Shearing as an Alternative Fragmentation Method

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EPIGENOMICS OF COMMON DISEASES

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Abstract

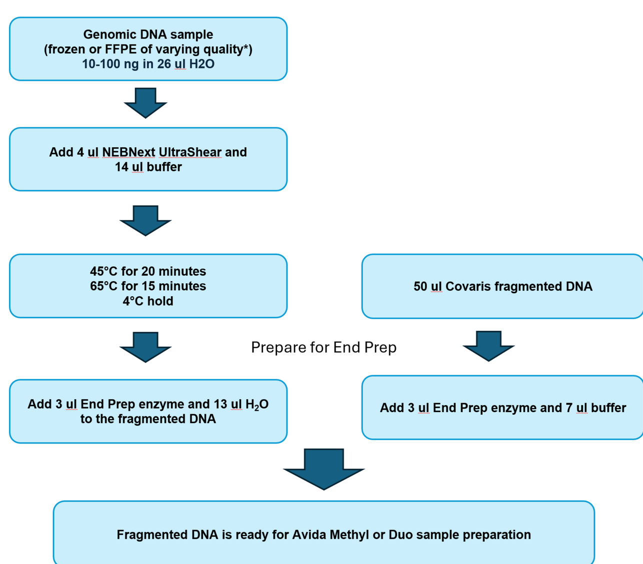
For genomic and epigenomic analysis, the Agilent Avida Methyl and Duo NGS workflows were optimized to maximize DNA recovery and offer high sensitivity through an innovative, high-performance target enrichment technology. The Avida Duo workflow facilitates multiomic analysis by capturing both DNA variants and methylation alterations from a single sample input, eliminating the need for sample splitting. The standard library preparation protocols for Avida Methyl and Duo workflows rely on mechanical shearing for DNA fragmentation. Although this shearing method is effective, it is costly, requires extra instrumentation, poses challenges for automation integration, and may lead to sample loss and potential DNA damage.

Here, we describe the incorporation of an enzymatic fragmentation method (that maintains methylation markers) into library preparation for the Avida Methyl and Duo workflows using genomic DNA (gDNA) from a cell line, formalin-compromised (fc) samples, and formalin-fixed paraffin embedded (FFPE) samples. Our results demonstrate that the methylation markers derived from libraries based on enzymatic shearing are retained at the same level compared to those from mechanical shearing, using both Avida Methyl and Duo workflows. Furthermore, sequencing metrics such as on-target rate, coverage, and uniformity are consistent across the two shearing methods. We verified the detection of variants with allele frequencies as low as 1% using a multiplex reference fc standard in the Avida Duo workflow. Finally, a tumor FFPE DNA sample subjected to both enzymatic and mechanical fragmentation, followed by DNA and methylation analysis in parallel. This FFPE sample shows comparable genomic alteration and methylation changes for the two fragmentation methods.

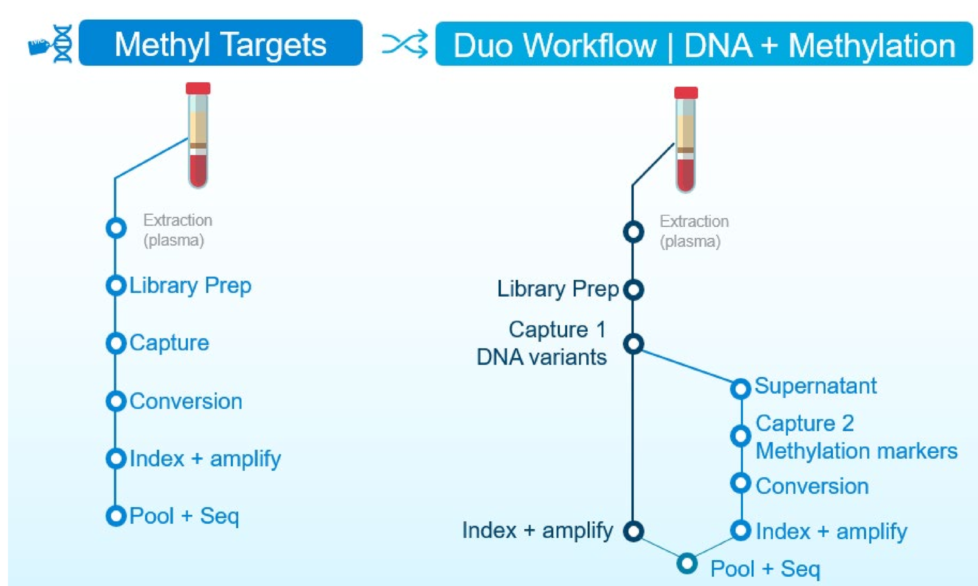
The Avida technology offers a simple, rapid, and streamlined process to evaluate both DNA and methylation changes. The demonstration of an enzymatic shearing method that retains methylation profiles upstream of the library preparation provides researchers more flexibility and compatibility for use in automated workflows.

Avida Workflow Overview and Capture Technology

A. Fragmentation workflow



B. Schematic for Methyl and Duo workflows



C. Avida capture technology

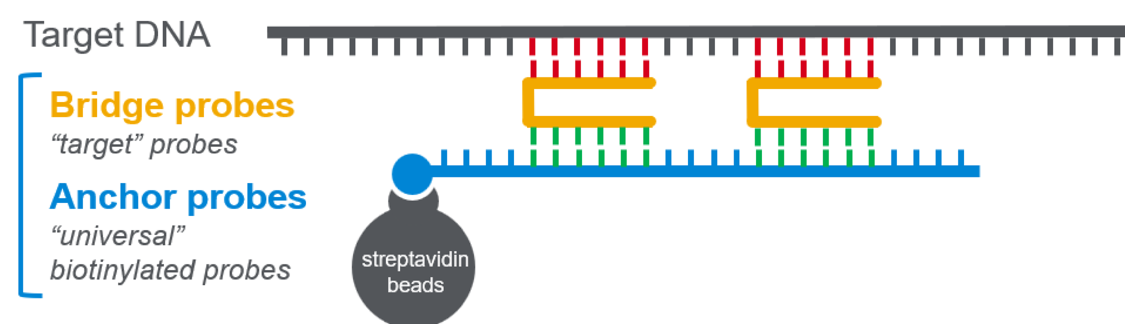


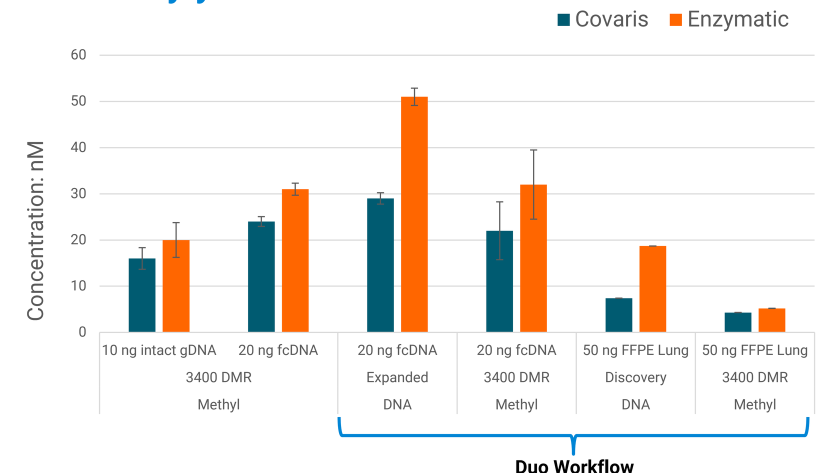
Figure 1. (A) Overview of the enzymatic and Covaris fragmentation steps. (B) Schematic presentation of the Avida Methyl and Duo workflows. (C) Proprietary Avida capture technology demonstrating a faster, more efficient hybridization reaction using a dual probe system. The captured target is stabilized when multiple short bridge probes hybridize synergistically to both the target DNA and the biotinylated anchor probe. *This enzymatic fragmentation method upstream of the Avida Methyl and Duo workflows is not recommended for samples with a DIN that is lower than 3.

Experimental Design

10 ng of intact gDNA (NA24385, Coriell Life Sciences), 20 ng of fcDNA (formalin-compromised HD799, Horizon Discovery), and 50 ng of DNA derived from a FFPE lung sample (BioChain Institute Inc) were fragmented using either enzymatic or mechanical (Covaris) shearing to achieve a mean size of approximately 200 bp (Figure 1A). For the Methyl workflow, the fragmented samples were captured after ligation using the Avida Methyl 3400 differentially methylated region (DMR) Cancer Panel (876 Kb). For the Duo workflow, the fragmented and ligated DNA samples first underwent the DNA capture process using either the Avida DNA Expanded (345 Kb) or Avida DNA Discovery Cancer Panel (2.67 Mb). The hybridization supernatant was then subjected to a second capture utilizing the Methyl 3400 DMR Cancer Panel (Figure 1B). The FFPE DNA with a DNA integrity number (DIN) of 6.7 is a medium quality sample (DIN between 3 and 8). All enriched libraries were sequenced on an Illumina NovaSeq 6000. Data for the DNA Expanded Cancer panel was down sampled to 25 million read pairs, the DNA Discovery Cancer panel data was down sampled to 40 million read pairs, and the Methyl 3400 DMR Cancer panel data was down sampled to 10 million read pairs. All metrics shown here were calculated after UMI deduplication for both DNA strands, except for the on-target rate.

Yield and Insert Size

A. Library yield



B. Median insert size

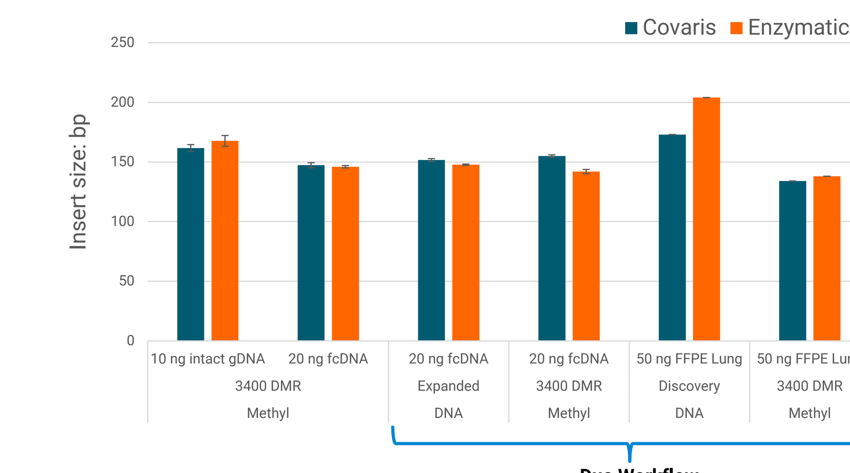
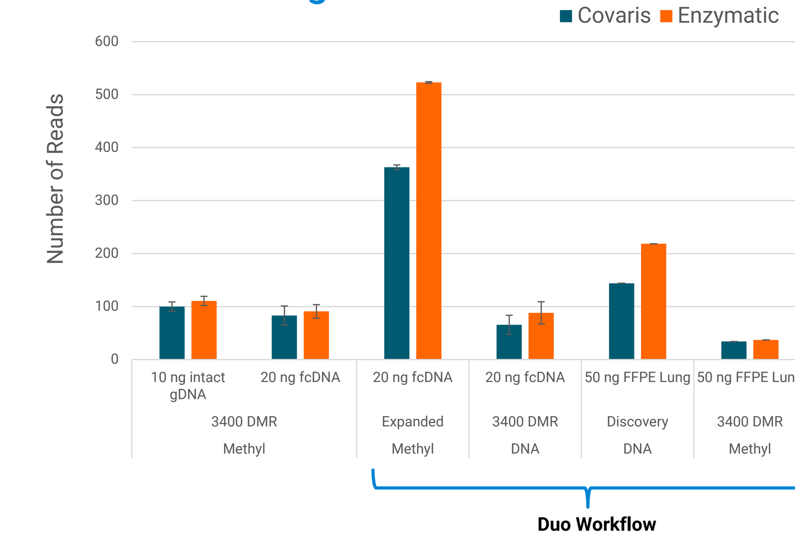


Figure 2. (A) Enhanced library yields with enzymatic shearing. Enzymatic shearing produces greater post-capture library yields (from 1.2 to 2.3-fold higher) than Covaris shearing across different sample types (intact, formalin-compromised, and FFPE), input amounts (10-50 ng), panels (DNA Expanded Cancer, DNA Discovery Cancer, and Methyl 3400 DMR Cancer), and different workflows. (B) Both shearing methods produce similar insert sizes across all parameters tested.

Mean Coverage, On-Target Rate and Uniformity

A. Mean coverage



B. On-target and uniformity

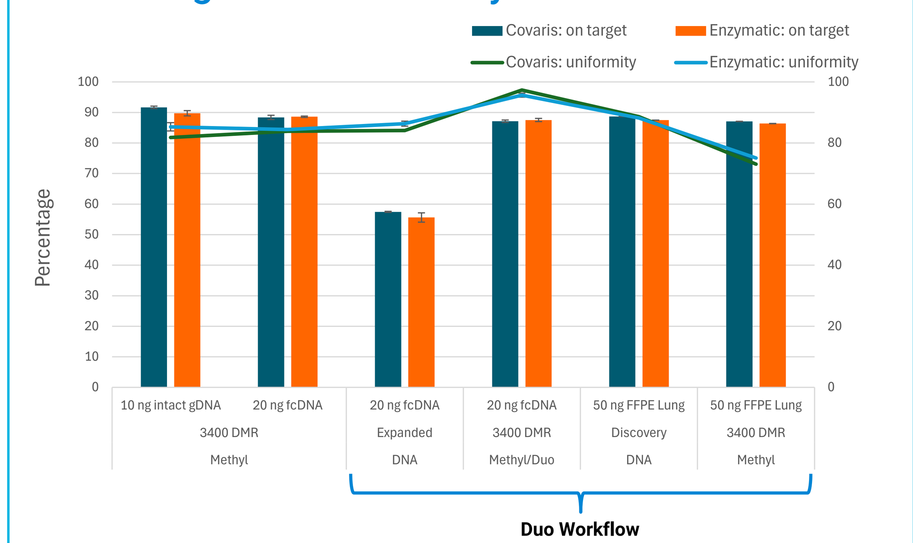


Figure 3. (A) Samples fragmented by enzyme compared to Covaris shearing show an increase of 1.1 to 1.4-fold in mean coverage across the target regions. (B) Both shearing methods generated similar on-target rate and uniformity across all samples with different panels and workflows. On-target is defined as the number of reads that map to within the 100 bases flanking the target region. Uniformity is measured by the fraction of targeted regions with coverage greater than 50% of the mean coverage across all regions. The lower on-target rate of the DNA Expanded Cancer panel is due to the inclusion of intronic regions for fusion detection. Intronic sequences are less complex and repetitive, making probes targeting these regions prone to cross-hybridization.

AT/GC Coverage Across the GC Content

AT/GC coverage: 10 ng intact gDNA using Duo DNA workflow and Expanded Cancer panel

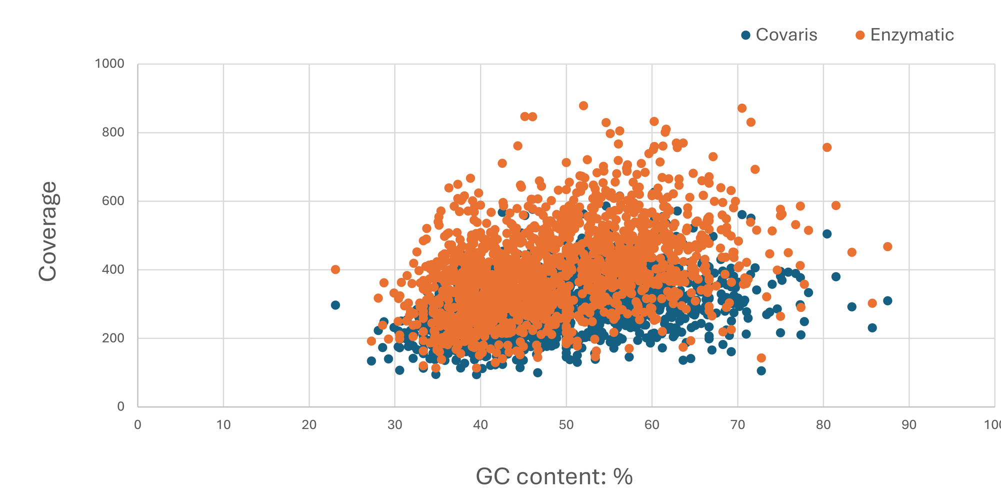


Figure 4. This data represents the coverage of the target regions as a function of their %GC using 10 ng of intact gDNA enriched with the DNA Expanded Cancer panel. It reveals that both shearing methods exhibit even coverage profiles across the entire GC content range of 20% to 90%, with enzymatic shearing generating higher read counts.

Variant Detection

Table 1. Variant detection in the 20 ng HD799 enriched with Expanded Cancer panel: Duo DNA

			Covaris			Enzymatic		
Gene	Variant	Expected %AF	# reads containing variant	# total reads	% VAF	# reads containing variant	# total reads	% VAF
EGFR	T790M	1	21	2447	0.86	27	3620	0.75
EGFR	ΔE746 - A750	2	27	2065	1.31	48	3420	1.40
EGFR	L858R	3	96	2307	4.16	91	3529	2.58
KRAS	G12D	6.25	37	923	4.01	48	1313	3.66
PIK3CA	E545K	9	103	1518	6.79	140	2228	6.28
KIT	D816V	10	85	1110	7.66	105	1721	6.10
BRAF	V600E	10.5	209	2047	10.21	439	2899	15.14
NRAS	Q61K	12.5	121	1540	7.86	193	2097	9.20
KRAS	G13D	15	113	927	12.19	154	1338	11.51
PIK3CA	H1047R	17.5	316	1625	19.45	435	2076	20.95
EGFR	G719S	24.5	277	1404	19.73	594	2422	24.53

Table 2. Variant detection in the 50 ng FFPE Lung enriched with Discovery Cancer panel: Duo DNA

			Covaris			Enzymatic		
Gene	Variant	Expected %AF	# reads containing variant	# total reads	% VAF	# reads containing variant	# total reads	% VAF
KRAS	G12D	25.5	44	134	32.80	57	213	26.50

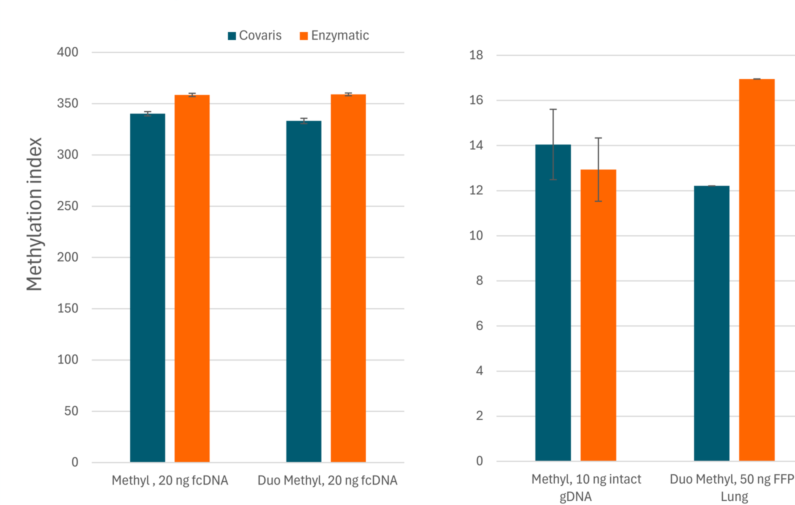
Table 1. All 11 variants verified by Horizon Discovery, with allele frequencies ranging from 1% to 24.5%, were detected at comparable rates in the 20 ng HD799 samples from both shearing methods. These samples were prepared using the Duo DNA workflow with the Avida Expanded Cancer Panel at a sequencing depth of 21,700X.

Table 2. The detection of one variant in the FFPE lung samples sheared by both methods shows an expected VAF of 25.5%. These samples were prepared using the Duo DNA workflow with the Avida Discovery Cancer Panel at a sequencing depth of 4,500X.

Both tables demonstrate that enzymatic shearing generated higher total read depth and a higher number of reads containing variants compared to Covaris fragmentation.

Methylation Assessment

A. Methylation index scores



B. DMR methylation levels: 20 ng fcDNA

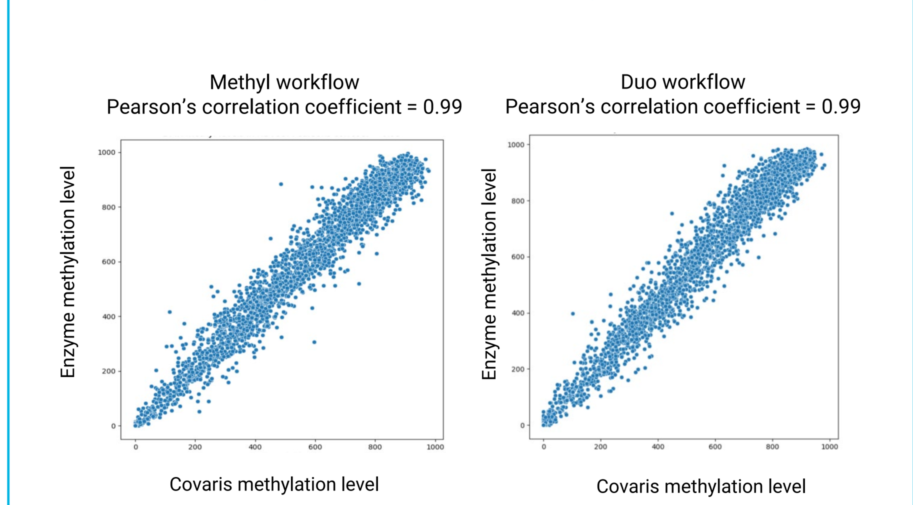


Figure 5. The Avida technology is designed for methylation assessment using the novel Avida soft conversion treatment. Unlike the traditional methods, this bisulfite treatment is formulated to minimize DNA damage, preserving the integrity of the DNA samples for a higher recovery of converted molecules. As shown in (A) and (B), the Methyl and Duo workflows demonstrate similar performance. Figure (A) shows the methylation index scores are similar in the both shearing methods using both workflows. Samples include 20 ng fcDNA and 50 ng FFPE Lung. Figure (B) shows high correlation of methylation levels in both shearing methods in the 20 ng fcDNA for both workflows. The methylation index score for the Avida Methyl 3400 DMR Cancer Panel is a metric that quantifies cancer-specific, hyper-methylated DNA molecules within pre-selected biomarker regions of a sample. This score provides a measurement of methylation levels, aiding in the detection and analysis of cancer-related epigenetic changes.

Conclusion

The Avida Methyl and Duo workflows are optimized to extract the most information from the limited material often found in FFPE samples. By integrating enzymatic fragmentation (NEB's UltraShear) with these workflows, we demonstrate that methylation markers are retained similarly to mechanical shearing in intact, formalin-compromised, and FFPE gDNA samples. This study shows that enzymatic fragmentation generates slightly higher library yields, while maintaining similar median fragment size, on-target rates, uniformity, SNP detection rates, and GC coverage compared to mechanical shearing.

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