Targeted Genomic and Epigenomic Dual Analysis of the Same Region for an Entire Gene in Tumor DNA

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Introduction

Genomic and methylation analysis for the same region

Current whole-genome technologies of simultaneous genomic and epigenomic analysis can be costly, requiring deep sequencing for low tumor content and often exhibiting limited coverage in high-GC regions. To address these limitations, we developed a novel strategy for simultaneous mutational and epigenome analysis of the same region within a single gene from a single DNA input. This approach offers a more affordable solution with improved coverage uniformity, particularly in high-GC regions.

Using the *ARID1A* gene as a model, we implemented the Agilent Avida Duo target enrichment system, enabling sequential capture and acquisition of both genomic and methylation information across the entire gene sequence.

ARID1A

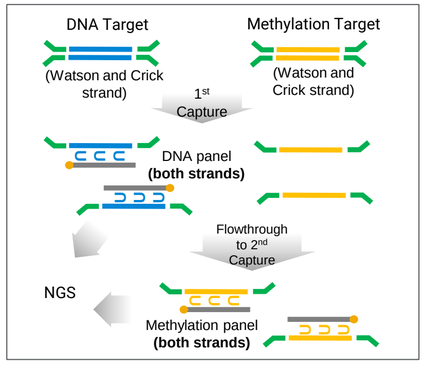
- *ARID1A* acts as a tumor suppressor, regulating cell growth and division.
- Mutations in *ARID1A* are implicated in various cancers.
- Further research is needed to fully elucidate the roles of both genetic and epigenetic changes across the entire *ARID1A* gene in cancer.

The Agilent Avida Duo workflow

- Agilent Avida target enrichment technology employs a novel indirect synergistic hybridization system, providing enhanced specificity. This allows for sequential capture using the flow-through from the initial capture with a second panel.
- Leveraging the PCR-free library preparation chemistry, the Agilent Avida Duo workflow enables dual analysis of genome alterations and methylation, capturing targets from two different target regions or a single target region.
- The high capture efficiency of the Avida technology enables direct capture from unamplified DNA, preserving crucial DNA methylation information.

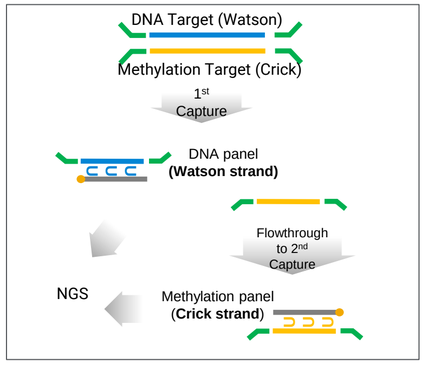
Technology

Standard method captures different regions



Left: The standard target capture method for the Avida Duo workflow captures different target regions for genomic alteration and methylation analysis. Two sets of bridge probes (blue and yellow) target distinct genomic regions- DNA target (blue) and methylation target (yellow), capturing both Watson and Crick strands for a given target. This strategy is optimized for cfDNA and other low input applications (a few ng DNA).

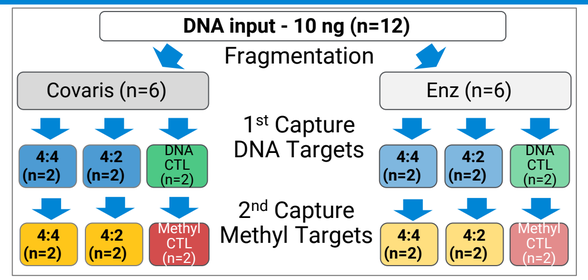
Modified method captures the same region



Right: The modified target capture method targets the same region for both DNA (blue) and methylation (yellow) analysis. Two sets of bridge probes (blue and yellow) target the same region, capturing either the Watson or the Crick strand. Note that this approach was not tested with less than 10 ng gDNA.

**All patients provided informed written consent prior to tissue collection and the study protocol was approved by the Research Ethics Committees (REC) at Aga Khan University Hospital (Ref: 2018/REC-80) and AIC Kijabe Hospital (KH IERC-02718/0036/2019). Permit to conduct the research was also sought from the National Commission for Science, Technology, and Innovation in Kenya. The research followed recognized ethical guidelines as defined by the Declaration of Helsinki and the U.S. Common Rule.*

Experimental Design



ARID1A panel performance was compared across two MT/LT ratios and two gDNA fragmentation methods. A modified target capture method, targeting the same ARID1A region, was evaluated with the standard method targeting different regions as a control.

We designed 2 custom panels (90 kb), the first targeting the Watson strand (- panel) for DNA variant analysis and a second panel targeting the Crick strand (+ panel) for methylation analysis.

As more challenging intronic targets are included, we used 2 design strategies, a) ~75% high-uniqueness (MT, "More Than" uniqueness cut off) and b) ~25% low-uniqueness (LT, "Less Than" uniqueness cut off, primarily intronic low-complexity regions) and tested 2 blends of this (MT:LT, 4:4 or 4:2) to provide a more comprehensive or a more specific target coverage.

As controls, the Agilent Avida DNA Focused Cancer Panel (27 kb, Cat# 5280-0049) and a custom methylation panel (TMS 200, 60 kb) were used for the standard Avida Duo workflow (Agilent, Cat# G9440A).

As starting material, 10 ng gDNA (Cat# 5190-8849) was fragmented by either sonication or enzymatic shearing in a 50 µL volume, using two replicate reactions per condition.

Four breast tumor samples (20-50 ng gDNA) that were approved for research use* (Tang, et al.) were analyzed using Covaris fragmentation, 4:2 panel mix capture.

Sequencing read budgets for the *ARID1A* panels were 10 M read pairs for the DNA panel, and 5 M read pairs for the Methyl panel. Sequencing read budgets for the control panels were 5 M read pairs for both DNA and Methyl panels.

Results

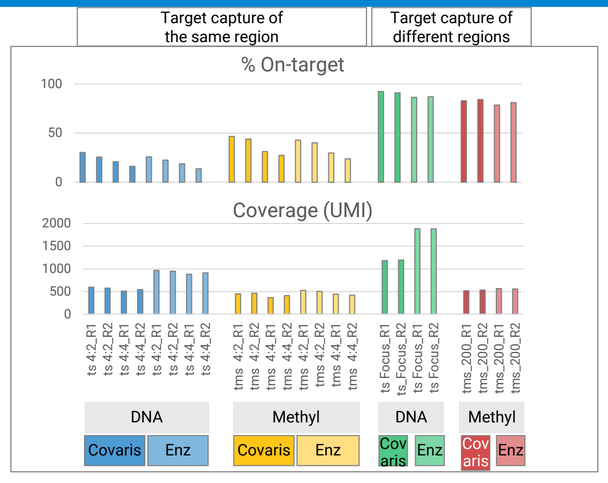


Figure 1. Sequencing performance: panel MT/LT ratio and fragmentation method comparison.

Our custom *ARID1A* panels, using a modified design approach, target the same region: the "-" strand panel enriches DNA, whereas the "+" strand panel enriches methylation. The 4:2 panel showed slightly higher recovery and on-target rate than the 4:4 mix.

Control panels used in the standard target capture strategy are targeting both strands, in different regions, and mainly on exonic regions (all MT regions). The recovery and on-target are much higher than the custom *ARID1A* panel covering the full gene sequence including difficult to map intronic regions.

For both *ARID1A* and control panels, enzymatic shearing (Enz) fragmentation yielded higher recovery, but slightly lower on-target rate compared to sonication (Covaris).

Two replicates (R1, R2) were examined for each condition.

Results

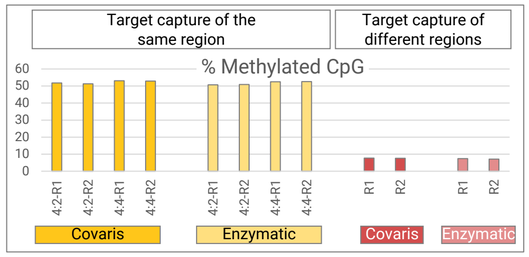


Figure 2. Avida soft conversion performance: comparison of panel ratios and fragmentation methods

The reported values represent the percentage of cytosine (C) that remained unconverted after conversion treatment. The levels of methylated CpG conversation rate were similar across different panel ratios (4:4, 4:2) and fragmentation methods (Covaris, dark yellow/ red and enzymatic shearing (Enzymatic, light yellow/ light red). Two replicates (R1, R2) were examined for each condition. The Avida soft conversion rate for all samples was between 98.9% and 99.3%.

Sample	Gene:pshort	Vaf pct	Effect_pred
gDNA1	ARID1A:p.Q503fs	43.1	frameshift
	ARID1A:p.Q585*	22.3	stop_gained
gDNA2	ND	NA	NA
gDNA3	ARID1A:p.N1705S	29.6	missense
	ARID1A:p.S613C	2.8	missense
	ARID1A:p.P1562T	1.1	missense
	ARID1A:p.G92G	4.5	synonymous
	ARID1A:p.E34G	1.4	missense
gDNA4	ARID1A:p.T572T	44.7	synonymous
	ARID1A:p.G369G	1.1	synonymous

Table 1. ARID1A genomic and methylation analysis from breast tumor gDNA

Genomic variants were detected in gDNA (20-50 ng) from breast tumor tissue samples (n=4). Median coverages were 3935X, 10695X, 1539X, and 2053X respectively.

A total of 1366 CpGs are covered by the *ARID1A* panel. The median coverage of each CpG are 298X, 570X, 97X, and 140X, of the four samples, respectively. Evaluation of the methylation percentage of each CpG showed differentiating methylation signals between tumor samples. The conversion rate is >99.4%.

Conclusions

The Agilent Avida Duo workflow offers a cost-effective solution for multiomic analysis, enabling the investigation of both acquired mutations/genomic variants and DNA methylation alterations from a single sample input, eliminating the need for sample splitting.

- Established a new approach to analyze both genomic and methylation changes within the *same* region, where optimal sequencing budgets can be set for the individual modalities independently.
- Method captures only one strand of DNA at a time, allowing for simultaneous analysis of both modalities in a defined region.
- Tested various combinations of high- and low-uniqueness sequence regions to optimize both target recovery and on-target rate. This adaptable approach allows for customization based on the specific research needs.
- Demonstrated the feasibility of integrating enzymatic fragmentation into the Avida Duo workflow, achieving acceptable recovery and on-target rates.
- Detected acquired mutations/genomic variants and methylation changes in the breast tumor samples (gDNA), demonstrating the feasibility of the Agilent Avida Duo reagent kit for dual DNA and methylation analysis of the same region for the entire *ARID1A* gene.

References

Tang, W.; Zhang, F.; Byun, J. S.; Dorsey, T. H.; Yfantis, H. G.; Ajao, A.; Liu, H.; Pichardo, M.S.; Pichardo, C. M.; Harris, A. R.; Yang, X. R.; Figueroa, J. D.; Sayed, S.; Makokha, F. W.; Ambs, S. Population-specific Mutation Patterns in Breast Tumors from African American, European American, and Kenyan Patients. Cancer Res. Commun. 2023, 3 (11), 2244-2255.
https://doi.org/10.1158/2767-9764.CRC-23-0165

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