Application Note

TAGOMICS PARTNERS WITH AGILENT TECHNOLOGIES TO OFFER A NOVEL SOLUTION FOR MULTIOMIC ANALYSIS

Tagomics has developed Interlace™, a seamless solution for multiomic profiling that enables the identification of disease-associated DNA biomarkers from a range of sample types. Through this partnership, Tagomics has integrated the Agilent SureSelect Library Preparation and Target Enrichment System* into its Interlace multiomic workflow. This enables the combination of a novel approach to epigenetic profiling with genomics, all within a single unified workflow, delivering unparalleled biological insights for biomarker discovery, diagnostic development, and precision medicine applications.

TAGOMICS

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- **Low input – optimised insights from as little as 10 ng**
- **Streamlined workflow – robust, reproducible, and fully integrated**
- **Novel insights – a comprehensive report that provides unique multiomic biomarkers**

INTRODUCTION

Tagomics' Interlace™ platform offers a seamless solution for multiomic profiling. It integrates the company's novel epigenetic profiling approach, which enables highly efficient genome-wide enrichment of unmethylated sites, with whole-genome or targeted sequencing, in one streamlined workflow.

Multiomic DNA analysis provides a more complete picture of cellular function and disease state, but challenges associated with the complexity of integrating multiple workflows and interpreting diverse and heterogeneous data outputs remain.

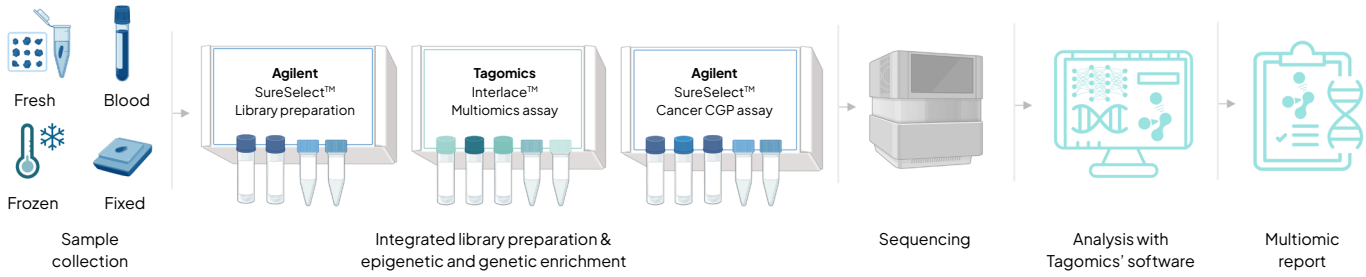

Figure 1. *A single workflow enables customers to conduct both comprehensive genomic profiling (covering 679 cancer-relevant genes from the Agilent SureSelect Cancer CGP assay) and genome-wide epigenomic analyses, providing novel, clear, and clinically informative insights. The results are summarised in a comprehensive report.*

TECHNICAL SPECIFICATIONS

Tagomics' Interlace platform addresses the challenges associated with multiomic DNA analysis by enabling seamless integration of genome-wide epigenetic profiling with either targeted or whole genome profiling into a single workflow (Fig. 1). It uses proprietary single-pot chemistries that avoid harsh chemicals, preserving the underlying DNA sequence and generating comprehensive epigenomic and genomic readouts from as little as 10 ng of input DNA, or 1 ng for the epigenetic approach alone (Fig.2).

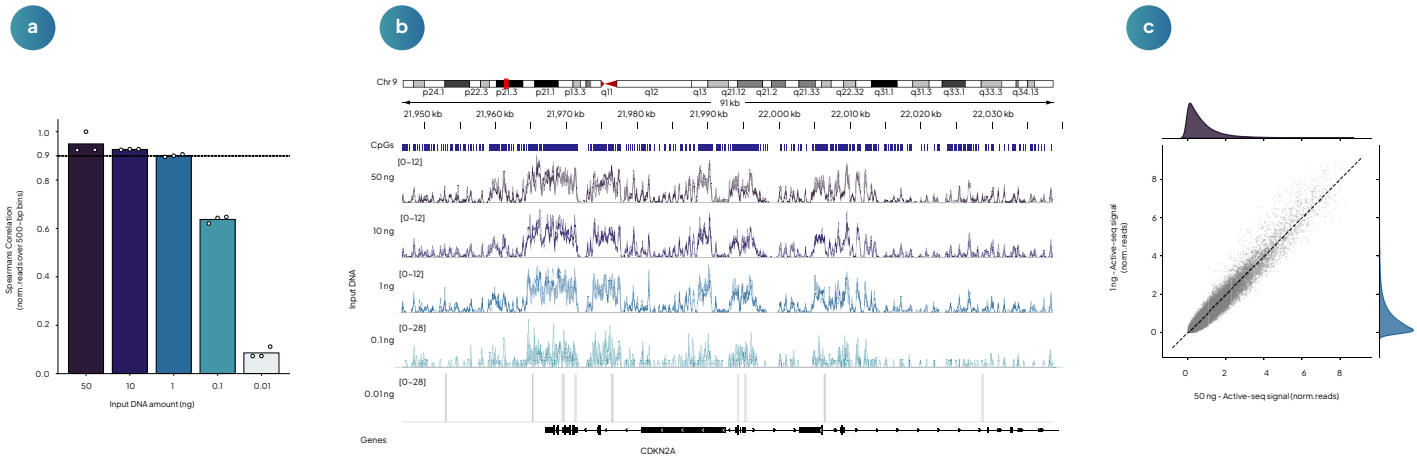

Figure 2. Panel a. *The effect of DNA input on the epigenomic profile was evaluated by comparing varying amounts of genomic DNA. Technical replicates were processed in triplicate using 50 ng, 10 ng, 1 ng, 0.1 ng, and 0.01 ng of input genomic DNA. The figure shows values of the calculated Spearman correlation between the replicates and one of the 50 ng input samples; calculations were performed genome-wide using 500bp bins and normalised read count. Epigenomic profiles have a Spearman correlation greater than 0.9 with as little as 1 ng of input material.* **Panel b.** *Genome browser view of the epigenetic signal at the CDKN2A gene locus (chr9:21,953,009-22,040,190). Peaks correlate with enrichment at unmethylated CpG sites. Each row displays normalised read coverage tracks for all technical triplicates. The coverage profiles demonstrate consistency across varying input DNA amounts, down to 1 ng.* **Panel c.** *Triplicate samples with a DNA concentration of 50 ng and triplicate samples with a concentration of 1 ng DNA were processed using Tagomics' epigenetic workflow. The joint plot illustrates the relationship between the average normalised reads of the triplicates processed with 50 ng of DNA input and those processed with 1 ng of DNA input using 500 bp windows, across the genome. The signal distribution for each sample is displayed along the margins (top and right) of the graph using density plots.*

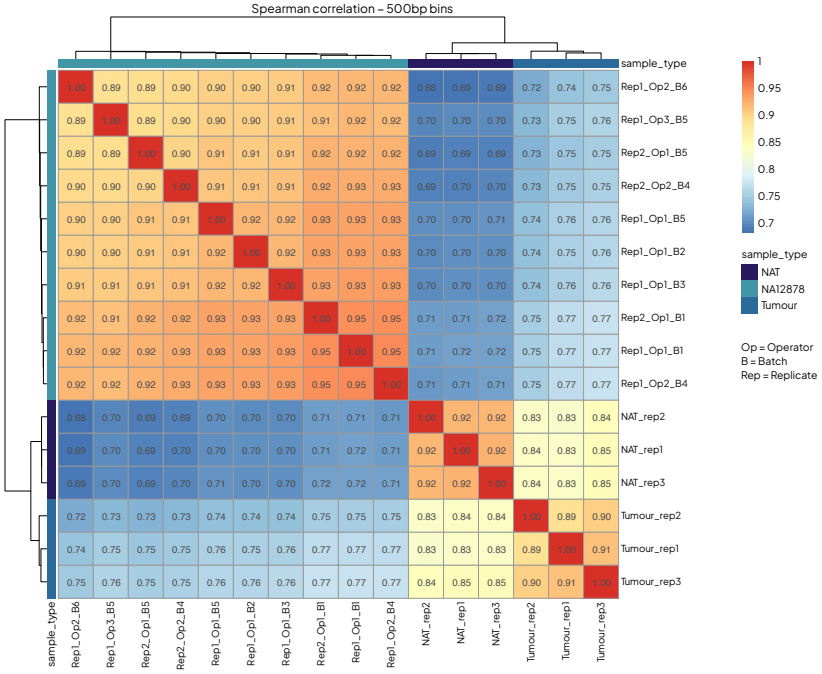

Figure 3. *Highly reproducible genome-wide epigenomic profiling across different sequencing runs, processed in distinct batches and by different operators. The Figure shows Spearman correlation analysis of the epigenetic using 500bp windows across the entire genome. The three separate clusters detected correspond to the three different sample types (DNA from the NA12878 cell line, patient tumour and normal adjacent tissue).*

Generation of a robust epigenetic profile requires as few as 75 to 100 million paired-end reads (150 bp read length) per sample to achieve Spearman correlation of over 0.9 (Fig. 3). It is compatible with a wide range of sample types, includingfresh frozen and formalin-fixed, paraffin-embedded (FFPE) tissue samples and plasma (cfDNA) (Figs. 4 and 5a). The resulting epigenetic profiles provide a unique and detailed map of unmodified CpG sites across the genome, including essential genomic features such as promoters, enhancers, and cell type-specific regulatory regions, which often undergo epigenetic changes during disease progression (Fig. 5b).

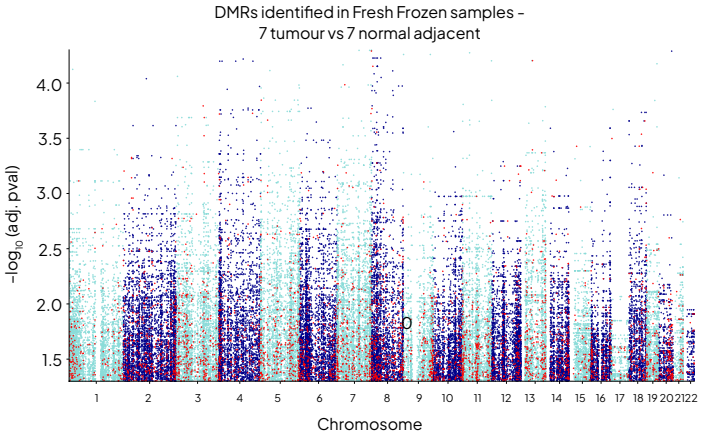

Figure 4. *Tagomics' Interlace platform was used to generate data for seven colorectal cancer (CRC) patients using 50 ng of input DNA from fresh frozen tumour and matched normal adjacent tissue samples. cfDNA (10ng) samples collected from the same patients and from 11 healthy controls were also processed. The epigenomic profiles were utilised to perform differential methylation analysis in the tissue samples. The epigenetic signal intensity across the genome was computationally segmented into different regions. Regions with a statistically significant change in the epigenetic signal between cancer and normal tissues were identified as Differentially Methylated Regions (DMRs). Using this approach,* ***51,165*** *DMRs were identified. The Manhattan plot shows the genomic location of each tissue DMR (with an adjusted p-value < 0.05) represented as blue dot (displayed by alternate dark and light blue dots to differentiate the distribution across the 22 somatic chromosomes). The red dots highlight the subset of* ***4,081*** *DMRs showing greater than 1.5-fold signal difference in the cfDNA samples (CRC vs healthy cohorts).*

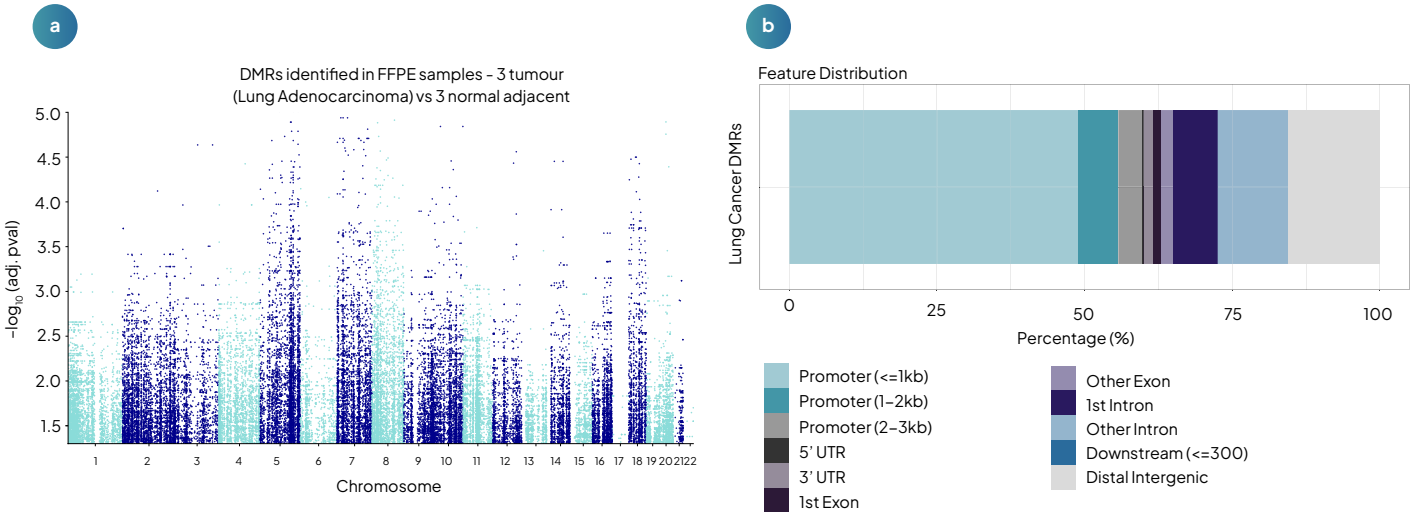

Figure 5. Panel a. *Multiomic data was generated for three lung cancer patients (adenocarcinoma) using tumour and matched normal adjacent tissue samples that had been preserved in FFPE. 100 ng of DNA with a DIN value between 2 and 6 was used as input. The epigenomic profiles of the tumour and normal adjacent tissue were compared and 36,080 tumour-specific DMRs were identified. The Manhattan plot shows the genomic location of each tissue DMR (with an adjusted p-value < 0.05) represented as blue dots (displayed by alternate dark and light blue dots to differentiate the distribution across the 22 somatic chromosomes).* **Panel b.** *Distribution of the DMRs identified in lung cancer patients (FFPE tissue samples) across different genomic features is displayed. The majority of the epigenetic changes take places in promoter regions.*

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Tagomics has partnered with Agilent Technologies to combine the Agilent SureSelect Library Preparation and Cancer CGP Assay with Tagomics' Interlace workflow. The SureSelect Cancer CGP assay supports a wide range of input volumes from various sample types and allows for efficient detection of key somatic variants in solid tumours, including single nucleotide variants, copy number variants, insertions and deletions, translocations, gene fusions, and immuno-oncology biomarkers such as tumour mutational burden and microsatellite instability. This combined solution retains the sensitive variant calling of the SureSelect Cancer CGP DNA assay (Fig. 6) while additionally offering a comprehensive, genome-wide epigenomic profile of the same sample.

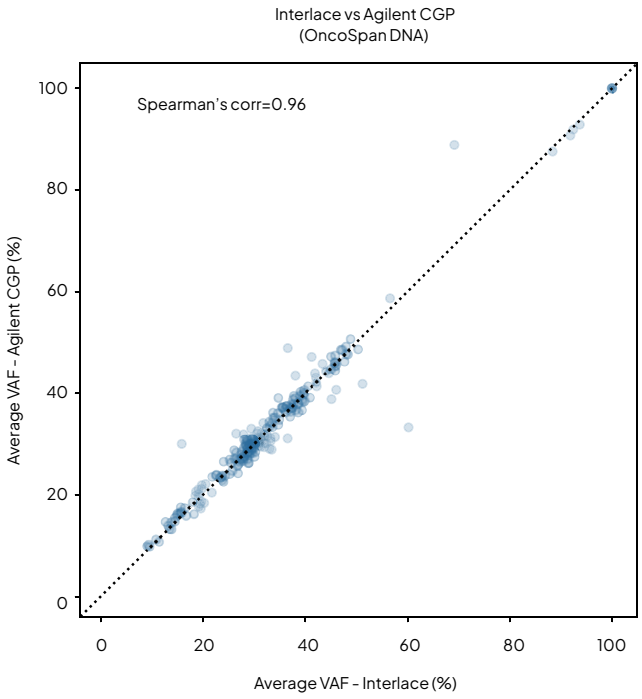

Figure 6. *The scatter plot illustrates the correlation between the average variant allele fraction (VAF) of three replicates, called on a reference genome DNA (HD827 OncoSpan gDNA, Horizon Discovery Biosciences), processed either with Tagomics' Interlace workflow or with the Agilent SureSelect Cancer CGP assay*, independently. Each workflow was performed in triplicate using 50 ng of input DNA. The Spearman correlation between of the average VAF detected using the two platforms is 0.96.*

REPORTING

The genomic and epigenomic profiles of each sample are captured in a comprehensive report, generated using Tagomics' proprietary software. This analytical pipeline facilitates scalable and reproducible scientific workflows and has optimised client computing and data storage to eliminate unnecessary analysis runs. The multi-sectioned report provides details on sample quality, protocol performance, genetic and epigenetic profiles, and clinical insights into any clinically relevant biomarkers.

CASE STUDY

Overlaying molecular phenotypes to inform associations between multiple omics holds enormous promise in the diagnosis and clinical management of disease. Multiomic analysis is currently being used to improve sensitivity and specificity of diagnostic tests and provide deeper biological insight into disease causality and potential therapeutic efficacy. The complementarity of genetic (Fig. 7a) and epigenetic (Fig. 7b) testing can enable personalised medicine and accelerate the process of drug development with rich biological data to underpin target discovery and patient-specific data that can facilitate patient stratification. Here, we present a case study that demonstrates the power of Interlace in identifying a combined epigenetic signal in CDKN2A and somatic mutation in KRAS, correlated to increased risk of cancer metastasis (Fig. 8). In this particular case study, Interlace provides unique additivity and demonstrates the significance of genome-wide multiomic profiling with no a priori knowledge of the patient's disease status.

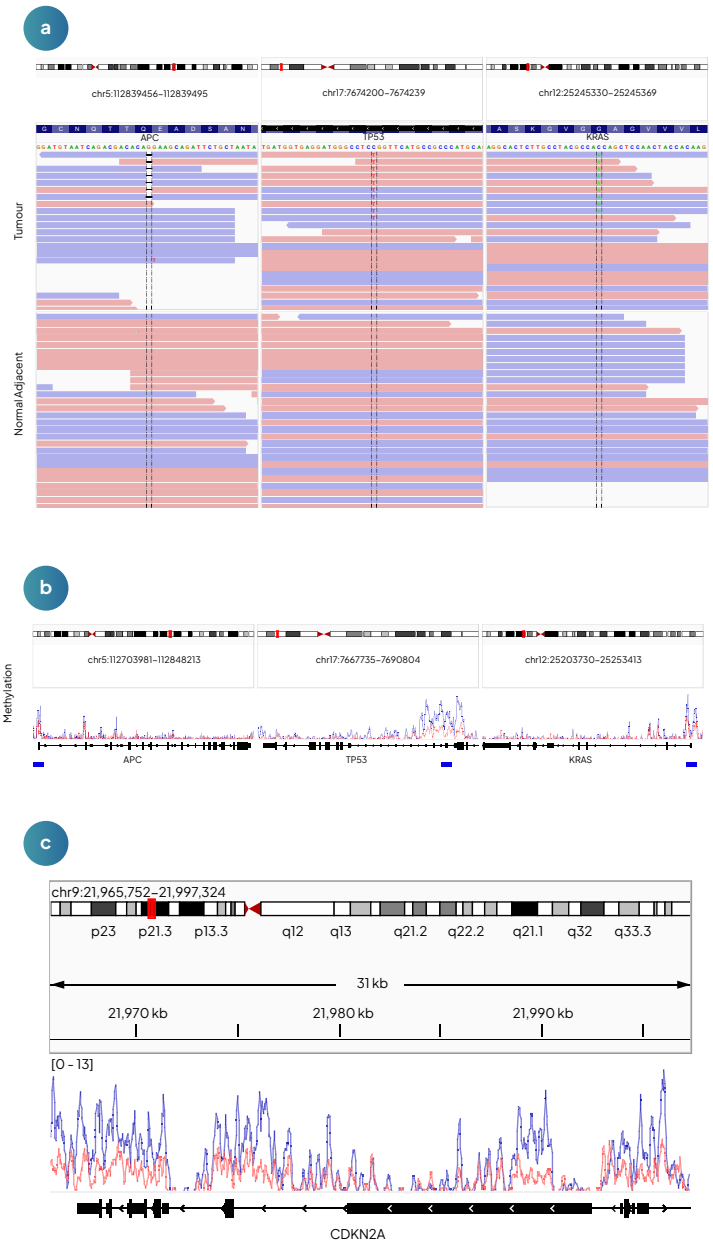

Figure 7. Panel a. *Panel a shows three different mutations identified in a colorectal cancer (CRC) patient (stage IIA) using Interlace and 50 ng of input DNA from fresh frozen tumour and matched normal adjacent tissue samples. In the top panel sequencing reads generated from the tumour sample are displayed, while the panels at the bottom displays sequencing reads of the normal adjacent tissue from the same patient. The figure is divided in 3 sections; on the left a frameshift mutation in the APC gene (AG>A) is shown; in the centre the (c.142G>A COSV52987957) variant in the TP53 gene is shown; and on the right is a missense variant in the KRAS gene (c.35G>T COSV55497419). In colorectal cancer, KRAS G12V is a predictive biomarker for the use of seven therapeutics (afatinib, dacomitinib, erlotinib, gefitinib, osimertinib, cetuximab, and panitumumab). Furthermore, the identified TP53 (COSV52987957 c.142G>A) variant in this patient is a predictive biomarker for use of idelalisib, lenalidomide, and rituximab. The observed APC frameshift is rare though APC is altered in 65.55% of colorectal carcinoma patients.* **Panel b.** *The epigenetic profile is shown for the same genes described in panel a. The blue trace represents the epigenetic signal for the normal adjacent tissue while the red trace represents the epigenetic signal from the cancer tissue. Peaks correlate with enrichment at unmethylated CpG sites. Promoter regions are marked by blue boxes and noticeably the cancer tissue shows a hypermethylated signal compared to the normal adjacent tissue.* **Panel c.** *The figure shows the epigenetic profile around the CDKN2A gene (chr9:21,953,009-22,040,190) from the same colorectal cancer patient from panel a. The blue trace represents the epigenetic signal for the normal adjacent tissue while the red trace represents the epigenetic signal from the cancer tissue. No somatic variants were identified in this gene, but a consistently distinctive hypermethylation signal is detected at the promoter region of this gene. The KRAS mutation, in combination with hypermethylation of the CDKN2A gene is associated with an increased risk of metastasis in colorectal cancer (Miranda et al, Br J Cancer. 2006, 95, 1101–1107). In this patient, extensive hypermethylation of the CDKN2A gene in the tumour (red profile) is indeed detected as compared to the normal adjacent tissue (blue profile). Interlace provides unique additivity and demonstrates the significance of unbiased, genome-wide multiomic profiling with no a priori knowledge of the patient's disease status.*

tagomics.com

Granta Park, The Cori Building, Little Abington, Cambridge
CB21 6GQ

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