Case Study

biomodal

Multiomic 5-letter sequencing allows reading of modified cytosine bases and simultaneous measurement of genomic mutations in cancer cells

Overview

Researchers in Dr Sam Aparicio's group at the British Columbia Cancer Research Centre (BCCRC) and the University of British Columbia, BC, Canada, utilised 5-letter sequencing technology, duet multiomics solution +modC, to investigate 'epigenetic rewiring' in breast cancer cells.

In this study, **duet multiomics solution +modC** helped to reveal:

- the epigenetic landscape of untransformed diploid breast epithelial cells with wild-type, p53$^{-/-}$BRCA1$^{-/-}$ and p53$^{-/-}$BRCA2$^{-/-}$ genetic backgrounds
- significant activation of stem cell enhancers through reduced DNA methylation in p53$^{-/-}$BRCA1$^{-/-}$ cells only
- similar activation of stem cell enhancers in a triple negative breast cancer (TNBC) patient xenograft sample
- the epigenetic rewiring caused by BRCA1$^{-/-}$, identifying it as a crucial gene for this type of cancer pathogenesis

Challenge

In this case study, we highlight the Aparicio group's research on decoding the relationship between genomic mutational background and epigenomic, or nongenomic, transcriptomic contributions to the fitness of cancer cells.

This research study measures both the state of the genome in cancer cells, as well as decodes the state of the epigenome and the transcriptome. Making it vital to capture the epigenetic information encoded in modified cytosine bases in DNA, as a component of their investigations.

BC CANCER RESEARCH

Provincial Health Services Authority

About BCCRC

The BC Cancer Research Centre's mission is to pursue world-class research that aims to transform the lives of patients by exploring basic mechanisms and technology developments in all areas of cancer research including cancer control, clinical studies and trials, cancer surveillance, and population health and services. The research portfolio also supports facilities and platforms in genomics, bioinformatics, imaging, drug development, and tissue banking.

The Aparicio group studies the genomic and phenotypic behaviour of breast and other cancers. They integrate leading technologies to support their efforts to better understand how cancer clones evolve and to identify novel strategies for cancer treatment and predictors of response.

"The challenges we have faced in the past in making these measurements involve the need to work with limiting amounts of material because our research is decoding this in single cells where you are dealing with very limited copies of the information.

Also, the need to have error correction in the measurements of the genome so that sequencing errors can be minimised is key for us."

Dr Sam Aparicio

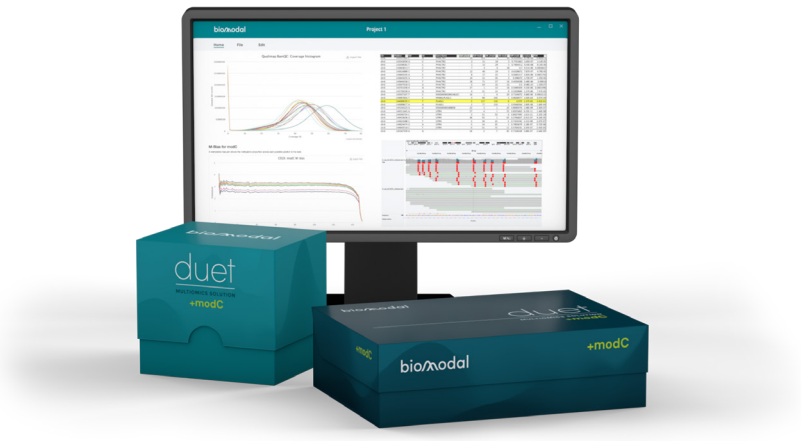
Solution

OVERCOMING CHALLENGES TO PERFORM SIMULTANEOUS GENOMIC AND EPIGENETIC SEQUENCING OF BREAST CANCER CELLS

To overcome previous challenges associated with the utilisation of low-sample volumes and multiple workflows, Dr Gurdeep Singh, postdoctoral fellow, at the Aparicio lab employed **duet multiomics solution +modC** to simultaneously investigate the genetic and epigenetic landscape of breast cancer cells known to exhibit homologous recombination deficiency (HRD) which drives genomic instability and cancer pathogenesis. This cancer cell trait or driving mechanism is involved in both triple-negative breast cancer (TNBC) and high-grade serous ovarian cancer.

duet multiomics solution +modC enabled Dr Singh to investigate epigenetic rewiring - known to play a critical role in cancer pathogenesis, cancer advancement, and cancer drug resistance - in breast tumour cells.

HRD mutational instability is known to be dependent on BRCA and p53 mutations. In this study, Dr Singh used untransformed diploid 184hTERT breast epithelial cell lines deficient in genes often mutated in HRD cancer ($p53^{-/-}BRCA2^{-/-}$ and $p53^{-/-}BRCA1^{-/-}$), with wild-type genomic background (WT184hTERT) cells as a control.



duet multiomics solution +modC empowers researchers by helping them gain complete genetic and modified cytosine (modC) information from a single low-volume DNA sample. The streamlined workflow provides standard genomic calls plus modC without errors and biases associated with other enzymatic or bisulfite treatments.

> ***"Having a single-workflow method that allows reading of modified cytosine bases and simultaneous measurement of genomic mutations is a game-changer for us."***
>
> ***Dr Sam Aparicio***

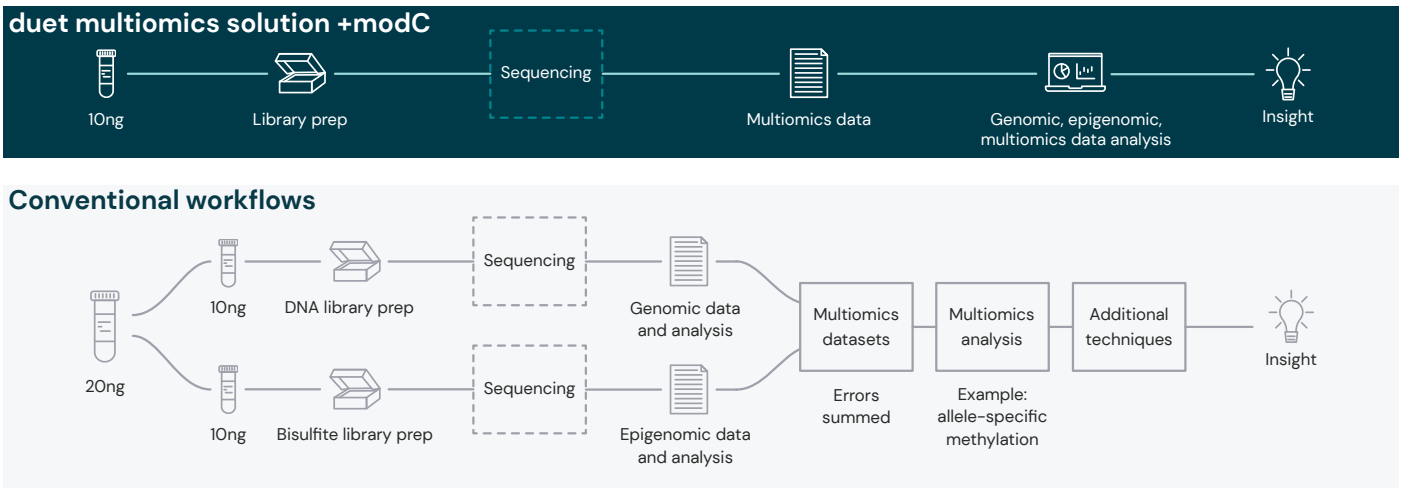


Figure 1. The duet multiomics solution +modC single workflow vs conventional genomic and epigenomic sequencing workflows. (Top panel) Following the pre-sequencing workflow, and device-agnostic sequencing, the post-sequencing bioinformatics pipeline aligns epigenetic and genomic sequencing data for analysis, interrogation, and insight.

(Bottom panel) Conventional epigenomic and genomic sequencing methods require multiple workflows, are more prone to errors, require more DNA sample (20ng), and multiple datasets to gain insights.

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Method

DECODING EPIGENETIC REWIRING USING DUET MULTIOMICS SOLUTION +MODC

Firstly, DNA methylation using Nanopore (PromethION) sequencing was used to compare and confirm the DNA methylation landscape seen with **duet multiomics solution +modC** for WT184hTERT. The resulting data revealed strong Pearson correlation.

The next step was to interrogate the genomic and epigenetic landscape of all the cell types using **duet multiomics solution +modC**. The single workflow approach enabled researchers to glean greater insights from small amounts of sample DNA (Fig1).

Interestingly, using **duet multiomics solution +modC**, revealed that only the p53$^{-/-}$BRCA1$^{-/-}$ 184hTERT, and not the untransformed diploid 184hTERT breast epithelial cell lines (WT184hTERT) or the p53$^{-/-}$BRCA2$^{-/-}$ 184hTERT, showed significant activation of stem cell enhancers through reduced DNA methylation, and hence cancer-associated epigenetic reprogramming.

In a second step, Dr Singh used **duet multiomics solution +modC** on a reference TNBC patient-derived xenograft (PDX) sample, which also showed significant activation of stem cell enhancers through DNA methylation changes.

Results

EMPOWERING GAME-CHANGING RESEARCH IN A SINGLE WORKFLOW

In this study, the Aparacio lab used **duet multiomics solution +modC** to analyse in vitro breast cancer cell lines, then compared these findings to cells from a patient biopsy. They found strong correlation and alignment from the resultant comparative genomic and epigenetic data and were able to inform their research on cancer progression in triple-negative breast cancer.

The findings illustrate that BRCA1$^{-/-}$ is crucial for HRD-specific cancer pathogenesis, where it also drives genomic instability signatures, and while BRCA2$^{-/-}$ drives genomic instability, it alone may not be able to drive the necessary epigenetic rewiring for cancer progression.

Researcher Spotlight

Dr Sam Aparicio, BM, BCh, PhD, FRCPath, FRSC

Dr Samuel Aparicio is the Nan & Lorraine Robertson Chair in Breast Cancer Research, holds the Canada Research Chair (Tier 1) in Molecular Oncology, and is the recipient of the 2014 Aubrey J Tingle Prize. He is also Head of the Department of Breast and Molecular Oncology at BC Cancer Research, part of the Provincial Health Services Authority, and a Professor in the Department of Pathology and Laboratory Medicine at UBC.

Dr Gurdeep Singh, PhD

Dr Gurdeep Singh is Post-Doc in Dr Samuel Aparicio's lab at BC Cancer, decoding the epigenetic basis of cancer pathogenesis and drug-resistance using CpG methylation & epigenomic landscape, and defining/testing the responsible transcriptional regulators. Dr Singh received his PhD in 2021 from The University of Toronto where he identified the genome sequence code that confers enhancer activity in embryonic stem cells, and other tissues, using functional genomics experiments and computational approaches.

"We are already seeing very exciting data emerge from cancer genomes and pre-cancer genomes.

Also, in our work involving measuring DNA fragments in patient plasma as a monitoring tool, conducting high-efficiency short-read modC will increase the sensitivity of circulating tumour DNA assays."

Dr Sam Aparicio

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