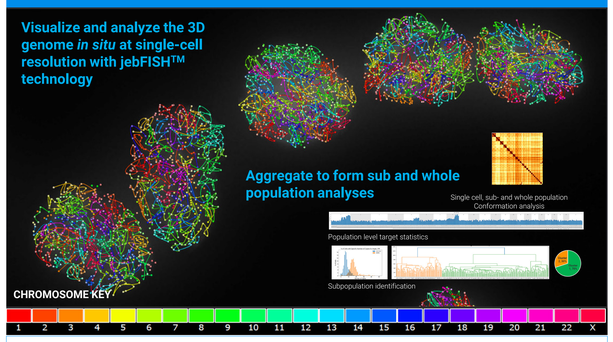
Integrated spatial multiomic profiling of 3D genome architecture and transcriptome-wide gene expression in breast cancer models

Yi Cui[2], Huy Nguyen[1], Brian Smart[1], Austin Nguyen[1], Andrea Floris[1], Serdar Tulu[1], David Castillo[1], Sierra McKinzie[2], Isabel Lee[2], Shanshan He[2], Shyamtanu Chattoraj[1], Jude Dunne[1] and Joseph Beechem[2]

[1]Bruker Spatial Genomics Inc., USA; [2]Bruker Spatial Biology, USA

Introduction



- Here we present a novel jebFISH™ protocol on the PaintScape™ platform and the CosMx® Spatial Molecular Imager (SMI) Whole Transcriptome (WTX) assay used to characterize differences in genome structure, gene expression and phenotype at subcellular resolution between ER+ breast cancer cell line MCF7 and ER- normal breast cell line MCF10a.
- ER+ cell lines often exhibit selective amplification of chromosomal regions (e.g. 17q23 and 20q23 in MCF7) containing distant estrogen response elements (DEREs), which facilitate long range chromatin interactions causing transcriptional repression of tumor-suppressor genes and activation of oncogenes.[1]
- ER activation induces upregulation in key proliferative and differentiation gene targets, including CCND1, MYC through both proximal EREs and long-range DEREs that coordinate enhancer-promoter interaction.[2-3]
- Luminal epithelial and non-invasive identity of MCF7 is maintained due to high expression of GATA3, a core luminal transcription factor co-expressed and co-regulated with ER.[4]
- The ER-GATA3 axis maintains luminal epithelial programs by maintaining high CDH1 expression while transcriptionally suppressing EMT drivers such as SNAI1 and SNAI2, despite higher genomic copy number of these two genes in MCF7.[5-6]
- Loss of ER/GATA3 signaling is associated with EMT activation and more aggressive, less differentiated tumor phenotypes[7]
- Selective copy gain, rearrangement, and altered 3D architecture of those oncogenes and their regulatory elements cause dysregulation of oncogenic signaling pathways and increases cancer cell proliferation providing positive selection for cancer progression.

Technology and Methods

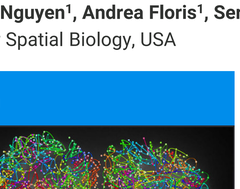
A The PaintScape System powered with jebFISH™ technology to visualize in-situ 3D genome organization in situ in single cells

What is the PaintScape System?

- An instrument platform that combines state-of-the-art optics, sophisticated bioinformatics, and a reagent system based on powerful **jebFISH** chemistry for direct ***in situ* visualization** of the 3D genome in individual cells

What is jebFISH?

- **High-plex, efficient** chemistry for *in situ* visualization of the 3D genome in single cells (in cell lines and fresh frozen tissue) using a proprietary multiplex optical **barcoded** chemistry
- Proprietary **jebSmart™** (smart barcode) method to allow precision loci identification and localization



B OncoPaint™ HuCL Cancer Pathways Kit 1 Panels

Each gene region is painted with 15 loci.

The MDM2 gene region shown as an example.

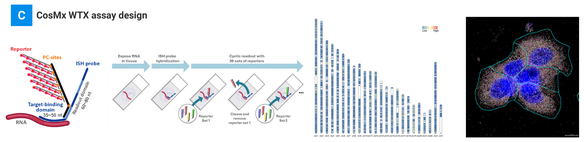
p-arm
q-arm

Kit 1 Panel Modules
- Cell Cycle and Apoptosis
- DNA Repair
- Transcriptional Regulation
- Chromatin Modification
- Chromatin Structure

PaintScape tracing of 1050 target loci in MCF10a. Each chromosome is painted as described in methods, section B

OncoPaint™ Panel Modules Used: Transcriptional Regulation, DNA Repair, Cell Cycle and Apoptosis, Chromatin Structure and A/B ploidy panels

C CosMx WTX assay design



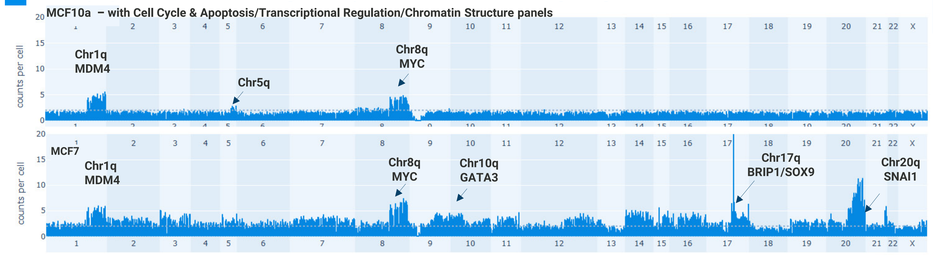
CosMx RNA detection schematic

WTX chromosome coverage

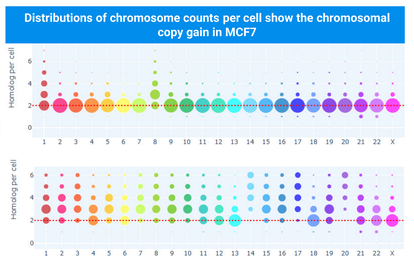
WTX RNA detection in cultured MCF7 cells

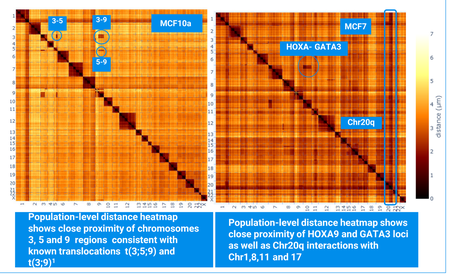
PaintScape system characterizes and directly visualizes 3D genome organization and structural genotype of ER+ MCF7 cells

A PaintScape system identifies differential 3D genome amplification and conformation of oncogenic regions between normal and ER+ breast cancer cells



Distributions of chromosome counts per cell show the chromosomal copy gain in MCF7

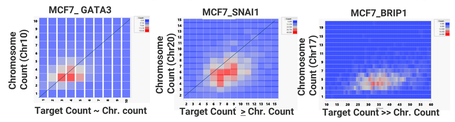




Population-level distance heatmap shows close proximity of chromosomes 3, 5 and 9 regions consistent with known translocations t(3;5;9) and t(3;9)[1]

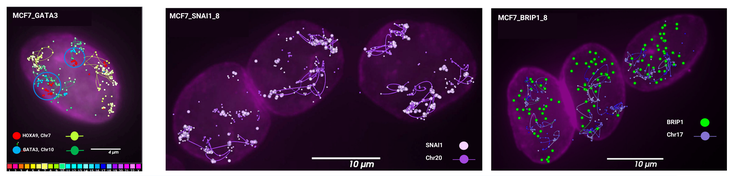
Population-level distance heatmap shows close proximity of HOXA9 and GATA3 loci as well as Chr20q interactions with Chr1,8,11 and 17

B PaintScape system identifies cells with target amplification in context of chromosomal copy number



Select genes (GATA3, SNAI1 and BRIP1) showing target counts similar to, slightly in excess of and largely in excess of chromosome count.

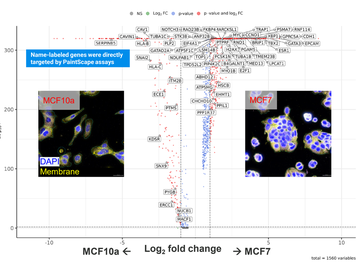
C PaintScape system identifies *in situ* 3D genome conformation in ER+ breast cancer cells



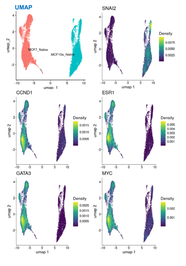
PaintScape directly visualizes cells with a range of target amplifications , GATA3, SNAI1 and BRIP1.
Note that GATA3 shows interaction with HOXA9 locus. Note that BRIP1 shows ecDNA like amplification relative to Chr17[8]

CosMx WTX assay characterizes unique gene expression profile of ER+ BC cell MCF7 *in situ* and identified key signaling pathways and unique cell states compared to normal breast cell MCF10a

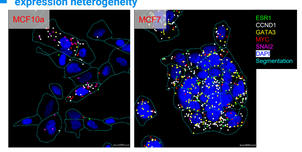
A MCF7 and MCF10a cells show transcriptome-wide differences, with key Estrogen-Responsive Genes (ERGs) differentially expressed



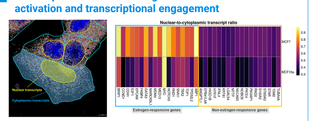
B MCF7 cells possess ER-DRIVEN molecular markers with specific oncogenic functions



C CosMx WTX reveals inter- and intrapopulation expression heterogeneity

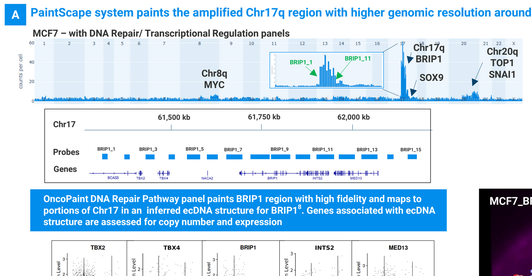


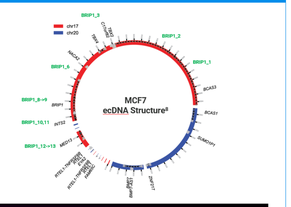
D ER+ MCF7 cells display spatial nuclear accumulation of ERG transcripts consistent with ligand-induced chromatin activation and transcriptional engagement



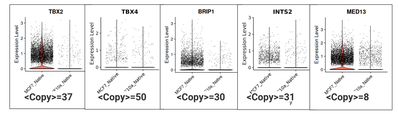
PaintScape 3D genomics and CosMx WTX characterizes ecDNA element driven oncogenic expression in MCF7

A PaintScape system paints the amplified Chr17q region with higher genomic resolution around BRIP1

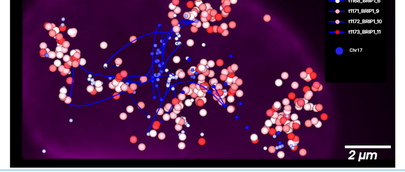




OncoPaint DNA Repair Pathway panel paints BRIP1 region with high fidelity and maps to portions of Chr17 in an inferred ecDNA structure for BRIP1[9]. Genes associated with ecDNA structure are assessed for copy number and expression

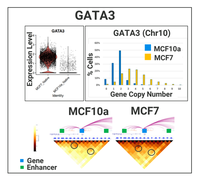


Single cell *in situ* visualization of ecDNA around BRIP1 locus on chr17 shows targets aggregated in a hub like manner[10]. Genes associated with this ecDNA such as TBX4 and INST2 do not show elevated expression despite high copy numbers indicating possible loss or hijacking of enhancer regions enabling dysregulated expression. Genes such as MED13 show elevated expression and may benefit from presence in ecDNA structures.



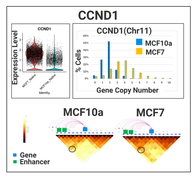
PaintScape 3D genome structural data yields insights into differential gene expression as measured by CosMx WTX

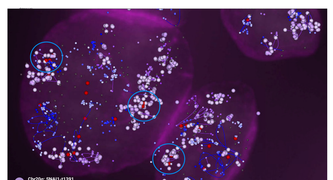
A PaintScape *in situ* visualization contextualizes differences in gene expression between cell lines at cell population level



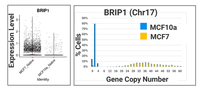
Differentially expressed genes in MCF7 such as GATA3 and CCND1 can be understood in terms of copy number gain and promoter-enhancer interaction proximity.

Gene Locus Distance Maps showing Topologically associated domains (TADs)

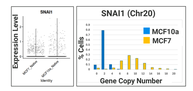




Close interaction between Chr17-Chr20 elements



SNAI1 and BRIP1 are amplified in MCF7 and do not show commensurate increase in gene expression.



PaintScape visualizes Chr 17q-20q interactions between DERE elements known to exhibit promoter-enhancer hijacking in ecDNA elements and known to dysregulate expression[1,8]

Conclusions

The PaintScape system enables high-resolution identification, characterization, and direct *in situ* visualization of single-cell 3D genome architecture in single cells. CosMx WTX profiles spatial gene expression at a single-cell level revealing disease state specific oncogenic function. Here, the combined approach of PaintScape and CosMx reveals how disruptions in genome organization drive disease mechanisms in ER+ breast cancer by linking altered chromatin topology to dysregulated expression of key oncogenic pathway genes. Distinct structure-function correlations are revealed:

- **Copy number variation including amplification and deletions *in situ* in single cells and sub-population**
  - Specific gain in Chr17q and 20q regions containing DEREs in MCF7 BC cells
  - Focal amplifications of important oncogenes BRIP1, MYC, SNAI1
  - Loss in CDKN2A gene on Chr 9
- **Single cell ploidy variation of whole chromosomes and sub-chromosomal regions**
  - Higher variation in sub-population of cells with variable chromosome ploidy in cancer cells compared to normal cells
  - Chr17 and Chr20 show much higher chromosome ploidy in cancer cells
- **Inter-chromosomal interactions, potential translocations and simultaneous proximity of multi-loci interactions *in situ* in single cells**
  - Close proximity of chromosomes 3, 5 and 9 regions in MCF10a consistent with known translocations t(3;5;9) and t(3;9)
  - Close proximity of GATA3 (Chr10) and HOXA (Chr7) loci in MCF7 causing dysregulated gene expression of GATA3 and certain HOXA genes in MCF7
- **ecDNA copy number, structure, nuclear locations and *in situ* interactions**
  - A fraction of amplified DERE targets of Chr17q and Chr20q regions were further from Chr17 or Chr20 territory suggesting possible ecDNA nature of those amplified targets
  - BRIP1 gene region shows very high copy number due to ecDNA amplification in MCF7
  - A significant fraction of the BRIP1 ecDNA formed self-interacting hub like structure in single MCF7 cells
  - BRIP1 ecDNA closely interacts with amplified DEREs on Chr20q including SNAI1 oncogene
- **3D genome structure, spatial gene expression and multiomic structure-function correlations**
  - CosMx WTX identified elevated expression of ER-driven oncogenes GATA3 and CCND1 in MCF7 cells, consistent with enhanced estrogen-dependent luminal signaling and proliferative activity
    - PaintScape 3D genome data shows increased enhancer-promoter interactions and strengthen intra-TAD interactions for these genes revealing mechanism of higher expression
  - PaintScape system characterized and directly visualized unique ecDNA structure containing relevant oncogenes BRIP1, TBX2, MED13 in MCF7
    - CosMx WTX shows higher expression of these genes in MCF7 compared to MCF10a revealing functional relevance of ecDNA amplification which promotes opportunistic enhancer hijacking within ecDNA elements and promoting dysregulated expression

PaintScape™ System and CosMx WTX enables *in situ* direct visualization of 3D genome changes and spatial gene expression in single breast cancer cells

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