# PaintScape™ enables multiomic in-situ direct visualization of spatial 3D genome architecture of single cells in intact fresh frozen Glioblastoma tissue in native tumor microenvironments

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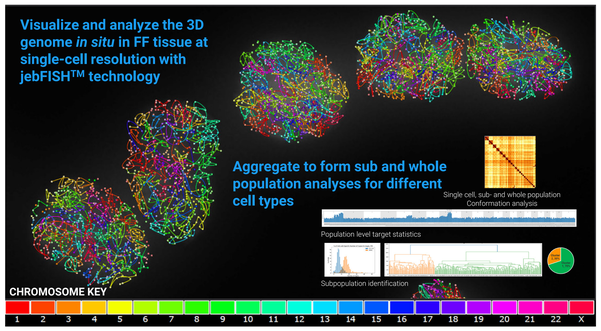
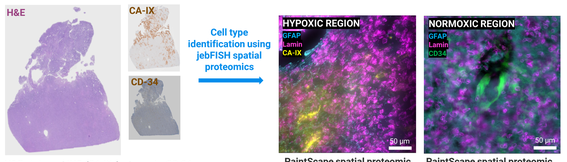
## Introduction




- Here we present a novel jebFISH™ tissue genome painting protocol on the PaintScape™ platform to reveal the 3D genome organization *in situ* of fresh frozen (FF) glioblastoma (GBM) tissue at single cell, sub-population, and population levels.
- GBM is the most aggressive and common type of brain tumor and is characterized by high intra- and inter-tumoral molecular heterogeneity. Over 70% of GBM patients exhibit genetic signatures, such as Chr7 amplification and Chr10 loss of heterozygosity (LOH)[1]. Also, GBM patient outcome is strongly impacted by tumor microenvironments, with hypoxia noted as a significant detrimental factor[2].
- Chromosome conformation capture analysis on bulk tissue has identified the role of the 3D genome in GBM[3], but further understanding of GBM requires tools that can study the genomic complexity with single cell resolution and spatial context.
- Here we use the PaintScape System to directly visualize the genome organization of GBM tissue *in situ* in single cells of a 30-µm FF tissue section. In addition, hypoxic and normoxic regions of the tissue section were identified to perform further structural genome organization analysis and comparison of tumor cells located in those regions and reveal differences or similarities in the respective structural genome landscapes of these sub-regions.
- The powerful nature of PaintScape-derived data enables even further filtering to focus on subpopulations of cells with specific genome organization traits for even deeper analysis of combinations of parameters and combinations of specific cell subsets that were not possible to compare before. For example, here, the 3D genome landscapes of a subset of tumor cells with Chr 7 trisomy from both hypoxic and normoxic regions of the GBM tissue is selected and further compared—revealing specific structural genome differences that contribute to the molecular heterogeneity of GBM tumor cells.

## Technology and Methods

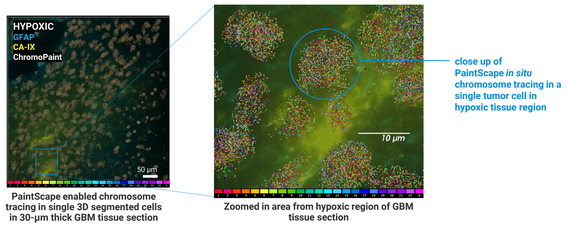
**A** Multiomic jebFISH™ workflow on PaintScape™ platform on 30-µm thick FF tissue sections




H&E stain and IHC (CA-IX for hypoxic, CD-34 for normoxic) **of sequential sections**

PaintScape spatial proteomic image of hypoxic region

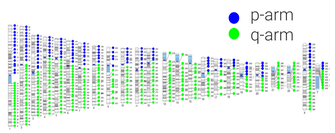
PaintScape spatial proteomic image of normoxic region




PaintScape enabled chromosome tracing in single 3D segmented cells in 30-µm thick GBM tissue section

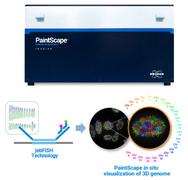
Zoomed in area from hypoxic region of GBM tissue section

**B** ChromoPaint™ FF PanChromo MPX Panel Loci




**419-plex panel used in jebFISH painting of GBM tissue**

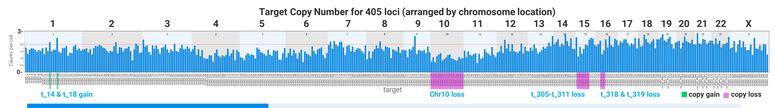
**C** jebFISH workflow on PaintScape platform




## PaintScape system directly visualizes and characterizes *in situ,* single cell 3D genome organization and structural genotype of Glioblastoma from 30 µm thick fresh frozen tissue section

### PaintScape characterizes CNVs, subpopulation ploidy, and 3D genome organization in FF GBM tissue at a single-cell level

**C**

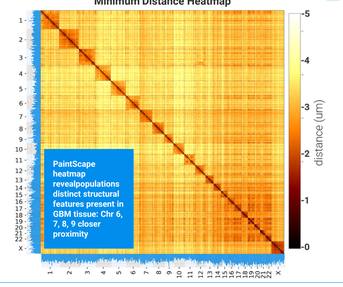



Data reveals specific copy number gains and losses across the genome in GBM related genes and additional variable regions that may influence disease biology

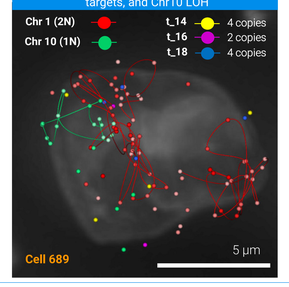
**Copy # increase:** Chr1: t_14 and t_18 → Regions contain highly expressed GBM markers (LRIG2 & SMG5)[4,5]

**Chromosome loss:** Chr10 → Common occurrence in GBM[1,7]. Contains key GBM genes (PTEN & ANXA7)[6,7]

**D**



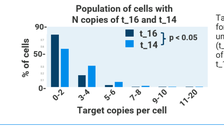

**E**




**Single cell 3D genome in GBM tissue**

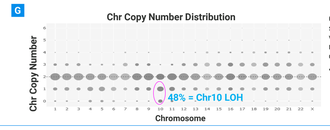
- PaintScape identified locus-specific CNVs across the genome in GBM related genes, while also exposing additional regions that may influence GBM
- Coexisting subpopulations with variable ploidy; including common GBM perturbation: Chr10 LOH[1] → intratumoral heterogeneity
- PaintScape enables discovery of distinct 3D genome landscapes of single cells *in situ* in FF tissue

**F**




Target count distribution per cell for **Target 14** (t_14) **gain** and unamplified neighbor Target 16 (t_16) shows higher percentage of cells with increased number of t_14 counts.

**G**



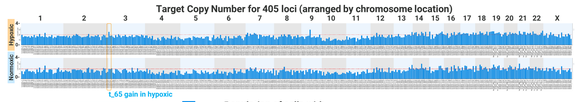

Coexistence of various sub-populations of cells with varying chromosomal ploidy reflects heterogenous nature of GBM

- **Chr10 loss in 48% of cells**

## PaintScape characterizes 3D genome and conformational changes in hypoxic and normoxic GBM tissue environments

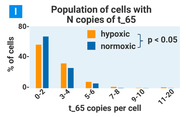
### PaintScape system characterizes CNVs, subpopulation ploidy, and 3D genome organization of hypoxic and normoxic regions in GBM tissue

**H**



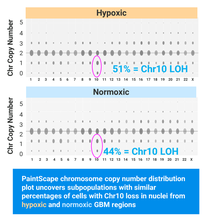

**Hypoxic regions show CNVs in chromosomal regions containing GBM and hypoxia related genes, with gain and loss trends consistent with expected expression**

**I**



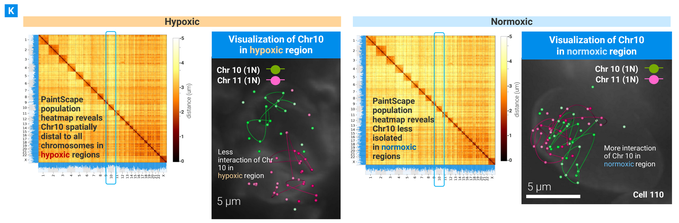

- Copy increase in Target 65 (t_65). The target region contains CHL1 (neural cell adhesion molecule) → upregulated in GBM & hypoxia[8,9]
- Target count distribution for t_65 gain in **hypoxic** vs **normoxic** regions. **Hypoxic** shows higher percentage of cells with increased number of t_65 counts.

**J**




**PaintScape chromosome copy number distribution plot uncovers subpopulations with similar percentages of cells with Chr10 loss in nuclei from hypoxic and normoxic GBM regions**

**K**




**Hypoxic vs Normoxic**

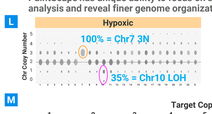
- In **hypoxic** regions (darker heatmap), most of genome interacts more than in normoxic (lighter heatmap)
- In **hypoxic** regions, Chr10 interacts less with rest of genome (lighter Chr10 strip in heatmap) than in normoxic
- Chr10 contains key GBM genes PTEN and ANXA7[6,7]
- Decreased Chr10 interactions may facilitate further repression of PTEN → facilitating upregulation of HIF-1 to respond to **hypoxic** environments[10]

## Single cell 3D genome conformational differences in Chr7 trisomy subpopulations in hypoxic and normoxic GBM tissue

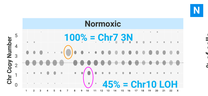
### As GBM is known to often have Chr7 copy gains, PaintScape was used to focus on specific cell subpopulations with Chr7 trisomy for deeper 3D genome analysis in the two tissue sub-regions

PaintScape has unique ability to focus on specific cells or cell subpopulations for further analysis and reveal finer genome organization differences with single-cell detail

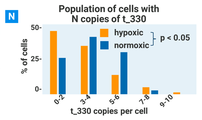
**L**



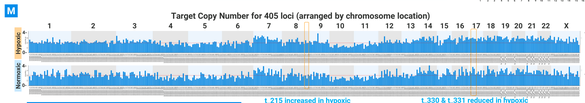

In **Chr7 3N subpopulations**, the percentage of cells with Chr10 LOH (commonly lost with Chr7 gain[1]) is slightly reduced in **hypoxic** regions. Of note, when a cell has Chr7 3N, Chr10 is not always lost—further demonstrating the genetic heterogeneity across GBM tissue. **PaintScape enables cell-to-cell comparison** of this heterogeneity.




**N**



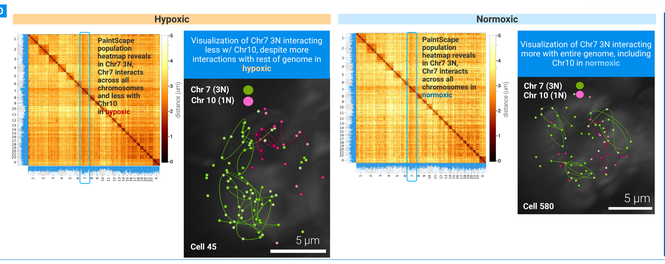

**M**




**Chr7 trisomy cells show CNVs in chromosomal regions containing GBM and hypoxia related genes**

- Focused filtering on Chr7 3N validates GBM-associated gains and losses (e.g. Chr10 LOH) and concurrently reveals new genomic alterations previously not associated with GBM (e.g. Target_215, containing a long non-coding RNA: RORB-AS1)
- Copy decrease in Targets 330 and 331 ( t_330 & t_331, respectively). Region contains TMEM98 (Transmembrane Protein) → oncogenic driver in many cancers, expression is downregulated in **hypoxia**[11,12]
- Target count distribution for t_330 reduction in **hypoxic** vs **normoxic** regions. **Normoxic** shows higher percentage of cells with increased t_331 counts.

**O**



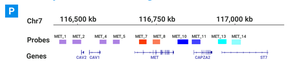

**Chr7 3N nuclei: Hypoxic Vs Normoxic**

- **In nuclei with Chr7 trisomy, Chr7 interacts widely across the genome in hypoxic** and normoxic **with reduced interaction with Chr10 in hypoxic**
- **Reduced Chr7-Chr10 interactions in hypoxic regions may further facilitate response to low oxygen (PTEN on Chr10 reduction → HIF1 stabilization)[10]**
- **Chr7 trisomy and Chr10 LOH is the most common CNV co-aneuploidy pair in GBM and all cancers[1]**
- **Coexistence of genetically distinct sub-populations including Chr7 trisomy with Chr10 LOH and Chr7 trisomy without Chr10 LOH → contributes to tumor heterogeneity**

## PaintScape characterizes MET gene region amplification and conformational changes in hypoxic and normoxic regions

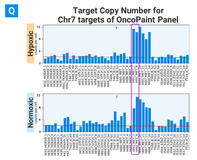
### PaintScape OncoPaint™ Pathway panel identifies, characterizes, and visualizes MET gene amplification in single cells

**P**



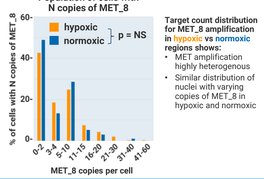

**Probes targeting MET gene region from an OncoPaint panel were used to visualize the MET gene and neighboring regions in GBM tissue.** [NOTE: Only MET gene region of panel shown]

**Q**




- **High amplification of MET gene region targets (MET_7-MET_14) in hypoxic and normoxic GBM tissue.** (boxed in magenta)
- **Note that target regions upstream to MET gene do not show high amplification**

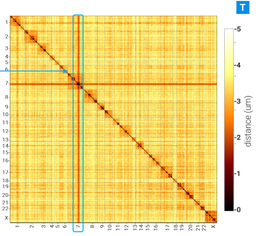
**R**




**Target count distribution for MET_8 amplification in hypoxic vs normoxic regions shows:**

- MET amplification highly heterogenous
- Similar distribution of nuclei with varying copies of MET_8 in hypoxic and normoxic

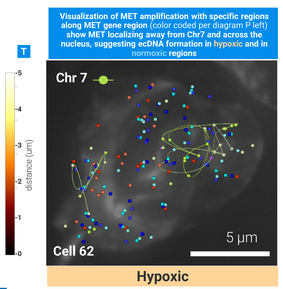
**S**




**MET amplification in single nuclei**

- **MET gene and gene region highly amplified in hypoxic and normoxic regions of GBM patient tissue**
- **MET amplification interacts genome wide, suggesting ecDNA formation**
- **Coexistence of subpopulations with varying amounts of MET gene region amplification**
- **Visualization shows MET amplification across the nucleus in clusters with neighboring genes CAPZA2 & ST7**
- **MET::CAPZA2/ST7 ecDNA seen in GBM and may serve as oncogeneic drivers[11]**

**T**




## Conclusions

The PaintScape™ System enables first-of-its-kind identification, characterization, and direct *in situ* visualization of single cell spatial 3D genome structure of various tissue microenvironments in 30-µm fresh frozen (FF) glioblastoma (GBM) tissue. The multiomic jebFISH protocols for tissue samples enable identification of specific cell types or tissue regions for further direct visualization and analysis of 3D genome organization. The power of the single-cell nature of the data allows for unprecedented filtering, analysis, and comparison of genomic landscapes or specific chromosomal aberrations in specific single cells as well as specific subpopulations of cells.

**Distinct structural genotypes and genome conformations revealed by jebFISH on the PaintScape System:**

1. **Canonical GBM genomic features including Chr10 loss and subpopulations**
2. **Hypoxic vs. normoxic:**
   - **CNVs in GBM and hypoxia related genes and 3D genome differences (Chr10 further from other chromosomes in hypoxic)**
   - **Chr7 3N cells: CNVs in GBM and hypoxia related genes and 3D genome differences (Chr7 and Chr10 differential interactions)**
3. **High levels of MET amplification forming ecDNA with neighboring genes CAPZA2 and ST7 in hypoxic and normoxic regions**

**PaintScape™ enables *in situ* direct visualization of 3D genome in FF Glioblastoma tissue in spatially resolved tumor microenvironments**

## References

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