

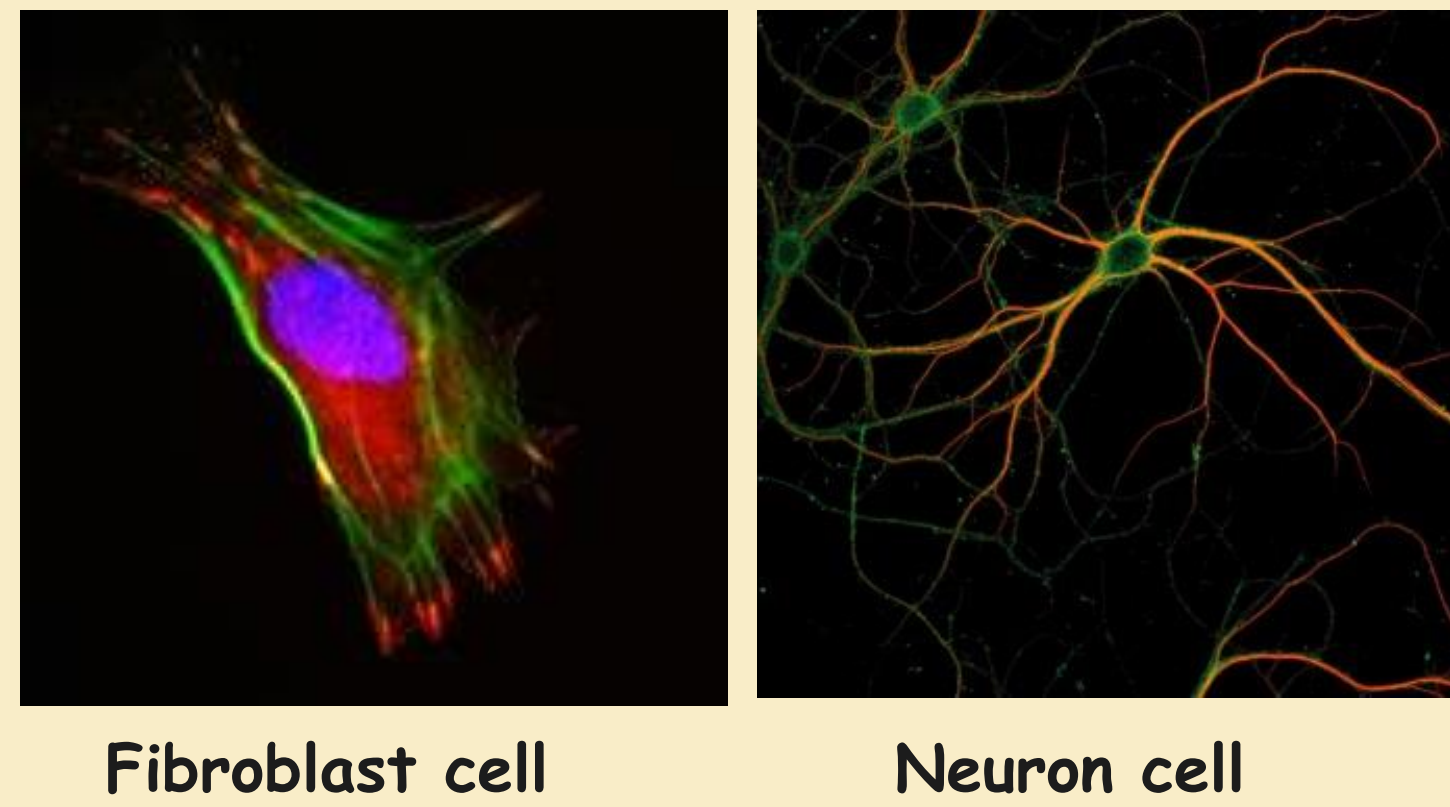
# EPIGENETIC MAPPING OF HUMAN CHROMOSOMES IN NANOCHANNEL ARRAYS



Tsilil Gabrieli, Yael Michaeli, Tamar Shahal, Hila Sharim, Yuval Ebenstein  
School of Chemistry, Tel Aviv University, Tel Aviv, Israel

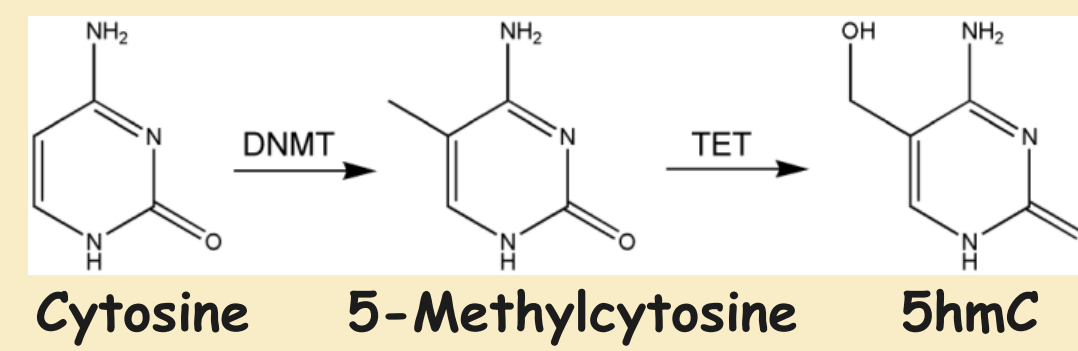
600kb human DNA molecule (blue) labeled with genetic barcode (red) and showing the corresponding 5hmC pattern (green).  
The molecule is aligned to the hg19 sequence reference of chromosome 5 (LIGHT BLUE)

Deletion

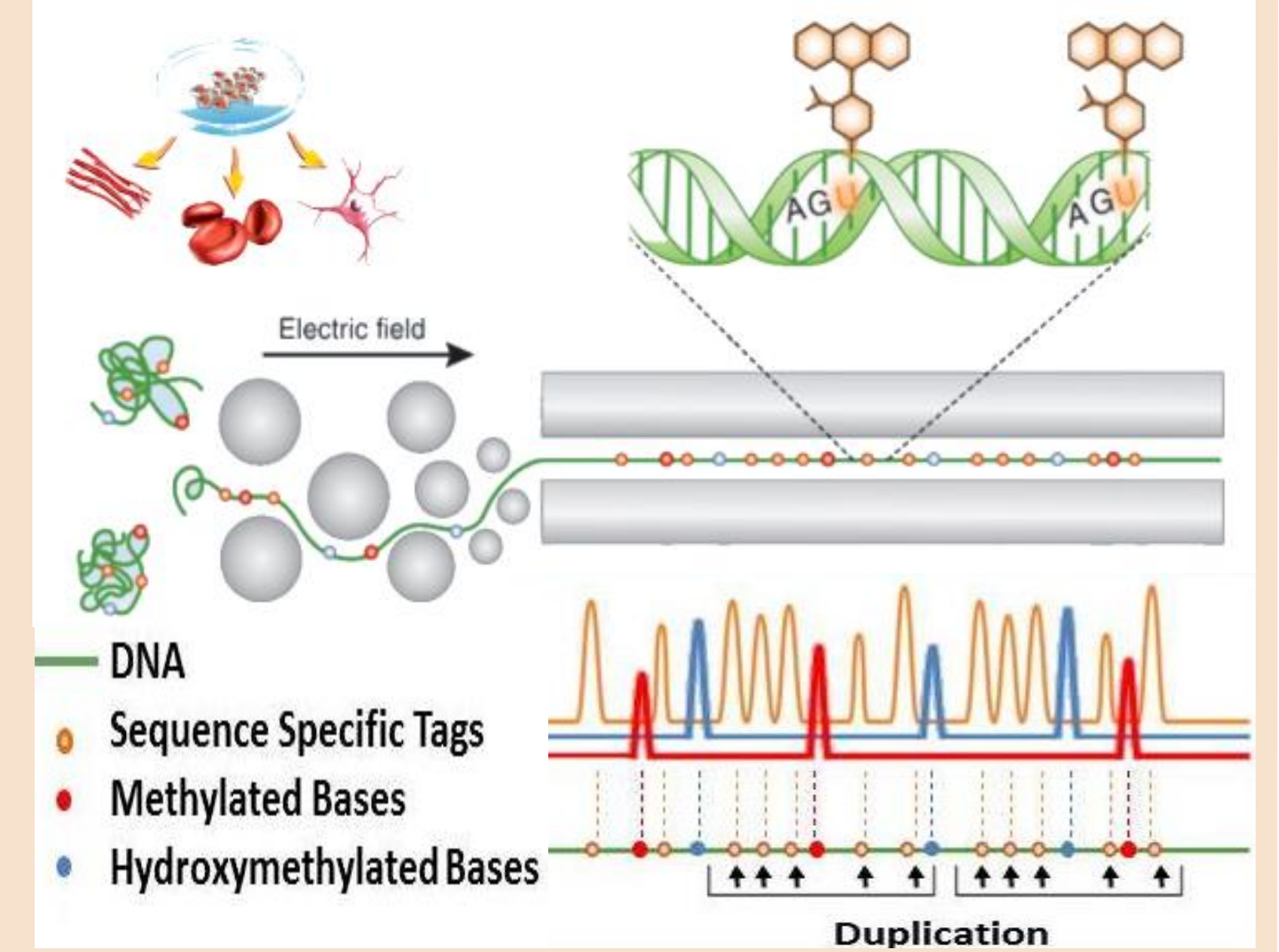


Cells from the same organism share a common genetic sequence, but their epigenetic profile determines their morphology and function.

Our work focuses on DNA Epigenetics:  
Chemical DNA modifications (methylation, hydroxymethylation)

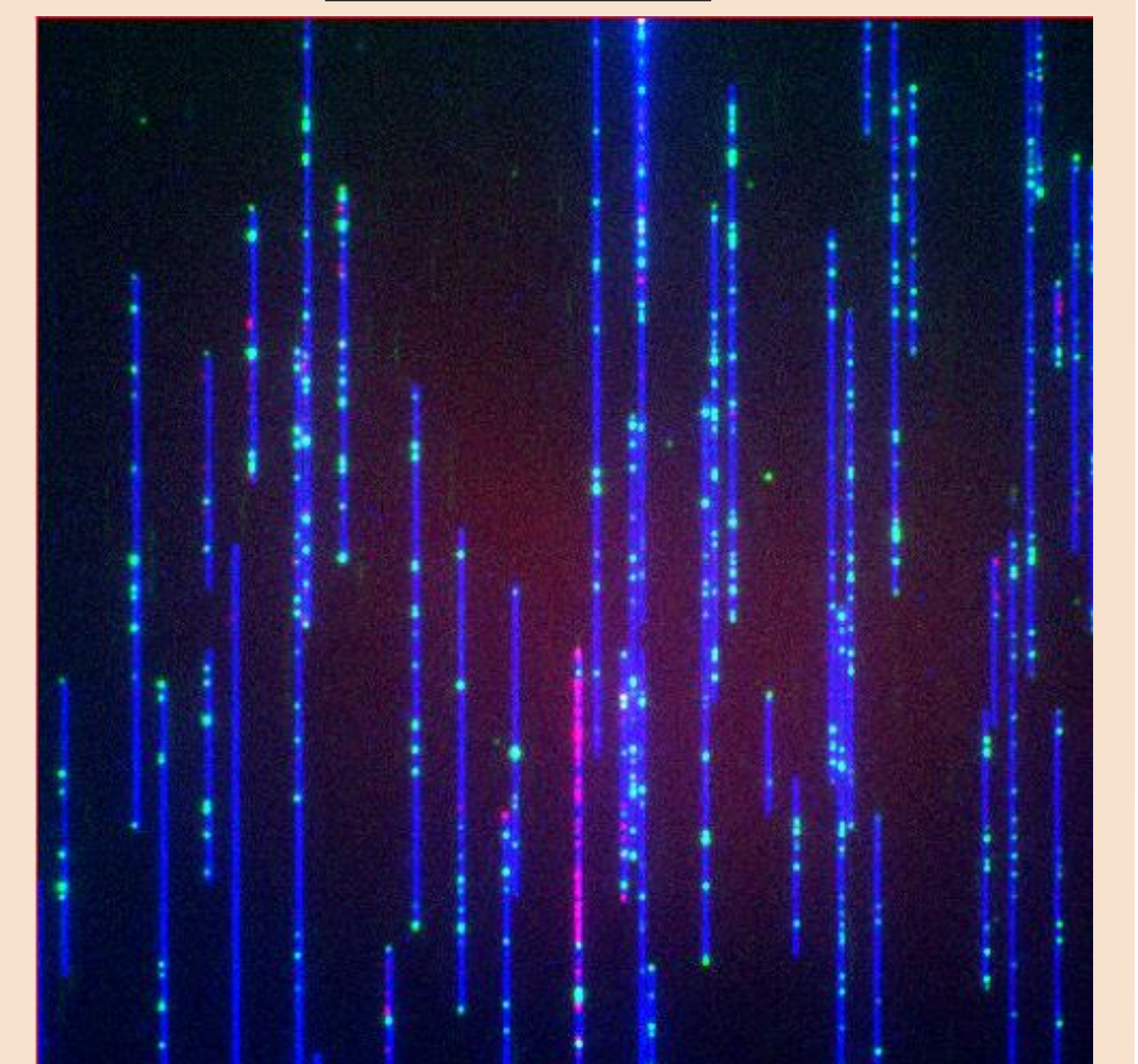


DNA is stretched in 45 nm wide silicon nanochannels



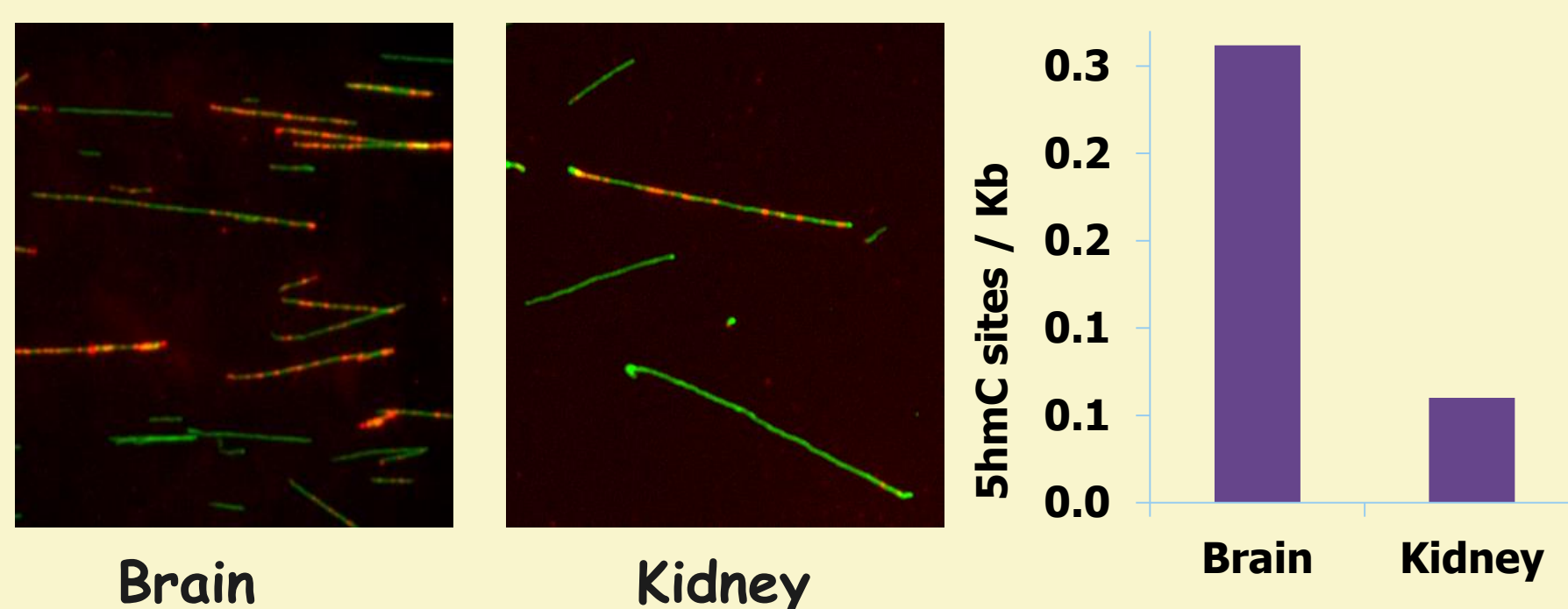
Dual-color labeled DNA extracted from human peripheral blood cells, was electrokinetically squeezed into an array of silicon nanochannels and imaged on a fluorescence microscope.

Dual colored DNA is stretched in nanochannels



Green: Genetic barcode Red: 5hmC

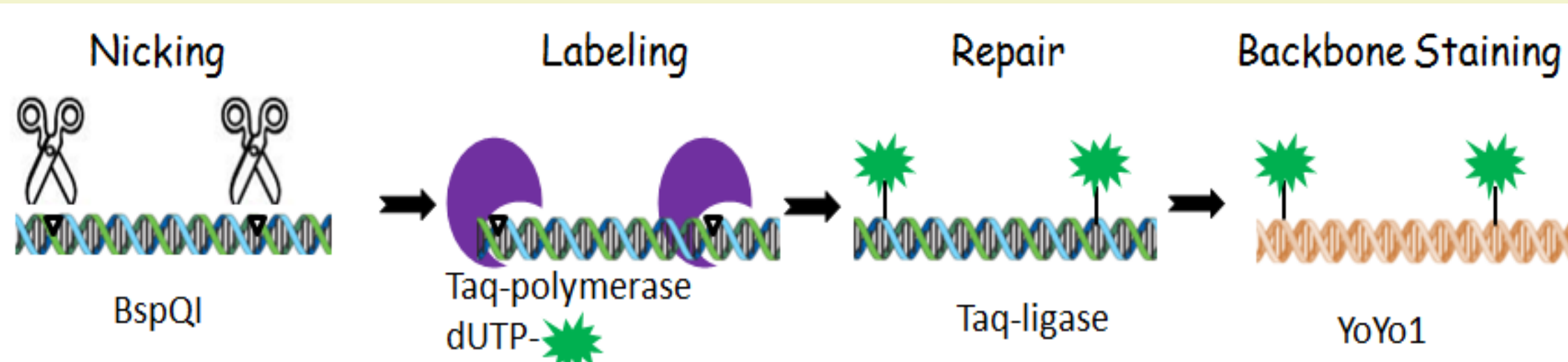
Labeling and quantification of 5hmC on DNA extracted from mouse tissues



DNA (green) with 5hmC moieties (red) stretched on modified glass surfaces and analyzed

DNA labeling

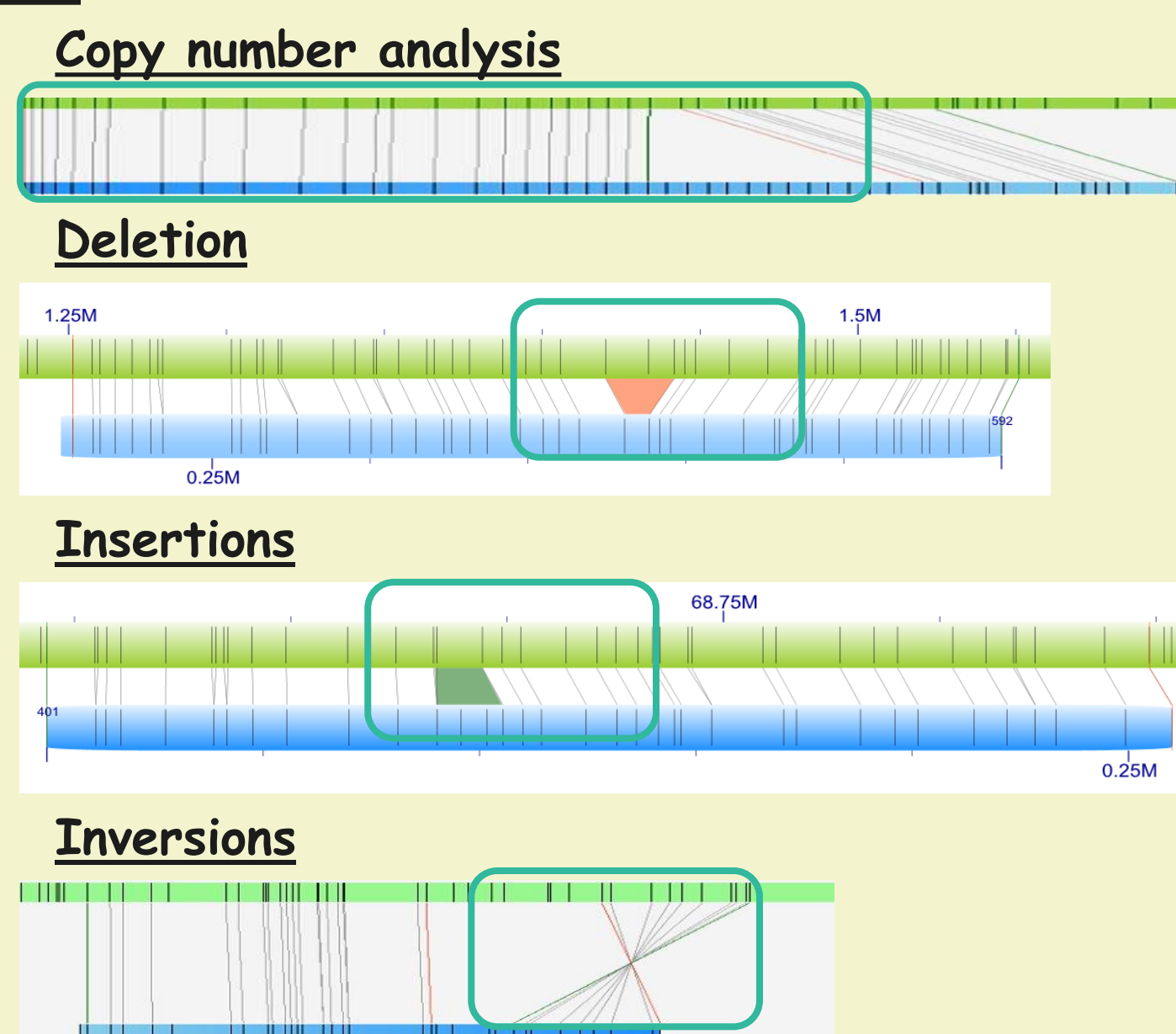
We start with mapping DNA molecules to the reference genome by generating a sequence specific barcode along individual DNA molecules. To do this we utilize nick translation with fluorescent nucleotides.



Structural variations

DNA optical mapping allows access to information that is lost using next generation sequencing such as:  
Detection of structural variations

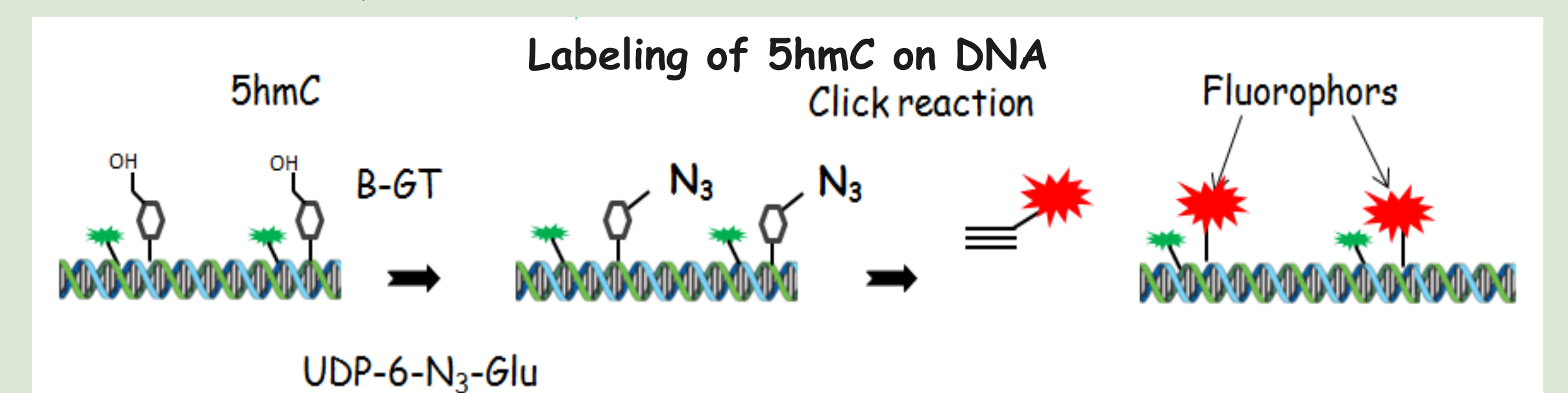
Green: Reference  
Blue: Query



5hmC mapping

Chemo-enzymatic fluorescent labeling of 5hmC

T4 β-glucosyltransferase is used to tag 5hmC sites with a fluorescent reporter molecule. The enzyme is fed with a synthetic cofactor UDP-6-N<sub>3</sub>-Glu, resulting in covalent attachment of a functional azide at the 5hmC site. This azide is further reacted with a DBCO functionalized fluorophore via a copper-free "click" chemistry reaction to generate the fluorescently labeled 5hmC.

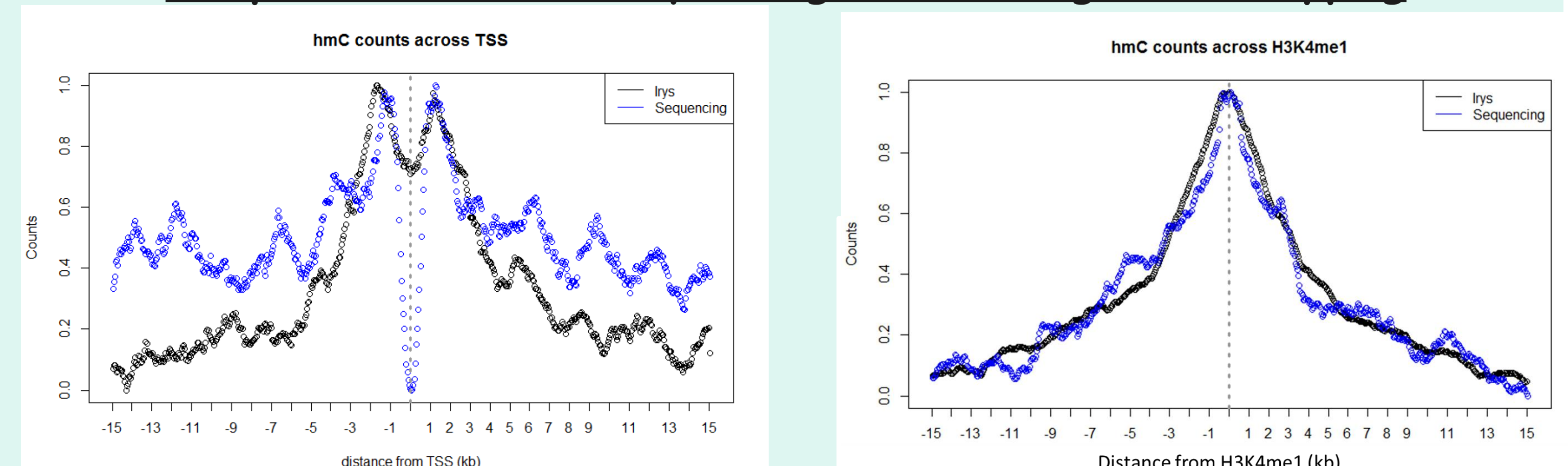


Double labeled molecule- BspQI and 5hmC

Alignment of double labeled single DNA molecules to an *in-silico* map in correlation with published genomic information



Comparison of 5hmC sequencing and 5hmC genome mapping



Funding

