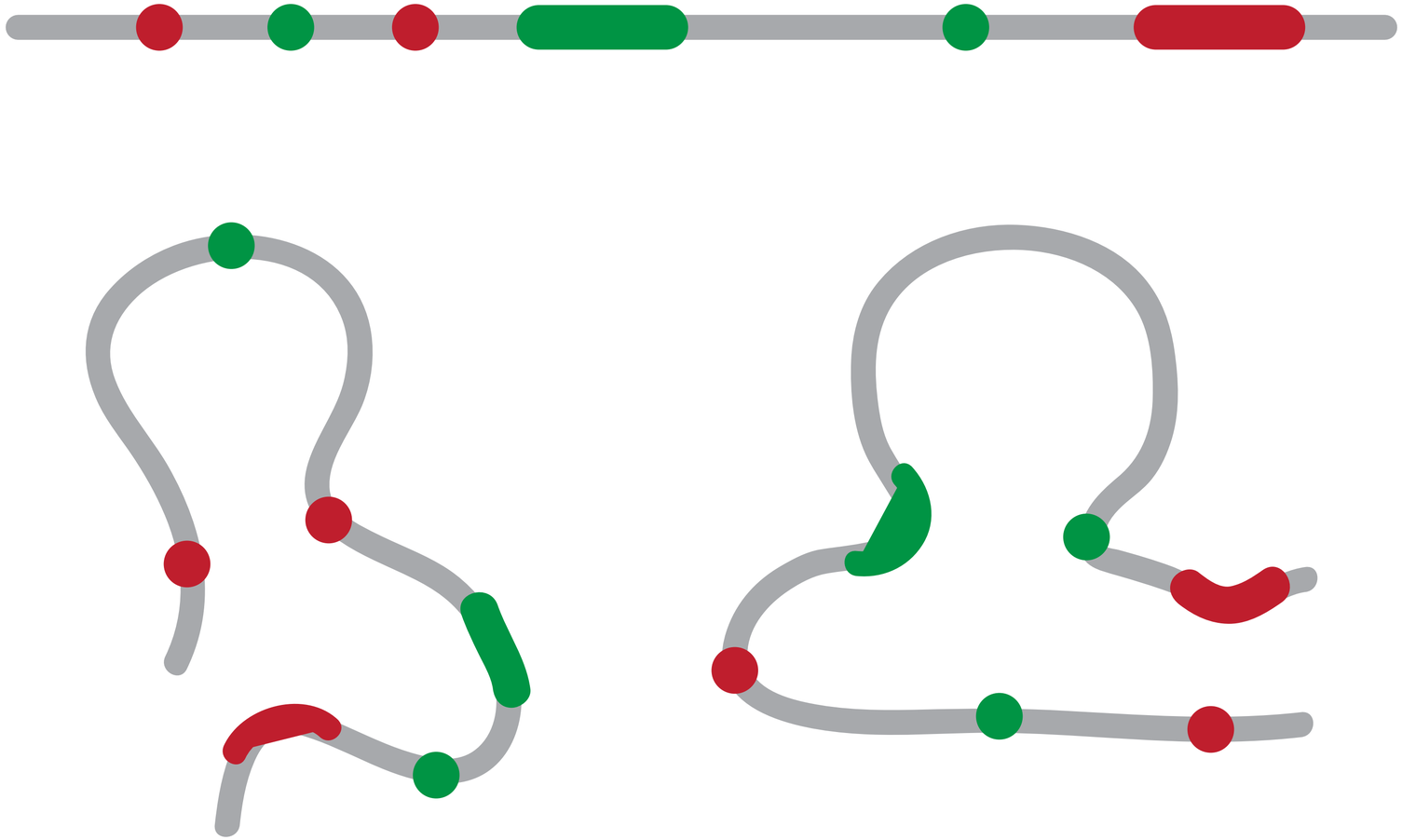

HUMAN “The Movie” by Yann Arthus-Bertrand

Structure determination of genomes and genomic domains.

Marc A. Marti-Renom
CNAG-CRG · ICREA

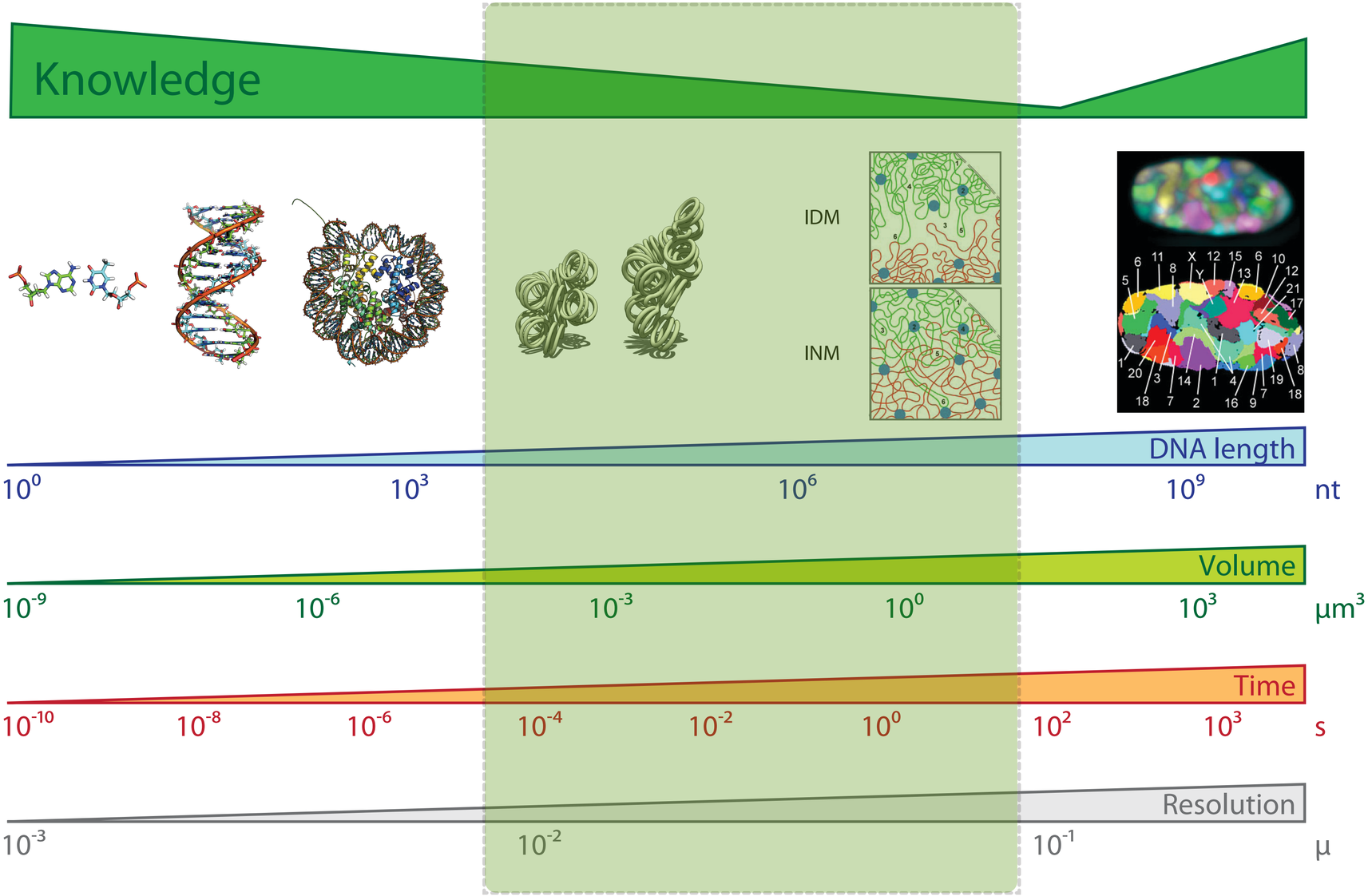
http://marciuslab.org
http://3DGenomes.org
http://cnag.crg.eu

cnag CRG ICREA



Resolution Gap

Marti-Renom, M. A. & Mirny, L. A. PLoS Comput Biol *7*, e1002125 (2011)



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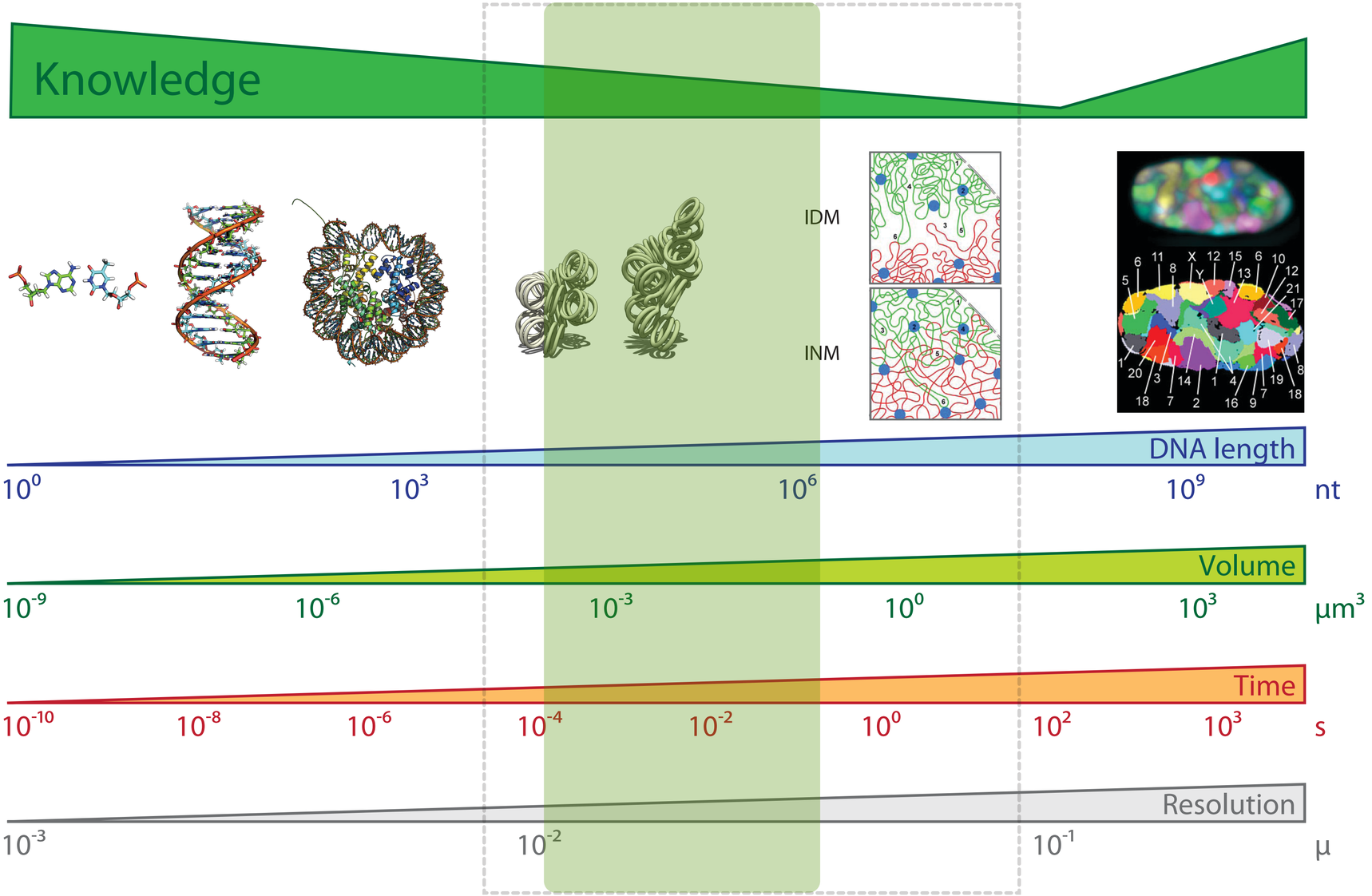
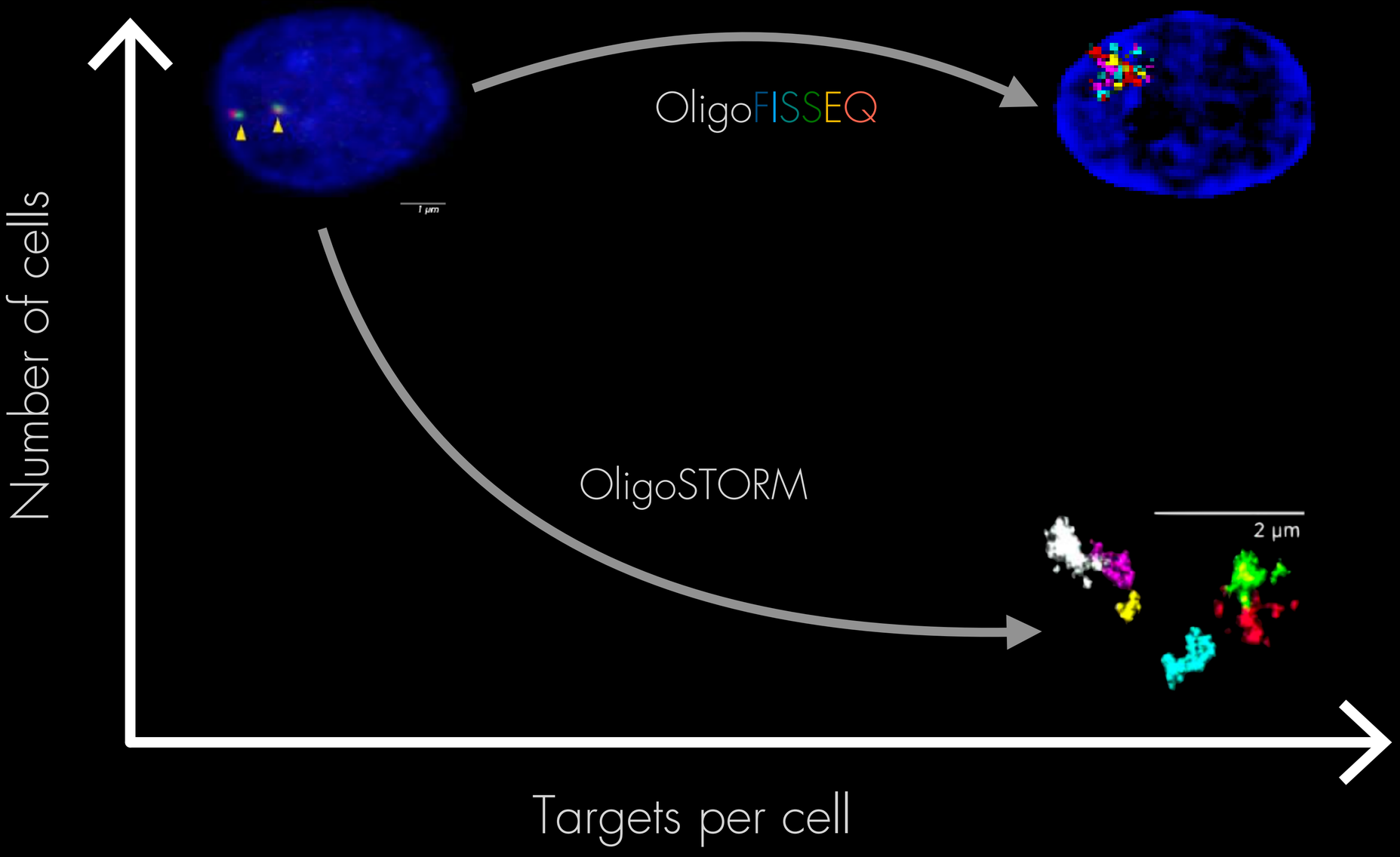



Number of cells
OligoFISSEQ
OligoSTORM
2 µm
Targets per cell

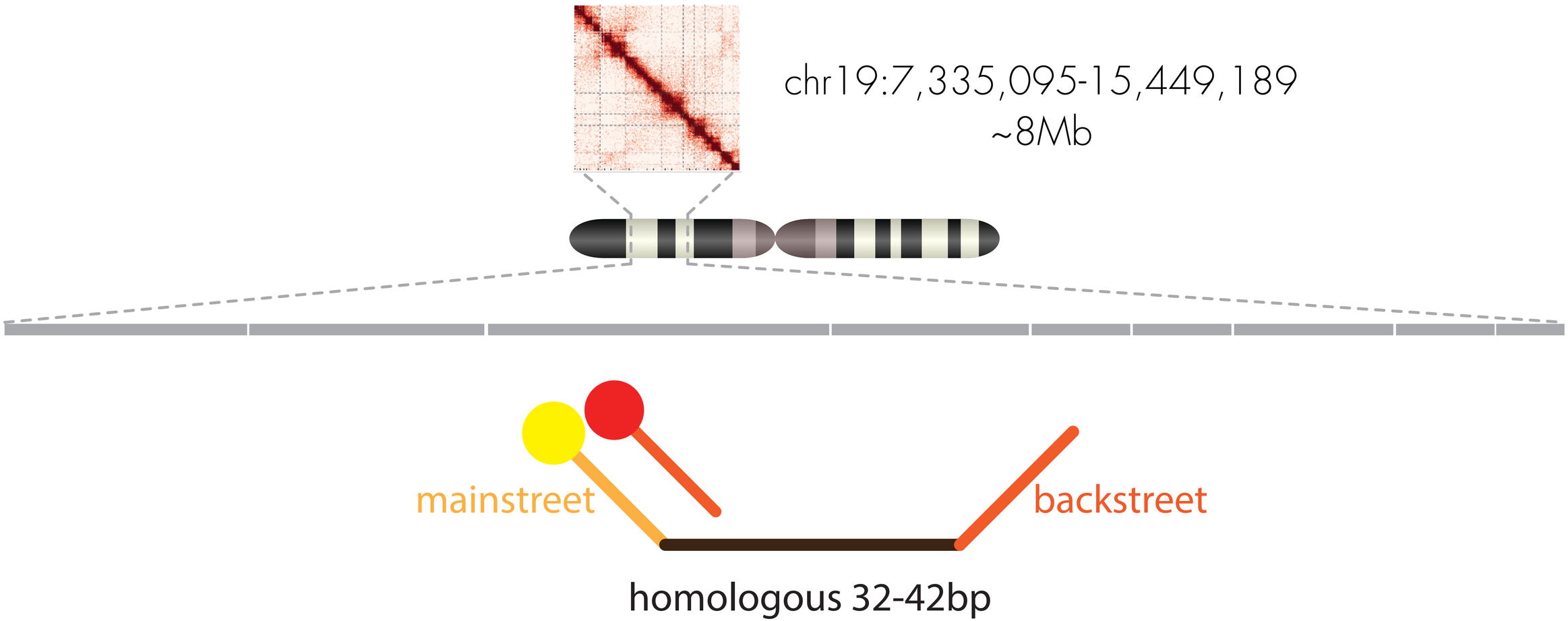
Chromosome walking with super-resolution imaging and modeling

Guy Nir
Irene Farabella
Cynthia Perez-Estrada
with Wu Lab (HMS, Boston) & Aiden Lab (UT, Texas)

PLOS Genetics (2018) 14(12) e1007872

High-resolution imaging

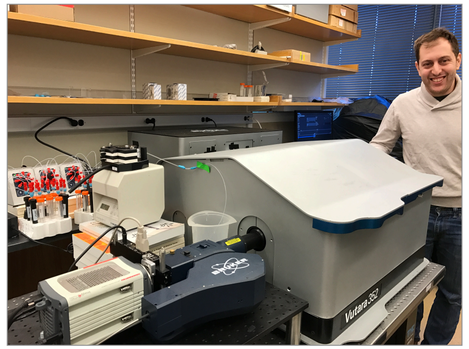
Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



Beliveau et al. Nat. Comm. 2015

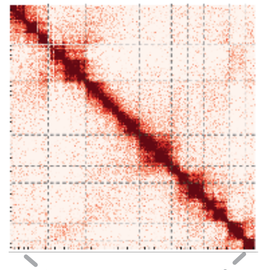
High-resolution imaging

Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells

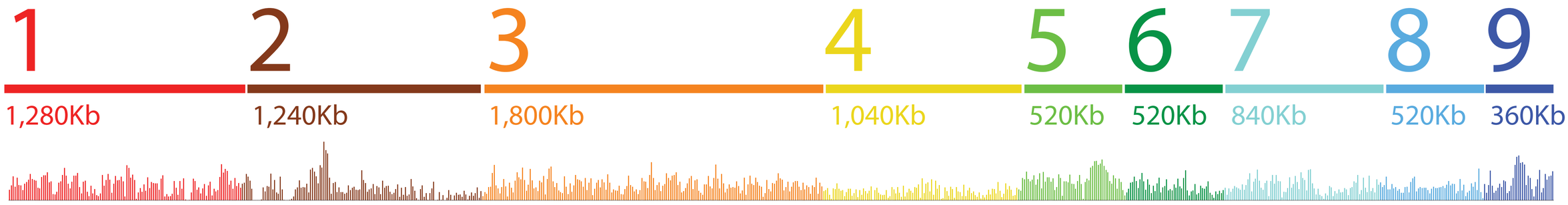


Guy Nir Harvard Med School

Bodgan Bintu Harvard
Carl Ebeling Bruker
Jeff Stuckey Bruker
John Schreiner Zero Epsilon
Steve Callahan Zero Epsilon

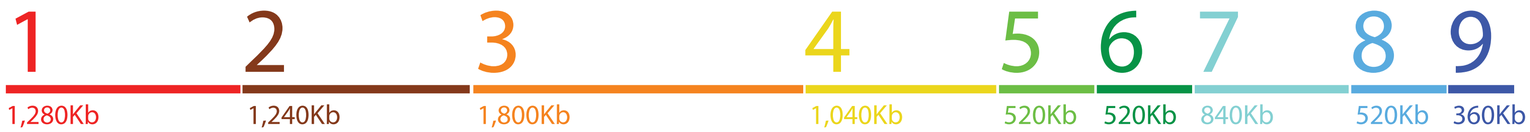


chr19:7,335,095-15,449,189
~8Mb



High-resolution imaging

Tracing chr19:7,335,095-15,449,189 ~8Mb

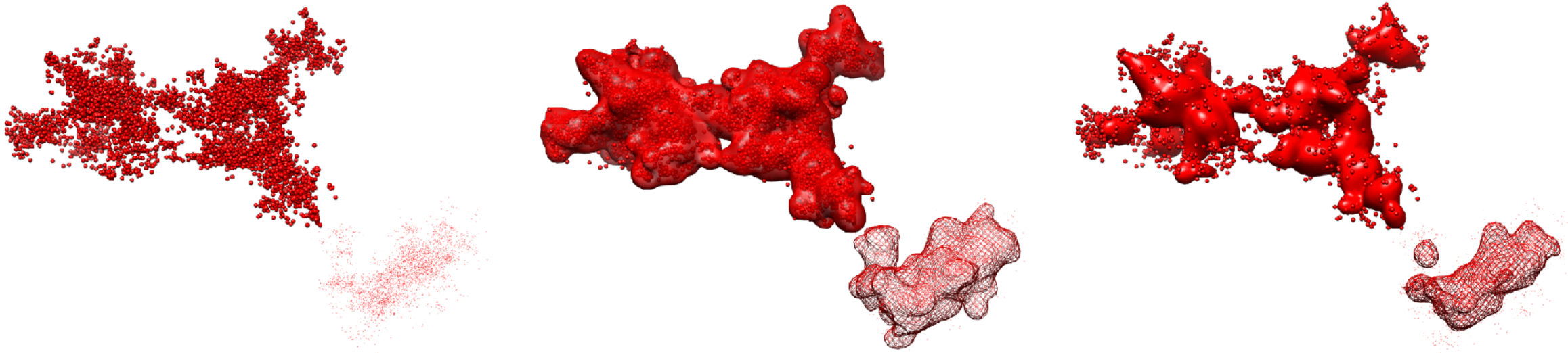


Cell-02

High-resolution imaging

XYZ points convolution into a density map

$$\rho(x,y,z) = \sum_N \frac{Z_N}{\left(\sigma\sqrt{2\pi}\right)^3} e^{-\frac{(x-x_n)^2+(y-y_n)^2+(z-z_n)^2}{2\sigma^2}}$$

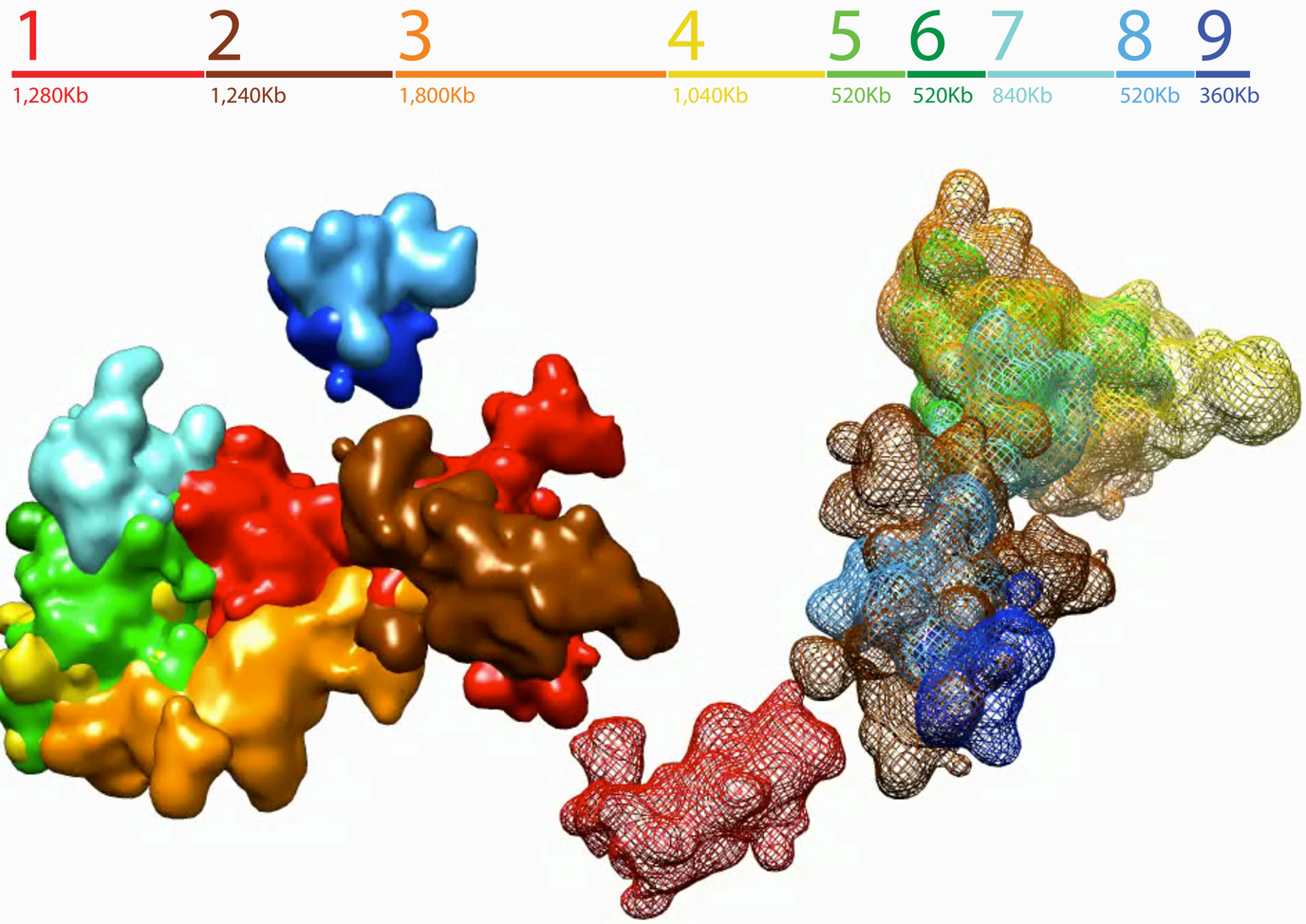


Cell-02 · Segment 1

Farabella et al, J Appl Crystallogr. 2015

Density maps

Cell-02 · Density map @ 50nm

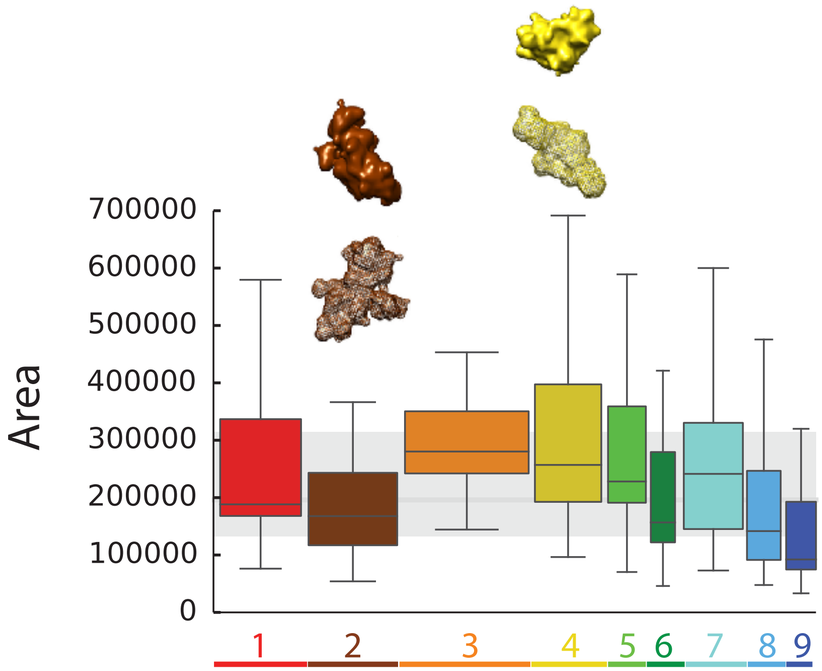


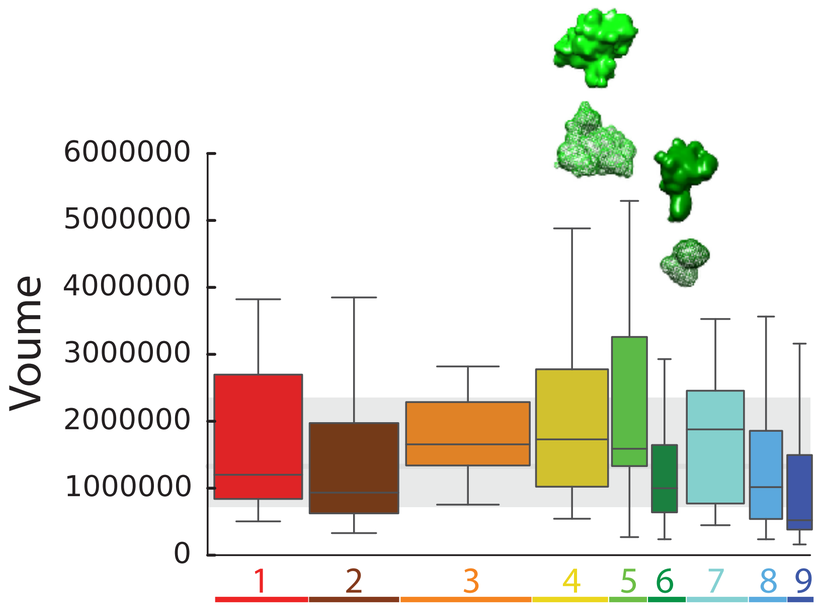
Area (nm^2)
Volume (nm^3)
Sphericity
Overlap (%)
Distance (nm)

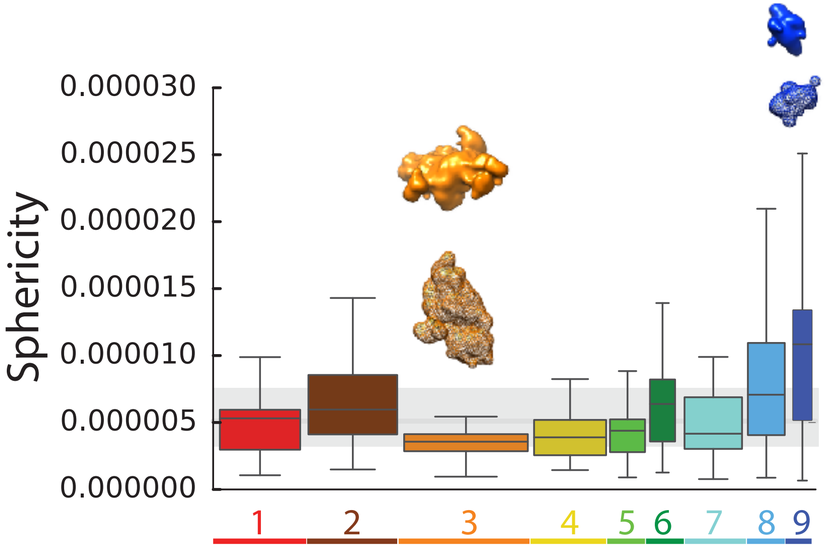
Farabella et al, J Appl Crystallogr. 2015

Structural features

Area, Volume and Sphericity of 19 cells each with 2 homologous resolved



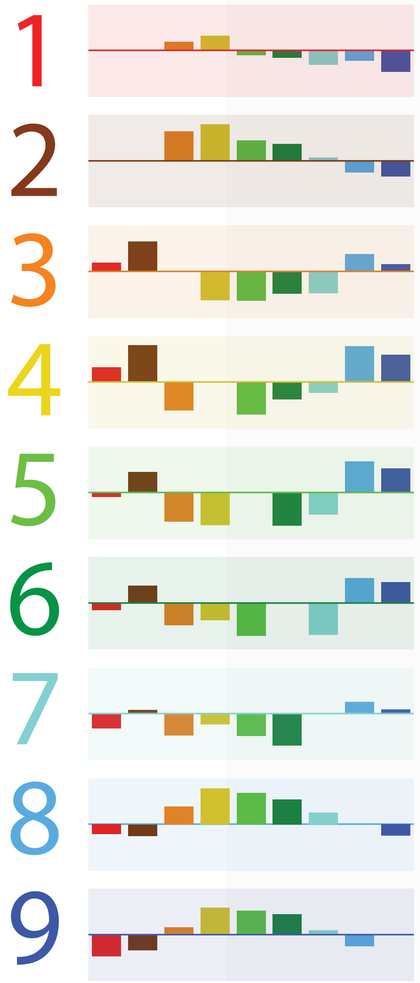


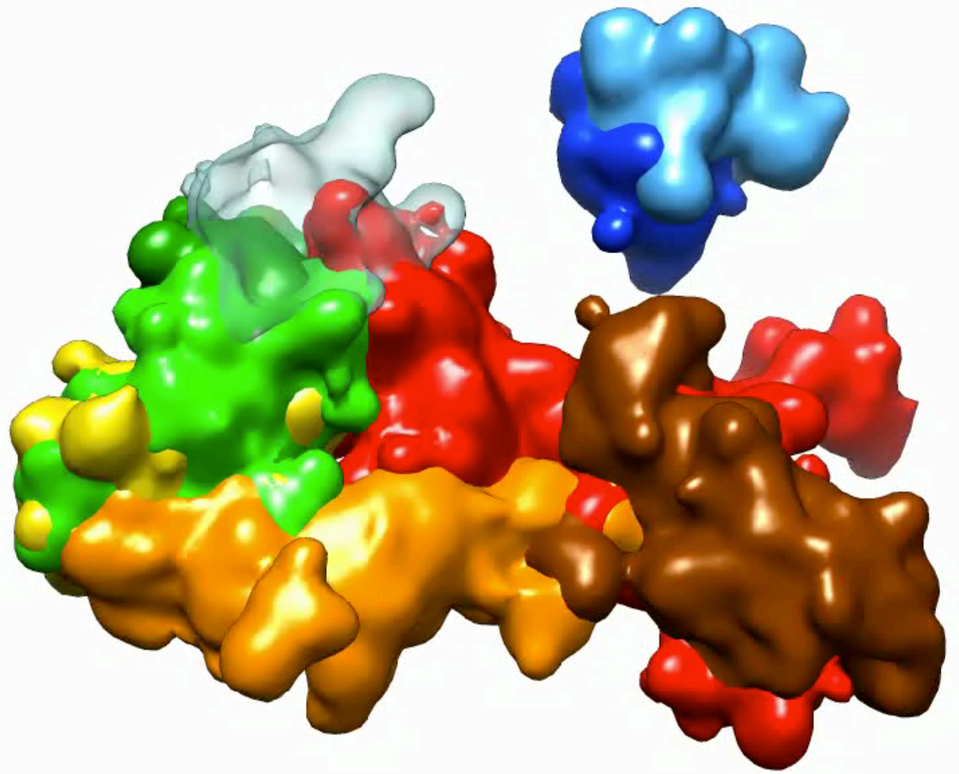


Spatial arrangement

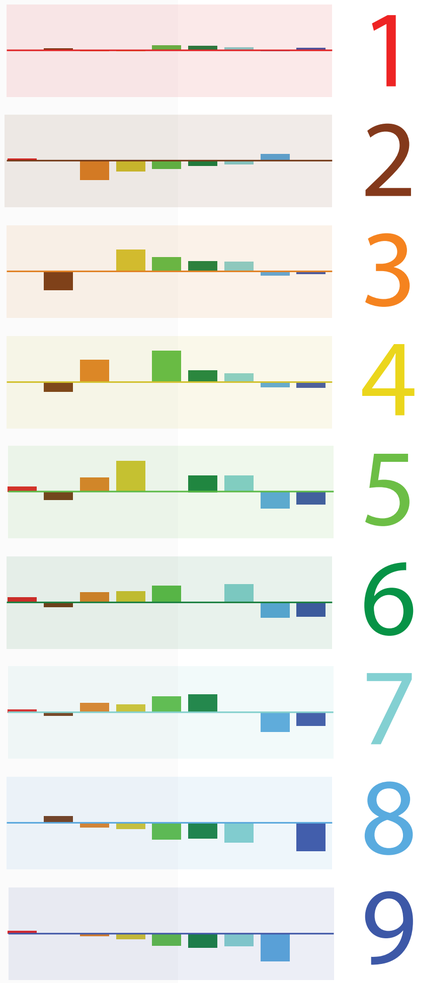
Distance and overlap of 19 cells each with 2 homologous resolved

Diff. distance



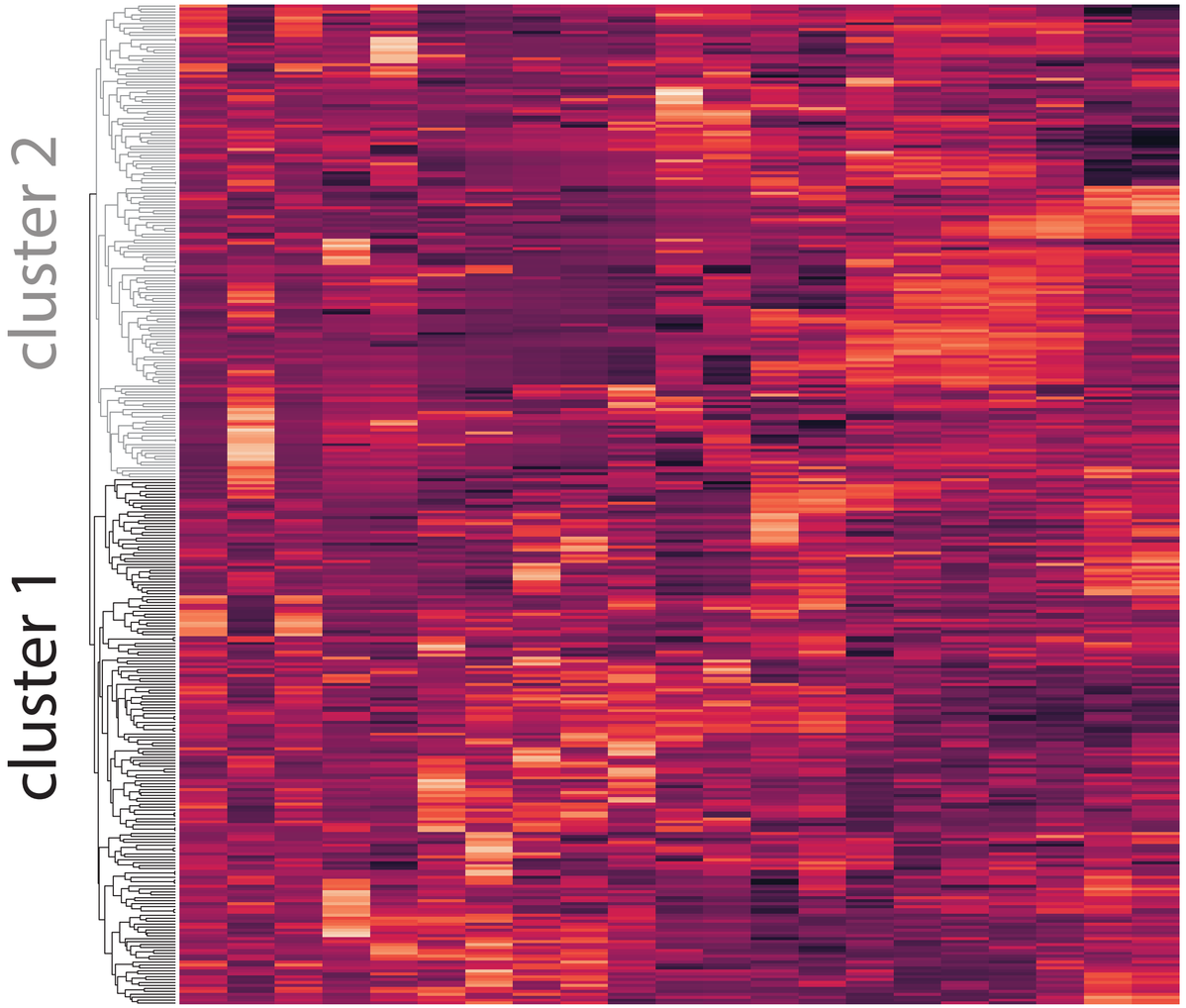


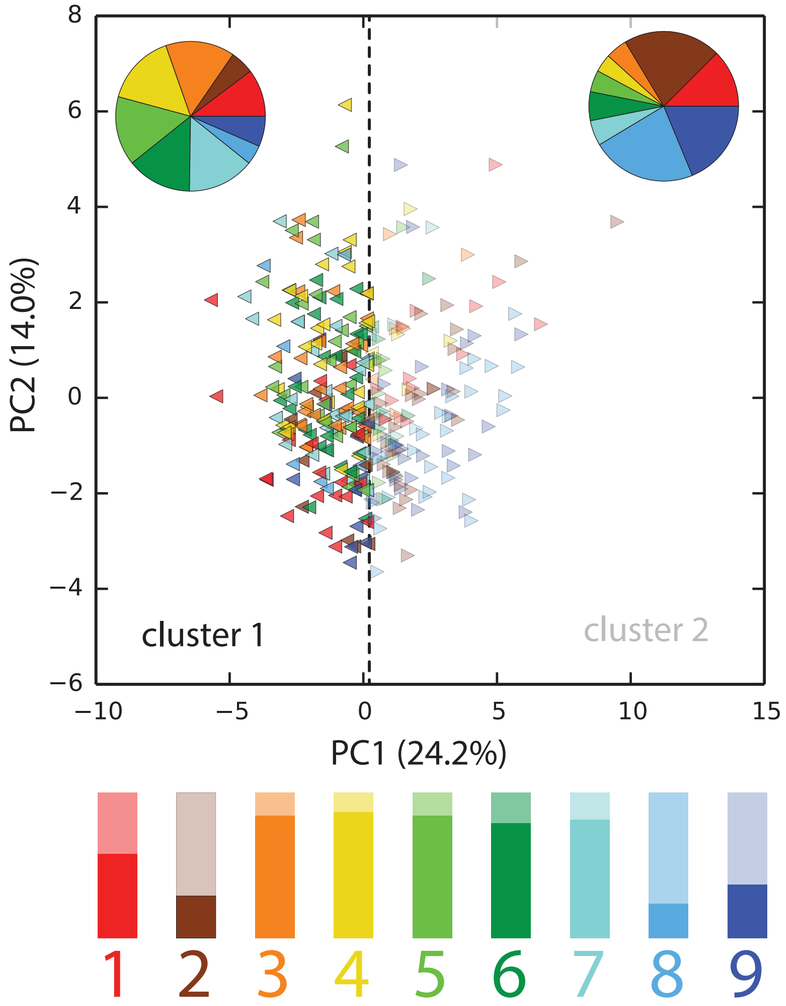
Diff. overlap



Structural clustering

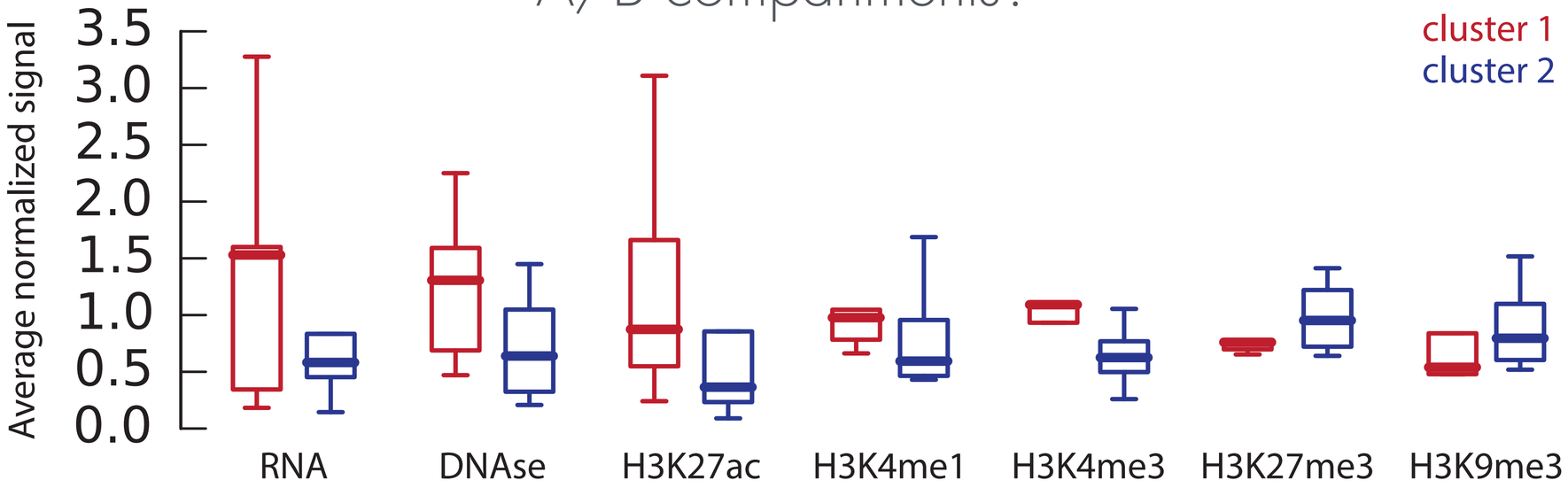
19 cells each with 2 homologous and 9 segments each (342)

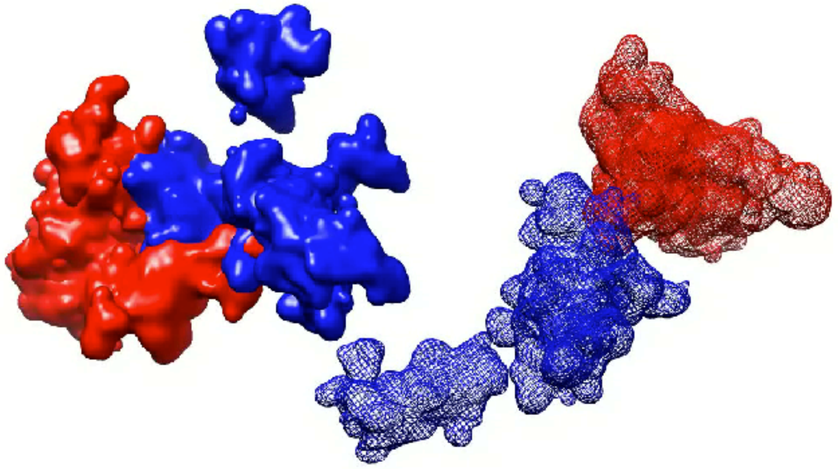


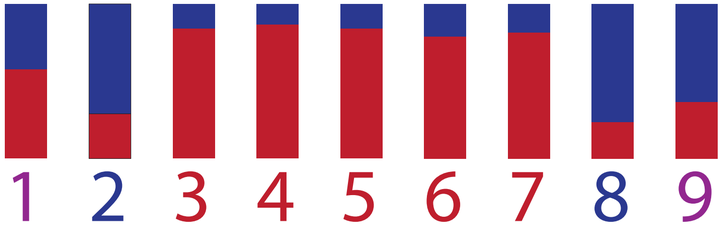


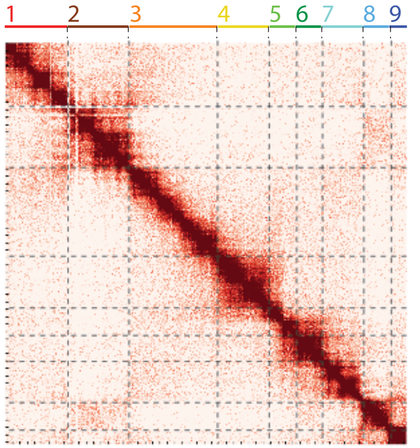
Cluster properties

A/B compartments?





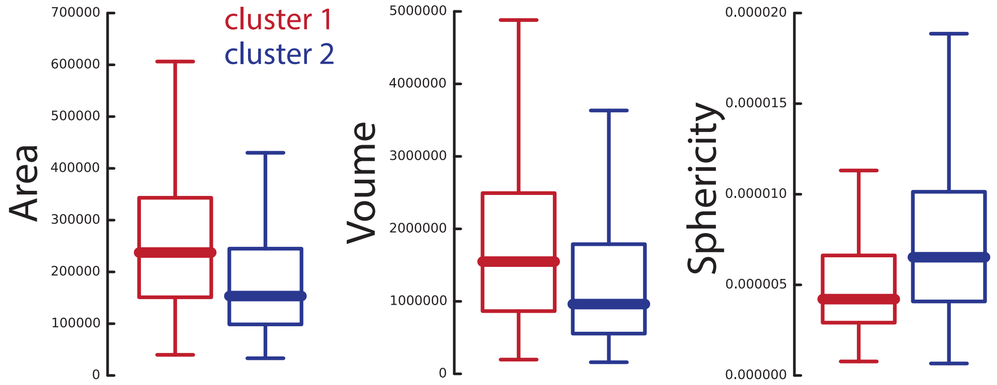


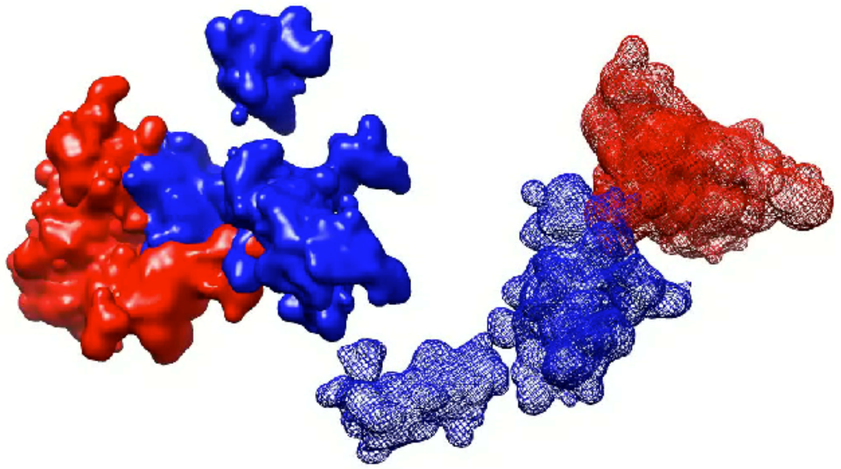


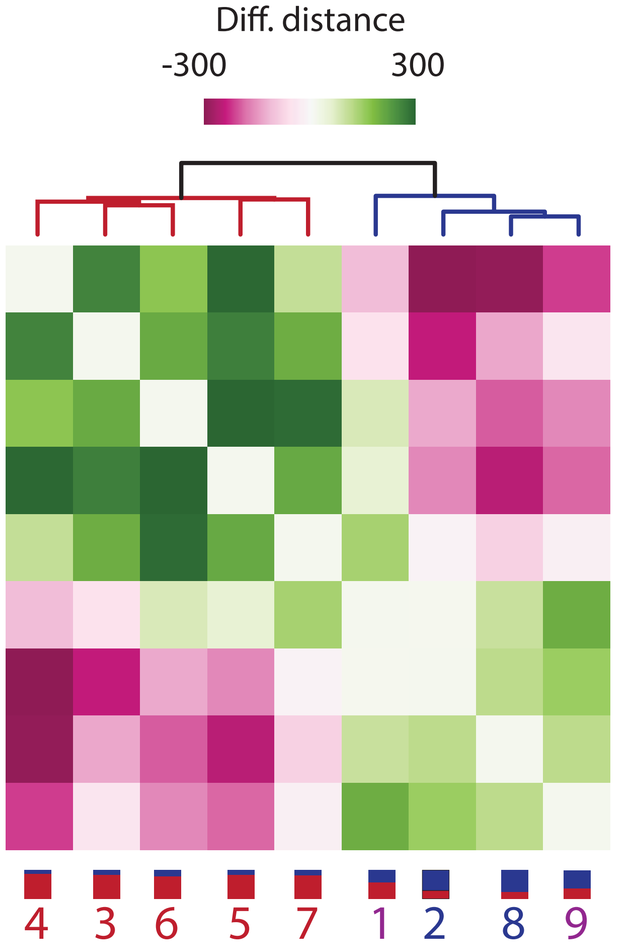
PGP1 ChIP-seq and Hi-C data from ENCODE and Lieberman-Aiden Lab, respectively

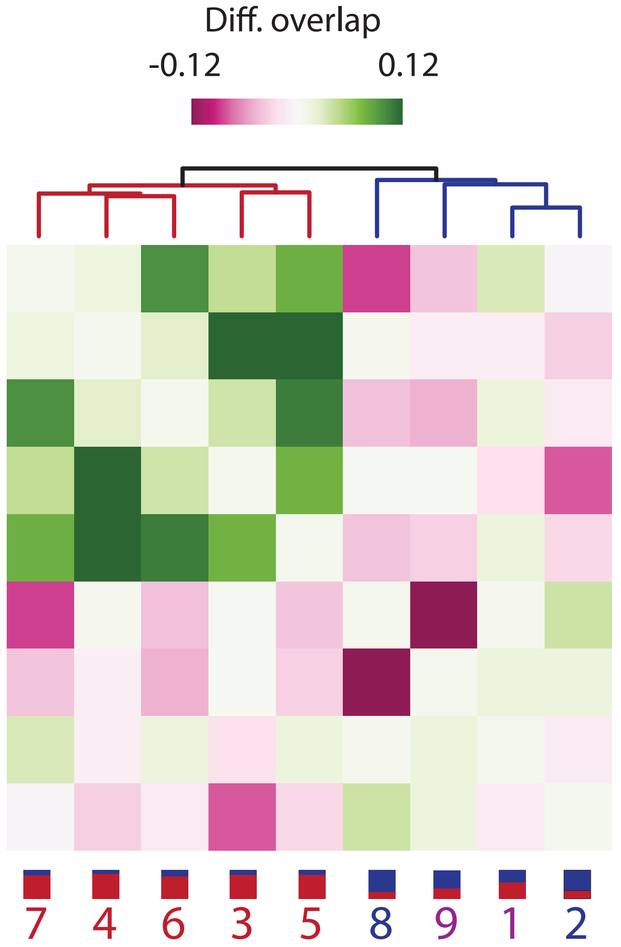
Cluster properties

A/B compartment properties



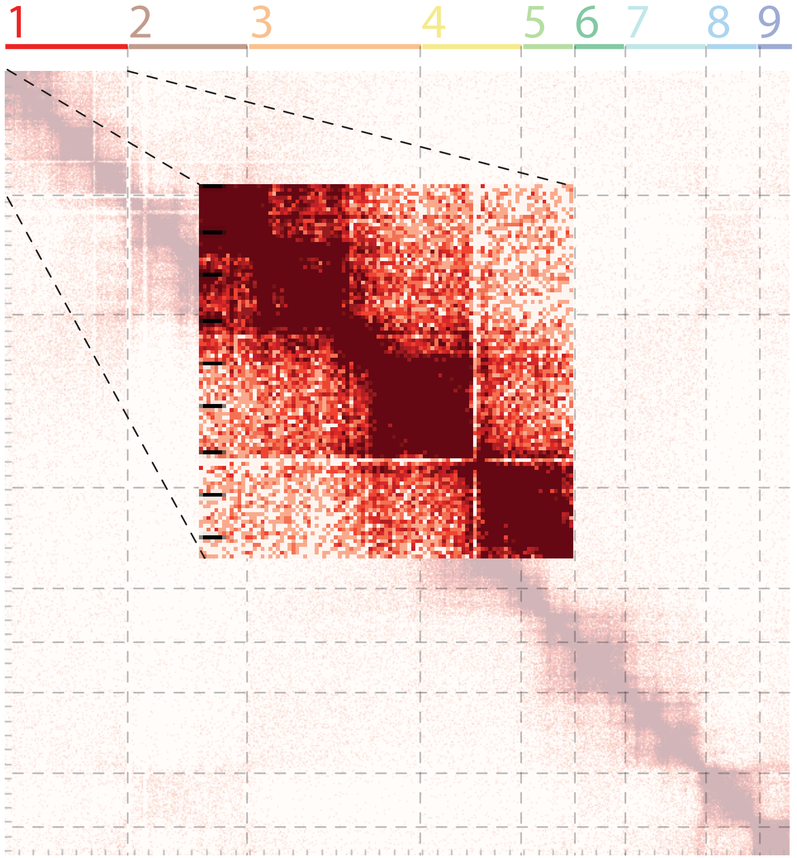




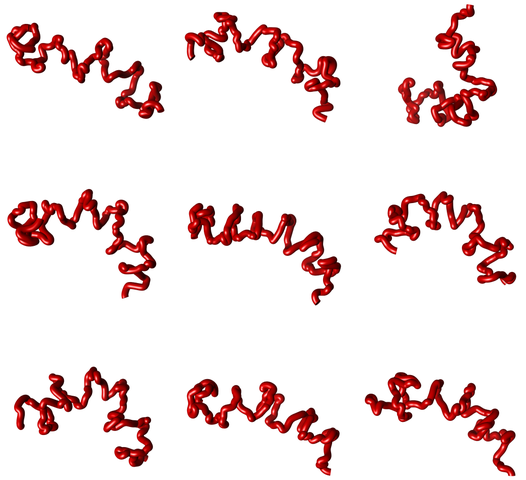


Increasing resolution

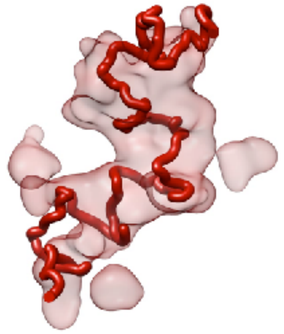
Rigid body fitting 3D structures based on Hi-C data



Segment 3 3D models

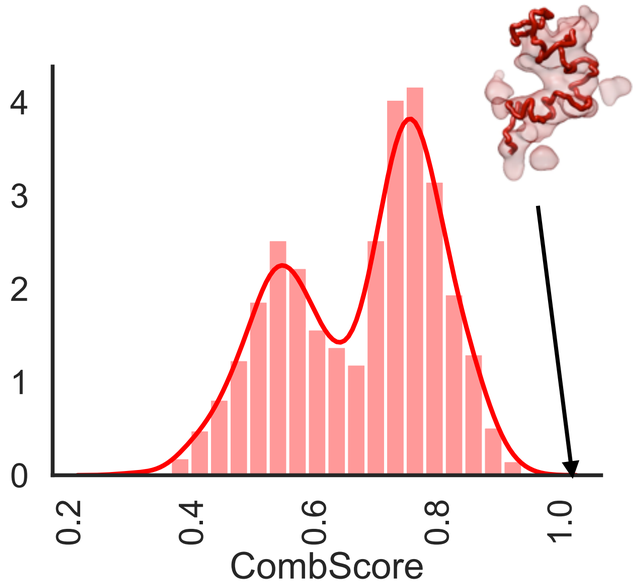


Segment 3 density map



$$\mathrm{ConS} = 1 - \frac{d_{P,COM}}{\max\left(d_{P,COM}\right)}$$

$$\mathrm{CCC} = \frac{\sum_{i=1}^{M}\left[\rho_i^{EM} - \bar{\rho}^{EM}\right]\left[\rho_i^{P} - \bar{\rho}^{P}\right]}{\sqrt{\sum_{i=1}^{M}\left[\rho_i^{EM} - \bar{\rho}^{EM}\right]^2 \sum_{i=1}^{M}\left[\rho_i^{P} - \bar{\rho}^{P}\right]^2}}$$



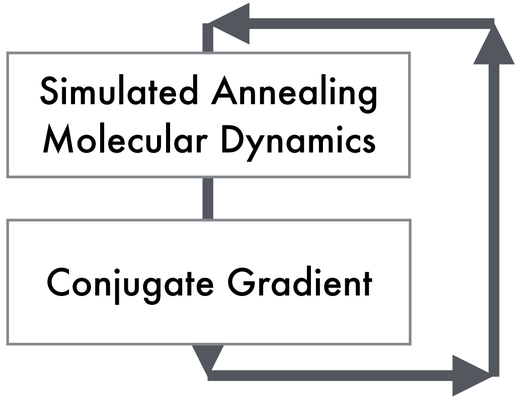
TADbit

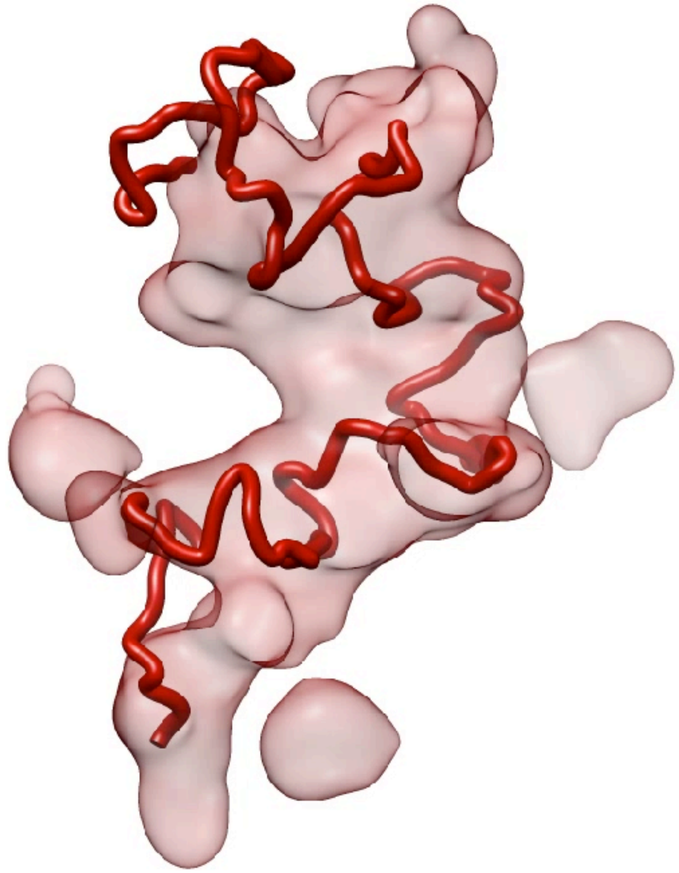
Serra, Baù, et al. PLOS CB 2017
http://www.3DGenomes.org

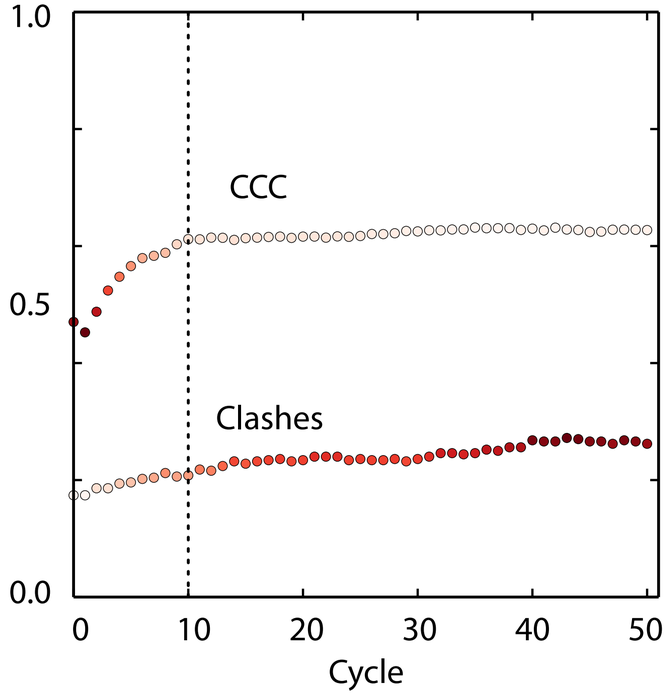
Farabella et al, J Appl Crystallogr. 2015
Roseman, 2000; Wriggers & Chacon, Structure 2001

Increasing resolution

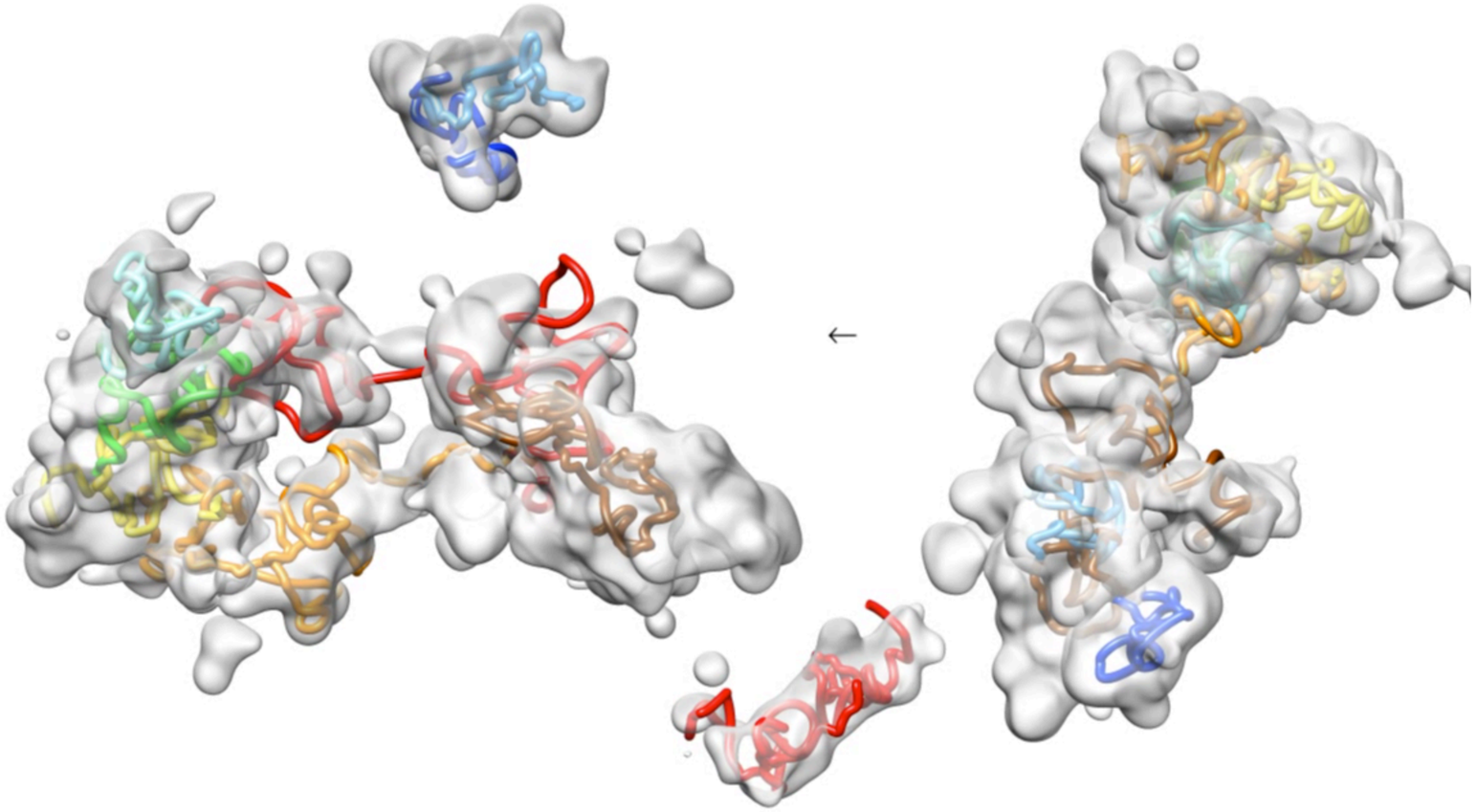
Flexible fitting 3D structures based on Hi-C data





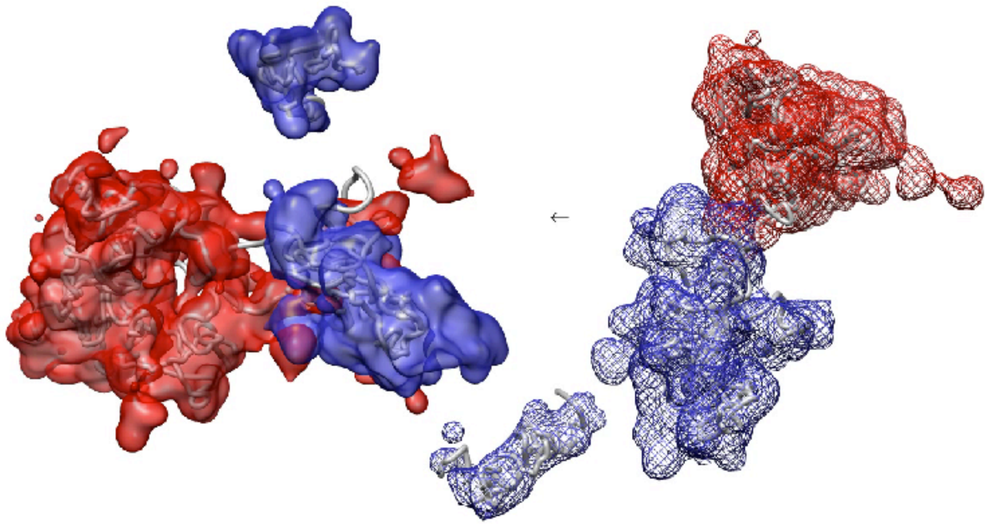


Chromosome walking path @10Kb resolution

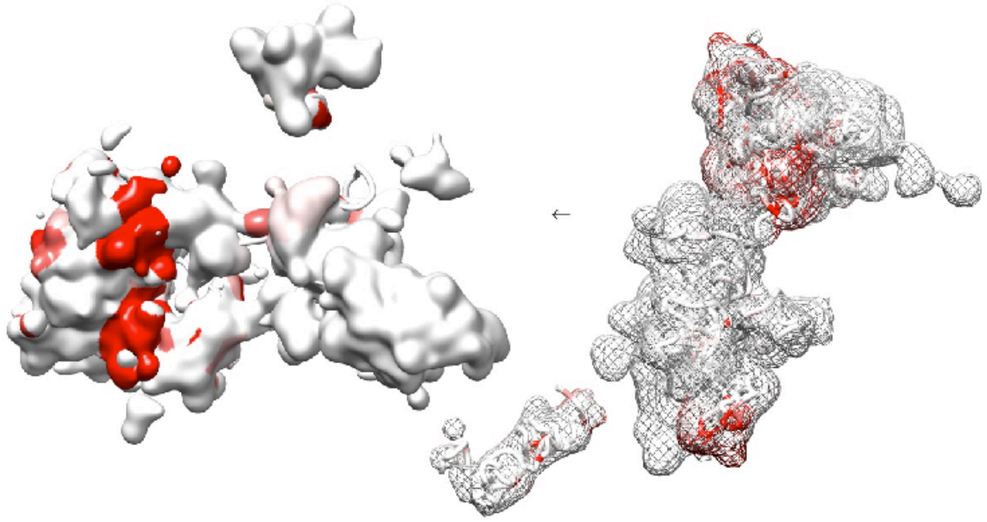


Mapping “omics”

3D organization of local structures



Both in “active” cluster
One in each cluster
Both in “inactive” cluster



μ mean value per 10kb μ+2σ

H3K4me1

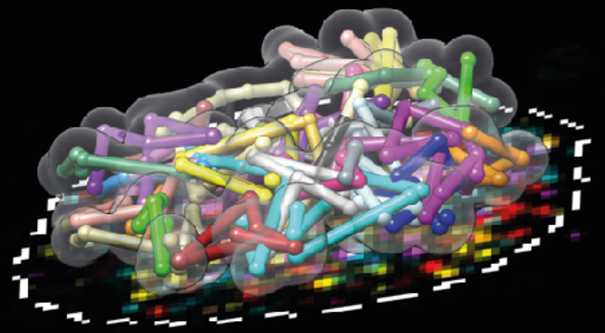
OligoSTORM

- Is a set of technologies for in-situ chromosome walking at super-resolution
- Is highly designable: can target any region of the genome (except repeats?)

- Combined with micro-fluidics can do up to tens of rounds (steps)
- Combined with modeling + Hi-C produces traces of chromosomes at 10Kb resolution

- Can be pipelined with other approaches such OligoFISSEQ for increased speed (next)

Chromosome tracing with OligoFISSEQ



Marc A. Marti-Renom

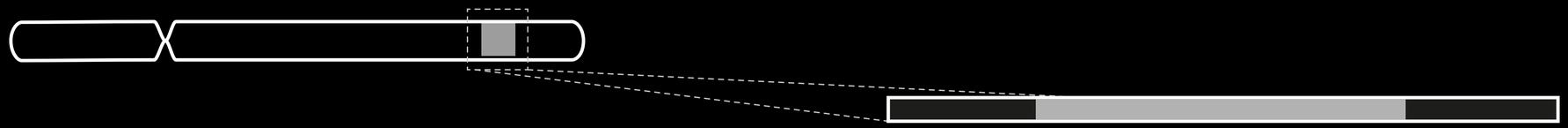
CNAG-CRG · ICREA

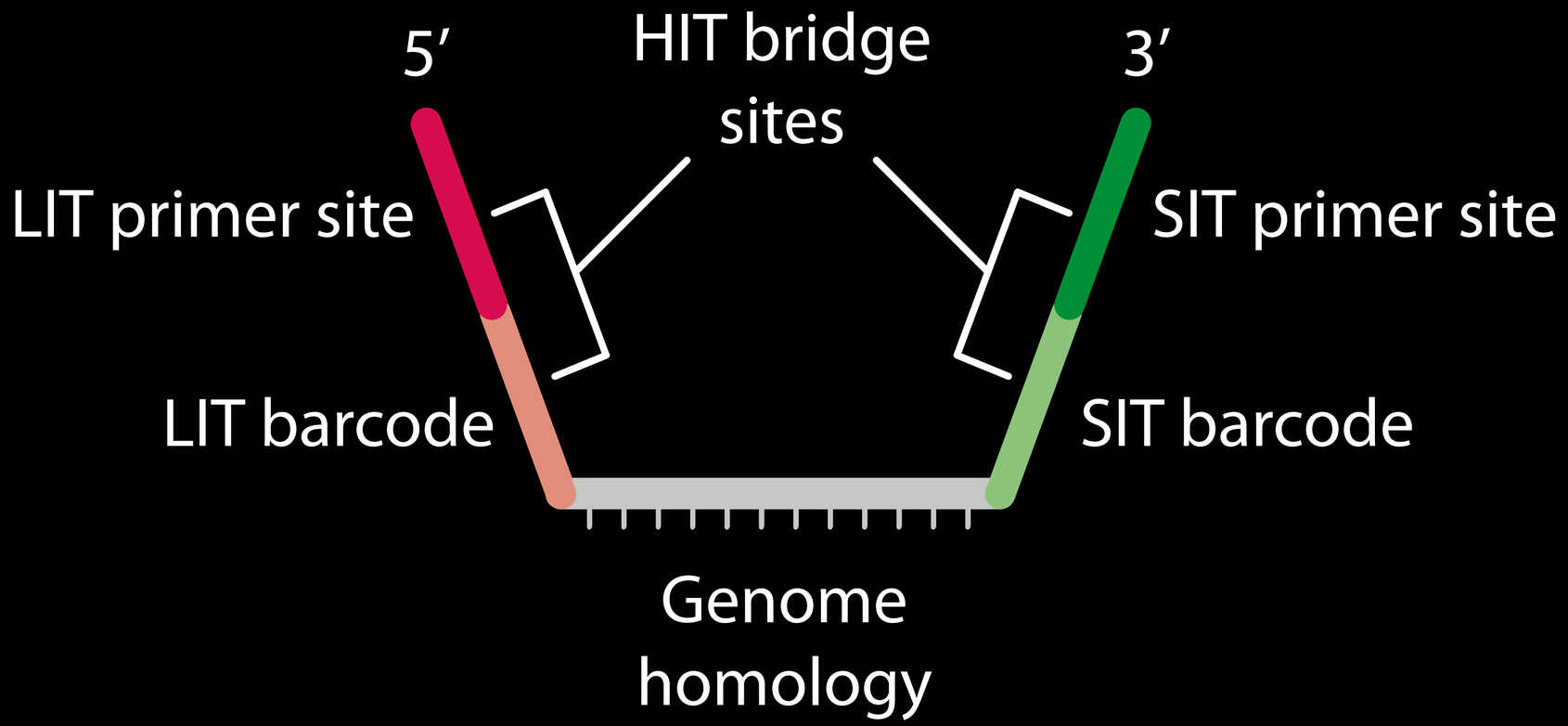
Huy Nguyen
Shyamtanu Chattoraj
David Castillo

in collaboration with the Wu Lab (HMS)

Nature Methods (2020) 17 p822

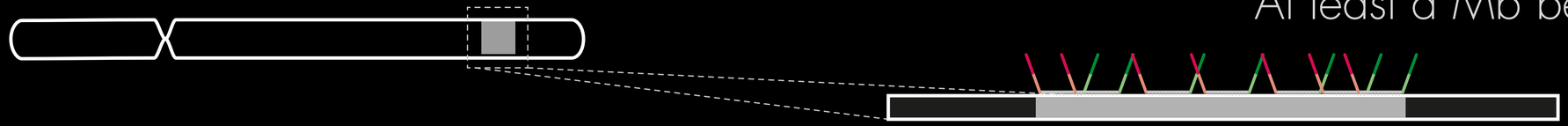
OligoFISSEQ



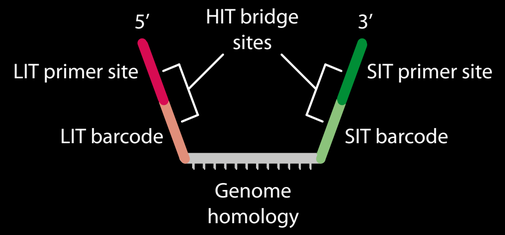


OligoFISSEQ

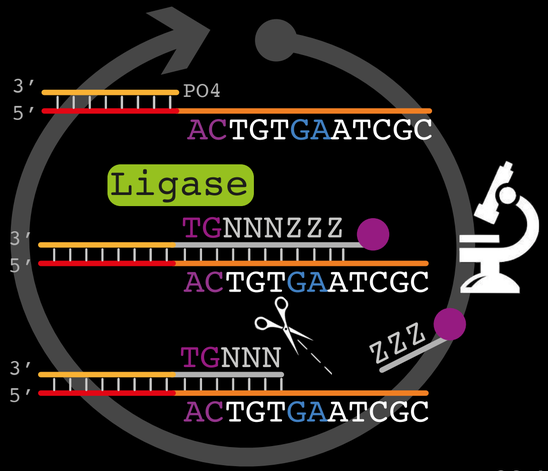
From tens of kb to Mb
Min. of few 100s oligos/target
At least a Mb between targets

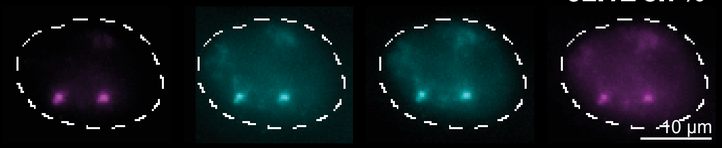


OligoFISSEQ

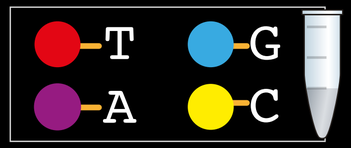


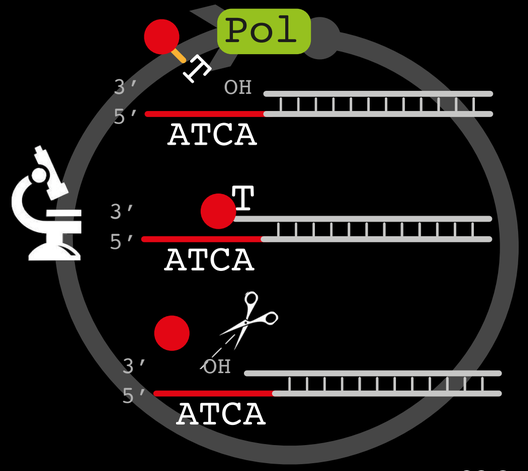
Ligation based Identification of Targets

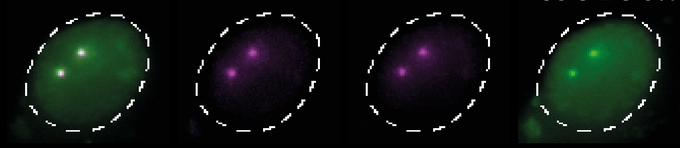




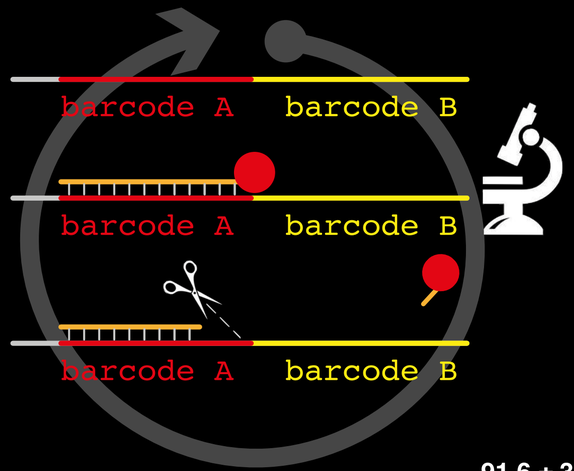
Synthesis based Identification of Targets

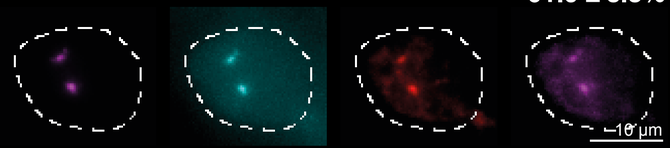






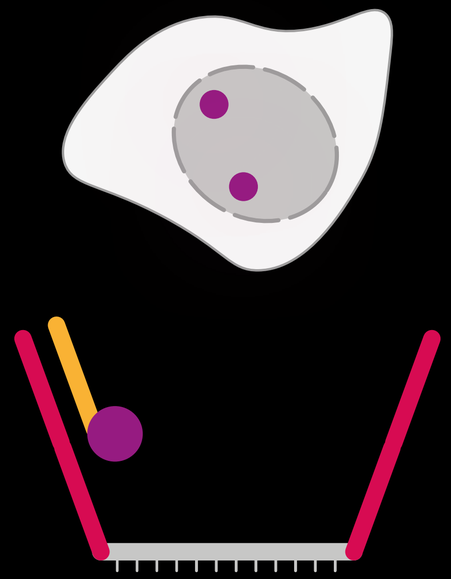
Hybridization based Identification of Targets





OligoFISSEQ scales exponentially!

Sequential hybridization

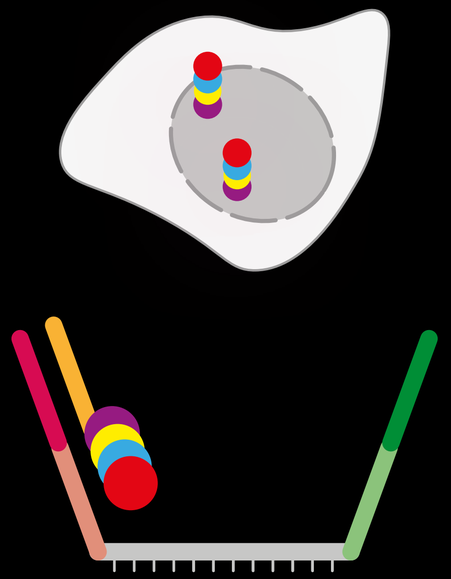


# of targets = F*N

F = # of fluorophores

N = # of seq. rounds

Barcode sequencing



# of targets = F^N

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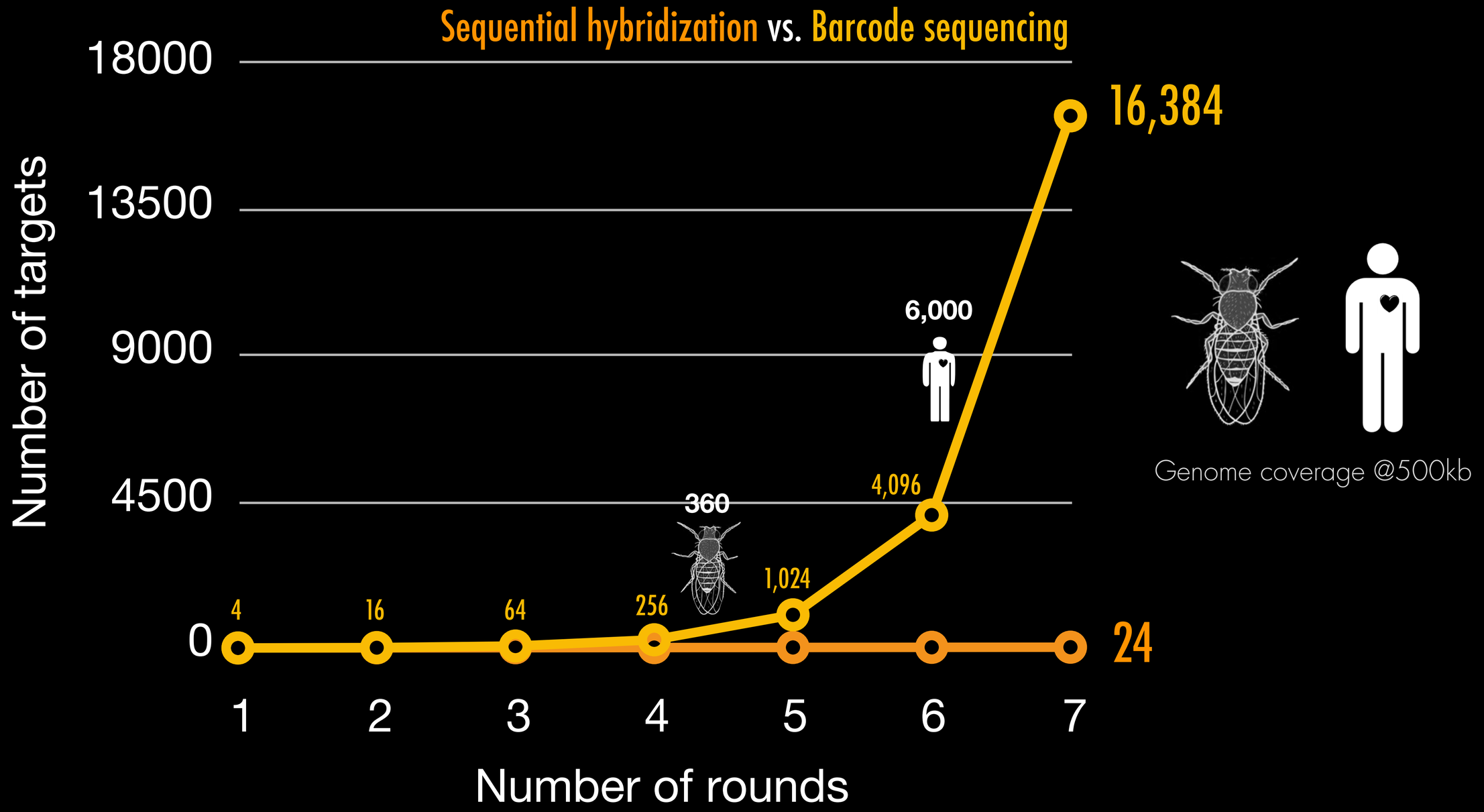
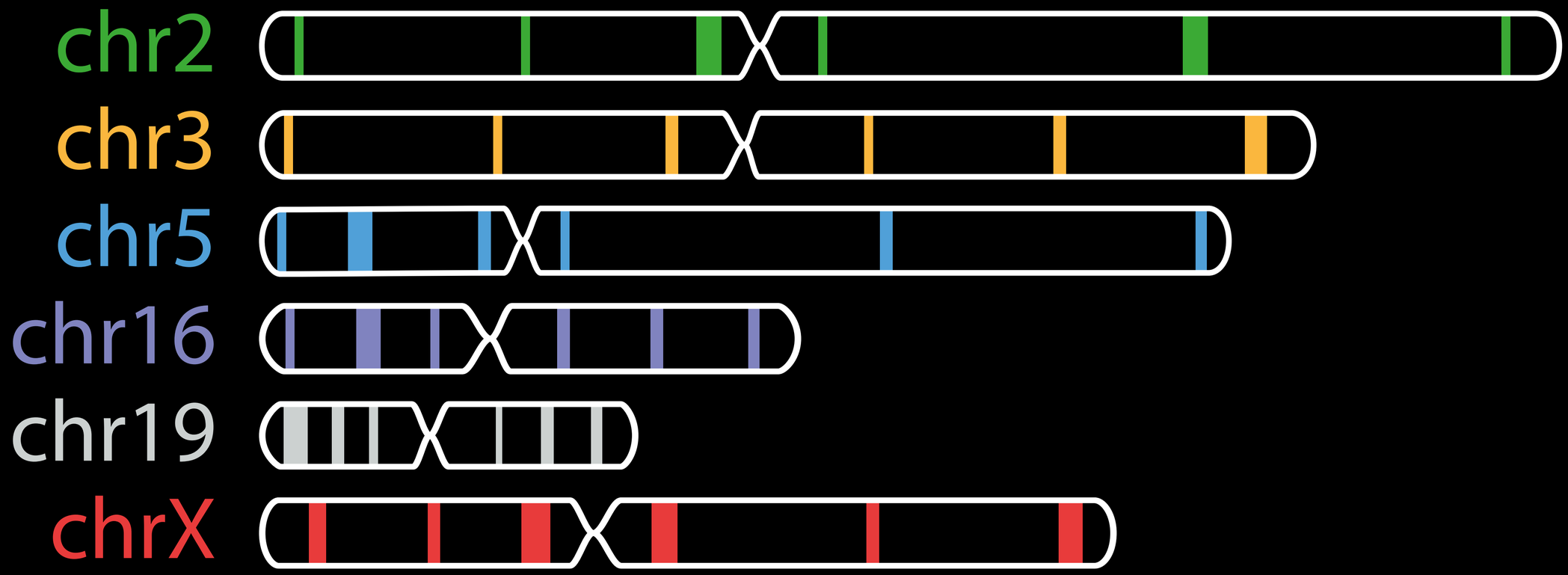
OligoFISSEQ scales exponentially!

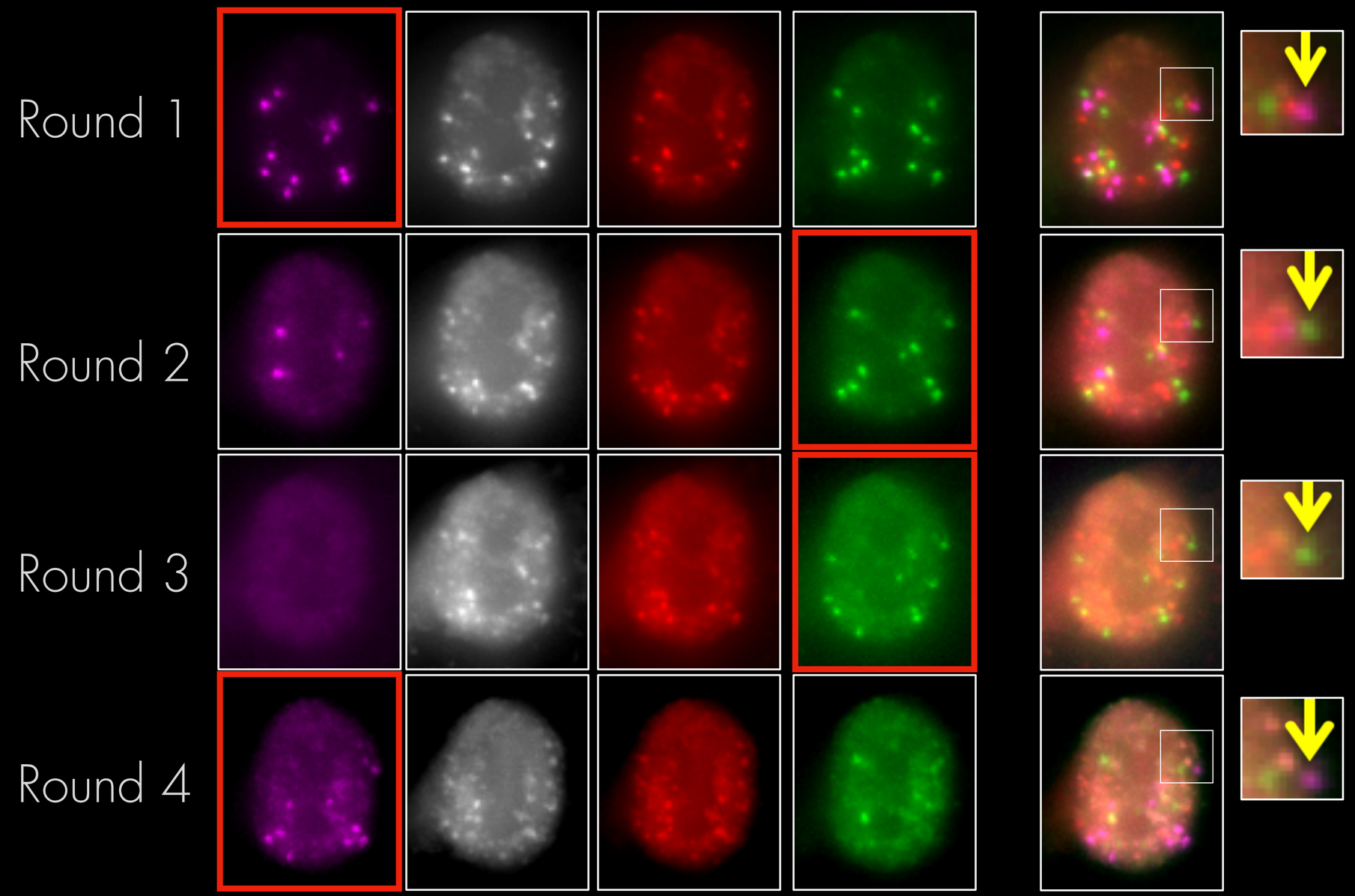


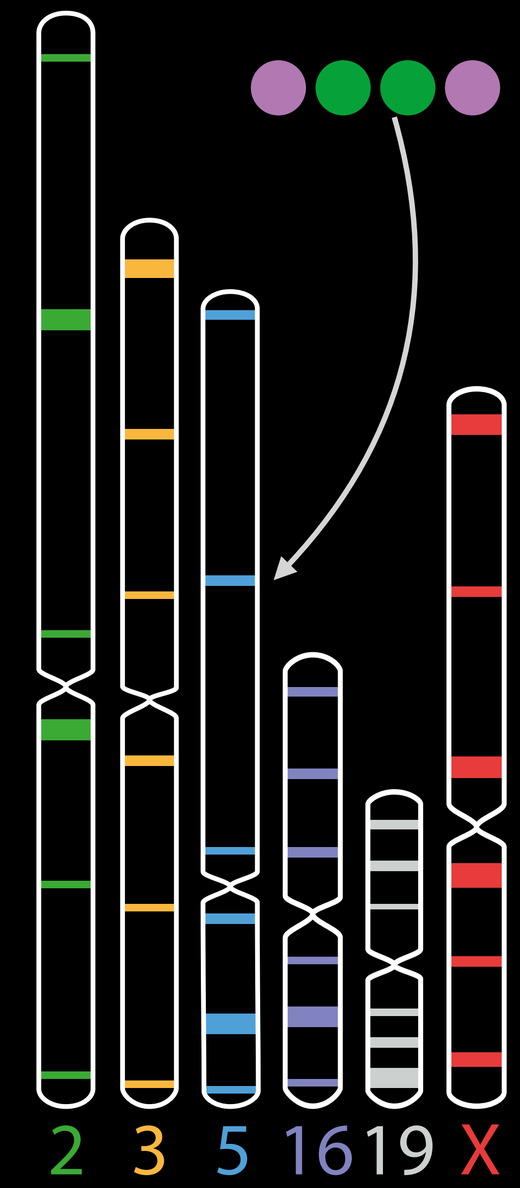
Proof-of-principle

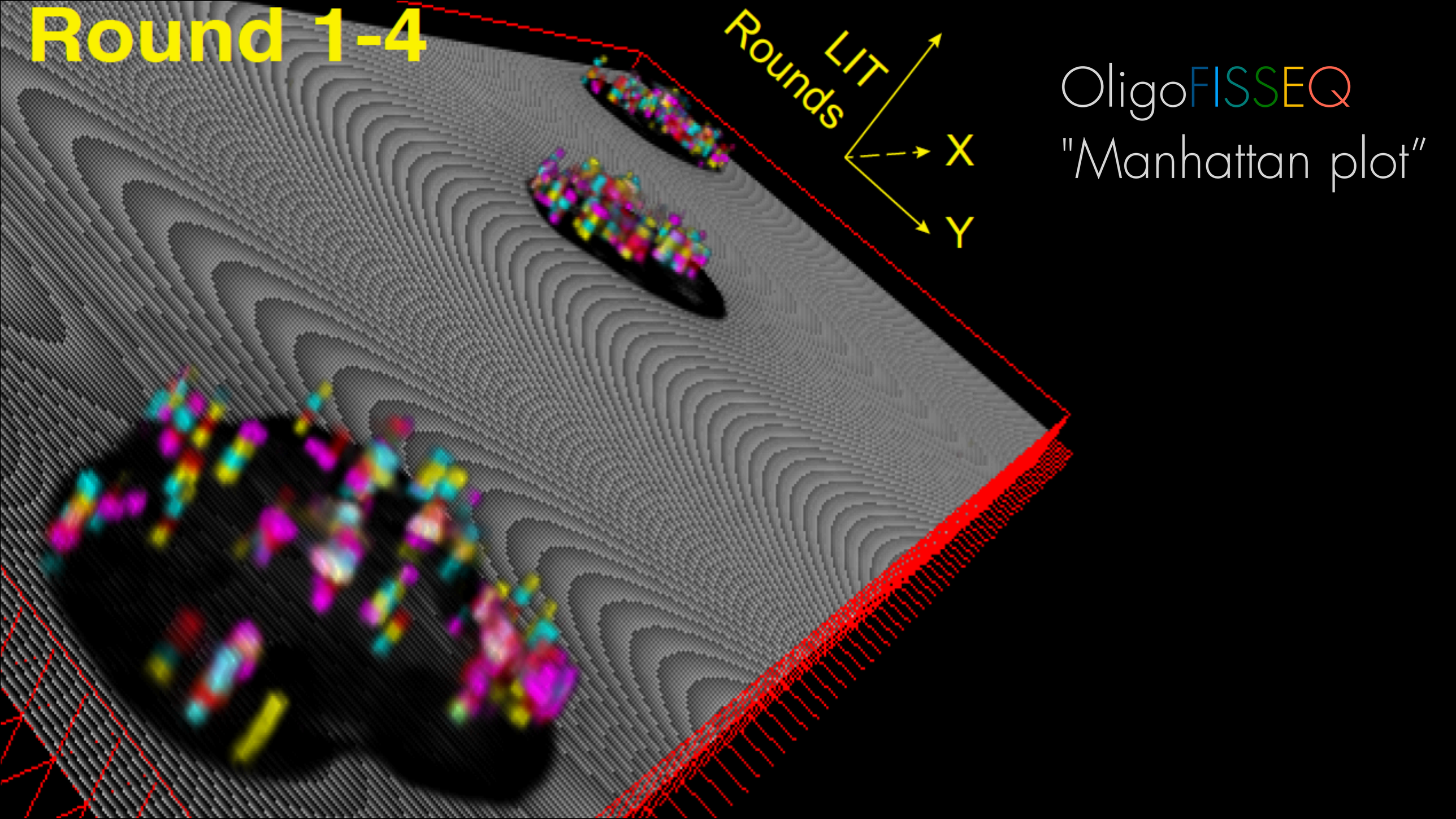


600kb-1Mb/target (876 kb average)
5,000 oligos/target
7-70Mb between targets

Detecting a given target



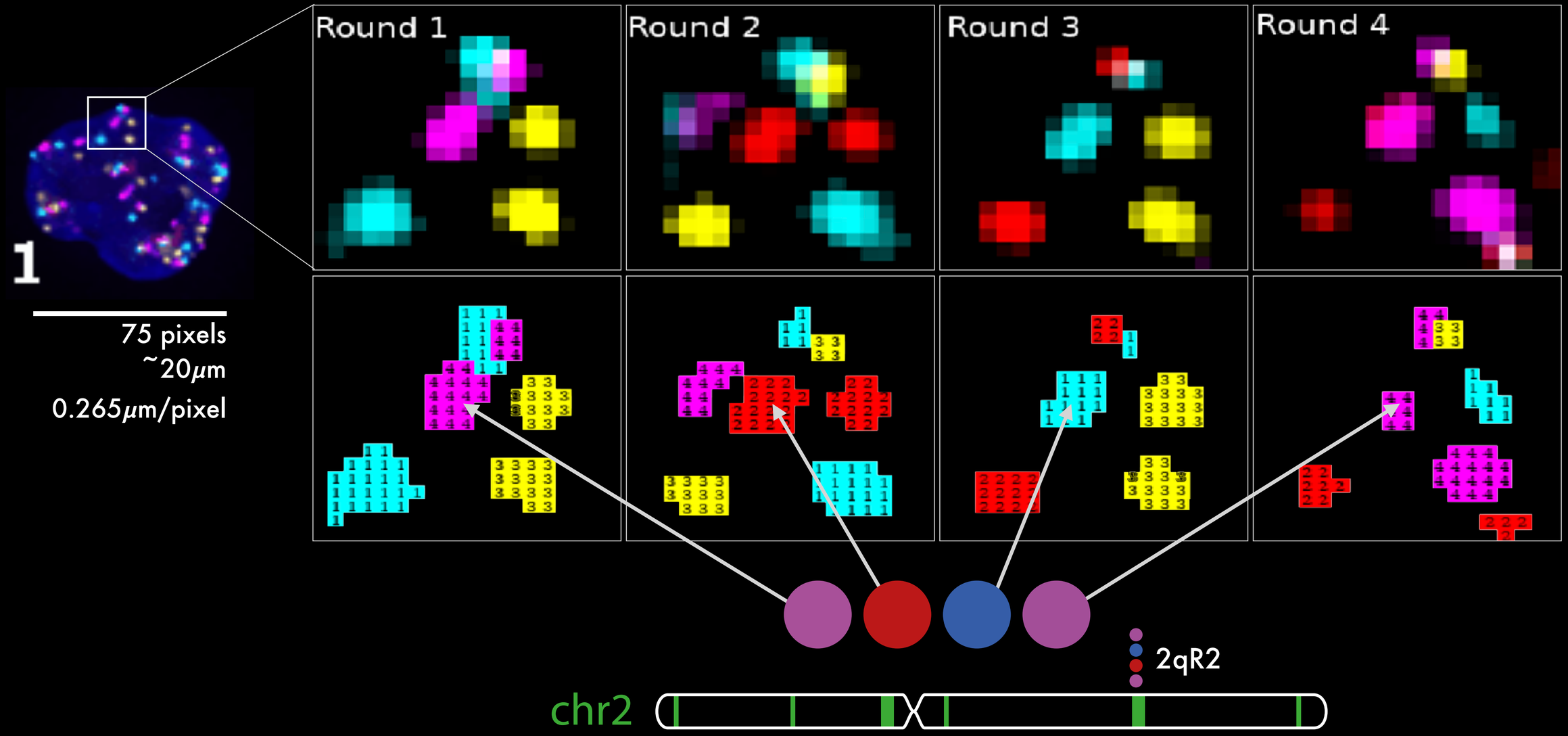




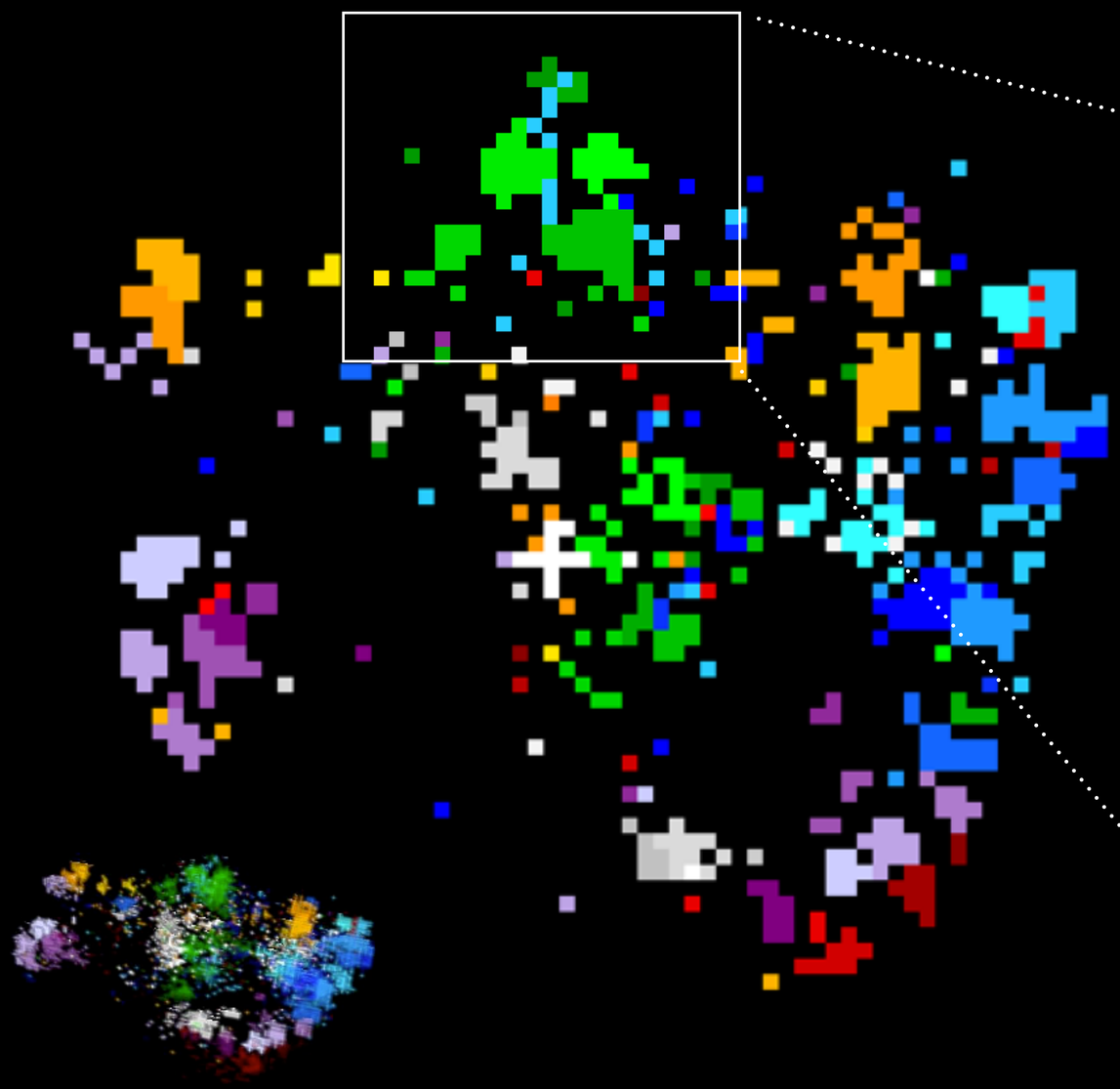
OligoFISSEQ
"Manhattan plot"

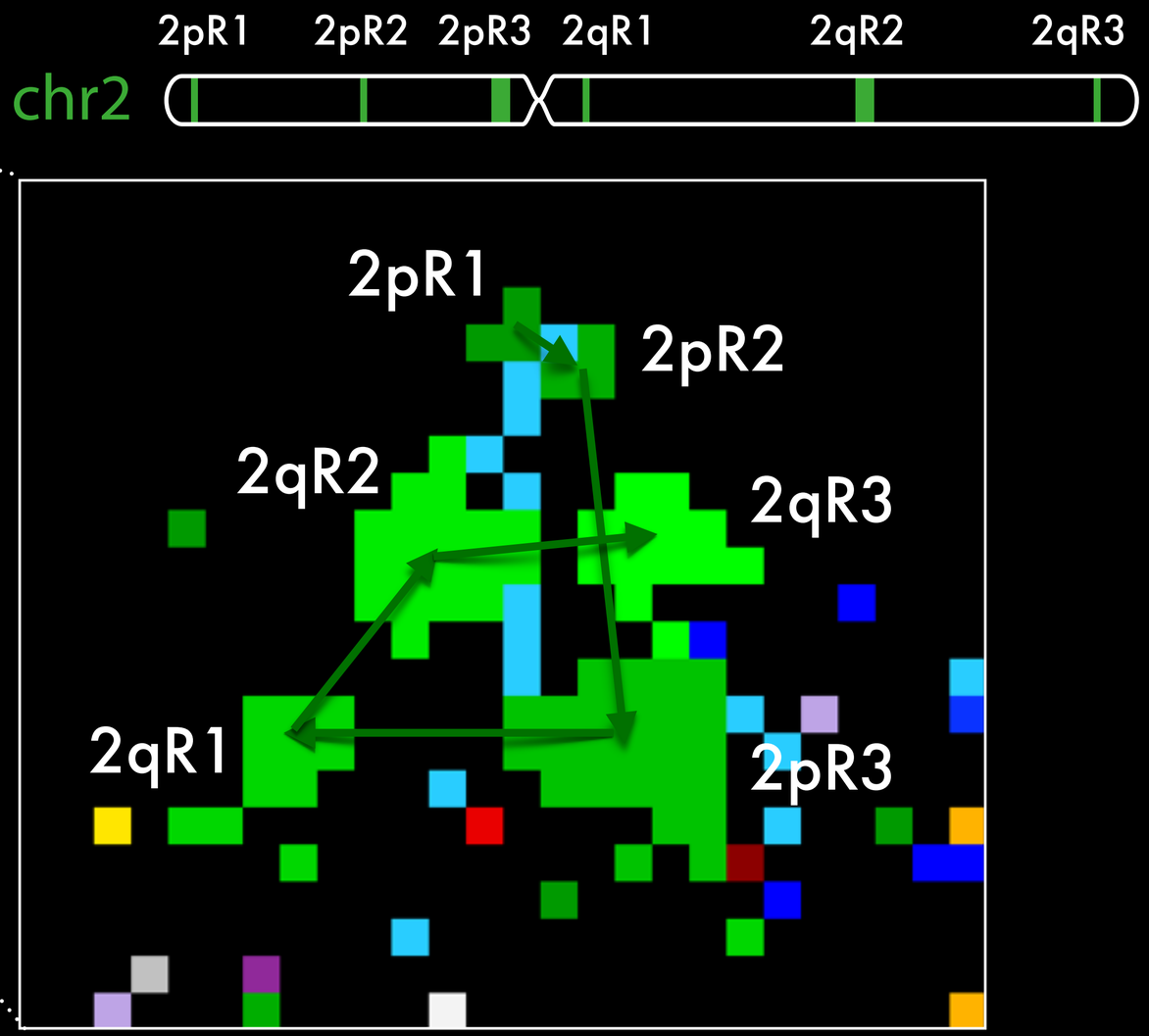
In OligoFISSEQ every pixel matters & make "patches"

4 rounds / 4 channels

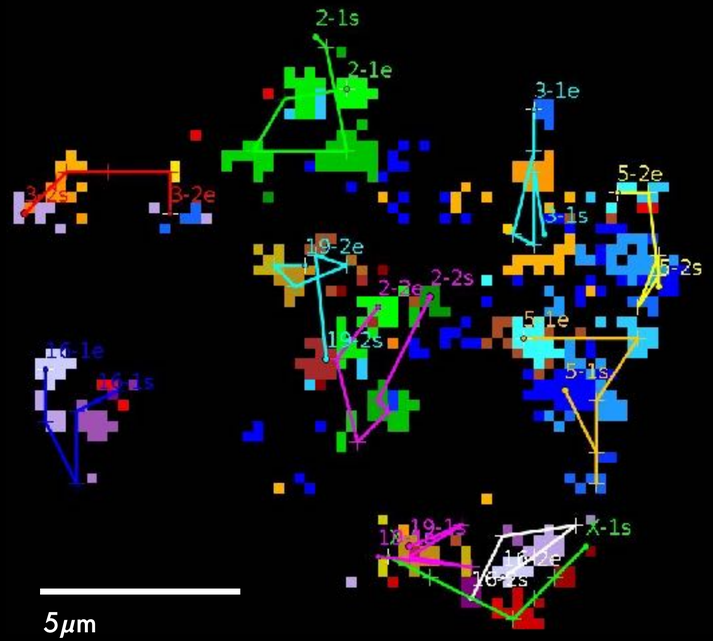


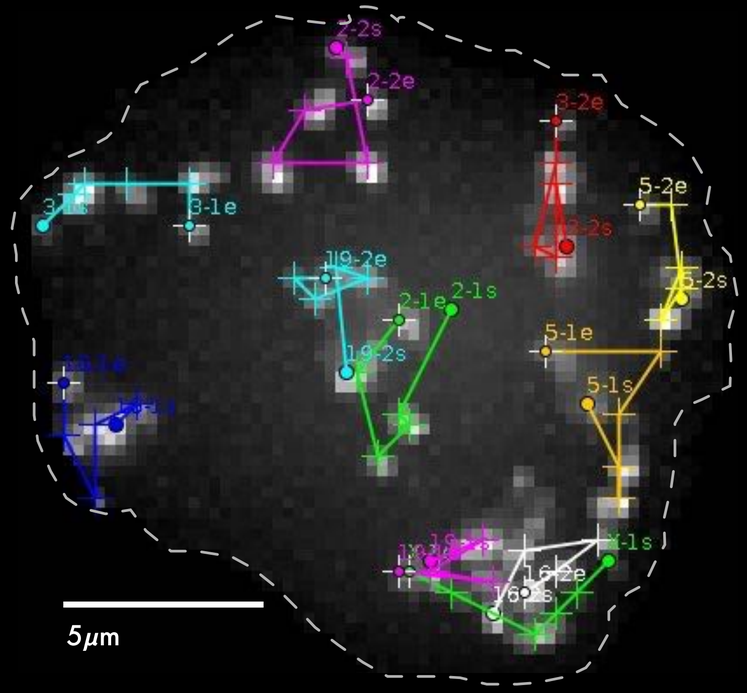
In OligoFISSEQ every pixel matters & make "patches"

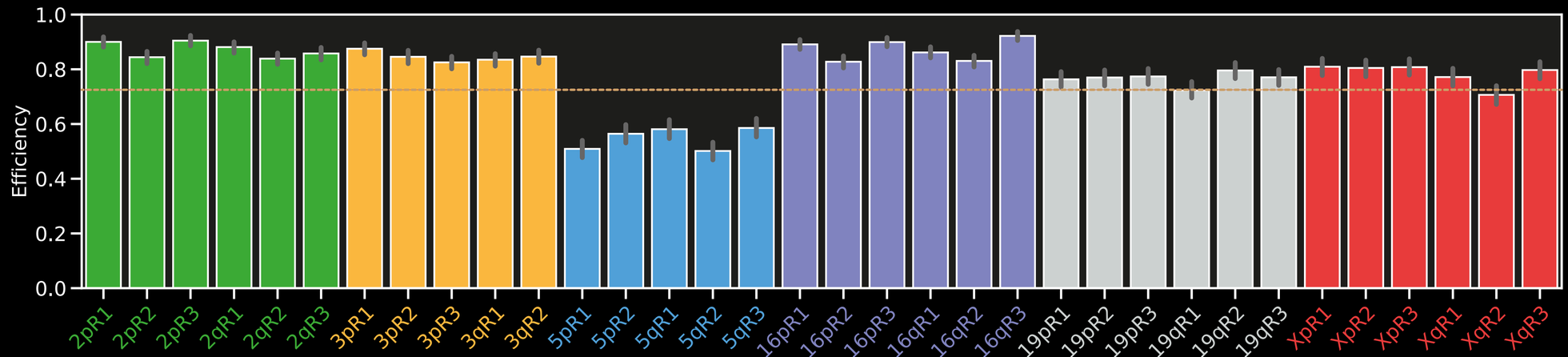




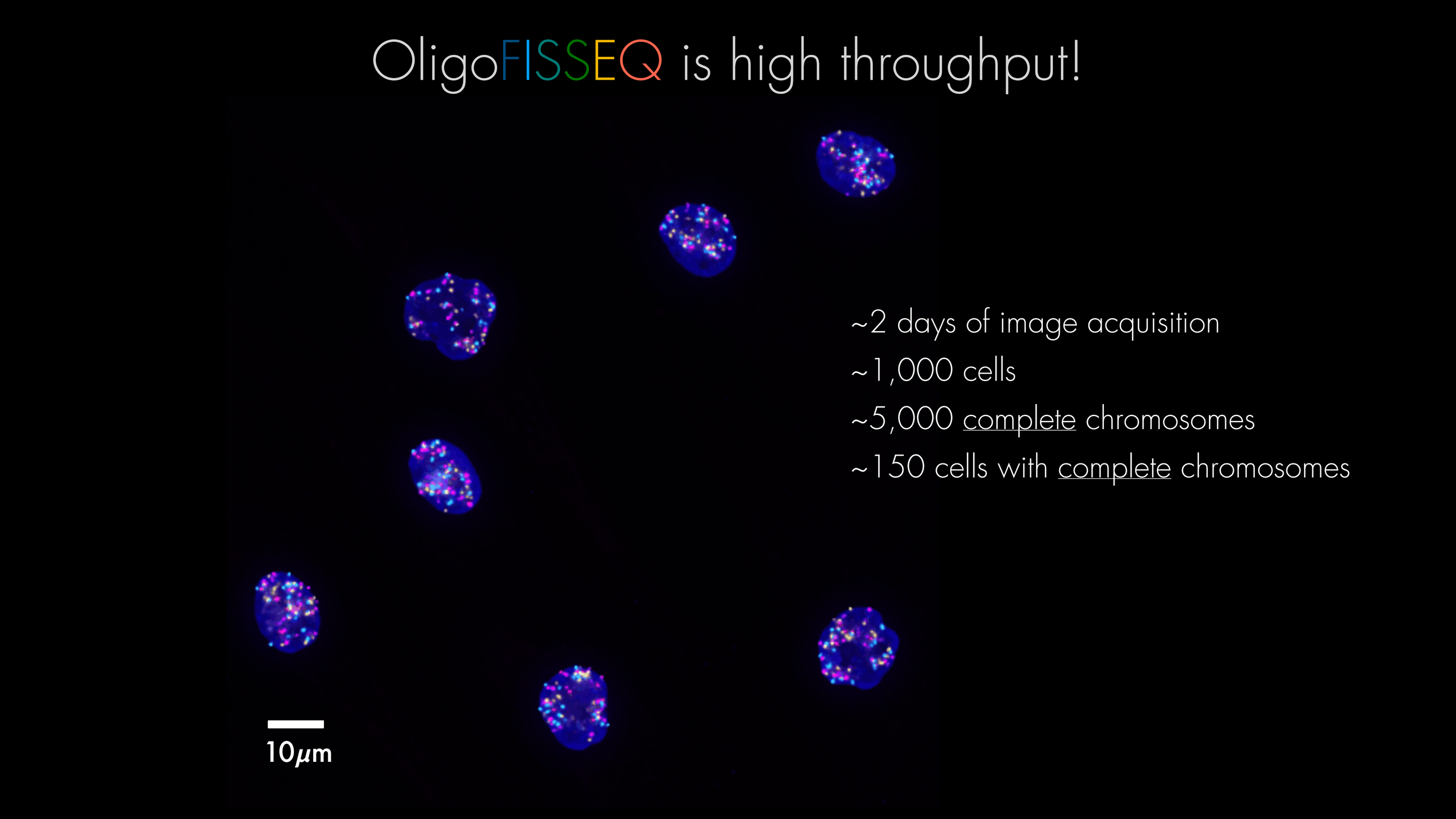
OligoFISSEQ barcode efficiency







OligoFISSEQ is high throughput!



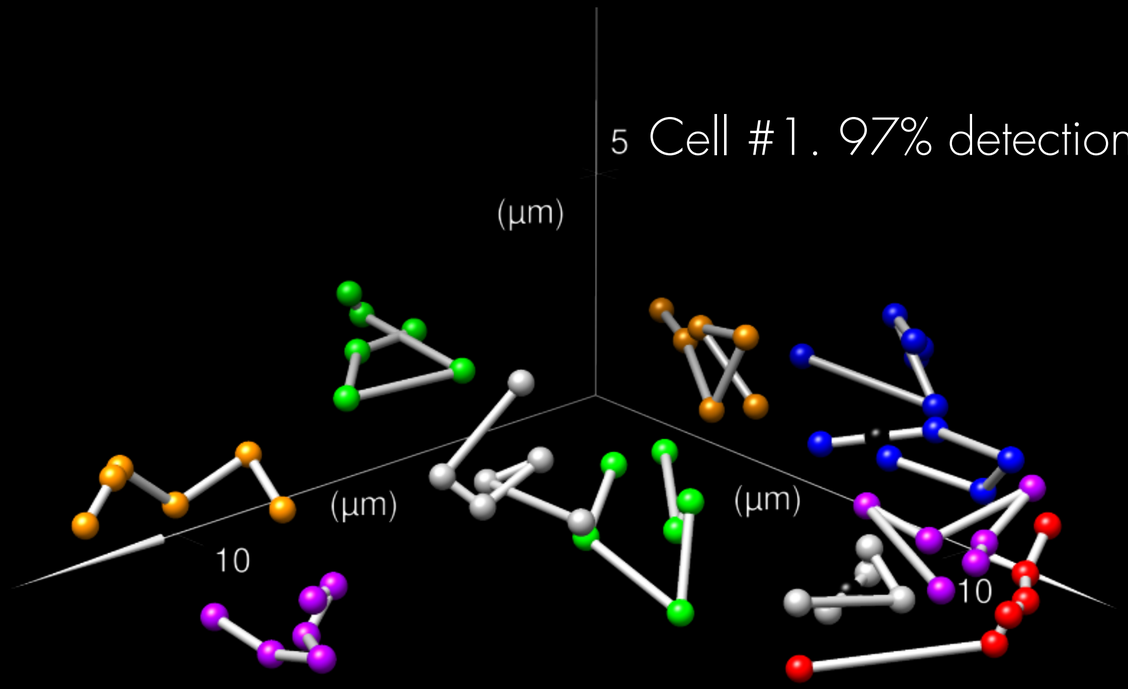
~2 days of image acquisition

~1,000 cells

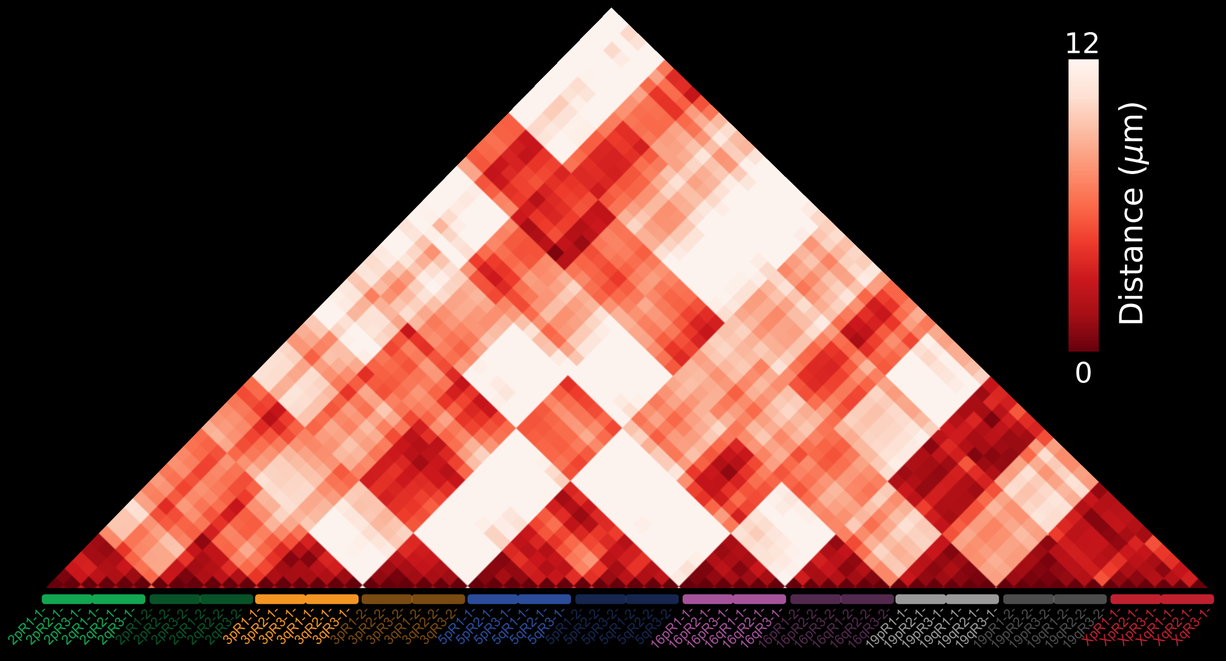
~5,000 complete chromosomes

~150 cells with complete chromosomes

Single cell homolog resolved tracing of chromosomes

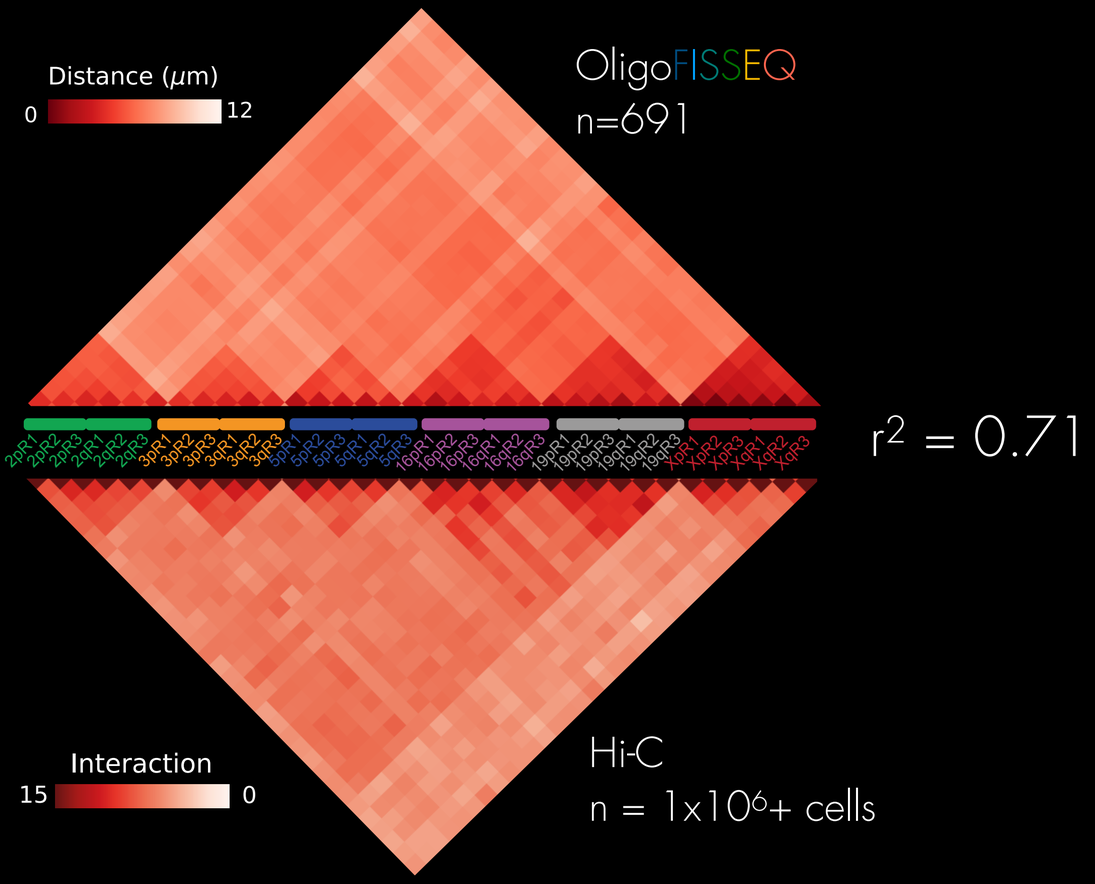


Cell #1. 97% detection

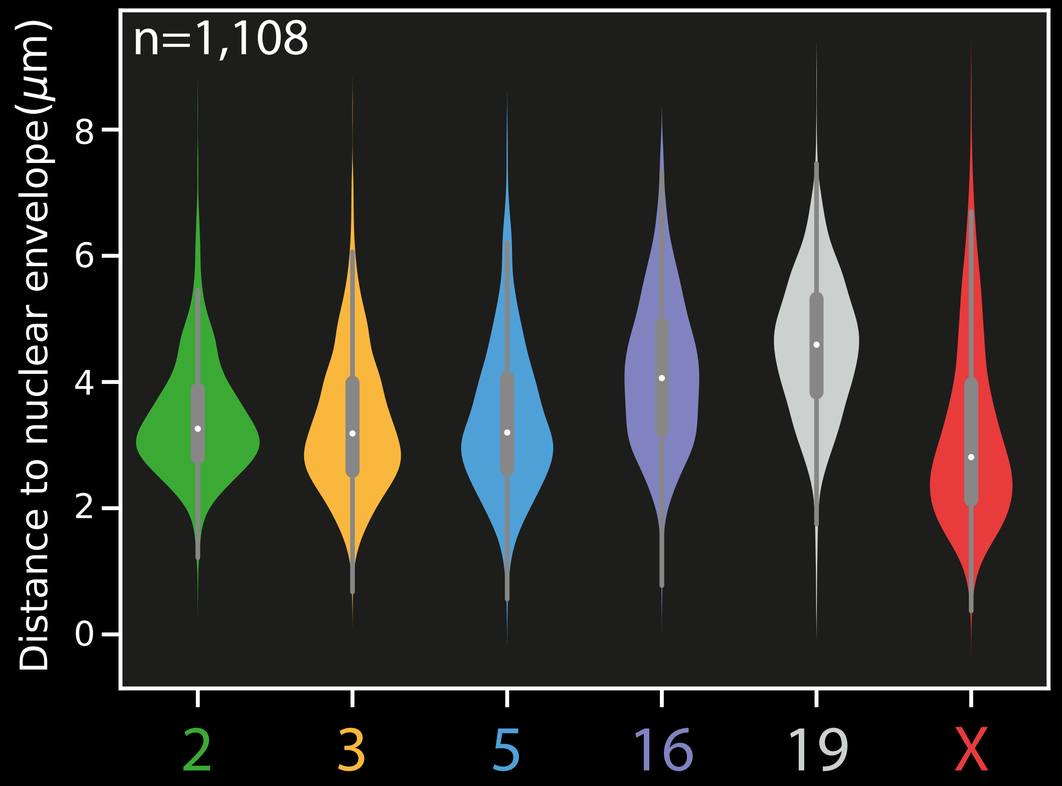


Do OligoFISSEQ tracing maps show known features?

Hi-C contact maps & Radial position of chromosomes



$r^2 = 0.71$

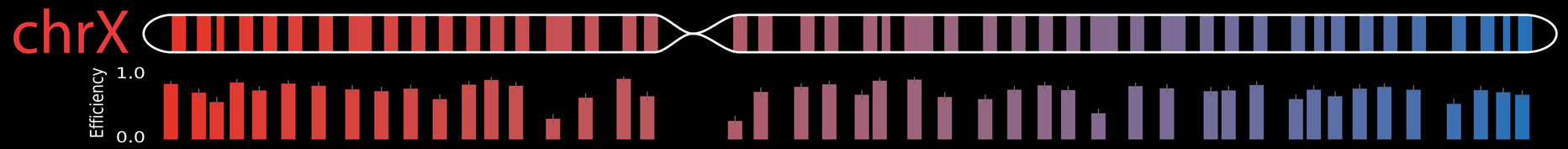


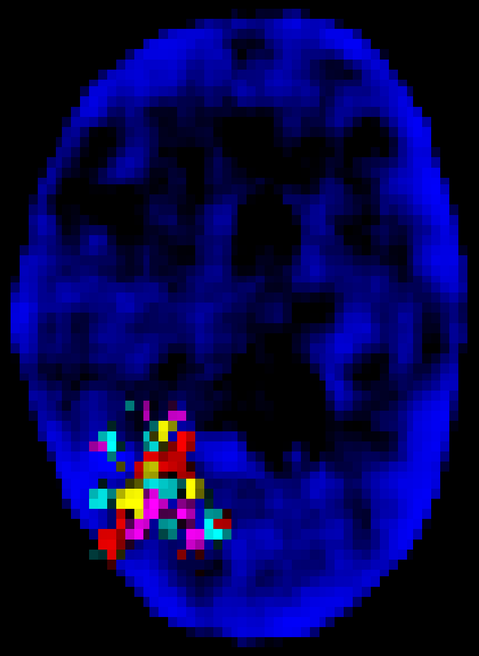
Are the chromosomes randomly located inside the nucleus?

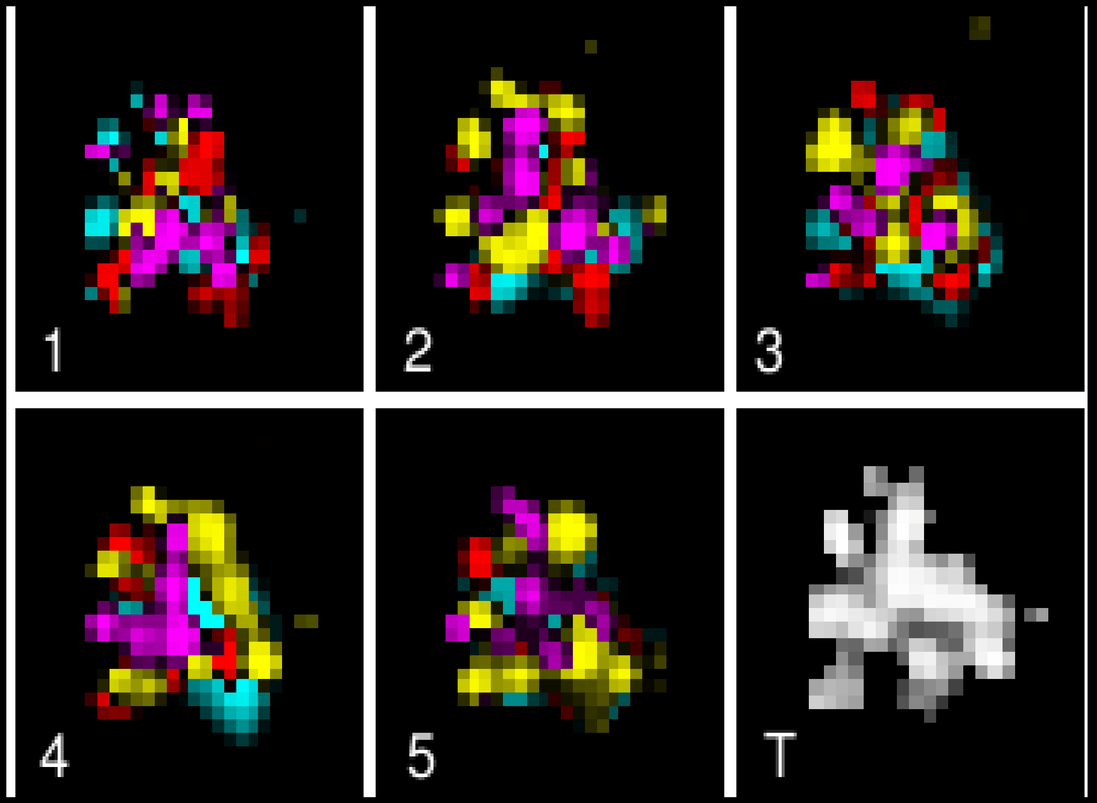
Are there preferred configurations in the cell population?

OligoFISSEQ tracing of (almost) entire chromosomes

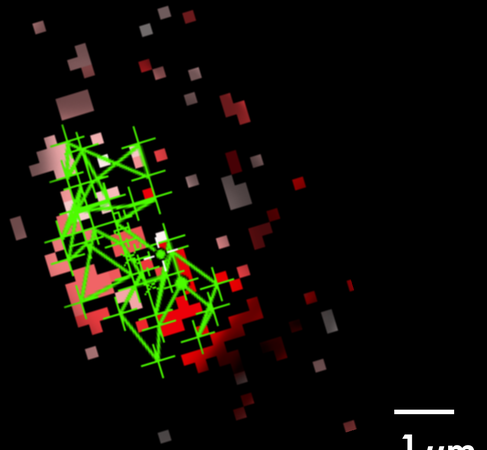
46 Plex in chromosome X





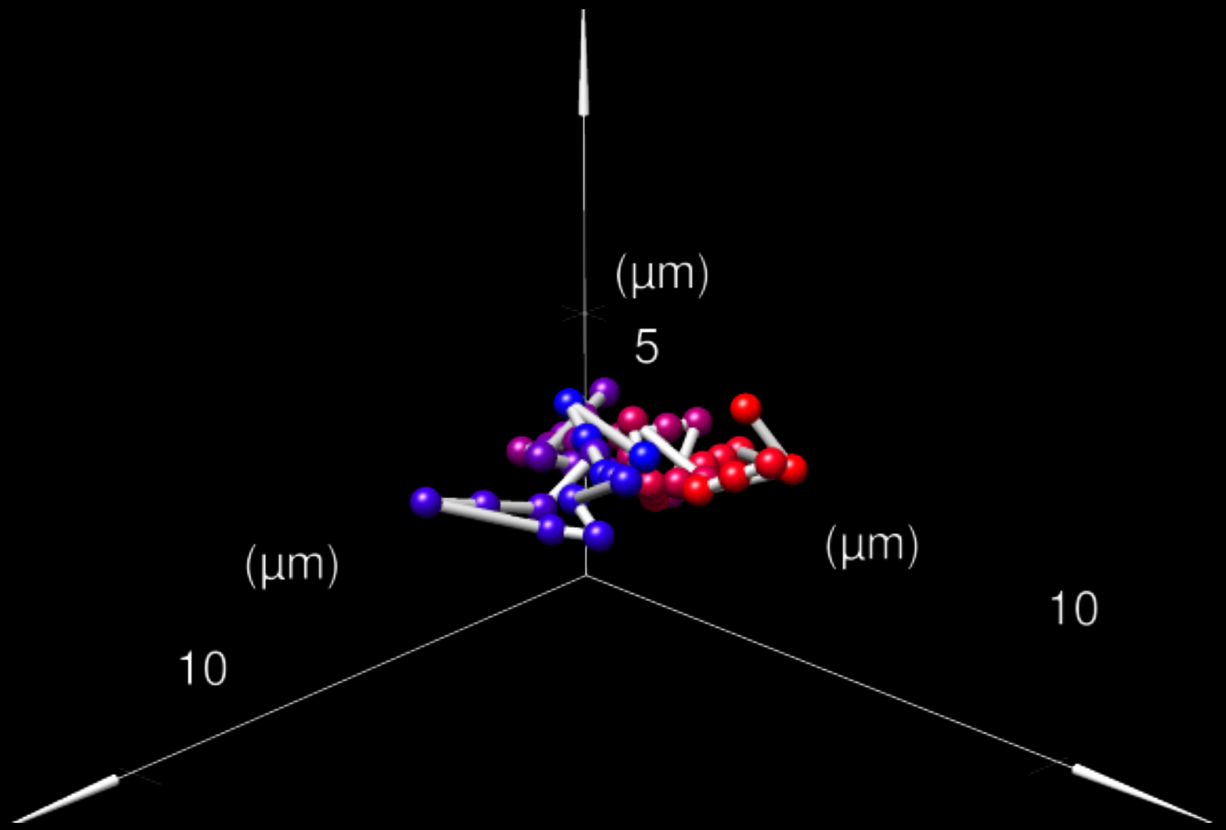


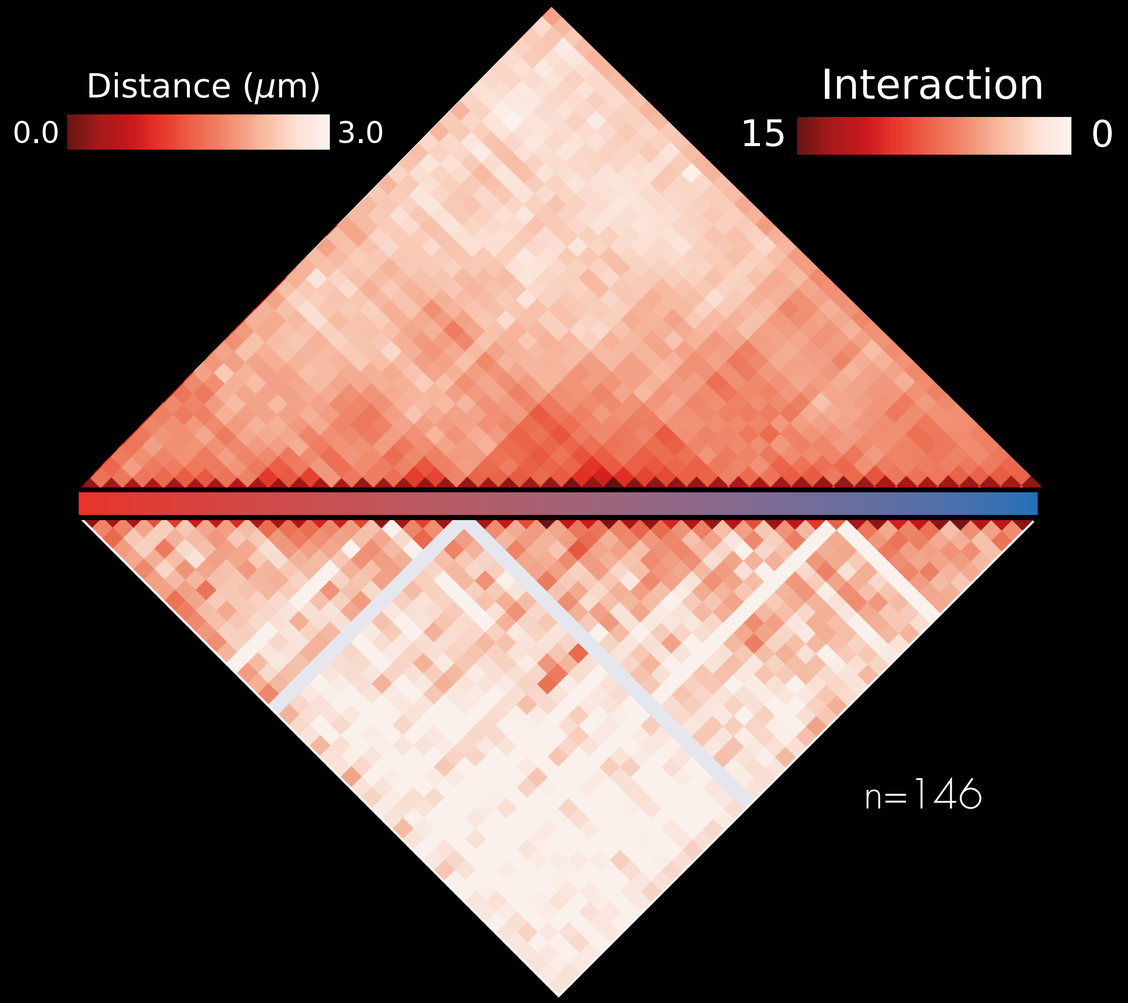
5 rounds
445 kb/probe
2,000 Oligopaints/probe
2 Mb between loci



OligoFISSEQ tracing of (almost) entire chromosomes

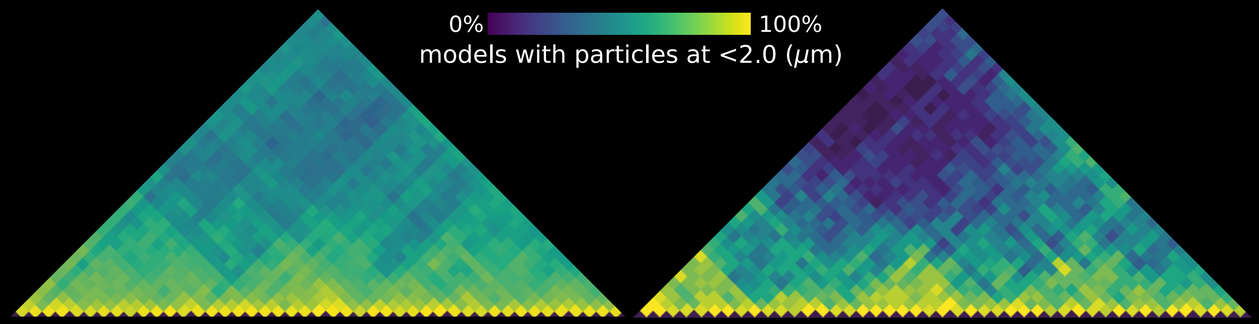
46 Plex in chromosome X





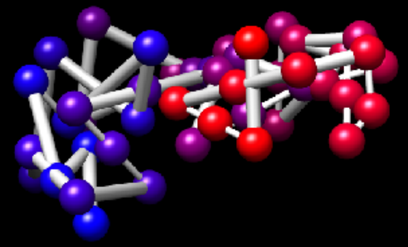
OligoFISSEQ tracing of (almost) entire chromosomes

46 Plex in chromosome X



Cluster 1 (n=156)

Cluster 2 (n=20)



OligoFISSEQ beyond chromosome tracing

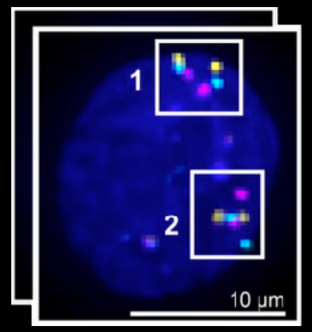
OligoFISSEQ pipelined with OligoSTORM

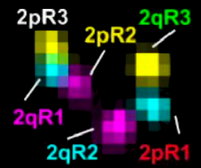
1 **OligoSTROM**
1 round
(2h/round)

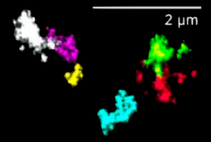
2 **OligoFISSEQ**
2 round
(3h/round)

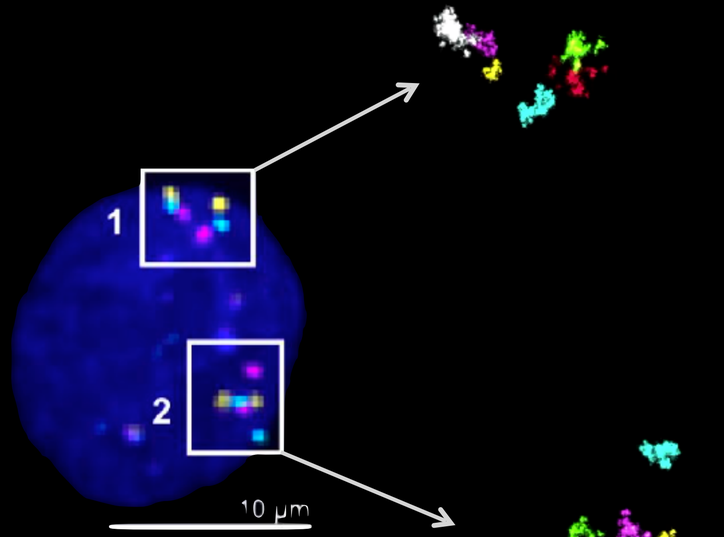
3 **Decoding**
OligoFISSEQ

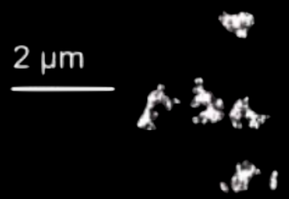
4 **Mapping**
OligoSTROM



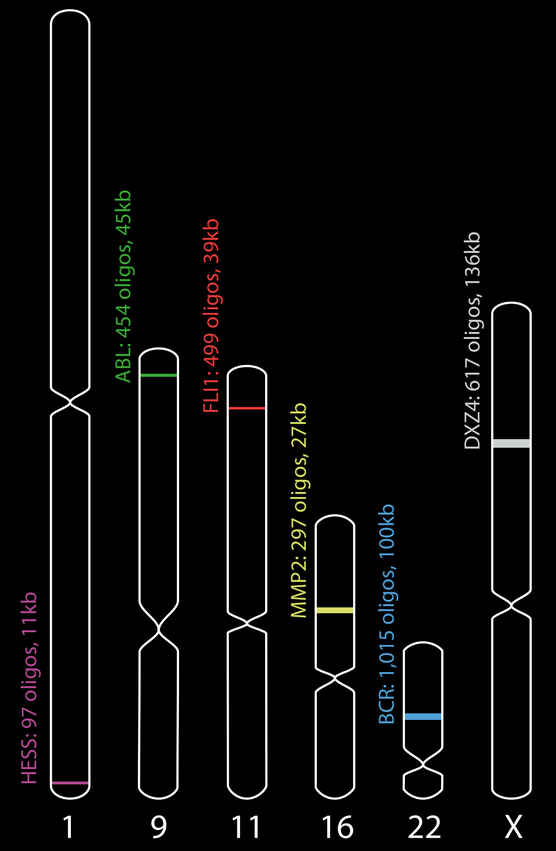


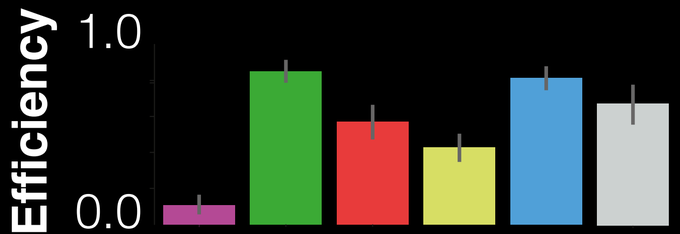


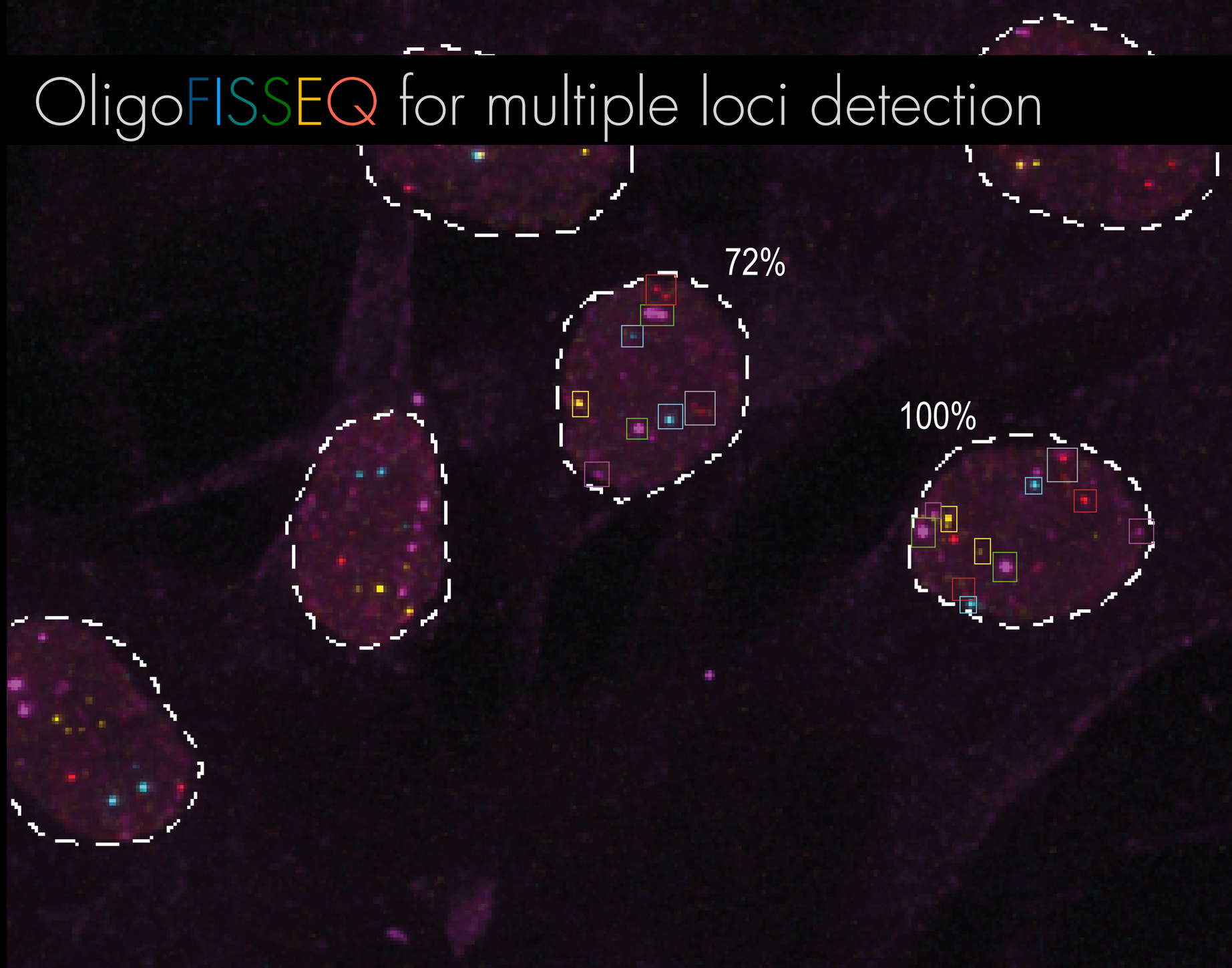




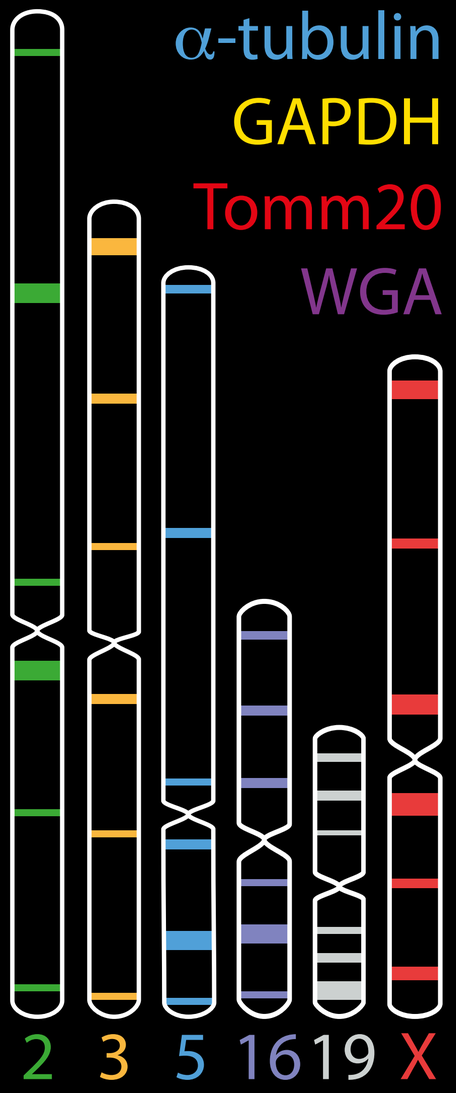
OligoFISSEQ for multiple loci detection



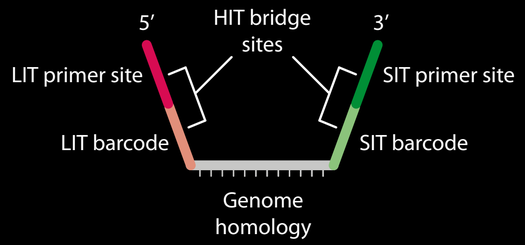




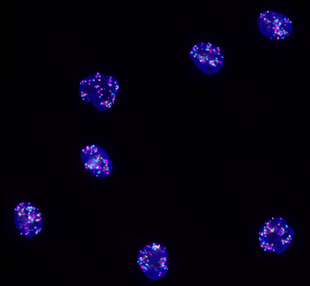
OligoFISSEQ + protein immunofluorescence



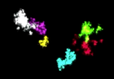
OligoFISSEQ



- Is a set of technologies for in-situ genome mapping
- Is highly versatile: mainstreet and backstreet



- Used with wide-field microscopy permits the analysis of thousands of cells.
- Identifies sub-clusters with specific conformational characteristics



- Can be pipelined with other approaches
  - OligoSTORM
  - Protein immunofluorescence
  - RNA…

http://marciuslab.org
http://3DGenomes.org

@marciuslab
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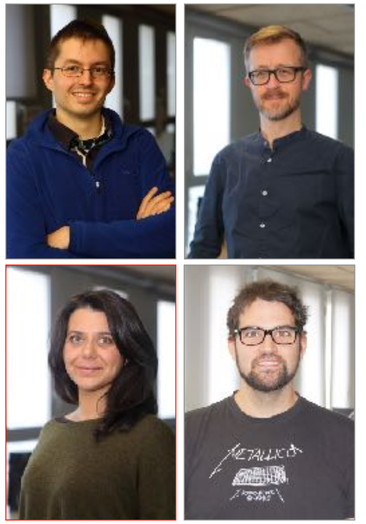

In collaboration with the Wu Lab — Ting Wu, Nir Guy, Huy Nguyen & Shyamtanu Chattoraj

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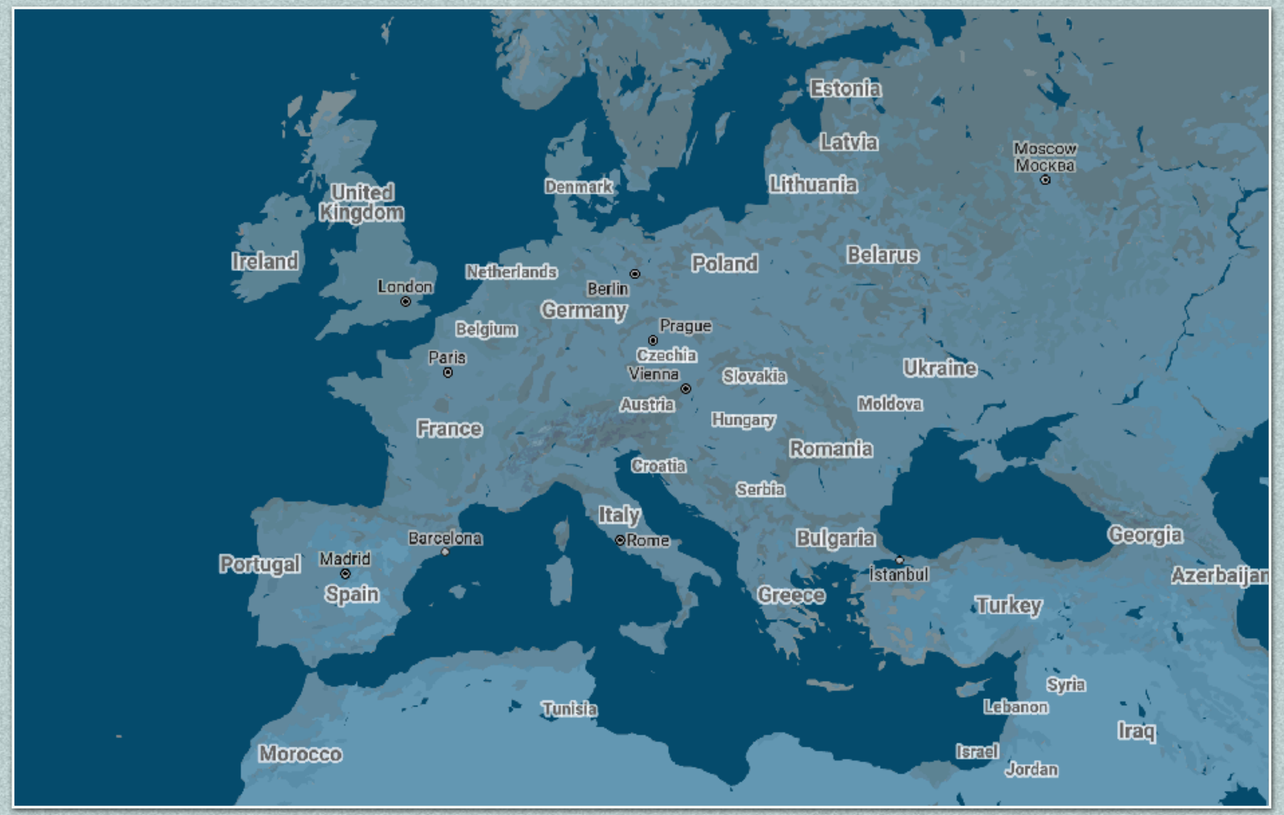

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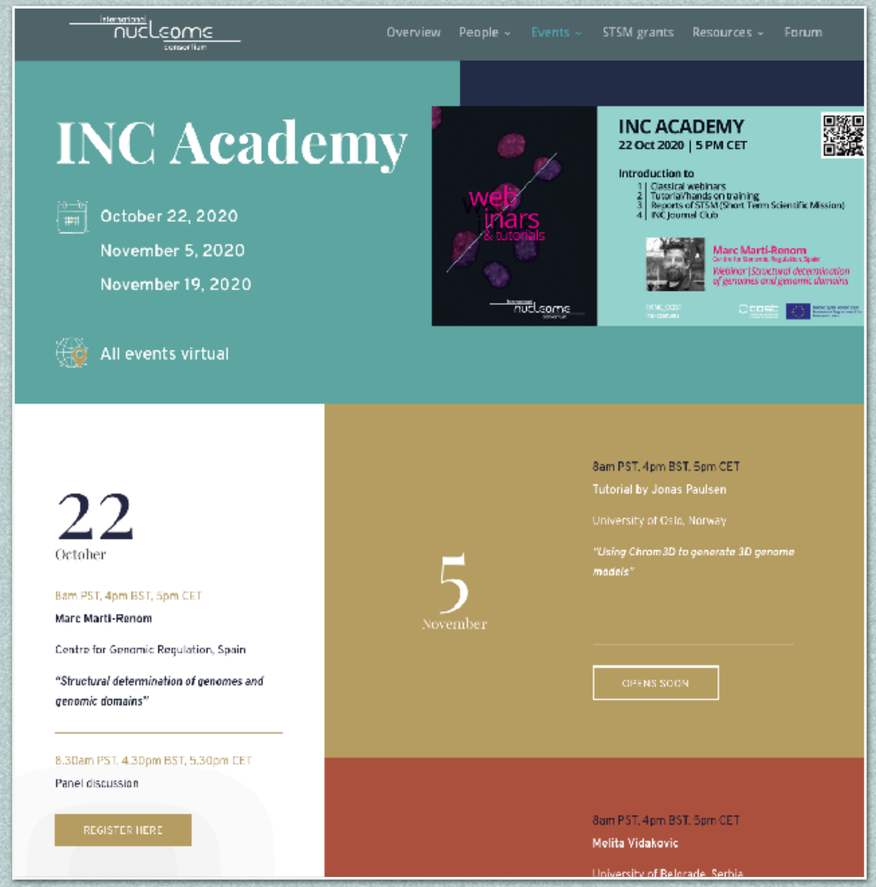

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