HUMAN “The Movie” by Yann Arthus-Bertrand

Structure determination of genomes and genomic domains by image tracing

Marc A. Marti-Renom

CNAG-CRG · ICREA

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

cnag CRG ICREA

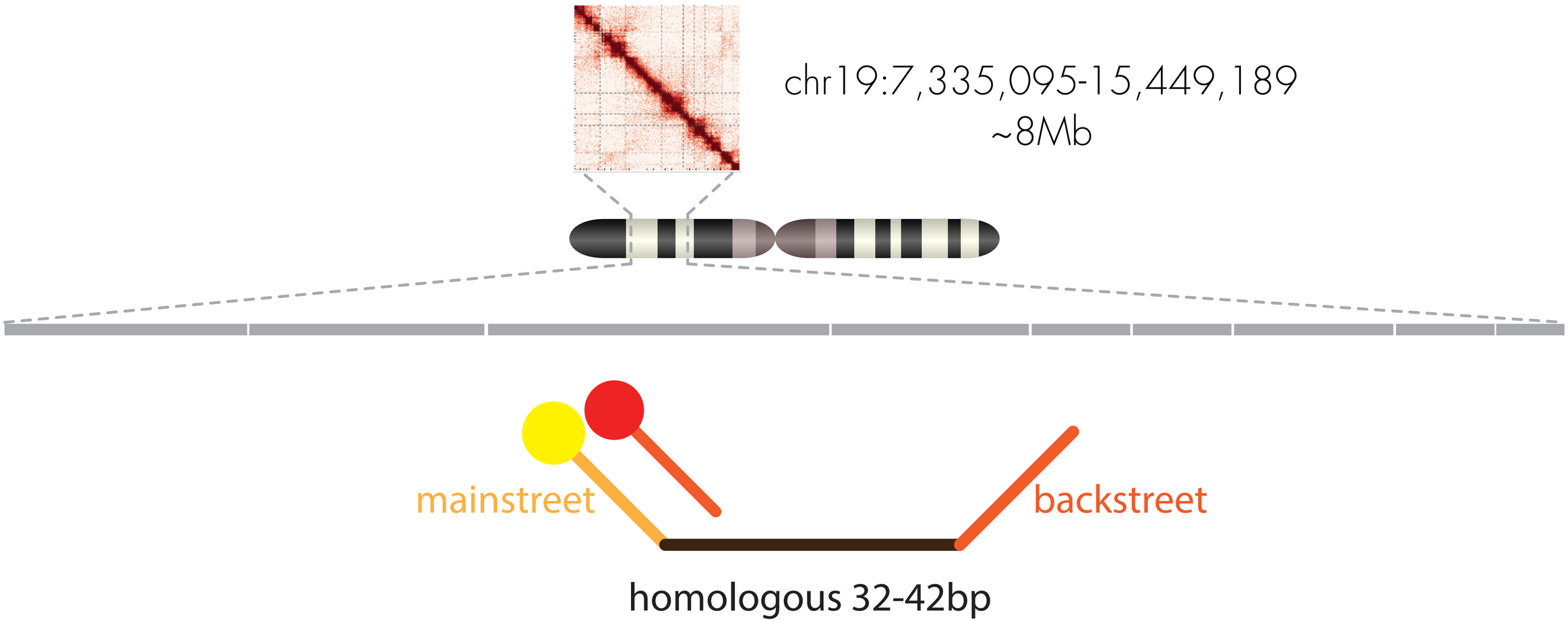
Chromosome walking with super-resolution imaging and modeling

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

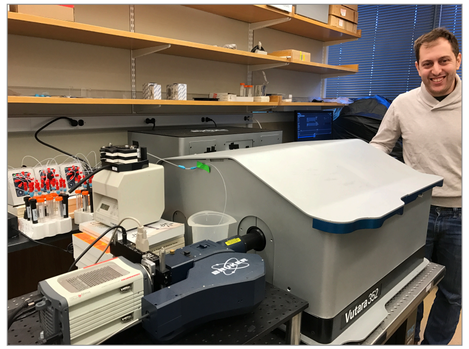
Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



Beliveau et al. Nat. Comm. 2015

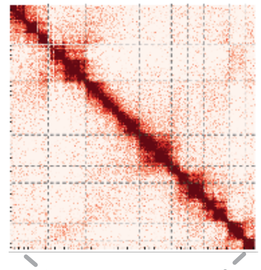
High-resolution imaging

Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells

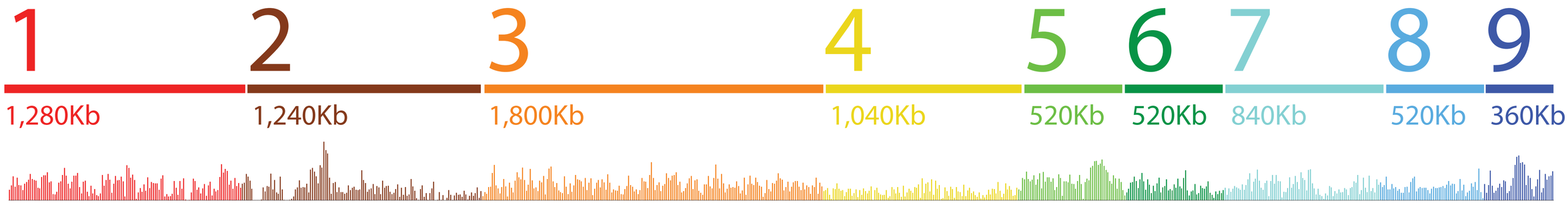


Guy Nir Harvard Med School

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

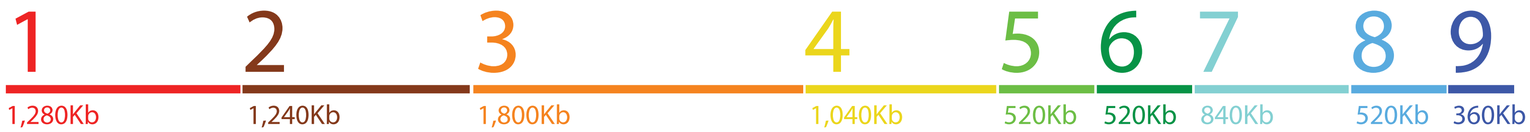


chr19:7,335,095-15,449,189
~8Mb



High-resolution imaging

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

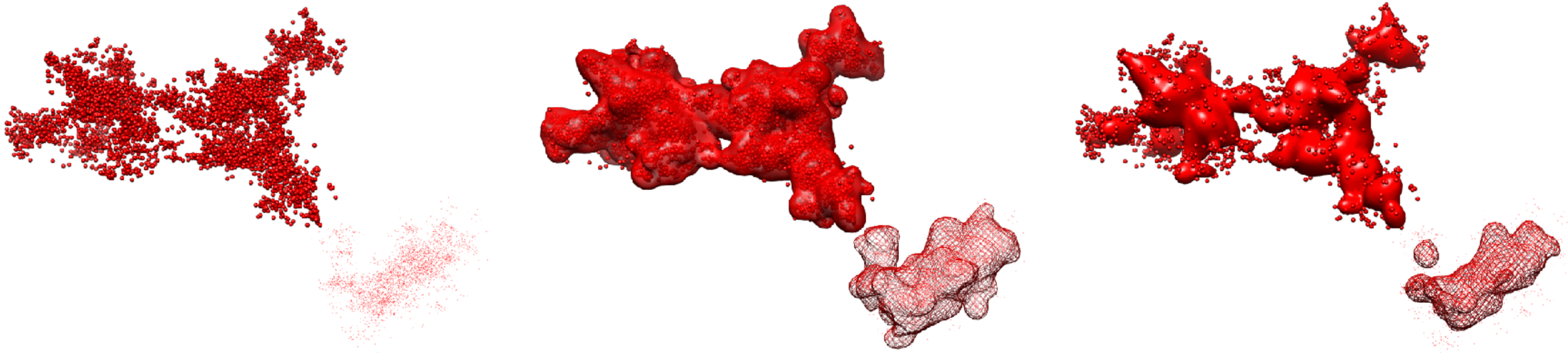


Cell-02

High-resolution imaging

XYZ points convolution into a density map

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

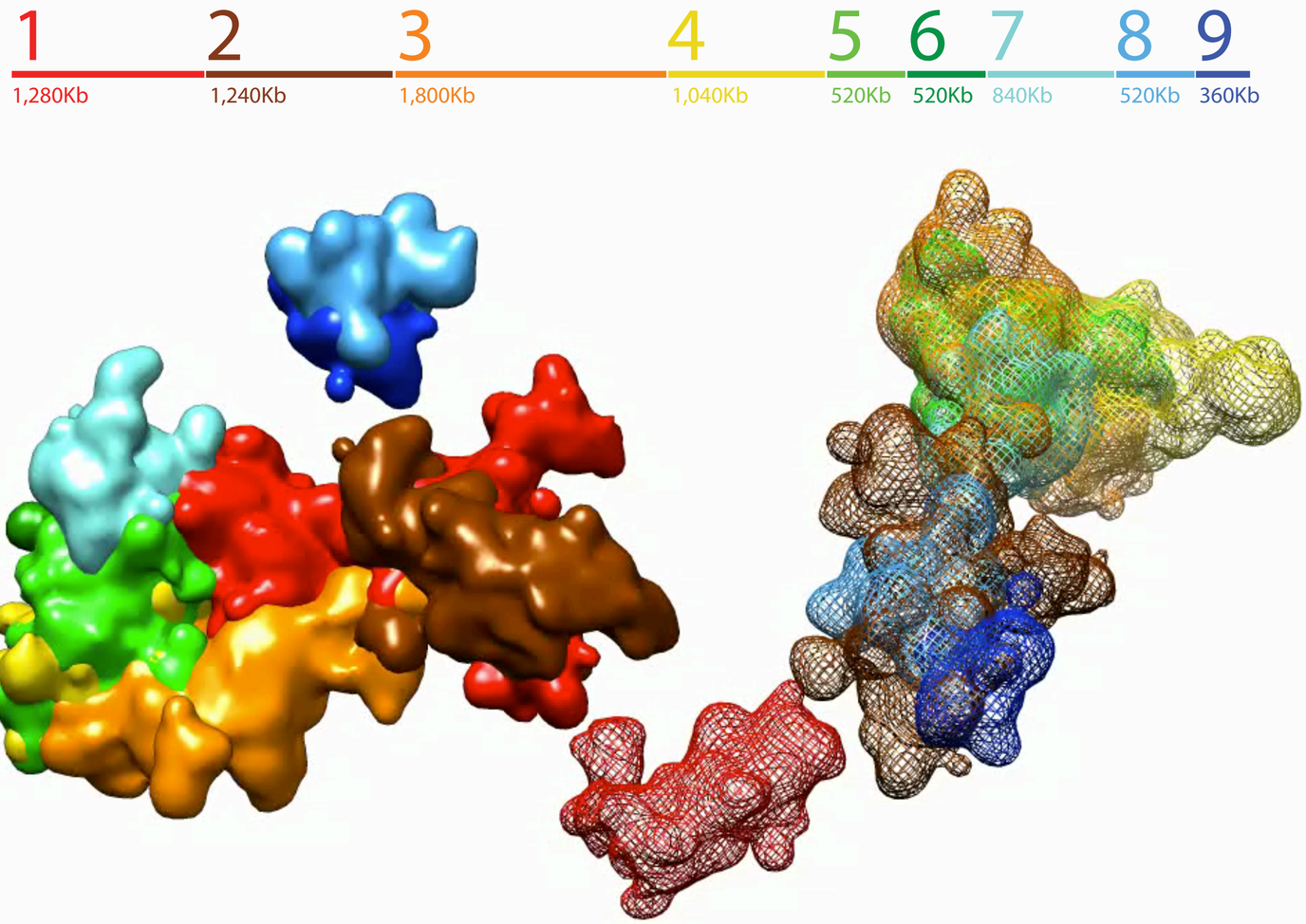


Cell-02 · Segment 1

Farabella et al, J Appl Crystallogr. 2015

Density maps

Cell-02 · Density map @ 50nm

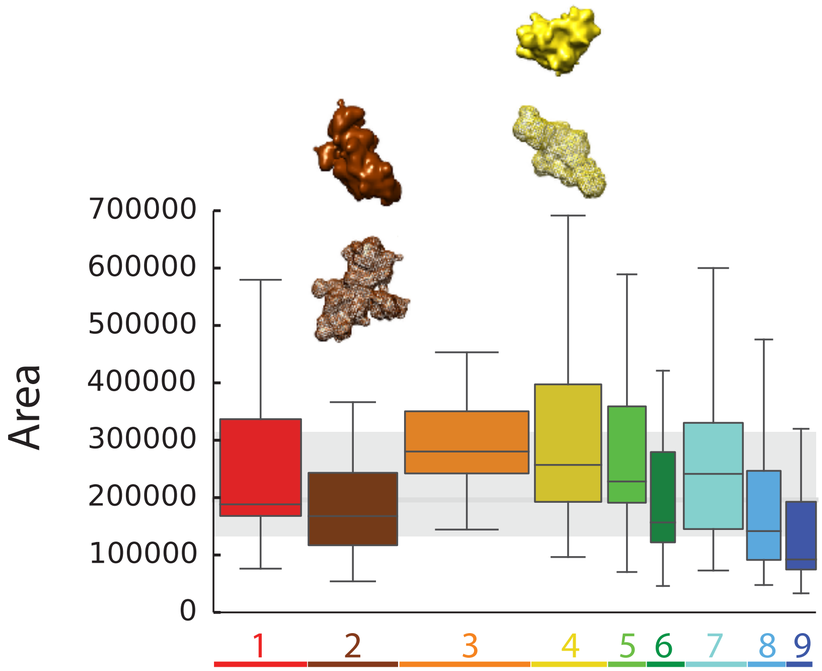


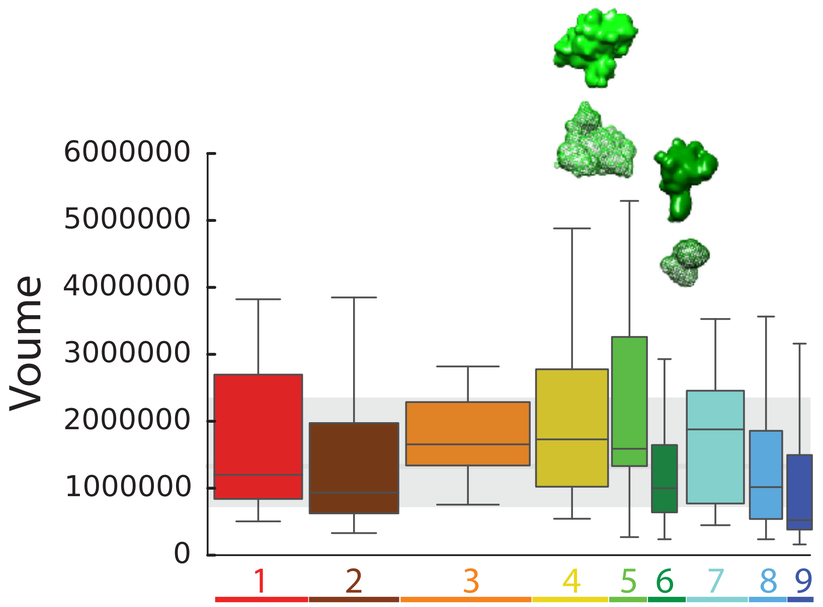
Area (nm^2)
Volume (nm^3)
Sphericity
Overlap (%)
Distance (nm)

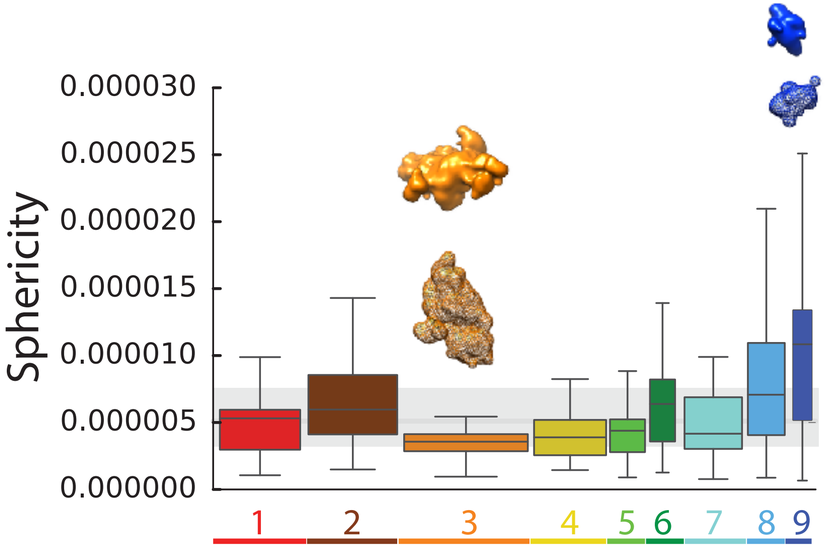
Farabella et al, J Appl Crystallogr. 2015

Structural features

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



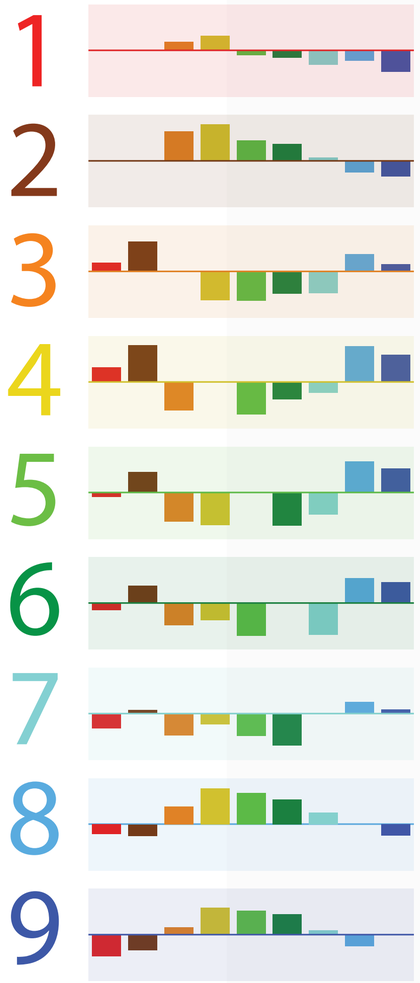


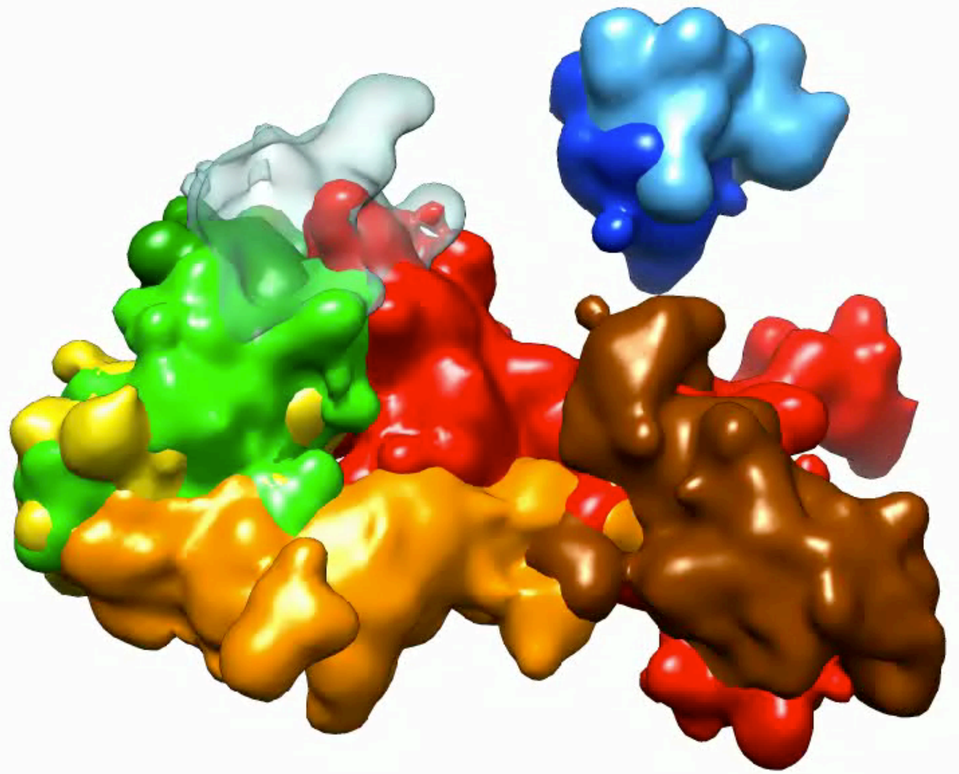


Spatial arrangement

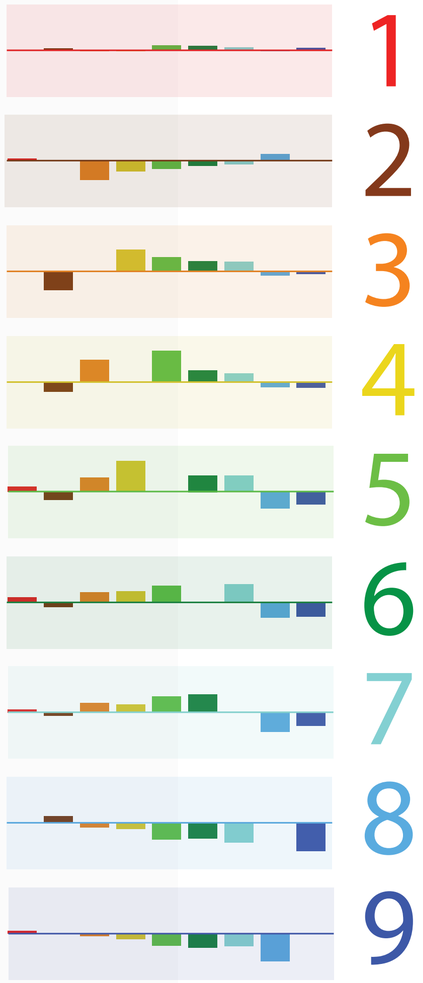
Distance and overlap of 19 cells each with 2 homologous resolved

Diff. distance



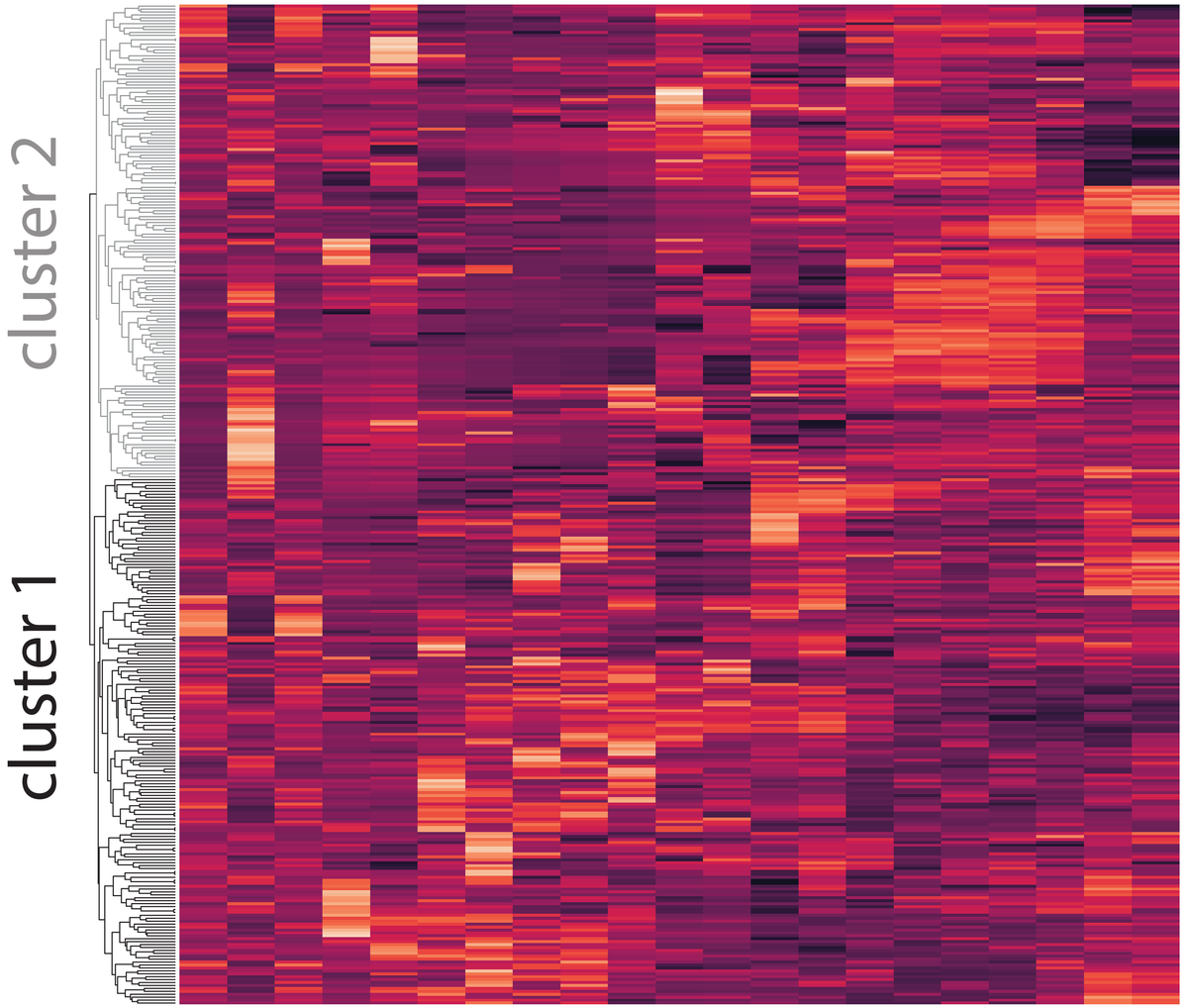


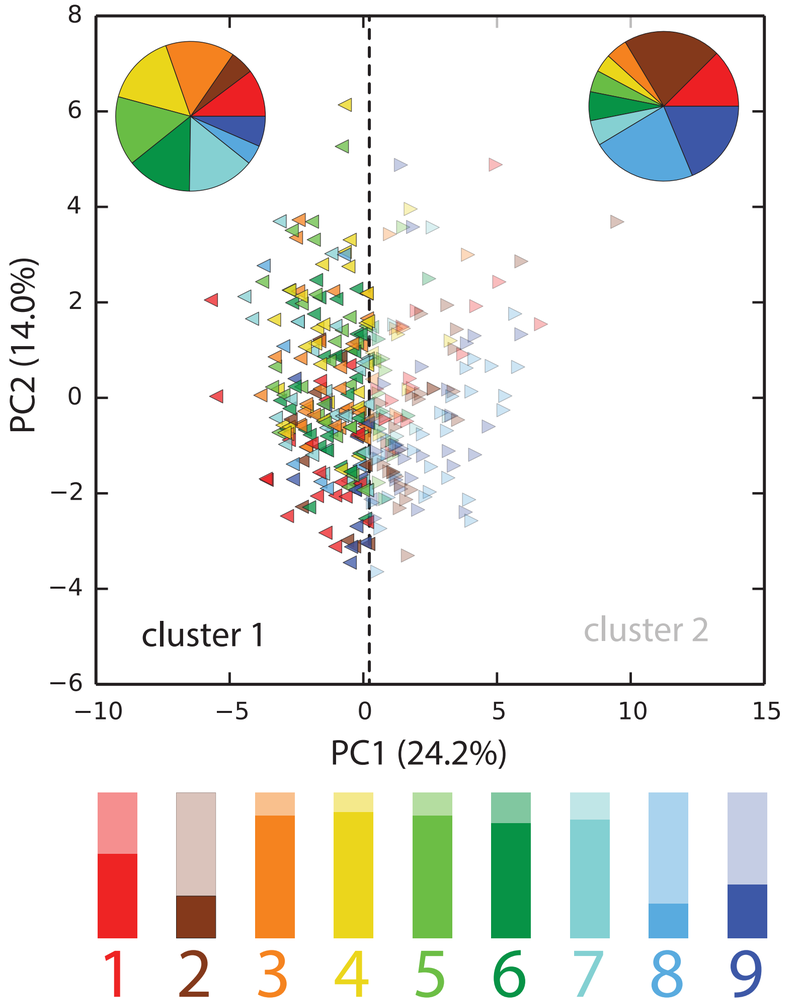
Diff. overlap



Structural clustering

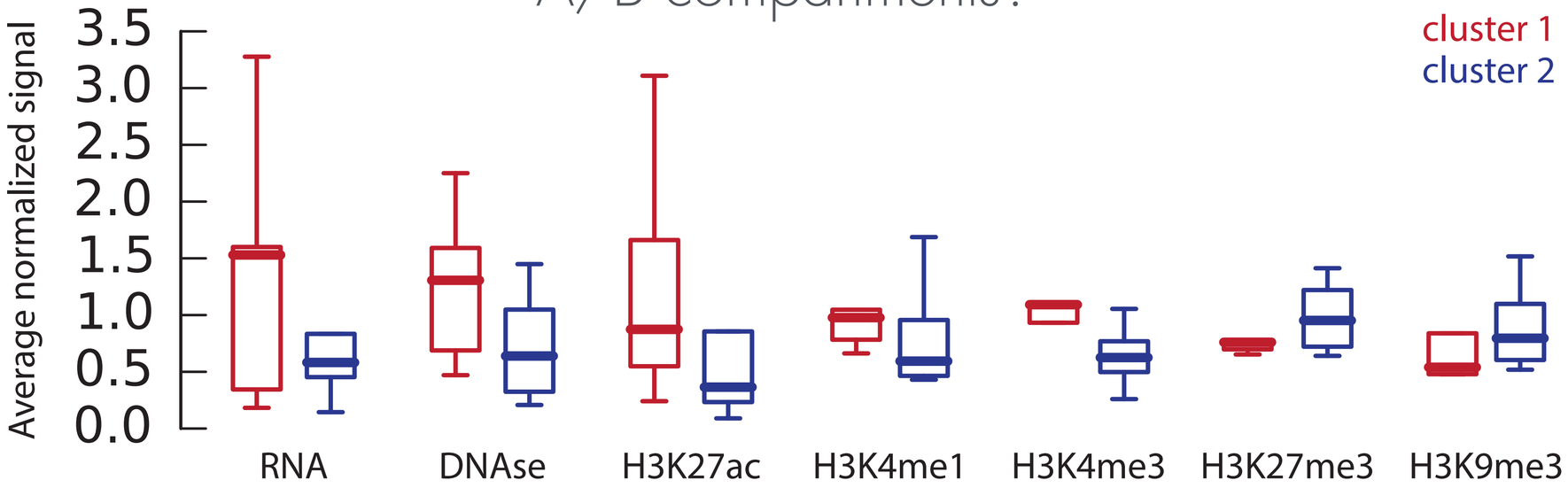
19 cells each with 2 homologous and 9 segments each (342)

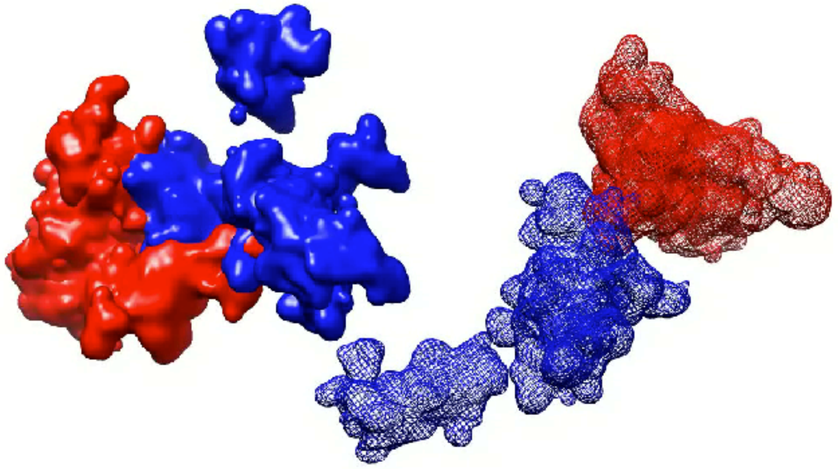


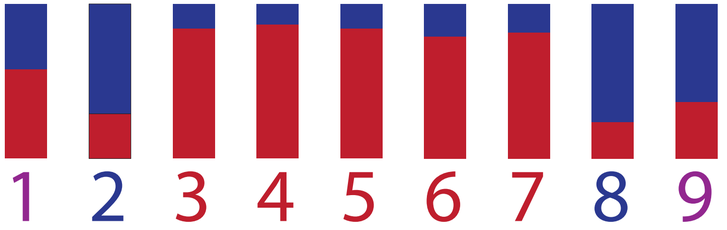


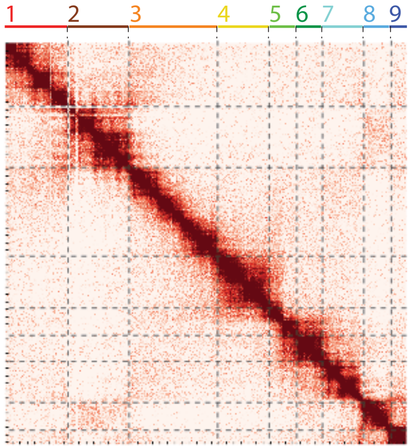
Cluster properties

A/B compartments?





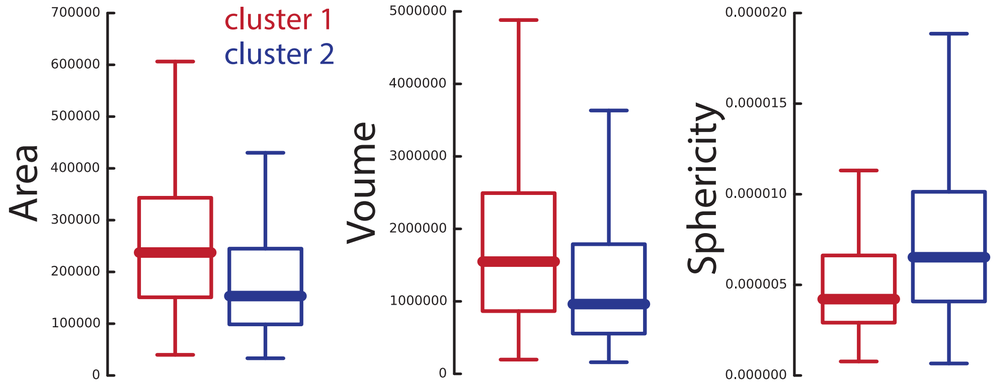


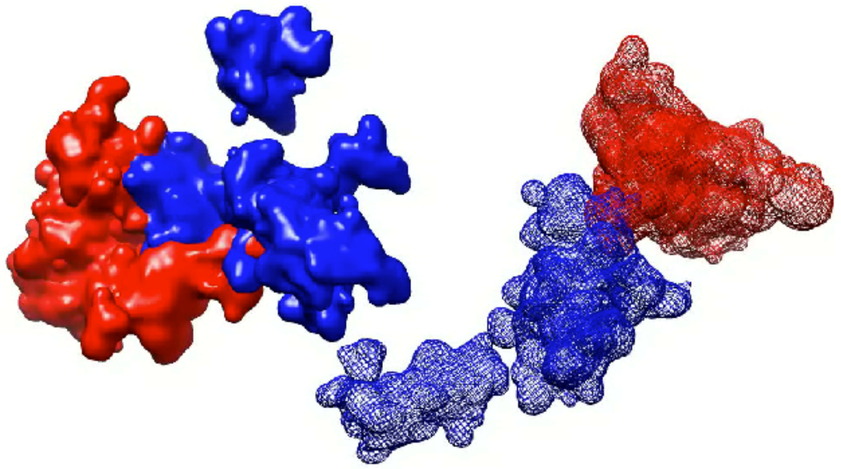


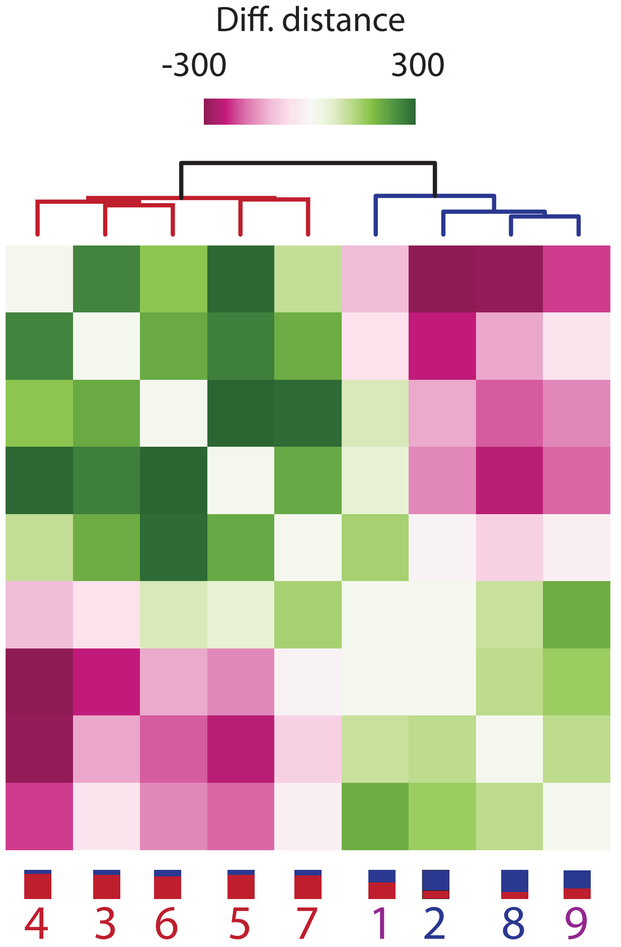
PGP1 ChIP-seq and Hi-C data from ENCODE and Lieberman-Aiden Lab, respectively

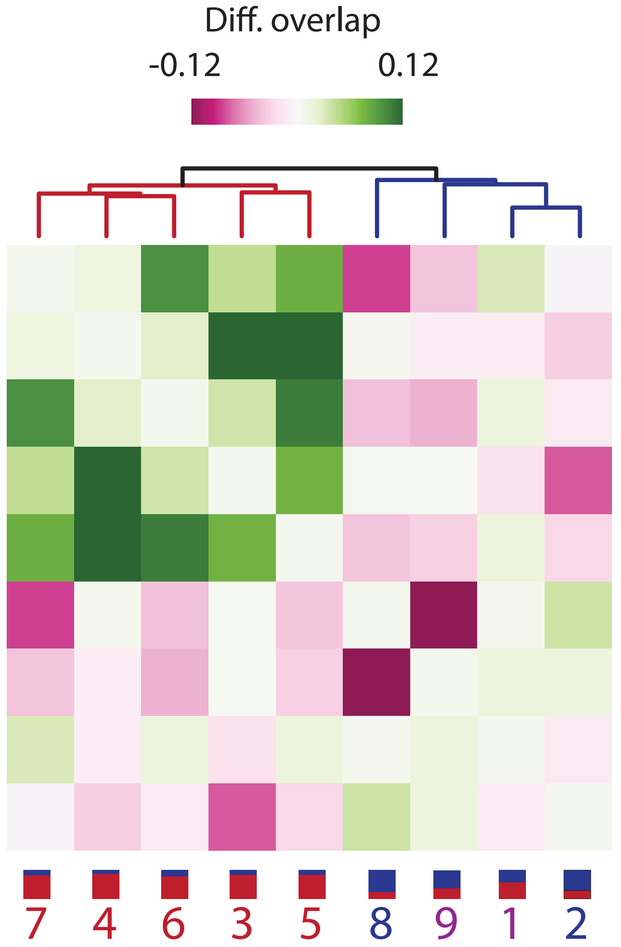
Cluster properties

A/B compartment properties



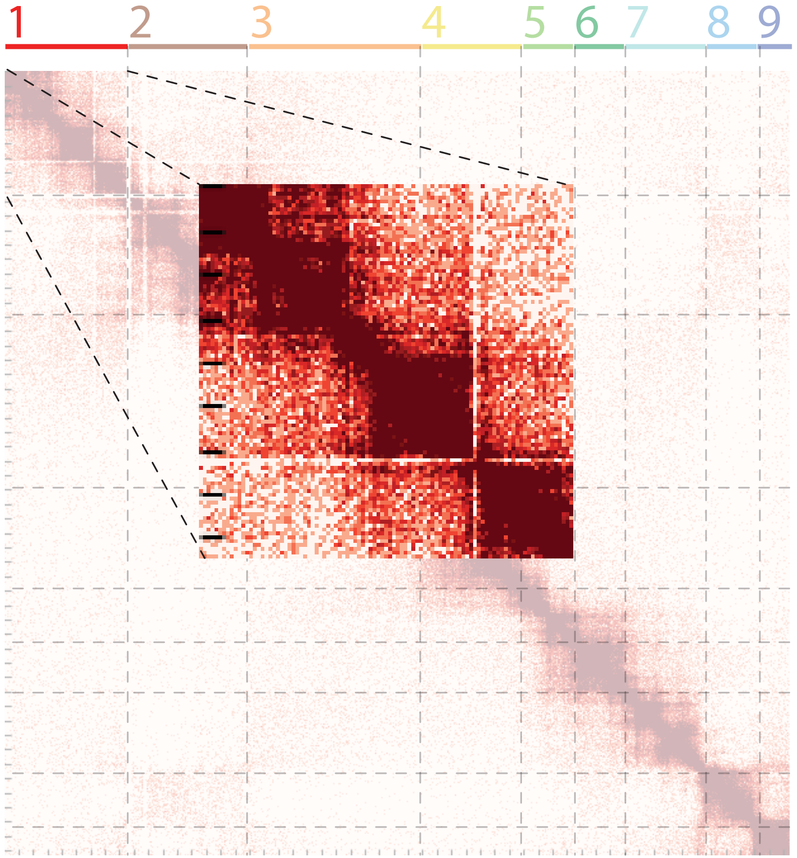




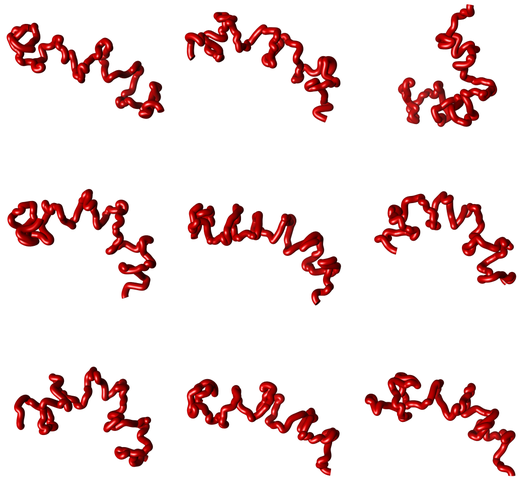


Increasing resolution

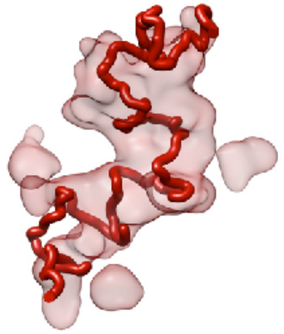
Rigid body fitting 3D structures based on Hi-C data



Segment 3 3D models

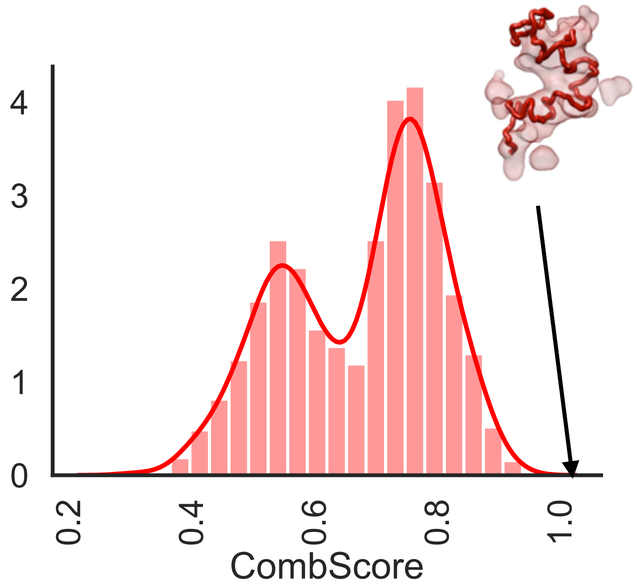


Segment 3 density map



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

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



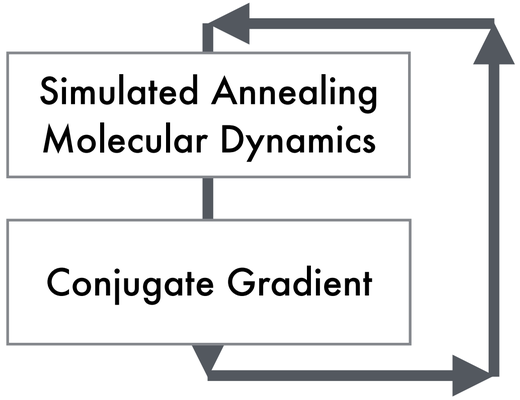
TADbit

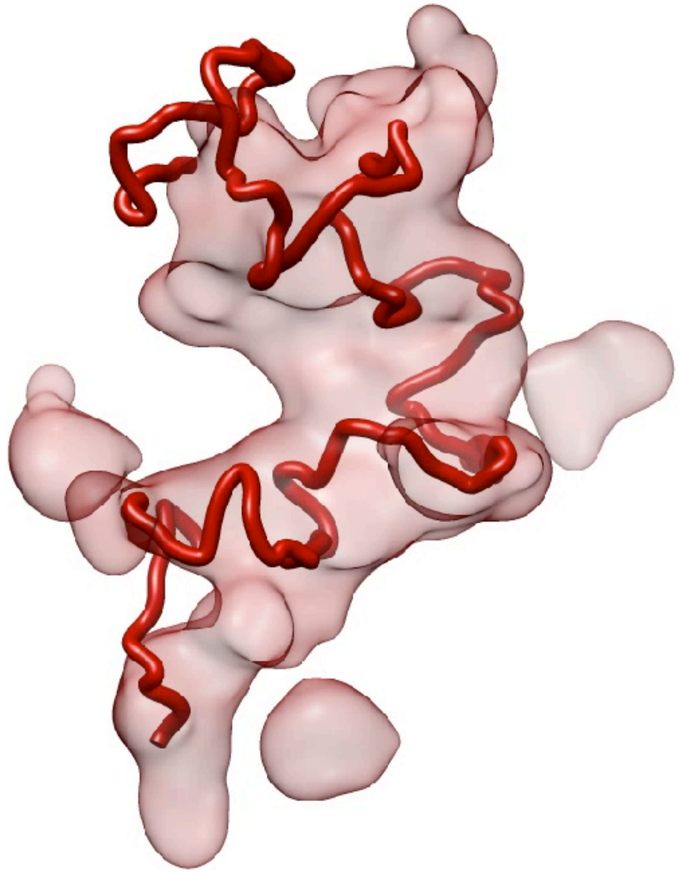
Serra, Baù, et al. PLOS CB 2017
http://www.3DGenomes.org

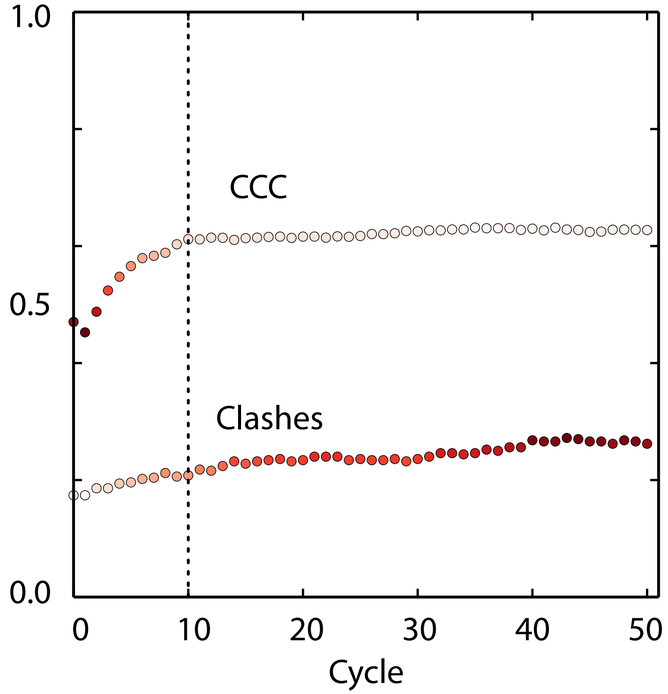
Farabella et al, J Appl Crystallogr. 2015
Roseman, 2000; Wriggers & Chacon, Structure 2001

Increasing resolution

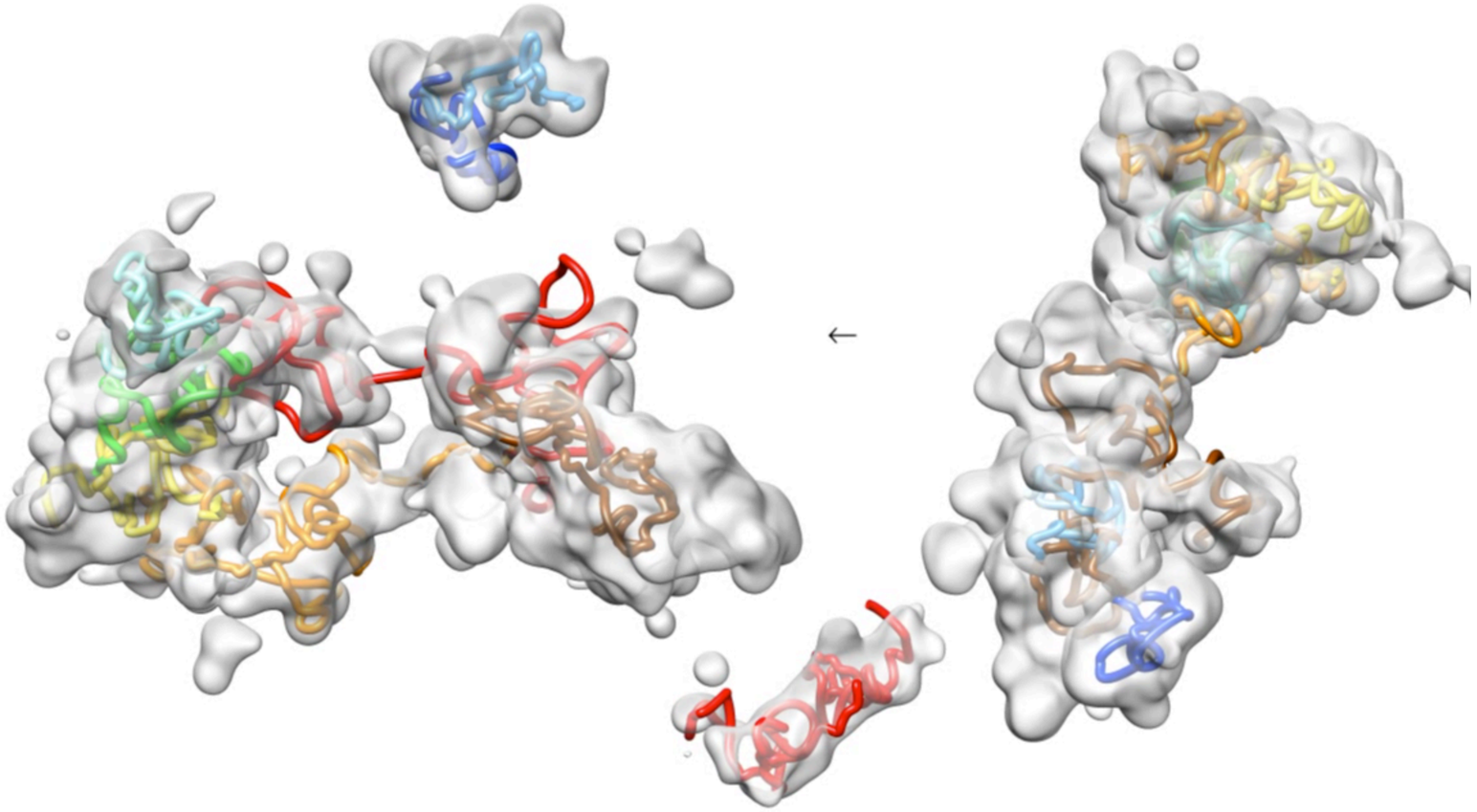
Flexible fitting 3D structures based on Hi-C data





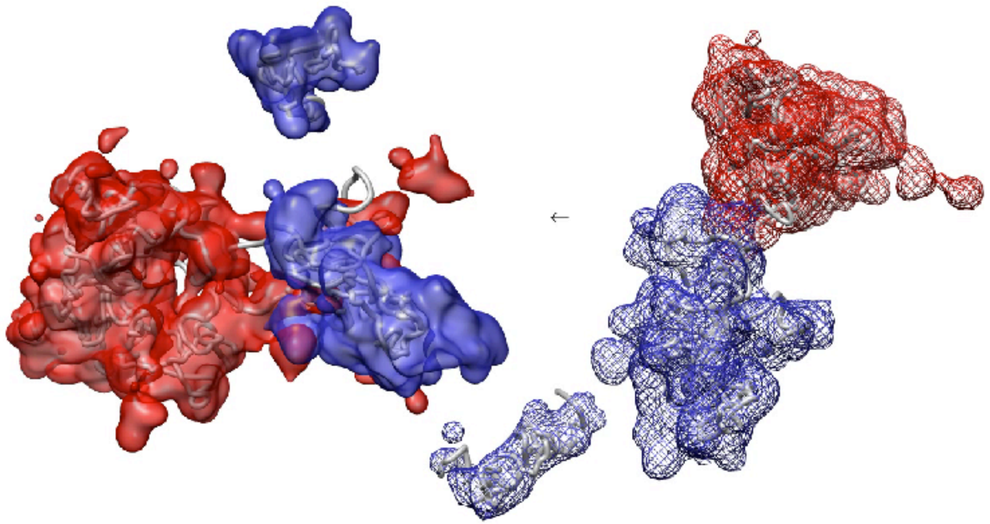


Chromosome walking path @10Kb resolution

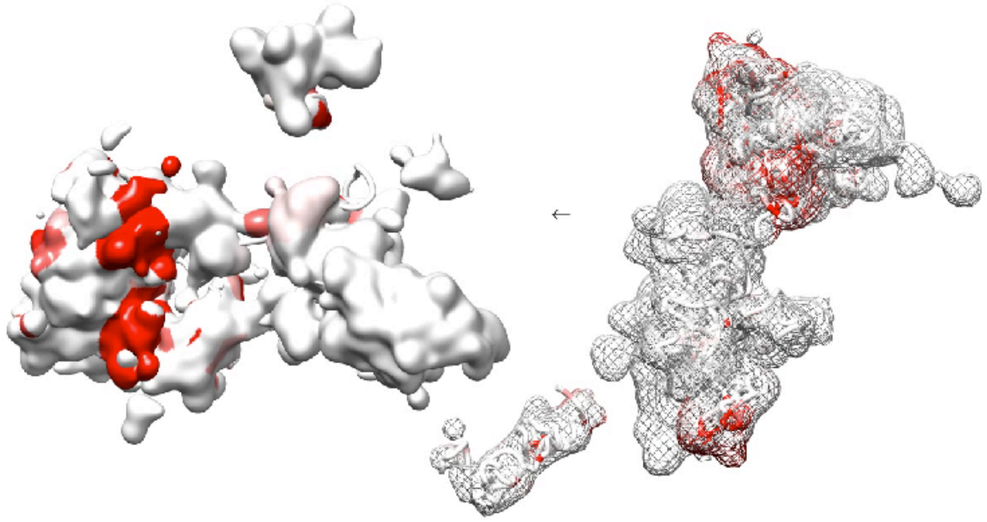


Mapping “omics”

3D organization of local structures



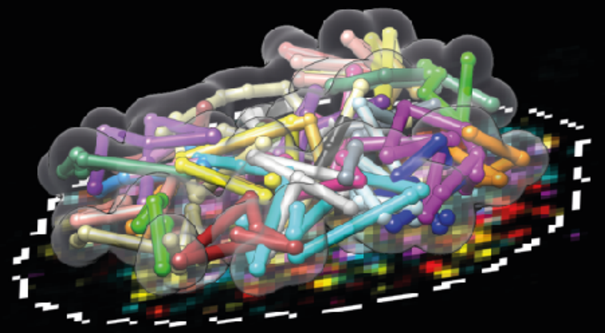
Both in “active” cluster
One in each cluster
Both in “inactive” cluster



μ mean value per 10kb μ+2σ

H3K4me1

Chromosome tracing with OligoFISSEQ



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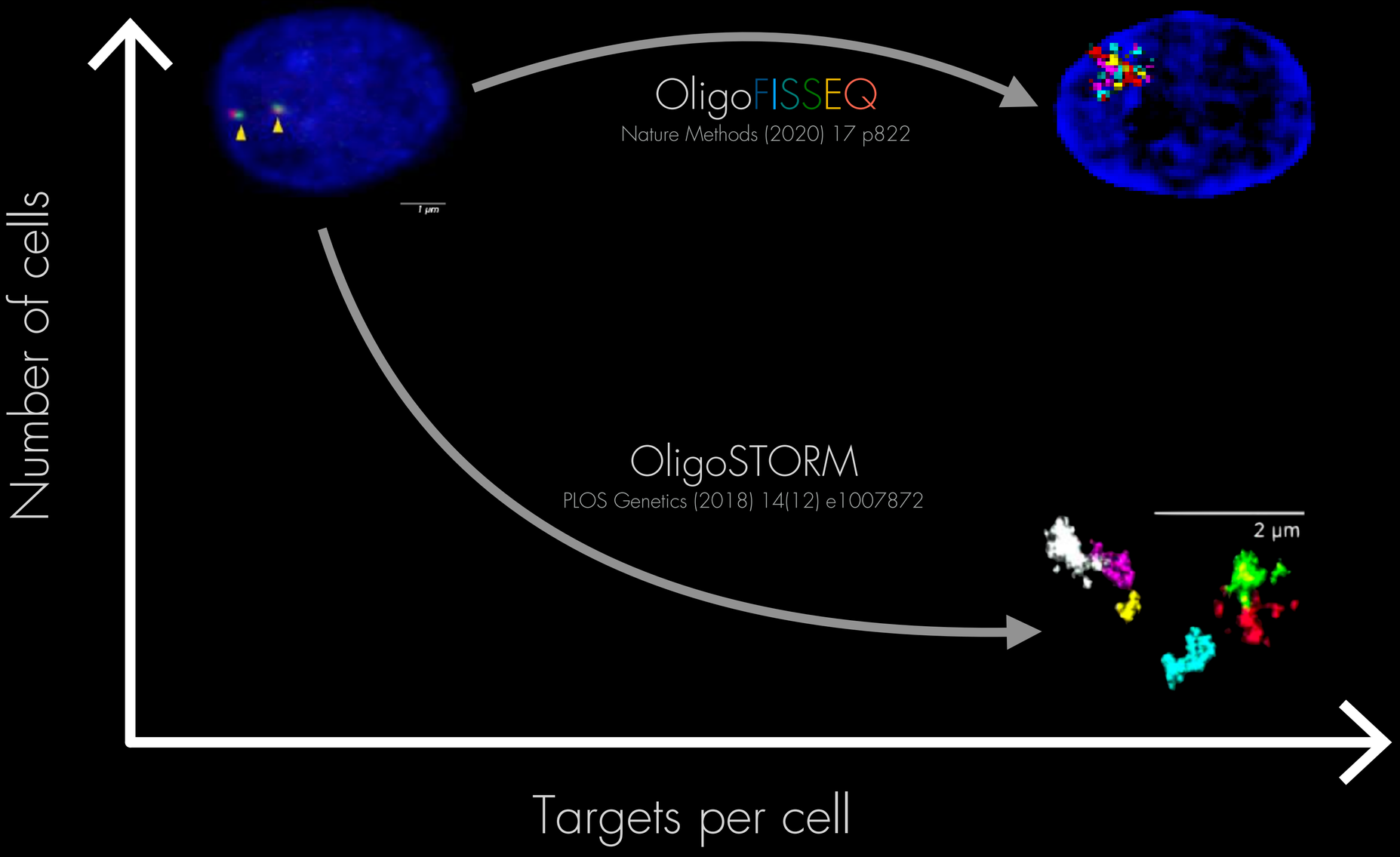
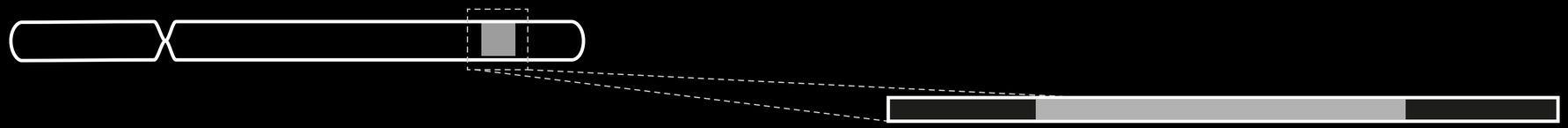
Huy Nguyen
Shyamtanu Chattoraj
David Castillo

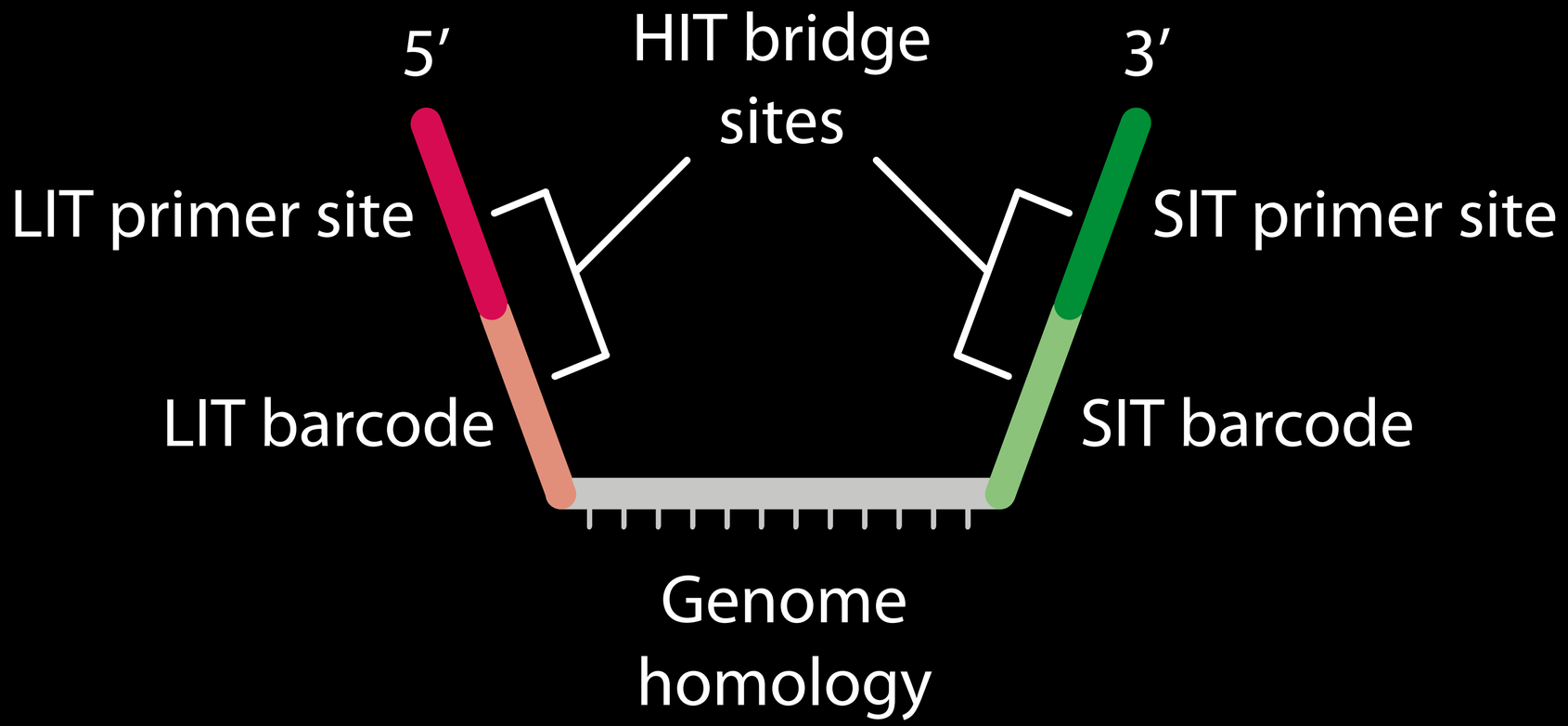
in collaboration with the Wu Lab (HMS)

Nature Methods (2020) 17 p822


OligoFISSEQ
Nature Methods (2020) 17 p822
1 µm
OligoSTORM
PLOS Genetics (2018) 14(12) e1007872
2 µm
Number of cells
Targets per cell

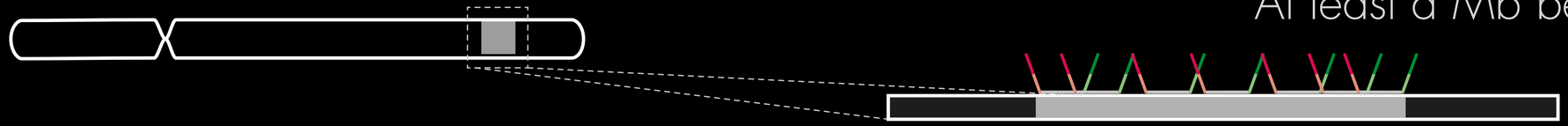
OligoFISSEQ



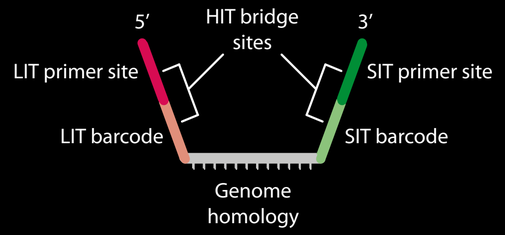


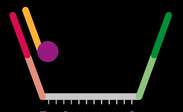
OligoFISSEQ

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

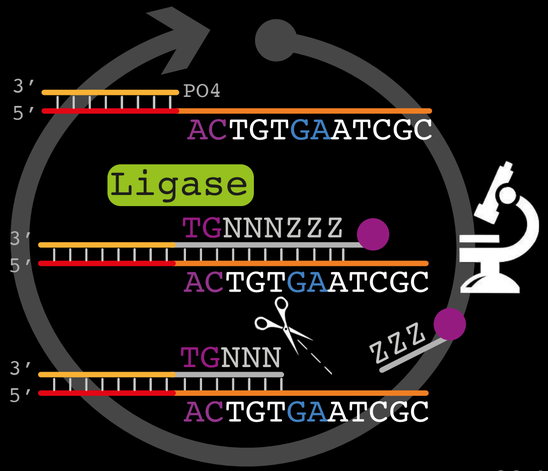


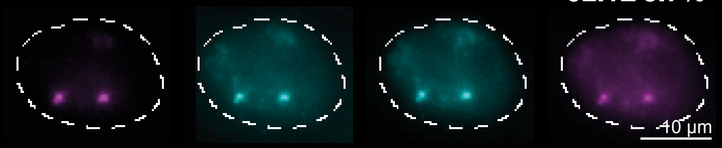
OligoFISSEQ

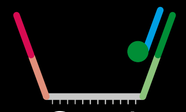




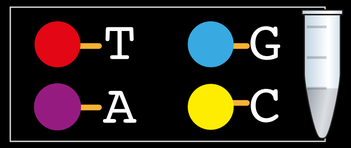
Ligation based Identification of Targets

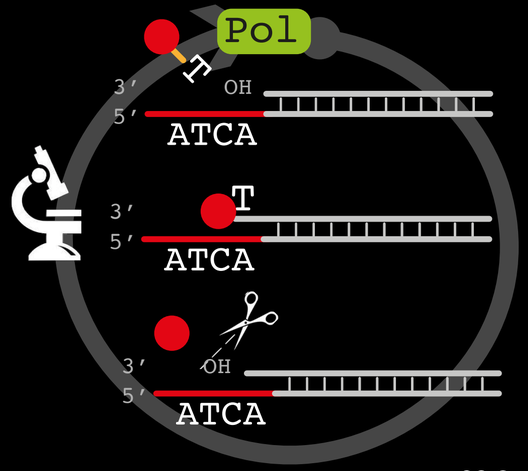


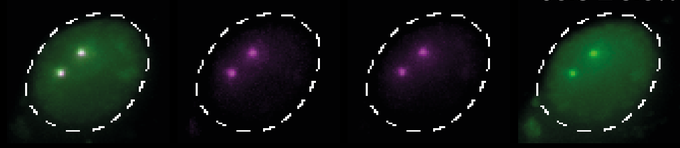




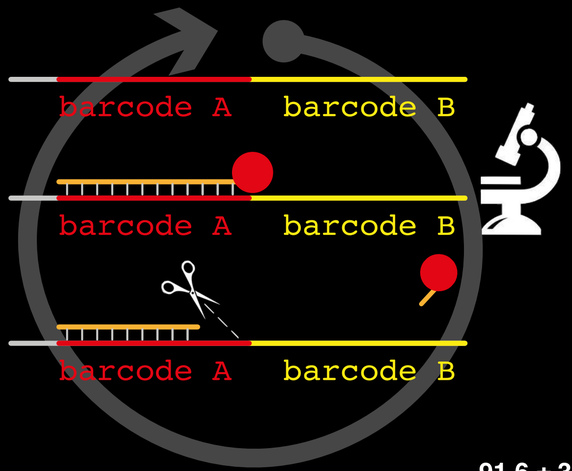
Synthesis based Identification of Targets

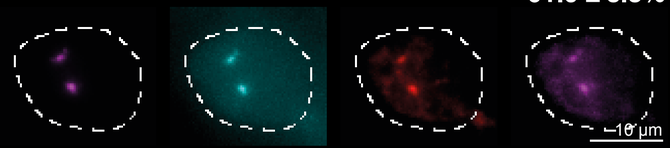






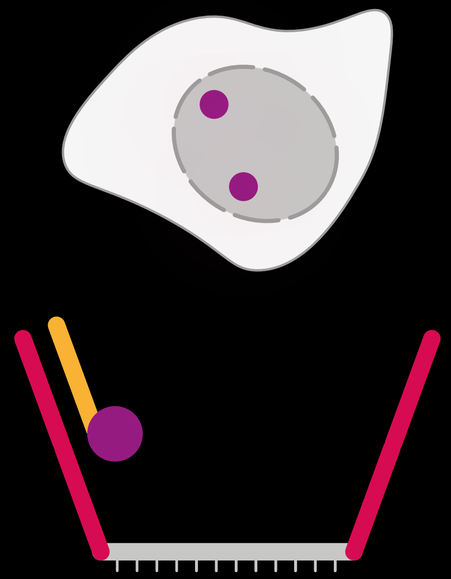
Hybridization based Identification of Targets





OligoFISSEQ scales exponentially!

Sequential hybridization

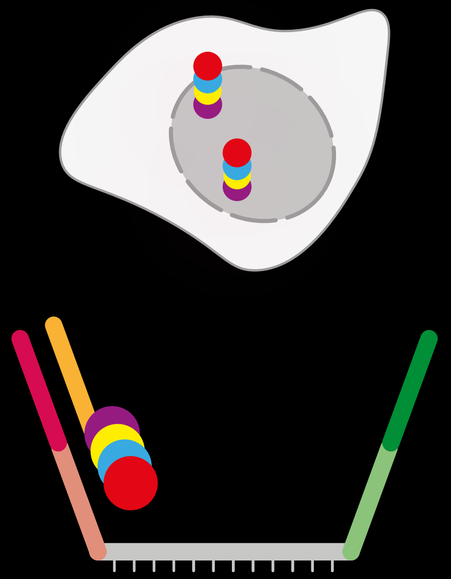


# of targets = F*N

F = # of fluorophores

N = # of seq. rounds

Barcode sequencing



# of targets = F^N

F = # of fluorophores

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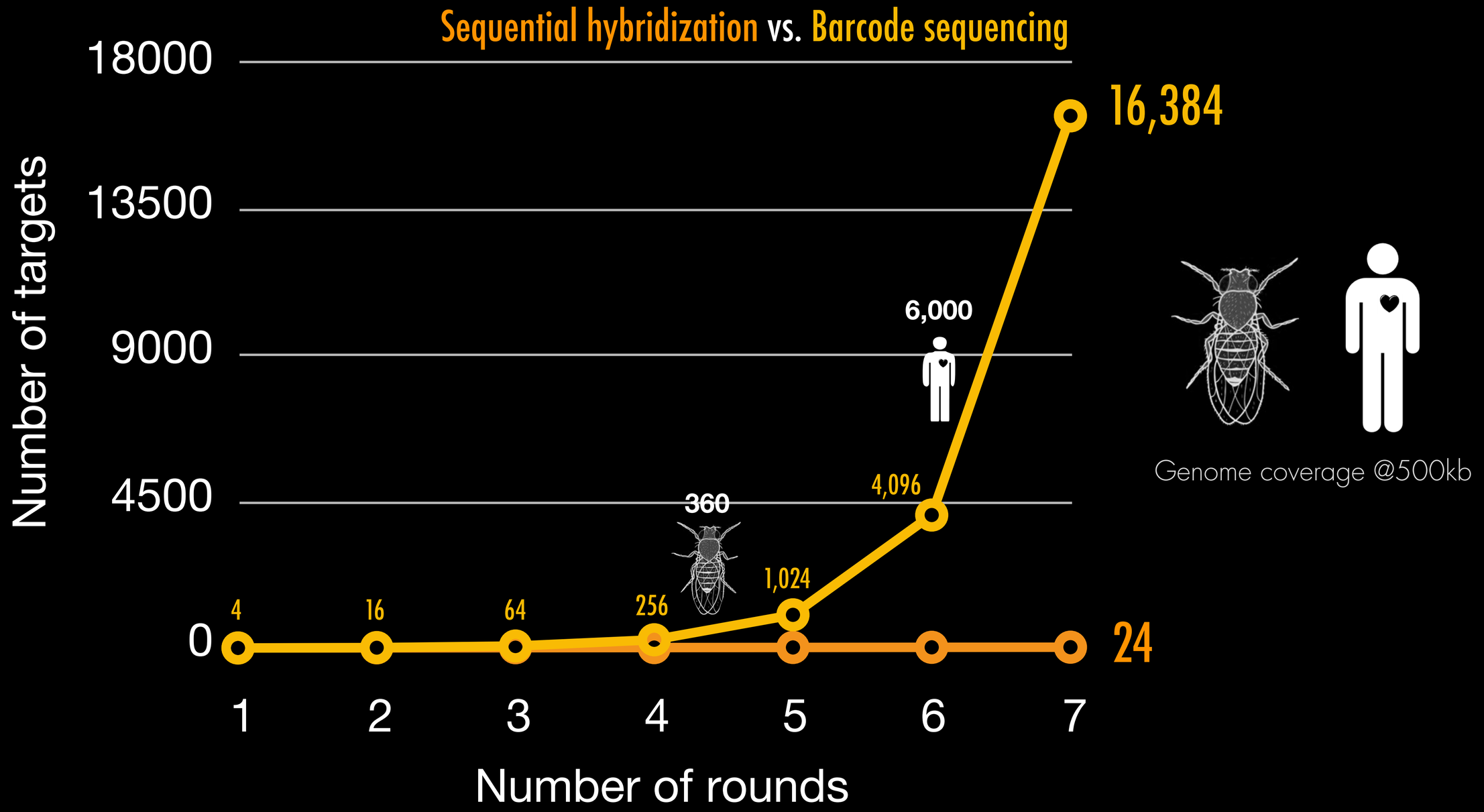
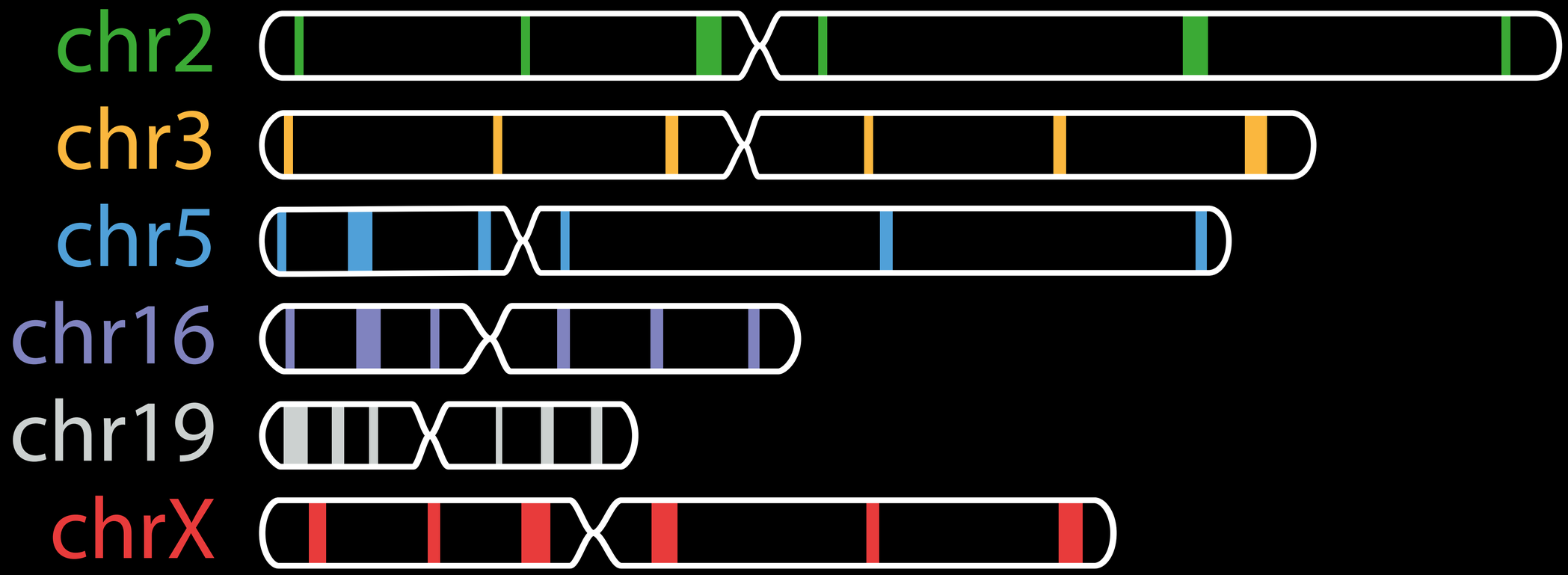
OligoFISSEQ scales exponentially!

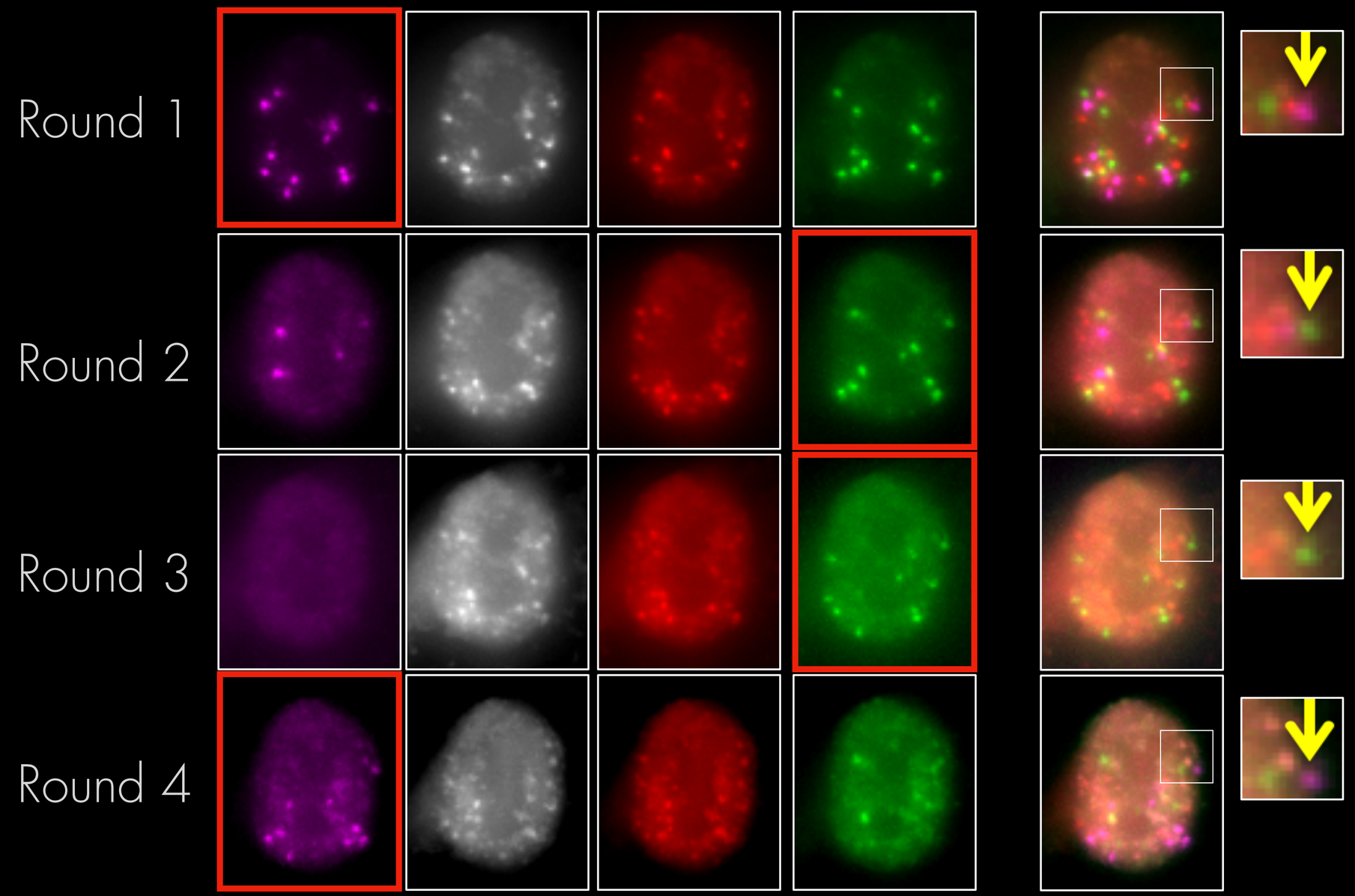


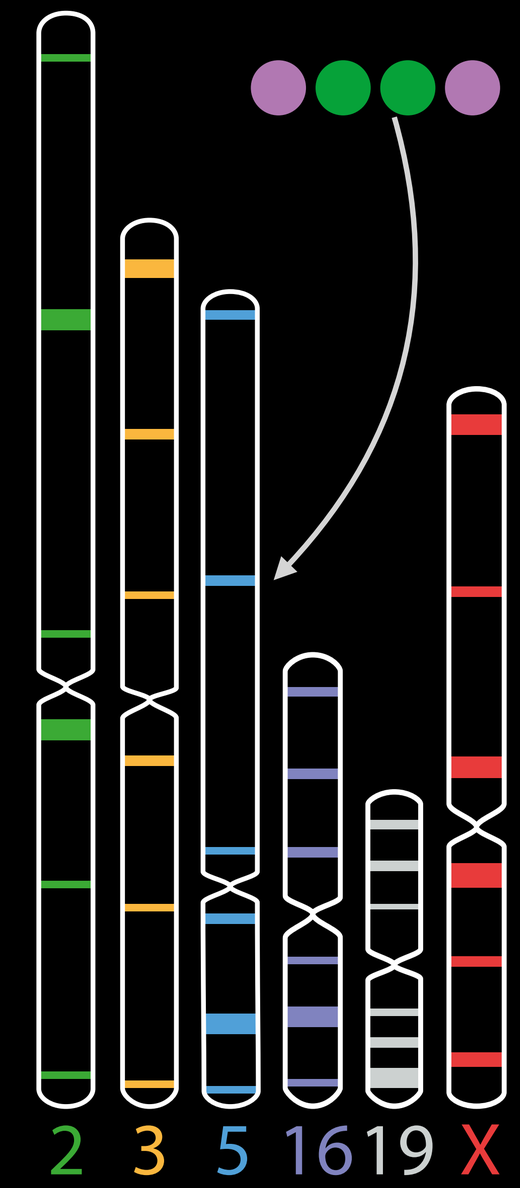
Proof-of-principle

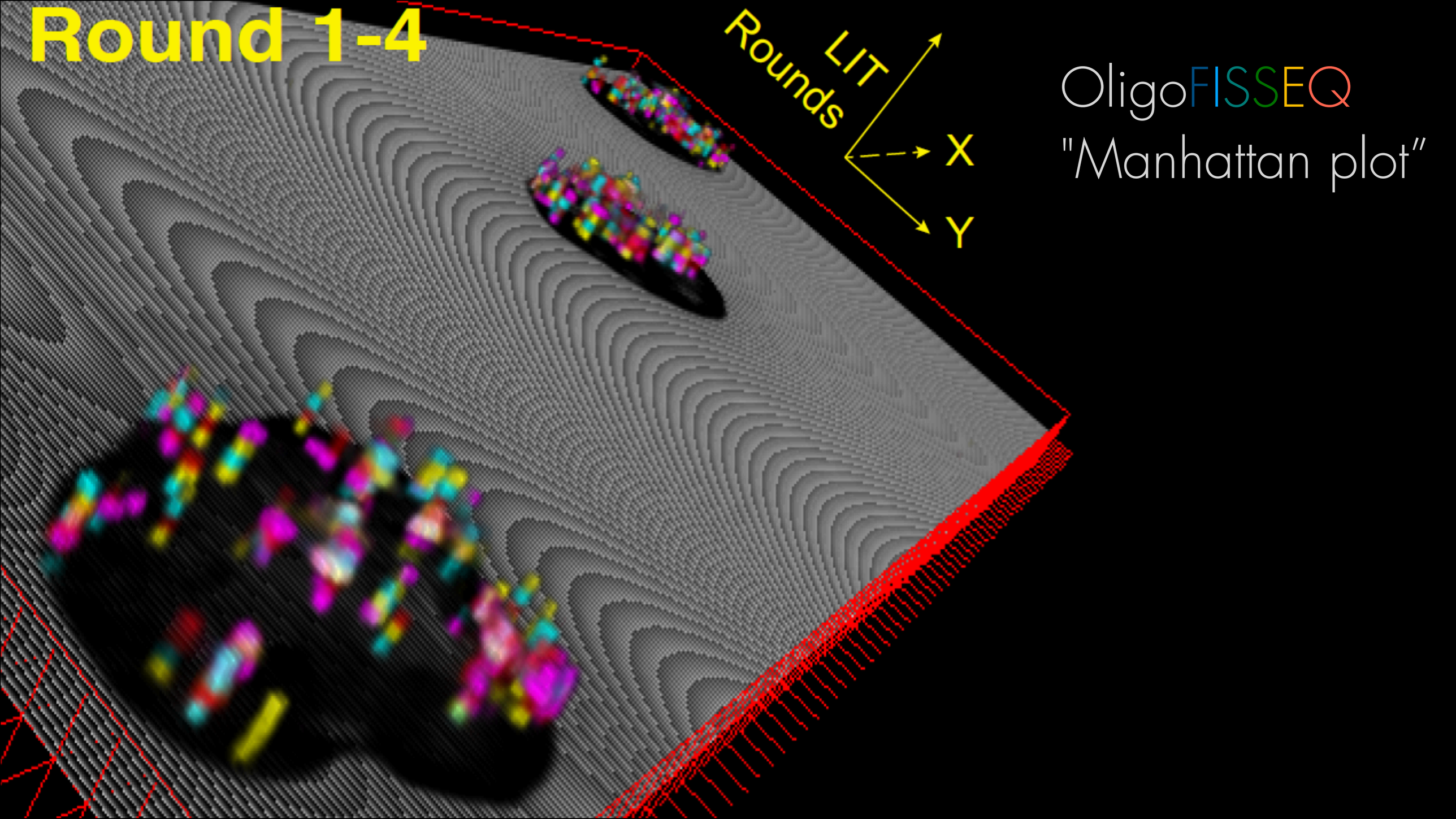


600kb-1Mb/target (876 kb average)
5,000 oligos/target
7-70Mb between targets

Detecting a given target



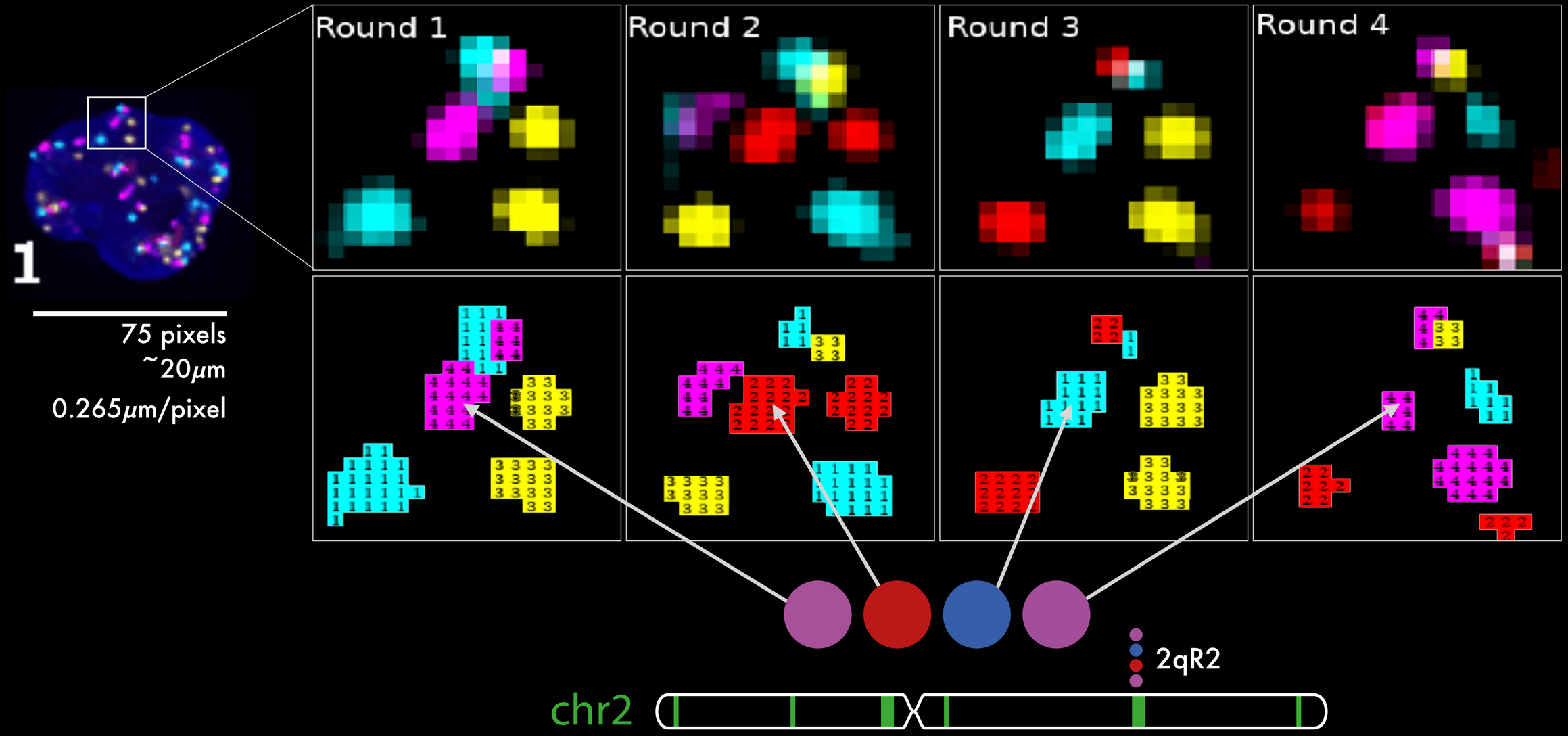




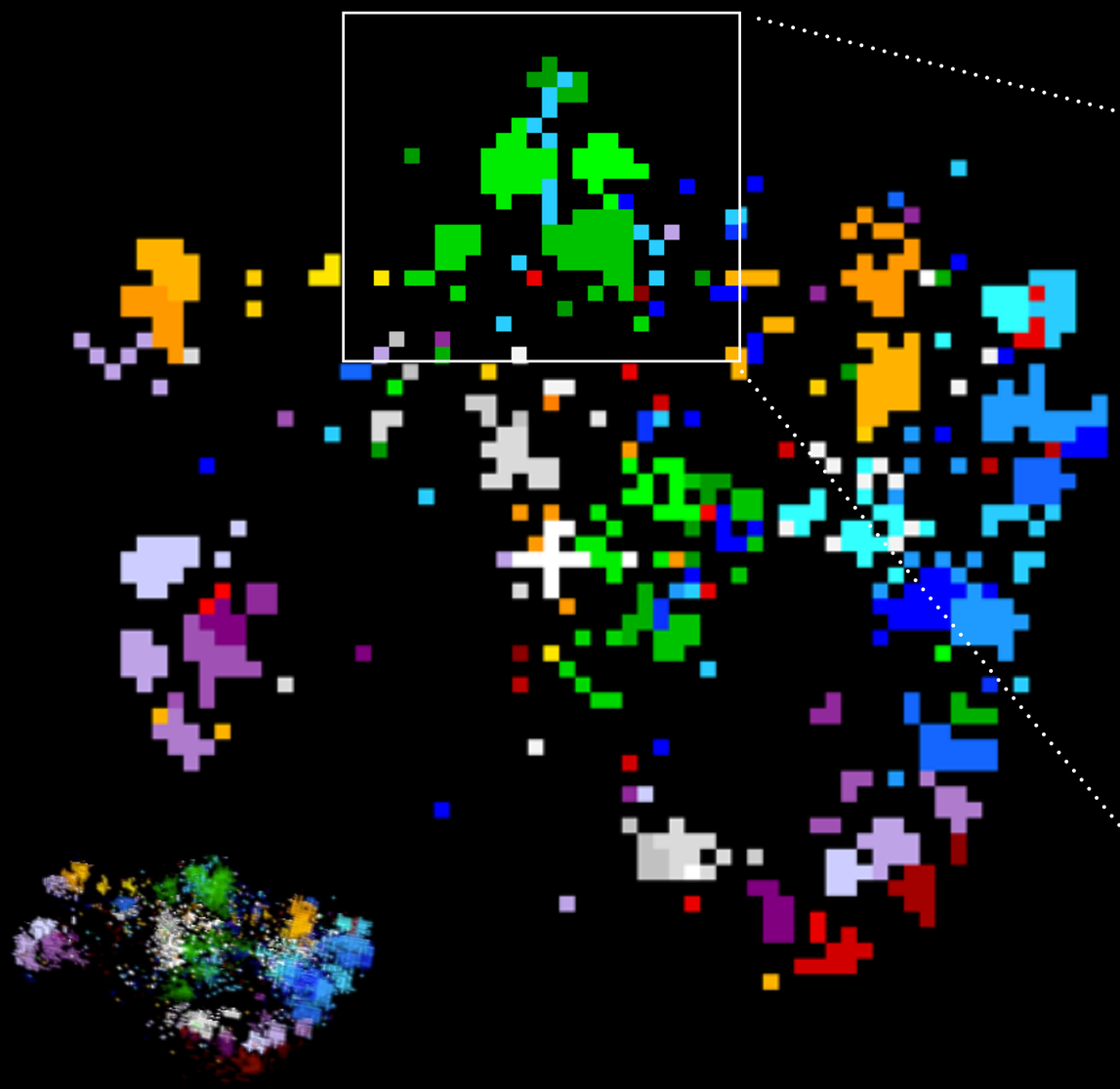
OligoFISSEQ
"Manhattan plot"

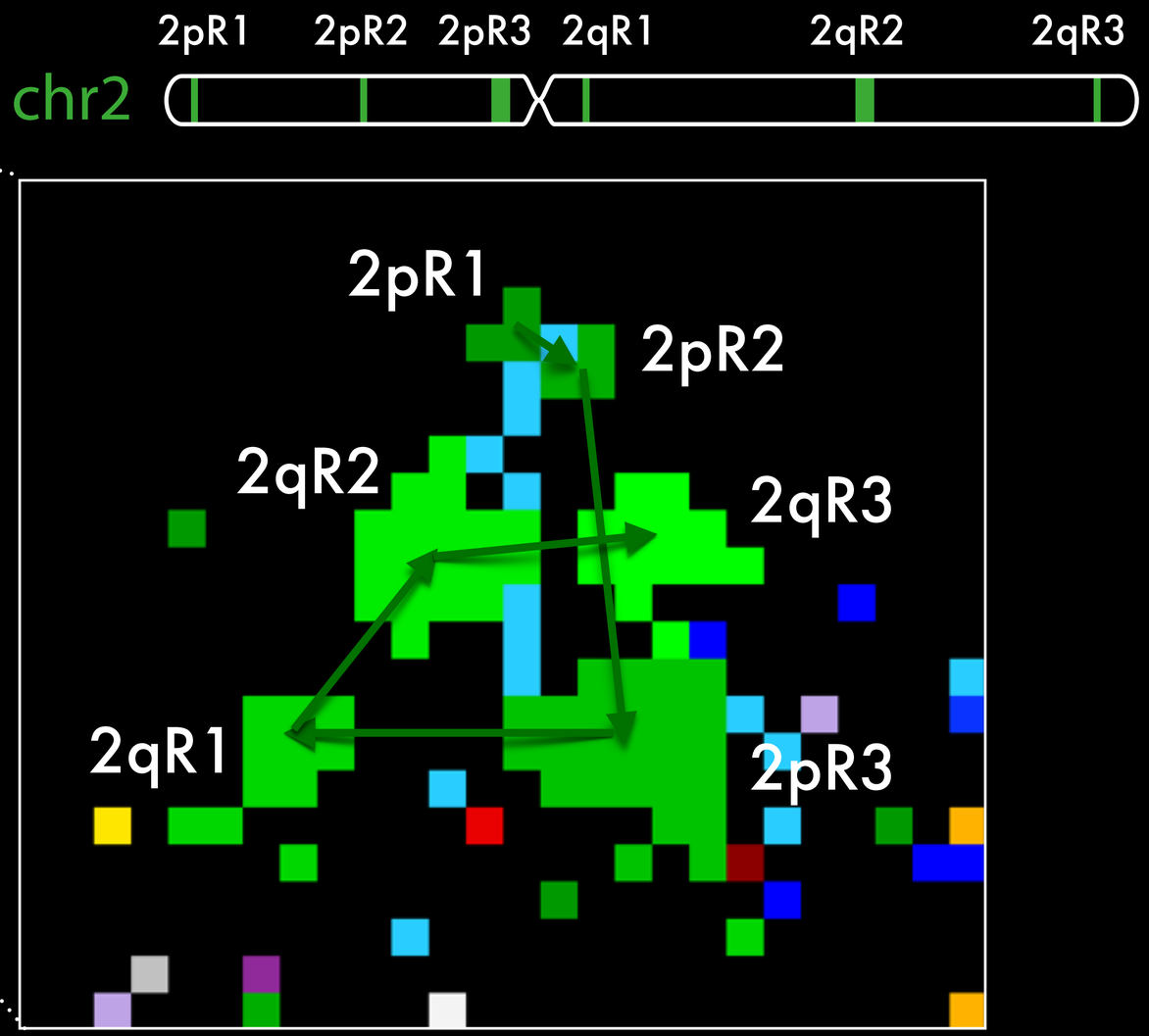
In OligoFISSEQ every pixel matters & make "patches"

4 rounds / 4 channels

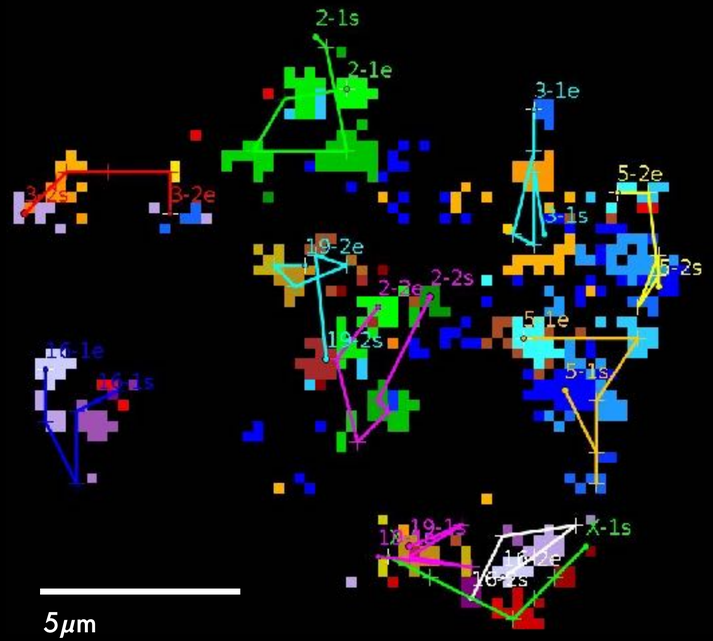


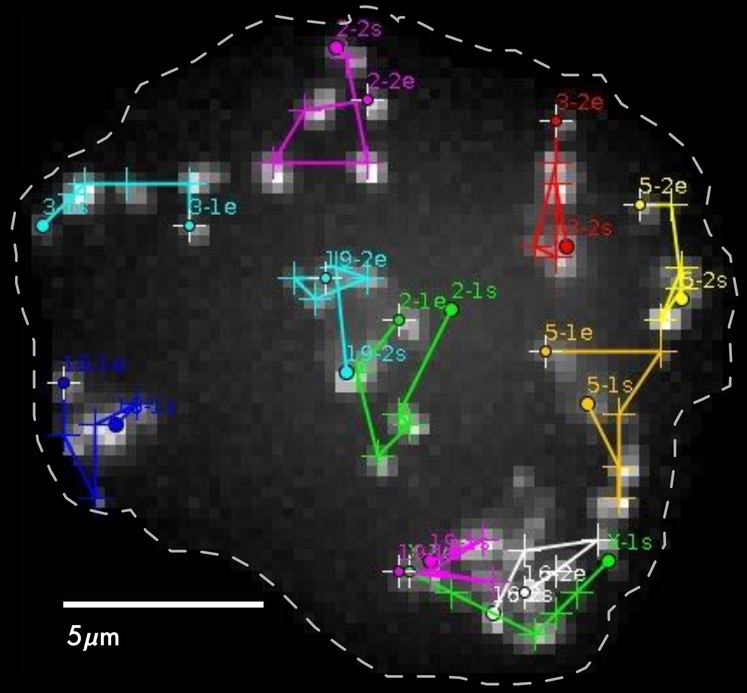
In OligoFISSEQ every pixel matters & make "patches"

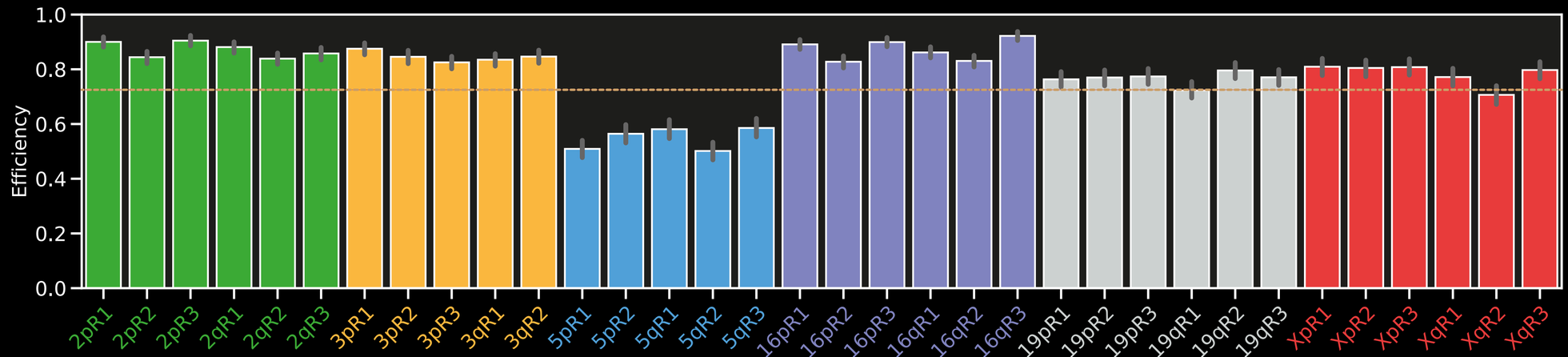




OligoFISSEQ barcode efficiency

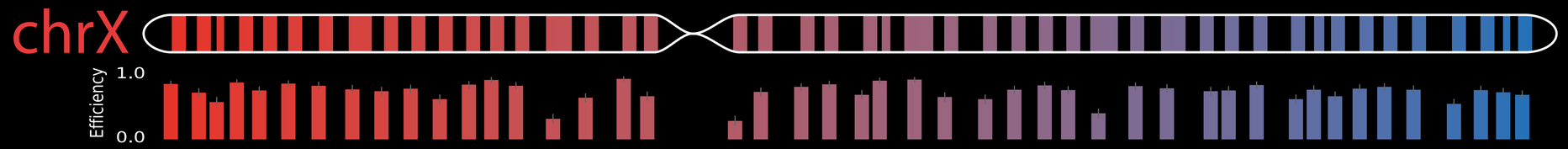


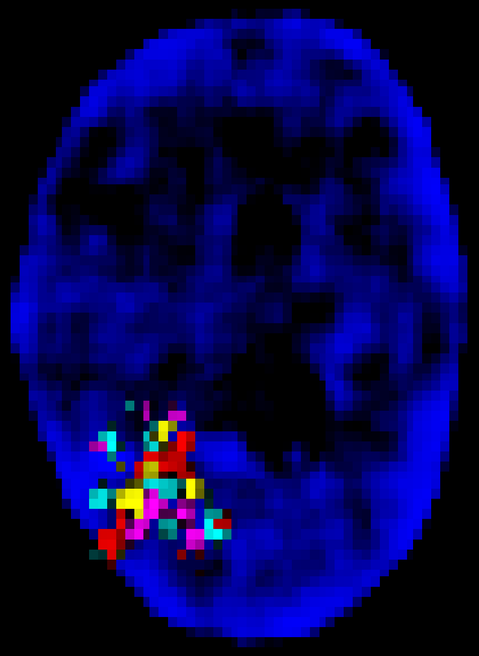


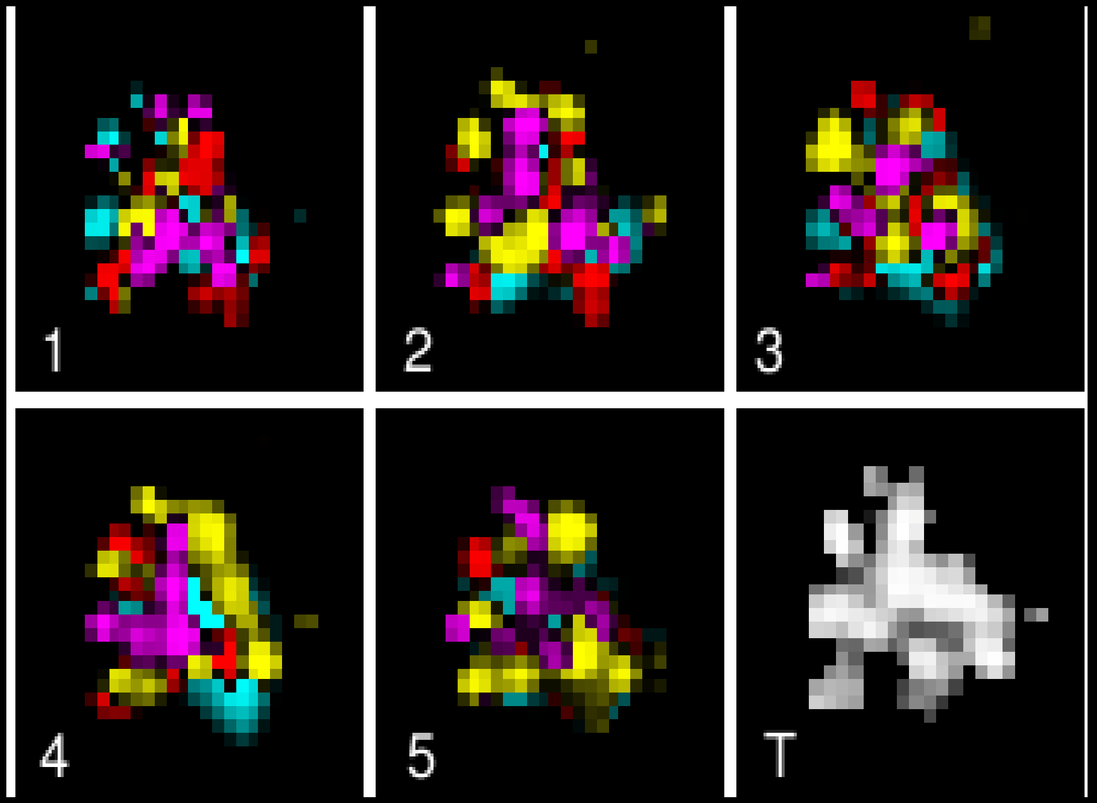


OligoFISSEQ tracing of (almost) entire chromosomes

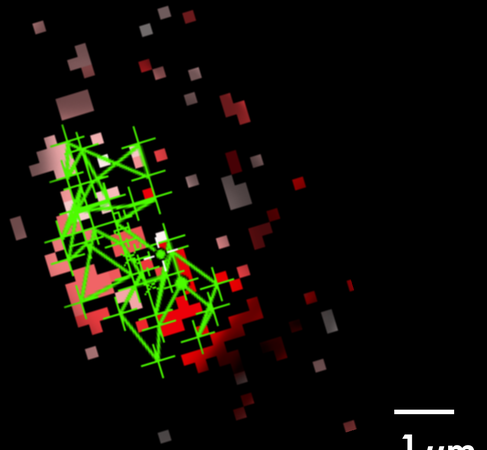
46 Plex in chromosome X



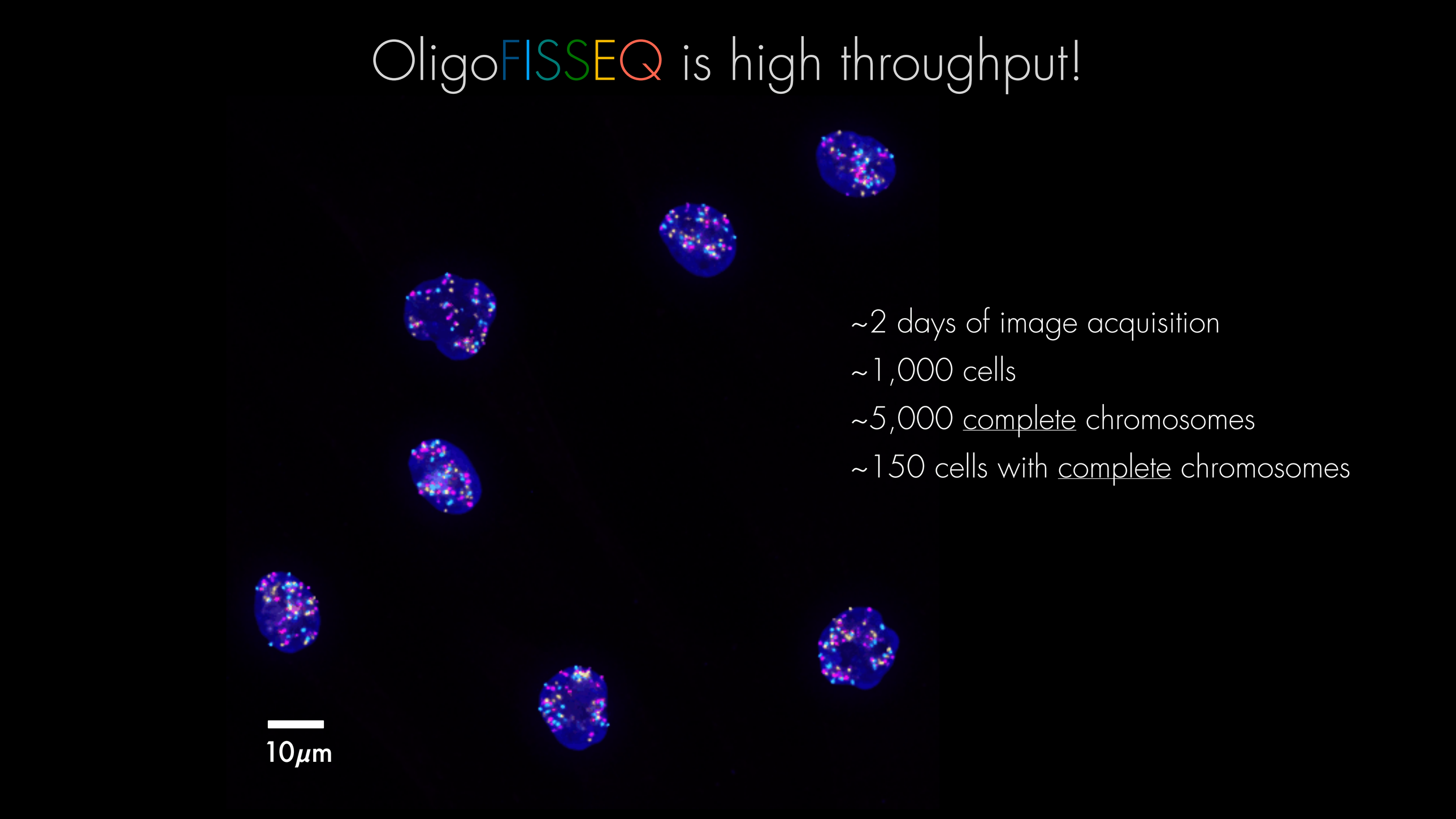




5 rounds
445 kb/probe
2,000 Oligopaints/probe
2 Mb between loci



OligoFISSEQ is high throughput!



~2 days of image acquisition

~1,000 cells

~5,000 complete chromosomes

~150 cells with complete chromosomes

OligoFISSEQ beyond chromosome tracing

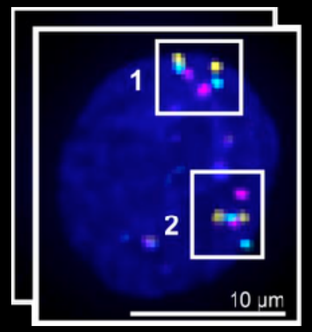
OligoFISSEQ pipelined with OligoSTORM

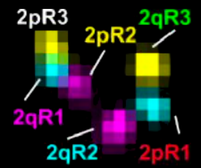
1 **OligoSTROM**
1 round
(2h/round)

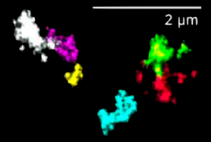
2 **OligoFISSEQ**
2 round
(3h/round)

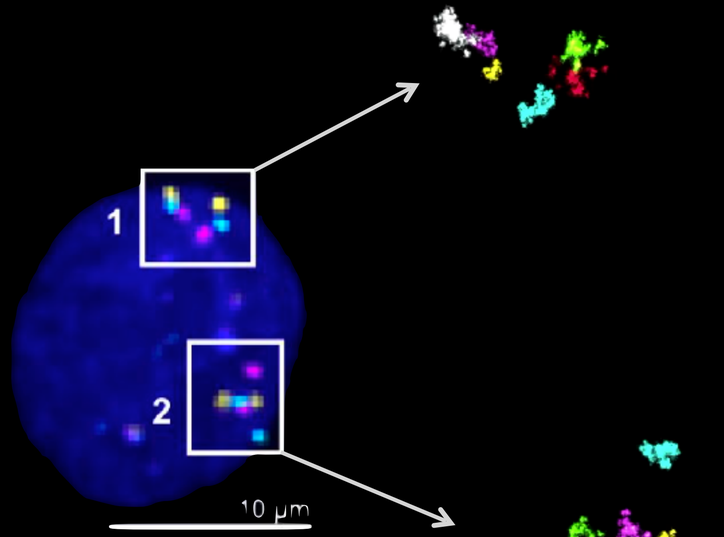
3 **Decoding**
OligoFISSEQ

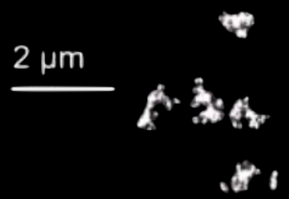
4 **Mapping**
OligoSTROM



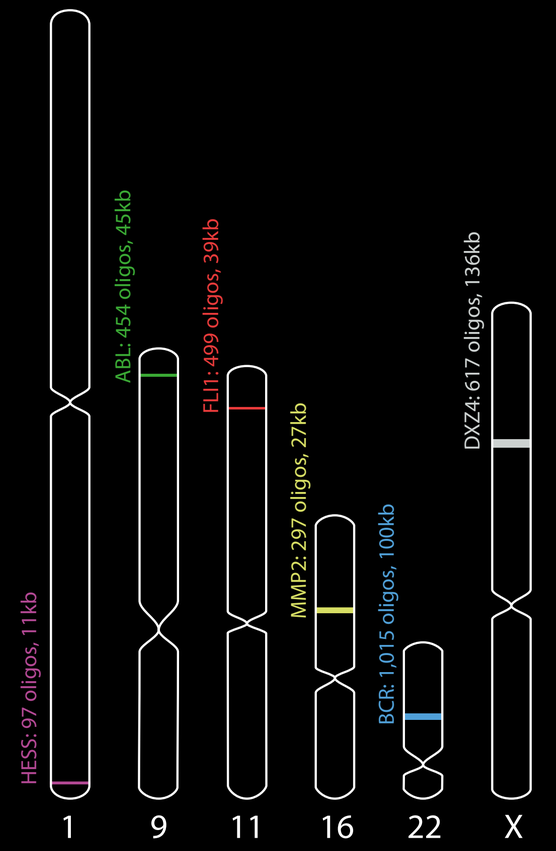


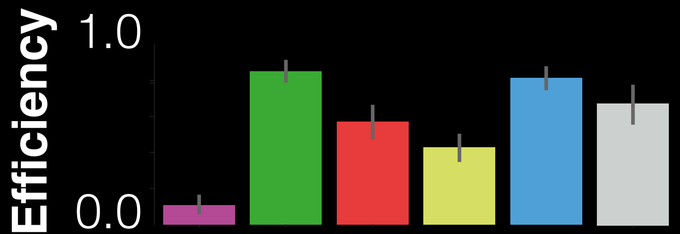


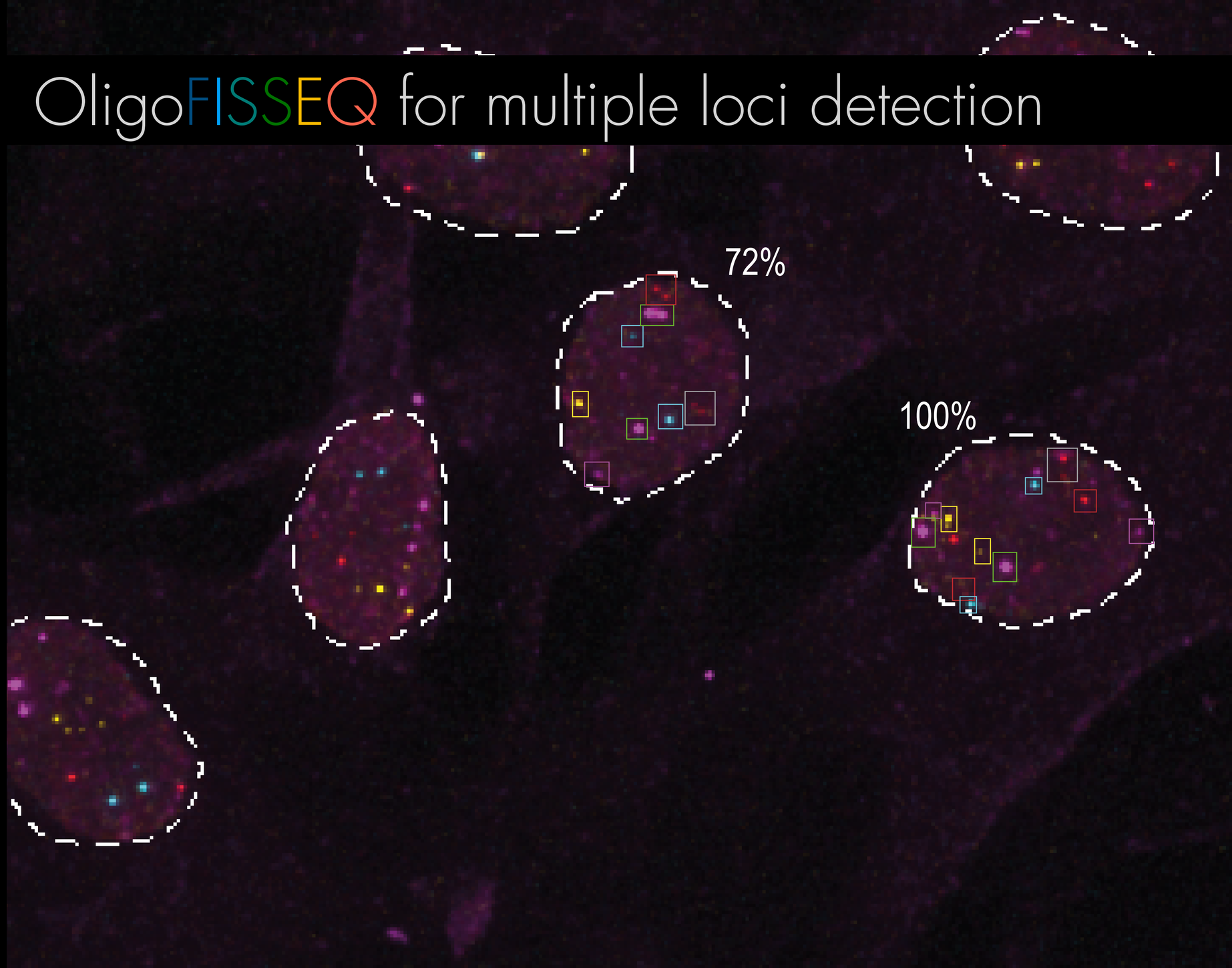




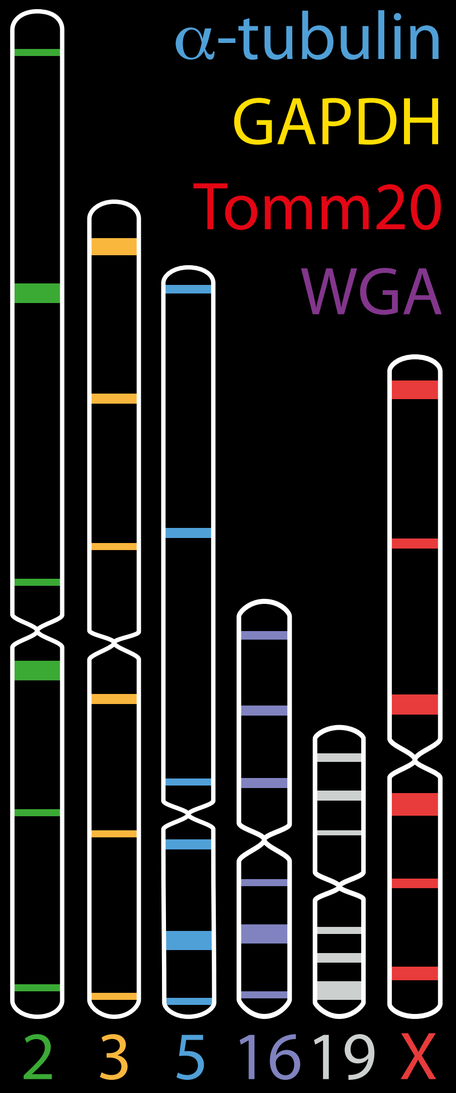
OligoFISSEQ for multiple loci detection



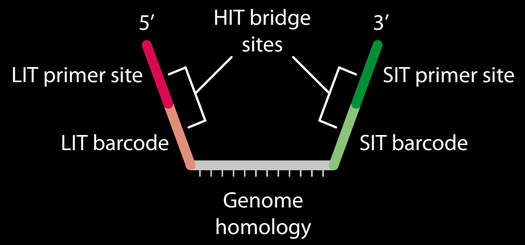




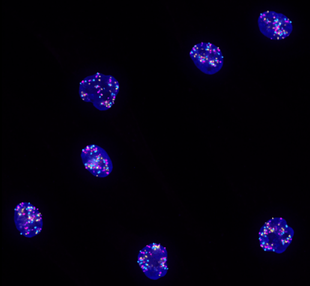
OligoFISSEQ + protein immunofluorescence



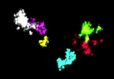
OligoFISSEQ



- 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…

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.: Conflict of Interest Statement :.
Since September 2021, Marc A. Marti-Renom serves as a consultant to Acuity Spatial Genomics, Inc., and receives compensation for these services.