



Structure determination of genomes and genomic domains by image tracing

**Marc A. Martí-Renom, Structural Genomics Team Leader
at Centro Nacional de Análisis Genómico (CNAG)**

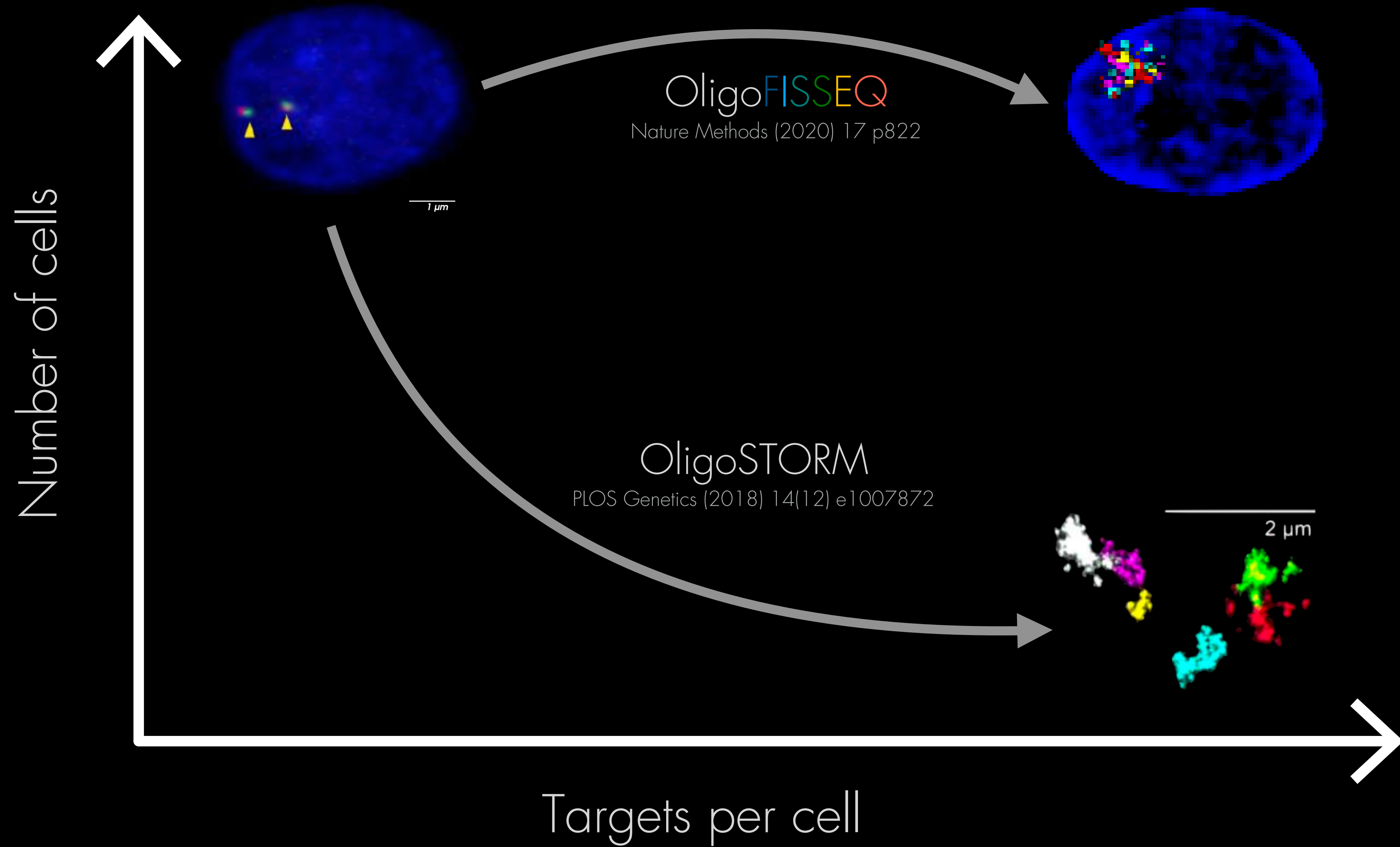




Photo by David Oliete - www.davidoliete.com

Chromosome walking with super-resolution imaging and modeling



Guy Nir

Irene Farabella

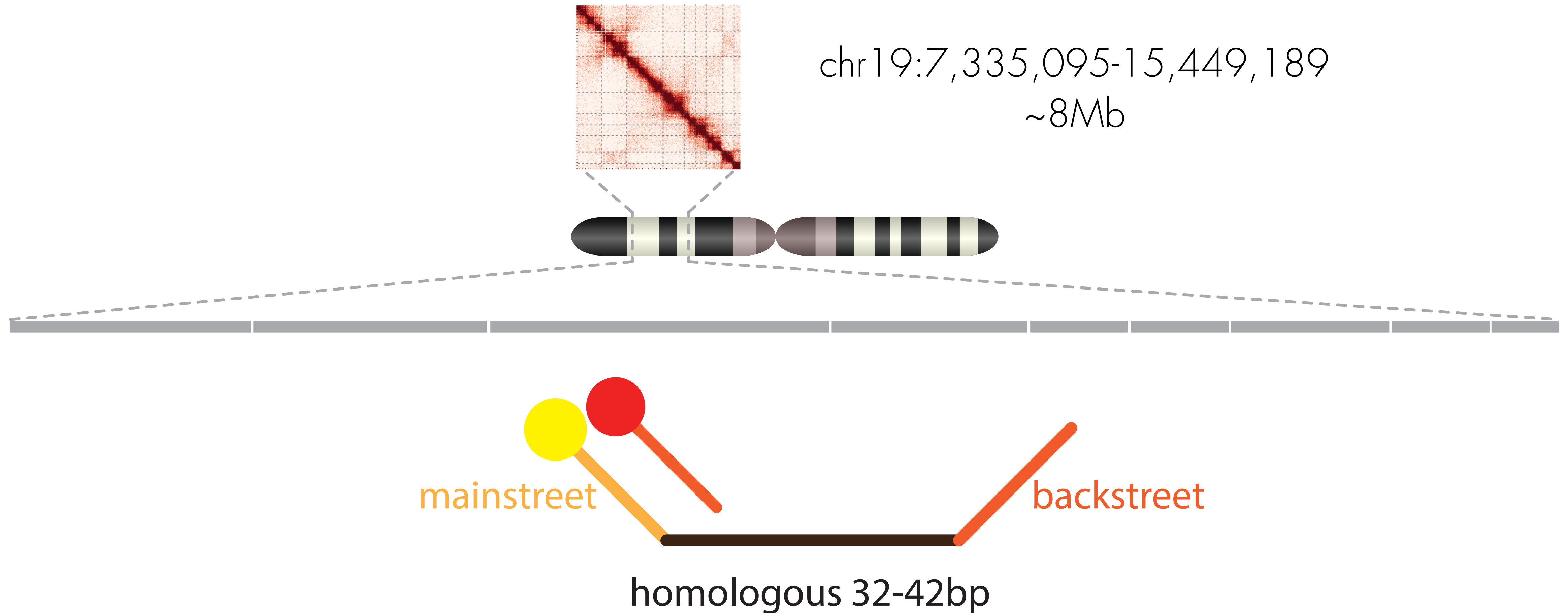
Cynthia Perez-Estrada

with Wu Lab (HMS, Boston) & Aiden Lab (UT, Texas)

PLOS Genetics (2018) 14(12) e1007872

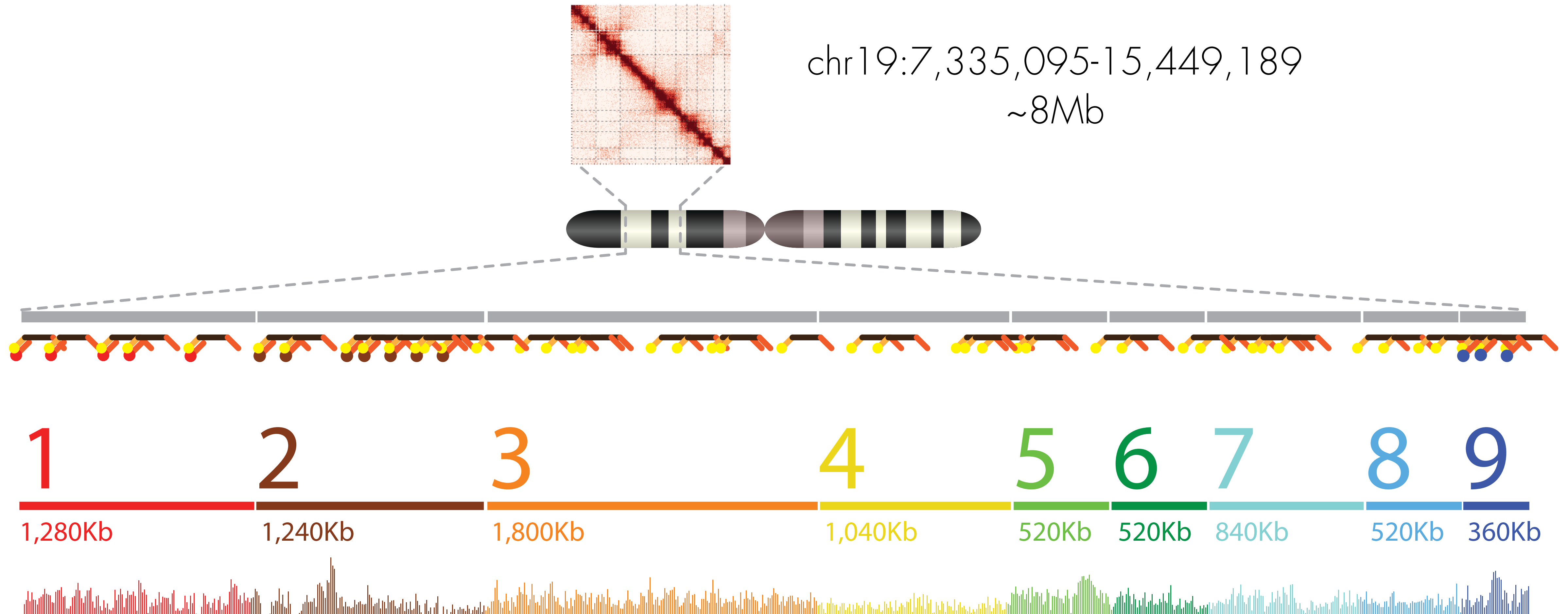
High-resolution imaging

Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



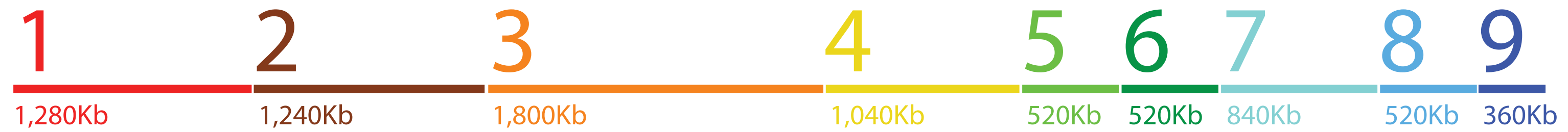
High-resolution imaging

Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



High-resolution imaging

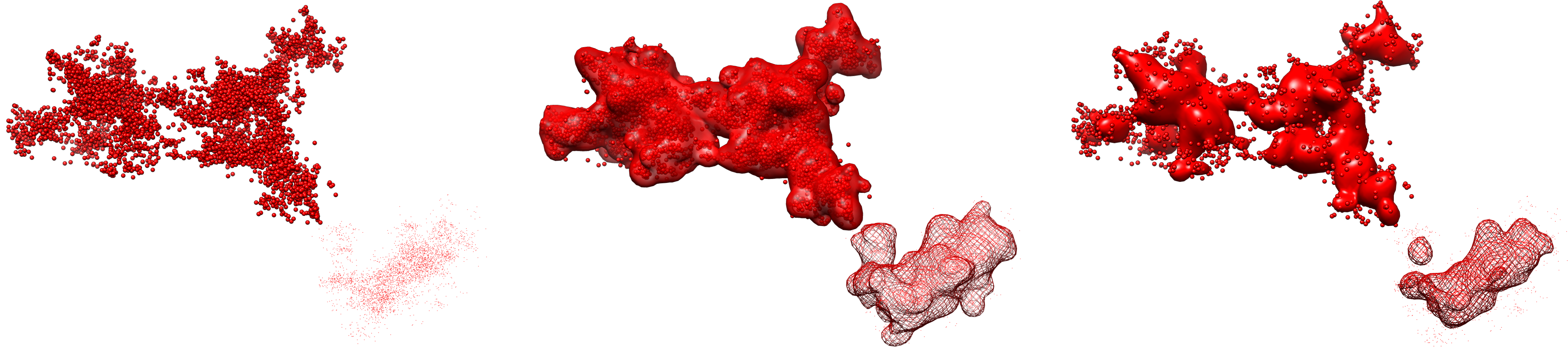
Tracing chr19:7,335,095-15,449,189 ~8Mb



High-resolution imaging

XYZ points convolution into a density map

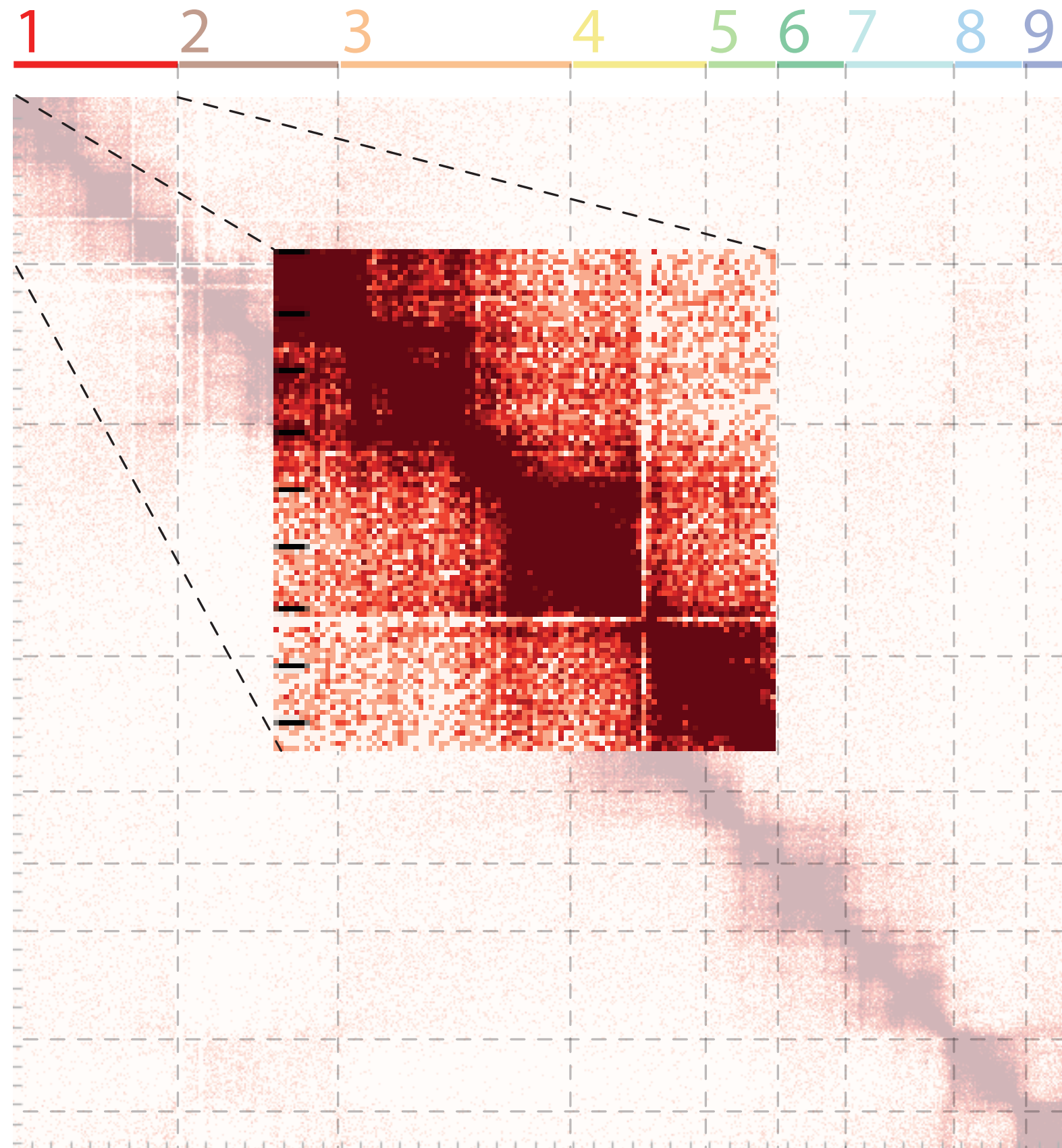
$$\rho(x, y, z) = \sum_N \frac{Z_N}{(\sigma\sqrt{2\pi})^3} e^{-\frac{(x-x_n)^2 + (y-y_n)^2 + (z-z_n)^2}{2\sigma^2}}$$



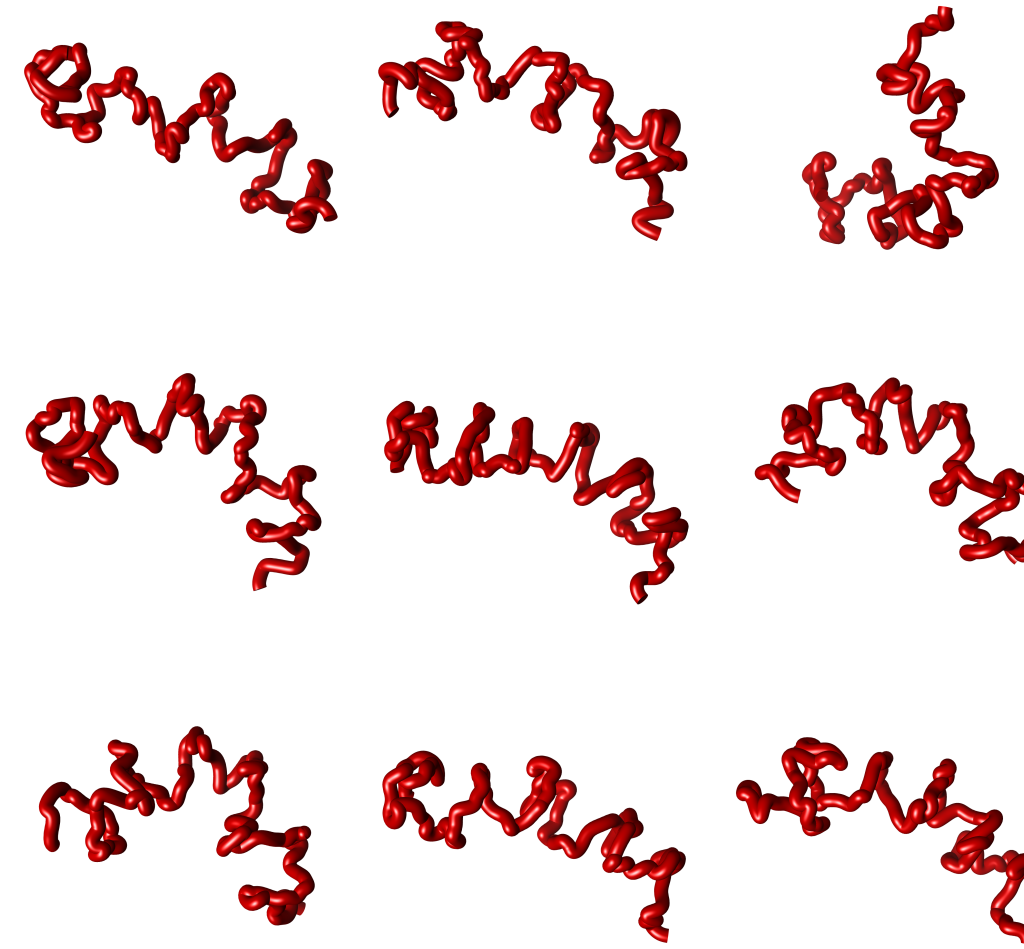
Cell-02 · Segment 1

Increasing resolution

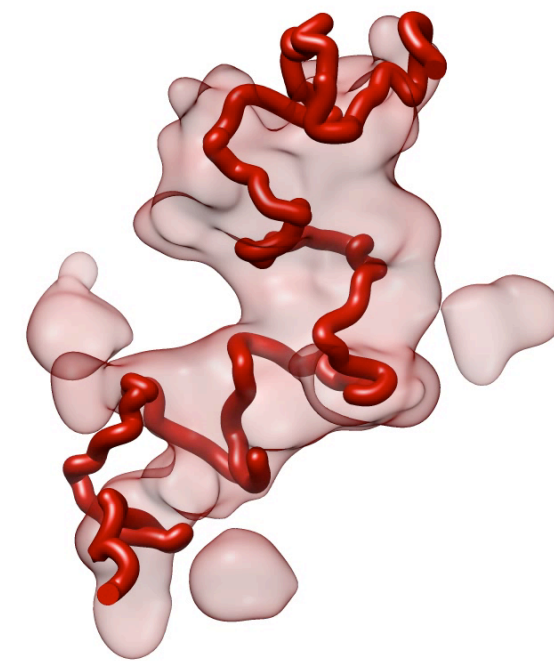
Rigid body fitting 3D structures based on Hi-C data



Segment 3 3D models

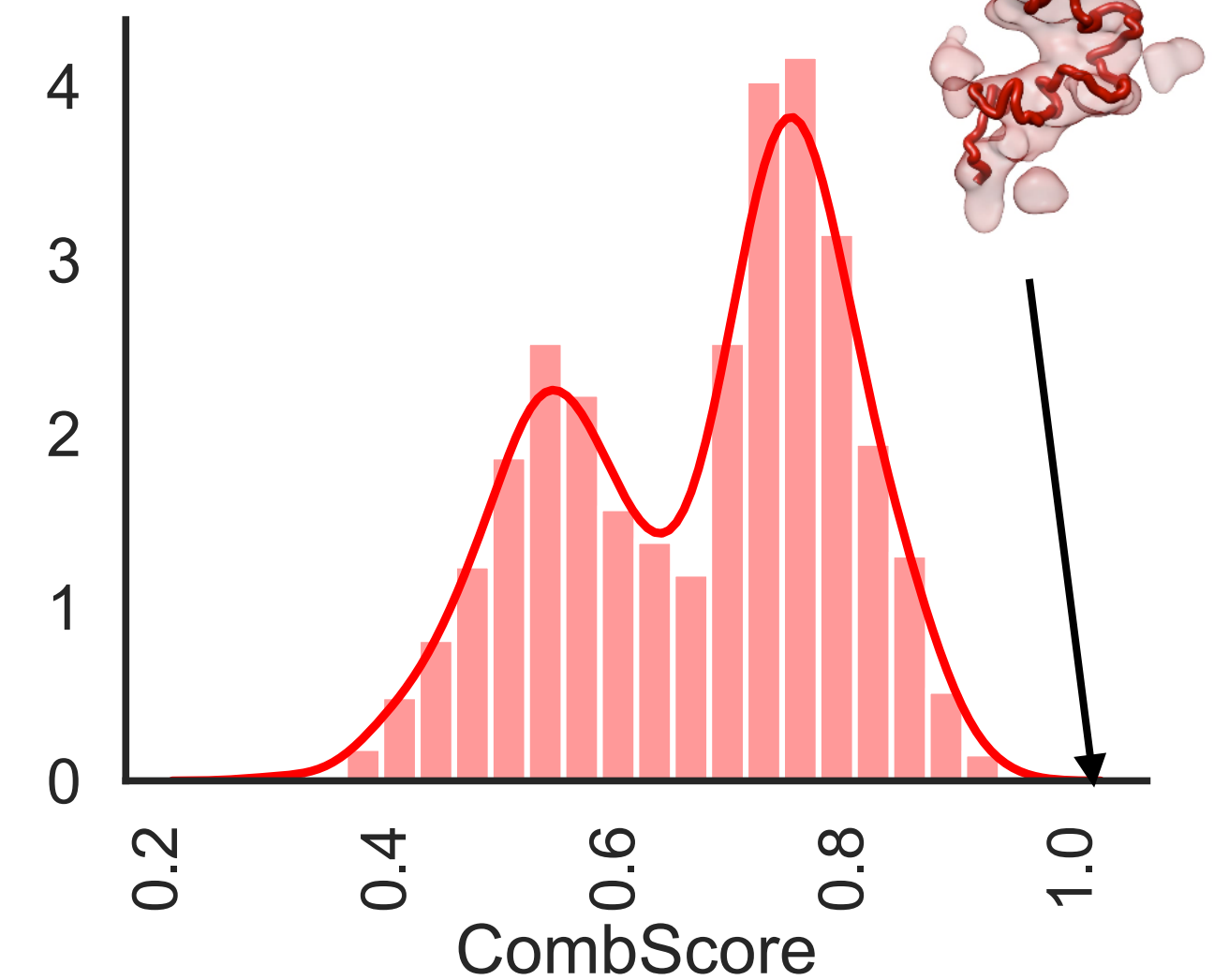


Segment 3 density map



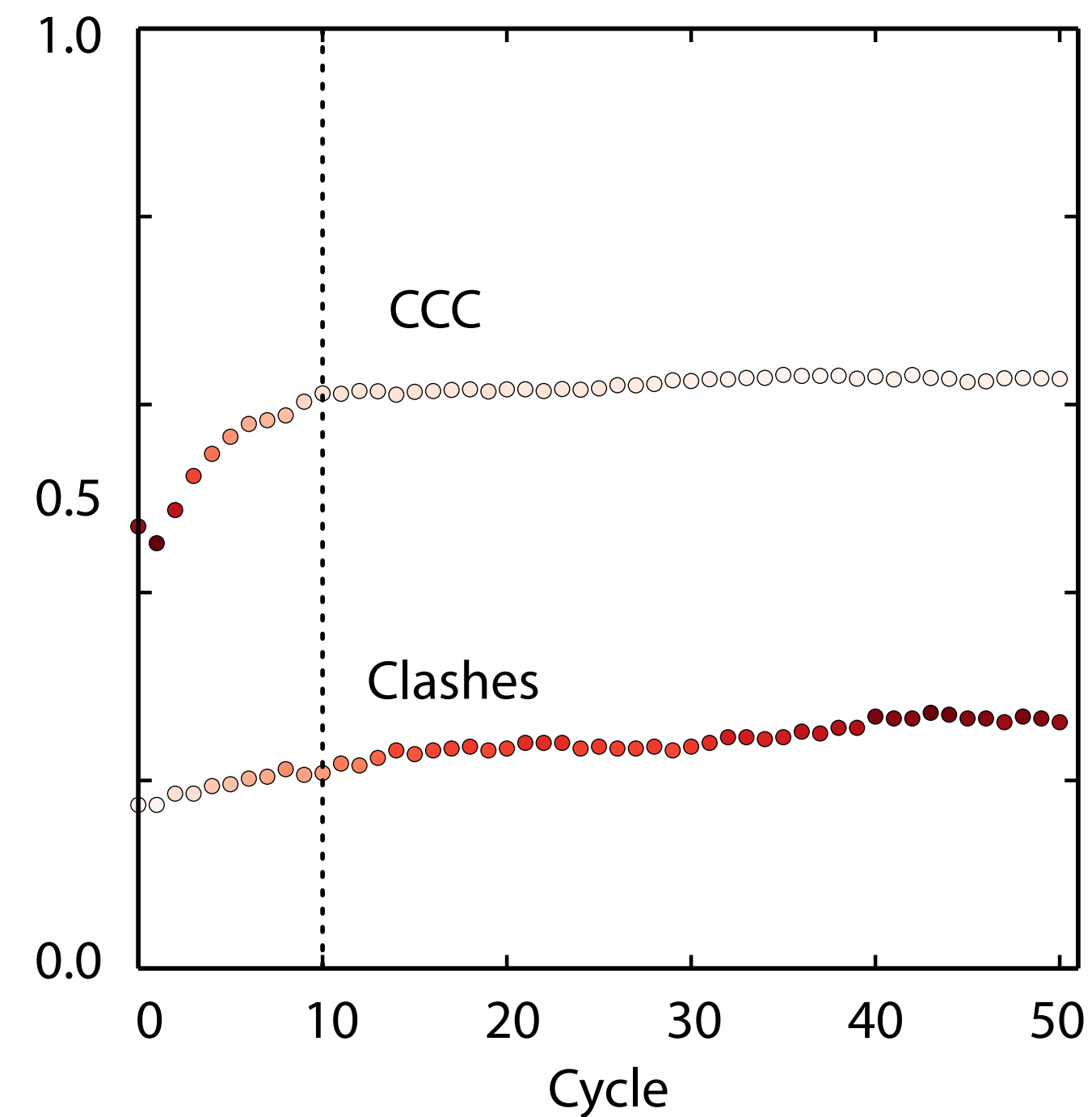
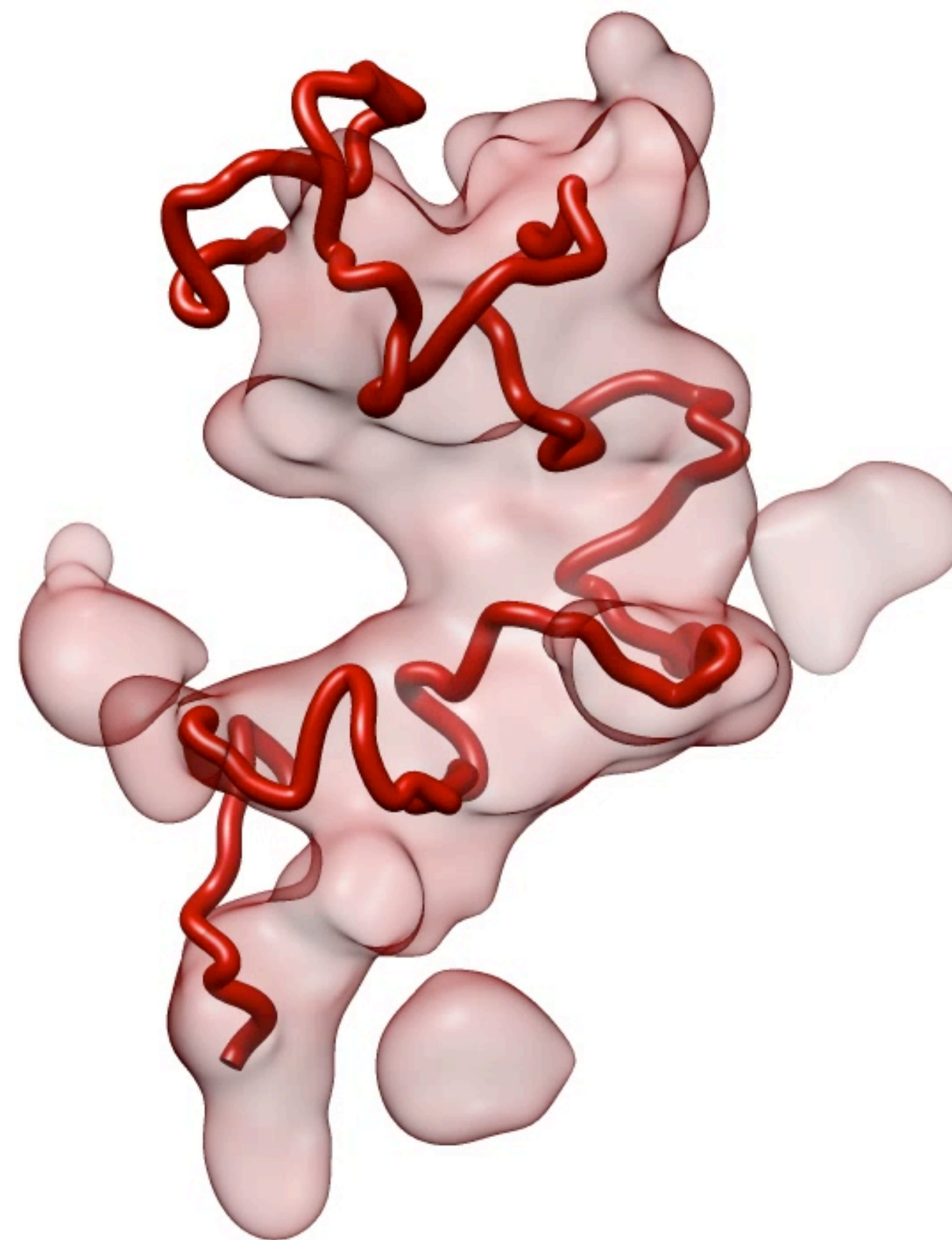
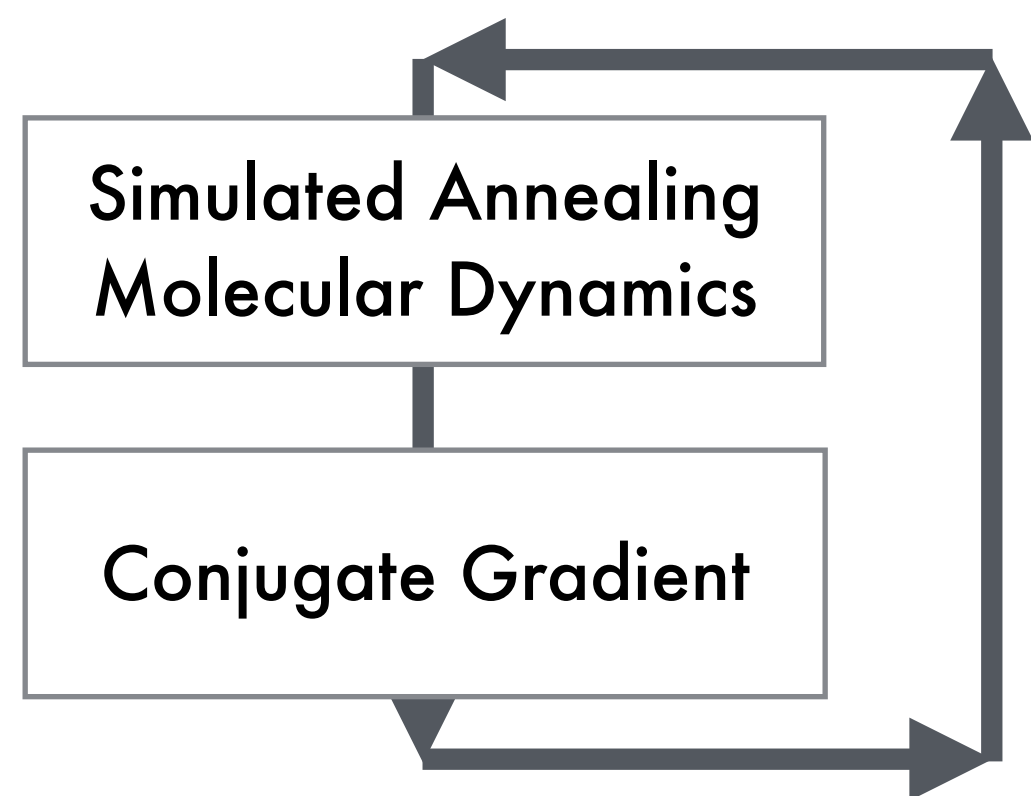
$$\text{ConS} = 1 - \frac{d_{P,\text{COM}}}{\max(d_{P,\text{COM}})}$$

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

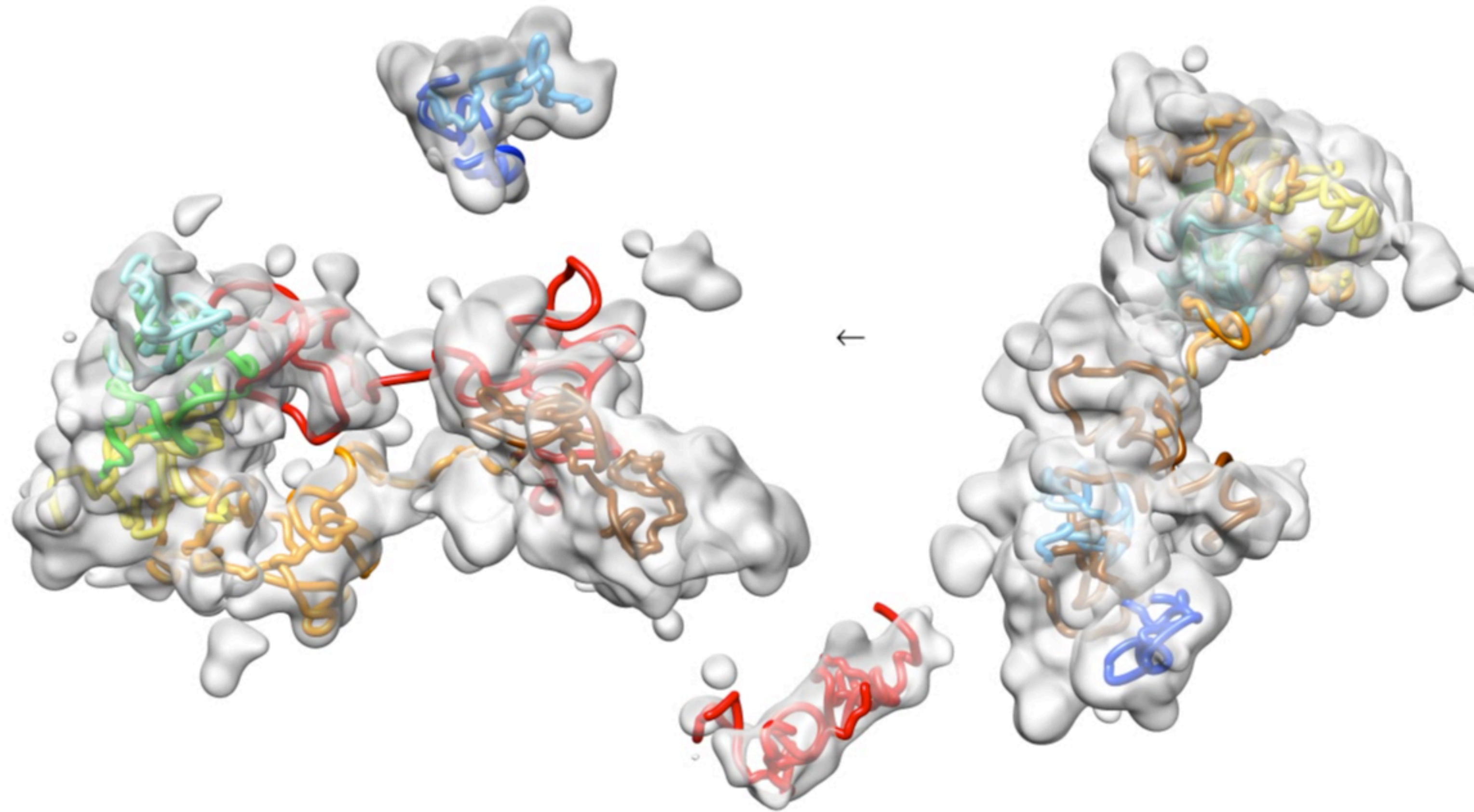


Increasing resolution

Flexible fitting 3D structures based on Hi-C data

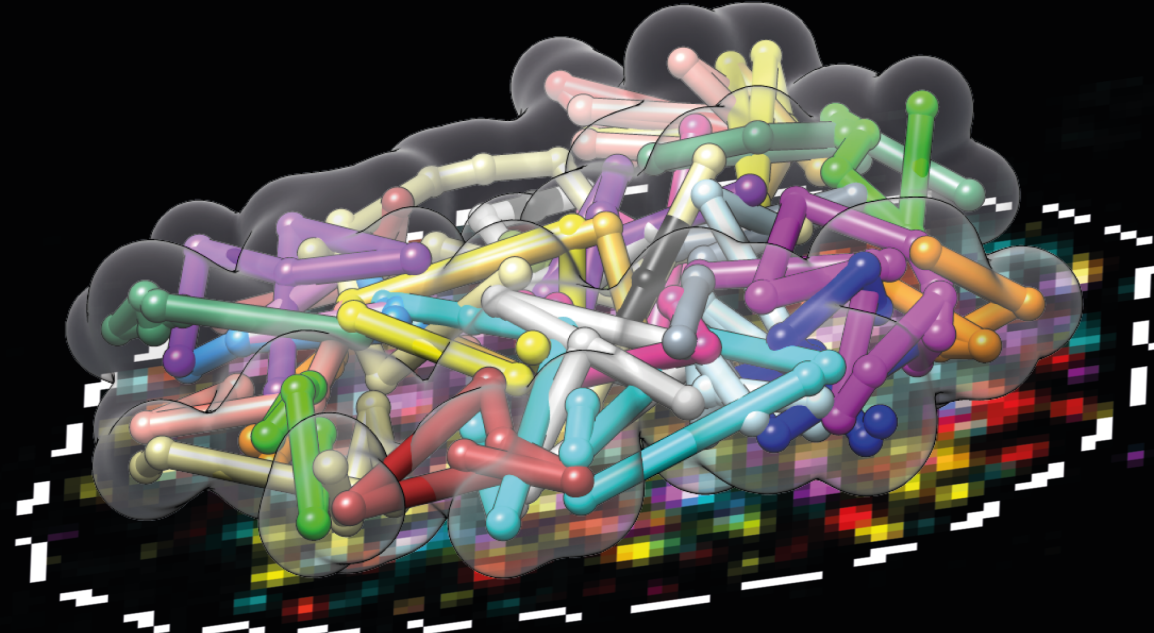


Chromosome walking path @10Kb resolution





Chromosome tracing with OligoFISSEQ

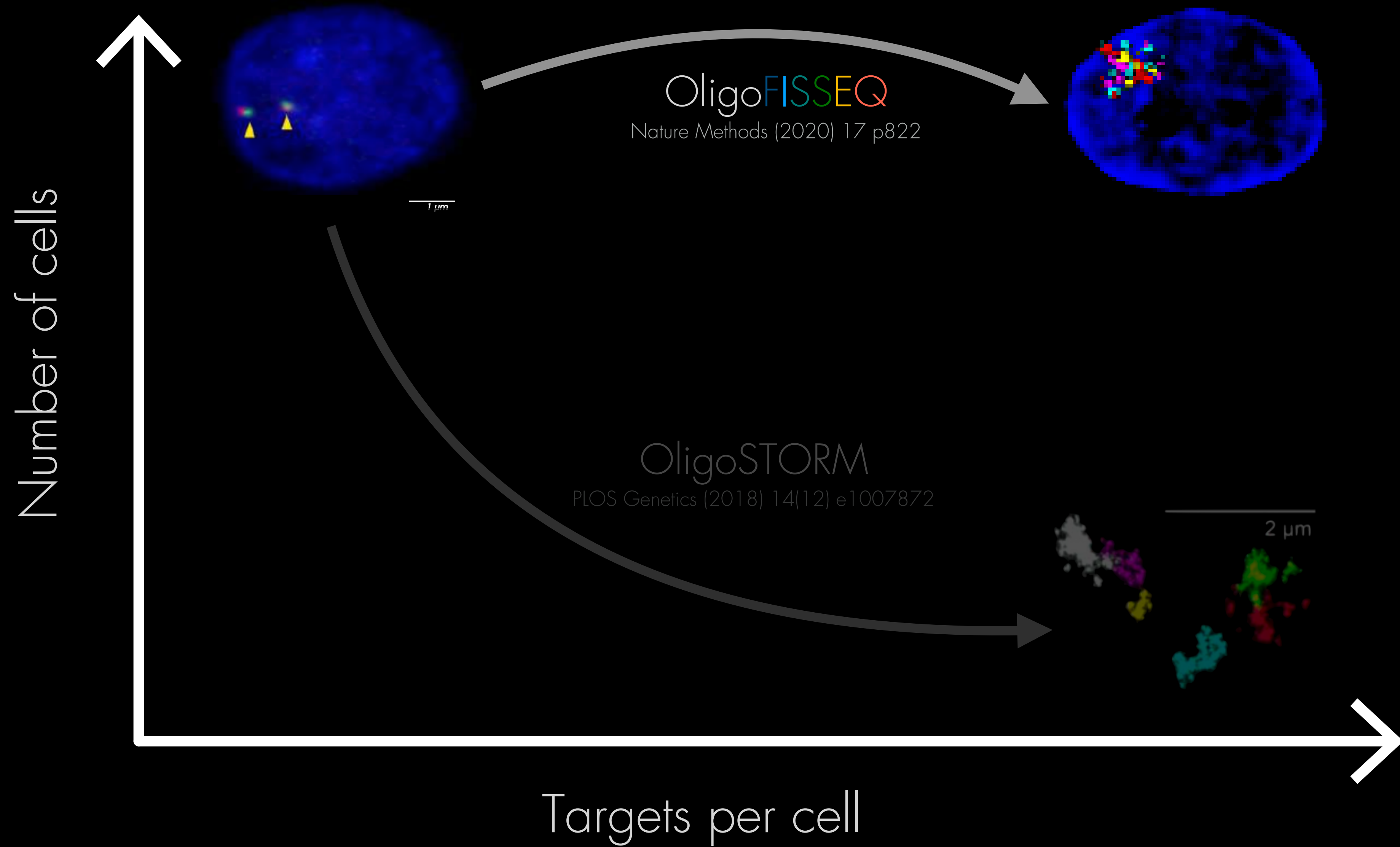


Marc A. Marti-Renom
CNAG-CRG · ICREA

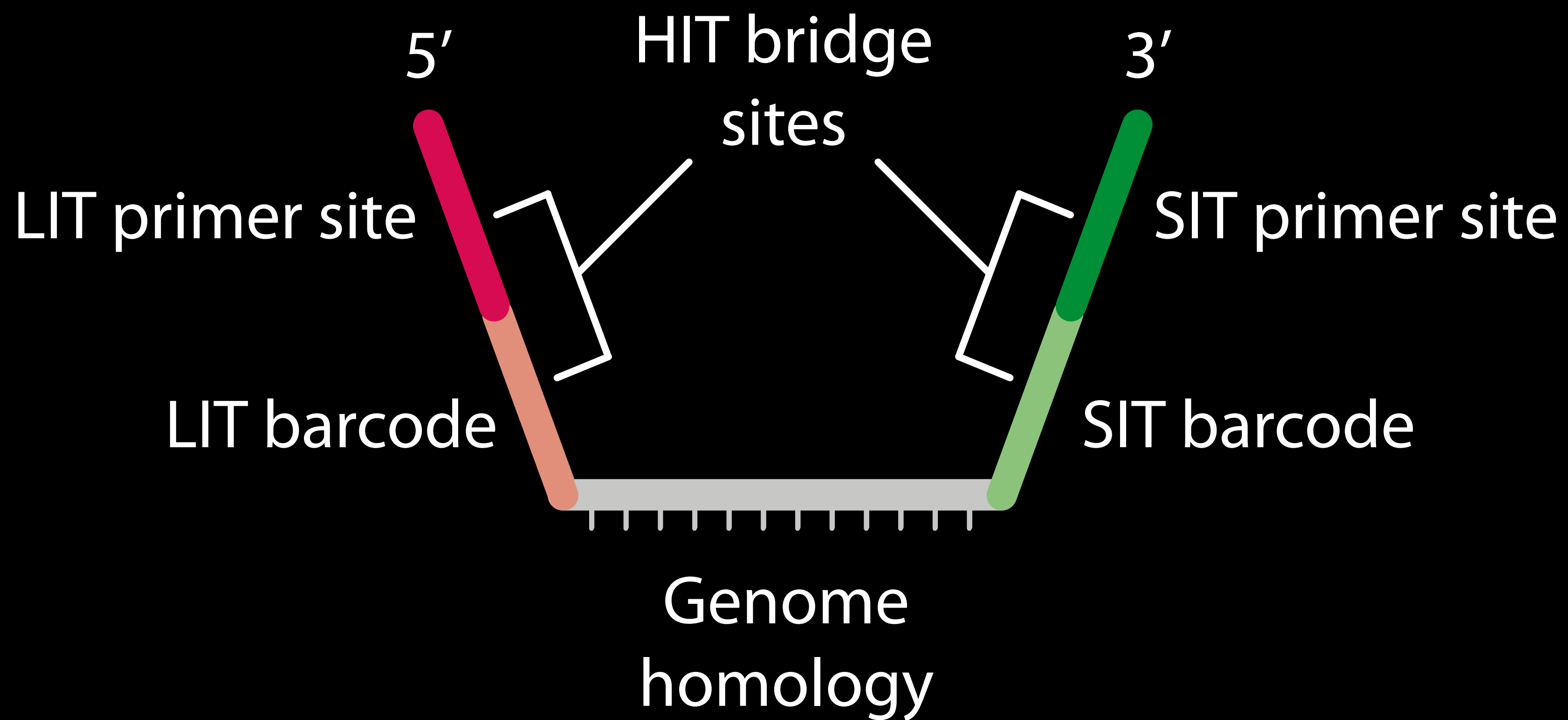
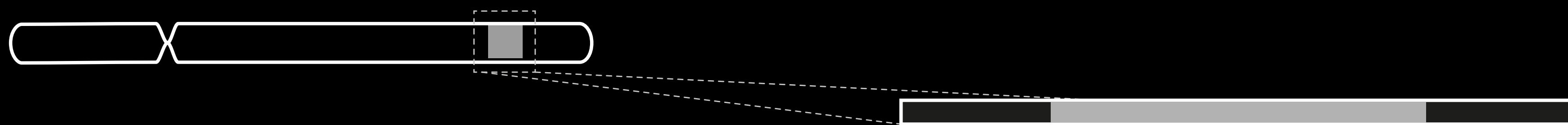


Huy Nguyen
Shyantanu Chattoraj
David Castillo

in collaboration with the Wu Lab (HMS)
Nature Methods (2020) 17 p822

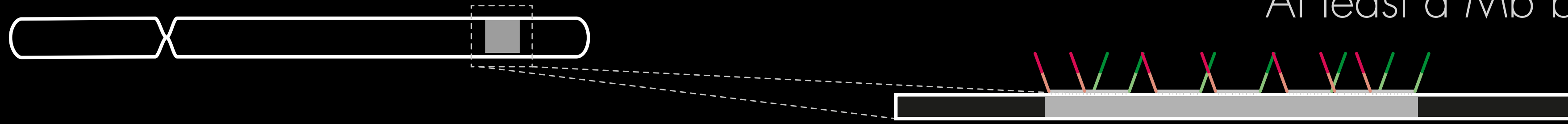


OligoFISSEQ

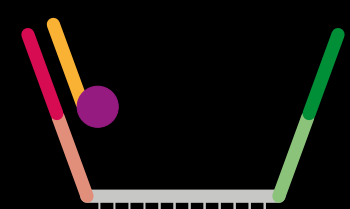
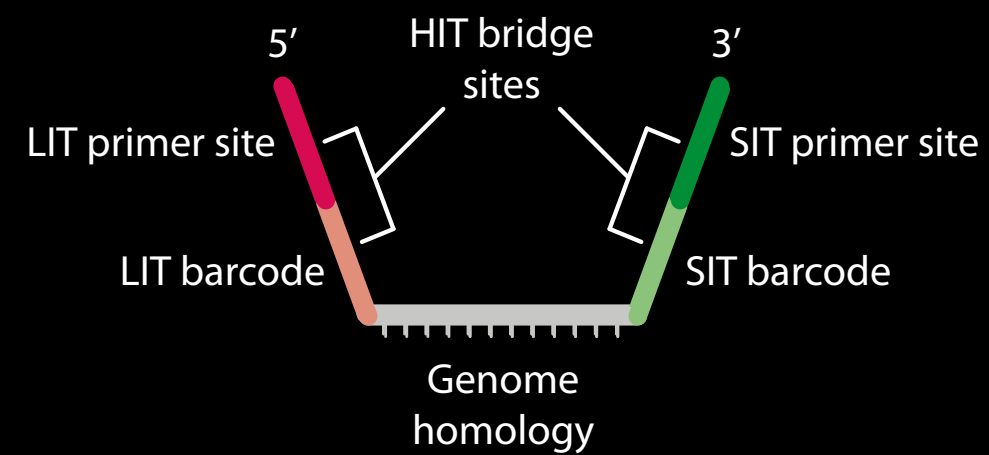


OligoFISSEQ

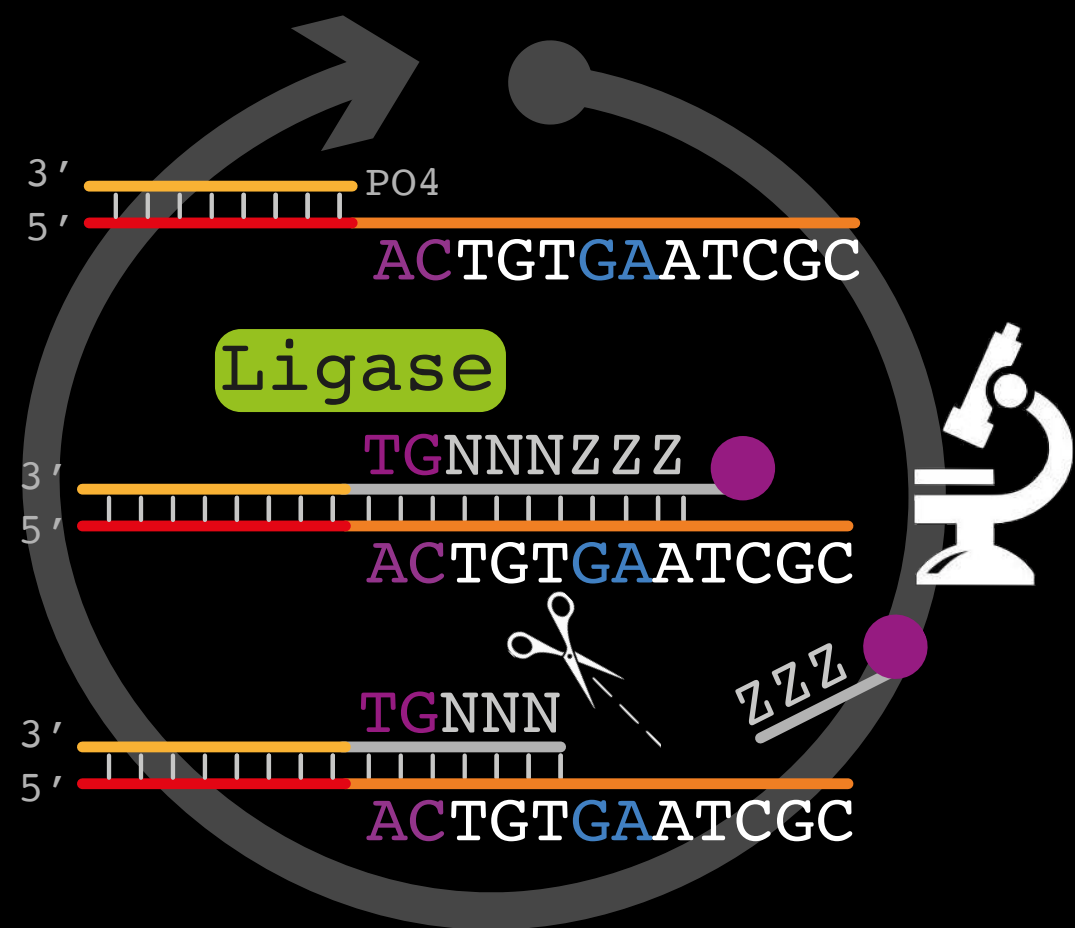
From tens of kb to Mb
Min. of few 100s oligos/target
At least a Mb between targets



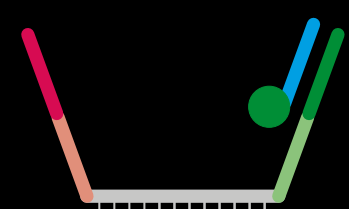
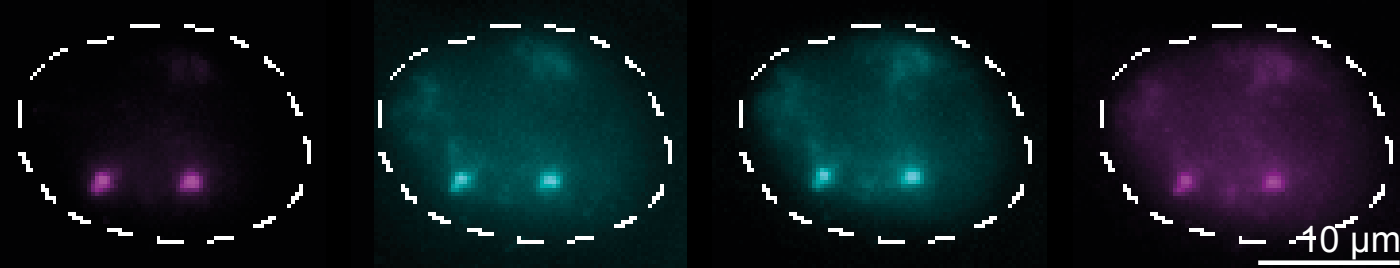
OligoFISSEQ



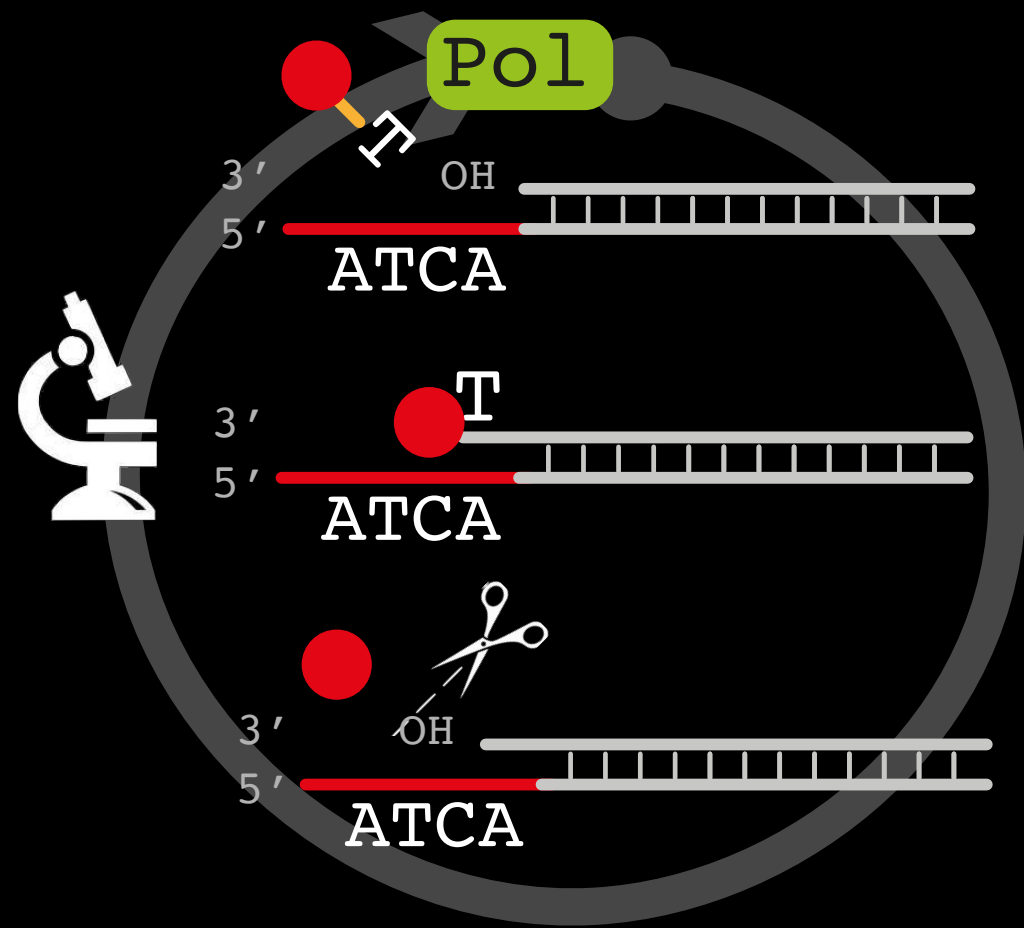
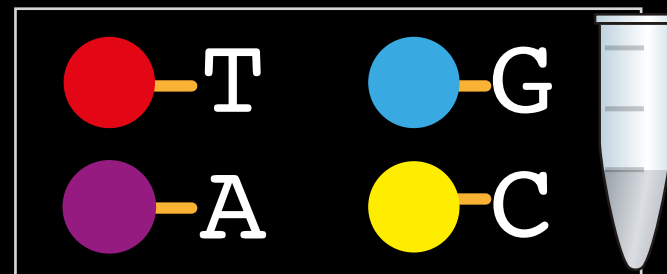
Ligation based Identification of Targets



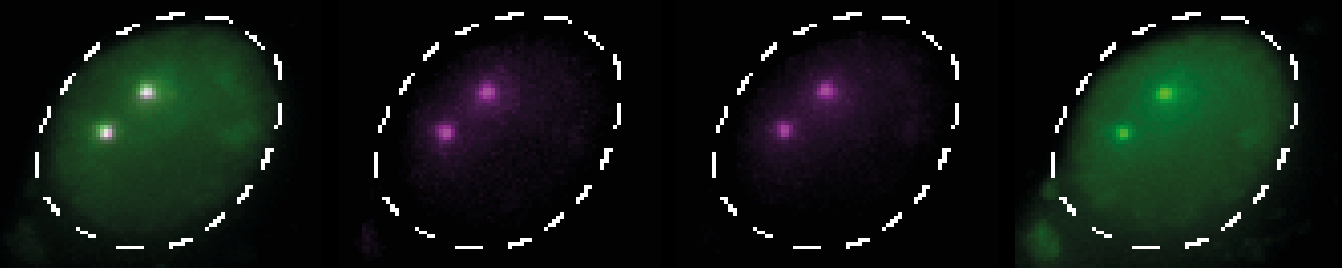
92.1 ± 5.7%



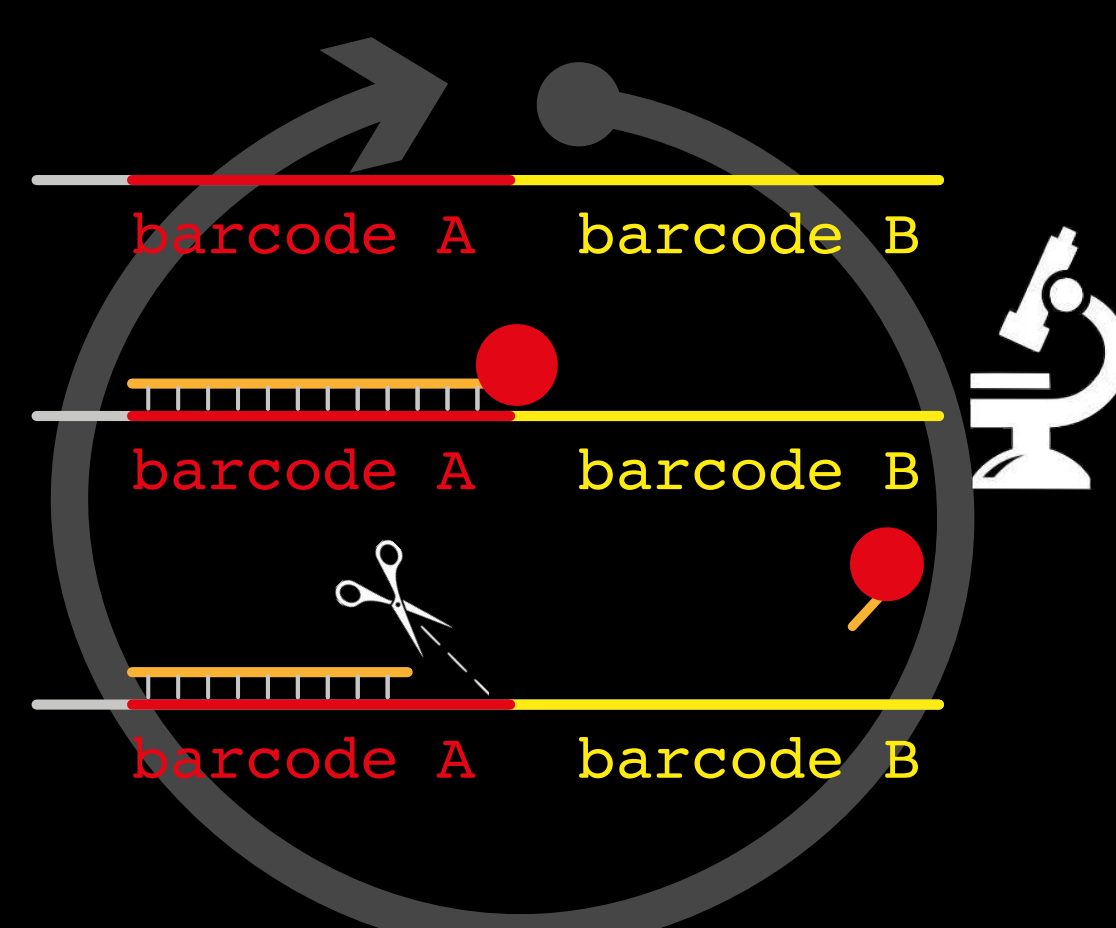
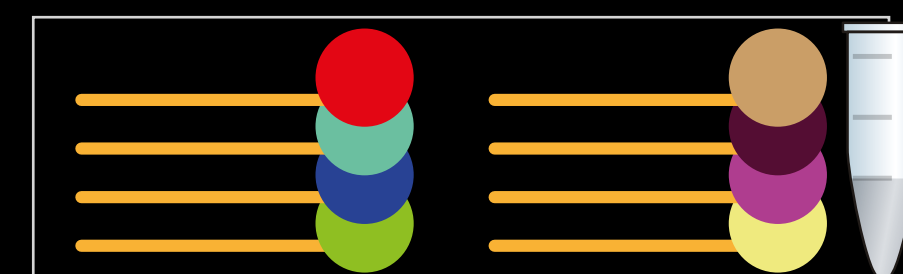
Synthesis based Identification of Targets



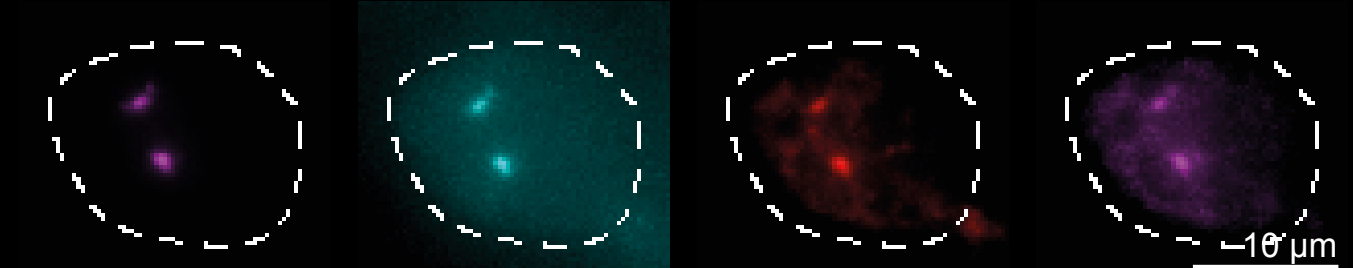
90.8 ± 5.6%



Hybridization based Identification of Targets

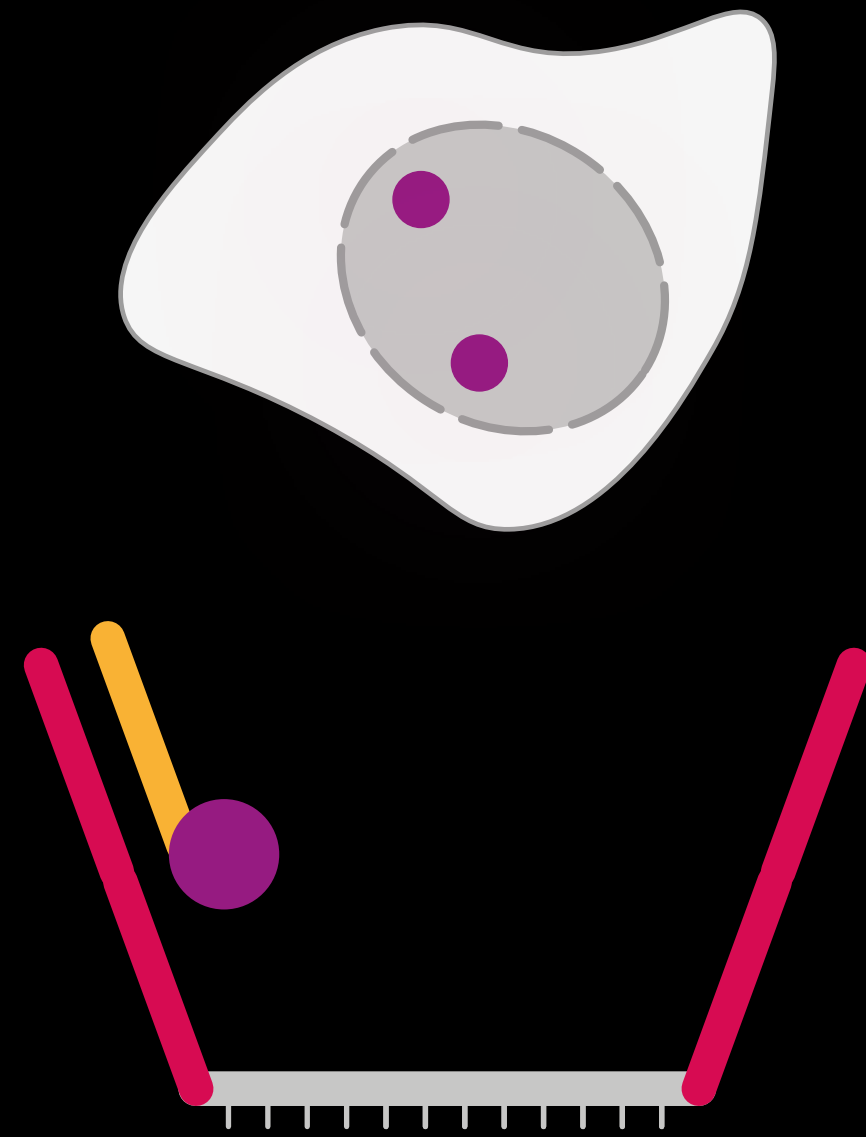


91.6 ± 3.8%



OligoFISSEQ scales exponentially!

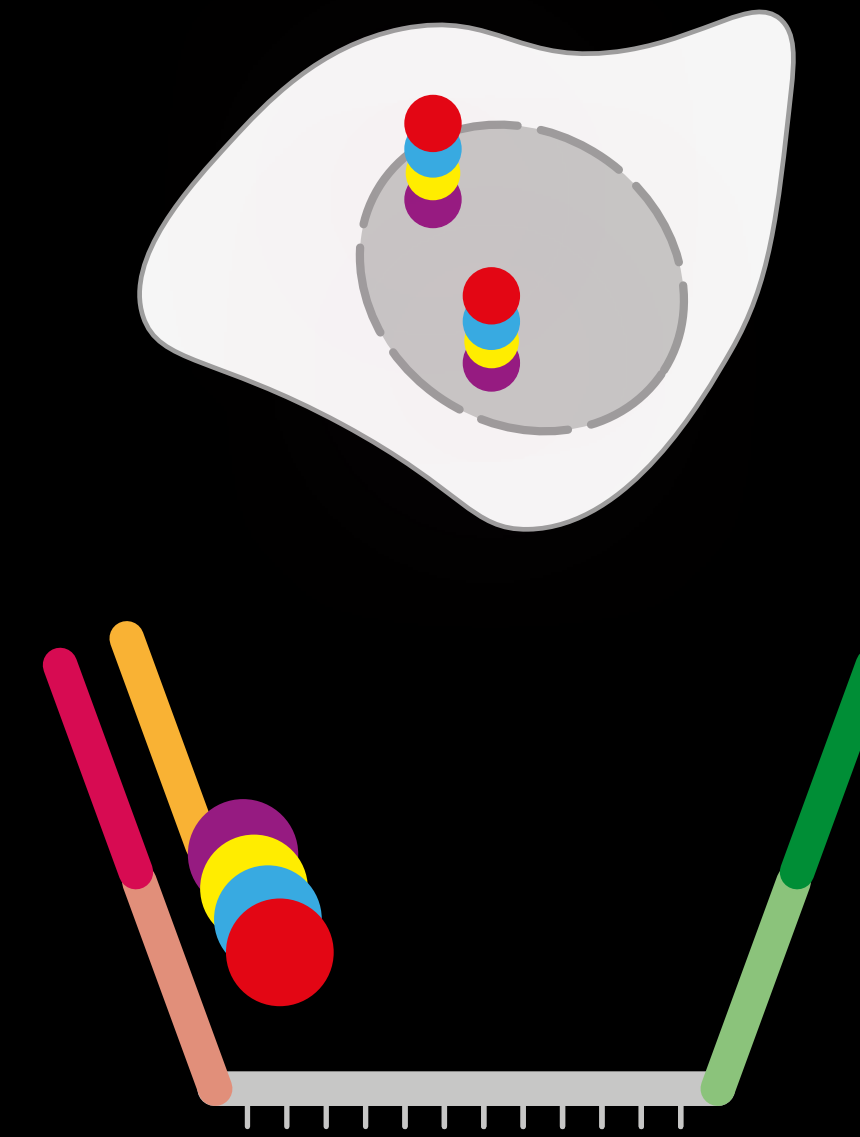
Sequential hybridization



$$\# \text{ of targets} = F * N$$

$F = \# \text{ of fluorophores}$
 $N = \# \text{ of seq. rounds}$

Barcode sequencing

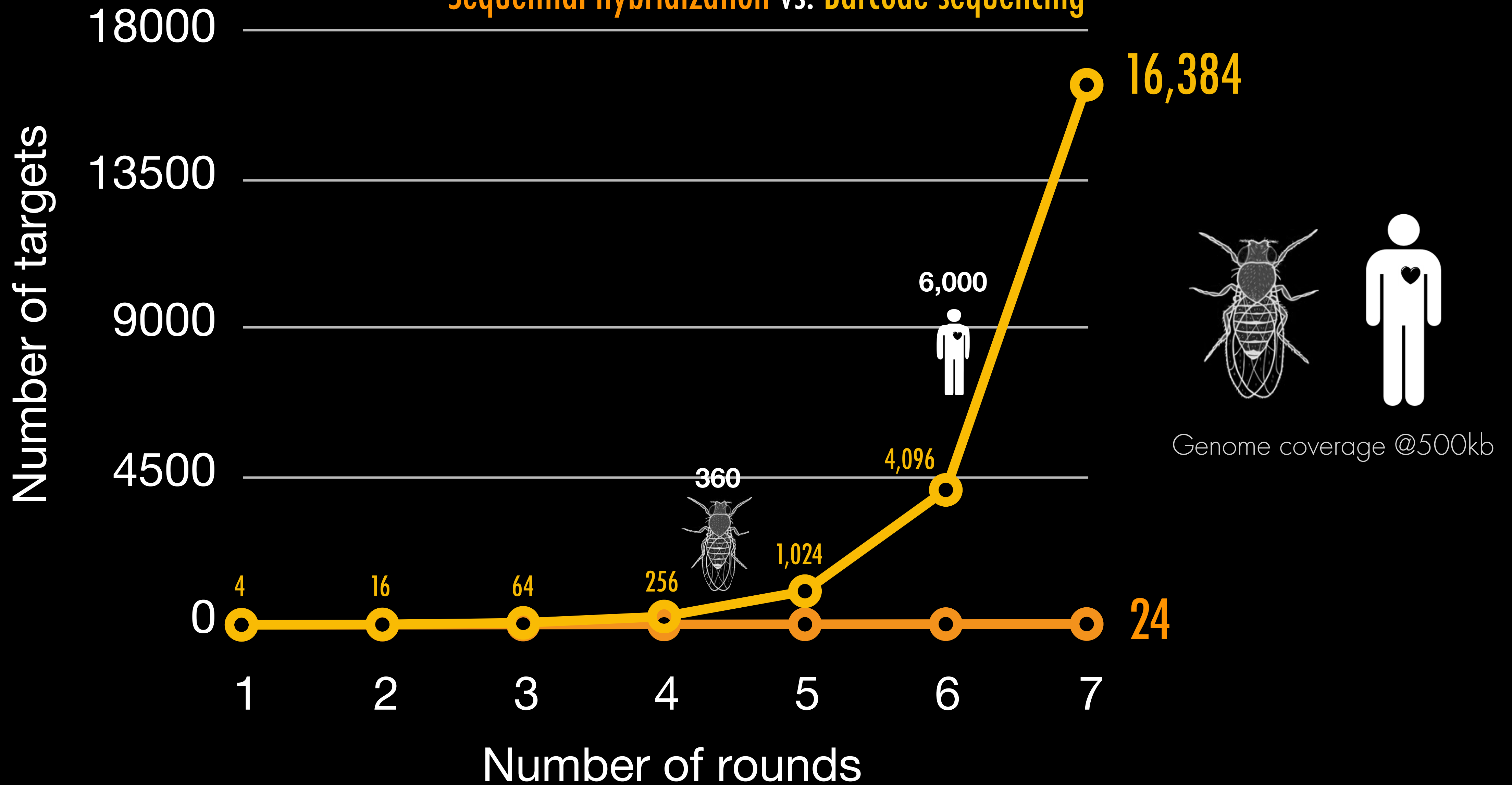


$$\# \text{ of targets} = F^N$$

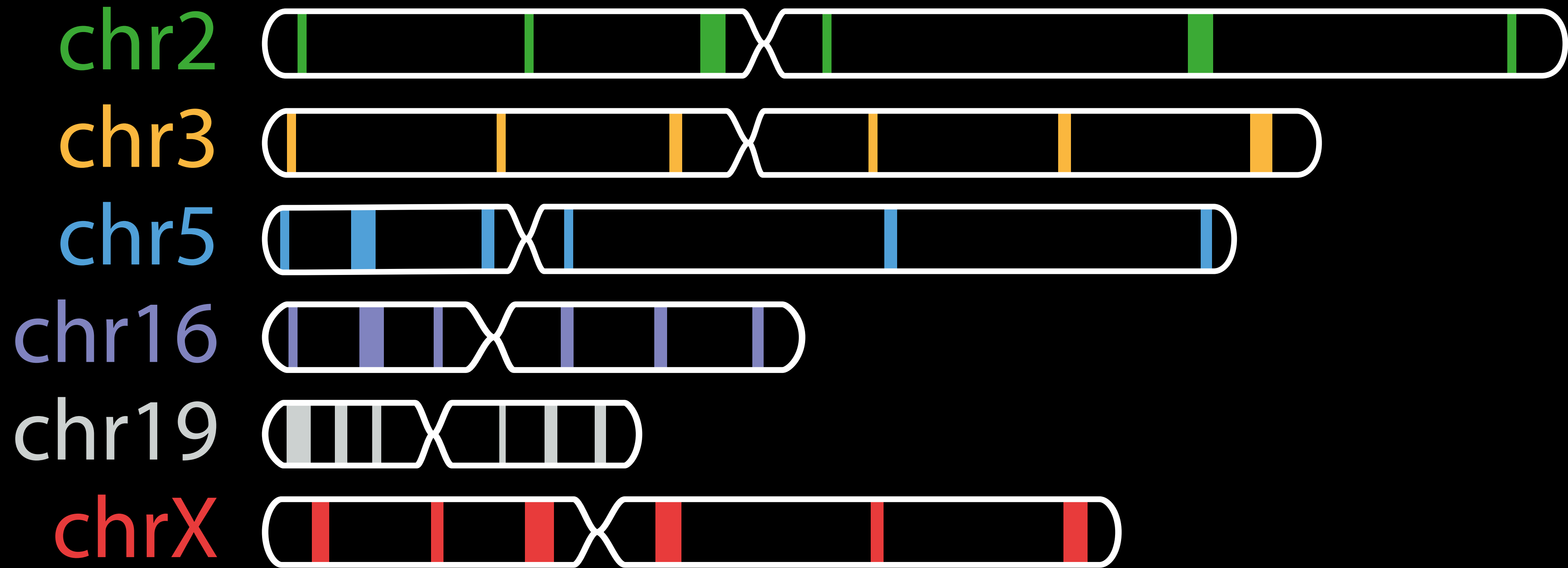
$F = \# \text{ of fluorophores}$
 $N = \# \text{ of seq. rounds}$

OligoFISSEQ scales exponentially!

Sequential hybridization vs. Barcode sequencing



Proof-of-principle

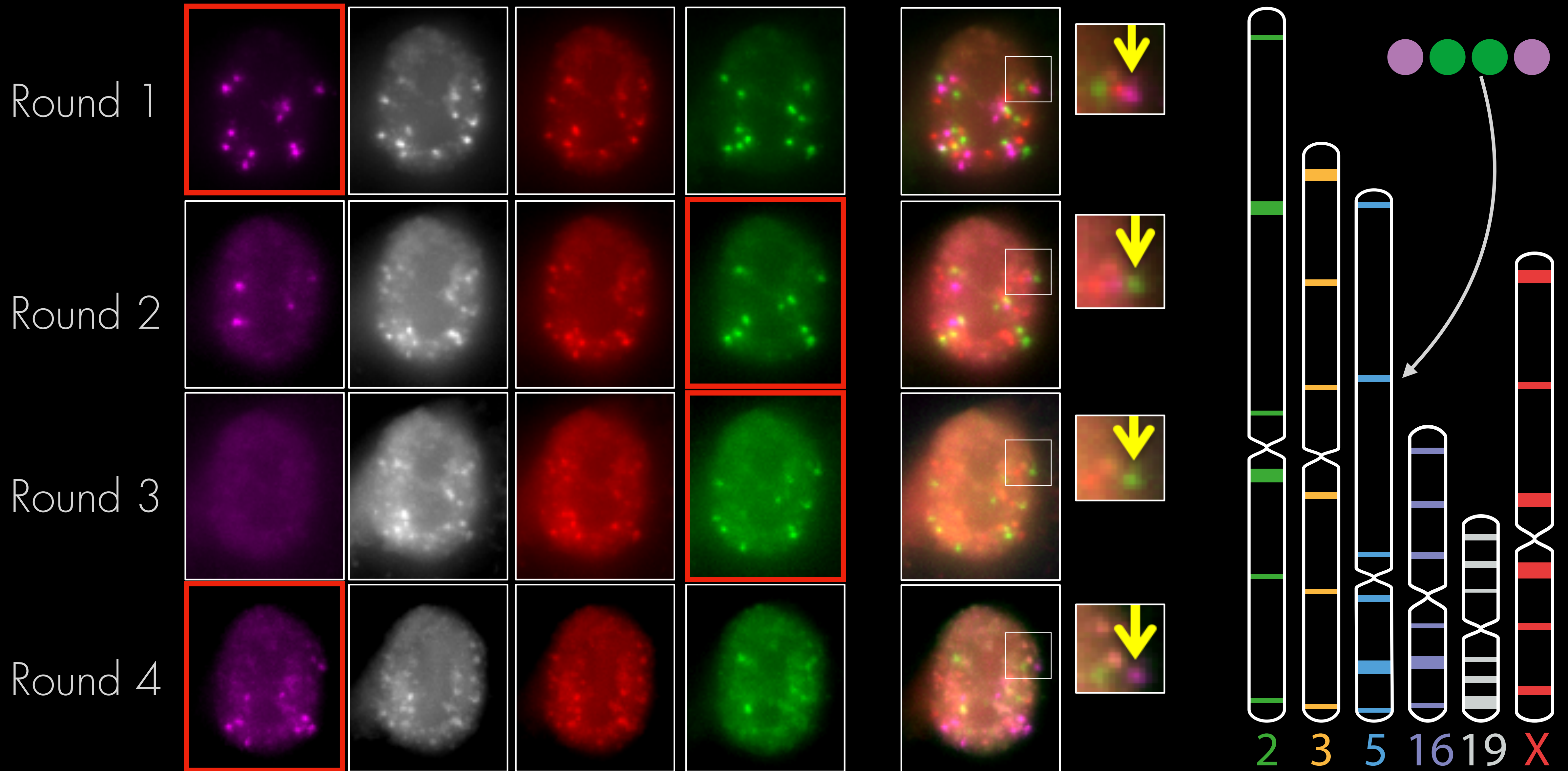


600kb-1Mb/target (876 kb average)

5,000 oligos/target

7-70Mb between targets

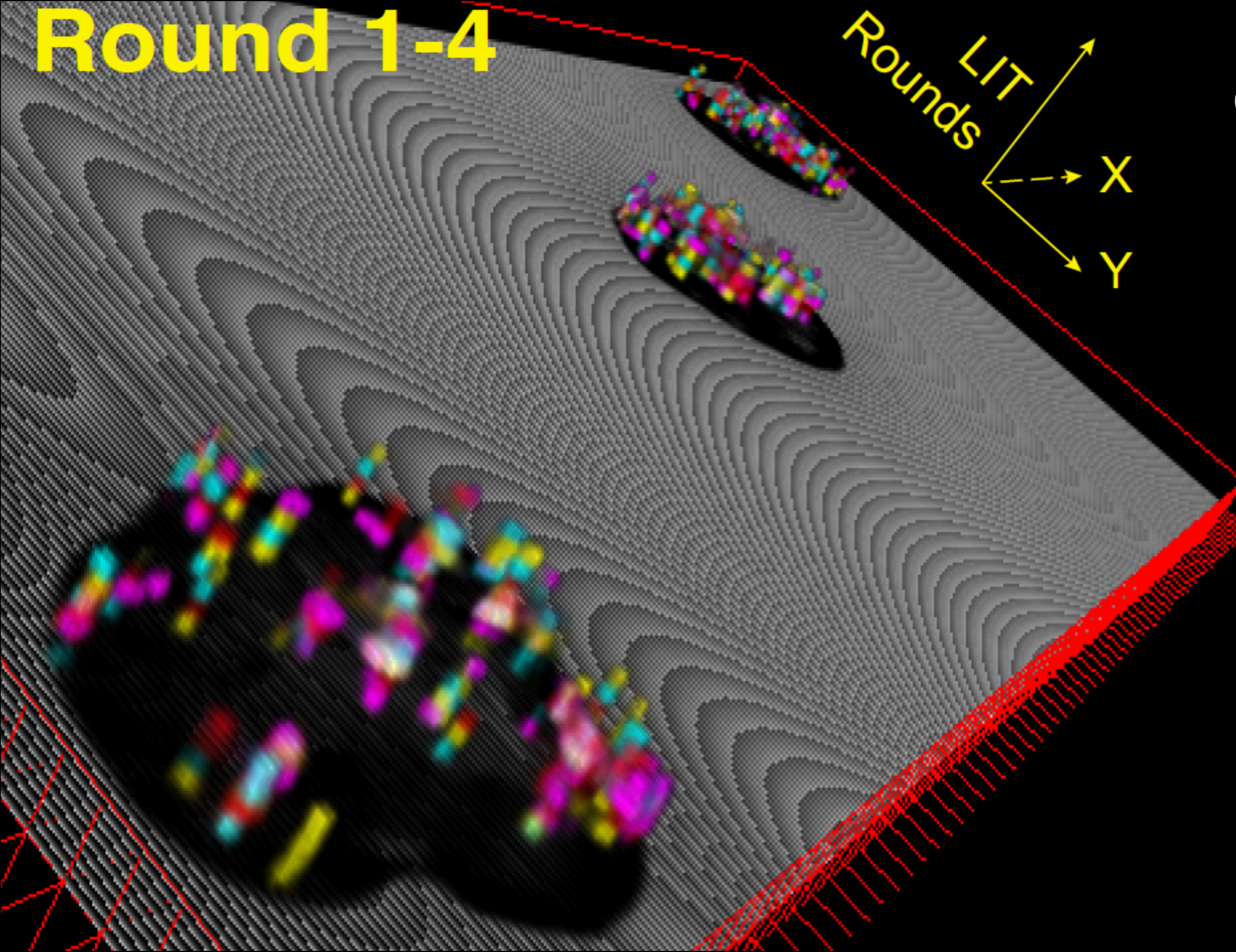
Detecting a given target



Round 1-4

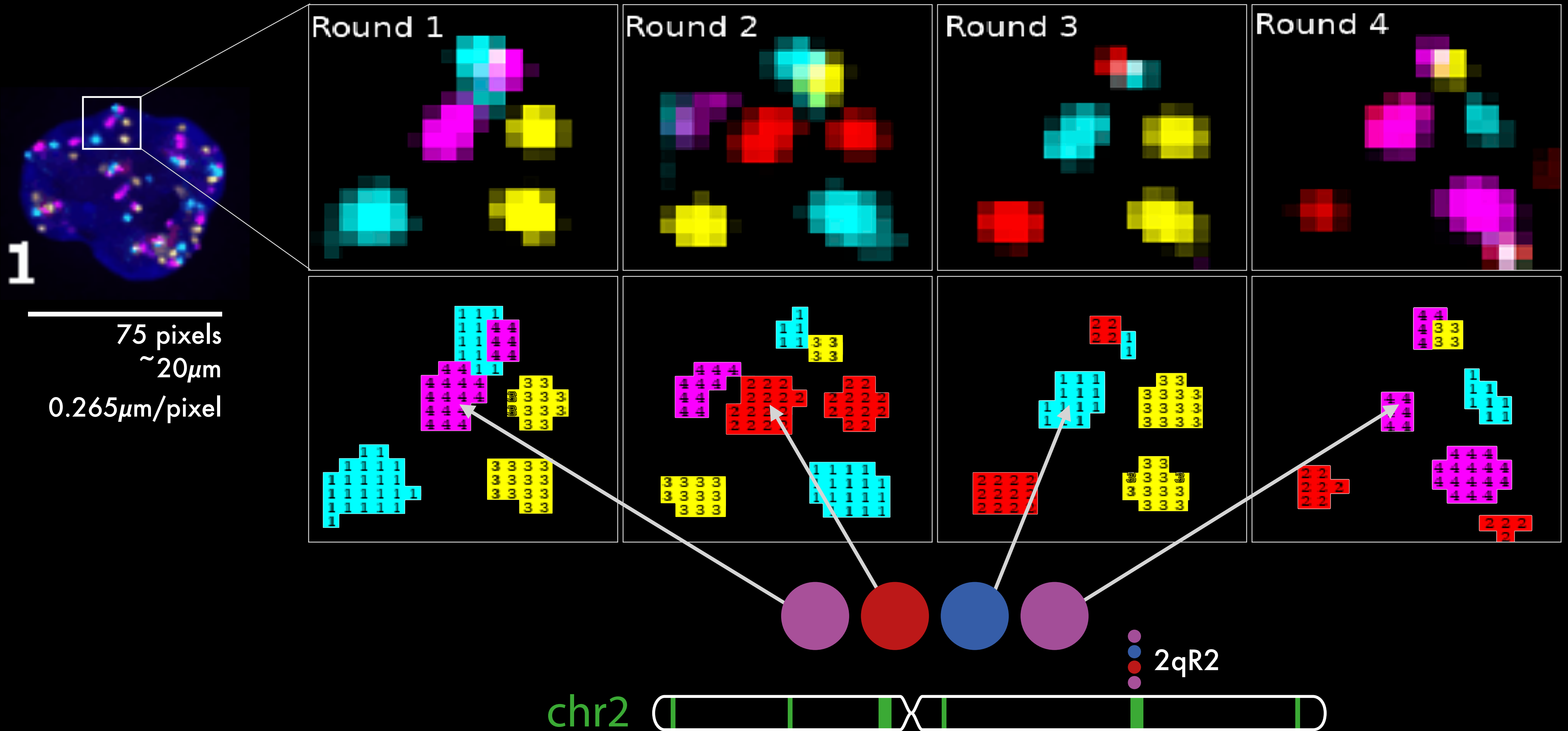
LIT
Rounds
X
Y

OligoFISSEQ
"Manhattan plot"

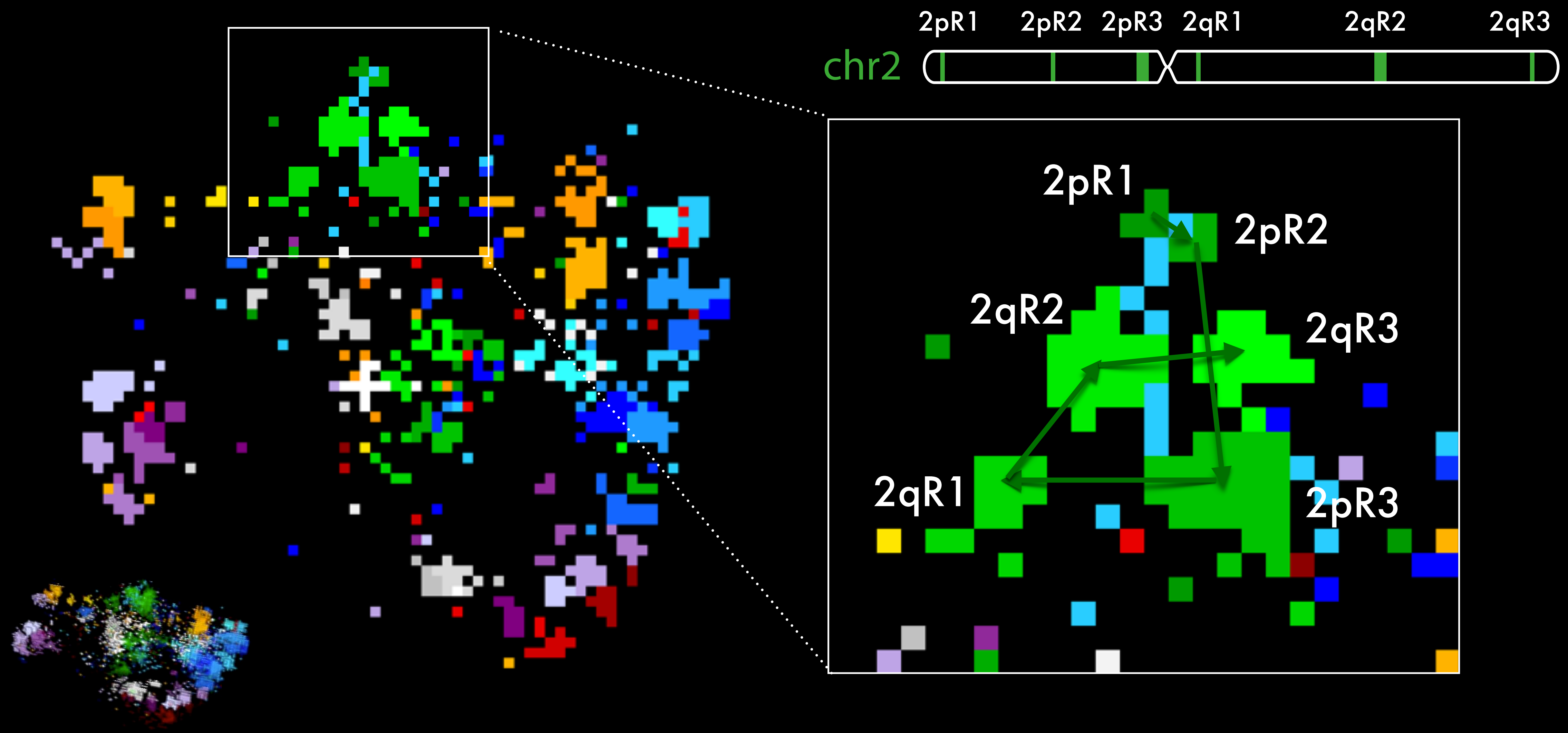


In OligoFISSEQ every pixel matters & make "patches"

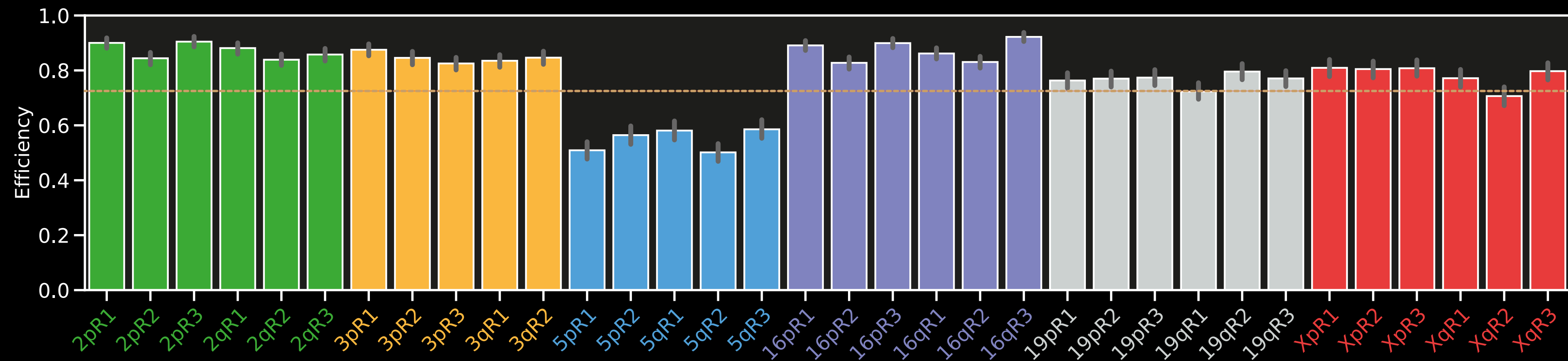
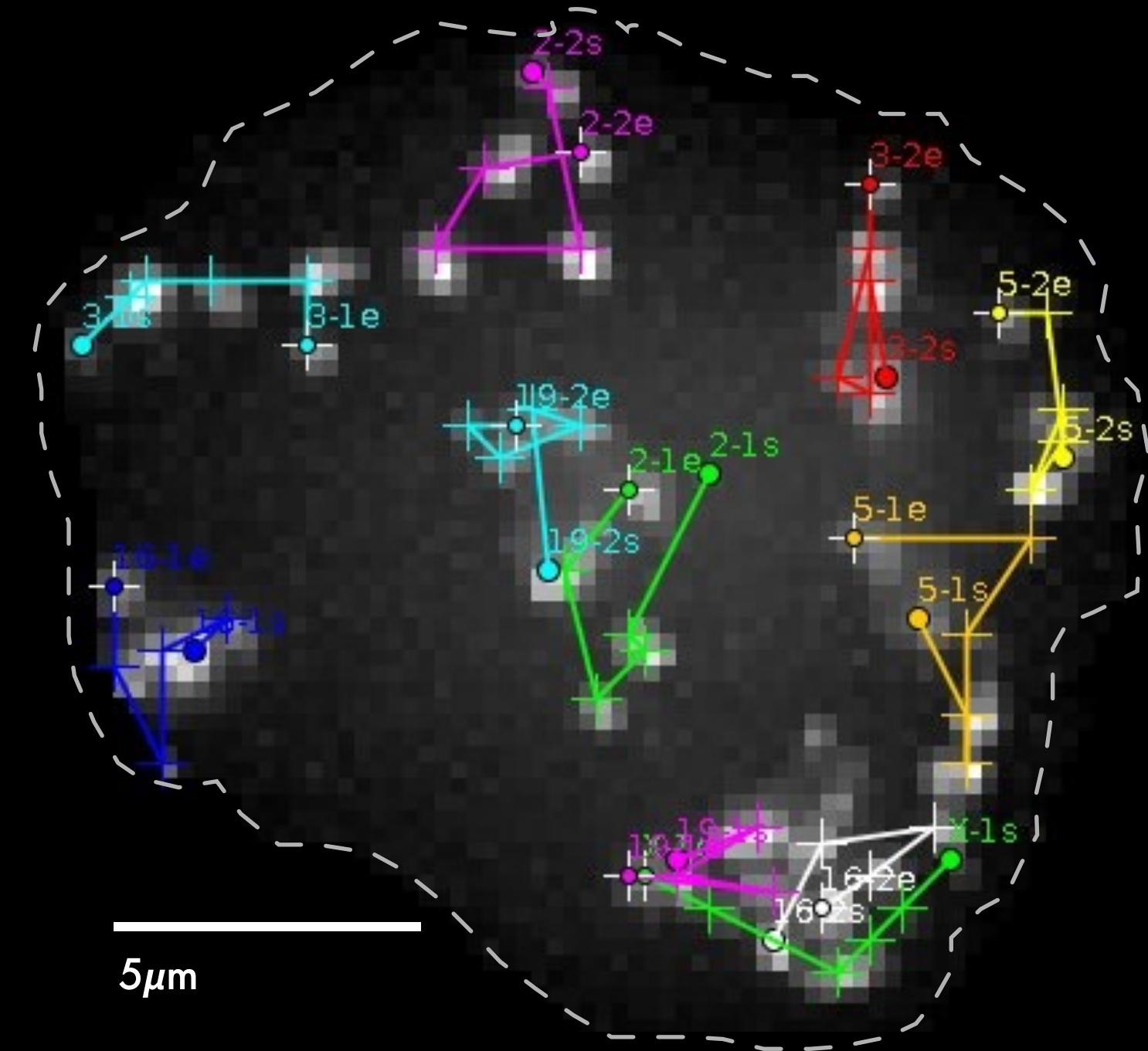
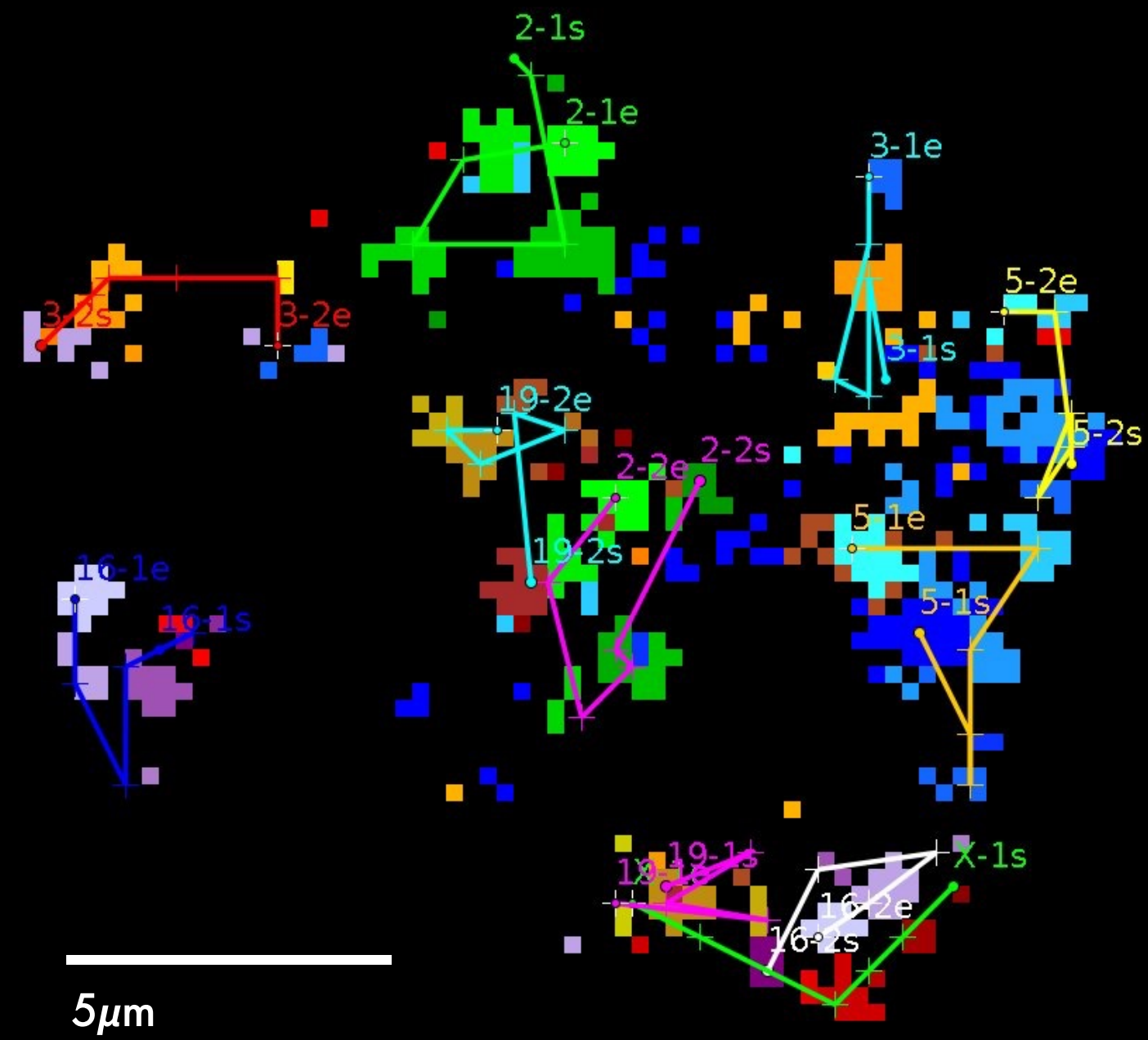
4 rounds / 4 channels



In **OligoFISSEQ** every pixel matters & make "patches"

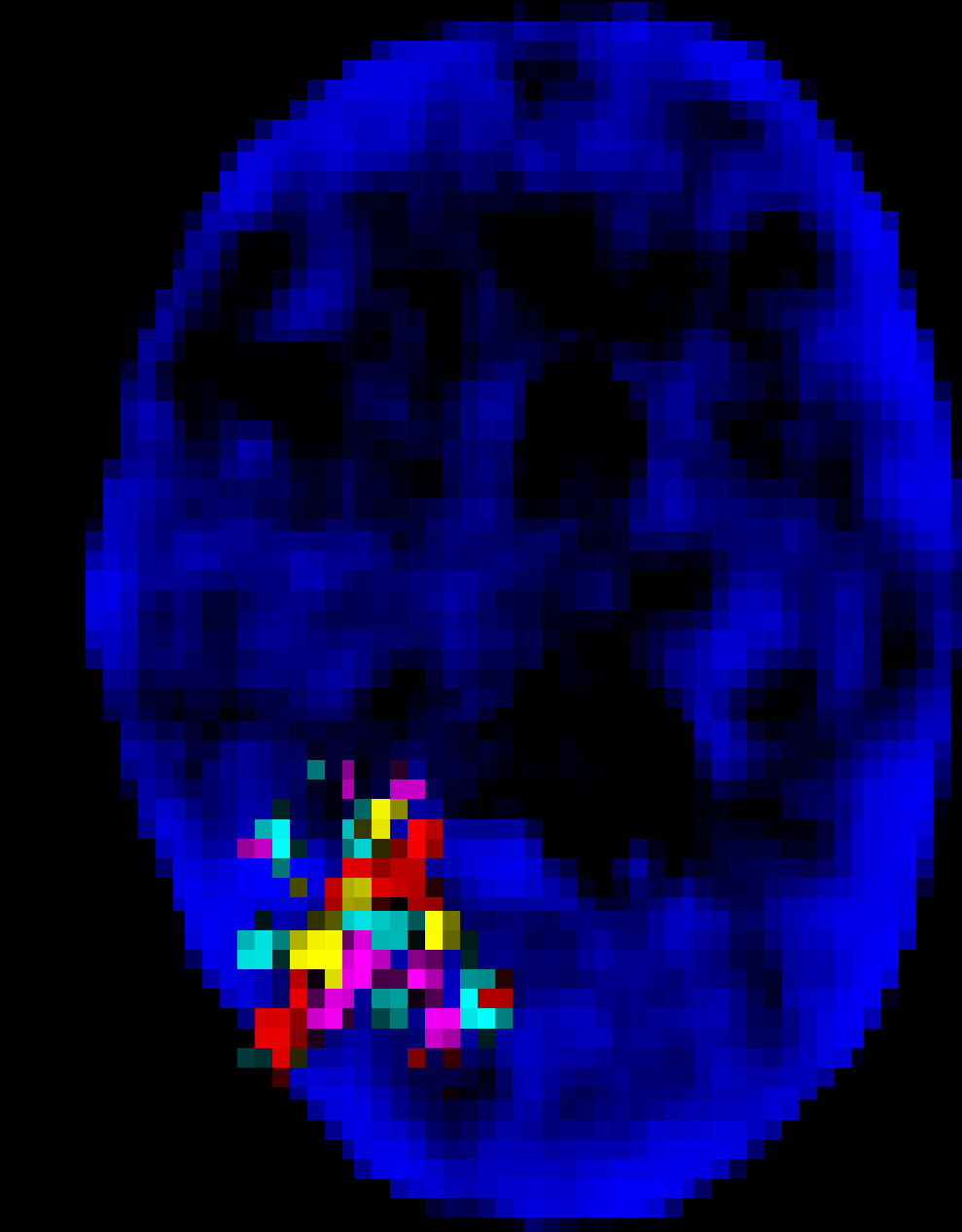
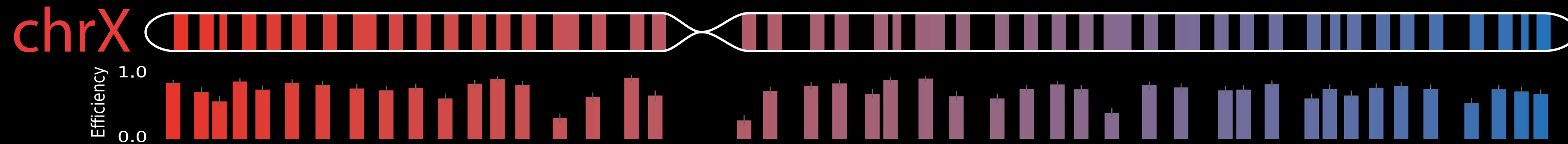


OligoFISSEQ barcode efficiency

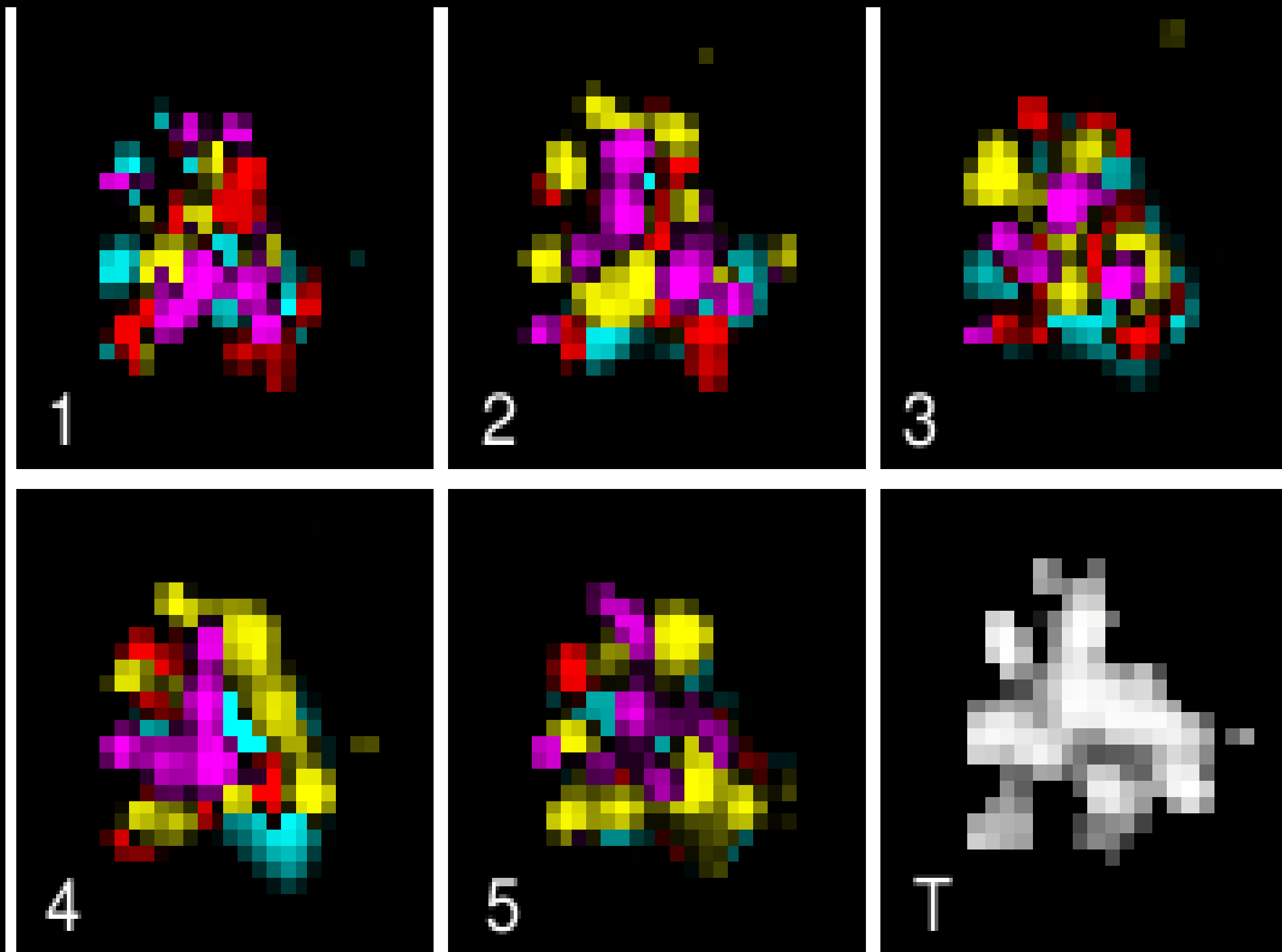


OligoFISSEQ tracing of (almost) entire chromosomes

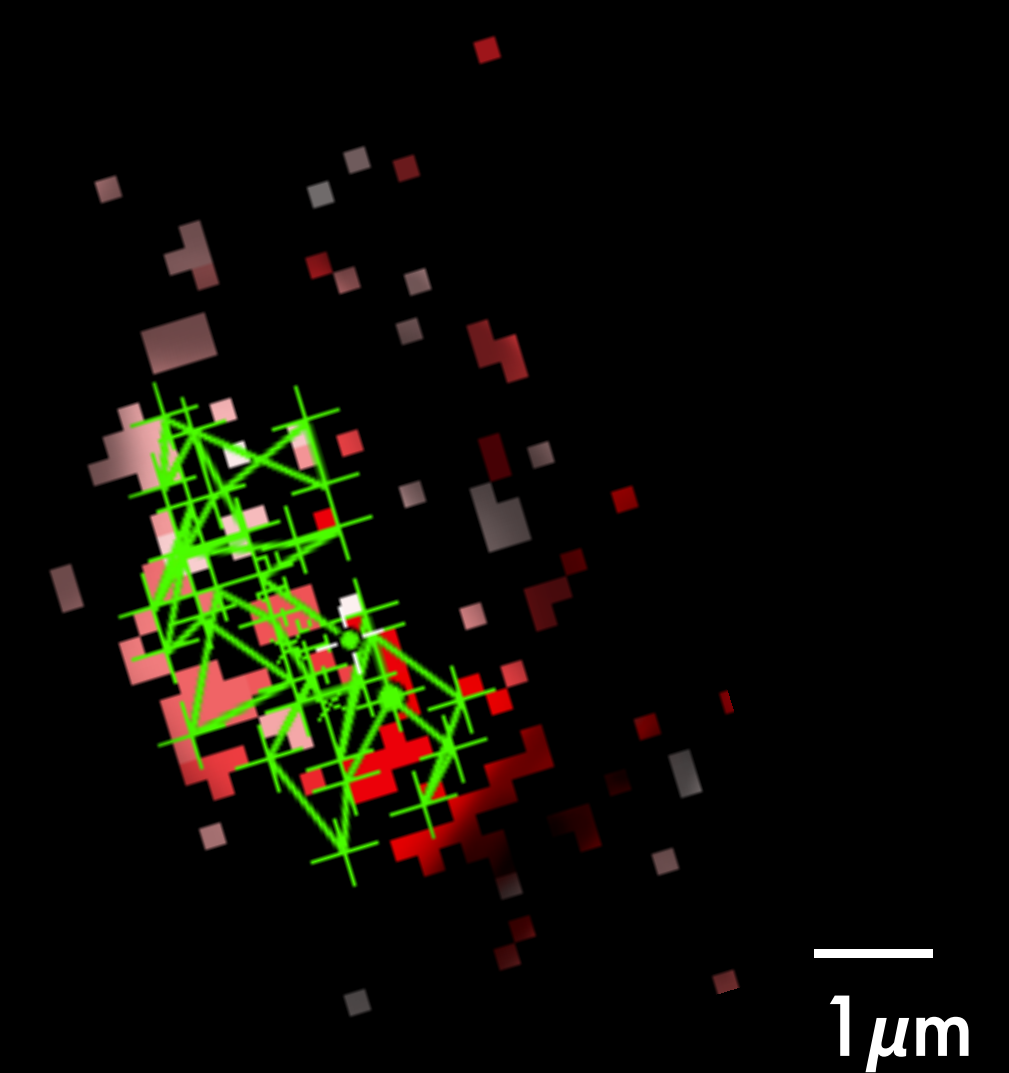
46 Plex in chromosome X



5 μ m

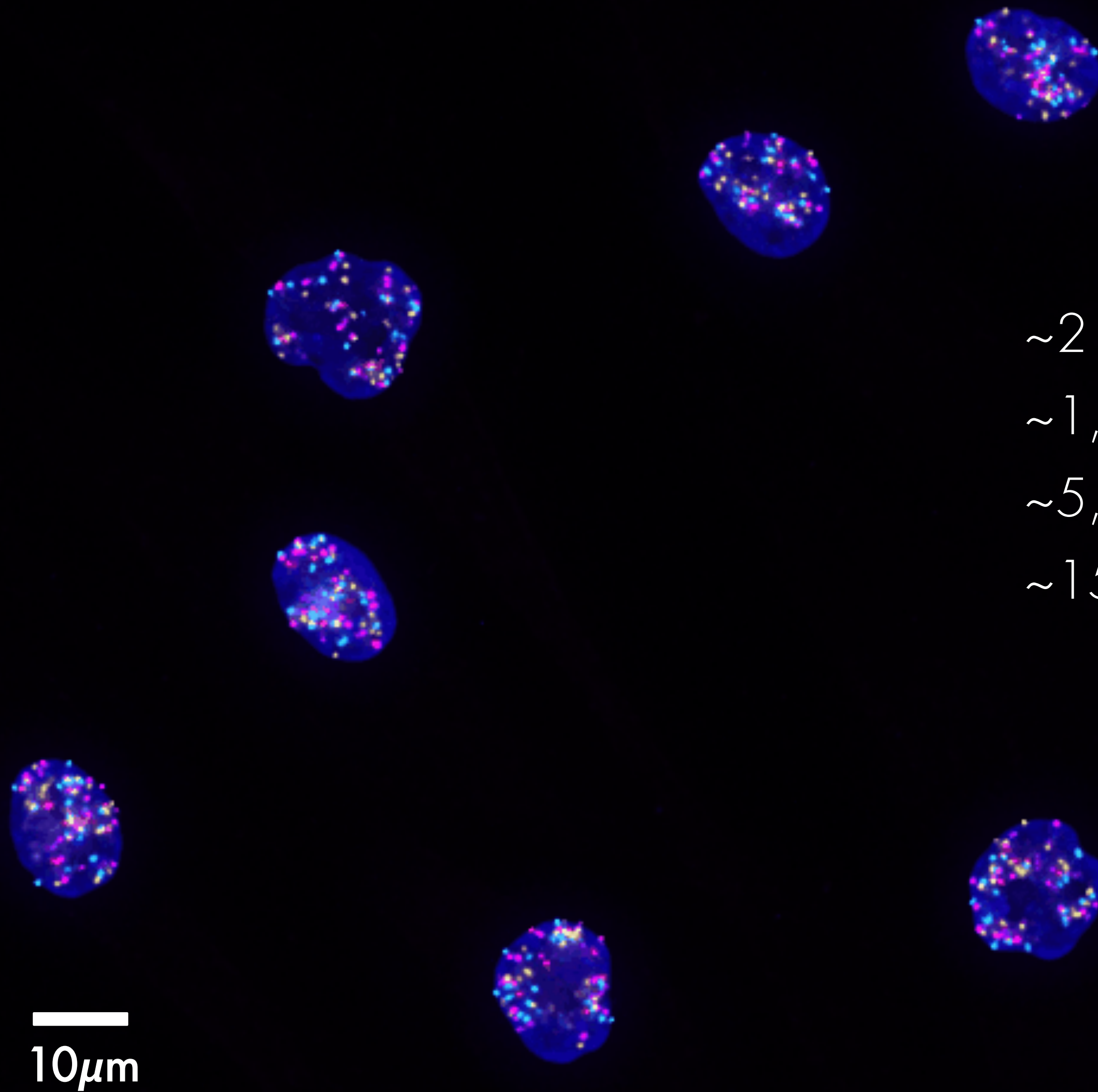


5 rounds
445 kb/probe
2,000 Oligopaints/probe
2 Mb between loci



1 μ m

OligoFISSEQ is high throughput!



~2 days of image acquisition

~1,000 cells

~5,000 complete chromosomes

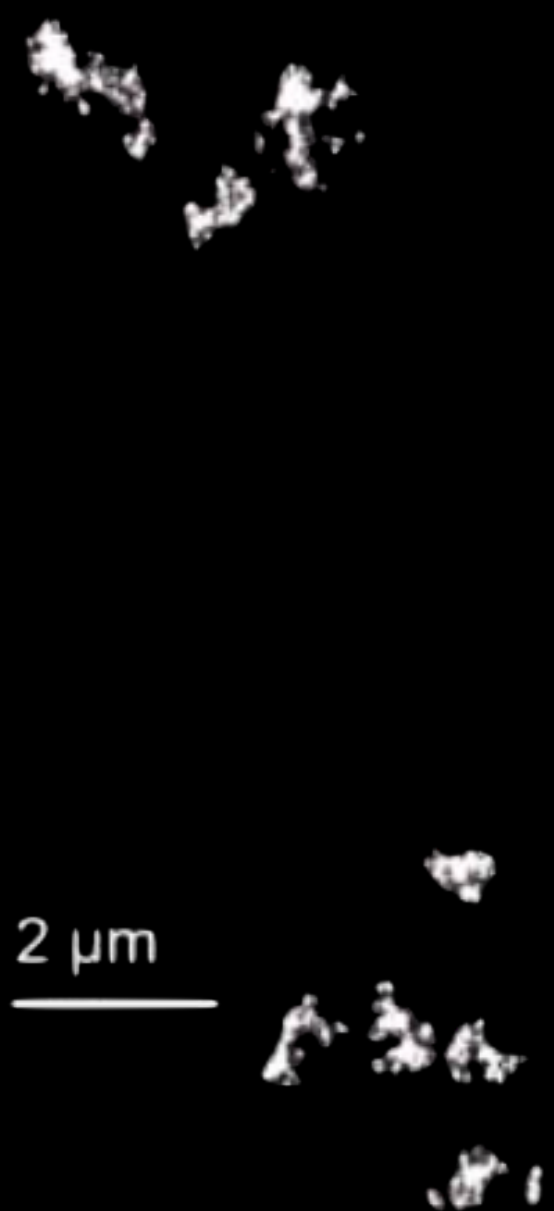
~150 cells with complete chromosomes

OligoFISSEQ pipelined with OligoSTORM

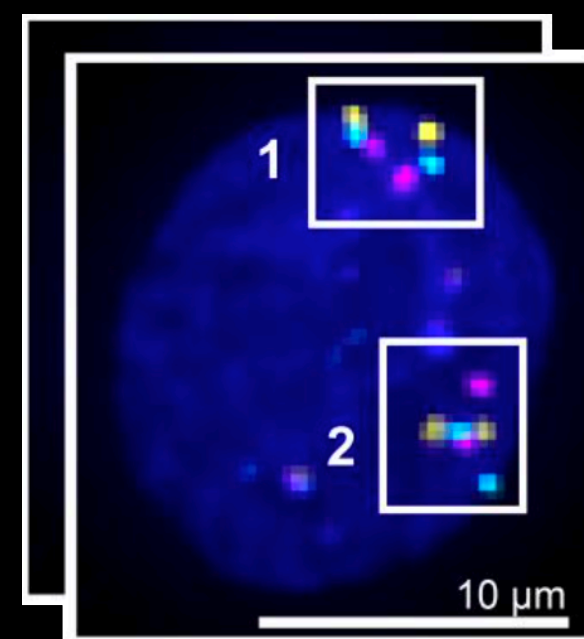


1 2 3 4

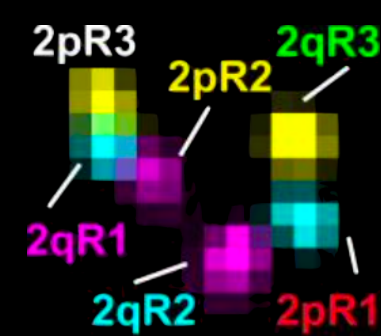
OligoSTROM
1 round
(2h/round)



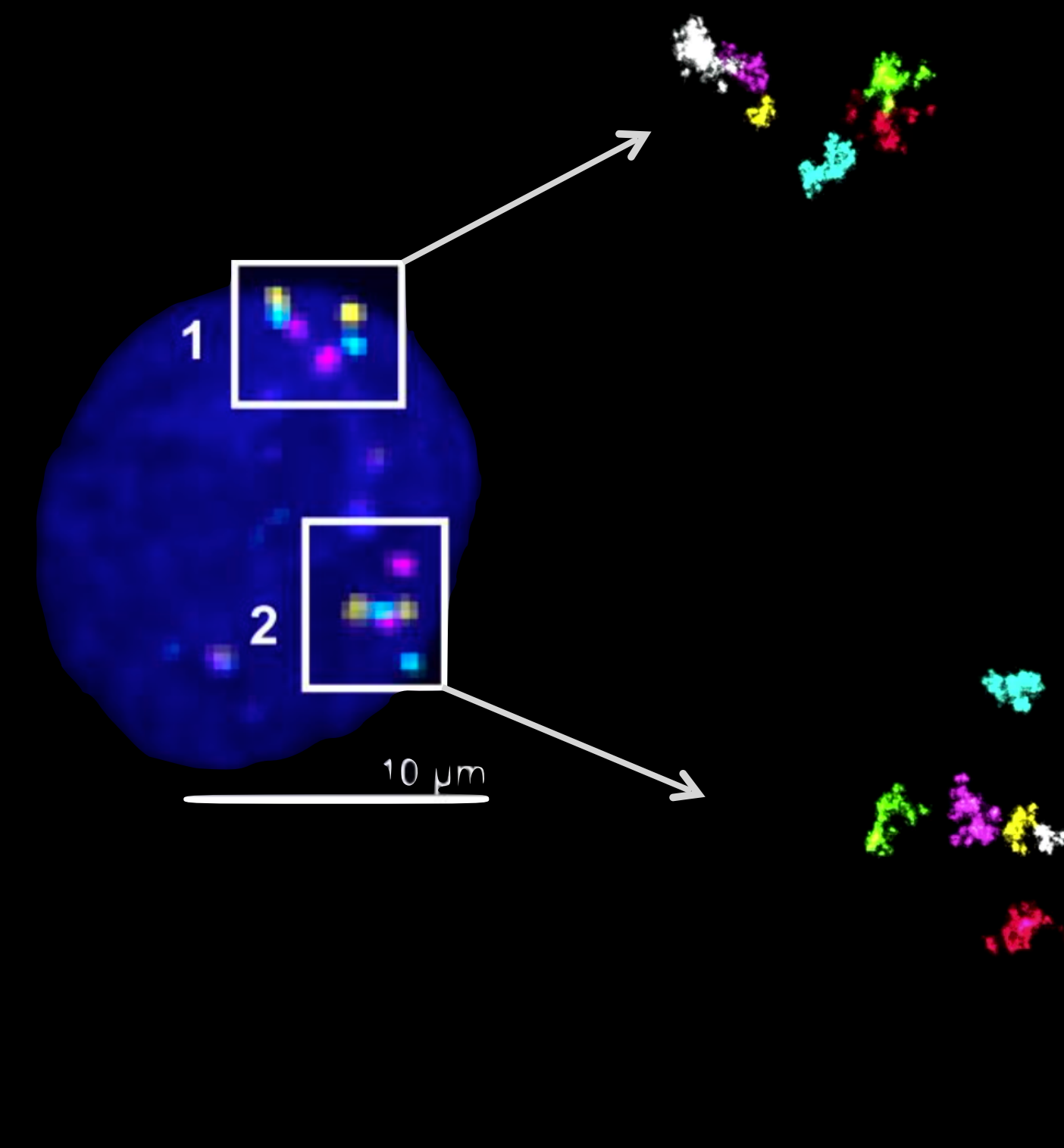
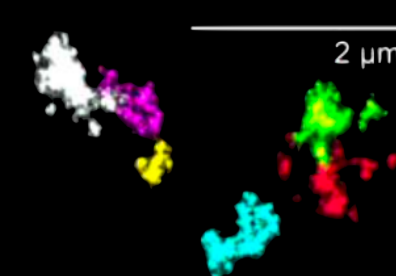
OligoFISSEQ
2 round
(3h/round)



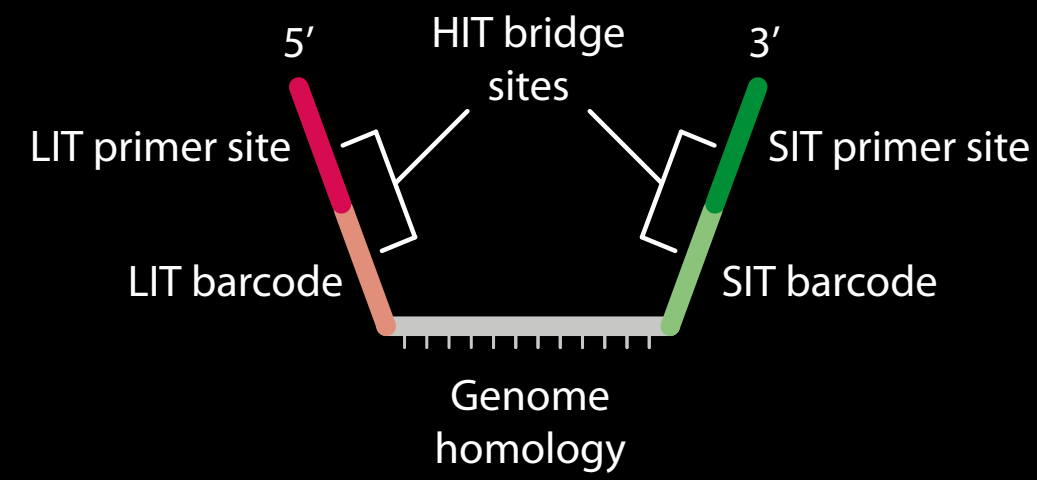
Decoding
OligoFISSEQ



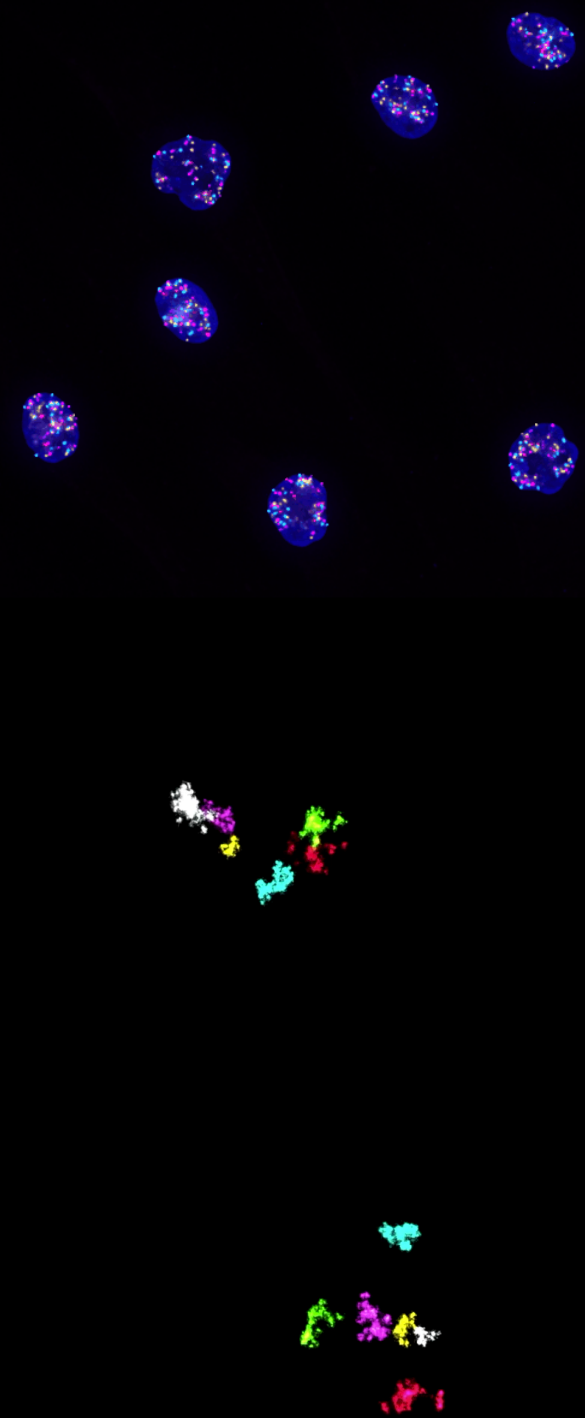
Mapping
OligoSTROM



OligoSTORM + OligoFISSEQ



- Is a set of technologies for in-situ genome mapping
- Is highly versatile: mainstreet and backstreet
- Used with wide-field microscopy allows for the analysis of thousands of cells.
- Identifies sub-clusters with specific conformational characteristics
- Can be pipelined with other approaches
 - OligoSTORM
 - Protein immunofluorescence
 - RNA...



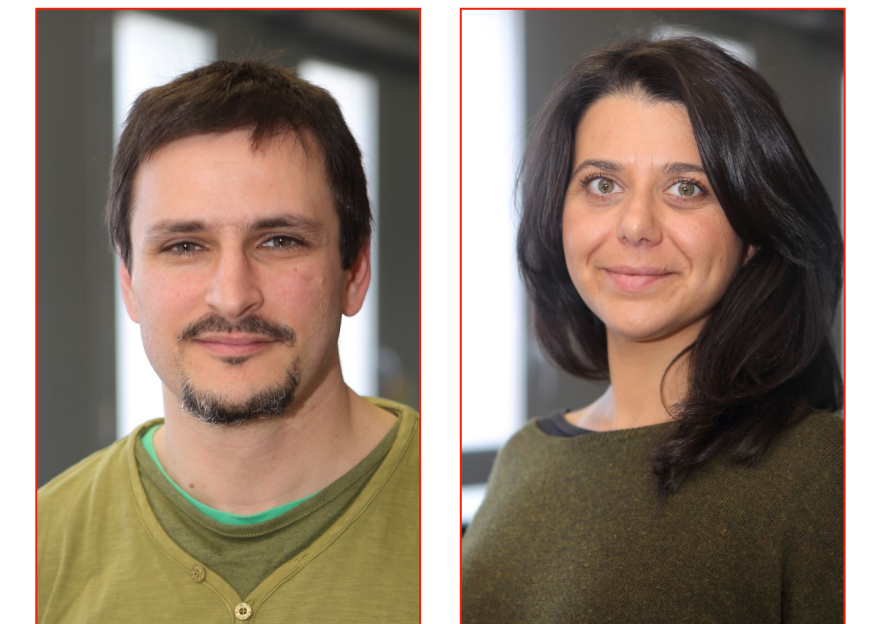
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<http://3DGenomes.org>

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Irene Farabella

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.: Conflict of Interest Statement :.

Between Sep 2021 and Apr 2023, Marc A. Marti-Renom served as a consultant to Acuity Spatial Genomics, Inc., and received compensation for these services.