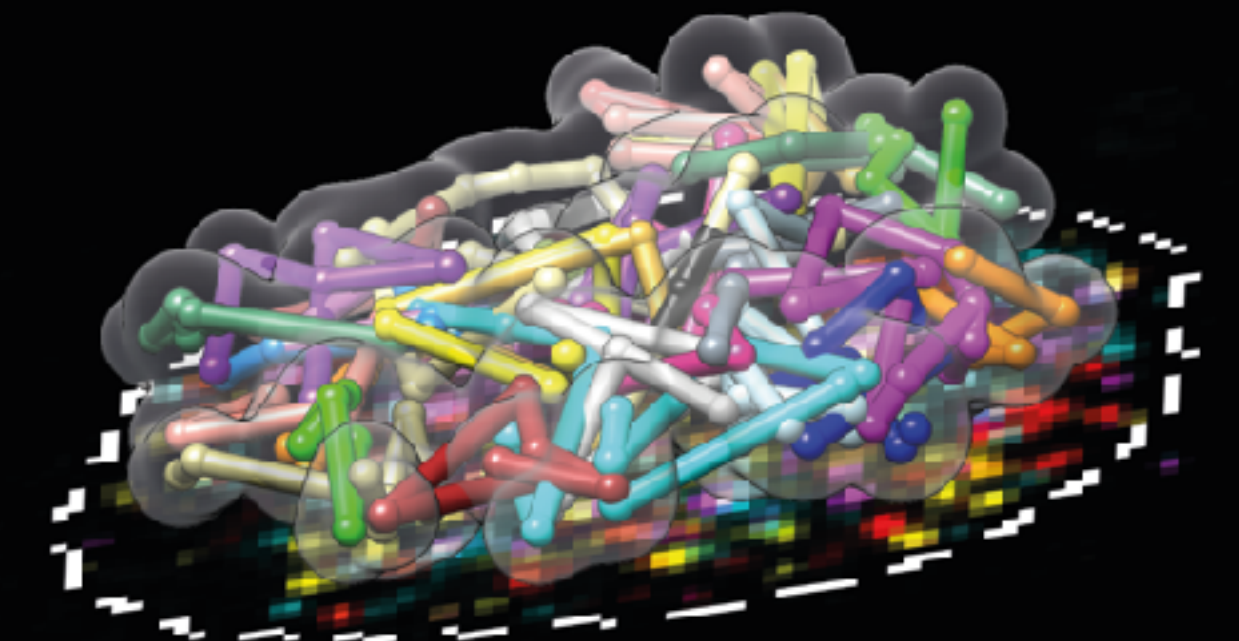




Chromosome tracing with OligoFISSEQ



Marc A. Marti-Renom

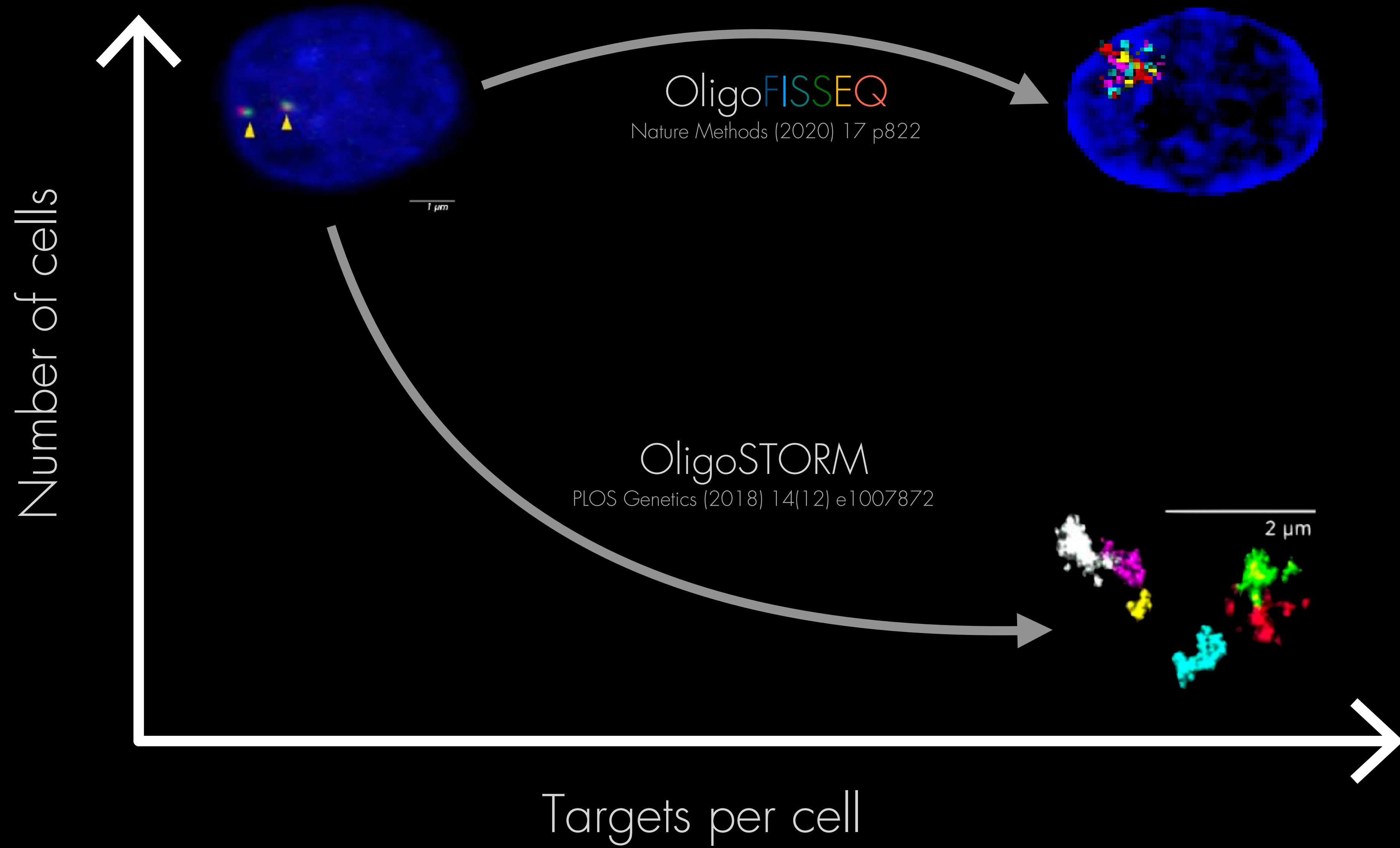
CNAG-CRG · ICREA



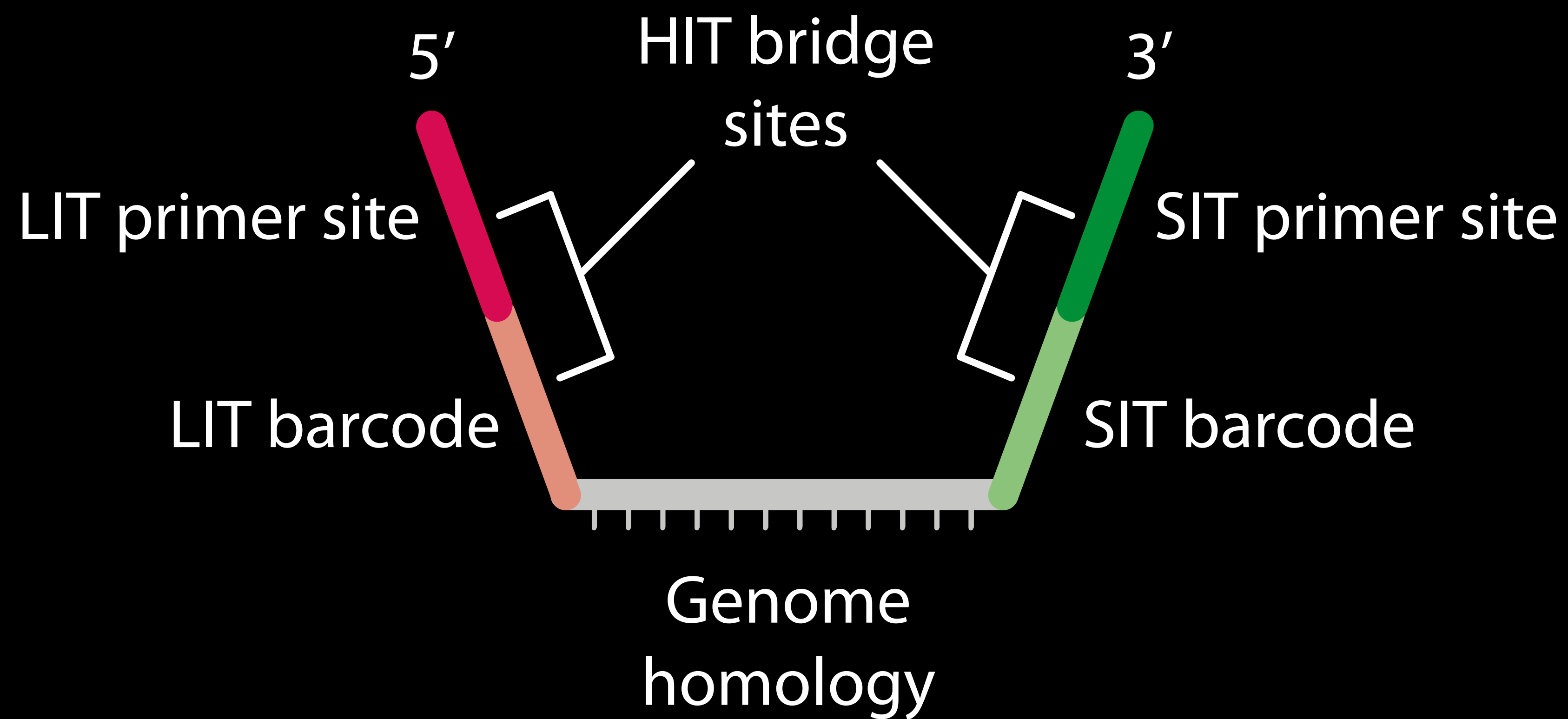
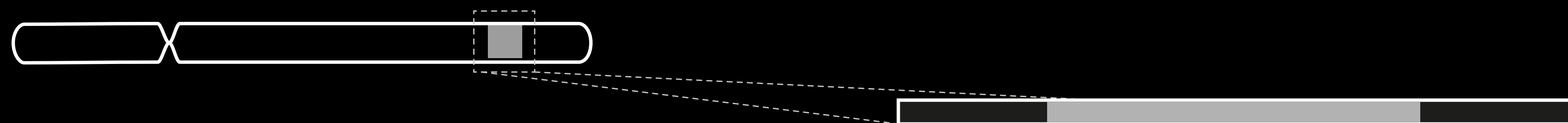
Huy Nguyen
Shyamtanu Chatteraj
David Castillo

in collaboration with the Wu Lab (HMS)

Nature Methods (2020) 17 p822

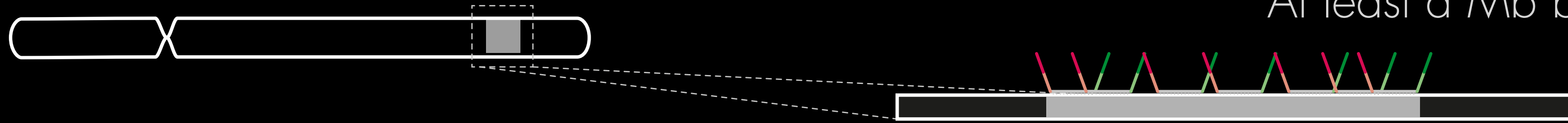


OligoFISSEQ

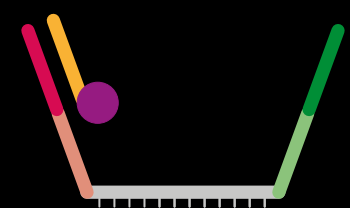
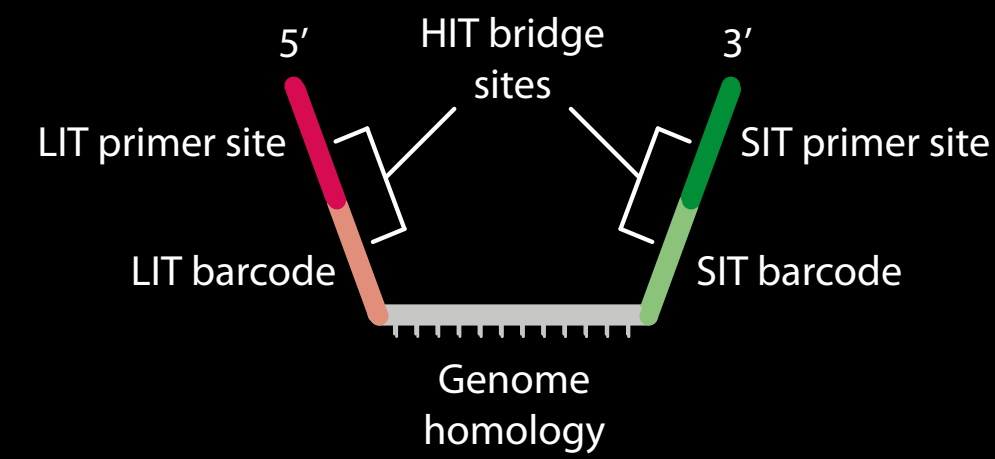


OligoFISSEQ

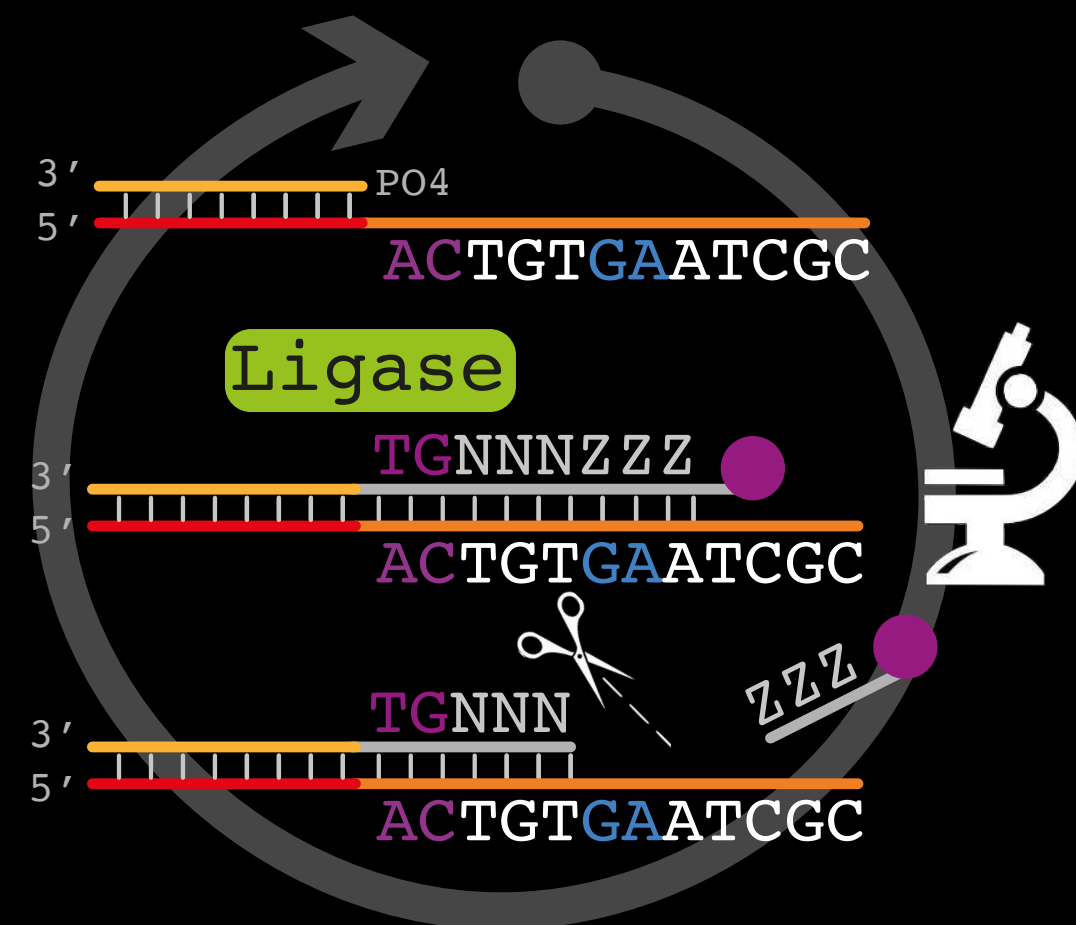
From tens of kb to Mb
Min. of few 100s oligos/target
At least a Mb between targets



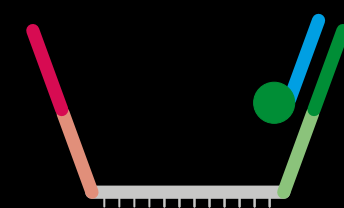
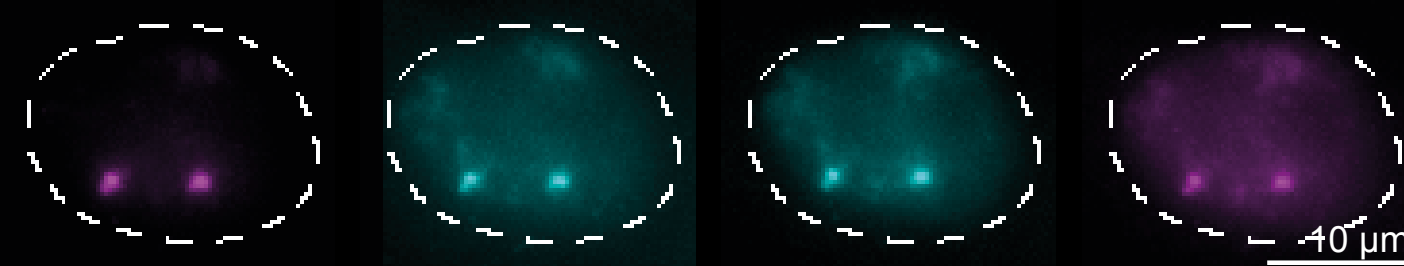
OligoFISSEQ



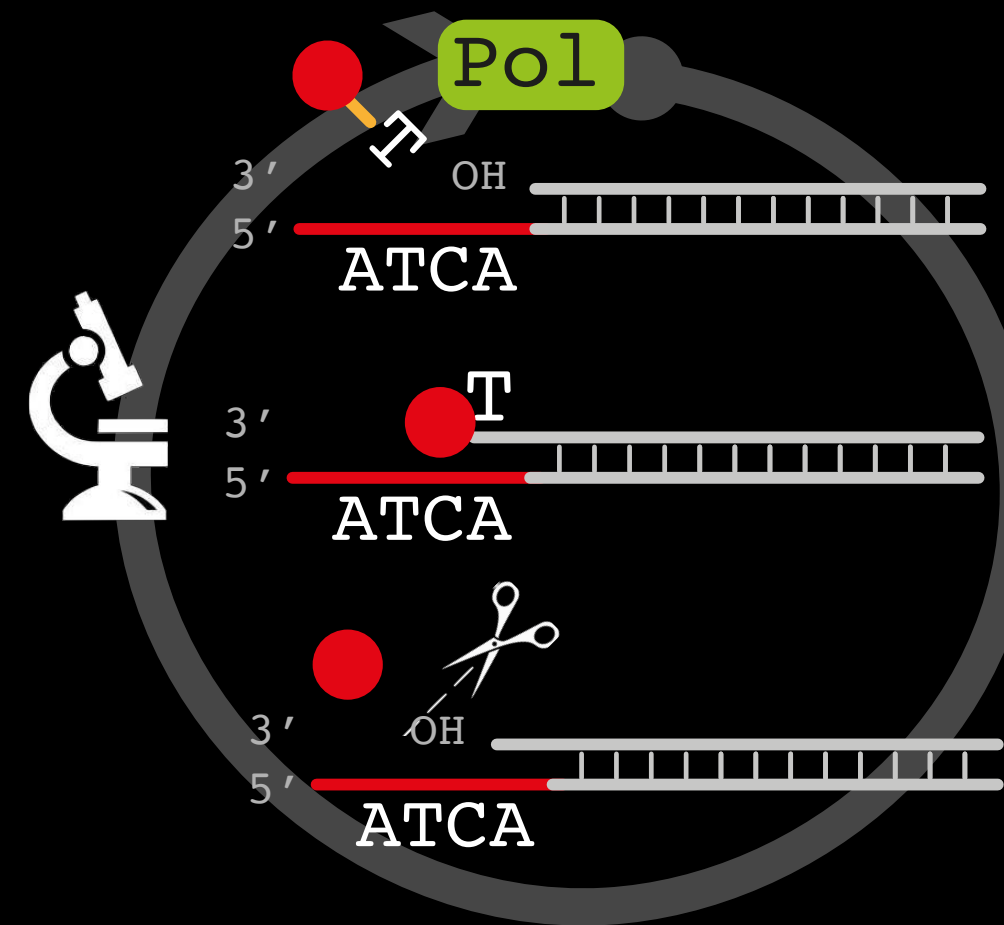
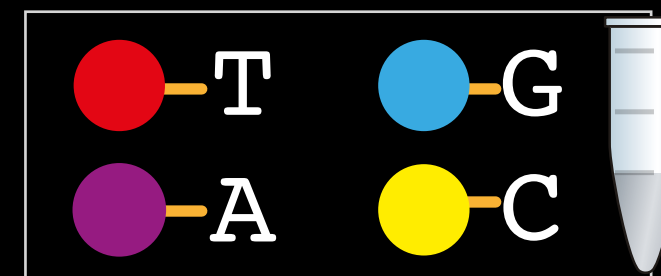
Ligation based Identification of Targets



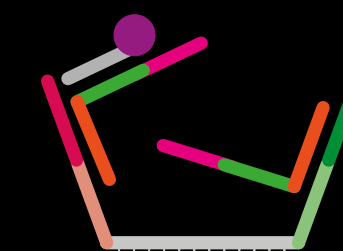
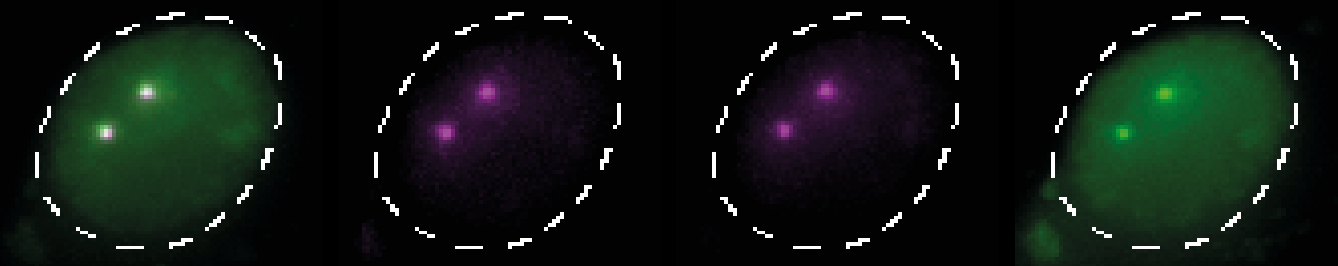
$92.1 \pm 5.7\%$



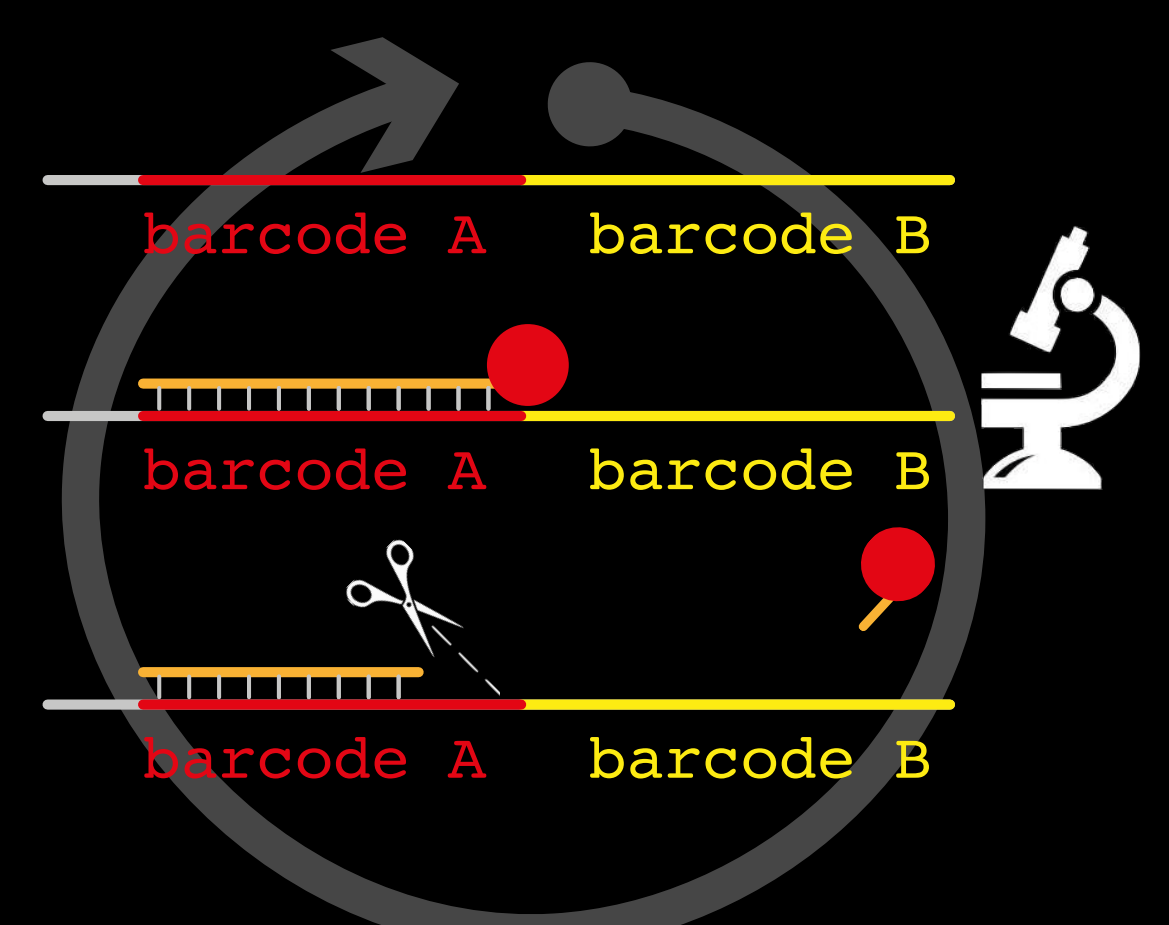
Synthesis based Identification of Targets



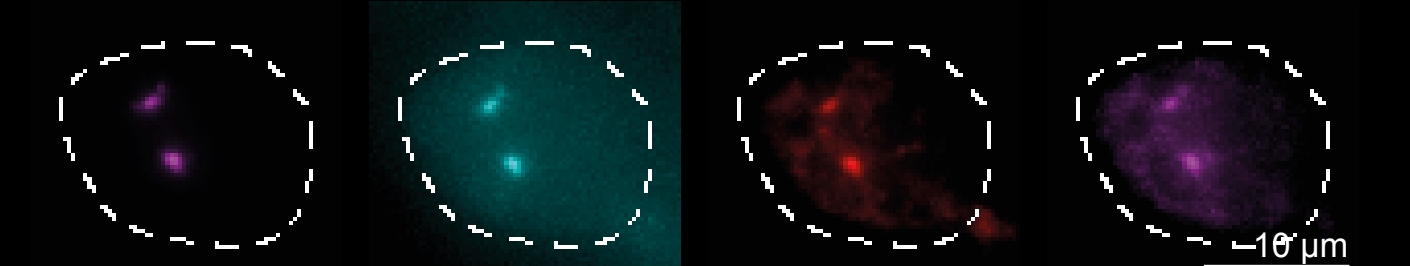
$90.8 \pm 5.6\%$



Hybridization based Identification of Targets

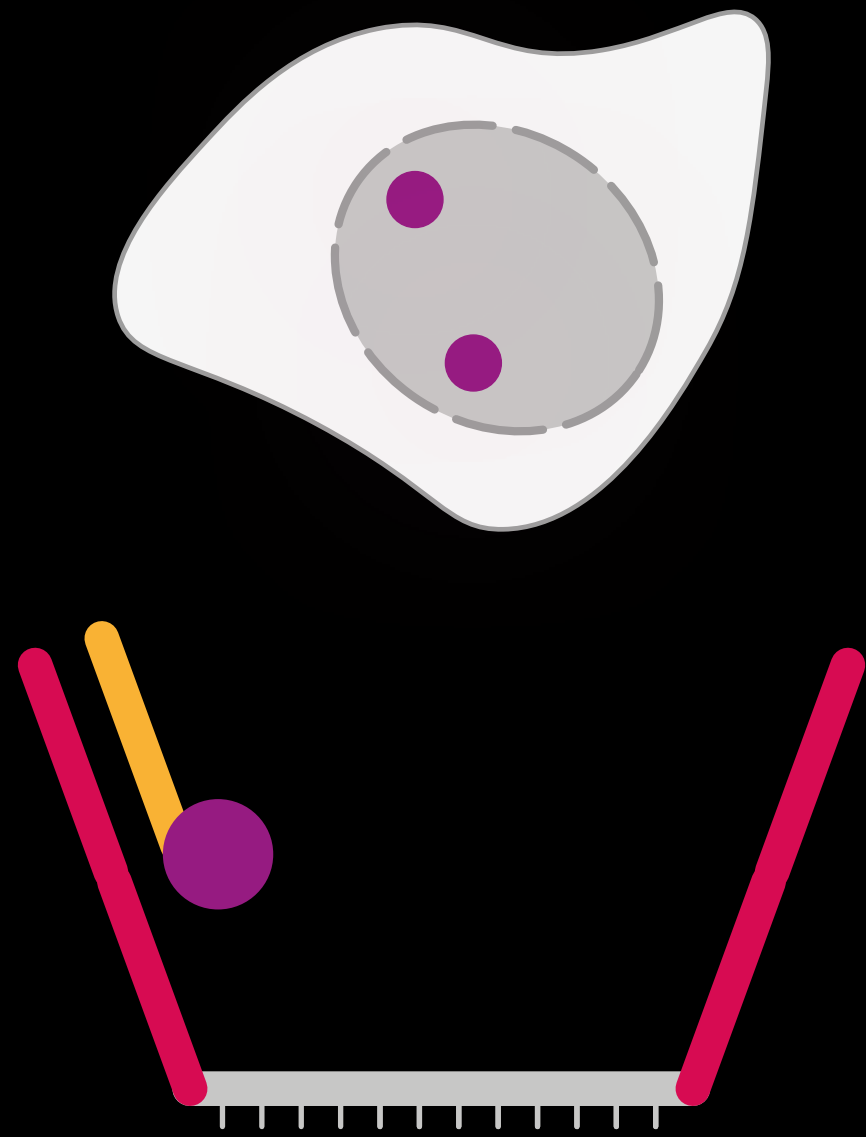


$91.6 \pm 3.8\%$



OligoFISSEQ scales exponentially!

Sequential hybridization

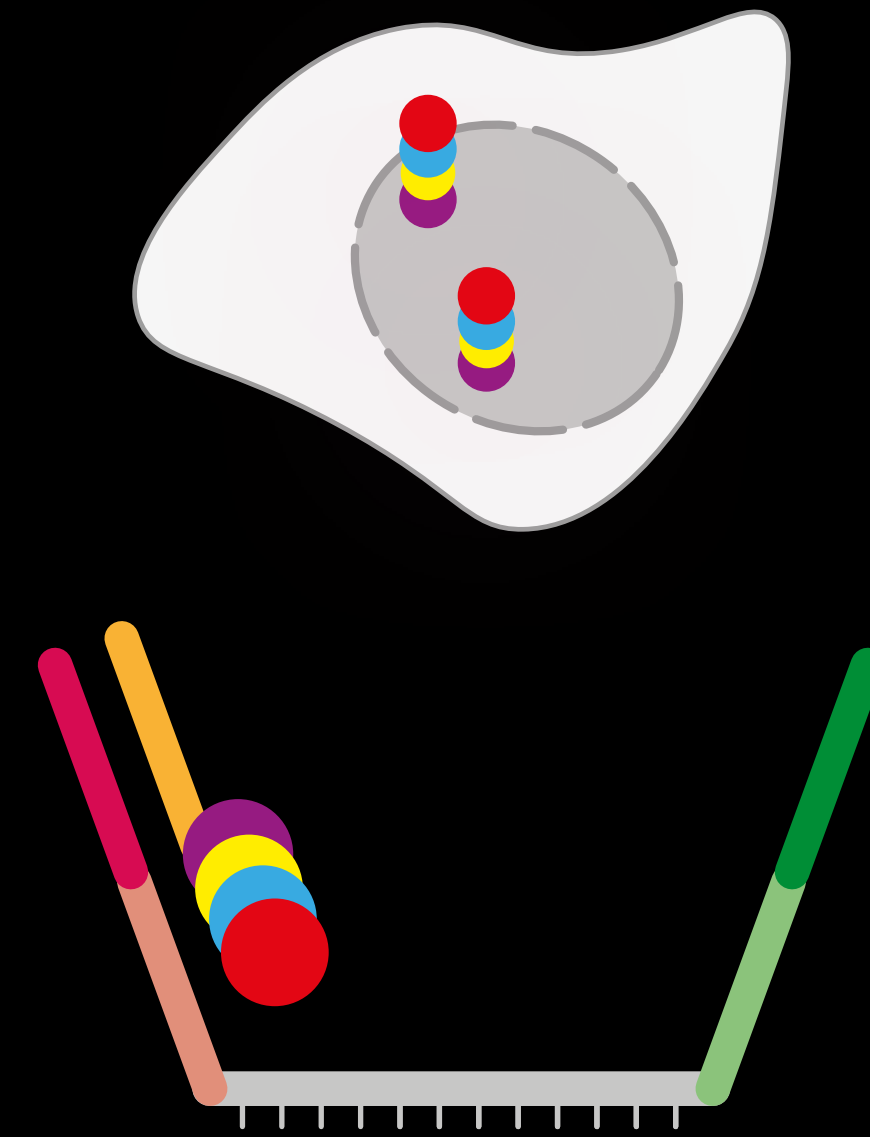


of targets = $F * N$

F = # of fluorophores

N = # of seq. rounds

Barcode sequencing

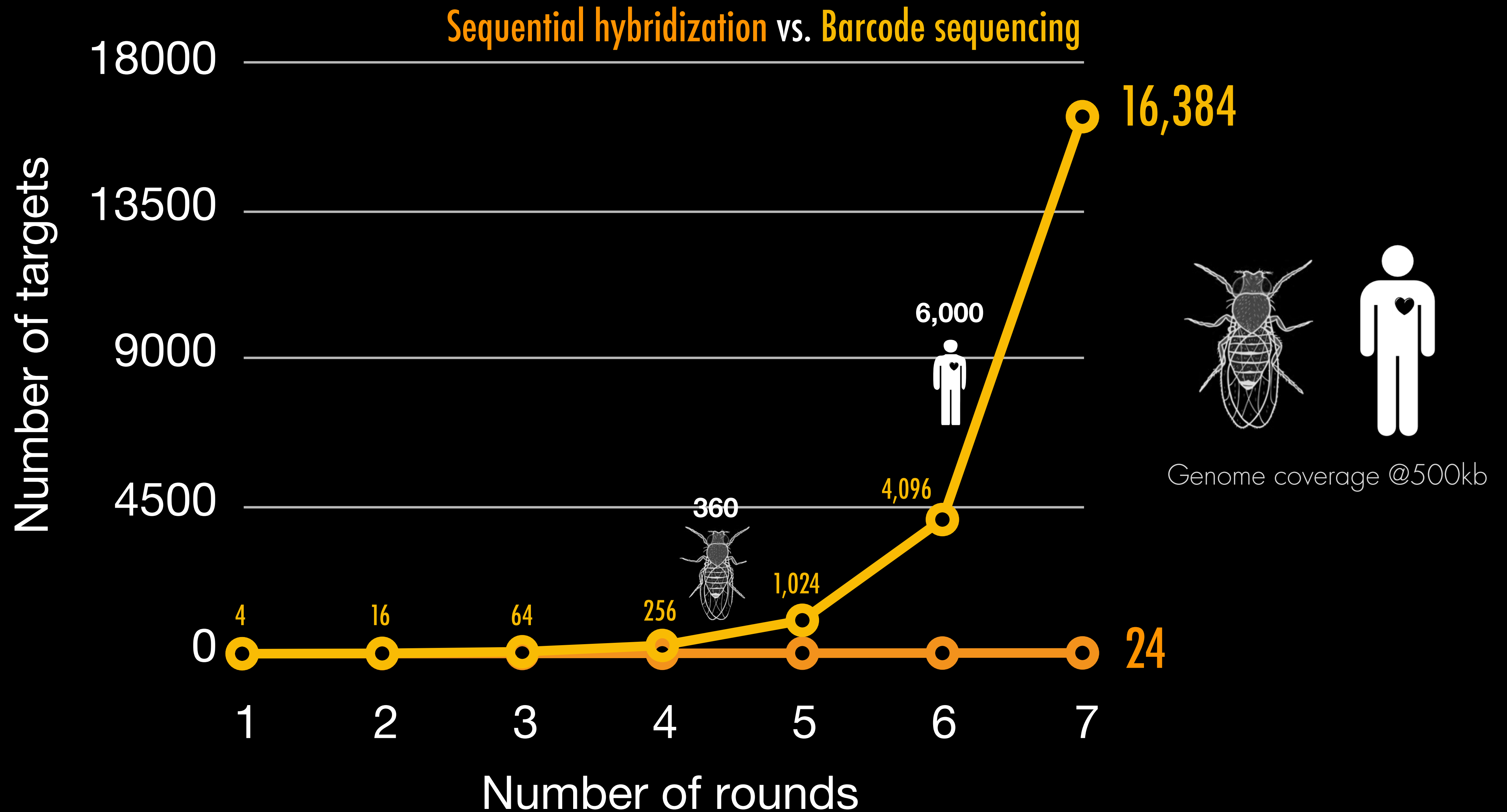


of targets = F^N

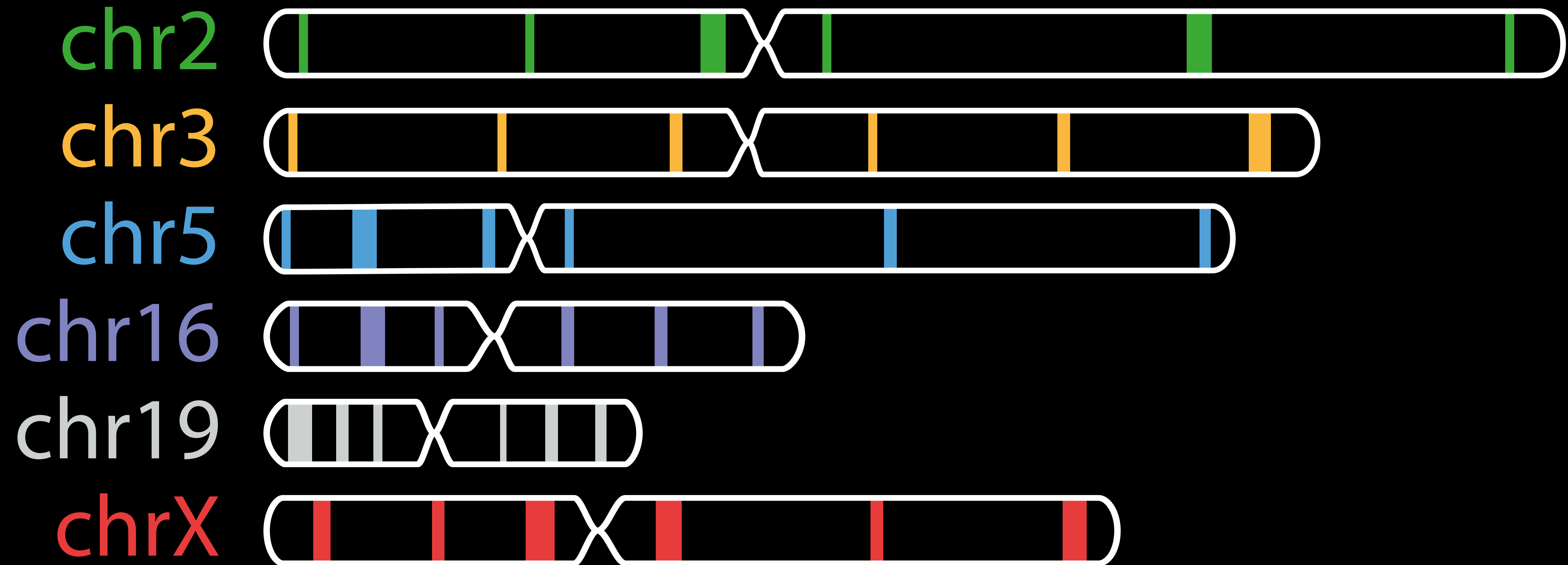
F = # of fluorophores

N = # of seq. rounds

OligoFISSEQ scales exponentially!



Proof-of-principle

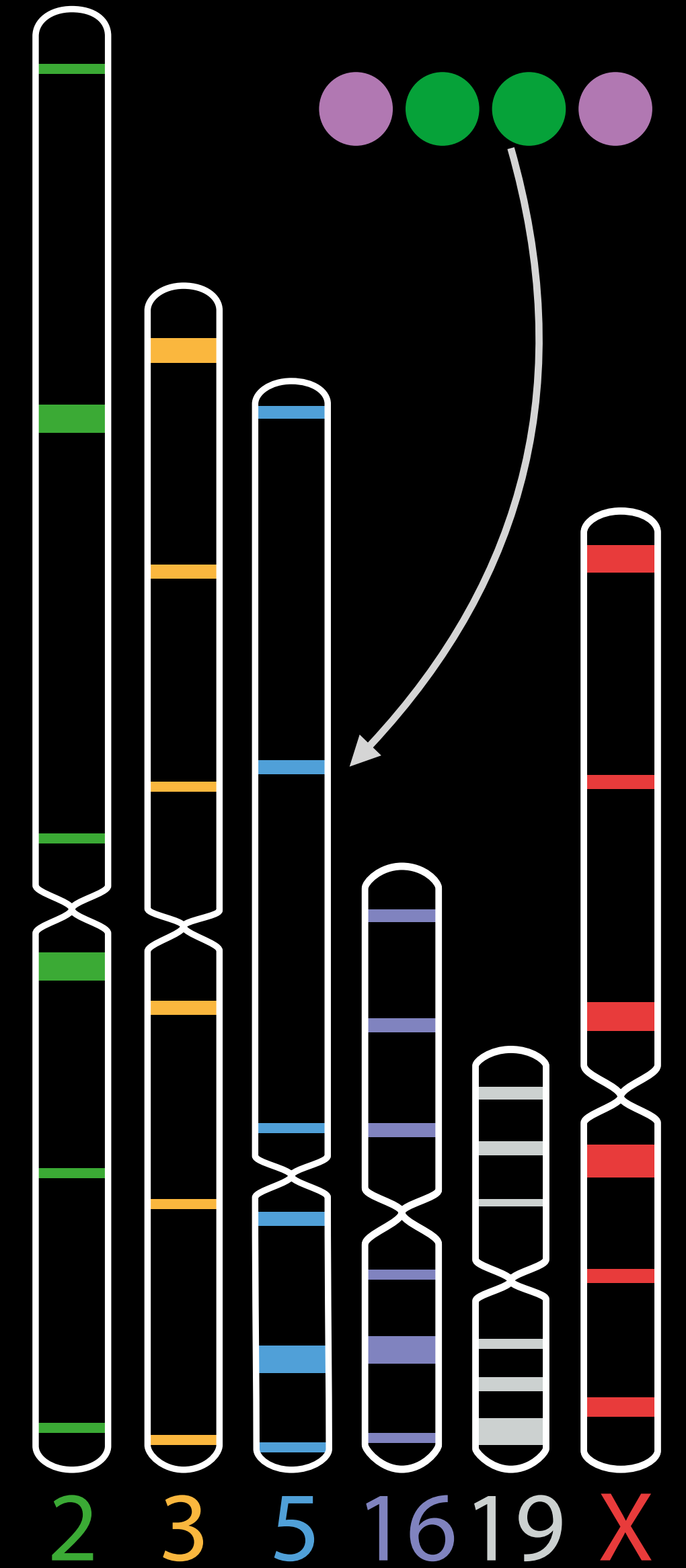
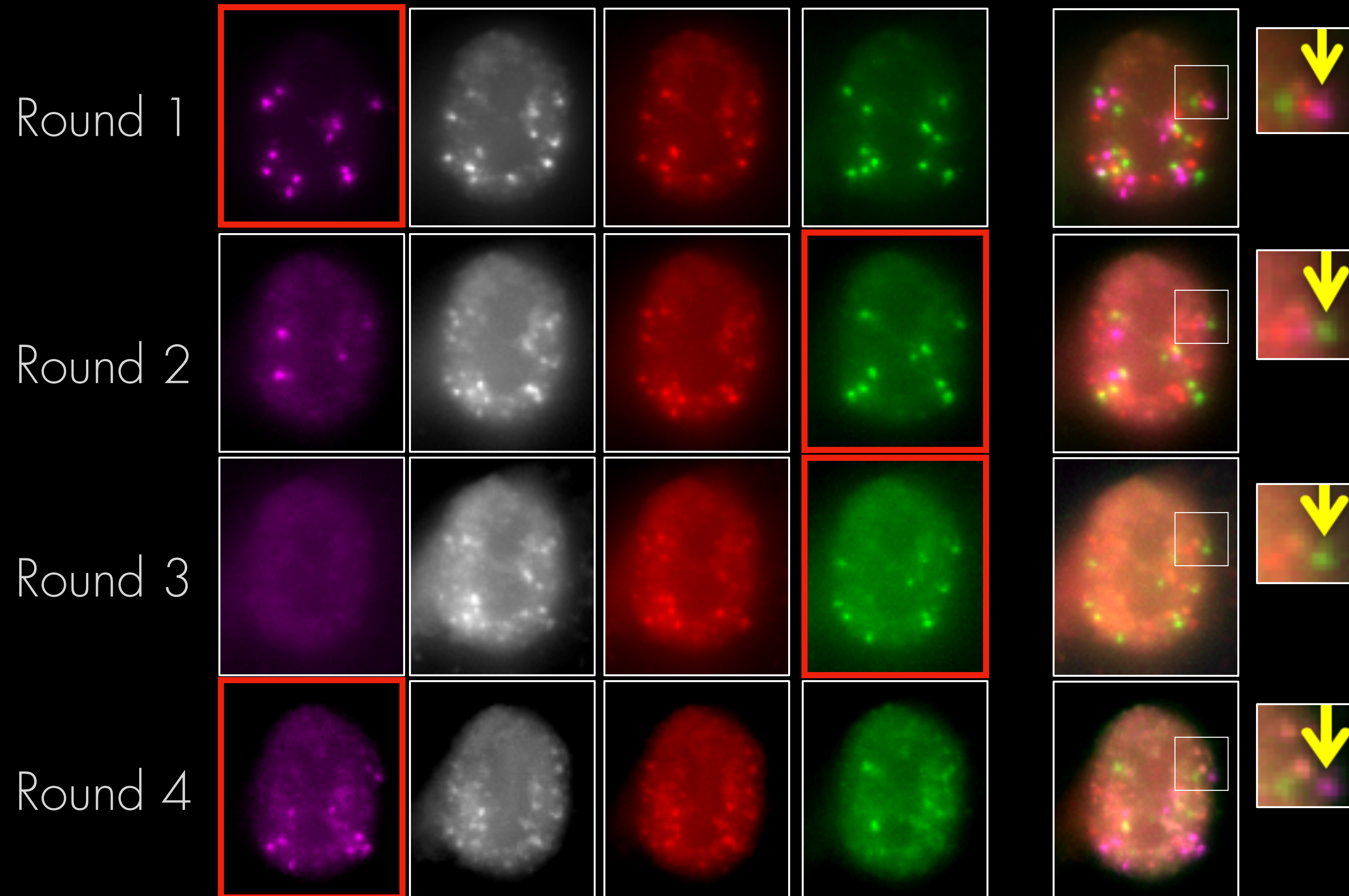


600kb-1Mb/target (876 kb average)

5,000 oligos/target

7-70Mb between targets

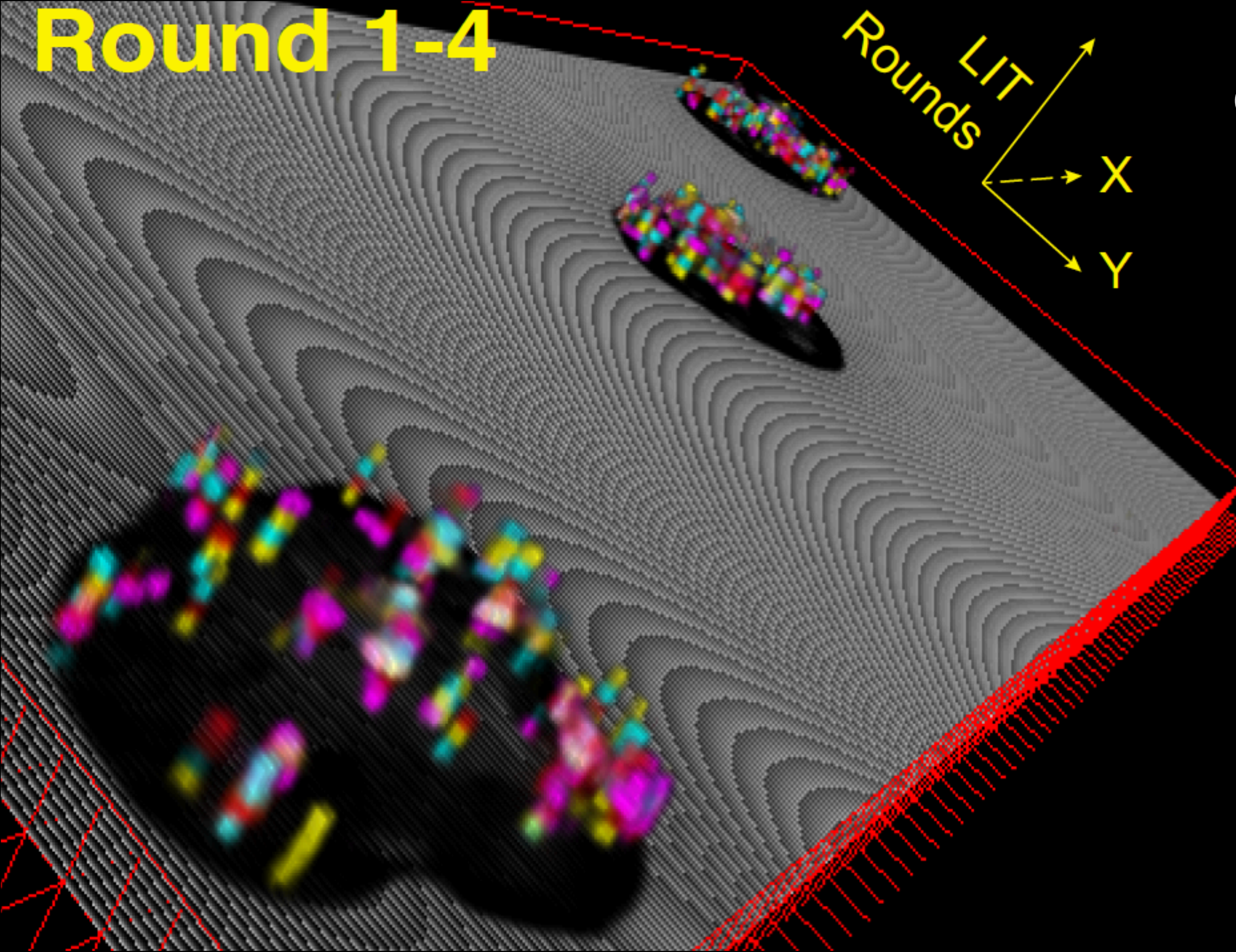
Detecting a given target



Round 1-4

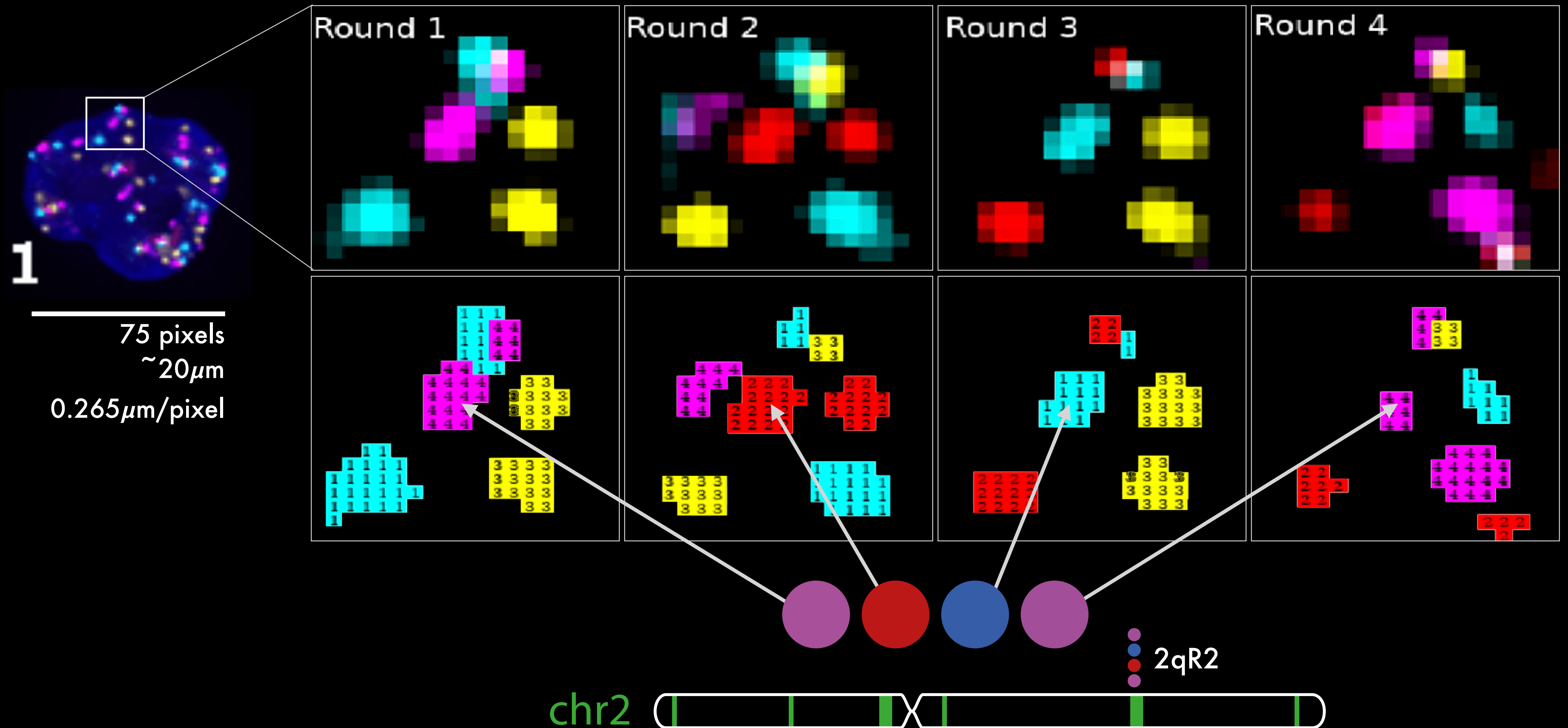
LIT
Rounds
X
Y

OligoFISSEQ
"Manhattan plot"

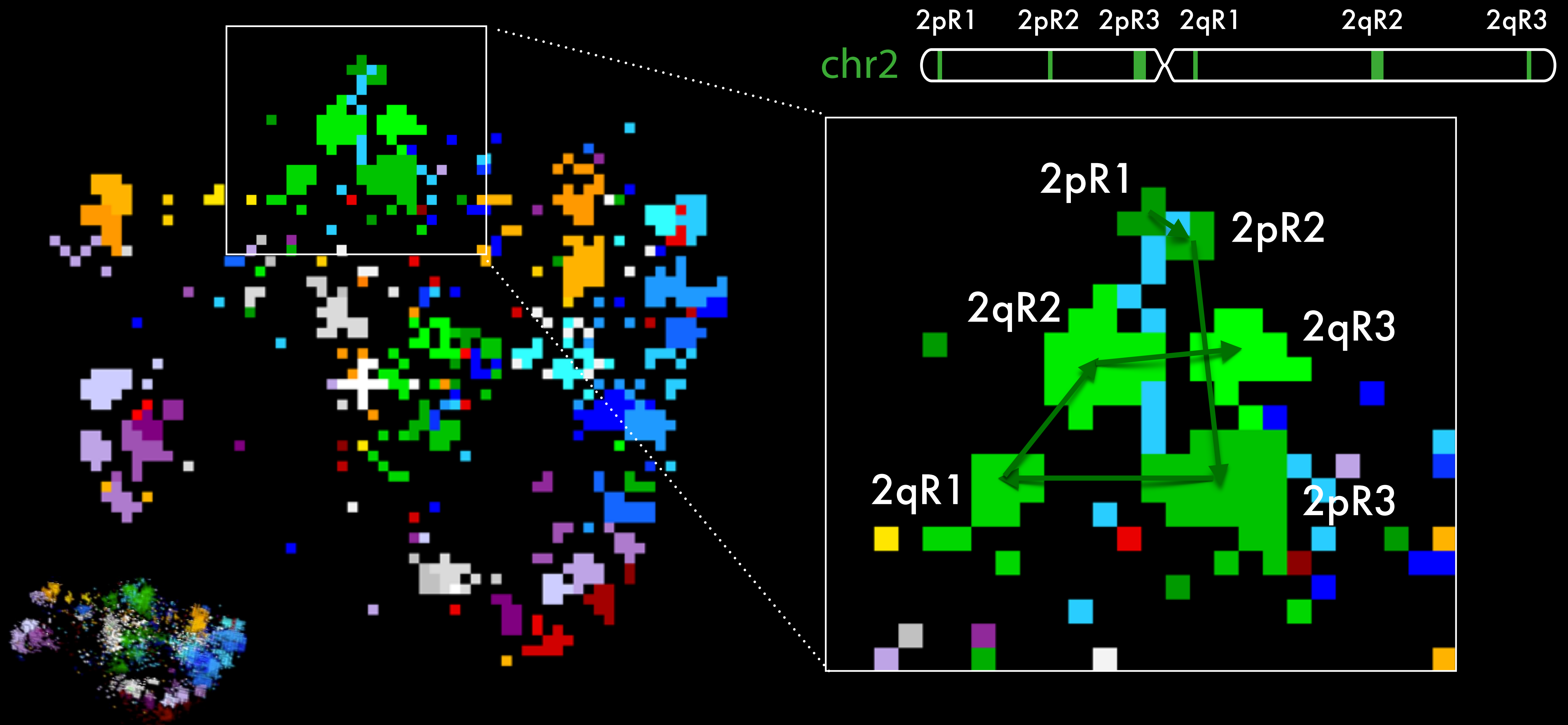


In OligoFISSEQ every pixel matters & make “patches”

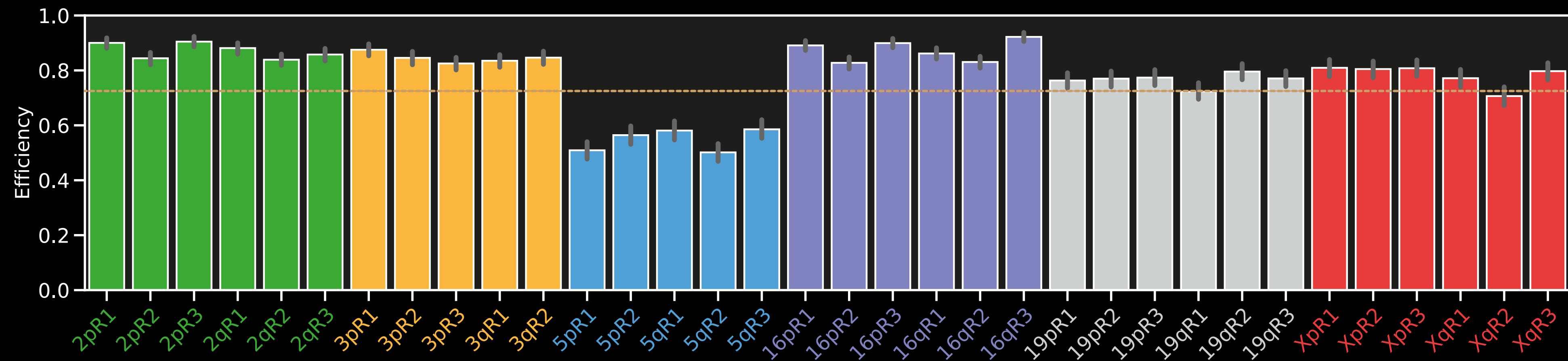
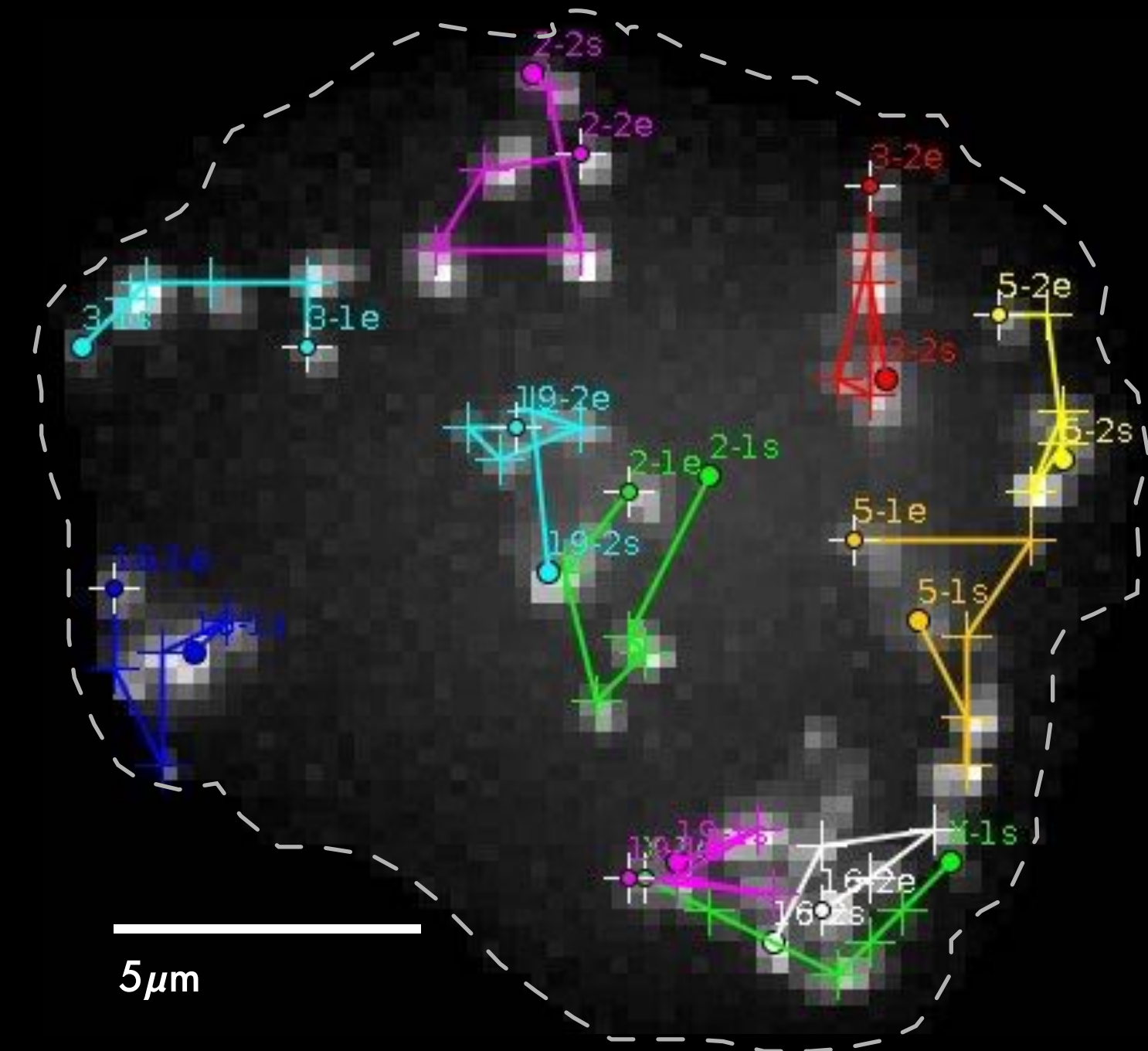
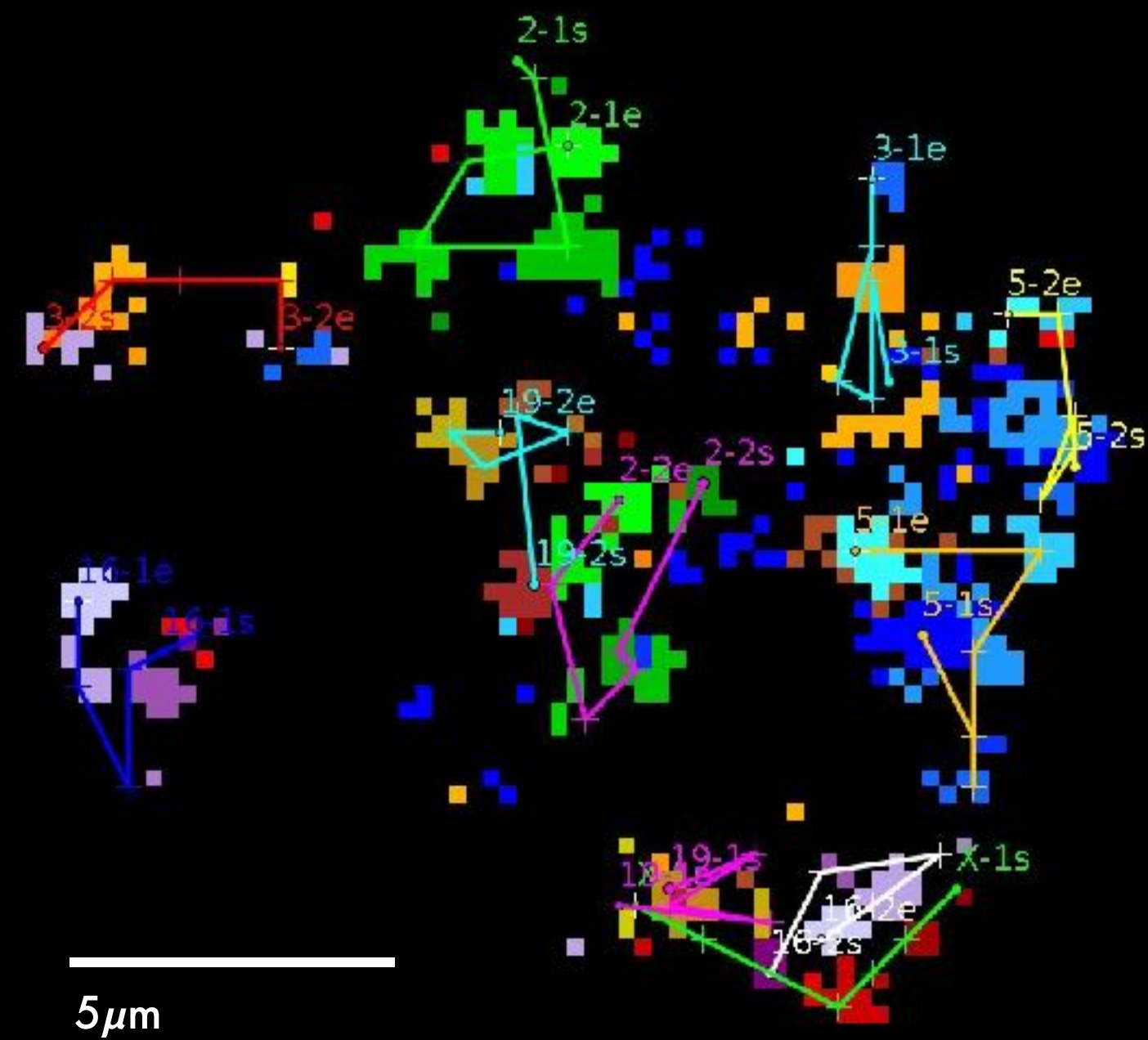
4 rounds / 4 channels



In OligoFISSEQ every pixel matters & make “patches”

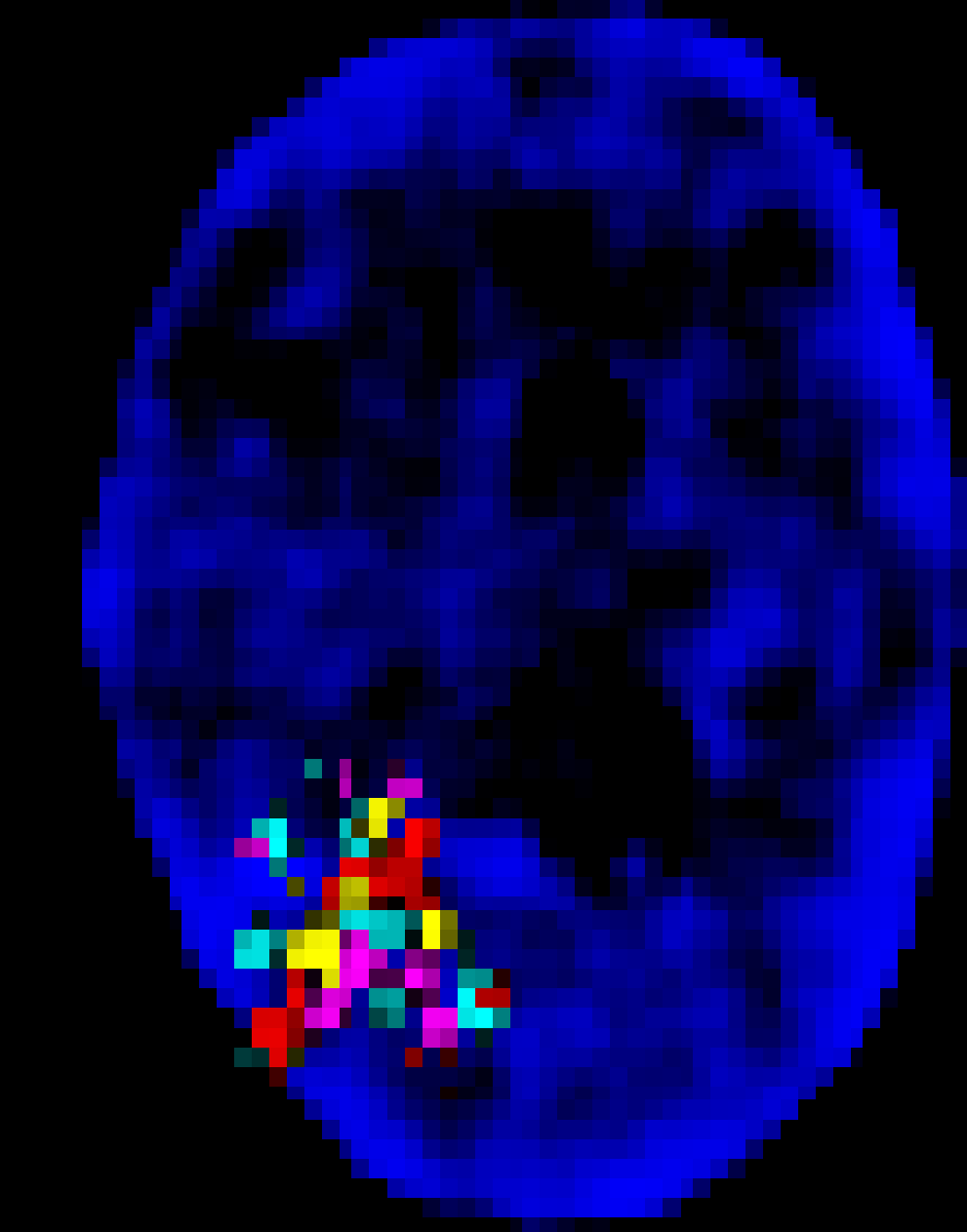
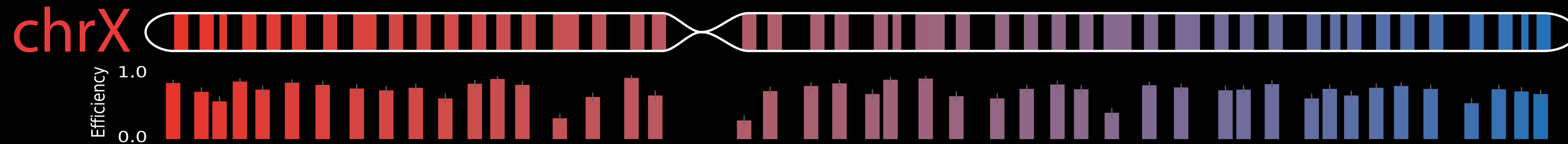


OligoFISSEQ barcode efficiency

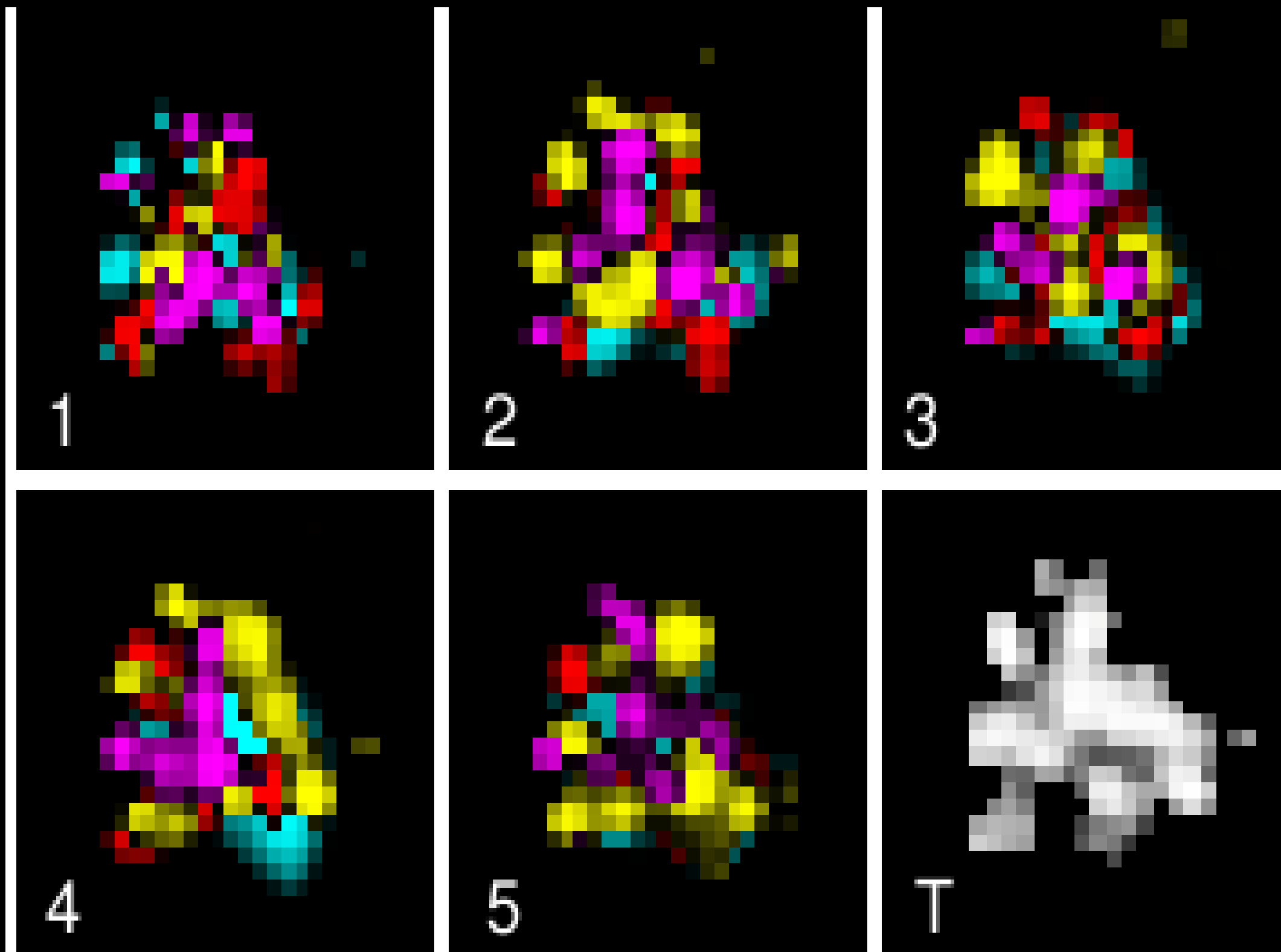


OligoFISSEQ tracing of (almost) entire chromosomes

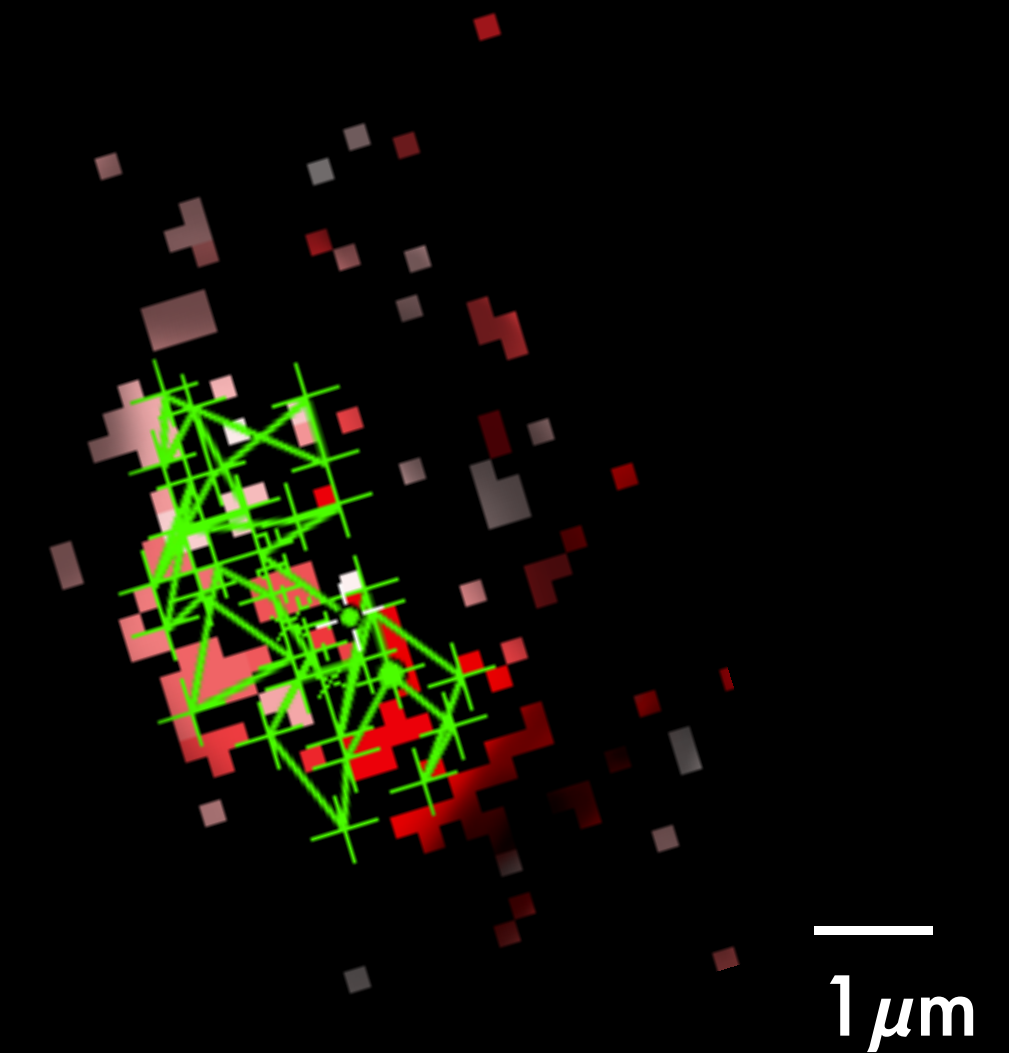
46 Plex in chromosome X



5 μ m

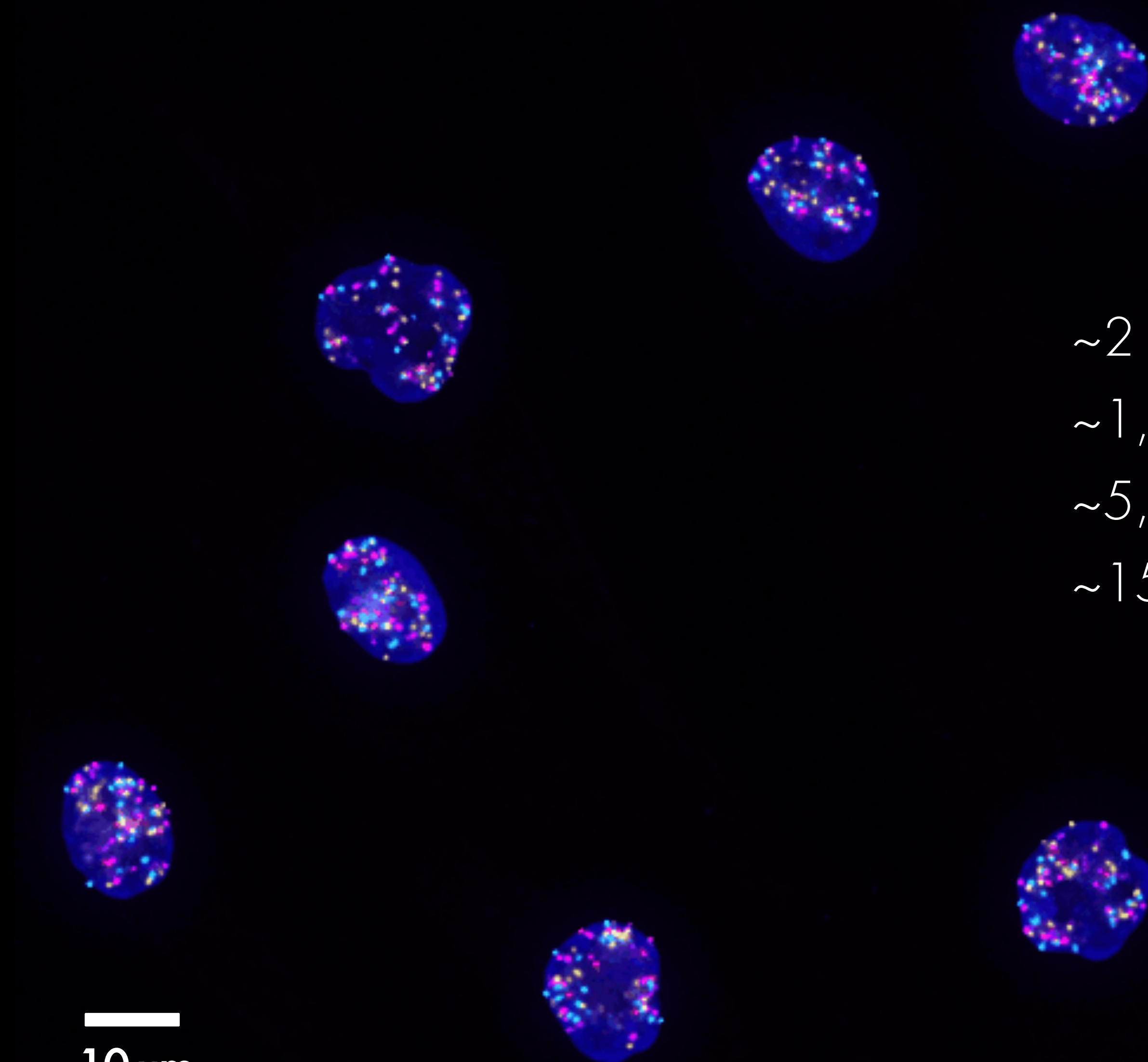


5 rounds
445 kb/probe
2,000 Oligopaints/probe
2 Mb between loci



1 μ m

OligoFISSEQ is high throughput!



~2 days of image acquisition

~1,000 cells

~5,000 complete chromosomes

~150 cells with complete chromosomes

10 μ m

OligoFISSEQ beyond chromosome tracing

OligoFISSEQ pipelined with OligoSTORM

chr2 

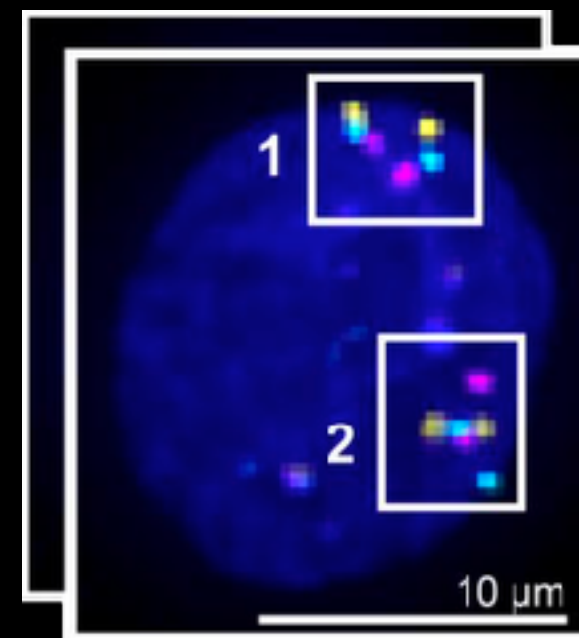
1

OligoSTROM
1 round
(2h/round)



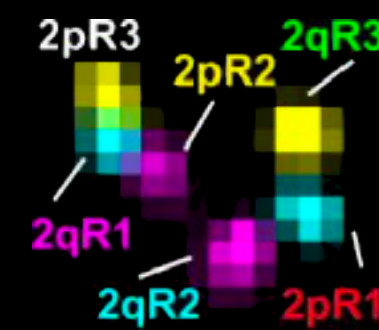
2

OligoFISSEQ
2 round
(3h/round)



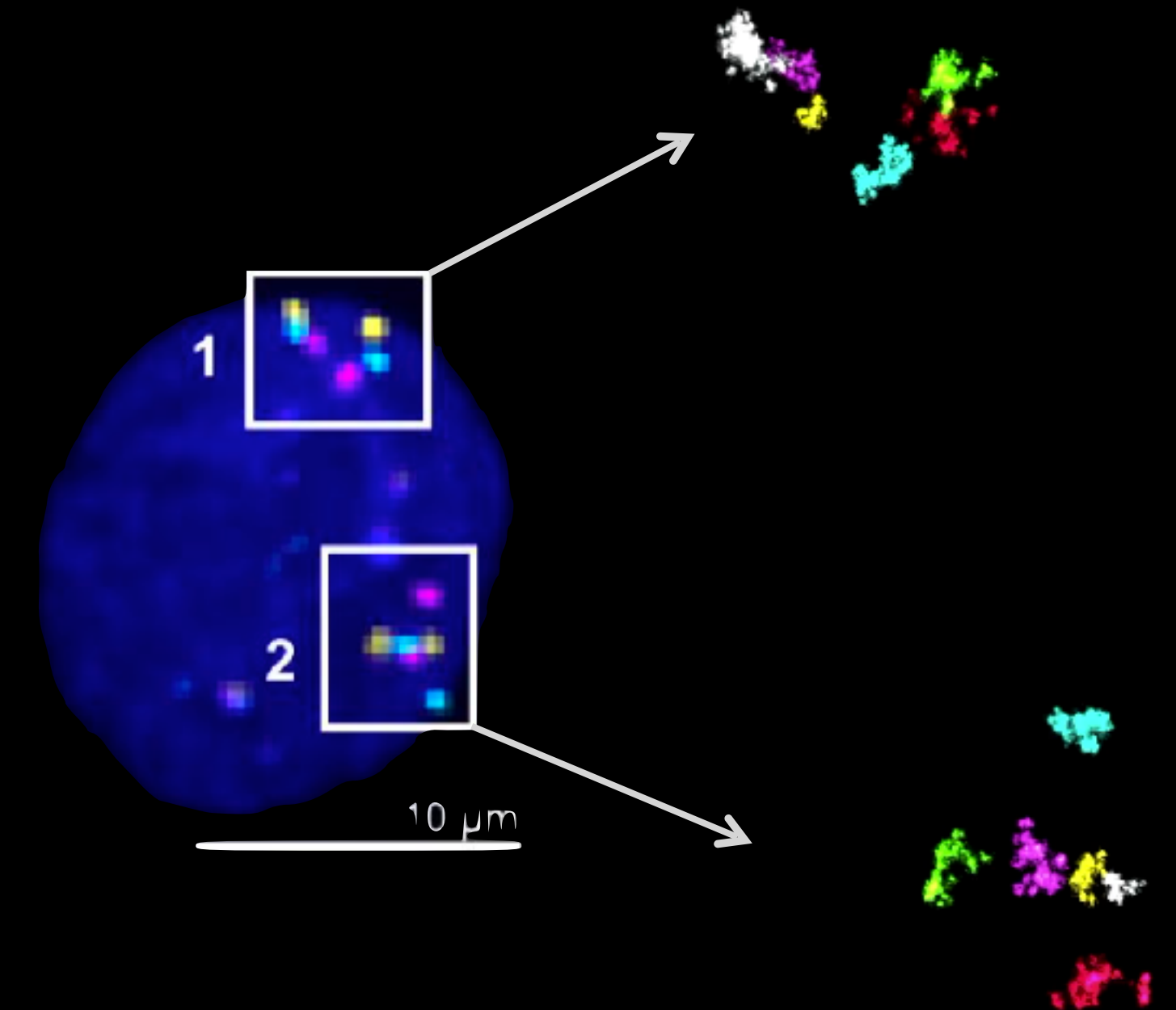
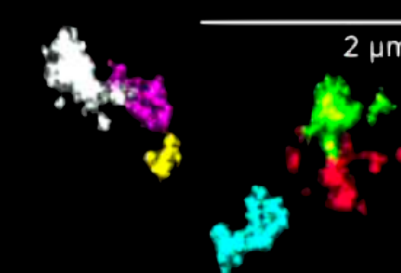
3

Decoding
OligoFISSEQ

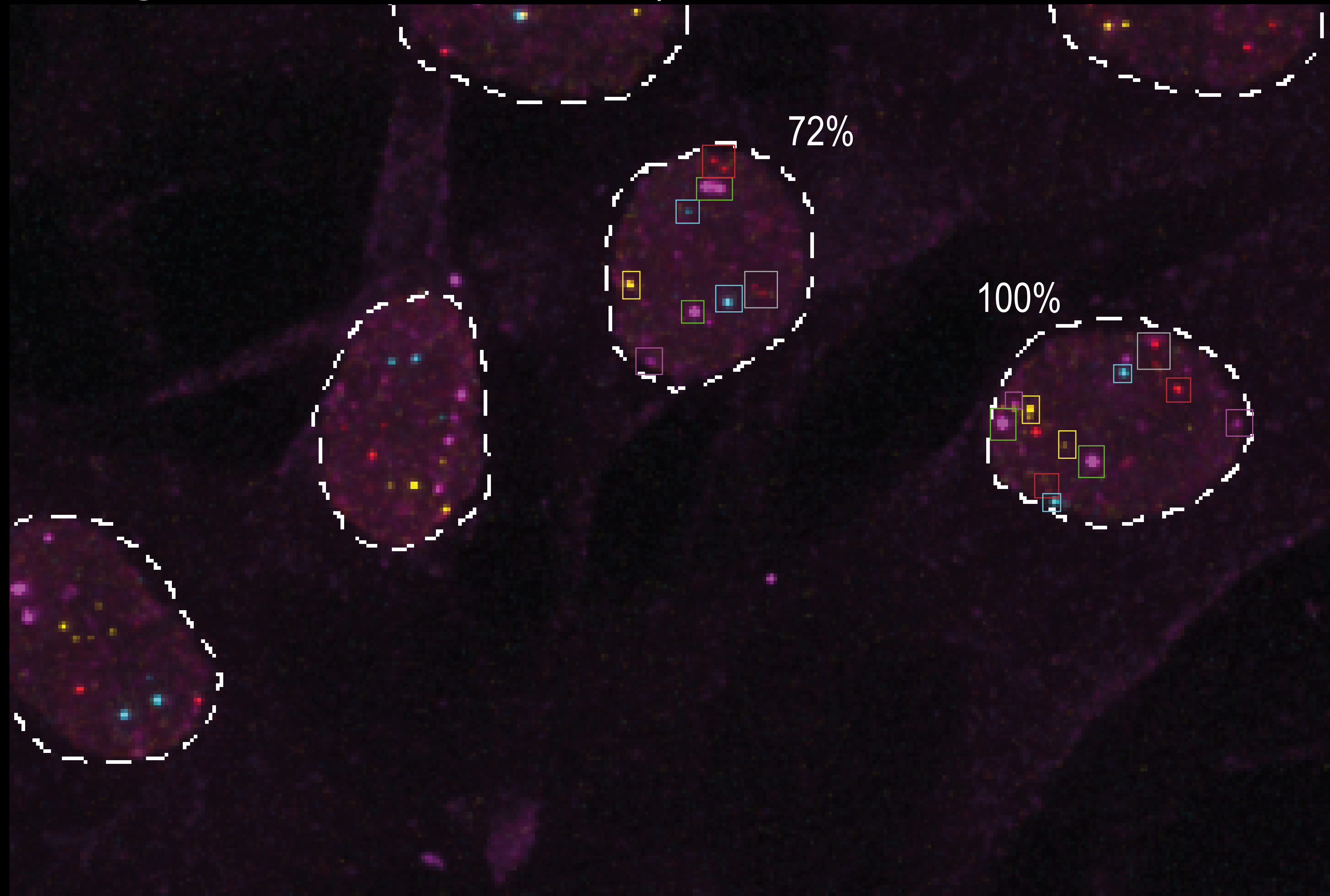
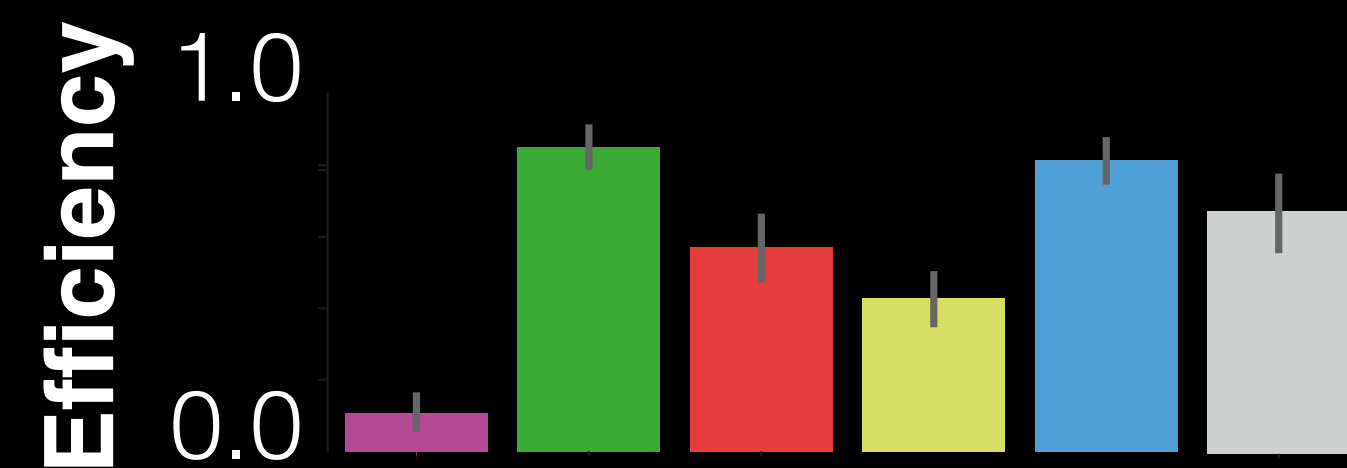
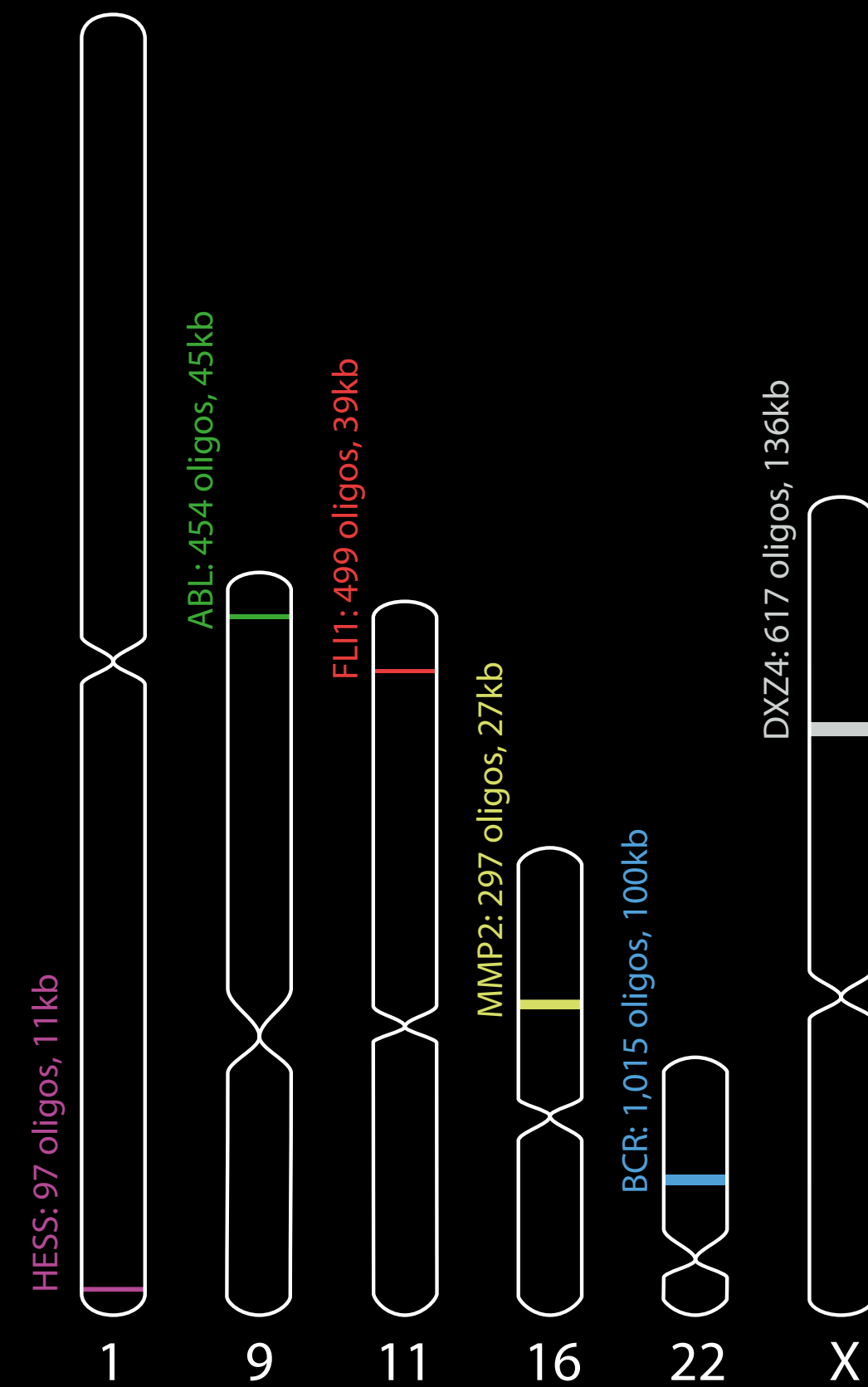


4

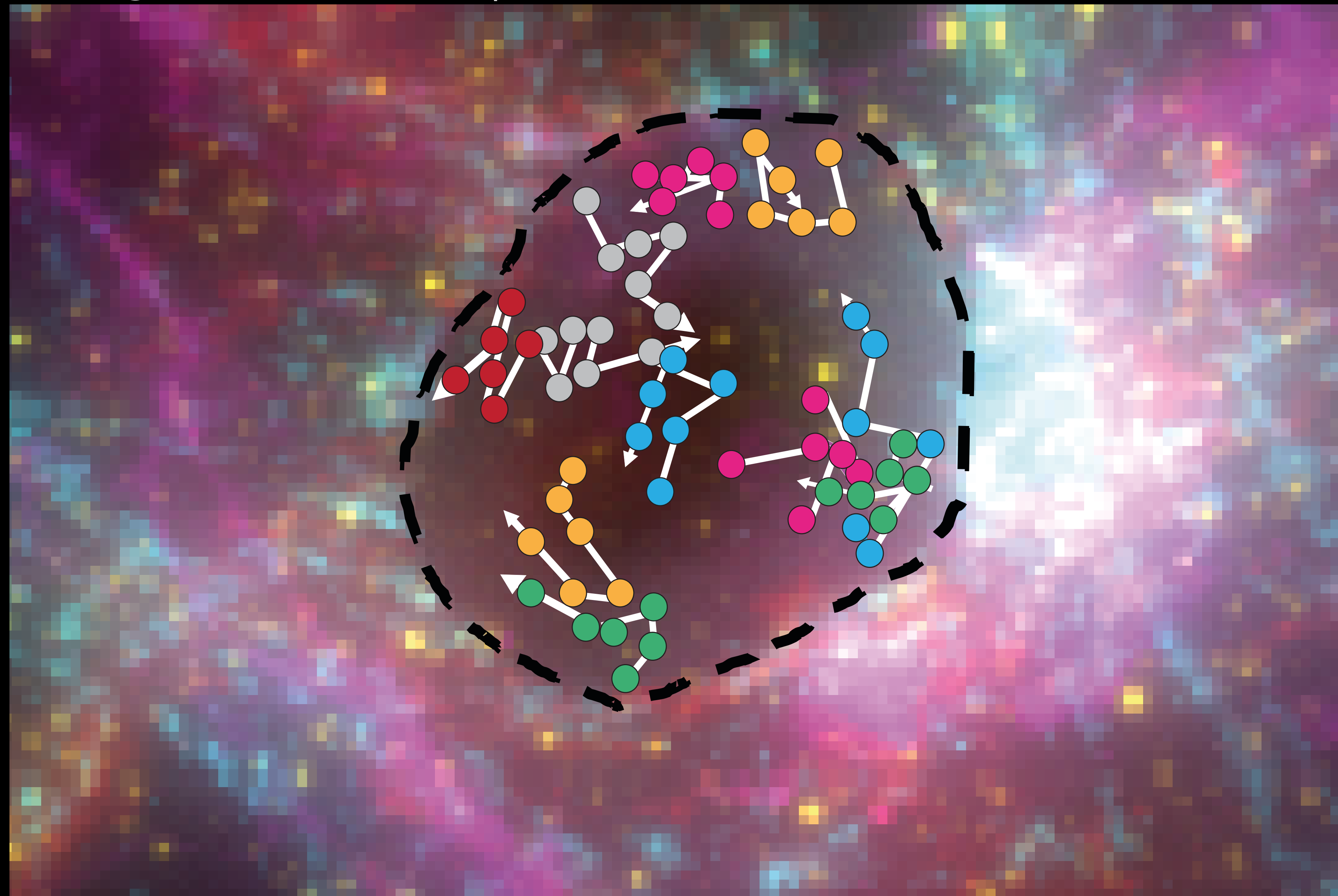
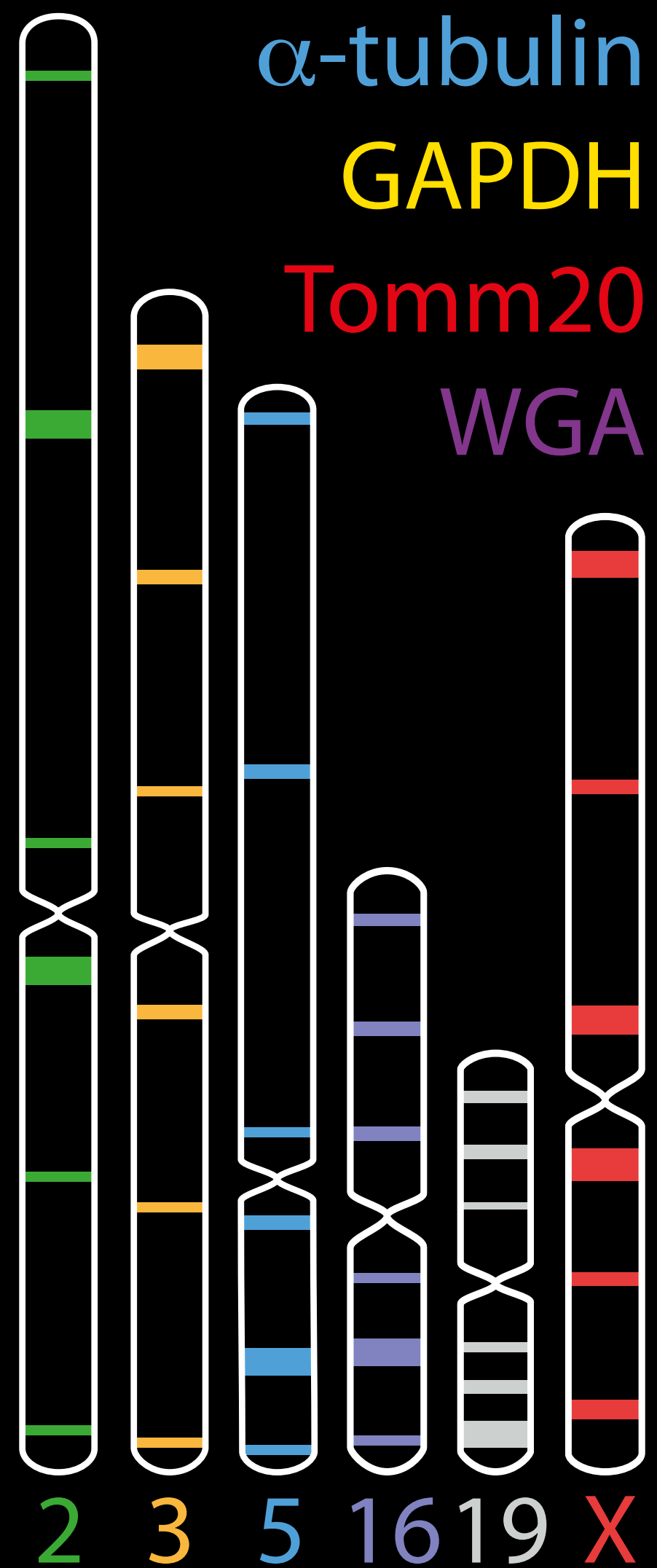
Mapping
OligoSTROM



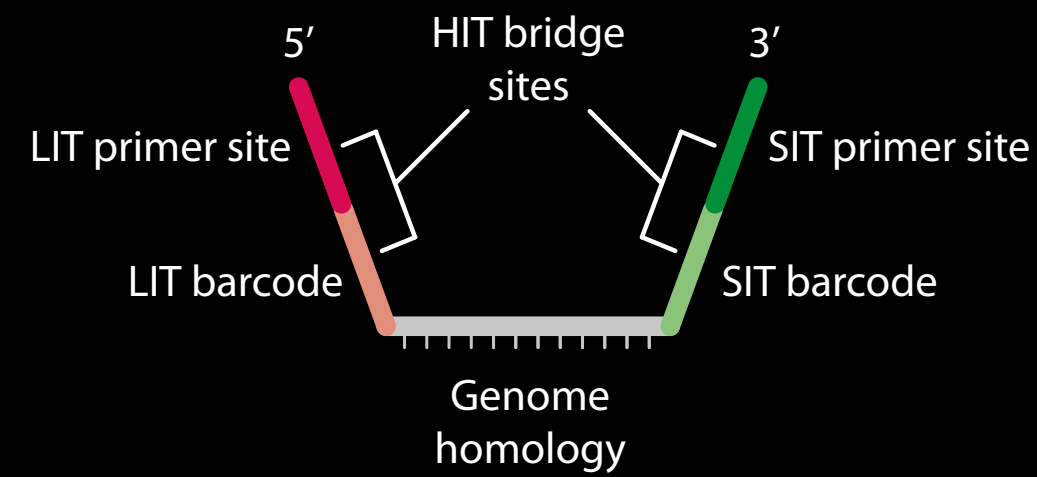
OligoFISSEQ for multiple loci detection



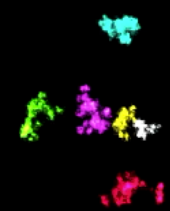
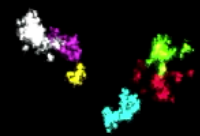
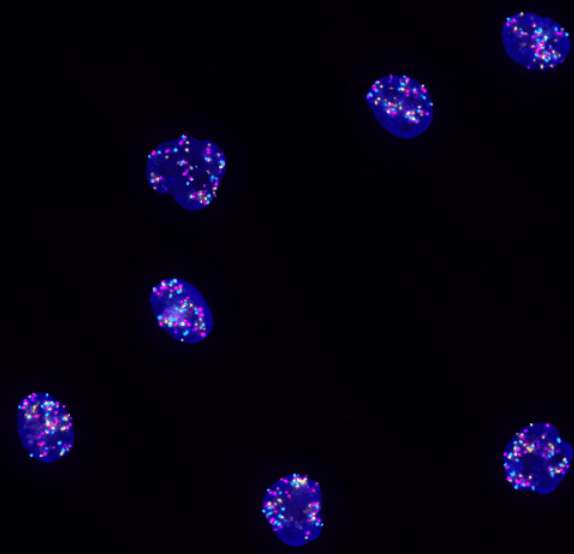
OlgoFISSEQ + protein immunofluorescence



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>

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Regulation

ICREA

David Castillo

Yasmina Cuartero

Silvia Galan

Rodrigo Jara

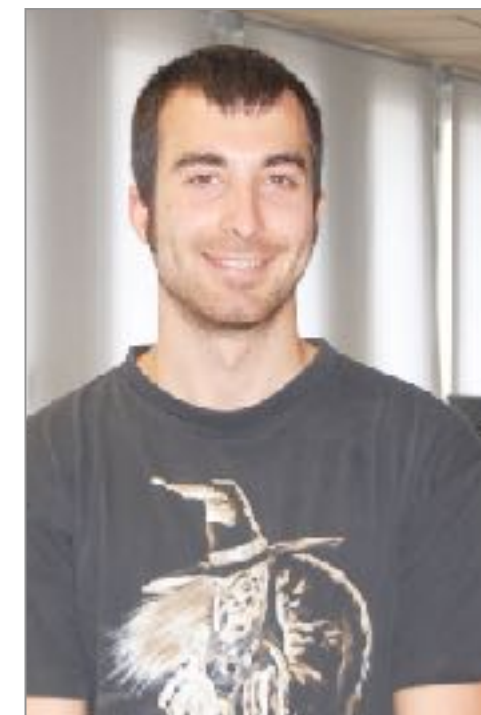
Iana Kim

Maria Marti-Marimon

Francesca Mugianesi

Julen Mendieta

Aleksandra Sparavier



Marco Di Stefano
Irene Farabella
Mike Goodstadt
Juan A. Rodriguez



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

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