

Structure determination of genomes and genomic domains by image tracing

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



Complex genome organization

Cavalli, G. & Misteli, T. Functional implications of genome topology. Nat Struct Mol Biol 20, 290–299 (2013).





Number of cells

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





homologous 32-42bp

Beliveau et al. Nat. Comm. 2015

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



High-resolution imaging Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



High-resolution imaging Tracing chr19:7,335,095-15,449,189 ~8Mb 1 2 3 4 5 6 7 8 9

1,800Kb

| 1,280Kb | 1,240Kb |
|---------|---------|

1,040Kb

520Kb 520Kb 840Kb

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Kb 520Kb 360Kb

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Farabella et al, J Appl Crystallogr. 2015



Density maps Cell-02 · Density map @ 50nm



Area (nm^2) Volume (nm³) 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



Area



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)







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

89

Cluster properties A/B compartment properties











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







Increasing resolution Flexible fitting 3D structures based on Hi-C data





Chromosome walking path @10Kb resolution



Mapping "omics" 3D organization of local structures









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









LIT barcode

HIT bridge 3' sites SIT primer site SIT barcode

Genome homology



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

OligoFISSEQ



LIT primer site

LIT barcode



OligoFISSEQ



OligoFISSEQ scales exponentially!

Sequential hybridization



of targets = F*NF = # of fluorophores N = # of seq. rounds

Barcode sequencing



of targets = FNF = # of fluorophores N = # of seq. rounds



Proof-of-principle



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

Round 1

Round 2

Round 3

Round 4



Detecting a given target





OligoFISSEQ "Manhattan plot"

--**≻** X



In OligoFISSEQ every pixel matters & make "patches" 4 rounds / 4 channels















OligoFISSEQ barcode efficiency



OligoFISSEQ tracing of (almost) entire chromosomes 46 Plex in chromosome X

chrX (













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 <u>complete</u> chromosomes ~150 cells with <u>complete</u> chromosomes

OligoFISSEQ beyond chromosome tracing

OligoFISSEQ for multiple loci detection

72%









OligoFISSEQ + protein immunofluorescence







OligoFISSEQ pipelined with OligoSTORM chr2

OligoSTROM 1 round

(2h/round)

OligoFISSEQ 2 round (3h/round)

 \bigcirc

Decoding OligoFISSEQ

3



1 30

1 1











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- Is a <u>set of technologies</u> for in-situ genome mapping Is <u>highly versatile</u>: mainstreet and backstreet
- - Used with wide-field microscopy allows for the analysis of thousands of cells. Identifies <u>sub-clusters</u> with specific conformational characteristics

- Can be <u>pipelined</u> with other approaches
 - OligoSTORM \bullet
 - Protein immunofluorescence
 - RNA...

OligoFISSEQ



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



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





.: Our current sponsors :.





National Human Genome Research Institute

.: Conflict of Interest Statement :.



https://www.chromdesign.eu/epic-conference/



EpIC - EpiGene3Sys meets INC-Spain



David S. Kong MIT Media Lab **United States**

ChromDesign: MSCA interdisciplinary training network, grant agreement 813327 **INC-Spain:** Spanish network of the International Nucleome Consortium funded by



