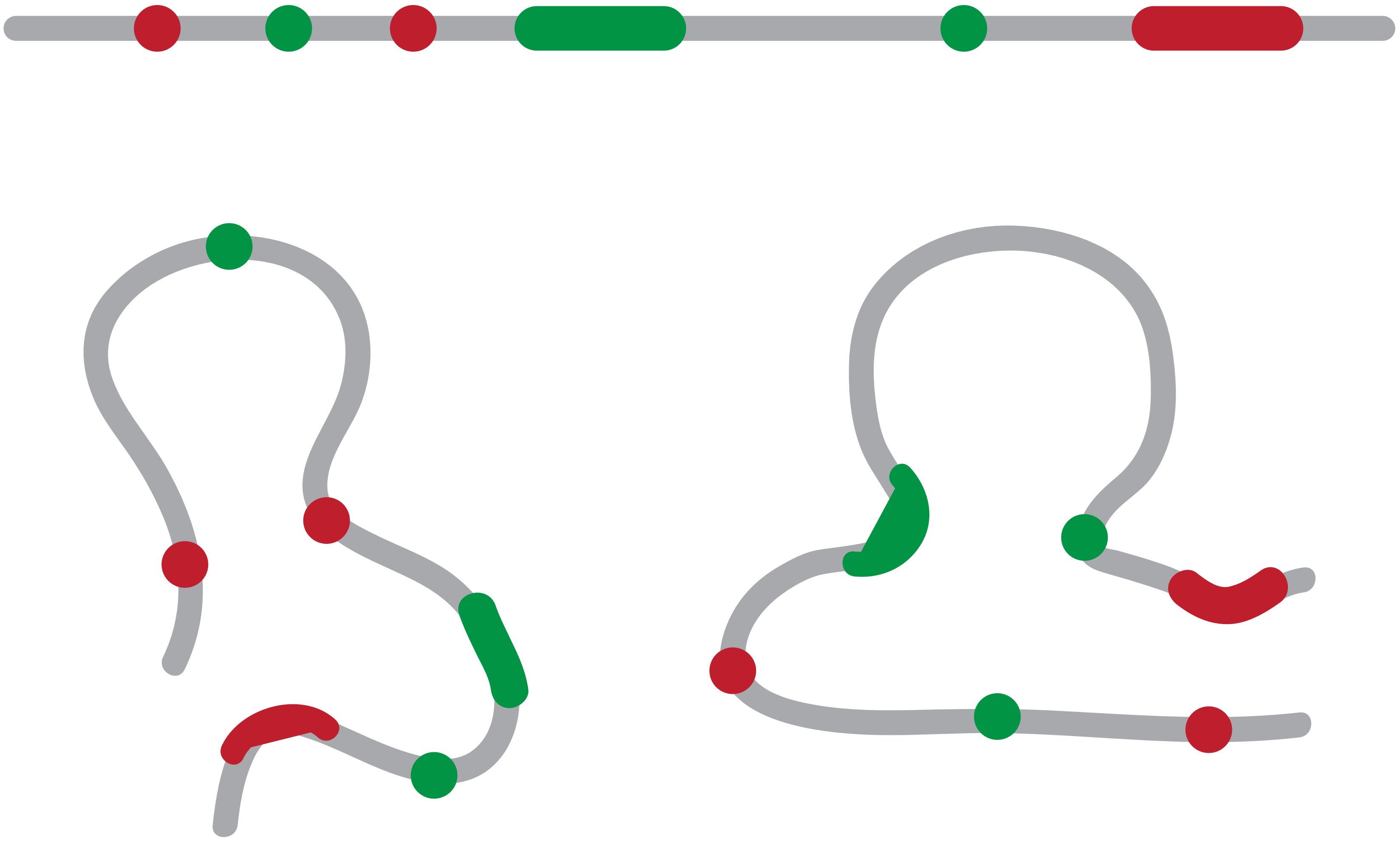
3DGenomics for genome engineering

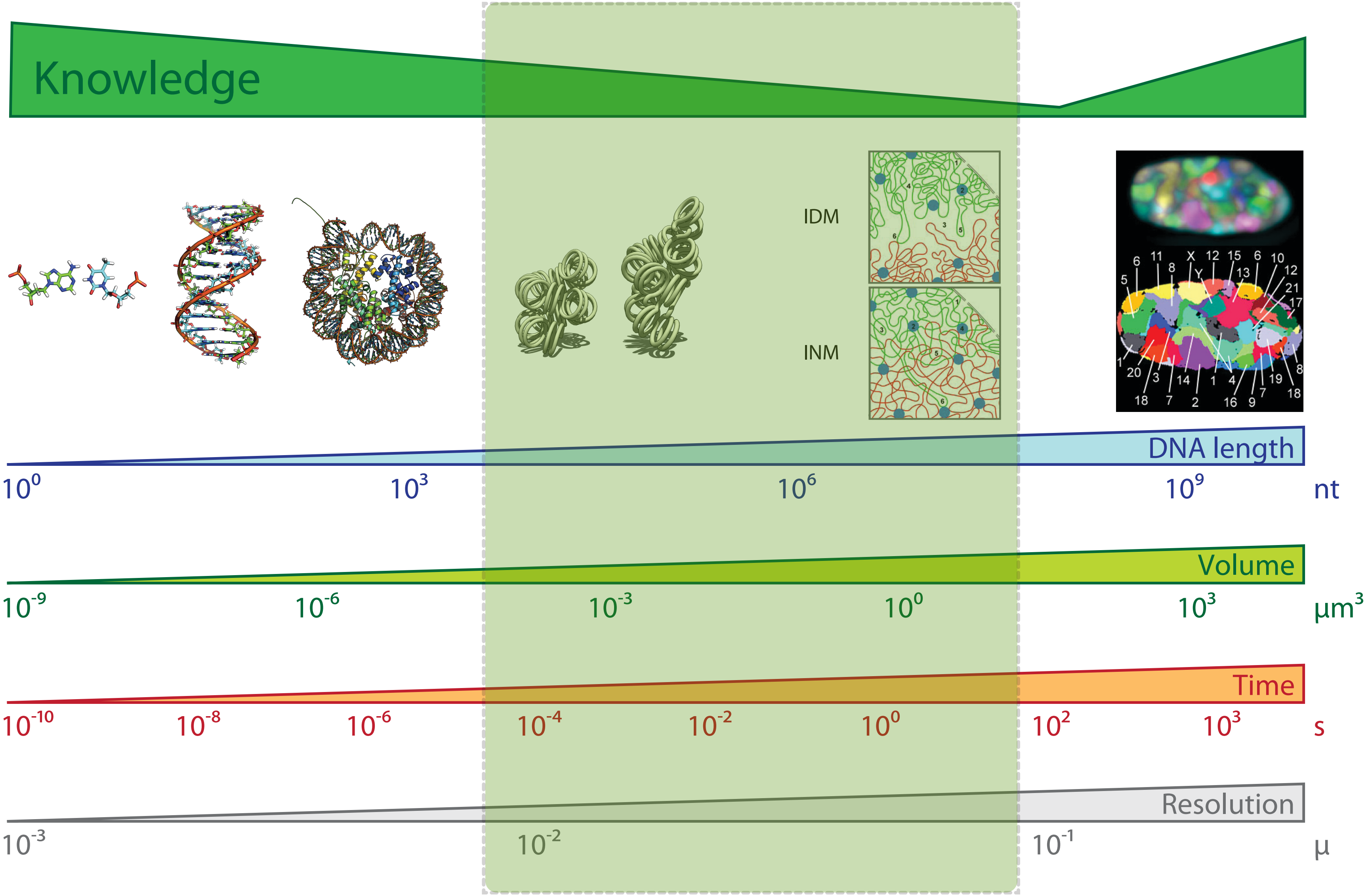
Marc A. Marti-Renom
Structural Genomics Group (ICREA, CNAG-CRG)

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



Resolution Gap

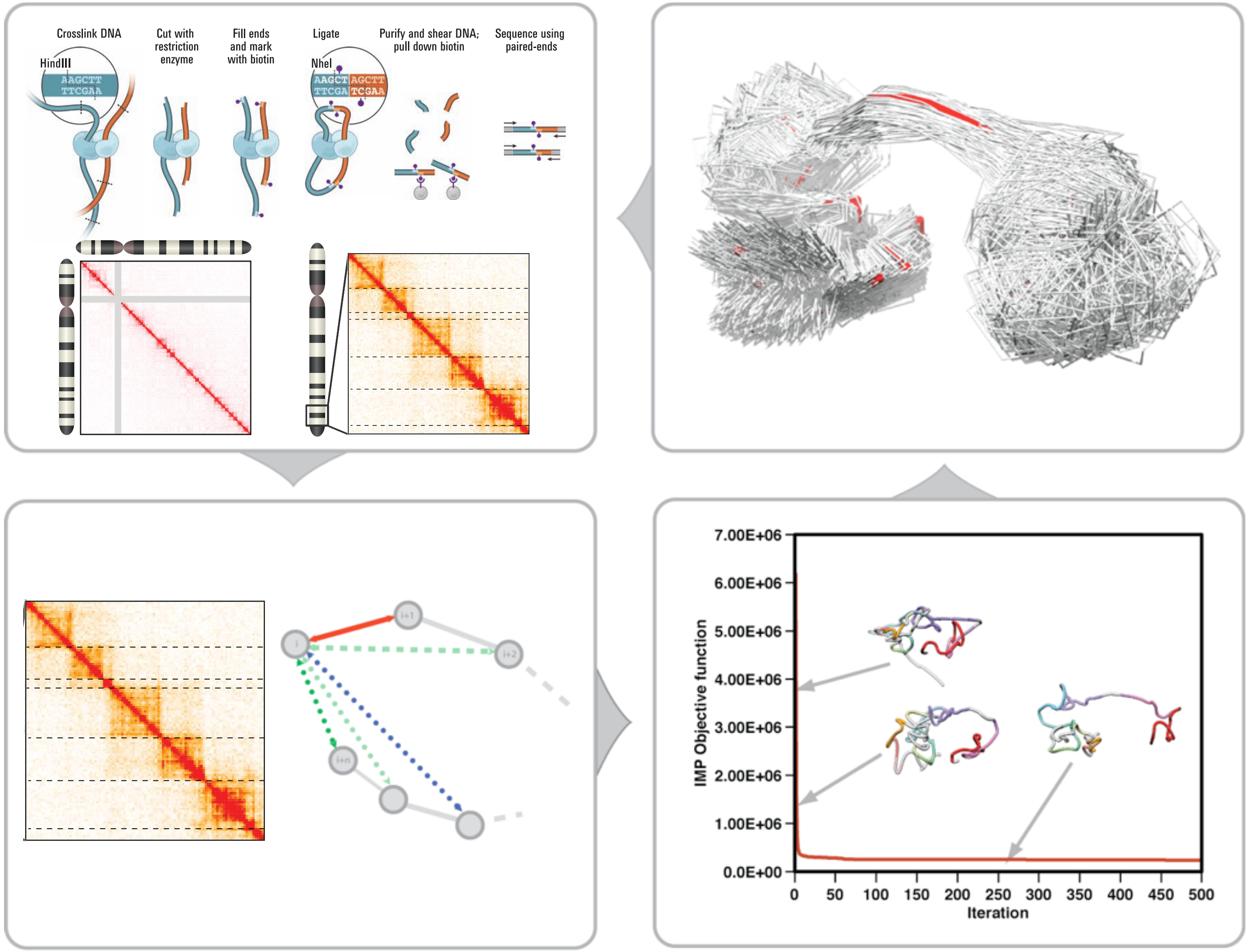
Marti-Renom, M. A. & Mirny, L. A. PLoS Comput Biol 7, e1002125 (2011)



Hybrid Method

Baù, D. & Marti-Renom, M. A. Methods 58, 300–306 (2012).

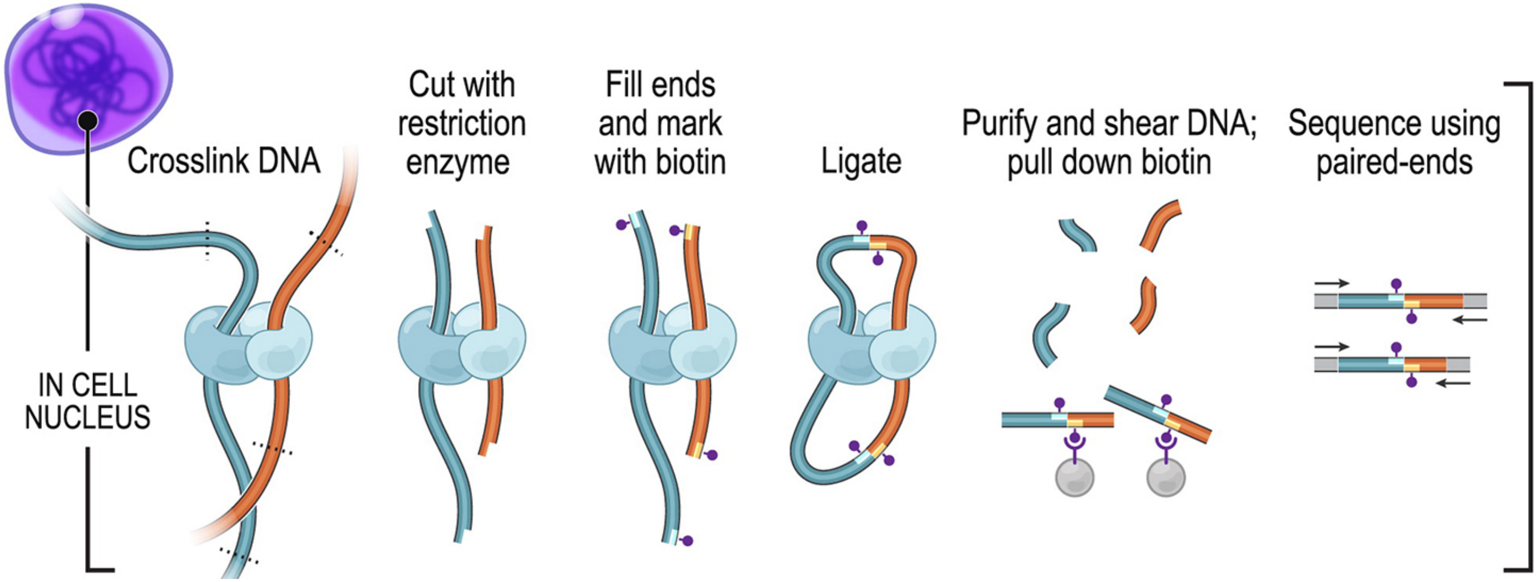
Experiments



Computation

Chromosome Conformation Capture

Dekker, J., Rippe, K., Dekker, M., & Kleckner, N. (2002). Science, 295(5558), 1306–1311.
Lieberman-Aiden, E., et al. (2009). Science, 326(5950), 289–293.



IN CELL NUCLEUS

Crosslink DNA

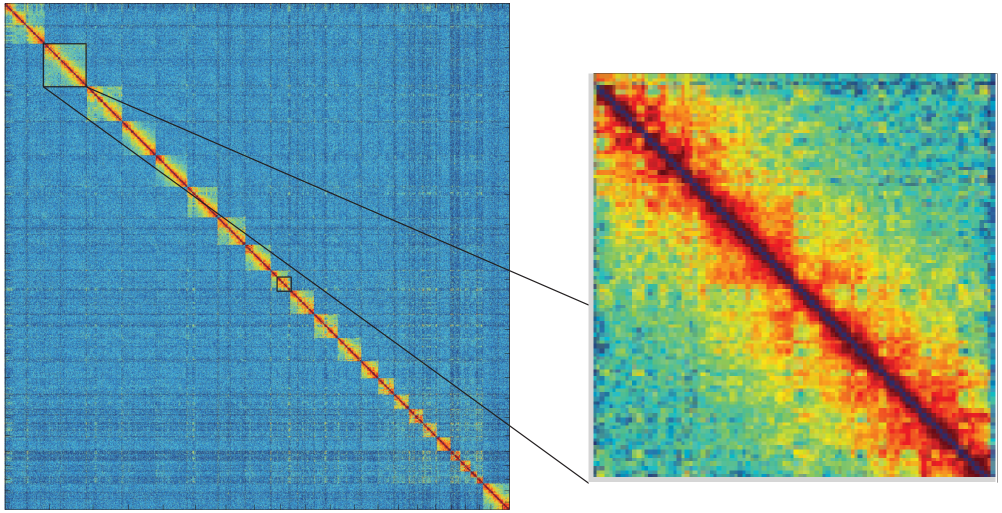
Cut with restriction enzyme

Fill ends and mark with biotin

Ligate

Purify and shear DNA; pull down biotin

Sequence using paired-ends



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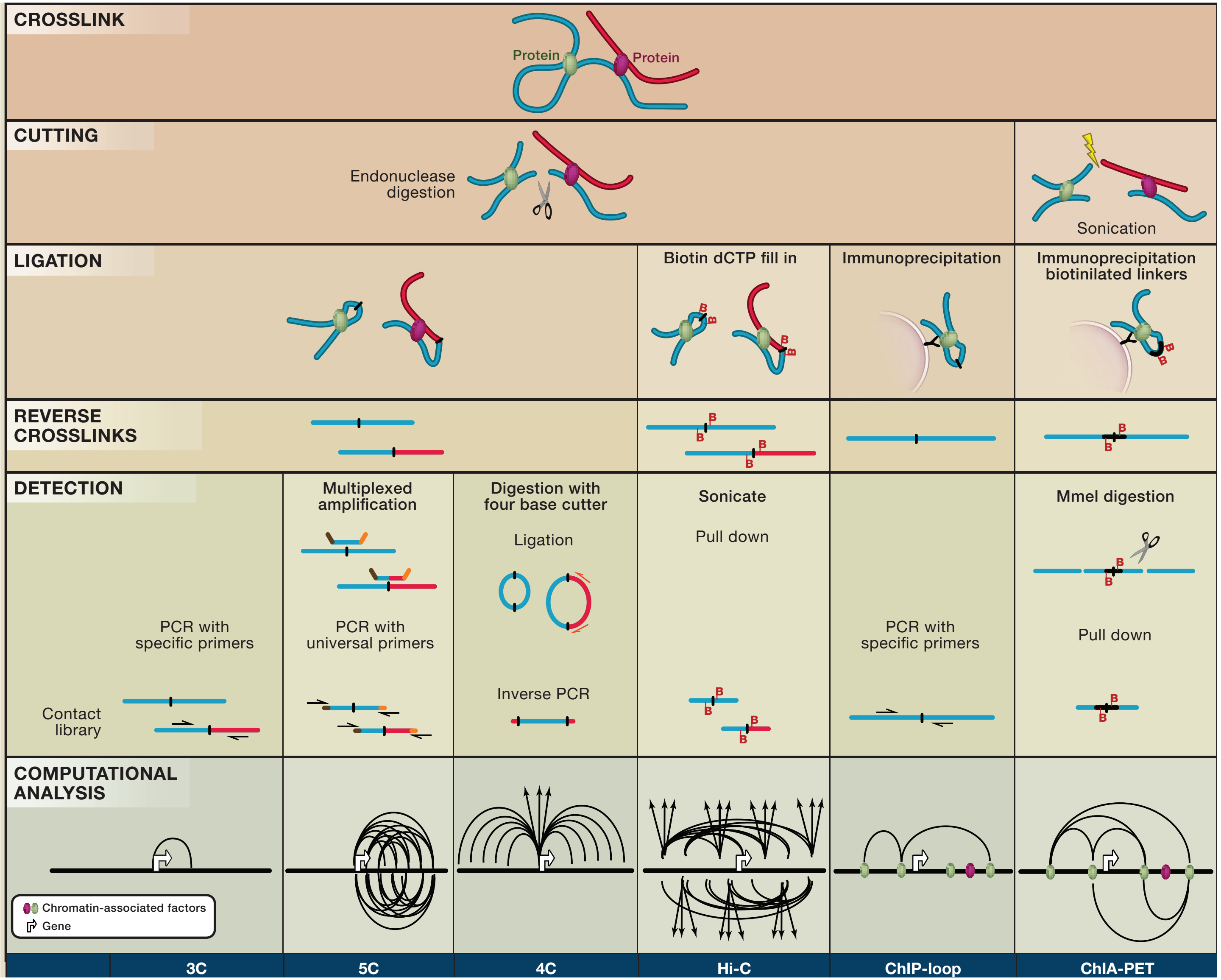


CROSSLINK

Protein

Protein

CUTTING

Endonuclease digestion

Sonication

LIGATION

Biotin dCTP fill in

Immunoprecipitation

Immunoprecipitation biotinilated linkers

REVERSE CROSSLINKS

DETECTION

PCR with specific primers

Contact library

Multiplexed amplification

PCR with universal primers

Digestion with four base cutter

Ligation

Inverse PCR

Sonicate

Pull down

PCR with specific primers

MmeI digestion

Pull down

COMPUTATIONAL ANALYSIS

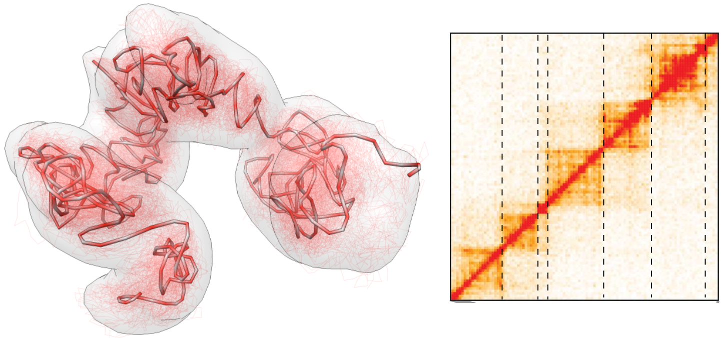
Chromatin-associated factors

Gene

3C | **5C** | **4C** | **Hi-C** | **ChIP-loop** | **ChIA-PET**

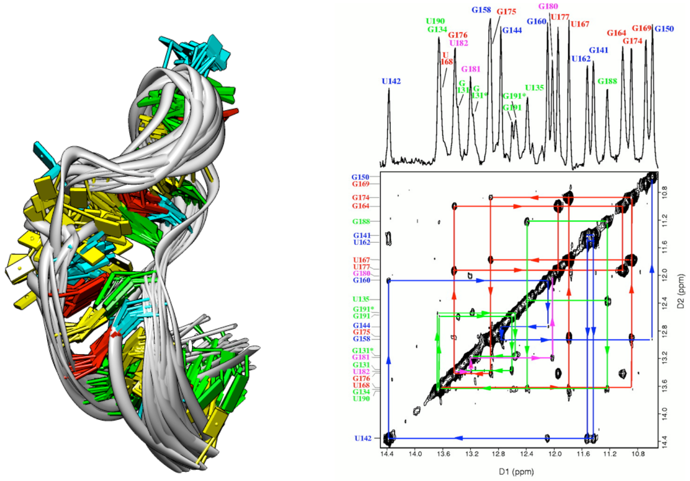
Restraint-based Modeling

Baù, D. & Marti-Renom, M. A. Methods 58, 300–306 (2012).



Chromosome structure determination

3C-based data



Biomolecular structure determination

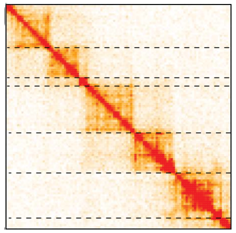
2D-NOESY data

TADbit

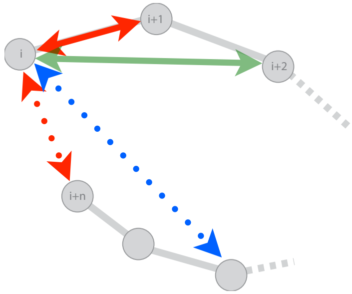
http://3DGenomes.org

```
@FORJUSP02AJWD1
CCGTCAATTCATTTAAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGT
+
AAAAAAAAAAAA::99@::::??@@::FFAAAAACCAA::::BB@@?A?
```

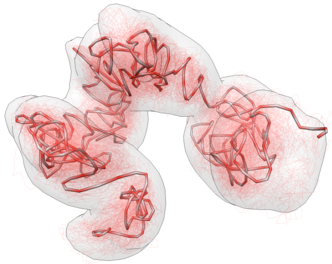
FastQ files to Maps



Map analysis



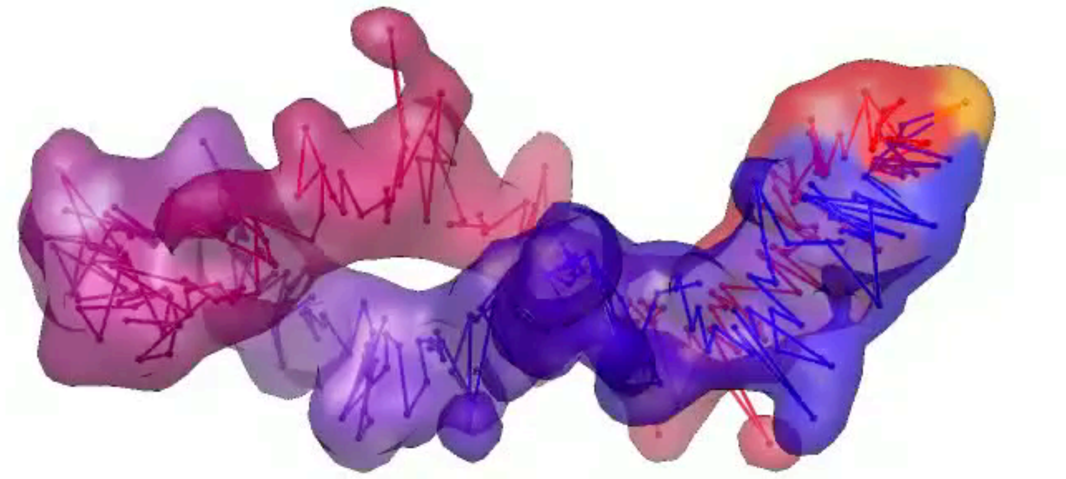
Model building



Model analysis

TADbit previous applications…

Baù, D. et al. Nat Struct Mol Biol (2011)
Umbarger, M. A. et al. Mol Cell (2011)
Le Dily, F. et al. Genes & Dev (2014)
Trussart M. et al. Nature Communication (2017)
Cattoni et al. Nature Communication (2017)
Stadhouders R. et al. Nature Genetics (2017) in press



Job Dekker

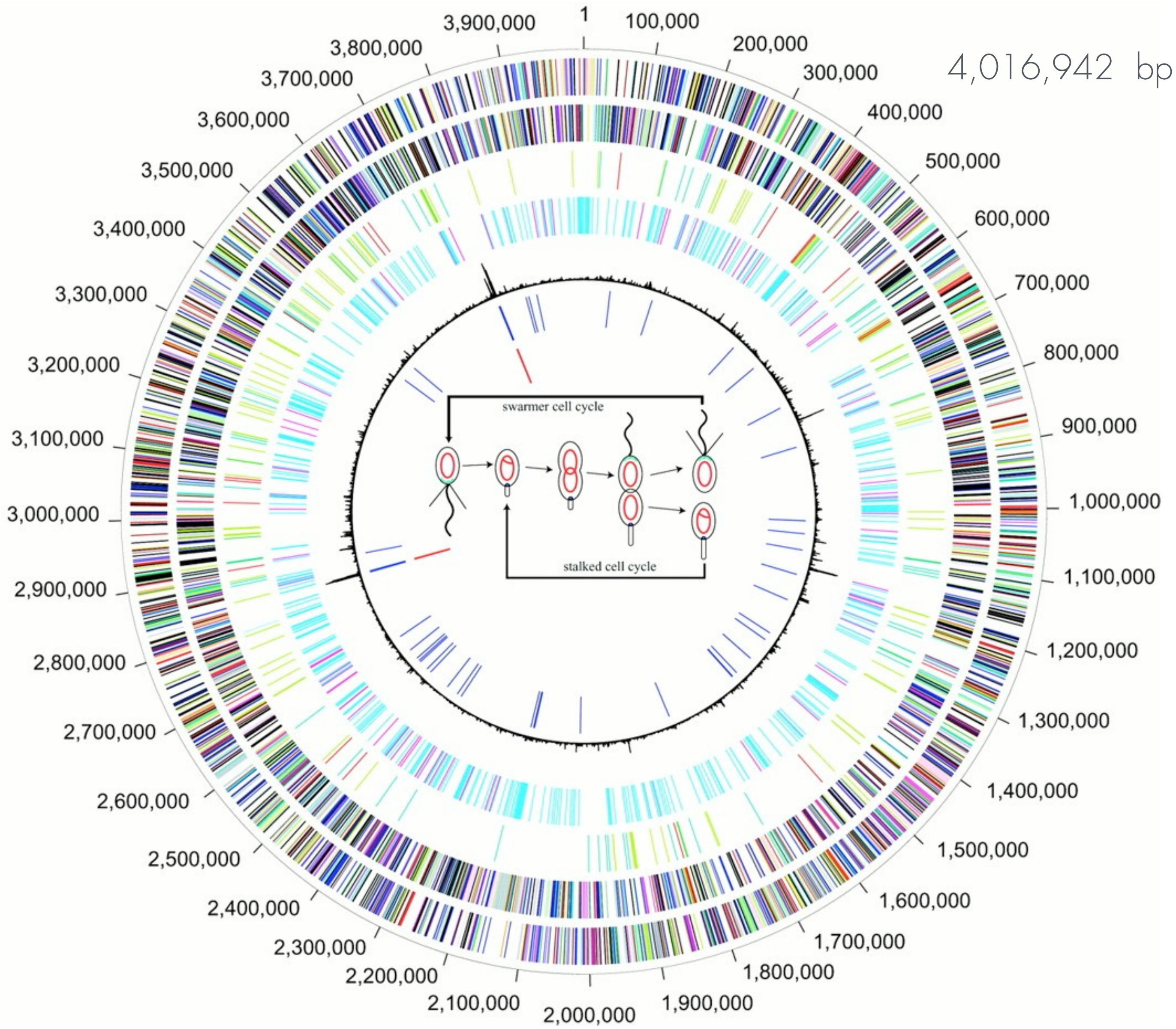
George M. Church

Lucy Shapiro

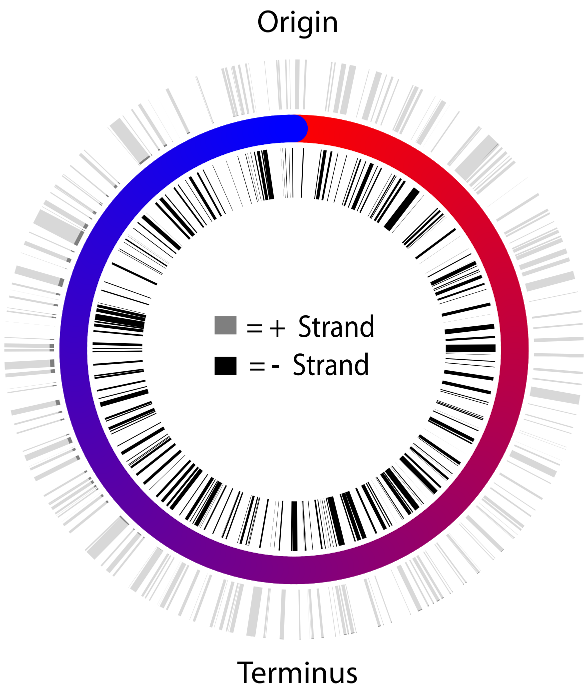
The 3D architecture of Caulobacter Crescentus

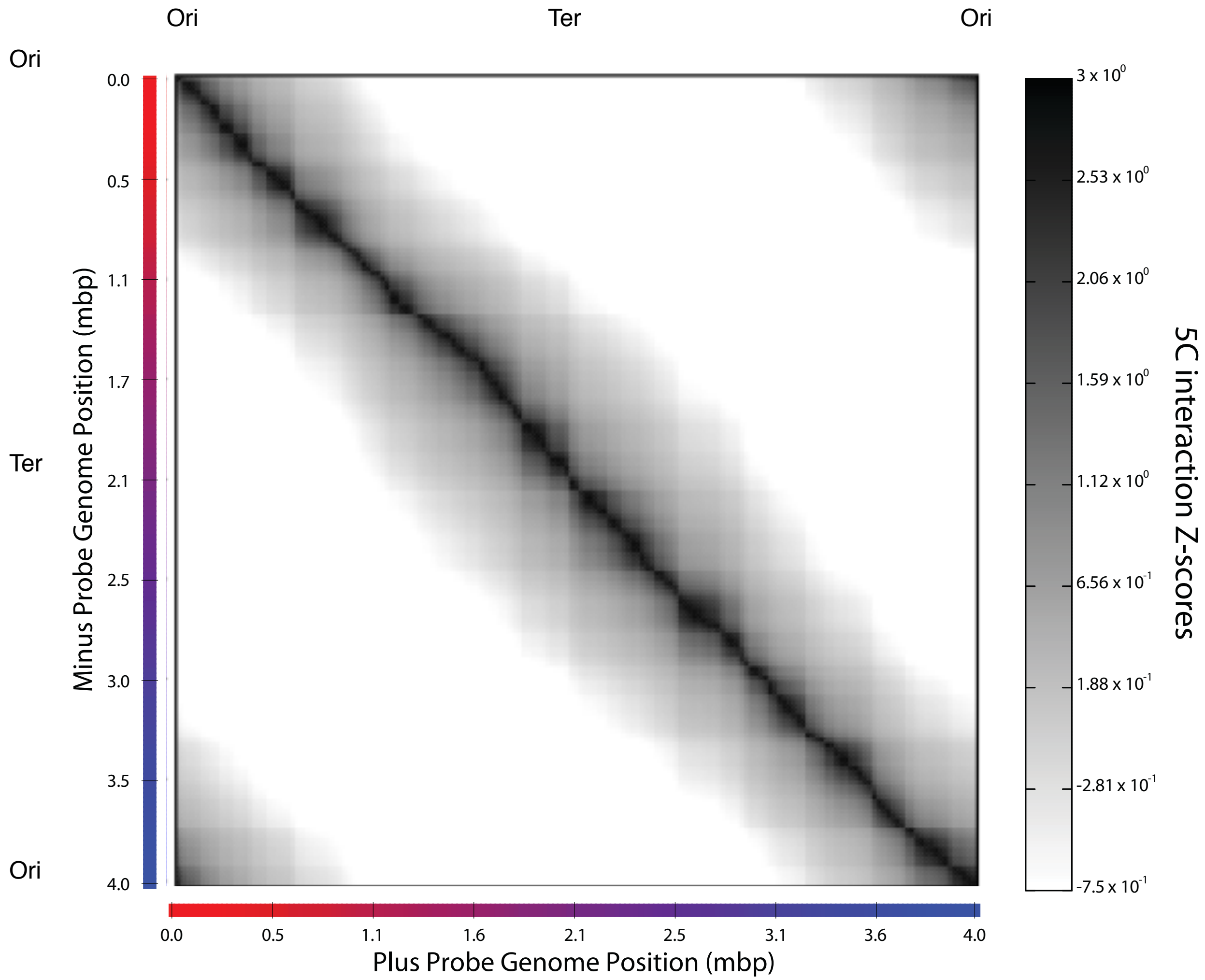
Nierman W C et al. PNAS 2001 ***98****:4136-4141*

4,016,942 bp & 3,767 genes



The 3D architecture of Caulobacter Crescentus





169 5C primers on + strand

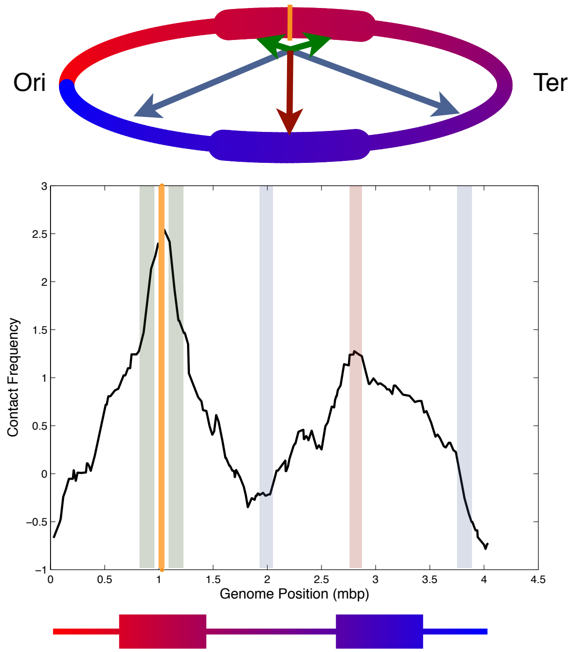
170 5C primers on – strand

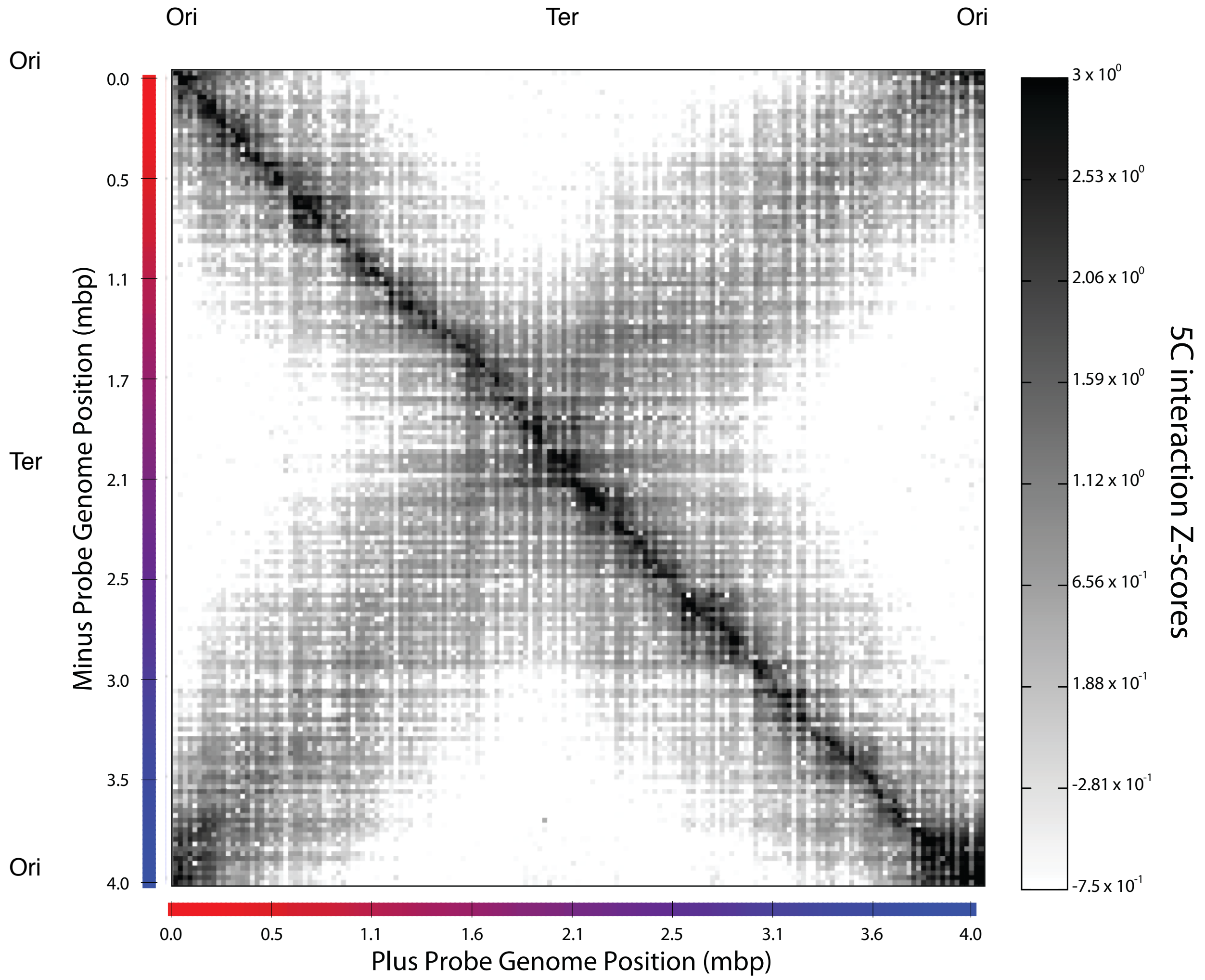
28,730 chromatin interactions

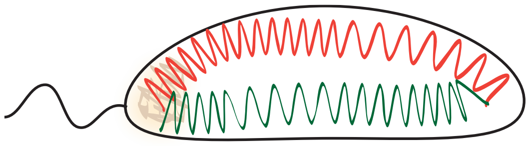
~13Kb

5C interaction matrix

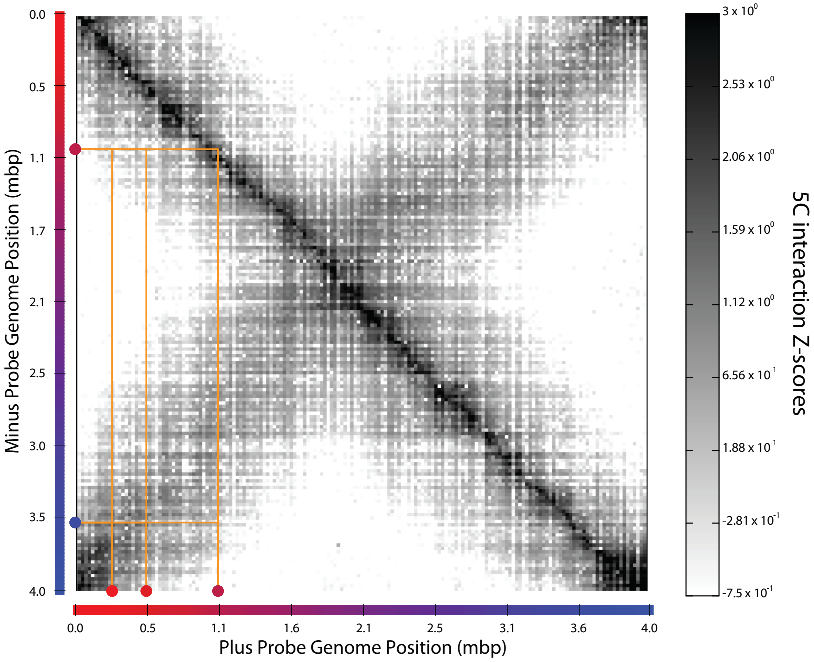
ELLIPSOID for Caulobacter cresentus



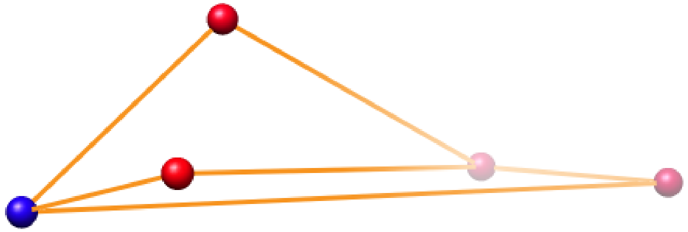


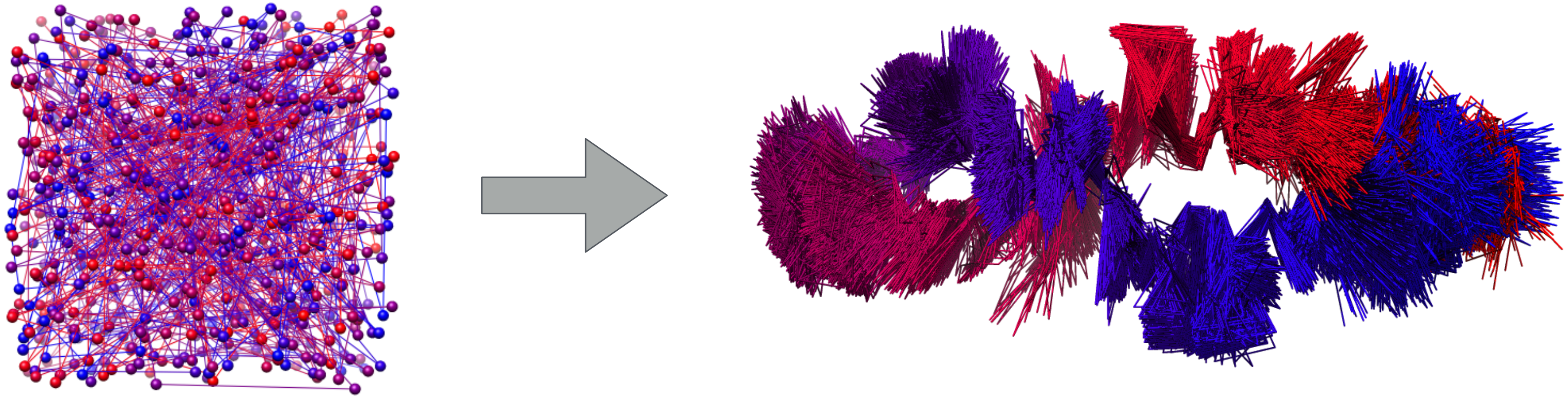


3D model building with the 5C + TADbit



339 mers





Genome organization in Caulobacter crescentus

Arms are helical

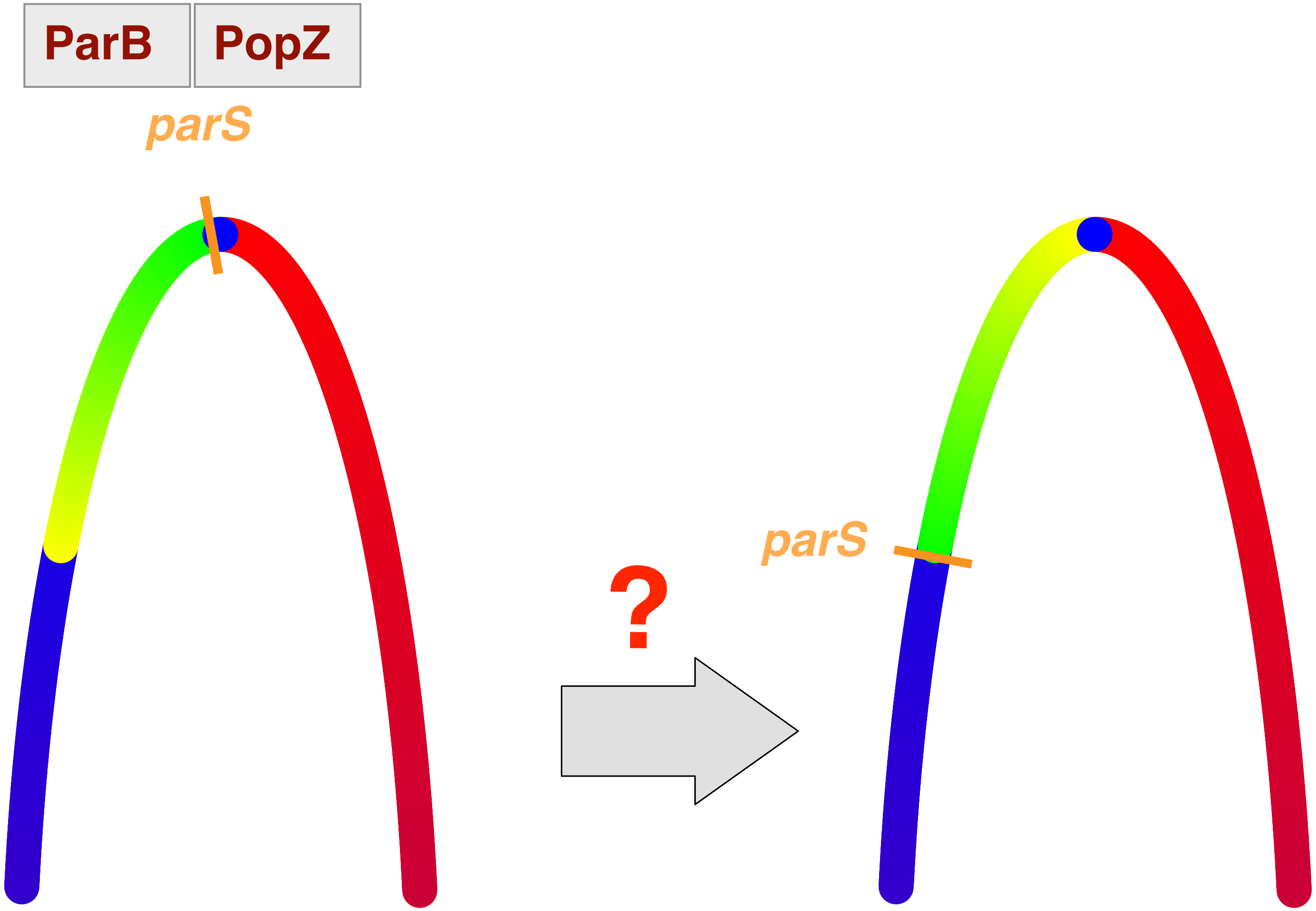

dif site 47±17Kb from Ter

parS sites 25±17Kb from Ori

Resolution of chromosomes

Centromer-like

Moving the parS sites 400 Kb away from Ori



ParB **PopZ**

parS

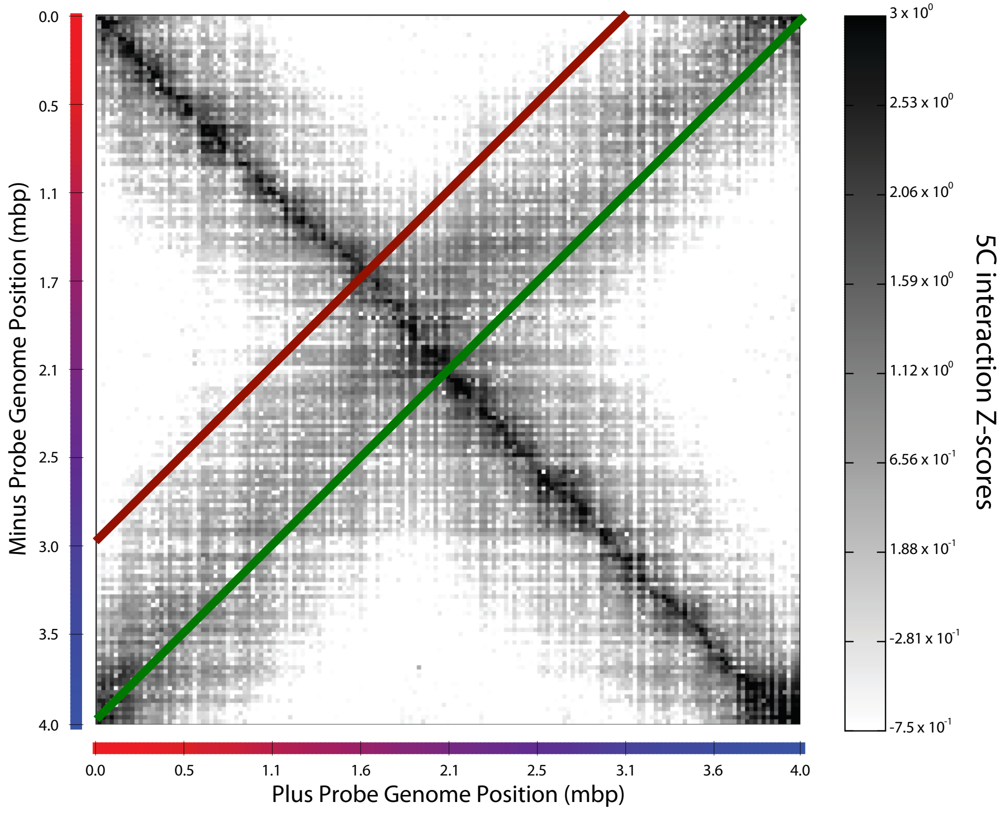
parS

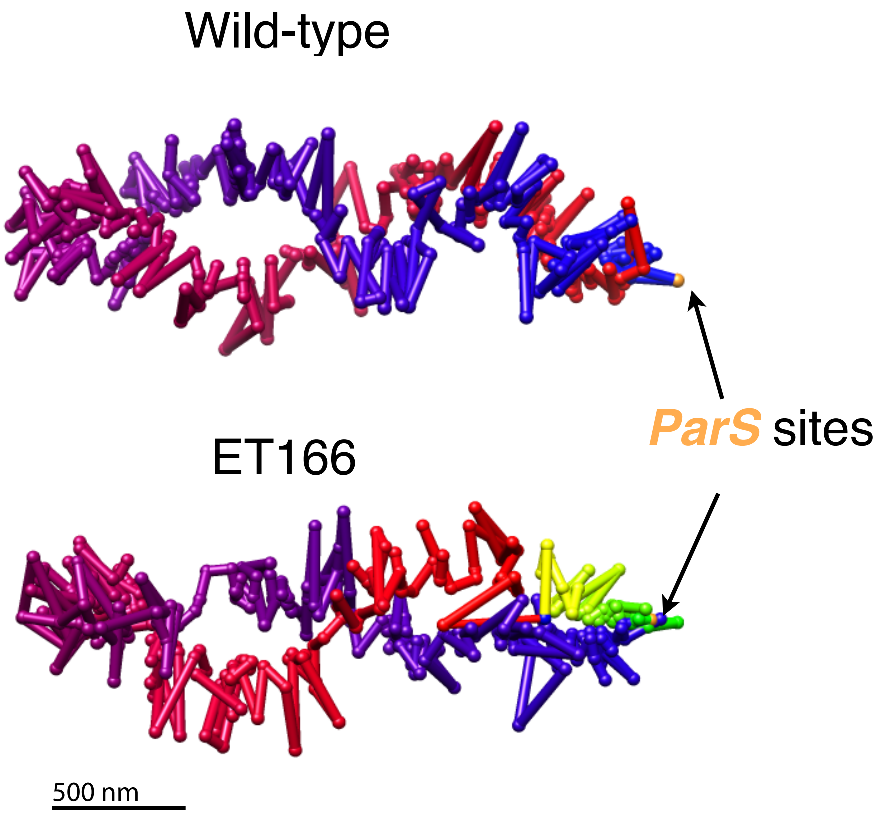
?

Wild-type

ET166

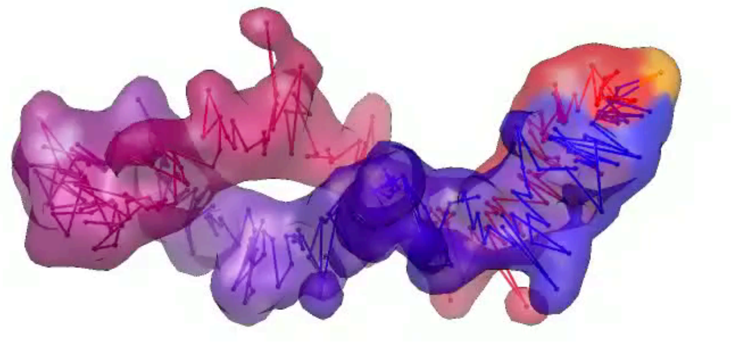
Moving the parS sites results in whole genome rotation!

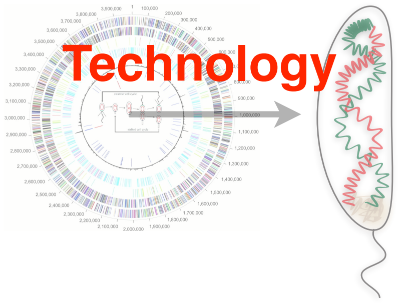


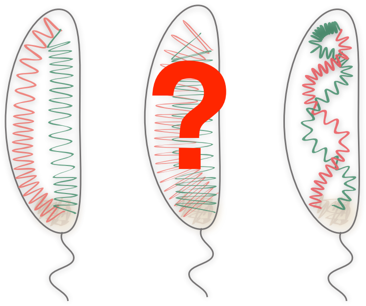


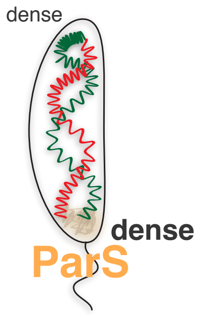
Arms are **STILL** helical

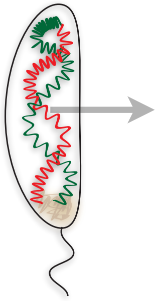
From Sequence to (Structure) to Function



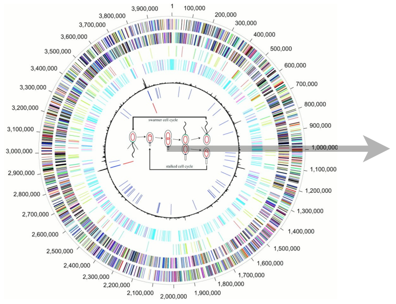








Genome Modification



Function!

Structure alteration and disease

three examples from
the Mundlos (2) and Young (1) Labs…

Chromosome Conformation Capture

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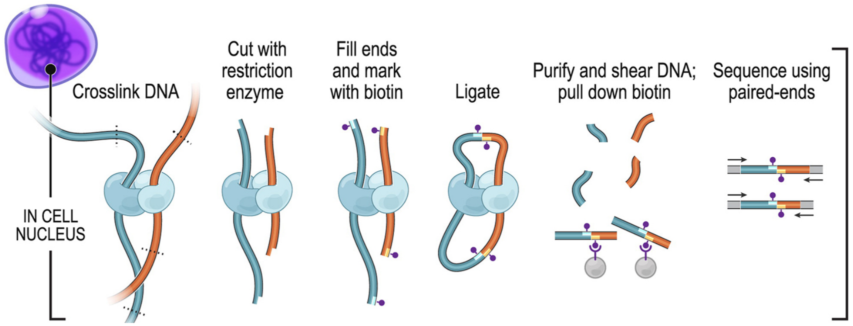
Lieberman-Aiden, E., et al. (2009). Science, 326(5950), 289–293.



Crosslink DNA

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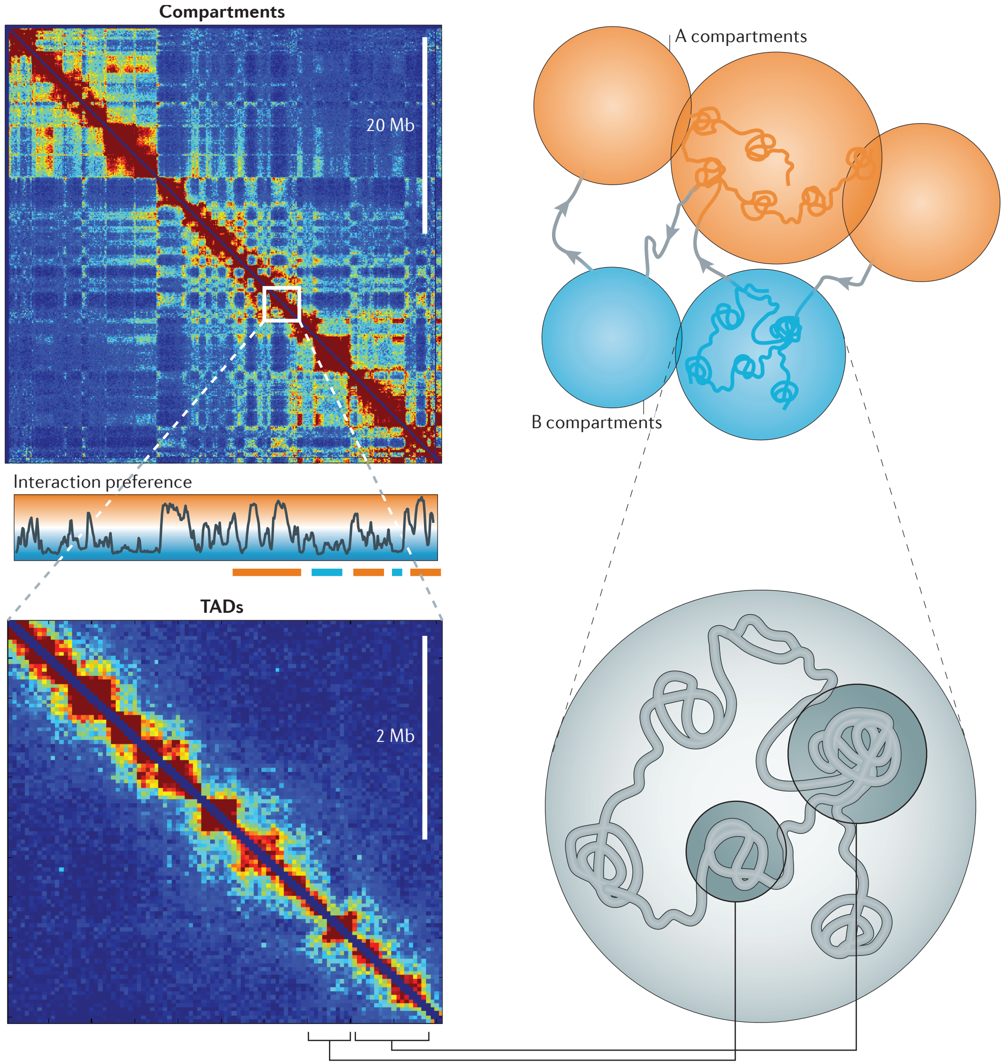
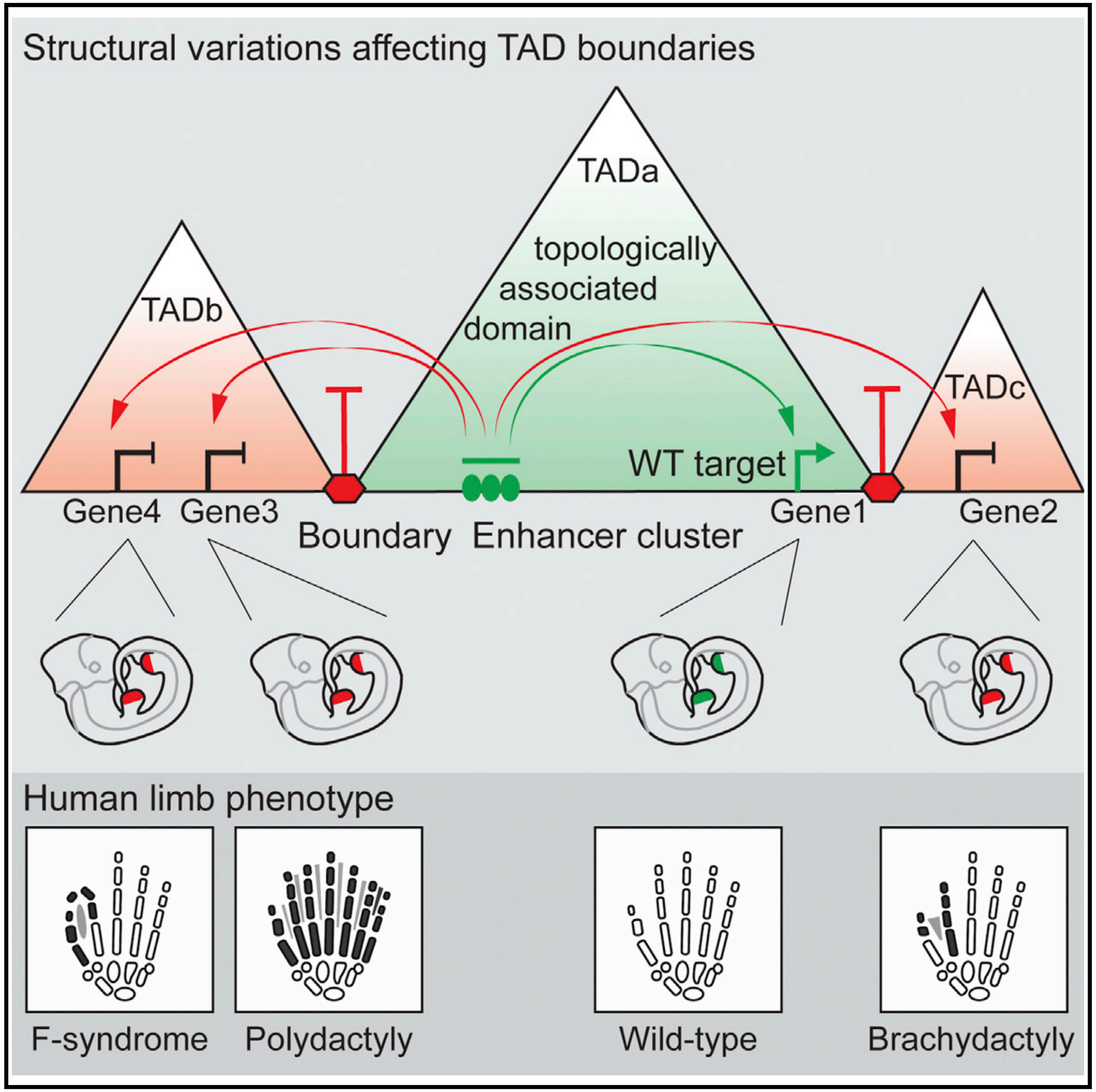


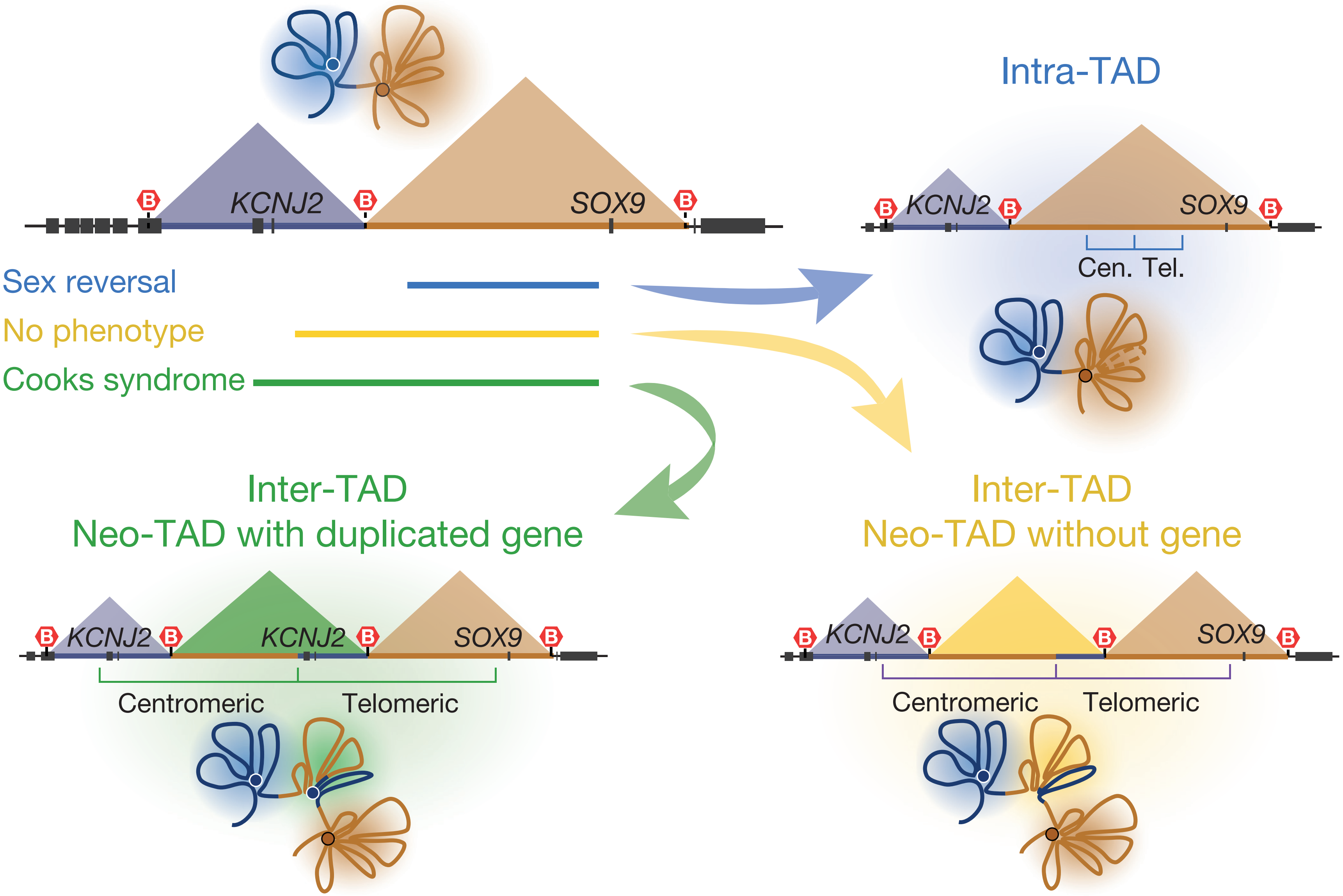
Structure alteration and disease

Lupiáñez, et al. (2015). Cell, 1–15.



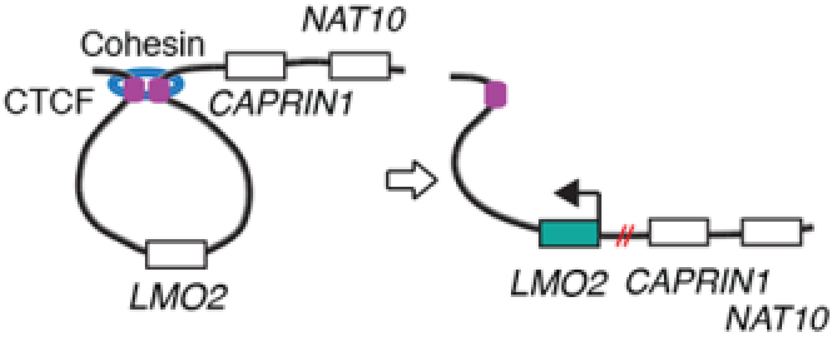
Structural alteration and disease

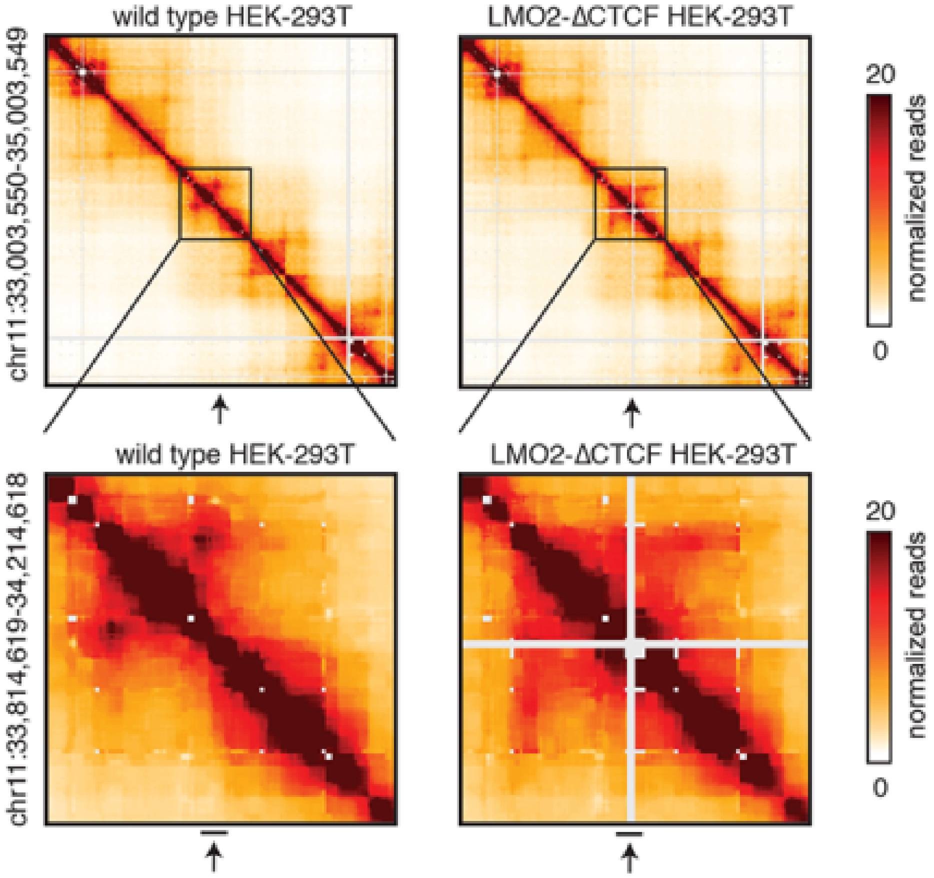
Franke, M., et al. (2016). Nature, 1–15.



Structural alteration and disease

Hnisz, D. et al. (2016). Science. 25;351(6280):1454-1458



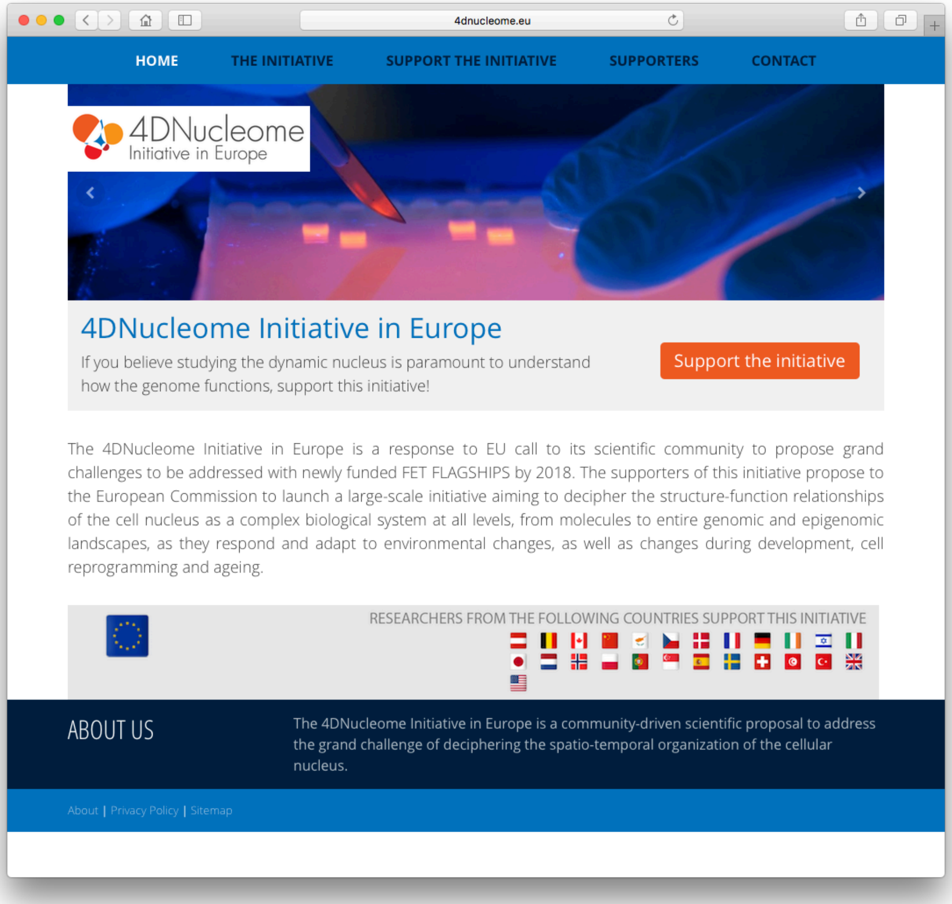


4DNucleome
Initiative in Europe

FET FLAGSHIPS

Future Pilot?

http://www.4dnucleome.eu



4DNucleome Initiative in Europe

If you believe studying the dynamic nucleus is paramount to understand how the genome functions, support this initiative!

The 4DNucleome Initiative in Europe is a response to EU call to its scientific community to propose grand challenges to be addressed with newly funded FET FLAGSHIPS by 2018. The supporters of this initiative propose to the European Commission to launch a large-scale initiative aiming to decipher the structure-function relationships of the cell nucleus as a complex biological system at all levels, from molecules to entire genomic and epigenomic landscapes, as they respond and adapt to environmental changes, as well as changes during development, cell reprogramming and ageing.

RESEARCHERS FROM THE FOLLOWING COUNTRIES SUPPORT THIS INITIATIVE

ABOUT US

The 4DNucleome Initiative in Europe is a community-driven scientific proposal to address the grand challenge of deciphering the spatio-temporal organization of the cellular nucleus.

David Castillo
Yasmina Cuartero
Irene Farabella
Silvia Galan
Mike Goodstadt
Francesca Mugianesi
Julen Mendieta
Juan Rodriguez
François Serra
Paula Soler
Aleksandra Sparavier
Yannick Spill
Marco di Stefano

Caulobacter 3D Genome in collaboration with Job Dekker, Jorge Church and Luci Shapiro

http://marciuslab.org
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