Bare with me for the first slides… 😁

and ask **any** question at **any** time

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3.000.000.000 nucleotides

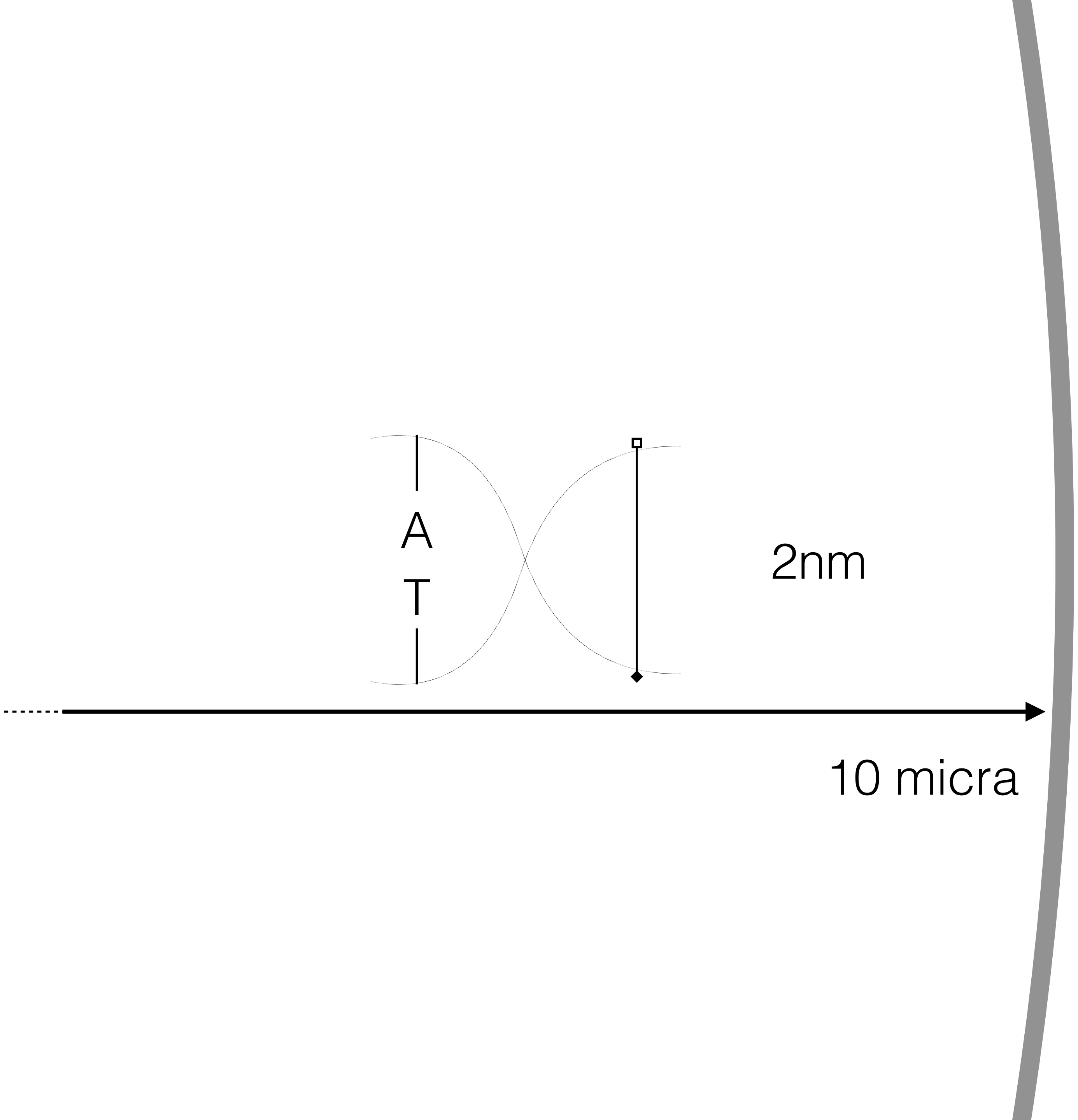
(1.000.000 pages in 12pt Helvetica)

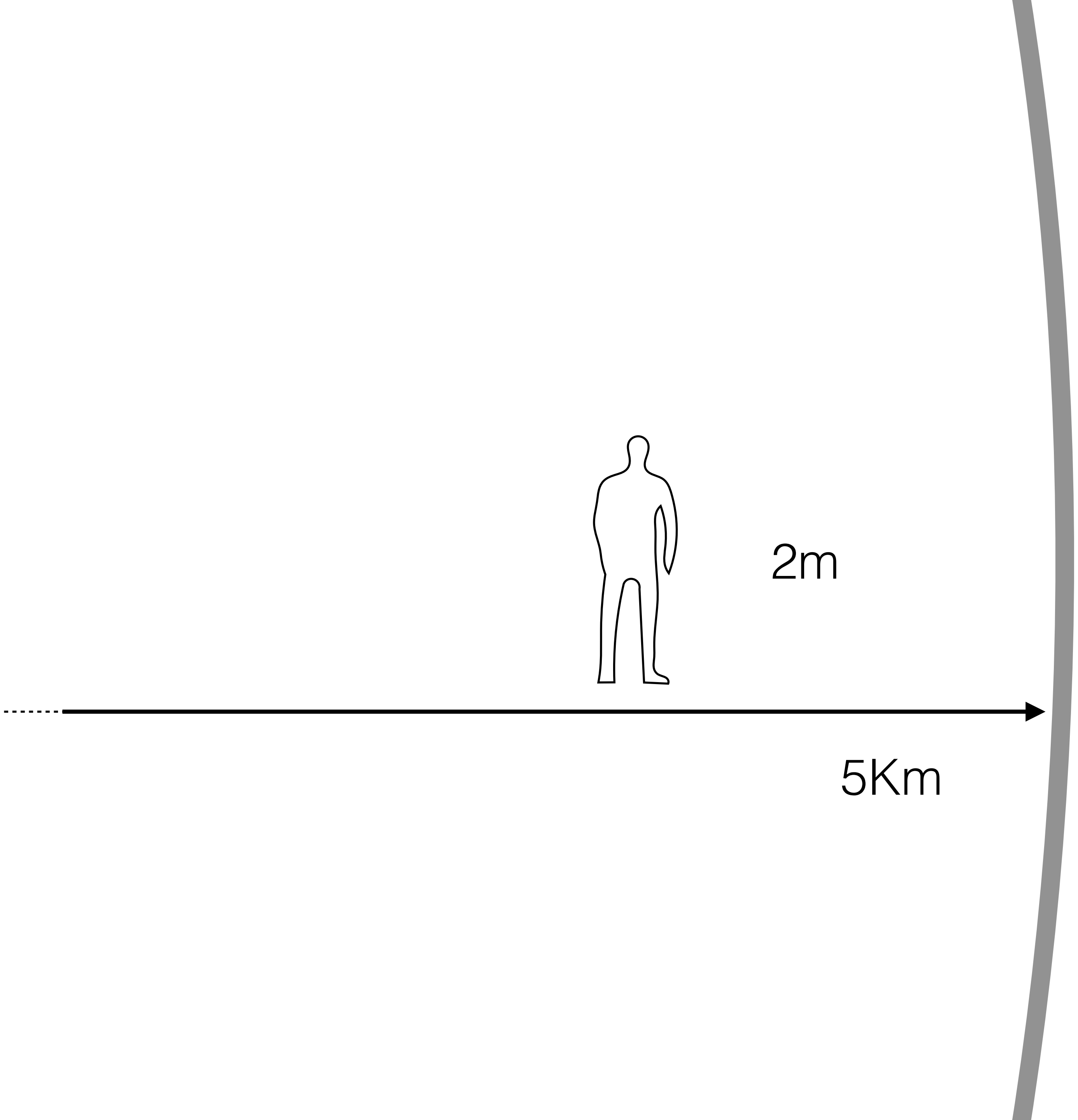
2 meters of DNA!

In sum… a human body has aprox

60.000.000.000Km of DNA

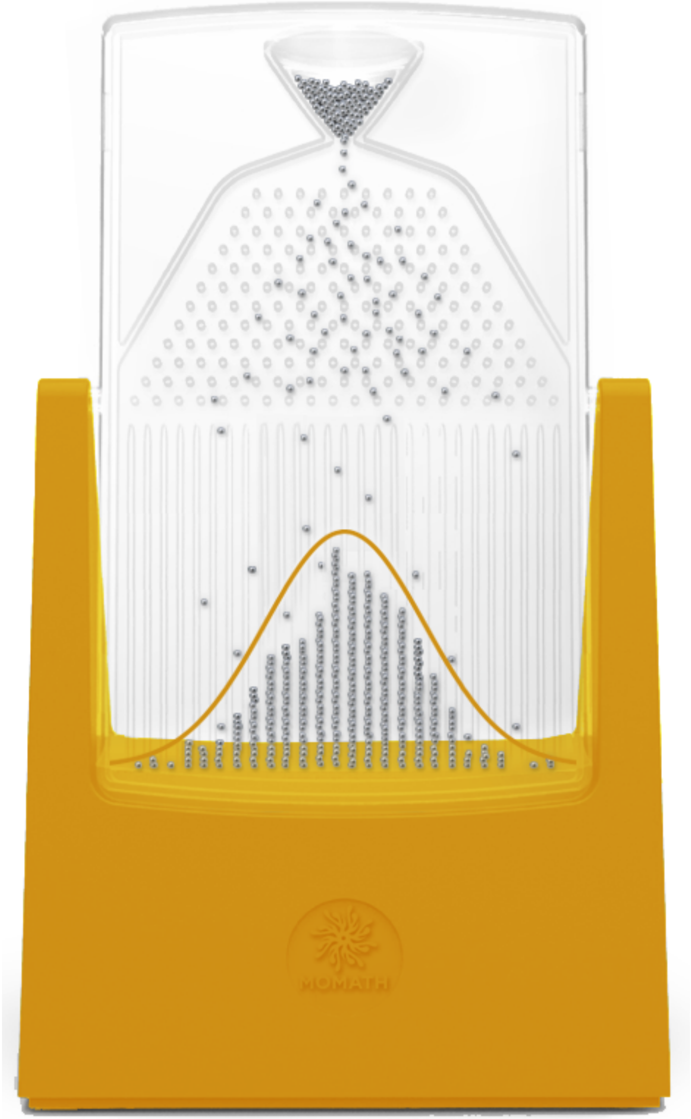
95.000 round trips to the moon!!

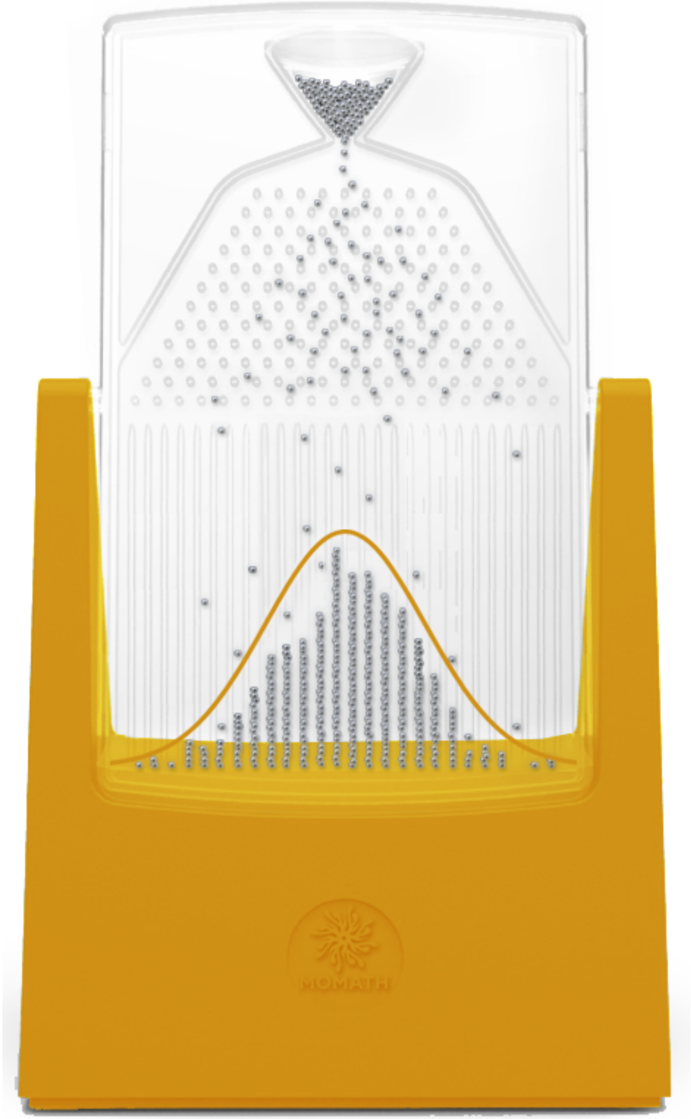

A
T
2nm
10 micra

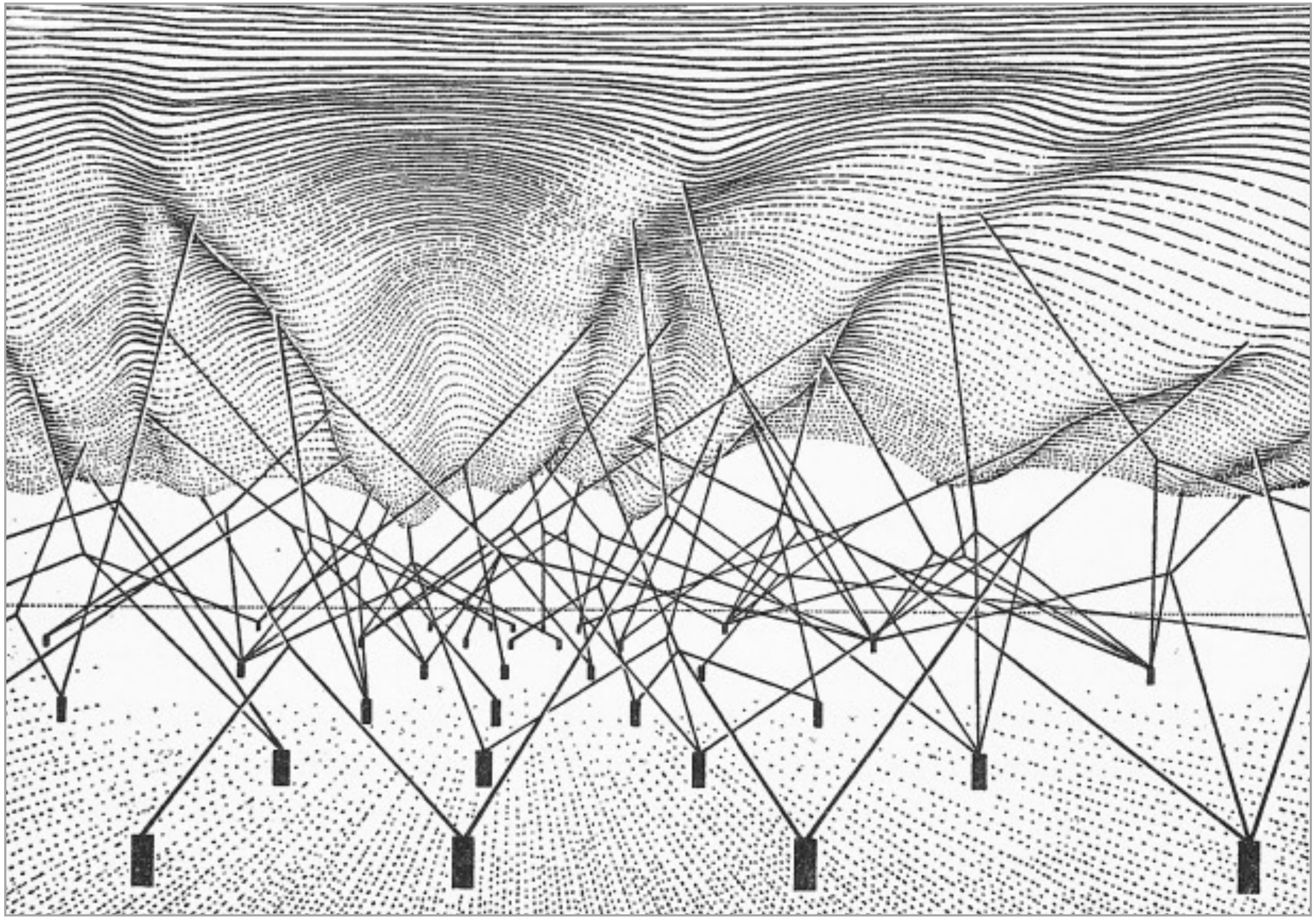

2m
5Km

Cavalli lab videos

https://www.youtube.com/@cavallilabvideos8738


MOMATH





Conrad Waddington (1957)

So…

The genome is large…, very large…, huge!

In fact, it is the largest biomolecule in our cells…

The genome is a physical object that lives in space and time

The genome is very dynamic!

ask Leonid for details…

The genome can be seen as a "probabilistic machine"

Spatio-temporal regulatory landscape of sex-determination

or… identifying 3D enhancer hubs

Marc A. Marti-Renom

CNAG-CRG · ICREA

BioRxiv 2022

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

Juan A. Rodríguez
CNAG-CRG
now @GLobe I. Denmark

Irene Mota
MDC Berlin

Dario Lupiañez
MDC Berlin
Now @CABD

Sex determination: a 3,000 year-old enigma

Mythology

Hermaphrodite primeval men

Plato´s symposium, 385-370 BC

Theories

Left-right theory

Alexandrian manuscripts, 1st cent. BC

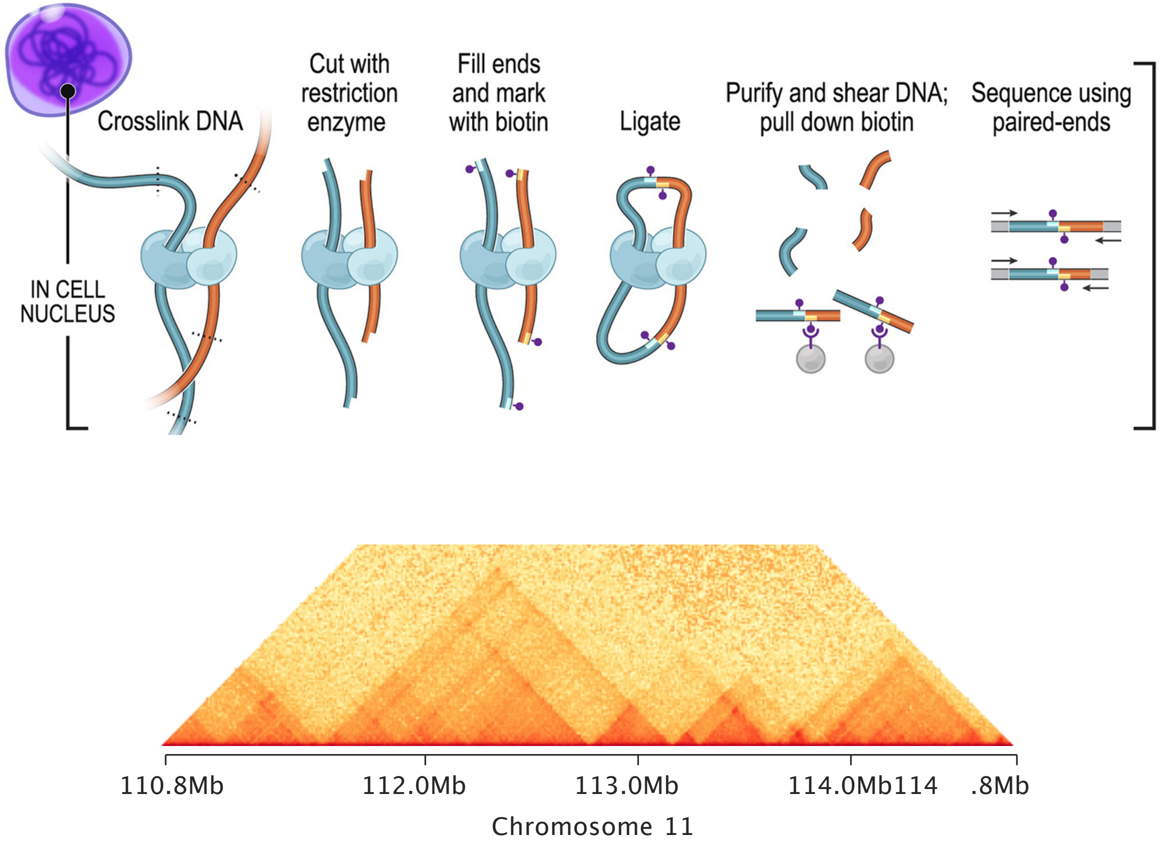
Genetics

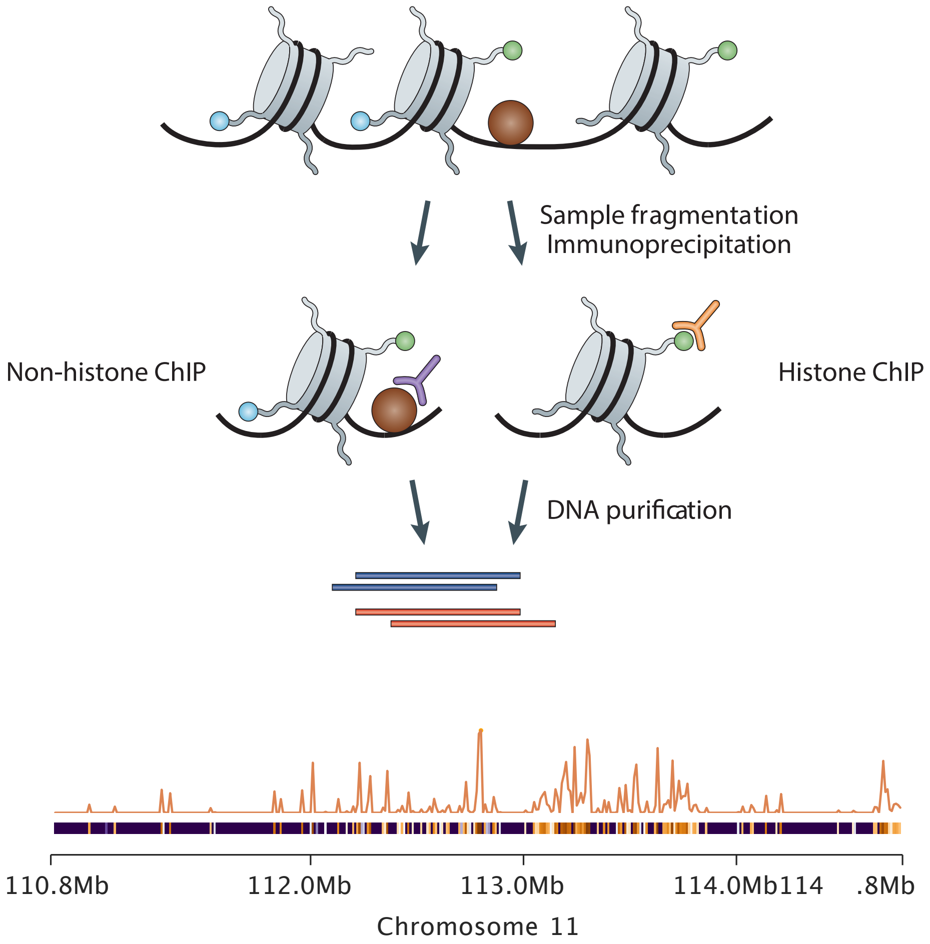
Discovery of *Sry* gene

Koopman et al., Nature, 1991

(Goodfellow & Lovell Badge labs)

Hi-C & ChIP-seq

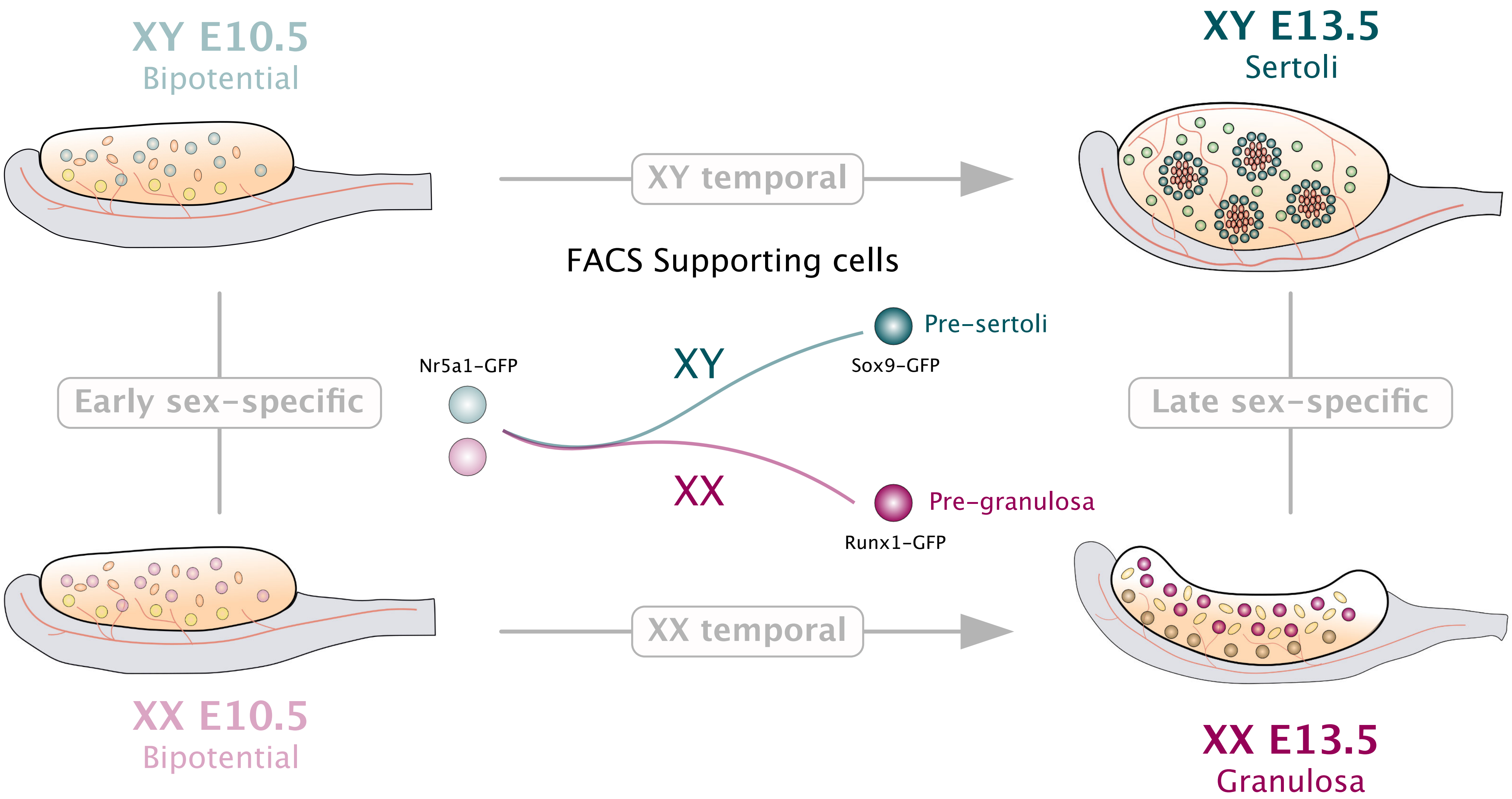




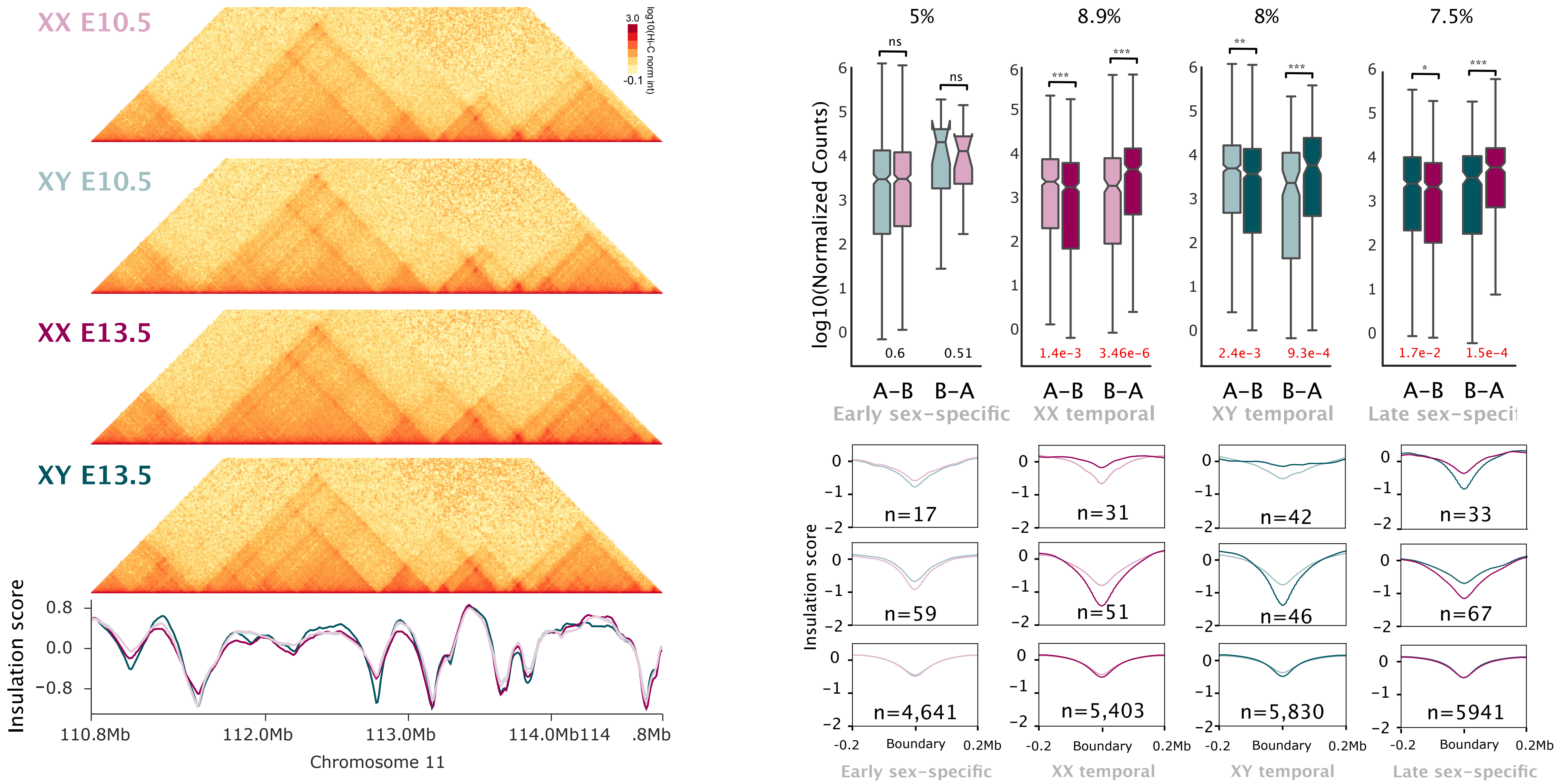
Dekker, J., Rippe, K., Dekker, M., & Kleckner, N. (2002). Science, 295(5558), 1306–1311.
Lieberman-Aiden, E., et al. (2009). Science, 326(5950), 289–293.

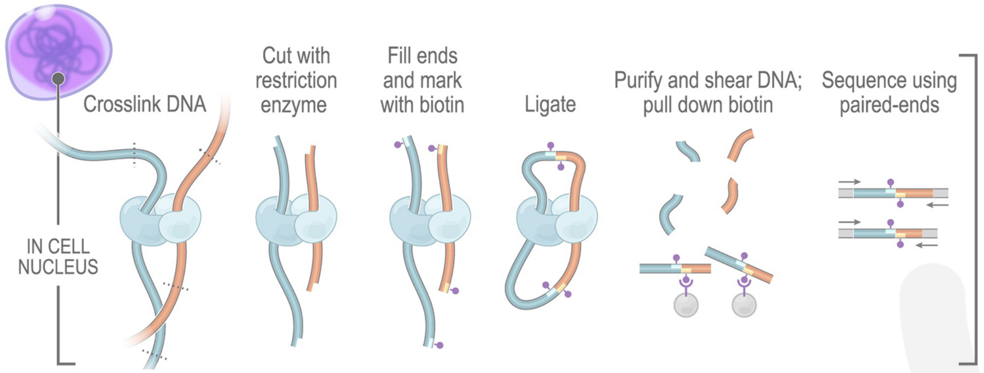
Solomon, M. J., Larsen, P. L. & Varshavsky, A. (1988) Cell 53, 937–947.
Park, P.J. (2009) Nature Reviews Genetics 10, 669–680.

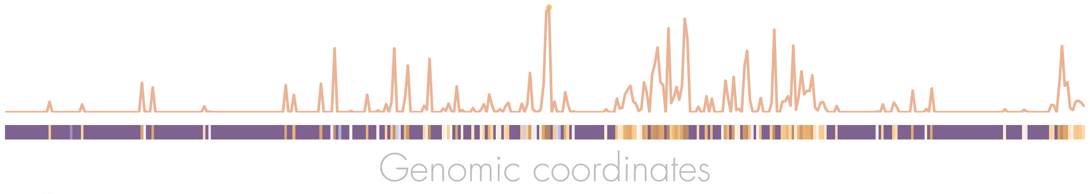
Sex-determination as a model for "bipotential" commitment



No major structural (apparent) differences



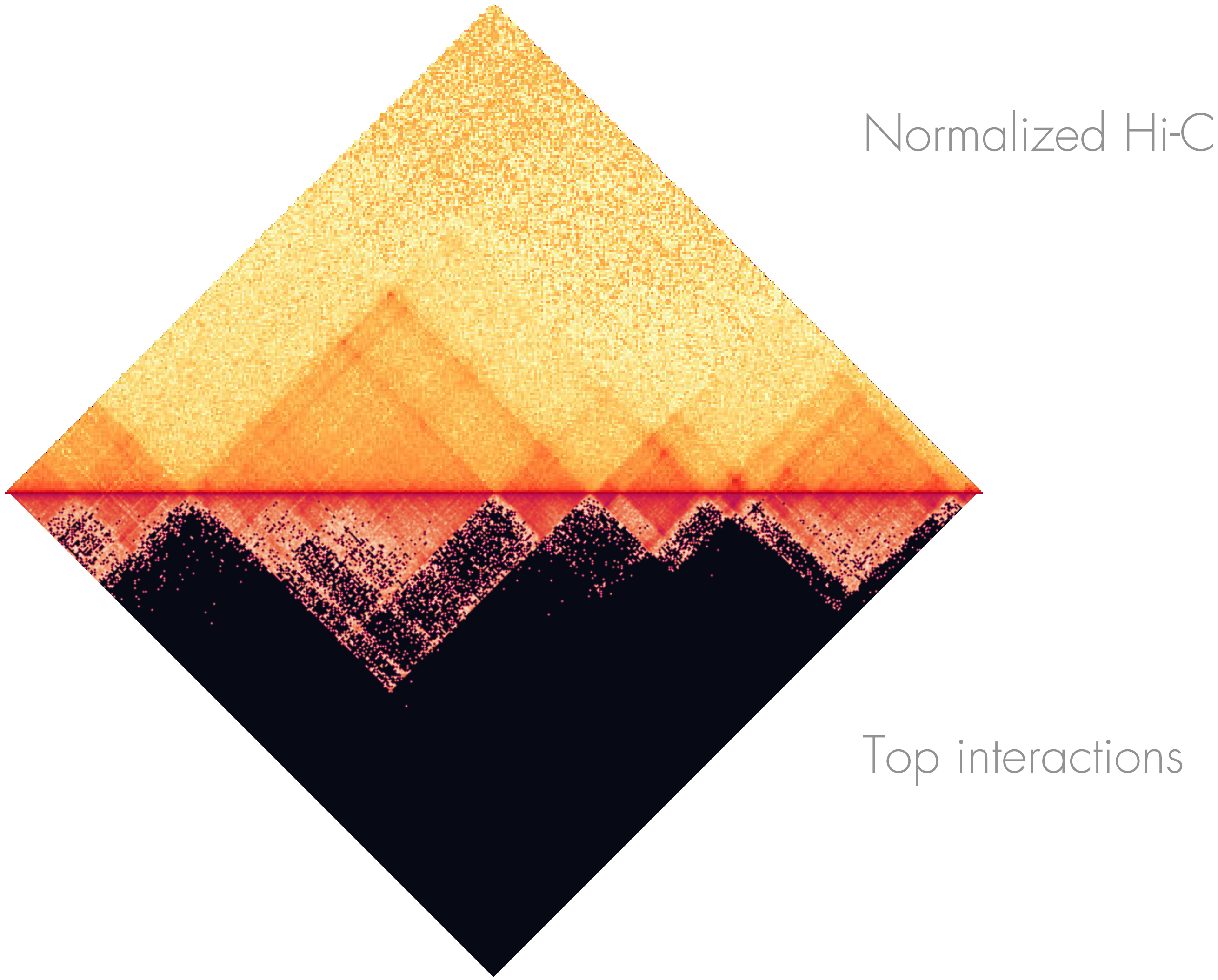

Crosslink DNA
Cut with restriction enzyme
Fill ends and mark with biotin
Ligate
Purify and shear DNA; pull down biotin
Sequence using paired-ends
IN CELL NUCLEUS


Genomic coordinates


METALoci

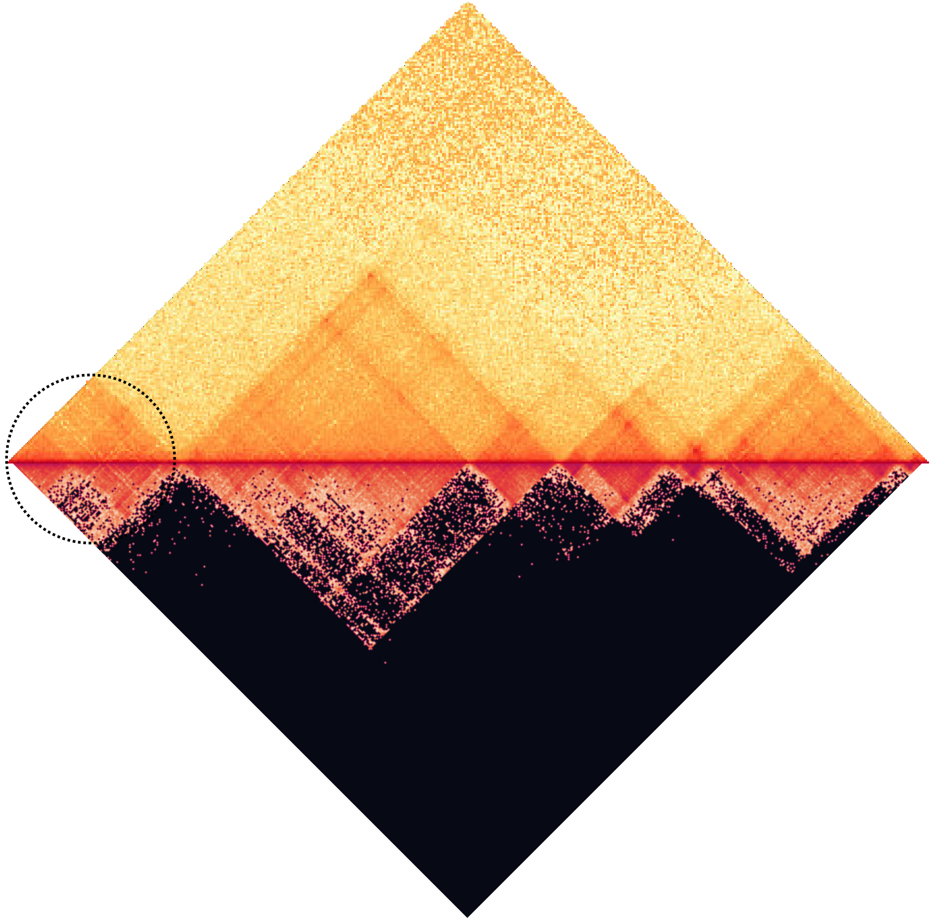
Hi-C normalization and interaction selection

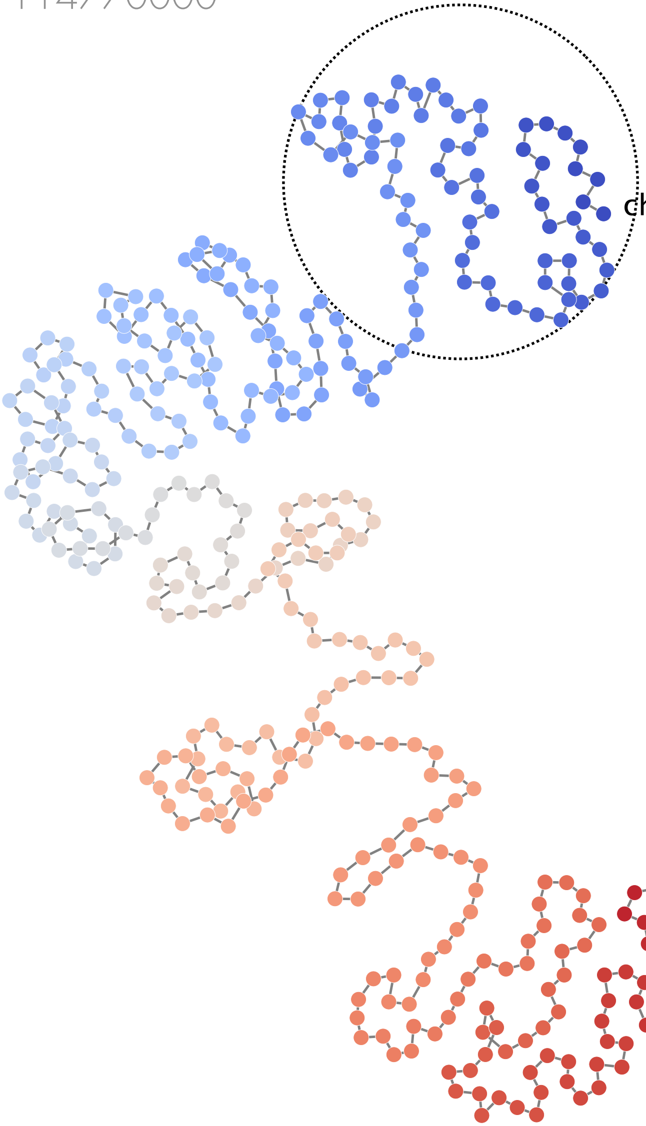
chr11:110780000-114770000



Spatial lay-out of significant interactions

chr11:110780000-114770000

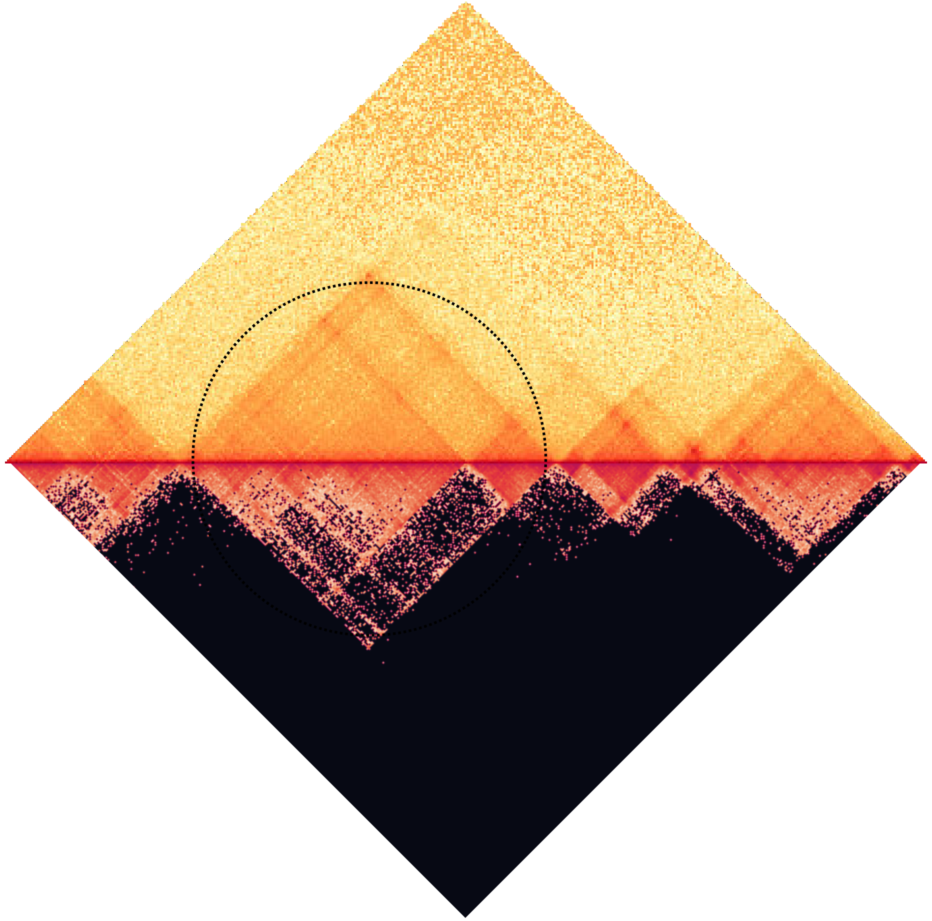


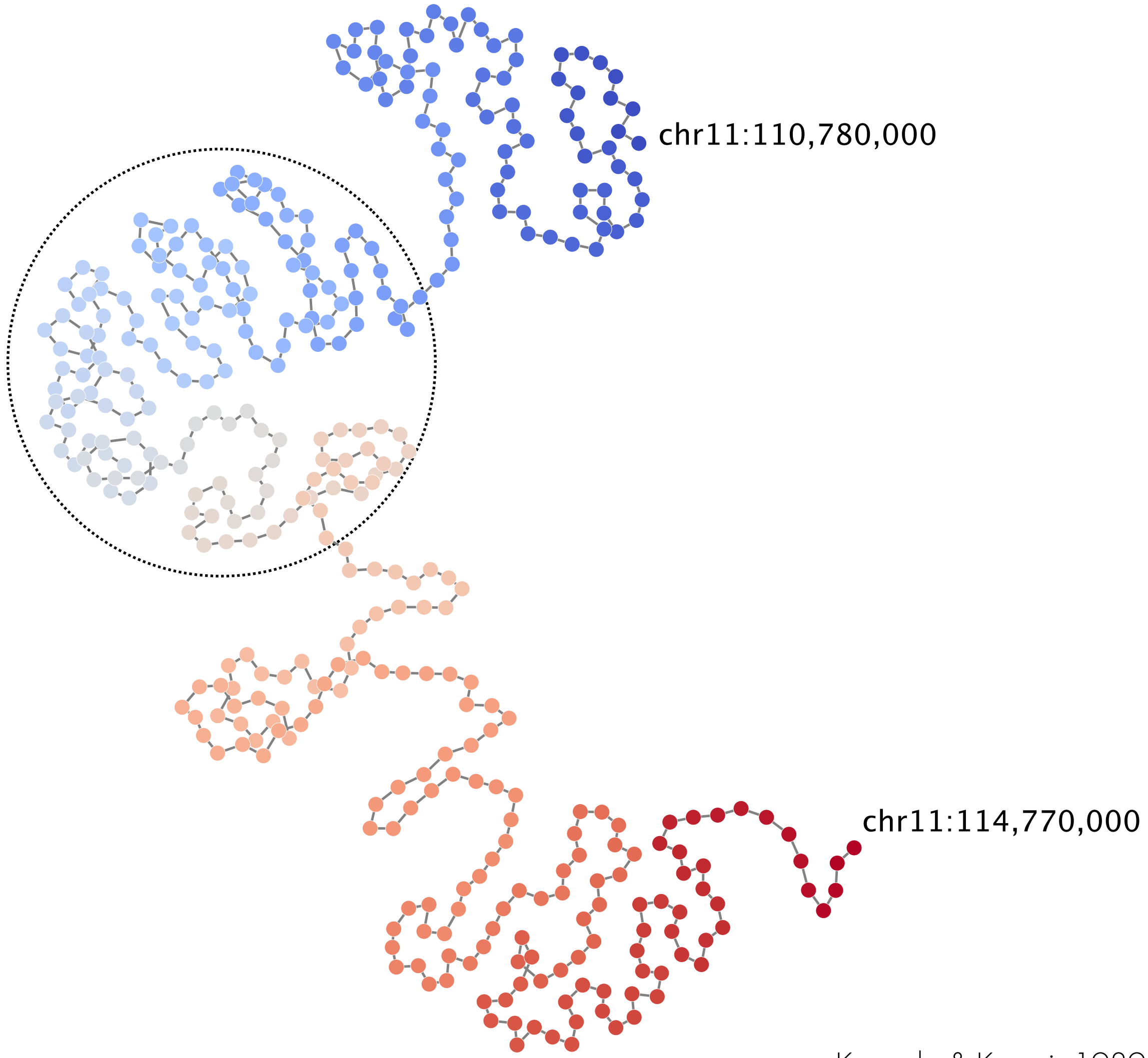


Kamada & Kawai, 1989

Spatial lay-out of significant interactions

chr11:110780000-114770000

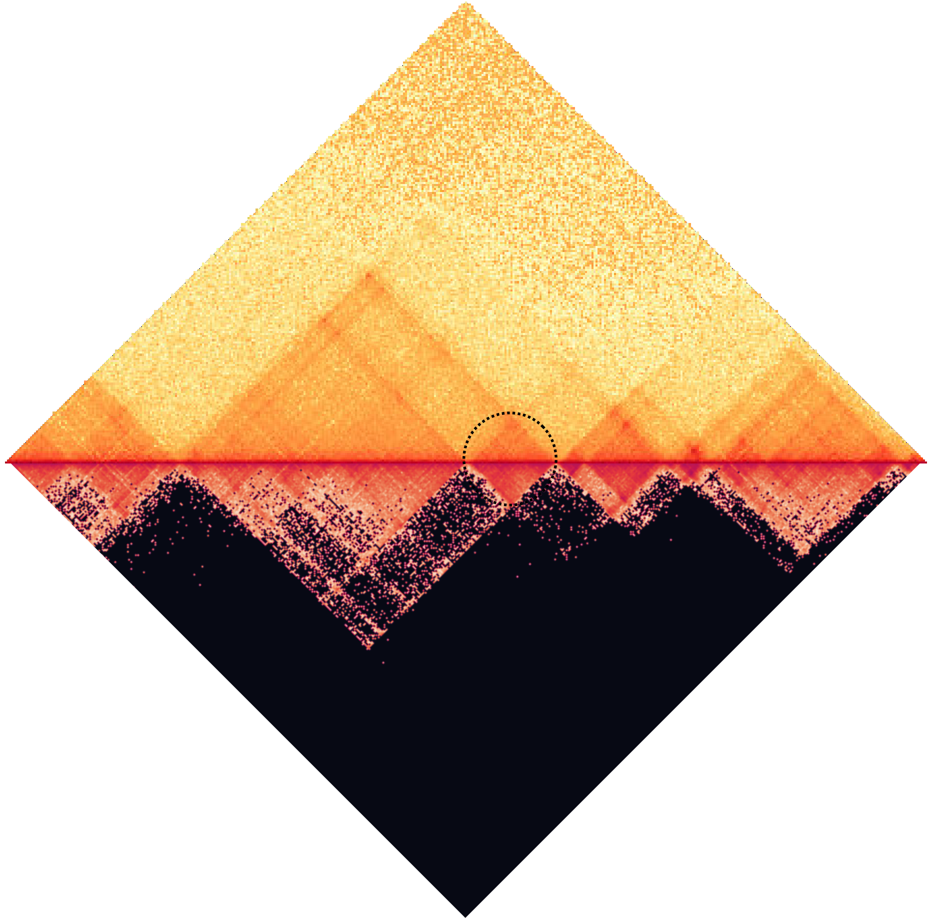


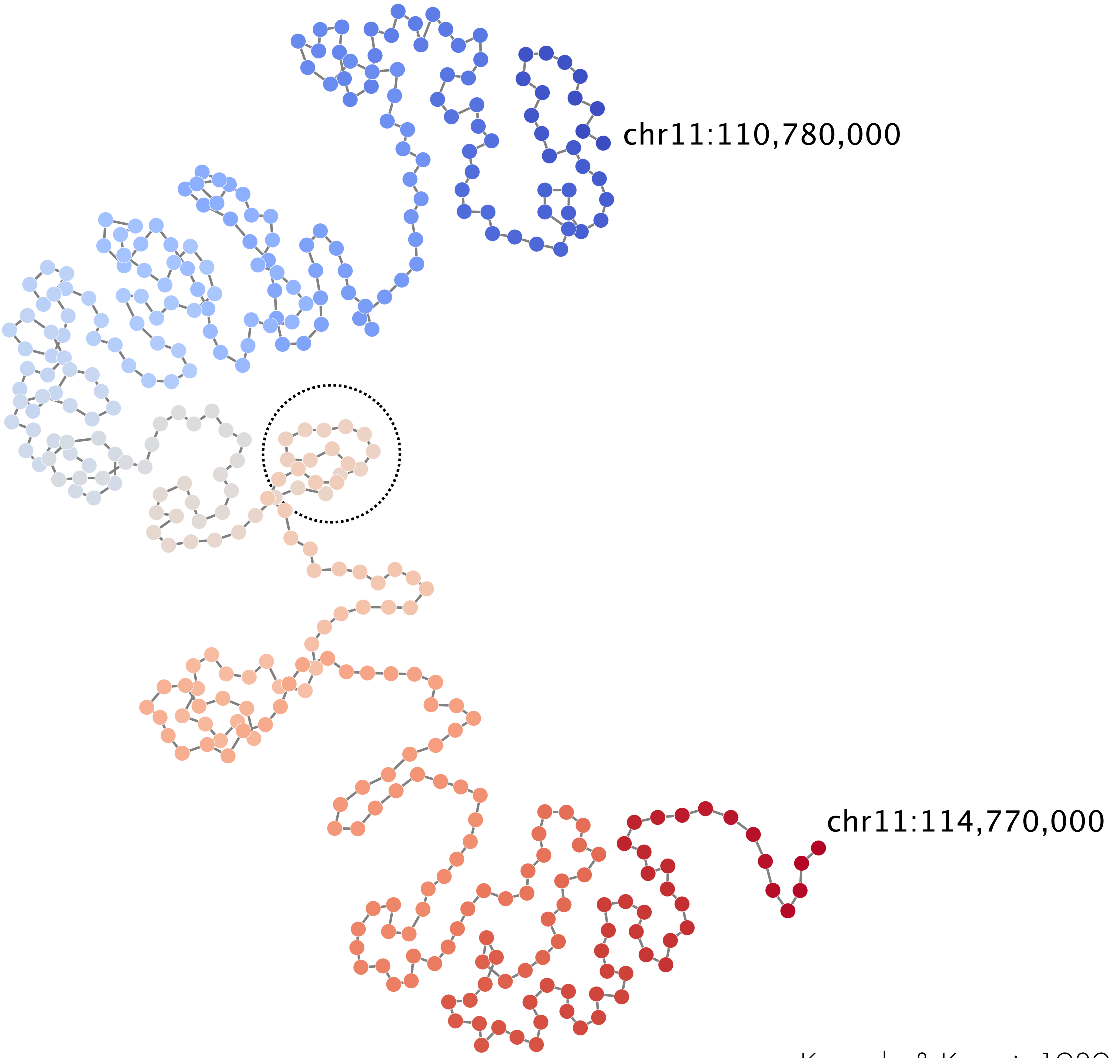


Kamada & Kawai, 1989

Spatial lay-out of significant interactions

chr11:110780000-114770000

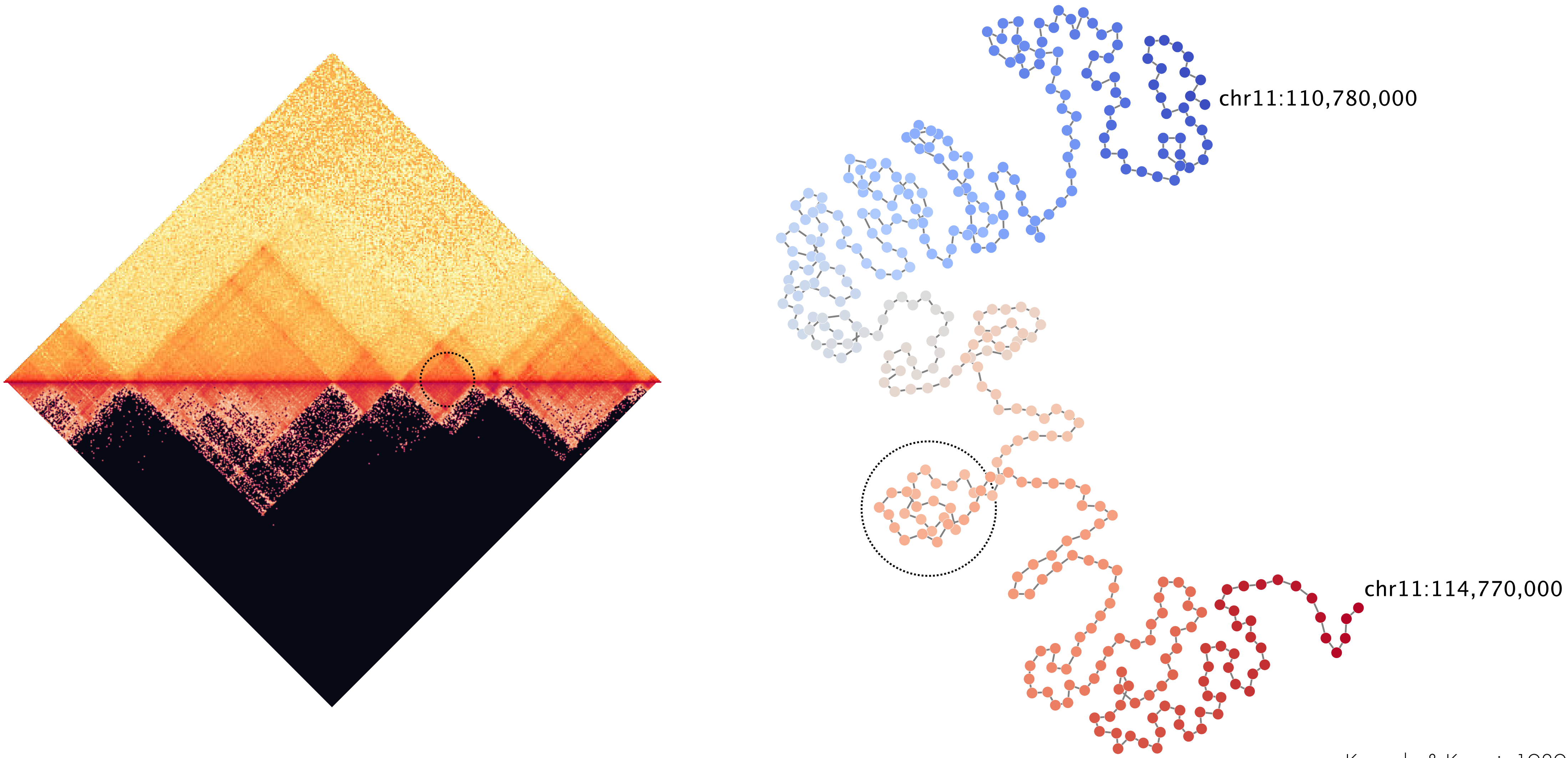




Kamada & Kawai, 1989

Spatial lay-out of significant interactions

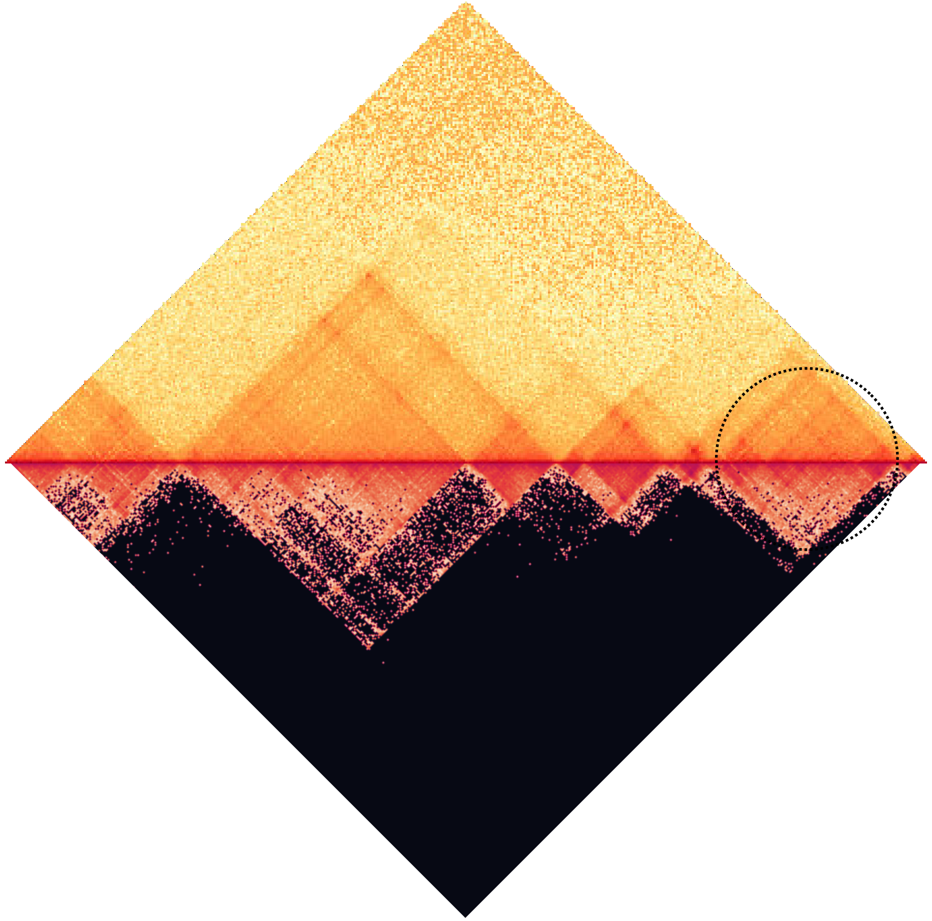
chr11:110780000-114770000

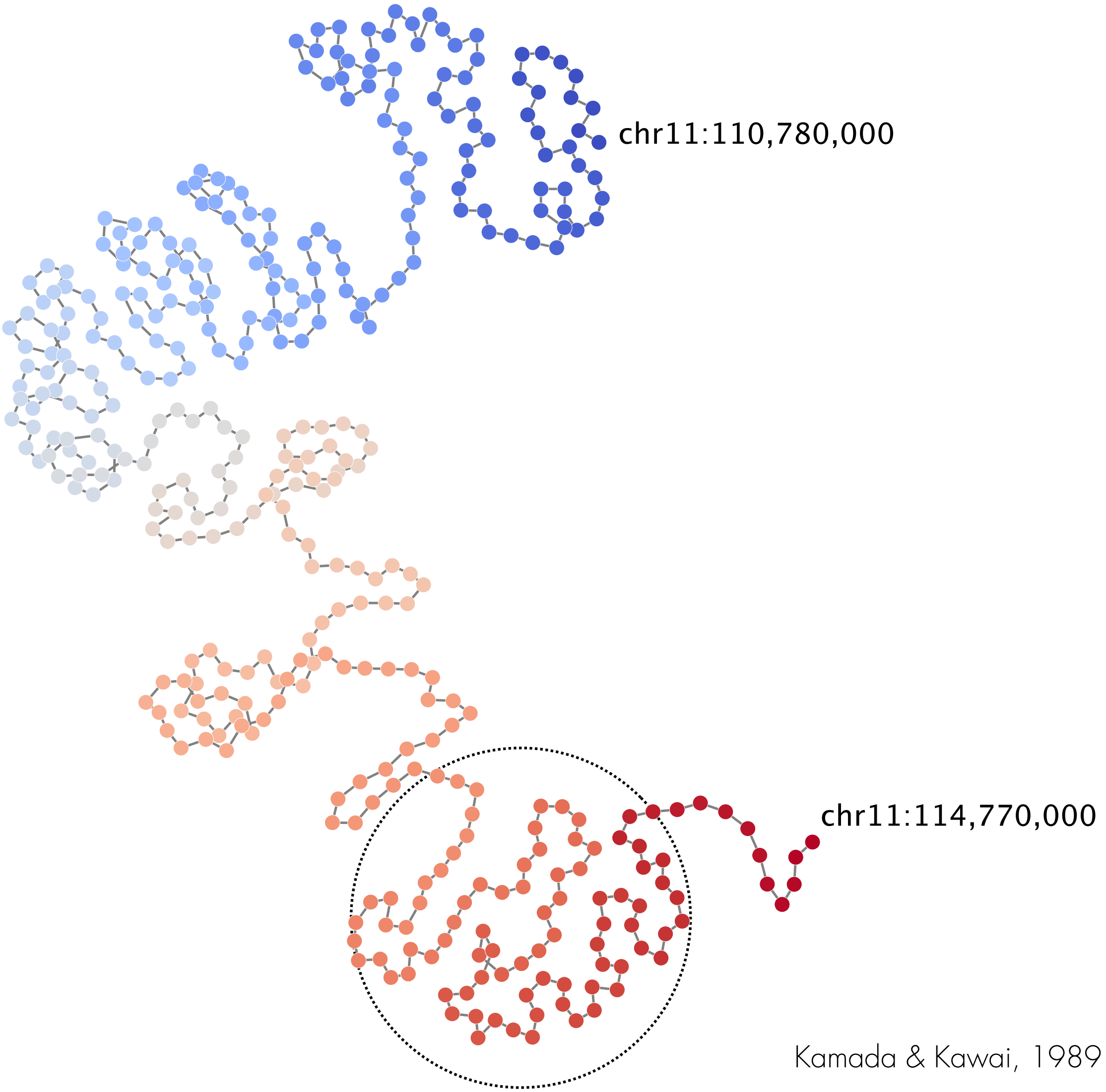


Kamada & Kawai, 1989

Spatial lay-out of significant interactions

chr11:110780000-114770000

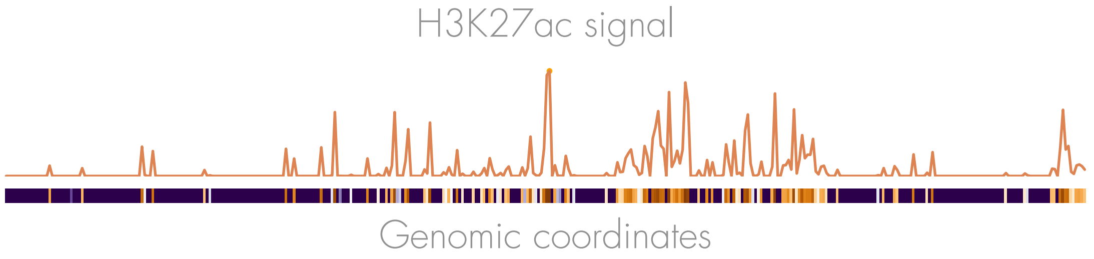


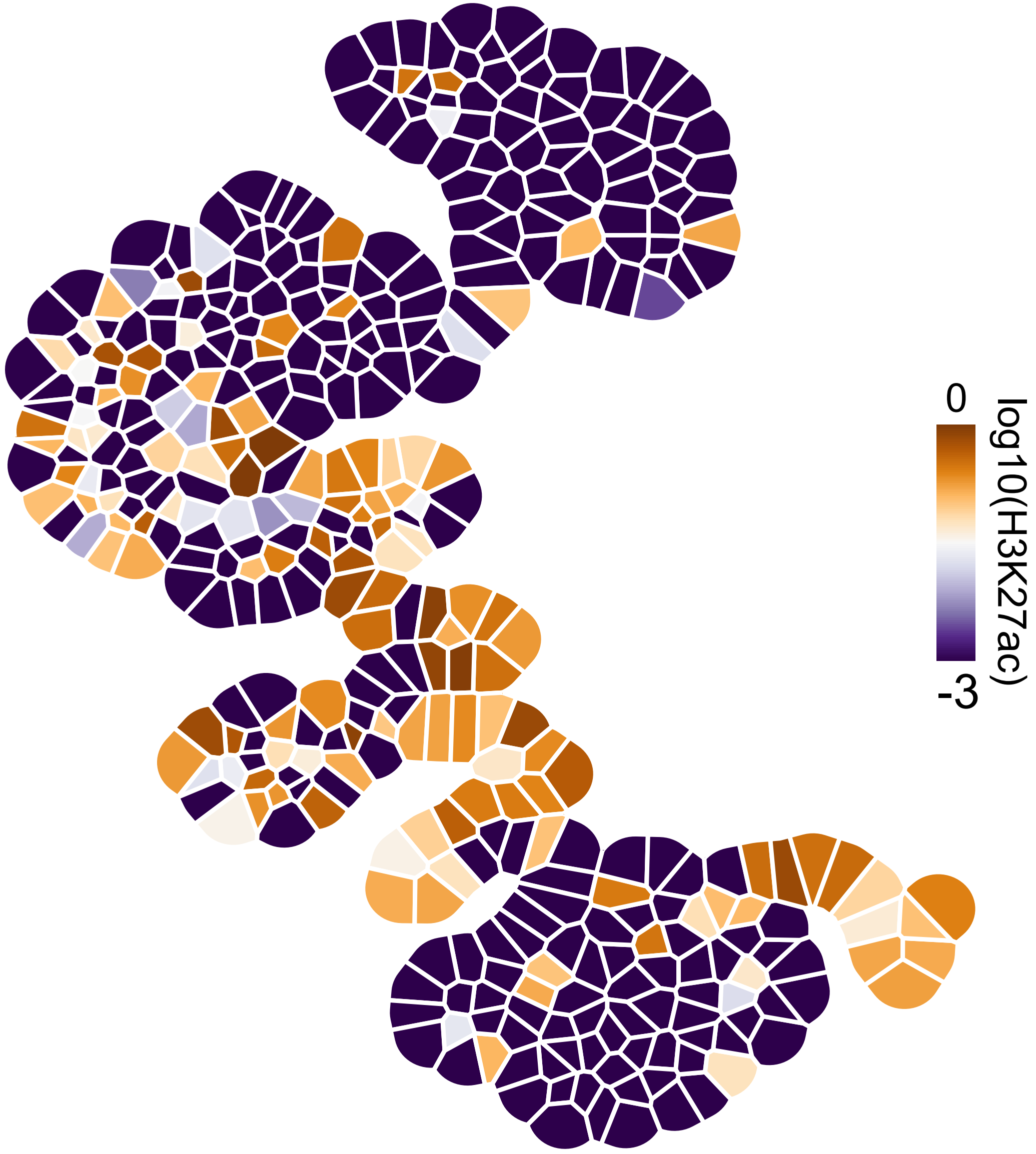


Kamada & Kawai, 1989

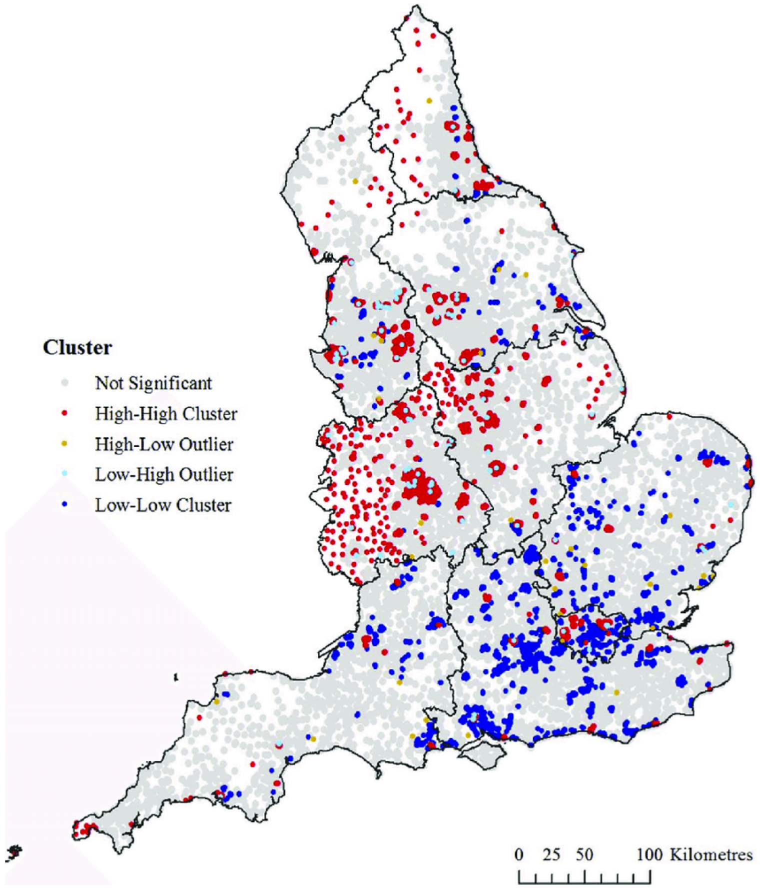
Marker (H3K27ac) into 2D mapping

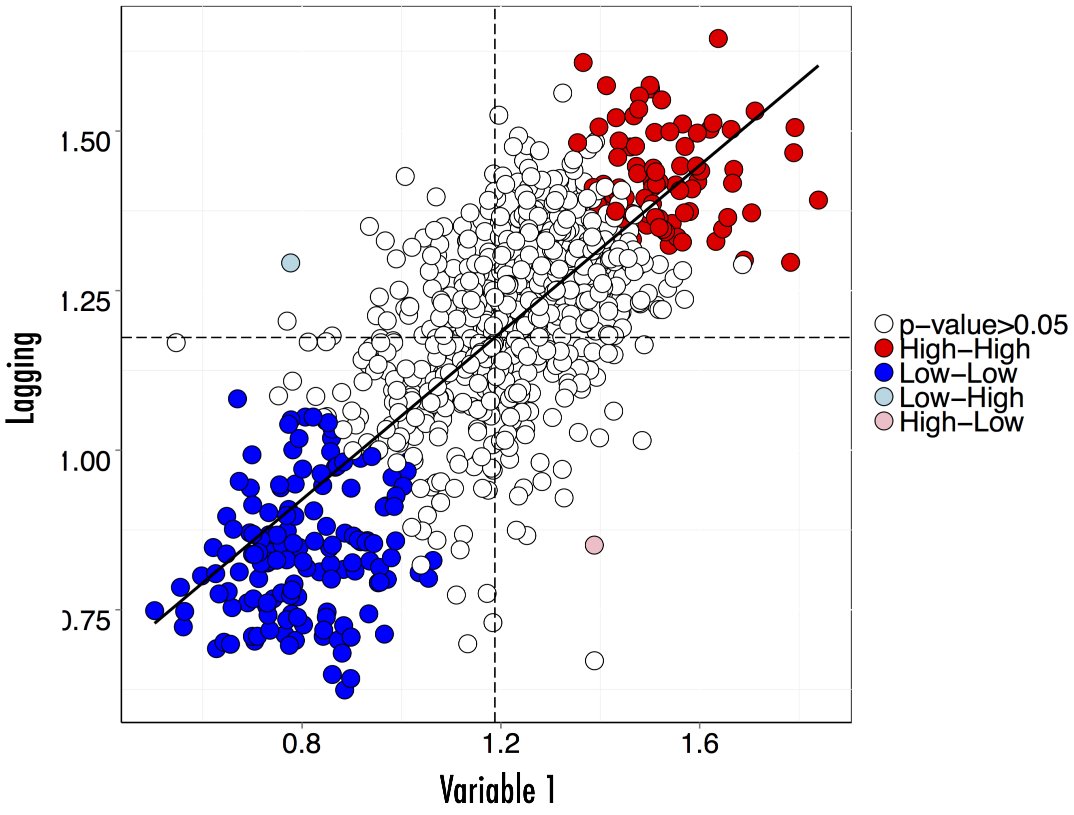
chr11:110780000-114770000



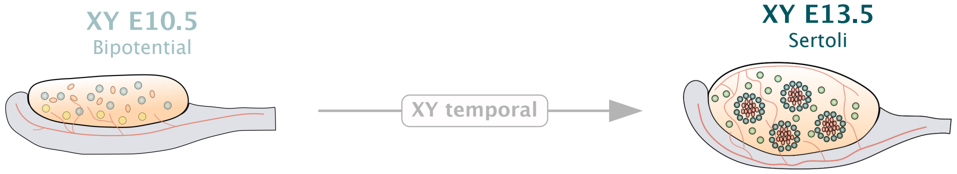


Local Moran Index

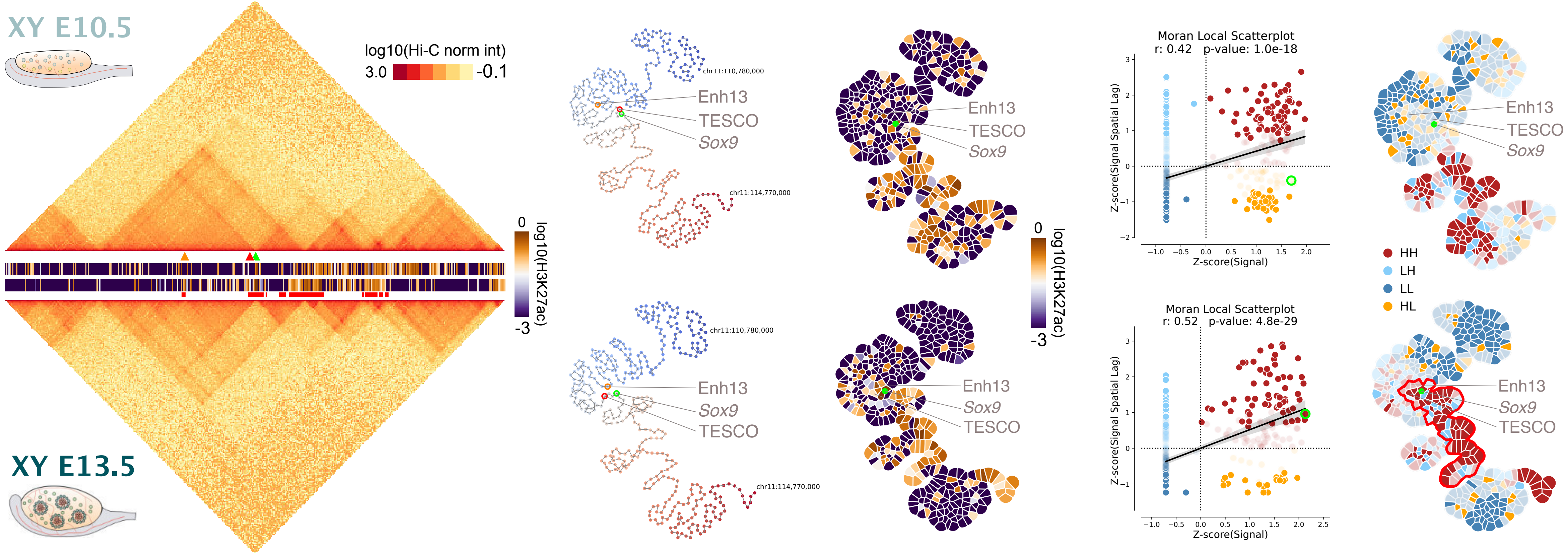




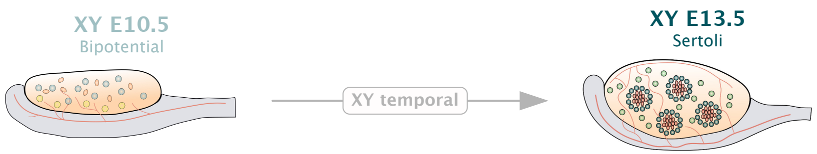
Quantifying regulatory environments bin by bin

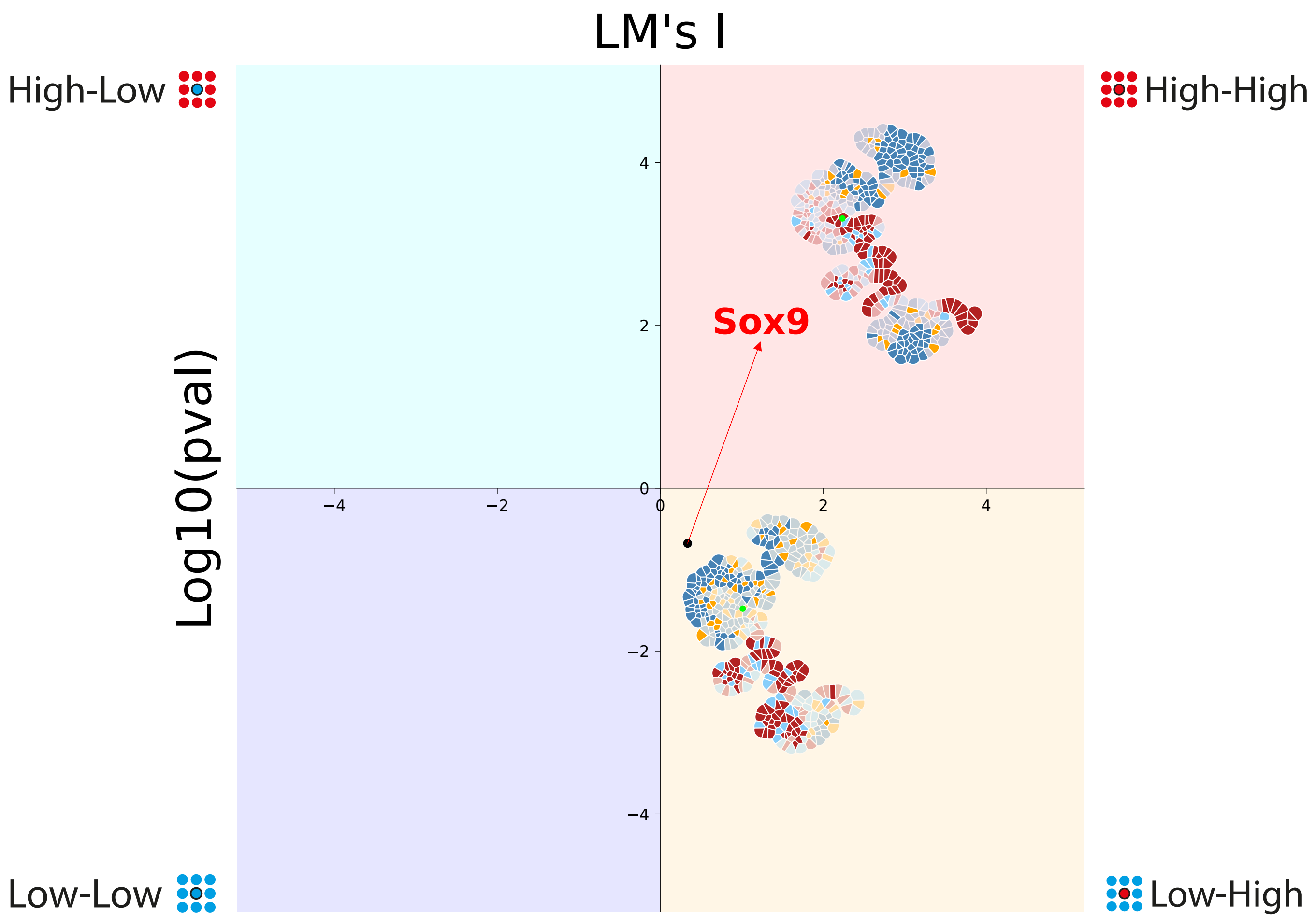


Sox9 locus chr11:110,780,000-114,770,000

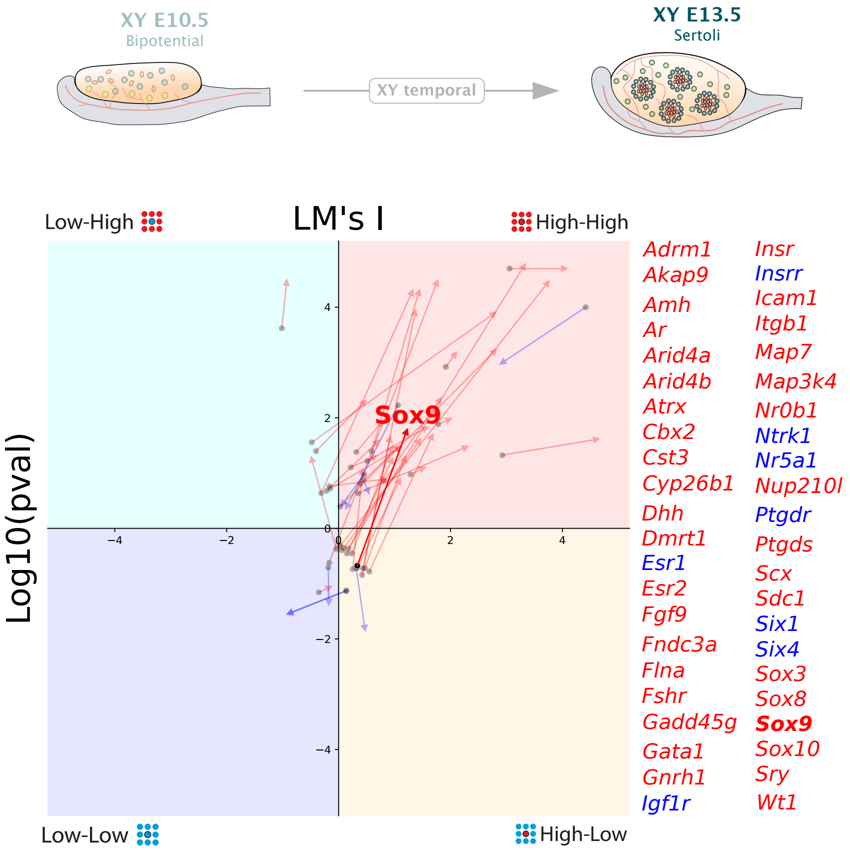


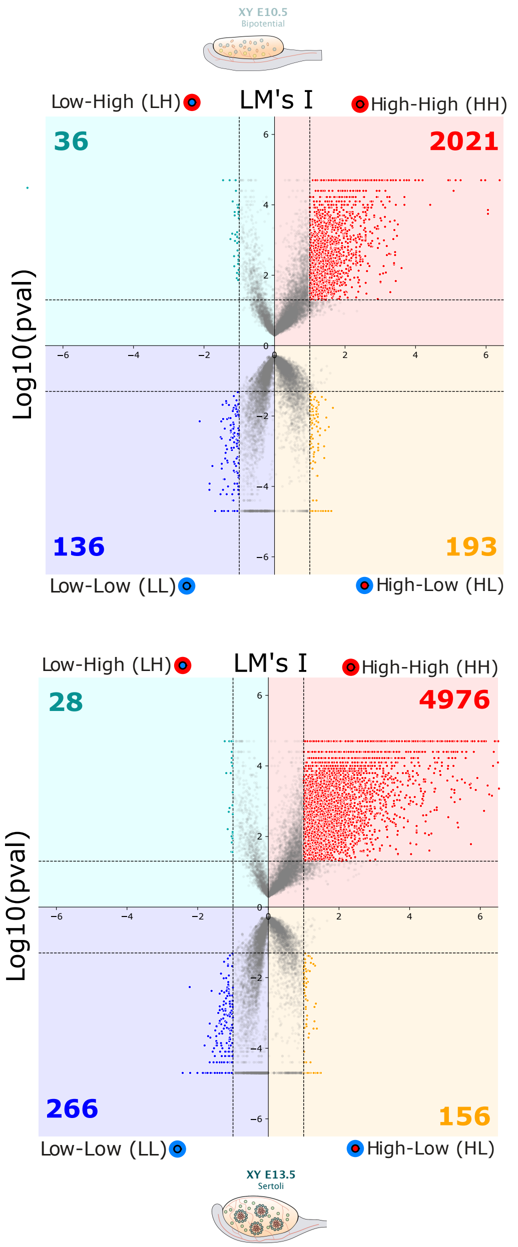
LMI Trip for Sox9 gene

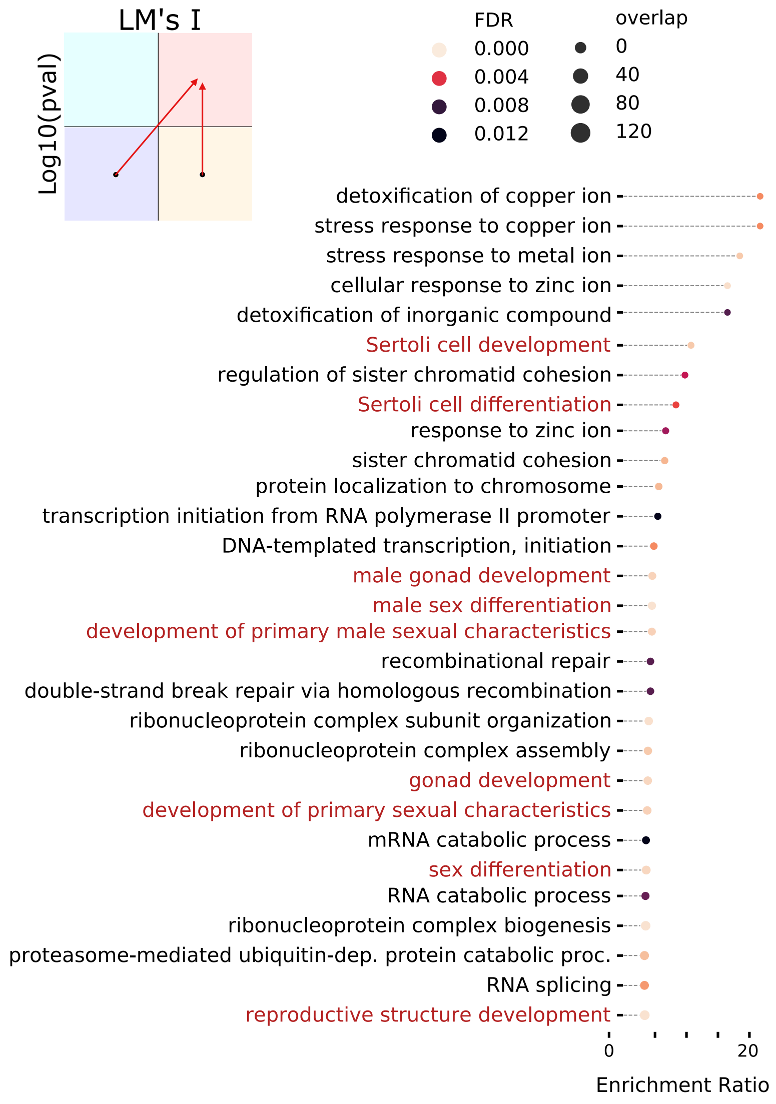




All genes LMI Trip





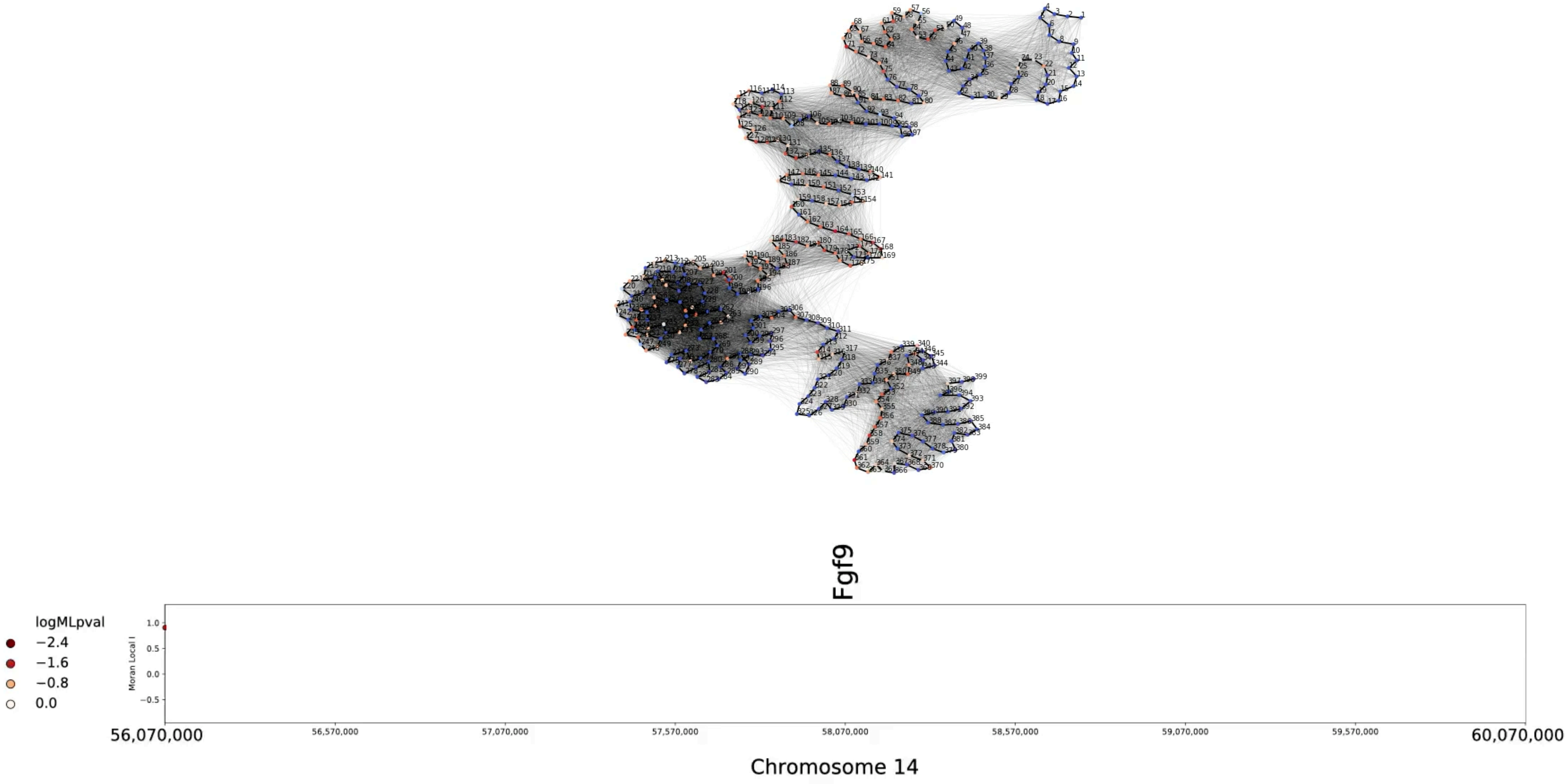


Now that we know the genes….

Can we identify regulatory elements using METALoci

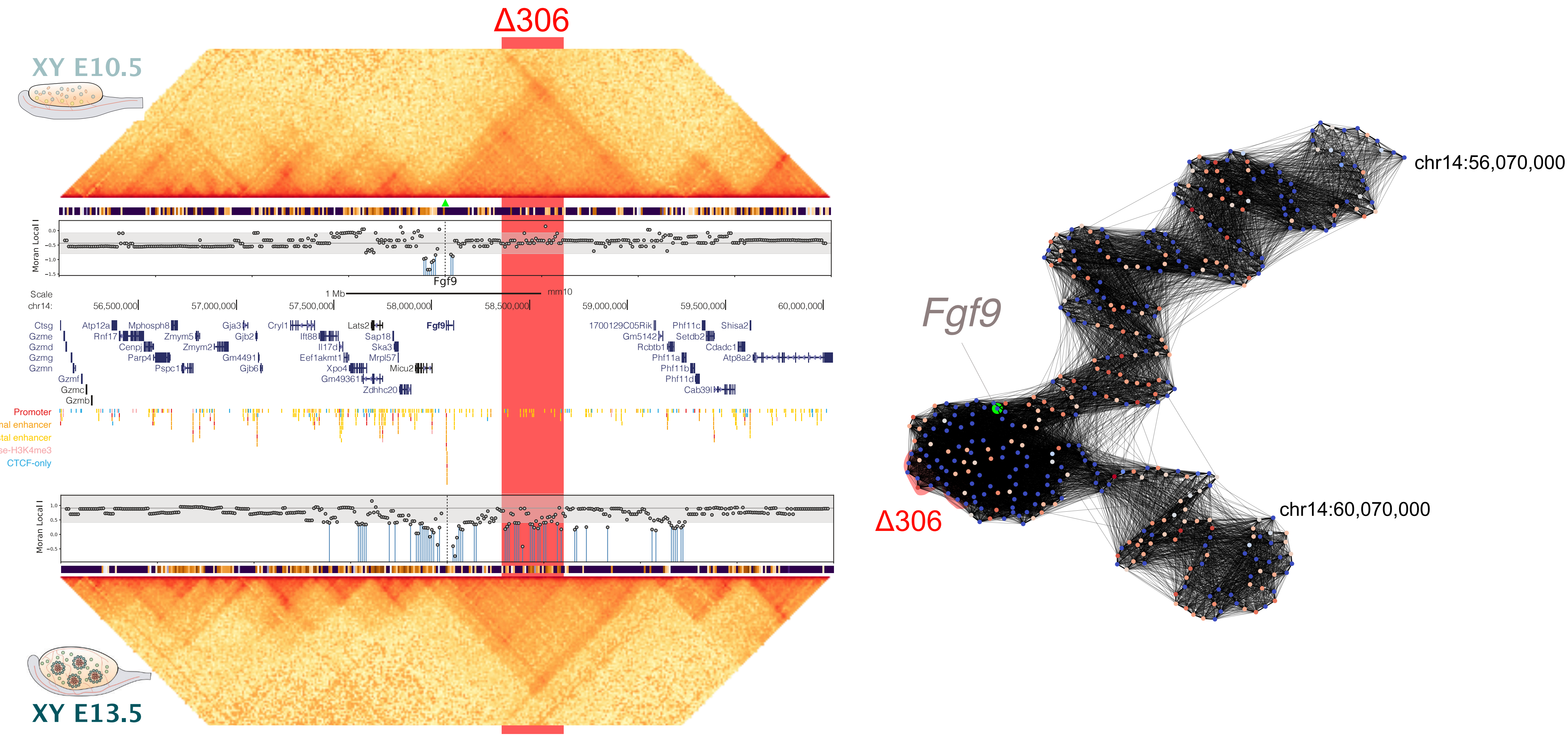
METALoci predictive mode

Fgf9 locus chr14:56,070,000-60,070,000



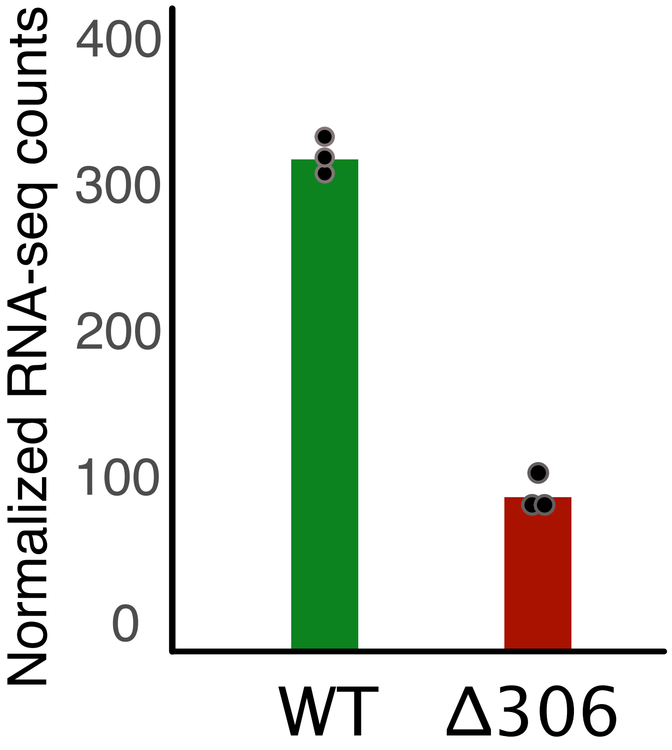
METALoci predictive mode

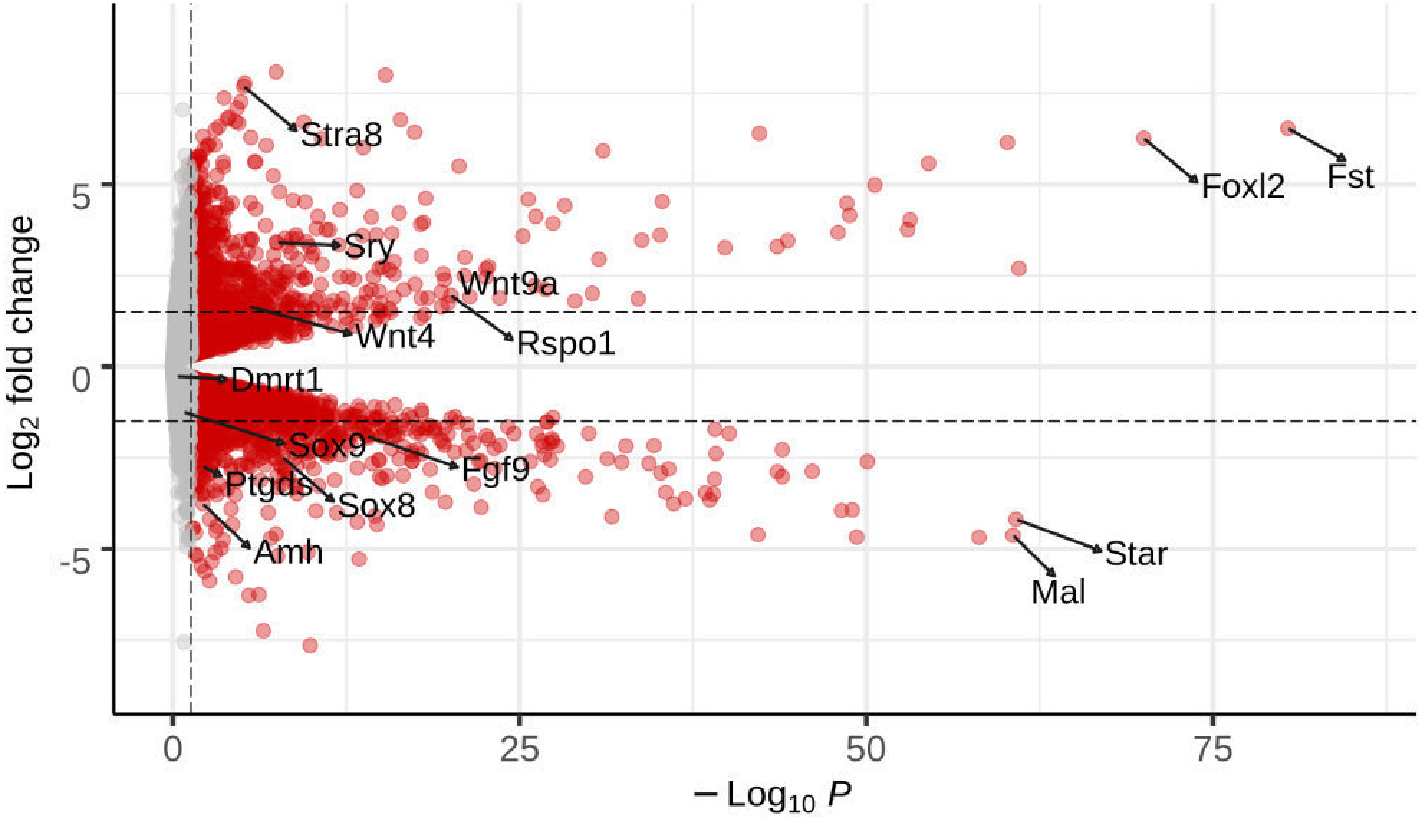
Fgf9 locus chr14:56,070,000-60,070,000



METALoci predictive mode

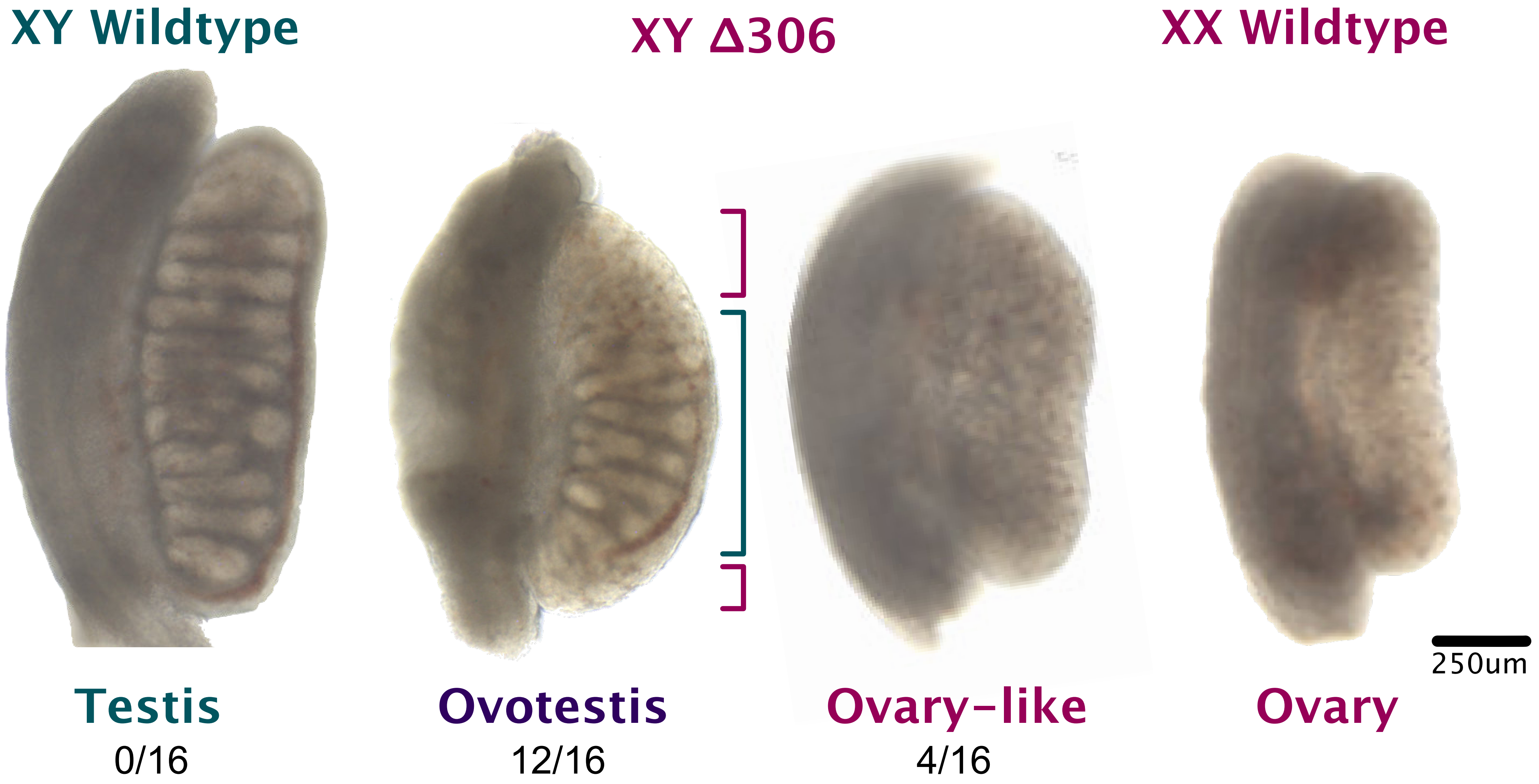
Fgf9 XY Δ306 mutant





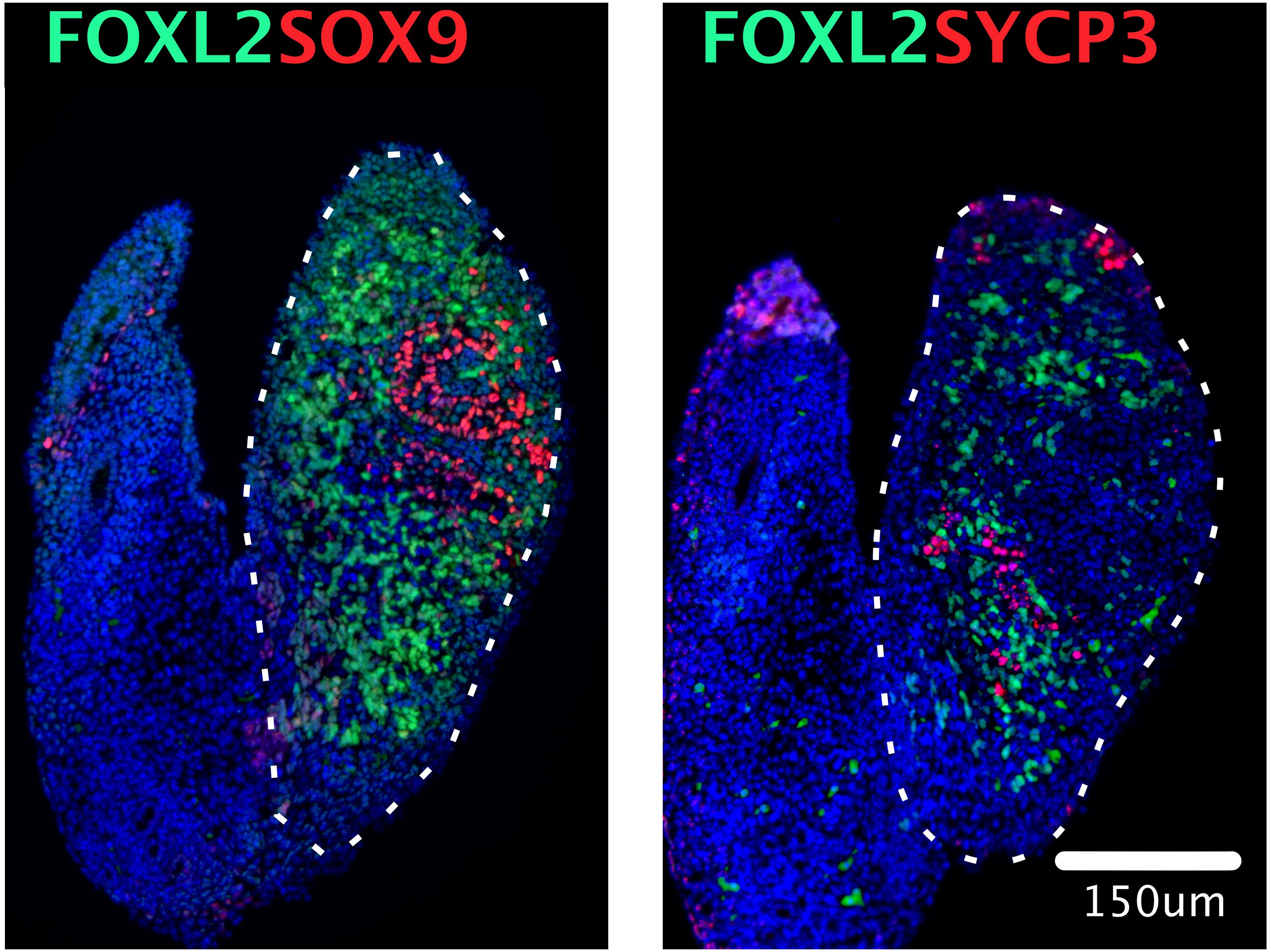
METALoci predictive mode

Fgf9 XY Δ306 mutant



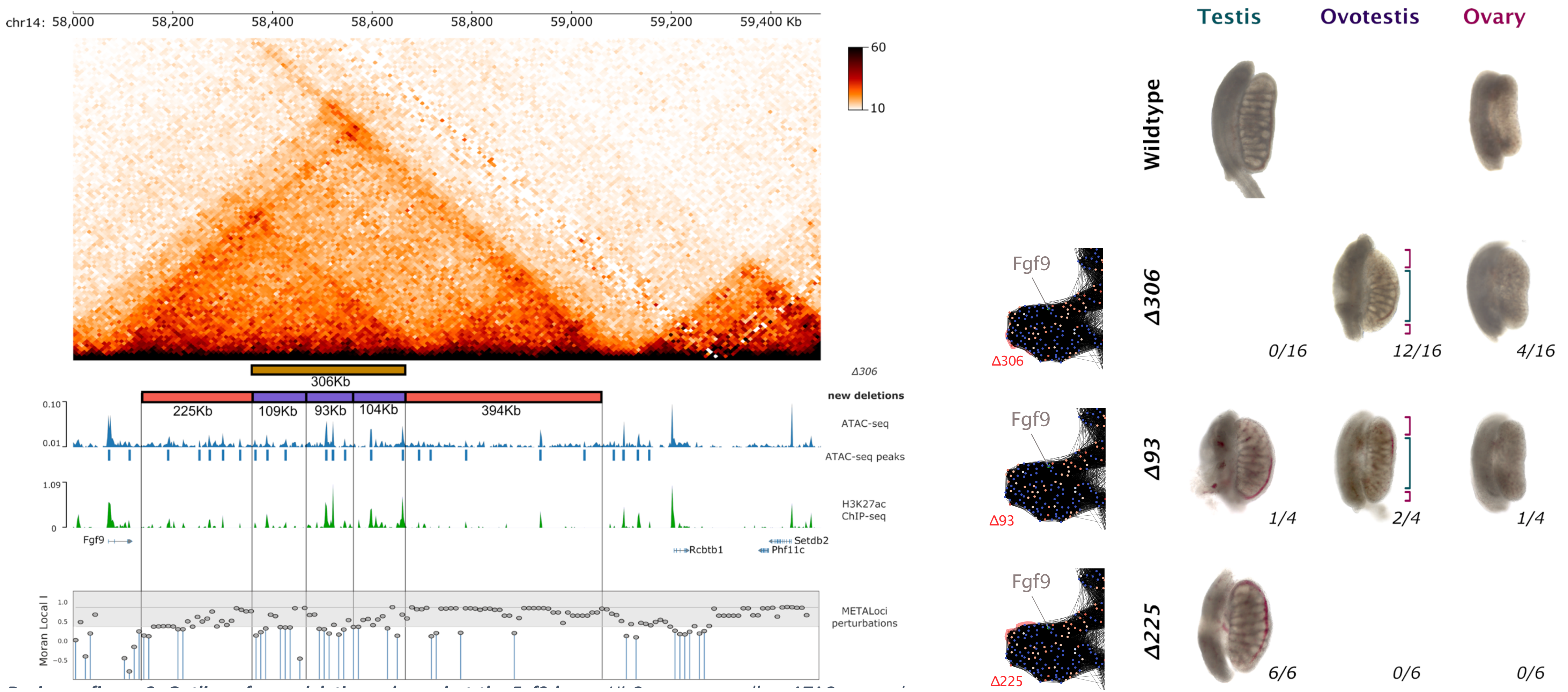
METALoci predictive mode

Fgf9 XY Δ306 mutant



METALoci predictive mode

Fgf9 XY additional mutants



Take home messages:

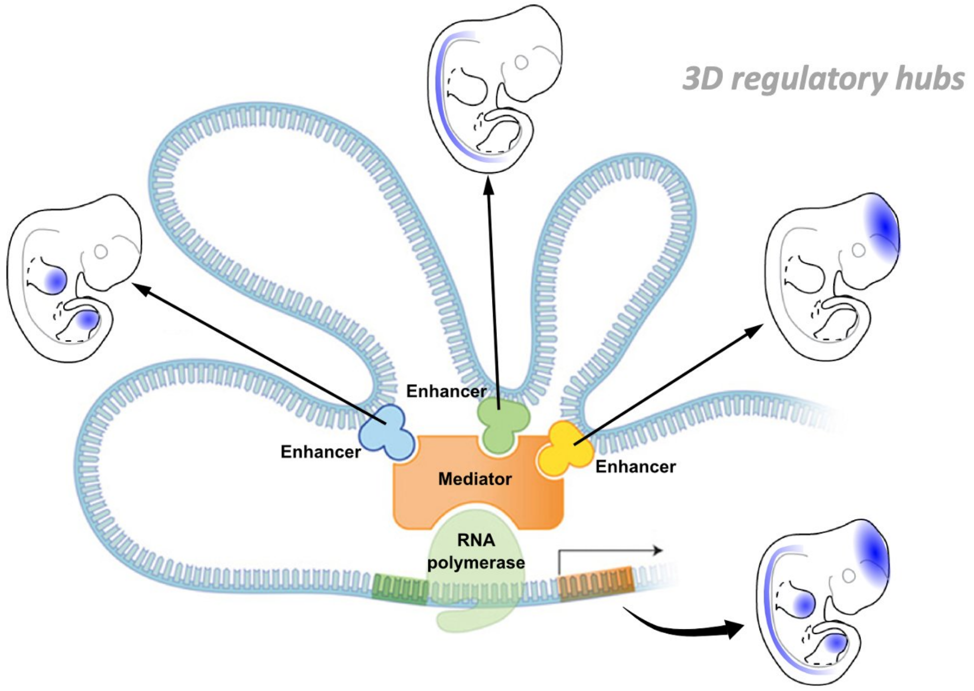
First characterization of the 3D regulatory landscape of sex determination

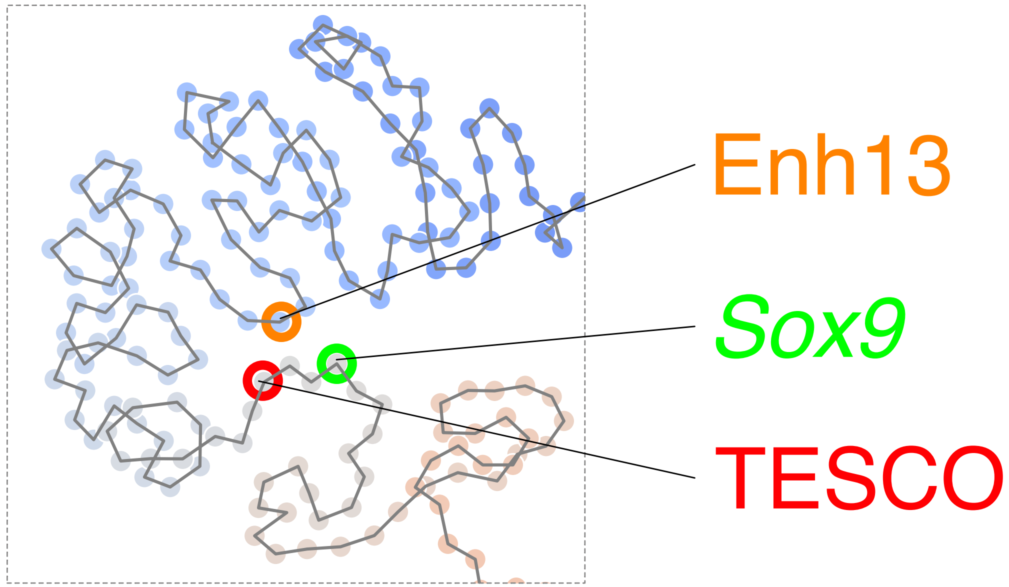
METALoci is an unbiased approach to quantify gene regulatory activity

METALoci is a predictive tool to identify critical regulatory loci

Discovery of a novel non-coding region controlling sex determination

Take home messages:





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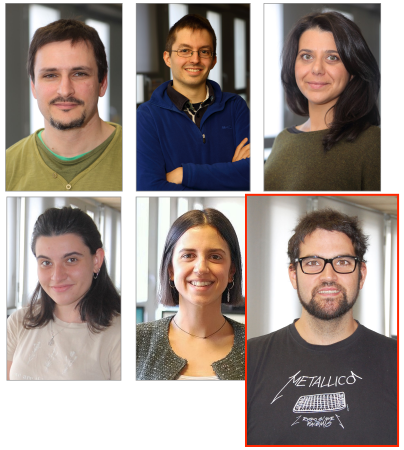

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.: Conflict of Interest Statement :.

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