Photo by David Oliete - www.davidoliete.com

Spatio-temporal regulatory landscape of sex-determination



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CNAG-CRG · ICREA

BioRxiv 2022

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

cnag CRG ICREA

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

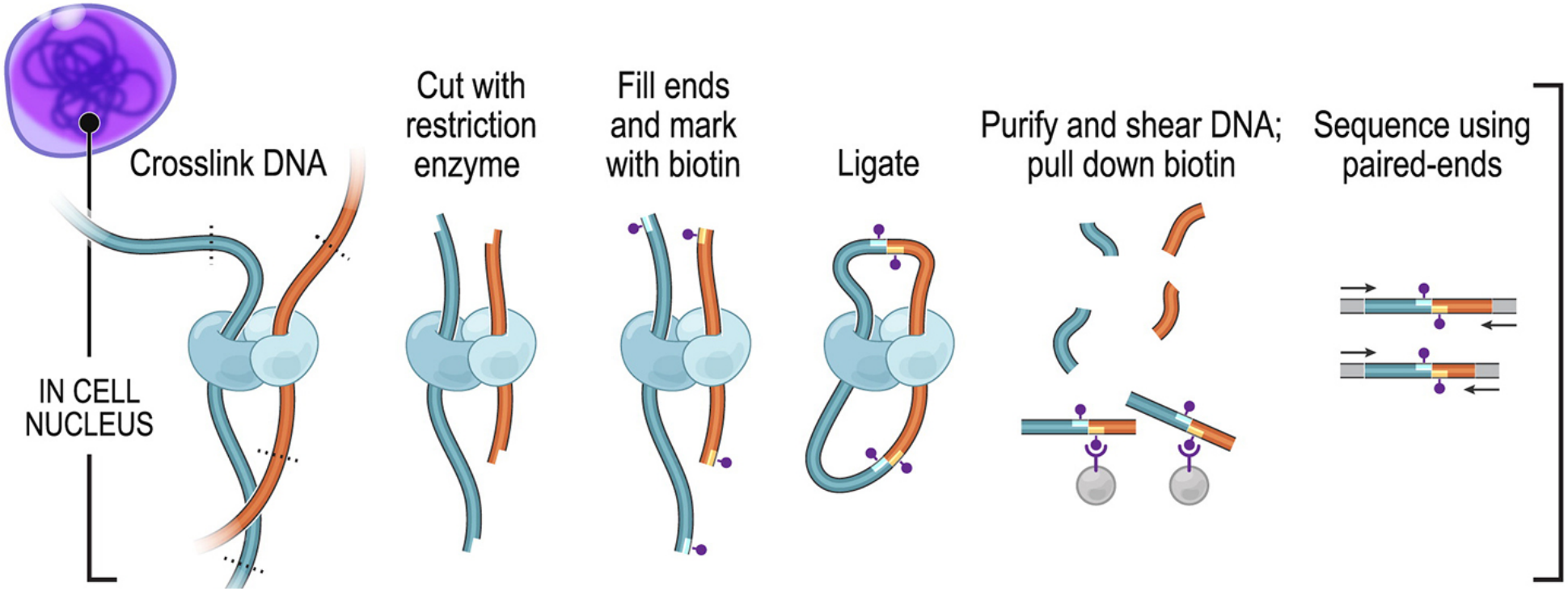
Discovery of *Sry* gene

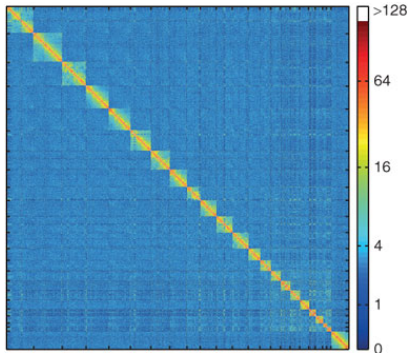
Koopman et al., Nature, 1991

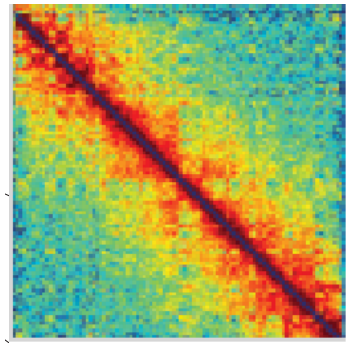
(Goodfellow & Lovell Badge labs)

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.

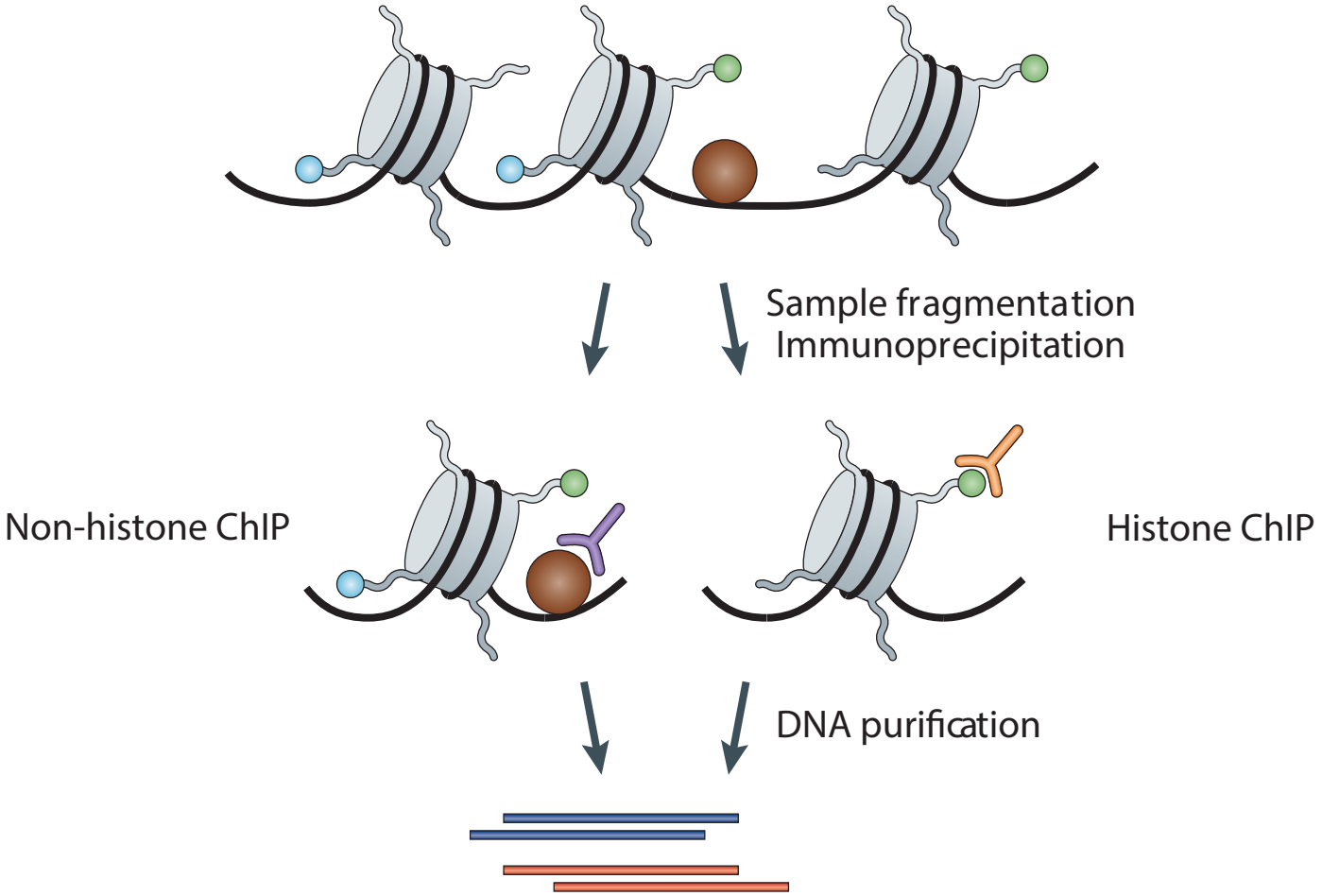




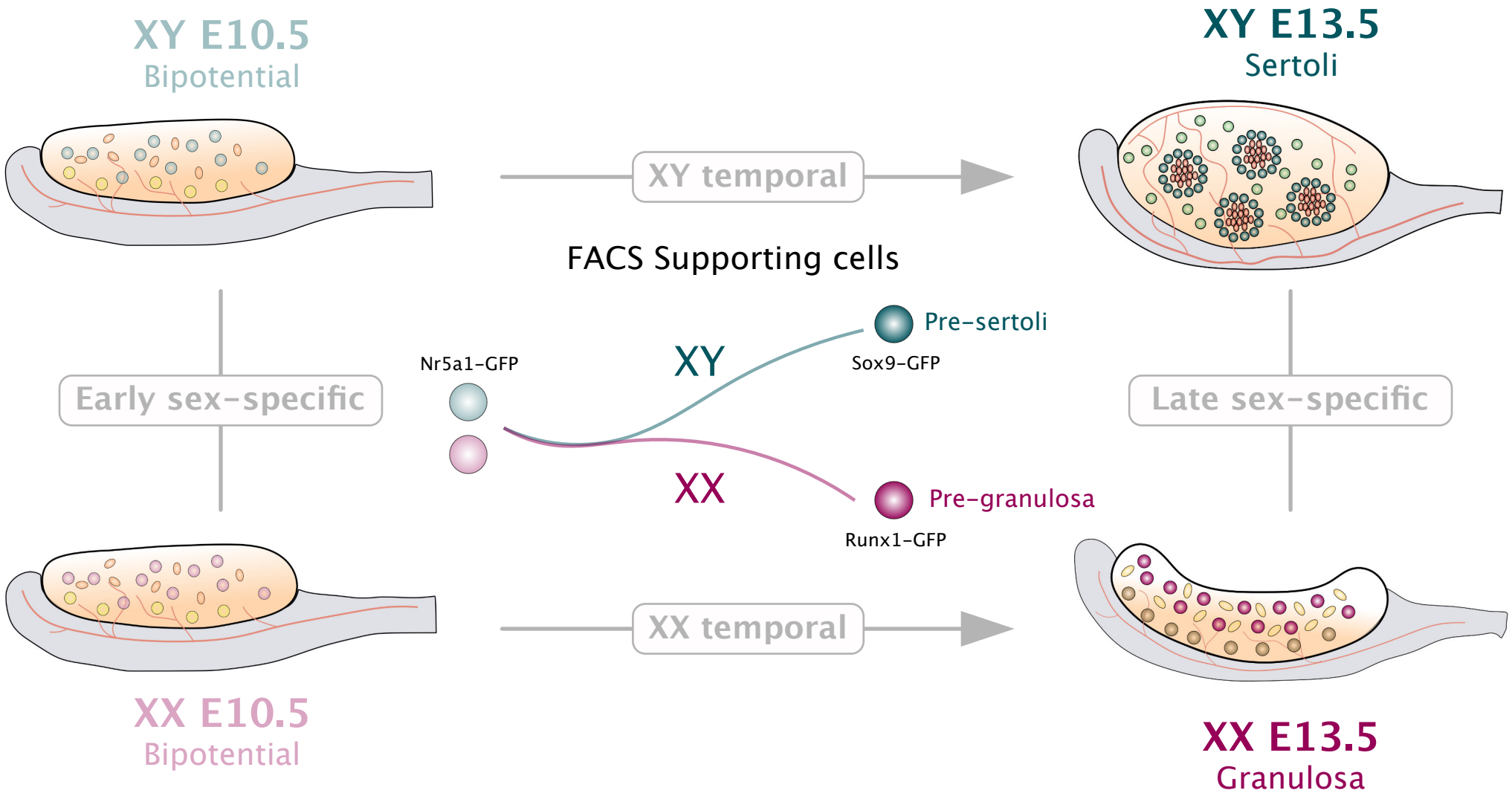


Chromatin Immunoprecipitation (ChIP)

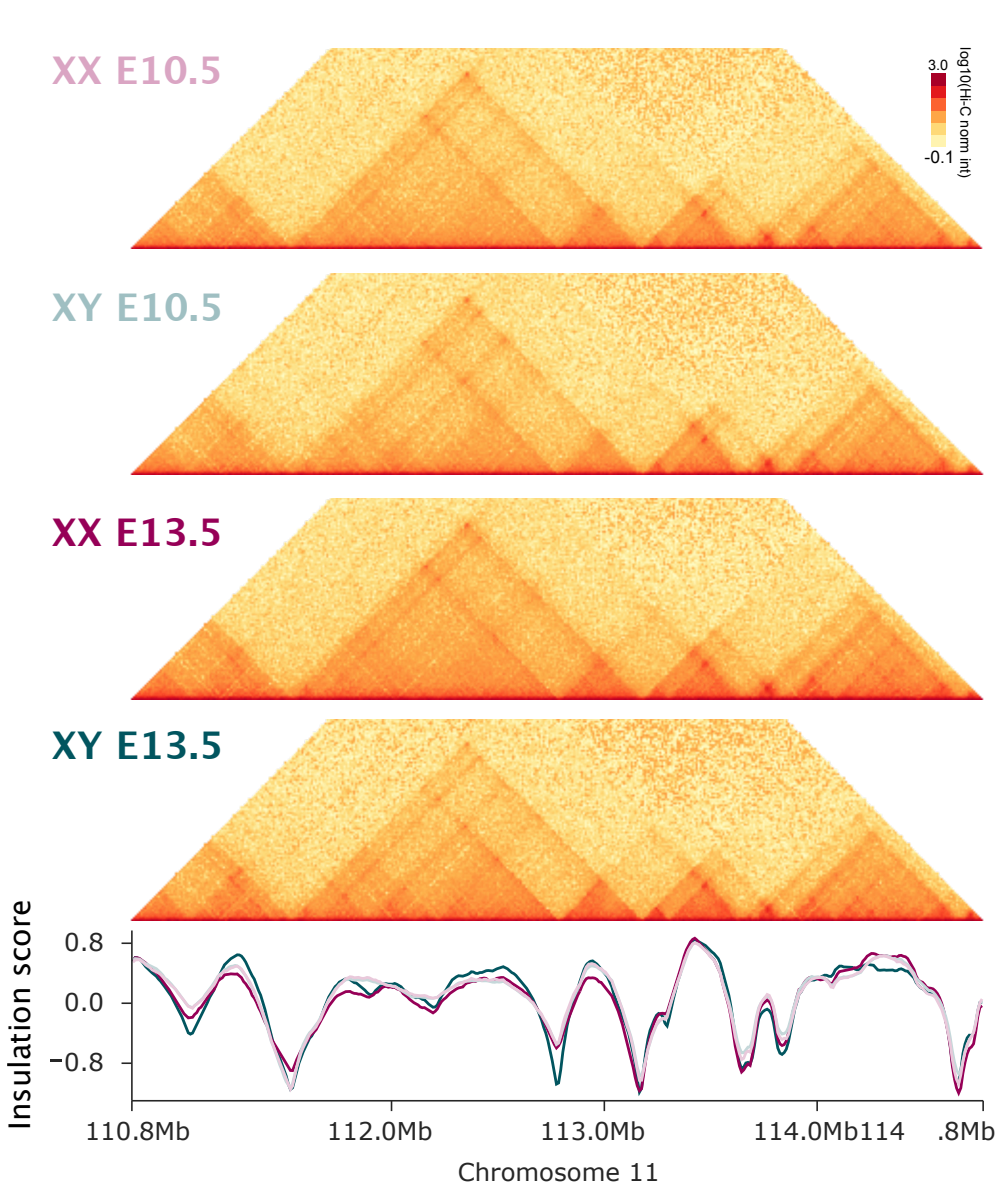
Solomon, M. J., Larsen, P. L. & Varshavsky, A. (1988) Cell 53, 937–947.
Park, P.J. (2009) Nature Reviews Genetics 10, 669–680.

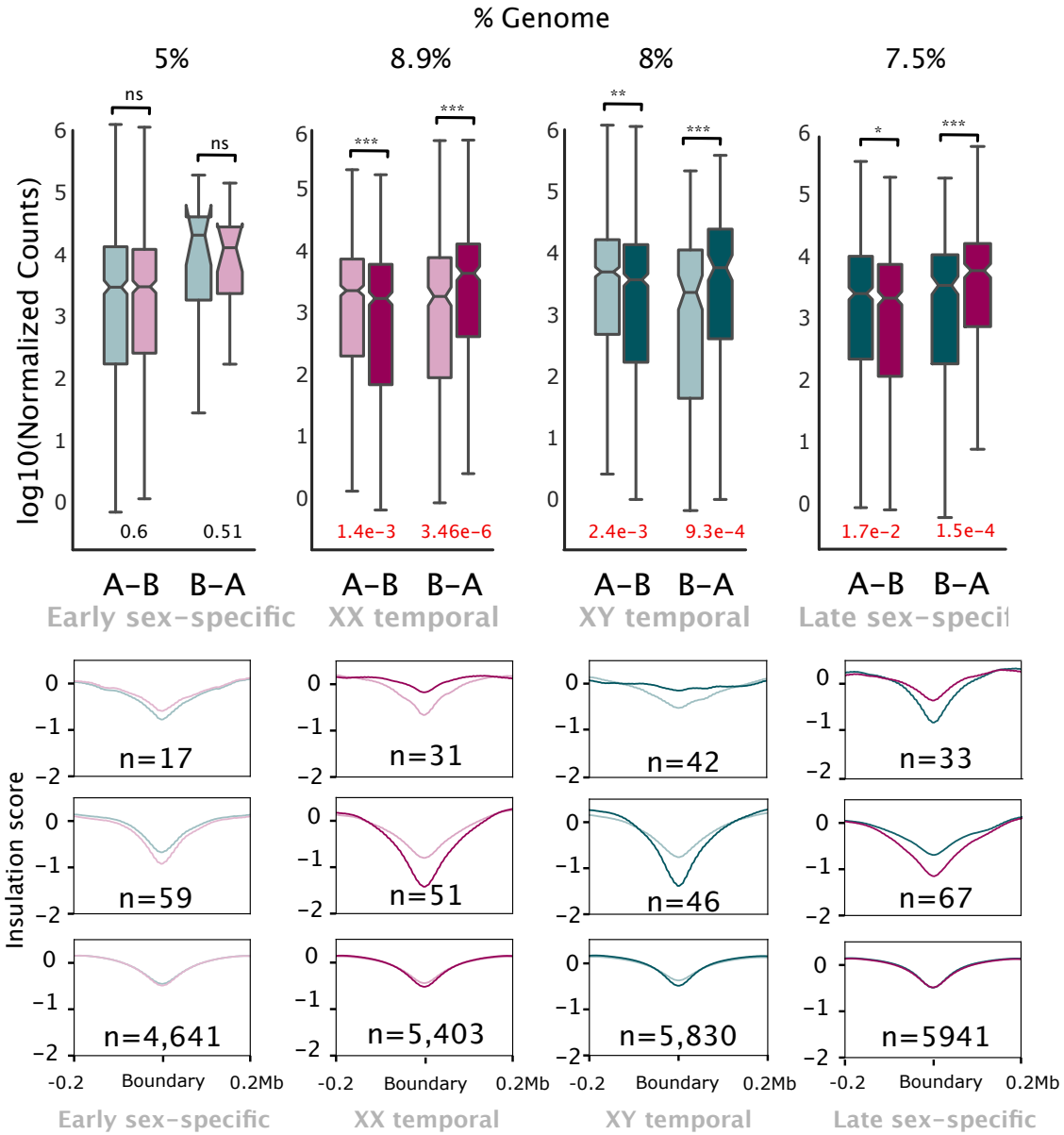


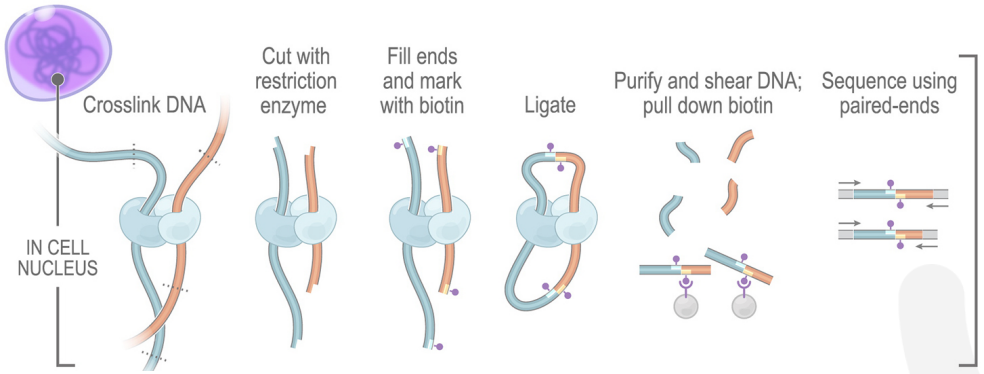
Sex-determination as a model for "bipotential" commitment

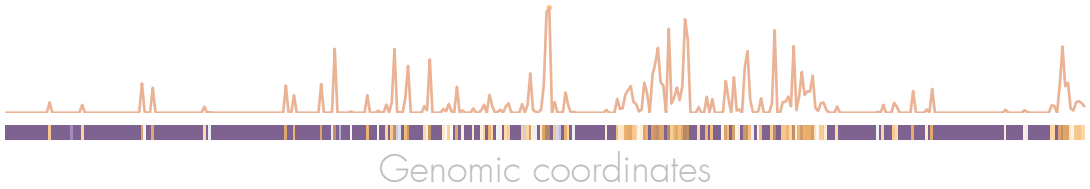


No major structural (apparent) differences





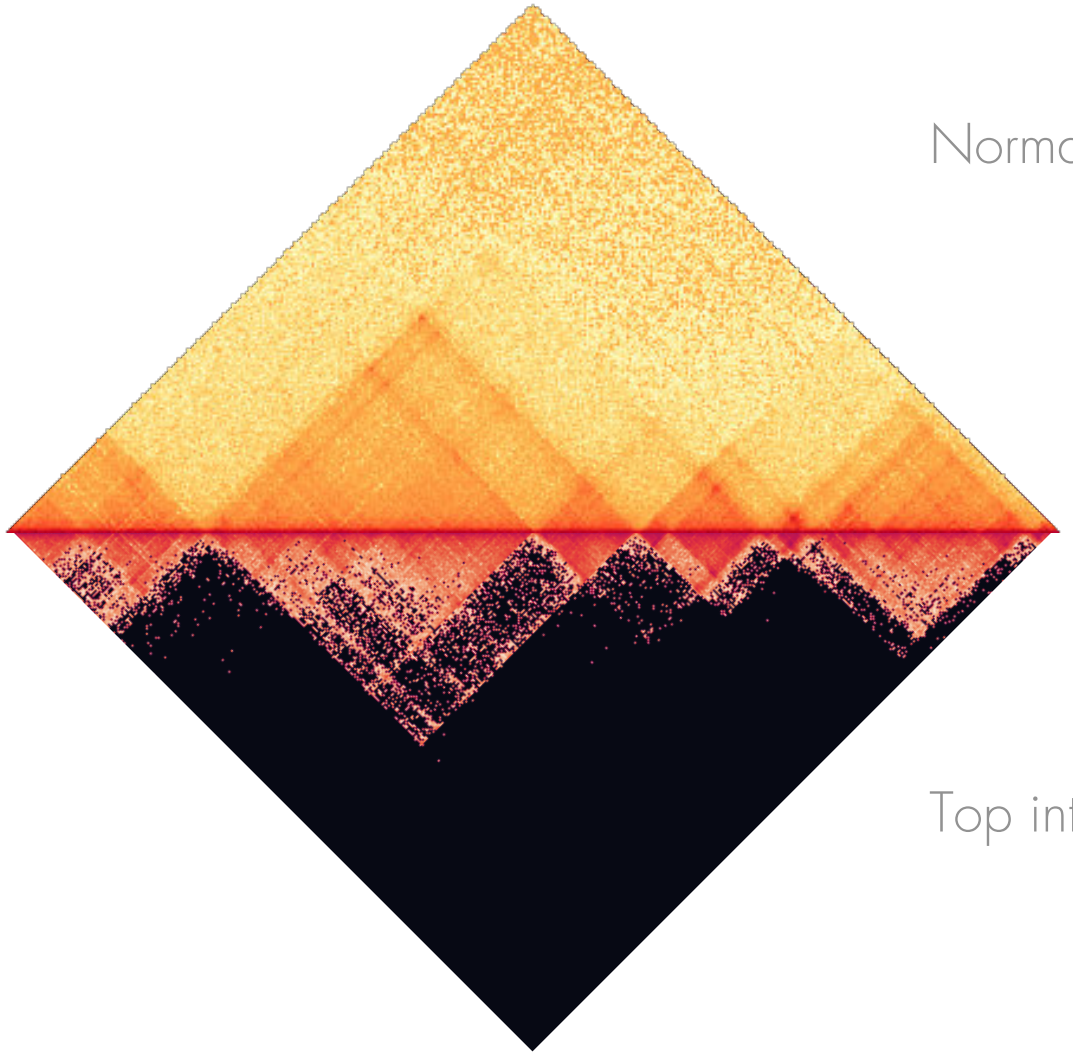

Crosslink DNA
IN CELL NUCLEUS
Cut with restriction enzyme
Fill ends and mark with biotin
Ligate
Purify and shear DNA; pull down biotin
Sequence using paired-ends


Genomic coordinates

ETALoci

Hi-C normalization and interaction selection

chr11:110780000-114770000

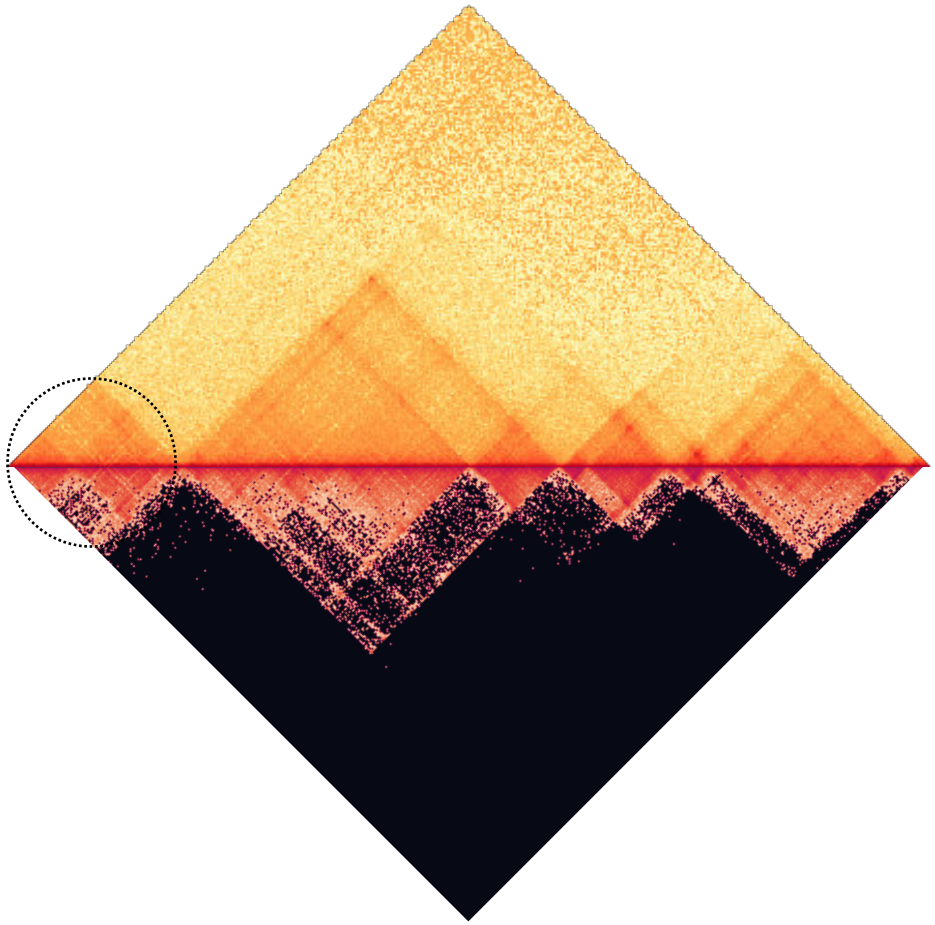


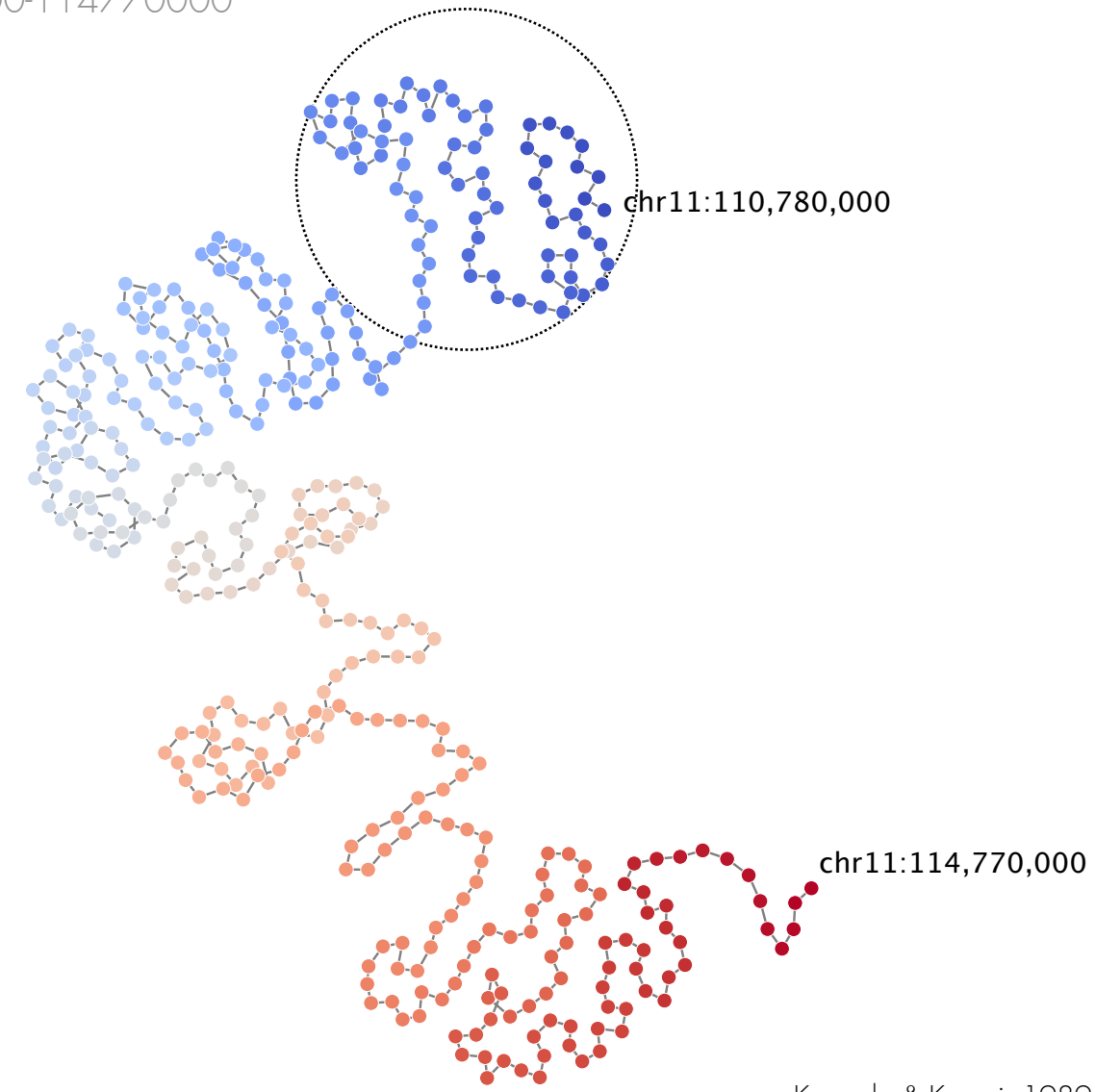
Normalized Hi-C

Top interactions

Spatial lay-out of significant interactions

chr11:110780000-114770000

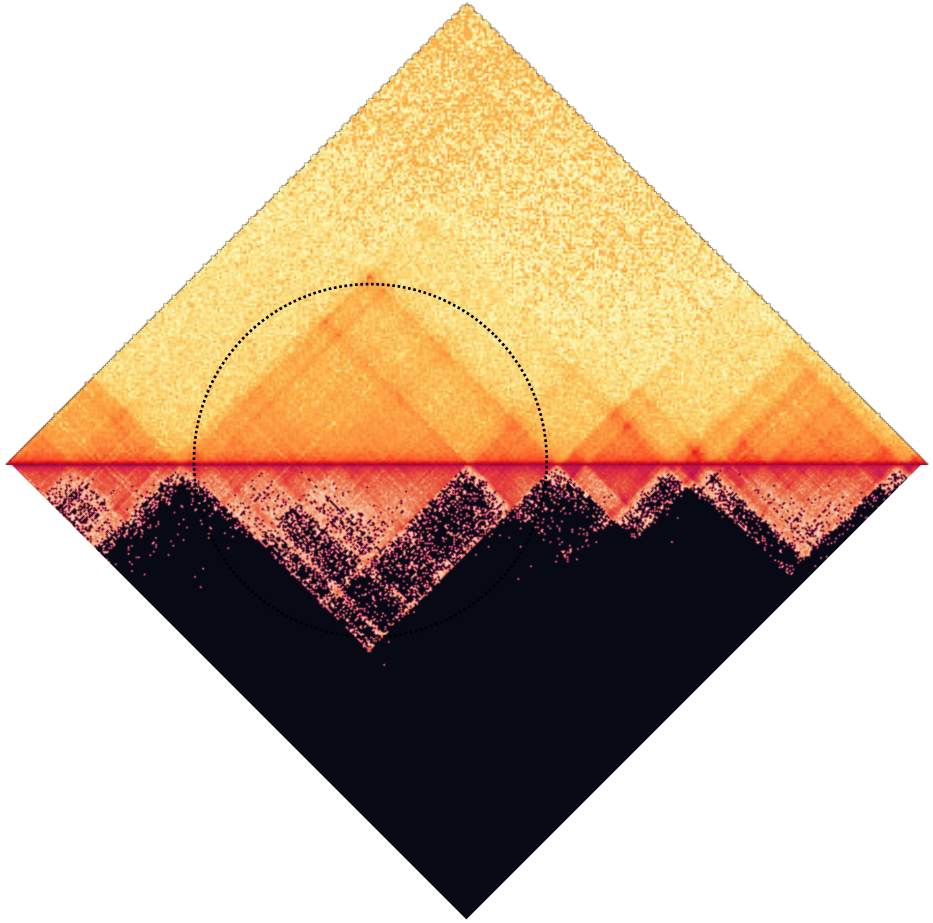


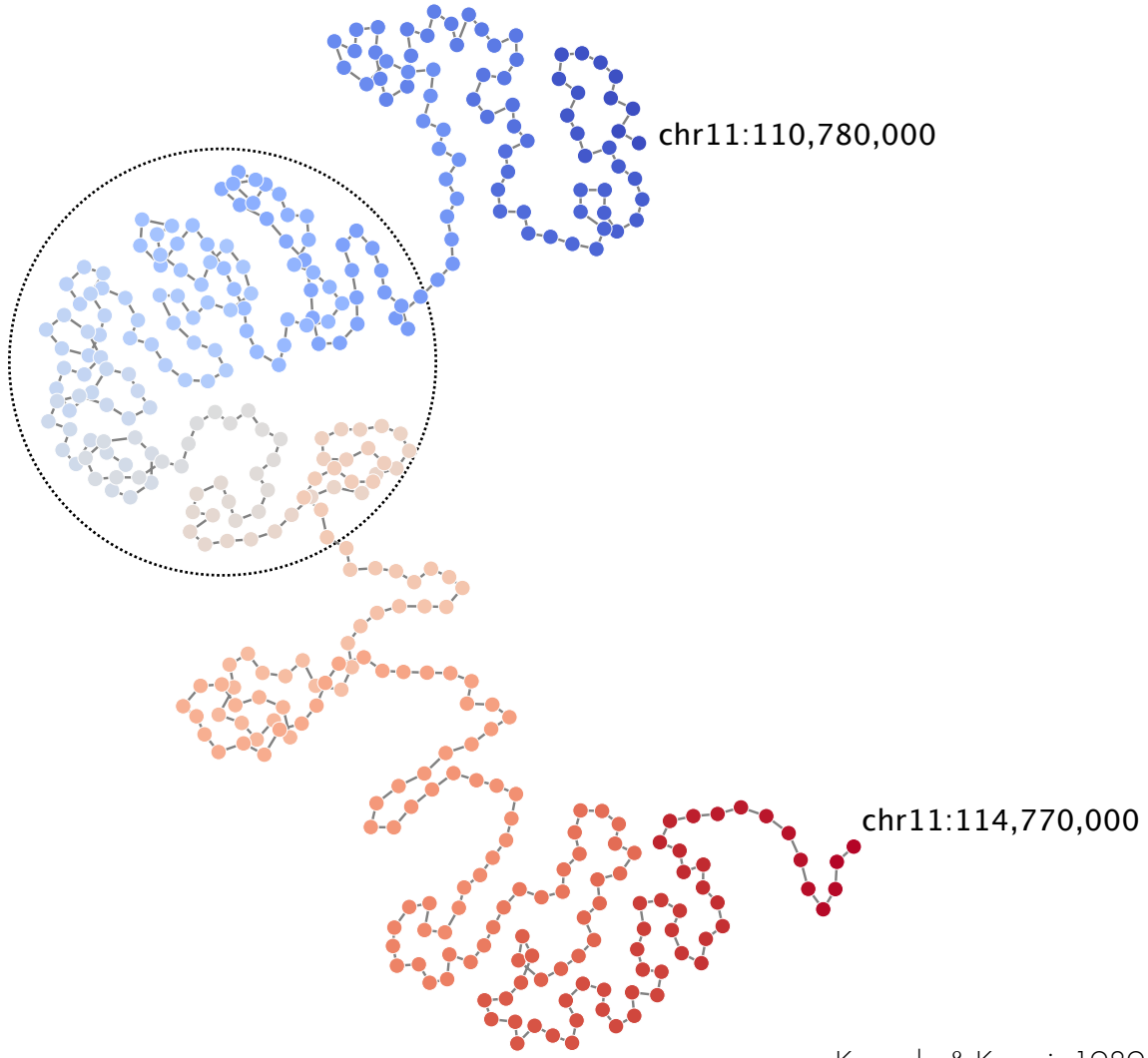


Kamada & Kawai, 1989

Spatial lay-out of significant interactions

chr11:110780000-114770000

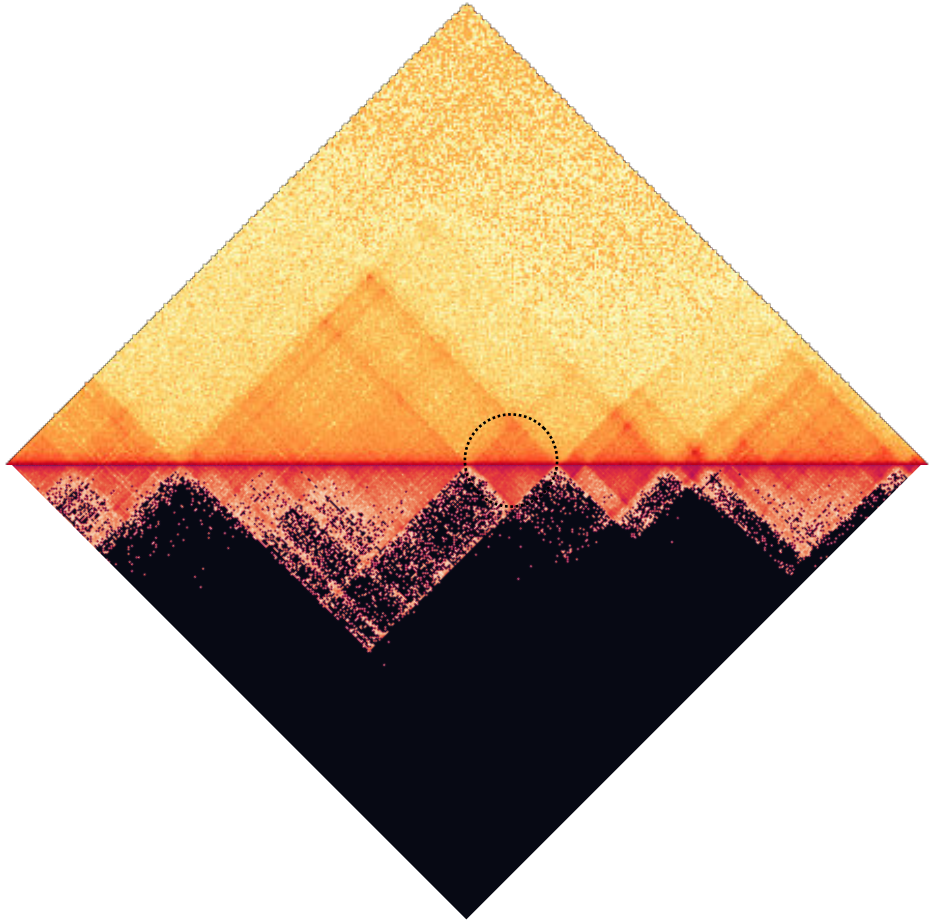


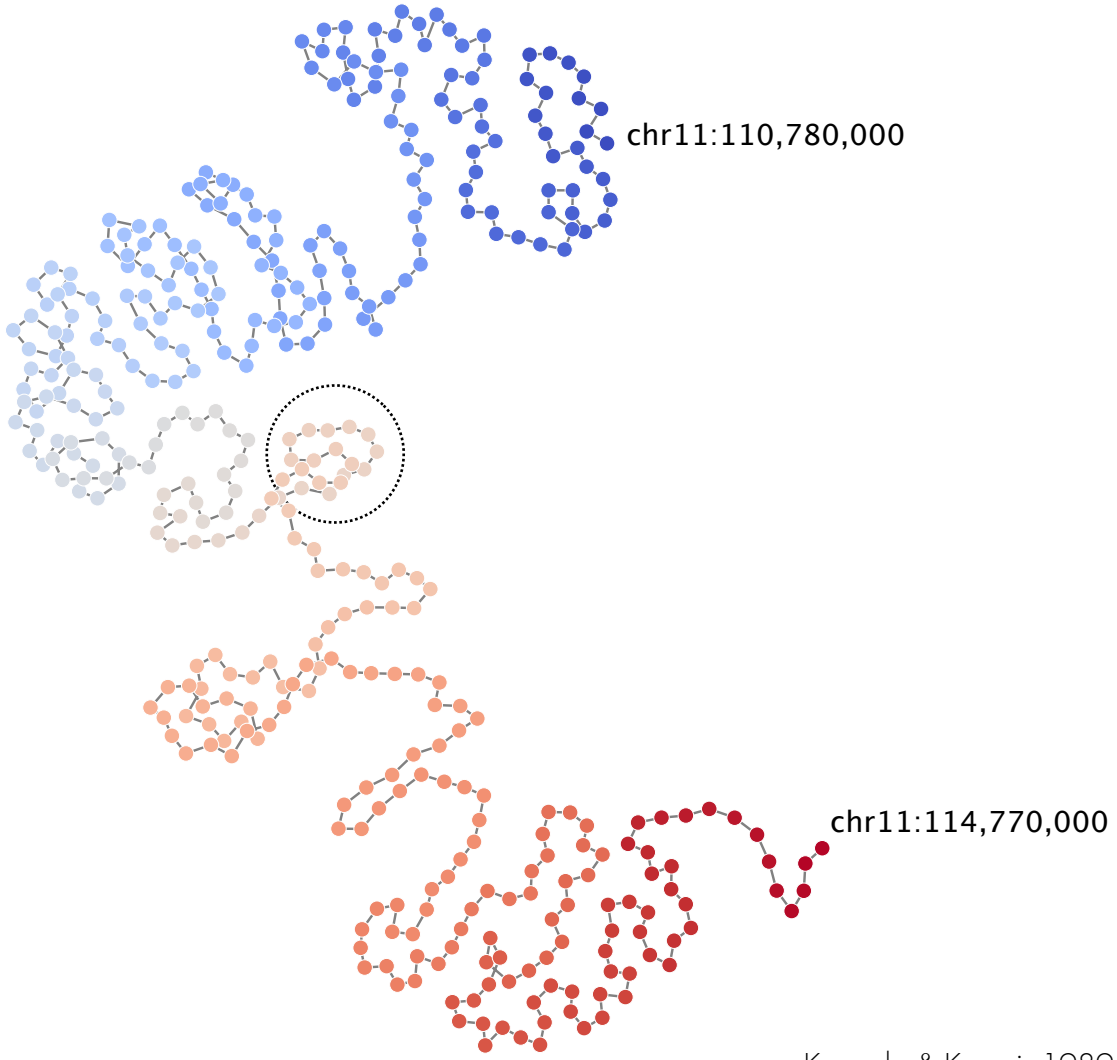


Kamada & Kawai, 1989

Spatial lay-out of significant interactions

chr11:110780000-114770000

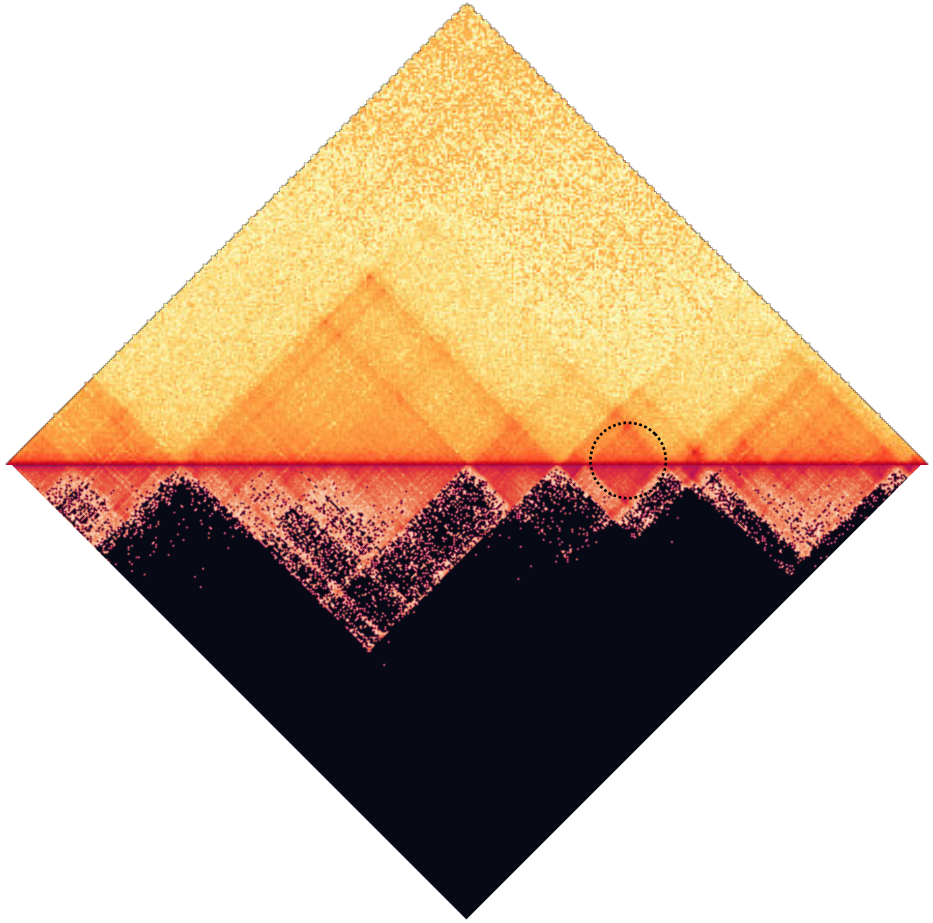


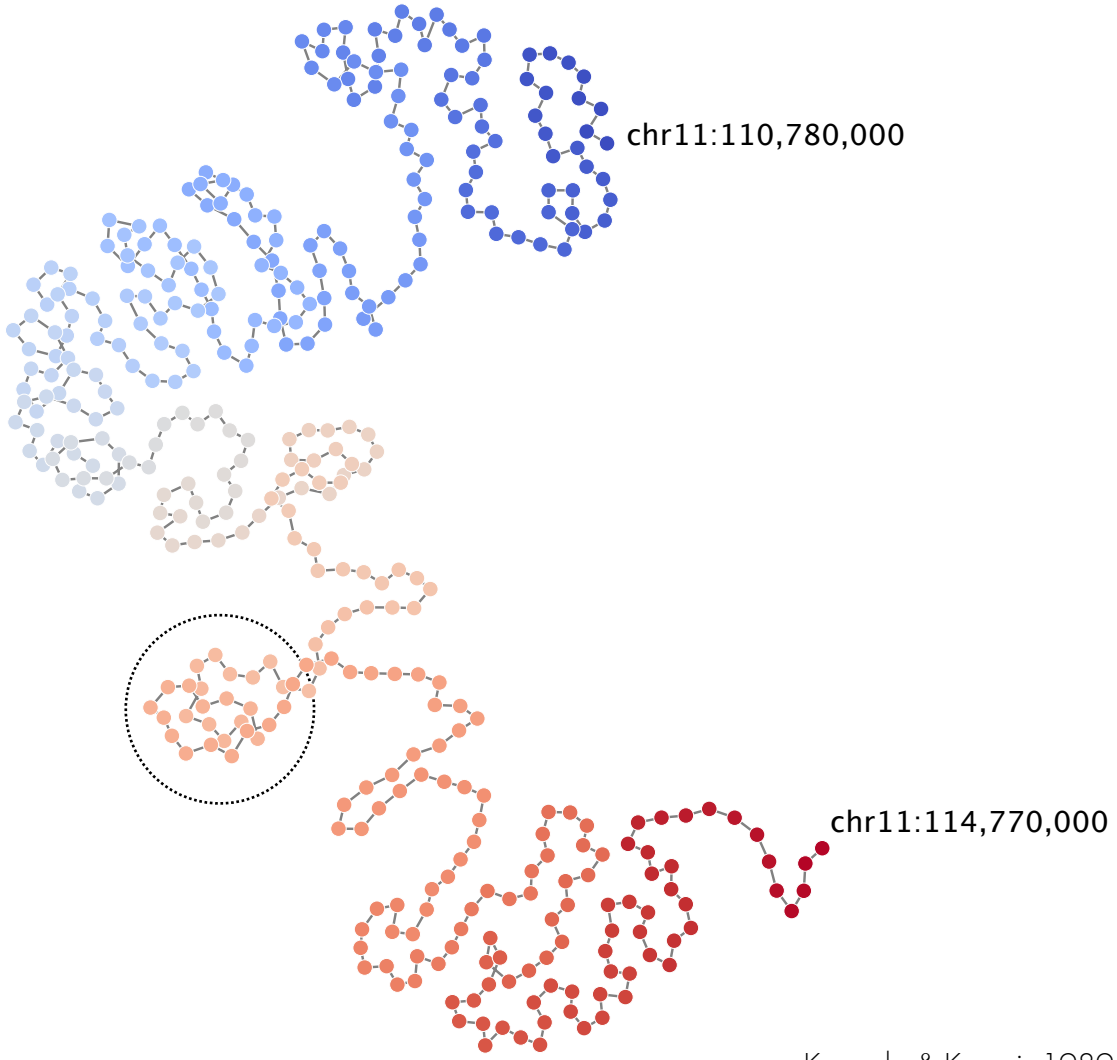


Kamada & Kawai, 1989

Spatial lay-out of significant interactions

chr11:110780000-114770000

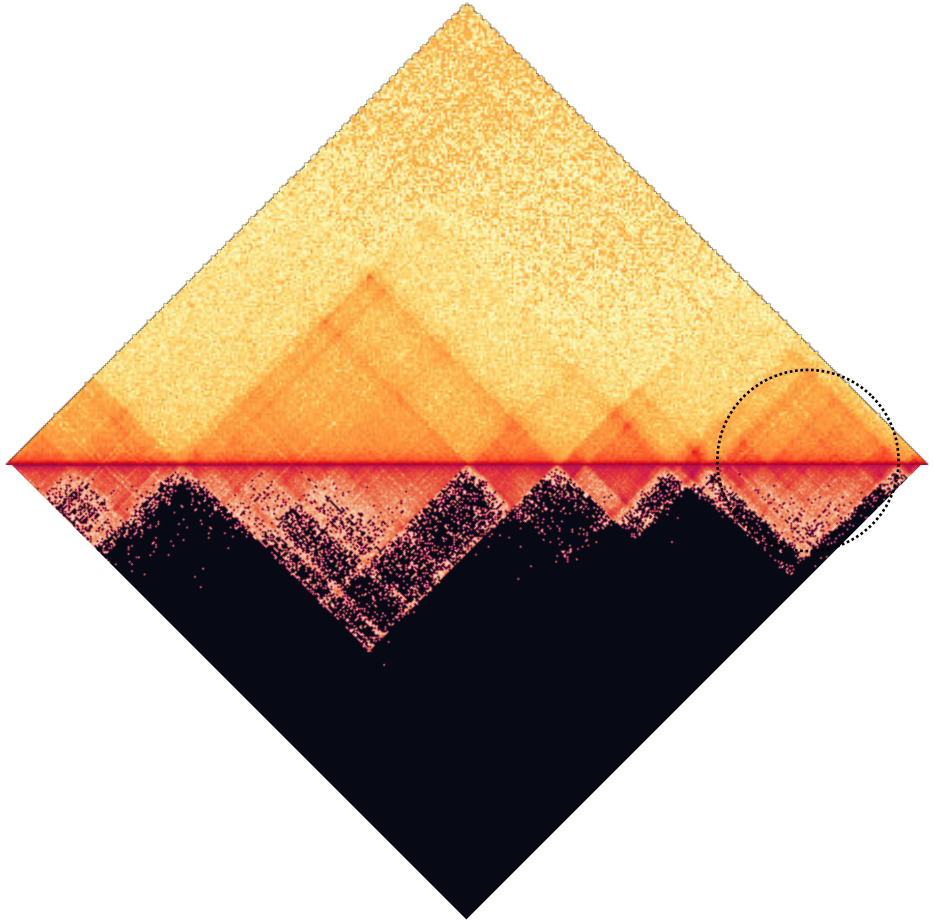


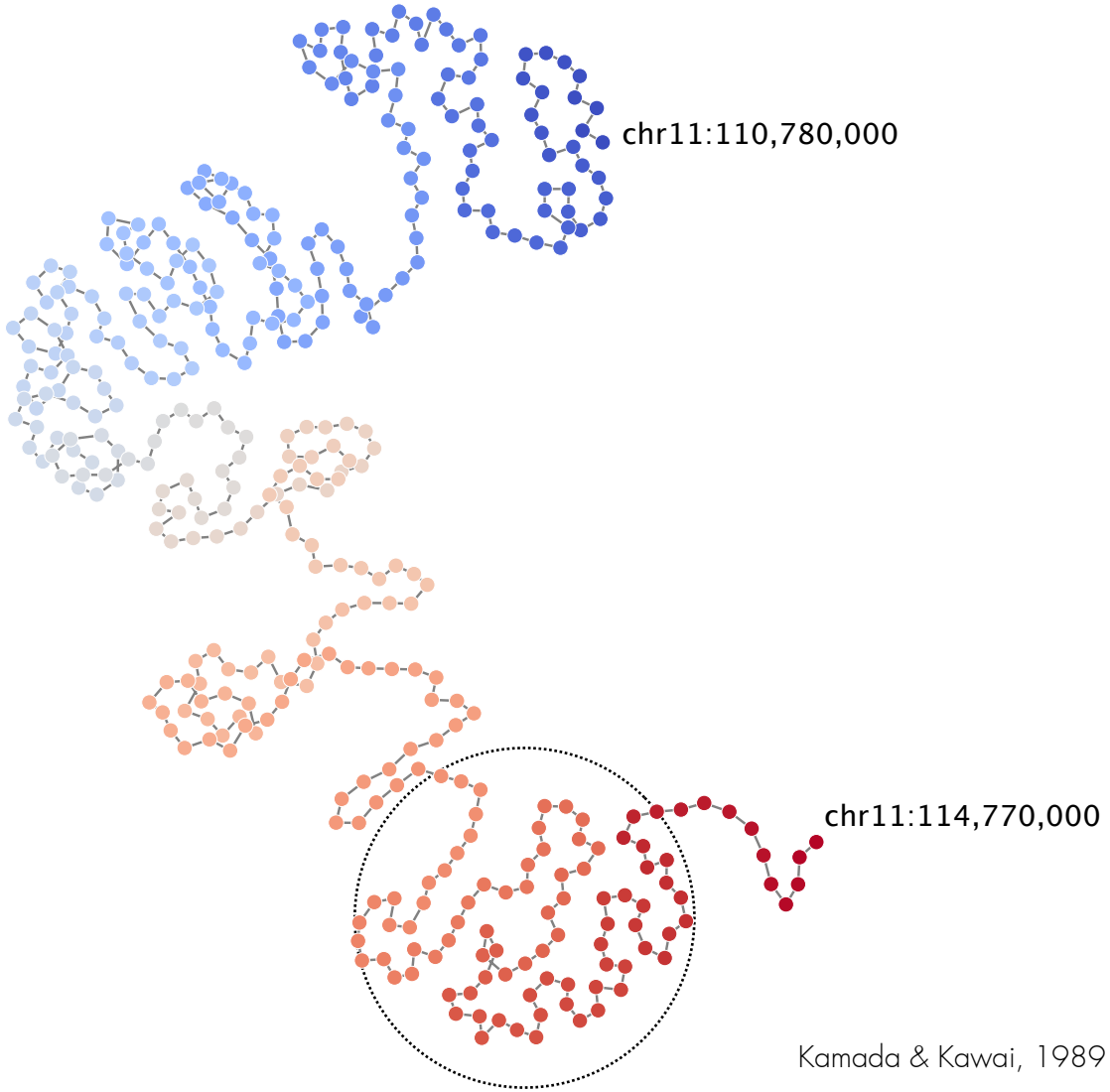


Kamada & Kawai, 1989

Spatial lay-out of significant interactions

chr11:110780000-114770000

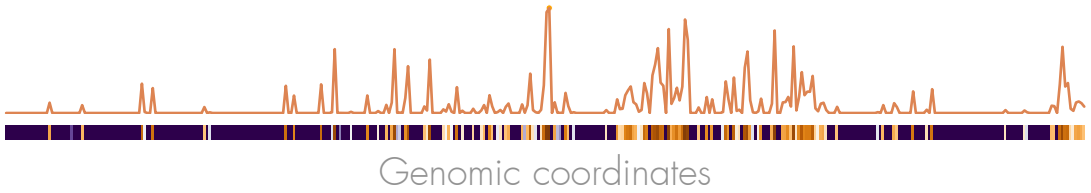


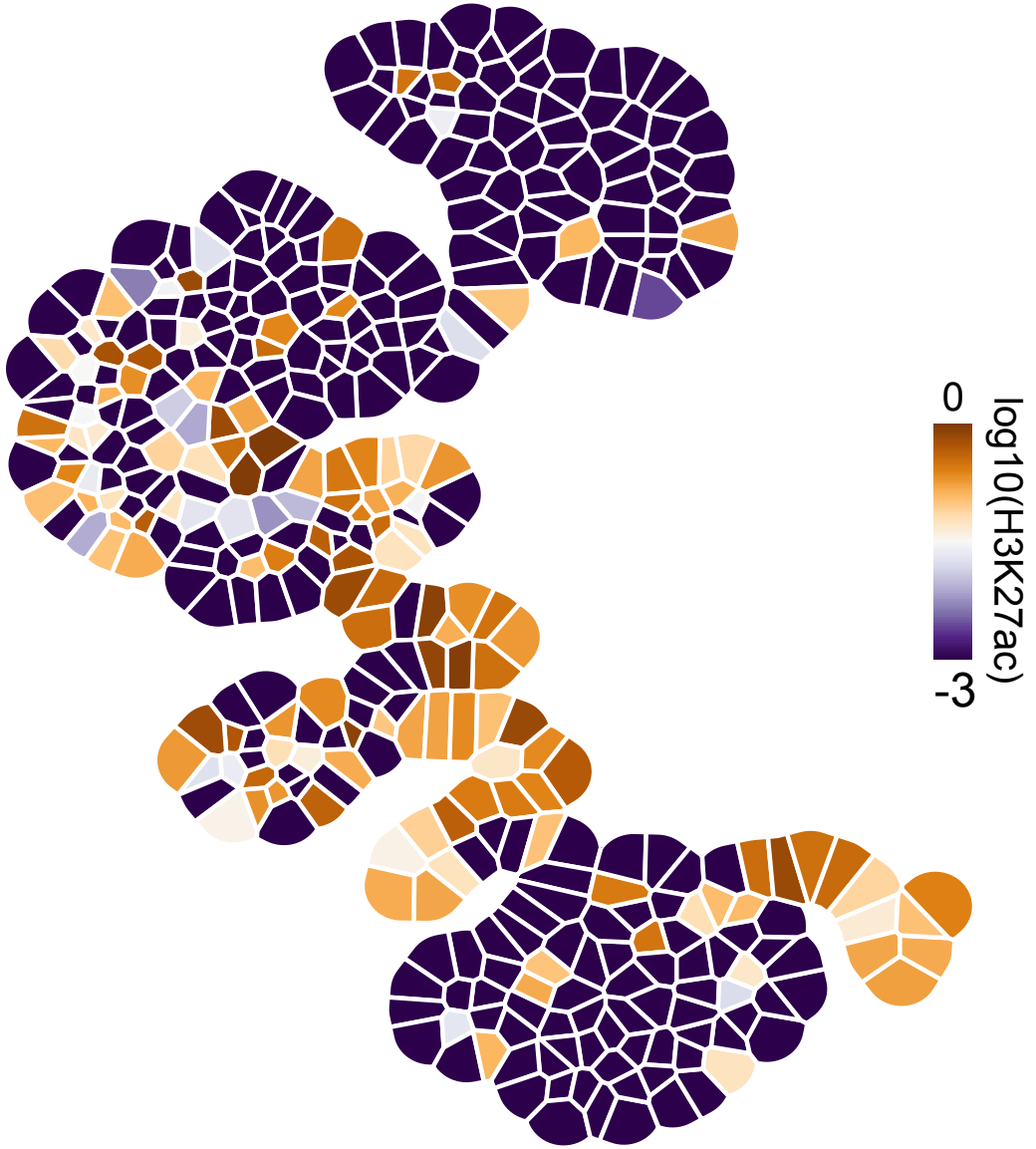


Kamada & Kawai, 1989

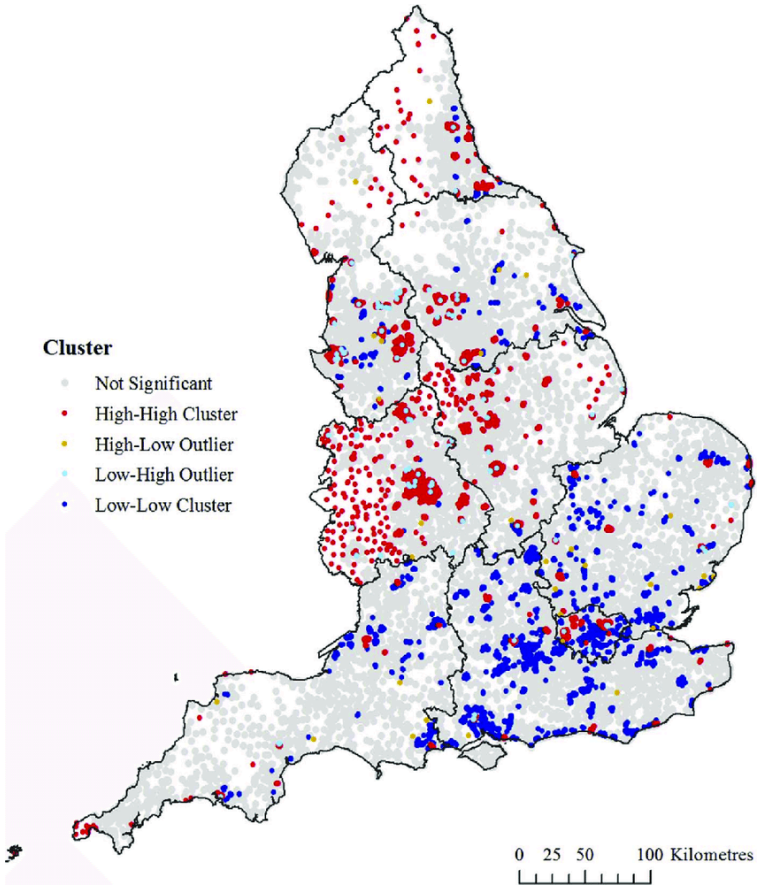
Marker (H3K27ac) into 2D mapping

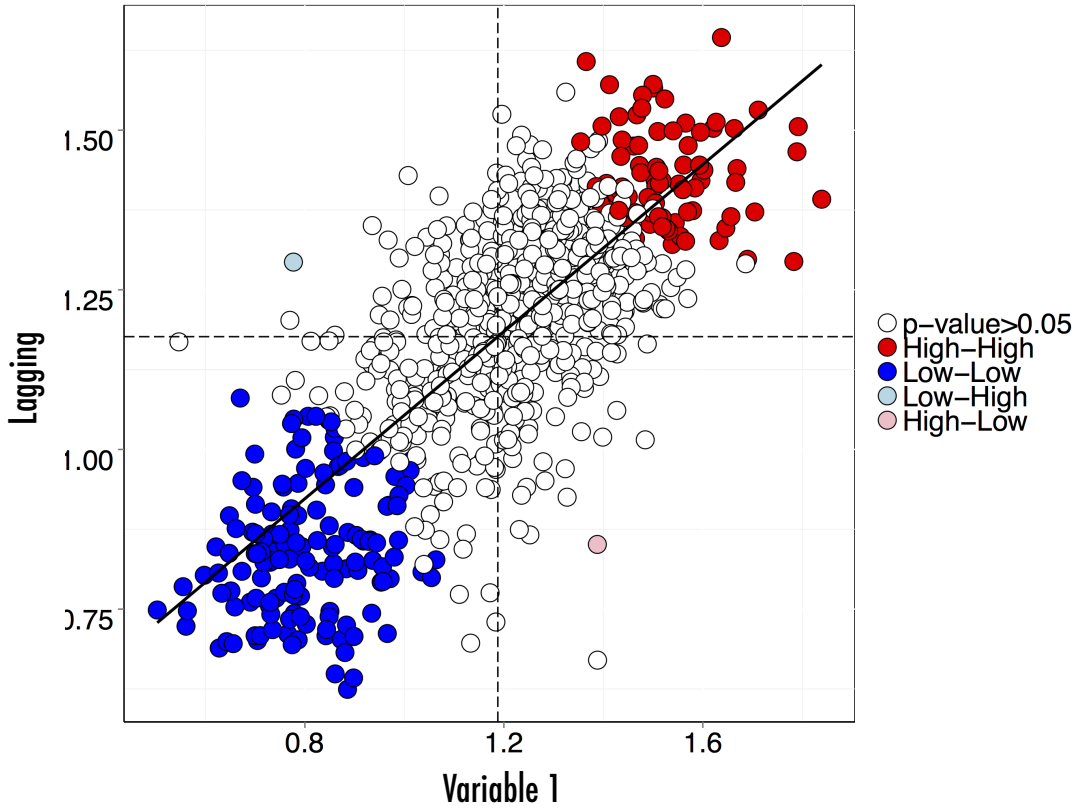
chr11:110780000-114770000





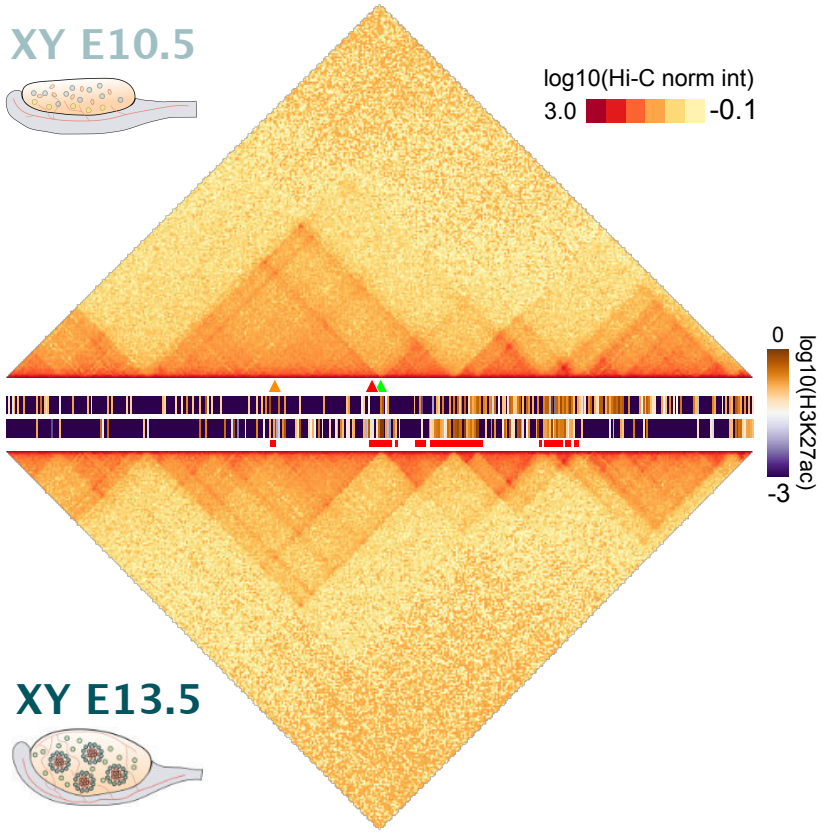
Local Moran Index

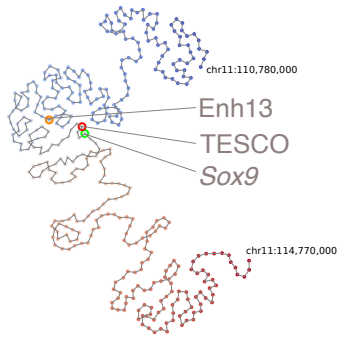


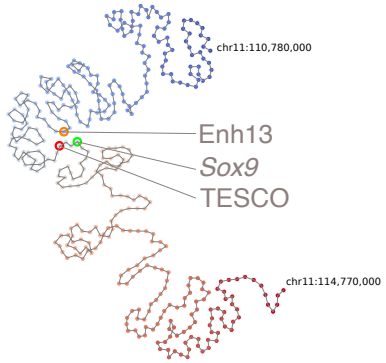


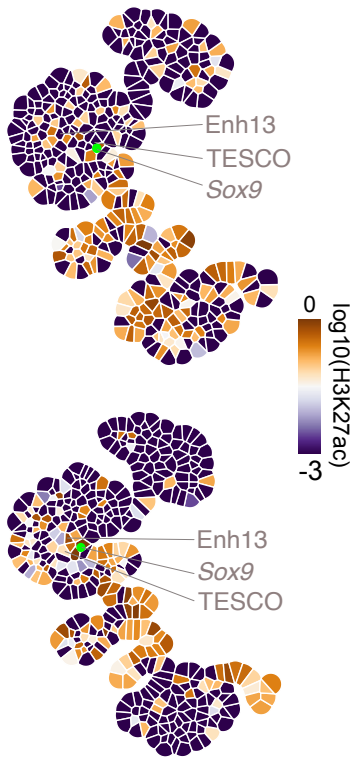
Quantifying regulatory environments bin by bin

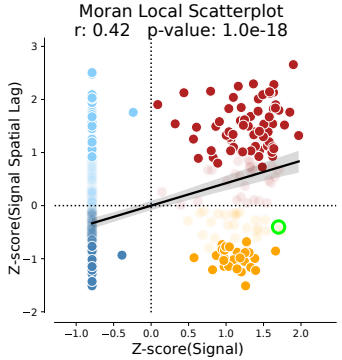
Sox9 locus chr11:110,780,000-114,770,000

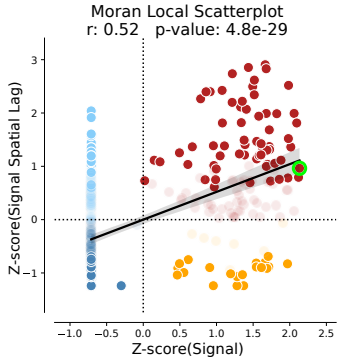


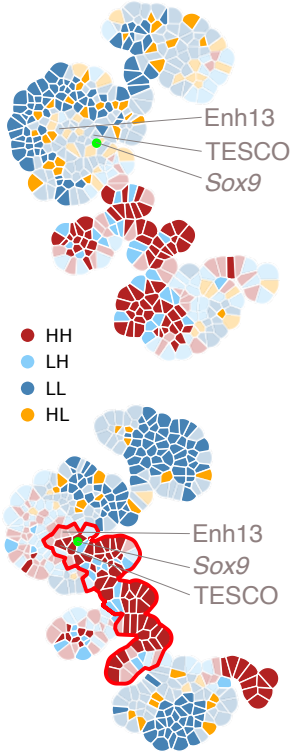




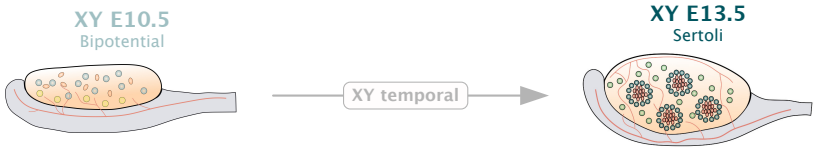


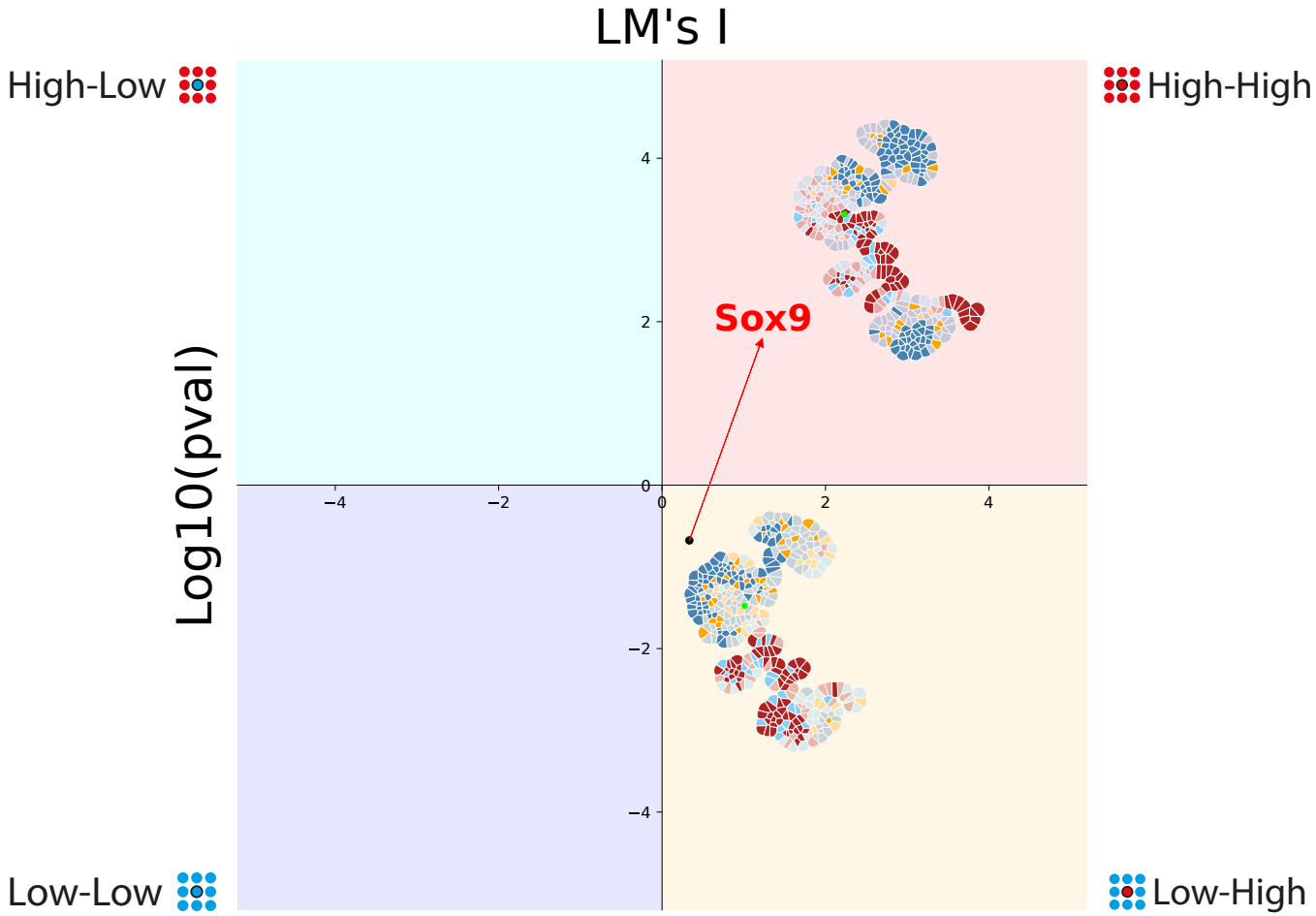




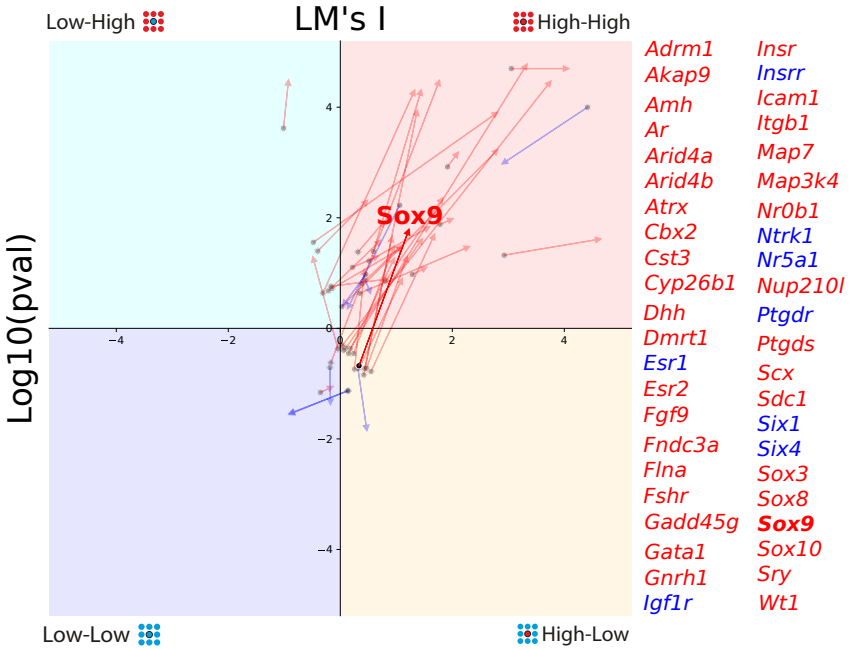


LMI Trip for Sox9 gene

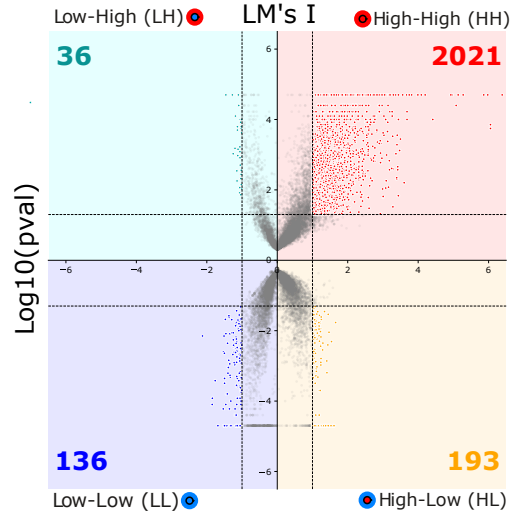


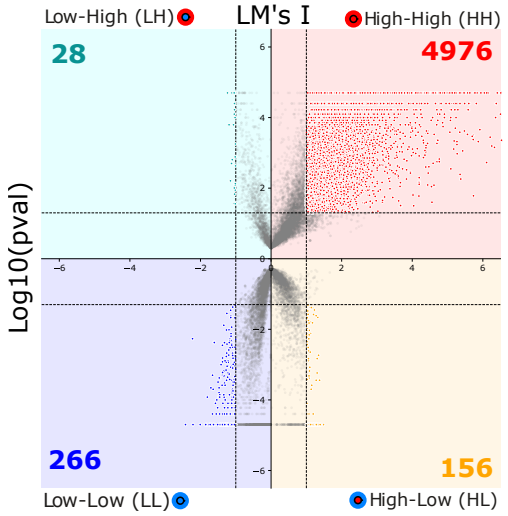


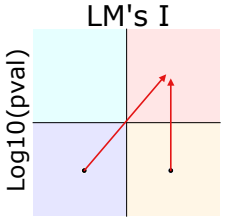
All genes LMI Trip

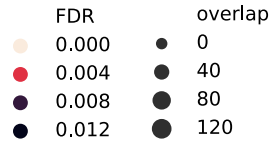


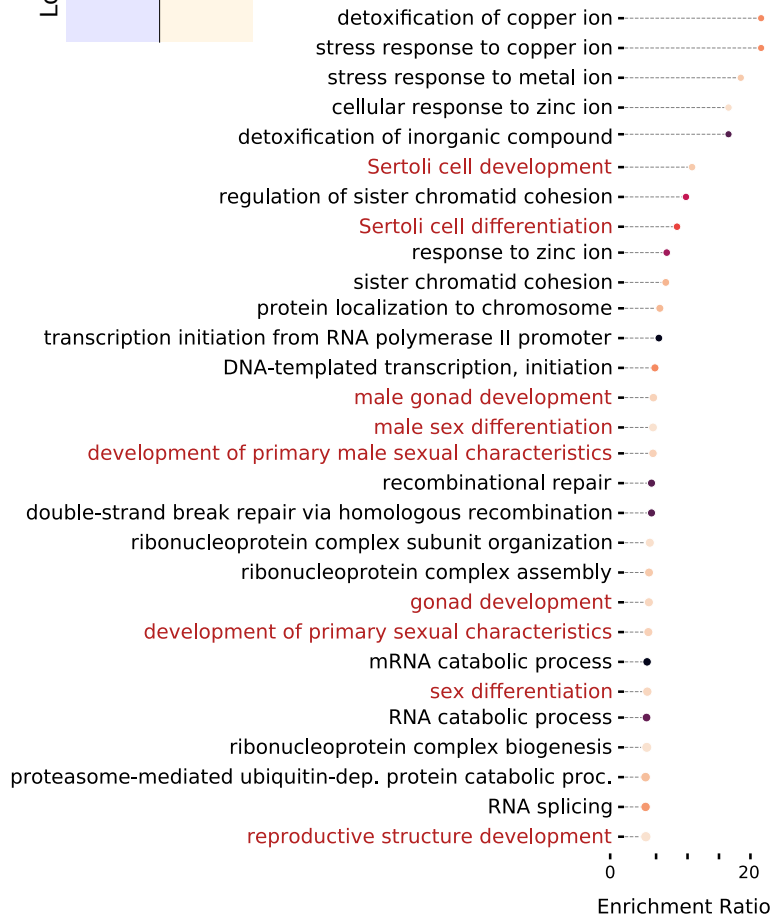
Adrm1
Akap9
Amh
Ar
Arid4a
Arid4b
Atrx
Cbx2
Cst3
Cyp26b1
Dhh
Dmrt1
Esr1
Esr2
Fgf9
Fndc3a
Flna
Fshr
Gadd45g
Gata1
Gnrh1
Igf1r
Insr
Insrr
Icam1
Itgb1
Map7
Map3k4
Nr0b1
Ntrk1
Nr5a1
Nup210l
Ptgdr
Ptgds
Scx
Sdc1
Six1
Six4
Sox3
Sox8
Sox9
Sox10
Sry
Wt1



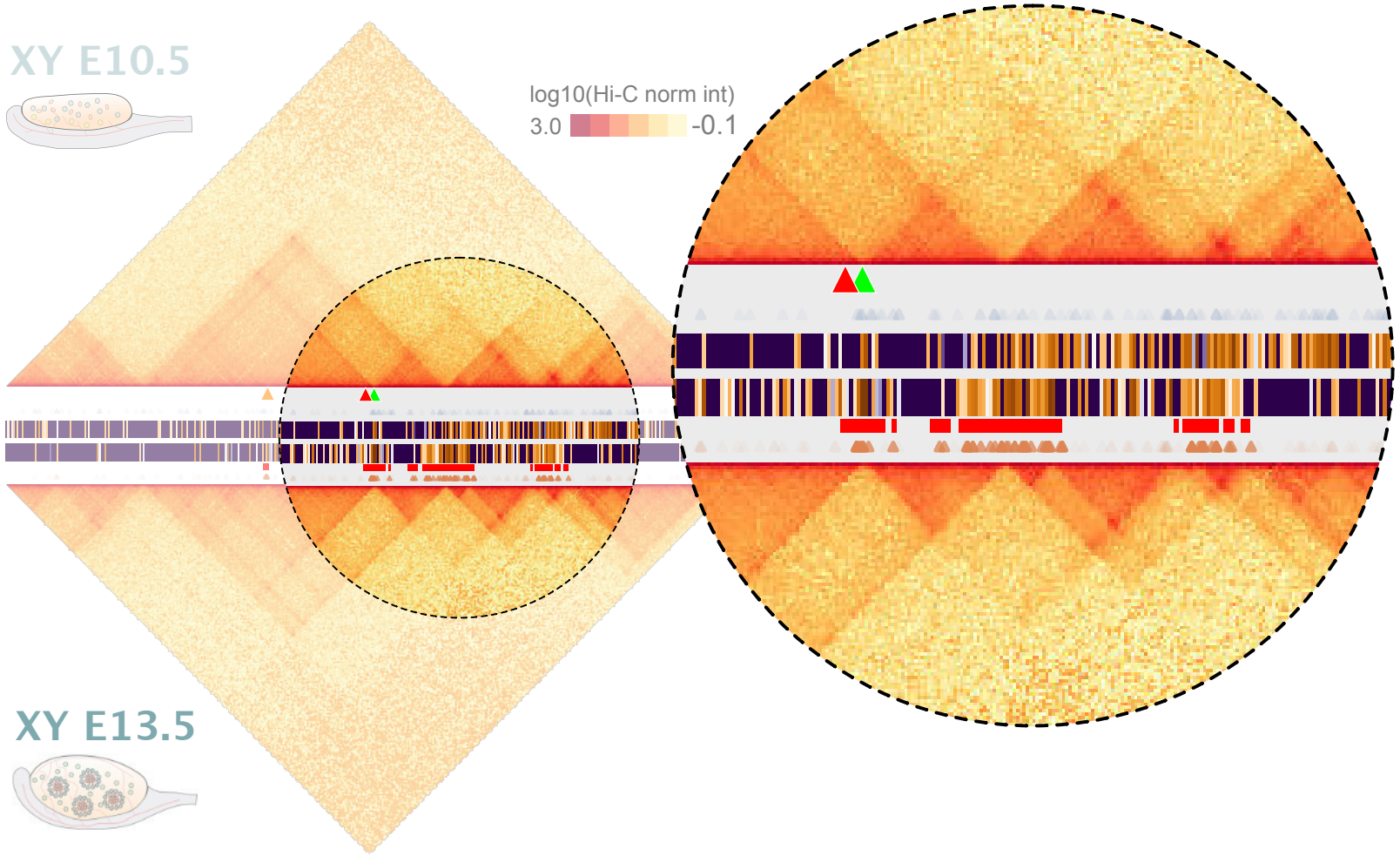






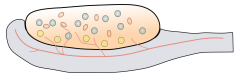


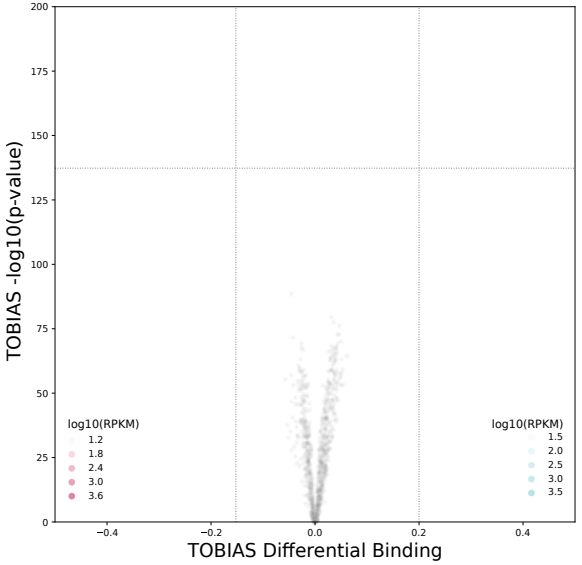
Are there specific Transcription Factors within the metaloci?



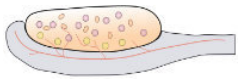
TF footprints

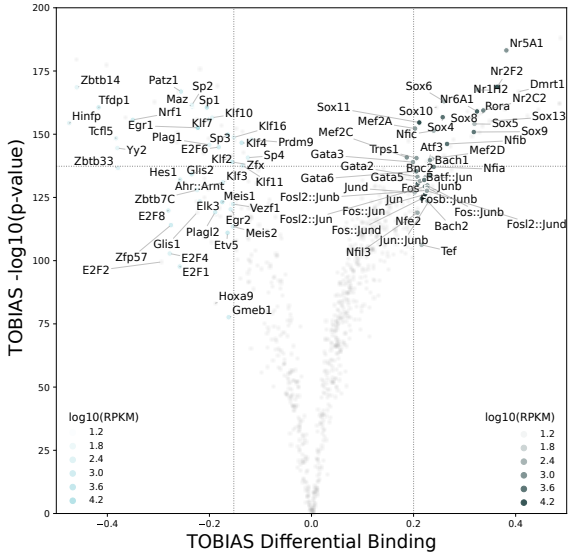
XY E10.5

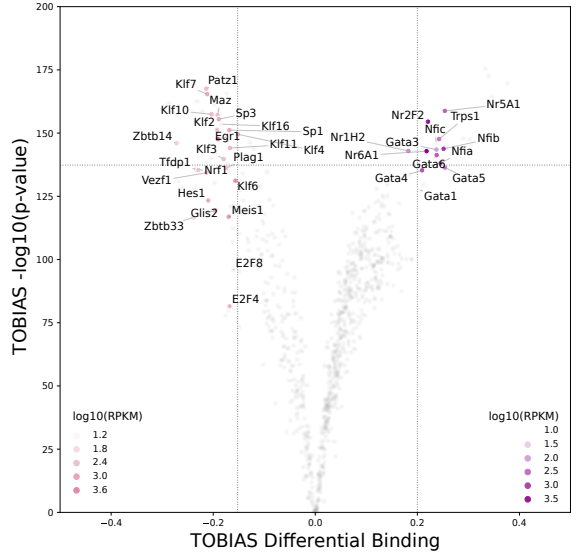




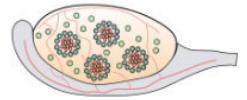
XX E10.5

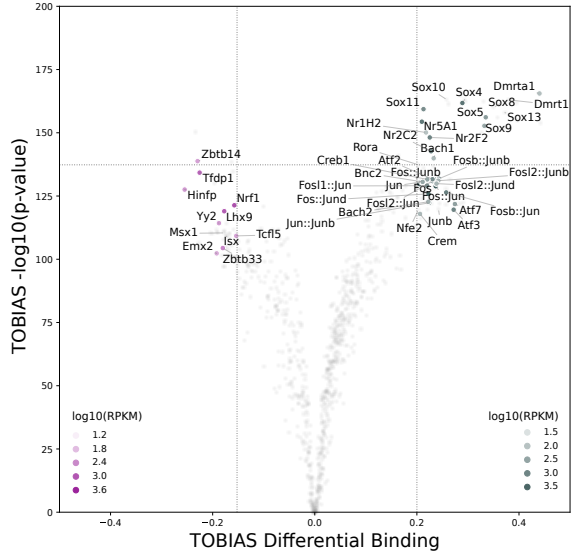




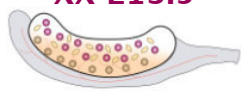


XY E13.5

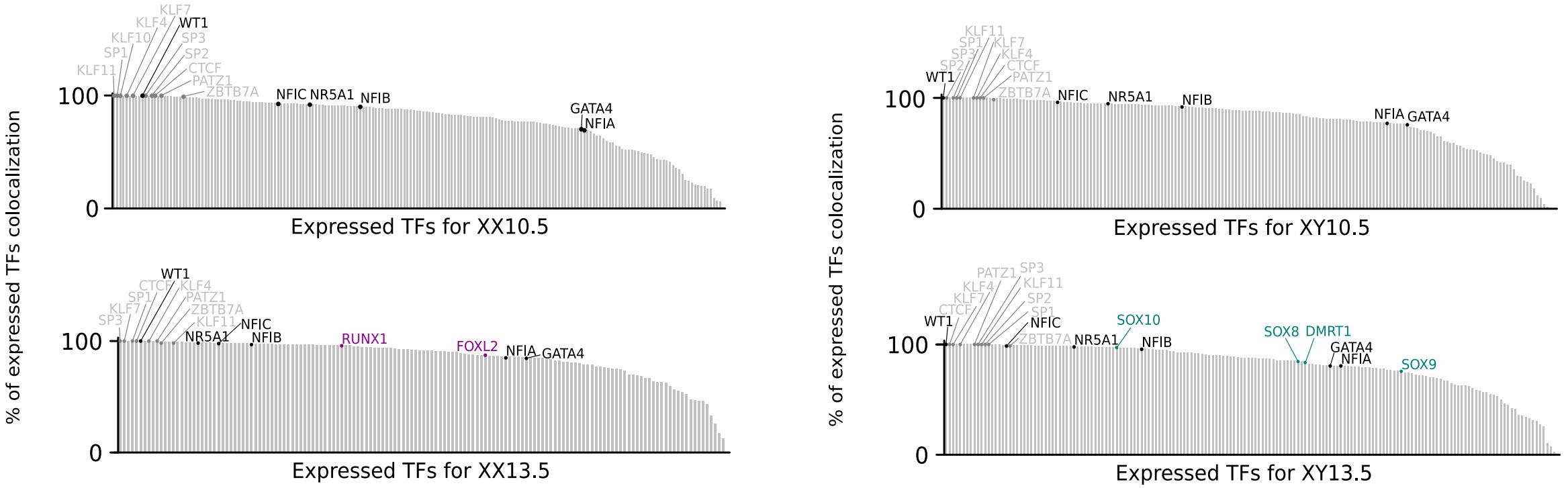




XX E13.5



"Stripe" TFs



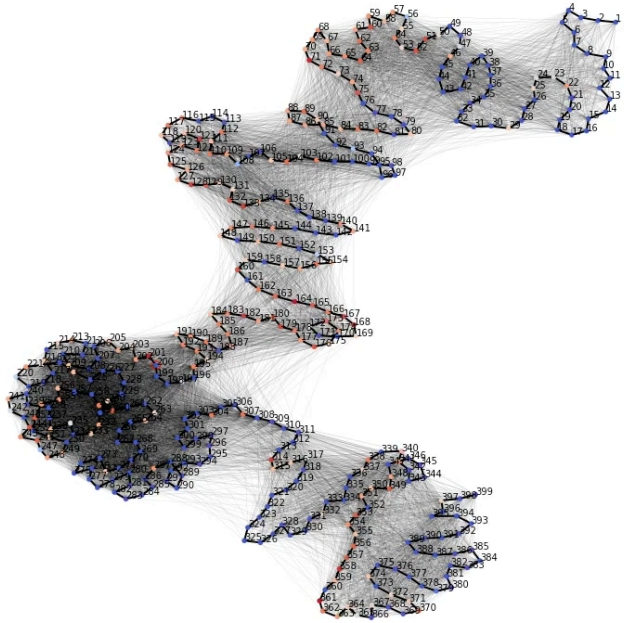
General · Gonad Specific · Female · Male

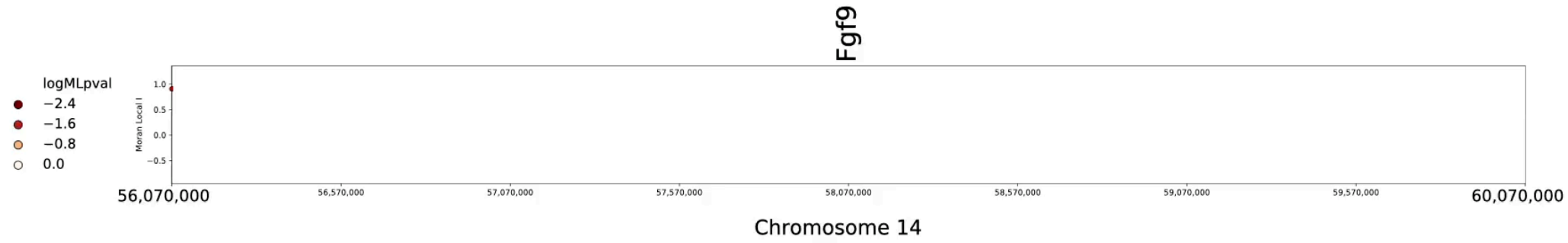
Now that we know the genes….

Can we identify regulatory elements using 

METALoci predictive mode

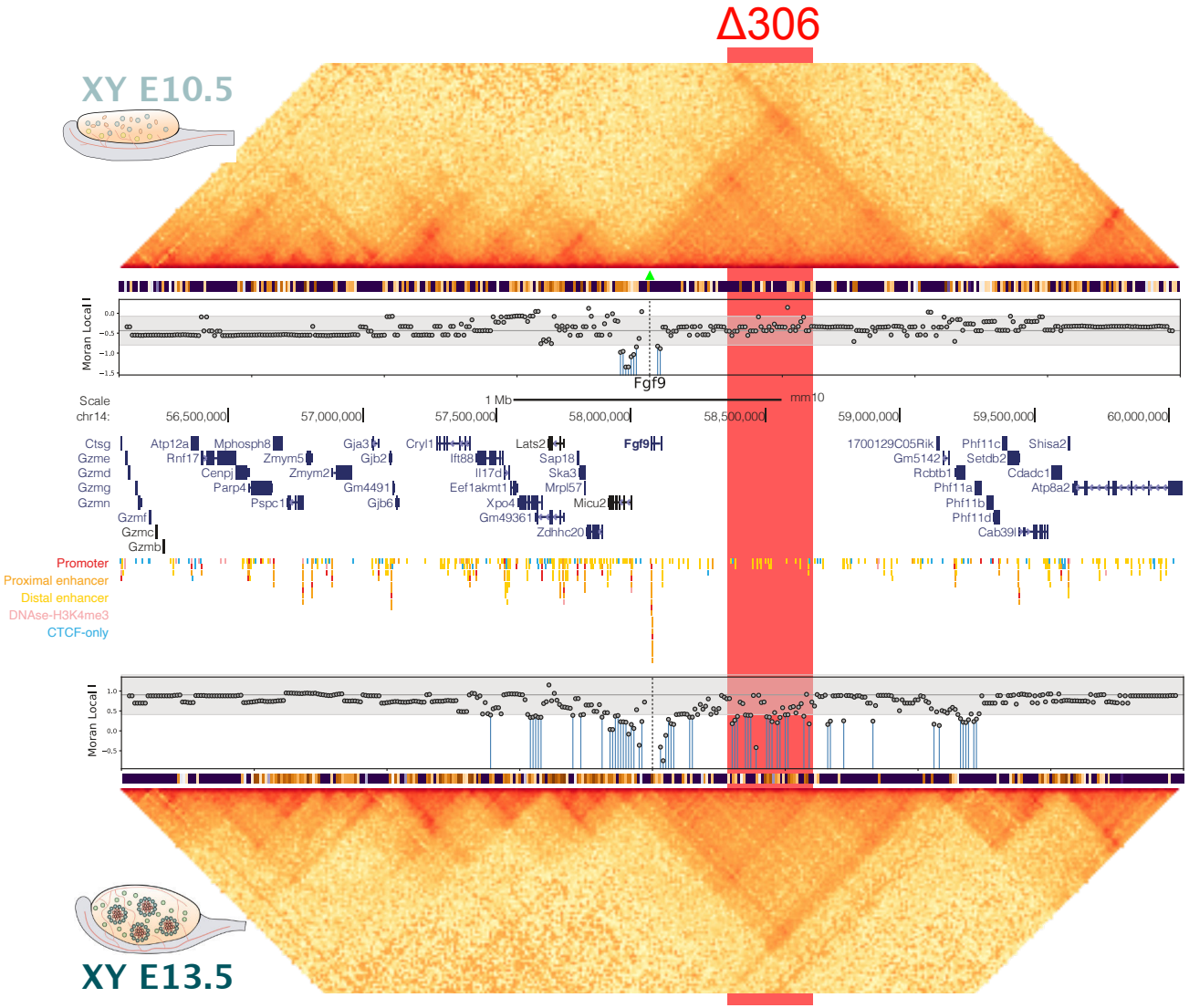
Fgf9 locus chr14:56,070,000-60,070,000

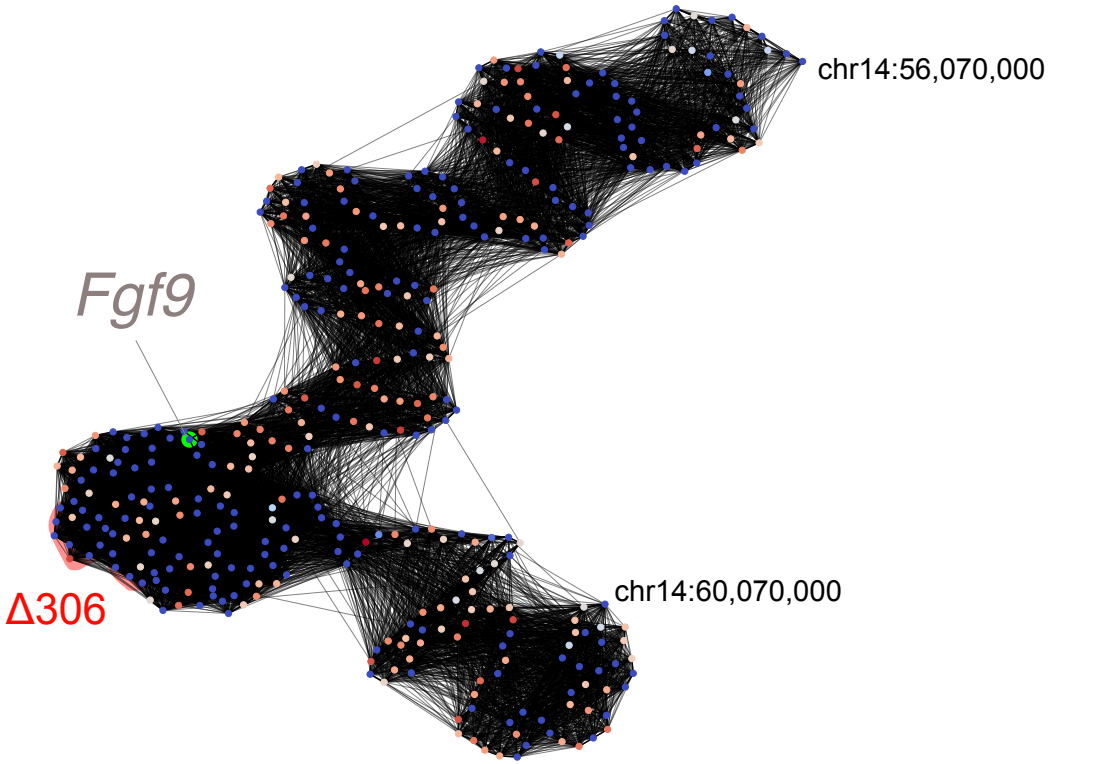




METALoci predictive mode

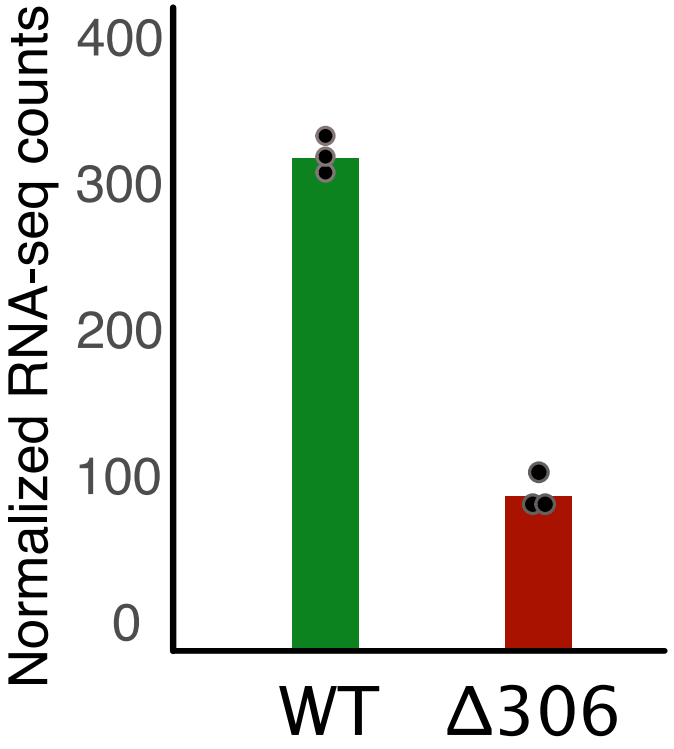
Fgf9 locus chr14:56,070,000-60,070,000

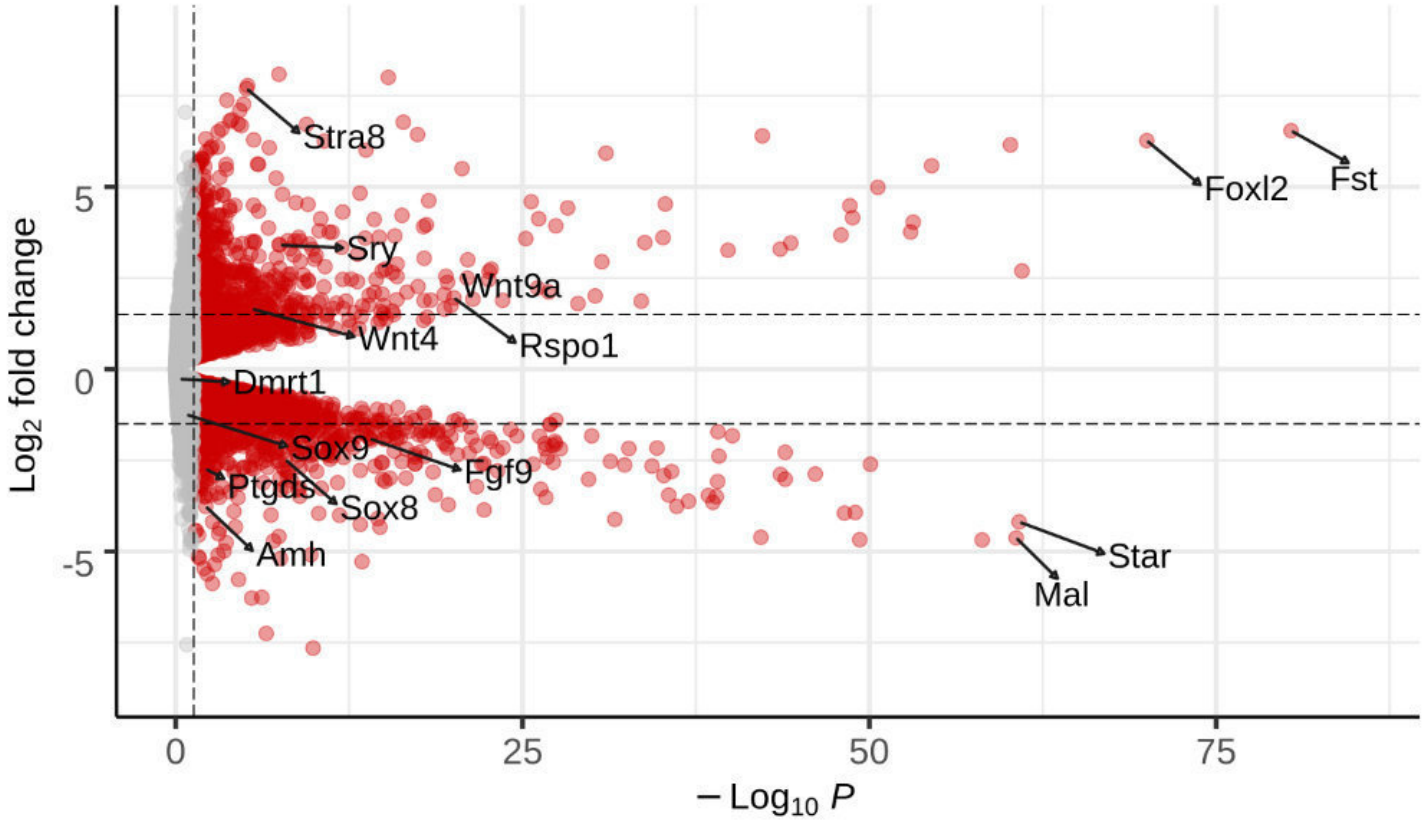




METALoci predictive mode

Fgf9 XY Δ306 mutant





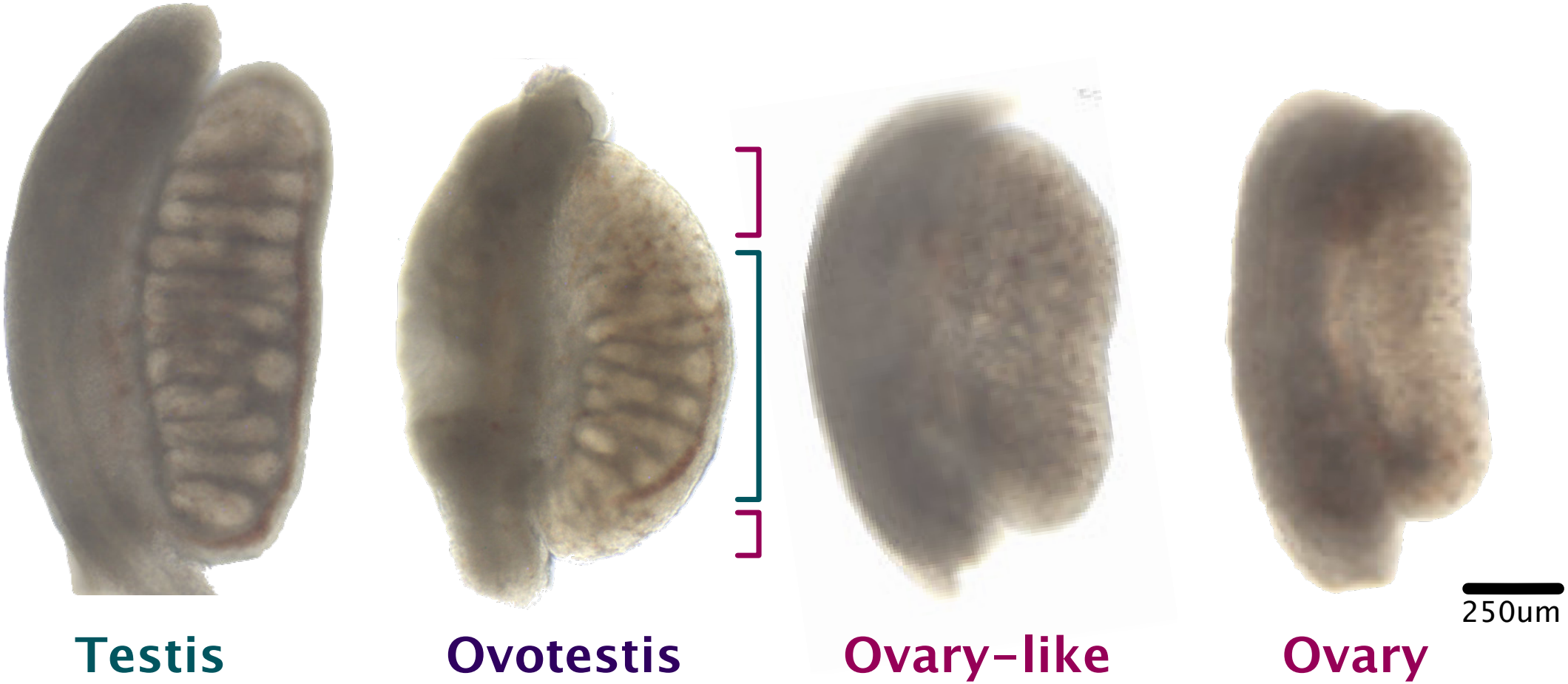
METALoci predictive mode

Fgf9 XY Δ306 mutant

XY Wildtype

XY Δ306

XX Wildtype



Testis

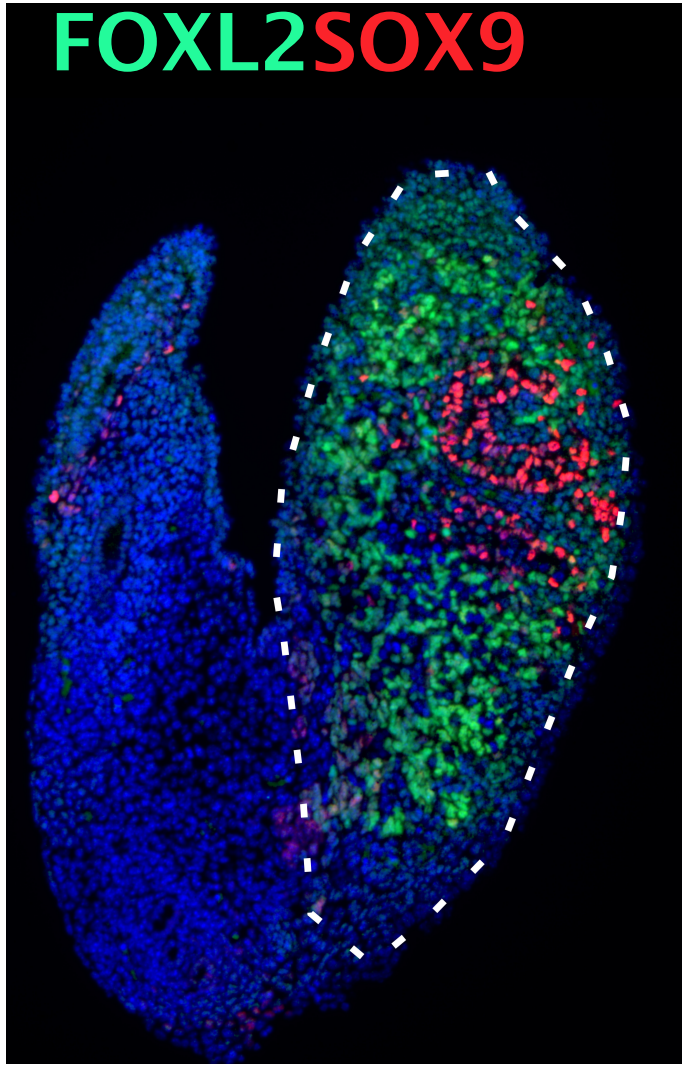
Ovotestis

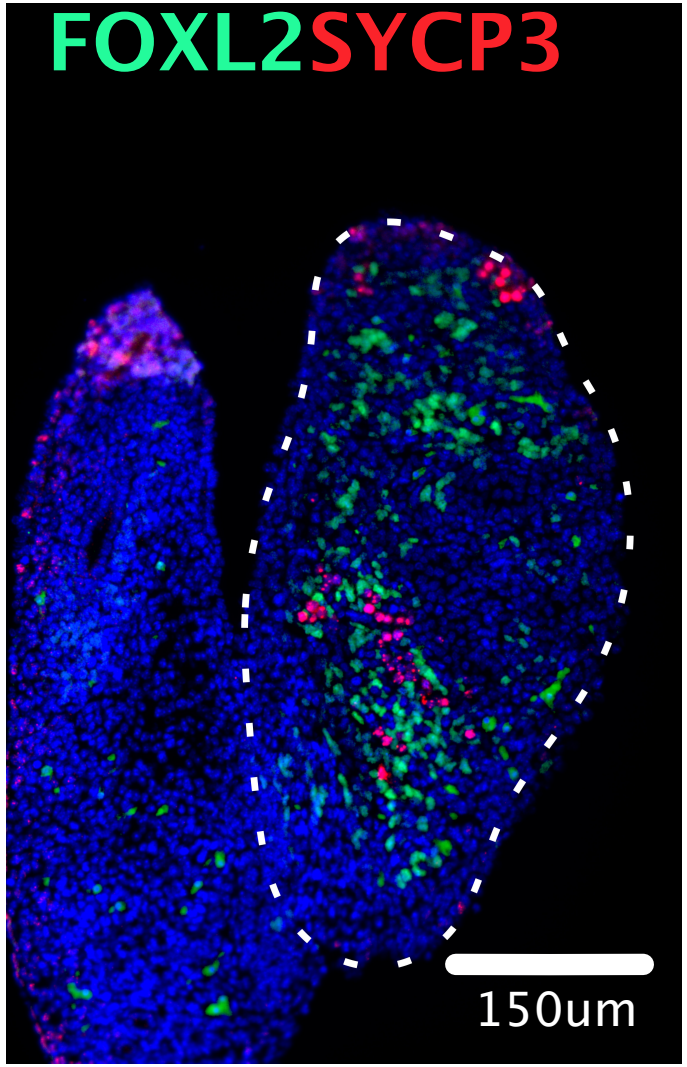
Ovary-like

Ovary

METALoci predictive mode

Fgf9 XY Δ306 mutant





Take home messages:

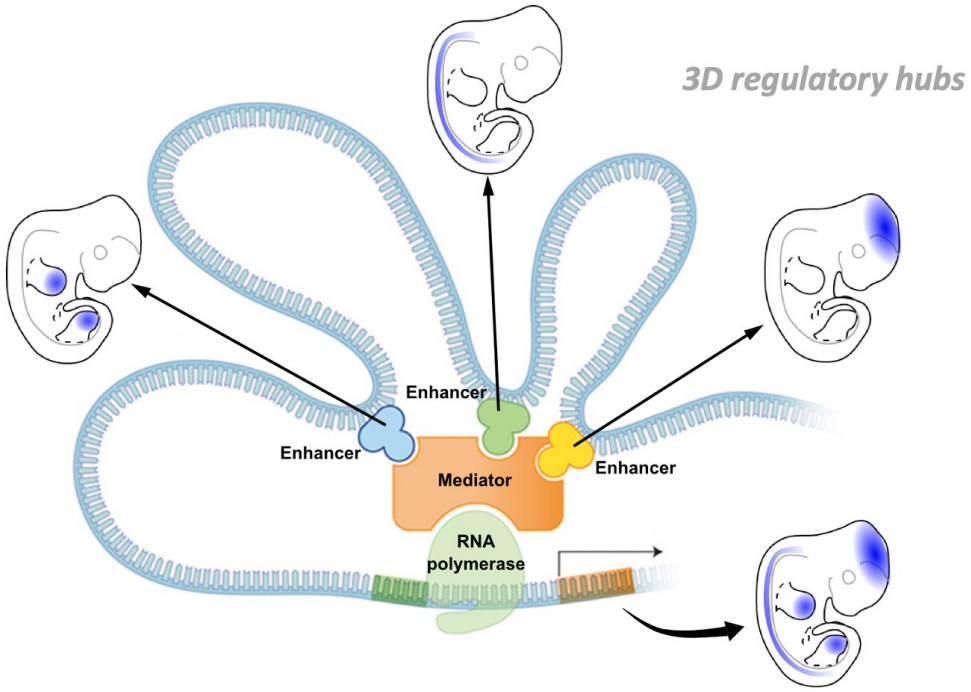
First characterization of the 3D regulatory landscape of sex determination

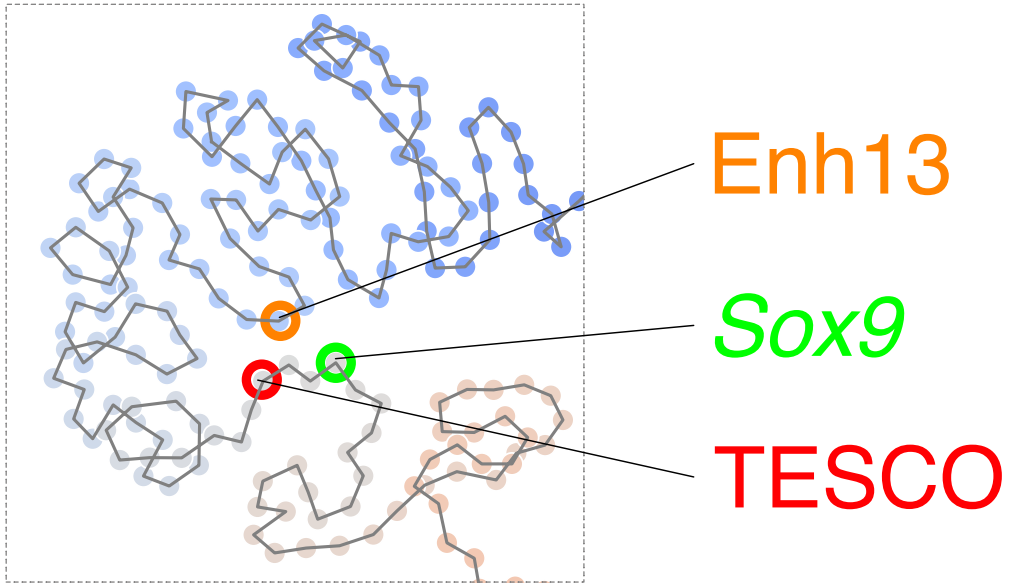
METALoci is an unbiased approach to quantify gene regulatory activity

METALoci is a predictive tool to identify critical regulatory loci

Discovery of a novel non-coding region controlling sex determination

Take home messages:





http://marciuslab.org
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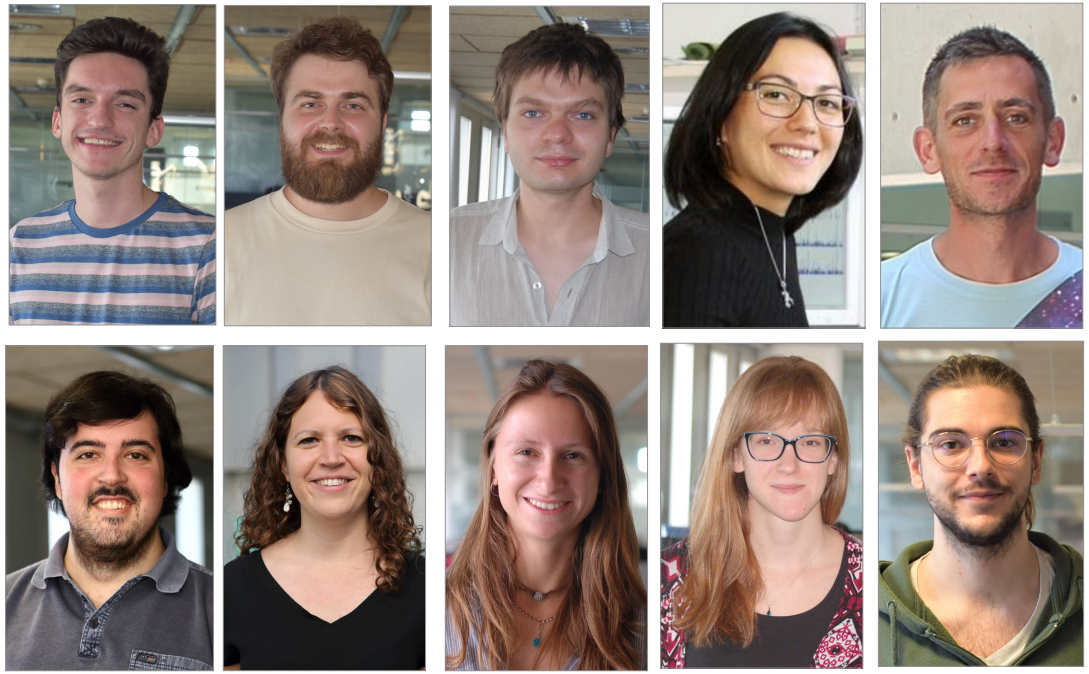

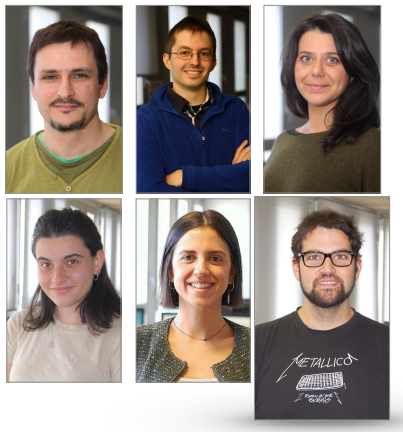

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In collaboration with the Capel Lab (Duke U.) and Lupiañez Lab (MDC Berlin)

.: Our current sponsors :.

European Commission
Generalitat de Catalunya
Obra Social "la Caixa"
Fundació La Marató de TV3
National Human Genome Research Institute

.: Conflict of Interest Statement :.
Marc A. Marti-Renom serves as a consultant to Acuity Spatial Genomics, Inc., and receives compensation for these services.