

Spatio-temporal regulatory landscape of sex-determination

or a case for
Structure-Based Genome Editing (SBGE)

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CNAG · CRG · ICREA

BioRxiv

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

cnag CRG ICREA



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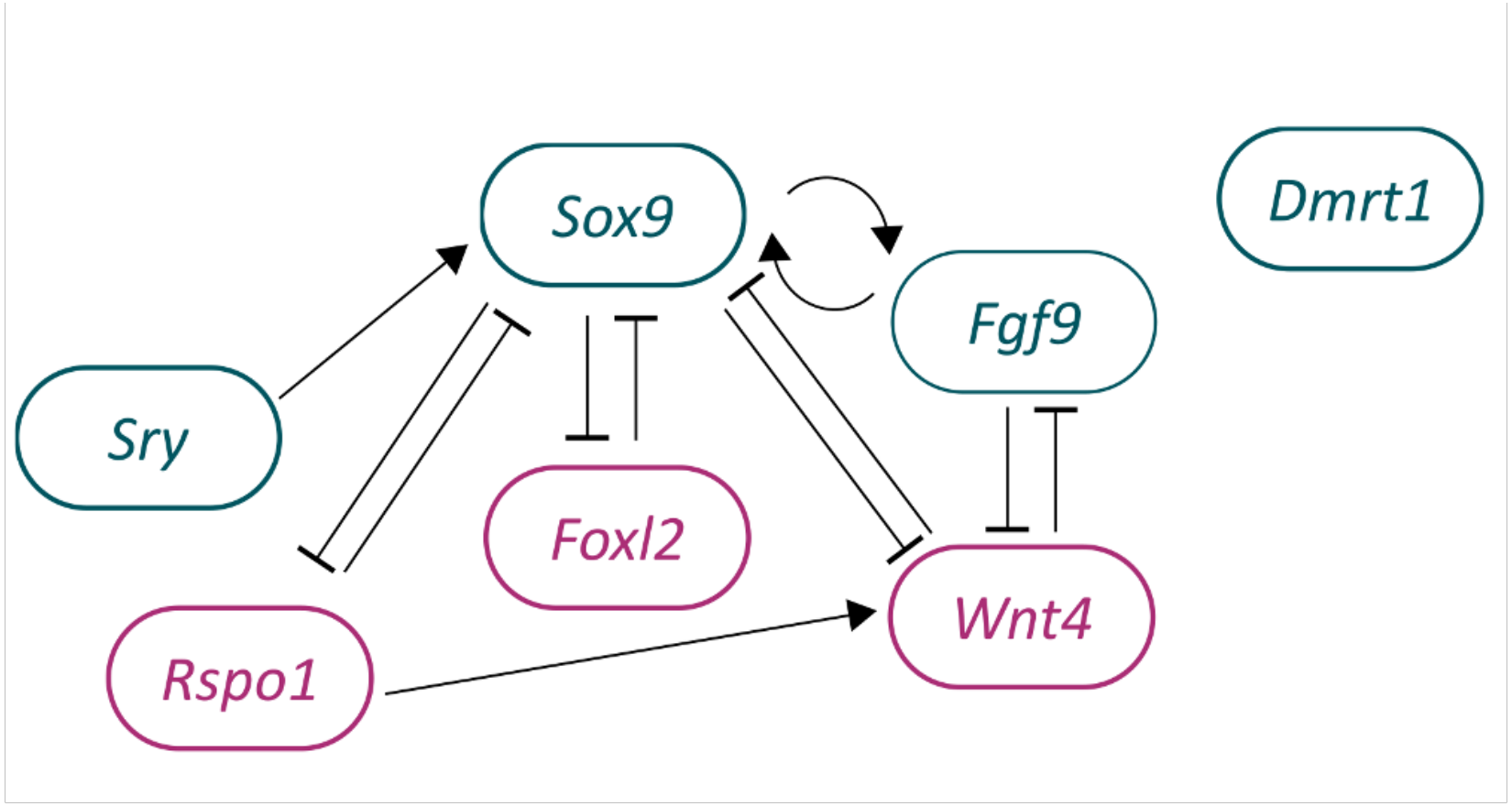


Irene Mota
MDC Berlin



Dario Lupiañez
MDC Berlin
Now @CABD

Sex determination: a 3,000 year-old enigma



Genetics

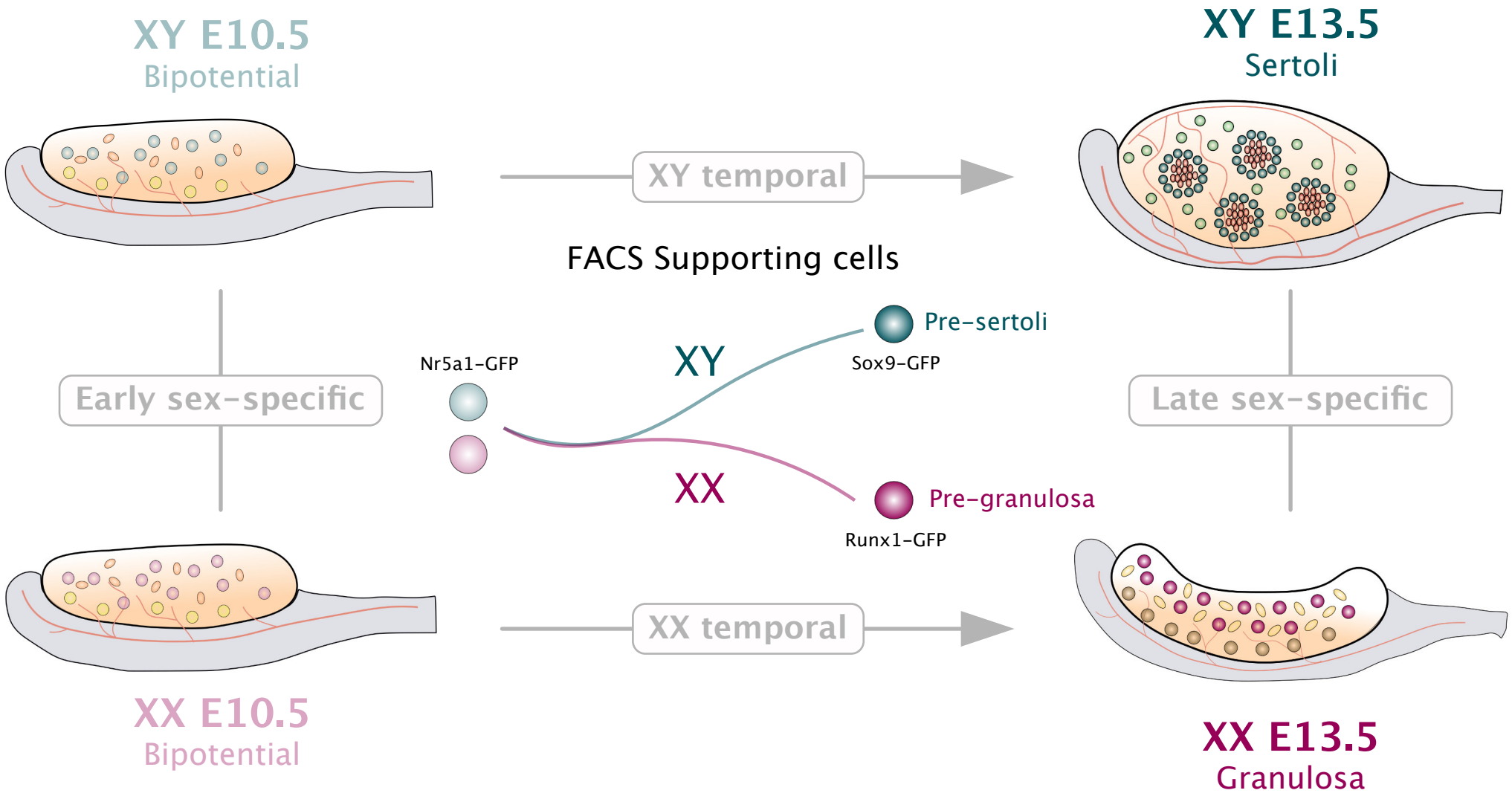


Discovery of *Sry* gene

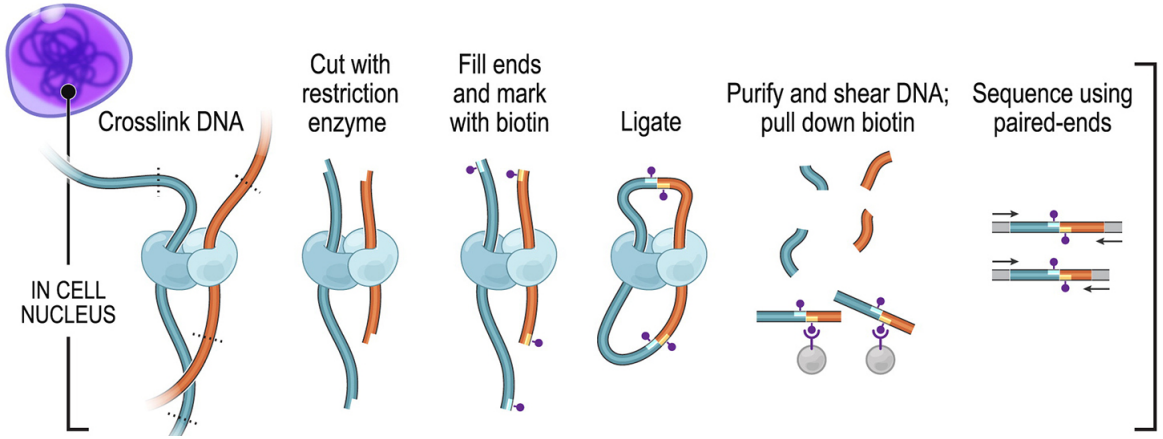
Koopman et al., Nature, 1991

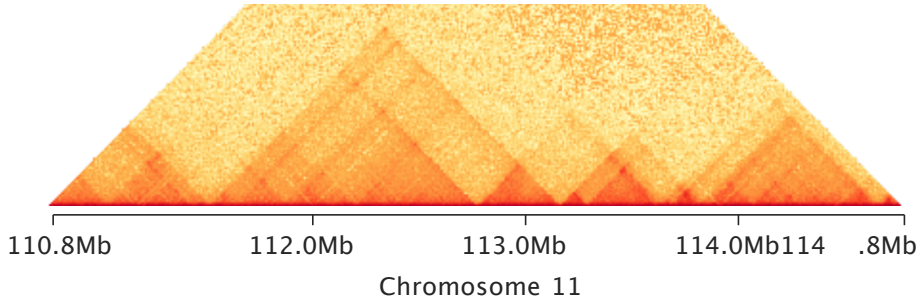
(Goodfellow & Lovell-Badge labs)

Sex-determination as a model for "bipotential" commitment

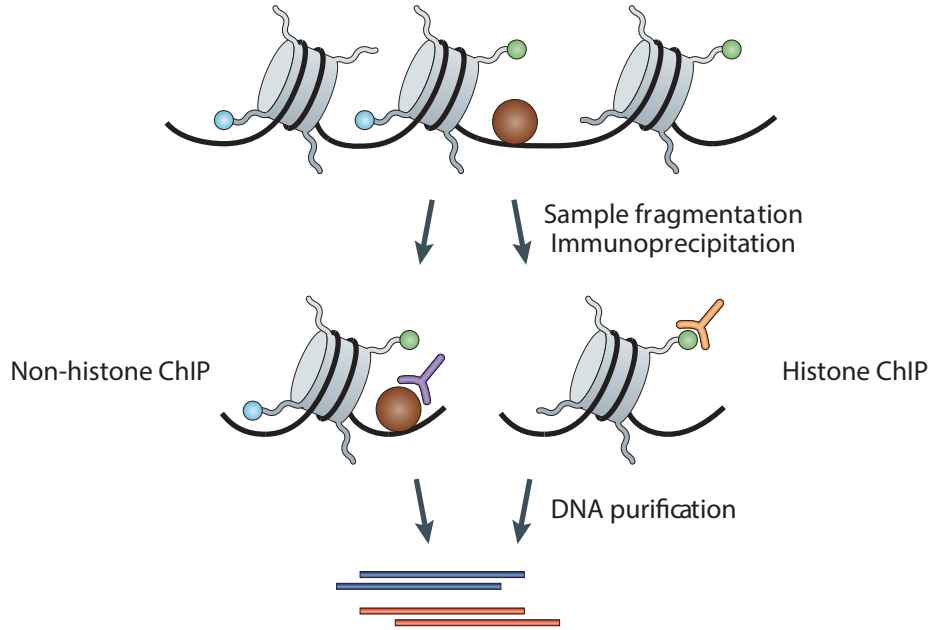


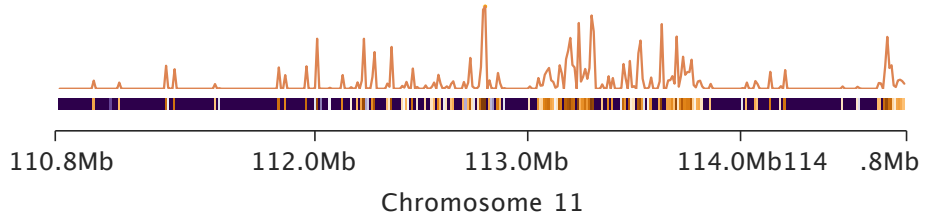
Hi-C & ChIP-seq





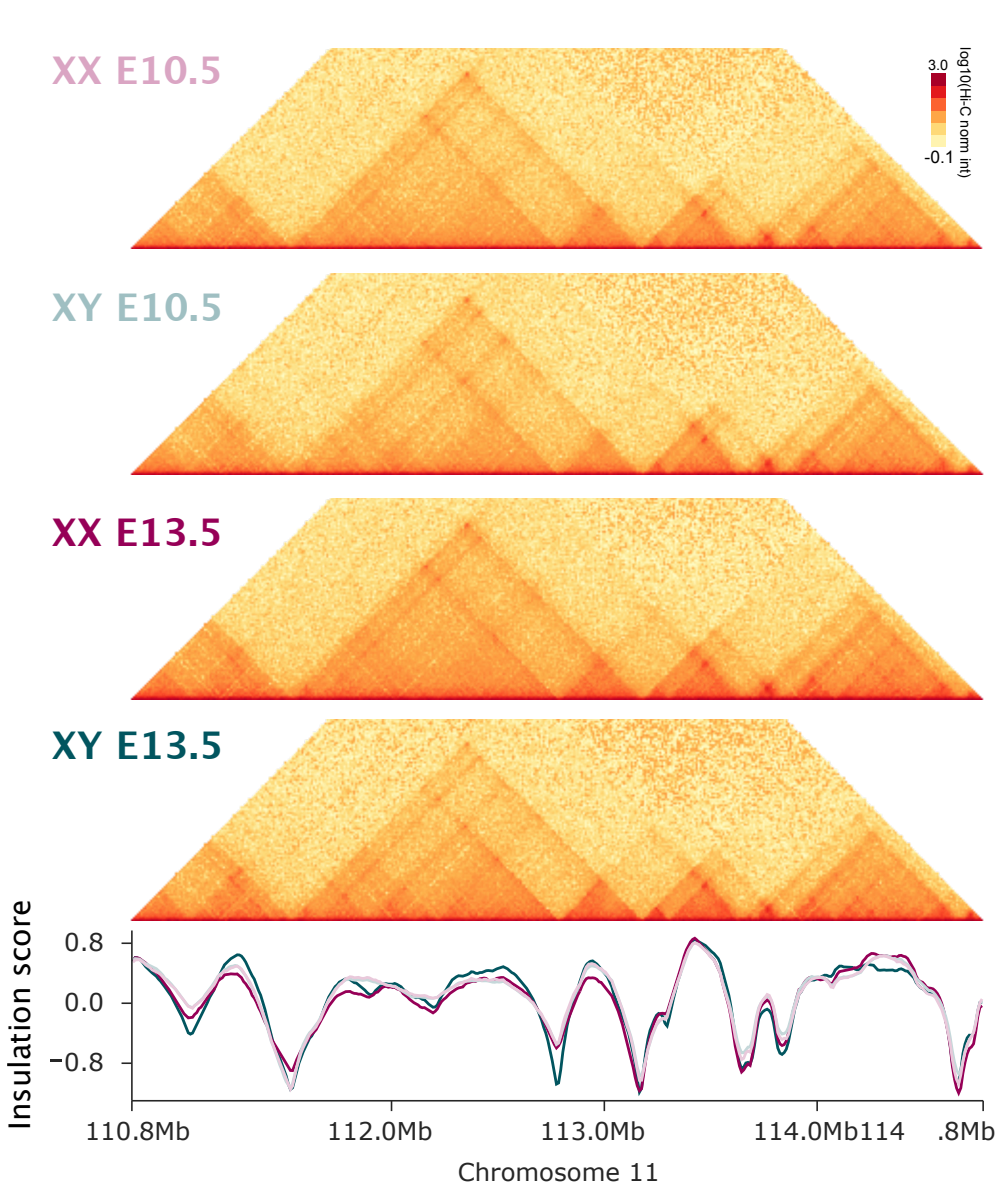
Dekker, J., Rippe, K., Dekker, M., & Kleckner, N. (2002). Science, 295(5558), 1306–1311.
Lieberman-Aiden, E., et al. (2009). Science, 326(5950), 289–293.

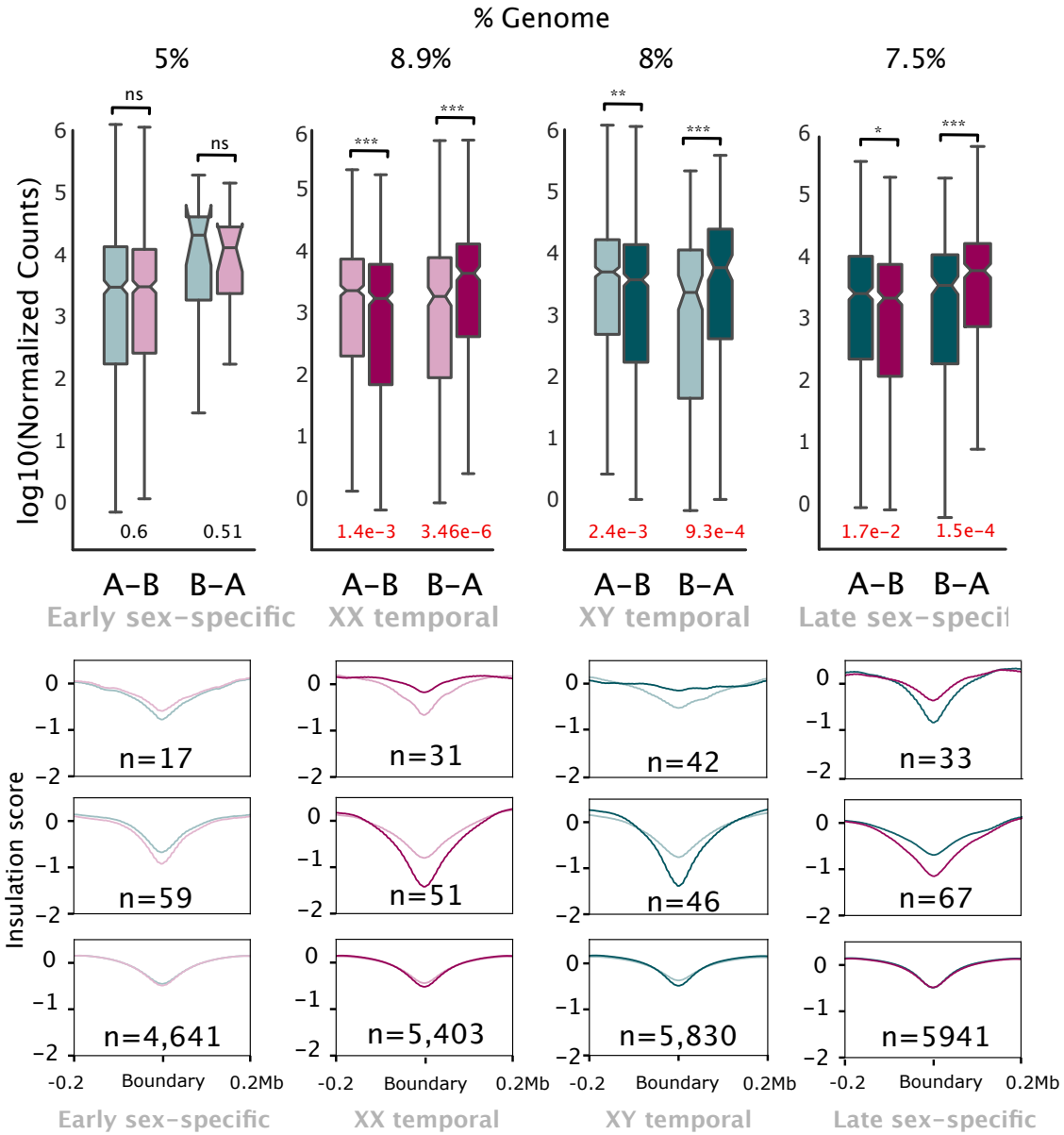


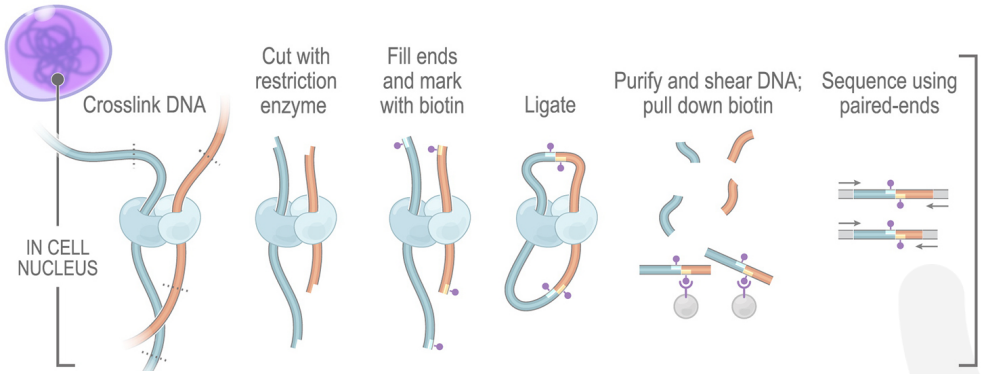


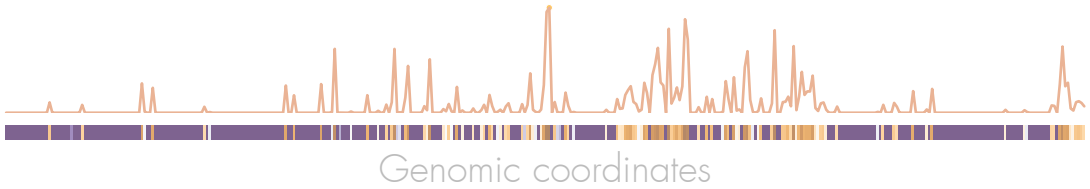
Solomon, M. J., Larsen, P. L. & Varshavsky, A. (1988) Cell 53, 937–947.
Park, P.J. (2009) Nature Reviews Genetics 10, 669–680.

No major structural (apparent) differences





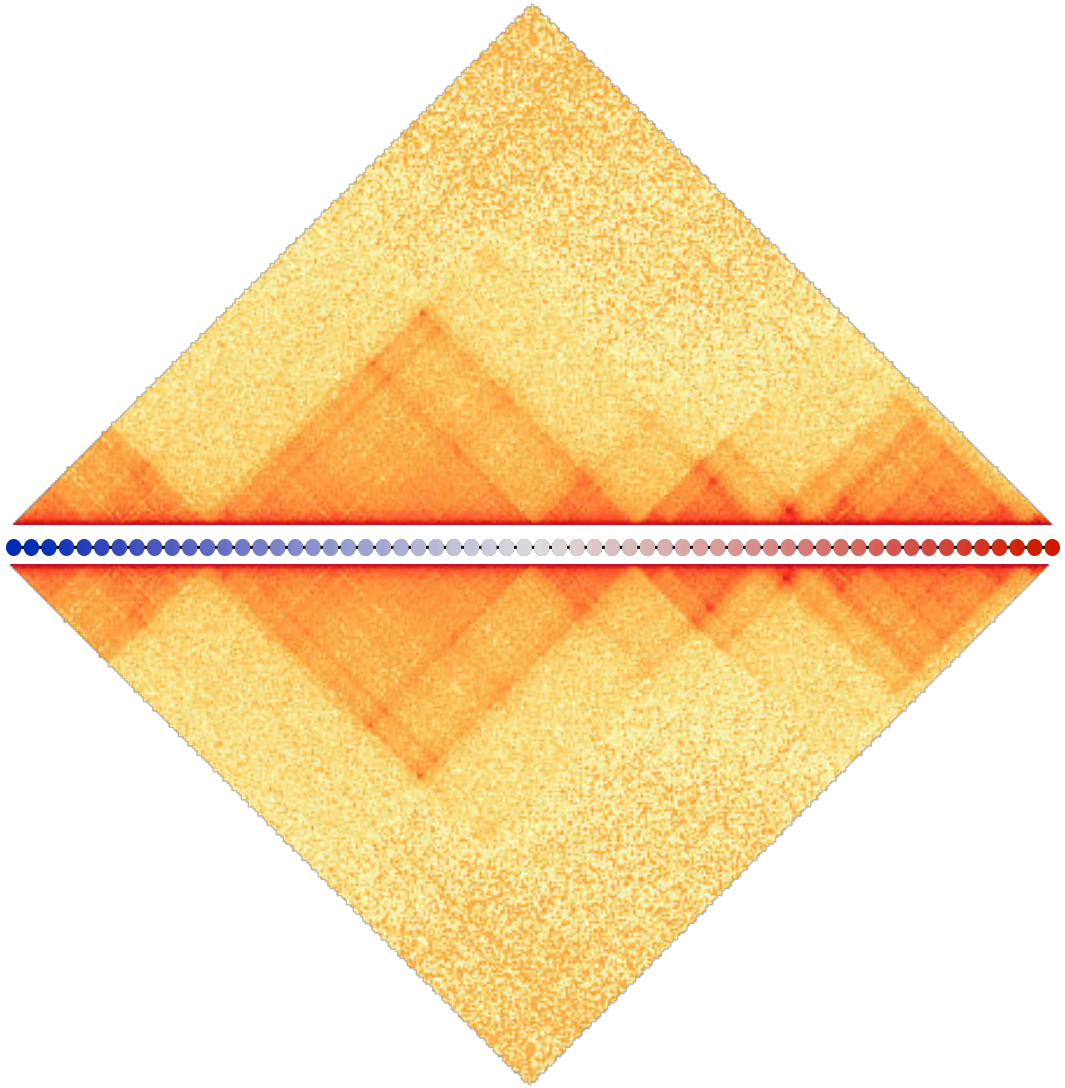

Crosslink DNA
IN CELL NUCLEUS
Cut with restriction enzyme
Fill ends and mark with biotin
Ligate
Purify and shear DNA; pull down biotin
Sequence using paired-ends

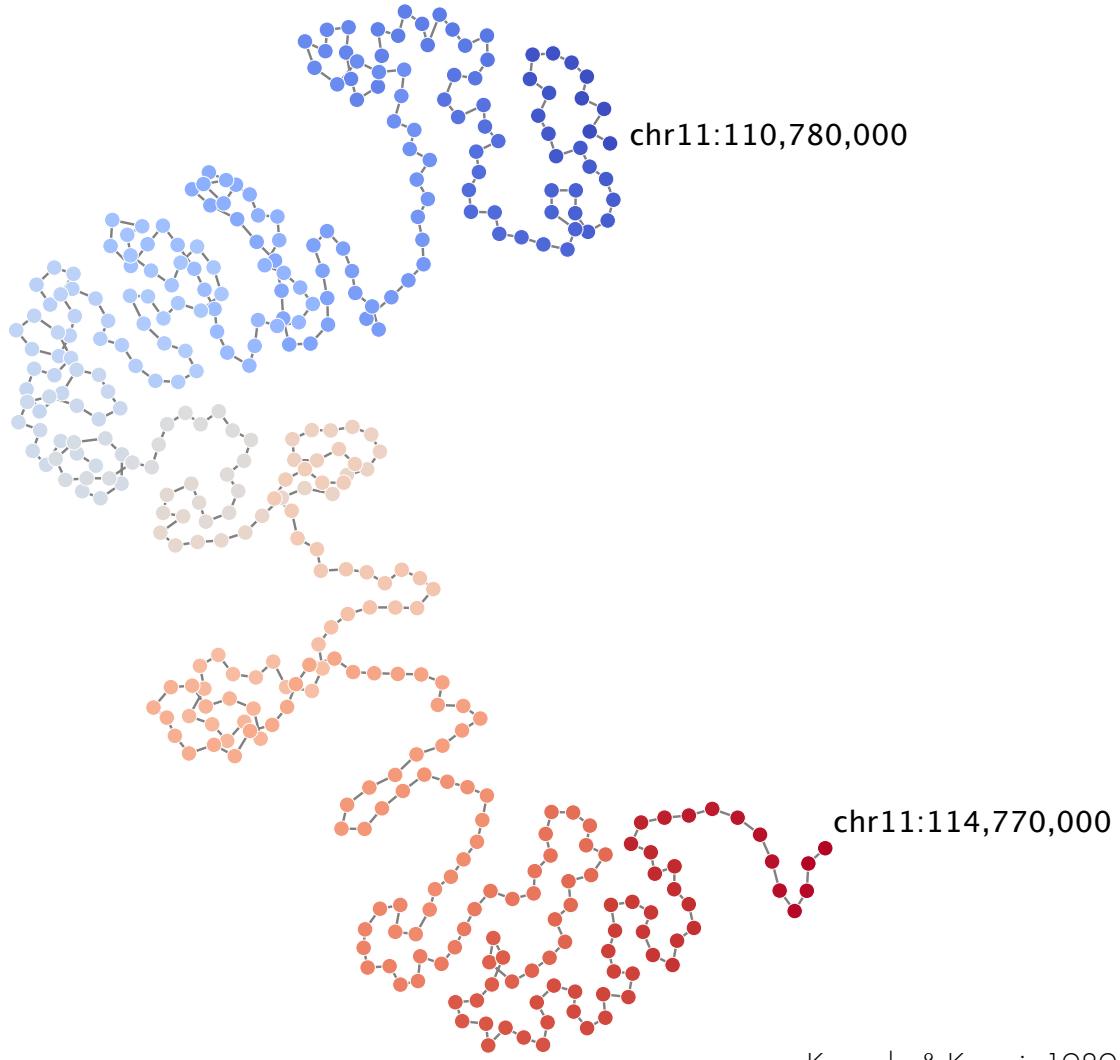

Genomic coordinates

ETALoci

Spatial lay-out of significant interactions

chr11:110780000-114770000 @10kb

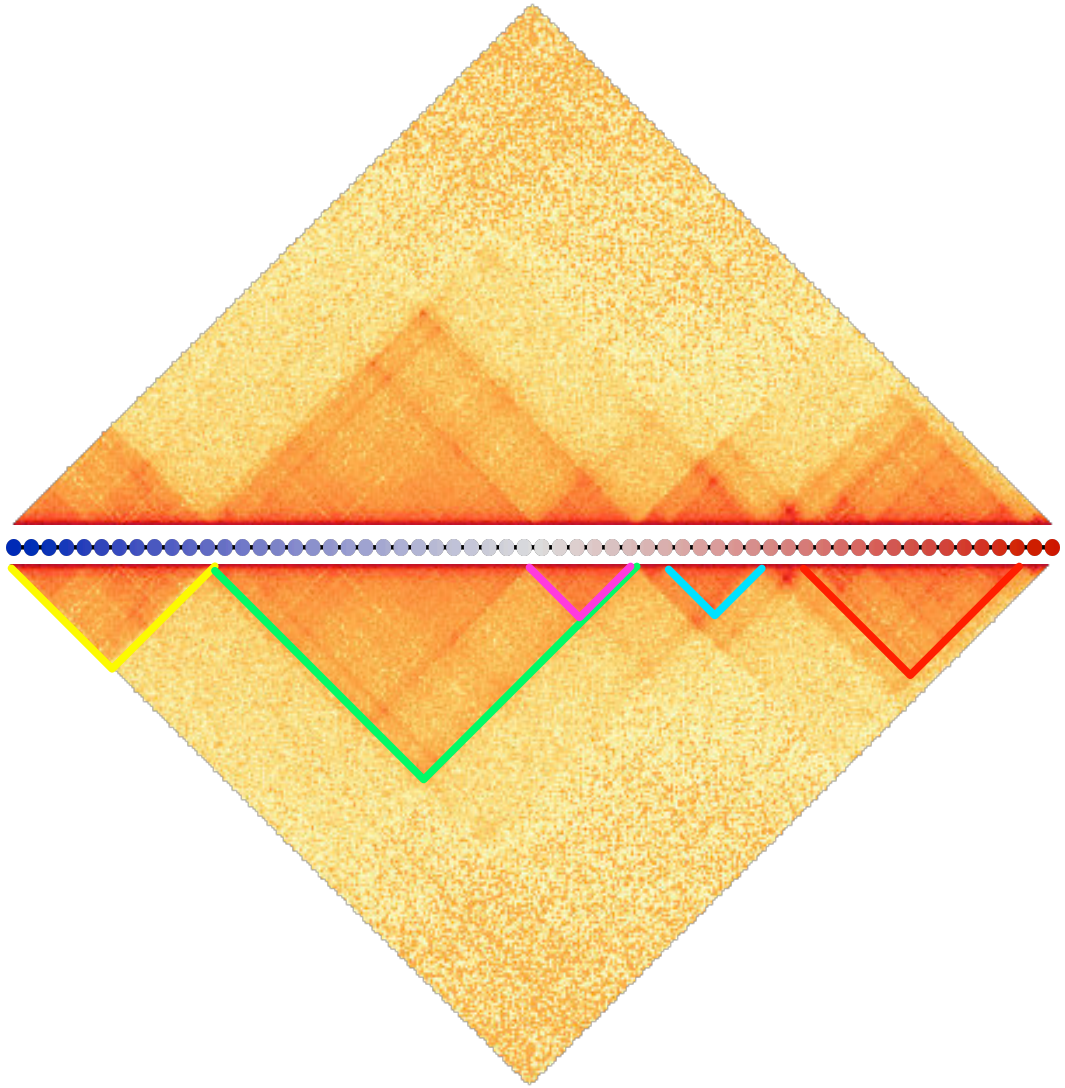


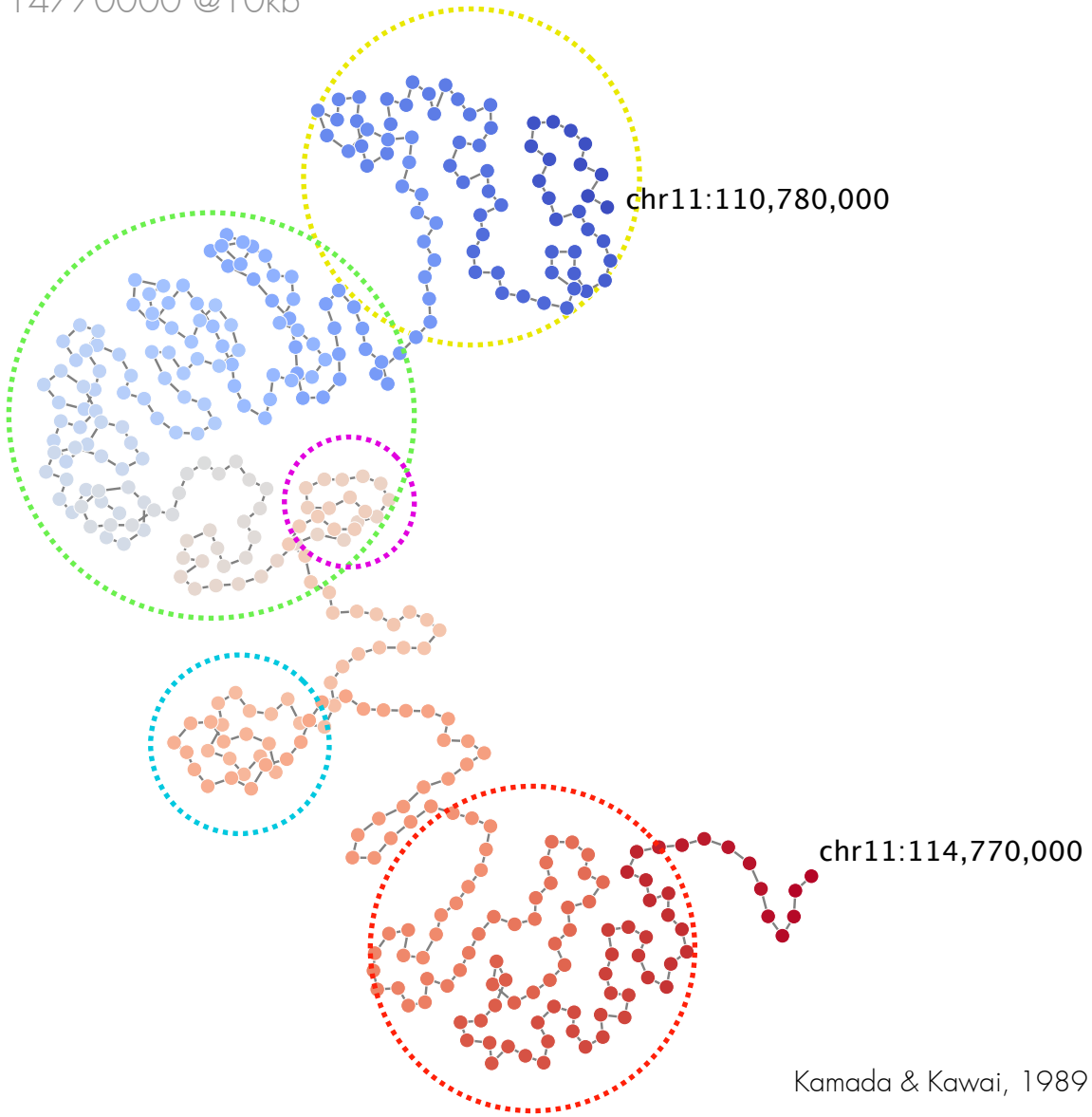


Kamada & Kawai, 1989

Spatial lay-out of significant interactions

chr11:110780000-114770000 @10kb

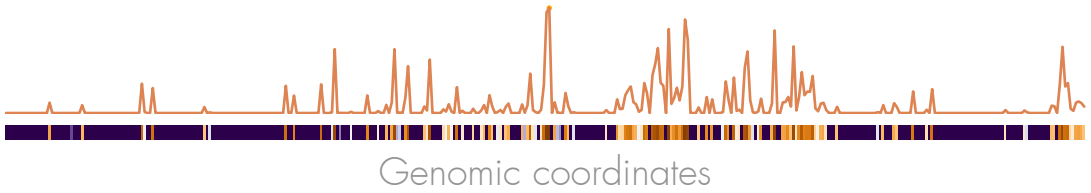


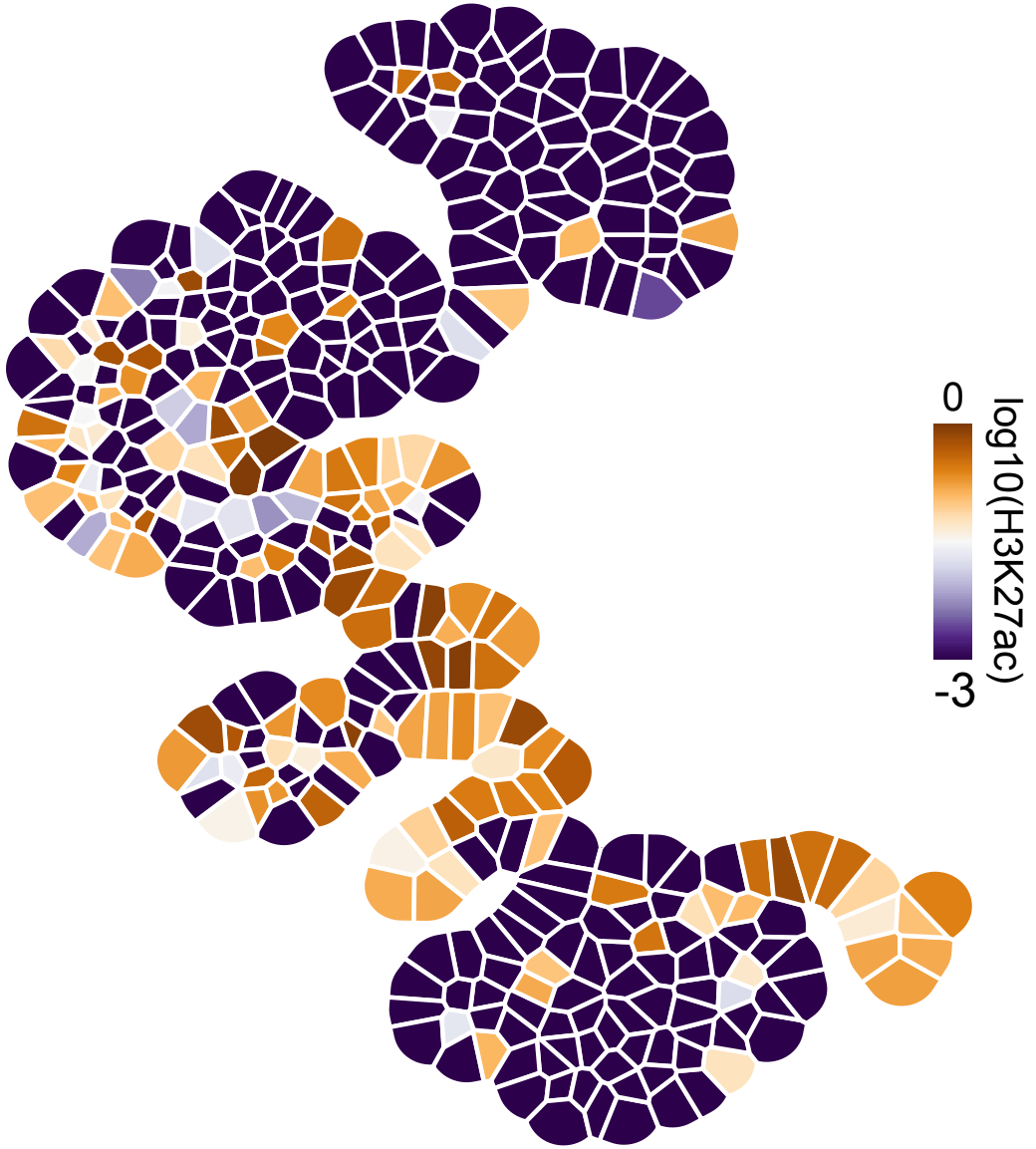


Kamada & Kawai, 1989

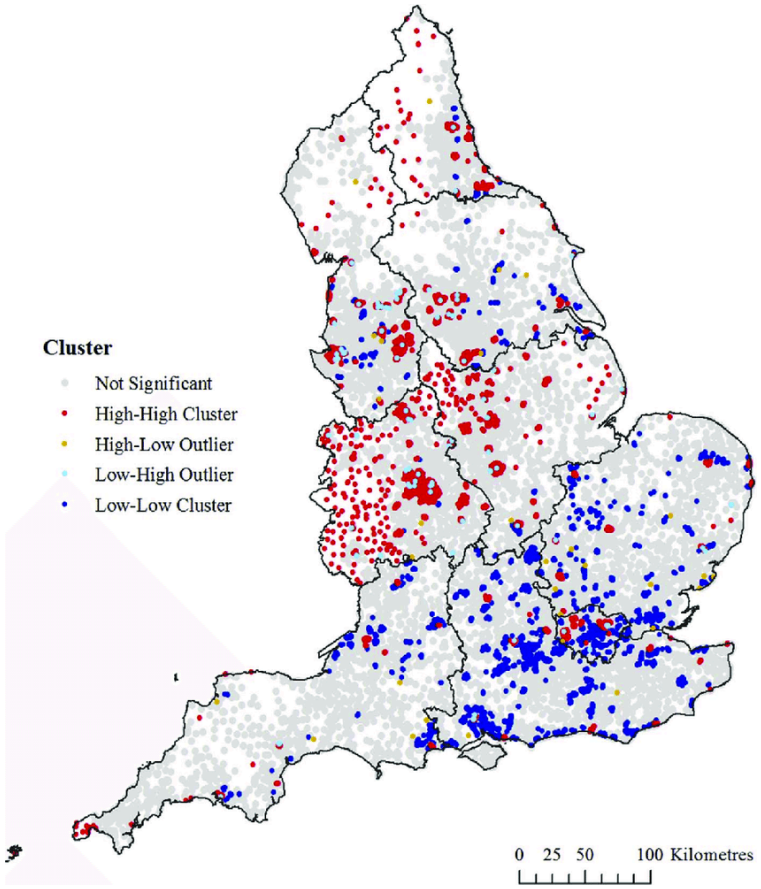
Marker (H3K27ac) into 2D mapping

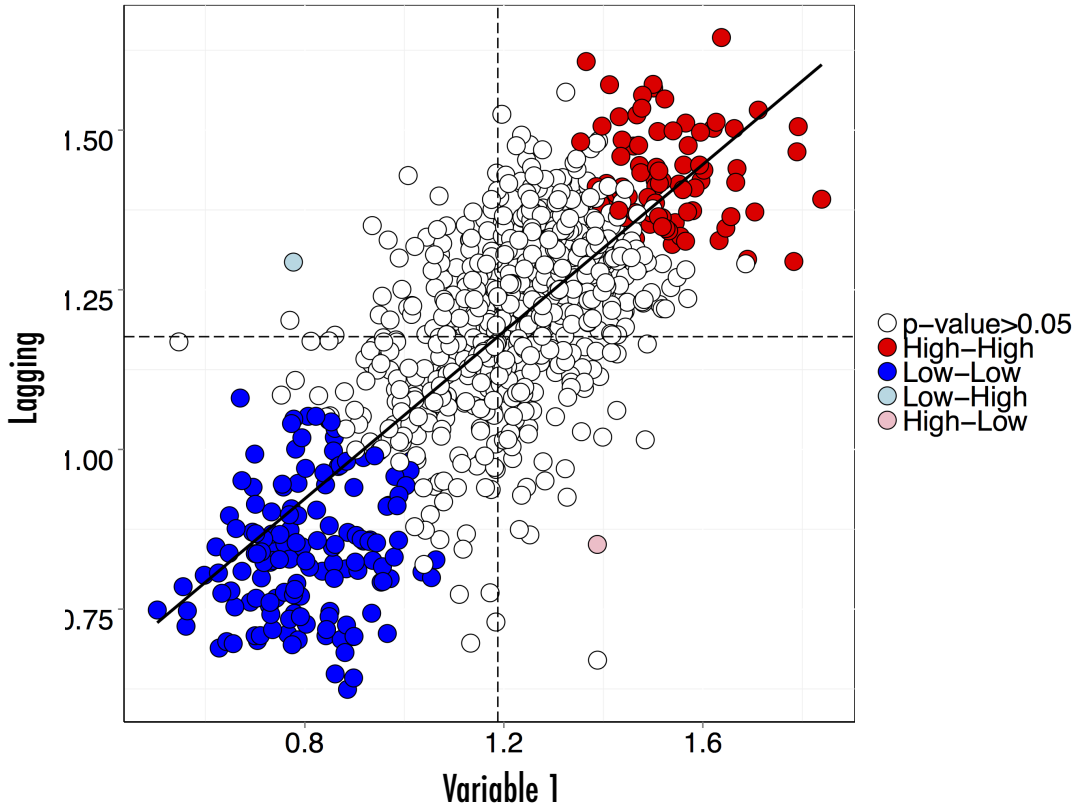
chr11:110780000-114770000





Local Moran Index

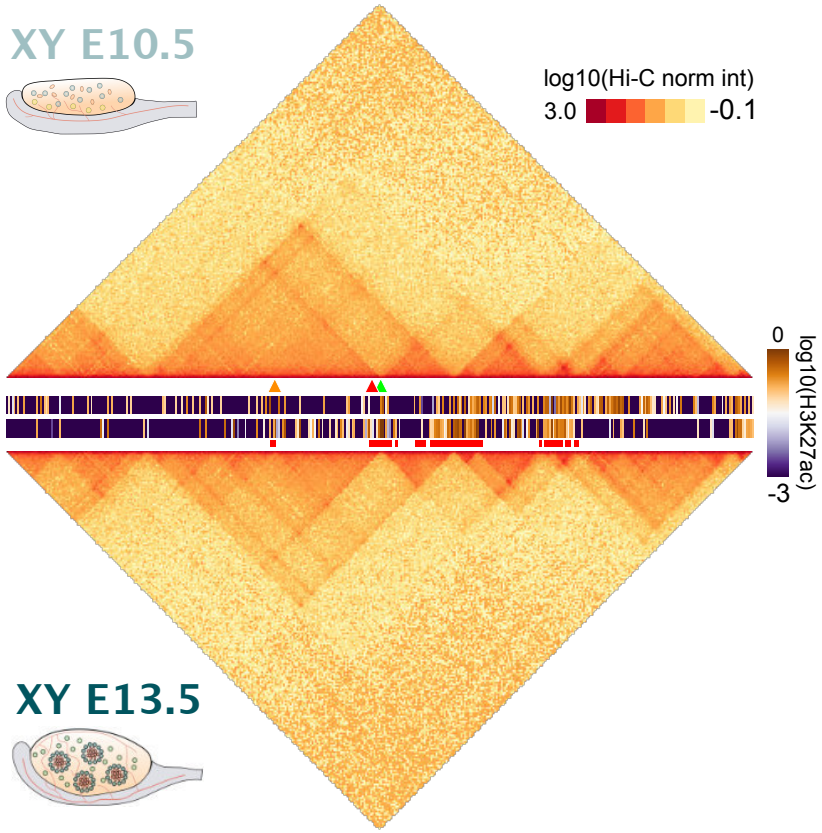


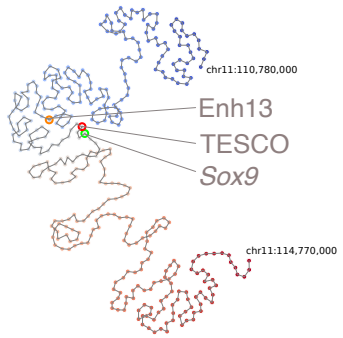


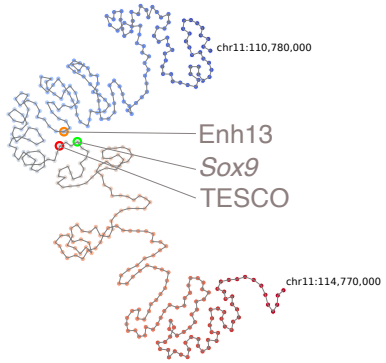
Quantifying regulatory environments bin by bin

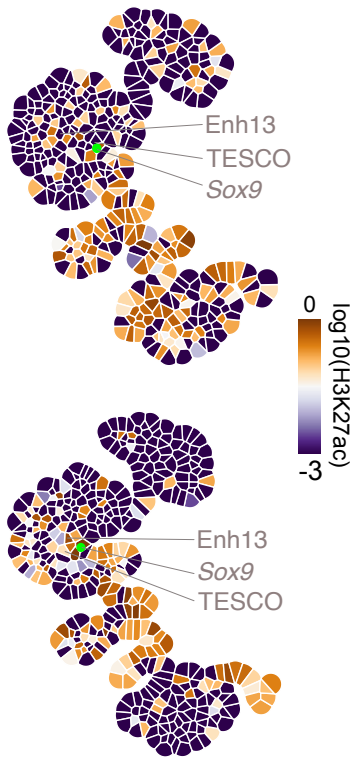


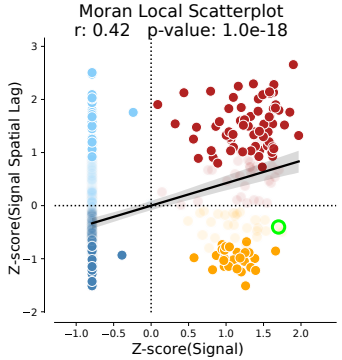
Sox9 locus chr11:110,780,000-114,770,000

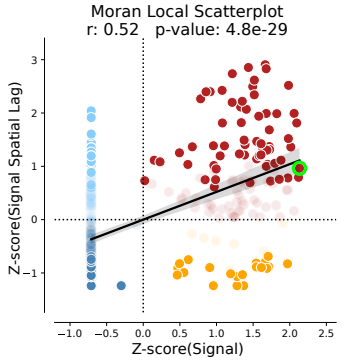


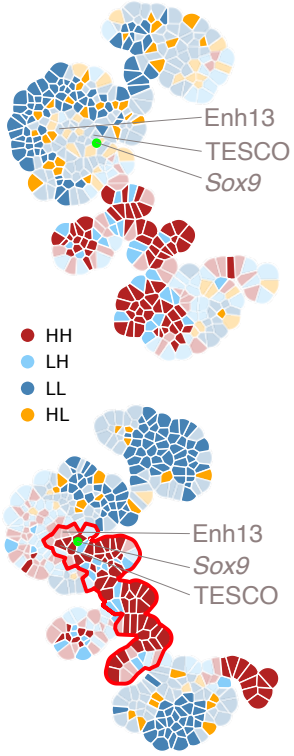




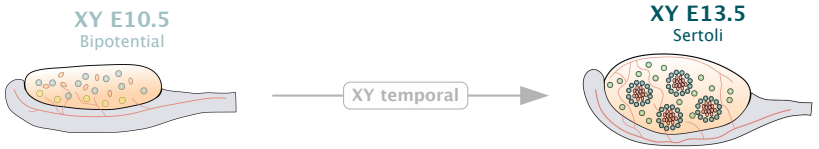


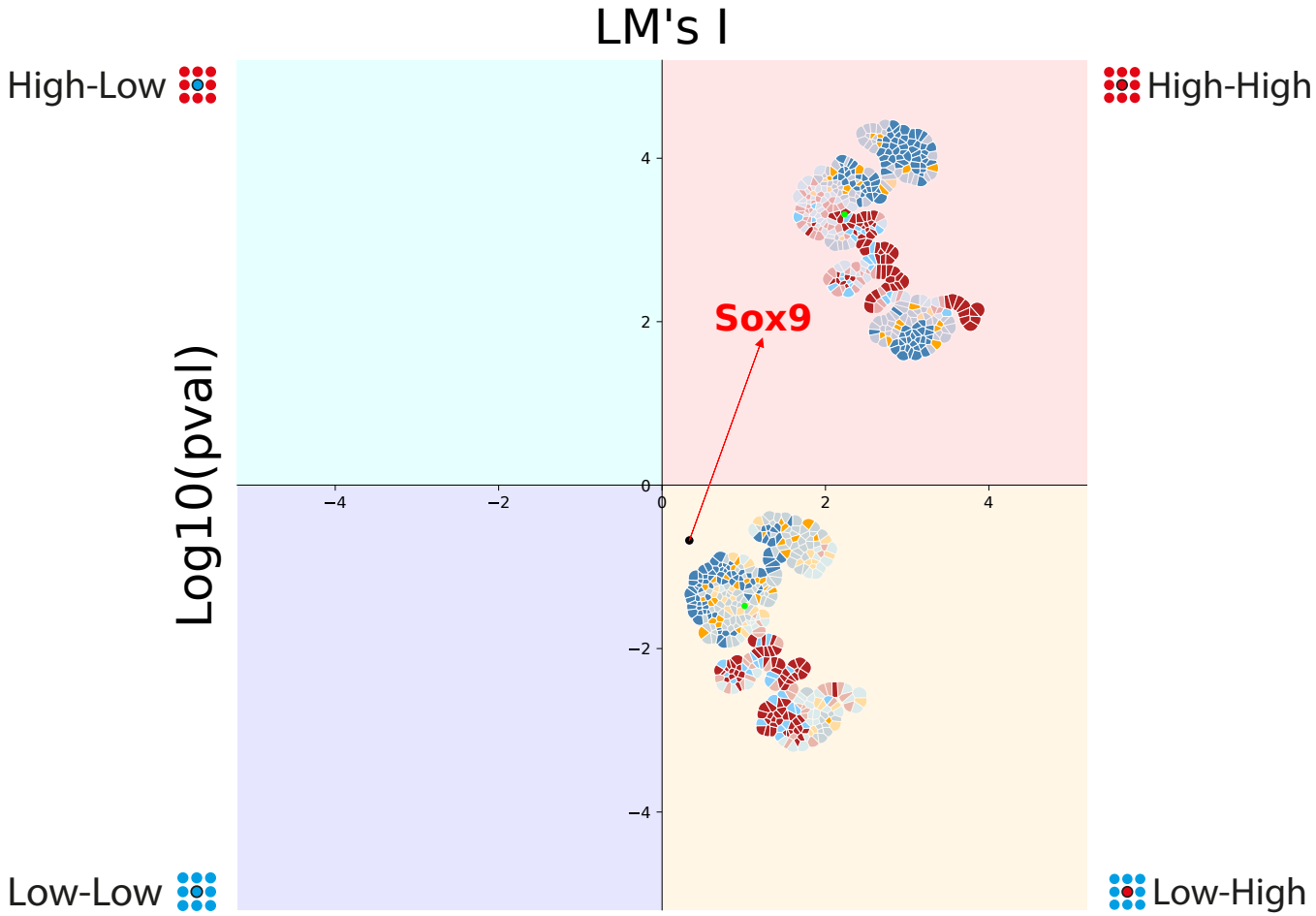






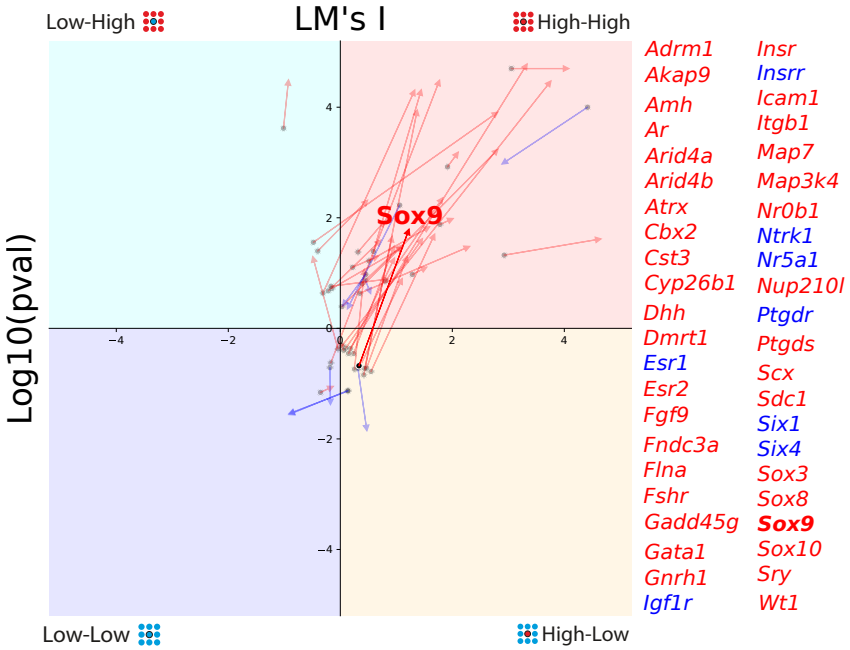
LMI Trip for Sox9 gene



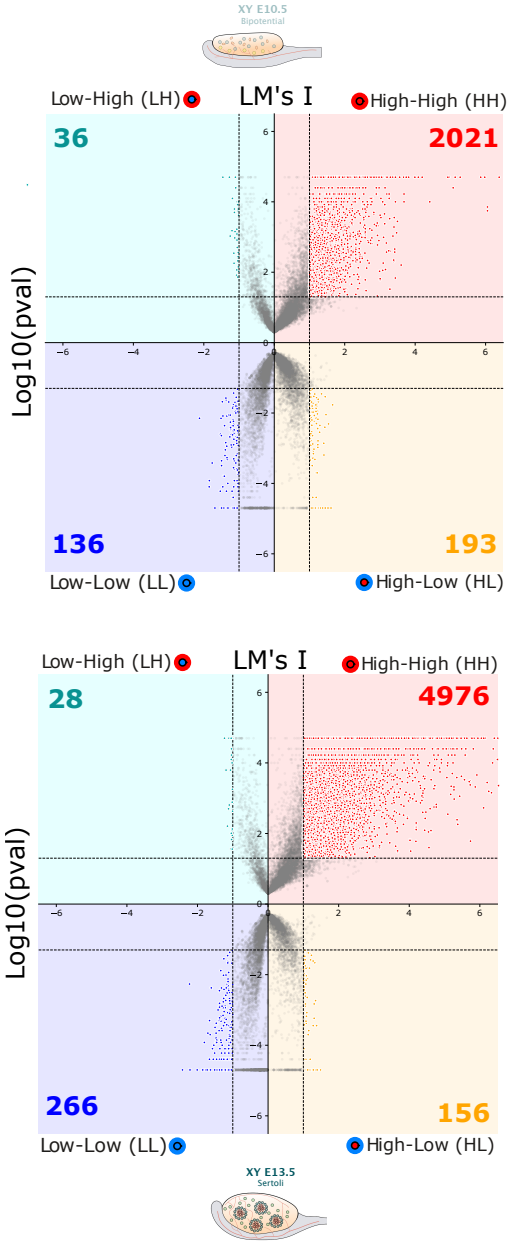


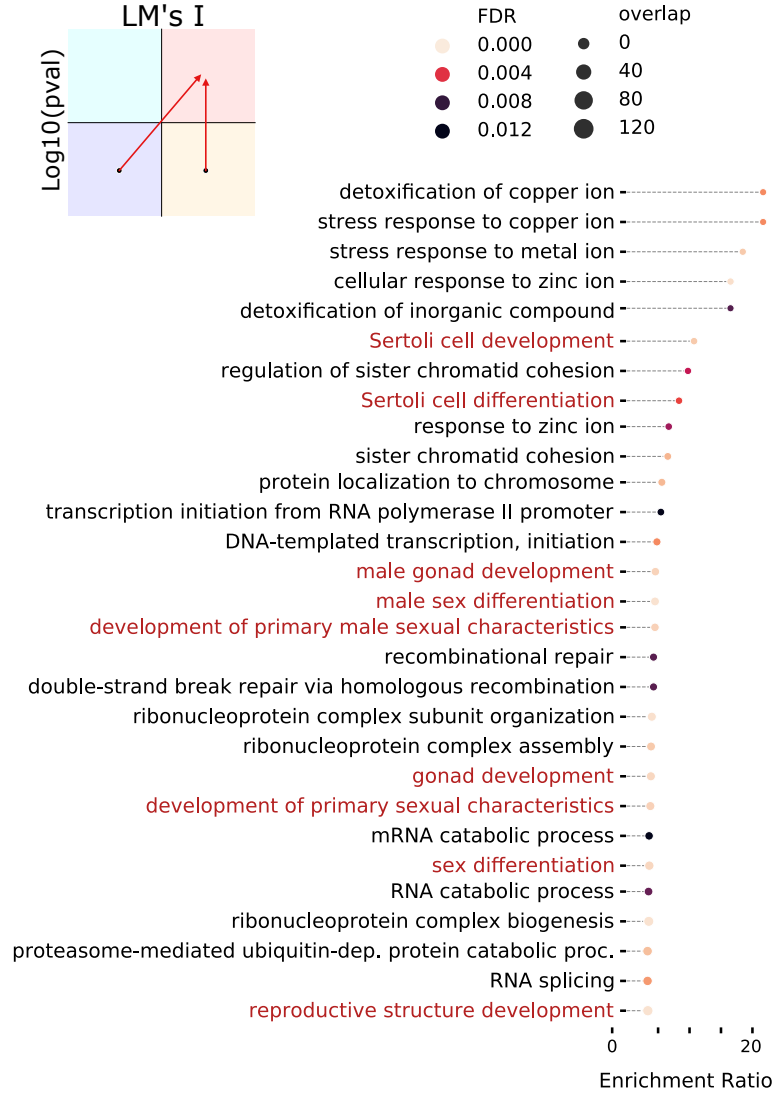
All genes LMI Trip





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



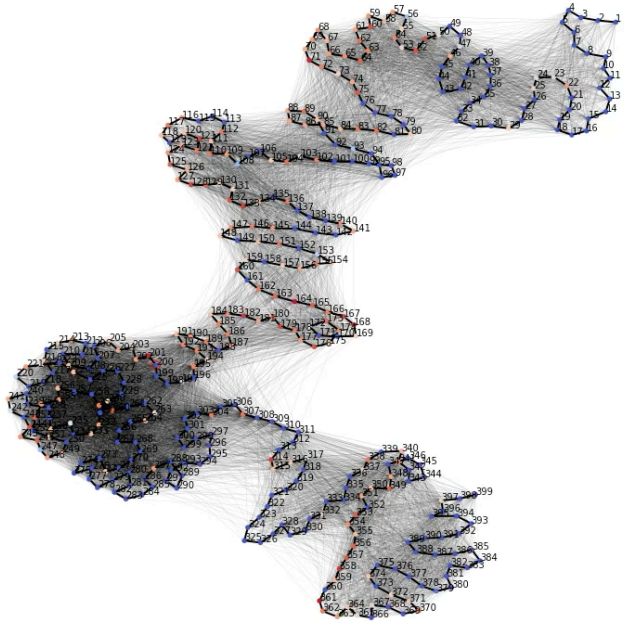


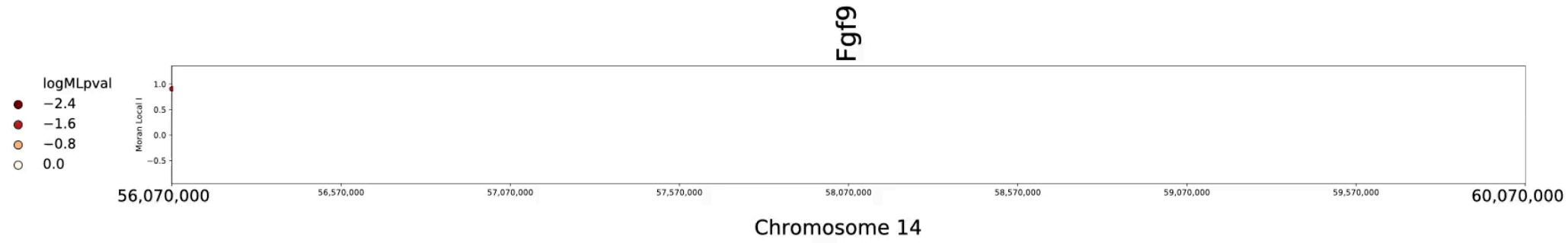
Now that we know the genes….

Can we identify regulatory elements using 

METALoci predictive mode

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

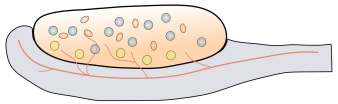


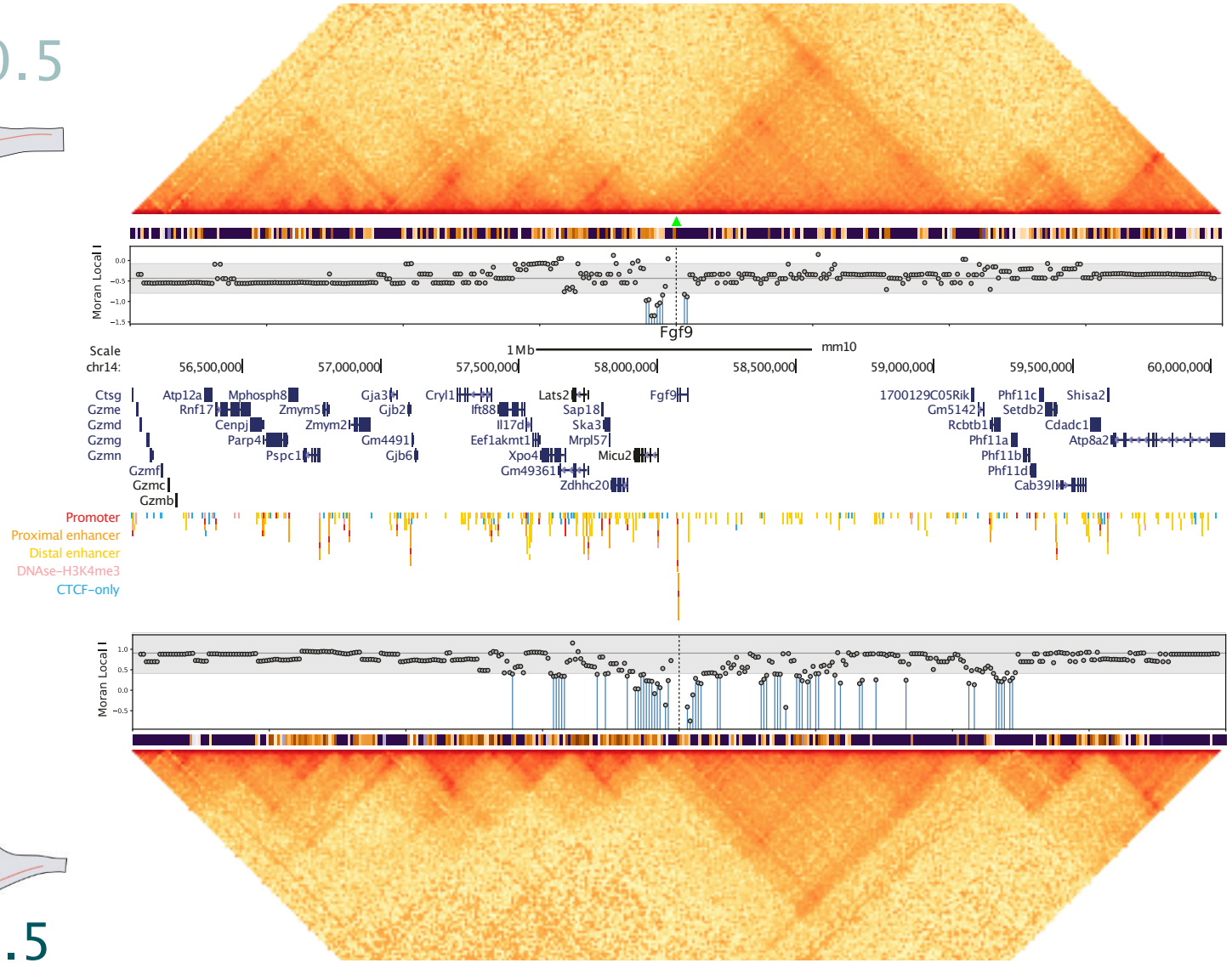


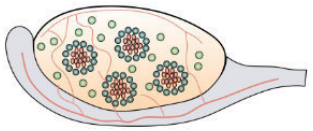
METALoci predictive mode

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

XY E10.5

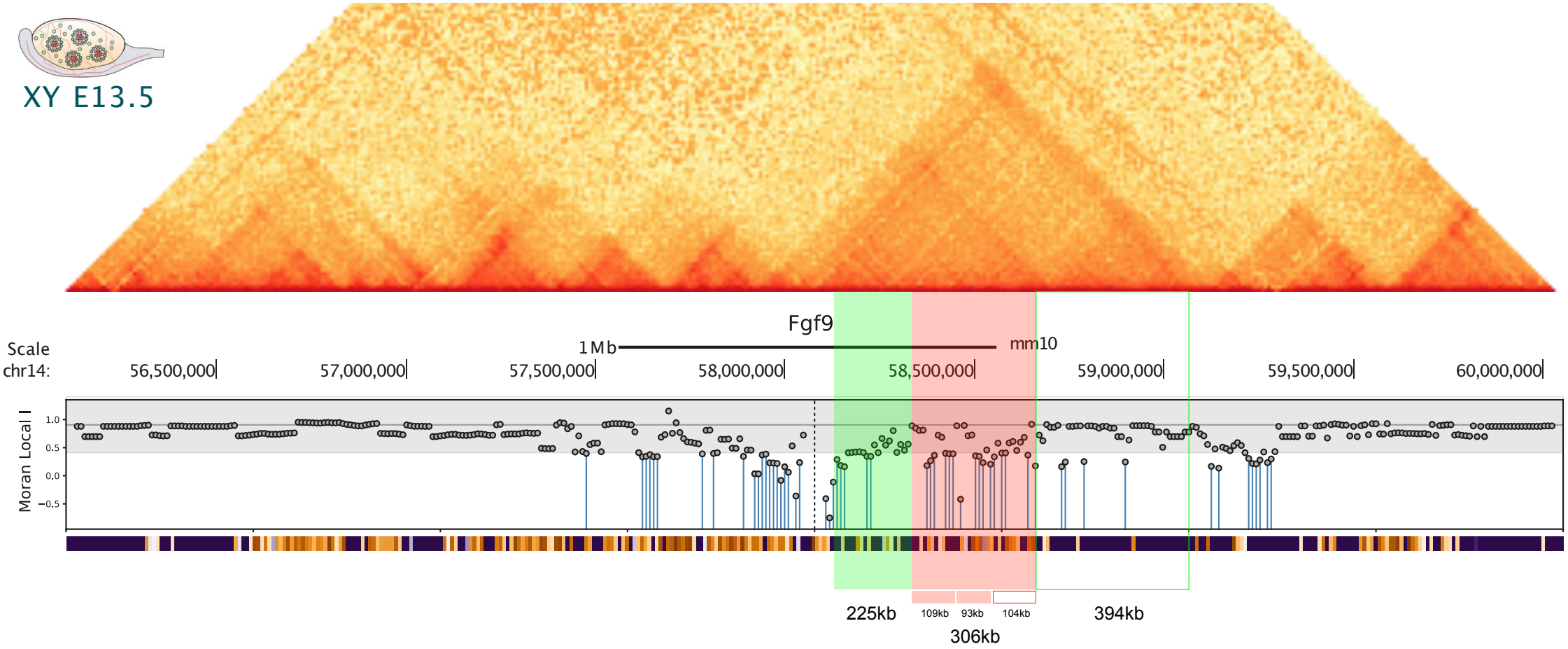






XY E13.5

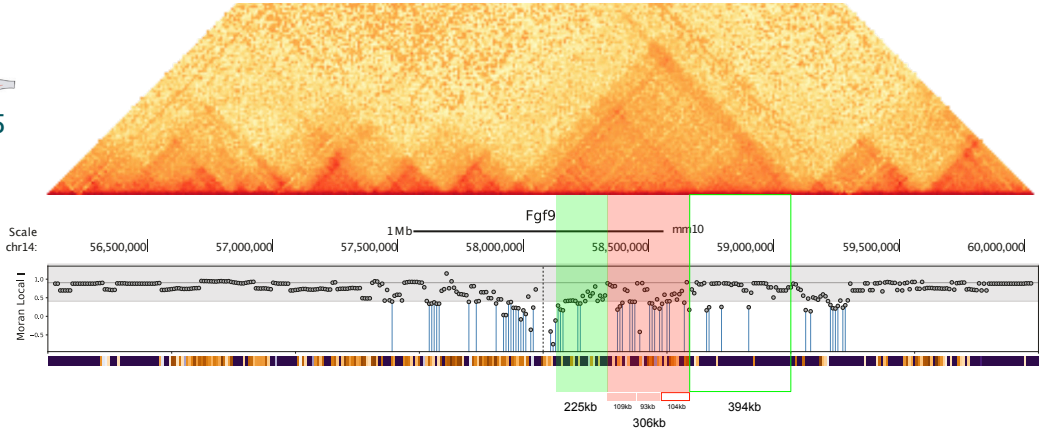
In silico prediction of regulatory regions at the Fgf9 locus



Phenotype confirms METALoci predictions



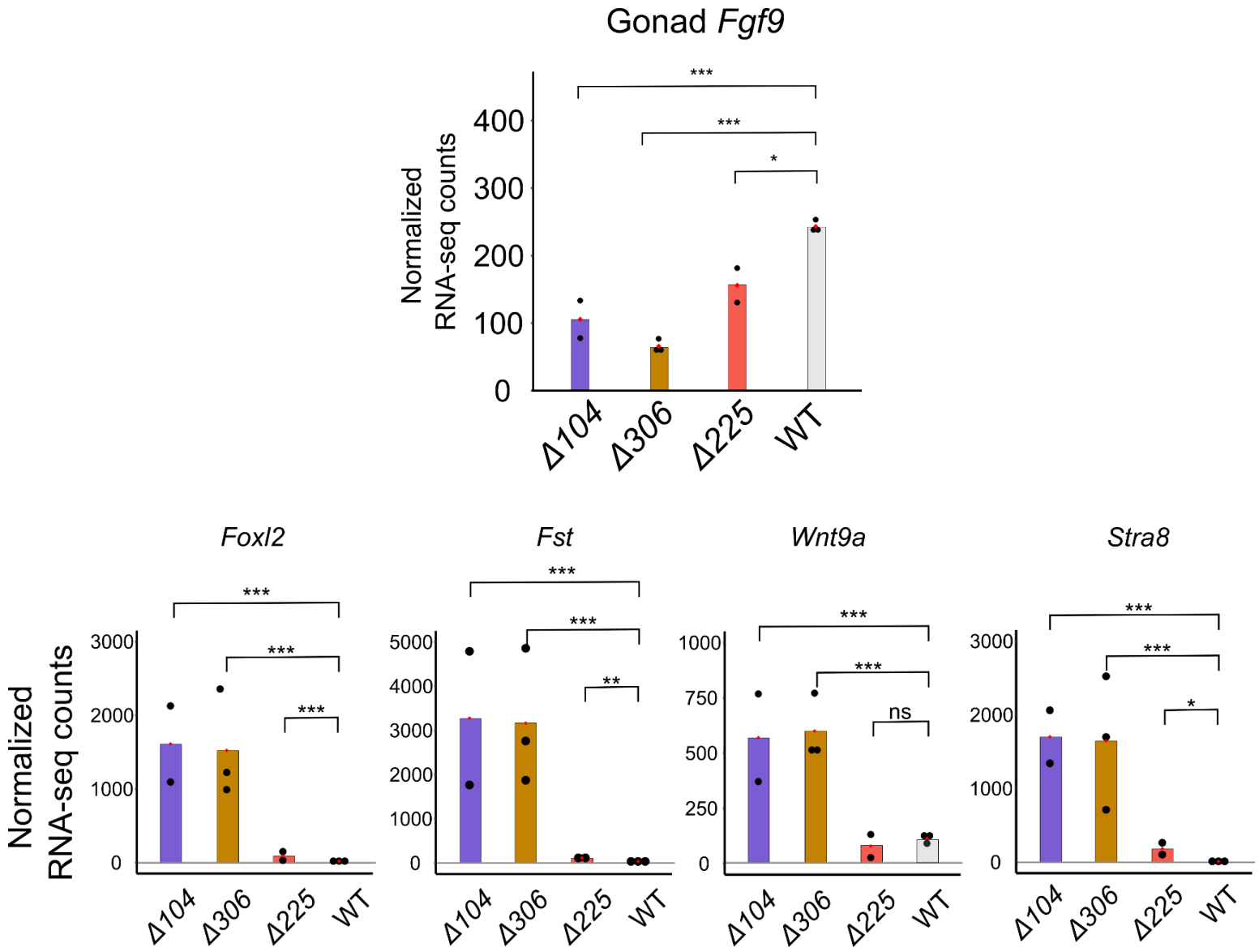
XY E13.5



	WT	Δ225	Δ306	Δ93	Δ104
Testis		6/6	0/16	1/4	0/2
Ovotestis		0/6	12/16	2/4	2/2
Ovary-like		0/6	4/16	1/4	0/2
Ovary		0/6	0/16	0/4	0/2

250um

RNA-seq confirms METALoci predictions



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

WE ARE HIRING!
Talk to me :)

@marciuslab
@mamartirenom

http://marciuslab.org

cnag

CRG Centre for Genomic Regulation

ICREA





Juan A. Rodríguez

Irene Mota

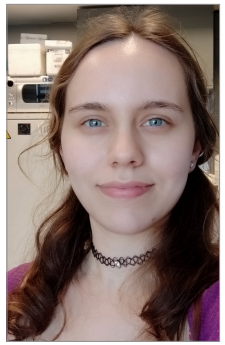
In collaboration with the Capel Lab (Duke U.) and Lupiañez Lab (CABD)














Alexander Barclay
Nikolai Bykov
Iana Kim
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