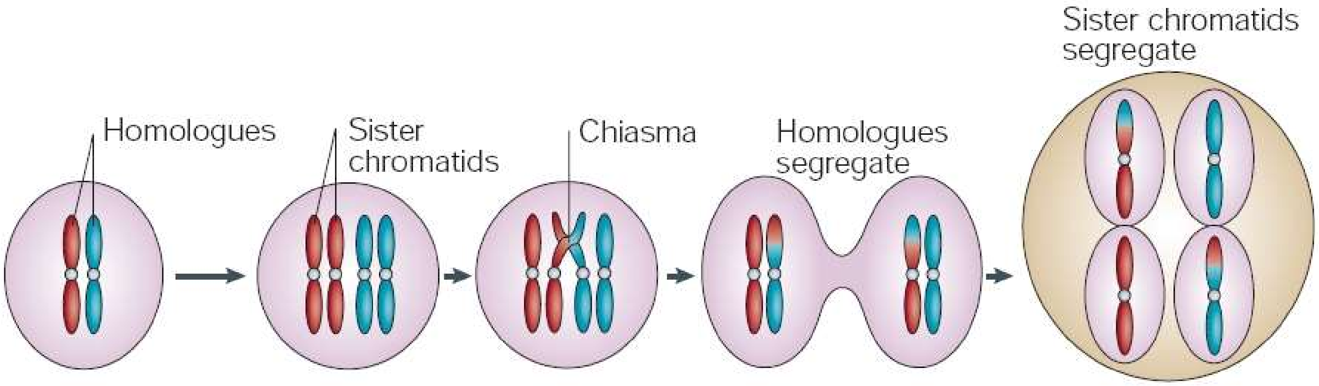
Multi-layered control of meiotic recombination initiation in mammals

Florencia Pratto
Dan Camerini-Otero lab
NIDDK - NIH

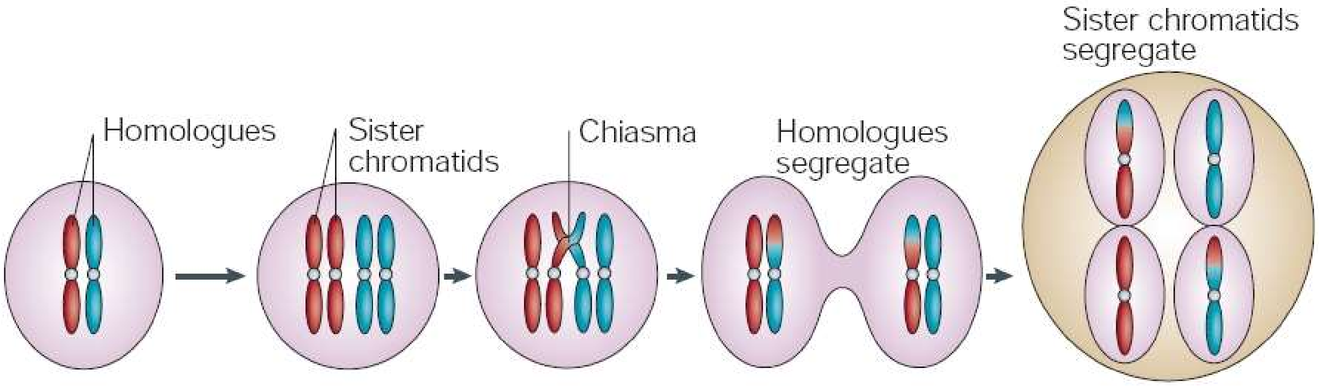
DNA Repair Interest Group

Meiosis



Adapted from
Marston and Amon, Nat Rev Mol Cell Biol 2005

Meiosis



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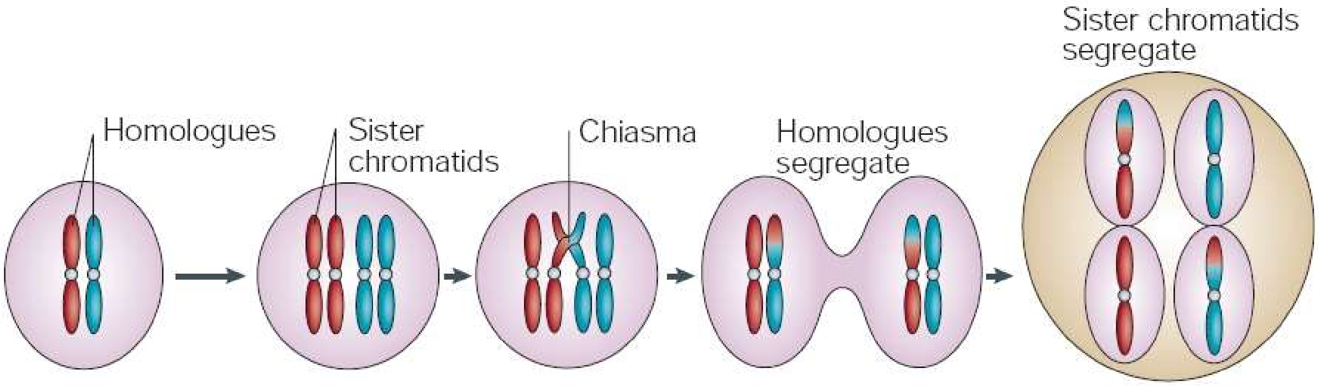
Prophase I:
Meiotic Recombination

Key role of meiotic recombination:

- Proper chromosome segregation
- Increase genetic diversity

Meiosis



Prophase I:
Meiotic Recombination

Adapted from
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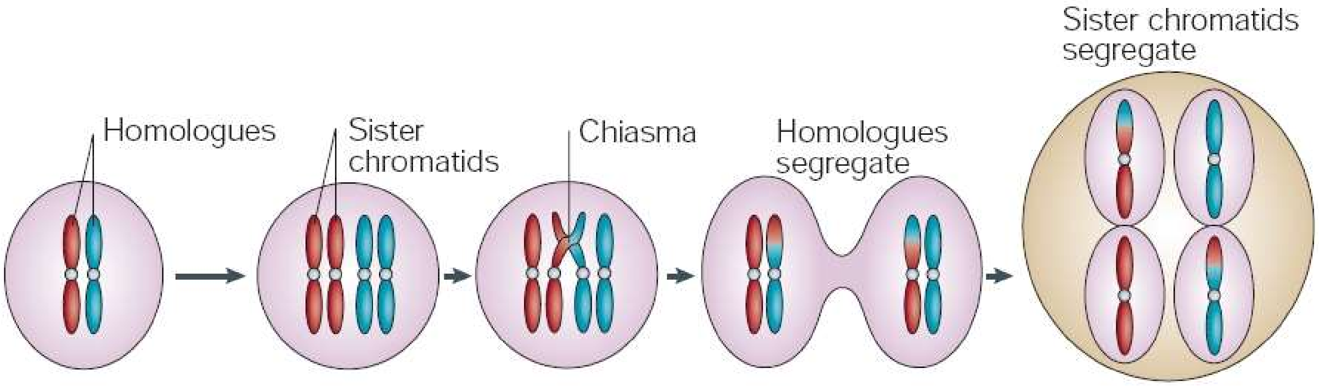
Error-prone process

Defective segregation causes a majority of aneuploidies

- 20 – 60 % of human eggs have a chromosomal abnormality

Gruhn et al. Science 2019

Meiosis



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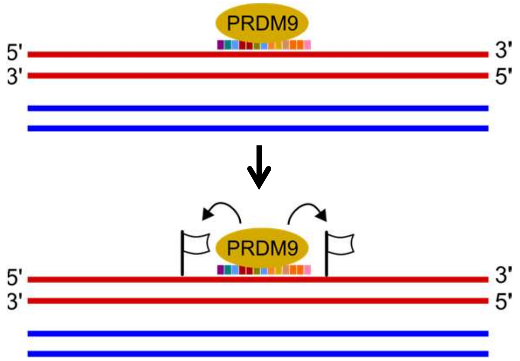
Prophase I:
Meiotic Recombination

Initiated by hundreds of programmed double-strand breaks

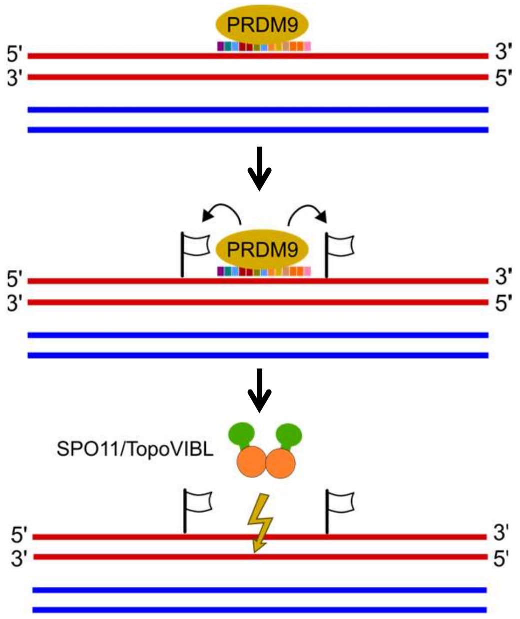
Meiotic recombination initiation in mammals



PRDM9: A meiosis-specific H3K4 methyltransferase is essential for defining hotspot locations

Baudat et al., 2010 Science
Myers et al., 2010 Science
Parvanov et al., 2010 Science

Meiotic recombination initiation in mammals



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SPO11/TOPOVIBL complex introduces programmed double-strand breaks (DSBs)

Romanienko et al., 2000 Mol Cell
Baudat et al., 2000 Mol Cell
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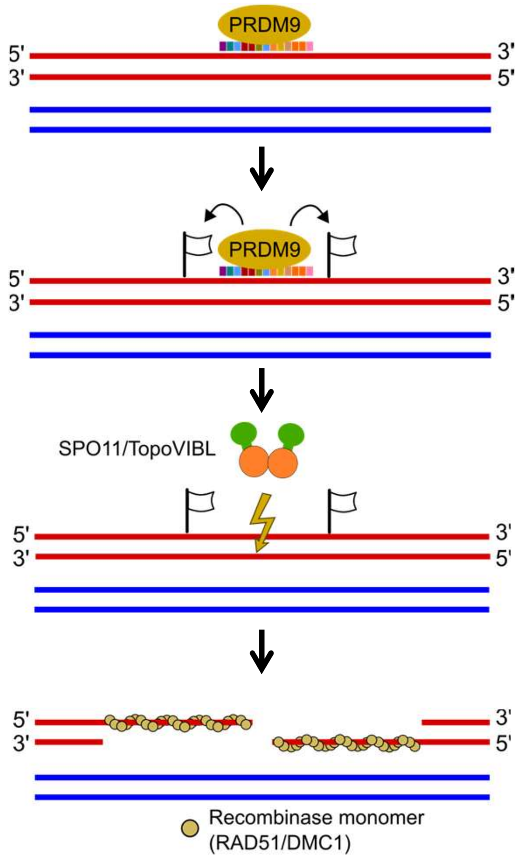

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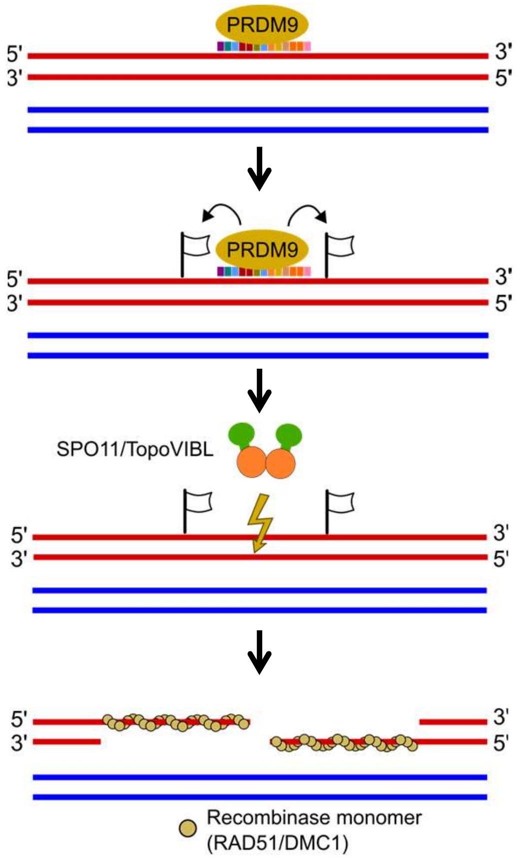
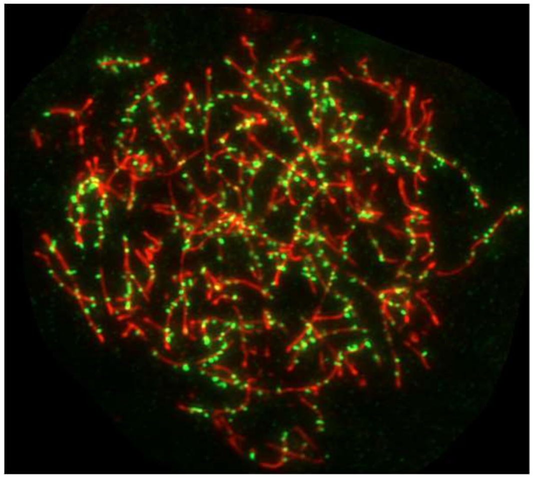
The DNA ends undergo 5′-to-3′ resection.
The 3′ ssDNA tail is bound by strand exchange proteins (RAD51/DMC1) to initiate repair.

Meiotic recombination initiation in mammals

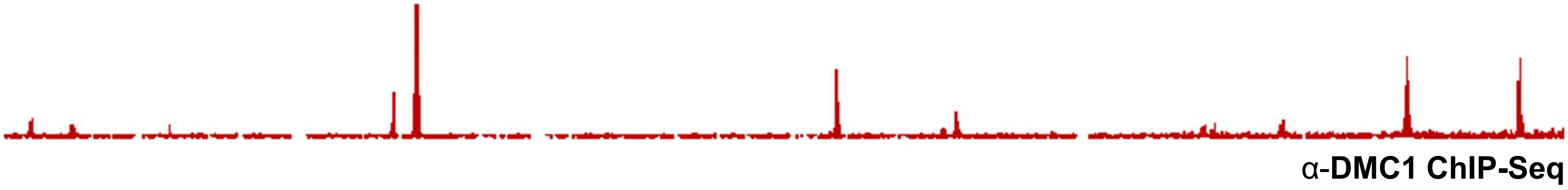


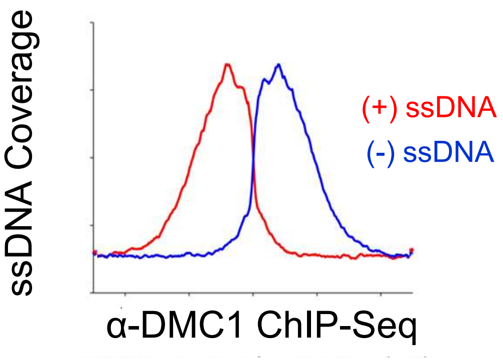
DMC1 (DSBs) / SYCP3 (Axis)



Immunofluoresence microscopy

Meiotic DSB hotspots can be identified using a variant of ChIP-seq





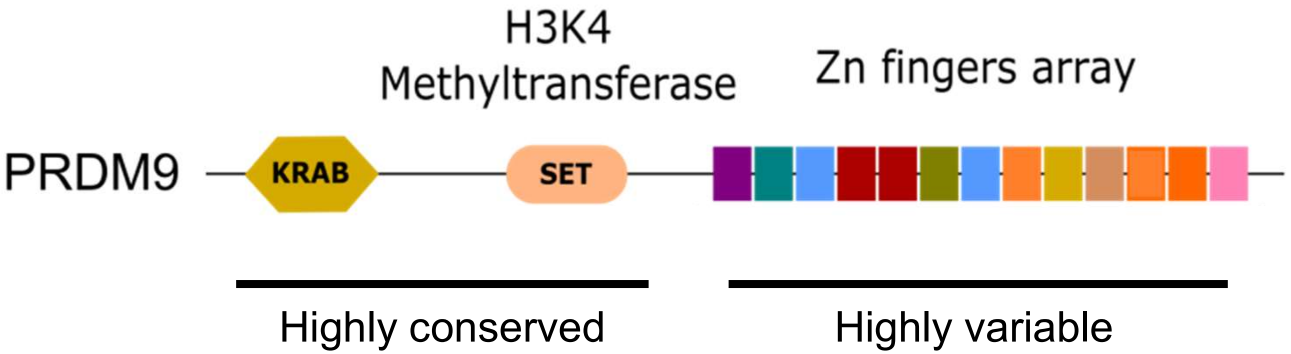
Single-Stranded DNA Sequencing (SSDS)

DSB hotspot detection is sensitive and highly specific:

- Up to 70% of total signal is at peak
- SSDS yields resected DNA either side of a DSB hotspot

Khil et al.,2012 Genome Res
Brick et al., 2012 Nature
Brick & Pratto et al.,2018 Meth. Enzym

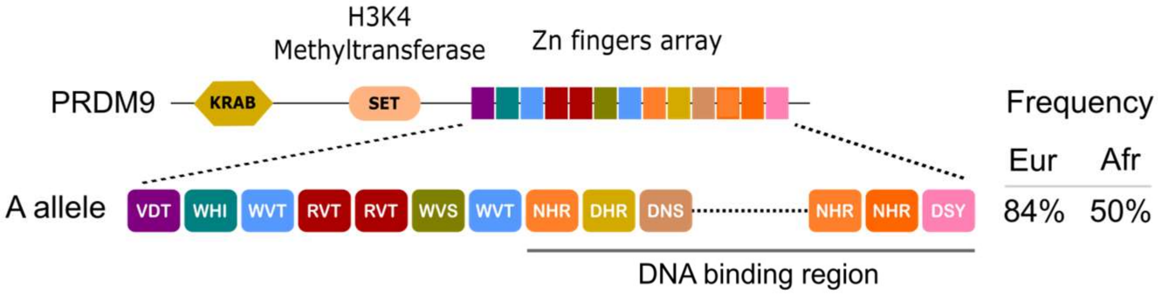
PRDM9 directs DSBs in mammals



- Prdm9 is under strong positive selection and is one of the fastest evolving proteins in mammals
- Several dozens alleles of PRDM9 are known in humans
- Only DNA-binding ZNF domain is hyper-variable, the rest is conserved

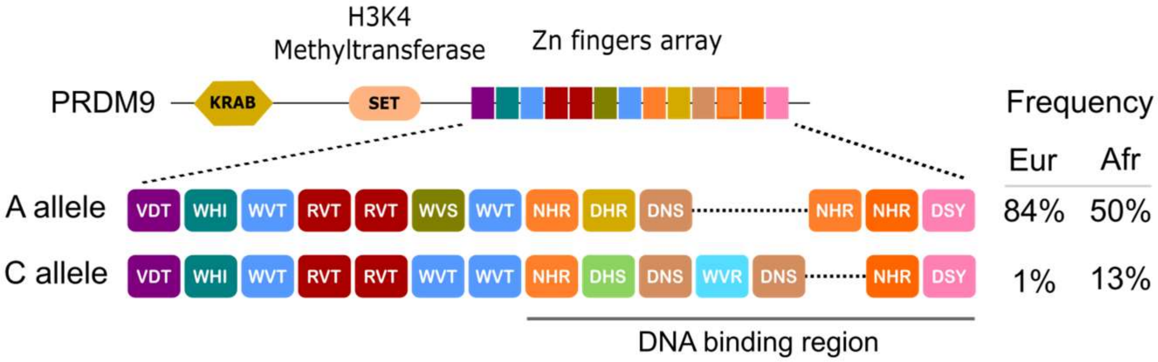
Oliver et al., PLoS Genetics 2009
Berg et al., Nature Genetics 2010

PRDM9 alleles in humans



*Allele frequencies form
Berg et al., 2010*

PRDM9 alleles in humans



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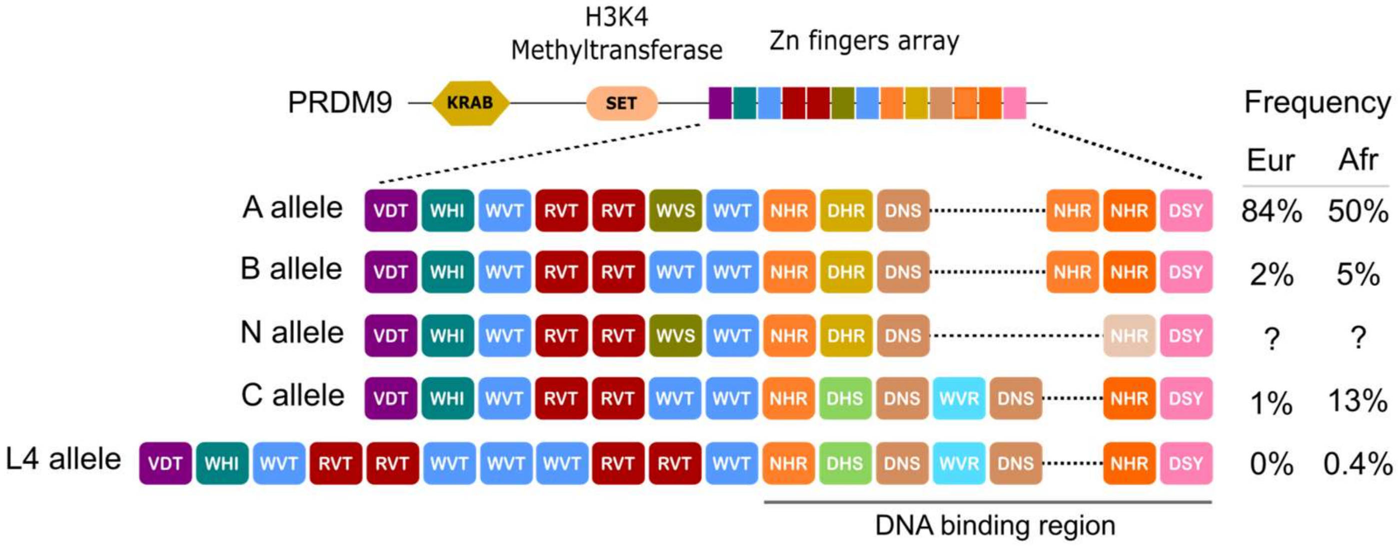

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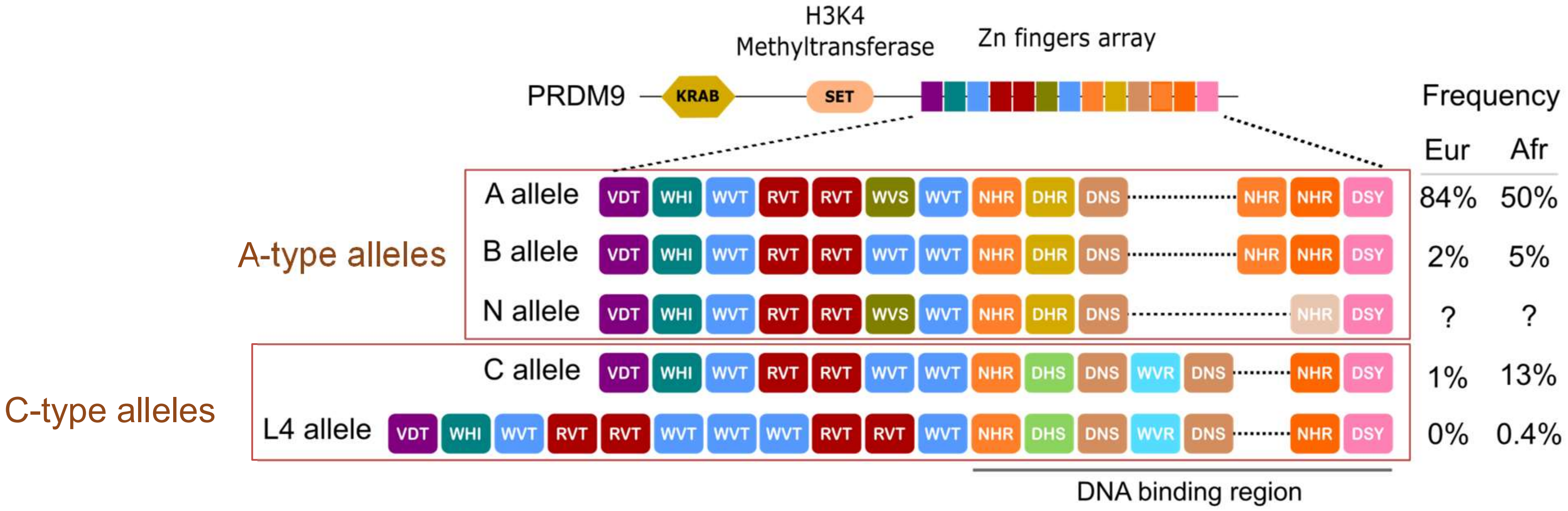

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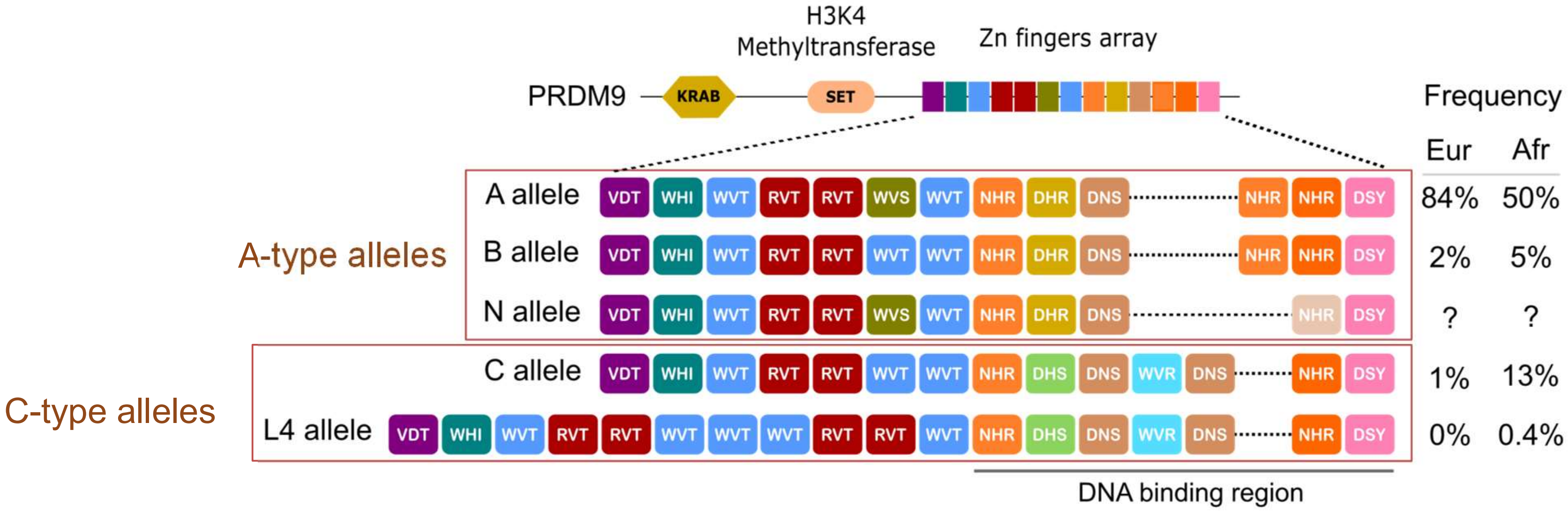


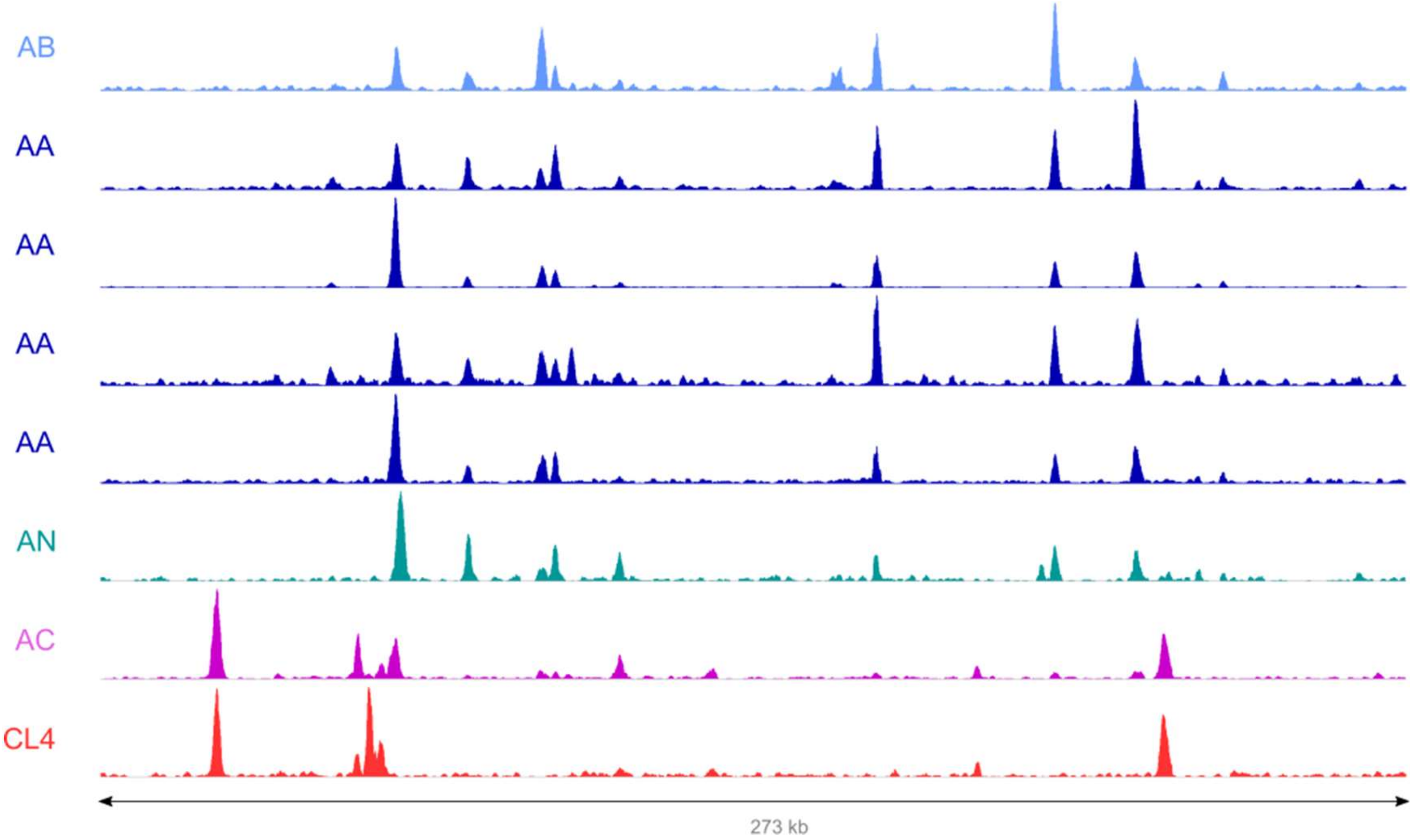
Samples

Sample	PRDM9 alleles	# Hotspots
AA_1	A / A	28,165
AA_2	A / A	34,278
AB	A / B	24,049
AC	A / C	38,945

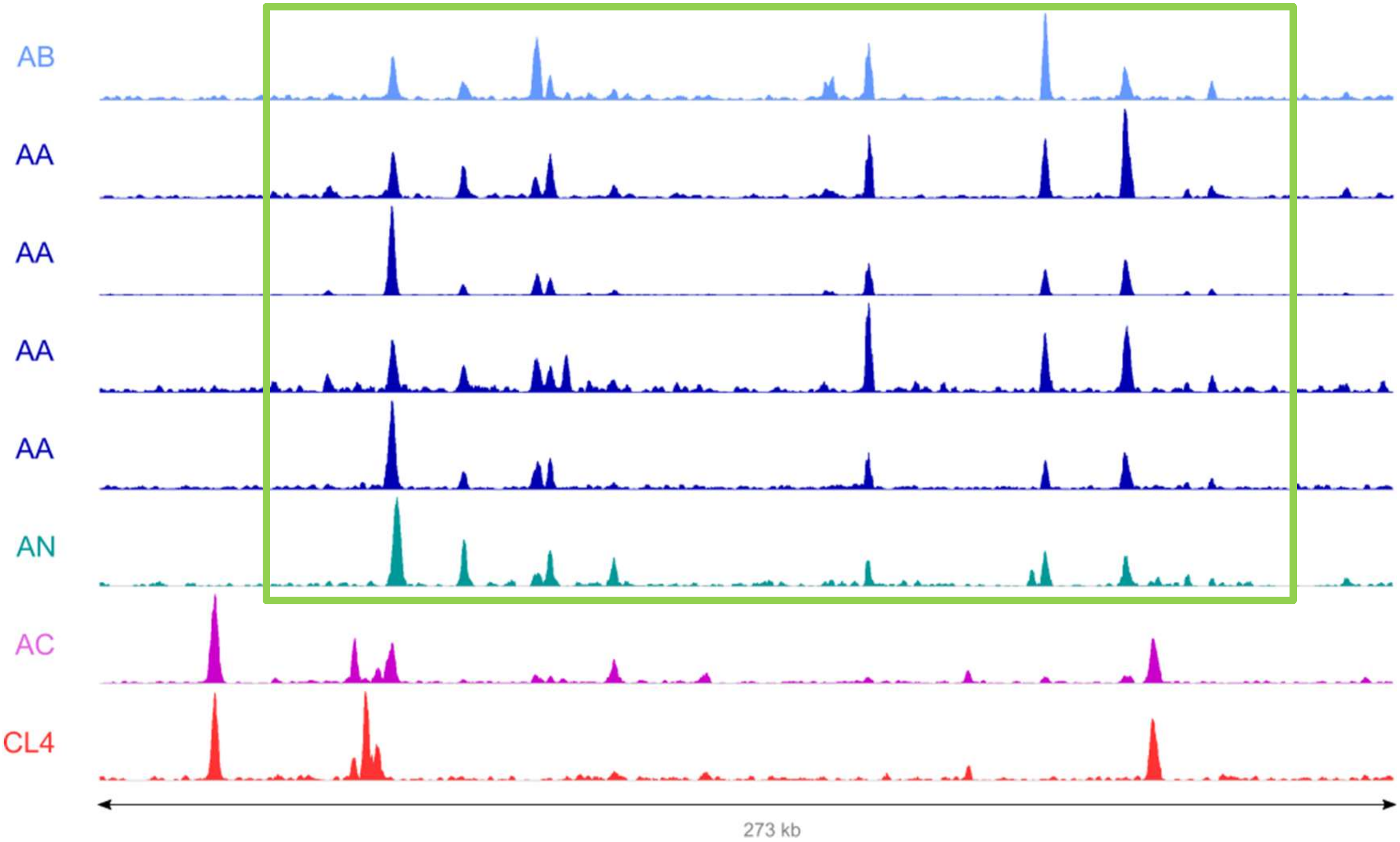
Sample	PRDM9 alleles	# Hotspots
AA_3	A / A	19,093
AA_4	A / A	23,966
CL4	C / L4	15,734
AN	A / N	29,609

*Allele frequencies form
Berg et al., 2010*

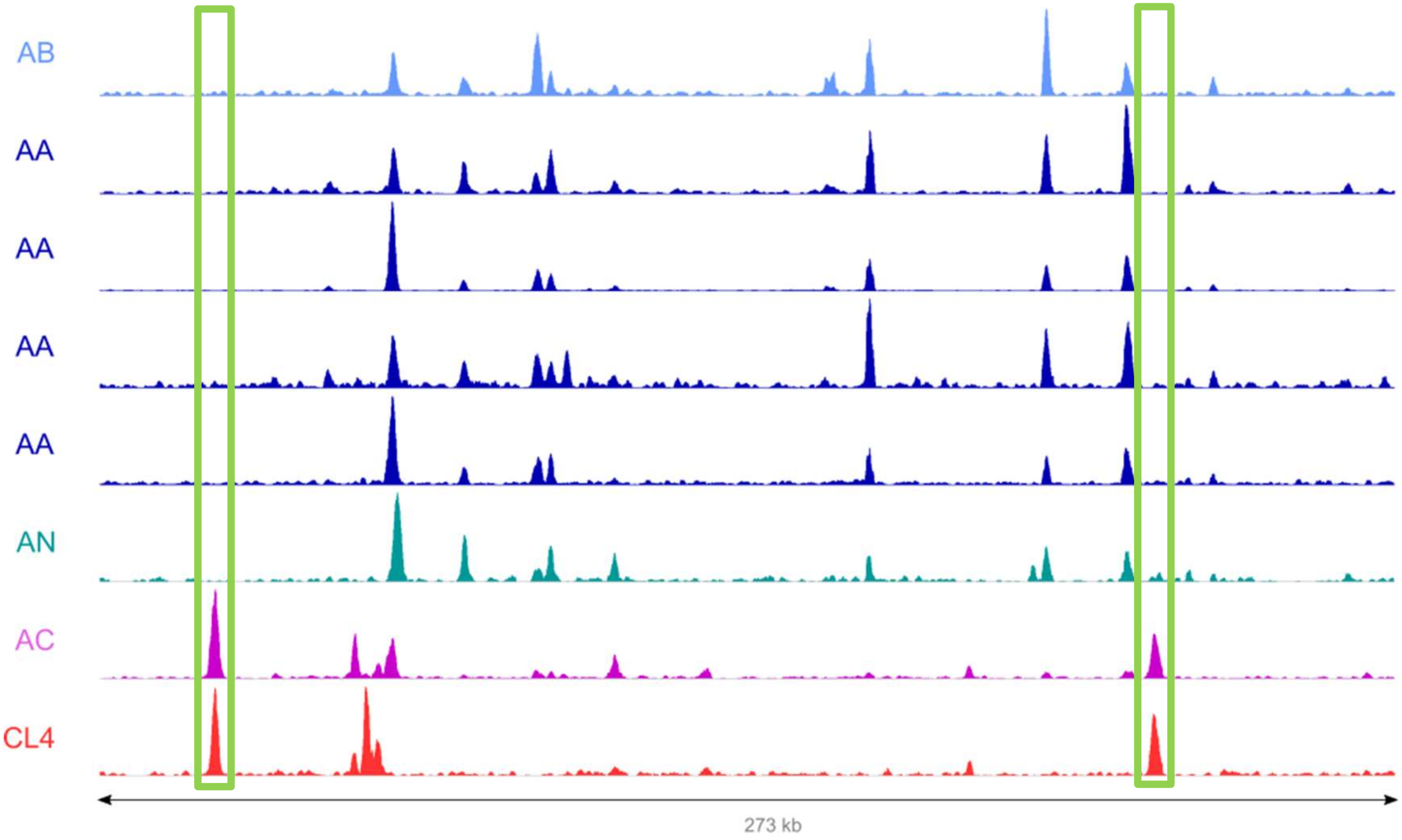
Overview of DSB maps from human males



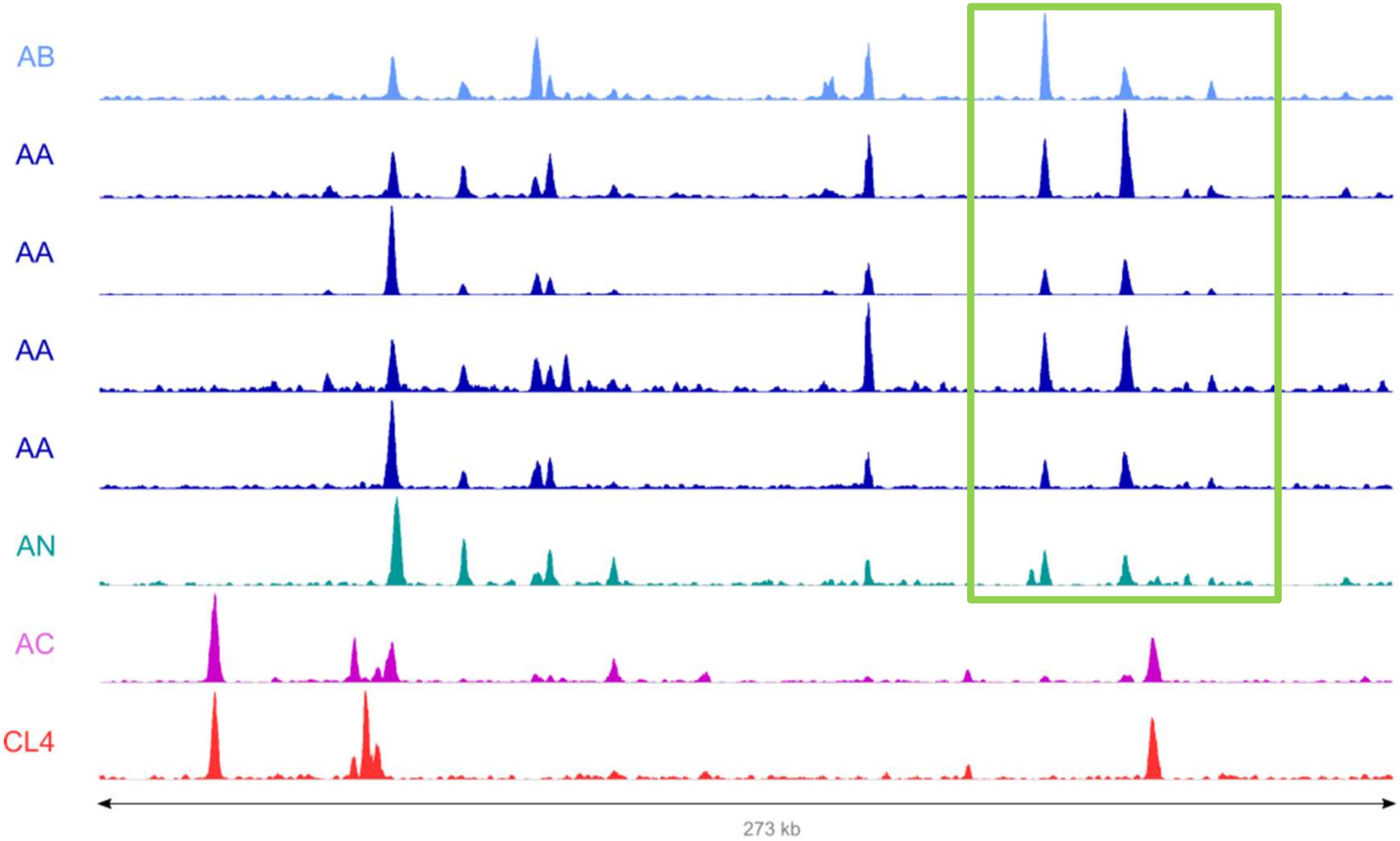
Hotspot from A-type individuals are broadly similar



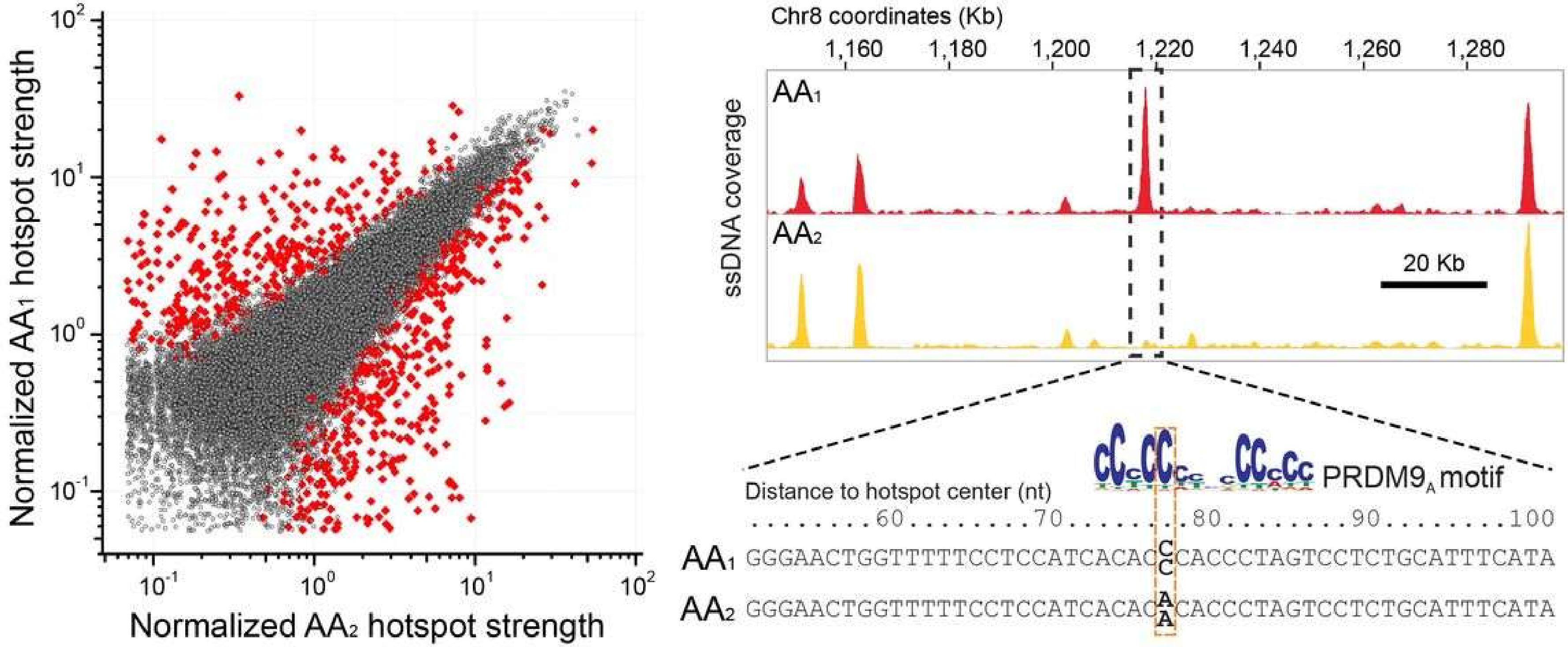
PRDM9-C allele defines a different set of hotspots



Inter-individual variation independent of PRDM9



About 5% of DSB hotspots are variable between two individuals with the same *PRDM9* genotype



Pratto et al., Science 2014

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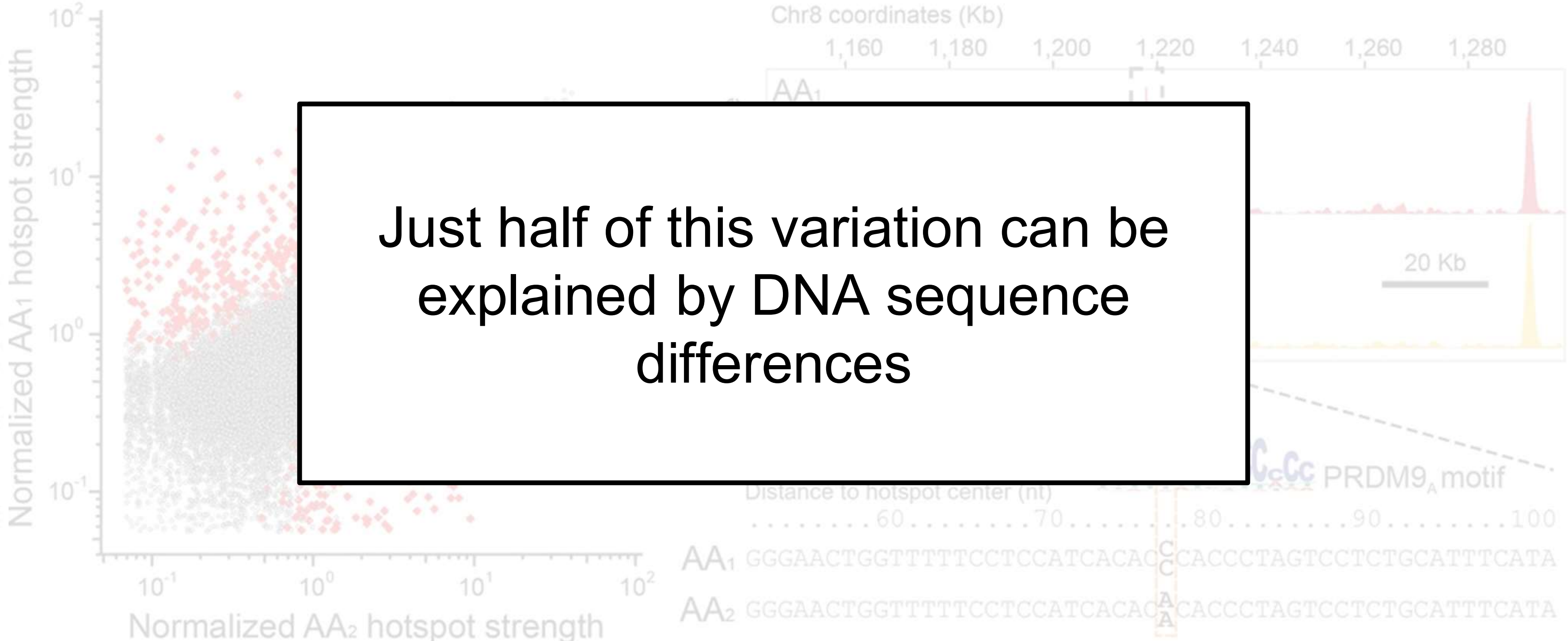
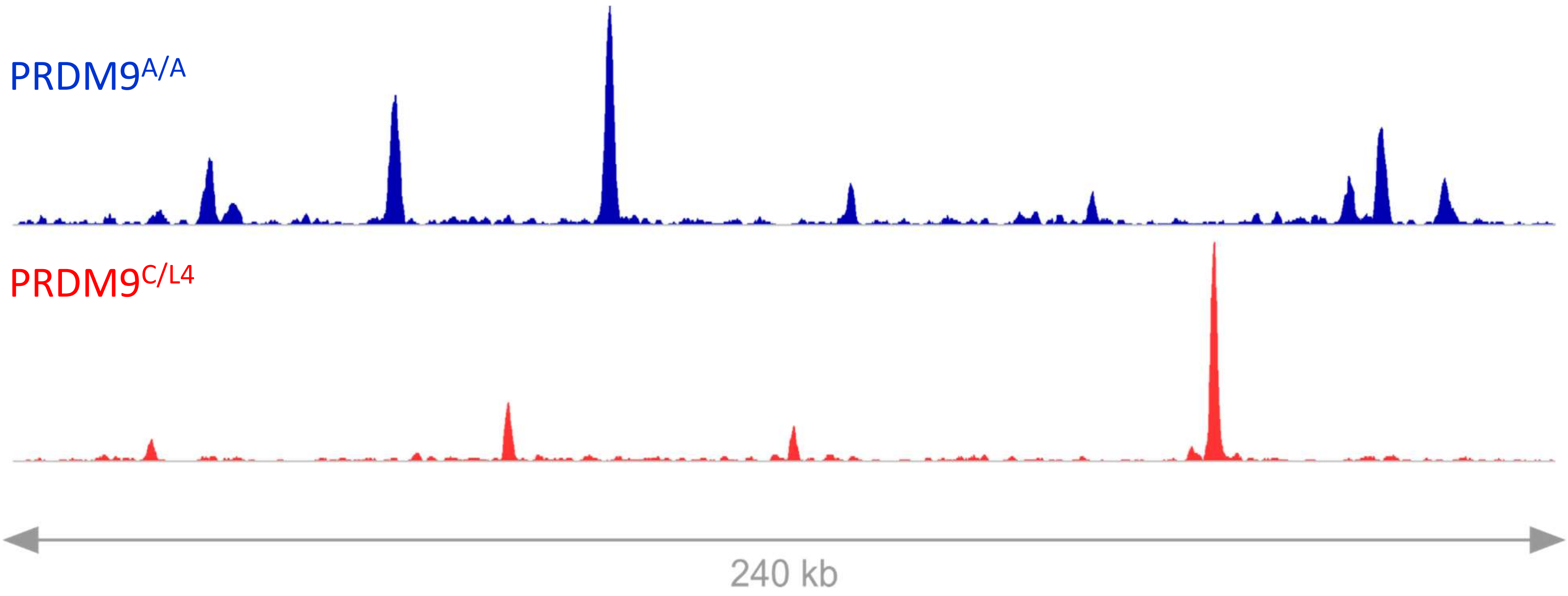


Just half of this variation can be explained by DNA sequence differences

Pratto et al., Science 2014

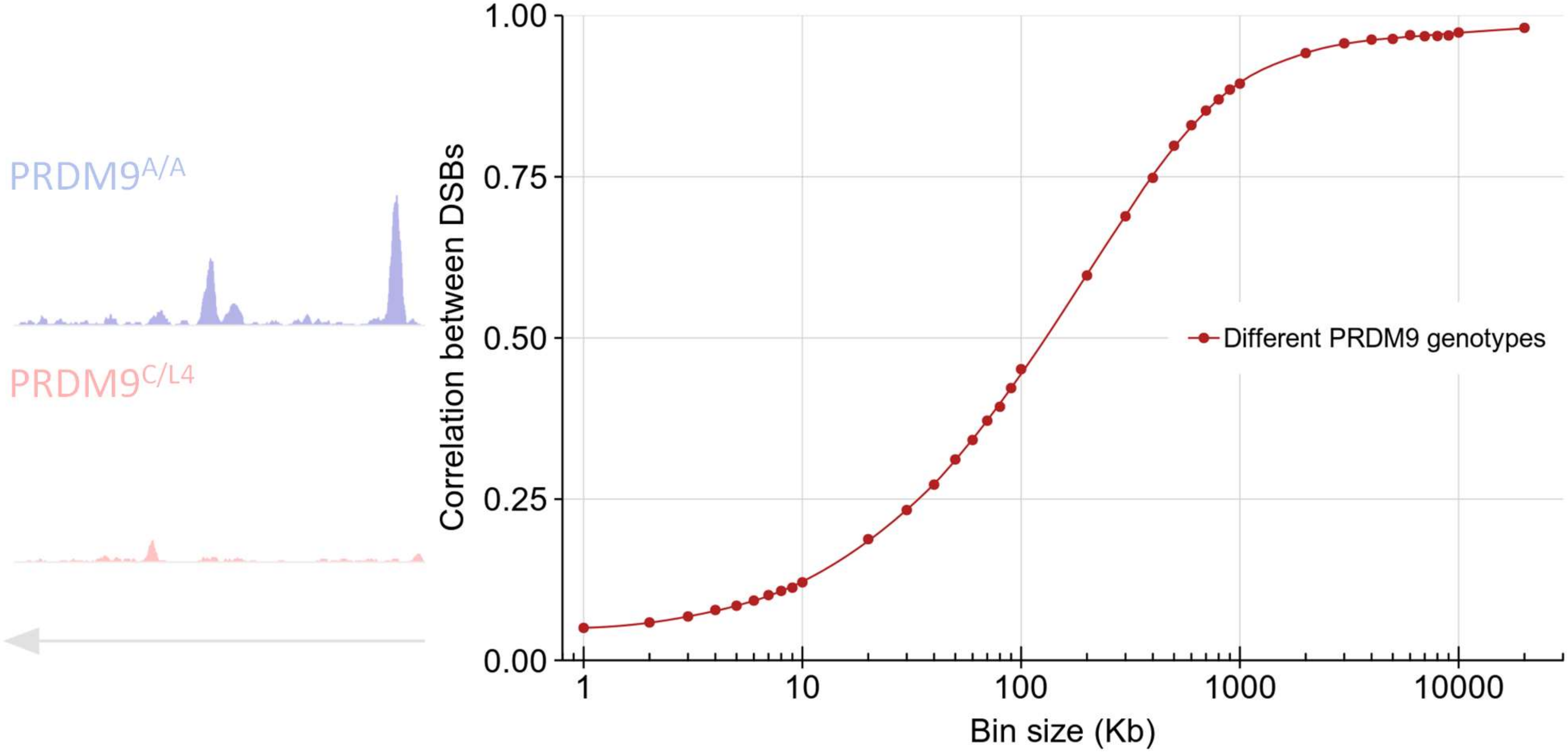
Local control of meiotic DSB formation

PRDM9 genotype dictates local recombination patterning



Baudat et al., Berg et al., Parvanov et al., Science 2010
Brick et al., Nature 2012

Global control of meiotic DSB formation



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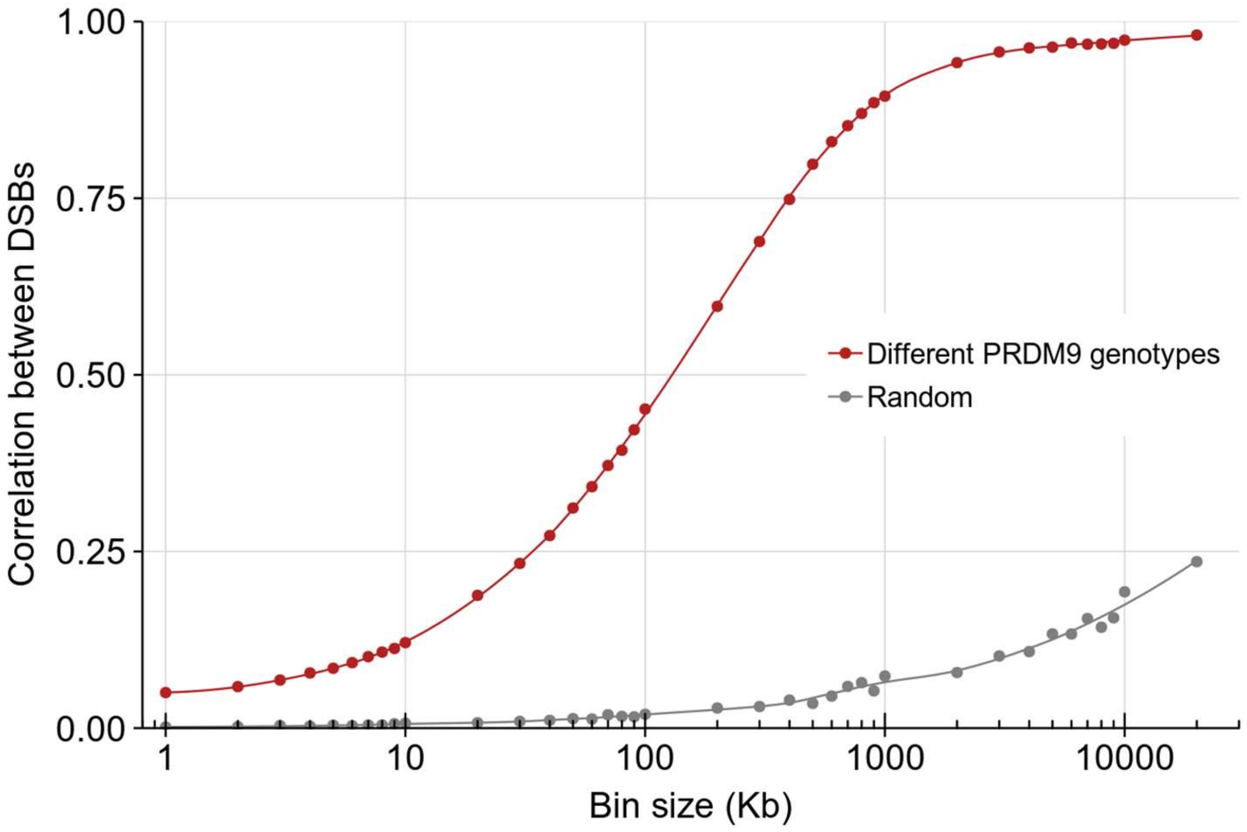

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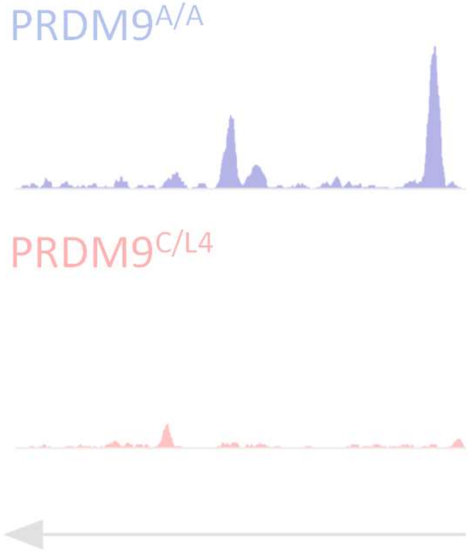
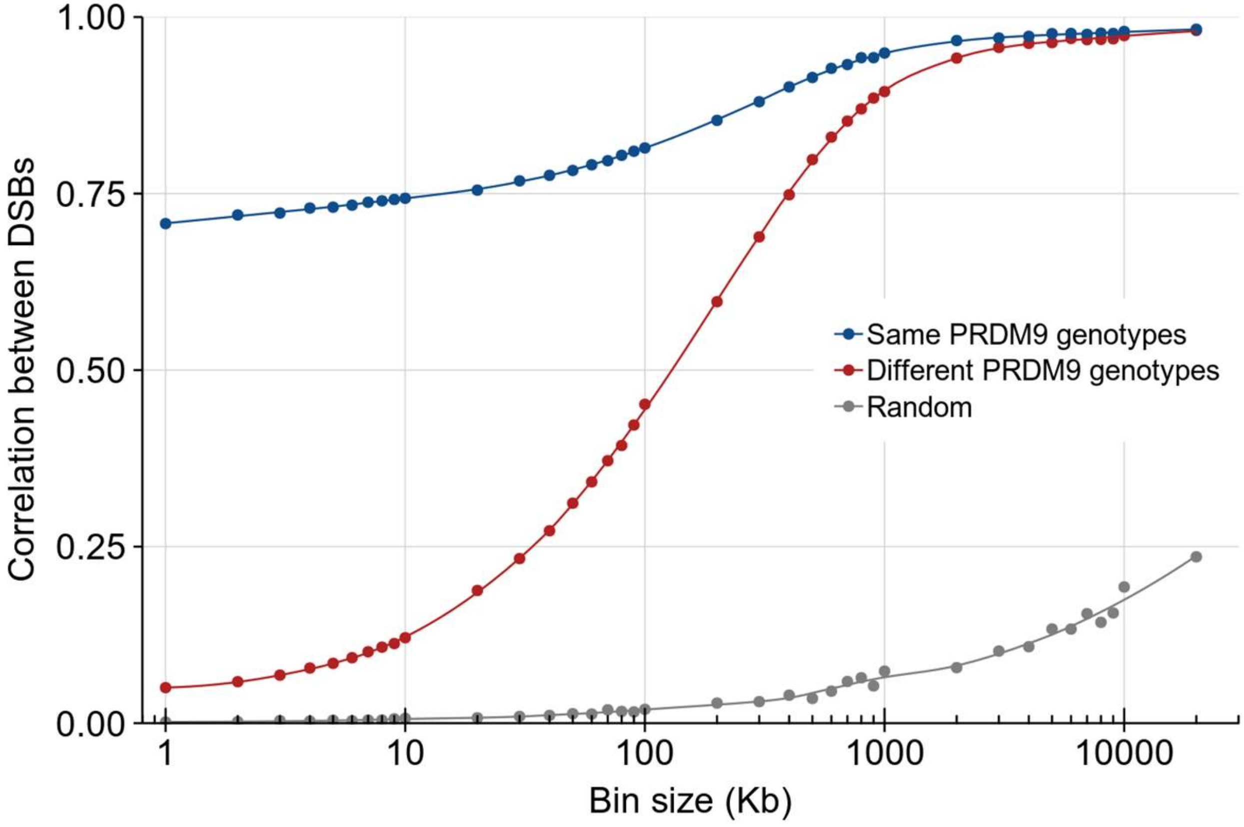
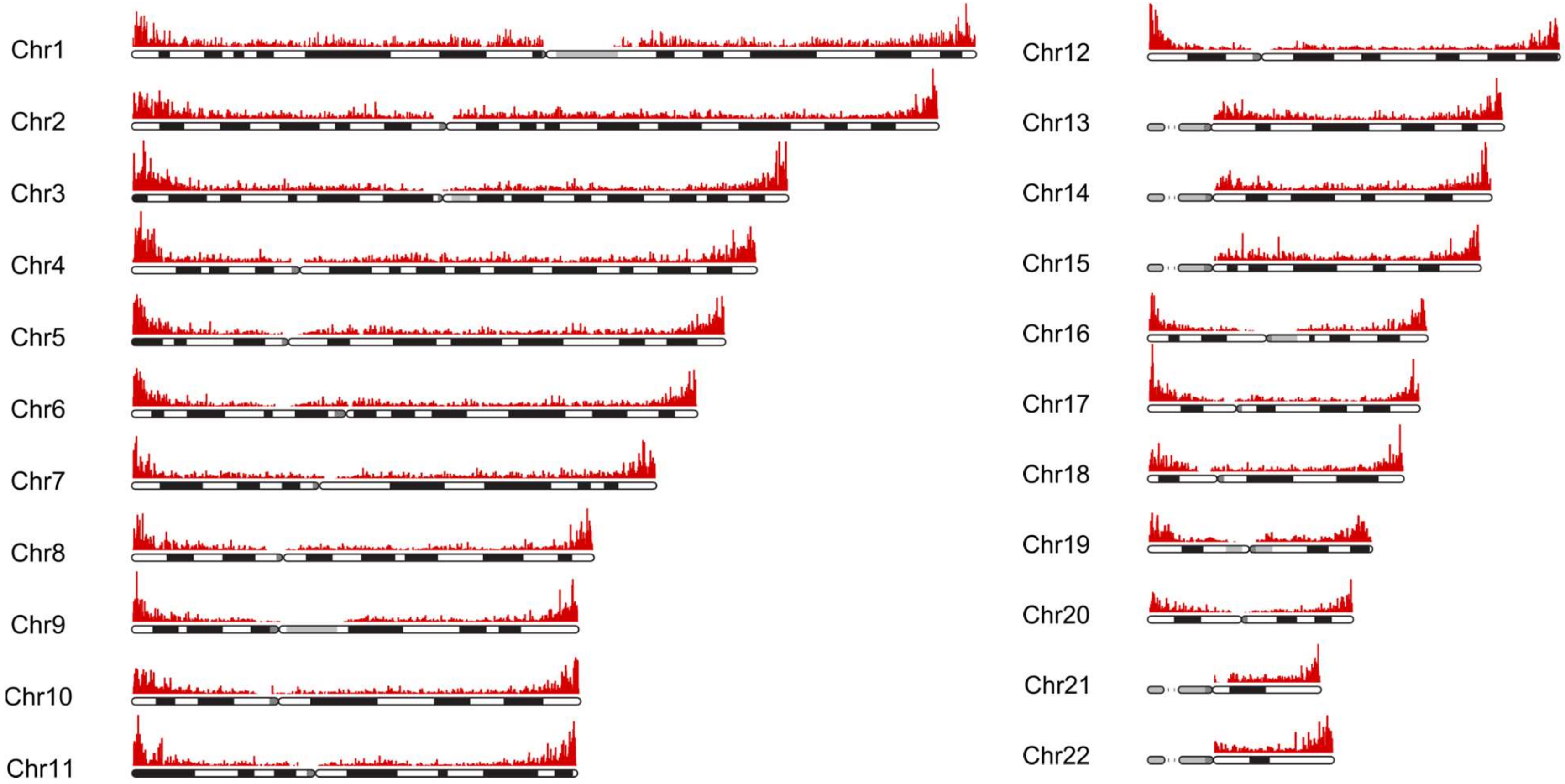




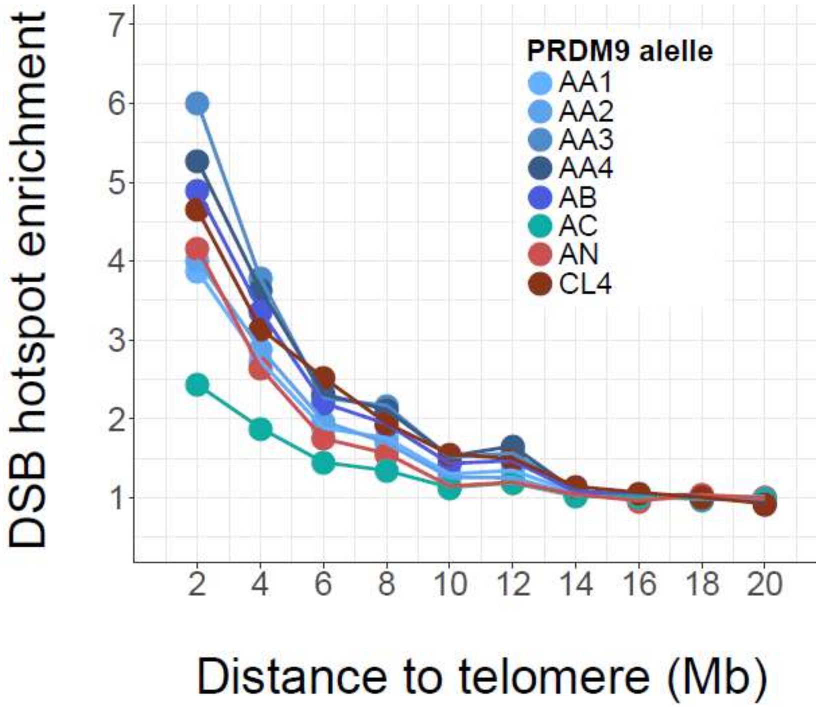
DSBs are enriched at subtelomeric regions



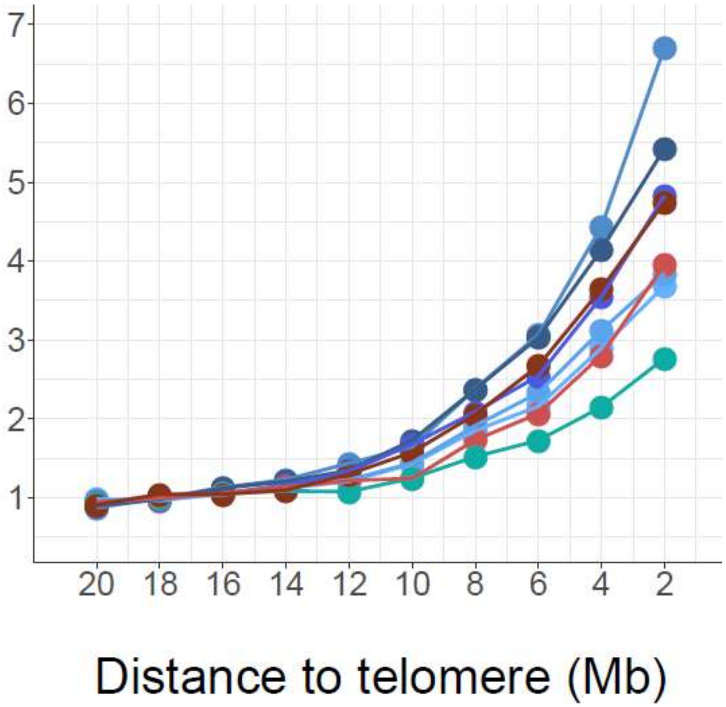
Pratto et al., Science 2014

DSBs are enriched at subtelomeric regions

- independently of the *PRDM9* genotype -

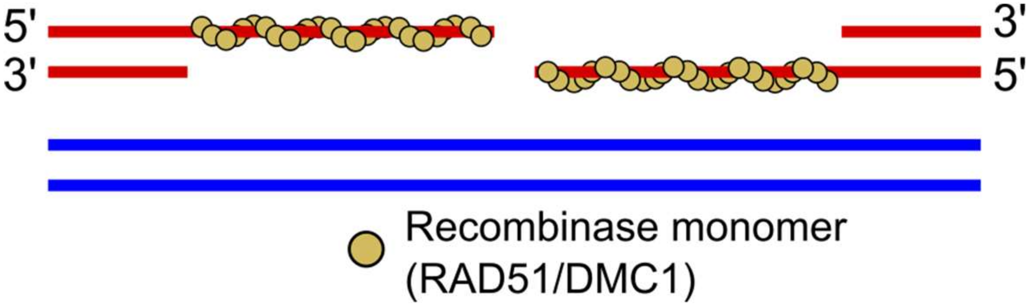


p-arm



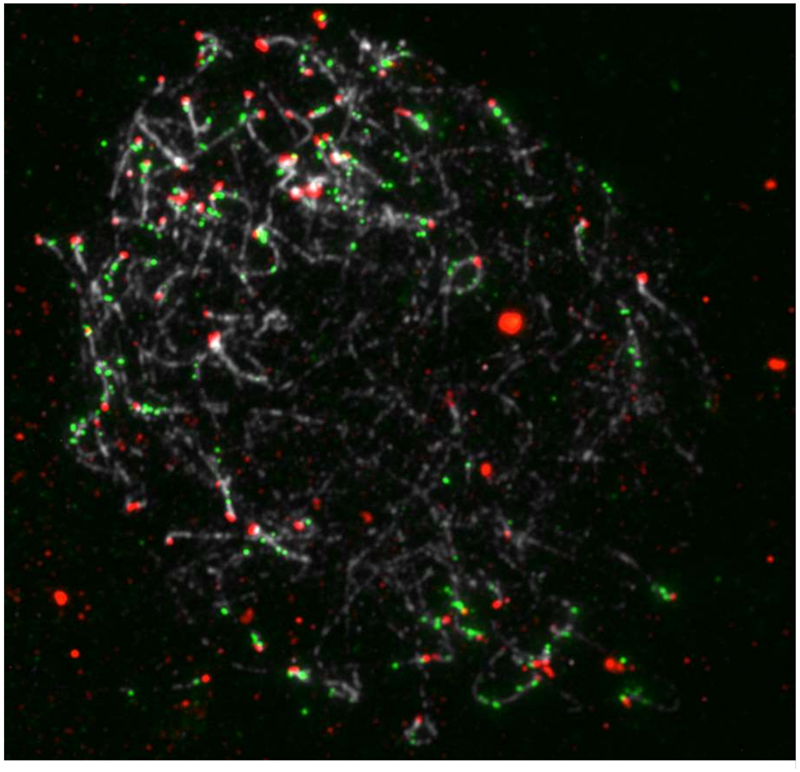
q-arm

DMC1-SSDS signal reflects both DSB frequency and lifespan of the filament



Spatiotemporal bias of DSB formation in human males

Early zygotene



SYCP3 (axis)

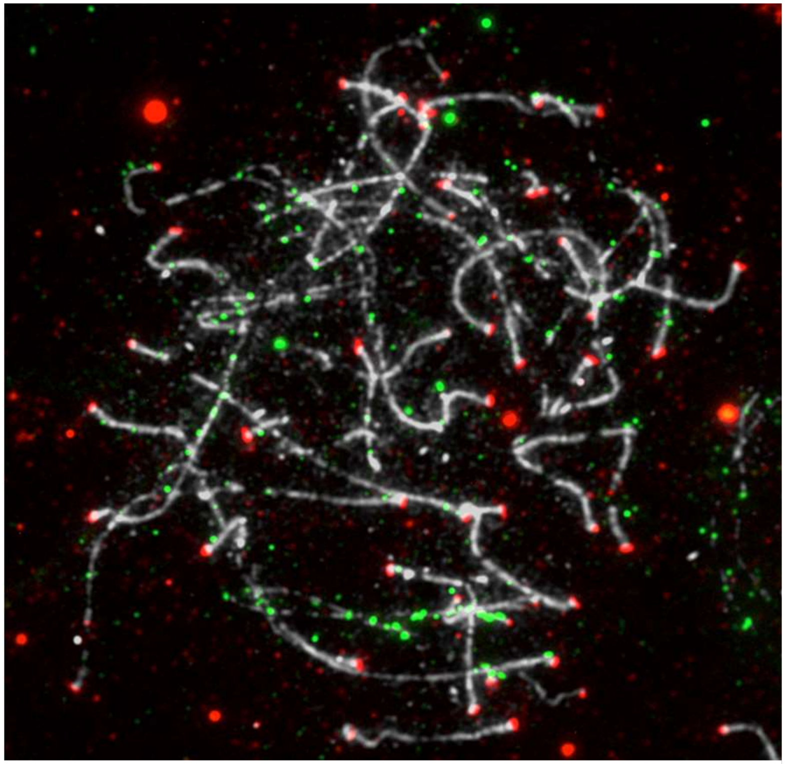
TRF2 (telomeres)

DMC1 (DSBs)

Pratto et al., Science 2014

Spatiotemporal bias of DSB formation in human males

Late zygotene



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TRF2 (telomeres)

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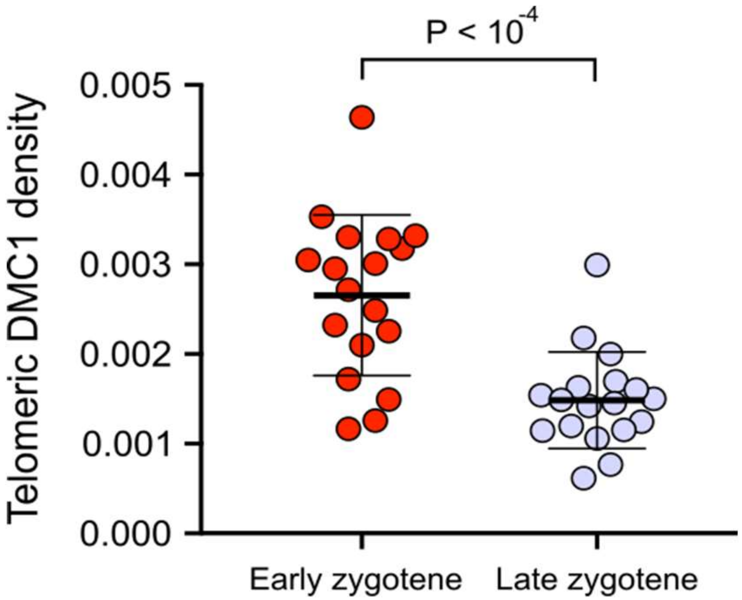
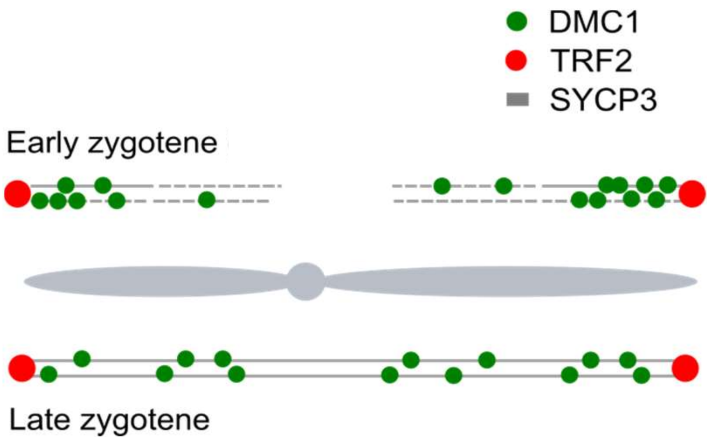
Pratto et al., Science 2014

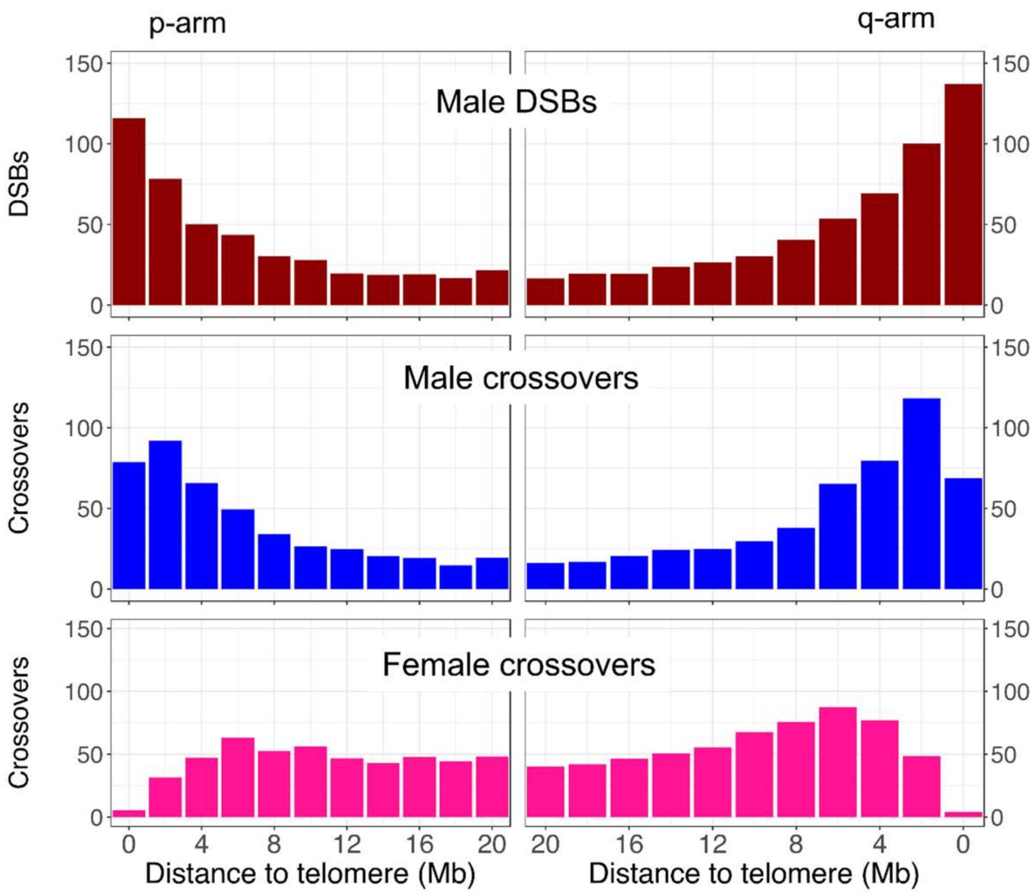
Spatiotemporal bias of DSB formation in human males





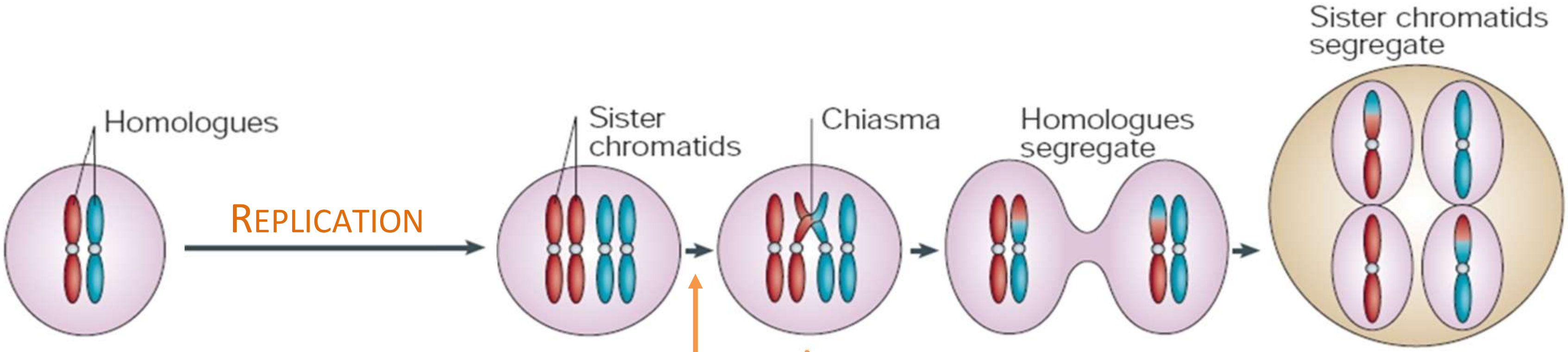
DSB formation close to telomeres in early zygotene shapes the global DSB distribution in our genome-wide maps

DSBs distribution resembles that of male crossovers



DSBs: Pratto et al., Science 2014
Crossovers: Bhérer et al., Nat. Comms. 2016

Meiotic recombination is preceded by replication



REPLICATION

PRDM9, SPO11
DSB FORMATION

CROSSOVERS

Adapted from Marston and Amon, Nat Rev Mol Cell Biol 2005

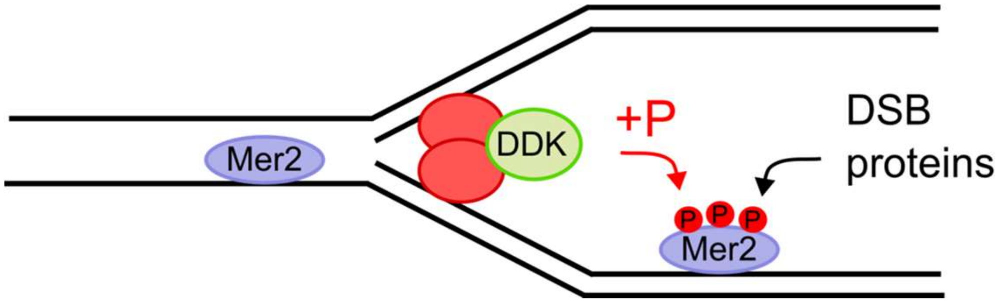
Temporo-spatial coupling of replication and DSB formation in yeast

- In ***S. cerevisiae***, delaying replication in a chromosome segment delays DSB formation by the same margin.

Borde et al., Science 2000

- Recruitment of DSB promoting factors to the replication machinery could confer a head start toward DSB formation.

Murakami and Keeney, Cell 2014



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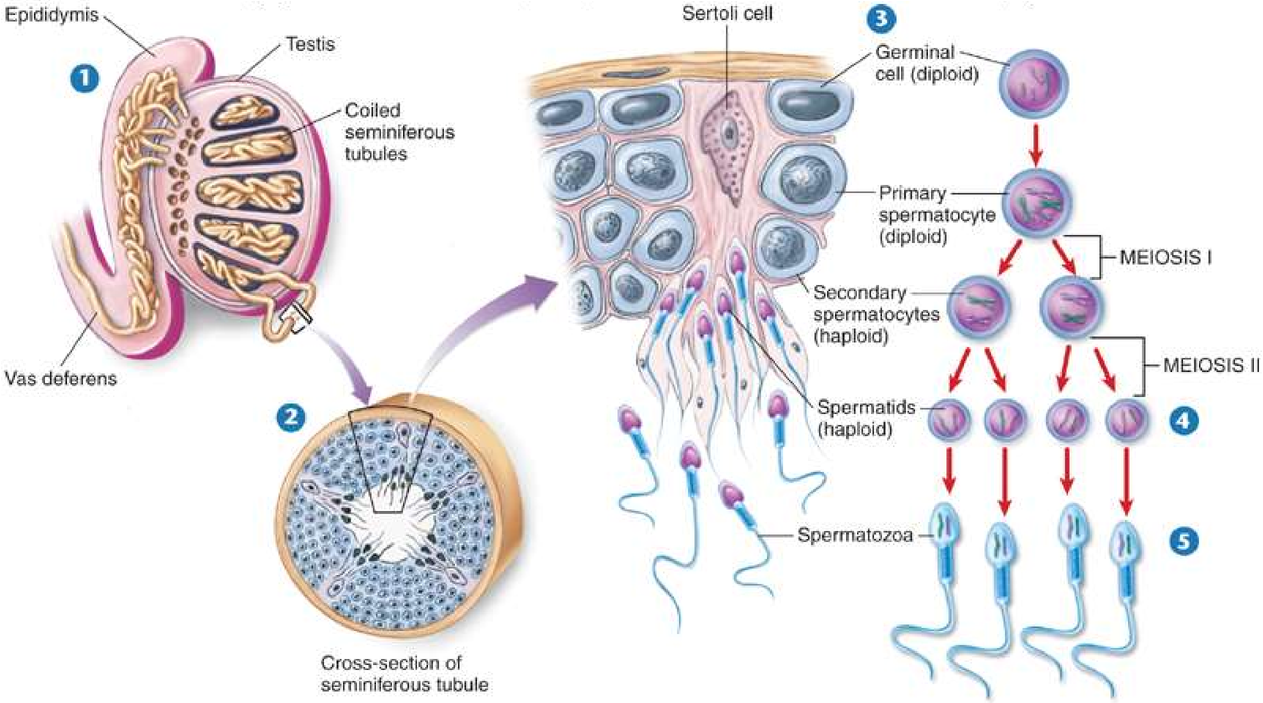
  Borde et al.,, Science 2000
- Recruitment of DSB promoting factors to the replication machinery could confer a head start toward DSB formation.
  Murakami and Keeney, Cell 2014
- In ***S. pombe*** and **barley** changes in replication timing are mirrored by changes in DSBs and crossover distribution

Higgins et al., Plant Cell 2012
Wu and Nurse, Mol Cell 2014

Mammalian meiosis cannot be studied *in vitro*

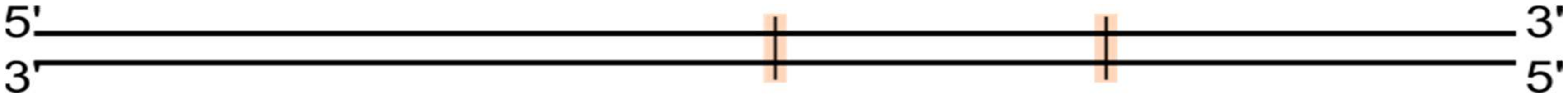


Only 1% of cells are undergoing meiotic replication

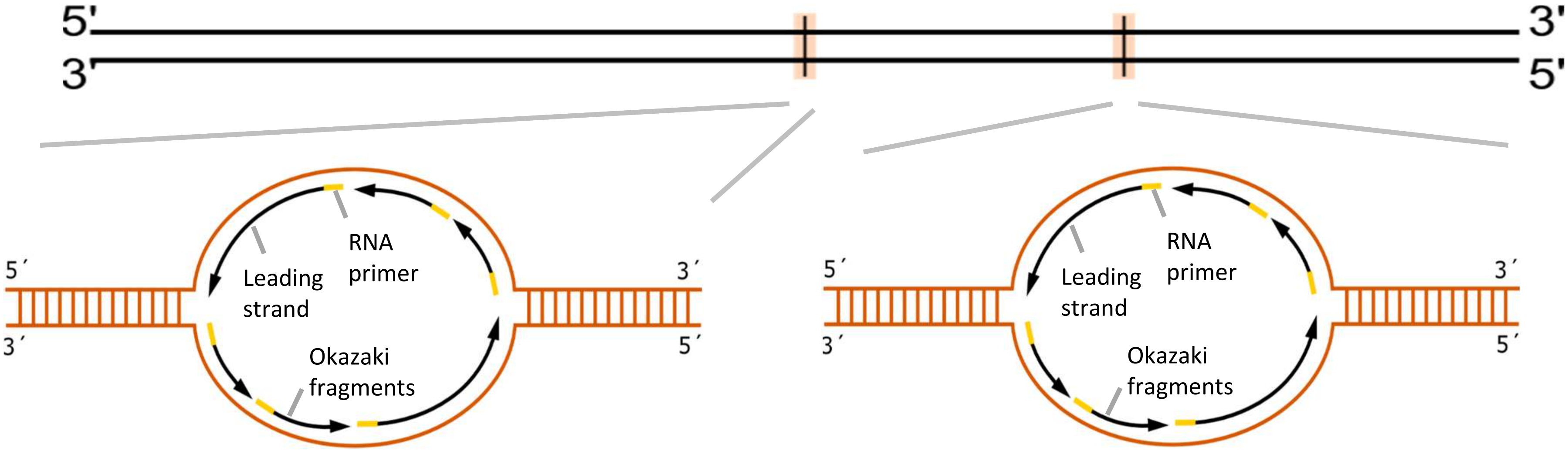
Tripartite approach to study meiotic replication

- Develop method to map origins of replication from tissue
- Quantify replication timing (RT) genome-wide in meiotic S-phase
- Integrate origins and RT into *in silico* model

Replication initiates at origins



Replication origins can be identified by leading strand capture



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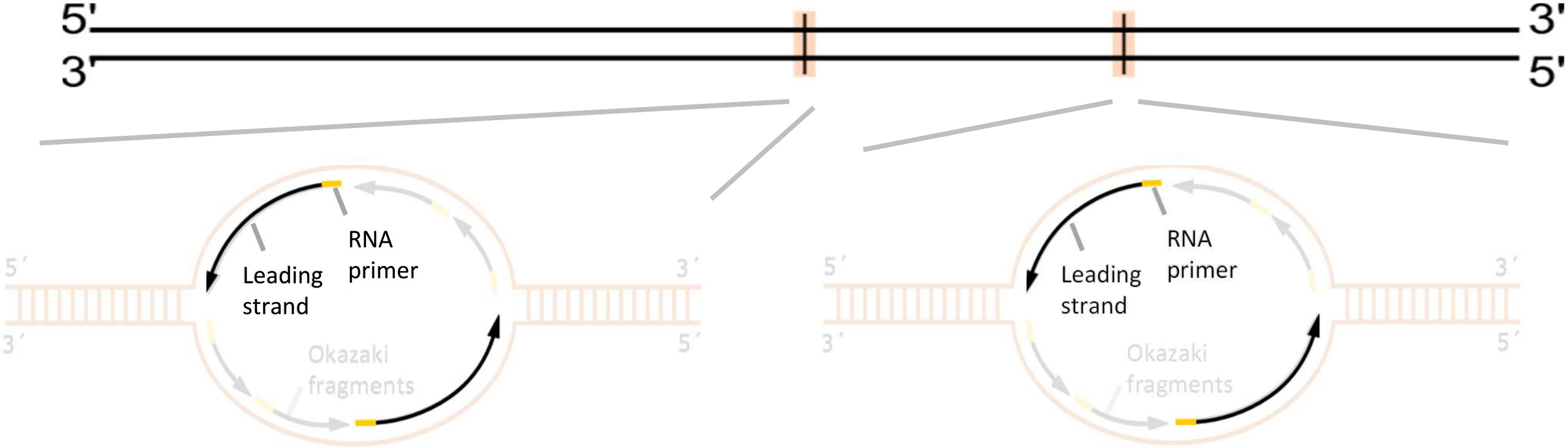
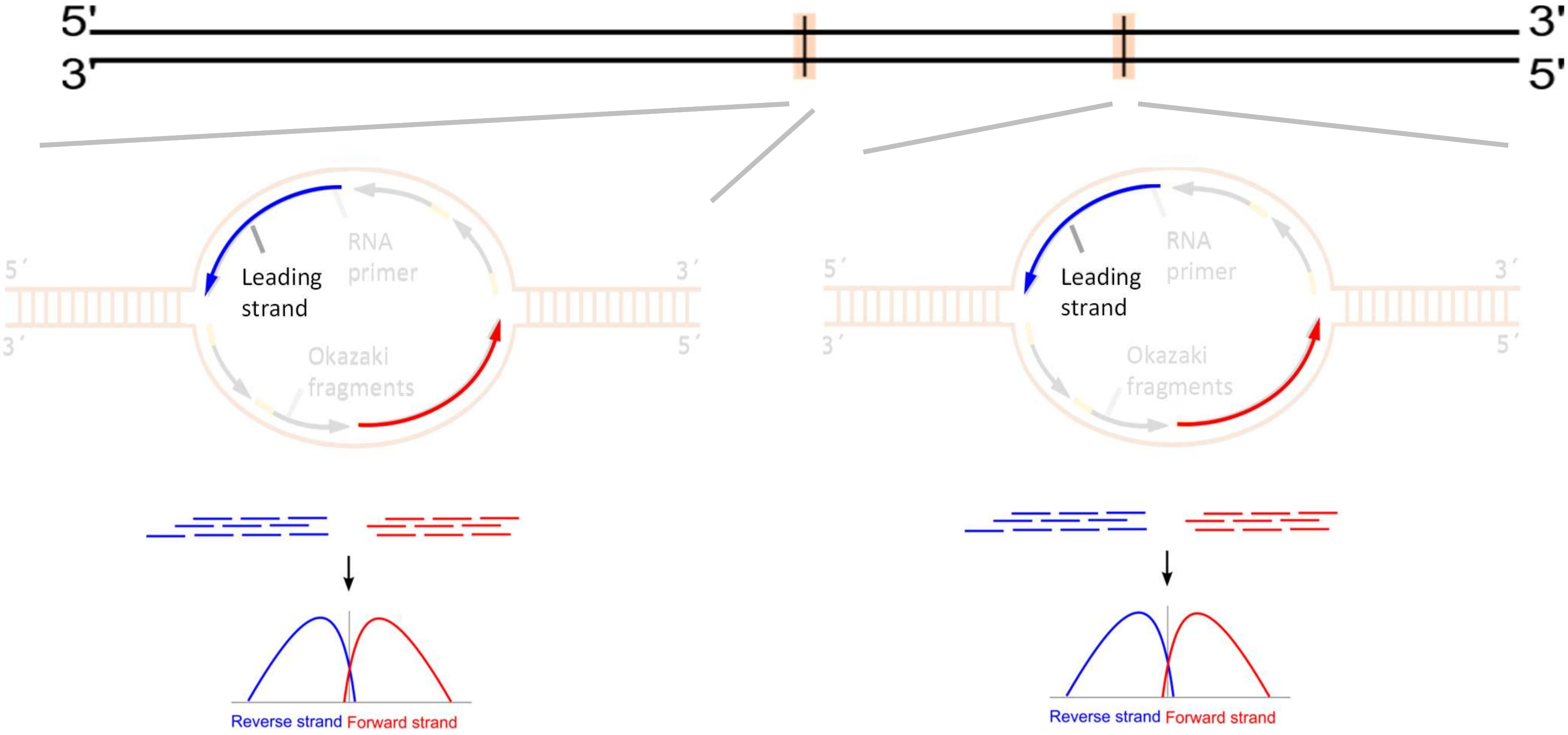
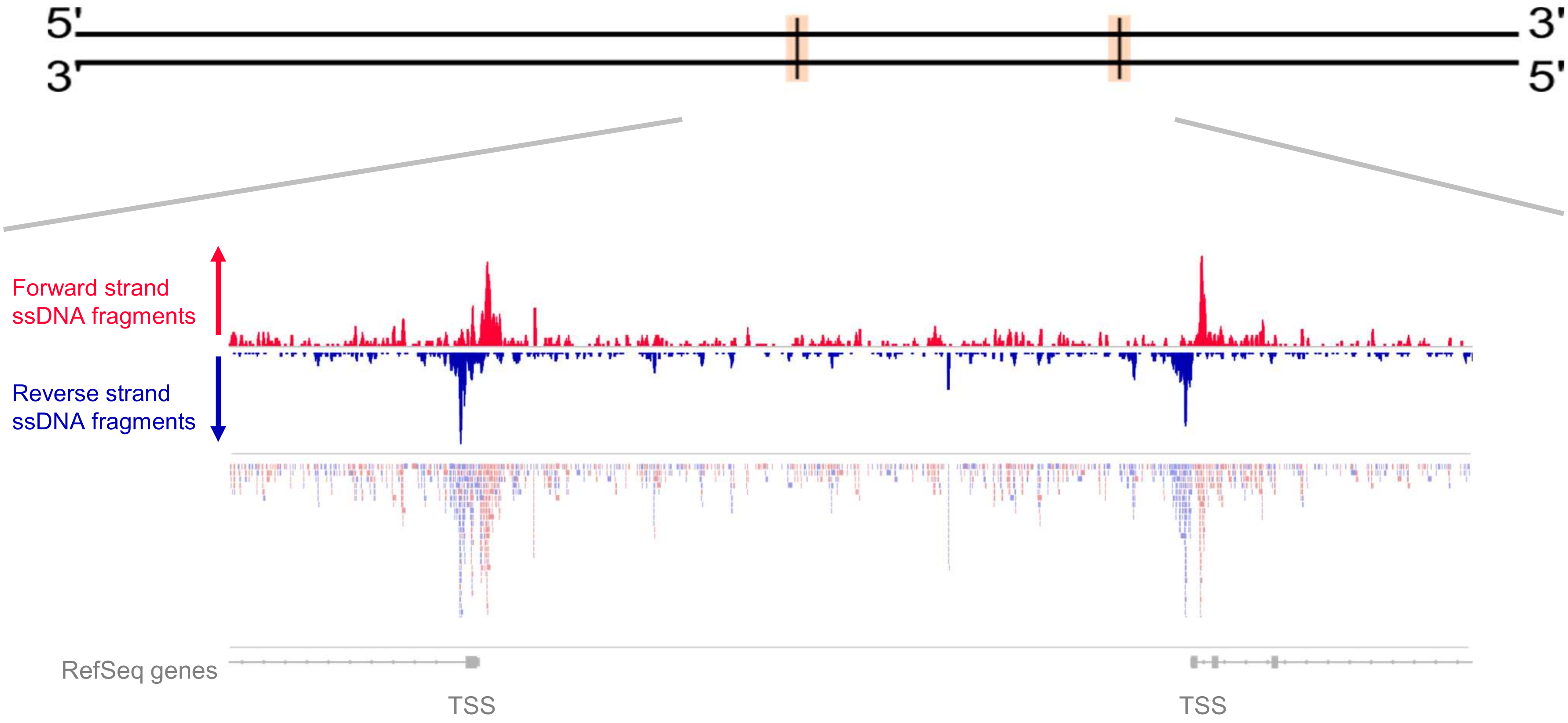


Leading strand capture with ssDNA-Seq

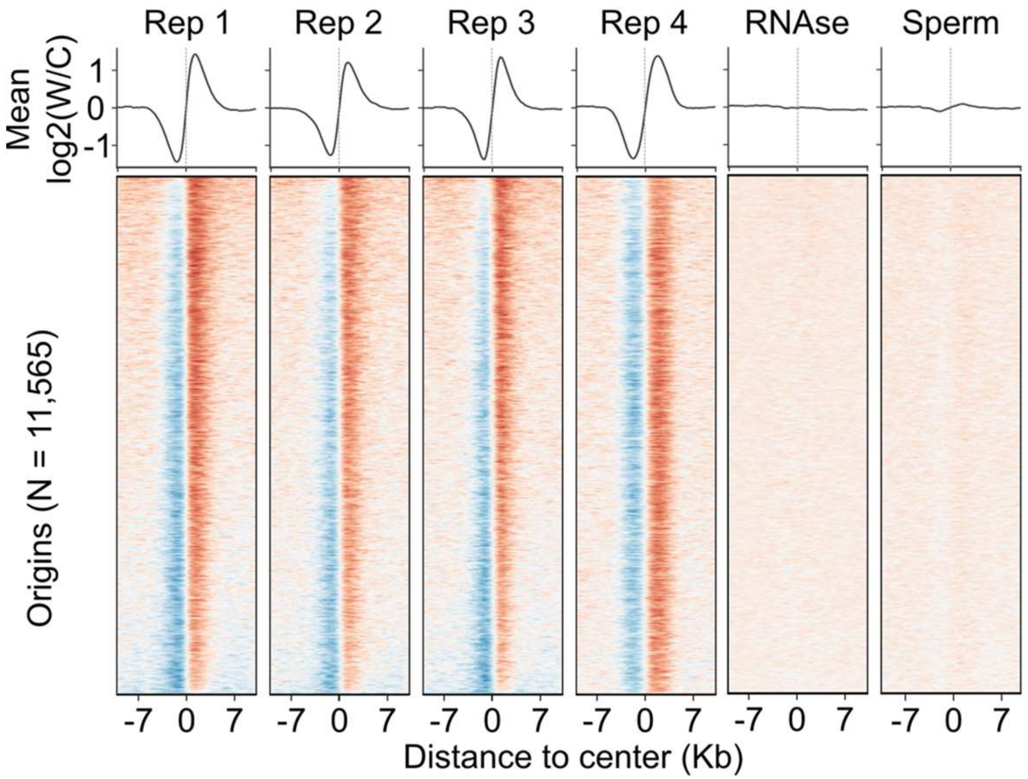
- greatly increases accuracy of origin mapping -

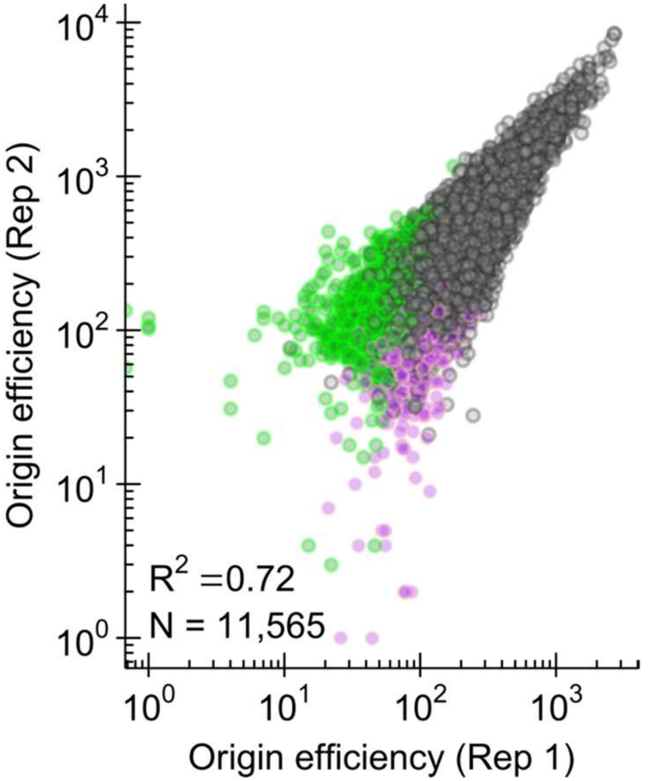


Characteristic ssDNA asymmetry at origins



Reproducible leading strand asymmetry at ~11,500 origins





Determining replication timing in meiosis

Isolate DNA from
S-phase
meiocytes
(2C - 4C)

Lam et al., Nat Comm 2019

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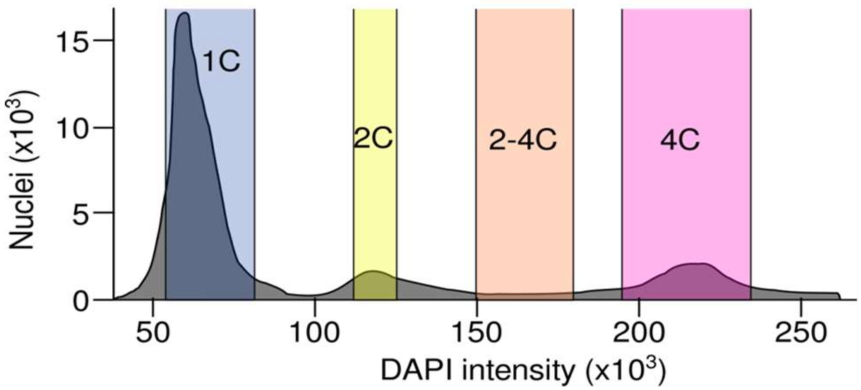
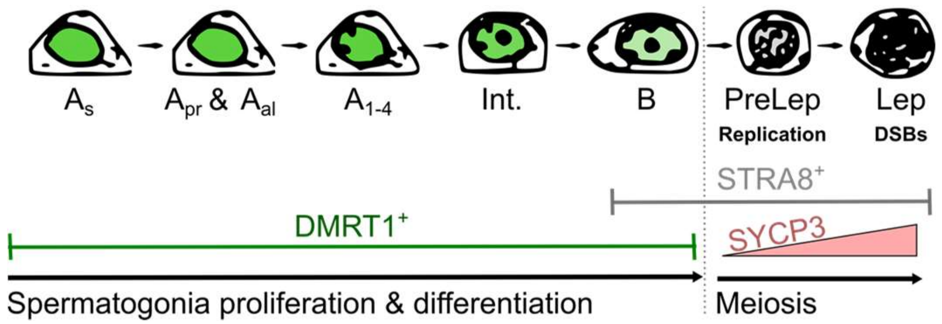
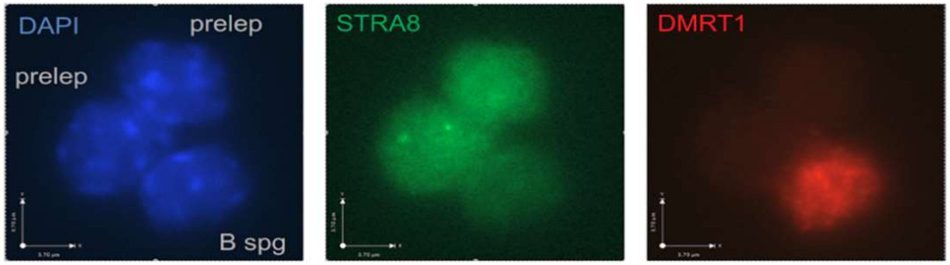


Isolation of Meiotic S-phase nuclei

Isolate DNA from S-phase meiocytes (2C - 4C)

Lam et al., Nat Comm 2019





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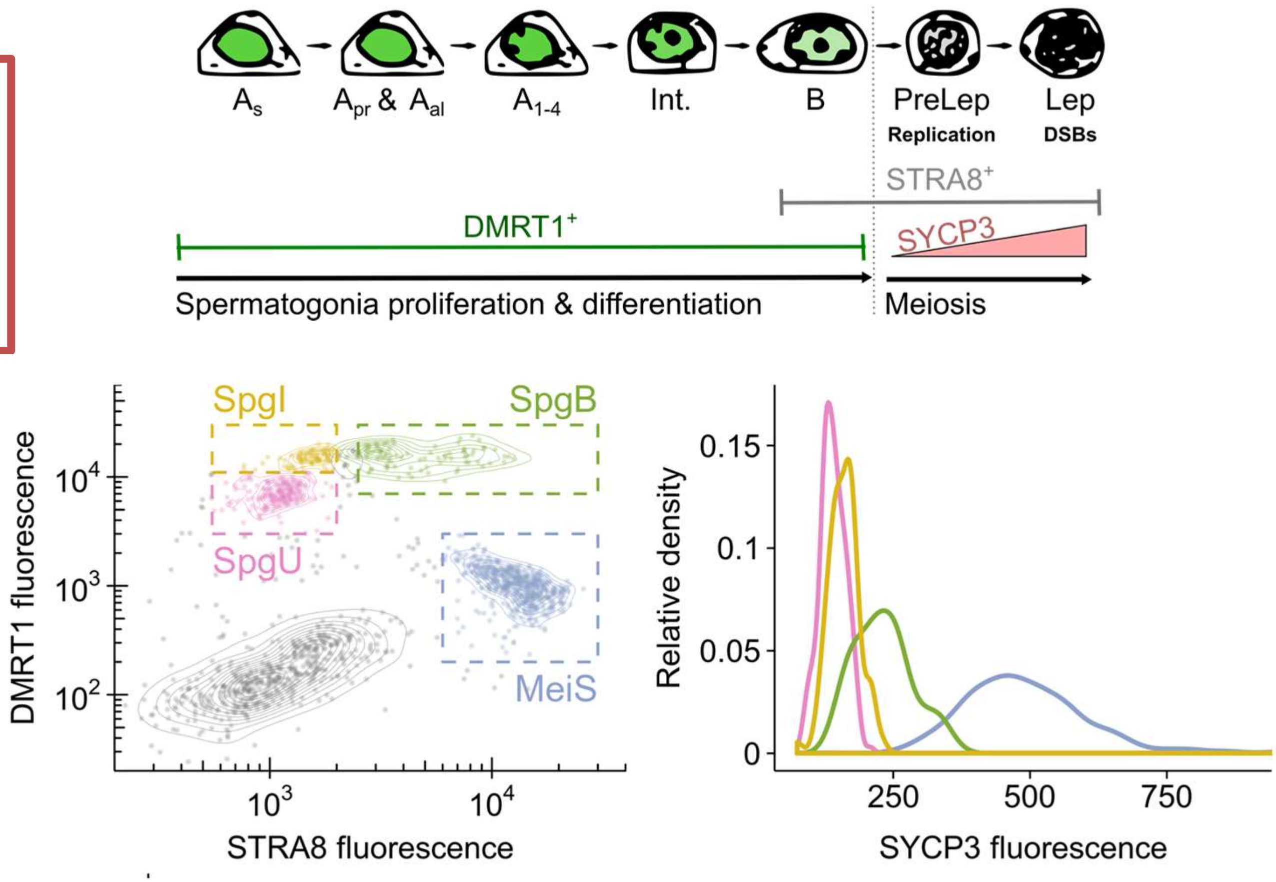
Lam et al., Nat Comm 2019



Determining replication timing in meiosis

Isolate DNA from
S-phase
meiocytes
(2C - 4C)

→

Whole genome
sequencing

Lam et al., Nat Comm 2019

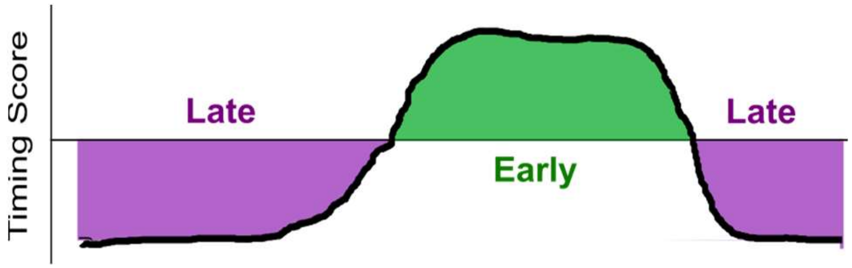
Determining replication timing in meiosis

Isolate DNA from S-phase meiocytes (2C - 4C) ➡ Whole genome sequencing ➡ Replication timing inference from WGS data

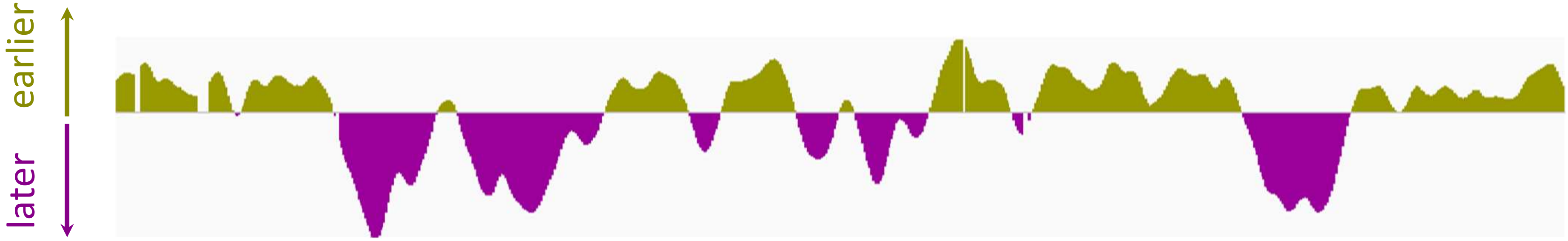
Lam et al., Nat Comm 2019

Koren et al, Cell 2014

For a population of S phase cells, DNA of early-replicating regions is overrepresented relative to later regions



RT-Seq reveals early and late replicating regions



Replication Timing on mouse chr19

Modelling DNA replication

Start point: *11,000+ origins of replication from whole testis* → ***Target:*** *Replication Timing profiles*

Search for combination of parameters that give the best fit to the experimental data

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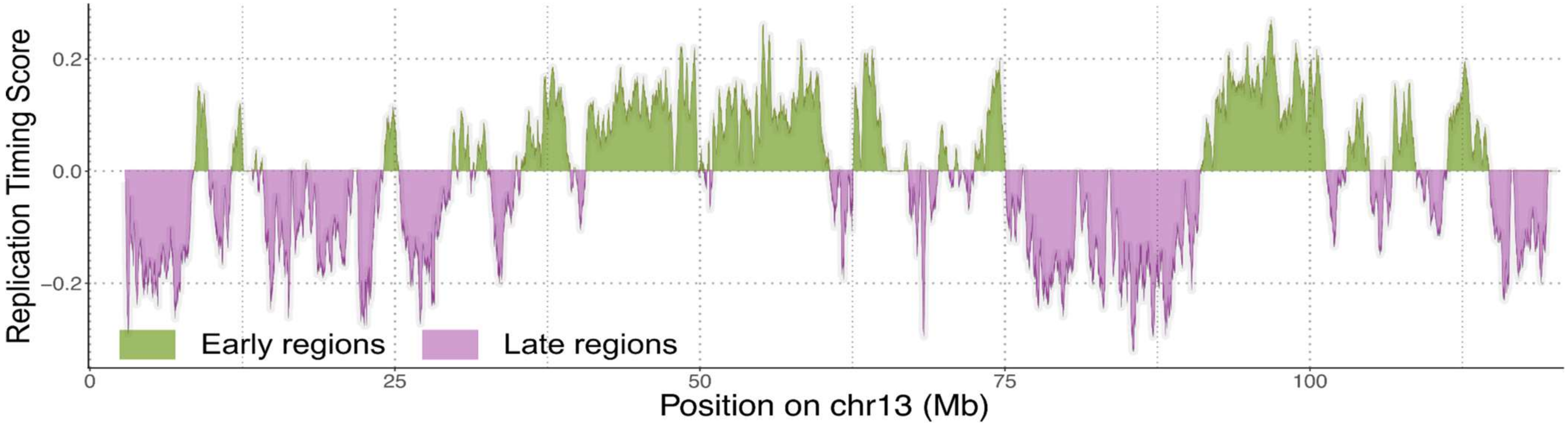
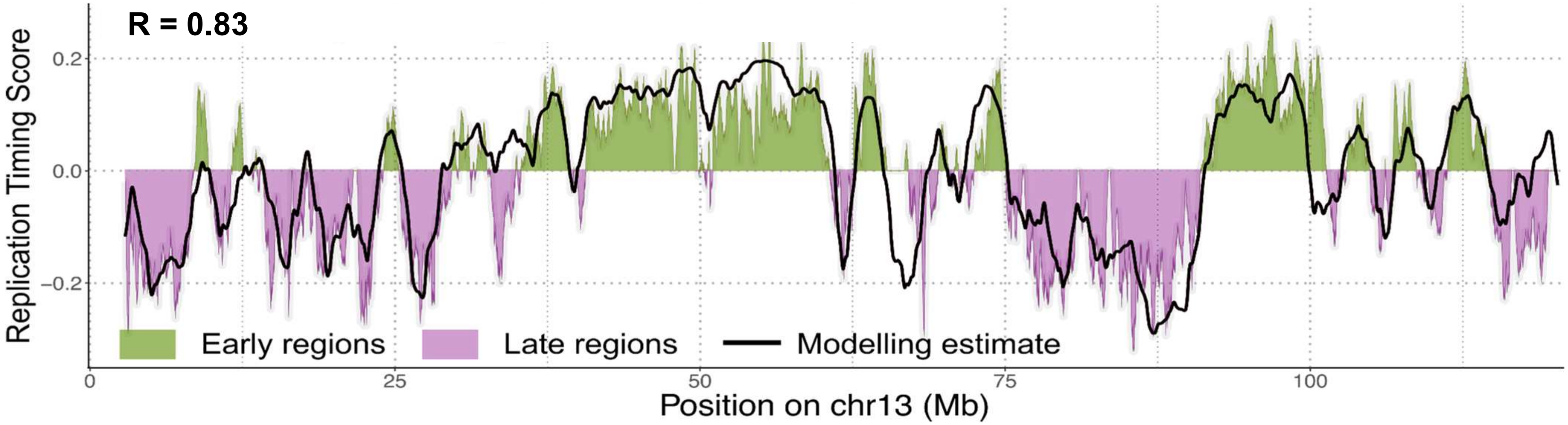


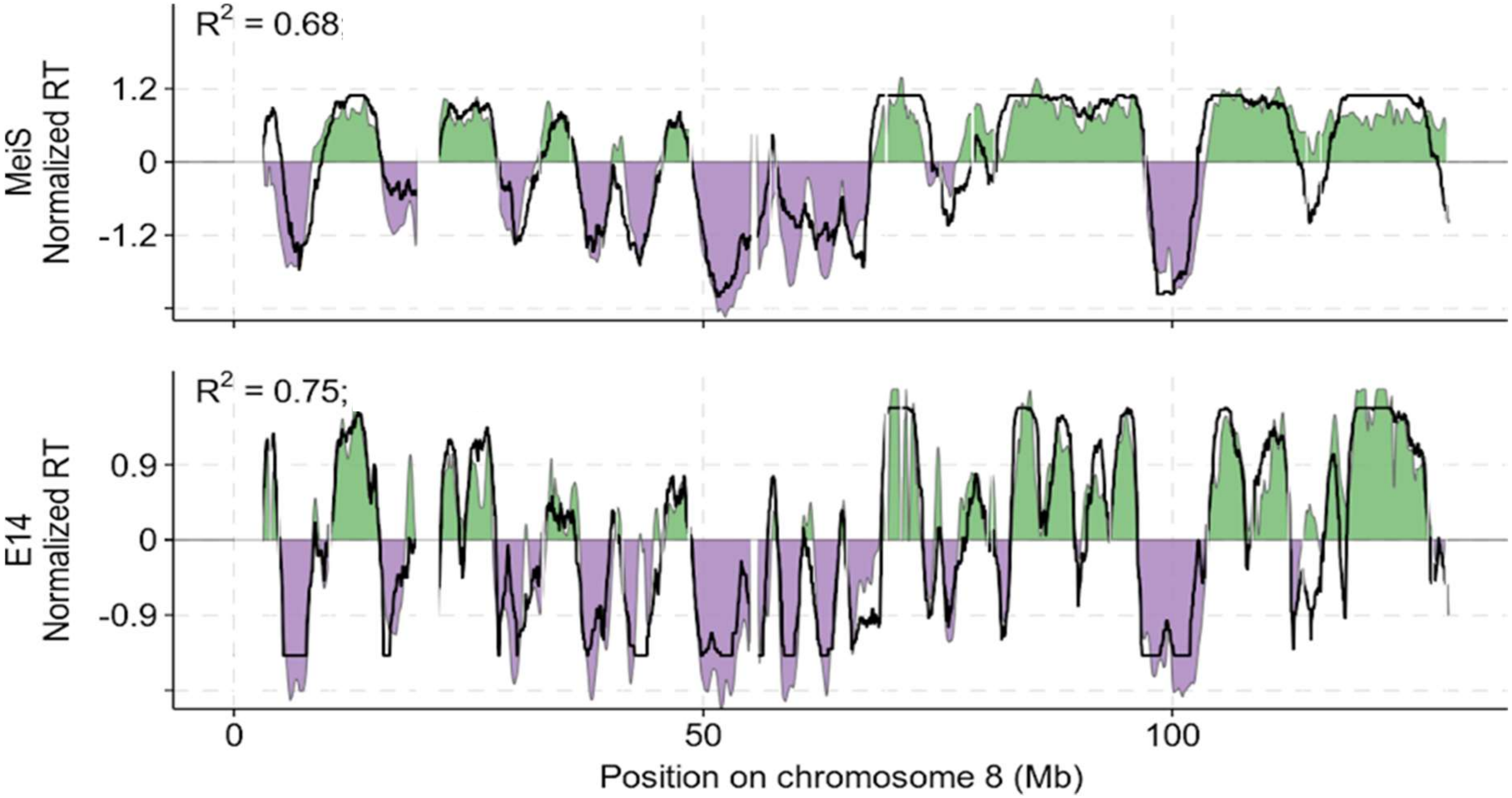
In silico modelling recapitulates replication timing

Start point: *11,000+ origins of replication from whole testis* → ***Target:*** *Replication Timing profiles*

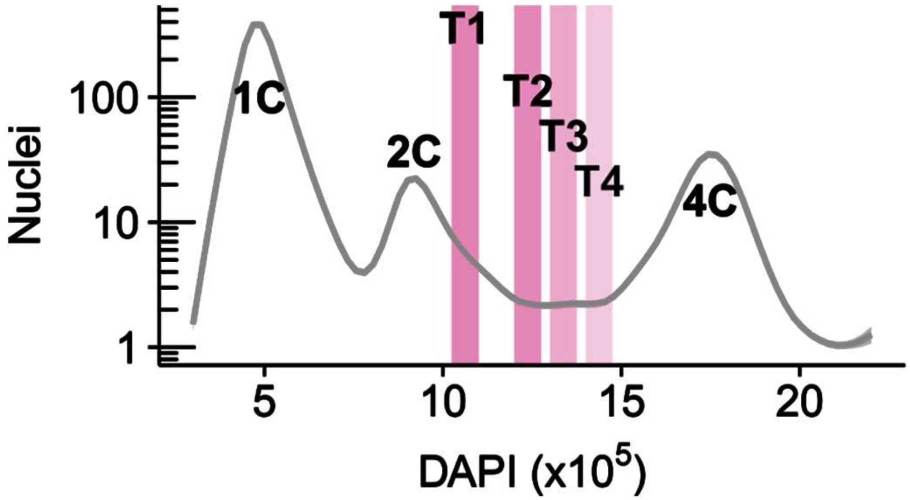
Search for combination of parameters that give the best fit to the experimental data

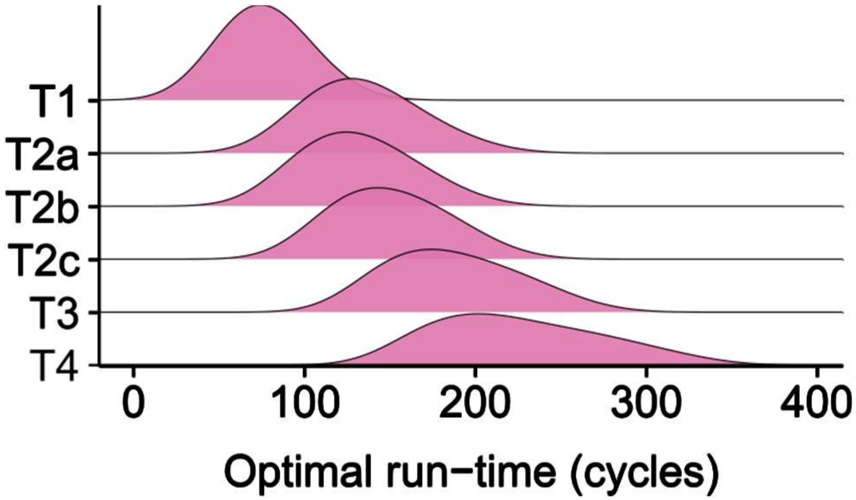


Origins from testis can model RT in other cell types

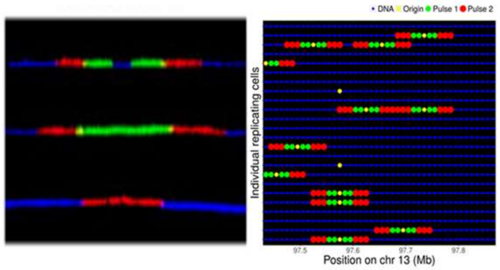


Modelling yields meaningful properties of replication





Model estimates agree with other data



DNA combing

Inter-origin distances

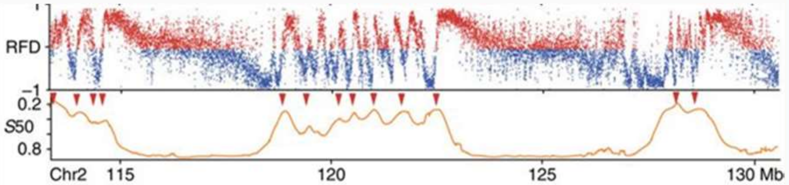
RT-Sim: ~100-200 Kbp

Real fibers: ~100-150 Kbp

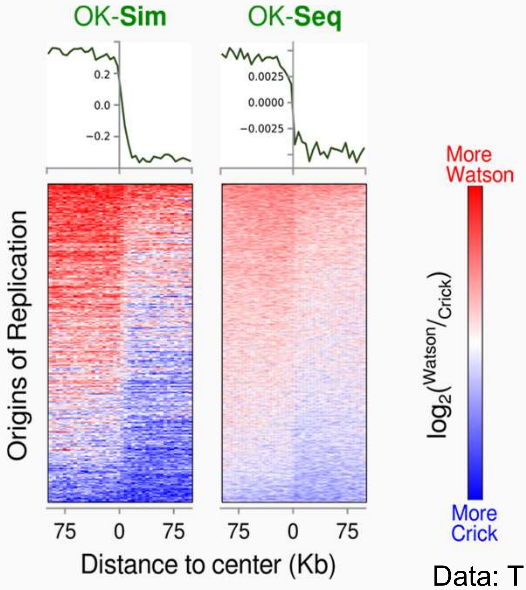
Techer et al., J. Mol Biol. 2013

Okazaki-fragment sequencing

Genomic replication patterning

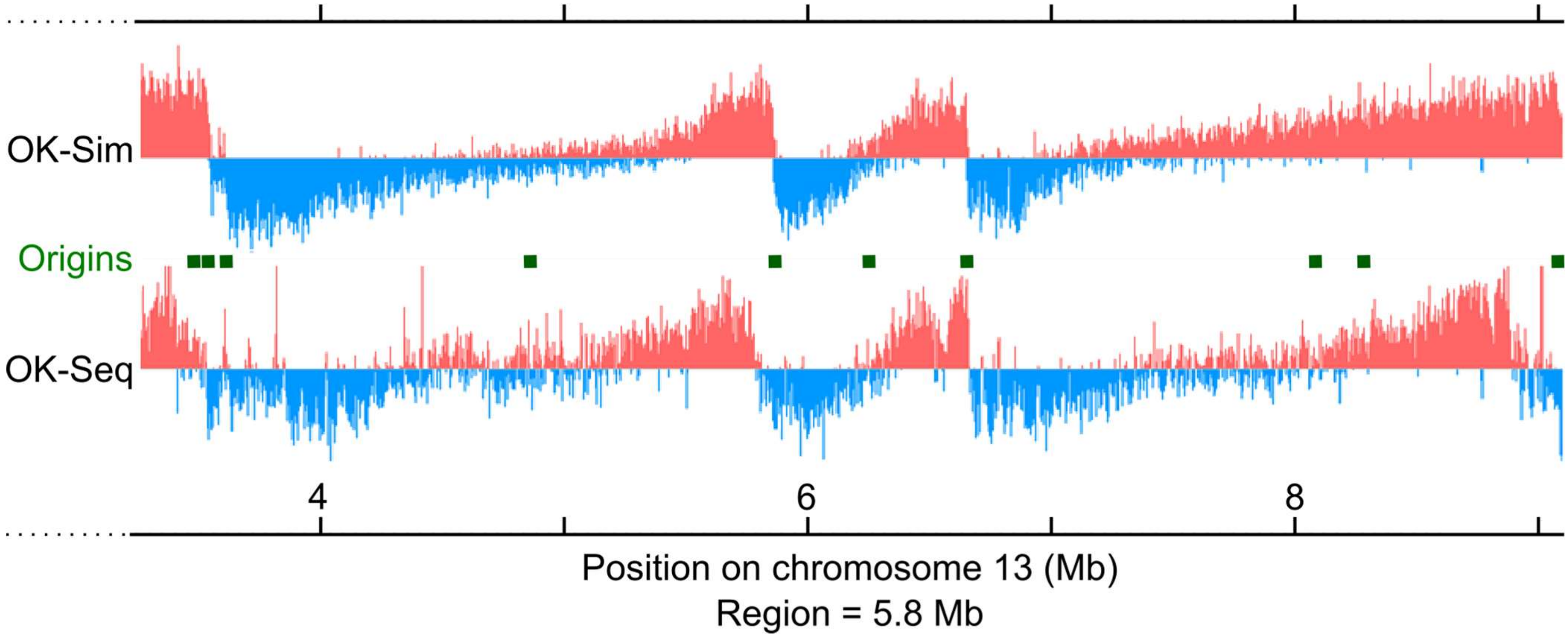


Petryk et al., Nat. Comm. 2016

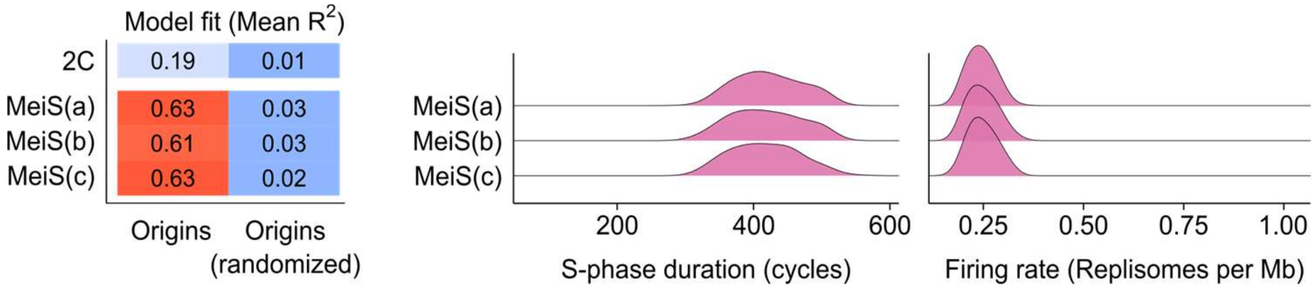


Data: Tubbs et al., Cell 2018

Simulated Okazaki-sequencing coverage



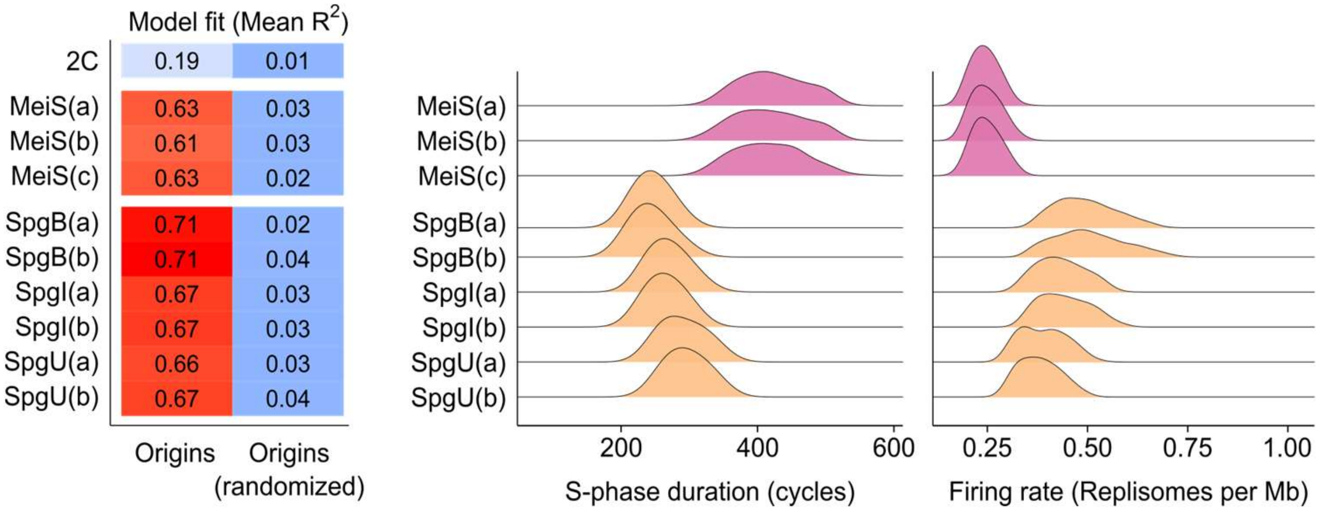
Modelling yields consistent results across meiotic replicates



Model fit (Mean R^2)

	Origins	Origins (randomized)
2C	0.19	0.01
MeiS(a)	0.63	0.03
MeiS(b)	0.61	0.03
MeiS(c)	0.63	0.02

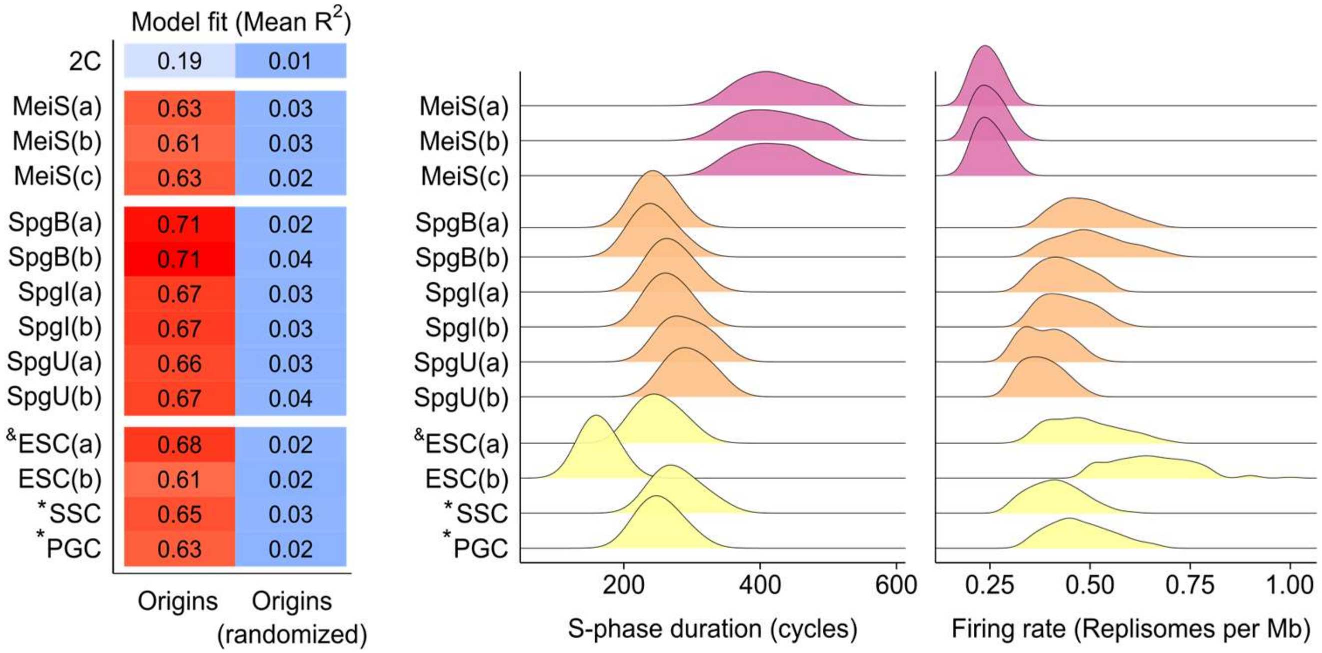
Meiotic replication is notably slower than in pre-meiotic germ cells



Model fit (Mean R^2)

	Origins	Origins (randomized)
2C	0.19	0.01
MeiS(a)	0.63	0.03
MeiS(b)	0.61	0.03
MeiS(c)	0.63	0.02
SpgB(a)	0.71	0.02
SpgB(b)	0.71	0.04
SpgI(a)	0.67	0.03
SpgI(b)	0.67	0.03
SpgU(a)	0.66	0.03
SpgU(b)	0.67	0.04

Meiotic replication is notably slower than in all germ cells



Model fit (Mean R^2)

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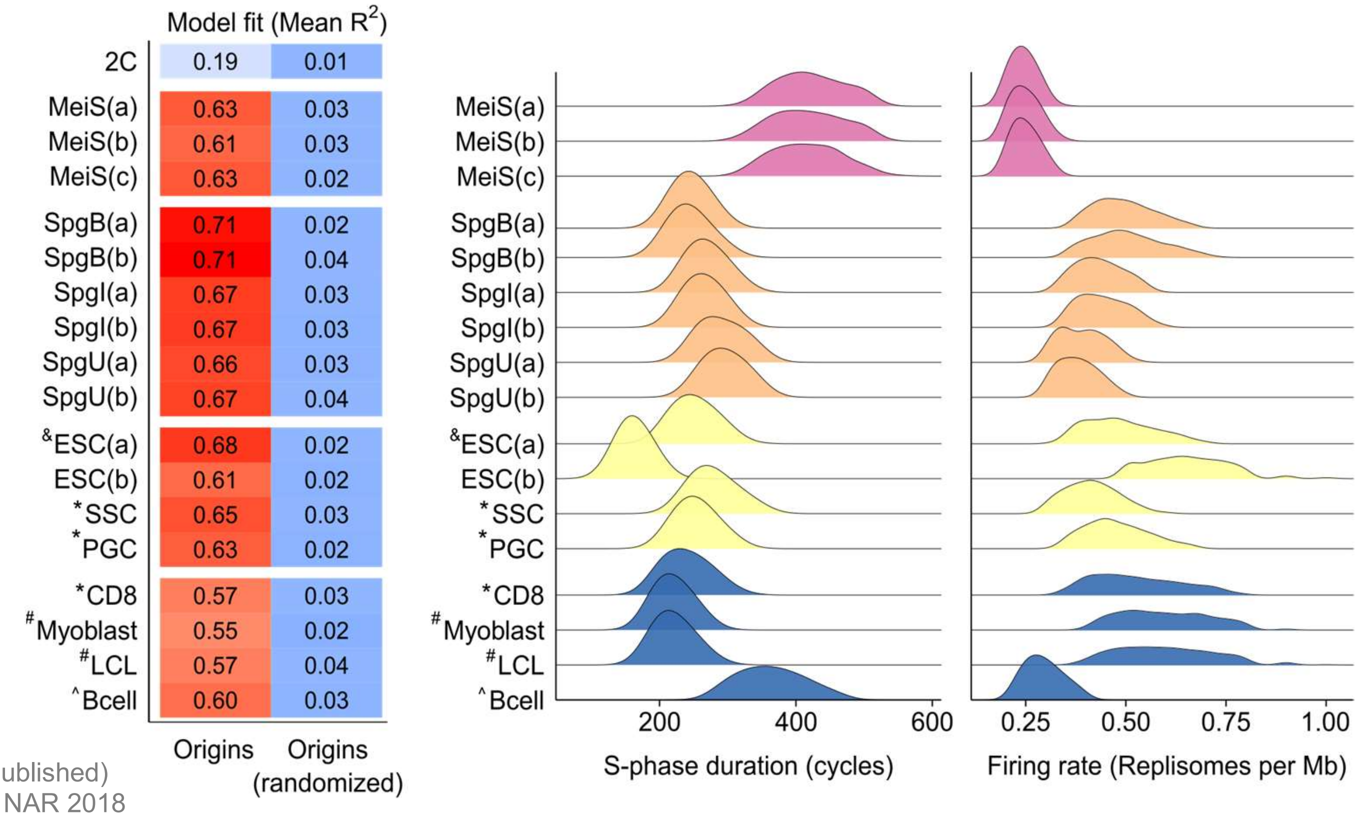
SpgB(a)	0.71	0.02
SpgB(b)	0.71	0.04
SpgI(a)	0.67	0.03
SpgI(b)	0.67	0.03
SpgU(a)	0.66	0.03
SpgU(b)	0.67	0.04
&ESC(a)	0.68	0.02
ESC(b)	0.61	0.02
*SSC	0.65	0.03
*PGC	0.63	0.02

& Dey et al (unpublished)
* Yehuda et al., NAR 2018

Activated B-cell replication is as slow as meiotic replication



Model fit (Mean R^2)

	Origins	Origins (randomized)
2C	0.19	0.01
MeiS(a)	0.63	0.03
MeiS(b)	0.61	0.03
MeiS(c)	0.63	0.02
SpgB(a)	0.71	0.02
SpgB(b)	0.71	0.04
SpgI(a)	0.67	0.03
SpgI(b)	0.67	0.03
SpgU(a)	0.66	0.03
SpgU(b)	0.67	0.04
[&]ESC(a)	0.68	0.02
ESC(b)	0.61	0.02
[*]SSC	0.65	0.03
[*]PGC	0.63	0.02
[*]CD8	0.57	0.03
[#]Myoblast	0.55	0.02
[#]LCL	0.57	0.04
[^]Bcell	0.60	0.03

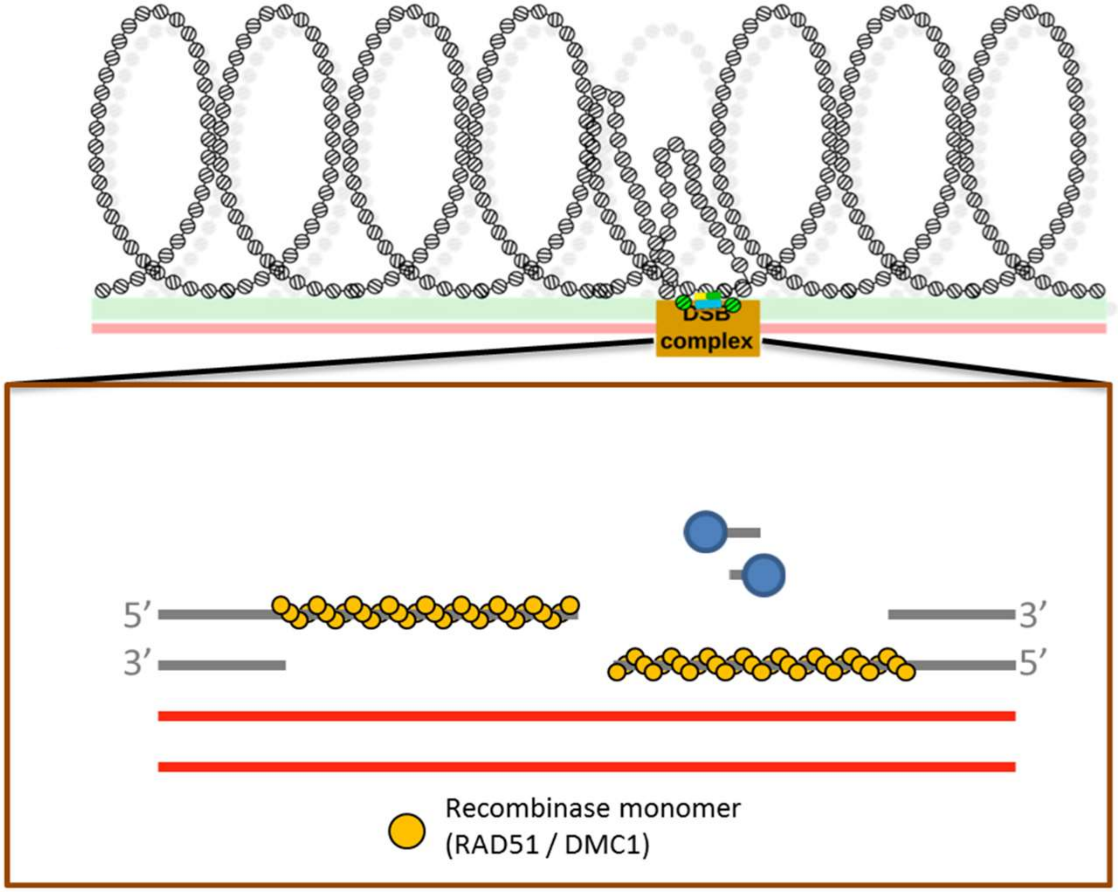
[&] Dey et al (unpublished)

[*] Yehuda et al., NAR 2018

[#] Replication Domain DB

[^] Tubbs et al., Cell 2018

Measures of hotspot usage / activity

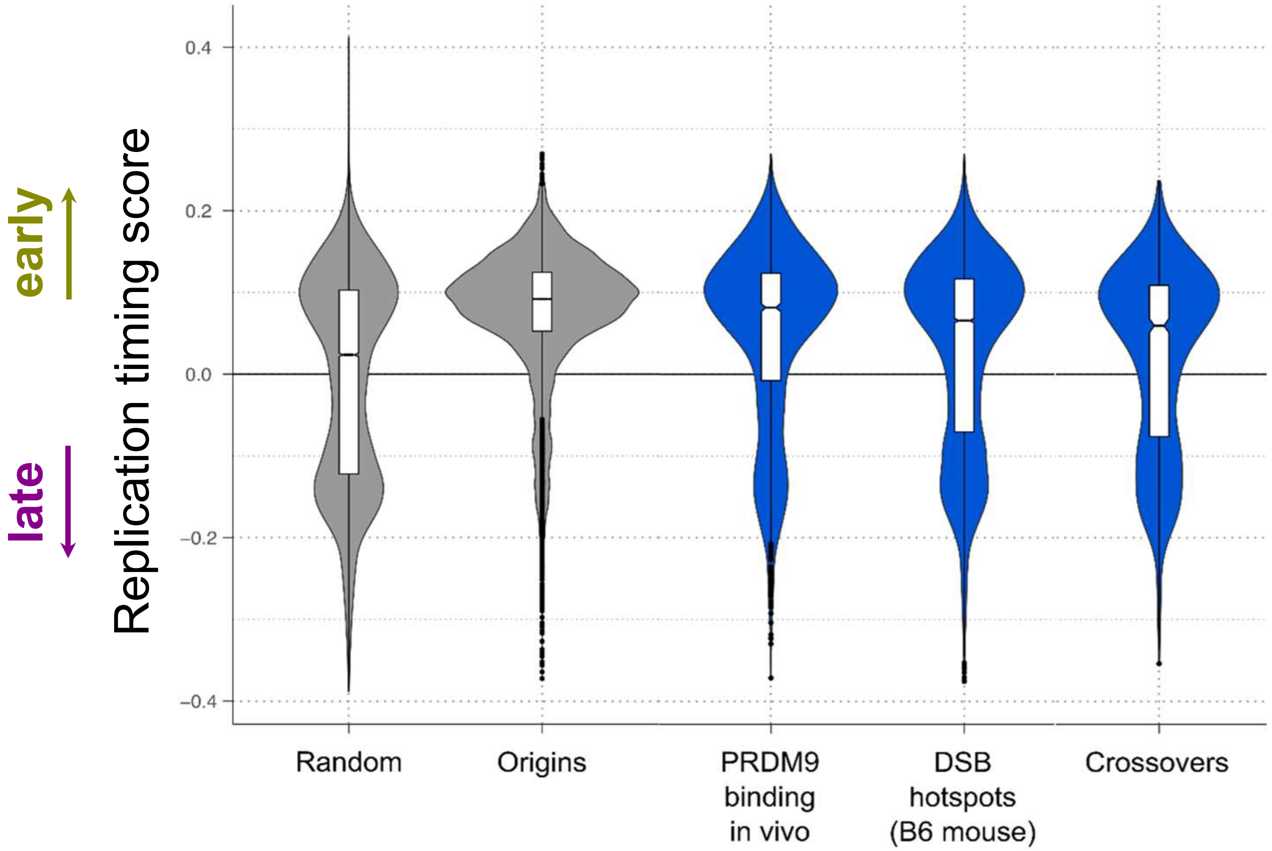


Frequency of PRDM9 binding
PRDM9 ChIP-Seq
H3K4me3 ChIP-Seq
(Grey et al., Genome Res 2017)

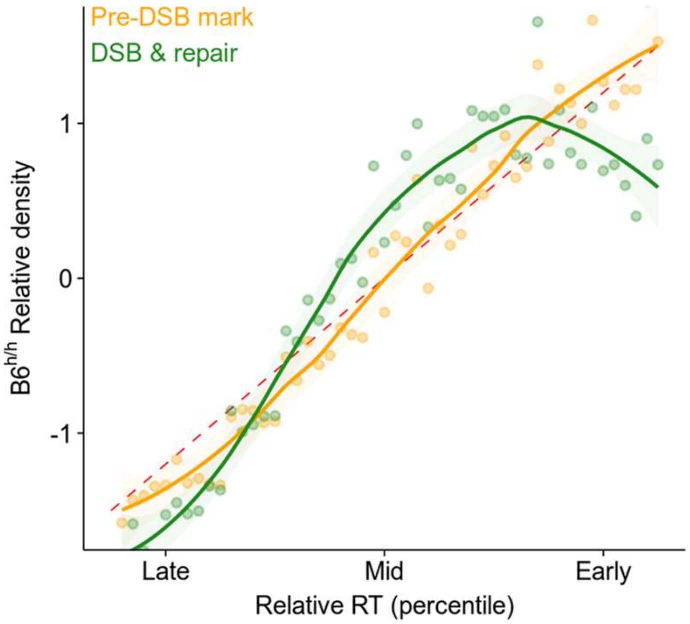
Frequency of "cutting":
SPO11-oligo
(Lange et al., Cell 2016)

Frequency of DSBs formation and repair:
DMC1-SSDS

Elevated recombination in early replicating regions



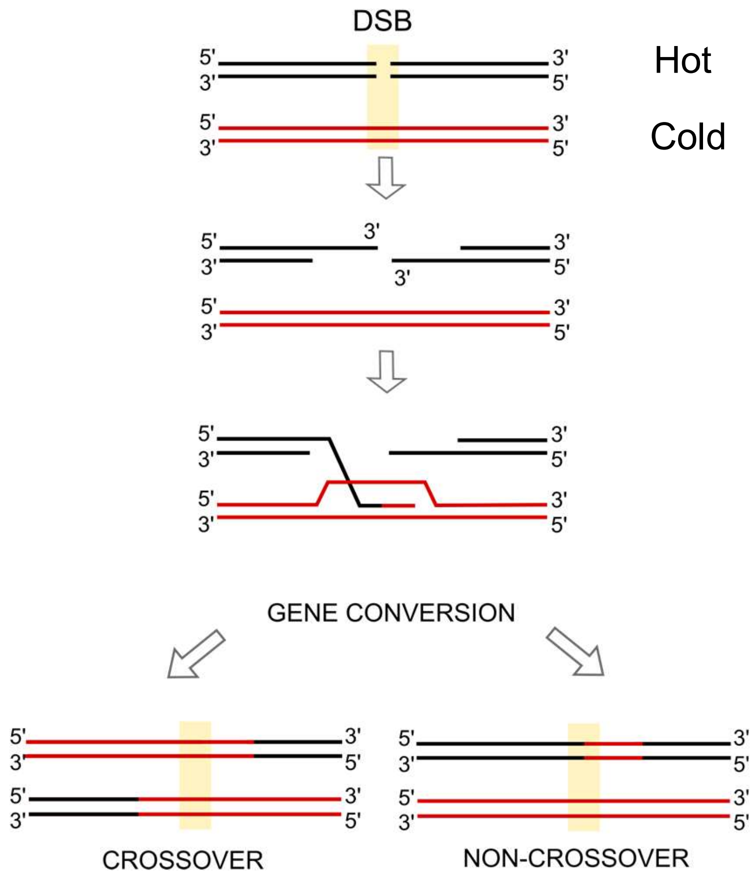
PRDM9-mediated H3K4me3 and repair intermediates are enriched in early replicating DNA



SSDS & H3K4m3 data:
Davies et al., Nature 2016

- Genome is binned into 50 equal parts by RT
- Genome-wide data is measured in all windows with a given RT

The “former” Hotspot Paradox

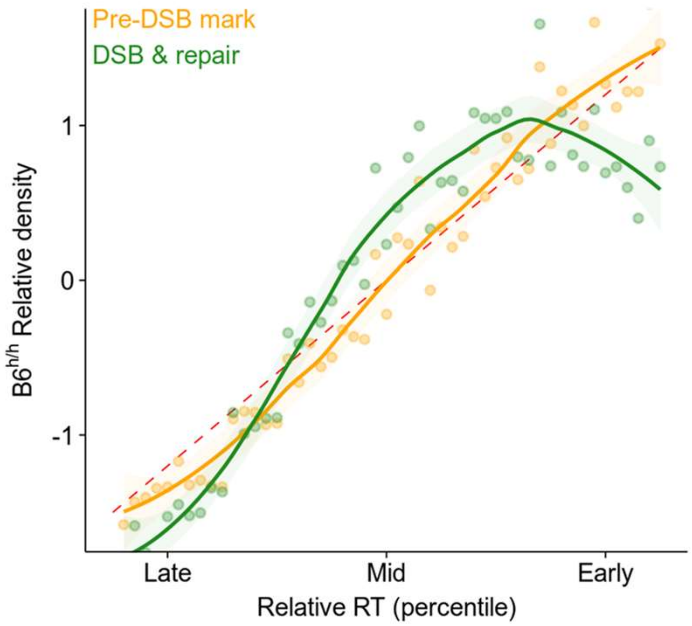


- Upon DNA repair good PRDM9 binding sites are eroded
- Drives PRDM9 evolution
- Leads to speciation in mice

Smagulova, Brick et al. Genes Dev. 2016
Davies et al. Nature 2016

PRDM9-mediated H3K4me3 and repair intermediates are enriched in early replicating DNA

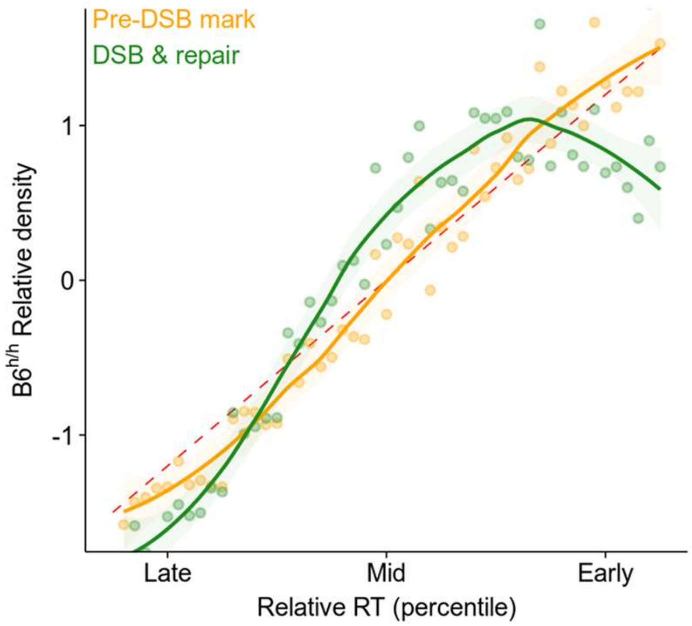
Humanized PRDM9



SSDS & H3K4m3 data:
Davies et al., Nature 2016

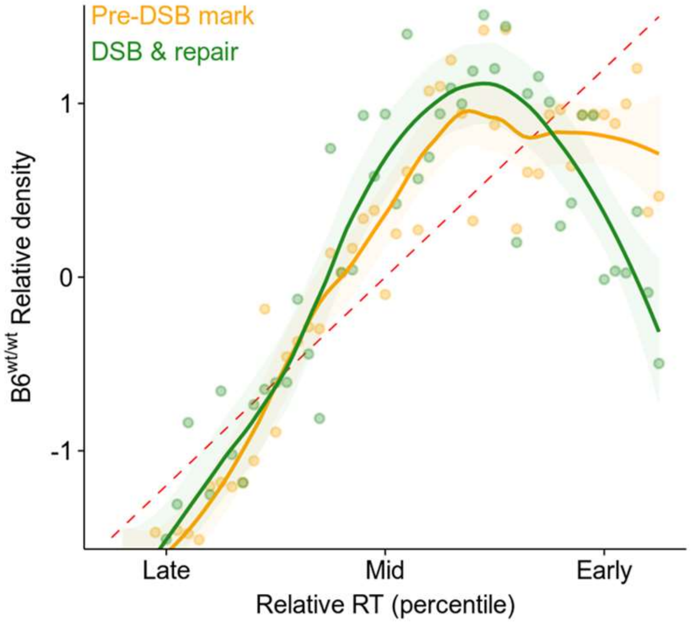
PRDM9-mediated H3K4me3 and repair intermediates are enriched in early replicating DNA

Humanized PRDM9



SSDS & H3K4m3 data:
Davies et al., Nature 2016

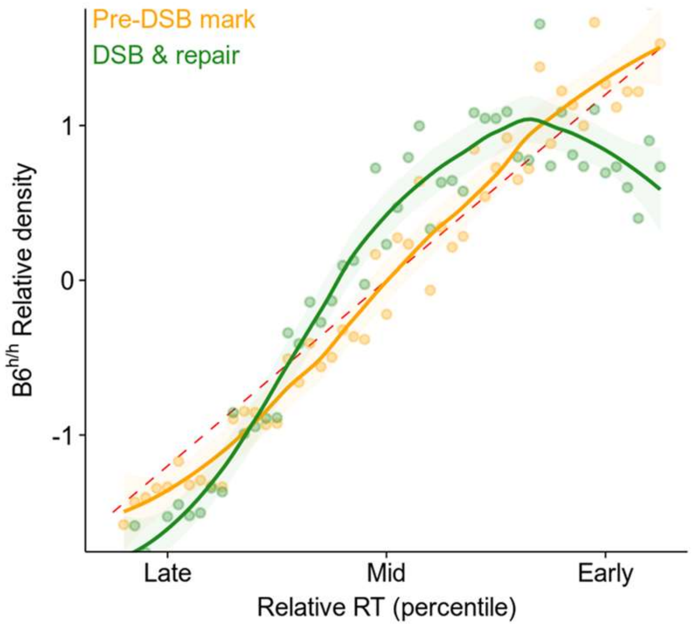
wild-type PRDM9



SSDS: Brick et al., Nature 2012
H3K4m3 : Baker et al., PLOS Gen 2016

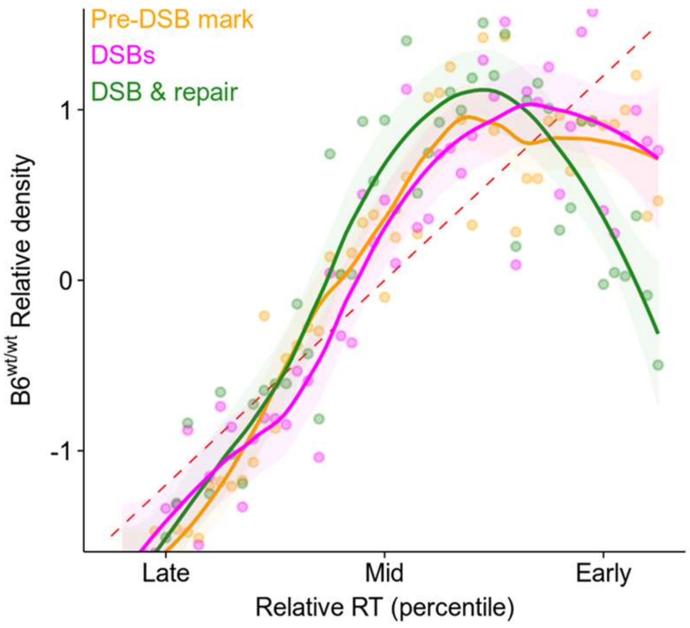
And so are DSBs, thus repair is altered in early replicating DNA

Humanized PRDM9



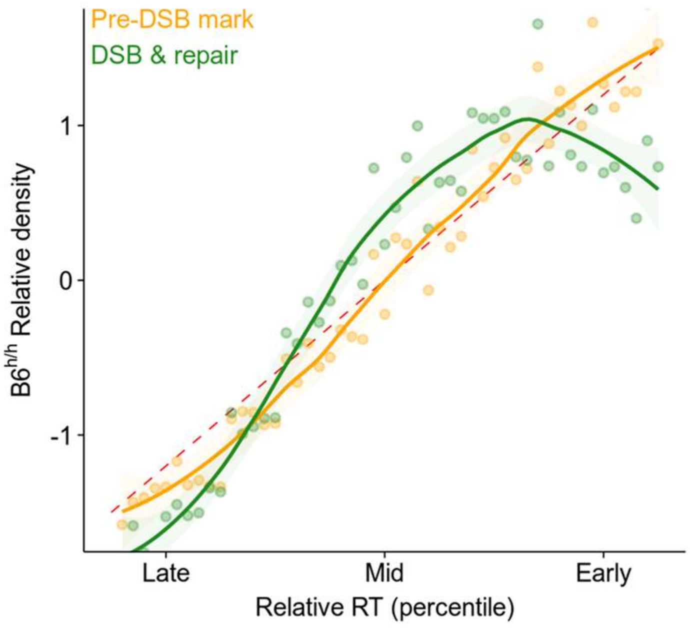
SSDS & H3K4m3 data:
Davies et al., Nature 2016

wild-type PRDM9

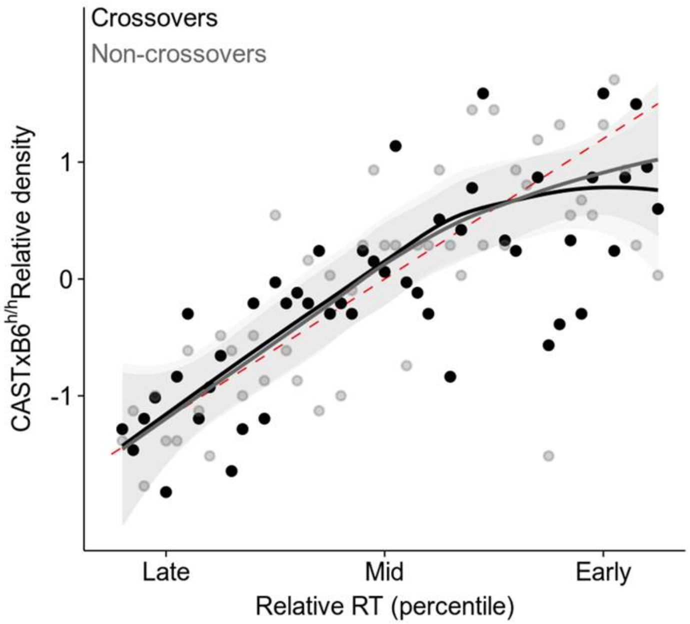


Spo11: Lange et al., Cell 2015
SSDS: Brick et al., Nature 2012
H3K4m3 : Baker et al., PLOS Gen 2016

CO and NCO are slightly depleted in the earliest replicating DNA

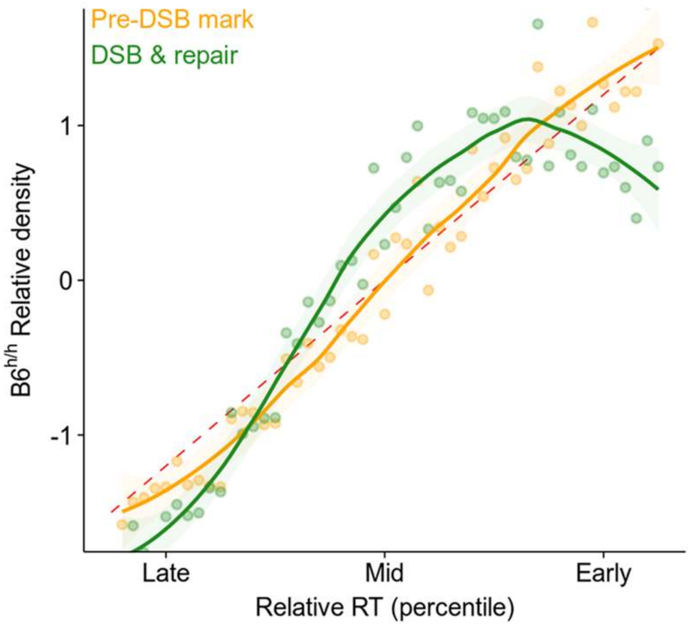


SSDS & H3K4m3 data:
Davies et al., Nature 2016



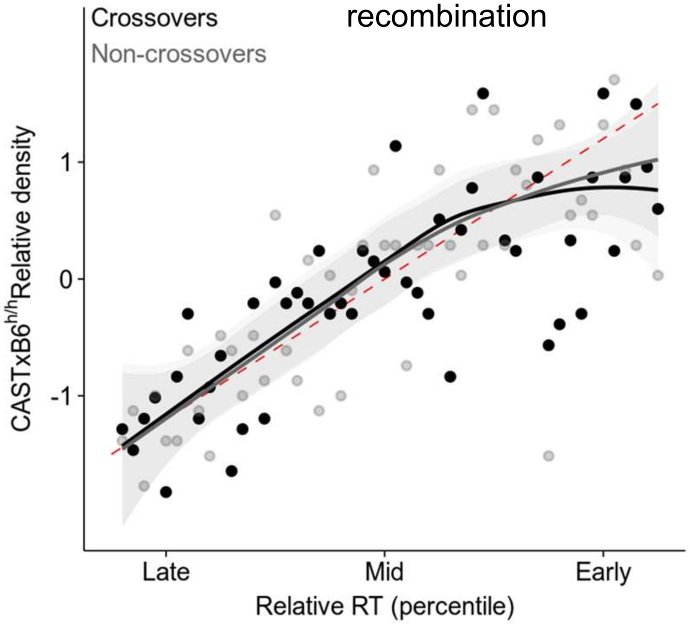
CO & NCO data:
Li et al., Nat. Comms. 2019

CO and NCO are slightly depleted in the earliest replicating DNA



SSDS & H3K4m3 data:
Davies et al., Nature 2016

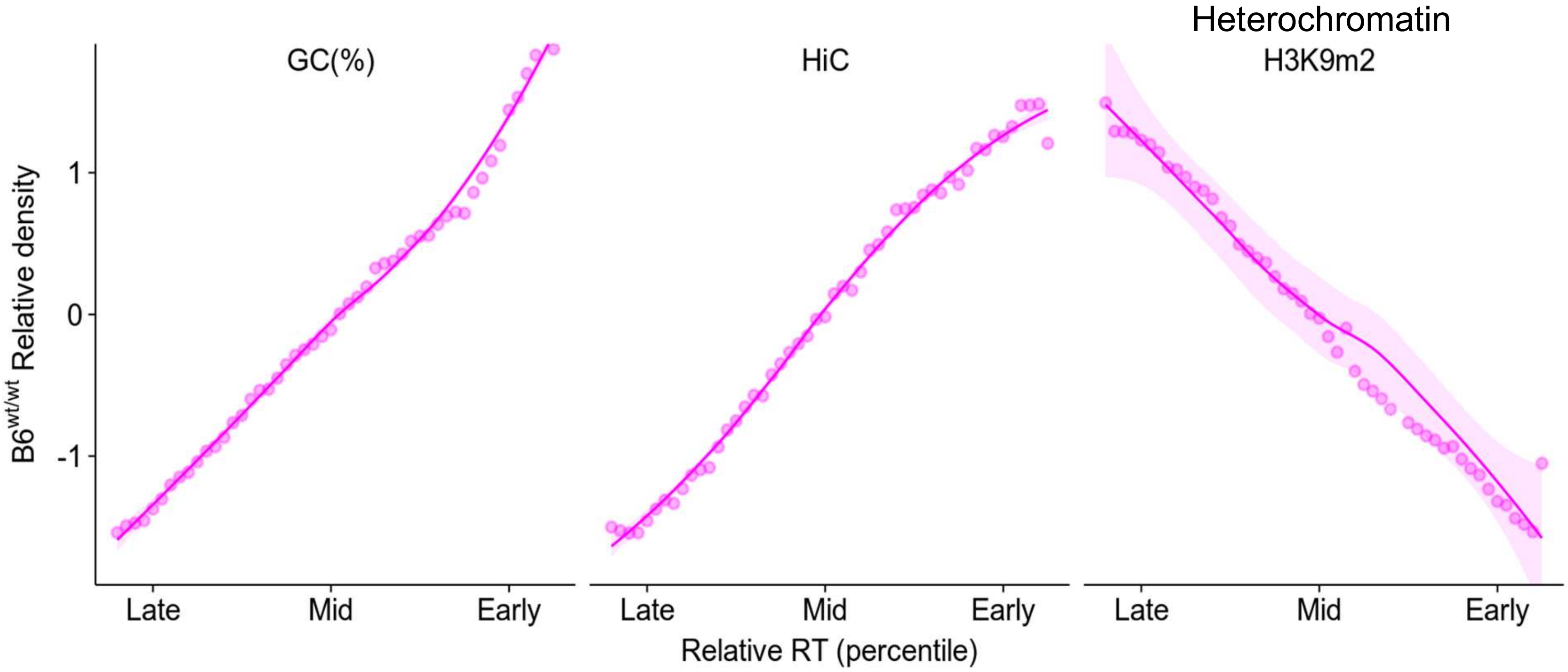
Detected CO and NCO are products of interhomolog recombination



Intersister?

CO & NCO data:
Li et al., Nat. Comms. 2019

Replication timing correlates with other things



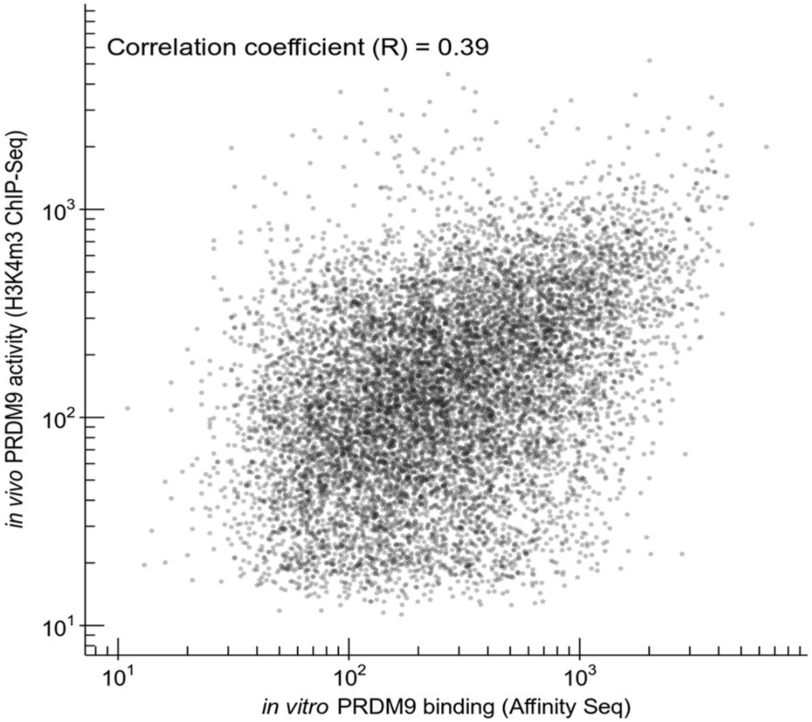
Patel et al., 2019 NSMB

Lam et al, 2019 Nature Comms

Can RT estimates help us to predict hotspot usage ?

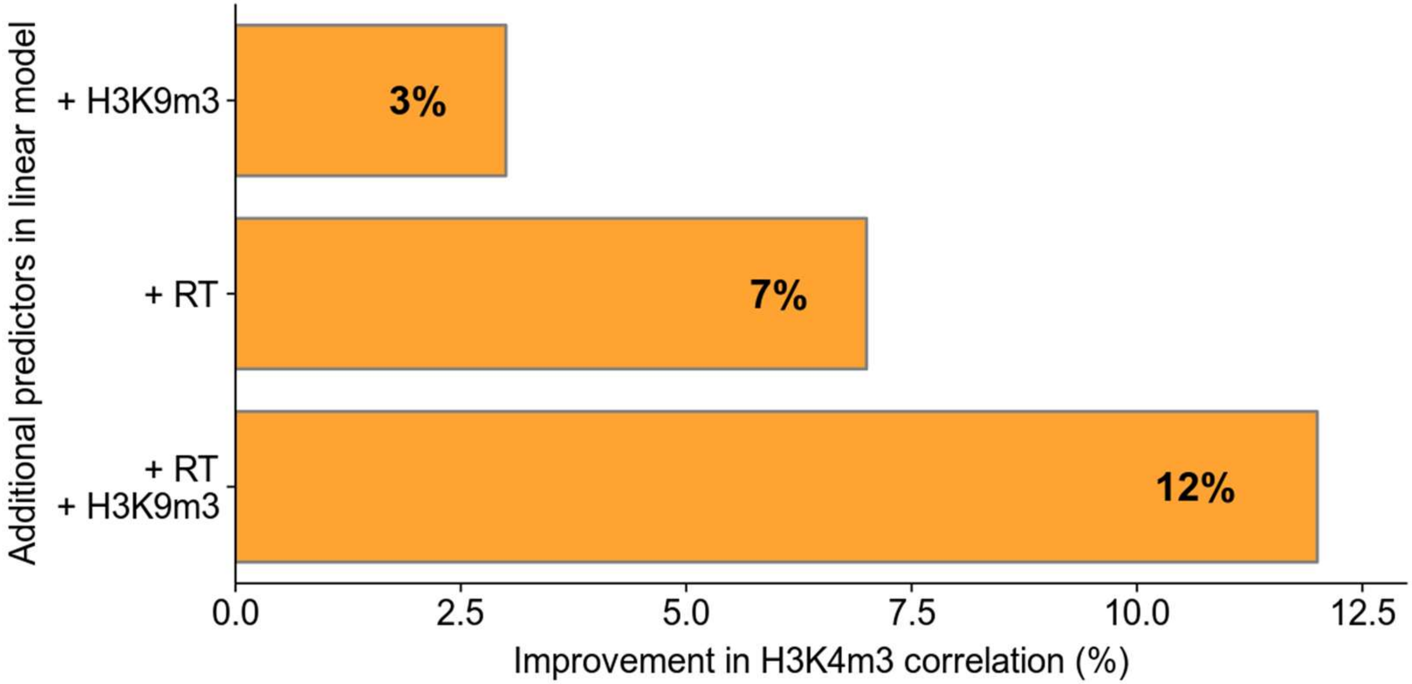
Estimate of PRDM9 binding in vitro
Affinity-Seq (Affy-Seq)

Estimates of in-vivo PRDM9 activity
H3K4me3 ChIP-Seq



Walker et al., Epigenetics Chom 2015
Baker et al., Genome Res. 2014

Chromatin structure and replication timing both help predict hotspot usage and their effect is additive



Circling back to the question, what is driving subtelomeric DSBs in human males?

Isolate DNA from S-phase meiocytes (2C - 4C)

➡

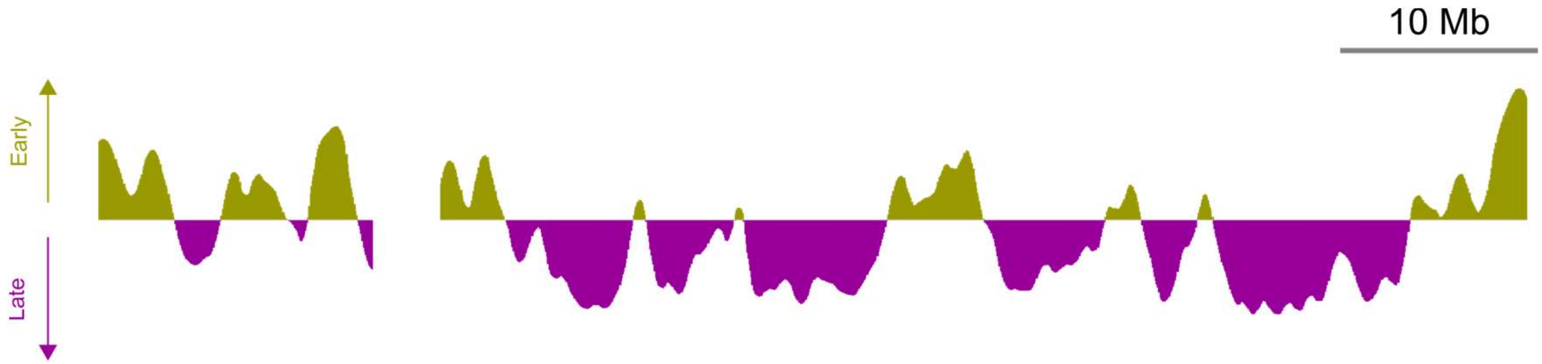
Whole genome sequencing

➡

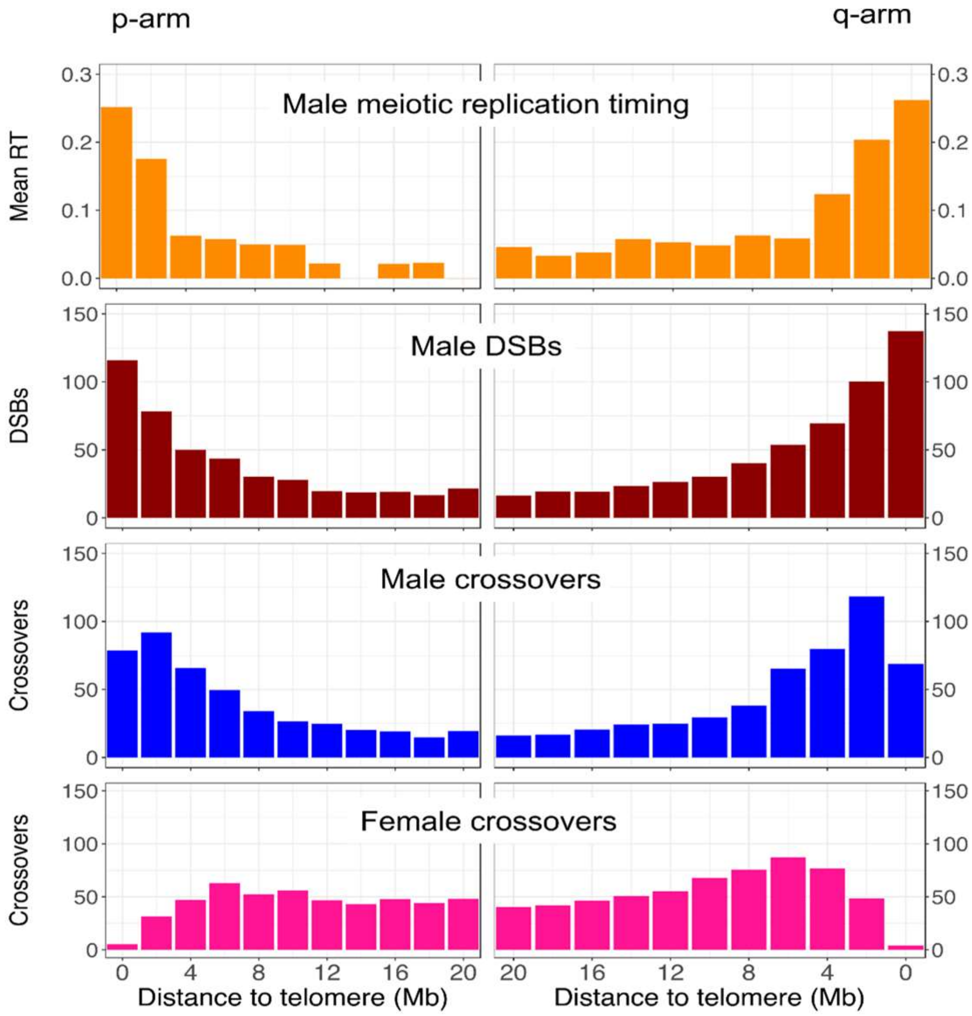
Replication timing inference from WGS data

Lam et al., Nat Comm 2019

Koren et al, Cell 2014

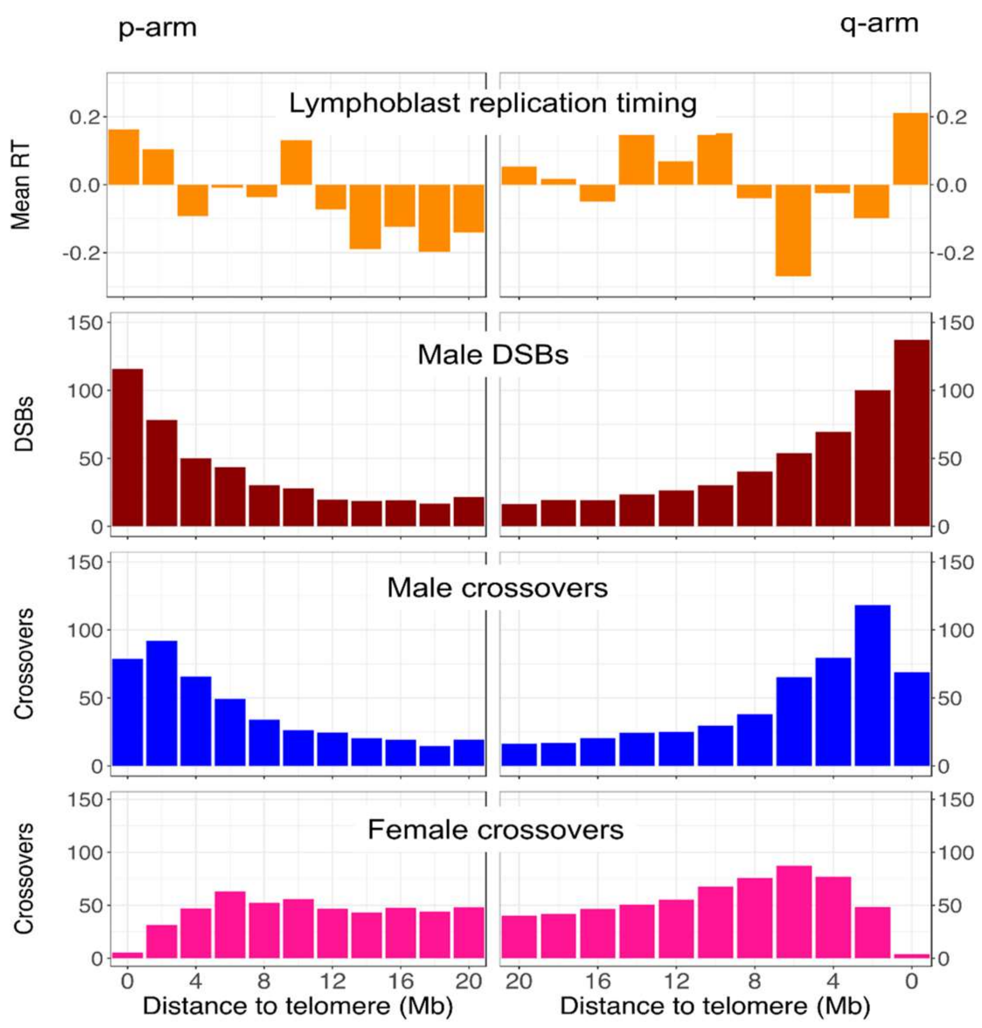


In human males, sub-telomeric DNA is replicated notably early



In human males, sub-telomeric DNA is replicated notably early

But not in other cell-types



Conclusions

- We have developed a tripartite approach to interrogate mammalian meiotic replication

- Meiotic DNA replication is distinct and fewer origins are used than in other cells

- DSBs are more frequent and stronger in early replicating regions, but dynamics of repair are altered – Likely inter-sister recombination

- Early replication of distal regions in meiosis may underlie increases in meiotic recombination in human males

Acknowledgements

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Gang Cheng
Gabriel Lam

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