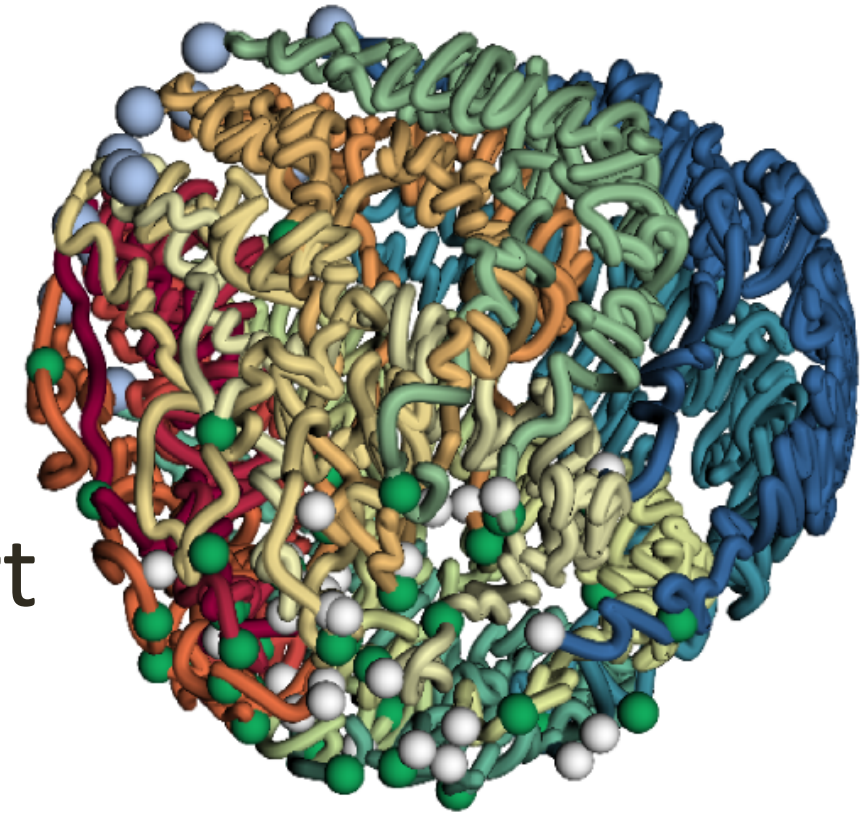
Reconstructing the 3D architecture of the genome



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Together, let's beat cancer.

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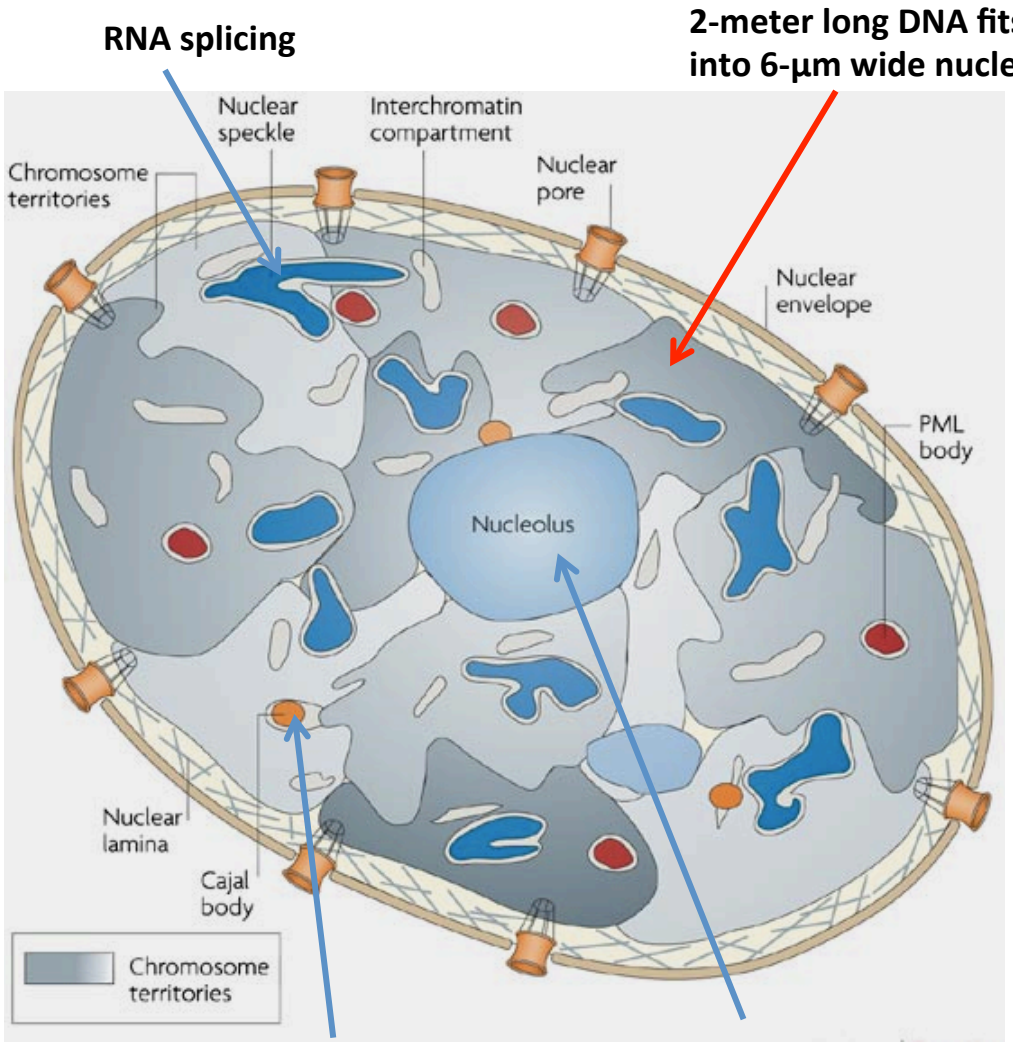
How does genome architecture influence genome function?



- Nuclear compartmentalization
- Nuclear lamina
- Transcription factories
- Chromosome conformation
  - Long-range looping
  - Chromatin domains
  - Chromosome territories

Lanctot et al. *Nature Rev. Genetics* 2007

Tools for capturing chromosome conformation

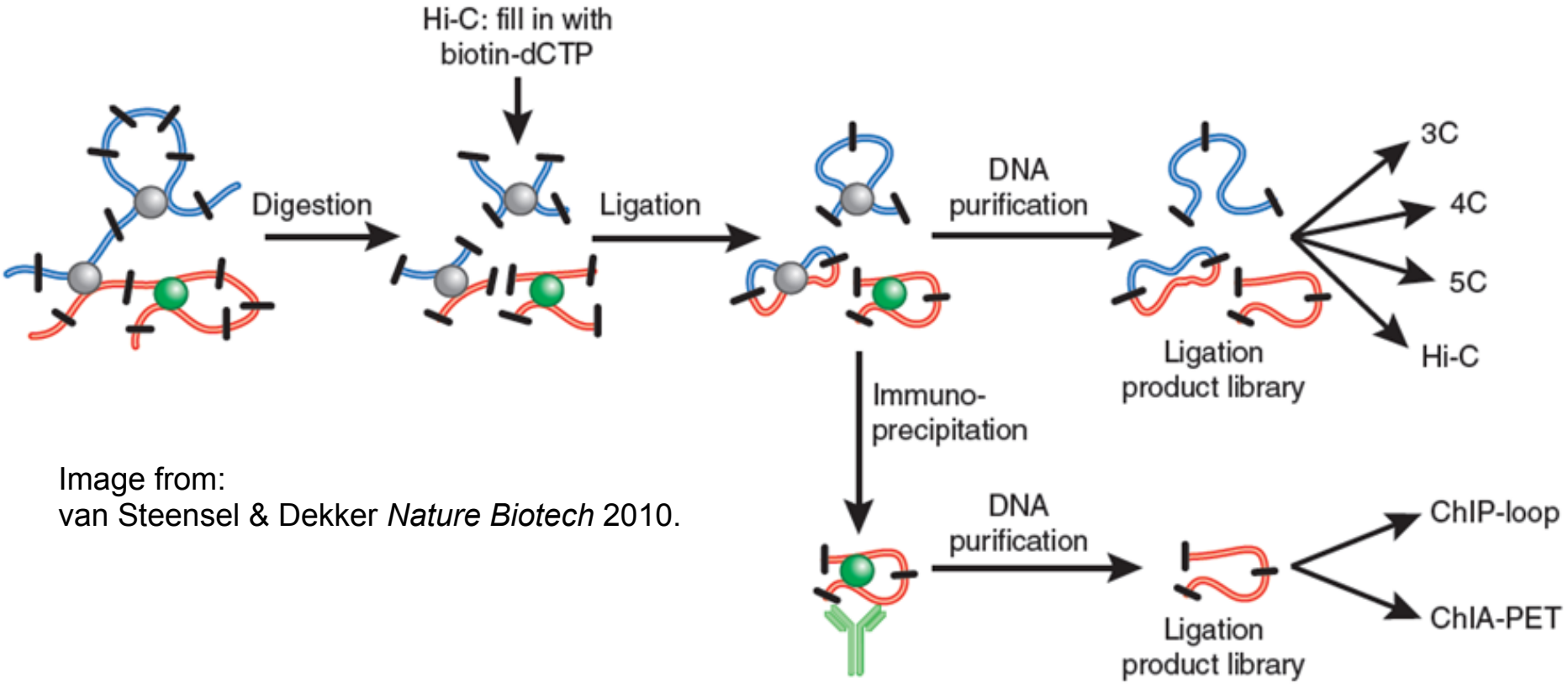
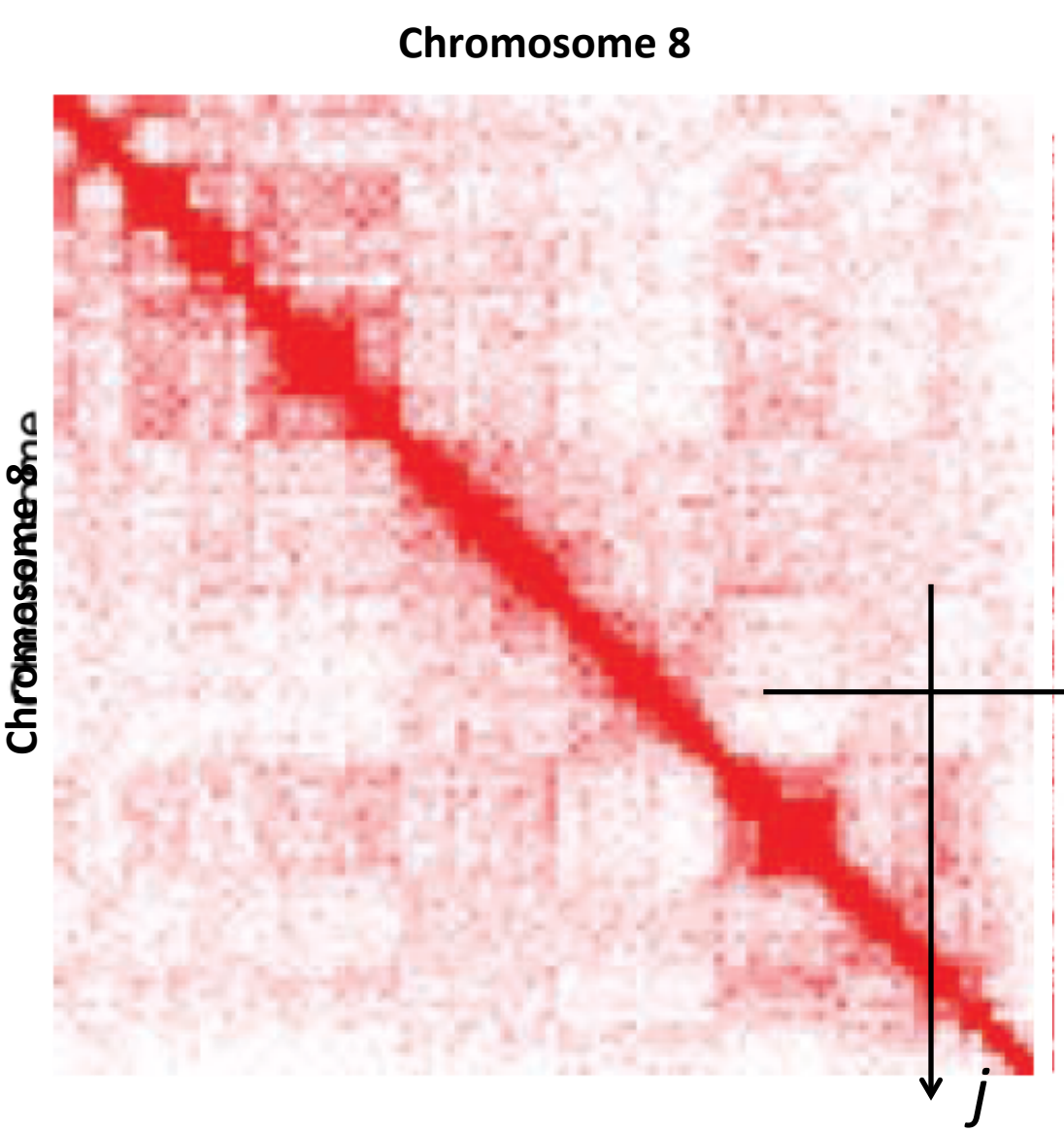


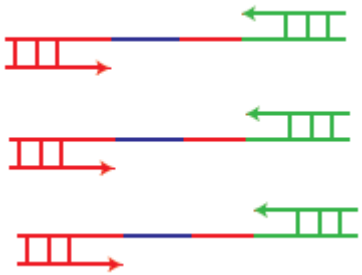
Image from:
van Steensel & Dekker *Nature Biotech* 2010.

3C	ChIA-PET	Hi-C	
Dekker et al. *Science* 2002	Fullwood et al. *Nature* 2009	L.-Aiden et al. *Science* 2009	Duan et al. *Nature* 2010

Output of conformation capture is a contact matrix



paired-end reads



C(i,j) = How many times locus ***i*** is linked to locus ***j*** by a paired-end read?

Inter-chromosomal contact

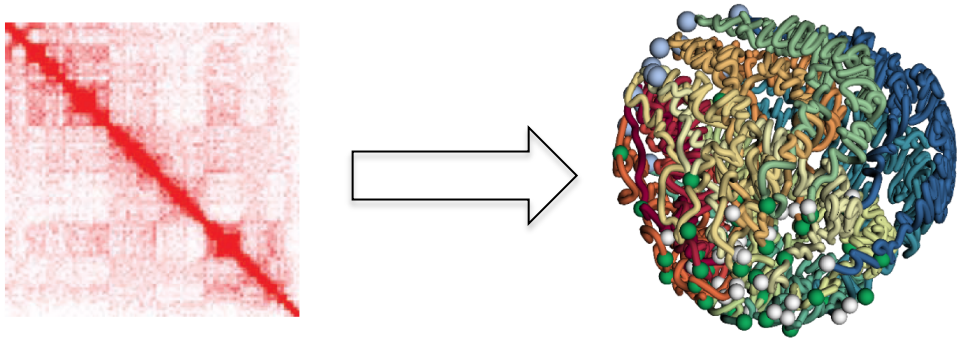
Intra-chromosomal contact

Outline

1. How to reconstruct the 3D structure of the genome from Hi-C data?
2. How genome architecture is related to gene expression regulation: the case of *P. falciparum*
3. Cheap identification of centromeres from metagenomic Hi-C data

Part 1

PASTIS, a tool to infer the 3D structure of the genome from HiC data



Reconstructing the 3D structure of the genome from Hi-C data

Two main approaches:

1. **Consensus methods** that infer a unique « average » structure

   [Duan et al. 2010; Tanizawa et al. 2010; Bau et al. 2011; Zhang et al. 2013; Ben-Elazar et al. 2013]

2. **Ensemble methods** that yield a population of structures

   [Rousseau et al. 2011; Khalor et al 2011; Hu et al 2013]

Modelisation

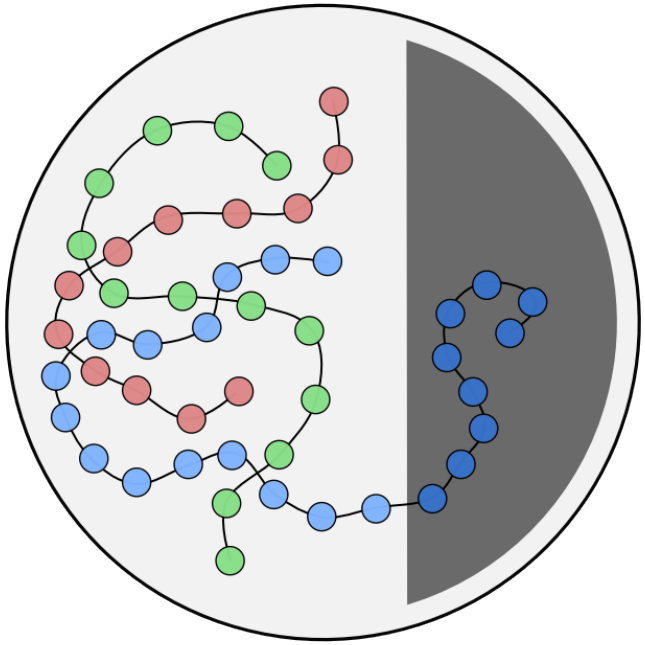
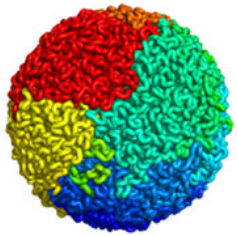


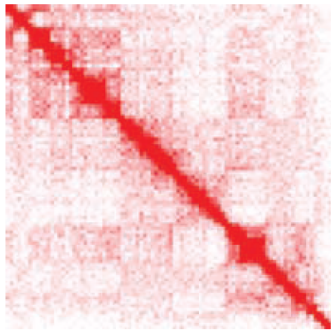
Figure: **Beads on a string model** Chromosomes are modeled as a series of beads. Nucleus is assumed to be spherical.

- Chromosomes are modeled as a series of beads.
- Each bead is spaced 10kb apart.

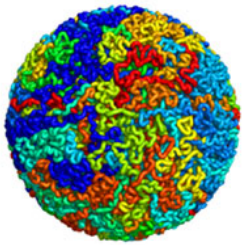
From interaction frequency to 3D distance

Fractal globule





Equilibrium



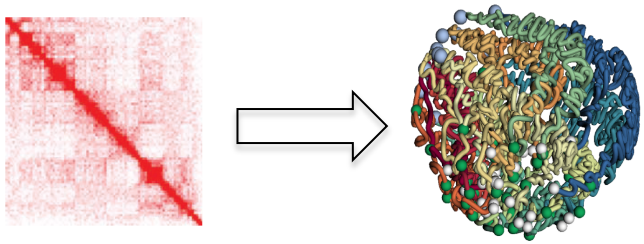
- $c \sim s^{-1}$
- $d \sim s^{1/3}$
- Valid for human.

- $c \sim s^{-3/2}$
- $d \sim s^{1/2}$ for $s < s_{\max}^{2/3}$
- Valid for budding yeast, and small organism

Default counts-to-distance transfer function

$$\delta_{ij} = \gamma c_{ij}^{-1/3}, \tag{6}$$

Metric MDS-based method



- Let $\mathbf{X} \in R^{n \times 3}$ be the coordinates of each bead.
- Let $\mathbf{C} \in R^{n \times n}$ be the contact count matrix and $\mathcal{D}$ the set of non-zeros entries.
- Let Θ the count-to-distance transfer function.

Optimization problem

$$\begin{aligned} \underset{\mathbf{x}_1,\ldots,\mathbf{x}_n}{\text{minimize}} \quad & \sigma(\mathbf{X}, \mathbf{C})) \\ \text{subject to} \quad & \text{some bio} \\ & \mathbf{x}_i^T \mathbf{x}_i \leq r \end{aligned}$$

- MDS1 [Duan et al., 2010]

$$\sigma(X, C) = \sum_{i,j} (\|x_i - x_j\|_2 - \Theta(c_{ij}))^2$$

- MDS2 [Ay et al., 2014]

$$\sigma(X, C) = \sum_{i,j} \frac{(\|x_i - x_j\|_2 - \Theta(c_{ij}))^2}{\Theta(c_{ij})^2}$$

- ChromSDE [Zhang et al., 2013]

$$\sigma(X, C) = \sum_{i,j} \frac{(\|x_i - x_j\|_2^2 - \Theta(c_{ij})^2)^2}{\Theta(c_{ij})} - \lambda \sum_{i,j \notin \mathcal{D}} \|x_i - x_j\|_2^2$$

Nonmetric MDS-based method

Idea

If two loci i and j are observed to be in contact more often than loci k and ℓ, then i and j should be closer in 3D space than k and ℓ

$$c_{ij} \geq c_{k\ell} \Leftrightarrow \|x_i - x_j\|_2 \leq \|x_k - x_\ell\|_2 \tag{4}$$

$$\begin{aligned} \underset{\mathbf{x}_1,\ldots,\mathbf{x}_n,\Theta}{\text{minimize}} \quad & \sigma(\mathbf{X}, \mathbf{C}, \Theta)) \\ \text{subject to} \quad & \Theta \quad \text{decreasing} \\ & \text{some biologically motivated constraints} \\ & \mathbf{x}_i^T \mathbf{x}_i \leq r_{\max}^2, \quad \text{(all beads should lie in the nucleus)} \end{aligned}$$

(Ben-Eleazar et al, 2013)

Poisson model

The idea

Let's assume that $c \sim Poisson(\beta d^{\alpha})$, where c is the interaction count, d the euclidean distance, and β and α unknown parameter.

Likelihood

$$\ell(\mathbf{X}, \alpha, \beta) = \prod_{i<j\leq n} \frac{(\beta d_{ij}^{\alpha})^{c_{ij}}}{c_{ij}!} exp(-\beta d_j^{\alpha}) \tag{5}$$

Optimization problem

$$\underset{\mathbf{x}_1,\ldots,\mathbf{x}_n,\alpha,\beta}{\text{minimize}} \quad \sigma(\mathbf{X}, \mathbf{C}, \alpha, \beta) = -\log(\ell(\mathbf{X}, \mathbf{C}, \alpha, \beta))$$

subject to some biologically motivated constraints

$$\mathbf{x}_i^T \mathbf{x}_i \leq r_{\max}^2, \quad \text{(all beads should lie in the nucleus)}$$

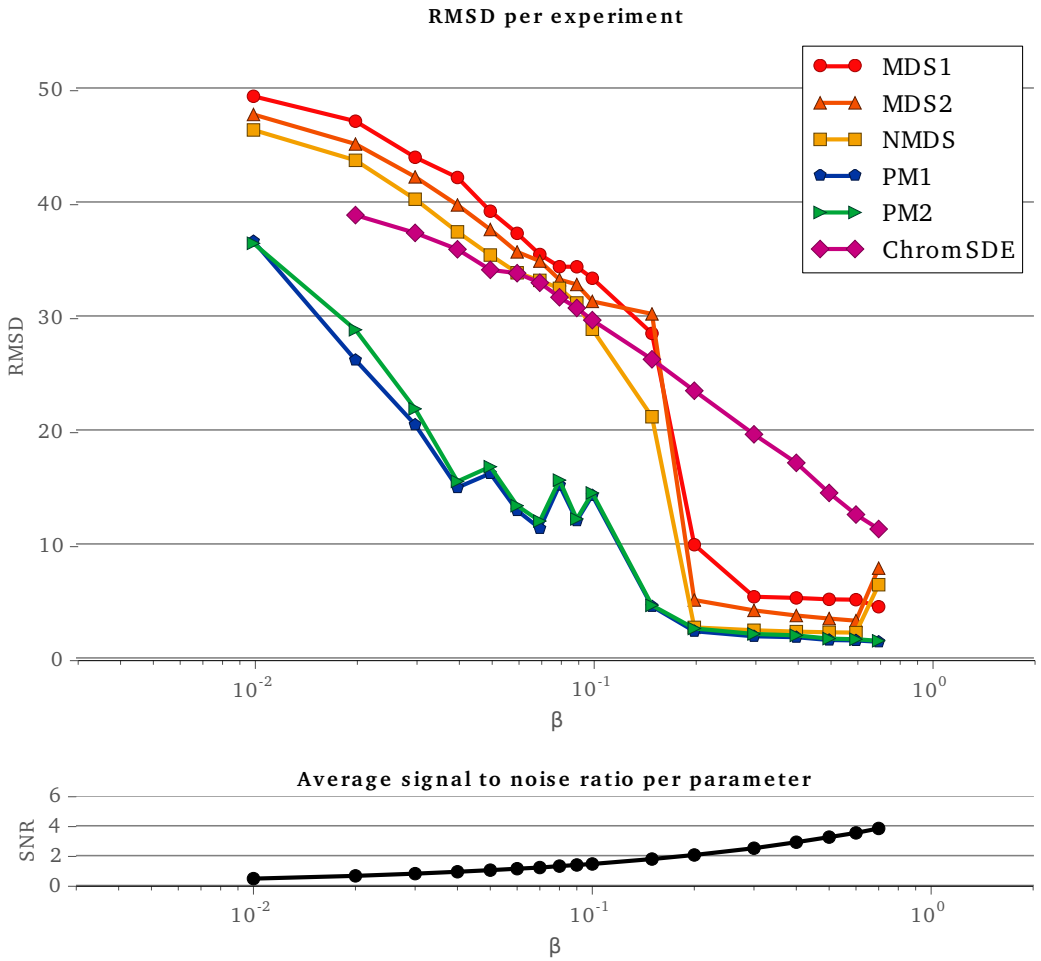
Data

- **Generated Datasets**

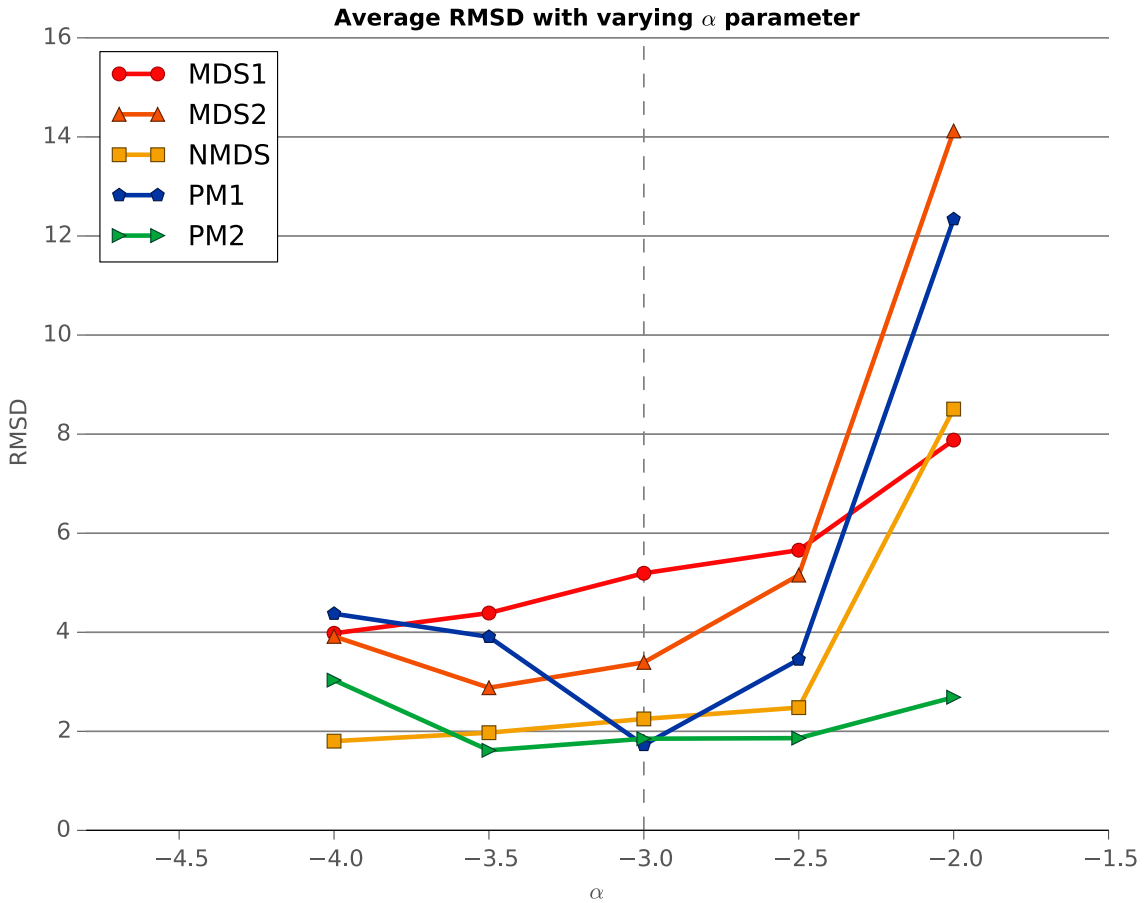
$$c_{ij} = P(\beta d_{ij}^{\alpha}), \tag{7}$$

  where
  - $\alpha = -3$ and β varies between 0.01 and 0.7.
  - α varies between -4 and -2 and β between 0.4 and 0.7.
- **Publicly available datasets** mouse embryonic stemcells at 100 kb, 200 kb, 500 kb, 1 Mb, normalized using ICE [Imakaev et al., 2012]

Performance as a function of coverage



Robustness to parameter misspecification



Mouse embryonic stem cells

- Stability across enzyme replicates

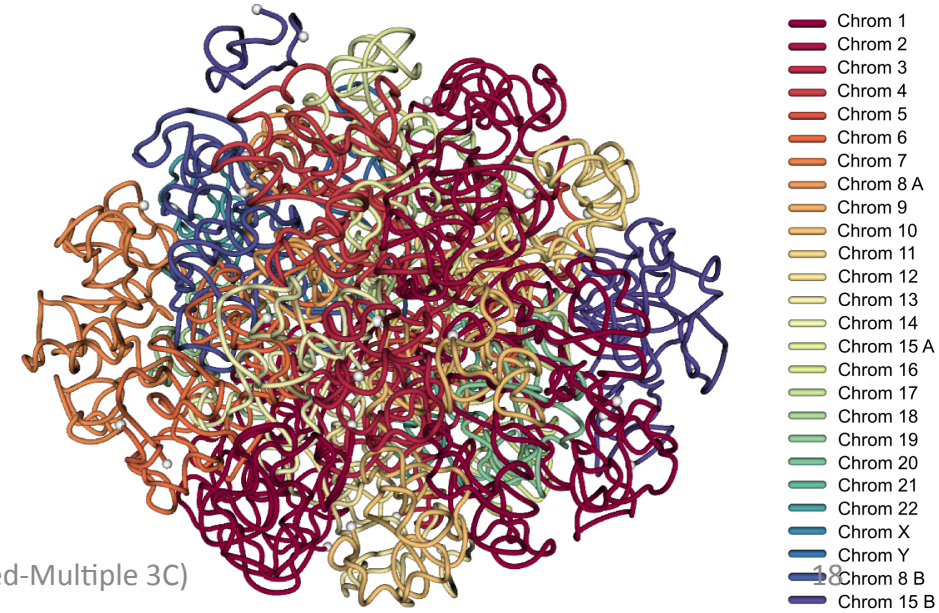
Resolution	1 Mb		500 kb		200 kb		100 kb	
	RMSD	Corr	RMSD	Corr	RMSD	Corr	RMSD	Corr
MDS1	13.13	0.945	10.00	0.942	5.64	0.940	5.07	0.736
MDS2	5.54	0.964	5.68	0.959	3.74	0.945	2.53	0.676
NMDS	5.80	0.965	5.67	0.959	3.73	0.946	2.52	0.666
PM1	7.28	0.931	7.14	0.913	4.01	0.891	**2.51**	0.664
PM2	**4.92**	**0.976**	**4.66**	**0.968**	**3.42**	**0.958**	2.76	**0.771**

- Stability across resolution

	MDS1	MDS2	NMDS	PM1	PM2
RMSD	14.86	12.92	12.98	13.03	**11.48**
Correlation	0.781	0.754	0.738	0.737	**0.807**

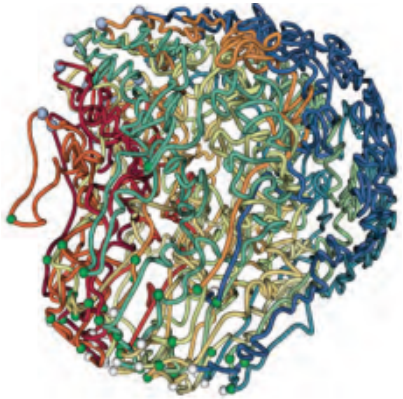
Conclusion

- Poisson model outperforms MDS-based models, particularly at low coverage (or high resolution)
- Code available at http://cbio.mines-paristech.fr/pastis
- N. Varoquaux, F. Ay, W. S. Noble and J.-P. Vert, "A statistical approach for inferring the three-dimensional structure of the genome », B*ioinformatics*, 30(12):i26-i33, 2014.
- Extension to aneuploidy?

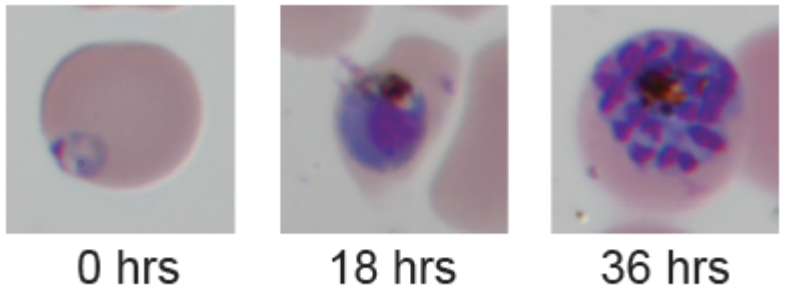


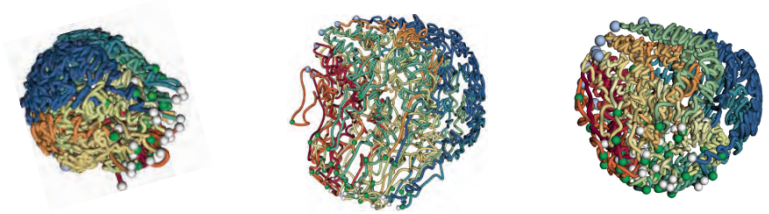
Part 2

The spatial organization of the *P. falciparum* genome

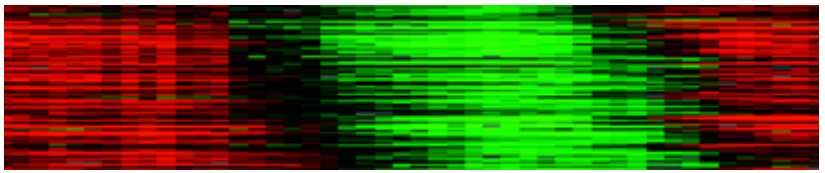


Modeling the dynamic genome architecture of a malaria parasite *(Plasmodium falciparum)*



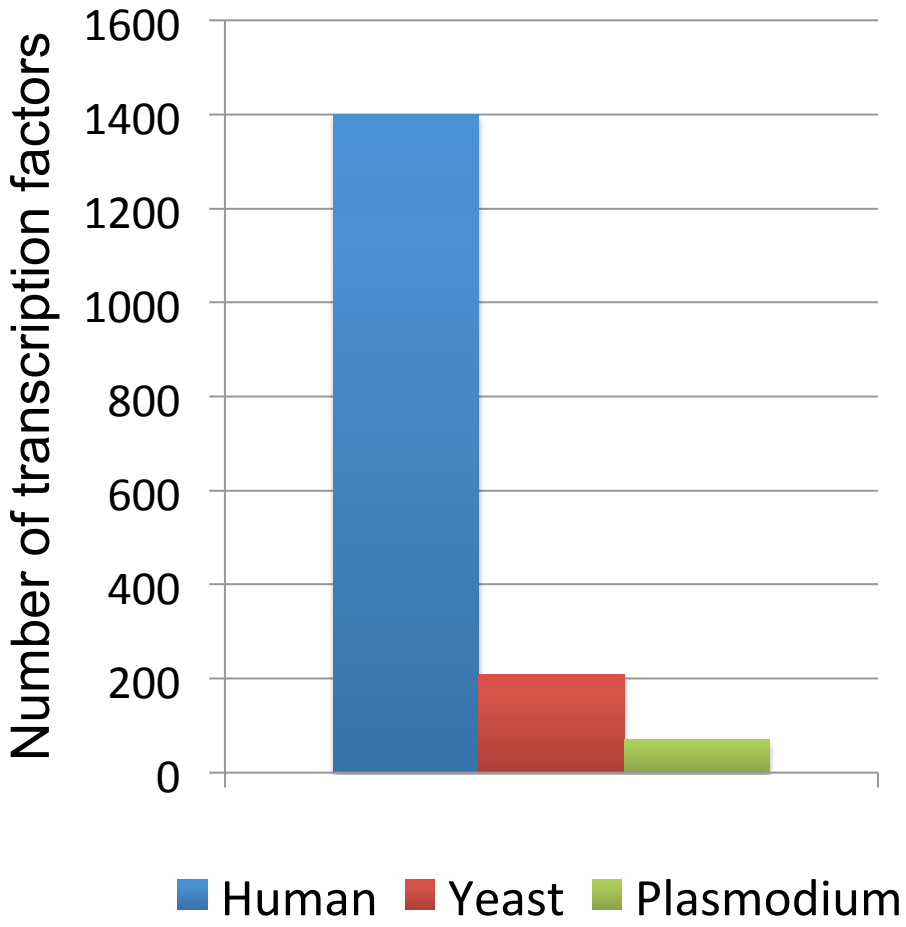


Gene expression



Ay*, Bunnik*, Varoquaux* et al. *Genome Research,* 2014.

How *Plasmodium* regulates gene expression is mysterious



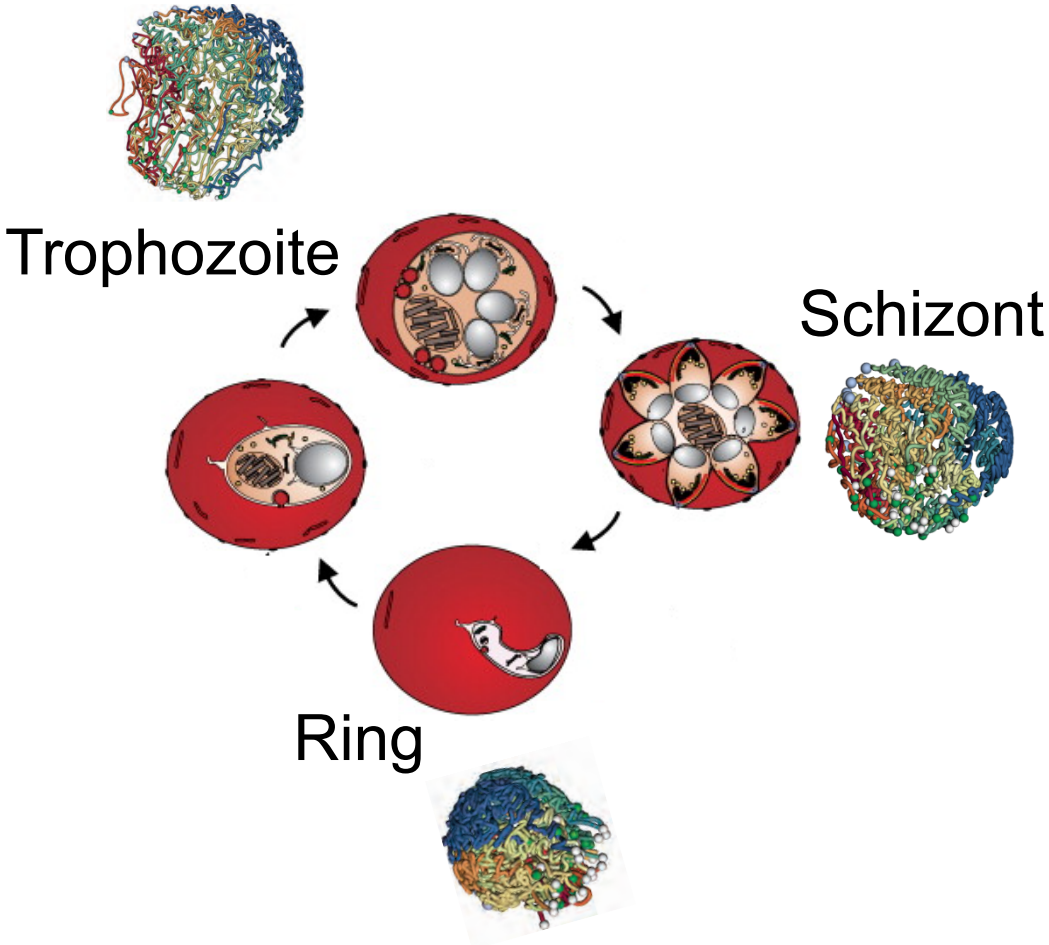
Very few transcription factors.

- 27 ApiAP2 plant-like TFs
  (Balaji et al. *NAR* 2005)
- 71 hits from homolog protein sequence search using HMMER

(Coulson et al. *Genome Research* 2004)

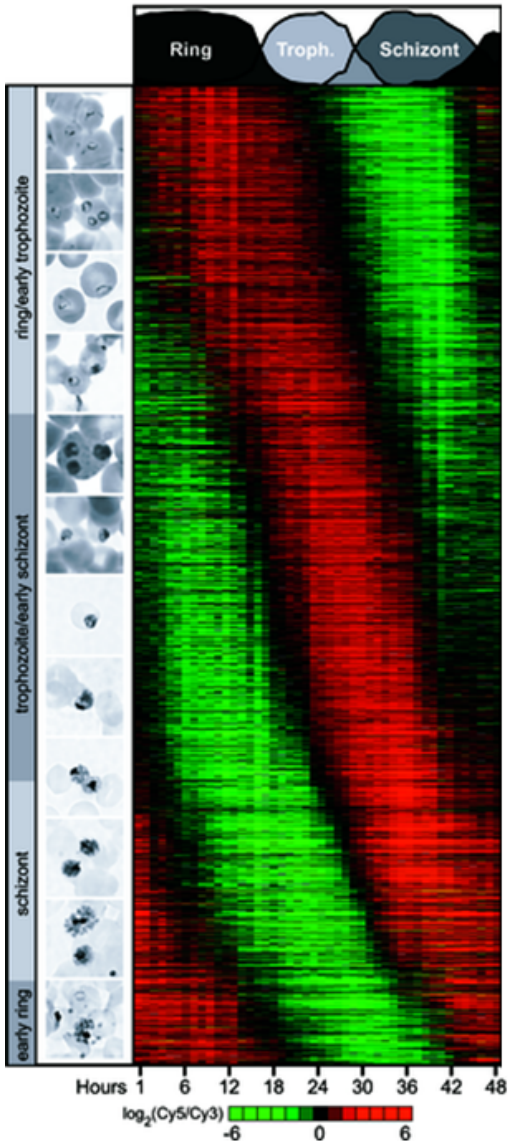
Hughes & de Boer *Genetics* 2013.

Genome architecture as an alternative mechanism for regulating gene expression?

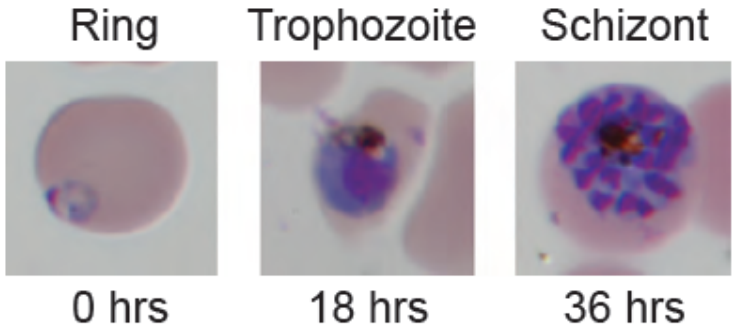


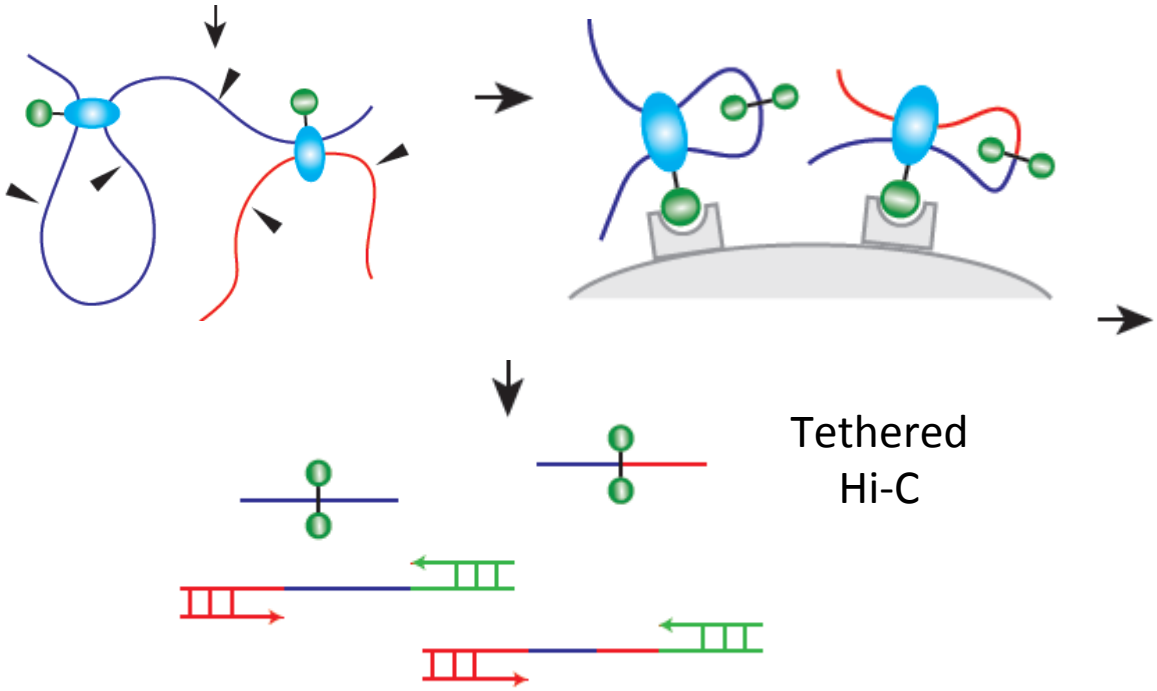
Erythrocytic cycle

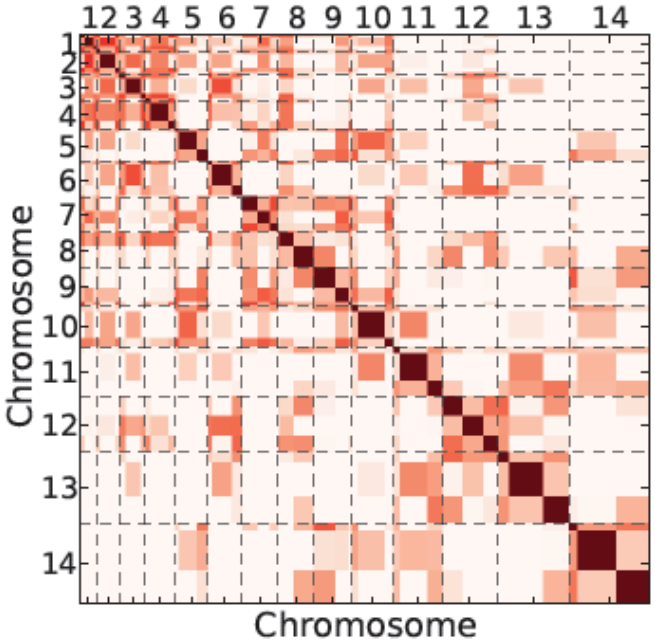
?



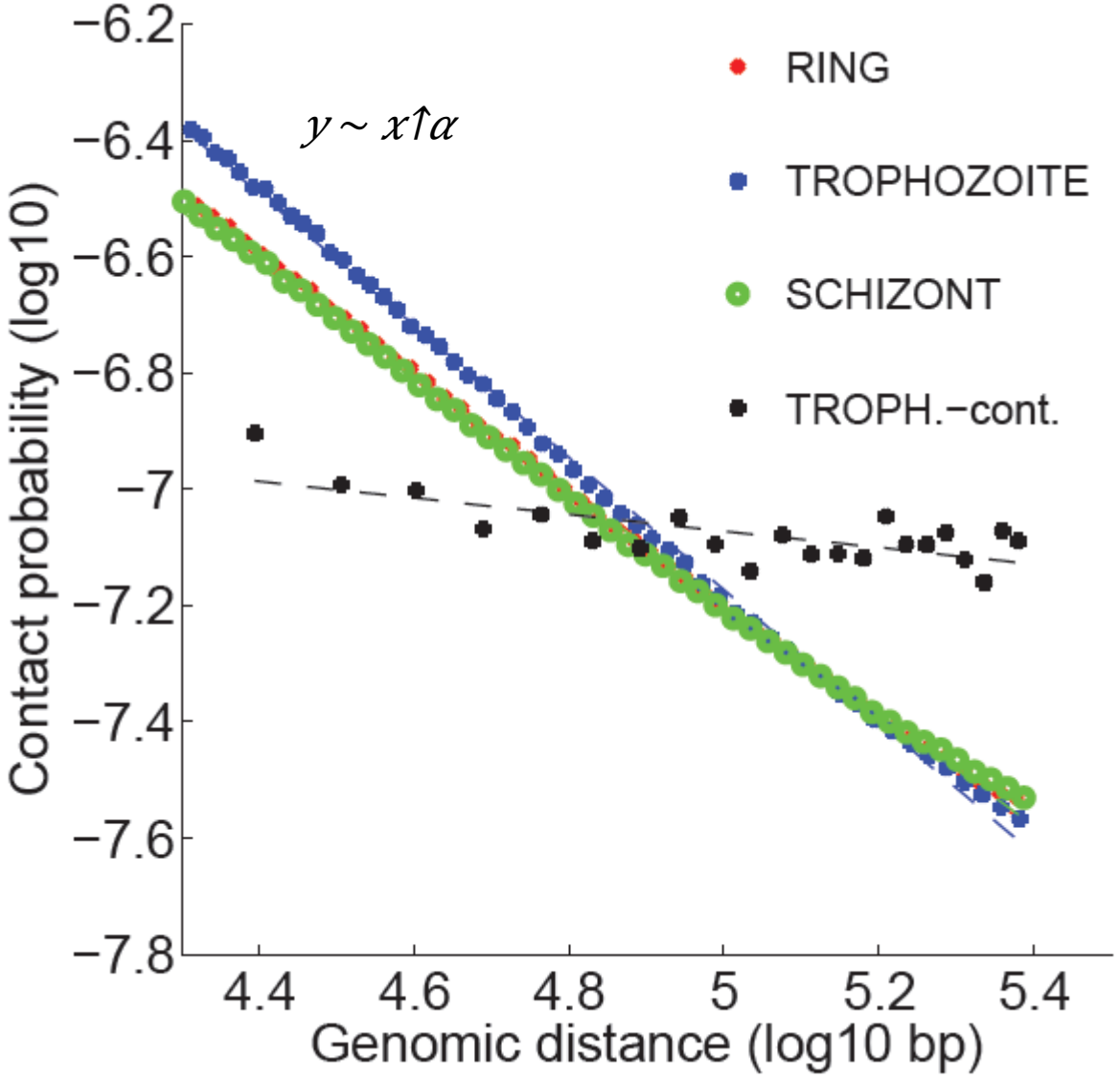
We assayed genome architecture at 3 time points in the erythrocytic cycle







Plasmodium contact frequencies suggest a fractal globule architecture



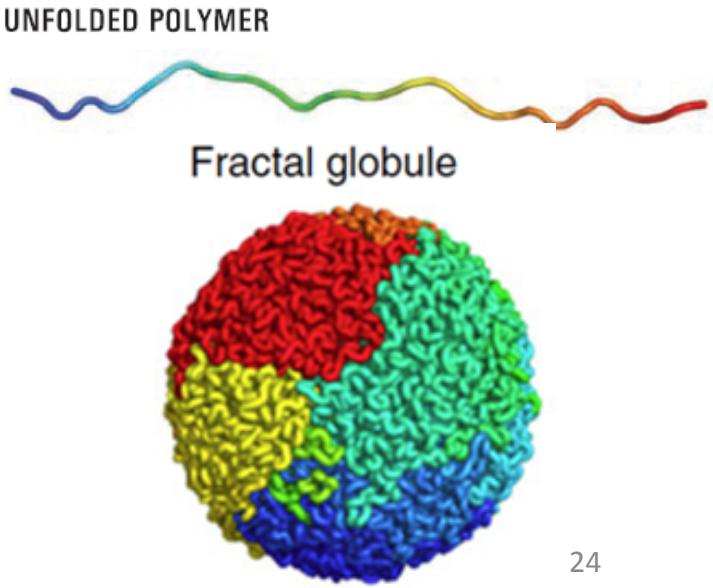
$\alpha =$

-0.98

-1.14

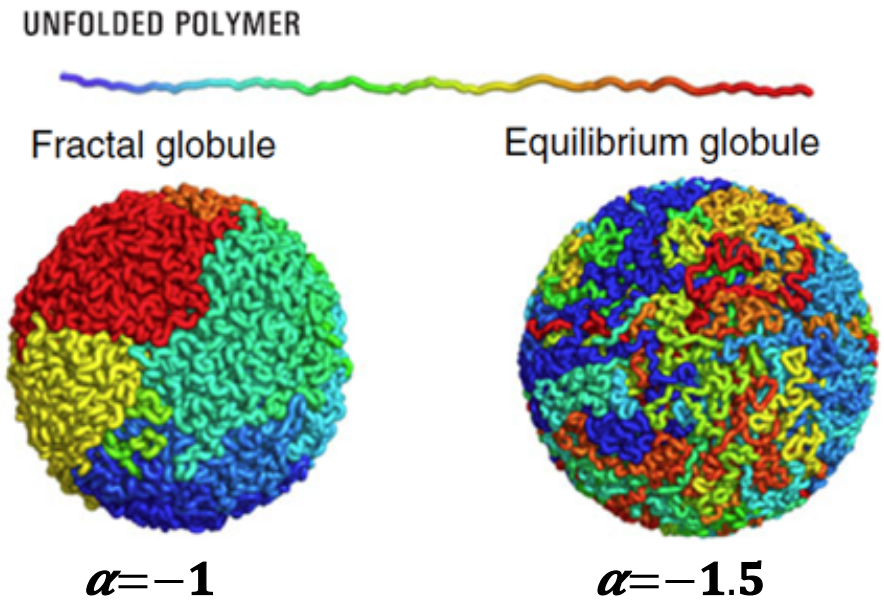
-0.96

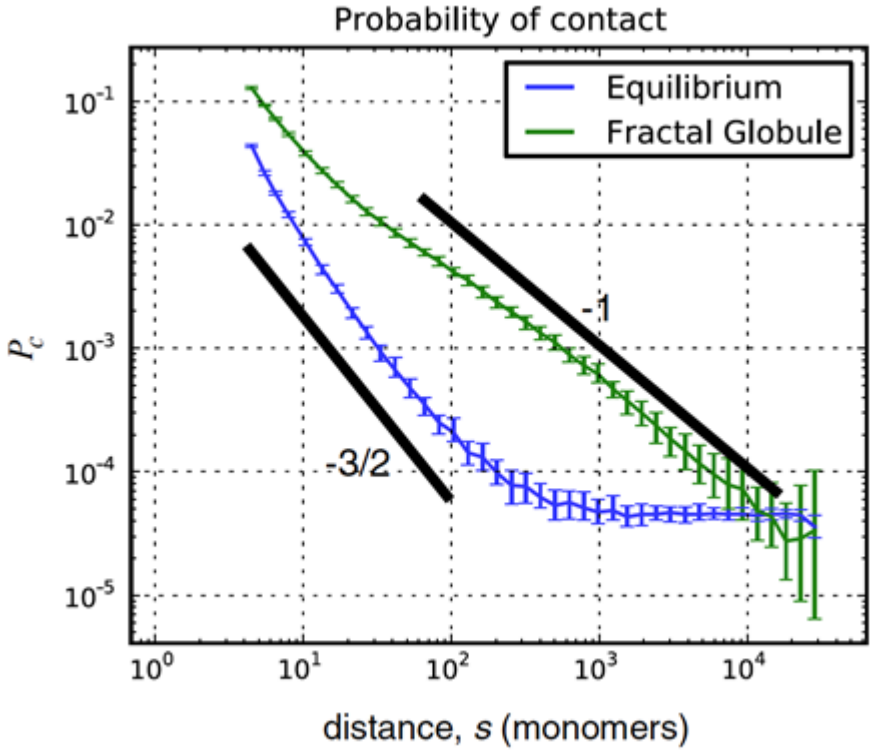
-0.14



Scaling parameter for the Trophozoite stage is indicative of more intermingled chromatin

$y \sim x^{-1.14}$





Lieberman-Aiden et al. *Science* 2009

We use the observed contact counts to infer a 3D model

- Model the genome as beads at 10 kbp resolution.
- Estimate Euclid e m ruler derived from intra-chromoso tions.
- Find 3D coordinates that ield the ex ected distances:

Distance according to the model

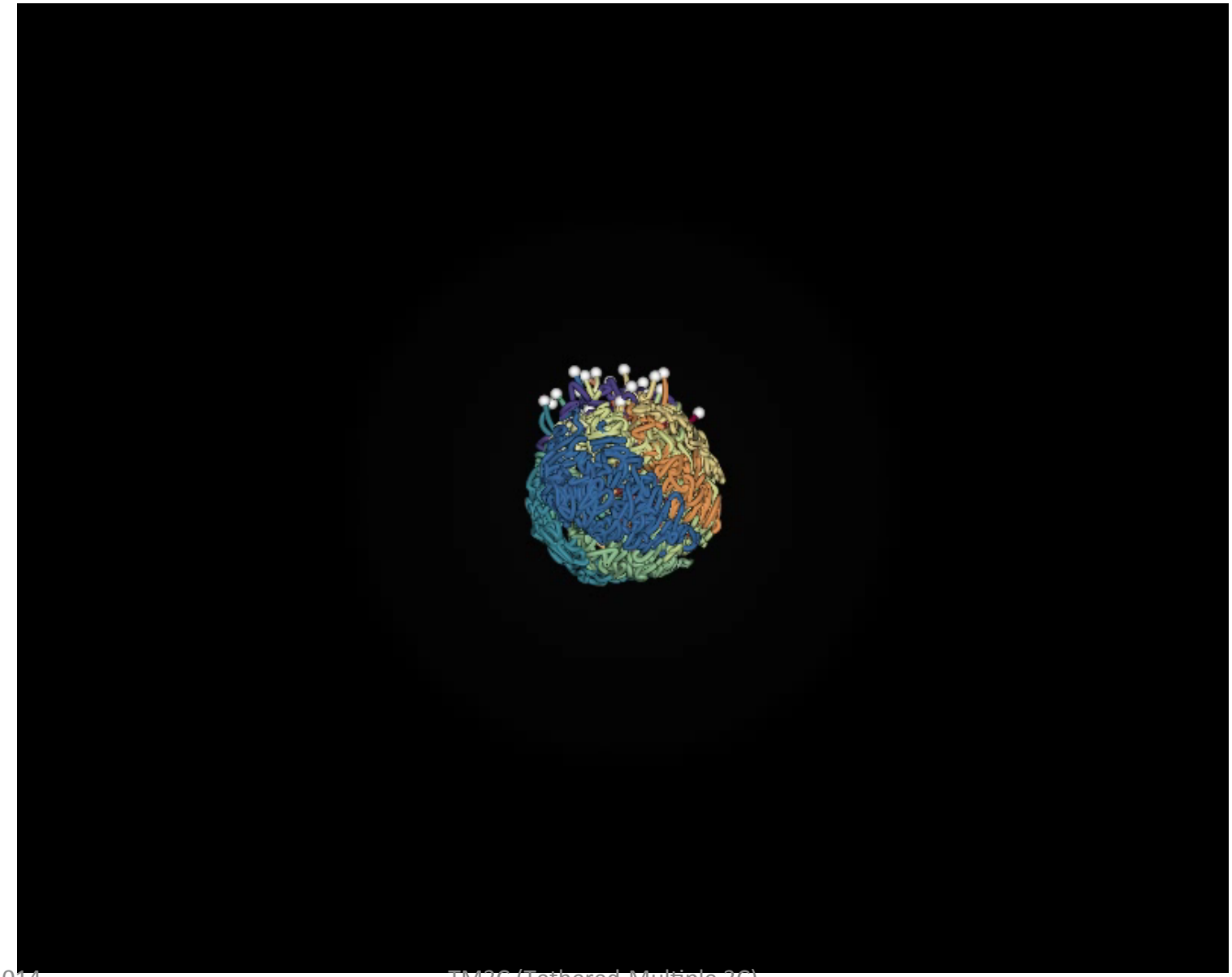
Distance according to the data

$$\underset{\mathbf{X}}{\text{minimize}} \sum_{\delta_{ij} \in \mathcal{D}} \frac{1}{d_{ij}^2} \left(d_{ij} - \delta_{ij} \right)^2 \qquad \mathbf{X} \in R^{3 \times n}$$

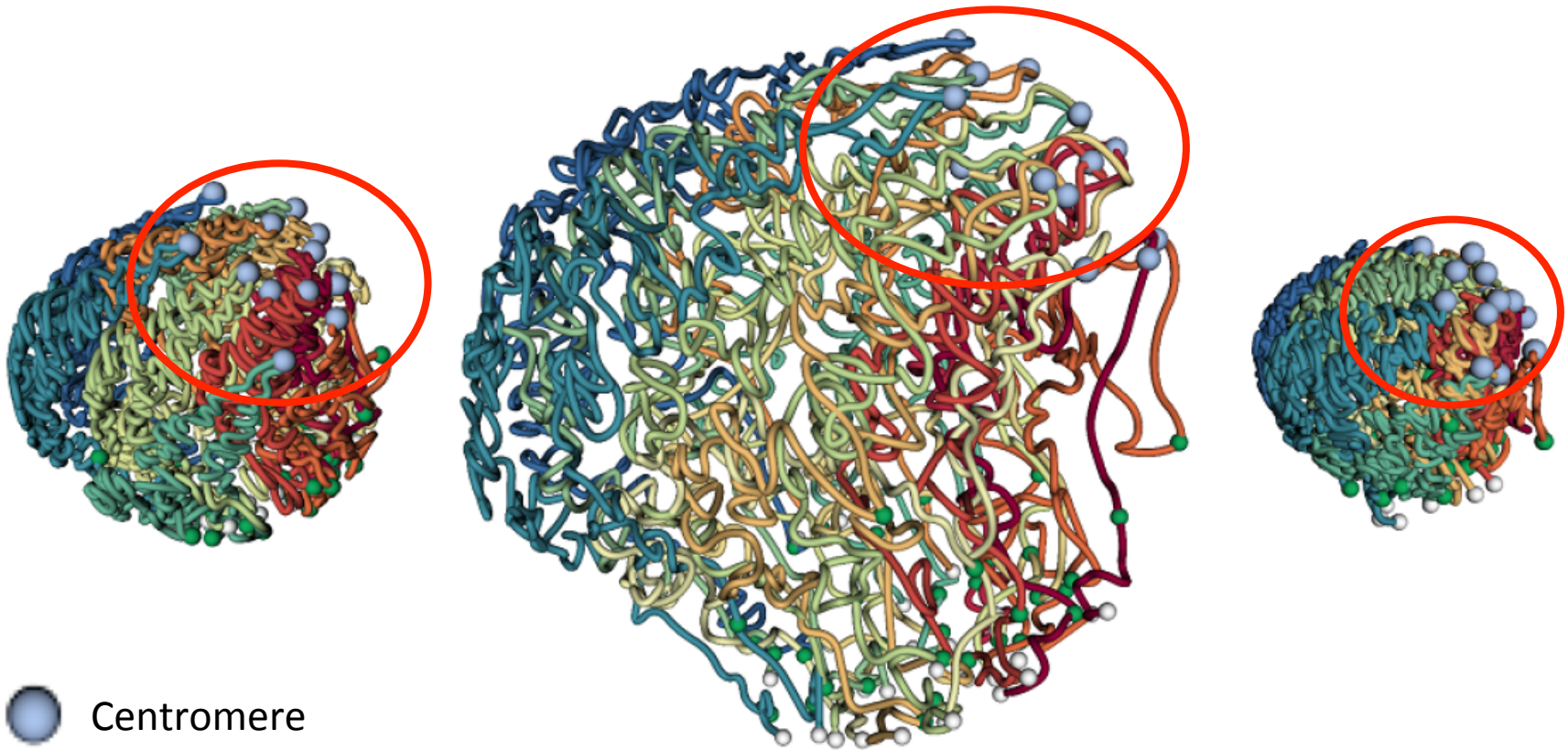
$$\mathcal{D} = \{ \delta_{ij} | \delta_{ij} \neq 0 \}$$

- Include constraints reflecting physical and biological prior knowledge.
  1. All loci must lie within a spherical nucleus centered on the origin.
     ,, (Weiner et al. Cell Microbiology, 2011).
  2. Two adjacent loci must not to be too far apart.
     1000 bp of chromatin occupies a distance between 6.6 to 9.1 nm (Bystricky et al. *PNAS*, 2004).

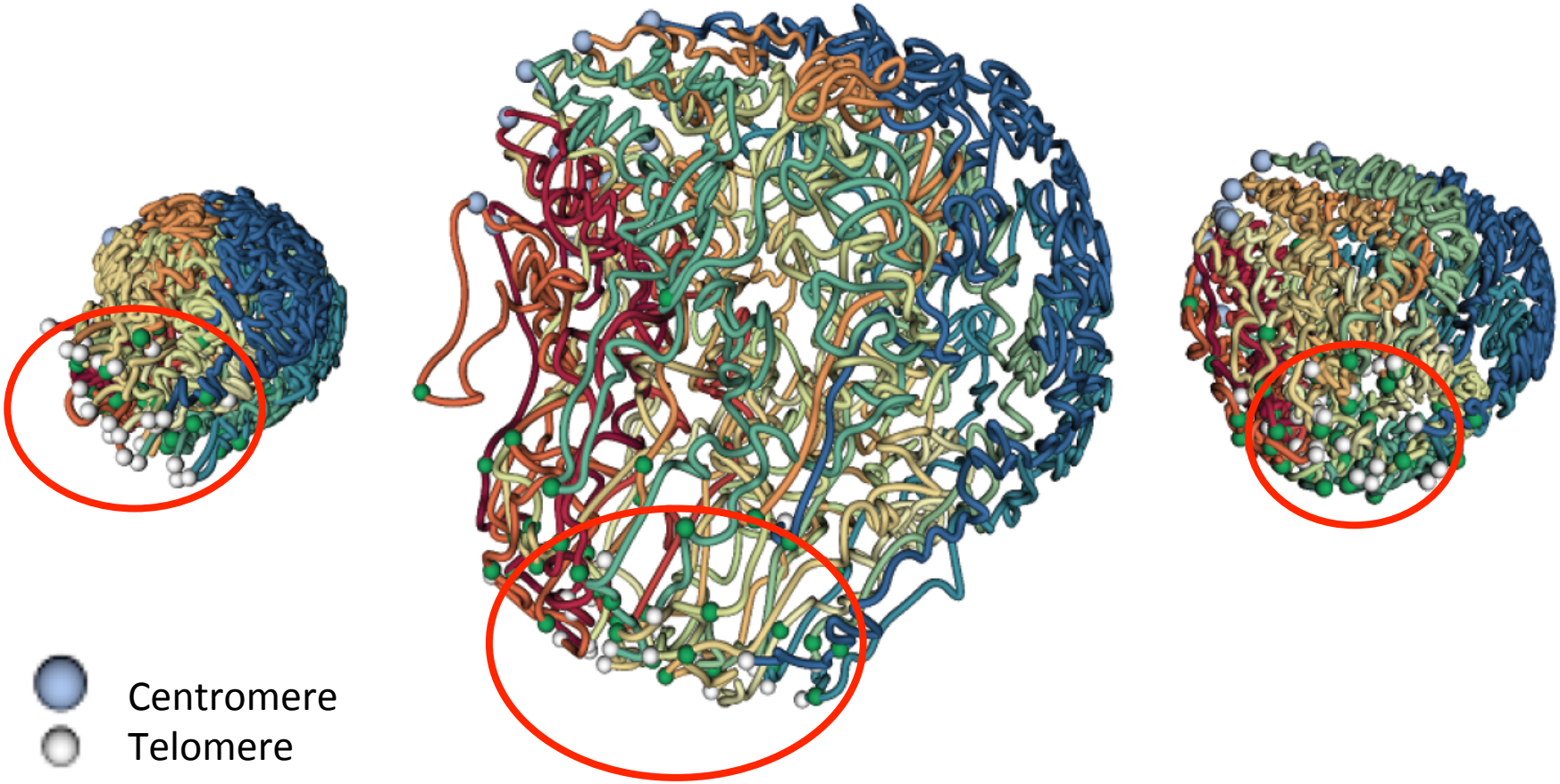
P. falciparum genome structure dynamic



Centromeres colocalize in 3D



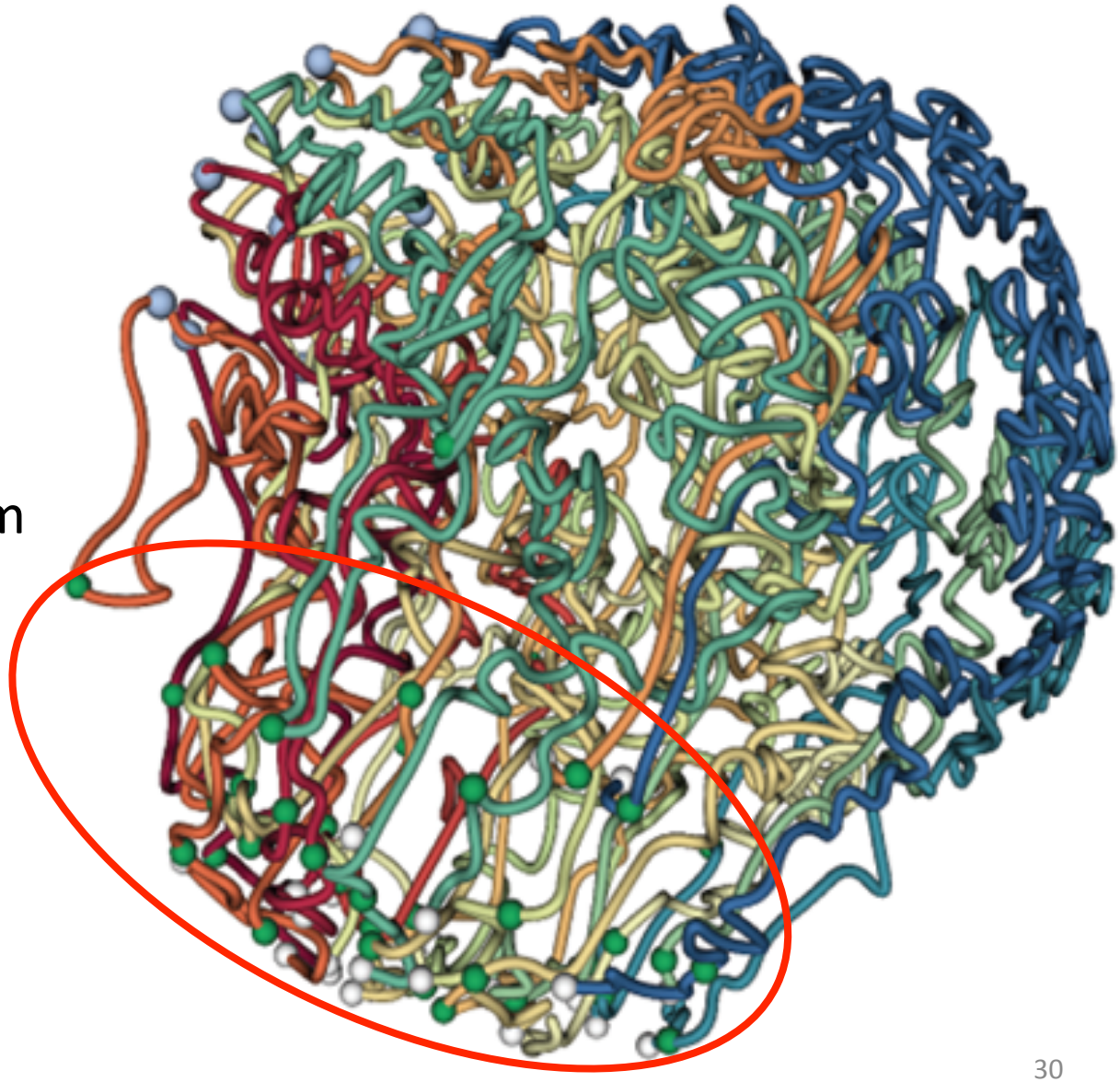
Telomeres colocalize in 3D



Virulence gene clusters colocalize in 3D

- *Plasmodium* encodes 60 virulence genes.
- Exactly one gene is expressed per cell.
- Regulatory mechanism of repression involves H3K36me3.

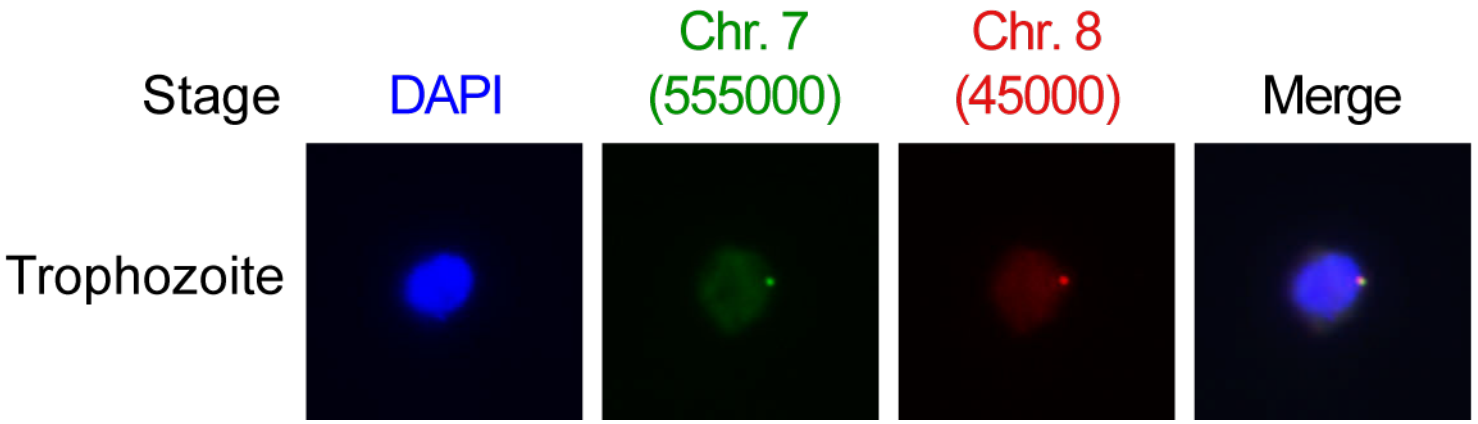
Jiang et al. *Nature* 2013.

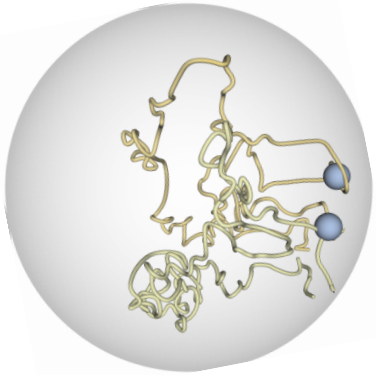


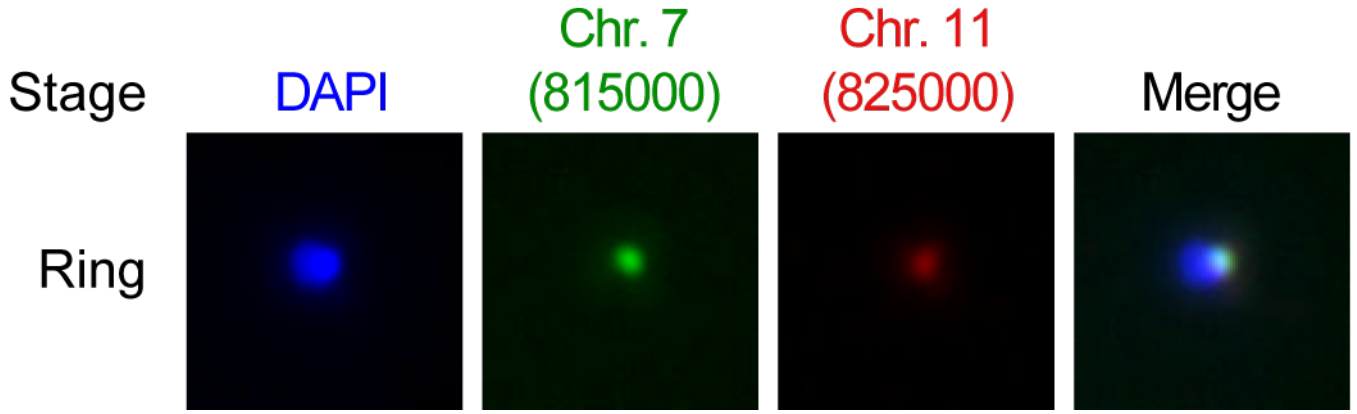
Centromere
Telomere
Virulence gene cluster

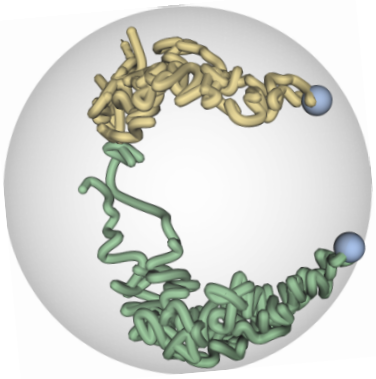
DNA FISH confirms selected contacts

Inter-chromosomal pair of virulence genes



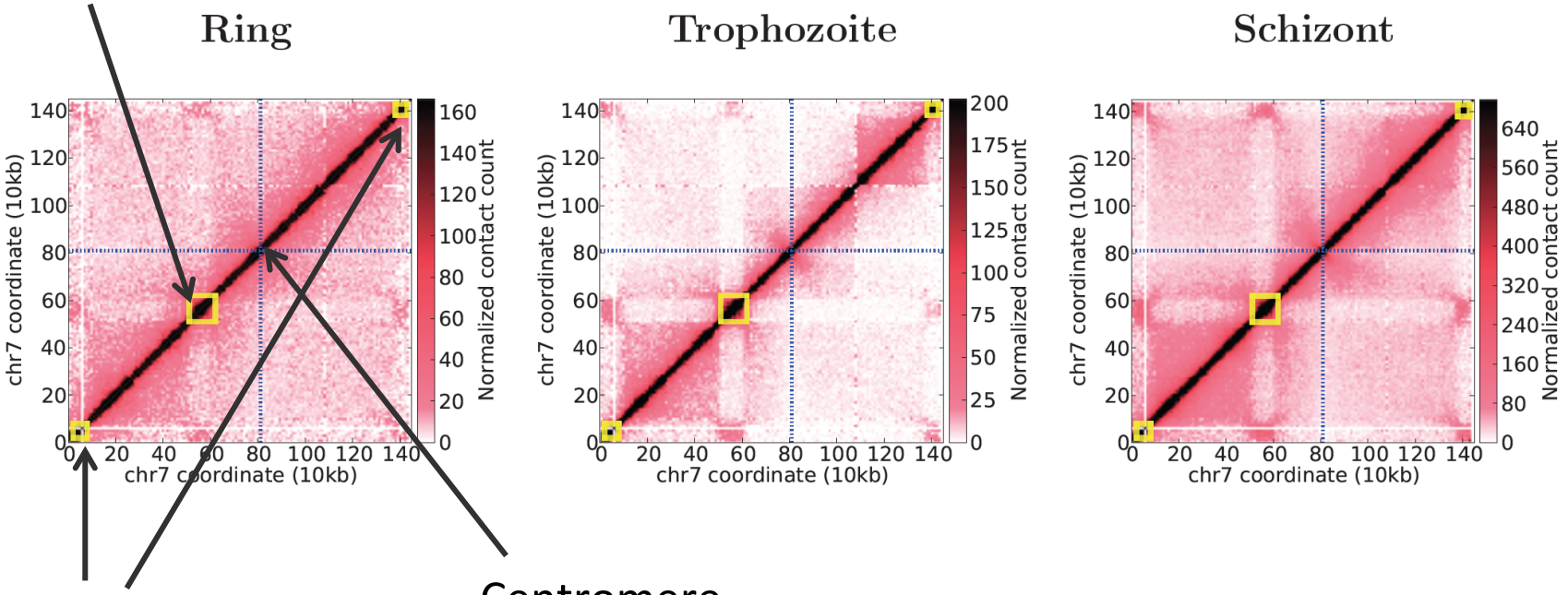






Clusters of virulence genes exhibit domain-like behavior at all stages

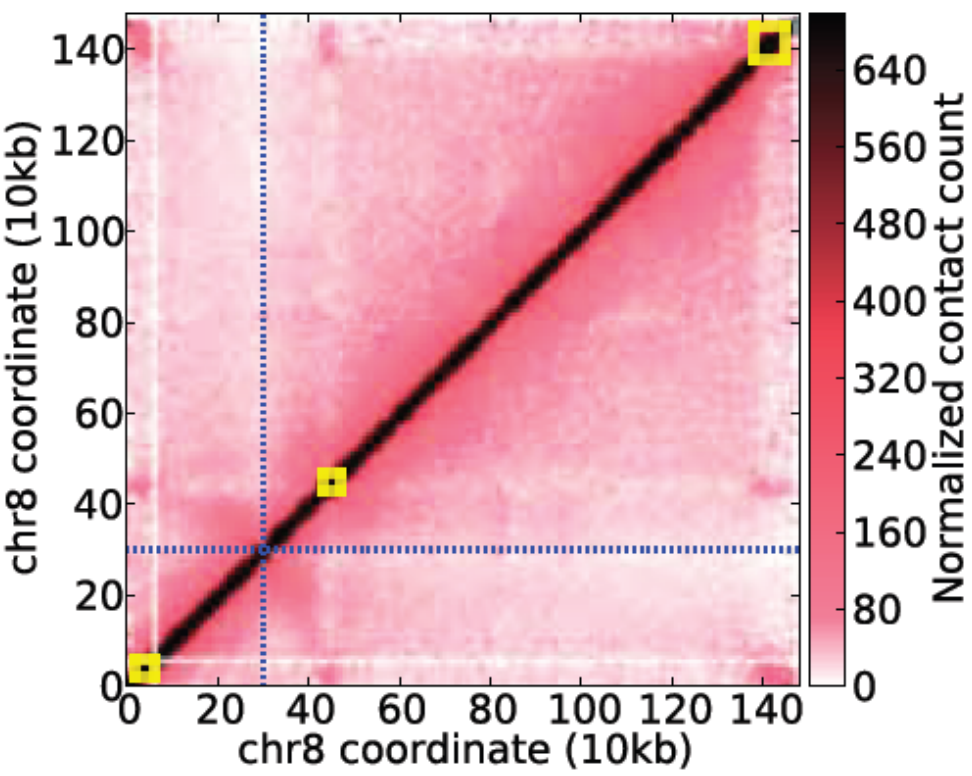
Internal virulence gene clusters



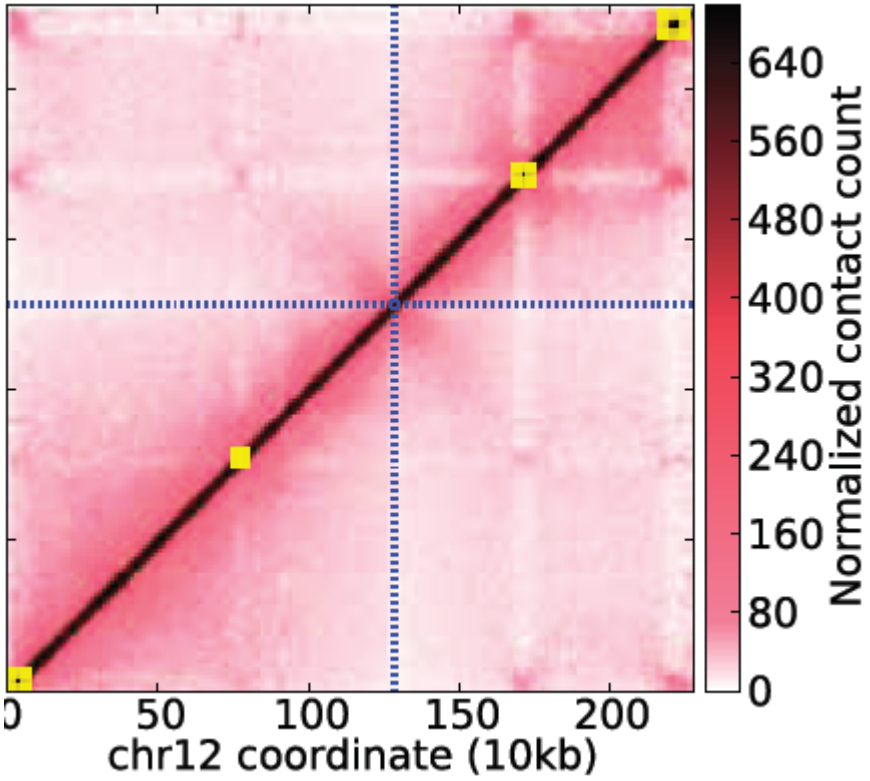
Chromosome 7

The pattern is consistent across chromosomes

Chromosome 8

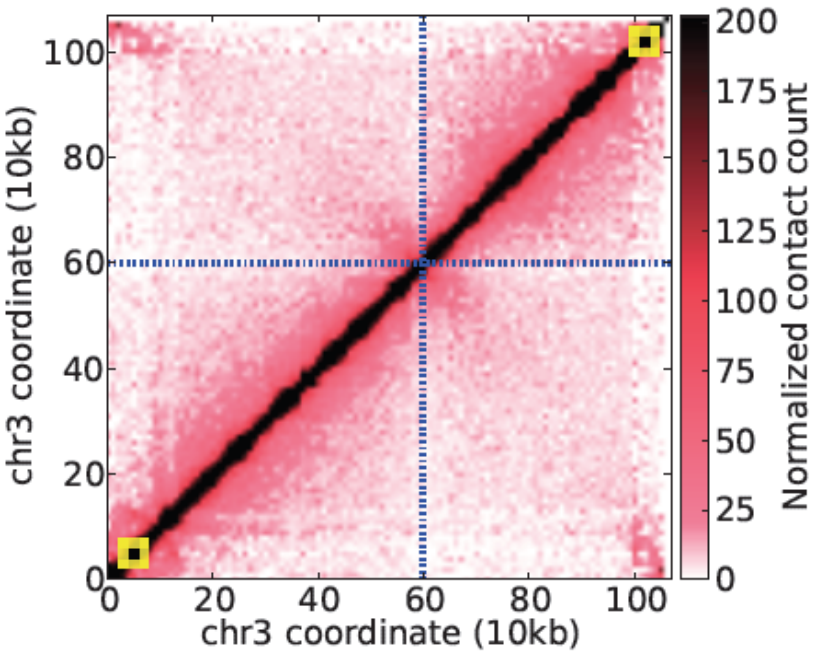


Chromosome 12

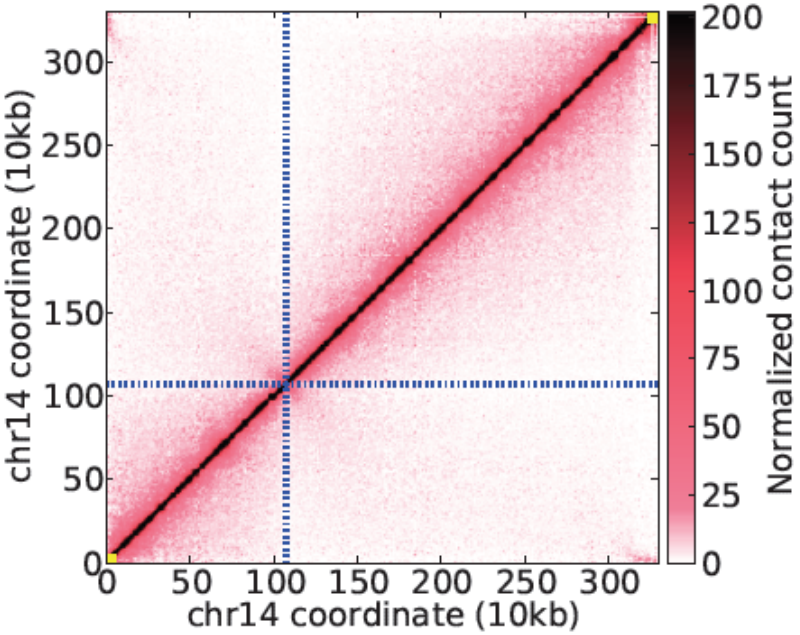


… and absent in chromosomes with no internal virulence gene clusters

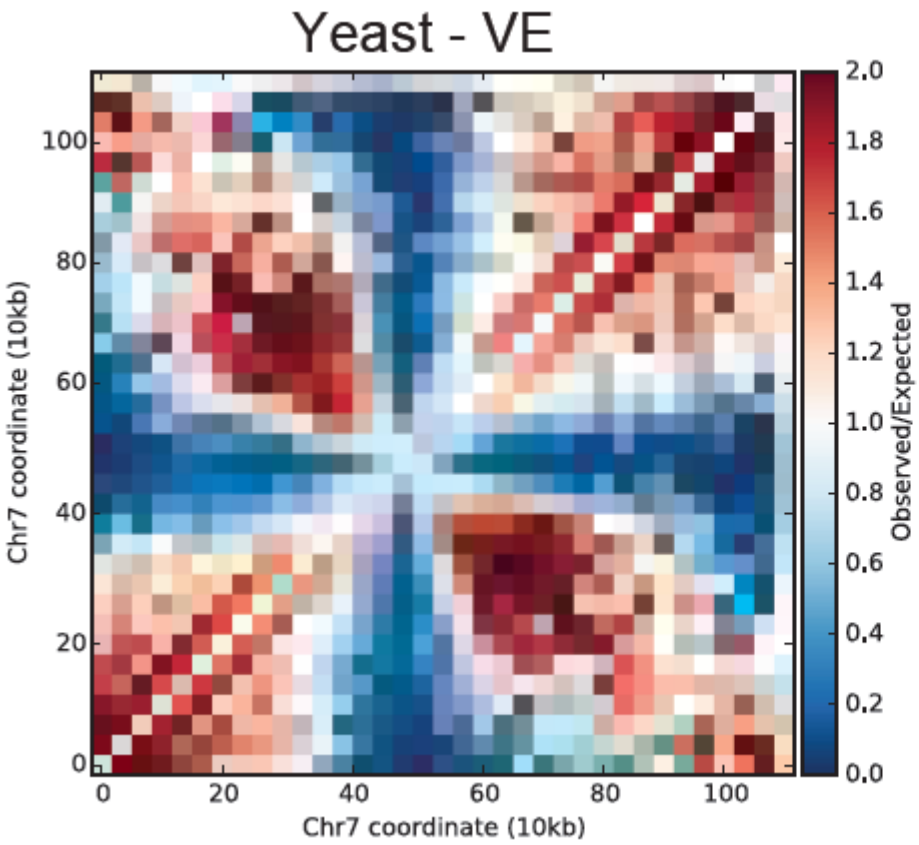
Chromosome 3

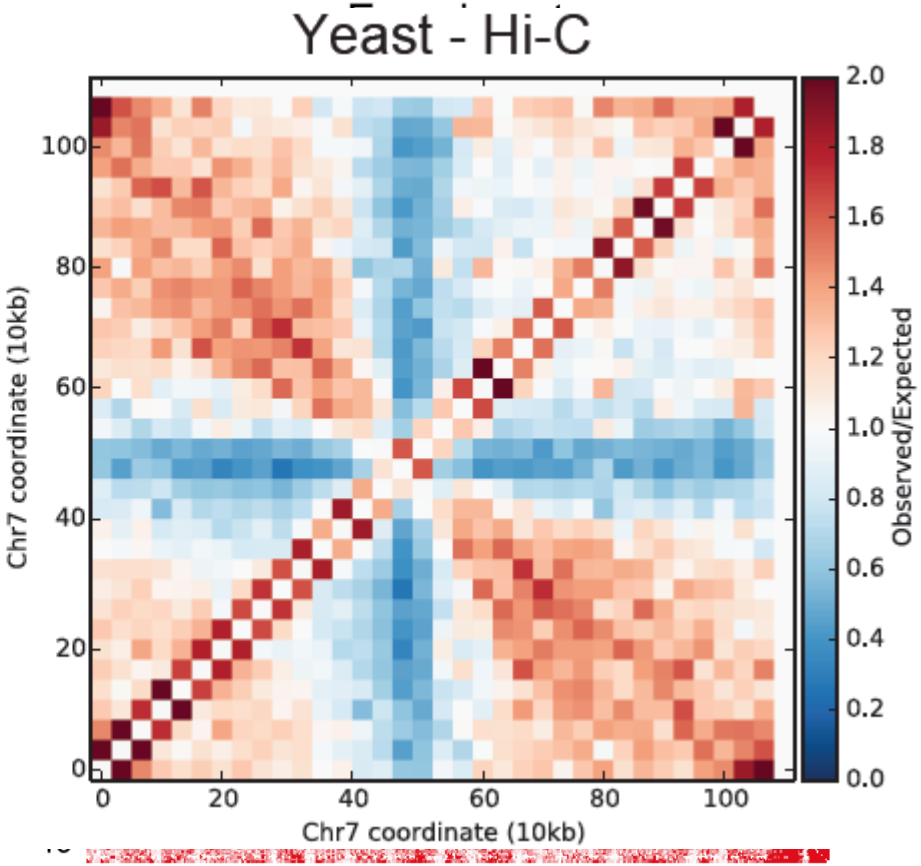


Chromosome 14



Simulated and observed chromatin contacts are highly concordant in yeast…



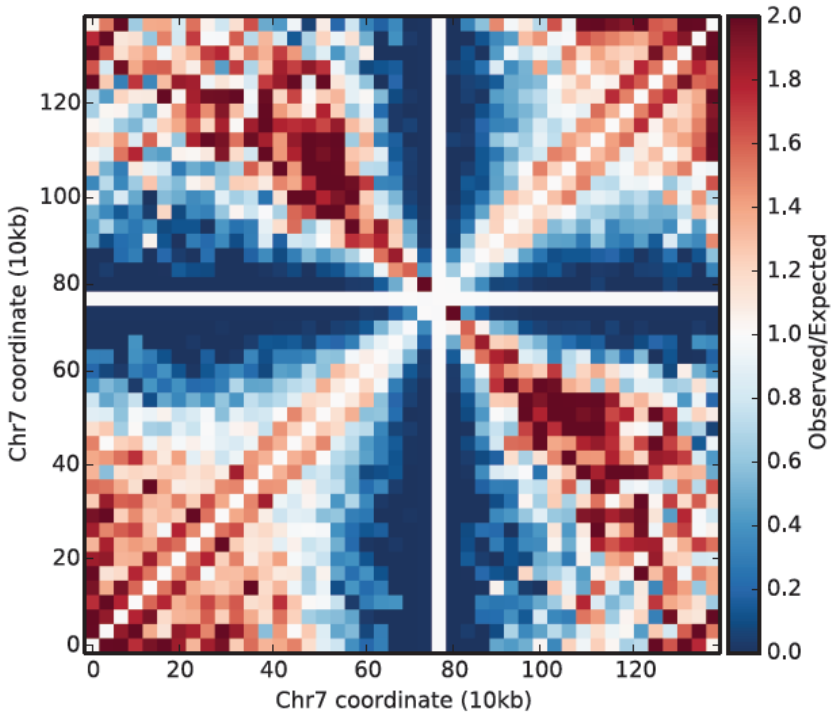


Tjong et al. *Genome Research* 2011.

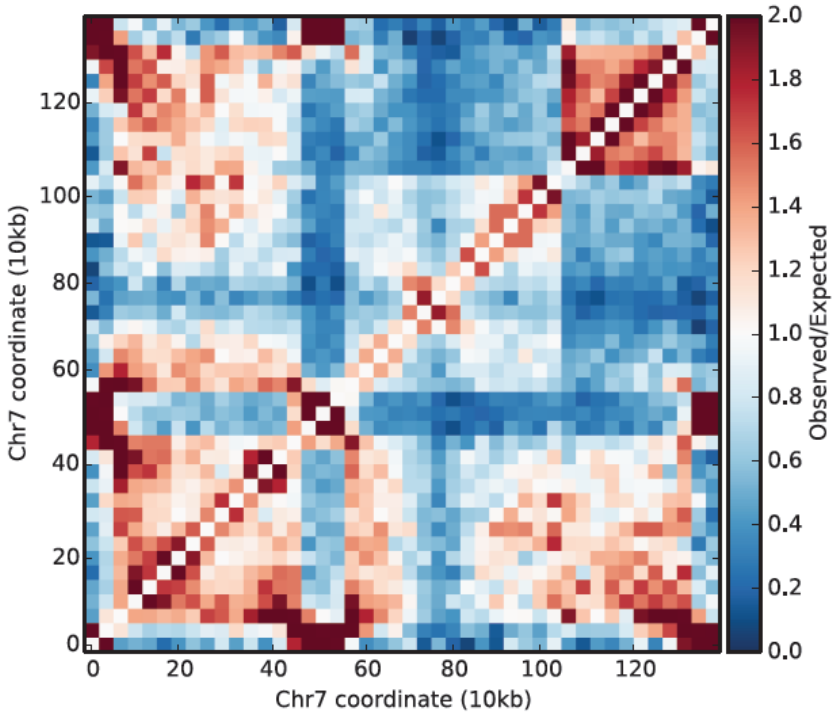
Duan et al. *Nature* 2010.

… but volume exclusion modeling does not capture *Plasmodium* architecture

Simulated

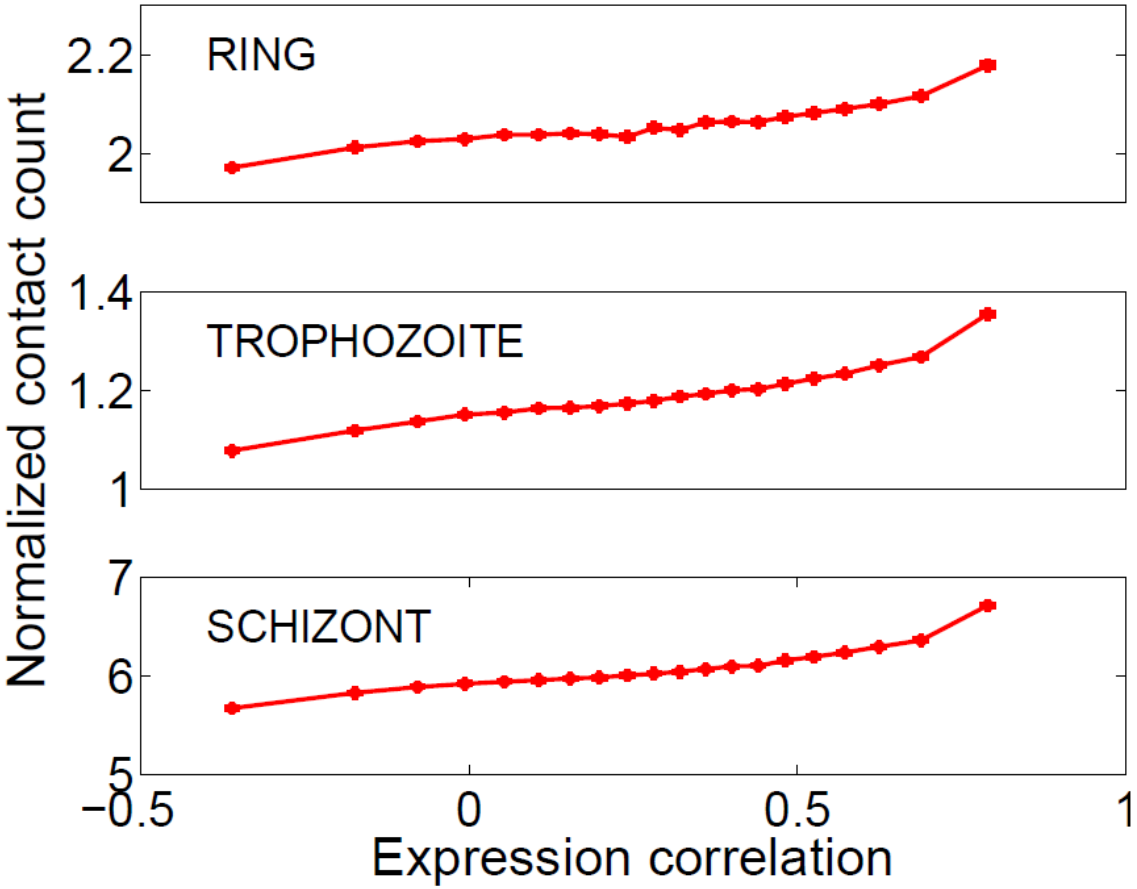


Observed



Chromosome 7, trophozoite stage

Genes that are close together exhibit correlated expression profiles

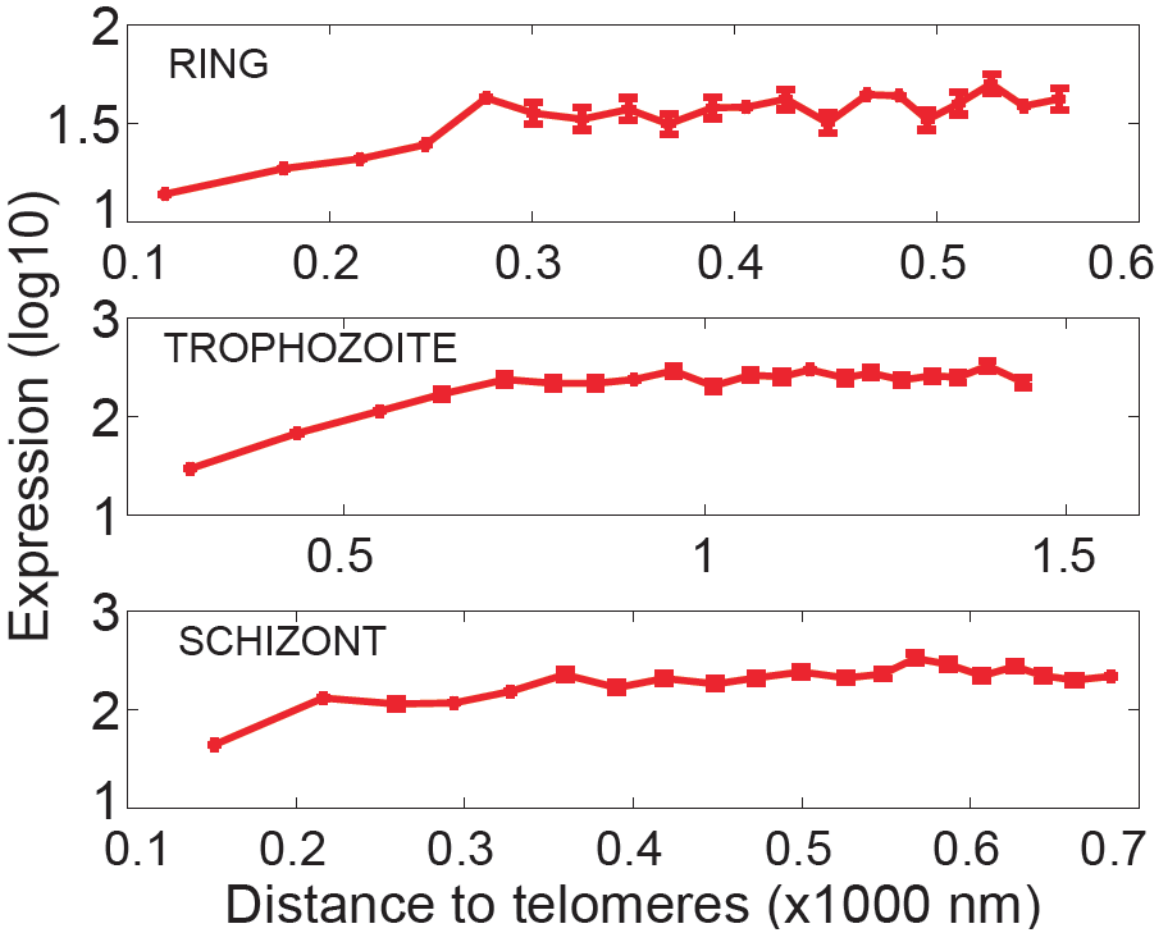


- Only inter-chromosomal gene pairs.
- Correlation between expression vectors:
  - Le Roch et al. *Science* 2003.
  - Otto et al. Mol. Microbiology 2010.
  - Lopez-B. et al. *BMC Genomics* 2011.
  - Bunnik et al. *Genome Biology* 2013.

Closer in 3D distance → more similar expression profile.

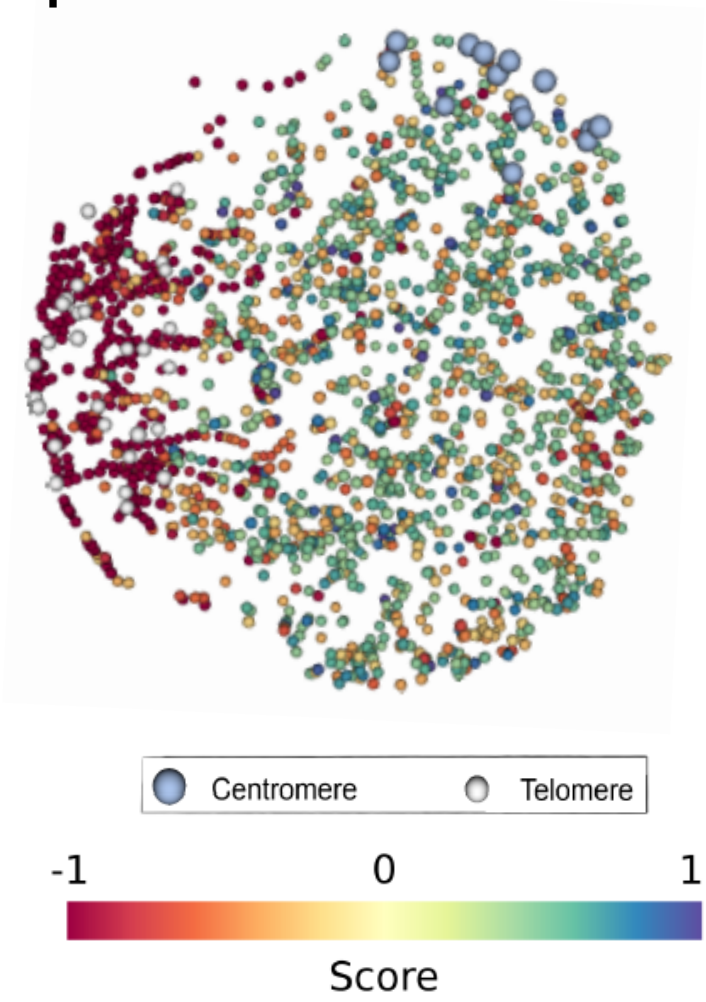
More in 3D distance → more similar expression profile.

Telomeres have a repressive effect on gene expression



Gene expression variation exhibits a gradient across the structure

Trophozoite



Telomeric

- Antigenic variation
- Sexual stage genes

Non-telomeric

- Translation
- Trophozoite genes

Kernel Canonical Correlation Analysis (kCCA)

Conclusions

- ✓ **Plasmodium genome architecture exhibits strong clustering of:**
  - centromeres, telomeres, virulence genes, and highly transcribed rDNA units.

- ✓ **Changes in power-law fits and chromosomal territories support a closed-open-closed model of the chromatin.**

- ✓ **Virulence gene clusters exhibit domain-like behavior.**

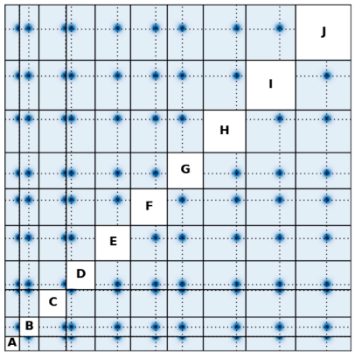
- ✓ **Genes with similar expression profiles tend to be in close spatial proximity.**

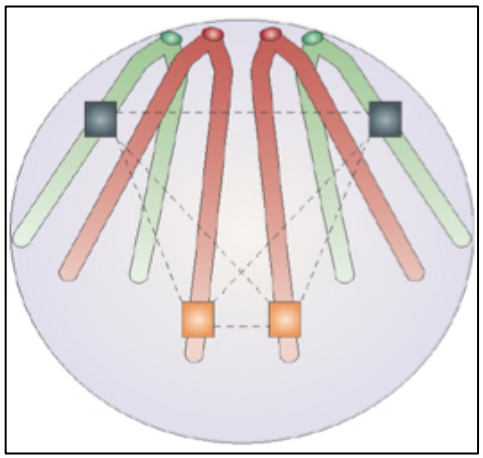
***Plasmodium* species may be excellent model organisms to study the impact of genome structure on gene regulation.**

F. Ay, E. M. Bunnik, N. Varoquaux, S. M. Bol, J. Prudhomme, J.-P. Vert, W. S. Noble and K. G. Le Roch, "Three-dimensional modeling of the P. falciparum genome during the erythrocytic cycle reveals a strong connection between genome architecture and gene expression » *Genome Research*, 24:974-988, 2014.

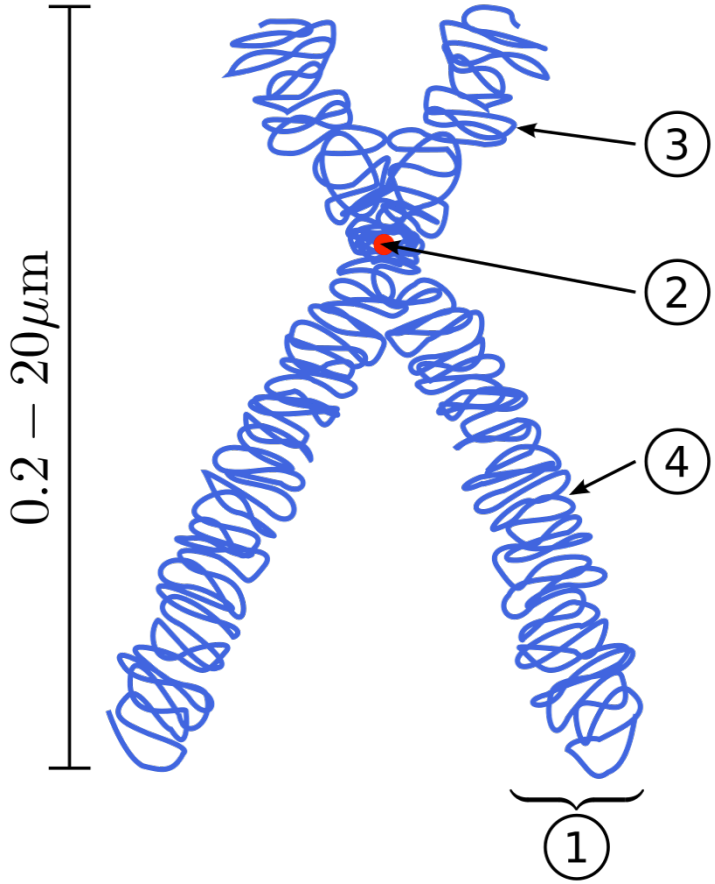
Part 3

Centromere calling from metagenomic Hi-C data

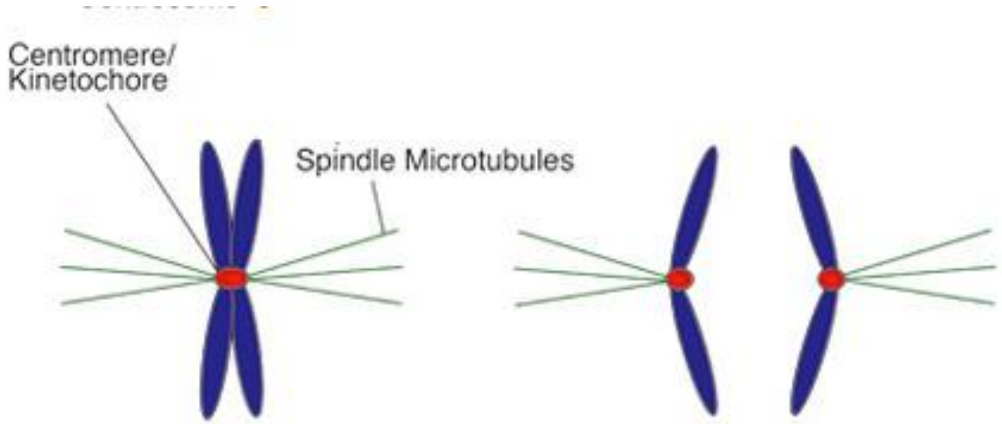




Centromeres (CEN)



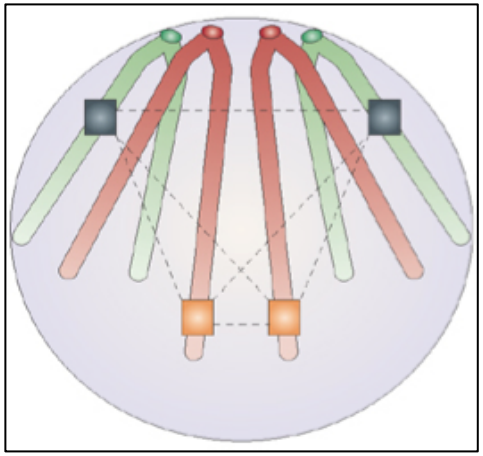
- part of a chromosome that links sister chromatid



Where are yeast CENs?

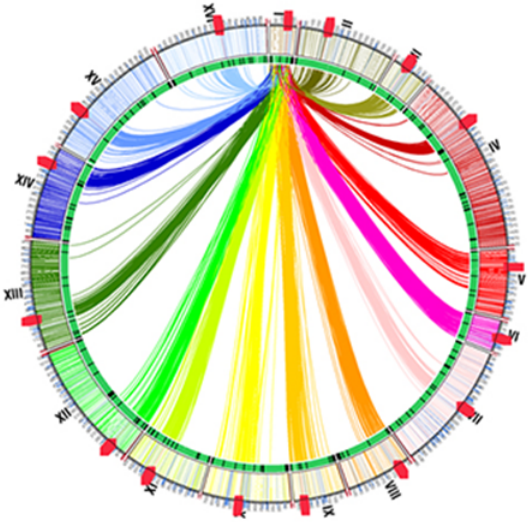
- Important for bioengineering (billion dollar industry)
- Still unknown for major yeast species
- Point centromeres: hard to find using traditional methods
  - CEN consists of 2-3 binding site motifs
  - Virtually no heterochromatin

CENs are strongly clustered in 3D for yeasts and some other species

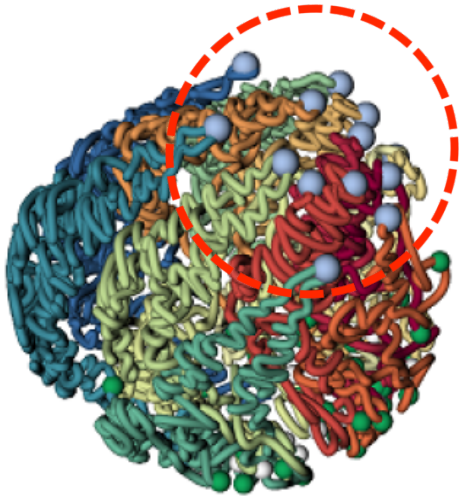


S. cerevisae: Jin, et al.
J. Cell Sci, 2000.

Barzel and Kupiec,
Nat Rev Genet, 2008

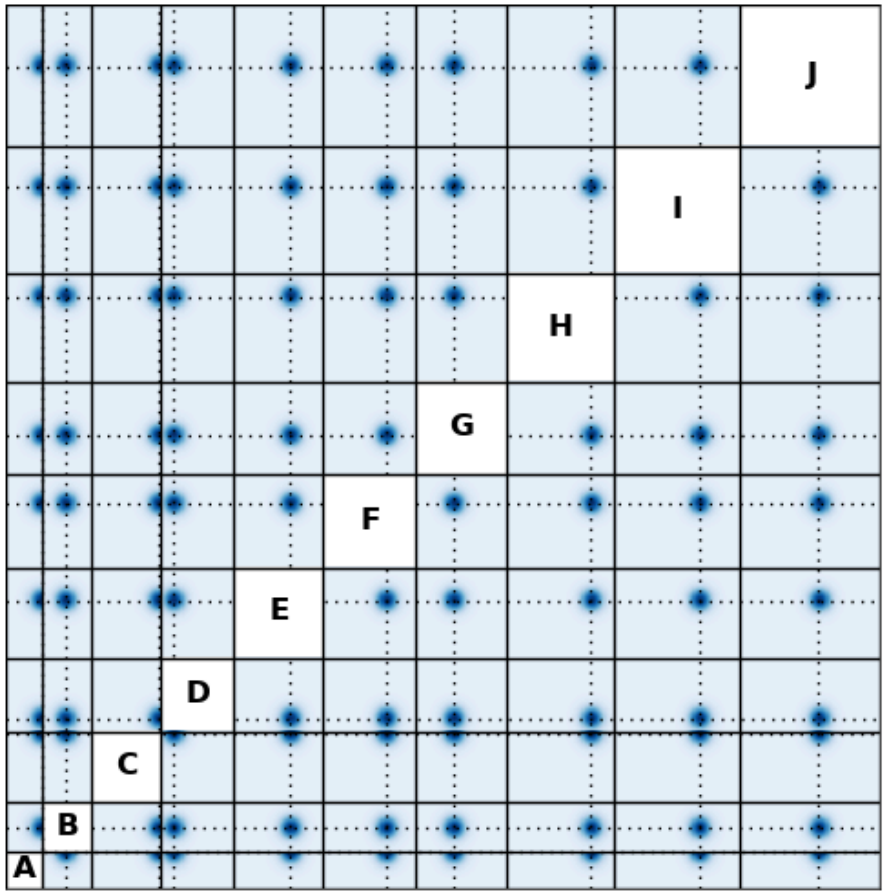


S. cerevisae:
Duan, *et al*. Nature, 2010

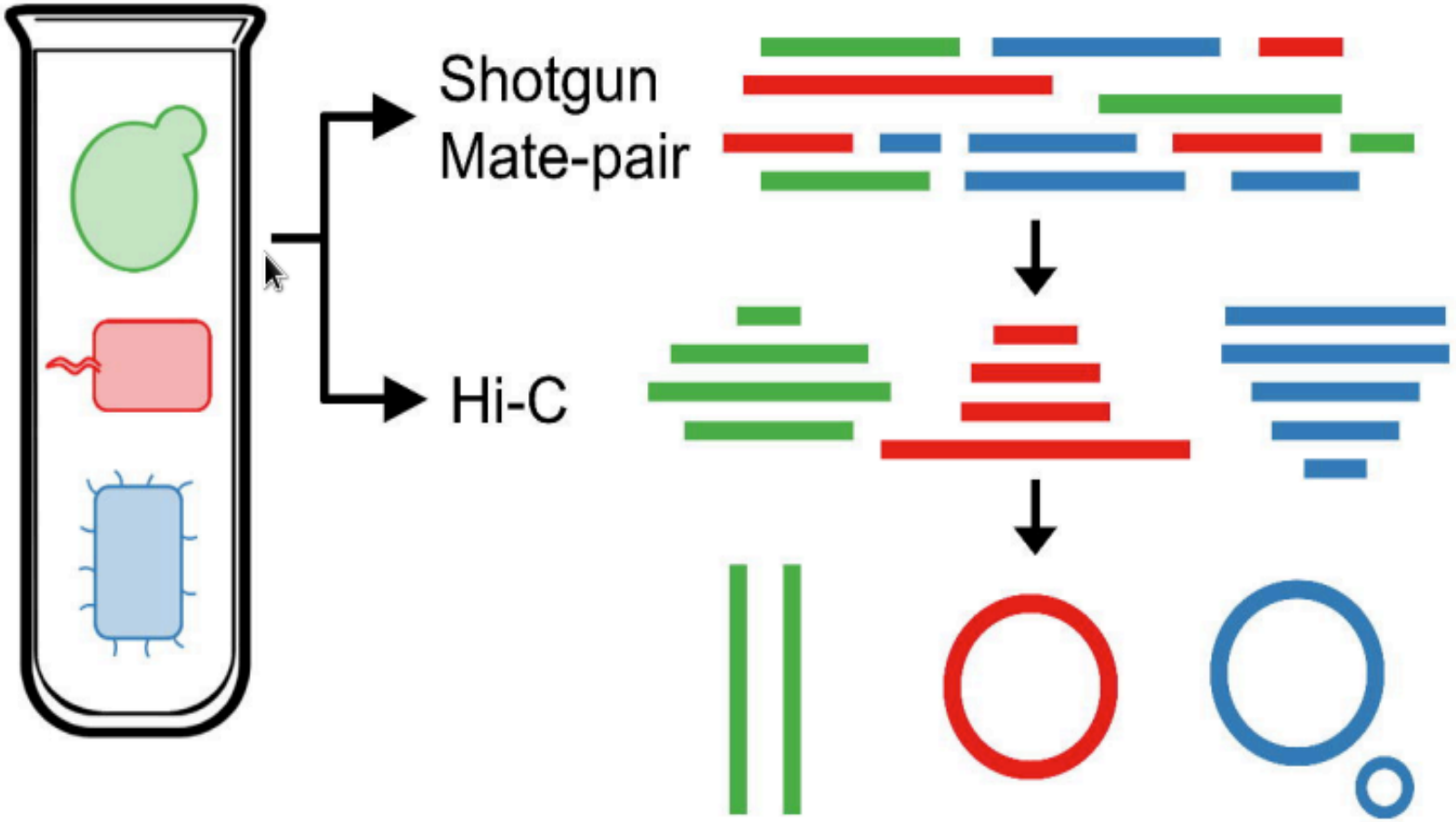


P. falciparum:
Ay, *et al.* Genome Res., 2014a

Idea: use Hi-C data to call CENs



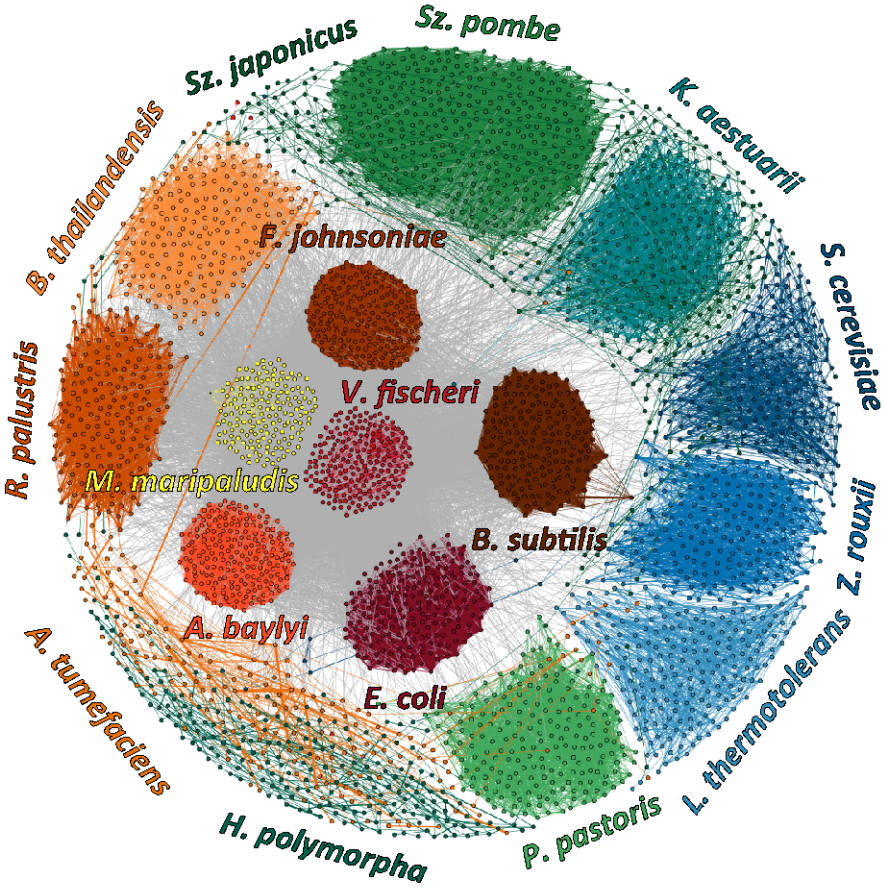
Data: Hi-C on metagenomic samples



Two datasets

Mixture of Yeasts (M-Y)
16 yeast strains

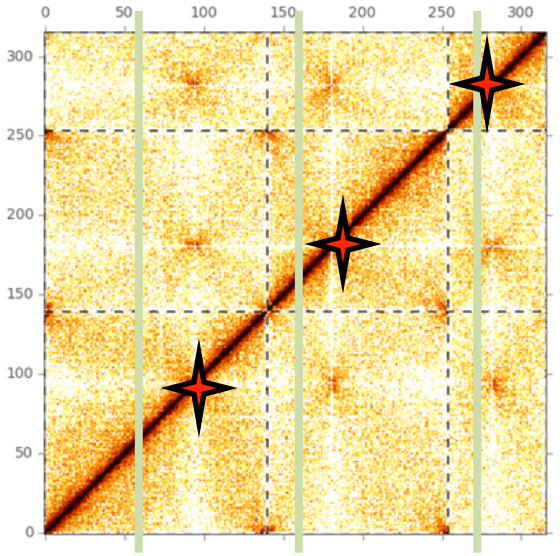
Mixture of 3 Domains (M-3D)
8 yeasts, 9 bacteria, and 1 archaeon



Burton, Liachko, *et al*. 2014

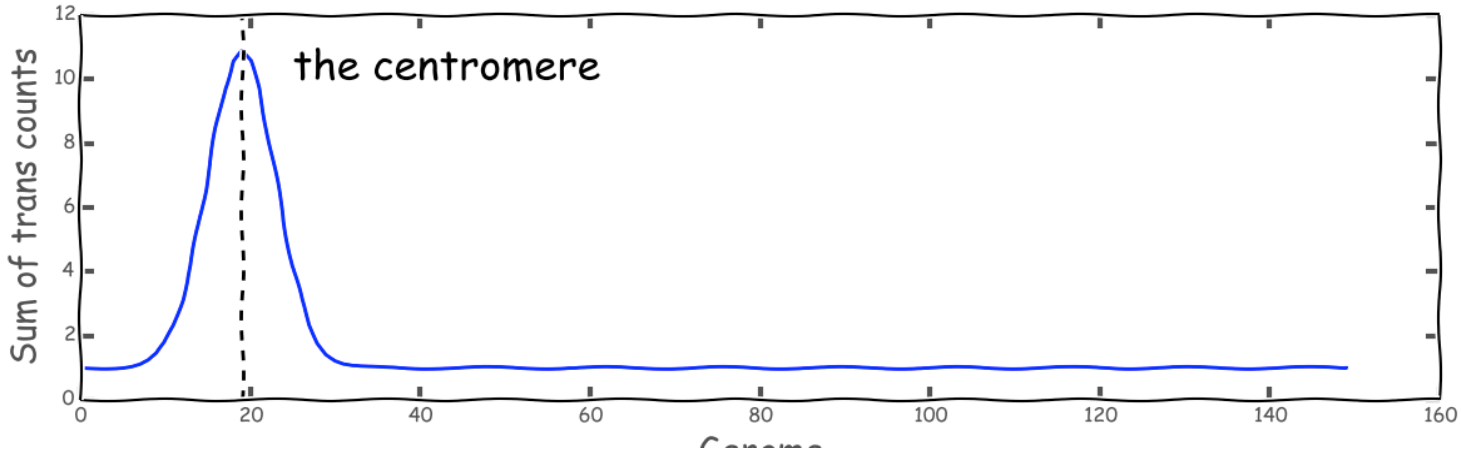
Outline of centromere identification for multiple yeasts from Hi-C contact maps

1. Align paired-end reads to a concatenated reference genome.
2. Post-process mapping results and create a contact map for each organism.
3. Pool data from all replicates (e.g., different restriction enzymes) for each organism.
4. Normalize contact maps for experimental/technical biases (10, 20, 40 kb).
5. Make “initial guesses” for each centromere using marginalized inter-chromosomal (trans) contact counts.
6. Starting from the initial guesses find a centromere position for each chromosome that best explains the observed inter-chromosomal contact count pattern.

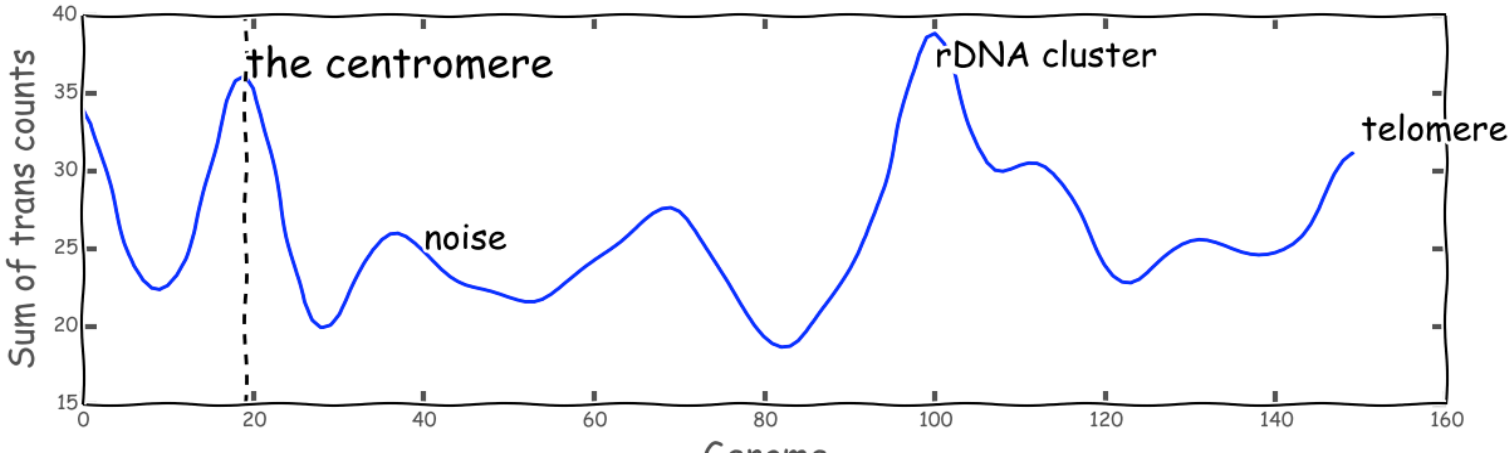


Initial guess of CEN localization

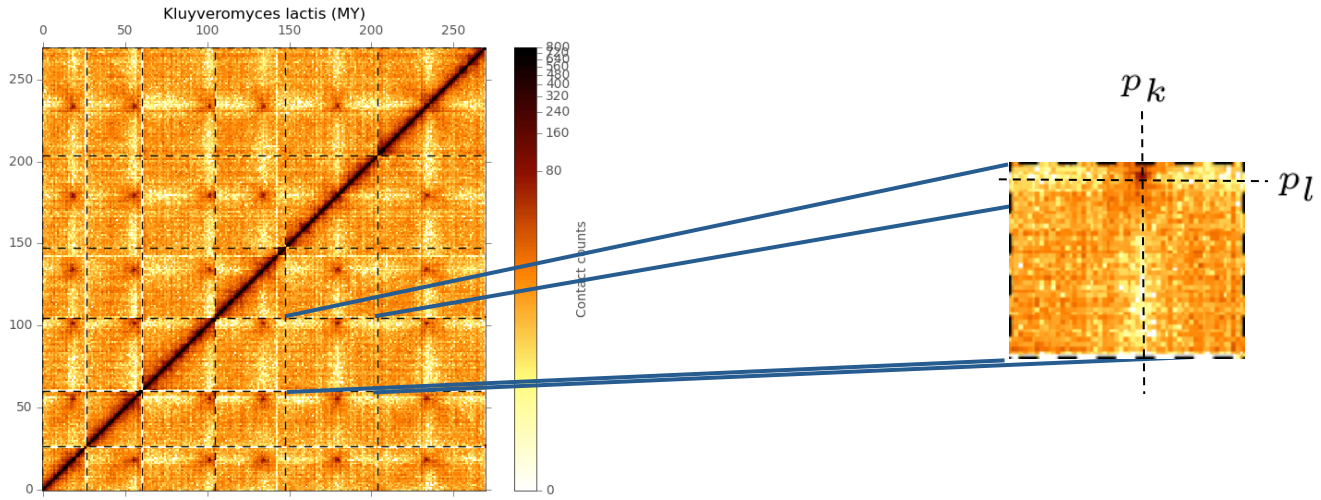
Ideal



Observed



Optimization from initial guess



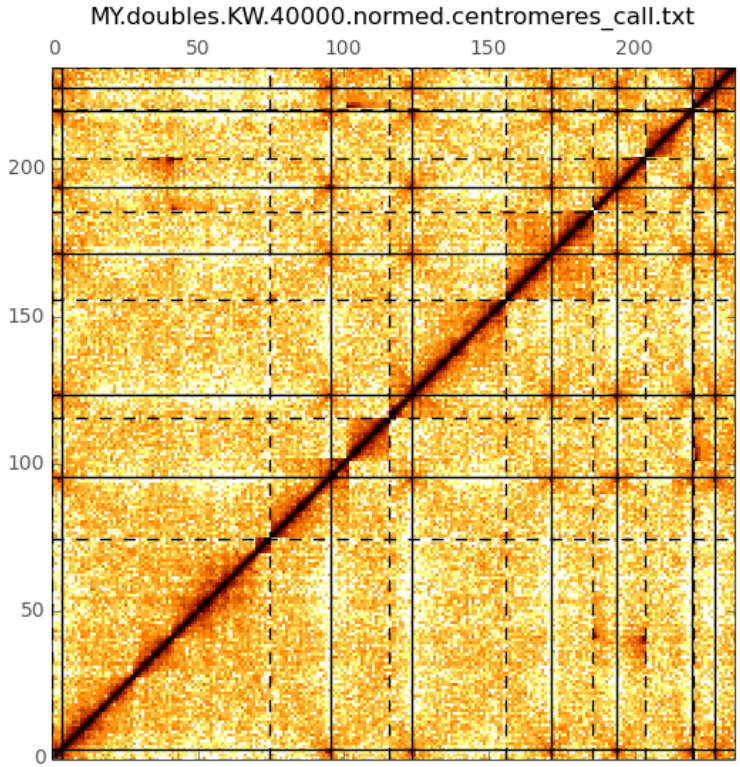
Gaussian centered in pairs of centromeres

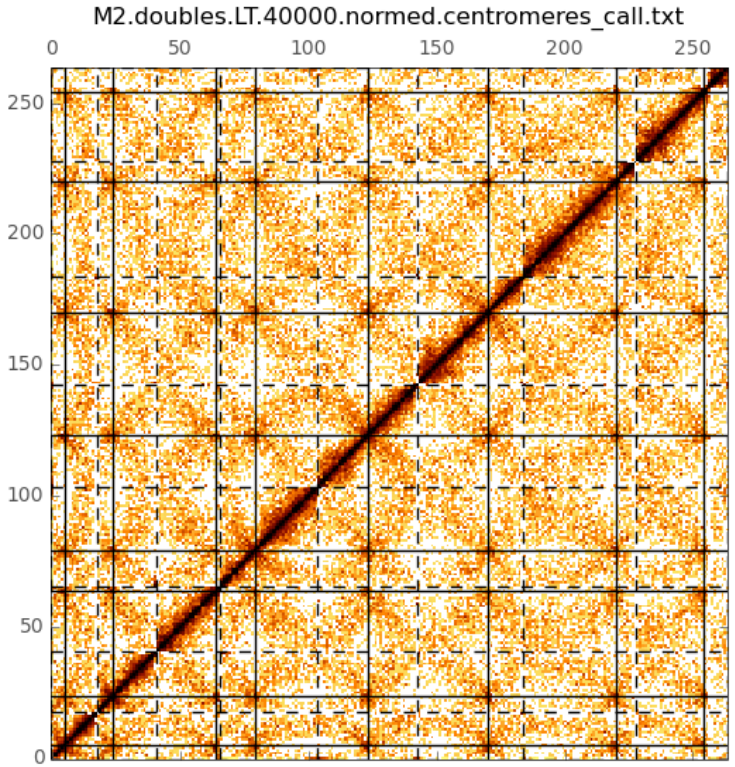
Data

$$\underset{\mathbf{P}}{\text{minimize}} \quad \sum_{(i,j)\in\mathcal{D}} \left(c_{ij} - a \sum_{k,l} \mathbb{1}_{[i\in\mathcal{D}_k]} \mathbb{1}_{[j\in\mathcal{D}_l]} e^{\frac{(i-p_k)^2-(j-p_l)^2}{2\sigma^2}} \right)^2$$

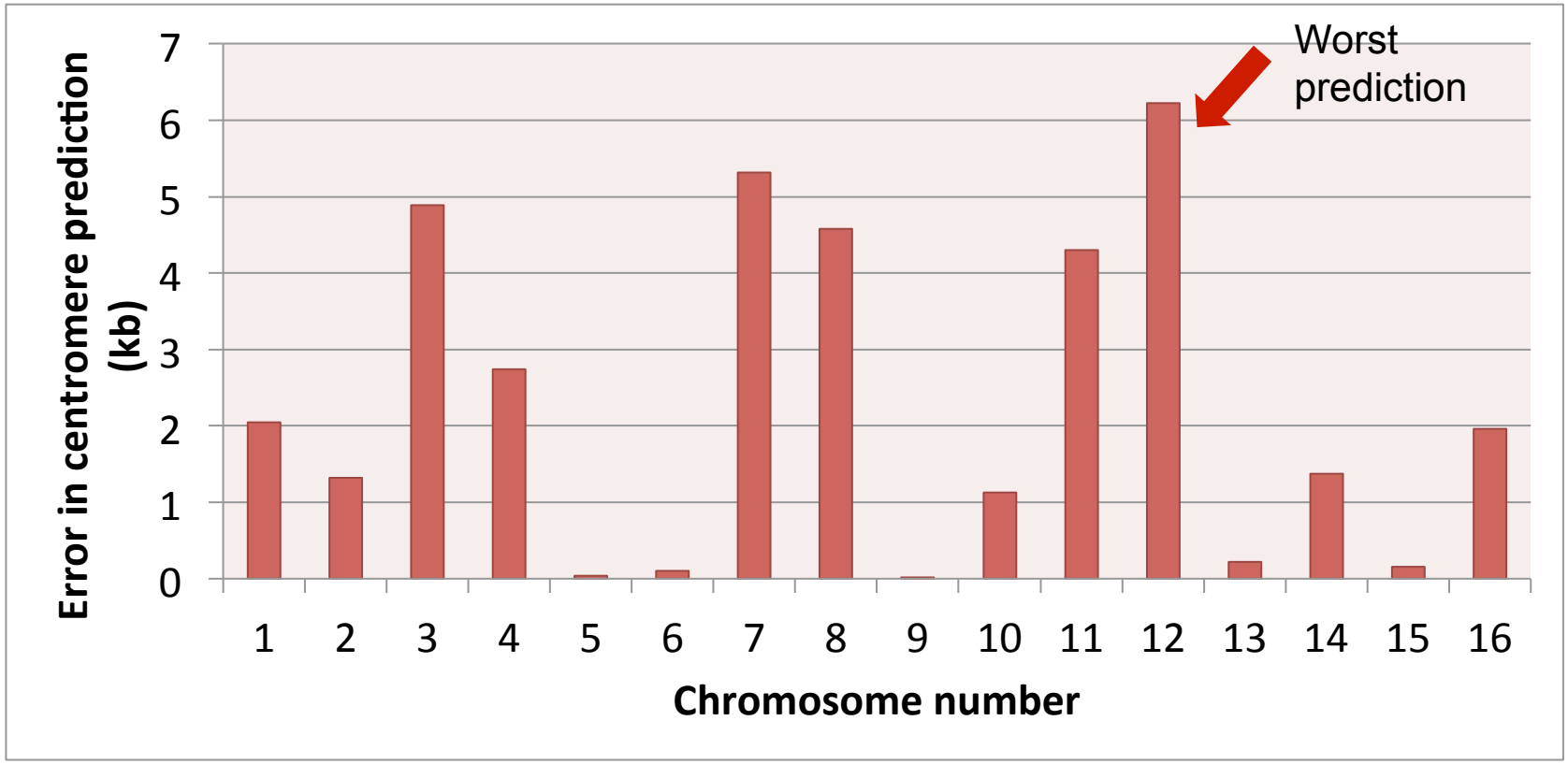
$$\text{subject to} \quad p_l \in \mathcal{D}_l \quad \forall l \in [1,\ldots,K]$$

Results


MY.doubles.KW.40000.normed.centromeres_call.txt
0
50
100
150
200
200
150
100
50
0


M2.doubles.LT.40000.normed.centromeres_call.txt
0
50
100
150
200
250
250
200
150
100
50
0

Accuracy on S. cerevisiae



10 out of 16 predictions are within ~2 kb of the known mid-point of the centromere

Hi-C data from Duan, *et al*. Nature, 2010

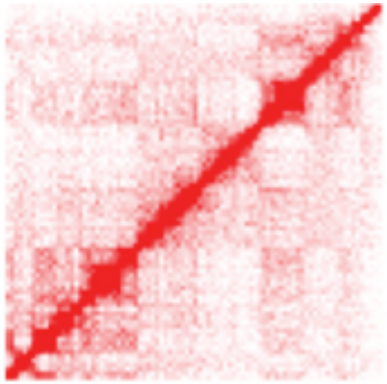
Average prediction error per genome

Organism	**Our method**	**Marie-Nelly et al. (*Bioinformatics*, 2014)**
L. kluyveri (M-Y)	**< 5 kb**	~ 312 kb
S. pombe (M-PE)	**< 11 kb**	~ 574 kb
S. mikatae (M-Y)	**< 6 kb**	~ 124 kb
*S. bayanus** (M-Y)	**< 6 kb**	~ 113 kb
K. lactis (M-Y)	**< 9 kb**	~ 19 kb

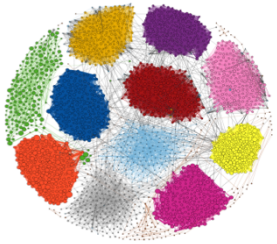
* only validated on the partial ground truth available

~5-10M paired-end reads enough per species (that's $50-$100)!

Conclusion : The many uses of Hi-C

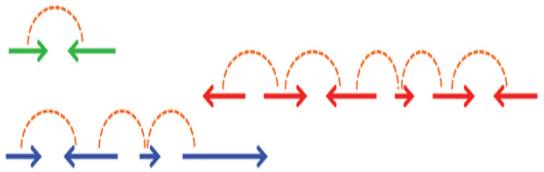


Lieberman-Aiden, *et. al*.
Science, 2009



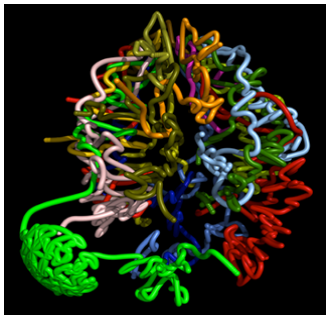
Organismal Deconvolution

Burton, Liachko, *et al*. G3, 2014



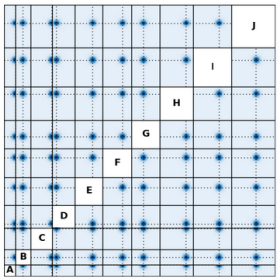
Genome scaffolding

Burton, *et al*.
Nature Biotech, 2013

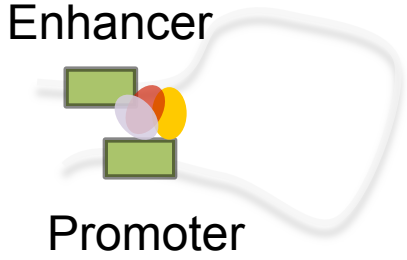


3D model of genome

Duan, *et al*. Nature, 2010 *(S. cerevisae),*
Ay, *et al*. Genome Res., 2014a *(P.falciparum)*



Centromere calling



Long-range chromatin contacts

Ay, *et al*. Genome Res., 2014b

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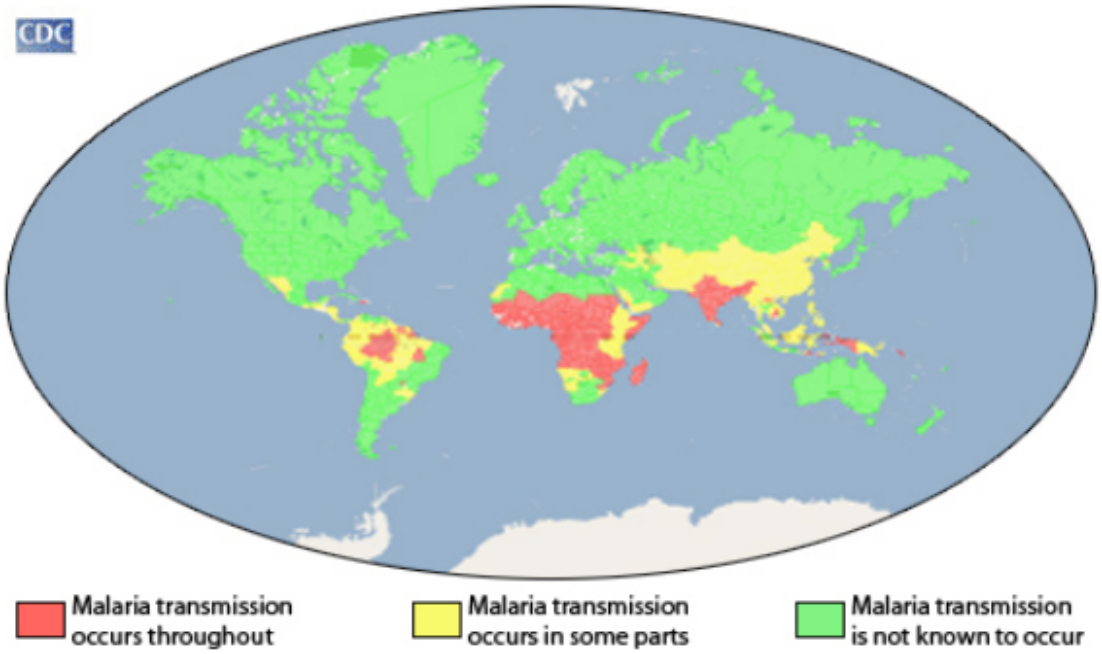
INVESTISSEMENTS D'AVENIR
SEVENTH FRAMEWORK PROGRAMME
erc

APPENDIX

- *Plasmo-3D*

Malaria facts

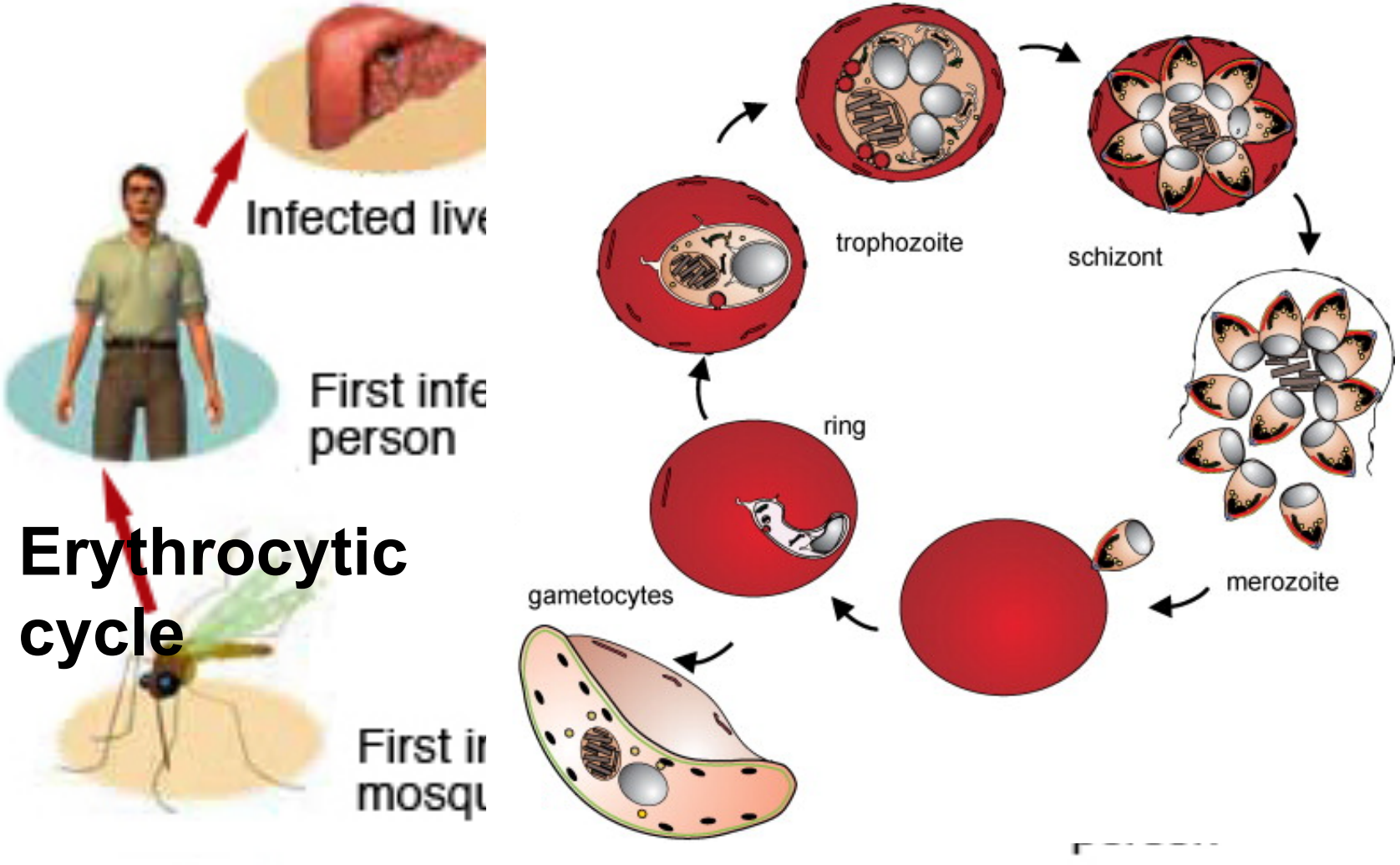
- About 3.3 billion people are at risk of malaria. [1]
- In 2010, ~219 million cases and ~660 000 deaths. [1]
- In sub-Saharan Africa over 75% of cases were due to *P. falciparum*. [2]
- Almost every malarial de



[1] http://www.cdc.gov/malaria/about/facts.html

[2] World Malaria Report 2008, WHO.

Malaria transmission cycle



The first malaria parasite genome was published over a decade ago

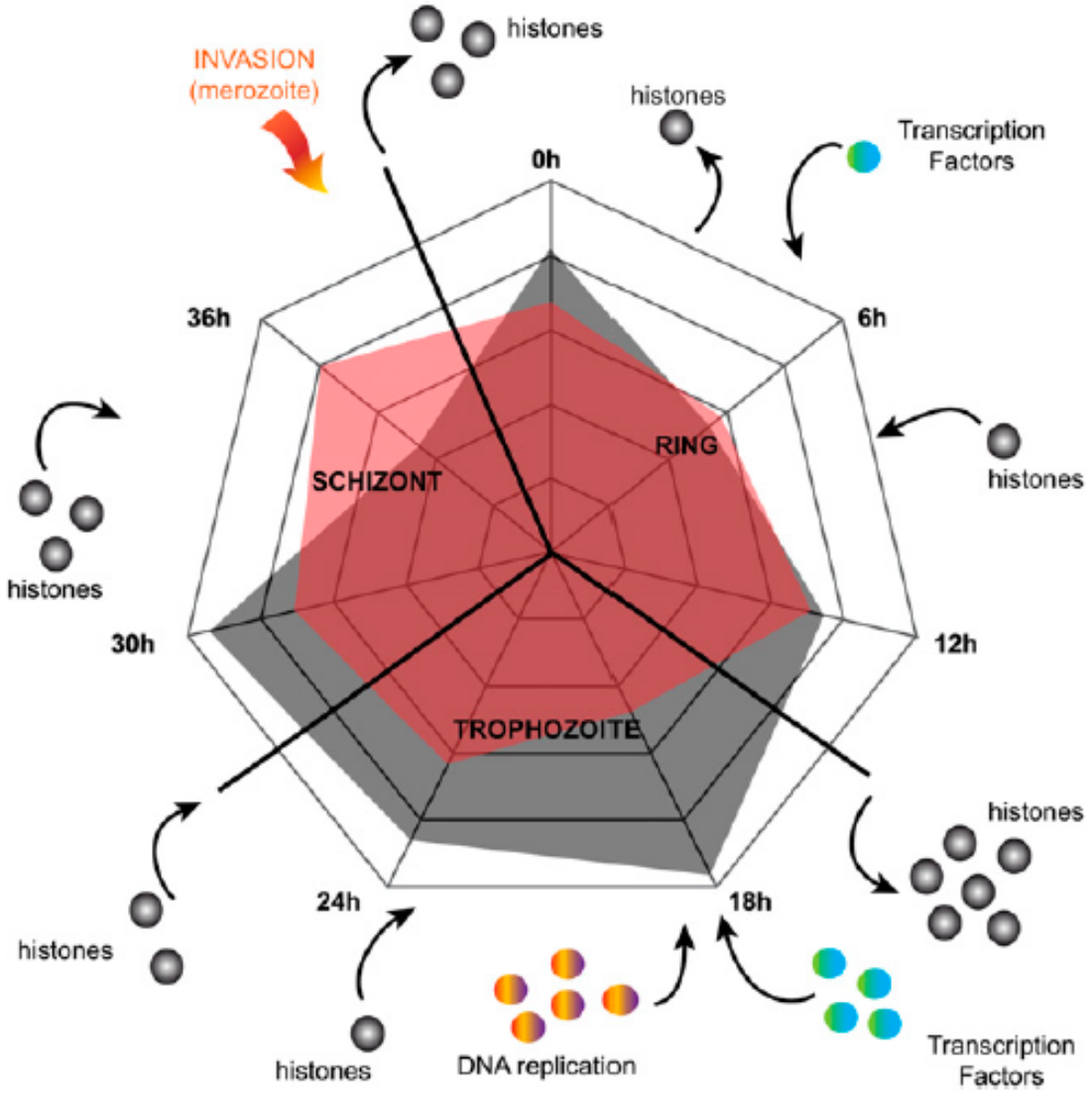
- 23.26 Mb in size.
- Haploid.
- 14 nuclear chromosomes, 1 mitochondrion, 1 plastid.
- ~6372 genes and 5524 protein coding genes.
- The most AT rich genome to date (~80%).
- 47% are still hypothetical proteins.

M. J Gardner et al. *Nature* 2002

Plasmodium chromatin architecture goes through systematic changes

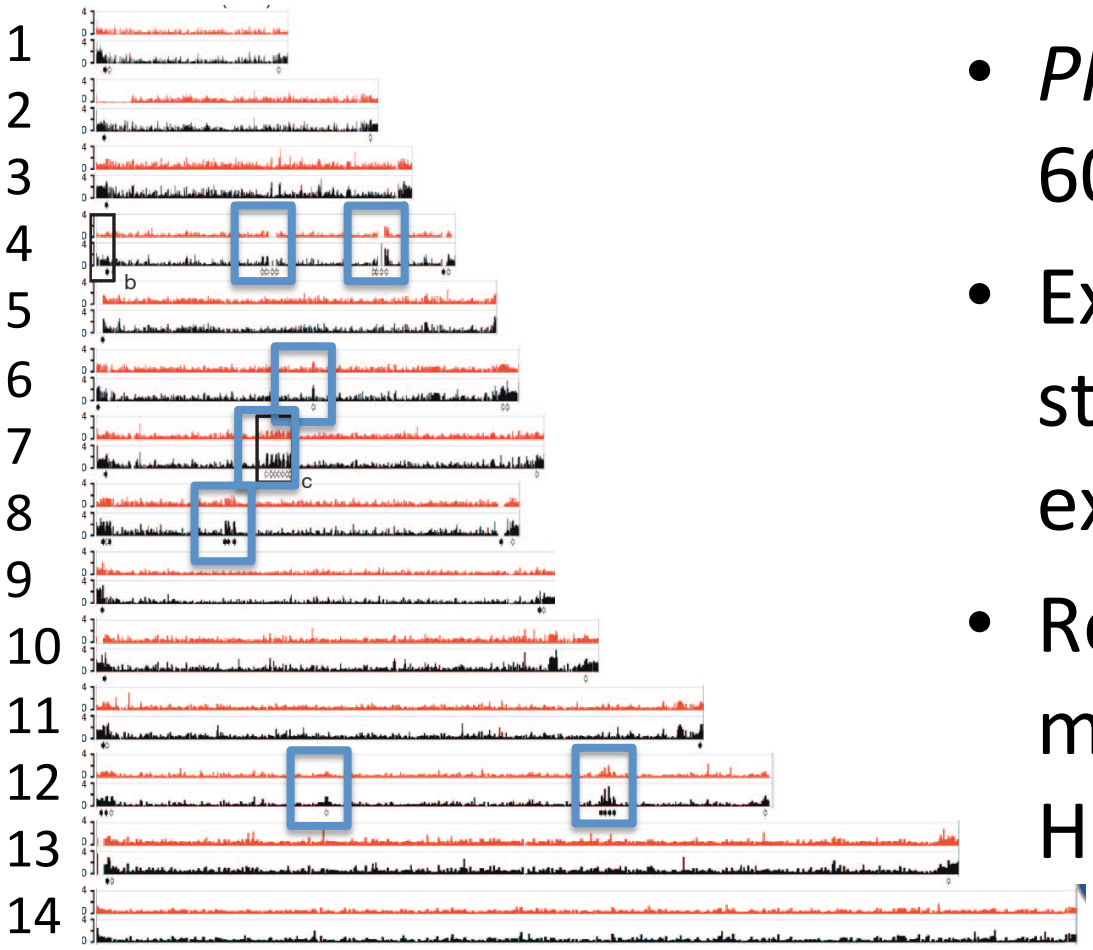
FAIRE (Open chromatin)

MAINE (Closed chromatin)



Ponts et al.
Genome Research 2010.

Virulence genes are tightly regulated for precise expression patterns



- *Plasmodium* encodes 60 virulence genes.
- Exactly one gene is stochastically expressed per cell.
- Regulatory mechanism involves H3K36me3.

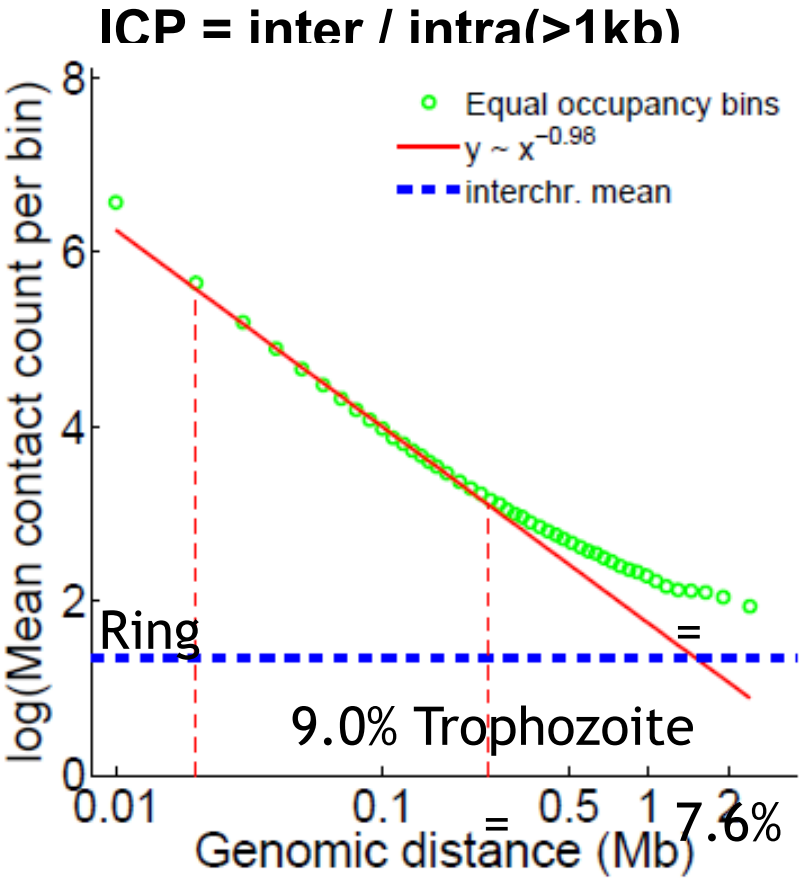
Jiang et al. *Nature* 2013.

Our modified Hi-C protocol works for the AT-rich *Plasmodium* genome

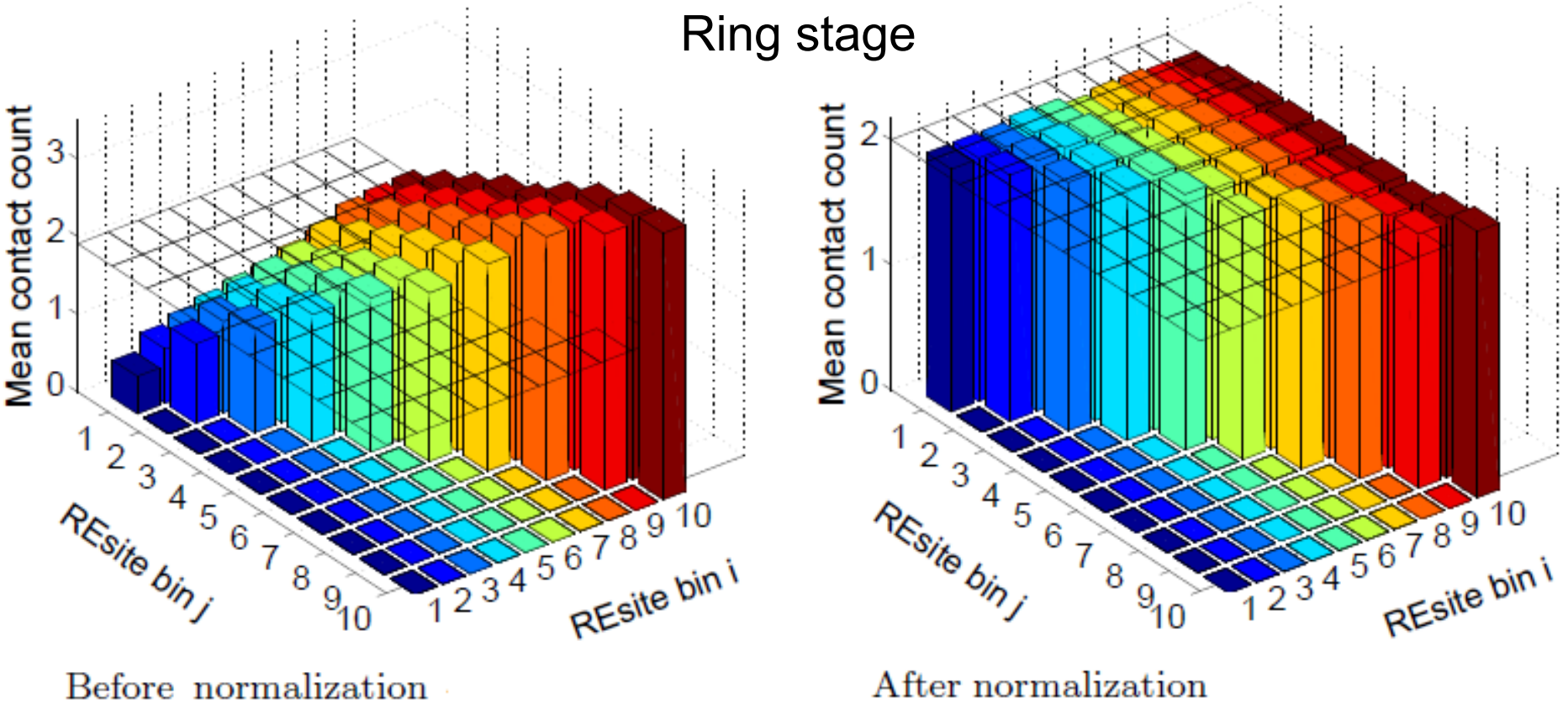
1) ICP index: Relatively low numbers of **inter-chromosomal** contacts from crosslinked samples with respect to random expectation and non-crosslinked control.

2) Contact probability between two **intra-chromosomal** loci exhibits a log-linear decay with increasing genomic distance.

3) The percentage of long-range contacts (either interchromosomal or intrachromosomal >20 kb) is higher than control and comparable to previous Hi-C data from other organisms.



Our data exhibits characteristics biases of Hi-C

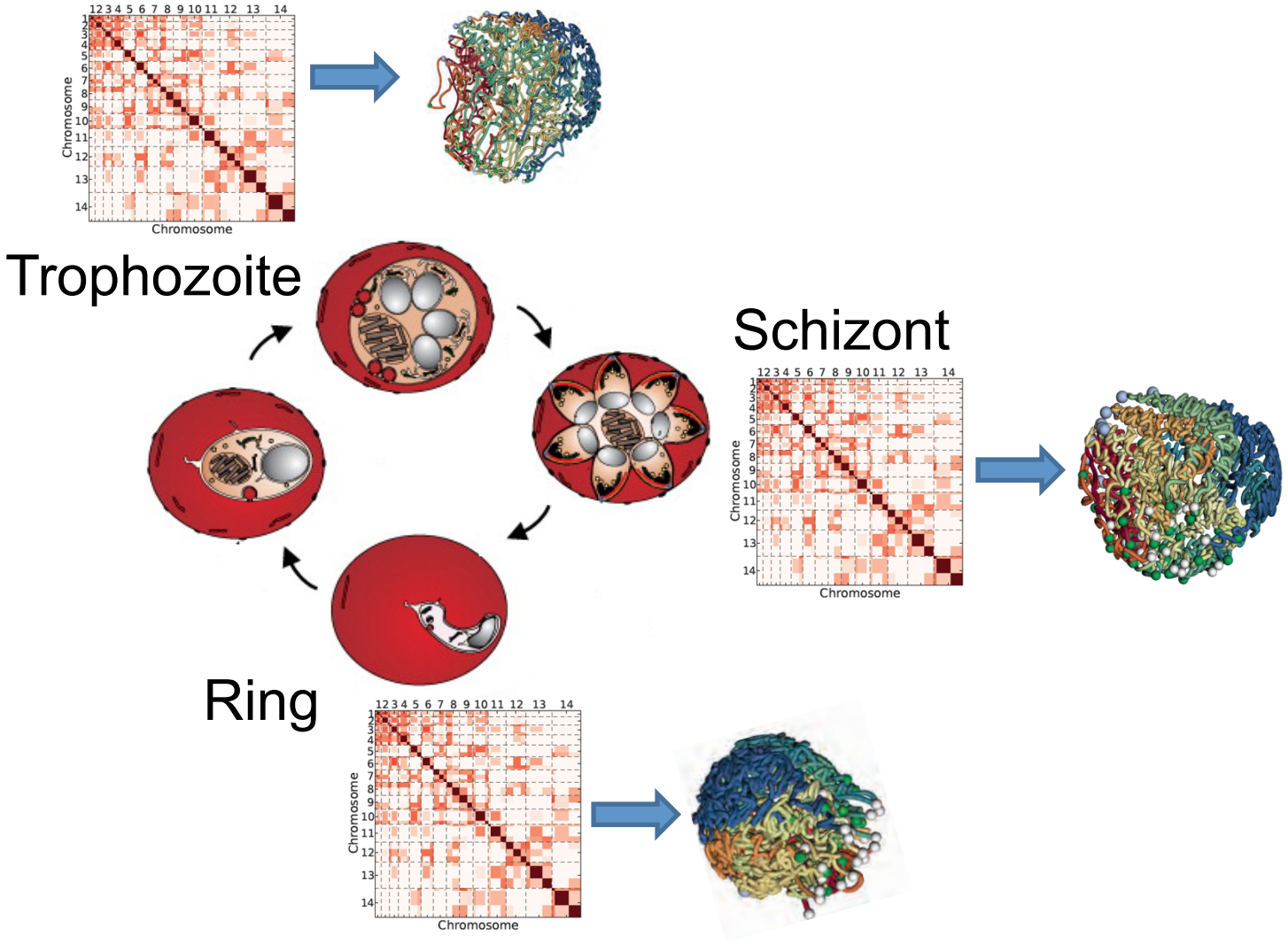


Larger number of restriction enzyme (RE) cut sites → More contacts

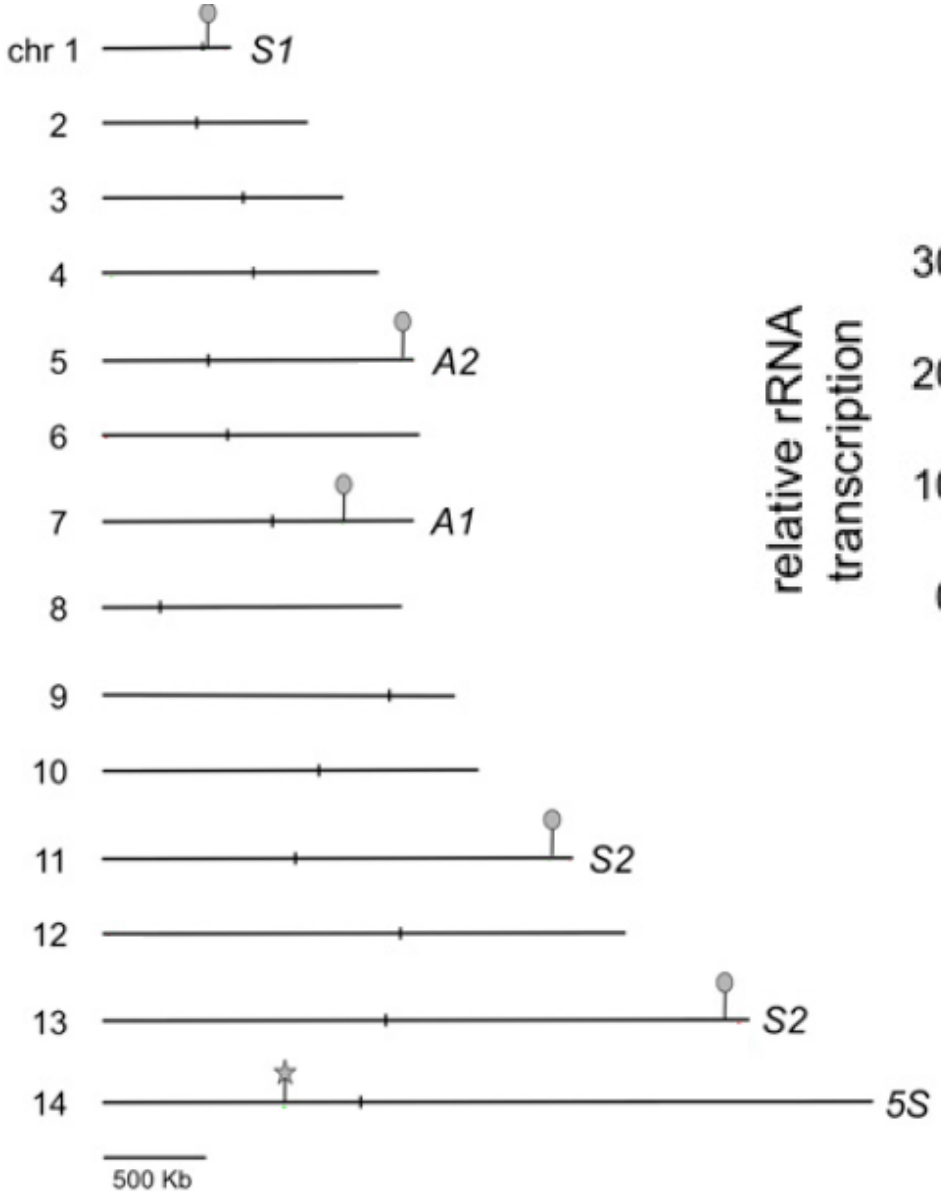
Other biases: GC content, mappability, visibility (in general).

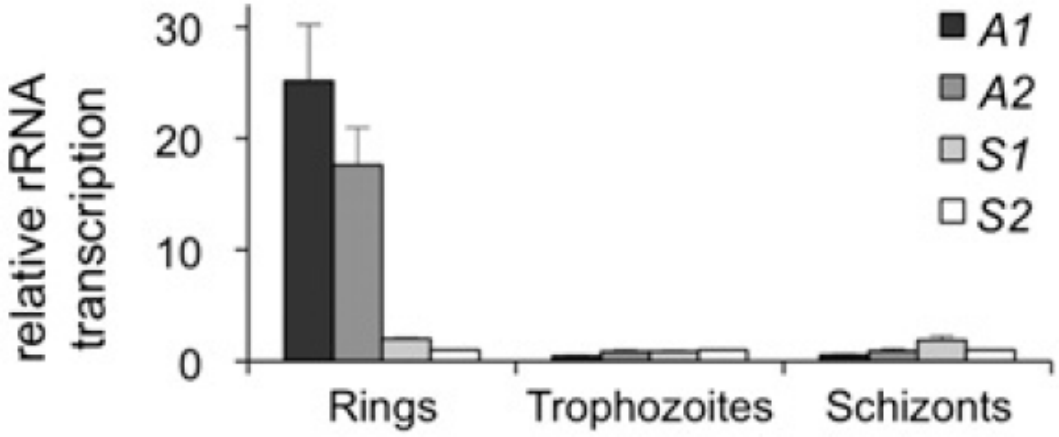
Imakaev et al. *Nature Methods* 2012.

Modeling genome architecture using Hi-C contact maps



Highly transcribed rRNA genes colocalize

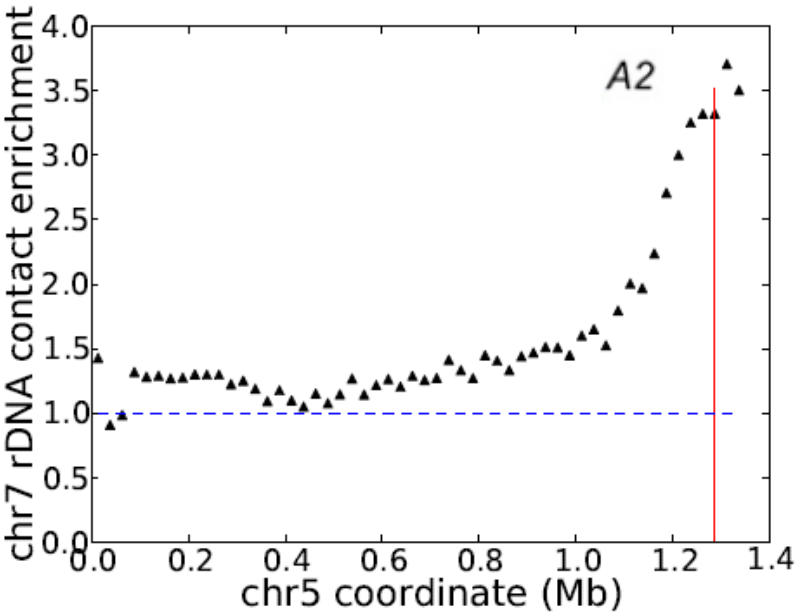




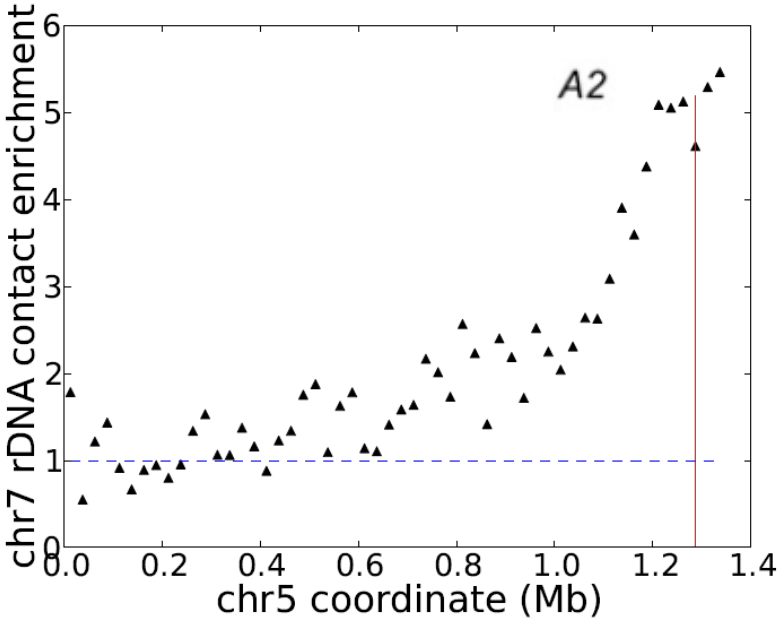
Mancio-Silva et al. *PNAS* 2010.

Highly transcribed rRNA genes colocalize

Virtual 4C plots using the A1 rDNA unit on chromosome 7 as the bait



Our data – Ring stage



B15C2 cells - Ring stage

Lemieux et al. *Mol. Microbiology* 2013

How unexpected/non-random is *Plasmodium* genome architecture?

So far

- ✓ Assayed 3 time points using Hi-C.
- ✓ Generated consensus 3D models.
- ✓ Validated our models using FISH and prior knowledge.
- ✓ Showed that simple volume exclusion does not explain *Plasmodium* genome architecture.
- ✓ Demonstrated existence of domain-like structures shaped around virulence genes.

Videos

Future directions

- ✓ **Mechanism behind formation of repressive/active compartments in *Plasmodium*.**
- ✓ **Causality between virulence gene clustering and transcriptional silencing.**
- ✓ **Interruption of genome architecture changes to interfere with parasite cell cycle for development of antimalarial drugs.**