Analyzing data from capture-based next generation sequencing assays

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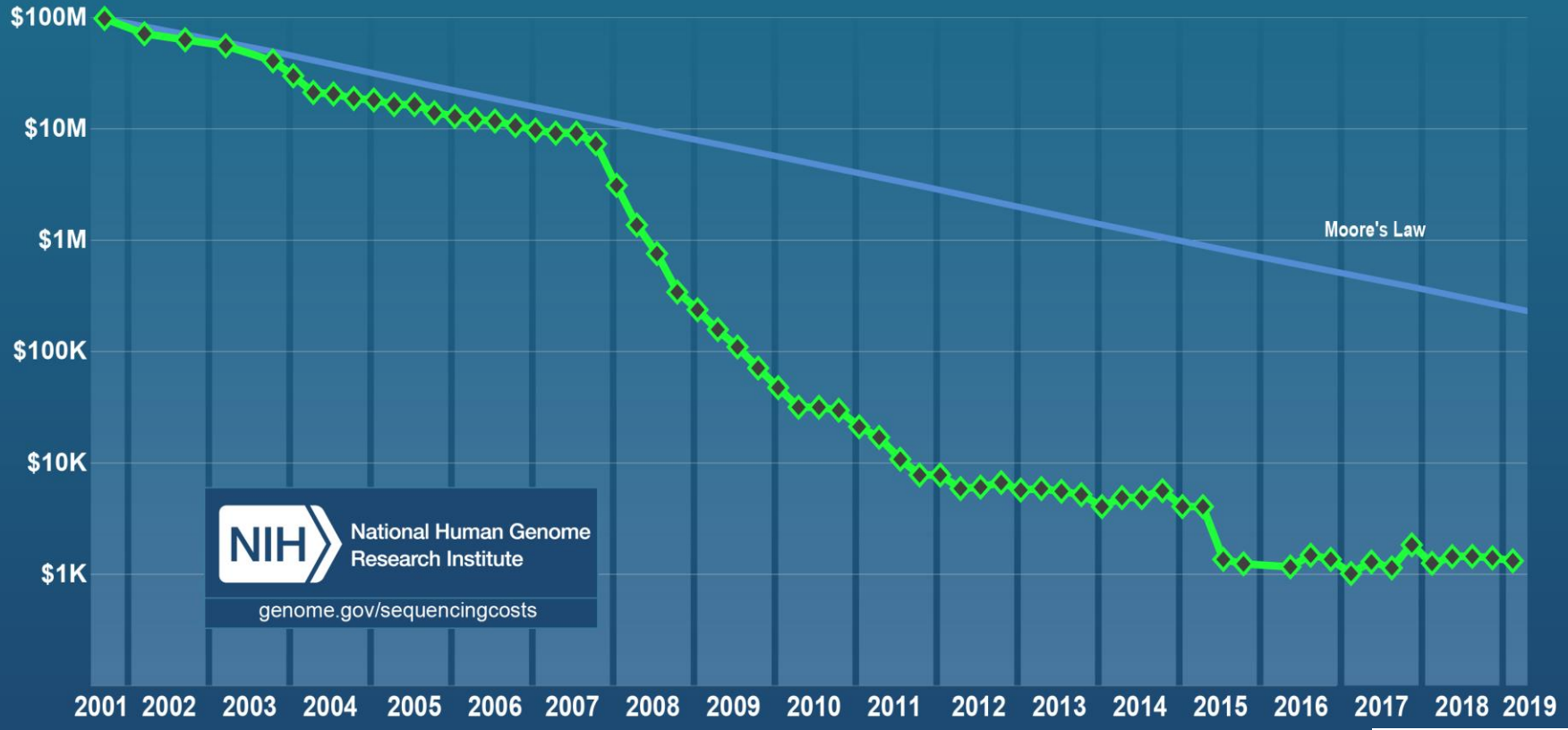
EMORY UNIVERSITY

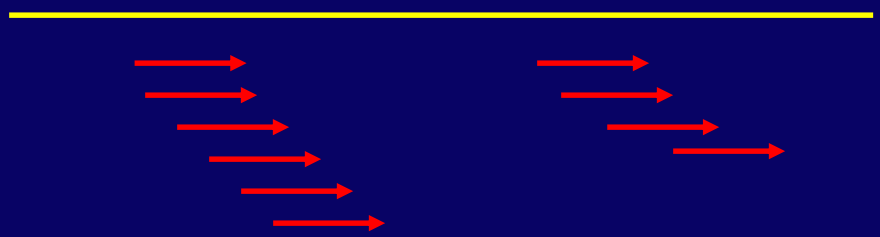
Cost per Genome



Different strategies of using sequencing technologies

- DNA-seq:
  - Whole genome sequencing
  - Uniform coverage
  - Flat

- ChIP-seq, Dnase-seq, ATAC-seq…
  - Capture-based
  - Selected “genome-wide” sequencing
  - Subset
  - Peaky



What is ChIP-seq

- Combination of chromatin immunoprecipitation (ChIP) with ultra high-throughput massively parallel sequencing.
- Allows mapping of protein–DNA interactions *in vivo* on a genome scale.
- Enables mapping of transcription factors binding, DNA binding proteins (HP1, Lamins, HMGA etc), RNA Pol II occupancy or Histone modification marks at genome scale.
- The typical ChIP assay usually take 4–5 days, and require approx. 1 -10 million cells.

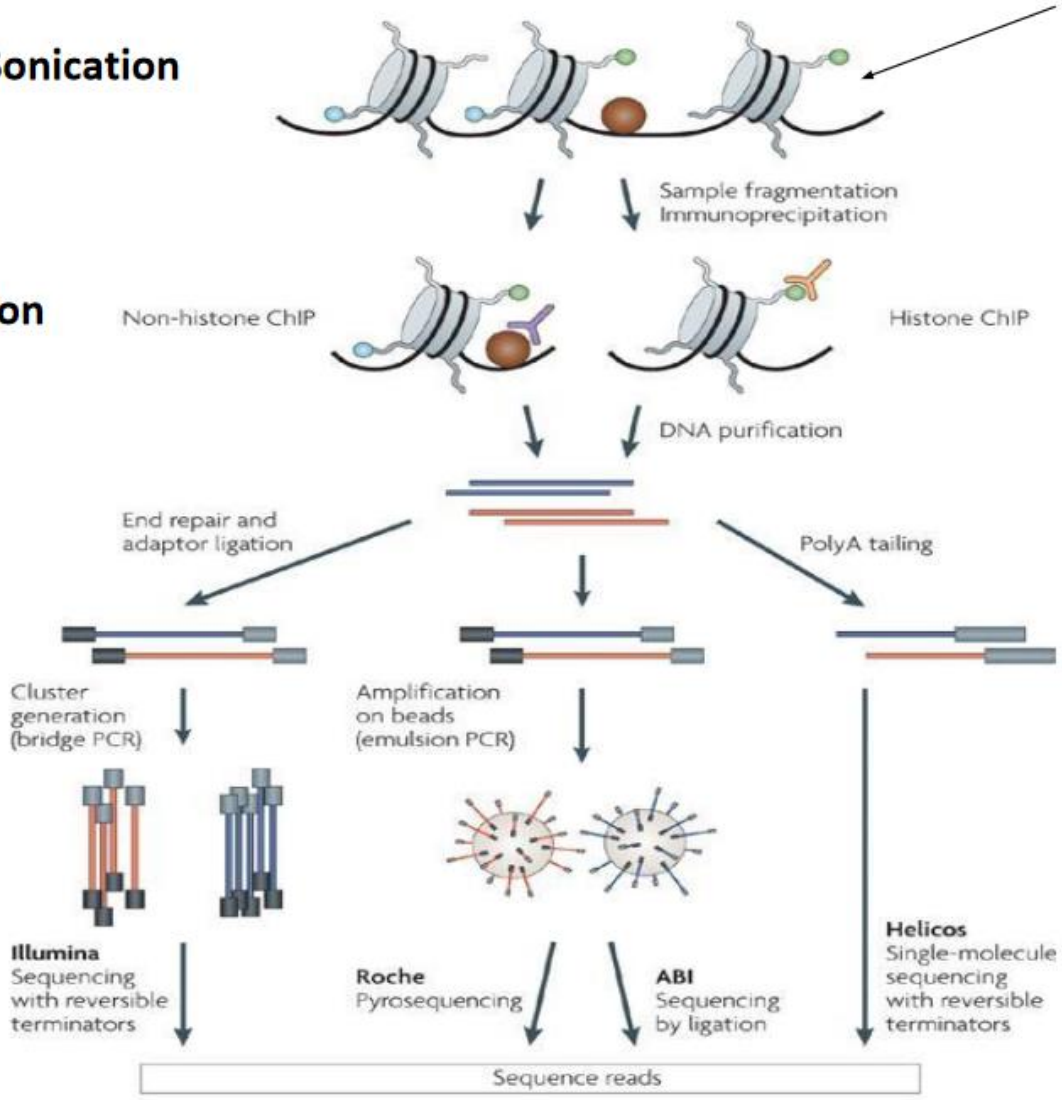
ChIP-seq methodology

Cross-link cells

Isolate genomic DNA

Sonication

Immuno-precipitation



Park 2009 Nat. Rev Genet.

ChIP sequencing

Resource

Cell

High-Resolution Profiling of Histone Methylations in the Human Genome

Artem Barski,[1,3] Suresh Cuddapah,[1,3] Kairong Cui,[1,3] Tae-Young Roh,[1,3] Dustin E. Schones,[1,3] Zhibin Wang,[1,3] Gang Wei,[1,3] Iouri Chepelev,[2] and Keji Zhao[1,*]
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[2]Department of Human Genetics, Gonda Neuroscience and Genetics Research Center, University of California, Los Angeles, Los Angeles, CA 90095, USA
[3]These authors contributed equally to this work and are listed alphabetically.
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DOI 10.1016/j.cell.2007.05.009

Genome-Wide Mapping of in Vivo Protein-DNA Interactions

David S. Johnson,[1]* Ali Mortazavi,[2]* Richard M. Myers,[1]† Barbara Wold[2,3]†

putational discovery of binding motifs feasible, this dictates the quality of regulatory site annotation relative to other gene anatomy landmarks, such as transcription start sites, enhancers, introns and exons, and conserved noncoding features (*2*). Finally, if high-quality protein-DNA interactome measurements can be performed rou-

www.sciencemag.org SCIENCE VOL 316 8 JUNE 2007

Genome-wide profiles of STAT1 DNA association using chromatin immunoprecipitation and massively parallel sequencing

ature.com/naturemethods

Gordon Robertson[1], Martin Hirst[1], Matthew Bainbridge[1], Misha Bilenky[1], Yongjun Zhao[1], Thomas Zeng[1], Ghia Euskirchen[2], Bridget Bernier[1], Richard Varhol[1], Allen Delaney[1], Nina Thiessen[1], Obi L Griffith[1], Ann He[1], Marco Marra[1], Michael Snyder[2] & Steven Jones[1]

[1]British Columbia Cancer Agency Genome Sciences Centre, 675 West 10th Avenue, Vancouver, British Columbia V5Z 4S6, Canada. [2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520, USA. Correspondence should be addressed to S.J. (sjones@bcgsc.ca).

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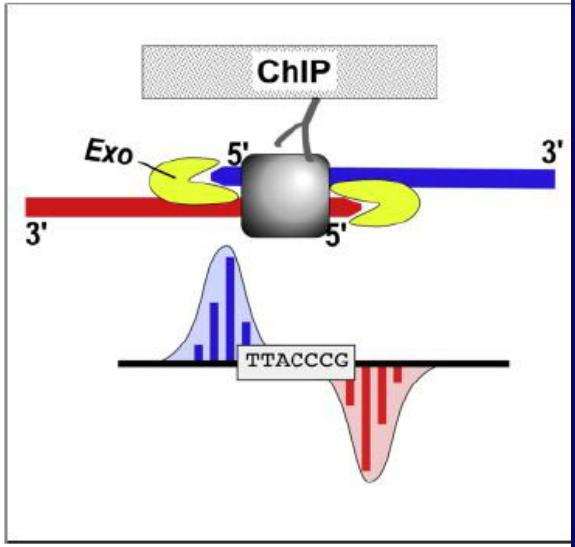
Advances in technologies for protein-DNA interaction

- ChIP-chip : combines ChIP with microarray technology.

- ChIP-PET : ChIP with paired end tag sequencing

- ChIP-exo : ChIP-seq with exonuclease digestion



- CLIP-seq / HITS-CLIP : cross-linking immunoprecipitation high throughput sequencing
- ATAC-seq : Assay for Transposon Accessible Chromatin
- Sono-seq : Sonication of cross linked chromatin sequencing.
- Hi-C: High throughput long distance chromatin interactions

Statistical aspects and best practices

These guidelines address :

- Antibody validation
- Experimental replication
- Sequencing depth
- Data and metadata reporting
- Data quality assessment
- Replicates

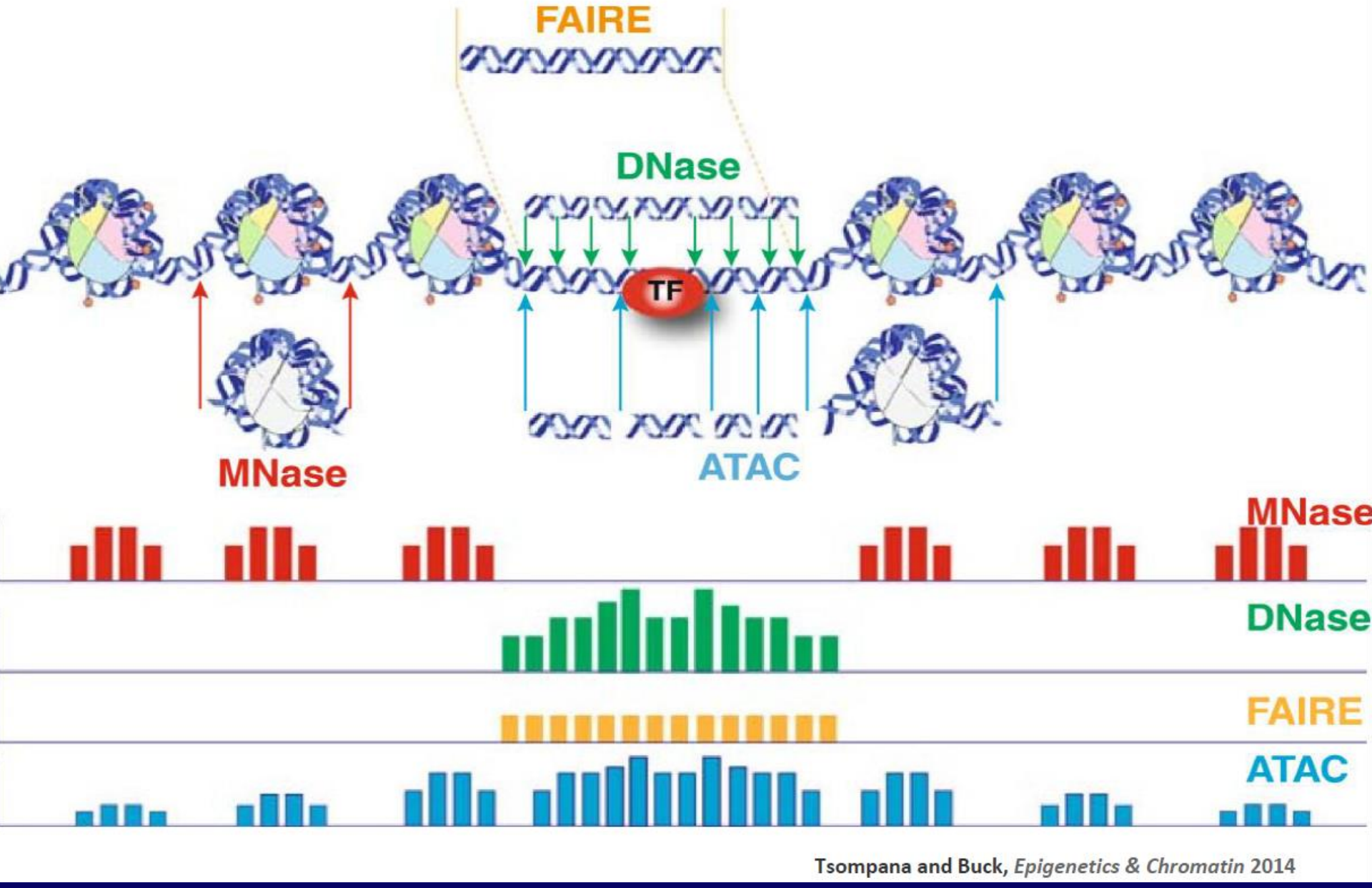
Experimental guidelines:

- Landt *et al.*, "ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia." *Genome Res. 2012.*
- Marinov *et al.*, "Large-scale quality analysis of published ChIP-seq data." 2014 *G3*
- Rozowsky *et al.*, "PeakSeq enables systematic scoring of ChIP-seq experiments relative to controls" *Nat Biotechnol.* 2009

Statistical aspects:

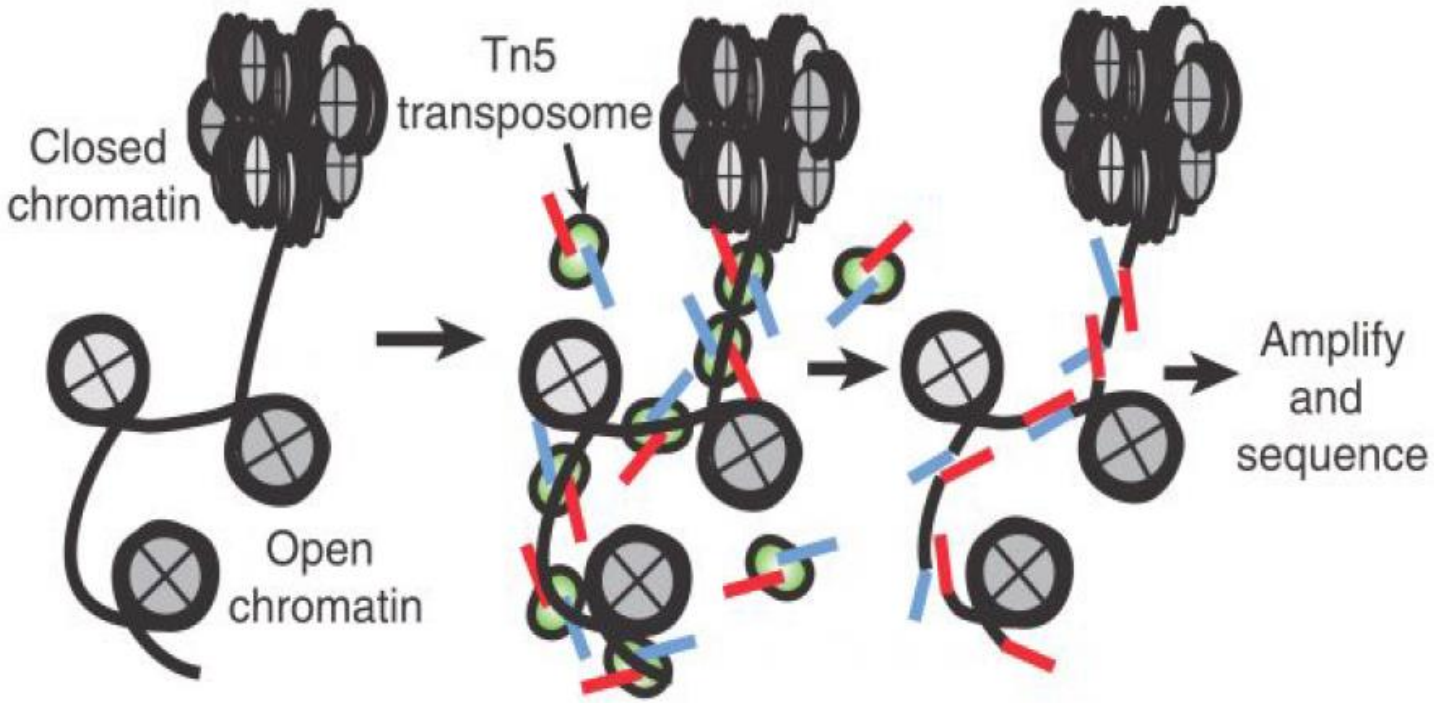
- Cairns *et al.*, "Statistical Aspects of ChIP-Seq Analysis." *Adv. in Stat Bioinf.*, 2013.
- Carroll TS *et al.*, "Impact of artifact removal on ChIP quality metrics in ChIP-seq and ChIP-exo data." *Front Genet.* 2014
- Bailey *et al.*, "Practical guidelines for the comprehensive analysis of ChIP-seq data." *PLoS Comput Biol.* 2013.
- Sims et al., "Sequencing depth and coverage: key considerations in genomic analyses." *Nat. Rev. Genet.* 2014.

Detecting Chromatin Accessibility



Tsompana and Buck, *Epigenetics & Chromatin* 2014

ATAC-seq

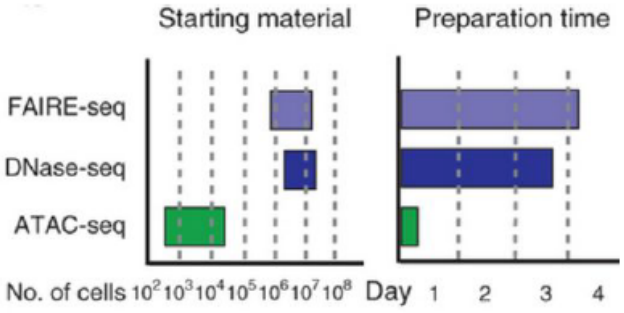


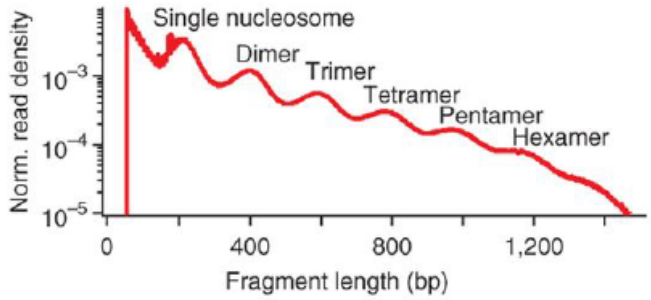
- Enables measurement of chromatin structure modifications (nucleosome free regions) on gene regulation.
- Does not require antibodies or tags that can introduce potential bias.
- Hyperactive Tn5 transposase is used to fragment DNA and integrate into active regulatory regions.
- During ATAC-seq, 500–50,000 unfixed nuclei are tagged *in vitro* with sequencing adapters by purified Tn5 transposase.
- Can also detect nucleosome packing, positioning and TF footprints.

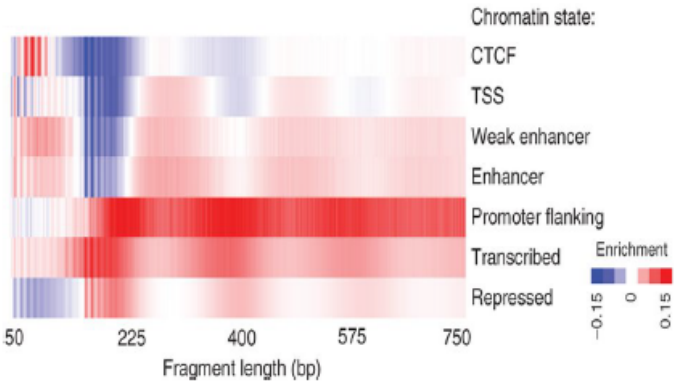
JD Buenrostro et al, Nature Methods, 2013.

ATAC-seq

- Two-step protocol
  - Insertion of Tn5 transposase with adaptors
  - PCR amplification
- Needs ~500-50,000 cells
- Paired-end reads produce information about nucleosome positioning.
- Insert size distribution of fragments has a periodicity of ~200bp, suggesting that fragments are protected by multiplies of nucleosomes
- Different fragmentation patterns can be associated with different functional states (eg. TSSs are more accessible than promoter flanking or transcribed regions)







[1] JD Buenrostro et al, *Nature Methods*, 2013.

Using model-based methods to analyze ChIP-seq data

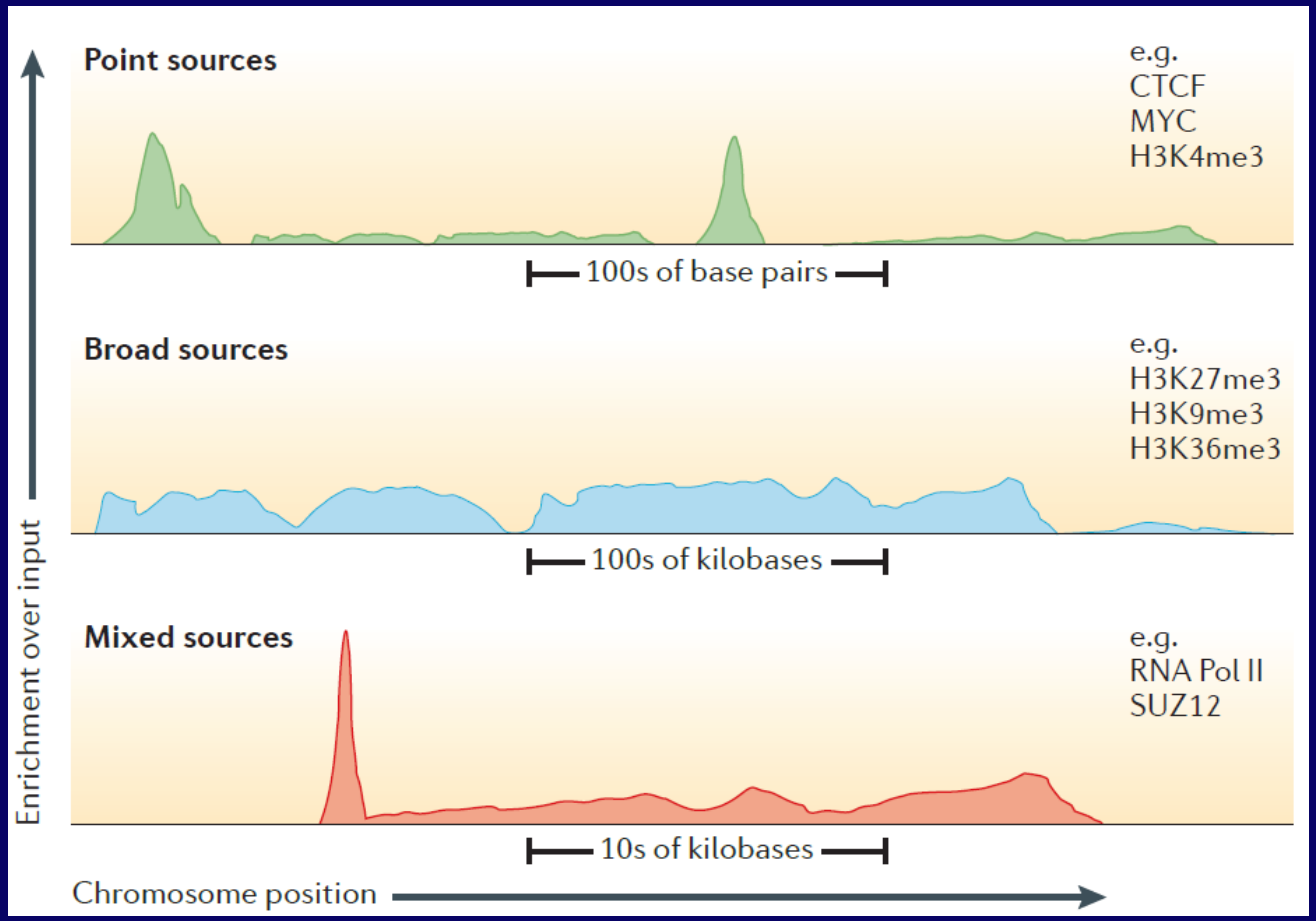
Outline

- Hidden Markov model for peak detection
- Hierarchical Hidden Markov model for combining ChIP-seq and ChIP-chip data, or analyze multiple ChIP-seq data
- Hybrid Monte Carlo strategy for Motif finding

Peak calling tools

- MACS
- HOMER
- CisGenome
- PeakSeq
- HPeak

etc.



Sims et al. Nature Review Genetics 2014.

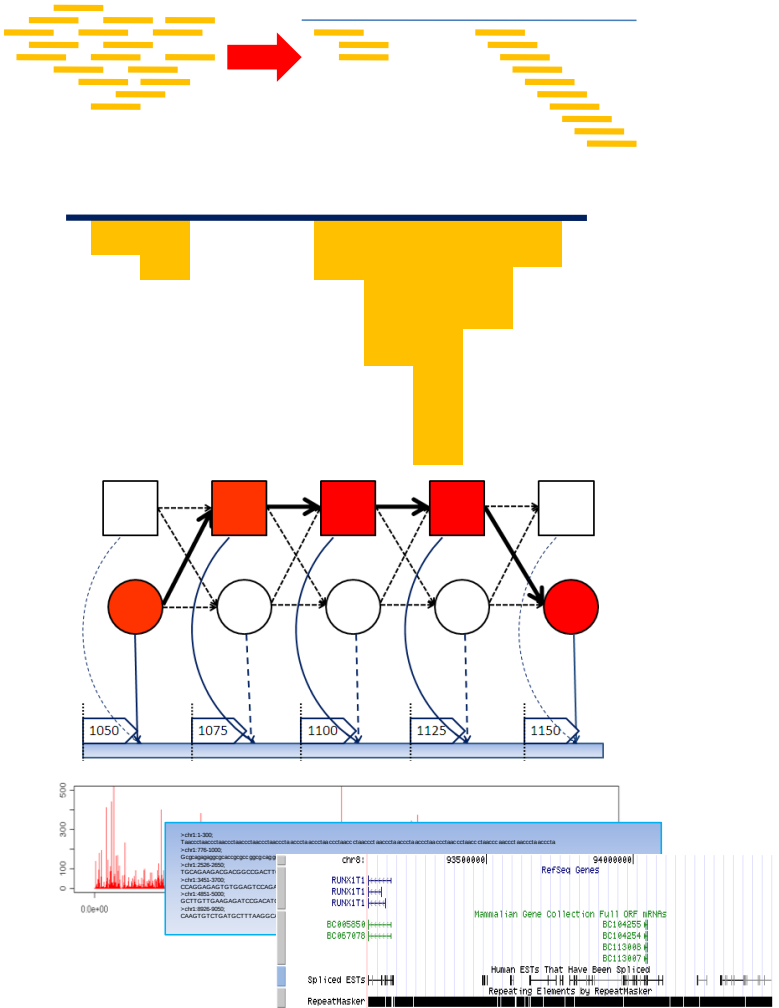
HPeak algorithm

Align reads to genome, get summary statistics, estimate model parameters.

Get read coverage for each bin on all chromosomes.

Build HMM to infer whether a bin belongs to peak or background.

Post-processing on identified peaks.



GP and ZIP distribution

- Do not require mean equal to variance which is useful to model over-dispersion and under-dispersion.

$$\mathrm{P}(\mathrm{Y}=\mathrm{y}\mid\lambda,\phi)=\left(\frac{\lambda}{1+\phi\lambda}\right)^{y}\frac{(1+\phi\lambda)^{y-1}}{y!}\exp\left\{\frac{-\lambda(1+\phi\lambda)}{1+\phi\lambda}\right\}$$

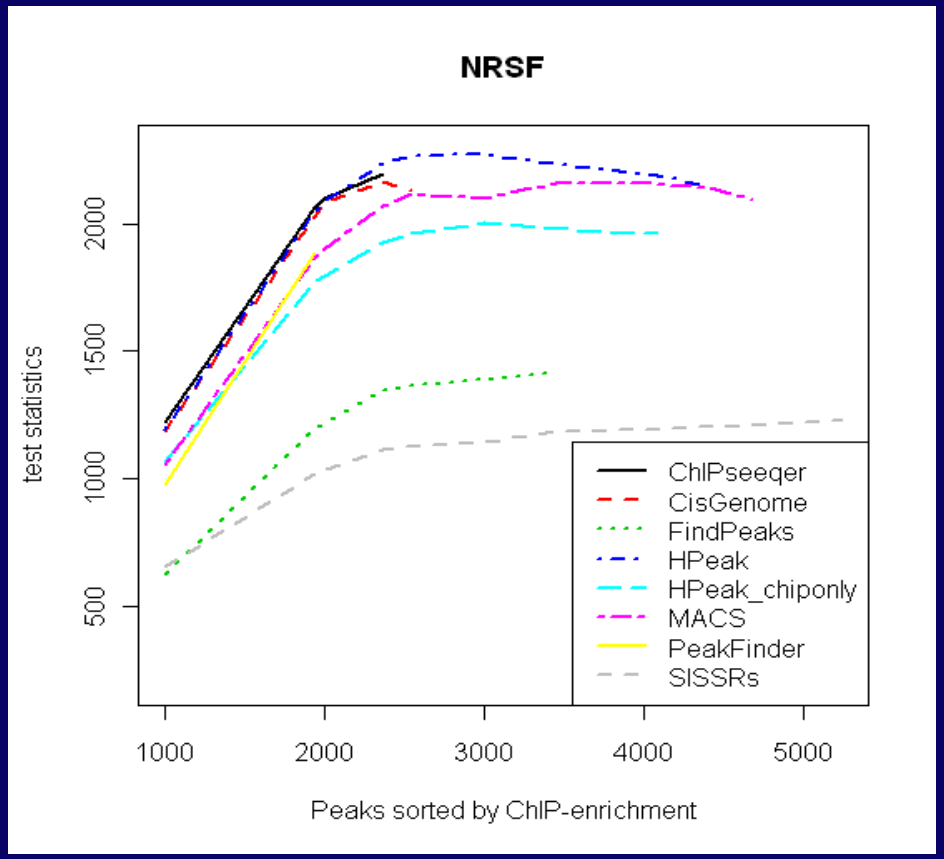
$$E(Y)=\lambda$$

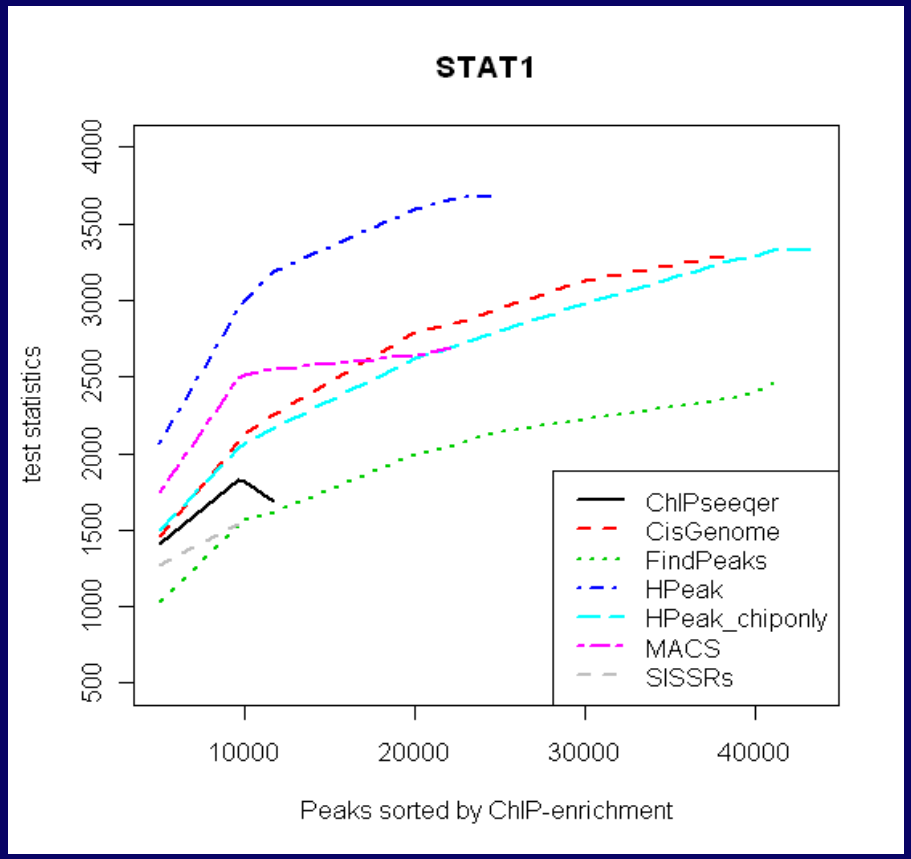
$$Var(Y)=\lambda(1+\phi\lambda)^{2}$$

- Zero-inflated Poisson distribution

$$f(Y\mid\pi,\mu)=\begin{cases}(1-\pi)+\pi e^{-\mu} & \text{if } x=0\\ \dfrac{\pi e^{-\mu}\mu^{x}}{x!} & \text{if } x=0\end{cases}$$

Motif enrichment results for NRSF and STAT1 data





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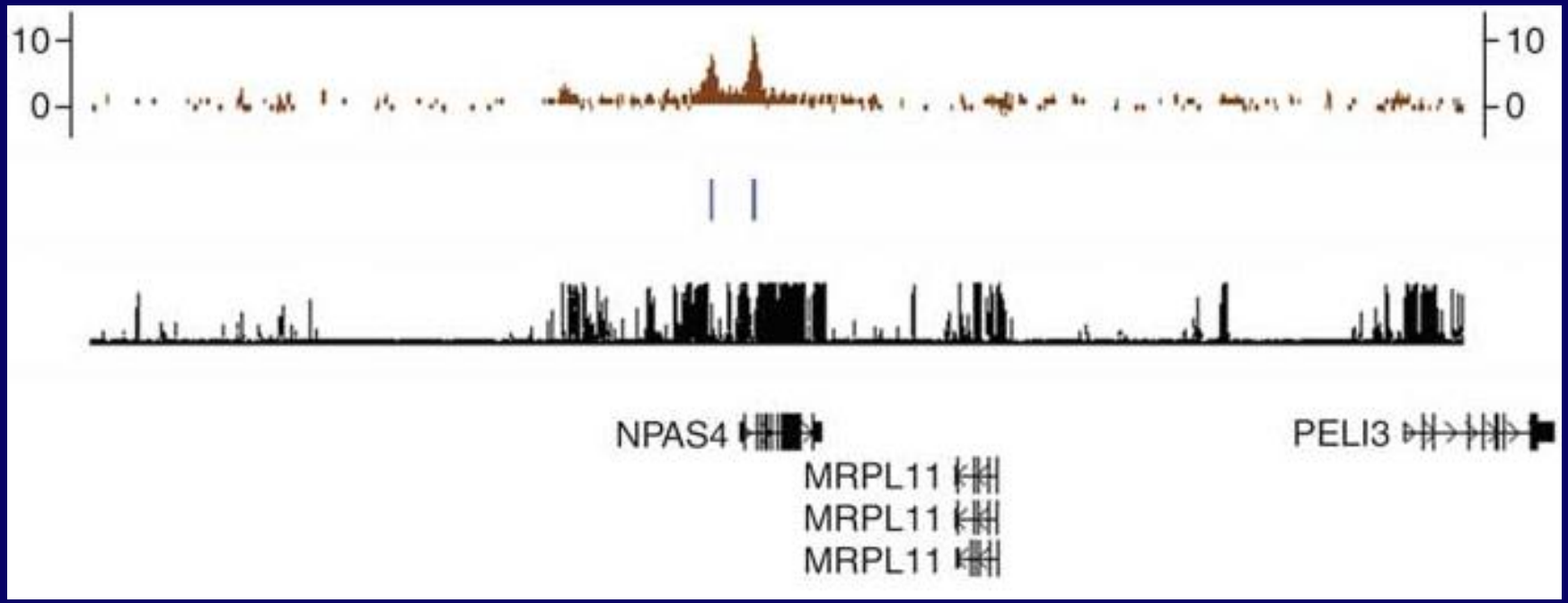
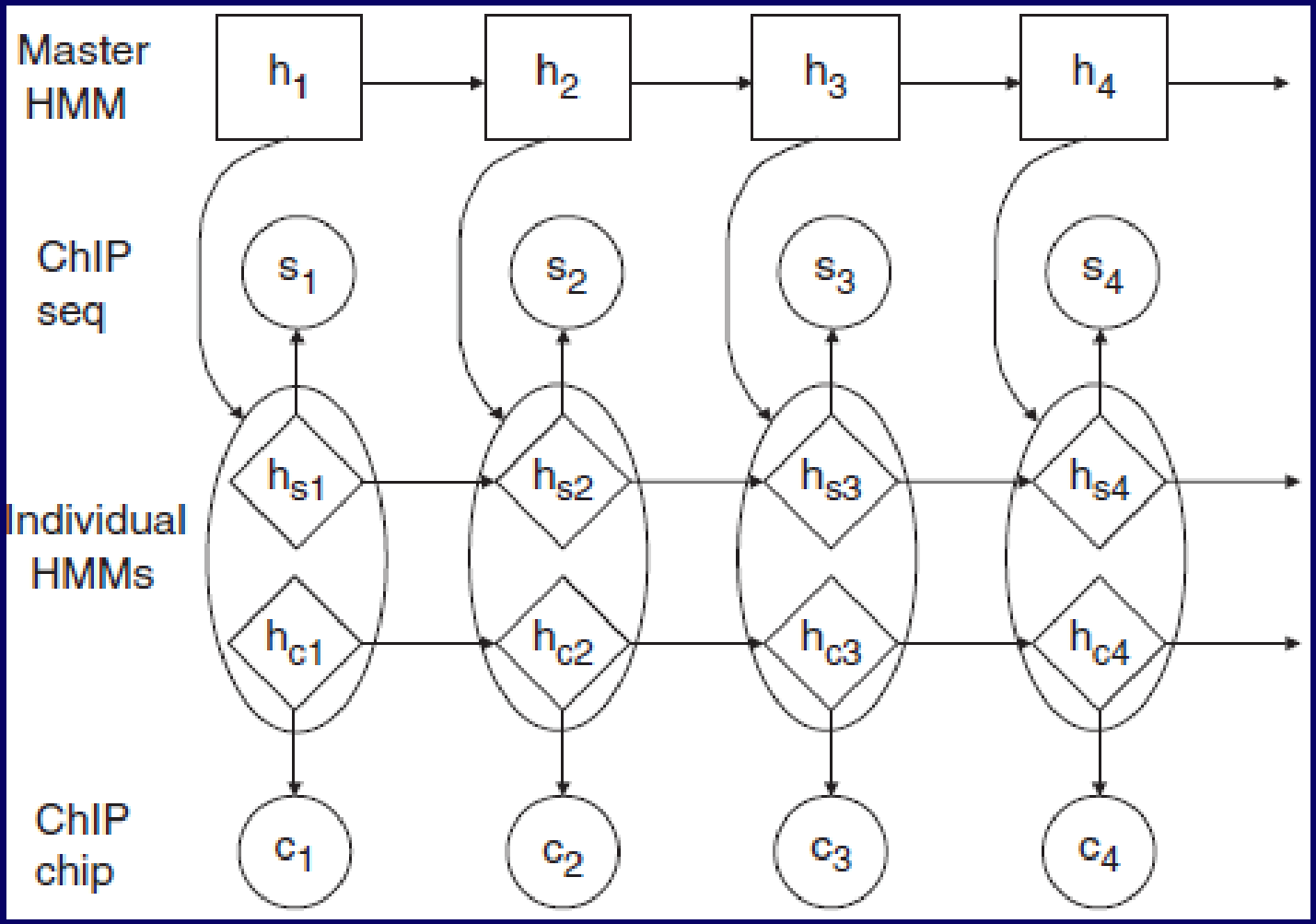
Joint analysis of ChIP-chip and ChIP-seq



Ji et al. *Nat Biotechnology*, 2008

Hierarchical HMM

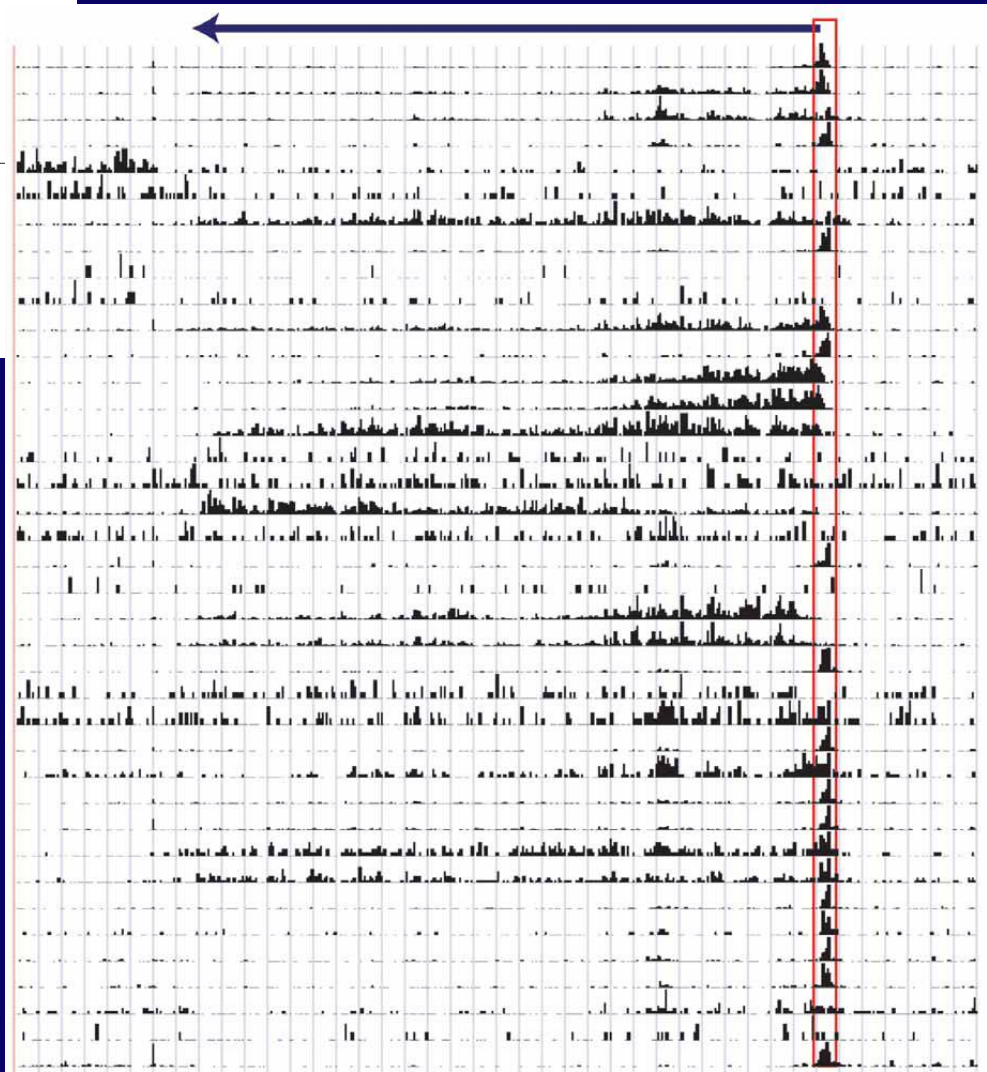


ChIP-Seq compendium

nature genetics

Combinatorial patterns of histone acetylations and methylations in the human genome

Zhibin Wang[1,5], Chongzhi Zang[2,5], Jeffrey A Rosenfeld[3–5], Dustin E Schones[1], Artem Barski[1], Suresh Cuddapah[1], Kairong Cui[1], Tae-Young Roh[1], Weiqun Peng[2], Michael Q Zhang[3] & Keji Zhao[1]



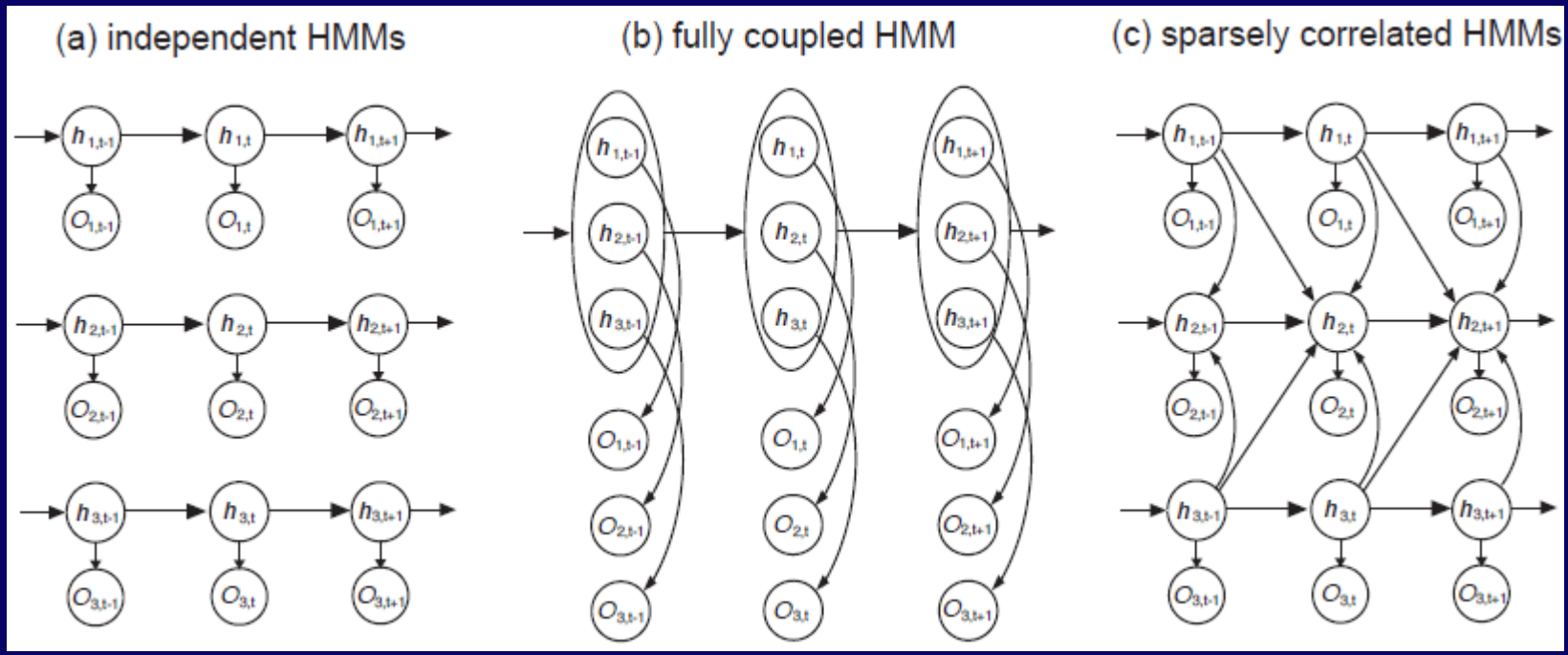
The problem

- N series of data, each can be modeled by an HMM,
- The goal is to infer the hidden states for all series,
- Suppose there are k states for each chain, then the total number of possible states for the whole datasets is k^N, the size of the transition matrix is k^{2N},
  - Independent: ignore correlation among the data series,
  - A single HMM to model all data together: intractable for large N.

Our goal

- Allow coupling among the chains,
- The goal is to borrow information across different experiments/datasets,
- Limit the amount of coupling allowed to reduce computation cost

Our scheme


(a) independent HMMs
(b) fully coupled HMM
(c) sparsely correlated HMMs

Our learning plan

- Perform inference one series a time,
- Incorporate knowledge of hidden states in other series into the learning process,
- Assume sparsity in the correlation matrix.

Our model

- Use an inhomogeneous HMM to incorporate correlation,
- Define the transition kernel for series *j* and time *t* as:

$$K_j(t) = \begin{pmatrix} 1-p_{jt} & p_{jt} \\ 1-q_{jt} & q_{jt} \end{pmatrix}$$

$$p_{jt} = Pr(h_{j,t} = 1 | h_{j,t-1} = 0) \text{ and } q_{jt} = Pr(h_{j,t} = 1 | h_{j,t-1} = 1).$$

$$\log\left(\frac{p_{jt}}{1-p_{jt}}\right) = \beta^p_{j0} + \sum_{k \neq j}\left(\beta^p_{jk} h_{k,t-1} + \beta^c_{jk} h_{k,t}\right)$$

$$\log\left(\frac{q_{jt}}{1-q_{jt}}\right) = \gamma^p_{j0} + \sum_{k \neq j}\left(\gamma^p_{jk} h_{k,t-1} + \gamma^c_{jk} h_{k,t}\right)$$

Our algorithm I

- Estimate regression parameters
  - Conditional on the current states, run penalized logistic regression to get model parameters,
  - LASSO penalty

$$
\begin{aligned}
y_t &= h_{j,t} \\
x_t &= (h_{1,t-1}, \ldots, h_{j-1,t-1}, h_{j+1,t-1}, \ldots, h_{N,t-1}, h_{1,t}, \ldots, h_{j-1,t}, h_{j+1,t}, \ldots, h_{N,t})
\end{aligned}
$$

$$
\min_{(\beta_{j0}, \vec{\beta}_j^p, \vec{\beta}_j^c)} \left\{ -\ell(\beta_{j0}, \vec{\beta}_j^p, \vec{\beta}_j^c) + \lambda P(\vec{\beta}_j^p, \vec{\beta}_j^c) \right\}
$$

$$
P(\vec{\beta}_j^p, \vec{\beta}_j^c) = \sum_{k \neq j} |\beta_{jk}^p| + \sum_{k \neq j} |\beta_{jk}^c|.
$$

Our algorithm II

- Estimate transition kernel
  - Use the regression parameters estimated in step 1 and the current states of chains other than *j*, to get log odds for chain *j* at all time point *t*, then get estimated transition kernel.

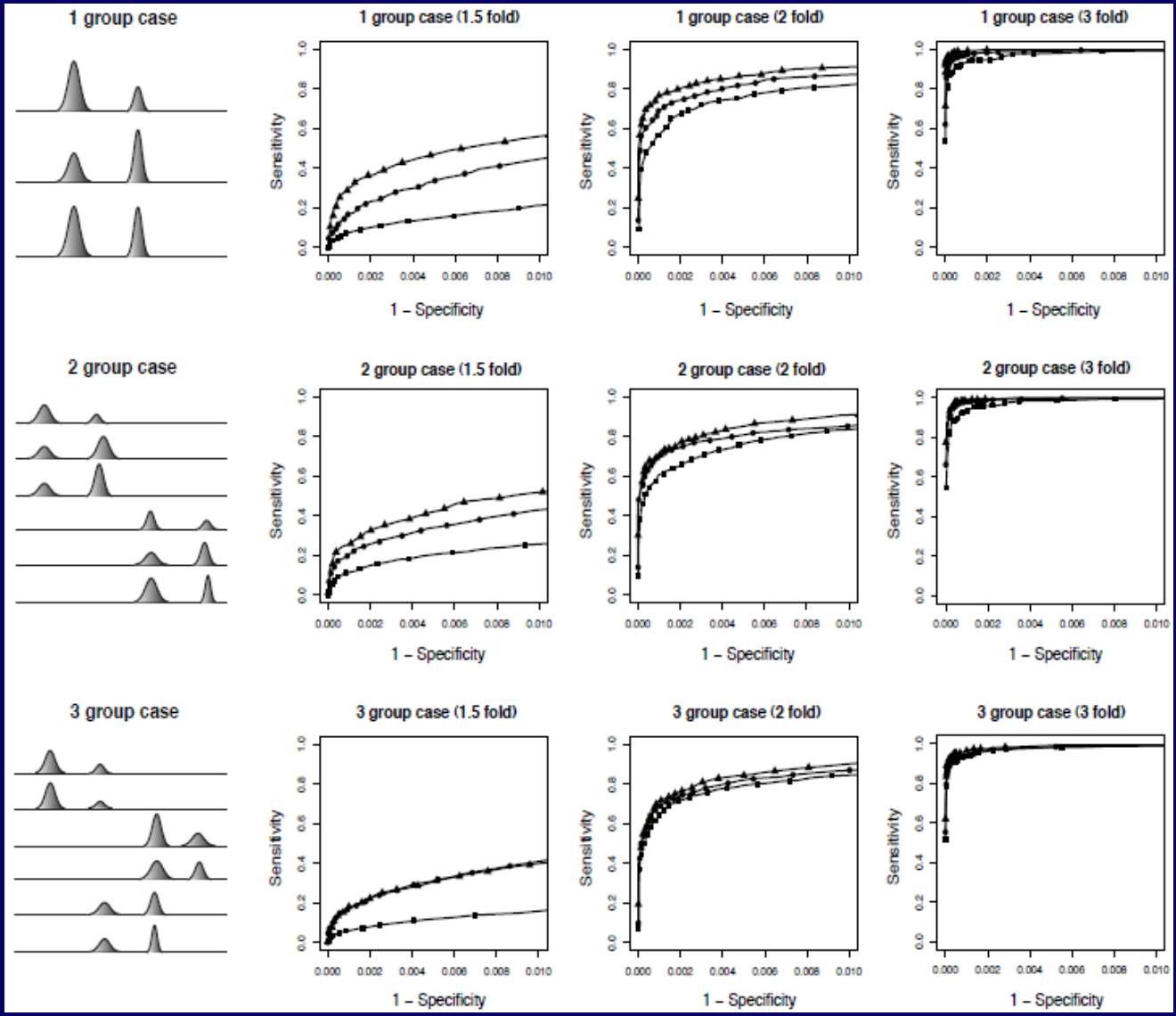
$$\log\left(\frac{p_{jt}}{1-p_{jt}}\right) = \beta^p_{j0} + \sum_{k\neq j}\left(\beta^p_{jk}h_{k,t-1} + \beta^c_{jk}h_{k,t}\right)$$

$$\log\left(\frac{q_{jt}}{1-q_{jt}}\right) = \gamma^p_{j0} + \sum_{k\neq j}\left(\gamma^p_{jk}h_{k,t-1} + \gamma^c_{jk}h_{k,t}\right)$$

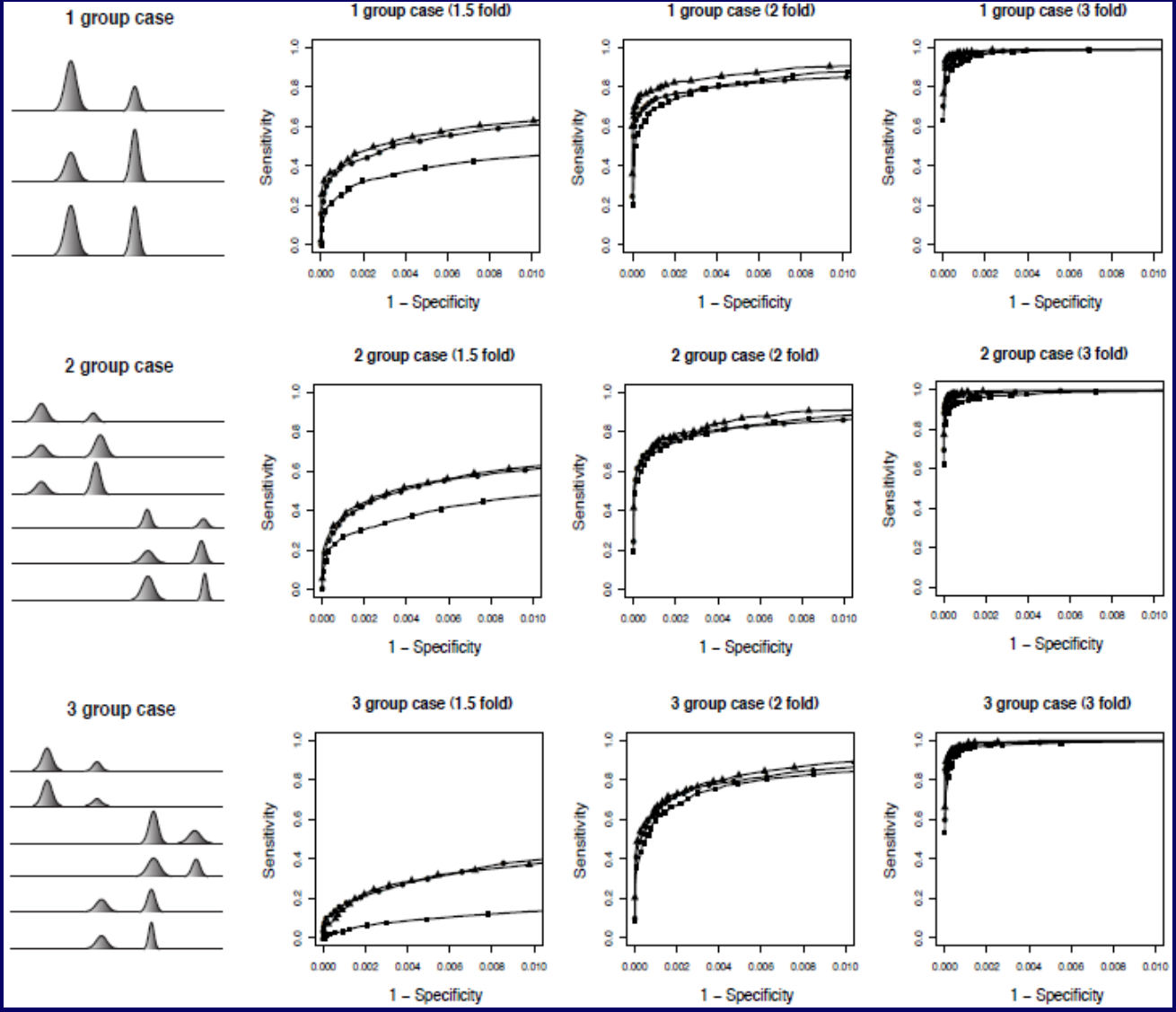
Our algorithm III

- Infer hidden states
  - Use the transition kernel estimated in step 2, current emission probabilities and observed data to run regular HMM (forward-backward algorithm) to get updated hidden states,
- Estimate the emission probabilities
  - Use the hidden states estimated in step 3 and observed data to update emission probabilities.

Simulation studies



Simulation studies



Joint inference of multiple ChIP-seq data

- JAMIE
  - Joint analysis of multiple ChIP-chip data
  - Wu, Ji Bioinformatics 2010
- HHMM
  - Joint analysis of ChIP-seq and ChIP-chip data
  - Choi et al. Bioinformatics 2009
- scHMM
  - Joint analysis of multiple ChIP-seq data
  - Choi et al. Bioinformatics 2013

Outline

- Hidden Markov model for peak detection
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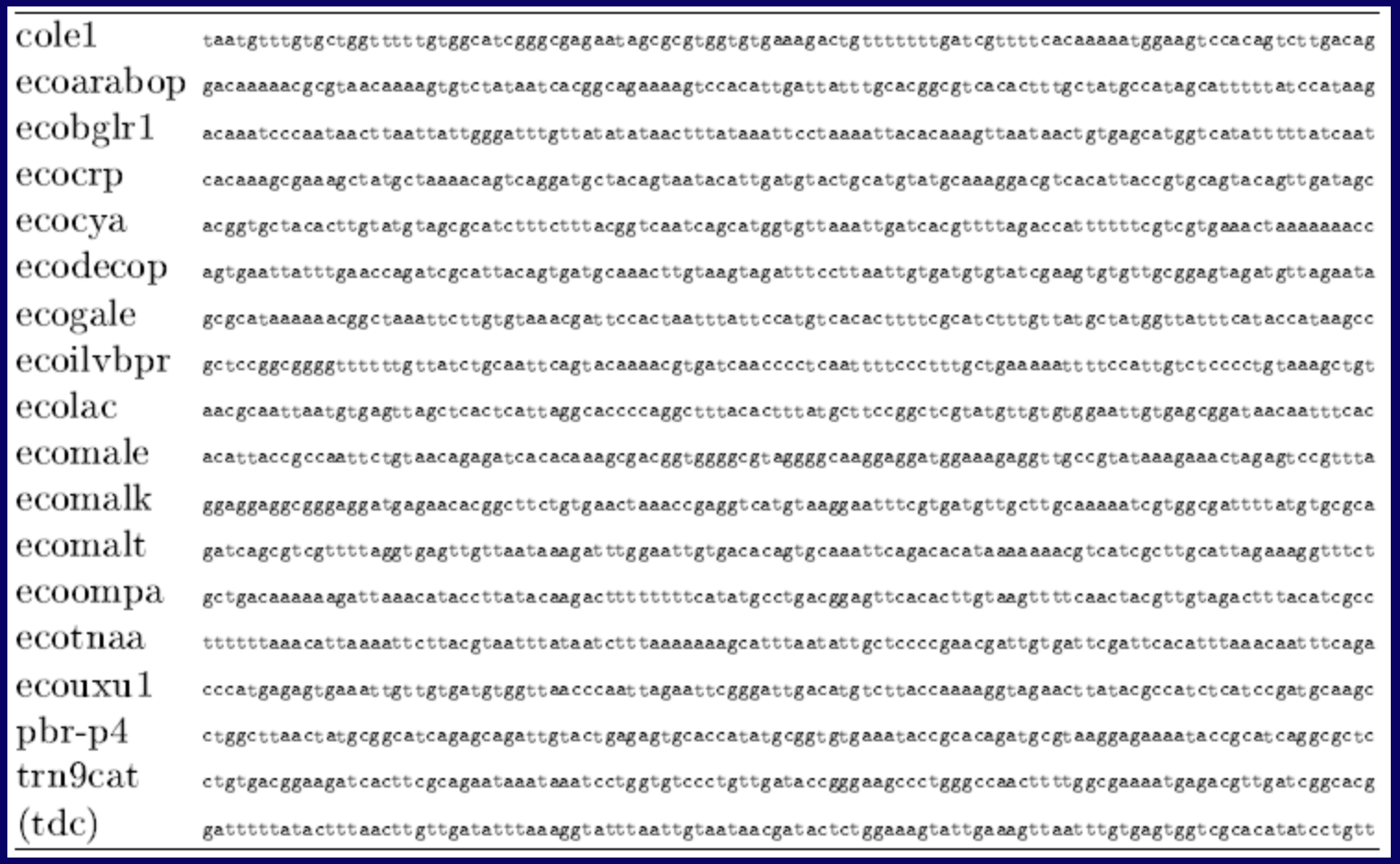
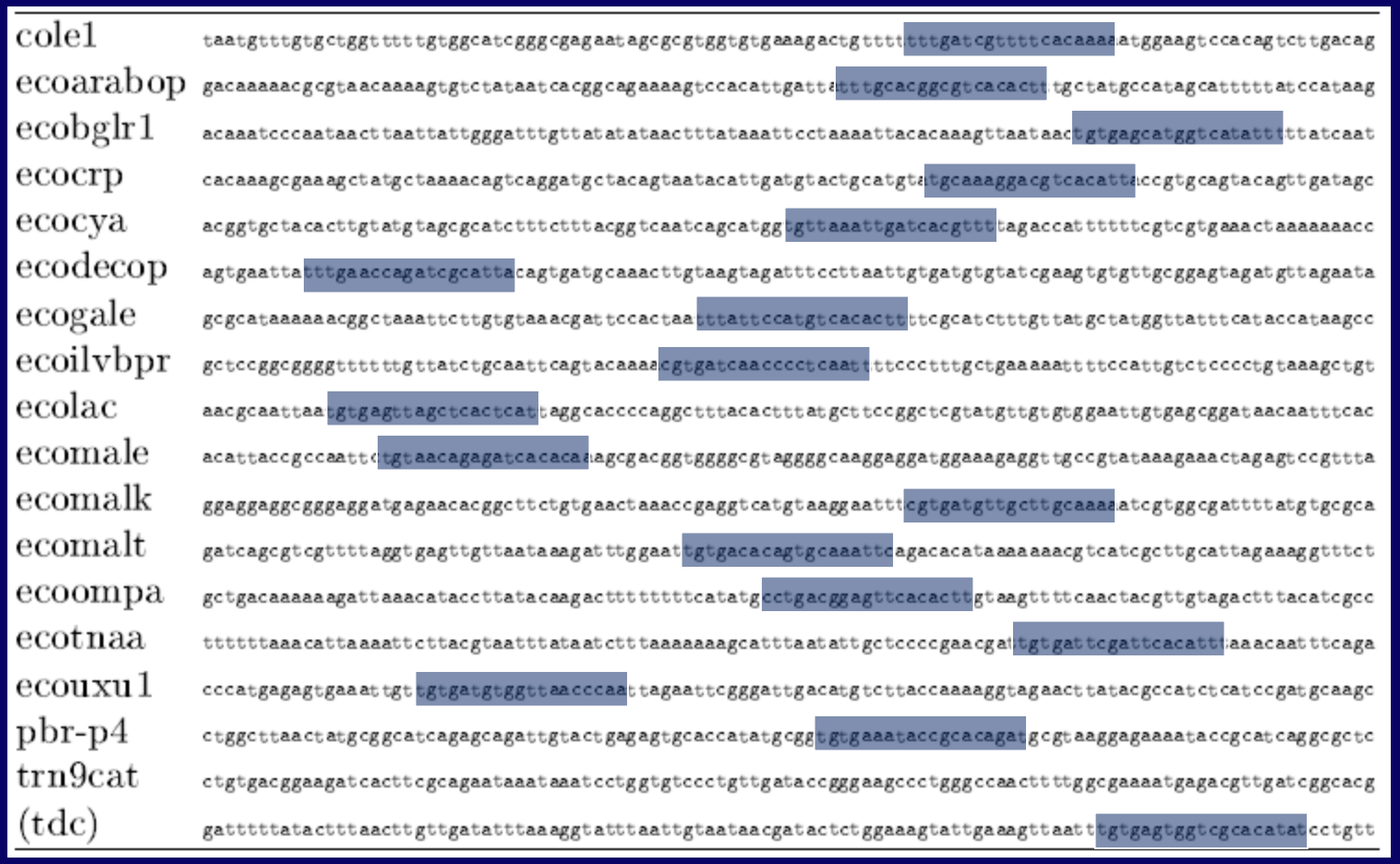
- Hybrid Monte Carlo strategy for Motif finding

Example: cyclic receptor protein (CRP)

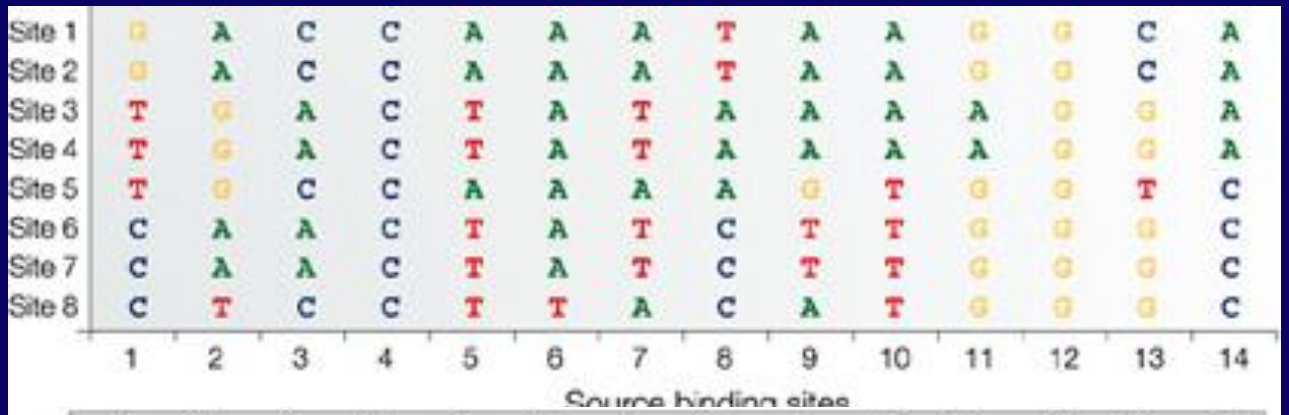


Stormo and Hartzell, 1989

Example: cyclic receptor protein (CRP)

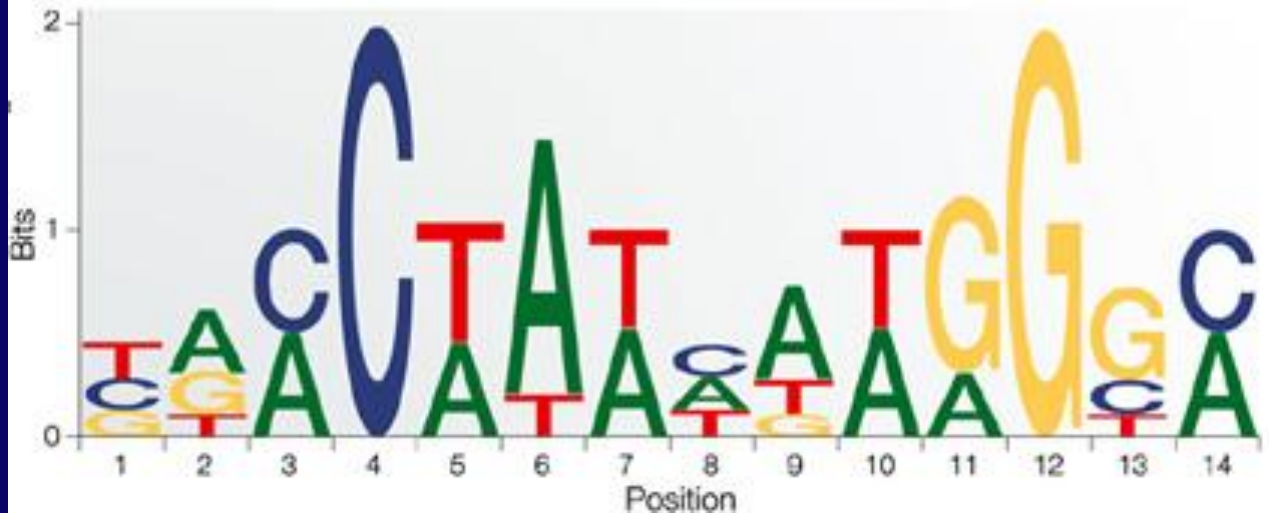


Transcription factor binding site (TFBS)



	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	0	4	4	0	3	7	4	3	5	4	2	0	0	4
C	3	0	4	8	0	0	0	3	0	0	0	0	2	4
G	2	3	0	0	0	0	0	0	1	0	6	8	5	0
T	3	1	0	0	5	1	4	2	2	4	0	0	1	0
	T	T	A	C	A	T	A	A	G	T	A	G	T	C

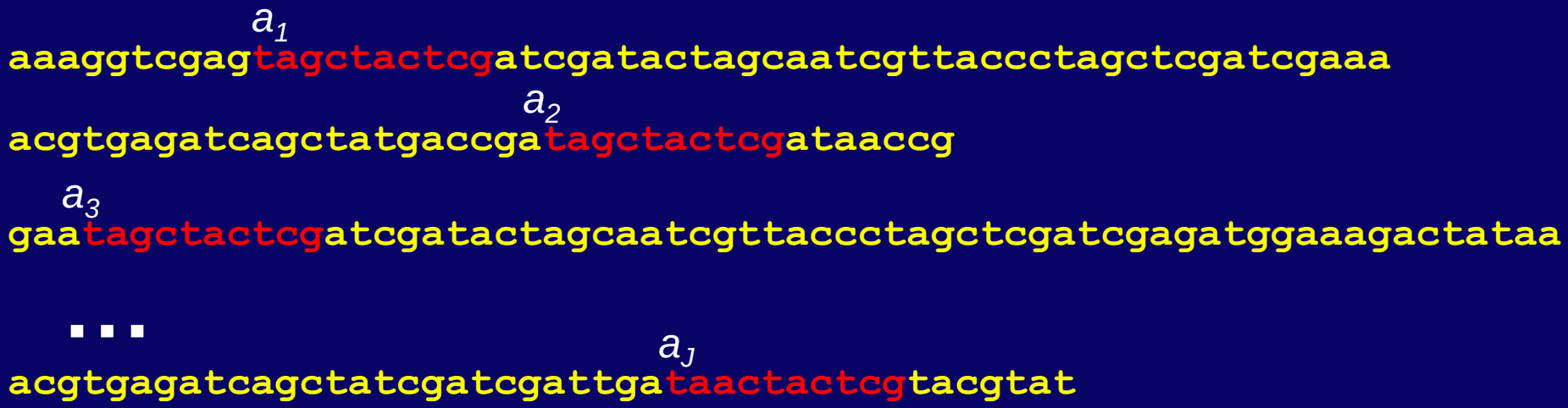
Σ = 5.23, 78% of maximum



Existing *de novo* motif finding algorithms

• Consensus	Hertz *et al.* 1990
• Gibbs Motif Sampler	Lawrence *et al.* 1993
• MEME	Bailey and Elkan 1994
• AlignACE	Roth *et al.* 1998
• BioProspector	Liu *et al.* 2001
• MDScan	Liu *et al.* 2002
• Mobydick	Bussemaker *et al.* 2000
…	
Review	Tompa *et al.* 2005

Motif identification model



Alignment variable $A = \{a_1, a_2 ..., a_J\}$

Posterior distributions

- The posterior conditional distribution for alignment variable ***A***

$$p(a_j = l \mid \boldsymbol{\theta}_0, \boldsymbol{\Theta}, \boldsymbol{R}_j, \boldsymbol{A}_{-j}) \propto \prod_{k=1}^{4} \theta_{0k}^{h_k(\boldsymbol{R}_j)} \prod_{i=1}^{w} \prod_{k=1}^{4} \left(\frac{\theta_{ik}}{\theta_{0k}} \right)^{h_k(r_{j,l+i-1})} \propto \prod_{i=1}^{w} \prod_{k=1}^{4} \left(\frac{\theta_{ik}}{\theta_{0k}} \right)^{h_k(r_{j,l+i-1})}$$

DNA sequence data $\boldsymbol{R} = (\boldsymbol{R}_1, \ldots, \boldsymbol{R}_J)$

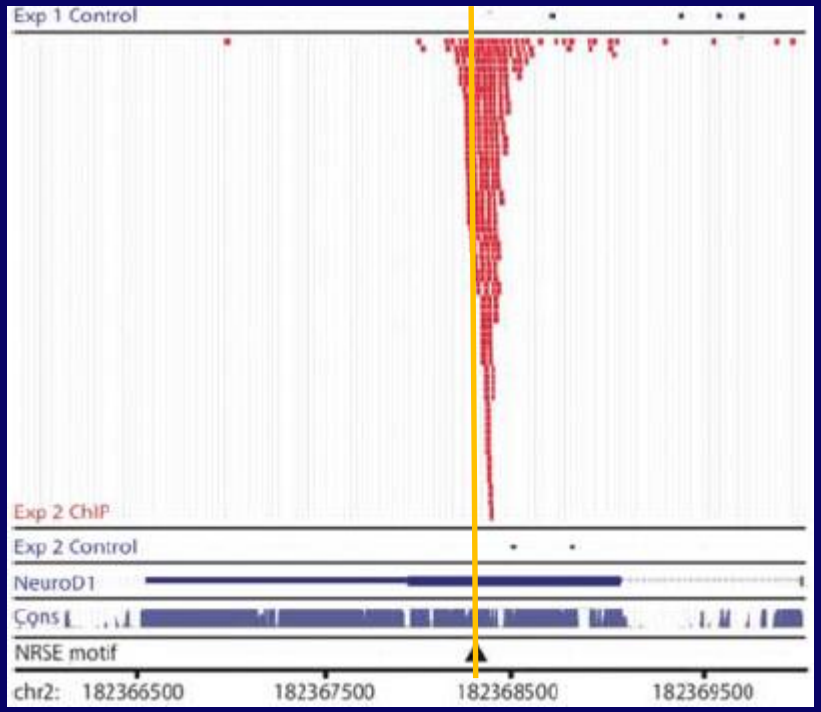
Lawrence *et al. Science* 1993, Liu *et al. JASA* 1995

Why *de novo* motif search

- The only option when the TF binding motif pattern is unknown.
- Reassuring to be able to rediscover the known TFBS motif.
- Many “known” motif patterns are biased and inaccurate.
- Multiple co-factors are often required in transcription regulation in eukaryotes.
- Binding specificity for some TFs may change under different conditions.

Challenges faced

- How to handle large number of input sequences?
- How to utilize sequencing depth information?



Johnson *et al. Science*

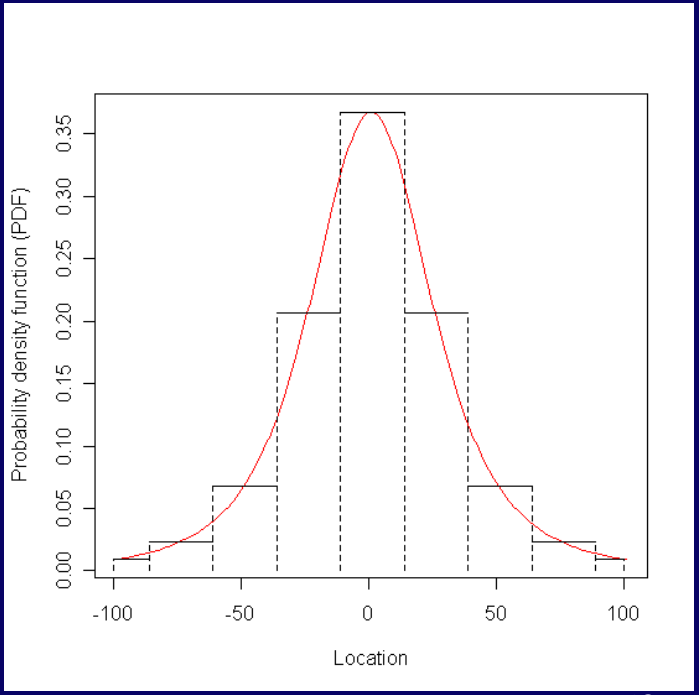
Features of our new algorithm

- Incorporate sequencing depth information in the statistical model.
- Generalize the product multinomial model to allow inter-dependent positions within the motif.
- Adopt a hybrid Monte Carlo strategy to speed up the traditional Gibbs sampler-based algorithm.

The informative prior

- The prior is symmetric and centered at the peak summit.
- The prior probabilities stem from Student's *t*-distribution with df=3.

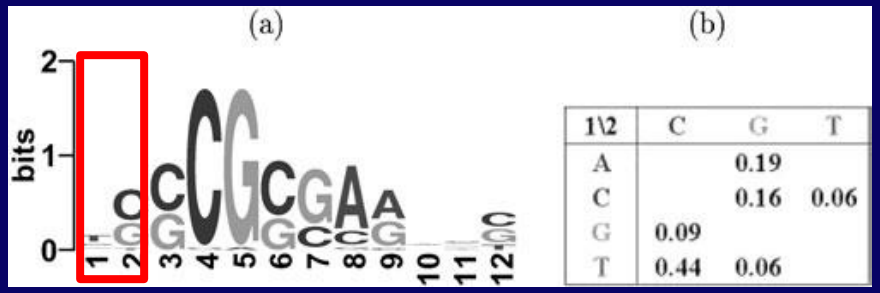
$$p(a_j = l) \propto t_3\left(\text{int}\left[\frac{|l + w/2 - s_j| + u/2}{u}\right]\right)$$



Modeling inter-dependent positions

- Zhou and Liu

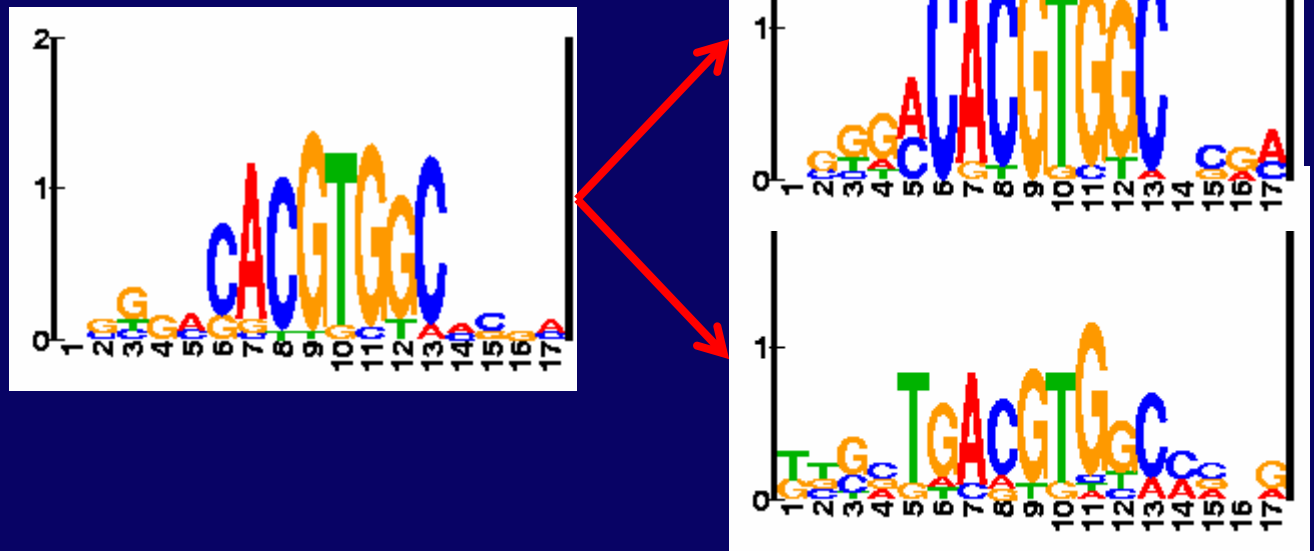
  Bioinformatics 2005



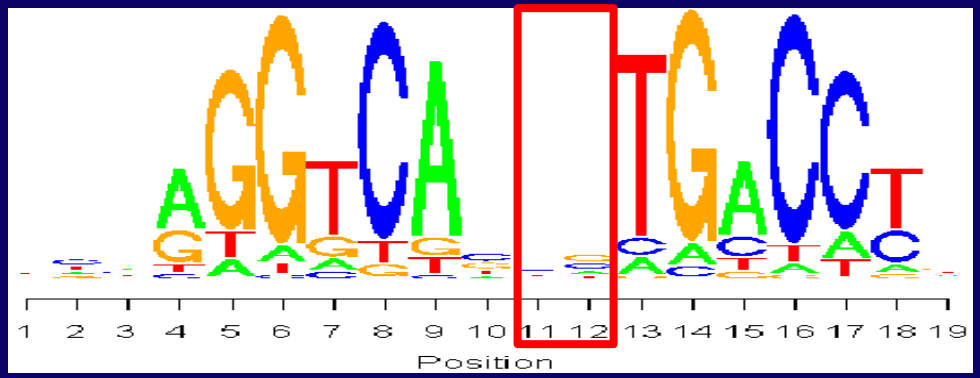
1\2	C	G	T
A		0.19	
C		0.16	0.06
G	0.09		
T	0.44	0.06	

- Barash *et al.*

  RECOMB 2003

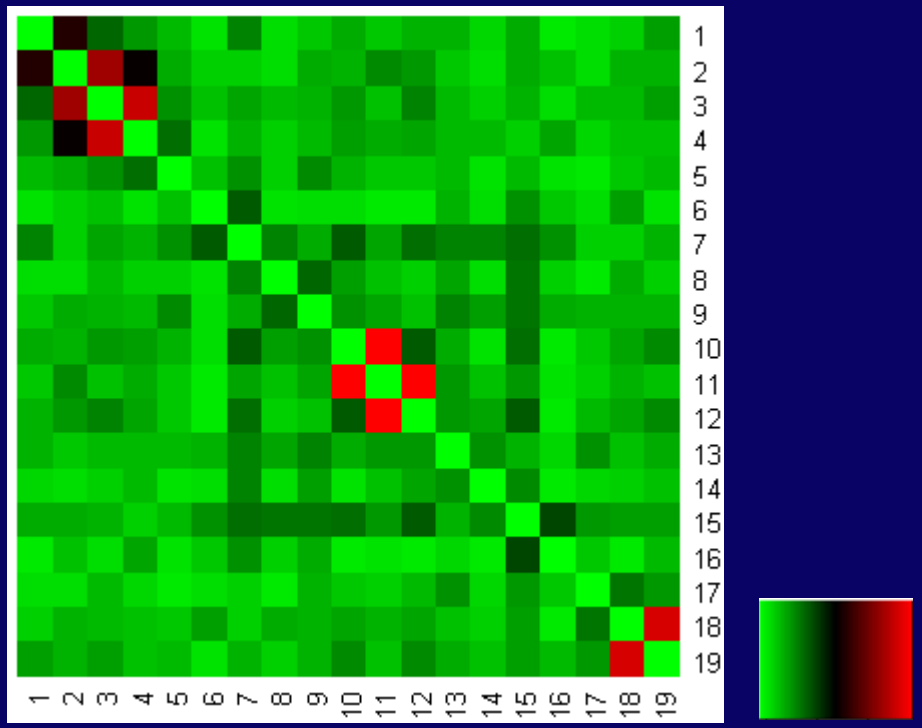


Detect intra-dependent position pairs



$$d_{ij} = \sum_{x=1}^{4}\sum_{y=1}^{4}\left|\hat{\eta}_{xy}(r_i, r_j) - \hat{\eta}_x(r_i)\hat{\eta}_y(r_j)\right|$$

	A	C	T	G	
A	0.03 (0.04)	0.15 (0.25)	0.28 (0.16)	0.03 (0.03)	0.49
C	0.00 (0.00)	0.01 (0.01)	0.00 (0.00)	0.00 (0.00)	0.01
T	0.05 (0.04)	0.34 (0.24)	0.06 (0.17)	0.03 (0.03)	0.48
G	0.00 (0.00)	0.02 (0.01)	0.00 (0.01)	0.00 (0.00)	0.02
	0.08	0.52	0.34	0.06	1



Prioritized hybrid Monte Carlo

- Subject each sequence to either stochastic sampling or greedy search.
- Input sequences are not created equal.
- ChIP-enrichment is indicative of binding affinity.

Implementation

- **H**ybrid **M**otif **S**ampler (HMS).
- Gibbs sampler type iterative procedure.
- Run multiple chains to avoid trapping in local mode.

Performance comparison

- Two established and popular motif discovery tools:
  - MEME (Bailey and Elkan 1994),
    - EM-based motif finding algorithm,
    - widely used.
  - MDscan (Liu *et al.* 2002),
    - designed to analyze ChIP-chip data,
    - combines word enumeration and probability matrix updating,
    - take into account ChIP-chip ranking,
    - very fast.

Real data analysis

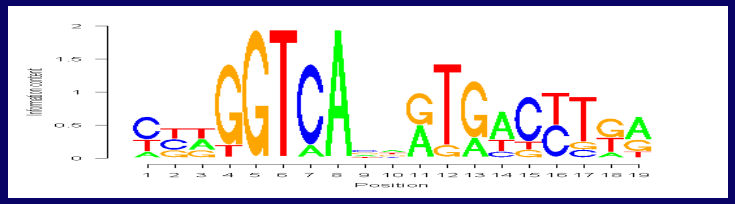
TF	Cell type	Antibody	# of peaks	Coverage	Reference
NRSF	Jurkat T cell	Monoclonal 12C11	4,982	1.4 MB	Johnson et al. (2007)
STAT1	HeLa S3 cell	Polyclonal	27,470	8.1 MB	Robertson et al. (2007)
CTCF	CD4+ T cell	Upstate 07-729	22,159	7.4 MB	Barski et al. (2007)
ER	**MCF7 cell**	**ER α (HC-20)**	**10,072**	**2.5 MB**	

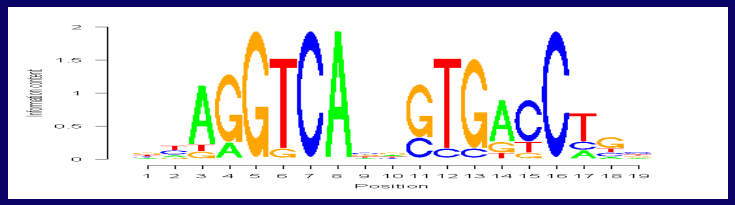
Performance evaluation

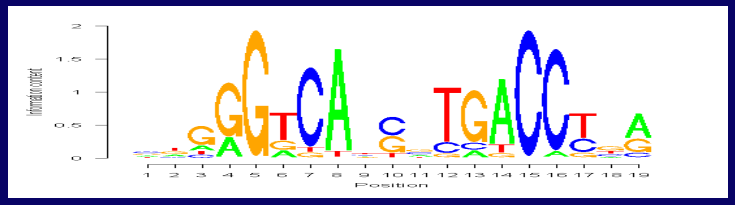
- Cross validation
  - Randomly separate all peaks into two halves: training and testing.
  - Run motif finding algorithms on the training data to predict the motif pattern.
  - Scan testing data using the identified motif pattern and compare to a set of control sequences.
- Testing
  - Using Chi-square test statistics to quantify motif enrichment .
  - Estimate FDR and plot FDR versus Chi-square test statistics.

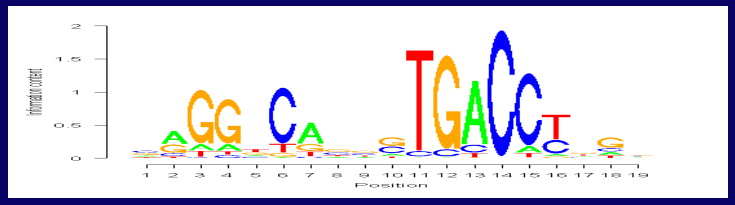
Compare ER motif patterns

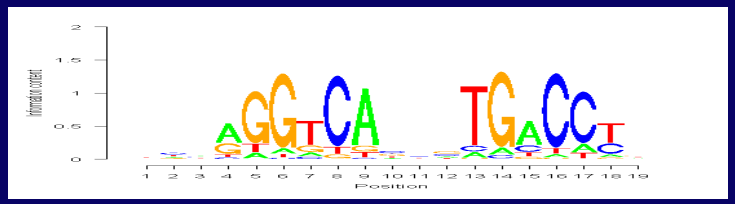
- V$ER01*
- V$ER02*
- V$ER03*
- MEME
- HMS







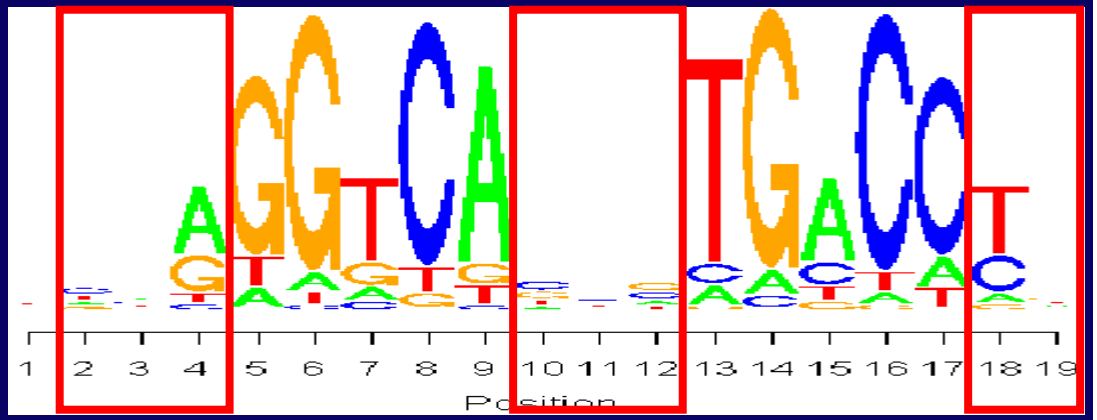


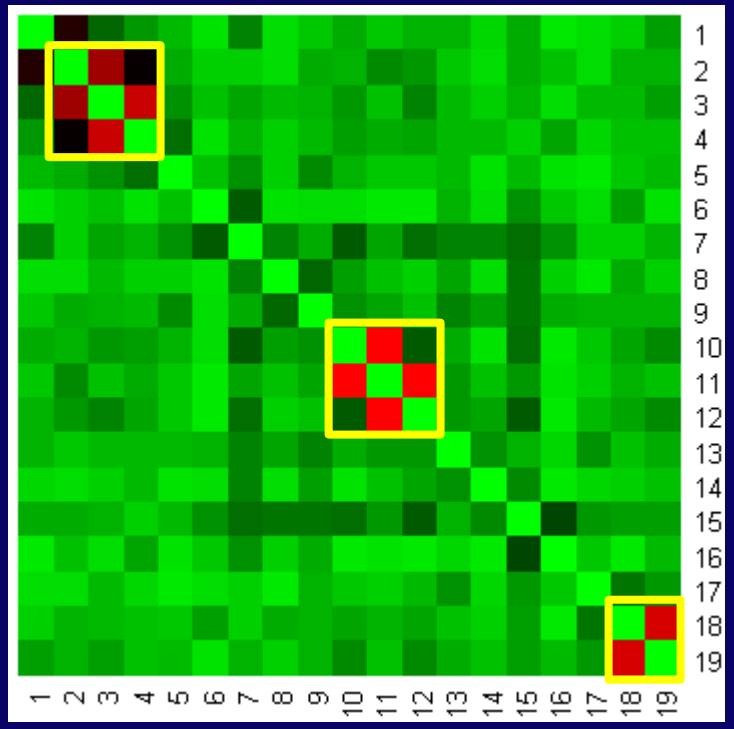


*

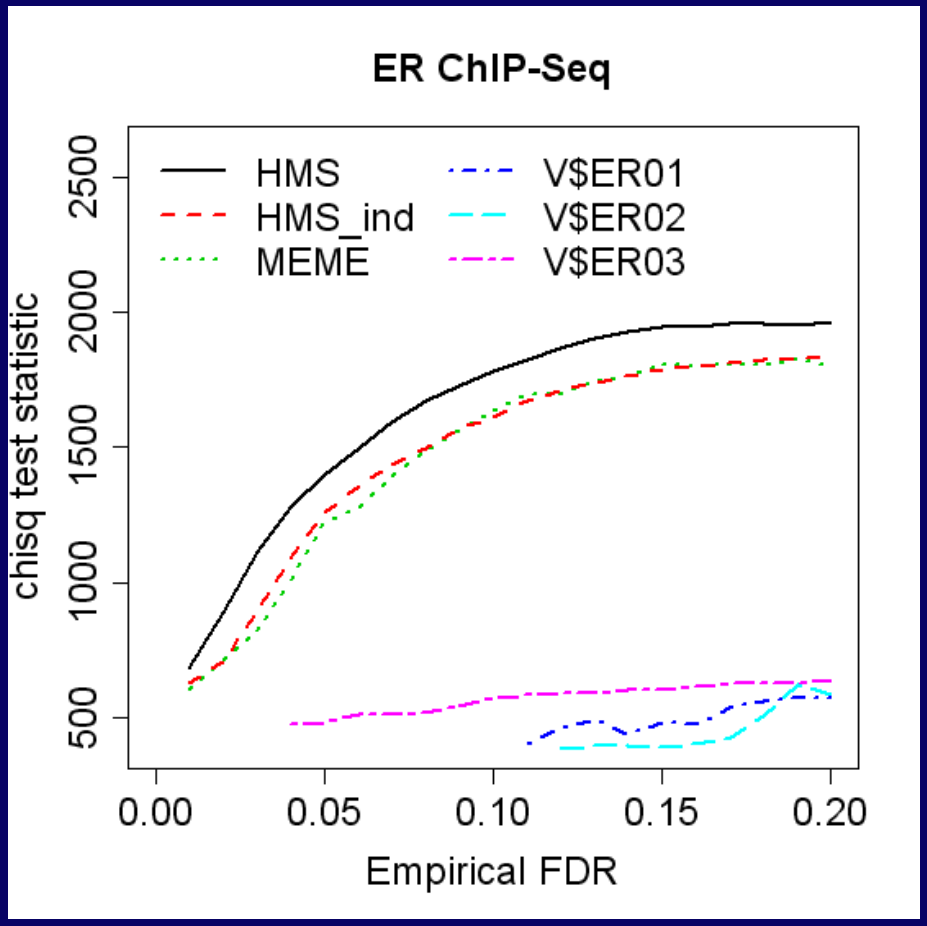
Genomatix
understanding gene regulation

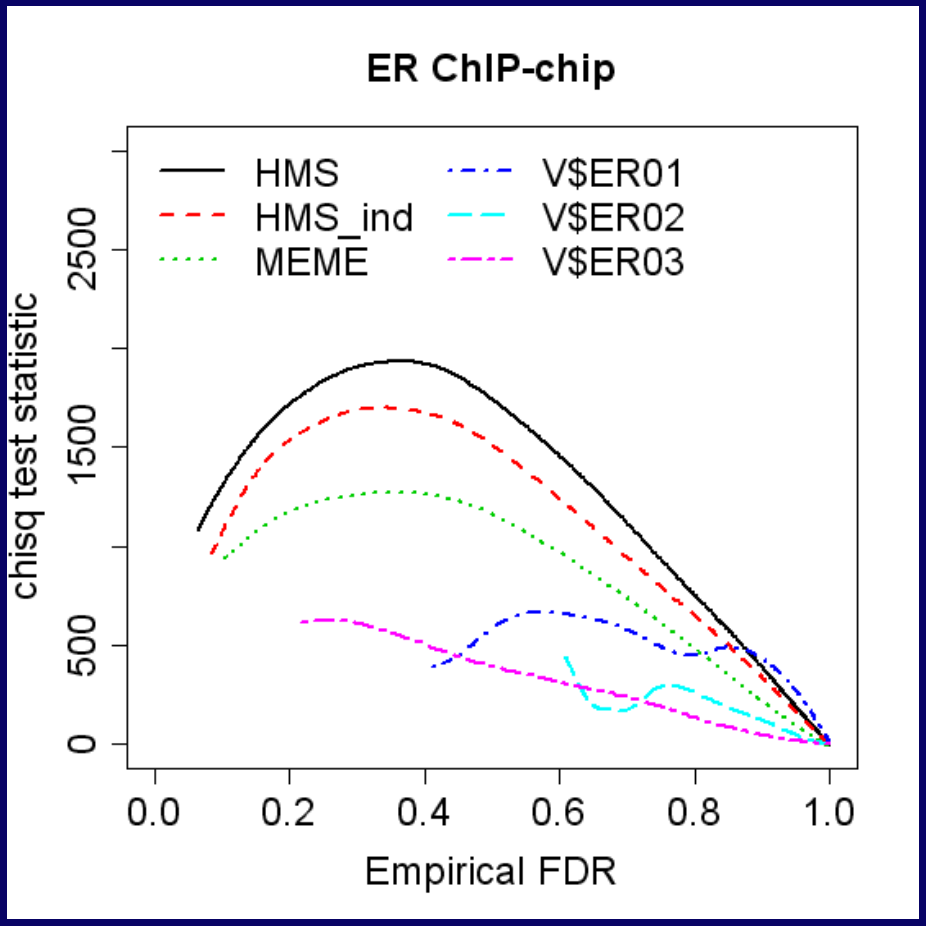
Positions show inter-dependency inside the ER motif





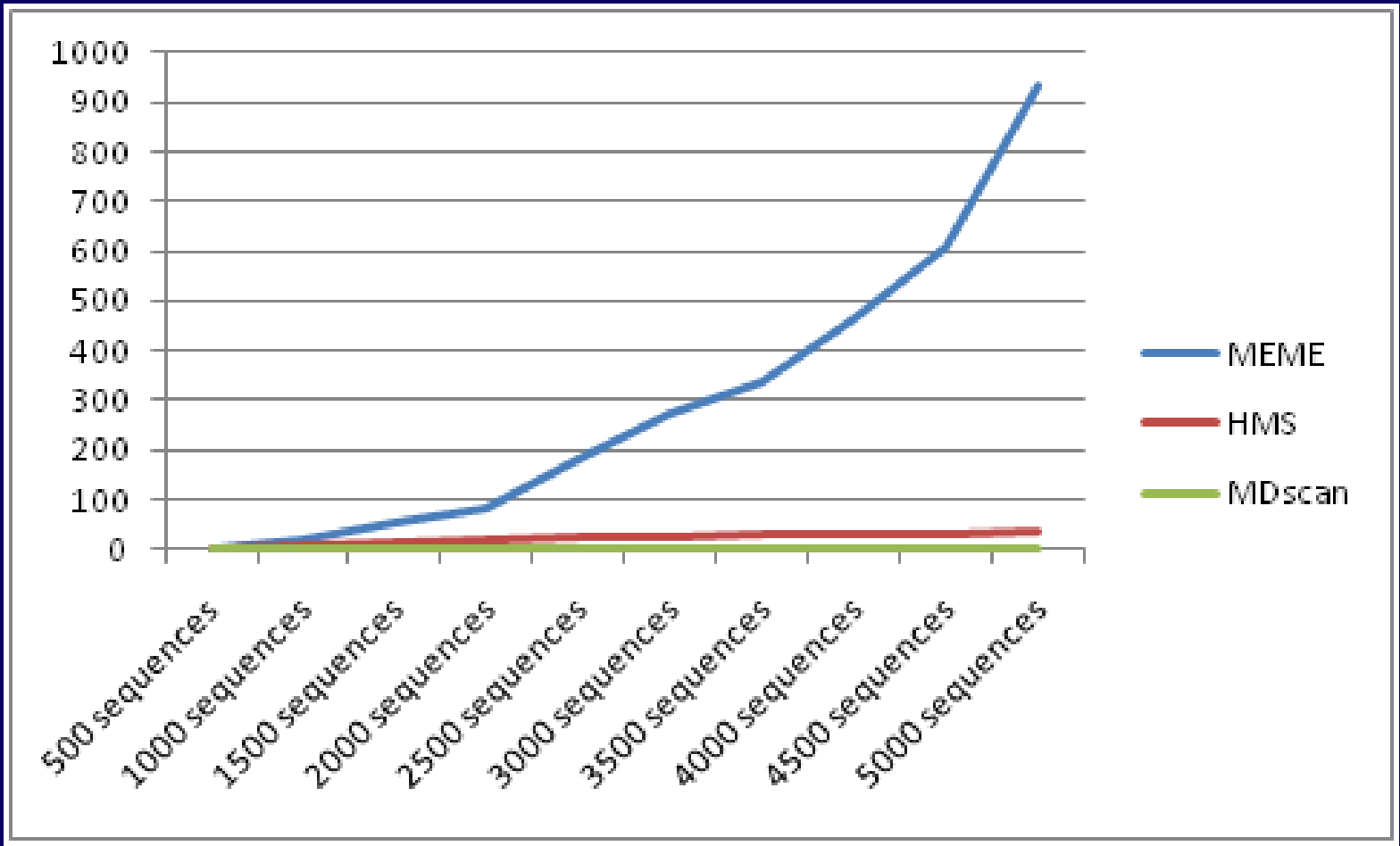
Compare ER motif enrichment





Carroll *et al. Nature Genetics* 2006

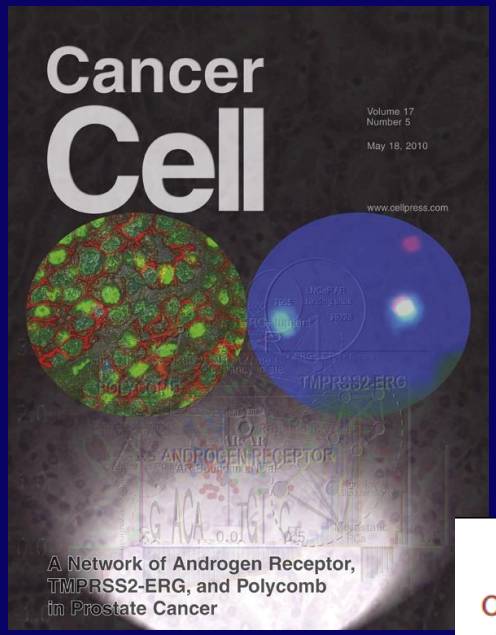
Computation time



Summary

- ChIP-Seq data offers abundant information and provides much improved opportunity for studying protein-DNA interaction.
- There are many biological and technical factors that affect the ChIP-Seq data we observe, careful modeling is critical in order to process ChIP-Seq data efficiently and thoroughly.
- New sequencing data are different from microarray, ChIP-chip data. Methods developed there do not work well for analyzing sequencing data, new models and algorithms need to be developed.

Apply to cancer genomics



Cancer Cell
Article

An Integrated Network of Androgen Receptor, Polycomb, and TMPRSS2-ERG Gene Fusions in Prostate Cancer Progression

Jindan Yu,[1,3,6,7] Jianjun Yu,[1,3] Ram-Shankar Mani,[1,3] Qi Cao,[1,3] Chad J. Brenner,[1,3] Xuhong Cao,[1,2,3] Xiaoju Wang,[1,3] Longtao Wu,[7] James Li,[1,3] Ming Hu,[1,5] Yusong Gong,[1,3] Hong Cheng,[1,3] Bharathi Laxman,[1,3] Adaikkalam Vellaichamy,[1,3] Sunita Shankar,[1,3] Yong Li,[1,3] Saravana M. Dhanasekaran,[1,3] Roger Morey,[1,3] Terrence Barrette,[1,3] Robert J. Lonigro,[1,6] Scott A. Tomlins,[1,3] Sooryanarayana Varambally,[1,3,6] Zhaohui S. Qin,[5] and Arul M. Chinnaiyan[1,2,3,4,6,*]

Reference

- Qin ZS, Yu J, Shen J, Maher CA, Hu M, Kalyana-Sundaram S, Yu J, Chinnaiyan AM. (2009) HPeak: An HMM-based Algorithm for Defining Read-enriched Regions in ChIP-Seq Data. *BMC Bioinformatics.* **11** 369.

  http://www.sph.umich.edu/csg/qin/HPeak/
- Choi H, Nesvizhskii A, Ghosh D, Qin ZS. (2009) Hierarchical Hidden Markov Model with Application to Joint Analysis of ChIP-chip and ChIP-seq Data. *Bioinformatics* **25** 1715-1721.

  http://sourceforge.net/projects/chipmeta/
- Hu M, Yu J, Taylor, JMG, Chinnaiyan AM, **Qin ZS.** (2010) On the Detection and Refinement of Transcription Factor Binding Sites Using ChIP-Seq Data. *Nucleic Acids Res.* **38** 2154-2167.

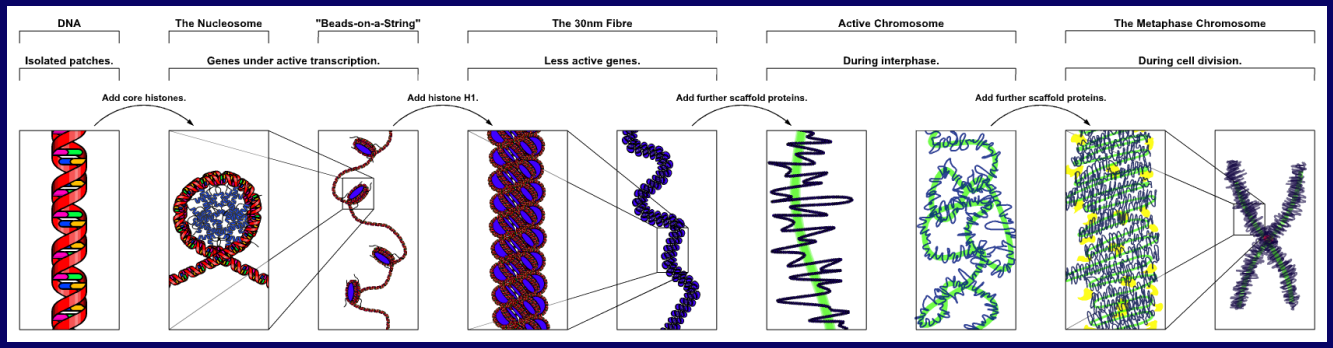
  http://www.sph.umich.edu/csg/qin/HMS/
- Hu M, Zhu Y, Taylor JMG, Liu JS, Qin ZS (2011). Using Poisson mixed-effects model to quantify exon-level gene expression in RNA-seq. *Bioinformatics.* **28** 63-68.

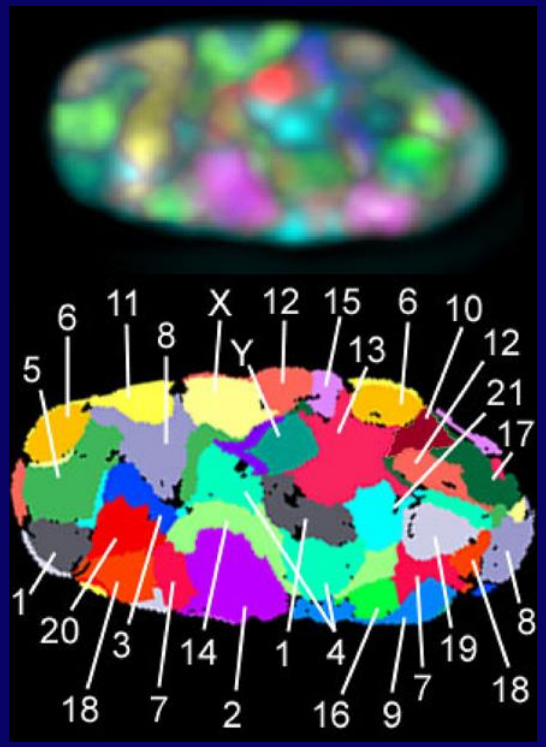
  http://www.stat.purdue.edu/~yuzhu/pome.html

Statistical model to infer chromosomal structures from Hi-C data

Chromosome folding

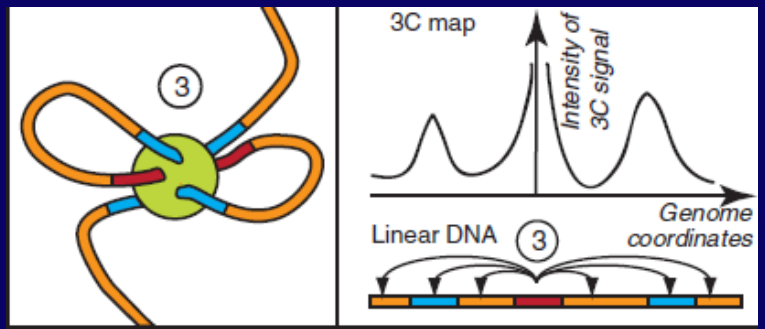
How can a two meter long polymer fit into a nucleus of ten micrometer (10^{-5} m) diameter?





http://en.wikipedia.org/wiki/Chromosome

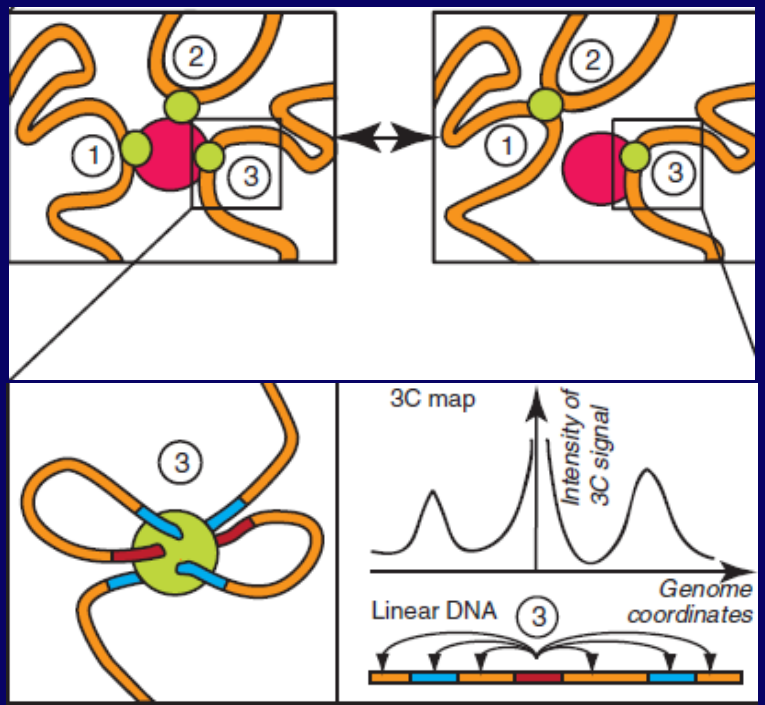
Chromosome Conformation Capture (3C)
Dekker et al. *Science* 2002



Fine scale: (0-kb)

Naumova and Dekker *J of Cell Science* 2010

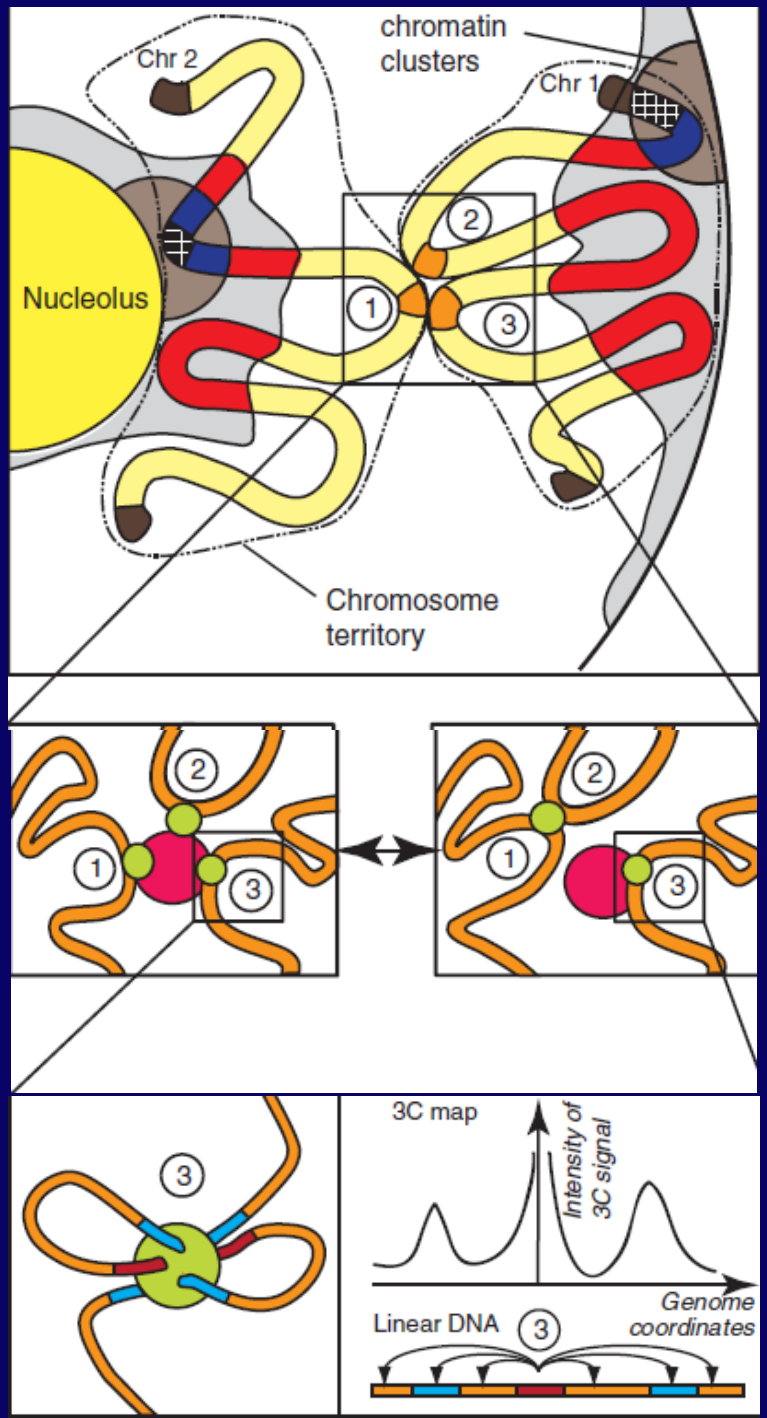
3C-on-chip/Circular 3C (4C)
5C



Intermediate: (0-Mb)

Fine scale: (0-kb)

Naumova and Dekker *J of Cell Science* 2010



Whole genome

Intermediate: (0-Mb)

Fine scale: (0-kb)

Naumova and Dekker *J of Cell Science* 2010


Science
9 October 2009 | $10
nature biotechnology
Arranging genomes in 3D
Pigeonpea staple sequenced
Deeper RNA sequencing
Cell
Volume 148
Number 3
February 3, 2012
Genome in 3D
Hypoxia in Disease

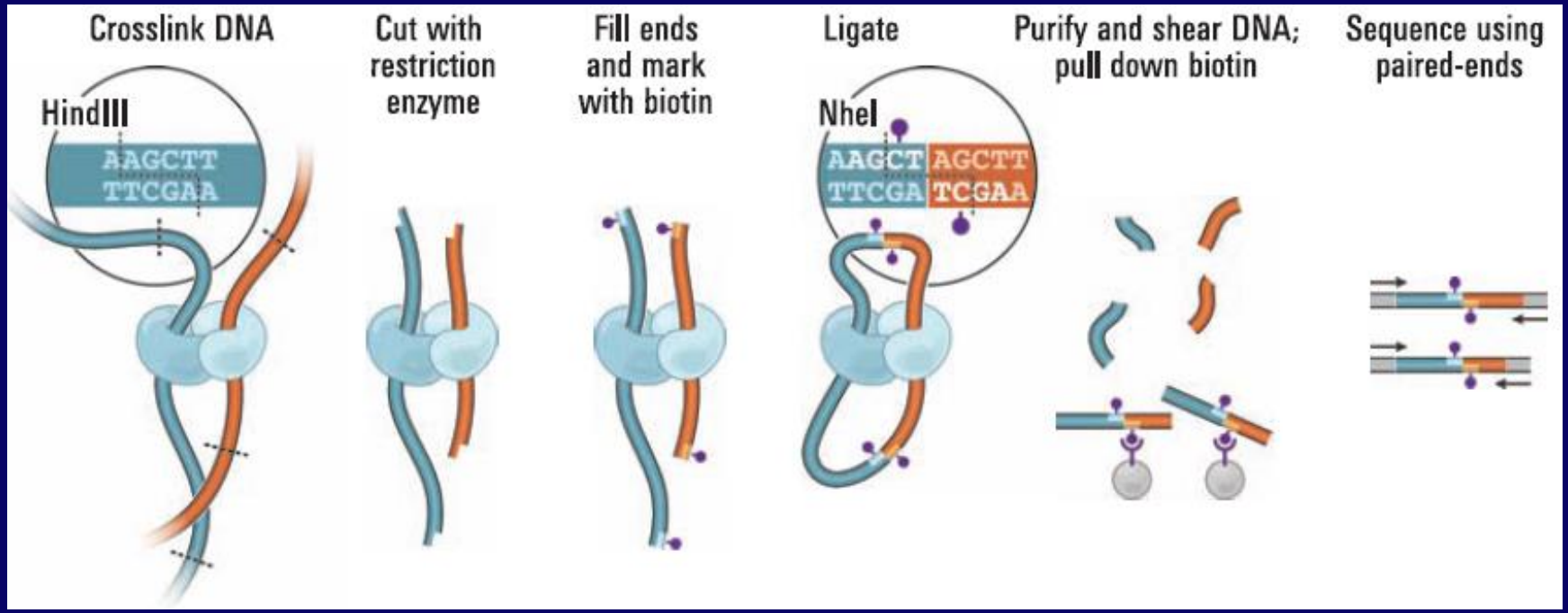
Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome

Erez Lieberman-Aiden,[1,2,3,4]* Nynke L. van Berkum,[5]* Louise Williams,[1] Maxim Imakaev,[2] Tobias Ragoczy,[6,7] Agnes Telling,[6,7] Ido Amit,[1] Bryan R. Lajoie,[5] Peter J. Sabo,[8] Michael O. Dorschner,[8] Richard Sandstrom,[8] Bradley Bernstein,[1,9] M. A. Bender,[10] Mark Groudine,[6,7] Andreas Gnirke,[1] John Stamatoyannopoulos,[8] Leonid A. Mirny,[2,11] Eric S. Lander,[1,12,13]† Job Dekker[5]†

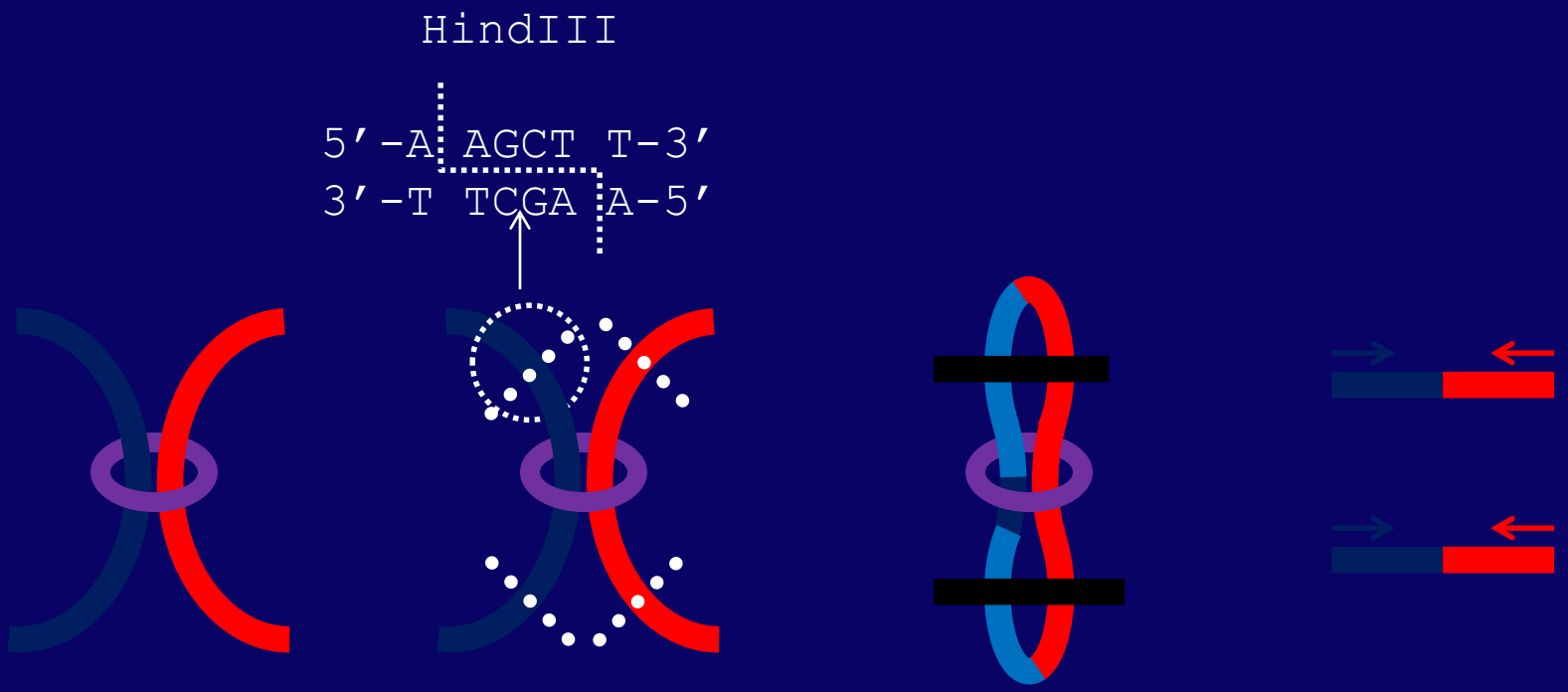
We describe Hi-C, a method that probes the three-dimensional architecture of whole genomes by

We created a Hi-C library from a karyotypically normal human lymphoblastoid cell line (GM06990) and sequenced it on two lanes of an Illumina Genome Analyzer (Illumina, San Diego, CA), generating 8.4 million read pairs that could be uniquely aligned to the human genome reference sequence; of these, 6.7 million corresponded to long-range contacts between segments >20 kb apart.

We constructed a genome-wide contact matrix M by dividing the genome into 1-Mb regions ("loci") and defining the matrix entry m_{ij} to be the number of ligation products between locus i and locus j (*10*). This matrix reflects an ensemble



Hi-C: one cell



Cross-link DNA

Restriction enzyme cut

Ligation and shear

Paired-end sequencing

Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome

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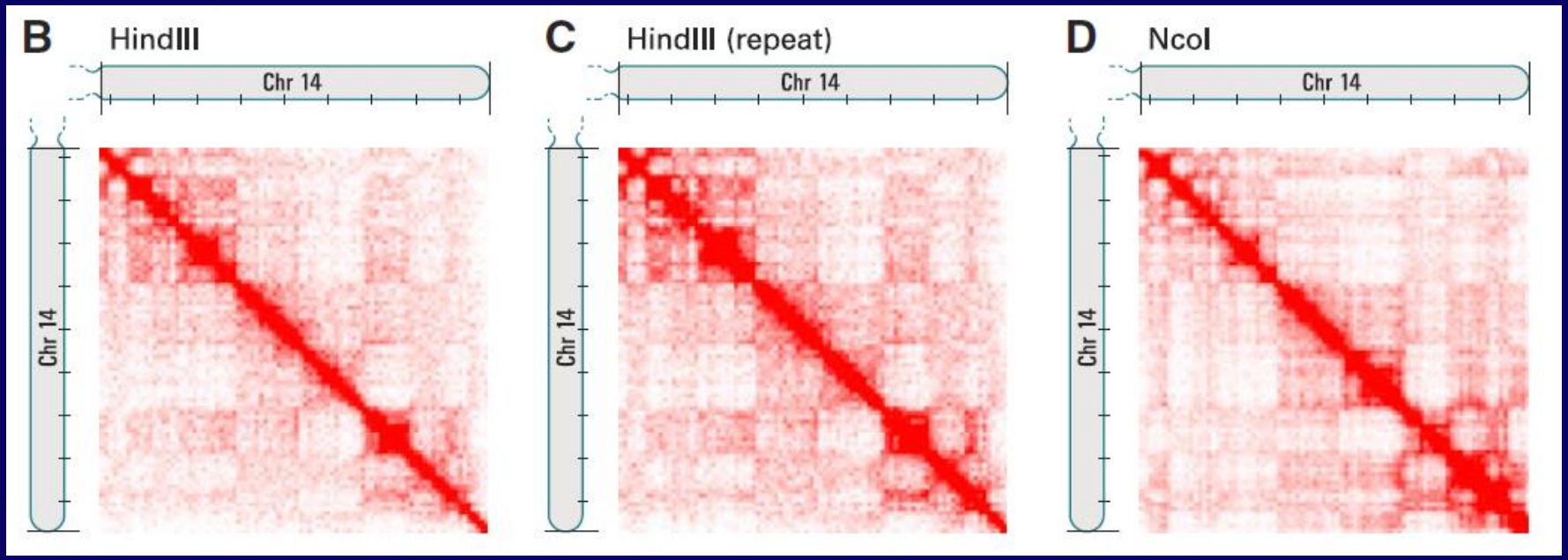
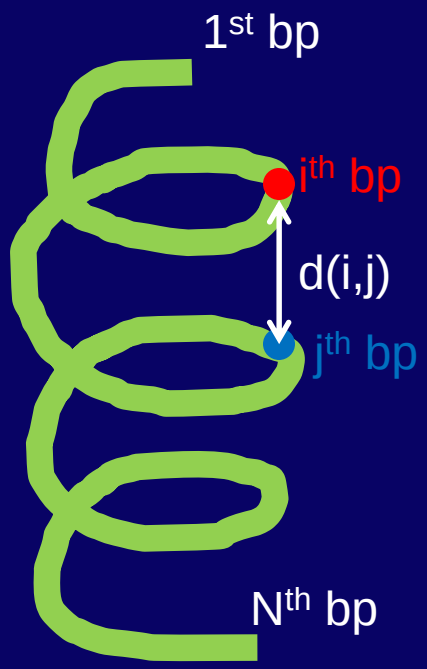
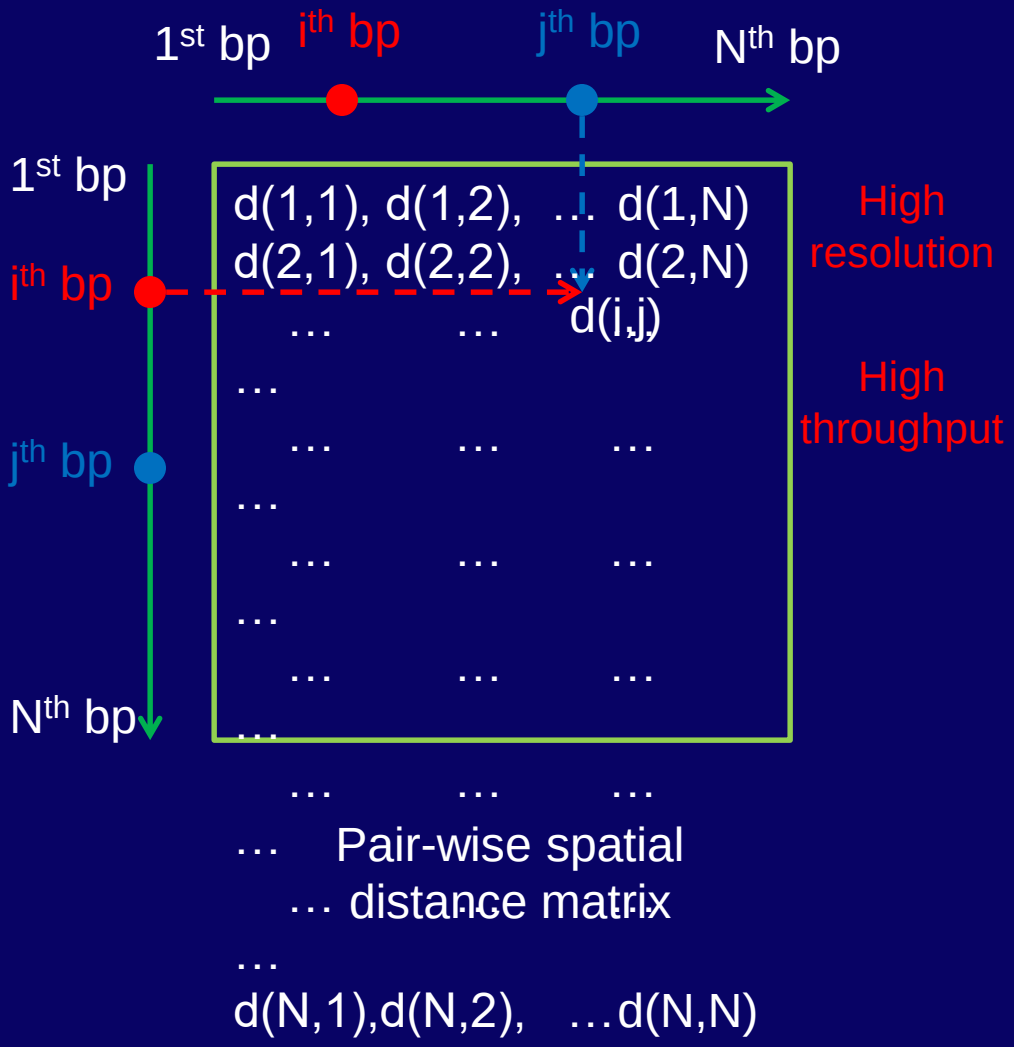


	chr1	chr2	chr3	chr4	chr5	chr6	chr7	chr8	chr9	chr10	chr11	chr12	chr13	chr14	chr15	chr16	chr17	chr18	chr19	chr20	chr21	chr22	chrX	chrY
chr1	2242	788	611	388	557	617	705	412	471	538	681	536	157	268	409	492	542	176	635	327	164	502	221	11
chr2	0	860	312	199	292	345	373	253	241	242	354	272	82	140	224	238	293	92	317	193	71	245	101	6
chr3	0	0	621	145	237	255	281	204	186	227	251	206	49	94	160	181	238	65	244	133	55	193	101	3
chr4	0	0	0	277	148	130	189	114	101	127	165	124	38	83	103	110	144	48	139	78	41	128	58	1
chr5	0	0	0	0	622	212	263	170	168	176	261	204	50	91	161	173	223	65	226	105	50	187	82	3
chr6	0	0	0	0	0	731	317	207	204	199	256	222	62	127	174	193	281	73	244	150	59	198	95	4
chr7	0	0	0	0	0	0	806	197	216	241	315	232	67	150	206	232	267	83	281	147	76	227	95	8
chr8	0	0	0	0	0	0	0	434	130	164	210	170	35	86	135	150	155	66	180	94	43	147	79	4
chr9	0	0	0	0	0	0	0	0	517	163	210	188	43	76	117	157	196	49	196	91	36	175	58	1
chr10	0	0	0	0	0	0	0	0	0	482	228	197	53	83	144	151	201	66	226	104	44	173	68	6
chr11	0	0	0	0	0	0	0	0	0	0	872	238	58	138	176	217	257	95	289	174	58	221	118	8
chr12	0	0	0	0	0	0	0	0	0	0	0	607	63	105	134	191	236	60	210	143	57	160	82	6
chr13	0	0	0	0	0	0	0	0	0	0	0	0	110	27	47	44	59	11	72	20	10	37	15	1
chr14	0	0	0	0	0	0	0	0	0	0	0	0	0	242	85	78	98	29	113	62	34	83	46	0
chr15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	437	114	181	45	172	93	46	128	61	2
chr16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	538	198	52	227	95	39	169	82	9
chr17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	781	61	243	126	68	184	75	2
chr18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	134	64	42	23	66	34	8
chr19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	825	143	63	207	89	3
chr20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	386	42	105	64	5
chr21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	144	45	23	1
chr22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	521	74	4
chrX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	170	0
chrY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

Hi-C Data Representation



3D chromosomal structure

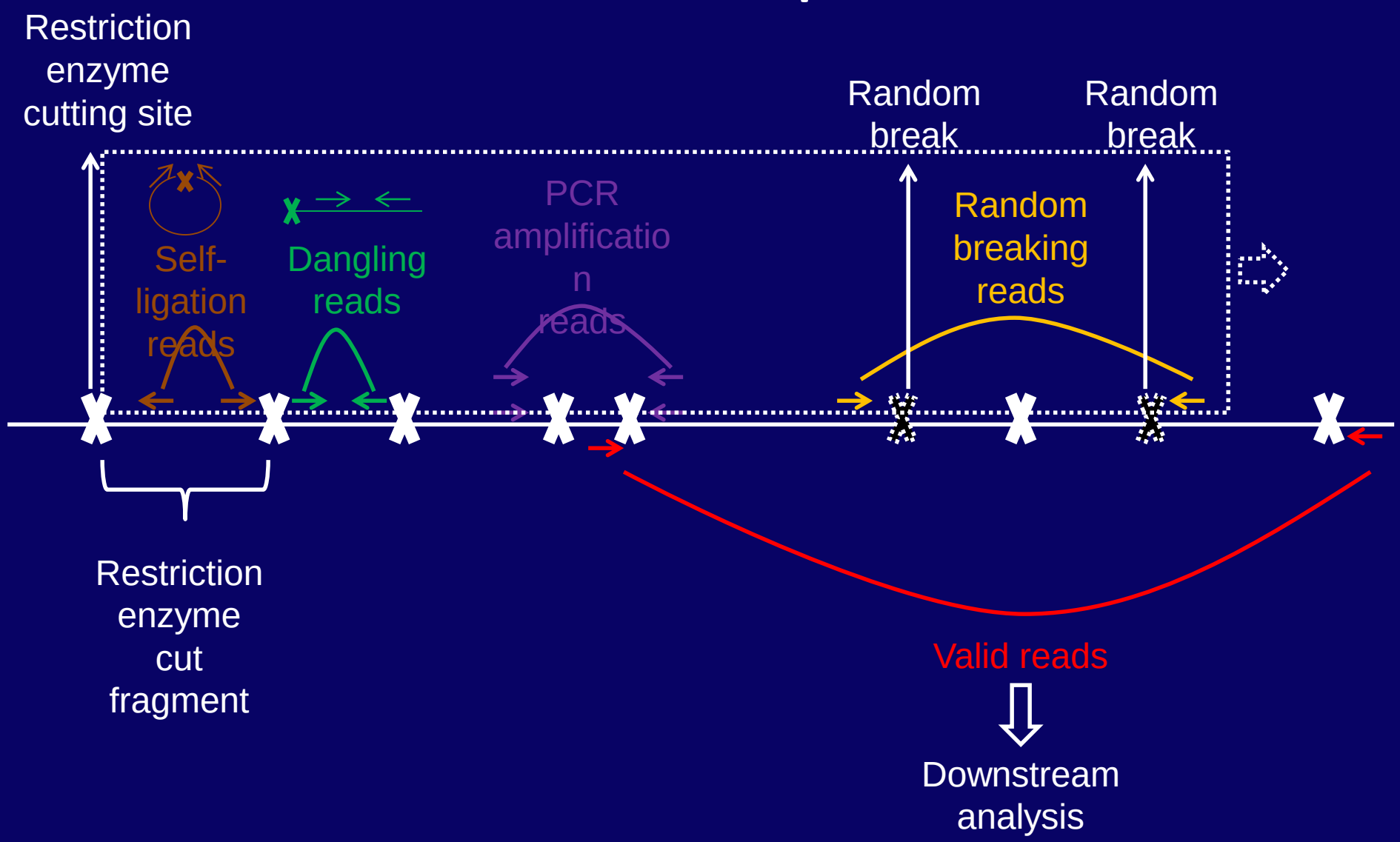


Pair-wise spatial distance matrix

Challenges

- Quality control and pre-processing of the reads,
- Any bias in the data? and if so, how to normalize?
- Whether it is possible, and if so, how, to infer the 3-dimesnional chromosomal structure based on the Hi-C data?

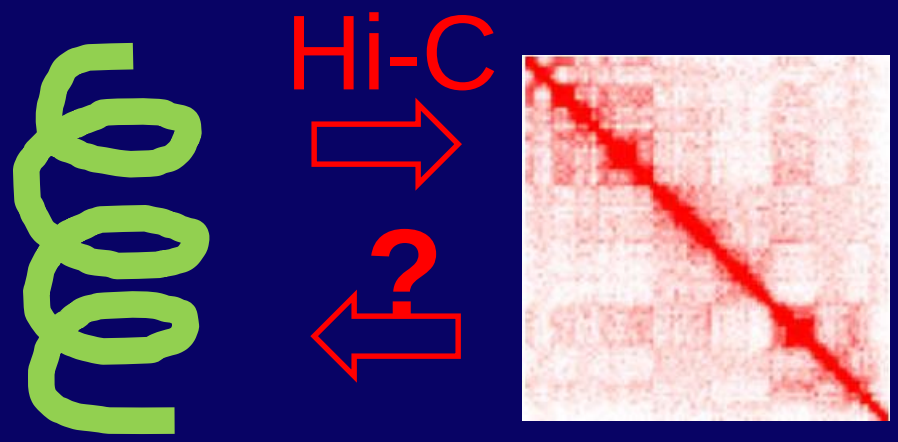
Hi-C Data Preprocess



Methods for Hi-C Bias Reduction

- Normalization (equal 'visibility', no assumption on biases)
  - Iterative correction and eigenvector decomposition (ICE) (Imakaev, et al, 2012)
  - Sequential component normalization (SCN) (Cournac, et al, 2012)
- Correction (posit a statistical model on biases)
  - Yaffe & Tanay's method (Yaffe & Tanay, 2011)
    Fragment level (4KB, 10^{12}), 420 parameters
  - **HiCNorm (Hu et al, 2012)**
    Any resolution level
    1MB, 10^6, 3 parameters

3D structure prediction



- Challenges:
  - Sequencing uncertainties
  - Biases: enzyme, GC content, mappability

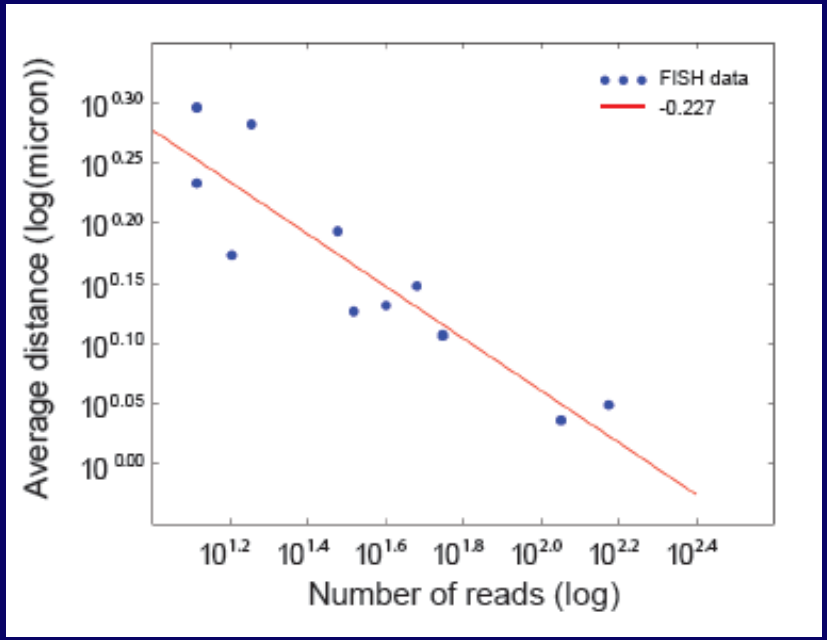
Yaffe and Tanay, 2011

What does the number mean?

- The Hi-C experiment is conducted on millions of cells,
- A captured pair-end read is from one cell,
- A number in the matrix (loci *i* and *j*) indicates the frequency of capture (link *i* and *j)* in the cell population,
- Do those numbers say anything about 3D distance?

Motivation and the key assumption

- Number of paired-end reads spanning the two loci is inversely proportional to the 3D spatial distance between them (obtained from fluorescence in situ hybridization(FISH)).



Lieberman-Aiden et al, 2009

Existing methods

- Optimizations-based method (Baù, et al, 2010, Duan, et al, 2010)
  - Biophysical properties of chromatin fiber.
  - No consideration of systematic biases.
  - No statistical inference.

- Statistical method: MCMC5C (Rousseau et al, 2011)
  - Normal model for count data.
  - No consideration of systematic biases.

Model

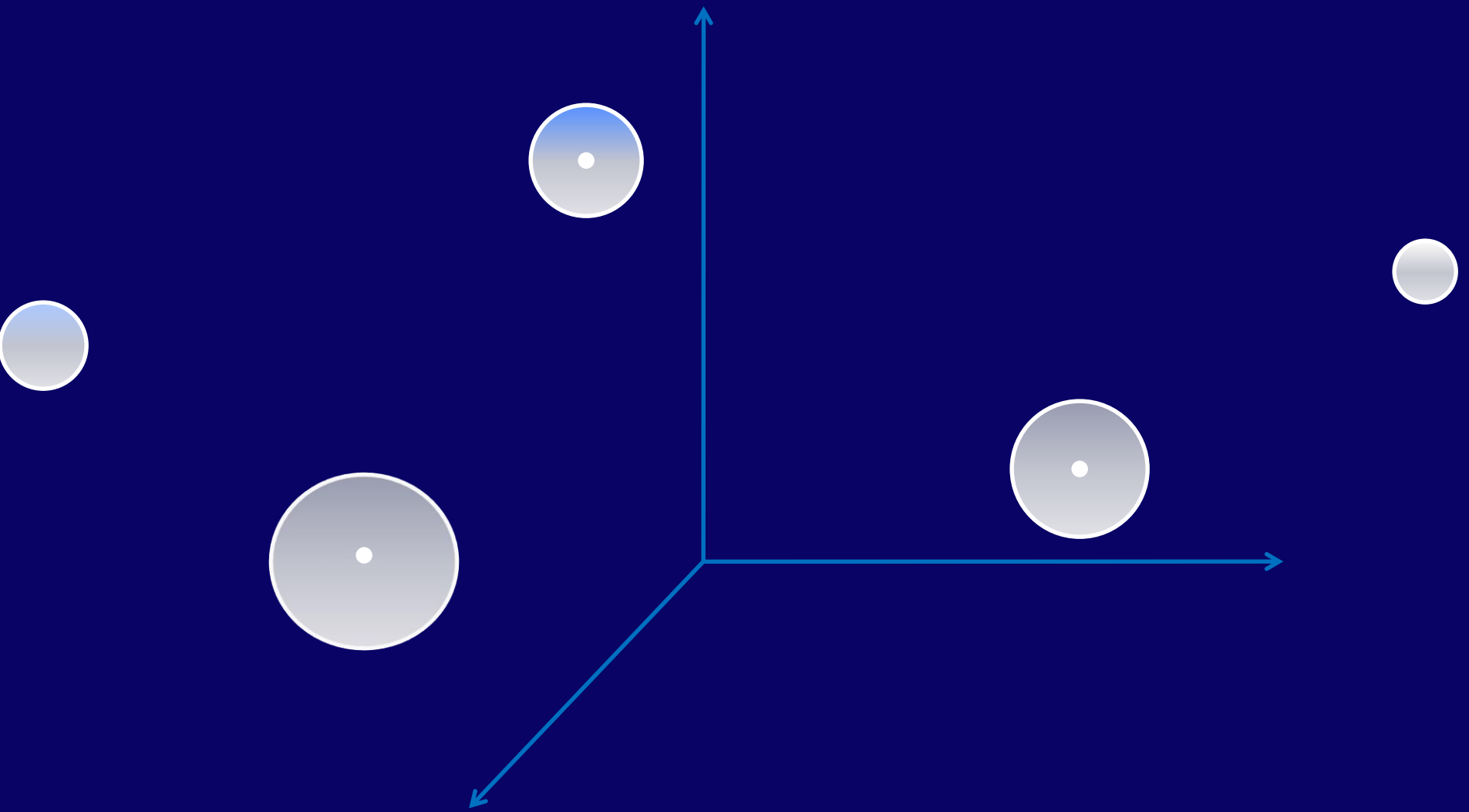
**ACGTAGCTAGATACTGTAGTACATCGATAGCGTAGTTTGGAACCTGAGGGTAAACC
TGGAGGGGATCATG**

Model

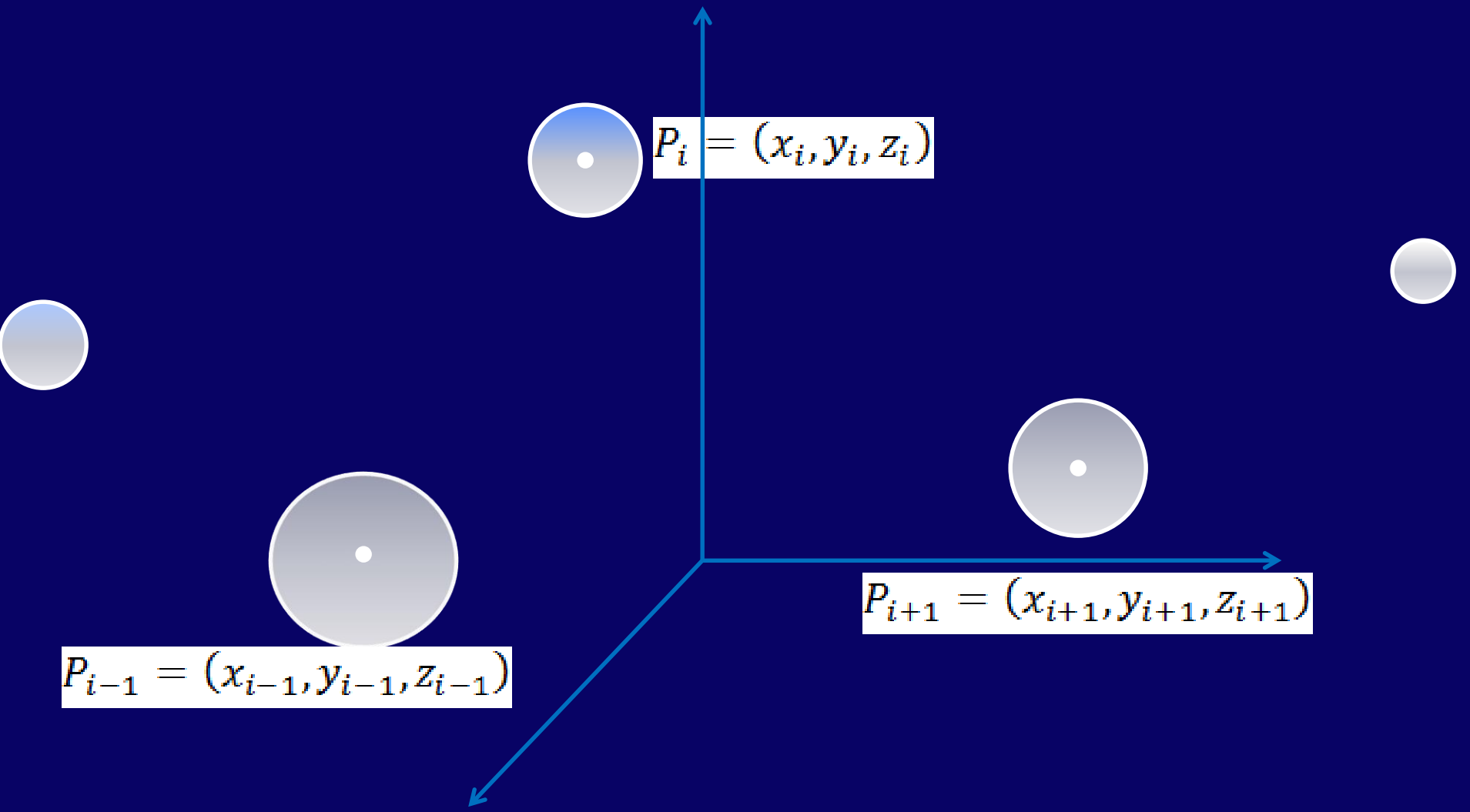
ACGTAGCTAGATACT GTAGTACATCGATAG CGTAGTTTGGAACCT GAGGGTAAACCTGG AGGGGAT

Model

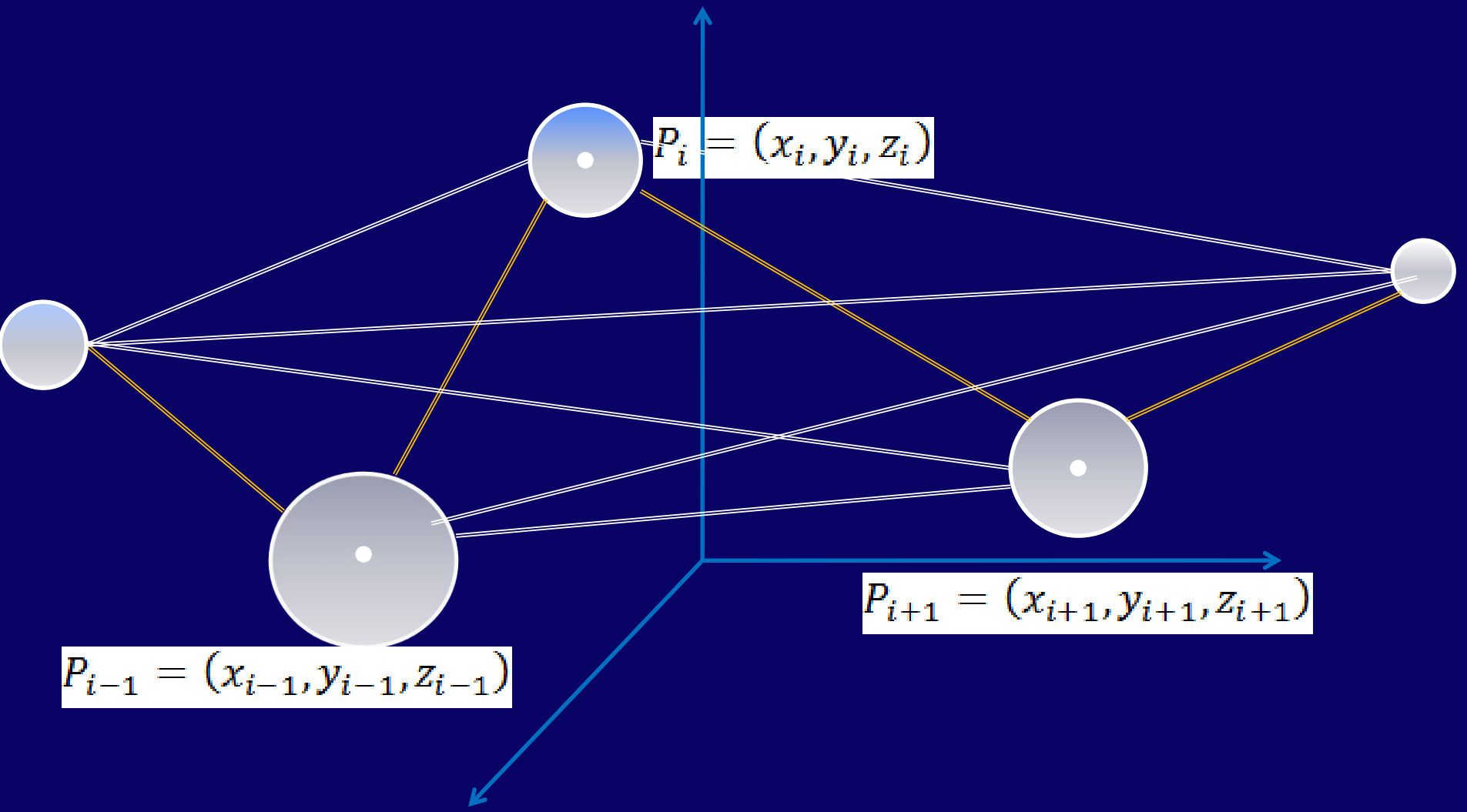
Beads-on-string



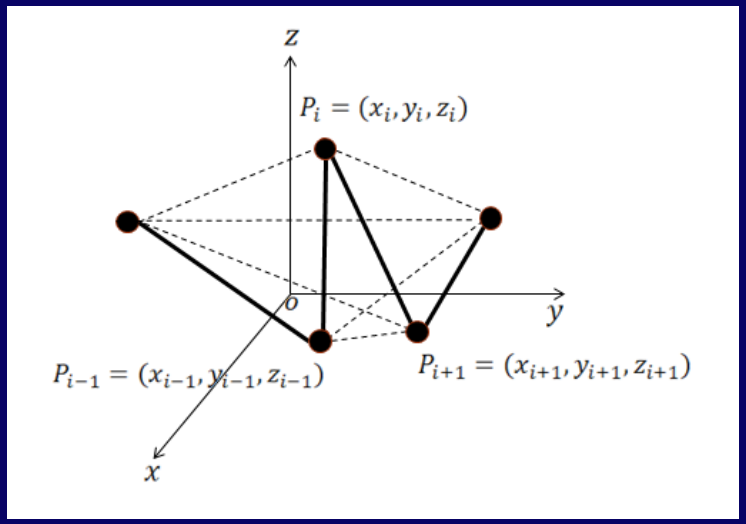
Beads-on-string



Beads-on-string



Bayesian statistical model



u_{ij} : number of reads between loci i and j.

d_{ij} : 3D Euclidian distance between loci i and j.

enz_i : number of enzyme cut site in locus i.

gcc_i : mean GC content in locus i.

map_i : mean mappability score in locus i.

$$u_{ij} \sim Poisson(\theta_{ij})$$

$$\log(\theta_{ij}) = \beta_0 + \beta_1 \log(d_{ij}) + \beta_{enz} \log(enz_i enz_j)$$

$$+ \beta_{gcc} \log(gcc_i gcc_j) + \beta_{map} \log(map_i map_j)$$

Bayesian Statistical Model

- Likelihood: $\binom{N}{2}$ data points, $3N + 5$ parameters

$$L(u_{ij}, 1 \le i < j \le N | x_i, y_i, z_i, 1 \le i \le N, \beta_0, \beta_1, \beta_e, \beta_g, \beta_m) = \prod_{1 \le i < j \le N} \frac{e^{-\theta_{ij}} \theta_{ij}^{u_{ij}}}{u_{ij}!}$$

$$\log(\theta_{ij}) = \beta_0 + \beta_1 \log\left(\sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}\right) + \beta_e \log(e_i e_j) + \beta_g \log(g_i g_j) + \beta_m \log(m_i m_j)$$

Statistical Inference

- Algorithm: Bayesian 3D constructor for Hi-C data (BACH)
  - Initialization 1: use Poisson regression to obtain the initial values of model parameters.
  - Initialization 2: use sequential important sampling to get the initial 3D chromosomal structure .
  - Refinement: use Gibbs sampler with hybrid Monte Carlo to refine the initial values for parameters.

SIS in BACH: Outline

- Goal: use sequential importance sampling to sequentially put *N* loci into 3D space, i.e. sample from:

$$\pi(x_i, y_i, z_i, 1 \le i \le N | u_{ij}, 1 \le i < j \le N)$$

- Bridging distributions:

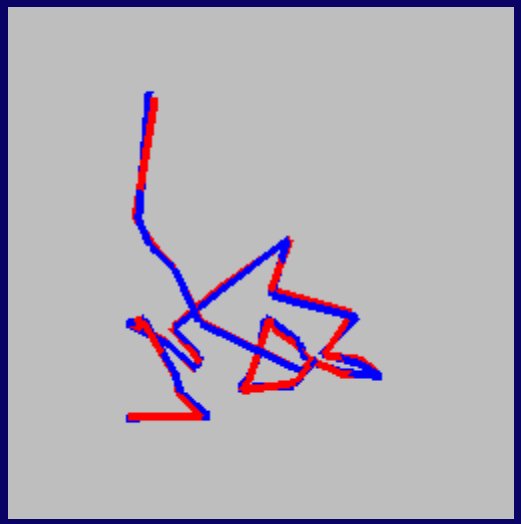
$$\pi_t(x_i, y_i, z_i, 1 \le i \le t | u_{ij}, 1 \le i < j \le t)$$

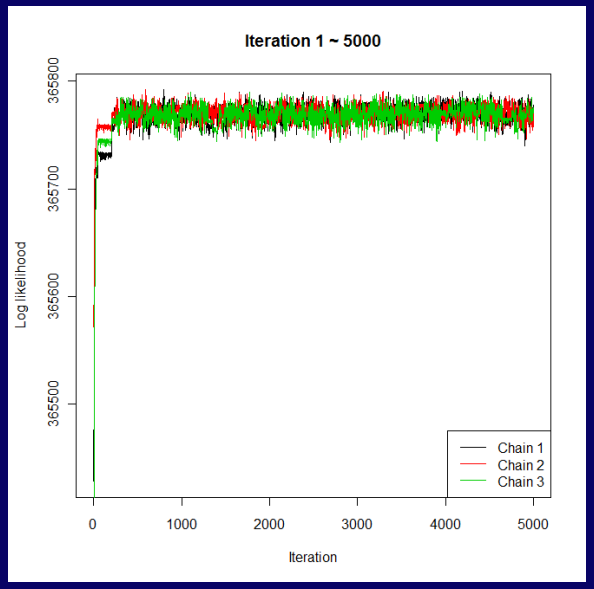
- Proposal distributions (given the previous *t*-1 loci, put the *t* th locus in to 3D space):

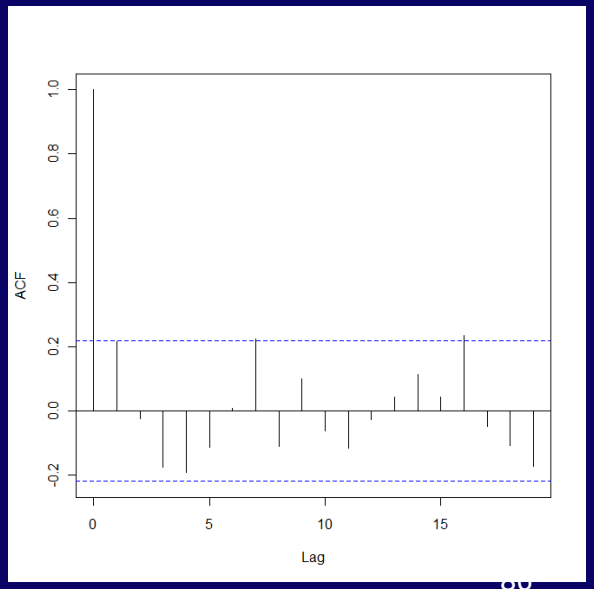
$$g_t(x_t, y_t, z_t | x_i, y_i, z_i, 1 \le i \le t-1, u_{ij}, 1 \le i < j \le t)$$

Simulation study

- Use random walk to simulate a 3D structure with 33 loci (red lines). Simulate Hi-C contact map from the posited model.
- Predicted 3D structure (blue lines) aligns well with true 3D structure (RMSD = 0.0091).



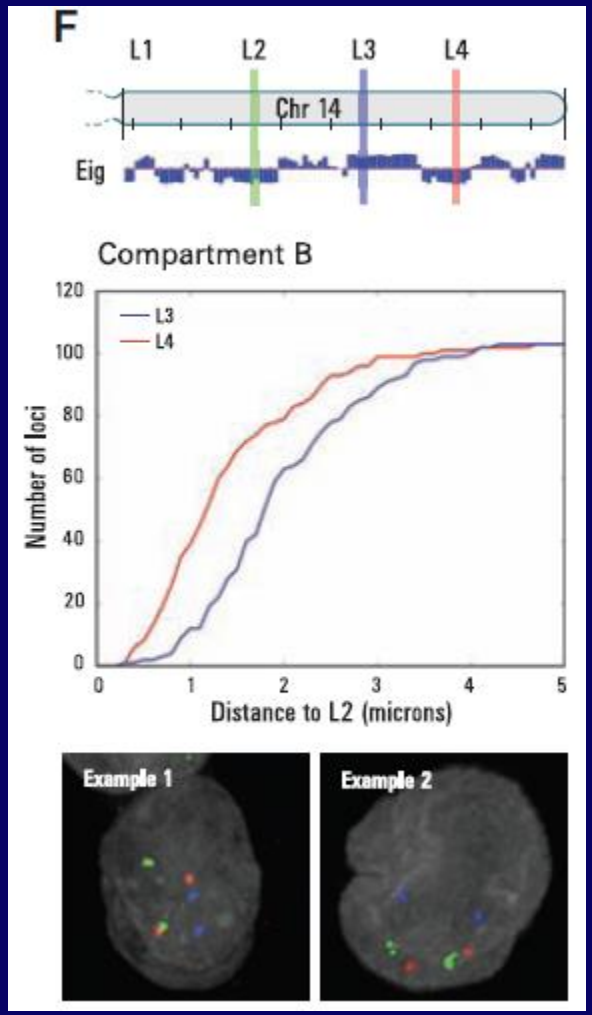


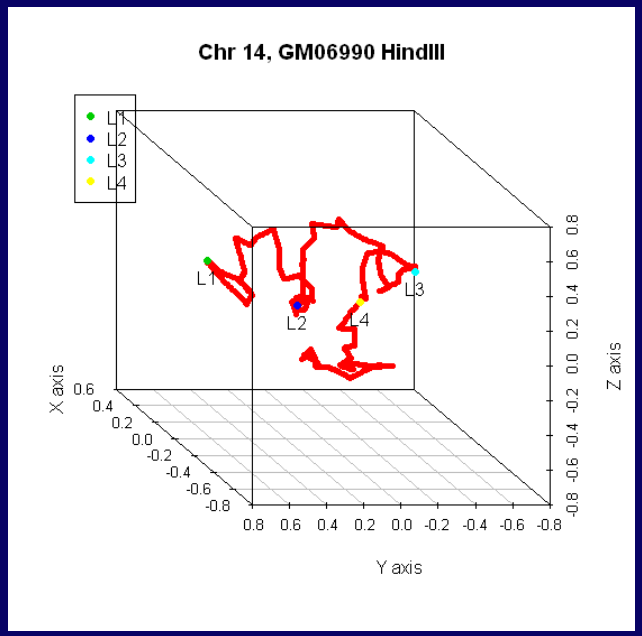


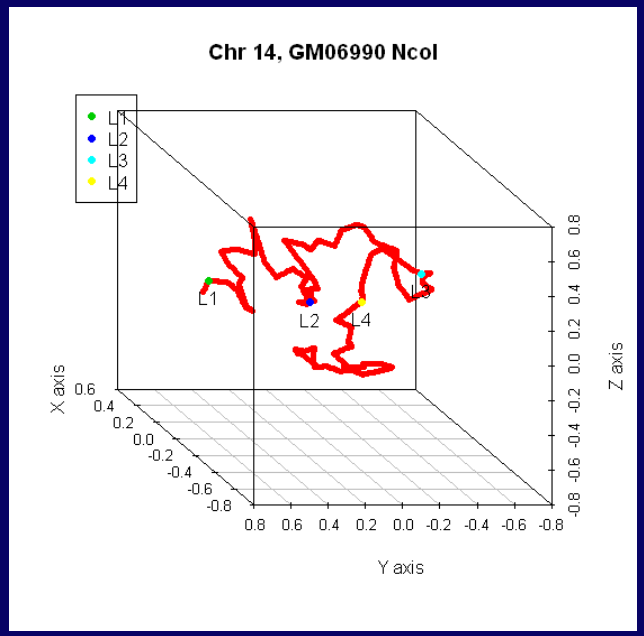
Human Hi-C data

Cell line	Restriction enzyme	# of reads (million)
GM06990	HindIII	4.1
GM06990	HindIII	4.4
GM06990	HindIII	4.9
GM06990	HindIII	5.4
GM06990	NcoI	8.8
GM06990	NcoI	10.1
K562	HindIII	12.1
K562	HindIII	9.7

Real Hi-C data from Lieberman-Aiden et al. 2009

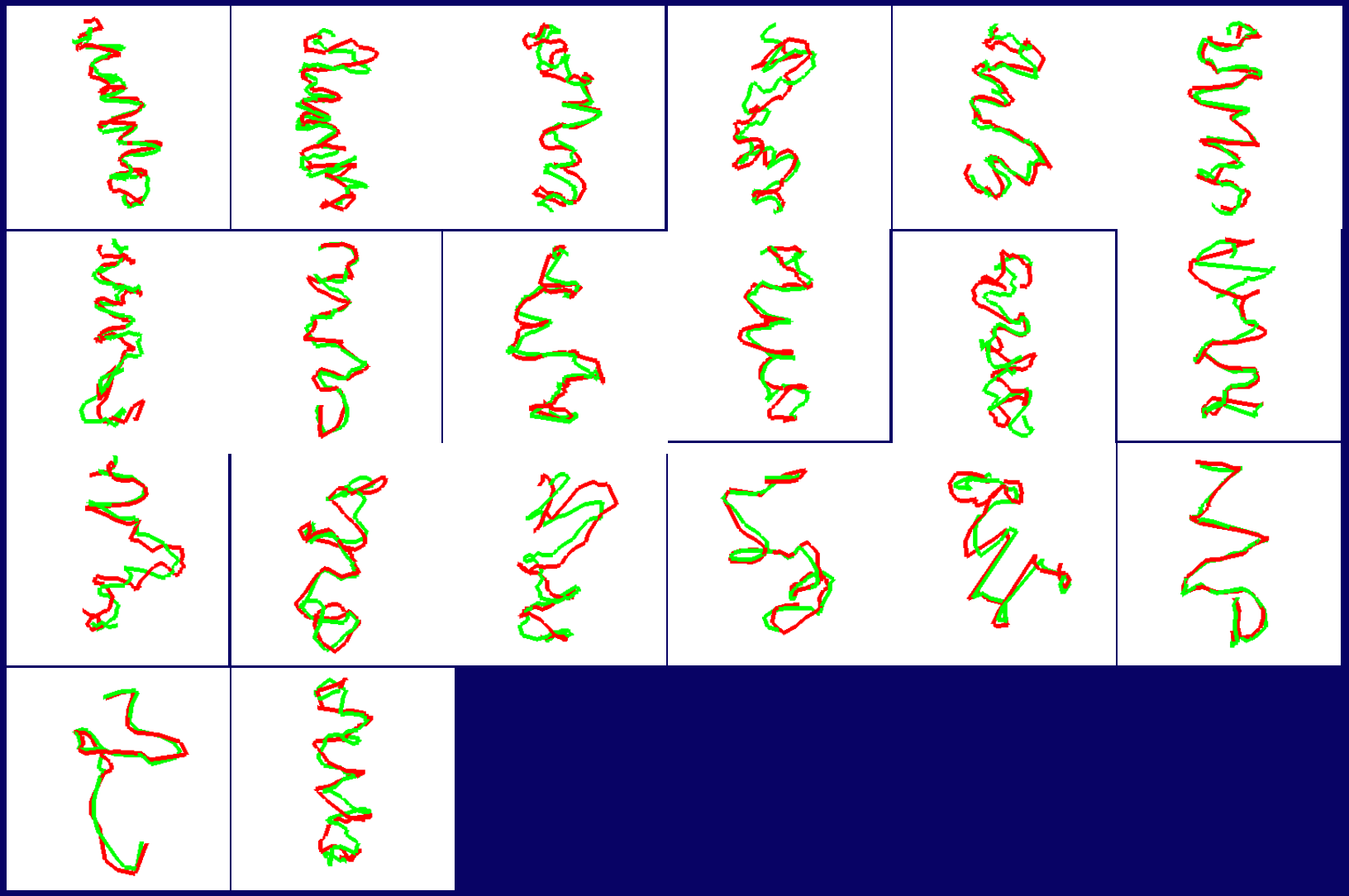






d(L2, L4) = 1.4042, d(L2, L3) = 1.9755, significant

mESC: Hind3 vs. Nco1



Whole Chromosome 3D Model

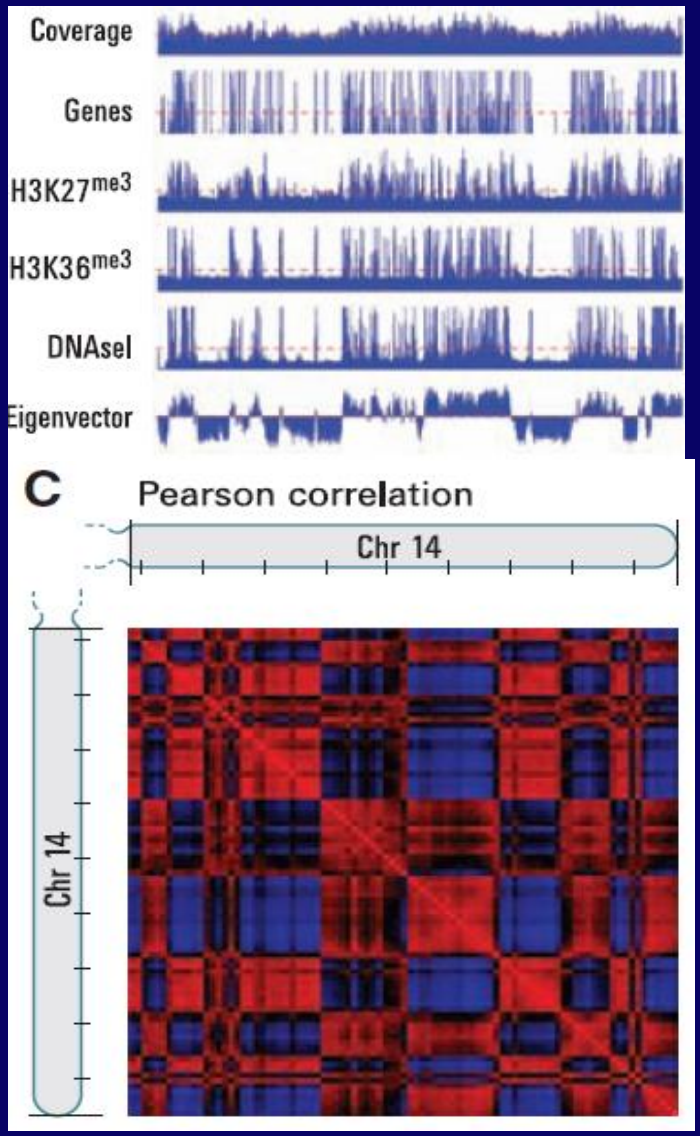
- Two compartments
  - Compartment A: gene rich, active transcription
  - Compartment B: gene poor, inactive transcription

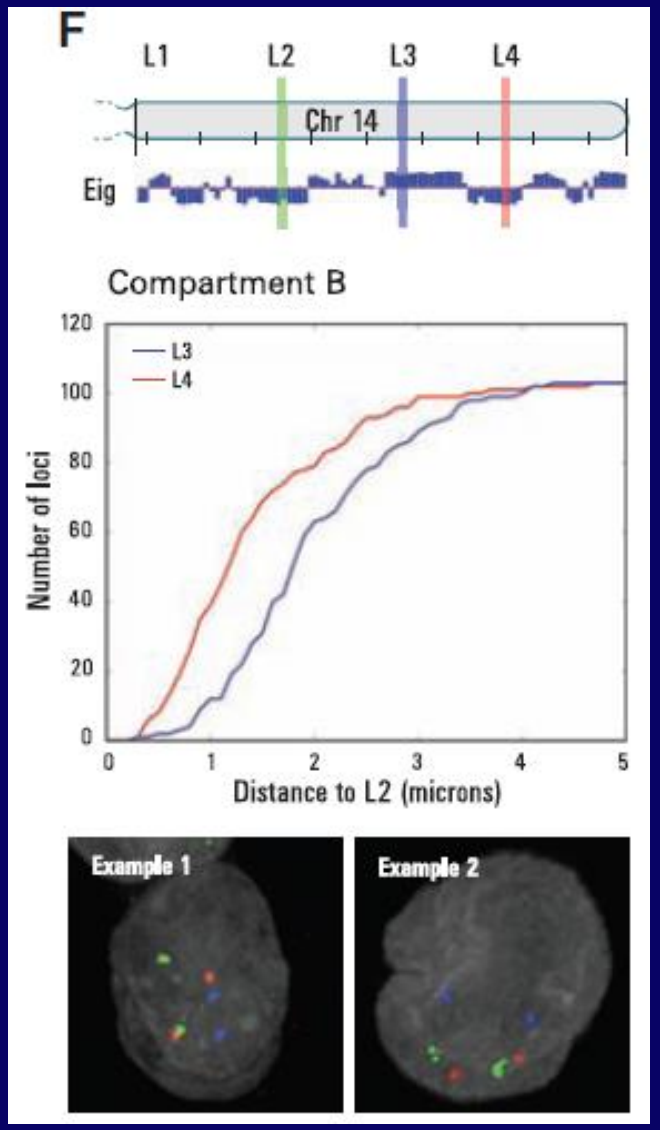
- Same compartment: strong chromatin interactions, spatially close

- Different compartments: weak chromatin interaction, spatially isolated

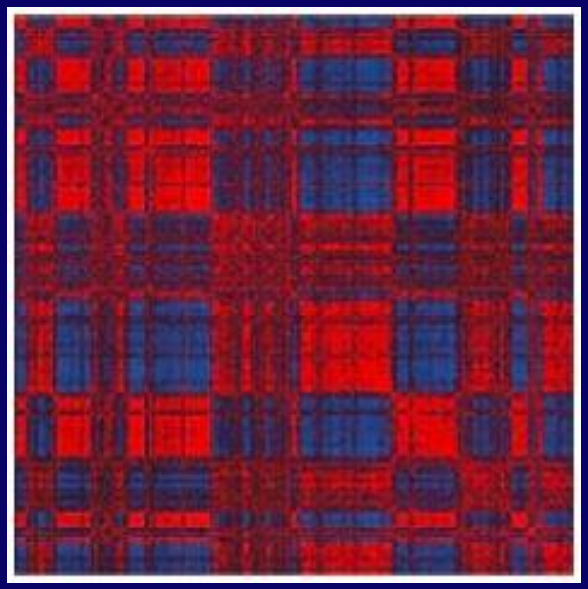
Lieberman-Aiden, et al, 2009

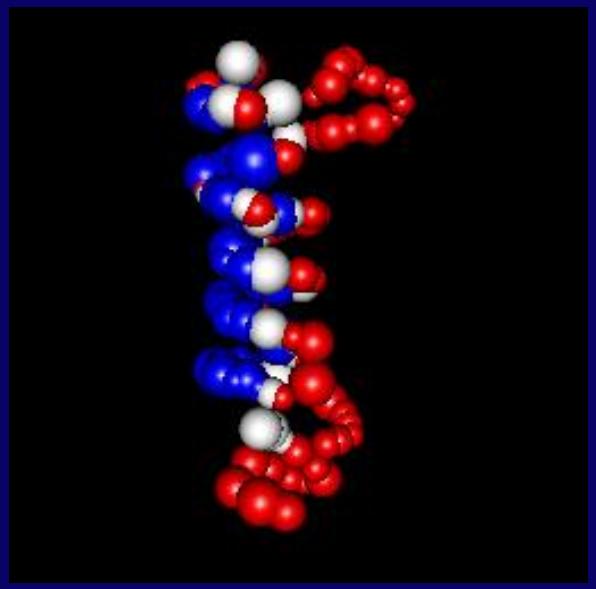
Two compartment model

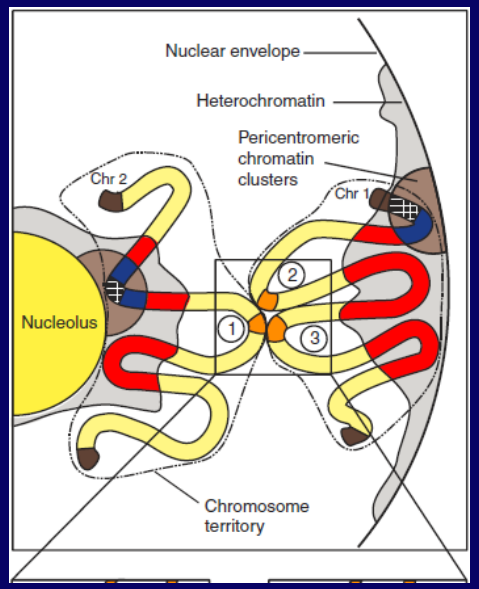




Whole Chromosome Model



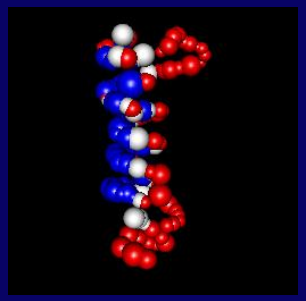




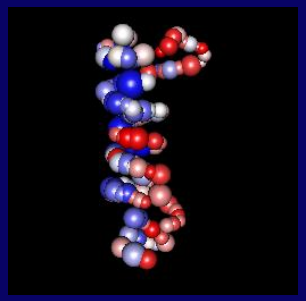
Lieberman-Aiden, et al, 2009
Naumova and Dekker, 2010

Other Features (Chromosome 2)

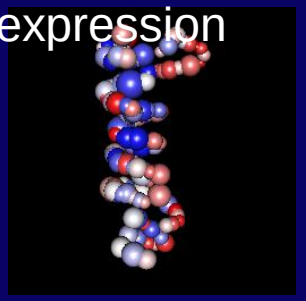
Compartment



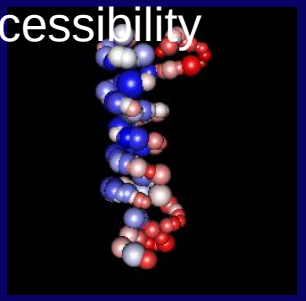
Gene density



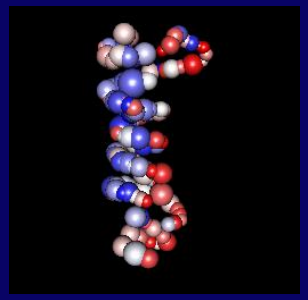
Gene expression



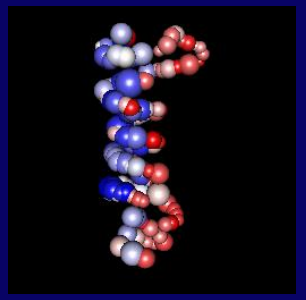
Chromatin accessibility



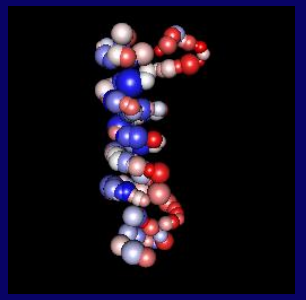
RNA polymerase II



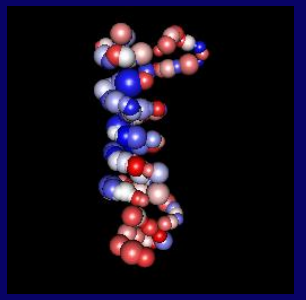
DNA replication time



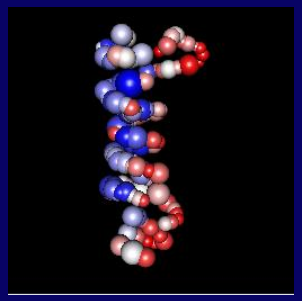
H3K36me3



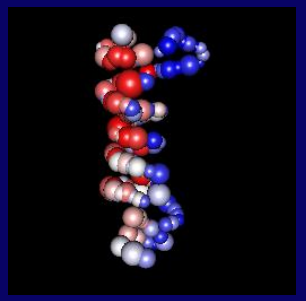
H3K27me3



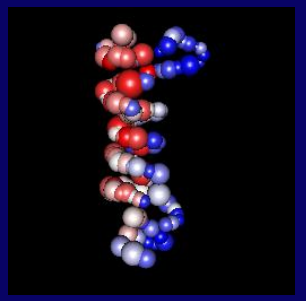
H3K4me3



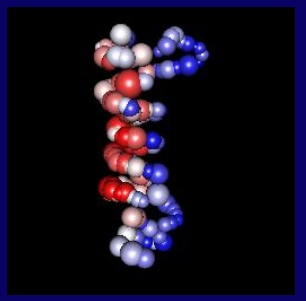
H3K9me3



H3K20me3



Lamina interaction



Conclusions

- BACH--Reconstruct chromosome 3D structures
- Remove systematic biases
- Consistent with FISH data
- Elongation of chromatin is highly associated with genetic/epigenetic features.
- Separation of compartments of A and B can be visualized.

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