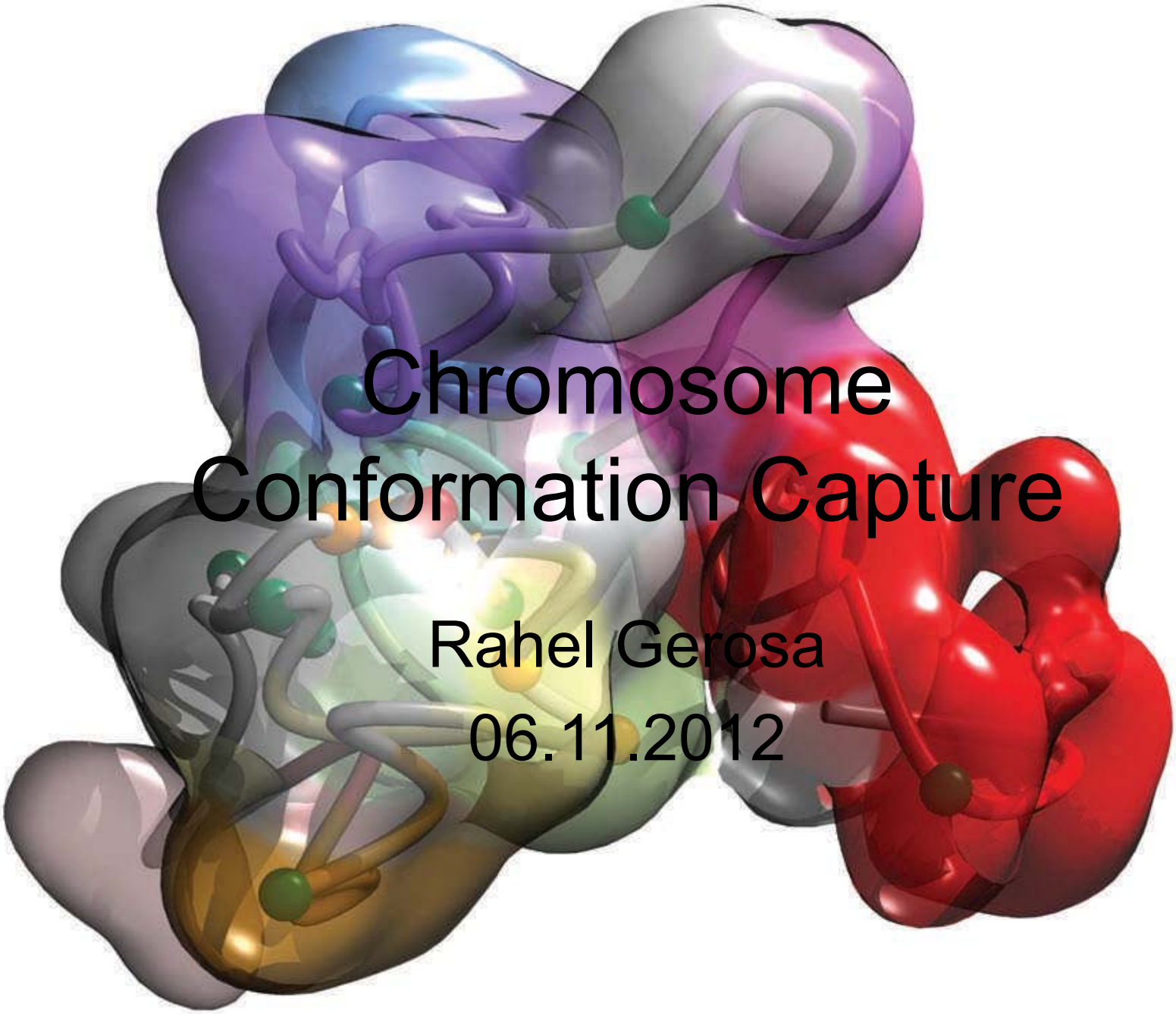
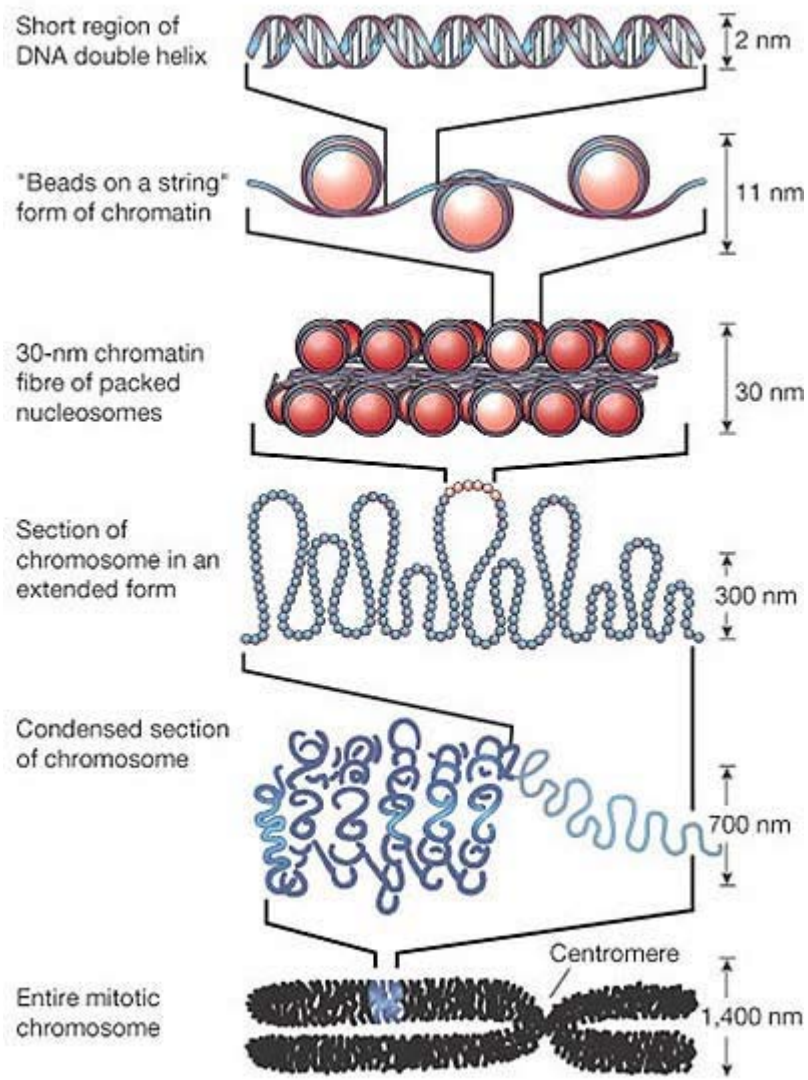


Chromosome Conformation Capture

Rahel Gerosa

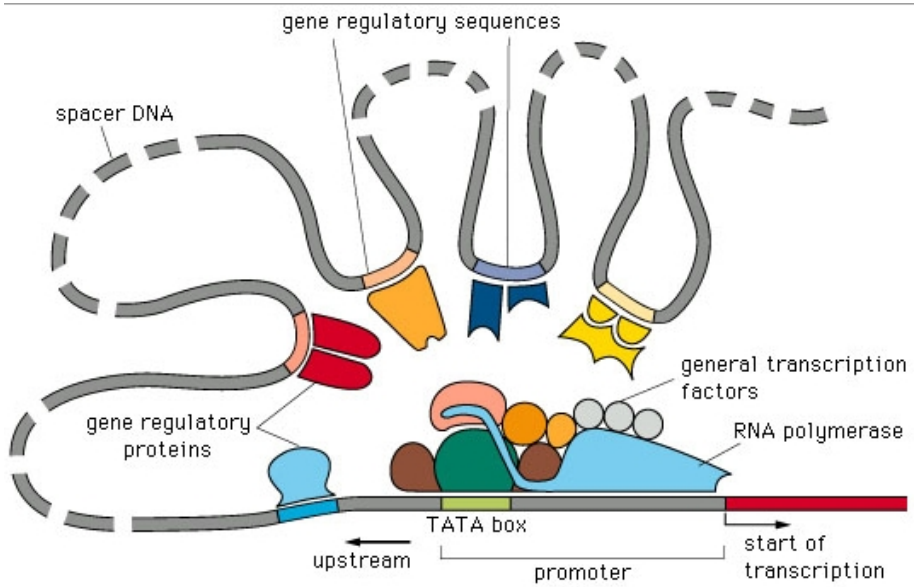
06.11.2012

Chromosomes



http://www.oocities.org/ganmni/

Gene regulation



http://pbil.univ-lyon1.fr/baobab/article.php3?id_article=278

Analysis of chromosome conformation

Analysis of chromosome conformation is complicated by technical limitations:

- Electron microscopy
  - Laborious and not easily applicable
- Light microscopy
  - Resolution of 100 to 200 nm → insuffisient to identify the contact of two loci
  - If the interaction is short-lived, microscopy can miss it altogether
- DNA binding proteins fused to GFP
  - Only a few positions can be examined simultaneously
- FISH
  - Requires severe treatement → may affect chromosome organization

→ **High-throughput method to analyze the overall spatial organization of chromosomes**

History

Capturing Chromosome Conformation

Job Dekker,[1]* Karsten Rippe,[2] Martijn Dekker,[3] Nancy Kleckner[1]

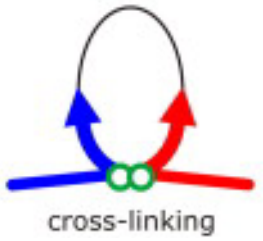
- «You start out with a difficult problem – where are two loci in three dimensions – and you convert it through a series of molecular steps to a simple problem, just sequencing a piece of DNA.»

  Job Dekker

- Detect frequency of interaction between any two genomic loci
- 3C method was the first to identify some of the long-range interactions (in yeast)

Chromosome Conformation Capture (3C)

1. Cross-linking

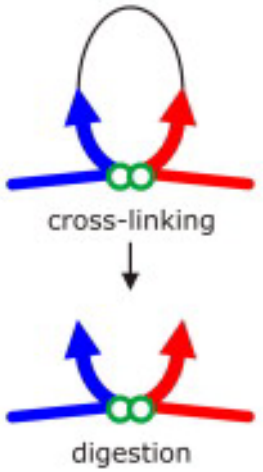


- Treating cells with formaldehyde
- Cross-linking of physically touching segments throughout the genome via contacts between their DNA-bound proteins (for example enhancer and promoter regions)
- Method also possible with isolated nuclei but interactions could be lost during the isolation procedure

Tolhuis B. et al., (2012). Methods

Chromosome Conformation Capture (3C)

1. Cross-linking



2. Restriction Digest
   - DNA is cut up with restriction enzymes or sheared by sonication, separating the non-interacting DNA from the interacting cross-linked chromatin

Small loci: frequently cutting enzymes (4bp)

Larger loci: larger cutters (6bp)

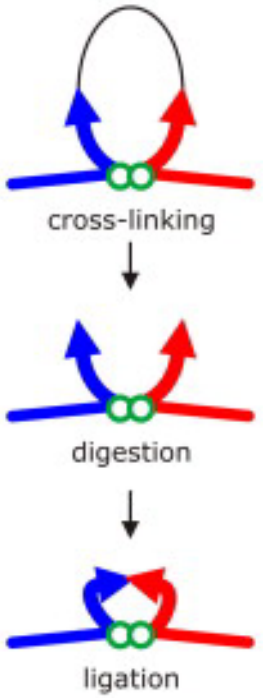
Tolhuis B. et al., (2012). Methods

Chromosome Conformation Capture (3C)

1. Cross-linking

2. Restriction Digest

3. Intramolecular Ligation

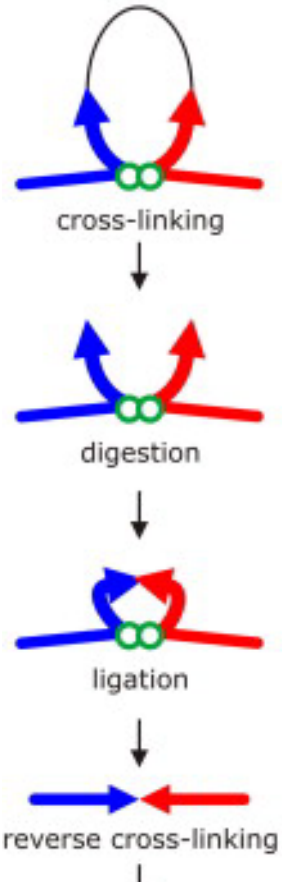


   - Ligation procedure joins restriction ends of cross-linked segments
   - Ligation conditions must favor the ligation of the intramolecular cross-linked DNA fragments

Tolhuis B. et al., (2012). Methods

Chromosome Conformation Capture (3C)

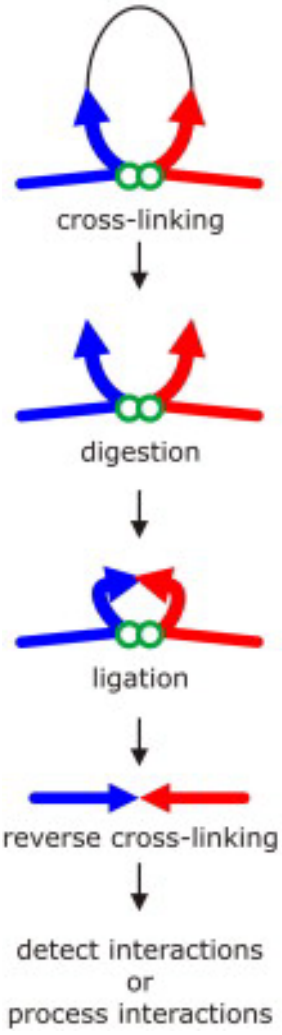
1. Cross-linking
2. Restriction Digest
3. Intramolecular Ligation
4. Reverse Cross-links
   - High temperature and proteinase K
   - Resulting pool of linear DNA fragments = 3C library



Tolhuis B. et al., (2012). Methods

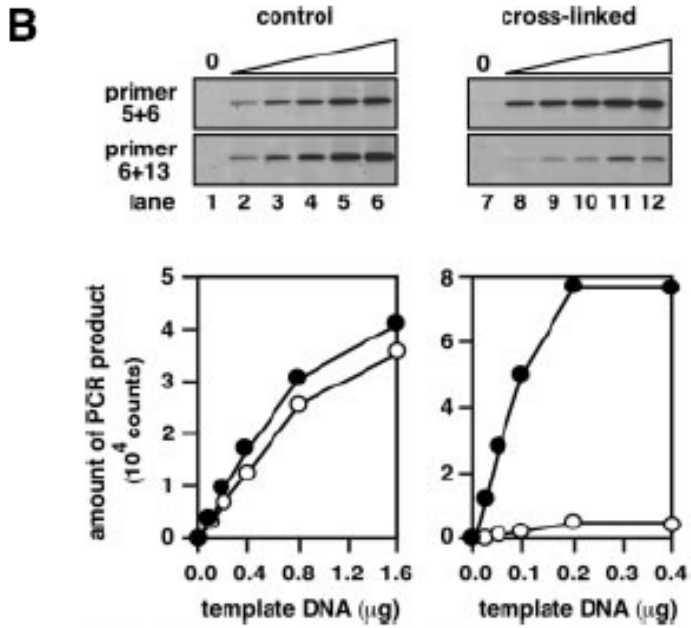
Chromosome Conformation Capture (3C)

1. Cross-linking
2. Restriction Digest
3. Intramolecular Ligation
4. Reverse Cross-links
5. Quantiation
   - qPCR with locus-specific markers
     → frequency of ligation events



Tolhuis B. et al., (2012). Methods

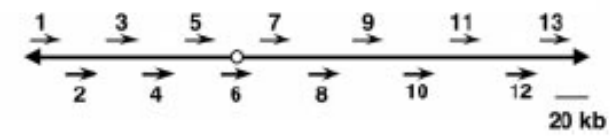
Chromosome Conformation Capture (3C) methodology

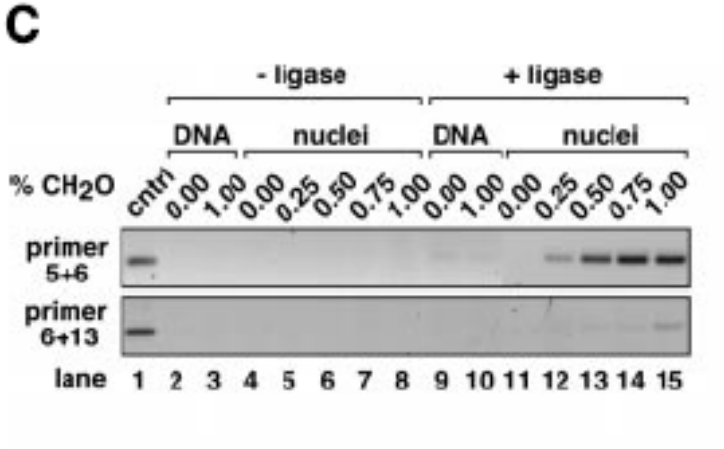


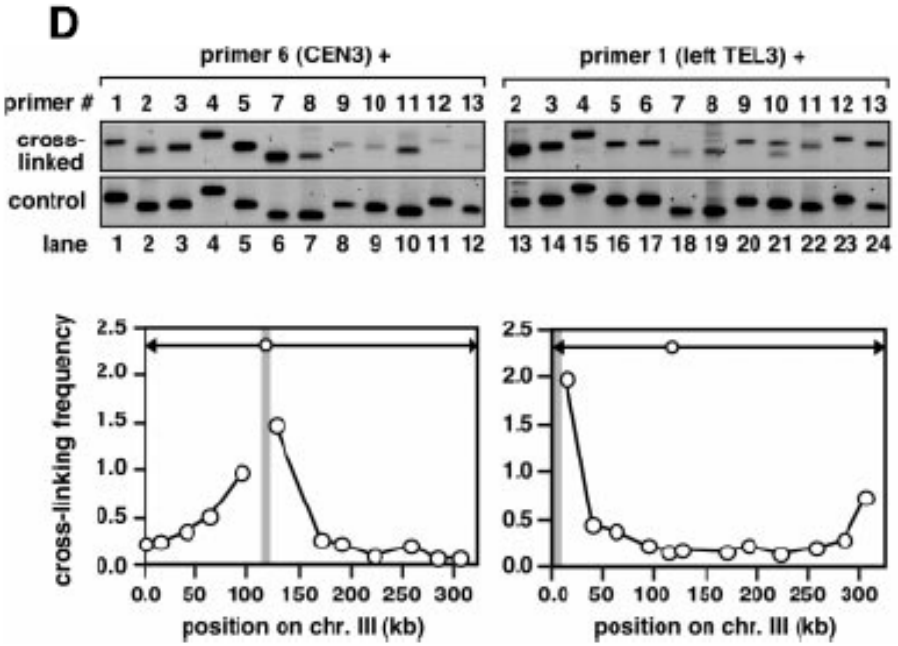
Primer 5 and 6

Primer 6 and 13

Primers Chromosome III

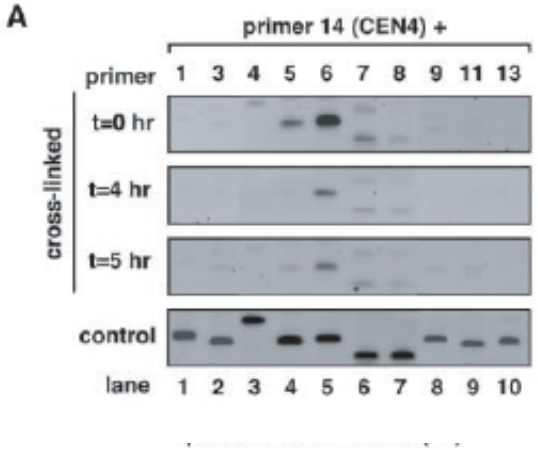


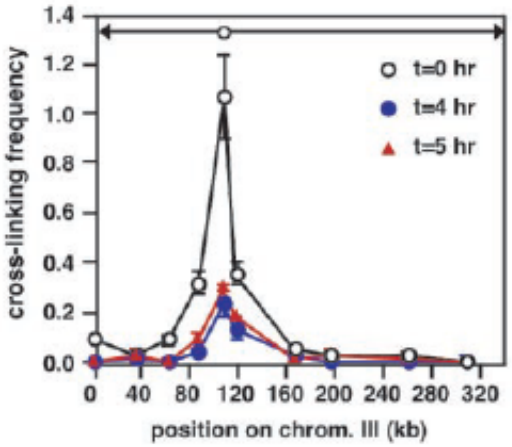


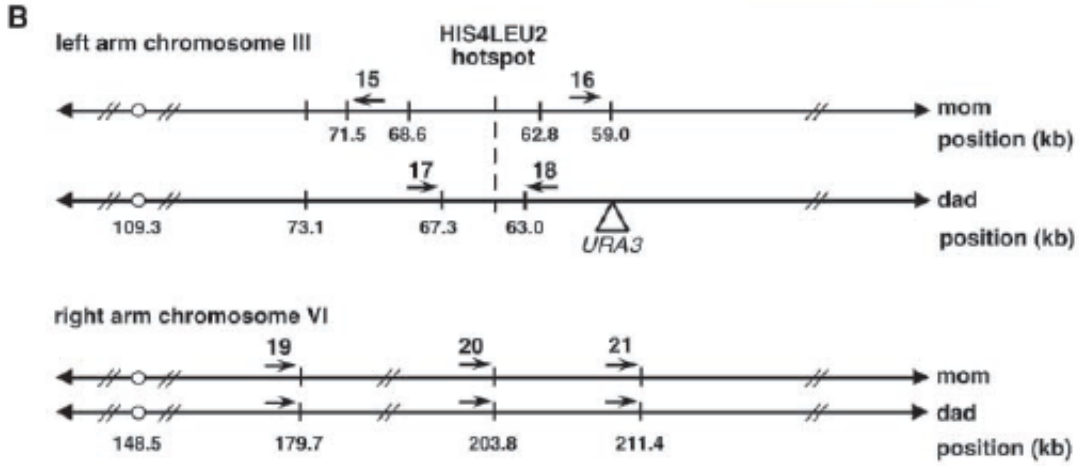


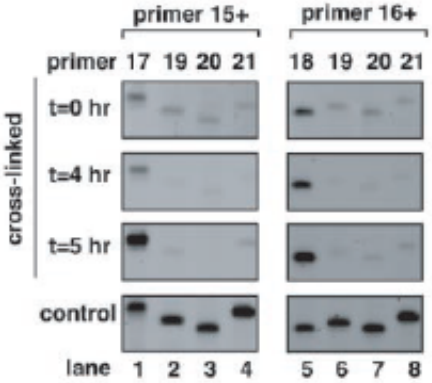
Dekker J. et al. (2002) Science

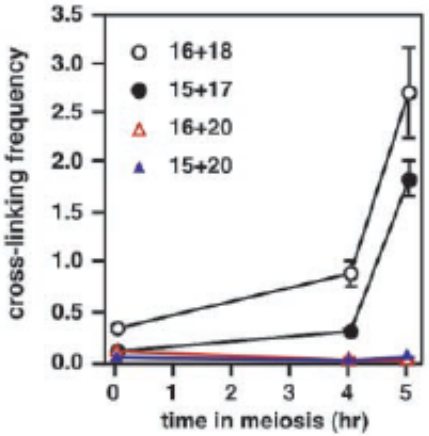
Analysis of nuclear dynamics during meiosis





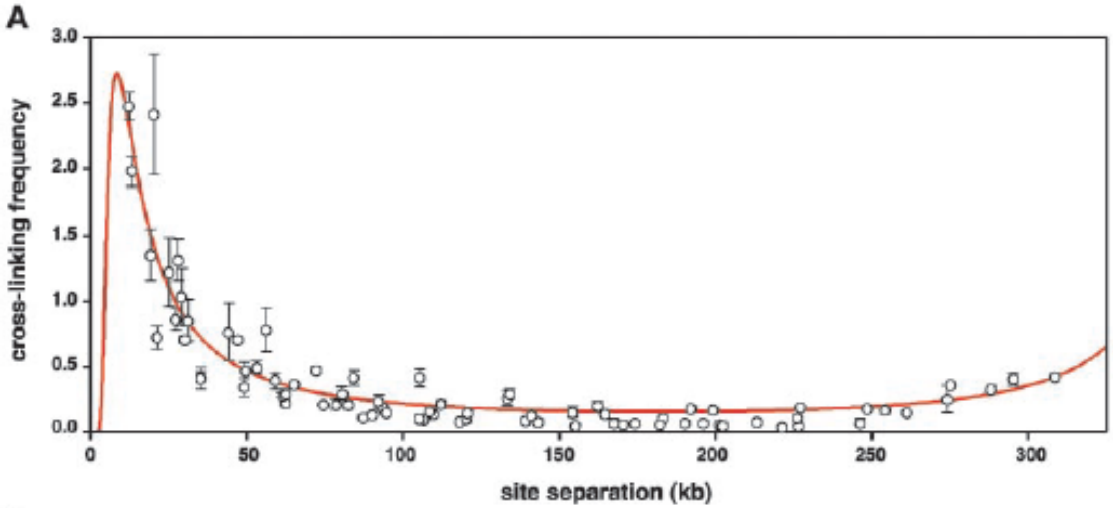


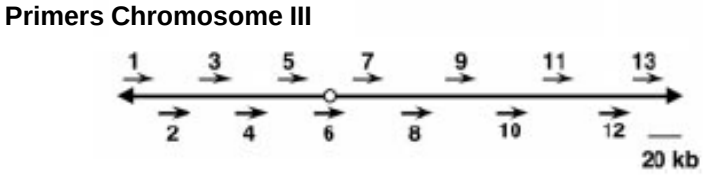


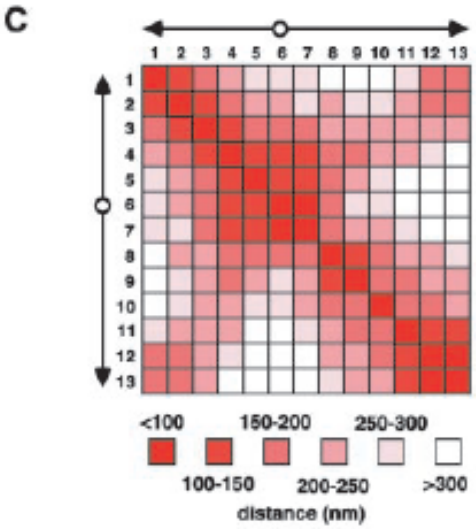


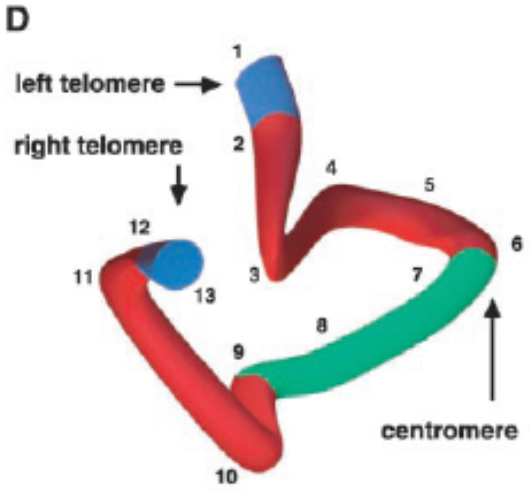
Dekker J. et al. (2002) Science

Analysis of the structure of chromosome III during interphase









blue=subtelomeric regions
red=GC-rich domains
green=AT-rich domain

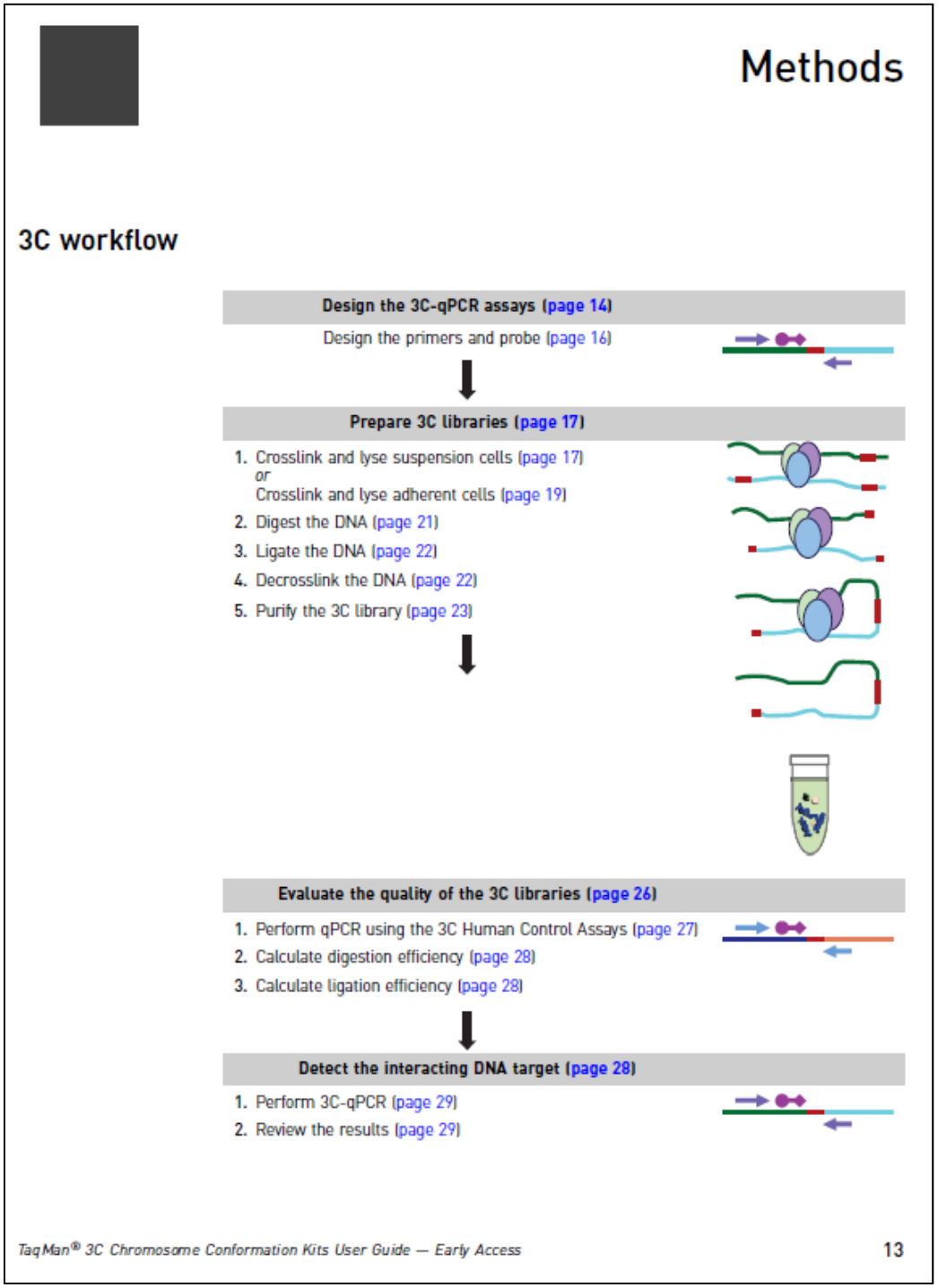
Dekker J. et al. (2002) Science

Method suitability and challanges

- 3C can detect interactions between regions located several thousand to several hundred thousand base pairs away
- Requires previous knowledge of interactiong DNA sequences, as **sequence-specific primers** for amplification are needed
- Generation of a matrix of interaction frequencies between sites on a chromosome → spatial disposition and information about physical properties
- This methodology can be applied to the spatial organization of entire genomes in organisms from bacteria to human
- Some hybrid DNA molecules produced by this technique are the result of random interctions, particularly between loci that are just a few kilobases apart on the same chromosome → needs bioinformatics and replicated experiments

- → improving 3C !!!

Life Technologies launched a kit that bundles together reagents for 3C experiments



3C workflow

Design the 3C-qPCR assays (page 14)

Design the primers and probe (page 16)

Prepare 3C libraries (page 17)

1. Crosslink and lyse suspension cells (page 17)
   or
   Crosslink and lyse adherent cells (page 19)
2. Digest the DNA (page 21)
3. Ligate the DNA (page 22)
4. Decrosslink the DNA (page 22)
5. Purify the 3C library (page 23)

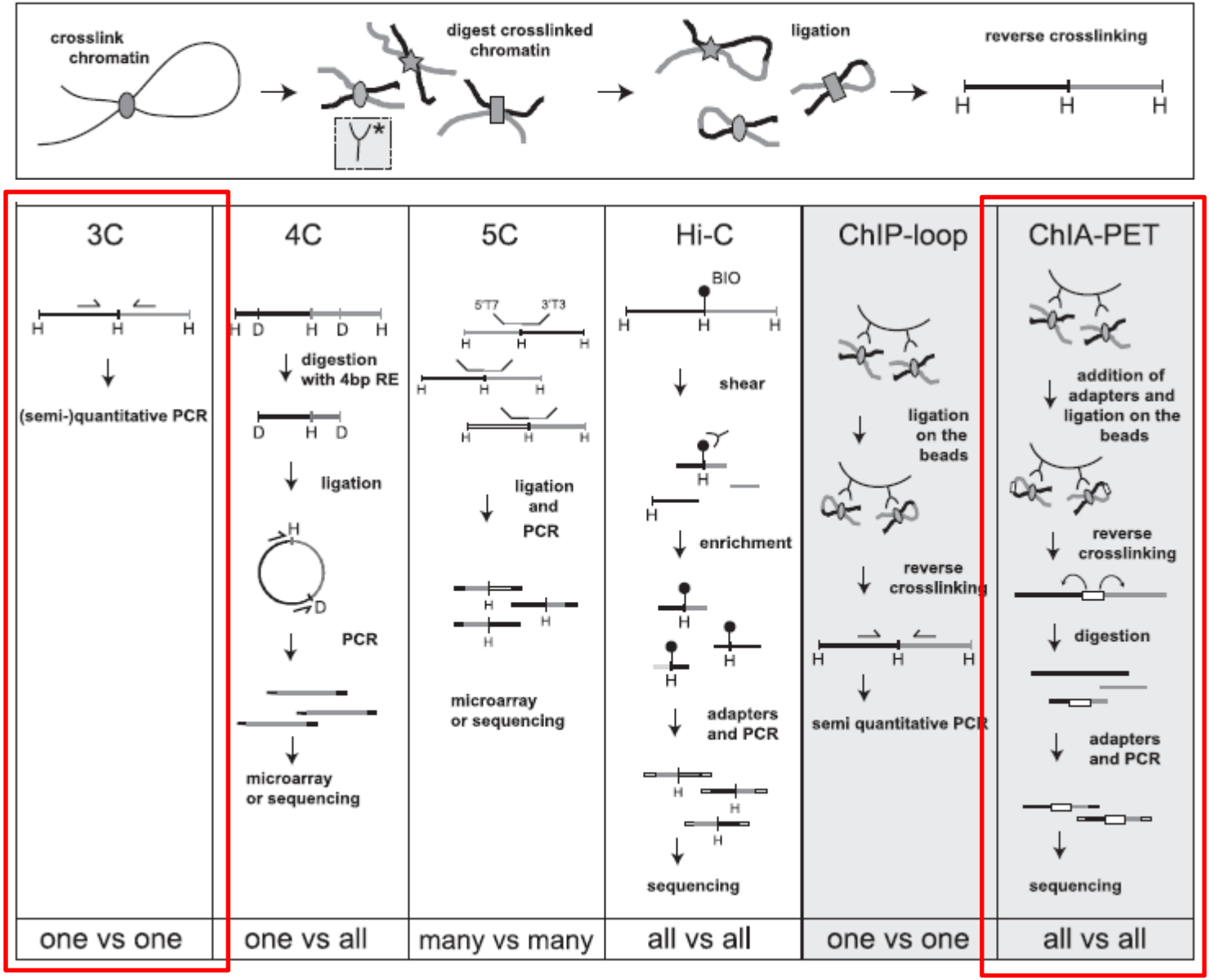
Evaluate the quality of the 3C libraries (page 26)

1. Perform qPCR using the 3C Human Control Assays (page 27)
2. Calculate digestion efficiency (page 28)
3. Calculate ligation efficiency (page 28)

Detect the interacting DNA target (page 28)

1. Perform 3C-qPCR (page 29)
2. Review the results (page 29)

3C-derived methods



E. De Wit, W. de Laat, (2012) Genes & Development

ARTICLES

NATURE|Vol 462|5 November 2009

An oestrogen-receptor-α-bound human chromatin interactome

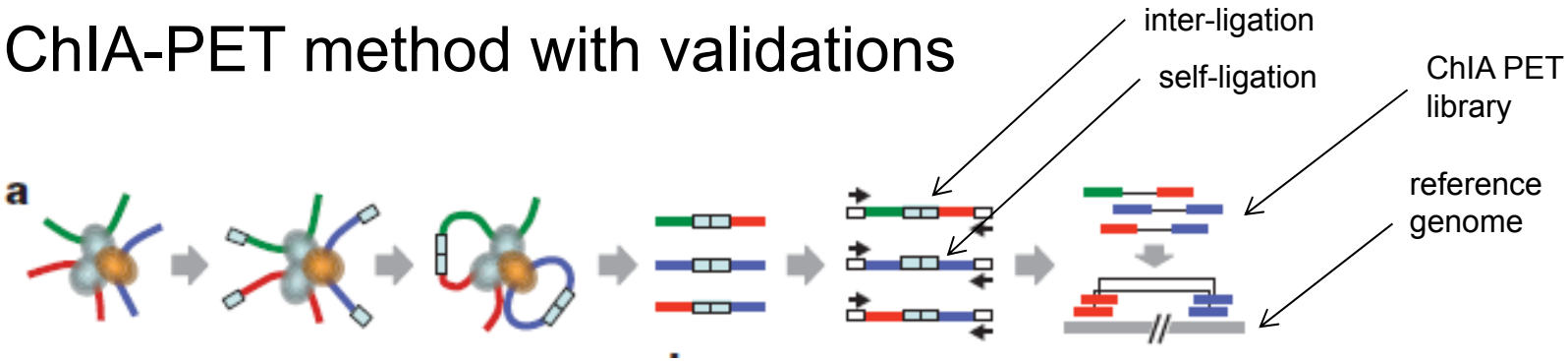
Melissa J. Fullwood[1], Mei Hui Liu[1], You Fu Pan[1], Jun Liu[1], Han Xu[1], Yusoff Bin Mohamed[1], Yuriy L. Orlov[1], Stoyan Velkov[1], Andrea Ho[1], Poh Huay Mei[1], Elaine G. Y. Chew[1], Phillips Yao Hui Huang[1], Willem-Jan Welboren[2], Yuyuan Han[1], Hong Sain Ooi[1], Pramila N. Ariyaratne[1], Vinsensius B. Vega[1], Yanquan Luo[1], Peck Yean Tan[1], Pei Ye Choy[1], K. D. Senali Abayratna Wansa[1], Bing Zhao[1], Kar Sian Lim[1], Shi Chi Leow[1], Jit Sin Yow[1], Roy Joseph[1], Haixia Li[1], Kartiki V. Desai[1], Jane S. Thomsen[1], Yew Kok Lee[1], R. Krishna Murthy Karuturi[1], Thoreau Herve[1], Guillaume Bourque[1], Hendrik G. Stunnenberg[2], Xiaoan Ruan[1], Valere Cacheux-Rataboul[1], Wing-Kin Sung[1,3], Edison T. Liu[1], Chia-Lin Wei[1], Edwin Cheung[1,4,5] & Yijun Ruan[1,4]

- Genomes have a high-level 3D structure → DNA elements separated by long genomic distances can interact functionally
- TF bind regulatory elements distant from gene promoters
- Chromatin **interactions and their impact on transcription** have not been investigated in a **genome-wide** manner
  → with ChIA-Pet a **de novo** detection of global chromatin interactions is possible (3C and its variants are incapable)
  → map of chromatin bound by estrogen receptor α (ER-α) in the human genome
- Using ChIA-PET ER- α bound chromatin interactions in estrogen-treated human breast adenocarcinoma cells (MCF-7) could be characterized and generated the **first human chromatin interactome map**

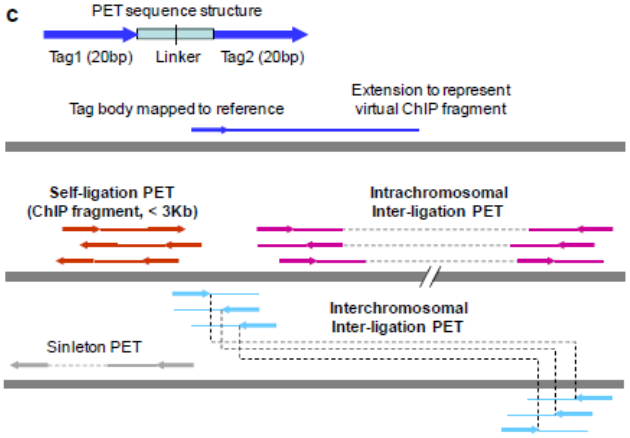
Oestrogen receptor α (ER-α)

- ER-α is a nuclear receptor activated by estrogen → ligand-activated TF
- ER-α is involved in the regulation of gene expression and affect cellular proliferation and differentiation in target tissues
- Binds the DNA as a homodimer
- Over half of all breast cancers overexpress ER- α → therefore used cell line: human breast adenocarcinoma cells (MCF-7)
- Most TF binding sites of ER-α are distal to transcriptional start sites of target genes → which of these distal binding sites are non-functional and which are involved in transcriptional activity through a remote control mechanism?

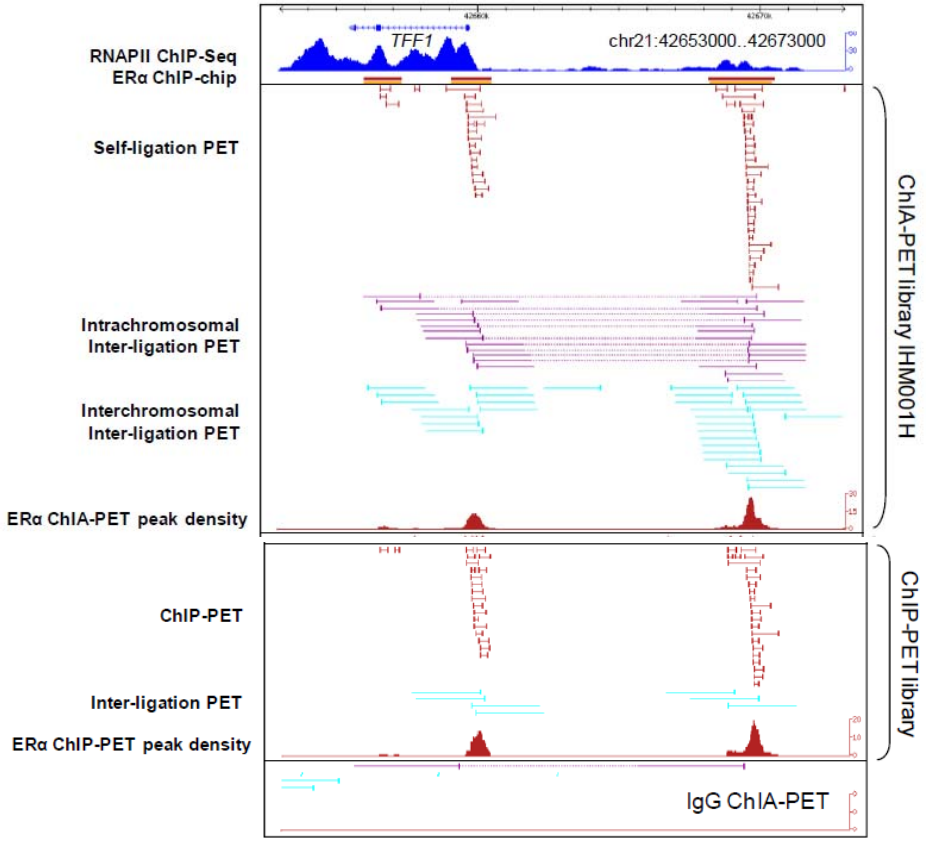
ChIA-PET method with validations



DNA fragments are sonicated, ChIP-enriched, processed by linker ligation, proximity ligation, PET extraction, sequencing and mapping → to reveal interacting loci



- Constructed two ChIA-PET libraries from independent ER-α ChIP-enriched estrogen-treated MCF-7 cells
- Negative control ChIP-PET library from the same ChIP sample (DNA was reverse crosslinked before proximity ligation) → to evaluate levels of false positive chromatin interactions
- Additional control: IgG which binds to chromatin nonspecifically



ER-α ChIA-PET data

Control library

ChIA-PET method with validations

Proximity ligation: **non-specific** chimeric DNA ligations between **different chromatin complexes** can occure, therefore linker nucleotide barcodes in the ChIA-PET method were used → the PET sequences with linker composition of A and B (AB) are considered as having derived from undesired chimeric ligation products

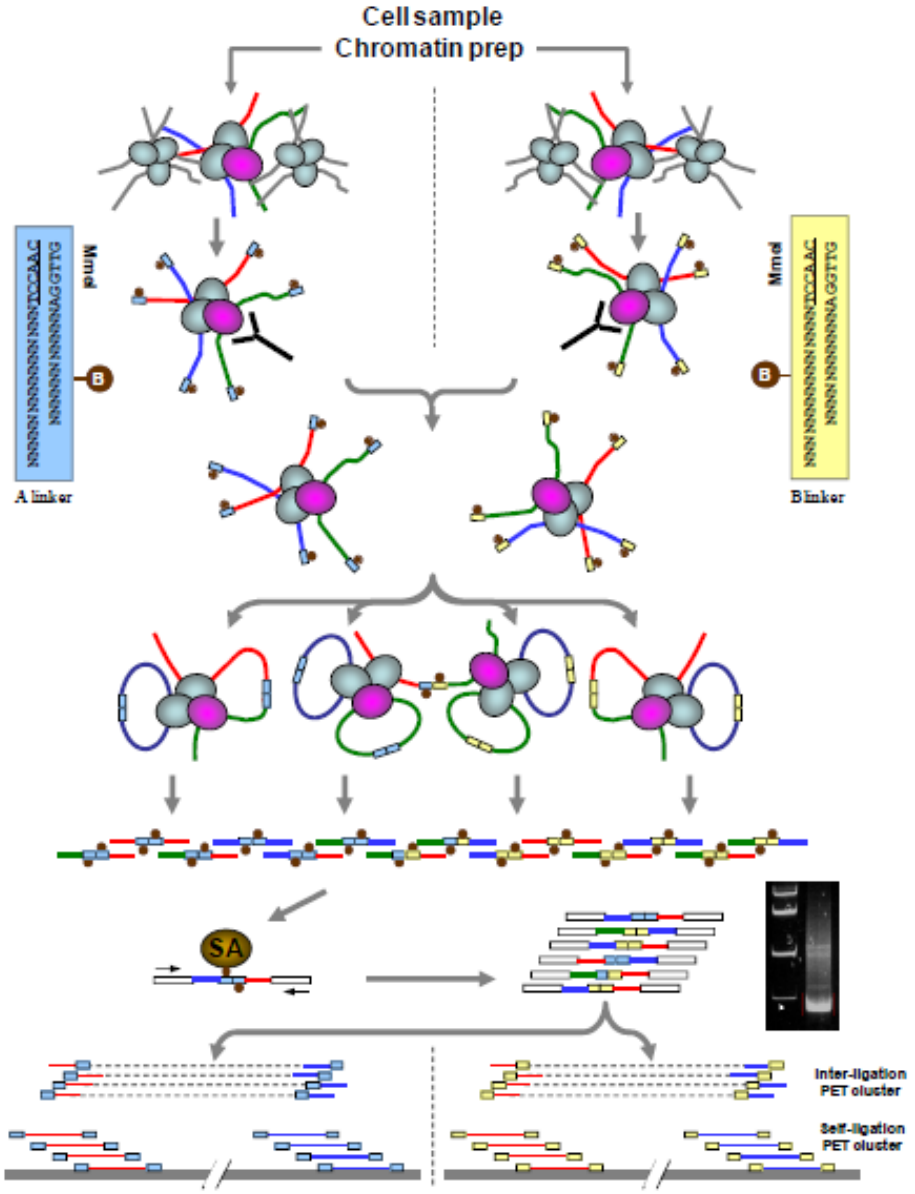


Table 1 | Summary statistics of library PET sequences

				Self-ligation		Intrachromosome inter-ligation		Interchromosome inter-ligation	
Library code	Library identity	Total PET	Unique PET	PET	PET clusters*	PET	PET clusters†	PET	PET clusters†
Small-scale testing of the ChIA-PET method									
IHM001N	ChIA-PET	715,369	271,648	78,706	2,701	16,677	176	176,265	0
IHM001H	ChIA-PET	764,899	293,754	103,740	3,405	17,718	215	172,296	0
IHM043	ChIP-PET	1,118,509	745,251	634,993	1,158	7,386	2‡	102,872	1
SHC007	ChIP-PET	361,241	214,668	192,511	489	2,196	0	19,961	0
IHM062	ChIA-PET (IgG)	436,248	217,708	40,847	0	11,254	0	165,607	0
Analysis of chimaeras									
IHH015M	ChIA-PET (AA+BB)	4,246,429	2,049,719	953,384	3,909	129,492	2,183	966,843	3
IHH015C	ChIA-PET (chimaeras)	5,904,476	1,790,714	15,490	35	98,805	0	1,676,419	0
Large-scale ChIA-PET analysis									
IHM001F	ChIA-PET	31,828,194	4,638,633	1,249,081	14,560	234,400	1,451	3,155,152	15
IHH015F	ChIA-PET	19,590,581	6,125,099	1,841,684	6,665	348,057	3,543	3,935,358	4

ChIA-PET data mapped at satellites and structural variation sites were removed.
* Self-ligation PET clusters for identifying binding sites (FDR < 0.01, PET count at least 5).
† Inter-ligation PET clusters for identifying interactions include at least two (small-scale) or three (chimaeras and large-scale analysis) overlapping PETs (FDR < 0.05). Interchromosomal interactions were subjected to manual curation.
‡ One interaction has a genomic span of less than 5 kb, suggesting that it results from extra-long self-ligation PETs, and the other has a genomic span of more than 10 Mb and PET counts of only 2, and so could be non-specific.

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putative ER-α **binding sites**

inter-ligation = duplex interactions
(two anchor regions coupled through a loop)

Many nerby duplex interactions are interconnected linking three or more anchors to aggregated complex interactions

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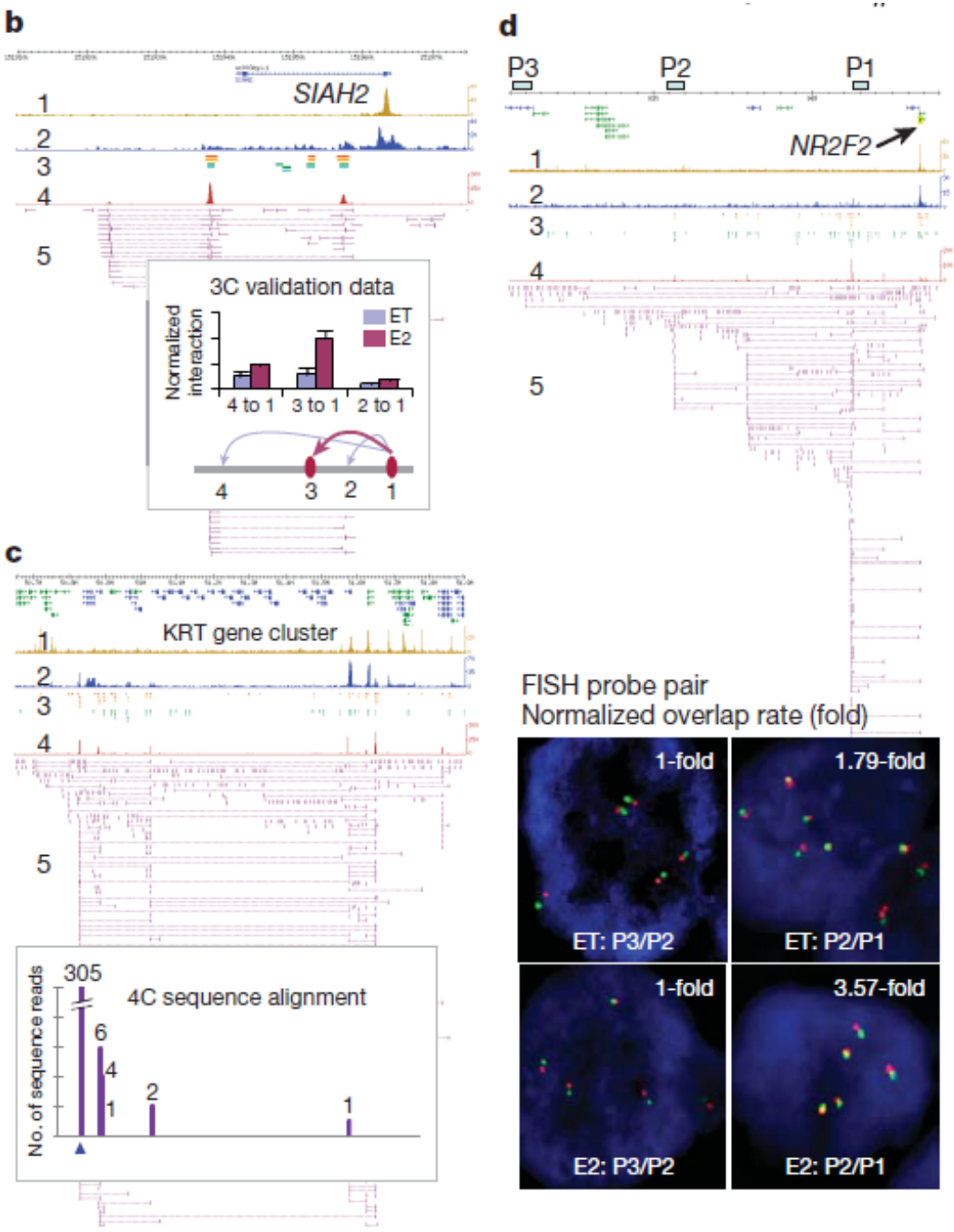
potential distant **interactions** bound by ER-α

ChIA-PET results verification

- Putative intrachromosomal interactions were validated by 3C, 4C and FISH
- 3C and FISH showed higher levels of chromatin interactions in estrogen-treated conditions than in untreated conditions, indicationg that the interactions are estrogen dependent



ER-α binding sites reproducibility

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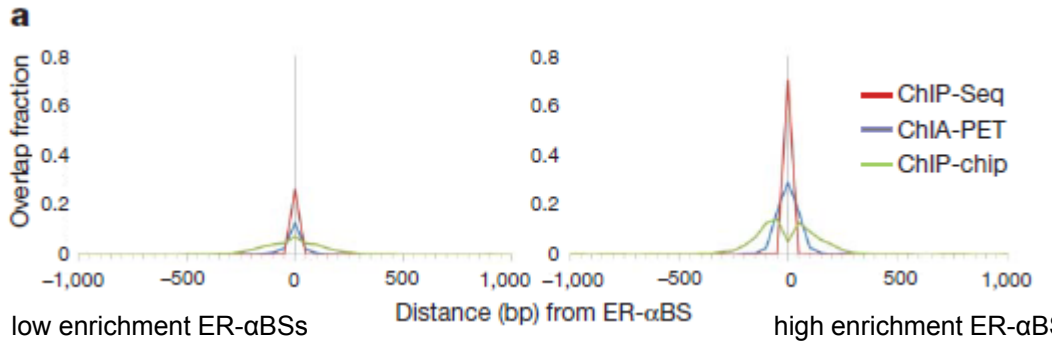
IHM001N	ChIA-PET	715,369	271,648	78,706	2,701	16,677	176	176,265	0
IHM001H	ChIA-PET	764,899	293,754	103,740	3,405	17,718	215	172,296	0
IHM043	ChIP-PET	1,118,509	745,251	634,993	1,158	7,386	2‡	102,872	1
SHC007	ChIP-PET	361,241	214,668	192,511	489	2,196	0	19,961	0
IHM062	ChIA-PET (IgG)	436,248	217,708	40,847	0	11,254	0	165,607	0
Analysis of chimaeras									
IHH015M	ChIA-PET (AA+BB)	4,246,429	2,049,719	953,384	3,909	129,492	2,183	966,843	3
IHH015C	ChIA-PET (chimaeras)	5,904,476	1,790,714	15,490	35	98,805	0	1,676,419	0
Large-scale ChIA-PET analysis									

None of the putative **inter**chromosomal interactions was reproduceble
→ Taken together, ChIA-PET method is highly reliable
→ Data suggest that ER-α functions primarily is of an **intra**chromosomal mechanism
→ Subsequent analysis therefore focused on intrachromosomal interactions

interactions (but few **inter**chromosomal interactions)

→ these ER-α binding sites also intersected well with previously reported ER-α binding maps



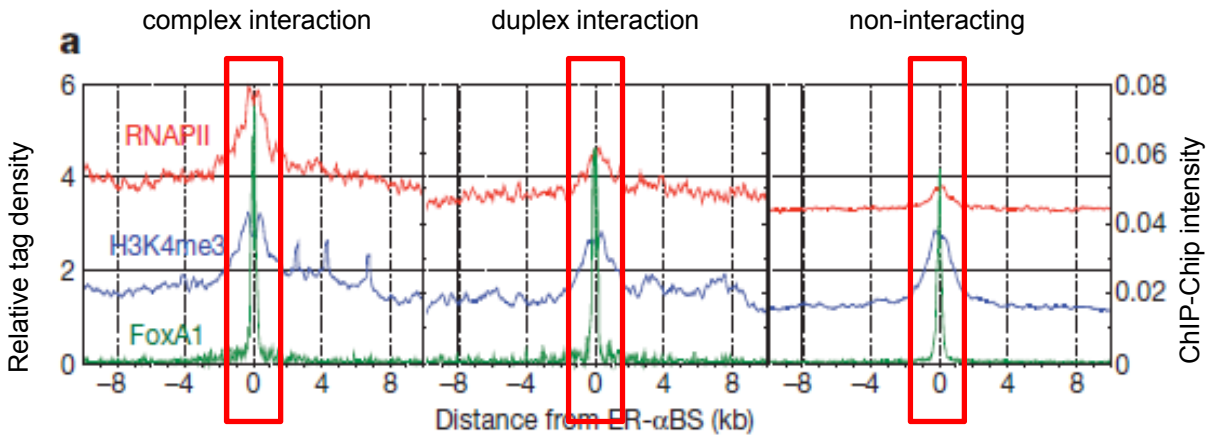
low enrichment ER-αBSs
5-49 PETs/site

high enrichment ER-αBSs
>50 PETs/site

→ More reliable than low-enrichment sites

Therefore: ER-α binding sites identified in this study show high reproducibility

ER-αBSs and their impact on transcription



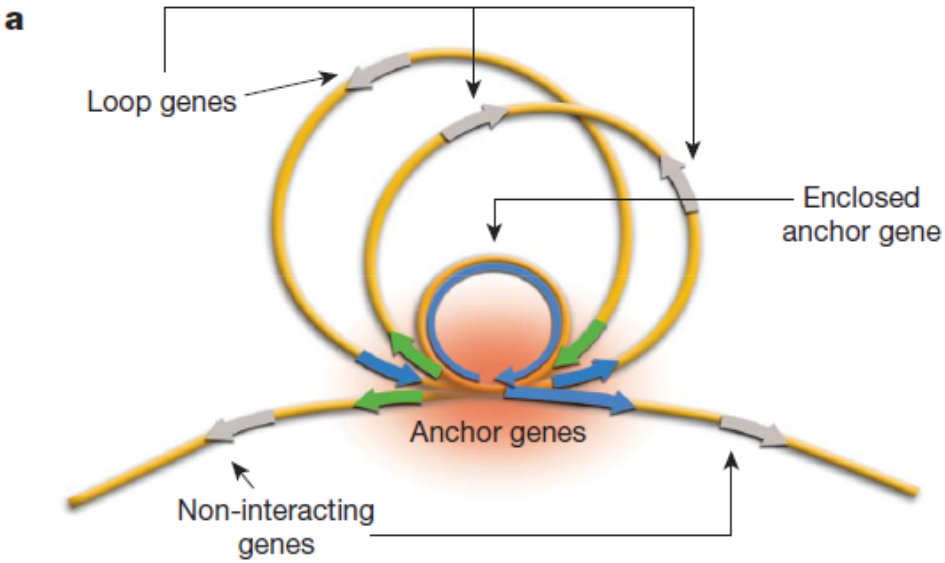
Enrichment around ER- α binding sites

Genome-wide ChIP-seq or ChIP-Chip data maps from MCF-7 cells under estrogen induction:

- H3K4me3 that marks active promoters
- RNAPII whose presence is a strong evidence for active transcription
- FoxA1 which is an important ER- α cofactor

Generally H3K4me3, RNAPII and FoxA1 marks showed enrichment around ER-α binding sites

Proposed ER-α-bound chromatin interaction and transcription regulation mechanism

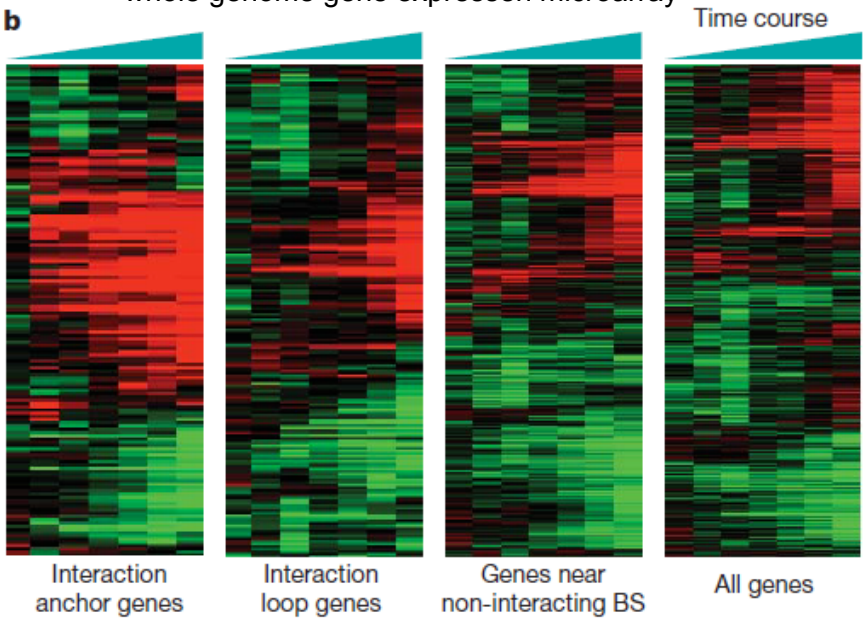


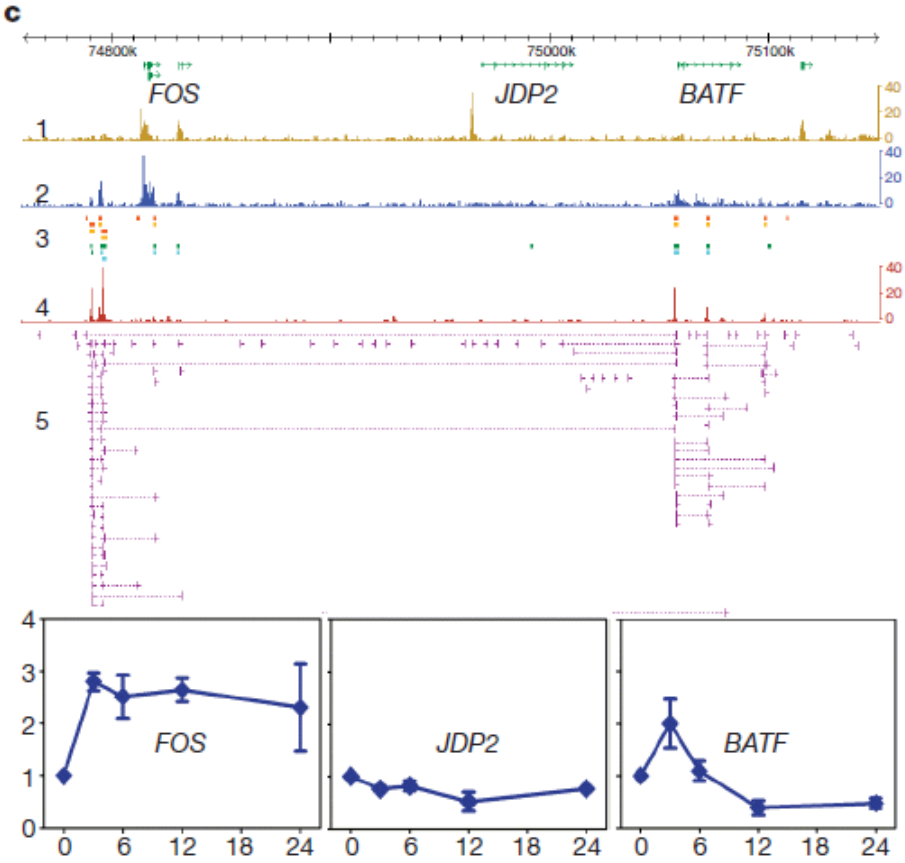
- Multiple ER-α binding sites may function as anchor regions forming chromatin looping structures in 3D space
- Genes close to interaction anchors are considered to be anchor genes and genes in the interaction loop regions to be loop genes
- A gene was considered to be associated with an interaction region if the TSS of the gene was wihtin 20kb of the interaction boundaries → includes many known and validated ER-α target genes

→ These observations suggest that direct ER-α binding might be initiated primarily at one or multiple distal sites, which then subsequently recruit other binding sites as anchor to form an interaction complex to ultimately engage the transcriptional machinery at gene promoters

Transcription regulation of ER-α interaction associated genes by estrogen induction

whole genome gene expressen microarray





- most anchor genes are upregulated (60%) by estrogen induction, and far less

→ indicating that anchor genes are significantly associated with gene upregulation

→ genes associated with non-interacting ER-αBS are less activated than genes associated with interaction ER-αBSs

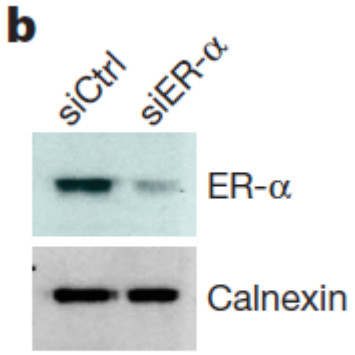
↳ There exists an association between chromatin interactions and gene transcriptional activation

- Enclosed anchor genes are activly transcriping
- FOS and BTF are enclosed anchor genes and are upregulated as shown by RNAPII marks and RT-qPCR
- JDP2 is a loop gene and is downregulated
- Dimerization partners of Jun to form AP1 → regulating ER-depentent transcription

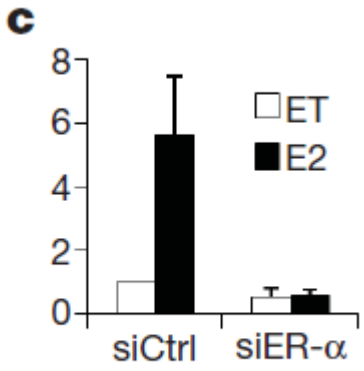
ER-α-bound chromatin interactions are required for transcription activation

siRNA knockdown experiment

RT-qPCR on GREB1 expression

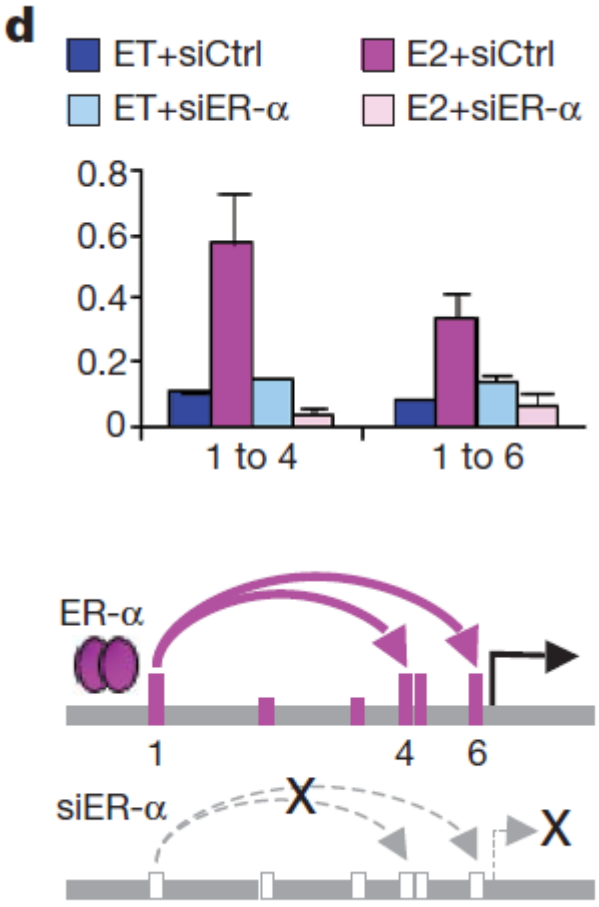


3C assay at GREB1



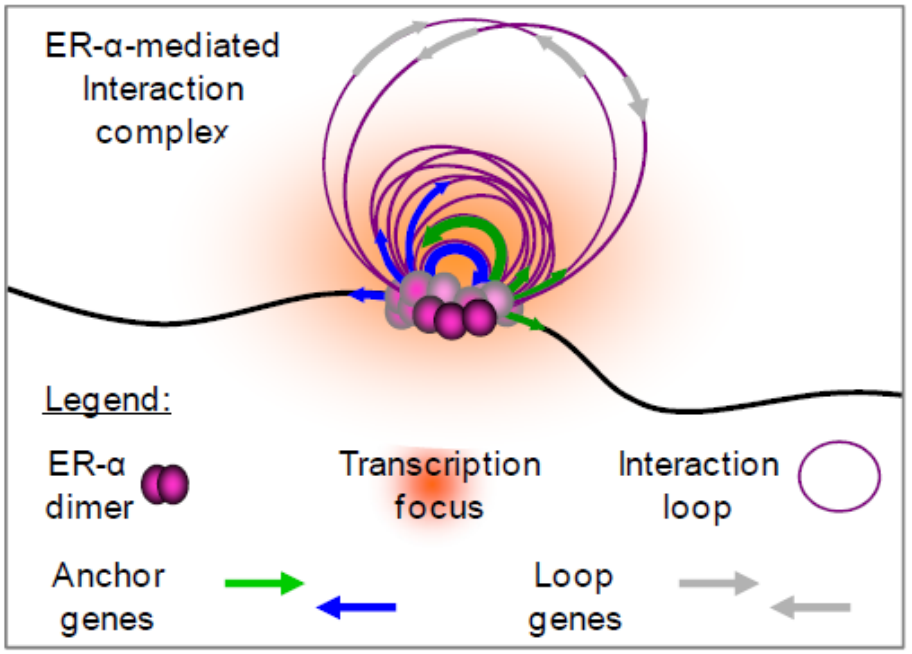
abolished interaction

siER-α knockdown abolishes chromatin interations and turns off transcription



The ER-α chromatin interaction model

- Speculate the ER-α protein dimers bind to distal regulatory elements and initiate long-range chromatin interactions involving promoter regions of target genes
- Multiple small and gene-centric loops could pachage genes near the anchoring center → could increase the local ER-α protein concentration → attract more TFs, cofactors and transcriptional machineries (e.g. RNAPII) → enhanced transcriptional activity
- Large interaction loops link together distant genes for coordinated regulation



Conclusion

- ChIA-PET mapping strategy is an **unbiased whole-genome** approach for **de novo** analysis of chromatin interactions → study higher-order organization of chromosomal structures and functions
- Globally addresses the 3D chromatin interaction mechanism by which TFBSs regulate transcription
- ER- α function: ER- α protein dimers are recruted to multiple ER-αBSs, which interact wiht one another and possibly with other factors such as FoxA1 and RNAPII to form chromatin looping structures around target genes
- Tightly enclosed chromatin interaction centres could help achieve and maintain high local concentrations of transcription components for efficient cycling of transcriptional machinery on target gene templates
- ER-α binding sites are at gene promoters for long-range chromatin interactions → ER-α might function by extensive chromatin looping to bring genes together for transcription regulation

Thanks for your attention