Chromatin conformation dynamics during T cell development

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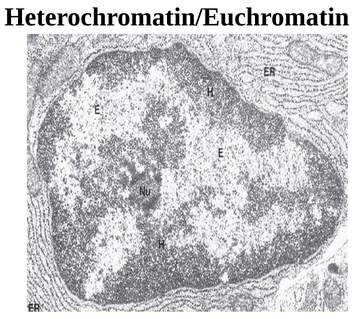
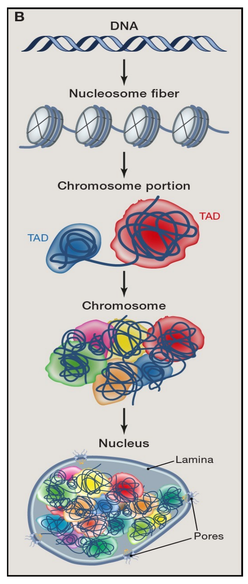
1 Background

*** Metazoan chromatin is highly compact BUT organized.**

Heterochromatin/Euchromatin

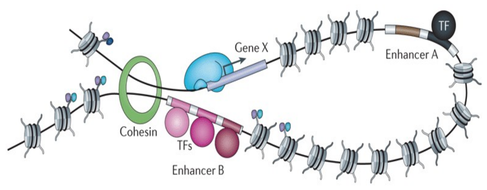




*** A striking correlation exists between chromatin topology and underlying gene activity.**

Example I: Enhancer-promoter loops

Chromatin loop



Chromatin loops bring genes into direct contact with distal regulatory elements, such as enhancers.

Example II: Topological Associated Domains (TADs)

Two TADs

Sexton et al 2012

The genome is organized into discretely folded megabase-sized regions, denoted as topologically associated domains (TADs), which seem to correlate well with transcriptional activity and histone modifications.

How does chromatin topology change during development ?

2 TAD developmental dynamics

How do TADs change during development ?

Noordermeer et al., 2011

Hox gene cluster

H3K27me3/repressed

H3K4me3/active

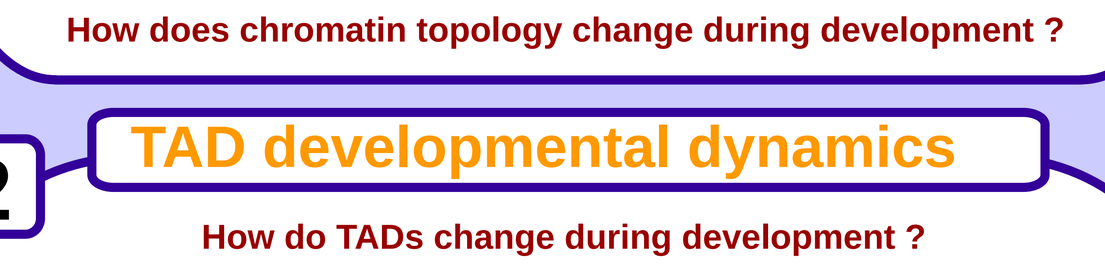
Dixon et al., 2012

*** Facultative TADs model can explain mouse Hox gene expression, whereby genes are sequentially activated during development, and according to anterior- posterior body position, in order along the chromosomal fiber.**

*** Constitutive TADs model suggests that TADs are developmentally stable which is supported by the comparaison of TADs in disparate mouse and human cell lines.**

Method I: Cap – Hi-C

Crosslinking → **Digestion** → **Ligation** → **DNA shearing** → **Sequence Capture** → **Sequencing**



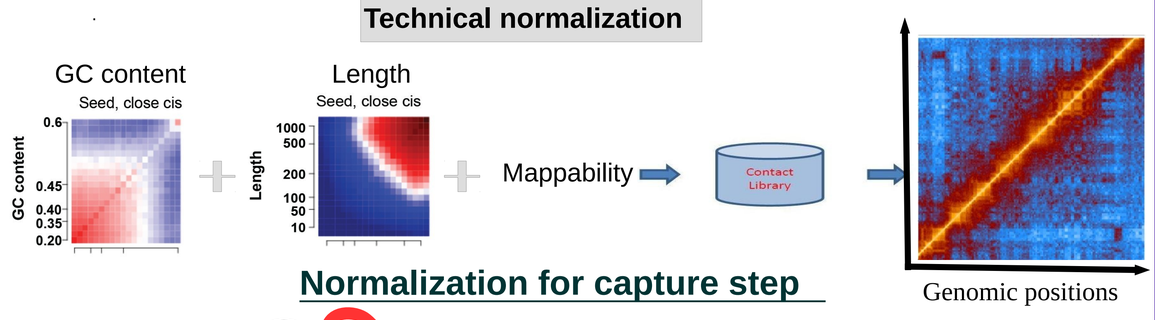
An adaptation of Hi-C to (i) track at high-resolution the conformation of specific TADs surrounding differentially expressed genes ; (ii) simultaneously detect all interactions between promoters and distal regulatory elements, across an important developmental transition of T cell : The differentiation from CD4/CD8 double negative (DN) to double positive (DP) cell.

Method II: Analysis

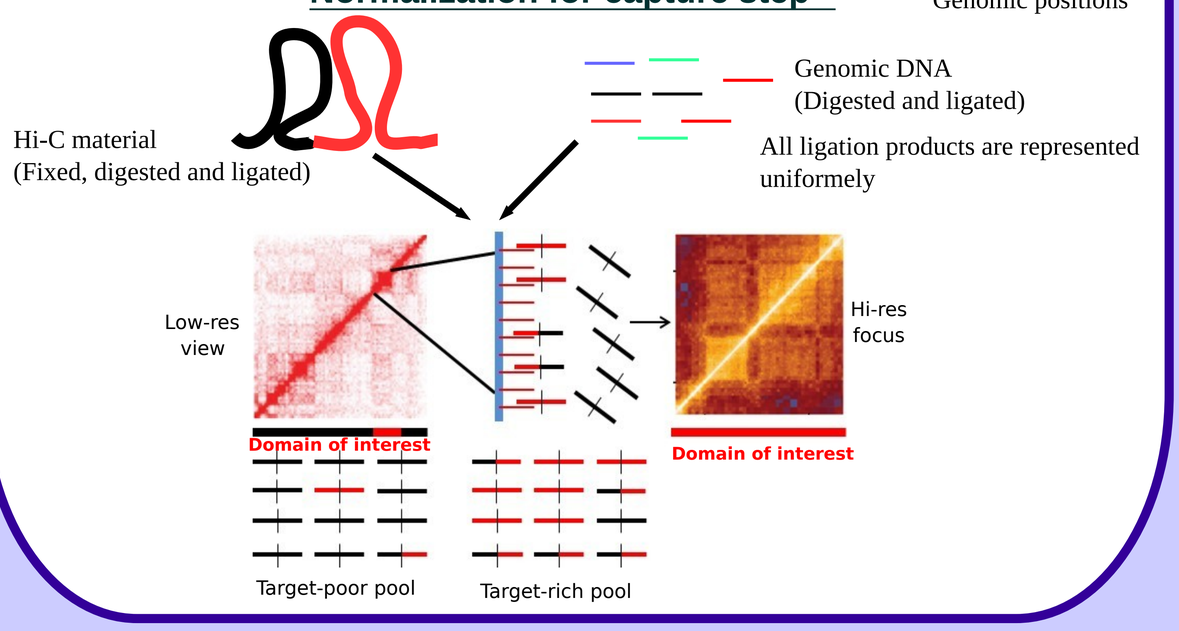
Hi-C Pipeline

Mapping reads → **Filtering reads** → **Technical normalization**

- **Filtering reads**: **Unique mapping**; **PCR replicates**; **True Hi-C products**

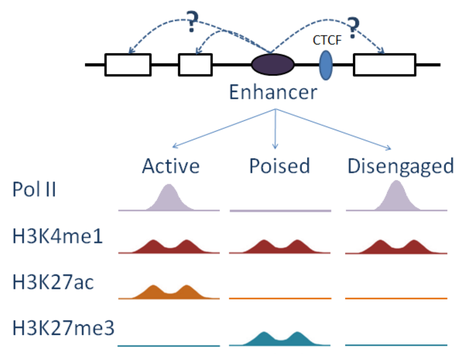


Normalization for capture step



3 Enhancer-promoter loop developmental dynamics

Target genes? Timing of loops? Chromatin state?

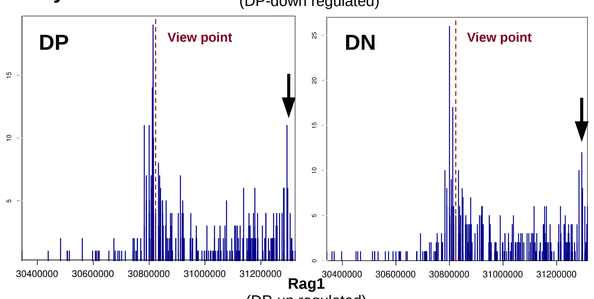


Known features of enhancers :

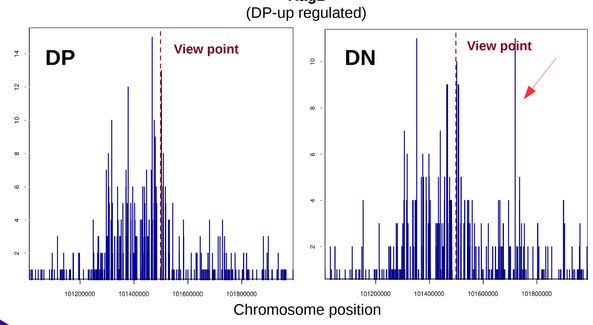
- *** Distant location from TSSs.**
- *** Gene target not necessarily closest on linear chromosome.**
- *** Epigenetic marks such as histone H3 Lys4 mono-methylation (H3K4me1).**
- *** Enrichment for transcription factor binding sites (TFBS).**
- *** Some marks distinguish « active » from « poised » enhancers.**

Preliminary Results

Il17rb
(DP-down regulated)



Rag1
(DP-up regulated)



Promoter Cap-C on DP and DN cells. Numbers of sequences are shown for 500 Kb Windows spanning promoters of two differentially-expressed genes, Il17rb (top ; expressed in DN) and Rag1 (bottom ;expressed in DP).

A constitutive interaction with Il17rb is shown by black arrows ; a DN-specific interaction with Rag1 is shown by a red arrow.

References

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