Protein-Mediated 3D Genome Architecture by HiChIP

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Introduction

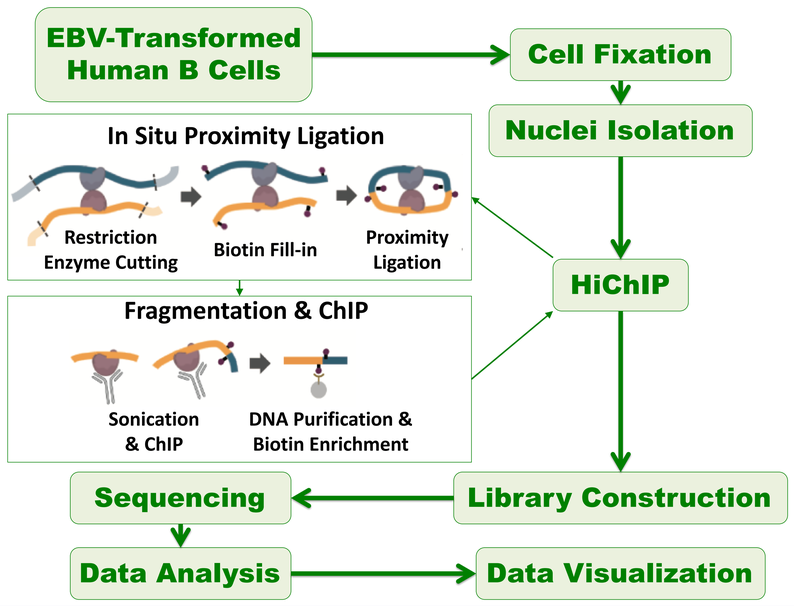
- Three-dimensional (3D) chromatin loops bring distant promoters and enhancers into close proximity to regulate gene transcription[1].
- Protein factors, such as cohesion and histone, facilitate the formation of the 3D chromatin conformation. Studying protein-mediated 3D genome architecture can help to understand how target genes influence human diseases[2,3].
- **HiChIP** is a new protein-mediated chromatin conformation capture method with improved efficiency and lower starting material requirements than ChIA-PET[4,5].

Method	HiChIP	ChIA-PET
Input Cells	1-10M	> 100M
Processing Time	2-4 Days	6 days
% Long-range Interaction	> 20%	1-2%
% In Loops	> 4%	< 0.5%

- The ***objective*** of this study is to comprehensively map the protein-mediated 3D genome architecture in EBV-transformed human B cells and primary immune cells using HiChIP. We examined protein factors **CCCTC-binding Factor** (CTCF) and **Histone 3 lysine 27 Acetylation** (H3K27ac) as structural and functional regulation units.

Methods

- **HiChIP**: Intact nuclei were digested using **MboI** restriction enzyme. The DNA fragment ends were filled and labeled with biotin. After proximity ligation, DNA was fragmented and ChIP enrichment was performed using CTCF or H3K27ac antibodies. HiChIP library was generated on streptavidin beads using Nextera DNA Library Prep Kit.
- **Sequencing and Data Analysis:** Libraries were sequenced on the Illumina NextSeq 500 sequencer. HiChIP raw reads were processed and analyzed through the **hichipper**[6] pipeline. Intrachromosomal loops are defined with a minimum length of 5KB and a maximum length of 2MB. Anchor and looping patterns are visualized by **DNAlandscapR**[7].



References

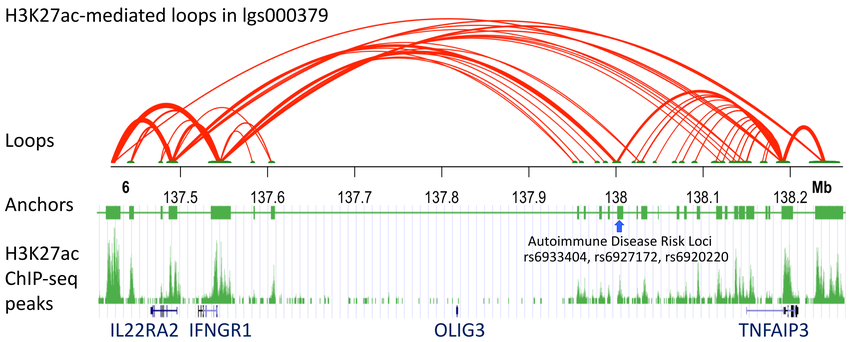
1. Schmitt et al. Genome-wide mapping and analysis of chromosome architecture. *Nature Reviews* (2016).
2. Tang et al. CTCF-mediated human 3D genome architecture reveals chromatin topology for transcription. *Cell* (2015).
3. Hnisz et al. Activation of proto-oncogenes by disruption of chromosome neighborhoods. *Science* (2016).
4. Mumbach et al. HiChIP: efficient and sensitive analysis of protein-directed genome architecture. *Nature Methods* (2016).
5. Fang et al. Mapping of long-range chromatin interactions by proximity ligation-assisted ChIP-seq. *Cell Research* (2016).
6. The hichipper pipeline is at https://github.com/aryeelab/hichipper
7. DNAlandscapR is at http://dnalandscaper.aryeelab.org

Results

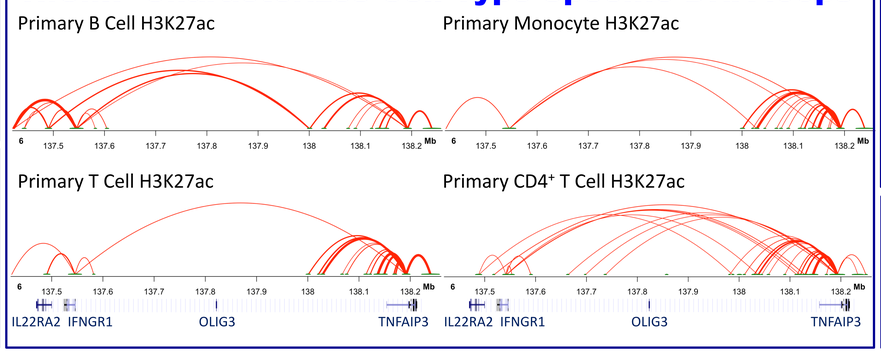
HiChIP Efficiently Generates High-Quality Informative Data in EBV Cells and Primary Cells

Cell Type	Input Cells	Anchor Protein	Average % Long-range Interaction
EBV	1-10M	H3K27ac	**24.79**
EBV	10M	CTCF	**20.02**
Primary B Cells	10M	H3K27ac	**18.45**
Primary T Cells	10M	H3K27ac	**15.64**
Primary Monocytes	10M	H3K27ac	**19.08**
Primary CD4⁺ T Cells	4M	H3K27ac	**15.10**
Primary CD4⁺ T Cells	4M	CTCF	**16.67**

HiChIP Reproducibly Identifies Functional Targets Associated with GWAS Variants

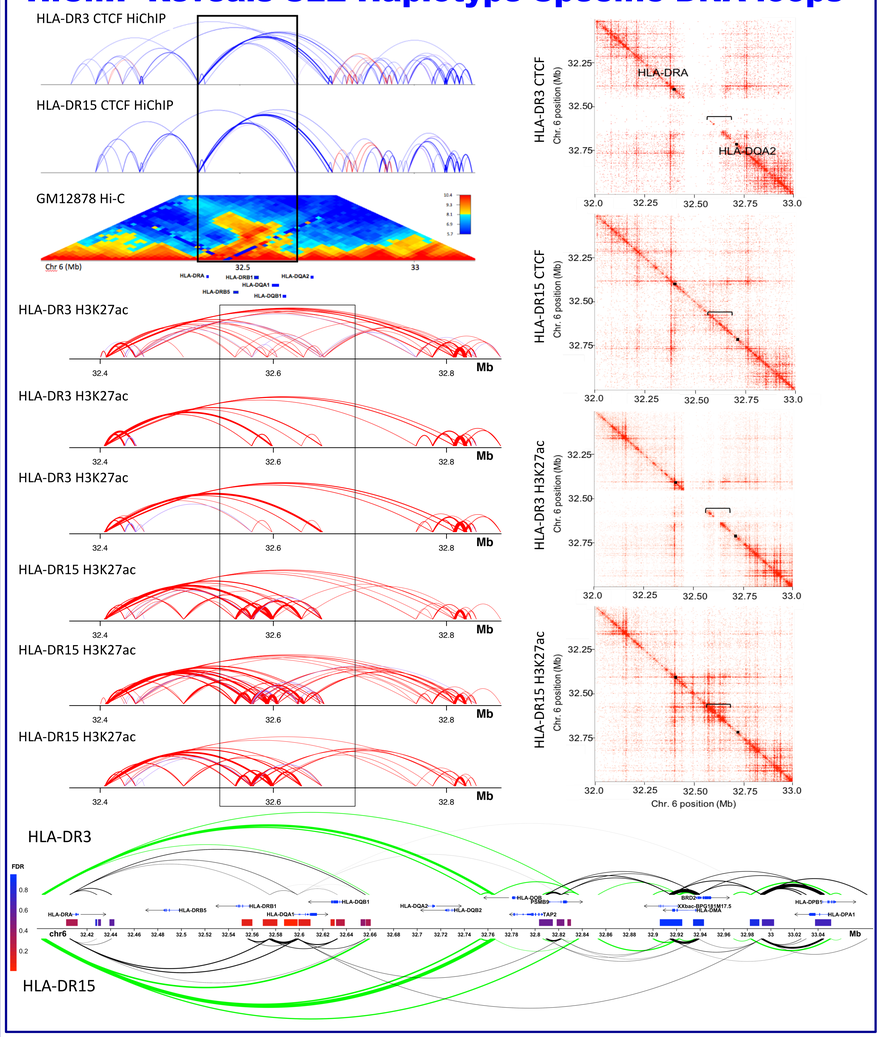


HiChIP Characterizes Cell Type-Specific DNA loops



Results

HiChIP Reveals SLE Haplotype-Specific DNA loops



Discussion

- HiChIP is a rapid and efficient method to measure protein-mediated 3D genome architecture in EBV-transformed human B cells and primary immune cells.
- HiChIP yields high-quality informative reads using input cell numbers 10-100 fold lower than traditional methods like ChIA-PET, and it is sensitive to identify haplotype-specific and cell type-specific 3D chromatin interactions.
- HiChIP can help to identify and characterize functional targets associated with GWAS variants in non-coding regions. Our data may also help to discern potential causal risk SNPs within a large linkage disequilibrium (LD) region.
- In the future, we will perform HiChIP on primary human cells obtained from SLE patients and healthy individuals to investigate how 3D chromatin topology is associated with SLE genetic variants and disease pathology.

Acknowledgements

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