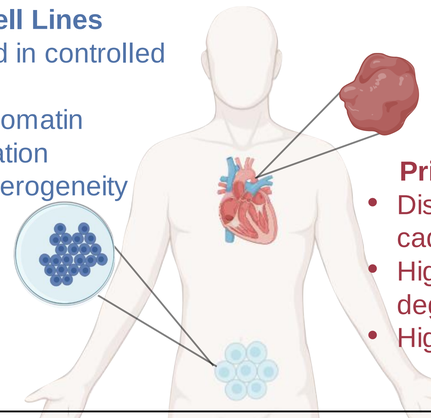
Dual Cross-Linking Improves Ability to Map Chromatin Loops

Shaiza Pasha[1,3], Ragini Mahajan[2,3], Muhammad Shamim[2,3], Erez Lieberman Aiden[1,2,3], Greg Morrison[1,2]
[1]University of Houston, [2]Rice University, [3]Baylor College of Medicine

Introduction

- **Hi-C** is a method that maps the 3D architecture and folding pattern of the genome, generating annotations of features, like DNA **loops** and domains, that bring distal elements in proximity and **influence their regulation**.
- Cross-linking samples is the first step in Hi-C, which is akin to "shooting glue through the nucleus". The kinds and amounts of cross-linkers used can affect the quality of contacts observed in the Hi-C data.
- While Hi-C has been generally robust, the quality of the data can be dependent on the inherent state of the input sample. In this study, we compare **how cross-linking variations affect Hi-C data generated from standard cell-lines versus novel primary tissues, and find if dual cross-linking can improve ability to map loops.**

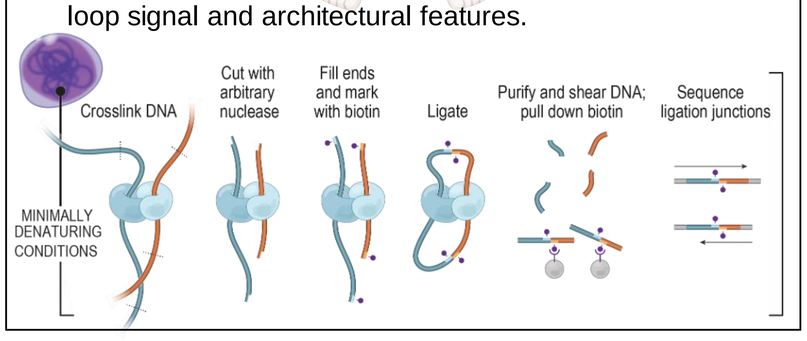
Cell Lines
- Cultured in controlled settings
- Low chromatin degradation
- Low heterogeneity



Primary Tissue
- Dissected from cadavers/patients
- Higher chromatin degradation
- High heterogeneity

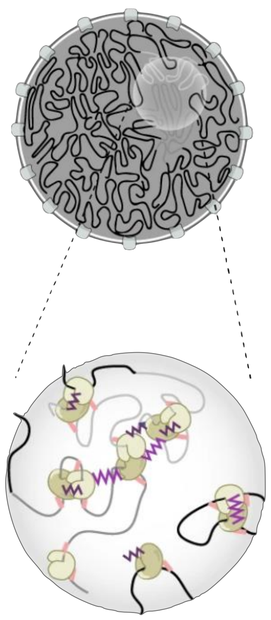
Methods

- We test cross-linking with **1% Fa** for 10 minutes (control) or **1% Fa + 3mM DSG** for 30 minutes on GM12878 cultured cells and human aorta tissue.
- We then process samples using **Intact Hi-C**, generating contact maps and quality statistics using the Juicer 2 pipeline. We then compare results between GM12878 and aorta tissue to see differences in quality statistics, loop signal and architectural features.

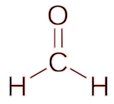


Results

Cross-Linking Chemistry

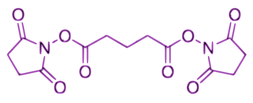


Formaldehyde (Fa)



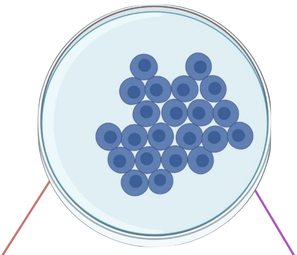
DNA-protein interactions

DSG

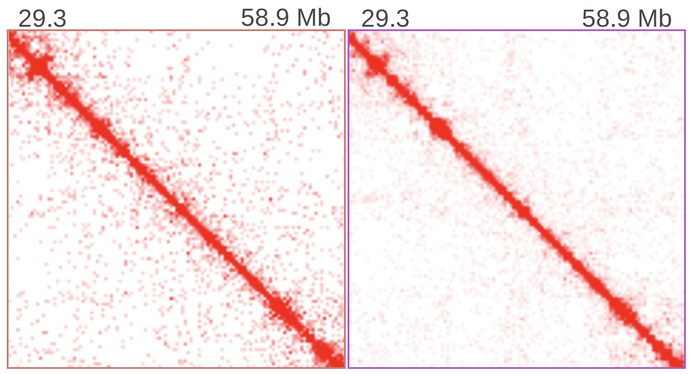


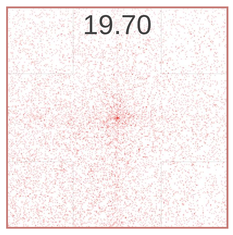
protein-protein interactions

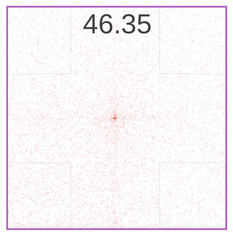
GM12878 Cell-Line



Fa | Fa + DSG



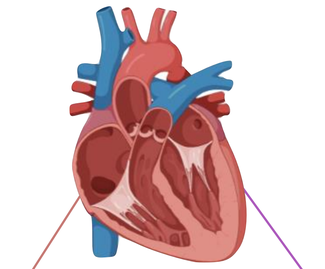




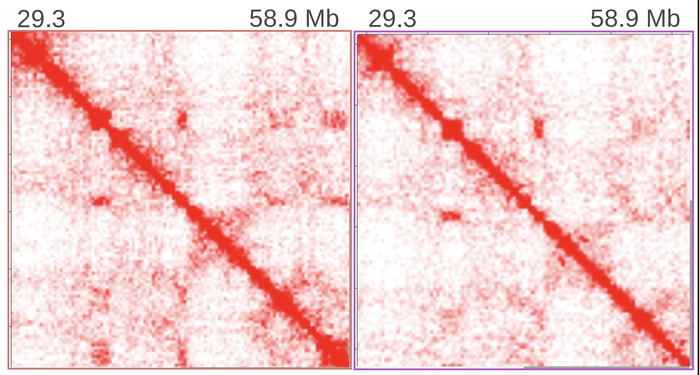
Sequenced Reads	5M
Inter-chromosomal	10.99%
Long Range	23.54%
APA	19.7
DNA Conc. (ng/uL)	14.7

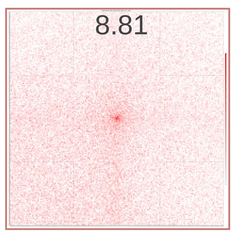
Sequenced Reads	5M
Inter-chromosomal	4.51%
Long Range	17.08%
APA	46.35
DNA Conc. (ng/uL)	73.8

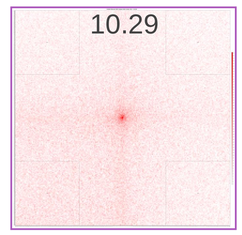
Primary Aorta Tissue



Fa | Fa + DSG







Sequenced Reads	13M
Inter-chromosomal	20.25%
Long Range	29.65%
APA	8.809
DNA Conc. (ng/uL)	10.8

Sequenced Reads	13M
Inter-chromosomal	12.65%
Long Range	27.48%
APA	10.295
DNA Conc. (ng/uL)	24.6

Discussion

- Dual cross-linking on GM12878 cells improves noise statistics, showing a lower % of inter-chromosomal contacts than its Fa-only counterpart. This trend is also observed in the aorta tissue. Along with a lower inter-chromosomal %, we observe higher DNA yield in the Fa+DSG samples, both in GM12878 and aorta tissue. We suspect that this could be due to greater chromatin stability as a result of the double cross-linking.
- Aggregate Peak Analysis (APA) of GM12878 vs Aorta tissue shows an increase in APA score in Fa+DSG samples, signaling improvement of looping signal in both, GM12878 cells and aorta tissue.
- Our findings suggest that dual cross-linking **improves overall quality** of both tissues and cell-lines comparably in terms of noise levels and DNA yields, while also **improving loop signal** recognition**.**
- For future work, we will test this method on a broader panel of cell lines and human tissues to gauge robustness for new standards.

Acknowledgements and References

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References: Oksuz, A.B. et al., *Nat. Methods*, **18** 1046–1055 (2021), Rao, S.S.P. et al., *Nature Cell Biology*, **159** 1665-1680 (2014), Images made by BioRender & Keynote.