Capturing complex enhancer regulatory hubs? Try Tri-C.

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A recent study in Nature Genetics from *Oudelaar et al.* (2018) describes Tri-C, a technique developed to study multi-way chromatin interactions at the allelic level from regulatory elements whilst maintaining high sensitivity and resolution. The method is used to explore the combinatorial interactions between multiple regulatory elements underlying precise gene regulation of the globin locus.

Gene expression is regulated in time and space through the interplay between gene promoters, distal regulatory enhancer elements and three-dimensional chromatin architecture. It is increasingly clear that complex relationships exist between enhancers and promoters to ensure both precise and robust gene expression profiles. Many developmental genes are regulated by multiple enhancer elements, such as in a redundant manner for the purposes of robust gene regulation in the correct tissue (Barolo, 2012)) or in a combinatorial fashion to drive diverse patterns of activity during development, such as at the HoxD gene cluster(Montavon et al., 2011).

Chromosome conformation capture methods have been widely used to observe long-range contacts, or chromatin loops, between distal enhancers and target gene promoters, suggesting that physical contact between these elements is an important step in gene regulation. Unravelling the cis-regulatory architecture of the genome requires a quantitative assessment of chromatin interactions at the highest resolution in several developmental contexts. Significant efforts have been made in this context, with recent advances in our understanding of genome topologies both locally(Schwartzman et al., 2016), globally(Bonev et al., 2017; Rao et al., 2014) and at the single-cell(Nagano et al., 2013) levels. Nevertheless, even the highest resolution Hi-C contact maps where regulatory topologies can be observed, are based on pair-wise interactions and thus, they cannot be used to identify potential hierarchical or combinatorial interactions between multiple regulatory elements underlying precise gene regulation. Furthermore, it is not possible to distinguish between simultaneous contacts among multiple loci and pairwise contacts in different sub-populations of cells. To address this issue, several groups have begun to develop methods to assay multi-way chromatin interactions. Combining third generation sequencing platforms (PacBio or Oxford Nanopore) with chromosome conformation methods(Allahyar et al., 2018; Olivares-Chauvet et al., 2016) has expanded the genome topology toolkit, revealing multiple simultaneously interacting fragments in a single long read and thus moving the field beyond standard pair-wise contact matrices. Complementary approaches using FISH-based methods confirm the existence of multi-way contacts and provide insight into chromatin

elements that co-occupy nuclear space (Beagrie et al., 2017). These methods are informing exciting new biology, for example revealing ‘enhancer hubs’ that can accommodate two B-globin genes simultaneously (Allahyar et al., 2018).

A recent study in Nature Genetics from *Oudelaar et al.* (2018) present Tri-C, an alternative technique developed to study multi-way chromatin interactions at the allelic level from specific regulatory elements. Due to the relatively low output of third generation sequencing platforms, coverage of multi-way contacts remains a challenge. Instead Tri-C combines the high resolution and quantitative nature of chromatin contact mapping methods with Illumina sequencing to maximise coverage of multi-way contacts. Tri-C libraries are prepared using a 4-cutter restriction enzyme (providing a resolution of ~450bp) and bait fragment ligations are enriched using an oligo-based capture method instead of bait-specific PCR. Tri-C is a new, complementary offering to the existing multi-way C methodologies mentioned above, providing high coverage of primarily 3-way contacts.

Focussing on the mouse globin loci, Oudelaar and colleagues used Tri-C to generate contact matrices from the mean number of normalised unique interactions in 10 erythoid and 7 embryonic stem cell (ES) replicates in mouse. Erythroid specific interactions between the α-globin genes and the strongest α-globin enhancer (R2) were detected as well as several multiway contacts with the remaining enhancers, including R1, R3, Rm and R4, representative of their expression in erythroid cells. Meanwhile, ES cells lacked any discernible structure, indicative of the lack of α-globin expression in ES cells. *Oudelaar et al.* also explored the potential for multi-way contacts between CTCF sites at individual alleles, with the aim of identifying whether stable loops form between multiple CTCF binding sites or whether the CTCF interactions are more dynamic and heterogeneous across alleles. Using Tri-C, the authors found broad regions of multi-way interactions around the CTCF sites at the a-globin locus, however there were no significant enrichments of specific interactions between multiple CTCF binding sites within this region. They suggest that the lack of punctate multi-way CTCF interactions argues against a stable CTCF-based architecture. Other methods, including a multiplexed super resolution FISH methodology have identified cooperative multi-way interactions between CTCF binding sites(Allahyar et al., 2018; Bintu et al., 2018), as well as general, cooperative higher order chromatin interactions that exist in single cells which include but are not preferential for CTCF sites(Bintu et al., 2018). Such complementary approaches for the investigation of regulatory architecture will be important avenues for future understanding.

The emerging view is that pairwise interactions do not capture the full complexity of chromatin structure, especially when considering population based contact methods. Inevitably future goals will involve maximising resolution of interacting elements without sacrificing the number of interacting partners that can be detected, and Tri-C takes important steps towards this aim. Characterization of complex multi-way cis-regulatory architecture will dramatically contribute to our understanding of precise and robust gene expression control.

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