GENOMICS

Capturing promoter-enhancer interactions in high throughput

Capturing the interaction of promoters with their regulatory elements allows a high-resolution view into the regulatory landscape of chromatin.

The question at the heart of research in the laboratory of Douglas Higgs at Oxford University centers on how gene expression is regulated during development. In collaboration with Jim Hughes, also at Oxford, his team sought to uncover general mechanistic principles by which *cis*-regulatory elements control their target genes. A variety of methods to probe interactions of genomic regions already exist, and when the project got under way four years ago, the researchers expected them to give the desired answers.

"We started with the standard 3C method," recalls Higgs, referring to a chromosome conformation capture method that yields the interactions between two particular genomic loci. But the researchers felt they needed a less biased approach. They tried 4C, a method that tests the interactions of one locus with the rest of the genome, but it did not meet their needs either. "We found it rather cumbersome, and the analysis required a lot of data manipulation," says Higgs. Moving to high-throughput 3C methods that query all genome-wide interactions, the researchers were not satisfied with the low resolution. Finding interactions in 1-megabase windows is not informative when one is looking for them at kilobase resolution.

"We needed a method that asks, for each promoter, which other part of the genome they are interacting with, particularly regions that have the hallmark of an enhancer," explains Higgs.

The team borrowed a strategy used to enrich genomic sequences, in which the sequences are hybridized to short oligonucleotides that comprise the target sequences. The ensuing Capture-C technique starts with a 3C library; chromatin is then cross-linked and digested with a frequently cutting enzyme, and the chromatin ends are ligated. Next, the loci of interest and their interaction partners are captured by the oligonucleotides, amplified and sequenced.

Choosing the right oligonucleotides proved to be the most challenging part of Capture-C, recalls Hughes. The researchers developed software to design oligos

that most efficiently captured the targeted regions without also bringing down a lot of background. Once that was done, Hughes says, the analysis was straightforward.

Capture-C worked as expected with the mouse α - and β -globin loci, regions well characterized by other 3C methods. Analyzing 455 promoters, the team was able to see general principles of *cis* regulation: they confirmed that enhancers do not necessarily regulate the promoter closest to them and saw that interactions take place within defined boundaries. Higgs likens the interactions within such domains to "sampling of the chromatin." He envisions a process whereby enhancers scan a domain and then preferentially bind to certain promoters. Hughes adds that although it is not yet clear what determines the boundaries, some domains are separated by known chromosome boundary elements.

Their next step will be to use genome editing to remove these potential boundary elements to understand in more detail how the chromosome is structured and what the functional implications of removing such structures are.

Taking advantage of Capture-C's ability to query many loci in a single experiment, Higgs and Hughes plan to follow the dynamics of interactions in hematopoietic stem cells as they differentiate into mature blood cells.

Capture-C will also be a boon to scientists following up on single-nucleotide polymorphisms (SNPs) that emerged as strongly associated with certain diseases. For those SNPs that fall into *cis*-regulatory regions, the method will identify the genes they control. Higgs hopes to use Capture-C in conjunction with other genomic data to "complete the circle between genome wide association study, gene expression and resulting phenotype."

Since they started presenting the method, Hughes says, people have discussed other applications he never thought of, adding, "It will be fascinating to see what the field does with it."

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RESEARCH PAPERS

Hughes, J.R. Analysis of hundreds of *cis*-regulatory landscapes at high resolution in a single, high-throughput experiment. *Nat. Genet.* **46**, 205–212 (2014).