

MICROARRAYS

ChIP-chip put to the test

A multi-laboratory comparative analysis of chromatin immunoprecipitation on array (ChIP-chip) provides an objective opportunity to compare tiling array platforms, amplification procedures and analytical algorithms.

“It really helps to have real definitive information about a technique, and what it is and isn’t able to do,” says Kevin Struhl of Harvard Medical School; “otherwise you just get people’s impressions and biases.” To obtain such definitive information about ChIP-chip, eight research groups embarked on a massive systematic analysis, which they recently reported in *Genome Research*.

At the core of this analysis were two carefully crafted test samples composed of human genomic DNA and about 100 ‘spike-in’ sequences, which were mixed in a range of known molar ratios. The people testing the samples, however, had no idea of the identity, quantitative range or even number of spike-ins. “It is nice that everything was done in a ‘blind’ way because it was really objective,” says Xiaole Liu of the Dana-Farber Cancer Institute, who contributed to the data interpretation.

This systematic approach demonstrated that the arrays work extremely well. “What was particularly impressive is not only that people called the targets but they were pretty good about calling the magnitude of enrichment,” comments Struhl.

Notably, all array platforms tested were comparable. “When this started, everyone thought it was going to be a big battle between the platforms,” recalls Jason Lieb of the University of North Carolina at Chapel Hill, another co-author on the study. “But it didn’t turn out that way.” The authors found interesting differences between the long-oligonucleotide platforms (Nimblegen and Agilent were tested) and the short-oligonucleotide Affymetrix platforms, in terms of the number of probes per genomic locus needed to have a high-confidence call. In terms of overall result quality, however, the high density of probes on the Affymetrix platform made up for the lower specificity associated with individual probes. “We had more variation within platforms, among

different groups using different algorithms, than we did between microarray platforms,” notes Lieb.

Thanks to a careful study design, it was possible to evaluate how different steps in the ChIP-chip procedure contribute to its variability. This is the first time, for example, that analysis algorithms (13 in total in this study) could be objectively tested. Different amplification protocols were also scrutinized independently, because one of the two test samples had to be amplified before being put on an array. The results are of interest to anybody using ChIP-chip.

This in-depth technical analysis comes at a time when arrays meet competition from the new high-throughput sequencing technologies, and the ChIP-seq approach has recently emerged as an alternative to ChIP-chip. But the two techniques have never been seriously compared, and the authors suggest that a similar systematic analysis of ChIP-seq would be very useful to better understand the underlying technical issues. Liu indicates that the test samples are hosted at the Dana-Farber Cancer Institute and available for anybody interested.

In any case, this analysis shows that DNA microarrays still have a bright future in association with ChIP. In particular, for smaller genomes that can be tiled at high resolution on a single array, the arrays remain cost-effective and more accessible than sequencing. Even for mammalian samples, the issues of cost and accessibility come into play. As Struhl points out, “while sequencing methods have some advantages for mapping and dealing with repetitive DNA for example, you really have to sequence a lot if you want to have serious quantitative information.” And that sequencing capacity is beyond the reach of most labs at the moment. As Lieb sums up, “some people have proclaimed the death of microarrays, but I wouldn’t be so fast about that.”

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Johnson, D.S. *et al.* Systematic evaluation of variability in ChIP-chip experiments using predefined DNA targets. *Genome Res.* **18**, 393–403 (2008).