

No Formaldehyde, No Problem for New Genome-Wide Chip Method

January 21, 2014

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Transcription factors (TFs) interpret regulatory information encoded in genomes to direct gene expression, thereby influencing essentially every cellular process. Mutations in TFs or the DNA sequences recognized by TFs are often found in human diseases (Lee and Young, 2013; Maurano et al., 2012). Mapping the genome-wide distributions of TF recognition sites is thus of significant interest for understanding basic cellular processes as well as the potential bases of many diseases. The gold-standard method for surveying protein-genome interactions is chromatin immunoprecipitation (ChIP), which has been combined with hybridization to tiled microarrays (ChIP-chip) or high-throughput sequencing (ChIP-seq) to give whole-genome pictures of protein binding. ChIP generally involves the treatment of cells with formaldehyde (X-ChIP), which covalently fixes protein-DNA and protein-protein interactions. Cells are then disrupted and chromatin is solubilized via sonication. While X-ChIP-chip and X-ChIP-seq studies have yielded many important insights, there are limitations associated with these techniques. For example, formaldehyde crosslinking preferentially generates protein-protein crosslinks (O'Neill and Turner, 2003) and can lead to false-positive ChIP signals due to crosslinking to the transcription machinery at highly expressed loci (Teytelman et al., 2013). Native ChIP (N-ChIP), which does not use formaldehyde crosslinking, may offer solutions to some of these issues.

To this end, graduate student Siva Kasinathan and colleagues in the laboratory of Dr. Steven Henikoff (Basic Sciences Division) in collaboration with researchers at Harvard University undertook a systematic comparison of crosslinking ChIP (X-ChIP) methodologies with an N-ChIP methodology first developed for genome-wide localization of chromatin remodeling enzymes (Zentner et al., 2013). The researchers found that this approach, termed Occupied Regions of Genomes from Affinity-purified Naturally Isolated Chromatin (ORGANIC), outperformed multiple X-ChIP methods. "Compared to other genome-wide ChIP methods, ORGANIC profiling and subsequent data analysis is relatively straightforward. This method also circumvents several of the limitations associated with X-ChIP approaches, most notably those associated with formaldehyde crosslinking," says Mr. Kasinathan.

In ORGANIC profiling, nuclei are isolated from cells and treated with micrococcal nuclease (MNase). This enzyme nicks single-stranded DNA exposed by breathing of the DNA helix, generating a double-strand break, and subsequently "nibbles" exposed DNA ends until it reaches an obstruction, usually a protein. The researchers first applied ORGANIC profiling to the budding yeast TF Reb1, which had been mapped previously by X-ChIP-chip and ChIP-exo, a high-resolution modification of X-ChIP-seq (Rhee and Pugh, 2011). ORGANIC detected more Reb1 binding sites than either X-ChIP-chip or ChIP-exo. The authors assessed the quality of the Reb1 binding sites by searching for the presence of a consensus DNA sequence known to support Reb1 binding. Of the ORGANIC Reb1 sites, 99.3% contained a consensus motif, while only 59.6% of ChIP-exo peaks contained such a motif. Similar results were obtained for the TF Abf1.

A widely cited rationale for crosslinking in ChIP experiments is the expectation that proteins will rearrange during chromatin preparation and immunoprecipitation steps. To address this, the researchers mixed yeast and fly nuclei prior to MNase digestion with the expectation that if Reb1 redistributed, substantial amounts of fly DNA would be recovered in the immunoprecipitation. They found only background levels of *Drosophila* DNA in mixed Reb1 immunoprecipitations, arguing against TF rearrangement. The authors also confirmed that the Reb1 and Abf1 sites detected by ORGANIC were occupied *in vivo* by analyzing genome-wide DNase I footprinting data. DNase I cleaves within accessible DNA, and protein-occupied regions of DNA are seen as local depletions of accessibility ("footprints"). Both Reb1 and Abf1 sites showed robust DNase I footprints, confirming their *in vivo* occupancy. Furthermore, ORGANIC sites were not biased towards regions of accessible chromatin, in contrast to ChIP-exo sites. This feature of ORGANIC obviates the need to normalize ChIP samples to input, which can be costly for large genomes due to the need for deep whole-genome sequence coverage.

Lastly, the researchers performed ORGANIC profiling of the *Drosophila* transcription factors GAGA factor (GAF) and Pipsqueak (Psq). When compared to X-ChIP-chip, ORGANIC detected an order of magnitude more sites with consensus TF-binding DNA sequences, demonstrating its high sensitivity and specificity, as well as its applicability to larger genomes. The authors hope to apply ORGANIC to genome-wide mapping of human proteins. "Our ultimate goal is to map the genome-wide distributions of disease-relevant human proteins in order to understand how transcription is dysregulated in disease," says Mr. Kasinathan.

[Kasinathan S, Orsi GA, Zentner GE, Ahmad K, Henikoff S](#). 2013. High-resolution mapping of transcription factor binding sites on native chromatin. *Nat Methods* Epub 15 Dec 2013.

See also: [Lee TI, Young RA](#). 2013. Transcriptional Regulation and Its Misregulation in Disease. *Cell* 152(6):1237-1251.

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[O'Neill LP, Turner BM](#). 2003. Immunoprecipitation of native chromatin: NChIP. *Methods* 31(1):76-82.

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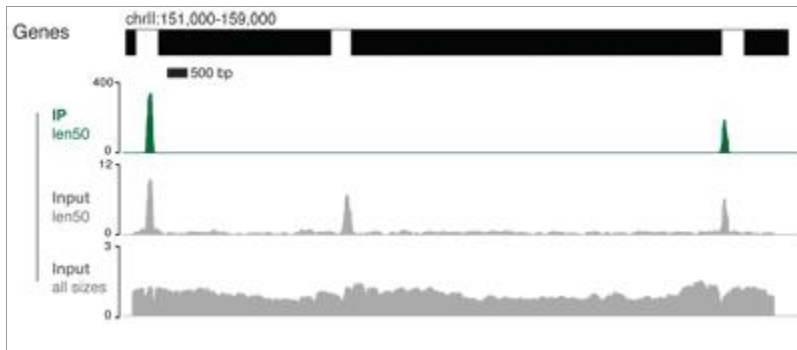


Image provided by Siva Kasinathan.

Tracks of Reb1 and input signal along a segment of yeast chromosome II showing robust enrichment of Reb1 (green) relative to input (grey). For tracks labeled with “len50,” only Reb1-bound and input fragments 1-100 bp in length were considered.