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Computational Genomics: Module 7

ChIP-seq

(1) Description of the technology:

ChIP-seq, or chromatin immunoprecipitation sequencing, is a technology used to find binding locations of proteins on the genome. These proteins are mainly transcription factors, as they bind to the genome and change expression rates of other genes. First, DNA is cross-linked to the proteins to form DNA-protein complexes. Samples are then fragmented with an exonuclease. An antibody specific for a specific protein bound to the DNA is used to immunoprecipitate the DNA-protein complex. The DNA is then extracted and sequenced, giving reads that were bound by the protein of interest.

(2) Paper that uses this technique:

Tu, Xiaoyu, et al. "Reconstructing the Maize Leaf Regulatory Network Using ChIP-Seq Data of 104 Transcription Factors." *Nature Communications*, vol. 11, no. 1, 2020, doi:10.1038/s41467-020-18832-8.

(3) Summary of paper question, and how the technique was used to answer it:

These scientists describe the problem to find transcription factor binding studies in plants, as there are too few studies describing results to produce a general picture of the complex network. They used large-scale ChIP-seq (104 transcription factors expressed in maize leaf) to reconstruct its transcription regulatory network, and then train machine-learning models to predict the transcription factor binding and co-localization.

shRNA-seq

(1) Description of the technology:

shRNA-seq is a method that uses shRNA to knock-down target mRNA translation. This is because shRNA is processed into siRNAs (small interfering RNAs) in the cell, which in turn knocks down target gene expression. RNAseq following knockdown allows for expression levels of other proteins to be understood, therefore the relationship between one protein's decreased expression and the rest of the cell's expression rates. Knockdown of transcription factors can provide large changes in the fold of expression rates of other proteins.

(2) Paper that uses this technique:

Aarts, Marieke, et al. "Coupling ShRNA Screens with Single-Cell RNA-Seq Identifies a Dual Role for MTOR in Reprogramming-Induced Senescence." *Genes & Development*, vol. 31, no. 20, 2017, pp. 2085–2098., doi:10.1101/gad.297796.117.

(3) Summary of paper question, and how the technique was used to answer it:

To investigate the mechanism of action of OSKM-induced senescence, these scientists combined shRNA screening and single cell RNA-seq. An shRNA library of ~58,000 shRNAs

was transduced into IMR90 fibroblasts after they were given a retroviral vector expressing OSKM. The integrated shRNAs were identified and their enrichment was assessed using NGS. They found that shRNAs targeting four genes (CDKN1A, MTOR, MYOT, and UBE2E1) would result in a consistent bypass of OSKM-induced senescence.

DNase-seq

(1) Description of the technology:

DNase-seq is a method that uses the DNase I enzyme for sequencing regions that are readily accessible by said endonuclease. This provides information on the accessibility of that genomic region, as in, whether or not the DNA is bound by proteins, or wrapped up by chromatin/nucleosome structures. Sequences obtained from sequencing after DNase I activity are thought to be more accessible than sequences that do not appear.

(2) Paper that uses this technique:

Zhong, Jianling, et al. "Mapping Nucleosome Positions Using DNase-Seq." *Genome Research*, vol. 26, no. 3, 2016, pp. 351–364., doi:10.1101/gr.195602.115.

(3) Summary of paper question, and how the technique was used to answer it:

These scientists describe the use of DNase I to precisely map the (translational) positions of in vivo nucleosomes genome-wide. Previously, DNase I was used to probe genomic regions devoid of nucleosomes. Here, they revealed a distinctive DNase I cleavage profile that showed that potential binding sites within nucleosome-associated DNA are often centered preferentially on an exposed major or minor groove. The DNase I activity was shown in the yeast genome, and the positions of nucleosomes were obtained.

CLIP-seq

(1) Description of the technology:

CLIP-seq, or cross-linking immunoprecipitation sequencing is a method that combines UV cross-linking with immunoprecipitation and finally sequencing to analyse protein interactions with RNA. Interaction sites can be identified by mapping the reads back to the transcriptome.

(2) Paper that uses this technique:

Potts, Anastasia H., et al. "Global Role of the Bacterial Post-Transcriptional Regulator CsrA Revealed by Integrated Transcriptomics." *Nature Communications*, vol. 8, no. 1, 2017, doi:10.1038/s41467-017-01613-1.

(3) Summary of paper question, and how the technique was used to answer it:

These scientists assessed the contribution of the CsrA protein to overall gene expression in *E. coli*. UV crosslinking immunoprecipitation and sequencing was used to identify RNAs that

interact directly with CsrA in vivo, providing hundreds of RNAs that interact with CsrA. In their paper, they describe the use of many integrated transcriptomics approaches to study post-transcriptional regulation. They came to the conclusion that their results, including that of the CLIP-seq, highlight the important role of post-transcriptional regulation in general, and more specifically, the importance of the Csr system in regulating bacterial gene expression.