GENOMICS INFORMATICS PROTEOMICS METABOLOMICS

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G A A T C T G A T C C T T G A A C T A C C T T C C A A G G T G

Agilent ChIP-on-chip Genome-Wide Location Analysis

Where the proteome meets the genome

Agilent's ChIP-on-chip (chromatin immunoprecipitation-on-chip) is a powerful technology driving the next generation of microarray applications. This emerging platform goes beyond gene expression to explore gene regulation activity and allows determination of the precise location on the DNA sequence where a protein is bound. It pairs chromatin immunoprecipitation (ChIP) and Agilent's 60-mer DNA microarrays to provide comprehensive and genome-wide insight into transcriptional regulation and epigenomic events. ChIP-on-chip enhances the understanding of vital processes like cell proliferation, cell fate determination, oncogenesis, inflammation, apoptosis, and gene silencing.

Transcription is a complex process that requires multiple interactions and orchestrated binding of numerous components. Thus, traditional gene expression analyses provide only partial glimpses into gene regulation, whereas ChIP-on-chip reveals a high-resolution map of transcriptional activities, including activation and repression.

By partnering our unparalleled microarray expertise with exclusively patented technology from the Whitehead Institute, Agilent enables you to obtain insightful and robust profiles of DNA-protein binding and promoter occupancy across entire genomes. Acquire more complete and reliable information for networks of *in vivo* gene expression in specific cells, tissues, or entire organisms in only a matter of weeks—information that previously would have taken years to collect.

Agilent Technologies

Features and Benefits

Versatile User-Defined Microarray Formats

The inherent flexibility of our microarrays allows users to define content—up to ~244K customizable features per microarray—for any tiling density, genomic region, and organism of interest. Whatever design or configuration you want—we can make and quickly deliver.

Superior Microarray Performance

Proprietary microarray technology using optimized 60-mer oligonucleotide probes and a convenient two-color labeling system delivers higher sensitivity, accuracy, and greater reproducibility than one-color systems. These unique features allow sensitive measurements of weak- and infrequent-binding events, as well as direct comparisons of samples on the same microarray.

The Agilent Probe Advantage

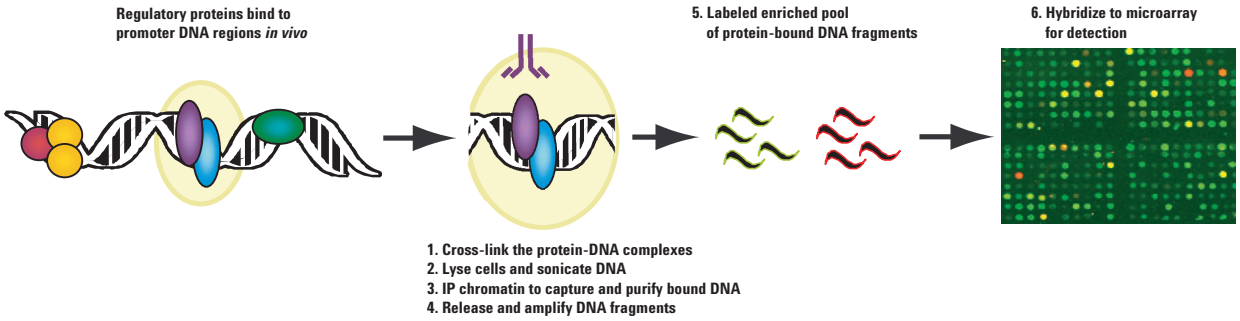
Unlike other companies, Agilent provides optimized and validated probe design that delivers the high signal-to-noise ratios that are essential for the success of ChIP-on-chip experiments. We carefully design our probes using stringent criteria* and do not sacrifice clean, robust data for higher-density microarrays.

- 60-mer oligonucleotide probes provide robust hybridization, critical for the sensitivity and specificity that ChIP-on-chip demands.
- Average probe spacing parameters are specifically optimized for the ChIP-on-chip method as compared to other microarray applications.
- Repeat regions are masked to significantly reduce nonspecific noise.

* Probes are designed with criteria including optimal Tm, unique sequence, and self-structure prediction.

Agilent SurePrint Technology

SurePrint features a flexible, industrial-scale inkjet printing process that synthesizes oligonucleotide probes *in situ* onto 1" x 3" slides. The maskless process allows quick iteration of microarray designs required in today's continuously evolving genomic research environment. This provides researchers with easy access to high-quality arrays loaded with relevant and rich content.



1. Rapid fixation of cells chemically cross-links DNA binding proteins to their genomic targets *in vivo*.
2. Cell lysis releases the DNA-protein complexes, and sonication fragments the DNA.
3. Immunoprecipitation (IP) purifies the protein-DNA fragments, with specificity dictated by antibody choice.
4. Hydrolysis reverses the cross-links within the released DNA fragments, and amplification is performed.
5. Label enriched pool with Cy dye.
6. Hybridization of DNA onto Agilent-validated or user-customized microarrays featuring 60-mer probes.

The ChIP-on-chip process enables the observation of a few to 50-fold the representation of protein-bound (occupied) DNA promoter fragments of interest in the final DNA pool.

ChIP-on-chip Genome-Wide Location Analysis

Comprehensive Genome-Wide and Focused Coverage

Each Agilent ChIP-on-chip microarray features a total of ~244,000 60-mer probes. Probes are spaced every ~100-300 bp across regions of interest in both coding and noncoding DNA sequences. Available formats include whole genome and focused promoter regions. In addition, we provide multi-array formats on single slides to facilitate workflow. Focused microarray designs include human and mouse promoter sets. Whole genome designs include human, mouse, Drosophila, *C. elegans*, and Arabidopsis microarray sets.

Access to Probe Sequence and Annotation

Complete access to public databases, probe sequences, and annotation files for convenient extraction of biological information.

Specifications

Microarrays per slide	One to four
Slide format	1" x 3" (25 mm x 75 mm)
Total number of features	243,504
Agilent internal quality control probes	5000
Feature size	65 µm
Oligonucleotide probe length	60-mer
Probe orientation	Varies
Average resolution	1 kb
Average probe spacing	~100-300 bp
Sequence source	Yeast: UCSC sacCer1 (October 2003 build)ENCODE - Human: ENCODE Human Promoter formats: UCSC hg17/NCBI release 35 (May 2004 build) Mouse Promoter formats: UCSC mm7/ NCBI release 35 (August 2005 build)
Starting sample input	0.5×10^7-1×10^8 cells
Type of labeling	Random priming using Klenow with Cy3 and Cy5 nucleotides
DNA required for labeling	2 µg
DNA required for hybridization	5 µg per channel
Hybridization volume	500 µL
Overall assay time	3 days from DNA amplification 6 days from chromatin immunoprecipitation
Storage for microarrays	Room temperature

Applications of Agilent ChIP-on-chip

- Uncover and validate gene regulation and regulatory networks by comprehensive determination of promoter occupancy.
- Identify and characterize molecular events associated with processes for transcription, DNA replication and repair, as well as with chromatin modifications and DNA methylation.
- Elucidate modes of action and potential therapeutic activities of compounds and target genes by mapping gene regulatory networks relevant to disease and pathophysiological states.
- Validate and augment existing gene expression data with authentic binding events.
- Identify, assess, and monitor biomarkers responsive to protein-DNA binding events to serve as bioassays or toxicant signatures for toxicogenomics.
- Uncover and profile off-target events as well as validate primary and secondary effects in screening of candidate compounds, siRNAs, therapeutics, etc.

ChIP-on-chip Product Formats

Product name	No. of arrays in set	Probe coverage and description	# of probes
Yeast (*S. cerevisiae*)	1	~12MB,	244,000
Yeast (*S. cerevisiae*) 4-array pack	4 per slide	Each slide contains four whole genome (~12MB) arrays.	41,776 per array
Human Promoter Set	2	-5.5 KB to +2.5 KB (relative to tss†) (~148MB)	~25 per gene
Mouse Promoter Set	2	-5.5 KB to +2.5 KB (relative to tss†) (~135MB)	~25 per gene
Human CpG Islands	1	covers 27,800 CpG Islands	~240,000
Drosophila Whole Genome	2	~475,000 probes covering 133MB	~240,000 per array
Arabidopsis Whole Genome	2	~475,000 probes covering 120MB	~240,000 per array
C. elegans Whole Genome	2	~475,000 probes covering 100MB	~240,000 per array
Human ENCODE	1	30MB, Chromosomes 1-22	~153,000

Our custom offerings include both Agilent-designed and customer-designed microarrays.

Custom Microarrays

- Fly (*Drosophila melanogaster*)
- Human
- Mouse
- Plant (*Arabidopsis thaliana*)
- Rat
- Worm (*Caenorhabditis elegans*)
- Yeast (*Schizosaccharomyces pombe, Saccharomyces cerevisiae*)
- Zebrafish (*Danio rerio*)

eArray is a Secure, Web-Based Application Tool That Enables You to:

- Create custom designs in a secure, online environment
- Search and access catalog microarray probe sequences and up-to-date annotations
- Submit array designs directly to Agilent manufacturing
- Download annotation files for use in image and data analysis applications
- Work collaboratively and share designs with colleagues

For more information, go to http://earray.chem.agilent.com/earray

Agilent ChIP Analytics Software

ChIP Analytics software combines annotated, algorithmic array data processing with easily manipulated text file output and high-speed statistical modeling functions. It provides an intuitive user interface for visually exploring and analyzing data from Agilent ChIP-on-chip microarrays. ChIP Analytics accepts data outputs from Agilent Feature Extraction software or Molecular Devices GenePix software and analyzes the significance of protein binding events without extensive processing and manipulation.

It performs user-configurable heuristics for binding event identification based on p-values and adjacent probes, inter- and intraarray intensity normalization, and error modeling. Powerful algorithms employ neighborhood probe voting with multiple probes to generate reliable data with greater true-binding events and fewer false positives.

ChIP Analytics Features at a Glance

- Simultaneous analysis of sets of microarrays with annotations
- Output reports containing probe, sequence, and gene detail
- Option of UCSC track report in .BED format for convenient viewing within the UCSC Genome Browser
- QC report and peak detection visualization for convenient, at-a-glance visualization of key experimental elements
- Support for replicates

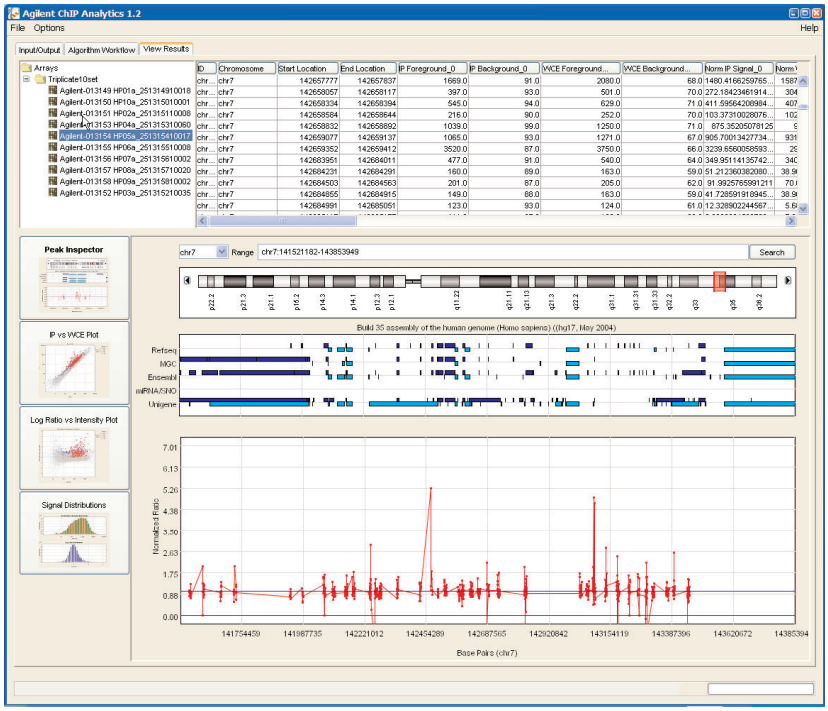
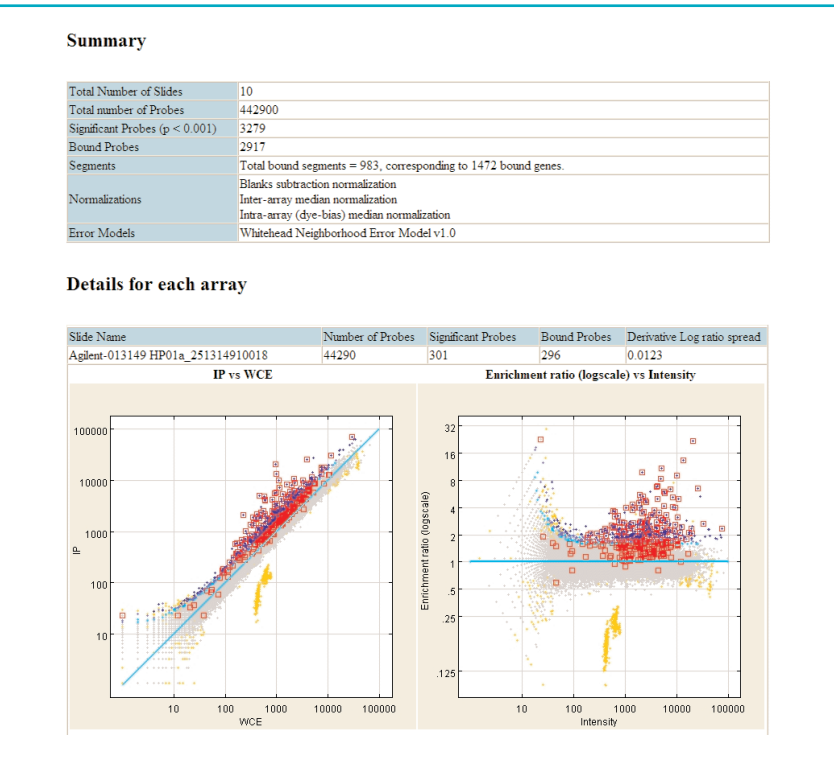


Figure 1. The peak detection visualization feature allows inspection of the regions of interest and a display of nearby genomic features.



Summary

Total Number of Slides	10
Total number of Probes	442900
Significant Probes ($p < 0.001$)	3279
Bound Probes	2917
Segments	Total bound segments = 983, corresponding to 1472 bound genes.
Normalizations	Blanks subtraction normalization Inter-array median normalization Intra-array (dye-bias) median normalization
Error Models	Whitehead Neighborhood Error Model v1.0

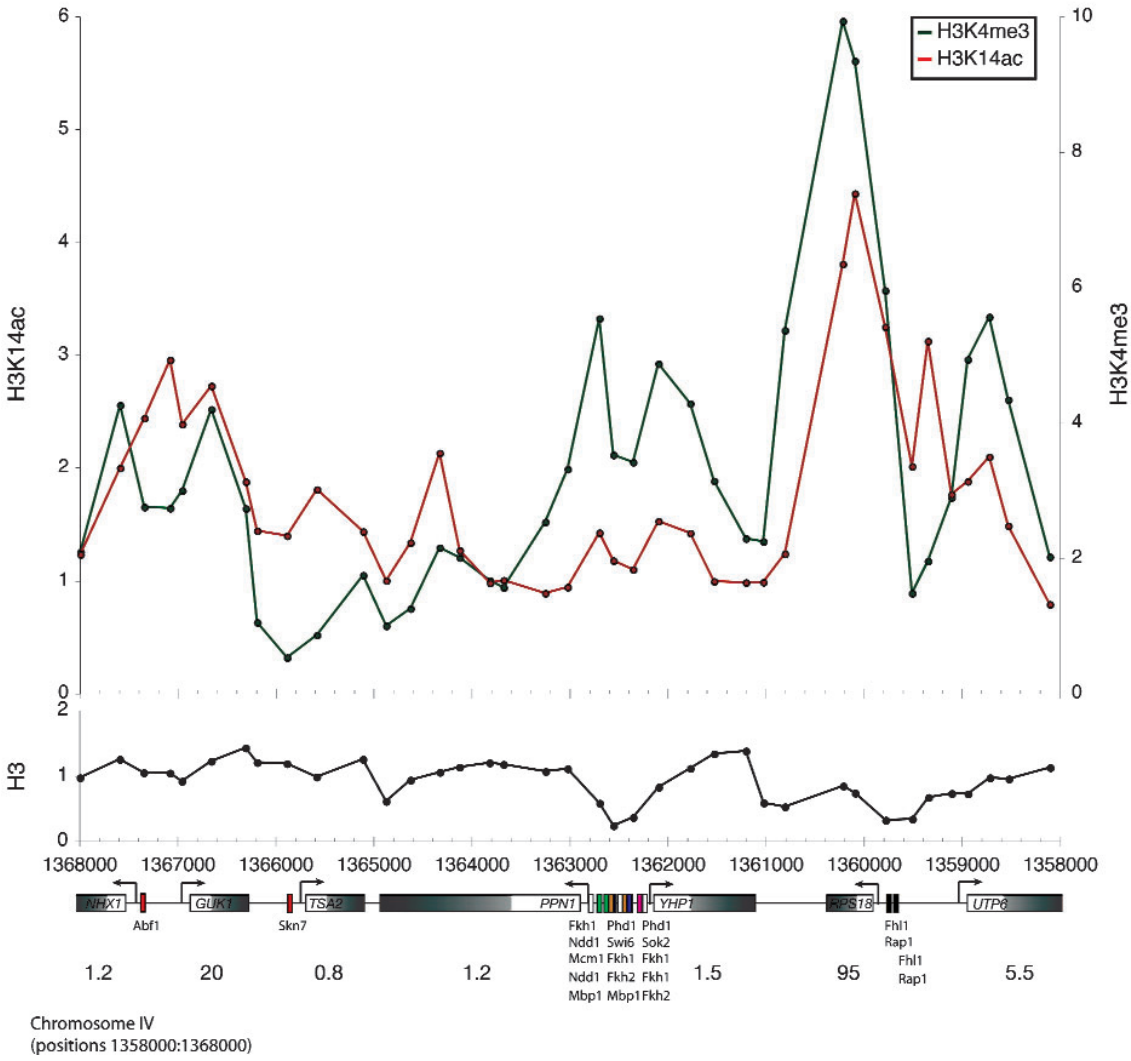
Details for each array

Slide Name	Number of Probes	Significant Probes	Bound Probes	Derivative Log ratio spread
Agilent-013149 HP01a_251314910018	44290	301	296	0.0123

Figure 2. QC Report

Generate Comprehensive and High-Resolution Maps of Genome Regulation

Nucleosome modifications, known to be associated with transcriptional regulation, were profiled across the yeast genome using ChIP-on-chip to produce high-resolution genome maps of histone acetylation and methylation (Pokholok et al., 2005). Agilent's yeast DNA microarray, featuring 44,290 total probes, was used to generate systematic binding maps for histones. These results have provided substantial insight into nucleosome occupancy and histone modification.

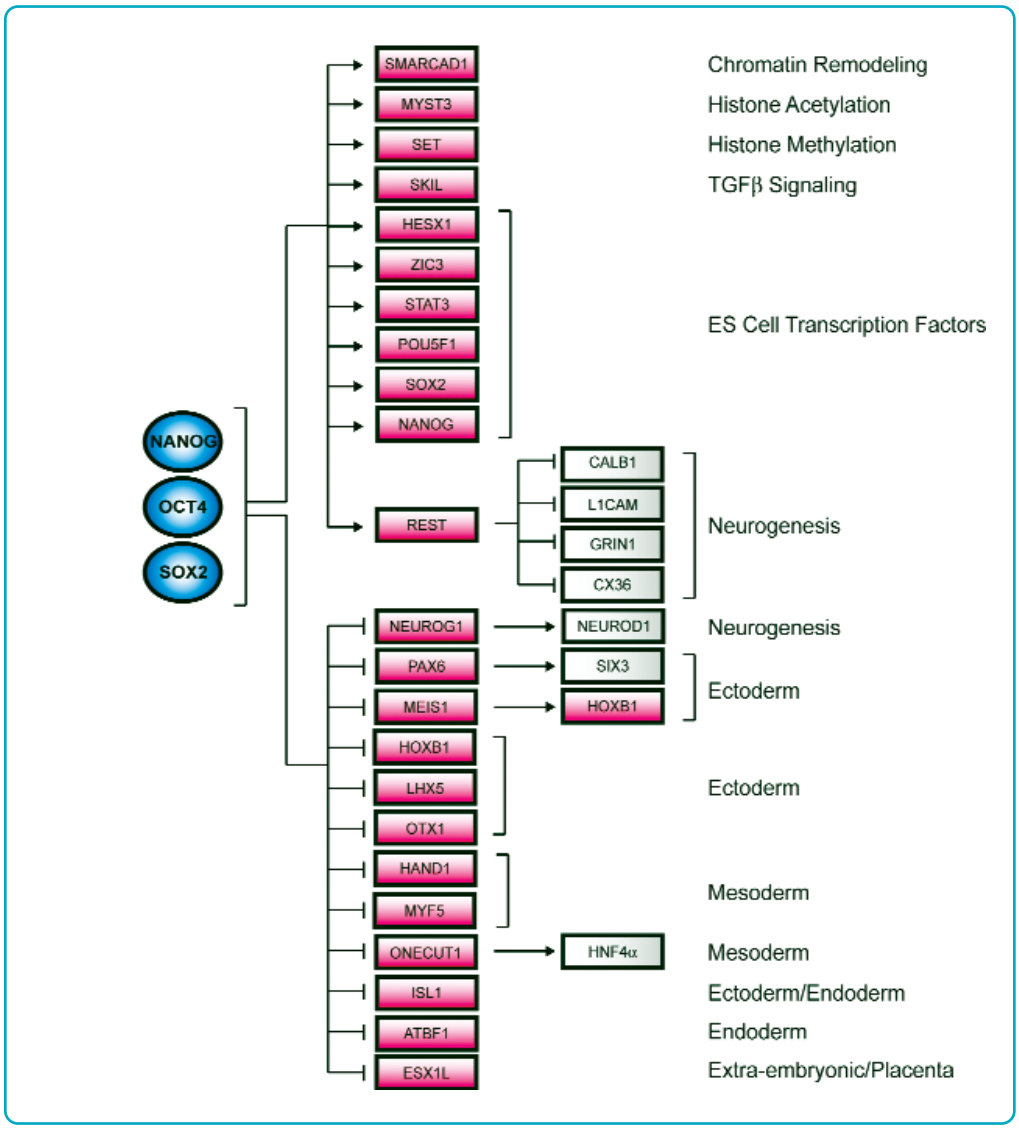


High-Resolution Genome-Wide Map of Nucleosome States

Location analysis map for a region of Chromosome IV. Genomic positions of probe regions are arrayed along the x axis, with the ratio of enrichment for probes along the y axis. ORFs are depicted as gray rectangles, and arrows indicate the direction of transcription. Conserved binding sites for transcriptional regulators are depicted as colored boxes. Numbers beneath genes represent transcriptional activity (mRNA/h). Enrichment values are shown from trimethylated histone H3 lysine 4, acetylated histone H3 lysine 14, and histone H3. Peaks within the profile indicate occupied sites.

Gain Relevant Insight into System-Specific Networks

Understanding transcriptional networks in embryonic stem (ES) cells is crucial for understanding the multiple interactions that control cell development. Agilent ChIP-on-chip technology was used to identify the genomic targets of three transcription factors essential for human ES development - OCT4, SOX2, and NANOG (Boyer et al, 2005). The transcriptional regulatory network model generated is an extensive map that identifies both active and inactive genes responsible for controlling ES cell identity and fate determination. This invaluable insight has provided a greater clarification of current models and hypotheses, and importantly, allows identification of potential therapeutic targets and screening of drug candidates for a wide field of diseases and tissue types.



Core Transcriptional Regulatory Network in Human Embryonic Stem Cells

Using multiple expression datasets, a model for the core transcriptional regulatory network was constructed by identifying and integrating target genes that encode transcription factors and chromatin regulators. Regulators are represented by blue ovals, gene promoters by red rectangles, and putative downstream target genes by gray rectangles.

References

Learn more about the biological utility and performance of Agilent's ChIP-on-chip platform:

Publications using the Agilent ChIP-on-chip platform

Polycomb complexes repress developmental regulators in murine embryonic stem cells. Laurie A. Boyer et al., (2006), *Nature* **441(18),** 349-53.

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Agilent Reprint Publication 5989-3841EN

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Agilent Reprint Publication 5989-3720EN

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Direct Isolation and Identification of Promoters in the Human Genome. Tae Hoon Kim et. al., (2005), *Genome Res.* **15(6),** 830-9.

Control of Pancreas and Liver Gene Expression by HNF Transcription Factors. Duncan T. Odom et al., (2004), *Science* **303(5662),** 1378-1381.

About Agilent's Integrated Biology Solutions

Agilent Technologies is a leading supplier of life science research systems that enable scientists to understand complex biological processes, determine disease mechanisms, and speed drug discovery. Engineered for sensitivity, reproducibility, and workflow productivity, Agilent's integrated biology solutions include instrumentation, microfluidics, software, microarrays, consumables, and services for genomics, proteomics, and metabolomics applications.

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