Automating epigenetic assays: Generating high-resolution ChIP-Seq profiles using the SX-8G IP-Star Platform

Ignacio Mazon[1], Manuela Saathoff[1], Henriette O'Geen[2], Lorigail Echipare[2], Sushma Iyengar[2], Sharon Squazzo[1], Peggy Farnham[2], Dominique Poncelet[1]

[1]Diagenode, Avenue l'Hopital, GIGA center, 3rd Floor, Liège, Belgium. [2]University of California Davis Genome Center, 4617 GBSF, 451 Health Sciences Dr., Davis, CA 95616, USA

Introduction

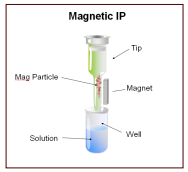
Chromatin immunoprecipitation followed by next generation sequencing (ChIP-Seq) is a powerful technique for generating genome-wide profiles of epigenetic modifications and transcription factor binding sites. "Manual ChIP assays" use labor-intensive, complex protocols that can introduce experimental variability and limit day-to-day and lab-to-lab reproducibility. The IP-Star Automation System reduces variability and eliminates the time consuming steps of manually performed ChIP assays.

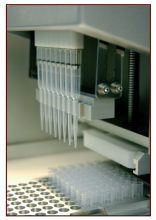
The following data demonstrates that the IP-Star is a flexible platform that is amenable to a broad range of ChIP assays in a variety of cell types and experimental sample conditions. The IP-Star can automate ChIP assays for both abundant histone modifications as well as site-specific transcription factors. We developed custom IP-Star protocols by varying standard ChIP assay parameters including incubation duration, incubation temperature, number of washes, buffer types and stringency, all within the automated program.

In addition, we performed automated ChIP, followed by next generation sequencing (Illumina Genome Analyzer) to map the epigenomic profiles of six major histone modifications in human primary T cells.

Our results demonstrate the reproducibility and versatility of the IP-Star Automation System in generating high-resolution ChIP-Seq profiles – a critical requirement for reliable epigenomic studies.

Overview of the SX-8G IP-Star Automation System





Reproducibility: Standardized system provides consistency between runs and users, increasing reproducibility of ChIP results

Speed and accuracy: Full walk-away automation saves time and eliminates tedious manual steps, minimizing cross-contamination, sample-carryover, and false positive rates

Sample efficiency: Minimal sample loss and smaller reaction volumes allows for less input material

High-throughput capability: Run up to 16 samples in parallel

Flexible and open system: SX-8G IP-Star® software provides an easy-to-use user interface, with open programming to easily adapt any magnetic bead based manual protocol

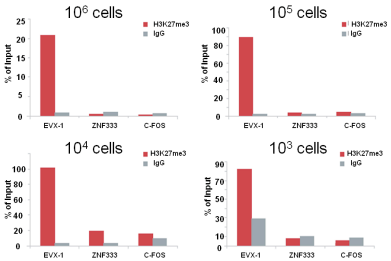
Simple assay set up: Simple reaction set up in minutes

Compatibility: The system is compatible with popular downstream applications including QPCR, microarrays, and high-throughput sequencing

IP-Star Decreases Variability versus Manual ChIP

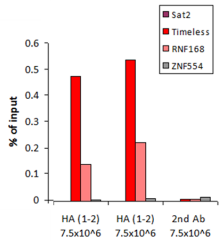
ChIP assays using an antibody specific for H3K9me3 and non-specific IgG were performed **(A)** manually by three different operators in duplicate and **(B)** four independent runs using the IP-Star. QPCR was performed using SAT2 as a positive primer set for H3K9me3. Results are shown as a percentage of input. Standard deviation was calculated for both the H3K9me3 and IgG ChIP samples.

Auto-ChIP with Varying Amounts of Input



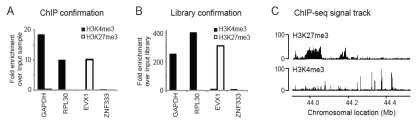
Auto-ChIP assays using an antibody specific for H3K27me3 were performed using chromatin isolated from 10^6 to 10^3 HeLa cells. QPCR was performed with EVX1 as a positive primer set. ZNF333 and c-FOS primer sets were used as negative controls for H3K27me3. Red bars represent H3K27me3 and gray bars represent IgG control sample as a percentage of input.

Auto-ChIP of an Epitope Tagged Transcription Factor



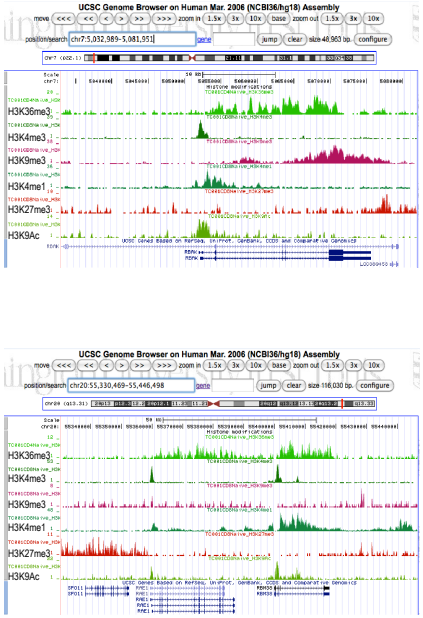
Auto-ChIP assays using an antibody specific for the HA epitope tag were performed using chromatin isolated from MCF7 cells expressing HA-E2F1. QPCR was performed with Timeless and RNF168 as positive primer sets for HA-E2F1. ZNF554 and SAT2 primer sets were used as negative controls. Results are shown as a percentage on input.

Auto-ChIP-Seq in Ntera2 Cells



Auto-ChIP assays using antibodies specific for H3K4me3 and H3K27me3 were performed using 1 µg of chromatin from Ntera2 cells and the IP-Star. Illumina Genome Analyzer Libraries were prepared and QPCR confirmed specific enrichment over input in **(A)** the Auto-ChIP samples and **(B)** the Auto-ChIP-Seq libraries. Black bars represent H3K4me3 enrichment and white bars represent H3K27me3 enrichment. GAPDH and RPL30 are positive control primer sets for H3K4me3 and negative control primer sets for H3K27me3. EVX1 was used as a positive primer set for H3K27me3 and a negative primer set for H3K4me3.The ZNF333 primer set was used as a negative control for both histone marks. **(C)** Auto-ChIP-Seq binding patterns for H3K4me3 and H3K27me3 are shown for a region on chromosome 17 encompassing the HOXB gene cluster. The peak height is plotted along the y-axis; chromosomal coordinates (hg18 coordinates) are shown on the x-axis. Samples were sequenced at the DNA Technologies Core at UC Davis.

Auto-ChIP-Seq of Six Histone Marks in Primary T Cells



Auto-ChIP assays were performed on primary human CD4 and CD8 T cells isolated by negative magnetic separation of peripheral blood mononuclear cells. Each Auto-ChIP sample was performed using Diagenode's Auto-Histone kit reagents and contained 1 ug of input chromatin. Illumina Genome Analyzer libraries were prepared and the samples were sequenced at the DNA Technologies Core at UC Davis.

Antibody	Vendor	Cat #	Amount
H3K36me3	Abcam	ab9050	2 ug
H3K4me3	CST	9751S	5 ul
H3K9me3	Diagenode	pab-069-050	2 ug
H3K4me1	Diagenode	pab-037-050	2 ug
H3K27me3	CST	9733S	15 ul
H3K9Ac	CST	9649S	10 ul

Summary of IP-Star Automated ChIP Assays

- IP-Star increases reproducibility versus manually performed ChIPs.
- Auto-ChIP can be performed on a wide range of input chromatin amount.
- IP-Star can efficiently ChIP histone marks and site-specific transcription factors.
- Auto-ChIP samples are compatible with Illumina Genome Analyzer sequencing.