SOLiD™ ChIP-Seq Kit for ChIP & ChIP-Seq with Low Cell Number Samples

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ABSTRACT

We developed a SOLiD™ ChIP-seq kit, which offers an optimized ChIP workflow and an efficient SOLiD ChIP-seq library construction from relatively low cell number samples. ChIP procedure is usually laborious, time consuming, and typically requires large starting cell numbers. We use MAGnify™ Chromatin Immunoprecipitation System, which is suitable for fast enrichment of chromatin complexes and DNA recovery, for ChIP workflow in the kit. It is able to use lower starting cell numbers (10,000-300,000 cells) for ChIP thus preserving precious samples such as primary cells, stem cells, biopsies. This new approach also allows faster throughput to investigate different chromatin and transcription time-course events as well as enable antibody screening to determine ChIP compatibility. In addition, we develop a sensitive ChIP-seq library construction procedure which enables users to construct a complex library using as low as 1 ng ChIP DNA. Combining with the SOLiD system's ultra high sequencing throughput, SOLiD ChIP-seq kit offers a highly sensitive, hypothesis-neutral approach to accurately characterize protein-DNA interactions at genome-wide scale.

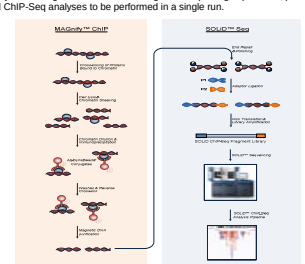
INTRODUCTION

Next-generation sequencing technology is quickly being applied in a myriad of creative ways to answer genome-wide questions such as specific protein-DNA associations in combination with distinct patterns of histone modifications. Currently, the chromatin immunoprecipitation assay (ChIP) is the most powerful method utilized to selectively enrich for DNA sequences bound by a particular protein in living cells and ChIP-sequencing (ChIP-Seq) experiments are predictive to be a key driver for the uptake of next-generation sequencing tools in basic and clinical research.

Here we describe the SOLiD™ based ChIP-Sequencing (ChIP-Seq) combines chromatin immunoprecipitation (ChIP) with massively parallel DNA sequencing, offering a highly sensitive, hypothesis-neutral approach to accurately characterize the protein-DNA interactions of an entire genome. Using the SOLiD™ ChIP-Seq kit, we are characterizing transcriptionally permissive histone H3 modifications in breast cancer cell lines utilizing the SOLiD platform.

The SOLiD™ ChIP-Seq system introduces the capacity to generate ChIP profiles from low starting amount. These features are important and relevant where sensitive and accurate screening of large numbers of samples is necessary, such as in clinical research and analysis. The SOLiD™ System provides a level of throughput and sensitivity that cannot be achieved with current hybridization technologies or other next-generation sequencing platforms. The SOLiD™ System's ability to generate over 400 million sequence tags, to provide a large dynamic range, and to take advantage of multiplexing capabilities, permits multiple hypothesis-neutral ChIP-Seq analyses to be performed in a single run.

A.



B.

Workflow Step	MAGnify™ ChIP Timeline	Conventional ChIP Timeline
Pre-clearing	N/A	1 -2 hrs
Antibody/Chromatin Incubation	2 hrs	Overnight
Bead Pulldown	1 hr	2 hrs
Washes	30 min (2 buffers)	1 – 3 hours (4 buffers)
Reverse Crosslinking	1.5 hrs	Overnight
Proteinase K Digestion		2 hrs
DNA Elution from Beads		15 – 30 min
DNA Purification		2 hrs - Overnight
Average Time	5 hrs	36 – 48 hrs

A. Overview of SOLiD™ ChIP-Seq System B. Comparison of workflow improvements vs. conventional protocols. Overall, The SOLiD™ Chromatin Immunoprecipitation System provides a streamlined, optimized assay for the enrichment of chromatin complexes and DNA recovery using magnetic bead capture technology.

RESULTS

Figure 1. ChIP enrichment from 10,000 cells

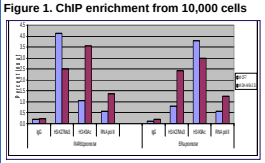


Figure 1. *ChIP from 10,000 cells.* Sheared chromatin from MCF7 or MDA-MB231 cells was prepared from 1 million cells in 50ul lysis buffer and dilute to 10,000 cells per ChIP according to the MAGnify™ Protocol. 1ug of IgG, H3-K27Me3, H3-K9Ac antibodies or 3ul of RNA pol II antibody were used per ChIP experiment. A fraction (10%) of the sonicated chromatin was set aside as input control. Optimized qPCR primers were used to amplify to different loci: RARb1 promoter or ERa promoter. Percent input was calculated by $100 \times 2^{(Ct_{adjusted\ input} - Ct_{enriched})}$. Input DNA Ct was adjusted from 10% to 100% equivalent by subtracting 3.33 Cts.

Figure 3. EGF-induced RNA polymerase II ChIP

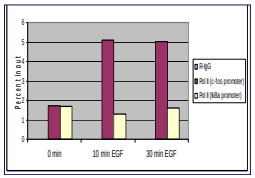


Figure 3. *EGF-induced RNA polymerase II.* 293Gt cells were serum-starved for 12 hours followed by EGF treatment (50ng/ml) for 10 minutes. Cells were counted and diluted to 50,000 cells per ChIP. 3ml of RNA polymerase II antibody or IgG antibody as a negative control was used. A fraction (1%) of the sonicated chromatin was set aside as input control. Optimized qPCR primers were used to amplify the c-fos promoter and data presented as percent input.

Figure 5. Optimized chromatin shearing for ChIP-ready libraries

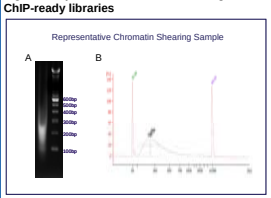


Figure 5. *Optimal Chromatin Shearing for ChIP-Seq ready libraries.* All samples are from MCF7 cells crosslinked with 1% formaldehyde for 10 minutes and processed per SOLiD ChIP-Seq protocol. A) MCF7 crosslinked chromatin (1 million cells in 50ml Lysis Buffer) loaded 10ul on gel. Ran aliquot of samples on 2% E-Gel with 100bp DNA ladder. B) Representative sample ran on Bioanalyzer 2100.

Figure 2. Primer efficiency with SOLiD™ ChIP-Seq kit

			A.E
MCF7	Slope	-3.8476	1.819
	Y-inter	26.96	
	R2	0.9958	
MDA	Slope	-3.0931	2.105
	Y-inter	20.539	
	R2	0.9697	
PC3	Slope	-3.4072	1.932
	Y-inter	28.063	
	R2	0.998	
Hela	Slope	-3.681	1.869
	Y-inter	27.777	
	R2	0.9954	

Figure 2. *Optimal Primer Efficiency with SOLiD™ ChIP-Seq Kit.* 100ng to 10pg gDNA was analyzed on AB StepOne instrument with ChIP-validated RARb1 primer pair (top). Table above displays primer efficiency of ChIP Input DNA recovered from 4 different cell lines using the SOLiD ChIP-Seq Kit and RARb1 promoter primer pair.

Figure 4. Histone H3 methylation profile in stem cells

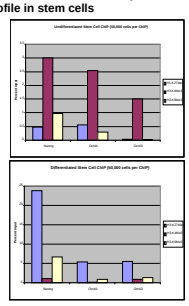


Figure 4. *Histone H3 Methylation profile in Stem Cells.* Sheared chromatin from RSC-1 embryonic stem cells or differentiated into neuronal cells was prepared from 3 million cells in 150ul lysis buffer and diluted to 50,000 cells per ChIP. 2ug of antibody (IgG, H3-K27Me3, H3-K4Me3, or H3-K9Me3) were used per ChIP experiment. A fraction (10%) of the sonicated chromatin was set aside as input control. Optimized qPCR primers were used to amplify the Nanog promoter or two distinct regions of the Oct4 promoter. ESC were characterized as Oct4+ and SSEA4+ but SSEA1- and the NSC were grown in neural induction medium including bFGF and were Nestin+ but beta-tubulin negative

Figure 6. ChIP-qPCR control

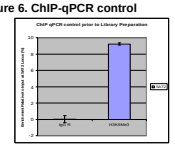
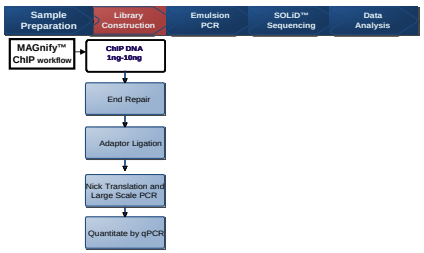


Figure 6. *ChIP-qPCR control prior to library preparation.* Sheared chromatin was diluted to 100,000 cells per ChIP and enrichment of H3-K9Me3 at the SAT2 locus was analyzed by qPCR. H3-K9Me3 is a maker for heterochromatin and a useful control for ChIP workflow.

Figure 7. SOLiD™ ChIP-Seq kit workflow

A.

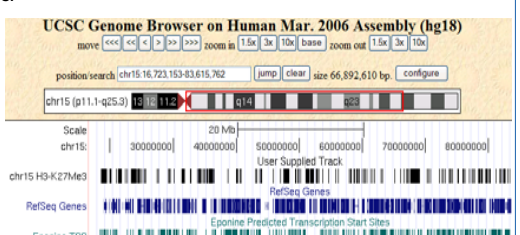


A. *Flow Chart of ChIP-Seq workflow.* Sample preparation begins with ChIP DNA prepared according to MAGnify™ protocol using 100,000 cells per ChIP using the H3-K27Me3 antibody. Subsequently the ChIP DNA enters library construction where libraries were made between 1-10ng, including input, and sequenced on a SOLiD (V3) platform in quad chambers. Templated bead generation for each library was performed according to SOLiD System 3.0 User Guide standard protocols. Each sample was deposited on one quadrant of the slide at a target bead density of 60,000–70,000 beads per panel. We generated two slides and processed them similarly to assess the reproducibility of the system. High-throughput sequencing was performed using the SOLiD System and analysis of 50-bp reads was carried out. The short sequence reads from the SOLiD System are mapped against genomic sequences using the SOLiD System alignment tools available through the Applied Biosystems software development community (http://info.appliedbiosystems.com/solidsoftwarecommunity) or third-party tools compatible with SOLiD sequencing data.

B.

Library	Total # of Beads	# of Matching Beads	Throughput (Mbp)
Chromatin input 5ng	82,777,712	29,972,094	1,498.60
Chromatin input 10ng	82,126,147	28,470,110	1,423.51
ChIP 1IP 1ng H3-K27Me3	86,001,613	26,033,473	1,301.67
ChIP 5IPs 5ng H3-K27Me3	72,908,620	21,824,844	1,091.24
		Total Throughput:	5,315.02

C.



B. *Table representing Mapping Statistics.* Fraction of uniquely mapped reads (0-5 mismatches) for SOLiD™ ChIP-Seq and control input libraries. Uniquely mapped reads represent those reads mapping to a single, unique location with 5 mismatches in color space. Data can then be visualized with a tool such as the University of California, Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway) to identify and quantify the regions of sequence that bind to the protein of interest. **C.** *Illustrated in the UCSC Genome* Browser is H3-K27Me3 ChIP enrichment over control input on chromosome 15 from 1ng input libraries.

Figure 8. SOLiD ChIP-Seq of Histone H3-K27Me3 enrichment

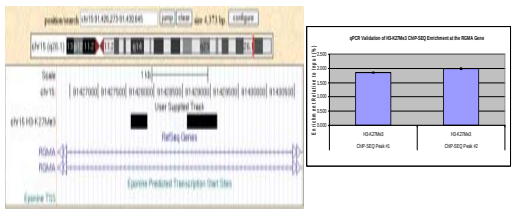


Figure 8. A. *UCSC Genome Browser view of a chromosomal locus near the RGMA gene.* SOLiD™ reads from MAGnify™ H3-K27Me3 ChIP were mapped and normalized against the input control. B. *Validation of the ChIP-Seq peak enrichment of H3-K27Me3 at the RGMA gene was observed by ChIP-qPCR.* Consistent with the ChIP-Seq results, we observed enrichment of H3-K27Me3 by qPCR. SYBR qPCR primers targeting the two peak regions observed in Figure 7A were designed and used for detection.

CONCLUSIONS

The SOLiD™ ChIP-Seq kit provides a faster and more reproducible solution for ChIP-seq and also includes all reagents needed to perform ChIP for your antibody of interest.

- **Combined with the ChIP-Seq workflow optimized on the SOLiD™ system, fingerprints of protein-DNA interactions can be systematically revealed and compared**
- ***Reduce cell number*** **per ChIP experiment (10,000-300,000 cells recommended) and reduce protocol time**
- **Improve reproducibility due to *optimized magnetic DNA purification* (avoid columns and phenol/chloroform steps)**
- **Easily increase throughput with small volumes, magnetic protocol and magnet compatible with multi-channel pipetting**
- **Increase confidence in results due to optimized and reproducible components, and antibodies qualified in chromatin immunoprecipitation**
- **Reduce experimental error with novel Dynabeads® Protein A/G Mix - worry less about antibody compatibility and non-specificity**
- ***Broader applications*****: Easier to screen more antibodies; Evaluate kinetics of recruitment and/or precious samples; compatible with massive parallel sequencing**

In summary, SOLiD™ ChIP-Seq kit is a seamless, easy workflow that provides robust enrichment and deep coverage for cost-effective sequencing to generate genome wide ChIP profiles from low amounts of starting material. The SOLiD™ System's industry-leading throughput and data analysis capabilities are well suited for analyzing DNA samples precipitated using the SOLiD™ ChIP-Seq System. Optimization of through SOLiD™ ChIP-Seq system provides a powerful, streamlined, and complete pipeline for studying protein-DNA interaction with unprecedented scope and depth.

REFERENCE

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ORDERING INFORMATION

Product †	Catalog #
SOLiD™ ChIP-Seq Kit	4449640
SOLiD™ ChIP-Seq Kit with ChIP Magnet*	4449638
DynaMag™-PCR*	49-2025

*The magnet (DynaMag™-PCR Magnet) needs to be ordered only once.

† For Research Use Only, not intended for any animal or human therapeutic or diagnostic use.