SOLiD™ ChIP-Seq kit for ChIP & ChIP-sequencing from low number of cell & tissue 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. We also developed a simple and effective sample preparation method for a variety of tissues, such as brain, heart, kidney and liver. This new approach 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 and multiplex sampling capacity with barcodes, SOLiD ChIP-Seq kit offers a highly sensitive, hypothesis-neutral approach to accurately characterize protein-DNA interactions at genome-wide scale.

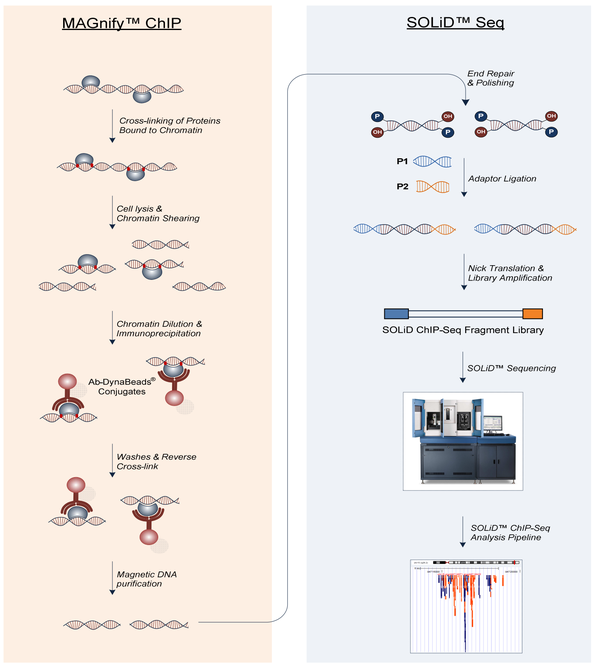
INTRODUCTION

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. ChIP-sequencing (ChIP-Seq) using next-generation sequencing methods is predictive to be a key driver for the uptake of next-generation sequencing tools in biological research.

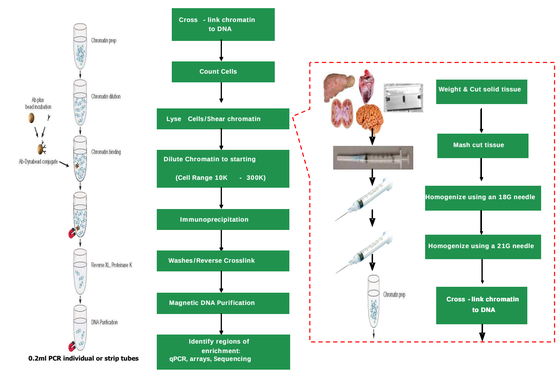
Here we describe the SOLiD™ ChIP-Seq kit, which includes MAGnify™ ChIP kit with SOLiD™ ChIP-Seq library construction reagents. Combining with SOLiD massively parallel sequencing, it offers a highly sensitive, hypothesis-neutral approach to accurately characterize the protein-DNA interactions of an entire genome. MAGnify™ ChIP system offers a faster, optimized ChIP workflow enabling lower starting cell numbers (10,000-300,000 cells) thus preserving precious samples such as primary cells and stem cells. We also developed a simple but efficient sample preparation method for a variety of tissues, such as brain, heart, kidney and liver. ChIP profiles from these tissues were generated with less than 1mg tissue per ChIP. In addition, we developed a sensitive SOLiD ChIP-Seq library construction procedure to prepare complex libraries using as low as 1 ng MAGnify™ ChIP DNA. These features are important and relevant where sensitive and accurate screening of large numbers of samples is necessary, such as in biological research and analysis.

Using the SOLiD™ ChIP-Seq kit and SOLiD™ system, we are characterizing transcriptionally permissive histone H3 modifications in breast cancer cell lines. 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™ 4 System generates up to 700 million sequence tags per flowcell and provides multiplexing capability with sample barcoding. It permits multiple hypothesis-neutral ChIP-Seq analyses be performed in a single run.

A. Overview of SOLiD™ ChIP-Seq Workflow



B. Illustration of MAGnifyTM ChIP workflow for cells and solid tissue samples



A new solid mammalian tissue collection procedure is shown in the inserted box.

C. Comparison of SOLiD™ ChIP-Seq ChIP workflow with conventional protocols.

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

Overall, The SOLiD™ Chromatin Immunoprecipitation System (MAGnify™ ChIP) provides a streamlined, optimized assay for the enrichment of chromatin complexes and DNA recovery using magnetic bead capture technology.

D. Advantages of the new tissue collection protocol

Workflow Step	MAGnify™ ChIP Tissue Collection	Home - brew Tissue Collection
Material Required per ChIP	< 1mg	~ 30mg
Collect and Weigh Tissue	5 min	5 min
Homogenize	5 min $60 1ml Syringes $10 18G Needles $10 21G Needles	20 - 40 min $2,300 Mechanical Homogenizer $180 Consumables
Crosslink and Glycine Treat	15 min	20 min
Washes	30 min	20 min
Lysis	5 min 1 Buffer All Sterile & Disposable Less anxiety	60 min $130 Glass 2 Buffers Homogenizer Risk for Cross - contamination
Time	1 hrs	>2 hours
Customer Cost per ChIP	<1¢ + No Equipment Cost	~50¢ + $2,430 Equipment Cost

RESULTS

Figure 1. ChIP enrichment from 10,000 cells

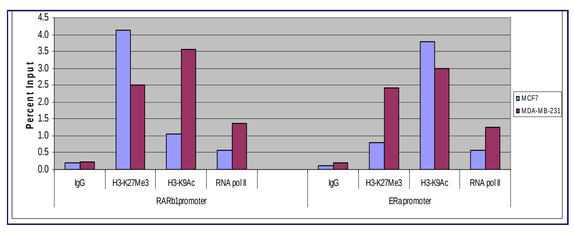


Figure 1. *ChIP from 10,000 cells.*

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. 1µg of IgG, H3-K27Me3, H3-K9Ac antibodies or 3µl 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 x $2^{(Ct_{adjusted\ input} - Ct_{enriched})}$. Input DNA Ct was adjusted from 10% to 100% equivalent by subtracting 3.33 Cts.

Figure 2. EGF-induced RNA polymerase II ChIP

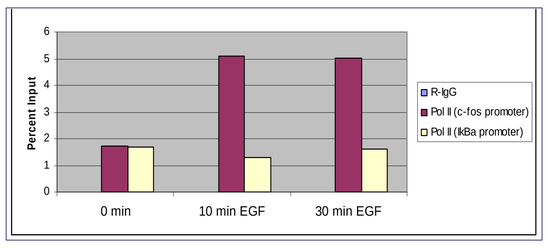
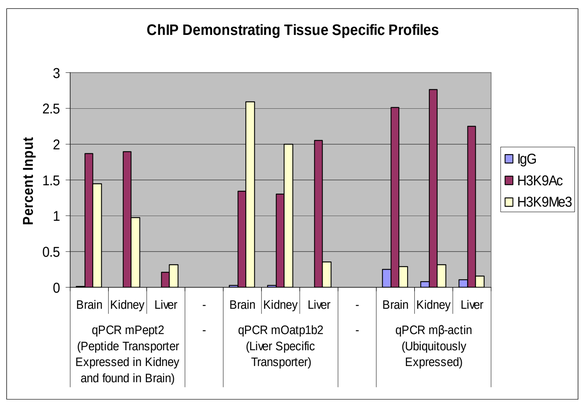


Figure 2. *EGF-induced RNA polymerase II*.
293Gt cells were serum-starved for 12 hours followed by EGF treatment (50ng/ml) for 10 minutes. Cells were counted and diluted to 50,000 cells per ChIP. 3ml of RNA polymerase II antibody or IgG antibody as a negative control was used. A fraction (1%) of the 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 3. Tissue ChIP profiles with pharmacologically relevant, organ specific targets.

A.



B.

	Pept2	Oatp1b2	β-actin	
Brain	High	Low	High	mRNA expression*
Kidney	High	Low	High	mRNA expression*
Liver	Low	High	High	mRNA expression*

* (Imai et al, *Mol Pharmacol* 75:568–576, 2009)

Figure 3. *Tissue ChIP Profiles with Pharmacologically Relevant, Organ Specific Targets.*
A. Sheared chromatin from mouse brain, kidney or liver was prepared from 150mg in 450ul lysis buffer and dilute to 0.83mg per ChIP according to the MAGnify™ Solid Tissue ChIP Protocol. All chromatin was sheared per standard SOLiD™ ChIP-Seq shearing. 1µg negative control IgG, H3-K9Me3 or H3-K9Ac antibody was used per ChIP experiment. A fraction (10%) of the sonicated chromatin was set aside as input control. Optimized qPCR primers were used to amplify mouse Pept2, Oatp1b2 and β-actin loci with data presented as percent input.
B. Table summarizing published mRNA expression data from total RNA isolated from mouse liver, kidney and cerebrum. The laboratory performed reverse transcription then PCR with primers specific for mouse Pept2, Oat1b2 and β-actin cDNA. The published agarose gel band intensity is presented here as either high or low to indicate relative mRNA expression levels in the different tissues examined.

Figure 4. Tissue chromatin shearing for ChIP-Seq ready library preparation

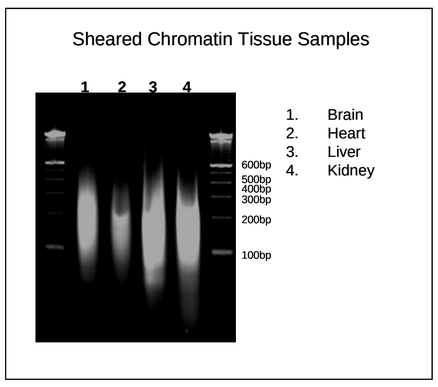


Figure 4. *Tissue Chromatin Shearing for ChIP-Seq ready library preparation.*
All samples are collected using the MAGnify™ solid tissue protocol, crosslinked with 1% formaldehyde for 10 minutes, and processed per standard SOLiD™ ChIP-Seq shearing. 0.5µl mouse brain, heart, liver and kidney crosslinked chromatin (50mg per 150µl Lysis Buffer) was ran on a 2% EX-Gel with 100bp DNA ladder.

Figure 5. SOLiD™ ChIP-Seq mammalian tissue libraries

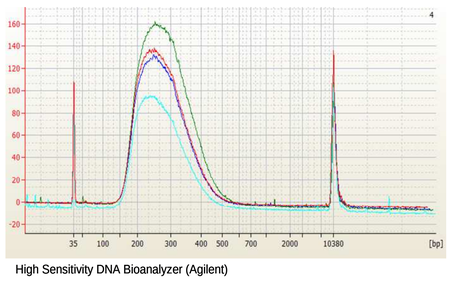


Figure 5. *SOLiD™ ChIP-Seq libraries from Mammalian Tissue.*
1ng ChIP input DNA, measured by Qubit Quant-iT dsDNA High Sensitivity Assay Kit, was processed per standard SOLiD™ ChIP-Seq library protocol. Libraries from mouse brain, heart, kidney and liver ChIP DNA were amplified 15 cycles and run on a High Sensitivity Bioanalzyer chip.

Figure 6. SOLiD™ ChIP-Seq results

System	Library (ChIP DNA amount)	Total # of Beads	# of Uniquely Mapped	% of Uniquely Mapped
SOLiD 3	Chromatin input (5 ng)	82,777,712	29,972,094	36.2%
	ChIP IP H3-K27Me3 (1 ng)	86,001,613	26,033,473	30.3%
SOLiD 4	Chromatin input (5 ng)	109,954,420	66,212,482	60.2%
	ChIP IP H3-K27Me3 (1 ng)	107,174,859	49,322,428	46.0%

Figure 6. *Significant improvement on SOLiD 4 system*.
ChIP DNA prepared according to MAGnify™ protocol using 100,000 cells per ChIP with H3-K27Me3 antibody.1ng ChIP DNA was used to construct ChIP-Seq library.Templated bead generation for each library was performed according to SOLiD System User Guide standard protocols. Each sample was deposited on one quadrant of the slide. We generated sequencing data from both SOLiD 3 and SOLiD 4 system and processed them similarly to assess the improvement of the system. 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/).

Figure 7. Validation of SOLiD ChIP-Seq of Histone H3-K27Me3 enrichment

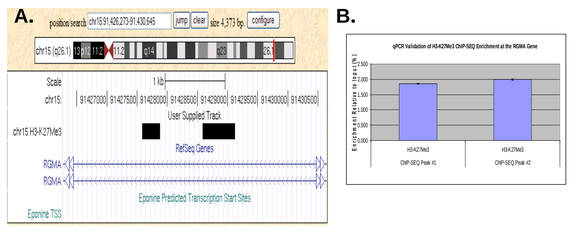


Figure 7. A. *UCSC Genome Browser view of a chromosomal locus near the RGMA gene*. SOLiD™ reads from MAGnify™ H3-K27Me3 ChIP were mapped and normalized against the input control. B. *Validation of the ChIP-Seq peak enrichment of H3-K27Me3 at the RGMA gene was observed by ChIP-qPCR*. Consistent with the 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.

- **Reduce cell number per ChIP experiment (10,000-300,000 cells recommended) and reduce protocol time**
- ***Tissue ChIP-Seq*: a simple and cost effective chromatin preparation solution for < 1 mg solid mammalian tissues compatible with SOLiD™ ChIP-Seq kit**
- **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 with novel Dynabeads® Protein A/G Mix and ChIP-qualified antibodies**
- **With SOLiD's highest sequencing density in all NGS platforms and barcoding capacity, fingerprints of genome-wide protein-DNA interactions can be cost-effectively revealed on the SOLiD™ system**

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PRODUCT INFORMATION

Product †	Catalog No.
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.

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