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Rapid Chromatin Preparation from Solid Mammalian Tissues for Low Cell ChIP Assays

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ABSTRACT

In this presentation we demonstrate a novel, rapid, and low input per ChIP tissue collection protocol for researchers utilizing solid mammalian tissue to study protein-DNA interaction via the **MAGnify™ ChIP System**. We also illustrate **SOLiD™ ChIP-Seq assay** feasibility with solid mammalian tissue by utilizing MAGnify™ ChIP DNA to generate fragment libraries for future use in massively parallel DNA sequencing. This simple, user-friendly protocol provides multiple benefits over the standard homebrew method for ChIP solid tissue collection. Thousands of dollars in up-front cost of specialized tissue equipment are eliminated and the once standard 50 cent assay is now reduced to less than 1 cent per reaction. The new protocol cuts processing time in half. It reduces reported homebrew amount of tissue from approximately 30mg to less than 1mg per ChIP reaction when paired with the powerful MAGnify™ ChIP system. We reduce anxiety associated with cross-contamination by implementing only sterile, disposable items. This sterility is especially a concern now that ChIP DNA feeds directly into **a** highly sensitive, hypothesis-neutral approach to accurately characterize protein-DNA interactions at genome-wide scale via the SOLiD™ ChIP-Seq Kit.

INTRODUCTION

To date, the most widely used and powerful method to identify regions of the genome associated with specific proteins is the Chromatin Immunoprecipitation (ChIP) assay. Determining how proteins interact with DNA to regulate gene expression is essential to fully understand many biological processes, cancers and disease states. In a ChIP assay, protein-DNA complexes are crosslinked, immunoprecipitated and then purified. This material is then ready for downstream analyses using technologies/platforms/methods such as qPCR, genome-wide analyses using promoter-tiling arrays, or massively parallel sequencing. We developed the MAGnify™ ChIP system: 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. In addition, we developed a sensitive SOLiD™ library construction procedure to produce complex libraries using as low as 1 ng MAGnify™ ChIP DNA. Through SOLiD™ ChIP-Seq, we characterized transcriptionally permissive histone H3 modifications in breast cancer cell lines utilizing the SOLiD™ platform.

From this, an exciting new customer-driven challenge emerged. Researchers wished to attempt low input MAGnify™ ChIP assays on precious solid mammalian tissue samples but required a tissue collection protocol radically different from the standard homebrew method. Tissue ChIP profiles with pharmacologically relevant, organ specific targets were generated with less than 1mg tissue per ChIP. SOLiD™ ChIP-Seq libraries were produced from 1ng MAGnify™ tissue ChIP input DNA.

MATERIALS AND METHODS

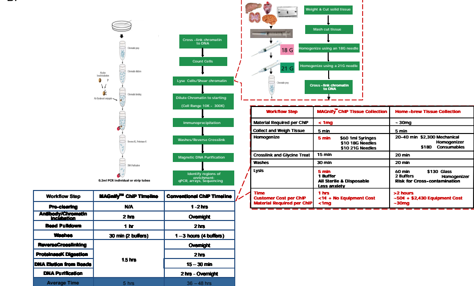
Three week old mouse brain, liver, heart and kidney were collected from male Ncr nude mice, weighed, and then minced. The plastic sheath was retained on a needle and used as a sterile, disposable mortar and pestle when paired with a 50ml conical tube. A specific, optimized gradient of gauge needles effectively homogenized these tissues and fed directly into the MAGnify™ ChIP and SOLiD™ ChIP-Seq Kits.

A.

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. Currently, an optimized cell culture collection protocol is provided. Here, a novel solid mammalian tissue collection protocol compatible with the MAGnify™ workflow is showcased.

B.

MAGnify™ ChIP Workflow with Cell Culture Collection



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	38 – 48 hrs

Supplementary MAGnify™ ChIP Solid Mammalian Tissue Collection

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 2 Buffers $130 Glass Homogenizer Risk for Cross-contamination
Time Customer Cost per ChIP Material Required per ChIP	1 hrs <1¢ + No Equipment Cost <1mg	>2 hours ~50¢ + $2,430 Equipment Cost ~30mg

RESULTS

Figure 1. Cancer Cell Line ChIP-qPCR Control

Figure 1. *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 2. SOLiD™ ChIP-Seq Workflow

Figure 2. *Flow Chart of ChIP-Seq workflow for Cancer Cell Line Run.* 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.

Figure 3. SOLiD™ ChIP-Seq of Histone H3-K27Me3 Cancer Cell Line Enrichment at RGMA

3A. 3B.

Figure 3. 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.

Figure 4. SOLiD™ ChIP-Seq of Histone H3-K27Me3 Cancer Cell Line Enrichment

Figure 4. *Illustrated in the UCSC Genome* Browser is H3-K27Me3 ChIP enrichment over control input on chromosome 15 from 1ng input libraries.

Figure 5. SOLiD™ ChIP-Seq Cancer Cell Line Mapping Statistics

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

Figure 5. *Significant improvement on SOLiD™ 4 system.*
ChIP DNA prepared according to MAGnify™ protocol using 100,000 cells per ChIP for SOLiD™ 3 or 50,000 cells per ChIP for 4.0. 5ng Input ChIP DNA and one H3K27Me3 IP was used to construct ChIP-Seq libraries. 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 reproducibility 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 6. Histone H3 Methylation ChIP from < 1mg Solid Mammalian Tissue

Figure 6. *Histone H3 Methylation ChIP from < 1mg Solid Mammalian Tissue (~50,000 cells).* Sheared chromatin from mouse brain, heart, kidney and 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 the mouse promoter GAPDH promoter. Percent input was calculated by 100 x 2^(Ct adjusted input — Ct enriched). Input DNA Ct was adjusted from 1% to 100% equivalent by subtracting 6.64 Cts.

Figure 7. Polymerase II ChIP from < 1mg Solid Mammalian Tissue

Figure 7. *Polymerase II ChIP from < 1mg Solid Mammalian Tissue (~50,000 cells).* Sheared chromatin from mouse brain, heart, kidney and 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 of IgG negative control antibody or 3µl 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 the mouse βMaj-globin region and data presented as percent input.

Figure 8. Tissue ChIP Profiles with Pharmacologically Relevant, Organ Specific Targets.

8A.

8B.

	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 8. *Tissue ChIP Profiles with Pharmacologically Relevant, Organ Specific Targets.* (8A) 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. (8B) 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 9. Tissue Chromatin Shearing for ChIP-ready Libraries

Figure 9. *Tissue Chromatin Shearing for ChIP-ready libraries.* 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 10. SOLiD™ ChIP-Seq Mammalian Tissue Libraries

Figure 10. *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 Bioanalyzer chip.

CONCLUSIONS

We currently offer the MAGnify™ and SOLiD™ ChIP-Seq kits, providing a faster and more reproducible solution for lower input cell ChIP and ChIP-seq.

Reduce cell number **per ChIP (10,000-300,000 cells recommended) and reduce protocol time**

Combined with the ChIP-Seq workflow optimized on the SOLiD™ system, fingerprints of protein-DNA interactions can be systematically revealed and compared

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

We now offer a novel, rapid chromatin preparation solution for solid mammalian tissues compatible with low cell MAGnify™ ChIP assays and able to produce libraries utilizing the SOLiD™ ChIP-Seq kit.

Eliminates greater than $2,400 in up-front tissue equipment cost

A 50 ¢ assay is reduced to less than 1 ¢ per tissue ChIP preparation

Reduces tissue processing time by greater than half

Reduces homebrew amount of tissue from approximately 30mg to less than 1mg (50,000 cells) per ChIP reaction when paired with the powerful MAGnify™ ChIP system

Lessens anxiety by implementing only sterile, disposable items. Cross-contamination is especially a concern now researchers require ChIP DNA to feed massively parallel DNA sequencing

SOLiD™ ChIP-Seq libraries from 1ng ChIP tissue input DNA for future analysis on the SOLiD™ platform

In summary, this method reduces time, cost, and sample per assay, while providing an innovative, user-friendly approach to processing mammalian tissue for MAGnify™ ChIP. This novel protocol capitalizes on existing MAGnify™ ChIP and SOLiD™ ChIP-Seq system advantages to produce pharmacologically relevant ChIP-qPCR profiles and ChIP-sequencing libraries from low amounts of starting mammalian tissue.
By utilizing less tissue per assay, studying protein-DNA interaction through ChIP and ChIP-seq is opened to researchers examining abundant as well as precious cancer, disease, animal-model, and biopsy solid mammalian tissues.

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ORDERING INFORMATION

Product †	Catalog #
SOLiD™ ChIP-Seq Kit	4449640
SOLiD™ ChIP-Seq Kit with ChIP Magnet*	4449638
DynaMag™-PCR*	492025
MAGnify ™ ChIP Kit	492024

*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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