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

Kyusung Park¹, Loni Pickle¹, Vasiliki Anest¹, Zhoutao Chen¹, George Marnellos¹, Dorothy Markowitz¹, Dan Krissinger¹, Nisha Mulakken², Darryl Leon², Rob Bennett¹, Life Technologies, Carlsbad¹, CA, 92008, Foster City², California 94404 For questions contact: Loni.Pickle@lifetech.com



ABSTRACT

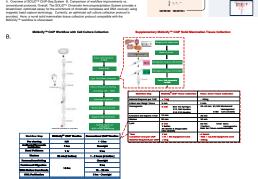
In this presentation we demonstrate a novel, rapid, and low input per ChIP tissue collection protocol for researchers utilizing solid mammalian tissue to study protein-DNA interaction via the MAGnify™ ChIP System. We also illustrate SOLID™ ChIP-Seq assay feasibility with solid mammalian tissue by utilizing 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-Sea 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 aPCR, 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 MAGnifv™ tissue ChIP input DNA.

Deck # 5 *** Dong MATERIALS AND METHODS 20005 20005 Three week old mouse brain liver heart and kidney were collected from male Nor nude mice weighed, and then minced. The plastic sheath was ----retained on a needle and used as a sterile, 1922-02-14 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 SOI iDTN ChIP-Sea Kite A. Overview of SOLIDE CNR-Sen System. B. Commerison of worldlow improvements of



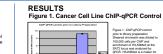




Figure 2. SOLiD™ ChIP-Seq Workflow



Figure 9. Tissue Chromatin Shearing for ChIP-ready Libraries



ChIP-wardy Branies. All samples are collected using the MAGnity™ solid tissue protocol, crosslinked with 1% formaldehyde for 10 minutes, and processed per standard SOLID™ ChilP-Seq shearing. 0.5µ mouse brain, heart, liver and fothery crosslinked chromatin (50mp per 150µ Lysis Buffer) was ran on a 2% EX-Gel with 150%. with 100bo DNA ladder

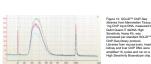


Figure 10. SOLiD™ ChIP-Seg Mammalian

High Sensitivity DNA Biganahger (Aplient) ing Kidney ChilP Input DNA SOLID ChilP-Seq Libr ing Heart ChiP Input DNA SOLID ChiP-Sep Library

Tissue Libraries

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

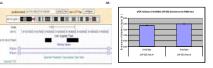


Figure 3. A. UCSC Genome Browser view of a chromosomal locus near the RGMA gene. SOLID** reads from MAGnifv** H3-K27Me3 ChIP were apped and normalized against the input control. B. Veideleion of the ChIP-See peak enrichment of H3-R27Ms3 at the RGMA game was observed by CNP-gPCR: Consistent with the CNP-Seq results, we observed enrichment of HS-K27Me3 by gPCR. SYBR gPCR primers targeting the two peak regions observed in Figure 7A were designed and used for detection.

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

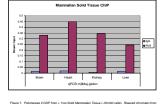
System	L ibrary (ChiPDNA Amount) 1	otal # of Beads	# of Unique Mapped	% of Uniquely Mapped
SOLiD™3	Chromatin input (5ng)	82,777,712	29,972,094	36.2%
	ChiPIP H3-K27Me3 (1ng)	86,001,613	26,033,473	30.3%
SOLiD™4	Chromatin input (5ng)	109,954,420	66,212,482	60.2%
	ChiPIP H3-K27Me3 (1ngl	107,174,859	49,322,428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

Figure 3. Spriftcet improvement on SCLOP* 4 system.

COVER 1994, present description (SLOP 4.4 system).

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



mouse brain, heart, kichey and liver was prepared from 150mg in 450ul lysis buffer and distate to 0.85mg per ChIP according to the MAGRINIy⁵⁰ Solid Tissue ChIP Protocol. All chromatin was shared per standard SOLID⁵⁰ ChIP-Seq sharings, tug of log registree cortrol artibody or 3µl RNA Pel II artibody were used per

ChIP experiment. A fraction (10%) of the sonicated chromatin was set aside as input control. Optimized

nPCR primare were used to emplify the mouse RMajuriphin region and data presented as negretal innu-

Low

Figure 6. Histone H3 Methylation ChIP from < 1mg Solid Mammalian Tissue

UCSC Genome Browser on Human Mar. 2006 Assembly (hg18)

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Figure 4. Illustrated in the UCSC Genome Browser is H3-K27Me3 ChIP

enrichment over control input on chromosome 15 from 1ng input libraries.

Cancer Cell Line Enrichment

Templated bead generation for each library was performed according to SQLiD^{TI}

System 3.0 User Guide standard protocols. Each sample was deposited on one quadrant of the slide at a baroat bead density of 60.000–70.000 beads per panel

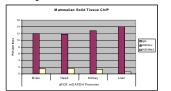
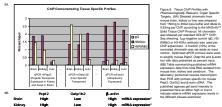


Figure 6. Historie H3 Methylation ChiP from < Ting Solid Mammellan Tissue (-50,000 cells). Sheared chromatin from mouse brein, hear Richey and liver was prepared from 150mg in 450ut lysis buffer and dilute to 0.63mg per ChIP according to the MAGnityTM Solid Tissue ChIP
Protocol. All chromatin was aheared per standard SOLIDTM ChIP-Sec ahearing. Up negative control log. H3-K9MG or H3-K9A antibody was used per ChIP experiment. A fraction (10%) of the sonicialed chromatin was set aside as injust control. Optimized dPCR primers were used to amplify the mouse GAPOR promoter. Percent input was calculated by 100 x 2*(C1 adjusted input — C1 ernichad). Input DNA C1 was adjusted from 1% to 100% equilibrier by substraction 6.64 Cbs.

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



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-

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-Seg 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-Seg 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-seg 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-Sea Kit 4449640 SOLiD™ ChIP-Seq Kit with ChIP Magnet* 4449638 DynaManTM-PCR* 492025 MAGnify ™ ChIP Kit 492024

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

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