Systematic Optimization of Parameters Involved in Preparation of Chromatin and Chromatin Immunoprecipitation (ChIP) Workflow

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ABSTRACT

The ChIP protocol is a fundamental tool for discoveries in the area of epigenomics and gaining insight into epigenetic phenomenon. Despite the fact that many vendors of life science research tools offer products to support ChIP, very little innovation has occurred around the ChIP method itself to make this process more reproducible and reliable. Issues surrounding antibody specificity and performance are critical to high resolution genomic mapping of chromatin events. In addition, reliable high-throughput options are not readily available for ChIP despite the ability to automate parts of the workflow. Chromatin preparation, for example, remains a laborious process and the field lacks accepted criteria to make determinations on the quality of chromatin suitable for ChIP.

We have engaged in an iterative process of examining parameters that influence chromatin fragmentation on a variety of devices and under various buffer systems, and have developed a protocol for optimizing this process for researchers new to the area of chromatin biology. As a leading antibody supplier, we have produced tools that allow researchers to evaluate the specificity of histone modifications antibodies in their own laboratories utilizing a simple dot blot western blotting approach. In addition, we have developed a new high-throughput and low-cell capable protocol that simplifies the process, reduces variability and significantly improves signal-to-noise ratio. This protocol is particularly suited for new users/beginners, and can utilize chromatin derived from cells and tissue. We present data comparing the effect of different sonication protocols on ChIP, analysis of user-to-user variation, as well as methods to optimize and control for factors that introduce variability in ChIP.

MATERIALS and METHODS

Various antibodies (both monoclonals and polyclonals) were selected from the Merck Millipore catalog (see below for ChIP validated versions) and evaluated by Dot Blot screening similar to methods described in Egelhofer, et.al. NSMB 2011. Dot blots were prepared on Immobilon® PVDF FL membranes using 100 ng and 10 ng of each peptide (Absurance, Cat. #16-668). Chromatin was prepared using a variety of sonicators and the EpiPure™ Chromatin Preparation Kit (Cat. #17-10082). Chromatin was analyzed using agarose gel electrophoresis and Agilent Bioanalyzer chips. ChIP reactions were performed with a variety of protocols, including the Magna ChIP™ HiSens (Cat. #17-10460), HT96 (#17-10077) and other ChIP kits for comparison.

Merck Millipore ChIPab+ Kits --every lot tested in functional ChIP assays

17-10246 ChIPAb+™ H2A.Bbd	17-10099 ChIPAb+™ H4K8Ac
17-10048 ChIPAb+™ H2AZ	17-10121 ChIPAb+™ H4K12Ac
17-10054 ChIPAb+™ H2B	17-10101 ChIPAb+™ H4K16Ac
17-650 ChIPAb+™ H2BK119ub	17-651 ChIPAb+™ H4K20me1
17-10245 ChIPAb+™ H3.3	17-671 ChIPAb+™ H4K20me3
17-10046 ChIPAb+™ H3	17-600 ChIPAb+™ CREB
17-10254 ChIPAb+™ H3	17-10044 ChIPAb+™ CTCF
17-615 ChIPAb+™ H3Ac	17-10061 ChIPAb+™ E2F-1
17-10141 ChIPAb+™ H3T3P	17-10062 ChIPAb+™ E2F-3
17-675 ChIPAb+™ H3K4un	17-10034 ChIPAb+™ EED
17-10050 ChIPAb+™ H3K4Ac	17-663 ChIPAb+™ EED
17-676 ChIPAb+™ H3K4me1	17-603 ChIPAb+™ ER α
17-677 ChIPAb+™ H3K4me2	17-662 ChIPAb+™ EZH2
17-614 ChIPAb+™ H3K4me3	17-10267 ChIPAb+™ FOXA1
17-678 ChIPAb+™ H3K4me3	17-10258 ChIPAb+™ FOXA2
17-658 ChIPAb+™ H3K9Ac	17-608 ChIPAb+™ HDAC1
17-609 ChIPAb+™ H3K9Ac	17-10199 ChIPAb+™ HDAC1
17-10241 ChIPAb+™ H3K9/K18Ac	17-10237 ChIPAb+™ HDAC2
17-680 ChIPAb+™ H3K9me1	17-10238 ChIPAb+™ HDAC3
17-648 ChIPAb+™ H3K9me2	17-646 ChIPAb+™ HP1γ
17-681 ChIPAb+™ H3K9me2	17-10264 ChIPAb+™ JHDM1B
17-625 ChIPAb+™ H3K9me3	17-10262 ChIPAb+™ JMJD1C
17-10242 ChIPAb+™ H3K9me3	17-10263 ChIPAb+™ JMJD6
17-685 ChIPAb+™ H3S10P	17-604 ChIPAb+™ LEF1
17-10139 ChIPAb+™ H3T11P	17-10252 ChIPAb+™ LSF
17-10051 ChIPAb+™ H3K14Ac	17-10260 ChIPAb+™ N-CoR
17-10111 ChIPAb+™ H3K18Ac	17-10060 ChIPAb+™ NFκB p65 (RelA)
17-10112 ChIPAb+™ H3K23Ac	17-613 ChIPAb+™ p53
17-683 ChIPAb+™ H3K27Ac	17-10131 ChIPAb+™ pCREB (Ser133)
17-643 ChIPAb+™ H3K27me1	17-641 ChIPAb+™ REST
17-10108 ChIPAb+™ H3K27me2	17-10456 ChIPAb+™ REST
17-622 ChIPAb+™ H3K27me3	17-672 ChIPAb+™ RNA Pol II 8WG16
17-10269 ChIPAb+™ H3S28P	17-620 ChIPAb+™ RNA Pol II CTD4H8
17-10498 ChIPAb+™ H3K36me1	17-10057 ChIPAb+™ SMRT
17-10032 ChIPAb+™ H3K36me3	17-656 ChIPAb+™ SOX-2
17-10125 ChIPAb+™ H3K79me2	17-10256 ChIPAb+™ SOX-2
17-10130 ChIPAb+™ H3K79me3	17-601 ChIPAb+™ Sp1
17-10047 ChIPAb+™ H4	17-661 ChIPAb+™ Suz12
17-630 ChIPAb+™ H4Ac	17-10098 ChIPAb+™ TBP
17-10250 ChIPAb+™ H4R3me2s	17-10109 ChIPAb+™ TCF-4
17-10045 ChIPAb+™ H4K5Ac	

Parameters Influencing ChIP Success

Selection of Highest Quality Antibodies

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Preparation & Optimization of Chromatin

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Optimization of Signal to Noise in IP

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Use of Spike In Antibody Controls for Data Normalization & Analysis

RESULTS

Validation of Histone Antibodies using AbSurance™ Dot Blot Macroarrays (Merck Millipore, Cat. #16-667, 16-665, 16-668)

Absurance Histone H3 Array (Cat. # 16-667)

	1	2	3	4	5	6	7	8	9	10	11	12	
A	H3 1-19 unmod	H3 1-19 R2me1	H3 1-19 R2me2a	H3 1-19 R2me2s	H3 1-19 T3P	H3 1-19 K4ac	H3 1-19 K4me1	H3 1-19 K4me2	H3 1-19 K4me3	H3 1-19 R8me1	H3 1-19 R8me2a	H3 1-19 R8me2s	100 ng
B	H3 1-19 unmod	H3 1-19 R2me1	H3 1-19 R2me2a	H3 1-19 R2me2s	H3 1-19 T3P	H3 1-19 K4ac	H3 1-19 K4me1	H3 1-19 K4me2	H3 1-19 K4me3	H3 1-19 R8me1	H3 1-19 R8me2a	H3 1-19 R8me2s	10 ng
C	H3 1-19 K9ac	H3 1-19 K9me1	H3 1-19 K9me2	H3 1-19 K9me3	H3 1-19 S10P	H3 1-19 T11P	H3 7-26 unmod	H3 7-26 K14ac	H3 7-26 R17me1	H3 7-26 R17me2a	H3 7-26 R17me2s	H3 7-26 K18ac	100 ng
D	H3 1-19 K9ac	H3 1-19 K9me1	H3 1-19 K9me2	H3 1-19 K9me3	H3 1-19 S10P	H3 1-19 T11P	H3 7-26 unmod	H3 7-26 K14ac	H3 7-26 R17me1	H3 7-26 R17me2a	H3 7-26 R17me2s	H3 7-26 K18ac	10 ng
E	H3 16-34 unmod	H3 16-34 K23ac	H3 16-34 K27ac	H3 16-34 K27me1	H3 16-34 K27me2	H3 16-34 K27me3	H3 16-34 R26me1	H3 16-34 R26me2a	H3 16-34 R26me2s	H3 16-34 S28P	H3 26-44 unmod	H3 26-44 K36ac	100 ng
F	H3 16-34 unmod	H3 16-34 K23ac	H3 16-34 K27ac	H3 16-34 K27me1	H3 16-34 K27me2	H3 16-34 K27me3	H3 16-34 R26me1	H3 16-34 R26me2a	H3 16-34 R26me2s	H3 16-34 S28P	H3 26-44 unmod	H3 26-44 K36ac	10 ng
G	H3 26-44 K36me1	H3 26-44 K36me2	H3 26-44 K36me3	H3 26-44 Y41P	H3 47-65 unmod	H3 47-65 K56ac	H3 71-89 unmod	H3 71-89 K79me1	H3 71-89 K79me2	H3 71-89 K79me3	Rat IgG 10 ng	Sheep IgG 10 ng	100 ng
H	H3 26-44 K36me1	H3 26-44 K36me2	H3 26-44 K36me3	H3 26-44 Y41P	H3 47-65 unmod	H3 47-65 K56ac	H3 71-89 unmod	H3 71-89 K79me1	H3 71-89 K79me2	H3 71-89 K79me3	Mouse IgG 10 ng	Rabbit IgG 10 ng	10 ng

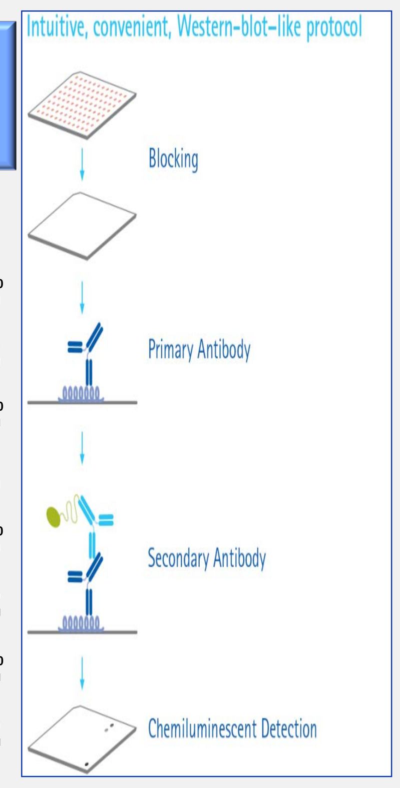
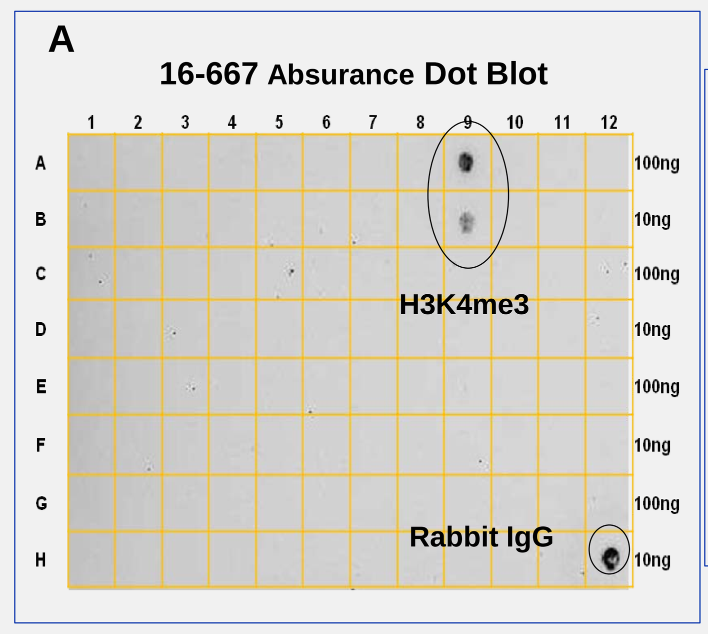
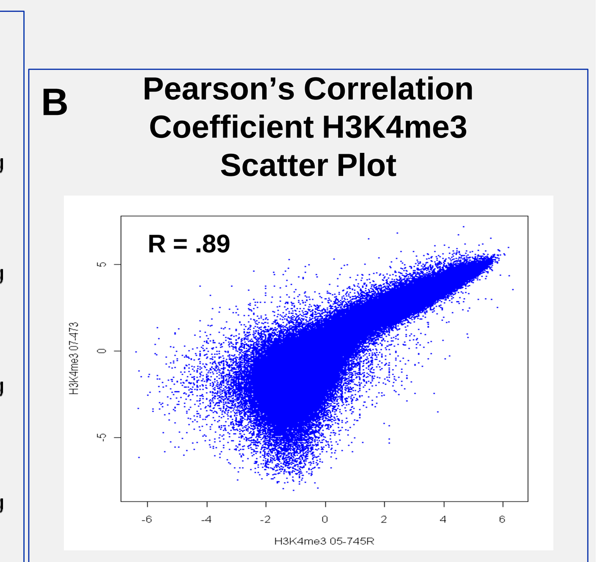


Figure 1: Validation of Anti-H3K4me3 Rabbit Monoclonal Antibody, 17-614





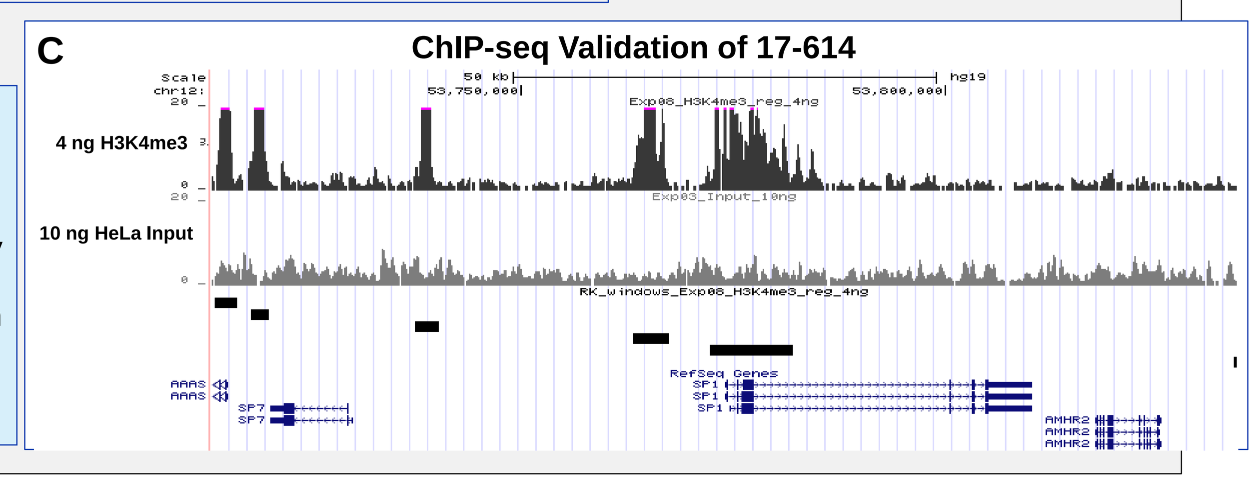


Figure 1. Antibody validation is a critical component of successful ChIP experiments. In this example, two independently derived anti-Trimethyl Histone H3 (Lys4) antibodies (05-745R/17-614 rabbit monoclonal and 07-473 rabbit polyclonal) were evaluated for Dot Blot efficiency using Absurance peptide membranes (Data shown for 17-614 Fig 1A). Each antibody was used to isolate HeLa chromatin for ChIP:chip analysis using 17-1001 Magna ChIP2 microarray kit with correlation mapping of signal at each feature shown (Fig. 1B). Antibodies were further validated using Magna-ChIP-Seq 17-1010 using HeLa chromatin with data from 17-614 shown overlayed on refseq genes with peak identification using a custom MACS algorithm (black bars, Fig. 1C). Region shows peaks in the region of the human Sp1 promoter, demonstrating poised or active gene transcription as this and other genes in the area.

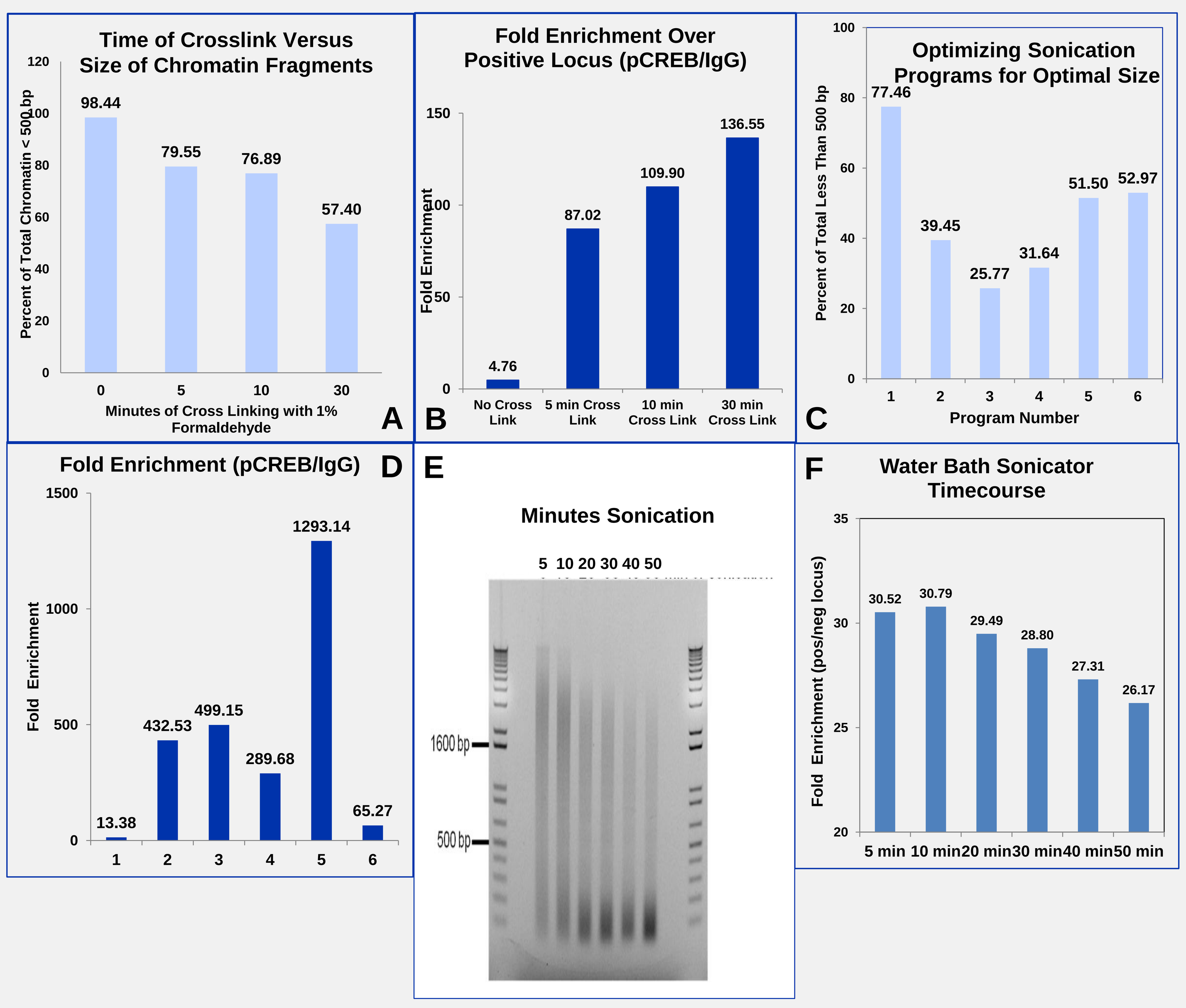


Figure 2: Analysis of Crosslinking, Fragment Size and Protocol Timecourse in ChIP Optimization Using 17-10082 EpiPure Chromatin Prep Kit

Figure 2. Chromatin optimization using the EpiPure Chromatin Preparation Kit. A. HeLa cells where crosslinked for various times at 1% CHO and analyzed for percent material below 500 bp following cross-link reversal and protein extraction. B. Material from A was evaluated in ChIP using 17-10131 pCREB antibody and qPCR analysis of the c-fos CRE relative to IgG ChIP. C, D. Various programs were utilized on a single sonicator (Branson Sonifier 450) and evaluated for Percent chromatin below 500 bp and evaluation of these chromatin samples at 100,000 cell equivalents per ChIP as in B. Timecourse of fragmentation was performed with a Qsonica sonicator, and analyzed by gel electrophoresis (E) and ChIP (F).

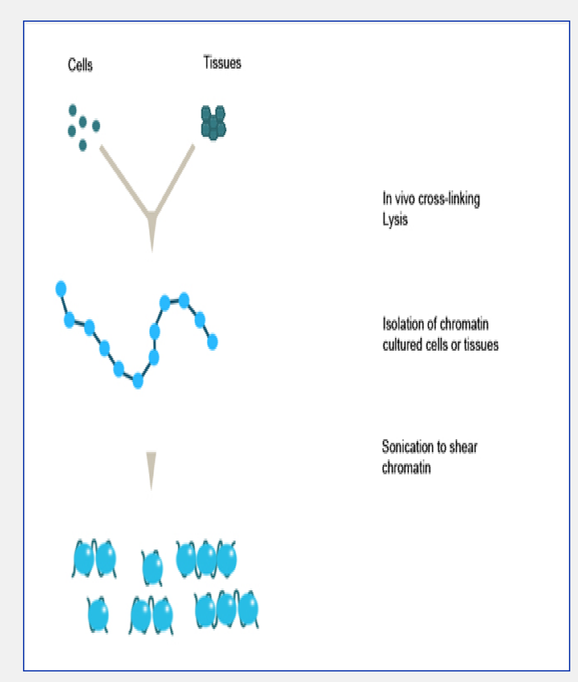
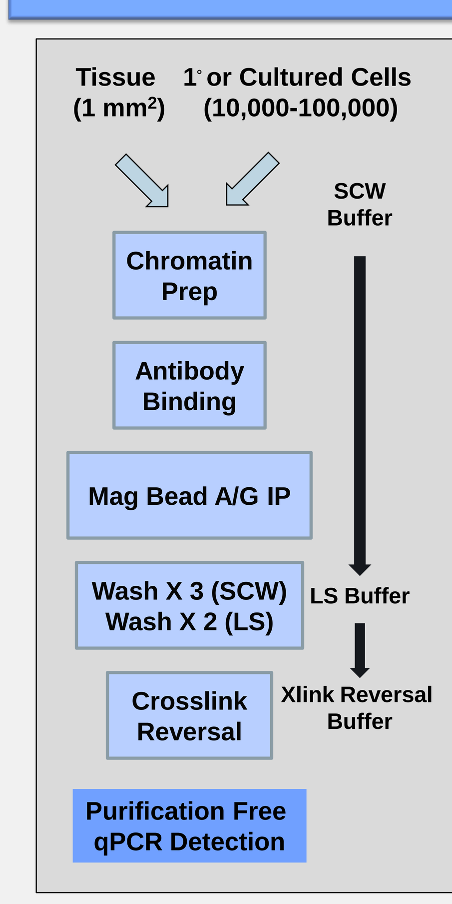
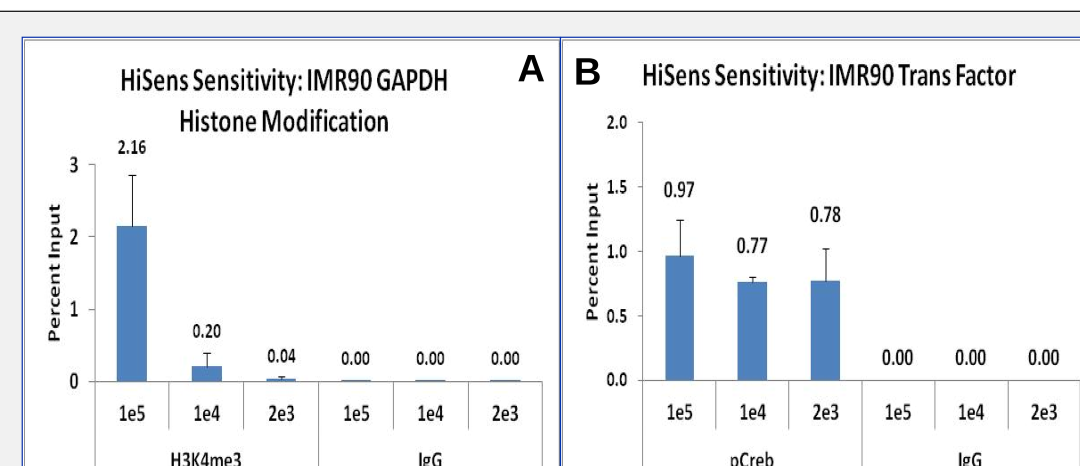


Figure 3: Magna ChIP™ HT96 & HiSens—A simplified, one buffer, high signal/low noise ChIP solution for 1e6 to 1e4 cells from 1 to 96 samples





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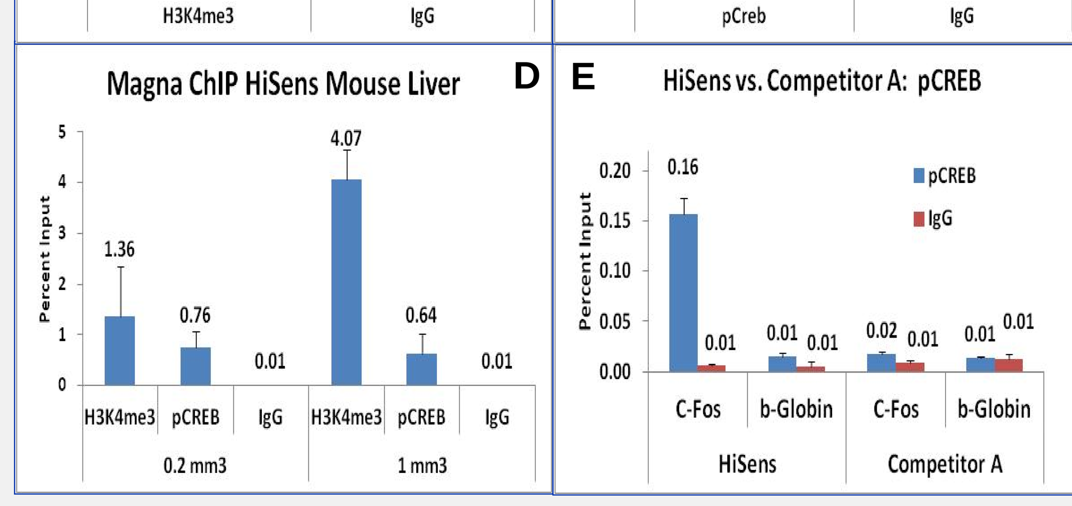
Antibody	IMR90 cell	Percent Input	Pos/Neg	Pos/IgG
H3K4me3	1e5	2.16	40.43	2160.21
	1e4	0.20	17.21	204.12
	2e3	0.04	3.54	44.41
pCreb	1e5	0.97	5.65	972.71
	1e4	0.77	4.60	770.12
	2e3	0.78	5.35	781.01
IgG	1e5	0.00		
	1e4	0.00		
	2e3	0.00		



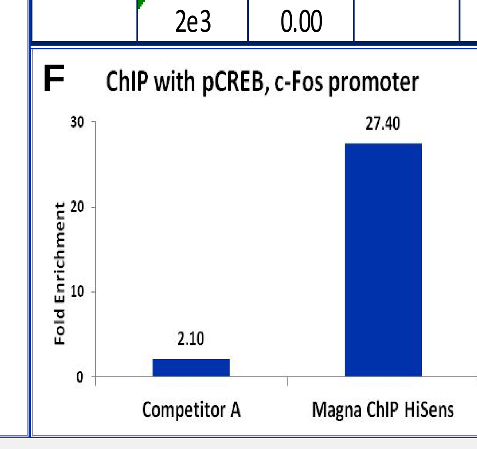


Figure 3. Analysis of sensitivity (A,B,C) performance in tissue (D) and comparative example (E,F) of 17-10460 Magna ChIP HiSens and 17-10077 Magna ChIP HT96. Performance across two antibodies, with varying amounts of chromain as indicated where evaluated.

CONCLUSIONS

•A variety of parameters contribute to ChIP variability, including antibody quality, Sonication conditions, and variations in ChIP Protocol

•Using the EpiPure Chromatin Preparation system, quality chromatin can be Produced with minimal optimization on a variety of sonication devices

•The Magna ChIP HiSens simplifies the ChIP procedure, reduces background to near Zero levels, and improves user to user reliability

www.emdmillipore.com/epigenetics

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