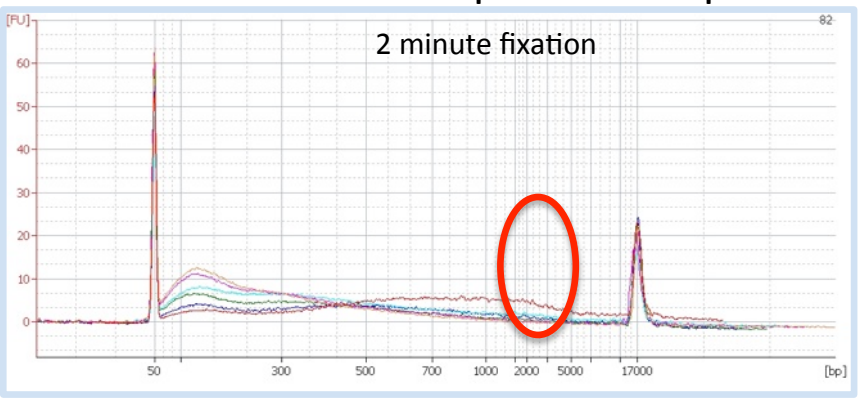
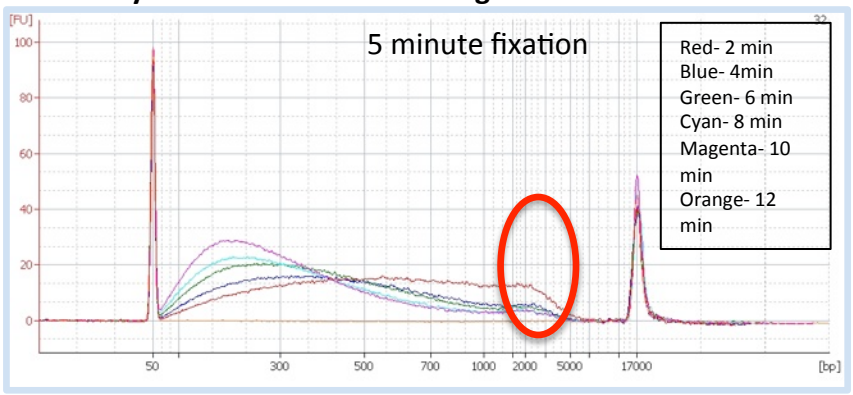
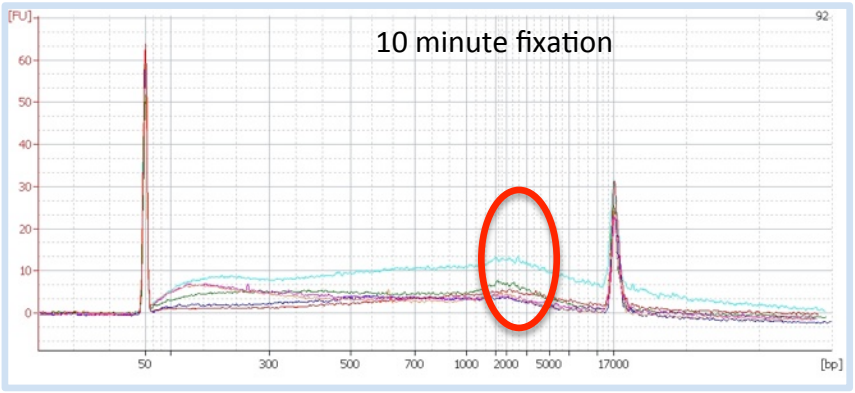
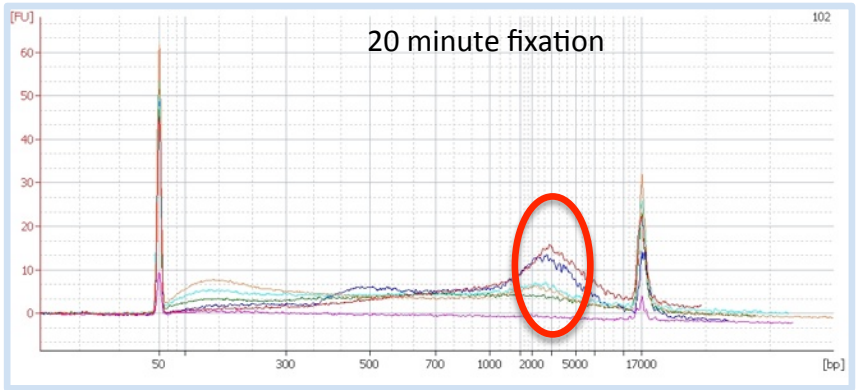
Over Fixation Effects on Chromatin Shearing

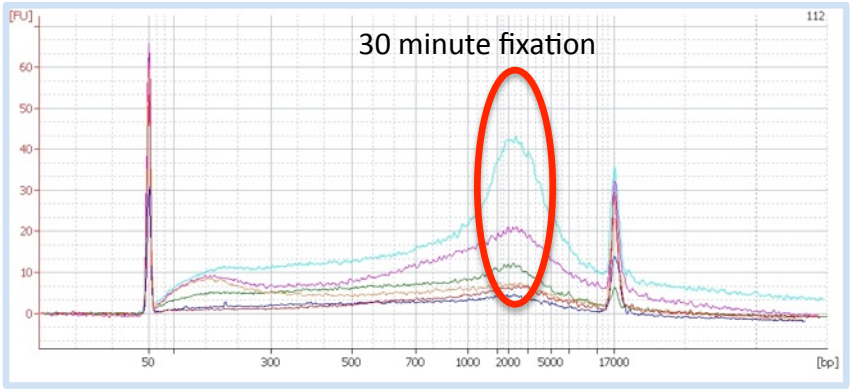
Reduced Sample Fixation time provides increased availability of chromatin for shearing

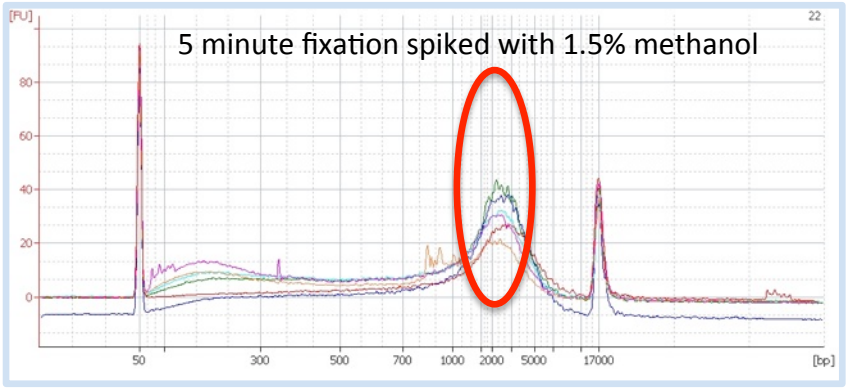






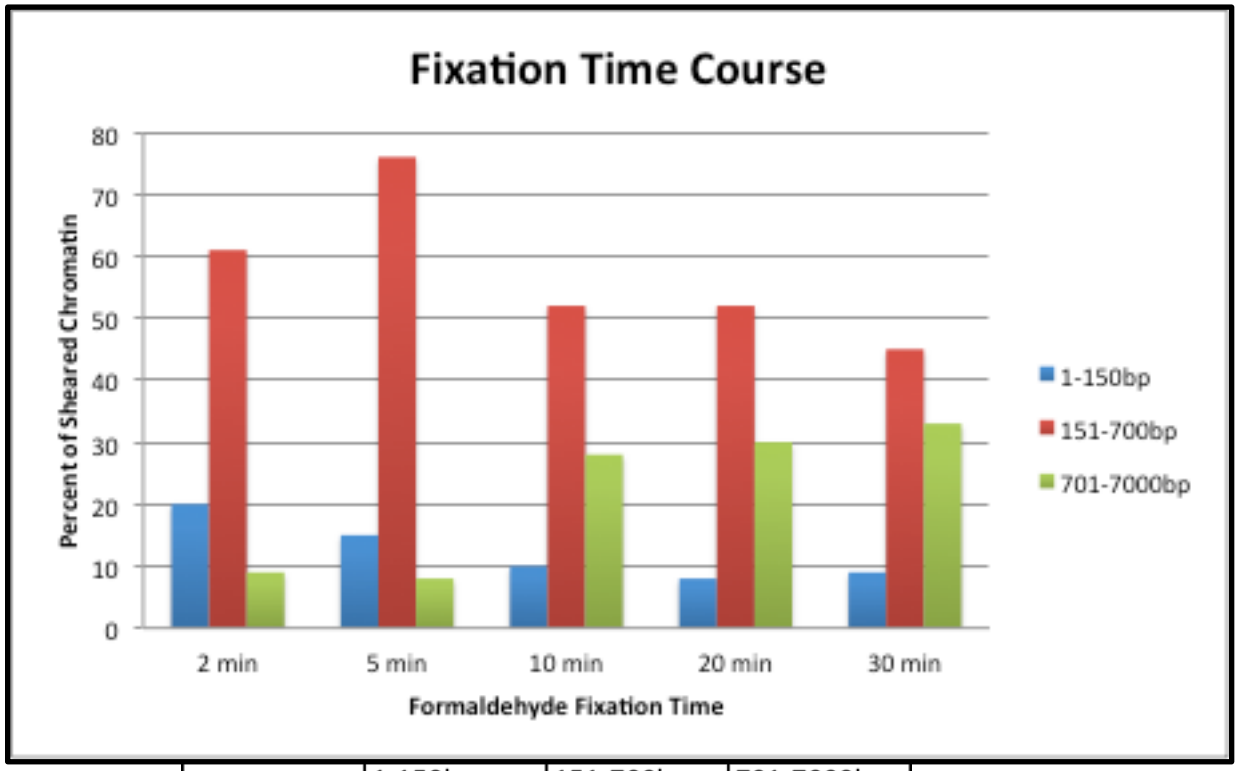






Six samples of ~2x10^7 MS4221(lymphoblast) were cross-linked for 2-20 minutes, and the nuclei prepared according to the Covaris *Tru*ChIP Kit protocol. The fix chromatin from 2x10^6 cells were then sheared for 2-12 minutes using a setting of 2%dc/3i/200cpb, reverse cross-linked overnight, and the DNA purified before analyzing DNA fragment size by agarose gel and an Agilent Bioanalyzer. Fixation time of greater than 5 minutes, as well as the presence of as low as 1.5% methanol resulted in an accumulation of large MW DNA.

Effect of Cross Linking Time on Cell Based Chromatin Shearing Efficiency

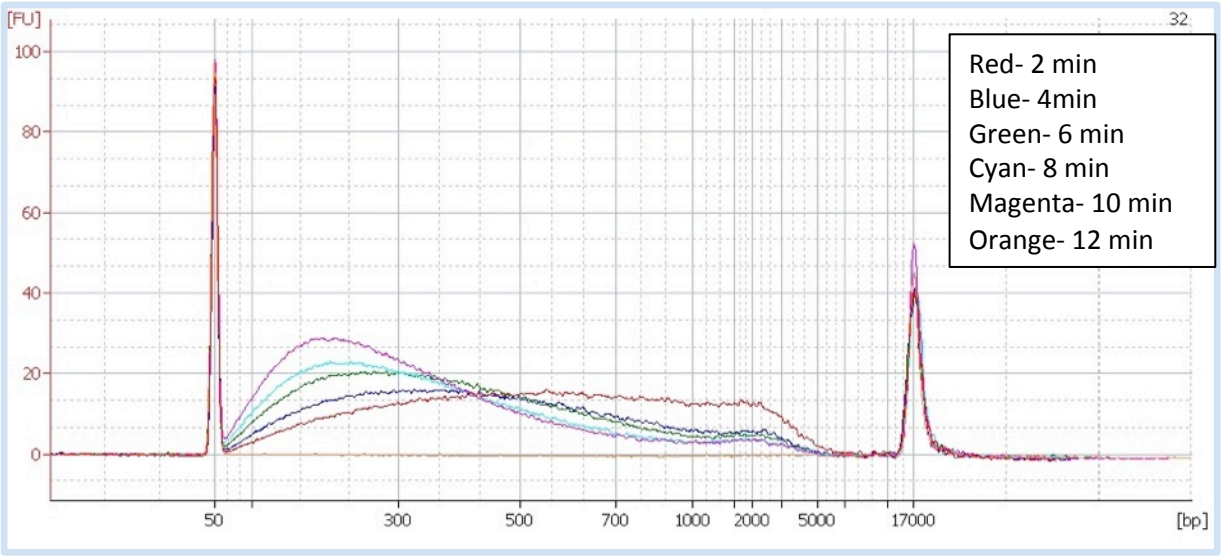


	1-150bp	151-700bp	701-7000bp
2 min	20	61	9
5 min	15	76	8
10 min	10	52	28
20 min	8	52	30
30 min	9	45	33

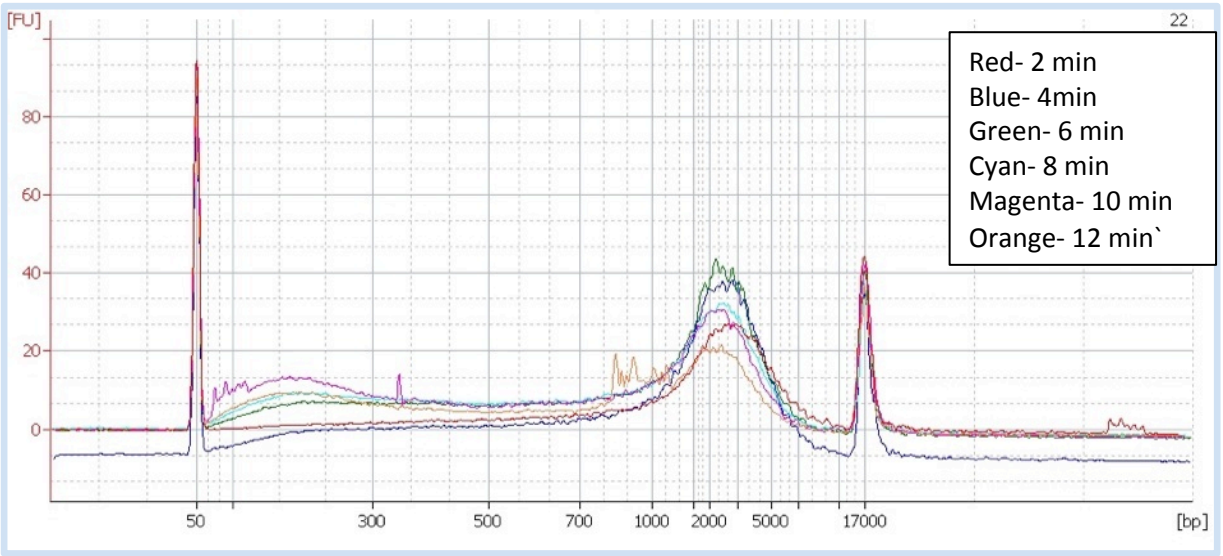
The 8 minute AFA chromatin shearing time point of the fixation time course were run on an Agilent Bioanalyzer, and the fragment size distribution analyzed. The 5 minute cross-linked cells generated the greatest yield of fragments from 151-700bp. Over cross-linking is indicated by the increase in the accumulation of fragments ranging from 701 - 7000 bp.

Effect of Methanol on Shearing Efficiency

Fresh methanol-Free Formaldehyde

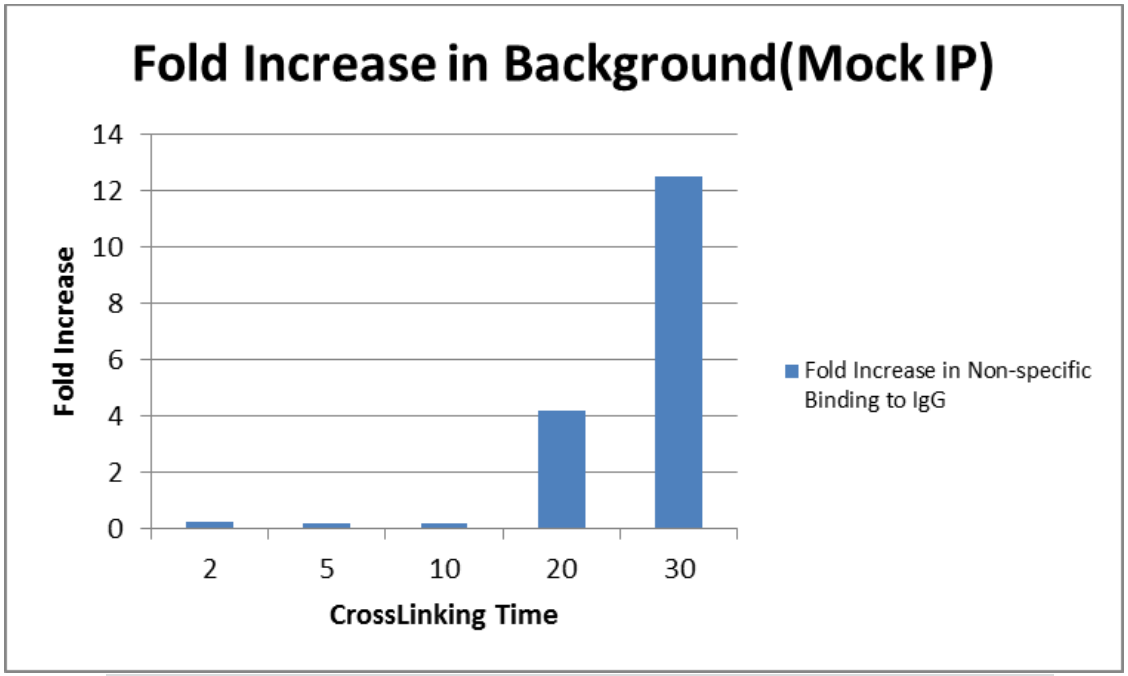


Fresh methanol-Free Formaldehyde Spiked with 1.5% Methanol



Aliquots of 2x10^7 cells were fixed for 5 minutes in either fresh 1% formaldehyde, or fresh 1% formaldehyde plus 1.5% methanol. The nuclei were prepared and the chromatin sheared according to the *tru*ChIP Low Cell SDS Chromatin Shearing Kit protocol. Purified DNA from the sheared chromatin was then analyzed on an Agilent Bioanalyzer for the determination of fragment length.

Fixation-Time Dependent Background Signal

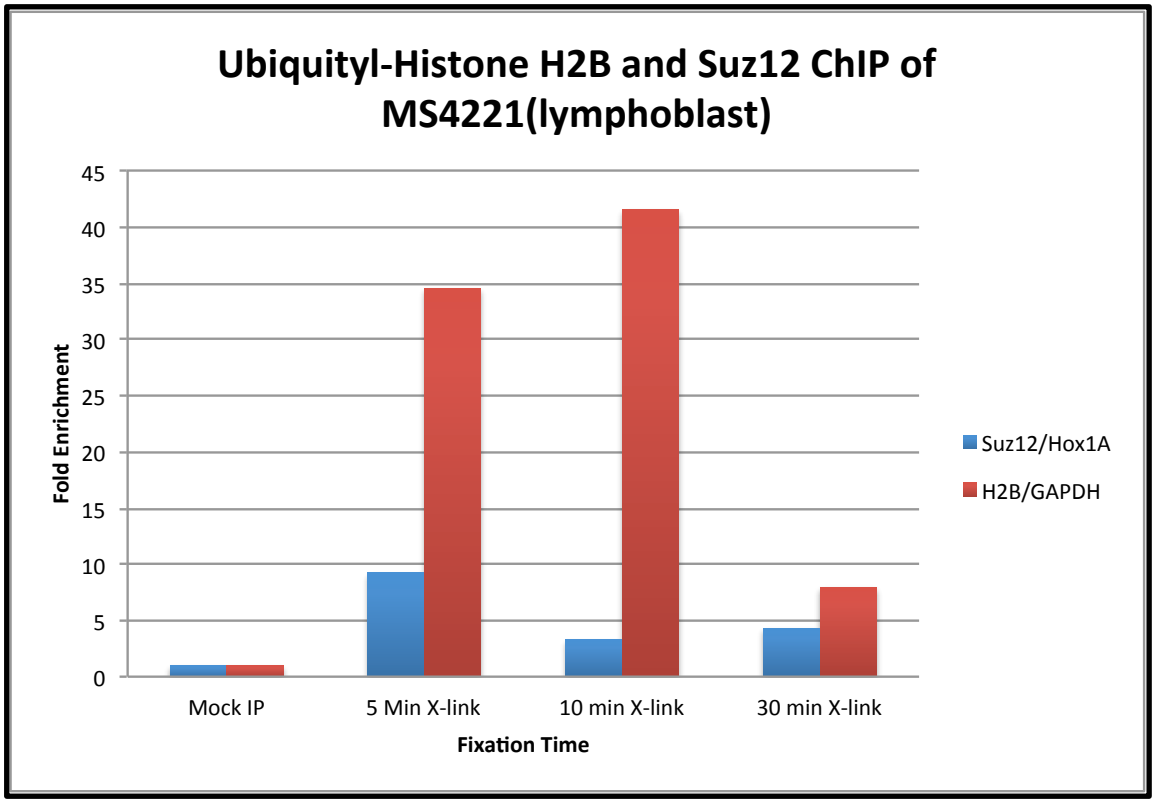


Fixation Time(min)	Input Ct	MockIP Ct	(ΔCt[Mock]-ΔCt[Input])	(2-ΔΔCt)
2	30.19	32.12	1.92	0.26
5	29.21	31.73	2.52	0.17
10	29.75	32.11	2.36	0.19
20	28.40	26.34	-2.06	4.17
30	28.40	24.76	-3.65	12.51

- Non-specific binding of epitopes to Antibody increases with crosslinking time
- Implications
  - False positive IP signal in both ChIP-Seq and ChIP-qPCR
  - Missing of loose epitope interactions due to increase in background

The 8 minute AFA chromatin shearing time point of the fixation time course were IP'd using normal mouse IgG, and qPCR carried out using GAPDH promoter primers. Fold increase over input was then calculated for each fixation time point.

ChIP for Modified Histones and Rare Transcription Factors Using the Same Shearing Protocol



	Suz12/Hox1A	H2B/GAPDH
Mock IP	1	1
5 Min X-link	9.25	34.57
10 min X-link	3.29	41.57
30 min X-link	4.3	7.98

$2x10^6$ cell aliquots of the 5, 10, and 30 minute cross-linked MS4221 chromatin were sheared by 10 minutes of AFA. Aliquots representing sheared chromatin from ~$5x10^5$ cells were used for ChIP anaylsis with anti-ubiquityl H2B and Suz12 antibodies, and 5ng of DNA from each IP was used for qPCR of the GAPDH and Hox1A promoters respectively. Fold enrichment of ubiquityl-Histone H2B and Suz12 over input DNA was calculated demonstrating significant enrichment of ubiquityl-Histone H2B and Suz12 binding sites using the same protocol.