Publication:
Katherine W. Jordan, Fei He, Monica Fernandez de Soto, Alina Akhunova & Eduard Akhunov Differential chromatin accessibility landscape reveals structural and functional features of the allopolyploid wheat chromosomes. Genome Biol 21, 176 (2020). https://doi.org/10.1186/s13059-020-02093-1

We have a limited understanding of how the complexity of the wheat genome influences the distribution of chromatin states along the homoeologous chromosomes. Using a differential nuclease sensitivity (DNS) assay, we investigated the chromatin states in the coding and transposon element (TE) -rich repetitive regions of the allopolyploid wheat genome.

Files:
GSE153289_MNase_score_rep1.bw
MNase differential score between library 1 and library 2

GSE153289_MNase_score_rep2.bw
MNase differential score between library 3 and library 4

GSE153289_MRF.bw
MNase resistant footprints detected by iSeg

GSE153289_MSF.bw
MNase susceptible footprints detected by iSeg

GSE153289_family.soft
metadata file

GSE153289_family.xml
metadata file

GSE153289_series_matrix.txt
metadata file

Source: GEO https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE153289

Extract protocol:
Wheat cultivar Chinese Spring was grown in greenhouse conditions with 16:8-hour light:dark cycle. Two-week old leaf tissue was collected and immediately flash frozen in liquid nitrogen. Nuclei were isolated using a modified protocol by Vera et al. Briefly 4 g of frozen tissue were ground using mortar and pestle under liquid nitrogen and were cross linked for 10 minutes in ice cold fixation buffer (15 mM PIPES-NaOH, pH 6.8, 80 mM KCl, 20 mM NaCl, 0.32mM sorbitol, 2 mM EDTA, 0.5 mM EGTA, 1 mM DTT, 0.15 mM spermine, 0.5 mM spermidine, 0.2 200 µM PMSF, and 200 µM phenanthroline, and 1% formaldehyde). The cross-linking was stopped by adding glycine to a final concentration of 125 mM and incubating at room temperature for 5 minutes. Nuclei were isolated by adding Triton-X 100 to a final volume of 1% and rotated for 5 minutes, then filtered through 1 layer of miracloth. Nuclear suspensions were divided in 2 aliquots and then suspended in 15mL of 50% volume:volume Percoll:PBS cushion, then centrifuged for 15 minutes at 4°C at 3,000 x g. Nuclei were transferred from the Percoll interphase to a new tube, diluted 2X in PBS buffer, and pelleted by centrifugation for

15 minutes at 4°C 2,000 x g. Pellets were resuspended in 15mL of ice cold MNase digestion buffer (50 mM HEPES-HCl, pH 7.6, 12.5% glycerol, 25mM KCl, 4mM MgCl2, 1mM CaCl2), and pelleted again by centrifugation for 15 minutes at 4°C 2,000 x g. Pellets were resuspended in 2 mL of MNase digestion buffer. A 100 µL aliquot of the resuspended nuclei were stained with 1µg/mL DAPI in PBS buffer, and quantified using hemacytometer on a confocal microscope. The remaining nuclei were split into 60µL aliquots containing 3,000 nuclei each and flash frozen in liquid nitrogen. Nuclei were digested by micrococcal nuclease (NEB) using 100 U/mL (heavy) and 10 U/mL (light) for 20 minutes at room temperature. Digestion was terminated by adding 10mM EGTA. To break the cross-links, digestions were treated overnight at 65°C in 1% SDS and 100 µg/mL proteinase K. DNA was extracted using phenol-chloroform extraction and precipitated in ethanol. Digested DNA was resuspended in 40 µg/mL RNaseA (Qiagen), and run on a 1% agarose gel to confirm the heavy and light digest.
!Sample_extract_protocol_ch1 = Two separate biological replicates of nuclei were thawed to room temperature and split into 8 separate 60ul aliquots. For each replicate, 4 separate light digestions (10 U/ml) and 4 separate heavy digestions (100U/ml) were carried out for 20 minutes at room temperature. Digestions were stopped with the addition of 0.5M EGTA. DNA was extracted in the same manner described above, and then the 4 samples of each like digestion were combined to produce 2 replicate light digestions, and 2 replicate heavy digestions, resulting in 4 total libraries. Prior to library preparation digested DNA samples were subjected to 100-200 bp size selection using the Pippin prep system (Sage Science). The DNA-seq libraries were constructed from 500 ng of size selected DNA with the GeneRead DNA library I core kit (Qiagen, cat #180434) and GeneRead Adapter I set B (Qiagen, cat # 180986) according to Qiagen protocol with one exception: seven PCR cycles were performed for the library enrichment. The sizes of resulting libraries were validated on the 7500 DNA Bioanalyzer chip. To test the quality of library preparations, two out of four barcoded libraries prepared using the high and low concentrations of MNase were pooled in the equimolar amounts and sequenced with 2 x 75 bp Illumina MiSeq run using MiSeq 150 cycles reagent kit v3. Then each of the four libraries was sequenced on 2 lanes of HiSeq 2500 system (8 lanes total) using a 2 x 50 bp sequencing run producing a total of 1,749,823,029 reads

Data processing:
Raw fastq files were run through quality control using Illumina NGSC Toolkit v2.3.3, and aligned to Chinese Spring RefSeqv1 genome using the HISAT2 v2.0.5 alignment program. Paired end reads were retained if 70% of the read length had a quality cutoff score of > 20. Only uniquely mapped reads were retained for further analysis.
BED files were made from each alignment, using the Bedtools v2.26.0 bamtobed conversion to get the coordinates where reads align, then depth of reads was measured in 10bp intervals using bedmap --count option. The read coverage (number of reads that map) for each 10bp interval was normalized by taking the total number of reads mapped for the whole genome and then dividing by million.
To get the differential MNase score for each 10bp interval, we subtracted the normalized depth of coverage of the heavy digest from the normalized depth of coverage of the light digest. For instance, for each 10 bp interval on a chromosome we obtain normalized depth of coverage for both light and heavy digests for each replicate, and then calculate the differential depth for each replicate (2 reps). We performed a segmentation analysis using the iSeg algorithm to identify distinctly accessible (hypersensitive) and inaccessible (hyper-resistant) regions of the genome. A biological cutoff for genome-wide significance of sd = 1.5, was used to identify regions either accessible or inaccessible to MNase digest. Replicates were run separately and regions that were found to surpass the biological cutoff in both replicates were considered either accessible (hypersensitive, MSF), or inaccessible (hyper-resistant, MRF).

All processed files are in bigWig format. MSF.bw has MNase susceptible footprints detected by iSeg. MRF.bw has MNase resistant footprints detected by iSeg. MNase_score_rep1.bw has MNase differential score between library 1 and library 2. MNase_score_rep2.bw has MNase differential score between library 3 and library 4.