Supplemental Material

Thermal stress accelerates *Arabidopsis thaliana* mutation rate

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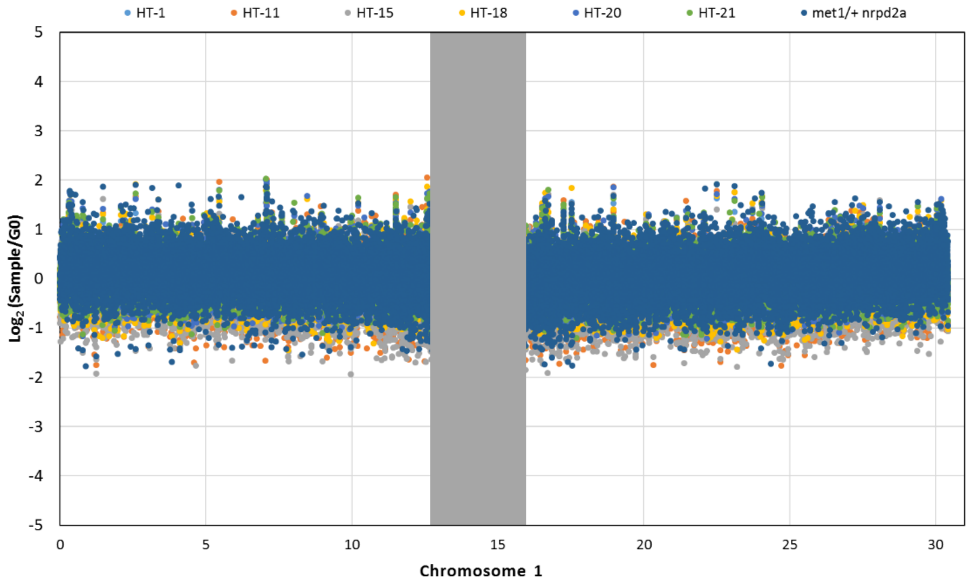
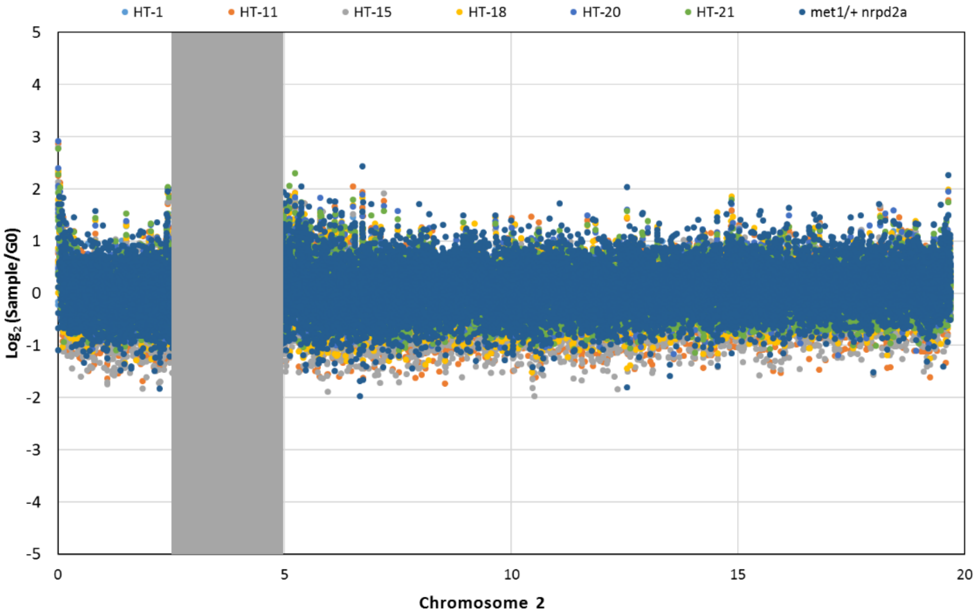
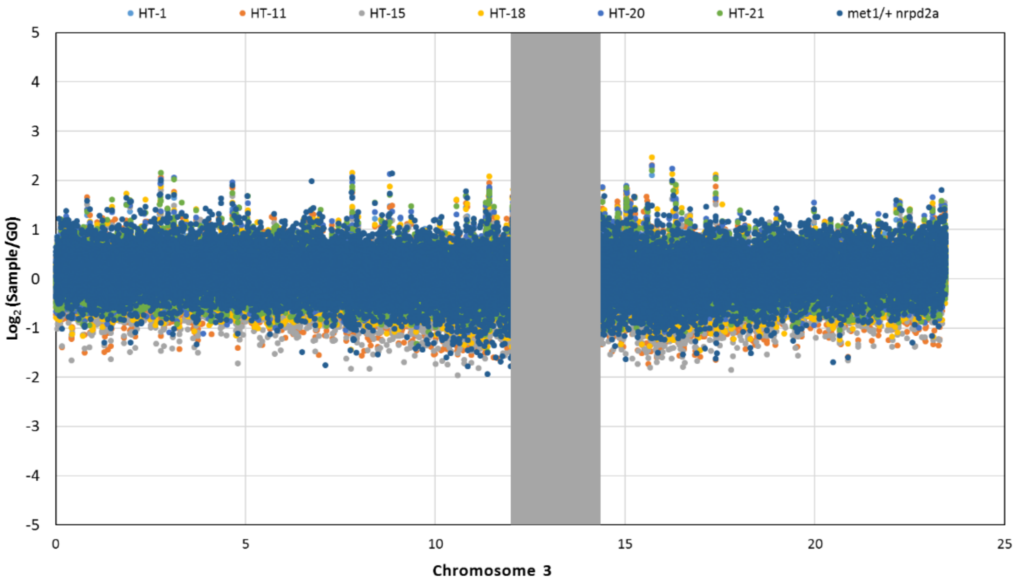
A


HT-1
HT-11
HT-15
HT-18
HT-20
HT-21
met1/+ nrpd2a
5
4
3
2
1
0
-1
-2
-3
-4
-5
Log$_2$ (Sample/G0)
0
5
10
15
20
25
30
Chromosome 1

B

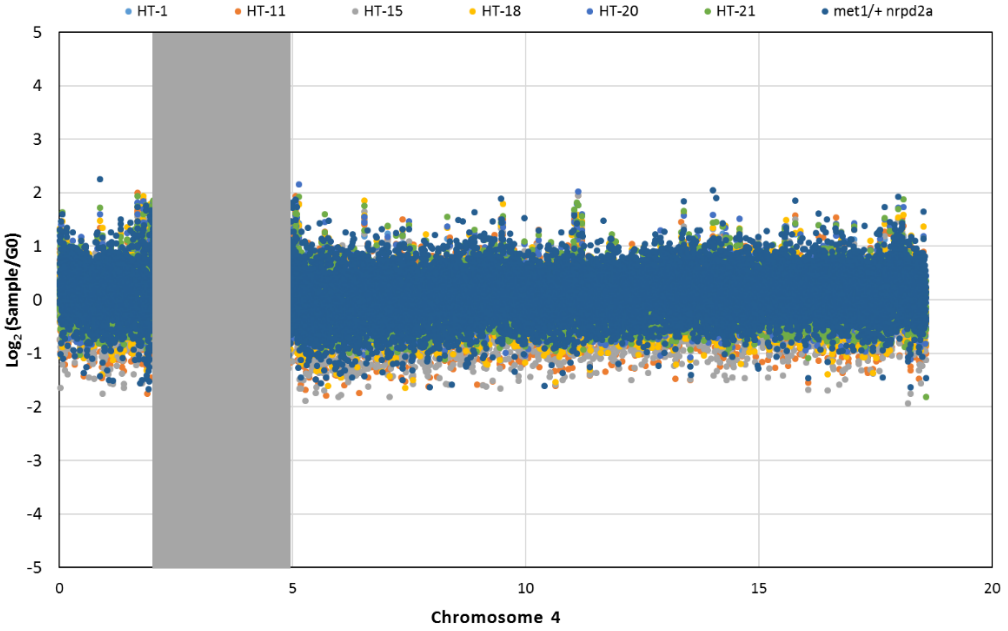

HT-1
HT-11
HT-15
HT-18
HT-20
HT-21
met1/+ nrpd2a
5
4
3
2
1
0
-1
-2
-3
-4
-5
Log$_2$ (Sample/G0)
0
5
10
15
20
Chromosome 2

C



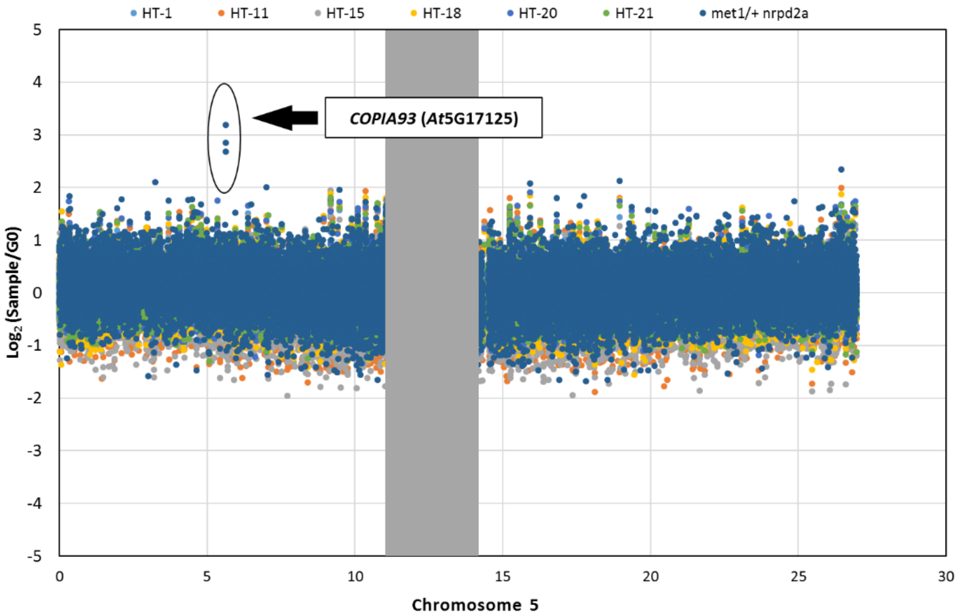
● HT-1
● HT-11
● HT-15
● HT-18
● HT-20
● HT-21
● met1/+ nrpd2a
5
4
3
2
1
0
-1
-2
-3
-4
-5
Log_2 (Sample/G0)
0
5
10
15
20
25
Chromosome 3

D

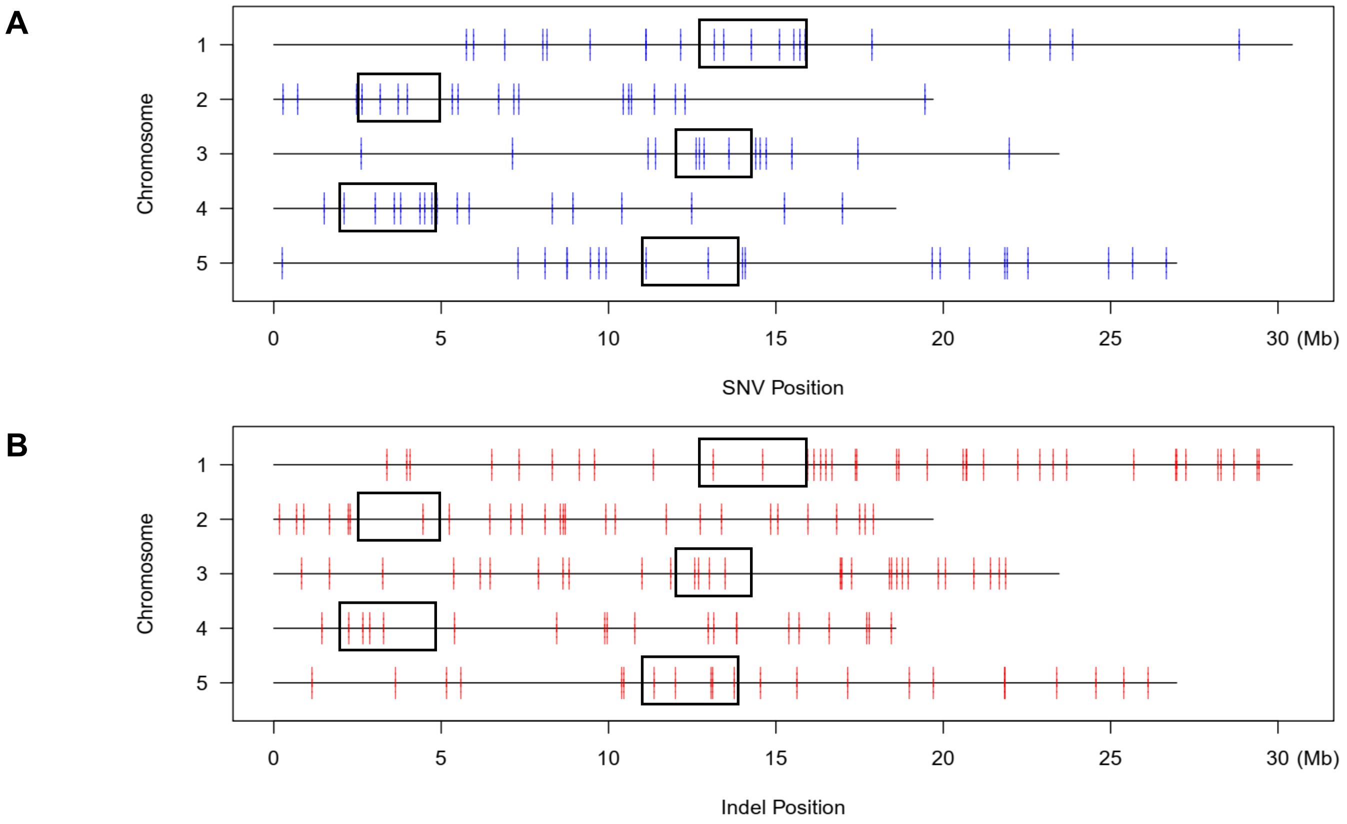


● HT-1
● HT-11
● HT-15
● HT-18
● HT-20
● HT-21
● met1/+ nrpd2a
5
4
3
2
1
0
-1
-2
-3
-4
-5
Log_2 (Sample/G0)
0
5
10
15
20
Chromosome 4

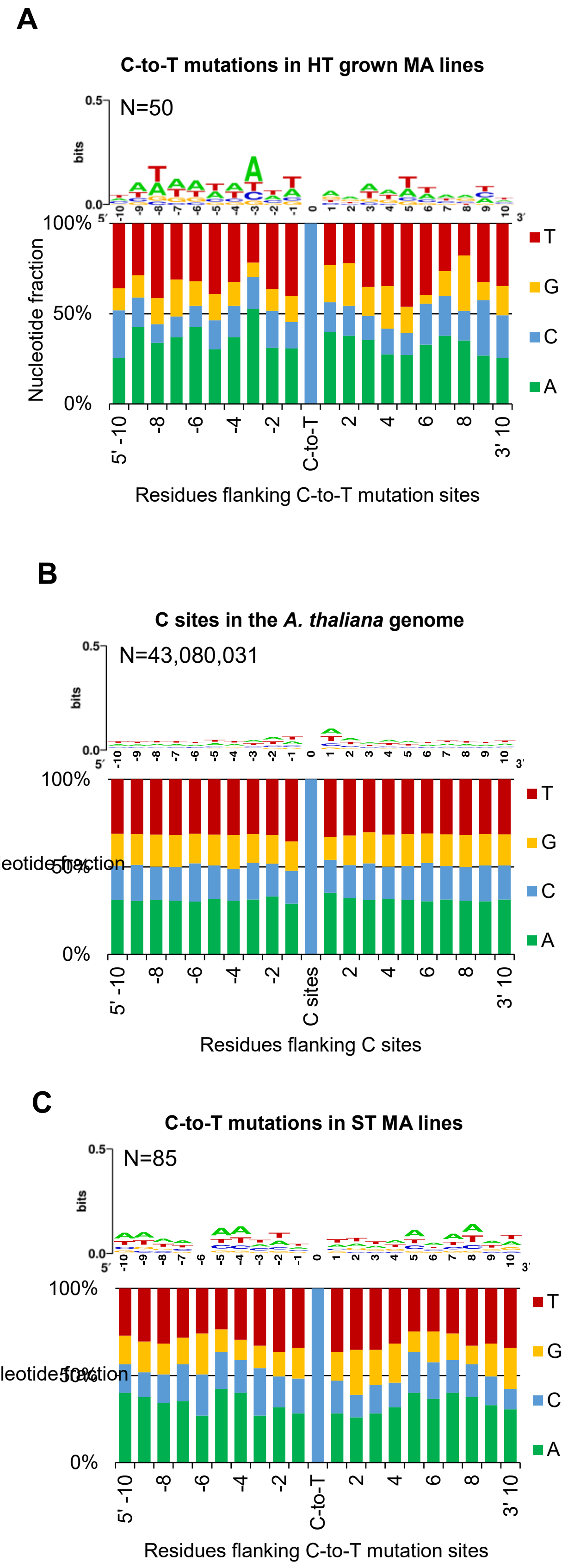
E



Supplemental Fig. 1. Genomes of HT-grown MA plants lack detectable Copy Number Variants (CNVs). Dot scatter-plots show $\log_2$ coverage ratio of reads from G11 HT MA line plants, together with a positive control dataset (*met1/+ nrpd2a* (Mirouze et al. 2009)), as compared with G0 ancestor reads (Jiang et al. 2014), and presented chromosome-by-chromosome (A–E, Chromosomes 1–5). The blue dots cluster around 0 and are the most visible because the *met1/+ nrpd2a* data that they represent were the last entered into the plots. Other dot colors indicate data from individual G11 HT MA line plants, as indicated, and represent consecutive 1000 bp windows from across TAIR10. The greyed-out sections of chromosome represent centromeres. The circled and black arrowed dots in (D) represent the CNV-generating transposition of *At*COPIA93 (positive control (Mirouze et al. 2009)). Any CNVs would be expected to result in a $\log_2$ score value of ≥1. Dots with $\log_2$ score values ≥1 were therefore visually checked using IGV (see Methods). However, none were verified as being genuine CNVs, indicating that HT growth does not detectably increase the frequency of larger-scale indel mutations during genome replication.

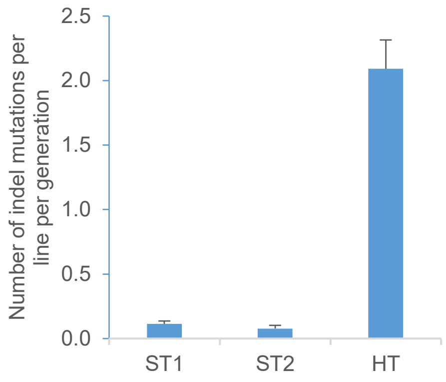


Supplemental Fig. 2. HT-induced mutations appear to be evenly spread across *Arabidopsis* chromosomes. Chromosomal distribution of SNVs (A) and indel (B) mutations in G11 HT-grown MA line plants. Chromosomal locations of mutations on the five *Arabidopsis* chromosomes (represented as horizontal black lines, length in megabases as shown) are represented by single vertical colored lines ((A) 92 SNVs, blue; (B) 138 indels, red). Peri-centromeric regions of each chromosome are indicated with rectangular black boxes.

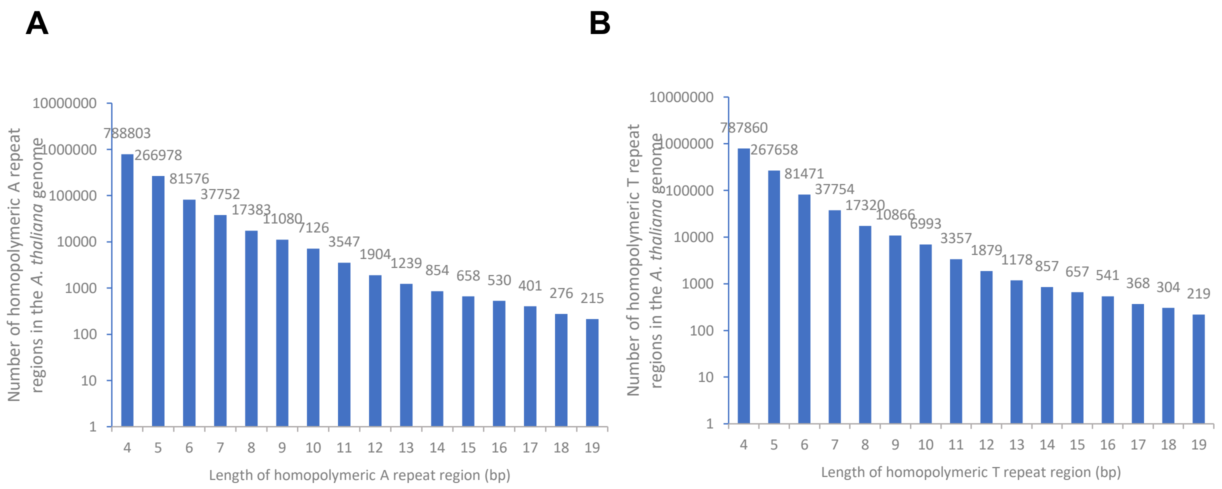


Supplemental Fig. 3. HT-induced GC-to-AT transitions may occur preferentially in particular sequence locations. Nucleotide flanking sequences ten bases upstream (5’) and downstream (3’) of

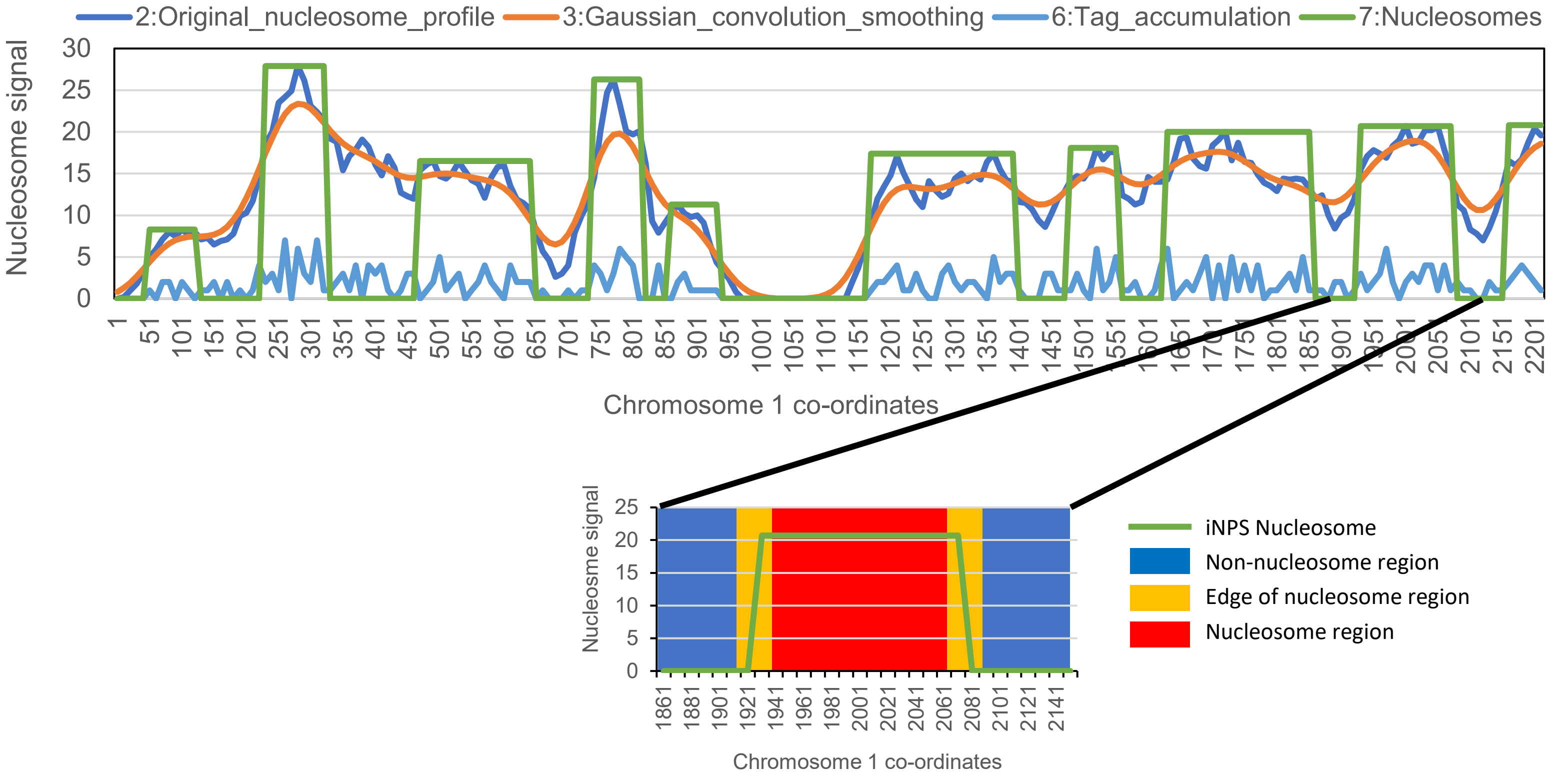
GC-to-AT transitions mutation site are shown as stacked columns and as sequence logos (Schneider and Stephens 1990; Crooks et al. 2004). (A) The combined 18 C-to-T and 32 reverse complemented G-to-A to C-to-T mutations in G11 HT MA line plants. (B) All C and all reverse complemented G sites in TAIR10. (C) The combined 47 C-to-T and 38 reverse complemented G-to-A to C-to-T mutations identified in ST-grown MA line plants (Ossowski et al. 2010; and Jiang et al. 2014). Comparison of the sequence logos (A–C) showed enrichment for an A residue at the 5′ -3 position to the mutated GC-to-AT transitions (A) in HT-grown plants versus TAIR10 (B) and ST-grown plants (C). A second residue, a G, at the 3′ +8 position to the mutated GC-to-AT transitions in HT-grown plants (A) showed no enrichment versus TAIR10 (B) but did however show enrichment versus ST-grown plants (C), see main Results section: HT growth boosts SNV frequency and maintains GC-to-AT bias, for further details. Sequence logos provide graphical representations of the relative frequencies of individual nucleotide residues with respect to position within flanking sequences. Individual residues are shown as stacked letters, along with the number of sites (combined C and reverse complemented G-to-A to C-to-T) considered (shown as N). The letters are ordered from most (top) to least (bottom) frequent in each stack. Overall stack indicates sequence conservation at that position (measured in bits), while the letter height indicates the relative frequency of each nucleotide (A, T, C or G) at that position (Crooks et al. 2004).



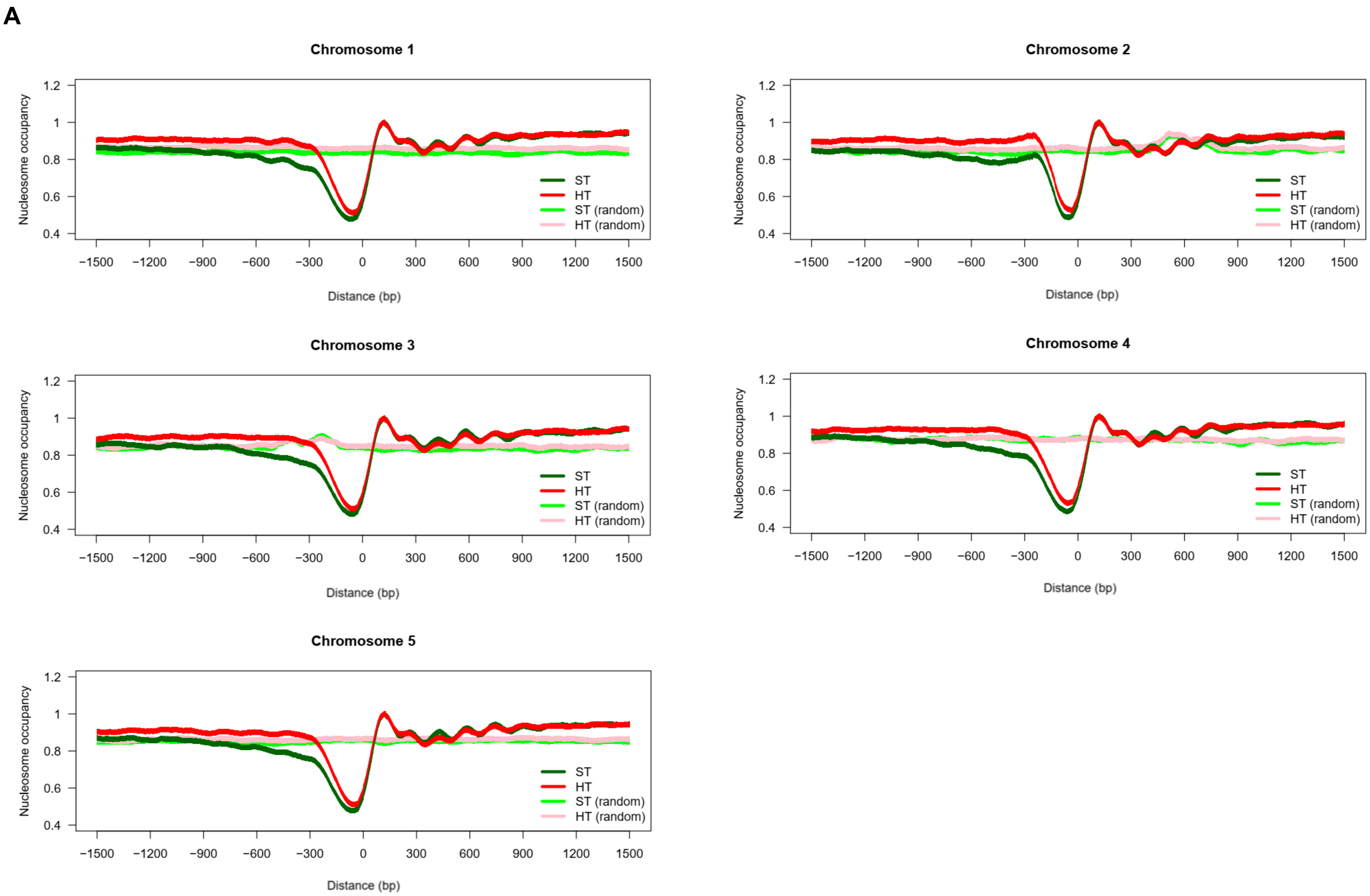
Supplemental Fig. 4. HT-growth increases the frequency of indel mutations. Comparison of the frequency of indels (insertions and deletions) in G11 HT MA lines with that of ST controls (ST1, data from Ossowski et al. 2010; ST2, data from Jiang et al. 2014). Error bars indicate SEM from 5 (ST1 data), 9 (ST2 data), or 6 (HT data) biological replicates.



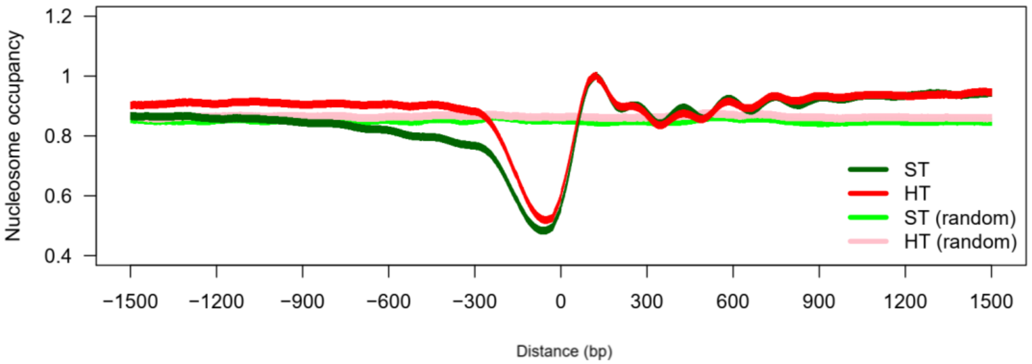
Supplemental Fig. 5. The frequency of homopolymeric repeat stretches within the *Arabidopsis* genome decreases with increasing stretch length. The frequency of homopolymeric A (A) or T (B) repeat stretches in the TAIR10 reference genome is plotted with respect to stretch length (data from (Belfield et al. 2018)).



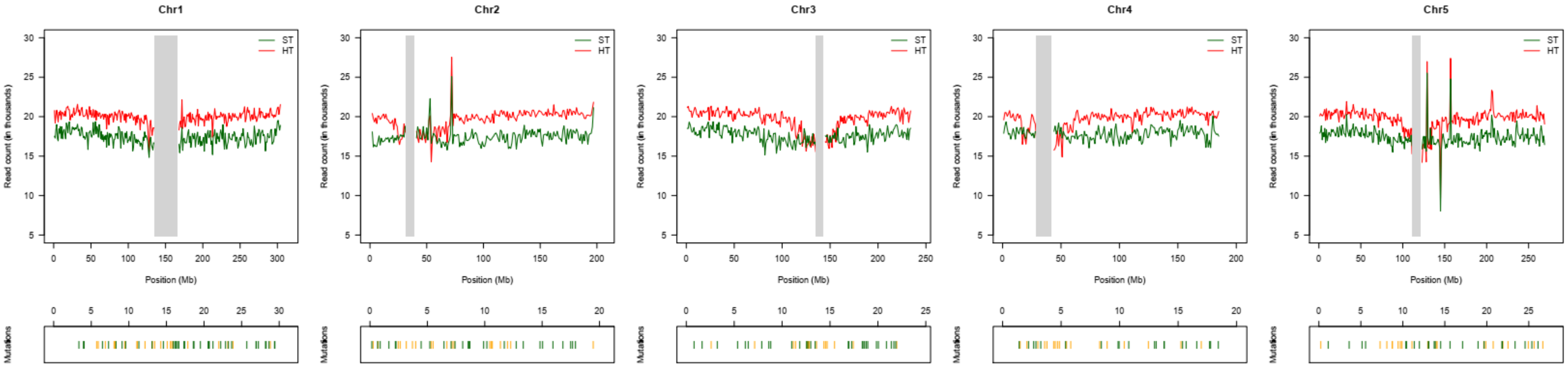
Supplemental Fig. 6. Nucleosome occupancy in an example segment of *A. thaliana* Chromosome 1. Illumina sequencing was performed on MNase-digested genomic DNA from ST- and HT-grown plants. The top panel shows nucleosome occupancy of a 2.2kb region of Chromosome 1 (from ST-grown plants) predicted by nucleosome-positioning algorithm iNPS (Chen et al. 2014) from the MNase-seq data. The bottom panel shows a zoomed-in region (co-ordinates 1,861 – 2,141bp) of Chromosome 1 showing a single predicted nucleosome. Nucleosomic regions are classified as: nucleosome (red block), non-nucleosome (blue block) and edge of nucleosome (yellow block) regions.


A
Chromosome 1
Chromosome 2
Chromosome 3
Chromosome 4
Chromosome 5
Nucleosome occupancy
1.2
1
0.8
0.6
0.4
Distance (bp)
−1500
−1200
−900
−600
−300
0
300
600
900
1200
1500
ST
HT
ST (random)
HT (random)

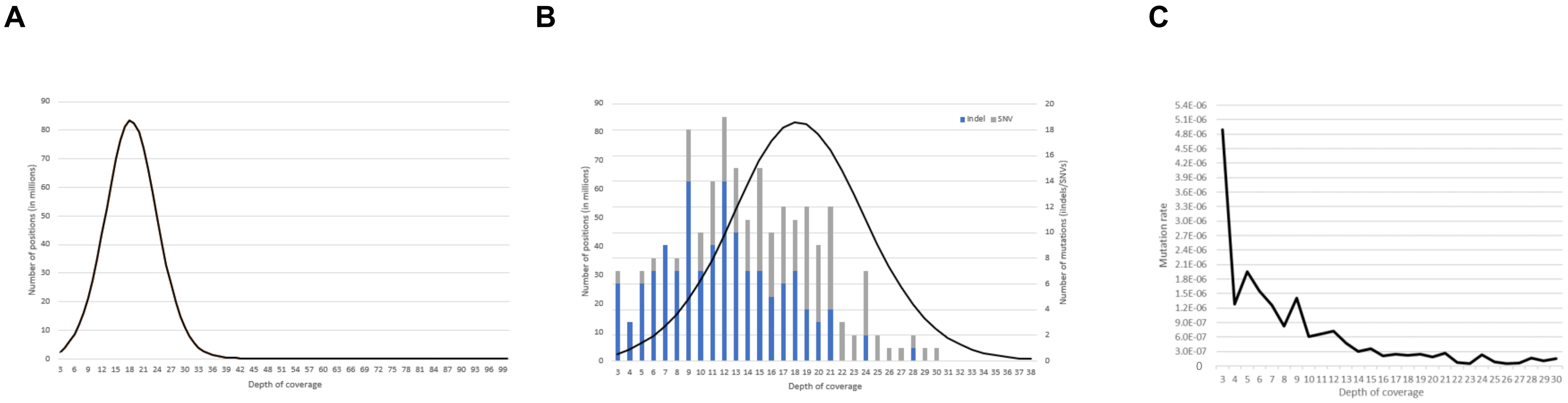
B



Supplemental Fig. 7. Comparison of the effect of growth temperature on nucleosome occupancy at Transcription Start Sites (TSS) versus at random positions across the genome. (A) Relative nucleosome occupancy in the vicinity of the TSS of all *A. thaliana* genes. Occupancy plots show the average nucleosomal coverage with respect to distance (bp) from the TSS in HT-grown (dark red trace), versus ST-grown (dark green trace) plants, presented on a chromosome-by-chromosome basis (plots show merged +ve and -ve DNA strands). Comparison traces show average nucleosome coverage with respect to distance (bp) from randomly chosen chromosomal positions in HT-grown (pink) and ST-grown (light green) plants. (B) Merged data for Chromosomes 1–5. Data are normalized to the highest value for each chromosome. The sites of the randomly chosen positions were determined by using the number of TSS sites (28,496) to divide the length of the genome (120Mb/28,496=~4.2kb), then taking points every ~4.2kb from the beginning of each of the 5 Chromosomes.



Supplemental Fig. 8. MNase-seq read-density and mutation distribution along chromosome arms. Nucleosome read count density in 100-kb tiles is plotted for HT-grown (red) and ST-grown (green) plants. Nucleosome-enriched pericentromeric regions are greyed out. Also shown are the chromosomal locations of HT-induced mutations (SNVs, yellow vertical lines; indels, green vertical lines).



Supplemental Fig. 9. The relationship between read coverage depth and frequency of detected mutation. (A) Numbers of TAIR10 genomic positions averaged across six G11 HT MA lines (x-axis) and plotted against the read coverage (y-axis). (B) A zoomed-in view of (A) overlaid with a histogram display of the 230 mutations (138 indels, blue bars; 92 SNVs, grey bars (right y-axis); Supplementary Table 2) detected in the six G11 HT MA lines. (C) A plot of mutation rates versus depth of coverage for the 230 mutations found in six G11 HT MA lines.

Line	Illumina paired-end read length (bp)	Raw sequencing data			Aligned sequencing data			
		Number of reads	Total number of bases sequenced	Genome fold coverage*	Number of Reads	Total number of bases sequenced	Genome fold coverage*	Total number of sites covered (million)
CT-2	100	35,822,644	3,582,264,400	30.1	30,499,439	3,049,943,900	25.6	118,674,753
CT-4	100	32,273,474	3,227,347,400	27.1	28,048,073	2,804,807,300	23.5	118,682,089
CT-6	100	32,426,492	3,242,649,200	27.2	27,711,819	2,771,181,900	23.3	118,565,154
CT-10	100	32,260,792	3,226,079,200	27.1	27,689,284	2,768,928,400	23.2	118,674,324
CT-12	100	32,337,888	3,233,788,800	27.1	26,396,211	2,639,621,100	22.2	118,674,951
HT-1	100	33,932,676	3,393,267,600	28.5	27,462,253	2,746,225,300	23.0	118,471,548
HT-11	100	33,743,230	3,374,323,000	28.3	27,818,608	2,781,860,800	23.3	118,381,764
HT-15	100	35,694,470	3,569,447,000	30.0	29,549,929	2,954,992,900	24.8	118,141,545
HT-18	100	34,000,868	3,400,086,800	28.5	27,134,763	2,713,476,300	22.8	118,436,561
HT-20	100	33,885,734	3,388,573,400	28.4	26,989,657	2,698,965,700	22.7	118,483,579
HT-21	100	34,807,454	3,480,745,400	29.2	27,953,947	2,795,394,700	23.5	118,462,564

Supplemental Table 1. Whole genome sequencing data summary table. Genomic DNA from five independent G6 cold temperature (CT) MA line plants (CT-2, -4, -6, -10, -12) and six independent G11 high temperature (HT) MA line plants (HT-1, -11, -15, -18, -20, -21) were sequenced using 100bp paired-end Illumina sequencing technology. For each sample, >32 million raw sequencing data reads (>3.2 gigabases of data) were aligned to the TAIR 10 *A. thaliana* reference genome, resulting in a 22.2–25.6-fold sequencing coverage depth (aligned data) for each genome. The total number of TAIR10 sites having aligned sequence data coverage ≥3 and ≤100 is shown. *The TAIR10 genome size of 119,146,348 bp was used to determine genome fold coverage.

MA lineage	Coordinates	Mutation size	TAIR10 reference	Mutation	Mutation location	Flanking indel mutation sequence*	Indel context
HT-21	Chr1:15949261	-2	TA	-	Intergenic	CATTG**TA**TATATATATATATATATATTAAA	Dinucleotide repeat
HT-15	Chr1:16142592	2	-	TA	Intergenic	GCAAG**ta**TATATATATATATATATATAATTAT	Dinucleotide repeat
HT-1	Chr1:16342316	-2	GA	-	Intergenic	GTGAG**GA**GAGAGAGAGAGAGAGAGAGTCAA	Dinucleotide repeat
HT-20	Chr1:16498351	-2	AT	-	Intergenic	GAAAA**AT**ATATATATATATATATATATATACATA	Dinucleotide repeat
HT-15	Chr1:17377490	-2	TA	-	Intergenic	TTTCT**TA**TATATATATATATATATATATATATATATATGTAG	Dinucleotide repeat
HT-21	Chr1:17421944	-2	AT	-	Intergenic	TATAA**AT**ATATATATATATATATATTTTTT	Dinucleotide repeat
HT-18	Chr1:18605859	-2	TA	-	Intergenic	ATTTT**TA**TATATATATATATATTAAA	Dinucleotide repeat
HT-18	Chr1:18672003	2	-	AT	Intergenic	CCTTA**at**ATATATATATATATATATATATGTGAA	Dinucleotide repeat
HT-1	Chr1:20593095	-2	CT	-	Intergenic	TGACC**CT**CTCTCTCTCTCTCTCTCTTTGTT	Dinucleotide repeat
HT-21	Chr1:22232997	2	-	AT	Intergenic	TATGA**at**ATATATATATATATATGGTAC	Dinucleotide repeat
HT-1	Chr1:29440434	-2	TA	-	Intergenic	ATAAG**TA**TATATATATATTTAA	Dinucleotide repeat
HT-11	Chr2:10205930	2	-	TA	Intron	GCTTT**at**ATATATATATATATATATATCCTTG	Dinucleotide repeat
HT-20	Chr2:13377446	-2	AT	-	Intergenic	TCTTA**AT**ATATATATATATATATATATATAACAT	Dinucleotide repeat
HT-15	Chr2:14846624	2	-	AT	Intergenic	GTTTG**at**ATATATATATATATATATATATATATATTATCA	Dinucleotide repeat
HTHT-11	Chr2:15061965	2	-	AT	UTR	CATAA**at**ATATATATATATATATACGTA	Dinucleotide repeat
HT-1	Chr2:1664191	-2	TA	-	Intergenic	AATAC**TA**TATATATATATATATATTTAT	Dinucleotide repeat
HT-20	Chr2:2284706	2	-	AT	Intergenic	ACCGA**at**ATATATATATATATATATATATATATTATGG	Dinucleotide repeat

HT-21	Chr2:6455752	2	-	TA	Intergenic	TTCTC**ta**TATATATATATATATAATTTG	Dinucleotide repeat
HT-15	Chr2:683661	2	-	TC	UTR	CTCTT**tc**TCTCTCTCTCTCTCTCTCTCTTTAT	Dinucleotide repeat
HT-21	Chr2:894394	-2	AT	-	Intergenic	TATAC**AT**ATATATATATATATATATATTTTT	Dinucleotide repeat
HT-11	Chr3:11857854	-2	AT	-	Intergenic	TAAAA**AT**ATATATATATATATATATATATATAATGT	Dinucleotide repeat
HT-1	Chr3:12575169	-2	TA	-	Intergenic	TTATT**TA**TATATATATATATAAACAC	Dinucleotide repeat
HT-1	Chr3:12691899	2	-	TA	Intergenic	TCTCT**ta**TATATATATATATATAAGAGA	Dinucleotide repeat
HT-15	Chr3:13483796	2	-	AT	Intergenic	ATATA**at**ATATATATATATATATGTTAT	Dinucleotide repeat
HT-18	Chr3:16925630	-2	TA	-	Intergenic	AAGAT**TA**TATATATATATATATATATATATATAATTAA	Dinucleotide repeat
HT-15	Chr3:18395159	2	-	AT	Intron	TAAAT**at**ATATATATATATATATATATATATATATATCTTTG	Dinucleotide repeat
HT-1	Chr3:18782079	2	-	AT	Intergenic	AAAGA**at**ATATATATATATTTCGT	Dinucleotide repeat
HT-15	Chr3:20067393	2	-	CT	Intergenic	CCCTA**ct**CTCTCTCTCTCTCTCTCTCTCGCTA	Dinucleotide repeat
HT-21	Chr3:21676382	-2	TA	-	Intergenic	CTCTC**TA**TATATATATATATATATCAAA	Dinucleotide repeat
HT-11	Chr3:21869819	-2	AT	-	Intergenic	GTAGA**AT**ATATATATATATATATATGTTAA	Dinucleotide repeat
HT-11	Chr3:6172463	-2	AT	-	Intergenic	ATTTG**AT**ATATATATATATATATCTATA	Dinucleotide repeat
HT-20	Chr3:7905919	-2	TA	-	Intergenic	AATTG**TA**TATATATATATATAATGTT	Dinucleotide repeat
HT-20	Chr3:832839	2	-	TA	Intergenic	CTATC**ta**TATATATATATATCTCC	Dinucleotide repeat
HT-20	Chr3:8644732	2	-	AT	Intergenic	ATTTA**at**ATATATATATATATATATATACATT	Dinucleotide repeat
HT-21	Chr4:15394126	-2	AT	-	UTR	GAGAG**AT**ATATATATATATATAGAGA	Dinucleotide repeat
HT-18	Chr4:17720802	-2	TA	-	Intergenic	AGTTT**TA**TATATATATATATATATATATAGAAAG	Dinucleotide repeat

HT-18	Chr4:18453389	-2	AT	-	Intergenic	CACAC**AT**ATATATATATATATATATATATATACTCA	Dinucleotide repeat
HT-21	Chr4:2245472	-2	TA	-	Intergenic	AACAT**TA**TATATATATATATTATT	Dinucleotide repeat
HT-21	Chr5:13111696	-2	TA	-	Intergenic	TATAT**TA**TATATATATATATATATAAAAAG	Dinucleotide repeat
HT-18	Chr5:13863189	-2	AT	-	Intergenic	AATCA**AT**ATATATATATATATATATATATATATTAAAC	Dinucleotide repeat
HT-20	Chr5:19701445	-2	TA	-	Intergenic	ACAAT**TA**TATATATATATATATATATATTTAT	Dinucleotide repeat
HT-21	Chr5:5165091	-2	TA	-	Intergenic	TTAGG**TA**TATATATATATATATATAGTTAA	Dinucleotide repeat
HT-21	Chr5:5594780	2	-	CT	UTR	TTTCC**ct**CTCTCTCTCTCTCTCTCTCCTAG	Dinucleotide repeat
HT-21	Chr1:11339645	-1	A	-	Intron	CTATT**A**AAAAAAAAAAAAAAATGAAA	Homopolymeric
HT-15	Chr1:13126991	-1	A	-	Intergenic	TATCT**A**AAAAAAATCAAC	Homopolymeric
HT-15	Chr1:14606433	-1	A	-	Intergenic	GCCCT**A**AAAAAAAAAATTGAC	Homopolymeric
HT-1	Chr1:16680566	-1	A	-	Intergenic	TACAC**A**AAAAAAAAAATGTGT	Homopolymeric
HT-21	Chr1:19526965	-1	A	-	Intergenic	AAAAG**A**AAAAAAAAAAGTAAA	Homopolymeric
HT-15	Chr1:20686277	-1	A	-	Intergenic	AAGAT**A**AAAAAAAAAGAAGA	Homopolymeric
HT-1	Chr1:20699996	-1	T	-	Intergenic	TATCA**T**TTTTTTTTTTTTAGAAA	Homopolymeric
HT-21	Chr1:21206782	-1	T	-	Intergenic	ACACA**T**TTTTTTTTTTTTATGGA	Homopolymeric
HT-11	Chr1:22891358	-1	T	-	Intergenic	TCACA**T**TTTTTTTTTTTTTGTTAA	Homopolymeric
HT-1	Chr1:23286686	-1	A	-	UTR	CTCTG**A**AAAAAAAAAAAAACTTTG	Homopolymeric
HT-21	Chr1:23690770	-1	T	-	UTR	TGACC**T**TTTTTTTTTCTATA	Homopolymeric
HT-15	Chr1:25695984	2	-	TT	Intergenic	TTCTC**tt**TTTTTTTTTTTTATTTT	Homopolymeric
HT-15	Chr1:26950761	-1	A	-	Intron	TGCTT**A**AAAAAAAAAAAAGTATA	Homopolymeric
HT-20	Chr1:26977659	-1	T	-	Intron	AGATC**T**TTTTTTTTTACGTC	Homopolymeric
HT-21	Chr1:27252098	1	-	A	Intron	AAAAG**a**AAAAAAAAAACAAGT	Homopolymeric
HT-1	Chr1:28209515	-1	A	-	UTR	ACAAG**A**AAAAAAAAAAAATTATA	Homopolymeric
HT-15	Chr1:28297417	-1	T	-	Intron	TTGGC**T**TTTTTTTTTTAATAA	Homopolymeric
HT-11	Chr1:28683950	-1	T	-	Intergenic	TTATG**T**TTTTTTTAGTTG	Homopolymeric

HT-21	Chr1:29383045	-1	A	-	Intergenic	GATGG**A**AAAAAAAAAAAAACTAAG	Homopolymeric
HT-11	Chr1:3381213	-1	T	-	Intergenic	TTCAA**T**TTTTTTTTTTGTGTT	Homopolymeric
HT-20	Chr1:3975532	-1	A	-	Intergenic	CCATT**A**AAAAAAAAAAAAACAGGA	Homopolymeric
HT-18	Chr1:4076288	-1	A	-	Intergenic	CCGTC**A**AAAAAAAAAACAATC	Homopolymeric
HT-21	Chr1:6512154	-1	A	-	Intergenic	TAGAT**A**AAAAAAAAAAGGAAA	Homopolymeric
HT-21	Chr1:7333806	-1	A	-	Intergenic	AAAAG**A**AAAAAAAAAAAATTACC	Homopolymeric
HT-20	Chr1:8319580	-1	T	-	Intergenic	GTTAC**T**TTTTTTTTTTGTCGT	Homopolymeric
HT-1	Chr1:9132314	-1	T	-	Intergenic	TCACG**T**TTTTTTTTTAATAT	Homopolymeric
HT-1	Chr1:9582862	-1	T	-	Intergenic	AGTAA**T**TTTTTTTTGTTAG	Homopolymeric
HT-18	Chr2:11728791	1	-	A	Intergenic	AAAAG**a**AAAAAAAAAGCAAA	Homopolymeric
HT-1	Chr2:12740680	-1	G	-	Intergenic	ACTTA**G**GGGGGGGGGGTATTG	Homopolymeric
HT-1	Chr2:15963389	-1	T	-	Intergenic	TCATA**T**TTTTTTTTTGATTA	Homopolymeric
HT-1	Chr2:16816144	-1	T	-	Intergenic	ATTTG**T**TTTTTTTTTTTTGTTTT	Homopolymeric
HT-20	Chr2:169643	-1	A	-	Intergenic	TTGAC**A**AAAAAAAAAAAAAGCATT	Homopolymeric
HT-1	Chr2:17503175	-1	T	-	Intergenic	TTTAC**T**TTTTTTTTTGGGTA	Homopolymeric
HT-20	Chr2:17670545	1	-	T	Intergenic	CACAG**t**TTTTTTTTTTTTTTTTTTAAAGG	Homopolymeric
HT-1	Chr2:17916933	-1	C	-	Intron	ATACA**C**CCCCCACTAA	Homopolymeric
HT-1	Chr2:2227013	-2	AA	-	Intergenic	TATAT**AA**AATCT	Homopolymeric
HT-20	Chr2:4458123	-1	T	-	Intergenic	TATTG**T**TTTTTTTTTTTTTTTAACAC	Homopolymeric
HT-21	Chr2:5240832	-1	G	-	TE	TCCGT**G**GGGGGGGGGTGGCA	Homopolymeric
HT-21	Chr2:7084619	-1	T	-	Intergenic	GTTAC**T**TTTTTTTTTTTTTTAGTCA	Homopolymeric
HT-20	Chr2:7422128	-1	A	-	Intergenic	AAATT**A**AAAAAAAAAAAGAGAG	Homopolymeric
HT-21	Chr2:8104061	-1	A	-	Intergenic	GAAGG**A**AAAAAAAAAAAGTGAT	Homopolymeric
HT-1	Chr2:8560007	-1	A	-	Intergenic	AAGTT**A**AAAAAAAAGTAGA	Homopolymeric
HT-21	Chr2:8647449	-1	A	-	Intergenic	CACAT**A**AAAAAAAAAAGGTTA	Homopolymeric
HT-20	Chr2:8706400	-1	T	-	Intron	CAAAC**T**TTTTTTTTTTTTTAATTA	Homopolymeric
HT-11	Chr2:9919932	-1	A	-	Intergenic	AAAAG**A**AAAAAAAAAACATAT	Homopolymeric
HT-1	Chr3:11004372	1	-	T	Intergenic	TTTTC**t**TTTTTTTTTTAATAT	Homopolymeric

HT-11	Chr3:1664694	-7	AAAAAAA	-	Intron	AGGTGAAAAAAAAAAAAAAAA**AAAAAAA**GTGAA	Homopolymeric
HT-21	Chr3:16968246	-1	T	-	Intergenic	TACAA**T**TTTTTTTTTTAATCT	Homopolymeric
HT-1	Chr3:16975618	-1	T	-	Intergenic	ACTTA**T**TTTTTTTTATTTA	Homopolymeric
HT-18	Chr3:17260053	-1	A	-	Intergenic	AAATT**A**AAAAAAAAAAAAAAGAGGA	Homopolymeric
HT-21	Chr3:18619801	-1	T	-	Intergenic	TCTAA**T**TTTTTTTTTTCTTTC	Homopolymeric
HT-18	Chr3:18950904	-1	T	-	Intergenic	TAATA**T**TTTTTTTTTTTTTGAGGA	Homopolymeric
HT-20	Chr3:19852394	-1	A	-	Intergenic	CAAAC**A**AAAAAAAAAAATCAAA	Homopolymeric
HT-11	Chr3:20914031	1	-	A	Intergenic	AATGT**a**AAAAAAAAAACTCGA	Homopolymeric
HT-20	Chr3:21412423	-1	A	-	Intergenic	AAAGG**A**AAAAAAAAAACAATT	Homopolymeric
HT-18	Chr3:3253223	-1	A	-	Intergenic	AAAAG**A**AAAAAAAAAAAGAAGA	Homopolymeric
HT-15	Chr3:5373167	-1	A	-	Intergenic	TTTAT**A**AAAAAAAAAAAAATCAAA	Homopolymeric
HT-20	Chr3:6463425	-1	T	-	Intergenic	ACAAC**T**TTTTTTTTTTAGTTA	Homopolymeric
HT-1	Chr3:8824254	1	-	A	Intron	TAAAG**a**AAAAAAAAACAGTT	Homopolymeric
HT-18	Chr4:10786560	1	-	A	Intergenic	GCAAT**a**AAAAAAAAAAAAAAAGTTCA	Homopolymeric
HT-11	Chr4:12985506	-1	A	-	Intergenic	GAAAT**A**AAAAAAAACTGAT	Homopolymeric
HT-1	Chr4:13148371	-1	T	-	Intron	ACACA**T**TTTTTTTAACTC	Homopolymeric
HT-21	Chr4:13828476	-14	AAAAAAAAAGAAAA	-	UTR	AAAAA**AAAGAAAAAAAAAA**GAGCT	Homopolymeric
HT-15	Chr4:1439209	-1	A	-	Intergenic	ACAAT**A**AAAAAAAAAAATAATG	Homopolymeric
HT-20	Chr4:15698587	-1	A	-	Intergenic	CAAAG**A**AAAAAAAAAAAGAGTA	Homopolymeric
HT-20	Chr4:16597704	-1	A	-	Intergenic	CTAAG**A**AAAAAAACCCTC	Homopolymeric
HT-21	Chr4:17794647	-1	T	-	Intergenic	TTGTA**T**TTTTTTTTTTGTCTT	Homopolymeric
HT-1	Chr4:2659645	-1	T	-	Intergenic	TTTTC**T**TTTTTTTTTTCAATC	Homopolymeric
HT-15	Chr4:2867176	-1	A	-	Intergenic	TAGAG**A**AAAAAAAAATGCAT	Homopolymeric
HT-21	Chr4:3282646	-1	A	-	Intergenic	AAAAC**A**AAAAAAAAAAATTGAA	Homopolymeric
HT-15	Chr4:5403159	-1	T	-	Intergenic	ACTTA**T**TTTTTTTTTTTTGTATT	Homopolymeric
HT-15	Chr4:9886010	-1	A	-	Intergenic	AAAAG**A**AAAAAAAAAATATAT	Homopolymeric
HT-11	Chr4:9960777	-1	A	-	Intergenic	GGATG**A**AAAAAAAAAAATAGAC	Homopolymeric
HT-1	Chr5:10391521	-1	T	-	Intergenic	ATTAC**T**TTTTTTTTCATAT	Homopolymeric

HT-20	Chr5:10457533	-1	A	-	Intergenic	AACCG**A**AAAAAAACTCGT	Homopolymeric
HT-21	Chr5:11365113	-1	G	-	Intergenic	GTGGA**G**GGGGGGGGGGCTTGG	Homopolymeric
HT-18	Chr5:1142548	-1	T	-	Intergenic	AATTG**T**TTTTTTTTTTAATTA	Homopolymeric
HT-11	Chr5:12004467	-1	T	-	Intergenic	CTACG**T**TTTTTTTTTTGTCTT	Homopolymeric
HT-20	Chr5:13062411	-1	A	-	Intergenic	ACAAT**A**AAAAAAAAATAAAA	Homopolymeric
HT-15	Chr5:13754181	-1	A	-	Intergenic	ATTAC**A**AAAAAGATCA	Homopolymeric
HT-20	Chr5:14537685	-1	A	-	Intron	ATTCG**A**AAAAAAAAAACAATG	Homopolymeric
HT-15	Chr5:15630622	-1	A	-	Intergenic	CAAAT**A**AAAAAAAAAAGTTAG	Homopolymeric
HT-11	Chr5:17148361	-1	A	-	Intergenic	AAAAT**A**AAAAAAAAAAGGTTT	Homopolymeric
HT-18	Chr5:18986634	-1	T	-	Intergenic	ACAGA**T**TTTTTTTTTATCAT	Homopolymeric
HT-18	Chr5:21837733	-11	AGACCTACCTT	-	Intergenic	CGTCT**AGACCTACCTT**TTAAC	Homopolymeric
HT-11	Chr5:23393276	-1	T	-	Intergenic	CGATC**T**TTTTTTTTTTTTTATCAA	Homopolymeric
HT-1	Chr5:24565073	-1	A	-	Intergenic	AAAAT**A**AAAAAAAATTGTA	Homopolymeric
HT-20	Chr5:25400022	-1	T	-	Intergenic	TAAAC**T**TTTTTTTTTTTTTACATT	Homopolymeric
HT-11	Chr5:26126363	1	-	T	UTR	ATTCC**t**TTTTTTTTTTAGTTA	Homopolymeric
HT-21	Chr3:13017805	-22	TGGGGGGTGGCTTC ACTGTAAT	-	TE	ATCGA**TGGGGGGTGGCTTCACTGTAAT**CCTAC	Random
HT-20	Chr3:18460777	1	-	A	Intergenic	TTCTT**a**ATTAT	Random
HT-18	Chr5:21837749	2	-	CG	Intergenic	TTAAC**cg**TCCTT	Random
HT-18	Chr5:21837751	5	-	TACAT	Intergenic	AACTC**tacat**CTTGA	Random
HT-21	Chr4:8452944	-1	G	-	UTR	GTAGT**G**GGAGAAA	Random
HT-21	Chr5:3633511	-3	TGT	-	Intergenic	TTAGT**TGT**TGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTCAAGTC	Trinucleotide repeat
HT-1	Chr1:14270590	0	G	A	TE		
HT-1	Chr1:15110486	0	C	G	Intergenic		
HT-1	Chr1:23197490	0	G	A	Intergenic		
HT-1	Chr2:275106	0	C	G	Intergenic		
HT-1	Chr2:3179661	0	A	C	Intergenic		
HT-1	Chr2:7327359	0	G	A	Pseudogene		

HT-1	Chr2:10442268	0	A	T	Intergenic		
HT-1	Chr2:12002525	0	A	C	Intergenic		
HT-1	Chr3:2611048	0	G	C	Intron		
HT-1	Chr3:12619911	0	T	A	Intergenic		
HT-1	Chr3:21971906	0	A	T	Intergenic		
HT-1	Chr4:3789271	0	G	A	TE		
HT-1	Chr4:4723209	0	G	A	Intergenic		
HT-1	Chr4:5480807	0	G	T	TE		
HT-1	Chr5:8755519	0	T	A	Intron		
HT-1	Chr5:8770714	0	G	A	Intergenic		
HT-1	Chr5:9460202	0	G	A	Intergenic		
HT-1	Chr5:9931692	0	G	A	Intergenic		
HT-1	Chr5:11126531	0	C	T	Intergenic		
HT-1	Chr5:21915294	0	G	A	Intergenic		
HT-11	Chr1:5759844	0	G	A	Intron		
HT-11	Chr1:8166201	0	G	A	Intergenic		
HT-11	Chr1:11117631	0	G	A	Intergenic		
HT-11	Chr1:13165211	0	C	T	Intergenic		
HT-11	Chr1:15879385	0	C	T	Intergenic		
HT-11	Chr2:715687	0	G	A	Intergenic		
HT-11	Chr2:2639933	0	C	G	TE		
HT-11	Chr2:3721045	0	A	G	Intergenic		
HT-11	Chr2:3994058	0	C	T	Intergenic		
HT-11	Chr2:5510145	0	C	T	TE		
HT-11	Chr2:7171329	0	C	T	TE		
HT-11	Chr2:11373558	0	G	A	Intergenic		
HT-11	Chr2:19454254	0	T	A	CDS		
HT-11	Chr3:7136342	0	C	T	Intron		

HT-11	Chr3:12862151	0	A	C	TE		
HT-11	Chr3:13596662	0	G	C	Intergenic		
HT-11	Chr4:1507960	0	C	T	Intergenic		
HT-11	Chr4:2101311	0	C	T	Intergenic		
HT-11	Chr4:4512917	0	A	C	TE		
HT-11	Chr4:15261455	0	C	T	Intergenic		
HT-11	Chr5:7300002	0	C	A	Pseudogene		
HT-11	Chr5:19675362	0	G	A	Intergenic		
HT-11	Chr5:26671119	0	T	A	Intergenic		
HT-15	Chr1:9454252	0	A	T	Intergenic		
HT-15	Chr1:11127336	0	G	A	Intergenic		
HT-15	Chr1:12161374	0	G	A	Intergenic		
HT-15	Chr1:13447138	0	G	A	TE		
HT-15	Chr1:15720647	0	G	A	Pseudogene		
HT-15	Chr3:14534689	0	C	G	Intergenic		
HT-15	Chr4:8319421	0	C	T	Intergenic		
HT-15	Chr4:8942472	0	G	A	Intergenic		
HT-15	Chr4:12484695	0	G	A	Intergenic		
HT-15	Chr5:12981924	0	G	A	Intron		
HT-15	Chr5:14002473	0	C	T	Intergenic		
HT-15	Chr5:25663357	0	A	T	Intergenic		
HT-18	Chr1:5970757	0	A	T	Intergenic		
HT-18	Chr1:17874078	0	G	T	Intergenic		
HT-18	Chr1:21970368	0	G	T	Intron		
HT-18	Chr1:28843265	0	G	T	Intergenic		
HT-18	Chr4:10400859	0	C	T	Intergenic		
HT-18	Chr4:16988590	0	G	C	CDS		
HT-18	Chr5:14087316	0	C	T	TE		

HT-18	Chr5:19910144	0	G	A	Intergenic		
HT-18	Chr5:20785553	0	A	G	CDS		
HT-18	Chr5:21837746	0	A	G	Intergenic		
HT-18	Chr5:22534434	0	C	T	Intergenic		
HT-20	Chr1:8042278	0	A	T	Intergenic		
HT-20	Chr1:15538681	0	G	A	Intergenic		
HT-20	Chr1:23872766	0	A	G	CDS		
HT-20	Chr2:10603285	0	C	G	Intergenic		
HT-20	Chr3:11408296	0	G	A	Intergenic		
HT-20	Chr4:3035490	0	G	A	Intergenic		
HT-20	Chr4:3606221	0	A	G	Intergenic		
HT-20	Chr4:5840966	0	C	A	CDS		
HT-20	Chr5:250133	0	C	A	Intergenic		
HT-20	Chr5:24944428	0	C	T	CDS		
HT-21	Chr1:6900138	0	T	C	Intergenic		
HT-21	Chr2:2471240	0	A	G	Intergenic		
HT-21	Chr2:5334363	0	T	G	TE		
HT-21	Chr2:6718575	0	A	C	TE		
HT-21	Chr2:10690015	0	C	T	CDS		
HT-21	Chr2:12286260	0	G	A	CDS		
HT-21	Chr3:11187461	0	G	A	TE		
HT-21	Chr3:12718778	0	G	A	TE		
HT-21	Chr3:14397126	0	A	G	Intergenic		
HT-21	Chr3:14718365	0	G	A	TE		
HT-21	Chr3:15478326	0	G	C	Intergenic		
HT-21	Chr3:17451934	0	T	C	Intron		
HT-21	Chr4:4370464	0	G	A	TE		
HT-21	Chr4:4880703	0	C	T	TE		

HT-21	Chr5:8107226	0	T	C	CDS		
HT-21	Chr5:9712336	0	G	A	TE		
CT-10	Chr1:23761255	0	A	G	Intergenic		
CT-10	Chr1:24306494	-1	T	-	Intergenic		
CT-2	Chr1:57619	0	G	A	CDS		
CT-2	Chr2:15883885	-1	A	-	UTR		
CT-2	Chr2:8555726	0	G	A	Intergenic		
CT-2	Chr5:23097639	0	T	C	CDS		
CT-2	Chr5:5707226	0	G	A	Intron		
CT-4	Chr2:13356157	0	A	C	Intron		
CT-4	Chr2:5586514	0	C	A	Pseudogene		
CT-4	Chr3:17006305	0	T	C	Intron		
CT-4	Chr3:17465651	0	T	G	CDS		
CT-4	Chr4:3775263	0	T	A	Intergenic		
CT-4	Chr5:19360667	0	T	G	TE		
CT-6	Chr1:13104509	0	C	T	Intergenic		
CT-6	Chr3:13532744	0	C	T	Intergenic		

Supplemental Table 2. Entire list of all detected HT- and CT-induced mutations. List shows each individual DNA sequence mutation identified in the six genome-sequenced G11 HT MA plants and five genome-sequenced G6 CT MA plants. * The column titled “Flanking indel mutation sequence” shows deletions in red uppercase text and insertions in red lowercase text. Also shown in underlined text are the dinucleotide repeats, trinucleotide repeat, and homopolymeric sequences the indel mutations are associated with.

Species	Organism	Organism and genotype	Description	Environmental temperature	Number of indel mutations reported	Number of SNV mutations reported	Indel/ SNV ratio	Reference
EUBACTERIA	Bacteria	*Micrococcus sp. KBS0714*	Wild type	ST	83	170	0.488	(Long et al. 2018)
EUBACTERIA	Bacteria	*Kineococcus radiotolerans*	Wild type	ST	122	280	0.436	(Long et al. 2018)
EUBACTERIA	Bacteria	*Bacillus subtilis*	Wild type	ST	29	69	0.420	(Sung et al. 2012)
EUBACTERIA	Bacteria	*Deinococcus radiodurans*	DNA repair-deficient	ST	35	127	0.276	(Long et al. 2015)
EUBACTERIA	Bacteria	*Vibrio fischeri ES114*	Wild type	ST	60	219	0.274	(Dillon et al. 2017)
EUBACTERIA	Bacteria	*Escherichia coli*	Wild type	ST	20	73	0.274	(Shewaramani et al. 2017)
EUBACTERIA	Bacteria	*Vibrio cholerae 2740-81*	DNA repair defective	ST	273	1022	0.267	(Dillon et al. 2017)
EUBACTERIA	Bacteria	*Mycobacterium smegmatis MC2 155*	Wild type	ST	207	856	0.242	(Kucukyildirim et al. 2016)
EUBACTERIA	Bacteria	*Escherichia coli*	Wild type	ST	17	74	0.230	(Shewaramani et al. 2017)
EUBACTERIA	Bacteria	*Escherichia coli*	Wild type	ST - 37°C	342	1636	0.209	(Chu et al. 2018)
EUBACTERIA	Bacteria	*Escherichia coli*	Wild type	CT - 28°C	214	1041	0.206	(Chu et al. 2018)
EUBACTERIA	Bacteria	*Arthrobacter sp. KBS0703*	Wild type	ST	88	451	0.195	(Long et al. 2018)
EUBACTERIA	Bacteria	*Mesoplasma florum L1*	Wild type	ST	101	527	0.192	(Sung et al. 2012)
EUBACTERIA	Bacteria	*Ruegeria pomeroyi DSS-3*	Wild type	ST	30	161	0.186	(Sun et al. 2017)
EUBACTERIA	Bacteria	*Pseudomonas aeruginosa*	Wild type	ST	8	44	0.182	(Dettman et al. 2016)
EUBACTERIA	Bacteria	*Escherichia coli*	Wild type	CT - 25°C	191	1108	0.172	(Chu et al. 2018)
EUBACTERIA	Bacteria	*Vibrio cholerae 2740-80*	Wild type	ST	22	138	0.159	(Dillon et al. 2017)
EUBACTERIA	Bacteria	*Caulobacter crescentus NA1000*	Wild type	ST	41	259	0.158	(Long et al. 2018)
EUBACTERIA	Bacteria	*Pseudomonas aeruginosa*	DNA repair defective	ST	108	690	0.157	(Dettman et al. 2016)
EUBACTERIA	Bacteria	*Staphylococcus epidermidis ATCC 12228*	Wild type	ST	45	294	0.153	(Sung et al. 2016)
EUBACTERIA	Bacteria	*Rhodobacter sphaeroides ATCC17025*	Wild type	ST	16	107	0.150	(Long et al. 2018)
EUBACTERIA	Bacteria	*Gemmata obscuriglobus DSM5831*	Wild type	ST	29	197	0.147	(Long et al. 2018)
EUBACTERIA	Bacteria	*Flavobacterium sp. KBS0721*	Wild type	ST	85	580	0.147	(Long et al. 2018)
EUBACTERIA	Bacteria	*Burkhoderia cenocepacia HI2424*	Wild type	ST	33	245	0.135	(Dillon et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - PFM35 uvrA*	DNA repair defective	ST	40	316	0.127	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Staphylococcus aureus ATCC 25923*	Wild type	ST	33	274	0.120	(Long et al. 2018)

EUBACTERIA	Bacteria	*Agrobacterium tumefaciens*	Wild type	ST	50	425	0.118	(Sung et al. 2016)
EUBACTERIA	Bacteria	*Escherichia coli - PFM40 alkA tagA*	DNA repair defective	ST	30	265	0.113	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli*	DNA repair defective	ST	34	306	0.111	(Lee et al. 2012)
EUBACTERIA	Bacteria	*Colwellia psychrerythraea 34H*	Wild type	ST	44	400	0.110	(Long et al. 2018)
EUBACTERIA	Bacteria	*Escherichia coli - PFM133 umuDC dinB polB*	DNA repair defective	ST	27	252	0.107	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - PFM180 xthA nfo*	DNA repair defective	ST	36	339	0.106	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - PFM2*	Wild type	ST	24	246	0.098	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli*	Wild type	ST	9	93	0.097	(Lee et al. 2012)
EUBACTERIA	Bacteria	*Escherichia coli - PFM88 ada ogt*	DNA repair defective	ST	24	250	0.096	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - ED1a*	Wild type	ST	37	407	0.091	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Vibrio fischeri ES115*	DNA repair defective	ST	382	4313	0.089	(Dillon et al. 2017)
EUBACTERIA	Bacteria	*Escherichia coli*	Wild type	ST	12	140	0.086	(Lee et al. 2012)
EUBACTERIA	Bacteria	*Escherichia coli - PFM101 umuDC dinB*	DNA repair defective	ST	23	269	0.086	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Janthinobacterium lividum KBS0711*	Wild type	ST	9	106	0.085	(Long et al. 2018)
EUBACTERIA	Bacteria	*Escherichia coli - PFM91 nfi*	DNA repair defective	ST	25	335	0.075	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - IAI1*	Wild type	ST	31	422	0.073	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Pseudomonas fluorescens*	DNA repair defective	ST	2201	31106	0.071	(Long et al. 2014)
EUBACTERIA	Bacteria	*Lactococcus lactis DSMZ20481*	Wild type	ST	56	813	0.069	(Long et al. 2018)
EUBACTERIA	Bacteria	*Deinococcus radiodurans*	Wild type	ST	19	388	0.049	(Long et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - PFM2m*	Wild type	ST	13	277	0.047	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - PFM22 nth nei*	DNA repair defective	ST	6	209	0.029	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - PFM6 mutY*	DNA repair defective	ST	6	485	0.012	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - PFM94 mutM mutY*	DNA repair defective	ST	6	4282	0.001	(Foster et al. 2015)
EUBACTERIA	Bacteria	*Escherichia coli - PFM61 mutT*	DNA repair defective	ST	1	2234	0.000	(Foster et al. 2015)
MULTICELLULAR EUKARYOTES	Plant	*Arabiposis thaliana*	Wild type	HT	138	92	1.500	This study
MULTICELLULAR EUKARYOTES	Plant	*Arabiposis thaliana - Atmsh2-1*	DNA repair defective	ST	4663	4048	1.152	(Belfield et al. 2018)

MULTICELLULAR EUKARYOTES	Fly	*Chironomus riparius*	Wild type	ST	25	26	0.962	(Oppold and Pfenninger 2017)
MULTICELLULAR EUKARYOTES	Plant	*Arabiposis thaliana*	Fast-neutron irradiated wild type	ST	44	64	0.688	(Belfield et al. 2012)
MULTICELLULAR EUKARYOTES	Plant	*Arabiposis thaliana*	Wild type	ST	30	72	0.417	(Jiang et al. 2014)
MULTICELLULAR EUKARYOTES	Honey bee	*Apis mellifera*	Wild type	ST	8	27	0.296	(Yang et al. 2015)
MULTICELLULAR EUKARYOTES	Plant	*Oryza sativa*	Wild type	ST	14	51	0.275	(Yang et al. 2015)
MULTICELLULAR EUKARYOTES	Plant	*Prunus interspecific crosses (P. davidiana × P. persica)*	Wild type	ST	28	144	0.194	(Xie et al. 2016)
MULTICELLULAR EUKARYOTES	Plant	*Prunus intraspecific crosses (P. persica and P. mira)*	Wild type	ST	18	96	0.188	(Xie et al. 2016)
MULTICELLULAR EUKARYOTES	Plant	*Arabiposis thaliana*	Wild type	ST	17	99	0.172	(Ossowski et al. 2010)
MULTICELLULAR EUKARYOTES	Plant	*Arabiposis thaliana*	Wild type	ST	7	44	0.159	(Jiang et al. 2014)
MULTICELLULAR EUKARYOTES	Fly	*Drosophila melanogaster*	Wild type	ST	188	1203	0.156	(Huang et al. 2016)
MULTICELLULAR EUKARYOTES	Nematode	*Caenorhabditis elegans - N2*	Wild type	ST	7	60	0.117	(Meier et al. 2014)
MULTICELLULAR EUKARYOTES	Mouse	*Mus musculus*	Wild type	ST	7	63	0.106	(Uchimura et al. 2015)
MULTICELLULAR EUKARYOTES	Water flea	*Daphnia pulex - ASEX*	Wild type	ST	58	569	0.102	(Keith et al. 2016)
MULTICELLULAR EUKARYOTES	Bumblebee	*Bombus terrestris*	Wild type	ST	2	23	0.087	(Liu et al. 2017)
MULTICELLULAR EUKARYOTES	Fly	*Drosophila melanogaster*	Wild type	ST	60	732	0.082	(Schrider et al. 2013)
MULTICELLULAR EUKARYOTES	Human	*Homo sapiens*	Wild type	ST	1282	17812	0.072	(Besenbacher et al. 2016)
MULTICELLULAR EUKARYOTES	Fly	*Drosophila melanogaster*	Wild type	ST	49	786	0.062	(Sharp and Agrawal 2016)
MULTICELLULAR EUKARYOTES	Western chimpanzee	*Pan troglodytes verus*	Wild type	ST	169387	3957360	0.043	(Venn et al. 2014)
MULTICELLULAR EUKARYOTES	Fly	*Drosophila melanogaster*	Wild type	ST	7	174	0.040	(Keightley et al. 2009)
MULTICELLULAR EUKARYOTES	Mouse	*Mus musculus*	Wild type	ST	4	118	0.034	(Uchimura et al. 2015)
MULTICELLULAR EUKARYOTES	Fly	*Drosophila melanogaster*	Wild type	ST	22	732	0.030	(Schrider et al. 2013)
MULTICELLULAR EUKARYOTES	Water flea	*Daphnia pulex - CYC*	Wild type	ST	4	164	0.024	(Keith et al. 2016)
MULTICELLULAR EUKARYOTES	Mouse	*Mus musculus*	Mutator *Pold1*$^{exo/exo}$ line C	ST	28	1304	0.021	(Uchimura et al. 2015)

MULTICELLULAR EUKARYOTES	Mouse	*Mus musculus*	Mutator *Pold1*$^{exo/exo}$ line D	ST	21	1472	0.014	(Uchimura et al. 2015)
MULTICELLULAR EUKARYOTES	Water flea	*Daphnia pulex*	Wild type	ST	6	477	0.013	(Flynn et al. 2017)
MULTICELLULAR EUKARYOTES	Butterfly	*Heliconius melpomene*	Wild type	ST	0	9	0.000	(Keightley et al. 2015)
UNICELLULAR EUKARYOTES	Protozoan parasite	*Plasmodium falciparum - 3D7*	Wild type	ST	164	15	10.933	(Hamilton et al. 2017)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - rad27Δ*	DNA replication defective	ST	220	175	1.257	(Serero et al. 2014)
UNICELLULAR EUKARYOTES	Fission Yeast	*Schizosaccharomyces pombe*	Wild type	ST	335	344	0.974	(Behringer and Hall 2015)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - msh2Δ*	DNA repair defective	ST	706	965	0.732	(Serero et al. 2014)
UNICELLULAR EUKARYOTES	Green algae	*Chlamydomonas reinhardtii*	Wild type	ST	13	20	0.650	(Sung et al. 2012)
UNICELLULAR EUKARYOTES	Algae	*Bathycoccus prasinos*	Wild type	ST	10	22	0.455	(Krasovec et al. 2017)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	95	279	0.341	(Liu and Zhang 2019)
UNICELLULAR EUKARYOTES	Green algae	*Chlamydomonas reinhardtii - CC-2391*	Mutator *strain*	ST	405	1215	0.333	(Ness et al. 2015)
UNICELLULAR EUKARYOTES	Green algae	*Chlamydomonas reinhardtii - CC-2937*	Wild type	ST	149	508	0.293	(Ness et al. 2015)
UNICELLULAR EUKARYOTES	Fission Yeast	*Schizosaccharomyces pombe*	Wild type	ST	119	422	0.282	(Farlow et al. 2015)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - mec1Δ tel1Δ*	DNA reapir and replication defective	ST	6	24	0.250	(Serero et al. 2014)
UNICELLULAR EUKARYOTES	Ichthyosporean	*Sphaeroforma arctica JP610*	Wild type	ST	79	323	0.245	(Long et al. 2018)
UNICELLULAR EUKARYOTES	Algae	*Ostreococcus mediterraneus*	Wild type	ST	11	54	0.204	(Krasovec et al. 2017)
UNICELLULAR EUKARYOTES	Algae	*Micromonas pusilla*	Wild type	ST	14	71	0.197	(Krasovec et al. 2017)
UNICELLULAR EUKARYOTES	Green algae	*Chlamydomonas reinhardtii - CC-2344*	Mutator *strain*	ST	181	946	0.191	(Ness et al. 2015)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	76	403	0.189	(Liu and Zhang 2019)
UNICELLULAR EUKARYOTES	Green algae	*Chlamydomonas reinhardtii - CC-1952*	Wild type	ST	66	366	0.180	(Ness et al. 2015)
UNICELLULAR EUKARYOTES	Protozoa	*Paramecium tetraurelia*	Wild type	ST	5	29	0.172	(Sung et al. 2012)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - pol32Δ*	DNA replication defective	ST	8	47	0.170	(Serero et al. 2014)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	21	125	0.168	(Liu and Zhang 2019)

UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	20	132	0.152	(Liu and Zhang 2019)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	HT - 37-42°C	18	123	0.146	(Huang et al. 2018)
UNICELLULAR EUKARYOTES	Algae	*Ostreococcus tauri*	Wild type	ST	13	91	0.143	(Krasovec et al. 2017)
UNICELLULAR EUKARYOTES	Green algae	*Chlamydomonas reinhardtii - CC-1373*	Wild type	ST	222	1696	0.131	(Ness et al. 2015)
UNICELLULAR EUKARYOTES	Yeast	*Rhodotorula toruloides ATCC10788*	Wild type	ST	83	714	0.116	(Long et al. 2016)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	19	166	0.114	(Liu and Zhang 2019)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - mre11Δ*	DNA recombination defective	ST	5	49	0.102	(Serero et al. 2014)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	11	112	0.098	(Liu and Zhang 2019)
UNICELLULAR EUKARYOTES	Green algae	*Chlamydomonas reinhardtii - CC-2342*	Wild type	ST	73	824	0.089	(Ness et al. 2015)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	17	198	0.086	(Liu and Zhang 2019)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - WT*	Diploid	ST	83	1183	0.070	(Sharp et al. 2018)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - clb5Δ*	Cell cycle defective	ST	7	156	0.045	(Serero et al. 2014)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - WT*	Haploid	ST	29	716	0.041	(Sharp et al. 2018)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - pif1Δ*	Maintenance of mitochondrial genome and telomere length defective	ST	3	75	0.040	(Serero et al. 2014)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - tsa1Δ*	Oxidative stress defective	ST	19	512	0.037	(Serero et al. 2014)
UNICELLULAR EUKARYOTES	Diatom	*Phaeodactylum tricornutum*	Wild type	ST	5	146	0.034	(Krasovec et al. 2019)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	1	33	0.030	(Lynch et al. 2008)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	26	873	0.030	(Zhu et al. 2014)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	0	29	0.000	(Nishant et al. 2010)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae - cac1Δ cac3Δ*	Nucleosome deposition defective	ST	0	40	0.000	(Serero et al. 2014)
UNICELLULAR EUKARYOTES	Yeast	*Saccharomyces cerevisiae*	Wild type	ST	0	41	0.000	(Serero et al. 2014)

Supplemental Table 3. Details of indel/SNV-ratio datasets used for Fig. 5C.

A

	Number of ST MNase-sequenced bases in nucleosomic categories			
Chromosome	**Nucleosome**	**Edge-of-nucleosome**	**Non-nucleosome**	**Total**
Chr1	12,725,320	5,527,610	12,174,741	30,427,671
Chr2	8,421,820	3,643,330	7,633,139	19,698,289
Chr3	10,066,850	4,370,270	9,022,710	23,459,830
Chr4	7,975,490	3,454,000	7,155,566	18,585,056
Chr5	11,453,920	4,990,800	10,530,782	26,975,502
Total	**50,643,400**	**21,986,010**	**46,516,938**	**119,146,348**
Percentage	42.5%	18.5%	39.0%	100%
Percentage	61.0%		39.0%	100%

B

	Number of HT MNase-sequenced bases in nucleosomic categories			
Chromosome	**Nucleosome**	**Edge-of-nucleosome**	**Non-nucleosome**	**Total**
Chr1	12,919,480	5,437,410	12,070,781	30,427,671
Chr2	8,549,770	3,582,010	7,566,509	19,698,289
Chr3	10,221,150	4,295,670	8,943,010	23,459,830
Chr4	8,086,600	3,391,510	7,106,946	18,585,056
Chr5	11,636,520	4,895,280	10,443,702	26,975,502
Total	**51,413,520**	**21,601,880**	**46,130,948**	**119,146,348**
Percentage	43.2%	18.1%	38.7%	100%
Percentage	61.3%		38.7%	100%

Supplemental Table 4. MNase-digested genomic DNA WGS data summary. MNase-digested genomic DNA extracted from plants grown at ST (A) and HT (B) was aligned to the TAIR10 reference using the improved nucleosome-positioning algorithm (iNPS (Chen et al. 2014)) to determine nucleosome occupancy profiles. Number of bases reported in nucleosome, edge-of-nucleosome and non-nucleosome regions in the genomes of plants are listed for Chromosomes 1–5. Also shown are the percentages of bases in nucleosomal, edge-of-nuclesome and non-nucleosomal categories, as well as the result of combining mutations located in nucleosome and edge-of-nucleosome regions into a single nucleosome-associated category.

A

Mutation	ST mutation count	ST mutation nucleosome category	Observed % of mutations in nucleosome category	Predicted % of mutations in nucleosome category
Indel	8	Nucleosome	44.4%	42.5%
	4	Edge-of-nucleosome	22.2%	18.5%
	6	Non-nucleosome	33.3%	39.0%
Total	**18**		**100%**	**100%**
SNV	68	Nucleosome	47.6%	42.5%
	24	Edge-of-nucleosome	16.8%	18.5%
	51	Non-nucleosome	35.7%	39.0%
Total	**143**		**100%**	**100%**

B

Mutation	ST mutation count	ST mutation nucleosome category	Observed % of mutations in nucleosome category	Predicted % of mutations in nucleosome category
Indel	12	Nucleosome-associated	66.7%	61.0%
	6	Non-nucleosome	33.3%	39.0%
Total	**18**		**100%**	**100%**
SNV	92	Nucleosome-associated	64.3%	61.0%
	51	Non-nucleosome	35.7%	39.0%
Total	**143**		**100%**	**100%**

C

Mutation	HT mutation count	HT mutation nucleosome category	Observed % of mutations in nucleosome category	Predicted % of mutations in nucleosome category
Indel	35	Nucleosome	26.5%	43.2%
	18	Edge-of-nucleosome	13.6%	18.1%
	79	Non-nucleosome	59.8%	38.7%
Total	**132**		**100%**	**100%**
SNV	43	Nucleosome	46.7%	43.2%
	18	Edge-of-nucleosome	19.6%	18.1%
	31	Non-nucleosome	33.7%	38.7%
Total	**92**		**100%**	**100%**

D

Mutation	HT mutation count	HT mutation nucleosome category	Observed % of mutations in nucleosome category	Predicted % of mutations in nucleosome category
Indel	53	Nucleosome-associated	40.2%	61.3%
	79	Non-nucleosome	59.8%	38.7%
Total	**132**		**100%**	**100%**
SNV	61	Nucleosome-associated	66.3%	61.3%
	31	Non-nucleosome	33.7%	38.7%
Total	**92**		**100%**	**100%**

Supplemental Table 5. Frequencies of indel and SNV mutations in different nucleosome categories. HT-induced (Supplemental Table 2) and ST (Ossowski et al. 2010; Jiang et al. 2014) mutations were overlaid onto the HT and ST MNase-seq nucleosome DNA profiles generated with iNPS (Chen et al. 2014) (see Methods and Supplemental Fig. 6). Shown are the observed genome-wide frequencies of ST indel and SNV mutations in different categories, versus the predicted (random distribution) expectation frequencies (see Supplemental Table 4A) in nucleosome, edge of nucleosome, and non-nucleosome regions (A). (B) Combined ST mutations located in nucleosome and edge-of-nucleosome regions shown as a single nucleosome-associated category (see Supplemental Table 4A). (C) and (D) Analyses of HT-induced indel and SNV mutations (as in (A) and (B), see Supplemental Table 4B predicted (random distribution) expectation frequencies).

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