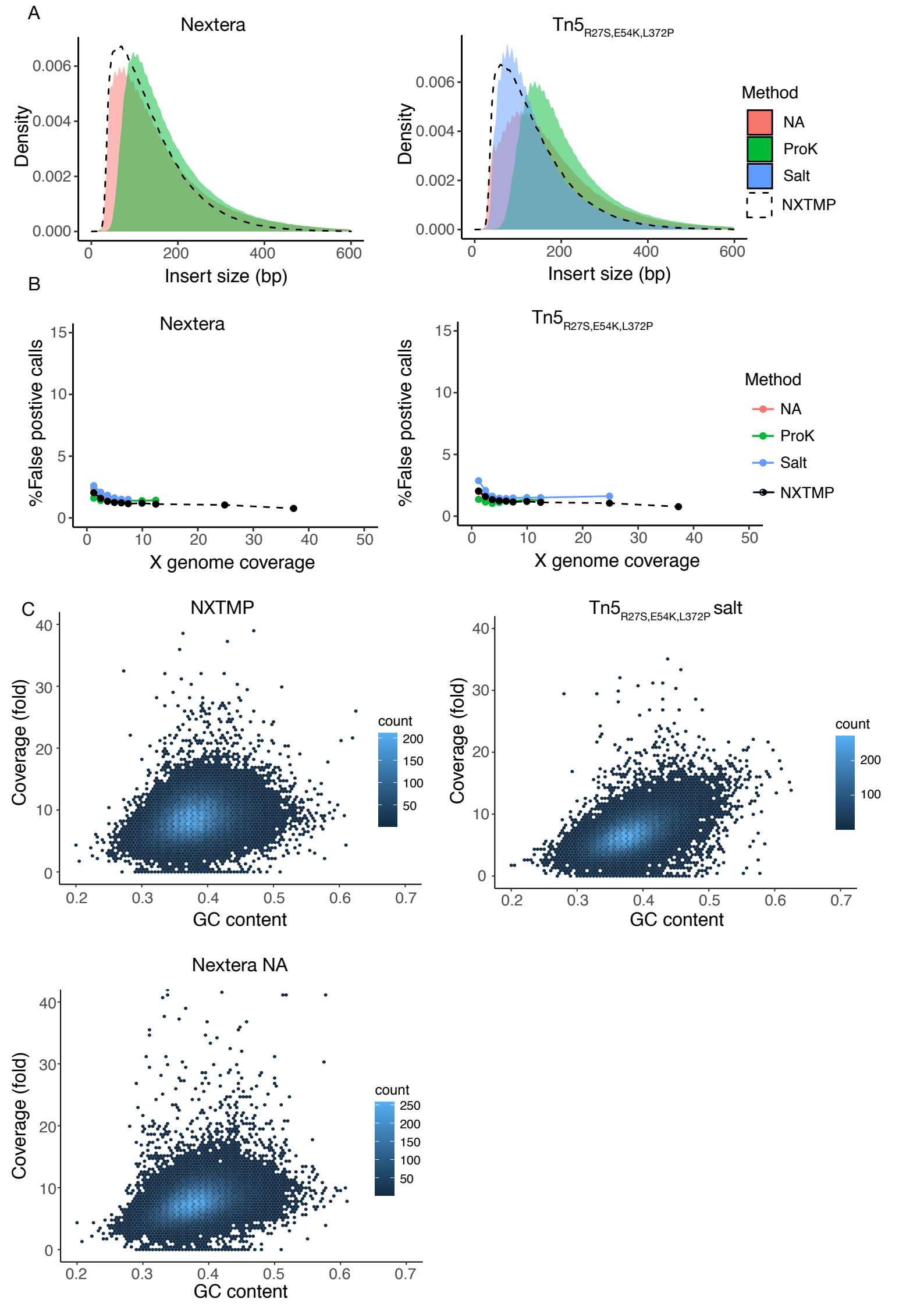
A
Nextera
Tn5R27S,E54K,L372P
Density
Insert size (bp)
Method
NA
ProK
Salt
NXTMP
B
%False postive calls
X genome coverage
C
NXTMP
Tn5R27S,E54K,L372P salt
Nextera NA
Coverage (fold)
GC content
count

Figure S1. Insert size, false positive calling-rate and GC-bias in extraction-free libraries. Salt (blue) indicates nucleosome dissociation by incubation with 1.125 M NaOAc after tagmentation; ProK (green) indicates nucleosome dissociation by incubation with Proteinase K at 65°C after tagmentation; NA (salmon) indicates no nucleosome dissociation step. NXTMP (black dashed line) is a library processed from 150 pg extracted genomic DNA with the Nextera XT kit and serves as reference. A) Distribution of fragment insert sizes. Final cleanup used 1.8X Ampure beads. The Salt condition is omitted for Nextera due to insufficient reads. B) Fraction of false positive SNP calls as a function of sequencing depth, with false positive call rate = number of mis-called SNPs/total number of SNPs. C) GC content related coverage bias in libraries prepared from extracted genomic DNA with the Nextera XT kit (NXTMP, upper left panel), from zymolyase and heat treated cells with the Nextera XT kit (Nextera NA, lower left panel) or with the extraction-free protocol using $Tn5_{R27S,E54K,L372P}$ and nucleosome dissociation by salt (upper right panel). GC content of the *S. cerevisiae* reference genome was determined for 500 bp sliding windows with 250 bp overlap, and per-base GC content was calculated as the average of overlapping windows.

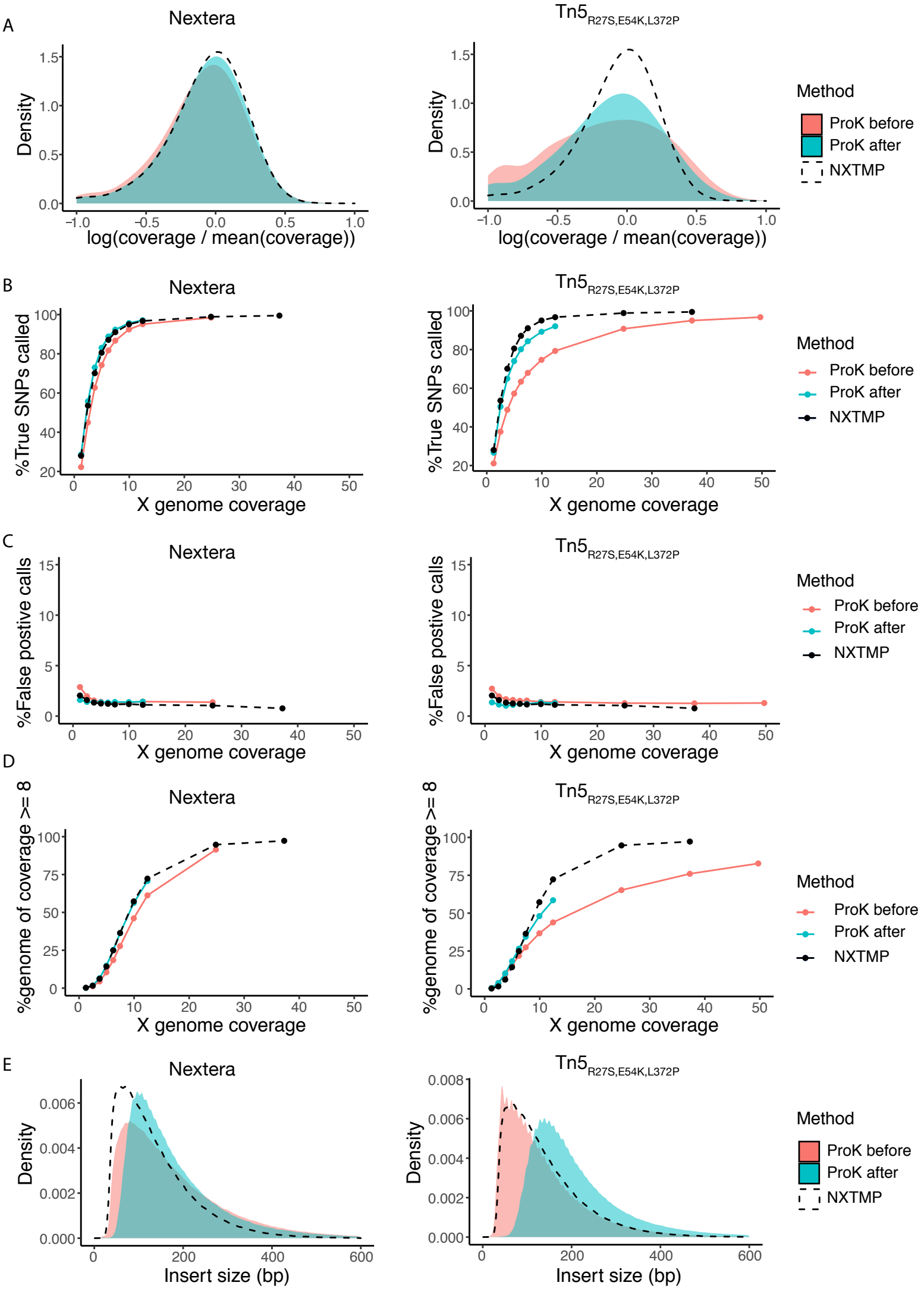
A
Nextera
Tn5R27S,E54K,L372P
Density
log(coverage / mean(coverage))
Method
ProK before
ProK after
NXTMP
B
%True SNPs called
X genome coverage
C
%False postive calls
D
%genome of coverage >= 8
E
Insert size (bp)

Figure S2. Nucleosome dissociation after tagmentation yields better library quality than pre-tagmentation. Samples starting from 240,000 cells were processed using commercial Nextera enzyme and buffers (left panels) or homemade $Tn5_{R27S,E54K,L372P}$ enzyme (right panels). Nucleosome dissociation by incubation with Proteinase K at 65° was performed before (salmon) or after (turquoise) tagmentation. A) Coverage bias distribution (log scale), with bias calculated as coverage at a base divided by average genome coverage. The salt condition is omitted for Nextera due to insufficient reads for this sample. B) Fraction of YJM789 SNPs called as a function of sequencing depth. The reference set of true positive SNPs (52,373 SNPs) is derived from variant calling on a sample prepared from extracted genomic DNA with the Nextera XT kit (NXTMP), and additionally referenced against previously published YJM789 polymorphisms. C) Fraction of false positive SNP calls as a function of sequencing depth, with false positive call rate = number of mis-called SNPs/total number of SNPs. D) Fraction of the genome covered at least 8X as a function of sequencing depth. E) Distribution of fragment insert sizes. Final cleanup used 1.8X Ampure beads. NXTMP (black dashed line) is a library processed from 150 pg extracted genomic DNA with the Nextera XT kit and serves as reference.

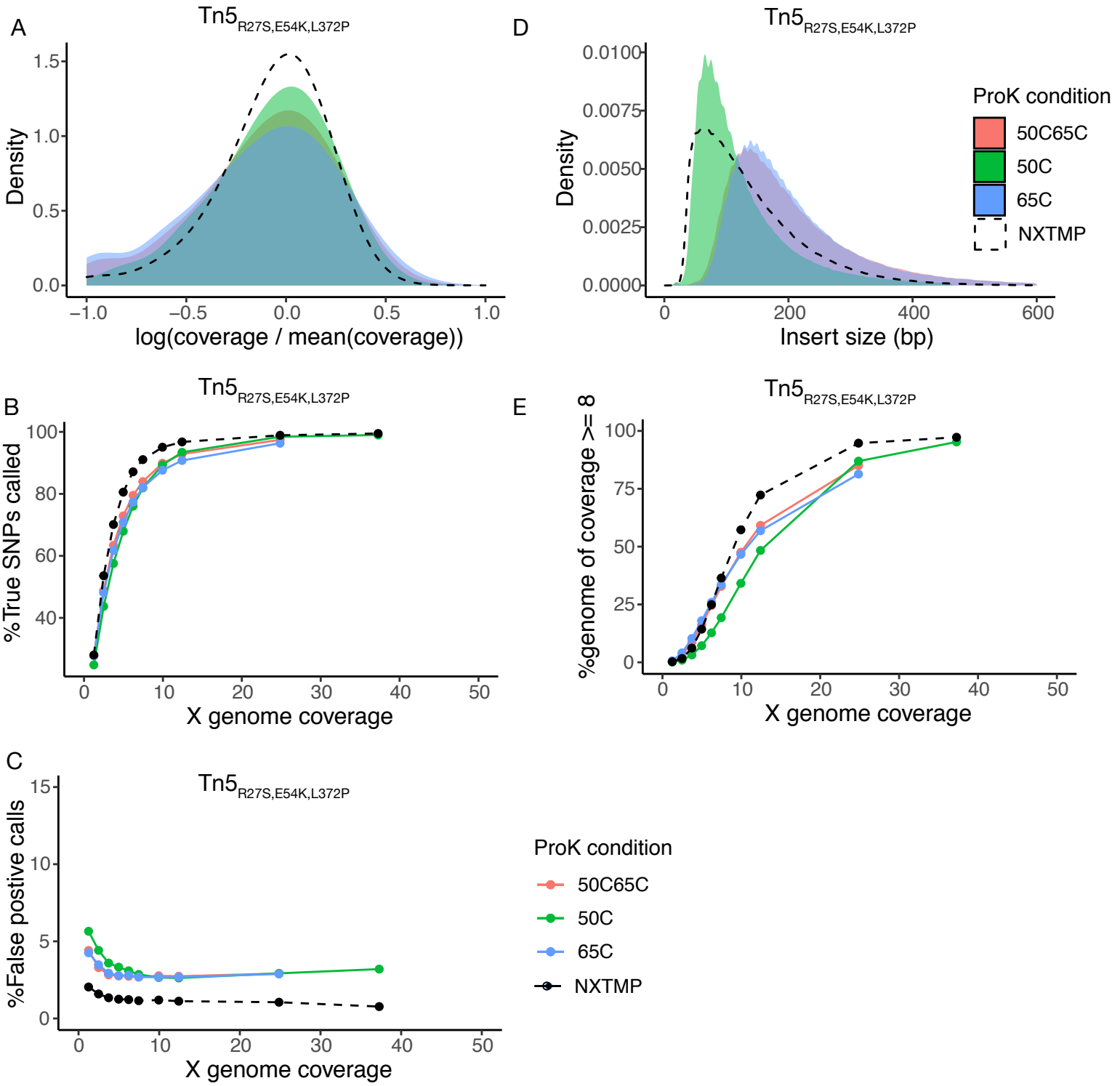

Figure S3. Nucleosome dissociation with variable Proteinase K incubation conditions. Samples starting from 240,000 cells were processed using homemade Tn5$_{R27S,E54K,L372P}$ enzyme and included nucleosome dissociation by incubation with Proteinase K (after tagmentation) at 65° (blue), 50° (green) or 50° followed by 65° (salmon). A) Coverage bias distribution (log scale), with bias calculated as coverage at a base divided by average genome coverage. The salt condition is omitted for Nextera due to insufficient reads for this sample. B) Fraction of YJM789 SNPs called as a function of sequencing depth. The reference set of true positive SNPs (52,373 SNPs) is derived from variant calling on a sample prepared from extracted genomic DNA with the Nextera XT kit (NXTMP), and additionally referenced against previously published YJM789 polymorphisms. C) Fraction of false positive SNP calls as a function of sequencing depth, with false positive call rate = number of mis-called SNPs/total number of SNPs. D) Distribution of fragment insert sizes. Final cleanup used 1.8X Ampure beads. E) Fraction of the genome covered at least 8X as a function of sequencing depth. NXTMP (black dashed line) is a library processed from 150 pg extracted genomic DNA with the Nextera XT kit and serves as reference.

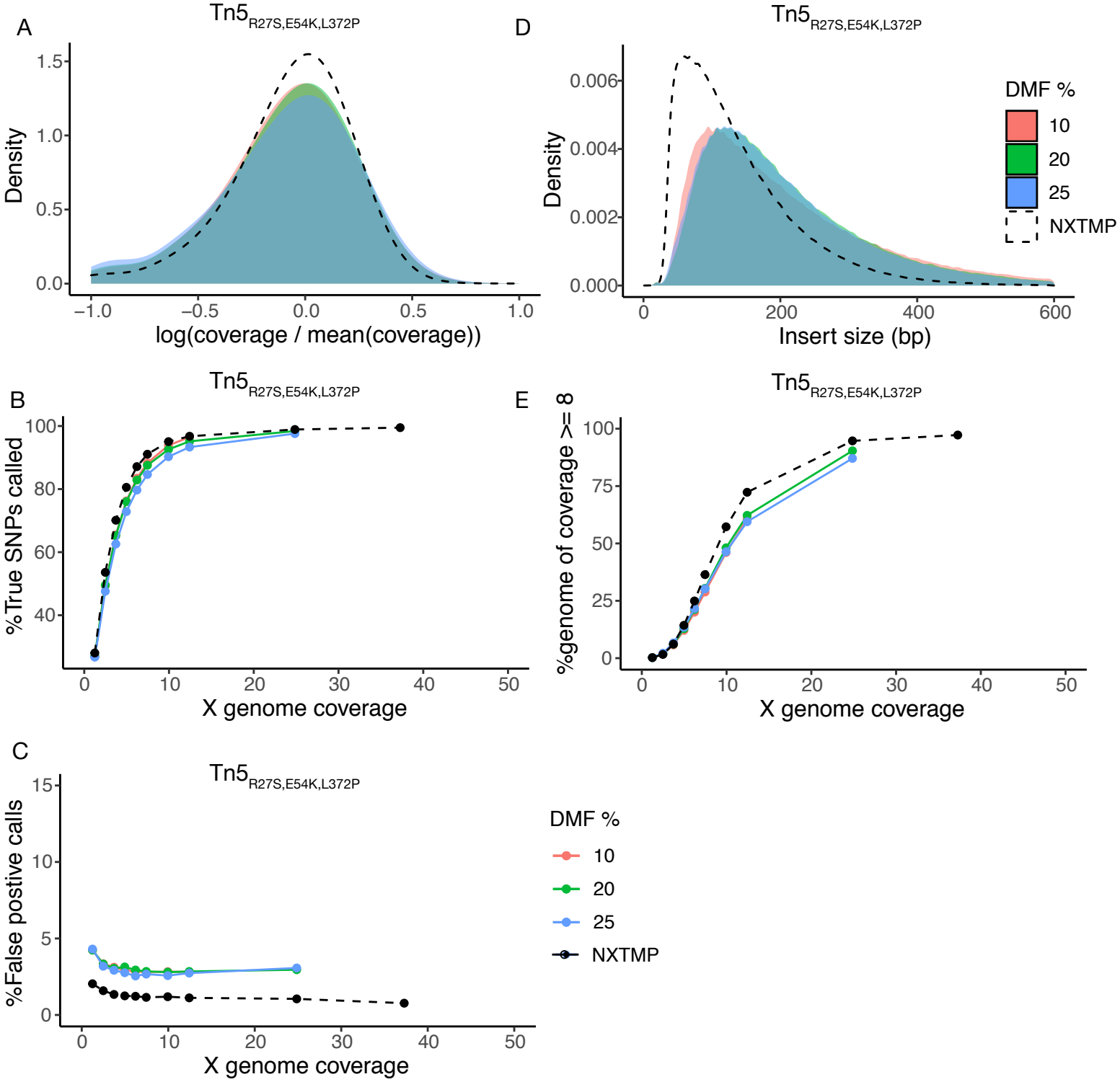

Figure S4. Lower DMF content in the tagmentation buffer improves coverage and variant calling. Samples starting from 500,000 cells were processed using homemade $Tn5_{R27S,E54K,L372P}$ enzyme without a nucleosome dissociation step and with varying DMF percentage in the tagmentation buffer: 25% (blue), 20% (green) or 10% (salmon). A) Coverage bias distribution (log scale), with bias calculated as coverage at a base divided by average genome coverage. The salt condition is omitted for Nextera due to insufficient reads for this sample. B) Fraction of YJM789 SNPs called as a function of sequencing depth (indicated as average genome coverage). The reference set of true positive SNPs (52,373 SNPs) is derived from variant calling on a sample prepared from extracted genomic DNA with the Nextera XT kit (NXTMP), and additionally referenced against previously published YJM789 polymorphisms. C) Fraction of false positive SNP calls as a function of sequencing depth, with false positive call rate = number of mis-called SNPs/total number of SNPs. D) Distribution of fragment insert sizes. Final cleanup used 1.8X Ampure beads. E) Fraction of the genome covered at least 8X as a function of sequencing depth. NXTMP (black dashed line) is a library processed from 150 pg extracted genomic DNA with the Nextera XT kit and serves as reference.

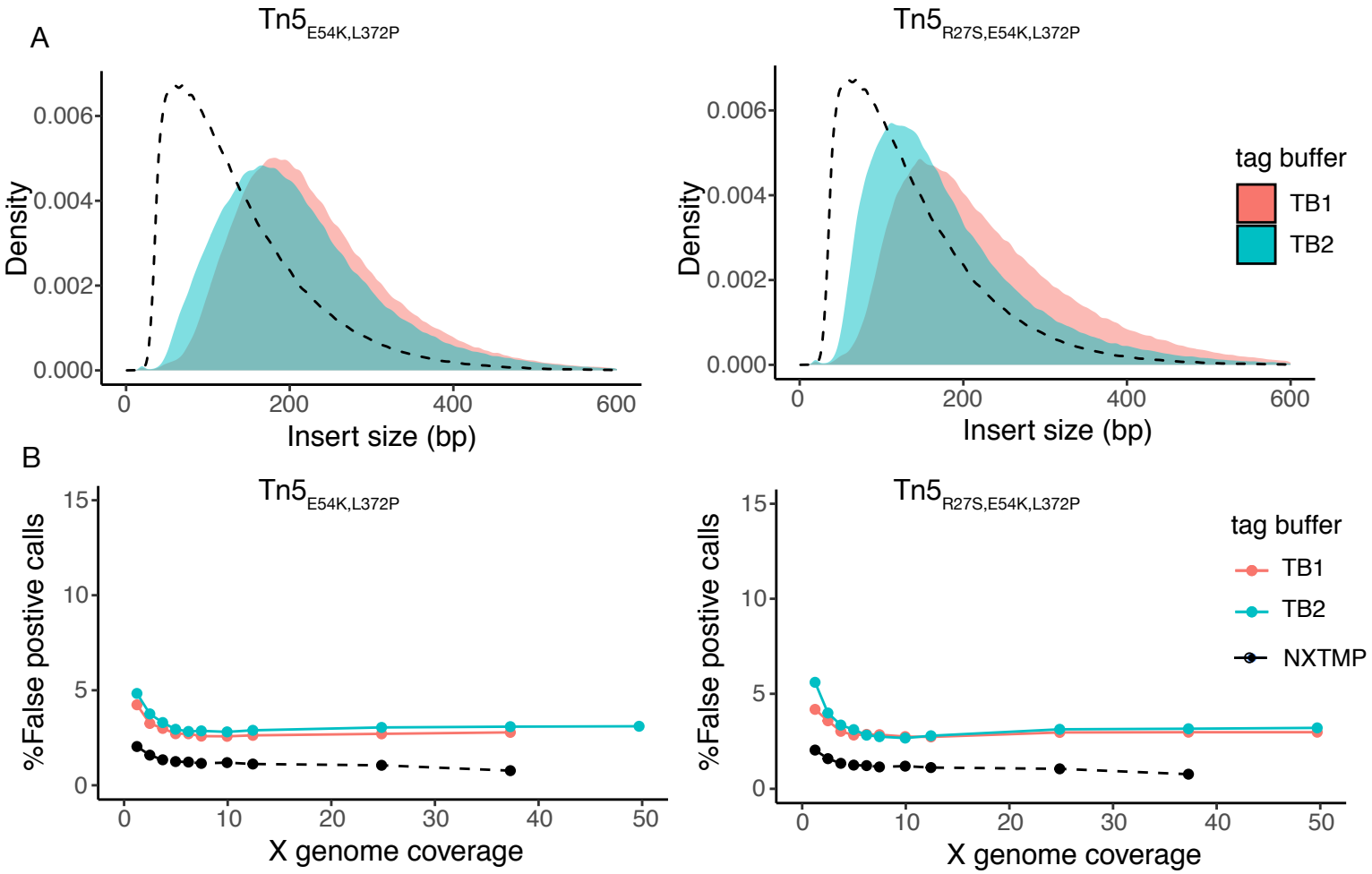

Figure S5. Insert size and false positive calling-rate of libraries prepared with extraction-free protocol using homemade enzymes. Samples were prepared from 100µl saturated overnight culture with nucleosome dissociation by ProK treatment at 50° followed by 65° with tagmentation in TB1 buffer (salmon) or optimized TB2 buffer (turquoise), and with homemade $Tn5_{E54K,L372P}$ or $Tn5_{R27S,E54K,L372P}$ enzyme. A) Distribution of fragment insert sizes. Final cleanup used 0.8X Ampure beads. B) Fraction of false positive SNP calls as a function of sequencing depth, with false positive call rate = number of mis-called SNPs/total number of SNPs. NXTMP (black dashed line) is a library processed from 150 pg extracted genomic DNA with the Nextera XT kit and serves as reference.

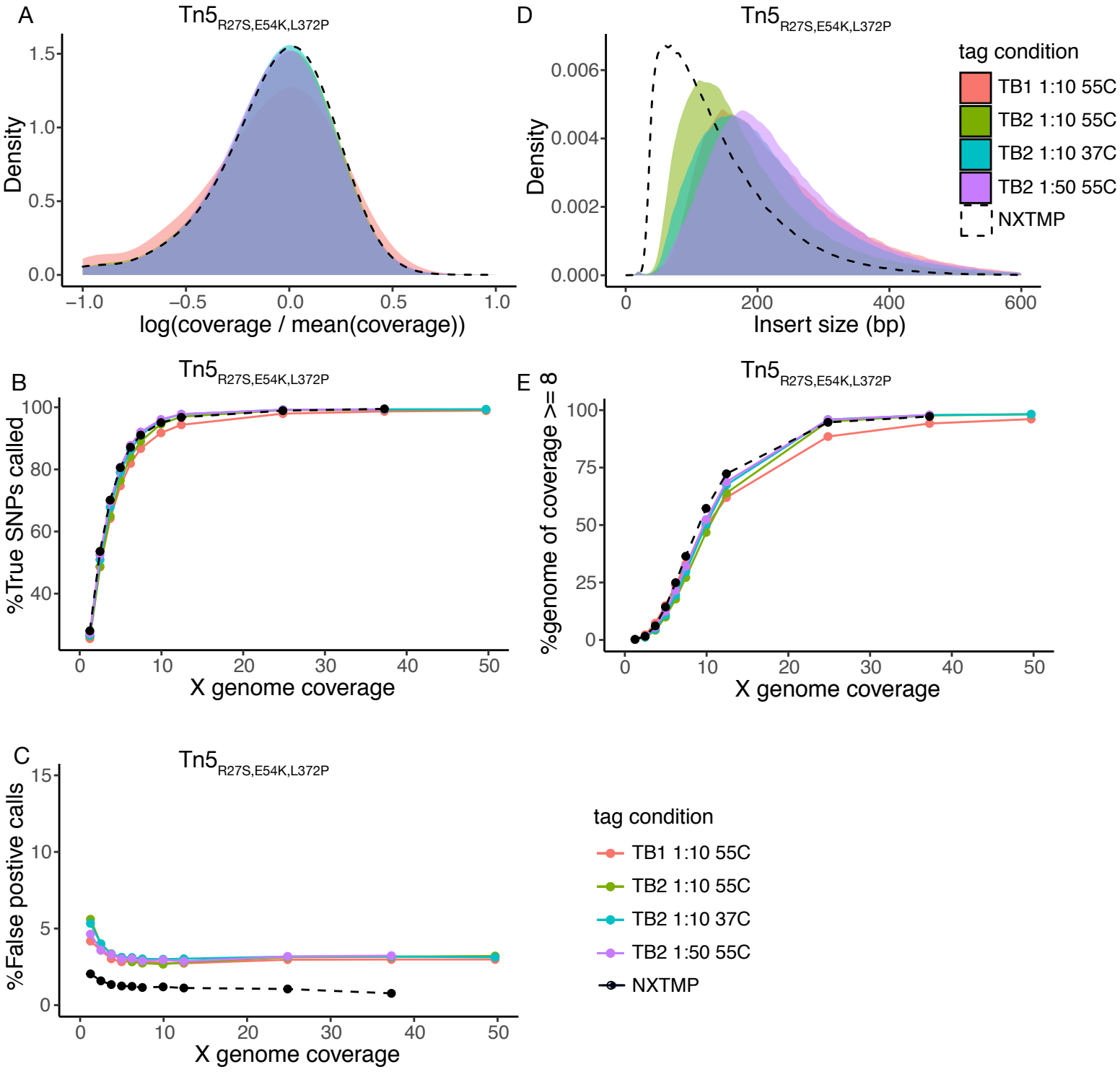

Figure S6. Lower temperature or enzyme concentration during tagmentation increase insert size. Samples were prepared from 100µl saturated overnight culture with nucleosome dissociation by Proteinase K treatment at 50° followed by 65° using homemade $Tn5_{R27S,E54K,L372P}$ enzyme and varying tagmentation conditions: tagmentation for 3 min at 55° in TB1 buffer, with 1:10 Tn5 dilution (salmon, standard condition); tagmentation for 3 min at 55° in TB2 buffer, with 1:10 Tn5 dilution (green); tagmentation for 3 min at 37° in TB2 buffer, with 1:10 Tn5 dilution (turquoise); tagmentation for 3 min at 55° in TB2 buffer, with 1:50 Tn5 dilution (purple). A) Coverage bias distribution (log scale), with bias calculated as coverage at a base divided by average genome coverage. The salt condition is omitted for Nextera due to insufficient reads for this sample. B) Fraction of YJM789 SNPs called as a function of sequencing depth. The reference set of true positive SNPs (52,373 SNPs) is derived from variant calling on a sample prepared from extracted genomic DNA with the Nextera XT kit (NXTMP), and additionally referenced against previously published YJM789 polymorphisms. C) Fraction of false positive SNP calls as a function of sequencing depth, with false positive call rate = number of mis-called SNPs/total number of SNPs. D) Distribution of fragment insert sizes. Final cleanup used 0.8X Ampure beads. E) Fraction of the genome covered at least 8X as a function of sequencing depth. NXTMP (black dashed line) is a library processed from 150 pg extracted genomic DNA with the Nextera XT kit and serves as reference.

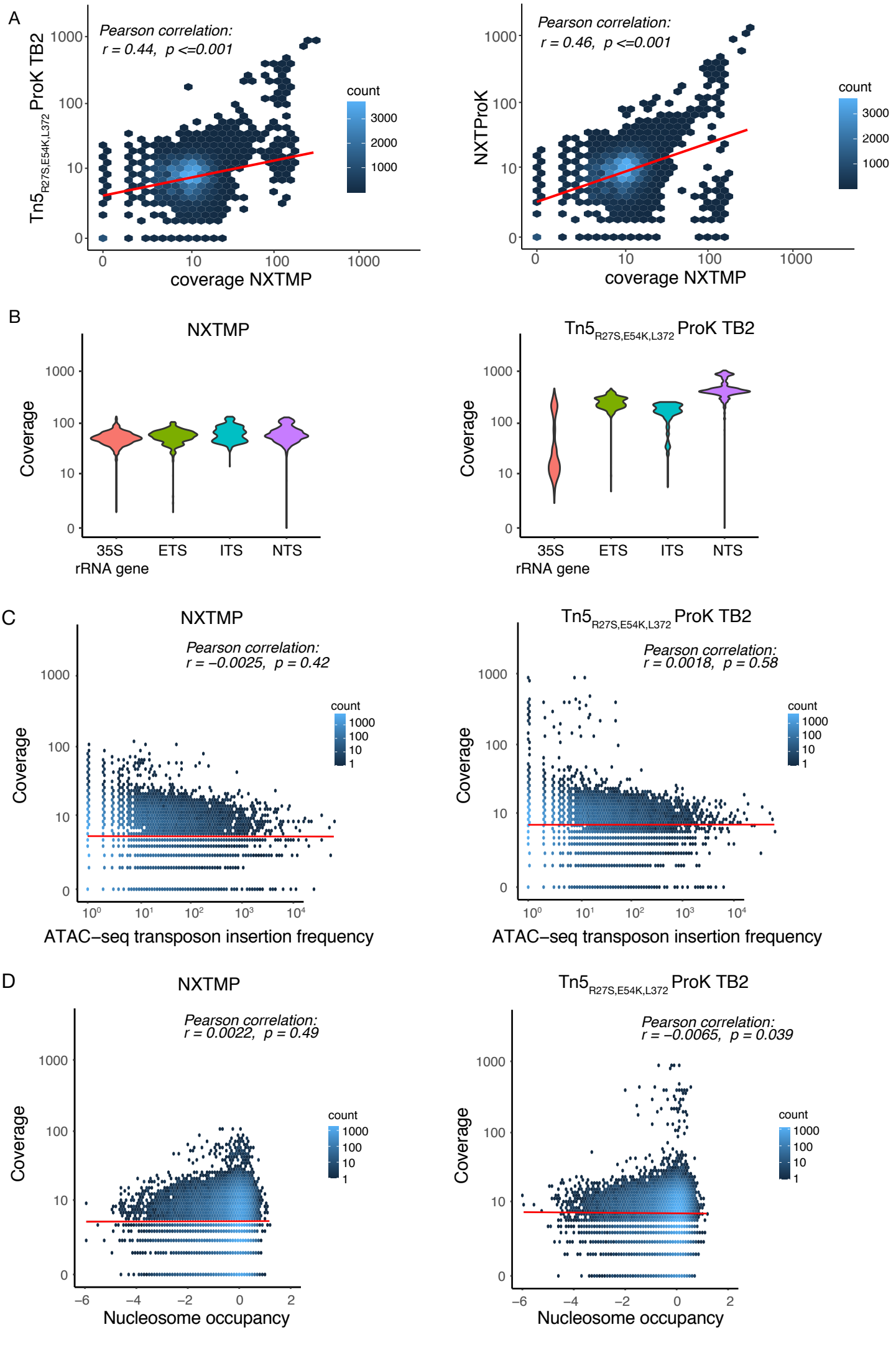
A
Pearson correlation:
r = 0.44, p <=0.001
Tn5R27S,E54K,L372 ProK TB2
coverage NXTMP
count
3000
2000
1000
Pearson correlation:
r = 0.46, p <=0.001
NXTProK
coverage NXTMP
B
NXTMP
Tn5R27S,E54K,L372 ProK TB2
Coverage
35S
ETS
ITS
NTS
rRNA gene
C
Pearson correlation:
r = −0.0025, p = 0.42
Pearson correlation:
r = 0.0018, p = 0.58
ATAC−seq transposon insertion frequency
D
Pearson correlation:
r = 0.0022, p = 0.49
Pearson correlation:
r = −0.0065, p = 0.039
Nucleosome occupancy

Figure S7. No correlation between genome-wide coverage and nucleosome occupancy in extraction-free libraries. A) Left panel: Correlation of coverage at individual bases between standard (NXTMP) and extraction-free library preparation using homemade enzymes ($Tn5_{R27S,E54K,L372P}$ Prok TB2). Right panel: Correlation of coverage at individual bases between standard (NXTMP) and extraction-free library preparation using Nextera enzyme and commercial reagents. B) Coverage across sequence elements at the yeast rDNA locus in samples with standard (left panel) or extraction-free (right panel) library preparation. C) Correlation between per-base sequencing coverage and ATAC-seq insertion frequency in samples with standard (left panel) or extraction-free (right panel) library preparation. D) Correlation between per-base sequencing coverage and nucleosome occupancy in samples with standard (left panel) or extraction-free (right panel) library preparation. NXTMP = library prepared with Nextera XT kit from 150 pg genomic DNA. $Tn5_{R27S,E54K,L372P}$ Prok TB2 = library prepared from 100ul saturated overnight culture with nucleosome dissociation by ProK treatment at 50° followed by 65° and tagmentation in TB2 buffer using $Tn5_{R27S,E54K,L372P}$ (From Fig. 4). NXT ProK = library prepared from 240,000 cells with nucleosome dissociation by Proteinase K treatment at 65° (from Fig. 2).

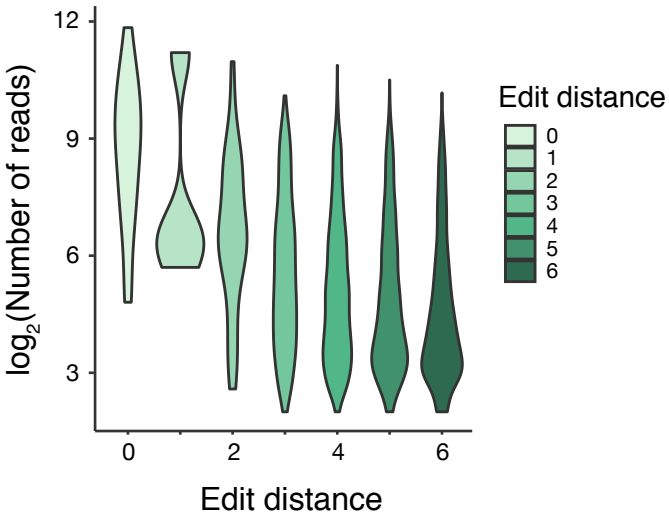

Figure S8. Lower Cas9 cleavage activity at CIRCLE-seq off-target sites with higher edit distances. Violin plots depict distribution of sequencing coverage for each edit distance in the CIRCLE-seq off-target dataset. Due to the nature of the assay, the number of reads across sites in CIRCLE-seq data reflects Cas9-cleavage activity.