OMTN, Volume *26*

Supplemental information

The roles of DNA methylation and hydroxymethylation at short interspersed nuclear elements in the hypothalamic arcuate nucleus during puberty

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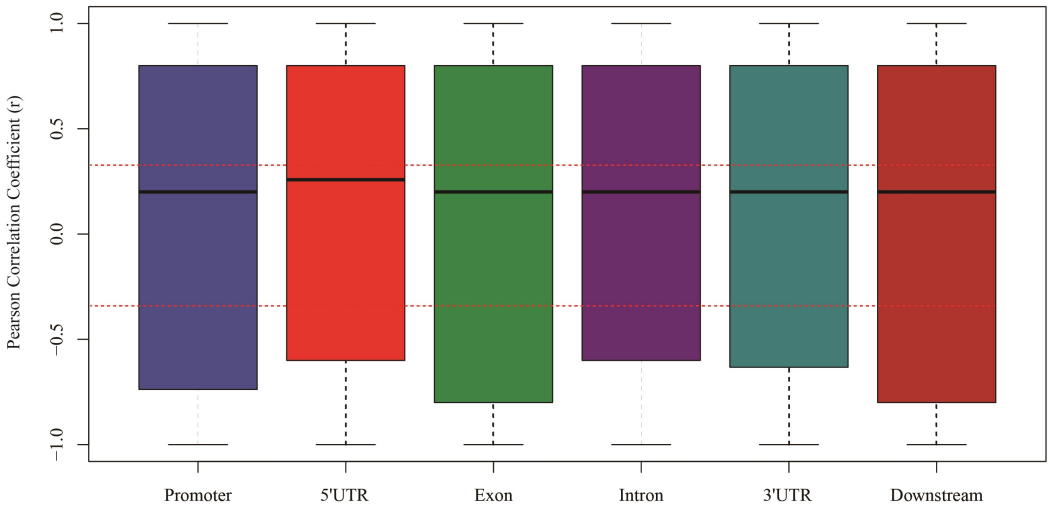
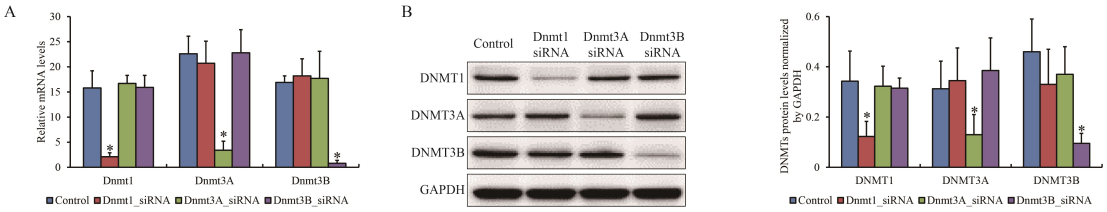
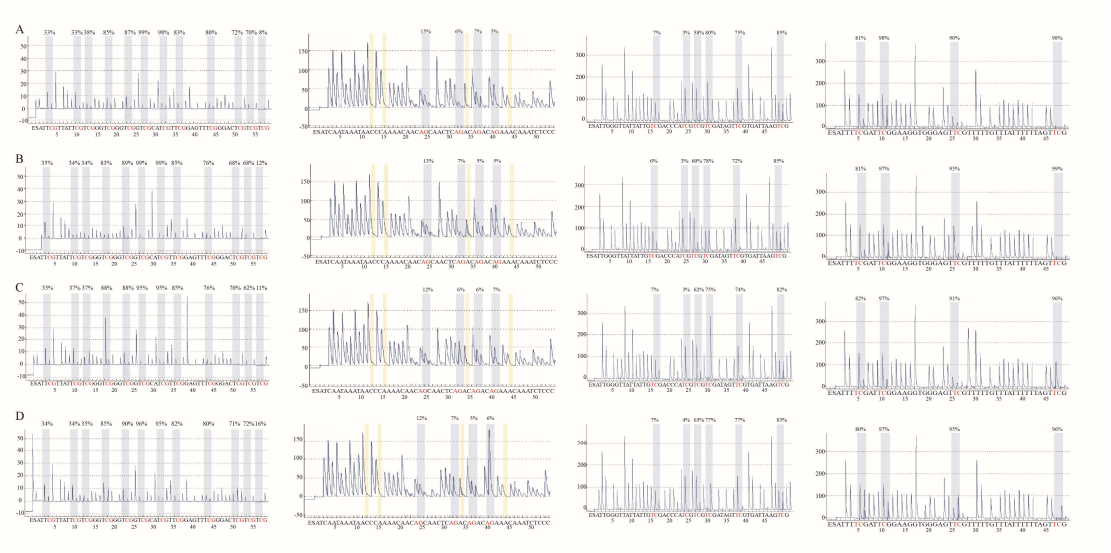


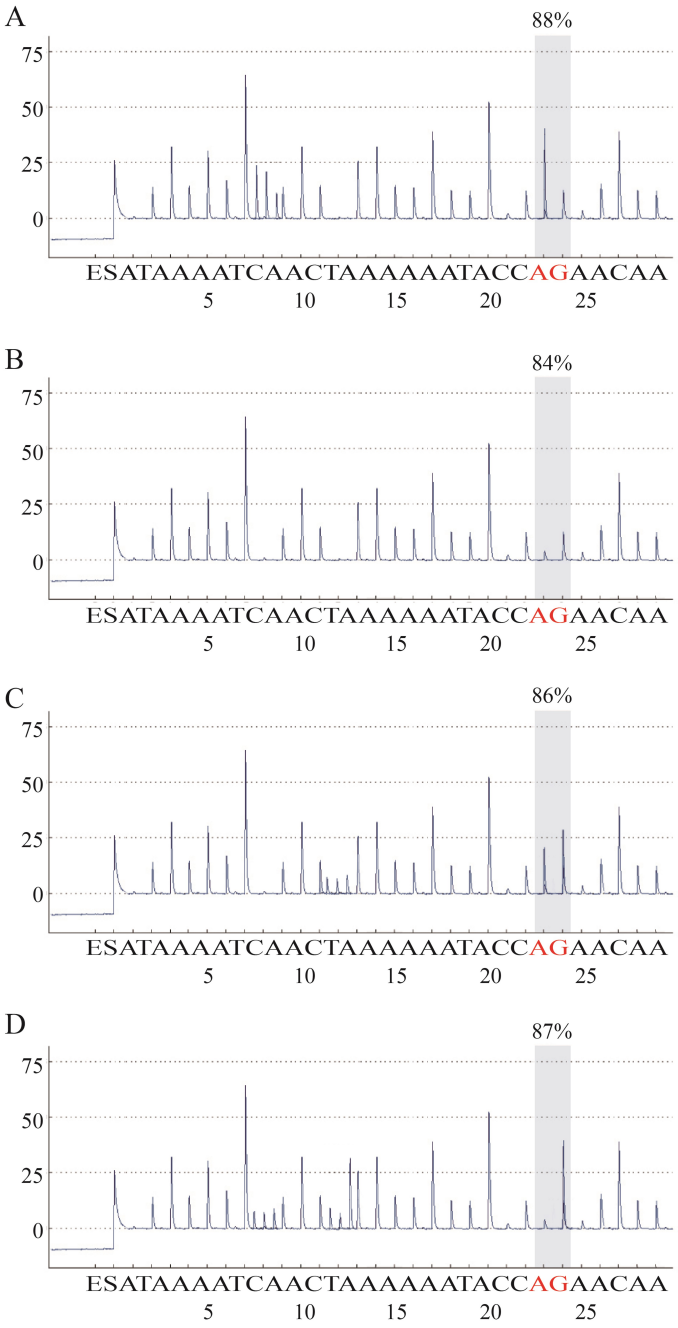
Supplementary figure 1. The Pearson correlation analysis between DNA hydroxymethylation of retroelements and adjacent genes expression in pubertal ARC.



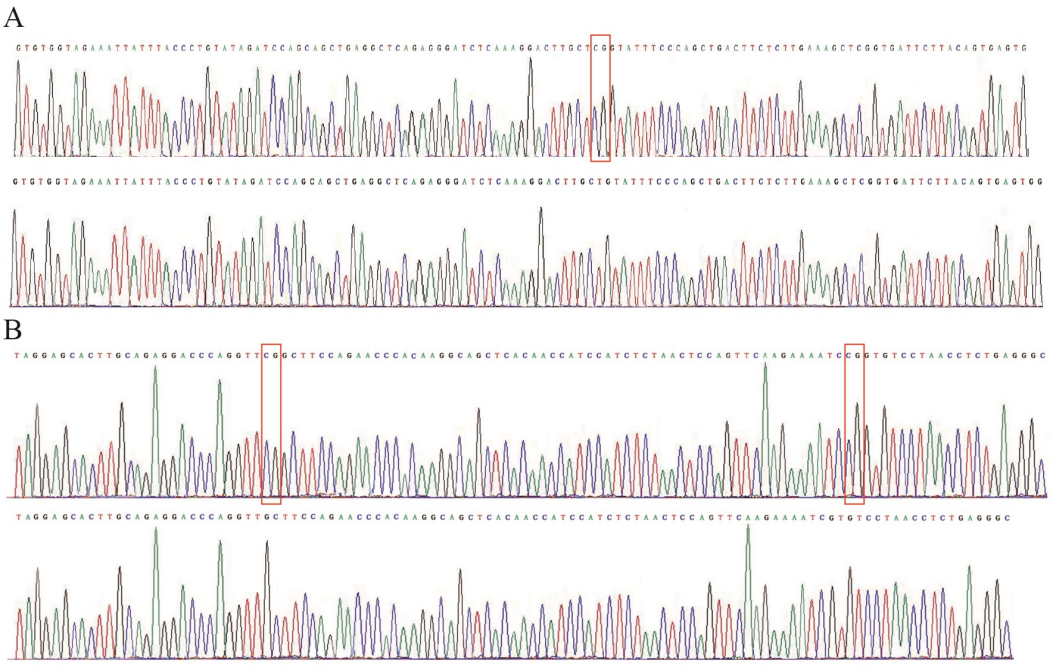
Supplementary figure 2. The efficacy of DNMTs siRNA confirmed by qPCR (A) and WB (B). The qPCR data and WB data by gray analysis is presented as the mean ± SEM of three separate experiments. "*" represents the significant difference with a *p*-value less than 0.05 by Student's t-test.



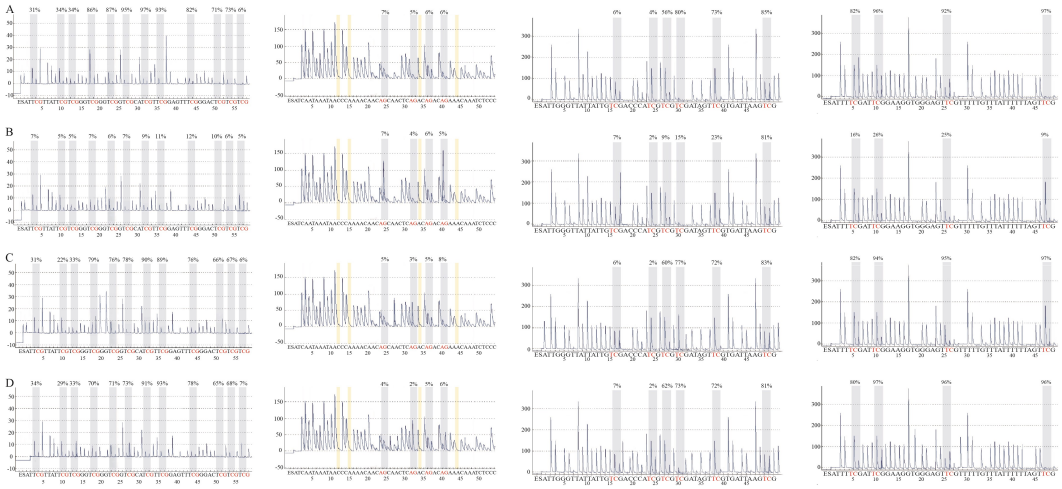
Supplementary figure 3. Pyrosequencing data of DNA methylation of two CpG islands at HTR6 promoter in GT1-7 cells induced by DNMTs siRNA. Wild type (A), *Dnmt1* siRNA (B), *Dnmt3a* siRNA (C), *Dnmt3b* siRNA (D). Red "A/G" or "T/C" represent the detected CpG loci. The percentages represent the probable proportion of cytosine (sense strands) or guanine (antisense strands) at the certain sites. This interpretation also applies to Supplementary figure 3, 5, 6 and 9.



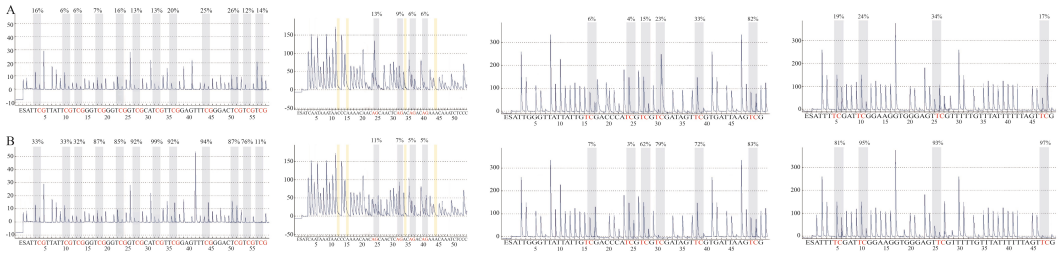
Supplementary figure 4. Pyrosequencing data of DNA methylation of SINE at HTR6 promoter in GT1-7 cells induced by DNMTs siRNA. Wild type (A), *Dnmt1* RNA interference (B), *Dnmt3a* siRNA (C), *Dnmt3b* siRNA (D).



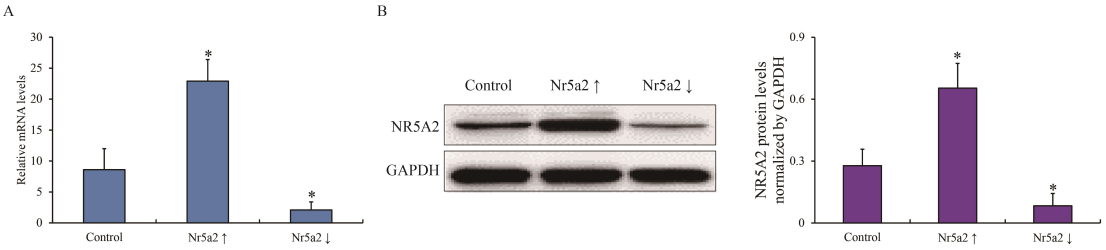
Supplementary figure 5. Sanger sequencing data of CpG loci deletion of SINEs by CRIPSPR-Cas9 system. Sequences of SINEs containing with CpG loci highlighted by red frames in HTR6 promoter (A) and enhancer (B) are shown (wild type above, knockout below).



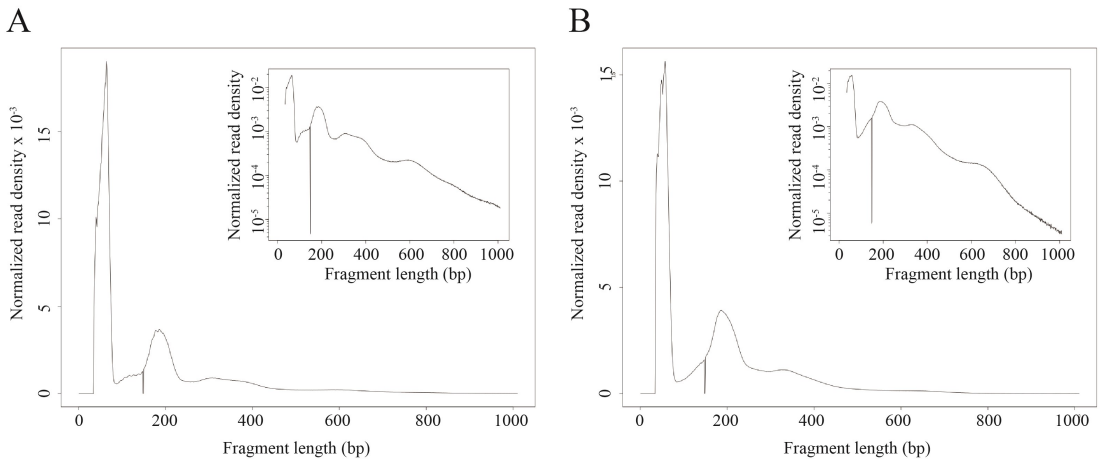
Supplementary figure 6. Pyrosequencing data of DNA methylation of two CpG islands at HTR6 promoter in GT1-7$^{\text{SINE-CpG-KO1}}$ cells induced by DNMTs siRNA. Wild type (A), *Dnmt1* siRNA (B), *Dnmt3a* siRNA (C), *Dnmt3b* siRNA (D).



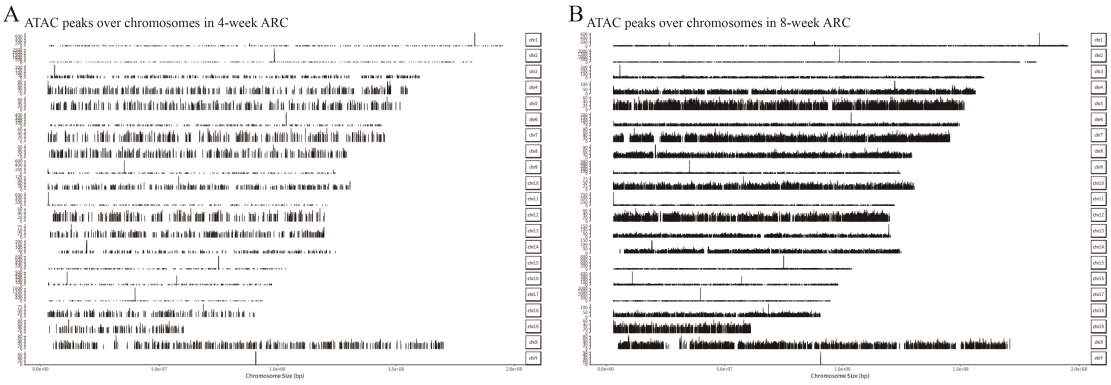
Supplementary figure 7. Pyrosequencing data of DNA methylation of two CpG islands at HTR6 promoter in GT1-7$^{\text{SINE-CpG-KO1}}$ cells induced by L-tryptophan. Wild type (A), L-tryptophan (B).



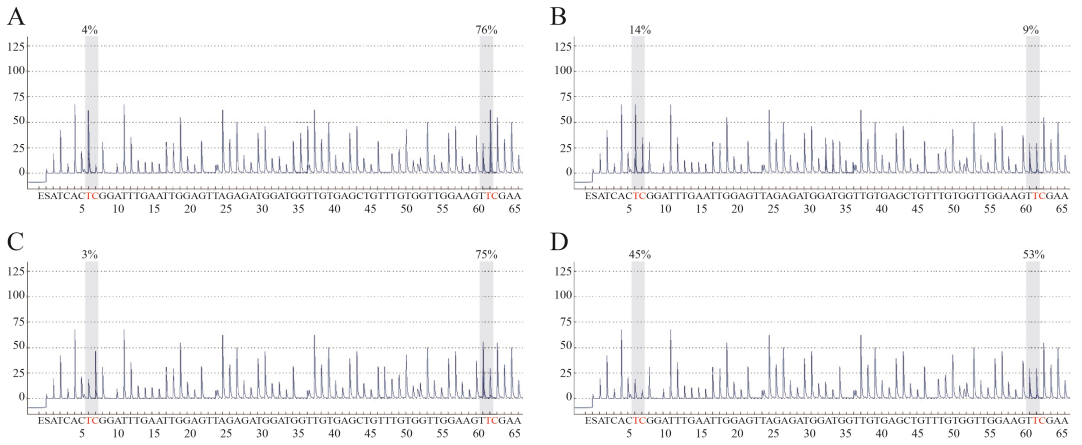
Supplementary Figure 8. The efficacy of *Nr5a2* siRNA or ectopic expression in GT1-7 cells confirmed by qPCR (A) and WB (B). The qPCR data and WB data by gray analysis is presented as the mean ± SEM of three separate experiments. "*" represents the significant difference with a *p*-value less than 0.05 by Student's t-test.



Supplementary figure 9. Quality control of different fragment size distribution for ATAC-seq data. 4-week ARC (A), 8-week ARC (B).



Supplementary figure 10. The overview of ATAC peaks across all chromosomes at 4- (A) and 8- (B) week ARC.



Supplementary figure 11. Pyrosequencing data of DNA hydroxymethylation of two CpG loci at SINE in enhancer region in GT1-7 cells induced by estradiol. 5mC in normal control GT1-7 cells (A), 5hmC in normal control GT1-7 cells (B), 5mC in GT1-7 cells treated with estradiol (C), 5hmC in GT1-7 cells treated with estradiol (D).

Supplementary table 1. DMREs at promoter regions of DEGs. Column B and C indicate the coordinates of the differential methylation peak. Column F and G indicate the coordinates of the annotated REs. Column L and M indicate the coordinates of the adjacent genes. All coordinates are referred to GRCm38.

Supplementary table 2. DHMREs at enhancers around DEGs. DEGs with significantly differential hydroxymethylated RE at up/downstream enhancers and chromatin accessibility are listed. All coordinates are referred to GRCm38.

Supplementary table 3. All nucleotides used in this study are listed above. gRNA: guide RNA; BSP: bisulfite PCR.

Purpose	Sequence
Dnmt1 siRNA	5'- GGUAGAGAGUUACGACGAATT -3'
Dnmt3a siRNA	5'- GCGUCACACAGAAGCAUAUTT -3'
Dnmt3b siRNA	5'- GCAUGAAGGCCAGAUCAAATT -3'
Nr5a21 siRNA	5'- GAUUGUUGCCUCUAGAAGUTT-3'
gRNA for SINE at HTR6 promoter	5'- GGATCTCAAAGGACTTGCTCGG -3'
Donar DNA1	5'- GTGTGGTAGAAATTATTTACCCTGTATAGATCCAGCAGCTGAGG CTCAGAGGGATCTCAAAGGACTTGCTGTATTTCCCAGCTGACTT CTCTTGAAAGCTCGGTGATTCTTACAGTGAGTGG -3'
gRNA for SINE at *Kiss1* promoter	5'- ACTCCAGTTCAAGAAAATCCG -3'
Donar DNA2	5'- TAGGAGCACTTGCAGAGGACCCAGGTTGCTTCCAGAACCCACA AGGCAGCTCACAACCATCCATCTCTAACTCCAGTTCAAGAAAAT CGTGTCCTAACCTCTGAGGGC -3'
qPCR for *Kiss1*	F: 5'- GTGTCGCCACCTATGGGGAGCC -3' R: 5'- TCAGGCGACTGCGGGAGGCACACAGG -3'
qPCR for *Htr6*	F: 5'- ACTGTAATAGCACCATGAACCCTATCAT -3' R: 5'- AAGCTGGGCTGTGAGCTGCAGGCCCGAGGTGC -3'
ChIP for *Htr6* promoter	F: 5'- ATGTGGGCAGGGGAGCCACAGGCGA -3' R: 5'- GCGGGGCTGGAGACTACAGGCAGCGG -3'
DNase I/ ChIP assay for enhancer	F: 5'- AGTTAGGAGCACTTGCAGAGGACCCAGG -3' R: 5'- TTTATTTTTATTTCAGTATATGAGTATTT -3'
DNase I/ ChIP assay for *Kiss1* promoter	F: 5'-AAGAGAACACTGAGACACCCAGGGAG -3' R: 5'- CCGTGTGTCATGGTAAGTAAGTCAGT -3'
BSP for *Htr6* promoter1	F: 5'- TTTTTTAGGTTTAGGGTATTATAA -3' R: 5'- CACAAAAACCCACAACACTTCAACCA -3' S: 5'- GAGGGGTTTGATTTTG -3'
BSP for *Htr6* promoter2	F: 5'- TTAGTGTTTAGGGGAGAAAGGAGAT -3' R: 5'- AAATAAAACTCCTAACCAAAAACAC -3' S1: 5'- CAATAAAATAACCCAAAA -3' S2: 5'- TTTTGGGTTATTTTATTG -3'

	S3: 5’- TTAGAGAGATTGTTT -3’
BSP for SINE at *Htr6* promoter	F: 5’- GTGTGGTAGAATTATTTATTTTGT -3’
	R: 5’- CCACTCACTATAAAAATCACC -3’
	S: 5’- AACTTTCAAAAAAA -3’
BSP for SINE at *Kiss1* promoter	F: 5’- AGTTAGGAGTATTTGTAGAGGATTTAGG -3’
	R: 5’- TTTATTTTTATTTTAGTATATGAGTATTT -3’
	S: 5’- TTTAGAGGTTAGGATAT -3’