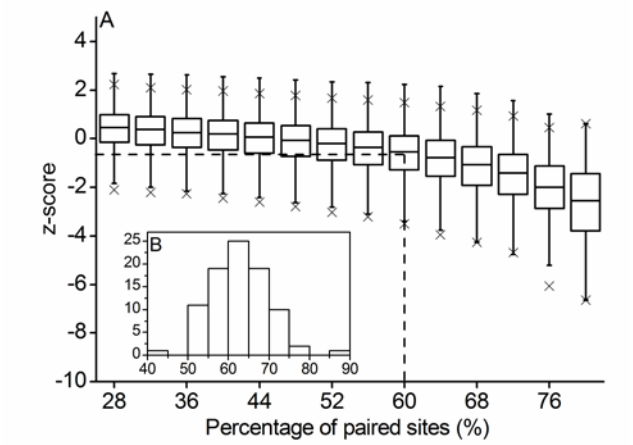

Figure S1. The correlation between z-score and percentage of paired sites. A z-score of -0.65 means that there are about 60% sites in a sliding window are base paired (indicated by dashed lines). B shows the distribution of percentage of paired sites inferred from tRNA.

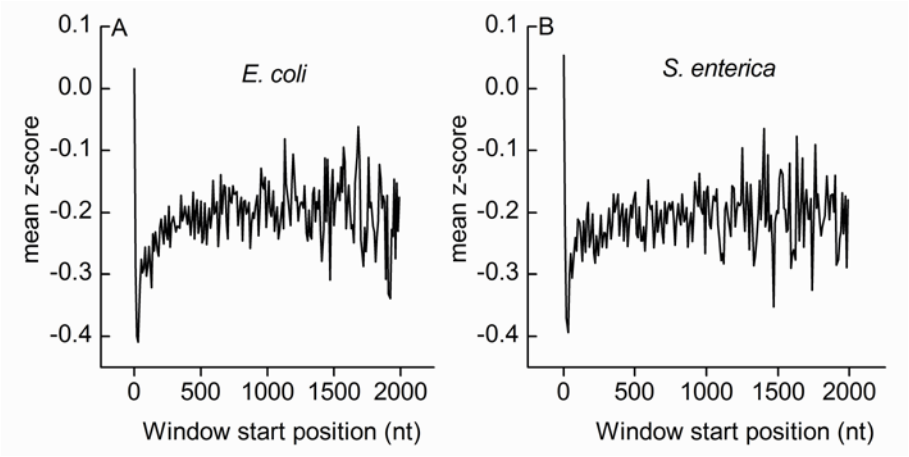

Figure S2. Mean z-score values along mRNA in *E. coli* and *S. enterica*. A high structural stability near 30-80 nucleotides interval is observed in both *E. coli* and *S. enterica*.

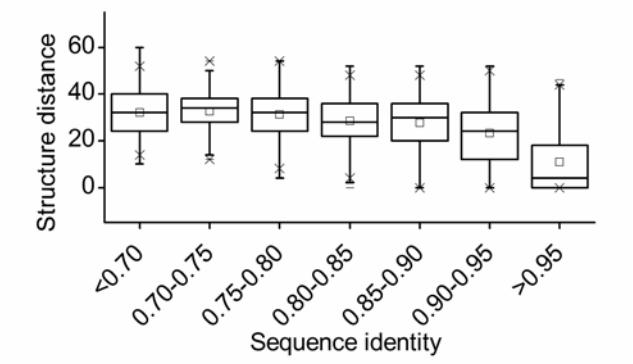

Figure S3. The correlation between structure distance and sequence identity of random sequence. Random sequence was generated by shuffle orthologs, while maintaining codon usage, amino acid sequences and sequence identity of orthologs.

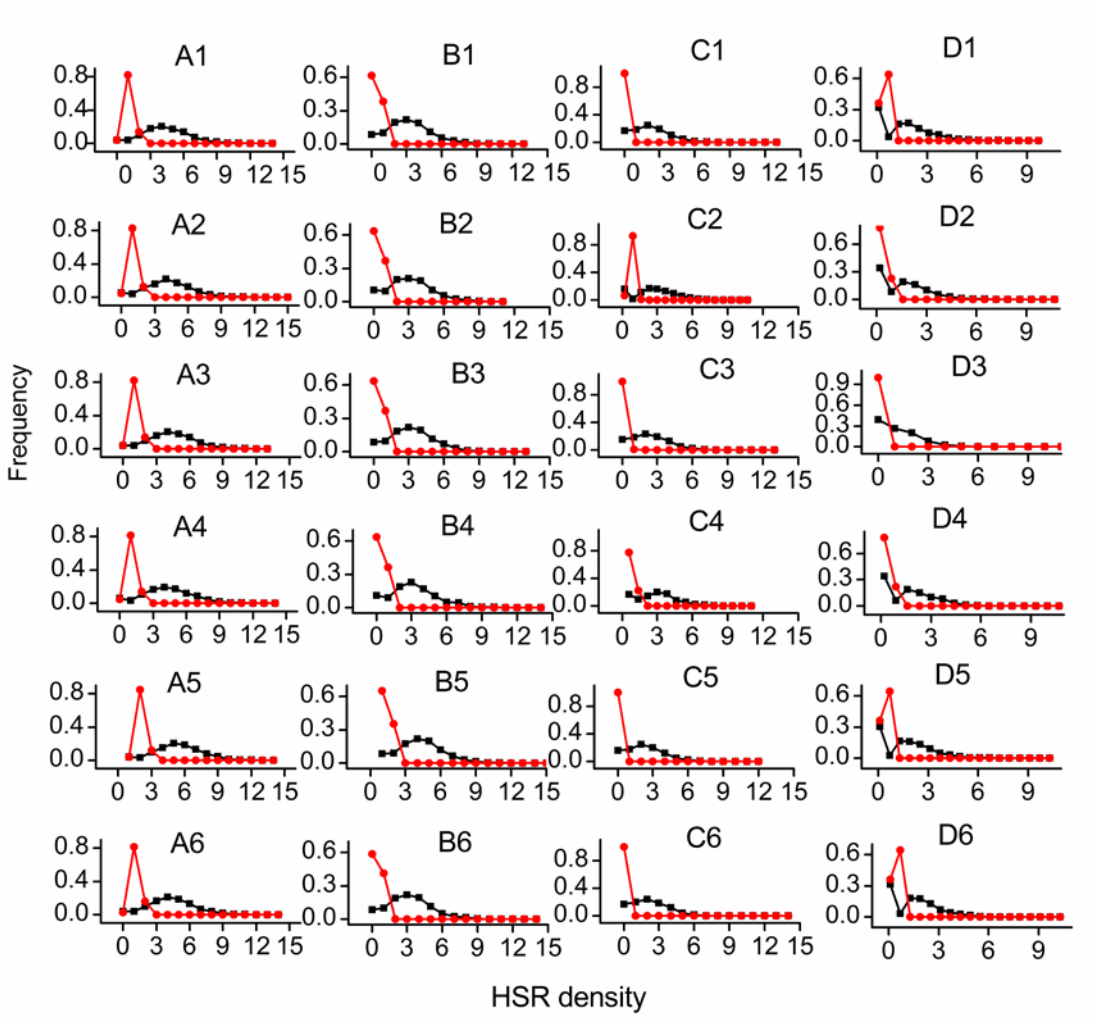

Figure S4. The distribution of HSR density in different species. 1) Different species are shown. 1: *Escherichia coli* 2: *Salmonella enterica* 3: *Yersinia pestis* 4: *Shigella flexneri* 5: *Vibrio cholera* 6: *Aeromonas hydrophila*. 2) Other thresholds are shown. A: -0.45, B: -0.65, C: -0.85, D: -1.2. HSR density in native sequences was indicated by black line, HSR density in random sequences was indicated by red line.

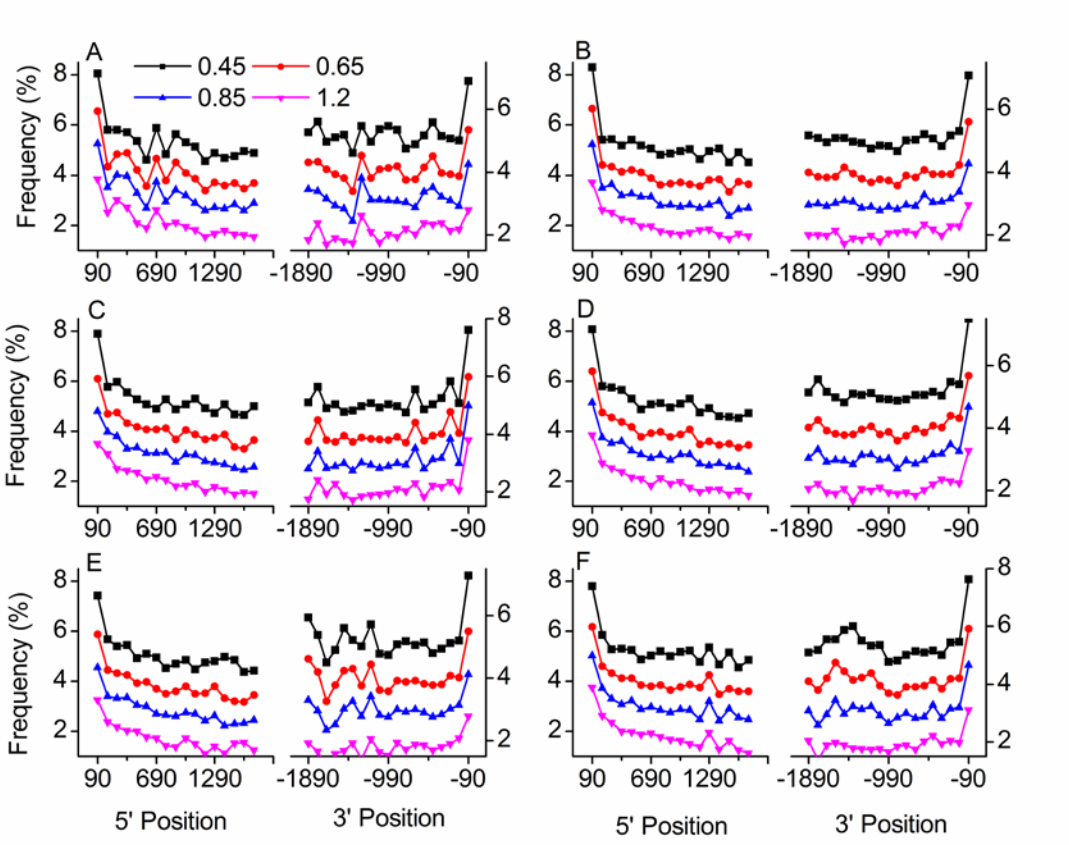

Figure S5. The locations of HSRs along mRNA. 1) Different species are shown. A: *Yersinia pestis* B: *Salmonella enterica* C: *Shigella flexneri* D: *Escherichia coli* E: *Aeromonas hydrophila* F: *Vibrio cholera*. 2) Different thresholds are shown. Black line:-0.45, red line: -0.65, blue line: -0.85, pink line: -1.2.

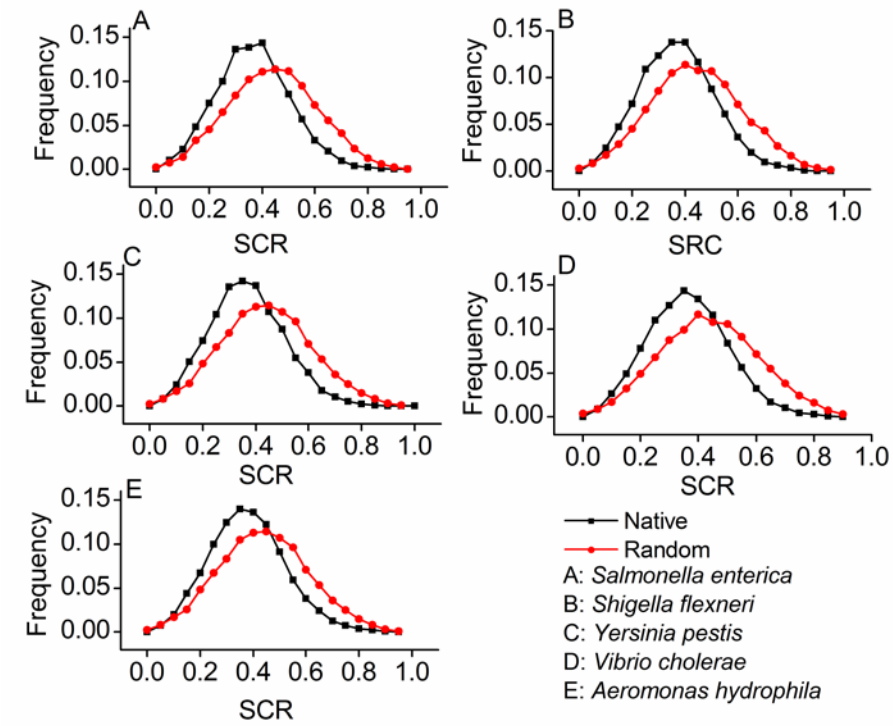

Figure S6. The distribution of SCR values of HSRs in other species. Native: native HSRs; Random: random HSRs, generated by shuffling native HSRs keeping codon usage and amino acid sequence unchanged, keeping similar MFE to the native HSRs.

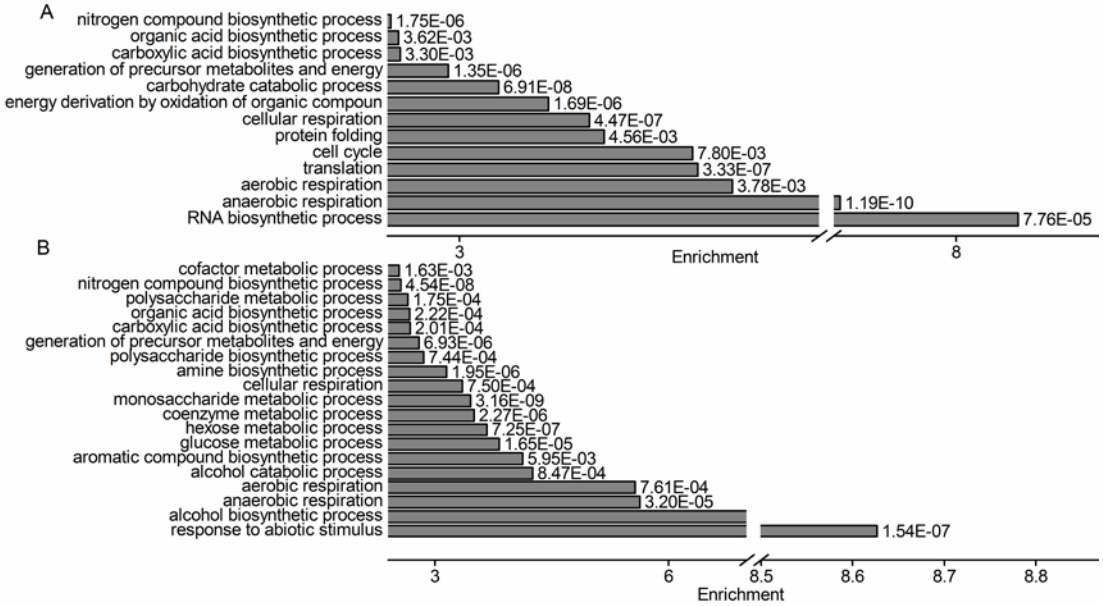

Figure S7. The most enriched terms between the top (A) and bottom (B) groups of HSR density. The result is based on the conserved HSRs. Conserved HSRs refer to the HSRs which exists in both *E. coli* and *S. enterica.*

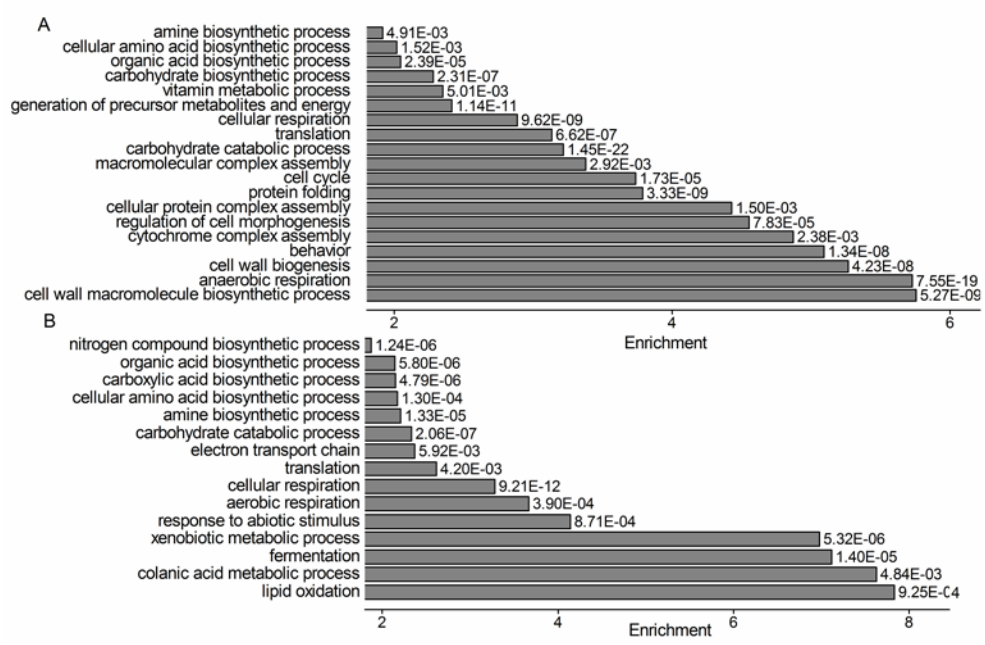

Figure S8. The most enriched terms between the top (A) and bottom (B) groups of HSR density. The threshold of -0.45 was used to define HSRs.

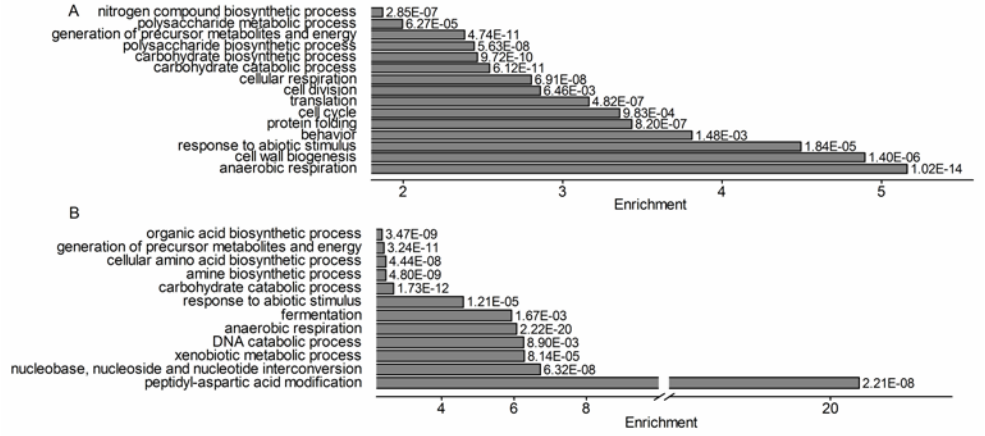

Figure S9. The most enriched terms between the top (A) and bottom (B) groups of HSR density. The threshold of -0.85 was used to define HSRs.

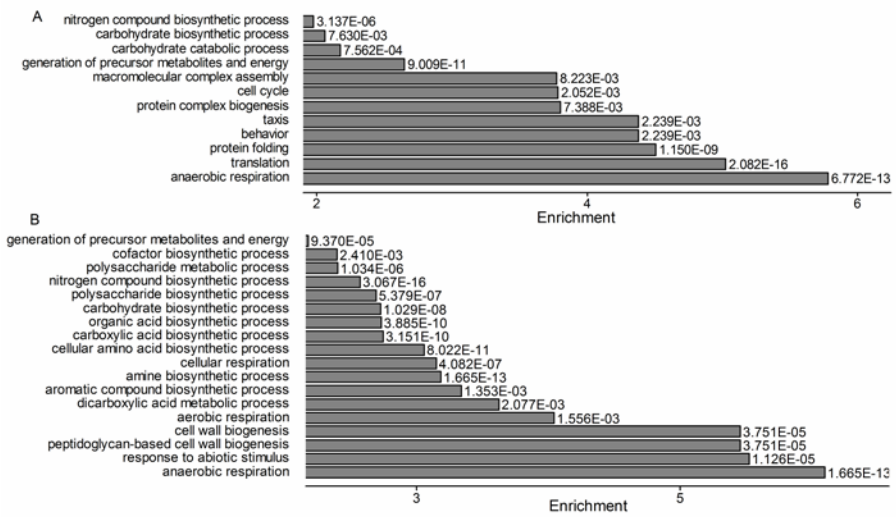

Figure S10. The most enriched terms between the top (A) and bottom (B) groups of HSR density. We excluded those HSRs which contain conserved secondary structures predicted by RNAz, the left HSRs were used to GO analysis.

Table S1. Summary of four species

Species	No. of CDS[a]	Orthologs[b]	Sequence identity[c]	All HSRs	Conserved HSRs
E. coli	4,152	-	-	14,841	-
S. enterica	4,233	2,948	0.78	14,286	2,766
S. flexneri	4,112	3,037	0.96	13,517	5,344
Y. pestis	3,770	-	-	14,043	-
A. hydrophila	4,025	-	-	12,471	-
V. cholerae	3,455	-	-	10,663	-

[a] Sequences with length < 200 were excluded.

[b] Sequences in *E. coli* were used to search for orthologs in other species. Only one to one orthologs were included. Insertions and deletions change the positions of HSRs vastly, thus the alignments with insertions or deletions >10 were discard.

[c] The value was obtained by averaging sequence identity of all pairs of orthologs.

Table S2. Comparison of HSR densities among different gene categories

Category	Cell processes	Transport	Information transfer	Metabolism	Regulation
Cell processes		0.922	0.437	**0.035**[a]	**0.020**
Transport			0.419	**0.015**	**0.010**
Information transfer				0.168	0.074
Metabolism					0.333
Regulation					

[a] Wilcox test between every two categories was performed, the significant differences (P <0.05) are highlighted in bold.