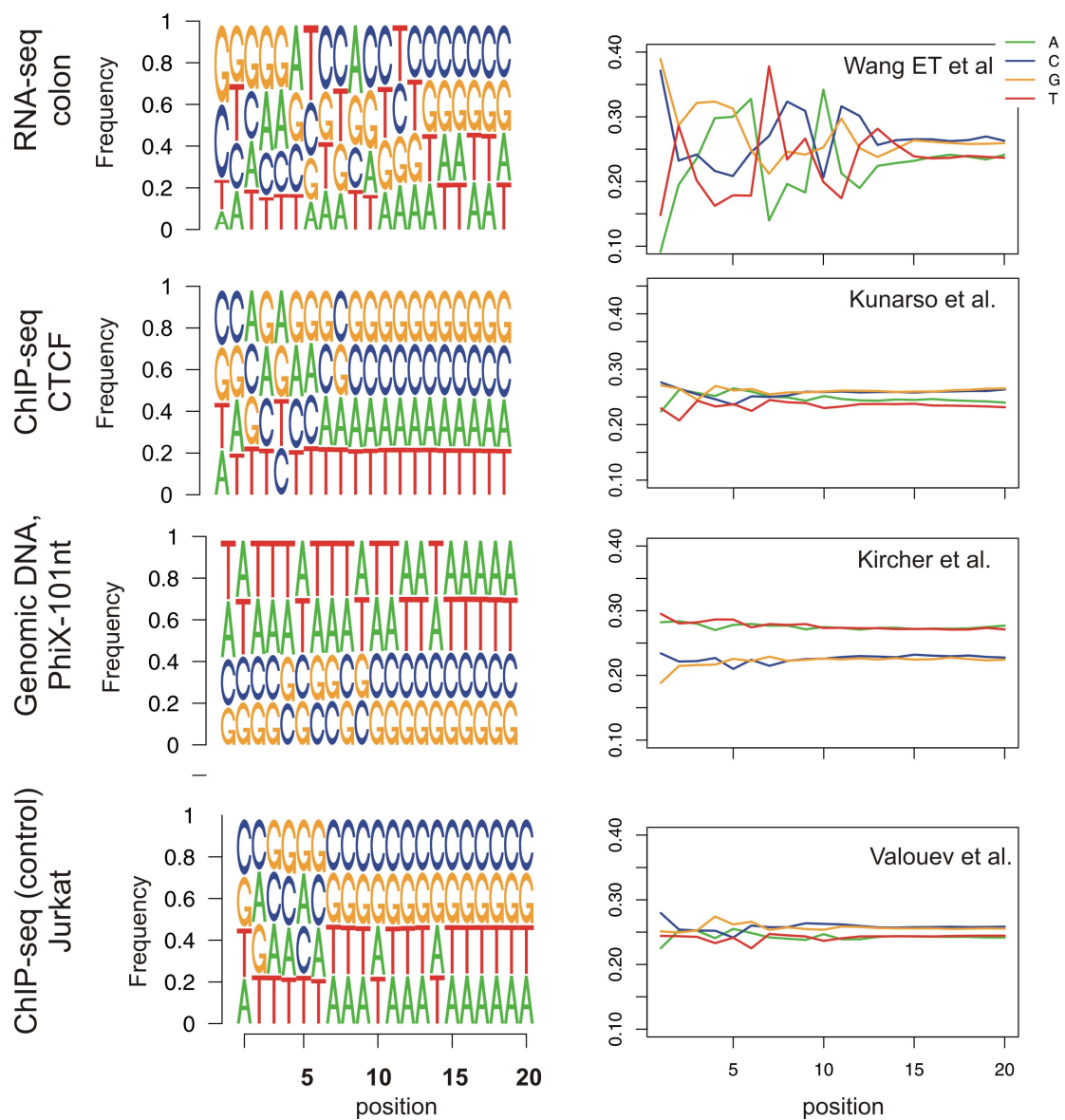


**Supplementary Materials:**  
**Detection and removal of biases in the  
analysis of next-generation sequencing reads**

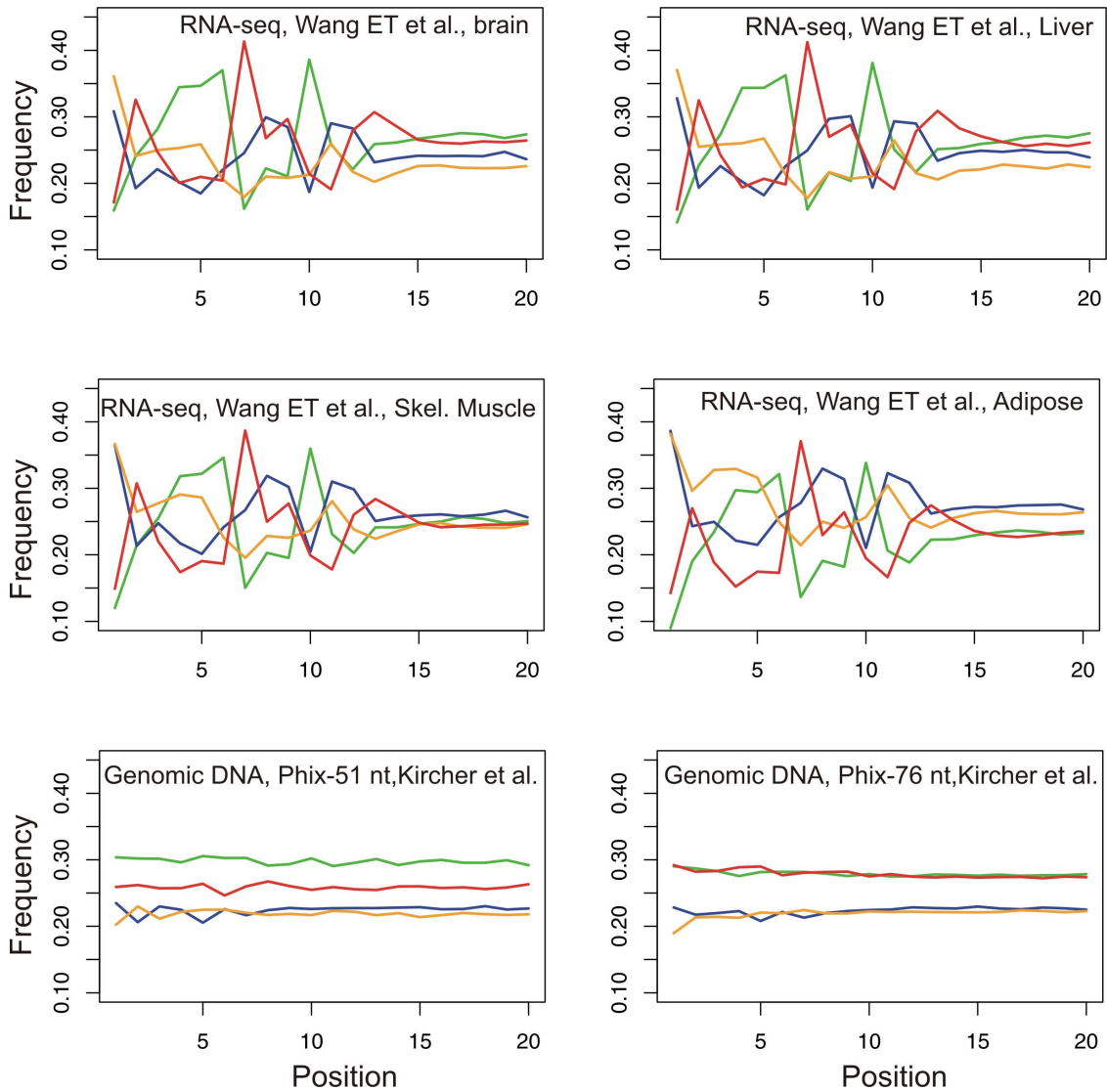
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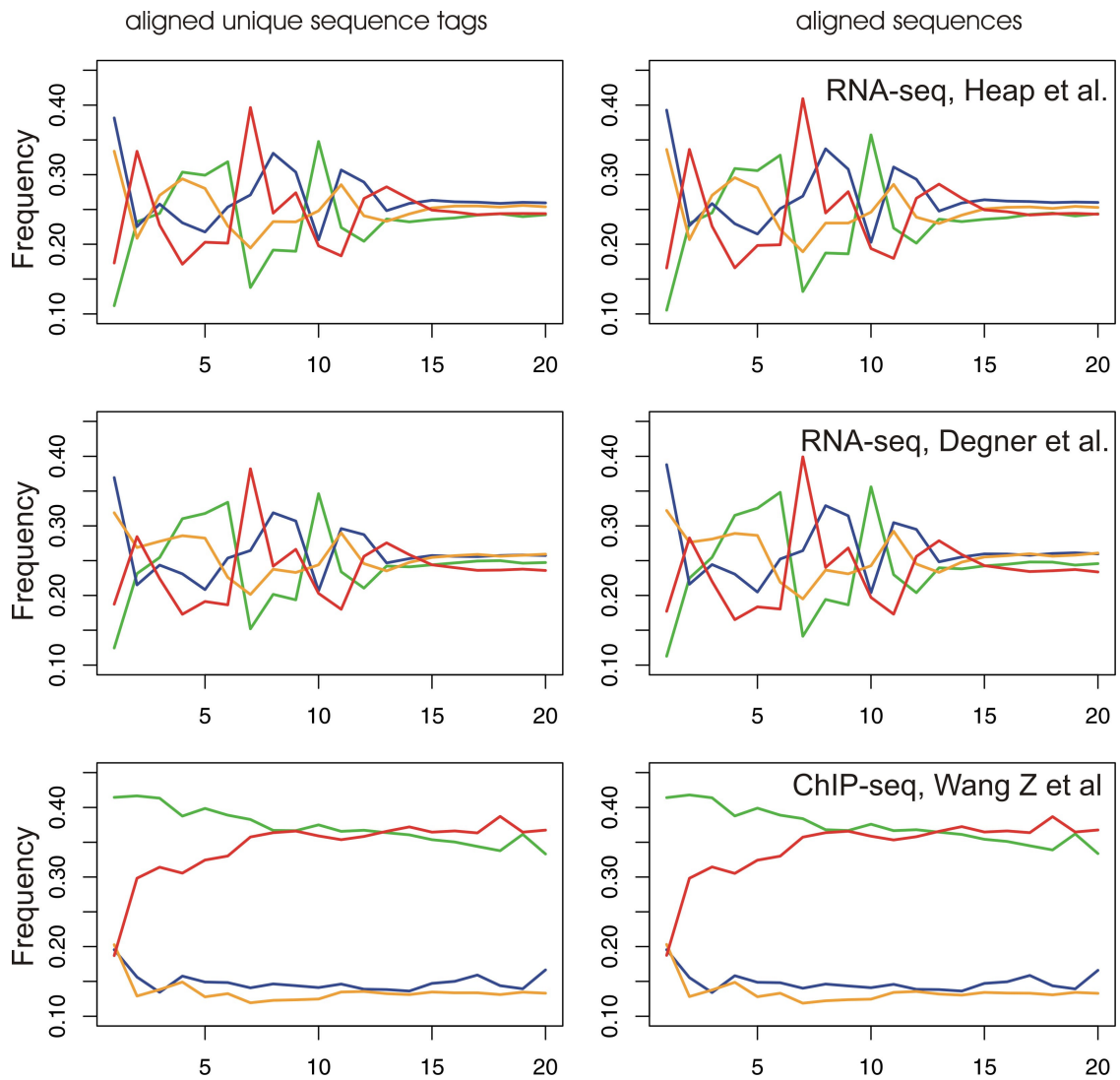
<sup>2</sup>These authors contributed equally.



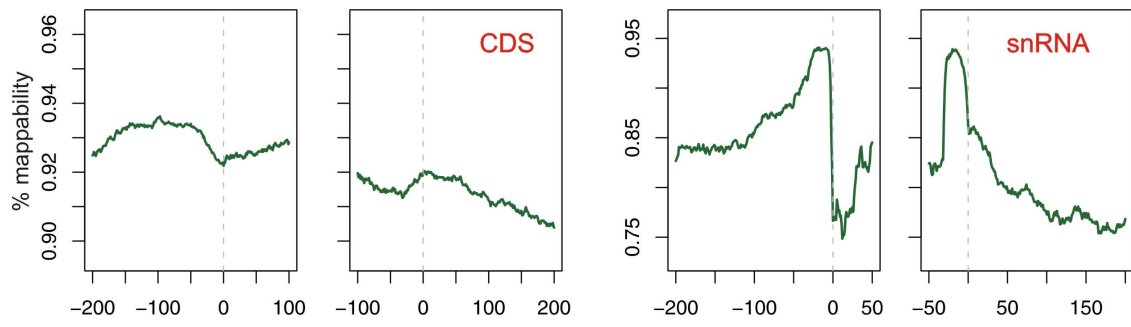
**Supplementary Figure 1:** Sequence logos and positional nucleotide plots for several additional examined datasets, as in Figure 1.



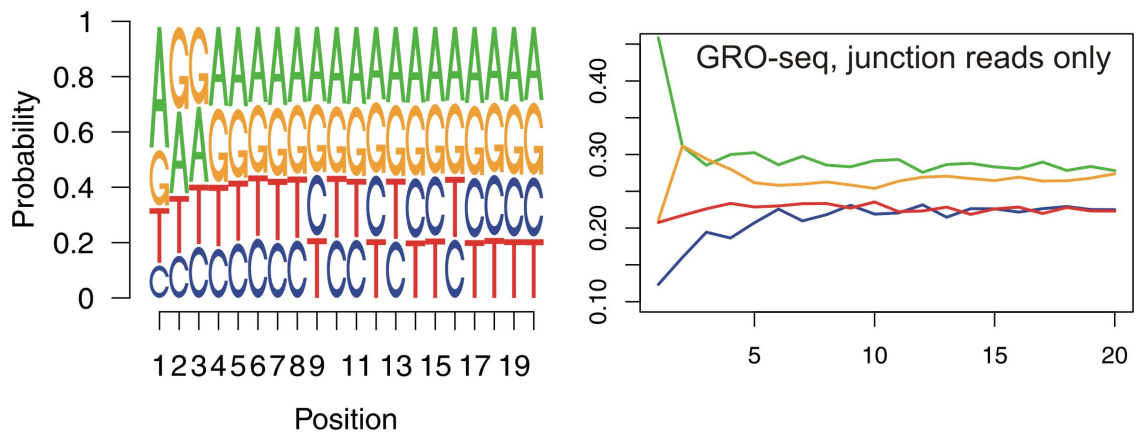
**Supplementary Figure 2:** Positional nucleotide plots for several additional examined datasets, as in Figure 1 and in Supplementary Figure 1.



**Supplementary Figure 3:** Comparison of nucleotide composition when examining only unique sequence tags (left column), as opposed to all sequences (as shown in Figure 1 in the manuscript). This analysis demonstrates that the bias in nucleotide composition is not likely to have occurred as a result of biased PCR amplification of few specific amplicons.



**Supplementary Figure 4:** Mappability density values within the regions surrounding the coding sequence start and end sites, and snRNAs.



**Supplementary Figure 5:** Sequence logos and positional nucleotide plots for junction reads in GRO-seq data. The similarity of these plots to the ones in Figure 4C-D demonstrates that the bias is presumably not due to biased hydrolysis of BrU containing residues, as it is present also in reads originating from contaminating mRNA.