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Protocol

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GLORI for absolute quantification of transcriptome-wide m^{6}A at single-base resolution

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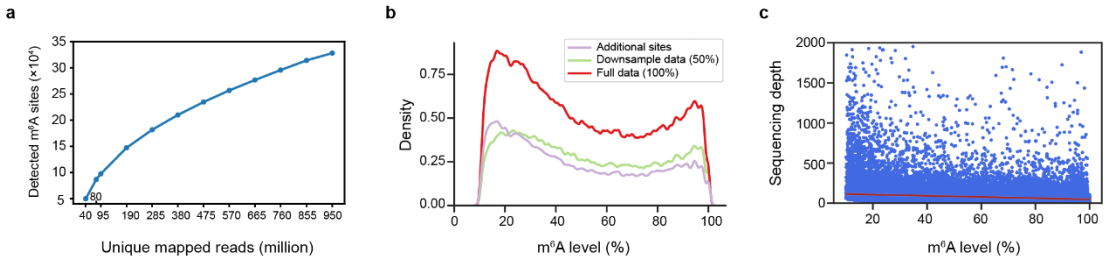
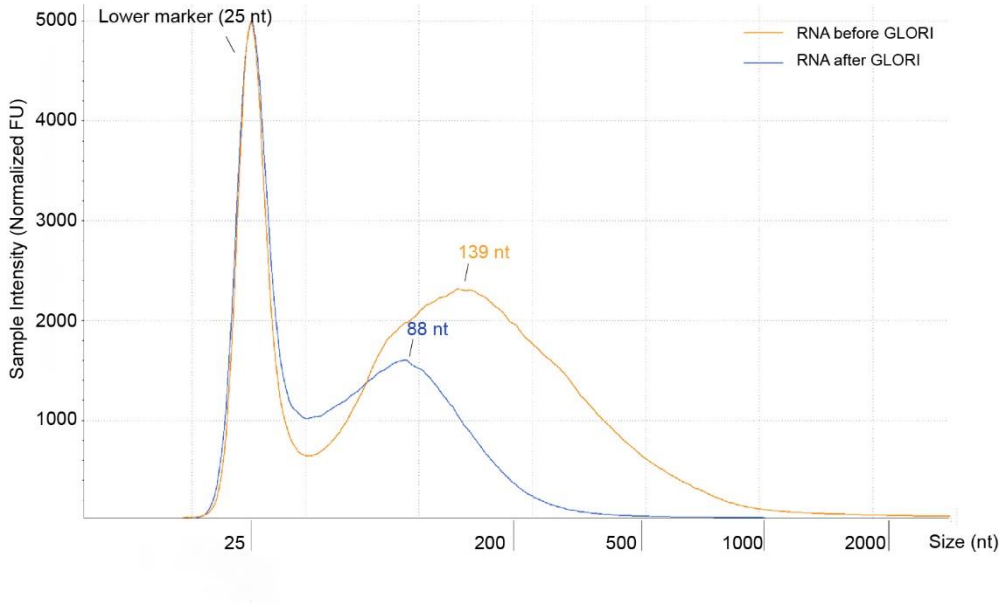


Supplementary Fig. 1 | The effects of sequencing depth on the number of detected m^{6}A sites and m^{6}A stoichiometry. (a) The number of detected m^{6}A sites at various sequencing depths in a merged dataset. We combined two replicates (GSM6432590 and GSM6432591) with two additional replicates generated in this manuscript (GSE233875) to create the merged dataset. (b) The distribution of m^{6}A stoichiometry for the sites detected in the full dataset, downsampled dataset, and additional sites between these two datasets (*n* = 28,206 Additional sites, *n* = 31,857 Downsampled data, *n* = 59,400 Full data). (c) Scatterplot shows the correlation of m^{6}A level and sequencing depth (*n* = 59,400).



Supplementary Fig. 2 | Size distribution analysis of RNA before and after GLORI treatment. The plot shows the peak of RNA before and after GLORI. Fragmented RNA is first protected by glyoxal, and deaminated by mixture of glyoxal and nitrite, then deprotected. The size distribution of RNA before and after GLORI treatment is finally checked using Agilent 2100 Bioanalyzer. Orange line represents the size distribution of RNA before GLORI, while blue line represents that after GLORI. RNA fragment length was

reduced from ~140 nt to ~90 nt after chemical treatment. The lower marker shown in the plot is 25 nt.