

# Supplemental Information

## Glyoxals as *In Vivo* RNA Structural Probes of Guanine Base Pairing

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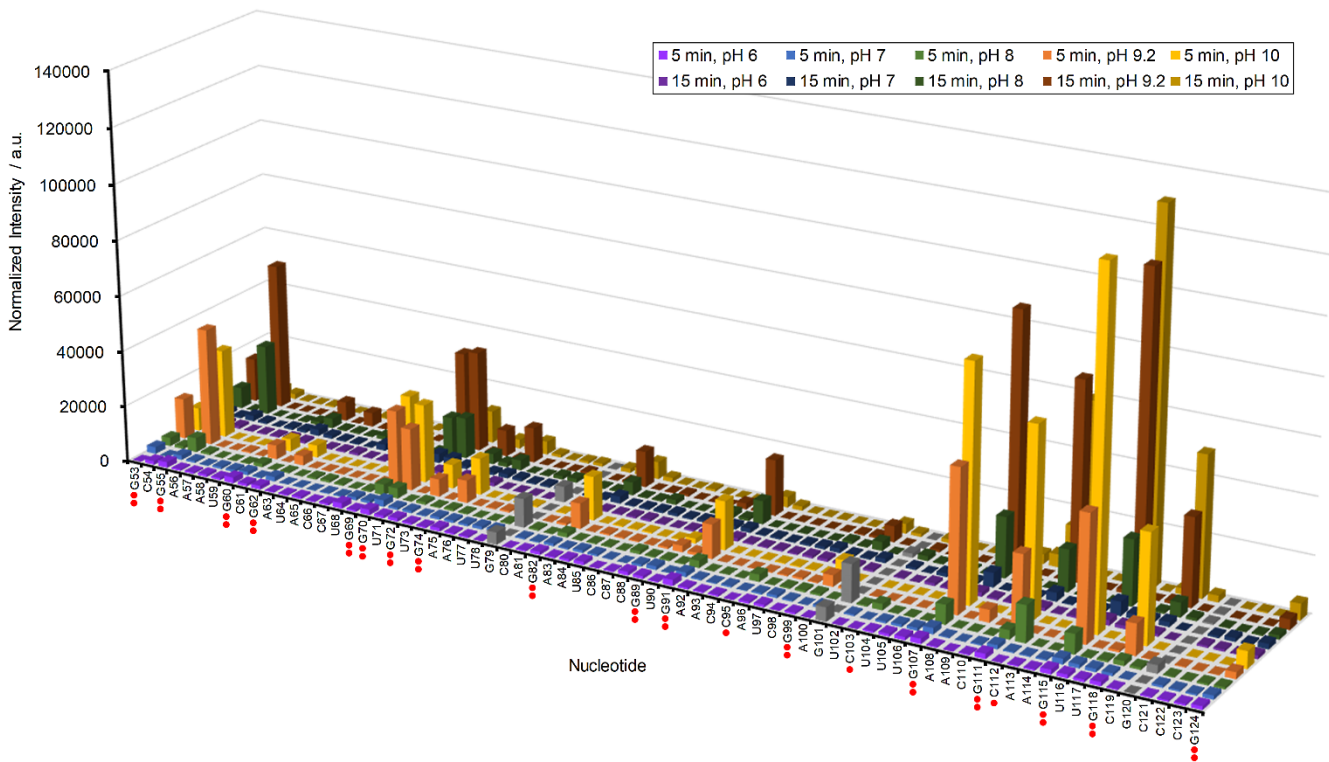
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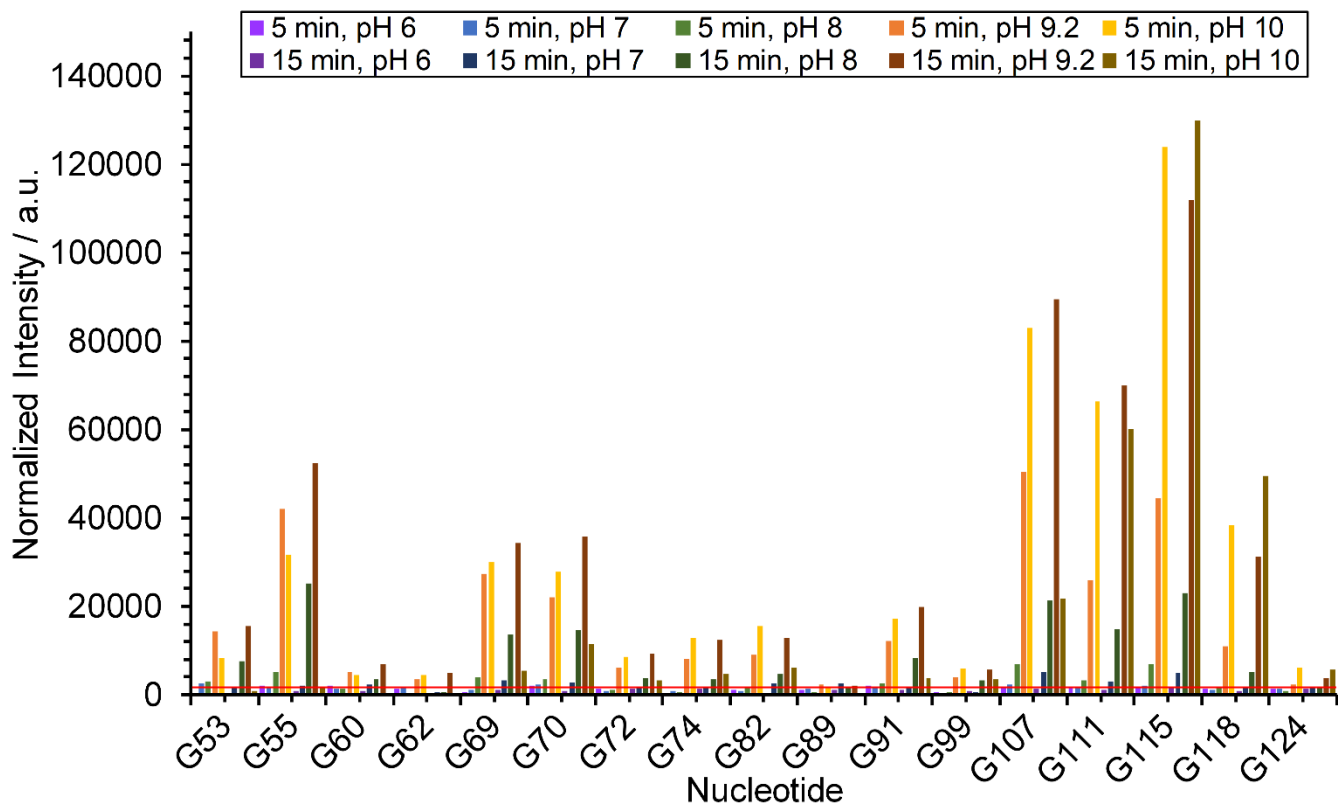
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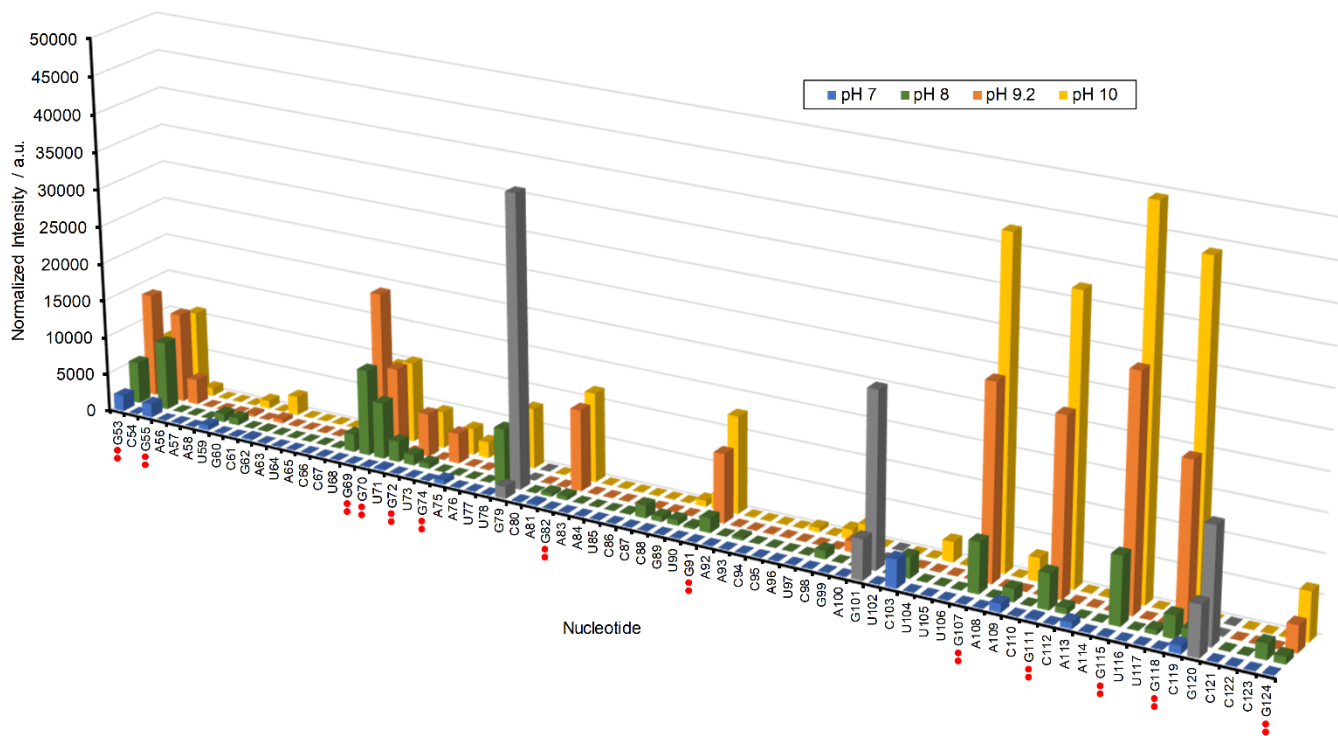
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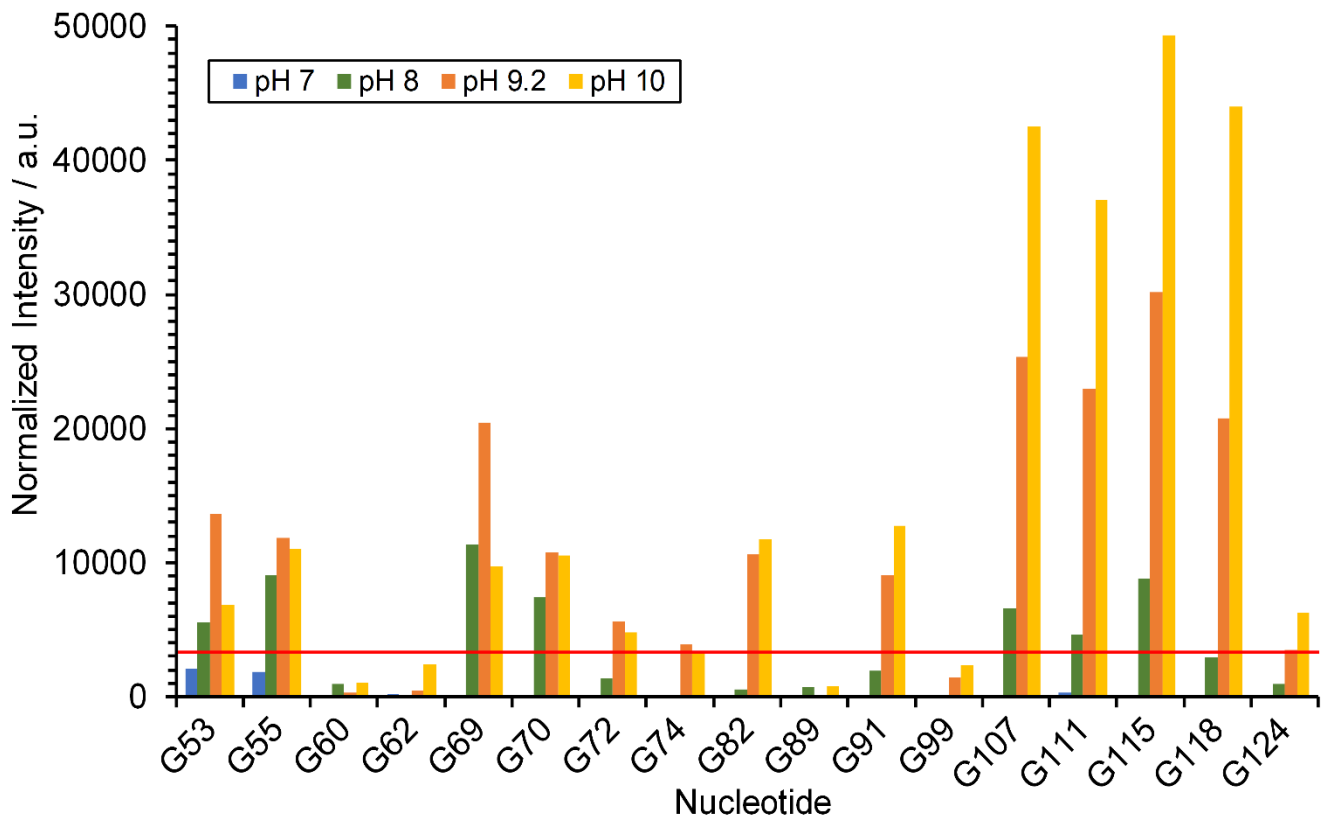
**Supplemental Figure S1.** Modification of rice 5.8S rRNA *in vitro* by glyoxal. All nucleotides within the examined range (G53-G124) are shown. A red dot indicates glyoxalated cytidines at buffer pH 8 and 5 min reaction time, whereas two red dots indicate glyoxalated guanosines. While uridines are shown, glyoxal cannot react with uridines (see Fig. 2 in main text). Therefore, no uridine residues are given red dots. Normalized intensities for each nucleotide are grouped by reaction time, with 5 min reaction times given a lighter color and 15 min reaction times given a darker color. G79, G101, and G120, which are the residues that give natural reverse transcriptase stops, are colored grey.



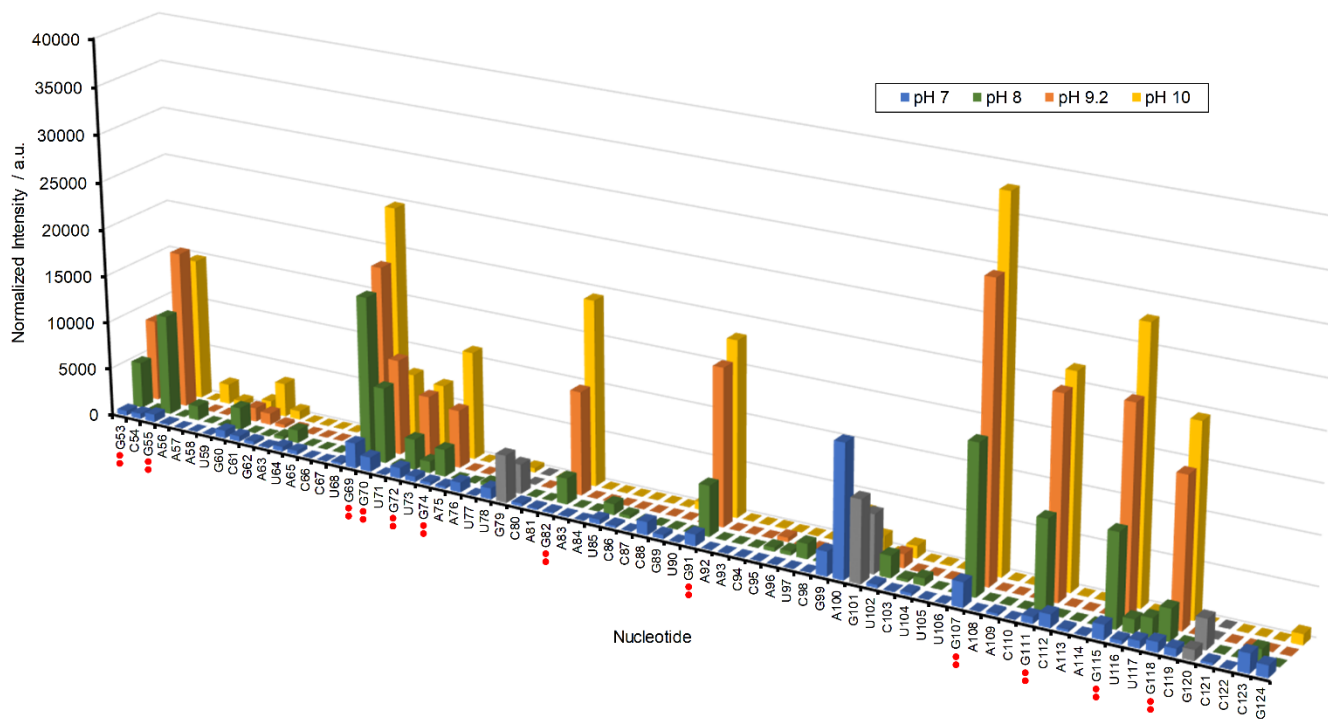
**Supplemental Figure S2.** *In vitro* modification of guanosine residues with rice 5.8S rRNA by glyoxal. Normalized band intensities of all guanosine nucleotides within the examined range (G53-G124) are shown except for the three guanosines that give natural reverse transcriptase stops (G79, G101, and G120). The baseline value above which glyoxalation is considered significant, 1806, is shown as a red line.



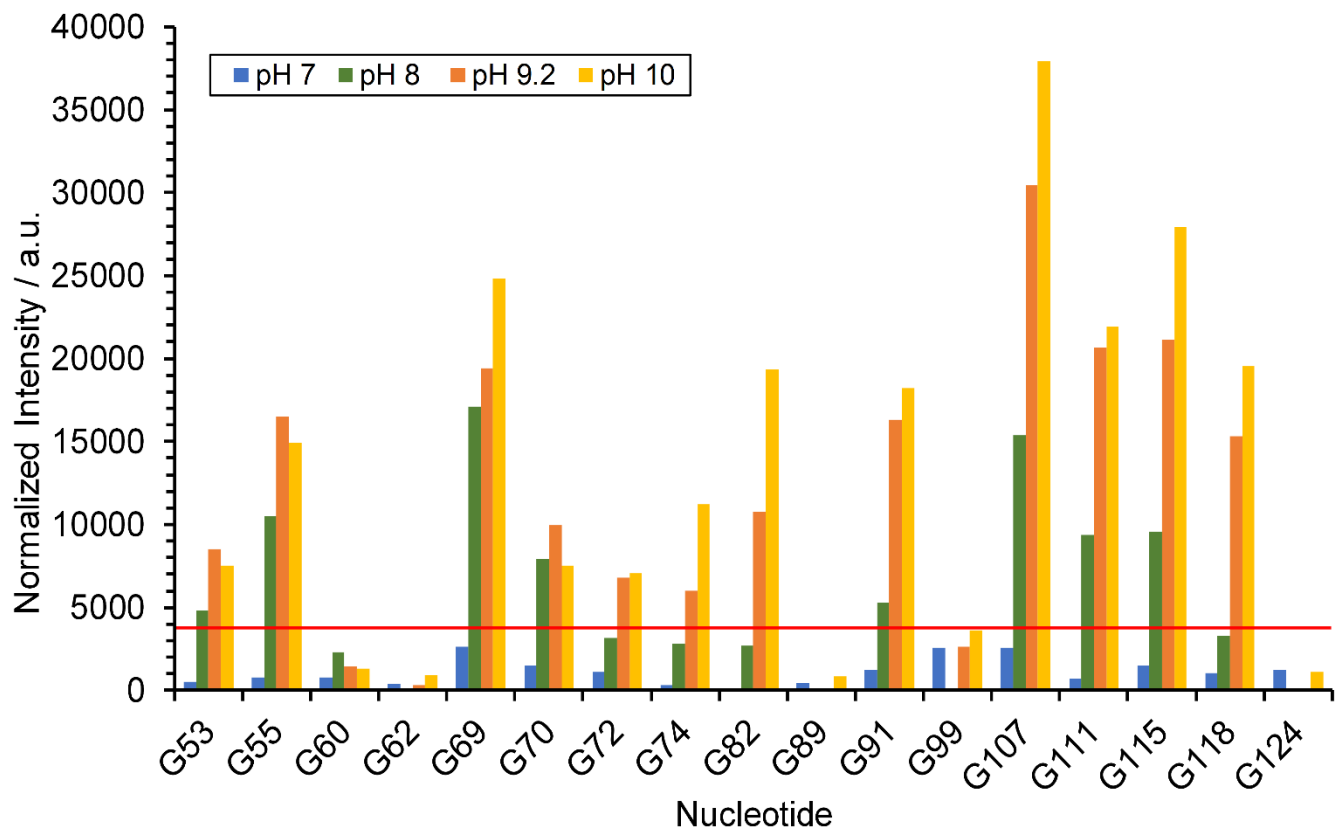
**Supplemental Figure S3.** Modification of rice 5.8S rRNA *in vitro* by methylglyoxal. All nucleotides within the examined range (G53-G124) are shown. Two red dots indicate significantly glyoxalated guanosines. Uridines, while shown, cannot be glyoxalated (see Fig. 2 in main text). G79, G101, and G120, which are the residues that give natural reverse transcriptase stops, are colored grey.



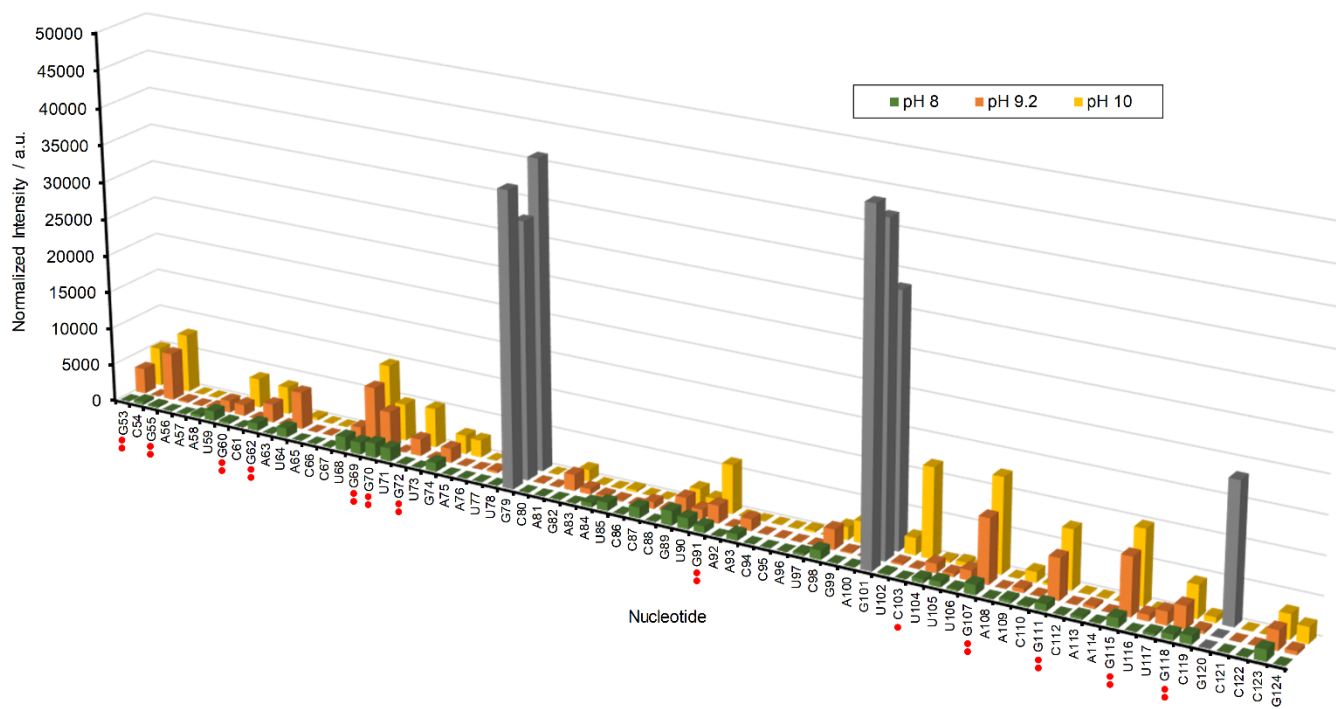
**Supplemental Figure S4.** Modification of guanosine residues with rice 5.8S rRNA *in vitro* by methylglyoxal. Normalized band intensities of all guanosine nucleotides within the examined range (G53-G124) are shown except for the three guanosines that give natural reverse transcriptase stops (G79, G101, and G120). The baseline value above which glyoxalation is considered significant, 3268, is shown as a red line.



**Supplemental Figure S5.** Modification of rice 5.8S rRNA *in vitro* by phenylglyoxal. All nucleotides within the examined range (G53-G124) are shown. Two red dots indicate significantly glyoxalated guanosines. Uridines, while shown, cannot be glyoxalated (see Fig. 2 in main text). G79, G101, and G120, which are the residues that give natural reverse transcriptase stops, are colored grey.

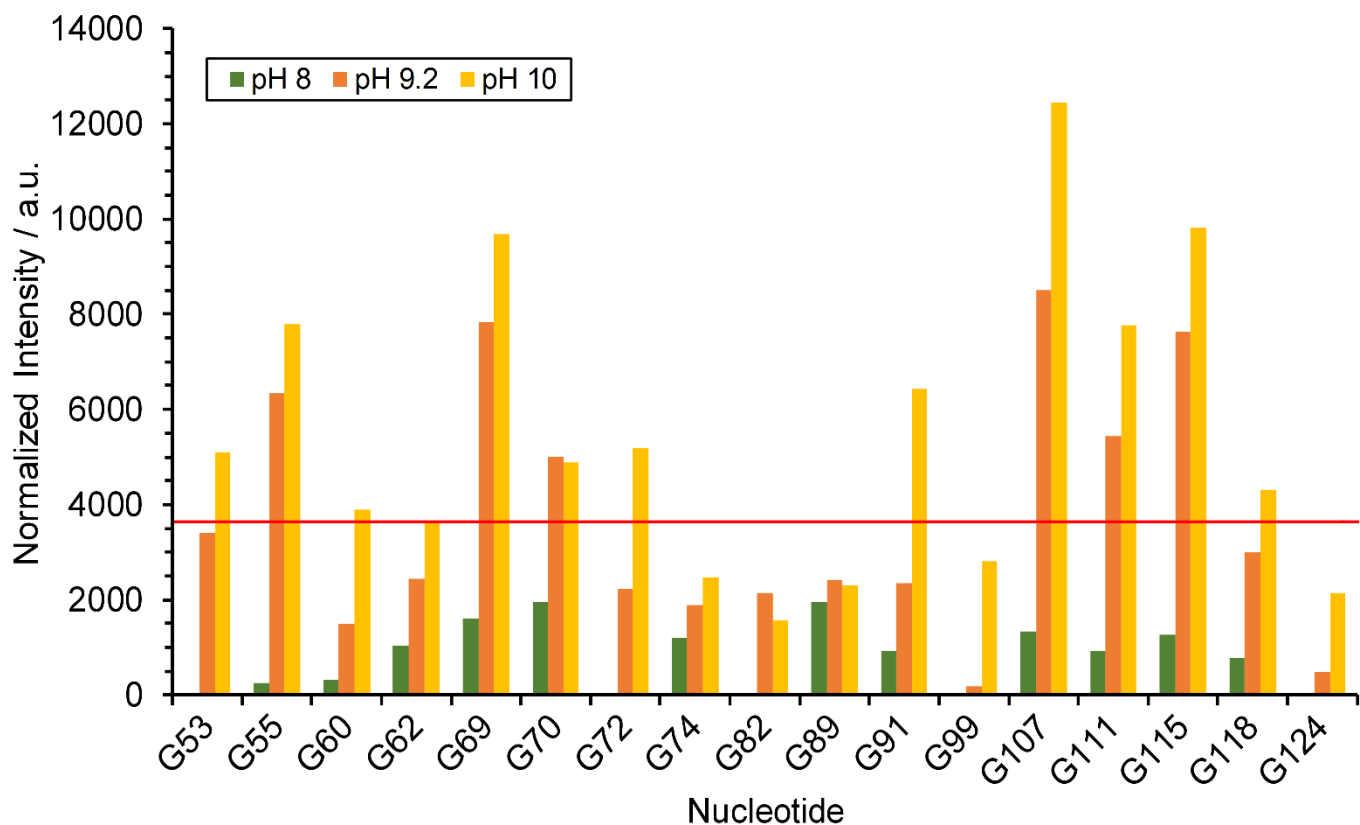


**Supplemental Figure S6.** Modification of guanosine residues with rice 5.8S rRNA *in vitro* by phenylglyoxal. Normalized band intensities of all guanosine nucleotides within the examined range (G53-G124) are shown except for the three guanosines that give natural reverse transcriptase stops (G79, G101, and G120). The baseline value above which glyoxalation is considered significant, 3910, is shown as a red line.

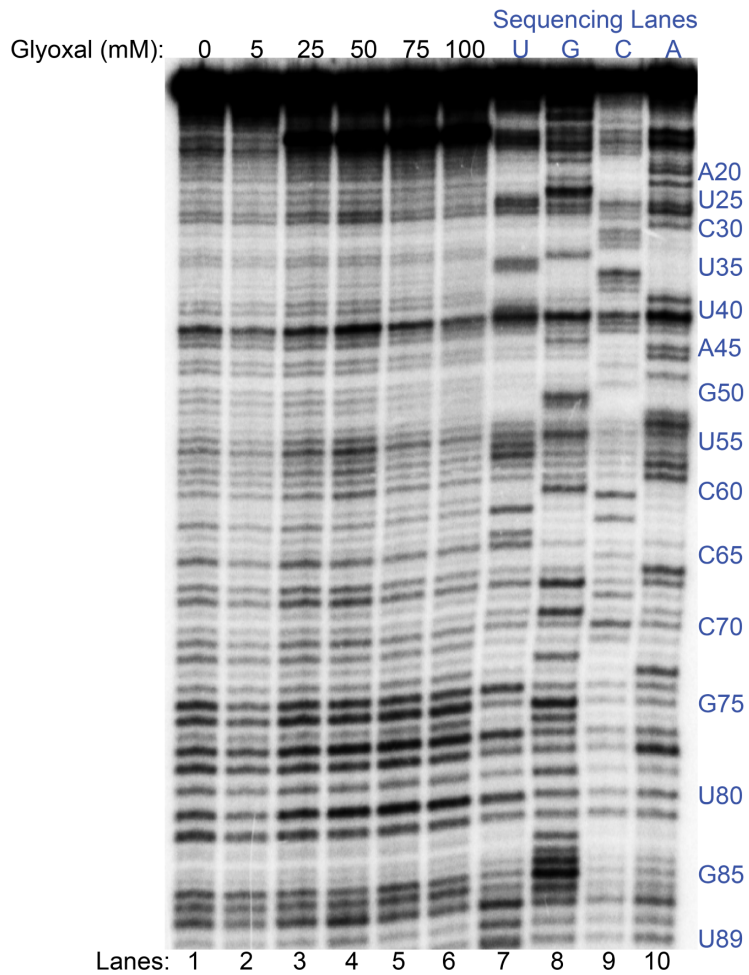


**Supplemental Figure S7.** Modification of rice 5.8S rRNA *in vitro* by dimethylglyoxal. All nucleotides within the examined range (G53-G124) are shown. Two red dots indicate significantly glyoxalated guanosines. Uridines, while shown, cannot be glyoxalated (see Fig. 2 in main text). G79, G101, and G120, which are the residues that give natural reverse transcriptase stops, are colored grey.





**Supplemental Figure S8.** Modification of guanosine residues with rice 5.8S rRNA *in vitro* by dimethylglyoxal. Normalized band intensities of all guanosine nucleotides within the examined range (G53-G124) are shown except for the three guanosines that give natural reverse transcriptase stops (G79, G101, and G120). The baseline value above which glyoxalation is considered significant, 3605, is shown as a red line.



**Supplemental Figure S9.** Glyoxal modification of *B. subtilis* 5S *in vivo* analyzed by denaturing PAGE after reverse transcription to generate cDNAs. Lane 1 is the control (0 mM glyoxal) while lanes 2-6 show reactions from 5 mM to 100 mM glyoxal. Lanes 7-10 show dideoxy sequencing of U, G, C, and A.