SUPPORTING INFORMATION FOR:

RNA Post-transcriptional Modifications in Two Large Subunit Intermediates Populated in *E.coli* Cells Expressing Helicase Inactive R331A DbpA

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Table SI 1. Mutation rates for the OH^5C 2505 nucleotide treated with $KMnO_4$.

	$KMnO_4$		No Chemical Treatment
	3 minutes	6 minutes	
Mutation rate [a]	0.466 ± 0.005	0.453 ± 0.005	0.007 ± 0.001

[a] The mutation rates for the samples treated with $KMnO_4$ for 3 or 6 minutes and the untreated control sample. The errors shown for each experiment are calculated using the equation[1] $\sqrt[2]{mutation\ rate} \Big/ \sqrt[2]{read\ depth}$

Table SI 2. Mutation rates for the $m^3\Psi$ 1919 nucleotide treated with CMCT plus $NaHCO_3$, or only with $NaHCO_3$.

		Chemical Treatment		
	Particle	CMCT and OH^-		OH^-
Mutation rate [a]	35S	0.094 ± 0.006	0.100 ± 0.006	0.158 ± 0.006
	45S	0.076 ± 0.004	0.078 ± 0.003	0.106 ± 0.003
	50S	0.602 ± 0.011	0.607 ± 0.010	0.703 ± 0.012

[a] The errors shown here are for each experiment and are calculated using the equation[1]

$$\sqrt[2]{mutation\ rate} \Big/ \sqrt[2]{read\ depth}$$

Table SI 3. Mutation rates' confidence intervals of 23S rRNA modified nucleotides.

Modified [a] Nucleotide	Enzyme [b]	95% Confidence Interval [c]		
		35S	**45S**	**50S**
Ψ 748	RluA	0.026 ± 0.013	0.030 ± 0.001	0.029 ± 0.001
Ψ 957	RluC	0.242 ± 0.032	0.247 ± 0.009	0.261 ± 0.016
Ψ 1915	RluD	0.013 ± 0.001	0.011 ± 0.003	0.039 ± 0.007
[d] m^3Ψ 1919	RluD/RlmH	0.097 ± 0.006	0.077 ± 0.001	0.605 ± 0.006
Ψ 1921	RluD	0.042 ± 0.004	0.024 ± 0.001	0.156 ± 0.008
Ψ 2461	RluE	0.090 ± 0.006	0.098 ± 0.002	0.108 ± 0.004
OH^5C 2505	RlhA	0.070 ± 0.003	0.108 ± 0.002	0.265 ± 0.008
m^2A 2507	RlmN	0.083 ± 0.000	0.097 ± 0.001	0.090 ± 0.011
Ψ 2508	RluC	0.105 ± 0.022	0.118 ± 0.008	0.080 ± 0.000
Ψ 2584	RluC	0.324 ± 0.000	0.322 ± 0.001	0.322 ± 0.014
Ψ 2608	RluF	0.029 ± 0.002	0.025 ± 0.003	0.034 ± 0.001
Ψ 2609	RluB	0.067 ± 0.005	0.055 ± 0.002	0.070 ± 0.000

[a] 23S rRNA modified nucleotides investigated in this study.
[b] Enzymes incorporating the modifications.
[c] 95% confidence interval was calculated using the equation:

$$Avg_{(i)(p)} \pm 1.96 \times Std_{(i)(p)}/\sqrt{n}$$

$Avg_{(i)(p)}$ is the average mutation rate for nucleotide i of particle p, $Std_{(i)(p)}$ is the standard deviation error for the nucleotide i of particle p. The average and the standard deviation were calculated using Equations 2 and 3 in the Materials and Methods section of the paper. n is the number of independent experiments, which is 2.
[d] The RluD enzyme performs the U 1919 to Ψ isomerization, while the RlmH enzyme methylates Ψ 1919 at position N3.

[1] Busan, S., and Weeks, K. M. (2018) Accurate detection of chemical modifications in RNA by mutational profiling (MaP) with ShapeMapper 2, *RNA 24*, 143-148.