Supplementary Information (SI) for Analyst.

Supporting Information

Exploiting Pfu DNA Polymerase's differential bypass capacity for AP vs. deoxyuracil sites: a novel strategy for sensitive uracil-DNA glycosylase detection

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Table S1 Oligonucleotide sequence information.

Name	Sequence (5'→3')
U-Probe	TAATCTGCCGGTGGTGTTGCTGTATCTCGGCTCTCTGACCACAGTAGUAGCTCGATGTAATGCTTGCGTGGCAAACCACGTGAGTCCTGAAAGACGGCA
AP-Probe	TAATCTGCCGGTGGTGTTGCTGTATCTCGGCTCTCTGACCACAGTAGAPAGCTCGATGTAATGCTTGCGTGGCAAACCACGTGAGTCCTGAAAAGACGGCA
T-Probe	TAATCTGCCGGTGGTGTTGCTGTATCTCGGCTCTCTGACCACAGTAGTAGCTCGATGTAATGCTTGCGTGGCAAACCACGTGAGTCCTGAAAGACGGCA
U-Probe-79	TAATCTGCCGGTGGTGTTGCCTCTCTGACCACAGTAGUAGCTCGATGTAATGCTTGCGT GTGAGTCCTGAAAGACGGCA
U-Probe-119	TAATCTGCCGGTGGTGTTGCACCTACCTGCTGTATCTCGGCTCTCTGACCACAGTAGUAGCTCGATGTAATGCTTGCGTGGCAAACCACGTTCGTAACAGTGAGTCCTGAAAGACGGCA
U-Probe-139	TAATCTGCCGGTGGTGTTGCGCCAGAAACAACCTACCTGCTGTATCTCGGCTCTCTGACCACAGTAGUAGCTCGATGTAATGCTTGCGTGGCAAACCACGTTCGTAACAACTCTTAGTTGTGAGTCCTGAAAGACGGCA
U-25-Probe	TAATCTGCCGGTGGTGTTGCTGTAUCTCGGCTCTCTGACCACAGTAGTAGCTCGATGTAATGCTTGCGTGGCAAACCACGTGAGTCCTGAAAGACGGCA
U-32-Probe	TAATCTGCCGGTGGTGTTGCTGTATCTCGGCUCTCTGACCACAGTAGTAGCTCGATGTAATGCTTGCGTGGCAAACCACGTGAGTCCTGAAAGACGGCA
U-64-Probe	TAATCTGCCGGTGGTGTTGCTGTATCTCGGCTCTCTGACCACAGTAGTAGCTCGATGTAATGCUTGCGTGGCAAACCACGTGAGTCCTGAAAGACGGCA
2U-Probe-1	TAATCTGCCGGTGGTGTTGCTGTATCTCGGCTCTCUGACCACAGTAGUAGCTCGATGTAATGCTTGCGTGGCAAACCACGTGAGTCCTGAAAGACGGCA
2U-Probe-2	TAATCTGCCGGTGGTGTTGCTGTAUCTCGGCTCTCTGACCACAGTAGUAGCTCGATGTAATGCTTGCGTGGCAAACCACGTGAGTCCTGAAAGACGGCA
2U-Probe-3	TAATCTGCCGGTGGTGTTGCTGTAUCTCGGCTCTCTGACCACAGTAGTAGCTCGATGTAATGCUTGCGTGGCAAACCACGTGAGTCCTGAAAGACGGCA
2U-Probe-4	TAATCTGCCGGTGGTGTTGCTGTATCTCGGCTCTCTGACCACAGTAGUAGCTCGATGTAATGCUTGCGTGGCAAACCACGTGAGTCCTGAAAGACGGCA
Forward Primer	TGCCGTCTTTCAGGACTCAC
Reverse Primer	TAATCTGCCGGTGGTGTTGC

Table S2 UDG reaction buffer formula.

Buffer's name	Fomula of 1 × Buffer
UDG Buffer	20 mM Tris-HCl(pH=8.0)，1 mM DTT，1 mM EDTA
Buffer C	20 mM Tris-HCl(pH=8.0)， 1 mM DTT，1 mM EDTA，10 mM (NH4)2SO4，10 mM KCl，2 mM MgSO4，0.002%Tween-20
Buffer D	20 mM Tris-HC(pH=8.0)，0.5 mM DTT，0.5 mM EDTA，5 mM (NH4)2SO4， 5 mM KCl，1 mM MgSO4，0.05%Triton X-100

Table S3 Differences between qPCR for feasibility analysis.

Group	UDG	Template	Polymerase
1	+	DNA-AP-99	Taq-Pol
2	−	DNA-U-99	Taq-Pol
3	+	DNA-AP-99	Pfu-Pol
4	−	DNA-U-99	Pfu-Pol

Table S4 Precision (RSD) of the proposed method determined by three independent samples analyzed in parallel three times for three consecutive days.

	UDG（U/mL）	Intra-day RSD (%, n=3)	Inter-day RSD (%, n=3)
Low	1×10^{-4}	1.6	0.39
Middle	1×10^{-3}	5.1	3.1
High	1×10^{-2}	0.33	2.9

Table S5 Comparison of different fluorescence methods for UDG detection.

Methods	Quantitative Range (U/L)	LOD (U/L)	Assay Duration	Number of Probes	Operational Complexity
A label-free and enzyme-free DNA machine[1]	0.0025~0.020	4.4×10^{-4}	>175 min	4	Three-step reaction
Toehold-mediated strand displacement reaction-dependent fluorescent strategy[2]	0.0002~0.0080	2.7×10^{-5}	>305 min	3	Four-step reaction
Catalytic single-molecule Förster resonance energy transfer biosensor[3]	0.005~0.5	3.67×10^{-3}	>145 min	3	One-step reaction
Exponential real-time rolling circle amplification[4]	0.0001~0.075	5.5×10^{-5}	>365 min	3	Four-step reaction
This work	0.0001~0.01	1×10^{-4}	>210 min	3	Two-step reaction

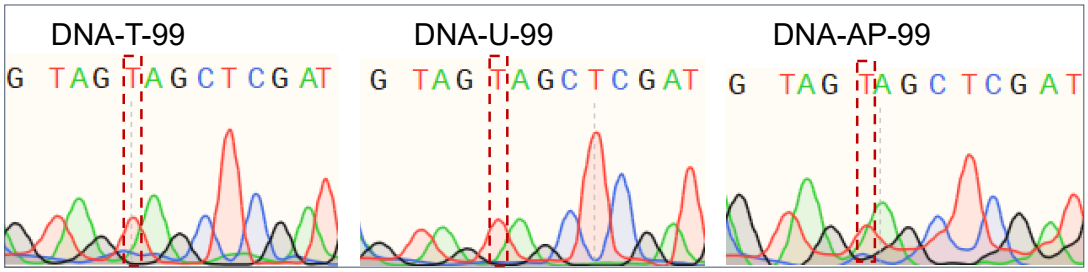

Fig. S1 Sanger sequencing detected amplification products of DNA-T-99, DNA-U-99, and DNA-AP-99, with corresponding locus highlighted in red.

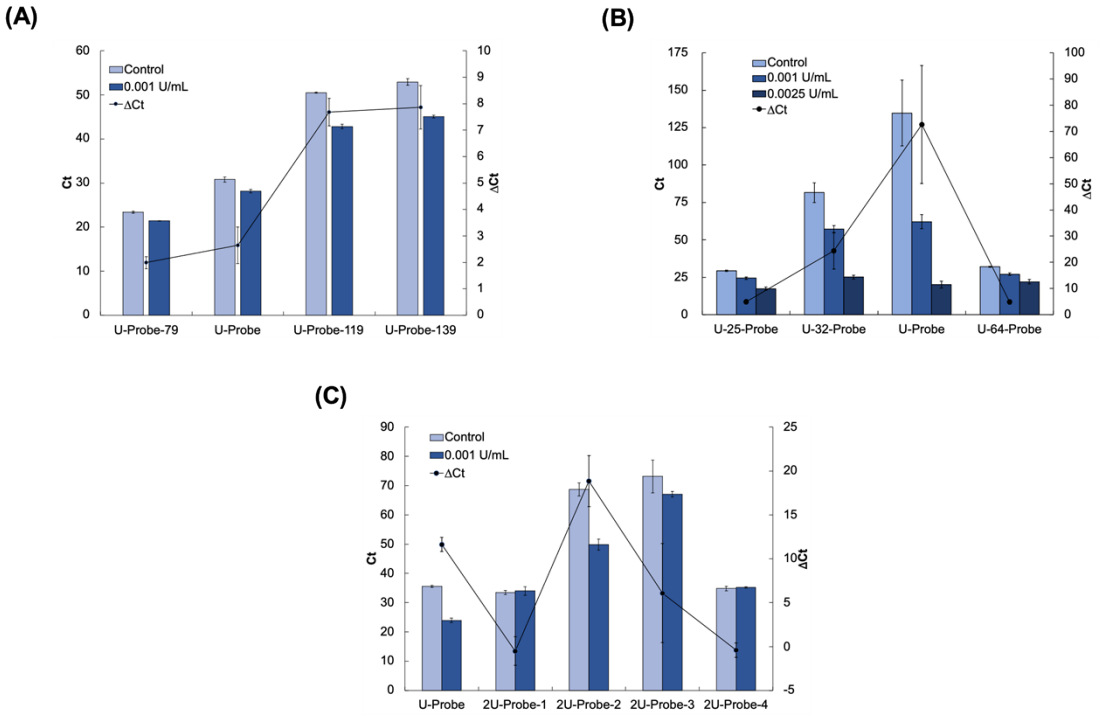

Fig. S2 Probe design optimization. (A) Length of the probe, U-Probe is 99 nt long; (B) Position of dU on the probe, U-Probe's dU is at the 48th loci from 5' end; (C) 2U-Probe means two dU residues on the probe.

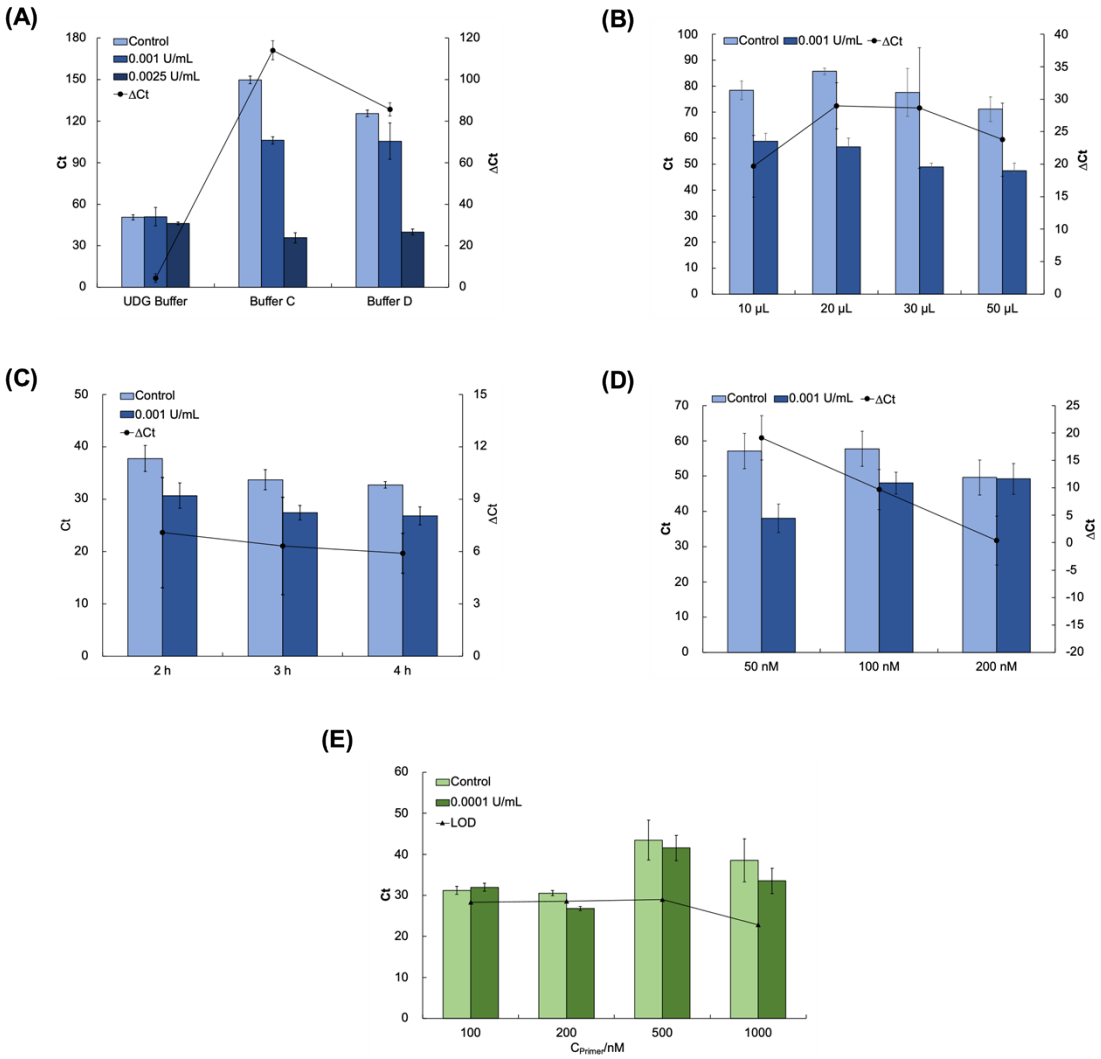

Fig. S3 Condition optimization results. (A) The Buffer of UDG Reaction. (B) The volume of UDG reaction. (C) The time of UDG reaction. (D) The concerntration of templates used in UDG rection. (E) The concerntration of primers in qPCR.

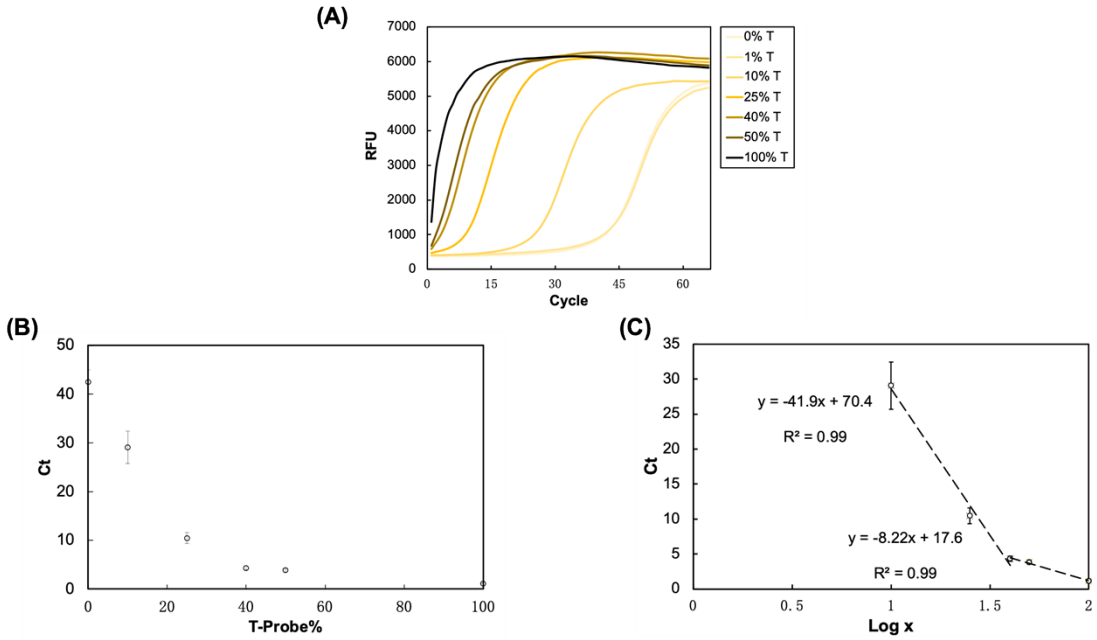

Fig. S4 (A) The original fluorescence curve of dT/dU mixed chains. (B) The variation of Ct value with percentage of T-Probe. (C) The standard curve of T-Probe/U-Probe mixtures.

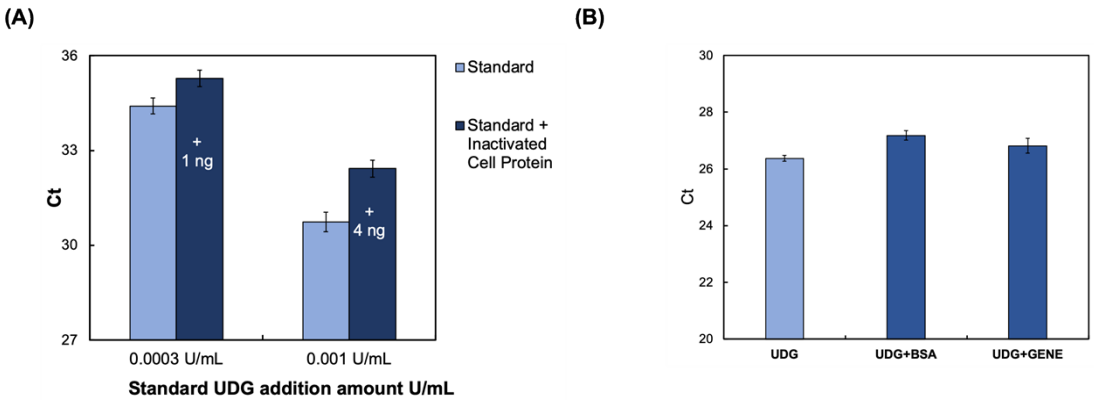

Fig. S5 The influence of matrix effect on UDG detection. (A) The effect of thermally inactivated cell lysates. (B) The impact of simulated cellular environment.

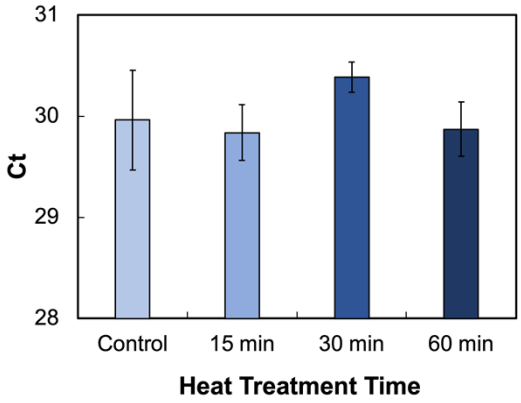

Fig. S6 The effect of time of heat treatment on UDG activity.

Reference

1. Y. S. Wu, L. Wang, J. Zhu and W. Jiang, *Biosens. Bioelectron.*, 2015, **68**, 654-659.
2. Y. S. Wu, L. Wang and W. Jiang, *Biosens. Bioelectron.*, 2017, **89**, 984-988.
3. Q. Zhang, C. C. Li, F. Ma, X. L. Luo and C. Y. Zhang, *Biosens. Bioelectron.*, 2022, **213**.
4. Y. Xu, Y. X. Cui, Q. G. Zhao, A. N. Tang and D. M. Kong, *Anal. Methods*, 2018, **10**, 2405-2410.