Electronic Supplementary Information (ESI)

A novel analysis principle by AP site-mediated T7 RNA polymerase transcription regulation for uracil-DNA glycosylase activity sensing

Weichen Gao,‡[a] Jin Xu,‡[b] Guowei Lian,[a] Xiaojun Wang,[c] Xiaoqun Gong,[a] Dianming Zhou,[c,*] and Jin Chang[a,*]

[a]*School of Life Sciences, Tianjin University and Tianjin Engineering Center of Micro-Nano Biomaterials and Detection-Treatment Technology (Tianjin), Tianjin 300072, China*

**Corresponding authors. E-mail:jinchang@tju.edu.cn*

[b]*Tianjin Hospital, 406 jiefangnan Road, Tianjin 300211, China*

[c]*Department of Toxicology, Tianjin Centers for Disease Control and Prevention, Tianjin 300011, China*

**Corresponding authors. E-mail: dianmingzhou@foxmail.com*

Fig.S1. Real-time fluorescence curve of T7 RNA polymerase transcription. (solid line: without UDG; dotted line: with 250 U/mL UDG)

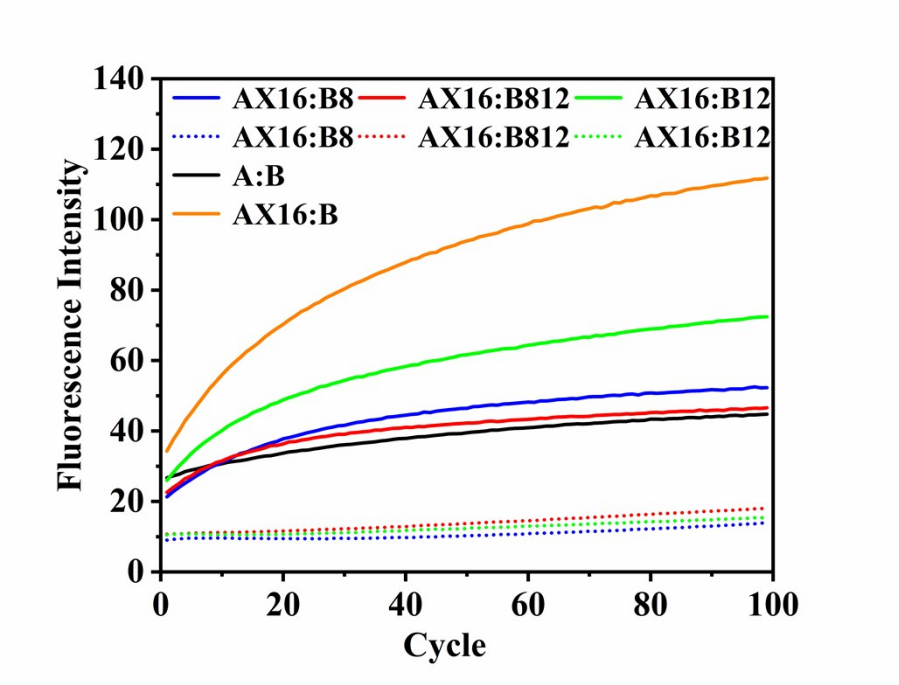


Fig.S2. Dependency of the sensing method on UDG excision time. (Endpoint fluorescence for 99min, UDG = 2.5 U/mL)

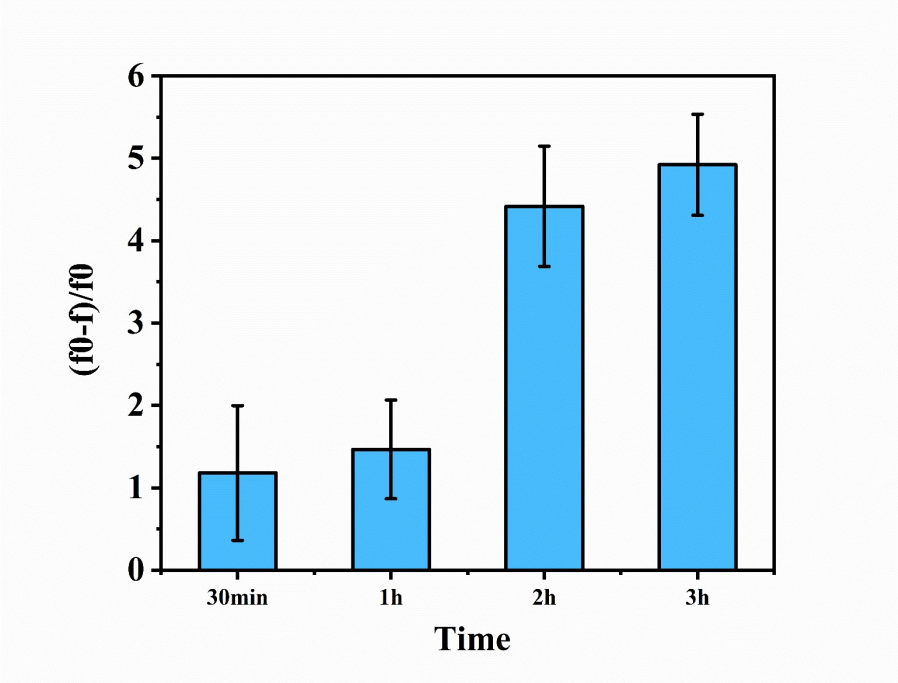


Fig.S3. Specificity of transcription regulation-based UDG analysis method. (2.5 U/mL UDG, 2.5 U/mL hAAG, 10 U/mL UGI, 1 mg/mL BSA)

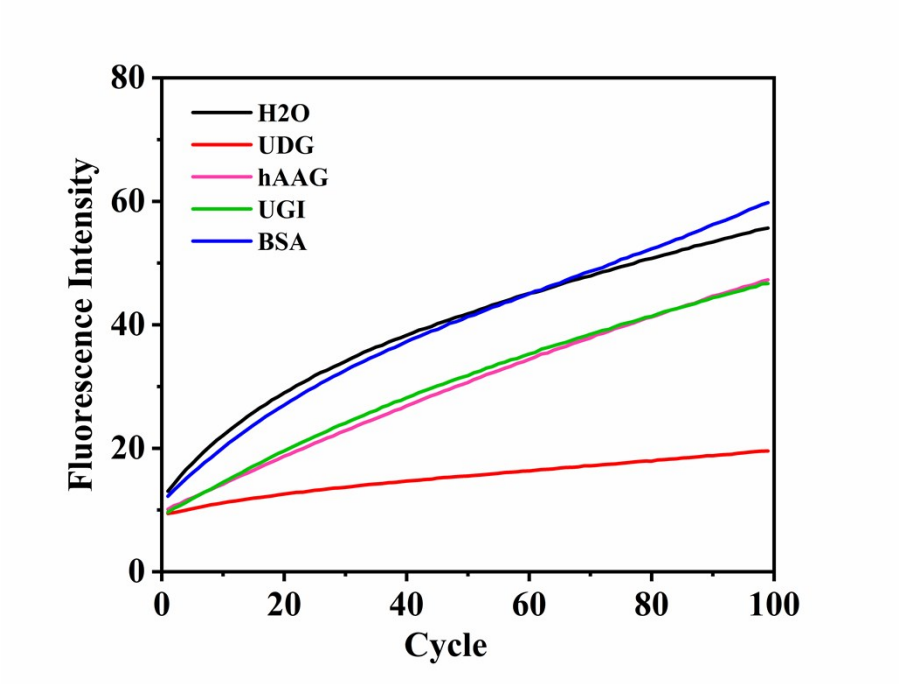
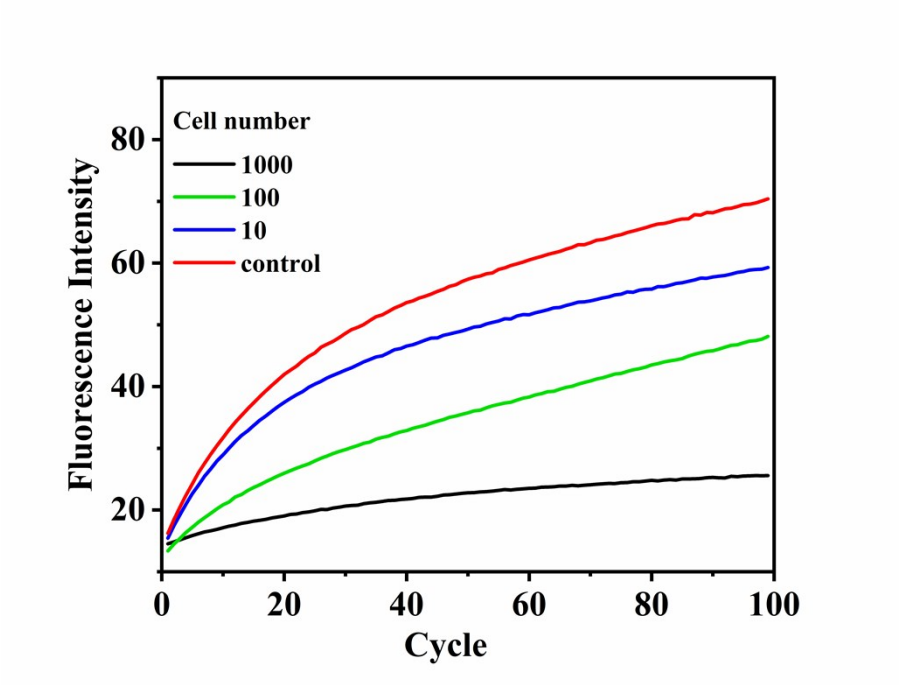


Fig.S4. Real sample analysis using HeLa cell lysate.



Effect of uracil replacement at template T region on transcription

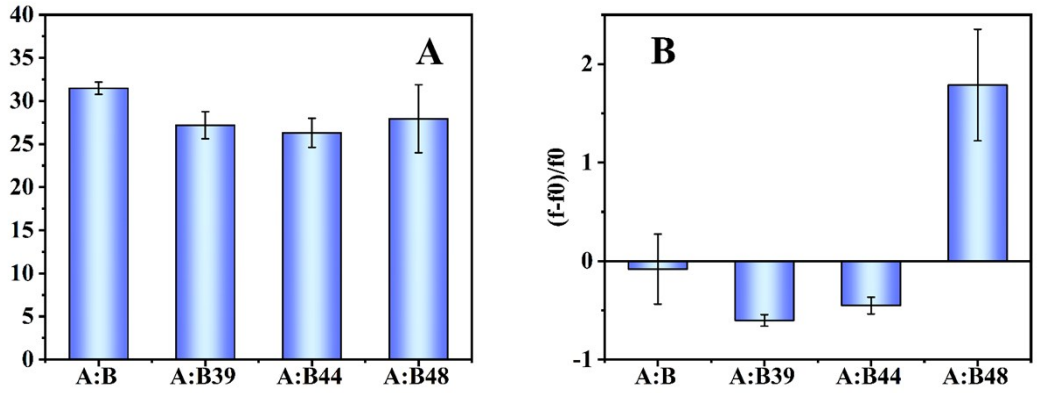
The designed probe sequences are shown in Table S1. The U marked in red font indicates that thymine is replaced by uracil. The A chain was hybridized with B39, B44, and B48, respectively, and the transcription result under the action of T7 RNA polymerase is shown in Figure S5A. As expected, uracil substitutions have no significant effect on transcription. These hybrid strands were treated with UDG and then reacted with T7 RNA polymerase, showing unexpected results (Figure S5B). A: B39 and A: B44 showed inhibition on transcription, while A: B48 showed promotion on transcription. This finding indicates that some AP sites in the T region of the B chain can also affect transcription.

Table S1. Sequences (5'-3') of synthetic oligonucleotides used in this study:

Abbreviation	Sequence (5'-3')
A	TAATACGACTCACTATAGGA
B39	GGATCCATTCGTTACCUGGCTCTCGCCAGTCGGGATCCTATAGTGAGTCGTATTA
B44	GGATCCATTCGUTACCTGGCTCTCGCCAGTCGGGATCCTATAGTGAGTCGTATTA
B48	GGATCCAUTCGTTACCTGGCTCTCGCCAGTCGGGATCCTATAGTGAGTCGTATTA

U represents uracil deoxyribonucleotide residues.

Fig.S5. Effect of uracil replacement at template T region on transcription.



We have made a table to compare the present work with other UDG detection methods in the Supplementary information. As shown in Table S2, this strategy makes substrate probes design easier and no need for multi-enzyme system. Simplified experiment operation and has good sensitivity.

Table S2. Comparison of this work with other UDG detection methods.

Methods	Sensitivity	Specificity	Probe design	Enzyme system	Ref.
Detection method of radioactive label based on gel electrophoresis	~10U/mL	Good	No need	No enzyme	1
Sensor based on colorimetry	~10^{-2}U/mL	Perfectly	Complex	No enzyme	2, 3
Chemiluminescence detection method	~10^{-1}U/mL	Good	Complex	No enzyme	4
Fluorescent labeling strategy based on chain displacement reaction	~10^{-4}U/mL	Perfectly	Complex	Single enzyme	5
Enzyme-assisted amplification strategy based on excision repair	~10^{-3}U/mL	Good	Simple	Multi-enzyme system	6
Amplification strategy assisted by TdT enzyme and T7 Exo	~10^{-4}U/mL	Perfectly	Simple	Multi-enzyme system	7
UDG detection strategy based on AP site regulation of T7 RNA polymerase	~10^{-4}U/mL	Perfectly	Simple	Single enzyme	This study

References:

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