DNA repair-retarded isothermal CRISPR amplification for rapid, sensitive base excision repair enzyme assay

Hyogu Han[1,2], Se Hee Jang[1,3], Jun Ki Ahn[1]*, Chang Yeol Lee[4]*

1. Material & Component Convergence R&D Department, Korea Institute of Industrial Technology (KITECH), Ansan, Korea
2. Department of Chemistry, Gangneung-Wonju National University, Gangneung, 25457, Korea
3. Department of Medical Device Engineering and Management, College of Medicine, Yonsei University, Seoul 03722, Korea
4. Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon, 34141, Korea

***Corresponding Authors:**

Jun Ki Ahn, PhD
Material & Component Convergence R&D Department
Korea Institute of Industrial Technology (KITECH)
Ansan, 15588, Korea
jkahn@kitech.re.kr

Chang Yeol Lee, PhD
Bionanotechnology Research Center
Korea Research Institute of Bioscience and Biotechnology (KRIBB)
Daejeon, 02114, Korea
lcyeol8457a@kribb.re.kr

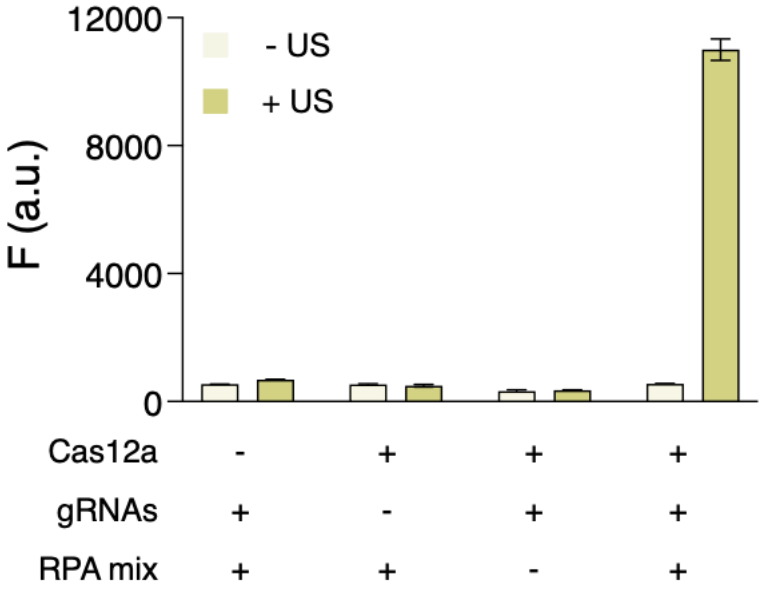

Fig. S1. Validation of one-pot, isothermal CRISPR amplification. When all key components for RPA and CRISPR/Cas12a system were present, the combined system resulted in high fluorescence signal. [US] = 100 fM.

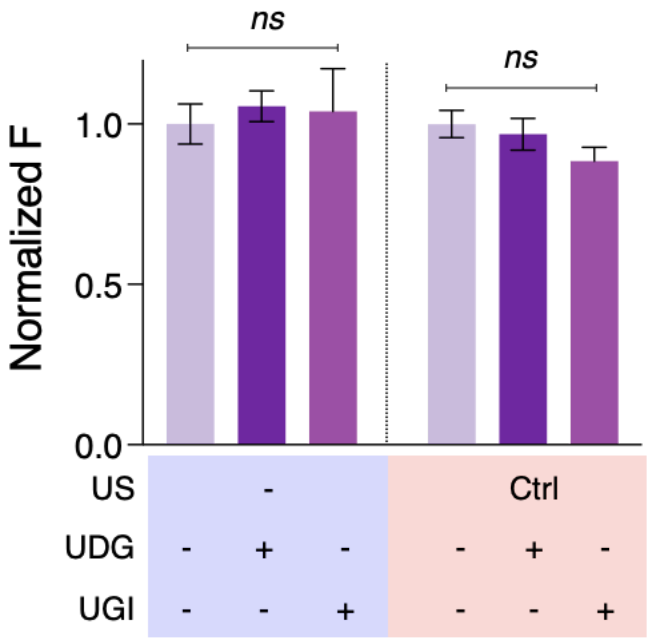

Fig. S2. Effect of UDG and UGI on URECA. To ensure that UDG and UDG inhibitor (UGI) do not affect URECA, we compared isothermal CRISPR amplification reaction efficiencies under different conditions. To prevent UDG-catalyzed reactions, a control UDG substrate (Ctrl) was used (**Table S2**). In the absence (light blue) and presence (light red) of Ctrl, UDG and UGI did not significantly affect the reaction efficiencies (*ns*: $p > 0.05$). Normalization was made to the signal without both UDG and UGI. [UDG] = 1 U/mL, [UGI] = 2 U/mL.

(a) **Fig. 1**

Substrate	US		Ctrl	
UDG	–	+	–	+
F (a.u.)	9903	1229	10625	10009
	10051	1538	10751	10106
	10189	1587	11004	10282

(c) **Fig. 2(b)**

Target	Blank	UDG	hAAG	Fpg	Endo IV	rSAP
F (a.u.)	10755	1996	11393	8732	10077	8812
	11475	2367	11949	9193	10860	9020
	11853	2598	12285	9764	11169	9106

(b) **Fig. 2(a)**

[UDG] (U/mL)	0	0.001	0.005	0.01	0.05	0.1	0.5	1
F (a.u.)	10585	10218	7802	7174	5065	4411	2087	1923
	10701	10202	8355	7233	5823	4580	2289	2410
	10797	10124	8580	7362	5998	4987	3140	1716

Fig. S3. Raw data of (a) Fig. 1 and (b and c) Fig. 2. Fluorescence signals were measured at 525 nm in triplicate. Those of Fig. 2 were converted to fluorescence signal changes ($\Delta F/F_0$; $\Delta F = F_0 - F$, where F_0 is the fluorescence signal at blank).

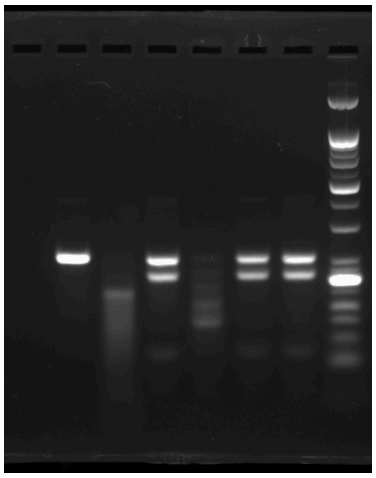

Fig. S4. Full image of the gel electrophoretic analysis result in Fig. 1(b)**.**

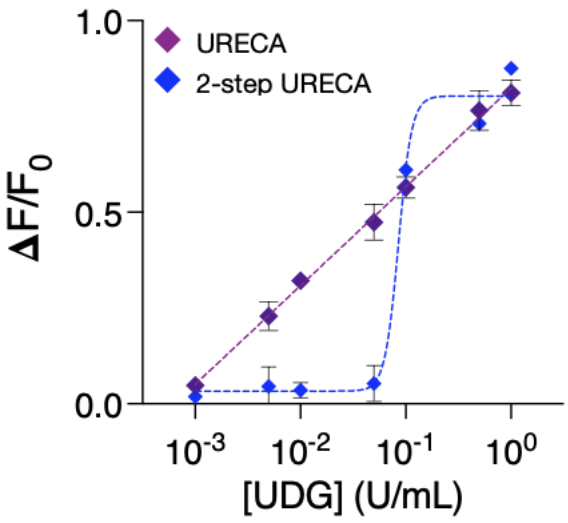

Fig. S5. Comparison of the sensitivity between URECA and 2-step URECA for UDG activity assay. While ssDNAs activate Cas12a in URECA, PAM-adjacent dsDNAs activate Cas12a in 2-step URECA. Specifically, in 2-step URECA, RPA amplifies UDG-treated US and a gRNA specific to the PAM-adjacent dsDNA sequence in the amplicon ($gRNA_{PAM}$, **Table S2**) subsequently activates Cas12a to further amplify detection signal. When testing serially diluted UDG samples with 2-step URECA, fluorescence signal changes ($\Delta F/F_0$) increased with increasing UDG concentrations and its LOD was determined to be 7.4×10^{-2} U/mL based on B + 3σ. ΔF is defined as F_0-F, where F_0 is the fluorescence signal at blank. B and σ are the signal and standard deviation at blank, respectively. Though 2-step URECA relies on dsDNA-activated Cas12a with stronger collateral cleavage activity than ssDNA-activated one, URECA (9.17×10^{-4} U/mL) still outperformed 2-step URECA in sensitivity.

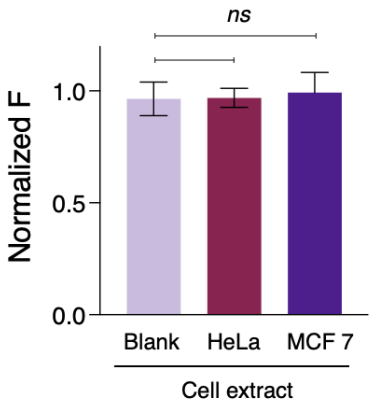

Fig. S6. Effect of cell extracts on URECA. To ensure that the cellular components other than UDG do not interfere with URECA, we treated a control UDG substrate (Ctrl, **Table S2**) with cell extracts (HeLa and MCF 7) and implemented isothermal CRISPR amplification reaction. The cell extracts did not compromise the reaction (*ns*: $p > 0.05$), which would enable reliable cellular UDG activity assay with URECA. Normalization was made to the signal without cells. [Cell] = 10^4 cells/mL.

Table S1. Comparison with previous UDG activity assays based on isothermal NA amplification and CRISPR/Cas system.

System	Assay time (min)	LOD (U/mL)	Characteristics	Reference
Primer remodeling combined with RCA	90	4.5×10^{-5}	Complicated, lengthy preparation of starting materials	[1]
Excision repair-initiated EXPAR	145	1×10^{-4}	Several assay steps	[2]
NEAR	70	3×10^{-3}	-	[3]
Transcription amplification of fluorogenic RNA aptamers	> 160	6.3×10^{-6}	Several assay steps	[4]
Transcription amplification	220	2.5×10^{-4}	-	[5]
CRISPR/Cas12a activation by toehold-mediated strand displacement	80 or 160	2.5×10^{-4} or 2.5×10^{-6}	-	[6]
Terminal extension activating CRISPR/Cas12a reaction	210	5×10^{-6}	Several assay steps	[7]
Target-induced PCR coupled with CRISPR/Cas12a system	> 180	3.5×10^{-6}	- Involvement of thermal cycling - Requirement of PAM site - Several assay steps	[8]
EXPAR coupled with CRISPR/Cas12a system	210	3.1×10^{-5}	Several assay steps	[9]
URECA	50	9.17×10^{-4}	-	This work

Table S2. Oligonucleotide sequences used in this work.

Probe	Sequence (5'-3')[a]
US[c]	AAC GUC UGC AGU UAA GGU UAT TUT GCA CAG UTG AAA AAU AAA CUG UAA AUC AUA TUC CTC CCC ATG TCG TAG GTA CTC CTT AAA GTT AGT ATT TTT ATA TGT AGT TTC TGA AGT AGA TAT GGC AGC ACA TAA TGA CAT ATT TGU ACU GCG UGU AGU AUC AAC AAC AGU AAC AAA UAG UTG GUT ACC CCA ACA AAU GCC
RPA forward primer	CAT TTG TTG GGG TAA CCA ACT ATT TGT TAC T
RPA reverse primer	CGT CTG CAG TTA AGG TTA TTT TGC ACA GTT G
Forward gRNA[b]	*UAA UUU CUA CUA AGU GUA GAU* GUA CUG CGU GUA GUA UCA AC
Reverse gRNA[b]	*UAA UUU CUA CUA AGU GUA GAU* GAA UAU GAU UUA CAG UUU AUU U
$gRNA_{PAM}$[b]	*UAA UUU CUA CUA AGU GUA GAU* AGG AGT ACC TAC GAC ATG GG
Ctrl	AAC GTC TGC AGT TAA GGT TAT TTT GCA CAG TTG AAA AAT AAA CTG TAA ATC ATA TTC CTC CCC ATG TCG TAG GTA CTC CTT AAA GTT AGT ATT TTT ATA TGT AGT TTC TGA AGT AGA TAT GGC AGC ACA TAA TGA CAT ATT TGT ACT GCG TGT AGT ATC AAC AAC AGT AAC AAA TAG TTG GTT ACC CCA ACA AAT GCC
F/Q probe	FAM-TTA TT-BHQ1

[a] Except red, the colors of oligonucleotide sequences correspond to those of the domains depicted in Scheme 1.

[b] The direct repeat sequences are in italic.

[c] The recognition site of $gRNA_{PAM}$ is highlighted in red and the PAM site is red-boxed in US.

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