Supporting Information

Simultaneous Elimination of Carryover Contamination and Detection of DNA with Uracil-DNA-Glycosylase-Supplemented Loop-Mediated Isothermal Amplification (UDG-LAMP)

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METHODS

Materials and Reagents. LAMP reaction reagents, including Loopamp DNA Amplification Kit and Loopamp Fluorescent Detection (FD) Reagent, were purchased from SA Scientific (San

Antonio, TX). LAMP DNA primers were ordered from Integrated DNA Technologies (Coralville, IA). Bst 2.0 DNA polymerase and low molecular weight DNA ladder were obtained from New England Biolabs (Ipswich, MA). Cod uracil-DNA-glycosylase (UDG) was purchased from ArcticZymes (Plymouth Meeting, PA). 2'-deoxyuridine-5'-triphosphate (dUTP) sodium salt was acquired in the form of a 100 mM solution from Affymetrix (Santa Clara, CA). Nuclease-free water (not DEPC-treated) and TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) were purchased from Life Technologies (Carlsbad, CA). Purified *Salmonella enterica serovar* Typhimurium genomic DNA was acquired from ATCC (Manassas, VA) and reconstituted in-house in TE buffer at 20 ng/μL, as measured by a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Agarose (Low-EEO/Multi-Purpose/Molecular Biology Grade) was purchased from Fisher BioReagents (Fair Lawn, NJ) and TBE buffer (0.089 M Tris base, 0.089 M boric acid (pH 8.3) and 2 mM Na_2EDTA) was purchased from National Diagnostics (Atlanta, GA). GelStar nucleic acid gel stain was obtained from Lonza (Basel, Switzerland).

UDG-LAMP Reaction Assembly. The reaction mix was assembled in a laminar flow hood in a laboratory separate from where the amplification and the detection steps were performed to prevent unwanted carryover contamination. A typical UDG-LAMP reaction mix (9 μL) contained the following: 1× Loopamp DNA Amplification Reaction Mix (20 mM Tris-HCl, 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Tween20, 0.8 M betaine, and 1.4 mM dNTP), 1.4 mM dUTP (1:1 dUTP-to-dTTP ratio), 0.2% BSA, 3.2 μM each of FIP and BIP primers, 0.4 μM each of F3 and B3 primers, 0.8 μM each of LF and LB primers (see Supporting Information Table S1 for sequences), 0.36 μL FD reagent, 0.64 U/μL Bst 2.0 DNA polymerase, 0.005 U/μL UDG, 0.5 μL genomic target DNA diluted from the stock with TE buffer to obtain the desired concentration, and 0.4 μL contaminant DNA diluted from previous LAMP reactions with TE buffer to obtain the desired concentration. Of note, during the reaction assembly, the reagents were kept cold with a 96-well PCR cold block (Eppendorf, Hauppauge, NY). Also, the two enzymes were added only after the other reagents had been assembled and well mixed (except for targets and contaminants) in order to ensure the activity of these enzymes. Finally, contaminant DNA was added in a fume hood in a separate laboratory to prevent exposing the reaction preparation facility with unwanted carryover contamination (see Supporting Information Table S2 for detailed composition and assembly of the reaction mixture).

Dose Estimation for Carryover Contamination. The mass yield of the LAMP reaction generated from 1×10^3 copies of target in the absence of UDG, which served as the source of carryover contaminants, was conservatively estimated to be 0.25 μg/μL, as typical LAMP reactions can generate up to 0.4 to 0.8 μg/μL of DNA amplicons. This source reaction was diluted by 1×10^8 to 1×10^{12}-fold and 0.4 μL of the diluted product was spiked in each UDG-LAMP reaction. The mass of carryover contaminant therefore corresponds to approximately 1×10^{-15} to 1×10^{-19} g.

UDG-LAMP Reaction Conditions and Detection. UDG-LAMP reactions were conducted in standard Eppendorf PCR tubes and in a bench-top thermocycler (DYAD 220 Peltier Thermal Cycler, MJ Research, Inc., Waltham, MA), first at 25 °C for 5 min and then at 65 °C for 60 min. Upon completion of the reaction, each sample was immediately evaluated without opening the reaction tube. Although calcein fluorescence can be clearly observed by naked eye under ambient light, images acquired under UV illumination within an imaging system can be more consistent due to the suppression of uneven background lighting. We therefore imaged each tube with a Kodak Gel Logic 200 imaging system equipped with Kodak molecular imaging software (Carestream Health, Rochester, NY) with a single exposure at 0.01 s exposure time.

Gel Electrophoresis. Amplified UDG-LAMP products were analyzed via gel electrophoresis using a 2% agarose gel pre-stained with 1× GelStar (Lonza). Each sample consisted of 1 μL LAMP reaction product, 2 μL 10× bromophenol blue loading dye, and 7 μL 1× TBE. In parallel, each lane of ladder contains 1 μL low molecular weight DNA ladder, 2 μL loading dye, and 7 μL 1× TBE. Electrophoresis was performed in 1× TBE buffer at 140 V for 40 min. Gel images were taken with Kodak Gel Logic 200 imaging system.

Table S1. LAMP primer sequences for *S.* Typhimurium *invA* gene

Primer	Sequence (5' to 3')
FIP	GACGACTGGTACTGATCGATAGTTTTTCAACGTTTCCTGCGG
BIP	CCGGTGAAATTATCGCCACACAAAACCCACCGCCAGG
F3	GGCGATATTGGTGTTTATGGGG
B3	AACGATAAACTGGACCACGG
FL	GACGAAAGAGCGTGGTAATTAAC
BL	GGGCAATTCGTTATTGGCGATAG

Table S2. Recipe for typical UDG-LAMP reactions with calcein fluorescence detection

Step 1 – Master Mix Assembly			
Component	**Final Concentration**	**Stock Concentration**	**10× Reaction Volume**
Tris-HCl (pH 8.8)	20 mM	40 mM	50 µL 2× reaction mix (RM)
KCl	10 mM	20 mM	
$MgSO_4$	8 mM	16 mM	
$(NH_4)_2SO_4$	10 mM	20 mM	
Tween20	0.1%	0.2%	
Betaine	0.8 M	1.6 M	
dNTP	1.4 mM	2.8 mM	
dUTP	1.4 mM	100 mM	1.4 µL
BSA	0.2%	2%	10 µL
Distilled H_2O (DW)			1.6 µL
FIP	3.2 µM	80 µM	4 µL
BIP	3.2 µM	80 µM	4 µL
F3	0.4 µM	40 µM	1 µL

B3	0.4 µM	40 µM	1 µL
LF	0.8 µM	40 µM	2 µL
LB	0.8 µM	40 µM	2 µL
Fluorescence Detection Reagents (FD)			4 µL
Bst 2.0 Polymerase	0.64 U/µL	8 U/µL	8 µL
UDG	0.005 U/µL	0.5 U/µL	1 µL
		TOTAL	**90 µL**

Step 2 – Negative Controls, Target Samples, and Carryover Contaminants			
Component	**Final Concentration**	**Stock Concentration**	**1× Reaction Volume**
Master Mix (Step 1)			8.1 µL
Target or distilled H_2O (DW)			0.5 µL
Contaminant or distilled H_2O (DW)			0.4 µL
		TOTAL	**9 µL**