Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

An efficient template-independent polymerase chain displacement reaction for the detection of Salmonella Typhimurium

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Experimental

Enzymes and reagents

SD DNA polymerase and SD reaction buffer were purchased from Bioron GmbH (Ludwigshafen, Germany). SYBR Green I (20× stock solution in dimethyl sulfoxide) was purchased from Thermo Fisher Scientific Inc. (Waltham, USA). DNA marker and dNTPs, 59 bp DNA template and PCR primers were purchased from Shanghai Sangon biological engineering technology & services Co., Ltd. (Shanghai, China). Their sequences are shown in Table 1. The primers were analyzed using the software Primer Premier 6.0 to avoid potential primer-dimer and hairpin issues. The genomic DNA of *Salmonella* and other bacteria were provided by Guangdong Institute of Microbiology (Guangdong, China).

Table S1 Sequences of the primers and an artificial template

Oligonucleotide	Sequence (5′ → 3′)
DNA template	CTGAGTCGCTTGGTCTATCTGTCACTCGTCTGTCATCTGACATAGGACCACATAGCGAC
Primer F0	CTGAGTCGCTTGGTCT
Primer R0	GTCGCTATGTGGTCCT
Primer F2	GCTGTGTCGCTTCTGTTTTGCTGTGTCGCTTCTGTTTTCTGAGTCGCTTGGTCT
Primer R2	CTCGCATCGCATCTCTTTTCTCGCATCGCATCTCTTTTGTCGCTATGTGGTCCT
Primer Fu	GCTGTGTCGCTTCTGT
Primer Ru	CTCGCATCGCATCTCT

TTR-PCDR amplification

All amplification reactions were carried out on a Bio-Rad CFX96 real-time PCR system (Bio-Rad Laboratories Ltd., America). The images of gel electrophoresis were scanned by the Gel Image Analysis System JY02S (Beijing, China). A 59 bp DNA template was used to perform the TTR-PCDR amplification. Assay components were optimized, and this system consisted of 10 U of SD DNA polymerase, 1×SD reaction buffer, 3.5 mM $MgCl_2$, 0.4 mM dNTPs (each), 30 nM primers F2 and R2 (each), 1 μM primer Fu and Ru (each), 2 μL template DNA, SYBR Geen I (20×) 0.5 μL. The total reaction volume was 25 μL. The amplification protocol included an initial denaturation 92 °C for 2 min, followed by 35 cycles of 92 °C for 20 s, 48 °C for 30 s, and 68 °C for 20 s. The product melting curve analysis protocol was 92 °C for 1 min, 48 °C for 20 s, then an increase of 0.5 °C/s to 92 °C for 30 s. All analyses were performed in duplicate. All reactions were performed in triplicate. Water was used for the no template control (NTC).

Real-time quantitative TTR-PCDR to detect Salmonella

To evaluate the specificity of the primer pairs of *Salmonella*, genomic DNA of *Salmonella Typhimurium* CMCC50071, *Salmonella Enteritidis* CMCC50041 and other bacteria (*Staphylococcus aureus* ATCC6538, *Escherichia coli* ATCC25922, *Listeria monocytogenes* CMCC54003) were extracted according to the previous reports[1, 2], and their concentration was quantified by a fluorometer. These extracted bacterial DNA were then examined by real-time quantitative TTR-PCDR.

The primers are designed to target the *Salmonella ttr* gene, because the *ttr* locus is identical to all *Salmonella* strains[3]. Additionally, the *ttr* locus locates in the pathogenicity island 2 and encodes the tetrathionate reductase which plays an important role in tetrathionate respiration.

Its function to respire tetrathionate plays a key role in the life cycle of *Salmonella*, so the *ttr* gene is also genetically stable in all *Salmonella* strains[3, 4]. In this work, the sequences of tailed tandem repeat primers were thus 5'-CAG AGA TGG TGG CAG GAA GTA AAC AGA GAT GGT GGC AGG AAG TAA AGG TAG TCA GGA AGT CAC GAA TG-3' and 5'-CAG GAG GCA GAG ACA AGA GGA AAC AGG AGG CAG AGA CAA GAG GAA AGC ACA GGT AAT GGC AAT CAG T-3', corresponding to nucleotides 2814 to 3484 of the *ttr* gene. The universal primers were 5'-CAG AGA TGG TGG CAG GAA GT-3' and 5'-CAG GAG GCA GAG ACA AGA GG-3'. The TTR-PCDR reaction mixture for amplification contained 10 U of SD DNA polymerase, 1×SD reaction buffer, 3.5 mM $MgCl_2$, 0.4 mM dNTPs (each), 30 nM tailed tandem repeat primers (each), 1 μM universal primers (each), 2 μL genomic DNA, SYBR Geen I (20×) 0.5 μL. The total reaction volume was 25 μL. The amplification protocol included an initial denaturation 92 °C for 2 min, followed by 40 cycles of 92 °C for 20 s, 55 °C for 30 s, and 68 °C for 30 s. The product melting curve analysis protocol was 92 °C for 1 min, 55 °C for 20 s, then an increase of 0.5 °C/s to 92 °C for 30 s.

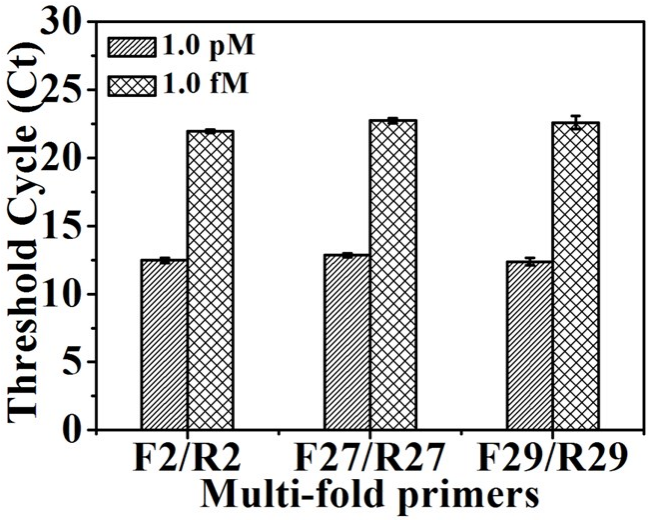

Fig. S1 The multi-fold PCR when using the primer pairs of F2/R2 with different length of connecting bases. F27/R27 and F29/R29 have 7 bases and 9 bases, respectively. Reaction assays contained the following amounts of DNA template: 1.0 pM and 1.0 fM.

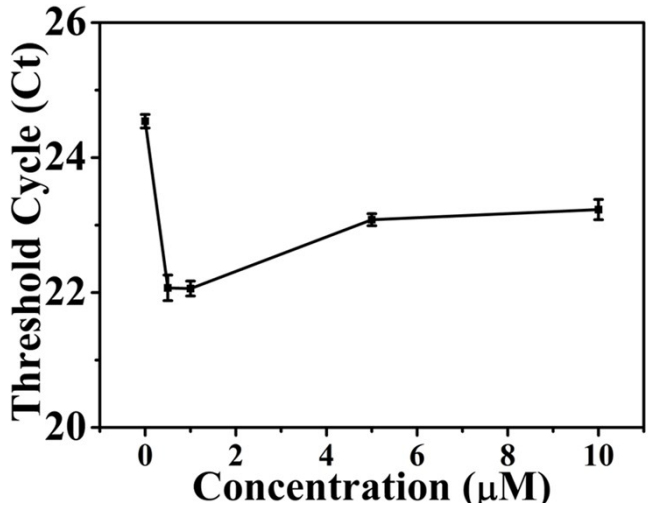

Fig. S2 The multi-fold PCR with different concentrations of the universal primers. Reaction assays contained the amounts of DNA template are 10 fM.

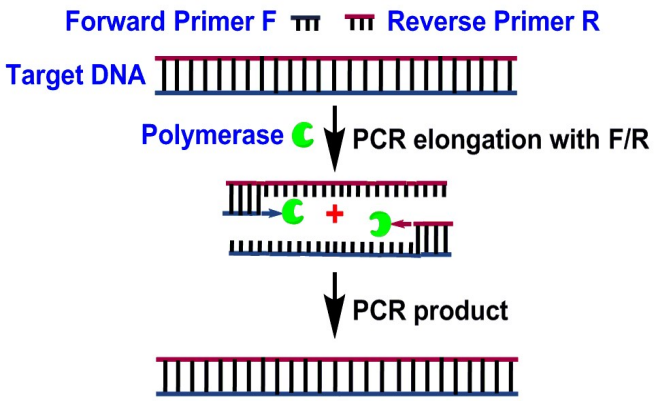

Fig. S3 Schematic diagram of the PCR. The PCR uses a pair of universal primers (F and R) and produces one kind of PCR product.

Effect of Non-specific amplification

The PCR amplification process was generally accompanied by non-specific PCR amplification, which by-products are similar to DNA of PCR product[5-7]. In this work, lambda-DNA[8] and calf thymus DNA[9] were employed to stimulate non-specific PCDR amplification product and DNA contamination because of its duplex structure. As shown in Fig. S4, the fluorescence intensity of blank samples, samples in presence of lambda-DNA and samples in presence of calf thymus DNA almost remained same compared with samples in the presence of template DNA. These results demonstrated the good specificity of this assay.

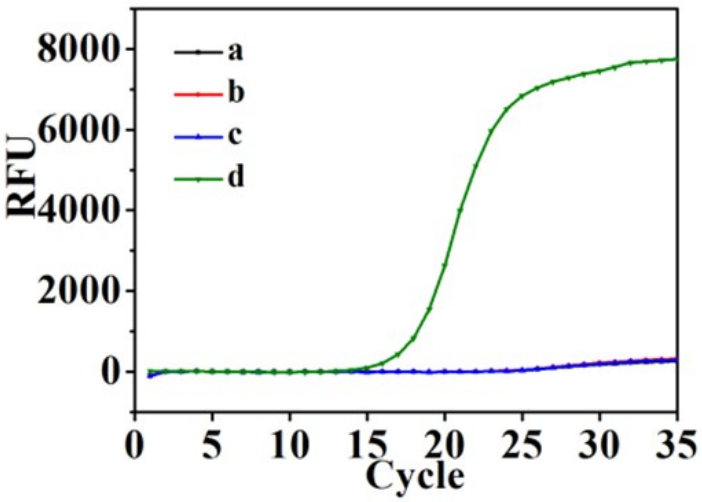

Fig. S4 Effect of non-specific contamination of TTR-PCDR amplification; (a) the TTR-PCDR product in the absence of template DNA; (b) the TTR-PCDR product in the presence of 0.1pM lambda-DNA; (c) the TTR-PCDR product in the presence of 0.1 pM calf thymus DNA; (d) the TTR-PCDR product in the presence of 10.0 fM template DNA.

Reference

1. D. Kallifidas, H.-S. Kang and S. F. Brady, *J. Am. Chem. Soc.*, 2012, **134**, 19552–19555.
2. Y. K. Lee, H. W. Kim, C. L. Liu and H. K. Lee, *J. Microbiol. Method.*, 2003, **52**, 245–250.
3. M. Hensel, A. P. Hinsley, T. Nikolaus, G. Sawers and B. C. Berks, *Mol. Microbiol.*, 1999, **32**, 275-287.

4. B. Malorny, E. Paccassoni, P. Fach, C. Bunge, A. Martin and R. Helmuth, *Appl. Environ. Microbiol.*, 2004, **70**, 7046–7052.
5. J. Li, B. Yao, H. Huang, Z. Wang, C. Sun, Y. Fan, Q. Chang, S. Li, X. Wang and J. Xi, *Anal. Chem.*, 2009, **81**, 5446–5451.
6. T. Chen and F. E. Romesberg, *J. Am. Chem. Soc.*, 2017, **139**, 9949–9954.
7. P. T. Monis, S. Giglio and C. P. Saint, *Anal. Biochem.*, 2005, **340**, 24–34.
8. C. Plesa, J. W. Ruitenberg, M. J. Witteveen and C. Dekker, *Nano Lett.*, 2015, **15**, 3153–3158.
9. E. M. Golenberg, A. Bickel and P. Weihs, *Nucleic Acids Res.*, 1996, **24**, 5026–5033.