SUPPLEMENTARY INFORMATION

High-specificity Double-stranded DNA Detection with "Humanoid" Molecular Beacon and TALEs

Yu Wang,‡[a] Jialei Baia,‡[a] Xiaochen Qu,[a] Yifei Gao,[b] Jiang Wang,[a] Shuang Li,[a] Longxing Fan,[ac] Haiping Wei,[d] Sha Liu,[ae] Yuan Peng,[a] Zihan Gao,[a] Yulin Zhu,[a] Zhixian Gao*[a] and Baoan Ning*[a]

[a] Tianjin Key Laboratory of Risk Assessment and Control Technology for Environment and Food Safety, Institute of Environmental and Operational Medicine, Academy of Military Medical Science, Academy of Military Science, Tianjin 300050, P.R. China

[b] School of chemistry,University of New South Wales, Sydney, Australia

[c] MOE Key Laboratory for Analytical Science of Food Safety and Biology College of Chemistry, Fuzhou University, Fujian 350108, P. R. China

[d] School of Tourism Management, Tianjin Vocational Institute, Tianjin 300350, P.R. China

[e] School of Public Health, Hebei Medical University, Shijiazhuang 050017, P.R. China

SUPPLEMENTARY FIGURES

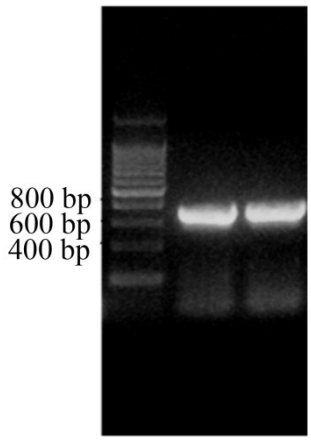

Fig. S1 E. coli 16S rDNA amplification electropherogram, marker: 200bp DNA Leader.

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              QAHG
Sbjct  1621  QAHG  1632
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Fig. S2 Assembled TALEs repeat unit amino acid sequences.

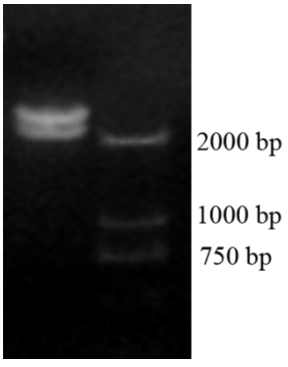

Fig. S3 Double enzyme digestion of pMD18-T-TALEs.

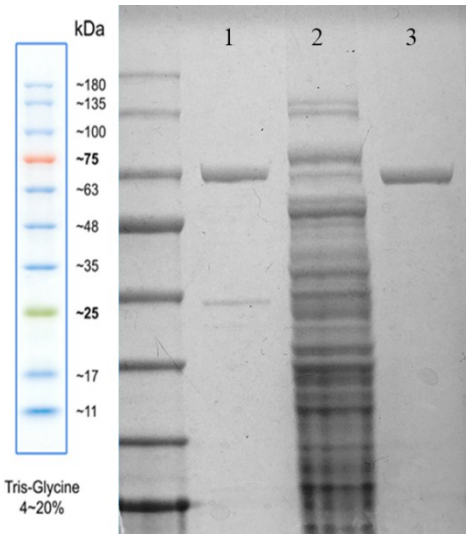

Fig. S4 TALEs renaturation results: 1 was bacterial broken precipitate; 2 was bacterial broken supernatant; 3 was renatured soluble TALEs.

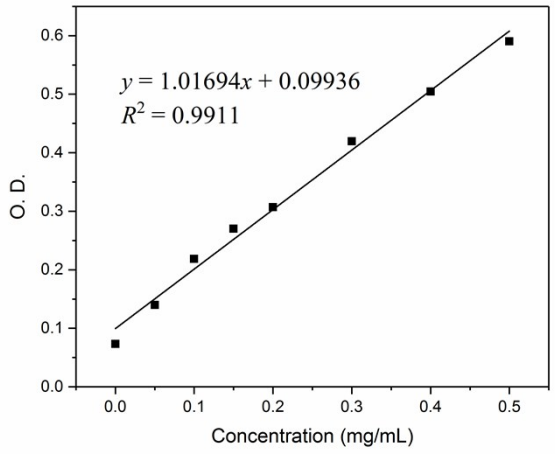

Fig. S5 Protein quantitative standard curve of TALEs.

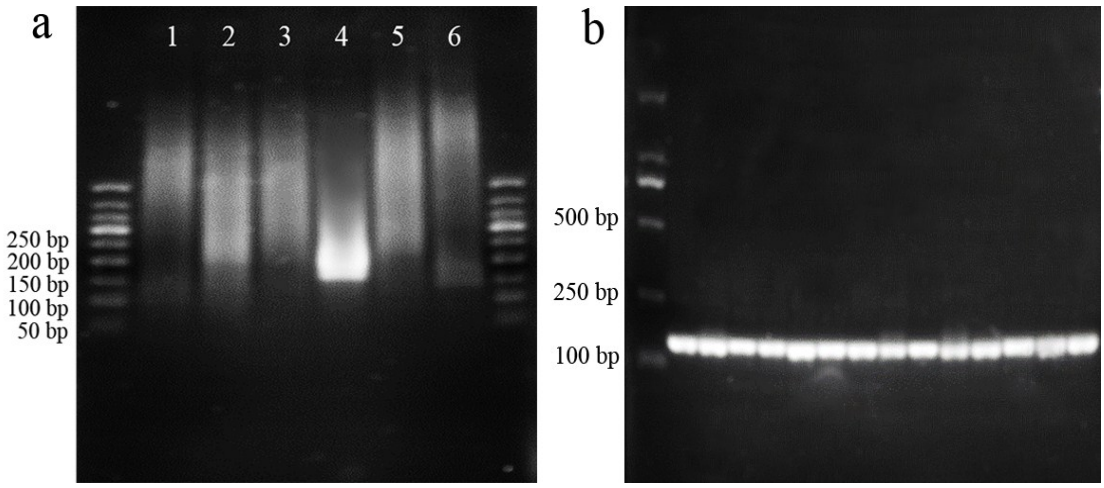

Fig. S6 (a) RPA amplification results for different primers; (b) electrophoresis identification after purification of RPA-F/R4 amplification product.

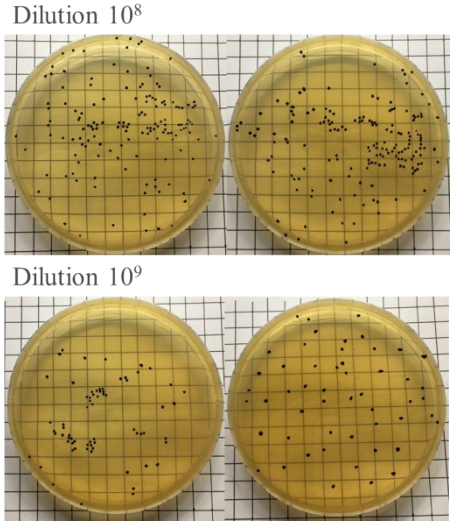

Fig. S7 Plate count of E.coli.

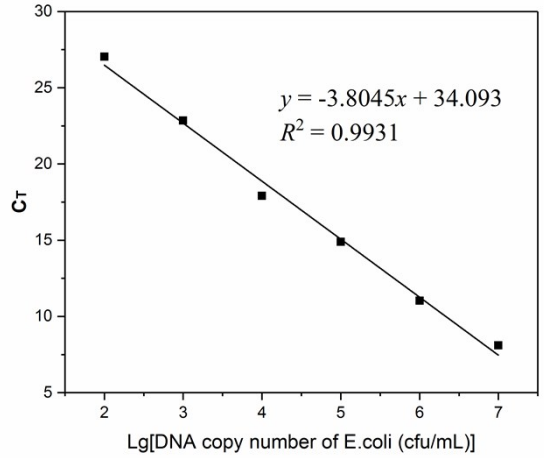

Fig. S8 Standard curve of RT-PCR E. coli detection.

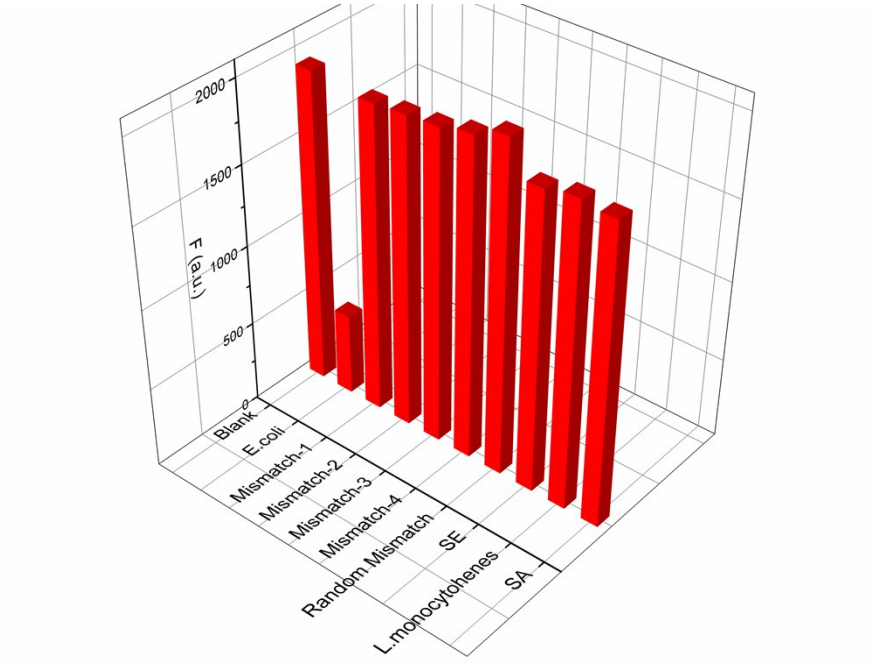

Fig. S9 Highly specific detection of E. coli 16 S rDNA allows for the differentiation of other bacteria and mismatched DNA.

SUPPLEMENTARY TABLES

Table S1. Sequence of RPA primers

Primer	DNA Sequence （5’ — 3’）
RPA-F1	GCCTAACACATGCAAGTCGAACGGTAACAG
RPA-R1	CTCCATCAGGCAGTTTCCCAGACATTACTC
RPA-F2	CCAAATTGAAGAGTTTGATCATGGCTCAGA
RPA-R2	GGTCTTGCGACGTTATGCGGTATTAGCTAC
RPA-F3	CCAAATTGAAGAGTTTGATCATGGCTCAGA
RPA-R3	CCTCTTTGGTCTTGCGACGTTATGCGGTA
RPA-F4	GCCTAACACATGCAAGTCGAACGGTAACAG
RPA-R4	CCCTCTTTGGTCTTGCGACGTTATGCGGTA
RPA-F5	GCCTAACACATGCAAGTCGAACGGTAACAG
RPA-R5	ACCTACTAGCTAATCCCATCTGGGCACATC
RPA-F6	CCAAATTGAAGAGTTTGATCATGGCTCAGA
RPA-R6	CCAGACATTACTCACCCGTCCGCCACTCGT

Table S2. DNA Sequence of gel retardation and Specific assays

Name	DNA Sequence （5’ — 3’）
Correct DNA	TGGCGGACGGGTGAGTAA
Mismatch DNA	TCCCCACGCTTTCGCACC
Mismatch-1	TAGCGGACGGGTGAGTAA
Mismatch-2	TGATGGACGGGTGAGTAA
Mismatch-3	TGCTTGACGGGTGAGTAA
Mismatch-4	TGCTAAACGGGTGAGTAA
Random Mismatch	ATCGGTCCTGACTTAACG

Table S3. Primer sequences for PCR

Name	DNA Sequence （5’ — 3’）
E. coli-F	TGGCGGACGGGTGAGTAA
E. coli-R	TCCCCACGCTTTCGCACC
TALEs-F	GCGCGGATCCGTAGATTTGAGAACTTTGG
TALEs-R	GATGTAAGCTTGTGCCACTCGATGTGATGT
qPCR-F	CTACAGGTGAAGGTGGAATGG
qPCR-R	TTCCTCTCTTTCCTCTGCGC

Table S4. DNA Sequence of “humanoid” molecular beacon (H-MB)

Name	DNA Sequence （5’ — 3’）
Head	GATCAACTACTACTTGGT (FAM) AAGGGTTTTTTTTTTTT TTCCCTTT (BHQ-1) GTTTCTCTAATTAAGGC
Right arm	GCCTTAATTAGAGAAACTTTACTCACCCGTCCGCCA
Left arm	TGGCGGACGGGTGAGTAATCCAAGTAGTAGTTGATC
Right leg	TGGCGGACGGGTGAGTAA
Left leg	TTACTCACCCGTCCGCCA

Table S5. Comparison between TALEs with other methods in the literature.

Methods	Linear range (cfu/mL)	LODs (cfu/mL)	Recognition element	Measure process	Ref.
EIA	7.8×10–7.8×10^6	3.4×10	Ag-Ab	Complex	1
ELISA	1.0×10^4–1.0×10^7	1.0×10^4	Ag-Ab	Complex	2
Raman Spectroscopy	-	10	Ag-Ab	Complex	3
QD-Apt	1.0×10–1.0×10^4	-	Apt	Complex	4
RT-PCR	2.0×10^2–6×10^5	2.0×10^2	DNA	Complex	5
PCR	1.2×10^3–1.2×10^8	1.2×10^3	DNA	Medium	6
TALEs	3.0–1.0×10^9	3.0	DNA-TALEs	Simple	This work

Table S6. Recovery of E. coli at different concentration levels in mineral water samples (n =3).

Sample	Spiking levels (cfu/mL)	ΔF (a.u.)	Amount measured (cfu/mL)	Recovery (%)
1	1×10	1766.1	0.942×10	94.2
2	1×10^4	1310.3	1.057×10^4	105.7
3	1×10^5	1161.5	1.047×10^5	104.7

References

1 Q. Guo, J. J. Han, S. Shan, D. F. Liu, S. S. Wu, Y. H. Xiong and W. H. Lai, *Biosens. Bioelectron.* , 2016, **86**, 990-995.

2 J. Khang, D. Kim, K. W. Chung and J. H. Lee, *Talanta*, 2016, **147**, 177.

3 L. Wang, P. Li, Z. Zhang, Q. Chen, Z. P. Aguilar, H. Xu, L. Yang, F. Xu, W. Lai and Y. Xiong, *Food Control*, 2014, **36**, 119-125.

4 X.-c. S. Z. Y. C. L. Y. L. L. G. Y. Zhou, *Sensor. Actuat. B-Chem.* , 2015, **221**, 985-992.

5 R. R. Hu, Z. Z. Yin, Y. B. Zeng, J. Zhang, H. Q. Liu, Y. Shao, S. B. Ren and L. Li, *Biosens. Bioelectron.* , 2015, **78**, 31.

6 P. Zhu, D. R. Shelton, S. Li, D. L. Adams, J. S. Karns, P. Amstutz and C. M. Tang, *Biosens. Bioelectron.* , 2011, **30**, 337-341.