Supporting information

Csm6-DNAzyme tandem assay for one-pot and sensitive analysis of lead pollution and bioaccumulation in mice

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Table S1. Oligonucleotide sequences

Number	Oligonucleotide	Sequences (5’ to 3’)
1	Sub (Sub-F2)	**rArArArA**GGAAGTGGTAACGTCAATTG
2	Sub-E	**rArArArArArA**GGAAGTGGTAACGTCAATTG
3	Sub-F0	**rArArArA**GGAAGTGGTAACGTCAATTG
4	Sub-F1	**rArArArA**GGAAGTGGTAACGTCAATTG
5	Sub-F3	**rArArArA**GGAAGTGGTAACGTCAATTG
6	Sub-H2	**rArArArA**GGAAGTGGTAACGTCAATTG
7	Sub-OMe2	**rArArArA**GGAAGTGGTAACGTCAATTG
8	Dz-C 9-4t	GTTACCACT GAAGTAGCGCCGCCG TTTT
9	Dz-C 9-6t	GTTACCACT GAAGTAGCGCCGCCG TTTTTT
10	Dz-C 9-8t	GTTACCACT GAAGTAGCGCCGCCG TTTTTTTT
11	Dz-C 9-10t	GTTACCACT GAAGTAGCGCCGCCG TTTTTTTTTT
12	Dz-C 9-12t	GTTACCACT GAAGTAGCGCCGCCG TTTTTTTTTTTT
13	Dz-C 9-14t	GTTACCACT GAAGTAGCGCCGCCG TTTTTTTTTTTTTT
14	Dz-C 8-10t	TTACCACT GAAGTAGCGCCGCCG TTTTTTTTTT
15	Dz-C 10-10t	CGTTACCACT GAAGTAGCGCCGCCG TTTTTTTTTT
16	Dz-C 11-10t	ACGTTACCACT GAAGTAGCGCCGCCG TTTTTTTTTT
17	Dz-C 12-10t	GACGTTACCACT GAAGTAGCGCCGCCG TTTTTTTTTT
18	Dz-C 13-10t	TGACGTTACCACT GAAGTAGCGCCGCCG TTTTTTTTTT
19	Sub-O	ACTCAC **/iBHQ1dT/** AT**rA**GGAAG **/i6FAMdT/** GATGGACGTG
20	Dz-O	GTCCATCACT GAAGTAGCGCCGCCG TATAG
21	C5 reporter	**6FAM-rCrCrCrCrC-BHQ1**

* The bold, red-marked letters represent the ribonucleotide at this position, the blue marked letters represent the ribonucleotides with 2’-F modification, the deep red marked letters represent the ribonucleotides with 2’-H, the green marked letters represent the ribonucleotides with 2’-OMe, and all other sequences are deoxyribonucleotides.

Table S2. Sequences of plasmids containing DNA sequences encoding for *Thermus thermophilus* Csm6 (TtCsm6) and *Enterococcus italicus* (EiCsm6)

Proteins	Sequences
TtCsm6	atggaagatctggatgcactgtgggaacgttaccgtgaagcggttcgtgcaggcggtaacccgcaggcgct gtatcaggaaatggtttggccggcgctgctggcgctgtggcgtgaaaaaccgcgtgtttacccgttcccgcag gccttcgctgtctctgttcacaccctgggtaccagcccggaagcgaccgcgctggccatcctgggcgcgggc gctgaacgtgtttacgtgctgcacaccccggaatccgcgcgcttcctgccgcgcctgcgccaggacaccggt aaagacctgtacccggtggaaatcggtaaatctgatgtggaagcgatttatcgcgaagttaaacgtctgctgg aaaaacacccggaagttccggtggcactggatctgaccagcgggactaaagctatgtccgctggcctggcg gcagccggcttcttcttccagcgtttttatccgaaagttcgtgttgtttacgtggataacgaggactacgatccgga actgcgccgtccgcgtgctggtaccgaaaaactgcgcatcctgccgaacccgcacgaagcgctggcggaa gtagatgcgctgttcgcaaaagaactctatggcaaaggtgaattcggccaggccgcagcgtacttccgcggt atggttggccgcaccggtaaccaggcgtacgcactgtatgcgctgctggcagaaatgtaccgtgcatggcgt gcactggactttggtgaagccctgaaagcgggccgtaaacttctgggccagctgagccagaacgtgtggctg aaccacccgctgaacgcccgccgtgaagcgctggaagcgcaggttgctctgctggaagcggtagatcgttt cctgaaagcccgcgacttcgctctgaaagaaggtgtttacggcctggcgcgtacgctgctgcacctggcaca ggaagctaaagaagaagcggcggtgctggccgcactgtatgcataccgcgctctggaactgctgctgcagg aacgtctggcgctgctgggccgtcgtgctgaagctccgggtctgagcccggaagaagccgaagccctgcgt aaagctctggcggaactgctgggcgtcctgccggaagaagtgcgcctgccggcgaaactgggtctgctgga cctgctggcattcctgcgcctgaaaggcgacgaagctctgggccgcctgagcctggctgaactgcgcggtctt gcaggcgcgctgaaaggtcgtaactccgcgctgctggtgcacggctttgacgtgccgagcccgaaagcagt agaaggcatcgcacgcctggcgcagggcctgctgcaggacctggaagcgcgcaccgcgctgggtccgct gtctccggaaccggtgccgctgggtttctaa
EiCsm6	atgaaaatcctgttcagcccgatcggtaacaccgatccgtggcgcaacgatcgtgacggcgcgatgctgca catcgtgcgtcactaccagccggaccgtgttgttctgtttttcaccgaaagcatctggcagggtaaccagcactt ctccggccagcaggcgttcgattgggttaaaattatccagagcatcaacgaaaactgccagatcgaaatcaa atgcgacaccatcgaagttgaaaacgacttcgatgcgtacaaagacctgttccaccagtacctggttgaaga aaaacgtaaatacccgaacgcggaaatctttctgaacgtgacctccggtaccccgcagatggaaaccaccc tgtgcctggaatacgttacctacccggacaaaatgcgctgcatccaggtgagcaccccgctgaaaacctcta acgcgaaaactaaatatgcgcaggcggattgccaggaagttgatctggaaatcgttaacgaagaagaatct cagcagccgagccgttgccataaaatcgcgatcctgtctttccgtgaagctatcgtgcgtaaccagatcaaat ccctgctggataactacgattacgaagcggccctgcagctggttgcgagccagaaatccttccgtaacggca aagaaatccgtaaaaaactgaaagaactgatcgatgatatcaaaatgcaccgcgtgttcagctacctgatca aacagtatccgcgcaacgaaaaactgcagaaagcgctgctgcacaccatcctgctggaaatgcgccacca gcgcggtgacatcgcggaaaccctgatccgtgtgaaaagcatcgcggaatacatcgttgaacagtacatcc agaaaaactatccgtacctgatcatctacaaagaagataaaccgtacttcaacgtgagctacagccaggaa ctgaccgaatcttacctggcgctgatggactctcgtaacaagaaaaccaacaaaaagatgaccgttgatagc ctggaccgtattctgggtttcccggcttaccgtgacttcctgcagctgctggaagcgagcaacgaaatgacca acgaaatgaacaaagttaacgaaatcaacaacctgcgtaacaaagttgcgcacaacctggactccctgaa cctggatcgtgataaaaacggtcgtaaaatcaccaacgcggttaccgcggttcgtaccatgctgctggcggttt tcccggaagtgcaggaaaacgatttccactacctgaaacagtttaaccagtctatcaaagaactgctgtaa

Table S3. Detection of Pb^{2+} spiked in the fresh egg and tap water samples

Samples	Added (nM)	Found (nM)	Recovery (%)	RSD (%) n=3
Fresh egg	10	9.01	90.09	3.12
	30	27.13	90.43	1.51
	50	52.66	105.32	1.71
Tap water	10	9.34	93.42	2.35
	30	27.09	90.29	2.71
	50	56.09	112.17	3.14

Table S4. Comparisons of detection performance of different fluorescent assays for Pb^{2+} sensing

Strategy	Recognition	One-step	One-tube	Separation required	Elaborate probe design	Linear range	LOD	Application	Ref.
Csm6-DNAzyme tandem assay	**DNAzyme**	**Yes**	**Yes**	**No**	**No**	**0.1-100 nM**	**70 pM**	**Water, eggs and mice**	**This work**
Magnetic separation-based Cas12a-DNAzyme	DNAzyme	No	No	Yes	Yes	0.01-10 nM	53 pM	Water	*ACS Sens.*, 2020, 5, 970-977
Cas12a/Cas14a-DNAzyme	DNAzyme	No	No	Yes	Yes	0.24-48 nM	480 pM	Water	*Anal. Chim. Acta,* 2022, 1192, 339356
Cas12a-G-quadruplex assay	G-quadruplex	No	Yes	No	No	0.1 nM- 5 μM	2.6 nM	Tea Beverage and milk	*Food Chem.*, 2022, 378, 131802
Cell-free paper-based biosensor	Allosteric transcription factor	No	Yes	No	No	1-250 nM	0.1 nM	Water	*J. Hazard. Mater.*, 2022, 438, 129499
Tetrahedral DNA Nanostructure-based DNAzyme	DNAzyme	Yes	Yes	No	Yes	0-500 nM	0.9125 nM	Tobacco leaf extracts	*J. Clean. Prod.*, 2022, 362, 132544
Label-free sequential DNAzyme	DNAzyme	No	Yes	No	Yes	0.1-10	0.22	Water and eggs	*ACS Sens.*, 2018, 3, 2660-2666

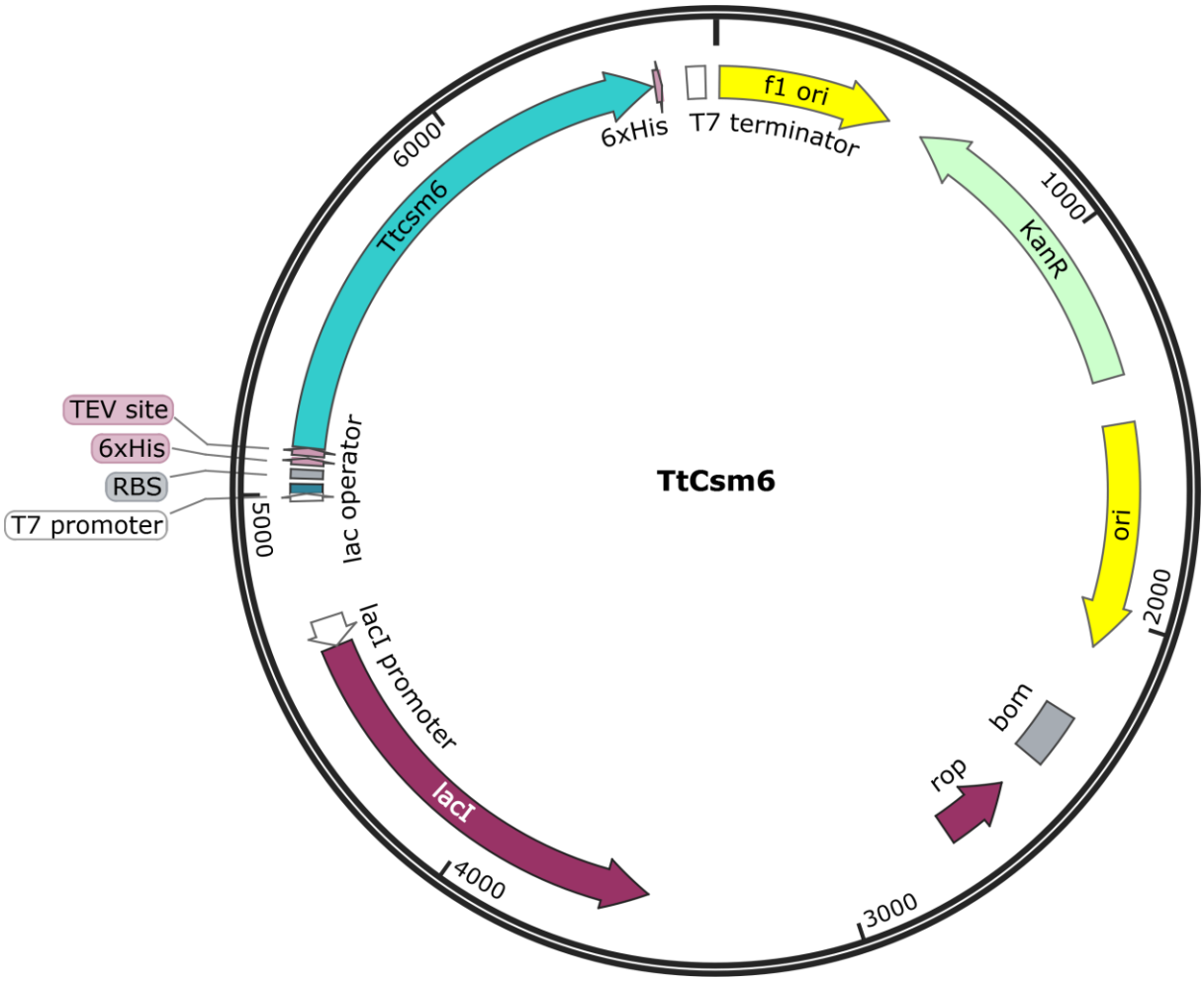

Figure S1. Plasmid vector for expression of TtCsm6.

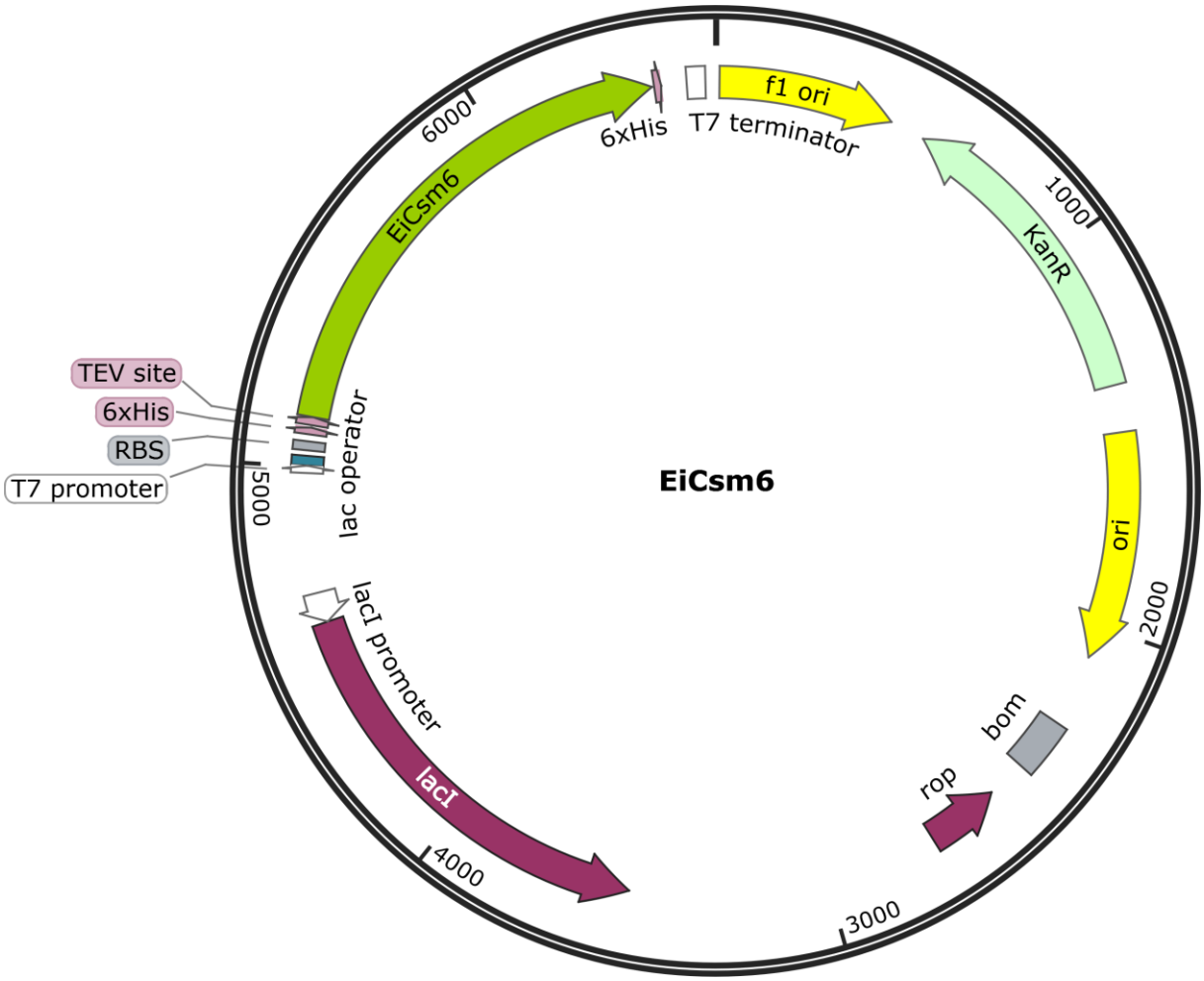

Figure S2. Plasmid vector for expression of EiCsm6.

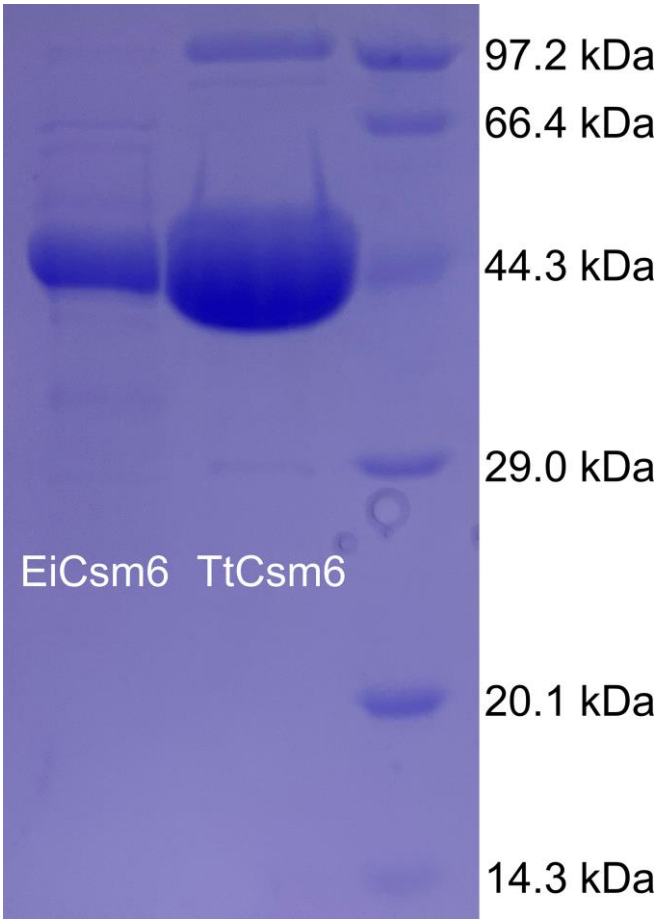

Figure S3. SDS-PAGE analysis of purified Csm6 proteins.

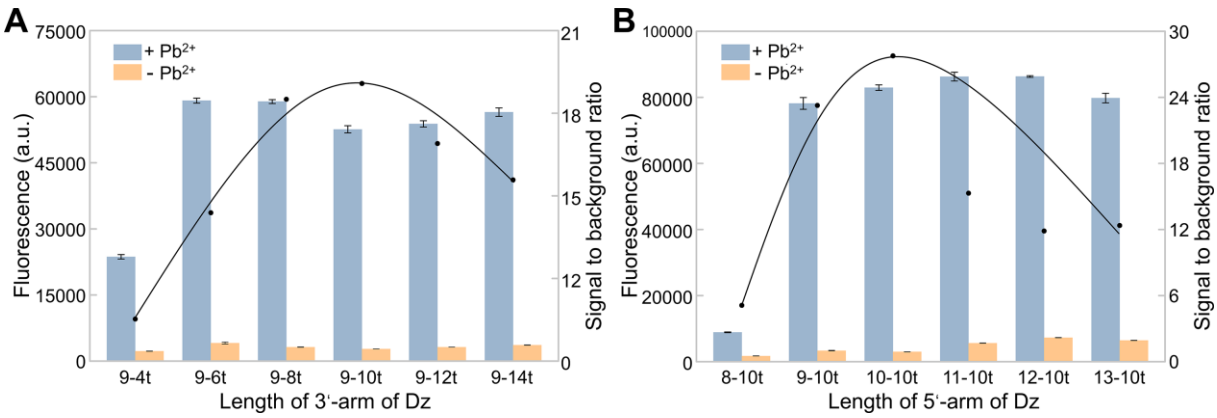

Figure S4. Optimization of the lengths of two arms of DNAzyme strand. Fluorescence signal and signal-to-background ratio of the cDNAzyme assay response to Pb^{2+} using **Sub** probes that hybridized with **Dz** with different arm lengths (3'-part) (A), different arm lengths (5'-part) (B). The excitation wavelength was 480 nm, with the corresponding emission wavelength of 510-600 nm. The concentrations of Pb^{2+}, **Sub** and **Dz** were 150 nM, 400 nM and 400, respectively.

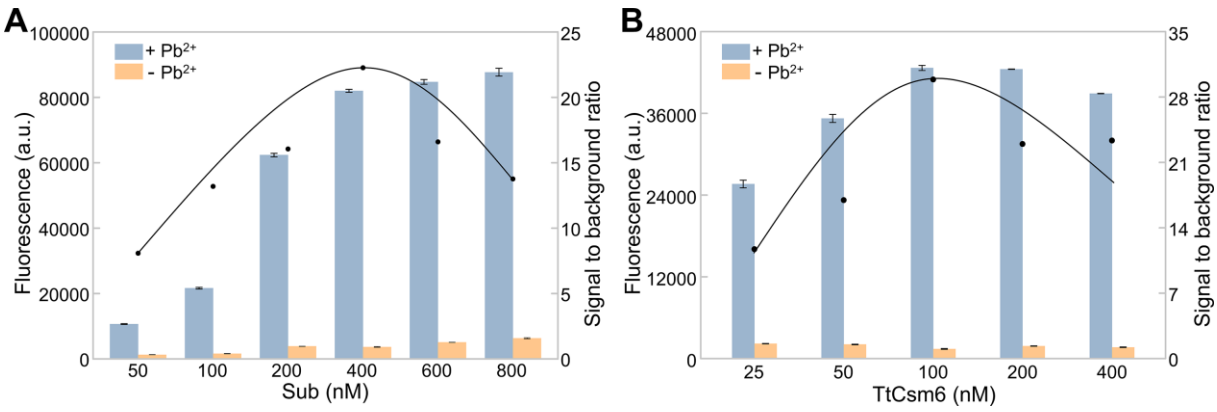

Figure S5. Optimization of the concentration of substrate and TtCsm6. Fluorescence signal and signal-to-background ratio of the cDNAzyme assay response to Pb^{2+} using different concentrations of **Sub** (A), using different concentration of TtCsm6 (B). The excitation wavelength was 480 nm, with the corresponding emission wavelength of 510-600 nm. The concentrations of Pb^{2+}, **Sub** and **Dz** were 150 nM, 400 nM and 400, respectively.

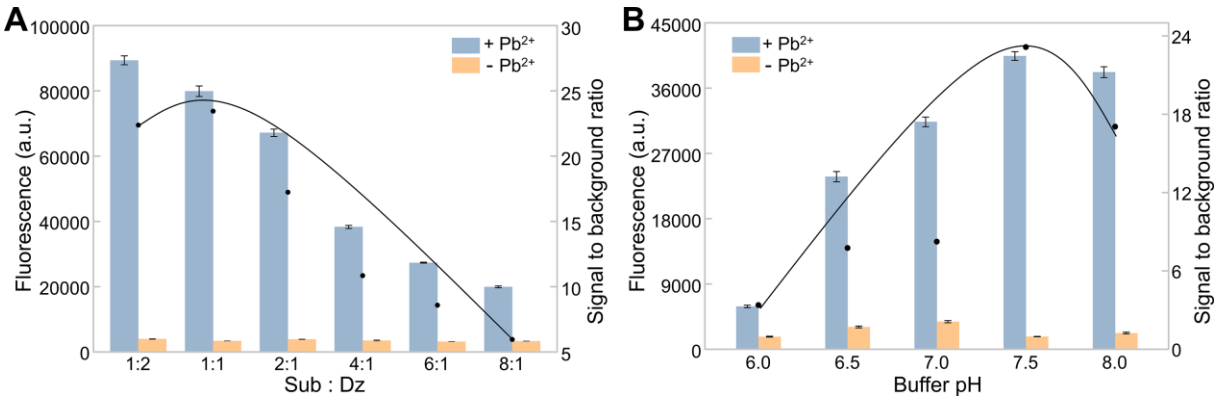

Figure S6. Optimization of the amount of DNAzyme strand and buffer pH. Fluorescence signal and signal-to-background ratio of the cDNAzyme assay response to Pb^{2+} using different ratios of **Sub** to **Dz** (A), using different buffer pH (B). The excitation wavelength was 480 nm, with the corresponding emission wavelength of 510-600 nm. The concentrations of Pb^{2+}, **Sub** and **Dz** were 150 nM, 400 nM and 400, respectively.

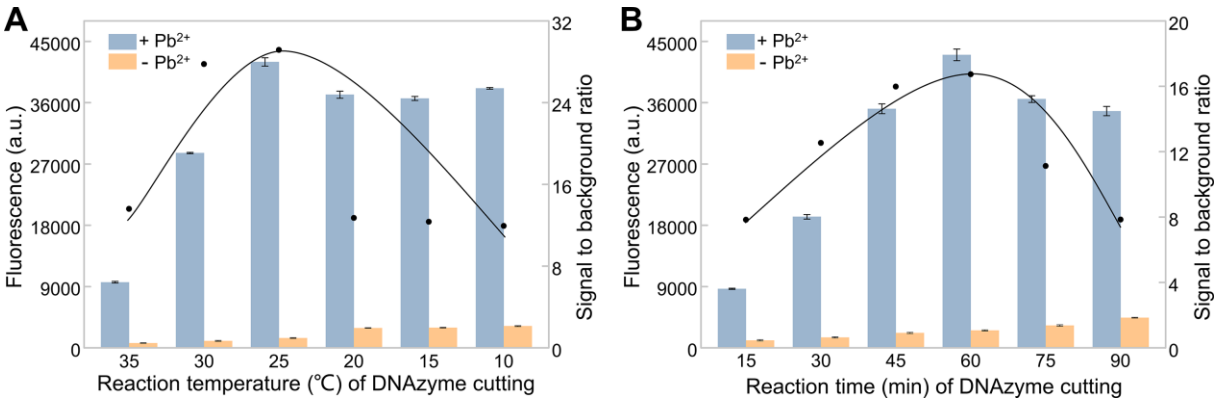

Figure S7. Optimization of the reaction temperature and time of DNAzyme cutting. Fluorescence signal and signal-to-background ratio of the cDNAzyme assay response to Pb^{2+} using different reaction temperature (A) and time (B) of DNAzyme cutting. The excitation wavelength was 480 nm, with the corresponding emission wavelength of 510-600 nm. The concentrations of Pb^{2+}, **Sub** and **Dz** were 150 nM, 400 nM and 400, respectively.

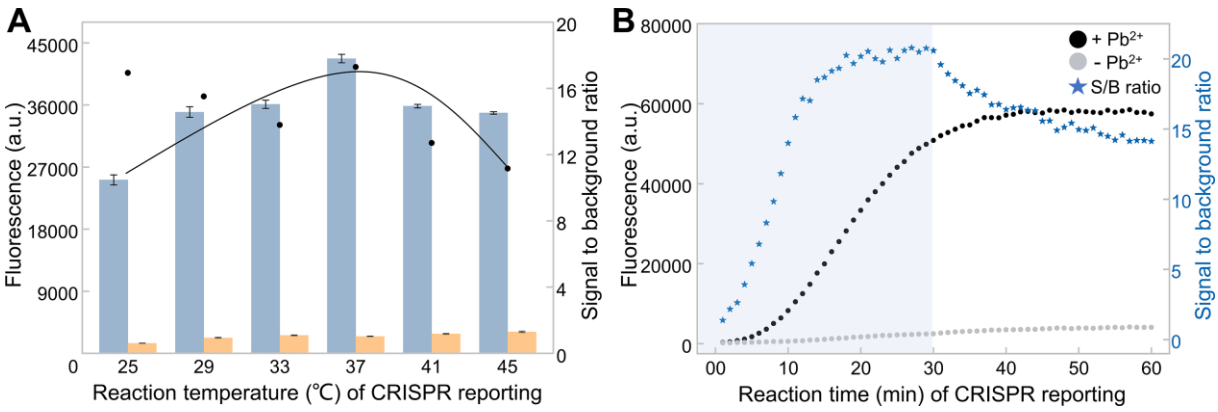

Figure S8. Optimization of the reaction temperature and time of CRISPR reporting. Fluorescence signal and signal-to-background ratio of the cDNAzyme assay response to Pb^{2+} using different reaction temperature (A) and time (B) of CRISPR reporting. The excitation wavelength was 480 nm, with the corresponding emission wavelength of 510-600 nm. The concentrations of Pb^{2+}, **Sub** and **Dz** were 150 nM, 400 nM and 400, respectively.

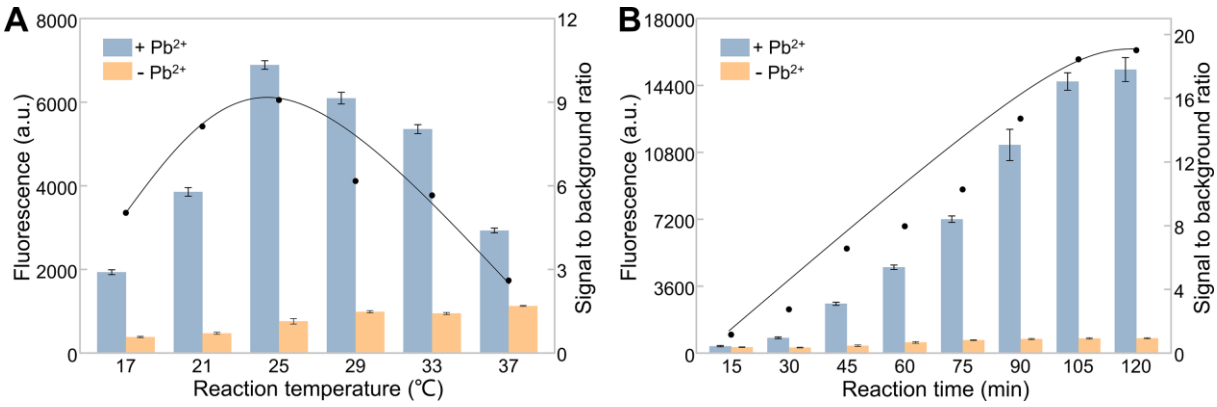

Figure S9. Optimization of the one-step cDNAzyme assay for Pb^{2+} detection. Fluorescence signal and signal-to-background ratio of the cDNAzyme assay response to Pb^{2+} using different reaction temperature (A) and reaction time (B). The excitation wavelength was 480 nm, with the corresponding emission wavelength of 510-600 nm. The concentrations of Pb^{2+}, **Sub** and **Dz** were 150 nM, 400 nM and 400, respectively. The reaction time was 1 h.