Electronic Supplementary Information (ESI)

Multiplex bacterial assay using element labeled strategy for 16s rRNA strands detection

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Experimental section

Chemicals and materials. Europium chloride hexahydrate, thulium (III) chloride hexahydrate, yetterbium nitrate pentahydrate, praseodymium chloride, lutetium chloride hexahydrate and holmium chloride hexahydrate were purchased from Shanghai Aladdin Biochemical Technology Co. Ltd. The maleimido-mono-amide-DOTA was purchased from Macrocyclics, Inc. The streptavidin functionalized magnetic beads (Dynabeads® M-280 Streptavidin) and DynaMag™-2 separation magnet were purchased from Invitrogen Co. (Shanghai, China). High purity deionized water used in all experiment was prepared by Merck Millipore Simplicity UV (Merck Millipore, China). The bacteria samples were purchased from China General Microbiological Culture Collection Center. The S1 nuclease was purchased from Takara Inc. Other reagents used in this work were of analytical grade or higher.

The buffers were prepared as follows. The PBS was purchased from Mediatch, Inc. The PBST used as washing buffer was PBS contained 0.02% Tween 20 (v/v).

The oligonucleotide probes functionalized with thiol group (-SH) and the DNA modified with biotin was purchased from Sangon Inc. (Beijing, China). The sequences of all oligonucleotides are listed in Table S1-S3 (the target sequences are complementary to the protecting probes).

Table S1. Sequence information of protecting probes

Name	Target bacterium	Sequence (5'-3')
BAC	Bacillus subtilis	AACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGT
LAC	Lactobacillus	TTGCTCCATCAGACTTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCC
EUB	Escherichia colibacillus	TAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACTAAAG
BL	Bifidobacterium longum	GTGCTTATTCAACGGGTAAACTCACTCTCGCTTGCTCCCCGATA
BA	Bifidobacterium adolescentis	GTACACTCACCCCGTTGGGCTTGCTCCCAGTCAAAAGCGC
BB	Bifidobacterium breve	GCACGTAGTTAGCCGGTGCTTATTCGAAAGGTACACTCAACACA

*The sequences with underline were complementary to the signal probes, and the sequences in red were complementary to the capture probes.

Table S2. Sequence information of capture probes

Name	Target bacterium	Sequence (5'-3')
BAC-cap	Bacillus subtilis	ACCAGAAAGCCACGGCTAACTACGTGCCA
LAC-cap	Lactobacillus	GGAGGCAGCAGTAGGGAATC
EUB-cap	Escherichia colibacillus	CTTTAGTTTGAACCATGCG
BL-cap	Bifidobacterium longum	TACCCGTTGAATAAGCAC
BA-cap	Bifidobacterium adolescentis	CTTCGGGGTGAGTGTAC
BB-cap	Bifidobacterium breve	AGCACCGGCTAACTACGTGC

Table S3. Sequence information of signal probes

Name	Target bacterium	Sequence (5'-3')
BAC-Eu	Bacillus subtilis	GCGGTAATACGTAGGTGGCAAGCGTTTTTTT-SH
LAC-Tm	Lactobacillus	ACGCAAGTCTGATGGAGCAATTTTTT-SH
EUB-Ho	Escherichia colibacillus	TCAAACAACCATCCGGTATTATTTTTT-SH
BL-Yb	Bifidobacterium longum	SH-TTTTTTATCGGGGAGCAAGCGAGAGTGAGT
BA-Pr	Bifidobacterium adolescentis	SH-TTTTTGCGCTTTTGACTGGGA
BB-Lu	Bifidobacterium breve	SH-TTTTTTGTGTTGAGTGTACCT

Synthesis of element labeled probes. The oligonucleotide probes functionalized with thiol group (-SH) were mixed with maleimido-mono-amide-DOTA (MMA-DOTA) in 0.5 M CH3COONH4 solution (pH 5.8) at 37 ℃ for 2 hours. Then the product was purified to remove the unreacted DNA and maleimido-mono-amide-DOTA by running the SDS-PAGE. The purified product was mixed with the rare earth element ions in CH3COONH4 solution (pH 5.8) at 37 ℃ for 1 hour. And a desalt procedure was used to remove the overused ions by dialysis. The final labeled probes were stored at 4 ℃ for immediately use or -20 ℃ for long time storage.

Modification of magnetic beads. The magnetic beads were modified with the capture

probes of the target oligonucleotides. The streptavidin functionalized magnetic beads were mixed with 100 uL DNA modified by biotin at the end, in the volume ratio of 40 : 1. The reagent was dissolves in PBS buffer. The reaction was under room temperature for 30 min to Immobilize the capture probes on the surface of the magnetic beads. Then, the modified beads were washed three times by PBST to remove the unreacted DNA strands and were store at 4 ℃.

Assay procedure. The bacteria samples were centrifuged to remove the culture medium or other substrate. After 1 mL lysis buffer was added to the sample, the mixture was decomposed by ultrasound for 15 min. Take 20 uL bacterial supersonic lysates to mix with 10 uL 2 umol/L protecting probes in PBS buffer. After incubating 1 hour, 60 UI S1 nuclease (diluted in 70 uL reacting buffer) was added to hydrolyze the single oligonucleotides strands for 1 hour. To end the hydrolysis, 150 uL stop solution was add, and the mixture was heated up to 95 ℃ for 15min. As for the standards, just take 20 uL standard solution to mixed with protecting probe. And then go through the enzymatic treatment as same as the bacterial supersonic lysates.

After the pretreatment above, 50 uL sample was mixed with capture probes and labeled probes. The hybridization procedure was at 37 ℃ for 30 min. Each tube was washed three times by PBST. Then, 200 uL ultrapure water was added into the tube and heated to 95℃ for 20 min. The solution was pumped into ICP-MS for determination.

Verification of the counting performance. To test to counting performance, the quantification results were correlated to the counts of bacterial numbers and compared with the results obtained by direct cell count method. The bacterial strains were first multiplied in the culture medium in appropriate conditions. Then, each bacterial strain was divided into two samples. One sample was then stained trypan blue solution to recognize the active cells before counted by the cell counter. The suspension would be diluted if the cell concentration was too high. Meanwhile, the other suspensions of the pure single strains were mixed. The mixture would be pretreated and detected afterwards according to the assay procedure describe above.

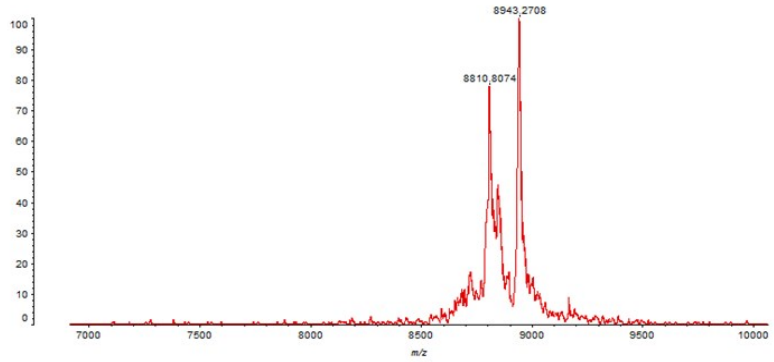
ICP detection. A Thermo ICP-MS iCAPQ (Thermo Fisher Scientific, USA) was used for determination of the rare earth element labeled DNA probes. The operating parameters were optimized and listed in Table S4.

Table S4. Operating parameters of ICP-MS

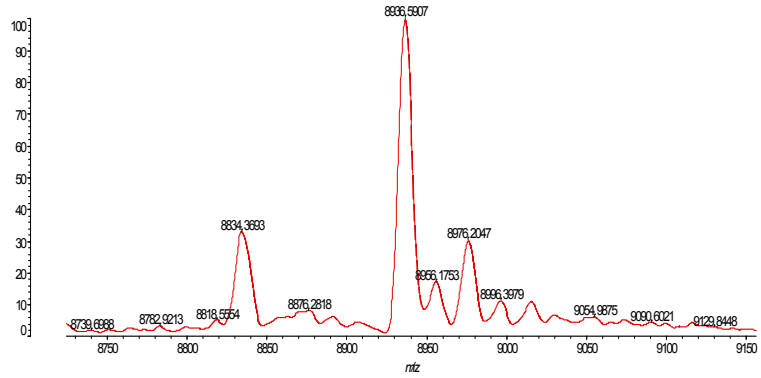
Parameters	Value
cool gas flow(L/min)	14
auxiliary gas flow(L/min)	0.8
nebulizer gas flow(L/min)	0.92
sample uptake(s)	43
dwell time (ms)	0.02

number of repeats per sample	3
PC detector voltage(V)	1275
RF power(W)	1548.61
analogue detector voltage(V)	-1950

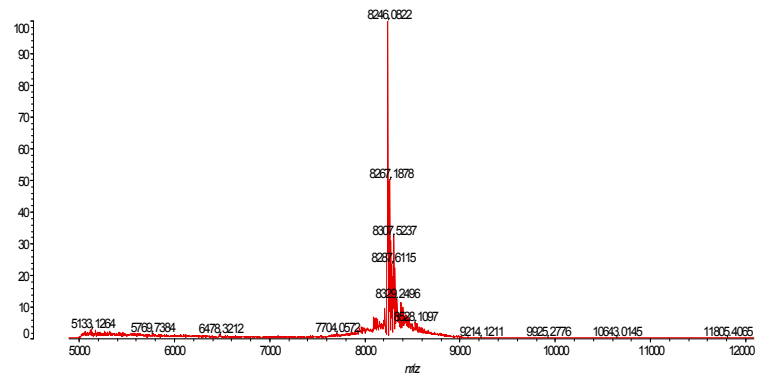
(a)



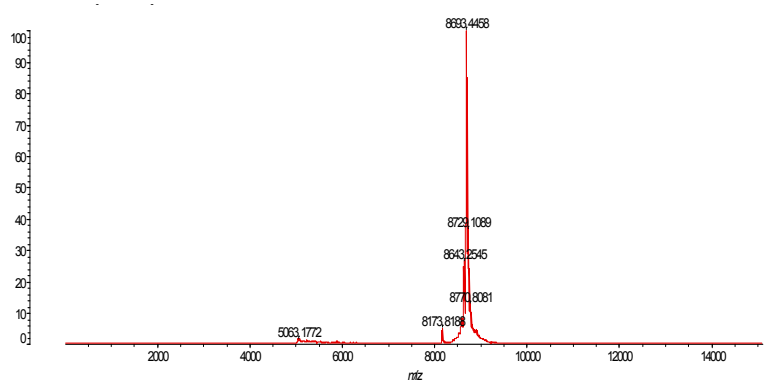
(b)



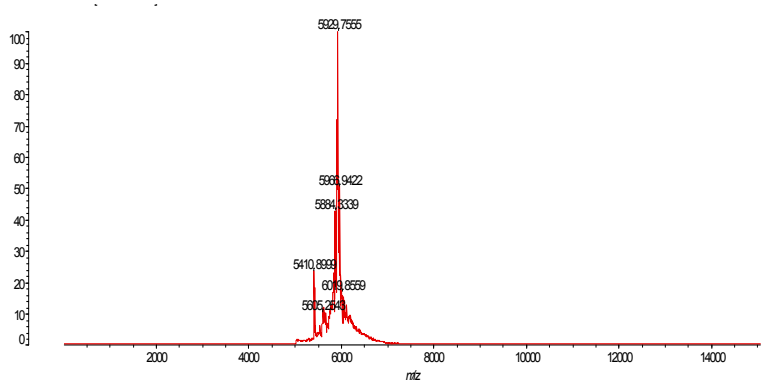
(c)



(d)



(e)



(f)

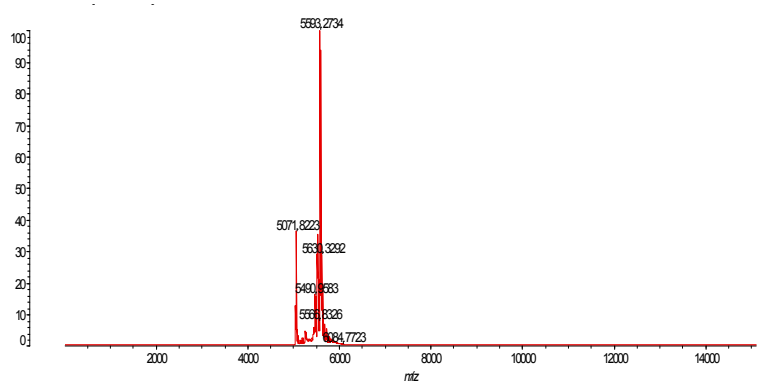
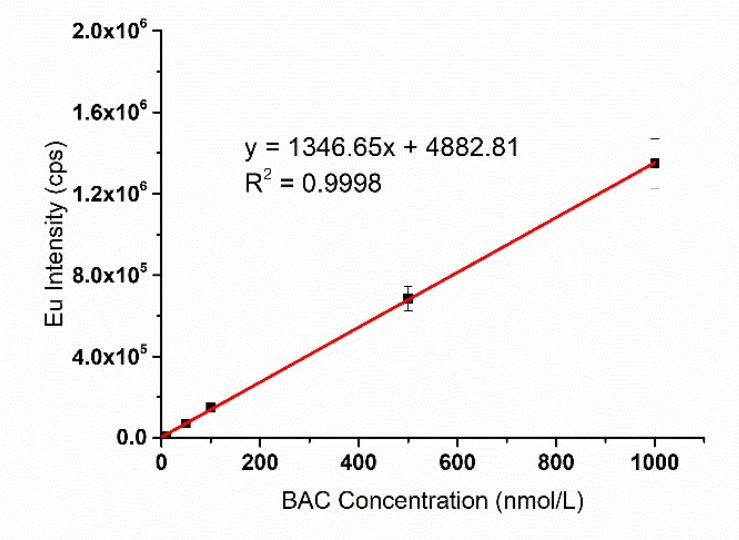
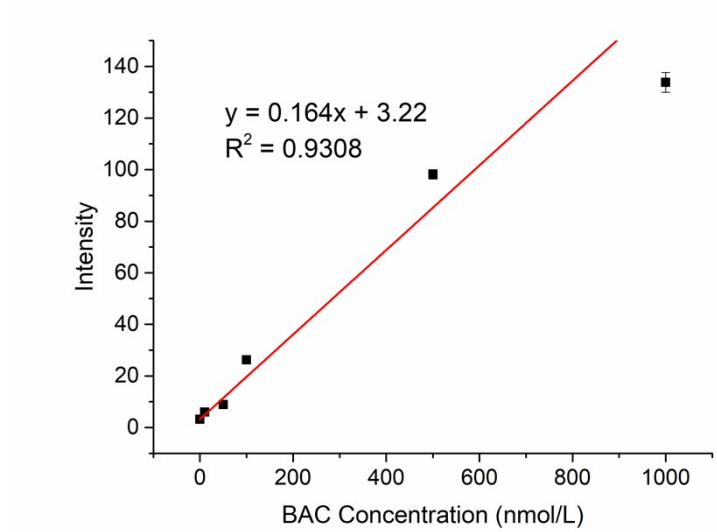


Fig. S1 The MALDI spectrum of the synthesis signaling labels. (a) the MALDI spectrum of BAC; (b) the MALDI spectrum of LAC; (c) the MALDI spectrum of EUB; (d) the MALDI spectrum of BL; (e) the MALDI spectrum of BA; (f) the MALDI spectrum of BB.

(a)


y = 1346.65x + 4882.81
R2 = 0.9998
Eu Intensity (cps)
BAC Concentration (nmol/L)

(b)


y = 0.164x + 3.22
R2 = 0.9308
Intensity
BAC Concentration (nmol/L)

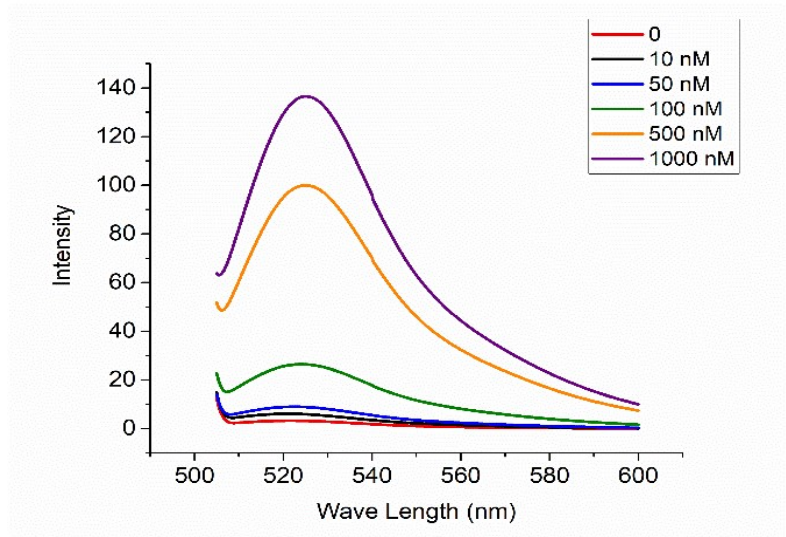

0
10 nM
50 nM
100 nM
500 nM
1000 nM
Intensity
Wave Length (nm)

Fig. S2 the comparison with fluorescent method. (a) the quantification result of specific sequence BAC by elemental labeled probe. (b) the quantification result of specific sequence BAC by fluorescent probe (FAM labeled).

Discussion

As shown in Fig. S2, the BAC was detected by the ICP-MS method and fluorescent method at the same time. All the regents are the same except the signaling probes. Our method showed comparable performance in the low concentration of the target. However, when the concentration reached 1000 nmol/L, our method could still contain linear correlation while the fluorescent method could not keep the good linear correlation as in low concentration. It is obvious that the fluorescent response became lower than the linear predicted. Although this could be solved by diluting the target, it might cost time to have a second test for the same target. Using elemental tags and ICP-MS as detector could have wider linear range and save time when the concentration of the targets are quite different in a wide range.