Supplementary Material (ESI) for Molecular BioSystems

Visual Detection of DNA from Salmonella and Mycobacterium using Split DNAzymes

SUPPORTING INFORMATION

Supporting Information Contents

Materials. Autoclaved MilliQ water was used for all buffers, stock solutions of oligonucleotides and Polymerase Chain Reaction (PCR). Oligonucleotides were manufactured from a local vendor (1st Base, Singapore) with standard TOP or HPLC purifications (depending on length) (Table S1). Hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) was from Kasei Chemical. 2,2'-Azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), hemin, Triton X-100, Magnesium chloride, sodium chloride, dimethyl sulfoxide (DMSO), Tris(hydroxymethyl) aminomethane (Tris-Base), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) were from Sigma-Aldrich (Singapore). Absorbance readings were taken on a SmartSpec3000 Spectrophotometer (Bio-Rad Laboratories, Singapore) at 419nm.

Asymmetric PCR conditions. 10x modified PCR buffer was prepared to a concentration of 400 mM NaCl, 100 mM Tris base in MillQ water. The PCR buffer was then adjusted to pH 8.3 using HCl. PCR amplifications were carried out using conditions optimised with a C1000 thermal cycler (Bio-Rad).

***Salmonella enterica* target amplification:** A 50µL PCR reaction was prepared using 45 ng of genomic DNA with 1.5mM $MgCl_2$ and 1x in house PCR Buffer. 0.8mM forward Primers, 0.053mM reverse primers & 2.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems) were added to the reaction volume. Cycling conditions included an initial denaturation at 95°C for 3 mins, followed by 70 cycles of 95°C 1min, 53.1°C 1min, 72°C 1min.

***Mycobacterium smegmatis* target amplification:** A 50µL PCR reaction was prepared using 50 ng of mycobacterium DNA with 2.0mM $MgCl_2$ and 1X in House PCR buffer. 0.8µM reverse primers, 0.08µM forward primers, 2.5 units of AmpliTaq Gold Polymerase (Applied Biosystems) were added to the reaction volume. Cycling conditions included an initial denaturation at 95°C for 3 mins, followed by 70 cycles of 95°C 1 min, 56.3°C 1 min, 72°C 1 min.

Non-target control amplification: An Emilin1 gene segment from human genomic DNA was amplified. A 50 µL PCR reaction was prepared using 50 ng of HapMap Genomic DNA sample (extracted from immortalized cell lines, Cat. No: GM7034, Corriell Laboratories) with 2.0mM $MgCl_2$ and 1x in house PCR buffer. 0.01µM forward primers, 1.0µM reverse primers and 2.5 units of AmpliTaq Gold DNA polymerase were added to the reaction volume. Cycling conditions included an initial denaturation at 95°C for 10 mins, followed by 70 cycles of 95°C 1min, 63°C 1min, 72°C 1min.

Table S1. Primers and probe sequences used in this study. Amplicon sizes were designed to be as small as possible (Fig S1). The sizes of the resulting ssDNA amplicons were 119bp for *invA* segment in salmonella DNA, 72bp for rRNA segment from mycobacterium and 50bp for the *Emilin1* segment in human DNA.

	Identity[1]	**Sequences**[2]
+ve Ctrl	Native G-quadruplex	5'- *GGGTAGGGCGGGTTGGG*
Salmonella *invA* target	F Primer	5'-TCGTCATTCCATTACCTACC
	R Primer	5'-AAACGTTGAAAAACTGAGGA
	α Probe	5'-ACCAATATCG-$(OCH_2CH_2)_3OPO_3$-*GGGTAGGG*
	β Probe	5'-*GGGTTGGG*-$(OCH_2CH_2)_3OPO_3$-CCAGTACG
Mycobacterium rRNA gene target	F Primer	5' -GCATCTAGTTCGTAAGAGTGTGG
	R Primer	5' -ACATCAATTTGTCCGCAACA
	α Probe	5'-GGTGGCGCAT-$(OCH_2CH_2)_3OPO_3$-*GGGTAGGG*
	β Probe	5'-*GGGTTGGG*-$(OCH_2CH_2)_3OPO_3$-TGTTGCGG
Non-Target *Emilin1*	F Primer	5' - TCTGCTGAGGCTCTCCTGTT
	R Primer	5' - CTGCTTTGAAGTCCACGTAGC
Synthetic target (Salmonella)	34 bases	5' - CTGAATATCGTACTGGCGATATTGGTGTTTATGG
	50 bases	5' –TGATCGCACTGAATATCGTACTGGCGATATTGGT GTTTATGGGGTCGTTC
	84 bases	5' – ATCTGGTTGATTTCCTGATCGCACTGAATAT CGTACTGGCGATATTGGTGTTTATGGGGTCGTTC TATATTGACAGAATC

[1] F – forward primer, R – reverse primer

[2] The sequences used for the split G-quadruplex, also known as the PW17 aptamer, are shown in italics. The split segments are shown in blue and red, and were conjugated with a triethylene glycol linker with the α and β probes, respectively. The synthetic target sequences complementary to the α & β split probes are also shown in green and pink, respectively.

Visual assay. The final DNAzyme assay buffer comprised 50mM HEPES, pH 7.4 (adjusted with ammonium hydroxide), 50mM $MgCl_2$, 8.33mM Tris, pH 8.3, 87mM NaCl, 150mM NH_4OAc, 0.03% Triton X-100 and 1% DMSO. Split aptamer probes; 1μM each for salmonella α & β Probe, 0.5μM each for α & β mycobacterium probes were incubated with 50μl PCR products and visual reaction buffer for 10 mins, followed by incubation with hemin at 125nM for another 10 mins. The colour change started occurring instantly (within one minute) upon finally adding, to a final concentration of 1mM, H_2O_2 and ABTS. The final reaction volume was 60μl. The absorbance readings (419nm) were taken within 5 mins of incubation at room temperature. Beyond 20 min, the background (negative control tubes) also started turning deeper shades of green. The PCR tubes were photographed using a Samsung (ST50) digital camera 12.2-mega pixel under fluorescent light white balance. This assay was demonstrated using ABTS as the substrate. 3,3'-diaminobenzidine tetrahydrochloride (DAB) may alternatively be applied, but better visual outputs were obtained by us with ABTS.

Supporting experiments and results

1. Effects of potassium ions and target length on visual output.

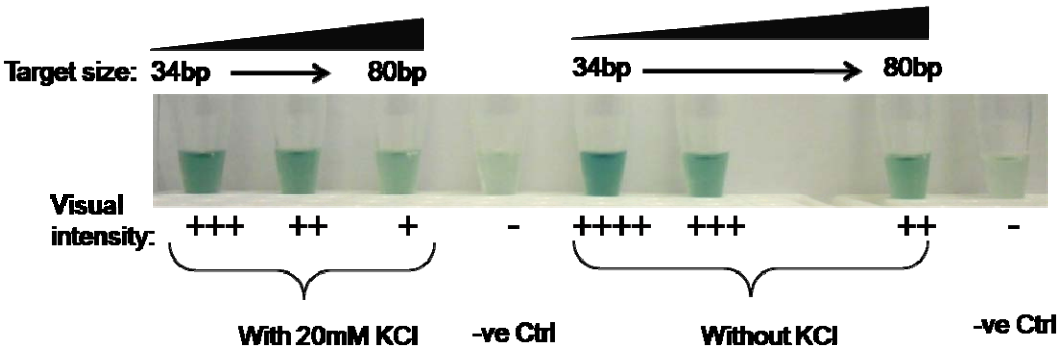

Figure S1. Different lengths of single stranded targets covering 34 bases, 50 bases and 80 bases of *invA* (at 1µM concentrations) were tested against the corresponding salmonella α/β probes, in the presence or absence of 20mM potassium chloride. The final tube (in the fourth position) for each experimental set served as the negative control, and contained no target DNA. (All tubes were imaged 5 min after the final reagent addition, in a final volume of 222 µL in 1.5mL eppendorf tubes)

The shorter oligonucleotide sequences displayed higher intensities. The presence of potassium ions also diminished the signal strength. Observing this trend, we designed our targets, produced by asymmetric PCR, to be as short as possible; 72 bases for mycobacterium, and 119 bases for salmonella. We also removed KCl from our reaction buffers to avoid interference.

2. Order of reagent addition affects visual output for the split DNAzyme assay

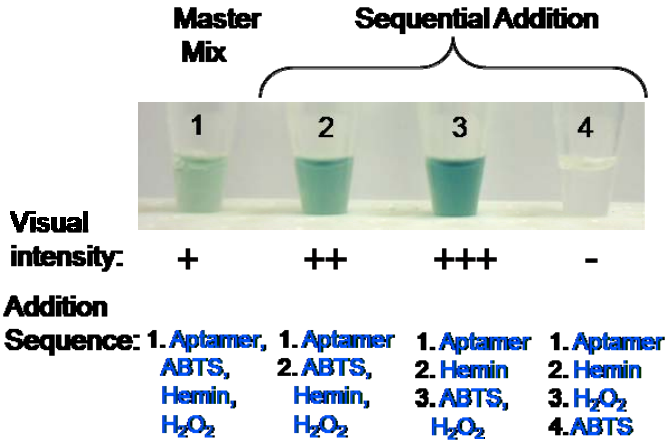

Figure S2. Different sequences of addition of split aptamer, hemin, ABTS & H_2O_2 to corresponding synthetic *invA* target sequence (34 base target). Master mix (tube 1) comprises simultaneous addition of α/β aptamers, hemin, ABTS and H_2O_2. The addition order for the remaining tubes were as follows: Tube 2 - α/β aptamers (10 mins), followed by hemin, ABTS and H_2O_2; Tube 3: α/β aptamers (10 mins), hemin

(10 mins), followed by ABTS & H_2O_2. Tube 4: α/β aptamers (10 mins), hemin (10 mins), H_2O_2 (5 mins), ABTS. (All tubes were imaged 5 min after the final reagent addition, in a final volume of 222 µL in 1.5mL eppendorf tubes)

Incubation of split α/β aptamers and hemin separately (Tube 3), generated the highest signal intensity. As a result, separate incubation of aptamers and hemin was adopted in our visual assay workflow. Although such a sequential addition process may enhance visual readout and discrimination ability, it was still possible to add hemin, ABTS & H_2O_2 simultaneously (Tube 2) and still be able to visually discriminate presence or absence of target sequence.

3. Mycobacterium DNA detection assay with the pre-addition of α/β probes within the asymmetric PCR mix.

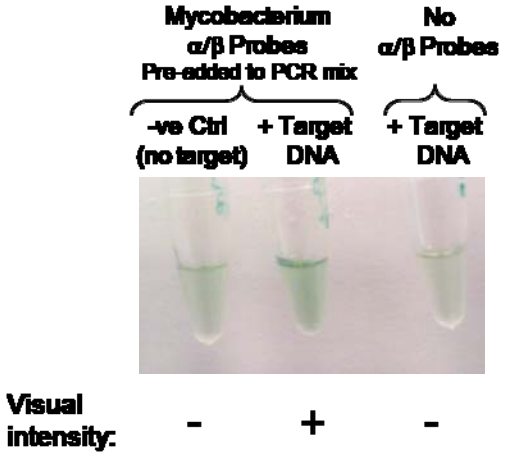

Figure S3. Assay detection with α/β probes pre-added prior to PCR for target DNA amplification. PCR tubes were imaged with 60µl of samples

4. Assay sensitivity - detection down to 10µl PCR volumes.

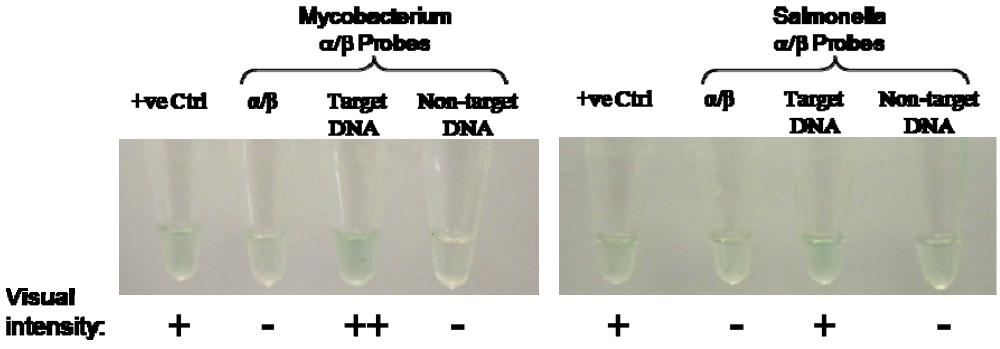

Figure S4. Assay detection using reactions downscaled to 10µL PCR volumes. Positive controls contained 0.3 µM of native DNAzyme. PCR tubes were imaged with a total of 12 µL of samples.

The positive PCR reactions for salmonella and mycobacterium contained approximately 2ng/µl or a total of ~20 ng of target DNA (with a concentration of 115nM), as estimated by absorbance 260/280nm measurements (after ethanol precipitation, and resuspension of PCR product in water). Going below PCR volumes of 10µl makes the readouts challenging to discern just by eye, because of the small volumes involved.