Sandwich assay for mixed-sequence recognition of double-stranded DNA (dsDNA): Invader-based detection of targets specific to foodborne pathogens†

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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

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Definition of zipper terminology. The following describes the relative arrangement between two monomers positioned on opposing strands in a duplex. The number *n* (e.g., +1) describes the distance measured in number of base pairs and has a positive value if a monomer is shifted toward the 5'-side of its own strand relative to a second reference monomer on the other strand. Conversely, *n* has a negative value if a monomer is shifted toward the 3'-side of its own strand relative to a second reference monomer on the other strand.

Protocol – Invader synthesis. Invader probes were synthesized via standard machine-assisted solid-phase DNA synthesis as previously described,[S1] using extended coupling times (4,5-dicyanoimidazole as activator, 15 min, ~98% coupling yield) during incorporation of the 2'-*O*-(pyren-1-yl)methylribonucleotide A^{Bz},[S2] C^{Bz},[S2] G^{iBu},[S3] or U[S4] phosphoramidites. The manufacturer's recommendations for incorporation of the 5'-amino-modifier C6 (C_6-NH_2; 6-(4-monomethoxytritylamino)hexyl-(2-cyanoethyl)-(*N*,*N*-diisopropyl)-phosphoramidite, Glen Research) were followed. Biotin-labeled signaling probes were synthesized using the 3'-biotin TEG CPG support (1-(4,4'-dimethoxytrityloxy)-3-*O*-(*N*-biotinyl-3-aminopropyl)-triethyleneglycolyl-glyceryl-2-*O*-succinyl-lcaa-CPG, Glen Research). Cleavage from solid support and removal of protecting groups was accomplished using 32% aq. ammonia (55 °C, 12 h). Invaders were purified via ion-pair reverse phase HPLC (XTerra MS C18 column) using a triethylammonium acetate - water/acetonitrile gradient, followed by detritylation (80% aq. AcOH), and precipitation (NaOAc/acetone, -18 °C for 12-16 h). The identity of synthesized ONs was established through MALDI-mass spectrometry (positive ion mode, Quadrupole ToF Tandem MS equipped with a MALDI source), while purity (>85%) was verified by analytical RP-HPLC.

Table S1. MALDI-MS of modified oligonucleotides used in this study.[a]

ON	Sequence	Observed *m/z* $[M+H]^+$	Calculated *m/z* $[M+H]^+$
CSU	5'-**U**A**U**GCCATT**U**GAAA	4910	4908
CSL	3'-b-A**U**A**C**GGTAAA**C**TTT	5508	5506
CCU	5'-AGC**U**A**U**AAGAG**U**TC	4934	4933
CCL	3'-TCGA**U**A**U**TCTCA**A**G-C_6NH_2	5080	5077
ESU	5'-AA**C**AGTTC**U**AT**C**AG	4921	4921
ESL	3'-b-TTG**U**CAAGA**U**AG**U**C	5494	5494
ECU	5'-G**C**A**U**GGCTCT**U**GAT	4930	4930
ECL	3'-CG**U**A**C**CGAGAA**C**TA-C_6NH_2	5110	5110
SSU	5'-**C**ACGTTCGGG**C**A**A**T	4953	4951
SSL	3'-b-G**U**GCAAGCCCG**U**T**A**	5495	5493
SCU	5'-TCGT**U**AT**U**GGC**G**AT	4945	4945
SCL	3'-AGCAA**U**AA**C**CGC**U**A-C_6NH_2	5080	5080

[a] **A**, **C**, **G** and **U** denote 2'-*O*-(pyren-1-yl)methyl-adenosine/cytidine/guanosine/uridine, respectively. "b" and "C_6NH_2" denotes 3'-biotin TEG and 5'-amino-modifier C6 units, respectively.

Protocol – sandwich assay. Pre-annealed Invader capture probes (100 μL/well, 100 nM in 1x PBS; 1x PBS stock solution: 0.29g of Na_2HPO_4, 0.06g $NaH_2PO_4 \cdot H_2O$, 1.57g NaCl and 0.08g KCl in 200 mL nanopure water, pH 7.4 adjusted with 1.0M HCl and 1.0M NaOH) were added to clear amine-reactive 96-well plates (Costar DNA-Bind, Fisher Scientific, #07-200-586) and incubated for ~18h at rt. Following removal of the supernatant, plates were blocked with bovine serum albumin (100 μL/well, 1% m/v BSA in 0.1x PBS) for 1.5h at rt to reduce non-specific binding, and then rinsed three times with a wash solution (0.1% Tween 20 in 0.05x PBS, 2 min/rinse). Separately pre-annealed Invader signaling probes (50 μL/well, 100 nM in 1x PBS) and dsDNA targets (50 μL/well, variable concentration in 1x PBS) were added and incubated for 1h at 37 °C. In some control experiments, either the capture or signaling probes were absent as specified. After removing the supernatant and rinsing the plates four times with the wash

solution (2 min/rinse), plates were treated with ELISA-grade streptavidin-horseradish peroxidase conjugate (100 μL/well of 0.033% solution in 0.1x PBS, Life Technologies, cat# SNN4004; 3:1 HRP:SA by weight) for 30 min at rt. The supernatant was removed, the plates were rinsed five times with the wash solution (2 min/rinse), and a solution of QuantaBlu was added (100 μL/well, prepared as recommended by the manufacturer; Thermo Scientific, cat#:15169). After incubating for 30 min at rt, QuantaBlu stop solution (50 μL/well) was added, and the supernatant was transferred to a black 96-well reading plate (Costar, Fisher Scientific, #07-200-590). The generated signal was quantified using a fluorescence plate reader (BioTek FLx800, excitation 340±30 nm, emission 420±40 nm). Six wells were used per data point. Signal intensities were corrected by subtracting the emission from completely untreated wells (i.e., empty wells). No outlier treatment was performed. Averaged measurements were normalized relative to background wells (i.e., wells that were not exposed to capture/signal/targets, but which otherwise underwent full treatment including BSA blocking) to give "fold signal above background"-values.

Although reasonable intra-plate consistency was observed, we did observe considerable plate-to-plate variability in "fold signal above background"-values for identical samples (note variation in S/N-values for 1 nM target in Figs. 2 and S1). This is attributed to considerable plate-to-plate variability in signal readings for background wells, which likely reflects insufficient blocking.

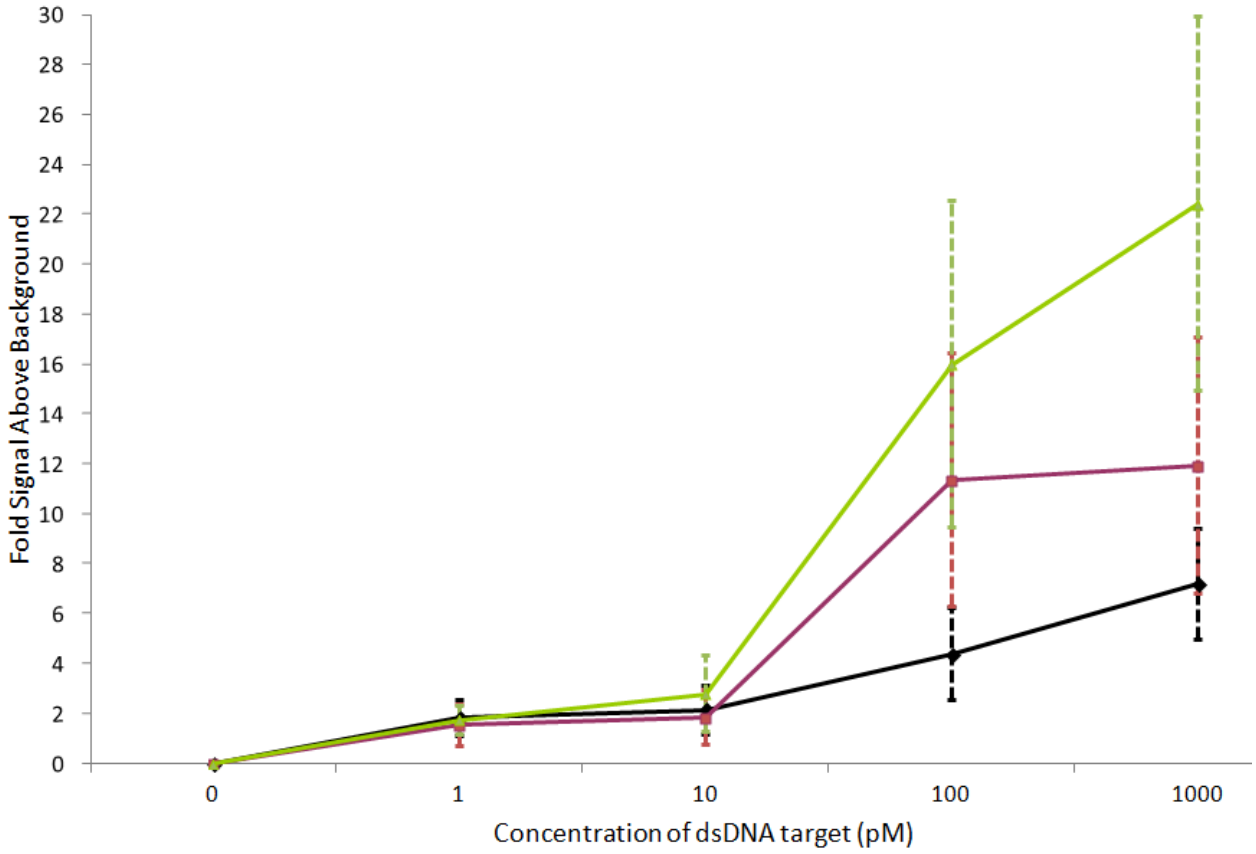

Figure S1. Detection of mixed-sequence dsDNA targets specific to *Salmonella enterica* (green), *E. coli* O157:H7 (red) and *Campylobacter jejuni* (black), using the Invader-based sandwich assay. Bars denote standard deviation.

The reasons for the different dose-response profiles of the three sets of Invader capture/signalling probes are not fully understood. One potential explanation is that they are the result of probe-specific effects, e.g., an influence of probe-target thermostability on recognition efficiency (Table 2). Alternatively, it may be a consequence of the plate-to-plate variability in signal that is observed for the background wells (see discussion in "Protocol – sandwich assay" section).

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References

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