Supplementary Information

Isothermal detection of RNA with restriction endonucleases

Lei Yan, Shizuka Nakayama, Saron Yitbarek, Isabel Greenfield and Herman O. Sintim*

Table S1. DNA sequences

Template	
T1	5'AGAGTCCACCAAGCTTCAGATTAG3'
T2	5'AGAGTCCACCAAGCTCTAGATTAG3'
T3	5'CAGGCAAGAGTCCACCAAGCTCTAGATTAG3'
mir16	5'UAGCAGCACGUAAAUAUUGGCG3'
Targeted sequence on *E.coli* K12 MG1655 ribosome RNA	5'...AGUCGACCGCCUGGGGAGUACGGCCGCAA GGUUAAAAC...3' (16S RNA 873~910)
Alzheimer's disease-related gene segment; perfect matching target	5'AGCAGTTGGCTTCGCCCAGGGT**G**CACCAGG ACACGGTT3'
Alzheimer's disease-related gene segment; C mismatch SNP	5'AGCAGTTGGCTTCGCCCAGGGT**C**CACCAGGA CACGGTT 3'
Alzheimer's disease-related gene segment; A mismatch SNP	5'AGCAGTTGGCTTCGCCCAGGGT**A**CACCAGGA CACGGTT 3'
Alzheimer's disease-related gene segment; T mismatch SNP	5'AGCAGTTGGCTTCGCCCAGGGT**T**CACCAGGA CACGGTT 3'
Probe A	
A1	5'TCTGAAGCT$^{FAM[a]}$TTGATC$^{Dabsyl[b]}$3'
A2	5'CTAGAGCT^{FAM}TTGATC^{Dabsyl}3'
A3	5'ATCTGAAGCT^{FAM}TTGATC^{Dabsyl}3'
A4	5'AATCTGAAGCT^{FAM}TTGATC^{Dabsyl}3'
A5	5' TAATCTGAAGCT^{FAM}TTGATC^{Dabsyl}3'
A6	5'CTAATCTGAAGCT^{FAM}TTGATC^{Dabsyl}3'
probe A for mir16	5'CGCCAATATTT^{FAM}TTGATC^{dabsyl}3'
probe A for *E. coli* ribosomal RNA	5'TGCGGCCGT^{FAM}TTGATC^{dabsyl} 3'
probe A for Alzheimer's disease related gene	5' GTGCACCCT^{FAM}TT GATC^{dabsyl} 3'
Probe B	
B1	5'AAAAGATCAAATTGGTGGACTCTAAAA3'
B2	5'AAAA(S)$^{[c]}$GATCAAATTGGTGGACTCTAAAA3'
B3	5'AAAA(dS) $^{[d]}$GATCAAATTGGTGGACTCTAAAA3'
B4	5'AAAA(Ac)$^{[e]}$GATCAAATTGGTGGACTCTAAAA3'
B5	5'AAAA(OMe)$^{[f]}$GATCAAATTGGTGGACTCTAAAA3'
B6	5'TGTAACGGAAAACCGTTACA(S)GATCATATTGGT

	GGACTCT3’
B7	5’TG(S)GATCGGAAAACC(S)GATCCA(S)GATCATAT TGGTGGACTCT3’
B8	5’TG(S)GATCGGCA(S)GATCGCAAAAGC(S)TGCC(S) GATCCA(S)GATCATATTGGTGGACTCT3’
B9	5’CA(S)GATCGCAAAAGC(S)GATCTGTG(S)GATCGG AAAACC(S)GATCCA(S)GATCATATTGGTGGACTCT3 ’
B10	5’CT(S)GATCCGAAAACG(S)GATCAGCA(S)GATCGC AAAAGC(S)GATCTGTG(S)GATCGGAAAACC(S)GAT CCA(S)GATCATATTGGTGGACTCT3’
B11	5’TG(S)GATCGGCT(S)GATCCGAAAACG(S)GATCAG CA(S)GATCGCAAAAGC(S)GATCTGGA(S)GATCGGA AAACC(S)GATCTCCC(S)GATCCA(S)GATCATATTGG TGGACTCT3’
probe B for mir16	5’TG(S)GATCGGAAAACC(S)GATCCA(S)GATCATAT ACGTGCTGCTA3’
probe B for *E. Coli* ribosomal RNA	5’TG(S)GATCGGAAAACC(S)GATCCA(S)GATCATAT ACTCCCCAGGCGGTCGACT3’
B12	5’AAAGATCAAATTGGTGGACTCTAAAA3’
B13	5’AAGATCAAATTGGTGGACTCTAAAA3’
B14	5’AGATCAAATTGGTGGACTCTAAAA3’
B15	5’GATCAAATTGGTGGACTCTAAAA3’
B16	5’GGGG(S)GATCAAATTGGTGGACTCTAAAA3’
B17	5’CCCC(S)GATCAAATTGGTGGACTCTAAAA3’
B18	5’TTTT(S)GATCAAATTGGTGGACTCTAAAA3’
B19	5’TG(S)GATCGGAAAACC(S)GATCCA(S)GATCATAT TGGTGGACTCTTGCCTG3’
B20	5’TG(S)GATCGGAAAACC(S)GATCCA(S)GATCATAT TGGTGGACTCTTGC3’
B21	5’TG(S)GATCGGAAAACC(S)GATCCA(S)GATCATAT TGGTGGA3’
probe B for Alzheimer’s disease related gene	5’TG(S)GATCGGAAAACC(S)GATCCA(S)GATCATAT GGGCGAAGCCA3’

[a]. T^{FAM} = Fluorescein-conjugated dT. [b]. ^{Dabsyl} = Dabsyl group conjugated at 3’ end. [c]. (S) = phosphorothioate linkage. [d]. (dS) = phosphorodithioate linkage. [e] (Ac) = phosphoroacetate linkage. [f]. (OMe) = methyl phosphate linkage.

Table S2. Components of junction probes

	Template	Probe A	Probe B
JP1	T1	A1	B1
JP2	T1	A1	B2
JP3	T1	A1	B3
JP4	T1	A1	B4

JP5	T1	A1	B5
JP6	T1	A1	B6
JP7	T1	A1	B7
JP8	T1	A1	B8
JP9	T1	A1	B9
JP10	T1	A1	B10
JPS1 = JP1	T1	A1	B1
JPS2	T1	A1	B12
JPS3	T1	A1	B13
JPS4	T1	A1	B14
JPS5	T1	A1	B15
JPS6	T1	A1	B16
JPS7	T1	A1	B17
JPS8	T1	A1	B18
JPS9	T1	A2	B7
JPS10	T1	A3	B7
JPS11	T1	A4	B7
JPS12	T1	A5	B7
JPS13	T1	A6	B7
JPS14	T3	A1	B19
JPS15	T3	A1	B20
JPS16	T3	A1	B7
JPS17	T3	A1	B21

Synthesis of oligonucleotides: All natural oligonucleotides were synthesized on 1 μmol scale on a DNA synthesizer (Model 392, Applied Biosystems, Foster City, CA) using standard β-cyanoethylphosphoramidite coupling protocol with DMT-on mode according to the manufacturer's manual. Deprotection and cleavage of the oligonucleotides from the CPG support were carried out by incubating the CPG powders in ammonium hydroxide for 8 h at 55 °C. Synthesis, deprotection and cleavage of fluorophore labeled and modified oligonucleotides were synthesized on 0.2 μmol scale on the same synthesizer using β-cyanoethylphosphoramidite coupling protocol with appropriate modifications according to manufacturer's protocols (Glen research, Sterling, VA). The oligonucleotides were first purified by reverse-phase semi-preparative HPLC (model 210 proster, Microsorb-MW 100-5C_{18} column 250 x 10 mm, Varian, Palo Alto, CA) using solvent A (0.1 M triethylammonium acetate, pH 7.0) and solvent B (acetonitrile) (10% A to 30% A in 20 min, then 30% A to 100% A in 30 min) with a flow rate of 3 mL/min. The retention times of the oligonucleotides were between 19-23 min. The HPLC-purified "DMT-on" oligonucleotides were de-tritylated by using Glen-Pak^{TM} cartridge according to the manufacturer's manual. All the purified oligonucleotides were dried in vacuo by Speedvac (Model SAVANT DNA 120, Thermal Fisher Scientific), and then dissolved in sterile H_2O. All oligonucleotide sequences are listed in Supporting Information Table S1. The oligonucleotides were quantified by UV absorbance. The optical densities were

measured at 260 nm by using a quartz cuvette with a 1 cm path length on a Jasco (Easton, MD) V-630 UV-Vis spectrophotometer. The sample concentration was calculated by using the oligo calculation software at www.idtdna.com.

Bacteria strains and RNA: Materials and reagents were sterilized by autoclaving at 120˚C for 20 min. MicroRNA miR-16 was provided by Azco Biotech (San Diego, CA).

E. coli K12 MG1655 strain bacteria were incubated in Luria–Bertani (LB) medium on a shaker at 37 ˚C until OD_{600} reached 0.77. 30 mL of the mixture was centrifuged at 5,500 g for 15 min at 4 ˚C. The supernatant was discarded. And the pallet was lysed in TE buffer containing lysozyme (10 mM Tris-HCl, pH 7.4; 1 mM EDTA; 0.4 mg/mL lysozyme). Total RNA was extracted from the suspension by using a total RNA extraction kit according to the manufacture's manual. (http://www.promega.com/tbs/tm279/tm279.pdf)

V. harveyi MM32 strain bacteria ware cultured the same way as *E. Coli* K12 MG1655 except in an autoinducer bioassay (AB) medium. AB medium preparation: The basal medium contained 0.3 M NaCl and 0.005 M $MgSO_4$. After adjusting the pH to pH 7.5 with KOH, it was sterilized by autoclaving (121 °C, 20 min). After cooling, the following sterile compounds were added: 1% (v/v) of 1 M potassium phosphate buffer pH 7.0 (K_2HPO_4/KH_2PO_4), 1% (v/v) of a 0.1 ML-arginine solution, 2% (v/v) of a 50% (v/v) glycerol solution, and 2% (v/v) of a 10% (w/v) vitamin-free solution of casamino acids (Difco, Becton Dickinson & Co., Sparks, MD, USA). For solid media, 2% (w/v) agar (Difco) was added prior to autoclaving the basal medium.

Crude lysate sample for detection was obtained by first filtering 1mL of the supernatant of the TE lysate through a 3 kDa filter, and dissolving the biomolecules on the surface of the filter in 200 μL Dnase and Rnase free water.

General fluorescence assay: All structure components are listed in Supporting Information Table S2. Probes and/or template in 60 μL of NE buffer 1X (components differ by enzymes; 10X buffers were supplied with enzymes from New England Biolab® (Ipswich, MA) were mixed at 30 °C, and the fluorescence at time = 0 min (referred to as t0) was recorded. REase was then added to the solution and fluorescence was taken.

Fluorimeter parameters: **Figure 1**: λ_{ex} = 494 nm, λ_{em} = 522 nm, $slit_{ex}$ = $slit_{em}$ = 5 nm, PMT = 600 V. **Figure 2**: λ_{ex} = 494 nm, λ_{em} = 522 nm, $slit_{ex}$ = $slit_{em}$ = 5 nm, PMT = 600 V. **Figure 3(a)**: λ_{ex} = 494 nm, λ_{em} = 522 nm, $slit_{ex}$ = $slit_{em}$ = 5 nm, PMT = 600 V. **Figure 3(b)**: λ_{ex} = 494 nm, λ_{em} = 522 nm, $slit_{ex}$ = $slit_{em}$ = 10 nm, PMT = 600 V.

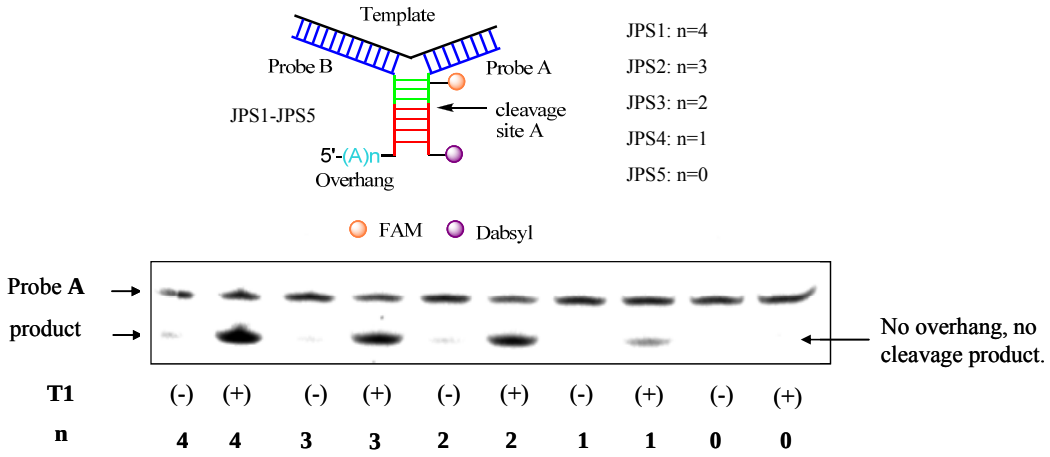

Figure S1. Only JP structures that have 5' overhang tail next to the cognate recognition sequence (colored red) are cleaved by the REase, BfuCI. Note that there is no cleavage product for JPS5 (n = 0).

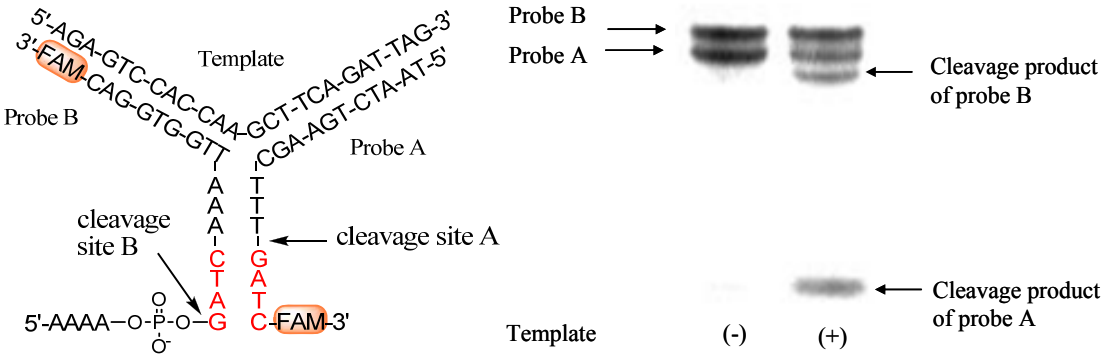

Figure S2. REase BfuCI can cleave both sites A and B, although site B is adjacent to a single strand overhang.

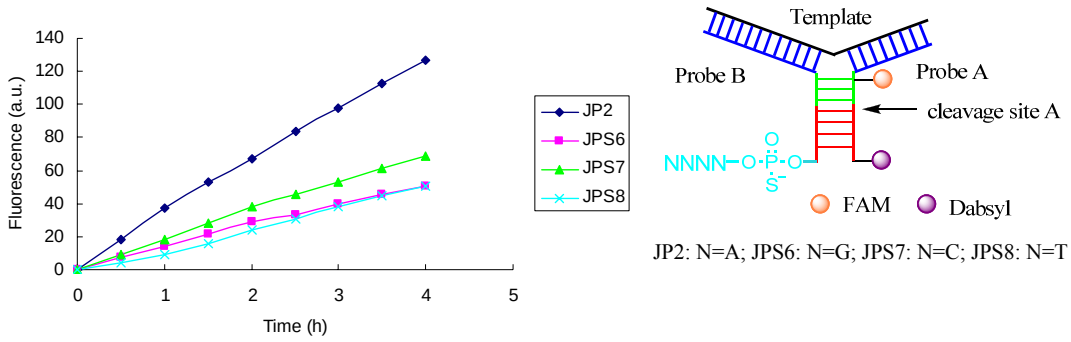

Figure S3. All probes are modified at site B with phosphorothioate. The sequence contents of the probe B 5'-overhangs are however different. [Probes A] = [probe B] = 1 μM, [DNA template] = 0.2 μM, [BfuCI] = 0.033 U/μL. Fluorescence data is normalized. λ_{ex} = 494 nm, λ_{em} = 522 nm, $slit_{ex}$ = $slit_{em}$ = 5 nm, PMT = 600 V.

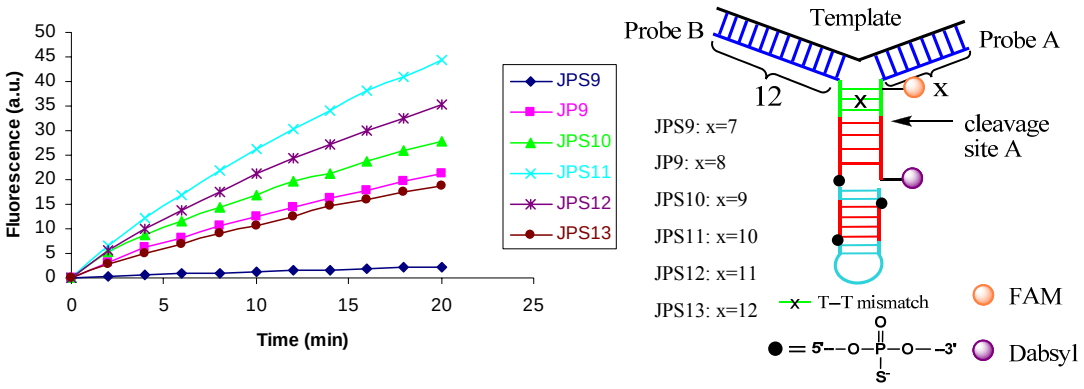

Figure S4. The base-pairing numbers between template and probes A (designated as x) influence the cleavage of JP tripartite by BfuCI. [Probes A] = [probe B] = 200 nM, [DNA template] = 1 nM, [BfuCI] = 0.1 U/μL, assay temperature = 35 ºC. λ_{ex} = 494 nm, λ_{em} = 522 nm, $slit_{ex}$ = $slit_{em}$ = 10 nm, PMT = 600 V.

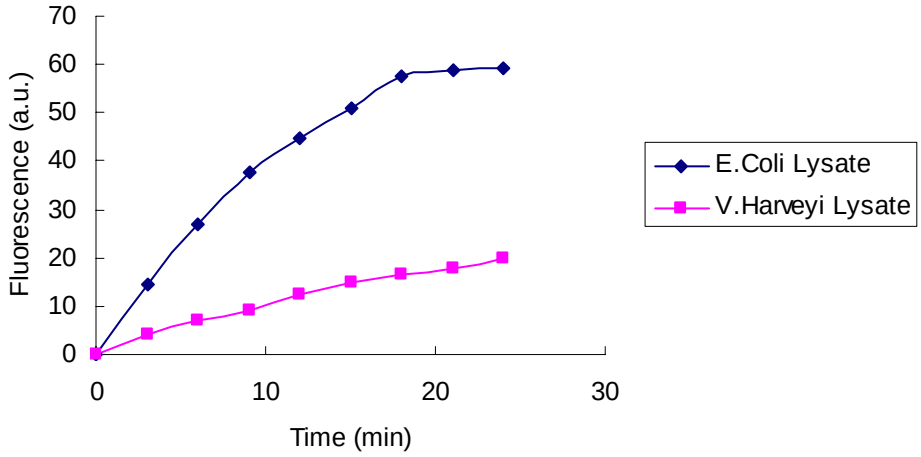

Figure S5. Ribosomal RNA in crude *E. coli* lysate was detected without separating out other macromolecules such the protein, RNA and carbohydrate debri. The cfu of *E. coli* and *V. harveyi* were similar. Of note, the crude lysate should not contain EDTA as Mg^{2+} is critical for the REase. Fluorescence data is normalized (λ_{ex} = 494 nm, λ_{em} = 522 nm, $slit_{ex}$ = $slit_{em}$ = 10 nm, PMT = 600 V). Concentrations of probes A and B = 200 nM, assay temperature = 30 ºC.

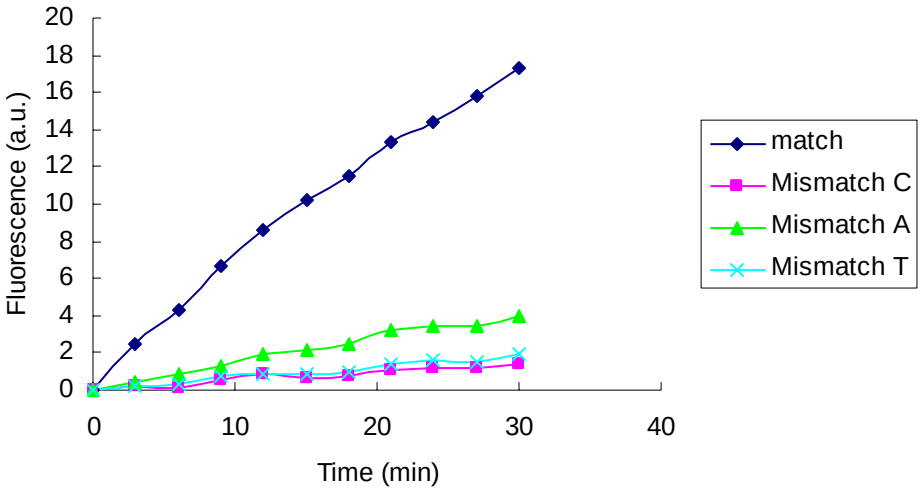

Figure S6. Detection of gene related to Alzheimer disease. Alleles of several SNPs of the gene encoding microtubule associated protein tau show association with increased cerebrospinal fluid levels of tau/ptau and might be linked to Alzheimer's disease.[1] These alleles can be detected by the JP platform. [Probes A] = [probe B] = 50 nM, [template] = 10 nM. λ_{ex} = 494 nm, λ_{em} = 522 nm, $slit_{ex}$ = $slit_{em}$ = 10 nm, PMT = 600 V.

Reference:

1. J. S. K. Kauwe, C. Cruchaga, K. Mayo, C. Fenoglio, S. Bertelsen, P. Nowotny, D. Galimberti, E. Scarpini, J. C. Morris, A. M. Fagan, D. M. Holtzman, A. M. Goate, *Proc. Natl. Acad. Sci. U. S. A.* 2008, **105**, 8050-8054.