Supplementary Table 1. Examples of DNA polymerase and nicking enzyme-involved sensors for biomolecule detection[a]

No. of example (Ref, Fig.)	Detection process[b]	Enzyme used	Role of enzyme	Features of strategy	Target	Sensing approach (The type of biosensor)	LOD	Linear range
1 (39; Figure 2a)	1. Use of a double-hairpin molecular beacon as probe DNA 2. Uncoiled one stem of beacon upon exposure of target 3. Binding of trigger DNA to an uncoiled region and bidirectional polymerization by KFP, producing an extended strand containing G-rich and Nt.BbvCl-specific regions 4. Nicking site recognition on the extended strand and nick-site cleavage by Nt.BbvCl 5. Binding of KFP to a nicking site and G-rich region in signal DNA released by SDA 6. Colorimetric detection	• Klenow fragment polymerase (3′-5′ exo-) • Nt.BbvCl endonuclease	• SDA which peels off target from the probe, leading to TRR • SDA which peels off G-rich region from the extended region, leading to signal probe amplification • Generation of a nicking site where KFP binds	• Enhanced LOD by TRR (steps 1-3) and signal DNA amplification (steps 4,5) • Use of probe DNA containing a target recognition domain, an Nt.BbvCI recognition sequence, sequences complementary to trigger DNA, and C-rich sequences which can fold into G-quadruplex structures responsible for the formation of HRP-mimicking DNAzyme	K-Ras gene mutation	Colorimetric assay, use of hemin/G-quadruplex HRP-mimicking DNAzyme (absorbance)	4 pM	15 pM to 370 pM
2 (35; Figure 2b)	1. Use of a hairpin molecular beacon as probe DNA 2. Uncoiled stem of beacon upon target exposure 3. Binding of trigger DNA to an uncoiled region and bidirectional polymerization by KFP, producing an extended strand containing a Nt.BbvCl-specific region and a primer region for RCA 4. Recognition of a nicking site on an extended strand and cleavage by Nt.BbvCl 5. Binding of KFP on a nicking site and primer region in signal DNA released by SDA 6. RCA with signal DNA (primer) and padlock DNA (template) for exponential amplification of RCA product with repeated	• Klenow fragment polymerase (3′-5′ exo-) • Nt.BbvCl endonuclease	• Nick-mediated SDA for peeling off target and signal DNA • Generation of a nicking site	• Enhanced LOD by three different cycling reactions: TRR (steps 1-3), signal DNA amplification (steps 4-6), and RCA (steps 6,7) • Use of padlock DNA-containing sequences complementary to signal DNA and the extended strand originated from step 3	p53 gene mutation	Fluorescence-based assay, use of quencher (fluorescent)	1 pM	100 pM to 40 nM

	Nt.BbvCl-specific and primer regions to be recycled							
3 (36; Figure 2c)	1. Use of linear DNA as probe DNA 2. Binding of a target to probe DNA, producing an extended strand containing an Nt.BstNBI-recognition site and trigger DNA 3. Recognition of nicking site on an extended strand and Nt.BstNBI-mediated cleavage 4. Binding of phi29 to a nicking site and trigger DNA amplification by SDA 5. Binding of amplified trigger DNA to signal DNA, forming DNAzyme in the presence of Mg^{2+} and digesting signal DNA to two fragments (biotin-modified and dye-labeled fragments) 6. Separation of biotin-modified fragments by streptavidin-coated magnetic bead and fluorescence measurement of dye-labeled fragment	• Phi29 polymerase (3′-5′ exo+) • Nt.BstNBI	• Nicking-mediated polymerization • Generation of a nicking site on which phi29 polymerase binds	• Enhanced LOD by trigger amplification reaction via SDA (steps 1-4) and signal DNA amplification via DNAzyme cleavage reaction (steps 5,6) • Composition of probe DNA:target-recognition domain and a nicking domain for Nb.BbvCI recognition. • Use of signal DNA containing biotin and AMCA dye at each terminus • Separation time (step 6): 45 min	miRNA	Fluorescence-based assay, use of AMCA dye (fluorescent)	0.27 fM	1 fM to 50 pM

4 (31; Figure 2d)	1. Use of hairpin molecular beacon as probe DNA 2. Uncoiled stem of beacon upon exposure of target 3. Binding of trigger DNA to an uncoiled region and polymerization by phi29, producing an extended strand containing an Nt.BbvCl-specific region and signal DNA 4. Recognition of nicking site on an extended strand and cleavage of this site by Nt.BbvCl 5. Binding of phi29 to the nicking site and primer region in signal DNA and release by SDA 6. TMSD reaction using short DNA fragments instead of enzyme	• Phi29 polymerase (3′-5′ exo+) • Nt.BbvCl endonuclease	• Nicking-mediated extension, leading to peel off a DNA fragment which triggers TMSD • Generation of a nicking site where phi29 polymerase binds	• Use of TMSD causes rapid amplification efficiency, flexible design, excellent thermostability, and robust resistance to complex environment. • Use of probe DNA containing target-recognition domains, an amplification domain to produce triggers, and a nicking domain for Nb.BbvCI recognition. • Assay time: 30 min	miRNA	Fluorescence-based assay, use of quencher, (fluorescent)	0.18 fM	1
5 (32)	1. Use of linear DNA as probe DNA 2. Binding of target to probe DNA, producing an extended strand containing an Nt.BbvCl-specific region and trigger DNA 3. Recognition of nicking site on an extended strand and cleavage by Nt.BbvCl 4. Binding of a Vent (exo-) to a nicking site and primer region in trigger DNA release by SDA 5. Consecutive binding of trigger DNA to two different hairpin DNAs (signal DNA) labeled with a fluorescence dye and quencher, leading to fluorescence emission and release of trigger DNAs to be recycled	• Vent (exo-) polymerase • Nt.BbvCl endonuclease	• Nicking-mediated polymerization • Generation of a nicking site on which Ven polymerase binds	• Use of probe DNA containing a nicking site for Nb.BbvCI recognition and an amplification domain to produce trigger DNA • Use of two hairpin DNA as signal DNA • Assay time: > 1 h	miRNA	Fluorescence-based assay, use of quencher (fluorescent)	3 fM	10 fM to 100 pM

6 (69)	1. Use of linear DNA as probe DNA 2. Binding of target to probe DNA, thereby producing an extended strand containing an Nt.BstmAI-specific region and trigger DNA 3. Recognition of nicking site on an extended strand and cleavage by Nt.BSmAI 4. Binding of phi29 to the nicking site and trigger DNA amplification by SDA 5. Binding of amplified trigger DNA to hairpin DNA (signal DNA) immobilized on a GO electrode and consecutive binding of an invading stacking primer to the stem region of hairpin DNA, generating an untied stem and an ECL signal-on of a Ru complex/TPrA 6. SDA of bound DNA fragment and release of trigger DNA to be recycled	• Phi29 polymerase (3′-5′ exo+), high fidelity • Nt.BstmAI	• Nicking-mediated polymerization • Invading primer-mediated polymerization • Generation of a nicking site on which phi29 polymerase binds	• Enhanced LOD by trigger DNA amplification reaction (steps 1-4) and signal DNA amplification reaction (steps 5-6) via SDA • Use of signal on-off mode depending on the distance from the Ru complex and the electrode surface because GO acts as a quencher of the Ru complex	miRNA	ECL (chemiluminescence)	0.14 fM	0.5 to 1 pM
7 (112)	1. Use of a hairpin molecular beacon as probe DNA 2. Untied stem of beacon upon exposure to target 3. Binding of trigger DNA to an untied region and polymerization by KFP, peeling off target by RCA and producing an extended strand containing a KpnI-specific region 4. Recognition of a specific sequence site on an extended strand by KpnI and cleavage into two fragments 5. Reuse of separated quencher-attached DNA by binding with probe DNA and fluorescence measurement of dye-labeled fragment	• Klenow fragment polymerase (3′-5′ exo-) • Restriction endonuclease Kpn I	• The trigger DNA-mediated SDA, generating ds DNA and displacing target • Cleavage of ds DNA to two dsDNA fragments with cohesive ends	• Enhanced LOD by TRR via SDA (steps 1-3) and signal DNA amplification via a KpnI-based dsDNA cleavage reaction (steps 4,5) • Polymerization time: 1 h • Restriction enzyme cleavage time: 3 h	p53 gene	Fluorescence-based assay, use of quencher (fluorescent)	0.1 pM	0.1 pM to 200 nM

8 (52; Figure 2e)	1. Use of a hairpin molecular beacon as probe DNA labeled with dye and quencher at ends. Binding of target to the hairpin beacon, opening the large hairpin structure and forming a small hairpin. 2. ICSDP by KFP, displacing the target to be recycled 3. Recognition of nicking site on an extended strand and cleavage by Nt.BbvCI. Binding of KFP on a nicking site and trigger DNA amplification by reverse SDA 4. Binding of amplified trigger DNA to hairpin DNA (probe DNA), opening the large hairpin structure 5. Producing an extended strand by KFP and emitting a fluorescence	• Klenow fragment polymerase (3′-5′ exo-) • Nt.BbvCI	• ICSDP • Nicking-mediated reverse SDA • Generation of a nicking site on which KFP binds	• Enhanced LOD by target recycling reaction via ICSDP (steps 1,2) and trigger DNA amplification via reverse SDR (step 3) • Rapid read-out by simultaneous binding of a hairpin beacon to target and amplified trigger DNA • Use of only one probe DNA in one reaction without the addition of any DNA • High signal-to-noise ratio because of self-assembly of the hairpin beacon • Assay time: > 30 min	p53 gene	Fluorescence-based assay, use of quencher (fluorescent)	1 nM	1 to 100 nM
9 (37)	1. Use of a magnetic bead-attached hairpin molecular beacon as probe DNA 2. Binding of target to the hairpin beacon, opening the hairpin structure 3. Binding an HRP-tagged trigger DNA to the untied stem 4. Production of the extended dsDNA strand KFP-based SDA and the release of target to be recycled 5. Recognition of nicking site on an extended strand and cleavage by Nt.BbvCI 6. Binding of KFP to a nicking site and target amplification by SDA 7. Magnetic separation of the extended strand 8. Release of the HRP-tagged strand from extended dsDNA strand by binding a signal DNA to the extended strand 9. CL measurement by mixing of HRP-attached strand with luminol/H_2O_2+PIP	• Klenow fragment polymerase (3′-5′ exo-) • Nb.BbvCI	• Nicking-mediated reverse SDA • Generation of a KFP-binding nicking site	• Enhanced LOD by TRR via trigger-induced SDA (steps 1-4) and target amplification by nicking-mediated SDA (steps 5,6) • Enabling multiplexed detection • Assay time: > 2 h	miRNA	CL, use of HRP/luminol/H_2O_2+PIP (chemiluminescence)	5.8 fM-8.3 fM	10 fM to 100 pM

[a]Abbreviations: LOD, limit of detection; KFP, Klenow fragment polymerase; SDA, strand displacement amplification; TRR: target recycling reaction; TMSD, toehold-mediated strand displacement; RCA, rolling circle amplification; HRP, horseradish peroxidase; miRNA, microRNA; AMCA, aminomethylcoumarin acetate; GO, graphene oxide; ECL,

electrochemiluminescence; TPrA, tripropylamine; ICSDP, isothermal circular strand-displacement polymerization; PIP, *p*-iodophenol; ; CL, chemiluminescence

[b] Number indicates the order of procedure for the detection of biomolecule.

Ref 112: Xu H, Jiang Y, Liu D, et al. Twin target self-amplification-based DNA machine for highly sensitive detection of cancer-related gene. Anal Chim Acta. 2018; 1011:86-93.

Supplementary Table 2. Examples of DNA exonuclease-involved sensors for biomolecule detection[a]

No. of example (Ref, Fig.)	Detection process[b]	Enzyme used	Role of enzyme	Features of strategy	Target	Sensing approach (The type of biosensor)	LOD	Linear range
1 (34; Figure 3b)	1. Use of a hairpin molecular beacon as probe DNA 2. Binding of target to hairpin beacon, creating a 5′-phosphorylated hairpin structure and digestion by λ exonuclease to generate trigger DNA 3. Binding of trigger DNA to the P1 strand of the P1:P2 hybrid and subsequent binding of additional NMM to P2 strand 4. Recognition of nicking site on a P1:trigger DNA hybrid and cleavage by Nt.BbvCI, generating trigger DNA to be recycled	• λ exonuclease • Nt.BbvCl	• Recognition of 3′ phosphate of dsDNA and cleavage of dsDNA in the direction of 5′ to 3′ to generate trigger DNA • Generation of nicking site	• Use of SDA strategy using different binding affinities of DNA fragment among P1, P2 and trigger DNA	T4 PNK activity	Fluorescence-based assay, use of NMM (fluorescent)	6.6×10^{-4} U/ml	0.005 to 5 U/ml
2 (33; Figure 3c)	1. Use of linear, 5′-phosphorylated antisense strand-protruding dsDNA as probe DNA 2. Binding of target to dsDNA, creating dsDNA with a 5′-phosphorylated sense strand, and cleavage of the sense strand by λ exonuclease 3. Formation of a hairpin structure in the remaining antisense strand and extension of strand by KFP 4. Recognition of the nicking site on the extended dsDNA and cleavage by Nt.BbvCI, leading to amplification of trigger DNA 5. Binding of trigger DNA to additional ssDNA and trigger DNA-mediated SDA by Nt.BbvCI and KFP	• λ exonuclease • Nt.BbvCI • Klenow fragment polymerase (3′-5′ exo-)	• Recognition of 3′ phosphate of dsDNA and cleavage of dsDNA from 5′ to 3′ to generate trigger DNA • Generation of nicking site • Nicking-mediated polymerization	• Enhanced LOD by two-step trigger DNA amplification by nicking-mediated SDA (step 3-4 and step 5)	T4 PNK activity	Fluorescence-based assay, use of SYBR green I (fluorescent)	2×10^{-4} U/ml	0.001 to 1 U/ml

3 (18)	1. Use of a hairpin molecular beacon incorporated with ATMND as probe DNA 2. Binding of target to overhang at the 3′-terminus of the hairpin beacon, creating a ds region with blunt end at its 3′-terminus to be cleaved by exonuclease III, releasing ATMND and emitting fluorescence	• Exonuclease III	• Target recycling	• Use of specific bonding and the fluorescence quenching property of ATMND to the C-C mismatch contained DNA duplex • Simple process requiring only one-step mixing without the addition of any exogenous reagents. • Assay time: > 45 min	Mismatched DNA	Fluorescence-based assay, the use of ATMND (fluorescent)	6 pM	10 pM to 1 μM
4 (22)	1. Use of a hairpin molecular beacon as probe DNA 2. Binding of target to hairpin (HP1), creating a G-quadruplex structure 3. Binding of the additional hairpin (HP2) to the G-quadruplex structure, releasing target 4. Binding of additional hairpin beacon 3 to HP1:HP2 duplex, generating a nicked dsDNA 5. Recognition of nicking site on a dsDNA and cleavage by exonuclease III, leading an amplification of the HP1:HP2 duplex to be recycled and release of the signal probe 6. Colorimetric detection using hemin/G-quadruplex HRP-mimicking DNAzyme	• Exonuclease III	• Target recycling	• Enhanced LOD by TRR (step 1-3) and trigger DNA/signal DNA amplification by exonuclease III-assisted cleavage (step 4,5) • Assay time: 90 min	Human α-thrombin	CL (chemiluminescence)	0.92 pM	1 pM to 1 nM
5 (20; Figure 4a)	1. Use of stem-loop structure with 5′-protruding termini as probe DNA 2. Binding of target to probe DNA, exposing a G-quadruplex for hemin attachment and changing a 3′-protruding terminus, which is resistant to exonuclease III digestion. In the absence of target, no conformational change in probe DNA and cleavage by exonuclease III 3. Binding of hemin to target:probe DNA duplex, which is responsible for the formation of HRP-mimicking DNAzyme	• Exonuclease III	• Removal of background signal	• High signal-to-noise ratio	p53 gene	Colorimetric assay, use of hemin/G-quadruplex HRP-mimicking DNAzyme (absorbance)	1 pM	1.25 pM to 125 nM

6 (21; Figure 4b)	1. Use of two different hairpins (HP1 and HP2) as probe DNA 2. Binding of target to HP1, opening the hairpin structure and sequential binding of HP2 to HP1:target duplex with 3′-protruding ends, which is resistant to exonuclease III digestion. In the absence of target, cleavage of HP1 and HP2 by exonuclease III 3. Toehold-mediated SDR, releasing a target to be recycled 4. Fluorescence measurement by adding SYBR green I dye	• Exonuclease III	• Removal of background signal	• Enhanced LOD by TRR and toehold-mediated SDR (step 4,5) • High signal-to-noise ratio • HP1/HP2 incubation time: 10 min • Enzyme treatment time: 25 min • Dyeing time: 10 min	p53 gene	Fluorescence-based assay, use of SYBR green I (fluorescent)	5.34 pM	0.01 to 1 nM
7 (24)	1. Use of a hairpin (HP1) with a 3′-protruding terminus as probe DNA, which is resistant to exonuclease III digestion 2. Binding of target to HP1, changing 3′-blunted end of HP1, which is digested by exonuclease III, resulting in a trigger DNA amplification and target recycling 3. Binding of the amplified trigger DNA to another hairpin (H2, signal DNA) immobilized on the electrode, opening the loop structure and producing blunt-end H2, which is digested by exonuclease III, resulting in trigger DNA recycling and signal DNA amplification 4. Liberation of quadruplex-forming signal DNA on the electrode and generation of G-quadruplex-hemin complexes with the help of K^+ and hemin to give an electrochemical response due to the oxidation of Fe(II) in hemin.	• Exonuclease III	• Target recycling • Trigger DNA recycling • Signal DNA amplification	• Label-free detection	HIV gene	Electrochemical assay (potentiometric)	4.83 fM	0.01 pM to 10 pM

8 (25; Figure 4d)	1. Use of stem-loop structures immobilized on the AuNCs@GNRs of GCE substrates as probe DNA 2. TMSD and binding of target to probe DNA, opening the loop structure 3. Target-triggered exonuclease III digestion, resulting in dissociation of target DNA from probe:target DNA duplex 4. Transport of target DNA from one location to another on the electrode surface driven by exonuclease III, giving rise to TRR 5. Addition of ferrocene-labeled signal DNA and electrochemical detection of the change of oxidation current response of ferrocene	• Exonuclease III	• Target recycling • DNA walking	• Use of DNA walking machine • Use of electrode with porous structure and large surface area • High signal-to-noise ratio	Mismatched DNA	Electrochemical assay (potentiometric)	0.6 fM	1 fM to 100 pM
9 (43)	1. Use of DNA duplex (DNA1:DNA2) with different lengths as probe DNA 2. Binding of target to ssDNA region of the DNA1:DNA2 duplex 3. Target-mediated SDA by KFP and Nt.BbvCI, release, and amplification of DNA2 4. Binding of the amplified trigger DNA on a hairpin (DNA3) and trigger DNA-mediated exonuclease III digestion of DNA3 and the release of the trigger DNA for recycling 5. Recognition of nicking site on an extended strand and cleavage of this site by Nt.BbvCl 6. Change in G-quadruplex structure of amplified trigger DNA and binding NMN or hemin to its structure to emit fluorescence and colorimetric signal	• Klenow fragment polymerase (3′-5′ exo-) • Nt.BbvCI • Exonuclease III	• Target-mediated SDA for a trigger DNA release • Nick-mediated SDA for a trigger DNA amplification • Generation of a nicking site • Trigger DNA amplification • Signal DNA amplification	• Enhanced LOD by trigger DNA amplification reaction by target-mediated SDA (step 1-3) and trigger DNA/signal DNA amplification by exonuclease III-mediated cleavage (step 4,5) • High signal-to-noise ratio • Assay time: 4 h	HIV and HCV DNA	• Fluorescence-based assay (fluorescent) • Colorimetric assay (absorbance)	10 fM 0.5 pM	10 fM to 5 pM 0.5 pM to 1 nM

[a] Abbreviations: LOD, limit of detection; NMM, N-methyl mesoporphyrin IX; SDA, strand displacement amplification; PNK, polynucleotide kinase; KFP, Klenow fragment polymerase; ATMND, 2-amino-5,6,7-trimethyl-1,8-naphthyridine; TRR: target recycling reaction; HRP, horseradish peroxidase; CL, chemiluminescence; HIV, human immunodeficiency virus; AuNCs, gold nanocages; GNRs; graphene nanoribbons; GCE, glassy carbon electrode; HCV, hepatitis C virus; NAD, nicotinamide adenosine dinucleotide; aTF, allosteric transcription factor; ThT, thioflavin T; 4-HBA, 4-hydroxybenzoic acid.

[b] Number indicates the order of procedure for the detection of biomolecule.

Supplementary Table 3. Examples of DNA ligase-involved sensors for biomolecule detection[a]

No. of example (Ref, Fig.)	Detection process[b]	Enzyme used	Role of enzyme	Features of strategy	Target	Sensing approach (The type of biosensor)	LOD	Linear range
1 (19; Figure 5a)	1. Use of dumbbell hairpin with two stem-loop structures and nicking sites as probe DNA 2. In the presence of NAD^+(cofactor), NAD^+ binding DNA ligase induces a removal of the nick in the dumbbell hairpin, which is resistant to both exonuclease I and III digestion. In the absence of NAD^+, nick remains, which is digested by exonucleases I and III. 3. Integration of SYBR green I dye into the stem of a ligated dumbbell hairpin, emits a fluorescence signal	• DNA ligase • Exonuclease I • Exonuclease III	• Binding of NAD+ • Nick filling • Digestion of probe DNA with nick site (5′-3′) • Digestion of probe DNA with nick site (3′-5′)	• Use of ligase for the detection of NAD^+, a cofactor of enzymatic activity	NAD^+	Fluorescence-based assay, use of SYBR green 1 (fluorescent)	0.1 nM	0.5 to 1500 nM
2 (58; Figure 5b)	1. Use of short capture probe (CP):long signal probe (SP) labeled with methylene blue dye duplex immobilized on an electrode. 2. In the presence of Ag^+, the conformational change of SP into a folded structure via cytosine-Ag^+-cytosine interactions and the ligation of a nick in folded SP by T4 DNA ligase. In the absence of Ag^+, no change in duplex. 3. Denaturation and removal of unligated SP and annealing for reconstruction of remaining probe	• T4 DNA ligase • Nt.CviPII	• Ligation of a nick into Ag^+-triggered folded DNA. • Formation of a nick at a ligated site in CP:SP hairpin duplex	• Enables the reuse of electrode by conformational change of a ligated CP:SP hairpin via endonuclease-assisted nick formation at a ligated site	Ag^+	Electrochemical assay, use of methylene blue (potentiometric)	0.1 nM	0.5 to 100 nM

3 (59; Figure 5c)	1. Use of linear DNA as a probe 2. In the presence of target DNA, probe and target DNA hybridize, with probe existing in a opened circular form. In the absence of target, no change of probe DNA 2. Change of linear probe to closed circular probe by T4 DNA ligase 3. Newly added primer DNA-triggered RCA by DNA polymerase and target DNA release and recycling 4. Filling the gap of amplified DNAs between the two electrodes	• T4 DNA ligase • Phi29 DNA polymerase	• Ligation of a nicked circular probe, which initiates an RCA reaction • Primer DNA-triggered RCA	• Addition of primer to facilitate the RCA reaction	Pathogen DNA	• Electrical assay, addition of $HAuCl_4$) (Conductometric) • Colorimetric assay (absorbance)	• 0.5 nM • 1 μM	-
4 (38)	1. Use of nicked dsDNA as starting material, which binds to the DNA binding domain of aTF 2. In the presence of target, an effector-binding domain of aTF binds to the target and is then dissociated from nicked dsDNA. In the absence of target, aTF still binds to nicked dsDNA 3. The nick in liberated dsDNA is filled by T4 DNA ligase. DNA occupied by aTF cannot repair its nick 4. Incorporation of RCA and nicking enzyme-triggered DNA amplification	• T4 DNA ligase • Nb.BbvCl	• Circularization of nicked dsDNA • Cutting amplified specific sequences to generate G-quadruplexes and additional primers	• Enhanced LOD by T4 DNA ligase–mediated circularization and phi29 DNA polymerase–mediated RCA reaction	4-HBA	Fluorescence-based assay, use of peroxidase-mimicking DNAzyme or G-quadruplex-bound ThT dye (fluorescent)	3.48 nM or 1.73 nM	10-100 nM or 5 to 200 nM

[a] Abbreviations: LOD, limit of detection; NAD, nicotinamide adenosine dinucleotide; RCA, rolling circle amplification; aTF, allosteric transcription factor; ThT, thioflavin T; 4-HBA, 4-hydroxybenzoic acid.

[b] Number indicates the order of procedure for the detection of biomolecule.