Hairpin self-assembly powered by exonuclease III for highly sensitive and cross-validated miRNA-155 detection

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Materials and reagents

NEB (New England Biolabs, Beijing, China) provided the Exo III and 10×NEB buffer1 (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.4 at 25℃) utilized in the studies. Sangon Bioengineering Co., Ltd. produced all the sequences in the experiment, which were made into 10 mM storage solutions using 1×TE buffer (1 mM Tris, 1 mM EDTA, pH 8.0 at 25°C). RNA sequences were stored in DEPC-treated water. Human serum was acquired from Beijing Zhongke Chenyu Biotechnology Co., LTD. The supplier of SYBR Green II was Shanghai Acmec Biochemical Co., LTD. The solvent for the reagents used in this experiment was Milli-Q water (resistance value >18.25 MQ cm). An extra 0.4U/mL RNase inhibitor and RNase-free water were added to exonuclease III diluent. The nucleic acid sequence information used in this experimental design is shown in Table S1.

Table S1 Nucleic acid sequences used in experiments

Name	Sequence (5'-3')
miRNA-155	UUA AUG CUA AUC GUG AUA GGG GU
Hp	CTA ATC GTG ATA GGA ACG CCA TAC CTA TCA CGA TTA GCA TTA A
H1 (FAM modification)	BHQ-GTG ATA GGA ACG CCT GTA CAC TAA TCG GCG TTC CTA TCA CGA TTA G -FAM
H1	GTG ATA GGA ACG CCT GTA CAC TAA TCG GCG TTC CTA TCA CGA TTA G
H2 (TAMRA modification)	TAMRA- CTG TAC ACT AAT CGT GAT AGG AAC GCC GAT TAG TGT ACA GGC GTT C-BHQ
H2	CTG TAC ACT AAT CGT GAT AGG AAC GCC GAT TAG TGT ACA GGC GTT C
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A

miRNA-198	GGU CCA GAG GGG AGA UAG GUU C
Let-7a	UGA GGU AGU AGG UUG UAU AGU U
miRNA-155-1	UAA AUG CUA AUC GUG AUA GCG GU
miRNA-155-2	UAA UUG CUA AUC GUG UUA GCG GU

Experimental instruments

Fluorescence detection was obtained by scanning with a fluorescence spectrometer (PerkinElmer, FL 8500, USA), setting the excitation wavelength to 492 nm, the emission wavelength range to 505-650 nm, and the excitation and emission slits to 5 nm. A ChemiDoc XRS chemiluminescence apparatus (Bio-Rad, USA) was used to conduct non-denaturing polyacrylamide gel electrophoresis. The incubation of the samples in the experiment was carried out in a constant temperature metal bath with a shading function.

Optimizing the conditions for experimentation

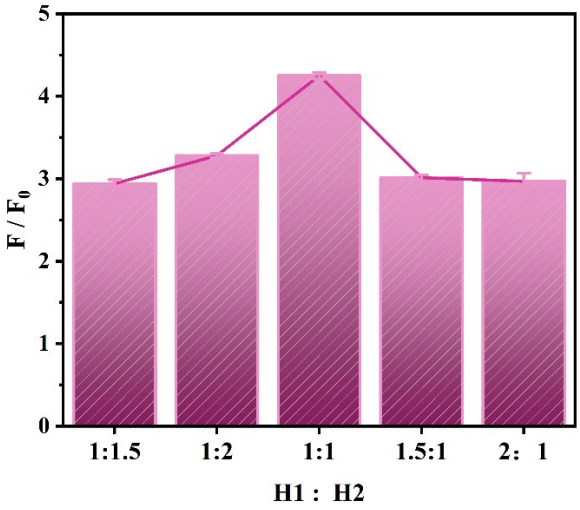

Figure S1. The ratio of double H1-H2 reaction. The standard deviation of the three independent measurements is shown by the error bar. [Hp] = 50 nM, [miRNA-155] = 50 nM, [H1] = 50 nM, [H2] = 50 nM.

Target miRNA-155 detection sensor-system sensitivity

To further illustrate the amplification process of exonuclease III and the contribution of the catalytic hairpin assembly process to sensitivity, we studied the detection limits under exonuclease III amplification only and under dual-mode fluorescence systems, as shown in Figure S2.

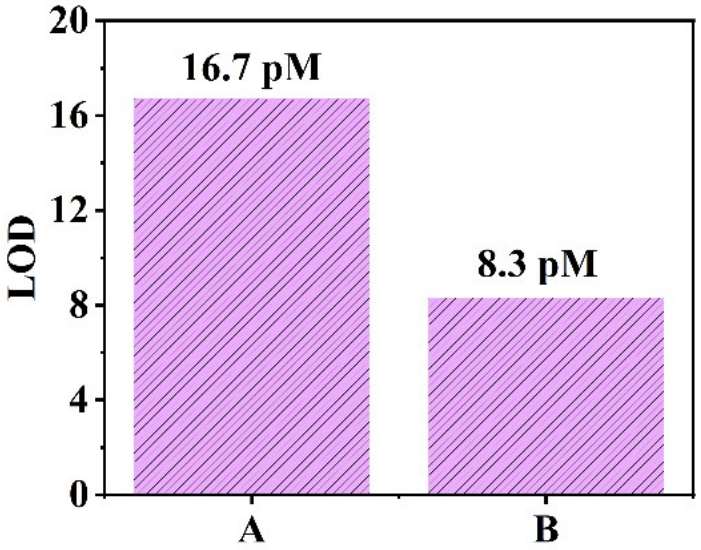

Figure S2. (A)Detection limit of single-mode green fluorescence system. (B) Detection limits of dual-mode fluorescence systems.

Table S2 Assess the different sensors used to detect nucleic acids.

Analysis method	Detection technique	Linear range	LOD	Reference
Ce-BNNSs	Fluorescence	0.1-70 nM	50 pM	[1]
G-quadruplex DNAzyme	colorimetric	1-100 nM	0.7 nM	[2]
DNA/Cu NPs and G/hemin complex	Fluorescence	0.5-100 nM	0.2 nM	[3]

CHA	Fluorescence	5 pM-0.5 nM	2.3 nM	[4]
Metal-Dielectric Nanostructures	SERS	0.5 nM-50 nM	485 pM	[5]
entropy-driven catalytic	ICP-MS	0.02 nM-1 nM	8.4 pM	[6]
Toehold-mediated strand displacement reaction-propelled	Fluorescence	5–50 nM	64 pM	[7]
CRISPR/Cas12a Using an Allosteric Inhibitory	Fluorescence	50 pM-10 nM	28 pM	[8]
localized catalytic hairpin assembly and hybridization chain reaction	Fluorescence	0.1 nM-60 nM	37 pM	[9]
exonuclease Ⅲ-assisted two-stage strand displacement reaction	Fluorescence	25 pM - 125 nM	25 pM	[10]
MoS_2 nanosheet-powered CRISPR/Cas12a	Fluorescence	0.1 nM 13.33 nM	381.78 pM	[11]
Enzyme-assisted target recycling and CHA	Fluorescence	25 pM-100 nM	8.3 pM	This work

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