CRISPR/Cas12a-coupled multiplexed amplification system for ultrasensitive detection of miRNA-155

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Reagents and apparatus

All the oligonucleotides were synthesized by Sangon Biotech (Shanghai) Co., Ltd (Table S1). T4 DNA Ligase, Nb.BbvCI and Klenow Fragment were obtained from New England Biolabs, Inc (Beijing). Cas12a (Cpf1) was provided by Magigen Biotechnology (Guangzhou) Co., Ltd. CRISPR Cas12/13 HybriDetect strips was purchased from Warbio (Nanjing). The ERA nucleic acid amplification kit was purchased from GenDx Biotech (Suzhou) Co., Ltd. The relevant reagents of polyacrylamide gel electrophoresis (PAGE) mainly included 5X TBE Buffer, 4S Green Plus Nucleic Acid Stain, 30% Acryl/Bis Solution and ddH_2O, which were provided by Sangon Biotech (Shanghai) Co., Ltd. Tetramethylethylenediamine (TEMED) was available form Macklin Biochemical (Shanghai) Co., Ltd. Reagents for real sample detection experiments mainly included miRNA reverse transcription kit (Stem-loop Method) and PCR Master Mix ,which were also obtained from Sangon Biotech (Shanghai) Co., Ltd. RNAsimple Total RNA Kit (DP419) was provided by TIANGEN (Beijing).

Fluorescence experiment results were collected by fluorescence spectrophotometer, (FL970, PerkinElmer). The experiment of polyacrylamide gel electrophoresis (PAGE) was obtained by vertical electrophoresis apparatus (Mini-PROTEAN, Bio-rad), and the result map was collected by a gel imager (UVsolo Imager, JENA).

Table S1 The DNA sequences used in this study

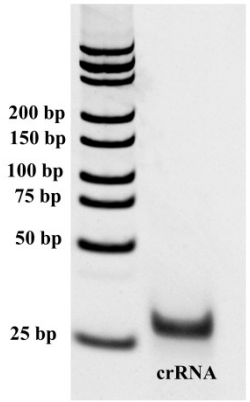
Name	Sequence (5'-3' direction)
miRNA-155	UUAAUGCUAAUCGUGAUAGGGGU
RPA template 1	CATTGAGCTGCGGGAGCTGGCACCCGCTGGACCCCTATCAC
RPA template 2	P-GATTAGCATTAACCTCAGCCGCGTGCAACCCCACACGGCAGCTGGTCCCTG
PRA template 1 (PAM)	CATTGAGCTGCGGGAGCTGGCACCCGCTGGTTTTACCCCTATCAC
Forward primer	CATTGAGCTGCGGGAGCTGGCACCCGCTGG
Reverse primer	CAGGGACCAGCTGCCGTGTGGGGTTGCACGCG
crRNA template	AATGCTAATCGTGATAGGGGATCTACACTTAGTAGAAATTA CCCTATAGTGAGTCGTATTAATTTC
T7 promoter	GAAATTAATACGACTCACTATAGGG
crRNA	UAAUUUCUACUAAGUGUAGAUCCCCUAUCACGAUUAGCAUU
Cleavage reporter	HEX-TATTATT-BHQ1

(FQ-reporter)	
Bio-DNA-FAM	FAM-TTA TTA TT-Biotin
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
Let 7a	UGAGGUAGUAGGUUGUAUAGUU
Random RNA	UUGUACUACACAAAAGUACUG
Stem-loop RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGG
(miRNA-155)	ATACGACACCCCT
155-Forward	GCGCTTAATGCTAATCGTGAT
155-Reverse	GTGCAGGGTCCGAGGT
Stem-loop RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTG
(U6)	GATACGACAAAATA
U6-Forward	CTCGCTTCG GCAGCACATA
U6-Reverse	GTGCAGGGTCCGAGGT

"p" means the phosphate modification.

Preparation of crRNA

crRNA was synthesized by T7 transcription in vitro. Firstly, the mixture of 20 μL containing T7 promoter (10 μM), crRNA template (10 μM) and RNase-free H_2O were annealed at 95 ℃ for 5 min and cooled down to room temperature slowly. Then 10 μL of NTP Mix (100 mM) and 1.5 μL of T7 Mix were added, the transcription reaction was performed at 37℃ overnight. Afterwards, 2 μL of DNase I was added into the reaction liquid and maintained at 37 ◦C for 2 h to degrade the DNA template. Finally, the product was purified by a miRNA purification kit and quantified with a NanoDrop 2000C (Thermo Fisher). And the obtained crRNA was characterized by gel electrophoresis analysis, Polyacrylamide gel were used for feasibility analysis experiments. The composition of polyacrylamide gel was shown in Table S3. After the reaction gel was prepared, mix the sample into loading buffer (6×) in a ratio of 5:1. After mixing evenly, 10 μL mixture was added into the gel hole. Electrophoretic reaction was performed for 50 min at 110 V in 1×TBE buffer. The 4S Red Plus nucleic acid staining solution was diluted 10,000 times with ddH_2O to prepare the dye solution. The steps of crRNA synthesis was consistent with our previous work[22].



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61 **Fig. S1** Gel electrophoresis analysis of crRNA

62 **Table S2** Comparison with other methods for miRNA detection.

method	signal amplification	detection range	LOD (M)	Ref.
Electrochemistry	hydroquinone-mediated enzymatic reduction	100 fM – 1 nM	22 fM	[35]
Electrochemistry	T7 exonuclease aided-digestion amplification	50 fM - 500 pM	34.8 fM	[36]
Colorimetry	catalytic hairpin self-assembly reaction combined with gold nanoparticle	0 pM - 5000 pM	20.7 pM	[37]
Colorimetry	CRISPR-Cas12a system combined with glucose oxidase (GOx) catalysis	50 fM - 50 pM	36.4 fM	[38]
Fiber-optic surface plasmon resonance	phenylboronic acid functionalized Au nanoparticles (PBA-AuNPs)	100 fM – 100 nM	0.27 pM	[39]
Fluorescence	nicking-enhanced rolling circle amplification (SMB-NRCA).	1 pM - 6 nM	1 pM	[40]
Fluorescence	CRISPR-Cas12a coupled with strand displacement amplification	250 fM – 40 pM	150 fM	[41]
Fluorescence	CRISPR/Cas12a-coupled multiplexed amplification system	200 fM to 1 nM	68.69 fM	this work

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64 **Table S3 Preparation of 10% non-denaturing polyacrylamide gel**

ingredient	volume（mL）
Acryl/Bis 30% Solution (29:1)	5 mL

4×TBE buffer	3.5 mL
ddH2O	6 mL
APS (1 mg/mL)	150 μL
TEMED	10 μL

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