Supporting Information

Simultaneously sensitive detection of multiple miRNAs in serum based on the strand displacement amplification

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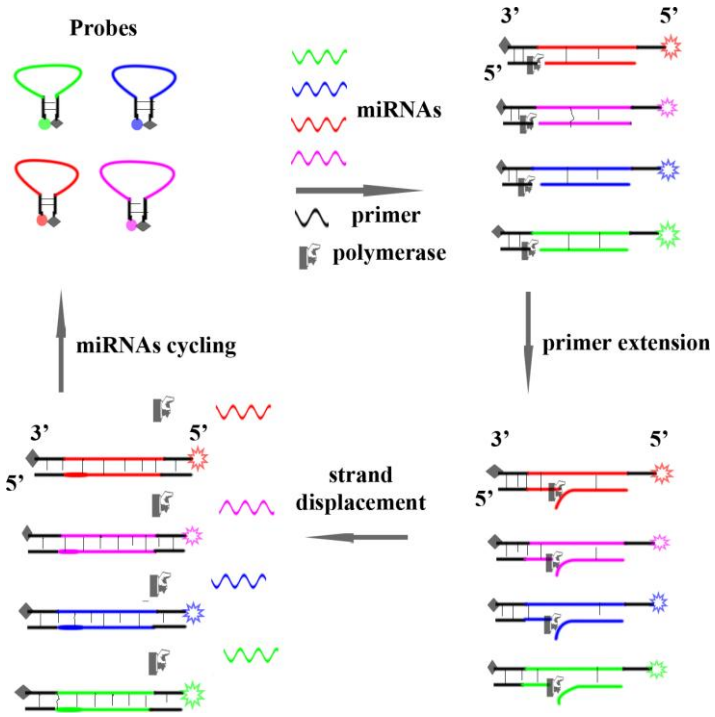

We reported an efficient strategy based on a strand displacement amplicication for serum miRNA detection. In such a system, a multiplexed, sensitive and quick detection of miRNAs could be achieved through a combination of fluorescence labeled probes, a common primer and a polymerase. This could be potentially used in clinical field to achieve early disease diagnosis and prognosis.

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1.1 General information: The oligonucleotides and dNTPs were purchased from Invitrogen Technology(Shanghai, China). *Bst* DNA Polymerase, large fragment was purchased from NEB(New England Biolabs Inc) company. RiboLock™ RNase Inhibitor was purchased from Fermentas Inc. Trizol for miRNA extraction was purchased from Invitrogen Technology(Shanghai, China). Fluorescence was measured by LS55 Perkin Elmer. All measurements were performed at room temperature.

1.2 Protocol of the strand displacement amplification for miRNA detection: This reaction was performed in 1× ThermoPol™ Reaction Buffer, which contained 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$ and 0.1 % Triton X-100 at pH 8.8@ 25°C. A 100 μL sample was incubated in a water bath at 37 ℃for 30 mins. In details, 200 μM dNTPs, 50 nM hairpin probes, 500 nM short primer at final concentrations, 40 U RNase inhibitor, 20 U Bst polymerase and different concentrations of miRNAs were used per reaction system.

1.3 Multiplexed detection of miRNAs: DEPC water, 10× ThermoPol™ Reaction Buffer, 200 μM dNTPs, 50 nM hairpin probes, 500 nM short primer, 40 U of RNase inhibitor, and 20 U of Bst polymerase were sequentially add to each reaction system. In the initial test, the miRNAs diluted to final concentrations at 10 nM was then added, including miR21, miR155, miR199a and miR141. Same volume of water in stead of miRNA solution was used as the negative control. The 1× ThermoPol™ Reaction Buffer contained 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$ and 0.1 % Triton X-100 at pH 8.8 and 25°C. A 100 μL well-mixed sample was incubated in a water bath at 37 ℃ for 30 min, and the fluorescence of each sample was then measured via using an LS55 Perkin Elmer spectrometer at corresponding excitation wavelengths.

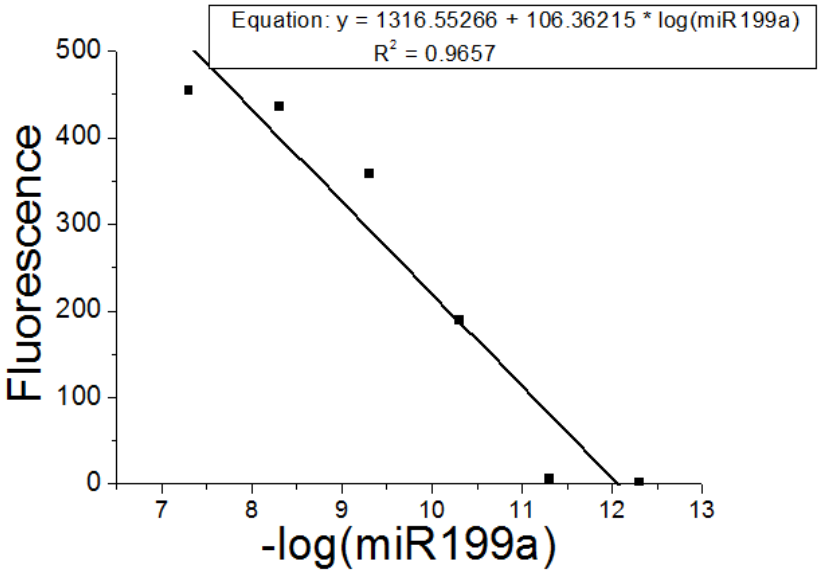
1.4 Extraction of miRNAs from human serum: Total RNA was extracted with Trizol reagent according to the instructions provided by the company. Briefly, 1 mL of Trizol was used to extract 200 μL human serum, which was obtained after separating blood into its solid and liquid components after clotting. After the incubation, add 200 μL chloroform to each sample, shake vigorously by vortex for 15 seconds and incubate at room temperature for3 minutes. Centrifuge 15,000 x g for 15 minutes at 4°C. Transfer the upper aqueous phase of the sample into a new 1.5 mL microcentrifuge tube via pipetting. Add 0.6 mL of 100% isopropanol to the aqueous phase. Add 1 mL 75% ethanol to the pellet and centrifuge the tube at 7500 × g for 5 minutes at 4°C. Resuspend the RNA pellet in 20 μL RNase-free water. The concentration of extracted RNAs was measured via using Nanodrop machine and 3 μg of RNA was used in the following detection via using the strand displacement reaction. The entire process should prevent RNA degradation. After extraction, the subsequent procedure was the same as described above.

1.5 **Table S1** Sequences of oligomers used in strand displacement for detection of miRNAs.

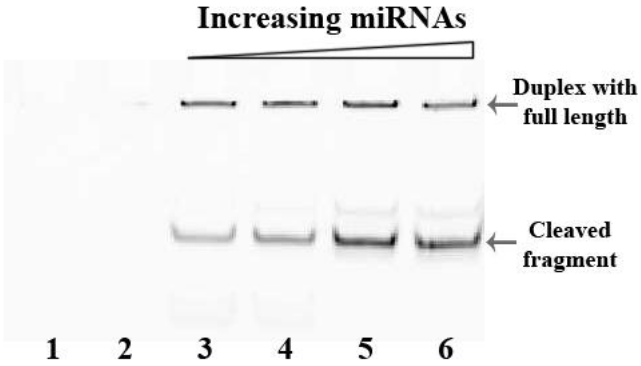
Oligomer	Sequence(from 5'to 3')
miR141(RNA)	UAACACUGUCUGGUAAAGAUGG
miR429(RNA)	UAAUACUGUCUGGUAAAACCGU
miR199a(RNA)	ACAGUAGUCUGCACAUUGGUUA
miR21(RNA)	UAGCUUAUCAGACUGAUGUUGA
miR155(RNA)	UUAAUGCUAAUCGUGAUAGGGGU
RNA199m(RNA)	ACAGUATUCUGCACAUUTGUUA
Probe-199a1(DNA)	/FAM/-CCCAACCCA TAACCAATGTGCAGACTACTGT TTACTAG TGGGTTGGG-/BHQ/
Probe-141(DNA)	/Texas Red/-CCCAACCCA CCATCTTTACCAGACAGTGTTA TTACTAG TGGGTTGGG-/BHQ/
Probe-21(DNA)	/Cy3/-CCCAACCCA TCAACATCAGTCTGATAAGCTA TTACTAG TGGGTTGGG-/BHQ/
Probe-155(DNA)	/Cy5/-CCCAACCCA ACCCCTATCACGATTAGCATTAA TTACTAG TGGGTTGGG-/BHQ/
primer	CCCAACCCA

1.6 Table S2 Samples used in the multiplexed miRNA detection

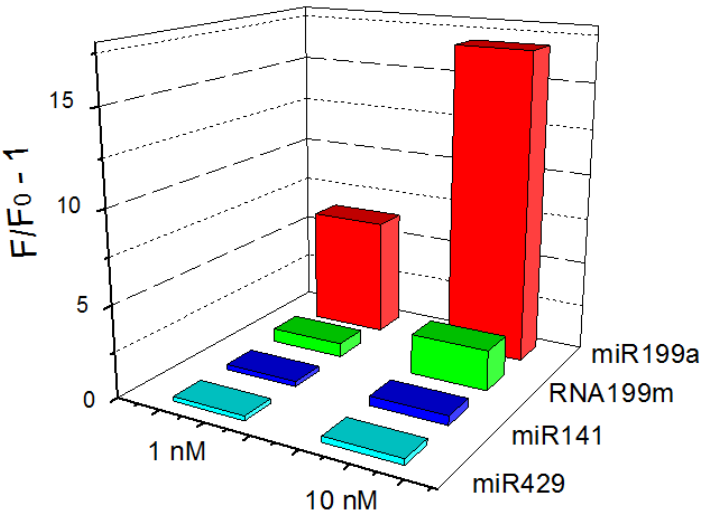
Name	Components (Final Conc.)
Sample 1	miR199(50 nM)
Sample 2	miR21(50 nM)
Sample 3	miR141(50 nM)
Sample 4	miR155(50 nM)
Sample 5	miR21(50 nM) and miR141(50 nM)
Sample 6	miR21(50 nM) and miR155(50 nM)
Sample 7	miR141(50 nM) and miR155(50 nM)
Sample 8	miR199(50 nM) and miR155(50 nM)
Sample 9	miR199(50 nM) and miR141(50 nM)
Sample 10	miR199(50 nM) and miR21(50 nM)
Sample 11	miR21(50 nM), miR141(50 nM) and miR155
Sample 12	miR199a(50 nM), miR141(50 nM) and miR155(50 nM)
Sample 13	miR199a(50 nM), miR21(50 nM) and miR155(50 nM)
Sample 14	miR199a(50 nM), miR21(50 nM) and miR141(50 nM)
Sample 15	miR199a(50 nM), miR21(50 nM), miR141(50 nM) and miR155(50 nM)



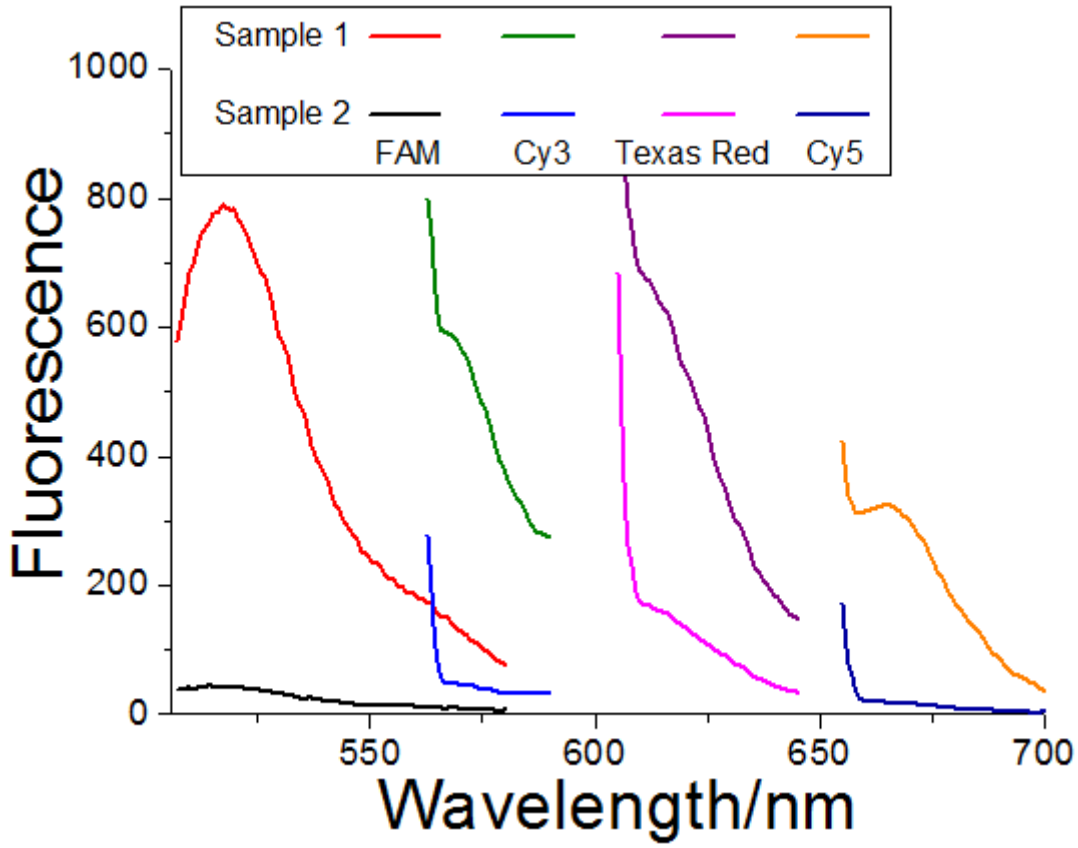
1.7 Figure S1: Fitting curve of the fluorescence enhancement for different amounts of miR199a. The curve was plotted with the fluorescence enhancement over background vs log(miR199a).



1.8 Figure S2: A 20% non-denaturing PAGE analysis of the products by the isothermal strand-displacement polymerization reaction and the following SpeI degestion was carried out in 1×TBE (pH=8.3) at 80V constant voltage for about 1 h. After polymerization, 10 U SpeI was directly added in the reaction. The digestion was conducted at 37 °C for 30 mins. Gels were scanned at a common excitation wavelength(488 nm) using an Image Master VDS-CL(Amersham Biosciences). Gel electrophoresis of digestion products. Lane 1, control without addition of *Bst* Polymerase; lane 2, control without addition of miR199a; lane 3, 100 pM miR199a; lane 4, 1 nM miR199a; lane 5, 10 nM miR199a; lane 6, 100 nM miR199a.



1.9 Figure S3: Specificity test of the newly developed method. Bars represent the fluorescence ratio (F/F0 − 1) upon the different miRNAs targets, including miR199a, RNA199m, miR141 and miR429. Probe-199a1 labeled with FAM was used as the polymerization template.



1.10 Figure S4: Initial multiplexed detection of samples consisting of four miRNAs. Sample 1 contained 10 nM miR199a, 10 nM miR21, 10 nM miR155 and 10 nM miR141, while sample 2 contained no added miRNAs. Four hairpin probes with their perfectly complementary miRNAs are labeled with different fluorephores. Probe-199a1 for miR199a(FAM, blue emission at 518 nm); Probe-21 for miR21(CY3, orange emission at 580 nm); Probe-141 for miR141(Texas Red, red emission at 613 nm); Probe-155 for miR155(Cy5, red emission at 663 nm).