Supporting information

Detection of NSCLC biomarker miRNAs via localized catalytic hairpin self-assembly and laser-induced fluorescence on a microfluidic chip

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S1. Immobilization of hairpin probes onto magnetic beads

MBCPs were formed by functionalizing magnetic beads (MBs) with Cps. To verify the binding ability of Cps to MBs, the concentration of Cps in the solution before and after the reaction was measured by a Nanodrop nucleic acid spectrophotometer, and the loading efficiency was calculated using **Equation S1** [1]:

$$\text{loading efficiency} = \frac{initially\ added\ Cp - remained\ Cp\ in\ supernatant}{theoretical\ binding\ capacity} \quad \text{(S1)}$$

The calculated loading efficiency of MBs is 94.2 ± 3.6% (n=3), suggesting sufficient Cps were successfully immobilized on MBs, and MBCPs can be used to capture target for detection.

S2. Optimization of LCHA conditions

To optimize the parameters of LCHA amplification, the influence of the amount of MBCPs, the target capture temperature and time, as well as the LCHA reaction temperature on the sensitivity of the LCHA-based assay were investigated.

In this proposed method, LCHA relies on MBCPs to target miRNA molecule to expose the trigger for amplification; however, excessive MBCPs would result in material waste, such that it is imperative to optimize the amount of MBCPs for LCHA amplification. **Figure S2A** shows the fluorescence intensity of LCHA product solutions with different volumes of added MBCP suspension after 90 minutes of amplification, when 500 μL of DNA-20a solution (100 nmol·L^{-1}) was added in the sample solution as target molecules. The fluorescence intensity increases with the volume of MBCP

suspension until 50 μL, then becomes stable despite further increased volume. The low fluorescence intensity at the low volumes of MBCP is attributed to limited amount of DTPs with opened Hp2 for fluorescence due to the lack of MBCPs to hybridize with. When more than 50 μL of MBCP suspension was used, the added target molecules were fully bonded to MBCPs, such that the fluorescence intensity doesn't increase any more. Therefore, the optimized volume of MBCP suspension in 1 mL of sample solution is 50 μL when 500 μL of DNA-20a solution (100 nmol·L^{-1}) is used as target. This is the recipe used for subsequent experiments.

Figure S2B shows the fluorescence intensity of LCHA product solutions with different target capture temperatures, the best target capture temperature is 37 °C, which shows the highest fluorescence intensity. When the temperature is low, an increase in temperature enhances the ability of base complementary pairing, thereby strengthening the binding affinity between the target molecules and Cps on MBCPs and resulting in an amplified fluorescence intensity. However, high target capture temperature weakens the base complementary pairing capability, leading to a decrease in target-Cp binding affinity and a reduction in fluorescence intensity. **Figure S2C** shows that the fluorescence intensity of LCHA product solutions increases with the target capture time until 60 minutes then slightly drops. The target capture temperature was fixed at 37 °C. The decline of the fluorescence intensity after 60 minutes might be attributable to the saturation of the capture reaction and hydrolysis of a few probes [2,3]. Hence the optimal target capture time is 60 min at 37 °C.

Figure S2D shows that the fluorescence intensity peaks at the LCHA temperature

of 37 °C. LCHA temperature is the temperature of the amplification process where MBCPs with exposed triggers bind with DTPs. Similar to the target capture process, an increase in LCHA temperature enhances the complementary pairing ability of bases and strengthens the hybridization ability of the triggers on MBCPs and the Hp1 on DTPs, leading to an increase in fluorescence intensity when the temperature is below 37 ℃. On the contrary, at excessively high temperature, base complementary pairing weakens, resulting in a decrease in base pairing ability and fluorescence intensity. Therefore, the optimal temperature for LCHA amplification is also 37 °C.

Table S1. Sequence information of DNA and miRNA species related to the detection of miR-20a

Name	Sequence（5'→3'）
DNA-20a	TAAAGTGCTTATAGTGCAGGTAG
miR-20a	UAAAGUGCUUAUAGUGCAGGUAG
Cp-20a	**TAAAGTGCTTACGACTATTAATAA**TTT CTAGCTGCAGAATAAGCACTTTA
Cp-20a-biotin	**TAAAGTGCTTACGACTATTAATAA**TTTCTAGCTGCAGAA TAAGCACTTTATTTTTTTTT-biotin
Cp-20a-FAM	6-FAM- **TAAAGTGCTTACGACTATTAATAA**TTTCTAGCTGCAGAA TAAGCACTTTATTTTTTTTT-biotin
trigger-20a	TAAAGTGCTTACGACTATTAATAA
H1-20a	TTATTAATAGTCGTAAGCACTTTA CCATGTGTAGATAAAGTGCTTACGAC
H1-20a-FAM	FAM- TTATTAATAGTCGTAAGCACTTTA CCATGTGTAGATAAAGTGCTTACGAC
H2-20a	ACTTTATCT(FAM)ACACATGGTAAA GTGCTTACGACCCATGTGT(BHQ-1)AGA
S1-20a	AGTCTGAATTCCTGGAGATACATGGCATTTGCTACACG CCCTATTAGAAGGTCCGATTTACTTTATCT(FAM)ACACATGGT AAAGTGCTTACGACCCATGTGT(BHQ-1)AGATGCCGTAGCA
S2-20a	GCAGTTGACGCGACAGTCGTTCAAGCCTTTCGGACCTTC TAATAGGGCGTGTAGCATTATGCGAGGGTCCAATACTCTGTTCCGG
S3-20a	GGCTTGAACGACTGTCGCGTCAACTGCTTACGACACTACGTA ACGGTCGAGGACTGTTGCTACGGCTTTTTTTTTTTTCCGACGTAGCTG
S4-20a	TGCCATGTATCTCCAGGAATTCAGACTTTCAGTCCTCGACCG TTACGTAGTGTCGTTTCCGGAACAGAGTATTGGACCCTCGCAT
Hp1-20a	CAGCTACGTCTTTTTTATTAATAGTCGTAAGCACTTTACCATGT GTAGATAAAGTGCTTACGAC
S3-20a-FAM	6-FAM- GGCTTGAACGACTGTCGCGTCAACTGCTTACGACACTACGTA ACGGTCGAGGACTGTTGCTACGGCTTTTTTTTTTTTCCGACGTAGCTG
miR-17	CAAAGUGCUUACAGUGCAGGUAG
miR-18a	UAAGGUGCAUCUAGUGCAGAUAG
miR-19a	AGUUUUGCAUAGUUGCACUACA

Note:

(1) The single underlined part is the complementary sequence of hairpin probes, and

the bold part is the trigger sequence in Cp-20a.
(2) In S1-20a, S2-20a, S3-20a, S4-20a, and Hp-20a, the parts with the same color indicate complementary sequences. The wavy line portion in S1-20a represents Hp2-20a (with a sequence identical to H2-20a), and the double underlined part in Hp1-20a is the same as the sequence of H1-20a.
(3) Cp-20a is designed based on the sequence of miR-20a, while H1-20a and H2-20a are designed according to the trigger sequence on Cp-20a. The design of Cp-20a, H1-20a, and H2-20a has been pre-verified through simulation using NUPACK (v4.0).

Table S2. Sequence information related to miR-223

Name	Sequence（5'→3'）
DNA-223	TGTCAGTTTGTCAAATACCCCA
miR-223	UGUCAGUUUGUCAAAUACCCCA
H1-223	TAAAAAATACTGGACAAACTGACACGATGTGTAGATGTCA GTTTGTCCAGT
H2-223	AACTGACATCT(FAM)ACACATGGTGTCAGTTT GTCCAGTCCATGTGT(BHQ-1)AGA
Hp1-223	CAGCTACGTCTTTTTAAAAAATACTGGACAAACTGACACGATGTG TAGATGTCAGTTTGTCCAGT
Cp-223-biotin	**TGTCAGTTTGTCCAGTATTTTTTA**TTT TCGGGTATTTGACAAACTGACATTTTTTTTT-biotin
S1-223	AGTCTGAATTCCTGGAGATACATGGCATTTGCTACACGCCCTATT AGAAGGTCCGATTTAACTGACATCT(FAM)ACACATGGTGTCAGTTTG TCCAGTCCATGTGT(BHQ-1)AGATGCCGTAGCA
S2-223	GCAGTTGACGCGACAGTCGTTCAAGCCTTTCGG ACCTTCTAATAGGGCGTGTAGCATTATGCGAGGGTCCAA TACTCTGTTCCGG
S3-223	GGCTTGAACGACTGTCGCGTCAACTGCTTACGACACTACGTAACG GTCGAGGACTGTTGCTACGGCTTTTTTTTTTTTCCGACGTAGCTG
S4-223	TGCCATGTATCTCCAGGAATTCAGACTTTCAGTCCTCGACCGTTA CGTAGTGTCGTTTCCGGAACAGAGTATTGGACCCTCGCAT

Note:

(1) The single underlined part is the complementary sequence of hairpin probes, and the bold part is the trigger sequence in Cp-223.

(2) In S1-223, S2-223, S3-223, S4-223, and Hp-223, the parts with the same color indicate complementary sequences. The wavy line portion in S1-223 represents Hp2-223 (with a sequence identical to H2-223), and the double underlined part in Hp1-20a is the same as the sequence of H1-223.

(3) Cp-223 is designed based on the sequence of miR-223, while H1-223 and H2-223 are designed according to the trigger sequence on Cp-223. The design of Cp-223, H1-223, and H2-223 has been pre-verified through simulation using NUPACK (v4.0).

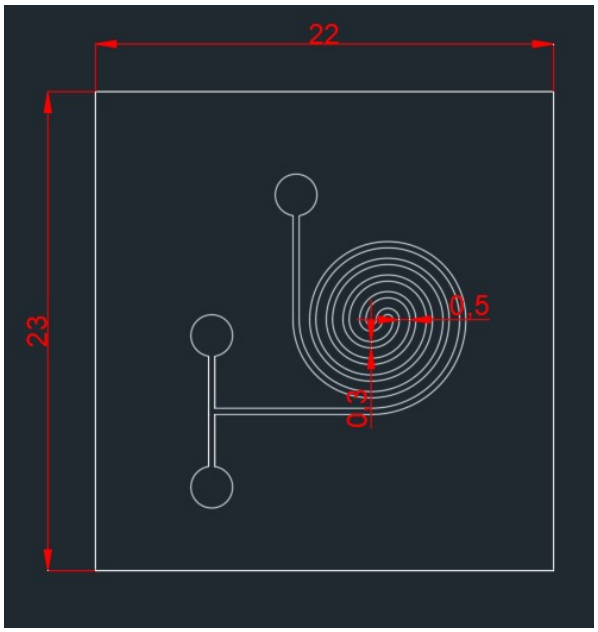

Figure S1. Design of the microfluidic chip

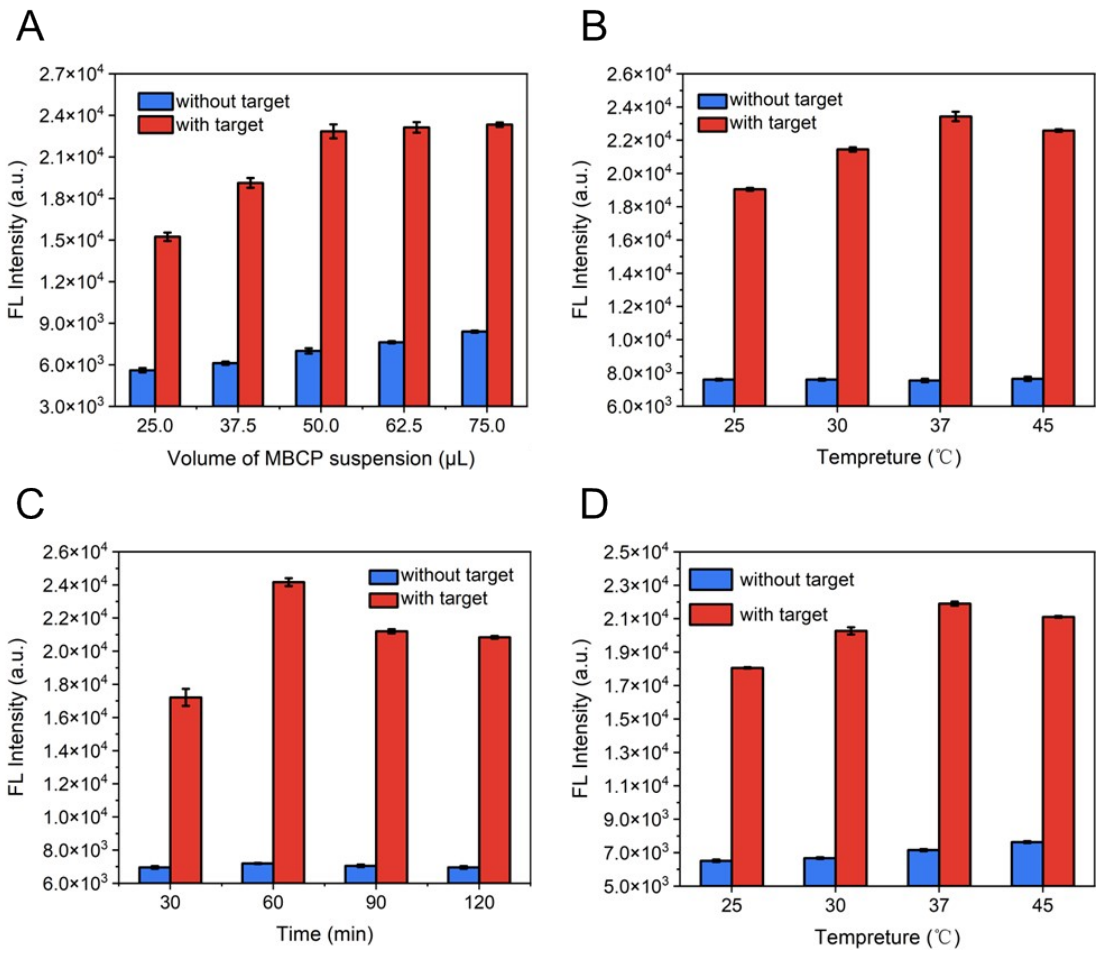

Figure S2 (A) Fluorescence intensity of the LCHA product solutions with different volumes of added MBCP suspension after 90 minutes of LCHA amplification at 37 °C. (*n*=3). (B) Fluorescence intensity of the LCHA product solutions with different target capture temperatures. (*n*=3) The target capture time was fixed at 60 minutes for all the samples. (C) Fluorescence intensity of the LCHA product solutions with different target capture time. (*n*=3) The target capture temperature was fixed at 37 °C for all the samples. (D) Fluorescence intensity of the LCHA product solutions with different LCHA amplification temperatures. (*n*=3) The total LCHA amplification time was fixed at 90 minutes for all the samples. For red bars, 500 μL of 100 nmol·L^{-1} DNA-20a solution was added in each sample solution, while no DNA solution was added for blue

bars.

References

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