Supplementary material for

LNA-Modified Magnetic Bead Platform with Surface HCR for Streamlined Capture and Amplification of Serum miR-182 in Lung Cancer Diagnosis

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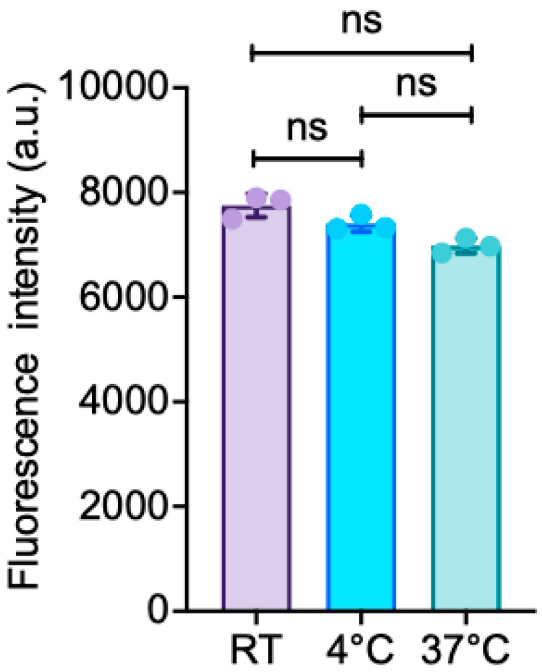

Figure S1. Effect of HCR incubation temperature on the fluorescence signal. HCR was performed at room temperature (RT), 4 °C, and 37 °C. Data are presented as mean ± SD from three independent experiments (n = 3). No significant differences were observed among the three conditions (one-way ANOVA, $p > 0.05$).

Table S1. Nucleic acid sequences of HCR and primer sequences used in RT-PCR

Name	Sequence
microRNA 182	5'-UUUGG-CAAUG-GUAGA-ACUCA-CACU-3'
HCR hairpin 1 (H1)	5'-AATGG-TAGAA-CTCAC-ACTCA-GATCA-GTGTG-AGTTC-TACCA-TTGCC-AAA-3'
HCR hairpin 2 (H2)	5'-GATCT-GAGTG-TGAGT-TCTAC-CATTT-TTGGC-AATGG-TAGAA-CTCAC-ACT-3'
HCR hairpin 2-FAM (H2-FAM)	5'-GATCT-GAGTG-TGAGT-TCTAC-CATTT-TTGGC-AATGG-TAGAA-CTCAC-ACT-3'-(FAM)
LH1-biotin	5'-AATGG-TAGAA-CTCAC-ACTCA-GATCA-GTGTG-AGTTC-TACCA-TTGCC-A*AAAA-AAA-3'-(TEG-Biotin)
182-RT-primer	5'-GTCGT-ATCCA-GTGCA-GGGTC-CGAGG-TATTC-GCACT-GGATA-CGACA-GTGTG-3'
182-forward primer	5'-GCGTT-TGGCA-ATGGT-AGAAC-T-3'
182-reverse primer	5'-AGTGC-AGGGT-CCGAG-GTATT-3'

* The underlined nucleic acid sequence was replaced with LNA.

Biotin is used to immobilize the LH1 hairpin probe on streptavidin-coated magnetic beads through the biotin–streptavidin interaction.
The LNA residues are located in the sticky end of LH1 and enhance target binding affinity and mismatch discrimination toward miR-182.

Table S2. Thermodynamic parameters and Tm values of the intermolecular duplexes.

Name	Condition	Na^+ (mM)	Mg^{2+} (mM)	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/(mol·K))	Tm (°C)
miR-182/H1	Detection	137	5	42.6	207.3	606.4	78.7
miR-182/H1	Electrophoresis	10	0	34.3	207.4	635.4	61.2
H1/H2	Detection	137	5	68.6	307.0	876.4	82.2
H1/H2	Electrophoresis	10	0	56.3	307.3	920.3	65.8

Note：1. Thermodynamic parameters were calculated using the UNAFold web server in " Hybridization of Two Different Strands of DNA or RNA" mode with an oligonucleotide concentration of 1 μM for all simulations. This concentration corresponds to the electrophoresis experiments and was used as a standardized model concentration for the hybridization conditions.

2. For the electrophoresis condition (1×TBE), [Mg^{2+}] was set to 0 mM, and [Na^+] was set to 10 mM (the minimum allowable input value in UNAFold) to approximate the low-salt environment.

3. The miR-182/H1 duplex was simulated using the unmodified H1 sequence, as the standard UNAFold model does not support LNA thermodynamic parameters. The LNA-modified LH1 is expected to form a more stable duplex; thus, these values represent a conservative estimate.

Table S3. The microRNA sequences used for specificity testing

Name	Sequence
microRNA 15	5'-UAGCAGCACAUAAUGGUUUGUG-3'
microRNA 21	5'-UAGCUUAUCAGACUGAUGUUGA-3'
microRNA 27	5'-AGGGCUUAGCUGCUUGUGAGCA-3'
microRNA 96	5'-UUUGGCACUAGCACAUUUUUGCU-3'
microRNA 155	5'-UUAAUGCUAAUCGUGAUAGGGGUU-3'
microRNA 183	5'- GUGAAUUACCGAAGGGCCAUAA-3'
microRNA 197	5'-CGGGUAGAGAGGGCAGUGGGAGG-3'
microRNA 205	5'-UCCUUCAUUCCACCGGAGUCUG-3'
microRNA 301	5'-GCUCUGACUUUAUUGCACUACU-3'

Table S4. Basic information of study subjects

		Lung cancer (LC)	Non-lung cancer (NC)		*P* value
		LC(N=31)	BLD (N=10)	HC(N=20)	
SEX	Male	14(45.2%)	4(40.0%)	12(60.0%)	0.478
	Female	17(54.8%)	6(60.0%)	8(40.0%)	
Age		57±10	62±8	55±9	0.172
BMI		23.0±3.5	24.0±3.6	23.0±3.3	0.835
Smoking status	Current or past	8(25.8%)	4(40.0%)	7(35.0%)	0.632
	Never	23(74.2%)	6(60.0%)	13(65.0%)	
Drinking status	Current or past	6(19.4%)	1(10.0%)	4(20.0%)	0.769
	Never	25(80.6%)	9(90.0%)	16(80.0%)	
Pathological type	LUAD	30(96.8%)	N.A.	N.A.	
	LUSC	1(3.2%)	N.A.	N.A.	
Stage	Early	27(87.1%)	N.A.	N.A.	
	Advanced	4(12.9%)	N.A.	N.A.	

Table S5. Concentrations of miR-182 derived from the MFI or the positive%.

MFI (pM)		Positive% (pM)	
LC	NC	LC	NC
1.52	2.12	1.38	1.79
1.25	1.81	1.05	2.76
63.85	0.98	38.42	0.71
4.32	*N.D.*	3.41	*N.D.*
2.06	1.28	2.19	2.13
4.36	0.92	3.69	1.79
1.15	2.03	1.49	1.99
1.28	*N.D.*	1.69	*N.D.*
5.25	*N.D.*	7.16	*N.D.*
2.69	1.08	3.90	0.93
53.23	*N.D.*	25.00	*N.D.*
20.35	0.91	21.73	1.41
3.34	1.94	2.65	1.20
1.17	1.20	1.05	0.92
10.74	1.95	9.03	1.50
1.39	4.05	1.32	1.86
2.44	1.58	3.65	2.87
1.86	*N.D.*	1.42	*N.D.*
2.91	*N.D.*	3.69	0.53
5.85	*N.D.*	4.03	0.65
1.81	2.72	1.81	1.87
40.06	*N.D.*	46.58	*N.D.*
3.88	1.52	3.90	1.09
0.83	*N.D.*	0.89	0.53
0.98	2.59	1.11	2.94
1.00	1.71	3.90	1.18
3.29	*N.D.*	3.36	0.96
2.11	2.99	1.19	0.63
36.00	*N.D.*	34.88	1.08
3.15	2.91	1.14	4.08
22.91		25.00	

N.D.: Not detected

Table S6. Optimal cutoff, sensitivity, specificity and confusion matrices of three method for microRNA 182 in lung cancer diagnosis.

Readout	Optimal cutoff	Sensitivity	Specificity	TP/FN/TN/FP
RT-qPCR	1.724 (R.L.)	64.52%	90.00%	20/11/27/3
MFI	3.073 (pM)	48.39%	96.67%	15/16/29/1
positive%	3.154 (pM)	54.84%	96.67%	17/14/29/1

Table S7. Comparison table of operation steps between MB-LH1-HCR and RT-PCR

MB-LH1-HCR	RT-PCR
Step 1: Capture and Separation	**Step 1: Nucleic Acid Isolation**
Operation 1: Add incubation buffer to the centrifuge tube	Operation 1: Add DEPC water to the centrifuge tube
Operation 2: Add MB-LH1 solution to the centrifuge tube	Operation 2: Add 500 μL serum to the centrifuge tube
Operation 3: Add 1 μL serum to the centrifuge tube	Operation 3: Add lysis binding buffer to the centrifuge tube
Operation 4: Vortex and let stand	Operation 4: Vortex to mix
Operation 5: Place on a magnetic rack	Operation 5: Add proteinase K solution to the centrifuge tube
Operation 6: Remove supernatant	Operation 6: Vortex to mix
Operation 7: Add buffer	Operation 7: Add magnetic bead binding buffer to the centrifuge tube
Operation 8: Transfer to a new centrifuge tube	Operation 8: Vortex to mix and incubate
Operation 9: Vortex and place on a magnetic rack	Operation 9: Add absolute ethanol to the centrifuge tube
Operation 10: Remove supernatant	Operation 10: Vortex and place on a magnetic rack
Step 2: HCR	Operation 11: Remove supernatant
Operation 11: Add HCR reaction mixture	Operation 12: Add absolute ethanol to the centrifuge tube
Operation 12: Vortex and incubate	Operation 13: Add lysis binding buffer to the centrifuge tube
Operation 13: Place on a magnetic rack	Operation 14: Vortex and let stand
Operation 14: Remove supernatant	Operation 15: Place on a magnetic rack
Operation 15: Add buffer	Operation 16: Remove supernatant
Operation 16: Vortex and place on a magnetic rack	Operation 17: Add absolute ethanol to the centrifuge tube
Operation 17: Remove supernatant	Operation 18: Add lysis binding buffer to the centrifuge tube
Operation 18: Add buffer	Operation 19: Vortex and let stand
Operation 19: Vortex and measure on the instrument	Operation 20: Place on a magnetic rack
	Operation 21: Remove supernatant
	Operation 22: Add 80% ethanol solution to the centrifuge tube
	Operation 23: Vortex and let stand
	Operation 24: Place on a magnetic rack
	Operation 25: Remove supernatant
	Operation 26: Add 80% ethanol solution to the centrifuge tube
	Operation 27: Vortex and let stand
	Operation 28: Place on a magnetic rack
	Operation 29: Remove supernatant
	Operation 30: Add absolute ethanol to the centrifuge tube
	Operation 31: Vortex and let stand
	Operation 32: Place on a magnetic rack
	Operation 33: Remove supernatant
	Operation 34: Dry in a metal bath at 65°C
	Operation 35: Add elution buffer to the centrifuge tube
	Operation 36: Incubate in a metal bath at 65°C
	Operation 37: Let stand, then place on a magnetic rack
	Operation 38: Collect the supernatant
	Step 2: Reverse Transcription
	Operation 39: Add the above supernatant to the tube
	Operation 40: Add gDNA Wiper Mix to the centrifuge tube
	Operation 41: Pipette to mix
	Operation 42: Incubate in a water bath at 42°C for 2 min
	Operation 43: Add Stem-loop primer (2 μM) to the centrifuge tube
	Operation 44: Add 10× RT Mix to the centrifuge tube
	Operation 45: Add HiScript II Enzyme Mix to the centrifuge tube
	Operation 46: Add RNase-free ddH_2O to the centrifuge tube
	Operation 47: Pipette to mix
	Operation 48: Perform reverse transcription on the instrument
	Step 3: PCR
	Operation 49: Add the cDNA obtained from the above reverse transcription and PCR reaction mixture to an RNase-free centrifuge tube
	Operation 50: Add the reverse transcription solution to the tube
	Operation 51: Add RNase-free ddH_2O to the tube
	Operation 52: Add 2× miRNA Unimodal SYBR qPCR Master Mix to the tube
	Operation 53: Add Forward Primer (10 μM) to the tube
	Operation 54: Add Reverse Primer (10 μM) to the tube
	Operation 55: Pipette to mix
	Operation 56: Perform PCR on the instrument

Table S8. Intra-day and inter-day precision of the MB-LH1-HCR-flow cytometry assay for serum miR-182 detection based on mean fluorescence intensity (MFI)

	miR-182 concentration （pM, n=5）					Average (pM)	CV （%）
Intra-day 1	9.23	10.85	9.13	9.13	8.90	9.45	8.4
Intra-day 2	10.01	8.97	9.93	10.65	9.23	9.76	6.9
Intra-day 3	11.37	10.40	10.37	9.30	10.59	10.41	7.1
Intra-day 4	9.87	10.08	10.40	8.98	9.87	9.84	5.4
Intra-day 5	10.29	10.09	9.97	10.16	8.85	9.87	5.9
Average CV							6.7
Inter-day 1	10.54	9.21	9.45	10.18	8.27	9.53	9.3
Inter-day 2	11.57	10.08	11.23	9.10	10.19	10.43	9.4
Inter-day 3	11.63	9.41	10.44	9.33	10.43	10.25	9.1
Inter-day 4	10.72	10.15	9.25	9.26	8.96	9.67	7.7
Inter-day 5	10.01	11.17	8.95	10.28	9.36	9.96	8.6
Average CV							8.8

Note: Each concentration value in the table represents the mean of five technical replicates. Intra-day precision was assessed in five replicate series, each consisting of five assay-level values obtained on the same day. Inter-day precision was assessed in five replicate series, each consisting of five assay-level values obtained on five separate days. For each series, the coefficient of variation (CV) was calculated as the standard deviation of the five assay-level means divided by their mean and multiplied by 100%. The average CV is the arithmetic mean of the CV values from the five series.

Table S9. Intra-day and inter-day precision of the MB-LH1-HCR-flow cytometry assay for serum miR-182 detection based on the percentage of positive beads

	miR-182 concentration （pM, n=5）					Average (pM)	CV （%）
Intra-day 1	9.07	10.10	10.12	9.16	9.50	9.59	5.3
Intra-day 2	10.04	9.90	10.41	10.98	9.40	10.15	5.8
Intra-day 3	10.48	9.48	11.31	9.43	10.07	10.16	7.7
Intra-day 4	10.32	9.18	9.19	10.21	9.67	9.71	5.6
Intra-day 5	10.17	9.22	9.05	10.51	9.27	9.65	6.8
Average CV							6.2
Inter-day 1	9.96	8.92	9.29	9.26	11.13	9.71	9.1
Inter-day 2	9.34	11.16	9.71	11.73	9.93	10.37	9.8
Inter-day 3	8.98	9.02	10.46	11.26	9.71	9.88	9.9
Inter-day 4	8.55	10.49	9.80	10.66	10.01	9.90	8.4
Inter-day 5	9.59	9.14	10.28	8.87	10.24	9.62	6.6
Average CV							8.8

Note: Each concentration value in the table represents the mean of five technical replicates. Intra-day precision was assessed in five replicate series, each consisting of five assay-level values obtained on the same day. Inter-day precision was assessed in five replicate series, each consisting of five assay-level values obtained on five separate days. For each series, the coefficient of variation (CV) was calculated as the standard deviation of the five assay-level means divided by their mean and multiplied by 100%. The average CV is the arithmetic mean of the CV values from the five series.