Electronic Supplementary Material (ESI) for Analyst.
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Supporting Information

An ultrasensitive microchip electrophoresis assay based on separation assisted double cycling signal amplification strategy for microRNA quantification in cell lysate

Kaiji Wei,[a] Jingjin Zhao,*[,a] Xiaoshu Luo,[b] Senghui Qiu,[b] Fuyun He,[b] Shuting Li[a] and Shulin Zhao*[a]

[a]State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, Guangxi Normal University, Guilin, 541004, China

[b]College of Electronic Engineering, Guangxi Normal University, Guilin, 541004, China

E-mail: zhaoshulin001@163.com; jzhao12@163.com.

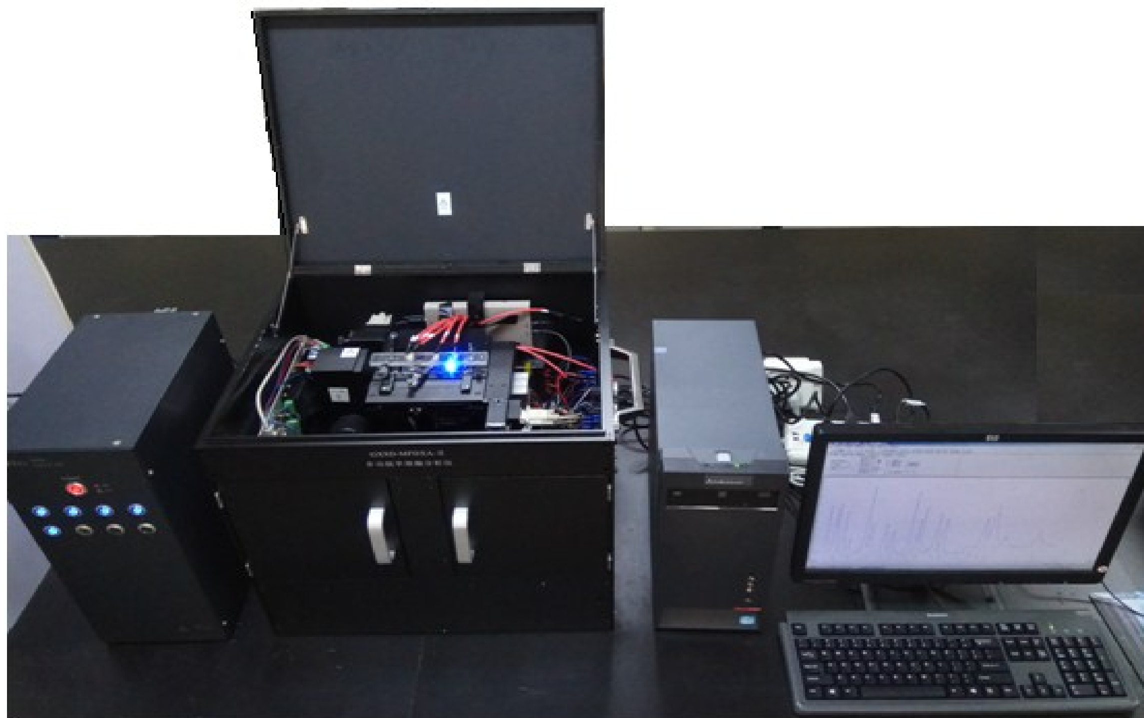

Figure S1. The MCE-LIF detection system developed by our laboratory.

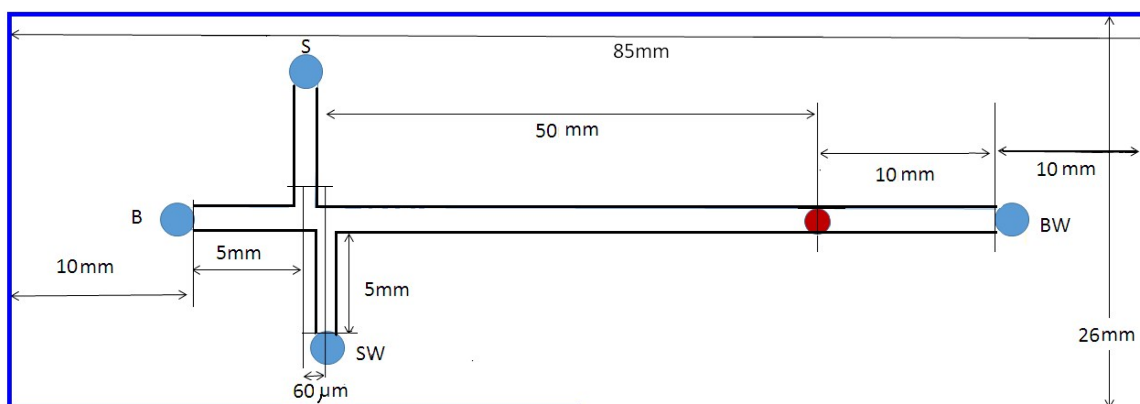

Figure S2. Dimensions and layout of the glass microchip. S: sample reservoir; B: buffer reservoir; SW: sample waste reservoir; BW: buffer waste reservoir; R: the oxidizer reagent reservoir.

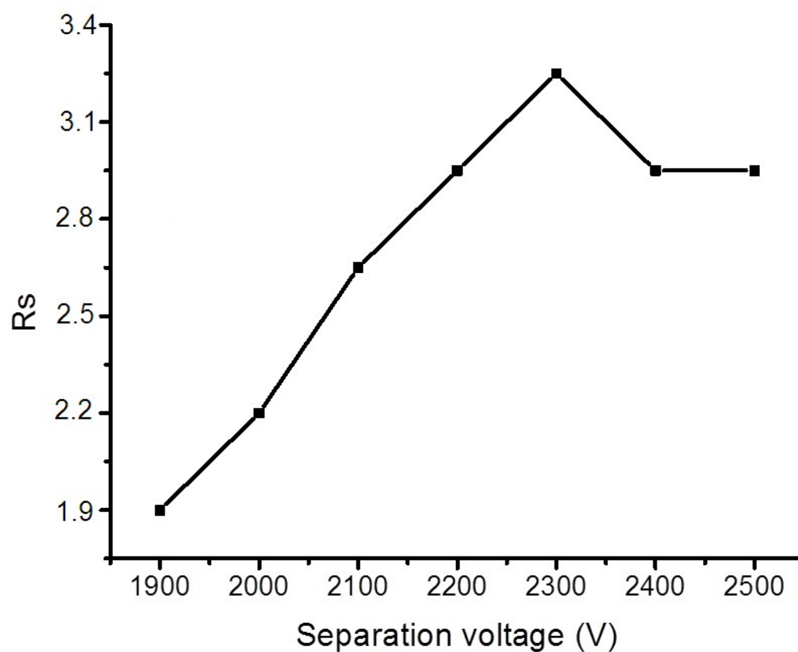

Figure S3. Influence of application voltage on the separation. The electrophoresis buffer is 30 mM borate solution at pH 9.2 with 30 mM SDS.

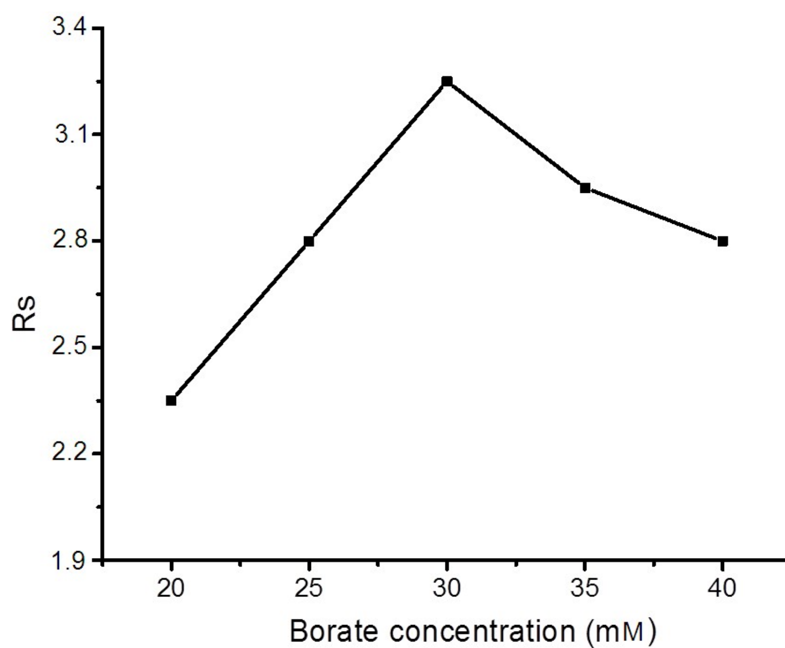

Figure S4. Influence of electrophoresis buffer concentration on the separation. The electrophoresis buffer comprises of borate solution of different concentrations at pH 9.2 and 30 mM SDS.

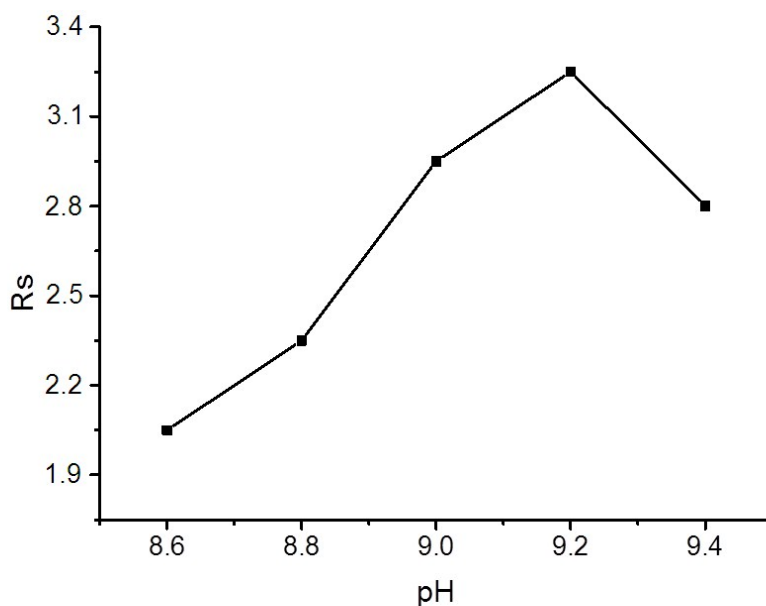

Figure S5. Influence of electrophoresis buffer pH on the separation. The electrophoresis buffer comprise of 30 mM borate and 30 mM SDS.

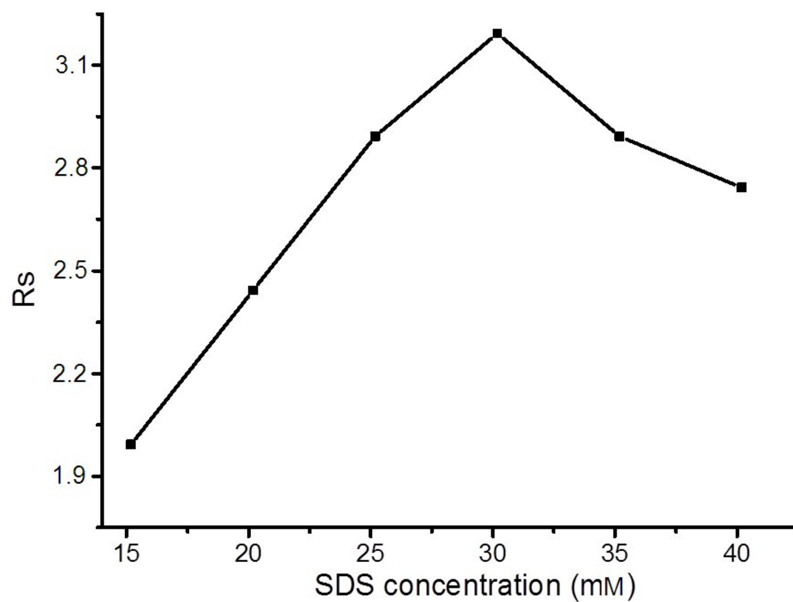

Figure S6. Influence of SDS concentration on the separation. The electrophoresis buffer contained 30 mM borate solution at pH 9.2, with different concentrations of SDS.

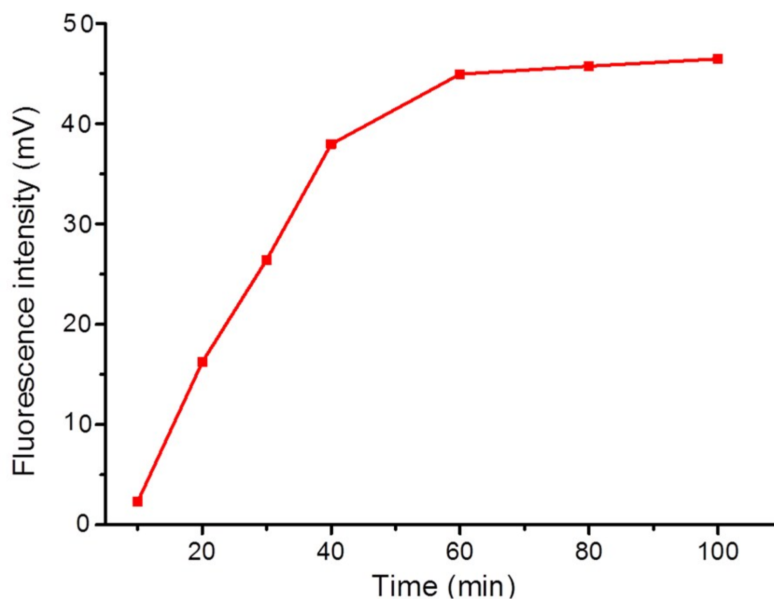

Figure S7. Influence of incubation time on the fluorescence intensity. $C_{P1/P2}$=2.0×10^{-9} M; C_{miRNA} = 2.0×10^{-11} M and C_{MB}= 5.0×10^{-9} M. The amount of T7 Exo is 25 U.

Table S1. DNA/RNA fragment sequences used in this work*

Name	Sequences (5'to 3') description
miRNA-141	UAACACUGUCUGGUAAAGAUGG
P1	*CCATCTTTACCAGACAGTG*AGGGTTAAAA
P2	CCCGCTAACCCTCACTGTCTAAAA
MB	FAM-TTAGACAGTGAGGGTTAGGTTACCTCACTG
miR-141-1a	UAACACUGUCUGGUAAAGAUG**A**
miR-141-1b	UAACACUGUCUGGUA**C**AGAUGG
miR-141-1c	UAACACUGUC**A**GGUAAAGAUGG
miR-141-2a	UAACACUGU**A**UGG**C**AAAGAUGG
miR-141-2b	**U**AACAC**G**GUCUGGUA**C**AGAUGG

* The italicized sequences of miRNA-141 and P1 are completely complementary. The underlined sequences of P1 and P2 are complementary and hybridizable, and the red sequence of P2 is complementary and hybridizable with MB. The underlined sequence in MB is its stem.

Table S2. Comparison of different methods for miRNA detection

Methods	Detection limit	Dynamic range	References
T 7 exonuclease-assisted cyclic enzymatic amplification method coupled with rolling circle amplification	12 fM	25 fM-1 pM	1
WS_2 nanosheet mediated fluorescence quenching and duplex-specific nuclease signal amplification	300 fM	1 pM-10 nM	2
Hairpin probe-based circular exponential amplification assay	380 fM	1 pM -10 nM	3
Non-enzymatic target recycling amplification	80 fM	0.1 pM-10 nM	4
Target-primed and branched rolling-circle amplification	10 fM	25 fM -2.5 pM	5
Target induced $adenosine_2$–coralyne–$adenosine_2$ formation	0.53 nM	0 -100 nM	6
Target-fueled DNA walker	58 fM	100 fM-1 nM	7
MCE assay based on separation assisted double cycling signal amplification strategy	8 fM	20 fM–20 pM	This work

Reference

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