Supporting information

Highly sensitive and multiplexed miRNAs analysis based on digitally encoded silica microparticles coupled with RCA-based cascade amplification

Shengquan Liu [a], Han Fang[a], Chengjiao Sun[a], Nana Wang[a], Jiong Li*[a]

a. Key Laboratory for Nano-Bio Interface Research, Nano-Bio-Chem Centre, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Suzhou, 215123, China.

*Corresponding authors: Jiong Li

E-mail: jli2006@sinano.ac.cn

Telephone: +86-512-62872598

Table S1. Sequences of oligonucleotides and miRNAs

Name	Sequence (5′ to 3′)
Padlock probe-Let-7a	p- CTACT ACCTC A GGAG ATGAG TGACT GACGA GCTGA GGCCT ATGTG CATTC GCTGA GG A AC TAT AC AAC
Capture probe-Let-7a	NH_2-AAAAAAAAAAAGGCCTATGTG CATTCGCTGA TGCGACCT
Padlock probe-MiRNA-21	p- CTG ATAAGCTA GGAGATGAGT GACTGACGA G CTGAGG G CAT TCTCCATGT G CTGAGG TCAA C ATCAGT
Capture probe-miR-21	NH_2-AAAAAAAAAAA GGGCATTCTC CATGTGCTGATGCGACCT
RCA primer	TCG TCA GTCACT CA TCTCC
UT	Cy3-AGGTCGCA
Designed sequence c for let-7a	TCAGC GAATG CACAT AGGCC
Let-7a	U GAGGU AGUAG GUUGUAUAGUU
Let-7b	UGAGGUAGUAGGUUGUGUGGUU
Let-7c	UGAGGUAGUAGGUUGUAUGGUU
MiRNA-21	UAGCUUAUCAGACUGAUGUUGA’

Table S2. The Comparison of Existing Amplification Methods for miRNA analysis

Detection method	LOD	Dynamic range	References
RCA	10 pM	-	1
Branched RCA	18 fM	3	2
Dumbbell probe-mediated RCA (D-RCA)	1 fM	8	3
RCA-assisted NESA	100 fM	4	4
P-ERCA	0.2 aM	3	5
Loop mediated isothermal amplification (LAMP)	1 pM	7	6
Exponential amplificaiton reaction (EXPAR)	0.1 aM	10	7
Duplex specific nuclease signal amplfication	100 fM	5	8
RCA based cascade amplification	0.5 fM	5	This work

References:

1. S. P. Jonstrup, J. Koch and J. Kjems, *RNA*, 2006,**12**, 1747.

2. Y. Cheng, X. Zhang, Z. Li, X. Jiao, Y. Wang and Y. Zhang, *Angew. Chem. Int. Ed.*, 2009, **48**, 3268.

3. Y. T. Zhou, Q. Huang, J. M. Gao, J. X. Lu, X. Z. Shen and C. H. Fan, *Nucleic Acids Res*, 2010, **38**, e156.

4. J. J. Li, Y. Z. Chu, B. Y. Lee and X. S. Xie, *Nucleic Acids Res.* 2008,**36**, e36.

5. H. Y. Liu, L. Li, L. L. Duan, X. Wang, Y. X. Xie, , L. L. Tong, Q. Wang and B. Tang, *Anal. Chem.* 2013,**85**, 7941.

6. C. Li, Z. Li, H. Jia and J. Yan, *Chem. Commun.*, 2011,**47**, 2595.

7. H. Jia, Z. Li, C. Liu and Y. Cheng, *Angew. Chem. Int. Ed.* 2010,**49**,5498.

8. B. Yin, Y. Liu and B. Ye, *J. Am. Chem. Soc.* 2012,**134**, 5064.

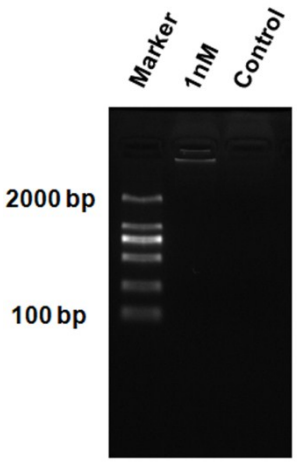
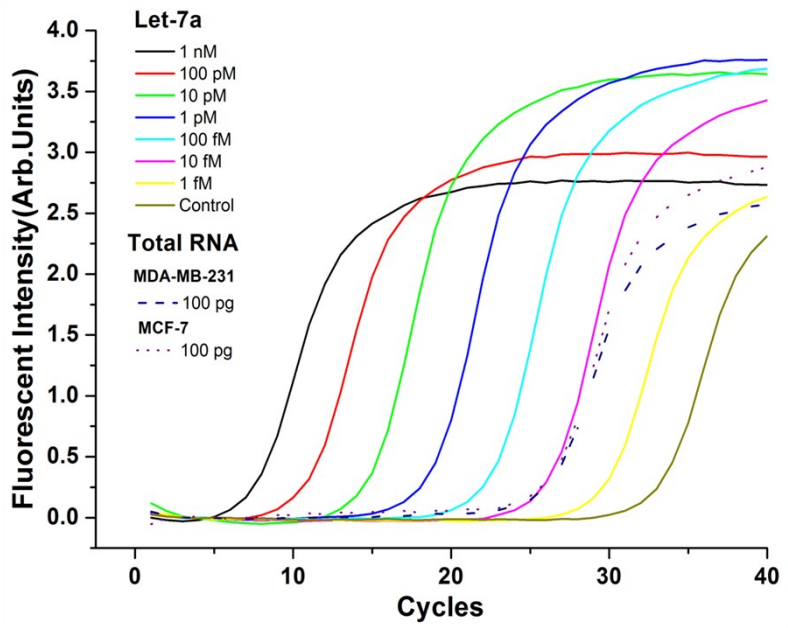


Fig. S1 Agarose gel electrophoresis image of the RCA product, with let-7a of 1 nM and DNA marker of 100–2000 bp.

Fig. S2

A



B

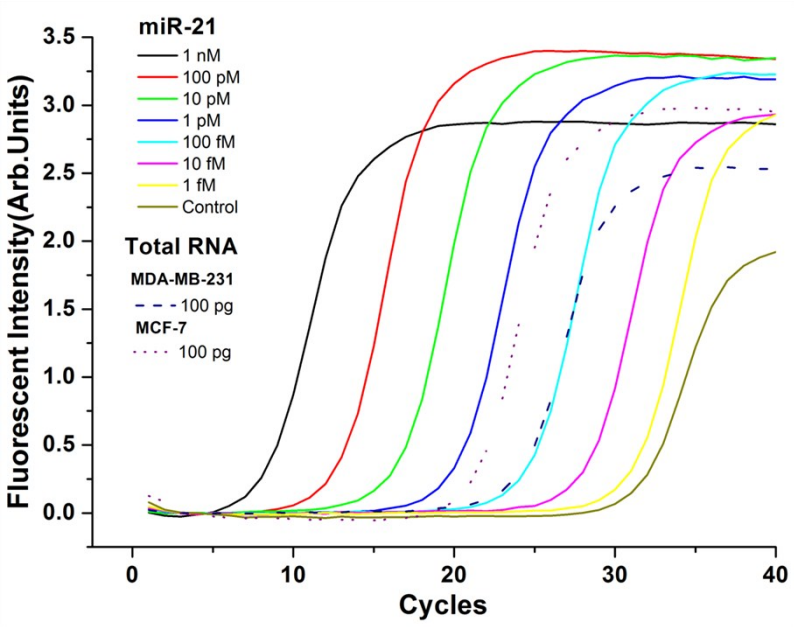


Fig. S2 (A) qRT-PCR analysis of let-7a with synthetic standards of concentrations ranging from 1 fM to 1 nM, and 100 pg total RNA from MDA-MB-231 and MCF-7 cell lines. (B) qRT-PCR analysis of miR-21 with synthetic standards of concentrations ranging from 1 fM to 1 nM, and 100 pg total RNA from MDA-MB-231 and MCF-7 cell lines.