Electronic Supplementary Information

A reverse transcription-free real-time PCR assay for rapid miRNAs quantification based on effects of base stacking

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Experimental Section

Sequences of mature and precursor miRNAs including the let-7 family, mir-21, mir-23a and mir-29b were selected from the Sanger Center miRbase (http://microrna.sanger.ac.uk/sequences), and sequence of U6 was obtained from NCBI database (http://www.ncbi.nlm.nih.gow). Synthetic mature miRNAs were purchased from Shanghai GenePharm. All of the DNA primers were purchased from Invitrogen. Oligonucleotides were purified by polyacrylamide gel electrophoresis. Real-time PCR was performed on LightCycler ® 480 Real-Time PCR Instrument (Roche). The 10 μL PCR usually included 5 μL 2×RT-PCR Master Mix (Brilliant III SYBR Green QPCR Master Mix, Agilent Technologies), 1 μL target RNA, 0.14 μM universal primer, 0.7 μM specific primer. The reactions were incubated in a 96-well plate at 95 °C for 3 min, followed by 35 cycles of 95 °C for 10 s, 50–56 °C for 10 s and 72 °C for 10 s. U6 snRNA served as endogenous control to normalize expression of miRNAs. All reactions were run in triplicate and all solutions were prepared in DEPC-treated deionized water (TaKaRa Biotechnology Co.Ltd.)

Total RNA was extracted from two cell lines by use of AccuZol reagent (Bioneer, Korean) according to the manufacturer's instructions. In brief, AccuZolTM (1 mL per 10 cm^2 of culture dish area) was added to lyse cells directly in the culture dish. After that, add 200 μL of chloroform per 1 mL of AccuZolTM and shake vigorously for 15 s and incubated at room temperature on ice for 5 min. The samples were centrifuged at 12, 000 g for 15 min at 4 °C and the upper aqueous phase were carefully removed without disturbing the lower phase into a fresh tube. The same

volumes of isopropyl alcohol were added and mixed well. The samples were incubated at 15–30 °C for 20 min and centrifuged at 12, 000 g at 4 °C for 10 min. Following centrifugation, the supernatants were removed completely and RNA pellet was washed with 80% ethanol. Finally, RNA pellet was air dried and eluted in nuclease-free water. The two cell lines HLF and A549 were obtained from Professor Yimin Zhu's lab (Suzhou institute of Nano-tech and Nano-bionics, Chinese Academy of Sciences, Suzhou, China). Further small RNAs enrichment was unnecessary in our experiments. The sequences of RNA and DNA oligonucleotides used were listed as follows:

Primer name	sequence
P-U-3	5′-CACACACACACACACACGCA-3′
P-U-4	5′-CACACACACACACACACCGCA-3′
P-U-5	5′-CACACACACACACACACTCGCA-3′
P-U-6	5′-CACACACACACACACACGTCGCA-3′
P-U-7	5′-CACACACACACACACACGGTCGCA-3′
mir-21-3	5′-TCAACATCAGTCTGATAAGCTATGC-3′
mir-21-4	5′-TCAACATCAGTCTGATAAGCTATGCG-3′
mir-21-5	5′-TCAACATCAGTCTGATAAGCTATGCGA-3′
mir-21-6	5′-TCAACATCAGTCTGATAAGCTATGCGAC-3′
mir-21-7	5′-TCAACATCAGTCTGATAAGCTATGCGACC-3′
Let-7f-5	5′-AACTATACAATCTACTACCTCATGCGA-3′
Let-7a-5	5′-AACTATACAACCTACTACCTCATGCGA-3′
Let-7b-5	5′-AACCACACAACCTACTACCTCATGCGA-3′
Let-7d-5	5′-AACTATGCAACCTACTACCTCTTGCGA-3′
mir-23a-5	5′-GGAAATCCCTGGCAATGTGATTGCGA-3′
mir-29b-5	5′-AACACTGATTTCAAATGGTGCTATGCGA-3′
106a-5	5′-CTACCTGCACTGTAAGCACTTTTTGCGA-3′
17-5	5′-CTACCTGCACTGTAAGCACTTTGTGCGA-3′
U6-1-5	5′-TGTGCTGCCGAAGCGAGCACTGCGA-3′

Micro-RNA	sequences
let-7a	5′-UGAGGUAGUAGGUUGUAUAGUU-3′
let-7b	5′-UGAGGUAGUAGGUUGUGUGGUU-3′
let-7d	5′-AGAGGUAGUAGGUUGCAUAGUU-3′
let-7f	5′-UGAGGUAGUAGAUUGUAUAGUU-3′

mir-21	5′-UAGCUUAUCAGACUGAUGUUGA-3′
mir-17	5′-CAAAGUGCUUACAGUGCAGGUAG-3′
mir-106a	5′-A AAAGUGCUUACAGUGCAGGUAG-3′

Amplification efficiency produced by different lengths of bridging sequence

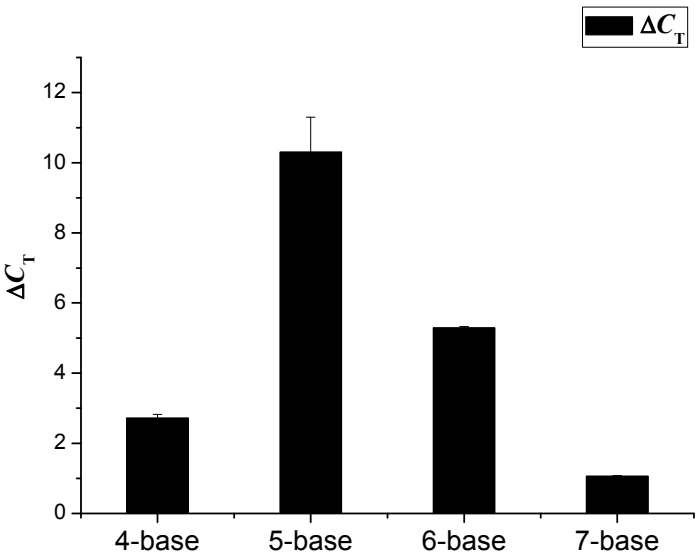

Fig. S1. Comparison of ΔC_T values **(**targets versus negative control) among 4–7 mer of bridging sequences. The reactions were performed with 5 μl 2×RT-PCR Master Mix (Brilliant III SYBR Green QPCR Master Mix, Agilent Technologies), 5 nM target RNA (mir-21), 0.14 μM universal primer (P-U-4, P-U-5, P-U-6, P-U-7, respectively), 0.7 μM miRNA specific-primer (mir-21-4, mir-21-5, mir-21-6, mir-21-7, respectively)

Distinguishing mir-17 and mir-106a

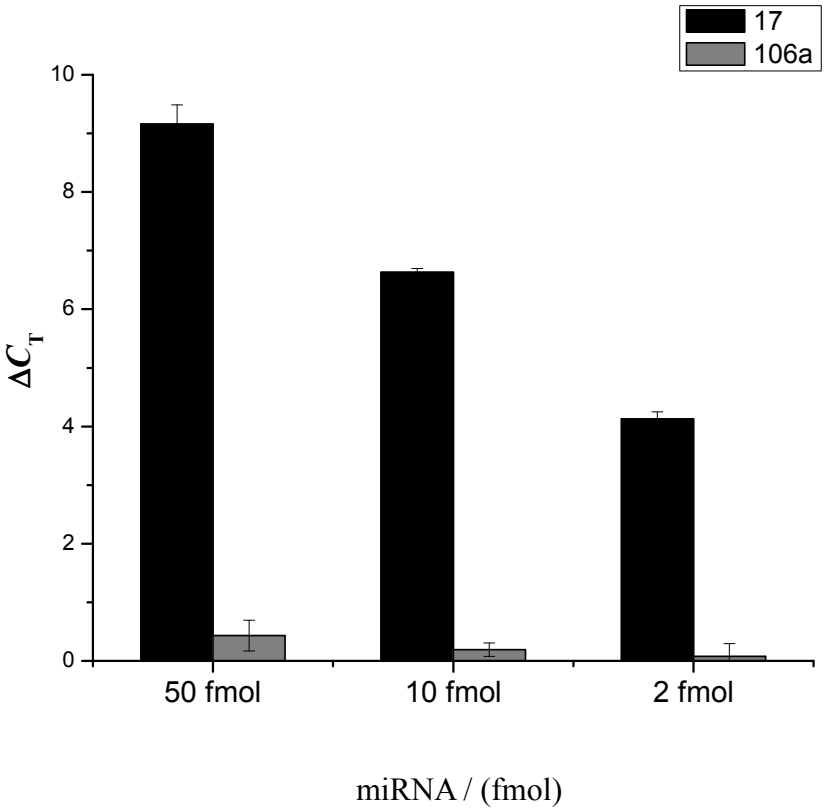

Fig. S2. The detection of 5' end single base mismatch based on base stacking effects. 2, 10, 50 fmol of synthetic mir-17 miRNA was used to estimate the specificity of the reverse transcription-free real-time PCR ΔC_T values ($\Delta C_T = C_{T\ \text{negative control}}, - C_{T\ 17},$), respectively.