

Electronic supplementary information (ESI)

MicroRNA Detection in Twenty Minutes Using Power-Free Microfluidic Chip: Coaxial Stacking Effect Enhances the Sandwich Hybridization

Hideyuki Arata, Hiroshi Komatsu, Aishan Han, Kazuo Hosokawa and Mizuo Maeda

1. Calculation of the melting temperatures (T_m) concerning miR-21

We used the HyTher program provided on the website by Prof. SantaLucia, Jr.'s lab at Wayne State University.

<http://ozone3.chem.wayne.edu/cgi-bin/login/login/loginVerify.cgi>.

Chemical conditions

Monovalent cation : 0.825 M (Na⁺ contained in 5×SSC)

Top strand (miR-21): 1 nM

Bottom strand (probe DNAs): 1 μM

Sequences (5' to 3')

Sandwich hybridization and full duplex

	Sandwich hybridization	MiR-21Flu/ Pro21full
Top strand	uag cuu auc aga cug aug uug a	uag cuu auc aga cug aug uug a
Bottom strand	tca aca tca gt s ctg ata agc ta (s: coaxial stacking)	tca aca tca gtc tga taa gct a
T_m	73.4 °C	70.7 °C

Hybridization with individual probe DNAs

	MiR-21/ProFlu21	MiR-21/ Pro21
Top strand	uag cuu auc ag	acu gau guu ga
Bottom strand	ctg ata agc ta	tca aca tca gt
T_m	37.6 °C	42.0 °C

2. Experiment with a gap base between the two probes

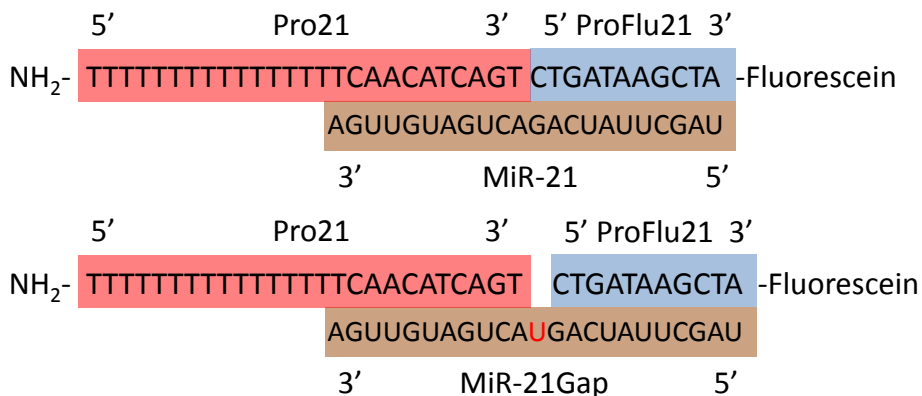


Fig. S1 Construction and sequences of sandwich hybridization by natural miR-21 (top, the results are described in the main text.) and sandwich hybridization by an artificial variant of miR-21 with additional U base at the center of its original sequence (MiR-21Gap). The additional base induces a single base gap between the two probe DNAs (bottom).

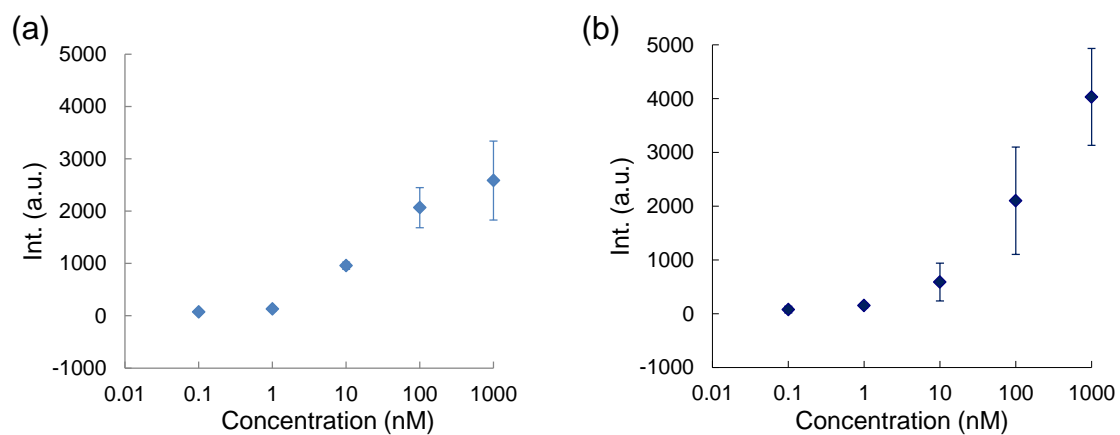


Fig. S2 Detection of miR-21Gap. Fluorescent intensity vs. miRNA concentration by (a) protocol 1 and (b) protocol 2, which are the same data as in the main text.