

Electronic Supplementary Information

Highly improved specificity for hybridization-based microRNA detection by controlled surface dissociation

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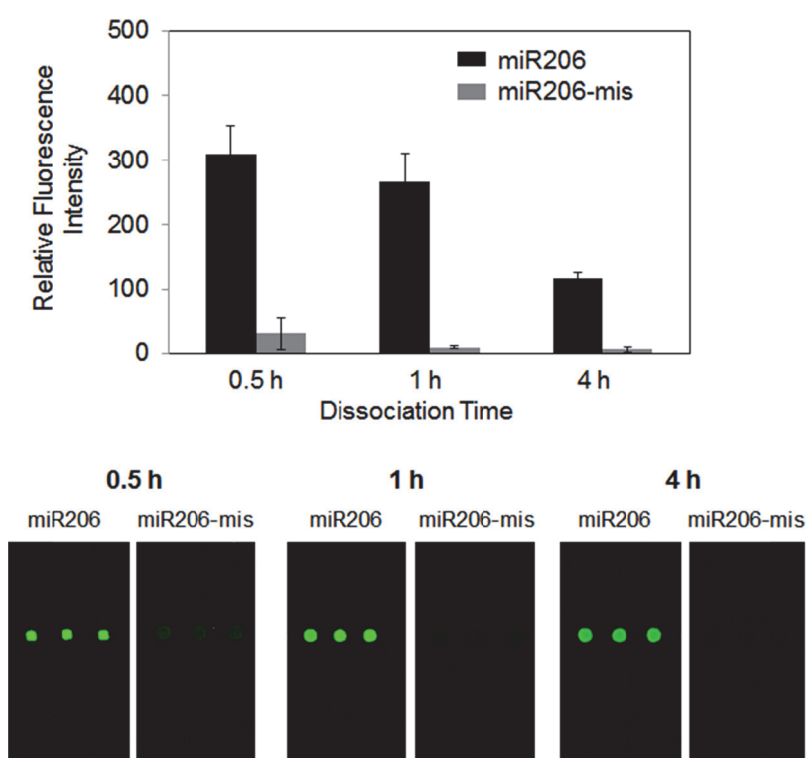


Fig. S1 Specific signals for miR206 (black bars) and miR206-mis (gray bars) against the miR206-specific DNA capture probe obtained by various surface dissociation time at 57 °C under low salt concentration. Fluorescence images of this experiment are shown under the graph.

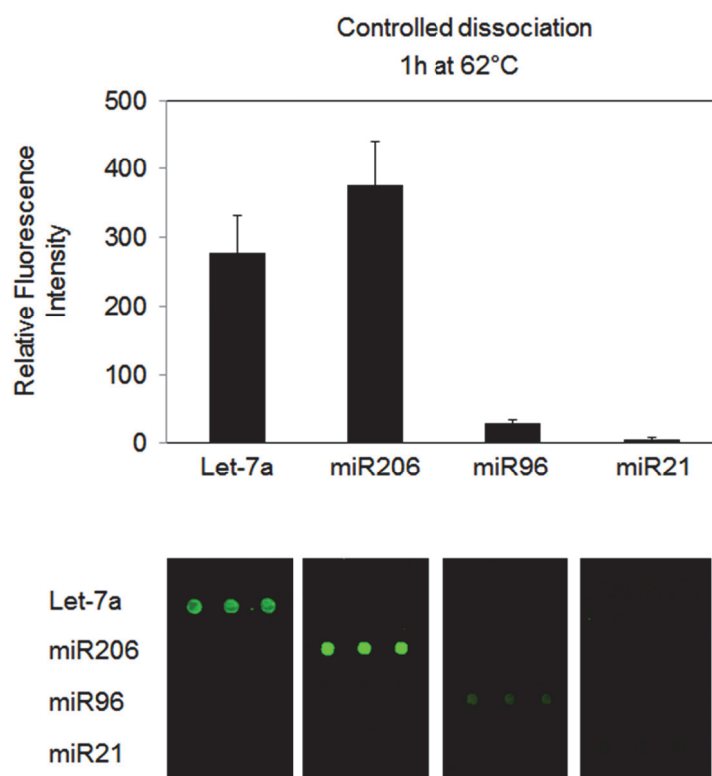


Fig. S2 Fluorescence signals of four target miRNAs (Let-7a, miR21, miR96, and miR206) against complementary DNA capture probes at the same dissociation condition (1 h at 62 °C) after low-stringent hybridization at 37 °C for 16 h. Fluorescence images of four target miRNAs are shown under the graph.

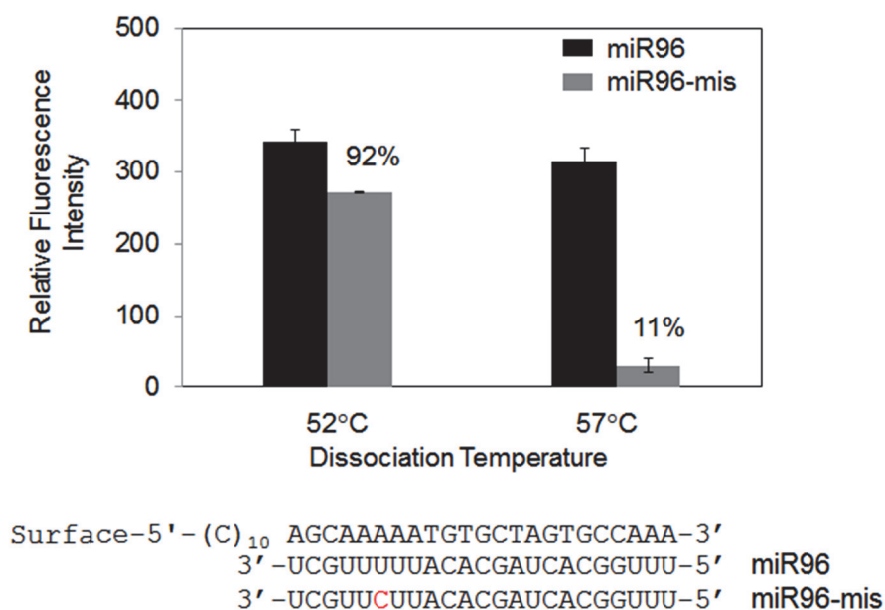


Fig. S3 Specific fluorescence signals for miR96 (black bars) and single base mismatched miR96-mis (gray bars) against the miR96-specific DNA capture probe obtained by surface dissociation (1 h) at various temperatures after low-stringent hybridization at 37 °C. Cross-hybridization levels of miR96-mis to miR96-specific capture probe are indicated. The miR96-specific DNA capture probe and miR96 miRNAs are given under the graph.

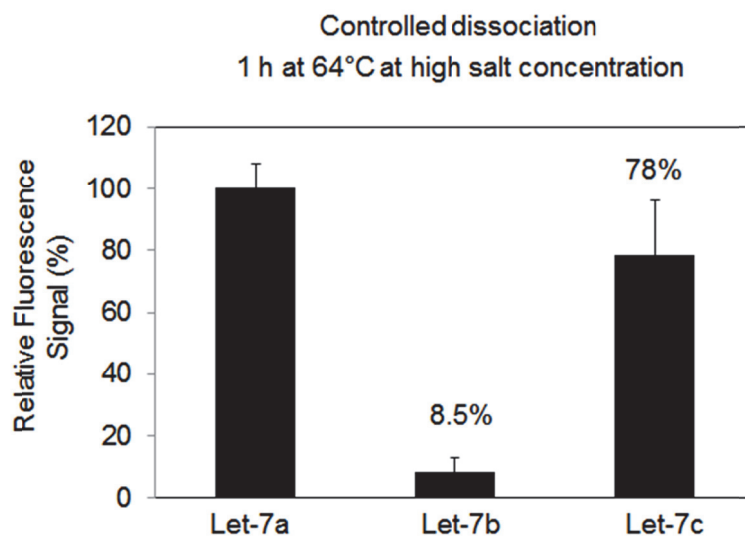


Fig. S4 Relative fluorescence signals (%) of three Let-7 family miRNAs (Let-7a, Let-7b, and Let-7c) by using Let-7a-specific LNA-modified probe. MiRNAs were first hybridized to the LNA probe at 42 °C for 16 h during equilibrated hybridization, and washed at 64 °C for 1 h under a 2X SSC washing buffer solution.

Discussion: Washing stringency was increased for optimal discrimination condition for Let-7a detection. Here lowered salt concentration of the washing solution at the same condition (64 °C for 1 h) afforded highly specific Let-7a detection against Let-7c as shown in Figure 4 in the text.

Determination of optimal discrimination (dissociation) condition: To determine optimal dissociation conditions for specific miRNA detection with various capture probes, washing temperature can be first optimized with fixed salt concentration (2X SSC) and duration (1 h). Dissociation step of 1 h generally afforded high specificity for miRNA detection. In addition, this duration (1 h) was practically reliable, whereas shorter washing steps cause higher variations and longer incubations reduced overall miRNA signals. Higher temperature than

64 °C for stringent dissociation, however, is practically difficult to maintain and therefore caused high variations for miRNA detections. In this case, higher stringency can be obtained by reducing salt concentration as we described above in miRNA detection with LNA modified probes.