Supplementary Data

A Simple and Highly Sensitive Fluorescence Assay for MicroRNAs

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Cell culture

The culture condition for the cell lines is: 5% CO\textsubscript{2}/95% air, 37°C. The growth medium for each cell line is as follows.

HeLa: ATCC-formulated Eagle's Minimum Essential Medium (Catalog No. 30-2003) + 1% penicillin-streptomycin + 10% (vol/vol) fetal bovine serum (FBS)

H1299 (Lung cancer cell): ATCC-formulated RPMI-1640 Medium (Catalog No. 30-2001) + 1% (vol/vol) penicillin-streptomycin + 10% (vol/vol) fetal bovine serum (FBS)

MRC-5 (Normal cell): ATCC-formulated Eagle's Minimum Essential Medium (Catalog No. 30-2003) + 1% penicillin-streptomycin + 10% (vol/vol) fetal bovine serum (FBS)

First, remove and discard the culture medium. Second, rinse the cell with 0.25% (w/v) typsin-0.53mM EDTA solution. Add 3 mL of Trypsin-EDTA solution to 75 mL flask and observe cells under an inverted microscope until cells are dispersed (~10min). Then, add 7 mL of growth medium and aspirate cells by gently pipetting. Lastly, transfer aliquots of the cell suspension to new flask and incubate at 37°C.\textsuperscript{1-3}

MiRNA extraction

MiRNA extraction from the harvested cells used the miRNeasy Micro Kit from Qiagen (Germany). The extraction procedure followed the manufacturer’s quick-start protocol\textsuperscript{4} and handbook\textsuperscript{5} of miRNeasy Micro Kit.

First, add 700 μL QIAzol Lysis Reagent to the harvested cells and vortex for 1 min to disrupt and homogenize the cells. Incubate at r.t. for 5 min. Add 140 μL chloroform and vortex vigorously for 15s, and place on the benchtop at r.t. for 3min. Centrifuge at 4 °C and transfer the upper aqueous phase to a new tube. Second, add 525 μL 100% ethanol and mix well by pipetting, transfer the sample to the RNeasy MinElute spin column in a 2 mL collection tube. Centrifuge for 15s at r.t. Discard the flow-through. Third, pipet 500 μL buffer RPE onto the RNeasy MinElute spin column to wash and centrifuge. Discard the flow-through again. Fourth, pipet 500 μL of 80% ethanol onto the RNeasy MinElute spin column to wash. Discard the collection tube with the flow-through. Fifth, place the RNeasy MinElute spin column into a new 2 mL collection tube, open the lid of the spin column, centrifuge to dry the membrane. Discard the collection tube with the flow-through. Finally, place the
RNeasy MinElute spin column in a new 1.5 mL collection tube. Add 10 μL RNase-free water directly to the center of the membrane. Centrifuge to elute the RNA, and then freeze the RNA for later use.

**MiRNA quantification**

MiRNA quantification was achieved by the quantitative reverse transcription-PCR (qRT-PCR) using NCode™ EXPRESS SYBR® GreenER™ miRNA qRT-PCR Kits Universal, which includes NCode™ VILO™ miRNA cDNA Synthesis Kit, EXPRESS SYBR® GreenER™ qPCR SuperMix Universal and ROX Reference Dye (Life technologies). The quantification procedure followed the instruction provided by the manufacturer.⁶

Using the NCode™ VILO™ miRNA cDNA Synthesis Kit to synthesize the cDNA:

First, combine 5x Reaction Mix, 10x SuperScript Enzyme Mix, total RNA, and top up to 20 μL by adding DEPC-treated water in a tube on ice. Second, vortex to mix and incubate tube at 37°C for 60 min to polyadenylate and reverse-transcribe miRNA, and the first-strand cDNA was synthesized. Last, terminate the reaction at 95°C for 5 min. Hold the reaction at 4°C until use.

Using the EXPRESS SYBR® GreenER™ qPCR SuperMix Universal to quantify miRNA:

First, combine EXPRESS SYBR GreenER Qpcr SuperMix Universal, 10 μM miRNA-specific forward primer, 10 μM Universal qPCR Primer, 25 μM ROX Reference Dye, undiluted cDNA, and top up to 20 μL by adding DEPC-treated water in a tube on ice. Second, prepare no-template control to test for DNA contamination of the enzyme/primer mixes, and gently mix well. Last, put into the real-time instrument, run the program, collect data and analyze the results.

Cycling program: 50°C for 2 min → 95°C for 2 min → 40 cycles of 95°C for 15 s and 60°C for 1 min.
Table S-1. Sequences of synthetic oligonucleotides used in the project

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’- 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe</td>
<td>5’-/5Biosg/T_{9} AAC TAT ACA ACC TAC TAC TCT AT_{36}-FAM/-3’</td>
</tr>
<tr>
<td>Let-7a (miRNA)</td>
<td>5’-UGA GGU AGU AGG UUG UAU AGU U-3’</td>
</tr>
<tr>
<td>Let-7f (miRNA)</td>
<td>5’-UGA GGU AGU AGA UUG UAU AGU U-3’</td>
</tr>
<tr>
<td>Let-7d (miRNA)</td>
<td>5’-AGA GGU AGU AGG UUG CAU AGU U-3’</td>
</tr>
<tr>
<td>dT21</td>
<td>5’-TTT TTT TTT TTT TTT TTT-3’</td>
</tr>
</tbody>
</table>

Table S-2. Composition of all buffers used in the project

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1× Binding and Washing Buffer (1× B&amp;W)</td>
<td>5.0 mM Tris-HCl, 0.5 mM EDTA, 1.0 M NaCl, pH 7.5</td>
</tr>
<tr>
<td>2× Binding and Washing Buffer (2× B&amp;W)</td>
<td>10 mM Tris-HCl, 1.0 mM EDTA, 2.0 M NaCl, pH 7.5</td>
</tr>
</tbody>
</table>
| Hybridization Buffer (for Mg\textsuperscript{2+} Optimization) | 50 mM Tris-HCl, pH 7.54:  
Vary concentration of MgCl\textsubscript{2} in buffer from 5.0 to 40 mM |
| Hybridization Buffer (for pH Optimization)  | 50 mM Tris-HCl, 25 mM MgCl\textsubscript{2}:  
Vary pH of buffer from 6.5 to 9.0               |
| Optimized Hybridization Buffer              | 50 mM Tris-HCl, 25 mM MgCl\textsubscript{2}, pH 8.0 |
Figure S-1. The fluorescence intensities of the remained BF oligonucleotides solution before and after linking BF oligonucleotides to the MBs.

Figure S-2. Optimization of DSN dosage from 0.01 U to 0.5 U. Condition: 20 mM Mg$^{2+}$, pH 7.5, 55°C, 30 min incubation.