Electronic Supplementary Material (ESI) for Analyst.

Supplementary Data

A Simple and Highly Sensitive Fluorescence Assay for MicroRNAs

Wei Shen, Kiat Huei Yeo, and Zhiqiang Gao*

Department of Chemistry, National University of Singapore, Singapore 117543.

* Corresponding author, Tel: +65 6516 3887, Fax: +65 6779 1691, e-mail: chmgaoz@nus.edu.sg

Cell culture

The culture condition for the cell lines is: 5% CO_2/95% air, 37℃. 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℃.[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[4] and handbook[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 ℃ and transfer the upper aqueous phase to a new tube. Second, add 525 μL100% 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 μLRNase-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℃ for 60 min to polyadenlyate and reverse-transcribe miRNA, and the first-strand cDNA was synthesized. Last, terminate the reaction at 95℃ for 5 min. Hold the reaction at 4℃ 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℃ for 2 min → 95℃ for 2 min →40 cycles of 95℃ for 15 s and 60℃ for 1 min.

Table S-1. Sequences of synthetic oligonucleotides used in the project

Name	Sequence (5'- 3')
Probe	5'-/5Biosg/T_9 AAC TAT ACA ACC TAC TAC CTC AT_9/36-FAM/-3'
Let-7a (miRNA)	5'-UGA GGU AGU AGG UUG UAU AGU U-3'
Let-7f (miRNA)	5'-UGA GGU AGU AG**A** UUG UAU AGU U-3'
Let-7d (miRNA)	5'-**A**GA GGU AGU AGG UUG **C**AU AGU U-3'
dT21	5'-TTT TTT TTT TTT TTT TTT TTT-3'

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

Buffer	Composition
1× Binding and Washing Buffer (1× B&W)	5.0 mM Tris-HCl, 0.5 mM EDTA, 1.0 M NaCl, pH 7.5
2× Binding and Washing Buffer (2× B&W)	10 mM Tris-HCl, 1.0 mM EDTA, 2.0 M NaCl, pH 7.5
Hybridization Buffer (for Mg^{2+} Optimization)	50 mM Tris-HCl, pH 7.54: Vary concentration of $MgCl_2$ in buffer from 5.0 to 40 mM
Hybridization Buffer (for pH Optimization)	50 mM Tris-HCl, 25 mM $MgCl_2$: Vary pH of buffer from 6.5 to 9.0
Optimized Hybridization Buffer	50 mM Tris-HCl, 25 mM $MgCl_2$, pH 8.0

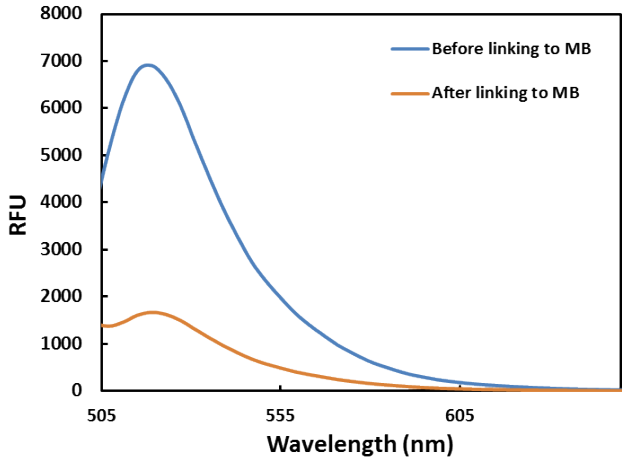

Figure S-1. The fluorescence intensities of the remained BF oligonucleotides solution before and after linking BF oligonucleotides to the MBs.

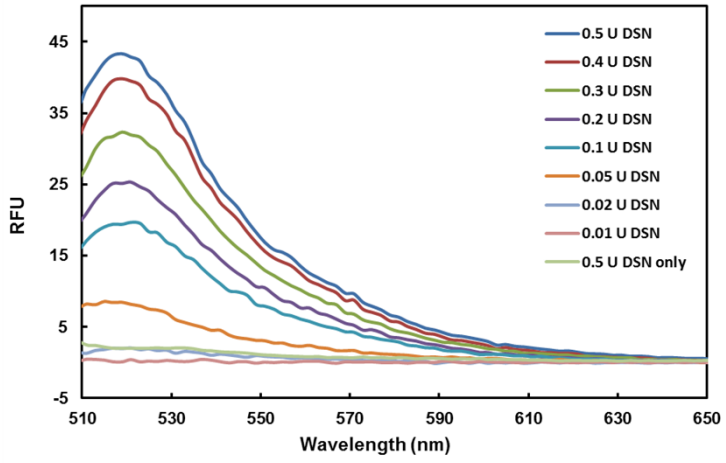

Figure S-2. Optimization of DSN dosage from 0.01 U to 0.5 U. Condition: 20 mM Mg^{2+}, pH 7.5, 55℃, 30 min incubation.

1. HeLa. http://www.atcc.org/Products/All/CCL-2.aspx#culturemethod (accessed 9th September, 2014).
2. H1299. http://www.atcc.org/products/all/CRL-5803.aspx#culturemethod (accessed 9th September, 2014).
3. MRC-5. http://www.atcc.org/Products/All/CCL-171.aspx#culturemethod (accessed 9th September, 2014).
4. Protocol. http://www.qiagen.com/resources/resourcedetail?id=a7967938-edf9-4d36-a9a3-9a3d08d2c50f&lang=en (accessed 9th September, 2014).
5. Handbook. http://www.qiagen.com/resources/resourcedetail?id=9dfe7ebc-45a2-4b1e-9ea3-d7c5ac264e00&lang=en (accessed 9th September, 2014).
6. NCode™ EXPRESS SYBR® GreenER™ miRNA qRT-PCR Kit universal. http://www.lifetechnologies.com/order/catalog/product/A11193051?ICID=search-productMI (accessed 9th September, 2014).