Multiplexed microRNA TG-FRET assay with isothermal and amplification-free single-step

Peng Miao

Human genome encodes more than 2000 distinct microRNAs (MicroRNA database: <http://www.mirbase.org/>), a class of single-strand noncoding RNAs with a short length of 19 to 25 nucleotides [1]. They take part as regulatory factors in almost all biological processes from cell proliferation, differentiation to death. Since the alternation in the microRNA expression level can be associated with a number of pathological conditions like diabetes, cardiac hypertrophy and cancer development, actual expression levels of one or more microRNAs can be regarded as next-gen-

eration biomarkers for disease diagnosis and prognosis. Therefore, various methods and materials have been developed for the analysis of microRNAs [2].

Currently, northern blotting, *in situ* hybridizations, quantitative reverse transcription polymerase chain reaction (qRT-PCR), oligonucleotide microarrays and various electrochemical/fluorescent biosensors are the main strategies to measure aberrant microRNA expression levels. The low abundance of microRNA usually requires signal amplification steps in these methods to achieve desired sensitiv-

Figure 1 Schematic illustration of the multiplexed microRNA TG-FRET assay. Reprinted with permission from Ref. [5]. Copyright 2015, Wiley-VCH Verlag GmbH & Co. KGaA.

Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, China
Email: miaopeng@sibet.ac.cn

ity, which may demand complex procedure or equipment [3,4]. The measurement may also become time-consuming. To facilitate sensitive, convenient and multiplexed detection of microRNAs, Professor Niko Hildebrandt's group at the University of Paris-Sud has succeeded in developing a multiplexed microRNA time-gated fluorescence resonance energy transfer (TG-FRET) assay with isothermal and amplification-free single-step [5].

In this work, Prof. Hildebrandt *et al.* designed two pairs of oligonucleotides (FRET and adaptor oligos). As shown in Fig. 1, two adaptor oligos contained sequences complementary with parts of target microRNA or DNA, which could be ligated by RNA ligase or DNA ligase. Two FRET oligos were labelled with a luminescent terbium complex Lumi4-Tb (Tb) donor or a dye acceptor on adaptable polynucleotide overhangs, respectively. The remaining sequences specifically hybridized with corresponding adaptor oligos. Therefore, after mixing these oligos and target, stable FRET-adaptor oligos and semistable adaptor-target complexes with an adaptor nick were formed. Further ligation made stable double-strands, which promised photoluminescence measurement of target microRNA or DNA based on TG-FRET from Tb to dyes. Three organic dyes including Cy3.5, Cy5 and Cy5.5 were used and this single-donor/triple-acceptors approach allowed the detection of three microRNAs simultaneously.

This reported assay method does not require any washing, separation or signal amplification steps. Moreover, the oligos can be easily redesigned for other desired targets and the separation of FRET and adaptor oligos reduces the cost for versatile assays [6]. This general strategy is a successful implementation of nucleic acid analysis. Further applications may include diagnosis and prognosis of inherited diseases, drug resistance assessment, food security monitoring, etc.

**Received 9 November 2015; accepted 13 November 2015;
published online 18 November 2015**

- 1 Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res*, 2011, 39: D152–D157
- 2 Dong HF, Lei JP, Ding L, *et al.* MicroRNA: function, detection, and bioanalysis. *Chem Rev*, 2012, 113: 6207–6233
- 3 Jamali AA, Pourhassan-Moghaddam M, Dolatabadi JEN, *et al.* Nanomaterials on the road to microRNA detection with optical and electrochemical nanobiosensors. *Trac-Trends Anal Chem*, 2013, 55: 24–42
- 4 Degliangeli F, Pompa PP, Fiammengio R. Nanotechnology-based strategies for the detection and quantification of microRNA. *Chem-Eur J*, 2014, 20: 9476–9492
- 5 Jin ZW, Geißler D, Qiu X, *et al.* A rapid, amplification-free, and sensitive diagnostic assay for single-step multiplexed fluorescence detection of microRNA. *Angew Chem Int Edit*, 2015, 54: 10024–10029
- 6 Roy R, Hohng S, Ha T. A practical guide to single-molecule FRET. *Nat Methods*, 2008, 5: 507–516