描述：HaiGene 的 HG TaqMan miRNA 定量 PCR 试剂盒，采用特异性的正向 miRNA 引物和接头引物进行 PCR 扩增，检测荧光基团采用 FAM 标记的特异性 miRNA TaqMan 探针（原理见图 1）。使用该试剂盒可以特异性、高灵敏度的检测低至 100 个细胞的 miRNA 分子。HaiGene 的 TaqMan miRNA 定量 PCR 试剂盒共有一万余种，试剂盒编号为 TAPXXXXX，每一个 mircoRNA 分子对应一个检测试剂盒（包含特异性的 TaqMan 探针、正向引物、反向引物、定量试剂），可在 HG TaqMan miRNA qPCR kit.xls 中查询。如果您研究的 mircoRNA 分子不在我们的列表中，请来信咨询，我们将及时给您优化设计。

内参基因可选择使用：

（1）TaqMan RNU6B miRNA 定量 PCR 试剂盒（货号：TAP01501）适用于人、鼠等哺乳动物组织、细胞样品；

（2）TaqMan hsa-miR16 miRNA 定量 PCR 试剂盒（货号：TAP01511）适用于来源于人、鼠全血样品。

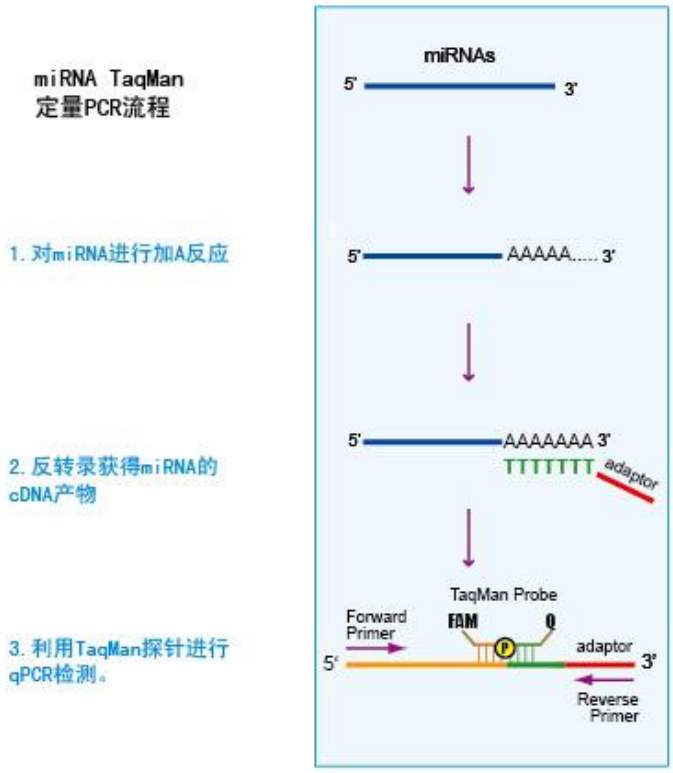


图 1，HaiGene TaqMan miRNAs 定量检测原理

组分：

名称	(100T×20 μl)
5×Golden HS TaqMan qPCR Mix	400μl
20×miRNA TaqMan Assay	100 μl

储存：避光置于-20°C，可保存 2 年；避免反复冻融

1 配制反应体系(20μl)

5×Golden HS TaqMan qPCR Mix	4 μl
20×miRNA TaqMan Assay	1 μl
*cDNA 模板	1～2.5 μl
ddH_2O	Up to 20 μl

2 进行 Real-Time PCR 反应

通常采用两步法，程序如下：

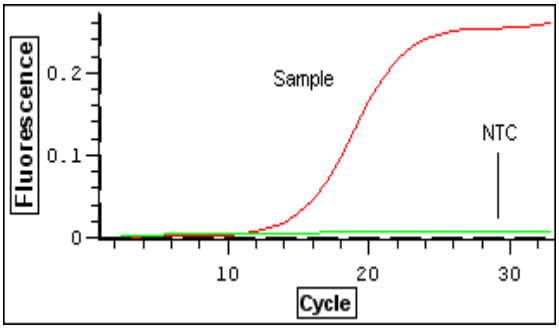
Stage 1:　95℃　15 min（热启动，不可缩短时间）

Stage 2:　95℃　10 s

60℃　30 s　40cycles

（收集信号采用 FAM 染料通道，Rox 矫正信号选择 None）

3 反应结束后确认 Real-Time PCR 的 cDNA 样品和 NTC 阴性对照的扩增曲线。



Material and Methods

The miRNA was extracted from the tissue or cell samples using Tissue&Cell microRNA Extraction Kit (HaiGene, Harbin, China) according to manufacturer's protocol. To investigate the miR-21 expression level in tissue samples, cDNA synthesis was carried out according to the protocol of TaqMan miRNA cDNA Synthesis Kit (HaiGene, Harbin, China). Briefly, the reaction mix consisting of 100 ng of miRNA, 1μl of 5×TaqMan miRNA RT Solution A, was performed to add the polyA tail at 37 ℃ for 30min, 85℃ for 5min. Followed with adding 2μl of 10×TaqMan miRNA RT Solution B, 2μl of 10×TaqMan miRNA RT Primer, and 11μl of ddH_2O, which incubated at 30 ℃ 5min, 55℃ 60min followed by enzyme inactivation at 95 ℃ for 5 minutes. Quantification of miRNA was performed by HG TaqMan miRNA PCR Kit (HaiGene, Harbin, China) . RealTime PCR was performed in 20 μl with 2 μl of cDNA product, 1 μl of miRNA TaqMan Assay, and 4μl of 5×Golden HS TaqMan qPCR Mix under the following conditions: 95℃ for 15min, 40 cycles of 95℃ for 10s, and 60℃ for 30s in LightCycler 480 (Roche). MiR-21 levels were normalized to U6B RNA levels using the 2(-ΔCt) model. Real-time PCR assays were performed in duplicate for each sample, and the mean value was used for the calculation of miRNA expression levels. The statistical analyses were done using Microsoft Excel (Microsoft) both to calculate the SD and to test for statistically significant differences between the samples using a T test. A value $P < 0.05$ was considered statistically significant.

References:

[1] Development of a robust, low cost stem-loop real-time quantification PCR technique for miRNA expression analysis. Mol Biol Rep. 2013. PMID: 23307300.

[2] Quantitative stem-loop RT-PCR for detection of microRNAs. Methods Mol Biol. 2011. PMID: 21533691.

[3] MicroRNA detection in prostate tumors by quantitative real-time PCR (qPCR). J Vis Exp. 2012. PMID: 22643910.

[4] Circulating microRNA-196a as a candidate diagnostic biomarker for chronic hepatitis C. Mol Med Rep. 2015. PMID: 25738504.

[5] Serum microRNA-21 as marker for necroinflammation in hepatitis C patients with and without hepatocellular carcinoma. PLoS One. 2011. PMID: 22066022.

[6] Diagnostic microRNA markers to screen for sporadic human colon cancer in stool: I. Proof of principle. Cancer Genomics Proteomics. 2013. PMID: 23741026.

[7] Stem-loop RT-qPCR for microRNA expression profiling. Methods Mol Biol. 2012. PMID: 22144190.

[8] Novel real-time PCR assay of microRNAs using S-Poly(T), a specific oligo(dT) reverse transcription primer with excellent sensitivity and specificity. PLoS One. 2012. PMID: 23152780.