目录

试剂盒组分和储存

货号：QPM-050	**规格: 50次反应**
货号：QPM-051	**规格: 100次反应**

U6 snRNA荧光定量校正试剂盒

U6 snRNA 荧光定量校正试剂盒包含以下组分。**请将下列组分避光储存于–20℃。**

试剂	规格		储存
	50rxns	100rxns	
荧光定量PCR反应液（2×）*	1.0ml	2.0 ml	4℃
U6 snRNA正反向引物套装（5μM）	80μl	160μl	-20℃
U6 snRNA反转录引物（10μM）	15μl	30μl	-20℃
Taq DNA聚合酶（5U/μl）	20μl	40μl	-20℃
灭菌双蒸水	1.0 ml	1.0 ml	4℃

*包含dNTP，Mg^{2+},SYBR Green I，Rox.

附属产品

附属产品

U6 snRNA荧光定量校正试剂盒中的一些试剂和其他一些配套试剂盒同样能在吉玛公司购得。订购信息如下，如需获得更多信息，请访问我们的网址(**www.genepharma.com**).

产品名称	Amount	Catalog no.
Hairpin-it™ miRNAs qPCR Quantitation Kit	20μl×50 rxns	QPM-010
	20μl×100 rxns	QPM-011

简介

综述

简介

U6 snRNA荧光定量校正试剂盒是一种灵敏特异的方法，能够校正和对总RNA样品中各个miRNA做整体的表达分析。

miRNA是动植物，病毒编码的由19-23个碱基组成的单链小RNA。成熟的miRNA会进入RNA诱导的沉默复合体（RISC）并且促使该复合体诱导mRNA的转录抑制或是特异靶mRNA的酶切降解。

U6小核RNA在剪接体的装配以及催化mRNA剪接的过程中会经历主要的构象变化，他会结合特异蛋白Prp24p和一套7个LSm2p-8p蛋白来形成U6小核核糖核蛋白复合体。而该剪接复合体在真核细胞中参与mRNA前体的剪接。

描述

用本试剂盒进行荧光定量相对校正实验包括两步，U6 snRNA逆转录反应和实时定量PCR反应。U6 snRNA的特异逆转录引物本试剂盒已经提供。

用户可以使用U6 snRNA荧光定量校正试剂盒在不同的样品间将特定的microRNA相对于U6 snRNA的表达比率标准化。

相对定量的计算基础

microRNA表达的相对定量是由实验中得到的阈值循环数（Ct）计算而来。阈值循环数是指在某一个循环时，ΔRn出现具有统计学意义的增长并且第一次被检测到。这样，在PCR反应时，含有更高初始模板浓度的样品就会比低模板浓度的样品更早到达检测阈值，并得到一个更小的阈值循环数。

一次理想的microRNA定量实验中，PCR反应的每个循环中，产物都会成倍增长。因此阈值循环数每相差1个单位就等于2倍的初始模板浓度的差距。例如，如果Ct值增加了1个单位，那么初始模板浓度就减少一倍；如果Ct值减少一个单位，那么就说明初始模板浓度增加了一倍。因此这种属性被应用于计算目标基因和内参基因之间表达的相对定量值。更多信息请参照手册中**数据分析**部分。

试剂盒组份

介绍

这部分介绍了本试剂盒中试剂更详细的信息。

荧光定量 PCR 反应液（2×）.

2×荧光定量反应液是一种独特的缓冲液体系，包含$MgCl_2$, dNTPs和SYBR Green I ，Rox染料。此反应液已经被证实能在严格的实验标准下良好工作。

注意请将 2×荧光定量反应液避光储存。

U6 snRNA 引物套装（5μM）

精心设计的 U6 snRNA 引物套装已经被证实能在严格的实验标准下良好工作，U6 snRNA 的数据信息提供如下。

Species	GeneBank Accession#	Primer Tm	Amplicon length
Homo sapiens	M14486	58~62℃	70~100 bp

U6 snRNA 逆转录引物（10μM）

精心设计的 U6 snRNA 逆转录引物，我们提供浓度为 10μM 的溶液。我们推荐用 **60nM 终浓度的逆转录引物**进行逆转录反应，即**先将逆转录引物稀释十倍至 1μM，然后取 1.2μl 至 20μl 逆转录反应体系中**。

Taq DNA 聚合酶（5U/μl）

大多数 PCR 反应中 DNA 聚合酶的终浓度通常为 0.05U/μl 每个反应。

用户自备材料

逆转录酶	进行 miRNA 的检测和定量，本试剂盒中提供的反转录引物是必需的，我们推荐使用传统的逆转录酶，如 MMLV。
RNA酶抑制剂	为了保持逆转录酶的活力，我们推荐在逆转录体系中加入 RNA 酶抑制剂。终浓度通常为 0.25 U/μl。
Rox 校正染料	为了使孔与孔之间的荧光信号标准化，我们推荐您在用 ABI PRISM 7000/7300/7500/7900, MX3000p/4000 时，往 PCR 体系中加入 Rox 校正染料。本试剂盒中的 2×荧光定量反应液中已经加入 Rox。
光学PCR板或PCR管	为了消除 PCR 管和 PCR 板对荧光信号检测的影响，我们推荐使用具有良好光学性能的 PCR 板或者 PCR 管。

方法

U6 snRNA逆转录反应操作

介绍

由于 miRNA 表达的相对定量需要一个管家基因作为内参因此，U6 snRNA 荧光定量校正试剂盒是必需的。该试剂盒是用于标准化 miRNA 相对于 U6 snRNA 的表达水平。相关的 miRNA 逆转录试剂以及 U6 逆转录试剂需要同时加入逆转录体系中，然后用荧光定量 PCR 同时检测这两种 RT 产物

U6 snRNA 逆转录引物的操作

我们推荐用 **60nM 终浓度的逆转录引物**进行逆转录反应，即**先将逆转录引物稀释十倍至 1μM，然后取 1.2μl 至 20μl 逆转录反应体系中**。

配制U6 snRNA逆转录反应体系

U6 snRNA 荧光定量校正试剂盒的模板可以是总 RNA，RNA 模板用量推荐使用至少 1μg 或更多，除了要在 42℃是保持稳定以及高逆转录效率之外，对您所选择的逆转录酶没有其他特殊的要求。我们推荐一个标准的 20μl 逆转录体系。

下列表格可作为逆转录体系的参照

Component	Final Con.	Vol /1 rxns
5×RT Buffer	1×	4μl
dNTP（10mM）	0.375mM	0.75μl
U6 snRNA/miRNA RT Primer（1μM）	60nM	1.2μl
RNasin（40U/μl）	0.5U/μl	0.25μl
MMLVReverse Transcriptase（200U/μl）	40U	0.2μl
RNA Sample （Total RNA）*	1μg	Xμl
RNase Free H_2O	—	To 20μl

***为了实验结果的稳定性，我们推荐将U6 snRNA和miRNA逆转录引物在同一管逆转录体系中进行反应。在进行逆转录反应前，请先用移液器混匀体系。**

U6 snRNA逆转录反应操作（接上页）

U6 snRNA 和 miRNA 共用逆转录程序

标准逆转录反应程序：

30 min at 16_℃，30~45 min at 42_℃，5 min at 85_℃

将所有的试剂盒组份，反应液和样品至于冰上。在配制完成后立即将反应体系置于已经预热的热循环仪上开始逆转录反应。

U6 和 miRNA 荧光定量 PCR 反应操作

U6 snRNA 和 miRNA 逆转录产物的操作

请先将 cDNA 产物用灭菌纯水进行 2-3 倍的稀释，混匀后从中取 2μl 作为荧光定量 PCR 反应的模板，同时检测 U6 和 miRNA 时都可参照此原则。如逆转录 cDNA 暂时不用，可将其存放于-20℃，三天内可保持稳定。

荧光定量 PCR 反应体系的配制

下列表格提供了 20μl 荧光定量 PCR 反应体系的配制方法

Component	Final Con.	Vol /1 rxns
2×Real-time PCR Master Mix [1]	1×	10μl
U6 snRNA Primer set(5μM) [2]	0.12μM	0.48μl
U6 snRNA RT product	—	2μl
Taq DNA polymerase（5U/μl）	0.5 U/μl	0.2μl
dd H_2O	—	To 20μl

[1] 2×反应液包括 Mg^{2+}, dNTP，SYBR Green I 和 Rox.

[2]U6 snRNA 引物套装包含了 PCR 正反向引物。

[3] 我们推荐每个样品至少做 3 个复孔。

如果要做多重反应，先配制一个通用的反应液，分装后再分别加入单独的组份。

U6 snRNA 和 miRNA 荧光定量 PCR 反应操作

请按照下列反应程序进行荧光定量PCR反应：

95℃ for 3minute hold, 40 cycles of:

95℃, 15 seconds ,**62℃, 40~60 seconds**

NOTE

荧光采集在62℃.

ROX Reference Dye

加入 ROX 染料能使一些适用的机器荧光信号标准化，通常厂商提供的 ROX 的浓度为 25μM，下列表格可以用来作为 ROX 用量的参考。

Instrument	Amount of ROX per 20μl reaction	Final ROX Conc.
ABI 7000, 7300, 7900HT	0.4μl	500 nM
ABI7500 Mx3000P, Mx4000	0.04μl	50 nM

NOTE

为了加样准确，可以先将ROX稀释10倍后取0.4μl使用。

IMPORTANT

1. 如果做多重的反应，可先配制通用的组份，分装后再加入单独的组份。配制反应混合液是十分重要的，能够减少加样误差。
2. 请先确认所有的组份都在管底，如有需要可稍稍离心。

数据分析

校正和标准化

设置一个校正样品

在做 microRNA 相对定量之前，首先必须确定校正样品，通常校正样品可以是正常的或是没经过实验处理的样品。

用 U6 snRNA 作为标准化内参

microRNA相对Ct法定量，标准化基因通常为管家基因或者一些经过表达谱分析验证过的miRNA。可以用microRNA的Ct值减去标准化基因的Ct值得到校正样品和其他样品的$\triangle C_T$。

IMPORTANT

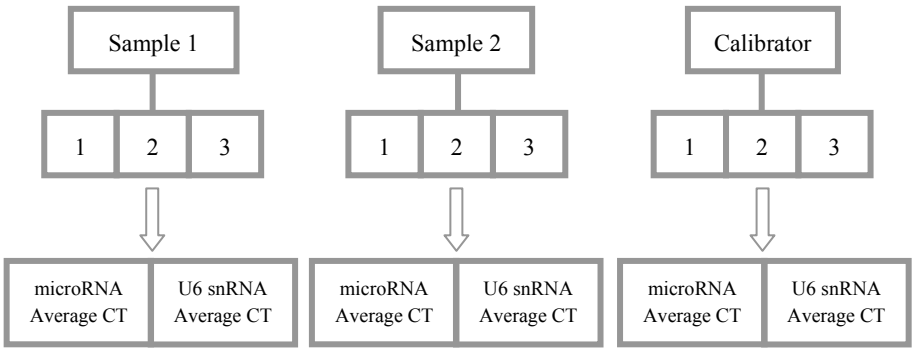
我们推荐任何一个样品的标准化基因的Ct值不能大于或等于30，如果该基因的Ct值确实稳定在30或以上，您可以考虑增加用于逆转录的初始总RNA的量。

计算 microRNA 相对于 U6 snRNA 的表达比率

第一步

设计 microRNA 相对定量实验

每个样品对应每个基因至少要做 2-3 个复孔。



第二步

进行荧光定量 PCR 反应来得到每个反应的 microRNA 和 U6 的 Ct 值。

Sample 1		Sample 2		Calibrator	
hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
30.40	23.63	24.21	22.66	26.21	24.60
30.35	23.40	24.60	22.56	26.15	24.31
30.41	23.52	24.66	22.48	26.35	24.72

数据分析（接上页）

IMPORTANT

注意复孔间的 Ct 值差异不要大于 0.5. 否则我们推荐重复实验。

第三步

计算样品和校正样品三复孔的平均值。

	Sample 1		Sample 2		Calibrator	
	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
Average C_T	30.39	23.51	24.49	22.56	26.24	24.54

第四步

miRNA的平均Ct值减去U6 snRNA 的Ct平均值，计算各个样品的△C_T值。

Sample 1 $\triangle CT = C_{T(miR\text{-}16)} - C_{T(U6snRNA)} = 30.39 - 23.51 = 6.88$

	Sample 1		Sample 2		Calibrator	
	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
Average C_T	30.39	23.51	24.49	22.56	26.24	24.54
ΔC_T	6.88	–	1.93	–	1.70	–

第五步

样品的ΔCT值减去校正样品的ΔCT值，计算得到各个样品的ΔΔCT值。

$\Delta\Delta CT_{(sample1)} = \Delta CT_{(sample1)} - \Delta CT_{(calibrator1)} = 6.88 - 1.70 = 5.18$

	Sample 1		Sample 2		Calibrator	
	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
Average C_T	30.39	23.51	24.49	22.56	26.24	24.54
ΔC_T	6.88	–	1.93	–	1.70	–
$\Delta\Delta C_T$	5.18	–	0.23	–	0.00	–

第六步

计算相对表达比率

Relative Expression Ratio $_{(sample1)} = 2^{-\Delta\Delta CT\,(sample1)} = 2^{-5.18} = 0.027$

	Sample 1		Sample 2		Calibrator	
	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
Average C_T	30.39	23.51	24.49	22.56	26.24	24.54
ΔC_T	6.88	–	1.93	–	1.70	–
$\Delta\Delta C_T$	5.18	–	0.23	–	0.00	–
$2^{-\Delta\Delta CT}$	0.027	–	0.85	–	1.0	–

附录

技术服务

万维网

您可以用您的网页浏览器访问我们的网站，您可以得到以下信息：

- □ 查找相应产品的货号
- □ 了解我们的新产品
- □ 咨询产品和相应的文章
- □ 下载产品手册和相关文章的 PDF 版本

吉玛网址www.genepharma.com

质量控制

产品已经用Hela的总RNA进行过功能性测试。

联系我们

如果您想获取更多的的技术支持，请致电，写信，电子邮件或是传真。

公司总部：
中国上海张江高科技园区哈雷路1011号
电话：86-21-51320195
传真：86-21-51320295
电子邮件：service@genepharma.com

Kit Contents and Storage

Cat. No. QPM-050	**Size: 50rxns**
Cat. No. QPM-051	**Size: 100 rxns**

U6 snRNA Real-time PCR Normalization Kit Reagents

U6 snRNA Real-time PCR Normalization Kit Reagents box includes the following items. Store the components at –20℃. **Reagents must be stored in the dark.**

Reagent	Amount		Storage
	50rxns	100rxns	
Real-time PCR Master Mix（2×）*	1.0ml	2.0 ml	4℃
U6 snRNA primer Set（5μM）	80μl	160μl	−20℃
U6 snRNA RT primer（10μM）	15μl	30μl	−20℃
Taq DNA polymerase （5U/μl）	20μl	40μl	−20℃
Sterilized ddH_2O	1.0 ml	1.0 ml	4℃

*Include dNTP Mixture，Mg^{2+},SYBR Green I，Rox.

Accessory Products

Accessory Products

Some of the reagents supplied in the U6 snRNA Real-time PCR Normalization Kit as well as other products suitable for use with the kit are available separately from Genepharma. Ordering information is provided below. For more information, refer to our Web site (**www.genepharma.com**).

Item	Amount	Catalog no.
Hairpin-it™ miRNAs qPCR Quantitation Kit	20μl×50 rxns	QPM-010
	20μl×100 rxns	QPM-011

Introduction

Overview

Introduction

The U6 snRNA Real-time PCR Normalization Kit is a sensitive and specific method using real-time PCR for the normalization and profiling of microRNAs (miRNA) from total RNA samples.

MiRNAs are small, single-stranded, ~19–23 nt RNA molecules encoded in the genomes of plants, animals, and viruses. Mature miRNAs enter the RNA-induced silencing complex (RISC) and guide the RISC to induce translational repression or endonucleolytic cleavage of specific target mRNAs.

The U6 small nuclear RNA (snRNA) undergoes major conformational changes during the assembly of the spliceosome and catalysis of mRNA splicing. It associates with the specific protein Prp24p, and a set of seven LSm2p-8p proteins, to form the U6 small nuclear ribonucleoprotein (snRNP). The spliceosome performs pre-mRNA splicing in eukaryotes.

Description of U6 snRNA Real-time PCR Normalization Kit

The Real-time PCR normalization assay of miRNAs using U6 snRNA as a housekeeping gene includes two steps, U6 snRNA RT reaction and real-time PCR. The U6 specific RT primer is supplied in the U6 snRNA Real-time PCR Normalization Kit, the RT-PCR kit (Cat # QPG-090) is available separately.

Users can use the U6 snRNA Real-time PCR Normalization Kit to normalize the expression ratio of a specific microRNA relative to the U6 snRNA between different samples.

Basic of Relative Quantification Calculation

Relative quantification of microRNA gene expression is calculated from the threshold cycle (C_T) values obtained in experiments. The threshold cycle is the cycle at which a statistically significant increase in ΔRn is first detected. Thus, wells with higher initial template concentrations reach the threshold value at lower cycle numbers during PCR than wells containing lower initial template concentrations.

For an ideal microRNA quantification assay, each cycle in the PCR reaction corresponds to a twofold increase in PCR product. Therefore a change in threshold cycle number of 1 equates to a two fold difference in initial template concentration. For example, if the CT increases by one, the initial template concentration decreases two fold. Conversely, if the CT decreases by one, the initial template concentration doubles. This property is used in the calculation of relative quantification values for the cytokine and internal control target sequences in each well.

For more information, see the **Data Analyzing** chapter in this manual.

Components of the Kit

Introduction

This section provides more information about the reagents supplied in the U6 snRNA Real-time PCR Normalization Kit.

Real-time PCR Master Mix 2×Conc.

The 2×Real-time PCR Master Mix consists of a proprietary buffer system, $MgCl_2$, dNTPs, SYBR Green I and Rox reference dye. The mix has been confirmed to be work well in restrict lab experiments.

It's very important for you to store the 2×Real-time PCR Master Mix in the dark.

U6 snRNA primer set 5μM

The pre-designed U6 snRNA primer set has been confirmed to be work well in restrict lab experiments. Data base information for U6 snRNA is provided below.

Species	GeneBank Accession#	Primer Tm	Amplicon length
Homo sapiens	M14486	58~62℃	70~100 bp

U6 snRNA RT primer 10μM

The pre-designed U6 snRNA RT primer is provided as a 10 μM solution. We recommended the final concentration of the RT primer 60nM for a reverse transcription reaction. **Please dilute the RT primer for 10 folds first and use 1.2μl per RT reaction.**

Taq DNA polymerase 5U/μl

For most PCR reaction, the final concentration of DNA polymerase was ussually 0.05U/μl per reaction volume.

User-Supplied Required Materials

Reverse Transcriptase	For detection and quantification of the miRNAs in your sample, a RT reaction use the miRNA specific RT primer provided in the kit was needed. We recommended using traditional Reverse Transcriptase such as MMLV.
RNase Inhibitor	To keep the activity of Reverse Transcriptase, adding RNase Inhibitor in your RT reaction was recommended. The final concentration usually was 0.25 U/μl.
Rox reference dye	To normalize the fluorescence difference from well to well, adding Rox reference dye in your qPCR system was recommended when using the Real-Time PCR instruments such as ABI PRISM 7000/7300/7500/7900, MX3000p/4000.
Optical PCR plates or tubes	As to eliminate the impact on fluorescence detection caused by PCR tubes or plates, using optical PCR plates and tubes was recommended to corporate the requirements of the Real-Time PCR instruments such as ABI PRISM 7000/7300/7500/7900, MX3000p/4000.

Method

Performing U6 snRNA RT Reaction

Introduction

The Hairpin-it™ miRNAs real-time Quantitation assay of miRNAs includes two steps, stem–loop RT reaction and real-time PCR detection. To profiling microRNA's expression relative to a said housekeeping gene, U6 snRNA or 5S rRNA qPCR Normalization Kit should be needed. The U6 snRNA normalization kit is used to normalize the expression level of microRNAs relative to U6 snRNA, which is a small nuclei RNA abounded in cells. Same volume RNA sample of microRNA assay and U6 normalization assay is asked to add in the RT reaction system, then, the RT product is quantified using real-time PCR.

Handling U6 snRNA RT Primer

Please dilute the RT primer for 10 folds first and use 1.2μl per RT reaction.

Preparing The U6 snRNA RT Reaction Mix

The template for the U6 snRNA Real-time PCR Normalization Kit can be total RNA or cell lysate. The RNA template's input should be at least 1μg or more as for as the requirement of the experiment. Other than the stability at 42℃, there is no special requirement of Reverse Transcriptase for the RT reaction. We recommended a standard 20μl RT reaction size.

The following table provides RT reaction Mix volumes for a standard 25μl reaction size.

Component	Final Con.	Vol /1 rxns
5×RT Buffer	1×	4μl
dNTP（10mM）	0.375mM	0.75μl
U6 snRNA/miRNA RT Primer（1μM）	60nM	1.2μl
RNasin（40U/μl）	0.5U/μl	0.25μl
MMLVReverse Transcriptase（200U/μl）	40U	0.2μl
RNA Sample （Total RNA）*	1μg	Xμl
RNase Free H_2O	—	To 20μl

*It's important to keep the RNA sample volume or quantity of U6 normalization RT assay and miRNA RT assay coincidence. Mix the RT reaction reagents and Mix before RT reaction.

Continued on next page

Performing U6 snRNA RT Reaction, continued

Performing miRNAs RT Reaction

Standard RT Reaction Program

30 min at 16_℃，30~45 min at 42_℃，5 min at 85_℃

NOTE

Keep all components, reaction mixes and samples on ice. After assembly, transfer the reaction mixes to a thermal cycler preheated to the cDNA synthesis temperature and immediately begins RT reaction.

Performing miRNAs & U6 snRNA Real-Time PCR Reaction

Handling the U6 snRNA RT product

For the U6 snRNA Real-time PCR Normalization Kit, pipet 2μl U6 snRNA RT reaction product as the template for real-time PCR step subsequently. Store the surplus microRNA RT reaction product at –20℃.

Preparing Real-Time Reaction Mix

The following table provides Real-time PCR reaction Mix volumes for a 20μl reaction size. Note that three repeat tubes be performed in an experiment at least.

Component	Final Con.	Vol /1 rxns
2×Real-time PCR Master Mix [1]	1×	10μl
U6 snRNA Primer set(5μM) [2]	0.2μM	0.8μl
U6 snRNA RT product		2μl
Taq DNA polymerase（5U/μl）	0.5 U/μl	0.2μl
dd H_2O		To 20μl

[1]The 2×Master Mix contains Mg^{2+}, dNTP,SYBR Green I and Rox dye.

[2]The U6 snRNA Primer set includes PCR forward and reverse primers.

For multiple reactions, prepare a master mix of common components, add the appropriate volume to each tube or plate well, and then add the unique reaction components.

Performing miRNAs & U6 snRNA Real-time PCR Reaction

Program the real-time PCR instrument to perform PCR amplification as shown below.

95℃ for 3minute hold, 40 cycles of:

95℃, 15 seconds ,**62℃, 40~60seconds**.

Continued on next page

NOTE

Fluorescence detection step is at 62℃.

ROX Reference Dye

ROX Reference Dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments that are compatible with that option. ROX is often supplied at a 25μM concentration. Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per 20μl reaction	Final ROX Conc.
ABI 7000, 7300, 7900HT	0.4μl	500 nM
ABI7500 Mx3000P, Mx4000	0.04μl	50 nM

NOTE

To accurately pipette 0.04 μl per reaction, dilute ROX 1:10 immediately before use and use 0.4μl of the dilution.

IMPORTANT

1. For multiple reactions, prepare a master mix of common components, add the appropriate volume to each tube or plate well on ice, and then add the unique reaction components (*e.g.,* template). Preparation of a master mix is *crucial* in qRT-PCR to reduce pipetting errors.
2. Make sure that all components are at the bottom of the tube/plate; centrifuge briefly if needed.

Data Analyzing

Calibrator and Normalizor

Settling a Calibrator

Before a microRNA relative quantification assay, you should ascertain which sample to be the calibrator. Usually a calibrator is the sample from normal cell or cells without any treatment.

Using U6 snRNA as Normalizor

For relative quantification of microRNA using comparative C_T method, the normalizor is another gene except microRNA, which is often the said housekeeping gene. Substrate the normalizor C_T value from a microRNA C_T value to calculate the $\triangle C_T$ for calibrator and samples in each microRNA profiling assay.

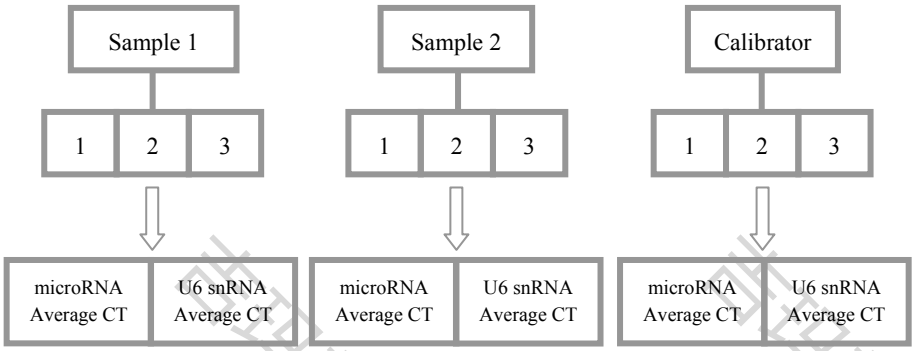
IMPORTANT

We recommend normalizor C_T values do not greater than or equal to 30 for any sample. If the normalizor C_T values is consistently equal to or greater than 30, we recommends that you increase the amount of initial total RNA (converted to cDNA) used in each reaction.

Calculating microRNA Expression Ratio Relative to U6 snRNA

Step 1
Designing microRNA relative quantitation assay

It's important to caring two or three repeats for each reaction at least.



Step 2
Run real-time PCR to obtain the C_T value for microRNA and U6 snRNA for each reaction.

Sample 1		Sample 2		Calibrator	
hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
30.40	23.63	24.21	22.66	26.21	24.60
30.35	23.40	24.60	22.56	26.15	24.31
30.41	23.52	24.66	22.48	26.35	24.72

continued on next page

Data Analyzing Continued

IMPORTANT

Attention that the CT value difference between repeat reactions should not greater than 0.5. Otherwise, you should delete the invalid data or carry out the experiment again.

Step 3

Average the C_T values for triplicate wells of the samples and calibrator.

	Sample 1		Sample 2		Calibrator	
	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
Average C_T	30.39	23.51	24.49	22.56	26.24	24.54

Step 4

Subtract the U6 snRNA values from the miRNAs C_T values to calculate $\triangle C_T$ for the calibrator and samples in each reaction.

Sample 1 $\triangle CT = C_{T\,(miR\text{-}16)} - C_{T\,(U6snRNA)} = 30.39 - 23.51 = 6.88$

	Sample 1		Sample 2		Calibrator	
	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
Average C_T	30.39	23.51	24.49	22.56	26.24	24.54
ΔC_T	6.88	–	1.93	–	1.70	–

Step 5

Subtract the ΔCT(calibrator) values from the ΔCT (samples) values to calculate their ΔΔCT (sample) values.

$\Delta\Delta CT_{(sample1)} = \Delta CT_{(sample1)} - \Delta CT_{(calibrator1)} = 6.88 - 1.70 = 5.18$

	Sample 1		Sample 2		Calibrator	
	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
Average C_T	30.39	23.51	24.49	22.56	26.24	24.54
ΔC_T	6.88	–	1.93	–	1.70	–
$\Delta\Delta C_T$	5.18	–	0.23	–	0.00	–

Step 6

Calculate the relative expression ratio.

Relative Expression Ratio $_{(sample1)} = 2^{-\Delta\Delta CT\,(sample1)} = 2^{-5.18} = 0.027$

	Sample 1		Sample 2		Calibrator	
	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA	hsa-miR-16	U6 snRNA
Average C_T	30.39	23.51	24.49	22.56	26.24	24.54
ΔC_T	6.88	–	1.93	–	1.70	–
$\Delta\Delta C_T$	5.18	–	0.23	–	0.00	–
$2^{-\Delta\Delta CT}$	0.027	–	0.85	–	1.0	–

Appendix

Technical Service

World Wide Web

Visit the GenePharma Web Resource using your World Wide Web browser. At the site, you can:

- □ Download manuals in Adobe Acrobat (PDF) format
- □ Explore our catalog with full color graphics
- □ Get the scoop on our hot new products and special product offers
- □ Request catalog and product literature

The Genepharma URL is **www.genepharma.com**

Quality Control

The product is tested functionally by qRT-PCR using total HeLa RNA as template. Kinetic analysis must demonstrate a linear dose response with decreasing target concentration and detection of U6 snRNA in 1 pg of total HeLa RNA.

Contact Us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.genepharma.com).

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