MicroRNA Applications

QuantiMiR
Small RNA Quantitation System

System Biosciences
What QuantiMir does:

The new QuantiMir RT Kit tags and converts small RNAs into cDNA, ready to use as template for real-time qPCR

- Measure and profile MicroRNA expression levels from any tissue source
- Detect and quantify siRNAs from knockdown experiments
- Validate the existence of newly discovered MicroRNA species

- No more messy Northerns or RNase protection assays! Say “good-bye” to radioactivity!
- Stop wasting your precious RNA on a single MicroRNA detection per RT reaction!
How QuantiMir Works

Single tube 3 Step Assay

1. Tag small RNA
2. Anneal adapter
3. Convert to cDNA

DONE!

Less than 2 hr process!
Start with as little as **200 pg** total RNA:
No purification or fractionation required

- Biopsies
- LCM
- Rare samples
High Specificity

Determine Tissue Expression Profiles

Normalize with U6 snRNA present in every tissue

miR-1
Heart + Sk. Muscle only

miR-122a
Liver only
Application: Validate New MicroRNAs

End-point RT-PCR using QuantiMir cDNA

Novel MicroRNAs
Application: MicroRNA Quantitation

Profile MicroRNA Expression in Cancer

- Breast
- Lung
- Colon
- Ovary
- Lymph Node

Log \Delta Ct

miR-9-1, miR-155, miR-125b, miR-125b, miR-145, miR-7, miR-17-3p, miR-18a, miR-20a, miR-92

Normal
Tumor

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Easy Forward Primer Design

Only need to design 1 Primer

Hsa-miR-16

Mature sequence MIMAT0000069

- Accession: MIMAT0000069
- ID: hsa-miR-16
- Sequence: 14 - uagcagcagauauuuuggc - 35
- Evidence: experimental; cloned [1,5,7], Northern [1,6]

Simple: Directly use sequence of mature miRNA as forward primer in oligo design.

The mature miRNA sequence 5’ – uagcagcagauauuuuggc – 3’ can be simply converted to a DNA sequence and used directly as the forward primer for end-point and qPCR analysis.

Forward primer for hsa-miR-16:
5’ – TAGCAGCAGTAAATATTGGCG – 3’
Tm= 58.9 °C, 45% GC and length =22 bases.
QuantiMir Kit Contents

Kit contains everything you need, including miR-16 and U6 snRNA + Controls

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 µl</td>
<td>5X PolyA polymerase Buffer</td>
<td>10 µl Poly A Reaction (enough for 20 reactions)</td>
</tr>
<tr>
<td>10 µl</td>
<td>PolyA polymerase</td>
<td></td>
</tr>
<tr>
<td>20 µl</td>
<td>25 mM MnCl₂</td>
<td></td>
</tr>
<tr>
<td>30 µl</td>
<td>5 mM ATP</td>
<td></td>
</tr>
<tr>
<td>10 µl</td>
<td>3’ Oligo dT Adaptor</td>
<td>20 µl RT Reaction (enough for 20 reactions)</td>
</tr>
<tr>
<td>80 µl</td>
<td>5X Reverse Transcriptase Buffer</td>
<td></td>
</tr>
<tr>
<td>20 µl</td>
<td>Reverse Transcriptase</td>
<td></td>
</tr>
<tr>
<td>30 µl</td>
<td>0.1 M Dithiothreitol (DTT)</td>
<td></td>
</tr>
<tr>
<td>40 µl</td>
<td>dNTP Mix</td>
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</tr>
<tr>
<td>25 µl</td>
<td>3’ Universal Reverse PCR Primer</td>
<td>End-point or qPCR Assay (enough for 500 reactions)</td>
</tr>
<tr>
<td>50 µl</td>
<td>5’ Human U6 Control Forward Primer (10 µM)</td>
<td></td>
</tr>
<tr>
<td>50 µl</td>
<td>5’ Human miR-16 Control Forward Primer (10 µM)</td>
<td></td>
</tr>
<tr>
<td>1.2 ml</td>
<td>RNase–free Water</td>
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</tbody>
</table>

End up with 20 µl cDNA, enough to perform at least 40 Real-time qPCR reactions per RT reaction.
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