miQPCR: A novel approach for expression profiling of mature microRNAs.

March 23, 2009

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Part 1: miQPCR; A novel approach for expression profiling of mature microRNAs

Part 2: Effect of RNA degradation on microRNA expression
Why are we interested in expression profiling microRNAs?

- miRNAs post-transcriptionally control the expression of genes involved in cell development, differentiation and metabolism.

- miRNAs dysfunction is involved in the biogenesis of human diseases including cancer and leukemia.

- Thus changes in miRNAs expression profiling are important to understand biology (e.g. cell Stemness) and may have great value as biomarkers to monitor disease progression and therapy response in various disease states, including cancer and leukemia.
Mature miRNAs are short single strand RNAs ~22 nts long

Mature microRNA sequence is contained in the precursor

Mature microRNA do not have common sequences such as CAP or polyA tail to be used as anchor

The predicted Tm of mature miRNAs versus complementary DNA varies between 40°C to 80°C

miRNAs are characterized by closely related family members that often differ of a single nucleotide (e.g. let-7 family)
miRNA-profiling tools developed at the Uni-Heidelberg/EMBL

Tm normalized LNA-based microarray platform for expression profiling of mature miRNA (miCHIP)

miCHIP R-library, for normalization of miCHIP hybridization (Bioconductor)

RT-PCR approach for expression profiling of mature miRNAs (miQPCR)
miCHiP; General principles

Codelink activated slides (GE) coated with N-hydroxycsiccinimide Capure probes with NH2-modification

Sample preparation
Sample A

Direct labeling of small RNA

Data Analysis

Expression profile clustering

Castoldi et al., RNA 2006
miCHIP, microarray platform for microRNA expression profiling

**hsa-miR-7**  
CAACAAAATCACTAGTCTTCCA  
Tm 50°C

**hsa-miR-92**  
ACAGGCCGGGACAAAGTGCAATA  
Tm 72°C

**hsa-miR-7**  
CAACAAAATCACTAGTCTTCCA  
Tm 72°C

(Modified from Exiqon)
0 Good correlation with several profiling platform

(ABI, Exiqon, Luminex and Northern blot)

• Primer/probes availability is lagging behind array design
• Primers/probes are not available for all the organisms
• Newly cloned microRNAs can be detected only by northern blot

➢ Designed an “Open” RT-PCR platform for expression profiling of mature microRNA named “miQPCR”
• Reverse transcription, 500 ng/total RNA

• RT-PCR (SYBR-GREEN) uses 2-5 ng/cDNA (250-100 PCR/sample)

• Simple and cost effective primer design
Dynamic Range of Let-7a-Syn expression

let-7a  UGAGGUAGUAGGUUGUAUAGUU

Dynamic Range, Detection of 0.4 aMol
Cross Hybridization Let-7 family Members

<table>
<thead>
<tr>
<th>Let-7a</th>
<th>Let-7b</th>
<th>Let-7c</th>
<th>Let-7d</th>
<th>Let-7e</th>
<th>Let-7g</th>
<th>Let-7i</th>
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</thead>
<tbody>
<tr>
<td>100</td>
<td>7.33</td>
<td>4.70</td>
<td>4.28</td>
<td>18.11</td>
<td>0.05</td>
<td>0.06</td>
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</table>

Percentage value (Against Let-7a spike)
miQPCR: Dynamic Range

Dynamic Range miQPCR (OVER 40 CYCLES)

- **Linear Range**, 5 folds = ~2.3 Cts, miR-122:
  - 5 → 1 ng, ~2.7 Cts
  - 1 → 200 pg, ~1.7 Cts
  - 200 pg → 40 pg, ~2.3
  - 40 pg → 8 pg, ~2.4
  - 8 pg → 1.6 pg, ~1.7

<table>
<thead>
<tr>
<th>Ct number (Remember that lower Ct means higher expression)</th>
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<tbody>
<tr>
<td>40</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>5 ng</td>
</tr>
<tr>
<td>1 ng</td>
</tr>
<tr>
<td>200 pg</td>
</tr>
<tr>
<td>40 pg</td>
</tr>
<tr>
<td>8 pg</td>
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<tr>
<td>1.6 pg</td>
</tr>
<tr>
<td>320 fg</td>
</tr>
<tr>
<td>64 fg</td>
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</table>

- miR-122
- miR-16
- miR-194
- miR-192

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miQPCR specificity

Amplicons Expected sizes

- R3A ~115 mer
- R87 ~110 mer
- R11 ~100 mer
- RNU6 ~75 mer
- miRNA ~65 mer

miQPCR specificity

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miQPCR correlation with other platforms

Expression Profiling across different platforms

<table>
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<tr>
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<th>mmu-miR-122</th>
<th>mmu-miR-16</th>
<th>mmu-miR-192</th>
<th>mmu-miR-194</th>
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<tbody>
<tr>
<td>miChip</td>
<td>-7.25</td>
<td>0.87</td>
<td>-3.29</td>
<td>-4.75</td>
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<tr>
<td>ABI</td>
<td>-6.20</td>
<td>1.90</td>
<td>-5.90</td>
<td>-5.10</td>
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<tr>
<td>miQPCR</td>
<td>-6.245</td>
<td>1.78</td>
<td>-3.055</td>
<td>-4.605</td>
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Log2 Ratio

miQPCR correlation with other platforms
miQPCR protocols optimized for 500 ng of total RNA (up to 250 individual qPCR)

- miQPCR detects as little as 0.4 amol of synthetic microRNA
- miQPCR primer design discriminate between closely related microRNAs
- Abundant microRNAs can be detected using as little as 320 fg of cDNA-equivalent
- miQPCR display good correlation with different microRNAs profiling methods (miCHIP and TaqMan)
Part 1: miQPCR; A novel approach for expression profiling of mature microRNAs

- Part 2: Effect of RNA degradation on microRNA expression
Liquid N. Flash frozen
Liver and Duodenum
Stored -80C

Stored and sliced
in dry ice

At time 0 (T0) samples
Were transferred in ice

RNA extracted at different time point
T0, T30, T60, T120 and T240 mins.

RNA integrity assessed
by using Bioanalyzer
Time course effect on RNA integrity

**Figure 1**

<table>
<thead>
<tr>
<th>LIVER</th>
<th>M</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
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</tbody>
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- T0, RIN 7.2
- T30, RIN 4.8
- T60, RIN 4.0
- T120, RIN 2.8
- T240, RIN 3.0

<table>
<thead>
<tr>
<th>DUODENUM</th>
<th>M</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
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</table>

- T0, RIN 8.5
- T30, RIN 4.0
- T60, RIN 3.3
- T120, RIN 2.7
- T240, RIN 2.5
How RNA integrity affects Sample Clustering

Figure 2
How RNA integrity affect Matrix plot analysis

Figure 3
Correlation among degradation and regulated probes (2 folds)

Figure 4
How RNA quality affects miRNAs expression by NB

Figure 5
Figure 6
• microRNAs are affected by degradation

• Microarray expression profiling of degraded RNA (miCHIP) generates “false positive” calls

• Expression profiling of microRNA by RT-PCR is marginally affected by RNA degradation
Thank you! Questions?