Label-Free Technology for the Amplified Detection of MicroRNA Based on the Allosteric Hairpin DNA Switch and Hybridization Chain Reaction

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Table S1. DNA and RNA sequences used in this work.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Hairpin DNA</td>
<td>5'-ATTGACCGCTGTGTGACGCAACACTCAATAAACTATAACAACCT ActACCTCAGTTATGAGTGTTCGATTCGCGTG-3'</td>
</tr>
<tr>
<td>C1-let7</td>
<td>CAATAAC-3’</td>
</tr>
<tr>
<td>C2-let7</td>
<td>5'-TTAACCCACGCGCAATCGAACACTGTTATGAGTGTTCGATT CGCGTG-3'</td>
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<tr>
<td>Hairpin DNA-G5</td>
<td>ACTACCTCAGTTATGAGTGTTCGATTCGCGTG-3’</td>
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<tr>
<td>C1-let7-G5</td>
<td>5'-TTACCTAGGATTACGGCGTGGGTTAACACGCCAATCCCTAGTAA TTAGATGTTACTAGGATTC CACCA-3'</td>
</tr>
<tr>
<td>C2-let7-G5</td>
<td>GCGTG-3’</td>
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<tr>
<td>Hairpin DNA-G13</td>
<td>ACTACCTCAGTTATGAGTGTTCGCGTG-3’</td>
</tr>
<tr>
<td>C1-let7-G13</td>
<td>5'-TTAACCCACGCGCAATCCCTAGTAA TTAGATGTTACTAGGATTC ACTTTG-3’</td>
</tr>
<tr>
<td>C2-let7-G13</td>
<td>GCGTG-3’</td>
</tr>
<tr>
<td>let-7 a</td>
<td>5'-UGAGGUAGUAGGUUGUUAUAGUU-3’</td>
</tr>
<tr>
<td>let-7 b</td>
<td>5'-UGAGGUAGUAGGUUGUUGUGUU-3’</td>
</tr>
<tr>
<td>let-7 c</td>
<td>5'-UGAGGUAGUAGGUUGUUGUUGUU-3’</td>
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<tr>
<td>let-7 d</td>
<td>5'-AGAGGUAGUAGGUUGCAAAGUUGU-3’</td>
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<tr>
<td>let-7 e</td>
<td>5'-UGAGGUAGGUAGGUUGUUAUAGU-3’</td>
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<tr>
<td>let-7 f</td>
<td>5'-UGAGGUAGUAGGUUGUUAUAGU-3’</td>
</tr>
<tr>
<td>let-7 g</td>
<td>5'-UGAGGUAGUAGGUUGUUAUAGU-3’</td>
</tr>
<tr>
<td>let-7 i</td>
<td>5'-UGAGGUAGUAGGUUGUUGUGCAGUUGU-3’</td>
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<tr>
<td>Hairpin DNA-miRNA 21</td>
<td>ATAAGCTATGAGTGTTCGATTCGCGTG-3’</td>
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<tr>
<td>miRNA 21</td>
<td>5'-UAGCUUAUCAGACUGAUGUUGA-3’</td>
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</table>
Figure S1. CL intensity vs the amount of SA-MPs. Experimental conditions: aMB probe, C1 and C2 sequences were 10, 20 and 30 pmol. 20 mM Mg$^{2+}$ ions in BA and 100 fmol let 7a, respectively. The detection procedure was carried out as described in the Experimental section.

Figure S2. CL intensity vs the amount of aMB probes. Experimental conditions: SA-MPs were 20 μg, aMB probe, C1 and C2 sequences were 10, 20 and 30 pmol. 20 mM Mg$^{2+}$ ions in BA and 100 fmol let 7a, respectively. The detection procedure was carried out as described in the Experimental section.
Figure S3. CL intensity vs the amount of C1. Experimental conditions: SA-MPs were 20 μg, aMB probe and C2 sequences were 10 and 20 pmol. 20 mM Mg$^{2+}$ ions in BA and 100 fmol let 7a, respectively. The detection procedure was carried out as described in the Experimental section.

Figure S4. CL intensity vs the amount of C2. Experimental conditions: SA-MPs were 20 μg, aMB probe and C1 sequences were 10 and 20 pmol. 20 mM Mg$^{2+}$ ions in BA and 100 fmol let 7a, respectively. The detection procedure was carried out as described in the Experimental section.
**Figure S5.** CL intensity vs the amount of SA-MPs. Experimental conditions: aMB probe and let 7a were 10 and 1 pmol, respectively. The detection procedure was carried out as described in the Experimental section.

**Figure S6.** CL intensity vs the amount of aMB probes. Experimental conditions: SA-MPs were 20 μg, let 7a was 1 pmol, respectively. The detection procedure was carried out as described in the Experimental section.
Figure S7. Log-Log calibration data for the target miRNA 21. Experimental conditions: SA-MPs were 20 μg, hairpin DNA-miRNA 21 switch probe, C1 and C2 sequences were 10, 20 and 20 pmol, 20 mM Mg²⁺ ions were in BA, respectively. The detection procedure was carried out as described in the Experimental section.
Figure S8. CL intensity and CL ratio vs different temperatures for let 7a (a) and miRNA 21 (b). Experimental conditions: SA-MP was 20 μg, both let-7a and miRNA 21 were 100 fmol, hairpin DNA switch probe, C1 and C2 sequences were 10, 20 and 20 pmol, 20 mM Mg$^{2+}$ ions were in BA, respectively. The detection procedure was carried out as described in the Experimental section.

Table S2. Comparison of sensitivity for different RNA assay methods.

<table>
<thead>
<tr>
<th>Analytical method</th>
<th>Label</th>
<th>target RNA</th>
<th>Detection Limit</th>
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</thead>
<tbody>
<tr>
<td>Electrochemical detection</td>
<td>Alkaline Phosphatase (ALP)</td>
<td>p185 BCR-ABL</td>
<td>1 fM $^1$</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>Poly(dimethylsiloxane)</td>
<td>Dengue virus</td>
<td>50 pM $^2$</td>
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<tr>
<td>Fluorescence</td>
<td>SYBR Green I,</td>
<td>Let-7a</td>
<td>10 fM $^3$</td>
</tr>
<tr>
<td>Surface plasmon resonance (SPR)</td>
<td>Label-free</td>
<td>miR24, 96, 1, 424</td>
<td>10 pM$^4$</td>
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<tr>
<td>Fluorescence</td>
<td>Cy3</td>
<td>Let-7a, miR21, 96, 206, 31</td>
<td>10 fM $^5$</td>
</tr>
<tr>
<td>Silicon photonic microring resonators</td>
<td>Label-free</td>
<td>Let-7a</td>
<td>150 fmol $^6$</td>
</tr>
<tr>
<td>Polyacrylamide gel electrophoresis/ MS</td>
<td>Label-free</td>
<td>RNase P, 7SL RNA and U6 snRNA,</td>
<td>100 fmol $^7$</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>Label-free</td>
<td>Nat-siRNAATGB2</td>
<td>35 amol $^8$</td>
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<tr>
<td>Colorimetry / Fluorescence</td>
<td>Methyl / Rhodamine Red</td>
<td>Genomic RNA</td>
<td>1 pmol $^9$</td>
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<tr>
<td>Electrochemical detection</td>
<td>HRP</td>
<td>miRNA-21</td>
<td>60 fmol $^{10}$</td>
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<tr>
<td>SPR</td>
<td>Label-free</td>
<td>mRNA from L. Innocua iap</td>
<td>200 pM $^{11}$</td>
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<td>SPR imaging</td>
<td>Label-free</td>
<td>16S ribosomal RNA</td>
<td>20 fmol $^{12}$</td>
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<td>Nanomechanical detection</td>
<td>Label-free</td>
<td>Messenger RNA of aldolase A</td>
<td>10 pM $^{13}$</td>
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<td>CL technique (this work)</td>
<td>Label-free</td>
<td>22 bases RNA</td>
<td>0.1 fmol (1 pM)</td>
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