A simplified pyrosequencing protocol based on linear-after-the-exponential (LATE)-PCR using whole blood as starting material directly

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**Supplementary Table**

Supplementary Table S1. Sequences of LATE-PCR primers

<table>
<thead>
<tr>
<th>Name&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sequence</th>
<th>Conc. (μM)</th>
<th>Tm value (°C)</th>
<th>Tm&lt;sup&gt;L&lt;/sup&gt; - Tm&lt;sup&gt;X&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt; (°C)</th>
<th>Tm&lt;sup&gt;A&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt; (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>677-PX</td>
<td>TGTCA TCCCTATTGGCAGGTTAC</td>
<td>1</td>
<td>65.9</td>
<td>5.8</td>
<td>77.0</td>
</tr>
<tr>
<td>677-PL</td>
<td>AGATCCGGGGACGATGGGGCAAG</td>
<td>0.1</td>
<td>71.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1298-PX</td>
<td>GGACTACTACCTCTTCTACCTGA</td>
<td>1</td>
<td>63.5</td>
<td>6.5</td>
<td>75.7</td>
</tr>
<tr>
<td>1298-PL</td>
<td>GGTTCCCCTCCAGCATCTACCTAC</td>
<td>0.1</td>
<td>70.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPYD-PX</td>
<td>GGG&lt;sup&gt;d&lt;/sup&gt;TATAAGCCTATGAATTGGATG</td>
<td>1</td>
<td>62.7</td>
<td></td>
<td>7.2</td>
</tr>
<tr>
<td>DPYD-PL</td>
<td>TGGCCCTGGACAAAGCTCCTTCTGA</td>
<td>0.1</td>
<td>69.9</td>
<td></td>
<td>73.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> PX mean excess primer, PL mean limiting primer; <sup>b</sup> Tm<sup>L</sup> and Tm<sup>X</sup> mean the Tm value of limiting primer and excess primer, respectively; <sup>c</sup> Tm<sup>A</sup> mean the Tm value of amplicons; <sup>d</sup> underlined letters were artificially mismatched bases.
Supplementary Figure Captions

Supplementary Fig. S1. The principle of linear-after-the-exponential (LATE)-PCR.

Supplementary Fig. S2. Pyrograms for genotyping MTHFR C677T in a set of five typical samples in triplicate.

Supplementary Fig. S3. The genotyping results of the 3 polymorphisms from the same sample using the whole blood LATE-PCR based pyrosequencing(A), conventional PCR based pyrosequencing(B) and commercial Sanger sequencing(C).
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