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Introduction
For researchers needing to increase their throughput or share a cycler with other users, there is a strong demand for faster, real-time PCR.

- Fast, real-time PCR can be achieved by:
  - Reduced DNA polymerase activation time
  - Shortened amplification cycles
  - Combined annealing and extension steps
  - Use of a dedicated fast-cycling instrument
  - Shortened IF step in one-step RT-PCR

Until now, fast, real-time PCR using standard reaction chemistry has been hampered by reduced sensitivity and increased variability of quantification data (1). We demonstrate how the combination of a newly developed fast-cycling PCR buffer with a rapid-activating host DNA polymerase allows significant reduction of PCR cycling times without sacrificing specificity and sensitivity.

Ultrafast cycling for end-point PCR analysis
Although fast results in PCR can be achieved on cyclers with rapid ramping rates, even faster results are possible by reducing cycling times. The QIAGEN® Fast Cycling PCR Kit, which integrates the fast-cycling PCR buffer with HotStarTaq Plus DNA Polymerase, provides significant time savings of up to 78% in endpoint PCR. Fast results can be accomplished on all cyclers, including cyclers not capable of fast ramping rates.

The PCR buffer maximizes amplification of nonspecific products, primer-dimer formation, and background smear in every PCR cycle. QiLightTM, an additive that facilitates efficient amplification of “difficult” (e.g., GC-rich) templates, is also provided with the kit.

PCR cycling times calculated for different fragment lengths

<table>
<thead>
<tr>
<th>Fragment length</th>
<th>Fragment</th>
<th>QIAGEN Fast Cycling</th>
<th>Standard cycling</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 bp</td>
<td>15 min</td>
<td>500 bp</td>
<td>20 min</td>
</tr>
<tr>
<td>500 bp</td>
<td>20 min</td>
<td>1000 bp</td>
<td>30 min</td>
</tr>
<tr>
<td>1000 bp</td>
<td>29 min</td>
<td>2000 bp</td>
<td>68 min</td>
</tr>
<tr>
<td>2000 bp</td>
<td>63 min</td>
<td>5000 bp</td>
<td>155 min</td>
</tr>
</tbody>
</table>

*Total time required for a PCR run of 35 cycles. The specified PCR cycling times do not include ramping times, which are cycle-dependent.

Specific and reliable results

- Resolution of small differences in copy number
- High annealing specificity is maintained by a balanced combination of KCl and NH4Cl in the buffer. The binding of primers to imperfectly matching sequences on the template is suppressed.

- We have developed a fast-cycling PCR buffer that significantly reduces denaturation, annealing, and extension times. A novel additive, Q-Bond, dramatically increases the binding affinity of DNA polymerase to single-stranded DNA. This turns the 3-step process of template denaturation, primer annealing, and DNA polymerase binding in standard-cycling PCR (A) into a faster 2-step process (B).

- High annealing specificity is maintained by a balanced combination of KCl and NH4Cl in the buffer. The binding of primers to imperfectly matching sequences on the template is suppressed.

- Specific primer annealing

- When the TaqMan® PCR buffer is used on 18% agarose gel, the primer and DNA polymerase bind sequentially to the template, increasing primer annealing time. Q-Bond increases the affinity of DNA polymerase for short, high-melting DNA, enabling primer annealing time to be less than 30 s.

Fast SYBR Green quantification with high specificity
In biological systems, minute changes in transcript abundance often lead to strong biological effects. Therefore, a method for reliable and reproducible discrimination between similar copy numbers is critical. With QIAGEN® SYBR Green Kits, even small differences in the amount of low-copy targets can be clearly distinguished.

- Achieving high specificity with SYBR Green based real-time PCR is important, since SYBR Green binds all double-stranded DNA. QIAGEN SYBR Green Kits promote specific primer annealing and provide a stringent hot start, preventing the formation of nonspecific PCR products that can affect SYBR Green fluorescence. Quantification is specific and sensitive, even with low template amounts.

- Specific one-step RT-PCR

- Resolution of small differences in copy number

- High annealing specificity is maintained by a balanced combination of KCl and NH4Cl in the buffer. The binding of primers to imperfectly matching sequences on the template is suppressed.

Summary
A potent, fast-running, fluorogenic PCR buffer containing Q-Bond significantly reduces denaturation, annealing, and extension times.

- HotStarTaq DNA Polymerase possesses no enzyme activity prior to PCR, and is rapidly heat-activated in 3 or 5 minutes.

- The fluorogenic PCR conditions provide significant time savings of up to 78% in endpoint PCR and up to 60% in realtime PCR without compromising specificity and sensitivity.

- New QIAGEN chemistries enable fluorogenic on all cyclers, including those not capable of achieving rapid ramping rates.

References

Sample & Assay Technologies