Thermal cycler amplification robustness: a comparison of several models

Introduction

The ability of a thermal cycler to amplify difficult targets uniformly across a sample block is a critical factor for many researchers. Ideally, a thermal cycler should produce high yields for a variety of difficult-to-amplify targets, including GC-rich and long templates. This study compares the ability of several thermal cyclers to uniformly amplify a selection of difficult templates.

Table 1. Instruments tested for amplification robustness.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Model name</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo Fisher Scientific</td>
<td>Applied Biosystems™ ProFlex™ 96-Well PCR System</td>
<td>4484075</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>Applied Biosystems™ SimpliAmp™ Thermal Cycler</td>
<td>A24811</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>Applied Biosystems™ MiniAmp™ Plus Thermal Cycler</td>
<td>A37835</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>Applied Biosystems™ MiniAmp™ Thermal Cycler</td>
<td>A37834</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>Applied Biosystems™ 2720 Thermal Cycler</td>
<td>4359659</td>
</tr>
<tr>
<td>Agilent</td>
<td>SureCycler™ 8800 Thermal Cycler</td>
<td>G8800A</td>
</tr>
<tr>
<td>Bio-Rad</td>
<td>GeneMax™ Thermal Cycler</td>
<td>BYQ6067</td>
</tr>
<tr>
<td>Bio-Rad</td>
<td>C1000 Touch™ Thermal Cycler with 96-Well Fast Reaction Module</td>
<td>185-1196</td>
</tr>
<tr>
<td>Bio-Rad</td>
<td>T100™ Thermal Cycler</td>
<td>186-1096</td>
</tr>
<tr>
<td>Eppendorf</td>
<td>Mastercycler™ Nexus Gradient</td>
<td>6331 000.017</td>
</tr>
<tr>
<td>Eppendorf</td>
<td>Mastercycler™ Pro S</td>
<td>6325 000.510</td>
</tr>
<tr>
<td>SensoQuest</td>
<td>Labcycler Gradient</td>
<td>011-101, 012-103</td>
</tr>
<tr>
<td>Takara</td>
<td>Dice™ Touch</td>
<td>TP350</td>
</tr>
</tbody>
</table>

Materials and methods

The instruments tested in this study are shown in Table 1. The same equipment, methods, and reagents were used for each instrument. A successful PCR experiment is shown by a clear, neat band on an electrophoresis gel. The intensity of the bands are not measured or compared.
AT-rich template (28.2% GC content)
A single bulk reaction was prepared using Applied Biosystems™ AmpliTaq Gold™ 360 Master Mix (Cat. No. 4398876) according to the standard protocol, with primers targeting the RB1 oncogene. The forward primer (5´-TGTAAAACGACGGCCAGTCCTTAGGTGGAT-CAGCTGGGTG-3´) and reverse primer (5´-CAGCTATGACCTCAATTATTTGCATTGTGCAT-3´) were used at 0.2 μM, and the template, Human Genomic DNA (Roche, Cat. No. 1169112001), was used at 1 ng/μL. The bulk reaction was split among 32 wells to test for amplification consistency. The thermal cycling protocol consisted of a primary stage at 94°C for 10 min followed by a secondary stage consisting of 32 cycles at 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, before a final stage at 72°C for 7 min.

GC-rich template (80.4% GC content)
A single bulk reaction was prepared using AmpliTaq Gold 360 Master Mix with 360 GC Enhancer reagent and primers targeting the CCNE (cyclin-E1) gene. The forward primer (5´-TGTTAAACGACGGCCAGTACAGAGAATGGCCGTGAGT-3´) and reverse primer (5´-CAGGAAAACAGCTATGACCTTAAGCTGTTTCCATAGGGT-GACACA-3´) were used at 0.2 μM, and the human genomic DNA template was used at 1 ng/μL. The bulk reaction was split among 32 wells to test for amplification consistency. The thermal protocol was the same as that used for the AT-rich template.

Dual-target amplification
A single bulk reaction was prepared using AmpliTaq Gold 360 Master Mix according to the standard protocol, with primers that produce a high molecular weight (HMW) product and low molecular weight (LMW) product. The forward primer (5´-TGTAAAACGACGGCCAGTTCCTTGTGCAGCTCAGCCTCCA-3´) and reverse primer (5´-CAGGAAAACAGCTATGACCTTAAGCTGTTTCCCATAGGGTG-GACACA-3´) were used at 0.2 μM, and the human genomic DNA template was used at 1 ng/μL. The bulk reaction was split among 32 wells to test for amplification consistency. The thermal protocol was the same as that used for the AT-rich template.

Long template (12,342 bp)
A single bulk reaction was prepared using the Applied Biosystems™ SequalPrep™ Long PCR Kit (Cat. No. A10498) according to the standard protocol, with primers targeting the BRCA2 gene. The forward primer (5´-CTCCCCCAGAAAAGGGGACAAAGC-3´) and reverse primer (5´-ACAAACTCCACATACCATGACCTG-3´) were used at 2 μM, and the human genomic DNA template was used at 2.5 ng/μL. The bulk reaction was split among 32 wells to test for amplification consistency. The thermal protocol consisted of a primary stage at 94°C for 2 min followed by a secondary stage consisting of 35 cycles at 94°C for 10 sec, 61°C for 30 sec, and 68°C for 14 min and 20 sec, before a final stage at 72°C for 5 min.

Data acquisition and analysis
After amplification, long PCR products were loaded onto an Invitrogen™ E-Gel™ 1% Agarose Gel (Cat. No. G800801). All other assays were loaded onto an E-Gel 2% Agarose Gel (Cat. No. G800802). The reactions were subjected to electrophoresis under standard conditions, with the Invitrogen™ 100 bp DNA Ladder (Cat. No. 15628019), and gel images were acquired using a constant exposure time and aperture size. After imaging, bands were quantified using Thermo Scientific™ myImageAnalysis™ Software (Cat. No. 62237).

Results
The 13 instruments were tested using the four PCR experiments as listed above. In many cases, certain wells failed to amplify any PCR product. The wells that failed to amplify in each assay are tallied in Table 2.
Table 2. Tally of wells that failed to produce bands.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Instrument</th>
<th>AT-rich template</th>
<th>GC-rich template</th>
<th>Dual target, HMW</th>
<th>Dual target, LMW</th>
<th>Long template (BRCA2, 12,342 bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo Fisher Scientific</td>
<td>ProFlex</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>SimpliAmp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>MiniAmp Plus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>MiniAmp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>2720</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Agilent</td>
<td>SureCycler 8800</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Bioer</td>
<td>GeneMax*</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Bio-Rad</td>
<td>C1000 Touch</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Bio-Rad</td>
<td>T100*</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Eppendorf</td>
<td>Mastercycler Nexus Gradient*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Eppendorf</td>
<td>Mastercycler Pro S</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SensoQuest</td>
<td>Labcycler Gradient</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Takara</td>
<td>Dice Touch*</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

* Evaporation occurred in corner wells.

**Discussion**

Thermal cycler performance is crucial when amplifying difficult or long DNA templates. Temperature accuracy and stability make it possible to successfully amplify difficult or long DNA templates, which require extended hold times. Multiple or smeared bands, rather than a single target band, were observed when using some thermal cyclers. If the block temperature is not uniform, nonspecific binding during the annealing step may occur, generating unwanted amplicons in addition to the target amplicon. Refer to thermofisher.com/thermalcycleraccuracy for more information.

Evaporation can occur in corner wells during a long temperature hold protocol. A heated lid with positive and constant pressure minimizes sample evaporation and ensures well-to-well reproducibility. Learn more at thermofisher.com/thermalcyclerevaporation.

The ability of a thermal cycler to uniformly amplify reactions is pivotal. In this study, we have shown that the quality of PCR amplification varies between thermal cyclers. This was carried out as a side-by-side comparison while utilizing the same reaction chemistry.

For best results, we recommend using a thermal cycler that produces a high yield for difficult-to-amplify targets but also does not sacrifice integrity and consistency of amplification. The combined results for each assay category suggest that the Applied Biosystems™ thermal cyclers meet these criteria.
<table>
<thead>
<tr>
<th>Laboratory Equipment</th>
<th>Gel Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied Biosystems ProFlex PCR System</td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems SimpliAmp Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems MiniAmp Plus Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems MiniAmp Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems 2720 Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>Agilent SureCycler 8800 Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>Bioer GeneMax Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>Bio-Rad C1000 Touch Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>Bio-Rad T100 Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>Eppendorf Mastercycler Nexus Gradient</td>
<td></td>
</tr>
<tr>
<td>Eppendorf Mastercycler Pro S</td>
<td></td>
</tr>
<tr>
<td>SensoQuest Labcycler Gradient</td>
<td></td>
</tr>
<tr>
<td>Takara Dice Touch</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Gel images of PCR products following amplification of AT-rich template.
| Applied Biosystems ProFlex PCR System |
| Applied Biosystems SimpliAmp Thermal Cycler |
| Applied Biosystems MiniAmp Plus Thermal Cycler |
| Applied Biosystems MiniAmp Thermal Cycler |
| Applied Biosystems 2720 Thermal Cycler |
| Agilent SureCycler 8800 Thermal Cycler |
| Bioer GeneMax Thermal Cycler |
| Bio-Rad C1000 Touch Thermal Cycler |
| Bio-Rad T100 Thermal Cycler |
| Eppendorf Mastercycler Nexus Gradient |
| Eppendorf Mastercycler Pro S |
| SensoQuest Labcycler Gradient |
| Takara Dice Touch |

Figure 2. Gel images of PCR products following amplification of GC-rich template.
<table>
<thead>
<tr>
<th>Applied Biosystems ProFlex PCR System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied Biosystems SimpliAmp Thermal Cycler</td>
</tr>
<tr>
<td>Applied Biosystems MiniAmp Plus Thermal Cycler</td>
</tr>
<tr>
<td>Applied Biosystems MiniAmp Thermal Cycler</td>
</tr>
<tr>
<td>Applied Biosystems 2720 Thermal Cycler</td>
</tr>
<tr>
<td>Agilent SureCycler 8800 Thermal Cycler</td>
</tr>
<tr>
<td>Bioer GeneMax Thermal Cycler</td>
</tr>
<tr>
<td>Bio-Rad C1000 Touch Thermal Cycler</td>
</tr>
<tr>
<td>Bio-Rad T100 Thermal Cycler</td>
</tr>
<tr>
<td>Eppendorf Mastercycler Nexus Gradient</td>
</tr>
<tr>
<td>Eppendorf Mastercycler Pro S</td>
</tr>
<tr>
<td>SensoQuest Labcycler Gradient</td>
</tr>
<tr>
<td>Takara Dice Touch</td>
</tr>
</tbody>
</table>

Figure 3. Gel images of dual-target PCR products.
Figure 4. Gel images of long PCR products following amplification of the *BRCA2* gene.
Figure 5. Gel layout and plate coordinates of 2% E-Gel 48 Agarose Gel for amplification of GC-rich template, AT-rich template, and dual target (dimer). DNA refers to the 100 bp DNA Ladder; TE refers to TE buffer.

Figure 6. Gel layout and plate coordinates of 1% E-Gel 48 Agarose Gel for long-range amplification of BRCA2 template. DNA refers to the 100 bp DNA Ladder; TE refers to TE buffer.

Find out more at thermofisher.com/thermalcyclers