Taq the fidelity and robust amplification of activity of Deep Vent DNA Polymerase increases strain that carries the Deep Vent® E. coli and an Thermus aquaticus Polymerase gene from Source: E. coli strain that carries the An Taq DNA (Figure 1).

A wide range of PCR products can be generated, Taq fold higher fidelity than Polymerase (1). This ready-to-use mix offers two Taq DNA Polymerase LongAmp™ DNA Polymerase. The 3´ 2X Master Mix. These guidelines cover PCR reactions. Amplification of templates with high GC content, high secondary structure or low template concentrations may require further optimization.

PCR
The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (2). Taq DNA Polymerase is an enzyme widely used in PCR (3). The following guidelines are provided to ensure successful PCR using New England Biolabs' LongAmp Taq 2X Master Mix. These guidelines cover routine PCR reactions. Amplification of templates with high GC content, high secondary structure or low template concentrations may require further optimization.

Reaction setup:
We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to 94°C and begin thermocycling.

Thermocycling Conditions for a Routine PCR:

<table>
<thead>
<tr>
<th>STEP</th>
<th>TEMP</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94°C</td>
<td>30 seconds</td>
</tr>
<tr>
<td>30 Cycles</td>
<td>94°C</td>
<td>10–30 seconds</td>
</tr>
<tr>
<td></td>
<td>45–65°C</td>
<td>15–60 seconds</td>
</tr>
<tr>
<td></td>
<td>65°C</td>
<td>50 seconds/kb</td>
</tr>
<tr>
<td>Final Extension</td>
<td>65°C</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Hold</td>
<td>4–10°C</td>
<td></td>
</tr>
</tbody>
</table>

General Guidelines:
1. Template: The quality of the DNA template is essential for long-range PCR amplification. Recommended amounts of DNA template for a 50 µl reaction are as follows:

<table>
<thead>
<tr>
<th>DNA</th>
<th>UP TO 15 kb</th>
<th>ABOVE 15 kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic</td>
<td>1 ng–500 ng</td>
<td>10 ng–1 µg</td>
</tr>
<tr>
<td>Plasmid or Viral</td>
<td>1 pg–1 ng</td>
<td>10 pg–10 ng</td>
</tr>
</tbody>
</table>

Successful amplification above 20 kb largely depends on the quality of DNA templates and the primer sequences.

2. Primers:
Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. For amplicons larger than 20 kb, it is desirable to have primers with GC content above 50%, matched Tm above 60°C and primers at least 24 nucleotides in length. The final concentration of each primer in a PCR reaction may be 0.05–1 µM, typically 0.1–0.5 µM.

3. Mg++ and additives:
Mg++ concentration of 1.5–2.0 mM is optimal for most PCR products generated with LongAmp Taq DNA Polymerase. The final Mg++ concentration in 1X LongAmp Taq Master Mix is 2 mM. This supports satisfactory amplification of most amplicons. However, Mg++ can be further optimized in 0.5 or 1.0 mM increments using MgSO4.

Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (4) or formamide (5).

4. Denaturation:
An initial denaturation of 30 seconds at 94°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–4 minutes at 94°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minute denaturation at 94°C is recommended. During thermocycling a 10–30 second denaturation at 94°C is recommended.

5. Annealing:
The annealing step is typically 15–60 seconds. Annealing temperature is based on the Tm of the primer pair and is typically 45–65°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated Tm. We recommend using NEB’s Tm Calculator, available at www.neb.com/TmCalculator to determine appropriate annealing temperatures for PCR.

When primers with annealing temperatures above 60°C are used, a 2-step PCR protocol is possible (see #8).

6. Extension:
The recommended extension temperature is 65°C. Extension times are generally 30 seconds per kb. A final extension of 10 minutes at 65°C is recommended.

7. Cycle number:
Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.
8. **2-step PCR:**
When primers with annealing temperatures above 60°C are used, a 2-step thermocycling protocol is possible.

**Thermocycling Conditions for a Routine 2-Step PCR:**

<table>
<thead>
<tr>
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<td>Hold</td>
<td>4–10°C</td>
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</tr>
</tbody>
</table>

9. **PCR product:**
The majority of the PCR products generated using LongAmp Taq DNA Polymerase contain dA overhangs at the 3’–end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

**Notes:** LongAmp Taq 2X Master Mix is stable for fifteen freeze-thaw cycles when stored at –20°C. LongAmp Taq 2X Master Mix is also stable for three months at 4°C, so for frequent use, an aliquot may be kept at 4°C.

**FAQs:**
1. **What is the fidelity of the LongAmp Taq DNA Polymerase compared to Taq DNA Polymerase?**
The LongAmp Taq DNA Polymerase offers two fold higher fidelity than Taq.

2. **Can the extension step be carried out at 72°C when using LongAmp?**
Yes, LongAmp Taq DNA Polymerase can be used at 72°C. However, extension at 65–68°C is a better choice for most amplicons.

3. **What is the extension rate when using LongAmp?**
We recommend 50 seconds per kb for maximum yields. Extension rates such as 30 seconds per kb can be used for targets up to 4 kb using a 3-step PCR protocol. Shorter extension rates such as 15 seconds per kb can be used for targets up to 2 kb using a 3-step PCR protocol on a fast PCR machine.

4. **What type of DNA ends result from a primer extension reaction or a PCR reaction using LongAmp Taq DNA Polymerase?**
The majority of the PCR products generated using LongAmp Taq DNA Polymerase contain dA overhangs at the 3’–end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

5. **Why is the product a smear when visualized on an agarose gel?**
When PCR conditions are not optimal, a smear or high level of background is often observed. Try one or more of the following suggestions:
- use lower amount of enzymes
- use 65°C for extension
- raise annealing temperature
- try 2-step cycling protocols

6. **Can LongAmp 2X Master Mix be used to amplify GC-rich amplicons?**
Yes. The addition of DMSO up to 10% helps amplify GC-rich amplicons.

**References:**

**Companion Products Sold Separately:**
- LongAmp Taq (Mg-free) Reaction Buffer Pack #B0322S 6.0 ml
- LongAmp Taq Reaction Buffer Pack #B0323S 6.0 ml
- Crimson LongAmp Taq Reaction Buffer Pack #B0326S 6.0 ml
- Magnesium Sulfate (MgSO₄) Solution #B1003S 6.0 ml
- LongAmp Taq PCR Kit #E5200S 100 Reactions
- LongAmp Taq DNA Polymerase #M0323S 500 units #M0323L 2,500 units
- Crimson LongAmp Taq DNA Polymerase #M0326S 250 units #M0326L 1,250 units
- Deoxynucleotide Solution Set #N0446S 25 µmol of each
- Deoxynucleotide Solution Mix #N0447S 8 µmol of each #N0447L 40 µmol of each

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