1X One Taq Master Mix with GC Buffer:
80 mM Tris-So4 (pH 9.2)
2 mM MgSO4
20 mM (NH4)2SO4
0.2 mM dNTPs
5% Glycerol
5% DMSO
0.06% IGEPA® CA-630
0.05% Tween-20
25 units/ml One Taq DNA Polymerase

One Taq High GC Enhancer:
10 mM Tris-HCl
25% DMSO
25% Glycerol, pH 9.2 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 15 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.

Unit Assay Conditions: 1X Thermopol Reaction Buffer, 200 µM dNTPs including [3H]-dTTP and 200 µg/ml activated calf thymus DNA.

Heat Inactivation: No

Quality Control Assays

Buffer-dependent GC-rich (> 65% GC) PCR:
30 cycles of PCR amplification of 10 ng of human genomic DNA with 1X One Taq Master Mix with GC Buffer in a 25 µl reaction in the presence of 0.2 µM primers resulted in the buffer-dependent production of the 737 bp GC-rich product.

Enhancer-dependent High GC (> 70% GC) PCR:
30 cycles of PCR amplification of 10 ng of human genomic DNA with 1X One Taq Master Mix with GC Buffer in a 25 µl reaction in the presence of 0.2 µM primers and 20% One Taq High GC Enhancer resulted in the enhancer-dependent production of the 627 bp high GC product.

Note: Product specifications for individual components in the One Taq 2X Master Mix with GC Buffer are available separately.

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’ One Taq 2X Master Mix with GC Buffer. These guidelines cover routine PCR reactions. Specialized applications may require further optimization.

Reaction Setup:
We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (94°C).

General Guidelines:
1. Template:
   Use of high quality, purified DNA templates greatly enhances the success of PCR reactions. Recommended amounts of DNA template for a 50 µl reaction are as follows:
   
<table>
<thead>
<tr>
<th>DNA</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic</td>
<td>1 ng–1 µg</td>
</tr>
<tr>
<td>Plasmid or Viral</td>
<td>1 pg–1 ng</td>
</tr>
</tbody>
</table>

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. The final concentration of each primer in a PCR reaction may be 0.05–1 µM, typically 0.2 µM.

3. Mg++ and Additives:
   Mg++ concentration of 1.5–2.0 mM is optimal for most PCR products generated with One Taq DNA Polymerase. The final Mg++ concentration in 1X One Taq Master Mix with GC Buffer is 2 mM. This supports satisfactory amplification of most amplicons. However, Mg++ can be further optimized in 0.2 mM increments using MgSO4 (sold separately).
   Amplification of extremely difficult targets may be improved by the addition of 10–20% One Taq High GC Enhancer (included).

4. Denaturation:
   An initial denaturation of 30 seconds at 94°C is sufficient to amplify most targets from pure DNA templates. For difficult templates, 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 2–5 minute denaturation at 94°C is recommended to lyse cells.
   During thermocycling a 15–30 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 45–68°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated Tm.

<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>10 seconds</td>
</tr>
<tr>
<td>10 Cycles</td>
<td>45–68°C</td>
<td>15–60 seconds</td>
</tr>
<tr>
<td>Final Extension</td>
<td>68°C</td>
<td>1 minute/kb</td>
</tr>
<tr>
<td>Hold</td>
<td>4–10°C</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>

Amplification of a selection of sequences with varying GC content from human genomic DNA using One Taq 2X Master Mix with GC Buffer. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).
6. Extension:
The recommended extension temperature is 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°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 of 68°C or above are used, a 2-step thermocycling protocol (combining annealing and extension into one step) is possible.

9. PCR Product:
The majority of the PCR products generated using One Taq™ DNA Polymerase contain dA overhangs at the 3' end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

Notes:
One Taq™ 2X Master Mix with GC Buffer is stable for fifteen freeze-thaw cycles when stored at −20°C.

One Taq™ 2X Master Mix with GC Buffer is also stable for one month at 4°C, so for frequent use, an aliquot may be kept at 4°C.

References: