Applications
- Routine PCR amplification of DNA fragments up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- RT-PCR.
- Genotyping.
- Generation of PCR products for TA cloning.

DreamTaq Green PCR Master Mix (2X) composition
DreamTaq Green PCR Master Mix contains DreamTaq DNA polymerase, 2X DreamTaq Green buffer, dATP, dCTP, dGTP and dTTP, 0.4 mM each, and 4 mM MgCl₂. DreamTaq Green buffer is a proprietary formulation optimized for robust performance in PCR. It contains a density reagent and two dyes for monitoring ultraphoresis progress: the blue dye migrates with 3–5 kb DNA fragments in a 1% agarose gel and the yellow dye migrates faster than 10 bp DNA fragments in 1% agarose gel. The dyes have absorption peaks at 424 nm and 615 nm.

PROTOCOL
1. Gently vortex and briefly centrifuge DreamTaq Green PCR Master Mix (2X) after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 μL reaction:

<table>
<thead>
<tr>
<th>Component</th>
<th>#K1001</th>
<th>#K1002</th>
</tr>
</thead>
<tbody>
<tr>
<td>DreamTaq Green PCR Master Mix (2X)</td>
<td>200 nls of 50 μL</td>
<td>1000 nls of 50 μL</td>
</tr>
<tr>
<td>Forward primer</td>
<td>0.1-1.0 μM</td>
<td></td>
</tr>
<tr>
<td>Reverse primer</td>
<td>0.1-1.0 μM</td>
<td></td>
</tr>
<tr>
<td>Template DNA</td>
<td>10 μg - 1 μg</td>
<td></td>
</tr>
<tr>
<td>Water, nuclease-free</td>
<td>4 × 1.25 mL</td>
<td>20 × 1.25 mL</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td>50 μL</td>
<td></td>
</tr>
</tbody>
</table>

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 μL of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature, °C</th>
<th>Time</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95</td>
<td>1-3 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>30 s</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>Tm-5</td>
<td>30 s</td>
<td>25-40</td>
</tr>
<tr>
<td>Extension*</td>
<td>72</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72</td>
<td>5-15 min</td>
<td>1</td>
</tr>
</tbody>
</table>

*The recommended extension step is 1 min for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 min/kb.

6. Load 5-15 μL of PCR mixture directly on a gel.

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION
During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and ampiclons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:
- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination.

GUIDELINES FOR PRIMER DESIGN
Use primer design software or follow the general recommendations for PCR primer design as outlined below:
- PCR primers are generally 15–30 nucleotides long.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5 °C.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3'-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3'-end.
- When introducing restriction enzyme sites into primers, refer to the table “Reaction conditions for FastDigest enzymes” located on www.thermofisher.com/fastdigest to determine the number of extra bases required for efficient cleavage.

Estimation of primer melting temperature
For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:

\[ Tm = 4 \left( G + C \right) + 2 \left( A + T \right), \]

where G, C, A, T represent the number of respective nucleotides in the primer.

If the primer contains more than 25 nucleotides we recommend using specialized computer programs to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA
Optimal amounts of template DNA for a 50 μL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 μg for genomic DNA. Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

All routine DNA purification methods can be used to prepare the template e.g., Thermo Scientific™ GeneJET™ Genomic DNA Purification Kit (#K0721) or Thermo Scientific™ GeneJET™ Plasmid Miniprep Kit (#K0502). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually removes trace contaminants from DNA samples.

Primers
The recommended concentration range of the PCR primers is 0.1-1 μM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.

For degenerate primers and primers used for long PCR we recommend higher primer concentrations in the range of 0.3-1 μM.

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CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation
It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95 °C is sufficient.

Denaturation
A DNA denaturation time of 30 seconds per cycle at 95 °C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

Primer annealing
The annealing temperature should be 5 °C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.

Extension
The optimal extension temperature for DreamTaq DNA polymerase is 70-75 °C. The recommended extension step is 1 min/kb at 72°C for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 1 min/kb. For amplification of longer templates (>6 kb) a reduction of the extension temperature to 68°C is required to avoid enzyme inactivation during prolonged extension times.

Number of cycles
If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension
After the last cycle, it is recommended to incubate the PCR mixture at 72 °C for an additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product has to be cloned into a TA vector, e.g. using Thermo Scientific™ InTAclona™ PCR Cloning Kit (#K1213), the final extension step may be prolonged to 30 min to ensure the highest efficiency of 3'-dA tailing of the PCR product. If the PCR product has to be used for cloning using Thermo Scientific™ CloneJET™ PCR Cloning Kit (#K1231), the final extension step can be omitted.

Troubleshooting
For troubleshooting please visit www.thermofisher.com

CERTIFICATE OF ANALYSIS

Functional Assay
DreamTaq Green PCR Master Mix (2X) was tested for amplification of 20 kb DNA fragment.
Quality authorized by:  Jurgita Zilinskiene

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