end point PCR protocols and it contains premixed gel loading dye which allows direct sample loading on the gel. The loading dye in the Master Mix does not interfere with PCR performance and is compatible with downstream applications such as DNA sequencing, ligation and restriction digestion.

2. Package information

<table>
<thead>
<tr>
<th>Component</th>
<th>#F-170S 100 rxns</th>
<th>#F-170L 500 rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x Phire Tissue Direct PCR Master Mix</td>
<td>2 x 1.25 mL 10 x 1.25 mL</td>
<td>5 mL 2 x 1.25 mL</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>5 mL</td>
<td>2 x 12.5 mL</td>
</tr>
<tr>
<td>DNARelease Additive</td>
<td>3 x 100 µL</td>
<td>1.3 mL</td>
</tr>
<tr>
<td>Universal control primer mix (25 µM each)</td>
<td>40 µL 40 µL</td>
<td>40 µL</td>
</tr>
<tr>
<td>Water, nuclease-free</td>
<td>2 x 1.25 mL</td>
<td>10 x 1.25 mL</td>
</tr>
<tr>
<td>O’GeneRuler Express DNA Ladder</td>
<td>100 applications (50 µg)</td>
<td></td>
</tr>
</tbody>
</table>

3. Important notes

- Primer annealing temperatures for Phire are different from many common DNA polymerases (such as Taq DNA polymerases). Read Section 9.3 carefully.
- Use 98 °C for denaturation.
- Use 50 µL reaction volume for direct protocols.
- Add the sample directly into a PCR reaction instead of an empty tube.
- The Dilution & Storage protocol (see Section 6) is recommended:
  - When working with new sample materials or a new primer pair.
  - With difficult samples or long amplicons.
  - When performing multiple reactions from the same sample.

4. Guidelines for PCR

Carefully mix and spin down all tubes before opening to ensure homogeneity and improve recovery. The PCR setup can be performed at room temperature. Always add the sample last to the reaction. Read Section 5 carefully for sampling guidelines.

4.1 Positive control reaction with purified DNA

When optimizing the reactions, it is recommended to perform a positive control with purified DNA to ensure that the PCR conditions are optimal. If the positive control with purified DNA fails, the PCR conditions should be optimized before continuing further.

4.2 Negative control

It is recommended to add a no-template control to all Direct PCR assays.

5. Guidelines for sample handling

To obtain small and uniform samples, we recommend using 0.35–0.50 mm diameter punch. If the punch is not to be reused, it is very important to clean the cutting edge properly to prevent cross-contamination between samples. Use 2% NaOCl solution for cleaning and cross-contamination prevention.

Other ways to take a sample is by cutting with scalpel to obtain 0.35–0.50 mm sample. Scalpel must be cleaned properly to prevent cross-contamination between samples.

6. Choosing the protocol

This Master Mix is optimized for various tissue samples. Please visit www.thermoscientific.com/directpcr to see a list of tested tissues and recommendations for sample sizes. With a few exceptions, both Direct and Dilution & Storage protocols are compatible with all sample types and applications. However, when amplifying longer fragments (e.g. > 500 bp from fish tissue or > 1 kb from other tissues) the Dilution & Storage protocol is recommended. The Dilution & Storage protocol is also useful when multiple PCR reactions are performed from the same sample or in some challenging applications where template amount is critical and titration is needed. When working with new sample materials or a new primer pair, start with the Dilution & Storage protocol, as it allows several PCR reactions to be performed from the same sample if optimization is required. Samples in Dilution Buffer can be stored for up to 4 weeks in different temperatures (–20 °C, +4 °C or room temperature) before using in PCR.

6.1 Solid samples

Animal tissues

1. Direct protocol:
   - Take a sample of 0.35–0.5 mm in diameter from tissue with a sterile scalpel (or small peace, e.g. one Drosophila leg) or by a tissue puncher. Place the sample directly into the PCR reaction (50 µL of volume). It is recommended to place the sample into the liquid rather than into an empty tube. Make sure that you see the sample in the solution.

2. Dilution & storage protocol:
   - Before beginning, warm a heat block to 98 °C. Place the tissue sample into 20 µL of Dilution Buffer. Add 0.5 µL of DNARelease Additive. Mix by vortexing the tube briefly, and spin down the solution. If a larger sample is used, adjust the volume of the Dilution Buffer and DNARelease Additive accordingly. Make sure the sample is covered with the solution. Incubate the reaction for 2–5 minutes at room temperature and then place the tube into the pre-heated (98 °C) block for 2 minutes. Spin down the remaining tissue and store the supernatant at –20 °C if not used immediately. Usually 1 µL of supernatant is sufficient for a 20 µL PCR reaction. In some cases the supernatant may have to be diluted 1:10 or 1:100, or the PCR reaction performed in a 50 µL volume.

Buccal swabs (e.g. nylon flocked swab)

1. Direct protocol:
   - For Nylon flocked swabs take a 0.5 mm punch (or similar) and place it directly into a 50 µL PCR reaction. In some cases reducing the punch size to 0.35 mm may improve the results.

2. Dilution & storage protocol:
   - Place the buccal swab tip into a 1.5 ml tube containing 50 µL Dilution Buffer, 1.5 µL DNARelease Additive and 250 µL TE, pH 8.0. Rub the swab 5–10 times before removing it from the tube by gently pressing the brush against the side of the tube. Mix by vortexing and spin down. Incubate at 98 °C for 2 minutes, spin down again, and use 0.5 µL of the supernatant as a template for a 20 µL PCR reaction. Note: Swabbing technique and storage conditions (not thoroughly dried) may cause yield variation.

Hair

1. Direct protocol:
   - Take 2 to 5 hair bulks and place them directly into a 50 µL PCR reaction.

2. Dilution & storage protocol:
   - Place 2 to 5 hair bulks in a tube that contains 20 µL Dilution Buffer and 0.5 µL DNARelease Additive. Make sure the sample is covered with the solution. Mix by vortexing and spin down. Incubate for 2–5 minutes at room temperature followed by 2 minutes at 98 °C. Spin down again, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction.

Teeth

1. Direct protocol:
   - Place a sample of tooth approximately this size (1–5 mm) directly into a 50 µL PCR reaction.

2. Dilution & storage protocol:
   - Place an approximate 13–15 mg sample of tooth in a tube that contains 50 µL Dilution Buffer and 0.5 µL DNARelease Additive.
Formalin-fixed paraffin-embedded (FFPE) tissues

1. Direct protocol: take a 0.5 mm sample and place it directly into a 50 µL PCR reaction. In some cases reducing the punch size to 0.35 mm may improve sensitivity.

2. Dilution & storage protocol: Place a 2 mm punch sample into a tube containing 20 µL Dilution Buffer and 0.5 µL DNA Release Additive. Make sure the sample is covered with the solution.

Note:
Using finely crushed sample may yield better results, e.g. improved sensitivity. The sample can be crushed for example by grinding in liquid nitrogen with a mortar and pestle or a homogenizer.

Skin biopsies (non-fixed)

1. Direct protocol: take a 0.5 mm sample and place it directly into a 50 µL PCR reaction. In some cases reducing the punch size to 0.35 mm may improve sensitivity.

2. Dilution & storage protocol: Place a 2 mm punch sample into a tube containing 20 µL Dilution Buffer and 0.5 µL DNA Release Additive. Make sure the sample is covered with the solution.

Mix by vortexing and spin down. Incubate for 2–5 minutes at room temperature, followed by 2 minutes at 98 °C. Spin down again, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction.

Fingernails

1. Direct protocol: Place a small nail sample (approximately 1 x 2 mm, in other words <1 mg) directly into a 50 µL PCR reaction.

2. Dilution & storage protocol: Place an approximately 7 mg nail sample in a tube that contains 50 µL Dilution Buffer and 1.5 µL DNA Release Additive. Make sure the sample is covered with the solution.

Mix by vortexing and spin down. Incubate for 2–5 minutes at room temperature, followed by 2 minutes at 98 °C. Spin down again, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction.

Note:
Using finely diced sample may yield better results, e.g. improved sensitivity.

Formalin-fixed paraffin-embedded (FFPE) tissues

1. Direct protocol: Not recommended

2. Dilution & storage protocol: Place an approximately 7 mg nail sample in a tube that contains 50 µL Dilution Buffer and 1.5 µL DNA Release Additive. Make sure the sample is covered with the solution.

Mix by vortexing and spin down. Incubate for 2–5 minutes at room temperature, followed by 2 minutes at 98 °C. Spin down again, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction.

7. Gel electrophoresis

2X Phire Plant Direct PCR Master Mix contains a premixed gel loading dye. After PCR samples can be directly loaded on the electrophoresis gel for analysis. It is recommended to use the DNA Release Additive for gel electrophoresis as otherwise DNA debris present in the PCR products can cause DNA fragments to get trapped in the agarose gel wells. Add 1.5 µL of DNA Release Additive into a 50 µL PCR reaction.

8. Notes About Reaction Components

8.1 Phire Tissue Direct PCR Master Mix

2X Phire Tissue Direct PCR Master Mix has been optimized for Direct PCR from variety of tissues. It contains the dNTPs and exonuclease activity. When cloning fragments amplified with Phire Hot Start II DNA Polymerase blunt end cloning is recommended. If TA cloning is required, it can be performed by adding A overhangs to the blunt PCR product with Thermo Sci. Taq DNA Polymerase, for example (protocol available at www.thermoscientific.com/pcrcloning).

8.2 Dilution Buffer

The Dilution Buffer has been optimized to release DNA from a wide variety of different tissues when supplemented with DNA Release Additive (see Section 8.1). Samples in Dilution Buffer can be stored for up to 4 weeks in different temperatures (20 °C, 4 °C and room temperature) before using in PCR. Always transfer the supernatant into a new tube before storage. Long term storage is recommended at -20 °C.

8.3 DNA Release Additive

DNA Release Additive is required when PCR is performed directly from certain tissue samples. It contains the dNTPs and exonuclease activity. When cloning fragments amplified in these PCR products can cause DNA fragments to get trapped in the agarose gel wells. DNA Release Additive eliminates this problem. DNA Release Additive is also used in the Dilution & Storage protocol to improve the release of DNA from the tissue sample.

8.5 Primers

The recommendation for the final primer concentration is 0.5 µM. The results from primer Tm calculations can vary significantly depending on the method used. Always use the Tm calculator and instructions on our website www.thermoscientific.com/tmc to determine the Tm values of primers and optimal annealing temperature.

9. Notes about cycling conditions

9.1 Initial denaturation

In Direct PCR protocol, the initial denaturation step is extended to 5 minutes to allow the lysis of cells, making genomic DNA available for PCR.

9.2 Denaturation

Keep the denaturation time as short as possible. Usually 5 seconds at 98 °C is enough for most templates. Note that the denaturation time and temperature may vary depending on the ramp rate and temperature control mode of the thermal cycler.

9.3 Primer annealing

Note that the optimal annealing temperature for Phire Hot Start II DNA Polymerase may differ significantly from that of Taq-based polymerases. Always use the Tm calculator and instructions at www.thermoscientific.com/pcrwebtools to determine the Tm values of primers and optimal annealing temperature.

9.4 Extension

The extension is performed at 72 °C. The recommended extension time is 20 seconds for amplicons ≤1 kb, and 20 s/kb for amplicons >1 kb.

10. Control reactions

10.1 Direct PCR control reaction using the control primer mix

When using mammalian tissue samples (e.g. mouse, human tissue), we recommend performing Direct PCR control reactions with both Direct and Dilution & Storage protocols using the control primers supplied with this Master Mix. If you use the same tissue material as in the actual experiment. The universal control primer mix contains degenerate primers that amplify a 237 bp fragment of mammalian genomic DNA. The amplified region is highly conserved non-coding region upstream of the SOX21 gene1 and the primers are designed to amplify this region from a wide range of vertebrate species.

Please note that these control primers are not compatible with fish or insect samples. The recommended control primer sequences for Drosophila and zebrafish are available at www.thermoscientific.com/directpcr.

Table 3: Pipetting instructions for control reactions.

<table>
<thead>
<tr>
<th>Component</th>
<th>20 µL rxn</th>
<th>50 µL rxn*</th>
<th>Final conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>add to 20 µL</td>
<td>add to 50 µL</td>
<td>-</td>
</tr>
<tr>
<td>2X Phire Tissue Direct PCR Master Mix</td>
<td>10 µL</td>
<td>25 µL</td>
<td>1X</td>
</tr>
<tr>
<td>Universal control primer mix</td>
<td>0.4 µL</td>
<td>1.0 µL</td>
<td>0.5 µM</td>
</tr>
<tr>
<td>Sample/Direct protocol</td>
<td>-</td>
<td>-</td>
<td>Amount depends on the sample**</td>
</tr>
<tr>
<td>Sample/Dilution &amp; Storage protocol</td>
<td>1 µL</td>
<td>2.5 µL</td>
<td>-</td>
</tr>
</tbody>
</table>

*50 µL reaction volume is recommended for the direct protocol.
**0.5 µM punch or a small sample of tissue (see www.thermoscientific.com/directpcr)
12. Troubleshooting

No product at all or low yield

<table>
<thead>
<tr>
<th>Direct Protocol:</th>
<th>Inhibition (positive control works with custom and control primers from the kit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Try to titrate sample amount.</td>
<td>If not successful, try Dilution &amp; Storage protocol.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Direct Protocol:</th>
<th>Dilution &amp; Storage Protocol:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Try to titrate sample amount.</td>
<td>1:100 with H2O or TE buffer, and use 1 μL as a template in PCR.</td>
</tr>
</tbody>
</table>

Not enough template

<table>
<thead>
<tr>
<th>Direct Protocol:</th>
<th>Dilution &amp; Storage Protocol:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Try to titrate sample amount.</td>
<td>Make sure to perform the 2-minute incubation at 98°C.</td>
</tr>
<tr>
<td>Try incubating sample in Dilution Buffer at elevated temperature (up to 65°C) instead of room temperature.</td>
<td></td>
</tr>
</tbody>
</table>

Problems with primer design

Positive control with purified DNA and custom primers fails, whereas there is product with control primers.

Assess your primer design.

Incorrect cycling parameters

Make sure to follow the cycling parameters recommended in the protocol.

Incorrect annealing temperature

Optimize annealing temperature (run a temperature gradient). The optimal annealing temperature for Phire DNA Polymerase may differ significantly from that of Taq-based polymerases. Always use the Tm calculator and instructions on our website: www.thermoscientific.com/pcrwebtools.

Too short extension time

The recommended extension time is 20 seconds for amplicons ≤1 kb, and 20 s/kb for amplicons >1 kb.

Too less cycles

For difficult amplicons try using 40 cycles.

Samples trapped in wells

Make sure to add DNARelease Additive prior gel electrophoresis.

Non-specific products

For some DNA gets trapped in wells

Add DNARelease Additive prior loading on gel.

---

10. References


11. Shipping and storage

Upon arrival, store the components at -20 °C. The Dilution Buffer can also be stored at +4 °C once it is thawed.

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