

Direct PCR protocol for muscle tissue using Phire[®] Hot Start DNA Polymerase



Application protocol

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There are two alternative protocols available, (1) direct and (2) dilution protocol.

1. Direct protocol

Prepare a 20 µl PCR reaction with Phire[®] Hot Start DNA Polymerase (refer to pipetting instructions in the product manual). Cut as small a piece of muscle tissue as possible (less than 0.4 mg) using a sterile scalpel and place the sample into the 20 µl PCR reaction.

Use the recommended protocol for Phire Hot Start DNA Polymerase, but increase the initial denaturation time at 98°C to 5 minutes and use a minimum of 20 seconds in the extension step. Use 40 cycles.

Table 1. Cycling conditions.

Cycle step	Temperature	Time	Number of cycles
Initial denaturation	98°C	5 min	1
Denaturation	98°C	5 s	40
Annealing	X°C	5 s*	
Extension	72°C	20 s ≤ 1 kb 20 s/kb > 1 kb	
Final extension	72°C 4°C	1 min hold	1

* Depending on the primers; see instructions below and the product manual

The optimal annealing temperature depends on the primers. With Phire Hot Start DNA Polymerase, the optimal annealing temperature is typically over 60°C, but some primers may require lower temperatures. Temperature gradient is a useful tool in finding an optimal annealing temperature. It is recommended to use Finnzymes' T_m calculator to set up the protocol.

After PCR, spin down the reactions and take the supernatant to gel electrophoresis. With some tissue samples, adding the DNARelease[™] Additive (F-355) into gel loading dye may be required to improve the PCR product migration in the gel (see the application note for mouse tissue).

2. Dilution protocol

Place a small piece of tissue (~0.4 g) in 50 µl of 1x TE buffer (pH 8.0). Crush the tissue with 1 ml pipette tip and incubate at 50°C for 3 minutes.

After centrifuging, use 0.5–2 µl of supernatant as template in a 20 µl PCR reaction with Phire Hot Start DNA Polymerase. The required volume of supernatant depends on the amount and type of tissue material used.

Use the same PCR protocol and post-PCR treatments as mentioned in Section 1.

General notes

1. As in all direct PCR applications, it is highly recommended to include a positive control (purified DNA) to ensure that the PCR reaction conditions are optimal. Also a negative control without DNA template is recommended.
2. These protocols may require further optimization depending on the starting material. It is recommended to use as small a piece of tissue as possible.
3. These protocols are validated for PCR products up to 300 bp. Longer amplicons may require protocol optimization.

Finnzymes' Direct PCR allows amplification of DNA directly from various starting materials such as blood, mouse ear and tail tissues, plants, and FFPE tissue samples. For more information about the Direct PCR products and protocols, please visit www.finnzymes.com/directpcr.