

Direct PCR protocol for bird feathers using Phire[®] Hot Start DNA Polymerase



Application protocol

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Protocol

Cut a 1–2 mm piece of the quill tip, and place it directly into a 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.

General notes

1. The tip of the quill is the best starting material, most probably because it contains trace amounts of blood and other tissue. In some cases the rachis and/or barbs have also given positive results in Direct PCR, but they seem to give more inconsistent results. We therefore strongly recommend using the quill tip.
2. 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. A negative control without DNA template is also recommended.
3. This protocol may require further optimization depending on the starting material. It is recommended to use as small a piece of feather as possible.
4. These protocols are validated for PCR products up to 400 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.