

Thermo Scientific Direct PCR

Amplify without purification

Amplify without prior DNA purification

The Thermo Scientific™ Direct PCR approach offers outstanding convenience for DNA amplification by allowing PCR directly from unpurified samples. A tiny amount of source material is used in the PCR reaction without any purification steps, allowing significant savings in both time and cost.

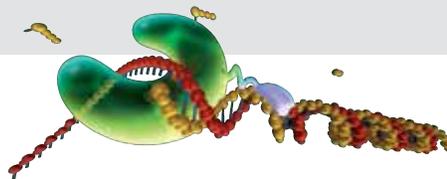
Benefits

- No need for time-consuming and expensive DNA purification
- Very little sample material required
- Minimized pipetting with master mix format and loading dyes pre-added
- Short PCR protocol times
- Robust hot-start DNA polymerases enable high yields of specific product
- Tested with a wide variety of sample types
- Rich composition—controls, water, and DNA ladder included

Based on specially engineered Thermo Scientific Phusion and Phire DNA polymerases

The Direct PCR approach is based on unique Thermo Scientific™ Phusion™ and Thermo Scientific™ Phire™ DNA polymerases. These engineered DNA polymerases have features superior to other PCR enzymes: they are highly robust and tolerant of many PCR inhibitors present in unpurified samples. Phusion and Phire DNA polymerases are therefore capable of reliably amplifying DNA in extremely challenging conditions—such as directly from crude samples.

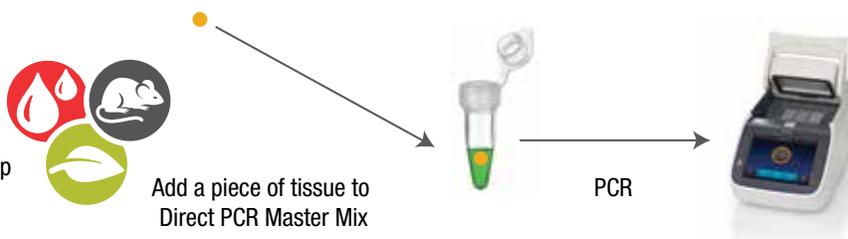
The superior performance of Phusion DNA Polymerase is based on fusion technology. The proofreading DNA polymerase is fused to a small dsDNA binding protein, creating an enzyme that generates PCR products with accuracy and speed unattainable with other DNA polymerases. Similar technology is utilized in low-fidelity Thermo Scientific™ Phire™ Hot Start II DNA Polymerase to give this PCR enzyme speed, robustness, and tolerance against various PCR inhibitors.



Two short protocols to suit your needs

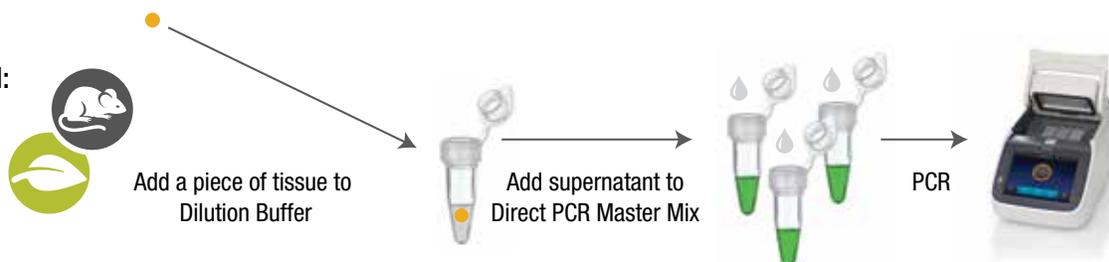
Direct protocol:

- Minimal hands-on time
- From sample to PCR in one step



Dilution and storage protocol:

- For multiple PCR reactions
- For long/difficult amplicons
- For sample storage



Efficient PCR from different plant materials across species

Thermo Scientific Phire Plant Direct PCR Master Mix

The Thermo Scientific™ Phire™ Plant Direct PCR Master Mix is designed to amplify DNA directly from plant samples. The master mix contains Phire Hot Start II DNA Polymerase and is specially formulated for performing PCR from samples with plant-derived inhibitors such as complex polysaccharides and polyphenols. It includes electrophoresis tracking dyes and a density reagent and allows direct loading of PCR products on gels, thereby simplifying direct PCR workflows.



Examples of materials tested

- Leaves: *Arabidopsis thaliana*, maize, rice, cotton, tobacco, grapevine
- Tissue on storage cards: maize, tomato
- Seeds: apple, carrot, pumpkin
- Fungi and moss: *Marschandia polymorpha*, *Agaricus* spp.
- Bacteria: gram +, gram -
- Yeast: *Saccharomyces cerevisiae*, *S. pombe*

Robust amplification from a variety of plant samples

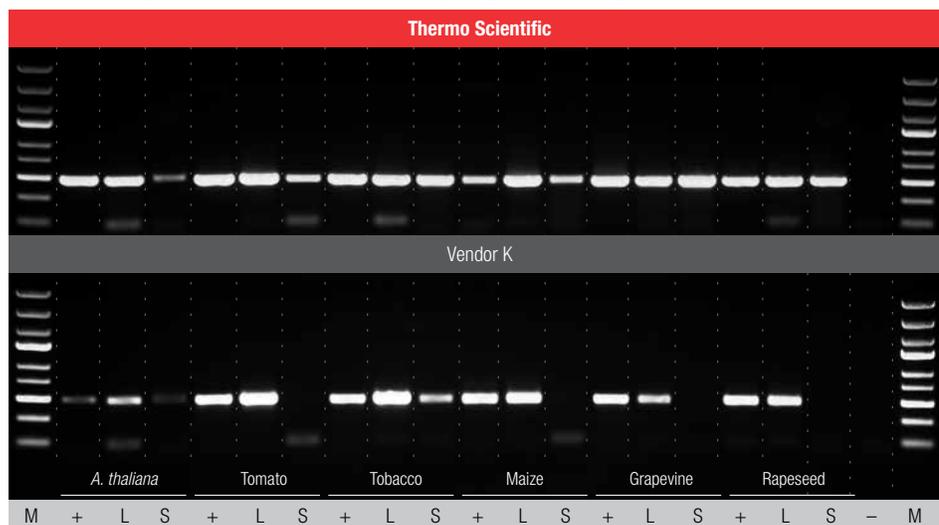


Figure 1. Amplification of a 0.5 kb DNA fragment directly from leaves and seeds from different plants. A 0.5 mm punch of leaf (L) or a small piece of crushed seed (S) was placed directly into 50 μ L of Phire Plant Direct PCR Master Mix. The same samples were amplified using a kit from Vendor K according to the supplier's instructions.

M: Thermo Scientific™ O'GeneRuler™ Express DNA Ladder

+: Control reaction with purified DNA

-: No-template control

Reliable results at a wide range of amplicon lengths

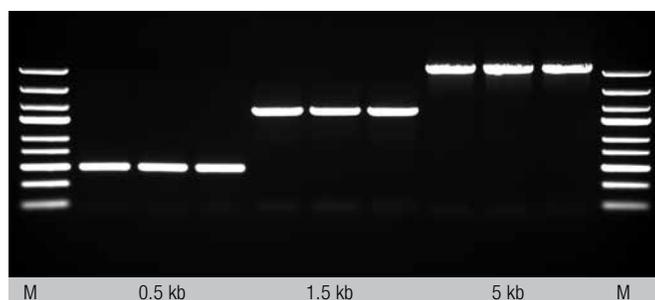


Figure 2. Different length amplicons (0.5 kb, 1.5 kb, and 5.0 kb) were amplified from dried *Laurus* spp. leaves. 0.5 mm punches of leaves were placed directly into Phire Plant Direct PCR Master Mix.

M: O'GeneRuler Express DNA Ladder

Dilution and storage protocol for retesting

For greater flexibility, Phire Plant Direct PCR Master Mix is optimized to work in direct or dilution and storage protocols. With the dilution and storage protocol, one tiny sample can be used for multiple PCR reactions, and experiments can be reproduced with great accuracy even after a month.

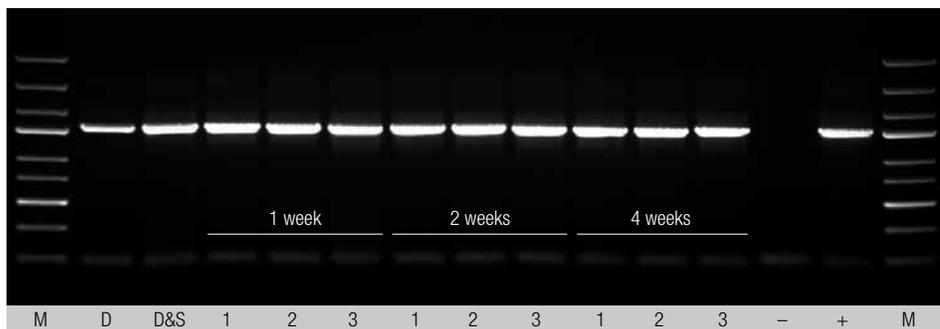


Figure 3. A 1.5 kb DNA fragment from tobacco leaves was amplified with Phire Plant Direct PCR Master Mix using the direct protocol (D) or the dilution and storage protocol (D&S).

With the D&S protocol, samples were stored in Dilution Buffer for 1, 2, or 4 weeks at different temperatures:

(1) –20°C, **(2)** 4°C, and **(3)** room temperature.

M: O'GeneRuler Express DNA Ladder

+: Control reaction with purified DNA

-: No-template control

Fast protocol from your sample to a loaded gel

In contrast to many other solutions for direct PCR, Phire Plant Direct PCR Master Mix offers a truly direct protocol. Samples can be added directly into PCR reaction mix with no prior incubation or extraction steps. Moreover, because of the advanced polymerase used, the cycling times are often half as long as those in other systems. The master mix includes electrophoresis tracking dyes and a density reagent to further reduce costs and processing time. Direct PCR reaction product can be directly loaded on a gel without adding a loading dye.

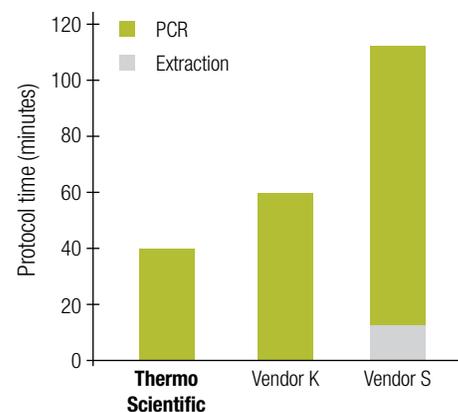


Figure 4. Phire Plant Direct PCR Master Mix saves time.

Kit component	F-160S	F-160L
2X Phire Plant Direct PCR Master Mix	2 × 1.25 mL	10 × 1.25 mL
Dilution Buffer	5 mL	2 × 12.5 mL
Control Primer Mix (25 µM each)	40 µL	40 µL
Water, nuclease-free	2 × 1.25 mL	10 × 1.25 mL
O'GeneRuler Express DNA Ladder	100 applications (50 µg)	

“The quality of PCR results was as good or better than with more time-consuming extraction methods, followed by PCR.”

Principal Investigator, Department of Biological Sciences, University of Manitoba, Winnipeg, Canada

PCR from a variety of animal and human tissues

Thermo Scientific Phire Tissue Direct PCR Master Mix

The Thermo Scientific™ Phire™ Tissue Direct PCR Master Mix has been developed for DNA amplification from animal and human tissues. The master mix contains Phire Hot Start II DNA Polymerase and is specially formulated for performing PCR in the presence of different tissue-derived inhibitors such as collagen, melanin and eumelanin (hair, skin), or myoglobin (muscle). It includes electrophoresis tracking dyes and a density reagent and allows direct loading of PCR products on gels, thereby simplifying direct PCR workflows. The master mix is supplied with Thermo Scientific™ DNARElease™ Additive, which can be used to improve the release of DNA from difficult tissues.

Outstanding robustness

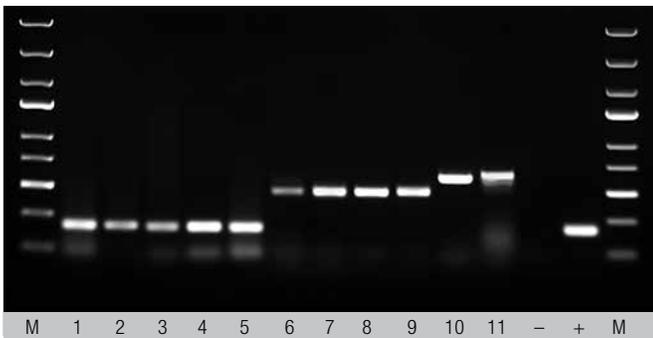


Figure 5. DNA fragments from different human and animal tissue samples were amplified with Phire Tissue Direct PCR Master Mix. Mouse (1) tail, (2) ear, and (3) hair; bird (4) muscle and (5) feather; human (6) hair, (7) tooth, (8) nail, and (9) saliva; (10) zebrafish muscle; and (11) fruit fly.

M: O'GeneRuler Express DNA Ladder

+: Positive control reaction

-: No-template control

Multiplex direct PCR

Phire Tissue Direct PCR Master Mix is suitable for multiplex reactions, enabling simultaneous determination of several markers in one reaction.

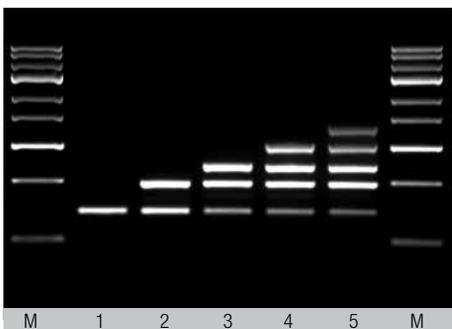


Figure 6. Different length amplicons, ranging from 185 to 650 bp, were amplified from mouse ear in 1-plex to 5-plex reactions with Phire Tissue Direct PCR Master Mix.

M: O'GeneRuler Express DNA Ladder



Examples of materials tested

- Mouse tissues: ear, tail, hair, liver, spleen, brain
- Cultured mouse cells: fibroblasts
- *Drosophila*: wing, whole insect
- Zebrafish: fin
- *Caenorhabditis elegans*: whole worm
- Dog: hair
- Human specimen: buccal swabs, fingernails, saliva, teeth, skin biopsies, hair, FFPE tissue

Dilution and storage protocol for retesting

For greater flexibility, Phire Tissue Direct PCR Master Mix is optimized to work in direct or dilution and storage protocols. With the dilution and storage protocol, one tiny sample can be used for multiple PCR reactions, and experiments can be reproduced at a later time.

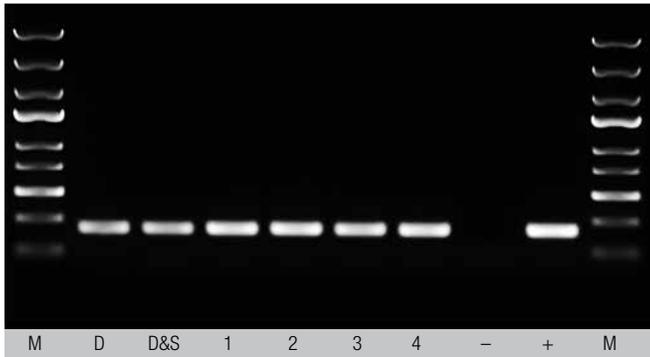


Figure 7. A 237 bp DNA fragment from mouse ear was amplified with Phire Tissue Direct PCR Master Mix using direct and dilution and storage protocols. The same fragment was amplified after samples were stored in Dilution Buffer for several weeks at 4°C. **(D)** Direct protocol, **(D&S)** dilution and storage protocol, and dilution and storage protocol after **1, 2, 3, and 4** weeks of storage in Dilution Buffer.

M: O'GeneRuler Express DNA Ladder

+: Control reaction with purified mouse DNA

-: No-template control

Fast protocol from your sample to a loaded gel

With the Phire Tissue Direct PCR Master Mix, samples are added directly into the PCR reaction mix without any prior DNA purification or extraction steps. The PCR cycling times are very short because of the special properties of Phire Hot Start II DNA Polymerase. The master mix includes electrophoresis tracking dyes and a density reagent and allows direct loading of PCR products on gels, reduces costs, and simplifies the workflow.

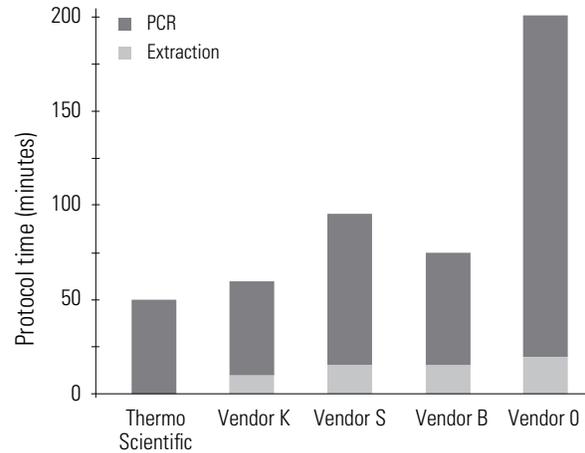


Figure 9. Phire Tissue Direct PCR Master Mix saves time.

Reliable results with a wide range of amplicon lengths

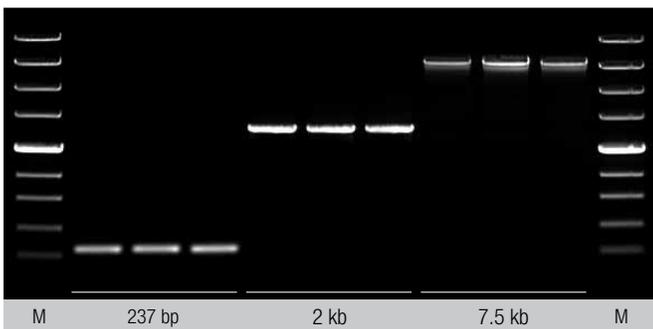


Figure 8. Different length amplicons (237 bp, 2 kb, 7.5 kb) were amplified directly from human saliva with Phire Tissue Direct PCR Master Mix.

M: Thermo Scientific™ ZipRuler™ Express DNA Ladder 2

Kit component	F-170S	F-170L
2X Phire Tissue Direct PCR Master Mix	2 × 1.25 mL	10 × 1.25 mL
Dilution Buffer	5 mL	2 × 12.5 mL
DNARelease Additive	3 × 100 µL	1.3 mL
Universal Control Primer Mix	40 µL	40 µL
Water, nuclease-free	2 × 1.25 mL	10 × 1.25 mL
O'GeneRuler Express DNA Ladder	100 applications (50 µg)	

“By side stepping the need to extract and clean up the DNA we can save up to half a day using the Direct PCR kit, great when we are working with a number of different strains.”

Research Technician, Nematode Biology Research, Bristol, UK

Excellent results from a broad spectrum of blood samples

Thermo Scientific Phusion Blood Direct PCR Master Mix

The Thermo Scientific™ Phusion™ Blood Direct PCR Master Mix is designed for amplification of DNA from whole blood at blood concentrations of up to 40% in the PCR reaction. This is achieved with specially modified Thermo Scientific™ Phusion™ Hot Start II High-Fidelity DNA Polymerase, which is resistant to PCR inhibitors present in blood, as well as to most widely used blood preservatives (e.g., heparin, EDTA, citrate). The master mix includes electrophoresis tracking dyes and a density reagent and allows direct loading of PCR products on gels, thereby simplifying direct PCR workflows.



Examples of materials tested

- Whole blood from: mouse, pig, cat, dog, cow, bird, human
- Blood preserved using heparin, citrate, EDTA
- Blood stored on Whatman 903™ and FTA™ cards

Direct PCR from a variety of blood samples

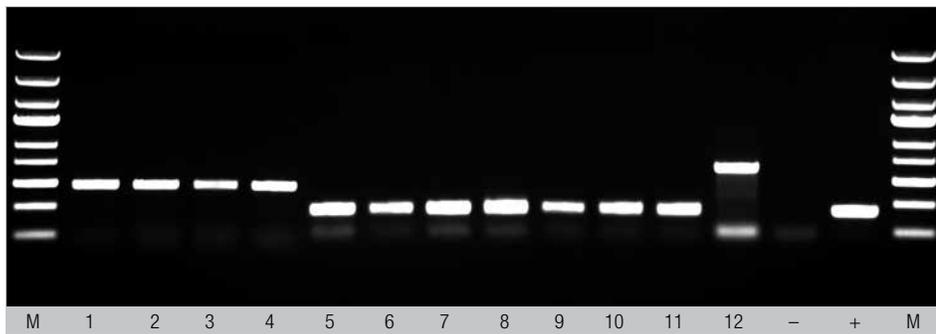


Figure 10. DNA was amplified from human and animal blood samples. Human blood preserved with (1) heparin, (2) citrate, (3) EDTA, or (4) stored on cards; (5) mouse; (6) cat; (7) dog; (8) cow; (9) bird; (10) sheep; (11) horse; and (12) fish.
M: O'GeneRuler Express DNA Ladder
+: Control reaction with purified DNA
-: No-template control

Kit component	F-175S	F-175L
2X Phusion Blood Direct PCR Master Mix	1 mL	5 × 1 mL
50 mM EDTA (pH 8.0)	0.5 mL	0.5 mL
50 mM MgCl ₂ solution	1.5 mL	1.5 mL
100 % DMSO	0.5 mL	0.5 mL
Universal Control Primer Mix	40 µL	40 µL
Water, nuclease-free	1.25 mL	4 × 1.25 mL
O'GeneRuler Express DNA Ladder	100 applications (50 µg)	

Extreme robustness with a wide range of amplicon lengths

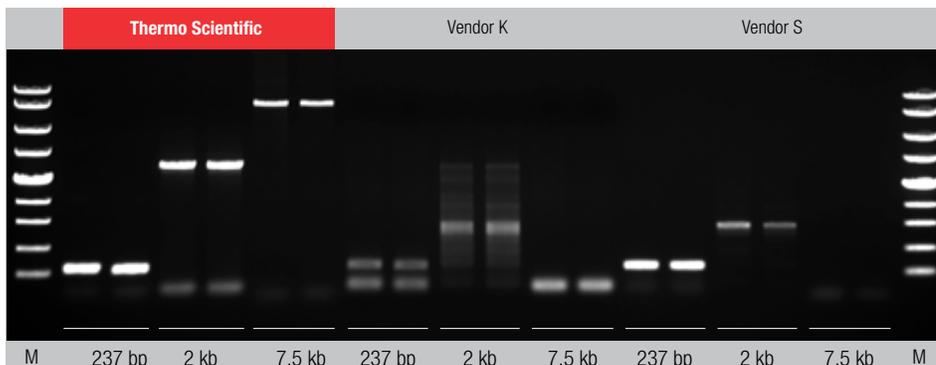


Figure 11. 237 bp, 2 kb, and 7.5 kb fragments were amplified directly from human blood (EDTA-treated) with Phusion Blood Direct PCR Master Mix and other vendors' kits. Blood was placed directly into reactions and PCR was performed according to suppliers' instructions.
M: ZipRuler Express DNA Ladder 2

Start your direct PCR experiment today

Direct PCR Tissue Puncher

The best direct PCR results are obtained by using a very small amount of tissue sample as starting material. The Thermo Scientific™ Tissue Puncher (0.3 mm) is a convenient tool for cutting small tissue-punch discs for direct PCR protocols. It is compatible with a variety of materials, including animal and plant tissues, as well as Whatman 903 and FTA cards.



Cat. No. F-200S

“Cleanest genotyping results I’ve ever had, in the shortest time, with the least amount of work.”

Research Assistant, Institute of Child Health, University College London, UK

Ordering Information

Product	Quantity		Cat. No.
	Direct protocol	Dilution and storage protocol	
Direct PCR Master Mixes			
Phire Tissue Direct PCR Master Mix	100 x 50 µL rxns	250 x 20 µL rxns	F-170S
	500 x 50 µL rxns	1,250 x 20 µL rxns	F-170L
Phire Plant Direct PCR Master Mix	100 x 50 µL rxns	250 x 20 µL rxns	F-160S
	500 x 50 µL rxns	1,250 x 20 µL rxns	F-160L
Phusion Blood Direct PCR Master Mix	100 x 20 µL rxns	NA	F-175S
	500 x 20 µL rxns	NA	F-175L
Direct PCR Kits			
Phire Animal Tissue Direct PCR Kit	200 x 50 µL rxns	500 x 20 µL rxns	F-140WH
Phire Plant Direct PCR Kit	200 x 50 µL rxns	500 x 20 µL rxns	F-130WH
Phusion Human Specimen Direct PCR Kit	200 x 20 µL rxns	200 x 20 µL rxns	F-150BID
Phusion Blood Direct PCR Kit	100 x 20 µL rxns	NA	F-547S
	500 x 20 µL rxns	NA	F-547L

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