



# Amplifying human genomic DNA from whole blood

## Introduction

This protocol is designed to amplify human genomic DNA from whole blood using Finnzymes' High Performance PCR solution. Freshly collected blood and blood stored at 4°C or -20°C are all suitable starting materials, and all common anticoagulants (EDTA, heparin and citrate) can be used. Whole blood volumes up to 5% of the total reaction volume have shown little or no inhibition even when amplifying large PCR products (up to 7.5 kb).

The protocol follows the standard recommendations for the Phusion™ Flash PCR Master Mix, with simple modifications of adding an initial 5-minute incubation step at 90°C and increasing the number of cycles from 30 to 35. The incubation step allows the lysis of leukocytes and makes genomic DNA available for PCR. This protocol works well with whole blood concentrations of 0.5-5% in the reaction.

## Materials and Methods

- Whole blood with EDTA (1.8 mg/ml), sodium citrate 3.2% (109 mM) or heparin (14 I.U/ml) as anticoagulant
- Primers for 0.7 kb fragment of Beta Glucuronidase gene:  
 Forward GCAGTGGCGCAATCTCGTCTC T<sub>m</sub> = 71.8°C  
 Reverse GGCCCAGGCTGCAACAATTC T<sub>m</sub> = 72.2°C
- Phusion™ Flash High-Fidelity PCR Master Mix, (Finnzymes Oy)
- Piko™ Thermal Cycler, (Finnzymes Oy)
- UTW™ tubes or plates, (Finnzymes Oy)

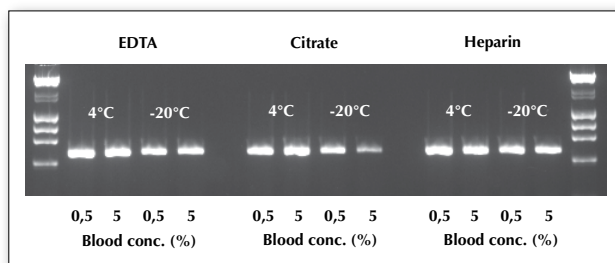
### Pipetting instructions

Component	20 µl react.	50 µl react.	Final conc.
H <sub>2</sub> O	add to 20 µl	add to 50 µl	
2x Phusion™ Flash PCR Master Mix	10 µl	25 µl	1x
Primer A	x µl	x µl	0.5 µM
Primer B	x µl	x µl	0.5 µM
Whole blood	up to 1 µl	up to 2.5 µl	up to 5%

### Cycling instructions

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Lysis of cells	90°C	5 min	90°C	5 min	1
Initial denaturation	98°C	10 s	98°C	10 s	1
Denaturation	98°C	0 or 1 s	98°C	0 or 1 s	35
Annealing*	-	-	X°C	5 s	
Extension	72°C	15 s/1 kb	72°C	15 s/1 kb	
Final extension	72°C 4°C	1 min hold	72°C 4°C	1 min hold	1

\* 2-step protocol is applicable when primer T<sub>m</sub> values are at least 69-72°C as calculated with Finnzymes' T<sub>m</sub> calculator. As a basic rule, for primers ≥ 20 nt, anneal at T<sub>m</sub> +3°C of the lower T<sub>m</sub> primer. For primers < 20 nt use an annealing temperature equal to the T<sub>m</sub> of the lower T<sub>m</sub> primer.



Example of 2-step PCR reactions with 0.5 and 5% concentrations of whole blood. A 0.7 kb human single copy genomic amplicon was amplified using the recommended protocol. Blood preserved with EDTA, heparin or citrate and stored at 4°C or -20°C was used as starting material. Fresh blood may also be used (data not shown). In this assay the total protocol time was 27 minutes.

### Note 1

For general troubleshooting guidelines, please refer to the Phusion Flash PCR Master Mix instruction manual. Special notes for this application are given below.

1. For some amplicons, blood preserved with citrate has proven to be somewhat inhibitory for PCR. It may be necessary to limit blood concentration to less than 5%.
2. For longer amplicons (> 2 kb) greater yields may be obtained by increasing the extension time to 20-30 s/kb. Also adding 5 cycles may improve the results.

### Note 2

Phusion and Phusion Hot Start High-Fidelity DNA Polymerases also work well for this application, but with certain limitations. For best results, follow the recommended protocols for the respective enzymes, but note these additional guidelines:

1. Include the 5-minute, pre-incubation at 90°C to lyse leukocytes.
2. Limit whole blood to 1% of total reaction volume.
3. Use Phusion GC Buffer.
4. Use 1-2 units enzyme per 50 µl reaction.
5. Up to 5 additional cycles may be required to achieve results equivalent to PCR using purified DNA as a template.

## High Performance PCR

High Performance PCR combines Finnzymes' highly processive proofreading Phusion™ DNA Polymerases, high-speed Piko™ Thermal Cyclers, and ultra-thin walled UTW™ tubes and plates. This solution enables the use of extremely short cycling protocols and improves DNA amplification from difficult starting materials.

Find out more at [www.highperformancepcr.com](http://www.highperformancepcr.com)