COBO TECHNOLOGIES
QUALITY CONTROL GENOME EDITING

PROFILase™ 2x Master Mix

Ammonium Buffer Based, 2.5 mM $MgCl_2$ final conc.

Cat. No.: C30104

Item	PROFILase 2x Master Mix, Ammonium Buffer Based, 5 mM $MgCl_2$
ID No.	5300150-1250
Cap colour	Blue
Size, rxn	5000
Volume	50 x 1.25 ml

Key Features

PROFILase 2x Master Mix is specifically formulated for efficient and robust INDEL analysis of nuclease-treated (CRISPR, ZFNs, TALENs) cells using the INDEL Detection by Amplicon Analysis (IDAA™) method[1].

- Optimised for IDAA tri-primer PCR amplification
- Convenient reaction set up at room temperature
- Increased reproducibility
- Increased specificity, sensitivity and product yield
- Designed to diminish the formation of non-specific product
- Fragment Analysis Grade™

PROFILase 2x Master Mix is an all-in-one 2x master mix containing PROFILase Hot Start DNA polymerase, ammonium buffer, dNTPs and magnesium chloride. Each reaction requires 12.5 µl of the 2x Master Mix. Simply add primers, template and water to a total reaction volume of 25 µl to successfully carry out PCR.

PROFILase Hot Start DNA Polymerase is a modified form of Taq DNA polymerase, which is activated by heat treatment. A chemical moiety is attached to the enzyme at the active site, which renders the enzyme inactive at room temperature. Thus, during setup and the first ramp of thermal cycling, the enzyme is not active and misprimed primers are not extended. The result is higher specificity, increased sensitivity and greater yields when compared to standard DNA polymerases.

Composition of PROFILase 2x Master Mix

- Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 5.0 mM $MgCl_2$, 0.2% Tween® 20
- 0.4 mM of each dNTP
- PROFILase Hot Start DNA Polymerase
- Enhancer

Recommended Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.

Option: Store at +4 °C for up to 6 months.

Quality Control

PROFILase Hot Start DNA Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity. The master mix is tested for functionality and absence of human gDNA. IDAA Functional Efficiency: IDAA/tri-primer PCR of human DNA with 85 to 105% efficiency measured by fragment analysis assay.

Unit Definition

One unit is defined as the amount of polymerase that incorporates 10 nmoles of dNTPs into acid-precipitable DNA in 30 minutes at 72 °C under standard assay conditions.

Primer Design Guidelines for Optimal IDAA tri-primer PCR

Design locus specific primers (For-extension, Reverse) to amplify a 150-550 bp product spanning the nuclease targeted cut site using Primer3 or similar software. Add the following 5' extension to the For-extension primer: 5'-AGCTGACCGGCAGCAAAATTG-3'

Universal FamFor (6-FAM 5'-labelled universal primer)

5'- 6-FAM-AGCTGACCGGCAGCAAAATTG-3'

NB! The 6-FAM fluorophore is light sensitive and should be stored in the dark at -20 °C.

Table 1. Stock concentrations of locus-specific PCR primers

		Primer stock conc.
Primer 1	Universal FamFor	25µM
Primer 2	For-extension	2.5µM
Primer 3	Reverse	25µM

Test new tri-primer PCR Primers

Test new IDAA tri-primer PCR primers using protocol specified below on control samples (non-nuclease treated cells) with roughly same cell concentration as the samples to be genotyped. There should be only one clear band and no smear, when analyzed by 3% (wt/vol) agarose gel electrophoresis.

Protocols

These protocols serve as a guideline for IDAA tri-primer PCR. PROFILase 2x Master Mix formulation has been designed for robust tri-primer amplification across different genomic loci from various organisms. The differing complexities of genomes and genomic loci may require adjustments to reaction conditions such as incubation times and temperatures for optimal amplification of certain complex loci.

Protocol-A (OPTIONAL) - Genomic DNA extraction using CoboXtract (C20101)

1. Add 50 µL of CoboXtract to PCR tube with cell pellet of $1x10^4$ – $3x10^5$ cells*.
2. Use a thermocycler with heated lid or equivalent heating block for the following incubations:
   a. Incubate 70°C x 10 minutes.
   b. Incubate 98°C x 10 minutes and cool down to room temperature.
3. Place tube on ice and proceed with IDAA tri-primer PCR protocol.

*If more cells are lysed or if the lysate becomes viscous add more CoboXtract.

Extracted genomic DNA can be stored at 4˚C for at least one week or at -20˚C for several months.

Protocol-B - IDAA Tri-primer PCR using PROFILase 2x Master Mix

Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Working on ice is not required.

Thaw the PROFILase 2x Master Mix and primer solutions. **It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts. Important:** Spin vials briefly before use.

1. Prepare the reaction mix. Table 2 shows the reaction mix set up for a final volume of 25 µl.

Table 2. Reaction mix and template DNA

Component	Vol./reaction*	Final concentration*
PROFILase 2x Master Mix	12.5 µl	1x
Primer1 (25µM) FAM	0.25 µl	0.25 µM
Primer2 (2.5µM)	0.25 µl	0.025 µM
Primer3 (25µM)	0.25 µl	0.25 µM
CoboXtract DNA or	1-2 µl	
Template DNA	Up to 50 ng	
PCR-grade H_2O	Add to 25 µl	-
TOTAL volume	25 µl	-

*Suggested starting conditions. Primer stock solution concentrations shown in brackets.
The final volume can be reduced to 12.5 µl by using half of the volumes suggested in Vol./reaction.

2. Mix the reaction mix thoroughly and dispense appropriate volumes into reaction tubes.
3. Add template DNA to the individual tubes containing the reaction mix.
4. Program the thermal cycler according to the manufacturer's instructions. **Each program must start with an initial heat activation step at 95°C for 15 minutes.** See table 3 for an example.
5. Place the tubes in the thermal cycler and start the reaction.

Table 3. Touch down thermocycling program

95ºC	15 minutes	
95ºC	30 seconds	x15*
72ºC*	30 seconds	
72ºC	30 seconds	
95ºC	30 seconds	x25
58ºC	30 seconds	
72ºC	30 seconds	
72ºC	30 minutes	
12 ºC	forever	

*Touch down 72ºC-1ºC pr cycle start from cycle one

Troubleshooting

The conditions given above have proven robust tri-primer amplification of a broad range of targets in multiple species. In the rare event that difficulty in specific amplicon generation is observed, standard optimization like slight increase in the annealing temperature used may be needed. However, targets with high G/C content, homopolymer tracts, long stretches of repeated sequences may be difficult to generate homogeneous uniform amplicons from.

References:

1. König S, Zhang Y, Wandall HH, Mussolino C, Bennett EP. Fast and Quantitative Identification of Ex Vivo Precise Genome Targeting-
Induced Indel Events by IDAA. Methods Mol Biol. 2019, 1961:45-66. doi: 10.1007/978-1-4939-9170-9_4.

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