TriLink BioTechnologies

mitoPrimers™

Primer	Catalog #	Sequence
A1 (L15997)	O-33001	5′ CAC CAT TAG CAC CCA AAG CT 3′
A2 (L16159)	O-33002	5′ TAC TTG ACC ACC TGT AGT AC 3′
A4 (L16209)	O-33003	5′ CCC CAT GCT TAC AAG CAA GT 3′
B1 (H16391)	O-33004	5′ GAG GAT GGT GGT CAA GGG AC 3′
B2 (H16237)	O-33005	5′ GGC TTT GGA GTT GCA GTT GAT 3′
B4 (H16164)	O-33006	5′ TTT GAT GTG GAT TGG GTT T 3′
C1 (L048)	O-33007	5′ CTC ACG GGA GCT CTC CAT GC 3′
C2 (L177)	O-33008	5′ TTA TTT ATC GCA CCT ACG TTC AAT 3′
C4 (L317)	O-33012	5′ CCC CCC CTC CCC CCG C 3′
D1 (H408)	O-33009	5′ CTG TTA AAA GTG CAT ACC GCC A 3′
D2 (H285)	O-33010	5′ GGG GTT TGG TGG AAA TTT TTT G 3′
D4 (H266)	O-33011	5′ GTT ATG ATG TCT GTG TGG AA 3′

Product Details

Phosphodiester DNA, Double RP-HPLC Purified
100 nmoles each

Store at or below -20°C

QC Analysis

Mass Spec; Pass
RP-HPLC; Pass
Quality control tested with NIST-certified SRM 2392.

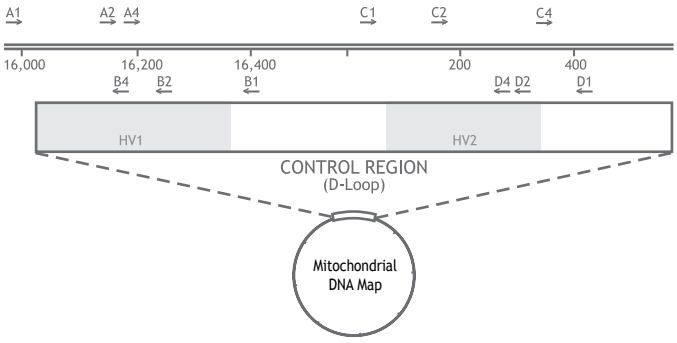
Product released by Quality Assurance

Product Overview

mitoPrimers™ are the standard primers used in the amplification and cycle sequencing of human mitochondrial DNA hypervariable region 1 (HV1) and hypervariable region 2 (HV2), aliquotted into convenient dilute-and-go 100 nmole vials. TriLink's mitoPrimers™ are manufactured in large batches and are quality control tested with NIST-certified SRM 2392. These features allow less in-house testing to assure quality by the end user.

Reconstitution to 100 µM Stock Solution

1. Pulse centrifuge to collect primer at the bottom of the tube.
2. Uncap tube and add 1 mL of deionized water or TE 8.0 buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA].
3. Vortex up to one minute to solubilize primer.
4. Pulse centrifuge to collect the liquid.
5. Distribute stock solution into smaller aliquots.
6. Store at or below -20°C.



Hypervariable Region 1 (HV1)

For/Rev Primer	Amplified Region
A1/B1	15998-16390
A1/B2	15998-16236
A2/B1	16160-16390
A4/B1	16210-16390
A1/B4	15998-16162

Hypervariable Region 2 (HV2)

For/Rev Primer	Amplified Region
C1/D1	49-407
C1/D2	49-284
C2/D1	178-407
C4/D1	318-407
C1/D4	49-265

Suggested PCR Amplification

This procedure can be used for the PCR amplification of HV1 and HV2. Forward and reverse primer pairs are shown in the Mitochondrial DNA Map on the reverse. Typically, the A1/B1 primer pair is used to amplify HV1 and the C1/D1 primer pair is used to amplify HV2.

1. Thaw CleanAmp™ PCR 2X Master Mix, primer stock solutions and BSA solution and place on ice. Note: Do not vortex CleanAmp™ PCR 2X Master Mix. Mix thoroughly by pipetting up and down and collect by pulse centrifugation.
2. Prepare 10 µM mitoPrimer™ working dilution. In a microcentrifuge tube, add 100 µL of mitoPrimer™ 100 µM stock solution and 900 µL deionized water or TE 8.0 buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA].
3. Prepare a reaction mixture containing all components except for the DNA template in a designated template-free zone. Add CleanAmp™ PCR 2X Master Mix, mitoPrimers™, BSA and sterile deionized water as shown in Table 2 into thin-walled PCR tubes. Keep on ice.
4. Mix the reaction mixture gently to protect the enzyme, by pipetting up and down. Do not vortex. Pulse spin as necessary to collect the liquid.
5. In an area designated for template use, add the appropriate volume of template DNA to reach a reaction volume of 15 µL.
6. Pulse spin to remove bubbles and collect reaction solution at bottom of PCR tube.
7. Place the tubes into a thermal cycler with a heated lid and perform the appropriate cycling conditions for standard thermal cycling:
   95°C @ 11 min
   [95°C @ 10 sec; 60°C @ 45 sec; 72°C @ 1 min] 28-33 cycles
   15°C @ 10 min
   Hold @ 4°C
8. Analyze a 2 µL aliquot of the completed reaction by agarose gel electrophoresis.

Table 1: Additional Reaction Components

Component	Vendor
CleanAmp™ PCR 2X Master Mix (L-5101)	TriLink
Bovine Serum Albumin (BSA)	Various
HL-60 Control DNA	Various

Table 2: Reaction Set Up

Component	Final Conc. (15 µL)	Volume per Reaction	Volume for 10 Reactions
CleanAmp™ PCR 2X Master Mix	1X	7.5 µL	75 µL
Forward mitoPrimer™ (10 µM)[1]	500 nM	0.75 µL	7.5 µL
Reverse mitoPrimer™ (10 µM)[1]	500 nM	0.75 µL	7.5 µL
BSA (10 µg/µL)	0.16 µg/µL	0.24 µL	2.4 µL
DNA template[2]	Variable	Variable	Variable
Sterile deionized water	Up to 15 µL	Up to 15 µL	Up to 150 µL
Total Reaction Volume	15 µL	15 µL	150 µL

[1] Refer to the Mitochondrial DNA Map on the reverse for the forward and reverse primers which can be paired together.
[2] Each experiment should include a no template control (NTC), a positive control template (such as HL-60 control DNA) and samples to be analyzed. Ideally, 0.5 ng of template should be used.