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LMNA gene amplification kit (STRIPS) for direct sequencing

REF MBK0010

96 REACTIONS /12 DNA samples

LMNA coding regions amplification for direct sequencing application

FOR RESEARCH USE ONLY

NAME AND INTENDED USE

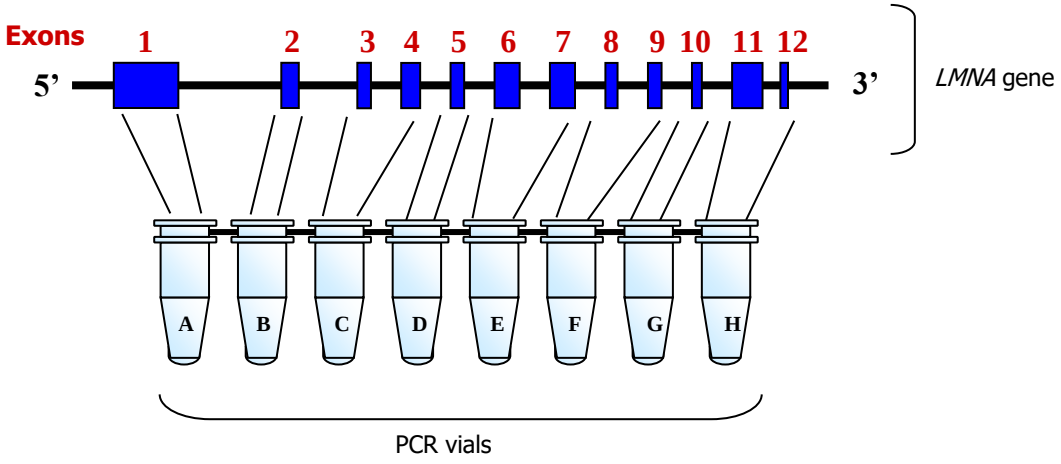
Ready-to-use strips for the simultaneous amplification of human LMNA gene exons with modified primers: amplicons can be used for direct sequencing with M13 universal primers.

INTRODUCTION

The *LMNA* gene codes for A-type lamins, proteins of nuclear envelope expressed in differentiated cells that are the major proteins lamin A and its smaller splice variant lamin C as well as other minor splice products. Mutations in lamin A and in lamin-binding proteins cause a broad spectrum of inherited human disorders named laminopathies. These pathologies include muscular dystrophy, cardiomyopathy, lipodystrophy, insulin-resistance, diabetes, and premature aging.
Nowadays numerous disease-causing mutations have been associated to *LMNA* and only in few cases it is possible to link a specific modification with a precise pathology. As a result, direct sequencing of the entire *LMNA* coding region is the only way to identify all these genetic defects.

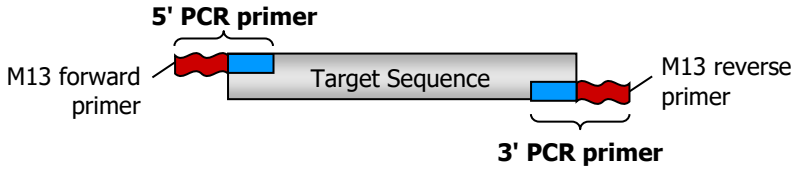
PRINCIPLE OF THE ASSAY

The kit is composed of 12 strips, one strip for every patient sample to be tested. The DNA sample must be added in all the 8-vials of a strip. Each vial contains all the reagents and primers necessary for the amplification of a specific *LMNA* gene region, that comprises one exon and its flanking regions or two exons and the intron included, as illustrated in the figure and table below.



PCR vial	A	B	C	D	E	F	G	H
Exon amplified	1	2	3-4	5	6-7	8-9	10	11-12
Amplicon lenght	678 bp	424 bp	649 bp	442 bp	644 bp	459 bp	400 bp	744 bp

PCR primers are composed of a specific *LMNA* target sequence with flanking priming sites for M13 Forward or Reverse primers.



The special composition of amplification primers allows the sequencing of all the *LMNA* coding regions using only two universal primers (M13 Forward and M13 Reverse). As consequence, only two sequencing reaction mixes are sufficient to obtain the entire coding sequence of the gene, eliminating the need to use several sequencing primers.

KIT CONTENTS

***LMNA* gene amplification strip:** n°12 strips. Each vial contains 8.8µl PCR mix.
Hot-Rescue DNA Polymerase (5U/µl): 30µl (150U)

OTHER SUPPLIES REQUIRED

- Disposable latex gloves.
- Precision pipettes.
- Sterile pipette tips.
- Tabletop centrifuge with microplate adapters.
- Optional: ExoSAP-IT (USB Corporation).

STORAGE

Store the kit at -20°C.

GENERAL PRECAUTIONS FOR PCR

The operator should always pay attention to:

- use pipette tips with filter;
- store positive material (specimens, controls and amplicons) separately from all other reagents and, if possible, add it to the reaction mix in a facility separated space;
- thaw all components and samples at room temperature before starting an assay;
- work on ice.

PROTOCOL

1.1 DNA ISOLATION

Various protocols or kits can be used to extract genomic DNA.

- Carry out the DNA isolation according to the chosen procedure making sure that the extracted DNA is free from PCR inhibitors.
- Determine the concentration of the isolated DNA by standard methods.
- Dilute the genomic DNA sample to the concentration of 50ng/µl with sterile deionized water or 10mM Tris pH 8.0.
- Store the diluted DNA at -20°C avoiding repeated freeze/thaw cycles.

1.2 PCR SET UP

- Thaw the necessary number of 8-vials PCR strip, calculating 1 strip for each DNA sample that must be tested and centrifuge briefly.
- In a sterile 1.5 ml vial, mix the DNA sample with the Hot-Rescue DNA Polymerase enzyme provided with the kit, following the pipetting scheme below:

FOR 8 REACTIONS (1 STRIP)	
Genomic DNA Sample 50 ng/µl	10.0 µl
Hot-Rescue DNA Polymerase 5U/ µl	2.0 µl

Note: for reaction blank use distilled water instead of DNA.

- Mix gently and centrifuge briefly.

- Aliquot 1.2µl of the mix into each of the 8 vials of a strip, from vial A to vial H. Centrifuge the strip briefly.

1.3 PCR RUN

- **Program the PCR thermal cycler with the following parameters*:***

1 cycle	95°C for 1 min
30 cycles	95°C for 30 sec
	72°C for 90 sec
1 cycle	72°C for 7 min
	cool down to 4°C

- Set the reaction volume to **10µl.**
- Perform the PCR run.

When the run is completed, proceed immediately to the next step or store the reaction at +4°C overnight or at –20°C for longer time.

1.4 AMPLICON PURIFICATION

Before proceeding with the sequencing reaction, excess of primers and nucleotides must be removed by a purification procedure.

We recommend ExoSAP-IT (not provided), following this procedure:

- centrifuge the PCR strip briefly;
- in each amplified reaction add 4 µl of ExoSAP-IT (USB Corporation);
- mix and centrifuge briefly;
- incubate the reaction in the PCR thermal cycler executing the program below:

Enzyme incubation	37°C for 15 min
Enzyme inactivation	80°C for 15 min
	cool down to 4°C

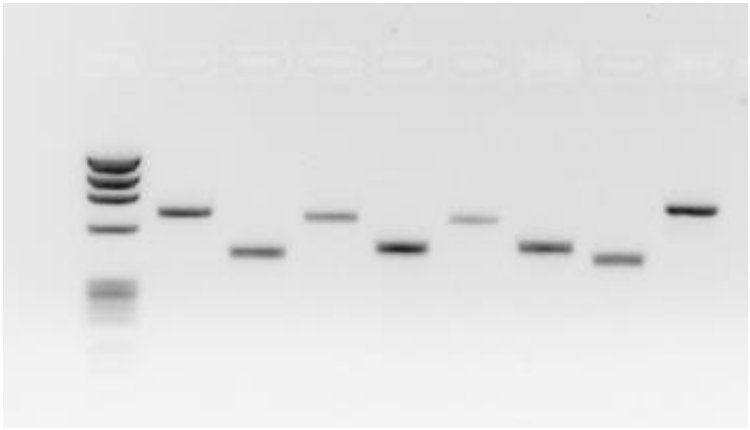
- adjust the reaction volume to 14 µl and perform the run;
- continue the protocol or store the amplicons at -20°C.

Alternative purification methods can be used but must be optimized by the operator.

1.5 AGAROSE GEL ELECTROPHORESIS

Before proceeding with the sequencing reaction, estimate the quality and the final yield of the amplified DNA by an agarose gel electrophoretic analysis.

- Mix 2μl of the purified PCR reaction with 10μl of sterile water and 5μl of DNA loading buffer.
- Load and separate PCR products on an 1.5% agarose gel, in the presence of a DNA standard specific for the low range (100-1000 bp). DNA bands can be stained and visualized with standard staining methods.



Lane 1: ΦX174 DNA/BsuRI DNA molecular weight marker (MBI Fermentas)
Lane 2-9: Purified amplicons A-H

1.6 DNA SEQUENCING

Each amplicon should be sequenced by M13 Forward and M13 Reverse primer (not provided).
M13 Forward primer allows the sequencing of 5' amplicon region while M13 Reverse primer to obtain the sequence of the 3' amplicon end.

M13 Forward primer: **TGTAAAACGACGGCCAGT**

M13 Reverse primer: **CAGGAAACAGCTATGACC**

REFERENCES

Available on library section: **http://www.diatheva.com/library.htm**