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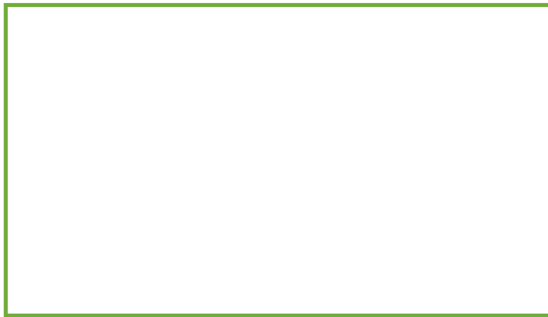


ZMPSTE24 gene amplification KIT for direct sequencing

MBK0025
108 REACTIONS /12 DNA samples

ZMPSTE24 coding regions amplification for direct sequencing application.

FOR RESEARCH USE ONLY

NAME AND INTENDED USE

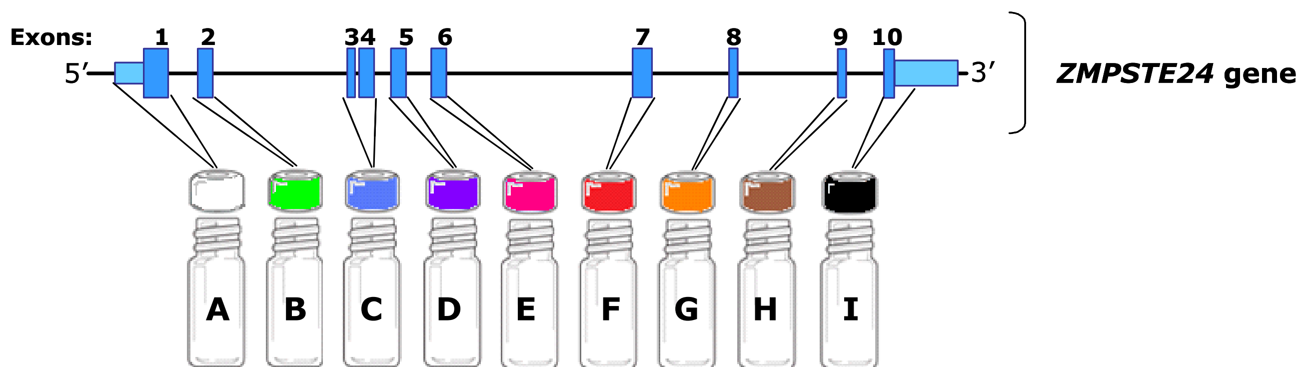
PCR mixes for the amplification of human ZMPSTE24 gene exons with modified primers: amplicons can be used for sequencing reactions with M13 universal primers.

INTRODUCTION

Zmpste24 (also called FACE-1) is a metalloproteinase involved in the maturation of lamin A, an essential component of the nuclear envelope. Both ZMPSTE24- and LMNA-deficient mice exhibit profound nuclear architecture abnormalities and multiple histopathological defects that determine an accelerated ageing process. Similarly, diverse human progeroid syndromes are caused by mutations in ZMPSTE24 or LMNA genes. In particular, defects in ZMPSTE24 gene are known to cause: mandibuloacral dysplasia with type B lipodystrophy (**MADB**) [MIM:608612] and lethal tight skin contracture syndrome [MIM:275210], also called restrictive dermopathy (**RD**).

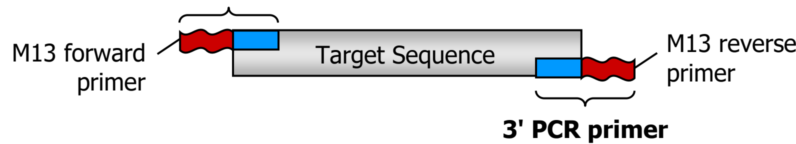
PRINCIPLE OF THE ASSAY

The kit is composed of 9 PCR mixes containing all reagents and primers necessary for the amplification of a specific ZMPSTE24 gene region that comprises: one exon and its flanking regions, the entire 5' UTR, partial 3'UTR, or two exons and the intron included, as illustrated in the figure and table below.



PCR mix	A	B	C	D	E	F	G	H	I
Sequence amplified	5'UTR(total) - Exo 1	Exo 2	Exo 3-4	Exo 5	Exo 6	Exo 7	Exo 8	Exo 9	Exo 10 - 3'UTR (partial)
Amplicon lenght	430 bp	457 bp	873 bp	473 bp	433 bp	475 bp	436 bp	419 bp	415 bp

PCR primers are composed of a specific ZMPSTE24 target sequence with flanking priming sites for M13 Forward or Reverse primers for subsequent sequencing reaction.



Their special composition allows the sequencing of all ZMPSTE24 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

1X Hot-Rescue DNA Polymerase:	30 µl (150U)
1X ZMPSTE24 amplification mix A:	110 µl
1X ZMPSTE24 amplification mix B:	110 µl
1X ZMPSTE24 amplification mix C:	110 µl
1X ZMPSTE24 amplification mix D:	110 µl
1X ZMPSTE24 amplification mix E:	110 µl
1X ZMPSTE24 amplification mix F:	110 µl
1X ZMPSTE24 amplification mix G:	110 µl
1X ZMPSTE24 amplification mix H:	110 µl
1X ZMPSTE24 amplification mix I:	110 µl

OTHER SUPPLIES REQUIRED

- Disposable latex gloves
- Precision pipettes
- Sterile pipette tips
- Sterile 1.5 ml vials
- Tabletop centrifuge
- Optional: ExoSAP-IT (USB Corporation)

STORAGE

Store the kit at -20°C. Repeated freeze-thawing should be avoid. For discontinued use (more than 5 freeze-thawing cycles) storage of mixes in working aliquots is recommended.

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.
- If necessary, dilute the genomic DNA sample 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 ZMPSTE24 amplification mixes and centrifuge briefly.
- Aliquot 8.8 μl of each ZMPSTE24 amplification mix (from A to I) into 9 PCR vials for every DNA sample to analyze.
- In a separated sterile 1.5 ml vial, prepare the **SAMPLE MIX** by adding the DNA sample to the provided Hot-Rescue DNA Polymerase, following the pipetting scheme below:

SAMPLE MIX FOR 9 REACTIONS (1 DNA SAMPLE)	VOLUME
Genomic DNA Sample 30-50 ng/reaction	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 1.2 µl of the **SAMPLE MIX** into each of the 9 PCR vials.
- Centrifuge 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
	67°C for 30 sec
	72°C for 60 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 to use ExoSAP-IT (not provided), following this procedure:
- centrifuge the PCR vials briefly;
- add 4 µl of ExoSAP-IT (USB Corporation) in each amplified reaction;
- mix and centrifuge briefly;
- incubate the vials in the PCR thermal cycler executing the program below:

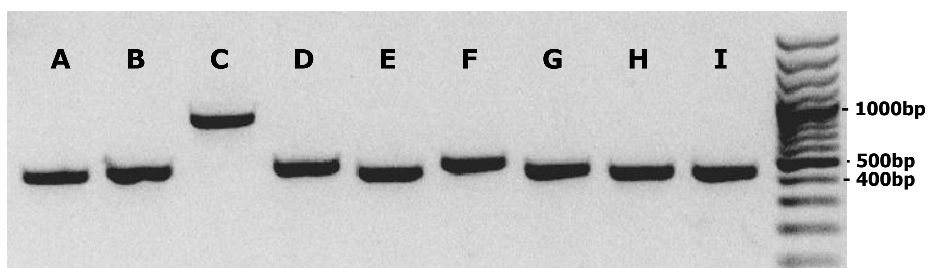
Enzyme incubation	37°C for 15 min
Enzyme inactivation	80°C for 15 min
	cool down to 4°C

- 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, the quality and the final yield of the amplified DNA must be estimated by an agarose gel electrophoretic analysis.
- Mix 2 µl of the purified PCR reaction with 8 µl of sterile water and 5 µl of DNA loading buffer.
- Separate the DNA on a 1.5% agarose gel containing ethidium bromide, in the presence of a DNA standard specific for the low range (100-1000 bp).



Lanes 1-9: Purified amplicons from amplification mixes A-I

Lane 10: Generuler 100bp plus DNA Ladder (Fermentas)

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 (M13-21): 5'-**TGT AAA ACG ACG GCC AGT**-3'

M13 Reverse primer: 5'-**CAG GAA ACA GCT ATG ACC**-3'

REFERENCES

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