EOSAL-CNV LDLR

LDLR Gene

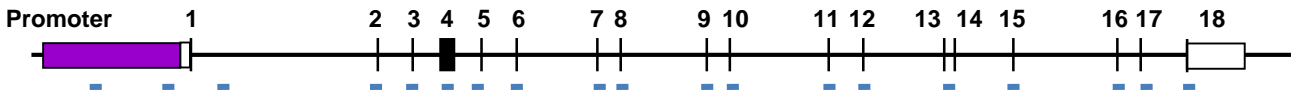**Promoter** 1   2 3 4 5 6   7 8   9 10   11 12   13 14 15   16 17   18

Diagram of LDLR gene (indicating promoter and exons) and location of the different analyzed fragments

EOSAL-CNV for RLDL gene allows the identification of CNVs affecting this gene in the promoter region or in its exons.

The system is based on the amplification and labeling of amplicons covering the regions of interest in a single PCR. This procedure is proportional for each amplicon to the starting number of copies in the sample.

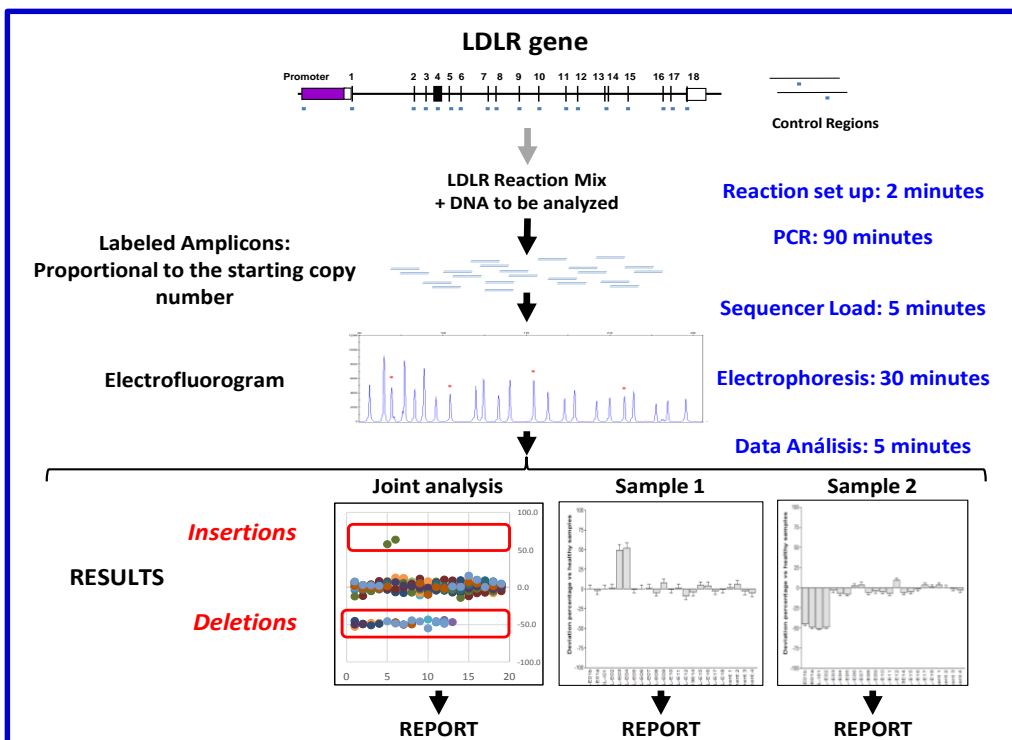**The protocol required is very simple and fast:**
- Add the problem DNA to the "ready to use" reaction mix.
- Perform PCR cycles.
- Analyze by a capillary sequencer the reaction products (no purification is required).
- Analyze data with EOSAL-CS for the detection of CNVs in each sample.

Mutations in the RLDL gene are responsible for over 90-95% of cases of Autosomal Dominant Hypercholesterolemia (ADH) with genetic diagnosis.

Traditionally, the disease caused by mutations in RLDL has been called Familial Hypercholesterolemia (OMIN 143890). This disease is characterized mainly by high levels of LDL-cholesterol and, associated with these, a high cardiovascular risk. The heterozygous form is present in 1 of every 80 or 250 individuals, depending on the population. The homozygous form has much higher levels of LDL-cholesterol and is much less frequent (1 in 160,000-250,000 individuals).

In the LDLR gene about 10% of the mutations described are CNVs; although in some populations the percentage can be much higher.

The main genes that cause HADs are LDLR, APOB and PCSK9. Being very important the study of CNVs of LDLR given that they represent up to 10% of cases. This percentage is much higher than that represented by the mutations in the other two genes together.

LDLR gene

Promoter 1   2 3 4 5 6   7 8 9 10 11 12 13 14 15   16 17 18

Control Regions

LDLR Reaction Mix + DNA to be analyzed

Reaction set up: 2 minutes

PCR: 90 minutes

Labeled Amplicons: Proportional to the starting copy number

Sequencer Load: 5 minutes

Electrofluorogram

Electrophoresis: 30 minutes

Data Análisis: 5 minutes

RESULTS

Insertions

Deletions

Joint analysis   Sample 1   Sample 2

REPORT   **REPORT**   **REPORT**

Fragment analyzed are based on sequence ENST00000558518.5.

REFS. RUO
(Research Use Only)
EOS-LDLR-24
EOS-LDLR-96

CONTACT:
www.seqplexing.com
e-mail:
info@seqplexing.com
Tel: +34 96 35432 63

EOSAL-CNV   LDLR

Refs. RUO
EOS-LDLR-24
EOS-LDLR-96
(24 and 96 reactions)

PCR REACTION.

➢ The following reactions should be performed:
• 3 normal controls in duplicate.
• 1 negative control,
• 1 positive control for reaction (optional).
• each sample in duplicate
  Prepare all DNAs at 10 ng/µl.

➢ Thaw the reaction mixture.
➢ Distribute 12.5 µl of the reaction mix in tubes (tubes, strips or plates) for thermocycler.
➢ Add 2.5 µl of DNA to the corresponding tube.

Perform the following cycles:

PCR CYCLES		
Temp ºC	Time	
95	15 min	
98	30 s	
62	30 s	**x 10 cycles**
72	50 s	
95	30 s	
65	30 s	**x 20 cycles**
72	50 s	
72	5 min	
4	∞	

FRAGMENT ANALYSIS BY CAPILLARY SEQUENCER (APPLIED BIOSYSTEMS/THERMO FISHER).

• Program the electrophoresis according to the equipment instructions.
• Prepare the loading mixture (5µl of Formamide and 0.05µl of Standard Size LIZ 500 for each sample): Distribute 5 µl in each well.
• Add 1.5 µL of the PCR products in the corresponding well.
• Denature the samples.
• Perform electrophoresis.

LIZ 500 size pattern (GeneScanTM 500 LIZ © size standard, Applied Biosystems, Ref 4322682).

DATA ANALYSIS.

Data analysis requires the following steps:
• Extract data of the run by GeneMapper Software (Applied Biosystems).
• Identify peaks using only LIZ500 sizes over 90 bp.
• Export the table with the data of the peaks obtained from all the samples analyzed.
• Analyze the data according to the software EOSAL-CS (www.seqplexing.com) following the instructions. Basically:
  - Import data file.
  - Identify controls samples and their duplicates.
  - Identify each problem sample and its duplicates.
  - Perform the analysis.

The results will show the deviation of each fragment analyzed in relation to normal values. Values over 35% indicate the presence of an insertion and lower than -35% a deletion. In the following figure some examples can be seen: