EOSAL-CNV HNPCC

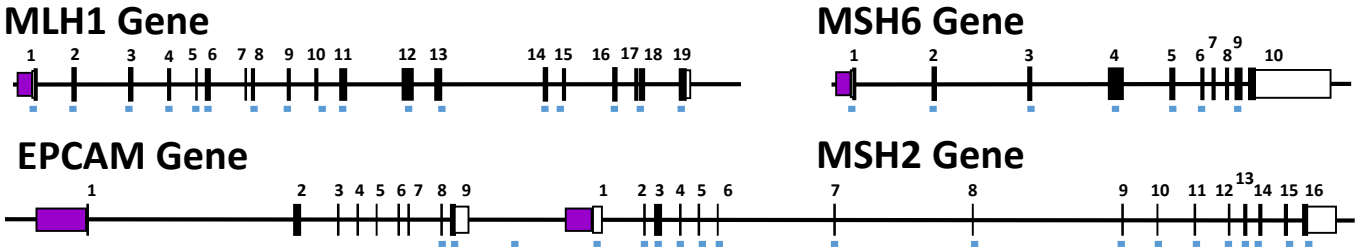


Diagram of MLH1, MSH6, EPCAM, and MSH2 genes (indicating promoter and exons) and location of the different analyzed fragments.

EOSAL-CNV for HPNCC allows the identification of CNVs that affect any of the exons of the MLH1 and MSH2 genes, and to exons 1-6 and 9 of MSH6 genes, and exons 8 and 9 of the EPCAM gene. This kit consists of two reaction mixtures one for the MLH1 and MSH6 genes and one for MSH2 and EPCAM.

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).

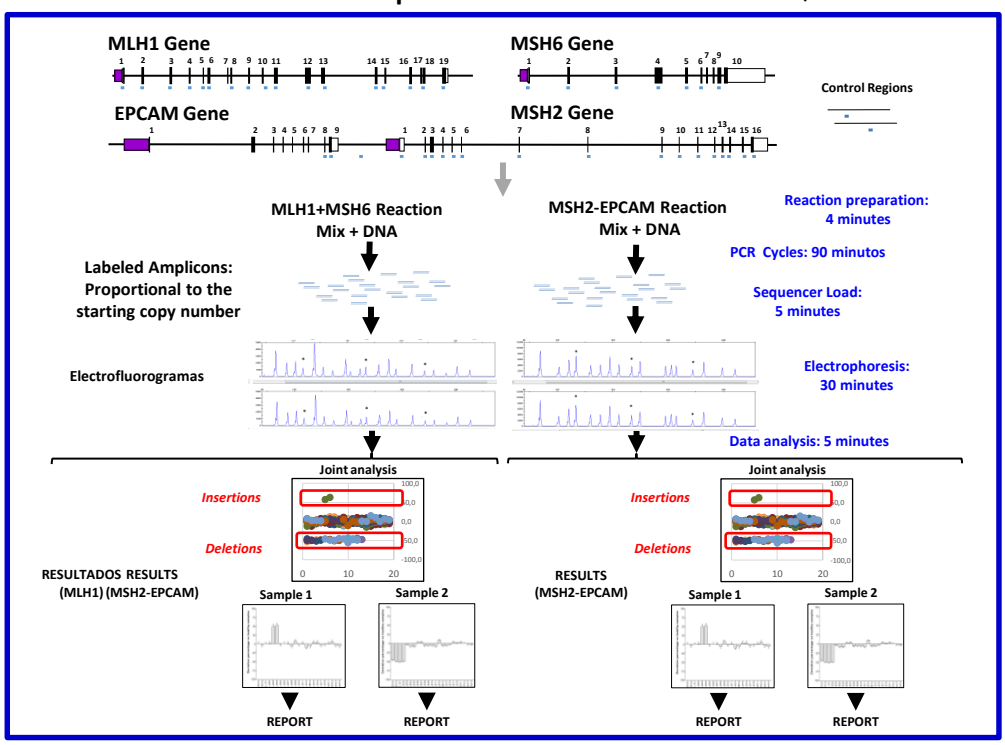
- Analyze data with EOSAL-CS for the detection of CNVs in each sample.

Mutations in MLH1 cause between 50-60% of cases of hereditary non-polyposis colon cancer (HNPCC). Mutations in MSH2 are responsible for 30-40%, in MSH6 about 7% and in EPCAM are present in 1-3%.

Of the cases identified with mutations in these genes; they present CNVs in 1% in MSH6, 10% that have mutations in MLH1, 20% when it is MSH2 and 100% in the case of carriers of EPCAM mutations.

The CNVs in the MLH1 and MSH2 genes can affect any region of these genes. EPCAM CNVs eliminate exons 8 and 9 of this gene and some can reach MSH2 (gene that is physically very close to EPCAM). Mutations that cause HPNCC that only affect EPCAM alter the regulation of MSH2 causing a decrease in the expression of this gene. MSH6 mutations can affect

Given the high percentage of patients with CNVs in these genes, it is essential to determine their presence in those who have a high risk of suffering from HNPCC and, especially, in those who do not have point mutations.



Fragments analyzed are based on sequences ENST00000231790 for MLH1, ENST00000233146 for C HEK2 and ENST00000263735 for EPCAM.

REFS. RUO
(for research use only)
EOS-HNPCC-24
EOS-HNPCC-96

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

EOSAL-CNV HNPCC

Refs. RUO
EOS-HNPCC-24
EOS-HNPCC-96
(24 and 96 reactions;)

PCR REACTIONS.

➢ 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 two reaction mixture.
➢ Distribute 12.5 µl of the reaction mix in tubes (tubes, strips or plates) for thermocycler. Reaction mixture 1 and 2 must be used separately.
➢ Add 2.5 µl of DNA to the corresponding tube.
Perform the following cycles:

PROGRAMA REACCIÓN		
Temp ºC	Tiempo.	
95	15 min	
98	30 seg	
62	30 seg	**x 10 ciclos**
72	50 seg	
95	30 seg	
65	30 seg	**x 20 ciclos**
72	50 seg	
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 reaction): 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. In the following figure some examples can be seen: