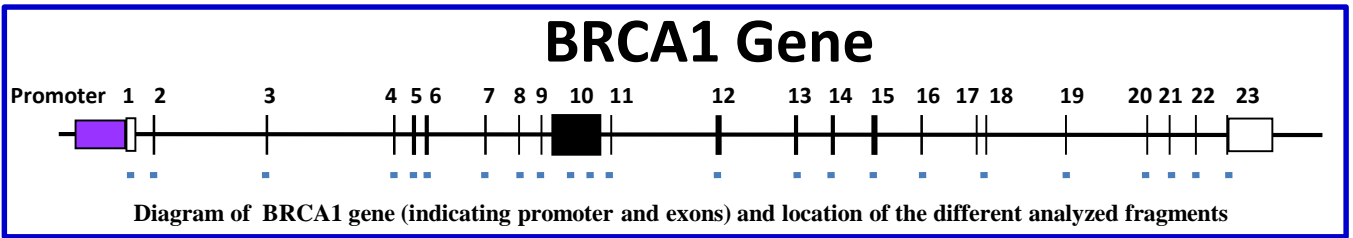


EOSAL-CNV BRCA1



EOSAL-CNV for BRCA1 gene allows the identification of CNVs affecting this gene in the promoter region or in 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:

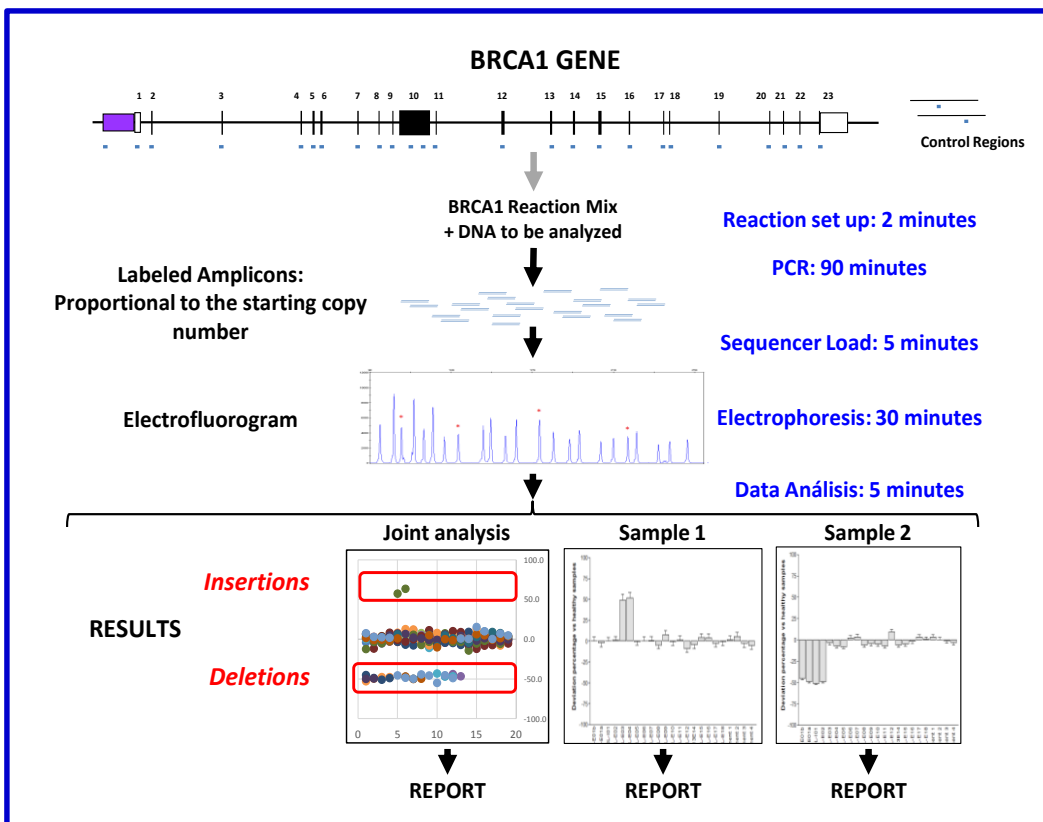
- Add the problem DNA to the “ready to use” reaction mix.
- Perform PCR cycles.
- Analyze by a capillary sequencer the products (no purification is required).
- Analyze data with EOSAL-CS for the detection of CNVs in each sample.

Mutations in BRCA1 gene (OMIM 113705) greatly increase the risk of developing Hereditary Breast Cancer (HBC), Ovary Cancer and increase the risk of other tumors (OMIN 604370). In addition, the alteration of the two copies of BRCA1 causes Fanconi Anemia (OMIN 617883).

Mutations in BRCA1 are important as the codified protein is involved in the DNA repair and maintenance.

Most cases of HBC are caused by mutations in this gene. About 10% of the mutations present in BRCA1 are CNVs, although in some populations it can reach 40%. The CNVs described can affect any area of the gene.

Given the high percentage of CNVs presents in BRCA1, it is essential to search for these mutations in those patients suspected of having HBC and Fanconi Anemia. Especially, it is necessary to determine the presence of CNVs in patients who do not present point mutations.



Fragment analyzed are based on sequence ENST00000357654.

REFS. RUO
(Research Use Only)
EOS-BRCA1-24
EOS-BRCA1-96

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

EOSAL-CNV BRCA1



Refs. RUO
 EOS-BRCA1-24
 EOS-BRCA1-96
 (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 / μ l.
- Thaw the reaction mixture.
- Distribute 12.5 μ l of the reaction mix in tubes (tubes, strips or plates) for thermocycler.
- Add 2.5 μ l of DNA to the corresponding tube.

Perform the following cycles:

PCR CYCLES		
Temp °C	Time	
95	15 min	
98	30 s	
62	30 s	x 10 ciclos
72	50 s	
95	30 s	
65	30 s	x 20 ciclos
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 (5 μ l of Formamide and 0.05 μ l of Standard Size LIZ 500 for each sample): Distribute 5 μ l in each well.
- Add 1.5 μ l of the PCR products in the corresponding well.
- Denature the samples.
- Perform electrophoresis.

LIZ 500 size pattern (GeneScan™ 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:

