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Detection of the rearrangement of exons 9-12 of the *BRCA1* gene with the use of multiplex PCR

PLOS One

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ABSTRACT

In this protocol we developed a method for the detection of the rearrangement of exons 9-12 of *BRCA1* based on multiplex PCR.

A multiplex PCR is performed for the detection of the normal and mutated alleles of exons 9-12 of *BRCA1* gene, 500 and 900 base pairs respectively, and a second PCR for the detection of the mutated allele. Both PCRs are observed by means of electrophoresis in 1.5% agarose gel.

MATERIALS

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- ☒ UltraPure Distilled Water **Invitrogen - Thermo Fisher Catalog #10977-015**
- ☒ DreamTaq DNA Polymerase **Contributed by users Catalog ##EP0701**
- ☒ dNTP Set 100 mM Solutions **Thermo Scientific Catalog #R0181**
- ☒ -N7-dGTP 7-Deaza-dGTP **Contributed by users Catalog # B0300**
- ☒ Betaine solution **Contributed by users Catalog #B0300**
- ☒ Specific oligonucleotides (Available upon request) **Integrated DNA Technologies**
- ☒ 10X DreamTaq Buffer **Thermo Scientific Catalog ##EP0703**

Keywords: BRCA1
rearreagments, PCR multiplex

- 1.- Wear clean gloves
- 2.- Measure DNA concentration in nanodrop
- 3.- Prepare aliquots of 50 ng / μ L DNA
- 4.- Clean and disinfect the PCR cabinet
- 5.- Gently mix the DNA samples and pass the reagents briefly on the vortex
- 6.-Centrifuge the DNA samples and reagents with a spin; 6- Keep reagents on ice
- 7.- Leave microtubes ready for preparation of the mix, keep the same ones identified and on ice.

PCR mix to detection of the rearrangement of exons 9-12 of ...

- 1 **Note:** This mix is to one reaction, you must consider how many reactions will be done.
Add ultrapure water to microtube of each mix (two mixes). Mix one 14.1 μ L ; Mix two 14.9 μ L
- 2 Add 10x DreamTaq Buffer which includes $MgCl_2$ at a concentration 20mM 3 μ L
- 3 - Add dNTPs 10Mm prepared with -N7-dGTP, 7-Deaza-dGTP 0.8 μ L
- 4 - Add Betaine solution 5M 6 μ L
- 5 - Add the oligonucleotides en each one mix: Add to mix one (B01del9-12, B02del9-12 and B03del9-12), 1 μ L 10 pmol , 0.8 μ L 10 pmol , 1 μ L 10 pmol respectively; Add to mix two (B01del9-12, B03del9-12) 1 μ L 10 pmol , 1 μ L 10 pmol
- 6 - Add the Taq DNA polymerase to the mix. Briefly vortex and centrifuge for some seconds.

🧪 0.3 µL 1.5 Units

- 7 - Add and aliquot of DNA sample to each tube 🧪 3 µL 150 ng
- 8 - Close perfectly the tubes and centrifuge for some seconds
- 9 - Put the reaction in the thermocycler
- 10 - Run the PCR following this conditions: 🌡️ 94 °C ⌚ 00:05:00 ; 🌡️ 94 °C ⌚ 00:00:30
🌡️ 50 °C ⌚ 00:00:30 - 🌡️ 72 °C ⌚ 00:00:35 10 cycles; 🌡️ 94 °C ⌚ 00:00:30 ,
🌡️ 55 °C ⌚ 00:00:40 , 🌡️ 72 °C ⌚ 00:00:35 25 cycles; then 🌡️ 72 °C ⌚ 00:10:00
and the last 🌡️ 4 °C ⌚ 00:15:00