Sep 24, 2019

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

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.4t8gwrw

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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.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0222709

MATERIALS

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

BEFORE STARTING

1.- Wear clean gloves
2.- Measure DNA concentration in nanodrop
3.- Prepare aliquots of 50 ng / uL 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 rearragement of exons 9-12 of *BRCA1* gene

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 µl** ; Mix two **14.9 µl**

2 Add 10x DreamTaq Buffer which includes MgCl2 at a concentration 20mM **3 µl**

3 - Add dNTPs 10Mm prepared with –N7-dGTP, 7-Deaza-dGTP **0.8 µl**

4 - Add Betaine solution 5M **6 µl**

5 - Add the oligonucleotides en each one mix: Add to mix one (B01del9-12, B02del9-12 and B03del9-12) , **1 µl** **10 pmol** , **0.8 µl** **10 pmol** , **1 µl** **10 pmol** respectively; Add to mix two (B01del9-12, B03del9-12) **1 µl** **10 pmol** , **1 µl** **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**