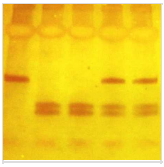

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Genotyping of the rs1695 polymorphism of the *GSTP1* gene by PCR-RFLP

PLOS One

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bqgqmtvw

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ABSTRACT

Glutathione S-transferases (GSTs) are enzymes that act in the cellular detoxification of harmful compounds. Due to the role of the GSTP1 isoenzyme in the brain, the presence of the single nucleotide polymorphism (SNP) rs1695 in the *GSTP1* gene may play an important role in the development of neurodegenerative diseases through oxidative stress, one of the main pathogenic mechanisms associated with the degeneration and death of motor neurons.Thus, this protocol provides a standardized methodology for genotyping the rs1695 polymorphism in the *GSTP1* gene through polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (PCR- RFLP). This polymorphism consists of the exchange of adenine for guanine at position 313 (codon 105), which causes the substitution of amino acid isoleucine (Ile) for valine (Val), resulting in a decreased Pi enzyme activity. Polymorphism genotyping techniques have been of great interest in determining the genetic association of several polymorphisms with different diseases, and therefore this protocol may facilitate future genetic studies.

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KEYWORDS

Glutathione S-transferase Pi 1, rs1695, SNP, PCR-RFLP, A313G

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MATERIALS TEXT

T100 PCR Thermal Cycler
Bio-Rad **1861096**

Photo-documenter L-PIX EX
Loccus Biotecnologia **03537895**

PureLink™ Genomic DNA Mini Kit **Invitrogen - Thermo Fisher Catalog #K182001**

Taq DNA Polymerase (W/ Buffer Free And Mgcl2 Solution) **Sinapse Inc Catalog #P1011-1**

dNTP Set (10mM) **Sinapse Inc Catalog #N9013**

Primers **Contributed by users**

100bp Ladder Ready-To-Use **Sinapse Inc Catalog #M1062**

50Bp Ladder Ready-To-Use 5 X 50 Ug **Sinapse Inc Catalog #M1042**

Mgcl2 (50mM) **Invitrogen - Thermo Fisher**

Silver Nitrate PA ACS 25g **Nox Lab Solutions Catalog #NR-00785**

Restriction enzyme Alw26I (BsmAI) (10 U/µL) **Invitrogen - Thermo Fisher Catalog #ER0031**

SAFETY WARNINGS

Please use personal protective types of equipment (such as gloves, safety glasses, a lab coat and masks) during

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all experiment, mainly when staining polyacrylamide gels, since the reagents are toxic and can cause consequences to the health.

BEFORE STARTING

Pay attention to all necessary precautions to perform a PCR assay.

DNA extraction

1 DNA samples were isolated from peripheral blood using DNA extraction kit (Invitrogen®), according to the manufacturer's instructions. *GSTP1* rs1695 variant was genotyped through PCR-RFLP method.

Primer design

2 The set of primers previously described by Harries *et al.* (1997) (forward: 5'-ACC CCA GGG CTC TAT GGG AA-3' and reverse: 5'-TGA GGG CAC AAG AAG CCC CT-3') were used to amplify the fragment of interest of the *GSTP1* gene.

Harries LW, Stubbins MJ, Forman D, Howard GC, Wolf CR (1997). Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer.. Carcinogenesis.

GSTP1 gene amplification by PCR

3 A conventional polymerase chain reaction (PCR) was performed to amplify the 176 base pairs (bp) region of the *GSTP1* gene. PCR mixture had a total of 25 µl per reaction, which contained 2.5 µL 10X PCR Buffer, 0.5 µL 0.2 mM dNTP Mix, 1 µL of primer mix, containing forward primer (1.0 uM) and reverse primer (1.0 uM) , 0.5 µL 50 mM $MgCl_2$, 0.5 µL (1.25 U) of Taq DNA polymerase, 18 µL of Milli-Q Water, and 2 µL of DNA.

PCR amplification was carried out with the Bio-rad thermocycler (T100^{TM} Thermal Cycler). Cycling conditions were 94°C for 5 min for initial denaturation, followed by 35 cycles of denaturation at 94 ° C for 1 min, annealing at 61 ° C for 1 min, and extension at 72° C for 1:30 min. A final polymerization step of 72°C for 7 min was performed to complete the elongation processes.

A mixture of 6µl of PCR product and 4 µl of 6× DNA loading buffer run on 12% polyacrylamide gel in 1X TBE buffer. A standard DNA ladder (100 bp) was used as a reference. After 240 min under 80V, the polyacrylamide gel was stained with silver nitrate. The presence of a 176 bp fragment confirmed the amplification of the PCR product, allowing subsequent steps to be carried out. Negative controls (PCR mix without DNA) were included in all PCR and electrophoresis assays.

Genotyping of *GSTP1* rs1695 polymorphism by PCR-RFLP

4 Amplified 176bp products were digested with *Alw26I* (Thermo Scientific^{TM}) restriction enzyme, using the Bio-rad thermocycler (T100^{TM} Thermal Cycler) under the following cycling conditions: 12 h (37ºC) and 20 min (65ºC). The reactions (final volume 30 µL) contained: 2 µL of 10X Buffer Tango, 17 µL of Milli-Q Water, 1U of the restriction enzyme, and 10 µL of the amplified product.

After this step,10 µL of the digested product and 4 µl of 6× DNA loading buffer run on 12% polyacrylamide gel in 1X TBE buffer. A standard DNA ladder (50bp) was used as a reference. After 360 min under 80V, the polyacrylamide gel was stained with silver nitrate. In all electrophoresis assays, positive controls (sample previously known as heterozygous genotype) were included. All gels were registered with the photo-documenter L-Pix Ex (Loccus Biotecnologia®).

Analysis and interpretation of the results

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5 The interpretation of the genotyping of *GSTP1* rs1695 polymorphism is based on the activity of the enzyme Alw26I. It recognizes the restriction site of the polymorphic variant (GTCTC(1/5)^) and promotes a cut in the 176 bp fragment, generating two fragments (85 and 91 bp). Thus, in the wild genotype (AA), which does not have the enzyme restriction site, the fragment remains unchanged (176 bp), while the heterozygous genotype (AG) is determined by the presence of three fragments (85, 91, and 176 bp). On the other hand, the mutant genotype (GG) is characterized by two fragments (85 and 91 bp), Figure 1.

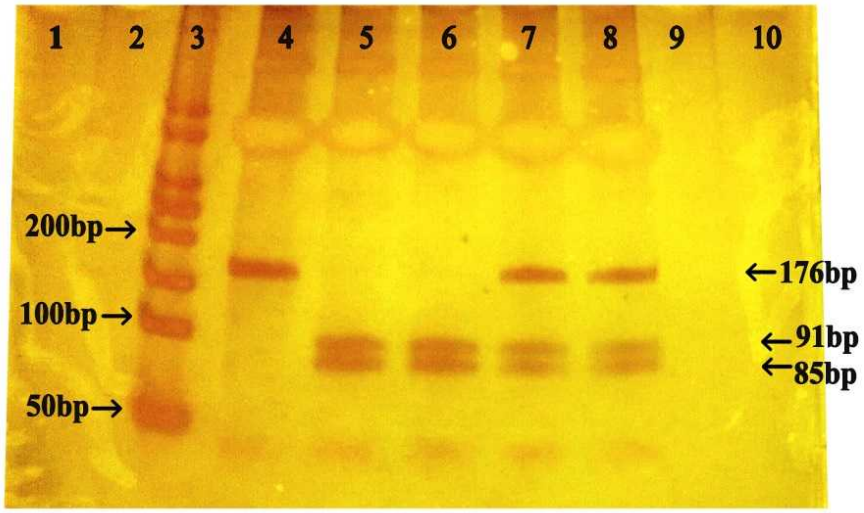

Figure 1.Original gel of the genotyping of the *GSTP1* rs1695 polymorphism by PCR-RFLP. Lanes 1, 2, 9, and 10: Well empty. Lane 3: Marker - Molecular DNA size marker 50bp (Sinapse® 50bp DNA ladder).Lane 4: Wild genotype (A/A). Lane 5 and 6: Mutant genotype (G/G). Lane 7: Heterozygous genotype (A/G). Lane 8: Positive control (sample previously known as heterozygous genotype, A/G).

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