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## Protocol: Phylogenetics analysis of TP53 gene in humans and its use in biosensors for breast cancer diagnosis

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**Protocol status:** In development

**We are still developing and optimizing this protocol**

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**Keywords:** TP53, Biosensors, Diagnosis of Breast Cancer, Phylogeny, AMOVA, breast cancer diagnosis biosensor, use in biosensor, biosensor, oligonucleotide probe, selective binding of the target analyte, tp53 gene, target analyte, phylogenetics analysis of tp53 gene, such as cell receptor, biological component, marked antibody, such as enzyme, antibody, detection, enzyme, direct detection, physical transducer, ligand, cell receptor, biological reaction,

## Abstract

Biosensors are small devices that use biological reactions to detect target analytes. Such devices combine a biological component with a physical transducer, which converts bio-recognition processes into measurable signals. Its use brings a number of advantages, as they are highly sensitive and selective, relatively easy in terms of development, as well as accessible and ready to use. Biosensors can be of direct detection, using a non-catalytic ligand, such as cell receptors and antibodies, or indirect detection, in which there is the use of fluorescently marked antibodies or catalytic elements, such as enzymes. They also appear as bio-affinity devices, depending only on the selective binding of the target analyte to the ligative attached to the surface (e.g., oligonucleotide probe).

## Troubleshooting

## METHODOLOGY

- 1 Protocol: Phylogenetic analysis of TP53 gene in humans and its use in biosensors for breast cancer diagnosis
- 2 Dataset: Initially, 301 sequences of a fragment of the human TP53 gene recovered from GENBANK (<https://www.ncbi.nlm.nih.gov/popset/430765060>) and participated in a PopSet made available by Hao, X.D and collaborators in 2013 (PopSet: 430765060).
- 3 Phylogeny
- 4 For the visualization of variable sites: Logos will be generated through the Weblogo3 program (Crooks, 2004). The analysis of the number of populations will be performed with the Structure 2.3 program (Pritchard, 2000) and two different methods are tested: a posteriori probability and ad hoc (k). The "a posteriori" probability will be calculated using an ancestry model with mixed alleles for 20,000 interactions in the burn-in period, followed by 200,000 Monte Carlo interactions via Markov Chain, increasing only the K value (number of populations), which will be from 1 to 10 according to Pritchard's methodology (2000).
- 5 For determine the most appropriate number of populations: The Evanno method (2005) will be used, using an ad hoc amount based on the second-order rate of the likelihood function between the successive values of K. Posteriori and k probability tests will initially be applied to the dataset in isolation.
- 6 Molecular variance
- 7 For the analysis of genetic variability: A project will be created with the Arlequin Software 3.1 (Excoffier et al., 2005). which aims to measure molecular diversity using standard estimators such as Theta (Hom, S, k, Pi), Tajima Neutrality test, paired and individual FST values, in addition to temporal divergence and demographic expansion indices (mismatch and Tau values) by molecular variance analysis (AMOVA) (Excoffier, 1992).
- 8 For the evolutionary molecular signal test: In this method, the distance matrix between all haplotype pairs will be used in a hierarchical variance analysis scheme producing estimates of variance components analogous to Wright's F statistics involving nonlinear transformations of the original information in estimates of genetic diversity. Mantel's Z statistic will be used to represent the divergence between possible microhabitats using the MULTIVAR (Mantel for Windows) program (Mantel, 1967).
- 9 References

- 10 CROOKS G.E., HON G, CHANDONIA JM, BRENNER SE WEBLOGO: A sequence logo generator, *Genome Research*, 14:1188-1190, (2004). EXCOFFIER, L. AND H.E. L. LISCHER (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*. 10: 564-567. EXCOFFIER, L; SMOUSE, P; QUATTRO, J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131, 479-491. G. EVANNO, S. REGNAUT et. al. Blackwell Publishing, Ltd. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Department of Ecology and Evolution, Biology building, University of Lausanne, CH 1015 Lausanne, Switzerland 2005. HAO XD, YANG Y, SONG X, et al. Correlation of telomere length shortening with TP53 somatic mutations, polymorphisms and allelic loss in breast tumors and esophageal cancer. *Oncol Rep*. 2013;29(1):226-236. doi:10.3892/or.2012.2098. MANTEL, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Res*. 27, 209-220. PRITCHARD, JK; STEPHENS, P; DONNELLY, P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155, 945-959.