A WAY TO SOLVE THE PROBLEM IN THE DISCRIMINATION OF MONOZYGOTIC TWINS

Laura Mañas García. Grau en Genètica. Universitat Autònoma de Barcelona

Introduction

What monozygotic twins are?

- An embryo is divided in two embryos instead of one.
- Sharing genotype → Genetically identical.
- Showing differences on phenotype → Caused by epigenetic alterations.

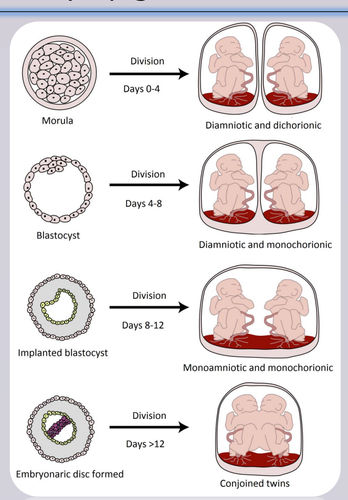
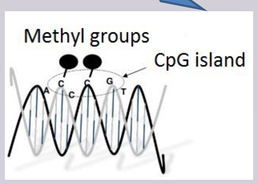


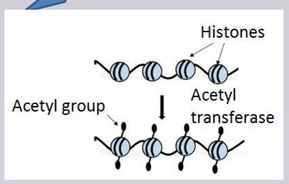
Figure 1: Depending on the time of zygote splitting, twins can be divided in groups.

How epigenetics can modify the phenotype?

Epigenetic alterations



DNA methylation



Histone acetylation

Epigenetic mechanisms are affected by

- Aging
- Environmental chemicals
- Development
- Drugs
- Diet

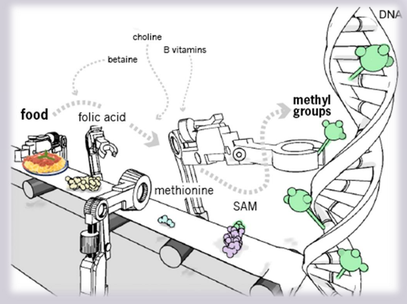
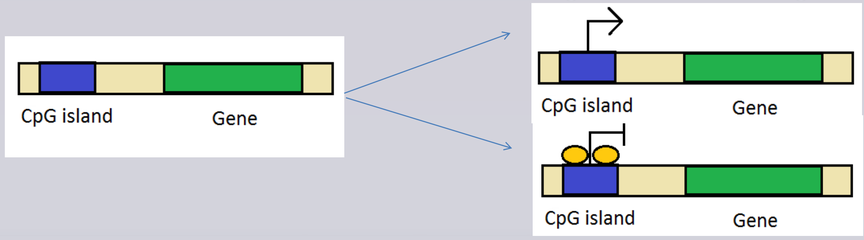


Figure 2: An example of how the diet can alter the DNA by adding methyl groups.

CpG island are clusters of cytosine-guanine repetitive sequence (CpG), their presence is around 1% of the genome. These clusters used to be close of the codificant region of DNA, concentrated on the promoter. Promoters are some specifics sequences of DNA localized on 5' that have the necessary information to activate or deactivate the gene that are regulating.



State of the art

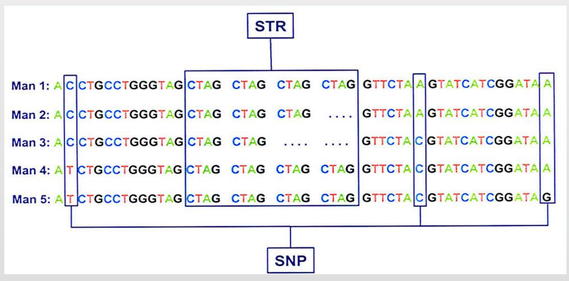
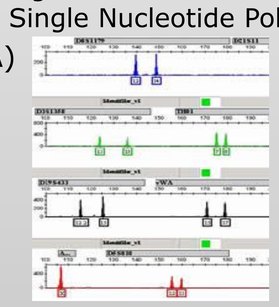


Figure 3: Short Tandem Repeats (STR) and Single Nucleotide Polymorphism (SNP).

A)



B)

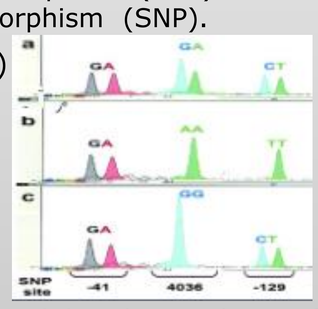


Figure 4: Results obtained by the analysis of STR (A) and SNP (B). From this results an individual profile is made.

These methods offer strong evidences for forensic studies, but there is one exception, the case of the monozygotic twins, where these methods offer no different profiles.

Hypothesis

Propose to realize a method based on the analysis of methylation patterns using Multiplex Methylation SNaPshot.

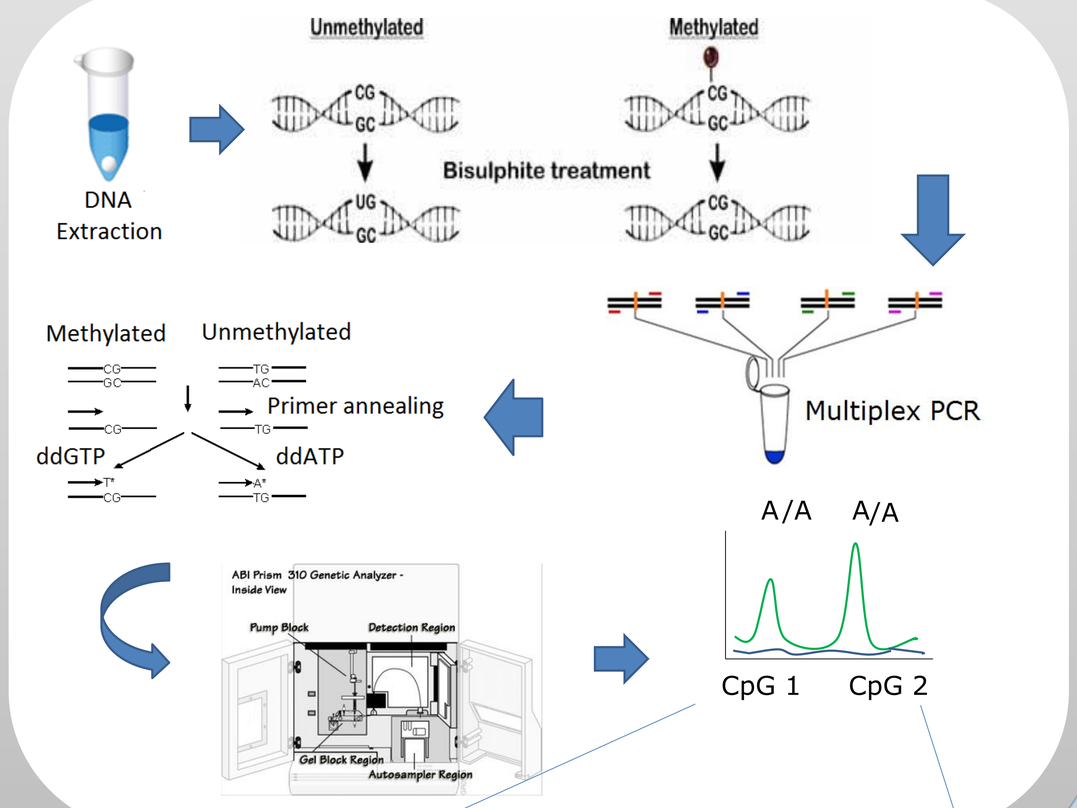
- Validate some candidate genes with samples of healthy monozygotic twins donors. →
  - ER
  - IGF2
  - MLH1
  - RUNX3
  - WIF1
- Validate the automation of this technique.

Work plan

Multiplex Methylation SNaPshot is the technique chosen in this project to study epigenetic changes between monozygotic twins.

It is based on the amplification of bisulfite-converted DNA and annealing of an Single-Base Extension (SBE) primer exactly one base pair upstream or downstream of the cytosine of a target CpG site. Thus, this method can measure the proportion of the methylated and/or unmethylated cytosine of the target CpG site simultaneously. 25 CpG sites are analyzed.

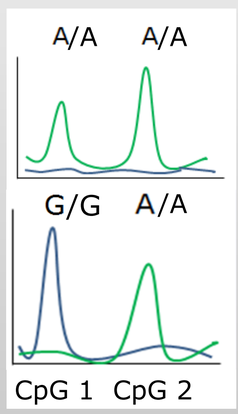
1. DNA extraction (n=40)
2. Sodium bisufite treatment
3. Amplification by multiplex PCR
4. Single-base extension
5. Detection of dye-labeled located on 5' by electrophoretic platforms
6. Results



What does this method offer?

- One of the most important advantages that this method offers is that the samples can be run on a fluorescence detection electrophoresis instrumentation, which is available in many forensic laboratories.
- In addition, it is a sensitive method that have the ability to be multiplex giving reliable results.
- It is easy because the first step only consist on the application of sodium bisulfite treatment and so on amplify DNA by a PCR.
- This technique has the potential to produce considerable savings in time and effort. Also is an inexpensive, technique for routinely.

Expected results



Each peak determinates any different CpG site analyzed.

When a peak shows :

- 'G' signal it will mean that in the complementary DNA strand there is a methylated cytosine
- 'A' signal it will mean that in the complementary DNA strand there is an unmethylated cytosine.

By the comparison of the analysis between each couple of twins, this method will show differences on the peaks, giving information about methylation status of each individual. So, monozygotic twins could be discriminate by this epigenetic differences.