EPSTEIN-BARR VIRUS AND MITOCHONDRIAL DNA: ELABORATION OF A PHYLOGENETIC TREE STUDYING MITOCHONDRIAL DNA SAMPLES AND STUDY OF THE GROWTH OF EPSTEIN-BARR INFECTED CELLS CULTURES

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INTRODUCTION

Have you ever heard about the kissing disease? Have you ever scratched yourself compulsively suffering of herpes? Has someone ever explained to you what the Epstein-Barr virus is ? The Epstein-Barr virus (**fig. 1**) infects nearly 95% of the worldwide population but hardly no one notices it. It is also known as HHV-4 (*Human Herpesvirus 4*).

My motivation when starting this project was to study genetics and biomedicine. When the CEXS UPF-IBE scientists gave me the opportunity of working on this with them, I thought it was an amazing occasion.

The objectives we want to reach with this project are:

- Get to know different lab methods and procedures. Knowing how these techniques help to understand many of the procedures that occur in biology.
- Investigate if there are infected cells in my organism using different methods such as PCR and saliva analysis.
- Observe the growth of different B lymphocytes cultures, and also extract their DNA.
- Make a comparison of different HRV mitochondrial genome parts in order to find the mutations and determine their haplogroups.
- Elaborate a phylogenetic tree.

HYPOTHESES

The initial hypotheses associated with the objectives discussed above are:

- Lab procedures are very difficult to be done and also to be understood, especially with a person with lack of experience on this field.
- I will have infected cells, because 95% of the population have them, and for statistics I have high probabilities of being infected too. Even though chances can be reduced because I am very young!! ;o)
- Infected lymphocytes will be different than non-infected ones, and they will die, because they are ill. We will have to feed them with some special food.
- My genome will be more alike to people who live near where I do, and as far as they live, as different it will be. Nonetheless, the history of migrations among populations can put me closer to an American than to an African, although Africa is physically closer than America.
- A phylogenetic tree is a tree which determines where your family DNA comes from, so I'll be near other European people.

MATERIALS AND METHODOLOGY

Variables: concentration in the liquids and mixtures, rpm on the centrifuge, quantity of DNA in a sample.

Materials: Pipettes, Eppendorf tubes, Centrifuge, Ethanol, Saliva samples, CR machine (thermocycler)

Methodology

We used *Hoffman & Winston's method (1987)* to extract the DNA from the saliva of the three people who worked in the project.

We used suspension cell cultures to observe the growth of 3 different cellular lines of infected lymphocytes. We also fed them with cellular media, counted them with a Neubauer's chamber and we extracted their DNA (**fig. 2**) with an *E.Z.N.A.*™ *Protocol for Cultured Cells*.

The std. amplif. PCR (**fig. 3**) was used to amplify millions of times only the small region of the mitochondrial genome (**figs. 4-5**) chosen through primers (HVR). The agarose gel revealed us the length of the molecules (electrophoresis). When we had all the sequences, we did an alignment. Doing this, we could determine the exact mutations. We were also able to build the phylogenetic tree.

RESULTS

When we started the cultures (contamination with bacteria), so we had to restart them. The three grew perfectly the 2nd time. Using different countries samples, we extracted the DNA. When we did the PCR, one of the samples, concretely 3 (J2S_1/4), didn't appear in the gel (**fig. 6**), so we repeated the extraction (all went correct).

We introduced all the sequences in a web (Blast), to make sure that they weren't contaminated and in another (Mitomaster) to know which haplogroup they had. When we had all the information, we used a program (PhyML) to elaborate the phylogenetic tree. Some of the results hadn't much credibility, due to the small fragment we introduced.

We made a conversion of the sequences to FASTA format, in order to make a Multiple Sequence Alignment, with a program called MAFFT, so we could compare them and find the different mutations.

In my case, my haplogroup is U5a1c, from the Clan Ursula, originally from northern Europe (**fig. 7**), that existed more than 16.000 years ago.

CONCLUSIONS

Taking into account everything learnt, my conclusions are:

- It's possible to extract DNA from a saliva, and it's also possible to sequence a chosen part of it. Recent studies have shown that saliva up to 74% of the cells derived from white blood cells (leukocytes), making a very attractive saliva for extracting DNA and RNA alternative source. It is possible to extract DNA from saliva samples and study important markers for the diagnosis of various diseases.
- It is possible to grow B lymphocytes in cultures because when they infect, they become immortal, so when they are frozen, they are not dead.

- You are not necessary near people from near origins in a phylogenetic tree, because migrations have occurred since millions of years ago.
- Finally, comparing the same part of DNA from different individuals, you can identify mutations and differences.

BIBLIOGRAPHY

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Electronic documents

- OMEGA bio-tech (2013) . *E.Z.N.A.™ Protocol for Cultured Cells.* Electronic document: http://omegabiotek.com/store/wp-content//D3396-Tissue-DNA-Kit-Combo-May2013-Online.pdf (02/02/15)
- Santpere G, Darre F, Blanco S, Alcami A, Villoslada P, Alba MM, Navarro A. *Genome-wide analysis of wild-type epstein-barr virus genomes derived from healthy individuals of the 1000 genomes project*. Genome Biology and Evolution. 2014; 6(4): 846-860. DOI 10.1093/gbe/evu054. Electronic document: http://hdl.handle.net/10230/23395 (29/10/14)

Webs

- Transformation of B lymphocytes: http://en.wikipedia.org/wiki/Epstein_Barr_virus#Transformation_of_B-lymphocytes (22/07/15)
- UNC Lineberger. *B-cell Immortalization Services*: http://unclineberger.org/research//tissueculture/b-cell-immortalization-services (28/09/15)

Other

- **Blog:** http://elmeudiaridebiogenetica.blogspot.com.es/

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- And finally, Ivan Nadal, whose work has been crucial in my project.

ANNEX

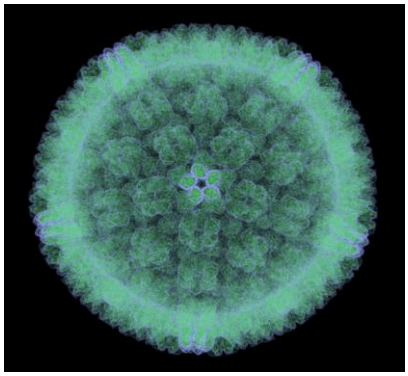

Fig. 1. Epstein-Barr virus

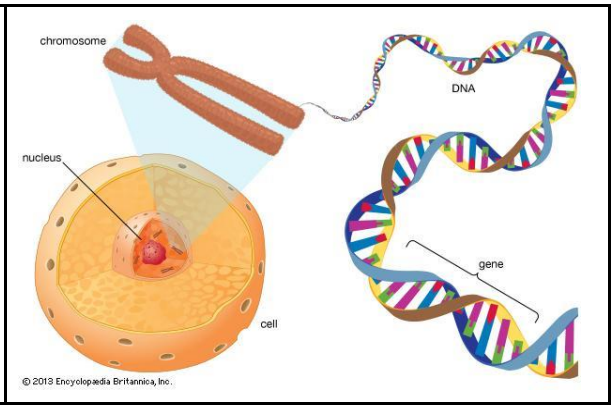

Fig. 2. Extraction of DNA cell.

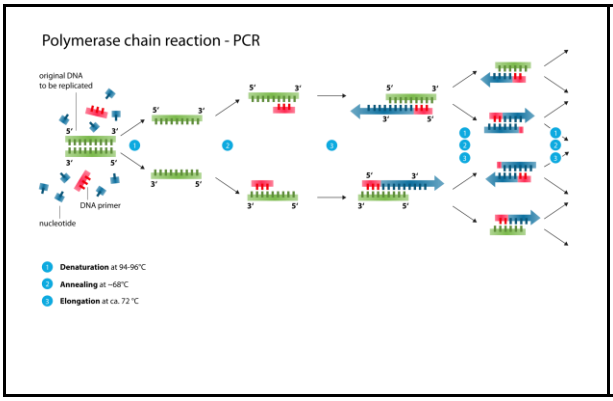

Fig. 3. Standard amplification PCR procedure

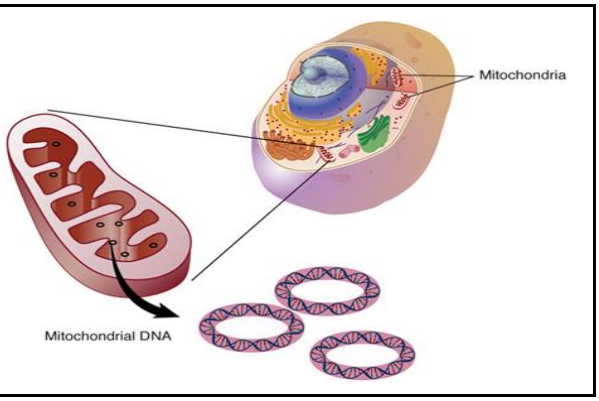

Fig. 4. Cell and mitochondrial DNA

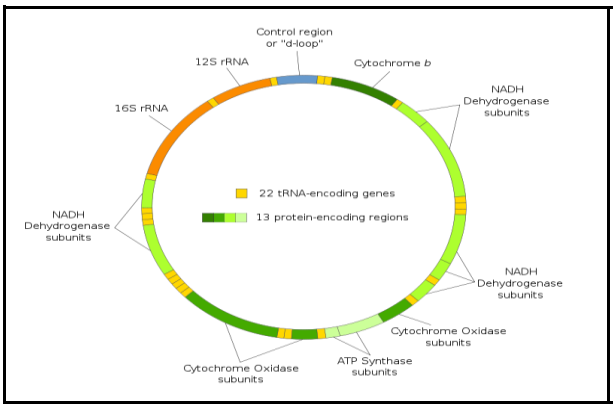

Fig. 5. Mitochondrial DNA structure.

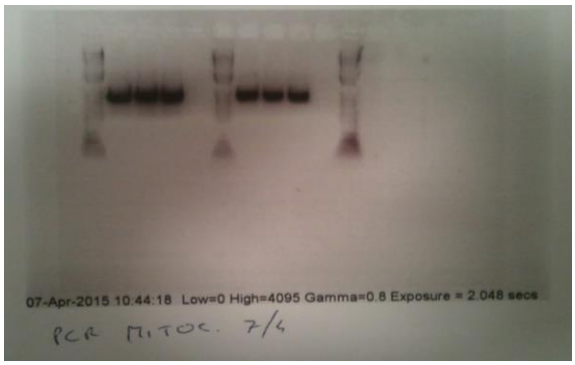

Fig. 6. PCR results.

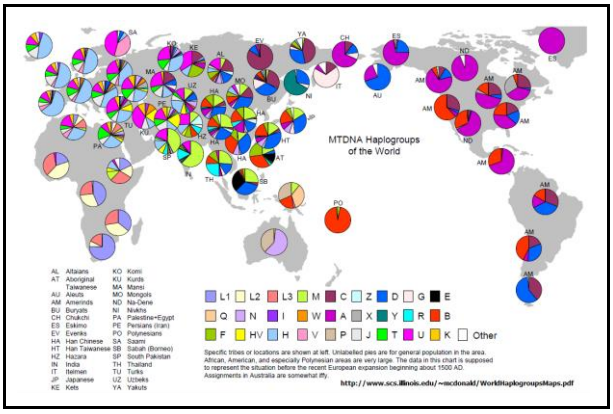

Fig. 7. World haplogroups