LONG-READ SEQUENCING TECHNIQUE AND ITS APPLICATION TO Y CHROMOSOME AS A POWERFUL TOOL IN FORENSIC INVESTIGATIONS

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In the past twenty years the statistical data on murders in USA stated that males are accounted for most of the violent crimes. The work of the forensic analyst, dealing with evidences coming for example from a vaginal swab form a rape case, is very hard , the samples present mixtures and most of the time the interpretation of the results are not enough to identify the perpetrators. The possibility to use markers on Y chromosome become a necessity when the other analysis fails. The purpose of this research study is to develop and optimize a method to sequence long reads including YSTRs and YSNPs together and demonstrate that this kind of analysis can represent a very powerful tool in forensic genetic analysis. This project represents one of the first studies to analyze these polymorphisms using the MinION, a portable sequencing device produced by Oxford Nanopore Technologies. The advantages for using these polymorphisms on Y chromosome are many; it will be possible to help solve crimes when male perpetrators belong to the same family and it could be used as an additional tool for the actual autosomic analysis. Moreover, the use of linked YSTR and YSNPs may provide additional insight into possible geographical localization of the suspect in those forensic cases in which there is no suspect and no witness. Finally, it would be possible to increase the power of discrimination between individuals and resolving complex mixtures, a task that is presently very difficult when using capillary electrophoresis. Since is impossible to detect small point mutations in the repetitive sequence. Such modifications in the repeat motif can instead be discovered by sequencing that region. In this project we will try to address these difficulties in forensic DNA analysis focusing on two main objectives. The goal of the first objective is optimizing the PCR for long fragments and make it suitable for forensic purposes. In this early step of analysis, the optimization of Long PCR will be tested at different DNA concentrations to ensure the accuracy of the amplification at low concentrations of DNA.
In the second part we will test the sequencing technique using the MinION device for long reads fragment of YSNP and YSTR. Finally, due to the information contained in the long-read sequences amplified from each donor, we will be able to detect the orientation and applicability of Y Haplotype and Haplogroup patterns and start creating a small database of contiguous Y chromosome polymorphisms.