Human DNA Quantification And Forensics

Human DNA is present in every cell except RBCs and can be found in body fluids like saliva, blood, semen, vaginal fluids, bones, teeth, hair and sweat. DNA has its individuality and DNA typing methodologies are subjected to scientific and legal scrutiny. DNA has been used as unique investigation material in forensics since Alec Jeffrey introduced RFLP in 1985 for identifing the unique markers in the genetic material.[4]

DNA Quantification estimates the amount of DNA present in the source of DNA evidences. It focuses on establishing the DNA concentration and evaluating the PCR inhibitors.[2] DNA quantification saves time by evaluating the Concentration of DNA that avoids redoing the experiment. Besides, it helps to know whether the amount of DNA in the sample is sufficient for the experimentation or not. It informs, if DNA sample is diluted and also, that you are using right DNA species. This process assures that whatever results you are getting is referring to DNA without contaminating RNA or proteins in the sample.[13]

Methods of DNA Quantification

1)UV Absorbance

The UV absorbance was first proposed by Warburg, O. and Christian W.(1942) , used to obtain qualitative and quantitative details of DNA/RNA.[6]

The purine and pyrimidine bases in DNA absorb UV light of specific wavelengths but the composite absorption occurs at 260 nm. A DNA solution of concentration 50 p,g/ml will yield an absorbency at 260 nm. [7]

Pros:

- Fast, Easy, Reagents are not required.
- Determination of proteins and phenols are possible[10]

Cons:

- Limited sensitivity, contaminants cause inaccuracies
- Differentiating nucleic acid species and determining the extent of DNA degradation isn’t possible.[10]

2)Fluorescence Measurements

DNA does not have fluorescence. It can possess the property of fluorescence with two dyes Hoechst 33258 (Brunk et al. 1979) and ethidium bromide. The maximum excitation and emission for both dyes shifts after binding to DNA. Hoechst 33258 appears to bind to A-T base pairs rich regions; whereas ethidium bromide binds between the stacked bases (Watson et al. 1987). [7]

edubirdie.com

Pros:

- selective detection of nucleic acid species
- 10 times more sensitive [10]

Cons:

- Requires calibration with standards
- molecular weight of DNA and level of contamination cannot be estimated.[10]

3)Yield Gel Measurements

Yield gels are small agarose gels containing ethidium bromide. DNA fragments are introduced to a electrophoretic separation at relatively high voltage. After the completion ofelectrophoresis, the gel is placed under UV light and a photograph is taken. [7]

Pros:

- Approximate molecular weight and concentration can be obtained
- More rapid[7]

Cons:

- It has poor sensitivity, and does not result in a quantitation that is specific to human DNA.
- Results are relatively accurate. [1]

Yield measurements

edubirdie.com

4)Slot Blot Assay

In this process, the genomic DNA is denatured and a tiny amount of sample is spotted onto a nitrocellulose membrane. Then, the immobilization of sdDNA is done on a nylon membrane. The targeted sequence is revealed by hybridization with a labeled 40-nucleotide probe complementary to a primate-specific ??satellite DNA sequence at the D17Z1 locus. [9]

Pros

- Does not require DNA digestion[11]
- Easy to analyze [8]

Cons

- Not automatable, requires more efforts
- Semi-quantitative[8]
- Slot-Blot Assay

5)Quantitative PCR Assays

Based on the principle of PCR amplification, the amount of PCR product amplified correlates with the initial DNA concentration. There are two categories of quantitative PCR methods.

- A)End-point PCR methods measure the amount of synthesis of amplified product during PCR at the end of the reaction. Usually, the fluorescence is emitted by the dyes that intercalate into the dsDNA. The yield of amplified DNA is detected from the amount of fluorescence emitted by dyes.
- B)Real-time PCR methods can quantify the amplified DNA during the exponential phase of PCR.[9]

Exponential phase by RT-PCR

6)Real-Time Quantitative PCR

Real-time quantitative PCR was developed earlier in 1990s, and analyzes the cycle-to-cycle change in a fluorescence signal due to the amplification of a target sequence during PCR. A PCR is monitored by fluorescence reporter by increasing the molecules as products accumulate with each round of amplification. [9]

Pros:

- Sensitivity in picogram range
- Ability to design in sequence-specificity[10]

Cons:

- Requires calibration with standards
- Requires expensive reagents and instrumentation
- Time-intensive assay [10]

Forensic Significance

The quantitation of DNA plays a central role in all areas and applications of forensic DNA analysis such as a discrete windows of DNA concentration are allowed amplification of the 13 CODIS STR loci with the commercial kits that are used for forensic casework and databank genotyping.[1]

DNA quantification is needed in detecting cell-free fetal DNA in the maternal circulation and also for procedures like NGS, PCR, RT-PCR, qPCR, cloning, etc.[12]

References

1. Timket, M., Swango, K., Orrego, C., Chong, M., & Buoncristiani, M. (2005). Quantitation of DNA for Forensic DNA Typing by qPCR (quantitative PCR): Singleplex and Multiplex Modes for Nuclear and Mitochondrial Genomes, and the Y Chromosome.. Ncjrs.gov. Retrieved 2 October 2020, from https://www.ncjrs.gov/pdffiles1/nij/grants/210302.pdf.
2. Ricci, U., Marchi, C., Previdere, C., & Fattorini, P. (2006). Quantification of human DNA by real-time PCR in forensic casework [Ebook]. International Congress Series 1288

edubirdie.com

(2006) 750 – 752. Retrieved 2 October 2020, from https://www.isfg.org/files/67dcf7d7d9946629c70003d18d286e67abe87cfd.0600077x_613929581202.pdf.
3. Standard for Training in Forensic DNA Quantification Methods. (2018). [Ebook] (1st ed.). Retrieved 2 October 2020, from https://www.nist.gov/system/files/documents/2018/12/17/osac_draft_standard_for_training_in_forensic_dna_quantification_methods.pdf.
4. Nurun Nahar Sultana, G., & Zakir Sultan, M. (2018). Mitochondrial DNA and Methods for Forensic Identification. Juniperpublishers.com. Retrieved 2 October 2020, from https://juniperpublishers.com/jfsci/pdf/JFSCI.MS.ID.555755.pdf.
5. LaSalle, H., Duncan, G., & McCord, B. (2011). An analysis of single and multi-copy methods for DNA quantitation by real-time polymerase chain reaction. Forensic Science International: Genetics, 5, 185-193. Retrieved 2 October 2020, from https://cnso.nova.edu/forms/george_duncan_an_analysis_of_single_and_multi-copy.pdf.
6. Pachchigar, K., Khunt, A., & Hetal, B. (2016). DNA QUANTIFICATION [Ebook]. Retrieved 2 October 2020, from http://www.researchgate.net/publication/318744511_DNA_QUANTIFICATION.
7. Samuel Baechtel, F. THE EXTRACTION, PURIFICATION AND QUANTIFICATION OF DNA [Ebook]. Retrieved 2 October 2020, from https://projects.nfstc.org/workshops/resources/literature/Extraction/05_The%20Extraction,%20Purification%20and%20Quatification%20of%20DNA.pdf.
8. DNA Extraction & Quantitation for Forensic Analysts. (2008). [Ebook]. Retrieved 2 October 2020, from https://www.sjsu.edu/people/steven.lee/courses/c2/s2/DNA%20Extraction%20and%20Quantitation%20for%20Forensic%20Analysts.pdf.
9. Li, R. (2011). Forensic biology (pp. 211-221). CRC PRESS.
10. Choosing the Right Method for Nucleic Acid Quantitation. Promega.in. (2020). Retrieved 3 October 2020, from https://www.promega.in/resources/pubhub/choosing-the-right-method-for-nucleic-acid-quantitation/.
11. Blouin, J., Rahmani, Z., & Chettouh, Z. (2020). Slot Blot Method for the Quantification of DNA Sequence and Mapping of Chromosome Rearrangements: Application to Chromosome 21. American Journal Of Human Genetics, 46, 518-526. Retrieved 3 October 2020.
12. Co.KG, B. (2020). DNA quantification - Berthold Technologies. Berthold Technologies GmbH & Co.KG. Retrieved 3 October 2020, from https://www.berthold.com/en/bioanalytic/applications/dna-quantification/.
13. Nucleic Acid Analysis. Promega.com. (2020). Retrieved 3 October 2020, from https://www.promega.com/nucleic-acid-analysis/.