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Qualitative and Quantitative Analysis of DNA from Human Saliva Respectively through Quantitative Polymerase Chain Reaction and UV-Visible Spectrophotometer: A Review

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Abstract: ***Saliva is significant proof that could be found in the location of the crime. The epithelial cells of saliva are the best source of DNA. Forensic DNA phenotyping is the future of Criminal investigations such as Victim or Suspect identification, Paternal Maternity identification, age determination, species identification, etc. Saliva can be found in different places or objects and under different conditions. Saliva could be found on cigarettes, chewing gum, bite marks, water bottle, etc. The quality and the quantity may be varied from sample to sample found in a different place. Environmental factors can also cause the degradation of a cell of saliva. High temperature, pH, and an enzyme found in the environment could be responsible for the denaturation of DNA in cells. In such cases, DNA quality and quantity can be altered. Salivary DNA could be extracted by the phenol-chloroform method. But advanced techniques like the Chelex-100 resin method are considered the best method to extract salivary DNA, providing better quality and quantity. DNA fragments are used for quantitative analysis of DNA through PCR. Digital PCR consumes less time to analyze DNA and made the procedure easy. UV spectrophotometer is widely used for measuring the quality of DNA from mid the twentieth century. The current review article highlights forensic research to measure DNA quality and quantity samples by qPCR, and UV-Vis Spectrophotometer methods from human saliva for further criminal investigations. This article also contributes knowledge to more effective PCRs used to measure DNA quantity.***

Keywords: Criminal Investigation, DNA profiling, Polymerase Chain Reaction, UV spectrophotometer, etc.

1. Introduction

Quantitative and qualitative DNA examination assumes a significant part in criminal examinations. The quantity and quality of DNA can affect forensic DNA phenotyping. The quality and quantity can vary between bodily fluids such as salivain different places and conditions. The identification between individuals is a vital part of Forensic Study. The repetitive sequence of DNA permits us to find out the difference between suspect and victim in a criminal investigation. Saliva might be found on different kinds of materials such as cigarette butts [1], bottles, glass, chewing gum, human skin [2], bite marks, etc. Salivary epithelial cells are an excellent source of DNA. Some new examination shows that proficient DNA could be separate from salivation [3]. Saliva kept in different environments can be useful to extract DNA [4]. The salivary DNA that is present on clothing, paper, or other objects is easier to collect and extract than the saliva present on human skin. There is the least method available to extract DNA from saliva. DNA contamination can also take place while extracting the DNA from saliva. For large amounts of DNA, the double swab technique is one of the reliable methods to collect saliva samples. [5].110µg DNA could be extracted per 2mL of Saliva sample [6].

Salivary DNA can be extracted by two following methods 1) phenol-chloroform extraction method, 2) Chelex extraction method, or modified chelex extraction method. Chelex extraction technique is a more reliable, easier, quick extraction technique than phenol extraction. This technique did not include any noxious solvents [7, 8, 9].

Visual instrumentation brought a new era for quantity and quality measurement of organic or inorganic compounds in the twentieth century. Advance UV-Visible spectrophotometer came into exits to measure the quality and quantity of organic or inorganic compounds like DNA, RNA, proteins, etc. The combination of UV-vis and chemometric classified algorithms are shown a promising result in the quantification and quality control of compounds [10]. Apart from that, alternative spectroscopic techniques like Near (IR) Infrared and Raman spectroscopy [11, 12, 13, 14] are also helpful for quality checking.

UV-vis spectroscopy is rapid and affordable technique among other spectroscopic methods for DNA quantitation. Apart from that, Horizontal gel electrophoresis, RT-PCR (Real Time-Polymerase Chain Reaction), and fluorescent dyes are also available for quantity check of DNA. These methods have a sharp link with quantification ratios. [15]

A UV-visible spectrophotometer with the computation of the ratio of OD differences with an appropriate limit between 1.6 and 1.8 is the universal approach for assessing DNA quality. The DNA quality, extracted from saliva provides results within an admissible range [16].

When compared to blood, using salivary DNA has certain technological benefits. A highly potential alternative source

of DNA is exfoliated buccal epithelial cells and other cells present in saliva [17]. Oral swabs, especially those taken from the cheeks in the mouth, provide the serologist with enough cells and DNA to determine the DNA profile. This might be a substitute for venipuncture for collecting controls. PCR is exceedingly responsive, and it can effectively type DNA samples ranging from 1 ng to 5 ng [18].

DNA Extraction:

There were several techniques available for extracting DNA from salivation considered: the phenol-chloroform technique of organic extraction [19] and a process involving 5% Chelex-100 resin [20]. Chelex is a polyvalent metal ion chelating resin with a high affinity. To separate DNA from a notable volume of saliva, a phenol-and chloroform-based [19] approaches along with the conventional Chelex-100-based method were tested [20]. DNA was separated from the whole saliva and a reference standard with a known focus to survey which extraction technique gave the most DNA yield and to break down the conceivable loss of DNA because of examining control. In comparison to the phenol-chloroform approach, the conventional Chelex technique was demonstrated to be the best extraction strategy. The traditional Chelex extraction procedure was used to extract DNA from the standard working solution and entire samples of saliva [7]. The organic extraction method of Chelex-100 resin is more successful than phenol-chloroform extracting DNA from saliva.

UV Spectrophotometer:

UV spectroscopy is utilized to check the Purity of DNA probes, a PCR inhibition test is used to determine the presence of inhibitors, and gel electrophoresis is utilized to decide intactness. The ratios of A260/A280 and A260/A230 are estimated by measuring the absorbance spectrum of a sample somewhere in the range of 200 and 320 nm and figuring the A260/A280 and A260/A230 proportions [21].

$$\frac{A260}{A280} == 1.7 - 2.0 \text{ for pure DNA}$$

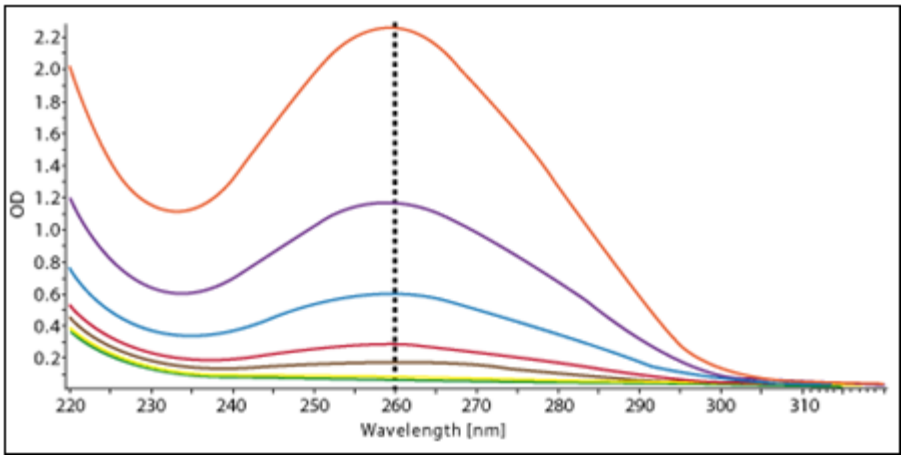

Figure 1: Absorbance spectrum of different concentrations of calf thymus DNA recorded on the POLAR star Omega. The detection range is between 220 and 310 nm and the resolutions were set at 1 nm. [48]

DNA loss due to the extraction process reduces the overall detection assay performance, accurate measurement of the amount of DNA is critical to optimizing the assay [22, 23]. While more precise DNA concentration measuring technologies, such as digital PCR, are available [24, 25, 26].

The assumptions and limits of different methods of concentration are different. For concentration measurements using UV spectroscopy, for example, two assumptions are required. First, DNA is the only molecule that absorbs light at a range of 260nm, which is double-stranded throughout. Measurements of light absorbency of DNA using UV spectroscopy were statistically superior to the other two methods. For several extracts, the A260/A280 and A260/A230 absorbance ratios were above the permissible limit, indicating that co-extracted RNA led the A260 test to overestimate DNA content. Absorbance-based DNA concentration tests commonly overestimate DNA concentrations in environmental samples due to contaminants in the sample matrix. [27]. The precision of absorbance-based DNA fixation tests is affected by sample tidiness [27, 28]. The Qubit and other bench top fluorometers provide relatively quick DNA concentration assays that are less impacted by DNA impurities than UV spectroscopy [29]. The DNA standards used determine the precision of the fluorometric DNA concentration measurements [30]. When compared to qPCR, fluorometric DNA concentration measurement techniques have the benefit of requiring no extra assay development for specific species. The lowest observed limit of quantification and the fewest samples with values below the technique were found in qPCR. The primary burden of using qPCR to quantify DNA focus is that strategy advancement, approval, and executions take a great deal of time and money. In any case, qPCR is the sole technique for deciding the grouping of DNA in a blended example.

qPCR:

Quantitive PCR is an assortment of strategies for estimating measures of explicit template DNA successions. In one methodology the limiting of a columnist dye (SYBR Green I) to twofold abandoned DNA is utilized to measure the advancement of the PCR at each pattern of amalgamation, and an example looking like remarkable or sigmoidal development is recorded until the fluorescence arrives at a level.

The purity of the DNA extract is of interest because of the potential impact of contaminants on downstream assay results. Proteins, polysaccharides, and RNA are among the contaminants that UV spectroscopy may identify in extracts [21]. Inhibition assays of PCR determine whether pollutants will interfere with the qPCR detection assay [31]. The majority of extract purity ratios (A260/A280 has a range of 1.8 to 2.0, while A260/A230 has a range of 1.8 to 2.2.) were outside of the permissible range (A260/A280 has a range of 1.8 to 2.0, while A260/A230 has a range of 1.8 to 2.2.), but no PCR inhibition was found in the most study. RNA in DNA extracts has little effect on downstream applications, although it does induce an overestimation of DNA quantities detected by UV spectroscopy [21].

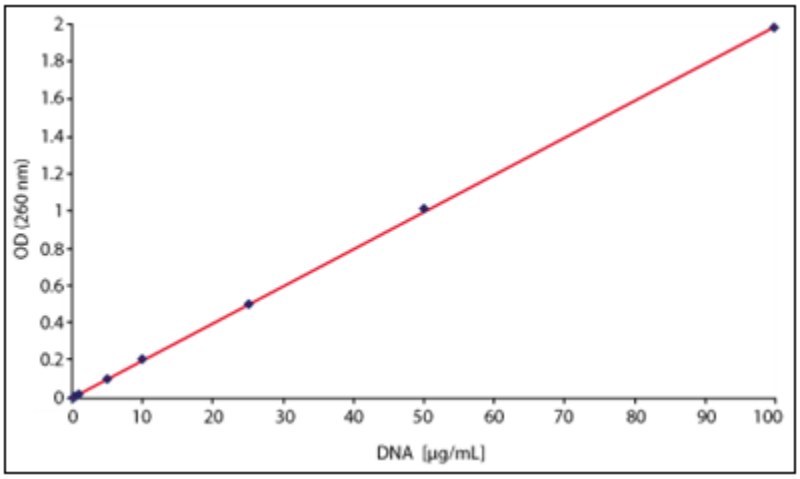

Figure 2: Linear regression fit performed on the DNA standard curve in the concentration range from 0.1 to 100 µg/mL [49]

High DNA concentration, however not really significant returns of absolute DNA, are needed for fruitful hereditary examination for SNP genotyping [32]. The spectrophotometric approach used in the quantitative assessment has a downside in that it doesn't take into account the assurance of whether or not the DNA source is human.

Limitations of UV Spectrophotometer:

The main drawback of DNA purity assessments through UV Spectrophotometer is the fact that there are no indications for various types of contaminants, and no data on how these contaminants affect downstream uses. Immaculateness examines are additionally at times utilized when explicit pollutants are of interest, like distinguishing humic corrosive by estimating the UV absorbance of an example at 320 nm. [23].

Limitations of PCR:

Hardly any examinations have investigated the connection between extricate quality and execution or potentially enhancement of PCR test. PCR inhibitors are frequently found in sample matrices such as blood, food, water, and soil [33]. PCR inhibitors can bring about bogus negatives or misjudges of biological quantities. The susceptibility of qPCR measures to restraint changes relies upon the polymerase, primer areas, and sequence of the target region [34, 35, 36]. DNA fragmentation can reduce the effectiveness of qPCR reactions or perhaps lead them to fail [37, 38], As a result, the process Limit of Detection is greater. Conventional agarose gel electrophoresis is frequently used when DNA extracts are tested for shearing [39, 40, 41]. For qPCR, a target length of 50-200 bp is appropriate [42].

2. Discussion

Even though various methods, for example, the utilization of clinical dressing cushions and cigarette papers, have been used with shifting levels of progress to recover spit and other follow proof from the skin, the most by and largely utilized methodology is the utilization of sterile swabs [43]. One of the most prevalent methods for quantifying DNA is to use UV absorbance. This methodology incorporates deciding the centralization of synthetic substances in a fluid by estimating the absorbance/transmission of light through it. UV spectroscopy was used to assess extract purity, an independent qPCR test was used to assess PCR inhibition, and gel electrophoresis was used to assess intactness. Even under identical circumstances, different qualities and quantities of DNA are recovered from various people from a similar place [44]. Pure DNA quality and sufficient quantity can lead to positive results to identify criminals. Still, it's possible that the quality and quantity of DNA are so low in some circumstances that it is required to try to enhance the extraction product yield. This is especially obvious on account of saliva stains. Better quality and quantity of DNA indicate the environmental condition also.

Below mentioned formula is used to compute the concentration of DNA:

"Concentration (µg/ml) = A260 reading x dilution factor x 50 µg/ml"

3. Conclusion

To sum it up, employing Chelex-100 resin to extract DNA from saliva is a more successful extraction technique. The updated Chelex process improves the extraction results even further. When managing little volumes of fluid salivation or spit stains where just a little amount of DNA can be

anticipated to be recovered, the modified Chelex approach is advised. Salivary DNA purity and concentration were determined to be near optimum, indicating that it might be used for gene amplification. Saliva might be a superb wellspring of DNA and could be used effectively in the field of forensic science. This innovative qPCR analysis approach does not require any PCR efficiency assumptions or calculations. The strategies used to examine DNA separate quality and the amount will be controlled by downstream application needs and normal wellsprings of downstream application inhibitors [45, 46, 47] while streamlining recognition tests and choosing which DNA extraction strategy to use. The A260/A280 proportion uncovers the virtue of the DNA test and might be assessed straightforwardly and in a similar measure of time as A260 alone. A total absorbance range in the scope of 220-1000 nm supports the recognition of contaminations and might be estimated in under one second for every well.

References

[1] M. N. Hochmeister, B. Budowle, J. Jung, U. V. Borer, C. T. Comey, and R. Dirnhofer, PCR-based typing of DNA extracted from cigarette butts. ht. J. Leg. Med., 104 (1991) 229-233.
[2] D. J. Sweet. Idrntificacidn de manchas de salivuhumanamedianteelandlisis de1 ADN, Doctoral Thesis. University of Granada.1995
[3] Quinque D, KittlerR, Kayser M, Stoneking M, Nasidze I. Evaluation of saliva as a source of human DNA for population and association studies. Anal Biochem2006; 353: 272-7.
[4] da Silva RH, Sales-Peres A, de Oliveira RN, de Oliveira FT, Sales-Peres SH. Use of DNA technology in forensic dentistry. J Appl Oral Sci 2007; 15: 156-61.
[5] D. J. Sweet. M. Lorente, J. A. Lorente, A. Valenzuela and E. Villanueva, An improved method to recover saliva from human skin: the double swab technique. J. Forensic Sci., (in press March 1997).
[6] Birnboim HC: DNA Stability with OrageneTM. Ottawa: DNA Genoteck, Inc.; 2004
[7] P. S. Walsh, D. A. Metzger and R. Higuchi, Chelex-100 as a medium for simpleextraction of DNA for PCR-based typing from forensic material. BioTechniques, 10 (1991) 506-513.
[8] J. M. Jung, CT. Comey, D. B. Baer and B. Budowle, Extraction study for obtaining DNA from bloodstains for PCR amplification and typing of the HLA-DQ alpha gene. Int. J. Leg. Med., 104 (1991) 145-148.
[9] P. Wiegand, B. Budowle, S. Rand and B. Brinkmann, Forensic validation of the STR systems SE33 and TCll. Int. J. Leg. Med., I05 (1993) 315-320
[10] X. Li, K. Xu, H. Li, S. Yao, Y. Li, and B. Liang, "Qualitative analysis of chiral alanine by UV-visible-shortwave near infrared diffuse reflectance spectroscopy combined with chemometrics, " RSC Advances, vol.6, no.10, pp.8395–8405, 2016.
[11] I. Bennani, M. A. Chentoufi, A. Cheikh, M. E. Karbane, and M. Bouatia, "Proposal of a simple and rapid method for the chemotherapy preparations analytical control by spectrophotometry UV-Vis method, " Journal of Oncology Pharmacy Practice, vol.27, no.1, pp.99–107, 2021.
[12] F. Nardella, M. Beck, P. Collart-Dutilleul et al., "A UV-Raman spectrometry method for quality control of anticancer preparations: Results after 18 months of implementation in hospital pharmacy, " International Journal of Pharmaceutics, vol.499, no.1-2, pp.343–350, 2016.
[13] F. Dziopa, G. Galy, S. Bauler et al., "A quantitative and qualitative method to control chemotherapeutic preparations by Fourier transform infrared–ultraviolet spectrophotometry, " Journal of Oncology Pharmacy Practice, vol.19, no.2, pp.121–129, 2013.
[14] L. Lê, M. Berge, A. Tfayli, P. Prognon, and E. Caudron, "Discriminative and quantitative analysis of antineoplastic taxane drugs using a handheld Raman spectrometer, " BioMed Research International, vol.2018, pp.1–7, 2018.
[15] Nishita DM, Jack LM, McElroy M, McClure JB, Richards J, Swan GE, Bergen AW: Clinical trial participant characteristics and saliva and DNA metrics. BMC Med Res Methodol 2009, 9: 71.
[16] Hansen TV, Simonsen MK, Nielsen FC, Hundrup YA: Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. Cancer Epidemiol Biomarkers Prev 2007, 16 (10): 2072–2076.
[17] García-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev2001; 10: 687-96.
[18] Dunncan GT, Tracey ML. Serology and DNA Typing. In: William G. Eckert, editor. Introduction to Forensic Sciences.2nd ed. New York: Elsevier; 1992. p.242-304.
[19] D. J. Walsh, A. C. Corey, R. W. Cotton, L. Forman, G. L. Herrin, C. J. Word and D. D. Garner, Isolation of deoxyribonucleic acid (DNA) from saliva and forensic science samples containing saliva. J. Forensic Sci., 37 (1992) 387-395.
[20] P. S. Walsh, D. A. Metzger and R. Higuchi, Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques, 10 (1991) 506-513.
[21] Wilfinger WW, Mackey K, Chomczynski P. In: DNA sequencing II optimizing preparation and cleanup. Sudbury KJ, editor. MA: Jones and Bartlett Publishers; 2006. Assessing the quantity, purity and integrity of RNA and DNA following nucleic acid purification; pp.291–312.
[22] Queipo-Ortuño MI, Tena F, Colmenero JD, Morata P, Queipo-Ortuno MI. Comparison of seven commercial DNA extraction kits for the recovery of Brucella DNA from spiked human serum samples using real-time pcr. Eur J Clin Microbiol Infect Dis.2008; 27: 109–114.
[23] Arbeli Z, Fuentes CL. Improved purification and PCR amplification of DNA from environmental samples. FEMS Microbiol Lett.2007; 272: 269–275. doi: 10.1111/j.1574-6968.2007.00764. x.
[24] Pinheiro LB, Coleman VA, Hindson CM, Herrmann J, Hindson BJ, Bhat S, Emslie KR. Evaluation of a droplet digital polymerase chain reaction format for

DNA copy number quantification. Anal Chem.2012; 84: 1003–1011. doi: 10.1021/ac202578x.
[25] Holden MJ, Rabb SA, Tewari YB, Winchester MR. Traceable phosphorus measurements by ICP-OES and HPLC for the quantitation of DNA. Anal Chem.2007; 79: 1536–1541. doi: 10.1021/ac061463b.
[26] Brennan RG, Rabb S, Holden MJ, Winchester MR, Turk GC. Potential primary measurement tool for the quantification of DNA. Anal Chem.2009; 81: 3414–3420. doi: 10.1021/ac802688x.
[27] Rothrock MJ Jr. Comparison of microvolume DNA quantification methods for use with volume-sensitive environmental DNA extracts. J Nat and Environ Sci.2011; 2: 34–38.
[28] De Mey M, Lequeux G, Maertens J, De Maeseneire S, Soetaert W, Vandamme E. Comparison of DNA and RNA quantification methods suitable for parameter estimation in metabolic modeling of microorganisms. Anal Biochem.2006; 353: 198–203. doi: 10.1016/j. ab.2006.02.014.
[29] Sauer P, Muller M, Kang J. Quantitation of DNA. Qiagen News.1998; 2: 23–26.
[30] Griffiths KR, Burke DG, Emslie KR. Quantitative polymerase chain reaction: a framework for improving the quality of results and estimating uncertainty of measurement. Anal Method.2011; 3: 2201. doi: 10.1039/c1ay05069a.
[31] Nolan T, Hands RE, Ogunkolade W, Bustin SA. SPUD: A quantitative PCR assay for the detection of inhibitors in nucleic acid preparations. Anal Biochem.2006; 351: 308–310. doi: 10.1016/j. ab.2006.01.051.
[32] Philibert RA, Zadorozhnyaya O, Beach SR, Brody GH: Comparison of the genotyping results using DNA obtained from blood and saliva. Psychiatric genetics 2008, 18 (6): 275–281.
[33] Wilson IG. Inhibition and facilitation of nucleic acid amplification. Appl Environ Microbiol.1997; 63: 3741–3751.
[34] Pontiroli A, Travis ER, Sweeney FP, Porter D, Gaze WH, Mason S, Hibberd V, Holden J, Courtenay O, Wellington EMH. Pathogen quantitation in complex matrices: a multi-operator comparison of DNA extraction methods with a novel assessment of PCR inhibition. PLoS One.2011; 6: e17916. doi: 10.1371/journal. pone.0017916.
[35] Huggett JF, Novak T, Garson JA, Green C, Morris-Jones SD, Miller RF, Zumla A. Differential susceptibility of PCR reactions to inhibitors: an important and unrecognized phenomenon. BMC Res Notes.2008; 1: 70. doi: 10.1186/1756-0500-1-70.
[36] Opel KL, Chung D, McCord BR. A study of PCR inhibition mechanisms using real time PCR. J Forensic Sci.2010; 55: 25–33. doi: 10.1111/j.1556-4029.2009.01245. x.
[37] Tung N-H, Aboudharam G, Drancourt M. Heat degradation of eukaryotic and bacterial DNA: an experimental model for paleomicrobiology. BMC Res Notes.2012; 5: 528. doi: 10.1186/1756-0500-5-528.
[38] Carbonero F, Nava GM, Benefiel AC, Greenberg E, Gaskins RH. Microbial DNA extraction from intestinal biopsies is improved by avoiding mechanical cell disruption. J Microbiol Methods.2012; 87: 125–127.
[39] Miller DN, Bryant JE, Madsen EL, Ghiorse WC. Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl Environ Microbiol.1999; 65: 4715–4724.
[40] Mumy KL, Findlay RH. Convenient determination of DNA extraction efficiency using an external DNA recovery standard and quantitative-competitive PCR. J Microbiol Methods.2004; 57: 259–268. doi: 10.1016/j. mimet.2004.01.013.
[41] Carrigg C, Rice O, Kavanagh S, Collins G, O'Flaherty V. DNA extraction method affects microbial community profiles from soils and sediment. Appl MicrobiolBiotechnol.2007; 77: 955–964. doi: 10.1007/s00253-007-1219-y.
[42] Nolan T, Hands RE, Bustin SA. Quantification of mRNA using real-time rt-PCR. Nat Protoc.2006; 1: 1559–1582. doi: 10.1038/nprot.2006.236.
[43] Rogers D and Newton M (2000). A physician's guide to clinical forensic medicine. In: Sexual assault examination (Stark MM, ed.). Humana Press Inc., New Jersey, 61-126.
[44] Muruganandhan J and Sivakumar G (2011). Practical aspects of DNA-based forensic studies in dentistry. J. Forensic Dent. Sci.3: 38-45
[45] Rajendhran J, Gunasekaran P. Strategies for accessing soil metagenome for desired applications. Biotechnol Adv.2008; 26: 576–590. doi: 10.1016/j. biotechadv.2008.08.002.
[46] Purohit MK, Singh SP. Assessment of various methods for extraction of metagenomic DNA from saline habitats of coastal gujarat (India) to explore molecular diversity. Lett Appl Microbiol.2009; 49: 338–344. doi: 10.1111/j.1472-765X.2009.02663. x.
[47] Manjula A, Sathyavathi S, Gunasekaran P, Rajendhran J. Comparison of seven methods of DNA extraction from termitarium for functional metagenomic DNA library construction. Ind Res.2011; 70: 945–951.
[48] Fig 1: Originalsource: https: //www.bmglabtech. com/fileadmin/04_Application/App_Notes_250-299/absorbance-spectrum-of-different-concentrations. png
[49] Fig 2: Original source: https: //www.bmglabtech. com/fileadmin/04_Application/App_Notes_250-299/linear-regression-dna-standard-curve. png