International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

The Benefits of the Absolute Quantifying Method with TaqMan Probeversus Other Methods

MimozaCanga MD PhD[1], Vito Antonio Malagnino MD DDS[2]

[1]University of Medicine Tirana, 9400 Albania

[2]Sapienza University of Rome, 00185 Italy

Abstract: ***Aim: To evaluate in an experimental way the best extraction method which maximally extracts the sample and avoids the issues or DNA degrading. Material and Method: The determination of the blood samples of the external environment is done by using the test Kastle Meyer. The determination of the extracted DNA is done by the method Absolute Quantifying with ABI Prism Real Time-PCR 7000. The complex of the reagents used for this aim is the type Quantifiler® Human DNA Quantification Kit. The software used for the analyses was: ABI PRISM® 7000; Sequence Detection System Software Kit version 1.0 The multiplying of the genetic markers is done by using the PCR method and the use of Thermal Cycler Gene Amp 9700. The complex of the genes used for this method is AmpF£STR® Identifier® PCR Amplification Kit. The analyzed locus are: D7S820, CSF1P0, D18S51, Amelogenin and FGA. The separation and the analyzing of these fragments is done by using the genetic analyzer ABI 310 (Applied Biosystem, P/N: 310-00-200/240-W) and softwer GeneMapper® ID Software v3.2 (Applied Biosystem, P/N: 4338951). Results: In the table we notice that samples are positive for hTERT gene. The negative result of this we think comes from the paper substract used for the collection of the sample, which is not appropriate for the deposit of the epithelial cells of the skin when is touched. Conclusion: The samples have been in different levels of damage of DNA molecules, this marker has shown high stability to environmental factors. the use of Quantifiler® Human DNA Quantification Kit increases the accuracy and eficasity in the discovery of human DNA presence and the determination of its amount wich makes this gene have a primary role compared with other markers used for the same purposes.***

Keywords: Kastle Meyer, DNA degrading, locus, markers

1. Introduction

The selection of the DNA extraction from the samples of the biological material has an important effect in the construction of the genetic profile. Various genetic locuses present different stages of polimorphism [2]-[6]. The similarity between the different individuals in the DNA level is near 99.9%, for this reason the areas where we look for differences is only 0.01%. The genetic markers with repeated long units are made of hundried and thousands bases of DNA satelites. STR as genetic markers multiply easily through the techique of PCR [2]-[6],[18]. When we have samples whose DNA is really degraded it is appropriate to use the markers of STR type but with shorter distance like STR[2].The conditionor the nature of the forensics samples is always doubtful[6]. There are genetic markers that other than polimorphic activity show a high resistance to the degrading factors [4],[-5].

2. Aim

In our study we have analyzed samples with different biologic material that went under direct or indirect factors of outer environment and other factors that can dammage DNA molecules.

3. Materials and Method

During the analyses of the STR markers we used various direct and indirect samples that were under different environmental factors. There are included deposited blood samples in substrats like Clothes and sponges. The other part of the biological material is taken from types as moldy clothes, blood samples collected on the different objects as concrete and glass. Microtraces with biologic material taken with a tampon from different surfaces like glass and textiles on which there were

skin epithelial cells. A main feature of the sample environment was the presence of humidity which has favoured the growth and development of different microorganisms that have enzymes that dammage the DNA in our samples. In the cases where the profile of DNA is used for the evaluation of the quantity and quality during the extraction, there are liquid samples used and after these samples are put in filter paper. After drying they are isolated and saved in low temperature environmentt= -20°C up to the moment of extraction. The determination of the blood samples of the external environment is done by using the test Kastle Meyer.

4. Results

The gene hTERT is only found in human DNA and this certain fact is used for to determine the quantity of DNA in the biologic material analyzed with the techique TaqMan probe.

Table 1: Presents the results from the blood samples.

N	1	2	3	4	5
Type of sample	Mold Covered	Skin	Skin	Skin	Skin
Methods	Chelex	Chelex	Chelex	Chelex	Chelex
Concen tration	-	0.326	0.326	0.326	0.326
Cycle	-	29	29	29	29
Multipli cation	-	1.2ng-10µL	1.2ng-10µL	1.2ng-10µL	1.2ng-10µL
ADN profile	-	-	-	-	-
Locus failed	-	-	-	-	-

Table 2: Presents the results from the blood samples

N	6	7	8	9	10
Sample Type	concrete material	half leather	spotted cotton material	glass surfaces	from earth
Method	Chelex	Chelex	Chelex	Chelex	Chelex
Concen tration	1.33	0.136	1.48	0.522	0.21
Cycle	29	31	31	33	33
Multipli cation	1.3ng-10µL	1.2ng-10µL	1.2ng-10µL	1.2ng-10µL	1.2ng-10µL
ADN profile	Total	Total	Total	Total	Total
Locus failed	D7S820	D7S820 CSF1PO DI8S51	-	-	-

This tables give data on the substract, extracting method, the percentage of the IST (ng/^L) after extraction, the cycle number whose border is used to analyze the percentage od IST, the replicated quantity of IST in ng/10^L, the way of percentage increase of IST for small samples. There are data for the injection of the sample expressed in seconds normal time is 5 seconds the gained profile or not of the IST, ist type whole, partial or mixed with two or more elements balancing or not of the parts of each alelle and data on dammaged locuses which are nor revealed during the analyses. The extraction of the IST form these samples is done with the Chelex method. The negative result of this sample is a consequence of high dammage of the IST molecule since the sample of the blood in the cotton tampon has molded in all ist surface. The smaller percentage of DNA isolated in these samples compared to the DNA samples of the table 1, we think that this can be explained with the fact that most of the these samples were biologic samples form the mouth or skin epithelium. To determine the quantity of the DNA extracted we have used the techique of Absolute Quantifying. With the spectrophotometric method we have determined and evaluated the total quantity of DNA in the analyzed amterial by including the free nucleotides, inhibitors and non human DNA. The technique of quantifying uses the DNA fragment of TaqMan Probe which is contemplater with the array of gene hTERT and in the case of analyses not only discoveres dhe human DNA but also does this with high sensitivity. By using this method in our samples we have reached to determine the contains of human DNA up to the values of 0.001ng/^L eventhough the minimal values of the prepared standards for the discovery is 0.136 ng/^L, so this value goes beyond the minimal border. The results show that this method is very sensitive and very accurate.

Table 3: Results of the analysis of samples of saliva and epithelial cells

N	1	2	3
Type of sample	Saliva	cotton	cotton
Methods	Chelex	Chelex	Chelex
Concen tration	0.031	0.031	0.001
Cycle	34	31	31
Multipli cation	Centrifugation	Centrifugation	Centrifugation
ADN profile	Partial	Total	Total
Locus failed	D7S820	-	-

Table 4: Results of the analysis of samples of saliva and epithelial cells

N	4	5	6	7
Type of sample	paper	cotton	cotton	cotton
Methods	Chelex	Chelex	Chelex	Chelex
Concen tration	-	0.007	0.006	0.006
Cycle	-	33	35	35
Multipli cation	Centrifugation	Centrifugation	Centrifugation	Centrifugation
ADN profile	Total	Partial	Partial	Partial
Locus failed	-	D18S51 FGA	D7S820 D18S51	D18S51 CSF1PO D7S851

On the other hand it is worth saying that the nature of table 3and 4 samples has more microorganisms compared with the table 1 samples so the probability of dammage in these samples is higher. The method of absolute quantifying was the best method and the most sensitive compared with the others. Such a success is demostrated even in the cases where the samples were dammaged in different levels. In order to obtain reliable results we have tried to to use the amplification of 1.2 ng/10^L [2],[3],[6],[20]-[25] in all the DNA samples. The samples where was only a small quantity of DNA with the insufficient maximal volume of 10^L, are centrifuged with a micron tube to reduce the buffer amount TE[4] and in the same time to increase the DNA concentration. For these samples is also increased the time of injection during the analyze in the capilar electrophoreses [14]. The samples with large amount of DNA they are diluted in the close amount of 1.2 ng/10^L. The blood samples Table 1 are collected from various substracts exposed to different weatehr conditions. Referring table 1we see that: 10 of the analyzed samples 4 of them have full DNA profile (samples nr: 7,8, 9, 10) 3 of them partial DNA profile (samples nr: 4 and 5), 3 samples did not give DNA profile (sample 1,2 and 3). The failure to give DNA profile in the last 3 samples is a result of DNA molecule dammaging and the presence of inhibitors in the sample (sample nr: 1, 2, 3). Sample nr. 1 did not give DNA profile because the substract has polluted blood in the presence of microorganisms and there was mold in this sample. This is seen from the absence of hTERT gene. The samples number 2, 3 which are deposited in skin material did not give DNA profile because of the fact that the gene hTERT is present and there is sufficient amount of DNA. The skin is a substract that is a multiplying inhibitor, so in this case thay failed to amplify [2],[3][9][12],[20]-[25].

The samples number 4 and 5 gave partial profile. We think this came from partial dammage of the DNA molecule. In these situations the first lucus that fail to analyze are the longest ones[5]. The genetic markers of these samples that failed to be analyzed are the locus: D7S820, D18S51,

CSF1PO and FGA. Based on gained results the STR genetic markers have shown high stability even in the cases where where the DNA molecule is dammaged [6],[9],[20]-[25]. Based on the results of table 2 we can say that the most problematic samples were the ones suspected of the presence of epithelial skin cells during touch of substract rubbing the same with mouth cell epithelium. From 10 analyzed samples 4 of them gave full DNA profile 3 of them gave partial profile and 3 of them failed to give DNA profile (sample 1,2 and 3).

As a result of the low amount of DNA the samples 2, 3 are centrufuged to increase the percentage of DANN and in the discovery of DNA in the genetic analyzer is increased the injection time of sample form 5 seconds to 8-10 seconds maximal time. A partial DNA profile was taken from samples 5 and 7 as a result of DNA damage. The failure to analyze the DNA profile for the samples are related to the fact that sample had insufficient amount of epithelial cells not adequate of the substract deposit and the DNA molecule was damaged. This can be argued with the fact that the gene hTERT is discovered here but is not discovered the genetic marker of Identifiler Kit. The saliva samples two of them have given full DNA profile samples 2 and 3. Referring to the data of Table 2 we think that the quantity and quality of extracted DNA in samples of skin epithelial cell in the saliva ones was better in the saliva.

5. Discussion

In the table we notice that samples are positive for hTERT gene. The negative result of this we think comes from the paper substract used for the collection of the sample, which is not appropriate for the deposit of the epithelial cells of the skin when is touched. Dixon et al. have analyzed the stability of the short repeated framents of the same locus by using the humidity as a dammaging factor of the blood and saliva samples[9]. From the results it is seen that STR segments show a high stability to the dammage mainly during the first week where is gained the full profile of DANN later the longer the time of izolation the more failure to show the profile. These results show that the percentage of isolated DNA by using the Chelex method is higher than the percentage of organic extraction (**p <0,001**). An examination of these curves shows that according to the Chelex extraction method detection the threshold of DNA is lower due to the higher concentration of DNA in these samples. It may be noted that time spent during the isolation of DNA with Chelex method is two times shorter than that of organic extractionhave worked with deposited samples in differents substracts[8],[12],[13],[27],[30]. In their studies in most of the samples used the most efficient method was the Chelex one. Our results are consistent with the results obtained from the study of Kubat et., al in the all the locus. The comparison of the results obtained from Jakovski Z et al., do not show a significant change in alelles with high frequency other than in locus D18S51 [15]. From the results taken for other samples where the gene hTERT was discovered it is noted that this genetic marker is very stable even in cases where DNA was greatly dammaged. This result is supported on the fact that this marker is discovered in other samples whereas other markers have failed to be discovered the set AmpFlSTR Identifiler so the samples 1, 2, 3. This results are gained from other authors [1].Buttler et al., and Timken et al. who have anayzed the DNA genetic markers, by stimulating the DNA damaging in different percentages of DNase I enzymes. They have achieved trusful results that the genetic marker of the type hTERT of Quantifiler Kit which is used for the determination of DNA quantity has shown high stability to the dammage, but the STR fragments are very stable to further analyses [2]-[6],[28].

6. Conclusion

The genetic marker hTERT which is used to determine the DNA profile, human or not, is done through two different samples: saliva and blood samples with epithelial cells which have been under the environmental conditions. Even though the samples have been in different levels of damage of DNA molecules, this marker has shown high stability to environmental factors.Based on the excellent results in this study we can say that the use of Quantifiler® Human DNA Quantification Kit increases the accuracy and eficasity in the discovery of human DNA presence and the determination of its amount wich makes this gene a primary role compared with other markers used for the same purposes. it is important to know that which of the genetic markers of the AmpFlSTR Identifier Kit reagents can withstand the environmental conditions where our samples are taken and how stable are they.

7. Acknowledgment

To perform and report this study no financial support from any companies or from a national or international funding source was received.

References

[1] Anderson, H., Nilsson, M., Budowle, B., Lundberg, H., & Allen M. 2006: Nuclear and mitochondrial DNA quantification of various materials. Forensic Science International 164, pp. 56.64.
[2] Buttler, M. J. 2011: Forensic DNA Typing: Methodology. Elsevier Publishers, USA.
[3] Buttler, M. J. 2010: Foundamentals of Forensic DNA Typing (3th ed). Elsevier Publishers, USA.
[4] Butler, M. J. 2005: Forensic DNA typing (2nd ed). Elsevier Publishers, USA.
[5] Butler, M.J. 2007: Short tandem repeat typing technologies used in human identity testing. Techniques 43: Suns, doi 10.2144/000112582.
[6] Buttler, M. J. 2006: Genetics and Genomics of Core Short Tandem Repeat Loci Used in Human Identity Testing. J. Forensic Sci, Vol. 51. No. 2, pp. 253.265.
[7] Buckleton, J., Triggs, M. Ch., & Walsh, J. S. 2005: Forensic DNA Evidence Interpretation, CRC Press, USA.
[8] Davoren, J., Vanek, D., Konjhodzic, R., Crews, J., Huffine, E., and Parsons, J. Th. 2007: Highly Effective DNA Extraction Method for Nuclear Short Tandem Repeat Testing of Skeletal Remains from Mass Graves. Croat Med J. August, 48(4): pp. 478485.
[9] Dixon, L. A., Dobbins, A. E., Pulker, H. K., Butler, J.

M., Vallone, P. M., Coble, M. D., Parson, W., Berger, B., Grubwieser, P., Mogensen, H. S., Morling, N., Nielsen, K., Sanchez, J. J., Petkovski, E., Carracedo, A., Sanchez.Diz, P., Ramos.Luis, E., Brion, M., Irwin, J. A., Just, R. S., Loreille, O., Parsons, T. J., Syndercombe.Court, D., Schmitter, H., Stradmann.Bellinghausen, B., Bender K., Gill, P. 2006: Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise. Forensic Science International, 164, pp. 33-44.

[10] Fung, W. K., &HuY, Q. 2008: Statistical DNA Forensics. UK. John Wiley & Sons Publishers. UK.

[11] Goodwin, W., Linacre, A., &Hadi, S. 2011: An Introduction to Forensic Genetics, John Wiley & Sons Publishers. UK.

[12] Hoff-Olsen, P., Mevag, B., Staalstrom, E., Hovde, B., Egeland, Th., Olaisen, B. 1999: Extraction of DNA from decomposed human tissue, an evaluation of five extraction methods for short tandem repeat typing. Forensic Science International, 105, pp. 171183.

[13] Hudlow, W. R., Chong, M. D., Swango, K. L., Timken, M. D., &Buoncristiani, I. 2008: A quadruplex real-time qPCR assay for the simultaneous assessment of total human DNA, human male DNA, DNA degradation and the presence of PCR inhibitors in forensic samples: A diagnostic tool for STR typing. Forensic Science International: Genetics, volume 2, Issue 2, pp. 108-125.

[14] Jacquie, T. K., & Lyndsey, B. 2008: Essentials of Nucleic Acid Analysis. LGC, Teddington, Middlesex, RSC Publishing. UK.

[15] Jakovski, Z.,Nikolova, K.,Furac, I.,Masic, M.,Janeska, B.,Kubat, M.2006: AlleleFrequencies for 15 STR loci in a population from the Republic of Macedonia.IntJLegal Med. Jan; 120 (1): 53 - 5. Epub 2005 Aug 9.

[16] Jeran, N., Hava, D., Ivanovi, V., Rudan, P. 2007: Genetic Diversity of 15 STR Loci A Population of Montenegro. Coll. Antropol. 31, 3, pp. 847-852.

[17] Keckarevic, D., and Pavicevic, D. S. 2009: University Belgrad, Faculty of Biology: Serbian Population Data. Personal communication. Kiely, F. T. 2006: Forensic Evidence: Science and the Criminal Law (2th ed). CRC Press. USA

[18] Kobilinsky, L., Levine, L., & Margolis-Nunno, H. 2007: Forensic DNA Analysis, chapter 4. Chelsea House Publishers. USA.

[19] Kubat, M., Skavic, J., Behluli, I., Nuraj, B., Bekteshi, T., Behluli, M., Klaric, I. M., Pericic, M. 2004: Population genetics of the 15 AmpFlSTRIdentifiler loci in Kosovo Albanians. Int J Legal Med. Apr; 118(2): 115-8. Epub 2004 Jan 23.

[20] President's DNA Initiative. 2006: DNA Analyst Training-Laboratory Training Manual User Guide, Protocols. Bloodstain Indication: Kastle-Meyer Test; Section 2.15, pp. 1-2.

[21] President'sDNA Initiative.2006: DNA Analyst Training. Laboratory TrainingManual User Guide, Protocols. Chelex®100 Non.Differential Extraction; Section 35pp. 1.5.

[22] President'sDNAInitiative.2006: DNAAnalystTraining.LaboratoryTrainingManual User Guide, Protocols. Chelex®100 Differential Extraction; Section 3.06, pp.1.5.

[23] President'sDNAInitiative.2006: DNA AnalystTraining.LaboratoryTraining Manual User Guide, Protocols. Quantifier Quantization F

[24] President'sDNAInitiative.2006: DNA Analyst Training. LaboratoryTrainingManual User Guide, Protocols. PCR Amplification and Electrophoresis of STRs; Section 4.01, 5.02, 6.01.

[25] President'sDNA Initiative. 2006: DNA Analyst Training. LaboratoryTrainingManual User Guide, Protocols. PCR Interpretation; Section 6.02, pp. 1.7.

[26] Semikhodskii, A. 2007: Dealing with DNA Evidence, chapters 1, 2, and 3. Taylor & Francis library. Publishers, USA.

[27] Thompson, J., Kibler, J., and Kupferschmid, Th. 2009: Comparing Five ForensicDNA Extraction Methods. Sorenson Forensics, Salt Lake City, UT 84115, Poster presentation. USA.

[28] Timken, M., Swango, K., Orrego, C., and Buoncristiani, M. 2005: A Duplex Real. Time qPCR Assay for the Quantification of Human Nuclear and Mitochondrial DNA in Forensic Samples: Implications for Quantifying DNA in Degraded Samples. *J Forensic Sci*, Sept. Vol. 50, No. 5. Paper ID JFS2004423.

[29] Vallone, P. M., Hill, C. R., & Butler, J. M. 2008: Demonstration of rapid multiplex PCR amplification involving 16 genetic loci. Forensic Science International, pp. 42. 45.

[30] Viltrop, T., Krjutskov, K., Palta, P., Metspalu A. 2010: Comparison of DNA extraction methods for multiplex polymerase chain reaction. Analytical Biochemistry 398 (2010) pp. 260-262.

Author Profile

Dr. Mimoza Canga is laureate at the University Sapienza,Roma- Italy. PhD University of Tirana. Currently works in the University of Vlora, Department of Public Health, Vlora-Albania.

Professor Vito Antonio Malagnino is laureate in the Faculty of the Medicine with excellent grades. Is specialized in odontostomatology University of Roma- Sapienza, Italy. He is the chief of the endodontics in Sapienza- University.