Application Note

Assessment of Y chromosome degradation level using the Investigator® Quantiplex® Pro RGQ Kit

Tomasz Kupiec, Milosz Janula, Andrzej Doniec

Section of Forensic Genetics, Institute of Forensic Research, Westerplatte 9, 31-033 Kraków, Poland

Introduction

As the importance of DNA profiling in forensic casework has increased, so too has the need for quantifying trace or degraded DNA samples. The latest DNA quantification kits not only offer an estimate of the total amount of amplifiable human autosomal DNA to ensure optimal amplification of target DNA for subsequent analysis, but also offer an estimate of the degree of DNA degradation in a sample. The ratio of the two autosomal targets, short (S) and long (L), provides a Degradation Index (DI), which is useful in predicting the quality of the DNA profiles prior to short tandem repeat (STR) typing.

Bones are a valuable source of DNA in forensic investigations, but are often exposed to unfavorable environmental factors that could increase the DNA degradation rate. Although the benefits of using autosomal STR DNA analysis in criminal investigations have been well documented over the past decade, its drawback becomes very evident when analyzing old or highly compromised samples, which often exhibit low levels of DNA or degraded DNA [1]. This arises because the short autosomal STR loci amplify and produce callable alleles, but the longer loci fail to amplify due to sample degradation, resulting in partial or no STR profiles [2].

Further improvements in quantification kits have led to the addition of a Y-chromosome target to some quantitative PCR (qPCR) assays. This allows simultaneous determination of the amount of male DNA and the total amount of human DNA in a sample. This quantification is highly crucial when analyzing forensic casework or sexual assault samples containing a very small amount of male DNA in a high female DNA background. By measuring the male component of a mixed sample, a more accurate determination of the amount of sample required for successful STR amplification is achieved. In addition, it allows for well-informed decision making regarding downstream STR analysis (autosomal or Y-STR).

Y-STR typing is more sensitive and can be implemented in conjunction with autosomal STRs to strengthen the forensic evidence, when profiling from challenging biological evidence [3]. The fact that Y-STR analysis focuses solely on short tandem repeats existing along the Y chromosome and provides information about a sample's male DNA composition, female contributions in evidentiary samples can be virtually eliminated. Here we present our analysis of the QIAGEN® Investigator Quantiplex Pro RGQ Kit, that not only allows measurement of autosomal DNA degradation, but also Y chromosome degradation, and discuss applications of the quantification kit in the analysis of casework samples extracted from bone, prior to autosomal STR and Y-STR typing.

Materials and methods

Sample preparation

Degraded DNA samples were prepared using human genomic DNA standard (DNA standard M1 included in the kit) at concentrations of 10 ng/µl and 20 ng/µl. Samples were degraded with DNase I (Sigma Aldrich) at a concentration of 0.02 U/µl and incubated for 10 and 20 min each (Table 1, n=4). Additionally, 11 DNA casework samples were extracted from bone tissues collected from skeletons at different stages of decomposition for up to 50 years after death.

Table 1. Parameters of digestion model and qPCR results obtained for artificially degraded samples

			Autosomal (ng/µl)				Y chromosome (ng/µl)			
No.	**DNA quantity (ng/µl)**	**Time of digestion (min)**	**Small**	**Small SD**	**Large**	**Large SD**	**Small**	**Small SD**	**Large**	**Large SD**
1	10	20	0.3003		0.0102		0.3016		0.0069	
			0.2584	0.0212	0.0092	0.0006	0.3034	0.0262	0.0062	0.0006
			0.2527		0.0087		0.2470		0.0054	
2	10	20	0.8809		0.0484		0.9008		0.0225	
			0.8349	0.0350	0.0429	0.0055	0.8943	0.0453	0.0255	0.0014
			0.7952		0.0350		0.8016		0.0223	
3	10	10	0.1148		0.0036		0.1540		0.0040	
			0.1112	0.0043	0.0031	0.0003	0.1618	0.0082	0.0038	0.0008
			0.1216		0.0038		0.1739		0.0055	
4	20	10	0.3584		0.0099		0.4277		0.0089	
			0.3586	0.0068	0.0103	0.0009	0.3711	0.0234	0.0096	0.0004
			0.3730		0.0120		0.4066		0.0098	

Quantitative PCR

QIAGEN DNA standard M1 was used for the preparation of a standard curve in each experiment. Per reaction, 2 µl of DNA sample was quantified using the Investigator Quantiplex Pro RGQ Kit on the Rotor-Gene® Q according to the manufacturer's instructions. The collected data was analyzed using the Q-Rex Software v1.0 and the corresponding Absolute Quantification HID Plug-in, and exported to a dedicated external program, Quantification Assay Data Handling Tool v3.0. The DI was determined for each sample.

STR analysis

To compare the sample DI for autosomal DNA and Y chromosome, STR amplification and analysis were performed on all bone samples (n=11) as well as on the artificially degraded samples. Samples were amplified using the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific) and the Yfiler™ Plus PCR Amplification Kit (Thermo Fisher Scientific). Based on the qPCR data, 1 ng DNA was included in each PCR reaction and amplified on a GeneAmp® PCR System 9700 (Thermo Fisher Scientific) for 29 cycles. A 3500 Genetic Analyzer (Thermo Fisher Scientific) with a 36 cm capillary array and POP-4™ polymer platform was used for the separation and detection of the PCR products. Data analysis was performed with the GeneMapper™ ID-X Software v1.4 (Thermo Fisher Scientific). STR amplification was not performed for two casework samples, due to the limited amount of DNA available (Table 2).

Table 2. qPCR results obtained for casework samples, samples 10 and 11 were not amplified due to insufficient DNA concentration

	Autosomal (ng/µl)		Y chromosome (ng/µl)	
No.	**Small**	**Large**	**Small**	**Large**
1	0.1928	0.0096	0.1500	0.0075
2	0.0071	0.0006	0.0077	0.0003
3	1.3639	0.0293	1.2089	0.0140
4	1.1356	0.0333	1.0657	0.0155
5	0.1414	0.0143	0.1069	0.0085
6	0.0555	0.0026	0.0568	0.0018
7	0.0939	0.0060	0.1003	0.0038
8	0.0519	0.0084	0.0403	0.0103
9	0.2522	0.0188	0.2392	0.0120
10	0.0009	undetermined	0.0015	undetermined
11	0.0026	undetermined	0.0022	undetermined

Results and discussion

A total of 9 casework samples were tested for their STR profile quality (analysis of two bone samples was not performed due to insufficient quantity of DNA). Figure 1 A–B shows the difference in relation to the two markers, autosomal DNA and Y chromosome, when the same samples were evaluated for their degradation index and percentage of identifiable loci. STR marker analysis resulted in the identification of 71–100% positive autosomal loci and 8–60% positive Y-chromosome loci. In terms of degradation, 8 out of 9 casework samples indicated moderate to severe degradation of autosomal DNA and Y chromosome (DI >10). Degradation was confirmed by data analysis using the QIAGEN Quantification Assay Data Handling Tool v3.0. The degradation indices were particularly elevated for the Y chromosome when compared to the autosomal DNA.

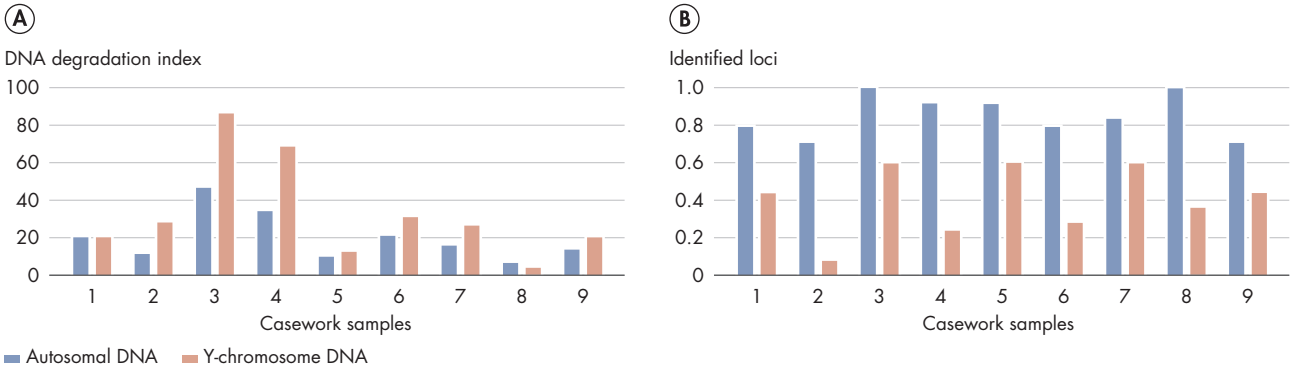

Figure 1. Comparison of DNA degradation index A **and percentage of identified loci** B **for autosomal DNA and Y chromosome, based on data obtained from casework sample analysis.**

The higher Y-chromosome degradation index is a subject of speculation. The fact that the Y-STRs are present on a single chromosome in contrast to the autosomal STRs that are spread over multiple autosomes, is presumably reflected in higher DI. An increase in DI values for autosomal and Y-STR targets directly correlates with a decrease in first-pass STR typing success. This difference in DI scores for autosomal and Y-chromosome DNA can result in a difference in the number of allelic dropouts and therefore, the two indices should be used separately to predict STR analysis success rate. In forensic casework, this information can be useful in optimizing downstream analysis, for instance, a sexual assault sample with a high DI score can be processed for Y-STR typing, in order to increase the success rate from such vital and limited casework samples.

Analysis of enzymatically degraded standard DNA samples showed a correlation between a reduction in DNA quantity and an increase in degradation index. Quantification and amplification results demonstrate that it is necessary to consider both DI and DNA concentration values when defining a downstream strategy for processing degraded samples, in order to maximize allelic recovery and overcome the degradation effect. We suggest increasing the input DNA volume to ensure a more complete profile post-amplification. Moreover, artificially degraded samples were characterized by a higher value of degradation index for the Y-chromosome compared to the autosomal targets (Figure 2). These results were concordant with the observations made for the casework samples, further supporting the idea that autosomal degradation index alone cannot be a measure of STR analysis success for degraded samples and suggesting the need for implementation of Y-chromosome degradation assessment in routine quantification in forensic laboratories.

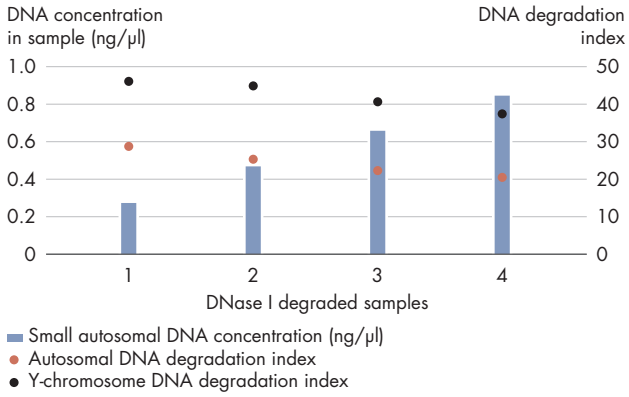

Figure 2. Comparison of DNA degradation index for autosomal DNA and Y chromosome and small DNA concentration based on data obtained from DNase I degradation model comprising the human genomic standard.

Conclusion

Challenging forensic samples, such as bone DNA, progressively degrades over time resulting in decreasing ability to gain a complete STR profile. Similarly, successful identification of casework samples exhibiting very high levels of DNA degradation may be complicated by their low amounts. A real-time quantitative PCR assay using the Investigator Quantiplex Pro RGQ Kit allows simultaneous quantification of total human and male DNA as well as an accurate assessment of autosomal DNA and Y-chromosome degradation. The degradation index reveals if the DNA in the sample is degraded and whether the sample should be processed for downstream STR analysis. Quantification of the longer Y-chromosome amplicon (359 bp) may refer to the long Y-STR sequences (>300 bp), and this information can be valuable in cases where standard autosomal DNA profiling is not informative. Several studies including ours, on different STR analysis success rates for autosomal and Y-chromosome markers in degraded samples, suggest the need for implementation of the Y-chromosome degradation index in routine quantification procedures in forensic laboratories.

References

1 Kayser, M. Forensic use of Y-chromosome DNA: a general overview. Hum Genet. (2017); 136(5): 621–635

2 Hughes-Stamm S.R., Ashton K.J., van Daal A. Assessment of DNA degradation and the genotyping success of highly degraded samples. Int J Legal Med. (2011); 125(3):341-8

3 Alshamali, F. et al. Y chromosome in forensic casework and paternity testing. International Congress Series (2004); 1261: 353–356

Ordering Information

Product	Contents	Cat. no.
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN RotorGene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316
Rotor-Gene Q 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9001660

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