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### Data Article

# Prolonged DNA hydrolysis in water: A study on DNA stability



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### ABSTRACT

This work provides a protocol for the *in vitro* production of damaged DNA samples. In particular, heat-mediated hydrolysis of the samples at 70 °C in ultrapure water was performed in 1.7 mL Eppendorf tubes sealed by Parafilm for 0–36 h. The chemical/physical features of the resulting samples are described. After normalization of the qPCR data, these were compared with those obtained from samples treated for 0–10 h in a previous study.

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## Specifications Table

Subject area	Biochemistry
More specific subject area	Molecular Genetics, Forensic Genetics
Type of data	graphs, figures
How data was acquired	Depurinated DNA samples were analyzed by NanoDrop ND-1000 (Thermo Fisher Scientific Inc.), agarose gel electrophoresis and Caliper LabChip GX (LifeSciences). qPCR was performed by using the The Quantifiler Human DNA Quantification kit (Applied Biosystems, Foster City, CA) in a in a CFX96 Real-Time System (Biorad) thermocycler. The release of the purinic moieties was assessed by MEKC (Micellar Electrokinetics Chromatography) using a MDQ (Beckman, USA) apparatus.
Data format	Raw, analyzed, normalized.
Experimental factors	Hydrolysis at 70 °C in ultrapure water for 0–36 h.
Experimental features	Chemical, physical and molecular characterization of the features of heavily depurinated DNA samples.
Data source location	University of Trieste, Trieste, Italy
Data accessibility	Data are provided within this article.

## Value of data

- Our data on damaged DNA samples are of interest in assessing the performance of new technologies in any field of the Molecular Biology;
- Heat-mediated hydrolysis of the DNA was prolonged up to 36 h (at 70 °C) leading to damaged samples which showed time-dependent features;
- Normalized qPCR data are essential to compare the intra and inter-laboratory outcome of the *in vitro* degradation of DNA samples;
- A reliable and reproducible protocol of heat-mediated hydrolysis of the DNA allows the commercial large-scale production of DNA samples with known (controlled) degree of degradation/modification useful in proficiency testing and in ISO/IEC 17025 validation procedures.

## 1. Data

The data include six figures. Fig. 1 shows the flowchart of the experiment. Fig. 2 reports the % of Guanine and Adenine released from the samples, as assessed by MEKC. Fig. 3 shows the correlation between the average purinic release and the  $OD_{260}/OD_{280}$  ratios of the samples. Fig. 4 describes the EtBr staining of the samples. Fig. 5 shows the traces obtained by the employment of Caliper LabChip GX. Fig. 6 illustrates the  $UV/qPCR$  ratios of the samples.

## 2. Experimental design, materials and methods

### 2.1. DNA preparation, hydrolysis and purification

DNA was extracted from the buffy coat obtained from 500 mL of peripheral blood of a donor. After DNA quantification by NanoDrop, 500  $\mu$ L aliquots of this human DNA, each one containing approximately 30  $\mu$ g, were hydrolyzed in ultrapure water as described elsewhere [1] for 0–36 h, in duplicate. Fig. 1 shows the flowchart of the procedure. After purification by Ultracel 3 K Amicon Ultra columns (Millipore, MA, USA), two samples were recovered: the filtrated sample (FS), which contains the released basic moieties, and the retained sample (RS), which contains the degraded samples.

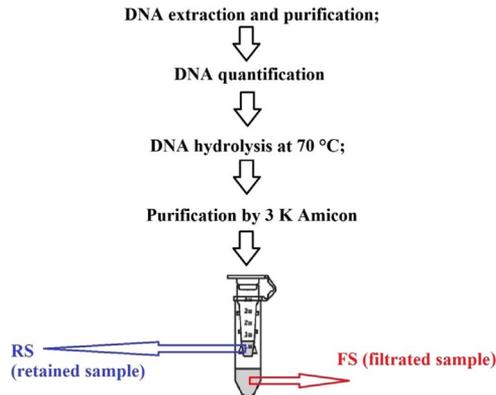


Fig. 1. Flowchart of the experiment.

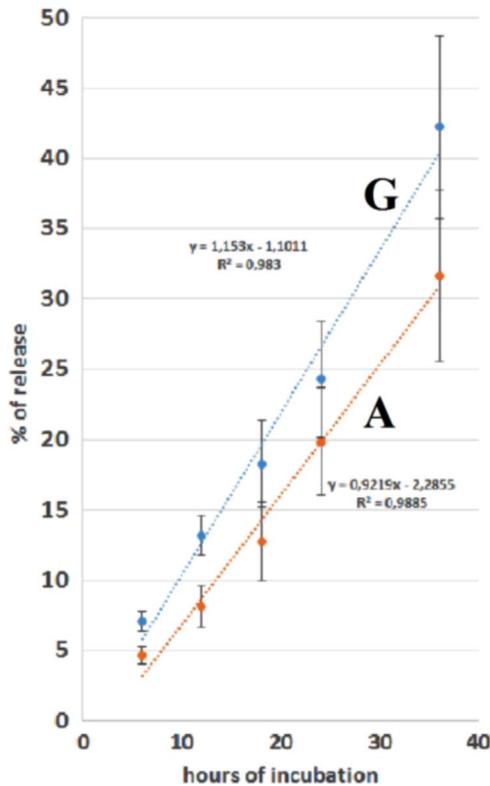
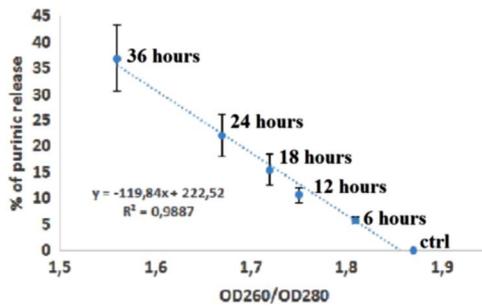


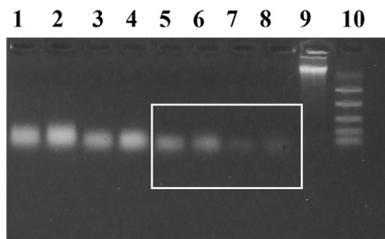
Fig. 2. Average % release ( $\pm$  standard deviation) of Guanine (G) and Adenine (A) from the samples (Y-axis). X-axis: length of incubation.

## 2.2. Evaluation of the purinic release

MEKC (Micellar Electrokinetics Chromatography) was performed by using a MDQ (Beckman, USA) apparatus. The analytical conditions of the MEKC system, as well the quantitative standards (QS)



**Fig. 3.** Correlation between the average OD<sub>260</sub>/OD<sub>280</sub> ratios (x-axis) and the average ( $\pm$  standard deviation) release of the purines (Y-axis) found in the samples.



**Fig. 4.** Agarose gel electrophoresis of samples. Lanes 1–2: 12 h; lanes 3–4: 18 h; lanes 5–6: 24 h; lanes 7–8: 36 h; lane 9: “time 0” control; lane 10: Easy ladder (Bioline). Analysis performed by loading 700 ng (as assessed by NanoDrop) of each replicate.

employed here, are described in ref. [2]. The FSs obtained from the separation through Ultracel 3 K Amicon Ultra columns were dried using a Concentrator 5301 (Eppendorf International, Germany) at 60 °C, redissolved by adding 12.5–25  $\mu$ L of 1% HCl and analyzed in replicate runs.

The concentrations of the released purines were calculated by comparison with the QS, which gave the following calibration data (from 12.5 to 330  $\mu$ M):  $y = 75.28x + 308.6$  ( $r^2 = 0.998$ ) for Guanine (G);  $y = 97.31x + 27.8$  ( $r^2 = 0.999$ ) for Adenine (A). Fig. 2 shows the % of the purinic release from the treated samples calculated as reported in ref. [3], by assuming that the human genome contains about 20.5% of G and about 29.5% of A [4]. Fig. 2 shows the results.

### 2.3. Spectrophotometric assessment of the samples

One microliter aliquots from each RS were quantified using the NanoDrop. Absorbances at 260 nm and 280 nm were determined for each of the samples by  $n = 6$  measurements. The OD<sub>260</sub>/OD<sub>280</sub> ratios were plotted against the average release of the purines (see Fig. 3).

### 2.4. Evaluation of the molecular weight of samples

1.3% agarose gel electrophoresis in TBE buffer (containing EtBr at concentration of 5 ng/mL) [5] was performed (see Fig. 4).

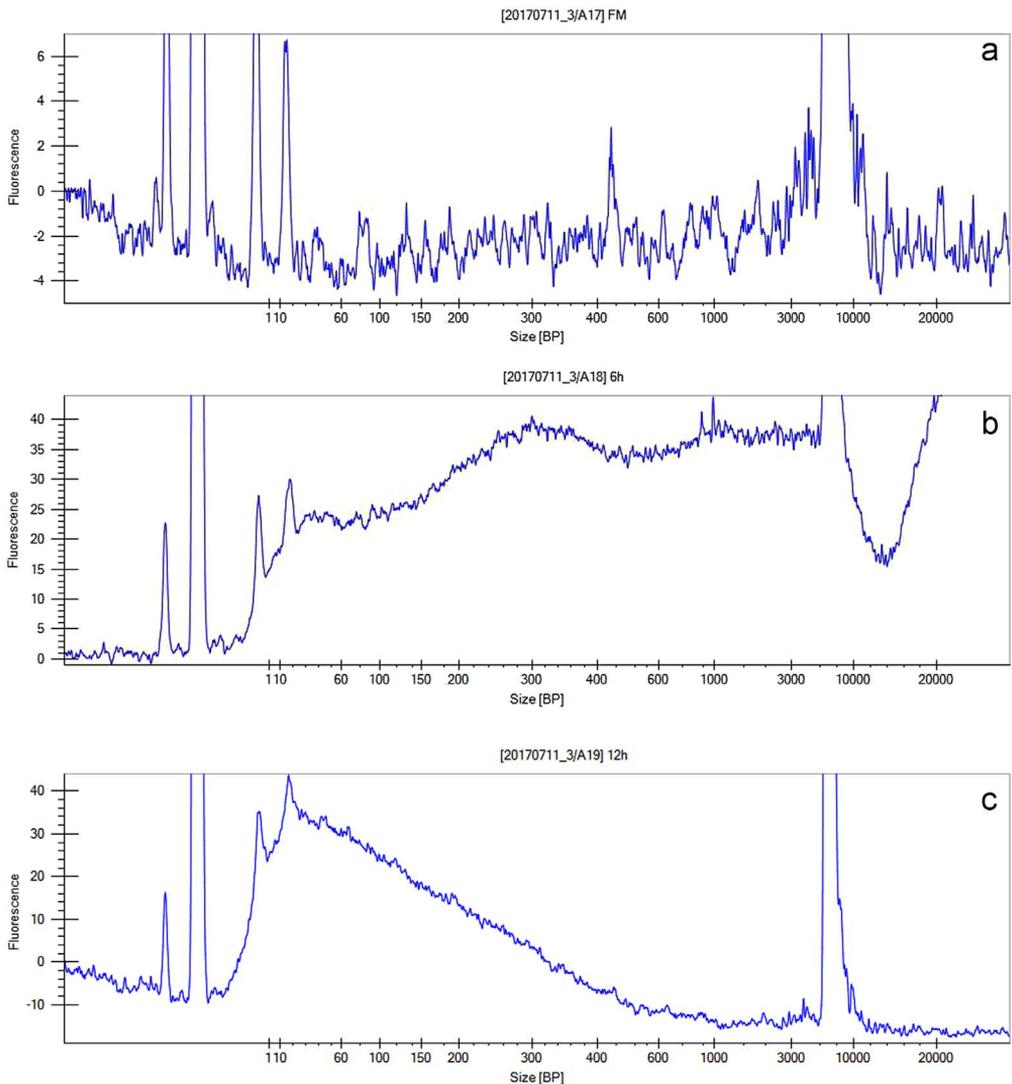
In addition, also the Caliper LabChip GX (LifeSciences) system was used following the manufacturer's recommendations (see Fig. 5).

### 2.5. qPCR analysis of the samples

The Quantifiler Human DNA Quantification kit (Applied Biosystems, Foster City, CA) [6] was employed as described in ref. [1]. The raw data (Cq) were then analyzed by the software (CFX Manager ver. 2.0), which gave the “concentration” of the samples. When no Cq was scored by the

software, the value “0” was assumed as the concentration of the sample [7] in the subsequent calculations.

For each set of samples, the average *UV/qPCR* ratio was calculated as described in ref. [3], following the MIQE Guidelines [8]. In particular, *UV* refers to the concentration of DNA assessed by NanoDrop, while *qPCR* is the “concentration” of the sample estimated by the Quantifiler kit. The data of the present study were then compared with those obtained from a different DNA sample treated for 0, 1, 2.5, 5, 7.5 and 10 h [3] (see Fig. 6). No statistical difference was found between the mean slopes  $\pm$  confidence interval at 95% level of probability.



**Fig. 5.** Analysis by Caliper LabChip GX (LifeSciences). **a:** control sample (“time 0” control); **b, c, d, e** and **f:** the same sample treated for 6, 12, 18, 24 and 36 h, respectively. The x-axis reports the molecular weight of the DNA increasing from left to right. The y-axis shows the relative units of fluorescence.

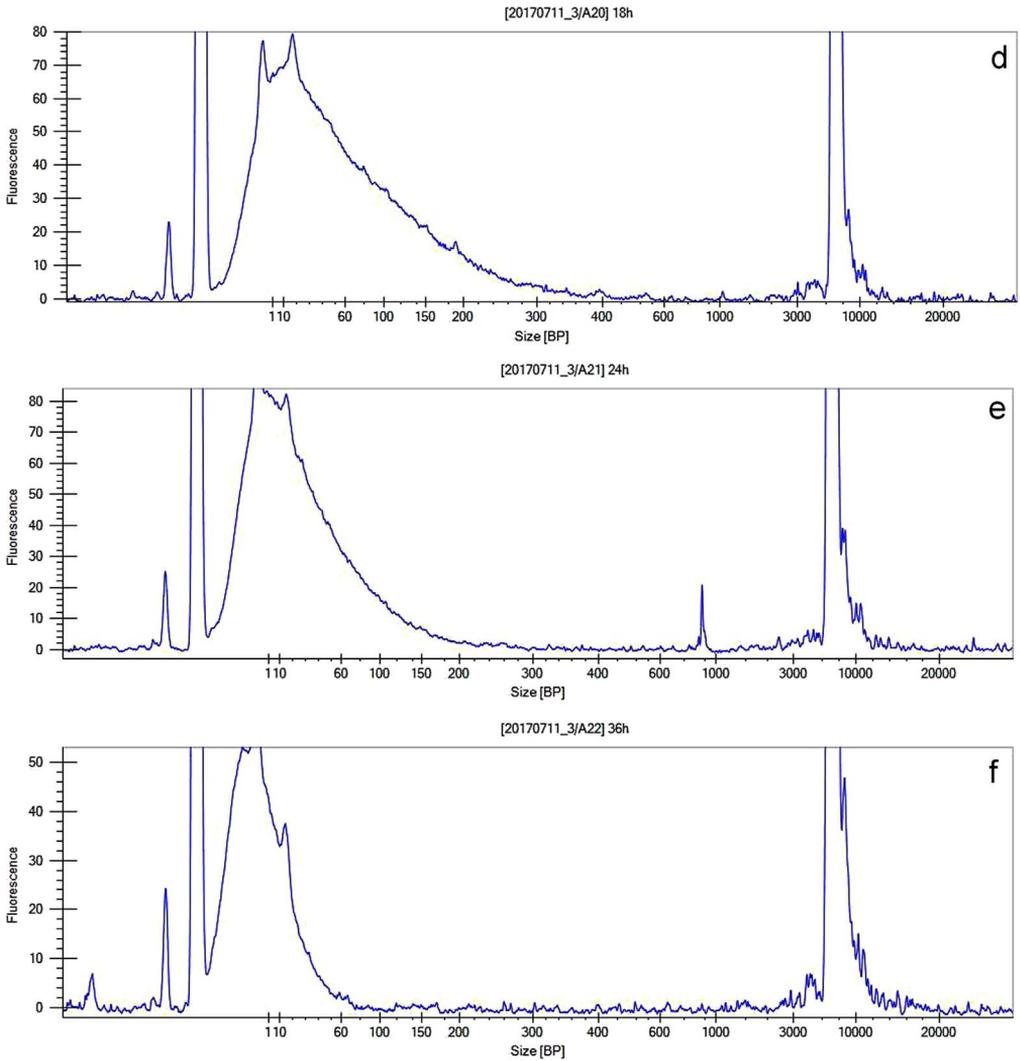


Fig. 5. (continued)

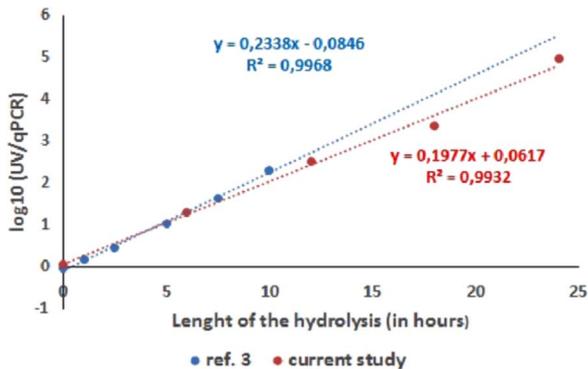


Fig. 6. UV/qPCR ratios of the samples analyzed in this study (red dots) and those of the samples tested in ref. [3] (blue dots). Since the samples incubated for 36 h did not give any Cq, this set of samples were not plotted.

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## Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2018.08.120>.

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