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Research article

Proteinase K challenged by a novel protease

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Abstract

Proteinase K is used in forensic DNA extraction methods for cell lysis and degradation of proteins. Here we compare Proteinase K with a novel protease. We conclude that there is no need to exchange Proteinase K in our methods.

Keywords: Proteinase K; DNA extraction; Protease; Cell lysis; Protein degradation

1. Introduction

DNA extraction is crucial for all downstream applications and greatly influences the chance to obtain a useful DNA profile from a trace sample. Proteinase K is widely used for cell lysis and degradation of proteins in forensic DNA extraction methods.

Here, a novel protease is investigated, referred to as Protease X. It has been developed by researchers at the Royal Institute of Technology in Stockholm, Sweden. The properties of Protease X are similar to Proteinase K with two major differences: Proteinase K has endolytic activity and an optimum at 56 °C whereas Protease X has both endolytic and exolytic activity with an optimum at 37 °C.

2. Materials and methods

Samples were prepared from human muscle tissue from one individual.

Proteinase K and Protease X were tested individually and as a mixture, and with incubation at 37 °C or 56 °C. Different incubation times, ranging from 5 min to 2 h, were tested. Five replicates and one negative control were run for each protease and incubation time.

The first sample set was purified using BioRobot® EZ1 (Qiagen) with the EZ1 DNA Investigator card and kit and with the Tip Dance protocol. The pre-treatment protocol included adding 10 μl protease (10 mg/μl) to the samples. In the mixture 5 μl of each protease was used.

The second sample set was incubated at 56 °C and purified by manual phenol–chloroform purification, followed by Centricon®-100 filtration (Millipore). 15 μl protease (10 mg/μl) were added to these samples, and 7.5 μl of each protease were used in the mixture.

Quantity and quality results were obtained using ABI 7300 Real Time PCR System with Quantifiler™ Human DNA Quantification Kit, GeneAmp® PCR System 9700 with AmpF*l*STR® SGM Plus® PCR Amplification Kit, Applied Biosystems 3130*xl* Genetic Analyzer and GeneMapper™*ID* software, version 3.1 (Applied Biosystems).

Statistical analyses of the results were performed with the software Minitab® 14 (Minitab Inc.).

3. Results and discussion

DNA recovery was significantly higher for samples treated with Proteinase K at 56 °C compared to Protease X or the mixture incubated at 37 °C (Fig. 1). No statistical difference was found between the latter two. The majority of samples generated complete DNA profiles.

When testing at shorter incubation times of 5 and 10 min, phenol–chloroform extraction was used. In Fig. 2, Protease X and the mixture incubated in 56 °C are compared to Proteinase K in 56 °C, Protease X in 37 °C and the mixture in 37 °C.

The quantification results from the second sample set are summarised in Fig. 3. One sample concentration was excluded from the mean value and standard deviation calculations because of a very aberrant result. All samples generated

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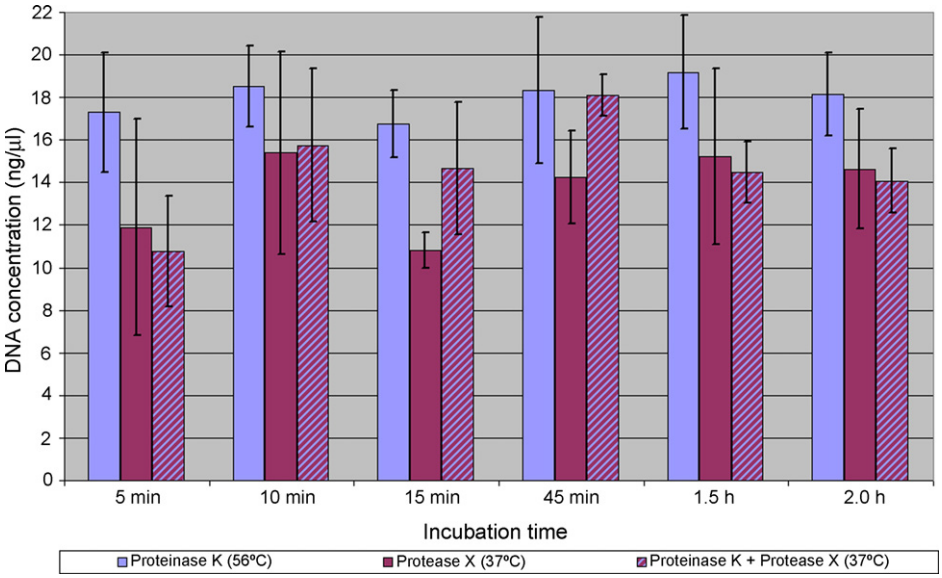

Fig. 1. Diagram over the quantification results from the comparison of Proteinase K, Proteinase X, and a mixture of Proteinase K and Protease X. Each column in the diagram represents the mean DNA concentration of five replicates. The vertical lines indicate the standard deviations for each mean value.

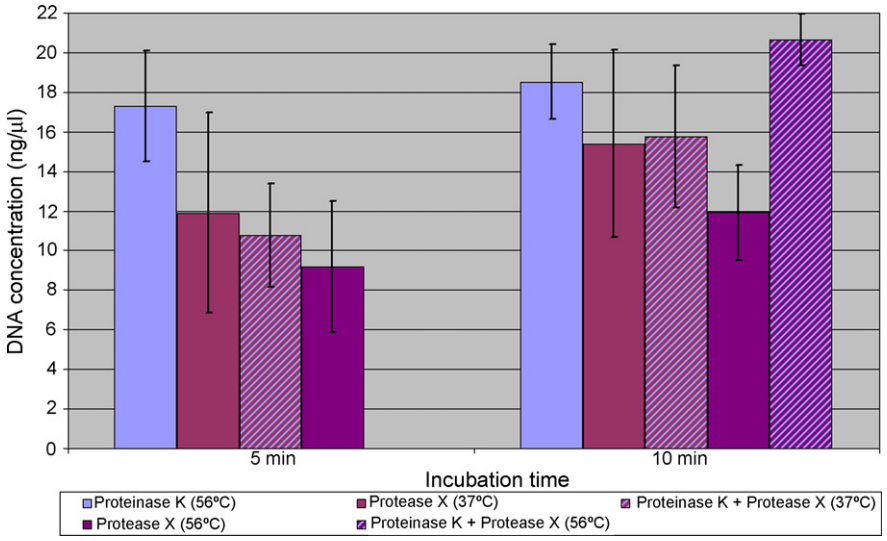

Fig. 2. The diagram shows the quantification results from the shorter incubation times from the Proteinase K and Protease X comparison test, also shown in Fig. 1. Included in this figure are also Protease X and the mixture incubated in 56 °C. Each column in the diagram represents the mean DNA concentration of five replicates. The vertical lines indicate standard deviations for each mean value.

complete DNA profiles. A two-way analysis of variance with Minitab® 14 showed that Proteinase K and the mixture of Proteinase K and Protease X obtained significantly higher DNA yields compared to Protease X, which can be due to the temperature optimum of Proteinase K. The mixture obtained slightly higher DNA concentrations than Proteinase K alone, but the difference was not significant. However, it indicates that there can be synergetic effects between Proteinase K and Protease X. The statistical analysis also showed that the time factor is negligible (data not shown).

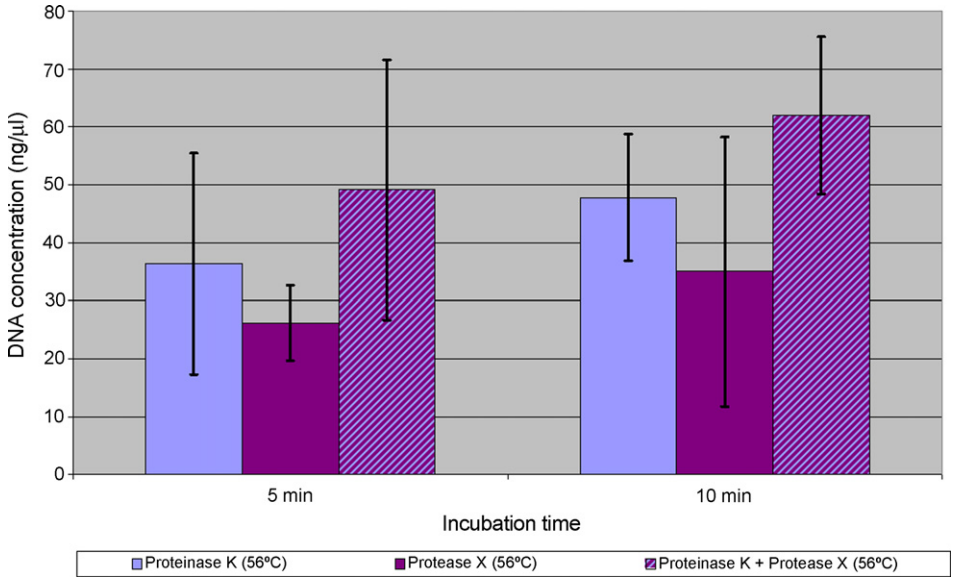

Fig. 3. Quantification results from the comparison test of Proteinase K, Protease X and a mixture of the two at incubation times 5 and 10 min in 56 °C. Each column in the diagram represents the mean value of five replicates, except the column for Protease X for 10 min, which shows the mean value of four replicates. The vertical lines mark the standard deviation.

4. Conclusions

For shorter incubation times at 56 °C, the mixture seems to be more efficient compared to Proteinase K alone. The difference is relatively small and not statistically significant in this test. We conclude that there is no need to exchange or complement Proteinase K in our DNA extraction methods.

Conflict of interest

None.