Next Generation DNase for Ultimate Performance in Molecular Biology Applications

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ABSTRACT

We have developed a new recombinant DNase I which is produced by GRAS recombinant host for mRNA synthesis workflow, by taking into account all quality attributes which are required for such enzyme and application.
In addition the new DNAse I mutant was engineered which is able to hydrolyze DNA at higher ionic strength and features increased thermolability if compared to conventional recombinant DNAse I. This next generation DNAse mutant could be an alternative for DNase I in various mRNA workflow applications where additional features of higher salt tolerance and thermolability are important. In our work we have compared the key enzymatic features of these enzymes and demonstrated various application scenarios which are common for different mRNA workflows.

INTRODUCTION

Deoxyribonucleases are hydrolytic enzymes which break down phosphodiester backbone of DNA. DNAse I family members require divalent cations such as Ca^{2+}, Mg^{2+} or Mn^{2+} for their catalytic activity and they release 5'-phosphates following DNA cleavage. DNase I is the most abundant serum endonuclease which hydrolyzes extracellular DNA into tri or tetra oligonucleotides[1, 2]. Conventional DNase I is the most widely used enzyme in molecular biology applications for DNA removal from RNA samples, in vitro transcription reactions, for DNA-free reagent preparation, nick translation reactions and DNase foot printing studies[3].

MATERIALS AND METHODS

Salt tolerance studies

The reaction was conducted at 37 ° C for 30 min in the presence of herring sperm DNA and sodium chloride was added to different concentrations (0-1M of NaCl). Reaction conditions: 40 mM Tris-Cl pH 8.0, 10 mM $MgCl_2$, 1 mM $CaCl_2$. 1U/µl of each DNase was diluted 250x in a final reaction mixture. The reaction was stopped by addition of 4% perchloric acid in a ratio 1:1, kept on ice for 30 min and centrifuged for 6 min at 14,000xg. Afterwards absorbance at 260 nm was measured. In alternative way plasmid DNA pUC19 was used instead of herring sperm DNA.

Thermolability experiments

DNases have been incubated for 30 min at different temperatures (4-95 ° C) before conducting the DNase assay. The DNase assay was conducted at 37° C for 30 min in the presence of herring sperm DNA. Reaction conditions: 40 mM Tris-Cl pH 8.0, 10 mM $MgCl_2$, 1 mM $CaCl_2$. 1U/µl of each DNase was diluted 250x in a final reaction mixture. In alternative way plasmid DNA pUC19 was used instead of herring sperm DNA.

Single and double stranded DNA hydrolysis assay

Reaction was performed with 30 bp DNA oligonucleotides which were single or double stranded and contained a fluorescent mark. 1U/µl of each DNase was diluted 125x in a final reaction mixture and reaction was conducted in the Varioskan Flash multimode reader at room temperature for 2 hours in the presence of 0.02 µM oligonucleotides.

In vitro transcription reactions

In vitro transcription reactions were performed with two kits according to the instruction manual: MEGAShortscript™ kit and TranscriptAID™ T7 High Yield Transcription kit. After IVT reactions were completed 1U or 5U of different DNases were added and incubated for 15 min at 37° C, then EDTA was added to 15 mM and DNases were heat inactivated by incubating them at 75° C for 10 min. Residual template DNA was measured by using Maxima™ SYBR Green qPCR Master Mix and a pair of specific primers targeting DNA template.

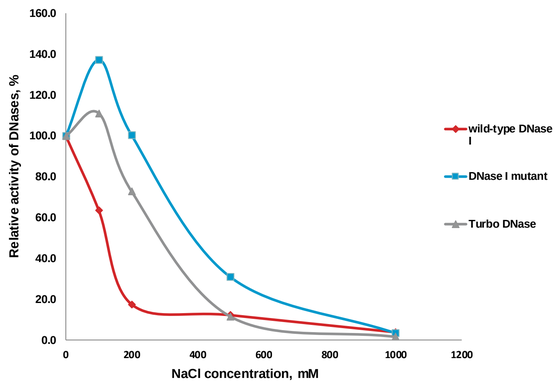
DNA removal in the presence or absence of RNA

DNA hydrolysis reaction was carried out in the presence of 1 µg plasmid DNA, 2U of each DNase and its reaction buffer. DNA hydrolysis was continued for 15 min at 37° C and residual DNA quantity was measured by means of qPCR. When DNA was hydrolyzed in the presence of high concentrations of RNA, 2 µg of *E. Coli* genomic DNA was added to 10 µg of yeast total RNA , 2U of each DNAse and its reaction buffer were supplemented to the final reaction mixture. The hydrolysis reaction was continued for 30 min at 37 ° C . Subsequently, 15 mM EDTA was added and DNases were heat inactivated by incubating them at 75° C for 10 min. Residual template DNA was measured by using Maxima™ SYBR Green qPCR Master Mix and a pair of specific primers targeting DNA template.

For Research Use Only. Not for use in diagnostic procedures.

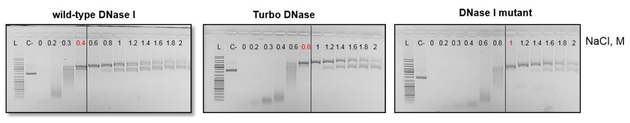
RESULTS

Figure 1. Salt tolerance studies show increased resistance to higher salt concentration of DNase I mutant



The salt tolerance experiments were conducted in the presence of DNase I, Turbo DNase™ and DNase I mutant. The reaction was conducted at 37° C for 30 min in the presence of herring sperm DNA and sodium chloride was added to different concentrations (0-1M of NaCl). Reaction conditions: 40 mM Tris-Cl pH 8.0, 10 mM $MgCl_2$, 1 mM $CaCl_2$.

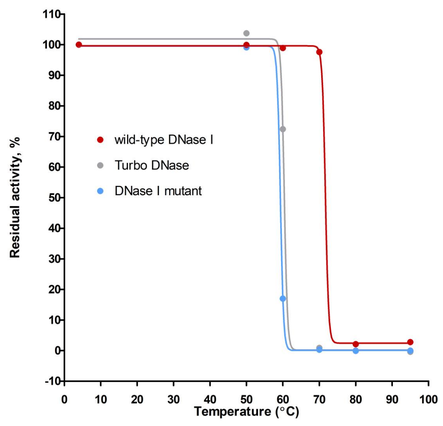
Figure 2. Higher salt tolerance of DNase I mutant was shown by incubation with plasmid DNA



Reaction was performed in the presence of 1 µg of plasmid DNA and samples incubated at 37° C for 10 min. Reaction was stopped by adding 6x DNA Loading Dye and SDS Solution and inactivated by heating at 70° C for 5 min. Samples were run on 1% agarose gels.

Figure 3. DNase I mutant shows higher thermolability as compared to wild-type DNase I

A



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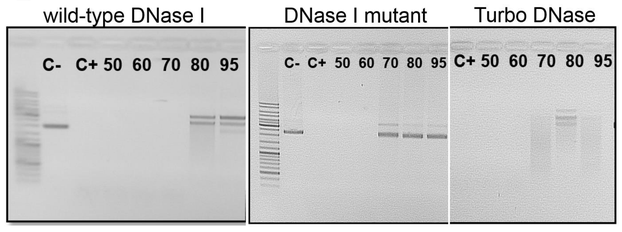


The thermolability experiments were conducted in the presence of DNase I, Turbo DNase™ and DNase I mutant which have been incubated for 30 min at different temperatures (4-95 ° C) before conducting the DNase assay. The DNase assay was conducted at 37° C for 30 min in the presence of herring sperm DNA. Reaction conditions: 40 mM Tris-Cl pH 8.0, 10 mM $MgCl_2$, 1 mM $CaCl_2$.

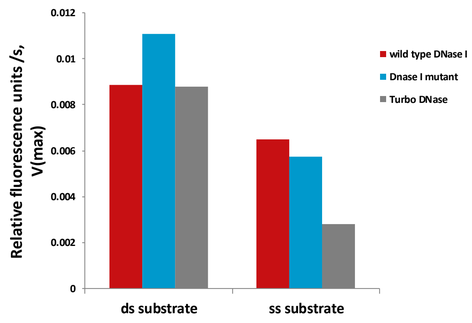
Samples have been incubated for 30 min at different temperatures (4-95 ° C) before conducting the DNase assay. Reaction was performed in the presence of 1 µg of plasmid DNA and samples incubated at 37° C for 10 min. Reaction was stopped by adding 6x DNA Loading Dye and SDS Solution and inactivated by heating at 70° C for 5 min. Samples were run on 1% agarose gels. C-: template DNA without DNase. C+: template DNA with DNase added.

Table 1. T_M50 values of wild-type DNase I, DNase I mutant and Turbo DNase™ as calculated from thermolability studies

Dnase mutants	Substrate: herring DNA or fluorescent DNA oligo
	T_M50, °C
Wild-type DNase I	71 ± 1
DNase I mutant	60 ± 1
Turbo DNase	65 ± 5

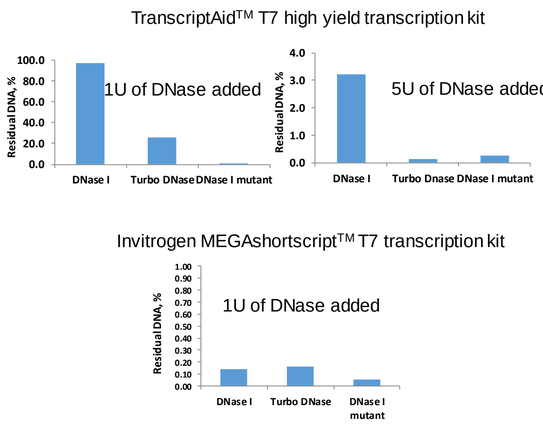
T_M50 values where calculated by approximating thermolability data to sigmoidal function reporting midpoints of DNAse inactivation curves. All datapoints were normalized to untreated samples before DNase assay.

Figure 4. DNase I mutant hydrolyzes single and double stranded DNA substrates at a similar rate as wild-type DNase I



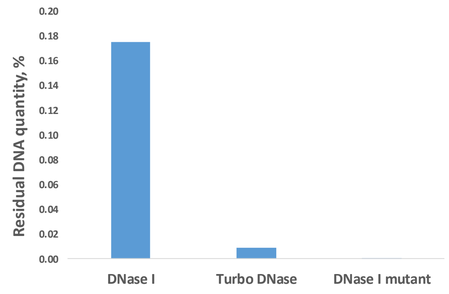
Reaction was performed with single stranded or double stranded fluorescently labeled oligonucleotides. Real-time fluorescence values were recorded for 2 hours and v_{max} values calculated with Varioskan Flash multimode reader .

Figure 5. DNase I mutant outperforms wild-type DNase I in efficient template DNA removal from in vitro transcription reactions



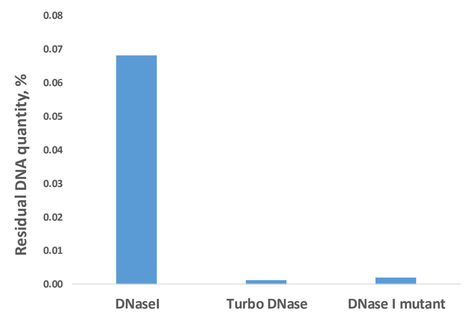
In vitro transcription reactions were performed with two kits: Megashortscript™ T7 transcription kit and TranscriptAid™ T7 high yield transcription kit. After IVT reactions were completed 1U or 5U of different DNases were added and incubated for 15 min at 37° C. Residual template DNA was measured by means of qPCR.

Figure 6. DNase I mutant shows highly efficient removal of plasmid DNA under standard reaction conditions



Reaction was carried out in the presence of 1 µg plasmid DNA, 2U of each DNase and its reaction buffer. DNA hydrolysis was continued for 15 min at 37° C and residual DNA quantity was measured by means of qPCR.

Figure 7. DNAse I mutant efficiently removes genomic DNA from RNA rich samples



2 µg of *E. Coli* genomic DNA was added to 10 µg of yeast total RNA , 2U of each DNAse and its reaction buffer were supplemented to the final mixture.The hydrolysis reaction was continued for 30 min at 37 ° C , DNases were inactivated and residual DNA quantity was measured by means of qPCR.

CONCLUSIONS

(1) Salt tolerance studies showed that the new DNAse I mutant is much more salt tolerant than wild-type DNAse I and has a comparable salt tolerance as Turbo DNase™. Wild-type DNAse I is completely inhibited in the presence of 0.4M NaCl while DNAse I mutant is inhibited in the presence of 1M NaCl.

(2) Thermolability experiments revealed that DNAse I mutant is more thermally labile than wild-type DNAse I and slightly more labile than Turbo DNase™ (T_M50 (wild-type DNase I) = 70° C, T_M50 (DNase I mutant) = 60° C, T_M50 (Turbo DNase) = 65° C).

(3) DNase I mutant hydrolyzes single and double stranded DNA substrates at a rate similar to wild-type DNase I.

(4) DNAse I mutant more efficiently removes DNA template from various in vitro transcription reactions and RNA samples as compared to wild-type DNase I.

REFERENCES

1. Hyone-Myong Eun, in Enzymology Primer for Recombinant DNA Technology, 1996.
2. Surya Pandey, Taro Kawai, in Biological DNA Sensor, 2014.
3. Tatiana Borodina, Marc Sultan et al., in Methods in Enzymology, 2011.

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TRADEMARKS/LICENSING