

A method of removing nucleic acid contamination in reverse transcription
and amplification reactions

The ~~disclosure~~present invention relates to the removal of contaminating DNA from reverse transcription reaction mixtures, hot-start DNA polymerase preparations and hot-start PCR reaction mixtures through the use of a DNase. The ~~disclosure~~invention also relates to the prevention of false positive results in nucleic acid amplification reactions through the use of a DNase, in particular amplification reactions which involve reverse transcription of the target sequence, a hot-start DNA polymerase and/or a barrier hot-start PCR set-up. The ~~disclosure~~invention also relates to an extremely thermolabile DNase suitable for use in such methods.

In a first aspect, the present invention refers to a DNase or an enzymatically active fragment thereof, said DNase having the sequence of SEQ ID No. 1 or a sequence which is at least 60% identical thereto, but wherein the proline residue at position 237 of SEQ ID No 1, or the equivalent proline in other sequences, has been modified, deleted or substituted, wherein said DNase or enzymatically active fragment thereof

(i) is irreversibly inactivated by at least 95% by heating at a temperature of 50°C for 5 mins in a buffer consisting of 25mM TrisHCl, pH 8.5, 5mM $MgCl_2$ and 1mM DTT, and

(ii) cleaves double stranded DNA, but has essentially no detectable activity towards single stranded DNA at concentrations of 0.01 to 0.05 U/ μ l.

In a second aspect, the present invention refers to a DNase or an enzymatically active fragment thereof, wherein said DNase has the sequence of SEQ ID No. 5 or a sequence which is at least 60% identical thereto, but wherein the proline residue at position 214 of SEQ ID No 5, or the equivalent proline in other sequences, has been modified, deleted or substituted, wherein said DNase or enzymatically active fragment thereof

(i) is irreversibly inactivated by at least 95% by heating at a temperature of 50°C for 5 mins in a buffer consisting of 25mM TrisHCl, pH 8.5, 5mM $MgCl_2$ and 1mM DTT, and

(ii) cleaves double stranded DNA, but has essentially no detectable activity towards single stranded DNA at concentrations of 0.01 to 0.05 U/ μ l.

In a third aspect, the present invention refers to a method of removing nucleic acid contamination from a reverse transcription reaction which method comprises use of a DNase as defined above.

In a fourth aspect, the present invention refers to a method of *in vitro* reverse transcription of a target nucleic acid, wherein said method includes a step of treating the reverse transcription reaction mixture with a DNase as defined above.

In a fifth aspect, the present invention refers to a method of hot-start PCR, wherein said reaction is a barrier hot-start PCR set up and/or involves a hot-start DNA polymerase, wherein said method includes a step of treating the hot-start PCR set up/mixture or the hot-start DNA polymerase with a DNase as defined above, prior to commencement of the hot start PCR.

In a sixth aspect, the present invention refers to a nucleic acid molecule encoding the DNase as defined above.

In a seventh aspect, the present invention refers to the use of a DNase as defined above as a decontaminating agent in a method of amplifying of a nucleic acid.

In an eighth aspect, the present invention refers to the use of a DNase as defined above as a decontaminating agent in a method of amplifying of a nucleic acid.

In a ninth aspect, the present invention refers to the use of a DNase as defined above in a method as defined above.

In a tenth aspect, the present invention refers to a method for the isolation and purification of a DNase or a fragment thereof as defined above, said method comprising expressing said DNase or fragment thereof in a suitable host cell, and subsequently separating the DNase from said host cells and/or the media in which said cells have been cultured.

In an eleventh aspect, the present invention refers to a kit or composition for carrying out a method as defined above said kit or composition comprising a DNase as defined above and/or a nucleic acid as defined above; and optionally one or more of the following

- (i) a nucleotide triphosphate;
- (ii) an oligonucleotide primer;
- (iii) a reverse transcription enzyme;
- (iv) a DNA polymerases;
- (v) a DNA ligase; and
- (vi) a restriction enzyme.

Nucleic acid amplification techniques such as polymerase chain reactions (PCR's) are one of the most powerful tools available in biotechnology, allowing preparation of a large number of copies of a target sequence from a sample containing only a small amount of nucleic acid. In the case of PCR, oligonucleotide primers complementary to their respective strands of a double stranded target sequence are added to the reaction mixture containing the target sequence and free nucleotides. Thermal cycling in the presence of a DNA polymerase results in amplification of the sequence between the primers. The ability of the amplified fragments created by the PCR process to act as templates for subsequent PCR cycles results in the rapid production of a considerable quantity of the target sequence. Even a single copy of the target sequence can yield sufficient nucleic acid to allow detection by, e.g. hybridization with a labelled probe or incorporation of a ³²P labelled deoxynucleotide triphosphates into the amplified segment.

Ligase amplification reaction (LAR) also known as ligase chain reaction (LCR), like PCR, uses repetitive cycles and alternating temperature to achieve an exponential increase in the number of copies of the target sequence. In this method, DNA ligase catalyses the joining of two oligonucleotides complementary to adjacent regions of one of the target DNA strands. Two other oligonucleotides complementary to the other strand can also be ligated. After denaturation, the original template strands and the two ligated pairs can act as templates for further hybridisation and ligation.

Strand displacement amplification (SDA) exploits the property of the enzymes involved in DNA excision DNA repair to replace a single nicked strand of DNA in a DNA duplex with a newly synthesised strand. To create a nicked single strand repeatedly an endonuclease restriction enzyme, e.g. HindI or BsoBI, is used which only nicks DNA on one strand of its recognition site when the opposite strand is hemiphosphorothiolated. The primers used in this method contain an appropriate recognition site and dATP α S is used in