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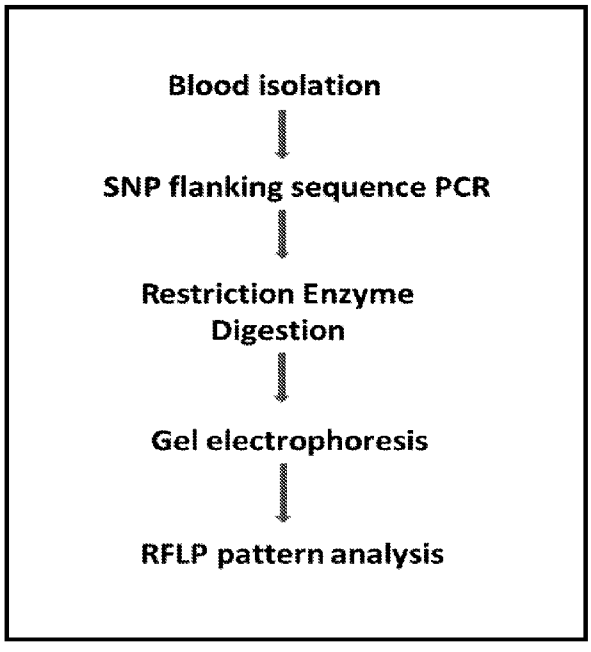
(54) Title: RAPID METHOD FOR GENOTYPING STING VARIANTS IN HUMAN INDIVIDUALS

Figure 1



(57) Abstract: The invention provides a method for determining the genetic polymorphism pattern in the "Stimulator of Interferon Genes" (STING) gene in a subject. The method comprises amplifying, in a sample obtained from a subject, a sequence of genomic DNA comprising a single nucleotide polymorphism (SNP) within the STING gene. The methods also comprises performing restriction fragment length polymorphism (RFLP) pattern analysis on the amplified DNA to determine the genetic polymorphism pattern in the STING gene in the sample. The invention also provides kits for distinguishing and detecting STING gene SNPs (i.e. STING genotyping) and STING protein variants expressed by humans.

[Continued on next page]

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Rapid Method for genotyping STING variants in human individuals

The present invention relates to the "Stimulator of Interferon Genes" (STING) gene, and to single nucleotide polymorphisms (SNPs) in the STING gene, as well as to the STING protein and variants of STING protein resulting from the SNPs. The invention particularly relates to methods and kits for distinguishing and detecting STING gene SNPs (i.e. STING genotyping) and STING protein variants expressed by humans. The present invention also relates to the uses of the methods and kits as a diagnostic tool to assess the suitability of individuals for STING agonist or antagonist therapy, and also to methods of treatment with a STING agonist or antagonist.

STING, also known as transmembrane protein 173, is an adaptor protein that binds cyclic dinucleotides (CDN) or agonist compounds, leading to the activation of IκB kinase (IKK) and TANK-binding kinase (TBK1), which when phosphorylated, activate Nuclear Factor kappa-light-chain-enhancer of activated B cells (NFκB) and interferon regulatory factor 3 (IRF3), respectively. These activated proteins translocate to the nucleus and induce transcription of genes encoding type I interferons (IFN) and inflammatory cytokines to promote an innate immune response against pathogens or neoplasms [1].

Cancer immunotherapy modulates and leverages the host's immune system to treat cancer, and, over the past decade, there have been significant advances in the field of cancer treatment. Numerous approaches have been explored to elicit or augment anticancer innate immunity and/or adaptive immunity. Recently, activation of STING, using STING agonists, has shown great potential to enhance antitumor immunity through the induction of a variety of pro-inflammatory cytokines and chemokines, including type I IFNs. A number of natural and synthetic STING agonists have been discovered and developed, and tested in preclinical models and in the clinic for the immunotherapy of various diseases, such as cancer and infectious diseases. For example, cyclic dinucleotides (CDNs), such as cyclic dimeric guanosine monophosphate (c-di-GMP), cyclic dimeric adenosine monophosphate (c-di-AMP), and cyclic GMP-AMP (cGAMP), are a class of STING agonists that can elicit immune responses.

Conversely, STING antagonists have also become an area of therapeutic interest, as they block overactive STING proteins in various autoimmune diseases. Recently, a

number of companies have developed various small molecule STING antagonists, such as C-176 and H-151.

Analysis of single nucleotide polymorphism (SNP) data from the 1000 Genome Project revealed that there are five major STING haplotypes that carry specific SNPs, resulting in variations in the STING polypeptide sequence. The major STING variant is R232, characterised by the presence of an arginine at the 232 position. This is the most prevalent human STING haplotype with an allele frequency of 57.6%, and is therefore considered to be the wild type. STING variant HAQ (R71H-G230A-R293Q), has an allele frequency of 20.4% and contains three SNPs. This HAQ variant has a histidine at the 71 position in place of an arginine, an alanine at the 230 position in place of glycine, and a glutamine at the 293 position in place of arginine. STING variant H232 (R232H) is characterised by a histidine at the 232 position in place of arginine, with an allele frequency of 13.7%. STING haplotype AQ (G230A-R293Q), contains an alanine at the 230 position in place of glycine, and a glutamine at the 293 position in place of arginine, with an allele frequency of 5.2%. Finally, STING variant Q (R293Q), contains a glutamine at the 293 position in place of arginine, and has an allele frequency of 1.5% [2].

Each of these STING protein variants differs in the production of IFNs upon activation. For example, the natural STING ligand cGAMP is a weaker activator of the H232 variant. Therefore, it will be appreciated that the therapeutic application of a STING agonist or an antagonist will be better directed if the identification of the STING alleles present in a specific patient who is about to undergo STING agonist or antagonist treatment is known.

Currently, there are three methods used for STING genotyping, namely Sanger sequencing, Taqman-PCR, and FRET hybridisation probe based melt curve analysis. Sanger sequencing requires highly advanced instrumentation, and is therefore a very expensive method for detecting SNPs. In addition, the laboratory protocols and interpretation of the results require significant dedicated hands-on operator time, resulting in a long turnaround time of approximately 37 hours. TaqMan-PCR and FRET hybridisation melt curve analysis are less time-consuming compared to Sanger sequencing, with a total turnaround time of approximately 3 to 5 hours. However, the initial cost of the instrumentation required for each of these methods is very high and is

not affordable by many laboratories. In addition, the FRET-based method also utilises two sets of probes, adding to the overall cost of this technique.

Accordingly, there remains a significant need in the art for an improved method for STING genotyping, i.e. for detecting the presence or absence of a genetic polymorphism pattern in the STING gene of a subject. In particular, there is a need for a method that provides a fast, cheap and convenient way by which the STING genotype of a patient can be determined. Accordingly, this will lead to improved diagnostic tools for assessing the suitability of STING agonist/antagonist therapy for patients suffering from diseases, such as cancer, chronic inflammation associated with fibrosis or autoinflammatory disease, or infectious diseases, such as those caused by viruses, bacteria, parasites and fungi.

Thus, according to a first aspect of the invention, there is provided a method for determining the genetic polymorphism pattern in the "Stimulator of Interferon Genes" (STING) gene in a subject, the method comprising:

- amplifying, in a sample obtained from a subject, a sequence of genomic DNA comprising a single nucleotide polymorphism (SNP) within the STING gene; and
- performing restriction fragment length polymorphism (RFLP) pattern analysis on the amplified DNA to determine the genetic polymorphism pattern in the STING gene in the sample.

According to a second aspect, there is provided an apparatus for determining the genetic polymorphism pattern in the "Stimulator of Interferon Genes" (STING) gene in a subject, the apparatus comprising:

- means for amplifying, in a sample obtained from a subject, a sequence of genomic DNA comprising a single nucleotide polymorphism (SNP) within the STING gene; and
- means for performing restriction fragment length polymorphism (RFLP) pattern analysis on the amplified DNA to determine the genetic polymorphism pattern in the STING gene in the sample.

According to a third aspect, there is provided a method for determining the efficacy of a treatment of a subject with a "Stimulator of Interferon Genes" (STING) agonist or a STING antagonist, the method comprising:

- determining the genetic polymorphism pattern in the Stimulator of Interferon Genes (STING) gene in a sample obtained from a subject using the method according to the first aspect or the apparatus of the second aspect; and
- determining the suitability of the subject for STING agonist or antagonist therapy based on the genetic polymorphism pattern in the STING gene.

In a fourth aspect of the invention, there is provided a diagnostic or prognostic tool for assessing the suitability of a subject for "Stimulator of Interferon Genes" (STING) agonist or antagonist therapy, comprising:

- determining the genetic polymorphism pattern in the Stimulator of Interferon Genes (STING) gene in a sample obtained from a subject using the method of the first aspect or the apparatus of the second aspect; and
- determining the suitability of the subject for STING agonist or antagonist therapy based on the genetic polymorphism pattern.

According to a fifth aspect, there is provided a method of treating a subject suffering from a disease characterised by underactive "Stimulator of Interferon Genes" (STING) protein or underexpressed STING gene, the method comprising:

- amplifying, in a sample obtained from a subject, a sequence of genomic DNA comprising a single nucleotide polymorphism (SNP) within the STING gene;
- performing restriction fragment length polymorphism (RFLP) pattern analysis on the amplified DNA to determine the genetic polymorphism pattern in the STING gene in the sample; and
- administering, or having administered, to the subject, a STING agonist, thereby treating the disease.

According to a sixth aspect, there is provided a method of treating a subject suffering from a disease characterised by overactive "Stimulator of Interferon Genes" (STING) protein or overexpressed STING gene, the method comprising:

- amplifying, in a sample obtained from a subject, a sequence of genomic DNA comprising a single nucleotide polymorphism (SNP) within the STING gene;
- performing restriction fragment length polymorphism (RFLP) pattern analysis on the amplified DNA to determine the genetic polymorphism pattern in the STING gene in the sample; and
- administering, or having administered, to the subject, a STING antagonist, thereby treating the disease.

Advantageously, the methods and apparatus of the first and second aspects of the invention enable the very quick and simple determination of the presence or absence of certain SNPs in the STING gene and, accordingly, the specific STING alleles carried by subjects using tissue or blood samples which can yield very accurate results within only a few hours. The methods and apparatus are inexpensive and have minimal requirements in terms of investment in instrumentation. In addition, genotyping can be easily carried out by simple visualisation of restriction fragments by gel electrophoresis, for which no specific software and little technical expertise is required. Considering the number of patients being tested per day per institution, the methods of the invention are both cost-effective and simple to perform. Therefore, the methods provide a very fast and cheap way by which the STING genotype of a patient can be determined. As such, the suitability of a patient for treatment with a STING agonist or antagonist can be readily determined, as per the third and fourth aspects of the invention. This means that the correct and most effective STING agonist or antagonist is administered to the subject in subsequent therapy, according to the fifth and sixth aspects of the invention.

The STING gene is located on chromosome 5 at position 31.2, and one embodiment of the coding sequence of STING is known and readily accessible at www.ncbi.nlm.nih.gov. The polymorphism pattern in the STING gene may comprise at least one polymorphism or polymorphic region in the STING gene. The polymorphism pattern in the STING gene comprises more than one, and preferably two, polymorphisms or polymorphic regions in the STING gene. The polymorphism pattern in the STING gene comprises three or more polymorphisms or polymorphic regions in the STING gene.

The term “polymorphism” can refer to the co-existence, within a population, of more than one form of a gene or portion thereof (e.g. an allelic variant). A portion of a gene of which there are at least two different forms, i.e. two different nucleotide sequences, is referred to as a “polymorphic region of a gene”. A specific genetic sequence at a polymorphic region of a gene is known as an allele.

The term “allele” can refer to the different sequence variants found at different polymorphic sites in DNA obtained from a subject. For example, each polymorphic region of the STING gene has at least two different alleles. The sequence variants of

each allele may be single or multiple base changes, including without limitation insertions, deletions, or substitutions, or may be a variable number of sequence repeats. Thus, a polymorphic region may be a single nucleotide (i.e. a single nucleotide polymorphism, or SNP), the identity of which differs in different alleles. In other embodiments, a polymorphic region can also be several nucleotides long.

The term "genotype", "allelic pattern" or "polymorphism pattern" can refer to the identity of an allele or alleles at one or more polymorphic sites. A genotype, allelic pattern or polymorphism pattern may consist of either a homozygous or heterozygous state at one or more polymorphic sites.

There are currently known to be five major STING gene haplotypes that carry specific SNPs, resulting in variations in the STING polypeptide sequence.

The term "haplotype" can refer to a set of genetic or DNA variants, such as SNPs, that are usually inherited together, and these sets of haplotypes are usually located on one chromosome. The alleles making up a haplotype can be located in different places on the chromosome but that are inherited together.

Hence, the polymorphism pattern in the STING gene may comprise at least one SNP, at least two SNPs, at least three SNPs, or at least four SNPs in the STING gene.

In a first embodiment, a STING variant is R232. In this embodiment, an individual has an arginine at position 232. This is the most prevalent human STING variant with an allele frequency of 57.6%, and is therefore considered to be the wild-type.

The SNP which encodes R232 is known as rs1131769. The alleles of STING rs1131769 SNP may be identified as (i) a G-allele, and (ii) an A-allele. Therefore, the method may comprise detecting or determining the G-allele or the A-allele of the STING rs1131769 SNP.

The nucleotide sequence encoding one embodiment of human STING variant R232 is referred to herein as SEQ ID No: 1, as follows:

GTTCATTTTTCACTCCTCCCTCCTAGGTCACACTTTTCAGAAAAAGAATCTGCATCCTGGAAACCAGAAG
AAAAATATGAGACGGGGAATCATCGTGTGATGTGTGTGCTGCCTTTGGCTGAGTGTGTGGAGTCCTGCTC

AGGTGTTAGGTACAGTGTGTTTGATCGTGGTGGCTTGAGGGGAACCCGCTGTTCAGAGCTGTGACTGCGG
CTGCACTCAGAGAAGCTGCCCTTGGCTGCTCGTAGCGCCGGGCCTTCTCTCCTCGTCATCATCCAGAGCA
GCCAGTGTCCGGGAGGCAGAAGATGCCCCACTCCAGCCTGCATCCATCCATCCCGTGTCCCAGGGGTCAC
GGGGCCCAGAAGGCAGCCTTGGTTCTGCTGAGTGCCTGCCTGGTGACCCTTTGGGGGCTAGGAGAGCCAC
CAGAGCACACTCTCCGGTACCTGGTGCTCCACCTAGCCTCCCTGCAGCTGGGACTGCTGTTAAACGGGGT
CTGCAGCCTGGCTGAGGAGCTGCGCCACATCCACTCCAGGTACCGGGGCAGCTACTGGAGGACTGTGCGG
GCCTGCCTGGGCTGCCCCCTCCGCCGTGGGGCCCTGTTGCTGCTGTCCATCTATTTCTACTACTCCCTCC
CAAATGCGGTCGGCCCGCCCTTCACTTGGATGCTTGCCCTCCTGGGCCTCTCGCAGGCACTGAACATCCT
CCTGGGCCTCAAGGGCCTGGCCCCAGCTGAGATCTCTGCAGTGTGTGAAAAAGGGAATTTCAACGTGGCC
CATGGGCTGGCATGGTCATATTACATCGGATATCTGCGGCTGATCCTGCCAGAGCTCCAGGCCCGGATTC
GAACTTACAATCAGCATTACAACAACCTGCTACGGGGTGCAGTGAGCCAGCGGCTGTATATTCTCCTCCC
ATTGGACTGTGGGGTGCCTGATAACCTGAGTATGGCTGACCCCAACATTCGCTTCCTGGATAAACTGCCC
CAGCAGACCGGTGACCGTGCTGGCATCAAGGATCGGGTTTACAGCAACAGCATCTATGAGCTTCTGGAGA
ACGGGCAGCGGGCGGGCACCTGTGTCCTGGAGTACGCCACCCCCTTGCAGACTTTGTTTGCCATGTCACA
ATACAGTCAAGCTGGCTTTAGCCGGGAGGATAGGCTTGAGCAGGCCAAACTCTTCTGCCGGACACTTGAG
GACATCCTGGCAGATGCCCCTGAGTCTCAGAACAACTGCCGCCTCATTGCCTACCAGGAACCTGCAGATG
ACAGCAGCTTCTCGCTGTCCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGGAAAAGGAAGAGGTTACTGT
GGGCAGCTTGAAGACCTCAGCGGTGCCCAGTACCTCCACGATGTCCCAAGAGCCTGAGCTCCTCATCAGT
GGAATGGAAAAGCCCCTCCCTCTCCGCACGGATTTCTCTTGAGACCCAGGGTCACCAGGCCAGAGCCTCC
AGTGGTCTCCAAGCCTCTGGACTGGGGGCTCTCTTCAGTGGCTGAATGTCCAGCAGAGCTATTTCCTTCC
ACAGGGGGCCTTGCAGGGAAGGGTCCAGGACTTGACATCTTAAGATGCGTCTTGTCCCCTTGGGCCAGTC
ATTTCCCCTCTCTGAGCCTCGGTGTCTTCAACCTGTGAAATGGGATCATAATCACTGCCTTACCTCCCTC
ACGGTTGTTGTGAGGACTGAGTGTGTGGAAGTTTTTCATAAACTTTGGATGCTAGTGTACTTAGGGGGTG
TGCCAGGTGTCTTTCATGGGGCCTTCCAGACCCACTCCCCACCCTTCTCCCCTTCCTTTGCCCGGGGACG
CCGAACTCTCTCAATGGTATCAACAGGCTCCTTCGCCCTCTGGCTCCTGGTCATGTTCCATTATTGGGGA
GCCCCAGCAGAAGAATGGAGAGGAGGAGGAGGCTGAGTTTGGGGTATTGAATCCCCCGGCTCCCACCCTG
CAGCATCAAGGTTGCTATGGACTCTCCTGCCGGGCAACTCTTGCGTAATCATGACTATCTCTAGGATTCT
GGCACCACTTCCTTCCCTGGCCCCTTAAGCCTAGCTGTGTATCGGCACCCCCACCCCACTAGAGTACTCC
CTCTCACTTGCGGTTTCCTTATACTCCACCCCTTTCTCAACGGTCCTTTTTTAAAGCACATCTCAGATTA

[SEQ ID No: 1]

The amino acid sequence of one embodiment of human STING variant R232 (bold, underlined) is referred to herein as SEQ ID No: 2, as follows:

MPHSSLHPSIPCPRGHGAQKAALVLLSACLVTLWGLGEPPEHTLRYLVLHLASLQLGLLLNGVCSLAEEL
RHIHSRYRGSYWRTVRACLGCPLRRGALLLLSIYFYYSLPNAVGPPFTWMLALLGLSQALNILLGLKGLA
PAEISAVCEKGNFNVAHGLAWSYYIGYLRLILPELQARIRTYNQHYNNLLRGAVSQRLYILLPLDCGVPD
NLSMADPNIRFLDKLPQQTGDRAGIKDRVYSNSIYELLENGQRAGTCVLEYATPLQTLFAMSQYSQAGFS
REDRLEQAKLFCRTLEDILADAPESQNNCRLIAYQEPADDSSFSLSQEVLRHLRQEEKEEVTVGSLKTSA
VPSTSTMSQEPELLISGMEKPLPLRTDFS

[SEQ ID No: 2]

Therefore, preferably the polymorphism pattern in the STING gene, which is determined, corresponds to STING variant R232. Preferably, the STING variant R232 comprises or consists of the amino acid sequence substantially as set out in SEQ ID No:2 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:1.

In a second embodiment, a STING variant is R71H-G230A-R293Q or "HAQ", in which three SNPs are present. This human STING variant has an allele frequency of 20.4%. In this embodiment, a histidine replaces an arginine at position 71; an alanine replaces a glycine at position 230; and a glutamine replaces an arginine at position 293.

The SNP which encodes R71H is known as rs11554776. The alleles of STING rs11554776 SNP may be identified as (i) a G-allele, and (ii) an A-allele. Therefore, the method may comprise detecting or determining the G-allele or the A-allele of the STING rs11554776 SNP.

The SNP which encodes G230A is known as rs78233829. The alleles of STING rs78233829 SNP may be identified as (i) a G-allele, and (ii) a C-allele. Therefore, the method may comprise detecting or determining the G-allele or the C-allele of the STING rs78233829 SNP.

The SNP which encodes R293Q is known as rs7380824. The alleles of STING rs7380824 SNP may be identified as (i) a G-allele, and (ii) an A-allele. Therefore, the method may comprise detecting or determining the G-allele or the A-allele of the STING rs7380824 SNP.

The nucleotide sequence encoding one embodiment of human STING variant HAQ is referred to herein as SEQ ID No: 3, as follows:

GTTCATTTTTCACTCCTCCCTCCTAGGTCACACTTTTCAGAAAAAGAATCTGCATCCTGGAAACCAGAAG
AAAAATATGAGACGGGGAATCATCGTGTGATGTGTGTGCTGCCTTTGGCTGAGTGTGTGGAGTCCTGCTC
AGGTGTTAGGTACAGTGTGTTTGATCGTGGTGGCTTGAGGGGAACCCGCTGTTCAGAGCTGTGACTGCGG
CTGCACTCAGAGAAGCTGCCCTTGGCTGCTCGTAGCGCCGGGCCTTCTCTCCTCGTCATCATCCAGAGCA
GCCAGTGTCCGGGAGGCAGAAGATGCCCCACTCCAGCCTGCATCCATCCATCCCGTGTCCCAGGGGTCAC
GGGGCCCAGAAGGCAGCCTTGGTTCTGCTGAGTGCCTGCCTGGTGACCCTTTGGGGGCTAGGAGAGCCAC
CAGAGCACACTCTCCGGTACCTGGTGCTCCACCTAGCCTCCCTGCAGCTGGGACTGCTGTTAAACGGGGT
CTGCAGCCTGGCTGAGGAGCTGCACCACATCCACTCCAGGTACCGGGGCAGCTACTGGAGGACTGTGCGG

GCCTGCCTGGGCTGCCCCCTCCGCCGTGGGGCCCTGTTGCTGCTGTCCATCTATTTCTACTACTCCCTCC
CAAATGCGGTCGGCCCGCCCTTCACTTGGATGCTTGCCCTCCTGGGCCTCTCGCAGGCACTGAACATCCT
CCTGGGCCTCAAGGGCCTGGCCCCAGCTGAGATCTCTGCAGTGTGTGAAAAAGGGAATTTCAACGTGGCC
CATGGGCTGGCATGGTCATATTACATCGGATATCTGCGGCTGATCCTGCCAGAGCTCCAGGCCCGGATTC
GAACTTACAATCAGCATTACAACAACCTGCTACGGGGTGCAGTGAGCCAGCGGCTGTATATTCTCCTCCC
ATTGGACTGTGGGGTGCCTGATAACCTGAGTATGGCTGACCCCAACATTCGCTTCCTGGATAAACTGCCC
CAGCAGACCGCTGACCGTGCTGGCATCAAGGATCGGGTTTACAGCAACAGCATCTATGAGCTTCTGGAGA
ACGGGCAGCGGGCGGGCACCTGTGTCCTGGAGTACGCCACCCCCTTGCAGACTTTGTTTGCCATGTCACA
ATACAGTCAAGCTGGCTTTAGCCGGGAGGATAGGCTTGAGCAGGCCAAACTCTTCTGCCAGACACTTGAG
GACATCCTGGCAGATGCCCCTGAGTCTCAGAACAACTGCCGCCTCATTGCCTACCAGGAACCTGCAGATG
ACAGCAGCTTCTCGCTGTCCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGGAAAAGGAAGAGGTTACTGT
GGGCAGCTTGAAGACCTCAGCGGTGCCCAGTACCTCCACGATGTCCCAAGAGCCTGAGCTCCTCATCAGT
GGAATGGAAAAGCCCCTCCCTCTCCGCACGGATTTCTCTTGAGACCCAGGGTCACCAGGCCAGAGCCTCC
AGTGGTCTCCAAGCCTCTGGACTGGGGGCTCTCTTCAGTGGCTGAATGTCCAGCAGAGCTATTTCCTTCC
ACAGGGGGCCTTGCAGGGAAGGGTCCAGGACTTGACATCTTAAGATGCGTCTTGTCCCCTTGGGCCAGTC
ATTTCCCCTCTCTGAGCCTCGGTGTCTTCAACCTGTGAAATGGGATCATAATCACTGCCTTACCTCCCTC
ACGGTTGTTGTGAGGACTGAGTGTGTGGAAGTTTTTCATAAACTTTGGATGCTAGTGTACTTAGGGGGTG
TGCCAGGTGTCTTTCATGGGGCCTTCCAGACCCACTCCCCACCCTTCTCCCCTTCCTTTGCCCGGGGACG
CCGAACTCTCTCAATGGTATCAACAGGCTCCTTCGCCCTCTGGCTCCTGGTCATGTTCCATTATTGGGGA
GCCCCAGCAGAAGAATGGAGAGGAGGAGGAGGCTGAGTTTGGGGTATTGAATCCCCCGGCTCCCACCCTG
CAGCATCAAGGTTGCTATGGACTCTCCTGCCGGGCAACTCTTGCGTAATCATGACTATCTCTAGGATTCT
GGCACCACTTCCTTCCCTGGCCCCTTAAGCCTAGCTGTGTATCGGCACCCCCACCCCACTAGAGTACTCC
CTCTCACTTGCGGTTTCCTTATACTCCACCCCTTTCTCAACGGTCCTTTTTTAAAGCACATCTCAGATTA

[SEQ ID No: 3]

The amino acid sequence of one embodiment of human STING variant HAQ (bold, underlined) is referred to herein as SEQ ID No: 4, as follows:

MPHSSLHPSIPCPRGHGAQKAALVLLSACLVTLWGLGEPPEHTLRYLVLHLASLQLGLLLNGVCSLAEEL
HIHSRYRGSYWRTVRACLGCPLRRGALLLLSIYFYYSLPNAVGPPFTWMLALLGLSQALNILLGLKGLA
PAEISAVCEKGNFNVAHGLAWSYYIGYLRLILPELQARIRTYNQHYNNLLRGAVSQRLYILLPLDCGVPD
NLSMADPNIRFLDKLPQQT**A**D**R**AGIKDRVYSNSIYELLENGQRAGTCVLEYATPLQTLFAMSQYSQAGFS
REDRLEQAKLFC**S**TLEDILADAPESQNNCRLIAYQEPADDSSFSLSQEVLRHLRQEEKEEVTVGSLKTSA
VPSTSTMSQEPELLISGMEKPLPLRTDFS

[SEQ ID No: 4]

Therefore, preferably the polymorphism pattern in the STING gene, which is determined, comprises a SNP which corresponds to STING variant R71H-G230A-R293Q or “HAQ”. Preferably, the STING variant R71H-G230A-R293Q or “HAQ” comprises or consists of the amino acid sequence substantially as set out in SEQ ID

No:4 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:3.

In a third embodiment, a STING variant is R232H or "H232", in which one SNP is present. This human STING variant has an allele frequency of 13.7%. In this embodiment, a histidine replaces an arginine at position 232.

The nucleotide sequence encoding one embodiment of human STING variant H232 is referred to herein as SEQ ID No: 5, as follows:

GTTCATTTTTCACTCCTCCCTCCTAGGTCACACTTTTCAGAAAAAGAATCTGCATCCTGGAAACCAGAAGAAAAATAT
GAGACGGGGAATCATCGTGTGATGTGTGTGCTGCCTTTGGCTGAGTGTGTGGAGTCCTGCTCAGGTGTTAGGTACAGT
GTGTTTGATCGTGGTGGCTTGAGGGGAACCCGCTGTTCAGAGCTGTGACTGCGGCTGCACTCAGAGAAGCTGCCCTTG
GCTGCTCGTAGCGCCGGGCCTTCTCTCCTCGTCATCATCCAGAGCAGCCAGTGTCCGGGAGGCAGAAGATGCCCCACT
CCAGCCTGCATCCATCCATCCCGTGTCCCAGGGGTCACGGGGCCCAGAAGGCAGCCTTGGTTCTGCTGAGTGCCTGCC
TGGTGACCCTTTGGGGGCTAGGAGAGCCACCAGAGCACACTCTCCGGTACCTGGTGCTCCACCTAGCCTCCCTGCAGC
TGGGACTGCTGTTAAACGGGGTCTGCAGCCTGGCTGAGGAGCTGCGCCACATCCACTCCAGGTACCGGGGCAGCTACT
GGAGGACTGTGCGGGCCTGCCTGGGCTGCCCCCTCCGCCGTGGGGCCCTGTTGCTGCTGTCCATCTATTTCTACTACT
CCCTCCCAAATGCGGTCGGCCCGCCCTTCACTTGGATGCTTGCCCTCCTGGGCCTCTCGCAGGCACTGAACATCCTCC
TGGGCCTCAAGGGCCTGGCCCCAGCTGAGATCTCTGCAGTGTGTGAAAAAGGGAATTTCAACGTGGCCCATGGGCTGG
CATGGTCATATTACATCGGATATCTGCGGCTGATCCTGCCAGAGCTCCAGGCCCGGATTCGAACTTACAATCAGCATT
ACAACAACCTGCTACGGGGTGCAGTGAGCCAGCGGCTGTATATTCTCCTCCCATTGGACTGTGGGGTGCCTGATAACC
TGAGTATGGCTGACCCCAACATTCGCTTCCTGGATAAACTGCCCCAGCAGACCGGTGACCATGCTGGCATCAAGGATC
GGGTTTACAGCAACAGCATCTATGAGCTTCTGGAGAACGGGCAGCGGGCGGGCACCTGTGTCCTGGAGTACGCCACCC
CCTTGCAGACTTTGTTTGCCATGTCACAATACAGTCAAGCTGGCTTTAGCCGGGAGGATAGGCTTGAGCAGGCCAAAC
TCTTCTGCCGGACACTTGAGGACATCCTGGCAGATGCCCCTGAGTCTCAGAACAACTGCCGCCTCATTGCCTACCAGG
AACCTGCAGATGACAGCAGCTTCTCGCTGTCCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGGAAAAGGAAGAGGTTA
CTGTGGGCAGCTTGAAGACCTCAGCGGTGCCCAGTACCTCCACGATGTCCCAAGAGCCTGAGCTCCTCATCAGTGGAA
TGGAAAAGCCCCTCCCTCTCCGCACGGATTTCTCTTGAGACCCAGGGTCACCAGGCCAGAGCCTCCAGTGGTCTCCAA
GCCTCTGGACTGGGGGCTCTCTTCAGTGGCTGAATGTCCAGCAGAGCTATTTCCTTCCACAGGGGGCCTTGCAGGGAA
GGGTCCAGGACTTGACATCTTAAGATGCGTCTTGTCCCCTTGGGCCAGTCATTTCCCCTCTCTGAGCCTCGGTGTCTT
CAACCTGTGAAATGGGATCATAATCACTGCCTTACCTCCCTCACGGTTGTTGTGAGGACTGAGTGTGTGGAAGTTTTT
CATAAACTTTGGATGCTAGTGTACTTAGGGGGTGTGCCAGGTGTCTTTCATGGGGCCTTCCAGACCCACTCCCCACCC
TTCTCCCCTTCCTTTGCCCGGGGACGCCGAACTCTCTCAATGGTATCAACAGGCTCCTTCGCCCTCTGGCTCCTGGTC
ATGTTCCATTATTGGGGAGCCCCAGCAGAAGAATGGAGAGGAGGAGGAGGCTGAGTTTGGGGTATTGAATCCCCCGGC
TCCCACCCTGCAGCATCAAGGTTGCTATGGACTCTCCTGCCGGGCAACTCTTGCGTAATCATGACTATCTCTAGGATT
CTGGCACCACTTCCTTCCCTGGCCCCTTAAGCCTAGCTGTGTATCGGCACCCCCACCCCACTAGAGTACTCCCTCTCA
CTTGCGGTTTCCTTATACTCCACCCCTTTCTCAACGGTCCTTTTTTAAAGCACATCTCAGATTA

[SEQ ID NO: 5]

The amino acid sequence of one embodiment of human STING variant H232 (bold, underlined) is referred to herein as SEQ ID No: 6, as follows:

MPHSSLHPSIPCPRGHGAQKAALVLLSACLVTLWGLGEPPEHTLRYLVLHLASLQLGLLLNGVCSLAEEL
RHIHSRYRGSYWRTVRACLGCPLRRGALLLLSIYFYYSLPNAVGPPFTWMLALLGLSQALNILLGLKGLA
PAEISAVCEKGNFNVAHGLAWSYYIGYLRLILPELQARIRTYNQHYNNLLRGAVSQRLYILLPLDCGVPD
NLSMADPNIRFLDKLPQQTGDRAGIKDRVYSNSIYELLENGQRAGTCVLEYATPLQTLFAMSQYSQAGFS
REDRLEQAKLFCRTLEDILADAPESQNNCRLIAYQEPADDSSFSLSQEVLRHLRQEEKEEVTVGSLKTSA
VPSTSTMSQEPELLISGMEKPLPLRTDFS

[SEQ ID NO: 6]

Therefore, preferably the polymorphism pattern in the STING gene, which is determined, comprises a SNP which corresponds to STING variant R232H or "H232". Preferably, the STING variant R232H or "H232" comprises or consists of the amino acid sequence substantially as set out in SEQ ID No:6 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:5.

In a fourth embodiment, a STING variant is G230A-R293Q or "AQ", in which two SNPs are present. This human STING variant has an allele frequency of 5.2%. In this embodiment, an alanine replaces a glycine at position 230; and a glutamine replaces an arginine a SNP at position 293.

The SNP which encodes G230A is known as rs78233829. The alleles of STING rs78233829 SNP may be identified as (i) a G-allele, and (ii) a C-allele. Therefore, the method may comprise detecting or determining the G-allele or the C-allele of the STING rs78233829 SNP.

The SNP which encodes R293Q is known as rs7380824. The alleles of STING rs7380824 SNP may be identified as (i) a G-allele, and (ii) an A-allele. Therefore, the method may comprise detecting or determining the G-allele or the A-allele of the STING rs7380824 SNP.

The nucleotide sequence encoding one embodiment of human STING variant AQ is referred to herein as SEQ ID No: 7, as follows:

GTTCATTTTTCACTCCTCCCTCCTAGGTCACACTTTTCAGAAAAAGAATCTGCATCCTGGAAACCAGAAG
AAAAATATGAGACGGGGAATCATCGTGTGATGTGTGTGCTGCCTTTGGCTGAGTGTGTGGAGTCCTGCTC

AGGTGTTAGGTACAGTGTGTTTGATCGTGGTGGCTTGAGGGGAACCCGCTGTTCAGAGCTGTGACTGCGG
CTGCACTCAGAGAAGCTGCCCTTGGCTGCTCGTAGCGCCGGGCCTTCTCTCCTCGTCATCATCCAGAGCA
GCCAGTGTCCGGGAGGCAGAAGATGCCCCACTCCAGCCTGCATCCATCCATCCCGTGTCCCAGGGGTCAC
GGGGCCCAGAAGGCAGCCTTGGTTCTGCTGAGTGCCTGCCTGGTGACCCTTTGGGGGCTAGGAGAGCCAC
CAGAGCACACTCTCCGGTACCTGGTGCTCCACCTAGCCTCCCTGCAGCTGGGACTGCTGTTAAACGGGGT
CTGCAGCCTGGCTGAGGAGCTGCCCCACATCCACTCCAGGTACCGGGGCAGCTACTGGAGGACTGTGCGG
GCCTGCCTGGGCTGCCCCCTCCGCCGTGGGGCCCTGTTGCTGCTGTCCATCTATTTCTACTACTCCCTCC
CAAATGCGGTCGGCCCGCCCTTCACTTGGATGCTTGCCCTCCTGGGCCTCTCGCAGGCACTGAACATCCT
CCTGGGCCTCAAGGGCCTGGCCCCAGCTGAGATCTCTGCAGTGTGTGAAAAAGGGAATTTCAACGTGGCC
CATGGGCTGGCATGGTCATATTACATCGGATATCTGCGGCTGATCCTGCCAGAGCTCCAGGCCCGGATTC
GAACTTACAATCAGCATTACAACAACCTGCTACGGGGTGCAGTGAGCCAGCGGCTGTATATTCTCCTCCC
ATTGGACTGTGGGGTGCCTGATAACCTGAGTATGGCTGACCCCAACATTCGCTTCCTGGATAAACTGCCC
CAGCAGACCGCTGACCCTGCTGGCATCAAGGATCGGGTTTACAGCAACAGCATCTATGAGCTTCTGGAGA
ACGGGCAGCGGGCGGGCACCTGTGTCCTGGAGTACGCCACCCCCTTGCAGACTTTGTTTGCCATGTCACA
ATACAGTCAAGCTGGCTTTAGCCGGGAGGATAGGCTTGAGCAGGCCAAACTCTTCTGCCAGACACTTGAG
GACATCCTGGCAGATGCCCCTGAGTCTCAGAACAACTGCCGCCTCATTGCCTACCAGGAACCTGCAGATG
ACAGCAGCTTCTCGCTGTCCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGGAAAAGGAAGAGGTTACTGT
GGGCAGCTTGAAGACCTCAGCGGTGCCCAGTACCTCCACGATGTCCCAAGAGCCTGAGCTCCTCATCAGT
GGAATGGAAAAGCCCCTCCCTCTCCGCACGGATTTCTCTTGAGACCCAGGGTCACCAGGCCAGAGCCTCC
AGTGGTCTCCAAGCCTCTGGACTGGGGGCTCTCTTCAGTGGCTGAATGTCCAGCAGAGCTATTTCCTTCC
ACAGGGGGCCTTGCAGGGAAGGGTCCAGGACTTGACATCTTAAGATGCGTCTTGTCCCCTTGGGCCAGTC
ATTTCCCCTCTCTGAGCCTCGGTGTCTTCAACCTGTGAAATGGGATCATAATCACTGCCTTACCTCCCTC
ACGGTTGTTGTGAGGACTGAGTGTGTGGAAGTTTTTCATAAACTTTGGATGCTAGTGTACTTAGGGGGTG
TGCCAGGTGTCTTTCATGGGGCCTTCCAGACCCACTCCCCACCCTTCTCCCCTTCCTTTGCCCGGGGACG
CCGAACTCTCTCAATGGTATCAACAGGCTCCTTCGCCCTCTGGCTCCTGGTCATGTTCCATTATTGGGGA
GCCCCAGCAGAAGAATGGAGAGGAGGAGGAGGCTGAGTTTGGGGTATTGAATCCCCCGGCTCCCACCCTG
CAGCATCAAGGTTGCTATGGACTCTCCTGCCGGGCAACTCTTGCGTAATCATGACTATCTCTAGGATTCT
GGCACCACTTCCTTCCCTGGCCCCTTAAGCCTAGCTGTGTATCGGCACCCCCACCCCACTAGAGTACTCC
CTCTCACTTGCGGTTTCCTTATACTCCACCCCTTTCTCAACGGTCCTTTTTTAAAGCACATCTCAGATTA

[SEQ ID No: 7]

The amino acid sequence of one embodiment of human STING variant AQ (bold, underlined) is referred to herein as SEQ ID No: 8, as follows:

MPHSSLHPSIPCPRGHGAQKAALVLLSACLVTLWGLGEPPEHTLRYLVLHLASLQLGLLLNGVCSLAEEL
RHIHSRYRGSYWRTVRACLGCPLRRGALLLLSIYFYYSLPNAVGPPFTWMLALLGLSQALNILLGLKGLA
PAEISAVCEKGNFNVAHGLAWSYYIGYLRLILPELQARIRTYNQHYNNLLRGAVSQRLYILLPLDCGVPD
NLSMADPNIRFLDKLPQQTADRAGIKDRVYSNSIYELLENGQRAGTCVLEYATPLQTLFAMSQYSQAGFS
REDRLEQAKLFCQTLEDILADAPESQNNCRLIAYQEPADDSSFSLSQEVLRHLRQEEKEEVTVGSLKTSA
VPSTSTMSQEPELLISGMEKPLPLRTDFS

[SEQ ID No: 8]

Therefore, preferably the polymorphism pattern in the STING gene, which is determined, comprises a SNP which corresponds to STING variant G230A-R293Q or "AQ". Preferably, the STING variant G230A-R293Q or "AQ" comprises or consists of the amino acid sequence substantially as set out in SEQ ID No:8 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:7.

In a fifth embodiment, a STING variant is R293Q or "Q", in which one SNP is present. This human STING variant has an allele frequency of 1.5%. In this embodiment, a glutamine replaces an arginine at position 293.

The SNP which encodes R293Q is known as rs7380824. The alleles of STING rs7380824 SNP may be identified as (i) a G-allele, and (ii) an A-allele. Therefore, the method may comprise detecting or determining the G-allele or the A-allele of the STING rs7380824 SNP.

The nucleotide sequence encoding one embodiment of human STING variant Q is referred to herein as SEQ ID No: 9, as follows:

GTTCATTTTTCACTCCTCCCTCCTAGGTCACACTTTTCAGAAAAAGAATCTGCATCCTGGAAACCAGAAG
AAAAATATGAGACGGGGAATCATCGTGTGATGTGTGTGCTGCCTTTGGCTGAGTGTGTGGAGTCCTGCTC
AGGTGTTAGGTACAGTGTGTTTGATCGTGGTGGCTTGAGGGGAACCCGCTGTTCAGAGCTGTGACTGCGG
CTGCACTCAGAGAAGCTGCCCTTGGCTGCTCGTAGCGCCGGGCCTTCTCTCCTCGTCATCATCCAGAGCA
GCCAGTGTCCGGGAGGCAGAAGATGCCCCACTCCAGCCTGCATCCATCCATCCCGTGTCCCAGGGGTCAC
GGGGCCCAGAAGGCAGCCTTGGTTCTGCTGAGTGCCTGCCTGGTGACCCTTTGGGGGCTAGGAGAGCCAC
CAGAGCACACTCTCCGGTACCTGGTGCTCCACCTAGCCTCCCTGCAGCTGGGACTGCTGTTAAACGGGGT
CTGCAGCCTGGCTGAGGAGCTGCGCCACATCCACTCCAGGTACCGGGGCAGCTACTGGAGGACTGTGCGG
GCCTGCCTGGGCTGCCCCCTCCGCCGTGGGGCCCTGTTGCTGCTGTCCATCTATTTCTACTACTCCCTCC
CAAATGCGGTCGGCCCGCCCTTCACTTGGATGCTTGCCCTCCTGGGCCTCTCGCAGGCACTGAACATCCT
CCTGGGCCTCAAGGGCCTGGCCCCAGCTGAGATCTCTGCAGTGTGTGAAAAAGGGAATTTCAACGTGGCC
CATGGGCTGGCATGGTCATATTACATCGGATATCTGCGGCTGATCCTGCCAGAGCTCCAGGCCCGGATTC
GAACTTACAATCAGCATTACAACAACCTGCTACGGGGTGCAGTGAGCCAGCGGCTGTATATTCTCCTCCC
ATTGGACTGTGGGGTGCCTGATAACCTGAGTATGGCTGACCCCAACATTCGCTTCCTGGATAAACTGCCC
CAGCAGACCGGTGACCGTGCTGGCATCAAGGATCGGGTTTACAGCAACAGCATCTATGAGCTTCTGGAGA
ACGGGCAGCGGGCGGGCACCTGTGTCCTGGAGTACGCCACCCCCTTGCAGACTTTGTTTGCCATGTCACA
ATACAGTCAAGCTGGCTTTAGCCGGGAGGATAGGCTTGAGCAGGCCAAACTCTTCTGCCAGACACTTGAG
GACATCCTGGCAGATGCCCCTGAGTCTCAGAACAACTGCCGCCTCATTGCCTACCAGGAACCTGCAGATG
ACAGCAGCTTCTCGCTGTCCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGGAAAAGGAAGAGGTTACTGT
GGGCAGCTTGAAGACCTCAGCGGTGCCCAGTACCTCCACGATGTCCCAAGAGCCTGAGCTCCTCATCAGT
GGAATGGAAAAGCCCCTCCCTCTCCGCACGGATTTCTCTTGAGACCCAGGGTCACCAGGCCAGAGCCTCC
AGTGGTCTCCAAGCCTCTGGACTGGGGGCTCTCTTCAGTGGCTGAATGTCCAGCAGAGCTATTTCCTTCC

ACAGGGGGCCTTGCAGGGAAGGGTCCAGGACTTGACATCTTAAGATGCGTCTTGTCCCCTTGGGCCAGTC
ATTTCCCCTCTCTGAGCCTCGGTGTCTTCAACCTGTGAAATGGGATCATAATCACTGCCTTACCTCCCTC
ACGGTTGTTGTGAGGACTGAGTGTGTGGAAGTTTTTCATAAACTTTGGATGCTAGTGTACTTAGGGGGTG
TGCCAGGTGTCTTTCATGGGGCCTTCCAGACCCACTCCCCACCCTTCTCCCCTTCCTTTGCCCGGGGACG
CCGAACTCTCTCAATGGTATCAACAGGCTCCTTCGCCCTCTGGCTCCTGGTCATGTTCCATTATTGGGGA
GCCCCAGCAGAAGAATGGAGAGGAGGAGGAGGCTGAGTTTGGGGTATTGAATCCCCCGGCTCCCACCCTG
CAGCATCAAGGTTGCTATGGACTCTCCTGCCGGGCAACTCTTGCGTAATCATGACTATCTCTAGGATTCT
GGCACCACTTCCTTCCCTGGCCCCTTAAGCCTAGCTGTGTATCGGCACCCCCACCCCACTAGAGTACTCC
CTCTCACTTGCGGTTTCCTTATACTCCACCCCTTTCTCAACGGTCCTTTTTTAAAGCACATCTCAGATTA

[SEQ ID No: 9]

The amino acid sequence of one embodiment of human STING variant Q (bold, underlined) is referred to herein as SEQ ID No: 10, as follows:

MPHSSLHPSIPCPRGHGAQKAALVLLSACLVTLWGLGEPPEHTLRYLVLHLASLQLGLLLNGVCSLAEEL
RHIHSRYRGSYWRTVRACLGCPLRRGALLLLSIYFYYSLPNAVGPPFTWMLALLGLSQALNILLGLKGLA
PAEISAVCEKGNFNVAHGLAWSYYIGYLRLILPELQARIRTYNQHYNNLLRGAVSQRLYILLPLDCGVPD
NLSMADPNIRFLDKLPQQTGDRAGIKDRVYSNSIYELLENGQRAGTCVLEYATPLQTLFAMSQYSQAGFS
REDRLEQAKLFCRTLEDILADAPESQNNCRLIAYQEPADDSSFSLSQEVLRHLRQEEKEEVTVGSLKTSA
VPSTSTMSQEPELLISGMEKPLPLRTDFS

[SEQ ID No: 10]

Therefore, preferably the polymorphism pattern in the STING gene, which is determined, comprises a SNP which corresponds to STING variant R293Q or "Q". Preferably, the STING variant R293Q or "Q" comprises or consists of the amino acid sequence substantially as set out in SEQ ID No:2 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:1.

The sample is preferably a biological bodily sample taken from the test subject. Determining the genetic polymorphism pattern in the STING gene in the sample is therefore preferably carried out *in vitro*. The sample may comprise tissue, blood, plasma, serum, spinal fluid, urine, sweat, saliva, sputum, tears, breast aspirate, prostate fluid, seminal fluid, vaginal fluid, stool, cervical scraping, amniotic fluid, intraocular fluid, mucous, moisture in breath, animal tissue, cell lysates, tumour tissue, hair, skin, buccal scrapings, nails, bone marrow, cartilage, prions, bone powder, ear wax, or combinations thereof. The sample may be a biopsy.

In another embodiment, the sample may be contained within the test subject, which may be an experimental animal (e.g. a mouse or rat) or a human, wherein the method is

an *in vivo* based test. Alternatively, the sample may be an *ex vivo* sample or an *in vitro* sample. Therefore, the cells being tested may be in a tissue sample (for *ex vivo* based tests) or the cells may be grown in culture (an *in vitro* sample). Preferably, the biological sample is an *ex vivo* sample.

The sample may be pretreated prior to being used in the invention (e.g. diluted, concentrated, separated, partially purified, frozen etc.). Preferably, the sample is a pretreated blood sample.

The sample may comprise blood, urine, or tissue. Most preferably, therefore, the sample comprises a blood sample. The blood may be venous or arterial blood. The apparatus may comprise a sample collection container for receiving the extracted sample. Blood samples may be assayed immediately. Alternatively, the blood sample may be stored at low temperatures, for example in a fridge or even frozen before the assay is conducted. Detection of SNPs in the STING gene may be carried out on whole blood. Preferably, however, the blood sample comprises blood serum. Preferably, the blood sample comprises nucleated cells.

The blood may be further processed before the STING assay is performed, i.e. determining the presence of SNPs in the STING gene. For instance, an anticoagulant, such as citrate (such as sodium citrate), hirudin, heparin, PPACK, or sodium fluoride may be added. Thus, the sample collection container may contain an anticoagulant in order to prevent the blood sample from clotting. Alternatively, the blood sample may be centrifuged or filtered to isolate the nucleated cell fraction of the blood or serum fraction, which may be used for analysis. Hence, it is preferred that the presence of SNPs in the STING gene is analysed or assayed in a nucleated cell fraction of the blood or a blood serum sample. It is preferred that the presence of SNPs in the STING gene is analysed *in vitro* from a blood serum sample or a nucleated cell fraction of the blood taken from the subject.

Amplification techniques for amplifying the sequence of genomic DNA comprising SNPs associated with STING are known to the skilled person and include, but are not limited to, cloning, polymerase chain reaction (PCR), polymerase chain reaction of specific alleles (PASA), polymerase chain ligation, nested polymerase chain reaction, and the like.

Thus, the detecting step may comprise amplification of the sample, for example PCR amplification. PCR involves amplifying DNA, preferably small amounts of DNA, to ease subsequent detection of the genetic polymorphic patterns. Many variations of the basic amplification protocol are well-known to those of skill in the art. PCR-based detection means include multiplex amplification of a plurality of polymorphisms or markers, simultaneously. For example, it is well-known to select PCR primers to generate PCR products that do not overlap in size and which can be analysed simultaneously. Alternatively, it is possible to amplify different markers with primers that are differentially labelled and thus can each be differentially detected. Of course, hybridization-based detection means allow the differential detection of multiple PCR products in a sample. Other techniques are known to allow multiplex analysis of a plurality of markers.

The skilled person would understand that the PCR reaction comprises a mixture of reagents that are well-known in the art in performing the PCR reaction. For example, the mixture may comprise a buffer, forward primer, reverse primer, template, polymerase and water.

The PCR may be Quantitative (Q)-PCR, droplet-digital PCR and Crystal™ Digital™ PCR. All of these techniques are routine in molecular biology and known to those skilled in the art.

Preferably, the activation of DNA polymerase and initial denaturation is carried out at a temperature of between 94 and 98°C, between 95 and 98°C, between 96 and 98°C, or between 97 and 98°C. Most preferably, the initial denaturation is carried out at a temperature of 98°C. Preferably, the activation of DNA polymerase and initial denaturation is carried out for a period of between 30 seconds and 5 minutes, or between 1 and 4 minutes, or between 2 and 3 minutes. Most preferably, the initial denaturation is carried out for a period of 2 minutes and 45 seconds.

Preferably, PCR is carried out for between 20 and 40 cycles, or between 25 and 35 cycles. Most preferably, PCR is carried out for 30 cycles. Preferably, the cycle includes the following steps:

- denaturation;
- annealing; and
- extension.

Preferably, the denaturation step is carried out at a temperature of between 94 and 98°C, between 95 and 98°C, between 96 and 98°C, or between 97 and 98°C. Most preferably, the denaturation step is carried out at a temperature of 98°C. Preferably, the denaturation step is carried out for a period of between 20 seconds and 2 minutes, between 20 seconds and 1 minute, or between 20 and 30 seconds. Most preferably, the denaturation step is carried out for 30 seconds.

Preferably, the annealing step is carried out at a temperature of between 40 and 60°C, or between 50 and 60°C. Most preferably, the annealing step is carried out at a temperature of 60°C. Preferably, the annealing step is carried out for a period of between 30 seconds and 2 minutes, or between 30 seconds and 1 minute. Most preferably, the annealing step is carried out for 30 seconds.

Preferably, the extension step is carried out at a temperature of between 70 and 75°C, between 71 and 74°C, or between 72 and 73°C. Most preferably, the extension step is carried out at a temperature of 72°C. Preferably, the extension step is carried out for a period of time between 30 seconds and 2 minutes, or between 30 seconds and 1 minute. Most preferably, the extension step is carried out for 30 seconds.

Preferably, PCR includes a final extension step. Preferably, the final extension step is carried out at a temperature of between 70 and 75°C, between 71 and 74°C, or between 72 and 73°C. Most preferably, the final extension is carried out at a temperature of 72°C. Preferably, the final extension is carried out for a period of time between 5 and 15 minutes, between 6 and 14 minutes, between 7 and 13 minutes, between 8 and 12 minutes, or between 9 and 11 minutes. Most preferably, the final extension is carried out for a period of 10 minutes.

Preferably, PCR uses a pair of primers, a forward primer and a reverse primer. The forward primer is designed so that it is complementary to a sequence of nucleotides upstream of the sequence of interest, whilst the reverse primer is designed so that it is complementary to a sequence of nucleotides downstream of the sequence of interest. The STING nucleotide sequence to which the primer sequences are capable of hybridizing to may be a DNA or RNA sequence. The RNA sequence may be a miRNA, mRNA or siRNA.

The term “primer” designates, within the context of the present invention, a nucleotide sequence of that can hybridize specifically to a target genetic sequence and serve to initiate amplification. Primers of the invention may be a single-stranded nucleotide sequence , with a length of between 10 and 50 nucleotides between 10 and 40 nucleotides, between 10 and 30 nucleotides, between 10 and 25 nucleotides between 11 and 50, between 11 and 40 nucleotides, between 11 and 30 nucleotides, between 11 and 25 nucleotides, between 13 and 50, between 13 and 40 nucleotides, between 13 and 30 nucleotides, between 13 and 25 nucleotides, between 14 and 50, between 14 and 40 nucleotides, between 14 and 30 nucleotides, between 14 and 25 nucleotides, between 15 and 50, between 15 and 40 nucleotides, between 15 and 30 nucleotides, between 15 and 25 nucleotides. Preferably, primers of the invention have a length of between 15 and 30 nucleotides. More preferably, primers of the invention have a length of between 15 and 25.

Preferably, primers are perfectly matched with the targeted sequence in the STING nucleotide sequence, i.e. having 100% complementarity, allowing specific hybridization thereto and substantially no hybridization to another region.

Preferably, the primers are designed to hybridize to sequences flanking the SNPs which correspond to amino acid residues 71, 230, 232 and 293. This is because substitutions at these residues correspond to the five major STING variants.

In an embodiment in which the SNP location corresponds to amino acid residue 71, the forward primer is provided herein as follows:

GTCTGTTTTGTAGATCGAGAAATGG

[SEQ ID NO: 11]

Thus, in a preferred embodiment, the forward primer comprises or consists of a nucleotide sequence substantially set out as SEQ ID No: 11, or a fragment or variant thereof. Preferably, the forward primer is capable of hybridizing to a sequence complementary to the nucleotide sequence as substantially set out in SEQ ID No: 11, or a fragment or variant thereof, and amplifies, with its paired reverse primer, the target sequence corresponding to amino acid residue 71.

In an embodiment in which the SNP location corresponds to amino acid residue 71, the reverse primer is provided herein as follows:

AGAATGGTCATGGATTTCTTGG

[SEQ ID NO: 12]

Thus in a preferred embodiment, the reverse primer comprises or consists of a nucleotide sequence substantially set out as SEQ ID No: 12, or a fragment or variant thereof. Preferably, the reverse primer is capable of hybridizing to a sequence complementary to the nucleotide sequence as substantially set out in SEQ ID No: 12, or a fragment or variant thereof, and amplifies, with its paired forward primer, the target sequence corresponding to amino acid residue 71. Preferably, the PCR product is 991 bp.

In an embodiment in which the SNP location corresponds to amino acid residue 230 or 232, the forward primer is provided herein as follows:

CAGCTAGGGACACTACAGCTCAGA

[SEQ ID NO: 13]

Thus in a preferred embodiment, the forward primer comprises or consists of a nucleotide sequence substantially set out as SEQ ID No: 13, or a fragment or variant thereof. Preferably, the forward primer is capable of hybridizing to a sequence complementary to the nucleotide sequence as substantially set out in SEQ ID No: 13, or a fragment or variant thereof, and amplifies, with its paired reverse primer, the target sequence corresponding to amino acid residues 230 and 232.

In an embodiment in which the SNP location corresponds to amino acid residue 230 or 232, the reverse primer is provided herein as follows:

CTGGCCTCCTGTACAATGAGAGT

[SEQ ID NO: 14]

Thus in a preferred embodiment, the reverse primer comprises or consists of a nucleotide sequence substantially set out as SEQ ID No: 14, or a fragment or variant thereof. Preferably, the reverse primer is capable of hybridizing to a sequence complementary to the nucleotide sequence as substantially set out in SEQ ID No: 14, or a fragment or variant thereof, and amplifies, with its paired forward primer, the target sequence corresponding to amino acid residues 230 and 232. Preferably, the PCR product is 501 bp.

In an embodiment in which the SNP location corresponds to amino acid residue 293, the forward primer is provided herein as follows:

CTCCATAGCCCCTTCTGACTCTT

[SEQ ID NO: 15]

Thus in a preferred embodiment, the forward primer comprises or consists of a nucleotide sequence substantially set out as SEQ ID No: 15, or a fragment or variant thereof. Preferably, the forward primer is capable of hybridizing to a sequence complementary to the nucleotide sequence as substantially set out in SEQ ID No: 15, or a fragment or variant thereof, and amplifies, with its paired reverse primer, the target sequence corresponding to amino acid residue 293.

In an embodiment in which the SNP location corresponds to amino acid residue 293, the reverse primer is provided herein as follows:

GGCTTAGTCTGGTCTTCCTCTTACC

[SEQ ID NO: 16]

Thus in a preferred embodiment, the reverse primer comprises or consists of a nucleotide sequence substantially set out as SEQ ID No: 16, or a fragment or variant thereof. Preferably, the reverse primer is capable of hybridizing to a sequence complementary to the nucleotide sequence as substantially set out in SEQ ID No: 16, or a fragment or variant thereof, and amplifies, with its paired forward primer, the target sequence corresponding to amino acid residue 293. Preferably, the PCR product is 314 bp.

Preferably, the RFLP pattern analysis comprises subjecting the genomic DNA to restriction enzyme digestion to produce at least one fragment; and subjecting the at least one fragment to gel electrophoresis. The presence of SNPs in the STING gene will result in fragments that display different migration profiles from the wild type fragment patterns.

The skilled person would understand that the restriction enzyme digestion reaction comprises a mixture of reagents that are well known in the art in performing the reaction. For example, the mixture may comprise the PCR product, a buffer, a restriction enzyme and water.

In one embodiment, the restriction enzyme is HhaI. Preferably, HhaI cuts the genomic DNA at the position corresponding to amino acid residue 71.

In one embodiment, HpaII may cut the genomic DNA at the position corresponding to amino acid residue 230. In another embodiment, the restriction enzyme is Eco91I.

Preferably, Eco91I cuts the genomic DNA at the position corresponding to amino acid residue 230.

In another embodiment, the restriction enzyme is Hin1II. Preferably, Hin1II cuts the genomic DNA at the position corresponding to amino acid residue 232.

In another embodiment, the restriction enzyme is HpaII. Preferably, HpaII cuts the genomic DNA at the position corresponding to amino acid residue 293.

The five major STING haplotypes are characterised by the amino acid present at the 71, 230, 232 and 293 positions. Depending on the amino acid present at these positions, and the restriction enzyme used, different length fragments are produced.

In an embodiment in which arginine is located at the 71 position, restriction enzyme digestion produces a 750 bp fragment and a 241 bp fragment. In an embodiment in which histidine is located at the 71 position, restriction enzyme digestion produces a 991 bp fragment.

In an embodiment in which glycine is located at the 230 position, restriction enzyme digestion produces a 317 bp fragment and a 184 bp fragment. In an embodiment in which alanine is present at the 230 position, restriction enzyme digestion produces a 501 bp fragment.

In an embodiment in which arginine is present at the 232 position, restriction enzyme digestion produces a 501 bp fragment. In an embodiment in which histidine is present at the 232 position, restriction enzyme digestion produces a 317 bp fragment and a 184 bp fragment.

In an embodiment in which an arginine is present at the 293 position, restriction enzyme digestion produces a 162 bp fragment, a 116 bp fragment, and a 36 bp fragment. In an embodiment in which glutamine is present at the 293 position, restriction enzyme digestion produces a 198 bp fragment and a 116 bp fragment.

Preferably, the restriction enzyme digestion is carried out at a temperature of between 35 and 40°C, between 36 and 39°C, or between 37 and 38°C. Most preferably, the restriction enzyme digestion is carried out at a temperature of 37°C.

Preferably, the restriction enzyme digestion is carried out for a period of between 5 minutes and 4 hours. More preferably, the restriction enzyme digestion is carried out for a period of between 10 minutes and 3 hours, or15minutes and 2 hours. Most preferably, the restriction enzyme digestion is carried out for a period of one hour.

In one embodiment, the fragment is electrophoresed on an agarose gel. Preferably, the concentration of the agarose in the gel is between 0.5% and 4%, or between 1 and 3.5%. Most preferably, the concentration of the agarose in the gel is between 2 and 3%.

Preferably, the fragment is electrophoresed on a polyacrylamide gel. Preferably, the concentration of the polyacrylamide in the gel is between 5 and 10%. More preferably, the concentration of the polyacrylamide in the gel is between 6 and 9%, or between 7 and 8%. Most preferably, the concentration of the polyacrylamide in the gel is 8%. Preferably, the polyacrylamide gel is placed in 0.5x TBE buffer.

Preferably, the gel is incubated in ethidium bromide staining solution for between 20 seconds and 5 minutes, or between 30 seconds and 4 minutes, or between 40 seconds and 3 minutes, or between 50 seconds and 2 minutes. Most preferably, the gel is incubated in ethidium bromide staining solution for one minute. Preferably, the gel is visualised using a UV gel documentation system.

The method of the fifth aspect may comprise administering, or having administered, to the subject, a STING agonist to treat a disease selected from a group consisting of: cancer, bacterial infection, viral infection, parasitic infection, fungal infection, immune-mediated disorder, central nervous system disease, peripheral nervous system disease, neurodegenerative disease, mood disorder, sleep disorder, cerebrovascular disease, peripheral artery disease and cardiovascular disease. Most preferably, however, cancer is treated with the STING agonist.

Examples of STING agonists include synthetic CDN STING agonists and small-molecule STING agonists. For example, cyclic dinucleotides (CDNs), such as cyclic dimeric guanosine monophosphate (c-di-GMP), cyclic dimeric adenosine monophosphate (c-di-AMP), and cyclic GMP-AMP (cGAMP), are a class of STING agonists that can elicit immune responses.

Examples of STING agonists also include small molecule modulators of STING, of which more information can be found in WO2018/234805, WO2018/234807, WO2019/243825, and WO2019/243823.

In an embodiment in which an individual has STING haplotype R232, HAQ, R232H, AQ or Q, a STING agonist is administered.

It will be appreciated that an 'agonist', an 'effector' or an activator, as it relates to STING, comprises a molecule, combination of molecules, or a complex, that stimulates STING.

The method of the sixth aspect may comprise administering, or having administered, to the subject, a STING antagonist to treat a disease selected from a group consisting of: autoimmune disease, liver fibrosis, fatty liver disease, non-alcoholic steatohepatitis (NASH), pulmonary fibrosis, lupus, sepsis, rheumatoid arthritis (RA), type I diabetes, STING-associated vasculopathy with onset in infancy (SAVI), Aicardi-Goutieres syndrome (AGS), familial chilblain lupus (FCL), systemic lupus erythematosus (SLE), retinal vasculopathy, neuroinflammation, systemic inflammatory response syndrome, pancreatitis, cardiovascular disease, non-alcoholic fatty liver disease, renal fibrosis, stroke and age-related macular degeneration (AMD).

Examples of STING antagonists include small-molecule STING antagonists, such as C-176 and H-151, and STING pathway antagonists.

Examples of STING antagonists also include small molecule modulators of STING.

It will be appreciated that an 'antagonist', as it relates to STING, comprises a molecule, combination of molecules, or a complex, that inhibits, counteracts, downregulates, and/or desensitizes STING. 'Antagonist' encompasses any reagent that inhibits a constitutive activity of STING. A constitutive activity is one that is manifest in the absence of a ligand/STING interaction. 'Antagonist' also encompasses any reagent that inhibits or prevents a stimulated (or regulated) activity of STING.

The "subject" or "individual" may be a vertebrate, mammal, or domestic animal. Hence, medicaments according to the invention may be used to treat any mammal, for example

livestock (e.g. a horse), pets, or may be used in other veterinary applications. Most preferably, the subject is a human being.

It will be appreciated that the invention extends to any nucleic acid or peptide or variant, derivative or analogue thereof, which comprises substantially the amino acid or nucleic acid sequences of any of the sequences referred to herein, including variants or fragments thereof. The terms "substantially the amino acid/nucleotide/peptide sequence", "variant" and "fragment", can be a sequence that has at least 40% sequence identity with the amino acid/nucleotide/peptide sequences of any one of the sequences referred to herein, for example 40% identity with the sequence identified as SEQ ID Nos: 1-16 and so on.

Amino acid/polynucleotide/polypeptide sequences with a sequence identity which is greater than 65%, more preferably greater than 70%, even more preferably greater than 75%, and still more preferably greater than 80% sequence identity to any of the sequences referred to are also envisaged. Preferably, the amino acid/polynucleotide/polypeptide sequence has at least 85% identity with any of the sequences referred to, more preferably at least 90% identity, even more preferably at least 92% identity, even more preferably at least 95% identity, even more preferably at least 97% identity, even more preferably at least 98% identity and, most preferably at least 99% identity with any of the sequences referred to herein.

The skilled technician will appreciate how to calculate the percentage identity between two amino acid/polynucleotide/polypeptide sequences. In order to calculate the percentage identity between two amino acid/polynucleotide/polypeptide sequences, an alignment of the two sequences must first be prepared, followed by calculation of the sequence identity value. The percentage identity for two sequences may take different values depending on:- (i) the method used to align the sequences, for example, ClustalW, BLAST, FASTA, Smith-Waterman (implemented in different programs), or structural alignment from 3D comparison; and (ii) the parameters used by the alignment method, for example, local vs global alignment, the pair-score matrix used (e.g. BLOSUM62, PAM250, Gonnet etc.), and gap-penalty, e.g. functional form and constants.

Having made the alignment, there are many different ways of calculating percentage of identity between the two sequences. For example, one may divide the number of

identities by: (i) the length of shortest sequence; (ii) the length of alignment; (iii) the mean length of sequence; (iv) the number of non-gap positions; or (v) the number of equivalenced positions excluding overhangs. Furthermore, it will be appreciated that percentage of identity is also strongly length dependent. Therefore, the shorter a pair of sequences is, the higher the sequence identity one may expect to occur by chance.

Hence, it will be appreciated that the accurate alignment of protein or DNA sequences is a complex process. The popular multiple alignment program ClustalW (Thompson et al., 1994, Nucleic Acids Research, 22, 4673-4680; Thompson et al., 1997, Nucleic Acids Research, 24, 4876-4882) is a preferred way for generating multiple alignments of proteins or DNA in accordance with the invention. Suitable parameters for ClustalW may be as follows: For DNA alignments: Gap Open Penalty = 15.0, Gap Extension Penalty = 6.66, and Matrix = Identity. For protein alignments: Gap Open Penalty = 10.0, Gap Extension Penalty = 0.2, and Matrix = Gonnet. For DNA and Protein alignments: ENDGAP = -1, and GAPDIST = 4. Those skilled in the art will be aware that it may be necessary to vary these and other parameters for optimal sequence alignment.

Preferably, calculation of percentage identities between two amino acid/polynucleotide/polypeptide sequences may then be calculated from such an alignment as (N/T)*100, where N is the number of positions at which the sequences share an identical residue, and T is the total number of positions compared including gaps and either including or excluding overhangs. Preferably, overhangs are included in the calculation. Hence, a most preferred method for calculating percentage identity between two sequences comprises (i) preparing a sequence alignment using the ClustalW program using a suitable set of parameters, for example, as set out above; and (ii) inserting the values of N and T into the following formula:- Sequence Identity = (N/T)*100.

Due to the degeneracy of the genetic code, it is clear that any nucleic acid sequence described herein could be varied or changed without substantially affecting the sequence of the protein encoded thereby, to provide a functional variant thereof. Suitable nucleotide variants are those having a sequence altered by the substitution of different codons that encode the same amino acid within the sequence, thus producing a silent (synonymous) change. Other suitable variants are those having homologous nucleotide sequences but comprising all, or portions of, sequence, which are altered by the substitution of different codons that encode an amino acid with a side chain of

similar biophysical properties to the amino acid it substitutes, to produce a conservative change. For example small non-polar, hydrophobic amino acids include glycine, alanine, leucine, isoleucine, valine, proline, and methionine. Large non-polar, hydrophobic amino acids include phenylalanine, tryptophan and tyrosine. The polar neutral amino acids include serine, threonine, cysteine, asparagine and glutamine. The positively charged (basic) amino acids include lysine, arginine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. It will therefore be appreciated which amino acids may be replaced with an amino acid having similar biophysical properties, and the skilled technician will know the nucleotide sequences encoding these amino acids.

All of the features described herein (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined with any of the above aspects in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

For a better understanding of the invention, and to show how embodiments of the same may be carried into effect, reference will now be made, by way of example, to the accompanying Figure, in which:-

Figure 1 is a flow diagram illustrating one embodiment of a STING genotyping method according to the invention;

Figure 2 shows SNP typing of human donor D1: STING R232/R232;

Figure 3 shows SNP typing of human donor D2: STING R232/HAQ;

Figure 4 shows SNP typing of human donor D3: STING H232/HAQ;

Figure 5 shows SNP typing of human donor D4: STING R232/H232;

Figure 6 shows SNP typing of human donor D5: STING HAQ/HAQ;

Figure 7 shows SNP typing of human donor D6: STING R232/H232;

Figure 8 shows SNP typing of human donor D7: STING H232/HAQ;

Figure 9 shows SNP typing of human donor D8: STING R232/R232;

Figure 10 shows SNP typing of human donor D9: STING R232/R232;

Figure 11 shows SNP typing of human donor D10: STING R232/R232;

Figure 12 shows SNP typing of human donor D11: STING R232/HAQ; and

Figure 13 shows SNP typing of human donor D12: STING R232/R232.

Examples

Referring to Figure 1, the inventors have designed a novel method for rapidly and reliably identifying the presence or absence of mutations in the STING gene and, accordingly, determining which STING alleles a subject carries using tissue or blood samples. The method involves PCR amplification of a SNP-containing sequence of genomic DNA obtained from a human subject. This is followed by RFLP analysis to identify the subject's STING variant according to the presence or absence of particular SNPs.

Materials and methods

Collection of blood

500µl of fresh whole blood was collected from individual healthy donors in Vacuette Na-Heparin coated vacuum tubes after obtaining signed consent (*as per Institutional Ethical Approval Committee guidelines*).

Genomic DNA isolation and measurement

Commonly available commercial kits can be used for isolation of genomic DNA as per the manufacturer's protocol. Such kits are marketed by Qiagen (QIAamp DNA Blood Mini Kit Cat #51104) and Promega (Wizard® Genomic DNA Purification Kit Cat #A1120). In this example, the inventors used the QIAGEN kit. DNA was quantified and measured by monitoring UV absorbance at 260nm using a spectrophotometer (Agilent technologies Cary 60 UV-Vis).

PCR for each SNP position

PCR was carried out for each SNP position according to the below mentioned PCR conditions and using the primer sets for each SNP amplification set out below.

a) Primer sets for each SNP amplification:

Primer sets for PCR of gDNA template		
Primer_ID	**Primer SNP**	**Sequence**
Fwd71	SNP_71_Fwd	GTCTGTTTTGTAGATCGAGAAATGG [SEQ ID No: 11]
Rev71	SNP_71_Rev	AGAATGGTCATGGATTTCTTGG [SEQ ID No: 12]
Fwd230	SNP_230/232_Fwd	CAGCTAGGGACACTACAGCTCAGA [SEQ ID No: 13]
Rev230	SNP_230/232_Rev	CTGGCCTCCTGTACAATGAGAGT [SEQ ID No: 14]

Fwd293	SNP_293_Fwd	CTCCATAGCCCCTTCTGACTCTT [SEQ ID No: 15]
Rev293	SNP_293_Rev	GGCTTAGTCTGGTCTTCCTCTTACC [SEQ ID No: 16]

b) PCR reaction table for each SNP PCR:

Phusion Pol hSTING cloning PCR rxn using gDNA template			
component	Conc.	Volume (ul)	Final conc.
buffer	5x	4.0	1x
dNTPs	10mM	0.4	200uM
primer Fwd	10uM	1.0	0.5uM
primer Rev	10uM	1.0	0.5uM
Template	50-100ng	1.0	
Phusion Pol	2U/ul	0.2	0.02U/ul
Water		12.4	
Total		20.0	

c) PCR cycling instructions:

Initial denaturation: 98°C for 2 min 45 seconds
Denaturation: 98°C for 30 seconds
Annealing: 60°C for 30 seconds
Extension: 72°C for 30 seconds
Cycles: 30 cycles
Final extension: 72°C for 10 minutes

Restriction enzyme digestion

The following reaction mix was prepared and incubated at 37°C for 1 hour. 5µl of the digested sample was electrophoresed on 8% polyacrylamide gel in 0.5X TBE buffer for resolution. The gel was incubated in ethidium bromide staining solution for a minute and visualized using a UV gel documentation system.

Reagent	**Volume**
PCR Product	5ul
FD Green 10x buffer	1ul
Restriction Enzyme	0.5ul (0.5U)
Water	3.5ul
Total	10ul

RFLP pattern analysis

Fragment Pattern expected in electrophoretic movement post digestion using gDNA template:

PCR prod size (bp)	Position Restriction Enzyme	Amino Acid	STING Variant	Fragments (bp)
991	71 HhaI	Arg(R71) His (H71)	R232/H232 HAQ	**750/ 241 991**
501	230 Eco91I	Gly(G230) Ala(A230)	R232/H232 HAQ/A230	**317/184 501**
501	232 Hin1II	Arg(R232) His (H232)	R232/HAQ H232	**501 317/184**
314	293 HpaII	Arg(R293) Gln(Q293)	R232/H232 HAQ/Q293	**162/116/36 198/116**

Results

In this example, the inventors used their newly designed method to identify the presence or absence of SNPs in the STING gene, and therefore determine the specific STING alleles carried by twelve healthy human donors. They first collected fresh whole blood from each individual healthy donor and then isolated and measured genomic DNA from the samples. PCR amplification of a SNP containing sequence of genomic DNA was then carried out to produce multiple copies of the DNA sample. The inventors then used restriction enzyme digestion to digest the DNA pieces into smaller fragments. Following this, the inventors carried out gel electrophoresis to separate the DNA fragments produced according to their size. Finally, restriction fragment length polymorphism (RFLP) pattern analysis was used to determine the specific STING alleles carried in the genotype of each subject.

Using this newly designed method, the inventors were able to quickly and reliably identify the SNPs present in the STING genes of healthy human donors, and therefore determine the STING variant of the individual. The inventors designed the method to identify SNPs that corresponded to the five major STING variants: R232, HAQ, H232, AQ and Q. Therefore, the PCR primers were designed to hybridize to sequences flanking the SNPs that correspond to amino acid residues 71, 230, 232 and 293, as substitutions at these residues correspond to the five major STING variants.

As illustrated in Figures 2-13, the inventors then used RFLP pattern analysis based on the electrophoretic movement of the DNA fragments to determine the STING variants

carried by each individual. For example, as illustrated in Figure 2, human donor D1 carries the STING alleles R232/R232. This is because RFLP pattern analysis of the PCR products revealed the presence of arginine at position 71, glycine at position 230, arginine at position 232 and arginine at position 293, based on the fragment lengths. Accordingly, human donor D1 carries the STING alleles R232/R232. In comparison, human donor D5 (Figure 6) carries the STING alleles HAQ/HAQ, since RFLP pattern analysis of PCR products revealed the presence of histidine at position 71, alanine at position 230, arginine at position 232 and glutamine at position 293. By identifying the SNPs and determining the STING variant, this method is extremely informative in revealing that both subjects would be suitable for treatment with a STING agonist.

Conclusions

In summary, therefore, the inventors have developed a novel method for determining the specific STING alleles carried by individuals using tissue or blood samples. In particular, this novel method is both rapid and reliable and can therefore yield accurate results within a few hours. In addition, this novel method does not require the need for expensive reagents and instruments. Therefore, considering the large number of patients being tested, this method will be both cost effective and feasible to perform. Accordingly, this method can therefore be used as a diagnostic tool to determine the most effective STING agonist therapy for a patient based on their STING genotype.

References

1. Cell Reports 11, 1018–1030, May 19, 2015 (*2015 Direct Activation of STING in the Tumor*)
2. PLoS ONE 8(10): e77846. doi:10.1371/journal.pone.0077846, 2013 (*2013 SNP of human STING*)
3. The Journal of Immunology, 2017, 198: 000–000 (*2016_The Common HAQ STING Is a Null AlleleHuman*)
4. Genes and Immunity (2011) 12, 263–269 (*2011_Identification and characterization of a loss-of-function STING SNPs*)

Claims

1. A method for determining the genetic polymorphism pattern in the "Stimulator of Interferon Genes" (STING) gene in a subject, the method comprising:

- amplifying, in a sample obtained from a subject, a sequence of genomic DNA comprising a single nucleotide polymorphism (SNP) within the STING gene; and
- performing restriction fragment length polymorphism (RFLP) pattern analysis on the amplified DNA to determine the genetic polymorphism pattern in the STING gene in the sample.

2. A method for determining the efficacy of a treatment of a subject with a "Stimulator of Interferon Genes" (STING) agonist or a STING antagonist, the method comprising:

- determining the genetic polymorphism pattern in the Stimulator of Interferon Genes (STING) gene in a sample obtained from a subject using the method according to claim 1; and
- determining the suitability of the subject for STING agonist or antagonist therapy based on the genetic polymorphism pattern in the STING gene.

3. The method according to either claim 1 or 2, wherein the polymorphism pattern in the STING gene, which is determined, corresponds to STING variant R232, optionally wherein the STING variant R232 comprises or consists of the amino acid sequence substantially as set out in SEQ ID No:2 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:1.

4. The method according to either claim 1 or 2, wherein the polymorphism pattern in the STING gene, which is determined, comprises a SNP which corresponds to STING variant R71H-G230A-R293Q or "HAQ", optionally wherein the STING variant R71H-G230A-R293Q or "HAQ" comprises or consists of the amino acid sequence substantially as set out in SEQ ID No:4 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:3.

5. The method according to either claim 1 or 2, wherein the polymorphism pattern in the STING gene, which is determined, comprises a SNP which corresponds to STING variant R232H or "H232", optionally wherein the STING variant R232H or "H232"

comprises or consists of the amino acid sequence substantially as set out in SEQ ID No:6 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:5.

6. The method according to either claim 1 or 2, wherein the polymorphism pattern in the STING gene, which is determined, comprises a SNP which corresponds to STING variant G230A-R293Q or "AQ", optionally wherein the STING variant G230A-R293Q or "AQ" comprises or consists of the amino acid sequence substantially as set out in SEQ ID No:8 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:7.

7. The method according to either claim 1 or 2, wherein the polymorphism pattern in the STING gene, which is determined, comprises a SNP which corresponds to STING variant R293Q or "Q", optionally wherein the STING variant R293Q or "Q" comprises or consists of the amino acid sequence substantially as set out in SEQ ID No:2 and/or is encoded by a nucleic acid sequence substantially as set out in SEQ ID No:1.

8. The method according to any preceding claim, wherein the amplification step comprises PCR, optionally

wherein the PCR comprises an initial denaturation step carried out at a temperature of between 94 and 98°C, or between 96 and 98°C, or between 97 and 98°C, and for a period of between 30 seconds and 5 minutes, or between 1 and 4 minutes, or between 2 and 3 minutes; and/or

wherein the PCR is carried out for between 20 and 40 cycles, or between 25 and 35 cycles, wherein a cycle includes a denaturation step, an annealing step and an extension step.

9. The method according to claim 11, wherein the denaturation step is carried out at a temperature of between 94 and 98°C, or between 95 and 98°C, or between 96 and 98°C, or between 97 and 98°C, and for a period of between 20 seconds and 2 minutes, or between 20 seconds and 1 minute, or between 20 and 30 seconds; and/or

wherein the annealing step is carried out at a temperature of between 40 and 60°C, or between 50 and 60°C, and for a period of between 30 seconds and 2 minutes, or between 30 seconds and 1 minute; and/or

wherein the extension step is carried out at a temperature of between 70 and 75°C, or between 71 and 74°C, or between 72 and 73°C, and for a period of between 30 seconds and 2 minutes, or between 30 seconds and 1 minute.

10. The method according to either claim 8 or 9, wherein the PCR includes a final extension step carried out at a temperature of between 70 and 75°C, or between 71 and 74°C, or between 72 and 73°C, and for a period of between 5 and 15 minutes, or between 6 and 14 minutes, or between 7 and 13 minutes, or between 8 and 12 minutes, or between 9 and 11 minutes.

11. The method according to any one of claims 8 to 10, wherein the PCR uses a forward primer and a reverse primer designed to hybridize to sequences flanking the SNPs which correspond to amino acid residues 71, 230, 232 and 293 of the STING variant.

12. The method according to claim 11, in which the SNP location corresponds to amino acid residue 71, wherein the forward primer comprises a nucleotide sequence substantially as set out in SEQ ID No: 11, or a variant or fragment thereof, and the reverse primer comprises a nucleotide sequence substantially as set out in SEQ ID No: 12, or a variant or fragment thereof.

13. The method according to claim 11, in which the SNP location corresponds to amino acid residue 230 or 232, wherein the forward primer comprises a nucleotide sequence substantially as set out in SEQ ID No: 13, or a variant or fragment thereof, and the reverse primer comprises a nucleotide sequence substantially as set out in SEQ ID No: 14, or a variant or fragment thereof.

14. The method according to claim 11, in which the SNP location corresponds to amino acid residue 293, wherein the forward primer comprises a nucleotide sequence substantially as set out in SEQ ID No: 15, or a variant or fragment thereof, and the reverse primer comprises a nucleotide sequence substantially as set out in SEQ ID No: 16, or a variant or fragment thereof.

15. The method according to any preceding claim, wherein the RFLP pattern analysis comprises subjecting the genomic DNA to restriction enzyme digestion to

produce at least one fragment; and subjecting the at least one fragment to gel electrophoresis.

16. The method according to claim 15, wherein the restriction enzyme digestion reaction comprises the genomic DNA, a buffer, a restriction enzyme and water.

17. The method according to claim 16, wherein the restriction enzyme is HhaI and cuts the genomic DNA at the position corresponding to amino acid residue 71.

18. The method according to claim 16, wherein the restriction enzyme is Eco91I and cuts the genomic DNA at the position corresponding to amino acid residue 230.

19. The method according to claim 16, wherein the restriction enzyme is Hin1II and cuts the genomic DNA at the position corresponding to amino acid residue 232.

20. The method according to claim 16, wherein the restriction enzyme is HpaII and cuts the genomic DNA at the position corresponding to amino acid residue 293.

21. The method according to claim 17, wherein restriction enzyme digestion produces a 750 bp fragment and a 241 bp fragment when an arginine is located at the 71 position, and/or a 991 bp fragment when a histidine is located at the 71 position.

22. The method according to claim 18, wherein restriction enzyme digestion produces a 317 bp fragment and a 184 bp fragment when a glycine is located at the 230 position, and/or a 501 bp fragment when an alanine is present at the 230 position.

23. The method according to claim 19, wherein restriction enzyme digestion produces a 501 bp fragment when an arginine is present at the 232 position, and/or a 317 bp fragment and a 184 bp fragment when a histidine is present at the 232 position.

24. The method according to claim 20, wherein restriction enzyme digestion produces a 162 bp fragment, a 116 bp fragment, and a 36 bp fragment when an arginine is present at the 293 position, and/or a 198 bp fragment and a 116 bp fragment when a glutamine is present at the 293 position.

25. The method according to any one of claims 15 to 24, wherein the restriction enzyme digestion is carried out at a temperature of between 35 and 40°C, or between 36 and 39°C, or between 37 and 38°C, and for a period of between 5 minutes and 4 hours, or between 10 minutes and 3 hours, or between 15 minutes and 2 hours.

26. The method according to any one of claims 15 to 25, wherein the gel electrophoresis step comprises electrophoresing the fragment on a polyacrylamide or agarose gel, wherein the concentration of the polyacrylamide in the gel is between 5 and 10%, or between 6 and 9%, or between 7 and 8% and the concentration of the agarose in the gel is between 0.5 and 4%, or between 2 and 3%.

27. An apparatus for determining the genetic polymorphism pattern in the "Stimulator of Interferon Genes" (STING) gene in a subject using the method of any one of claims 1 to 26, the apparatus comprising:

- means for amplifying, in a sample obtained from a subject, a sequence of genomic DNA comprising a single nucleotide polymorphism (SNP) within the STING gene; and
- means for performing restriction fragment length polymorphism (RFLP) pattern analysis on the amplified DNA to determine the genetic polymorphism pattern in the STING gene in the sample.

28. A diagnostic or prognostic tool for assessing the suitability of a subject for "Stimulator of Interferon Genes" (STING) agonist or antagonist therapy, comprising:

- determining the genetic polymorphism pattern in the Stimulator of Interferon Genes (STING) in a sample obtained from a subject using the method of any one of claims 1 to 26 or the apparatus of claim 27; and
- determining the suitability of the subject for STING agonist or antagonist therapy based on the genetic polymorphism pattern.

Figure 1

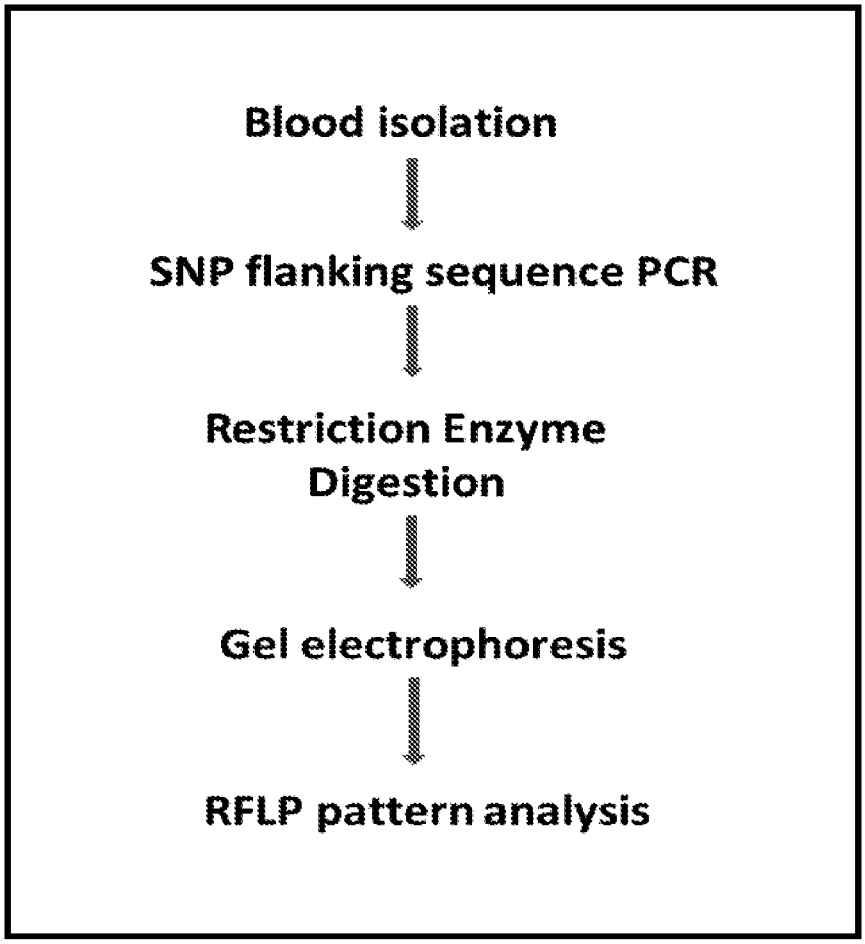
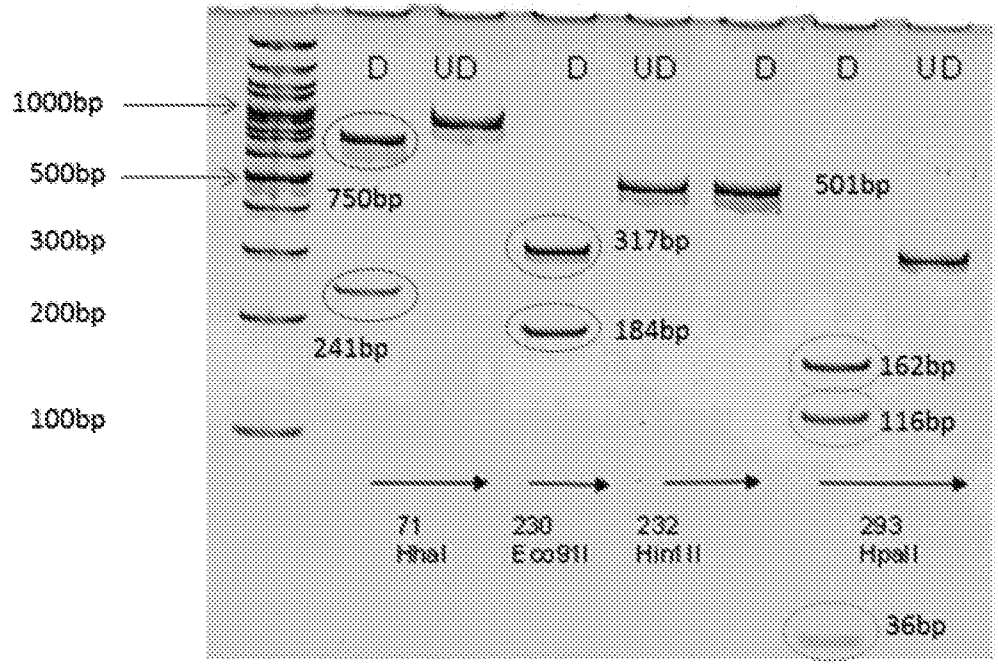

Blood isolation
SNP flanking sequence PCR
Restriction Enzyme Digestion
Gel electrophoresis
RFLP pattern analysis

Figure 2



PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	750/ 241
		His (H71)	HAQ	991
501	230 Eco91I	Gly(G230)	R232/H232	317/184
		Ala(A230)	HAQ	501
501	232 Hin1II	Arg(R232)	R232/HAQ	501
		His (H232)	H232	317/184
314	293 HpaII	Arg(R293)	R232/H232	162/116/36
		Gln(Q293)	HAQ	198/116

STING Alleles	
Allele-1	Allele-2
R232	R232

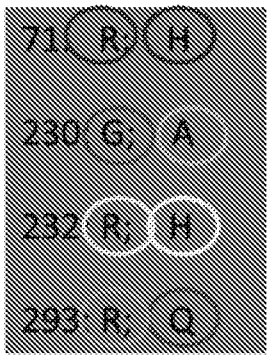
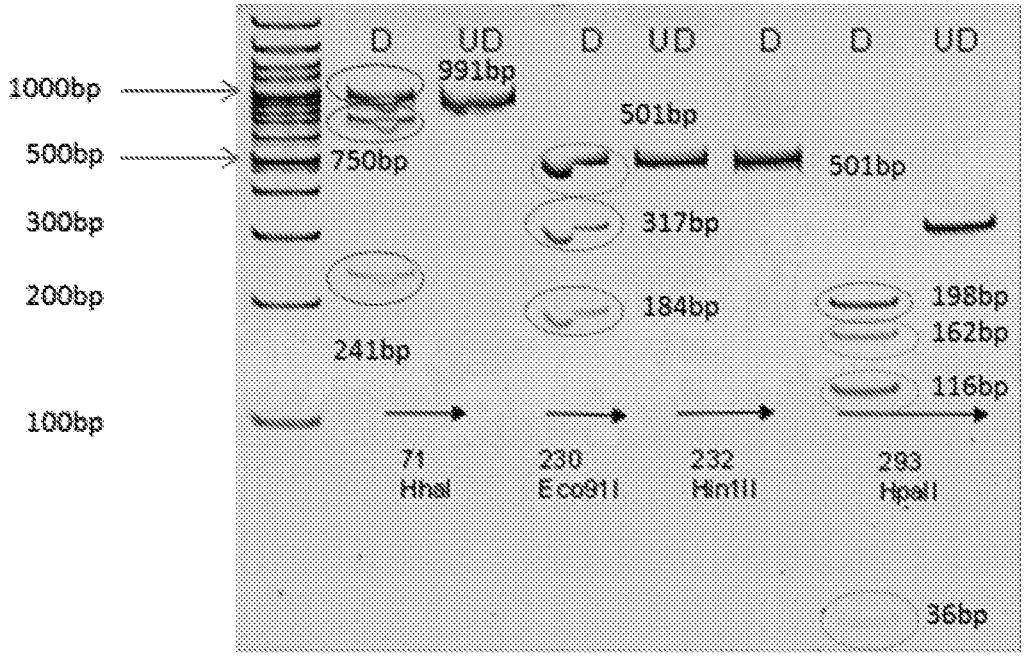


Figure 3



PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	**750/ 241**
		His (H71)	HAQ	**991**
501	230 Eco91I	Gly(G230)	R232/H232	**317/184**
		Ala(A230)	HAQ	**501**
501	232 HinIII	Arg(R232)	R232/HAQ	**501**
		His (H232)	H232	**317/184**
314	293 HpaII	Arg(R293)	R232/H232	**162/116/36**
		Gln(Q293)	HAQ	**198/116**

STING Alleles	
Allele-1	Allele-2
R232	HAQ

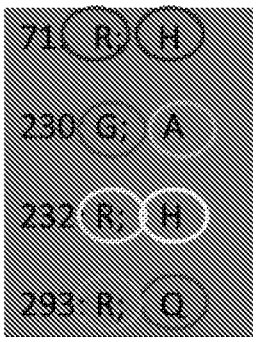
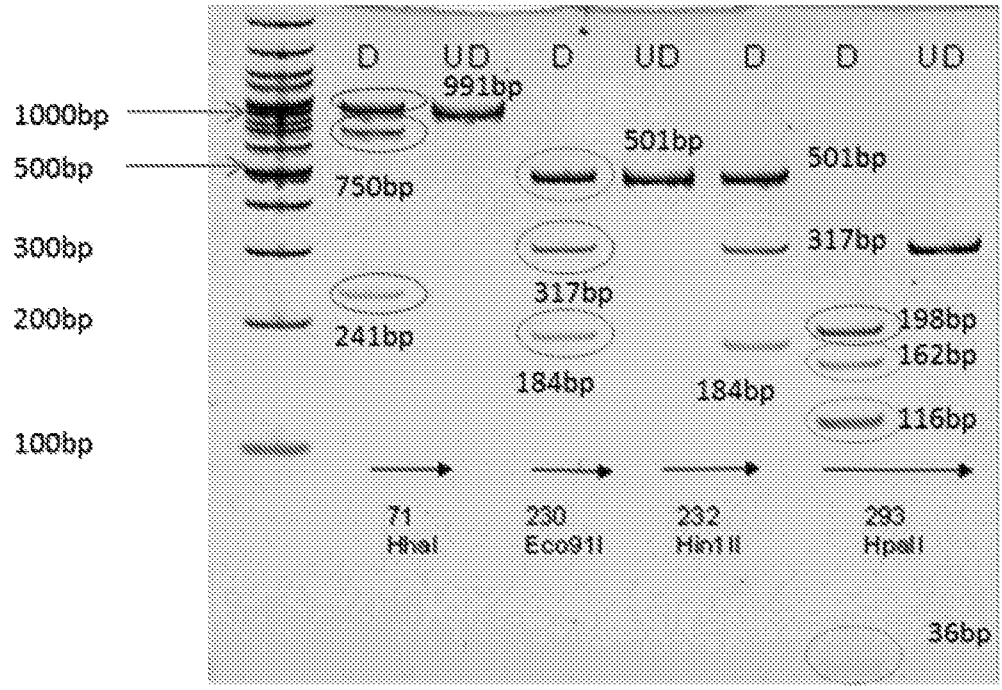


Figure 4



PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	750/ 241
		His (H71)	HAQ	991
501	230 Eco91I	Gly(G230)	R232/H232	317/184
		Ala(A230)	HAQ	501
501	232 HinIII	Arg(R232)	R232/HAQ	501
		His (H232)	H232	317/184
314	293 HpaII	Arg(R293)	R232/H232	162/116/36
		Gln(Q293)	HAQ	198/116

STING Alleles	
Allele-1	Allele-2
H232	HAQ

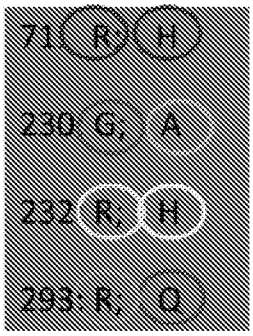
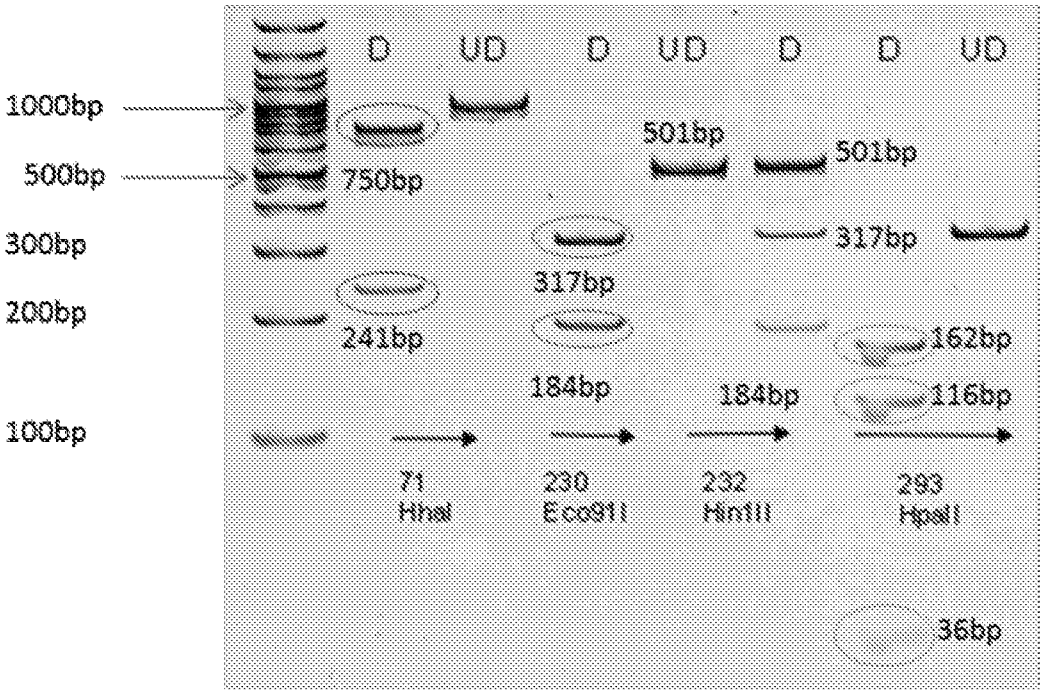


Figure 5



PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	**750/ 241**
		His (H71)	HAQ	**991**
501	230 Eco91I	Gly(G230)	R232/H232	**317/184**
		Ala(A230)	HAQ	**501**
501	232 Hin1II	Arg(R232)	R232/HAQ	**501**
		His (H232)	H232	**317/184**
314	293 HpaII	Arg(R293)	R232/H232	**162/116/36**
		Gln(Q293)	HAQ	**198/116**

STING Alleles	
Allele-1	Allele-2
R232	H232

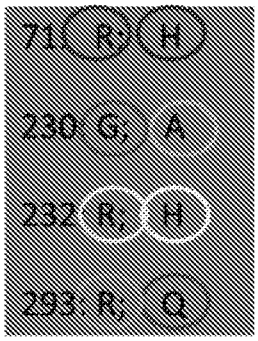
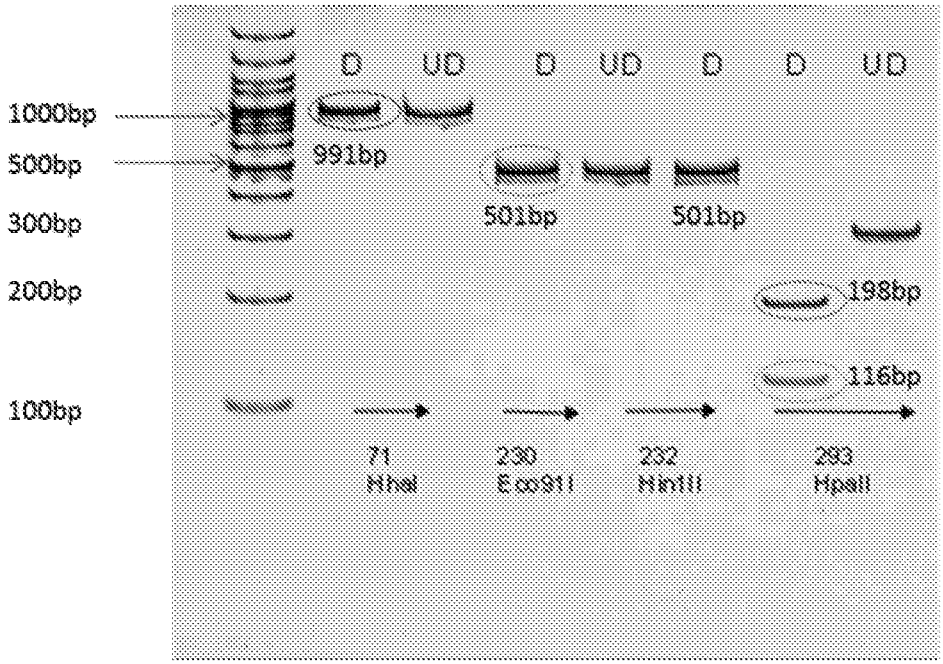


Figure 6



PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	750/ 241
		His (H71)	HAQ	991
501	230 Eco91I	Gly(G230)	R232/H232	317/184
		Ala(A230)	HAQ	501
501	232 HinIII	Arg(R232)	R232/HAQ	501
		His (H232)	H232	317/184
314	293 HpaII	Arg(R293)	R232/H232	162/116/36
		Gln(Q293)	HAQ	198/116

STING Alleles	
Allele-1	Allele-2
HAQ	HAQ

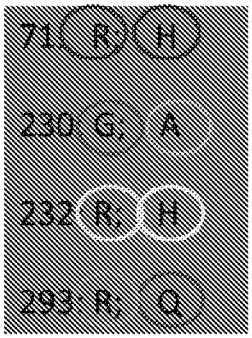
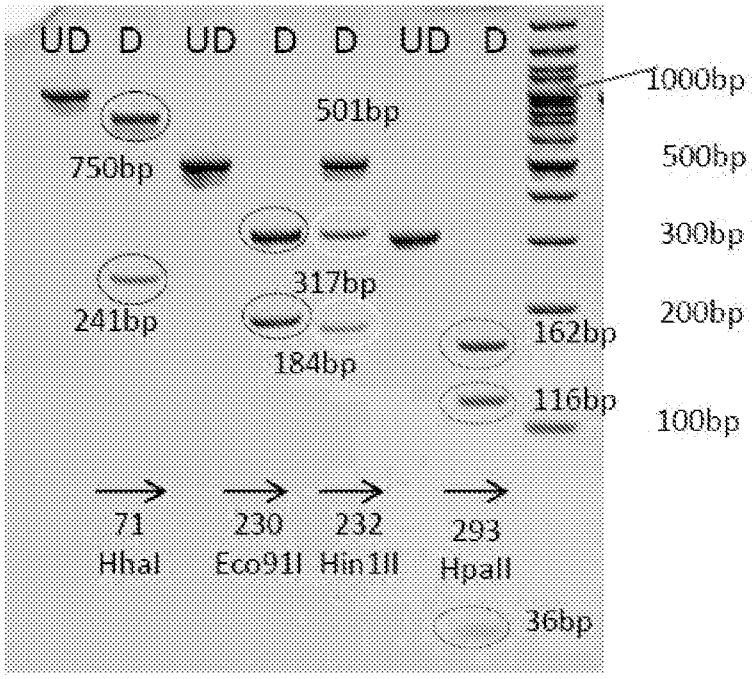
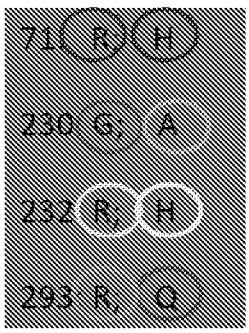


Figure 7



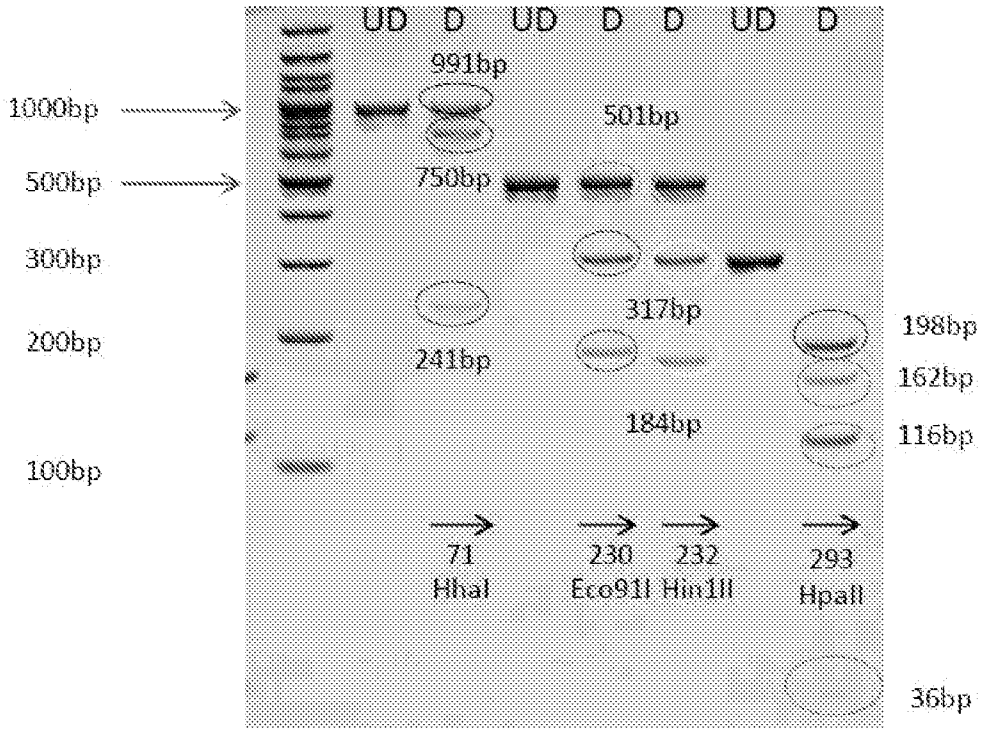
PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	**750/ 241**
		His (H71)	HAQ	**991**
501	230 Eco91I	Gly(G230)	R232/H232	**317/184**
		Ala(A230)	HAQ	**501**
501	232 Hin1II	Arg(R232)	R232/HAQ	**501**
		His (H232)	H232	**317/184**
314	293 HpaII	Arg(R293)	R232/H232	**162/116/36**
		Gln(Q293)	HAQ	**198/116**

STING Alleles	
Allele-1	Allele-2
R232	H232



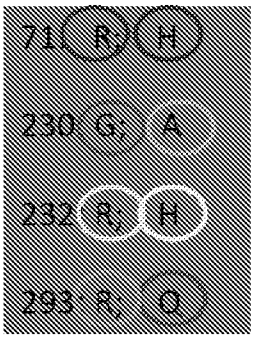
UD- Undigested
D- Digested

Figure 8



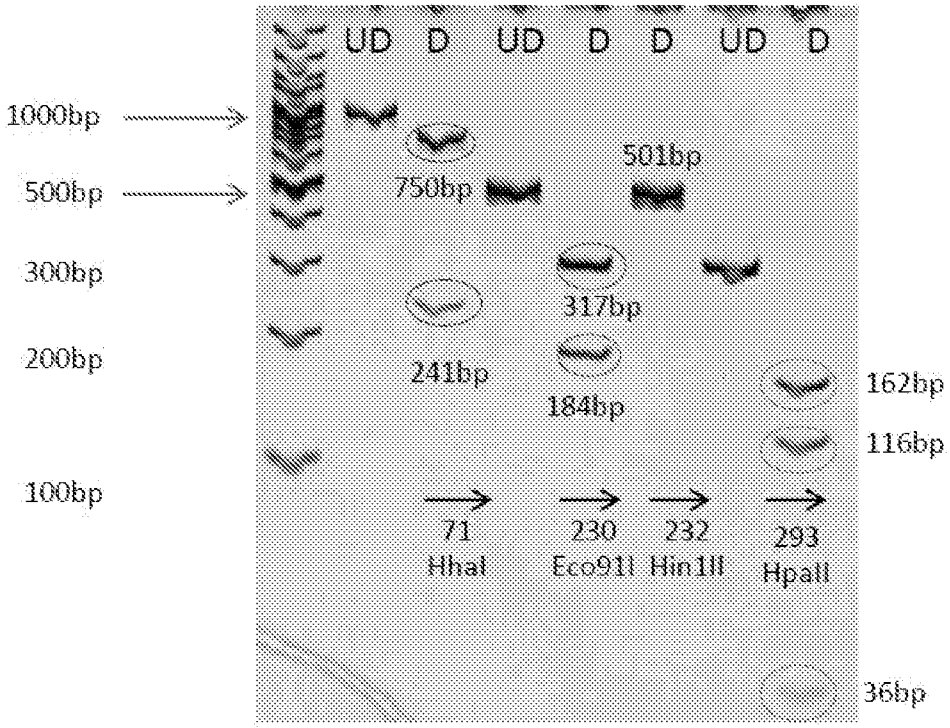
PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	**750/ 241**
		His (H71)	HAQ	**991**
501	230 Eco91I	Gly(G230)	R232/H232	**317/184**
		Ala(A230)	HAQ	**501**
501	232 Hin1II	Arg(R232)	R232/HAQ	**501**
		His (H232)	H232	**317/184**
314	293 HpaII	Arg(R293)	R232/H232	**162/116/36**
		Gln(Q293)	HAQ	**198/116**

STING Alleles	
Allele-1	Allele-2
H232	HAQ



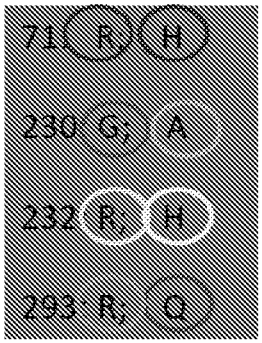
UD- Undigested
D- Digested

Figure 9



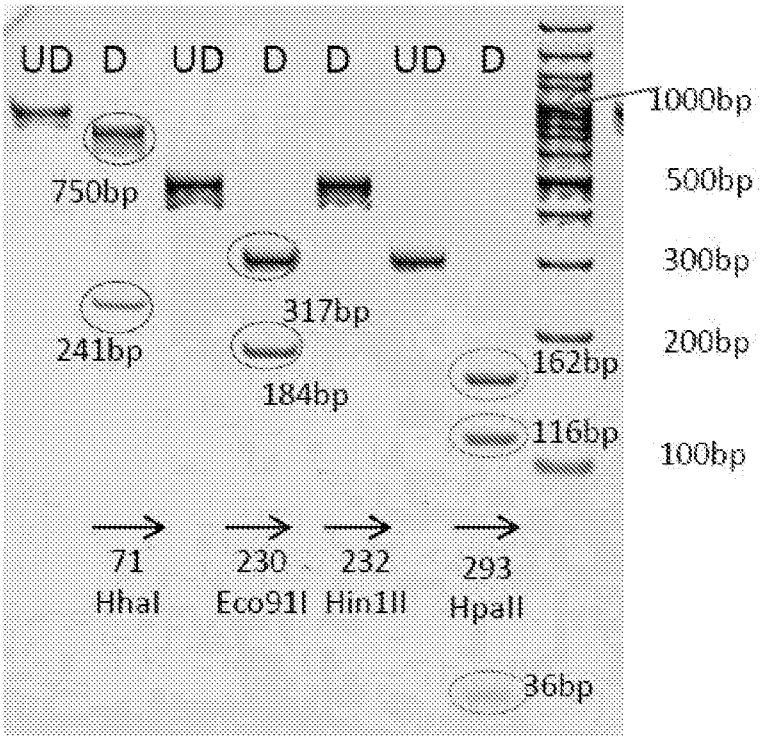
PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	**750/ 241**
		His (H71)	HAQ	**991**
501	230 Eco91I	Gly(G230)	R232/H232	**317/184**
		Ala(A230)	HAQ	**501**
501	232 Hin1II	Arg(R232)	R232/HAQ	**501**
		His (H232)	H232	**317/184**
314	293 HpaII	Arg(R293)	R232/H232	**162/116/36**
		Gln(Q293)	HAQ	**198/116**

STING Alleles	
Allele-1	Allele-2
R232	R232



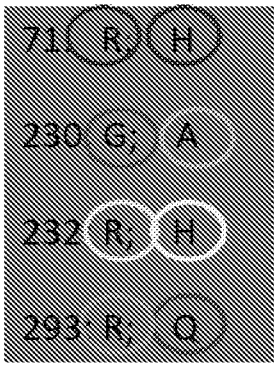
UD- Undigested
D- Digested

Figure 10



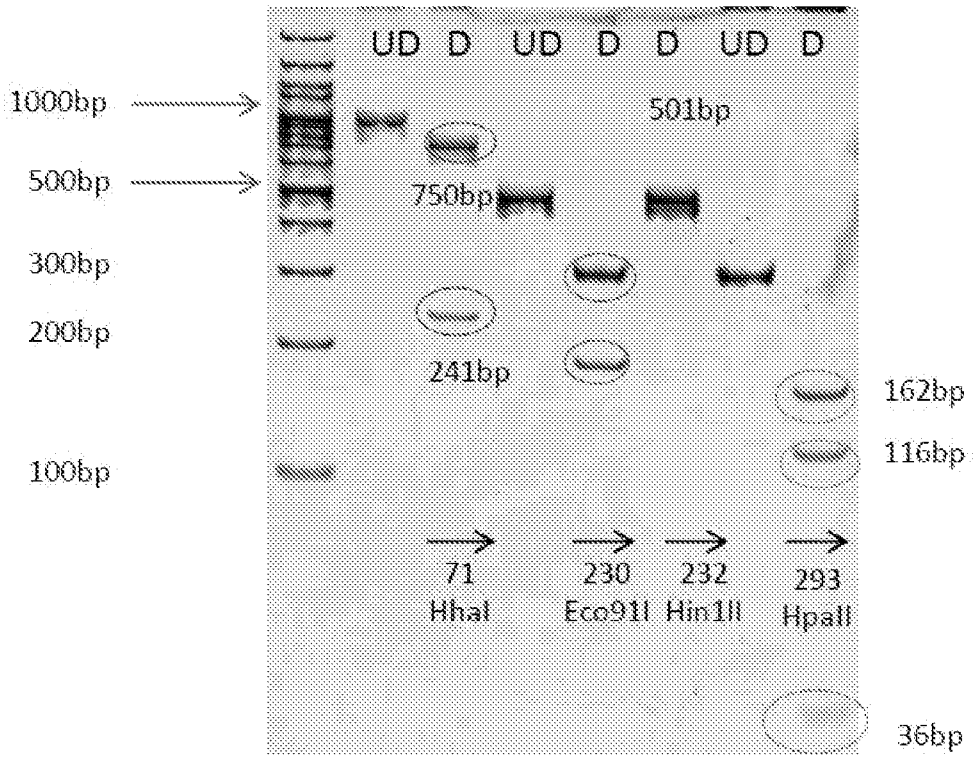
PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	**750/ 241**
		His (H71)	HAQ	**991**
501	230 Eco91I	Gly(G230)	R232/H232	**317/184**
		Ala(A230)	HAQ	**501**
501	232 Hin1II	Arg(R232)	R232/HAQ	**501**
		His (H232)	H232	**317/184**
314	293 HpaII	Arg(R293)	R232/H232	**162/116/36**
		Gln(Q293)	HAQ	**198/116**

STING Alleles	
Allele-1	Allele-2
R232	R232



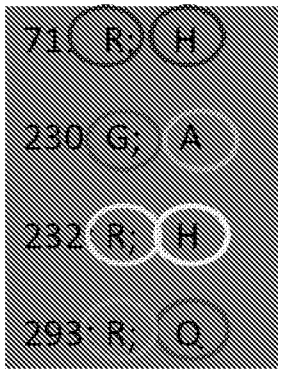
UD- Undigested
D- Digested

Figure 11



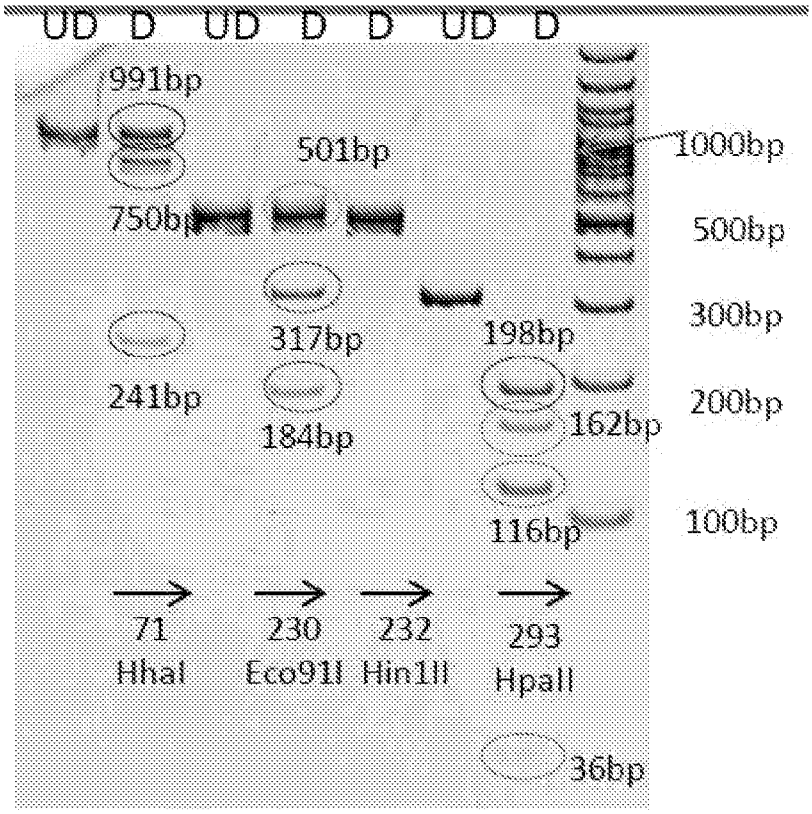
PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	**750/ 241**
		His (H71)	HAQ	**991**
501	230 Eco91I	Gly(G230)	R232/H232	**317/184**
		Ala(A230)	HAQ	**501**
501	232 Hin1II	Arg(R232)	R232/HAQ	**501**
		His (H232)	H232	**317/184**
314	293 HpaII	Arg(R293)	R232/H232	**162/116/36**
		Gln(Q293)	HAQ	**198/116**

STING Alleles	
Allele-1	Allele-2
R232	R232



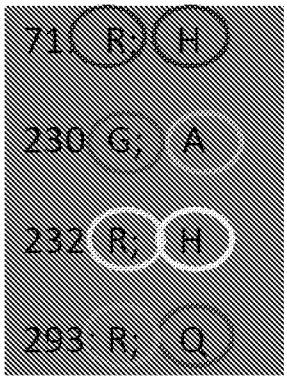
UD- Undigested
D- Digested

Figure 12



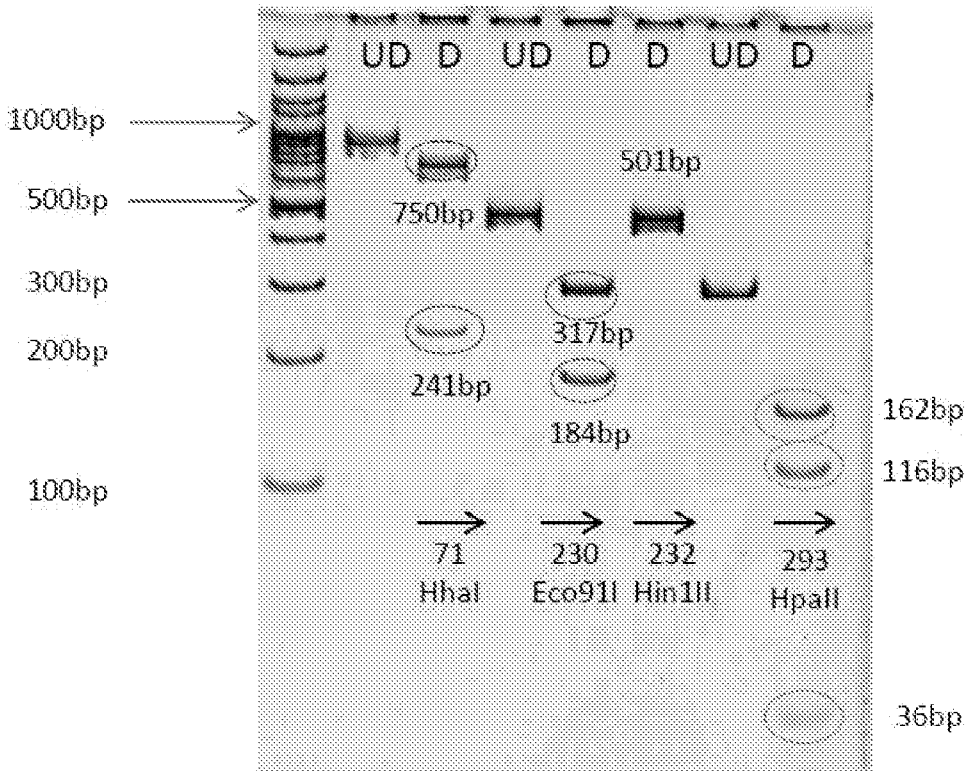
PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	**750/ 241**
		His (H71)	HAQ	**991**
501	230 Eco91I	Gly(G230)	R232/H232	**317/184**
		Ala(A230)	HAQ	**501**
501	232 Hin1II	Arg(R232)	R232/HAQ	**501**
		His (H232)	H232	**317/184**
314	293 HpaII	Arg(R293)	R232/H232	**162/116/36**
		Gln(Q293)	HAQ	**198/116**

STING Alleles	
Allele-1	Allele-2
R232	HAQ



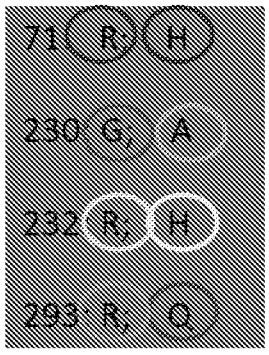
UD- Undigested
D- Digested

Figure 13



PCR prod size (bp)	Position RE	AA	STING Variant	Fragments
991	71 HhaI	Arg(R71)	R232/H232	**750/ 241**
		His (H71)	HAQ	**991**
501	230 Eco91I	Gly(G230)	R232/H232	**317/184**
		Ala(A230)	HAQ	**501**
501	232 Hin1II	Arg(R232)	R232/HAQ	**501**
		His (H232)	H232	**317/184**
314	293 HpaII	Arg(R293)	R232/H232	**162/116/36**
		Gln(Q293)	HAQ	**198/116**

STING Alleles	
Allele-1	Allele-2
R232	R232



UD- Undigested
D- Digested

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/054949

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6883
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/033700 A1 (PML SCREENING LLC [US]; THE UNIV PARIS SUD [FR] ET AL.) 13 February 2020 (2020-02-13)	1,2,27, 28
Y	abstract paragraph [00593] claims 42, 68	3-26

X	WO 2019/241746 A1 (FLAGSHIP PIONEERING INNOVATIONS V INC [US]) 19 December 2019 (2019-12-19)	1,2,27, 28
Y	abstract page 36, line 10 - line 18 claims 1,5	3-26

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 October 2021	14/10/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Barz, Wolfgang

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/054949

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	L JIN ET AL: "Identification and characterization of a loss-of-function human MPYS variant", GENES AND IMMUNITY, vol. 12, no. 4, 20 January 2011 (2011-01-20), pages 263-269, XP055315011, GB ISSN: 1466-4879, DOI: 10.1038/gene.2010.75 abstract table 1 figures 1-5	3-26

Y	GUANGHUI YI ET AL: "Single Nucleotide Polymorphisms of Human STING Can Affect Innate Immune Response to Cyclic Dinucleotides", PLOS ONE, vol. 8, no. 10, 21 October 2013 (2013-10-21), page e77846, XP055315013, DOI: 10.1371/journal.pone.0077846 abstract figure 1 discussion	3-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/054949

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2021/054949

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020033700 A1	13-02-2020	AU 2019316556 A1	18-02-2021
		CA 3108807 A1	13-02-2020
		US 2020165673 A1	28-05-2020
		WO 2020033700 A1	13-02-2020
WO 2019241746 A1	19-12-2019	EP 3806841 A1	21-04-2021
		MA 52897 A	21-04-2021
		US 2021283091 A1	16-09-2021
		WO 2019241746 A1	19-12-2019