

techniques, they should be producible at low cost, without constituting a hazard to the environment or staff handling them. Further, the presence of controls should not adversely affect the sensitivity of the diagnostic assays. These and other objectives are fulfilled by the present invention.

The inventors of the present invention *inter alia* succeeded in providing system controls for Next Generation Sequencing (NGS) reactions which may be used as positive and negative as well as extraction controls in a NGS reaction.

Before some of the embodiments of the present invention are described in more detail, the following definitions are introduced.

Summary

Disclosed herein is a Next Generation Sequencing-based diagnostic method comprising extracting nucleic acid material from a sample and at least one control sample, wherein the control sample is prepared prior to extraction, further comprising subjecting the extracted nucleic acid material to a Next Generation Sequencing method, wherein the control sample comprises sample material supplemented with a nucleic acid control which comprises at least one wild-type nucleic acid sequence and at least one mutant of said wild-type sequence.

Definitions

As used in the specification and the claims, the singular forms of “a” and “an” also include the corresponding plurals unless the context clearly dictates otherwise.

It needs to be understood that the term “comprising” is not limiting. For the purposes of the present invention, the term “consisting of” is considered to be a preferred embodiment of the term “comprising”. If hereinafter a group is defined to comprise at least a certain number of embodiments, this is also meant to encompass a group which preferably consists of these embodiments only. When a nucleic acid control or a target nucleic acid sequence comprises a nucleic acid (sequence) that means that the presence of additional residues in the sequence comprising the other is possible, e.g. in plasmid comprising control nucleic acid sequences.

The expressions “target nucleic acid sequence-specific NGS-based diagnostic method” or “NGS-based target nucleic acid detection method” indicate that these methods comprise Next Generation Sequencing, but that additional steps are possible, e.g. extraction and/or purification of nucleic acids from a given sample, any modification of the NGS-protocol as long as the sequencing reaction is conducted

CLAIMS

1. A Next Generation Sequencing-based diagnostic method comprising extracting nucleic acid material from a sample and at least one control sample, wherein the control sample is prepared prior to extraction, further comprising subjecting the extracted nucleic acid material to a Next Generation Sequencing method, wherein the control sample comprises sample material supplemented with a nucleic acid control which comprises at least one wild-type nucleic acid sequence and at least one mutant of said wild-type sequence.
2. The Next Generation Sequencing-based diagnostic method according to claim 1, wherein said nucleic acid control is selected from non-human derived nucleic acids and viral nucleic acids.
3. The Next Generation Sequencing-based diagnostic method according to claim 1 or 2, wherein said nucleic acid control is selected from nucleic acids that are derived from Tobacco Mosaic Virus.
4. The Next Generation Sequencing-based diagnostic method according to any one of claims 1 to 3, wherein said method is suitable for the detection of the presence or absence of sequences derived from an infectious agent or from an oncogene.
5. The Next Generation Sequencing-based diagnostic method according to any one of claims 1 to 4, wherein the presence or absence of a target sequence derived from an infectious agent or from an oncogene and the presence of at least one nucleic acid control sequence subsequent to the sequencing reaction are indicative of accomplished nucleic acid extraction from the samples.
6. The Next Generation Sequencing-based diagnostic method according to any one of claims 1 to 5, further comprising a contamination control.
7. The Next Generation Sequencing-based diagnostic method according to claim 6, wherein the contamination control comprises at least one nucleic acid control as

defined in any one of claims 1 to 3, and wherein the contamination control does not comprise sample material.

8. The Next Generation Sequencing-based diagnostic method according to claim 6 or 7 comprising subjecting the contamination control to a target nucleic acid-specific sequencing reaction.
9. The Next Generation Sequencing-based diagnostic method according to any one of claims 6 to 8, comprising subjecting the contamination control to a target nucleic acid specific sequencing reaction, wherein the absence of detection of a target nucleic acid-specific sequence in the contamination control and the detection of the presence of a control nucleic acid-specific sequence are indicative of the absence of contamination.
10. The Next Generation Sequencing-based diagnostic method according to any one of claims 1 to 9, wherein said control nucleic acid in the control sample is present in an amount of 0.1 to 10 fg subsequent to extraction of the nucleic acids from the samples.
11. The Next Generation Sequencing-based diagnostic method according to any one of claims 1 to 10, wherein the sample is a clinical sample derived from a human subject, and wherein the control nucleic acid is a plasmid comprising wild-type and/or mutant sequences of a non-human virus, selected from animal viruses not infecting humans, or plant viruses, or wherein the control nucleic acid has been artificially designed and has no equivalent in nature.