

Irrespective of benefits offered by NGS technologies a number of challenges that must be adequately addressed before they can be transformed from research tools to routine clinical practices. NGS technologies for diagnostic purposes should require as little manual steps, include adequate mechanisms for preventing contamination by nucleic acid material originating from other sources than the clinical sample that is subject to analysis at a given time point, and the methods should be fast and should be easily performed by staff working in a clinical laboratory.

Different NGS techniques have been developed, which involve physico-chemical mechanisms resulting in distinct methods used in the analysis of respective nucleic acid sequences. These techniques are generally known in the technical field. The most widely applied techniques are Ion semiconductor (Ion Torrent) sequencing, pyrosequencing and sequencing by synthesis (Illumina).

Summary

In an aspect, there is provided a method of preparing a DNA library comprising the steps: a) extracting nucleic acids from a sample, b) exposing the extracted nucleic acids to a mixture comprising UDG, a DNA polymerase and optionally a reverse transcriptase, and dUTP, c) incubating the mixture to decontaminate the mixture from carry over amplification products derived from prior amplification reactions, d) performing an amplification reaction in the presence of dUTP, wherein the decontamination reaction, optionally reverse transcription and DNA polymerase-conducted amplification reaction are performed in the same reaction mixture, wherein the DNA library is subsequently used in a next generation sequencing reaction.

In another aspect, there is provided a reagent composition for use in a next generation sequencing reaction, the composition comprising an enzyme mix comprising a UDG, a DNA polymerase, optionally a reverse transcriptase and Taq DNA Polymerase or a functional derivative thereof.

In yet another aspect, there is provided a method of decontaminating a reaction mixture for the amplification of nucleic acid templates comprising said nucleic acid templates, a DNA polymerase, a UDG enzyme and optionally a reverse transcriptase, and dUTP, and reagents for DNA polymerization, and optionally

reverse transcription, the method comprising inactivating the UDG enzyme after incubating the mixture for a period sufficient to decontaminate the mixture from carry over amplification products derived from prior amplification reactions, wherein the DNA library produced from the amplification reactions is subsequently used in a next generation sequencing reaction.

~~In still another aspect~~an embodiment, there is provided a method for the preparation of a DNA library comprising the steps: a) extracting nucleic acids from a sample, b) exposing the extracted nucleic acids to a mixture comprising a DNA polymerase and optionally a reverse transcriptase, c) performing an amplification reaction in the presence of dUTP, d) normalizing the obtained amplification products, wherein the normalization method comprises the following steps: (i) adding a buffer composition comprising an alkali metal salt and a solvent to the amplification mixture comprising amplification products, (ii) adding carrier particles to the amplification mixture comprising amplification products, (iii) incubating the mixture for a time sufficient for the DNA to bind to the carrier particles, (iv) washing the mixture with ethanol, (v) elution of normalized PCR products from the carrier particles.

Definitions

Having described the method of the invention generally, each aspect of this method will be described in greater detail.

As used herein, the nucleic acid being sequenced is referred to as the target nucleic acid (or the target). Target nucleic acids include but are not limited to DNA such as but not limited to genomic DNA, mitochondrial DNA, cDNA and the like, and RNA such as but not limited to mRNA, miRNA, and the like. The target nucleic acid may derive from any source including naturally occurring sources or synthetic sources. The nucleic acids may be PCR products, cosmid, plasmids, naturally occurring or synthetic library members or species, and the like. The invention is not intended to be limited in this regard. The nucleic acid may be from animal or pathogen sources including without limitation mammals such as humans, and microbes such as bacteria, viruses, fungi, parasites, and mycobacteria. In some embodiments, the nucleic acid is not a viral nucleic acid. The target nucleic acid can be obtained from

Claims

1. A method of preparing a DNA library comprising the steps:
 - a) Extracting nucleic acids from a sample,
 - b) Exposing the extracted nucleic acids to a mixture comprising UDG, a DNA polymerase and optionally a reverse transcriptase, and dUTP,
 - c) Incubating the mixture to decontaminate the mixture from carry over amplification products derived from prior amplification reactions,
 - d) Performing an amplification reaction in the presence of dUTP,

wherein the ~~the~~ decontamination reaction, optionally reverse transcription and DNA polymerase-conducted amplification reaction are performed in the same reaction mixture, wherein the DNA library is subsequently used in a next generation sequencing reaction.

2. The method according to claim 1, wherein the DNA polymerase is a *Thermus aquaticus* (Taq) DNA polymerase, or a functional derivative thereof, wherein the functional derivative of Taq polymerase has at least 80%, preferably at least 90%, more preferably at least 100% of the DNA polymerization activity of Taq polymerase.

3. The method according to any one of claims 1 and 2, wherein the extracted nucleic acids are fragmented prior to step b).

~~4. The method according to any one of claims 1 to 3, wherein the DNA library is subsequently used in a next generation sequencing reaction.~~

~~54.~~ A reagent composition for use in a next generation sequencing reaction, the composition comprising an enzyme mix comprising a UDG, a DNA polymerase, optionally a reverse transcriptase and Taq DNA Polymerase or a functional derivative thereof.

- ~~65.~~ The reagent composition according to claim ~~5-4~~ further comprising dUTP.

~~7. The reagent composition comprising Taq DNA Polymerase or a functional derivative thereof.~~

~~86. The reagent composition comprising reagents for reverse transcription and/or PCR.~~

~~97. A method of decontaminating a reaction mixture for the amplification of nucleic acid templates comprising said nucleic acid templates, a DNA polymerase, a UDG enzyme and optionally a reverse transcriptase, and dUTP, and reagents for DNA polymerization, and optionally reverse transcription.~~

~~10. The method according to claim 9, the method comprising inactivating wherein the UDG enzyme is inactivated after incubating the mixture for a period sufficient to decontaminate the mixture from carry over amplification products derived from prior amplification reactions, wherein the DNA library produced from the amplification reactions is subsequently used in a next generation sequencing reaction.~~

~~118. The method according to any one of claims 9 and 10, wherein the DNA polymerase is a *Thermus aquaticus* (Taq) DNA polymerase, or a functional derivative thereof.~~

~~129. The method according to any one of claims 9 to 11 and 8, wherein the extracted nucleic acids are fragmented prior to step b).~~

~~1310. The method according to any one of claims 1 to 3 and 7 to 9 to 12, wherein the DNA library is subsequently used in a next generation sequencing reaction is selected from the group consisting of Ion semiconductor sequencing, pyrosequencing and sequencing by synthesis.~~

~~14. A method for the preparation of a DNA library comprising the steps:~~

~~a) Extracting nucleic acids from a sample,~~

~~b) Exposing the extracted nucleic acids to a mixture comprising a DNA polymerase and optionally a reverse transcriptase,~~

~~c) Performing an amplification reaction in the presence of dUTP,~~

~~d) normalizing the obtained amplification products, wherein the normalization method comprises the following steps:~~

- ~~(i) adding a buffer composition comprising an alkali metal salt and a solvent to the amplification mixture comprising amplification products,~~
- ~~(ii) adding carrier particles to the amplification mixture comprising amplification products,~~
- ~~(iii) Incubating the mixture for a time sufficient for the DNA to bind to the carrier particles,~~
- ~~(iv) Washing the mixture with ethanol,~~
- ~~(v) Elution of normalized PCR products from the carrier particles.~~

~~15. The method according to claim 14, wherein the alkali metal salt is NaCl.~~

~~16. The method according to any one of claims 14 and 15, wherein the solvent is polyethylene glycol.~~

~~17. The method according to any one of claims 14 to 16, wherein the alkali metal salt is added in an amount of about 2.0 to about 5.0 M NaCl, preferably about 2.5 M NaCl.~~

~~18. The method according to any one of claims 14 to 17, wherein the solvent is PEG 8000.~~