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SPECIFICATION

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APPARATUS FOR IMMOBILIZED DNA LIBRARY PREPARATION, APPARATUS FOR GENE AMPLIFICATION, METHOD FOR TEMPERATURE CONTROL AND METHOD FOR COMPARING GENES SYSTEMATICALLY

INDUSTRIAL FIELD

The present invention relates to a reaction apparatus utilized to various reactions by heating a small amount of solution in a field of molecular biological and biochemistry such as gene engineering, protein engineering, cell engineering, embryology engineering and immunology engineering. Particularly, the present invention relates to a method for immobilized DNA library preparation, a method for gene amplification, a method for comparing genes systematically, an apparatus therefor and immobilized DNA library therefore.

BACKGROUND OF THE INVENTION

Conventionally, a polymeraze-chain-reaction (PCR) method has been broadly utilized as means for amplifying genes. In the PCR method, a tube-shaped plastic reaction container in which reaction solution is filled is inserted into an aluminum block and so on with a cap for preventing the reaction solution from evaporating. A temperature of the aluminum block is periodically changed to a thermal metamorphic temperature, an annealing temperature and a DNA synthetic temperature in order. Such

a thermal control cycle is repeated for a predetermined number.

However, in the conventional PCR method, a used device is a plastic reaction container of which thermal capacity is relatively large and thermal conductivity is relatively low. Further, the thermal control is operated with respect to an aluminum block itself. Therefore, it needs to spend a long time for heating/cooling.
A target duplicated amount of DNA is relatively low, since thermal control of the reaction solution is not operated completely.

A purpose of the present invention is to resolve the above drawbacks and to provide an apparatus for gene amplification with simple operation wherein a step of heating/cooling can be operated rapidly and a thermal cycle of the reaction can be shortened and a method for amplifying genes.

Another purpose of the present invention is to provide substrates for immobilizing DNA and a method for preparing immobilized DNA library suitable to the above apparatus.

Another purpose of the present invention is to provide a method for comparing genes systematically by the above apparatus.

DISCLOSURE OF THE INVENTION

An apparatus for preparing immobilized DNA library according to the present invention is an apparatus

comprising a reaction body on which a groove portion for receiving a container is provided and a cap portion provided at an upper portion of the reaction body with means for heating/cooling and means for cooling, wherein the apparatus is characterized in that the container is made of at least one substrate chemically modified.

A gene amplifying apparatus according to the present invention is an apparatus comprising a reaction body on which a groove portion for receiving a container is provided and a cap portion provided at an upper portion of the reaction body with means for heating/cooling and means for cooling, wherein the apparatus is characterized in that the container is made of at least one substrate chemically modified.

A gene amplifying apparatus according to the present invention is an apparatus comprising a reaction body on which a groove portion for receiving a container is formed and a cap portion provided at an upper portion of the reaction body with means for heating/cooling and means for cooling, wherein the apparatus is characterized in that the container is made of at least one substrate for immobilizing genes.

In these apparatus, it is preferable that the substrates are solid-state substrate and the container is a separable type and a cassette type.

In a method for temperature control by the apparatus for amplifying genes according to the present invention, the method is characterized that a signal from

a temperature measuring portion 61 is input to a computer control portion 64 and the computer control portion 64 compares with the signals and program chart previously input so that a thermal control portion 62 of means for heating/cooling 51 and a thermal control portion 63 of means for cooling 52 can be driven.

The substrates for immobilizing DNA according to the present invention are characterized in that the substrates are utilized in the above apparatuses.

A method for preserving the substrates in a refrigerator according to the present invention is characterized in that the substrates for immobilizing DNA is preserved in the refrigerator.

A method for comparing genes systematically according to the present invention is characterized in that a plurality of DNA-immobilized libraries are set in a container.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a plan view of a gene amplifying apparatus according to the present invention, wherein a cap is opened.

Fig. 2 shows a cross sectional view taken a line A-A in Fig. 1.

Fig. 3 shows a cross sectional view taken a line B-B in Fig. 1.

Fig. 4 shows a process how to make a container. Fig. 5 shows a cross sectional view of the gene amplifying

apparatus according to the present invention.

Fig. 6 shows a process how to control a temperature of the gene amplifying apparatus according to the present invention.

Fig. 7 shows a process how to produce immobilized cDNA library and to amplify gene.

Fig. 8 shows a porcess how to produce immobilized gDNA library and to amplify gene.

BEST MODE FOR CARRYING OUT THE INVENTION

A gene amplification apparatus according to the present invention will be described.

Fig. 1 shows a plane view of the gene amplification apparatus according to the present invention, wherein a cap is opened. Fig. 2 shows a cross sectional view of the gene amplification apparatus according to the present invention taken a line A-A in Fig. 1. Fig. 3 shows a cross sectional view of the apparatus taken along a line B-B in Fig. 1. Fig. 4 shows a process how to make a container. Fig. 5 shows a cross sectional view of the gene amplification apparatus according to the present invention.

As shown in Fig. 1 to Fig. 3, numeral 10 indicates a reaction body and numeral 11 indicates a grooved portion formed on the reaction body. It is preferable that the reaction body 10 is made of chemically stable polymer material such as acrylic resin and polystyrene resin. At least one grooved portion 11 is formed on the reaction body. A shape of the grooved portion is preferable cylindrical

or polygon pole such as triangle pole and square pole. It is preferable that a container 12 is made substrate 41 with excellent thermal conductive character, for example, of a diamond substrate, a ceramic substrate such as an aluminum nitride substrate and a silicone carbide substrate and a metallic substrate such as an aluminum substrate and a stainless steel substrate.

Alternatively, it is preferable a diamond-like carbon substrate of which a part is made of diamond, a carbon substrate and a carbon compound substrate. It is also preferable a plastic substrate such as a polycarbonate substrate and a fluorocarbon substrate. Further, it is preferable a substrate combined with the above described substrates.

The container 12 may have a bottom portion and is preferably attached to/detached from the grooved portion 11 easily.

The container 12 preferably comprises substrates 41 divided into a bottom portion and side wall portions. For example, in the case of a square pole container, the substrates can be divided to a bottom wall portion and four side wall portions. As shown in Fig. 4(a), each edge of the respective substrate 41 is from 5mm to 10mm and a thickness of the each substrate 41 is about 0.1mm. As shown in Fig.4 (b), a container 12 may be assembled by a bottom portion 43 and side wall portions 44 with a synthetic resin substrate holder 42 (see Fig. 4(c)).

In such a case, it is preferable that the container

12 comprises a bottom portion or at least one side wall portion, wherein genes are immobilized on at least one substrate of the portion.

Further, these containers 12 may be connected with each other (cassette type) so that a plurality of containers can be attached to/detached from a plurality of grooved portions 11 in one action so as to improve operating efficiency. A detailed description of the cassette type containers will be described at a description of immobilized DNA library.

Fig. 5 shows a cross sectional view of a gene amplification apparatus according to the present invention. Fig. 5(a) shows a reaction container 10 with a cap portion 50 mounted on an upper portion of the reaction container 10. Fig. 5(b) shows a cross sectional view taken a line C-C in Fig. 5(a). In Fig. 5, numerals 50, 51 and 52 indicates a cap portion, means for heating/cooling and means for cooling, respectively. It is preferable that the cap portion 50 is made of chemically stable polymeric material such as acrylic resin, polystyrene resin and etc.

Means for heating/cooling 51 is means for heating/cooling the container 12 by contacting with the container 12. It is preferable that means for heating/cooling 51 is Peltier element, an electric heater, an infrared lamp and so on. By directly contacting with the container 12, a temperature of the container 12 can be directly controlled. Upon comparing with a conventional amplification apparatus and the apparatus according to the

present invention, a thermal control accuracy of the present invention is superior. Means for cooling 52 is a unit comprising a pipe into which coolant or cool air is flown or a cooling fun so as to cool the container 12 rapidly. Fig. 6 shows a thermal control unit of a gene amplification apparatus according to the present invention. In Fig. 6, a temperature of the container 12 is controlled by a temperature control portion 62 of means for heating/cooling 51 and a thermal control portion 63 of means for cooling 62 in accordance with information from a temperature measuring portion 61. A signal from the temperature measuring portion 61 that is directly contacted with a bottom portion or a side wall portion of the container 12 is input to a computer control portion 64. The computer control portion 64 compares the input signal and a program chart (temperature increasing speed, maintained temperature, maintained time, temperature decreasing speed, and so on) previously input. As the result, the temperature control portion 62 of means for heating/cooling 51 and the temperature control portion 63 of means for cooling 52 can be driven.

EMBODIMENTS

Embodiments according to the present invention will be described with reference to Fig. 7 and Fig. 8. As a container 12, diamond chips 41 (5mm x 5mm, thickness 0.1mm) are utilized as diamond substrates. As shown in Fig. 4, one diamond chip 41 is utilized as a bottom portion and

four diamond chips are utilized as side wall portions. These diamond chips are assembled with a synthetic resin substrate holder 42 so as to form a container 12. The diamond chips 41 utilized as the side wall portions are chemically modified with organic carbonic acid. After assembling the container 12, the cap portion 50 is mounted on an upper portion of the reaction body 10.

(Preparing immobilized DNA library)

In the next, an example of preparing immobilized DNA library will be described with reference to Fig. 7 and Fig. 8. At first, in the case of mRNA (messenger RNA), oligo dT_{16-20} are immobilized on surfaces of the four diamond chips 41 contacting with solution in the container 12 by a chemical ester linkage reaction. In the case of gDNA (genomic DNA), oligonucleotide having restricted enzyme portion are immobilized on the surfaces of the diamond chips 41 by the chemical ester linkage reaction (see Fig.7 and Fig.8). The four diamond chips 41 have been chemically modified with organic carbonic acid. In order to immobilize, a 3' terminal of the oligo dT_{16-20} and a 5' terminal of the oligonucleotide are requested to connect with adenine(A) or cytosine(C) or guanine(G) which comprises amino radical. In Fig. 7 and Fig. 8, a mark "ADC-R-" indicates a diamond chip chemically modified with organic carbonic acid.

In the next, in the case of cDNA (complementary DNA), the total RNA solution containing mRNA is extracted from tissues and cells, and mRNA is hybridized with

oligo-dT_{16-20} at a low temperature of 0℃ to 4℃. After hybridization, cDNA is synthesized by Reverse Transcriptase (RT) at a suitable constant temperature of 37℃ to 60℃. The cDNA are reacted and immobilized so as to extend toward the terminal 5' of hybridized oligo-dT_{16-20}. Hybridized solution of synthesized immobilized cDNA and mRNA is heated up to 90℃ in order to dehybridize mRNA. Then, the reaction solution is exchanged to Tris-EDTA (TE) buffer solution. The reaction solution is cleaned with ethanol at a low temperature of 0℃ to 4℃ again. As the result, purified immobilized cDNA library in one chain DNA condition is prepared (see Fig. 7).

In the case of gDNA library, immobilized olegonucleotide having target restrictive enzyme (see Fig. 8) are immobilized on surfaces of the diamond chips 41 as similar as oligo-dT_{16-20}. In the next, in order to chemically immobilize, the reaction solution is exchanged to reaction solution including hybridized oligonucleotide with target restrictive enzyme at a low temperature of 0℃ to 4℃. After hybridization with olegonucleotide, the reaction solution is heated up to 37℃ so as to cut restrictive enzyme of semi-immobilized olegonucleotide.

After cutting the restrictive enzyme, the reaction container is cooled down at a low temperature of 0℃ to 4℃ again. The reaction solution is exchanged to reaction solution including gDNA of which target restrictive enzyme is cut and Ligase enzyme. The exchanged solution is heated up to 37℃ again so as to react Ligase reaction. AS the

result, gDNA library in one chain DNA condition can be prepared (see Fig. 8).

Immobilized cDNA library prepared on surfaces of the diamond chips 41 or immobilized gDNA library prepared on the surfaces of the diamond chips 41 can be set in the different containers so as to compare systematically for the purposes (1) to (3).

(1) To compare gene variation of the same kind of tissues and cells of the plurality of samples.

(2) To compare gene production and variation of each tissue and cell of the same sample.

(3) To compare gene production and variation of the original genes and genes after medical treatment, sergeant and so on with respect to the same sample.

For example, in the case of (1), the plurality of samples (a plurality of diamond chips having immobilized DNA library) are set in different containers so as to compare gene variation of the same kind of tissues and cells. These pluralities of containers are designated as one cassette. These cassettes are attached to a reaction body. If two or more than cassettes are prepared systematically, a plurality of samples can be compared effectively.

In the present invention, one cassette in which a plurality of containers are connected or a plurality of cassettes are set as an aggregation. Immobilized DNA library is set in each container of the aggregation, which is designated as a cassette type immobilized DNA library. In the present invention, these cassette type immobilized

DNA library are utilized to compare the above described purposes (1) and (2) systematically so that variation of genes can be searched effectively.

Even if the PCR method is utilized or not, the cassette type immobilized DNA library can be utilized semi-permanently in accordance with the request of the comparison data and so on by sufficiently cleaning the containers with TE buffer solution and ethanol solution (70~75%) and refrigerating and preserving the containers by immersing in ethanol solution (100%).

(Amplifying genes with immobilized DNA library)

Side wall portions of the container 12 are formed by cassette type immobilized DNA libraries of the above immobilized cDNA or gDNA. Inner surfaces of the container 12 are sufficiently cleaned with TE buffer solution. Then, a primer with respect to amplified DNA is set and PCR reaction solution including four kind of nucleotide and DNA polymerase is added. After adding the solution, the container 12 is thermally changed to a thermal metamorphic temperature for dividing two chain DNA into one chain DNA (95℃, about 1.5 minute), an annealing temperature for connecting the one chain DNA and DNA primer (45℃, about 1 minute) and a DNA amplification temperature for extending DNA chain by heat-resistant DNA polymerase (74℃, about 2 minute) in order. This thermal control is repeated 30times in order to operate the PCR method (see Fig. 7 and Fig. 8). In order to search genes of a plurality of samples one time for a relatively short time, it may provide a system in which

necessary number of apparatuses for preparing the cassette type immobilized DNA library and amplifying genes are juxtaposed. For example, in the case of 50 samples, five apparatuses for amplifying genes according to the present invention may be juxtaposed.

Opportunity for utilizing in the industry field

The apparatus according to the present invention can heat and cool reaction solution in a reaction container rapidly so that DNA can be amplified remarkably short time. The apparatus according to the present invention can be suitable to an automatic gene diagnosis apparatus. In a development of the automatic gene diagnosis apparatus, the most important technical problems are a preparation of a cassette type immobilized library utilized semi-permanently and a development of a gene amplification apparatus having excellent efficiency and specificity in view of gene amplification.

The both described problems can be resolved by providing a high performance automatic cassette type immobilized DNA library and a gene amplification apparatus with the most simple operation and the most compact size since thermal control from a step of preparation of the library in the PCR method can be controlled by the same substrates.

That is, in accordance with an apparatus according to the present invention, thermal control for amplifying genes can be directly controlled by utilizing diamond chips

immobilized DNA library in addition to thermal control for directly controlling DNA immobilized reaction.

In accordance with an apparatus according to the present invention, thermal control for PCR reaction solution can be directly controlled by utilizing substrates such as high conductivity diamond substrates so that thermal control efficiency of the PCR method can be remarkably improved and the necessary time can be remarkably shortened. In addition, the PCR efficiency such as specifically amplification of target DNA can be improved.

If the substrates according to the present invention are high thermal conductivity, a prepared immobilized DNA library becomes very stable, since the diamond substrates is stable with respect to chemical material/solution, excellent in view of heat-resistant and strong with respect to radiation exposure. Once a cassette type immobilized DNA library is prepared, it is possible to provide a DNA library repeatedly utilized for semi-permanent.

By utilizing high thermal conductivity of diamond substrates, DNA-immobilized chips having DNA immobilized on diamond substrates without chemically modified may be set in an amplification apparatus according to the present invention so as to operate the conventional PCR method.

What is claimed is:

1. An apparatus for preparing an immobilized DNA library comprising:

a reaction body on which a grooved portion for receiving a container is formed, and

a cap portion provided at an upper portion of said reaction body, said cap portion including means for heating/cooling and means for cooling,

wherein said container is formed by substrates chemically modified.

2. An apparatus for amplifying genes comprising:

a reaction body on which a grooved portion for receiving a container is formed, and

a cap portion provided at an upper portion of said reaction body, said cap portion including means for heating/cooling and means for cooling,

wherein said container is formed by substrates chemically modified.

3. An apparatus for amplifying gene comprising:

a reaction body on which a grooved portion for receiving a container is formed, and

a cap portion provided at an upper portion of said reaction body, said cap portion including means for heating/cooling and means for cooling,

wherein said container is formed by substrates for immobilizing DNA.

4. An apparatus as claimed in one of claims 1 through 3, wherein said substrates are solid-state substrates.

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5. An apparatus as claimed in one of claims 1 through 4, wherein said container can be divided.

6. An apparatus as claimed in one of claims 1 through 5, wherein said container is a cassette type.

7. A method by utilizing an apparatus as claimed in one of claims 1 through 6, wherein a signal from a temperature measuring portion 61 is input to a computer control portion 64, the computer control portion 61 compare the input signal and program chart previously input and then a temperature control portion 62 of means for heating/cooling 51 and a temperature control portion 63 of means for cooling 52 are driven.

8. A substrate for immobilizing DNA utilized by an apparatus as claimed in one of claims 1 through 6.

9. A method for refrigerating and preserving substrates for immobilizing DNA.

10. A method for comparing genes systematically by setting a plurality of DNA immobilized library in a container.

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