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(54) Titre : PROCEDE D'ANALYSE D'ACIDES NUCLEIQUES AMPLIFIES
(54) Title: METHOD FOR ANALYZING AMPLIFIED NUCLEIC ACIDS

(57) Abrégé/Abstract:
The invention relates to a method for analysing nucleic acids in a microfluidic device. Said method consists of the following steps: a) nucleic acids are amplified in a first chamber in the microfluidic device; b) the amplified nucleic acids are brought into contact with an additive comprising: i) monovalent cations and ii) an Mg2+ ion-binding agent, the additive being provided in a second chamber in the microfluidic device; and c) the amplified nucleic acids are hybridised on at least one probe oligonucleotide.

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WO 2007/147712 A1

(54) **Title:** METHOD FOR ANALYSING AMPLIFIED NUCLEIC ACIDS

(54) **Bezeichnung:** VERFAHREN ZUR ANALYSE VON AMPLIFIZIERTEN NUKLEINSÄUREN

(57) **Abstract:** The invention relates to a method for analysing nucleic acids in a microfluidic device. Said method consists of the following steps: a) nucleic acids are amplified in a first chamber in the microfluidic device; b) the amplified nucleic acids are brought into contact with an additive comprising: i) monovalent cations and ii) an Mg2+ ion-binding agent, the additive being provided in a second chamber in the microfluidic device; and c) the amplified nucleic acids are hybridised on at least one probe oligonucleotide.

(57) **Zusammenfassung:** Verfahren zur Analyse von Nukleinsäuren in einer mikrofluidischen Vorrichtung, aufweisend die folgenden Schritte: a) Amplifizieren von Nukleinsäuren in einer ersten Kammer in der mikrofluidischen Vorrichtung; b) in Kontakt Bringen der amplifizierten Nukleinsäuren mit einem Zusatz, aufweisend: i) monovalente Kationen und ii) ein Mg2+ Ionen-bindendes Agens, wobei der Zusatz in einer zweiten Kammer in der mikrofluidischen Vorrichtung vorgesehen ist; und c) Hybridisieren der amplifizierten Nukleinsäuren an mindestens ein Sonden-Oligonukleotid.

Description

Method for analyzing amplified nucleic acids

The invention relates to a method for analyzing amplified nucleic acids in a microfluidic device. The invention furthermore relates to an arrangement for carrying out such a method.

Prior Art

DNA analysis by hybridization is a known method in molecular biology (cf. "Gentechnische Methoden", ["Genetic Engineering Methods"], G. Gassen and G. Schrimpf, Spektrum Akademischer Verlag Heidelberg, 1999, pages 243 to 261). This technique plays an important part in the detection of specific nucleic acids, e.g. in the molecular diagnosis of single point mutations (single nucleotide polymorphism, SNP). In this case, a probe oligonucleotide comprising a sequence of e.g. approximately 20 nucleotides is used to bind to nucleic acids that differ only in a single nucleotide. It is noted that in the present context the expression "nucleic acids" is intended to encompass a nucleic acid sequence, e.g. a DNA sequence or RNA sequence. Given a suitable choice of the hybridization conditions (in particular temperature and salt concentration), the probe oligonucleotide selectively binds the non-mutated variant of the nucleic acid, while the nucleic acid variant having the single point mutation does not bind, or binds only weakly. Detection of single point mutations is thereby possible. On account of the small differences in terms of binding energy between the variant without a mutation (that is to say the wild type) and the mutant, the reaction conditions with regard to temperature and also composition and salt concentration of the reaction solution have to satisfy exact stipulations.

Since the corresponding nucleic acids in the sample material (e.g. blood) are usually not available in

sufficient quantity or concentration, it is necessary to amplify the nucleic acids to be examined. This amplification can be effected in a sequence-specific manner by various methods known in molecular biology, e.g. by SDA (strand displacement amplification), described in Walker, GT, *et al.*, "Strand Displacement Amplification, an isothermal, in vitro DNA Amplification Technique", Nucleic Acids Research, 1992, 20, 1961 to 96; by TMA (transcription mediated amplification); or by polymerase chain reaction (PCR), described in US 4 683 195, inter alia. One problem here is that the composition of the reaction solution of the amplification reaction, and hence the "amplification crude product", does not have the composition, and in particular salt concentrations, required for hybridization. For molecular diagnosis, it may additionally be necessary to selectively separate the hybrids formed in a subsequent process (melting) e.g. by increasing the temperature. In order to enable hybridization processes with a high yield, a high concentration of monovalent cations (e.g. Na^+ ions) is necessary, inter alia. Monovalent cations promote the formation of the double helix structure during the hybridization reaction.

However, the reaction mixtures of the amplification reactions, e.g. for a PCR reaction, contain a low concentration of monovalent cations. Furthermore, PCR reaction buffers have a relatively high concentration (a few mM) of Mg^{2+} ions, which, during the hybridization to detection probes, adversely affect the binding of probes and complimentary strands to form complete hybrids, can bring about an extension of the probes by polymerase activity and, during a subsequent melting process, bring about a stabilization of double strands and make melting more difficult, which leads to "washed out" melting curves at high temperatures.

In accordance with the prior art, amplification products are therefore purified before the hybridization reaction; in this case, all components that disturb a hybridization reaction (inter alia polymerase, primers, nucleotides, salts) are removed and the concentration of Na^+ ions is increased. This purification process is relatively complicated and is usually effected by non-specific binding of the nucleic acids to a solid phase (by means of so-called purification columns), washing of the amplification product on the column and dissolution from the solid phase or by phenol/chloroform extraction or similar methods. Particularly when carrying out nucleic acid analyses in microfluidic devices, wherein all the reaction processes proceed in an integrated manner and in a small space, the purification methods that are customary in the prior art are not appropriate since their realization is too complicated, under these circumstances.

Statement of Object

It is an object of the present invention to provide a simple and cost-effective method which enables the efficient conditioning of amplified nucleic acids for further method steps, requires no additional binding or washing steps and can be realized with a simple fluidics concept.

Description of the Invention

Expressed in general terms, the concept of the invention resides in amplifying a sample with nucleic acids and admixing the sample containing the amplification crude product with a suitable additive in order to condition the amplification crude product for further method steps, e.g. further analysis steps. Adding the additive avoids the need to purify the amplified nucleic acids. This method is particularly suitable for use in microfluidic devices, in which uncomplicated method sequences with a simple fluidics concept

are preferred. According to the invention, the additive comprises monovalent cations and an Mg^{2+} ion-binding agent. Such an additive is suitable in particular for conditioning amplified nucleic acids for a subsequent hybridization to probe oligonucleotides.

According to the invention, the object is achieved in particular by means of the method as described herein. Advantageous developments are also described herein. An associated arrangement for carrying out the method according to the invention is described herein. Developments of this arrangement are also described herein.

The present invention provides, in particular, a method for analyzing amplified nucleic acids in a microfluidic device, comprising the following steps:

a) amplifying nucleic acids in a first chamber in the microfluidic device;

b) contacting the amplified nucleic acids with an additive, comprising:

i) monovalent cations and

ii) an Mg^{2+} ion-binding agent,

wherein the additive is provided in a second chamber in the microfluidic device; and

c) hybridizing the amplified nucleic acids to at least one probe oligonucleotide.

In a specific embodiment, the invention recites to a method for analyzing nucleic acids in a microfluidic device, comprising the following steps: a) amplifying nucleic acids in a first chamber in the microfluidic device; b) contacting the amplified nucleic acids with an additive, comprising: i) monovalent cations and ii) an Mg^{2+} ion-binding agent, wherein the additive is provided in a second chamber in the microfluidic device; and c) hybridizing the amplified nucleic acids to at least one probe oligonucleotide, wherein the additive in the second chamber is provided as a dry reagent, and wherein the amplified nucleic acids can be contacted with the

additive, such that a mixture of amplified raw product and additive is available for hybridization.

The expression “microfluidic” denotes methods which comprise the handling of fluids having volumes in the microliters range. The microfluidic device is preferably embodied as a cartridge, that is to say as a flat structure

having the form of a card, with depressions formed therein which form channels and chambers or cavities through which liquids can be moved in accordance with predetermined reaction sequences or schemes.

Monovalent cations comprise e.g. Li^+, Na^+, Ka^+, and are present in the additive according to the invention preferably in the form of Na^+ ions. An Mg^{2+} ion-binding agent should be understood to mean all substances that bind Mg^{2+} ions, in particular complexing agents, e.g. Chelate complexing agents such as EGTA or EDTA. The additive used in the method according to the invention preferably comprises EDTA. Furthermore, the additive preferably comprises a binder, e.g. polyvinylpyrrolidone. Further auxiliaries, e.g. buffer substances, surface-active substances, or the like, can likewise be provided.

In accordance with one preferred aspect of the invention, the additive in the second chamber is provided as a dry reagent and is kept therein in storage-stable fashion.

Preferably, the probe oligonucleotides are immobilized as a microarray on a carrier in the microfluidic device.

In accordance with a first embodiment of the method of the present invention, the additive is transferred from the second chamber to the first chamber (the amplification chamber) in order to contact the additive with the amplified nucleic acids. If the additive is provided as a dry reagent in the second chamber in this embodiment of the method according to the invention, it is expedient to dissolve the additive using a solvent, e.g. water. The dissolved additive can then be transferred from the second chamber to the first chamber in order to be mixed there with the amplification crude product.

In accordance with a second embodiment of the present invention, after amplification has been effected, the amplified

nucleic acids in the reaction solution are transferred from the first chamber to the second chamber and then conducted as a mixture with the additive to the probe oligonucleotides. If the additive is provided as a dry reagent in the second chamber, it can be dissolved directly by the reaction solution pumped into the second chamber with the amplification crude product.

According to the invention, it is preferred for the nucleic acids to be amplified by PCR reaction.

In accordance with a further aspect of the present invention, the amplified nucleic acids hybridized to the probe oligonucleotides are then preferably detected. This detection can be effected for example using a label (a marking) of the amplified nucleic acids. The label can be an optical label; it can also be an enzymatic label, for example. An enzymatic reaction can be catalyzed by an enzymatic label, which reaction can be detected e.g. optically or electrochemically. According to the invention, preferably an electrochemical detection is carried out, which particularly preferably comprises a current measurement amplified by means of redox cycling.

In particular, the invention furthermore relates to an arrangement for carrying out the method according to the invention, which is provided in a microfluidic device, comprising a first chamber, which is designed for the amplification of nucleic acids, and a second chamber, in which the additive described above is kept in storage-stable fashion, wherein the second chamber can be connected in fluid communication via a connection to the first chamber. Preferably, the arrangement comprises a microarray arrangement having probe oligonucleotides immobilized on a carrier.

In a specific embodiment, the invention relates to an arrangement for carrying out the method as described above, which is provided in a microfluidic device, comprising: a) a first chamber, which is designed for the amplification of nucleic acids, and b) a second chamber, in which an additive, comprising i) monovalent cations and ii) an Mg^{2+} ion-binding agent, is kept in storage-stable fashion,

wherein the second chamber can be connected in fluid communication via a connection to the first chamber, wherein the additive is kept in the second chamber as a dry reagent.

The connection between the first and second chambers can be embodied in the form of a line or a channel, and

can preferably be selectively opened and closed, e.g. by means of a valve, such that fluid can selectively be transferred from the first to the second chamber, or from the second to the first chamber. Furthermore, means can be provided for introducing a solvent into the second chamber, e.g. in the form of an inlet channel to the second chamber.

Preferably, the microarray arrangement is assigned means for detecting hybridized nucleic acids, which enable an optical or electrochemical detection, for example. Electrochemical detection means which are designed for measuring currents and/or potentials are particularly preferred. The optical detection means can comprise e.g. a transparent region of the device, through which e.g. optical absorption or fluorescence excitation and detection can be read out. The electrochemical detection means preferably enable the measurement of potentials and/or currents and can comprise an electrode system onto which the probe oligonucleotides are immobilized (spotted) at each detection spot, as described for example in the documents DE 101 26 341 A1 or DE 100 58 397 A1.

Furthermore, corresponding means, e.g. in the form of corresponding chambers and/or channels, can be provided for storing and/or passing on reagents for the detection, e.g. enzyme or enzyme substrate, in the microfluidic device.

Means for supplying heat and/or dissipating heat are preferably assigned to the first chamber (that is to say the amplification chamber). Said means can comprise a region having increased thermal conductivity in the microfluidic device, which region can be realized for example by the microfluidic device being embodied in particularly thin-walled fashion in said region. However, it is also conceivable for an element

that generates or dissipates heat to be provided in the microfluidic device itself.

Exemplary embodiment

Further features and advantages of the present invention will become apparent from the following description of figures in association with the exemplary embodiments and with reference to the appended drawings, which are merely by way of example and illustrative.

In the figures:

figure 1 shows a diagrammatic illustration of the method according to the invention;

figure 2 shows a schematic illustration of an arrangement for carrying out the method of the invention in accordance with a first embodiment;

figure 3 shows a schematic illustration of an arrangement for carrying out the method of the invention in accordance with a second embodiment;

figure 4 shows an excerpt from a schematic illustration of a cartridge with the arrangement in accordance with figure 2;

figure 5 shows a comparative illustration of melting curves of hybridized nucleic acids without the use of the method according to the invention; and

figure 6 shows a comparative illustration of melting curves of hybridized nucleic acids with the use of the method according to the invention.

Figure 1 schematically illustrates the basic concept of the method according to the invention for analyzing nucleic acids

in a microfluidic device. In accordance with the prior art it is known to purify amplification crude products prior to hybridization. This constitutes a subtractive method, that is to say that disturbing components (polymerase, primers, nucleotides) are removed from the solution containing the amplification crude product. By contrast, the method according to the invention is an additive method, that is to say that an additive is added to the amplification crude product, which additive enables an improved hybridization reaction, or a subsequent improved, selective separation of the hybrids. In this case it is unimportant whether the additive is added to the amplification crude product or the amplification crude product is added to the additive; what is crucial primarily is that a mixture of amplification crude product and additive is used for the hybridization reaction.

Figure 2 shows an arrangement for carrying out the method according to the invention in accordance with a first embodiment with a first chamber 10a and a second chamber 20a. After the amplification reaction has taken place, the additive in solution is pumped from the second chamber 20a (the additive chamber) into the first chamber 10a (the amplification reaction chamber). If the additive in the second chamber is provided as a dry reagent, firstly a solvent (water) is pumped into the second chamber 20a in order to dissolve the dry reagent, and then the solvent is transferred to the first chamber 10a (amplification reaction chamber).

Figure 3 shows an arrangement for carrying out the method according to the invention in accordance with a second embodiment with a first chamber 10b and second chamber 20b. In this case, the opposite procedure to the method implementation described above takes place: after the amplification reaction, the amplification crude product is conducted from the first

chamber 10b (the amplification reaction chamber) into the second chamber 20b (the additive chamber) and intermixed there with the additive. If the additive is present as a dry reagent,

the amplification crude product is pumped over the dry reagent and dissolves the latter.

In both embodiments, the mixture of amplification crude product and additive is then transferred to the microarray for hybridization.

Figure 4 shows an arrangement in accordance with the embodiment shown in figure 2. Figure 4 shows a cartridge 101, made from a plastic material. Channels 130, 150, 160, chambers 110, 120, 140 and depressions 121 are provided in the cartridge 101. The plastic cartridge can be embodied simply with upwardly open channels, depressions and chambers and can be covered with a film after the reagents have been spotted on, whereby the channels, depressions and chambers incorporated into the surface of the cartridge are closed off. In the first chamber 110, which can be filled via the inlet channel 160, nucleic acids are amplified by PCR reaction, for example. This is done by inserting the cartridge into a corresponding device, such that the chamber 110 can be heated and/or cooled, e.g. by Peltier elements situated in the device. The additive, comprising EDTA and sodium chloride, is present as a dry reagent in the depressions 121 in the second chamber 120, which is embodied as a channel. At one of its ends, the chamber 120 can be closed by a valve 122. This can be embodied for example as a simple pinch valve. After the PCR reaction has taken place, water is pumped into the chamber 120, and the additive stored as a dry reagent is thereby dissolved. Afterward, the valve 122 is opened and the dissolved additive can then be pumped into the amplification chamber 110, where the dissolved additive intermixes with the amplification crude product, and the mixture is then pumped via the channel 130 into the hybridization chamber 140, in which a microarray with probe oligonucleotides immobilized on a carrier is provided. By a suitable choice of the fluidics, e.g. geometry or flow rates, it is possible to realize the effect whereby

firstly unchanged amplification product is conveyed at the beginning of the pumping process and additive substances are increasingly admixed only upon further pumping. Consequently, a possibly advantageous rising concentration gradient of additive substances can be pumped into the hybridization chamber. The hybridization reaction can then take place in this chamber. Excess solution is fed via the outlet channel 150 to a waste container. Further chambers and channels can be provided (not shown) on the cartridge in order e.g. to keep ready reagents for the detection of the bound nucleic acids, such as, for instance, enzyme or enzyme substrate.

Example

An injection-molded plastic card of the type shown in figure 4 was used for recording the curves shown in figures 5 and 6, in which card dry reagent was spotted on in the depressions 121. In order to evaluate the method according to the invention, the gene factor V wild type (FcV wild type) and the single point mutation factor V Leiden (FcV Leiden) were amplified by PCR so as to obtain a PCR product having a size of 168 bp. The gene product of the factor V gene is a protein of the blood coagulation cascade, and the mutation is described in Bertima *et al.*, Nature, 1994; 369(6475):64-7.

The DNA sample is introduced into the first chamber (amplification chamber) 110 and the PCR reaction is carried out. The channel 120 with the depressions 121 is filled with water, and the dry reagent is thereby dissolved. The additive spotted on as a dry reagent is dimensioned such that a solution comprising 0.23M NaCl, 0.1M EDTA results upon addition of approximately 25 µl of water. This ensures a sufficient concentration of monovalent cations and the minimization of the concentration of free Mg^{2+} by complexing. Furthermore, a buffer

substance is added to the additive, such that the solution is adjusted to a pH value of pH=8. After the end of the PCR reaction, as a result of the dissolved additive flowing

into the first chamber 110, the PCR crude product (amplification crude product) is mixed with the additive, and the mixture is forwarded into the chamber 140, in which a microarray arrangement is situated. Biotinylated PCR products are obtained by using biotinylated primers during the PCR reaction. Probe oligonucleotides which can be used to discriminate between FcV wild type and FcV Leiden are spotted on the microarray arrangement. The probe oligonucleotides are chosen such that some spots carry probe oligonucleotides which perfectly match FcV wild type, while other spots carry probe oligonucleotides which perfectly match FcV Leiden. If the PCR product contains FcV wild type sequences, for example, then they form a "perfect match" (a complete pairing of strand and counter strand) with the wild type probes, but a "single base mismatch", having a lower binding strength, with the FcV Leiden probes. The PCR products bound to probe molecules are rinsed with a washing solution containing a streptavidin-conjugated enzyme (alkaline phosphatase). The enzyme binds to the biotinylated PCR products bound on the microarray arrangement. The microarray arrangement is then rinsed with a substrate solution containing p-aminophenyl phosphate. The p-aminophenyl phosphate is converted to p-aminophenol by the alkaline phosphatase and the p-aminophenol formed is oxidized to form quinone imine in a redox reaction at electrodes of the microarray arrangement and the p-aminophenol/quinone imine redox pair is cyclized, which leads to a measurable current rise at the electrodes. This current rise (dI/dT) is proportional to the amount of bound PCR product. The temperature is then increased step by step over a temperature range by approximately 20°C to approximately 60°C, which leads to a progressive melting of the hybrids at relatively high temperatures (starting from approximately 25°C), wherein the PCR products with single point mutations which have a mismatch in the hybrid melt significantly more rapidly than the "perfect match" hybrids, the wild type hybrids in the example. This results in a detectable signal difference between the

wild type (perfect match) and the single point mutant (single base mismatch). The summary of a plurality of experiments is illustrated in the curves 31a, 31b, 32a, 32b shown in figures 5 and 6. The signal strength is illustrated as a function of the temperature. The curves 31a, 31b show the melting curves for FcV wild type and the curves 32a, 32b show the melting curves for FcV Leiden. It can readily be recognized that with the use of the method according to the invention with the added additive, the melting curves 31b, 32b (figure 6) are reproducible significantly better (that is to say lie closer together in each case for FcV wild type and FcV Leiden) and more distinctive signal differences result than without the use of the method according to the invention, 31a, 32a (figure 5). Primarily at relatively high temperatures (T>35°C), the hybrids melt significantly more cleanly. One advantageous property of this method could be that the concentration of NaCl and EDTA in the chamber 140 (the detection chamber) rises during the hybridization.

It is emphasized that the exemplary embodiment described is merely by way of example, and many kinds of variations with regard to the type and concentration of the additive, the type of detection and the reaction implementation are conceivable.

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CLAIMS:

1. A method for analyzing nucleic acids in a microfluidic device, comprising the following steps:

a) amplifying nucleic acids in a first chamber in the microfluidic device;

b) contacting the amplified nucleic acids with an additive, comprising:

i) monovalent cations and

ii) an Mg^{2+} ion-binding agent,

wherein the additive is provided in a second chamber in the microfluidic device; and

c) hybridizing the amplified nucleic acids to at least one probe oligonucleotide,

wherein the additive in the second chamber is provided as a dry reagent,

and wherein the amplified nucleic acids can be contacted with the additive, such that a mixture of amplified raw product and additive is available for hybridization.

2. The method as claimed in claim 1, wherein the monovalent cations are provided in the form of Na^+ ions.

3. The method as claimed in claim 1 or 2, wherein the Mg^{2+} ion-binding agent is EDTA.

4. The method as claimed in any one of claims 1 to 3, wherein, prior to contacting the additive with the amplified nucleic acids, a solvent is introduced into the second chamber.

5. The method as claimed in claim 4, wherein the additive is transferred in dissolved form from the second to the first chamber in order to contact the additive with the amplified nucleic acids.

6. The method as claimed in any one of claims 1 to 3, wherein the amplified nucleic acids are transferred from the first to the second chamber in order to contact the additive with the amplified nucleic acids.

7. The method as claimed in claim 6, wherein the amplified nucleic acids in solution as amplification crude product are transferred to the second chamber and then conducted as a mixture with the additive to the at least one probe oligonucleotide.

8. The method as claimed in any one of claims 1 to 7, wherein the nucleic acids are amplified by means of PCR reaction.

9. The method as claimed in any one of claims 1 to 8, wherein the at least one probe oligonucleotide is immobilized in the form of a microarray arrangement on a carrier.

10. The method as claimed in any one of claims 1 to 9, wherein the amplified nucleic acids hybridized to the probe oligonucleotide are detected.

11. The method as claimed in claim 10, wherein the detection is effected using a label of the amplified nucleic acids.

12. The method as claimed in claim 11, wherein the label is an optical label.

13. The method as claimed in claim 11, wherein the label is an enzymatic label.

14. The method as claimed in claim 13, wherein the label catalyzes an enzymatic reaction which is optically detectable.

15. The method as claimed in claim 13, wherein the label catalyzes an enzymatic reaction which is electrochemically detectable.

16. The method as claimed in claim 15, wherein the electrochemical detection involves a current measurement amplified by means of redox cycling.

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17. The method as claimed in any one of claims 1 to 16, wherein the additive comprises a binder.

18. An arrangement for carrying out the method as claimed in any of claims 1 to 17, which is provided in a microfluidic device, comprising:

a) a first chamber, which is designed for the amplification of nucleic acids, and

b) a second chamber, in which an additive, comprising

i) monovalent cations and

ii) an Mg^{2+} ion-binding agent,

is kept in storage-stable fashion,

wherein the second chamber can be connected in fluid communication via a connection to the first chamber,

wherein the additive is kept in the second chamber as a dry reagent.

19. The arrangement as claimed in claim 18, wherein the monovalent cations are provided in the form of Na^+ ions.

20. The arrangement as claimed in claim 18 or 19, wherein the Mg^{2+} ion-binding agent is EDTA.

21. The arrangement as claimed in any one of claims 18 to 20, wherein means are provided for transferring fluid selectively from the first to the second chamber.

22. The arrangement as claimed in any one of claims 18 to 20, wherein means are provided for transferring fluid selectively from the second to the first chamber.

23. The arrangement as claimed in claim 18, wherein means are provided for introducing a solvent into the second chamber.

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24. The arrangement as claimed in any one of claims 18 to 23, further comprising a microarray arrangement having probe oligonucleotides immobilized on a carrier.

25. The arrangement as claimed in any one of claims 18 to 24, further comprising means for optically detecting hybridized nucleic acids.

26. The arrangement as claimed in any one of claims 18 to 24, further comprising means for electrochemically detecting hybridized nucleic acids.

27. The arrangement as claimed in claim 26, wherein the electrochemical detection means are designed for measuring currents and/or potentials.

28. The arrangement as claimed in any one of claims 18 to 27, wherein means for supplying heat and/or dissipating heat are assigned to the first chamber.

FIG 1

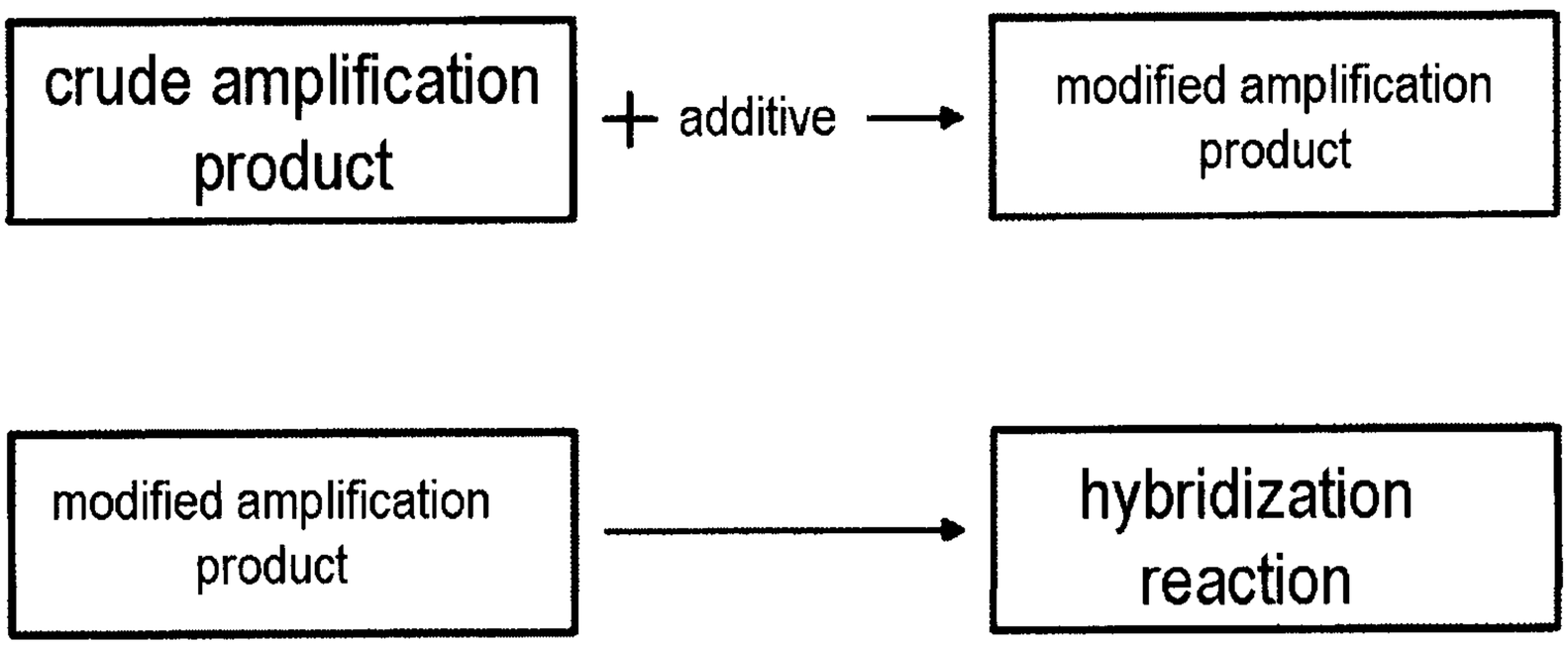
crude amplification product
+ additive
modified amplification product
modified amplification product
hybridization reaction

FIG 2

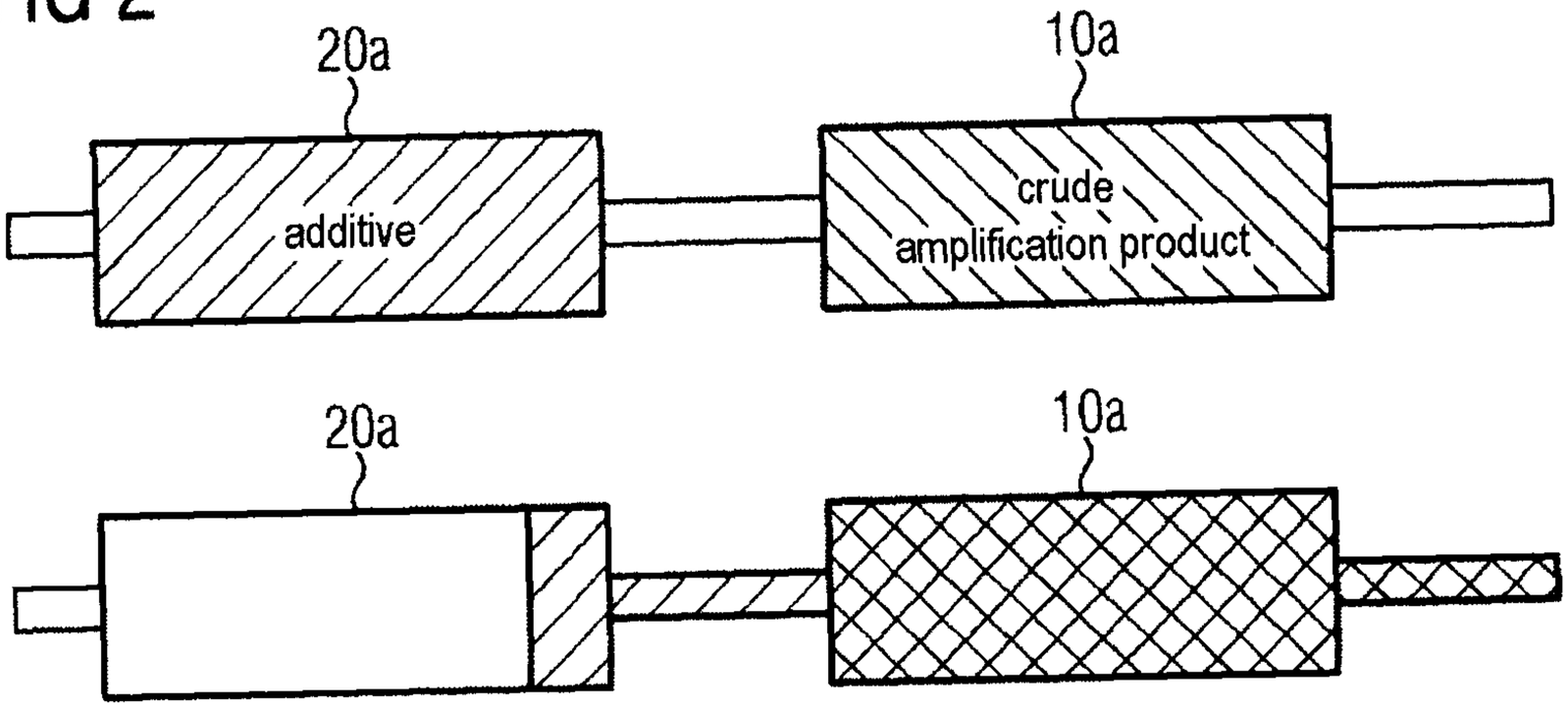
20a
10a
additive
crude
amplification product
20a
10a

FIG 3

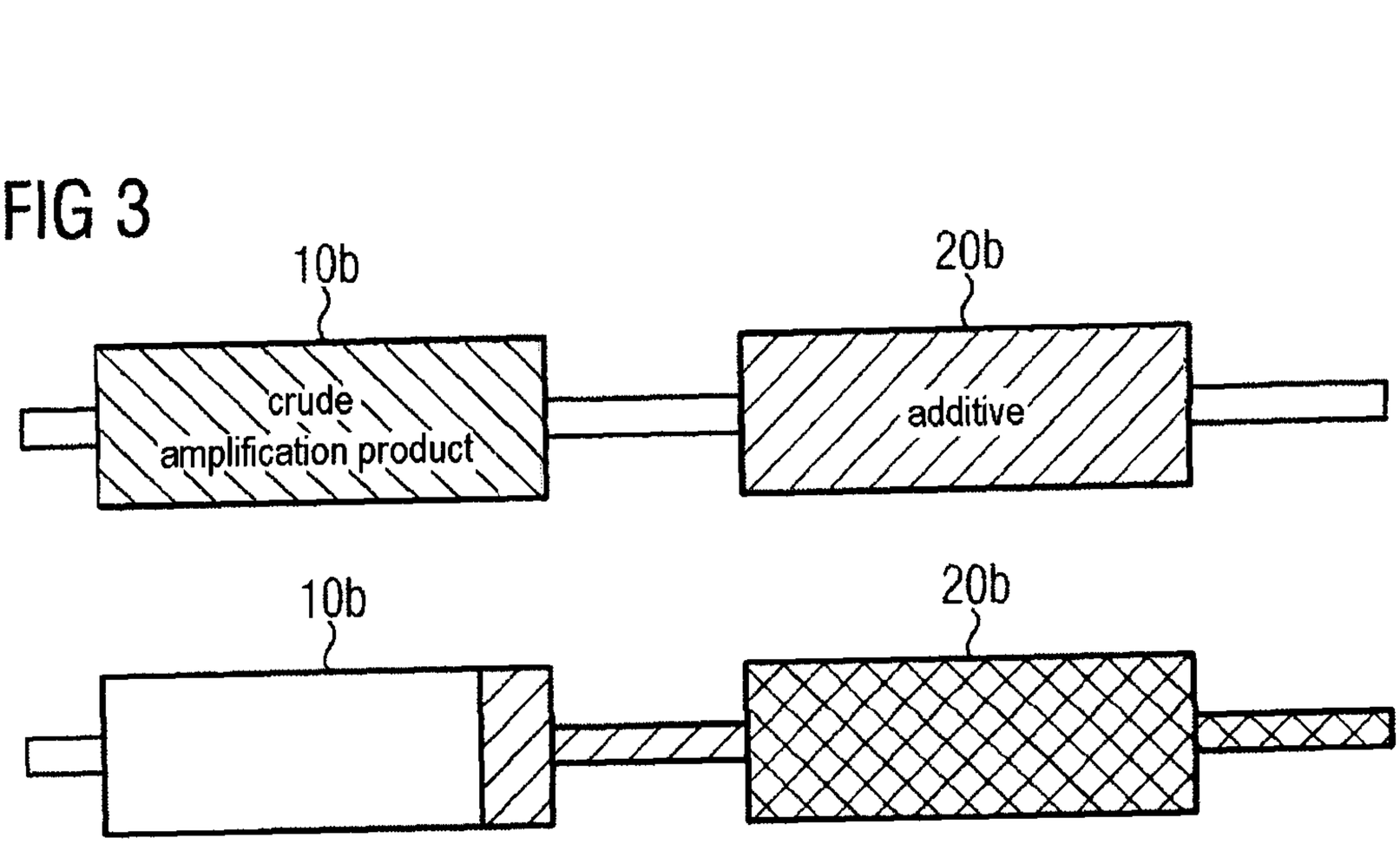
10b
20b
crude
amplification product
additive
10b
20b

FIG 4

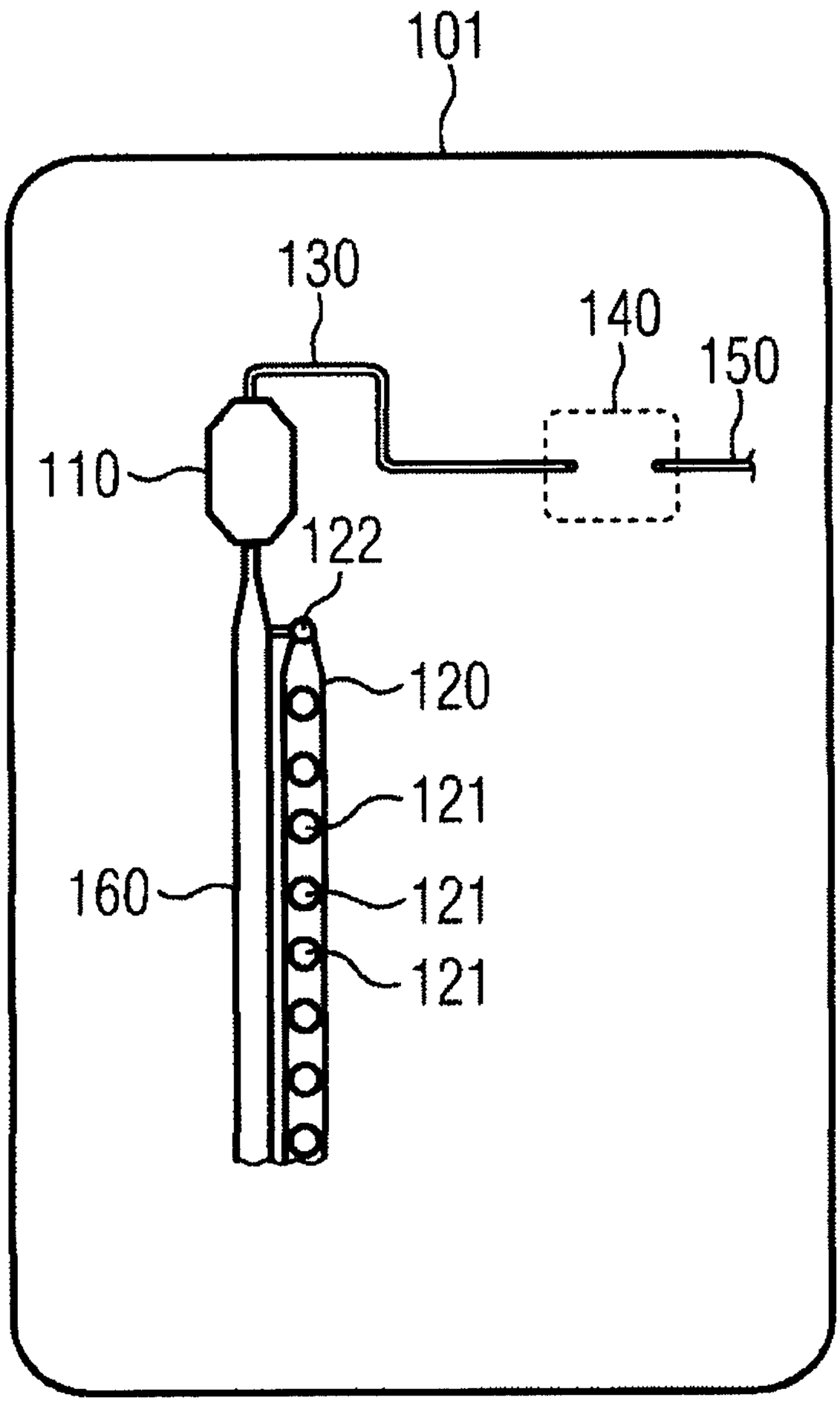

FIG 5

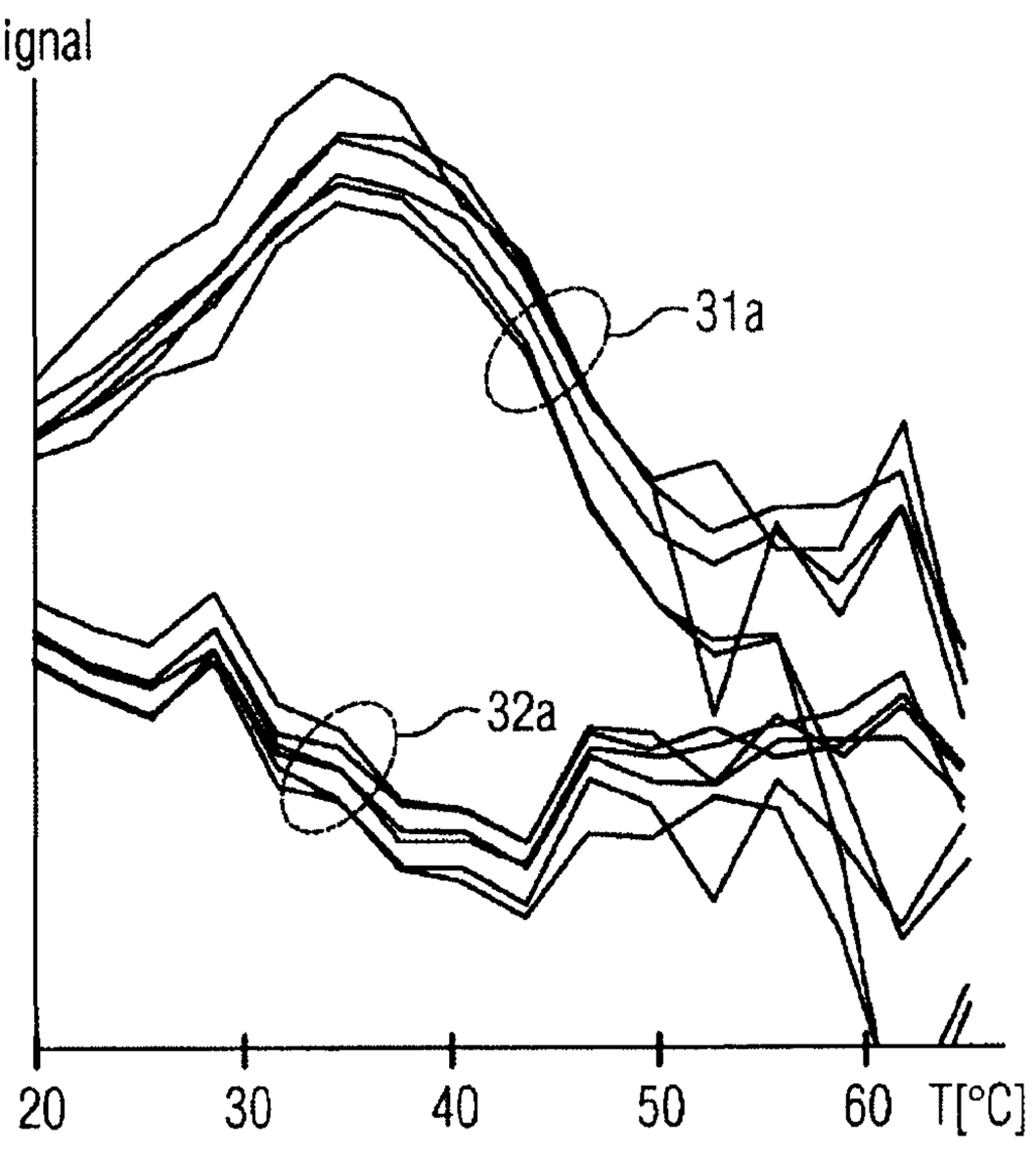

FIG 6