



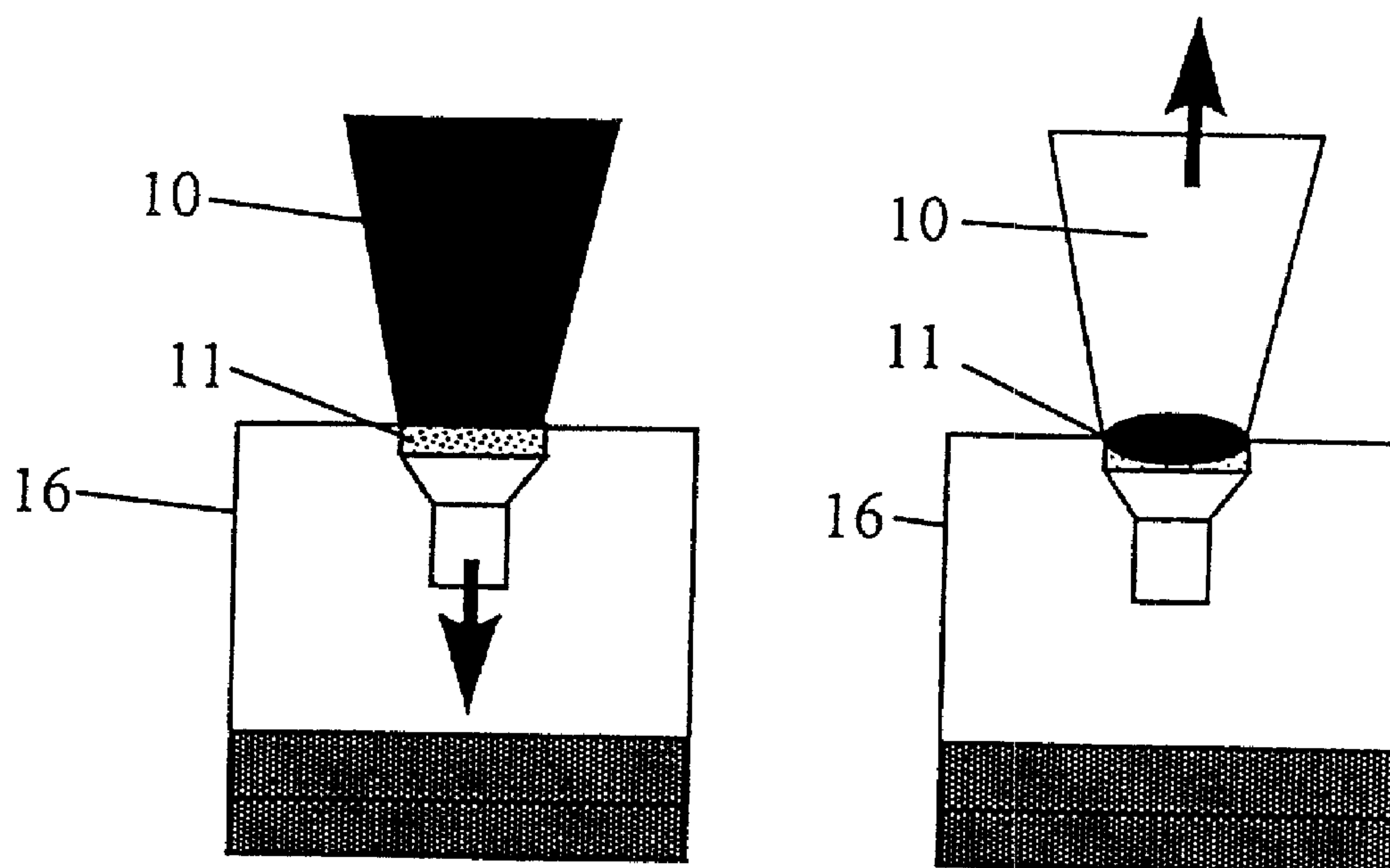
(86) Date de dépôt PCT/PCT Filing Date: 1999/04/20
(87) Date publication PCT/PCT Publication Date: 2000/05/04
(45) Date de délivrance/Issue Date: 2012/06/19
(85) Entrée phase nationale/National Entry: 2001/04/20
(86) N° demande PCT/PCT Application No.: EP 1999/002664
(87) N° publication PCT/PCT Publication No.: 2000/024927
(30) Priorité/Priority: 1998/10/23 (EP PCT/EP98/06756)

(51) Cl.Int./Int.Cl. *C12Q 1/68* (2006.01),
C07H 1/08 (2006.01), *C12M 1/00* (2006.01),
C12N 15/09 (2006.01)

(72) Inventeurs/Inventors:
GAUCH, SIMONE, DE;
BASTIAN, HELGE, DE;
ULLMANN, SUSANNE, DE;
OELMULLER, UWE, DE;
WEBER, MARTIN, DE;
FUHRMANN, GUIDO, DE;
SCHORR, JOACHIM, DE

(73) Propriétaire/Owner:

(54) Titre : PROCÉDES ET MOYENS POUR L'ISOLEMENT ET LA PURIFICATION D'ACIDES NUCLEIQUES SUR DES SURFACES
(54) Title: PROCESSES AND MEANS FOR THE ISOLATION AND PURIFICATION OF NUCLEIC ACIDS AT SURFACES



(57) Abrégé/Abstract:

The present invention involves new processes and equipment for the isolation and purification of nucleic acids on surfaces. The invention is aimed at processes which use surfaces, e.g. porous membranes, at which, out of the sample containing the nucleic acids, in a simple manner, nucleic acids can be immobilized and can be released by means of equally simple process steps, whereby the simple carrying out, according to the invention, particularly permits the performance of the processes fully automatically. A further aspect of the invention is aimed at the fact, that nucleic acids are bonded to an immobile phase, particularly a membrane, in such a way, that they can be released again from this phase without any complications in a following reaction step and, if applicable, can be used for further applications, e.g. restrictive digestion, RT, PCR or RT-PCR or in any other of the above mentioned analytical- or enzyme reactions. Finally, the invention is directed at a special isolation container with which the processes can be executed according to the invention.

(73) **Propriétaires(suite)/Owners(continued):**QIAGEN GESELLSCHAFT MIT BESCHRANKTER HAFTUNG, DE

(74) **Agent:** FETHERSTONHAUGH & CO.

Abstract

The present invention involves new processes and equipment for the isolation and purification of nucleic acids on surfaces. The invention is aimed at processes which use surfaces, e.g. porous membranes, at which, out of the sample containing the nucleic acids, in a simple manner, nucleic acids can be immobilized and can be released by means of equally simple process steps, whereby the simple carrying out, according to the invention, particularly permits the performance of the processes fully automatically. A further aspect of the invention is aimed at the fact, that nucleic acids are bonded to an immobile phase, particularly a membrane, in such a way, that they can be released again from this phase without any complications in a following reaction step and, if applicable, can be used for further applications, e.g. restrictive digestion, RT, PCR or RT-PCR or in any other of the above mentioned analytical- or enzyme reactions. Finally, the invention is directed at a special isolation container with which the processes can be executed according to the invention.

Processes and Means for the Isolation and Purification of Nucleic Acids at Surfaces

The present invention refers to new processes and equipment for the isolation and purification of nucleic acids on surfaces.

That the genetic origin and functional activity of a cell can be determined and researched by studying their nucleic acids has been known for a long time. The analysis of nucleic acids enable the direct access to the cause of activities of cells. Thus they are potentially superior to indirect, conventional methods such as e.g. verification of metabolic products. A strong increase in analyses of nucleic acids is therefore to be anticipated for the future. Molecular biological analyses are already employed in many areas, e.g. in the medical and clinical diagnostics, in the pharmaceutical industry with the development and evaluation of medicines, in food analysis as well as with the supervision of food production and the control of foodstuffs, in agriculture with the cultivation of plants and breeding of animals as well as in environmental analysis and many other areas of research. Examples include paternity analysis, tissue typing, identification of hereditary diseases, genome analysis, molecular diagnostics e.g. the identification of infectious diseases, transgenic research, basic research in areas of biology and medicine as well as multiple related areas.

The activities of genes can be determined directly through analysis of the RNA, especially the mRNA in cells. Metabolic diseases, infections or the development of cancer through recognition of faulty exprimated genes has become possible through the quantitative analysis of transcript patterns (mRNA patterns) in cells by means of modern molecular biological methods such as e.g. real time – reverse transcriptase PCR (“real time RT-PCR”) or gene expression chip analysis. For example, proof of genetic defects or the determination of the HLA type as well as other genetic markers has become possible through the analysis of the DNA from cells through molecular biological methods such as e.g. PCR, RFLP, AFLP or sequencing.

The analysis of genomic DNA and RNA is also used for the direct verification of infectious pathogens, such as viruses, bacteria etc.

In this, the general difficulty lies in the preparation of biological and clinical samples such that the nucleic acids contained can be employed directly in the respective analytical method. Precisely the direct utilization of nucleic acids with good yield and high quality and simultaneously high reproducibility is however important with high numbers of samples when the analysis is to be run automatically.

The state of the art already includes many processes for the purification of DNA. For example, it is known how to purify plasmid DNA for the purpose of cloning -- and other experimental processes as well -- according to the method of Birnboim [Methods in Enzymology 100 (1983) p 243]. In this process, a clarified lysate of bacterial origin is exposed to a cesium chloride gradient and centrifuged for a period of 4 to 24 hours. This step is usually followed by the extraction and precipitation of the DNA. This process is associated with the disadvantage that a very expensive apparatus is required on the one hand and, on the other hand, it takes a great deal

29620-1

of time, is cost-intensive and cannot be automated.

Other techniques in which clarified lysates are used to isolate DNA consist of ion-exchange chromatography [Colpan et al., J. Chromatog. 296 (1984) p 339] and gel filtration [Moreau et al., Analyt. Biochem. 166 (1987) 188]. These processes are offered primarily as alternatives to the cesium chloride gradients, but require an elaborate apparatus for the solvent supply as well as the precipitation of the DNA fractions such obtained, since these usually contain salts in high concentrations and are in extremely-diluted solutions.

Marko et al. [Analyt. Biochem., 121 (1982) p.382] and Vogelstein et al. [Proc. Nat. Acad. Sci. 76 (1979) p 615] recognized that if the DNA from extracts containing nucleic acids is exposed to high concentrations of sodium iodide or sodium perchlorate, the DNA alone will adhere to small glass scintillation tubes, fiberglass membranes or fiberglass sheets that have been finely particulated by mechanical means, while RNA and proteins do not. The DNA that has been bound in this manner can be eluted, for example, with water.

For example, in WO 87/06621, the immobilization of nucleic acids on a PVDF membrane is described. However, the nucleic acids bound to the PVDF membrane are subsequently not eluted; instead the membrane, together with all the bound nucleic acids is introduced directly into a PCR preparation. Finally, in this international patent application and in the other literature, it is revealed that hydrophobic surfaces or membranes must in general be wetted beforehand with water or alcohol, in order to be able to immobilize the nucleic acids with yields that are halfway satisfactory.

On the other hand, for a number of modern applications, such as, for example, the PCR, reversed transcription PCR, SunRise, LCR, branched-DNA, NASBA, or TaqMan* technologies and similar real-time quantification techniques for PCR, SDA, DNA- and RNA-chips and -arrays for the gene expression- and mutation analysis, differential display analysis, RFLP, AFLP, cDNA-synthesis, or subtractive hybridization, it is absolutely necessary to be able to release the nucleic acids directly from the solid phase. In this connection, WO 87/06621 teaches that, while the nucleic acids can indeed be recovered from the membranes used in the process, this recovery is fraught with problems and is far from suited to the quantitative isolation of nucleic acids. In addition, the nucleic acids obtained in this manner are, comparatively, extremely dilute -a circumstance that makes subsequent steps to concentrate and isolate them absolutely necessary.

All aqueous and other solutions of nucleic acids as well as all substances and samples, such as biological samples and materials, foodstuffs etc. shall be included in the term nucleic acid sample in the sense of the present invention. In the sense of the present invention, a sample containing nucleic acid or a substance shall be defined by a sample or sample preparation that contains nucleic acids. By biological substance or biological sample is meant, e.g. sample substance void of cells, plasma, body fluids - such as blood, saliva, urine, feces, sperm, cells, serum, fractions of leucocytes, crust phlogistica, smears, tissue samples of any kind, tissue parts and organs, food samples containing free or bonded nucleic acids or cells containing nucleic acids, plants or plant particles, bacteria, viruses, yeasts and other fungi, other eucaryotes and

*Trade-mark

29620-1

procaryotes etc. as they are set forth by the European Patent Application No.: 95909684.3, to which content is herewith referred - or free nucleic acids as well. By nucleic acids, in the sense of the present invention, are
5 meant all possible kinds of nucleic acids, as, e.g., ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) in all lengths and configurations as double-stranded, single-stranded, circular and linear, branched, etc., monomer nucleotides, oligomers, plasmides, viral and
10 bacterial DNA and RNA, as well as genomic or other non-genomic DNA and RNA from animal and plant cells or other eukaryotes, t-RNA, mRNA in processed and unprocessed form, hn-RNA, rRNA, and cDNA, as well as all other imaginable nucleic acids.

15 For the reasons stated above, the processes known from the state of the art do not constitute - particularly with regard to automation of the process for obtaining nucleic acids - a suitable starting point for a quantitative isolation of nucleic acids that is as simple as possible as
20 far as process engineering is concerned.

In one aspect, the invention relates to a process for the precipitation of plasmid nucleic acid, comprising: providing an isolation container comprising a membrane located therein, said membrane comprising pores having a pore size
25 larger than or equal to 0.45 micrometers and wherein said membrane comprises hydrophilized nylon, polyethersulfone, polycarbonate, polyacrylate, acrylatecopolymers, polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate, polytetrafluoroethylene, polyvinylidendifluoride,
30 polyethylenetetrafluoroethylene-copolymerisate, one

29620-1

polyethylenechlorotrifluoroethylene- copolymerisate, cellulose acetate, cellulose nitrate or polyphenylene sulfide; charging said isolation container with a solution containing plasmid nucleic acids; and precipitating said plasmid nucleic acids
5 contained in said solution with iso-propanol, wherein the volume ratio of the solution containing plasmid nucleic acids to iso-propanol is 2:1 to 1:1, whereby said plasmid nucleic acids bond with said membrane.

In another aspect, the invention relates to use of a
10 membrane comprising pores having a pore size larger or equal to 0.45 micrometers and wherein said membrane comprises hydrophilized nylon, polyethersulfone, polycarbonate, polyacrylate, acrylatecopolymers, polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate,
15 polytetrafluoroethylene, polyvinylidendifluoride, polyethylenetetrafluoroethylene-copolymerisate, one polyethylenechlorotrifluoroethylene- copolymerisate, cellulose acetate, cellulose nitrate or polyphenylene sulphide to bind plasmid nucleic acids precipitated with iso-propanol, wherein
20 the volume ratio of the solution containing nucleic acids to iso-propanol is 2:1 to 1:1.

29620-1

Further advantageous aspects and embodiments of the invention are evident from the dependent claims, the description, and the attached figures.

In this connection, the invention involves processes which use surfaces, e.g., porous membranes, on which the nucleic acids can be immobilized in a simple way from the test sample containing the nucleic acids and can again be released by means of simple procedural steps. In particular, the simple procedure, according to the invention, makes it possible to carry out the processes completely automatically.

A further aspect of the present invention is aimed at binding nucleic acids to an immobile phase, a membrane in particular, in such a way, that they can again be released from that phase, without complications, in the following reaction step and can, if applicable, come to additional use in e.g. restriction digestion, RT, PCR or RT-PCR or in any other of the above mentioned analytical or enzyme reactions.

A surface in the sense of the present invention is defined through any micro-porous separation layer. This can for example rest directly upon a substrate and as such be accessible only from one side or stand freely. A separation layer, accessible from both sides, therefore not resting with its entire surface upon an impermeable substrate but completely free or only supported at a few points, shall be referred to as a membrane in accordance with the present invention.

Isolation in the sense of the present invention is defined as any enrichment of nucleic acids during which the concentration of the nucleic acids is increased and/or the portion of the non-nucleic acid in a preparation or sample is decreased.

The invention provides for a process to isolate nucleic acids according to the following steps:

- charging of a membrane with at least one sample of nucleic acids;
- immobilization of the nucleic acids at the membrane;
- release of the immobilized nucleic acids from the membrane; and
- removal of the released nucleic acids by transfer through the membrane

whereby the membrane comprises nylon, polysulfone, polyethersulfone, polycarbonate, polyacrylate, acrylate-copolymer, polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate, polytetrafluorethylene, polyvinylidene difluoride, polyethylene tetrafluorethylene-copolymerisate, polybenzimidazoles, polyethylenechlorotrifluorethylene-copolymerisate, polyimides, polyphenylene sulfide, cellulose, cellulose mixed esters, cellulose nitrate, cellulose acetate, polyacrylnitriles, polyacrylnitrile-copolymers, nitrocellulose, polypropylene and/or polyester.

Other membranes, for example those mentioned in addition within this description, can be used for the process according to this invention.

Preferable the charging occurs from the top and the removal downwards, it could however be imagined that, for example, liquid transfer processes, for which a horizontally arranged column is charged with a solution containing nucleic acids from one side, penetrating the membrane after the immobilization of the nucleic acids at the membrane and is then taken off at the other end of the column.

The membrane can preferably be placed inside a container, e.g. the above mentioned or another column, fitted with a supply and take-off and cover the entire cross section of the container.

The membrane can be coated, whereby it can be rendered hydrophobic or hydrophilic by the coating.

To achieve a complete isolation of the nucleic acids, isolation processes known so far, particularly within columns, operate with relatively thick membranes or fleeces. This however, during the suction transfer of the solution through the membrane, leads to a relatively large, so-called dead space volume, namely the volume of the membrane, from which the nucleic acids can only be obtained with larger amounts of elution buffer. As a result, after the elution, the nucleic acids are present in a more diluted form which, for many applications, is undesirable or

disadvantageous. For this reason a preferred embodiment for carrying out the invention utilizes a membrane which is less than 1 mm, preferably less than 0.5 and particularly preferred less than 0.2, e.g. 0.1mm, thick.

The invention furthermore concerns a process for the isolation of nucleic acids according to the following steps:

- charging of a surface with at least one sample of nucleic acids;
- immobilizing the nucleic acids at the surface; and
- releasing of the immobilized nucleic acids from the surface with an elution medium.

This process is characterized in that the release is carried out at a temperature where the upper limit is 10°C or below and the lower limit is at the freezing point of the elution medium such that the elution medium does not freeze. Thus the inequation $10^{\circ}\text{C} \geq T \geq T_{S,EM}$ applies, whereby T describes the temperature of the release and $T_{S,EM}$ the freezing point of the elution medium. This is because it has been shown, against common belief, that the release of nucleic acids is definitely possible near the freezing point of the elution medium. An elution at such a low temperature even has the unexpected advantage that the nucleic acids are being treated gentler and that, where there are still signs of the presence of nucleases (DNases or RNases), these come to a virtual stand still near the freezing point, so that the degradation of the nucleic acids are reduced or completely suppressed.

Accordingly, during elution, the temperature should preferably be even lower, e.g. below 5°C. The lower limit can also be at 0°C or -5°C if the preparation, due to its ion content, is still liquid at that temperature. The upper limit should also be low if possible, e.g. approximately 5°C.

This process according to the present invention, sometimes demands a cooling of the elution buffer and can demand a cooling of further solutions, if applicable, cooling of the isolation container as well. Since cooling cannot always be assured reliably, particularly during examinations in the field, such as screening of persons in developing countries, the present invention is furthermore directed at an isolation container which permits an isolation of nucleic acids at low temperatures, independent of external cooling.

Thus the invention is furthermore directed at an isolation container for the isolation of nucleic acids comprising at least one upper part with one top opening, one bottom opening and one membrane which is located at the bottom opening and covers the entire cross section of the upper part; one lower part with an absorbant material; and a jacket to contain a coolant which surrounds the upper part at least in the area of the membrane. The jacket which contains the coolant permits the cooling of the membrane and the solutions to lower temperatures, such as the lysate, the washing buffers and the elution buffer which are brought onto the membrane so that the final elution can be conducted reliably within the desired temperature range, close to the freezing point of the elution buffer.

With one of the embodiments of the isolation container, the jacket comprises two compartments which are separated by a mechanically destructible partition, whereby a solution is contained in each of the compartments and the coolant is formed through the mixture of the both solutions after the destruction of the partition.

The partition can be destroyed by the experimenter in that he e.g. presses against the provided points of the outer wall of the jacket and thus ruptures the partition. Solutions suitable for the containment inside the compartments are known to specialists in the area of chemical refrigeration techniques. These can be adjusted to the desired temperatures and to the ambient temperatures expected during the utilization of the isolation container.

During the extraction of nucleic acids from biological samples, e.g. the samples mentioned above, it is frequently necessary to firstly lyse cells or secretions, to access the nucleic acids. The lysates thus obtained may contain, besides nucleic acids, large amounts of undesirable substances, e.g. proteins or lipids. A blockage of the membrane may result from a charge if the amount of undesirable substances within the lysate is too large which reduces the efficiency of the isolation of the nucleic acids and reduces the permeability of the membrane during washing or the elution. To avoid this undesirable effect, the invention is therefore also directed at a process during which undesirable substances are removed before they reach the membrane.

This process for the isolation of nucleic acids according to the present invention comprises the following steps:

- standardization of at least one sample of nucleic acids to bonding conditions which permit the immobilization of nucleic acids, contained in the at least one sample of nucleic acids, at a surface;
- charging of the surface with at least one sample of nucleic acids; and
- immobilization of the nucleic acids at the surface,

characterized in that prior and/or after the adjustment of the bonding conditions, a pre-purification is carried out.

The pre-purification can be carried out, for example, by demineralization or through filtration, centrifugation, enzymatic treatment, temperature influence, precipitation, and/or extraction of the nucleic acids solution and/or bonding of contaminants of the nucleic acids solution to surfaces. The pre-purification can also be carried out by mechanical particulation or homogenization of the nucleic acids solution where a lysate of an organic sample is concerned.

The standardized bonding conditions can hereby enable an immobilization of the RNA and/or DNA.

A pre-purification may be particularly necessary if an isolation is carried out on biological samples with severe contamination. The biological sample may comprise any imaginable substance which may be used directly or is in turn obtained from other biological samples. These can for example be blood, saliva, urine, feces, sperm, cells, serum, leucocyte fractions, crusta phlogistica, smears, tissue samples, plants, bacteria, fungi, viruses and yeasts as well as all other types of biological samples mentioned above.

If the biological sample contains a high proportion of undesirable substances, the process according to the present invention can naturally be employed to a particular advantage.

After immobilization of the nucleic acids out of the pre-purified sample of nucleic acids, the common isolation steps follow, i.e.:

- release of the immobilized nucleic acids from the surface;
- removal of the released nucleic acids from the surface.

A particular advantage of the processes according to the present invention lies in the fact that these can be combined with the chemical reactions to which the nucleic acids are subjected at the surface. A multitude of analytical techniques can thus be carried out on the nucleic acids isolated at the surface. To achieve free access, in doing so, it is possible to release the nucleic acids from the surface prior to the reaction. Alternatively however, a suitable reaction can also be carried out on nucleic acids bonded directly to the surface.

In one aspect, accordingly, the invention is directed at a process with pre-purification, as illustrated above, characterized in that the following step is carried out after the release step, preferably at least once:

- carrying out of at least one chemical reaction on the nucleic acids.

A particular advantage of this process lies in the fact that prior to the chemical reaction no decanting from the isolation container to a reaction container is required which might cause losses, but that isolation and reaction can occur in the same container.

In a further aspect, independent from pre-purification, the invention is directed at a process to carry out a nucleic acid amplification reaction with the following steps:

- charging of a surface with at least one sample of nucleic acids;
- immobilization of the nucleic acids at the surface; and
- carrying out of an amplification reaction with the nucleic acids.

Particularly in case of the small amounts of substance usually or necessarily worked with in amplification reactions, it is an advantage if the entire preparation of nucleic acids can be employed without losses due to decantation. This is also of particular advantage for an automation as all processes can be carried out in one container. In addition the amount of waste is reduced and the process becomes faster and more cost efficient.

With this the amplification reaction can be an isothermal or a non-isothermal reaction.

With this the amplification reaction can for example be a SDA reaction (strand displacement amplification), a PCR, RT-PCR, LCR or a TMA or a Rolling Circle Amplification.

A NASBA amplification reaction is also possible with this process according to the present invention.

Prior to the amplification reaction the nucleic acids can be released from the surface by means of a reaction buffer, where the elution medium is located on or within the membrane. Alternatively the amplification reaction can be carried out inside a reaction buffer that does not lead to a release of the nucleic acids from the surface.

This process preferably shows these further steps:

- if applicable, the release of the reaction products from the surface (as far as these were still bound during the reaction); and
- removal of the released reaction products from the surface.

A further aspect contains a process for the carrying out of chemical reactions on nucleic acids with the following steps:

- charging a surface with at least one sample of nucleic acids;
- immobilization of the nucleic acids at the surface;
- release of the immobilized nucleic acids from the surface;
- carrying out of at least one chemical reaction on the nucleic acids; and
- removal of the nucleic acids from the surface without prior immobilization.

With this process the nucleic acids are not bound to the membrane (immobilized) after the chemical reaction but are removed without bond. Although saving such an additional step might deteriorate the purity of the preparation but might be preferred due to time savings in time critical

applications and the simplification in certain forms of application. The process according to the invention offers a wide selection of chemical reactions. Chemical reaction in the sense of this invention shall be understood as any interaction of the nucleic acids with further substances (except with the surface, as this "reaction" occurs with all processes described herein), i.e. enzymatic modifications, hybridization with probes, chemical sequencing reactions, pH value modifications e.g. the basic depurination of RNA and acidic depurination of DNA, as well as bonding anti-bodies and protein additions. Generally any reaction, be it aimed at a modification of covalent bonds or hydrogen bonds, shall be included.

One advantage of the process according to the present invention is to be seen in the permanent spatial unification of a volume space in which different processes occur and a membrane to which nucleic acids may bond. In the simplest way this unification permits a manipulation of nucleic acids with an immediate subsequent membrane bond. This is of great advantage particularly for automated processes. Once bonded to the membrane, the nucleic acids are available for further treatment steps, e.g. - as illustrated above - for the isolation of potentially pure nucleic acids or for the carrying out of chemical reactions with nucleic acids. In a further aspect of the invention it is however also possible to subject the nucleic acids, bound to the membrane, immediately to another analysis to determine specific properties of the nucleic acids.

Thus the invention is furthermore directed at a process for the analysis of nucleic acids inside an isolation container with the following steps:

- providing an isolation container with a membrane located inside;
- charging of the isolation container with at least one sample of nucleic acids;
- immobilization of the nucleic acids on the membrane;
- transfer of the liquid components of the sample through the membrane; and
- analysis of at least one property of the nucleic acids on the membrane located inside the isolation container.

In a further process embodiment at least one chemical reaction, as described above, can be carried out on the nucleic acids, after the transfer of the liquid components. This can, for example, serve the purpose to enable the subsequent analysis of the nucleic acids. Examples for this application are hybridization with probes, the radioactive marking of nucleic acids bound to the membrane or the bond of specific anti-bodies. Auxiliary reactions such as the coloring of nucleic acids, e.g. with intercalating substances such as ethidium bromide shall also be understood as chemical reactions.

Different properties of the nucleic acids are open to a membrane bound analysis and have already been described for conventional membranes without a combined reaction container. Some of the properties that can be analyzed are the radioactivity of the nucleic acids or their

ability to bond with molecules, whereby the molecules can be e.g. anti-bodies, nucleic acid bonding colorant molecules or colorant molecules bonding to nucleic acids or proteins.

This process presents a considerable simplification of the analysis of nucleic acids as a manipulation of the exposed membrane is no longer necessary. Rather, it is located within the isolation container.

An irreversible bond of the nucleic acids to the membrane, e.g. for the subsequent analysis steps, is also contained within the frame of the present invention. The durable or irreversible bond allows the manipulation of the membrane and the nucleic acids bound hereto in a manner not open to reversibly bound nucleic acids.

In a further aspect the invention is aimed at the quantitative precipitation of nucleic acids.

With previously known methods for the pure-preparation of 100 µg or more (hereafter referred to as "large scale") of plasmid-DNA, based on anion exchange chromatography, the plasmid DNA is eluted from the column with a high salt concentration buffer in the last step. To separate the Plasmid DNA from the salt for one and to concentrate it for the other, it is precipitated by means of alcohol (e.g. iso-propanol) and centrifuged in a suitable container. The centrifuge pellet is washed with a 70% ethanol to remove the remaining traces of the salt and then subjected to a centrifugation again. The pellet of the second centrifugation is typically dissolved in a small amount of a low salt concentration buffer and the plasmid-DNA is processed further in this form.

Furthermore, processes have been suggested in the state of the art, which transform the DNA, through an addition of chaotropic salts to the high salt concentration buffer, into a state which bonds on silica membranes. After washing accordingly, the DNA is released from the membrane with a low salt concentration buffer.

In a publication (Ruppert, A. et al., Analytical Biochemistry (1995), 230: 130-134) a similar application is described with which, on a small scale (isolation of less than 100 µg plasmid-DNA), a DNA which was precipitated with iso-propanol, bonds to PVDF membranes with a pore size smaller than 0.2 µm, thereafter washed with ethanol and subsequently eluted with TE (tris-EDTA). A description of such a method on a large scale is however non-existent.

The described precipitation of DNA with subsequent centrifugation is extremely time consuming (approximately 1 hour), in addition, the use of centrifuges is necessary. Besides the high time requirement for this procedure, the described last step in the plasmid preparation is particularly susceptible to faults. Every now and then a partial or complete loss of the DNA-pellet occurs. It appears that the kind (material) of which the centrifugation container is made plays a decisive role.

The also described application of chaotropic salts and subsequent bonding of nucleic acids to silica membranes is also time consuming, compounded by the fact that the introduction of chaotropic salts into the preparation might pose the danger of a contamination of the lastly

isolated DNA.

The described filtration of alcoholic precipitates on a small scale has the disadvantage of not being able to be transferred to the large scale in a linear fashion. Membranes employed according to the state of the art only permit the isolation of small amounts of nucleic acids because the membranes are rapidly saturated and no longer absorbant. Frequently a large part of the nucleic acids is thus lost during the removal of the precipitation buffer and during washing. To avoid such a loss, the invention is also aimed at a process for the precipitation of nucleic acids with the following steps:

- providing an isolation container in which at least one membrane has been placed;
- charging of the isolation container with one sample of nucleic acids;
- precipitation, with alcohol, of the nucleic acids contained in the sample, such, that the nucleic acids bond with at least the one membrane. The process is characterized in that the pore size of the at least one membrane is larger or equal to 0.2 micrometer.

Firstly the alcohols considered for the carrying out of the process according to the present invention, are all hydroxyl derivatives of aliphatic or acyclic saturated or unsaturated hydrocarbons.

Among the hydroxyl compounds, the C1 – C5 alkanols such as methanol, ethanol, n-propanol, n-butanol, tert.-butanol, n-pentanol or their mixtures are preferred. For the carrying out of the process according to the present invention iso-propanol is particularly preferred.

In this the alcohol can be mixed with this solution prior or after the charging of the isolation container with the solution containing nucleic acid. The volume ratio of the nucleic acid containing solution to alcohol, particularly iso-propanol, is preferably 2:1 to 1:1, particularly preferred 1.67:1 to 1:1 and e.g. 1.43:1.

The surface area of the membrane is preferably selected such that the total of the nucleic acids contained in the solution bonds with the membrane.

For the bonding of alcohol precipitated nucleic acids, which can be DNA and/or RNA, the invention is also aimed at the use of membranes with a pore size larger or equal to 0.2 μm .

The use of a 0.45 μm cellulose acetate – or cellulose nitrate filter or the use of several layers of 0.65 μm cellulose acetate – or cellulose nitrate filters is regarded as particularly advantageous. The procedure can be used for vacuum filtration as well as pressure filtration.

The process according to the present invention enables a time saving transfer of nucleic acids from a high salt concentration buffer system to a low salt concentration buffer system which is possible without extensive apparatus requirements. It is suitable as a substitute for the classical

alcoholic precipitation of DNA from a high salt concentration buffer which is typically performed with the aid of centrifugation steps. Due to the high efficiency of the method (low loss of yield) it is particularly suitable for large scale preparations. Furthermore, through the use of the process according to the present invention, no foreign substances are introduced into the already purified nucleic acids. In addition, the susceptibility to faults (loss of centrifugation sediment during the washing step is not possible here), in comparison to the classical method, is reduced.

The charging during the various processes described above is preferably carried out from above. In principal, diverse methods are available to transfer the different solutions, i.e. the immobilization buffers containing nucleic acids, washing buffers, eluates etc through the membrane. This can be gravitation, centrifugation, vacuum, positive pressure (from the charging side) and capillary forces.

Between the steps of immobilization and release, a washing step of the immobilized nucleic acids with at least one washing buffer can be carried out. The washing comprises the following steps for each washing buffer:

- application of the pre-determined amount of washing buffer onto the surface; and
- transfer of the washing buffer through the surface.

The charging and immobilization of the nucleic acids can again include the following steps:

- mixing of the sample of nucleic acids with an immobilization buffer;
- charging of the sample of nucleic acids together with the immobilization buffer onto the surface; and
- transfer of the liquid components through the surface, essentially in the direction of charging.

The procedures have the great advantage that they can be easily automated, with the result that at least one of the steps can be carried out completely automatically by means of an automated apparatus. It is also possible that all the steps of the procedures can be carried out in a controlled series of steps by an automated apparatus.

Particularly in such cases, but also during manual processing, it is possible to subject a multitude of nucleic acids to the isolation simultaneously. For example multiple isolation containers in the form of commercially available "Multi-well" containers with 8, 12, 24, 48, 96 or more isolation recesses can be used.

The removal of the nucleic acids can occur in two fundamentally different directions. For one, it is possible to transfer the removed (eluted) nucleic acids through the membrane and remove them

to that side of the membrane, which lies opposite to the side onto which the solution, containing the nucleic acids or lysate, was added. In this case, the nucleic acid is removed in the direction of the addition through the membrane. To remove the nucleic acids from the side of the addition onto the membrane or surface is the other possibility. The removal then occurs in the direction opposite to that of the addition or "to the same direction" from which was added; being the side of the addition. In that case the nucleic acids do not have to pass the membrane. With some of the processes according to the present invention, the removal of the nucleic acids occurs always through the membrane in the direction of the addition. Should the process be carried out with a surface placed upon a substrate impermeable to liquids, e.g. a plastic wall, the removal can obviously only occur in the direction towards the direction of the addition (being the opposite direction). In the case of some processes however, a removal in both directions is possible.

If the nucleic acids are eluted (released) from the surface essentially in a direction opposite to the one in which they were added and immobilized, the "same direction" is actually referred to for any direction with an angle smaller or equal to 180° compared to the direction of the addition, so that, in any event, during the elution, the nucleic acids do not penetrate the surface, e.g. a membrane, but are removed from the surface in a direction opposite to the direction of charging onto the surface. In preferred forms of carrying out the process, the other buffers, therefore that buffer in which the nucleic acids are located during charging, and, if applicable, a washing buffer, are transferred through the surface by means of suction or otherwise. If the isolation occurs on a membrane located inside a container, where the membrane covers the entire cross-section of the container, the charging preferably occurs from above. In this case the removal step occurs upwards again. Fig. 2 for example shows a funnel type isolation container which is charged from above and the removal of the nucleic acids occurs upwards.

It is to be understood, however, that also with a removal in a direction opposite to that of the addition, other arrangements are conceivable, i.e., a removal of the nucleic acids from below. It is, for example, conceivable that a buffer containing nucleic acids, such as a lysate buffer, can be drawn from a reaction container directly into an isolation device by means of a vacuum apparatus, so that the nucleic acids are bound to the underside of a membrane in the isolation device. In such a case, the removal of the nucleic acids from the surface takes place by means of an elution buffer drawn from below, which after release of the nucleic acids is then drained downward into a container. In this case, the removal of the nucleic acids therefore takes place in a downward direction.

Also, a lateral removal of the nucleic acids is possible, for example, when a column placed horizontally with a membrane located inside is charged in a flow-through process with a lysate and the horizontally placed column is subsequently rinsed with an elution buffer on the side of the membrane on which the nucleic acids are bound.

An example for the maximum angle of 180 degrees possible is an inclined surface with a surface suitable for the bonding of nucleic acids over which the various solutions or buffers flow downwards. Like all buffers, the elution buffer, too, comes from one side and flows down to the other side. In this case, the direction of the entering stream of the buffer and the exiting stream of

the buffer containing the nucleic acids form an angle of 180 degrees; the removal, however, always takes place on the same side of the surface as the immobilization.

In the process according to this invention, the sample containing nucleic acids described above is introduced into a solution which contains the appropriate salts or alcohol(s), then, in appropriate cases, solubilizes the preparation and passes the mixture achieved in this way, by means of a vacuum, through the use of a centrifuge, by means of positive pressure, by capillary forces, or by other appropriate procedures through a porous surface, by which process the nucleic acids are immobilized on the surface.

Suitable salts for the immobilization of nucleic acids on membranes or other surfaces and/or lysates of samples of nucleic acids include salts of metal cations such as the alkaline or alkaline earth metals with mineral acids, in particular alkaline or alkaline earth halogenides or sulfates or phosphates, with the halogenides of sodium, lithium or potassium or magnesium sulfate being especially preferred. Other metal cations, e.g. Mn, Cu, Cs or Al or the Ammonia cation can be used, preferably as salts of mineral acids.

Also suited for carrying out the process according to the invention are salts of mono- or polybasic acids or polyfunctional organic acids with alkaline or alkaline earth metals. These include, in particular, salts of sodium, potassium or magnesium with organic dicarboxylic acids -- such as oxalic, malonic or succinic acid -- or with hydroxy- or polyhydroxycarboxylic acids -- for example, preferably, with citric acid.

If it turns out to be more suitable for certain applications, the above listed substances for the immobilization of nucleic acids on the surfaces and/or to lyse samples of nucleic acids can with this be used on their own or in mixtures.

The use of so-called chaotropic agents has proved to be especially effective. Chaotropic substances are capable of disturbing the three-dimensional structure of hydrogen bonds. This process also weakens the intramolecular binding forces that participate in forming the spatial structures -- including primary, secondary, tertiary or quaternary structures -- in biological molecules. Chaotropic agents of this kind are known to the expert from the state of the art (Römpp, Lexikon der Biotechnologie, published by H. Dellweg, R.D. Schmid and W.E. Fromm, Thieme Verlag, Stuttgart 1992).

The preferred chaotropic substances for use with this invention are, for example, salts from the trichloroacetate, thiocyanate, perchlorate or iodide group or guanidinium hydrochloride and urea.

The chaotropic substances are used in a 0.01- to 10-molar aqueous solution, preferably in a 0.1- to 7-molar aqueous solution and most preferably in a 0.2- to 5-molar aqueous solution. The chaotropic agents mentioned above can be used alone or in combinations. In particular, a 0.01- to 10-molar aqueous solution, preferably a 0.1- to 7-molar aqueous solution and most preferably a 0.2- to 5-molar aqueous solution of sodium perchlorate, guanidinium hydrochloride, guanidinium isothiocyanate, sodium iodide, and/or potassium iodide is used.

The salt solutions used for the lysis, bonding, washing and /or elution in the process according to the invention preferably are buffered. Suitable as buffer substances are the following buffer systems, e.g. carboxylic acid buffers, in particular, citrate buffer, acetate buffer, succinate buffer, malonate buffer as well as glycine buffer, morpholinopropansulfonic acid (MOPS) or Tris(hydroxymethyl)aminomethane (Tris) in a concentration of 0.001 – 3 mol/l, preferably 0.005- 1 mol/l, particularly preferred 0.01 – 0.5 mol/l, especially preferred 0.01 – 0.2 mol/l.

The suitable alcohols for the conduct of the process according to the invention include, first of all, all the hydroxyl derivatives of aliphatic or acyclic saturated or unsaturated hydrocarbons. It is initially unimportant whether these compounds contain one-, two, three or more hydroxyl groups -- such as polyvalent C1-C5 alcohols, including ethylene glycol, propylene glycol or glycerine.

In addition, the usable alcohols according to the invention include the sugar derivatives, the so-called aldoses, as well as the phenols, such as polyphenols.

Among the hydroxyl compounds mentioned previously, the C1-C5 alcohols, such as methanol, ethanol, n-propanol, tertiary butanol and the pentanols are especially preferred.

In the sense of the present invention, the term hydrophilic applies to such materials or membranes which by virtue of their chemical nature mix easily with water or absorb water.

In the sense of the present invention, the term hydrophobic applies to such materials or membranes which by virtue of their chemical nature do not penetrate into water -- or vice versa -- and which are not able to remain in it.

By the word surface, in the sense of the present invention, is meant any microporous-separating layer. In the case of a membrane, the surface consists of a film of a polymer material. The polymer will preferably be composed of monomers with polar groups.

In another embodiment of the process according to the invention, the concept of surface in the broader sense includes a layer of particles or a granulate or even fibers such as e.g. silica gel fleeces.

In connection with the use of hydrophobic membranes, in the sense of the present invention, those membranes are preferred which consist of a hydrophilic substance and which can be rendered hydrophobic by a subsequent chemical treatment which is well known from the state of the art, such as hydrophobized nylon membranes which are commercially available.

For the purposes of this invention, hydrophobized membranes include, in general, those membranes which may or may not have been hydrophilic to begin with and are coated with the hydrophobic coating agents mentioned below. Hydrophobic coating agents of this kind cover hydrophilic substances with a thin layer of hydrophobic groups, such as fairly long alkyl chains or siloxane groups. Suitable hydrophobic coating agents are known in great number from the

state of the art; for purposes of the invention, these include paraffins, waxes, metallic soaps etc., if necessary with additives of aluminum or zirconium salts, quaternary organic compounds, urea derivatives, lipid-modified melamine resins, silicones, zinc-organic compounds, glutaric dialdehydes and similar compounds.

In addition, the hydrophobic membranes that can be used for purposes of the invention are membranes which are hydrophobic per se and those that have been made hydrophobic and whose basic material contains polar groups. According to these criteria, for example, materials from the following group -- particularly hydrophobized ones -- are suitable for use with the invention:

Nylon, polysulfones, polyether sulfones, cellulose nitrate, polypropylene, polycarbonates, polyacrylates and acrylate copolymers, polyurethanes, polyamides, polyvinyl chloride, polyfluorocarbonates, polytetrafluorethylene, polyvinylidene difluoride, polyethylenetetrafluorethylene copolymerisates, polyethylene chlorotrifluorethylene copolymerisates or polyphenylene sulfide as well as cellulose and cellulose mixed esters, cellulose acetate or nitric celluloses and polybenzimidazoles, polyimides, polyacrylnitriles, polyacrylnitrile copolymers, hydrophobized glass fibre membranes amongst which hydrophobized nylon membranes are particularly preferred.

Preferred hydrophilic surfaces include hydrophilic materials per se and also hydrophobic materials, which have been made hydrophilic. For instance the following substances can be used: hydrophilic nylon, hydrophilic polyether-sulfones, hydrophilic polycarbonates, hydrophilic polyesters, hydrophilic polytetrafluoro-ethylenes on polypropylene tissues, hydrophilic polytetrafluorethylenes on polypropylene fleeces, hydrophilized polyvinylidene difluorides, hydrophilized polytetrafluorethylenes, hydrophilic polyamides, nitric cellulose, hydrophilic polybenzimidazoles, hydrophilic polyimides, hydrophilic polyacrylnitriles, hydrophilic polyacrylnitrile copolymers, hydrophilic polypropylene, cellulose nitrate, cellulose mixed esters and cellulose acetate.

In connection with processes for bonding of nucleic acids the membranes mentioned above are partially known, although not in the context of this invention, from the present state of the art. A selection of materials for this process is however not yet known from the state of the art. The extensive experiments by the inventors have shown, however, that there are further membranes suitable for the bonding of nucleic acids.

The present invention is thus also aimed at the use of cellulose acetate, non carboxylated, hydrophobic polyvinylidene difluoride or solid, hydrophobic polytetrafluorethylene as material for adhesion and isolation of nucleic acids.

The term "solid" herein refers to a material which consists throughout of the relevant chemical compound and is neither coated nor placed as a coating on a substrate.

The material can be used in the form of a membrane, as granules, in the form of fibers or in any other suitable form. The fibers for example can be arranged as a fleece and the granules as a compressed frit.

The membranes that are used in the processes according to the invention described above (with the exception of iso-propanol precipitation) have, for example, a pore diameter of 0.001 to 50 μm , preferably 0.01 to 20 μm and most preferably 0.05 to 10 μm . In the case of the precipitation of nucleic acids with iso-propanol according to the process described above, the pore size has to be above 0.2 μm .

As washing buffers, the salts or alcohols described above, or phenols or polyphenols, can be considered. Also detergents and natural substances in the widest sense, such as albumin and milk powder, can be used for the washing steps. The addition of chaotropic substances is also possible. Polymers as well as detergents and similar substances can be added. In any event, the washing buffer and the substances contained therein should generally be able to bind, solubilize or react with the undesirable contaminants such, that these contaminants or their decomposite products are removed together with the washing buffer. The temperatures in the washing step will usually be within the range from 10 to 30° C, preferably room temperature, whereas higher or lower temperatures can also be used successfully. As such it might for example be indicated to cool the washing buffer for elutions performed at low temperatures, e.g. 2°C, to pre-cool the isolation container and the surface or membrane to the desired temperature value. An application for low temperatures is the cytoplasmic lysis, during which the cell nuclei initially remain undamaged. Higher temperatures of the washing buffer, on the other hand, cause a better solubilization of the contaminants that are to be washed out.

Suitable eluting agents for the elution of bound nucleic acids for the purposes of the invention are water or aqueous salt solutions. As salt solutions, buffer solutions that are known from the present state of the art are used, such as morpholinopropane sulfonic acid (MOPS), Tris (hydroxymethyl) aminomethane (TRIS), 2-[4-(2-hydroxyethyl) -1- piperazino]ethane sulfonic acid (HEPES) in a concentration from 0.001 to 0.5 mol/liter, preferably 0.01 to 0.2 mol/liter, most preferably 0.01- to 0.05-molar solutions. Also preferred for use are aqueous solutions of alkaline or alkaline earth metal salts, in particular their halogenides, including 0.001 to 0.5 molar, preferably 0.01 to 0.2 molar, most preferably 0.01 to 0.05 molar aqueous solutions of sodium chloride, lithium chloride, potassium chloride or magnesium dichloride. Also preferred for use are solutions of salts of the alkaline or alkaline earth metals with carboxylic or dicarboxylic acids, such as oxalic acid or acetic acid, solutions of sodium acetate or - oxalate in water, for example in the range of concentrations mentioned previously, for example 0.001 to 0.5 molar, preferably 0.01 to 0.2 molar, most preferably 0.01 to 0.05 molar.

The addition of auxillary substances like detergents and DMSO is also possible. If a chemical reaction is to be carried out with the eluted nucleic acids, be it directly at the membrane or in another reaction container, it is also possible to add such substances or other auxillary substances to the elution buffer that is to be used in the reaction preparation. Thus the addition of DMSO in low concentrations is common for many reaction preparations.

After a chemical reaction has been performed on nucleic acids those can also be eluted with the reaction buffer. After a SDA or a NASBA reaction the nucleic acid can for example be eluted with the reaction buffer or the reaction master mix.

Pure water is especially preferred as a means of elution, e.g., demineralized, double distilled, or ultrapure Millipore water.

Elution can be carried out successfully at temperatures from below 0° to 90° Celsius, for example, between 10° to 30° Celsius, and even at higher temperatures. Elution with steam is also possible. The lower limit of the elution temperature is, as described above, to be seen in the freezing point of the elution medium.

Because the process according to the invention can be carried out without problems even in the "field", i.e. away from laboratory installations and thus without extensive, electrically powered equipment, the invention is also aimed at the provision of isolation containers with which the process, according to the invention, can be carried out with a minimum of additional accessories. For this a reaction container, which contains a membrane, can be used. This can be brought into contact with an absorbant material, such as a sponge, to draw the various used buffers through the membrane. The sponge thus functions like a combination of a vacuum pump or a centrifuge in conjunction with a waste container. To obtain the eluate, the contact of the absorbant material with the membrane is interrupted such, that the eluate is not lost but removed and/or inspected further.

In this aspect the invention is specifically aimed at an isolation container for the isolation of nucleic acids with at least one cylindrical upper part with a top opening, a bottom opening and a membrane which is located at the bottom opening and covers the entire cross-section of the upper part; a lower part with an absorbant material; and a mechanism for the coupling of the upper part and the lower part, where, with established connection, the membrane is in connection with the absorbant material and with non-established connection the membrane is not in contact with the absorbant material.

The lower part is preferably a cylinder with a diameter equal to that of the upper part. In this way a simple tube with essentially constant diameter is obtained which can be manipulated like conventional reaction containers. This effect is achieved when the upper part or the upper part plus the lower part form a tube which can be placed inside a reaction container holder as utilized in laboratories. The mechanism can be a connection which permits a physical separation of upper part and lower part for example a bayonet coupling, a plug coupling or a screw coupling. Here a bayonet coupling has the advantage that it is easier to lock and unlock while the screw coupling permits a better, more water tight connection of upper and lower parts. Alternatively a predetermined breaking point between upper and lower parts can be provided for to permit at least a once-off separation of both parts and can be manufactured particularly cost efficient.

The mechanism can however also be a slide which can be inserted between the absorbant material and the membrane. This design also permits a separation of membrane and absorbant material.

To increase the process capacity and to make the process according to the invention even more economical it is furthermore possible to modify the isolation container described above in such a manner that several upper parts are located on top of the lower part. The lower part can at the same time serve as the support of the arrangement and be dimensioned such that a multitude of isolation processes can be carried out, in any event more than the connections for upper parts available in the lower part, before the drawing capacity of the absorbant material inside the lower part is exhausted.

The absorbant material in the lower part can show a sponge and/or granules. The granules can for example consist of superabsorbant material as it is known to specialists in the area of absorption technology (such as e.g. articles for hygiene).

The invention is simultaneously aimed at the use of the isolation container according to the present invention for the analysis of properties of nucleic acids and the isolation of nucleic acids.

With regard to the individual steps, the processes according to the invention are performed as follows:

If biological samples are the origin they firstly are to be lysed with a suitable buffer. Here additional processes can be introduced for the lysis, e.g. mechanical influence such as homogenization or ultra sound, enzymatic influence, change in temperature or additives. If necessary or desired, a pre-purification step can follow the lysis to remove any debris from the lysis. Subsequently, if not already carried out, the conditions, under which the immobilization of the nucleic acids at the surface is to be carried out, are standardized. Even after standardization of the bonding conditions a pre-purification step can follow either cumulative or alternative.

The pre-treated lysate of the sample used for the recovery of the nucleic acids or the originally free nucleic acid(s) (unless the original sample is biological) is/are pipetted, for example, into a (plastic) column, in which the membrane is fastened -- for example, on the bottom section. It is more efficient if the membrane is fastened to a frit, which serves as a mechanical support. The lysate is then transferred through the membrane, which can be achieved by applying a vacuum at the outlet of the column. On the other side the transfer can be accomplished by applying positive pressure from the lysate side. In addition -- as mentioned above -- the transport of the lysate can take place by centrifugation or by the effect of capillary forces. The latter can be produced, for example, with a sponge-like material which is brought in contact with the lysate or filtrate below the membrane. For a centrifugation the isolation container, open at the bottom, can be used inside a collection container for the transferred liquid.

The added washing step in the preferred embodiments of the invention can take place by having the washing buffer transferred through the surface or membrane, or by having it remain on the

same side of the surface as the nucleic acids. If the washing buffer is passed or drawn through, this can take place in a variety of ways, e.g., by a sponge mounted on the other side of the membrane, by a vacuum or positive pressure apparatus, or by a centrifuge or gravity.

The advantage of an arrangement using an absorbant material such as a sponge lies in a simple, safe and easy way to dispose of the filtrate -- in this case, only the sponge, which is now more or less saturated with the filtrate, needs to be changed. It should be clear at this point that the column can be operated continuously or in batches and that both these methods of operation can be carried out fully automated, until the membrane is completely saturated with nucleic acid. During the last step, if applicable, the elution of the nucleic acid occurs, which can for example be drawn off or removed by a pipette or removed upwards in some other way if no in situ analysis of the still bonded nucleic acids is to be carried out.

The desired nucleic acids are obtained in weak or no salt containing solutions in very small volumes which is of great advantage for all processes of molecular biological analysis as it is desirable to employ as small a volume as possible at simultaneously high concentration. To obtain as small a volume of eluate as possible, membranes that are as thin as possible are used as surfaces because only little liquid collects in them.

Moreover, the present invention has the advantage that, when the device is placed in a vertical position (the membrane then being in horizontal position), the space above the membrane can be used as a reaction area. Thus, it is possible, for example, that after the isolation and release of the nucleic acids produced by the process which is fundamental to this invention, not only not to remove them, but also to leave them in the isolation device and subject them to a molecular-biological application -- like restriction digestion, RT, PCR, RT-PCR, in vitro transcription, NASBA, rolling circle, LCR (ligase chain reaction), SDA (strand displacement amplification) or enzyme reactions like Rnase and Dnase-digestion for the complete removal of the nucleic acids not desired from time to time, to again bind the nucleic acids, which were produced by these reactions, to the membrane in accordance with the procedures on which this invention is based, or to retain them in the supernatant liquid, if applicable to wash them as described and subsequently to elute them, to isolate or analyze them, by means of, e.g., chromatography, spectroscopy, fluorometry, electrophoresis or similar measuring techniques.

The nucleic acids isolated pursuant to this invention are free from enzymes which decompose nucleic acids, and have a level of purity which is so high that they can immediately be treated and processed in the most varied ways.

The nucleic acids, which are produced according to this invention, can be used for cloning, and can serve as substrates for the most varied enzymes, such as DNA polymerases, RNA polymerases, e.g. T7 polymerase or T3 polymerase, DNA restriction enzymes, DNA-ligase, reverse transcriptase and others.

The nucleic acids produced by the process in this invention are especially well suited for amplification, particularly for the PCR, strand displacement amplification, the rolling circle procedure, the ligase chain reaction (LCR), SunRise, NASBA and similar procedures.

In addition, the processes according to this invention are particularly well suited for the preparation of nucleic acids for use in diagnostics, e.g. food analysis, toxicological tests, in medical and clinical diagnostics, pathogene diagnostics, gene expression analysis and in environmental analysis. The processes are particularly suitable for a diagnostic procedure which is characterized in that the nucleic acids purified by the process according to the invention are amplified in a subsequent step, and the nucleic acids amplified in this way are then subsequently or immediately detected (e.g. Holland, P.M. et al., 1991. *Proc. Natl. Acad. Sci.* 88, 7276 - 7280. Livak, K.J. et al., 1995. *PCR Methods Applic.* 4, 357 - 362; Kievits, T. et al., 1991. *J. Virol. Meth.* 35, 273 - 286; Uyttendaele, M. et al., 1994. *J. Appl. Bacteriol.* 77, 694 - 701).

Furthermore, the processes according to this invention are particularly well suited for the preparation of nucleic acids which can be subjected in a subsequent step to a signal amplification step based on a hybridization reaction, which is characterized especially in that the nucleic acids produced by the process according to the invention can be brought into contact with "branched nucleic acids," especially branched DNA and/or branched RNA and/or dendritic nucleic acids, as described in the following articles (e.g., Bresters, D. et al., 1994. *J. Med. Virol.* 43 (3), 262-286; Collins M.L. et al., 1997. *Nucl. Acids Res.* 25 (15), 2979-2984), and that the arising signal can be detected.

In the following an example for the ability to automate the process according to the invention is explained and examples are listed for the carrying out of the process with various surfaces and nucleic acids. Reference is made to the attached figures which illustrate the following:

Fig. 1 shows automatic equipment suitable for the performance of the process according to the invention in a schematic diagram.

Fig. 2 shows a first embodiment of an isolation container and collection tube for the performance of the process according to the invention.

Fig. 3 shows a second embodiment of an isolation container and collection tube for the performance of the process according to the invention.

Fig. 4 shows a third embodiment of an isolation container and collection tube for the performance of the process according to the invention.

Fig. 5 shows embodiments of isolation containers with an upper part according to the invention.

Fig. 6 shows the ethidium bromide stained gel of an electrophoretic separation of various samples according to the process of the invention.

Fig. 7 shows another ethidium bromide stained gel of an electrophoretic separation of various samples according to the process of the invention.

Fig. 8 shows another ethidium bromide stained gel of an electrophoretic separation of various samples according to the process of the invention.

Fig. 9 shows yet another ethidium bromide stained gel of an electrophoretic separation of various samples according to the process of the invention.

Fig. 10 again shows another ethidium bromide stained gel of an electrophoretic separation of various samples according to the process of the invention.

Fig. 11 finally shows another ethidium bromide stained gel of an electrophoretic separation of various samples according to the process of the invention.

The processes according to the invention are preferably performed automatically, either partially or completely, in other words, in all steps. An example for suitable automatic equipment is illustrated in Fig. 1, in which a main part 1 is equipped with control electronics and driving motors with a work platform 3 and a movable arm 2. Various elements are positioned on the work platform, such as area 4 to hold various containers. A vacuum manifold 5 serves to draw liquids from isolation containers, positioned above and open at the bottom or otherwise with the containers connected to the vacuum manifold. A shaker 6 is also provided, which e.g. can be used to subject the biological samples to lysis. The isolation container assemblies used are e.g. injection-molded parts with integrated isolation containers, in which the surfaces are placed according to the invention. Typically 8, 12, 24, 48, 96 or up to 1536 isolation containers can be used as these are for example seen in the formats of modern multi-well-trays. Even higher numbers of isolation containers might be possible on one assembly, if the corresponding standards are available. With the aid of Luer-adapters it is, however, also possible to make individual bottoms of the assembly available and to equip these with one or more isolation containers as needed. Isolation containers used individually without Luer-adapters are also included in the invention.

Under a vacuum and dispensing mechanism 8 the isolation container assemblies are placed in the automatic apparatus and via these, liquids can be taken in and drained off. In this assembly several single vacuum tubes can be used, so as to make the simultaneous processing of more than one isolation or reaction container possible. The vacuum and dispensing mechanism 8 therefore acts as a pipette. Vacuum and pressure are fed to the vacuum and dispensing mechanism 8 via tube 9.

To isolate the nucleic acids, reaction containers with cells may for example be placed in the shaker/holder 6, into which lysis buffers are introduced by means of the dispensing mechanism 8. After mixing, the cell lysates are transferred to isolation containers. The lysis buffer is subsequently drawn through the surfaces in the isolation containers. Subsequently, the surfaces may be washed with a washing buffer in order to remove cell lysate residue, in which also the

washing buffer is drained off downwards. Finally an elution buffer is dispensed into the isolation devices and after possibly additional shaking, the released nucleic acids are removed in an upwards direction and transferred to collection tubes.

Usually, disposable tips are used on the vacuum and dispensing mechanism 8 to prevent contamination of the samples.

Fig. 2 to 4 show different schematic examples for suitable isolation containers to be used in the present invention.

In Fig. 2 a funnel-shaped isolation container 10 is provided with a surface 11, e.g. a membrane, which is placed on a collection tube 12, which contains a sponge-like material 13 that serves to absorb the lysis and washing buffers. Under the sponge-like material 13 a superabsorbent layer 14 may be placed to improve the suction performance. Alternatively layer 14 may also contain a material which is able to convert water chemically, e.g. acrylate. The water is thereby also removed from the process. Lysate or another preparation of nucleic acids is placed in the funnel. The sponge-like material 13 draws the applied liquid through membrane 11. Prior to the addition of the elution buffer, the sponge is spaced some distance from the membrane, e.g. by a mechanism inside collection tube 12 (not visible in the fig.). This will prevent that the eluate buffer in the last step is also suctioned off through membrane 11. This buffer rather stays on the surface (Fig. 1b) and can be removed together with the nucleic acids in an upwards direction. When using this assembly, the vacuum mechanism 5 is not required in the automatic apparatus.

Fig. 3 shows another example of an isolation device, which, via a Luer-connection located at its bottom, is, by way of a Luer-adapter 17, connected to a collection container 16, which in this case does not contain a sponge, but is connected to a vacuum mechanism with the aid of a nozzle 18. Lysis and washing buffers may in this case be drawn through membrane 11 by applying a vacuum. When the eluate buffer is introduced, the vacuum remains turned off, so that the eluate can be removed in an upward direction. With the use of a Luer-connection, individual isolation containers can be removed from the isolation container assembly. It is to be understood however, that the vacuum collection container can also be combined with fixed isolation devices, e.g. multi-well containers with 8, 12, 24, 48, 96 or more individual containers.

Fig. 4 finally shows an embodiment which provides for a collection container, into which the buffers are drawn through gravity or centrifugation. The eluate buffer, which is used in small volumes, is not heavy enough in and of itself to penetrate membrane 11 and can again be removed in an upward direction.

Fig. 5 shows the embodiments of the isolation containers according to the invention.

Fig. 5 A shows an isolation container with a cylindrical upper part 20. This upper part is connected to the lower part 22 by means of a screw coupling 25. In place of the screw coupling, other forms of connections may be used as long as these permit a liquid-tight connection and support the membrane 11. In this example of an embodiment the membrane 11 is located directly

29620-1

at the bottom opening of the upper part 20. It may however be placed inwards or at an angle other than 90° to the wall of the upper part. The lower part shows a cylindrical form as well but may, with other embodiments, be developed differently. As such a rectangular form might be used which improves the stability of the upper part 20 on the substrate. An enlargement of the lower part 22 in relation to the upper part 20 is also possible, for example, if, with certain options of the process according to the invention, a larger space is required in the lower part 22 to completely contain the used solutions in the absorbant material.

Part B shows an alternative embodiment to the embodiment shown in fig. 5 A. In this the upper part 20 and the lower part 22 are permanently connected with one another or may also be made as one single component. Between the absorbant material 13 and the membrane 11, a slide 27 can be inserted into the isolation container through an opening 26 to separate the membrane 11 and the absorbant material 13. In this example slide 27 additionally shows a handle section 28 to ease the retraction of the slide 27. However, a slide may also be designed without this handle section. As shown in fig. 5 B, the absorbant material 13 slightly expands to bridge the space taken by the slide and to contact the membrane.

Fig. 5 C shows a further embodiment of the isolation container according to the invention. Here the lower part 23 is equipped with several sockets 30 to accommodate the upper parts 20 which permit the simultaneous processing of several preparations. In this example the upper parts 20 are connected to the lower part 23 by means of screw couplings 31. Although depicted smaller than the upper parts 20 in Fig. 5 A and B, it is to be understood that the upper parts can be of the same size (or larger or smaller) as or than these embodiments.

Fig. 5 D finally shows an isolation container with a jacket 32 containing cooling liquid and surrounding the membrane 11 on the outside. In this example upper part 20 and lower part 24 are plugged together. Another form of connection or a one-part embodiment are also possible. The jacket 32 comprises two compartments 33 and 34 which can be connected through a destructible partition 35. Both compartments 33, 34 are filled with substances, e.g. solutions, whose temperatures sink through mixing after the destruction of the partition 35.

The procedure described above is explained by the following examples. Different and various ways of using the procedures will be evident to the expert from the foregoing description and from the examples. Reference is explicitly made to the fact that these examples and the corresponding descriptions are presented solely for the purpose of illustration, and are not to be regarded as limitations on the invention.

Example 1

Isolation of total RNA from HeLa-cells

Commercially available hydrophobic nylon membranes (for example, a material from MSI: Magna*SH with a pore diameter of $1.2\ \mu\text{m}$ or a material from Pall GmbH: Hydrolon* with a pore

*Trade-mark

diameter of 1.2 μm) which have been made hydrophobic by means of a chemical post-treatment are placed in a plastic column in a single layer. The membranes are placed on a polypropylene frit which serves as a mechanical support. The membranes are fixed in the plastic column with a ring.

The column prepared in this manner is connected by means of a Luer connection to a vacuum chamber. All the isolation steps are conducted through the application of a vacuum.

For the isolation, 5×10^5 HeLa-cells are pelletized by centrifugation and the supernatant liquid is removed. The cells are lysed by the addition of 150 μl of a commercial guanidinium isothiocyanate buffer -- for example RLT buffer from the Qiagen Company -- in a manner familiar from the present state of the art. The lysis is promoted through roughly mixing by pipetting or vortexing over a period of about 5 s. Then 150 μl of 70% ethanol are added and mixed in by pipetting or by vortexing over a period of about 5 s.

The lysate is then pipetted into the plastic column and suctioned through the membrane by evacuating the vacuum chamber. Under the conditions thus created, the RNA remains bound to the membrane. Next, washing takes place with a first commercial washing buffer containing guanidinium isothiocyanate -- for example, with the RW1 buffer of QIAGEN -- and, after that, with a second washing buffer containing TRIS or TRIS and alcohol -- for example, with the RPE buffer of QIAGEN. In each case the washing buffers are suctioned through the membrane by evacuation of the vacuum chamber. After the final washing step, the vacuum is maintained for a period of about 10-min, in order to dry the membrane, after which the vacuum chamber is airted.

For the elution, 70 μl of RNase-free water is pipetted onto the membrane in order to release the purified RNA from the membrane. After incubation for one minute at a temperature in the range from 10 to 30° C, the eluate is pipetted off the membrane from the top and the elution step is repeated in order to make sure that the elution is complete.

The amount of isolated total RNA obtained in this manner is then determined by photometric measurement of the light absorption with a wavelength of 260 nm. The photometric determination of the ratio between the absorbance values at 260 and 280 nm measures the quality of the RNA such obtained.

The results of the two isolations with hydrophobic nylon membranes (Nos. 1 and 2) are compared in the following Table 1, comparative experiments, in which on the one hand a hydrophilic nylon (Nylaflo) (No. 3) as well as a silica membrane (No. 4) were used. The values reported in the table provide convincing support for the impressive isolation yield and separation effect of the materials used in accordance with the invention. They also show that silica-gel fleeces produce clearly less yield, which presumably can be attributed to its fleece-like structure and the ensuing absorption of the major portion of the eluate buffer.

Table 1. RNA yield and purity of the total RNA isolated in accordance with Example 1

No.	Type of membrane	Yield of Total RNA (μ g)	E ₂₆₀ /E ₂₈₀
1	Magna SH 1.2 μ m (hydrophobic nylon)	6.0	1.97
2	Hydrolon 1.2 μ m (hydrophobic nylon)	7.1	2.05
3	Nylaflo (hydrophilic nylon)	<0.2	Not determined
4	Hydrophilic silica membrane	<0.2	Not determined

The isolated RNA can also be analyzed on agarose gels that have been stained with ethidium bromide. For this purpose, for example 1.2%-formaldehyde-agarose gels are produced. The result is reproduced in Fig. 6.

In Fig. 6, line 1 embodies a total RNA that was isolated by means of a hydrophobic nylon membrane from Magna SH with a pore diameter of 1.2 μ m.

Line 2 depicts a total RNA that was isolated by means of a hydrophobic nylon membrane from Hydrolon with a pore diameter of 1.2 μ m.

Line 3 depicts the chromatogram of a total RNA that was isolated by means of a silica membrane.

In each case, 50 μ l of the total RNA isolates were analyzed.

Fig. 6 provides distinct evidence that, when a silica membrane is used, no measurable proportion of the total RNA can be isolated.

Example 2

Isolation of free RNA by binding the RNA to hydrophobic membranes by means of various salt/alcohol mixtures.

In this example, the lysate and washing solutions are lead across the hydrophobic membrane by applying a vacuum.

Hydrophobic nylon membranes (for example, 1.2 μ m Hydrolon from the Pall Company) are introduced into plastic columns that are connected to a vacuum chamber, in a manner analogue to that of Example 1.

100 μ l of an aqueous solution containing total RNA is mixed, by pipetting, with, respectively, 350 μ l of a commercially available lysis buffer containing guanidium isothiocyanate (for example, the RLT buffer from QIAGEN), 350 μ l of 1.2 M sodium acetate solution, 350 μ l 2 M

sodium chloride solution and 350 µl of 4 M lithium chloride solution, respectively, and mixed by pipetting.

Next, 250 µl of ethanol is added to each mixture and mixed in, likewise by pipetting. After that, the solutions containing RNA are pipetted into the plastic columns and suctioned through the membrane by evacuating the vacuum chamber. Under the conditions described, the RNA remains bound to the membranes. The membranes are then washed, as described in Example 1.

Finally, the RNA - also as described in Example 1 - is removed from the membrane by pipetting in an upward direction.

The volume of isolated total RNA was determined by photometric measurement of the light absorption at 260 nm. The photometric determination of the ratio between the light absorbance values at 260 and 280 nm measures the quality of the RNA such obtained.

Table 2: Isolation of free RNA by binding the RNA to hydrophobic membranes by means of various salt-alcohol mixtures

No.	Salt/Alcohol-mixture	Yield of Total RNA (µg)	E ₂₆₀ /E ₂₈₀
1	RLT buffer QIAGEN/ 35%-ethanol	9.5	1.92
2	0.6 M sodium acetate / 35%-ethanol	8.5	1.98
3	1 M sodium chloride/ 35%-ethanol	7.9	1.90
4	2 M lithium chloride/ 35%-ethanol	4.0	2.01

Example 3

Isolation of total RNA from HeLa-cells

According to example 1, plastic columns with various hydrophobic membranes are assembled. The column prepared in this manner is placed in a collection tube, the following isolation steps are conducted through centrifugation.

For the isolation, 5×10^5 HeLa-cells are pelletized by centrifugation and the supernatant substance is removed. The cells are lysated by the addition of 150 µl of a commercial guanidium isothiocyanate buffer - for example RLT buffer from QIAGEN - in a manner principally familiar from the state of the art. Lysis is promoted through repeatedly mixing by pipetting or vortexing over a period of about 5 s. Then 150 µl of 70% ethanol are added and mixed in by pipetting or by vortexing over a period of about 5 s.

The lysate is subsequently transferred into the plastic column and passed through the membrane by way of centrifugation at 10000 x g for 1 minute. Subsequently, washing takes place with a commercially available washing buffer containing guanidinium isothiocyanate— e.g. with the RW1-buffer of QIAGEN – followed by a second washing buffer containing Tris and alcohol— e.g. RPE-buffer by QIAGEN. The washing buffers are passed through the membrane by centrifugation. The last washing step takes place at 20000 x g for 2 minutes to dry the membrane.

For the elution, 70µl RNase-free water is pipetted onto the membrane in order to release the purified RNA from the membrane. After incubation for 1 - 2 minutes at a temperature in the range of 10 to 30° C, the eluate is pipetted off the membrane from the top. The elution step is repeated once in order to achieve a complete elution.

The volume of isolated total RNA obtained in this manner is then determined by photometric measurement of the light absorption at a wavelength of 260 nm. The photometric determination of the ratio between the light absorbance values at 260 and 280 nm measures the quality of the RNA.

The results of the isolations with different hydrophobic membranes are shown in Table 3.

3 – 5 parallel tests per membrane are carried out and the average value is calculated. By using a silica membrane, no measurable volume of total RNA can be isolated, if the eluate is obtained by removing it from the top of the membrane.

Table 3: RNA-yield of total RNA according to example 3 by binding to hydrophobic membranes

Manufacturer	Membrane	Material	RNA (µg)	E ₂₆₀ / E ₂₈₀
Pall	Hydrolon, 1.2 µm	hydrophobic nylon	6.53	1.7
Pall	Hydrolon, 3 µm	hydrophobic nylon	9.79	1.72
Pall	Fluoro Trans G	hydrophobic polyvinylidene difluoride	6.16	1.72
Pall	Fluoro Trans W	hydrophobic polyvinylidene difluoride	5.4	1.9
Pall	Bio Trace	hydrophobic polyvinylidene difluoride	4.3	1.97
Pall	Supor-450 PR	hydrophobic polyether sulfone	3.96	1.76
Pall	V-800 R	hydrophobic acrylate copolymer	6.26	1.72
Pall	Versapor-1200R	hydrophobic acrylate copolymer	6.23	1.68
Pall Sciences	Versapor-3000R	hydrophobic acrylate copolymer	3.54	1.74
GORE-TEX	OH 9335	hydrophobic polytetrafluor-ethylene	1.59	1.72
GORE-TEX	OH 9336	hydrophobic polytetrafluor-ethylene	2.15	1.65

29620-1

GORE-TEX	OH 9337	hydrophobic polytetrafluor-ethylene	3.6	1.59
GORE-TEX	QH 9316	hydrophobic polytetrafluor-ethylene	3.61	1.69
GORE-TEX	QH 9317	hydrophobic polytetrafluor-ethylene	2.87	1.70
Millipore	Mitex* Membrane	hydrophobic polytetrafluor-ethylene	1.98	1.62
Millipore	Durapore *	hydrophobic polyvinylidene difluoride	7.45	1.72
MSI	Magna-SH, 1.2 μ m	hydrophobic nylon	4.92	1.69
MSI	Magna-SH, 5 μ m	hydrophobic nylon	10.2	1.71
MSI	Magna-SH, 10 μ m	hydrophobic nylon	7.36	1.76
MSI	Magna-SH, 20 μ m	hydrophobic nylon	7.04	1.65
Sartorius	type 118	hydrophobic polytetrafluor-ethylene	7.6	1.61
Mupor	PM 12 A	hydrophobic polytetrafluor-ethylene	6.7	1.77
Mupor	PM 3 VL	hydrophobic polytetrafluor-ethylene	6.6	1.77

Example 3b

Isolation of total RNA from HeLa-cells through bonding onto hydrophilic membranes

According to example 1, plastic columns with various hydrophilic membranes are assembled. The column prepared in this manner is placed in a collection tube, the following isolation steps are conducted through centrifugation.

For the isolation, 5×10^5 HeLa-cells are used. The following isolation steps and the elution of the nucleic acid are carried out as described in example 3.

The volume of isolated total RNA obtained in this manner is then determined by photometric measurement of the light absorption at a wavelength of 260 nm. The photometric determination of the ratio between the light absorbance values at 260 and 280 nm measures the quality of the RNA.

The results of the isolations with different hydrophilic nylon membranes are shown in Table 3b. 2 – 5 parallel tests per membrane are carried out and the average value is calculated. By using a silica membrane, no measurable volume of total RNA can be isolated, if the eluate is obtained by removing it from the top from the membrane.

*Trade-mark

29620-1

Table 3b: RNA-yield of total RNA according to example 3b by binding to hydrophilic membranes

Manufacturer	Membrane	Material	RNA (µg)	E ₂₆₀ /E ₂₈₀
Pall	Loprodyne*	hydrophilic nylon	3.1	1.8
Pall	Loprodyne*	hydrophilic nylon	3.1	1.78
Pall	Biodyne A*	hydrophilic nylon	3.1	1.8
Pall	Biodyne A*	hydrophilic nylon	3.6	1.83
Pall	Biodyne B*	hydrophilic nylon	2.6	1.84
Pall	Biodyne B*	hydrophilic nylon	4.2	1.84
Pall	Biodyne C*	hydrophilic nylon	6.1	1.88
Pall	Biodyne C*	hydrophilic nylon	5.2	1.91
Pall	Biodyne plus*	hydrophilic nylon	3.3	1.87
Pall	I.C.E.-450*	hydrophilic polyether sulfone	6.36	1.8
Pall	I.C.E.-450sup*	hydrophilic polyether sulfone	3.07	1.71
Pall	Supor-800*	hydrophilic polyether sulfone	4.12	1.7
Pall	Supor-450*	hydrophilic polyether sulfone	4.69	1.69
Pall	Supor-100*	hydrophilic polyether sulfone	3.25	1.71
Pall	Hemasep V*	hydrophilic polyester	4.16	1.74
Pall	Hemasep L*	hydrophilic polyester	6.67	1.65
Pall	Leukosorb*	hydrophilic polyester	1.5	1.84
Pall	Premium Release*	hydrophilic polyester membrane	1.66	1.63
Pall	Polypro-450*	hydrophilic polypropylene	5.09	1.78
Gore-Tex	OH 9339*	hydrophilic polytetrafluor- ethylene	1.08	1.65
Gore-Tex	OH 9338*	hydrophilic polytetrafluor- ethylene	3.97	1.67
Gore-Tex	QH 9318*	hydrophilic polytetrafluor- ethylene	3.61	1.69
Millipore	Durapore*	hydrophilized polyvinylidene difluoride	5.6	1.69
Millipore	Durapore*	hydrophilized polyvinylidene difluoride	3.12	1.68
Millipore	LCR*	hydrophilized polytetrafluoro ethylene	3.14	1.66
Sartorius	Type 250*	hydrophilic polyamide	4.3	1.66
Sartorius	Type 113*	hydrophilic cellulose nitrate	1.8	1.86
Sartorius	Type 113*	hydrophilic cellulose nitrate	1.9	1.74
Infiltec	Polycon, 0.01*	hydrophilic polycarbonate	0.17	1.64
Infiltec	Polycon, 0.1*	hydrophilic polycarbonate	0.73	1.68
Infiltec	Polycon, 1*	hydrophilic polycarbonate	3.33	1.86

Example 4

Isolation of free RNA from an aqueous solution

According to Example 1 plastic columns with different hydrophobic membranes are assembled. 100 µl of an aqueous solution containing total RNA are mixed with 350 µl of a commercially available lysis buffer containing guanidinium-isothiocyanate – e.g. RLT-buffers by QIAGEN.

*Trade-mark

29620-1

Subsequently 250 µl of ethanol are added and mixed by pipetting. This mixture is then transferred to the column and passed through the membrane by way of centrifugation (10000 x g; 1 minute). The membranes are subsequently washed twice with a buffer – e.g. RPE by QIAGEN. Each buffer is passed through the membranes by way of centrifugation. The last washing step is carried out at 20000 x g to dry the membranes.

Subsequently, the RNA, as already described in Example 1, is eluted with RNase-free water and pipetted off the membrane from the top.

The volume of isolated total RNA obtained in this manner is then determined by photometric measurement of the light absorption at a wavelength of 260 nm. The photometric determination of the ratio between the light absorbance values at 260 and 280 nm measures the quality of the RNA.

The results of the isolation with various hydrophobic membranes are listed in Table 4 below.

3 – 5 parallel tests per membrane are carried out and in each case the average value is calculated.

By using a silica membrane, no measurable volume of total RNA can be isolated, if the eluate is recovered by removing it off the membrane from the top.

Table 4: Isolation of free RNA from an aqueous solution by binding to hydrophobic membranes

Manufacturer	Membrane	Material	RNA (µg)	E ₂₆₀ /E ₂₈₀
Pall	Hydrolon, 1.2µm*	Hydrophobic nylon	5.15	1.75
Pall	Hydrolon, 3 µm*	Hydrophobic nylon	0.22	1.79
Pall	Fluoro Trans G*	Hydrophobic polyvinylidene difluoride	5.83	1.79
Pall	Fluoro Trans W*	Hydrophobic polyvinylidene difluoride	5.4	1.84
Pall	Bio Trace *	Hydrophobic polyvinylidene difluoride	4	1.79
Pall	Emflon*	Hydrophobic polytetrafluor – ethylene	0.2	1.7
Pall	Supor-450 PR*	Hydrophobic polyether sulfone	5.97	1.71
Pall	Supor-200 PR*	Hydrophobic polyether sulfone	2.83	1.66
Pall	V-800 R*	Hydrophobic acrylate co-polymer	2.74	1.77
Gore-Tex	OH 9335*	Hydrophobic polytetrafluor – ethylene	4.35	1.63
Gore-Tex	OH 9336*	Hydrophobic polytetrafluor – ethylene	7.43	1.71
Gore-Tex	OH 9337*	Hydrophobic polytetrafluor – ethylene	5.96	1.62
Gore-Tex	QH 9316*	Hydrophobic polytetrafluor – ethylene	5.92	1.67
Gore-Tex	QH 9317*	Hydrophobic polytetrafluor – ethylene	8.7	1.66
Millipore	Fluoropore *	Hydrophobic polytetrafluor - ethylene	8.46	1.70
Millipore	Durapore, 0.65 µm*	Hydrophobic polytetrafluor - ethylene	4.23	1.8
MSI	Magna-SH, 1.2 µm*	Hydrophobic nylon	1.82	1.76
MSI	Magna-SH, 5 µm*	Hydrophobic nylon	0.6	1.78
Sartorius	Typ 118*	Hydrophobic polytetrafluor - ethylene	0.9	1.82
Sartorius	Typ 118*	Hydrophobic polytetrafluor – ethylene	5.4	1.74
Mupor	PM12A*	Hydrophobic polytetrafluor - ethylene	1.1	1.98

*Trade-mark

Example 4 b

Isolation of free RNA from an aqueous solution through binding to hydrophilic membranes

According to Example 1 plastic columns with different hydrophilic membranes are assembled.

100 µl of an aqueous solution containing total RNA are mixed with 350 µl of a commercially available lysis buffer containing guanidinium-isothiocyanate – e.g. RLT-buffers by QIAGEN. Subsequently 250 µl of ethanol are added and mixed by pipetting. This mixture is then transferred to the column and, according to example 4, passed through the membrane, washed and dried.

Subsequently, the RNA, as already described in Example 1, is eluted with RNase-free water and pipetted off the membrane from the top.

The volume of isolated total RNA obtained in this manner is then determined by photometric measurement of the light absorption at a wavelength of 260 nm. The photometric determination of the ratio between the light absorbance values at 260 and 280 nm measures the quality of the RNA.

The results of the isolations with various hydrophilic membranes are listed in Table 4b below.

2 – 5 parallel tests per membrane are carried out and in each case the average value is calculated.

By using a silica membrane, no measurable volume of total RNA can be isolated, if the eluate is recovered by removing it off the membrane from the top.

Table 4b: Isolation of free RNA out of an aqueous solution through binding to hydrophilic membranes

Manufacturer	Membrane	Material	RNA (µg)	E ₂₆₀ /E ₂₈₀
Pall	Loprodyne	hydrophilic nylon	2	1.8
Pall	Loprodyne	hydrophilic nylon	1.4	1.87
Pall	Biodyne A	hydrophilic nylon	4.5	1.93
Pall	Biodyne A	hydrophilic nylon	3.1	1.9
Pall	Biodyne B	hydrophilic nylon	1.7	1.94
Pall	Biodyne B	hydrophilic nylon	1.2	1.94
Pall	Biodyne C	hydrophilic nylon	3.7	1.93
Pall	Biodyne C	hydrophilic nylon	3.1	1.93
Pall	Biodyne plus	hydrophilic nylon	1.1	1.87
Pall	I.C.E.-450	hydrophilic polyether sulfone	1.92	1.82
Pall	I.C.E.-450sup	hydrophilic polyether sulfone	0.87	1.67
Pall	Supor - 800	hydrophilic polyether sulfone	3.93	1.74
Pall	Supor – 450	hydrophilic polyether sulfone	1.78	1.74
Pall	Supor – 100	hydrophilic polyether sulfone	1.04	1.68
Pall	Hemasep V	hydrophilic polyester	4	1.79
Pall	Hemasep L	hydrophilic polyester	0.47	2.1

Pall	Polypro – 450	hydrophilic polypropylene	5.09	1.78
Gore-Tex	OH 9339	hydrophilic polytetrafluor- ethylene	0.43	1.48
Gore-Tex	OH 9338	hydrophilic polytetrafluor- ethylene	3.63	1.64
Gore-Tex	QH 9318	hydrophilic polytetrafluor- ethylene	5.92	1.67
Millipore	Durapore	hydrophilized polyvinylidene difluoride	1.18	1.79
Millipore	LCR	hydrophilized polytetrafluor- ethylene	2.84	1.72
Sartorius	Type 250	hydrophilic polyamide	2.7	1.7
Sartorius	Type 111	hydrophilic cellulose acetate	1.6	1.85
Sartorius	Type 111	hydrophilic cellulose acetate	2.2	2.1
Sartorius	Type 111	hydrophilic cellulose acetate	0.3	2.01
Sartorius	Type 113	hydrophilic cellulose nitrate	4	1.88
Sartorius	Type 113	hydrophilic cellulose nitrate	3.8	1.87

Example 5

Isolation of total RNA from HeLa-cells depending on the membrane's pore size

According to Example 1 plastic columns have been assembled with hydrophobic membranes with different pore sizes.

According to Example 3, a cell lysate is made from 5×10^5 HeLa-cells and transferred to the columns. Subsequently the membranes are washed with the commercially available buffers RW1 and RPE of QIAGEN by aid of centrifugation. The last centrifugation step is carried out at 20000 x g for 2 minutes to dry the membrane. The elution is carried out as described in Example 1.

The results are listed in Table 5 below.

3 – 5 parallel tests are performed per membrane and the average value is calculated for each.

Table 5: RNA-yield of isolated total RNA by binding to hydrophobic membranes with different pore sizes

Manufacturer	Membrane	Material	Pore Size (μm)	RNA (μg)	E ₂₆₀ /E ₂₈₀
Infiltec	Polycon 0.01	Hydrophilic Polycarbonate	0.01	0.17	1.64
Pall	Fluoro Trans G	Hydrophobic polyvinylidene difluoride	0.2	6.16	1.72
Pall	Supor-450 PR	Hydrophobic polyether sulfone	0.45	3.96	1.76
Millipore	Durapore	Hydrophobic polyvinylidene difluoride	0.65	7.45	1.72
MSI	Magna-SH	Hydrophobic Nylon	1.2	4.92	1.69
MSI	Magna-SH	Hydrophobic Nylon	5	10.2	1.71
MSI	Magna-SH	Hydrophobic Nylon	10	7.36	1.76
MSI	Magna-SH	Hydrophobic Nylon	20	7.04	1.65

Example 6

Stability and Quality of Total-RNA isolated from HeLa cells

According to Example 1, plastic columns are assembled with hydrophilic membranes (e.g. Hydrolon, pore size 3 μm by the Pall Company).

According to Example 3, a cell lysate is made from 5×10^5 HeLa-cells and transferred to the columns. Subsequently the membranes are washed with the commercially available buffers RW1 and RPE of QIAGEN by aid of centrifugation. The last centrifugation step is carried out at 20000 x g for 2 minutes to dry the membrane. The elution is carried out as described in Example 1. The isolated total-RNA is incubated for 16 hours at 37°C and subsequently applied to a non-denaturalizing agarose gel and analyzed. It becomes evident that the RNA was not subjected to any degradation. The RNA isolated according to the method described above shows no contamination with nucleic acid degrading enzymes and is thus of a high quality.

Example 7

Isolation of free RNA out of aqueous solutions through binding at a hydrophilic membrane inside a 96-well-tray

A 96-well-tray with a hydrophilic polyvinylidene difluoride membrane (Durapore, 0.65 μm, from Millipore Company) is used.

5.3 ml of an aqueous solution containing total-RNA are mixed with 18.4 ml of a commercially

available lyse buffer containing guanidinium isothiocyanate e.g. RLT buffer from QIAGEN company. Subsequently 13.1 ml ethanol are added and mixed through pipetting. 350 µl of this mixture are added to every well and transferred through the membrane by applying a vacuum. The membranes are subsequently washed twice with a buffer e.g. RPE of QIAGEN company. The buffer is transferred through the membrane through application of a vacuum in every instance. After the last washing step the tray is dabbed off with a paper towel and then dried for 5 minutes through applying a vacuum.

Subsequently the RNA is eluted, as described in example 1, with RNase free water and pipetted off the membrane.

The volume of isolated total RNA obtained in this manner is then determined by photometric measurement of the light absorption at a wavelength of 260 nm and the average value as well as the standard deviation calculated for the entire tray. The average value is 8.4 µg with a standard deviation of 0.7 µg.

Example 8

Isolation of Total-RNA through capillary forces

A 96-well-tray with a hydrophilic polyvinylidene difluoride membrane (Durapore, 0.65 µm, from Millipore Company) is used.

33 µl of an aqueous solution containing total-RNA are mixed with 110 µl of a commercially available lyse buffer containing guanidinium – iso –cyanate e.g. RLT buffer from QIAGEN company. Subsequently 78 µl ethanol are added and mixed through pipetting. 45 µl of this mixture are added to every well. A household sponge with strong suction properties is wetted with water and the 96-well-tray placed with the bottom of the membrane on top of the sponge. The RNA mixture is transferred through the membrane by means of the capillary forces. The membranes are subsequently washed twice with a buffer e.g. RPE of QIAGEN company. The buffer is also transferred through the membrane by placing the tray on top of the sponge. After the last washing step the tray is dried for 5 minutes exposed to air.

Subsequently the RNA is eluted, as described in example 1, with RNase free water and pipetted off the membrane.

The volume of isolated total RNA obtained in this manner is then determined by photometric measurement of the light absorption at a wavelength of 260 nm and the average value as well as the standard deviation calculated for the entire tray. The average value is 5.9 µg with a standard deviation of 0.7 µg.

Example 9

Isolation of genomic DNA from an aqueous solution by means of a buffer containing guanidinium hydrochloride.

According to Example 1, plastic columns are assembled with hydrophobic membranes (e.g. Magna-SH, pore size 5 µm by the MSI Company). The purification is carried out with

commercial buffers from QIAGEN company.

200 µl of an aqueous solution containing genomic DNA derived from liver tissue are prepared in PBS buffer. 200 µl of a buffer containing guanidinium hydrochloride e.g. AL from QIAGEN company are added and mixed. Subsequently 210 µl ethanol are added and mixed by vortexing. The mixture is added to the column according to example 3 and transferred through the membrane by centrifugation. Subsequently the membrane is washed and dried with a buffer containing alcohol, e.g. AW from QIAGEN company. The elution is carried out as described in example 1. Three parallel tests are carried out and the average value is calculated.

The volume of isolated DNA is then determined by photometric measurement of the light absorption at a wavelength of 260 nm and amounts to approximately 30% of the original volume. The ratio of the absorption at 260nm to that at 280 nm amounts to 1.82.

Example 10

Isolation of genomic DNA out of an aqueous solution through binding at hydrophobic membranes by means of a buffer containing guanidinium-iso-thiocyanate.

According to the example 1 plastic columns with various membranes are assembled.

100 µl of an aqueous solution containing total- DNA are mixed with 350 µl of a lysis buffer (4 M GITC, 0.1 M MgSO₄, 25 mM Na citrate, pH 4) containing guanidinium isothiocyanate. Subsequently 250 µl ethanol are added and mixed by pipetting. The mixture is added to the column and transferred through the membrane by centrifugation (10000 x g; 1 minute). Subsequently the membranes are washed twice with a buffer e.g. RPE from QIAGEN company. The buffer is transferred through the membranes by centrifugation on each occasion. The last washing step is carried out at 20000 x g to dry the membranes. The elution is carried out as described in example 1. Three parallel tests are carried out per membrane and for each the average value is calculated.

The results are listed in table 6.

Table 6: Yield of DNA out of aqueous solution through binding to hydrophobic membranes

Manufacturer	Membrane	Material	DNA (µg)
Pall	Hydrolon 1.2 µm	Hydrophobic nylon	1.3
Pall	Supor-450 PR	Hydrophobic polyether sulfone	2.2
Millipore	Fluoropore	Hydrophobic polytetrafluor - ethylene	1.1
Millipore	Durapore	Hydrophobic polyvinylidene difluoride	1.2

Example 11

Isolation of genomic DNA from tissue

According to Example 1, plastic columns are assembled with hydrophobic membranes (e.g. Magna-SH, 5 µm by MSI). Purification is carried out with the commercially available buffers of QIAGEN.

180 µl of ATL-buffer are added to 10 mg of kidney tissue (mouse) and ground in a mechanical homogenizer. Subsequently proteinase K (approx. 0.4 mg eluted in 20 µl of water) are added and left to incubate for 10 minutes at 55°C. After adding 200 µl of a buffer containing guanidinium hydrochloride - e.g. AL by QIAGEN - and after a 10 minute incubation at 70°C, 200 µl of ethanol are added and mixed with this solution. This mixture is placed in the column and passed through the membrane by centrifugation. The membrane is then washed with alcohol containing buffers, e.g. AW1 and AW2 from QIAGEN company – and subsequently dried by way of centrifugation. The elution is carried out as described in Example 1. Three parallel tests are carried out and the average value is calculated.

The amount of isolated DNA is subsequently determined by photometric measurement of the light absorption at a wavelength of 260 nm and is on average 9.77 µg. The absorption ratio at 260 nm to 280 nm is 1.74.

Example 12

Isolation of genomic DNA from blood

According to Example 1, plastic columns are assembled with hydrophobic membranes (e.g. Magna-SH, 5 µm from MSI company). Purification is carried out with the commercially available buffers from QIAGEN.

To 200 µl blood 200 µl of AL and 20 µl QIAGEN protease are added, mixed in thoroughly and incubated at 56°C for 10 minutes. After an addition of 200 µl of ethanol the preparation is mixed, added to the column and transferred through the column by means of centrifugation. The membrane is washed with buffers containing alcohol – e.g. AW1 and AW2 from QIAGEN company – and dried through centrifugation. The elution is carried out as described in example 1. The amount of isolated DNA is subsequently determined by photometric measurement of the light absorption at a wavelength of 260 nm and amounts to 1.03 µg. The absorption ratio at 260 nm to 280 nm is 1.7.

Example 13

Isolation of total-RNA from a RNA-DNA-mixture

According to Example 1, plastic columns are assembled with hydrophobic membranes (e.g. Hydrolon 1.2 μm by the Pall Company).

275 μl of an aqueous solution containing total-RNA and genomic DNA are mixed with 175 μl of a lysis buffer, e.g. RLT buffer from QIAGEN company, containing guanidinium isothiocyanate. Subsequently 250 μl ethanol are added and mixed by pipetting. This mixture is added to the column and transferred through the membrane, washed and dried according to example 4. The penetrant from the first centrifugation step is transferred to a commercially available Mini-Spin-Column (e.g. QIAamp Mini-Spin-Column from QIAGEN company) and drawn through the membrane by centrifugation. The further washing steps are carried out as per example 4. Subsequently the nucleic acids are eluted with 140 μl RNase-free water by means of centrifugation (10000 $\times$ g, 1 minute) and analyzed in a non denaturizing agarose gel (fig.7). The method described here permits a separation of total-RNA and genomic DNA to a great extent.

Figure 7 shows an ethidium bromide stained gel of an electrophoretic separation of two different eluates.

Line 1: Isolation of total-RNA by means of hydrophobic nylon membrane;

Line 2: Isolation of genomic DNA from the penetrant by means of a QIAamp Mini-Spin-Column from QIAGEN company.

Example 14

Isolation of plasmid-DNA from aqueous solutions through binding to hydrophobic and hydrophilic membranes

According to example 1 plastic columns are assembled with various membranes.

100 μl of an aqueous solution (pCMV $\square$ from Clontech company) containing plasmid are mixed with 350 μl of a lyse buffer (4 M GITC, 0.1 M MgSO_4 , 25 mM sodium acetate, pH 4) containing guanidinium hydrochloride. Subsequently 250 μl iso-propanol are added and mixed by pipetting. The mixture is added to the column according to example 4 and transferred through the membrane, washed and dried. Finally the plasmid-DNA is eluted with RNase-free water as already described in example 1 and pipetted off the membrane.

The volume of isolated plasmid-DNA is then determined by photometric measurement of the light absorption at a wavelength of 260 nm.

The results of the isolations with the various membranes are listed in the following table. Three parallel tests are conducted per membrane and the average value is calculated for each.

Table 7: Yield of Plasmid-DNA out of an aqueous solution through binding on membranes

Manufacturer	Membrane	Material	Plasmid-DNA (μ g)
Pall	Hydrolon 1.2 μ m	Hydrophobic nylon	1.9
Pall	Fluoro Trans G	Hydrophobic polyvinylidene difluoride	2.2
Pall	I.C.E.-450	Hydrophilic polyether sulfone	0.8
Pall	I.C.E.-450sup	Hydrophilic polyether sulfone	1.5
Pall	Supor-450 PR	Hydrophobic polyether sulfone	4.7
Pall	Supor-200 PR	Hydrophobic polyether sulfone	4
Pall	Supor-800	Hydrophilic polyether sulfone	0.5
Pall	Supor-450	Hydrophilic polyether sulfone	0.9
Pall	Supor-100	Hydrophilic polyether sulfone	1
Pall	V-800 R	Hydrophobic acrylate-copolymer	1.5
Pall	Versapore – 1200R	Hydrophobic acrylate copolymer	0.2
Pall	Polypro – 450	Hydrophilic polypropylene	1.4
Gore-Tex	QH 9318	Hydrophilic polytetrafluor - ethylene	4.9
Gore-Tex	OH 9335	Hydrophobic polytetrafluor - ethylene	4.3
Millipore	Durapore, 0.65 μ m	Hydrophilized polyvinylidene difluoride	1.8
Millipore	Durapore, 0.65 μ m	Hydrophobic polyvinylidene difluoride	1.7
MSI	Magna-SH, 1.2 μ m	Hydrophobic nylon	1.1

Example 15

Immobilization of total-RNA from an aqueous solution with the use of different chaotropic agents

According to example 1 plastic columns are assembled with hydrophobic membranes.

Each 100 μ l of an aqueous solution containing total RNA are mixed with 350 μ l of different lysis buffers, which contain guanidinium iso thiocyanate (GITC) or guanidinium hydrochloride (GuHCl) in different concentrations. Subsequently 250 μ l ethanol are added and mixed by pipetting. The mixture is then transferred to the column and passed through the membrane by way of centrifugation (10000 x g; 1 minute). The membranes are subsequently washed twice with an alcohol-containing buffer, e.g. RPE from QIAGEN company. The buffer is passed through the membrane by way of centrifugation. The last washing step is performed at 20000 x g to dry the membranes. The elution is carried out as described in example 1. Twin tests are carried out and each average value is shown.

The results are listed in table 8.

Table 8: RNA yield out of an aqueous solution by way of chaotropic agents

Membrane	Chaotropic agents and concentration in Binding Preparation	Yield of Total RNA (μg)
Hydrolon 1.2 μm	GITC, 500mM	2.3
Hydrolon 1.2 μm	GITC, 1M	0.8
Hydrolon 1.2 μm	GITC, 3M	0.9
Fluoro Trans G	GITC, 500mM	0.4
Fluoro Trans G	GITC, 1M	1.25
Fluoro Trans G	GITC, 3M	0.6
Hydrolon 1.2 μm	GuHCl, 500mM	2.6
Hydrolon 1.2 μm	GuHCl, 1M	6.7
Hydrolon 1.2 μm	GuHCl, 3M	2.9
Fluoro Trans G	GuHCl, 500mM	0.4
Fluoro Trans G	GuHCl, 1M	1.25
Fluoro Trans G	GuHCl, 3M	0.6

Example 16

Immobilization of total RNA from an aqueous solution with the use of different alcohols

According to Example 1, plastic columns are assembled with hydrophobic membranes.

100 μl of an aqueous solution containing total RNA are mixed with 350 μl of a lysis buffer containing guanidinium isothiocyanate (concentration 4 M). Subsequently, different amounts of ethanol or isopropanol are added and loaded with RNase-free water up to 700 μl and mixed. This mixture is then transferred to the column and passed through the membrane and washed according to Example 4. The elution took place as in Example 1.

Twin tests are carried out and each average value is shown.

The results are listed in Table 9.

Table 9: RNA-yield from an aqueous solution with different alcohols in a binding preparation

Membrane	Alcohol and Concentration in Binding Preparation	Yield of Total RNA (μg)
Hydrolon, 1.2 μm	Ethanol, 5%	0.7
Hydrolon, 1.2 μm	Ethanol, 30%	2.85
Hydrolon, 1.2 μm	Ethanol, 50%	4.5
Durapore, 0.65 μm	Ethanol, 5%	0.4
Durapore, 0.65 μm	Ethanol, 30%	1.25
Durapore, 0.65 μm	Ethanol, 50%	0.6
Hydrolon, 1.2 μm	Isopropanol, 5%	0.35

Hydrolon, 1.2 μm	Isopropanol, 30%	4.35
Hydrolon, 1.2 μm	Isopropanol, 50%	3.2
Durapore, 0.65 μm	Isopropanol, 10%	1.35
Durapore, 0.65 μm	Isopropanol, 30%	4.1
Durapore, 0.65 μm	Isopropanol, 50%	3.5

Example 17

Immobilization of total RNA from an aqueous solution with various pH-values

According to Example 1, plastic columns are assembled with hydrophobic membranes.

100 μl of an aqueous solution containing total RNA are mixed with 350 μl of a lysis buffer containing guanidinium isothiocyanate (concentration 4 M). The buffer contains 25 mM of sodium citrate and is adjusted to different pH-values by way of HCl or NaOH. Subsequently 250 μl of ethanol are added and mixed. This mixture is then transferred to the column and passed through the membrane and washed according to Example 4. The elution also took place as in Example 1. Twin tests are carried out and each average value is shown.

The results are listed in Table 10.

Table 10: RNA-yield from an aqueous solution with various pH-values in a binding preparation

Membrane	pH-Value in Binding preparation	Yield of Total RNA (μg)
Hydrolon, 1.2 μm	pH 3	0.15
Hydrolon, 1.2 μm	pH 9	1.6
Hydrolon, 1.2 μm	pH 11	0.05
Fluoro Trans G	pH 1	0.45
Fluoro Trans G	pH 9	2.85
Fluoro Trans G	pH 11	0.25

Example 18

Immobilization of total RNA from an aqueous solution with various salts

According to Example 1, plastic columns are assembled with hydrophobic membranes.

100 μl of a total RNA containing aqueous solution are mixed with 350 μl of a lysis buffer containing salts (NaCl, KCL, MgSO_4). Subsequently 250 μl of H_2O or ethanol are added and mixed. This mixture is then transferred to the column and passed through the membrane, washed and eluted according to Example 4. Twin tests are carried out and each average value is shown.

The results are listed in Table 11.

Table 11: RNA-yield from an aqueous solution with various salts in a binding preparation

Membrane	Salt Concentration in Binding Preparation	Yield of Total RNA (µg)
Hydrolon, 1.2 µm	NaCl, 100 mM; without ethanol	0.1
Hydrolon, 1.2 µm	NaCl, 1 M; without ethanol	0.15
Hydrolon, 1.2 µm	NaCl, 5 M; without ethanol	0.3
Hydrolon, 1.2 µm	KCl, 10 mM; without ethanol	0.2
Hydrolon, 1.2 µm	KCl, 1 M; without ethanol	0.1
Hydrolon, 1.2 µm	KCl, 3 M; without ethanol	0.25
Hydrolon, 1.2 µm	MgSO ₄ , 100 mM; without ethanol	0.05
Hydrolon, 1.2 µm	MgSO ₄ , 750 mM; without ethanol	0.15
Hydrolon, 1.2 µm	MgSO ₄ , 2 M; without ethanol	0.48
Hydrolon, 1.2 µm	NaCl, 500 mM; with ethanol	2.1
Hydrolon, 1.2 µm	NaCl, 1 M; with ethanol	1.55
Hydrolon, 1.2 µm	NaCl, 2.5 M; with ethanol	1.35
Hydrolon, 1.2 µm	KCl, 500 mM; with ethanol	1.6
Hydrolon, 1.2 µm	KCl, 1 M; with ethanol	2.1
Hydrolon, 1.2 µm	KCl, 1.5 M; with ethanol	3.5
Hydrolon, 1.2 µm	MgSO ₄ , 10 mM; with ethanol	1.9
Hydrolon, 1.2 µm	MgSO ₄ , 100 mM; with ethanol	4.6
Hydrolon, 1.2 µm	MgSO ₄ , 500 M; with ethanol (sic!)	2

Translated as per German original

Example 19

Immobilization of total RNA from an aqueous solution by way of various buffer conditions

According to Example 1, plastic columns are assembled with hydrophobic membranes.

100 µl of an aqueous solution containing total RNA are mixed with 350 µl of a lysis buffer containing guanidinium isothiocyanate (concentration 2.5 M). The lysis buffer is mixed with various concentrations of sodium citrate, pH 7, or sodium oxalate, pH 7.2. Subsequently 250 µl of ethanol are added and mixed. This mixture is then transferred to the column and, according to Example 4, passed through the membrane, washed and eluted.

The results are listed in Table 12. Twin tests are carried out and each average value is shown.

Table 12: RNA-yield from an aqueous solution with various buffer concentrations in a binding preparation

Membrane	Na-Citrate/Na-Oxalate in the Lysis Buffer	Yield of Total RNA (µg)
Hydrolon, 1.2 µm	Na-Citrate, 10 mM	2.2
Hydrolon, 1.2 µm	Na-Citrate, 100 mM	2.4
Hydrolon, 1.2 µm	Na-Citrate, 500 mM	3.55
Supor-450 PR	Na-Citrate, 10 mM	1.1
Supor-450 PR	Na-Citrate, 100 mM	1.15
Supor-450 PR	Na-Citrate, 500 mM	0.2
Hydrolon, 1.2 µm	Na-Oxalate, 1 mM	1.5
Hydrolon, 1.2 µm	Na-Oxalate, 25 mM	1.05
Hydrolon, 1.2 µm	Na-Oxalate, 50 mM	0.9
Supor-450 PR	Na-Oxalate, 1 mM	1.9
Supor-450 PR	Na-Oxalate, 25 mM	1.3
Supor-450 PR	Na-Oxalate, 50 mM	1.7

Example 20

Immobilization of total DNA from an aqueous solution by way of various buffer substances

According to Example 1, plastic columns are assembled with hydrophobic membranes (e.g. Hydrolon 1.2 µm from Pall company).

100 µl of an aqueous solution containing total-DNA are mixed with 350 µl of a lysis buffer containing guanidinium isothiocyanate (4 M GITC, 0.1 M MgSO₄). The lysis buffer is mixed with various buffer substances (concentration 25 mM) and standardized to various pH values. Subsequently 250 µl of ethanol are added and mixed. This mixture is then transferred to the column and, according to Example 4, passed through the membrane, washed and eluted.

The results are listed in Table 13. Triple tests are carried out and the average value determined each time.

Table 13: DNA-yield from an aqueous solution with various buffer concentrations in a binding preparation

Buffer Substance	pH in Lysis Buffer	Yield of DNA (µg)
Sodium -citrate	pH 4	1.3
Sodium -citrate	pH5	0.6
Sodium -citrate	pH6	1.4

Sodium –citrate	pH7	0.5
Sodium –acetate	pH4	0.9
Sodium –acetate	pH5	1
Sodium –acetate	pH6	0.6
Sodium –acetate	pH 7	0.5
Potassium - acetate	pH 4	0.6
Potassium – acetate	pH5	0.9
Potassium – acetate	pH6	1.2
Potassium - acetate	pH7	1.4
Ammonia - acetate	pH4	0.7
Ammonia – acetate	pH5	0.3
Ammonia – acetate	pH6	5.7
Ammonia - acetate	pH7	1.5
Glycine	pH4	0.5
Glycine	pH5	1.1
Glycine	pH6	1.6
Glycine	pH7	1.1
Malonate	pH4	1.5
Malonate	pH5	0.3
Malonate	pH6	3.1
Malonate	pH7	1.6
Succinate	pH4	2.8
Succinate	pH5	2.3
Succinate	pH6	2.5
Succinate	pH7	4.7

Example 21

Immobilization of total RNA from an aqueous solution by means of phenol

As in Example 1, plastic columns with hydrophobic membranes (e.g., Hydrolon, 1.2 μm from the company Pall) are constructed.

An aqueous RNA solution is mixed with 700 μl of phenol and distributed across the membranes by means of centrifugation. As in example 4, the membranes are washed and the RNA eluted. Twin measurements were carried out, and in each case the average value is indicated.

The volume of isolated DNA (sic!) is then determined by photometric measurement of the light absorption at a wavelength of 260 nm and amounts to approximately 10.95 μg . The ratio of the absorption at 260nm to that at 280 nm amounts to 0.975.

Example 22

Washing of immobilized total-RNA under different salt concentrations

According to Example 1, plastic columns are assembled with hydrophobic membranes.

100 µl of an aqueous solution containing total RNA are mixed with 350 µl of a lysis buffer containing guanidinium isothiocyanate (concentration 4 M). Subsequently, 250 µl of ethanol are added and mixed. This mixture is then transferred to the column and passed through the membrane as well as washed according to Example 4. The membranes are then washed twice with a buffer which contains various concentrations of NaCl and 80% ethanol. The buffer is passed through the membrane each time by way of centrifugation. The last washing step is carried out at 20000 x g in order to dry the membranes. The elution also takes place according to Example 1. Twin tests are carried out and in each case the average value is shown.

The results are listed in Table 14.

Table 14: RNA-yield from an aqueous solution with NaCl in the washing buffer

Membrane	NaCl in the Washing Buffer	Yield of Total RNA (µg)
Hydrolon, 1.2 µm	NaCl, 10 mM	1.4
Hydrolon, 1.2 µm	NaCl, 50 mM	3.15
Hydrolon, 1.2 µm	NaCl, 100 mM	3
Durapore, 0.65 µm	NaCl, 10 mM	2.7
Durapore, 0.65 µm	NaCl, 50 mM	2.85
Durapore, 0.65 µm	NaCl, 100 mM	2.7

Example 23

Elution of immobilized total RNA under different salt and buffer conditions

According to Example 1, plastic columns are assembled with hydrophobic membranes.

100 µl of an aqueous solution containing total RNA are mixed with 350 µl of a lysis buffer containing guanidinium isothiocyanate (concentration 4 M). Subsequently 250 µl of ethanol are added and mixed. This mixture is then transferred to the column and passed through the membrane and washed according to Example 4.

For the elution, 70 µl of a solution containing NaCl, of a Tris/HCl-buffer (pH 7) or of a sodium oxalate solution (pH 7.2) are pipetted onto the membrane, in order to elute the purified RNA from the membrane. After 1 to 2 minutes of incubation, at a temperature between 10 – 30°C, the eluate is pipetted from the top off the membrane. The elution step is repeated once in order to achieve complete elution. Twin tests are carried out and in each case the average value is shown.

The results are summarized in Table 12.

Table 12: RNA-yield from an aqueous solution with NaCl, Tris/HCl or Na-oxalate in the elution buffer

Membrane	NaCl, Tris or Na-oxalate in the Elution Buffer	Yield of Total RNA (µg)
Hydrolon, 1.2 µm	NaCl, 1 mM	1.35
Hydrolon, 1.2 µm	NaCl, 50 mM	1.2
Hydrolon, 1.2 µm	NaCl, 250 mM	0.45
Durapore, 0.65 µm	NaCl, 1 mM	0.9
Durapore, 0.65 µm	NaCl, 50 mM	0.35
Durapore, 0.65 µm	NaCl, 500 mM	0.15
Hydrolon, 1.2 µm	Tris 1 mM	0.35
Hydrolon, 1.2 µm	Tris 10 mM	0.75
Durapore, 0.65 µm	Tris 1 mM	1.5
Durapore, 0.65 µm	Tris 50 mM	1
Durapore, 0.65 µm	Tris 250 mM	0.1
Hydrolon, 1.2 µm	Na-Oxalate, 1 mM	0.45
Hydrolon, 1.2 µm	Na-Oxalate, 10 mM	0.65
Hydrolon, 1.2 µm	Na-Oxalate, 50 mM	0.3
Durapore, 0.65 µm	Na-Oxalate, 1 mM	2
Durapore, 0.65 µm	Na-Oxalate, 10 mM	1.55
Durapore, 0.65 µm	Na-Oxalate, 50 mM	0.15

Example 24

Elution of immobilized RNA at various temperatures

According to Example 1, plastic columns are assembled with a hydrophobic membrane (e.g. Hydrolon, 3 µm from Pall company).

For the isolation 5×10^5 HeLa cells are used. The following isolation steps, as described in example 3, are carried out.

For the elution 70 µl of RNase-free water of various temperatures is pipetted onto the membrane to elute the RNA from the membrane. After an incubation of 1-2 minutes at the appropriate elution temperature, the eluate is pipetted off the membrane from above. The elution step is repeated once to achieve a complete elution.

Triple tests are carried out and in each case the average value is shown.

The results are summarized in table 16.

Table 16: Yield of RNA at various elution temperatures

Membrane	Elution Temperature	Yield of total RNA (µg)
Hydrolon, 3 µm	Ice cold	2.2
Hydrolon, 3 µm	40°C	3.2
Hydrolon, 3 µm	50°C	3.9
Hydrolon, 3 µm	60°C	3.7
Hydrolon, 3 µm	70°C	3.7
Hydrolon, 3 µm	80°C	2.9

Example 25

Elution of immobilized RNA by way of centrifugation

According to Example 1, plastic columns are assembled with a hydrophobic membrane (e.g. Hydrolon 1.2 µm from Pall company).

100 µl of an aqueous solution containing total-DNA are mixed with 350 µl of a lysis buffer containing guanidinium isothiocyanate – e.g. RLT buffer from QIAGEN. Subsequently 250 µl ethanol are added and mixed by pipetting. This mixture is then transferred to the column and, through centrifugation (10000 x g; 1 minute), passed through the membrane. Subsequently the membranes are washed twice with a buffer – e.g. RPE from QIAGEN company. The buffer is transferred through the membrane by centrifugation in each case. The last washing step is carried out at 20000 x g to dry the membrane.

For the elution 70 µl of RNase-free water is pipetted onto the membrane to release the RNA from the membrane. After an incubation period of 1 minute at a temperature within a range of 10 – 30°C the eluate is transferred through the membrane by way of centrifugation (10000 x g, 1 minute). The elution step is repeated again to assure a complete elution and the eluates are combined. 5 parallel tests are carried out and in each case the average value is shown.

The volume of isolated total RNA is then determined by photometric measurement of the light absorption at a wavelength of 260 nm and amounts, on average, to 6.4 µg. The ratio of the absorption at 260 nm to that at 280 nm amounts to 1.94.

Example 26

Use of total RNA in a “Real Time” quantitative RT-PCR with the use of 5'-nuclease PCR-assays for the amplification and detection of β-actin mRNA.

According to Example 3, plastic columns are assembled with a commercially available membrane (Pall, Hydrolon with a pore size of 3 µm).

To isolate RNA, 1 x 10⁵ HeLa-cells are used and the purification of total RNA is carried out as described in Example 1. The elution takes place with 2 x 70 µl of H₂O as described in example 1.

For the complete removal of remaining slight amounts of DNA, the sample is treated with a DNase prior to analysis.

A "single-container 'Real Time' quantitative RT-PCR" is carried out with the use of the commercially available reaction system of Perkin-Elmer (TaqMan™ PCR Reagent Kit) by using an M-MLV reverse transcriptase. By using a specific primer and a specific TaqMan-probe for β -actin (TaqMan™ β -Actin Detection Kits made by Perkin Elmer) the β -actin mRNA-molecules in the total RNA-sample, are first transcribed to β -actin cDNA and subsequently the total reaction is amplified and detected immediately, without interruption, in the same reaction container. The reaction preparations are produced according to the manufacturer's instructions. Three different volumes of isolated total RNA are used (1, 2, 4 μ l of eluate) and triple tests are carried out. As a control, three preparations without RNA are also tested.

The cDNA synthesis takes place at 37°C for one hour, immediately followed by a PCR which comprises 40 cycles. The reactions and the analyses are carried out on an ABI PRISM™ 7700 Sequence Detector supplied by Perkin Elmer Applied Biosystems. Every amplicon generated during a PCR-cycle produces a light-emitting molecule, which is generated by splitting from the TaqMan-probe. The total light signal that is generated is directly proportional to the amplicon volume that is being generated and hence to the original amount of transcript available in the total RNA sample. The emitted light is measured by the apparatus and evaluated by a computer program. The PCR-cycle, during which the light signal must first be detected over the background noise, will be designated as the „Threshold Cycle“ (ct). This value is a measure for the amount of specifically amplified RNA available in the sample.

For the employed volume 1 μ l of total RNA, isolated with the process described here, an average ct-value of 17.1 is obtained; for 2 μ l of total RNA the ct-value is 16.4 and for 4 μ l of total RNA the ct-value is 15.3. This results in a linear correlation between the total RNA employed and the ct-value. This indicates that the total RNA is free of substances that might inhibit the amplification reaction. The control specimens containing no RNA do not produce any signals.

Example 27

Use of total RNA in an RT-PCR for amplification and detection of β -actin mRNA.

According to Example 1, plastic columns are assembled with commercially available membranes (Pall company, Hydrolon with a pore size of 1.2 or 3 μ m; Sartorius company, Sartolon with a pore size of 0.45 μ m).

To isolate RNA, two different starting materials are used:

- 1) total RNA from liver (mouse) in an aqueous solution; purification and elution are carried out as described in example 4 and
- 2) 5×10^5 HeLa-cells, the purification of total RNA and the elution are carried out as described in example 3.

For each test 20 ng of isolated total RNA are used. As a control, RNA which was purified by way of RNeasy-Kits (QIAGEN) and a preparation without RNA are used.

A RT-PCR is performed with these samples under standard conditions. For the amplification two different primer pairs are used for the β -Actin-mRNA. A 150 Bp-sized fragment serves to verify the sensitivity, a 1.7 kBp-sized fragment assesses the integrity of the RNA. From the RT-reaction, 1 μ l is removed and introduced to the subsequent PCR. 25 cycles are performed for the small fragment and 27 cycles for the large fragment. The temperature for the addition reaction is 55°C. The amplified preparations are subsequently placed on a non-denaturizing gel and analyzed.

For the employed 20 ng volume of total RNA isolated in the process described above, the corresponding DNA-fragments can be verified in the RT-PCR. When using total RNA from mouse liver, no transcript can be verified, as the conditions used here are adjusted to human β -actin-mRNA. The control specimens which contain no RNA do not produce any signals.

Fig. 8 shows ethidium bromide stained agarose gels of an electrophoretic separation of RT-PCR reaction products.

A: Line 1 to 8: RT-PCR of a 150 Bp-fragment;

Line 1, 2: RNA from mouse liver out of an aqueous solution purified with the Hydrolon 1.2 μ m membrane;

Line 3, 4: RNA from HeLa-cells purified with the Sartolon membrane;

Line 5, 6: RNA from HeLa-cells purified with the Hydrolon 3 μ m membrane;

Line 7: RNA purified by way of RNeasy-Mini-Kit;

Line 8: Control without RNA.

B: Line 1 to 8: RT-PCR of a 1.7 kBp-fragment;

Line 1, 2: RNA from mouse liver out of an aqueous solution purified with the Hydrolon 1.2 μ m membrane;

Line 3, 4: RNA from HeLa-cells purified with the Sartolon membrane;

Line 5, 6: RNA from HeLa-cells purified with the Hydrolon 3 μ m membrane;

Line 7: RNA purified by way of RNeasy-Mini-Kit;

Line 8: Control without RNA.

Example 28

Use of total RNA in a NASBA-reaction (nucleic acid sequence based amplification) for amplification and detection of β -actin mRNA.

According to Example 1, plastic columns are assembled with commercially available membranes (Pall company, Hydrolon with a pore size of 1.2 or 3 μ m; Sartorius company, Sartolon with a pore size of 0.45 μ m).

To isolate RNA, two different starting materials are used:

- 1) total RNA from liver (mouse) in an aqueous solution; purification and elution are carried out as described in example 4 and
- 2) 5×10^5 HeLa-cells, the purification of total RNA and the elution are carried out as described in example 3.

A NASBA-reaction is performed under standard conditions (Fahy, E. et al., 1991. PCR Methods Amplic. 1, 25 – 33). For the amplification β -actin-specific primers are used.

20 ng each of the isolated total RNA are employed. As a control, RNA purified by way of the RNeasy-kit (QIAGEN company) and a preparation without RNA are carried out. Firstly the incubation takes place for 5 minutes at 65°C and for 5 minutes at 41°C. Subsequent to this step an enzyme mixture consisting of RNaseH, T7-polymerase and AMVV-RT are added and incubated for 90 minutes at 41°C. The amplified preparations are subsequently placed on a non-denaturizing gel and analyzed.

For the employed 20 ng volume of total RNA isolated in the process described above, a specific transcript can be verified (Fig. 9).

Fig. 9 shows an ethidium bromide stained agarose gel of an electrophoretic separation of NASBA-reactions.

Line 1 to 8: NASBA-reactions;

Line 1, 2: RNA from mouse liver out of an aqueous solution purified with the Hydrolon 1.2 μ m membrane;

Line 3, 4: RNA from HeLa-cells purified with the Sartolon membrane;

Line 5, 6: RNA from HeLa-cells purified with the Hydrolon 3 μ m membrane;

Line 7: RNA purified by way of RNeasy-Mini-Kit;

Line 8: Control without RNA.

Example 29

NASBA-reaction for the amplification and detection of β -actin mRNA on hydrophobic membranes.

According to Example 1, plastic columns are assembled with commercially available membranes (Pall company, Hydrolon with a pore size of 3 μ m; Supor-450 PR with a pore size of 0.45 μ m; Millipore company, Fluoropore with a pore size 3 μ m).

To isolate RNA, various amounts of HeLa-cells are used, the purification of the total RNA is carried out as described in example 3. The elution is carried out through the addition of 20 μ l NASBA reaction buffer. The NASBA reaction is subsequently carried out at the membrane. The reaction takes place at standard conditions (Fahy, E. et al., 1991 PCR Methods Amplic. 1, 25 – 33). For the amplification β -actin specific primers are employed.

The reaction container is firstly incubated for 5 minutes at 41°C in a waterbath. Subsequent to

this step an enzyme mixture consisting of RNaseH, T7-polymerase and AMVV-RT is added and incubated for 90 minutes at 41°C. The amplified preparations are subsequently placed on a non-denaturizing gel and analyzed.

For the employed amount of total RNA, isolated from 5×10^5 to 3×10^4 HeLa-cells, at the total RNA isolated according to the process described here, a specific transcript can be verified (Fig. 10).

Fig. 10 shows an ethidium bromide stained agarose gel of an electrophoretic separation of NASBA-reactions.

A: Line 1 to 4: RNA purified with the membrane Hydrolon 3 μ m out of HeLa-cells;

Line 1: 2.5×10^5 cells;

Line 2: 1.25×10^5 cells;

Line 3: 6×10^4 cells;

Line 4: 3×10^4 cells;

B: Line 1 to 3: RNA purified out of HeLa-cells;

Line 1: RNA purified with the Hydrolon 3 μ m membrane out of 2.5×10^5 HeLa cells;

Line 2: RNA purified with the Supor-450 PR membrane out of 5×10^5 HeLa cells;

Line 3: RNA purified with the Fluoropore 3 μ m membrane out of 5×10^5 HeLa cells.

Example 30

Isolation of plasmid-DNA on a hydrophobic membrane with the enzyme *AvaI*

According to example 1 plastic columns are assembled with hydrophobic membranes (e.g. Supor-200 PR from Pall company).

100 μ l of an aqueous solution (pCMV β from Clontech company) containing plasmid are mixed with 350 μ l of a lyse buffer (4 M GITC, 0.1 M MgSO₄, 25 mM sodium acetate, pH 4) containing guanidinium isothiocyanate. Subsequently 250 μ l iso-propanol are added and mixed by pipetting. The mixture is added to the column according to example 4 and transferred through the membrane, washed and dried.

100 μ l of a 1 x buffer for the restriction enzyme *AvaI* are applied to the membrane and

1) removed, transferred into a new reaction container and subsequently a restriction enzyme (e.g. *AvaI* from Promega company) is added;

2) a restriction enzyme (e.g. *AvaI* from Promega company) is added to the eluate in the column.

The reactions are incubated for one hour at 37°C and subsequently applied to a non-denaturizing gel and analyzed (Fig. 11).

Fig. 11 shows an ethidium bromide stained agarose gel of an electrophoretic separation of the plasmid pCMV β after restriction with *AvaI*.

Line 1: uncut plasmid;

Line 2, 3: elution with the reaction buffer for *AvaI*, restriction in new container;

Line 4, 5: Restriction with *AvaI* on the membrane.

Example 31

Pressurized filtration for the precipitation of DNA with isopropanol

The isolation of plasmid DNA including the elution step via anion exchange chromatography, is carried out according to standard protocols. The DNA is eluted from the column in a high salt concentration buffer. Subsequently 0.7 volume isopropanol is added to this DNA solution, the preparation is mixed and incubated for 1-5 minutes at room temperature. The filtration equipment used is a 0.45 μm cellulose acetate filter with a surface of 5 cm^2 inside a filtration cartridge (standard equipment for sterile filtration, e.g. Minisart from Sartorius company). This filter is plugged onto a syringe from which the plunger has been removed.

The syringe is now filled with the DNA-isopropanol mixture and the mixture pressed through the filter with the plunger. In this form of precipitate a high percentage of the DNA remains on the filter (can not penetrate the pores).

Now the plunger is removed from the syringe again, replaced and air is pressed through the filter. This step is repeated 1-2 times and serves the drying of the membrane.

Subsequently a low salt buffer is used to elute such that the buffer is filled into the body of the syringe and is pressed through the filter with the aid of the plunger. To increase the yield, this first eluate is filled into the syringe body once more and pressed through the filter with the aid of the plunger. The yields obtained typically range from 80% to 90% with this test arrangement (refer example 34).

Example 32

Vacuum filtration for the precipitation of DNA with isopropanol

As with the pressure filtration the plasmid-DNA is firstly isolated and 0.7 vol isopropanol is added. The filtration set-up is an apparatus constructed for vacuum filtration into which a 0.45 μm cellulose acetate filter with a surface of 5 cm^2 was fitted. 0.45 μm cellulose nitrate filters or several layers of 0.65 μm cellulose acetate or cellulose nitrate filters can also be used.

The isopropanol-DNA mixture is incubated for 1-5 minutes and then transferred onto the filtration set-up. By connecting the vacuum the solution is drawn through the filter. An appropriate amount of 70% ethanol is given onto the filter with the DNA-precipitates and washed by connecting the vacuum again. The elution of the DNA from the filter is carried out by adding a low salt buffer, a short incubation and once more, connection of the vacuum. The yield can be obtained through either again eluting from the filter with a second volume of a low salt buffer or through a renewed elution with the eluate from the first elution step.

Here as well the yields obtained typically range from 80% to 90% of the originally employed DNA.

Example 33

As method the vacuum filtration indicated in example 32 is used. The vacuum filtration instrument Sartorius 16315 is used as the filtration container. pCMV β , which had been isolated from DH5 α , is used as plasmid DNA.

15 ml QF buffer (high salt concentration buffer) are added to 500 μ g of plasmid and mixed. 10.5 ml isopropanol are added and mixed again. This is incubated for 5 minutes. The plasmid-DNA thus precipitated is transferred onto the membrane fastened into the filtration apparatus. Now the vacuum is connected and filtration begins. The membranes are washed with 5 ml of 70% ethanol (renewed application of vacuum). Thereafter 1 ml TE-buffer is pipetted onto the membrane, incubated for 5 minutes and the DNA eluted by applying vacuum. Subsequently a post-elution is carried out with 1 ml of TE-buffer. The total amounts of DNA are subsequently measured each in the penetrant, in the washing fraction and in the combined eluate (OD260). The following results are achieved:

Membrane	Amount	Penetrant	Washing Fraction	Eluate	Speed
PVDF 0.2 μ m	1	0 μ g DNA	0 μ g DNA	131 μ g DNA	very slow
Cellulose-Acetate 0.65 μ m	3	0 μ g DNA	0 μ g DNA	469 μ g DNA	fast
Cellulose-Nitrate 0.65 μ m	2	0 μ g DNA	0 μ g DNA	418 μ g DNA	fast

Calculated for the 500 μ g original amount, the following yields result in this test:

PVDF 0.2 μ m	26%
Cellulose acetate 0.65 μ m	94%
Cellulose nitrate 0.65 μ m	84%

Example 34

The pressure filtration as mentioned in example 34 is used as the method. The filtration apparatus employed is a commercial 0.45 μ m cellulose acetate filter (Minisart, Sartorius). PCMV β , which had been isolated from DH5 α , is used as plasmid DNA.

15 ml QF buffer (high salt concentration buffer) are added to 100, 200, 300, etc up to 900 μ g of plasmid and mixed. 10.5 ml isopropanol are added and mixed again. This is subsequently

incubated for 5 minutes. The plasmid-DNA thus precipitated is transferred into a syringe from which the plunger has been removed. Now the pressure filtration occurs with the aid of the syringe. The filter is washed with 2 ml of 70% ethanol and dried twice as described. The elution is carried out with 2 ml TE-buffer. A post-elution is carried out with the eluate. The total amounts of DNA are subsequently measured each with combined eluate (OD260). The following results are achieved:

Employed DNA-amount	Eluted DNA-amount	% yield
100 µg	100 µg	100%
200 µg	176 µg	88%
300 µg	257 µg	86%
400 µg	361 µg	90%
500 µg	466 µg	93%
600 µg	579 µg	97%
700 µg	671 µg	96%
800 µg	705 µg	88%
900 µg	866 µg	96%

Example 35

The pressure filtration as mentioned in example 32 is used as the method. The filtration apparatus employed is a commercial 0.45 µm cellulose acetate filter (Minisart, Sartorius) which is plugged onto a filtration chamber (QIAvac). As a reservoir the body of a syringe is attached to the other end of the filter. PCMVβ, which had been isolated from DH5α, is used as plasmid DNA. 15 ml QF buffer (high salt concentration buffer) are added to 500 µg of plasmid and mixed. 10.5 ml isopropanol are added and mixed again. This is incubated for 5 minutes. The plasmid-DNA thus precipitated is transferred onto the membrane fastened into the filtration apparatus. Now the vacuum is connected and filtration begins. The filter is not washed with 5 ml of 70% ethanol. The immediate elution is rather carried out with 2 ml buffer EB (QIAGEN). Post-elution is carried out once with the eluate. The total amount of DNA is measured (OD260). The following result is obtained:

Test Number	Eluted DNA	% Yield
1	434 µg	87%
2	437 µg	87%

29620-1

CLAIMS:

1. A process for the precipitation of plasmid nucleic acid, comprising:

providing an isolation container comprising a
5 membrane located therein, said membrane comprising pores
having a pore size larger than or equal to 0.45 micrometers
and wherein said membrane comprises hydrophilized nylon,
polyethersulfone, polycarbonate, polyacrylate,
acrylatecopolymers, polyurethane, polyamide,
10 polyvinylchloride, polyfluorocarbonate,
polytetrafluoroethylene, polyvinylidendifluoride,
polyethylenetetrafluoroethylene-copolymerisate, one
polyethylenechlorotrifluoroethylene- copolymerisate,
cellulose acetate, cellulose nitrate or polyphenylene
15 sulfide;

charging said isolation container with a solution
containing plasmid nucleic acids; and

precipitating said plasmid nucleic acids contained
in said solution with iso-propanol, wherein the volume ratio
20 of the solution containing plasmid nucleic acids to
iso-propanol is 2:1 to 1:1, whereby said plasmid nucleic
acids bond with said membrane.

2. The process according to claim 1, wherein said
iso-propanol is added to said solution containing plasmid
25 nucleic acids prior to charging said isolation container
with said solution containing plasmid nucleic acids.

29620-1

3. The process according to claim 1, wherein said iso-propanol is added to said solution containing plasmid nucleic acids after charging said isolation container with said solution containing plasmid nucleic acids.

5 4. The process according to any one of claims 1 to 3, wherein said membrane comprises a surface to which all said plasmid nucleic acids contained in said solution can bond.

5. The process according to claim 1, wherein the volume ratio of the solution containing plasmid nucleic
10 acids to iso-propanol is 1.67:1 to 1:1.

6. The process according to claim 1, wherein the volume ratio of the solution containing plasmid nucleic acids to iso-propanol is 1.43:1 to 1:1.

7. The process according to claim 1, wherein said
15 membrane comprises cellulose acetate or cellulose nitrate.

8. The process according to any one of claims 1 to 7, wherein said membrane comprises pores having a pore size of larger than 0.45 μm .

9. The process according to any one of claims 1 to 7,
20 wherein said membrane comprises pores having a pore size larger than 0.6 μm .

10. The process according to any one of claims 1 to 9, wherein said isolation container comprises a plurality of said membranes.

29620-1

11. Use of a membrane comprising pores having a pore size larger or equal to 0.45 micrometers and wherein said membrane comprises hydrophilized nylon, polyethersulfone, polycarbonate, polyacrylate, acrylatecopolymers, polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate, polytetrafluoroethylene, polyvinylidendifluoride, polyethylenetetrafluoroethylene-copolymerisate, one polyethylenechlorotrifluoroethylene-copolymerisate, cellulose acetate, cellulose nitrate or polyphenylene sulphide to bind plasmid nucleic acids precipitated with iso-propanol, wherein the volume ratio of the solution containing nucleic acids to iso-propanol is 2:1 to 1:1.
12. The use according to claim 11, wherein said plasmid nucleic acids precipitated with iso-propanol are DNA.
13. The use according to claim 11, wherein said membrane comprises cellulose acetate or cellulose nitrate.
14. The use according to any one of claims 11 to 13, wherein said membrane comprises pores having a pore size of larger than 0.45 μm .
15. The use according to any one of claims 11 to 13, wherein said membrane comprises pores having a pore size larger than 0.6 μm .
16. The use according to any one of claims 11 to 15, wherein a plurality of said membranes are used.

1/12

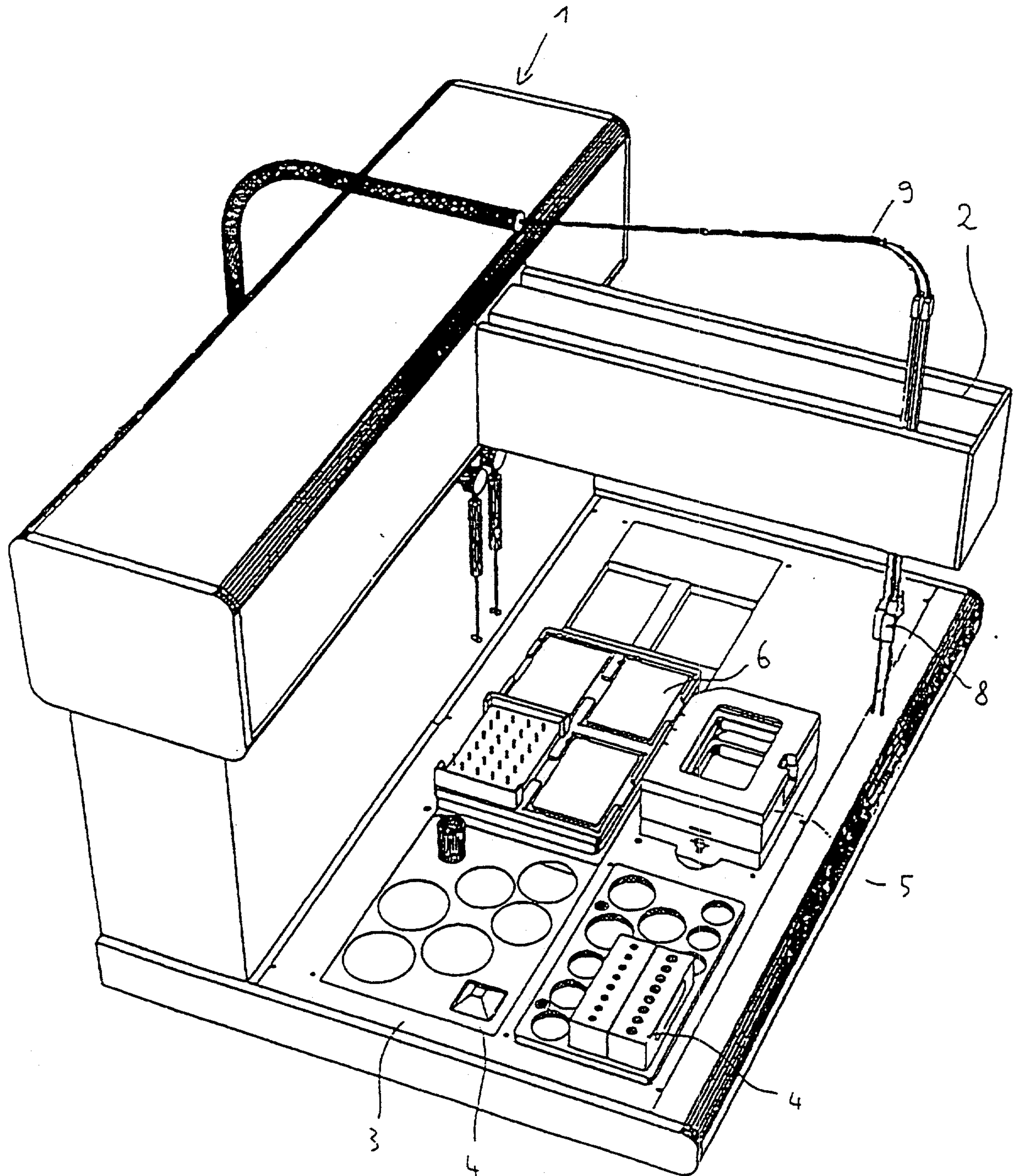


Fig. 1

2/12

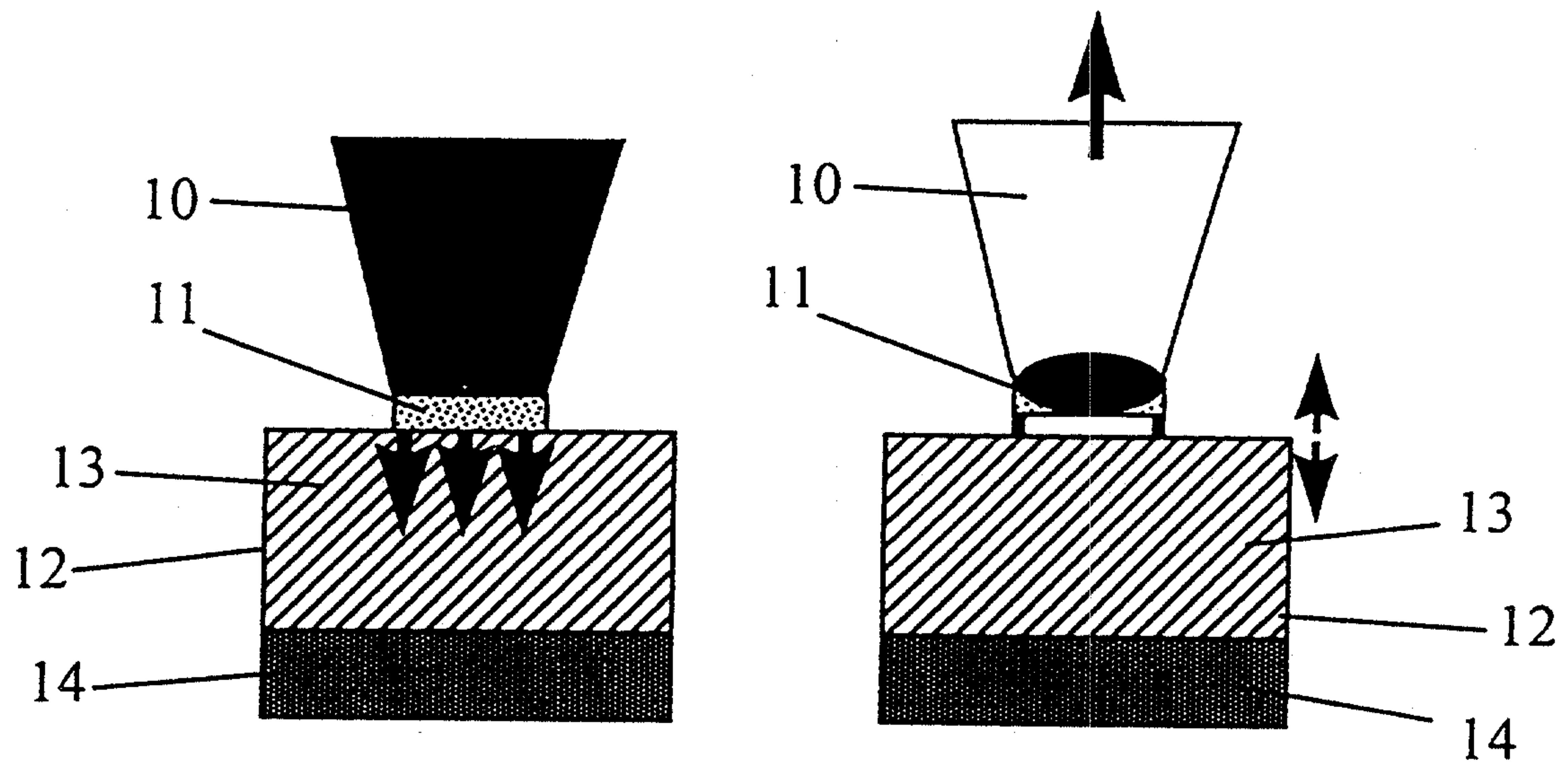


Fig. 2

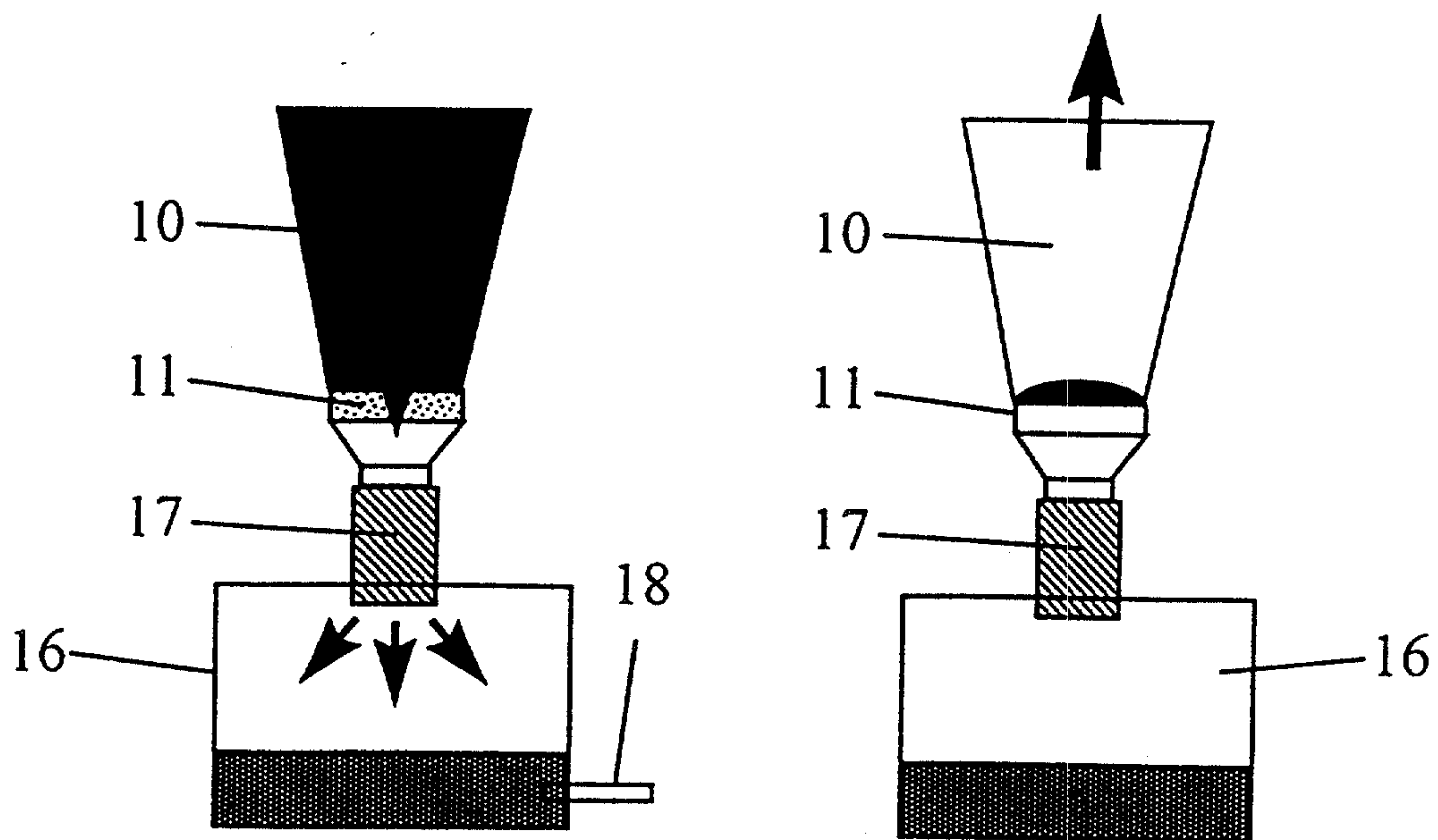


Fig. 3

3/12

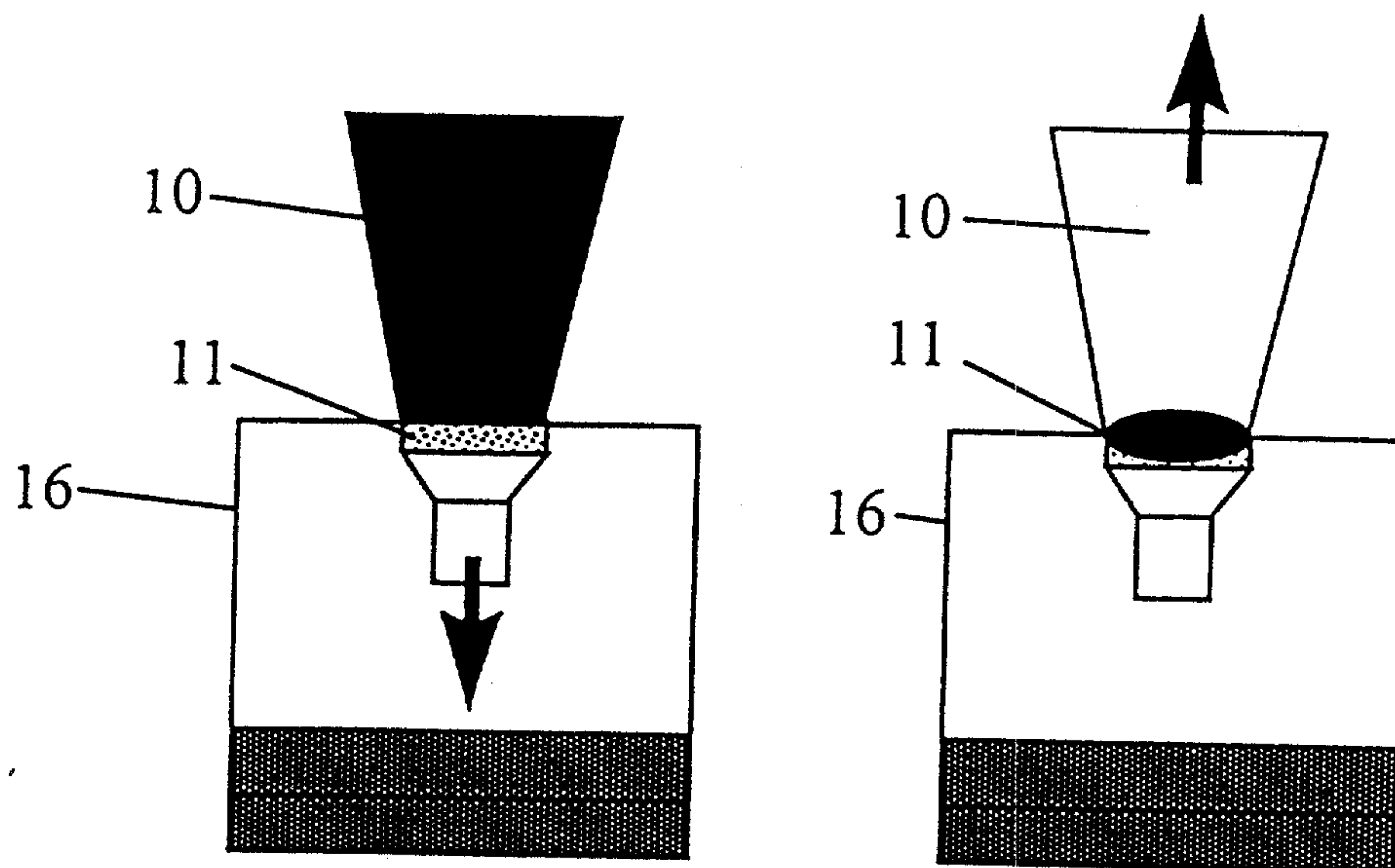


Fig. 4

4/12

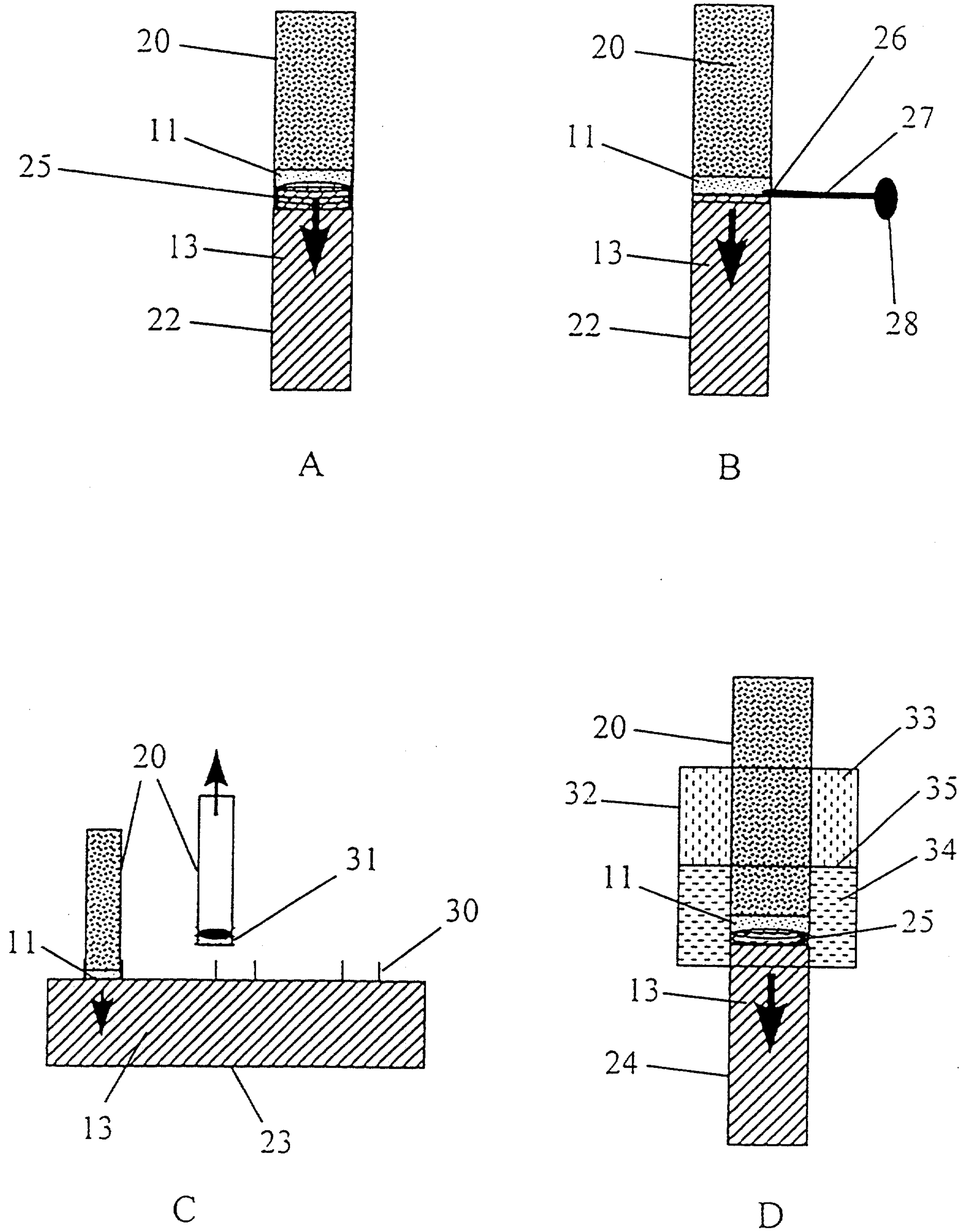


Fig. 5

5/12

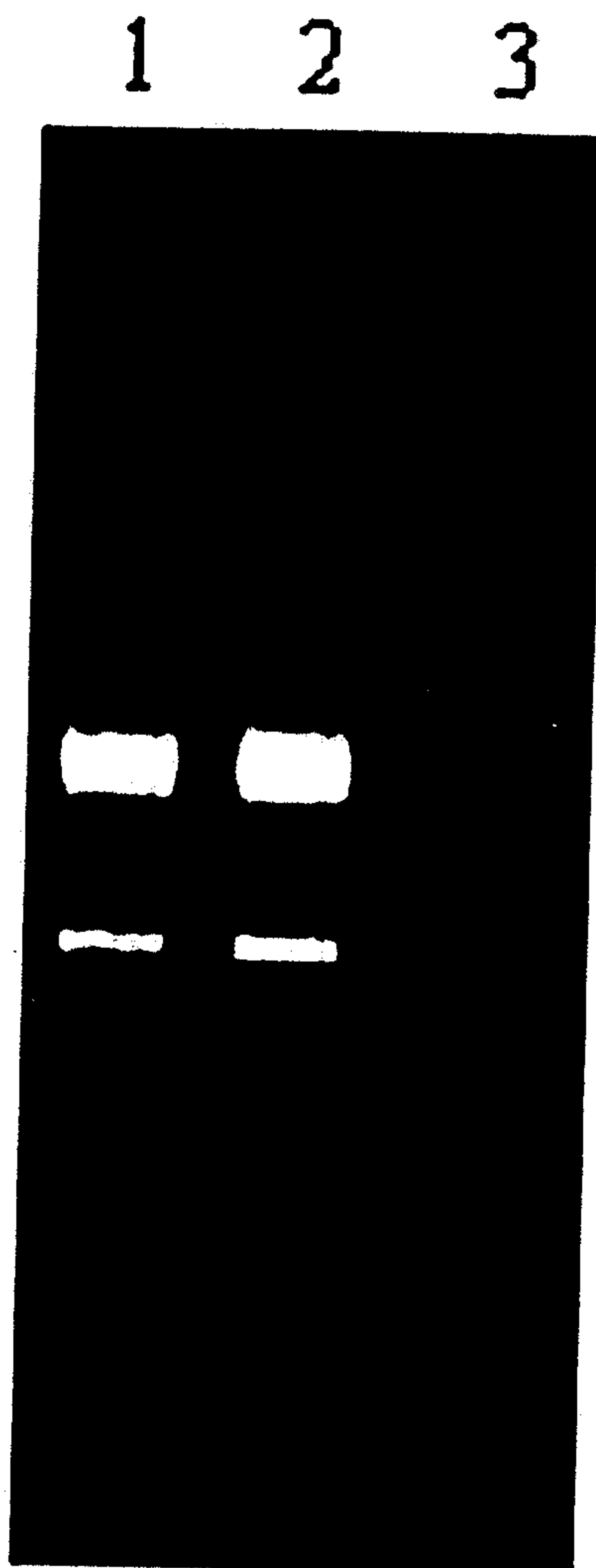


Fig. 6

6/12

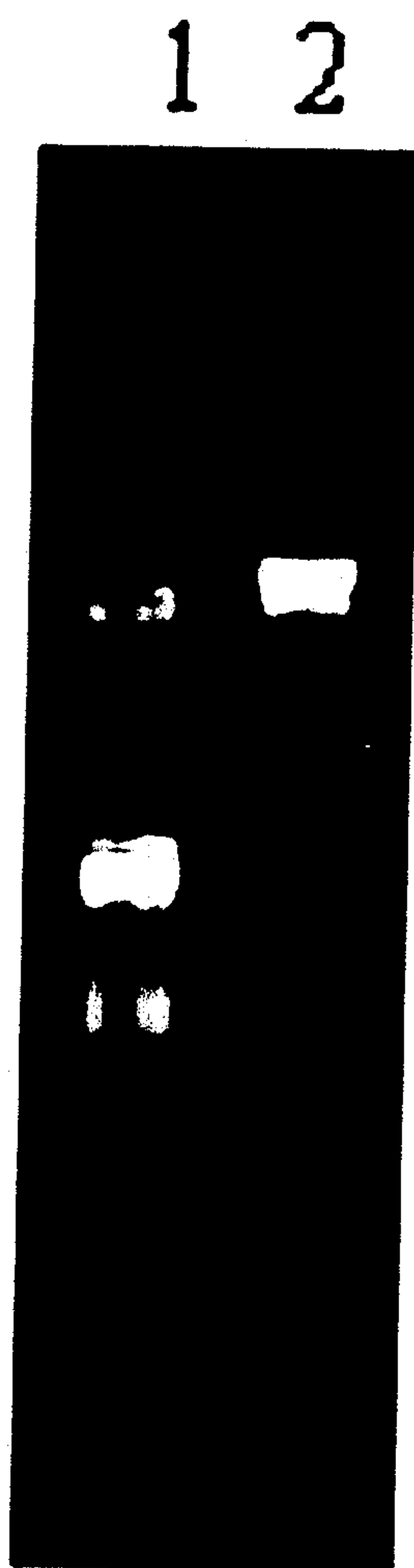


Fig. 7

7/12

M 1 2 3 4 5 6 7 8

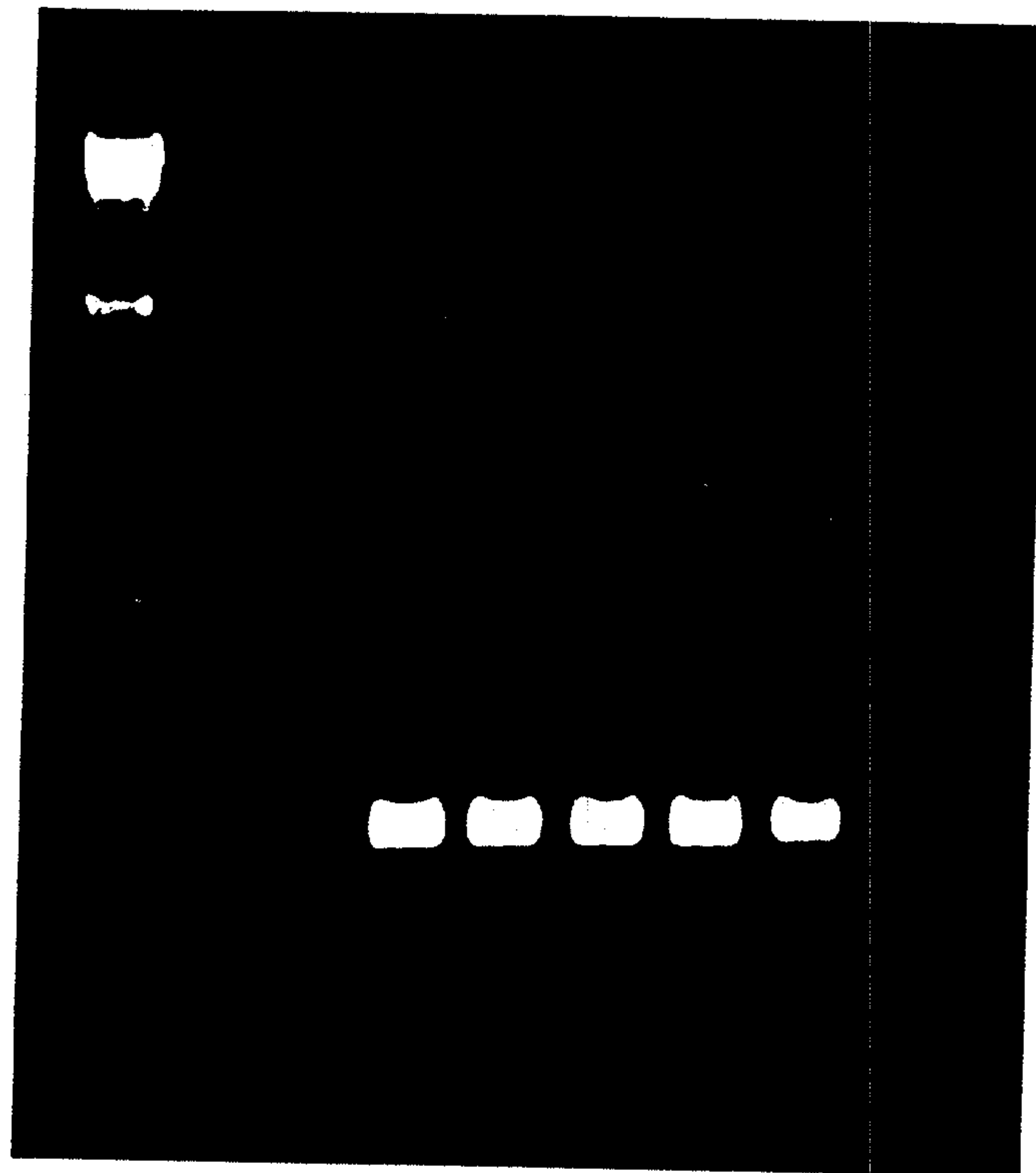


Fig. 8 A

8/12

M 1 2 3 4 5 6 7 8

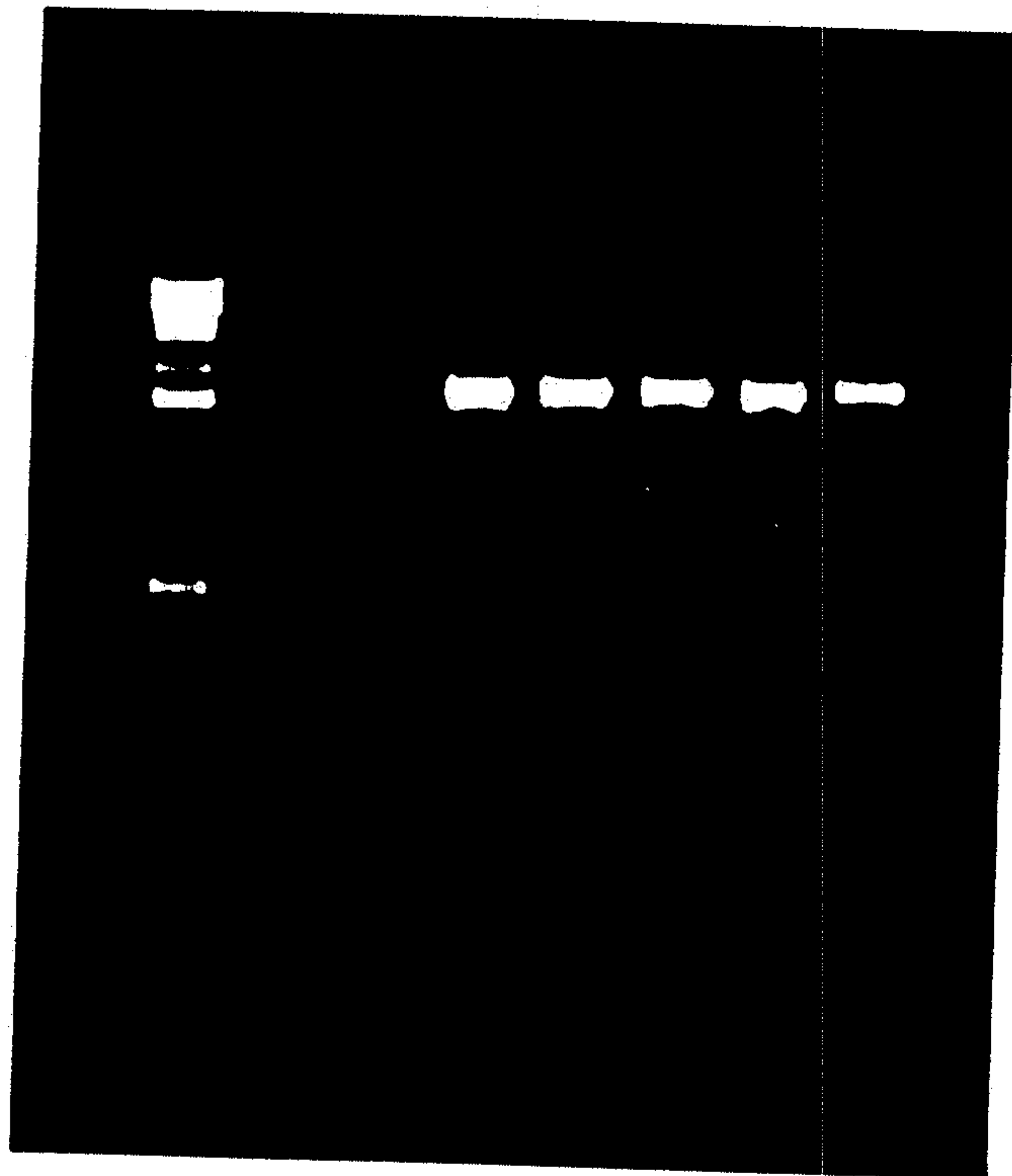


Fig. 8 B

9/12

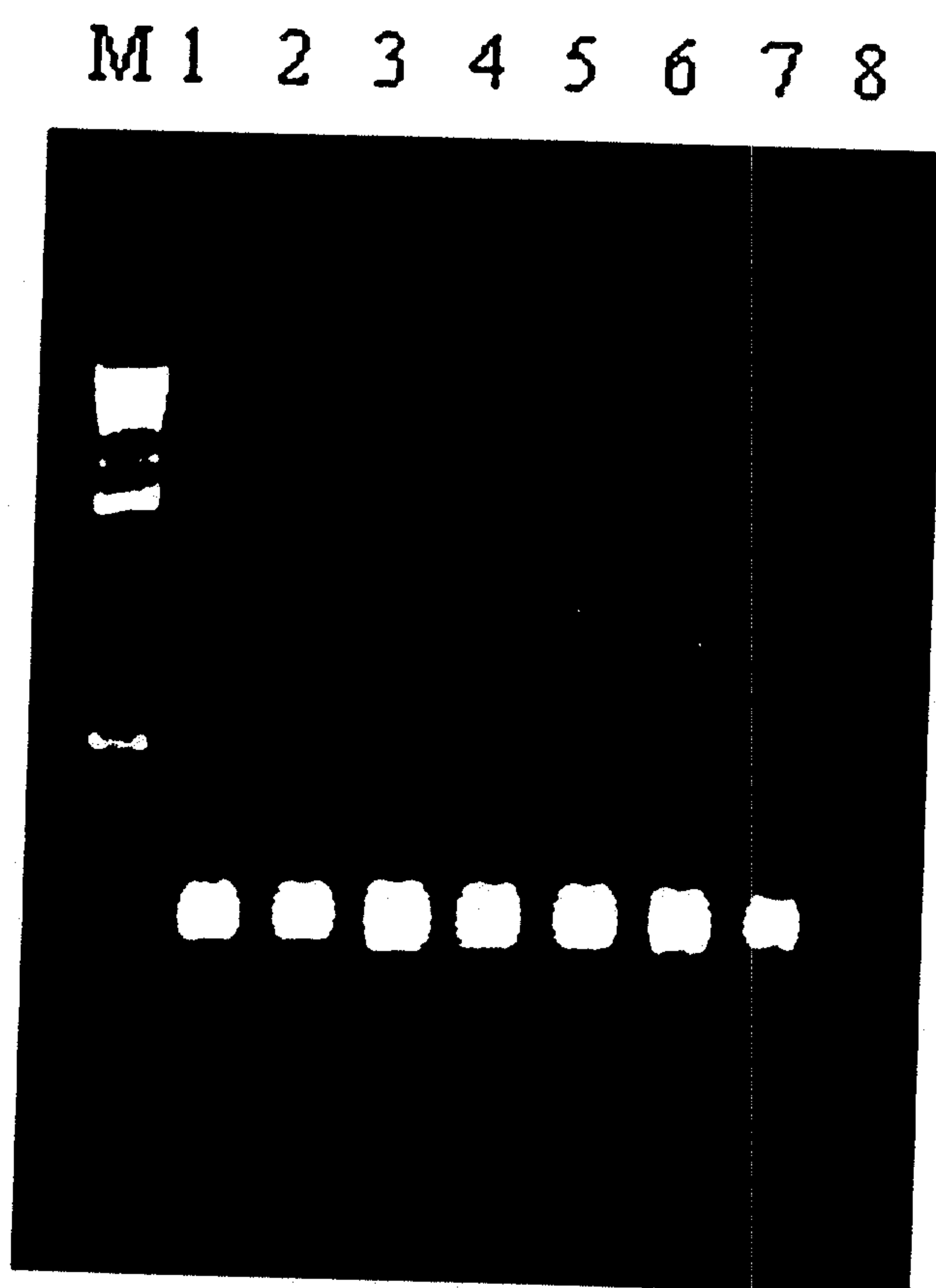


Fig. 9

10/12

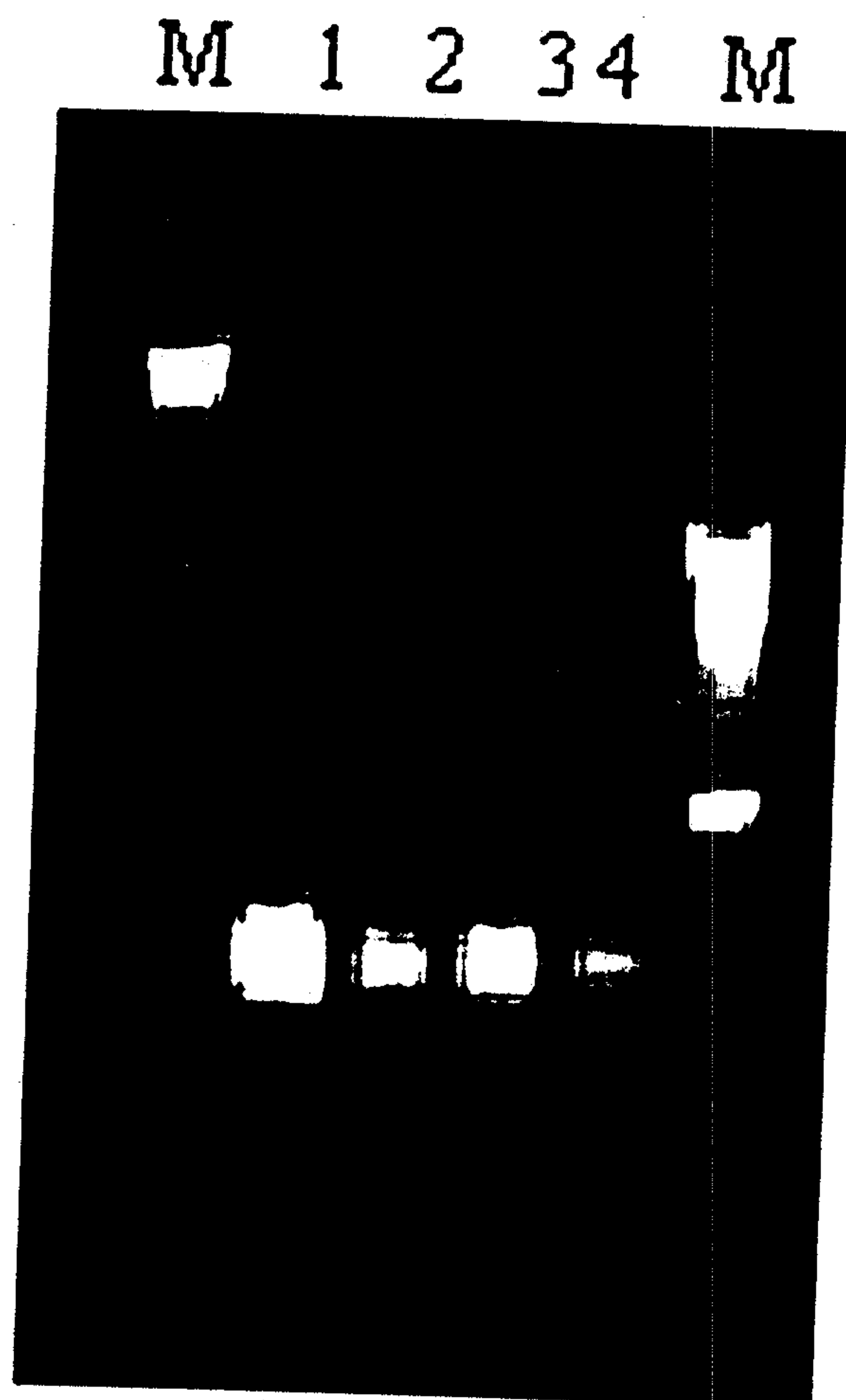


Fig. 10 A

11/12

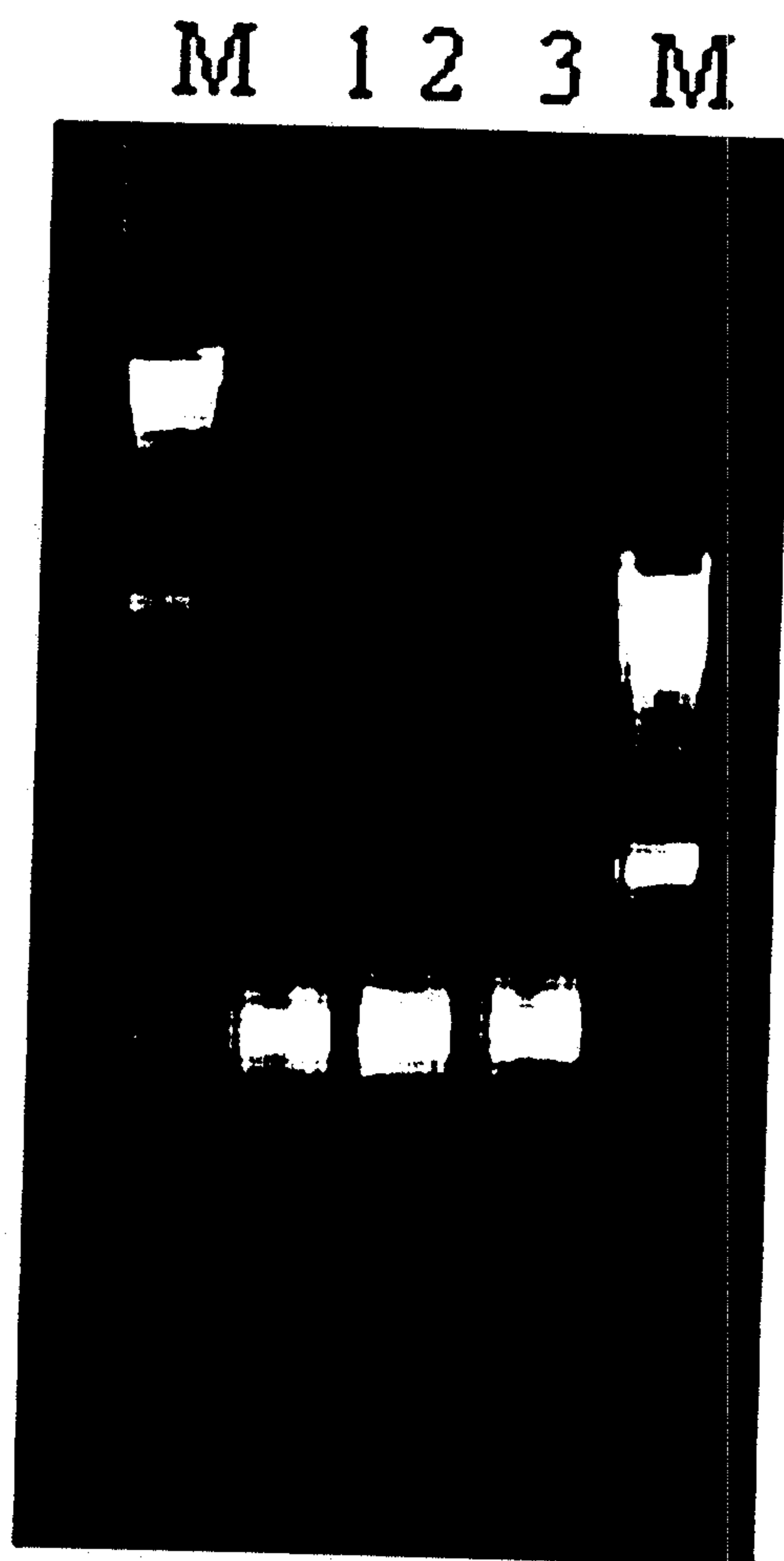


Fig. 10 B

12/12

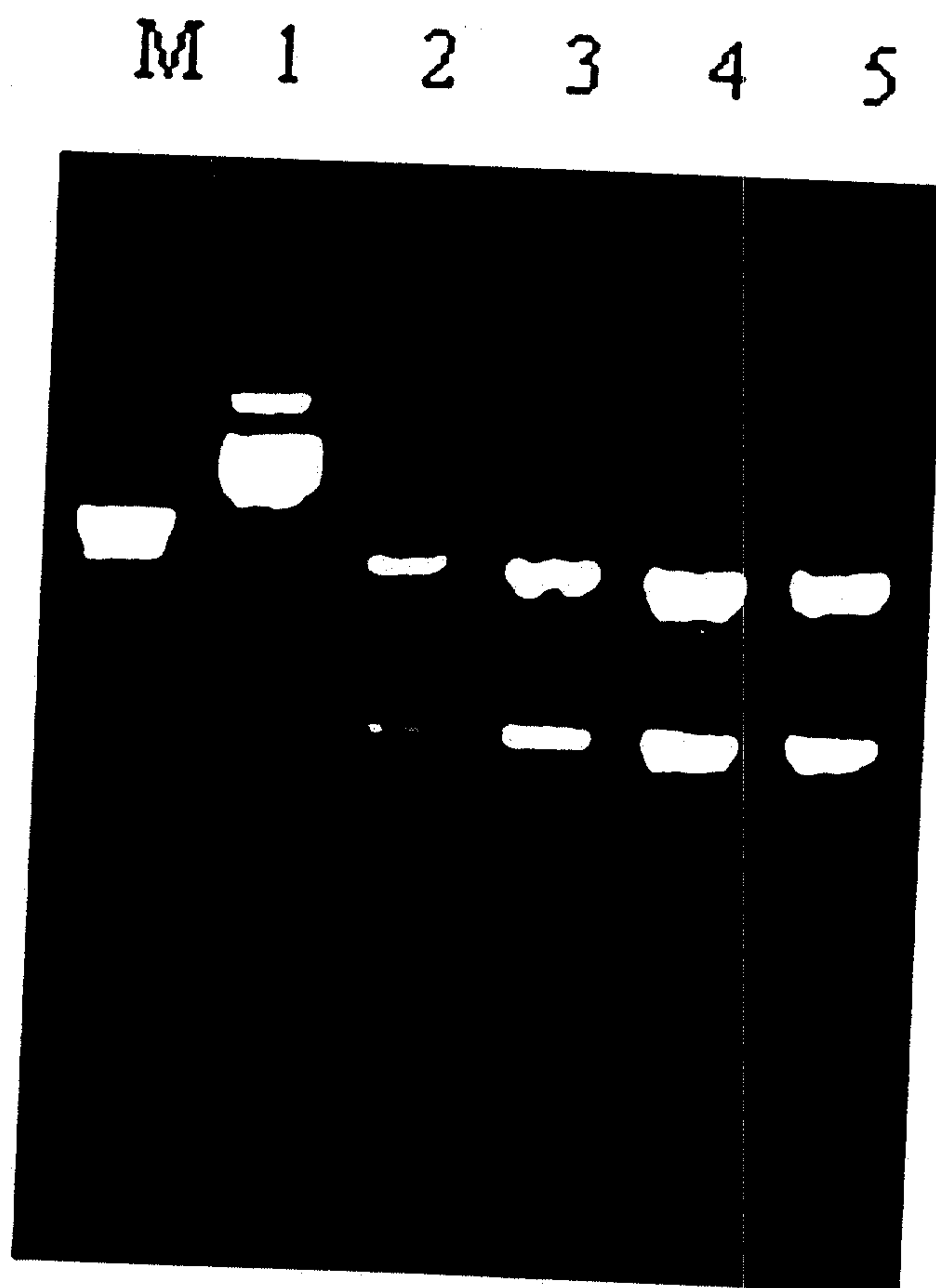


Fig. 11

