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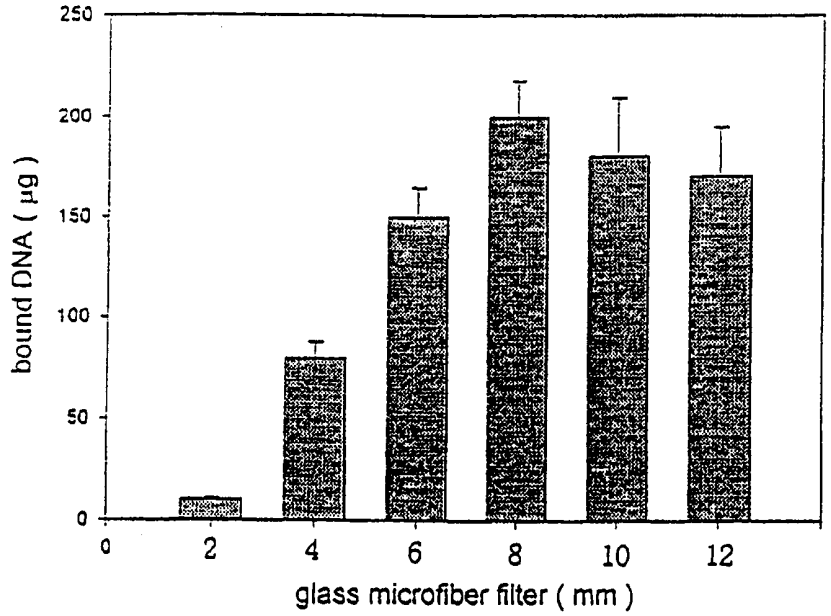

(57) Abstract

Disclosed are glass microfiber column kits for plasmid DNA preparation and DNA purification and the methods for preparing and purifying DNA. The column kits use resins which are formed of borosilicate glass microfiber membranes and/or particles. They are useful to prepare a large quantity of highly pure plasmid DNAs from bacteria cultures and to purify DNA from agarose gels and polyacryl amide gels, simply and quickly. The DNA preparations are so highly pure that they can be applied for various higher experiments, including gene cloning, transfection into mammalian cells and transduction, base sequencing analysis, PCR, radio–labeled DNA probe quantification, cell proliferation bioassay using radio–labeled DNA, etc.

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GLASS MICROFIBER COLUMN AND METHOD FOR THE PREPARATION AND PURIFICATION OF PLASMID DNA USING THE SAME

TECHNICAL FIELD

The present invention relates to glass microfiber (GF) columns and a method for preparing and purifying plasmid DNAs using the same. More particularly, the present invention relates to use of a combination of borosilicate or silicate glass microfiber with guanidine hydrochloride as a DNA binding material, thereby preparing and purifying a large quantity of highly pure DNA for a short period time.

BACKGROUND ART

Plasmid DNAs are one of the most fundamental materials in molecular biology. Therefore, the preparation of purified plasmid DNA is a technique which is the most frequently used in molecular biological research. Numerous manual preparation methods for plasmid DNAs are known in the art and are now used in many laboratories. However, these manual methods take too much time and are unreliable. On the other hand, financially well-off laboratories purchase DNA preparation kits, such as those sold by Qiagen, Germany and by Promega, U.S.A. Typically, these products take advantage of plasmid DNAs' binding to silica gel.

Vogelstein and Gillespie disclosed a method for isolating DNAs from agarose gel, in their article (1977). According to this method, DNAs are bound to flint glass which is in a form of large flint glass particles, medium flint glass particles or flint glass powders and the binding of DNA to the flint glass is identified in an NaI solution. A product based on this principle is already put on the market under the brand name of "glass milk", making easy the isolation of DNA from agarose gel. As far as DNA isolation effect, the flint glass

powders are found to have the most effective DNA binding power and the medium flint glass particles show a significant decrease in the DNA binding power. The worst are the large flint glass particles. In other words, as the flint glass particle size increases, the DNA isolation effect is reduced.

Besides, there are developed other materials, including large borosilicate glass particles, glass fiber filters and porous glass beads, which can be used to isolate DNA by virtue of their binding powers. However, these materials also suffer from their own disadvantages. For example, the large borosilicate glass particles are not effective in binding DNAs, like the large flint glass. The glass fiber filters are very weak in DNA binding, so that the DNA prepared from a small volume is too small to be used. For the porous glass beads, excellent DNA binding powers result, but it takes a long time to bind DNA. When reclaiming the DNA from the porous beads, DNA degradation takes place. Further, the reclaim rate is too low, so its amount is insufficient for quantitative analysis.

Certainly, silica gel is vastly effective in DNA binding, like the flint glass powders. However, it is problematic in mechanical handling and in DNA preparation yield. At any rate, silica gel is now utilized for DNA preparation column kits and commercialized by some companies, such as Qiagen and Promega. Because the products take a column passing manner based on gravity flow, their DNA preparation requires a long period of time. In addition, the resin slurries used are removed from the DNA by additional purification procedures. Further, the DNA solution obtained by use of the preexisting DNA preparation kits is not completely free of endonucleases, so self-digestion may occur upon storage for a long period of time. Another significant problem of the preexisting products is that they cannot guarantee the absence of protein impurities, such as endotoxins, in the DNA prepared. If an endotoxin is contained in the DNA solution prepared, cytotoxicity is caused when the DNA vector is transfected into mammalian cells, leading to cell death and immunological responses in the cells. For animal transfection, therefore, purer DNA preparations are required. Commercially available reagents for preparing the DNAs useful

for animal tests are very expensive. Thus, there is a strong demand for reagents which enable plasmid DNAs to be prepared with such a high purity as to allow animal transfection, but are not expensive.

Examples of DNA purification include isolation of radio-labeled probes, purification of PCR products, and removal of RNA and protein impurities such as endonucleases and endotoxins from DNA preparations. In addition, the DNA purification can be applied for cell proliferation assays. For instance, when the cells which have been grown in a medium containing isotopes are spun down in a DNA preparation column, the isotopes which are not taken in the cells pass through the column; only the isotopes taken in the cells are caught in the column. Therefore, the isotopes taken in cells can be quantified without any additional expensive apparatus.

DISCLOSURE OF THE INVENTION

With the background in mind, the intensive and thorough research on the preparation and purification of DNA, repeated by the present inventors, resulted in the finding that a combination of borosilicate and guanidine hydrochloride has a potent DNA binding capacity. The invention is based on the various advantages of borosilicate. Borosilicate is noble chemically and physically and stable in weak acid and weak alkali. Its 1.0 μm particles show an excellent wet absorption efficiency. Further, with a superior ability to remove impurities, borosilicate is not contaminated by microorganisms even during a long-term and provides accurate analysis results even though it is of small loading capacity. The excellency in DNA binding capacity of the present invention was proven by comparing with that of silica gel. It was found that the mixture of guanidine hydrochloride and borosilicate is 2-4 fold higher in DNA binding capacity than is silica gel. The present invention is accomplished by using the mixture of borosilicate and guanidine hydrochloride as a resin of a kit for DNA preparation and purification.

Therefore, it is an object of the present invention to overcome the above problems encountered in prior arts and to provide a column kit for plasmid DNA preparation and purification, with which a large quantity of highly pure DNA materials can be obtained easily in a short period of time.

It is another object of the present invention to provide a method for making such a column kit for plasmid DNA preparation and purification.

It is a further object of the present invention to provide a DNA preparation and purification method, which can be applied for the purification of DNA from agarose gel or polyacrylamide gel, purification of radio-labeled probes, preparation of PCR products, purification of prepared plasmid DNAs, preparation of genomic DNAs, preparation of RNAs, and quantification of isotopes taken in cells.

In the present invention, borosilicate glass microfiber membrane multilayer columns for mini, midi and maxi plasmid DNA preparation kits and borosilicate glass microfiber membrane/particle double layer column for DNA preparation are fabricated. Using the kits, plasmid DNAs were prepared with high purity and subjected to various assays, including transfection and transduction of the DNA purified into cells, ^{35}S dATP base sequencing and automatic base sequencing analysis, base sequencing analysis of the PCR products prepared, and expression of the DNA purified, so as to prove the superiority of the DNA preparation kit according to the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a is an exploded view showing a borosilicate glass microfiber mini column kit according to the present invention;

Fig. 1b is a cross sectional view showing an assembled state of the column kit of Fig. 1a;

Fig. 2 is a histogram in which plasmid DNA preparation yields are plotted against

the thickness of the borosilicate glass microfiber membrane;

Fig. 3a is an exploded view showing a borosilicate glass microfiber maxi column kit according to the present invention;

Fig. 3b is a cross sectional view showing an assembled state of the column kit of Fig. 3a;

Fig. 4a is an exploded view showing a glass microfiber membrane/particle midi column kit;

Fig. 4b is a cross sectional view showing an assembled state of the column kit of Fig. 4a;

Fig. 5 is a histogram in which plasmid DNA preparation yields are plotted against the thickness of the glass microfiber membrane/particle double layer of the midi column kit;

Fig. 6 is a histogram in which plasmid DNA preparation yields are plotted against the thickness of glass microfiber filter for borosilicate and silicate;

Fig. 7 is a photograph after the plasmid DNA prepared through a column kit of the present invention is digested with restriction enzymes and electrophoresed on an agarose gel;

Fig. 8 is a ^{35}S dATP sequencing autograph for a DNA prepared through a column kit of the present invention;

Fig. 9a is a microphotograph in which the expression of β-galactosidase in the cells transfected with a β-galactosidase gene-containing plasmid prepared by a conventional column kit, is identified by staining;

Fig. 9b is a microphotograph in which the expression of β-galactosidase in the cells transfected with a β-galactosidase gene-containing plasmid prepared by a column kit of the present invention, is identified by staining;

Fig. 10 is an auto-sequencing analysis graph obtained from the pGEM 7Z prepared through a column kit of the present invention using the T7 primer;

Fig. 11 is an auto-sequencing analysis graph obtained from the pGEM 7Z prepared through a column kit of the present invention using the SP7 primer;

Fig. 12 is a photograph after a PCR product obtained by using a plasmid DNA prepared through a column kit of the present invention as a template, is electrophoresed on an agarose gel;

Fig. 13 is a photograph after a plasmid DNA is treated with RNases, but not with ethanol and passed through a column kit of the present invention and then, electrophoresed; and

Fig. 14 is a photograph after a DNA band excised from an agarose gel is purified by a column kit of the present invention and electrophoresed.

BEST MODES FOR CARRYING OUT THE INVENTION

In the present invention, there are provided various DNA preparation and purification kits according to preparation capacity. For this, columns containing borosilicate glass microfiber membranes or a mixture of glass microfiber membranes and particles are first fabricated. For example, borosilicate glass microfiber multilayer columns for DNA mini and maxi preparation, and borosilicate glass microfiber matrix columns for DNA midi preparation are fabricated. With these column kits, highly pure plasmid DNAs are prepared at high yields. Because the column kits of the present invention are far superior in binding DNA, they can be utilized not only in preparing plasmid DNAs from bacteria, but also in purifying DNAs from various contaminants or matrix materials. For example, plasmid DNAs obtained in conventional manners can be further purified through the column kits of the present invention. In addition, the column kits can be applied for DNA purification from agarose gels and polyacryl amide gels. For PCR products, for example, after they are electrophoresed on agarose gels, desirable DNA bands are excised from the gels and passed through the column kits of the present invention. Of course, the

purification ability of the column kits of the present invention is also effective for the DNA bands in polyacryl amide gels.

The high quality DNA preparations obtained by the column kits of the present invention are very useful for higher molecular biological research, including gene cloning, transfection into mammalian cells and transduction, base sequencing analysis, PCR, radio-labeled DNA probe quantification, cell proliferation bioassay using radio-labeled DNA, etc.

The following examples are set forth to illustrate more clearly the principles and practice of this invention to one skilled in the art. As such, they are not intended to limit the invention, but are illustrative of certain preferred embodiments.

EXAMPLE I

Fabrication of GF Columns and Preparation of Plasmid DNA

Experiment Example 1: Fabrication of GF Membrane Multilayer Column for DNA Mini-Preparation Kit and Its Uses

As shown in Fig. 1a, first, borosilicate glass microfiber membranes were cut into circles with a diameter of 8 mm and stacked in a spin mini column 11 with a diameter of 13 mm and a depth of 31 mm to form a multilayer structure 13 2-10 mm tall. Thereafter, a ring 12 7.5 mm in diameter was inserted in the column to fix the multilayer GF structure, followed by subjecting this assembly to gas sterilization. For use, the GF column fabricated was inserted in a collecting tube 10. Fig. 1b shows a combined state of the column 11 in the collecting tube 10.

In order to examine the optimal thickness of the glass microfiber membrane multilayer at which DNA could be obtained with the highest purity at the maximal amount, there were fabricated various columns whose membrane multilayers were different in thickness by 2 mm. Using these columns, plasmid DNAs were prepared and the results are

depicted in Fig. 2. As seen in this graph, a maximal quantity (200 μg) was obtained when the membrane multilayer was 8 mm thick. A membrane thickness as thick as or thicker than 10 mm could not provide a further improvement to the DNA yield.

Experiment Example 2: Fabrication of GF Membrane Multilayer Column for DNA Maxi-Preparation Kit and Its Uses

A glass microfiber membrane multilayer column for a DNA maxi-preparation kit was fabricated in a similar manner to that of Experiment Example 1. In a large column 31 which was 86 mm deep with a diameter of 22 mm, as shown in Fig. 3a, was charged approximately 1 g of borosilicate glass microfiber membranes 33 which were cut into circles with a diameter of 22 mm, after which the circular membranes were fixed with a crisscross-patterned ring 32. For use, a 50 ml falcon tube may be used as a collecting tube 30. Fig. 3b shows an assembled state of the large GF column 31 in the collecting tube 30.

Using this large column, approximately 1.2 mg of plasmid DNA could be obtained from a 250 mL culture of *E. coli*.

Experiment Example 3: Fabrication of GF Membrane/Particle Column for Mini and Midi Kits and Their Uses

Borosilicate glass microfibers were mixed, together with a solution of 1 mM EDTA in 10 mM Tris/HCl pH 7.5, in a mixer to give a glass microfiber particle suspension. In a polypropylene column 22 with a height of 80 mm and a diameter of 15 mm, as shown in Fig. 4a, glass microfiber membranes 24 with a diameter of 8 mm were stacked to a height of 2 mm, followed by charging the glass microfiber particle suspension therein. This content was spun down with a swing rotor centrifuge to form a glass microfiber membrane/particle double column layer which was, then, fixed by use of a ring or frit 8 mm

in diameter and subjected to gas sterilization. For use, the column was adapted to an Effendorf tube 21 which was then set in a collecting tube 20. Fig. 4b shows an assembled state of the column.

Plasmid DNA preparation yields were examined against the depth of the glass microfiber membrane/particle double column layer and the results are given in Fig. 5. As seen, when the depth was 8 mm, a maximal yield was allowed.

Borosilicate glass microfiber membranes were cut into particles with a diameter of 3 mm or less with the aid of a paper cutter. These resins were charged at an amount of about 0.1 g in a mini column and about 0.42 g in a midi column, after which 4.2 M guanidine HCl was loaded on the columns and spun down to give active columns. The mini column enabled plasmid DNA to be prepared at an amount of about 100 μg while the midi column allowed the preparation of plasmid DNA at about 200 μg. In Table 1, below, are shown the preparation yield and purity of plasmid DNA obtained when using these columns.

TABLE 1

Plasmid DNA Purity and Preparation Yield

Columns	E.coli JM109 Cultures	OD_{260}/OD_{280}	Yields
GF matrix mini column	10 ml	1.7-1.9	ca. 100 μg
GF matrix midi column	100 ml	1.7-1.9	ca. 200 μg

Experiment Example 4: Preparation Yield of DNA prepared by Borosilicate GF column and by Silicate GF column

To examine the capacity difference between borosilicate and silicate, the DNA preparation yield of a borosilicate glass microfiber column was compared with that of

silicate glass microfiber column. The columns were fabricated as in Experiment Example 1 and used to prepare plasmid DNAs from *E. coli* cultures. The plasmid DNAs obtained were analyzed for their yield and purity and the results are depicted in Fig. 6. As seen from the graph, the silicate glass microfiber was smaller in DNA preparation yield by approximately 20% than the borosilicate glass microfiber column. The purity of the plasmid DNA obtained by use of the silicate glass microfiber column was measured to be 1.5 (OD_{260}/OD_{280}) while the plasmid DNA prepared by use of the borosilicate glass microfiber column ranged, in purity, from 1.7 to 1.8 as measured by a spectrophotometer.

Experiment Example 5: Preparation Yield and Purity of DNA prepared by Silica Gel Column and by Silica Gel/Silicate GF Double layer Column

As in Experiment Example 3, a borosilicate glass microfiber membrane/particle layer 2 mm thick was formed in each of five midi-columns, followed by forming a silica gel layer 6 mm thick thereon. For comparison, silica gel was charged to a height of 8 mm in additional five midi-columns. With the aid of these columns, plasmid DNA was prepared, and analyzed for purity and yield, as in Experiment Example 2. The results are given in Table 2, below, demonstrating that the recruitment of the borosilicate glass microfiber layer to the silica gel significantly increases the DNA preparation yield and purity compared with the silica gel alone.

In the following examples, all of the plasmid DNAs used were those which were prepared through the silicate glass microfiber columns.

TABLE 2

Plasmid DNA Purity and Preparation Yield

Columns	E.coli JM109 Cultures	OD_{260}/OD_{280}	Yields
Silica Gel	10 ml	1.7-1.9	ca. 100 μg
Silica gel/borosilicate GF	100 ml	1.7-1.9	ca. 200 μg

EXAMPLE II

Preparation of Plasmid DNA

Using the GF mini column kit, the GF midi column or the GF maxi column kit fabricated in Example I, plasmid DNA was prepared. First, an E. coli strain harboring a certain plasmid was cultured in a broth and the culture was centrifuged to give a pellet. This cell pellet was suspended in Buffer A (50 mM Tris, 10 mM EDTA pH 8.0, 100 μg/ml RNases A), lysed with Buffer B (20 mM NaOH, 1.0% SDS (w/v)), and neutralized with Buffer C (3.2 mM potassium acetate, pH 5.0). This neutralized lysate was centrifuged and the supernatant was loaded on the GF column kit which was previously activated with guanidine HCl, to bind the DNA to the column. After being deprived of nucleases, the bound DNA was washed and eluded. For the removal of nucleases, 4.2 M guanidine hydrochloride was used. As the washing solution, 10 mM Tris/HCl (pH 7.5), 50 mM NaCl, 0.1 mM EDTA, 70% ethanol was used.

EXAMPLE III

Molecular Biological Assay and Analysis

Experiment Example 1: Digestion with Restriction Enzyme

The plasmid DNA prepared in Example II was treated with restriction enzymes *EcoR* I and *Hind* III and Electrophoresed on an agarose gel along with an intact plasmid

DNA. As seen in Fig. 7, the prepared plasmid DNA was clearly digested by the endonucleases.

Experiment Example 2: ^{35}S dATP Sequencing Analysis

10 μg of the plasmid DNA prepared in Example II were reacted with a sequencing kit, such as that sold by United State Biochemical, identified as Sequenase™ version 2.0, using α ^{35}S dATP as a radio label, along with 10 μg of the plasmid DNA prepared through the kit of Qiagen as a control. The sequencing results were read from the sequencing gel by autoradiography. As seen in Fig. 8, the bands autographed from the plasmid DNA prepared through the column of the invention are clearly separated while the bands obtained through the kit of Qiagen were not effectively separated.

Experiment Example 3: Cell Transfection and Transduction

The plasmid DNA prepared through the column kit of the present invention was transfected and transduced in mammalian cells while the plasmid DNA prepared through the kit of Qiagen was used as a control. As shown in Fig. 9b, the plasmid DNA prepared through the column kit of the present invention was effectively expressed in cells as identified by stained proteins. In contrast, the control DNA showed a poor expression efficiency, as shown in Fig. 9a.

Experiment Example 4: Automatic base Sequencing Analysis

An automatic base sequencing analysis was performed using ABI PRIM™ Dye terminator cycle sequencing ready reaction kit, commercially available from Perkin Elmer. 0.4 μg of a template DNA prepared through the column of the invention were mixed with

3 p moles of a primer, along with 8 μl of a terminal ready reaction mix and adjusted to a total volume of 20 μl. This solution was subjected to 25 reaction cycles, each consisting of 96 °C/30 min, 50 °C/15sec and 60 °C/4 min, in a thermal cycler, such as that sold by Bio Rad, identified as "Gene Cycler™". The elongated DNA product was precipitated with ethanol, dried in a speed vac system and analyzed in an automatic sequencer, such as that sold by Applied Biosystem. As the template was used the plasmid pGEM7z in which a DNA fragment 1.2kb long was inserted. A T7 or SP6 primer was used.

Experiment Example 5: PCR Reaction and Purification of PCR Product

A PCR reaction was carried out using as a template the CMV-TNF plasmid DNA prepared through the column of Example I. A reaction mixture containing 20 ng of the CMV-TNF plasmid, 2 μl of 1 mM dNTP, 5 μl of 10x reaction buffer, 2U of Tag polymerase, and a TNF anti-sense primer in combination with a proximal promoter sense primer or a distal promoter sense primer, was pre-heated at 95 °C for 5 min and then, subjected to 30 reaction cycles, each consisting of 95 °C/1 min, 55 °C/1 min and 72 °C/1 min. and finally to extension reaction at 72 °C for 10 min. The reaction product was divided to two aliquots. One aliquot was mixed with an equal volume of 4.2 M guanidine HCl, passed through a micro DNA purification column and washed with a WA solution. This purified DNA was electrophoresed on an agarose gel while the other aliquot was used as a control. This electrophoresis result is shown in Fig. 12. As seen in this figure, no primer bands were not found in the lane for the PCR product purified by the micro DNA purification column.

Experiment Example 6: DNA Purification

From three cultures with a volume of 250 ml, plasmid DNAs were prepared using

the GF matric maxi columns fabricated in Example I. First, a typical method using a WA solution was applied for a first culture. The DNA from a second culture was obtained by ethanol precipitation without washing with a WA solution, and then dissolved in 500 μl of deionized water. For the final culture, DNA was obtained as in the second culture, aliquoted to a volume of 250 μl. 20 μl of RNase A (3 mg/ml) were added to each of the aliquots, reacted at 37 °C for 30 min, inactivated at 65 °C for 10 min, and passed through the micro columns. The DNA products thus obtained were electrophoresed on an agarose gel, as shown in Fig. 13. These results are given in Table 3, below. As demonstrated by the data of Table 3, the plasmid DNA, when being not treated with ethanol, was obtained at a great yield, but contaminated with RNA. On the other hand, the plasmid DNA which was obtained after the treatment with RNase A and the purification through the column, was completely free of RNA impurities and showed an increase of DNA purity (OD_{280}/OD_{260}) from 1.72 to 1.90. Accordingly, the present invention can overcome the serious disadvantages from which conventional DNA preparation methods suffer, that is, RNA contamination and low DNA yield and purity.

TABLE 3

	OD_{280}/OD_{260}	Conc.	Total DNA
WA washing	1.44	7.60	760 μg
no washing	1.68	10.80	1.08 mg
no washing/column purification	1.90	11.75	1.175 mg

Experiment Example 7: Isolation of DNA from Agarose Gel or Polyacrylamide Gel

A DNA solution was electrophoresed on an agarose gel/TAE or TBE, and a DNA band was excised from the gel and placed in a dialysis bag which was then charged with a TAE solution and subjected to electrophoresis to separate the DNA from the gel. The

TAE solution was added with an equal volume of 4.2 M guanidine hydrochloride and loaded on a GF mini column of the present invention. The column was spun and washed with a WA solution (10 mM Tris/HCl pH 7.5, 30 mM EDTA, 70% ethanol). Subsequently, the DNA was eluded with 50 μl of deionized water by spinning. The DNA was identified and analyzed by electrophoresis on agarose gels, as shown in Fig. 14.

Analysis results are summarized in Table 4, below. According to these results, the present invention has advantages as follows. First, the column kits of the present invention make it possible to prepare plasmid DNA simply and quickly. Second, the column kits of the present invention show extremely high DNA preparation yields. Compared with conventional spin column kits, the GF mini column kits of the present invention can yield DNA at an amount approximately 50-100 fold larger (ca. 200 μg). With the GF midi column kits of the present invention, as much as 400 μg of DNA can be obtained while the GF maxi column kits enjoyed 2.2 mg of plasmid DNA. Third, the plasmid DNA obtained through the column kits of the present invention ranged, in DNA purity (OD_{260}/OD_{280}), from 1.7 to 1.9 as measured by spectrophotometry. Together with the maximal plasmid DNA yields, the conditions under which plasmid DNAs can be prepared effectively, are given in Table 5, below. Finally, nucleases, which frequently appear in the DNA preparation from *E. coli*, and carbohydrates, which advent as contaminants in the DNA preparation from JM 109 strains, are never found in the DNA preparations obtained by use of the column kits of the present invention.

TABLE 4

Plasmid DNA preparation by GF midi column kit

Reagents	JM109 cultures	Plasmid	OD_{260}/OD_{280}	Max. Yield
Chaotropic salt	200 ml	pGEM 7Z	1.7-1.8	50-100μg
Guanidine HCl	200 ml	pGEM 7Z	1.7-1.9	200-400μg

TABLE 5

Plasmid DNA Preparation by GF Column Kits

Kits*	JM109 Cultures	Plasmid	OD_{260}/OD_{280}	GF resin	Yields
Mini	10 ml	pGEM 7Z	1.7-1.8	0.1 g	ca. 70μg
Midi	100 ml	pGEM 7Z	1.6-1.9	0.42 g	ca. 200μg
Maxi	250 ml	pGEM 7Z	1.7-1.9	1-1.34 g	832μg-2.2mg

* GF mini column and midi column: matrix type; GF maxi column: multilayer type

INDUSTRIAL APPLICABILITY

As described hereinbefore, the GF column kits of the present invention is useful to prepare a large quantity of plasmid DNAs from bacteria cultures, simply and quickly. The plasmid DNAs are so highly pure that they can be applied for various higher experiments, such as base sequencing analysis, transfection and transduction, cloning, etc., without further purification. In addition, the column kits of the present invention can be used for the purification of DNA from agarose gels or polyacryl amide gels, and the purification ability thereof can be further applied for the isolation of radio-labeled probes, the purification of PCR products, and the removal of RNA and protein impurities such as endonucleases and endotoxins from DNA preparations, and cell proliferation assays. Therefore, the present invention is very valuable in the biomedical industry.

Although the invention has been described in detail by referring to certain preferred embodiments, it will be understood that various modifications can be made within the spirit and scope of the invention. The invention is not to be limited except as set forth in the following claims.

CLAIMS

1. A glass microfiber multilayer column kit for the preparation and purification of DNA, comprising a column which contains a resin therein, said resin being formed of a multilayer of glass microfiber membranes.

2. A glass microfiber matrix column kit for the preparation and purification of DNA, comprising a column which contains a resin therein, said resin being formed of a double layer of glass microfiber membranes and glass microfiber particles.

3. A glass microfiber multilayer column kit as set forth in claim 1, wherein said column comprises a mini spin column tube; a multilayer resin consisting of glass microfiber membranes for binding DNA; a ring or frit for fixing the glass microfiber membranes in the mini spin column tube; and a collecting tube for collecting materials which pass through the glass microfiber membranes.

4. A glass microfiber matrix column kit as set forth in claim 2, wherein said column comprises a midi column tube; a bilayer resin consisting of glass microfiber membranes and glass microfiber particles for binding DNA; a 1.5 ml micro centrifuge tube for collecting DNA which passes through the bilayer resin, which is detachably adapted to the midi column tube; a collecting tube for collecting materials which pass through the bilayer resin, in which said midi column and said 1.5 ml micro centrifuge tube are assembled; a ring or frit for fixing the bilayer resin in said midi column tube.

5. A glass microfiber multilayer column kit as set forth in claim 1, wherein said glass microfiber membrane is formed of borosilicate or a mixture of silicate and borosilicate.

6. A glass microfiber multilayer column kit as set forth in claim 1, wherein said glass microfiber multilayer column tube is in a mini, midi or maxi size.

7. A glass microfiber matrix column kit as set forth in claim 2, wherein said glass microfiber membranes and glass microfiber particles are formed of borosilicate or a mixture of silicate and borosilicate.

8. A column kit as set forth in claim 1 or 2, wherein the glass microfiber matrix column tube is in a mini, midi or maxi size.

9. A method for preparing plasmid DNAs, in which the glass microfiber membrane multilayer column kit of claim 1 or the glass microfiber matrix column kit of claim 2 is used.

10. A method for isolating DNA from agarose gels or polyacryl amide gels, in which the glass microfiber membrane multilayer column kit of claim 1 or the glass microfiber matrix column kit of claim 2 is used.

11. A method for quantitatively analyzing intracellular isotopes, in which the glass microfiber membrane multilayer column kit of claim 1 or the glass microfiber matrix column kit of claim 2 is used.

12. A method for purifying DNA, in which the glass microfiber membrane multilayer column kit of claim 1 or the glass microfiber matrix column kit of claim 2 is used to remove RNA or protein contaminants from DNA.

13. A method for purifying radio-labeled probes, in which the glass microfiber

membrane multilayer column kit of claim 1 or the glass microfiber matrix column kit of claim 2 is used.

FIG. 1a

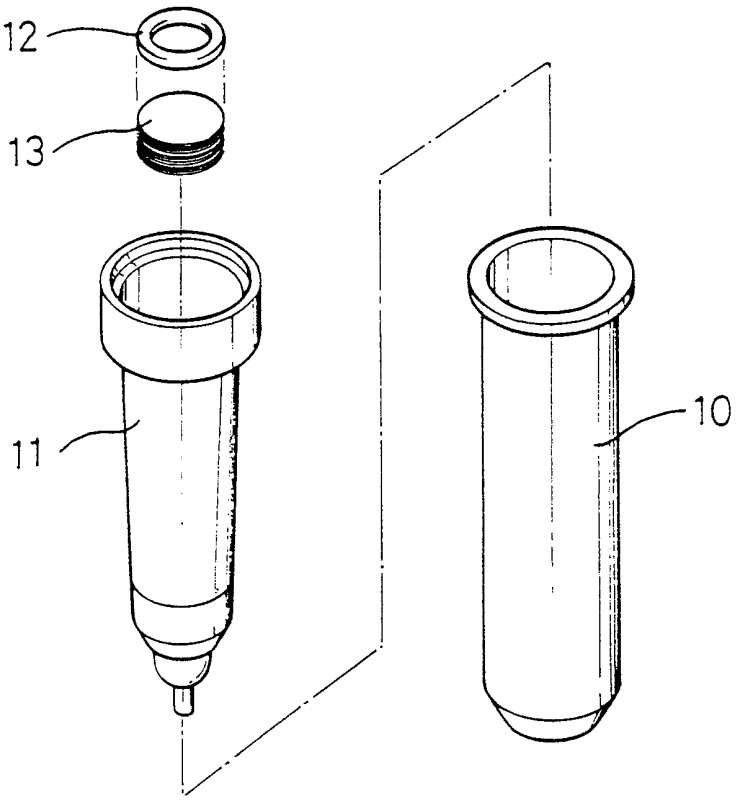


FIG. 1b

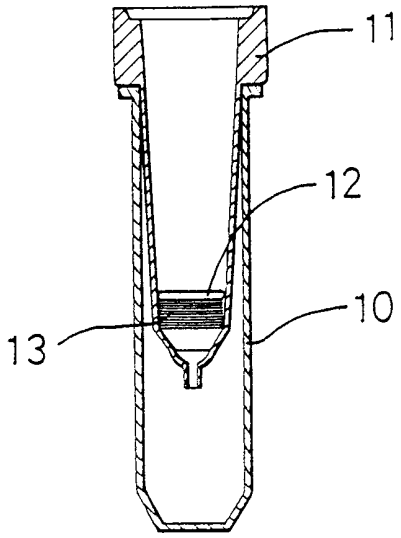


FIG. 2

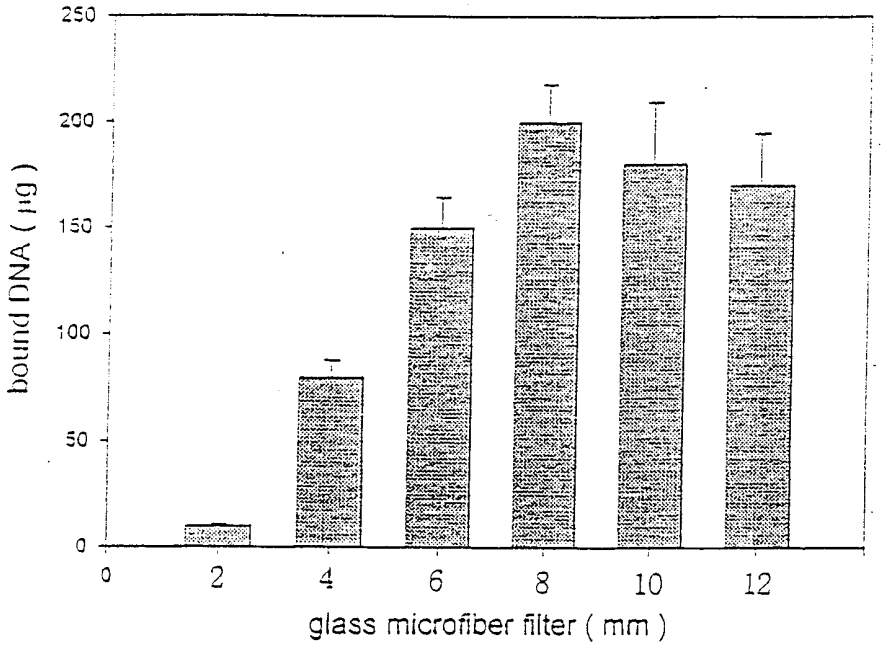

250
200
150
100
50
0
bound DNA (μg)
0
2
4
6
8
10
12
glass microfiber filter (mm)

FIG. 3a

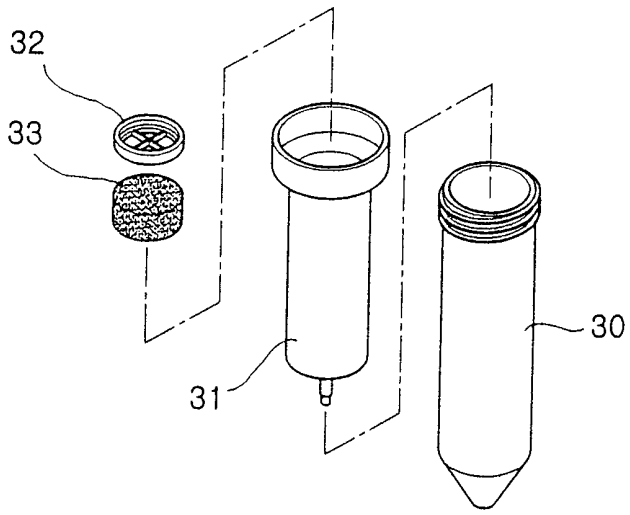


FIG. 3b

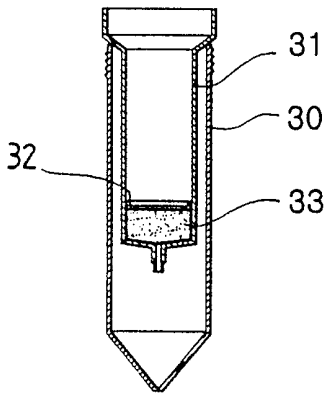


FIG. 4a

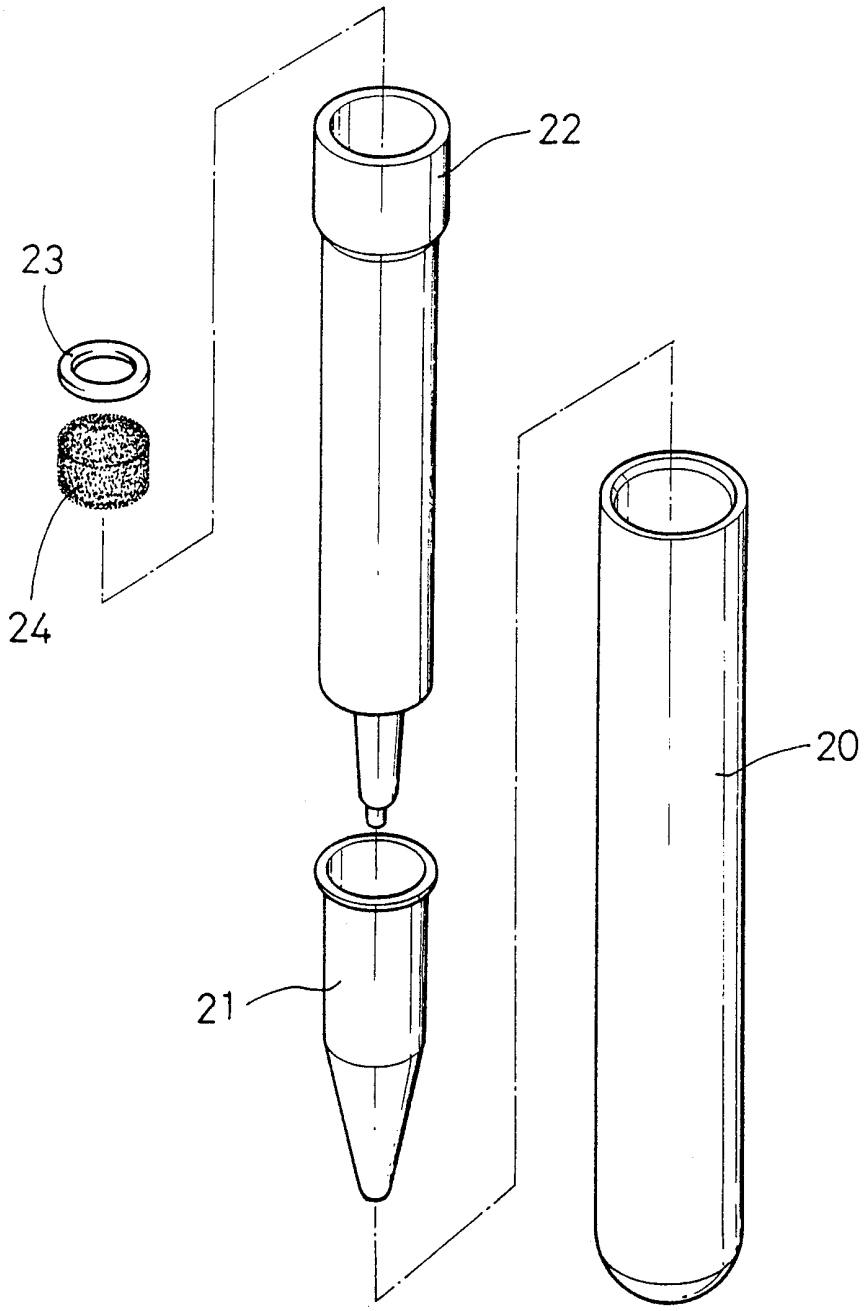


FIG. 4b

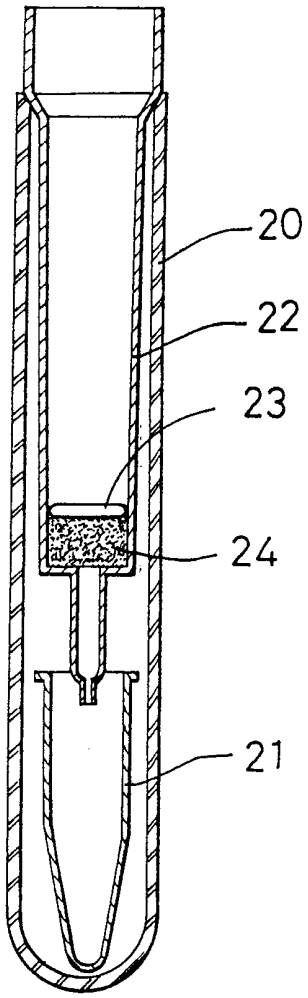


FIG. 5

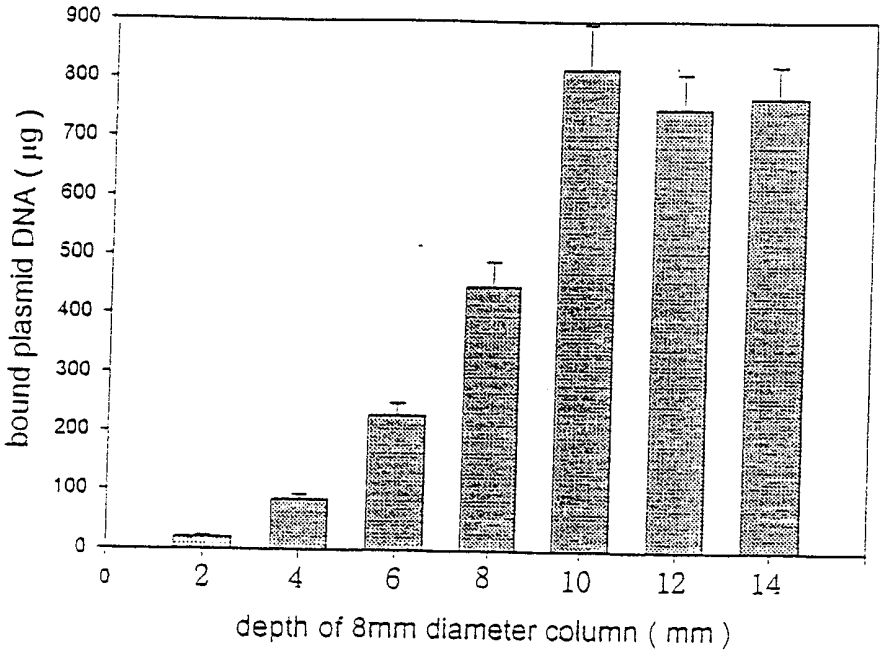

bound plasmid DNA (μg)
900
800
700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
depth of 8mm diameter column (mm)

FIG. 6

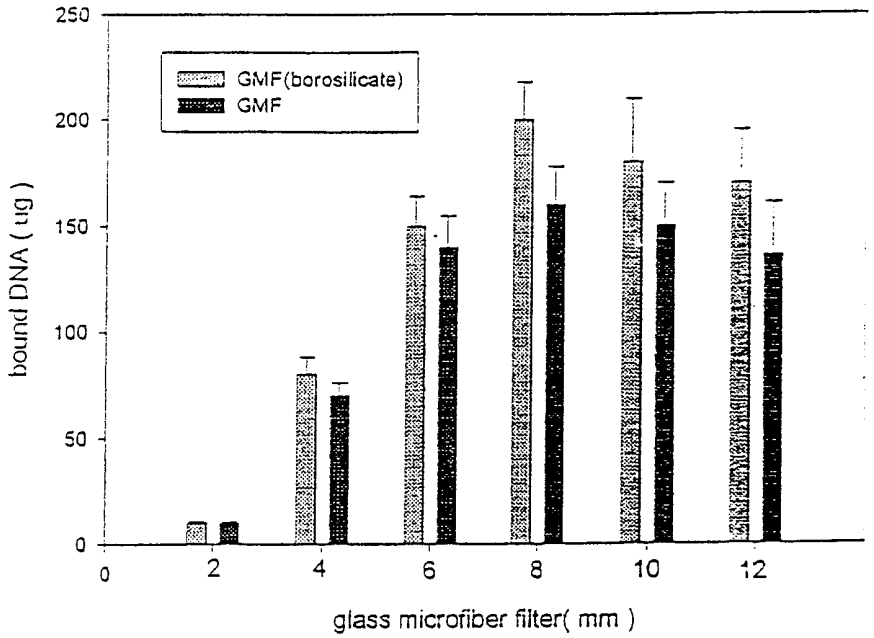
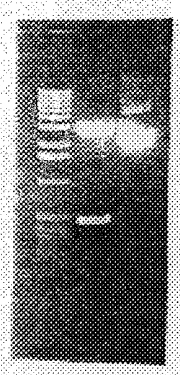

250
200
150
100
50
0
bound DNA (ug)
GMF(borosilicate)
GMF
0
2
4
6
8
10
12
glass microfiber filter(mm)

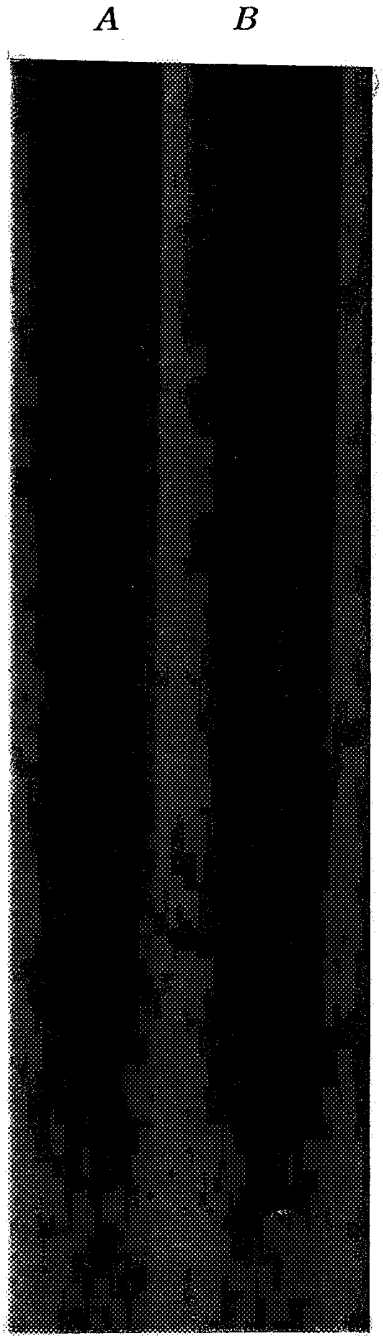
FIG. 7



Restriction Enzyme Digestion.

1% Agarose
M : 1kb marker
1 : EcoRI-Hind III digested plasmid 5ul
2 : uncut plasmid 4ul

FIG. 8



35S dATP Sequencing Analysis.

A : Invention

B : Prior Art

FIG. 9a

FIG. 9b

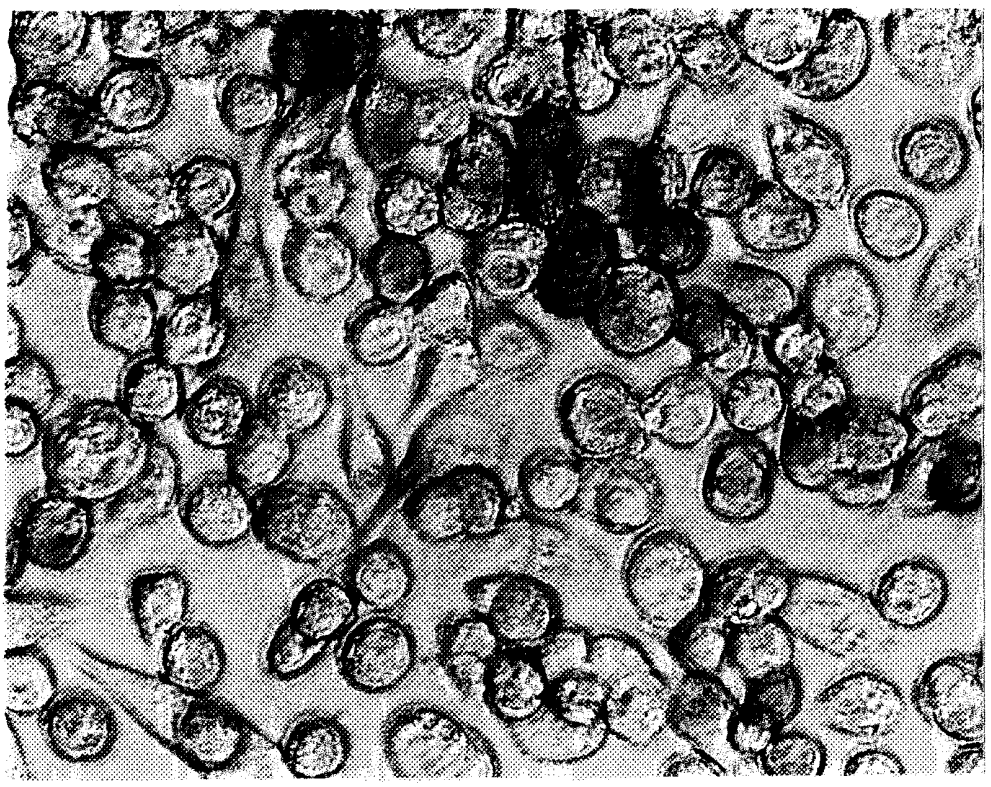


FIG. 10

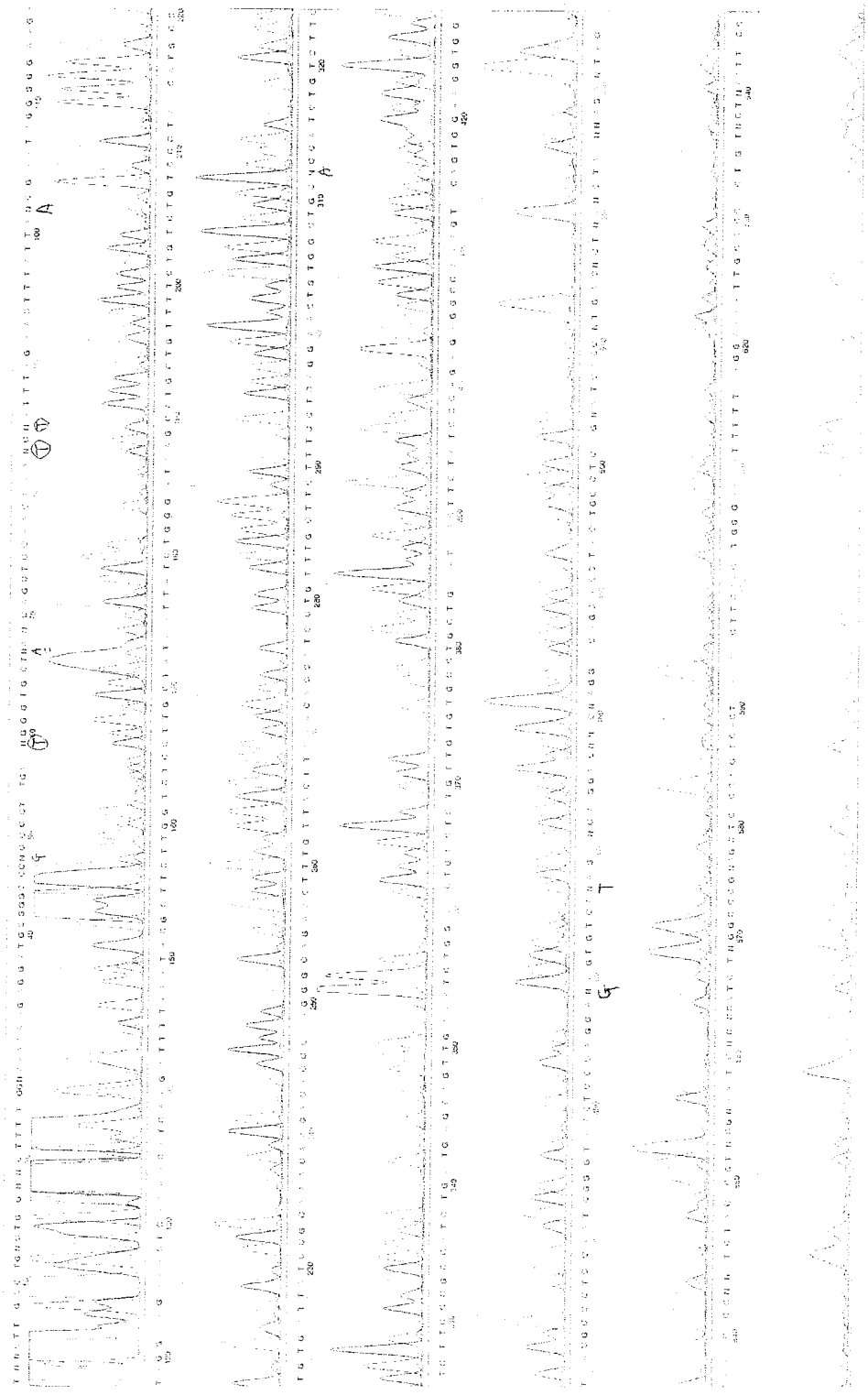


FIG. 11

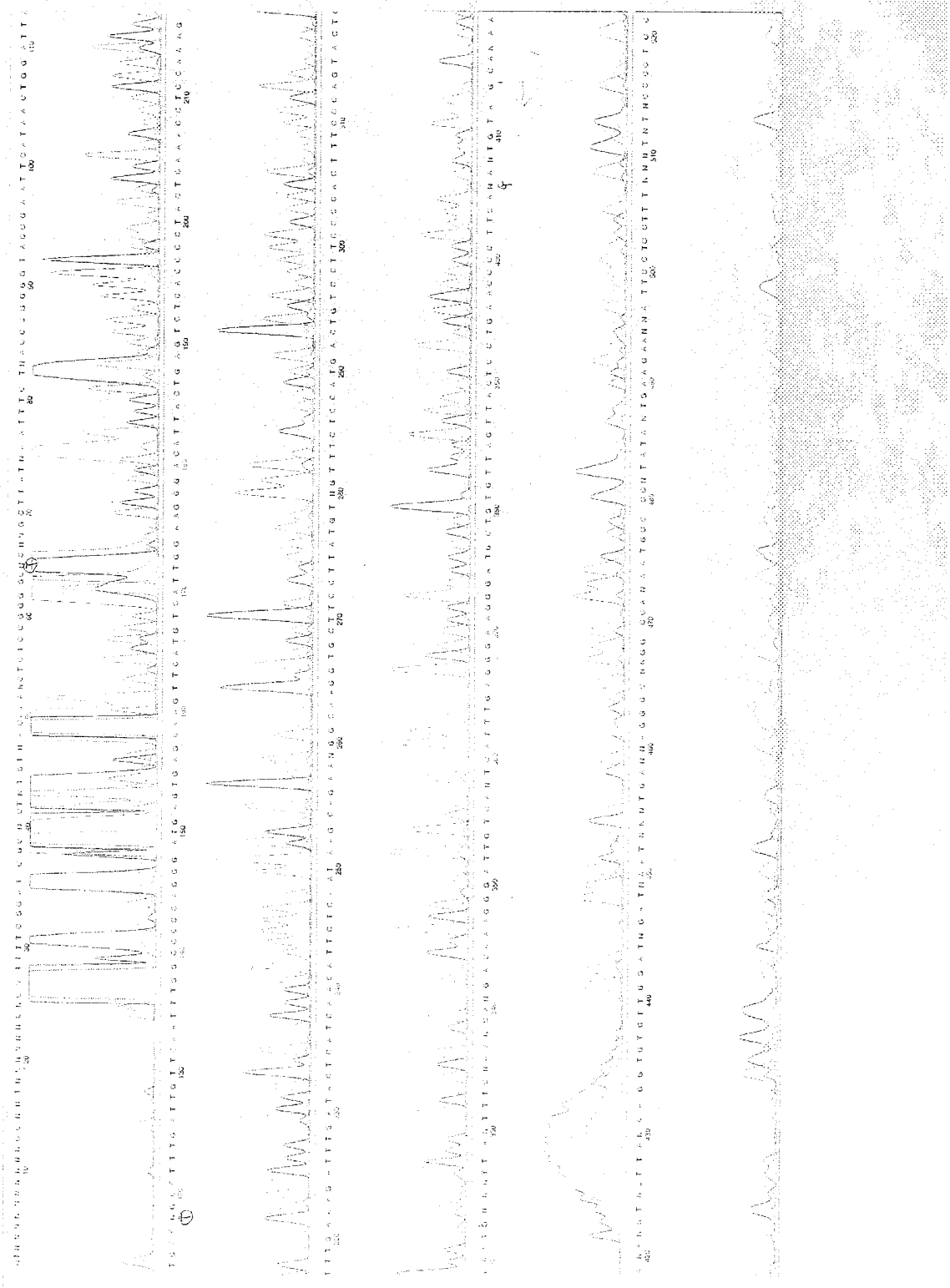


FIG. 12

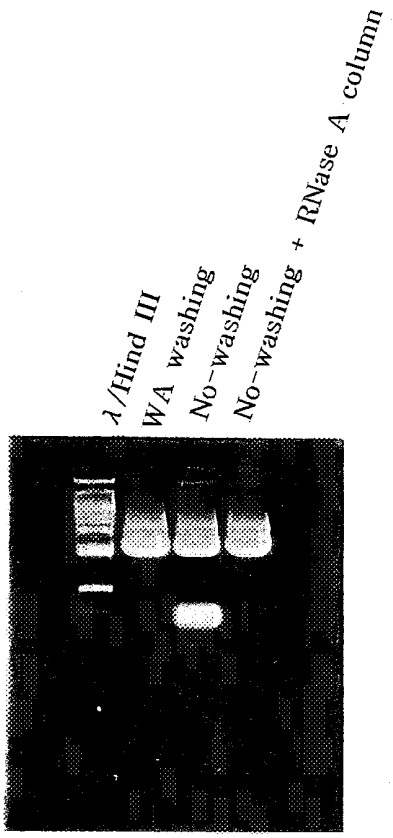

λ/Hind III
WA washing
No-washing
No-washing + RNase A column

FIG. 13

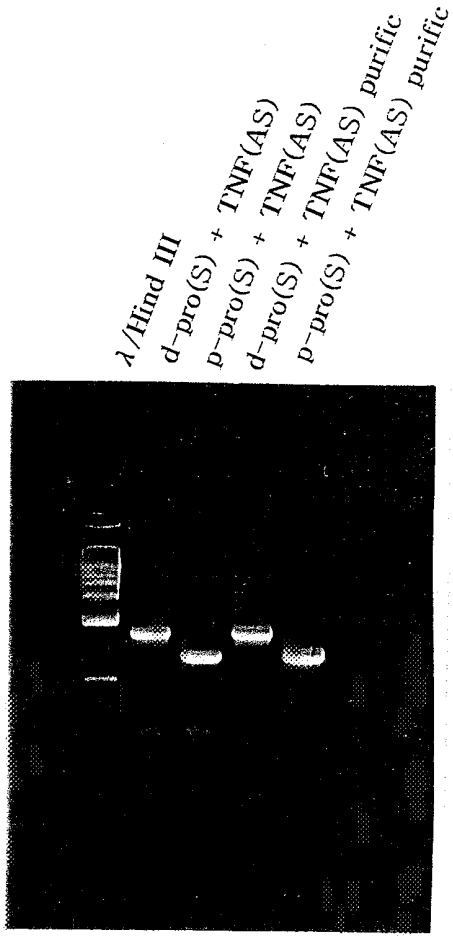
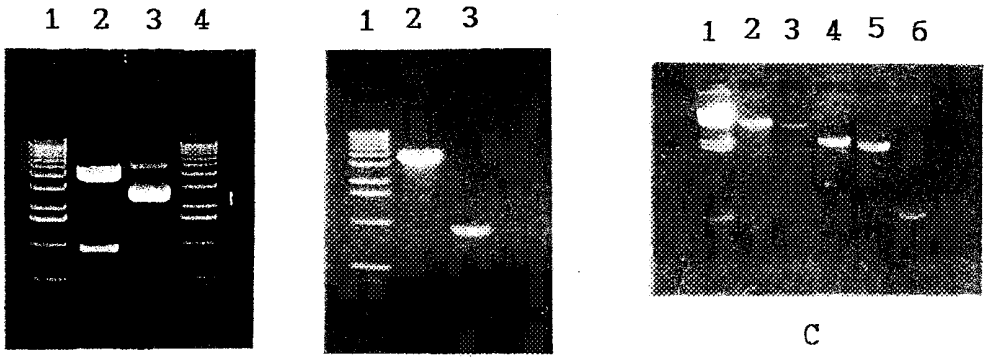

λ/Hind III
d-pro(S) + TNF(AS)
p-pro(S) + TNF(AS)
d-pro(S) + TNF(AS) purific
p-pro(S) + TNF(AS) purific

FIG. 14



A B C

A.
lane 1,4 : 1 kb DNA ladder(pBR322 Hinf 1)
lane 2 : CMV TNF α (Not 1 digestion)
lane 3 : CMV TNF α

B.
lane 1 : 1 kb DNA ladder(pBR322 Hinf 1)
lane 2 : CMV vector separated from gel.
lane 3 : TNF α separated from gel.

C.
lane 1 : DNA ladder (λ Hind III).
lane 2, 3, 4, 5, 6 : each DNA ladder of λ Hind III separated from gel.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00160

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6]: C 12 N 15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[6]: C 12 N 15/10

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/08 500 A1 (QIAGEN GMBH), 21 March 1996 (21.03.96), abstract; page 4, paragraph 3; page 5, paragraph 3 - page 6, paragraph 3; claims 1,6-9.	1
A	DE 41 39 664 A1 (DIAGEN INSTITUT FÜR MOLEKULARBIOLOGISCHE DIAGNOSTIK GMBH), 03 June 1993 (03.06.93), column 4, line 60 - column 5, line 10; column 8, lines 29-40, 53-66; examples; fig.2,4-6.	1-13
A	US 5 658 548 A (PADHYE et al.), 19 August 1997 (19.08.97), column 4, lines 17-19, 35-47; column 6, lines 44-59; column 12, lines 21-42; claims 1-4, 13-16.	1-12
A	US 5 660 984 A (DAVIS et al.), 26 August 1997 (26.08.97), columns 3-5; fig.1.	1-12

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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„E" earlier application or patent but published on or after the international filing date
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„P" document published prior to the international filing date but later than the priority date claimed

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„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 August 1999 (13.08.99)	24 August 1999 (24.08.99)

Name and mailing adress of the ISA/AT	Authorized officer
Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/200	Mosser Telephone No. 1/53424/437

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00160

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 649 853 A1 (BECTON DICKINSON AND COMPANY), 26 April 1995 (26.04.95), abstract; page 4, lines 2-5; example 2; claims 1,10.	1,2,9,10,13

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR 99/00160

In Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
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		EP B1 616638	10-04-1996
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		CA AA 2170604	09-03-1995
		EP A1 723549	31-07-1996
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