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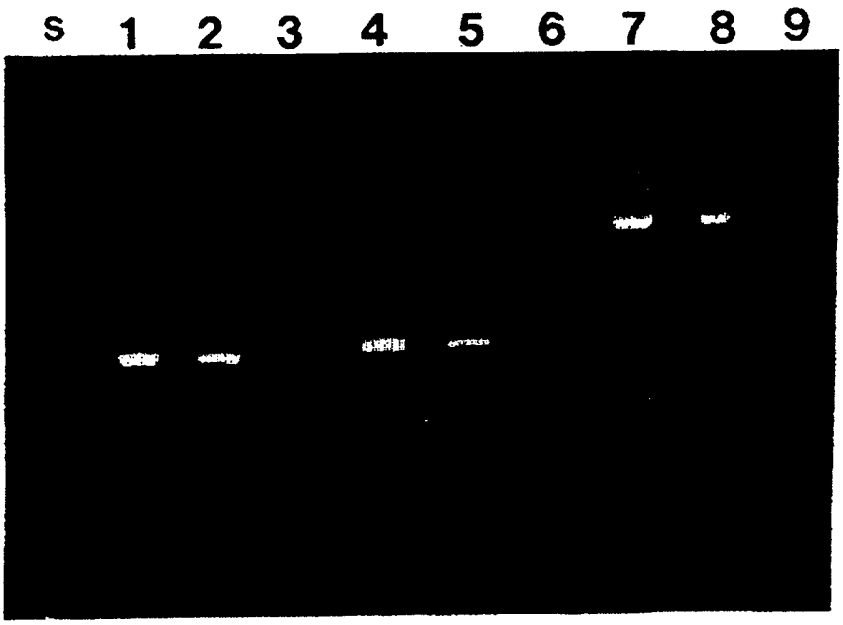

(57) Abstract

An apparatus for identifying, tracking and processing a biological sample (104) stored on a solid medium (105) including a sample recognising/recalling identification station (101), a processing station (103), a tracking station (102) that tracks the sample through the other stations (101, 103) and an interactive networking of the stations. Also disclosed is a cannula (1) connectable in fluid communication to an automatic fluid delivery system constructed with a primary fluid aspirating and dispensing port (6) and at least one auxiliary fluid aspirating and dispensing port (7). A method of removing a by-product from a biopolymer reaction mixture using a polymeric gel is also disclosed.

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APPARATUS AND METHOD FOR STORAGE, PURIFICATION OR REACTION AND PROCESSING OF A BIOPOLYMER

Field of the Invention

The present invention is directed to a system for storage, purification and reaction of biopolymers, for example, deoxyribonucleic acid (DNA). The components of the invention comprise: a solid storage medium for DNA, a cannula for use with automatic fluid delivery systems for aspirating and dispensing fluids associated with a solid material, an apparatus for separation of low molecular weight molecules from biopolymers, and a system for identifying a selected stored biological sample, processing the selected sample and tracking the sample to correlate the sample with the process results. The components of the system may be used together as a single system or, in some applications, independently as individual components of other systems. The invention is further directed to methods for utilizing the components of the system for purification and reaction of biopolymers.

Cross Reference to Related Applications

The present application is a continuation in part application of U.S. S\N 08\159,104, filed November 30, 1993, the disclosure of which is incorporated herein by reference.

Background of the Invention

In the past, blood DNA has been transported as purified DNA, liquid blood, frozen blood or blood dried onto paper. All of these methods have disadvantages. Transport of blood DNA as dried, purified DNA is most desirable, but it requires a high standard of technical assistance to be available at the places of collection. When technical assistance is not available at the collection place, whole blood or other crude samples are usually sent to a central facility where the DNA is purified. Transport of liquid blood involves the need for sterility of collection. This is extremely inconvenient under some circumstances, e.g., where the sample is a heel-prick taken from an infant. The transport of liquid blood or frozen blood also demands temperature control and an appropriate transport system other than the regular postal system. This is true even before the question of hygiene is considered. In addition, problems with pathogens such as the HIV virus generally rule out the transport of any potentially infective liquid or frozen sample except under proper and expensive supervision.

Blood dried on filter paper is a proven alternative to the above procedures and it has been shown that DNA can be extracted and isolated from dried whole blood spots in a form and in sufficient quantities for use in DNA analysis[1]. This procedure still suffers from a number of disadvantages. For example, there has been no deliberate and rapid destruction of most pathogens, and there has been no deliberate inhibition of the processes degrading the DNA other than that caused by desiccation. Slow desiccation or even a small degree of rehydration under conditions of high relative humidity will allow the growth of DNA-destroying microflora. Even in the presence of bacteriostatic agents of the type that do not denature proteins, there will be conditions that permit enzymic-autolytic breakdown of the DNA and some nonenzymic breakdown of the DNA. Enzymic-autolytic breakdown refers to the process whereby dying or damaged tissues, either human cells or parasite cells, activate enzymes that degrade their own components. Additionally, there is considerable difficulty desorbing very high molecular weight DNA from paper, if this is required. Surface adsorption effects can cause losses of DNA and this will cause the preferential loss of the least degraded DNA, i.e. the most desired class of DNA molecules. Thus, there is a need for safe, convenient, and minimally labor intensive means for storage of liquid DNA containing samples.

A further problem in the past has been the treatment of biopolymers, for example DNA, subsequent to dry storage on filter paper. Such stored samples were typically not capable of rapid processing through automatic fluid delivery systems due to the construction of prior art pipettes. During subsequent treatment of the compounds, the solid medium on which the compound was adsorbed, such as filter paper, could block the aspiration and dispensing port of the pipette preventing effective aspiration of reaction fluids. Hence, prior to treatment of the stored compound it was necessary to first remove the compound from the solid storage medium. The need for compound removal from the storage medium not only caused the loss of compounds such as DNA, but also caused increased laboratory costs due to loss of time, increased labor and increased reagent costs. If it was desirable to leave the compound in association with the solid medium (or if the solid medium was desirable in the reaction system) automatic fluid delivery systems were inefficient due to the need for manually preventing the solid from obstructing the end of the pipette.

After biopolymer synthesis, for example DNA amplification via PCR methodology, it is often necessary to isolate and analyze the product compound. A problem which can hinder the analysis and isolation of a biopolymer post synthesis is the separation of low molecular weight by-products from the desired product. For example, methods for analysis and isolation of DNA amplified through PCR technology generally require removal of the reaction by-products from the amplification reaction prior to analysis. This is readily appreciated by considering a relatively new technology for DNA analysis, capillary electrophoresis. This method has been used successfully for automated analysis of DNA following PCR amplification. However, prior to capillary electrophoresis, it is essential to remove salts which are by-products of the PCR reaction mixture.

Prior methods for removing by-products from a biopolymer produced by PCR reaction are laborious, relatively expensive and not ideal for multiple sample analyses. Moreover, these methods generally require relatively large sample volumes and are designed for manual rather than automated use.

The simultaneous processing of large numbers of biological, agricultural or environmental samples is becoming more common. DNA fingerprinting for forensic or medical diagnostic purposes is a typical example of this. Often, identification of a specific sample, for example a specific genotype, requires screening a large pool of samples. Rapid processing of the pooled samples is desirable to obtain results within a reasonable amount of time. As the sample quantity screened is increased there remains the need to maintain accurate correlation of a sample tested and the results generated for that sample.

Summary of the Invention

According to the present invention, a cannula is provided for use with an automated fluid delivery system. The cannula generally comprises: a structure including a shaft defining fluid channel; a mechanism for operatively connecting the cannula in fluid flow communication with the automatic fluid delivery system for selectively aspirating and dispensing fluid through the cannula; a primary fluid aspirating and dispensing port; and, at least one auxiliary fluid aspirating and dispensing port. The at least one auxiliary fluid aspirating and dispensing port is arranged, relative to the primary fluid aspirating and dispensing port, such that it is unlikely that both the primary port and the at least one auxiliary port will be

blocked simultaneously with solid material in the region to which fluid is dispensed and from which fluid is aspirated, in use.

A variety of arrangements to accomplish this are described. In one, the primary fluid aspirating and dispensing port is continuous with the at least one auxiliary fluid aspirating and dispensing port. This is provided, for example, by having one of the ports in an end of the cannula with the other connected to (or continuous with) the first port, but extending upwardly along the side of the cannula shaft. In an alternate embodiment, the ports are separated from one another and are geometrically located such that one piece of solid material is not likely to block both at the same time. In yet another embodiment, both the auxiliary port(s) and the primary port(s) are provided by means of a porous frit positioned in an end of the cannula. If the frit is geometrically configured in a non-flat orientation, for example if it is spherical, it is not likely the solid material could simultaneously block all ports through the porous frit.

Also according to the present invention, an automatic fluid delivery system for aspirating and dispensing a fluid from a fluid reaction system associated with a solid material is provided. In general, the automatic fluid delivery system comprises a cannula as described above, in association with the flow arrangement constructed and arranged to dispense reaction fluid through the cannula. The flow arrangement may be, for example a pump system, or the like provided in the automatic fluid delivery system.

Also according to the present invention, a biopolymer purification arrangement or apparatus and method are provided. In general, the biopolymer purification arrangement comprises a polymeric gel shaped to hold a volume of fluid. In use, the biopolymer reaction material to be purified is placed within the gel. Materials will diffuse at different rates through the gel. In the absence of an applied electric field, a large biopolymer such as DNA will tend to remain in the vessel for an extended period of time, and will not diffuse into the gel. Smaller materials, on the other hand, will tend to diffuse into the gel; the result being purification of the large biopolymer left within the receiving bore in the gel.

The invention relates to a solid medium for use in the storage of DNA, particularly DNA in blood samples, and to methods which comprise the use of this solid medium. In particular, the invention relates to a method for storage and

transport of DNA on the solid medium, as well as to methods which involve either (a) the recovery of the DNA from the solid medium or (b) the use of the DNA in situ on the solid medium (for example, DNA sequence amplification by the polymerase chain reaction).

The DNA in blood samples (hereinafter referred to as "blood DNA") is used for the purposes of diagnosis of genetic diseases, diagnosis and monitoring of blood-borne parasitic diseases such as malaria, the determination of paternity, and the monitoring of other unusual cell populations in the blood as can occur in some neoplasias. For these purposes, the term "blood DNA" is used to cover all sources of DNA commonly found in blood, and thus includes the DNA of the human patient from whom the blood sample was obtained, as well as the DNA in any other organisms circulating within his/her blood.

The present invention provides a material and method for the removal of by-products from reactions producing biopolymers that is convenient to use and is amenable for automated sampling and analysis of the biopolymers. It is particularly useful for the removal of by-products from PCR reactions prior to analysis of DNA by capillary electrophoresis in an automated process. It enables the products of multiple DNA amplification reactions to be processed at the same time, if this is required.

The medium is relatively straightforward to produce and can be made in bulk in sizes to suit requirements.

Brief Description of the Drawings

Figure 1 depicts an analysis described with respect to Experiment 3.2(c)(iii)

Figure 2 is a prospective view of a preferred pipette or cannula according to the present invention.

Figure 3 is an enlarged fragmentary cross-sectional view of a portion of the cannula shown in Figure 2.

Figure 4 is an end view of the cannula shown in Figures 2 and 3.

Figure 5 is an enlarged fragmentary cross-sectional view of a cannula according to an alternate embodiment of the present invention.

Figure 6 is an enlarged fragmentary cross-sectional view, generally analogous to Figures 3 and 5, of yet a second alternate embodiment of the present invention.

Figure 7 is a top plan schematic view of a vessel usable in purifications according to the present invention.

Figure 8 is a cross-sectional view of the arrangement shown in Figure 7, taken generally along line 8-8 thereof.

Figure 9 is a schematic view of a system for efficiently processing large quantity of biological samples, according to the present invention.

Detailed Description of the Invention

I. The Solid Medium and Method for DNA Storage

According to the present invention, there is provided a solid medium for storage of biopolymers, for example DNA, including blood DNA, which comprises a solid matrix having a compound or composition which protects against degradation of the biopolymer incorporated into or absorbed on the matrix.

Preferably, the solid matrix comprises a solid support, for example, an absorbent cellulose-based paper (such as filter paper) or a micromesh of synthetic plastics material, with the biopolymer-protecting compound or composition absorbed onto the solid support. Alternatively, the solid matrix may include a suitable binder material so that the matrix is in the form of a compressed tablet or pellet, with the biopolymer-protecting compound or composition incorporated into or absorbed onto the tablet or pellet.

In a preferred embodiment, the present invention provides a method for the storage of DNA, including blood DNA, which comprises applying the DNA to a solid medium. The solid medium of the invention comprises a solid matrix having a compound or composition which protects against degradation of DNA that is incorporated into or adsorbed on the matrix.

In one embodiment of the invention, particularly for long term storage of purified DNA, the DNA-protecting compound or composition comprises uric acid, together with a weak base to convert the uric acid to a urate salt and to provide an alkaline pH between 8.0 and 9.5.

In one particularly preferred aspect of the present invention, there is provided a solid medium for storage of blood DNA, which comprises a solid matrix having incorporated therein or absorbed thereon a composition comprising a weak base, a chelating agent and an anionic surfactant or detergent, and optionally uric acid or a urate salt. Preferably, the composition is such as to impose an

alkaline pH, such as a pH of between 8.0 and 9.5, on blood that is added to the matrix.

A further aspect of the present invention is the long term storage of DNA on the solid medium of this invention, by impregnating the solid medium or encasing the solid medium in a protective material, for example a plastics material such as polystyrene. The polystyrene may be subsequently removed when access to the stored DNA is required.

In practice, in the storage of Blood DNA the blood sample is applied as a blood spot to the solid medium of this aspect of the invention, where the components (more particularly the surfactant) will denature proteins and the majority of any pathogenic organisms in the sample. At the same time, however, the blood DNA will be protected from degradation factors, such as oxidation and ultraviolet (UV) light and processes of the type described above so that the relatively stable, and denatured, dried blood sample can then be transported to a diagnostic laboratory. There, the DNA can be extracted or the DNA can be used in situ on the solid medium.

Preferably, the composition used in this aspect of this invention comprises the following:

(a) a monovalent weak base (such as "Tris", i.e. tris-hydroxymethyl methane, either as the free base or as the carbonate);

(b) a chelating agent (such as EDTA, ethylene diamine tetracetic acid); and

(c) an anionic detergent (such as SDS, sodium dodecyl sulphate); and optionally

(d) uric acid or a urate salt.

By way of example, a particularly preferred solid medium according to this aspect of the invention comprises an absorbent cellulose-based paper such as filter paper having a minimal loading, per sq.cm. of paper, as follows:

(a) EDTA: 0.5 micromols (146.1 mg of free acid)

(b) Tris: 8 micromols (968.8 mg of free base)

(c) SDS: 1 mg; and optionally

(d) uric acid: 2 micromols (336.24 mg of acid).

Although not vital for the short-term storage of DNA on the solid medium, the use of uric acid or a urate salt in accordance with this invention has been found to be particularly desirable for the long term storage of DNA, as this component performs a number of functions. Firstly, it is converted into allantoin in acting as a "free-radical" trap that preferentially accepts free radicals, that would otherwise damage the base guanine in the DNA, (e.g. [2,3]). Such free radicals are generated by the spontaneous oxidation of, for example, thio groups in the denatured serum protein, and may also be generated by the large amount of iron in blood[4]. The uric acid also acts as a component of the buffering system in that it is a weak acid. It also acts as an erodible surface in that it is sparingly soluble so that DNA-containing material dried onto its crystals will be released as the urate beneath them erodes.

As previously described, the composition may include a base, optionally a monovalent weak base, in an appropriate amount to cause an alkaline pH between 8.0 and 9.5 to be imposed upon the blood that is placed upon the matrix. This is to ensure the proper action of the chelating agent in binding divalent metals. It is also to prevent the action of acid nucleases that are not so dependent on divalent metals. The base may be a weak organic base, such as Tris. Alternatively, an inorganic base such as an alkali metal carbonate or bicarbonate, for example sodium, lithium or potassium carbonate or bicarbonate, may be used.

The chelating agent is preferably a strong chelating agent such as EDTA, however a wide range of suitable strong chelating agents are commercially available. The function of the chelating agent is to bind divalent metal ions, magnesium and calcium, and also to bind transition metal ions, particularly iron. Both calcium and magnesium are known to promote DNA degradation by acting as co-factors for enzymes. Metal ions such as iron, that readily undergo oxidation and reduction, also damage nucleic acids by the production of free radicals[4].

The anionic surfactant or detergent is included in the composition of this aspect of the invention as the primary denaturing agent. Any strong anionic detergent that binds to and denatures proteins is suitable, and as well as SDS mentioned above other detergents such as sodium lauryl sarcosinate may also be used. This anionic detergent causes many pathogens to be inactivated due to the non-specific destruction of the secondary structure of their coat proteins, their

internal proteins, and any membranes they may be dependent upon. There are exceptions, since the anionic detergent does not inactivate the most resistant bacterial spores, nor does it inactivate some extremely stable enteric virions. However, these exceptions are agents that are already likely to be transferred by ordinary contact and there is currently no great concern that these agents constitute a risk from blood.

In tests leading to the present invention, it has been demonstrated that blood DNA can be extracted satisfactorily from detergent-treated paper and that uric acid salt protects purified DNA against degradation during storage for more than 60 months. In further tests, DNA on filter paper specially treated in accordance with this invention was purified in situ, then subjected to the polymerase chain reaction (PCR). Details of these tests are set out in the Examples.

II. The Cannula

The cannula component of the present invention provides for sample processing utilizing an automatic fluid delivery system (AFDS) and a fluid reaction system associated with a solid material. In the past, AFDSs were not amenable to use with a fluid reaction system associated with a solid material. The problem hindering such use was due to obstruction of fluid flow into and out of the prior art cannulas or pipettes by the solid material. The cannulas or pipettes of the instant invention are advantageous over prior art pipettes used with AFDSs by permitting fluid flow into and out of the cannula even when the fluid reaction system is associated with a solid material.

Herein, the term "fluid" includes both liquid and gaseous substances, as well as flowable suspensions. A "fluid reaction system" is any system wherein a starting material is transformed into the desired final product through the addition and/or removal of one or more fluid reagents. Typically, a fluid reaction system is conducted in a vessel of a selected size which may be varied according to the requirements of the fluid reaction system. The vessel size and composition may be of any size and material suitable for the process being performed. A suitable vessel composition is one which is non-reactive and impermeable to the reagents of the fluid reaction system. Fluid reaction systems amenable to utilization of the cannulas of the invention include, for example, PCR amplification, ELISA assay,

oligoligase assay (OLA) and ligase chain reaction (LCR). In general, the pipette or cannula is used to direct fluid into, or remove fluid from, the fluid reaction system. In fluid reaction systems having solid material therein, conventional pipettes were undesireable since they would become blocked by the solid material, when the pipette was used to "suck" fluid from the fluid reaction system.

The term "automatic fluid delivery system" as used herein includes hand-held and robotic fluid delivery systems. Typically, automatic fluid delivery systems are devices which dispense and remove fluid reagents to and from individual wells of multi-well reaction plates. Hand-held automatic fluid delivery systems comprise a single plunger handle with multiple fluid aspirating and dispensing ends to simultaneously aspirate and dispense fluid of a fluid reaction system from single or multiple fluid reaction vessels simultaneously. Robotic automatic fluid delivery systems are computer operated rather than hand-held and include such products, for example, as BIOMEK 2000 (Beckman Instruments, Fullerton, CA) and Zymark Benchmate (Zymark, Hopkinton, MA).

According to the invention, the cannula component is of a construction to inhibit obstruction of fluid flow into and out of the cannula even when the fluid reaction system is associated with a solid material. Herein, "associated" or "association with a solid material" means a solid particle which is present in the fluid reaction system such that it is capable of obstructing the aspirating or dispensing port of a cannula and hence inhibit proper functioning of the AFDS. A solid material associated with a fluid reaction may be present in the fluid reaction system to provide a chemical or mechanical function in the fluid reaction system. Examples of solid materials associated with fluids in a fluid reaction system which provide a chemical function include: solid material upon which a biological compound or biopolymer is adsorbed; a solid charcoal absorbent; C_{18} derivatized silca; ion exchange resins; and/or polystyrene divinyl benzene. An example of a solid material associated with a fluid reaction system which can provide a mechanical function is a magnetic stir bar.

The reference numeral **1** of Figure 2 depicts a pipette or cannula according to the present invention. A typical cannula **1** is a cylindrical tube. A useable cannula may, however, be of a geometric shape other than cylindrical. As shown at reference numeral **2** in Figure 6, the cannula includes a shaft or wall **2** defining

internal fluid flow channel **3** (Fig. 3). The cannula has a distal end **4** and a proximal end **5**. The distal end **4** is that end which is inserted into the fluid of a fluid reaction system. The proximal end **5** is operatively connected to the AFDS, in use.

The distal end **4** of the cannula wall **2** comprises at least two orifices. The orifices or "ports" are in fluid flow communication with the fluid channel **3**. The ports are a primary fluid aspirating and dispensing port **6** ("primary port") and at least one auxiliary fluid aspirating and dispensing port ("auxiliary port") **7**. The primary and auxiliary ports **6, 7** may be continuous or discontinuous. By "continuous" it is meant that there is no physical or mechanical separation between the primary port **6** and the auxiliary port **3**. Additionally, the location of the auxiliary port **7** relative to the primary port **6** varies with the particular embodiment of the cannula. Both the primary port **6** and auxiliary port **7** contact the fluid of the fluid reaction system.

The construction of the primary **6** and auxiliary **7** port of a cannula **1** according to the present invention is such that when a negative pressure is exerted by the AFDS, a single solid particle associated with a fluid of the fluid reaction system will not likely obstruct fluid flow into the distal end of the cannula **1**, by simultaneously blocking both ports. To inhibit a solid material from obstructing fluid flow into fluid channel **3**, it is preferred that the cannula **1** be constructed such that the primary port **6** and auxiliary ports **7** are positioned relative to one another in a preferred manner. The position of the ports **6, 7** should be such that at least a portion of one port will likely remain unobstructed by any solid material blocking the other port. According to the invention, the relative configurations and/or locations of the primary and auxiliary ports provide for this. For the embodiment shown in Figures 1 through 3, this is accomplished by positioning port **6** at an end of the cannula **1**, and by positioning port **7** along a side of shaft **2**.

According to the invention, the AFDS is connected to a cannula. The exact mechanism by which the cannula connects to an AFDS will vary with the AFDS used. For example, the connection for a cannula used with a ROSYS 3300, (ROSYS, Wilmington, DE) comprises .5 inch polypropylene tubing. Other methods of coupling include slip fittings and "screw on" connections. In Figure **2**, the coupler is shown at **8**. When functioning with a connected cannula, the AFDS

cyclicly exerts a positive and negative pressure through fluid channel 3. It is typically during the negative pressure phase that the fluid channel of the prior art pipette port opening becomes obstructed. The obstruction typically occurs as a result of the negative pressure drawing the solid material into the source of the negative pressure which, in prior art pipettes, is the pipette port opening in contact with the fluid of the fluid reaction system. Hence, a solid particle which is larger than the pipette port opening covers the pipette port opening and obstructs the negative pressure from effectively removing any remaining fluid.

Various embodiments of the cannula can be used to effect the stated goal. The various relative locations of the primary and auxiliary port are best explained by describing several embodiments of a cannula. In addition to embodiments discussed, it will be apparent that many changes and modifications can be made to the invention without departing from the spirit or scope of the invention.

Regardless of the embodiment of the cannula, it is generally preferred to ensure that an edge of the cannula which may come into contact with the solid material is smooth, i.e. is relatively free of "burrs" or other imperfections which could cause adherence of the solid material to the cannula even in the absence of negative pressure.

According to the invention, cannulas may be manufactured from materials commonly used to make fluid dispensing pipettes and tips which are known in the art. For any given system, however, the material the cannula is composed of should preferably be chemically inert to conditions encountered when contacting a reagent of the fluid reaction system. Materials suitable according to the invention include malleable metals, for example, stainless steel, titanium, aluminum, platinum, gold, nickel and other inert metals. In addition, a cannula may be manufactured out of plastic resins or glass. For a plastic cannula to be used in a fluid reaction system containing phenol, inert, phenol-resistant resins are preferred.

The dimensions for the disclosed cannula are for use with the above-listed robotic systems and the dimensions may be changed as needed for any specific system. The preferred length of the cannula is generally governed by the requirements of the AFDS used. The preferred diameter of the cannula is determined by the requirements of the AFDS and the diameter of the fluid reaction

system vessel. Of course, the cannula should be of a size which can be operably inserted into the vessel of the fluid reaction system, if needed.

In the embodiment of Figures 2 through 4, the ports **6, 7** were discontinuous with one another and were spaced on cannula **1** such that they would not likely be simultaneously blocked by a single particle. In an alternate embodiment of the cannula **14** (Figure 6), the primary port **25** is an orifice at the distal most aspect **26** of the fluid channel **15**. The opening of the primary port **25** is in a plane generally perpendicular to a longitudinal axis **27** of the fluid channel **15**. In this embodiment, the primary port diameter is approximately equal to an inside diameter of the fluid channel **15**, although such is not required. For a typical application involving the ROSYS 3300, the primary port inside diameter is about .010 to .1 mm, preferably .020 to .040 mm. For an application involving the ROSYS 3300 the length of the cannula would be about 6 cm to 20 cm, preferably 15 cm. As used her the term "about" refers to the quantitative dimensions stated and dimensions which are not necessarily quantitatively equal, but which are of a dimension which will effect a similar result.

Referring further to the embodiment of Figure 6, the cannula comprises at least one auxiliary port **24**. The auxiliary port **24** is continuous with (i.e. is connected to) the primary port **25** and is in the shape of an inverted V. The inverted V has an apex **11** and a base **28**. The apex **11** of the inverted V shape of the auxiliary port is up the cannula wall **19** from the base **28** in the direction away from the distal end **26**. For a preferred embodiment utilizing the ROSYS 3300, the width of the base **28** of the inverted V is about .010 to .030 mm, preferably about .020 mm and the height of the inverted V from the base **28** to the apex **11** is about 1.0 mm to 4.0 mm, preferably about 2.0 mm. One or more inverted V's may be placed circumferentially around the circumference of the cannula wall. When multiple inverted V's are used, the profile of the distal end of the cannula will have a "saw tooth" appearance. Therefore, according to this embodiment, a solid material in association with a fluid reaction system preferably does not likely simultaneously obstruct the primary **25** and auxiliary **24** port when a negative pressure is exerted. If, under negative pressure, the solid material adheres to auxiliary ports **24**, the primary port **25** and/or any additional auxiliary port **24** preferably remains unobstructed. If, on the other hand, the negative pressure

causes a solid material to adhere to the primary port **25**, preferably one or more auxiliary ports **24** remain unobstructed.

Another alternate embodiment of the invention is depicted on Figure 5. Here a cannula **35** comprising a primary port **36** at the distal end **37** of the fluid channel **38** is in a plane perpendicular to a longitudinal axis **39** of the fluid channel **38**. The port **36** is then fitted with a porous frit **40** of about 0.20 μm to 0.60 μm porosity, preferably about 0.45 μm. Because of the porosity of frit **40** multiple pores are present. Hence, any single pore of the frit **40** may be considered an auxiliary port to the primary port, which would be other ports in the same frit **40**. Frits suitable according to the invention can be manufactured from such materials as spun glass, stainless steel, titanium and resins. According to this embodiment, a non-flat portion of the spherical frit **40** remains exterior to the distal most aspect wall **41**. Application of a negative pressure, by the AFDS, to the fluid channel **38** in contact with a fluid reaction system containing a solid material will draw the solid against the frit **40**. Because of the non-flat (spherical) geometry of the inserted frit **40**, a solid material drawn against the frit **40** will adhere to the frit **40** tangentially and obstruct fluid flow at the tangential point of adherence. The remainder of the ports of the frit **40**, however, will remain unobstructed thereby allowing fluid to flow into pores of the frit which are not blocked by the adhering solid material. It will be understood that alternate, non-flat, geometries for frit **40** could be used.

The preferred embodiment for cannula construction will be described in detail in Example 5.

III. The Biopolymer Purification Apparatus

The biopolymer purification apparatus component of the present invention provides an apparatus which removes salts and other low molecular weight compounds from biopolymers in a biopolymer reaction mixture. After contacting a biopolymer reaction mixture with the biopolymer purification apparatus, the purified polymer may, for example, be used in in vitro and in vivo biochemical reactions or be subject to analysis by, for example, capillary electrophoresis.

As used herein, the term "biopolymer" means any polymeric molecule utilized in biological systems derived from the same or different monomers (e.g.,

polypeptides, proteins, DNA, RNA, carbohydrates and similar biological molecules). The biopolymers may be chemically synthesized, genetically engineered or be derived from natural products. The phrase "biopolymer reaction mixture" is used herein to define any biopolymer in a solution in combination with salts or other low molecular weight compounds. As used herein, the term "by-products" means compounds which are of a low molecular weight relative to a biopolymer including, for example, salts and/or residual reactants.

The biopolymer purification apparatus will be described with the aid of Figures 7 and 8. (Figure 7 is a top plan view and Figure 8 is a cross-sectional view.) Figures 7 and 8 show the biopolymer purification apparatus as preferably comprising a (rectangular) block **50** comprising a polymeric gel, preferably polyacrylamide. Within the block **50** are multiple wells or depressions **51** in the top surface **52**. The wells **51** are formed into a shape which can hold a volume of fluid. The method for forming the wells **51** includes any method known for shaping polymeric gels (e.g., glass plate castings, injection molding and poured molds). The typical appearance of a biopolymer purification apparatus is similar to that of a multi-well plate. As described below, a biopolymer reaction mixture is placed into a well **51**. After contact with the well for an amount of time, the low molecular weight by-products will diffuse from the well, as indicated by arrows **60**.

The biopolymer purification apparatus is flexible but dimensionally sufficiently stable for use with a robot controlled automatic fluid delivery system to locate and operate reliably on its micro-wells.

According to the invention, a volume of biopolymer reaction mixture is placed into a well **51** of the biopolymer purification apparatus. For a typical application, the size of a typical well **51** of the biopolymer purification apparatus will be about 0.5 mm to 6.0 mm deep, preferably about 4.0 cm deep and about 0.25 cm to 2.0 cm in diameter, preferably about 0.3 cm to 1.0 cm in diameter. After contact of the biopolymer reaction mixture with the biopolymer purification apparatus for a period of time, the by-products of the biopolymer reaction mixture diffuse out of the well **51** and leave the biopolymer relatively free from low molecular weight by-products. The amount of contact time between the biopolymer reaction mixture and the biopolymer purification apparatus will vary depending upon the application, but typically be about 5 to 40 minutes, preferably

10 to 30 minutes. What is required is a sufficient time for an acceptable level of purification to occur. As used herein, the term "about" in this context refers to the quantity of time stated and times which are not necessarily quantitatively equal but of a duration which will yield a similar result.

According to the invention, it is recognized that varying the concentration of the polymeric (polyacrylamide) components of the polymeric gel affects the amount of cross linkage between polymers. The amount of cross linkage directly affects the porosity of the gel. (An increased amount of acrylamide causes an increased amount of cross linking which decreases the porosity and hence, the size of molecule which can readily diffuse out of the well **51** into the remainder of the gel block **50**.) Therefore, by controlling the quantity of cross linkage, it is possible to control the size of molecules which will diffuse out or which will remain within the well. While a preferred embodiment of the invention provides for separation of biopolymers from low molecular weight by-products, the inventors foresee the use of a biopolymer purification apparatus, as described herein, for separation of molecules having a lesser discrepancy in their molecular weight but falling on opposite ends of a given polymeric gel pore size.

Polyacrylamide (a polymeric gel) is commonly used in gel electrophoresis to separate small biopolymer molecules. In gel electrophoresis, the biopolymer diffuses through the medium under the influence of an applied electric field. Hence, polyacrylamide gel is used to electrophoretically separate small DNA molecules by diffusion through the gel. In contrast, here the polyacrylamide gel is used as a medium for biopolymer purification without significant loss of the biopolymer by its diffusion into the gel. No applied electric field is used.

Although no mechanism is asserted, one possible reason for the result discovered in the invention is that in an electric field, as applied in gel electrophoresis, a biopolymer such as DNA is highly orientated and can thus be drawn into the gel to move through it. However, when such a biopolymer is not in an electric field, as in the present invention, it is randomly rotating and will have an effective molecular volume (or diameter) higher than it does in an applied electric field. Thus, the biopolymer is prevented from moving through the gel due to lack of an orientating electric field. Although the gel is a barrier to

biopolymers, it is not a barrier to smaller molecules and ions, which can readily diffuse into the gel material leaving the biopolymer behind (in the vessel).

The purified biopolymer solution can then be recovered from the vessel manually or by an automatic fluid delivery system. The biopolymer may be used in a subsequent biochemical processes or, for example, be analyzed. Production and use of the biopolymer purification apparatus is described in Example 6.

IV. Punch Technology

This aspect of the invention is a system for efficiently processing large quantities of biological samples. Referring to Figure 9, the system will be described with regard to the components (stations) comprising the system.

The system **100** provides for identifying a sample **101**, placing a sample into a reaction vessel for processing **103**, processing a sample; and tracking the sample **102** through processing **103**, permitting correlation of a sample with the corresponding process result. To be of greatest utility the system performs without cross-contamination or carryover between individual samples.

As used herein, the system **100** will be referred to as a Punch Technology System (PTS) or a "system". In its broadest embodiment the system **100** is capable of identifying, tracking and processing any biological sample stored therein. Interactive networking of the identification **101**, tracking **102** and processing **103** components of the invention is performed by the integral system data center (ISDC). The operations performed by the ISDC may be performed by a human being. Preferably, the ISDC is a computer hardware and software system which provides interactive commands to the PTS.

As used herein, the term "identify", "identification" and derivations thereof means the capacity to detect and store, or detect and send information about a sample to another component of the system, for correlation of process results with the appropriate sample. The term "tracking", "track" and derivations thereof, means the capacity to follow and correlate a sample with its processing result. The term "process", and "processing" means any procedure whereby a sample is subjected to reaction, purification, buffering, washing or other steps to transform a starting sample into a desired final product.

In a preferred embodiment, the PTS system has the ability to identify, process and track at least two and typically a large quantity of samples, quickly and efficiently. By "large quantity" it is meant that the system can preferably identify, process and track at least two, preferably at least 2 to 2,000 samples. The ability to obtain results from a large quantity of samples quickly and efficiently makes the system **100** especially suited for large scale screening operations. Such screening has great utility in medical diagnosis and treatment, pathology, biochemical research, drug screening, design molecular biology, forensic science, genetic engineering and other areas where large sample screening is required. Specific areas of use include, for example, antigen/antibody studies, histocompatibility, DNA fingerprinting and genetic diagnostics. In a preferred embodiment, the system will quickly and efficiently compare a single DNA sample, stored on a solid medium as described above, to a large database of DNA profiles also stored on a solid medium. See eg. "UK to set up DNA Database of criminals," 370 Nature 588 (Aug. 25, 1994).

In a preferred embodiment, the system **100** provides for an automated system whereby a stored biological sample, for example blood derived DNA, can be identified, processed and tracked providing a final DNA product which is suitable for PCR amplification or restriction enzyme digestion. A typical method for storage of biological samples processed according to the invention is a biological sample desiccated on a solid medium such as cellulose, nitrocellulose, plastic, glass fiber, anion exchange paper, filter paper, other treated paper, cotton patches or other medium used in the art. A preferred storage media for DNA samples is described in Section I of the present invention.

Figure 9 depicts a schematic of the punch technology system **100** of the present invention. Again, the system comprises the identification station **101**, tracking station **102** and processing station **103**.

Preferably, a sample to be identified, tracked and processed by the PTS is stored on a sample card **104**. Numeral **106** indicates the "spots" of individual samples stored on the sample card **104**. Preferably, the sample card **104** is "marked" with indicia **105** for identification by the identification station **101**.

Prior to application of the system, the sample cards **104** are placed into a "stacker" **107** which orientates the stored cards **108** in such a way as to be

accessible to the identification station **101**. A barrier **109** is placed between individual stored sample cards **108** while in the stacker **107**.

Arrow **110** indicates a path sample card may take when moving into the indentification station **101**. The identification station **101** recognizes the mark **106** of the selected card. The information obtained by the identification station is sent to the tracking station **102** for later correlation with results from the processing station **103**.

Arrow **111** depicts a path an identified sample card may take in moving to the tracking station **102**. The tracking station **102** follows a selected sample **105** removed from a sample card **104** as it moves through the processing station **103**. The tracking station **102** first locates a selected sample **105** on the sample card **104**. Then the tracking station **102** functions to remove the selected sample **105** from the sample card **104**. The selected sample **105** is received by a container in which the sample will be processed by the processing station **103**. The tracking station **102** tracks the location of the selected sample **105** from the time the selected sample card arrives at the tracking station **102** until sample processing is completed. After a selected sample card is removed from a sample card **104**, the sample card **104** preferably moves along a path depicted by arrow **112** to be collected at stacker **114**. A barrier **116** is placed between sample cards in stacker **114**, to prevent direct contact between collected sample cards in the stacker **114**. The sample cards in the stacker **114** can be returned to stacker **107** or some other storage location.

A sample **105** selected from a sample card may move along a path depicted by arrow **120** from the tracking station **102** to the processing station **103**. As described below, the processing station **103** processes a selected sample to achieve the desired final product result. The result for the processing station **103** is then correlated with the selected sample **105** tracked by the tracking station **102** from the selected sample card.

In some applications, the processes conducted at the processing station **103** may be conducted directly on the selected cartridge without removing the sample from the card.

A. The Identification Station

1. The Stored Sample

The PTS provides for identifying, processing and tracking a biological sample which has been stored on a solid medium. A medium particularly suited for the system comprises a solid medium whereby processing of the sample does not require a step of initially removing the sample from its constituent medium.

According to the invention, a suitable medium for use with a stored sample of DNA, for example, is the solid matrix described in Section I of the present invention. In the past, a DNA sample first had to be removed from a storage medium before PCR amplification and/or restriction enzyme digestion could be performed. While a number of methods for removing DNA from a solid medium are currently in use, many of these methods require extensive sample manipulation including: boiling, multiple pipetting and centrifugation steps, desalting chromatography, precipitation, membrane dialysis and other methods known in the art. Utilization of the solid medium described in Section I of the present invention of the provides a stored sample suitable for PCR amplification and/or restriction enzyme digestion without the need for first removing the sample from the solid matrix. Hence, the matrix of Section I of the present invention permits automated processing of a DNA sample without the problems associated with other sample storage systems.

In a preferred embodiment of the system, a sample is stored on a solid medium or "card" **104**. A card **104** may be of any size sufficient to hold a minimum of one drop of blood or other biological sample. A typical card would be of a rectangular shape. The dimensions of a typical card would be about 1 to 25 cm, preferably about 5 to 15 cm in length by about 1 to 20 cm, preferably about 5 to 10 cm. Cards of different geometrical shape and size may also be suitable in alternative applications of the invention.

The solid medium or card **104** is preferably of a constituency for storage of the sample without alteration of the starting sample. Preferably a biological sample **105**, for example DNA, from blood, tissue cultures, bacterial cultures or other liquid medium is placed on the solid medium **104**. Herein, the phrase "liquid medium" means a sample wherein the biological sample to be stored

on the solid medium is suspended or solubilized. In general, the liquid will evaporate from the solid medium leaving the DNA or other suspended or solubilized molecule adsorbed to the solid medium. A single biological sample or multiple biological samples in a liquid medium is/are placed on the card in an arrangement suitable for sample location by a card locator mechanism (described supra). The card is marked **106**, either before or after receiving the sample, for later recognition by the identifying mechanism (described in Section IV,A,Z). After the liquid medium contained sample has been placed on the card, the liquid medium evaporates and the card containing the desiccated sample is stored by suitable means known in the art, for example, file storage, polystyrene coated, -25° C and other methods which preserve a sample on a solid medium. A preferred method for storage of DNA, for example, is described in Section I.

2. **Identifying Mechanism**

The identification station of the PTS provides for the system to recognize and recall a sample card. Each card may contain at least one, or more, of the same or a different biological sample. Preferably, a single card contains one or more of only a single biological sample. Preferably, each sample card has a mark which is identified by the identification station of the system. The mark allows for recognition and recall of the biological sample makeup of a sample card. Recall of the biological sample makeup of the card may be made by the identifying mechanism at the time of identification. Alternatively, the identifying mechanism may send the identification information to the "card sample locator" (CSL) mechanism (described infra.) for recall of the biological sample makeup of a card.

To effect sample identification the identification station utilizes a "stacker" and an "identifying mechanism". The stacker provides a means for holding the cards prior to being identified by the identifying mechanism. A stacker may comprise a rack or cassette as used, for example in paper copying machines or other systems which hold paper or other "sheetlike" items. The stacker can take any of a variety of shapes provided it is of size and shape capable of holding the cards in an orientation permitting the identifying mechanism to identify a card.

The cards are placed in the stacker in such a way as to prevent crossover contamination between cards or samples. Prevention of crossover contamination may be effected by placing a barrier between each card, preferably a non clinging barrier such as cellulose paper, wax paper, plastic and other barriers known in the art. The barrier is removed from between the cards before or after identification depending on the location of the identifying mechanism.

The identifying mechanism of the system provides means for identifying the cards and the samples contained thereon. Specifically, the card contains a "mark." As used herein, "mark" means a code or other means for the identifying mechanism to recognize the card. In a preferred embodiment, the cards may contain a bar code and the identifying mechanism is a bar code reader as used in grocery store check out lines, DNA repositories, bar code scanners on gas chromatograph/mass spectrometry autosamplers and high pressure liquid chromatography (HPLC) autosamplers. Alternatively, the cards may be identified by machine readable numbers or by other methods.

The information identified by the identifying mechanism is then sent to the tracking station to track a sample on the sample card identified by the identifying mechanism for later correlation with the process results.

Once the sample card has been identified by the identifying mechanism, the tracking station is activated. A sample card may be identified by the identifying mechanism while in the stacker and subsequently moved to the tracking station. Alternately, a sample card may first be moved from the stacker across an identifying mechanism, identified and then moved to the tracking station.

Proper card identification at this point is necessary for effective functioning of the sample tracking component.

B. Tracking Station

The tracking station of the system provides for tracking of an individual sample from a time after the sample card is identified by the identifying station until completion of the final step of the process being performed by the processing station. The ability of the system to track an individual sample through an entire process assures the accuracy of the system in correlating the proper sample with the appropriate result when processing a large quantity of samples.

The sample tracking station has three main functions. It should first locate a sample on a card (possibly containing multiple samples). The tracking station then removes the located (selected) sample from the sample card for processing. (If the sample is removed for processing.) Finally, the tracking station maintains recall of the location of a selected sample as it moves through the processing station of the system. The three functions are termed the "card sample locator" (CSL) mechanism, the "punch mechanism" (PM) and the "receiving container sample locator" (RCSL) mechanism respectively.

After a sample card has been identified by the identifying station it moves to the tracking station. Once at the tracking station, the card preferably comes into contact with the card sample locator (CSL) mechanism. The CSL mechanism comprises a device capable of locating a spot on a sample card containing a single sample. Preferably, information permitting the CSL mechanism to locate which sample on the card is to be processed comes from the ISDC. CSL mechanisms suitable for use according to the method of the invention include, for example, devices such as laser indexing systems, mechanical indexing systems, and optical density systems. Laser and mechanical indexing systems are used, for example, on photocopiers and facsimile machines.

Once the desired sample is located on the card, the punch mechanism is activated to remove the sample from the sample card into a receiving container. According to the invention, a punch mechanism comprises a blade, cutting laser, solid punch and other devices capable of cutting through the solid medium containing the sample. The removed sample is then placed into a receiving container. The size of the card sample which is removed from the card is obviously of a size which fits into the receiving container.

A preferred embodiment of the punch mechanism comprises a circular blade. The appearance of the blade is similar to a skin biopsy punch or a cork bore. Typically, such blades are composed of stainless steel, titanium or other materials commonly used to make paper cutting blades. The diameter of the solid medium punched is about 2 mm to 10 mm, preferably about 4 mm in diameter. When using a blade which directly contacts the sample card (vs. for example, a laser puncher) containing a sample, the surface of the blade which has come in contact with the card is preferably heat sterilized to minimize cross contamination

of samples. Heat sterilization my accomplished using a propane flame or heating coil at a temperature of about 250 to 300°C, preferably about 275°C.

As stated above, the sample removed by the punch mechanism is placed into a receiving container. As used herein, the term "receiving container" includes a test tube, a single well of a microtiter plate, eppendorf tube, PCR tube and other reaction container used routinely by those skilled in the art to process biological samples.

The receiving container into which the removed sample is placed is detected by the "receiving container sample locator" (RCSL) mechanism. The RCSL may send the information of the location of a selected sample to the ISDC which may function to track the sample throughout the processing by denoting location and progress throughout the processing station.

After a sample is removed from a sample card the card is restacked in a second stacker or cassette. The cards are placed in the stacker in such a way as to prevent crossover contamination between cards or samples. As described above, prevention of crossover contamination may be effected by placing a barrier between each card. The receiving container which contains a biological sample is then moved to the sample processing station.

C. **Sample Processing Station**

The sample processing station of the PTS provides for purification, buffering, washing, PCR amplification or other processes necessary to transform a sample into the processed final product. After a sample has been placed into a receiving container, the sample may be treated in situ. Alternatively, the sample to be processed may first be removed from the solid media. The process that the processing station applies varies with the type of sample and desired treatment goal.

The processing station of the PTS may comprise an automated fluid delivery system with robotic mechanisms for moving the reaction container and for aspirating and delivering reaction fluids during sample processing. The use of automatic fluid delivery systems in the processing station is preferably performed utilizing the cannula system disclosed in Section II of this invention.

For some samples the processing station of the PTS may first subject a sample to a process for removing the sample from the solid medium (i.e. punched sample card). In this situation, the steps of the process would involve treating the sample with the appropriate reactants needed to desorb the stored sample from the solid medium. The sequential steps for desorbing a sample from its storage medium are known in the art.

Alternatively, the sample may remain adsorbed to the solid medium throughout the entire reaction process. For example, DNA adsorbed to a solid medium, as described earlier, may ultimately be amplified by PCR or digested by restriction enzymes in the same receiving container. In performing processes of this type the DNA is preferably rendered free of proteins and other impurities contained in the adsorbed sample. After the sample is punched and placed into a receiving container the processing station may render the DNA free of protein and other impurities using the protocol described in Example 3 and 4.

To perform PCR amplification of the purified DNA the sample must be subjected to PCR processing as described in Example 3 and 4. To perform this procedure the processing station is preferably equipped with four independently controlled heat/cold zones. Of these, one zone is preferably at about 4°C, a second zone is preferably at 54°C, a third zone is preferably at about 96°C and a fourth zone is preferably at about 37°C.

DNA amplification utilizing PCR technology is sensitive to cross contamination. Herein, "cross contamination" means contamination of one sample by another. Alternately, "cross contamination" means contamination of one reagent by another. Hence, to minimize occurrence of such contamination, the pipette tip used for aspirating and dispensing fluids during PCR amplification are preferably used for only a single reagent and only a single sample. Such prevention of cross contamination may be accomplished using automatic fluid delivery systems. Preferably, to accomplish this result, pipette tips analogous to automatic fluid delivery system cannulas described earlier in the invention may be produced for single application use. According to this aspect of the invention, after a single use of a pipette tip, the tip is disconnected from the automatic fluid delivery system. A new pipette tip is then placed on the automatic fluid delivery system before the AFDS moves to the next sample or reagent. Once installed, the

new pipette tip is used to dispense a single fluid and the process for pipette tip replacement is repeated.

After completion of processing, the processing results of a selected sample are correlated with the sample previously recognized by the identification station.

While the invention is susceptible of various modifications and alternative forms, specific embodiments of the invention will hereinafter be described in detail and shown by way of example. It should be understood, however, that the examples are not intended to limit the invention to the particular forms disclosed, but, on the contrary, the invention is to cover all modifications and alternatives falling in the spirit and scope of the invention as expressed in the appended claims.

Examples

EXAMPLE 1. Collection and Extraction of DNA:

Sodium dodecyl sulphate was applied to Whatman 3mm paper in a solution such that there was approximately 50μl per sq.cm. of a solution of 2% sodium dodecyl sulphate, 10mM EDTA, and 60mM tris (free base), i.e. approximately 1 mg of sodium dodecyl sulphate per sq.cm. The paper was then dried.

The treated paper was soaked with drops of blood from various primates. The blood-stained paper was dried, sent through the ordinary mail so that it spent at least three days in the mail, and then had the DNA extracted from it using standard procedures involving detergent-aided proteolysis and phenol extraction of the paper. The resultant DNA was then tested for its quality by being digested with restriction endonucleases and the fragments analyzed by agarose gel electrophoresis.

The DNA fragments were found to be as high in quality as DNA produced from fresh blood. This demonstrates that the DNA can be extracted from detergent-treated papers and that the DNA is of sufficient quality for most normal purposes.

EXAMPLE 2. Long-Term Storage of Semi-Purified or Purified DNA:

Storage of DNA, such as plasmids or other viral replicating forms, has been carried out using record cards made of absorbent paper previously soaked in a

solution of uric acid and tris (free base). The cards are subsequently plasticised for further protection. This procedure has been established for the long-term storage of clones from massed dot-blots when it is possible that the original material is required at some much later date, and when a great many such massed clonings are to be kept in orderly, low-volume, files. Samples have been stored successfully this way for about four years.

2.1 Preparation of DNA Record Cards

Record cards can be prepared in batches and stored until needed. Whatman No.1 paper about 10cm x 15cm in size and with appropriate places marked out with an "indian ink" (i.e. colloidal carbon ink-stamp) is suitable, and any special notes on the cards can be made with an ordinary "lead" pencil (i.e. Graphite pencil). Preferably, the cards are marked out in a regular pattern to assist in systematic storage and retrieval of DNA samples.

Marked cards are wrapped in clean paper, then foil, and autoclaved with a dry cycle. They are then treated with a solution of 40mM uric acid and 100mm tris (free base). The function of the urate is to protect the DNA from aging and to aid the desorption from the paper if required. These treated record cards can then be kept until required.

2.2 DNA Samples and Their Application.

DNA to be stored is taken up in a dilute alkaline buffer containing EDTA, e.g. TE buffer (Tris-EDTA pH 8.0). By way of example, approx 1 ml of bacterial culture containing plasmid, is treated by the alkaline lysis method, with one phenol extraction and one alcohol precipitation, to get approx 50μl of plasmid or other DNA in TE buffer. A 5μl aliquot of each DNA sample is used to make a spot on the urate treated record card.

2.3 Impregnation of DNA-Loaded Cards with Plastic.

Once a card is fully loaded with DNA samples, the DNA spots are thoroughly air-dried, then the card is dipped in 5ml of 12% w/v polystyrene in acid-free chloroform. This is preferably achieved by putting the card in a fitting polyethylene baglet and then pouring the polystyrene solution into it, spreading the

polystyrene solution to thoroughly coat the card and then stripping off the soiled polyethylene. The card is then allowed to dry at room temperature.

2.4 Storage Conditions.

The cards are conveniently stored in a sealed container in a refrigerator freezer (about -15°C) in the presence of drying agent such as silica gel and a few grains of dry sodium carbonate to remove any traces of acid vapours.

2.5 Using DNA on Plasticised Cards.

The storage container is allowed to rise to room temperature in order to minimize unnecessary wetting and drying cycles on long-term storage cards. The appropriate card is abstracted, the relevant DNA spot identified and a small sample of it cut out.

Since the 5μl spots are laid out in a regular fashion, it is quite practical to cut them across with a scalpel blade to remove a portion of the sample, e.g. one-quarter of a spot. This is then placed in a disposable plastic tube with approximately 5 ml of acid- free chloroform, capped and rotated on a blood rotator at room temperature for at least 30 minutes. This removes the protective polystyrene and effectively sterilizes the sample also. DNA may then be eluted from the sample or the sample can be treated and used in situ, for example in a DNA sequence amplification reaction (PCR) (see later).

In an example, the desorption of DNA samples, both single and double stranded, from Whatman No.1 paper soaked with a solution of 40mM uric acid, and 100mM tris (free base) was examined by using the plasmid pUC19 as a source of standard double stranded DNA and M13 as a source of single stranded DNA.

In both cases, samples of the DNA were dried onto paper from solutions in TE buffer and the paper was then sheathed in protective polystyrene, as described above. The paper was later chloroform extracted to remove the protective layers, the DNA extracted with fresh TE buffer and the efficiency of extraction estimated by observing the transformation of the E.coli strain JM101 by the extracted DNA. This procedure was carried out with both untreated paper and paper that had been pre-soaked and dried with the above urate solution. Much less than 10% of

transformationally active DNA was successfully recovered from the untreated paper, whereas the treated paper gave recoveries of approximately 100%.

Further DNA samples which had been applied to treated paper and sheathed as previously described, were tested after 36 months storage in a dry atmosphere at -15°C. The samples again had the plastic stripped off with chloroform, the DNA (only pUC19) recovered by simple extraction with TE buffer, and the DNA activity tested as previously described. Again the activity of the DNA was high, compatible with virtually no loss of activity.

EXAMPLE 3. In situ Use of Stored Blood DNA in PCR.

Blood DNA stored on filter paper treated in accordance with the present invention can be amplified in situ by the polymerase chain reaction (PCR) technique. The treated paper used in this Example was Whatman 3 mm paper treated with a solution comprising, per sq.cm of paper, 2 micromols uric acid, 8 micromols tris (free base), 0.5 micromols EDTA and 1 mg SDS. The stored blood DNA was treated to remove protein, then washed to remove phenol and add suitable ions, prior to DNA amplification.

3.1 Solutions.

Solution A:

One-phase Phenol solution. A suitable mixture is phenol, 50 gm containing 120 mg of 8-hydroxyquinoline that has been saturated with 10 ml of 1.0 M tris-acetate pH 8.0 and 1.0 ml 2-mercaptoethanol. After saturation by shaking at room temperature, the aqueous phase is thoroughly removed and discarded.

Solution B:

75% v/v Isopropanol, 25% v/v 0.1M potassium acetate at pH 7.8.

Solution C:

75% v/v Isopropanol, 25% v/v 0.01M magnesium acetate.

(Note that other alcohols or similar water-miscible solvents, e.g. n-propanol, may be substituted for isopropanol in these solutions.)

3.2 Method.

All steps are preferably carried out in a single tube made of a suitable phenol resistant material, e.g. polyethylene.

(a) Removal of Protein: a 0.5cm x 0.5cm square of blood DNA impregnated paper is treated with 1 ml of solution A, for example for approximately 1.5 hours at 45°C (this temperature and time is not critical). The dirty solution A is then aspirated to waste and the paper square quickly washed three times with 0.25 ml of more solution A. Each wash need be only a few seconds long and is immediately aspirated to waste.

(b) Removal of phenol and addition of suitable ions: the paper in its tube from step (a) above, is rapidly washed in three lots of 1 ml of solution B. Washes are at room temperature and are simple additions followed by aspiration to waste. The paper is then washed for 20 minutes at room temperature with solution C. (This is to saturate the DNA on the paper with Magnesium ions and remove the last of the phenol.) The solution C is aspirated to waste and the paper is solvent-dried with one wash of pure isopropanol and then vacuum dried.

The final DNA-paper should be quite white without any obvious remnants of the red-brown colour of blood. It is now ready for use in a PCR reaction mix.

(c) Amplification of DNA on treated paper:

The treated DNA-paper as described above has been shown to be a suitable substrate for DNA polymerase chain reaction (PCR) amplification of DNA.

(i) Specimens

Extracted DNA: DNA from 10 ml of blood obtained from a male volunteer was extracted by standard protocols.

Treated DNA Filter Paper: Blood specimens from the same volunteer were applied directly to treated filter paper with subsequent treatment as described above. The paper was cut into about 1mm^2 pieces for use in PCR reactions.

(ii) Targets for Amplification.

Target No. 1: Region of exon 2 of the n-Ras proto-oncogene on chromosome 1. The primers used are:

R1: 5' TGA CTG AGT ACA AAC TGG TGG TG 3' and

R2: 5' CTC TAT GGT GGG ATC ATA TTC A 3'.

The amplified DNA fragment obtained with these primers is 110bp in size.

Target No. 2: A male specific Y chromosome repeat sequence. The primers are:

007: 5' TGG GCT GGA ATG GAA AGG AAT GCA AAC 3' and

008: 5' TCC ATT CGA TTC CAT TTT TTT CGA GAA 3'.

The amplified DNA fragment obtained with these primers is 124 bp in size.

Target No.3: A male specific Y chromosome repeat sequence. The primers used are:

004: 5' GAA TGT ATT AGA ATG TAA TGA ACT TTA 3' and

006: 5' TTC CAT TCC ATT CCA TTC CTT TCC TTT 3'.

The amplified DNA fragment obtained with these primers is 250 bp in size.

(iii) PCR Protocol

Extracted DNA (1 μg) or about 1mm^2 fragments of treated DNA filter paper were placed into 0.5ml Eppendorf tubes and made to 25 μl in PCR reaction mixture consisting of:

67 mM Tris HCl (pH 8.8 @ 25°C)

16.6 mM ammonium sulfate

2 mM $MgCl_2$

0.01% (w/v) gelatin

0.1 mM deoxynucleotides (dATP, dTTP, dCTP, dGTP)

0.25 μg of each primer (for respective target)

0.25 U of Taq DNA polymerase.

The mixture was overlaid with 25 μl of light mineral oil and DNA amplification was performed by 30 cycles of amplification on a Perkin Elmer-Cetus "thermal cycler".

The first cycle consisted of:

DNA denaturation 6 min. @ 94°C

Primer-DNA annealing 1 min. @ 55°C

Taq DNA polymerase extension 1 min. @ 75°C

followed by 29 cycles as above except DNA denaturation was for 1 min @ 94°C and the extension time on the last cycle was 10 min @ 72°C before cooling of reaction mixture to 4°C.

After amplification, 10 μl aliquots of PCR mixture were analyzed by electrophoresis on 15% (w/v) polyacrylamide gels. Amplified target DNA was visualized by UV illumination of the ethidium bromide stained gel. An analysis of products from PCR of extracted DNA and treated DNA filter papers as shown in Figure 1, as follows:

Lanes 1-3: target No.1, lane 1: 1 μg DNA, lane 2: 1mm^2 filter, lane 3: no DNA control;

Lanes 4-5: target No.2, lane 4: 1 μg DNA, lane 5: 1 mm^2 filter; Lane 6: no DNA control;

Lanes 7-9: target No.3, lane 7: 1 μg DNA, lane 8: 1 mm^2 filter, lane 9: no DNA control; lane S: DNA size markers (pUC19/HpaII digest).

The results shown clearly demonstrate that the DNA has not been changed in any way as a result of its storage on filter paper in accordance with this invention, and that it can be used in situ as described herein.

EXAMPLE 4: Methods and Materials.

Additional blood samples were collected and stored. Blood proteins were extracted from the treated paper by a procedure using Solution A as in Example 3. Blood proteins were also extracted from samples using two procedures involving a modified Solution A (Solution A4 and Solution A7). Extractions were carried out on samples made with a 0.5 x 0.5 mm leak punch (B.Y.T. Co., Baycity, MI) and placed in 1.7 μl Eppendorf tubes.

The protein-free adsorbed DNA was then amplified using PCR methodology.

4.1 Sample Collection and Storage

All blood samples were stored on Watman No. 1 filter paper treated with sodium dodocyl sulfate, 10mM EDTA, and 60mM Tris (free Base). The paper was then dried and treated with 40mM Uric Acid and 100mM Tris. (Urate aids in protecting the DNA from aging and desorption from the paper (Flinders Technology, Australia). The treated filter paper was then impregnated with blood supplied from laboratory personnel and stored at 4°C in foil.

4.2 Extraction Procedures

Procedure 1: Add 500 μl to each tube *Solution A* (50ml of Ultra pure liqu Phenol, 10ml 1M TE buffer, 120mg 8-Hydroxyquinoline, 1ml 2-β Mercaptoethanol. Allow to sit 15 minutes to let aqueous and organic layer to separate. Remove aqueous layer. Incubate 1.5 hours at 55°C. Wash 1X with Solution A. Wash 3X with Solution B (75% v/v Isopropanol, 25% v/v Potassium Acetate at pH 7.8). Incubate at room temperature for 20 minutes with Solution C (75% v/v Isopropanol, 25% v/v Magnesium Acetate). Solvent dry paper with pure Isopropanol then Vacuum dry 10 minutes. Paper should be white and ready for PCR.

Procedure 2: Add 500 μl *to each tube Solution A4* (50ml Phenol/chloroform/Isoamyl, 10 ml 1M TE buffer, 120 mg 8- Hydroxyquinoline, no 2-β mercaptoethanol was added. Allow to sit 15 minutes, remove top layer). Incubate 1.5 hours at 55°C. Wash 1X with Solution A4. Wash 3X with Solution B (75% v/v Isopropanol, 25% v/v Potassium Acetate at pH 7.8). Incubate at room temperature for 20 minut vith Solution C (75% v/v Isopropanol, 25% v/v Magnesium Acetate). So nt dry paper with pu Isopropanol then Vacuum dry 10 minutes. Paper should be white and ready for PCR.

Procedure 3: Add 500 μl to each tube *Solution A7* (a suitable digestion buffer such as 10mM Tris, 5 mM EDTA, 0.5%SDS at pH 7.8 plus 3 μl Proteinase K). Incubate at 55°C for 1 hour. Deactivate enzyme at 95°C for 10 minutes. Incubate 1.5 hours at 55°C. Wash 1X with Solution A7. Wash 3X with Solution

B (75% v/v Isopropanol, 25% v/v Potassium Acetate at pH 7.8). Incubate at room temperature for 20 minutes with Solution C (75% v/v Isopropanol, 25% v/v Magnesium Acetate). Solvent dry paper with pure Isopropanol then Vacuum dry 10 minutes. Paper should be white and ready for PCR.

4.3 PCR Amplification

All reactions carried out in Perkin Elmer 9600 Thermocycler. To ensure Quality assurance all reactions are run with Positive and Negative control.

Amplitype ® HLA DQα (Perkin Elmer, Foster City, CA) 25μl Master Mix + 25μl 8 mM $MgCl_2$ + paper overlay with 40μl Mineral Oil in .5 μl Gene Amp Thin-walled tubes. Thermocycler parameters are: 94°C/30 sec, 94°C/10 sec, 60°C/10 sec, 72°C/10 sec (32X), 72°C/10 min, followed by 4°C soak.

Amplitype™ PM (Perkin Elmer, Foster City, CA) 40μl Master Mix + 40μl 6mM $MgCl_2$ + paper in Micro-Amp tubes. Thermocycler parameters are: 95°C/1 min, 95°C/30 sec, 63°C/30 sec, 72°C/30 sec (32X), 72°C/10 min, followed by 4°C soak. DQα and Amplitype™ PM were typed using protocol outlined by Perkin Elmer.

Gene Print™ STR system (TH01) (Promega, Madison, WI.) Amplification was done according to the Promega protocol-revised 9/93.

EXAMPLE 5 **Preparation of a Cannula.**

This example is for preparation of a preferred embodiment of a cannula according to the invention as depicted on Figures 2 and 3. A standard cannula for use with a ROSYS 3300 (ROSYS, Wilmington, Delaware) was obtained from ROSYS (Wilmington, Delaware). The length of the cannula is 15 cm. The inside diameter of the primary port **6** is .040 mm.

At a distance of 2 mm proximal from the primary port **6**, up the wall of the cannula, two auxiliary ports **7** of .020 mm in diameter were drilled at about 180° apart from each other using a titanium drill bit. The edges of the primary and axillary port **6, 7** were smoothed using a file.

The completed cannula was coupled to a ROSYS 3300 (ROSYS, Wilmington, Delaware) automatic fluid delivery system. Once connected, the automatic fluid delivery system with the cannula disclosed according to the invention was put to use for aspirating and dispensing fluids from a fluid reaction system associated with a solid. The solid was a small sample of the solid storage medium containing DNA as described in Example 1.

EXAMPLE 6. Preparation of a Biopolymer Purification Apparatus.

This example is for preparation of a standard poly-acrylamide gel to be used to form a biopolymer purification apparatus. Other hydrophilic polymers having similar properties can be used.

The polymer was prepared as follows:

12 ml water
2 g acrylamide
40 mg N,N'-methylene-bis-acrylamide
0.2 ml tris EDTA (500mm, pH 8.0)
12 ul TEMED
7 mg ammonium persulphate

The polymer reaction mixture was cast into rectangular blocks of a convenient size (e.g., 8.0 mm deep by 50 mm long and 30 mm wide) with regularly spaced small raised dimples (which create the micro-wells) in its bottom surface. The individual wells were about 4.0 mm deep and 4.0 mm in diameter. The poly-acrylamide is allowed to set in the mould about 30 minutes and then placed in distilled water overnight. The final product is approximately 14% water soluble plastic, but after desiccation the apparatus is about 12.2% dry weight of polymer.

For storage and distribution purposes, the apparatus may be sealed in plastic bags made from polyethylene, for example. The material of the apparatus has a very long shelf life stored with minimal care but preferably 25°C or below and in the dark.

Method for Using the Biopolymer Purification Apparatus

Typically, DNA reaction mixtures from DNA amplification by PCR are placed into separate micro-wells of the apparatus at room temperature, either manually or automatically by a cannula under robotic control.

After sufficient time for the level of contamination to be reduced (e.g., 2 to 30 minutes), the solutions of DNA in the micro-wells can be sampled either manually or by a robotic cannula and the DNA isolated or subjected to analysis by, for example, capillary electrophoresis.

The material of the apparatus can be cleaned for re-use by washing with distilled water overnight, however, the material is relatively cheap to produce and normally is discarded after use to avoid any possibility of contamination of subsequent samples.

It will be readily apparent to those skilled in the art that the above solid-state steps for purification and amplification of blood DNA are particularly suited for performance under automated conditions.

REFERENCES:

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2. Ames, et.al. (1981). Proc. Natl. Acad. Sci. (USA). 78 : 6858-6862.

3. Kwok Lai Lam, Fong, D., Lee, A. and Ken Ming D.Lui. (1984), J. Inorg. Biochem. 22 : 241-248.

4. Singer, B. and Fraenkel-Conrat, H. (1965). Biochemistry 4 : 226-223.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Flinders Technologies Pty Ltd.
(B) STREET: The Flinders University of South Australia
(C) CITY: Bedford Park
(D) STATE: South Australia
(E) COUNTRY: AU
(F) POSTAL CODE (ZIP):

(ii) TITLE OF INVENTION: Apparatus for Storage, Purification or Reaction of a Biopolymer and Method

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/320,041
(B) FILING DATE: 07-OCT-1994
(C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/159,104
(B) FILING DATE: 30-NOV-1993

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGACTGAGTA CAAACTGGTG GTG 23

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
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(ii) MOLECULE TYPE: DNA (genomic)

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(2) INFORMATION FOR SEQ ID NO:4:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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(2) INFORMATION FOR SEQ ID NO:5:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATGTATTA GAATGTAATG AACTTTA 27

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTCCATTCCA TTCCATTCCT TTCCTTT 27

CLAIMS:

1. A system for identifying, tracking and processing a biological sample stored on a solid medium, said system comprising:

(a) a solid medium having a biological sample stored thereon;

(b) an identification station which recognizes and recalls the biological sample stored on said solid medium;

(c) a processing station which processes said biological sample stored on said solid medium;

(d) a tracking station which tracks a biological sample stored on said solid medium from said identifying mechanism through said processing station; and

(e) an integral system data center, which provides interactive networking of said identification, tracking and processing stations.

2. The system according to claim 1 wherein said integral system data center is performed by a human being.

3. The system according to claim 1 wherein said integral system data center is a computer hardware and software system.

4. The system according to claim 1 wherein said integral system data center is performed by a human being and a computer hardware and software system.

5. The system according to claim 1 wherein said biological sample stored on said solid medium is a sample of DNA.

6. The system according to claim 1 wherein said solid medium for storage of said biological sample comprises a solid matrix having adsorbed or incorporated thereto an effective amount of a composition which prevents degradation of DNA.

7. The system according to claim 6 wherein said composition which prevents degradation of DNA comprises a protein denaturing agent.

8. The composition according to claim 6 further comprising a free radical trap.

9. The system according to claim 5 wherein DNA in said sample of DNA stored on said solid medium is purified in said processing station.

10. The system according to claim 1 wherein said identification station and tracking station is performed manually by a human being.

11. The system according to claim 1 wherein said processing station is a robotic automatic fluid delivery system.

12. The robotic automatic fluid delivery system according to claim 11 comprising:

(a) a flow arrangement constructed and arranged to aspirate and dispense a reaction fluid;

(b) a cannula operatively positioned in fluid flow communication with said flow arrangement; said cannula comprising:

(i) a wall defining a fluid channel;

(ii) a mechanism to operatively connect said cannula in fluid flow communication with said robotic automatic fluid dispensing system;

(iii) a primary fluid aspirating and dispensing port;

(iv) at least one auxiliary fluid aspirating and dispensing port, wherein said cannula primary and auxiliary fluid aspirating and dispensing ports are constructed and arranged with respect to one another for inhibition of likely blockage of fluid flow into or out of said fluid channel of said cannula when the robotic automatic fluid delivery system is aspirating and dispensing a fluid from a fluid reaction system.

13. A cannula for use with an automatic fluid delivery system for selectively aspirating and dispensing fluid; said cannula comprising:

(a) a wall defining a fluid channel;

(b) a mechanism to operatively connect said cannula in fluid flow communication with the automatic fluid delivery system;

(c) a primary fluid aspirating and dispensing port;

(d) at least one auxiliary fluid aspirating and dispensing port, wherein said cannula primary and auxiliary fluid aspirating and dispensing ports are constructed and arranged with respect to one another for inhibition of likely blockage of fluid flow into or out of said fluid channel of said cannula when said automatic fluid delivery system is aspirating and dispensing a fluid from a fluid reaction system.

14. The cannula according to claim 13 wherein said primary fluid aspirating and dispensing port is spaced at least about 2 mm from said at least one auxiliary fluid aspirating and dispensing port.

15. A cannula according to claim 13 wherein said primary fluid aspirating and dispensing port is continuous with said at least one auxiliary distal fluid aspirating and dispensing port.

16. A cannula according to claim 13 wherein said primary fluid aspirating and dispensing port and said at least one auxiliary fluid aspirating and dispensing port are components of a non-flat porous frit.

17. A cannula according to claim 13 including:

(a) an elongate shaft having a distal end;

(i) said primary fluid aspirating and dispensing port comprising an aperture in said distal end of said shaft; and,

(ii) said at least one auxiliary port being spaced from said distal end and said primary port, along said shaft.

18. A method for utilizing a polymeric gel to remove a by-product from a biopolymer in a biopolymer reaction mixture, said method comprising:

(a) providing a polymeric gel shaped to hold a volume of fluid;

(b) placing a volume of biopolymer reaction mixture to said shaped polymeric gel;

(c) contacting the biopolymer reaction mixture with said shaped polymeric gel for a period time sufficient for some diffusion of material from the biopolymer reaction mixture to occur, in the absence of an applied electric field; and,

(d) removing said biopolymer from the shaped polymeric gel.

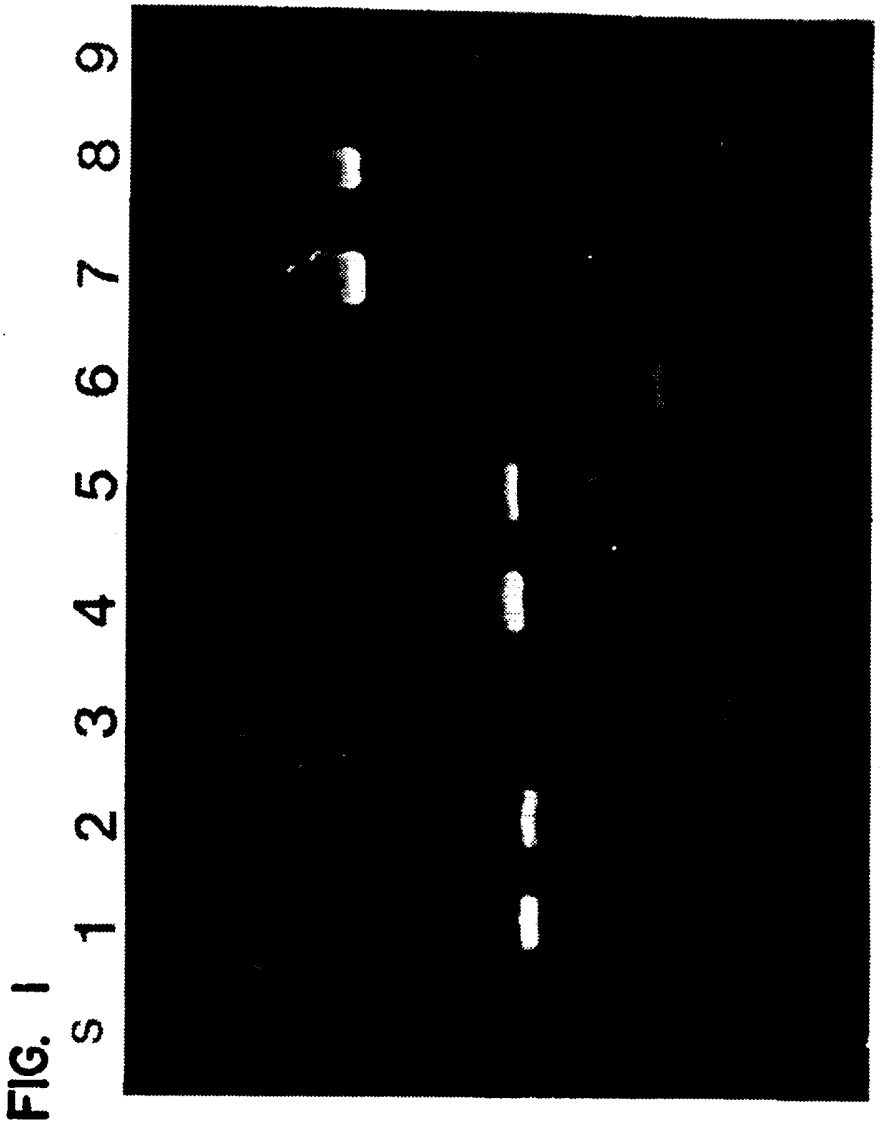


FIG. 1

FIG. 2

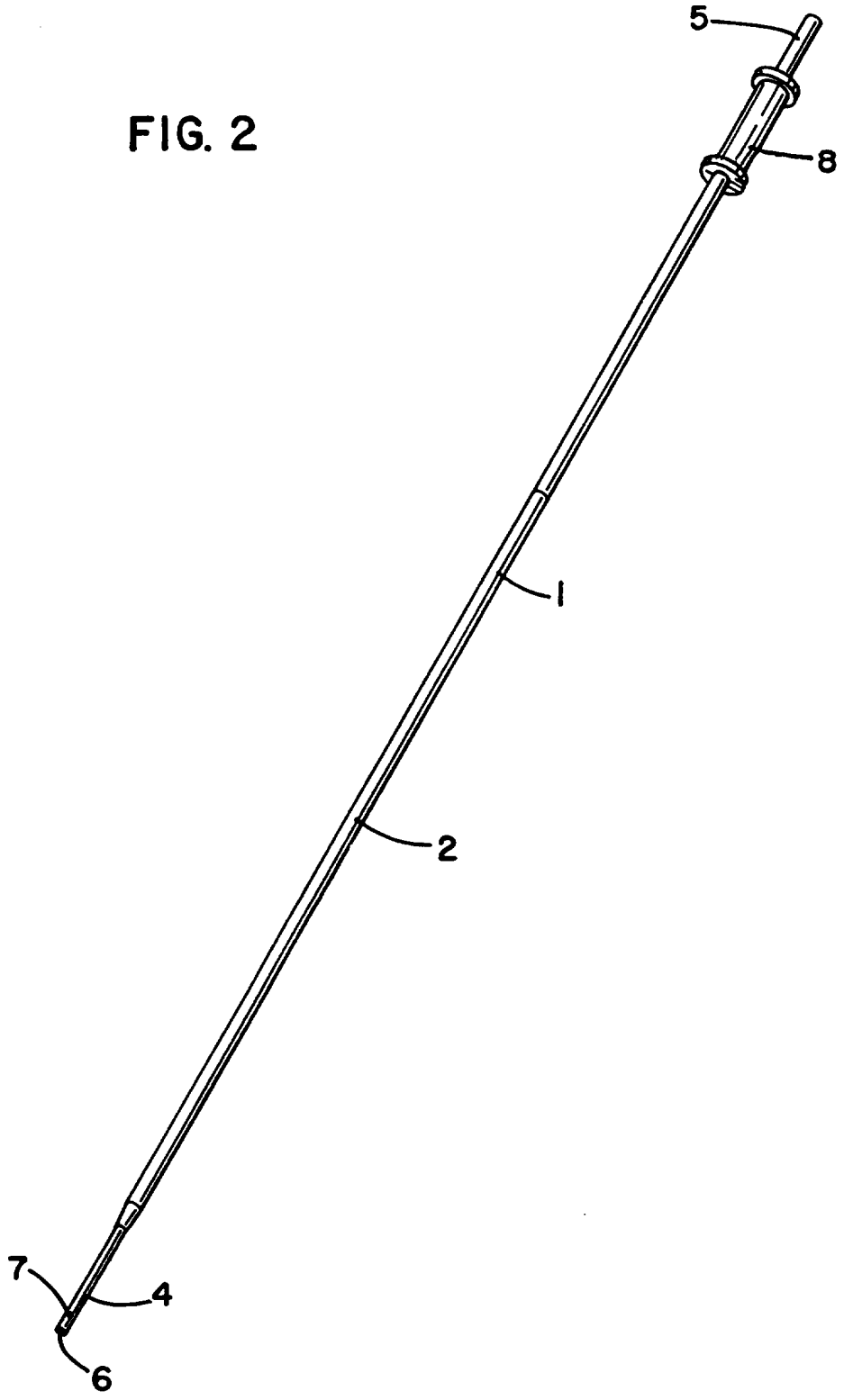


FIG. 3

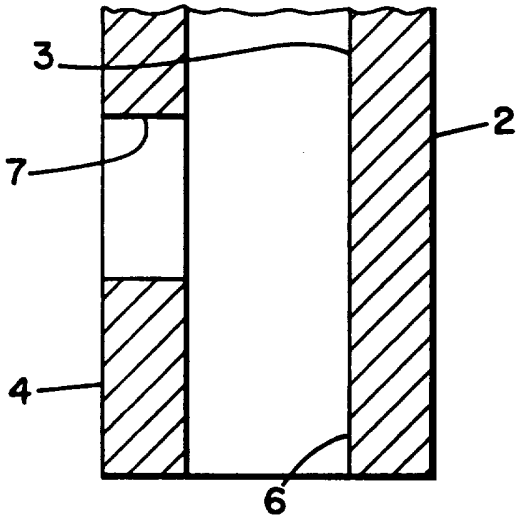


FIG. 4

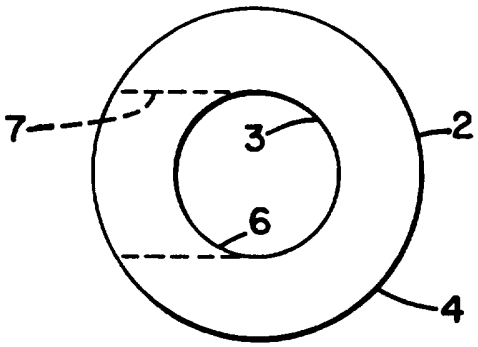


FIG. 5

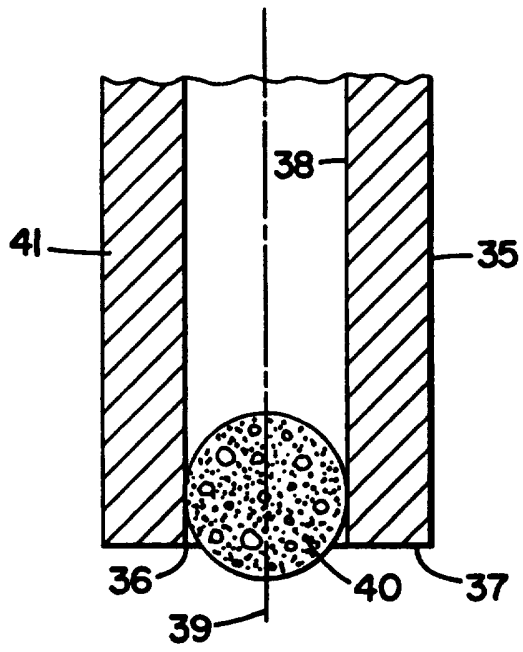


FIG. 6

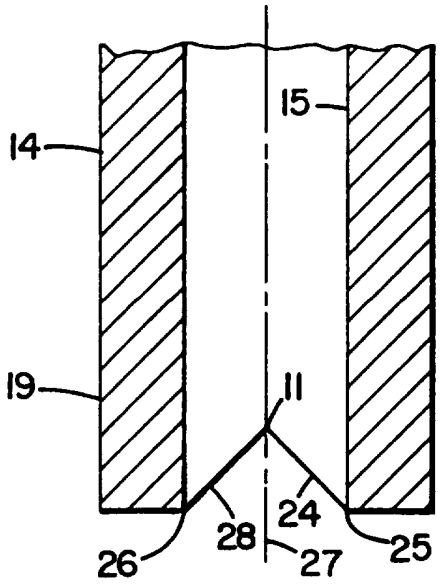


FIG. 7

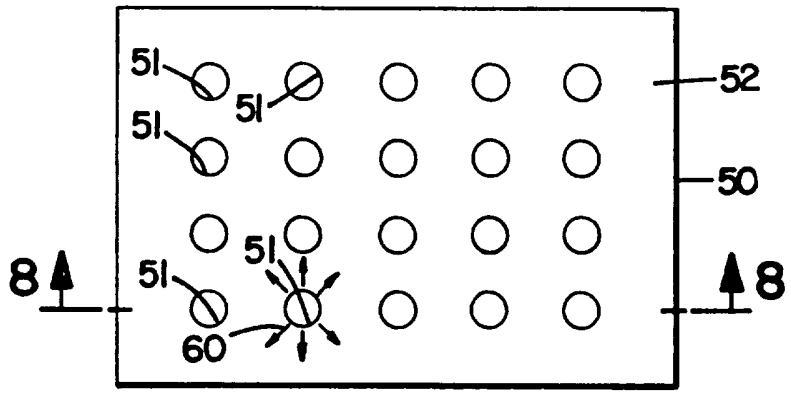
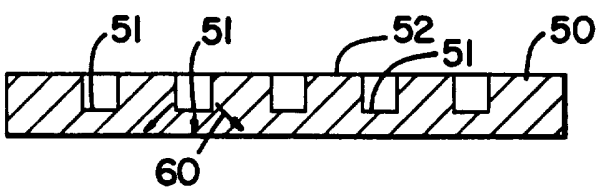


FIG. 8



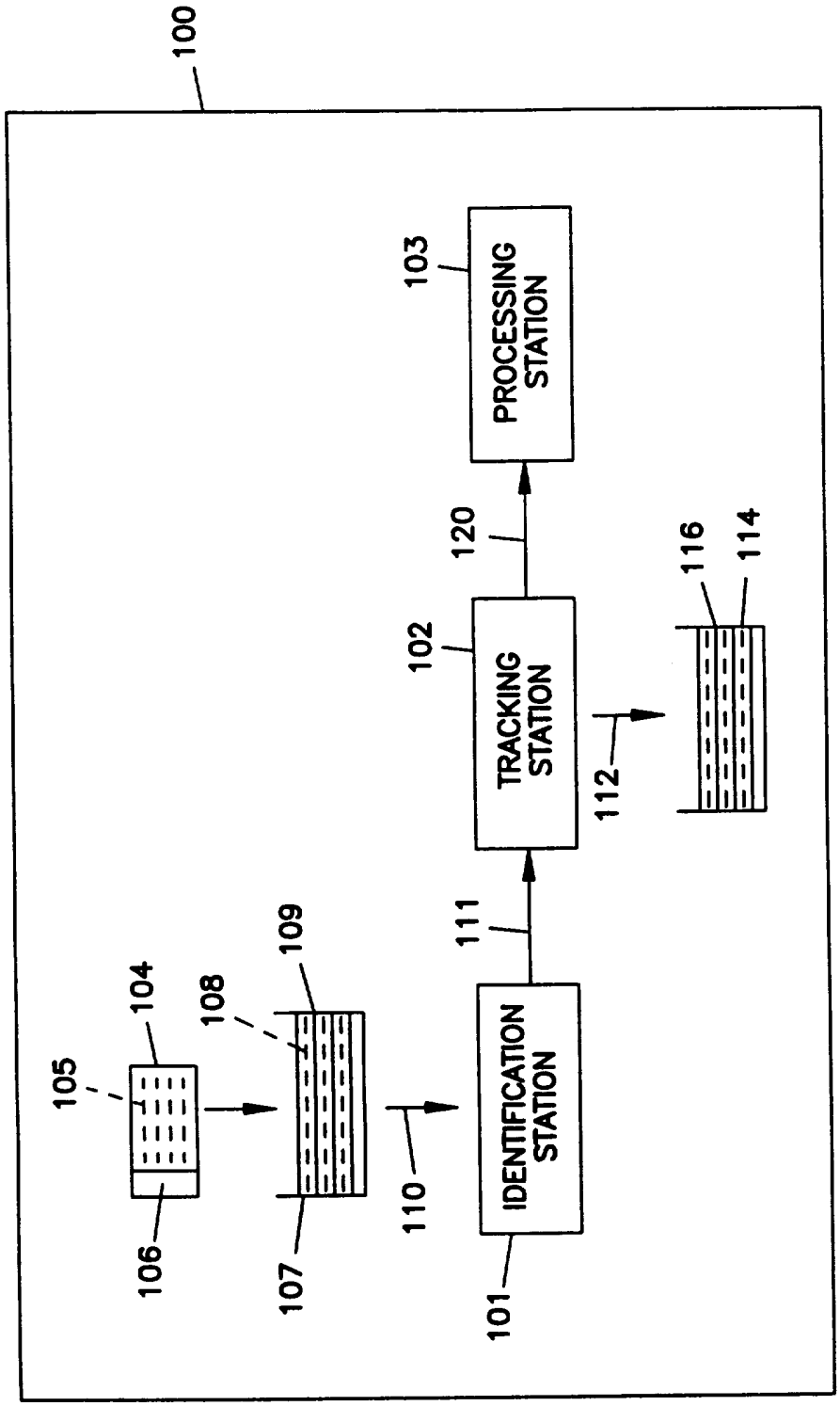

100
PROCESSING STATION
103
120
TRACKING STATION
102
116
114
112
111
IDENTIFICATION STATION
101
110
109
108
104
105
106
107

FIG. 9

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/IB 95/00989

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: G01N 35/00, 33/49, A61M 25/14, A61M 39/00, C12M 3/06, B01D 15/00, 15/08

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 G01N 35/00, 33/49, A61M 1/00, 25/00, 25/14, 39/00, A61B 1/00, 1/012, 1/015, B01L 3/00, A61B 17/00, 19/00, F16L 9/00, 11/00; B01D 15/00, 15/08; C12M 3/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU : IPC AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT WPAT, CHEMICAL ABSTRACTS, JAPIO; KEYWORDS : BIOPOLYMER #, BIO ( ) POLYMER #, DNA, NUCLEIC ( ) ACID #, NUCLEOTIDE #, PROTEIN #, REMOV:, PURIF:, FILTRAT:, FILTER:, POLY ( ) ACRYLAMIDE #, POLY: ACRYLAMIDE #, CHROMATOGRAPHY, GEL.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 8702802 A (CELL ANALYSIS SYSTEMS, INC.,) 7 May 1987 Abstract, pages 1-28, claims, drawings	1-5 6-12
P,Y	WO 9422580 A (MDS HEALTH GROUP LIMITED) 13 October 1994 Abstract, pages 4-7, drawings	1,2
Y	WO 8706008 A (BECKMAN INSTRUMENTS, INC) 8 October 1987 Abstract pages 1-14, page paragraph 2, page 46 paragraph 1	1,2

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"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

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 February 1996	05.03.96
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	Authorized officer C. BERKO Telephone No.: (06) 283 2169

Form PCT/ISA/210 (second sheet) (July 1992) copmak

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 9325912 A (MEDICAL RESEARCH COUNCIL) 23 December 1993 Whole document	5-10
Y	EP 122772 A (SCIENCE AND TECHNOLOGY AGENCY, MINISTER'S SECRETARIAT, DIRECTOR OF FINANCE DIVISION) 24 October 1984 Whole document	1-10
Y	WO 9315407 A (RUGGERI) 5 August 1993 Whole document	1,2,11,12
Y	US 4805469 A (COMMARMOT) 21 February 1989 Whole document	1,2
X Y	GB 970647 A (SHERIDAN) 23 September 1961 Page 1 lines 10-20, page 2 lines 42-51, figures 2 and 3	13 to 15 16,17
X Y	GB 1086821 (& FR 1427931) A (EDWARDS LABORATORIES) 11 October 1967 Page 2 lines 11-30, figure 2	13 to 15 16,17
X Y	GB 1316560 A (SHERIDAN) 9 May 1973 Page 1 lines 10-24, page 2 lines 7-21, figure 1	13 to 15 16,17
X Y	WO 81/02675 A (SONTEK INDUSTRIES INC) 1 October 1981 Page 1 lines 26-27, page 3 lines 4-8, page 4 line 24 to page 5 line 9, figures 1 and 2	13 to 15 16,17
X Y	WO 93/18801 A (SCHON) 30 September 1993 Figures 1 to 5	13 to 15 16
Y	WO 90/06150 A (W.L. GORE & ASSOCIATES) 14 June 1990 Page 2 lines 1-16, figures 2B and 4C	16
Y	FR 2651676 A (DERIEN) 15 March 1991 Figure 1 (ref 5)	16
X	EP 233620 A (KANEGAFUCHI KAGAKU KOGYO KAISHA) 26 August 1987	18
X	PATENTS ABSTRACTS OF JAPAN, C-643, page 72 JP 01-174505 (MITSUBISHI KASEI CORP) 11 July 1989	18
X	PATENT ABSTRACTS OF JAPAN, C-596, page 115 JP 01-27467 (TOYOBO CO LTD) 30 January 1989	18

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/ IB 95/00989

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN, C-1216, page 136 JP 06-79104 (KURITA WATER IND LTD) 22 March 1994	18
X	PATENT ABSTRACTS OF JAPAN, C-1020, page 5 JP 04-256441 (MITSUI TOATSU CHEM INC) 11 September 1992	18
X	DERWENT ABSTRACT NO. 87-105931, CLASS 503 JP 074705 (MITSUBISHI CHEM IND K.K.) 9 March 1987	18

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/IB 95/00989

Box 1 Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. Claims 1-12 are directed to a system for identifying tracking and processing a biological sample (in the form of DNA) including an interactive computer networking identification and recall system. This system is considered to constitute a first "special technical feature".

(continued in Supplementary Box)

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: II

2. Claims 13-17 are directed to a cannula that can be connected in fluid flow communication, to an automatic fluid delivery system and characterised by an arrangement of at least two aspirating and dispensing ports. This arrangement is considered to constitute a second separate "special technical feature"

3. Claim 18 is directed to a method for removing a by-product from a biopolymer in a reaction mixture utilizing a polymeric gel characterised by allowing a sufficient period of contact between the gel and the mixture for some diffusion of material from the mixture to occur without applying an electric field. The diffusion occurrence is considered to constitute a third separate "special technical feature".

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/IB 95/00989

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	8702802	DE	3688330	DE	3689856	EP	245466
		EP	248840	EP	571053	JP	7146289
		JP	7168102	JP	7181179	JP	7111425
		US	4741043	US	4887892	US	4998284
		US	5008185	US	5018209	US	5086476
		US	5109429	US	5134662	US	5281517
		US	5485527	WO	8702803	EP	311368
WO	9422580	AU	64223/94	CA	2093393	FI	954741
		NO	953965				
WO	8706008	EP	261202	FI	875113	JP	63502931
		US	5108703	US	5206568	US	5104621
		US	5125748	US	5139744	US	5369566
WO	9325912	AU	43439/93	AU	43440/93	WO	9325709
EP	122772	DE	3473512	JP	59192963	US	4632808
		US	4735776				
WO	9315407	IT	92500136				
US	4805469	FR	2600166				
GB	1316560	CA	929064	DE	2016607	GB	1314391
		US	3605750				
WO	8102675	AU	71504/81	CA	1152834	EP	48743
		JP	57500418	US	4351328		

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/ IB 95/00989

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9318801	AU	37507/93	DE	4208912	EP	631508
WO	9006150	AU	46617/89	JP	2142576		
EP	233620	AT	56633	CA	1283073	DE	3764972
		JP	62191042				

END OF ANNEX