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(54) **Title**: IMPROVEMENTS TO NUCLEIC ACID ELUTION

(57) **Abstract**: This invention relates to the storage on a solid matrix of genetic material, in particular DNA that has been purified prior to the application to the solid matrix. More specifically, the invention relates to a solid matrix for the storage of purified DNA, which matrix has been treated with a solution comprising plant polysaccharide inulin. One avantage of the invention is that an increased amount of DNA can be stored in the solid matrix of the present invention.

IMPROVEMENTS TO NUCLEIC ACID ELUTION

Field of the Invention

This invention relates to the storage on a solid matrix of genetic material, in particular DNA that has been purified prior to the application to the solid matrix.

Background of the Invention

Storing of nucleic acids, particularly DNA, is typically accomplished in solution using refrigeration either at 4°C for up to several days or –20°C or even lower temperatures for longer periods. This is costly and space consuming for static storage. It presents greater issues when nucleic acid samples require transportation and samples are often shipped in dry ice.

Burgoyne (WO 90/03959) described a method whereby biological samples, usually blood, could be applied to a solid matrix which combined reagents which lysed the cells. The released DNA was retained on the solid matrix. These samples could be stored for long periods at room temperature.

US 5,939,259 and WO 03/016552 describe techniques whereby the DNA associated with the solid matrix could be eluted for further study. In many cases however, recovery of the purified DNA applied to the solid matrix results in about 40% of the DNA recovered as determined by quantitative real time PCR. There is clearly a need for a simple method which stores DNA at room termperature for long periods of time but allows greater recovery of the applied DNA.

Summary of the Invention

It has been surprisingly observed that the addition of inulin (a polysaccharide

found in plants) to the solid matrix, greatly increases the percentage of the applied DNA that can be eluted from the solid matrix. This is particularly apparent when purified DNA is applied the solid matrix.

Brief Description of the Drawings

Figure 1 shows the struture of inulin.

Detailed Description of the Invention

A range of chemicals was added to a solid matrix to study their effect on the yields of DNA recovered from the solid matrix. In particular the solid matrix known as FTA Elute (Whatman) has proved to be particularly useful in the practice of the current invention. However, it is anticipated that other types of solid matrix can also be used with the invention. Many of the solid matrices are based on cellulose. The solid matrix was treated with a solution of test reagents diluted in 2M guanidine isothiocynate. Many chemicals had little or no effect on the yield of DNA recovered. Polyethylene glycol (PEG) at concentrations of about 10% had a small increase on the amount of DNA recovered. PEG is a long chain polymer of ethylene glycol subunits. PEG is prepared in a variety of molecuar weights defined by the average number of subunits per molecule. Polymers of MW 400, 1000 and 3350 were evaluated. It was observed that PEG 1000 produced the best recovery of applied DNA results (data not shown) and was used in the remainder of the tests but is referred to as PEG. At concentration of PEG at about 25% the results varied between experiments; this may imply small inconsistencies in the coating process of the solid matrix at these concentrations.

It was found that when the solid matrix was treated with the plant polysaccharide inulin then increases in the amount of DNA eluted from the solid matrix was observed. It

was found that adding inulin up to concentrations of 20% to the solid matrix increases the yield of DNA recovered from the solid matrix from 25-40%, without the addition of inulin to 80%. There were indications that adding 10% PEG in addition to the added inulin increased the yield of recovered DNA to approximately 85%.

Inulin is a naturally occuring polysaccharide found in many plants. Its structure is given in Figure 1. it is anticpated that simple modifications of inulin eg esterification would be possible and still achieve the improved elution of the applied purified DNA.

The purified DNA can be appiled to the solid matrix that has been treated with inulin in buffers that are routinely used in nucleic acid chemistry. Up to 10% PEG can also be included in the application buffer. The DNA prior to application to the solid matrix can be purified by a variety of standard laboratory techniques.

An important consideration is that the increased yield of recovering DNA is maintained with time ie. prolonged storage at room temperature. It has been found that DNA can be recovered with increased yield for at least twenty-three days. It is expected that this increased yield will occur with even longer storage periods. Room temperature is usually about 20°-25°C with a typical value of 20°C.

Examples

The present example is provided for illustrative purposes only, and should not be construed as limiting the present invention as defined by the appended claims.

Example 1 Matrix Chemistry Modification

The solid matrix was FTA Elute 903 matrix from Whatman.

1) A 4 M stock of guanidine thiocyanate was prepared and diluted to 2 M using various concentrations of test reagents.

2) 903 matrix (2 ¼" x 2 ¼") was placed into trays containing guanidine thiocyanate/ test reagent mixes and agitated gently for 10 seconds.

3) Matrices were dried for 10 min on a metal rack using two hair dryers (Simply Basic DS-727); one placed at a 30° angle 15 cm above the matrix, the other placed 25 cm below the matrix at a 30° angle such that the two hair dryers and the matrix were in alignment. Matrices were dried further without the air flow at 21 ± 2°C overnight.

4) Matrices were stored at room temperature in a desiccator until use.

Example 2 Application of DNA to test solid matrix

Human DNA (Roche) was spotted onto the test solid matix at concentration of 160ng/µl. Usually a pre-punched 5mm diameter disc of the matrix was used. Discs were dried at room temperature for a minimum of 3 hours.

Example 3 Elution of DNA from Solid matrix

1) Each dried disc was placed in a sterile 1.5 ml microfuge tube and washed with 500 µl dH_2O by pulse vortexing three times for a total of five seconds.

2) Discs were transferred to 0.5 ml microfuge tubes containing 100 µl of dH_2O, ensuring that discs were fully submerged.

3) Microfuge tubes were placed in a heat block for 30 min at 98°C.

4) Microfuge tubes were pulse vortexed for 60 sec and then briefly centrifuged.

5) Eluates were transferred to new 0.5 ml tubes, leaving discs behind. Eluates were stored at 4°C until quantification.

Example 4 Quantification of DNA in Eluates

Quantification of DNA in eluates was performed by QPCR using a 7900HT Thermal Cycler (Applied Biosystems). Reactions were set up using an RNase P assay and TAQMAN® Universal PCR Master Mix (Applied Biosystems). A four point standard curve was prepared using a serial dilution from 10 to 0.01 ng/µl of the same Roche DNA used for experiments. Early QPCR quantifications were performed in 96-well plates and were set up manually. Following the introduction and validation of a liquid handling robot, later quantifications were performed using 384-well plates. Both 96 and 384-well plates were validated and also tested against each other to check for consistency between methods.

Results

Results (Table 1) show that matrices impregnated with either inulin or PEG did result in increased DNA recovery compared to FTA Elute by itself. In addition to this, the use of a spotting buffer containing 10 % PEG resulted in an additional increase in DNA yield (85 % when used with FTA Elute + 20 % inulin).

		Spotting Buffer	
		DNA Only	10% PEG
FTA Elute Matrix	10% PEG	49	66
	20% Inulin	62	**85**
FTA Elute		42	48

Table 1: Percent recoveries of DNA (1 µg) applied to FTA Elute matrices impregnated with additional chemicals. Spotting buffers were mixed with DNA immediately prior to application to pre-punched 5 mm diameter discs. For each entry, n=4. Discs were dried and DNA eluted as described in section 1.1.

The matrix containing 20 % inulin showed the highest % recovery of applied purified DNA. Since PEG was also identified as a possible additive to matrix impregnation chemistry, FTA Elute impregnated with a combination of 20 % inulin and 10 % PEG was also prepared for further investigation .A spotting buffer containing 10 % PEG was confirmed as further increasing yields when used in conjunction with the modified matrix chemistry.

Results were also obtained from experiments where the discs with applied purified DNA had been stored in a dessicator at room temperature. The results showed that the discs could be stored for at least twenty-three days before DNA elution with similar increased recovery of DNA when the matrix had been treated with inulin.

Results also showed that the amount of DNA applied and recovered from the test

matrix could be as low as 1ng and as high as 1μg (the maximum tested) and the increased effect on inulin treatment was still observed.

It is to be understood that any feature described in relation to any one embodiment may be used alone, or in combination with other features described, and may also be used in combination with one or more features of any other of the embodiments, or any combination of any other of the embodiments. Furthermore, equivalents and modifications not described above may also be employed without departing from the scope of the invention, which is defined in the accompanying claims.

What is claimed is:

1. A solid matrix suitable for the storage of purified DNA which matrix has been treated with a solution comprising inulin.

2. The solid matrix of claim 1, which has also been treated with PEG.

3. The solid matrix of claim 1 or 2, wherein the inulin treatment is at a concentration of up to 20%.

4. The solid matrix of claims 1-3, which also contains PEG at up to 10%.

5. A method of increasing of the yield of purified DNA eluted from a solid matrix by treating the solid matrix with a solution comprising inulin prior to the addition of said nucleic acid.

6. The method of claim 5, wherein the solid matrix has also been treated with PEG or the DNA is applied in a buffer comprising PEG.

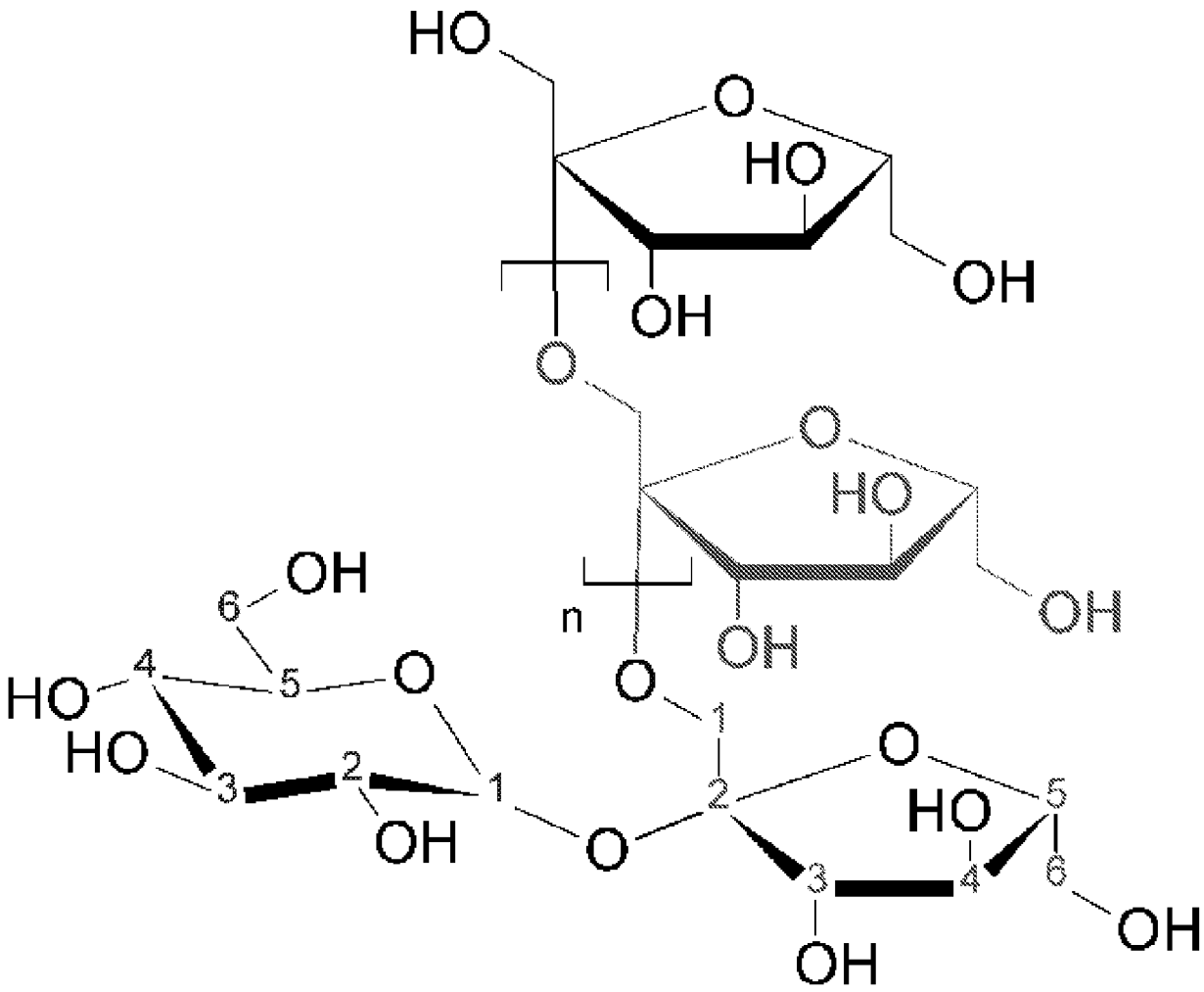

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/10 C12Q1/68
ADD.

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)
C12N C12Q

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

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

EPO-Internal, BIOSIS, COMPENDEX, Sequence Search, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/044211 A2 (WHATMAN INC [US]; BURGOYNE LEIGH A [AU]) 30 May 2003 (2003-05-30) the whole document	1-6
X	WO 03/016552 A2 (WHATMAN INC [US]; DAVIS JAMES C [US]; SMITH MARTIN A [US]; VERA-GARCIA) 27 February 2003 (2003-02-27) cited in the application the whole document	1-6

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[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

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"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

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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HINRICHS W L J ET AL: "Inulin is a promising cryo- and lyoprotectant for PEGylated lipoplexes", JOURNAL OF CONTROLLED RELEASE, ELSEVIER, AMSTERDAM, NL, vol. 103, no. 2, 21 March 2005 (2005-03-21), pages 465-479, XP004823759, ISSN: 0168-3659, DOI: DOI:10.1016/J.JCONREL.2004.12.011	1-6
A	DE JONGE JORGEN ET AL: "Inulin sugar glasses preserve the structural integrity and biological activity of influenza virosomes during freeze-drying and storage", EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 32, no. 1, September 2007 (2007-09), pages 33-44, XP002629865, ISSN: 0928-0987	1-6
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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		AU 2002366048 A1	10-06-2003
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