(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 December 2005 (01.12.2005)

PCT

(10) International Publication Number
WO 2005/113769 A1

(51) International Patent Classification[7]: C12N 15/10, C12Q 1/68

(21) International Application Number: PCT/US2005/017046

(22) International Filing Date: 16 May 2005 (16.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/571,120 14 May 2004 (14.05.2004) US

(71) Applicant *(for all designated States except US)*: **EXACT SCIENCES CORPORATION** [US/US]; 100 Campus Drive, Marlborough, MA 01752 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SHUBER, Anthony, P.** [US/US]; 6 Parker Road, Mendon, MA 01756 (US). **WHITNEY, Duncan, H.** [US/US]; 20 Read Road, Sudbury, MA 01776 (US).

(74) Agent: **WALLER, Patrick, R., H.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/113769 A1

(54) Title: METHOD FOR STABILIZING BIOLOGICAL SAMPLES FOR NUCLEIC ACID ANALYSIS

(57) Abstract: Disclosed is a method for preparing nucleic acid containing biological samples for a nucleic acid integrity assay and/or multiple mutation analysis by incubating the biological sample with a stabilization solution that includes a buffer, a chelating agent and a salt.

METHOD FOR STABILIZING BIOLOGICAL SAMPLES FOR NUCLEIC ACID ANALYSIS

RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 60/571,120, entitled "Method for Stabilizing a Biological Sample for a Nucleic Acid Integrity Assay" filed on May 14, 2004, the disclosure of which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The invention relates generally to assays to detect nucleic acid markers indicative of cancer and other diseases and more particularly to preparing nucleic acid-containing biological samples for use in these assays.

BACKGROUND OF THE INVENTION

Tissue and body fluid samples, including stools, contain shed cellular debris. In healthy patients, such debris is the result of apoptotic degradation as part of the normal cell cycle. Apoptosis reduces the integrity (intactness) of nucleic acids, proteins, and other cellular components in healthy individuals, so that only small fragments exist in the debris that results from the apoptotic process (e.g., exfoliated cellular debris).

In diseases in which cell cycle mechanisms are destroyed or impaired, cellular debris can include high-integrity cellular components, such as nucleic acids that have not been degraded by apoptosis. One class of disease in which cell cycle mechanisms are disrupted is cancer. An increased presence of high molecular weight nucleic acids in a biological sample therefore can reveal the presence of cancer in a patient from whom the biological sample was obtained. Disease detection assays known as nucleic acid integrity analysis assays have been developed that are based on the increased levels of non-degraded nucleic acid in a cancerous tissue or body fluid as compared to the level of non-degraded nucleic acid in a non-cancerous tissue or body fluid.

Nucleic acids in patient samples tend to degrade after they have been removed from the patient. This degradation can diminish the effectiveness of a nucleic acid integrity assay that scores a sample as diseased (e.g., cancerous) based on the presence of intact nucleic acids; if

the sample is excessively degraded, a sample that is actually positive may appear to be negative.

SUMMARY OF THE INVENTION

The invention is based in part on the discovery of a method for stabilizing nucleic acids in tissue and body fluid samples so as to facilitate analysis of the samples in a nucleic acid integrity assay. It has been unexpectedly found that contacting a patient sample with a stabilization solution stabilizes the DNA so that intact nucleic acids indicative of diseased cells are more effectively detected in a nucleic acid integrity assay.

Accordingly, in one aspect, the invention provides a method for preparing a nucleic acid sample for a nucleic acid integrity analysis assay. In one aspect, the invention provides a method for preparing a nucleic acid sample for a nucleic acid integrity analysis assay. The method includes providing a patient sample that includes shed cells or cellular debris and a nucleic acid and contacting the patient sample with a stabilization solution under conditions sufficient to stabilize the nucleic acid for nucleic acid integrity analysis. In one embodiment, the stabilization solution includes a buffer, a salt, and a chelating agent.

In some embodiments, the conditions are sufficient to detect at least a three-fold genomic equivalent (GE) increase in a nucleic acid integrity analysis of a patient sample having adenoma or cancer as compared to the GE detected in a nucleic acid integrity analysis of a sample from the patient that is not incubated with the stabilization solution. In one embodiment, the integrity analysis is performed by determining an amount of nucleic acid greater than about 200 bp in length using an assay that detects a nucleic acid (which can be a wild-type or mutant nucleic acid). The nucleic acid is present in a patient sample that includes shed cells or cellular debris. A patient is identified as having cancer or adenoma if the amount of nucleic acid is greater than an amount of nucleic acid expected to be present in a sample obtained from a patient who does not have cancer or adenoma.

The patient sample can be obtained from a patient that is, e.g., a vertebrate, including a mammal, a reptile, or an amphibian. A mammal can be, e.g., a human, a non-human primate (such as a gorilla or monkey, including a chimpanzee), a rodent (such as a mouse, rat, guinea pig, or gerbil) dog, cat, horse, pig, goat, sheep, or cow. The patient sample can be any body tissue or fluid that is suspected of containing DNA from a diseased cell (such as a precancerous or cancerous cell). In one embodiment, the patient sample is a stool sample.

In some embodiments, the patient sample can be obtained as part of a screen for, e.g., a disease or disease-associated condition that impairs, or could lead to impairment of, the proper function of the gastrointestinal system. Gastrointestinal diseases can include, e.g., those associated with the stomach, small intestine, and/or colon. The disease or condition can include cancers or precancerous conditions such as an adenoma. Other conditions include inflammatory bowel syndrome, inflammatory bowel disease, Crohn's disease, and others in which a genomic instability is thought to play a role.

In some embodiments, the patient sample is frozen and thawed prior to incubation with stabilization solution. In other embodiments, the patient sample is not frozen prior to incubation with the stabilization solution.

In general, the stabilization solution is added to the patient sample at a ratio of about 1 ml/gram of patient sample to about 20 ml/gram of patient sample. In some embodiments, the stabilization solution is provided at 1-15 ml/gram, 2-12 ml/gram, 3-11 ml/gram, or 4-7 ml/gram. However, higher or lower ratios may be used. When the patient sample is a stool sample, and the stabilization solution is 10 mM Tris-Cl, 1 mM EDTA, and 150 mM NaCL, a suitable ratio of stabilization solution to patient sample is 7 ml/gm.

In some embodiments, the patient sample and stabilization solution are incubated at about 4 to 28 degrees Centigrade. In some embodiments the temperature is 17 to 27 degrees Centigrade, e.g., about 20 to 25 degrees Centigrade. However, the sample and stabilization solution may be exposed to higher or lower temperatures (e.g., the sample and stabilization solution may be frozen). Also, a sample and buffer may be exposed to changing temperatures during transport and/or storage.

In various embodiments, the patient sample and stabilization solution are incubated at least 6 hours, e.g., at least 12 hours, at least 24 hours, or at least 36 hours.

In some embodiments, the buffer in the stabilization solution is 0.5 mM to 25 mM Tris, e.g., 5 mM to 15 mM Tris, 8 mM to 13 mM Tris or about 10 mM Tris. However, other buffers and/or concentrations may be used.

In some embodiments, the chelating agent in the stabilization solution is 0.01 to 2.5 mM EDTA, e.g., 0.75-1.25 mM EDTA, or 1mM EDTA. However, other chelating agents and/or concentrations may be used.

In some embodiments, the salt in the stabilization solution is 75 mM to 225 mM NaCl, e.g., 100 mM to 175 mM NaCl, or 150 mM NaCl. However, other salts (e.g., KCl etc.) and/or

In some embodiments, the stabilization solution is provided at pH 7.0 to 9.0, e.g., pH 7.5 to 8.5, or pH 8.0. However, higher or lower pHs may be used.

In some embodiments, the method further includes determining in the incubated patient sample an amount of nucleic acid greater than about 200 bp in length using an assay that detects wild-type or mutant nucleic acid, and identifying the patient as having cancer or adenoma if the amount is greater than an amount of nucleic acid expected to be present in a sample obtained from a patient who does not have cancer or adenoma (e.g., more than about 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 50 fold, or more greater than an amount expected in a normal individual). In one aspect, a DNA integrity assay may include interrogating a sample for the presence of long DNA fragments (e.g., longer than 200 nucleotides, longer than 500 nucleotides, longer than 1,000 nucleotides, etc.) at two or more different loci. In one embodiment, a patient is identified as having cancer or adenoma if two or more loci (e.g., 3, 4, or more loci) are positive for the presence of abnormally high levels of long DNA.

In one embodiment, the invention provides a method for preparing a nucleic acid sample for a nucleic acid integrity and/or multiple mutation analysis assays for diagnosing a carcinoma or adenoma. The method may include providing a patient stool sample that includes shed cells or cellular debris and a nucleic acid, and incubating the patient sample with a stabilization solution under conditions sufficient to stabilize the nucleic acid for nucleic acid integrity and/or multiple mutation analysis. The stabilization solution may be about pH 7.5 to about pH 8.5 and may include 0.5 mM to 25 mM Tris, 0.01 to 2.5 mM EDTA and 100 mM to 200 mM NaCl. For example, the stabilization solution can be 10 mM Tris-Cl pH 8.0, 1 mM EDTA, and 150mM NaCl. In one embodiment, the conditions are sufficient to detect at least a three-fold genomic equivalent (GE) increase in a nucleic acid integrity analysis of a patient sample having adenoma or cancer as compared to the GE detected in a nucleic acid integrity analysis of a sample from the patient that is not incubated with the stabilization solution.

Aspects of the invention may be used for transporting or storing biological samples after they are obtained and before they are processed for analysis. For example, methods of the invention may be used to stabilize nucleic acid in biological samples (e.g., stool samples) for about 12 hours, about 24 hours, about 36 hours, or longer (e.g., 4 days, 5, days, 6 days, 1 week, or longer), even in the absence of refrigeration or freezing.

Aspects of the invention may be particularly useful for detecting indicia of adenomas, early stage cancers, and/or other diseases that may be characterized by very low frequencies of

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.