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SERIAL NUMBER	077385,986	FILING DATE	07/20/89	CLASS	435
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				GROUP ART UNIT	185 187
				EXAMINER	

APPLICANTS

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****CONTINUING DATA**** *****
 VERIFIED THIS LN IS A CON OF 06/732,374 05/09/85 ABN
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Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
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TITLE

METHOD AND STRUCTURES EMPLOYING CHEMICALLY-LABELLED POLYNUCLEOTIDE PROBES

U.S. DEPT. of COMM. Pat. & TM Office - PTO-4361 (rev. 10-78)

ARTS OF APPLICATION LED SEPARATELY

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October 10, 1990	10/12/90	Total Claims: 27 Print Claim: 1
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Amount Due: 620. ⁰⁰ Date Paid: 10/31/90		Sheets Drwg.: 0 Figs. Drwg.: 0 Print Fig.: -
Label Area	ISSUE CLASSIFICATION	ISSUE BATCH NUMBER
	Class: 435 Subclass: 6	453
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REGULAR UTILITY

Form PTO-436
(Rev. 8/78)

SERIAL NUMBER (series of 1979)	732374	PATENT DATE	PATENT NUMBER			
SERIAL NUMBER	06/732,374	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
		05/09/85	435	6	22/85	Halenatt
		FILE 47				

APPLICANTS

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****CONTINUING DATA*******
 VERIFIED THIS APPLN IS A CIP OF 06/461,469 01/21/83, *Now abandoned*
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****FOREIGN/PCT APPLICATIONS*******
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Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS DRWGS	TOTAL CLAIMS	INDEP CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
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FILE
 METHODS AND STRUCTURES EMPLOYING CHEMICALLY-LABELLED POLYNUCLEOTIDE
 PROCES

U.S. DEPT of COMM. - Pat. & Office - PTO-436L (rev 10-78)

filed under Rule 47(a) to be submitted to the solicitor before issue.

PARTS OF APPLICATION FILED SEPARATELY					PREPARED FOR ISSUE	
					(Assistant Examiner)	(Docket Clerk)
AT ALLOWANCE					EXAMINED AND PASSED FOR ISSUE	
SHEETS DRWGS.	FIGURES DRWGS.	CLAIMS	CLASS	SUBCLASS	(Primary Examiner)	(Art Unit)
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					Date mailed	Date paid

RETENTION LABELS
Page 2 of 5

732374

Group 120

Entered

CONTENTS

- 1. Applications ^{papers}
- 2. *Letter (Re: from decl. omitted signature) June 4, 1988*
- 3. *Dec. Recepting Papers Under 147(a) Dec 12, 1985*
- 4. *Re: (3) 1-19-88 12/21*
- 5. *Title Report Feb 11, 1988*
- 6. *Power of atty (WE) Jan 19, 1988*
- 7. *Status Ltr. Jun 19, 1988*
- 8. *Ltr. re. Platty Feb 22, 88*
- 9. *Regime 3 mos August 15, 1988*
- 10. *Advt A August 15, 1988*
- 11. *Title Report Aug 15, 1988*
- 12. *Power of Atty (WE) August 15, 1988*
- 13. *Ltr re: Power 9-14-88*
- 14. *Ltr. 3 mos 11/29/88 11/7*
- 15. *Regime 3 mos May 19, 1989*
- 16. *Advt B (WE) May 19, 1989*
- 17. *Advisory Action 5-26-89 5/25*
- 18. *Appeal May 25, 1989*
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INITIALS

CONTENTS

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SEARCH NOTES

	Date	Ex'r
Reviewed parent file 461469	12/15/87	JAN
Biosis Search DNA, glass, Silane, couple	12/14/87	JAN
APS glass, treat, amorphous triethoxysilane DNA, RNA, nucleic acid. hybridize, probe.	12/14/87	JAN
Spoke to Koppenger	11/7/88	JAN
APS- RNA, DNA, Nonporous glass	11/7/88	JAN

SEARCHED

Class	Sub	Date	Ex'r
435	6, 7, 188 296, 300, 810.4	12/18/87	JAN
436	94, 501, 524, 527 531, 532	↓	
	update	11/7/88	JAN
	update	5/25/89	JAN

PRINT CLAIM(S):

INDEX OF CLAIMS

Claim	Date	Claim	Date
Final		Final	
Original	12/18/87	Original	12/18/87
1	✓	26	✓
2		27	
3		28	
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19	✓	44	
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23		48	
24	✓	49	
25	✓	50	

INTERFERENCE SEARCHED

Class	Sub	Date	Ex'r

SYMBOLS STATUS

- ✓ Rejected
- = Allowed
- (Through numeral) Canceled
- + Restriction requirement
- N Nonelected invention or species
- I Interference
- A Appeal
- O Object

INDEX OF CLAIMS

Claim		Date
Final	Original	
1	1	12/5/89
2	2	10/5/90
3	3	
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SYMBOLS

- ✓ Rejected
- Allowed
- (Through numeral) Cancelled
- + Restricted
- N Non-elected
- I Interference
- A Appeal
- O Objected

SEARCHED			
Class	Sub.	Date	Exmr.
Updated from parent case		12/1/89	AM
Updated plus		10/5/90	AM
536	27	↓	↓
436	800,810		
935	77,78,86,87		

SEARCH NOTES		
	Date	Exmr.
DIALOG & ABS search	12/1/89	AM
Updated	10/5/90	AM

INTERFERENCE SEARCHED			
Class	Sub.	Date	Exmr.
435	4,6,7,188,296,300,810	10/5/90	AM
436	94,501,524,527,531,532,800,810		
536	27		

07/385986

PATENT APPLICATION SERIAL NO. 07/385986

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

S 20578 08/01/89 385986

05-1135 020 101 382.00CH



732374

Filed PTO 1082
For Other Than A Small Entity

Attorney Docket No. Enz-7 CIP

Applicant(s): Jannis G. Stavrianopoulos et al.

For ⁵⁷⁰¹ METHODS AND STRUCTURES EMPLOYING CHEMICALLY-LABELLED PROBES

EXPRESS MAIL CERTIFICATION

"Express Mail" mailing label number B 82913141

Date of deposit May 9, 1985.

I hereby certify that this transmittal letter and the other papers and fees identified in this transmittal letter as being transmitted herewith are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and are addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Roderic L Satter

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

TRANSMITTAL LETTER FOR UNEXECUTED ORIGINAL PATENT APPLICATION

Sir:

Transmitted herewith for filing are the specification; claims; abstract; unexecuted declaration, for the above-identified patent application.

Also transmitted herewith are:

_____ sheets of:

Formal drawings.

Informal drawings. Formal drawings will be filed during the pendency of this application.

The filing fee has been calculated as shown below:

FOR	NUMBER FILED	NUMBER EXTRA	RATE	FEE
BASIC FEE				\$300
TOTAL CLAIMS	26 - 20 =	6	x \$10 =	\$ 60
INDEPENDENT CLAIMS	4 - 3 =	1	x \$30 =	\$ 30
<input type="checkbox"/> A MULTIPLE DEPENDENT CLAIM			+ \$100 =	\$
			TOTAL	<u>\$ 390</u>

A check in the amount of \$ 390.00 in payment of the filing fee is transmitted herewith.

The Commissioner is hereby authorized to charge payment of any additional filing fees required under 37 C.F.R. 1.16 in connection with the paper(s) transmitted herewith, or credit any overpayment of same, to Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.

Please charge \$ _____ to Deposit Account No. 06-1075 in payment of the filing fee. A duplicate copy of this transmittal letter is transmitted herewith.

Mary E. Bak 5/9/85

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300.00-101
60.00-JOINT-103
30.00-102

732374

APPLICATION

FOR

UNITED STATES LETTERS PATENT

Specification.

To ALL WHOM IT MAY CONCERN:

BE It Known, that we JANNIS G. STAVRIANOPOULOS, DOLLIE KIRTIKAR and
KENNETH H. JOHNSTON, BARBARA E. THALENFELD, citizens
of the United States of America, the USA, Canada and USA and residents,
515 West 59th Street 42-72 80th Street
respectively, of New York, N.Y. 10019; Elmhurst, New York 11373;
95 Horatio Street 250 East 39th Street
and of New York, New York 10014; and New York, New York 10016
have invented certain new and useful improvements in METHODS AND STRUCTURES EMPLOYING
CHEMICALLY LABELLED POLYNUCLEOTIDE PROBES

of which the following is a specification:

501
METHODS AND STRUCTURES EMPLOYING
CHEMICALLY-LABELLED POLYNUCLEOTIDE PROBES

This is a continuation-in-part of applicants' pending United States patent application, serial number 461,469, filed January 21, 1983.

Handwritten notes: "Mant 21" and "SU CC 11" with arrows pointing to the title and the "TECHNICAL FIELD OF INVENTION" section.

TECHNICAL FIELD OF INVENTION

The present invention relates generally to the detection of genetic material by polynucleotide probes. More specifically, it relates to a method for quantifiably detecting a targeted polynucleotide sequence in a sample of biological and/or nonbiological material employing a probe capable of generating a soluble signal. The method and products disclosed herein in accordance with the invention are expected to be adaptable for use in many laboratory, industrial, and medical applications wherein quantifiable and efficient detection of genetic material is desired.

BACKGROUND OF THE INVENTION

In the description, the following terms are employed:

Analyte - A substance or substances, either alone or in admixtures, whose presence is to be detected and, if desired, quantitated. The analyte

may be a DNA or RNA molecule of small or high molecular weight, a molecular complex including those molecules, or a biological system containing nucleic acids, such as a virus, a cell, or group of cells. Among the common analytes are nucleic acids (DNA and RNA) or segments thereof, oligonucleotides, either single- or double-stranded, viruses, bacteria, cells in culture, and the like. Bacteria, either whole or fragments thereof, including both gram positive and gram negative bacteria, fungi, algae, and other microorganisms are also analytes, as well as animal (e.g., mammalian) and plant cells and tissues.

Probe¹³ - A labelled polynucleotide or oligonucleotide sequence which is complementary to a polynucleotide or oligonucleotide sequence of a particular analyte and which hybridizes to said analyte sequence.

Label¹³ - That moiety attached to a polynucleotide or oligonucleotide sequence which comprises a signalling moiety capable of generating a signal for detection of the hybridized probe and analyte. The label may consist only of a signalling moiety, e.g., an enzyme attached directly to the sequence. Alternatively, the label may be a combination of a covalently attached bridging moiety and signalling moiety ~~or moiety~~ or a combination of a non-covalently bound bridging moiety and signalling moiety which gives rise to a signal which is detectable, and in some cases quantifiable.

Bridging Moiety¹³ - That portion of a label which on covalent attachment or non-covalent binding to a polynucleotide or oligonucleotide sequence acts as a link or a bridge between that sequence and a signalling moiety.

Signalling Moiety¹³ - That portion of a label which on covalent attachment or non-covalent binding to a polynucleotide or oligonucleotide sequence or

to a bridging moiety attached or bound to that sequence provides a signal for detection of the label.

Signal - That characteristic of a label or signalling moiety that permits it to be detected from sequences that do not carry the label or signalling moiety.

The analysis and detection of minute quantities of substances in biological and non-biological samples has become a routine practice in clinical, diagnostic and analytical laboratories. These detection techniques can be divided into two major classes: (1) those based on ligand-receptor interactions (e.g., immunoassay-based techniques), and (2) those based on nucleic acid hybridization (polynucleotide sequence-based techniques).

Immunoassay-based techniques are characterized by a sequence of steps comprising the non-covalent binding of an antibody and antigen complementary to it. See, for example, T. Chard, An Introduction To Radioimmunoassay And Related Techniques (1978).

Polynucleotide sequence-based detection techniques are characterized by a sequence of steps comprising the non-covalent binding of a labelled polynucleotide sequence or probe to a complementary sequence of the analyte under hybridization conditions in accordance with the Watson-Crick base pairing of adenine (A) and ^{thymine}thymidine (T), and guanine (G) and ^{cytosine}cytidine (C), and the detection of that hybridization. [M. Grunstein and D. S. Hogness, "Colony Hybridization: A Method For The Isolation Of Cloned DNAs That Contain A Specific Gene", Proc. Natl. Acad. Sci. USA, 72, pp. 3961-65 (1975)]. Such polynucleotide detection techniques can involve a fixed analyte [see, e.g., United States patent 4,358,535 to Falkow et al], or can involve detection

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7 of an analyte in solution [see U.K. patent application 2,019,408 A].

The primary recognition event of polynucleotide sequence-based detection techniques is the non-covalent binding of a probe to a complementary sequence of an analyte, brought about by a precise molecular alignment and interaction of complementary nucleotides of the probe and analyte. This binding event is energetically favored by the release of non-covalent bonding free energy, e.g., hydrogen bonding, stacking free energy and the like.

In addition to the primary recognition event, it is also necessary to detect when binding takes place between the labelled polynucleotide sequence and the complementary sequence of the analyte. This detection is effected through a signalling step or event. A signalling step or event allows detection in some quantitative or qualitative manner, e.g., a human or instrument detection system, of the occurrence of the primary recognition event.

The primary recognition event and the signalling event of polynucleotide sequence based detection techniques may be coupled either directly or indirectly, proportionately or inversely proportionately. Thus, in such systems as nucleic acid hybridizations with sufficient quantities of radiolabeled probes, the amount of radio-activity is usually directly proportional to the amount of analyte present. Inversely proportional techniques include, for example, competitive immuno-assays, wherein the amount of detected signal decreases with the greater amount of analyte that is present in the sample.

Amplification techniques are also employed for enhancing detection wherein the signalling event is related to the primary recognition event in a ratio greater than 1:1. For example, the signalling component of the assay may be present in a ratio of

10:1 to each recognition component, thereby providing a 10-fold increase in sensitivity.

A wide variety of signalling events may be employed to detect the occurrence of the primary recognition event. The signalling event chosen depends on the particular signal that characterizes the label or signalling moiety of the polynucleotide sequence employed in the primary recognition event. Although the label may only consist of a signalling moiety, which may be detectable, it is more usual for the label to comprise a combination of a bridging moiety covalently or non-covalently bound to the polynucleotide sequence and a signalling moiety that is itself detectable or that becomes detectable after further modification.

a The combination of bridging moiety and signalling moiety, described above, may be constructed before attachment or binding to the sequence, or it may be ^{sequentially} ~~sequentially~~ attached or bound to the sequence. For example, the bridging moiety may be first bound or attached to the sequence and then the signalling moiety combined with that bridging moiety. In addition, several bridging moieties and/or signalling moieties may be employed together in any one combination of bridging moiety and signalling moiety.

Covalent attachment of a signalling moiety or bridging moiety/signalling moiety combination to a sequence is exemplified by the chemical modification of the sequence with labels comprising radioactive moieties, fluorescent moieties or other moieties that themselves provide signals to available detection means or the chemical modification of the sequence with at least one combination of bridging moiety and signalling moiety to provide that signal.

Non-covalent binding of a signalling moiety or bridging moiety/signalling moiety to a sequence

involve the non-covalent binding to the sequence of a signalling moiety that itself can be detected by appropriate means, i.e., or enzyme, or the non-covalent binding to the sequence of a bridging moiety/signalling moiety to provide a signal that may be detected by one of those means. For example, the label of the polynucleotide sequence may be a bridging moiety non-covalently bound to an antibody, a fluorescent moiety or another moiety which is detectable by appropriate means. Alternatively, the bridging moiety could be a lectin, to which is bound another moiety that is detectable by appropriate means.

There are a wide variety of signalling moieties and bridging moieties that may be employed in labels for covalent attachment or non-covalent binding to polynucleotide sequences useful as probes in analyte detection systems. They include both a wide variety of radioactive and non-radioactive signalling moieties and a wide variety of non-radioactive bridging moieties. All that is required is that the signalling moiety provide a signal that may be detected by appropriate means and that the bridging moiety, if any, be characterized by the ability to attach covalently or to bind non-covalently to the sequence and also the ability to combine with a signalling moiety.

Radioactive signalling moieties and combinations of various bridging moieties and radioactive signalling moieties are characterized by one or more radioisotopes such as ^{32}P , ^{131}I , ^{14}C , ^3H , ^{60}Co , ^{59}Ni , ^{63}Ni and the like. Preferably, the isotope employed emits β or γ radiation and has a long half life. Detection of the radioactive signal is then, most usually, accomplished by means of a radioactivity detector, such as exposure to a film.

The disadvantages of employing a radioactive signalling moiety on a probe for use in the

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a identification of analytes are well known to those skilled in the art and include the precautions and hazards involved in handling radioactive material, the short life span of such material and the comparatively large ^{expenses} ~~expensive~~ involved in use of radioactive materials.

Non-radioactive signalling moieties and combinations of bridging moieties and non-radioactive signalling moieties are being increasingly used both in research and clinical settings. Because these signalling and bridging moieties do not involve radioactivity, the techniques and labelled probes using them are safer, cleaner, generally more stable when stored, and consequently cheaper to use. Detection sensitivities of the non-radioactive signalling moieties also are as high or higher than radio-labelling techniques.

Among the presently preferred non-radioactive signalling moieties or combinations of bridging/signalling moieties useful as non-radioactive labels are those based on the biotin/avidin binding system. [P. R. Langer et al., "Enzymatic Synthesis Of Biotin-Labeled Polynucleotides: Novel Nucleic Acid Affinity Probes", Proc. Natl. Acad. Sci. USA, 78, pp. 6633-37 (1981); J. Stavrianopoulos et al., "Glycosylated DNA Probes For Hybridization/Dection Of Homologous Sequences", presented at the Third Annual Congress For Recombinant DNA Research (1983); R. H. Singer and D. C. Ward, "Actin Gene Expression Visualized In Chicken Muscle Tissue Culture By Using In Situ Hybridization With A Biotinated Nucleotide Analog", Proc. Natl. Acad. Sci. USA, 79, pp. 7331-35 (1982)]. For a review of non-radioactive signalling and bridging/signalling systems, both biotin/avidin and otherwise, see D. C. Ward et al., "Modified Nucleotides And Methods Of

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Preparing And Using Same", European Patent application No. 63879.

Generally, the signalling moieties employed in both radioactive and non-radioactive detection techniques involve the use of complex methods for determining the signalling event, and/or supply only an unquantifiable positive or negative response. For example, radioactive isotopes must be read by a radioactivity counter; while signalling moieties forming insoluble "signals", i.e., precipitates, certain fluorescers, and the like [see, e.g., David et al., United States Patent No. 4,376,100] only provide detection not quantitation of the analyte present in the tested sample.

One step toward facilitating rapid and efficient quantitation as well as detection of the hybridization event was the work of Heller et al. in European Patent Applications No. 70685 and 70687 which describe the use of a signalling moiety which produces a soluble signal for measurable detection by a spectrophotometer. These European patent applications disclose the use of two different probes complementary to different portions of a gene sequence, with each probe being labelled at the end which will abut the other probe upon hybridization. The first probe is labelled with a chemiluminescent complex that emits lights of a specific wavelength. The second probe is labelled with a molecule that emits light of a different wavelength measurable by spectrophotometry when excited by the proximity of the first signalling moiety. However, this technique is performed in solution and can generate false positive results in the absence of the analyte if the two probes happen to approach too closely in solution and react with each other.

Similarly, U.K. Patent Application 2,019,408A, published October 31, 1979, discloses a method for

detecting nucleic acid sequences in solution by employing an enzyme-labelled RNA or DNA probe which, upon contact with a chromogen substrate, provides an optically readable signal. The analytes may be separated from contaminants prior to hybridization with the probe, or, alternatively, the hybrid probe-analyte may be removed from solution by conventional means, i.e., centrifugation, molecular weight exclusion, and the like. Like Heller's technique, this method is performed in solution.

There remains therefore a need in the art for a reliable, simple and quantifiable technique for the detection of analytes of interest in biological and non-biological samples.

SUMMARY OF THE INVENTION

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The ~~present~~ invention provides a solution for the disadvantages of presently available methods of detecting analytes by a novel combination of hybridization and immunological techniques. In accordance with the practice of the present invention, chemically labelled polynucleotide or oligonucleotide probes are employed to detect analytes by having the capacity to generate a reliable, easily quantifiable soluble signal.

Analytes to be detected by the detection processes of this invention may be present in any biological or non-biological sample, such as clinical samples, for example, blood urine, feces, saliva, pus, semen, serum, other tissue samples, fermentation broths, culture media, and the like. If necessary, the analyte may be pre-extracted or purified by known methods to concentrate its nucleic acids. Such nucleic acid concentration procedures include, for example, phenol extraction, treatment with chloroform-isoamyl alcohol or chloroform-octanol, column chromatography

(e.g., Sephadex, hydroxyl apatite), and CsCl equilibrium centrifugation. The analyte, separated from contaminating materials, if present, is, according to the present invention, fixed in hybridizable form to a solid support.

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^{Analytes}
~~In accordance with the practice of this invention, analytes~~ in a biological sample are preferably denatured into single-stranded form, and then directly fixed to a suitable solid support. Alternatively, the analyte may be directly fixed to the support in double-stranded form, and then denatured. The present invention also encompasses indirect fixation of the analyte, such as in in situ techniques where the cell is fixed to the support and sandwich hybridization techniques where the analyte is hybridized to a polynucleotide sequence that is fixed to the solid support. ^{It}
~~In the practices of this invention, it~~ is preferred that the solid support to which the analyte is fixed be non-porous and transparent, such as glass, or alternatively, plastic, polystyrene, polyethylene, dextran, polypropylene and the like. Conventional porous materials, e.g., nitrocellulose filters, although less desirable for practice of the method of the present invention, may also be employed as a support.

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It is also highly desirable that the analyte be easily fixed to the solid support. The capability to easily fix the analyte to a transparent substrate would permit rapid testing of numerous samples by the detection techniques described herein.

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Chemically-labeled probes ~~according to the invention~~ are then brought into contact with the fixed single-stranded analytes under hybridizing conditions. The probe ~~according to the present invention~~ is characterized by having covalently attached to it a chemical label which consists of a signalling moiety capable of generating a soluble

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signal. Desirably, the polynucleotide or oligonucleotide probe provides sufficient number of nucleotides in its sequence, e.g., at least about 25, to allow stable hybridization with the complementary nucleotides of the analyte. The hybridization of the probe to the single-stranded analyte with the resulting formation of a double-stranded or duplex hybrid is then detectable by means of the signalling moiety of the chemical label which is attached to the probe portion of the resulting hybrid. Generation of the soluble signal provides simple and rapid visual detection of the presence of the analyte and also provides a quantifiable report of the relative amount of analyte present, as measured by a spectrophotometer or the like.

The method of the present invention involving the colorimetric or photometric determination of the hybridized probes employs as the signalling moiety reagents which are capable of generating a soluble signal, e.g., a color change in a substrate in solution. Preferable components of the signalling moiety include enzymes, chelating agents and co-enzymes, which are able to generate colored or fluorescent soluble signals. Specifically, certain chromogens upon contact with certain enzymes are utilizable in the method of the present invention. The following Table I lists exemplary components for the signalling moiety of the present invention. Each chromogen listed is reactive with the corresponding enzyme to produce a soluble signal which reports the presence of the chemically-labeled probe analyte hybrid. The superscript notation (*) indicates that the chromogen fluoresces, rather than produces a color change.

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<u>ENZYME</u>	<u>CHROMOGEN</u>
alkaline phosphatase or acid phosphatase	*4-Methylumbelliferyl phosphate *bis (4-Methylumbelliferyl phosphate 3-0-methylfluorescein. *Flavone-3-diphosphate triammonium salt p-nitrophenyl phosphate 2Na.
peroxidase	*Tyramine hydrochloride *3-(p-hydroxyphenyl) Propionic acid *p-Hydroxyphenethyl alcohol 2,2'-Azino-Di-3-Ethylbenzthiazoline sulfonic acid (ABTS) ortho-phenylenediamine 2HCl 0-dianisidine *5-aminosalicylic acid p-cresol 3,3'-dimethyloxybenzidine 3-methyl-2-benzothiazoline hydrazone tetramethyl benzidine
β-D-galactosidase	0-nitrophenyl β-D-galactopyranoside 4-methylumbelliferyl-β-D-galactoside
glucose-oxidase	ABTS

As another aspect of the present invention, the signalling moiety may be attached to the probe through the formation of a bridging entity or complex. Likely candidates for such a bridging entity would include a biotin-avidin bridge, a biotin-streptavidin bridge, or a sugar-lectin bridge.

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Once the fixed probe-analyte hybrid is formed, the method may further involve washing to separate any non-hybridized probes from the area of the support. The signalling moiety may also be attached to the probe through the bridging moiety after the washing step to preserve the materials employed. Thereafter, another washing step may be employed to separate free signalling moieties from those attached to the probe through the bridging moiety.

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Broadly, ^{the invention provides} ~~the practices of this invention~~ provide hybridization techniques which provide the same benefits as enzyme linked immunosorbent assay techniques, i.e, the qualitative and quantitative determination of hybrid formation through a soluble signal. Various techniques, depending upon the chemical label and signalling moiety of the probe, may be employed to detect the formation of the probe-analyte hybrid. It is preferred, however, ~~in the practices of this invention,~~ to employ spectrophotometric techniques and/or colorimetric techniques for the determination of the hybrid. These techniques permit not only a prompt visual manifestation of the soluble signal generated by the signalling moiety on the double-stranded hybrid, but also permit the quantitative determination thereof, i.e., by the enzymatic generation of a soluble signal that can be quantitatively measured.

Yet another aspect of the method of the present invention involves generating the soluble signal from the probe-analyte hybrid in a device capable of transmitting light therethrough for the detection of the signal by spectrophotometric techniques. Examples of devices useful in the spectrophotometric analysis of the signal include conventional apparatus employed in diagnostic laboratories, i.e., plastic or glass wells, tubes, cuvettes or

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arrangements of wells, tubes or cuvettes. It may also be desirable for both the solid support to which the analyte is fixed and the device to be composed of the same material, or for the device to function as the support in addition to facilitating spectrophotometric detection.

A further aspect of the present invention provides products useful in the disclosed method for detection of a polynucleotide sequence. Among these products is a device containing a portion for retaining a fluid. Such portion contains an immobilized polynucleotide sequence hybridized to a polynucleotide or oligonucleotide probe. The probe, as described above, has covalently attached thereto a chemical label including a signalling moiety capable of generating a soluble signal. Also part of the device is a soluble signal, preferably a colored or fluorescent product, generatable by means of the signalling moiety. The portion of the device for containing the fluid is desirably a well, a tube, or a cuvette. A related product of the invention is an apparatus comprising a plurality of such devices for containing a fluid, in which at least one such device contains the above-described immobilized polynucleotide sequence, polynucleotide or oligonucleotide probe, signalling moiety, and soluble signal. Additionally the present invention provides for the novel product of a non-porous solid support to which a polynucleotide is directly fixed in hybridizable form. Such a fixed sequence may be hybridized to another polynucleotide sequence having covalently attached thereto a chemical label including a signalling moiety capable of generating a soluble signal. As indicated above, the support is preferably transparent or translucent. Such products could be advantageously employed in diagnostic kits and the like.

Other aspects and advantages of the present invention will be readily apparent upon consideration of the following detailed description of the preferred embodiments thereof.

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DETAILED DESCRIPTION

The following examples are illustrative of preferred embodiments of the method of the present invention. Specifically referred to therein are methods for fixing the analyte to a non-porous solid support, as well as illustrations of the use of soluble signals in polynucleotide probes as discussed above.

EXAMPLE 1

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For purposes of the present invention, ^{an}~~an~~ analyte is immobilized on a solid support, preferably a non-porous translucent or transparent support. To effect easy fixing of a denatured single-stranded DNA sequence to a glass support, ^{an}~~an~~ exemplary "fixing" procedure may involve pretreating the glass by heating or boiling for a sufficient period of time in the presence of dilute aqueous nitric acid. Approximately forty-five minutes in 5% dilute acid should be adequate to leach boron residues from a borosilicate glass surface. The treated glass is then washed or rinsed, preferably with distilled water, and dried at a temperature of about 115°C, for about 24 hours. A 10 percent solution of gamma-aminopropyltriethoxysilane, which may be prepared by dissolving the above-identified silane in distilled water followed by addition of 6N hydrochloric acid to a pH of about 3.45, will then be applied to the glass surface. The glass surface is then incubated in contact with the above-identified silane solution for about 2-3 hours at a temperature of about 45°C. The glass surface is then washed with an equal volume of water and dried overnight at a temperature of about 100°C. The resulting treated glass surface will now have

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available alkylamine thereon suitable for immobilizing or fixing any negatively charged polyelectrolytes applied thereto. [See Weetal, H. H. and Filbert, A. M., "Porous Glass for Affinity Chromatography Applications", Methods in Enzymology, Vol. XXXIV, Affinity Techniques Enzyme Purification: Part B. pp. 59-72, W. B. Jakoby and M. Wilchek, eds.]

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Such treated glass could then be employed in the practice of the method of the invention. For example, glass plates provided with an array of depressions or wells would have samples of the various denatured analytes deposited therein, the single-stranded analytes being fixed to the surfaces of the wells. Thereupon, polynucleotide probes provided with a chemical label may be deposited in each of the wells for hybridization to any complementary single-stranded analyte therein. After washing to remove any non-hybridized probe, the presence of any hybrid probe-analyte is ^{then} detectable, ~~according to the method of the present invention.~~ One detection technique as described herein involves the addition of an enzyme-linked antibody or other suitable bridging entity of the label for attachment to the probe. Subsequently a suitable substrate is added to elicit the soluble signal, e.g., a color change or chemical reaction, which is then measured colorimetrically or photometrically.

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EXAMPLE 2

A glass surface treated as described in Example 1 can be employed in the method of the present invention, wherein glucosylated DNA is employed as the labelled probe, and the signalling moiety comprises the combination of acid phosphatase and its substrate paranitrophenylphosphate.

In this procedure, glucosylated bacteriophage T₄ DNA, isolated from E.coli CR63 cultures

69 infected with phage T₄ AM82 [44³¹62³¹] and purified to be free of chromosomal DNA, or non-glucosylated, highly purified calf thymus DNA is delivered in 100μl portions to treated glass tubes in triplicate set. After 15-30 minutes at room temperature, the solution is removed and the tubes rinsed generously with PBS·Mg⁺⁺ buffer [100mM Na-K-PO₄, pH 6.5, 150mM NaCl and 10mM MgCl₂].

8 One set of tubes is checked for the presence of DNA by staining with ethidium bromide [100μl of 1 mg/ml solution, 30 minutes in the dark, at room temperature]. The staining solution is removed and the tubes rinsed and checked by UV light. Both glucosylated labelled and unlabelled DNA "probe" bound to the activated glass surface by the observed red fluorescence characteristic of ethidium bromide.

882 To another set of tubes is delivered fluorescein-labelled ConA [100μl of 0.1 mg/ml in PBS·Mg⁺⁺ buffer]. The Concanavalin A [ConA] is obtained and solubilized in 2.0M NaCl at a concentration of 50 mg/ml, and fluorescein-labelled by reacting ConA with fluorescein isothiocyanate at an FITC to protein molar ratio of 3 to 1 in 0.1M sodium borate solution at a pH of 9.2 and at a temperature of 37°C for 60 minutes. Any unreacted FITC is removed by gel filtration on Sephadex G-50. After 60 minutes at room temperature, the solution is removed and the tubes rinsed and checked under UV light. ConA bound only to glucosylated DNA in tubes containing T₄ DNA.

82 To the third set of tubes is delivered 100μl of unlabeled ConA in PBS·Mg⁺⁺ buffer. After 60 minutes at room temperature, the tubes are rinsed free of ConA with 0.2M Imidazole buffer pH 6.5.

8 Acid phosphatase is then added [0.005 units in 100μl at 0.2 percent phosphatase-free BSA] and the tubes are incubated at room temperature for 30 minutes. After rinsing with 0.15M NaCl to remove

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any unbound enzyme, 0.1mM paranitrophenylphosphate
in 0.2M ^{imidazole} ~~imidazole~~ at pH 6.5 is added and incubation
~~continued~~ ^{continued} for 60 minutes at 37°C. The enzyme reac-
tion is terminated by adding 1.0 ml of 0.5 percent
sodium bicarbonate and absorbance is determined at
A₃₀₀.

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The resulting observed test results indi-
cate that acid phosphatase, one component of the
signalling moiety gives a positive visible color
reaction, upon reaction with its chromogen, only in
tubes containing "probe" T₄ DNA and bridging moiety,
ConA, but ^{is} ~~was~~ washed off from the tubes which
contained ~~only~~ ConA or ConA and calf thymus DNA.

EXAMPLE 3

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In an example of the method of the present
invention, phage lambda DNA was employed as the
analyte, glucosylated DNA as the labelled probe,
ConA as the bridging entity and alkaline phosphatase
with paranitrophenylphosphate as the signalling
moiety. Bacteriophage lambda, obtained by heat
induction of E.coli stain W3350 lysogenic for λ C₁857
phage, was employed for the preparation of phage
lambda DNA. In these tests, the analyte, phage
lambda DNA, was immobilized on an activated glass
surface according to the following procedure. After
rinsing with buffer, glass tubes were coated with
100µl of coating solution [50 percent formamide, 5X
SSC, 100µg salmon sperm DNA 0.2 percent polyvinyl
pyrrolidone, 0.1 percent Triton X-100, 0.2 percent
BSA and 0.05 percent SDS] at 42°C for 90-120 minutes.
The coating solution was removed and the ¹⁴ surface was
covered with 100µl of coating solution containing
phage lambda DNA.

Phage lambda DNA employed as the probe is
nick translated with maltose-triose dUTP to introduce
glucosyl residues into the DNA. The glucosylated

minutes and rapidly cooled in ice bath immediately before use. The tubes were then incubated with probe at 42°C for 24 hours. The solution was removed and tubes were rinsed with PBS·Mg⁺⁺ buffer. As described above in example 2, ConA is added to the tubes in PBS·Mg⁺⁺ buffer. After 60 minutes at room temperature the tubes are rinsed with 0.2M Imidazole buffer.

Also as described in Example 2, the signaling moiety components, acid phosphatase and paranitrophenyl phosphate, are sequentially introduced into the tubes, to generate the detectable soluble signal. In these tests, the glucosyl moiety of the DNA probe is one bridging moiety of the chemical label, and reacts with and is strongly attracted to the second bridging moiety, ConA. The results indicated that acid phosphatase was not washed off from the tubes which contained glucosylated probe, whereas tubes containing non-labelled probe did not show any enzyme activity.

EXAMPLE 4

As in the above example employing a glucosylated DNA as the labelled probe, wherein the glucosyl moiety serves as part of the chemical label, comparable results may also be achieved, ^{by} ~~in the practice of this invention~~ employing a biotin-labeled DNA probe. When biotin is employed as a bridging moiety of the chemical label of the DNA probe, the presence of the biotin-labeled DNA probe would be elicited or detected by means of an avidin or strept-avidin-linked enzyme, since avidin is strongly reactive with or strongly bonds to biotin.

For example, a biotin-labeled DNA probe would readily be detected by an enzyme complex of the character avidin^{-biotin}-alkaline phosphatase. More specifically, the presence of the biotin-labeled DNA probe would readily be detected by contacting

a DNA probe would readily be detected by contacting the hybrid containing the biotin-labeled probe with the enzyme complex avidin-biotin-alkaline phosphatase, and bringing the resulting probe and avidin-biotin-alkaline phosphatase complex into contact with a suitable substrate which, upon contact with the enzyme, would produce a soluble signal that would be readily noticed or ^{be} capable of being determined, both qualitatively and quantitatively, by photometric and/or colorimetric means. If desired, instead of an avidin-biotin-enzyme complex, there could be used an antibody to biotin for attachment to the biotin moiety of the biotin-labeled DNA probe, followed by a complex comprising anti-antibody-enzyme in the manner described above.

a EXAMPLE 5

The advantages of ~~the practices~~ of this invention are also obtainable when the probe is immobilized on a non-porous plastic surface. When a plastic surface is employed, it is sometimes desirable to increase the effectiveness or uniformity of the fixation by pretreating the plastic surface.

Because polystyrene from various batches or sources exhibits different binding capacities, the adherence or fixing of DNA to a polystyrene surface is improved by treating the surface with an amino-substituted hydrophobic polymer or material. Previous experiments demonstrated that addition of dodecylamine (DDA) to polystyrene resulted in a uniform binding coefficient of polystyrene plates of different batches. Another technique for improving the fixing or uniformity of the plastic surface for fixing DNA involves treatment of the surface with polylysine (PPL).

In tests involving the fixing of DNA to a plastic surface, biotinylated DNA (b-DNA) was

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a denatured and aliquoted into Dynatech, Immulon II™
~~removable~~^{removable} wells. Samples were allowed to dry onto
the plastic surface at 37°C. The amount of bound
b-DNA was determined by sequential addition of goat
anti-biotin antibody and rabbit anti-goat antibody
complexed to the signalling moiety, alkaline phos-
phatase, followed by development with p-nitrophenyl
phosphate in diethanolamine buffer, pH 9.6. Enzymatic
activity was monitored at 405 nm utilizing the auto-
matic Dynatech Micro ELISA Scanner. This procedure
enables quantitation of the amount of bound DNA and
therefore the degree of biotinylation. To increase
the sensitivity of detection, a fluorogenic substrate
such as 4-methylumbelliferyl-phosphate, or its
analogues, with companion enzymes, may be used.

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a In a further example of the method, dena-
tured adenovirus 2 DNA, the analyte, was bound to
polystyrene plates as described above. After blocking
with Denhardt's formamide blocking buffer, several
biotinylated probes, β -adeno-2-DNA and lambda DNA
were hybridized to the immobilized DNA. To one set
of immobilized DNA, no probe was added. The extent
of hybridization was determined by means of ~~of~~ the
antibody-enzyme reaction as described above. It was
observed that only the homologous adeno-2 probe
hybridized. This technique demonstrated that in
vitro hybridization under these conditions is specific
and can be monitored quantitatively by the method of
the present invention.

Other methods for enabling fixation of single~~s~~
stranded analyte to a solid support for use in the
method of the present invention include the following.

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EXAMPLE 6

In further tests, radioactively-labeled
DNA was prepared by nick translation with [³H]dATP.
The labelled, non-biotinylated denatured DNA [2000 ng

9 to 5 ng] was applied to DDA-coated polystyrene plates. The test samples or plates were not allowed to dry. After incubation at 37°C for periods of 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 18 hours, samples were counted. Binding was maximal after two hours of incubation, however, 50 percent of the originally applied DNA bound regardless of the concentration, thereby indicating that there is an equilibrium between bound and unbound DNA.

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9 In other tests, polystyrene microfilter wells were nitrated using the procedure of Filipsson and Hornby, Biochem. J. 120, 215 (1970). The polystyrene wells were immersed for 20 minutes in a mixture of concentrated nitric and sulfuric acid [41 percent, v/v] cooled to 0°C. The wells were then washed thoroughly with water and subsequently heated to 70°C in a 6 percent solution of sodium dithionate in 2M potassium hydroxide. After 4 hours, the wells were washed thoroughly with 0.5M hydrochloric acid and distilled water.

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8 To produce 6-aminohexane linked polystyrene, 6-amino-caproic acid-N-hydroxysuccinimide ester hydrobromide [5 mg thereof dissolved in 0.2M dimethylformamide prepared by reacting 6-aminocaproic acid hydrobromide with N-hydroxysuccinimide and dicyclohexyl carbodiimide in dimethylformamide and recrystallized from isopropyl alcohol] was added to 0.1M sodium borate [0.4ml]. Amino-derivitized polystyrene microfilter wells filled with this solution were allowed to react at room temperature for 4 hours and then washed thoroughly with distilled water. The resulting treated wells absorbed H-labeled DNA from aqueous solution at pH less than 9.5.

An improved capability for fixing or immobilization of DNA to non-porous siliceous solid supports, such as glass and plastic, is also provided by treatment with a coating of an epoxy resin. For

example, treatment of glass or polystyrene surfaces with commercially available epoxy glues, such as a solution of epoxy glue in ethanol [1 percent w/v] serves this purpose. These epoxy solutions are applied to the surfaces or wells, and the solvent, ethanol, evaporated thereupon at a temperature of 37°C, thereby providing a polyamine polymeric coating on the treated surface. These surfaces were found to absorb ³H-labeled DNA from aqueous solution at pH less than 9.5.

EXAMPLE 7

Yet another example of the method of the present invention, including fixing the polynucleotide analyte sequence directly to a non-porous solid support, such as a conventional microtiter well, may be performed according to the procedures outlined below.

Conventional microtiter well plates can be pre-rinsed with 1M ammonium acetate (NH₄OAc), in an amount of 200µls/well. Analyte DNA would be diluted to 10-200ng/50ul in water or 10mM Tris-HCl at pH 7.5 and 1mM EDTA(TE). After boiling for 5 minutes and quick cooling in ice water, an equal volume of 2M NH₄OAc would be added and 50ul of analyte DNA is added per well, giving 5-100ng of analyte DNA per well. After open plate incubation for 2 hours at 37°C, the wells can be sealed and plates stored at 4°C. Alternatively, open plates can be incubated at 37°C until the wells are dry, at which point the plates can be sealed, and stored at 4°C for up to one-two months. Single-stranded analyte DNA is now fixed to the wells.

An alternative method to denature and then fix the analyte DNA to the well is to add 50ul of DNA in TE to wells at a concentration of 10-200ng/50ul. After adding 25ul at 0.9N NaOH and mixing, the plates can be incubated for 10 minutes at room temperature.

After adding 25ul of 4M NH_4OAc , the open plate may be incubated at 37°C for 4 hours or until dry and the plates sealed and stored at 4°C until ready to use.

To prepare the plates for hybridization, the wells would be rinsed twice with 0.3m NaCl, 0.03m sodium citrate (2X SSC) (200ul/well) buffer regardless of whether the plate was dried or not. Preferably, the wells can be rinsed once with 2X SSC/1% Triton X-100 after the two 2X SSC rinses. Plates should be blotted on absorbent paper before beginning each rinse.

To hybridize the fixed analyte with a probe, the following protocol would be followed. A nick translated probe would be heat denatured and added to a hybridization solution containing 30% formamide (deionized), 2X-4X SSPE (20X SSPE = 3.6 M NaCl, 0.2M NaPO_4 , pH 7.4, 0.02M EDTA) depending on the GC content of probe, 0.1% SDS, and 5.0% dextran sulfate to give a final concentration of 0.2-1.0 ug probe/ml. An alternative hybridization solution contains 30% formamide (deionized), 2X-4X SSPE, 1.0% Triton X-100, and 5.0% dextran sulfate and 0.2-1.0 ug probe/ml. 100ul of the selected hybridization mixture is added to each well. After sealing the plates, they are incubated at 37°C for a desired time.

The hybridization solution is poured out, or collected by aspiration for reuse if desired. The plates are rinsed twice with 2X SSC and 0.1% SDS or 2X SSC and 0.1% Triton X-100 according to whether the first or second hybridization solution identified above was employed. At this point two to four stringency rinses of SSC and detergent are preferably performed by heating the buffer to the desired temperature and adding it hot to the wells. Formamide and low SSC or SSPE can be used at 37-40°C to achieve the desired stringency. Following stringency washes,

wells are rinsed twice with 1X SSC or 1X SSC and 0.1% Triton X-100, and the plates are now ready for detection.

See Detection of the fixed hybridized analyte probe according to the invention may employ the procedure for commercially available ELISA assays using the sensitive DETEK® 1-alkaline phosphatase or DETEK® 1-horseradish peroxidase assays (Enzo Biochem, Inc.). Beginning at the blocking procedure, the standard method is employed except that after blocking, no rinsing step is used. Complex diluted in 1X complex dilution buffer is thereafter added as taught in these commercially available assays.

As will be apparent to those skilled in the art in the light of the foregoing disclosure, many alterations, modifications and substitutions are possible in the practice of this invention, without departing from the spirit or scope thereof. Consequently, only such limitations as appear in the appended claims should be placed upon the scope of the invention.

Can what is claimed in:

Claim 1

WHAT IS CLAIMED IS:

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1. A method for detecting a polynucleotide sequence which comprises:
fixing said polynucleotide sequence to a solid support in hybridizable form;
forming an entity comprising said polynucleotide sequence hybridized to a polynucleotide or oligonucleotide probe, said probe having covalently attached thereto a chemical label comprising a signalling moiety capable of generating a soluble signal; and
detecting said polynucleotide sequence by means of the generation of a soluble signal.

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2. The method according to claim 1, ^{wherein} characterized in that said detecting step comprises spectrophotometric techniques.

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3. The method according to Claim 1, ^{wherein} characterized in that said soluble signal is selected from the group consisting of a colored product, ^{and} or a fluorescent product.

Claim 3
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4. The method according to claim 1, ^{wherein} characterized in that said signalling moiety is selected from the group consisting of an enzyme, a chelating agent and a co-enzyme.

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5. The method according to Claim 1, ^{wherein} characterized in that said solid support is non-porous.

6. The method according to Claim 6, ^{wherein} characterized in that said solid support is transparent or translucent.

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a ^{wherein} 5. The method according to claim ¹,
~~characterized in that~~ said solid support is selected
from the group consisting of glass, plastic, poly-
styrene, polyethylene, dextran and polypropylene.

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a ^{wherein} 8. The method according to Claim 1,
~~characterized in that~~ said solid support is porous.

C
a ^{wherein} 6. The method according ^{to} claim 1,
~~characterized in that~~ said polynucleotide sequence
is directly fixed to said solid support.

a ^{wherein} 10. The method according to claim ⁶,
~~characterized in that~~ said polynucleotide sequence
is fixed to said solid support in single stranded
form.

a ^{wherein} 11. The method according to claim 1,
~~characterized in that~~ said signalling moiety is
attached to said polynucleotide or oligonucleotide
probe through the formation of a complex.

a ^{wherein} 12. The method according to claim ¹¹,
~~characterized in that~~ said complex is selected from
the group consisting of biotin and avidin, biotin
and streptavidin, and a sugar and a lectin.

a ^{wherein} 10 13. The method according to claim 1,
~~characterized in that~~ said forming step further com-
prises washing to remove said polynucleotide or oligo-
nucleotide probes that do not form said entity.

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14. The method in accordance with
Claim 13, characterized in that said forming step
further comprises attaching said signalling moiety
to said polynucleotide or oligonucleotide probe

Claims 11+12

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through the formation of a complex formed after said washing.

15. The method in accordance with Claim 14, characterized in that said forming step further comprises separating free signalling moieties from said signalling moiety-probe complexes.

¹³ 16. The method according to Claim 1, wherein said detecting step further comprises generating said soluble signal in a device capable of transmitting light therethrough for the detection of said soluble signal by spectrophotometric techniques.

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¹⁴ ¹³ 17. The method ^{according to} ~~in accordance with~~ Claim 16, ^{wherein} ~~characterized in that~~ said device is selected from the group consisting of a well, a tube, a cuvette and an apparatus which comprises a plurality of said wells, tubes or cuvettes.

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18. The method according to Claim 16, ^{wherein} ~~characterized in that~~ said soluble signal is selected from the group consisting of a colored product and a fluorescent product.

*Claim 15
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¹⁶ ¹³ 19. The method according to ~~claim 16~~ ¹³, ^{wherein} ~~characterized in that~~ said solid support and said device are composed of the same materials.

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20. A device which comprises:
means for containing a fluid
comprising:
(i) an immobilized polynucleotide sequence hybridized to a polynucleotide or oligonucleotide probe, said probe having covalently attached thereto a

Claims 17-19

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chemical label comprising a signalling moiety capable of generating a soluble signal, and

(ii) a soluble signal generated by means of said signalling moiety.

21. The device according to Claim 20, wherein said means for containing a fluid is selected from the group consisting of a well, a tube, and a cuvette.

22. The device according to Claim 21, wherein said soluble signal is selected from the group consisting of a colored or fluorescent product.

23. An apparatus comprising:
a plurality of means for containing a fluid, wherein at least one of said means comprises:
(i) an immobilized polynucleotide sequence hybridized to a polynucleotide or oligonucleotide probe, said probe having covalently attached thereto a chemical label comprising a signalling moiety capable of forming a soluble signal, and
(ii) a soluble signal generated by means of said signalling moiety.

24. A non-porous solid support having directly fixed thereto a polynucleotide sequence in hybridizable form.

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25. The support according to Claim 24, ~~wherein characterized in that~~ said polynucleotide sequence is hybridized to a polynucleotide or oligonucleotide probe, said probe having covalently attached thereto a chemical label comprising a signalling moiety capable of generating a soluble signal.

a ~~wherein~~ 26. The support according to Claim 24,
~~characterized in that said support is a transparent~~
or translucent support.

*add
A2*

*add
C2*

*add
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METHODS AND STRUCTURES EMPLOYING
CHEMICALLY-LABELED POLYNUCLEOTIDE PROBES

ABSTRACT OF THE DISCLOSURE

Polynucleotide sequences in a sample of biological or nonbiological material are detected by a method involving fixing of the sequences on a solid support and forming an entity between the fixed sequences and chemically-labeled polynucleotide or oligonucleotide probes having a sequence complementary to the fixed sequence for determining the identification and/or presence of the target polynucleotide sequences. The chemical label covalently or non-covalently attached to the probe comprises a signaling moiety capable of generating a soluble signal detectable by spectrophotometric assay techniques.

**DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHODS AND STRUCTURES EMPLOYING CHEMICALLY-LABELED POLYNUCLEOTIDE
the specification of which PROBES

(check is attached hereto.
one)

was filed on _____ as
Application Serial No. _____
and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I do not know and do not believe that the invention was ever patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application.

I do not know and do not believe that the invention was in public use or on sale in the United States of America more than one year prior to this application.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
<u> </u>	<u> </u>	<u> </u>	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
<u> </u>	<u> </u>	<u> </u>	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
<u> </u>	<u> </u>	<u> </u>	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

<u>461,469</u>	<u>Jan. 27, 1983</u>	<u>Pending</u>
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

<u> </u>	<u> </u>	<u> </u>
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

As a named inventor, I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

<u> </u>	<u>Charles B. Smith, Esq. - Reg. No. 16,763</u>
<u> </u>	<u>James F. Haley, Esq. - Reg. No. 27,794</u>
<u> </u>	<u>Mary E. Bak, Esq. - Reg. No. 31,215</u>
<u> </u>	<u>Samuel B. Abrams, Esq. - Reg. No. 30,605</u>

Send Correspondence to: Mary E. Bak, Esq.
Fish & Neave
29th Floor
875 Third Avenue
New York, New York 10022

Direct telephone calls to: Mary E. Bak
(212) 715-0600

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor Jannis G. Stavrianopoulos

Inventor's signature _____ Date _____

Residence 515 West 59th Street, New York, New York 10019

Citizenship United States of America

Post Office Address _____

Full name of second joint inventor Dollie Kirtikar

Second Inventor's signature _____ Date _____

Residence 42-72 80th Street, Elmhurst, New York 11373

Citizenship United States of America

Post Office Address _____

Full name of third joint inventor Kenneth H. Johnston

Third Inventor's signature _____ Date _____

Residence 95 Horatio Street, New York, New York 10014

Citizenship Canada

Post Office Address _____

Full name of fourth joint inventor Barbara E. Thalenfeld

Fourth Inventor's signature _____ Date _____

Residence 250 East 39th Street, New York, New York 10016

Citizenship United States of America

Post Office Address _____



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Mary E. Bak
c/o Fish & Neave
875 Third Avenue
New York, New York 10022

Applicant(s): Jannis G. Stavrianopoulo

Serial Number: et al 732,374

Filing Date: 5-9-85

Title: Methods And Structures
Employing Chemically (ETC)

Notice to File Missing Parts of Application-
Filing Date Granted

If all missing parts are filed within the period set below, the total amount owed by applicant as a large entity, small entity (verified statement filed), is \$ 100.00

1. The statutory basic filing fee is: missing insufficient. Applicant as a large entity, small entity, must submit \$ _____ to complete the basic filing fee and MUST ALSO SUBMIT THE SURCHARGE AS INDICATED BELOW.
2. Additional claim fees of \$ _____ as a large entity, small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due. NO SURCHARGE IS REQUIRED FOR THIS ITEM.
3. The oath or declaration is:
 - missing.
 - does not cover items omitted at the time of execution.
 An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Serial Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.
4. The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63 identifying the application by the above Serial Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.
5. The signature to the oath or declaration is: missing; a reproduction; by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Serial Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.
6. The signature of the following joint inventor(s) is missing from the oath or declaration: _____. Applicant(s) should provide, if possible, an oath or declaration signed by the omitted inventor(s), identifying this application by the above Serial Number and Filing Date. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.
7. The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$20.00 under 37 CFR 1.17(k), unless this fee has already been paid. NO SURCHARGE IS REQUIRED FOR THIS ITEM.
8. Other: \$100.00 surcharge is required.

A Serial Number and Filing Date have been assigned to this application. However, to avoid abandonment under 37 CFR 1.53(d), the missing parts and fees identified above in items 1 and 3-6 must be timely provided ALONG WITH THE PAYMENT OF A SURCHARGE OF \$100.00 for large entities or \$50.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e). Applicant is given ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE of this application, WHICHEVER IS LATER, within which to file all missing parts and pay any fees. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Direct the response to, and any questions about, this notice to the undersigned, Attention, Application Branch, and include the above Serial Number and Filing Date.

[Signature]
FC: [Signature]
(703) 597-3337

350⁰⁰117
2/2



ENZ 7 CIP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Jannis G. Stavrianopoulos et al.
Serial No. : 732,374
Filed : May 9, 1985
For : METHODS AND STRUCTURES EMPLOYING
CHEMICALLY-LABELLED POLYNUCLEOTIDE
PROBES

New York, New York
September 11, 1985

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Attention: G. Barnhart
Application Branch

PETITION FOR EXTENSION OF TIME
FOR RESPONDING TO NOTICE TO
FILE MISSING PARTS OF APPLICATION

Sir:

Pursuant to 37 C.F.R. § 1.136, applicants request that time for responding to the Notice To File Missing Parts of Application dated June 4, 1985 be extended for a period of three months from July 9, 1985, i.e., two months from the filing date of the application, up to and including September 9, 1985.

Pursuant to 37 C.F.R. § 1.17, applicants have enclosed a check in the amount of \$350.00 to cover the fee for this two month extension.

The Commissioner is hereby authorized to charge payment of any additional filing fees required by this paper under 37 C.F.R. § 1.16, or credit any overpayment of same, to

09/23/85 732374 1 117 350.00 CK

Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is enclosed.

Respectfully submitted,

Mary E. Bak

James F. Waley, Jr. (Reg. No. 27,794)
Mary E. Bak (Reg. No. 31,215)
Attorneys for Applicants
c/o FISH & NEAVE
875 Third Avenue, 29th Floor
New York, New York 10022
Tel.: (212) 715-0600

732874



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
0677321374	05/07/06	STAVRIANOUPOULOS	J ENZ-701P

MARY E. BAK
FISH & NEAVE
29TH FLOOR
875 THIRD AVENUE
NEW YORK, NY 10022

EXAMINER	
MOLEATT/07	
ART UNIT	PAPER NUMBER
1.27	4

DATE MAILED: 01/19/08

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

This application has been examined Responsive to communication filed on _____ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), _____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- 1. Notice of References Cited by Examiner, PTO-892.
- 2. Notice re Patent Drawing, PTO-948.
- 3. Notice of Art Cited by Applicant, PTO-1449
- 4. Notice of informal Patent Application, Form PTO-152
- 5. Information on How to Effect Drawing Changes, PTO-1474
- 6. _____

Part II SUMMARY OF ACTION

- 1. Claims 1-26 are pending in the application.
Of the above, claims _____ are withdrawn from consideration.
- 2. Claims _____ have been cancelled.
- 3. Claims _____ are allowed.
- 4. Claims 1-26 are rejected.
- 5. Claims _____ are objected to.
- 6. Claims _____ are subject to restriction or election requirement.
- 7. This application has been filed with informal drawings which are acceptable for examination purposes until such time as allowable subject matter is indicated.
- 8. Allowable subject matter having been indicated, formal drawings are required in response to this Office action.
- 9. The corrected or substitute drawings have been received on _____ . These drawings are acceptable; not acceptable (see explanation).
- 10. The proposed drawing correction and/or the proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been approved by the examiner. disapproved by the examiner (see explanation).
- 11. The proposed drawing correction, filed _____, has been approved. disapproved (see explanation). However, the Patent and Trademark Office no longer makes drawing changes. It is now applicant's responsibility to ensure that the drawings are corrected. Corrections **MUST** be effected in accordance with the instructions set forth on the attached letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474.
- 12. Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has been received not been received
 been filed in parent application, serial no. _____; filed on _____.
- 13. Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
- 14. Other

Serial No. 732374

-3-

Art Unit 127

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent.

Claims 1, 3, 4, 8-10 and 13 are rejected under 35 U.S.C. 102(a) as being anticipated by Falkow et al.

Falkow et al teach a method of detecting polynucleotide sequences in a test sample, by fixing the polynucleotide sequences to a nitrocellulose filter and incubating the filter with a chemical labeled probe (See column 4, lines 12-18), ^{and} forming hybrids where nucleic acid sequences are homologous. The filter is rinsed and assayed for the presence of a label which will be a soluble signal when enzymes or fluorescent compounds are utilized.

Claims 2 and 11-19 are rejected under 35 U.S.C. 103 as being unpatentable over Falkow et al as applied to claims 1, 3, 4, 8-10 and 13 above, and further in view of Kourilsky et al.

Kourilsky et al teach a liquid hybridization assay, which utilizes an enzyme labeled probe. The probe is labeled by the formation of a complex, for example, by coupling avidin to an enzyme, such a beta-galactosidase, and by coupling biotin to the probe. The enzyme-avidin and the probe-biotin are mixed and complex thus producing a labeled probe (See column 4, lines 36-61).

Serial No. 732374

-4-

Art Unit 127

Further, Kourilsky et al teach determining the presence of hybrids by the measurement of the generated soluble signal by optical density (O.D.) readings in a spectrophotometer. Further, Kourilsky et al teach measuring the O.D. in tubes. In the absence of unexpected results, it would be obvious to modify the method of Falkow et al by attaching the label to the probe utilizing the complexing technique of Kourilsky et al, ^{as a} matter of utilizing known methods of attaching labels to nucleic acids. Further, it would be obvious to measure the soluble signal in a tube with a spectrophotometer as a matter of utilizing one known detection system for another.

Claims 5-7 are rejected under 35 U.S.C. 103 as being unpatentable over Falkow et al in view of Gillespie et al or Weetall.

Falkow et al are applied as above.

Gillespie et al teach utilizing a glass filter to immobilize RNA in a hybridization assay. Weetall teaches the coupling of antigens, such as nucleotides (See column 3, lines 4-48) to siliceous material such as glass. In the absence ^{of unexpected results}, it would be obvious to select a nonporous material, such as glass, to immobilize nucleic acids, as suggested by Gillespie et al or Weetall and substitute this support into the method of Falkow et al for the nitrocellulose filter as a matter of substituting one support which will bind nucleic acids for another. It is admitted that the glass supports of both

Serial No. 732374

-5-

Art Unit 127

Gillespie et al and Weetall are porous; however, these references teach the use of glass to immobilize nucleic acids is known in the art. It is deemed to be obvious to select a nonporous support rather than a porous one as a mere matter of experimental design.

Claims 20-23 are rejected under 35 U.S.C. 103 as being unpatentable over Kourilsky et al in view of Falkow et al.

The references are applied as above. In the absence of unexpected results, it would be obvious to place the immobilized polynucleotide sequence hybridized to a probe with a chemical label as taught by Falkow et al into a tube as taught by Kourilsky et al for spectrophometric measurement of the soluble signal released in measuring the presence of the hybrid, as a mere matter of utilizing the tube as the means for measuring the signal. In regard to claim 23, it would be obvious to utilize a plurality of tubes containing the immobilized sequence hybridized to the probe, if one wished to perform a number of assays.

Claims 24-26 are rejected under 35 U.S.C. 103 as being unpatentable over Gillespie et al or Weetall in view of Falkow et al.

The references are applied as above. In the absence of unexpected results, it would be obvious to utilize a non-porous solid support composed of glass or

Serial No. 732374

-6-

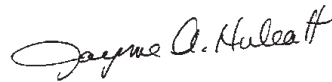
Art Unit 127

other siliceous materials to immobilize the polynucleotide sequences of Falkow et al because Gillespie et al or Weetall teach that nucleic acids will bind to glass. The choice of utilizing nonporous glass versus porous glass appears to be a matter of design choice.

Any inquiry concerning this communication should be directed to Jayme Huleatt at telephone number 703-557-1748.

HULEATT:pc

1/13/88



JAYME HULEATT
EXAMINER
ART UNIT 127

P.O. 892 (REV. 3-78)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUPART UNIT	ATTACHMENT TO PAPER NUMBER
		732374	127	4
NOTICE OF REFERENCES CITED		APPLICANT(S) STAVRIANOPOULOS et al		

U.S. PATENT DOCUMENTS							
*	DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE	
A	4358535	11/9/82	FALKOW et al	435	6x	12/8/80	
B	4581333	4/8/86	KOURILSKY et al	435	6	4/13/79	
C	4483920	11/20/84	GILLESPIE et al	435	6	5/17/82	
D	3652761	3/28/72	WEETALL et al	435	7x		
E							
F							
G							
H							
I							
J							
K							

FOREIGN PATENT DOCUMENTS								
*	DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG. PP. SPEC.	
L								
M								
N								
O								
P								
Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)	
R	
S	
T	
U	

EXAMINER <i>Jayme A. Halvath</i>	DATE 12/18/87
-------------------------------------	------------------

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

FORM PTO-122 (REV. 12-87)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	
TITLE REPORT			PAPER NO. <i>05</i>
A. APPLICATION FILE DATA			
1. SERIAL NO. <i>732374</i>		2. FILED <i>05/09/85</i>	
3. INVENTOR(S) - FULL NAME(S) <i>Jannis G. Starnianopoulos</i> <i>Dolli Kiritlan Ed</i>			
4. DIVISION OF			
5. CONTINUATION OF			
6. REISSUE OF			
7. SUBSTITUTE OF			
B. ASSIGNMENT RECORD DATA			
The assignment records reveal that the Title appears to be vested in:			
<input checked="" type="checkbox"/> (1.) Inventor(s) <input type="checkbox"/> (2.) As endorsed <input type="checkbox"/> (3.) As the record now stands, the patent, when granted, will issue in the name of the inventor(s). <input type="checkbox"/> (4.) Other			
EXAMINED UP TO AND INCLUDING <i>01/22/88</i>	THIS CERTIFICATE DATED		<i>02/16/88</i>
BRANCH CHIEF OF ASSIGNMENT SEARCH BRANCH DIANE G. RUSSELE			

7.9.88



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#6
C.M.
2/17/88

Applicants: Jannis G. Stavrianopoulos, et al.
Serial No. 732,374) Art Unit: 127
Filed May 9, 1985) Examiner:
For: METHODS AND STRUCTURES EMPLOYING CHEMICALLY-LABELLED
POLYNUCLEOTIDE PROBES

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

RECEIVED
FEB 1 1988
GROUP 120

Sir:
SUBSTITUTE POWER OF ATTORNEY

Enzo Biochem, Inc., a corporation of the State of New York, having its principal place of business at 325 Hudson Street, New York, New York 10013, the owner of the entire right, title and interest in and to the above-identified application and the invention disclosed and/or claimed therein hereby revokes any and all powers of attorney previously granted with respect to the above-identified application and appoints, as principal attorney, Charles J. Herron (Reg. No. 28,019), with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications to:

Charles J. Herron, Esq.
Corporate Patent Counsel
Enzo Biochem, Inc.,
325 Hudson Street
New York, New York 10013

and direct all telephone calls regarding this application to Charles J. Herron at telephone no. (212) 741-3838, extension 127.

Enzo Biochem, Inc.

Dean L. Engelhardt 1/13/88
Dean L. Engelhardt Date
V.P. Research (ENZ-7 CIP)

FIRST CLASS CERTIFICATE
I hereby certify that this correspondence is being deposited today with the U.S. Postal Service as first class mail in an envelope addressed to:
Commissioner of Patents & Trademarks, Washington, D.C. 20231
Charles J. Herron
Charles J. Herron, Esq.
Reg. No. 28,019
Date
Jan 14, 1988
Date



#7
107
2/17/88

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Jannis G. Stavrianopoulos, et al..

Serial No. : 732,374

) Art Unit: 127

Filed : May 9, 1985

) Examiner:

For : METHODS AND STRUCTURES EMPLOYING CHEMICALLY-LABELLED
POLYNUCLEOTIDE PROBES

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

STATUS INQUIRY

The above identified application was filed more than 2 1/2 years ago as a Continuation-In-Part of U.S. Serial Number 461,469, which was filed on January 21, 1983.

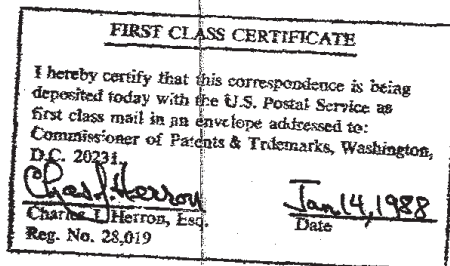
A Notice To File Missing Parts of the application under 37 CFR 1.53(d) was dated June 4, 1985 and applicants responded thereto on September 11, 1985.

No further activity is seen in our files. Applicants therefore respectfully inquire as to the status of the captioned application.

Respectfully submitted

Charles J. Herron

Charles J. Herron
Registration No. 28,019



Enz-7 CIP



**U.S. DEPARTMENT OF COMMERCE
Patent Office**

Address Only: COMMISSIONER OF PATENTS
Washington, D.C. 20231

Paper No. 8

Mailed

Huleatt Art Unit 127
06/732374 05/09/85
Jannis G. Stavrianopoulos, et al

Charles J. Herron, Esq.
Corporate Patent Counsel
Enzo Biochem, Inc.
325 Hudson Street
New York, New York 10013

MAILED

FEB 22 1988

GROUP 120

Responsive to the proposed communication re the power of attorney filed 1-19-88.

The above communication, signed by David Engelhardt, has not been entered for the reason checked below:

1. A report from the Assignment Branch dated 2-11-88, indicates that title appears to be vested in another.
2. The signature of _____, (assignee of part interest) (co-inventor) in this application, has been omitted. The paper will be entered upon receipt of confirmation signed by said co-inventor.
3. The person signing for the assignee has omitted his position, or is not an officer in the company.
4. The substitute power of attorney does not comply with Commissioner's Notice of April 23, 1957, 718 O. G. 219. It is being returned herewith.
5. There is no evidence in the records that the person appointed in the proposed power of attorney is registered to practice before the U.S. Patent Office.
6. The person signing the proposed power of attorney is without any authority of record in this application.
7. The signature of the applicant having part interest in this application has been omitted. R. 33.
8. The signature on the power is a facsimile and therefore not acceptable.
9. Other.

Kathryn P. Perry

PLEASE FURNISH YOUR ZIP CODE IN ALL CORRESPONDENCE

64V-127



RECEIVED GR...
AUG 17 1988
#9
MA
8/20/88

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Jannis G. Stavrianopoulos, et. al
Serial No. 732,374 Art Unit: 127
Filing Date: May 9, 1985 Examiner: J. Huleatt
Title: Method and Structures Employing Chemically-Labelled
Polynucleotide Probes

PETITION FOR EXTENSION OF TIME

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Applicant(s) requests that the time for taking action in this case be extended pursuant to 37 C.F.R. 1.136(a) for:

- () one month (\$56.00) (X) three months (\$390.00)
- () two months (\$170.00) () four months (\$610.00)

to July 19, 1988.

The fee set in 37 C.F.R. 1.17 for the extension of time is \$390.00.

- () Fee enclosed.
- (X) Charge fee to Deposit Account No. 05-1135.
- (X) Charge any additional fee required for this extension of time to Deposit Account No. 05-1135.

A duplicate copy of this paper is enclosed.

Also enclosed is a:

- (X) Response () Notice of Appeal () Appeal Brief

6 11229 08/16/88 732374 05-1135 110 117 390.00CH

FIRST CLASS CERTIFICATE
I hereby certify that this correspondence is being deposited today with the U.S. Postal Service as first class mail in an envelope addressed to: Commissioner of Patents & Trademarks Washington, D.C. 20231
Helen Tzagoloff
Helen Tzagoloff
Reg. No. 32317/988
Date: July 15, 1988

Respectfully submitted,

Helen Tzagoloff
Helen Tzagoloff
Registration No. 32,317

Date: _____
Case No.: Enz-7 CIP



182 4/19/88

RECEIVED GROUP 3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE 17 1988

Application of: Jannis G. Stavrianopoulos, et.al.

Serial No.: 732,374

Art Unit: 127

Filing Date: May 9, 1985

Examiner: J. Huleatt

Title: Method and Structures Employing Chemically-Labelled Polynucleotide Probes

Honorable Commissioner of Patents and Trademarks Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified patent application.

The fee has been calculated as shown below:

	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	ADDITIONAL FEE
TOTAL	28	MINUS	26	- 2	x 12	\$ 24
INDEP	2	MINUS	4	- 0	x 34	\$
() FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIMS					+110	\$
TOTAL ADDITIONAL FEE -----						\$ 24

(X) Charge Deposit Account No. 05-1135 in the amount of \$ 24

() A check in the amount of \$ _____ is attached.

(X) The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 05-1135: any filing fees under 37 CFR 1.16 for the presentation of extra claims and any patent application processing fees under 37 CFR 1.17.

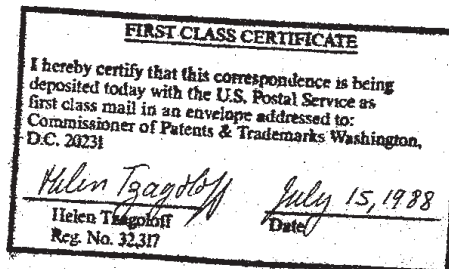
A duplicate copy of this paper is enclosed.

Date: July 15, 1988

Enzo Biochem, Inc. 325 Hudson Street New York, NY 10013 (212) 337-3355

Case No.: Enz-7 GIP

Helen Tzagoloff Registration No.: 32,317





IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

6AV-127
10/2
1/17
RECEIVED CRL
108171033
8/23/88

Re application of:
Jannis G. Stavrianopoulos, et. al..

Serial No.: 732,374

Art Unit: 127

Filed: May 9, 1985

Examiner: J. Huleatt

For: Methods and Structures Employing Chemically-Labelled
Polynucleotide Probes

July 15, 1988
New York, New York

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

RESPONSE UNDER 37 CFR 1.115

Dear Sir:

Please enter this response to the Office Action of January 19, 1988,
including the following amendments:

Amend The Specification As Follows:

Page 2, line 25, delete "or moiety."

Page 3, line 28, delete "thymidine" and insert -- thymine --.

Page 3, line 29, delete "cytidine" and insert -- cytosine --.

Page 5, line 19, delete "sequentially" and insert -- sequentially --.

Page 7, line 5, delete "expensive" and insert -- expenses --.

Page 9, line 16, delete "present".

6 11230 08/16/88 732374

05-1135 110 103

24.00CH

Enz-7 CIP

Page 9, line 20, delete "accordance with the practice of."

Page 10, line 3, delete "," after "is" and insert - - , - - after "present."

Page 10, lines 6 and 7, delete "In accordance with the practice of this invention, analytes" and insert - - Analytes - -.

Page 10, lines 17 and 18, delete "In the practices of this invention, it" and insert - - It - -.

Page 10, lines 31 and 32, delete "according to the invention."

Page 10, lines 34 and 35, delete "according to the present invention."

Page 13, lines 11 and 12, delete "the practices of this invention provide" and insert - - the invention provides - -.

Page 13, lines 19 and 20 delete "in the practices of this invention,".

Page 15, line 13, delete "on" and insert - - an - -.

Page 15, line 17, delete "on" and insert - - an - -.

Page 16, line 9, delete "the practice of".

Page 16, lines 19 and 20, delete "according to the method of the present invention".

Page 16, line 19, insert - - then - - before "detectable" and insert

----- after "detectable".

Page 18, line 2, delete "Imidazole" and insert - - imidazole - - .

Page 18, line 3, delete "contained" and insert -- continued -- .

Page 18, line 12, delete "was" and insert -- is -- .

Page 18, line 13, delete "contained" and insert -- contain -- .

Page 19, lines 24 and 25, delete "in the practices of this invention" and insert
-- by -- .

Page 19, line 34, after "avidin" insert - - - - (dash).

Page 19, line 36, delete "DNA probe would readily be detected by
contacting."

Page 20, line 8, after "or" insert -- be -- .

Page 20, line 17, delete "of the practices".

Page 21, line 2, delete "removeable" and insert -- removable -- .

Page 21, line 20, delete "B" and insert -- b -- .

Page 21, line 23, delete "of", second occurrence.

Page 22, line 27, insert a space after "isopropyl."

Cancel The Following Claims:

Cancel claims 20-23, inclusive

Amend The Claims As Follows:

Claim 2, line 2, delete "characterized in that" and insert -- wherein -- .

Claim 3, line 2, delete "characterized in that" and insert -- wherein -- .

Claim 3, line 3 delete "or" and insert - - and - -.

Claim 4, line 2, delete "characterized in that" and insert - - wherein - -.

Claim 5, line 2, delete "characterized in that" and insert - - wherein - -.

Claim 6, line 2, delete "characterized in that" and insert - - wherein - -.

Claim 7, line 2, delete "characterized in that" and insert - - wherein - -.

Claim 8, line 2, delete "characterized in that" and insert - - wherein - -.

Claim 9, line 2, delete "characterized in that" and insert - - wherein - -.

Claim 10, line 2, delete "characterized in that" and insert - - wherein - -.

Claim 11, line 2, delete "characterized in that" and insert - - wherein - -.

Claim 12, line 2, delete "characterized in that" and insert - - wherein - -.

Claim 13, line 2, delete "characterized in that" and insert - - wherein - -.

A Claim ¹⁴ ~~14~~ (Amended) The method according to [in accordance with] Claim ¹⁰ ~~10~~ ^{wherein said forming step} ~~10, which~~ [characterized in that said] forming [step], further comprises attaching said signalling moiety to said polynucleotide or oligonucleotide probe [through the formation of a complex formed] after said washing ^{step}.

B Claim ¹² ~~15~~ (Amended) The method according to [in accordance with] Claim ¹¹ ~~14~~, which [characterized in that said] further [step] comprises separating free signalling moieties from said attached signalling moieties [signalling moiety - probe complexes].

Claim 17, line 1, delete "in accordance with" and insert - - according to - -.

Claim 17, line 2, delete "characterized in that" and insert -- wherein --.

Claim 18, line 2, delete "characterized in that" and insert -- wherein --.

Claim 19, line 2, delete "characterized in that" and insert -- wherein --.

Claim 25, line 2, delete "characterized in that" and insert -- wherein --.

Claim 26, line 2, delete "characterized in that" and insert -- wherein --.

Enter the following New Claims:

~~27~~ ²⁷ A device for detecting a polynucleotide sequence according to the method of Claim 1, which device comprises a solid support, having said polynucleotide sequence fixed thereto in hybridizable form.

~~28~~ ¹⁸ 28. A kit for detecting a polynucleotide sequence, which comprises the device of Claim ~~27~~ ¹⁷ in packaged combination with a container of an oligonucleotide or polynucleotide probe, having covalently attached thereto a chemical label comprising a signalling moiety capable of generating a soluble signal.

~~29~~ ¹⁹ 29. The kit of Claim ~~28~~ ¹⁸, wherein said soluble signal is a colored product or a fluorescent product.

~~30~~ ²⁰ 30. A composition comprising a polynucleotide or oligonucleotide probe, said probe having covalently attached thereto a chemical label comprising a signalling moiety capable of generating a soluble signal and an immobilized polynucleotide sequence capable of being hybridized to said polynucleotide or oligonucleotide probe.

32
could

~~31. A composition according to Claim 30 wherein said soluble signal is selected from the group consisting of a colored product and a fluorescent product.~~

with ABC

~~32. The method according to Claim 1 wherein part of the solid support is modified to facilitate fixation of said polynucleotide sequence to said solid support.~~

Remarks

Reconsideration of the application in view of the above amendments and the following remarks is requested.

Claims 20, 21, 22 and 23 have been cancelled. Claims 2-15, claims 17-19, 25 and 26 have been amended. New claims 27-32 have been added to further define applicants' invention. No new matter has been introduced. Support for new claims may be found throughout the specification.

Claims 1-19, 24-26 and new claims 27-32 are active in the application.

The Rejections Under 35 U.S.C. 112, Second Paragraph

It is believed that the claims, as amended, are free of the ground of rejection under 35 U.S.C. 112, second paragraph.

The Rejection Under 35 U.S.C. 102

Claims 1,3,4,8-10 and 13 had been rejected under 35 U.S.C. 102 (a) as being anticipated by Falkow et al.

Claim 1, the claim of broadest scope, is directed to a method for detecting a polynucleotide sequence. The method comprises, in brief, fixing the sequence to a support and forming a hybrid with a polynucleotide probe. The hybrid is detected by means of a soluble signal which is generated by a signalling moiety. The latter is attached covalently to the probe.

Falkow et al. describe a hybridization method in which clinical samples are spotted onto an inert support, such as a nitrocellulose filter. It is especially suitable for screening of bacterial colonies for a specific

polynucleotide sequence. The cell number may be increased by placing the support on a nutrient medium. In order to allow diffusion of nutrients, the support has to be porous. The preferred method of labeling as disclosed is labeling with radionuclides (column 3, lines 25-27, column 6, line 6). This would allow for fast screening of many samples, as the authors point out in column 9, lines 1-5: "Numerous samples may be spotted on the same filter and processed simultaneously, greatly increasing clinical efficiency. The technique therefore offers significant opportunities for large scale epidemiological and surveillance studies". (Emphasis added.) In such a method, the detectable signal must be insoluble. Such a signal cannot be used for accurate quantitation of the reactants. The use of labels, other than radionuclides such as enzymes and fluorescent compounds, would also generate an insoluble signal, e.g., deposition of colored precipitates, according to this method.

Applicants' method, in which the polynucleotide signal is detected by means of a soluble signal, is not addressed to rapid screening of many colonies on a porous inert support. Applicants' method, by utilizing a solid support and a soluble signal allows for accurate quantitation of the target sequence by spectrophotometric techniques. There is no suggestion or disclosure in Falkow et. al., of accurately quantitating the target polynucleotide which is attached to a support (porous or non-porous) by means of a soluble signal. It is submitted that claim 1 is not anticipated by Falkow et. al. Claims 3,4,8,-10 and 13 are all narrower in scope than claim 1 and are allowable for the same reasons given above to distinguish claim 1.

The Rejections Under 35 U.S.C. 103

Claims 2 and 11-19 had been rejected under 35 U.S.C. 103 as being unpatentable over Falkow et. al., as applied to claims 1,3,4,8-10 and 13 above, and further in view of Kourilsky et. al. Claims 5-7 had been rejected under 35 U.S.C. 103 as being unpatentable over Falkow et. al., or Weetall. Claims 20-23 had been rejected under 35 U.S.C. 103 as being unpatentable over Kourilsky et. al., in view of Falkow et. al. Claims 24-26 had been rejected under 35 U.S.C. 103 as being unpatentable over Gillespie et. al. or Weetall in view of Falkow et. al.

Kourilsky et. al., describe a method of hybridization, in which the analyte is in solution. The unhybridized probe and the unbound signalling moiety have to be removed by cumbersome procedures before detection of label (see column 4, lines 40-45). In Applicants' method, a simple rinsing step is all that is necessary to remove the free probe and signalling moiety (see, for example, p.13). This is a significant improvement and an unexpected advantage over the method of Kourilsky et. al., both in terms of efficiency and in yielding a more accurate quantitative determination as a result of dispensing with the involved separation techniques of Kourilsky, et. al. where nonspecific loss of reactants is unavoidable.

In the office action, it is stated that it would be obvious to modify the method of Falkow et. al., by attaching the label to the probe utilizing the complexing technique of Kourilsky et. al., and it would be obvious to measure the soluble signal in a tube. Applicants submit that such a conclusion cannot be drawn. The methods of Falkow et. al., and Kourilsky et. al., are significantly different in fundamental aspects and one skilled in the art would not seek to modify them

as suggested. Of course, hindsight afforded by Applicants' own teaching is not permissible within the scope of 35 U.S.C. 103.

The Gillespie et. al., patent discloses a method of separating messenger RNA from other cellular components by passage of samples through a porous filter (nifrocellulose or glass fibers). This method has nothing to do with quantitation or detection of signal in hybridization of polynucleotide sequences.

The Weetall publication describes a procedure for attaching analytes, which can be nucleotides, to inorganic carriers, for example, glass, by means of an intermediate silane coupling agent. This procedure is well known in the art and applicants' claims do not encompass this procedure. As in the case with Gillespie et. al., this publication has nothing to do with detection of signal in hybridization assays.

The examiner has referred to unexpected results, whereas the references have not provided a basis even for a prima facie case of obviousness of the claimed invention as a whole, for none of the above references cited individually or in combination, disclose or suggest the present invention.

Summary & Conclusion

In summary claims 1-19, 24,25,26 and new claims 27-32 are active in the application. Claims 20-23 have been cancelled. No new matter has been added.

The Patent and Trademark Office is hereby authorized to charge Deposit Account No. 05-1135 for any fees required in connection with this communication and to credit any overpayment thereto.

This Response is accompanied by and includes a request for three months extension of time. The Patent and Trademark Office is hereby authorized to charge Deposit Account No. 05-1135 for the requisite fee of \$390.00 U.S. Dollars, as set by 37 CFR 1.17(c).

For all of the above reasons, Applicants submit that claims 1-19,24,25,26 and new claims 27-32, are all patentably distinguished from the cited prior art and allowable. A favorable and speedy reconsideration of their rejection is requested. If any of the claims are found not to be in condition for allowance for any reason, the Examiner is respectfully requested to telephone the undersigned at (212) 337-3355, extension 26 to identify a time at which a personal interview would be granted.

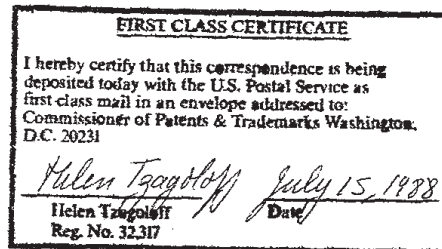
Respectfully submitted,



Helen Tzagoloff
Reg. No. 32,317

ENZO BIOCHEM, INC.
325 Hudson Street
New York, N.Y. 10013
(212) 337-3355

ENZ 7 CIP



Enz-7 CIP

FORM PTO-122 (REV. 12-87)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	
TITLE REPORT		PAPER NO. <i>11</i>	
A. APPLICATION FILE DATA			
1. SERIAL NO. <i>32374</i>		2. FILED <i>5/9/85</i>	
3. INVENTOR(S)—FULL NAME(S) <i>Cernis G. Stavrianopoulos</i> <i>Dalje Kirtikar</i> <i>Kenneth H. Johnston</i>			
4. DIVISION OF			
5. CONTINUATION OF			
6. REISSUE OF			
7. SUBSTITUTE OF			
B. ASSIGNMENT RECORD DATA			
The assignment records reveal that the Title appears to be vested in:			
<input checked="" type="checkbox"/> (1.) Inventor(s) <input type="checkbox"/> (2.) As endorsed <input type="checkbox"/> (3.) As the record now stands, the patent, when granted, will issue in the name of the inventor(s). <input type="checkbox"/> (4.) Other			
EXAMINED UP TO AND INCLUDING <i>05/08/85</i>		THIS CERTIFICATE DATED <i>05/30/85</i>	
BRANCH CHIEF OF ASSIGNMENT SEARCH BRANCH DIANE G. RUSSELE			

ngw



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RECEIVED GR...

AUG 17 1988

#12
M
9/18/88

Applicants: J. Stavrianopoulos, et al.
Serial No.: 732,374
Filed : May 9, 1985
For : Method and Structures Employing Chemically-Labelled Polynucleotide Probes

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

REVOCATION OF PREVIOUS POWERS OF ATTORNEY AND
APPOINTMENT OF NEW POWER OF ATTORNEY

Enzo Biochem, Inc., a corporation of the State of New York, having its principal place of business at 345 Hudson Street, New York, New York 10013, the owner of the entire right, title and interest in and to the above-identified application and the invention disclosed and/or claimed therein hereby revokes any and all powers of attorney previously granted with respect to the above-identified application and appoints, as principal attorney, Charles J. Herron (Reg. No. 28,019) with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Enzo Biochem, Inc. hereby appoints as associate attorneys in the above identified application Helen Tzagoloff (Reg.No. 32,317) and Serle I. Mosoff (Reg. No. 25,900) and whose address is Enzo Biochem, Inc., 345 Hudson Street, New York, New York, 10014.

Please address all communications to:

Charles J. Herron
Corporate Patent Counsel
Enzo Biochem, Inc.
345 Hudson Street
New York, New York 10014

and direct all telephone calls regarding this application to Helen Tzagoloff at telephone no. (212) 337-3355.

Enzo Biochem, Inc.

Dean L. Engelhardt 7/15/88

Dean L. Engelhardt (Date)
V.P. Research (Enz-7CIP)



**U.S. DEPARTMENT OF COMMERCE
Patent Office**

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Washington, D.C. 20231

Paper No. 413

Hueatt 185
06/732,374 05/09/85
Jannis G. Stavrianopoulos, et al

Mailed

Mary E. Bak
Fish & Neave
29th Floor
875 Third Avenue
New York, NY 10022

SEP 14 1988

GROUP 180

Responsive to the proposed communication re the power of attorney filed 8-15-88.

The above communication, signed by Dean C. Engelhardt, has not been entered for the reason checked below:

1. A report from the Assignment Branch dated 8/30/88, indicates that title appears to be vested in another.
2. The signature of _____ (assignee of part interest), (co-inventor) in this application, has been omitted. The paper will be entered upon receipt of confirmation signed by said co-inventor.
3. The person signing for the assignee has omitted his position, or is not an officer in the company.
4. The substitute power of attorney does not comply with Commissioner's Notice of April 23, 1957, 718 O. G. 219. It is being returned herewith.
5. There is no evidence in the records that the person appointed in the proposed power of attorney is registered to practice before the U.S. Patent Office.
6. The person signing the proposed power of attorney is without any authority of record in this application.
7. The signature of the applicant having part interest in this application has been omitted. R. 33.
8. The signature on the power is a facsimile and therefore not acceptable.
9. Other.

Kathryn P. Ferry

PLEASE FURNISH YOUR ZIP CODE IN ALL CORRESPONDENCE

732374



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
06/732,374	05/09/85	STAVRIANOUPOULOS	ENZ-7/CIF

MARY E. BAK
FISH & NEAVE
29TH FLOOR
875 THIRD AVENUE
NEW YORK, NY 10022

EXAMINER	
HOLEATT, J	
ART UNIT	PAPER NUMBER
183	14

DATE MAILED: 11/29/88

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

This application has been examined Responsive to communication filed on August 15, 1988 This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), — days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- 1. Notice of References Cited by Examiner, PTO-892.
- 2. Notice re Patent Drawing, PTO-948.
- 3. Notice of Art Cited by Applicant, PTO-1449
- 4. Notice of Informal Patent Application, Form PTO-152
- 5. Information on How to Effect Drawing Changes, PTO-1474
- 6. _____

Part II SUMMARY OF ACTION

- 1. Claims 1-32 are pending in the application.
Of the above, claims _____ are withdrawn from consideration.
- 2. Claims 20-23 have been cancelled.
- 3. Claims _____ are allowed.
- 4. Claims 1-19 and 24-32 are rejected.
- 5. Claims _____ are objected to.
- 6. Claims _____ are subject to restriction or election requirement.
- 7. This application has been filed with informal drawings which are acceptable for examination purposes until such time as allowable subject matter is indicated.
- 8. Allowable subject matter having been indicated, formal drawings are required in response to this Office action.
- 9. The corrected or substitute drawings have been received on _____. These drawings are acceptable; not acceptable (see explanation).
- 10. The proposed drawing correction and/or the proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been approved by the examiner. disapproved by the examiner (see explanation).
- 11. The proposed drawing correction, filed _____, has been approved. disapproved (see explanation). However, the Patent and Trademark Office no longer makes drawing changes. It is now applicant's responsibility to ensure that the drawings are corrected. Corrections **MUST** be effected in accordance with the instructions set forth on the attached letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474.
- 12. Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has been received not been received
 been filed in parent application, serial no. _____; filed on _____.
- 13. Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
- 14. Other

Serial No. 732374

-2-

Art Unit 182

Claim 32 is rejected under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited in accordance with the specification on pages 15 and 16, Example 1 which discloses treating glass supports by separate steps of boiling in nitric acid, contacting with dilute acid, rinsing in distilled water, drying at 115°C for 24 hours, treating with 10% gamma-aminopropyltriethoxysilane for 2-3 hours at 45°C, washing in water and drying overnight at a temperature of 100°C. See MPEP 706.03(n) and 706.03(z).

Claim 32 is directed to "modifying" part of the solid support to facilitate fixation of a polynucleotide sequence. The specification only teaches one such method of "modifying" the support and provides no guidance to one skilled in the art of any other modification methods that are comparable and will result in facilitation of fixation of polynucleotide sequences. Therefore, it is the examiner's position that the breadth of the claims are not supported by the one disclosed glass treatment method.

Claims 14, 15³⁰ and 31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Serial No. 732374

-3-

Art Unit 185

✓ Claim 14 is confusing and indefinite in the recitation of "... which forming further comprises...". Language such as "wherein said forming step further comprises" is suggested.

Claim 30 is indefinite in the recitation of "... an immobilized polynucleotide sequence capable of being hybridized to said polynucleotide or oligonucleotide probe." This phrase is indefinite as it exists in the composition. The probe and the immobilized polynucleotide sequence exist together in the composition, and as such, these sequences can already be hybridized, can be partially hybridized or can exist separately and not be hybridized. Therefore, it is unclear what composition is being claimed.

Claims 1, 3, 4, 8-10, 13 and 27-32 are rejected under 35 U.S.C. 102(a) as being anticipated by Falkow et al .

This rejection is repeated for essentially the same reasons as set forth in the rejection of claims 1, 3, 4, 8-10 and 13. In regard to new claims 27-32, Falkow et al, in column 2, lines 21-31 teach providing the reagents as commercial kits. In column 6, lines 67 and 68 and in column 7, lines 1-44, Falkow et al teach preparation of the "solid support" for fixation of the polynucleotide sequence. This section also discloses the composition containing the probe and the immobilized polynucleotide sequence.

Serial No. 732374

-4-

Art Unit 185

Applicants argue that the Falkow et al preferred method is labeling probes with radiosotopes and that the signal produced would be insoluble. This statement is agreed with; however, as stated in the previous office action, Falkow et al clearly teach (See column 3, lines 38-52 and column 4, lines 5-18) utilizing other known labels, such as fluorescers, chemiluminescers or enzymes. Particularly, column 3, lines 43-52, provide guidance to select an appropriate label other than a radiolabel.

Applicants further argue that the use of enzymes and fluorescent compounds would result in the generation of an insoluble signal; however, not all enzyme labels result in the production of an insoluble signal. For example Falkow et al suggest in column 4, lines 12-16, the use of hydrolases, such as esterases and glycosidases or umbelliferone. Applicants' examples disclose utilizing alkaline phosphatase which is a hydrolase (Examples 4 and 5) and an umbelliferone derivative (Example 5) which are involved in the generation of a soluble signal. These types of labels are suggested by Falkow et al. Therefore, the suggestion of such labels for probes would inherently result in the production of a soluble signal which would be measured by any available instrumentation. Thus, applicants' arguments have not been found to be persuasive and this rejection is maintained.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) and (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 2 and 11-19 are rejected under 35 U.S.C. 103 as being unpatentable over Falkow et al in view of Kourilsky et al.

This rejection is repeated for essentially the same reasons as set forth in the previous office action. Applicants argue that Kourilsky et al disclose cumbersome methods for removing unbound signal and unhybridized probe. Kourilsky et al were not applied to teach the liquid hybridization method but rather were applied to teach a probe labeled to an enzyme signaling moiety such as beta-galactosidase through the formation of a complex such as biotin-avidin (See column 4, lines 46-55). The labeled probe of Kourilsky et al could be

Serial No. 732374

-6-

Art Unit 185

utilized as the probe in method of Falkow et al where the polynucleotide sequence to be tested is immobilized on a solid support. It is maintained that this substitution would be obvious to one skilled in the art. The advantages of the Kourilsky et al probe are set forth in column 3, lines 3-28. Therefore, even though the basic manipulative steps of the Falkow et al and Kourilsky et al procedures are different, both procedures utilize labelled probes and thus the substitution of a superior probe (Kourilsky et al) into a more easily manipulated procedure (immobilized polynucleotide sequence of Falkow et al) is deemed to be obvious.

Claims 5-7 are rejected under 35 U.S.C. 103 as being unpatentable over Flakow et al in view of Gillespie et al or Weetall.

This rejection is repeated for essentially the same reasons as set forth in the previous office action and for the reasons set forth above. Gillespie et al and Weetall et al were applied to teach that nucleic acids can be fixed to a transparent or translucent support such as glass, not to teach detection of signals in hybridization assays. It is deemed to be an obvious matter of choice to select non-porous supports for fixing the nucleic acids, absent unexpected results. Therefore, to substitute other known solid supports,

Serial No. 732374

-7-

Art Unit 185

such as non-porous supports, for the nitrocellulose filter of Falkow et al is deemed to be an obvious matter of choice. All that is required of the support is that the nucleic acid attaches to the support and does not interfere with the method.

Claims 24-26 are rejected under 35 U.S.C. 103 as being unpatentable over Gillespie et al or Westall in view of Falkow et al.

This rejection is repeated for essentially the same reasons as set forth in the previous office action and for the reasons set forth above.

Applicants' requested amendment to page 19, line 34 could not be made. It is suggested that applicant request that "avidinbiotin" be changed to "avidin-biotin", if that is applicants' intent.

Applicant's amendment necessitated the new grounds of rejection. Accordingly, THIS ACTION IS MADE FINAL. See MPEP 706.07(a).

Applicant is reminded of the extension of time policy set forth in 37 CFR 1.136(a). The practice of automatically extending the shortened statutory period an additional month upon the filing of a timely first response to a final rejection has been discontinued by the Office. See 1021 TMOG 35.

Serial No. 732374

-8-

Art Unit 185

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 CFR 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

Any inquiry concerning this communication should be directed to Jayme A. Huleatt at telephone number 703-557-1748.

Huleatt:cmr
11/18/88

Jayme A. Huleatt
JAYME A. HULEATT
EXAMINER
ART UNIT 185



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

59 MAY 24 PM 3:04

*H/S
JM
5/24/89*

Inventor: Stavrianopoulos, J. et al. GROUP 280

Serial No.: 732,874 Art Unit: 127
Filing Date: May 9, 1985 Examiner: J. Huleatt
Title: Methods And Structures Employing Chemically-Labeled Polynucleotide Probes.

Honorable Commissioner of Patents and Trademarks Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified patent application.

The fee has been calculated as shown below:

	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	ADDITIONAL FEE
TOTAL	14	MINUS	22	= 0	x 12	\$ 0
INDEP	1	MINUS	1	= 0	x 34	\$ 0
() FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIMS					+110	\$
TOTAL ADDITIONAL FEE -----						\$ 0

- Charge Deposit Account No. 05-1135 in the amount of \$ 0.
- A check in the amount of \$ _____ is attached.
- The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 05-1135: any filing fees under 37 CFR 1.16 for the presentation of extra claims and any patent application processing fees under 37 CFR 1.17.

A duplicate copy of this paper is enclosed.

Date: May 18, 1989

05-1135 030 117 430.00CH
Helen Tsagoloff
Helen Tsagoloff
Registration No. 32,317

Enzo Biochem, Inc.
325 Hudson Street
New York, NY 10013
(212) 741-3838

Case No.: Enz-7 CIP

EXPRESS MAIL CERTIFICATE	
"Express Mail" Label No.:	NB 445 566 649
Deposit Date	<u>May 19, 1989</u>
I hereby certify that this paper and the attachments herein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington DC 20231.	
<i>Helen Tsagoloff</i> Helen Tsagoloff Reg. No. 32,317	<u>May 19, 1989</u> DGC



182

185

yp127,
16/B
(initials)
JM
5/24/89

EXPEDITED PROCEDURE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Jannis G. Stravrianopoulos, et al.,

Serial No.: 732,374

Art Unit: 427 185

Filed: May 9, 1985

Examiner: J. Huleatt

Title: Methods And Structures Employing Chemically-Labeled Polynucleotide Probes

May 19, 1989
New York, New York

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Response Under 37 CFR 1.116

RECEIVED
09 MAY 24 AM 8:08
GROUP 180

Dear Sir:

OK to
Enter
JGH
5/25/89

Please enter this response to the Office Action of November 29, 1988.

In The Specification

Page 19, line 34, delete "avidinbiotin" and insert - - avidin-biotin - - .

In The Claims

Cancel claims 5, 6, 8, 24-26, 30 and 31.

Amend The Claims As Follows:

Claim 1, line 4, after "a" insert - - transparent or translucent, non-porous - - .

Claim 7, line 1, delete "5" and insert - - 1 - - .

Claim 14 (Twice Amended) line 2, delete "which forming" and insert - - wherein said forming step - - .

Claim 14 (Twice Amended) line 4, after "washing", insert - - step - - .

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Claim 32 (Amended) The method according to Claim 1 wherein part of the solid support is modified [to facilitate fixation of said polynucleotide sequence to said solid support] by

boiling in dilute nitric acid;

washing with distilled water;

drying at about 115°C, for about 24 hours;

treating with 10% gamma-

aminopropyltriethoxysilane for about two to three hours at about 45°C;

washing with water; and

drying overnight at a temperature of about 45°C.

REMARKS

Reconsideration of the above-identified application is respectfully requested. The claims presented for further examination are believed to be in condition for allowance. The Examiner is respectfully requested to consider the above amendments and the discussion of the issues submitted herein.

The Rejection Under 35 U.S.C. 112, first paragraph

Claim 32 has been rejected because, as alleged by the Examiner, it is not enabled in accordance with the specification. The Examiner's position is as follows:

Claim 32 is rejected under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited in accordance with the specification on pages 15 and 16. Example 1 which discloses treating glass supports by separate steps of boiling in nitric acid, contacting with dilute acid, rinsing in distilled water, drying at 115°C for 24 hours, treating with 10% gamma-aminopropyltriethoxysilane for 2-3 hours at 45°C, washing in water and drying overnight at a temperature of 100°C. See MPEP 706.03(n) and 706.03(z).

Claim 32 is directed to "modifying" part of the solid support to facilitate fixation of a polynucleotide sequence. The

specification only teaches one such method of "modifying" the support and provides no guidance to one skilled in the art of any other modification methods that are comparable and will result in facilitation of fixation of polynucleotide sequences. Therefore, it is the Examiner's position that the breadth of the claims are not supported by the one disclosed glass treatment method.

Applicants have amended claim 32 in accordance with the Examiner's suggestions. It is respectfully submitted that the rejection on this ground has now been overcome.

The Rejection Under 35 U.S.C. 112, second paragraph

Claims 14, 15, 30 and 31 have been rejected as allegedly being indefinite. The Examiner's position is as follows:

Claims 14, 15, 30 and 31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 14 is confusing and indefinite in the recitation of ". . . which forming

further comprises . . .". Language such as "wherein said forming step further comprises" is suggested.

Claim 30 is indefinite in the recitation of . . . an immobilized polynucleotide sequence capable of being hybridized to said polynucleotide or oligonucleotide probe. This phrase is indefinite as it exists in the composition. The probe and the immobilized polynucleotide sequence exist together in the composition, and as such, these sequences can already be hybridized, can be partially hybridized or can exist separately and not be hybridized. Therefore, it is unclear what composition is being claimed.

Applicants have amended claim 14 in accordance with the Examiner's suggestion. Applicants have cancelled claims 30 and 31, rendering the rejection thereof moot.

It is respectfully submitted that amended claim 14 and claim 15, which depends therefrom, are now in condition for allowance and that the rejection thereof should be withdrawn.

The Rejection Under 35 U.S.C. 102

Claims 1, 3, 4, 8-10, 13 and 27-32 have been rejected under 35 U.S.C. 102(a) as being anticipated by Falkow *et al.* The rejection is for essentially the same reasons as have been set forth previously in the rejection of claims 1, 3, 4, 8-10 and 13. With regard to new claims 27-32, it is the Examiner's position that Falkow *et al.*, teach providing the reagents as commercial kits.

Applicants have made significant amendments to independent claim 1, the claim of broadest scope. The support has now been limited to a transparent or translucent, non-porous solid surface. Support for amended claim 1 can be found throughout the specification and in particular, on page 10, lines 18-22 and 28; on page 14, lines 33-34, and in examples 1 and 2. Falkow *et al.*, neither teach nor suggest a transparent or translucent, non-porous support as disclosed in the instant invention. In fact, Falkow *et al.*, by employing a porous support for the purpose of expanding the cell number, (see column 2, lines 3-7), teach away from Applicants' invention.

It is submitted in view of the amendment to claim 1 made hereunder, that the claim is not anticipated by Falkow *et al.*, and is novel and patentable. Since claims 3, 4, 8-10, 13 and 27-32 depend from and contain all the limitations of claim 1 as amended, these claims are also novel and patentable.

The Rejections Under 35 U.S.C. 103

Claims 2 and 11-19 have been rejected under 35 U.S.C. 103 as being unpatentable over Falkow *et al.*, in view of Kourilsky *et al.*, for essentially the same reasons as have been set forth in the previous office action.

Dependent claims 2 and 11-19 read on preferred embodiments of Applicants' invention. As pointed out above, Falkow *et al.*, teach away from Applicants' amended claim 1. It is noted that the Examiner acknowledges that the basic manipulative steps of the Falkow and Kourilsky procedures are different from the instant case. It is submitted that the combination of Falkow and Kourilsky would not result in Applicants' method in which the polynucleotide sequence is fixed to a solid, transparent or translucent, non-porous support.

It is believed that dependent claims 2 and 11-19 are patentable and novel and withdrawal of the rejection thereof is respectfully requested.

Claims 5-7 have been rejected under 35 U.S.C. 103 as being unpatentable over Falkow *et al.*, in view of Gillespie *et al.*, or Weetall. The Examiner's position is:

Gillespie *et al.*, and Weetall *et al.*, were applied to teach that nucleic acids can be fixed to a transparent or translucent support such as glass, not to teach detection of signals in hybridization assays. It is deemed to be an obvious matter of choice to select non-porous supports for fixing the nucleic acids, absent unexpected results. Therefore, to substitute other known solid supports, such as non-porous supports, for the nitrocellulose filter of Falkow *et al* is deemed to be an obvious

matter of choice. All that is required of the support is that the nucleic acid attaches to the support and does not interfere with the method.

Claims 5 and 6 have been cancelled, rendering the rejection thereof moot.

Claim 7 has been amended to depend from claim 1. This claim is addressed to alternative embodiments of the transparent or translucent, non-porous, solid support.

The Gillespie *et al.*, patent discloses a method of separating messenger RNA from other cellular components by passage of samples through a porous filter, such as glass fibers or nitrocellulose.

The Weetall publication describes a procedure for attaching analytes, which can be nucleotides, to inorganic carriers, for example, glass, by means of an intermediate silane coupling agent.

Neither reference suggests or discloses a method for quantitating or detecting signal in hybridization of polynucleotide sequence as taught in the instant case. It is submitted that Applicants' invention when considered in its entirety, is not obvious in view of the above references. It is further submitted that Applicants' amended claim 7 is patentable and novel and withdrawal of this rejection is requested.

Claims 24-26 have been rejected under 35 U.S.C. 103 as being unpatentable over Gillespie *et al.*, or Weetall in view of Falkow *et al.* These claims have been cancelled rendering the rejection thereof moot.

SUMMARY & CONCLUSION

Claims 1-4, 7, 9, 11-19, 27-29 and 32 are presented for further examination. Claims 1, 7, 14 and 32 have been amended. Claims 5, 6, 8, 24-26, 30 and 31 have been cancelled.

The Patent and Trademark Office is hereby authorized to charge Deposit Account No. 05-1135 for any fees required in connection with this Response and to credit any overpayment thereto.

This Response is accompanied by and includes a request for a three month extension of time. The Patent and Trademark Office is hereby authorized to charge Deposit Account No. 05-1135 for the requisite fee of \$ 390 U.S. Dollars, as set by 37 CFR 1.17(c).

Applicants submit that in view of the above amendments and discussion of the issues, each of Claims 1-4, 7, 9, 11-19, 27-29 and 32 is in condition for allowance. A favorable and speedy reconsideration of their rejection is requested.

Respectfully submitted,



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EXPRESS MAIL CERTIFICATE	
"Express Mail" Label No.:	NB 445 566 649
Deposit Date	May 19, 1989
I hereby certify that this paper and the attachments herein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington DC 20231.	
Helen Tzagoloff Reg. No. 32,317	May 19, 1989 Date

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UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
0677 329 377	05/10/79	STEVENS PRODUCTIONS	EN2701P

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29TH FLOOR
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NEW YORK, NY 10022

EXAMINER	
HULEATT	
ART UNIT	PAPER NUMBER
185	17

DATE MAILED: 05/26/79

Below is a communication from the EXAMINER in charge of this application
COMMISSIONER OF PATENTS AND TRADEMARKS

ADVISORY ACTION

THE PERIOD FOR RESPONSE:

- is extended to run 6 MONTHS from the date of the Final Rejection
- continues to run _____ from the date of the Final Rejection
- expires three months from the date of the final rejection or as of the mailing date of this Advisory Action, whichever is later. In no event however, will the statutory period for response expire later than six months from the date of the final rejection.

Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a), the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee. Any extension fee pursuant to 37 CFR 1.17 will be calculated from the date that the shortened statutory period for response expires as set forth above.

- Appellant's Brief is due in accordance with 37 CFR 1.192(a).
- Applicant's response to the final rejection, filed May 19, 1979, has been considered with the following affect, but it is not deemed to place the application in condition for allowance:

1. The proposed amendments to the claim and/or specification will not be entered and the final rejection stands because:
 - a. There is no convincing showing under 37 CFR 1.116(b) why the proposed amendment is necessary and was not earlier presented.
 - b. They raise new issues that would require further consideration and/or search. (See Note).
 - c. They raise the issue of new matter. (See Note).
 - d. They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.
 - e. They present additional claims without cancelling a corresponding number of finally rejected claims.

NOTE: _____

2. Newly proposed or amended claims _____ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.
3. Upon the filing of an appeal, the proposed amendment will be will not be, entered and the status of the claims in this application would be as follows:

Allowed claims: _____

Claims objected to: _____

Claims rejected: 1-4, 7, 9-19, 27-29 and 32

However:

 - a. The rejection of claims _____ on references is deemed to be overcome by applicant's response.
 - b. The rejection of claims _____ on non-reference grounds only is deemed to be overcome by applicant's response.
4. The affidavit, exhibit or request for reconsideration has been considered but does not overcome the rejection.
5. The affidavit or exhibit will not be considered because applicant has not shown good and sufficient reasons why it was not earlier presented.

The proposed drawing correction has has not been approved by the examiner.

Other

32
The rejection of the claim under 35 USC 112, first paragraph is maintained. The amended claim does not contain all of the steps of Example 1. Claims 1, 3, 4, 7-10, 13, 27-29 and 32 are rejected under 35 U.S.C. 103 as obvious over Falkow et al in view of Gillespie or Wheel et al. and claims 2 and 11-19 are rejected over Falkow in view of Roussell et al and Gillespie and Wheel et al under 35 USC 103. The rejection of claims 24-26 are maintained for the reasons of record.

Jayme A. Huleatt
JAYME A. HULEATT
PRIMARY EXAMINER
ART UNIT 185



~~EXPE~~
~~BOX AF~~

EXPED PROCEDURE
Group Art Unit: 127

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#18
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5/31/89

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Jannis G. Stravrianopoulos, et al.,

Serial No.: 732,374

Art Unit: 127

Filed: May 9, 1985

18C 5-20-89
83 1-55

Examiner: J. Huleatt

Title: Methods And Structures Employing Chemically-Labeled Polynucleotide Probes

May 25, 1989
New York, New York

Commissioner of Patents and Trademarks
Washington, D.C. 20231

NOTICE OF APPEAL TO THE BOARD OF PATENT APPEALS AND INTERFERENCES

Sir:

Applicant(s) hereby appeal(s) to the Board of Patent Appeals and Interferences from the decision(s) dated November 29, 1988 of the Primary Examiner finally rejecting claims 1-19 and 24-32

The item(s) checked below are appropriate:

1. Appeal Fee (for other than Small Entity) \$130.00
2. Fee enclosed
3. Fee not required (Fee paid in prior appeal)
4. Charge Fee to Deposit Account No. 05-1135.
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.
5. The Commissioner is hereby authorized to charge any additional fees which may be required by the paper, or credit any overpayment to Deposit Account No. 05-1135.

30 MAY 31 10:56
GROUP 180

Respectfully submitted,

Helen Tsagoloff
Helen Tsagoloff
Registration No. 32,317

05-1135 05/30/89 732374

05-1135 000 119

140 0000

Dated: May 25, 1989

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EXPRESS MAIL CERTIFICATE	
"Express Mail" Label No.:	NB 445 566 660
Deposit Date:	<u>May 25, 1989</u>
I hereby certify that this paper and the attachments herein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 110 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington DC 20231.	
<u>Helen Tsagoloff</u> Helen Tsagoloff Reg. No. 32,317	<u>May 25, 1989</u> Date