如何將研發成果轉換成專利

聖島國際專利商標聯合事務所
潘佳欣

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大綱

- 專利v.s.研發成果
- 申請專利的好處
- 申請專利的提案以及流程

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專利v.s.研發成果

專利v.s.研發成果(1)

專利 ↔ 研發成果

專利說明書 ↔ 學術論文

廣度 ↔ 深度

獨佔範圍 ↔ 學術地位

earn money ↔ earn respect

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專利v.s.研發成果(2)

- 申請專利是公開學術研究成果的另一種形式
- 學術研究成果利益化是世界趨勢
- 智慧財產權的下放

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申請專利的好處

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- 我趕著升等，等有時間再來申請!

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申請專利的好處

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申請專利的提案以及流程

學校網頁中與專利申請相關的資訊

- 專利申請流程
- 專利申請與專利說明書撰寫要點
- 專利申請表填寫範本
- 專利申請表
- 專利權讓渡協議書

來源：http://rd.csmu.edu.tw/front/bin/ptlist.phtml?Category=327

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研究成果專利申請表撰寫要點

- 研究成果專利申請表是撰寫專利說明書的基礎
- 研究成果專利申請表應提供哪些資訊？

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發明專利說明書的架構

發明說明 (bracketing 發明所屬技術領域 through 圖式簡單說明)

發明名稱	Title of the Invention
摘要	Abstract
發明所屬技術領域	Field of the Invention
先前技術	Background of the Invention
發明內容	Summary of the Invention Detailed Description of the Invention
實施方式	Example
圖式簡單說明	Brief Description of the Figures
主要元件符號說明	X
申請專利範圍(或稱請求項)	Claim
圖式	Figure

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專利說明書-以美國為例

US007488467B2

(12) **United States Patent**
Farber et al.

(10) **Patent No.: US 7,488,467 B2**
(45) **Date of Patent: Feb. 10, 2009**

申請訊息

(54) **HIGH THROUGHPUT GENETIC SCREENING OF LIPID AND CHOLESTEROL PROCESSING USING FLUORESCENT COMPOUNDS**

(75) Inventors: **Steven Farber**, Cherry Hill, NJ (US); **Michael Pack**, Philadelphia, PA (US); **Marnie Halpern**, Pikesville, MD (US)

(73) Assignees: **Trustees of the University of Pennsylvania**, Philadelphia, PA (US); **Carnegie Institute of Washington**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 655 days.

(21) Appl. No.: **10/341,538**

(22) Filed: **Jan. 13, 2003**

(65) **Prior Publication Data**

US 2003/0135869 A1 Jul. 17, 2003

Related U.S. Application Data

(63) Continuation of application No. 09/974,550, filed on Oct. 10, 2001, now abandoned.

(60) Provisional application No. 60/264,977, filed on Jan. 30, 2001, provisional application No. 60/238,928, filed on Oct. 10, 2000.

審查訊息

(51) **Int. Cl.**
A61K 49/00 (2006.01)

(52) **U.S. Cl.** **424/9.2**; 424/1.11; 424/9.1

(58) **Field of Classification Search** 424/1.11, 424/1.65, 9.1, 9.2, 1.37, 1.81; 436/71
See application file for complete search history.

審查訊息

(56) **References Cited**

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4,789,633	A *	12/1988	Huang et al.	435/458
6,299,858	B1 *	10/2001	Serbedzija et al.	424/9.2
2003/0129670	A1 *	7/2003	Tsien et al.	435/7.2

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Kamisaka et al (1999), Biochimica et Biophysica Acta, vol. 1438, pp. 185-198.*

Ikeda et al (Journal of Nutrition, 1989, vol. 119, No. 10, pp. 1383-1387).*

Hendrickson et al (Analytical Biochemistry, 1999, vol. 276, pp. 27-35).*

Farber et al (The Journal of Biological Chemistry, 1999, vol. 274, No. 27, pp. 19338-19346).*

Pack, M. et al., 1996, "Mutations affecting development of zebrafish digestive organs", Development (Cambridge), 123(1):321-328.

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技術摘要

(57) **ABSTRACT**

The present invention utilizes fluorescent lipids, particularly quenched phospholipid or cholesterol analogues, to facilitate screening for phenotypes representing perturbations of lipid processing; screening for genetic mutations that lead to disorders of phospholipid and/or cholesterol metabolism; and screening of compounds designed to treat disorders of phospholipid and/or cholesterol metabolism.

21 Claims, 13 Drawing Sheets

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(51) **Int. Cl.**
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Primary Examiner—D. L Jones
(74) *Attorney, Agent, or Firm*—Evelyn H. McConathy; Montgomery, McCracken, Walker & Rhoads, LLP

(57) **ABSTRACT**

The present invention utilizes fluorescent lipids, particularly quenched phospholipid or cholesterol analogues, to facilitate screening for phenotypes representing perturbations of lipid processing; screening for genetic mutations that lead to disorders of phospholipid and/or cholesterol metabolism; and screening of compounds designed to treat disorders of phospholipid and/or cholesterol metabolism.

21 Claims, 13 Drawing Sheets

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Claim項數
本案圖數

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發明名稱

發明領域

先前技術

先前技術

HIGH THROUGHPUT GENETIC SCREENING OF LIPID AND CHOLESTEROL PROCESSING USING FLUORESCENT COMPOUNDS

CONTINUING APPLICATION DATA

This application is a continuation of U.S. application Ser. No. 09/974,550, filed Oct. 10, 2001, which claims benefit under 37 C.F.R. 119(e) of U.S. provisional patent applications Ser. No. 60/238,928, filed Oct. 10, 2000, and 60/264,977 filed Jan. 30, 2001.

GOVERNMENT RIGHTS TO THE INVENTION

The invention was made with government support under grant F32 NS103265 awarded by the National Institute of Health. The government has certain rights to the invention.

FIELD OF THE INVENTION

The present invention generally relates to the fields of biochemistry and pharmacology and to the use of a genetic model organism labeled with fluorescent lipids to screen for drugs and genetic alterations related to phospholipid and/or cholesterol metabolism and, more particularly, to the use of optically clear zebrafish in conjunction with tagged or quenched lipids for studying lipid metabolism in vivo.

BACKGROUND OF THE INVENTION

Genetic analysis in zebrafish is a powerful approach for identifying genes that direct vertebrate development (**1-3**). Since the completion of the large-scale chemical mutagenesis screens in 1997, the phenotypic and molecular characterizations of many mutations have been reported (**4-16**). Analyses of mutations that affect early developmental processes, such as the specification of the embryonic axes and germ layers, have been particularly rewarding (**7**, **10**, **17-27**). Recently, related work with mutations that affect organogenesis has led to the recognition that the zebrafish is an important model system for biomedical research (**28-31**). Given the many aspects of organ physiology that have been conserved during vertebrate evolution, genetic screening to assay organ function in the optically transparent zebrafish is a valuable approach to understanding a variety of metabolic processes and disorders in vertebrates.

By zebrafish chemical mutagenesis screening, nine recessive lethal mutations that perturb development of the digestive organs were identified (**2**, **31**). Although the mutants were identified using morphological criteria, their phenotypic analysis suggests that in some cases the affected genes regulate developmental processes that are relevant to digestive physiology and other aspects of vertebrate metabolism.

Through the analysis of these and other zebrafish mutants, the limitations inherent to genetic screens that are based solely on morphological criteria became apparent. First, not all organs are readily distinguished in zebrafish larvae, and mutations that perturb organ morphology are often overlooked. Second, since it is difficult to visualize specific cell populations within many larval organs, mutations that affect the development or function of these cells can be overlooked as well. Third, despite the transparency of the zebrafish larva, the function of few organs can be effectively assayed by visual inspection alone.

For these reasons, it was concluded that, in most instances, morphology-based screens are best suited for the identification of genes that regulate specification and patterning of embryonic structures. By contrast, screens designed to address biomedical concerns are most effective when they assay physiological processes directly.

Within the past few years, the discovery and analysis of zebrafish mutants affecting organogenesis has confirmed an important role for the zebrafish in biomedical research. The ability to apply high throughput genetic analyses to vertebrate organ physiology using this model system is unprecedented and will undoubtedly, over time, lead to the discovery of many genes that regulate vertebrate organ development and physiology. Such zebrafish research will complement research in other vertebrate model systems.

By conducting a mutagenesis screen using fluorescent lipids, an undertaking not feasible with standard zebrafish screening strategies, the power of high throughput genetic analysis can be applied to lipid metabolism. This has important implications for human diseases such as, but not limited to, cancer, inflammatory and cardiovascular diseases, and congenital and acquired diseases of the intestine and liver.

The fluorescent phospholipase A_2 (PLA_2) substrates described in the present invention are the first prototypes in this class of reagents. Although lipid metabolism in the digestive tract is complex and involves multiple organs the present invention discloses a method of assaying this pathway since gall bladder fluorescence represents one of the last steps in lipid processing. Because they serve as reporters of lipid processing, the fluorescently-tagged reagents of the instant invention provide a sensitive assay for a wide range of digestive developmental and physiological processes including, but not limited to, swallowing; lipid digestion, absorption, and transport; esophageal sphincter function; intestinal motility; organogenesis of the mouth and pharynx, esophagus, intestine, liver, gallbladder and biliary system, and exocrine pancreas and ducts; and the cellular and molecular biology of PLA_2 regulation, polarized transport, and secretion.

Given the shared features of lipid processing in mammals and teleosts (**82**, **105**), zebrafish mutagenesis screens using lipid reporters can be used to identify genes with functions relevant to human lipid metabolism and disease. Moreover, since both mammals and teleosts metabolize lipids in an analogous manner, the high throughput screens and fluorescent lipids disclosed in the instant invention can be employed using a variety of vertebrate model systems, including but not limited to, rodents, amphibia, and fish. The present invention involves utilizing fluorescent lipids to screen for phenotypes representing perturbations of lipid processing; to screen for mutations of specific genes that lead to disorders of phospholipid and/or cholesterol metabolism; and to screen for compounds designed to treat disorders of phospholipid and/or cholesterol metabolism, such as, but not limited to cancer, inflammatory and cardiovascular disease, and congenital and acquired diseases of the intestine and liver.

ABBREVIATIONS

"PLA_2" means "phospholipase A_2"
"PLA_1" means "phospholipase A_1"
"PLB" means "phospholipase B"
"PLD" means "phospholipase D"
"PED6" means "N-((6-(2,4-dinitrophenyl)amino)hexanoyl)-1-palmitoyl-2-BODIPY-FL-pentanoyl-sn-glycero-3-phosphoethanolamine"
"hpf" means "hour post-fertilization"
"dpf" means "day post-fertilization"
"FRET" means "fluorescence resonance energy transfer"

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"PC" means "phosphatidylcholine"
"TLC" means "thin layer chromatography"
"$cPLA_2$" means "cytoplasmic PLA_2"
"$sPLA_2$" means "secretory PLA_2"
"COX" means "cyclooxygenase"
"APC" means "adenomatous polyposis coli"
"EP" means "early pressure"
"ENU" means "ethylnitrosourea"
"WT" means "wild-type"
"SLR" means "single locus rate"
"IVF" means "in vitro fertilization"
"BAC" means "bacterial artificial chromosome"
"PAC" means "P1-derived artificial chromosome"
"YAC" means "bacterial artificial chromosome"
"SSR" means "simple sequence report"
"CSGE" means "conformation sensitive gel electrophoresis"
"VLDL" means "very low density lipoprotein"
"EM" means "embryo medium"
"NBD cholesterol" means "nitrobenzoxadiazole cholesterol"

BRIEF DESCRIPTION OF THE DRAWINGS

圖式簡單說明

FIG. **1**. Schematic of PED6, a quenched PLA_2 substrate. A. The dinitrophenol on the phospholipid headgroup effectively quenches any emission resulting from excitation at 505 nm of the BODIPY-labeled acyl chain. B. When the BODIPY-labeled acyl chain is liberated by PLA_2 mediated cleavage, the quencher is separated from the fluorophore and emission (515 nm) is observed. C. NDB-labeled cholesterol in which the fluorophore replaces the terminal segment of cholesterol's alkyl tail.

FIG. **2**. Intestinal differentiation in 84 hpf larvae. A. Cross-section of posterior intestine. Desmin immunoreactivity of intestinal smooth muscle (green) and enteric neurons as indicated by zn6 immunoreactivity (red) demonstrate development of the enteric neuromuscular system. B. Transmission electromicrograph showing mature apical junctional complexes in enterocytes in the intestinal epithelium. Arrow points to desmosomes; A: Adherens junction. T: tight junction. C. Histochemical detection of enterocyte aminopeptidase activity (red); anterior intestinal cross section. D. Histological detection of goblet cell mucin (purple) in the posterior intestine. Also present at this developmental stage, immunoreactive pancreatic polypeptide in enteroendocrine cells.

FIG. **3**. PED6: a fluorescent lipid reporter. A. Bright-field image of a 5 dpf larva soaked in PED6 (3 ug/ml, 2 hr). B. Corresponding fluorescent image, with intestinal (arrow) and gall bladder (arrowhead) labeling. C. Larva soaked in BODIPY-C5-PC (0.2 ug/ml). In contrast to B, unquenched fluorescent lipid labels the pharynx (arrowhead), confirming that lipid is swallowed before gall bladder labeling (arrow).

FIG. **4**. Rate of fluorescence after PED6 labeling. Larvae (n=5) were placed in medium containing PED6 (0.17 mg/ml) and tricaine. Images were captured at various times and fluorescence intensity was determined in specific structures. Organ fluorescence intensity determined at specific times was normalized to the observed intensity at 45 min. Data are expressed as Mean±SEM.

FIG. **5**. Lipid processing. A. and B. Atorvastatin (ATR) inhibits processing of PED6 (A) but not of BODIPY-FL-C5 (Molecular Probes). B. Larvae were bathed in fluorophore (0.6 uM) in the presence or absence of atorvastatin (Lipitor tablet suspension containing 1 mg/ml) (arrowhead, gall bladder). C. Mouse digestive organs. D. Gall bladder fluorescence after processing (t=30 min) of PED6 (1 ug), administered by

gavage. Symbols: gb, ball bladder; d, common bile duct; lv, liver. Scale bars, 1.0 mm (C and D), 200 um (other images).

FIG. **6**. BODIPY FR-PC reveals both the substrate and PLA_2 cleavage product. Upon integration into cells, excitation at 505 nm results in an emission at 568 nm (orange) due to fluorescence resonance energy transfer (FRET). After cleavage by PLA_2 emission is observed only at 515 nm (green).

FIG. **7**. Fluorescence emission spectrum of mixed micelles of 0.05 mol % BODIPY FR-PC in mixed-lipid vesicles. The fluorescence emission spectrum of BODIPY FR-PC (0.5 μM in ethanol; excitation, 505 nm) showed peaks at 514 nm and 568 nm with a ratio of 1.0 indicating FRET. An excitation scan (emission, 568 nm) showed peaks at 507 and 560 nm with a ratio (507/560) of 1.2. Solid line: before addition of PLA_2. Dashed line: after addition of *N. naja* PLA_2. Vesicles were prepared by sonication of the dried lipids (from chloroform-methanol solution); 1.1 μM BODIPY FR-PC (0.05 mol %), 0.1 mM dimyristoylphosphatidycholine (46 mol %), 0.12 mM ditetradecylphospha-tidylmethanol (54 mol %), buffer (50 mM Tris, pH 8, 100 mM NaCl, 1 mM $CaCl_2$); excitation, 505 nm.

FIG. **8**. BODIPY FR-PC is effective in paramecium. Paramecium incubated (1 hr) in BODIPY FR-PC (2.5 ug/ml) results in labeling of lipid droplets (orange). Digestion of the lipid results in green fluorescence. Excitation 505 nm, emission LP520.

FIG. **9**. BODIPY FR-PC localizes to the intestinal epithelium. Fish were incubated (1-4 hr) in BODIPY FR-PC (2.5 ug/ml). BODIPY FR-PC metabolites are observed in gall bladder (green). Excitation 505 nm, mission LP520. A. Brightfield image. B. and C. Visualization of antigen presenting enterocytes in segment II of the larval zebrafish intestine after only 1 hr of labeling. Arrows mark segment II domain. D. 4 hr labeling with BODIPY FR-PC results in FRET effect throughout intestine.

FIG. **10**. Lipid processing in intestinal mutants. A and C. Bright field images of 5 dpf mlt and pie larvae. B and D. Fluorescent images corresponding to A and C. Normal lipid processing of PED6 (0.3 ug/ml, 2 hr) in mlt larva (arrowhead marks gallbladder). D and E. Abnormal lipid processing in PED6 labeled pie and slj larvae; fluorescence is present in the intestinal lumen (white arrowhead) and reduced in the gallbladder (red arrowhead).

FIG. **11**. Phospholipid processing and transport. A. Following uptake, PED6 is cleaved by PLA_2 liberating a labeled fatty acid. The fate and transport of this fatty acid remains unknown. B. TLC of fluorescent lipid standards. PED6 (black because it is quenched), D3803 (C5-BODIPY PC), and C5-fatty acid are easily resolved using a two solvent system silica gel plates (Whatman, LK5D). Solvent 1 (toluene, ether, ethanol, acetic acid; 25/15/2/0.2); Solvent 2 (chloroform, methanol, acetic acid, water; 25/15/4/2).

FIG. **12**. Red BODIPY-PC given by gavage labels the gall bladder. Adult fish was anesthetized in tricaine, injected with 80 ug of D3806 (a BODIPY PC 582/593 nm), allowed to recover for 1 hr and dissected on ice.

FIG. **13**. Larval zebrafish (5 dpf) labeled with NDB cholesterol—derivative labels gall bladder within 30 minutes of ingestion (3 ug/ml, solubilized with fish bile).

圖式簡單說明

DETAILED DESCRIPTION

The present invention relates to a mutagenesis screen to identify genes that regulate lipid metabolism using fluorescently-tagged or quenched lipids such as cholesterol or lipids that are substrates for phospholipases such as PLA_2. For

實施例說明

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申請專利範圍

We claim:

1. A method of assaying lipid or cholesterol metabolism, or a combination thereof, in a vertebrate's digestive system by a high through-put assay in a teleost. the method comprising:

providing fluorescently labeled , quenched or unquenched lipid reporters, wherein fluorescent moieties are covalently linked to the lipid and/or cholesterol being assayed, such that the resulting lipid reporters are absorbed by the vertebrate's digestive system or function as substrates for target enzymes in the vertebrate's digestive system:

bathing the teleosts in media comprising the labeled lipid reporters, such that the lipid reporters are ingested by the teleost into its digestive system:

screening for and detecting accumulated fluorescence in digestive organs of the teleost as an indicator of metabolic processing or uptake of the lipid reporter in the digestive system: and

quantifying the amount and rate of fluorescence from the processed or unprocessed fluorescently labeled lipid reporters in the digestive organs of the teleost, providing a high through-put readout of digestive organ function, physiology of the digestive system and organs, and metabolic lipid processing.

2. The method of claim **1**, wherein the fluorescently labeled lipid reporters are selected from phospholipase $A_2(PLA_2)$ substrates.

3. The method of claim **1**, wherein the fluorescently labeled lipid reporters are selected from the group consisting of quenched or unquenched fluorescent phosphatidylcholine (PC) analogues, and quenched or unquenched fluorescent fatty acids.

4. The method of claim **3**, wherein the quenched PC analogue is PED6.

5. The method of claim **1**, wherein the lipid reporters are NBD-labeled cholesterols.

6. The method of claim **1** wherein the teleost is an intact viable zebrafish.

7. The method of claim **6**, wherein the zebrafish is a zebrafish larvae, embryo or adult.

8. The method of claim **1**, comprising detecting the processed or unprocessed fluorescently labeled lipid reporters in the teleost's stomach,. intestine, liver, pancreas, biliary tract or gall bladder, or combinations thereof.

9. The method of claim **8**, comprising quantifying the resulting fluorescence in the stomach, intestine, liver, pancreas, biliary tract or gall bladder of the teleost.

10. The method of claim **1**, wherein quantifying the resulting fluorescence in the digestive organs of the teleost from digestive processing or uptake of the fluorescently labeled lipid reporters comprises a high-through-put screen for the effects of perturbing lipid processing of the teleost.

11. The method of claim **10**, wherein the effect of perturbing lipid processing or uptake in the teleost digestive system is visualized as organ-specific, accumulated fluorescence in the teleost's stomach, intestine, liver, pancreas, biliary tract or gall bladder, or combinations thereof.

12. The method of claim **10**, further comprising applying the findings in the teleost regarding the effects of perturbing the lipid processing or uptake to higher level vertebrates and humans.

13. The method of claim **1**, wherein quantifying the resulting fluorescence in the digestive organs of the teleost from digestive processing or uptake of the fluorescently labeled lipid reporters comprises a high-through-put screen for determining mutations of specific genes that lead to or result in disorders of lipid or cholesterol metabolism of the teleost.

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專利說明書-以我國為例

發明專利說明書

（本說明書格式、順序，請勿任意更動，※記號部分請勿填寫）

※申請案號：

※申請日：　　　　　　　　　　※IPC分類：

一、發明名稱：（中文/英文）

二、中文發明摘要：

三、英文發明摘要：

四、指定代表圖：

（一）本案指定代表圖為：圖（　　）。

（二）本代表圖之元件符號簡單說明：

五、本案若有化學式時，請揭示最能顯示發明特徵的化學式：

1

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六、發明說明：

【發明所屬之技術領域】

【先前技術】

【發明內容】

【實施方式】

【圖式簡單說明】

【主要元件符號說明】

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七、申請專利範圍：

1.

2.

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先前技術(Prior Art)

- 先前技術應包含下列內容：
  - 以文字並且輔助圖式呈現最相近前案的技術手段
  - 發明所欲解決的問題
- IDS (Information Disclosure Statement)

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發明內容

- 先前技術之待改善處
- 概述本發明最上位的技術概念、技術手段特徵以及各個請求標的
- 本發明與先前技術間之比較
  (手段、目的、功效)

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實施方式(Example)(1)

- HOW TO MAKE AND HOW TO USE
- 實施例必須包括下列內容：
  - 完整且詳細的實驗操作過程
  - 敘明實驗中所使用的試劑與儀器的來源/型號
  - 對實驗結果的描述以及結論
  - 搭配圖式和/或表格來說明更佳

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US 20060024827 A1-說明書首頁

(19) **United States**

(12) **Patent Application Publication**
Hwang et al.

(10) **Pub. No.: US 2006/0024827 A1**
(43) **Pub. Date: Feb. 2, 2006**

(54) **STROMA-FREE, SERUM-FREE, AND CHEMICALLY DEFINED MEDIUM AND METHOD FOR EX VIVO MONONUCLEAR CELL EXPANSION USING THE SAME**

(75) Inventors: **Shiaw-Min Hwang**, Hsinchu City (TW); **Chao-Ling Yao**, Tainan City (TW); **Tzu-Bou Hsieh**, Hsinchu City (TW); **I-Ming Chu**, Hsinchu City (TW)

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(21) Appl. No.: **11/192,960**

(22) Filed: **Jul. 29, 2005**

Related U.S. Application Data

(60) Provisional application No. 60/592,042, filed on Jul. 29, 2004.

Publication Classification

(51) **Int. Cl.**
C12N 5/02 (2006.01)
(52) **U.S. Cl.** **435/406**

(57) **ABSTRACT**

A stroma-free, serum-free, and chemically defined medium and a method for mononuclear cell expansion ex vivo using the same. An exemplary medium includes a basal medium, a serum substitute, and a cytokine formula.

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US 20060024827 A1-實施例

- Characteristics of the MNC Isolation From UCB
- Serum Substitutes Screening
- Cytokines Screening
- Comparison of Different Basal Media and Commercial Media

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US 7244425-說明書首頁

(12) **United States Patent**
Liu et al.

(10) **Patent No.:** **US 7,244,425 B2**
(45) **Date of Patent:** **Jul. 17, 2007**

(54) **ACID- AND BILE SALT-RESISTANT *LACTOBACILLUS* ISOLATES HAVING THE ABILITY TO LOWER AND ASSIMILATE CHOLESTEROL**

(75) Inventors: **Yu-Ju Liu**, Hsin-Chu (TW); **Chin-Chu Yu**, Kaohsiung (TW); **Ching-Fen Tan**, Hsin-Chu (TW); **Chii-Cherng Liao**, Hsin-Chu (TW)

(73) Assignee: **Food Industry Research and Development Institute**, Hsin-Chu (TW)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

G. H. Fleet et al. "Evolution of Yeasts and Lactic Acid Bacteria During Fermentation and Storage of Bordeau Wines". Applied and Environmental Microbiology 48:1034-1038, Nov. 1984.

S. E. Gilliland et al. "Assimilation of Cholesterol by *Lastobacillus acidophilus* ". Applied and Environmental Microbiology 49(2):377-381, Feb. 1985.

S. W. Gilliland et al. "Factors to Consider When Selecting a Culture of *Lactobacillus acidophilus* as a Dietary Adjunct to Produce a Hypocholesterolemic Effect in Humans". Journal Series of the Oklahoma Agriculture Experiment Station, Stillwater, OK, Paper No. 5596, Sep. 25, 1989.

K. K. Grunewald. "Serum Cholesterol Levels in Rats Fed Skim Milk Fermented by *Lactobacillus Acidophilus* ". Journal of Food Science 47:2078-2079, 1982.

L. L. Rudel et al. "Determination of cholesterol using o-phthalaldehyde". Journal of Lipid Research 14:364-366, 1973.

M. G. O'Sullivan et al. "Probiotic bacteria: myth or reality?" Trends

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US 7244425-實施例

- Isolation and Screening of *Lactobacillus* Isolates
- Acid Tolerance Test
- Bile Salt Tolerance Test
- Assay for the Ability of the Tested Bacterial Strains in Lowering Cholesterol
- Identification and Characterization of *Lactobacillus* Isolates

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實施方式(Example)(2)

- 實施例揭露不足
  —無法取得專利權
- 實施例揭露太多權利範圍太小
  —多出的貢獻給社會
- 實施例揭露的剛剛好
  —為上策

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實施方式(Example)(3)

- 多種不同的實施態樣來例證，或提供學理上的相關資料，會有助於取得較大的範圍
- 生物、化學相關領域專利申請案的審查必須回歸到實施例內容

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圖式/圖式簡單說明

物品或裝置：

立體組合圖、分解圖、剖視圖等

物質與方法：

結構式、流程圖、折線圖、電顯圖等

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申請專利範圍(1)

- 了解最相近的先前技術→先前技術
- 有別於先前技術的特徵所在→可專利性
- 限制條件越多，專利範圍越小
- 哪些是必要的元件或限制？

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申請專利範圍(2)

- 申請專利範圍的形式
- 獨立項/附屬項
- 申請專利範圍的架構

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申請專利範圍的形式(1)

- 組合式
- 特徵式(Jepson-type)：日本、大陸

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申請專利範圍的形式-組合式(1)

- 前言(preamble)

  →標的、適用範疇、與本發明相關但非必要之元件(element)

- 連接詞 (transition)

- 請求主體(body of claim)

  →發明之所有必要元件

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連接詞的種類

- 開放式連接詞：包含、包括
  (comprising, containing, including)
- 半開放式連接詞：基本上由……所組成
  (consisting essentially of ……)
- 封閉式連接詞：由……所組成
  (consisting of ……)

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申請專利範圍的形式-組合式(2)

- TW I241912

  具有降低與同化膽固醇能力的新穎耐酸與耐膽鹽乳桿菌(*Lactobacillus*)分離株

  一種用於消化整腸的藥學組成物,其包含有一如申請專利範圍第1項的乳桿菌物種(*Lactobacillus sp.*)之分離株。

前言

連接詞

請求主體

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申請專利範圍的形式-組合式(3)

- TW I272912

一種含有血管升壓素轉換酶抑制劑之機能性發酵乳的製備方法

1.一種含有血管升壓素轉換酶抑制劑之發酵乳的製備方法,其步驟包含:
(1)準備一能供製備發酵乳之原料乳,並於該原料乳中接種適量的乳酸菌;
(2)將步驟(1)中的該原料乳置於適合該乳酸菌生長的溫度下進行發酵反應;
(3)進一步於步驟(2)的該原料乳中,於接種該乳酸菌後0-2小時內,添加0.1-1.0%(w/w)的蛋白酶以及
(4)於該發酵反應之發酵時間達3-10小時後中止反應。

前言
連接詞
請求主體

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申請專利範圍的形式-特徵式(1)

- 前言(preamble)

  →標的、適用範疇、與習知技術共有之元件(element)

- 在前言與請求主體之間

  →...其特徵在於：...

- 請求主體(body of claim)

  →發明中非與習知技術共有之元件

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申請專利範圍的形式-特徵式(2)

- TW I288779 具單核球來源之經去分化的可程式化幹細胞及其製造與用途

一種用於生產具人類單核球來源之經去分化的可程式化幹細胞之方法,其特徵在於:

a)自人類血液中分離單核球;

b)單核球係於一適宜的培養基中增殖,該培養基含有細胞生長因子 M-CSF;

c)單核球係與第b)步驟同時或於其後培養於一種含有IL-3之培養基中;及

d)藉由自培養基中分離細胞,而得人類成年型經去分化的可程式化幹細胞。

前言

請求主體

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申請專利範圍的形式-特徵式(3)

- TW I274074

  低酒精啤酒般飲料及其製造方法

1.一種低酒精啤酒般飲料製造方法,其特徵在於,稀釋啤酒及/或發泡酒,使酒精濃度不足1v/v%;同時,配加賦與視覺及香味上有如啤酒般之特徵的添加劑。

前言

請求主體

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獨立項/附屬項

- 獨立項

→以「一種……(A/An……)」為開頭

→一個申請案可以有一個以上的獨立項

- 附屬項

→敘明被依附的請求項項號以及申請標的

→限定部分：增加技術特徵(進一步限定或增加非必要元件)

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申請專利範圍的架構-獨立項

前言 ← 連接語 → 本體

We claim:

1. A method of assaying lipid or cholesterol metabolism, or a combination thereof, in a vertebrate's digestive system by a high through-put assay in a teleost. the method comprising:

providing fluorescently labeled, quenched or unquenched lipid reporters, wherein fluorescent moieties are covalently linked to the lipid and/or cholesterol being assayed, such that the resulting lipid reporters are absorbed by the vertebrate's digestive system or function as substrates for target enzymes in the vertebrate's digestive system:

bathing the teleosts in media comprising the labeled lipid reporters, such that the lipid reporters are ingested by the teleost into its digestive system:

screening for and detecting accumulated fluorescence in digestive organs of the teleost as an indicator of metabolic processing or uptake of the lipid reporter in the digestive system: and

quantifying the amount and rate of fluorescence from the processed or unprocessed fluorescently labeled lipid reporters in the digestive organs of the teleost, providing a high through-put readout of digestive organ function, physiology of the digestive system and organs, and metabolic lipid processing.

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申請專利範圍的架構-附屬項(1)

2. The method of claim **1**, wherein the fluorescently labeled lipid reporters are selected from phospholipase A_2(PLA_2) substrates.

Claim 1 所載內容	+	Claim 2 所載內容

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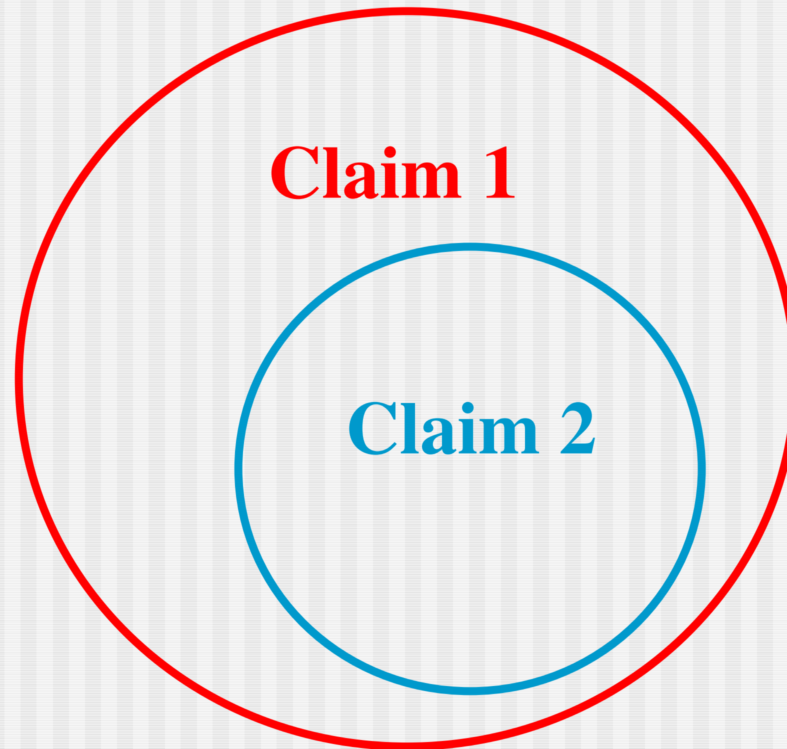
獨立項與附屬項的關係



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申請專利範圍的架構-附屬項(2)

6. The method of claim 1 wherein the teleost is an intact viable zebrafish.

7. The method of claim 6, wherein the zebrafish is a zebrafish larvae, embryo or adult.

Claim 1
所載內容
+
Claim 6
所載內容
+
Claim 7
所載內容

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獨立項與附屬項的關係

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謝謝您的參與!

若有任何問題，歡迎寫信至

siiplo@mail.saint-island.com.tw

kevin.chen@mail.saint-island.com.tw

(請於信件主旨中指名收信者)

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專利書目識別代碼(INID)

- 由專利資訊檢索國際合作委員會所訂定，以二位數阿拉伯數字來代表各個書目資料項目，以彌補各國專利文獻在語言文字及編排上的差異。

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國際專利分類(IPC)(1)

國際專利分類為一種編排各種專利類型的通用分類法，分成八個大部，由A~H開頭；

A- 人類生活必需品

B- 作業;運輸

C- 化學;冶金

D- 紡織;造紙

E- 固定建築物

F- 機械工程;照明;供熱;武器;爆破

G- 物理

H- 電學

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國際專利分類(IPC)(2)

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