

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 August 2004 (12.08.2004)

PCT

(10) International Publication Number
WO 2004/066823 A2

(51) International Patent Classification⁷:

A61B

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(21) International Application Number:

PCT/US2004/002535

(22) International Filing Date: 29 January 2004 (29.01.2004)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/443,681 30 January 2003 (30.01.2003) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:

US 60/443,681 (CON)
Filed on 30 January 2003 (30.01.2003)

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

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

Published:

— without international search report and to be republished upon receipt of that report

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



WO 2004/066823 A2

(54) Title: METHODS AND COMPOSITIONS FOR ANALYSIS OF MITOCHONDRIAL-RELATED GENE EXPRESSION

(57) Abstract: The invention provides arrays for analyzing the expression of mitochondrialrelated coding sequences. The invention allows the efficient analysis of expression levels across each of these coding sequences. The invention has important applications in the field of medicine for the screening and diagnosis of patients with ailments associated with aberrant mitochondrial function, as well as in the development of treatments therefore.

BACKGROUND OF THE INVENTION

The present application claims priority to co-pending U.S. Provisional Patent Application Serial No. 60/443,681 filed January 30, 2003. The entire text of the above-referenced disclosure is specifically incorporated herein by reference without disclaimer.

5 The government may own rights in the present invention pursuant to grant number Grant No. P60AG17231 from the National Institutes of Health, National Institute on Aging.

1. Field of the Invention

The present invention relates generally to the fields of molecular biology and medicine. More particularly, the invention relates to arrays of nucleic acids immobilized
10 on a solid support for selectively monitoring expression of mitochondrial-related genes from the nuclear and mitochondrial genomes and methods for the use thereof.

2. Description of Related Art

Global populations of individuals over the age of 65 have increased, with most
destined to live into their 80s. Given the average survival age of the elderly,
15 improvements in the health of the elderly are needed or the economy will be faced with a tremendous burden. The economy will be burdened with special needs for nursing care, transportation, housing, and medical arrangements. This burden can be reduced by improving overall health care. Substantial increases in research on diseases of aging are thus needed. Currently, less than one percent of the 1.14 trillion dollars the U.S. spends
20 each year on health care goes for research on Alzheimer's, arthritis, Parkinson's, prostate cancer and other age-related diseases. Unless more diseases of aging are delayed or conquered, mounting bills for illness will swamp even the most robust Medicare program.

Finding cures and alleviating symptoms of diseases would have a major positive
25 effect on the economy. According to studies by the Milken Institute, an investment of 175 million dollars in diabetes research now saves 7 billion dollars in medical costs. Work done by the University of Chicago supports this thinking, with studies reporting that the economic value of reductions in heart disease in people aged 70 to 80 could amount to 15 trillion dollars. Also, as exemplified by the work of others, diseases such as

polio, Alzheimer's and many other aging and age-related diseases can be conquered. Thus, research can do much to improve the quality of life for the elderly.

A major key to understanding, alleviating, or ameliorating diseases of the aging population lies in the genetic basis of aging. The sequence of the entire human genome 5 Anderson *et al.*, 1981) has been completed and will greatly advance the development of technologies beneficial in understanding the genetic basis of aging. The sequence of the entire mouse genome has recently been reported and will advance biomedical research on animal models representative of human diseases (Waterston, *et al.*, 2002). Studies at UTMB Galveston have recently shown that mitochondrial (mtDNA) is damaged three to 10 four times more frequently than nuclear DNA by a wide variety of agents, which induce reactive oxygen species (Mandavilli *et al.* 2002; Santos *et al.*, 2002; Ballinger *et al.*, 2000). Thus, mitochondrial DNA and its ability to transcribe mitochondrial specific genes represent a critical cellular target for reactive oxygen species-induced cell death.

There are two major hypotheses that deal with the role of mitochondrial integrity 15 and function in aging: firstly, the catastrophic demise of mitochondrial function is a primary mechanism in aging; and secondly, ROS generated in the mitochondria causes mitochondrial DNA damage, which in turn causes the release of more ROS, leading to further mitochondrial decline and age-associated pathologies (Harmon, 1972; Golden and Melov, 2001; Ames *et al.*, 1993; Finkel and Holbrook, 2000; Beckman and Ames, 1998; 20 Beckman and Ames, 1999; Zhang *et al.*, 1992).

Therefore, the integrity of the mitochondria is a major factor in the function of aged tissues, mitochondria-associated diseases, and responses of the mitochondria to oxidative stress or inflammatory agents - both environmental and internal. The mitochondrion provides the energy needed to carry out critical biological functions. Any 25 factor(s) that disrupt or compromise mitochondrial functions are of importance, because they relate to diseases including genetic diseases, environmental toxins, and responses to hormones and growth factors (Mitochondria and Free radicals in Neurodegenerative Diseases, 1997).

Most human genes are encoded by the nuclear DNA of the cell, but some are also 30 found in the mitochondrial DNA. Mitochondria are the "power plants" within each cell

and provide about 90 percent of the energy necessary for cells – and thus provide tissues, organs and the body as a whole with energy. Mutations of the mtDNA can cause a wide range of disorders – from neurodegenerative diseases to diabetes and heart failure. Scientists also suspect that injury to the genes within the mitochondria may play an
5 important role in the aging process as well as in chronic degenerative illnesses, such as Alzheimer's Parkinson's and Lou Gehric's disease (Golden and Melov, 2001; Ames *et al.*, 1993).

In the course of investigating mtDNA deletions in disease it became apparent that normal individuals can also be heteroplasmic for deleted mtDNA and that the fraction of
10 deleted DNA increases exponentially with age. These observations raised interest in the role played by mtDNA mutations in aging. One hypothesis is that continuous oxidative damage to mtDNA is responsible for an age-related decline in oxidative phosphorylation capacity (Golden and Melov, 2001; Finkel and Holbrook, 2001; Ventura *et al.*, 2002). Whether a causal relationship exists between mtDNA mutations and aging, however,
15 remains to be established.

What has been lacking in the art is a procedure allowing simultaneous and parallel determination of the activity of mitochondrial and nuclear genes that make the enzymes and structural protein of the mitochondrion. Analysis of the mRNA levels of each of these genes would provide insight as to the overall biochemical phenotype (picture) of
20 mitochondrial organellogenesis. Procedures have been available to determine the activity of a limited numbers of genes in one experiment. There are, however, several hundred mitochondrial-related genes. What is needed, therefore, is a method of analyzing the expression of these genes, thereby providing insight as to the roles mitochondrial proteins play in different disease states.

25

SUMMARY OF THE INVENTION

The invention overcomes the deficiencies in the art by providing methods and compositions for assessing the integrity and function of the mitochondria. Thus, the invention provides arrays comprising nucleic acid molecules comprising a plurality of
30 sequences, wherein the molecules are immobilized on a solid support and wherein at least

5% of the immobilized molecules are capable of hybridizing to mitochondrial-related acid sequences or complements thereof.

In some aspects of the invention, the array may further be defined as comprising at least 20, at least 40, at least 100, at least 200, or at least 400 nucleic acid molecules. In 5 other aspects the array of the invention comprises nucleic acid molecules comprising cDNA sequences. In further aspects of the invention, the nucleic acid molecules may comprise at least 17 nucleotides. These mitochondrial-related nucleic acid sequences may, for example, be from a mammal, a primate, a human, a mouse, a yeast, an arthropod such as a Drosophila, or a nematode such as *C. elegans*. In certain embodiments of the 10 invention, at least 25%, at least 35%, at least 50%, at least 75%, at least 85%, at least 95%, or at least 100% of the immobilized molecules are capable of hybridizing to mitochondrial-related nucleic acid sequences or complements thereof. In still a further aspect of the invention, at least one of the mitochondrial-related nucleic acid sequences is encoded by a mitochondrial genome.

15 In particular aspects of the invention, the immobilized molecules are capable of hybridizing to at least 5, at least 10, at least 15, at least 30, at least 60, at least 100, or at least 200 mitochondrial-related nucleic acid sequences or complements thereof. In further aspects of the invention, the immobilized molecules are capable of hybridizing to at least 300, at least 500, or at least 1000 mitochondrial-related nucleic acid sequences or 20 complements thereof. In further aspects of the invention, at least one of the mitochondrial-related nucleic acid sequences is encoded by a nuclear or mitochondrial genome.

In a further aspect, the invention provides a method for measuring the expression of one or more mitochondrial-related coding sequence in a cell or tissue, the method 25 comprising: a) contacting an array as described above with a sample of nucleic acids from the cell or tissue under conditions effective for mRNA or complements thereof from the cell or tissue to hybridize with the nucleic acid molecules immobilized on the solid support; and b) detecting the amount of mRNA or complements thereof hybridizing to mitochondrial-related nucleic acid sequences or complements thereof. In one 30 embodiment of the invention, the detecting in step (b) may be carried out

colorimetrically, fluorometrically, or radiometrically. In certain embodiments, the cell may be a mammal cell, a primate cell, a human cell, a mouse cell, or an yeast cell.

In yet another aspect, the invention provides a method of screening an individual for a disease state associated with altered expression of one or more mitochondrial-related nucleic acid sequences comprising: a) contacting an array, according to that described above, with a sample of nucleic acids from the individual under conditions effective for the mRNA or complements thereof from the individual to hybridize with the nucleic acid molecules immobilized on the solid support; b) detecting the amount of mRNA or complements thereof hybridizing to mitochondrial-related nucleic acid sequences; and c) screening the individual for a disease state by comparing the expression of the mitochondrial-related nucleic acid sequences detected with a pattern of expression of the mitochondrial-related nucleic acid sequences associated with the disease state. In one embodiment of the invention, the disease state may be selected from that provided in Table 1. In particular aspects, the disease state is cystic fibrosis, Alzheimer's disease, Parkinson's disease, ataxia, Wilson disease, Maple syrup urine disease, Barth syndrome, Leber's hereditary optic neuropathy, congenital adrenal hyperplasia diabetes mellitus, multiple sclerosis, or cancer, but is not limited to such.

In one embodiment of the invention, detecting the amount of mRNA or complements thereof hybridizing to mitochondrial-related nucleic acid sequences may be carried out colorimetrically, fluorometrically, or radiometrically. In further aspects of the invention, the individual may be a mammal, a primate, a human, a mouse, an arthropod, or an nematode but is not limited to such.

In still yet another aspect, the invention provides a method of screening a compound for its affect on mitochondrial structure and/or function comprising: a) contacting an array according to that described above, with a sample of nucleic acids from a cell under conditions effective for the mRNA or complements thereof from the cell to hybridize with the nucleic acid molecules immobilized on the solid support, wherein the cell has previously been contacted with the compound under conditions effective to permit the compound to have an affect on mitochondrial structure and/or function; b) detecting the amount of mRNA encoded by mitochondrial-related nucleic

acid sequences or complements thereof that hybridizes with the nucleic acid molecules immobilized on the solid support; and c) correlating the detected amount of mRNA encoded by mitochondrial-related nucleic acid molecules or complements thereof with the affect of the compound mitochondrial structure and/or function.

5 In one embodiment of the invention, the compound is a small molecule. In another embodiment of the invention, the compound is formulated in a pharmaceutically acceptable carrier or diluent. In still another embodiment of the invention, the compound may be an oxidative stressing agent, an inflammatory agent, or a chemotherapeutic agent.

10 In still yet another aspect, the present invention provides a method for screening an individual for reduced mitochondrial function comprising: a) contacting an array according to that described above, with a sample of nucleic acids from a cell under conditions effective for the mRNA or complements thereof from the cell to hybridize with the nucleic acid molecules immobilized on the solid support; b) detecting the amount of mRNA encoded by mitochondrial-related nucleic acid sequences or 15 complements thereof that hybridizes with the nucleic acid molecules immobilized on the solid support; and c) correlating the detected amount of mRNA or complements thereof with reduced mitochondrial function.

20 In certain embodiments of the invention, the detecting step as described above may be carried out colorimetrically, fluorometrically, or radiometrically. In still another embodiment, the individual is a mammal, a primate, a human, a mouse, an arthropod, or a nematode.

25 It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments 30 of the invention, are given by way of illustration only, since various changes and

modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. DNA microarray generated from PCR™ products using thirteen genes that code for mitochondrial proteins.

FIG. 2. Map of the *Mus musculus* mitochondrial DNA showing the location of the 13 peptides of the OXPHOS complexes.

FIG. 3. Map of the *Homo sapien* mitochondrial DNA showing the location of the 13 peptides of the OXPHOS complexes.

FIG. 4. The effects of rotenone, an inhibitor of mitochondrial Complex I, on the expression of mouse mitochondrial genes in AML-12 mouse liver cells in culture.

FIGS. 5A-5B. Analysis of mitochondrial DNA encoded gene expression. **FIG. 5A** - response to 3-nitropropionic acid, an inhibitor of Complex II - succinic dehydrogenase. The data show that inhibition of Complex II stimulates the synthesis of mitochondrial encoded mRNAs and the 23S and 16S ribosomal RNAs. **FIG. 5B** - analysis of mitochondrial DNA encoded gene expression in trypanosome infected heart tissue. The data show a decline in mRNA and ribosomal RNA levels at 37 days post infection.

FIGS. 6A-6C. Analysis of mitochondrial gene expression in mouse mutants. **FIG. 6A** - mitochondrial gene expression in livers of young Snell dwarf mouse mutants. **FIG. 6B** - analysis of mitochondrial gene expression in livers of aged Snell dwarf mouse mutants. **FIG. 6C** - RT-PCR analysis of Hsd3b5 expression levels in control versus dwarf Snell mice.

FIGS. 7A- 7D. Analysis of mitochondrial gene expression in heart muscle of trypanosome infected mice. FIG. 7A – control; FIGS. 7B-7D – three heart muscles from trypanosome infected mice.

FIGS. 8A-8D. The effects of 40% TBS thermal injury on mouse liver mitochondrial function in control (**FIG. 8A**) and three livers from thermally injured mice 24 hours after burn (**FIGS. 8B-8D**).

FIG. 9. Array analysis of the expression of the 13 mitochondrial DNA encoded genes in livers of thermally injured mice.
5

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention overcomes limitations in the art by providing methods and compositions for determining the integrity and function of the mitochondria. Arrays are 10 provided that allow simultaneous screening of the expression of mitochondrial-related coding sequences. The invention thus allows determination of the role of mitochondrial genes in various disease states. The ability to accumulate gene expression data for the mitochondria provides a powerful opportunity to assign functional information to genes of otherwise unknown function. The conceptual basis of the approach is that genes that 15 contribute to the same biological process will exhibit similar patterns of expression. This mitochondrial gene array thus provides insight into the development and treatment of disease states associated with effects on mitochondrial structure and/or function.

A. The Present Invention

20 Use of arrays, including microarrays and gene chips, provides a promising approach for uncovering mitochondrial gene function. A major factor in the age-associated gradual decline of tissue function has been attributed to the reduction or loss of mitochondrial integrity and function. Furthermore, this has been attributed to the age-associated increase in oxidative stress that targets mitochondrial DNA and proteins. One 25 aspect of the present invention is thus to determine the integrity of the mitochondria, both structure and function, as is indicated by the activity of the genes that code for mitochondrial enzymes and structural proteins.

Another aspect of the present invention is to identify the genetic expression patterns that govern aging. The mtDNA array can be used to determine specific patterns 30 of altered gene expression for mtDNA as well as the nuclear DNA that encodes the

mitochondrial proteins. In order to achieve this goal, mitochondrial and related nuclear genes can be used to generate an array of nucleic acids by immobilizing them on a solid support, including, but not limited to, a microscopic slide or hybridization filter. By screening a plurality of mitochondrial-related coding sequences (genes) in this manner, 5 associations between gene expression and various disease states may be determined.

The term "array" as used herein refers to any desired arrangement of a set of nucleic acids on a solid support. Specifically included within this term are so called microarrays, gene chips and the like. As used herein, the term "mitochondrial-related" coding sequence refers to those coding sequences necessary for the proper structure, 10 assembly, and/or function of mitochondria. Such mitochondrial-related coding sequences may be found on the nuclear and mitochondrial genomes. The term "plurality of mitochondrial-related coding sequences" refers to at least 13 mitochondrial encoded genes, which represents a minimum representative sampling for screening of gene expression associated with mitochondrial structure and/or function.

15 Patterns of mitochondrial gene expressions in younger and older animal tissue can be screened with the invention by including in arrays nucleic acids from genes that are expressed in different tissues such including, but not limited to, liver, brain, heart, skeletal and cardiac muscle, spleen, kidney, gut, and blood. The differences in the expression of the mitochondrial genes in younger and older animals will provide insight 20 into the regulatory processes of mtDNA in aging.

The arrays provided by the invention can also be used to study young versus aged tissues in mice, in response to a number of substances, for example, candidate drugs, inflammatory agents, heavy metals, and major acute phase reactants. The pathways associated with longevity and the effects of aging in responding to stress can thus be 25 analyzed. The genes encoding signaling pathway intermediates activated by mitochondrial damaging agents and the genes targeting these pathways may also be examined.

The arrays provided by the invention may also be used to identify the effects of aging on liver, brain, muscle and other tissues as well as various other cells in culture; for 30 example, to demonstrate that increased ROS due to mitochondrial damage in aged tissues

may be a basic factor in the persistent activation of signals mediating chronic stress; and to demonstrate that the response to stress and injury is a major process affected by aging. Previous studies suggest that each tissue in the body could exhibit specific age-associated decrements in its ability to manifest specific response(s) to stress. The invention could 5 thus be used to establish that responses to stress are intrinsic processes affected by aging even in the absence of disease, but whose decline can be accelerated by environmental factors and disease.

The arrays of the invention could also be used, for example, to investigate the role or effect of mitochondrial function in different diseases, including neurodegenerative 10 diseases (Alzheimer's and Parkinson's disease), diabetes mellitus, and others (Table 1). The arrays may also be used for the development of drugs and evaluation of their effects on mitochondrial function, and for the identification and detection of modulation of mitochondrial damage in different disease states. Table 1 lists some of the *Mus musculus* and corresponding *Homo sapiens* mitochondrial genes and the human diseases associated 15 with specific genetic defects. Accordingly, one aspect of the invention provides an array comprising nucleic acids corresponding to the accessions listed in Table 1. In one embodiment of the invention, nucleic acids of at least 5, 10, 13, 15, 20, 30 or 40 or more of the accessions given in Table 1 are included on an array of the present invention.

In another embodiment of the present invention, it is contemplated that the arrays 20 may be used to screen "knockout" or "knockin" genes affecting mitochondrial development or function. Well known technologies such as, but not limited to, the Cre-lox system, homologous recombination, and interfering RNAs (siRNA, shRNA, RNAi) are commonly used by those skilled in the art to alter gene expression in animals or cell lines. The arrays of the present invention could be used to monitor the degree of altered 25 gene expression which would indicate the success or failure of such experiments. For instance densitometric or fluorescent analysis of arrays of the present invention could determine the degree of expression reduction in a shRNA experiment where success or failure is measured by the degree of gene knockdown. Commonly the number of interfering RNA molecules hybridizing along a gene sequence determines the degree of expression reduction which could be compared to controls in an array experiment where 30

one or more genes could be altered. Therefore in this embodiment the arrays of the present invention could be used to monitor one or many genes with respect to their expression levels in gene expression altering experiments.

Overall, the invention has broad applicability in that it encompasses all factors
5 that will affect mitochondrial biogenesis and assembly (replication) and mitochondrial function under any physiological or pathophysiological conditions.

Table 1

<i>Mus musculus</i> Gene List			<i>Homo sapien</i> Gene List and Related Diseases		
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Related Disease</u>
-	I48884	-	MTOP_D1	Deficiency of complex I	
Abc7	U433892	ABAT	GABT_HUMAN		
Acadl	ACDL_MOUSE	ABC7	ABC7_HUMAN	X-linked sideroblastic anemia and ataxia (XLSSA/A)	
Acadm	A55724	ACAA2	S43440		
Acads	I49605	ACADL	A40559	LCAD deficiency	
Acadv1	ACDV_MOUSE	ACADM	I52240	MCAD deficiency	
Acat1	87870	ACADS	A30605	SCAD deficiency	
Acat2	87871	ACADSB	A55680		
Aco2	87880	ACADVL	ACDB_HUMAN	VLCAD deficiency	
Aif	AF100927	VLCAD			
Ak2	87973	ACAT1	JH0255	Deficiency of 3-ketothiolase (3KTD)	
Ak3	87979	ACAT			
Alas2	SYMSAL	T2			
Aldh2	I48966	THIL			
Ahd-5		ACO2	Q99798		
Ahd1		AFG3L2	Y18314		
And5		AGXT	P21549		
Ant1	S37210	AIF	AF100928		
Ant2	S31814	AK2	KAD2_HUMAN		
Aop1; Aop2	JQ0064	AK3	KIHUA3		
Ap5a1	JC1473	AKAP1	I39173		
Ap5b	P56480	AKAP84			
Ap5g1	AT91_MOUSE	AKAP84	I39173		
Ap5k	JC1412	AKAP1			
Atp5i		ALAS1	SYHUAL		
Ap7b	U38477	ALAS			

<i>Mus musculus</i> Gene List				<i>Homo sapien</i> Gene List and Related Diseases			
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>	
Bax	BAXA_MOUSE	ALAS2	SYHUAE				
Bckdha	S71881	ASB					
Bckdhb	S39807	ALDH2	DEHUE2				
Bcl2	B25960	Hs.1230					
D1Nds7		ALDH4	PUT2_HUMAN				
D1Nds7		ALDH5	A40872				
Bzrp	A53405	AMACR	CAB44062				
COII/NDS5	ND5 I76673	AMT	I54192				
Car5	S12579	AOP1	TDXM_HUMAN				
Cbr2	A28053	ARG2	ARG2_HUMAN				
Ckmtl	S24612	ATP5A1	PWHUA				
Cox4	S12142	ATP5A2	NNN10				
Cox5a	S05495	ATP5A1	NNN08				
Cox5b	A39425	ATP5A2	NNN09				
Cox6a1	COXD_MOUSE	ATP5B	A33370				
Cox6a2	S552088	ATPSB					
Cox6b	107460	ATP5BL1	NNNN06				
Cox6c2	S16083	ATP5BL2	NNNN07				
Cox7a2	I48236	ATP5C1	A49108				
Cox7c1	S10303	ATP5C2	NNNN03				
Cox7c	COXO_MOUSE	ATP5CL1	NNNN04				
Cox8a	COXR_MOUSE	ATP5CL2	NNNN05				
Cox8b	COXQ_MOUSE	ATP5D	S22348				
Cpo	A48049	ATP5E	AF077045				
Cps1	891996	ATP5F1	JQ1144				
Cpt2	A49362	ATP5G1	S34066				
Crat	CACP_MOUSE	ATP5G2	S34067				
Cs	88529	ATP5G3	I38612				

<i>Mus musculus</i> Gene List		<i>Homo sapien</i> Gene List and Related Diseases	
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>
Cycts	CCMS	ATP5I	AB028624
Cycrt	CCMST	ATP5J	JT0563
Cyp11a	88582	ATP5O	ATPO_HUMAN
Cyp11b1	A41552	OSCOP	
Cyp11b2	88584	ATP7B	S40525
Cyp24	S60033	BAX	BAXA_HUMAN
Cyp27	88594	BCAT2	BCAM_HUMAN
Dbt	S65760	BCKDHA	DEHUXA
BCKADE2		BCKDHB	A37157
Dci	S38770	BCL2	D37332
Dial	94893	BCL2L1	BCLX_HUMAN
Did	107450	BCLX	
Es9	95448	BCS1L	AF026849
Etfα	106092	BDH	A42845
Etfβ	106098	BID	BID_HUMAN
Etfdh	106100	BNIP3L	NIPL_HUMAN
Foxj1	S53524	BZRP-S	A49361
Fdxr	S60028	BZRP	I38105
Fech	A37972	C14ORF2	68MP_HUMAN
Fpgs	S65755	PLPM	
Frida	S75712	CA5	CRHUS
Gcdh	GCDH_MOUSE	CACT	Y10319
Gls	95752	CASQ1	A60424
Glud	S16239	CGI-114	T14770
Got2	S01174	CKMT1	A30789
Hadhd	JC4210	CKMT2	A35756
Hces	CCHL_MOUSE	CLPP	S68421
Hk1	A35244	CLPX	CLPX_HUMAN

<i>Mus musculus</i> Gene List		<i>Homo sapien</i> Gene List and Related Diseases			
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>	
Hmgcl	HMGL_MOUSE	COQ7	AF032900		
Hngcs2	B55729	CLK-1			
Hsc70t	96231	COX11	COXZ_HUMAN		
Hsd3b1	I49762	COX15	AF044323		
Hsd3b2	3BH2_MOUSE	COX17	Q14061		
Hsd3b3	3BH3_MOUSE	COX4	OLHU4		
Hsd3b4	3BH4_MOUSE	COX5A	OTHU5A		
Hsd3b5	3BH5_MOUSE	COX5B	OTHU5B		
Hsd3b6	3BH6_MOUSE	COX5BL4	NNN01		
Hsp60	HHMS60	COX6A1	OGHU6L		
HSPD1		COX6A2	OGHU6A		
Hsp70-1	Q61698	COX6B	OGHU6B		
Hsp74	A48127	COX6C	OGHU6C		
HspE1	A55075	COX7A1	OSHU7A		
Hspe1	CH10_MOUSE	COX7A2	OSHU7L		
Idh2	IDHP_MOUSE	COX7B	OSHU7B		
Maca	I59594	COX7C	OSHU7C		
Maob	96916	COX7RP	O14548		
Mcs	A37199	COX8	OSHU8		
Mint44	U69898	CPO	I52444	Hereditary coproporphyria (HCP)	
Mod2	97045	CPS1	JQ1348	Hyperammonemia, type I	
Mor1	DEMSSMM	CPT1A	I59351	Carnitine O-palmitoyltransferase I deficiency	
Mthfd	A33267	CPT1-L			
Mut	S03680	CPT1B	S70579		
Ndufa4	NUML_MOUSE	CPT2	A39018	Carnitine O-palmitoyltransferase II deficiency	
Ndufs6	NUMM_MOUSE	CPT1			
Nrt	S54876	CRAT	A55720	Carnitine O-acetyltransferase deficiency	
Oat	XNMSO	CS	AF047042		

***Mus musculus* Gene List**

<i>Homo sapien</i> Gene List and Related Diseases			
gene	Accession	gene	Accession
gene	Accession	gene	Accession
Ogdh	ODO1_MOUSE P11928	CYB5	CBHUS S00680
Oias1	P29080	CYC1	
Oias2		Hs.697	
Otc	OWMS 97499	CYP11A1	S14367
Poca	A47255	CYP11A	A25922
Pcx		CYP11B1	S11338
Pdha1	\$23506	CYP11B	
Pdhal	S23507	CYP11B2	B34181
Pla2g2a	I48342	CYP24	A47436
Polg	DPOG_MOUSE S68367	CYP27	A39740
Ppox	97937	CYP3	A41581
Rnmp		DBT	A32422
Rp123	1196612	DCI	A55723
Scp2	A40015	DEC R	S53352
Slc1a1	EAT3_MOUSE	DFN1	U66035
EAAC1		DGUOK	JC6142
Sod2	I57023	DHODH	PC1219
Star	A55455	DIA1	RDHUB5
Surf	B25394	DLAT	XXHU
Tfarm	P97894	DLTA	
Tst	THTR_MOUSE A31106	DLAT_h	S25665
Ucp	UNG_MOUSE	DLD	DEHULP
Ung		DLDH	
UNG1		LAD	
Vdac1	106919	DLST	PN0673
Vdac2	106915	DMGDH	M2GD_HUMAN
Vdac3	106922	DUT	DUT_HUMAN
Ywhaz	JC5384	ECGR1	P19971
			Myoneurogastrointestinal encephalopathy syndrome (MNGIE)

<i>Mus musculus</i> Gene List		<i>Homo sapien</i> Gene List and Related Diseases			
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	Related Disease	
mt-Atp6	PWMS6	ECHS1	ECHM_HUMAN		
MTATP6		EFE2	TFZ_HUMAN	Barth syndrome	
mt-Atp8	PWMS3	EFTS-LSB	I84606		
	ODMS1	ENDOG	NUCG_HUMAN		
mt-Co1	OBMS2	ETFA	A31998	Gluutaric aciduria, type IIa (GAIIa)	
mt-Co2	OTMS3	ETFB	S32482	Gluutaric aciduria, type IIb (GAIIb)	
mt-Co3	CBMS	ETFDH	Q16134	Gluutaric aciduria, type IIc (GAIIc)	
mt-Cyrb		FACL1	LCFA_HUMAN		
COB	QXMS1M	FACL2	JX0202		
mt-Nd1	QXMS2M	FARS1	AF097441		
mt-Nd2		FDX1	AXHU		
ND2	QXMS3M	FDX			
mt-Nd3		FDXR	A40487		
ND3	QXMS4M	FECH	A36403	Erythropoietic protoporphyrina (EPP)	
mt-Nd4		FH	UFHUM	Deficiency of fumarate hydratase	
ND4	QXMS4L	FPGS	A46281		
mt-Nd4l	QXMS5M	FRDA1	U43747		
mt-Nd5	QXMS5M	GAT	AF023466	Friedreich ataxia 1	
ND5	DEMSN6	GATM	S41734		
mt-Nd6		GCDH	GCDH_HUMAN		
ND6	12S_rRNA	GCK	A46157	Gluutaric aciduria, type I (GA-I)	
mt-Rnr1	16S_rRNA	HK4		Diabetes mellitus, type II (NIDDM)	
mt-Rnr2	tAla_1	HK4			
mt-Ta		tCys_1	Hs.1270		
mt-Tc		tAsp_1	Hs.1270		
mt-Td		tGlu_1	NIDDM		
mt-Te		tPhe_1	NIDDM		
mt-Tf		tGly_1	GCSH	GCHUH	Non-ketotic hyperglycinemia, type III (NKH3)
mt-Tg					

<i>Homo sapien</i> Gene List and Related Diseases			
<u>Gene</u>	<u>Accession</u>	<u>Gene</u>	<u>Accession</u>
<u>Gene</u>	<u>Accession</u>	<u>Gene</u>	<u>Accession</u>
mt-Th	tHis_1	GK	GLPK_HUMAN
mt-Ti	tIle_1	GKP2	GKPF_HUMAN
mt-Tk	tLys_1	GLDC	B39521
mt-Tl1	tLeu_1	GLUD1	DEHUE
mt-Tl2	tLeu_2	GLUDP1	A53719
mt-Tm	tMet_1	GOT2	XNHUDM
mt-Tn	tAsn_1	GPD2	GPDDM_HUMAN
mt-Tp	tPro_1	GST12	B28083
mt-Tq	tGln_1	HADHA	JC2108
mt-Tr	tArg_1	HADHB	JC2109
mt-Ts1	tSer_1	HCCS	G02133
mt-Ts2	tSer_2	HCHU	
mt-Tt	tThr_1	AF112968	
mt-Tv	tVal_1	HIBADH	D3HI_HUMAN
mt-Tw	tItp_1	HK1	A31869
mt-Ty	tTyr_1	HK2	JC2025
		HLCS	BPL1_HUMAN
		Hs.12357	
		HMGCL	A45470
		HMGCS2	S51103
		HSD3B1	DEHUHS
		HSDB3	
		HSD3B2	DEHUH2
		HSPA1L	B45871
		HSPA9	B48127
		GRP75	
		HSPD1	A32800

<i>Mus musculus</i> Gene List		<i>Homo sapien</i> Gene List and Related Diseases		
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>
GROEL				
HSPE1	S47532			
CPN10				
HTOM34P	Q15785			
HTOM	AF026031			
Hs.3816	A56650			
DH2	S57499			
DH3A	S55282			
DH3B	IDHB_HUMAN			
DH3G	IDHG_HUMAN			
IVD	A37033	Isovleric acidemia (IVA)		
KIAA0016	S66619			
TOM20				
KIAA0028	SYLM_HUMAN			
KIAA0123	Q10713			
KNP-I	JC4913			
LOC51081	JC7165			
LOC51189	JC7175			
LOC51629	NP_057100			
LOC56624	NP_063946			
MAOA	A36175	Brunner's syndrome		
MAOB	JH0817			
MCD	DCMC_HUMAN	Malonyl-CoA decarboxylase deficiency (MLYCD)		
MCSP	MCS_HUMAN			
MDH2	MDHM_HUMAN			
ME2.1	S53351			
ME2	A39503			
MFT	AF283645			

<i>Mus musculus</i> Gene List				<i>Homo sapien</i> Gene List and Related Diseases			
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>			
MIPEP	U80034						
MIP							
MLN64	S60682	MMSDH	MMSA_HUMAN	Methylmalonate semialdehyde dehydrogenase deficiency (MMSDHD)			
MPO	OPHUM						
MRRF	AA085690			Myeloperoxidase deficiency (MPOD)			
MTRRF							
RRF							
MT-ACT48	AF132950						
MTABC3	AF076775						
MTATP6	PWHU6			Leigh syndrome; Neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP); Leber's hereditary opticneuropathy (LHON); Familial bilateral striatal necrosis (FBSN)			
MTATP8	PWHU8						
MTATT	NNN20						
MTCH1	AF176006						
CHI-64							
MTCH2	NP_055157						
MTCO1	ODHU1						
				Leber's hereditary optic neuropathy (LHON); Alzheimer disease (AD); Myoclonus epilepsy; deafness, ataxia, cognitive impairment and Cox deficiency; Acquired idiopathic sideroblastic anemia (AISA)			
				Alzheimer disease (AD); Mitochondrial encephalomyopathies			
MTCO2	OBHU2						
MTCO3	OTHU3			Leber's hereditary optic neuropathy (LHON); Progressive encephalopathy (PEM); Mitochondrial encephalomyopathies			
MTCYB	CBHU						
				Leber's hereditary optic neuropathy (LHON); Mitochondrial Myopathy (MM); Parkinsonism/MELAS overlap syndrome			
COB							
MTDLOOP	NNN21						
MTERF	Y09615						

<i>Mus musculus</i> Gene List				<i>Homo sapien</i> Gene List and Related Diseases			
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>	<u>Related Disease</u>
MTHFD	MTHFD1	A31903					
MTHFD	MTHFD2	DEHUMT					
MTHSP1	NNN15						
MTHSP2	NNN16						
MTIF2	A55628						
MTLSP	NNN02						
MTND1	DNHUN1						
MTND2	DNHUN2						
MTND3	DNHUN3						
MTND4	DNHUN4						
MTND4L	DNHUNL						
MTND5	DNHUNS						
MTND6	DEHUN6						
MTOLR	NNN19						
MTTRF1	RF1M_HUMAN						
MTTRF1							
MTRNR1	12s_rRNA						
MTRNR2	16S_rRNA						
MTRNR3	NNN17						
MTTA	TAla						
MTTC	TCys						
MTTD	TAsp						
MTTE	TGlu						
MTTER	NNN18						

Mus musculus Gene List*Homo sapien* Gene List and Related Diseases

<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>
MTTF	TPhe			MELAS
MTFFH	NNN13			
MTTFL	NNN14			
MTTFX	NNN12			
MTTFY	NNN11			
MTTG	TGly			Hypertrophic cardiomyopathy; Progressive encephalopathy (PEM)
MTTH	THis			
MTTI	Tle			Fatal infantile hypertrophic cardiomyopathy (FIHC)
MTTK	TLys			MERRF; Cardiomyopathy and deafness; Myoneurogastrointestinal encephalopathy syndrome (MNGIE); Diabetes mellitus-deafness syndrome (DMDF)
MTTL1	tLeu_a			MELAS; MERRF/MELAS overlap syndrome; Mitochondrial myopathy (MM); Diabetes mellitus-deafness syndrome (DMDF); Pediatric MMC; Adult MMC; Deafness; Maternally inherited diabetes mellitus; Chronic progressive external ophthalmoplegia (CPEO) CPEO plus; Mitochondrial myopathy (MM)
MTTL2	tLeu_b			Chronic progressive external ophthalmoplegia (CPEO)
MTTM	TMet			Mitochondrial myopathy (MM)
MTTN	TAsn			Mitochondrial myopathy (MM)
MTTP	TPro			Alzheimer disease and Parkinson disease (ADPD)
MTTQ	TGln			
MTTR	TArg			
MTTS1	tSer_1			MERRF/MELAS overlap syndrome; Ataxia, myoclonus and deafness (AMDF); Deafness; Myoclonus epilepsy, deafness, ataxia, cognitive impairment and Cox deficiency; MM with RRF
MTTS2	t_Ser2			Diabetes mellitus-deafness syndrome (DMDF); Sensorineural hearing loss and retinitis pigmentosa (DFRP)
MTTT	TThr			Lethal infant mitochondrial myopathy (LIMM); Mitochondrial myopathy (MM)
MTRV	TVal			Ataxia, progressive seizures, mental deterioration, and hearing loss
MTRW	TTip			Dementia and chorea (DEMCHO)

<i>Mus musculus</i> Gene List		<i>Homo sapien</i> Gene List and Related Diseases		
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>
MTTY	TTyr	MTTX1	MTTXN_HUMAN	
MTX1		MTX2	AAC25105	
MTX2		MUT	S40622	
MUT		MUTYH	U63329	Methylmalonic acidemia (MUT, MUT type)
MUTYH		NDUFA10	O95299	
NDUFA10		NDUFA1	O15239	
NDUFA1		NDUFA2	O43678	
NDUFA2		NDUFA3	O95167	
NDUFA3		NDUFA4	NUML_HUMAN	
NDUFA4		NDUFA5	NUFM_Human	
NDUFA5		NDUFA6	P56556	
NDUFA6		NDUFA7	AAD05427	
NDUFA7		NDUFA8	NUPM_HUMAN	
NDUFA8		NDUFAB1	T00741	
NDUFAB1		NDUFB10	O96000	
NDUFB10		NDUFB1	O75438	
NDUFB1		NDUFB2	AAD05428	
NDUFB2		NDUFB3	O43676	
NDUFB3		NDUFB4	O95168	
NDUFB4		NDUFB5	O43674	
NDUFB5		NDUFB6	O95139	
NDUFB6		NDUFB7	NB8M_HUMAN	
NDUFB7		NDUFB8	JE0382	
NDUFB8		NDUFB9	S82655	
NDUFB9		B22		
B22		NDUFC1	O43677	
NDUFC1		NDUFC2	O95298	

<i>Mus musculus</i> Gene List				<i>Homo sapien</i> Gene List and Related Diseases			
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>			<u>Related Disease</u>	
NDUFS1	SI7854	NDUFS1	SI7854				
NDUFS2	JE0193	NDUFS2L	NUEM_HUMAN				
NDUFS3	O75489						
NDUFS4	NUYM_HUMAN						
NDUFS5	O43920						
NDUFS6	O75380						
NDUFS7	O75251						
NDUFS8	NUIM_HUMAN						
NDUFV1	A44362						
NDUFV2	A30113						
NDUFV3	NUOM_HUMAN						
NIFS	AAD09187						
NME4	NDKM_HUMAN						
NNT-PEN	G02257						
NOC4	NP_006058						
NRF1	A54868						
NTHL1	AB001575						
NTH1							
OAT	XNHUO						
OGDH	A38234						
OGG1	U96710						
OIAS	A91013						
OPA1	T00336						
OTC	OWHU						
OX1L	I38079						
OXCT	SCOT_HUMAN						
P43-LSB	I53499						

<i>Mus musculus</i> Gene List		<i>Homo sapien</i> Gene List and Related Diseases		
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>
P69	A42665			
P71	B42665			
PC	JC2460			Deficiency of pyruvate carboxylase, type I and II
PCCA	A27883			Propionic acidemia, type I (PA-1)
PCCB	A53020			Propionic acidemia, type II (PA-2)
PCK2	S69546			Hypoglycemia and liver impairment
PDHA1	DEHUPA			Pyruvate dehydrogenase deficiency; Leigh syndrome
PDHA2	DEHUPT			
PDHB	DEHUPB			Pyruvate dehydrogenase deficiency; Leigh syndrome
PDK1	I55465			
PDK2	I70159			
PDK3	I70160			
PDK4	Q16654			
PDX1	U82328			Pyruvate dehydrogenase deficiency
PEMT	PEMT_HUMAN			
PEMT2	PET112L	GATB_HUMAN		
	PHC	A53737		
	PLA2G1B	PSHU		
PLA2				
PPLA2				
PLA2G2A	PSHUYF			
PLA2L				
PLA2G4	A39329			
PLA2G5	U03090			
PMPCB	O75439			
PNUTL2	AF176379			
POLG2	U94703			

<i>Mus musculus</i> Gene List				<i>Homo sapien</i> Gene List and Related Diseases			
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>			<u>Related Disease</u>	
POLG	G02750						
Hs.1436							
POLRMT	HSU75370						
PPOX	PPOX_HUMAN						
PRAX-1	AF039571						
PRDX5	AAF03750						
ACR1							
AOEB166							
PMP20							
PRXV	S42366						
PRSS15							
LON-PEN							
LON							
PSORT	AAC05748						
PYCR1	A41770						
P5C							
RMRP	HSMRP						
RPL23L	RL23_HUMAN						
RPL23							
RPL3	R5HUL3						
RPMI12	RM12_HUMAN						
RPMI19	RLX1_HUMAN						
KIAA0104							
RPMI37	AAF36155						
RPMI3	R5HUL3						
RPMS12	RT12_HUMAN						
SCHAD	JC4879						
SCO2	AL021683					Fatal infantile cardioencephalomyopathy due to Cox deficiency	

<i>Mus musculus</i> Gene List				<i>Homo sapien</i> Gene List and Related Diseases
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>
SCP2	B40407			
SDH1	A34045			
IP				
SDH				
SDH2	JX0336			Leigh syndrome;Deficiency of succinate dehydrogenase
SDHC	D49737			Heredity paraganglioma, type III (PGL3)
SDHD	DHSD_HUMAN			Heredity paraganglioma, type I (PGL1)
SHMT2	B46746			
SLC1A1	EAT2_HUMAN			
EAAC1				
SLC1A3	JC2084			
SLC20A3	TXTP_HUMAN			
SLC25A12	Y14494			
SLC25A13	NP_055066			Citrullinemia, type II (CTLN2)
CTLN2				
SLC25A14	O95258			
SLC25A16	A40141			
GDA				
GT				
ML7				
SLC25A18	AY008285			
SLC25A4	A44778			Chronic progressive external ophthalmoplegia, type III (CPEO3);Mitochondrial myopathy and cardiomyopathy (MiMyCa)
ANT1				
SLC25A5	A29132			
ANT2				
T3				
SLC25A6	S03894			

<i>Mus musculus</i> Gene List				<i>Homo sapien</i> Gene List and Related Diseases			
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>			
ANT3							
SLC9A6	Q92581	KIAA0267					
SMAC	NP_063940	DSHUN					
SOD2		Y16610		Hereditary spastic paraplegia (HSP)			
SPG7		JN0568					
SSBP		I38896		Congenital lipoïd adrenal hyperplasia			
STAR		AU058953					
SUCLA2		P53597					
SUCLG1		T08812					
SUCLG2		S55874		Sulfocysteinuria			
SUOX		S63453					
SUPV3L1		S57749		Leigh syndrome			
SURF1		AB029948					
SerRSmt		SERS					
mtSerRS							
TAT	S10887			Tyrosine transaminase deficiency, type II (Richner-Hanhart syndrome)			
TCF6L1	JC1496						
TCF6L3	M62810						
TFAM	X64269						
TID1	TID1_HUMAN						
TIM17	IM17_HUMAN						
TIM17B	NP_005825						
TIM23	AF030162						
TIM44	IM44_HUMAN						
TK2	KIHUT						
TPO	OPHTUT			Iodide peroxidase deficiency (IPD)			

<i>Mus musculus</i> Gene List		<i>Homo sapien</i> Gene List and Related Diseases		
<u>gene</u>	<u>Accession</u>	<u>gene</u>	<u>Accession</u>	<u>Related Disease</u>
TR	THI2_HUMAN	TR3		
TST	ROHU	TST	S62767	
TUFM		TUFM	A60793	
UCP1		UCP2_HUMAN		
UCP2		UCP3	JC5522	
UCP3		UCP4_HUMAN		
UCP4	A60472	UNG		
DGU		DGU		
UDG		UDG		
UQCRB	A32450	UQCRB		
UQBP		UQBP		
UQCRC1	A48043	UQCRC1		
UQCRC2	A32629	UQCRC2		
UQCRCFS1	UCR1_HUMAN	UQCRCFS1		Mitochondrial myopathy (MM)
UQCRRH	S00219	UQCRRH		
UROS	A40483	UROS		
VDAC1	MMHUP3	VDAC1		
VDAC2	B44422	VDAC2		
VDAC3	S59547	VDAC3		
VDAC4	Q36732	VDAC4		
WARS2	AA227572	WARS2		
WFS	Y18064	WFS		DIDMOAD
YME1L1	AJ132637	YME1L1		
YWHAE	143E_HUMAN	YWHAE		
YWHAZ	PSHUAM	YWHAZ		PSHUAM

B. The Mitochondria**1. Role of mitochondrial integrity in tissue function: Critical factors in mitochondrial dysfunction and decline in tissue function**

It has been hypothesized that environmental factors accelerate the intrinsic processes of aging and the development of the aged phenotype. The overall results of past studies have suggested that aged tissues exhibit characteristics of chronic stress and a prolonged recovery from stress challenges. To understand the underlying basis for the development of these characteristics, the inventors have proposed that mitochondrial integrity and function may be severely affected in aged tissues due to oxidative metabolism (stress) which may lead to DNA damage and an increased production of ROS. Thus, in mitochondrial dysfunction a major factor responsible for many age-dependent changes is ROS. As a result of these homeostatic changes, there is an increase in the state of oxidative stress in aged tissues, which produces a chemical effect on the activity of signaling pathways and stress response genes. The age-associated increase of the pro-oxidant state based on continued and increased production of ROS by intrinsic and extrinsic factors enhance biological processes characteristic of chronic stress in aged tissues, and enhance development of age-associated diseases.

2. Mitochondrial Physiology

One of the primary functions of the mitochondria is the generation of cellular energy by the process of oxidative phosphorylation (OXPHOS). OXPHOS encompasses the electron transport chain (ETC) consisting of NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome c-coenzyme Q oxidoreductase (complex III) and cytochrome c oxidase (complex IV). Oxidation of NADH or succinate by the ETC generates an electrochemical gradient ($\Delta\psi$) across the mitochondrial inner membrane, which is utilized by the ATP synthase (complex V) to synthesize ATP. This ATP is exchanged for cytosolic ADP by the adenine nucleotide translocator (ANT). Inhibition of the ETC results in the accumulation of electrons in the beginning of the ETC, where they can be transferred directly to O_2 to give superoxide anion (O_2^-). Mitochondrial O_2^- is converted to H_2O_2 by superoxide dismutase (MnSOD), and H_2O_2 is

converted to H₂O by glutathione peroxidase (GPx1). The mitochondria is also the primary decision point for initiating apoptosis. This is mediated by the opening of the mitochondrial permeability transition pore (mtPTP), which couples the ANT in the inner membrane with porin (VDAC) in the outer membrane to the pro-apoptotic Bax and anti-apoptotic Bcl2. Increased mitochondrial Ca⁺⁺ or ROS and/or decreased Δψ or ATP tend to activate the mtPTP and initiate apoptosis (Wallace, 1999). Most of the above genes are components of the current microarrays.

3. The Mitochondrial Genome

The mouse (Anderson *et al.*, 1981) and human (Waterston *et al.*, 2002) mitochondrial genomes consist of a single, circular double stranded DNA molecule of 16,295 and 16,569 base pairs respectively, both of which has been completely sequenced (FIG.1 and 2). They are present in thousands of copies in most cells and in multiple copies per mitochondrion. The mouse and human mitochondrial genomes (Tables 2-3) contain 37 genes, 28 of which are encoded on one of the strands of DNA and 9 encoded on the other. Of these genes, 24 encode RNAs (Table 3) of two types, ribosomal RNAs required for synthesis of mitochondrial proteins involved in cellular oxidative phosphorylation, and 22 amino acid carrying transfer RNAs (tRNA). The mitochondrial genome thus encodes only a small proportion of the proteins required for its specific functions; the bulk of the mitochondrial polypeptides are encoded by nuclear genes and are synthesized on cytoplasmic ribosomes before being imported into the mitochondria; examples of these genes may be found in Table 1 and on the internet on websites such as the National Center for Biotechnology Information (NCBI) website and GenomeWeb. The mitochondrial genome resembles that of a bacterium in that the genes have no introns, and that there is a very high percentage of coding DNA (about 93% of the genome is transcribed as opposed to about 3% of the nuclear genome) and a lack of repeated DNA sequences.

Table 2**Homo sapiens mitochondrion, complete genome**

Location	Strand	Length	Gene	Product
3308..4264	+	319	ND1	NADH dehydrogenase subunit 1
4471..5514	+	348	ND2	NADH dehydrogenase subunit 2
5905..7446	+	414	COX1	Cytochrome c oxidase subunit I
7587..8270	+	228	COX2	Cytochrome c oxidase subunit II
8367..8573	+	69	ATP8	ATP synthase F0 subunit 8
8528..9208	+	227	ATP6	ATP synthase F0 subunit 6
9208..9988	+	260	COX3	Cytochrome c oxidase subunit III
10060..10405	+	115	ND3	NADH dehydrogenase subunit 3
10471..10767	+	99	ND4L	NADH dehydrogenase subunit 4L
10761..12138	+	459	ND4	NADH dehydrogenase subunit 4
12338..14149	+	604	ND5	NADH dehydrogenase subunit 5
14150..14674	-	175	ND6	NADH dehydrogenase subunit 6
14748..15882	+	378	CYTB	Cytochrome b

Mus musculus mitochondrion, complete genome

Location	Strand	Length	Gene	Product
2760..3707	+	316	ND1	NADH dehydrogenase subunit 1
3914..4951	+	346	ND2	NADH dehydrogenase subunit 2
5328..6872	+	515	COX1	Cytochrome c oxidase subunit I
7013..7696	+	228	COX2	Cytochrome c oxidase subunit II
7766..7969	+	68	ATP8	ATP synthase F0 subunit 8
7927..8607	+	227	ATP6	ATP synthase F0 subunit 6
8607..9390	+	261	COX3	Cytochrome c oxidase subunit III
9459..9803	+	115	ND3	NADH dehydrogenase subunit 3
9874..10167	+	98	ND4L	NADH dehydrogenase subunit 4L
10161..11538	+	459	ND4	NADH dehydrogenase subunit 4
11736..13559	+	608	ND5	NADH dehydrogenase subunit 5
13546..14064	-	173	ND6	NADH dehydrogenase subunit 6
14139..15282	+	381	CYTB	Cytochrome b

TABLE 3

<i>Mus musculus</i>		<i>Homo sapiens</i>			
24 RNA Genes		24 RNA Genes			
Ribosomal RNAs		Ribosomal RNAs			
Location	Product	Location	Product		
650..1603	+	12S ribosomal RNA	650..1603	+	12S ribosomal RNA
1673..3230	+	16S ribosomal RNA	1673..3230	+	16S ribosomal RNA
Transfer RNAs		Transfer RNAs			
Location	Product	Location	Product		
1..68	+	tRNA-Phe	579..649	+	tRNA-Phe
1025..1093	+	tRNA-Val	1604..1672	+	tRNA-Val
2676..2750	+	tRNA-Leu	3231..3305	+	tRNA-Leu
3706..3774	+	tRNA-Ile	4264..4332	+	tRNA-Ile
3772..3842	-	tRNA-Gln	4330..4401	-	tRNA-Gln
3845..3913	+	tRNA-Met	4403..4470	+	tRNA-Met
4950..5016	+	tRNA-Trp	5513..5580	+	tRNA-Trp
5018..5086	-	tRNA-Ala	5588..5656	-	tRNA-Ala
5089..5159	-	tRNA-Asn	5658..5730	-	tRNA-Asn
5192..5257	-	tRNA-Cys	5762..5827	-	tRNA-Cys
5260..5326	-	tRNA-Tyr	5827..5892	-	tRNA-Tyr
6869..6939	-	tRNA-Ser	7446..7517	-	tRNA-Ser
6942..7011	+	tRNA-Asp	7519..7586	+	tRNA-Asp
7700..7764	+	tRNA-Lys	8296..8365	+	tRNA-Lys
9391..9458	+	tRNA-Gly	9992..10059	+	tRNA-Gly
9805..9872	+	tRNA-Arg	10406..10470	+	tRNA-Arg
11539..11606	+	tRNA-His	12139..12207	+	tRNA-His
11607..11665	+	tRNA-Ser	12208..12266	+	tRNA-Ser
11665..11735	+	tRNA-Leu	12267..12337	+	tRNA-Leu
14065..14133	-	tRNA-Glu	14675..14743	-	tRNA-Glu
15238..15349	+	tRNA-Thr	15889..15954	+	tRNA-Thr
15350..15416	-	tRNA-Pro	15956..16024	-	tRNA-Pro

4. Mitochondrial DNA Mutations

Mitochondrial DNA mutations that develop during the course of a lifetime are called somatic mutations. The accumulation of somatic mutations might help explain how people who were born with mtDNA mutations often become ill after a delay of years or even decades. It is hypothesized that the buildup of random, somatic mutations depresses energy production and cause mitochondrial dysfunction that results in a decline in tissue function. This decline in the activity of proteins of the electron transport complexes involved in energy production within the mitochondria could be an important contributor to aging as well as to various age-related degenerative diseases. The characteristic hallmark of disease – a worsening over time – is thought to occur because long-term effects on certain tissues such as brain and muscle leads to progressive disease.

Other factors believed to contribute to the decline in mitochondrial energy production and its associated age-related diseases are, long-term exposure to certain environmental toxins, and accumulated somatic mutations. Mitochondria generate oxygen-free radicals that scientists believe may attack mitochondria and mutate mtDNA. Thus, somatic mutations of mtDNA contribute to the more common signs of aging (loss of strength, endurance, memory, hearing and vision) and some mtDNA mutations have been reported to increase with the age of the heart, skeletal muscle, liver, and brain regions controlling memory and motion (Melov *et al.*, 2000). Few of these mutations can be detected before the age of 30 or 40, but they increase exponentially with age after that.

Current theories propose that progressive age-associated declines in tissue function are caused by changes in biological processes that occur in the absence of disease, and that wear and tear are major factors that accelerate this decline in tissue function. Thus, it is important to demonstrate that the development of certain intrinsic biological processes may be the basis for the gradual age-associated decline in tissue function, and ultimately for organ failure and death, and that environmental insults are important factors which may accelerate the gradual decline in tissue function. The etiologic agents that bring about homeostatic changes that occur in aged cells and tissues, include factors that generate reactive oxygen species (ROS), such as cytokines and

oxidative phosphorylation. It is hypothesized that a gradual decline in tissue function is caused by the increase in the pro-oxidant state of aged tissues. Furthermore, this may be due to an elevated intrinsic oxidative stress that is mitochondrially derived, which causes an overall increase in the pro-oxidant state of aged tissues, and that such extrinsic factors as mitochondrial damaging agents intensify this pro-oxidant state. The working hypothesis states that aging increases the activity of stress factors (*e.g.*, cytokines, ROS), and that stabilization of this new level of activity produces chronic stress in aged tissues (Papaconstantinou, 1994; Saito *et al.*, 2001; Hsieh *et al.*, 2002).

5. Mitochondrial genes in degenerative diseases and aging

i) Mitochondrial Diseases

It is becoming increasingly apparent that mitochondrial dysfunction is a central factor in degenerative diseases and aging. The present invention provides a tool for identifying mitochondrial genes involved in aging and age-related diseases, but is not limited to such. Mitochondrial diseases have been associated with both mtDNA and nuclear DNA (nDNA) mutations. MtDNA base substitution mutations resulting in maternally inherited diseases can affect the structure and function of proteins and protein synthesis (mutations of rRNAs and tRNAs).

In comparison with the nuclear genome, the mitochondrial genome is a small target for mutation (about 1/200,000 of the size of the nuclear genome). Thus, the proportion of clinical disease due to mutations in the mitochondrial genome might therefore be expected to be extremely low. However, due to the large amounts of non-coding DNA in the nuclear genome, most mutations in the nuclear genome do not cause diseases. In contrast, the bulk of the mitochondrial genome is composed of coding sequence and mutation rates in mitochondrial genes are thought to be about 10 times higher than those in the nuclear genome, likely because of the close proximity of the mtDNA to oxidative reactions; the number of replications is higher; and mtDNA replication is more error-prone. Accordingly, mutation in the mitochondrial genome is a significant contributor to human disease.

Mitochondrial diseases can be caused by the same types of mutations that cause disorders of the nuclear genome *i. e.*, base substitutions, insertions, deletions and rearrangements resulting in missense or non-sense transcripts. An important aspect of the molecular pathology of mtDNA disorders, however, is whether every mtDNA molecule carries the causative mutation (homoplasmy) or whether the cell contains a mixed population of normal and mutant mitochondria (heteroplasmy). Where heteroplasmy occurs, the disease phenotype may therefore depend on the proportion of abnormal mtDNA in some critical tissue. Also, this proportion can be very different in mother and child because of the random segregation of mtDNA molecules at cell division.

The idea that defects in mitochondrial respiratory chain function might be the basis of disease has been considered for some time but it was not until 1988 that molecular analysis of mtDNA provided the first direct evidence for mtDNA mutations in neurological disorders, notably Leber's hereditary optic neuropathy. An example of a pathogenic mtDNA missense mutation is the ND6 gene mutation at nucleotide pair (np) 14459, which causes Leber's hereditary optic neuropathy (LHON) and/or dystonia. The np 14459 mutation results in a marked complex I defect, and the segregation of the heteroplasmic mutation generates the two phenotypes along the same maternal lineage (Jun *et al.*, 1994; Jun *et al.*, 1996).

A relatively severe mitochondrial protein synthesis disease is caused by the np 8344 mutation in the tRNALys gene resulting in myoclonic epilepsy and ragged red fiber (MERRF) disease. Mitochondrial myopathy with ragged red muscle fibers (RRFs) and abnormal mitochondria is a common feature of severe mitochondrial disease. A delayed onset and progressive course are common features of mtDNA diseases (Wallace *et al.*, 1988; Shoffner *et al.*, 1990). The severity as well as temporal characteristics of mtDNA mutations is illustrated by some of the most catastrophic diseases in which a the nt 4336 mutation in the tRNA^{Glu} gene is associated with late-onset Alzheimer (AD) and Parkinson Disease (PD) (Shoffner *et. al.*, 1993).

Degenerative diseases can also be caused by rearrangements in the mtDNA. Spontaneous mtDNA deletions often present with chronic progressive external ophthalmoplegia (CPEO) and mitochondrial myopathy, together with an array of other

symptoms (Shoffner *et. al.*, 1989). Maternal-inherited mtDNA rearrangement diseases are more rare.

Mitochondrial function also declines with age in the post-mitotic tissues of normal individuals. This is associated with the accumulation of somatic mtDNA rearrangement mutations in tissues such as skeletal muscle and brain (Corral-Debrinski *et al.*, 1991; Corral-Debrinski *et al.*, 1992a; Corral-Debrinski *et al.*, 1992b; Corral-Debrinski *et al.*, 1994; Horton *et al.*, 1995; Melov *et al.*, 1995). This same age-related accumulation of mtDNA rearrangements is seen in other multi-cellular animals including the mouse, where the accumulation of mtDNA damage is retarded by dietary restriction (Melov *et al.*, 1997). Some examples of human disorders that can be caused by mutations in the mtDNA are listed in Table 1.

ii) Aging and Age-Related Diseases

Several factors could cause mitochondrial energy production to decline with age even in people who start off with healthy mitochondrial and nuclear genes. Long-term exposure to certain environmental toxins is one such factor. Many of the most potent toxins known, play a role in inhibiting the mitochondria. Another factor could be the lifelong accumulation of somatic mitochondrial DNA mutations. The mitochondrial theory of aging holds that as an individual lives and produces ATP, the mitochondria generates oxygen free radicals that inexorably attack and mutate the mitochondrial DNA. This random accumulation of somatic mitochondrial DNA mutations in people who began life with healthy mitochondrial genes would ultimately reduce energy output below needed levels in one or more tissues if the individuals lived long enough. In so doing, the somatic mutations and mitochondrial inhibition could contribute to common signs of normal aging, such as loss of memory, hearing, vision, strength and stamina. In people whose energy output was already compromised (whether by inherited mitochondrial or nuclear mutations or by toxins or other factors), the resulting somatic mtDNA injury would push energy output below desirable levels more quickly. These individuals would then display symptoms earlier and would progress to full-blown

disease more rapidly than would people who initially had no deficits in their energy production capacity.

There is a plethora of evidence that energy production declines and somatic mtDNA mutation increases as humans grow older. Work by many groups has shown that the activity of at least one respiratory chain complex, and possibly another, falls with age in the brain, skeletal muscle, and the heart and liver. Further, various rearrangement mutations in mtDNA have been found to increase with age in many tissues—especially in the brain (most notably in regions controlling memory and motion). Rearrangement mutations have also been shown to accumulate with age in the mtDNA of skeletal muscle, heart muscle, skin and other tissues. Certain base-substitution mutations that have been implicated in inherited mtDNA diseases may accumulate as well. All of these reports agree that few mutations reach detectable levels before age 30 or 40, but they increase exponentially after that. Studies of aging muscle attribute some of this increase to selective amplification of mitochondrial DNAs from which regions have been deleted.

C. Arrays for Analysis of Mitochondrial-Related Gene Expression

The mitochondrial array is a complex resource that requires basic information and knowledge of procedures for constructing the genetic (DNA) sequences (components/targets) of each spot on the microarray; the preparation of DNA-probes needed to detect the mitochondrial gene products and the analysis of the resultant intensities of hybridization to the microarray chip. The arrays provided by the present invention have the potential to identify all of several hundred known mitochondrial genes identified. Further, additional genes may be added as desired and when they are identified.

The recent sequencing of the entire yeast, human, and mouse genomes has provided information on all of the mitochondrial genes of these organisms. This database has been used to search the mouse, rat and human genome databases for homologous genes. All of the known mitochondrial genes for mouse, rat and human have been identified. This information can be used for the construction of arrays for these species in accordance with the invention. In principle, DNA sequences representing all of the

mitochondrial-related genes of an organism can be placed on a solid support and used as hybridization substrates to quantify the expression of the genes represented in a complex mRNA sample in accordance with the invention. Thus, the present invention provides a DNA microarray of mitochondrial and nuclear mitochondrial genes. The mitochondrial gene array will play a crucial role in the analysis of mitochondrially associated diseases, both genetic and epigenetic; it will provide the resources needed to develop drugs and pharmaceuticals to counteract such diseases; it will provide information on whether drugs affect mitochondrial function; and it will provide information on how toxic factors, hormones, growth factors, nutritional factors and stress factors affect mitochondrial function.

1. DNA Arrays

DNA array technology provides a means of rapidly screening a large number of DNA samples for their ability to hybridize to a variety of single or denatured double stranded DNA targets immobilized on a solid substrate. Techniques available include chip-based DNA technologies, such as those described by Hacia *et al.* (1996) and Shoemaker *et al.* (1996). These techniques involve quantitative methods for analyzing large numbers of genes rapidly and accurately. The technology capitalizes on the complementary binding properties of single stranded DNA to screen DNA samples by hybridization (Pease *et al.*, 1994; Fodor *et al.*, 1991). Basically, a DNA array consists of a solid substrate upon which an array of single or denatured double stranded DNA molecules (targets) have been immobilized.

For screening, the array may be contacted with labeled single stranded DNA probes which are allowed to hybridize under stringent conditions. The array is then scanned to determine which probes have hybridized. In a particular embodiment of the instant invention, an array would comprise targets specific for mitochondrial genes. In the context of this embodiment, such targets could include synthesized oligonucleotides, double stranded cDNA, genomic DNA, plasmid and PCR products, yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), chromosomal markers or other constructs a person of ordinary skill would recognize as being able to selectively

hybridize to the mRNA or complements thereof of a mitochondrial-related coding sequence.

A variety of DNA array formats have been described, for example U.S. Patents 5,861,242 and 5,578,832, which are expressly incorporated herein by reference. A means for applying the disclosed methods to the construction of such an array would be clear to one of ordinary skill in the art. In brief, in one embodiment of the invention, the basic structure of an array may comprise: (1) an excitation source; (2) an array of targets; (3) a labeled nucleic acid sample; and (4) a detector for recognizing bound nucleic acids. Such an array will typically include a suitable solid support for immobilizing the targets.

In particular embodiments of the invention, a nucleic acid probe may be tagged or labeled with a detectable label, for example, an isotope, fluorophore or any other type of label. The target nucleic acid may be immobilized onto a solid support that also supports a phototransducer and related detection circuitry. Alternatively, a gene target may be immobilized onto a membrane or filter that is then attached to a microchip or to a detector surface. In a further embodiment, the immobilized target may be tagged or labeled with a substance that emits a detectable or altered signal when combined with the nucleic acid probe. The tagged or labeled species may, for example, be fluorescent, phosphorescent, or otherwise luminescent, or it may emit Raman energy or it may absorb energy. When the probes selectively bind to a targeted species, a signal can be generated that is detected by the chip. The signal may then be processed in several ways, depending on the nature of the signal.

DNA targets may be directly or indirectly immobilized onto a solid support. The ability to directly synthesize on or attach polynucleotide probes to solid substrates is well known in the art (see U.S. Patents 5,837,832 and 5,837,860, both of which are expressly incorporated by reference). A variety of methods have been utilized to either permanently or removably attach probes to a target/substrate (Stripping and reprobing of targets). Exemplary methods include: the immobilization of biotinylated nucleic acid molecules to avidin/streptavidin coated supports (Holmstrom, 1993), the direct covalent attachment of short, 5'-phosphorylated primers to chemically modified polystyrene plates (Rasmussen *et al.*, 1991), or the precoating of polystyrene or glass solid phases with

poly-L-Lys or poly L-Lys, Phe, followed by the covalent attachment of either amino- or sulphydryl-modified oligonucleotides using bi-functional crosslinking reagents (Running *et al.*, 1990; Newton *et al.*, 1993). When immobilized onto a substrate, targets are stabilized and therefore may be used repeatedly. In general terms, hybridization may be performed on an immobilized nucleic acid target molecule that is attached to a solid surface such as nitrocellulose, nylon membrane or glass. Numerous other matrix materials may be used, including, but not limited to, reinforced nitrocellulose membrane, activated quartz, activated glass, polyvinylidene difluoride (PVDF) membrane, polystyrene substrates, polyacrylamide-based substrate, other polymers such as poly(vinyl chloride), poly(methyl methacrylate), poly(dimethyl siloxane), photopolymers (which contain photoreactive species such as nitrenes, carbenes and ketyl radicals capable of forming covalent links with target molecules on substrates such as membranes, glass slides or beads).

Binding of probe to a selected support may be accomplished by any means. For example, DNA is commonly bound to glass by first silanizing the glass surface, then activating with carbodimide or glutaraldehyde. Alternative procedures may use reagents such as 3-glycidoxypropyltrimethoxysilane (GOP) or aminopropyltrimethoxysilane (APTS) with DNA linked *via* amino linkers incorporated either at the 3' or 5' end of the molecule during DNA synthesis. DNA may be bound directly to membranes using ultraviolet radiation. With nylon membranes, the DNA probes are spotted onto the membranes. A UV light source (Stratalinker,TM Stratagene, La Jolla, Ca.) is used to irradiate DNA spots and induce cross-linking. An alternative method for cross-linking involves baking the spotted membranes at 80°C for two hours in vacuum.

Specific DNA targets may first be immobilized onto a membrane and then attached to a membrane in contact with a transducer detection surface. This method avoids binding the target onto the transducer and may be desirable for large-scale production. Membranes particularly suitable for this application include nitrocellulose membrane (*e.g.*, from BioRad, Hercules, CA) or polyvinylidene difluoride (PVDF) (BioRad, Hercules, CA) or nylon membrane (Zeta-Probe, BioRad) or polystyrene base substrates (DNA.BINDTM Costar, Cambridge, MA).

2. Solid and Liquid Phase Array Assays

Genetic sequence analysis can be performed with solution and solid phase assays. These two assay formats are used individually or in combination in genetic analysis, gene expression and in infectious organism detection. Currently, genetic sequence analysis uses these two formats directly on a sample or with prepared sample DNA or RNA labeled by any one from a long list of labeling reactions. These include, 5'-Nuclease Digestion, Cleavase/Invader, Rolling Circle, and NASBA amplification systems to name a few. Epoch Biosciences has developed a powerful chemistry-based technology that can be integrated into both of these formats, using any of the amplification reactions to substantially improve their performance. These two formats include the popular homogeneous solution phase and the solid phase micro-array assays, which will be used in examples to demonstrate the technology's ability to substantially improve sensitivity and specificity of these assays.

Hybridization-based assays in modern biology require oligonucleotides that base pair (*i.e.*, hybridize) with a nucleic acid sequence that is complementary to the oligonucleotide. Complementation is determined by the formation of specific hydrogen bonds between nucleotide bases of the two strands such that only the base pairs adenine-thymine, adenine-uracil, and guanine-cytosine form hydrogen bonds, giving sequence specificity to the double stranded duplex.

In duplex formation between an oligonucleotide and another nucleic acid molecule, the stability of the duplexes is a function of its length, number of specific (*i.e.*, A - T, A - U, G - C) hydrogen bonded base pairs, and the base composition (ratio of G-C to A-T or A-U base pairs), since G-C base pairs provide a greater contribution to the stability of the duplex than does A-T or A-U base pairs. The quantitative measurement of a duplex's stability is expressed by its free energy (ΔG). Often a duplex's stability is measured using melting temperature (T_m) - the temperature at which one-half the duplexes have dissociated into single strands. Although ΔG is a more correct and universal measurement of duplex stability, the use of T_m s in the laboratory are frequently used due to ease of measurement. Routine comparisons using T_m are an economical and

sufficient way to compare this association strength characteristic, but is dependent on the nature and concentration of cations in the hybridization buffer. While many of the diagrams and charts in the site will use Tm rather than ΔG , these values were generated using constant parameters of 1X PCR buffer and 1 μ m primer

Arrays in accordance with the invention may be composed of a grid of hundreds or thousands or more of individual DNA targets arranged in discrete spots on a nylon membrane or glass slide or similar support surface and may include all mitochondrial-related coding sequences that have been identified, or a selected sampling of these. A sample of single stranded nucleotide can be exposed to a support surface, and targets attached to the support surface hybridize with their complementary strands in the sample. The resulting duplexes can be detected, for example, by radioactivity, fluorescence, or similar methods, and the strength of the signal from each spot can be measured. An advantage of the arrays of the invention is that a nucleic acid sample can be probed to detect the expression levels of many genes simultaneously.

D. Mitochondrial Nucleic Acids/Oligonucleotides

The present invention provides, in one embodiment, arrays of nucleic acid sequences immobilized on a solid support that selectively hybridize to expression products of mitochondrial-related coding sequences. Such mitochondrial-related coding sequences have been identified and include, for example, a coding sequence from the human or mouse mitochondrial genome. Sequences from the mouse mitochondrial genome are given, for example, by SEQ ID NO:1 to SEQ ID NO:13 herein.

Nucleic acids bound to a solid support may correspond to an entire coding sequence, or any other fragment thereof set forth herein. The term, "nucleic acid," as used herein, refers to either DNA or RNA. The nucleic acid may be derived from genomic RNA as cDNA, *i.e.*, cloned directly from the genome of mitochondria; cDNA may also be assembled from synthetic oligonucleotide segments. The nucleic acids used with the present invention may be isolated free of total viral nucleic acid.

The term "coding sequence" as used herein refers to a nucleic acid which encodes a protein or polypeptide, including a gene or cDNA. In other aspects of the invention, the

term, "coding sequence" is meant to include mitochondrial genes (*i.e.*, genes which reside in the mitochondria of a cell) as well as nuclear genes which are involved in mitochondrial structure, in mitochondrial function, or in both mitochondrial structure and mitochondrial function. Suitable genes include for example, yeast mitochondrial-related genes, *C. elegans* (nematode) mitochondrial-related genes, *Drosophila* mitochondrial-related genes, rat mitochondrial-related genes, mouse mitochondrial-related genes, and human mitochondrial-related genes. Many of the genes are known and are available at GenBank (a general database available on the internet at the National Institutes of Health website) and MitBase (see *e.g.*, a database for mitochondrial related genes available on the internet). Other coding sequences can be readily identified by screening libraries based on homologies to known mitochondrial-related genes of other species. Some particularly suitable mitochondrial-related genes are set forth in the examples of this application.

Allowing for the degeneracy of the genetic code, sequences that have at least about 50%, usually at least about 60%, more usually about 70%, most usually about 80%, preferably at least about 90% and most preferably about 95% of nucleotides that are identical to a mitochondrial-related coding sequence may also be functionally defined as sequences that are capable of hybridizing to the mRNA or complement thereof of a mitochondrial-related coding sequence under standard conditions.

Each of the foregoing is included within all aspects of the following description. In the present invention, cDNA segments may also be used that are reverse transcribed from genomic RNA (referred to as "DNA"). As used herein, the term "oligonucleotide" refers to an RNA or DNA molecule that may be isolated free of other RNA or DNA of a particular species. "Isolated substantially away from other coding sequences" means that the sequence forms the significant part of the RNA or DNA segment and that the segment does not contain large portions of naturally-occurring coding RNA or DNA, such as large fragments or other functional genes or cDNA noncoding regions. Of course, this refers to the oligonucleotide as originally isolated, and does not exclude genes or coding regions later added to it by the hand of man.

Suitable relatively stringent hybridization conditions for selective hybridizations will be well known to those of skill in the art. The nucleic acid segments used with the present invention, regardless of the length of the sequence itself, may be combined with other RNA or DNA sequences, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol.

For example, nucleic acid fragments may be prepared that include a short contiguous stretch identical to or complementary to a mitochondrial-related coding sequence, or the mRNA thereof, such as about 10-20 or about 20-30 nucleotides and that are up to about 300 nucleotides being preferred in certain cases. Other stretches of contiguous sequence that may be identical or complementary to any such sequences, including about 100, 200, 400, 800, or 1200 nucleotides, as well as the full length of the coding sequence or cDNA thereof. All that is necessary of such sequences is that selective hybridization for nucleic acids of mitochondrial-related coding sequences be carried out. The minimum length of nucleic acids capable of use in this regard will thus be known to those of skill in the art.

In principle, these oligonucleotide sequences can all selectively hybridize to a single gene such as a mitochondrial-related gene. Typically, however, the oligonucleotide sequences can be chosen such that at least one of the oligonucleotide sequences hybridizes to a first gene and at least one other of the oligonucleotide sequences hybridizes to a second, different gene.

As indicated above, the array can include a plurality of oligonucleotide sequences. For example, the array can include at least 5 oligonucleotide sequences, and each of the 5 oligonucleotide sequences can selectively hybridize to genes. In this case, a first oligonucleotide sequence would selectively hybridize to a first gene; a second oligonucleotide sequence would selectively hybridize to a second gene; a third oligonucleotide sequence would selectively hybridize to a third gene; a fourth oligonucleotide sequence would selectively hybridize to a fourth gene; and a fifth

oligonucleotide sequence would selectively hybridize to a fifth gene, and each of the first, second, third, fourth and fifth genes would be different from one another.

1. Oligonucleotide Probes and Primers

The various probes and targets used with the present invention may be of any suitable length. Naturally, the present invention encompasses use of RNA and DNA segments that are complementary, or essentially complementary, to a mitochondrial-related coding sequence. Nucleic acid sequences that are "complementary" are those that are capable of base-pairing according to the standard Watson-Crick complementary rules. As used herein, the term "complementary sequences" means nucleic acid sequences that are substantially complementary, as may be assessed by the same nucleotide comparison set forth above, or as defined as being capable of hybridizing to a mitochondrial-related coding sequence, including the mRNA and cDNA thereof, under relatively stringent conditions such as those described herein. Such sequences may encode the entire sequence of the mitochondrial coding sequence or fragments thereof.

Alternatively, the hybridizing segments may be shorter oligonucleotides. Sequences of 17 bases long should occur only once in the human genome and, therefore, suffice to specify a unique target sequence. Although shorter oligomers are easier to make and increase *in vivo* accessibility, numerous other factors are involved in determining the specificity of hybridization. Both binding affinity and sequence specificity of an oligonucleotide to its complementary target increases with increasing length. Oligonucleotide targets may also be attached to substrates such that each target selectively hybridizes to a separate region along a single gene for the purposes of identification and detection of gene mutations including, rearrangements, deletions, insertions, or single nucleotide polymorphisms (SNP) based on reduced probe signal compared to normal control signals.

E. Assaying for Relative Expression of Mitochondrial-Related Coding Sequences

The present invention, in various embodiments, involves assaying for gene expression. There are a wide variety of methods for assessing gene expression, most which are reliant on hybridization analysis. In specific embodiments, template-based amplification methods are used to generate (quantitatively) detectable amounts of gene products, which are assessed in various manners. The following techniques and reagents will be useful in accordance with the present invention.

Nucleic acids used for screening may be isolated from cells contained in a biological sample, according to standard methodologies (Sambrook *et al.*, 1989 and 2001). The nucleic acid may be genomic DNA or RNA or fractionated or whole cell RNA. Where RNA is used, it may be desired to convert the RNA to a complementary DNA using reverse transcriptase (RT). In one embodiment, the RNA is mRNA and is used directly as the template for probe construction. In others, mRNA is first converted to a complementary DNA sequence (cDNA) and this product is amplified according to protocols described below.

As used herein, "hybridization", "hybridizes" or "capable of hybridizing" is understood to mean the forming of a double or triple stranded molecule or a molecule with partial double or triple stranded nature. The term "anneal" as used herein is synonymous with "hybridize." The term "hybridization", "hybridize(s)" or "capable of hybridizing" encompasses the terms "stringent condition(s)" or "high stringency" and the terms "low stringency" or "low stringency condition(s)."

The phrase, "selectively hybridizing to" refers to a nucleic acid that hybridizes, duplexes, or binds only to a particular target DNA or RNA sequence when the target sequences are present in a preparation of DNA or RNA. By selectively hybridizing, it is meant that a nucleic acid molecule binds to a given target in a manner that is detectable in a different manner from non-target sequence under moderate, or more preferably under high, stringency conditions of hybridization. Proper annealing conditions depend, for example, upon a nucleic acid molecule's length, base composition, and the number of mismatches and their position on the molecule, and must often be determined

empirically. For discussions of nucleic acid molecule (probe) design and annealing conditions, see, for example, Sambrook *et al.*, (1989 and 2001).

As used herein "stringent condition(s)" or "high stringency" are those conditions that allow hybridization between or within one or more nucleic acid strand(s) containing complementary sequence(s), but precludes hybridization of random sequences. Stringent conditions tolerate little, if any, mismatch between a nucleic acid and a target strand. Such conditions are well known to those of ordinary skill in the art, and are preferred for applications requiring high selectivity. Non-limiting applications include isolating a nucleic acid, such as a gene or a nucleic acid segment thereof, or detecting at least one specific mRNA transcript or a nucleic acid segment thereof, and the like.

Stringent conditions may comprise low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C. It is understood that the temperature and ionic strength of a desired stringency are determined in part by the length of the particular nucleic acid(s), the length and nucleobase content of the target sequence(s), the charge composition of the nucleic acid(s), and to the presence or concentration of formamide, tetramethylammonium chloride or other solvent(s) in a hybridization mixture.

High stringency hybridization conditions are selected at about 5° C lower than the thermal melting point – Tm – for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of complementary strands, the presence of organic solvents, *i.e.*, salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. High stringency may be attained, for example, by overnight hybridization at about 68°C in a 6X SSC solution, washing at room temperature with a 6X SSC solution, followed by washing at about 68°C in a 6X SSC solution then in a 0.6X SSX solution or using commercially available proprietary hybridization solutions such as that offered by ClonTech™.

Hybridization with moderate stringency may be attained, for example, by: (1) filter pre-hybridizing and hybridizing with a solution of 3X sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at pH 7.5, 5X Denhart's solution; (2) pre-hybridization at 37° C for 4 hours; (3) hybridization at 37°C with amount of labeled probe equal to 3,000,000 cpm total for 16 hours; (4) wash in 2X SSC and 0.1% SDS solution; (5) wash 4X for 1 minute each at room temperature and 4X for 30 minutes each; and (6) dry and expose to film.

It is also understood that the ranges, compositions and conditions for hybridization are mentioned by way of non-limiting examples only, and that the desired stringency for a particular hybridization reaction is often determined empirically by comparison to one or more positive or negative controls. Depending on the application envisioned it is preferred to employ varying conditions of hybridization to achieve varying degrees of selectivity of a nucleic acid towards a target sequence. In a non-limiting example, identification or isolation of a related target nucleic acid that does not hybridize to a nucleic acid under stringent conditions may be achieved by hybridization at low temperature and/or high ionic strength. Such conditions are termed "low stringency" or "low stringency conditions", and non-limiting examples of low stringency include hybridization performed at about 0.15 M to about 0.9 M NaCl at a temperature range of about 20°C to about 50°C. Of course, it is within the skill of one in the art to further modify the low or high stringency conditions to suite a particular application.

Generally, nucleic acid sequences suitable for use in the arrays of the present invention (*i.e.*, those oligonucleotide sequences that selectively hybridize to mitochondrial-related genes) can be identified by comparing portions of a mitochondrial-related gene's sequence to other known sequences (*e.g.*, to the other sequences described in GenBank) until a portion that is unique to the mitochondrial-related gene is identified. This can be done using conventional methods and is preferably carried out with the aid of a computer program, such as the BLAST program. Once such a unique portion of the mitochondrial-related gene is identified, flanking primers can be prepared and targets corresponding to the unique portion can be produced using, for example, conventional PCR techniques. This method of identification, preparation of flanking primers, and

preparation of oligonucleotides is repeated for each of the mitochondrial-related genes of interest.

Once the oligonucleotide target sequences corresponding to the mitochondrial-related genes of interest are prepared, they can be used to make an array. Arrays can be made by immobilizing (*e.g.*, covalently binding) each of the nucleic acids targets at a specific, localized, and different region of a solid support. As described herein, these arrays can be used to determine the expression of one or more mitochondrial-related genes in a cell line, in a tissue or tissues of interest. The method may involve contacting the array with a sample of material from cells or tissues under conditions effective for the expression products of mitochondrial-related genes to hybridize to the immobilized oligonucleotide target sequences. Illustratively, isostopic or fluorometric detection can be effected by labeling the material from cells or tissue with a radioisotope which will be incorporated into the probe during or after reverse transcriptase (RT) reaction or fluorescent labeled nucleotide (A,T,C,G,U) (*e.g.*, fluorescein), washing non-hybridized material from the array after hybridization is permitted to take place, and detecting whether a ('labeled) mitochondrial-related gene transcripts hybridized to a particular target using, for example, phosphorimagers or laser scanners for detection of label and the knowledge of where in the array the particular oligonucleotide was immobilized. The arrays of the present invention can be used for a variety of other applications related to mitochondrial structure, function, and mutations as described herein.

F. Screening For Modulators of Mitochondrial Function

The present invention further comprises methods for identifying modulators of the mitochondrial structure and/or function. These assays may comprise random screening of large libraries of candidate substances; alternatively, the assays may be used to focus on particular classes of compounds selected with an eye towards structural attributes that are believed to make them more likely to modulate the function or expression of mitochondrial genes.

To identify a modulator, one generally may determine the expression or activity of a mitochondrial gene in the presence and absence of the candidate substance, a

modulator defined as any substance that alters function or expression. Assays may be conducted in cell free systems, in isolated cells, or in organisms including transgenic animals. It will, of course, be understood that all the screening methods of the present invention are useful in themselves notwithstanding the fact that effective candidates may not be found. The invention provides methods for screening for such candidates, not solely methods of finding them.

As used herein, the term "candidate substance" refers to any molecule that may potentially inhibit or enhance activity or expression of a mitochondrial or mitochondrial related gene. The candidate substance may be a protein or fragment thereof, a small molecule, a nucleic acid molecule or expression construct. It may be that the most useful pharmacological compounds will be compounds that are structurally related to a mitochondrial gene or a binding partner or substrate therefore. Using lead compounds to help develop improved compounds is known as "rational drug design" and includes not only comparisons with known inhibitors and activators, but predictions relating to the structure of target molecules.

The goal of rational drug design is to produce structural analogs of biologically active polypeptides or target compounds. By creating such analogs, it is possible to fashion drugs, which are more active or stable than the natural molecules, which have different susceptibility to alteration or which may affect the function of various other molecules. In one approach, one would generate a three-dimensional structure for a target molecule, or a fragment thereof. This could be accomplished by x-ray crystallography, computer modeling or by a combination of both approaches.

It also is possible to use antibodies to ascertain the structure of a target compound activator or inhibitor. In principle, this approach yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of anti-idiotype would be expected to be an analog of the original antigen. The anti-idiotype could then be used to identify and isolate peptides from banks of chemically- or biologically-produced peptides. Selected peptides would then serve as the pharmacore. Anti-

idiotypes may be generated using the methods described herein for producing antibodies, using an antibody as the antigen.

On the other hand, one may simply acquire, from various commercial sources, small molecule libraries that are believed to meet the basic criteria for useful drugs in an effort to "brute force" the identification of useful compounds. Screening of such libraries, including combinatorially generated libraries (e.g., peptide libraries), is a rapid and efficient way to screen large number of related (and unrelated) compounds for activity. Combinatorial approaches also lend themselves to rapid evolution of potential drugs by the creation of second, third and fourth generation compounds modeled of active, but otherwise undesirable compounds.

Candidate compounds may include fragments or parts of naturally-occurring compounds, or may be found as active combinations of known compounds, which are otherwise inactive. It is proposed that compounds isolated from natural sources, such as animals, bacteria, fungi, plant sources, including leaves and bark, and marine samples may be assayed as candidates for the presence of potentially useful pharmaceutical agents. It will be understood that the pharmaceutical agents to be screened could also be derived or synthesized from chemical compositions or man-made compounds. Thus, it is understood that the candidate substance identified by the present invention may be peptide, polypeptide, polynucleotide, small molecule inhibitors or any other compounds that may be designed through rational drug design starting from known inhibitors or stimulators.

Other suitable modulators include RNA interference molecules, antisense molecules, ribozymes, and antibodies (including single chain antibodies), each of which would be specific for the target molecule. Such compounds are described in greater detail elsewhere in this document. For example, an antisense molecule that bound to a translational or transcriptional start site, or splice junctions, would be an ideal candidate inhibitor.

In addition to the modulating compounds initially identified, the inventors also contemplate that other sterically similar compounds may be formulated to mimic the key portions of the structure of the modulators. Such compounds, which may include

peptidomimetics of peptide modulators, may be used in the same manner as the initial modulators.

G. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

Capability and Feasibility Studies

In order to demonstrate the capability of the present invention, a DNA microarray was generated from PCR products using thirteen genes that code for the mitochondrial proteins (FIG. 1). These genes were attached to nylon membranes by cross linking with UV radiation.

Positions #1 to #13 on array 1 (young) and array 2 (aged) contain the 13 mitochondrial gene targets. A hybridization study was carried out using samples from young vs aged mouse livers. The samples were labeled by reverse transcriptase incorporation of radiolabeled nucleotides and the results were observed by autoradiography. Intense and specific hybridization signals were detected at all positions indicating levels of transcript abundance.

The data showed a successful hybridization of a limited set of mitochondrial genes on the test array.

EXAMPLE 2

Location of *Mus Musculus* and *Homo sapiens* Mitochondrial Peptides and Proteins

FIGs. 2 and 3, are maps of the human and mouse (*Mus musculus*) mitochondrial genomes which show the location of the 13 peptides of the OXPHOS complexes, 22 tRNAs, and 2 rRNAs that are encoded by the mitochondrial genome, and that were used, in part, to prepare an array of the present invention.

Table 2 shows the location of the *Mus Musculus* and *Homo sapien* mitochondrial proteins (13 polypeptides). It gives their location (nucleotides), strand, length of polypeptide (number of amino acids) name of the gene, and the protein products which was used in part as targets for an array of the present invention. Table 3 shows the location of the *Mus musculus* and *Homo sapiens* mitochondrial 12S and 16S ribosomal RNAs and 22 tRNA.

EXAMPLE 3

Effects of Rotenone on Expression of Mouse Mitochondria Genes

The effects of rotenone, an inhibitor of mitochondrial Complex I, on the expression of mouse mitochondrial genes in AML-12 mouse liver cells in culture were examined (FIG. 4; Table 4). The microarrays show the mRNAs whose pool levels are up-regulated. Spots A1-G11 represent mitochondrial related nuclear encoded genes; spots G12-H12 represent the 13 genes encoded by mitochondrial DNA. It should be noted that in subsequent microarray designs (constructions) the mitochondrial DNA encoded genes G12-H12 were removed from the filters and arrayed separately. Thus, the G12-H12 spots were replaced with nuclear encoded genes. The following data suggest that the a number of genes are up-regulated in response to rotenone treatment: A11, ATP synthase lipid binding proteins; B8, ADP, ATP carrier protein; B9, cytochrome C oxidase chain VIIa; D12, chaperonin 10; E12, pyruvate carboxylase; H7, Complex I: Protein Dehydrogenase chain 3. E4 and E5 represent the 23S and 16S mitochondrial ribosomal RNAs. The data also suggest that inhibition of Complex I may stimulate the production of mRNAs of Complex I proteins (H7, H10), suggesting a compensatory response to the inhibitor.

Table 4-Micro array template for FIG. 4

real number	spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
PCR				
1	1	Acadl	ACDL_MOUSE	AcyL-CoA dehydrogenase, long-chain specific precursor (LCAD)
2	2	Acadm	A55724	AcyL-CoA dehydrogenase, medium-chain specific precursor (MCAD)
3	3	Acads	I49605	AcyL-CoA dehydrogenase, short-chain specific precursor
4	4	Aif	AF100927	Apoptosis-inducing factor
5	5	Alas2	SYMSAL	5-aminolevulinate synthase precursor
6	6	Aldh2	I48966	Aldehyde dehydrogenase (NAD+) 2 precursor
7	7	Ant1	S37210	ADP,ATP carrier protein, heart isoform T1
8	8	Ant2	S31814	ADP,ATP carrier protein, fibroblast isoform 2
9	9	Aop1;Aop2	JQ0064	MER5 protein
10	10	Atp5a1	JC1473	H+-transporting ATP synthase chain alpha

real number PCR	spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
11	11	Atp5g1	ATPL_MOUSE	ATP synthase lipid-binding protein P1 precursor (protein 9)
12	12	Atp7b	U38477	Probable copper transporting P-type ATPase
13	13	Bax	BAXA_MOUSE	Apoptosis regulator BAX, membrane isoform alpha
14	14	Bckdha	S71881	Branched chain alpha-ketoacid dehydrogenase chain E1-alpha
15	15	Bckdhb	S39807	3-methyl-2-oxobutanoate dehydrogenase (lipoamide)
16	16	Bcl2	B25960	Transforming protein bcl-2-beta
17	17	Bzrp	A53405	Peripheral-type benzodiazepine receptor 1
18	18	Car5	S12579	Carbonate dehydratase, hepatic
20	19	Ckmt1	S24612	Creatine kinase
21	20	Cox4	S12142	Cytochrome c oxidase chain IV precursor
23	21	Cox7a2	I48286	Cytochrome C oxidase polypeptide VIIa-liver/heart precursor
24	22	Cox8a	COXR_MOUSE	Cytochrome c oxidase chain VIII
25	23	Cpo	A48049	Coproporphyrinogen oxidase
26	24	Cpt2	A49362	Carnitine O-palmitoyltransferase II precursor
27	25	Crat	CACP_MOUSE	Carnitine O-acetyltransferase (carnitine acetyltransferase)
28	26	Cycs	CCMS	Cytochrome C, somatic
31	27	Dbt	S65760	Dihydrolipoamide transacetylase precursor
32	28	Dci	S38770	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor
33	29	Dld	107450	Dihydrolipoamide dehydrogenase (E3)
34	30	Fdx1	S53524	Adrenodoxin precursor
35	31	Fdxr	S60028	Ferrodoxin--NADP+ reductase precursor
124	32	Nrf1	NM_010938	Nuclear respiratory factor
37	33	Fpgs	S65755	Tetrahydrofolylpolyglutamate synthase precursor
38	34	Frida	S75712	Friedreich ataxia

real number PCR	spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
39	35	Gcdh	GCDH_MOUSE S16239	Glutaryl-CoA dehydrogenase precursor (GCD)
40	36	Glud	S01174	Glutamate dehydrogenase (NAD(P)+) precursor
41	37	Got2		Glutamate oxaloacetate transaminase-2
42	38	Hadh	JC4210	3-hydroxyacyl-CoA dehydrogenase, short chain-specific, precursor
43	39	Hccs	CCHLMOUSE	Cytochrome C-type heme lyase (CCHL)
44	40	Hk1	A35244	Hexokinase I
45	41	Hmgcl	HMGL_MOUSE B55729	Hydroxymethylglutaryl-CoA lyase
46	42	Hmgcs2		Hydroxymethylglutaryl-CoA synthase, mitochondrial
47	43	Hsc70t	96231	Heat shock protein cognate 70, testis
48	44	Hsd3b1	3BH1_MOUSE	3-beta hydroxy-5-ene steroid dehydrogenase type I
49	45	Hsp60	HHMS60	Heat shock protein 60 precursor
50	46	Hsp70-1	Q61698	Heat shock protein, 70K (hsp68) (fragment)
Blank	47	Blank	Blank	
52	48	HspE1	A55075	Chaperonin-10
53	49	Idh2	IDHP_MOUSE	Isocitrate dehydrogenase (NADP)
54	50	Mimt44	U699898	TIM44 - mitochondrial inner membrane import subunit
55	51	Mor1	DEMSMM	Malate dehydrogenase precursor, mitochondrial
56	52	mt-Rnr1	12S rRNA	12S rRNA
57	53	mt-Rnr2	16S rRNA	16S rRNA
58	54	Mthfd	A33267	Methylenetetrahydrofolate dehydrogenase (NAD+)
59	55	Mut	S08680	Methylmalonyl-CoA mutase alpha chain precursor
60	56	Nnt	S54876	NAD(P)+ transhydrogenase (B-specific) precursor
61	57	Oat	XNMSO	Ornithine--oxo-acid transaminase precursor
62	58	Oias1	25A1_MOUSE	(2'-5')oligoadenylate synthetase 1
64	59	Otc	OWMS	Ornithine carbamoyltransferase precursor
65	60	Pcx	A47255	Pyruvate carboxylase

real number	spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1 μM each primer
PCR				
66	61	Pdhal	S23506	Pyruvate dehydrogenase (lipoamide)
67	62	Pdhal	S23507	Pyruvate dehydrogenase (lipoamide)
69	63	Polg	DPOG_MOUSE	DNA polymerase gamma
70	64	Ppox	S68367	Protoporphyrinogen oxidase
71	65	Rpl23	1196612	L23 mitochondrial - related protein
72	66	Scp2	JU0157	Sterol carrier protein X
74	67	Sod2	I57023	Superoxide dismutase (Mn) precursor
75	68	Star	A55455	Steroidogenic acute regulatory protein precursor, mitochondrial
76	69	Tfam	P97894	Mitochondrial transcription factor A - mouse
77	70	Tst	THTR_MOUSE	Thiosulfate sulfurtransferase
79	71	Ung	UNG_MOUSE	Uracil-DNA glycosylase
80	72	Vdac1	106919	Voltage-dependent anion channel 1
81	73	Vdac2	106915	Voltage-dependent anion channel 2
82	74	Vdac3	106922	Voltage-dependent anion channel 3
83	75	Ywhaz	JC5384	14-3-3 protein zeta/delta
84(non-Mitop)	76	WS-3		
85(non-Mitop)	77	Slkd3		
93(non-Mitop)	78		L00923	Myosin 1
94	79	GAPDH	M32599	Glyceraldehyde 3-phosphate dehydrogenase (G3PDH)
108(non-Mitop)	80	Hsd3b5	L41519	3-ketosteroid reductase
119	81	APE 1	P28352	Apurinic/apyrimidinic endonuclease 1
122	82	Ogdh	U02971	2-Oxoglutarate dehydrogenase E1 component
123	83	ACADV	U41497	Acyl-Co A dehydrogenase very long chain

real number	spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
PCR				
Mito13	84	mt-Nd1	QXMS1M	Protein 1 (NADH dehydrogenase (ubiquinone) chain 1)
95	"	mt-Nd2	QXMS2M	Protein 2 (NADH dehydrogenase (ubiquinone) chain 2)
96	"	mt-Co1	ODMS1	Cytochrome c oxidase subunit I
97	"	mt-Co2	OBMS2	Cytochrome c oxidase subunit II
98	"	mt-Atp8	PWMS8	Protein A61 (H+-transporting ATP synthase protein 8)
99	"	mt-Atp6	PWMS6	ATPase 6 (H+-transporting ATP synthase protein 6)
100	"	mt-Co3	OTMS3	Cytochrome c oxidase subunit III
101	"	mt-Nd3	QXMS3M	Protein 3 (NADH dehydrogenase (ubiquinone) chain 3)
102	"	mt-Nd4L	QXMS4L	Protein 4L (NADH dehydrogenase (ubiquinone) chain 4L)
103	"	mt-Nd4	QXMS4M	Protein 4 (NADH dehydrogenase (ubiquinone) chain 4)
104	"	mt-Nd5	QXMS5M	Protein 5 (NADH dehydrogenase (ubiquinone) chain 5)
105	"	mt-Nd6	DEMSN6	Protein 6 (NADH dehydrogenase (ubiquinone) chain 6)
106	"	mt-Cytb	CBMS	Cytochrome b (ubiquinol--cytochrome c reductase subunit III)
107				

EXAMPLE 4**Effects of 3-Nitropropionic Acid and Trypanosome Infection on Expression of Mitochondrial Genes**

Analysis of mitochondrial DNA encoded gene expression in response to 3-nitropropionic acid (3NPA), an inhibitor of Complex II - succinic dehydrogenase was performed (FIG. 5A, Table 5). The 3 NPA treatments were at 6, 12 and 26 hours. The data showed that inhibition of Complex II stimulates the synthesis of mitochondrial encoded mRNAs and the 23S and 16S ribosomal RNAs.

In an example of overall gene down-regulation an analysis of mitochondrial DNA encoded gene expression in trypanosome infected heart tissue was also performed (FIG. 5B, Table 5). These data showed a decline in mRNA and ribosomal RNA levels at 37 days post infection.

Table 5- Microarray template for FIGS. 5A, 5B and 9

A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17							
1	mt-Rnr1	12S_rRNA	12S_rRNA	16S_rRNA	16S_rRNA							
2	mt-Rnr2											
3	mt-Nd1	QXMS1M										
4	mt-Nd2	QXMS2M										
5	mt-Co1	ODMS1										
6	mt-Co2	OBMS2										
7	mt-Atp8	PWMS8										
8	mt-Atp6	PWMS6										
9	mt-Co3	OTMS3										
10	mt-Nd3	QXMS3M										
11	mt-Nd4L	QXMS4L										
12	mt-Nd4	QXMS4M										
13	mt-Nd5	QXMS5M										
14	mt-Nd6	DEMSN6										
15	mt-Cytb	CBMS										
16	GAPDH	M32599										
17	β -actin	X03672										

EXAMPLE 5**Mitochondrial Gene Expression In Livers of Young and Aged Snell Dwarf Mouse Mutants**

Analysis of mitochondrial gene expression in livers of young Snell dwarf mouse mutants and aged Snell dwarf mouse mutants was performed (FIG. 6A, FIG. 6B, Table 6). The Snell dwarf mouse served as a genetic model of longevity because of its increased life-span (40%). These analyses of mitochondrial gene expression were designed to determine whether there are specific changes or differences in mitochondrial gene expression associated with longevity. Differences in mitochondrial gene activity in livers of 4 young control, and 4 young (long-lived) Snell dwarf mouse mutants were observed. The mitochondrial genes that change in the young dwarfs are: A2 - acyl CoA dehydrogenase; A5 - 5-aminolevulinate synthase; D8 - 3-beta hydroxy-5-ene-steroid dehydrogenase (Hsd3b1); D11, heat shock protein 70; E4 - carbonyl reductase (NADPH); F6 - sterol carrier protein X; G8 - 3-beta hydroxy-5-ene-steroid dehydrogenase (Hsd3b5).

15 G7 - GAPDH served as a positive control.

The differences in mitochondrial gene activity in livers of 3 aged controls and 3 aged long-lived Snell dwarf mouse mutants were also analyzed. The mitochondrial genes that change in the aged dwarfs are: A2, acyl-CoA dehydrogenase; A5 - 5-aminolevulinate synthase; E4 - carbonyl reductase (NADPH); F6 - sterol carrier protein X; and G8 - Hsd3b5.

Overall, the data suggest that there are major differences in steroid metabolism between aged control and aged long-lived dwarf mutants. FIG. 6C shows RT-PCR analysis of Hsd3b5 (G8) expression levels in the control versus dwarf Snell mice. mRNA levels confirmed that the levels of this gene are significantly decreased in the liver mitochondria of the aged dwarf.

Table 6-Microarray template for FIGs 6A and 6B

spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1 μM each primer
1	Acadl	ACDL_MOUSE	Acyl-CoA dehydrogenase, long-chain specific precursor (LCAD)
2	Acadm	A55724	Acyl-CoA dehydrogenase, medium-chain specific precursor (MCAD)
3	Acads	I49605	Acyl-CoA dehydrogenase, short-chain specific precursor
4	Aif	AF100927	Apoptosis-inducing factor
5	Alas2	SYMSAL	5-aminolevulinic synthase precursor
6	Aldh2	I48966	aldehyde dehydrogenase (NAD+) 2 precursor
7	Ant1	S37210	ADP,ATP carrier protein, heart isoform T1
8	Ant2	S31814	ADP,ATP carrier protein, fibroblast isoform 2
9	Aop1;Aop2	JQ0064	MER5 protein
10	Atp5a1	JC1473	H ⁺ -transporting ATP synthase chain alpha
11	Atp5g1	ATPL_MOUSE	ATP synthase lipid-binding protein P1 precursor (protein 9)
12	Atp7b	U38477	Probable copper transporting P-type ATPase
13	Bax	BAXA_MOUSE	apoptosis regulator BAX, membrane isoform alpha
14	Bckdha	S71881	branched chain alpha-ketoacid dehydrogenase chain E1-alpha
15	Bckdhb	\$39807	3-methyl-2-oxobutanoate dehydrogenase (lipoamide)
16	Bcl2	B25960	transforming protein bcl-2-beta

spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1µM each primer
17	Bzrp	A53405	peripheral-type benzodiazepine receptor 1
18	Car5	S12579	carbonate dehydratase, hepatic
19	Ckmt1	S24612	creatine kinase
20	Cox4	S12142	cytochrome c oxidase chain IV precursor
21	Cox7a2	I48286	cytochrome C oxydase polypeptide VIIa- liver/heart precursor
22	Cox8a	COXR_MOUSE	cytochrome c oxidase chain V ^{III}
23	Cpo	A48049	Coproporphyrinogen oxidase
24	Cpt2	A49362	carnitine O-palmitoyltransferase II precursor
25	Crat	CACP_MOUSE	carnitine O-acetyltransferase (carnitine acetylase)
26	Cycs	CCMS	cytochrome C, somatic
27	Dbt	S65760	dihydrolipoamide transacylase precursor
28	Dci	S38770	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor
29	Dld	1E+05	dihydrolipoamide dehydrogenase (E3)
30	Fdx1	S53524	adrenodoxin precursor
31	Fdxr	S60028	ferredoxin--NADP+ reductase precursor
32		Blank	
33	Fpgs	S65755	Tetrahydrofolylpolyglutamate synthase precursor
34	Frida	S75712	Friedreich ataxia
35	Gcdh	GCDH_MOUSE	Glutaryl-CoA dehydrogenase precursor (GCD)
36	Gldd	S16239	glutamate dehydrogenase (NAD(P)+) precursor
37	Got2	S01174	glutamate oxaloacetate transaminase-2
38	Hadhd	JC4210	3-hydroxyacyl-CoA dehydrogenase, short chain-specific, precursor
39	Hccs	CCHL_MOUSE	cytochrome C-type heme lyase (CCHL)
40	Hk1	A35244	hexokinase I
41	Hmgcl	HMGL_MOUSE	Hydroxymethylglutaryl-CoA lyase
42	Hmgcs2	B55729	Hydroxymethylglutaryl-CoA synthase, mitochondrial
43	Hsc70t	96231	heat shock protein cognate 70, testis
44	Hsd3b1	3BH1_MOUSE	3-beta hydroxy-5-ene steroid dehydrogenase type I
45	Hsp60	HHMS60	heat shock protein 60 precursor
46	Hsp70-1	Q61698	heat shock protein, 70K (hsp68) (fragment)

spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1µM each primer
47	Hsp74	A48127	heat shock protein 70 precursor
48	HspE1	A55075	chaperonin-10
49	Idh2	IDHP_MOUSE	isocitrate dehydrogenase (NADP)
50	Mimt44	U69898	TIM44 - mitochondrial inner membrane import subunit
51	Mor1	DEMSMM	malate dehydrogenase precursor, mitochondrial
52	Cbr2	A28053	carboxyl reductase (NADPH) - mouse
53	Cox6al	COXD_MOUSE	cytochrome C oxydase polypeptide VIa-heart precursor
54	Mthfd	A33267	Methylenetetrahydrofolate dehydrogenase (NAD+)
55	Mut	S08680	methylmalonyl-CoA mutase alpha chain precursor
56	Nnt	S54876	NAD(P)+ transhydrogenase (B-specific) precursor
57	Oat	XNMSO	ornithine--oxo-acid transaminase precursor
58	Oias1	25A1_MOUSE	(2'-5')oligoadenylate synthetase 1
59	Otc	OWMS	ornithine carbamoyltransferase precursor
60	Pcx	A47255	pyruvate carboxylase
61	Pdhα1	S23506	pyruvate dehydrogenase (lipoamide)
62	sdh1	bc013509	succinate dehydrogenase subunit b iron sulphur protein
63	Polg	DPOG_MOUSE	DNA polymerase gamma
64	sdh2	xm_127445	succinate dehydrogenase subunit a flavoprotein
65	sdhc	nm_025321	succinate dehydrogenase integral membrane protein CHI-3
66	Scp2	JU0157	sterol carrier protein X
67	Sod2	157023	superoxide dismutase (Mn) precursor
68	Star	A55455	steroidogenic acute regulatory protein precursor, mitochondrial
69	Tfam	P97894	mitochondrial transcription factor A - mouse
70	Tst	THTR_MOUSE	thiosulfate sulfurtransferase
71	Umg	UNG_MOUSE	uracil-DNA glycosylase
72	Vdac1	1E+05	voltage-dependent anion channel 1
73	Vdac2	1E+05	voltage-dependent anion channel 2
74	Vdac3	1E+05	voltage-dependent anion channel 3
75	Ywhaz	JC5384	14-3-3 protein zeta/delta
76	WS-3		

spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1µM each primer
77	Skd3		
78	GAPDH	L00923	Myosin 1
79	Hsd3b5	M32599	Glyceraldehyde 3-phosphate dehydrogenase (G3PDH)
80	APE 1	L41519	3-keytosteroid reductase
81	Ogdh	P28352	Apurinic/apyrimidinic endonuclease 1
82	ACADV	U02971	2-Oxoglutarate dehydrogenase E1 component
83	Slc1a1	U41497	Acyl-Co A dehydrogenase very long chain
84	Hprt	EAT3_MOUSE	Excitatory amino acid transporter 3
85	PplA2	J00423	Hypoxanthine phosphoribosyl transferase (HPRT)
86	Cab45	D78647	Phospholipase A2
87	NTRF1	U45977	Calcium-binding protein Cab45
88	Cox5b	NM_010938	Nuclear Respiratory Factor 1
89	Cox6a2	x53157	Cytochrome C oxidase subunit Vb
90	Atp5k	L06465	Cytochrome C oxidase subunit Via liver precursor
91	B-actin	S52977	ATP synthase H+ transporting chain e
92		X03672	beta-actin
93		M10624	Murine ornithine decarboxylase (MOD)
94	Tom40		Mitochondrial outer membrane protein
95	Gpam		Glycerol-3-phosphate acyltransferase
96	sthd	xm_134803	succinate dehydrogenase small subunit integral membrane protein

EXAMPLE 6**Mitochondrial Gene Expression In Heart Muscle Of Trypanosome Infected Mice**

Trypanosome infections are chronic, and long after the initial infection the parasite accumulates in the heart and other organs. In the heart the parasite causes severe cardiovascular disease that results in heart failure. Thus, mitochondrial gene expression in heart muscle of trypanosome infected mice was analyzed (FIGS. 7A-7D, Table 7). The microarray for this analysis is composed of 96 genes of nuclear origin. The 13 genes encoded by the mitochondrial DNA were removed from the microarray and treated separately (see FIG. 5B, Table 5). The microarray analysis shows mRNA levels in a 4-month old mouse heart mitochondria 3 days postinfection and 37 days postinfection. When normalized to GAPDH (G7) and β-actin (H8) the data show an overall decrease in mitochondrial gene expression after 37 days postinfection. This decrease in mitochondrial function is a basic factor in trypanosome mediated cardiovascular pathology and ultimately leads to heart failure.

15

Table 7-Microarray template for FIGS. 7 and 8.

Spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1 μM each primer
1	Acadl	ACDL_MOUSE A55724	Acyl-CoA dehydrogenase, long-chain specific precursor (LCAD)
2	Acadm	I49605	Acyl-CoA dehydrogenase, medium-chain specific precursor (MCAD)
3	Acads	AF100927	Acyl-CoA dehydrogenase, short-chain specific precursor
4	Aif	SYMSAL	Apoptosis-inducing factor
5	Alas2	I48966	5-aminolevulinic synthase precursor
6	Aldh2	S37210	Aldehyde dehydrogenase (NAD+) 2 precursor
7	Ant1	S31814	ADP,ATP carrier protein, heart isoform T1
8	Ant2	JQ0064	ADP,ATP carrier protein, fibroblast isoform 2
9	Aop1;Aop2	JC1473	MER5 protein
10	Atp5al	ATPL_MOUSE	H+-transporting ATP synthase chain alpha
11	Atp5g1	U38477	ATP synthase lipid-binding protein P1 precursor (protein 9)
12	Atp7b	BAXA_MOUSE	Probable copper transporting P-type ATPase
13	Bax	S71881	Apoptosis regulator BAX, membrane isoform alpha
14	Bcl2dha	S39807	Branched chain alpha-ketoacid dehydrogenase chain E1-alpha
15	Bcl2dhb	B25960	3-methyl-2-oxobutanate dehydrogenase (lipoamide)
16	Bcl2	A53405	Transforming protein bcl-2-beta
17	Bzrp	S12579	Peripheral-type benzodiazepine receptor 1
18	Car5		Carbonate dehydratase, hepatic

Spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1µM each primer
19	Ckmt1	S24612	Creatine kinase
20	Cox4	S12142	Cytochrome c oxidase chain IV precursor
21	Cox7a2	I48286	Cytochrome C oxydase polypeptide VIIa- liver/heart precursor
22	Cox8a	COXR_MOUSE	Cytochrome c oxidase chain VIII
23	Cpo	A48049	Coproporphyrinogen oxidase
24	Cpt2	A49362	Carnitine O-palmitoyltransferase II precursor
25	Crat	CACP_MOUSE	Carnitine O-acetyltransferase (carnitine acetylase)
26	Cyss	CCMS	Cytochrome C, somatic
27	Dbt	S65760	Dihydrolipoamide transacylase precursor
28	Dci	S38770	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor
29	Dld	1E+05	Dihydrolipoamide dehydrogenase (E3)
30	Fdx1	S53524	Adrenodoxin precursor
31	Fdxr	S60028	Ferrodoxin-NADP+ reductase precursor
32			Blank
33	Fpgs	S65755	Tetrahydrofolylpolyglutamate synthase precursor
34	Frda	S75712	Friedreich ataxia
35	Gcdh	GCDH_MOUSE	Glutaryl-CoA dehydrogenase precursor (GCD)
36	Glud	S16239	Glutamate dehydrogenase (NAD(P)+) precursor
37	Got2	S01174	Glutamate oxaloacetate transaminase-2
38	Hadh	JC4210	3-hydroxyacyl-CoA dehydrogenase, short chain-specific, precursor
39	Hces	CCHL_MOUSE	Cytochrome C-type heme lyase (CCHL)
40	Hk1	A35244	Hexokinase I
41	Hmgcl	HMGL_MOUSE	Hydroxymethylglutaryl-CoA lyase
42	Hmges2	B55729	Hydroxymethylglutaryl-CoA synthase, mitochondrial
43	Hsc70t	96231	Heat shock protein cognate 70, testis
44	Hsd3b1	3BH1_MOUSE	3-beta hydroxy-5-ene steroid dehydrogenase type I
45	Hsp60	HHMS60	Heat shock protein 60 precursor
46	Hsp70-1	Q61698	Heat shock protein, 70K (hsp68) (fragment)
47	Hsp74	A48127	Heat shock protein 70 precursor
48	HspE1	A55075	Chaperonin-10

Spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
49	Idh2	IDHP_MOUSE	Isocitrate dehydrogenase (NADP)
50	Mimt44	U69898	TIM44 - mitochondrial inner membrane import subunit
51	Mor1	DEMSMM	Malate dehydrogenase precursor, mitochondrial
52	Cbr2	A28053	Carboxyl reductase (NADPH) - mouse
53	Cox6a1	COXD_MOUSE	Cytochrome C oxydase polypeptide VIa-heart precursor
54	Mthfd	A33267	Methylenetetrahydrofolate dehydrogenase (NAD+)
55	Mut	S08680	Methylmalonyl-CoA mutase alpha chain precursor
56	Nnt	S54876	NAD(P)+ transhydrogenase (B-specific) precursor
57	Oat	XNMSO	Ornithine--oxo-acid transaminase precursor
58	Oias1	25A1_MOUSE	(2'-5')oligoadenylate synthetase 1
59	Otc	OWMS	Ornithine carboxamoyltransferase precursor
60	Pcx	A47255	Pyruvate carboxylase
61	Pdhα1	S23506	Pyruvate dehydrogenase (lipoamide)
62	Pdhα1	S23507	Pyruvate dehydrogenase (lipoamide)
63	Polg	DPOG_MOUSE	DNA polymerase gamma
64	Ppox	S68367	Protoporphyrinogen oxidase
65	Rpl23	1E+06	L23 mitochondrial - related protein
66	Scp2	JU0157	Sterol carrier protein X
67	Sod2	I57023	Superoxide dismutase (Mn) precursor
68	Star	A55455	Steroidogenic acute regulatory protein precursor, mitochondrial
69	Tfam	P97894	Mitochondrial transcription factor A - mouse
70	Tst	THTR_MOUSE	Thiosulfate sulfurtransferase
71	Ung	UNG_MOUSE	Uracil-DNA glycosylase
72	Vdac1	1E+05	Voltage-dependent anion channel 1
73	Vdac2	1E+05	Voltage-dependent anion channel 2
74	Vdac3	1E+05	Voltage-dependent anion channel 3
75	Ywhaz	JC5384	14-3-3 protein zeta/delta
76	WS-3		
77	Skd3	L00923	Myosin 1
78			

Spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
79	GAPDH	M32599	Glyceraldehyde 3-phosphate dehydrogenase (G3PDH)
80	Hsd3b5	L41519	3-ketosteroid reductase
81	APE 1	P28352	Apurinic/apyrimidinic endonuclease 1
82	Ogdh	U02971	2-Oxoglutarate dehydrogenase E1 component
83	ACADV	U41497	Acyl-Co A dehydrogenase very long chain
84	Slc1a1	EAT3_MOUSE	Excitatory amino acid transporter 3
85	Hprt	J00423	Hypoxanthine phosphoribosyl transferase (HPRT)
86	PplA2	D78647	Phospholipase A2
87	Cab45	U45977	Calcium-binding protein Cab45
88	NRF1	NM_010938	Nuclear Respiratory Factor 1
89	Cox5b	X53157	Cytochrome C oxidase subunit Vb
90	Cox 6a2	L06465	Cytochrome C oxidase subunit Via liver precursor
91	Afp5k	S52977	ATP synthase H+ transporting chain e
92	β-actin	X03672	Beta-actin
93		M10624	Murine ornithine decarboxylase (MOD)
94	Tom40		Mitochondrial outer membrane protein
95	Gpam		Glycerol-3-phosphate acyltransferase
96	Arg2		Arginase type II

EXAMPLE 7

Effects Of TBS Thermal Injury On Mouse Liver Mitochondrial Function

The effects of 40% TBS thermal injury on mouse liver mitochondrial function were examined (FIGS. 8A-8D, Table 7). In addition to a control (A), three livers from thermally injured mice 24 hours after burn were analyzed (B-D). The boxes indicate changes in levels of gene expression due to thermal injury. Some of the changes observed are as follows: A6 - aldehyde dehydrogenase (NAD^+)2; A8 - ADP/ATP carrier protein, fibroblast isoform 2; ; A9 - MER 5 protein; A10 - H^+ transporting ATP synthase chain α ; B8 - cytochrome c oxidase chain IV;; D6 - hydroxymethyl butyryl-CoA synthase; F7 - super oxide dismustase (Mn); H6, cytochrome oxidase subunit Vb; H8, β -actin.

A microarray analysis of the expression of the 13 mitochondrial DNA encoded genes in livers of thermally injured mice was performed. FIG. 9 provides the results of the analysis of 3 individual mice 24 hours after thermal injury. The data clearly showed that expression of mitochondrial DNA encoded mRNAs is not affected by thermal injury. I, control; II-IV, 24 hours after thermal injury.

EXAMPLE 8

Human Mitochondrial Microarray

In order to further demonstrate the capability of the present invention, a human DNA microarray was generated from PCR products using human cDNAs that code for mitochondrial proteins. These cDNAs were cloned into the pCR2.1 vector (Invitrogen). The genes were then attached to nylon membranes by cross linking with UV radiation and a hybridization study was conducted. The samples were labeled by reverse transcriptase incorporation of radiolabeled nucleotides and the results were observed by autoradiography. Intense and specific hybridization signals for specific target genes were detected at a number of positions indicating levels of transcript abundance. The data demonstrate successful and selective hybridization of human mitochondrial-related genes on the array. Table 8 represents an array of nuclear encoded genes for mitochondrial proteins and Table 9 represents an array of mitochondria encoded genes.

Table 8-Human Mito Chips (Nuclear Encoded Genes)

Plate 1																
		1	2	3	4	5	6	7	8	9	10	11	12			
A	1	1	2	3	4	5	6	7	8	9	10	11	12			
B	13	14	15	16	17	18	19	20	21	22	23	24				
C	25	26	27	28	29	30	31	32	33	34	35	36				
D	37	38	39	40	41	42	43	44	45	46	47	48				
E	49	50	51	52	53	54	55	56	57	58	59	60				
F	61	62	63	64	65	66	67	68	69	70	71	72				
G	73	74	75	76	77	78	79	80	81	82	83	84				
H	85													GAPDH	PPLA2	
														β-action	HPRT	MYOSIN

Plate 2																
		86	87	88	89	90	91	92	93	94	95	96	97			
A	86	87	88	89	90	91	92	93	94	95	96	97				
B	98	99	100	101	102	103	104	105	106	107	108	109				
C	110	111	112	113	114	115	116	117	118	119	120	121				
D	122	123	124	125	126	127	128	129	130	131	132	133				
E	134	135	136	137	138	139	140	141	142	143	144	145				
F	146	147	148	149	150	151	152	153	154	155	156	157				
G	158	159	160	161	162	163	164	165	166	167	168	169				
H	170													GAPDH	PPLA2	
														β-action	HPRT	MYOSIN

Plate 3

	A	171	172	173	174	175	176	177	178	179	180	181	182
	B	183	184	185	186	187	188	189	190	191	192	193	194
	C	195	196	197	198	199	200	201	202	203	204	205	206
	D	207	208	209	210	211	212	213	214	215	216	217	218
	E	219	220	221	222	223	224	225	226	227	228	229	230
	F	231	232	233	234	235	236	237	238	239	240	241	242
	G	243	244	245	246	247	248	249	250	251	252	253	254
	H												
									GAPDH	β -actin	HPRT	MYOSIN	PPLA2

Spot No.	Gene Name	Accession No.	Description	Related Disease
1	ACAA1	D16294	3-oxoacyl-CoA thiolase	
2	ACADL	M74096	long-chain-acyl-CoA dehydrogenase (LCAD)	LCAD deficiency
3	ACADM	AF251043	acyl-CoA dehydrogenase precursor, medium-chain-specific	MCAD deficiency
5	ACADSB	U12778	short/branched chain acyl-CoA dehydrogenase precursor	
4	ACADS	M26393	acyl-CoA dehydrogenase precursor, short-chain-specific	SCAD deficiency
6	ACADVL	D43682	acyl-CoA dehydrogenase, very-long-chain-specific-precursor (VLCAD)	VLCAD deficiency -
7	ACAT1	D90228	acetyl-CoA C-acetyltransferase 1 precursor	Deficiency of 3-ketothiolase (3KTD)
8	ACO2	U80040	probable aconitate hydratase, mitochondrial (citrate hydrolyase)	
9	AGAT	X86401	glycine amidinotransferase precursor	
10	AK2	U39945	adenylate kinase isoenzyme 2, mitochondrial (ATP-AMP transphosphorylase)	

Spot No.	Gene Name	Accession No.	Description	Related Disease
11	AK3	X60673	nucleoside-triphosphate--adenylate kinase 3	
12	ALDH2	X05409	aldehyde dehydrogenase (NAD+) 2 precursor	Alcohol intolerance, acute
13	ALDH4	U24267	Delta-1-pyrroline-5-carboxylate dehydrogenase precursor	Hyperprolinemia, type II (HPII)
14	ALDH5	M63967	aldehyde dehydrogenase (NAD+) 5 precursor	
15	AMT	D13811	glycine cleavage system T-protein precursor (aminomethyltransferase)	Non-ketotic hyperglycinemia, type II (NKH2)
16	ANT2	J02683	ADP, ATP carrier protein T2	
17	ANT3	J03592	ADP, ATP carrier protein T3	
18	AOP1	D49396	mitochondrial thioredoxin-dependent peroxide reductase precursor	
19	ARG2	U75667	arginase II precursor (non-hepatic arginase) (kidney type arginase)	
20	ATP5A1	X59066	H+-transporting ATP synthase, mitochondrial F1 complex	
21	ATP5B	X05606	H+-transporting ATP synthase, mitochondrial F1 complex	
22	ATP5D	X63422	H+-transporting ATP synthase, F1 complex, δ chain precursor	
23	ATP5F1	X60221	H+-transporting ATP synthase, complex F0, subunit B	
24	ATP5G3	U09813	ATP synthase, mitochondrial F0 complex, chain 9 (subunit C)	
25	ATP5I	NM_007100	H+-transporting ATP synthase, mitochondrial F0 complex	
26	ATP5J	M37104	ATP synthase, mitochondrial F0 complex, subunit F6	
27	ATP5O	X83218	ATP synthase oligomycin sensitivity conferral protein precursor	
28	BAX	L22473	apoptosis regulator BAX, membrane isoform α	
29	BCAT2	U68418	thyroid-hormone aminotransferase	
30	BCL2L1	Z23115	BCL2-like 1 - human	

Spot No.	Gene Name	Accession No.	Description	Related Disease
31	BCS1L	AF026849	BCS1 (yeast homolog)-like - human	
32	BDH	M93107	D-beta-hydroxybutyrate dehydrogenase precursor	
33	BID	AF042083	BH3 interacting domain death agonist (BID)	
34	BNIP3L	AF079221	bcl2/adenovirus e1b 19-kDa protein-interacting protein	
35	BZRP	M36035	peripheral benzodiazepine receptor	
36	BZRP-S	L21950	peripheral benzodiazepine receptor-related protein	
37	CACT	Y10319	Carnitine-acylcarnitine translocase (CACT)	Carnitine-acylcarnitine translocase deficiency
38	CASQ1	S73775	calsequestrin precursor, fast-twitch skeletal muscle	
39	CGI-114	AF151872	oligoribonuclease, mitochondrial precursor	
40	CKMT1	XM_007535	creatine kinase precursor	
41	CKMT2	JO5401	creatine kinase precursor, sarcomere-specific	
42	CLPX	AJ006267	putative ATP-dependent CLP protease ATP-binding subunit CPLX	
43	COQ7	AF032900	ubiquinone biosynthesis protein COQ7 (CLK1 homologue of <i>c. elegans</i>)	
44	COX11	AF044321	cytochrome c oxidase assembly protein COX11	
45	COX4	X54802	cytochrome-c oxidase chain IV precursor	
46	COX5A	NM_004255	cytochrome-c oxidase chain Va precursor	
47	COX5B	M19961	cytochrome-c oxidase chain Vb precursor	
48	COX6A2	NM_005205	cytochrome-c oxidase chain VIa precursor, cardiac	
49	COX6B	XM_009350	cytochrome-c oxidase chain VIb	
50	COX7A1	XM_009337	cytochrome-c oxidase chain VIIa precursor, cardiac and skeletal	
51	COX7RP	AB007618	cytochrome-c oxidase subunit VIIA-related protein	
52	CPO	Z28409	coproporphyrinogen oxidase	Hereditary coproporphyria (HCP)

Spot No.	Gene Name	Accession No.	Description	Related Disease
53	CPS1	XM_010819	carbamoyl-phosphate synthase (ammonia) precursor	Hyperammonemia, type I
54	CPT2	M58581	carnitine O-palmitoyltransferase II precursor	Carnitine O-palmitoyltransferase II deficiency
55	CRAT	X78706	carnitine O-acetyltransferase precursor	Carnitine O-acetyltransferase deficiency
56	CS	AF047042	citrate synthase, mitochondrial	
57	CYB5	NM_030579	cytochrome b5, microsomal form	
58	CYC1	NM_001916	ubiquinol-cytochrome-c reductase cytochrome c1 precursor	
59	CYP11A1	M14565	cholesterol monooxygenase (side-chain-cleaving)	
60	CYP3	NM_005729	cytochrome P450e	
61	DBT	X66785	peptidylprolyl isomerase 3 precursor	Maple syrup urine disease (MSUD)
62	DCI	Z25820	dihydrodipoamide S-(2-methylpropanoyl) transferase precursor	Deficiency of 2,4-dienoyl-CoA reductase
63	DECR	XM_005309	dodecenoyl-CoA δ-isomerase precursor	Mohr-Tranebaerg syndrome (MTS)
64	DFN1	U66035	2,4-dienoyl-CoA reductase precursor	
64			deafness dystonia protein	
65	DIA1	XM_010028	cytochrome-b5 reductase	
66	DLAT_h	X13822	dihydrodipoamide S-acetyltransferase heart	Dihydriopioamide dehydrogenase deficiency;
67	DLD	J03620	dihydrodipoamide dehydrogenase precursor	Leigh syndrome
68	DLST	XM_012353	dihydriopioamide S-succinyltransferase	Myoneurogastrointestinal encephalopathy syndrome (MNGIE)
69	ECGF1	M63193	thymidine phosphorylase precursor (TDRPASE)	

Spot No.	Gene Name	Accession No.	Description	Related Disease
70	ECHS1	XM_005677	enoyl-CoA hydratase, mitochondrial	
71	EFE2	X92762	tafazzins protein	Barth syndrome
72	EFTS	AF110399	mitochondrial elongation factor TS precursor (EF-TS)	
73	ENDOG	XM_005364	endonuclease G, mitochondrial	
74	ETFA	XM_007626	electron transfer flavoprotein alpha chain precursor	Glutaric aciduria, type IIa (GAIa) Glutaric aciduria, type IIc (GAIc)
75	ETFDH	NM_004453	electron transfer flavoprotein dehydrogenase	
76	FACL1	XM_010921	long-chain-fatty-acid-CoA ligase 1 (palmitoyl-CoA ligase)	
77	FACL2	NM_021122	long-chain-fatty-acid-CoA ligase 2	
78	FDX1	M34788	adrenodoxin precursor	
79	FDXR	J03826	ferredoxin--NADP+ reductase, long form, precursor	
80	GCDH	U69141	glutaryl-CoA dehydrogenase precursor (GCD)	
81	GCSH	XM_010661	glycine cleavage system protein H precursor	Glutaric aciduria, type I (GA-I)
82	GK	XM_010221	glycerol kinase (ATP: glycerol 3 - phosphotransferase)	Non-ketotic hyperglycinemia, type III (NKH3)
83	GLDC	XM_0111805	glycine dehydrogenase (decarboxylating) precursor	Glycerol kinase deficiency (GKD)
84	GLUD1	X07769	glutamate dehydrogenase (NAD(P)+) precursor	Non-ketotic hyperglycinemia, type I (NKH1)
85	GOT2	M22632	aspartate transaminase precursor	
86	GPD2	XM_002442	glycerol-3-phosphate dehydrogenase	
87	GST12	J03746	glutathione transferase, microsomal	Diabetes mellitus, type II (NIDDM)

Spot No.	Gene Name	Accession No.	Description	Related Disease
88	HADH _A	NM_000182	long-chain-fatty-acid beta-oxidation multienzyme complex alpha	Trifunctional enzyme deficiency;Maternal acute fatty liver of pregnancy (AFLP)
89	HADHB	NM_000183	long-chain-fatty-acid beta-oxidation multienzyme complex beta	Trifunctional enzyme deficiency
90	HCCS	U36787	cytochrome c - type heme lyase (holocytochrome-c synthase)	
91	HK1	X66957		Diabetes mellitus, type II (NIDDM)
92	HK2	NM_000189	hexokinase I	Biotin-responsive multiple carboxylase deficiency
93	HLC _S	XM_009757	biotin-[methylmalonyl-CoA-carboxyltransferase] ligase	Hydroxymethylglutaricaciduria (HMGCL)
94	HMGCL	L07033	hydroxymethylglutaryl-CoA lyase	Severe depletion of steroid formation
95	HSD3B1	M27137	3-beta hydroxy-5-ene steroid dehydrogenase type I	
96	HSPA1L	M11717	heat shock protein HSP70	
97	HSPA9	L15189	mitochondrial hsp70 precursor	
98	HSPD1	M22382	heat shock protein 60 precursor	
99	HSPE1	X75821	heat shock protein 10	
100	HTOM34P	U58970	Human putative outer mitochondrial membrane 34 kDa translocae	
101	HTOM	AF026031	putative mitochondrial outer membrane protein import receptor	
102	IDH2	X69433	isocitrate dehydrogenase (NADP+) precursor	
103	IDH3A	U07681	NAD(H)-specific isocitrate dehydrogenase α chain precursor	

Spot No.	Gene Name	Accession No.	Description	Related Disease
104	DH3B	U49283	isocitrate dehydrogenase (NAD), mitochondrial subunit β	
105	DH3G	Z68907	isocitrate dehydrogenase (NAD), mitochondrial subunit γ	Isovaleric acidemia (IVA)
106	IVD	M34192	isovaleryl-CoA dehydrogenase precursor	
107	KIAA0016	D13641	Mitochondrial import receptor subunit TOM20 homolog	
108	KIAA0028	D21851	Probable leucyl-tRNA synthetase	
109	KIAA0123	D50913	mitochondrial processing peptidase α subunit precursor	
110	LOC51081	AF077042	ribosomal protein S7 small chain precursor	
111	LOC51189	AB029042	ATPase inhibitor precursor	Brunner's syndrome
112	MAOA	M68840	amine oxidase (flavin-containing) A	
113	MAOB	XM_010261	amine oxidase (flavin-containing) B	
114	MDH2	XM_004905	malate dehydrogenase mitochondrial precursor (fragment)	
115	ME2.1	X79440	malate dehydrogenase (oxaloacetate-decarboxylating)	
116	ME2	M55905	malate dehydrogenase (NAD+) precursor	
117	MFT	AF283645	folate transporter/carrier	
118	MIPEP	U80034	mitochondrial intermediate peptidase	
119	MLN64	D38255	MLN 64 protein (steroidogenic acute regulatory protein related)	
120	MMSDH	M93405	methylmalonate-semialdehyde dehydrogenase (acylating)	Methylmalonate semialdehyde dehydrogenase deficiency (MMSDHD)
121	MRRF	AF072934	mitochondrial ribosome recycling factor 1	
122	MTABC3	AF076775	mammalian mitochondrial ABC protein 3	
123	MTCH1	AF176006	mitochondrial carrier homolog 1 isoform a	
124	MTCH2	AF176008	mitochondrial carrier homolog 2	
125	MTTERF	Y09615	transcription termination factor	
126	MTHFD1	J04031	methylenetetrahydrofolate dehydrogenase (NADP+)	

Spot No.	Gene Name	Accession No.	Description	Related Disease
127	MTHFD2	X16396	methylenetetrahydrofolate dehydrogenase (NAD ⁺)	
128	MTIF2	L34600	methionine tRNA synthetase initiation factor IF-2 precursor	
129	MTRF1	AF072934	mitochondrial translational release factor 1	
130	MTX1	XM_002192	metaxin 1 - human	
131	MTX2	XM_002547	metaxin 2 - human	
132	MUT	M65131	methylmalonyl-CoA mutase precursor (MCM1)	Methylmalonic acidemia (MUT-, MUT0 type)
133	MUTYH	U63329	mutY (E. coli) homolog - human	
134	NDUFA10	AF087661	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 10 (42kD)	
136	NDUFA2	AF047185	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 2 (8kD)	
137	NDUFA4	U94586	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 4 (9kD)	
138	NDUFA5	U53468	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 5 (13kD)	
139	NDUFA6	XM_010025	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 6 (14kD)	
140	NDUFA7	NM_005001	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 7 (14.5kD)	
141	NDUFA8	AF044953	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 8 (19kD)	
142	NDUFAB1	AF087660	acyl carrier protein, mitochondrial precursor (ACP)	
143	NDUFB1	AF054181	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 1 (7kD)	
144	NDUFB2	XM_004607	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 2 (8kD)	

Spot No.	Gene Name	Accession No.	Description	Related Disease
145	NDUFB3	NM_002491	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 3 (12kD)	
146	NDUFB4	AF044957	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 4 (15kD)	
147	NDUFB5	AF047181	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 5 (16kD)	
148	NDUFB6	XM_005532	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 6 (17kD)	
149	NDUFB7	M33374	NADH dehydrogenase (ubiquinone) B18 subunit (Complex I-B18)	
150	NDUFB8	XM_005701	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 8 (19kD)	
151	NDUFB9	S82655	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 9 (22kD)	
152	NDUFC2	AF087659	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2(14.5kD)	
153	NDUFS2	AF050640	NADH dehydrogenase (ubiquinone) Fe-S protein 2 (49kD)	
154	NDUFS3	AF067139	NADH dehydrogenase (ubiquinone) 30K chain precursor	
155	NDUFS5	AF020352	NADH dehydrogenase (ubiquinone) Fe-S protein 5 (15kD)	
156	NDUFS6	AF044959	NADH dehydrogenase (ubiquinone) 13kD-A subunit precursor	
157	NDUFS7	NM_024407	NADH dehydrogenase (ubiquinone) Fe-S protein 7 (20kD)	Leigh syndrome
158	NDUFS8	U65579	NADH dehydrogenase (ubiquinone) 23kD subunit precursor	Leigh syndrome
159	NDUFV1	AF053070	NADH dehydrogenase (ubiquinone) 51K chain precursor (fragment)	Alexander disease; Leigh syndrome
160	NDUFV2	M22538	NADH dehydrogenase (ubiquinone) 24K chain precursor	
161	NDUFV3	XM_009784	NADH dehydrogenase (ubiquinone) 9kD subunit precursor	
162	NIFS	XM_009457	cysteine desulfurase (homolog of nitrogen-fixing bacteria)	

Spot No.	Gene Name	Accession No.	Description	Related Disease
163	NME4	Y07604	nucleosid diphosphate kinase (NDP kinase)	
164	NNT-PEN	U40490	NAD(P)+ transhydrogenase (B-specific) precursor	
165	NOC4	XM_008056	neighbor of COX4 (NOC4)	
166	NRF1	NM_005011	nuclear respiratory factor 1	
167	NTHL1	AB001575	endonuclease III (E. coli) homolog	
168	OAT	M23204	ornithine--oxo-acid transaminase precursor	Ornithinemia with gyrate atrophy (GA)
169	OGDH	D10523	oxoglutarate dehydrogenase (lipoamide) precursor	Deficiency of α -ketoglutarate dehydrogenase
170	OGG1	U96710	8-oxoguanine DNA glycosylase	
171	OLAS	X02874	(2'-5') oligoadenylate synthetase E16	Optic atrophy (OPA1)
172	OPA1	XM_039926	Optic atrophy 1 protein, KIAA0567	Hyperammonemia, type II
173	OTC	K02100	ornithine carbamoyltransferase precursor	
174	OXATL	X80695	OXA1 homolog	
175	OXCT	U62961	Succinyl-CoA:3-ketoacid-coenzyme A transferase precursor	Deficiency of Succinyl-CoA:3-oxoacid-CoA transferase
176	P43-LSB	S75463	mitochondrial elongation factor-like protein P43	Propionic acidemia, type I (PA-1)
177	PCCA	X14608	propionyl-CoA carboxylase α chain precursor	Propionic acidemia, type II (PA-2)
178	PCCB	XM_051992	propionyl-CoA carboxylase β chain precursor	Hypoglycemia and liver impairment
179	PCK2	S69546	phosphoenolpyruvate carboxykinase (GTP) precursor	Deficiency of pyruvate carboxylase, type I and II
180	PC	U04641	pyruvate carboxylase precursor	Pyruvate dehydrogenase deficiency; Leigh syndrome
181	PDHA1	J03503	pyruvate dehydrogenase (lipoamide) α chain precursor	

Spot No.	Gene Name	Accession No.	Description	Related Disease
182	PDHA2	M86808	pyruvate dehydrogenase (lipoamide) α chain precursor, testis	
183	PDK1	L42450	pyruvate dehydrogenase kinase isoform 1	
184	PDK2	L42451	pyruvate dehydrogenase kinase isoform 2	
185	PDK3	L42452	pyruvate dehydrogenase kinase isoform 3	
186	PDK4	U54617	pyruvate dehydrogenase kinase isoform 4	
187	PDX1	U82328	pyruvate dehydrogenase complex protein X subunit precursor	Pyruvate dehydrogenase deficiency
188	PEMT	AF176807	phosphatidylethanolamine N-methyltransferase (PEMT)	
189	PET112L	AF026851	probable glutamyl-tRNA(gln) amidotransferase subunit b	
190	PHC	XM_039620	phosphate carrier isoform A (alternatively spliced, exon IIIA)	
191	PLA2G2A	M22430	phospholipase A2, group II A, platelet, synovial fluid	
192	PLA2G4	M72393	phospholipase A2, cytosolic, group IV	
193	PLA2G5	U03090	phospholipase A2, group V	
194	PMPCB	AF054182	mitochondrial processing peptidase β subunit precursor	
195	POLG2	U94703	mitochondrial DNA polymerase accessory subunit	
196	POLG	X98093	DNA polymerase γ (mitochondrial) DNA polymerase catalytic subunit	
197	POLRMT	U75370	mitochondrial RNA polymerase (DNA directed)	
198	PPOX	D38537	protoporphyrinogen oxidase (PPO)	Porphyria variegata (VP)
199	PRAX-1	AF039571	benzodiazepine receptor-associated protein 1	
200	PRDX5	AF110731	Peroxiredoxin 5 (antioxidant enzyme B166)	
201	PYCR1	M77836	pyrroline-5-carboxylate reductase	
202	RPL23L	Z49254	mitochondrial 60S ribosomal protein L23	
203	RPML12	X79865	mitochondrial 60S ribosomal protein L7/L12 precursor	
204	RPML3	X06323	ribosomal protein L3 precursor	
205	RPMS12	Y11681	mitochondrial 40S ribosomal protein S12 precursor	

Spot No.	Gene Name	Accession No.	Description	Related Disease
206	SCHAD	X96752	3-hydroxyacyl-CoA dehydrogenase, short chain-specific, precursor	
207	SCO2	AF177385	SCO2 homolog of <i>S. cerevisiae</i>	Fatal infantile cardioencephalomyopathy due to Cox deficiency
208	SCP2	M55421	sterol carrier protein 2	
209	SDH1	U17248	succinate dehydrogenase (ubiquinone) 27K iron-sulfur protein	
210	SDH2	L21936	succinate dehydrogenase (ubiquinone) flavoprotein precursor	Leigh syndrome; Deficiency of succinate dehydrogenase
211	SDHC	D49737	succinate dehydrogenase (ubiquinone) cytochrome b large subunit	Hereditary paraganglioma, type III (PGL3)
85	SDHD	AB006202	succinate dehydrogenase (ubiquinone) cytochrome b small subunit	Hereditary paraganglioma, type I (PGL1)
212	SerRSmt	AB029948	seryl-tRNA synthetase	
213	SHMT2	NM_005412	glycine hydroxymethyltransferase precursor	
214	SLC20A3	U25147	tricarboxylate transport protein precursor	
215	SLC25A12	Y14494	mitochondrial carrier protein aralar 1	
216	SLC25A16	M31659	mitochondrial solute carrier protein homolog	
217	SLC25A18	AY008285	solute carrier SLC25A18	
218	SLC9A6	AF030409	sodium/hydrogen exchanger 6 (Na ⁽⁺⁾ H ⁽⁺⁾) exchanger	
219	SOD2	X14322	superoxide dismutase (Mn) precursor	
220	SSBP	M94556	single-stranded mitochondrial DNA-binding protein precursor	
221	SUCLA2	XM_012310	succinyl-CoA ligase (ADP_forming), β-chain precursor	
222	SUCLG1	NM_003849	succinyl-CoA ligase (GDP_forming), α-chain precursor	
223	SUCLG2	AF058954	succinyl-CoA ligase (GDP_forming), β-chain precursor	

Spot No.	Gene Name	Accession No.	Description	Related Disease
225	SUOX	XM_006727	sulfite oxidase precursor, mitochondrial	Sulfocysteinuria
226	SUPV3L1	XM_005981	putative ATP-dependent mitochondrial RNA-helicase	
227	SURF1	NM_003172	Surfeit locus protein 1	Leigh syndrome
228	TAT	NM_000353	tyrosine transaminase (EC 2.6.1.5)	Tyrosine transaminase deficiency, type II (Richner-Hanhart syndrome)
229	TCF6L1	M62810	transcription factor 1 precursor	
230	TID1	AF061749	tumorous imaginal discs homolog precursor (HTTD-1)	
231	TIM17B	AF034790	translocase of inner mitochondrial membrane 17 (yeast) homolog B	
232	TIM17	AF106622	translocase of inner mitochondrial membrane 17 (yeast) homolog A	
233	TIM23	AF030162	inner mitochondrial membrane translocase TIM23	
234	TIM44	AF041254	translocase of inner mitochondrial membrane 44	
235	TK2	U77088	thymidine kinase	
236	TST	X59434	thiosulfate sulfurtransferase	
237	TUFM	L38995	translation elongation factor Tu precursor	
238	UCP2	U82819	uncoupling protein 2	
239	UCP3	U82818	uncoupling protein 3	
240	UCP4	NM_004277	uncoupling protein 4	
241	UNG	X15653	uracil-DNA glycosylase precursor	
242	UQCRB	NM_006294	ubiquinone-binding protein QP-C	
243	UQCRC1	NM_003365	ubiquinol--cytochrome-c reductase core I protein	
244	UQCRC2	NM_003366	ubiquinol--cytochrome-c reductase core protein II	
245	UQCRCFS1	XM_012812	ubiquinol--cytochrome-c reductase iron-sulfur subunit precursor	Mitochondrial myopathy (MM)
246	UQCRH	NM_006004	ubiquinol--cytochrome-c reductase 11K protein precursor	

Spot No.	Gene Name	Accession No.	Description	Related Disease
247	UROS	AF230665	uroporphyrinogen-III synthase	
248	VDAC1	L06132	voltage-dependent anion channel 1	
249	VDAC2	L06328	voltage-dependent anion channel 2	
250	VDAC3	NM_005662	voltage-dependent anion channel 3	
251	WARS2	XM_001388	tryptophanyl-tRNA synthetase 2	
252	WFS	AF084481	Transmembrane protein	Diabetes mellitus and insipidus with optic atrophy and deafness (DIDMOAD); Wolfram syndrome
253	YME1L1	AJ132637	ATP-dependent metalloprotease YME1	
254	YWHAE	U28936	14-3-3 protein epsilon (mitochondrial import stimulation factor)	

Table 9: Human Mito Chip (Mitochondria encoded)

Spot #	Genomic Accession	Description
1	MTCO1 V00662	Cytochrome-c oxidase chain I
2	MTCO2 V00662	Cytochrome-c oxidase chain II
3	MTCO3 V00662	Cytochrome-c oxidase chain III
4	MTCYB V00662	Ubiquinol--cytochrome-c reductase cytochrome b
5	MTND1 J01415	NADH dehydrogenase (ubiquinone) chain 1
6	MTND2 J01415	NADH dehydrogenase (ubiquinone) chain 2
7	MTND3 J01415	NADH dehydrogenase (ubiquinone) chain 3
8	MTND4 J01415	NADH dehydrogenase (ubiquinone) chain 4
9	MTND4L J01415	NADH dehydrogenase (ubiquinone) chain 4L
10	MTND5 J01415	NADH dehydrogenase (ubiquinone) chain 5
11	MTND6 J01415	NADH dehydrogenase (ubiquinone) chain 6
12	MT-ATP 6 J01415	ATP synthase subunit 6
13	MT-ATP 8 J01415	ATP synthase subunit 8
14	MTRNR1 J01415	mitochondrial ribosomal RNA, 12S
15	MTRNR2 J01415	mitochondrial ribosomal RNA, 16S
		Aminoglycoside-induced deafness; Nonsyndromic deafness Chloramphenicol resistance; Alzheimer disease and Parkinson disease (ADPD)

All of the compositions and/or methods and/or apparatus disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and/or apparatus and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein
5 by reference.

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10 U.S. Patent 4,883,750

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CLAIMS

1. An array comprising nucleic acid molecules comprising a plurality of sequences, wherein the molecules are immobilized on a solid support and wherein at least 5% of the immobilized molecules are capable of hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.
5
2. The array of claim 1, further defined as comprising at least 20 nucleic acid molecules.
3. The array of claim 1, further defined as comprising at least 40 nucleic acid molecules.
- 10 4. The array of claim 1, further defined as comprising at least 100 nucleic acid molecules.
5. The array of claim 1, further defined as comprising at least 200 nucleic acid molecules.
- 15 6. The array of claim 1, further defined as comprising at least 400 nucleic acid molecules.
7. The array of claim 1, wherein said nucleic acid molecules comprise cDNA sequences.
8. The array of claim 1, wherein each of said nucleic acid molecules comprises at least 17 nucleotides.
- 20 9. The array of claim 1, wherein the mitochondrial-related nucleic acid sequences are from a mammal.
10. The array of claim 9, wherein the mitochondrial-related nucleic acid sequences are from a primate.
11. The array of claim 9, wherein the mitochondrial-related nucleic acid sequences are from a human.
25

12. The array of claim 9, wherein the mitochondrial-related nucleic acid sequences are from a yeast.
13. The array of claim 9, wherein the mitochondrial-related nucleic acid sequences are from a mouse.
- 5 14. The array of claim 9, wherein the mitochondrial-related nucleic acid sequences are from *Drosophila*.
15. The array of claim 9, wherein the mitochondrial-related nucleic acid sequences are from the nematode, *C. elegans*.
16. The array of claim 1, wherein at least 25% of the immobilized molecules are capable of hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.
- 10 17. The array of claim 1, wherein at least 35% of the immobilized molecules are capable of hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.
- 15 18. The array of claim 1, wherein at least 50% of the immobilized molecules are capable of hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.
19. The array of claim 1, wherein at least 75% of the immobilized molecules are capable of hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.
- 20 20. The array of claim 1, wherein at least 85% of the immobilized molecules are capable of hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.
21. The array of claim 1, wherein at least 95% of the immobilized molecules are capable of hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.

22. The array of claim 1, wherein 100% of the immobilized molecules are capable of hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.
23. The array of claim 1, wherein at least one of said mitochondrial-related nucleic acid sequences is encoded by a mitochondrial genome.
5
24. The array of claim 1, wherein the immobilized molecules are capable of hybridizing to at least 5 mitochondrial-related nucleic acid sequences or complements thereof.
25. The array of claim 1, wherein the immobilized molecules are capable of hybridizing to at least 10 mitochondrial-related nucleic acid sequences or complements thereof.
10
26. The array of claim 1, wherein the immobilized molecules are capable of hybridizing to at least 13 mitochondrial-related nucleic acid sequences or complements thereof.
- 15 27. The array of claim 1, wherein the immobilized molecules are capable of hybridizing to at least 20 mitochondrial-related nucleic acid sequences or complements thereof.
28. The array of claim 1, wherein the immobilized molecules are capable of hybridizing to at least 30 mitochondrial-related nucleic acid sequences or
20 complements thereof.
29. The array of claim 1, wherein the immobilized molecules are capable of hybridizing to at least 60 mitochondrial-related nucleic acid sequences or complements thereof.
30. The array of claim 1, wherein the immobilized molecules are capable of hybridizing to at least 100 mitochondrial-related nucleic acid sequences or
25 complements thereof.

31. The array of claim 1, wherein the immobilized molecules are capable of hybridizing to at least 200 mitochondrial-related nucleic acid sequences or complements thereof.
32. The array of claim 1, wherein the immobilized molecules are capable of hybridizing to at least 300, at least 500, or at least 1000 mitochondrial-related nucleic acid sequences or complements thereof.
5
33. The array of claim 1, wherein at least one of said mitochondrial-related nucleic acid sequences is encoded by a nuclear genome.
34. The array of claim 1, wherein at least one of said mitochondrial-related nucleic acid sequences is encoded by a mitochondrial genome.
10
35. A method for measuring the expression of one or more mitochondrial-related coding sequence in a cell or tissue, said method comprising:
 - a) contacting an array according to claim 1 with a sample of nucleic acids from the cell or tissue under conditions effective for mRNA or complements thereof from said cell or tissue to hybridize with the nucleic acid molecules immobilized on the solid support; and
15
 - b) detecting the amount of mRNA or complements thereof hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.
- 20 36. The method of claim 35, wherein said detecting is carried out colorimetrically, fluorometrically, or radiometrically.
37. The method of claim 35, wherein the cell is a mammal cell.
38. The method of claim 35, wherein the cell is a primate cell.
39. The method of claim 35, wherein the cell is a human cell.
- 25 40. The method of claim 35, wherein the cell is a mouse cell.

41. The method of claim 35, wherein the cell is a yeast cell.
42. A method of screening an individual for a disease state associated with altered expression of one or more mitochondrial-related nucleic acid sequences comprising:
 - 5 a) contacting an array according to claim 1 with a sample of nucleic acids from the individual under conditions effective for the mRNA or complements thereof from said individual to hybridize with the nucleic acid molecules immobilized on the solid support;
 - b) detecting the amount of mRNA or complements thereof hybridizing to mitochondrial-related nucleic acid sequences; and
 - c) screening the individual for a disease state by comparing the expression of said mitochondrial-related nucleic acid sequences detected with a pattern of expression of said mitochondrial-related nucleic acid sequences associated with said disease state.
- 15 43. The method of claim 42, wherein said disease state is a disease state as listed in Table 1.
44. The method of claim 43, wherein the disease state is cystic fibrosis, Alzheimer's disease, Parkinson's disease, ataxia, diabetes mellitus, multiple sclerosis or cancer.
- 20 45. The method of claim 42, wherein said detecting is carried out colorimetrically, fluorometrically, or radiometrically.
46. The method of claim 42, wherein the individual is a mammal.
47. The method of claim 42, wherein the individual is a primate.
48. The method of claim 42, wherein the individual is a human.
49. The method of claim 42, wherein the individual is a mouse.

50. The method of claim 42, wherein the individual is a an arthropod.
51. The method of claim 42, wherein the individual is a nematode.
52. A method of screening a compound for its affect on mitochondrial structure and/or function comprising:
 - 5 a) contacting an array according to claim 1 with a sample of nucleic acids from a cell under conditions effective for the mRNA or complements thereof from said cell to hybridize with the nucleic acid molecules immobilized on the solid support, wherein the cell has previously been contacted with said compound under conditions effective to permit the compound to have an affect on mitochondrial structure and/or function;
 - 10 b) detecting the amount of mRNA encoded by mitochondrial-related nucleic acid sequences or complements thereof that hybridizes with the nucleic acid molecules immobilized on the solid support; and
 - 15 c) correlating the detected amount of mRNA encoded by mitochondrial-related nucleic acid molecules or complements thereof with the affect of the compound mitochondrial structure and/or function.
- 20 53. The method of claim 52, wherein the compound is a small molecule.
54. The method of claim 52, wherein the compound is formulated in a pharmaceutically acceptable carrier or diluent.
55. The method of claim 52, wherein the compound is an oxidative stressing agent or an inflammatory agent.
- 25 56. The method of claim 52, wherein the compound is a chemotherapeutic agent.

57. The method of claim 52, wherein said detecting is carried out colorimetrically, fluorometrically, or radiometrically.
58. A method for screening an individual for reduced mitochondrial function comprising:
 - 5 a) contacting an array according to claim 1 with a sample of nucleic acids from a cell under conditions effective for the mRNA or complements thereof from said cell to hybridize with the nucleic acid molecules immobilized on the solid support;
 - 10 b) detecting the amount of mRNA encoded by mitochondrial-related nucleic acid sequences or complements thereof that hybridizes with the nucleic acid molecules immobilized on the solid support; and
 - c) correlating the detected amount of mRNA or complements thereof with reduced mitochondrial function.
- 15 59. The method of claim 58, wherein said detecting is carried out colorimetrically, fluorometrically, or radiometrically.
60. The method of claim 58, wherein the individual is a mammal.
61. The method of claim 58, wherein the individual is a primate.
62. The method of claim 58, wherein the individual is a human.
- 20 63. The method of claim 58, wherein the individual is a mouse.
64. The method of claim 58, wherein the individual is an arthropod.
65. The method of claim 58, wherein the individual is a nematode.

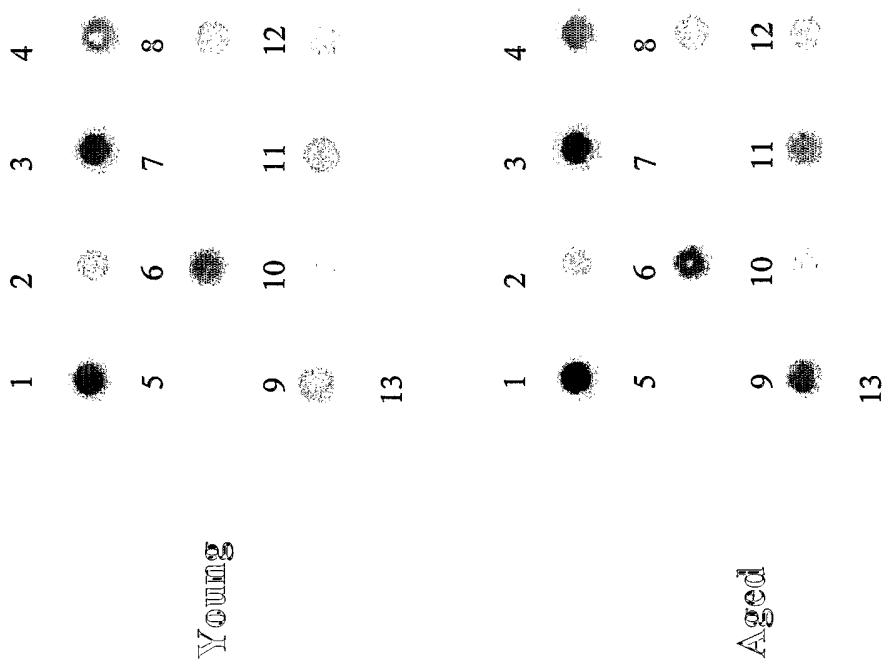


FIG. 1

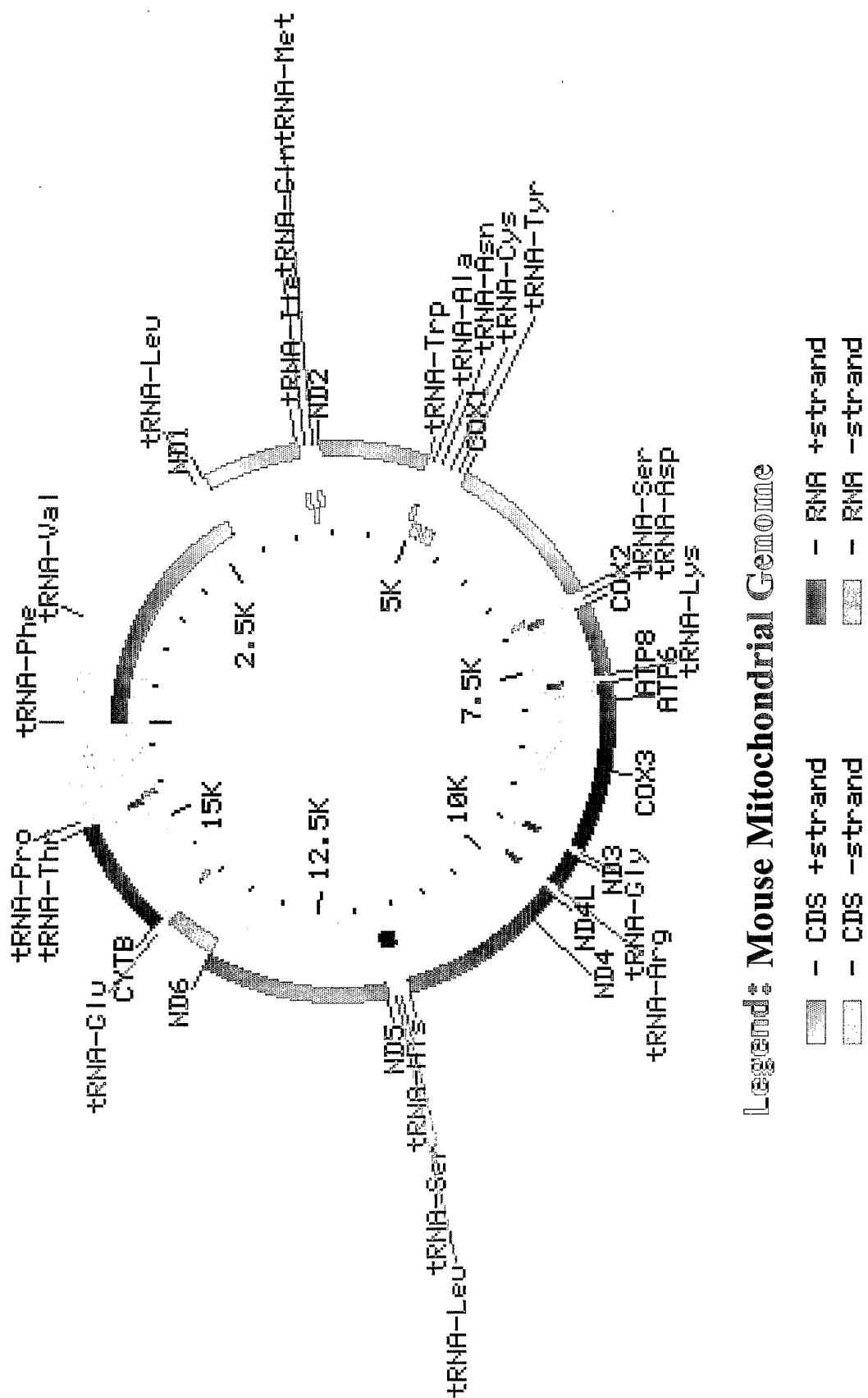


FIG. 2

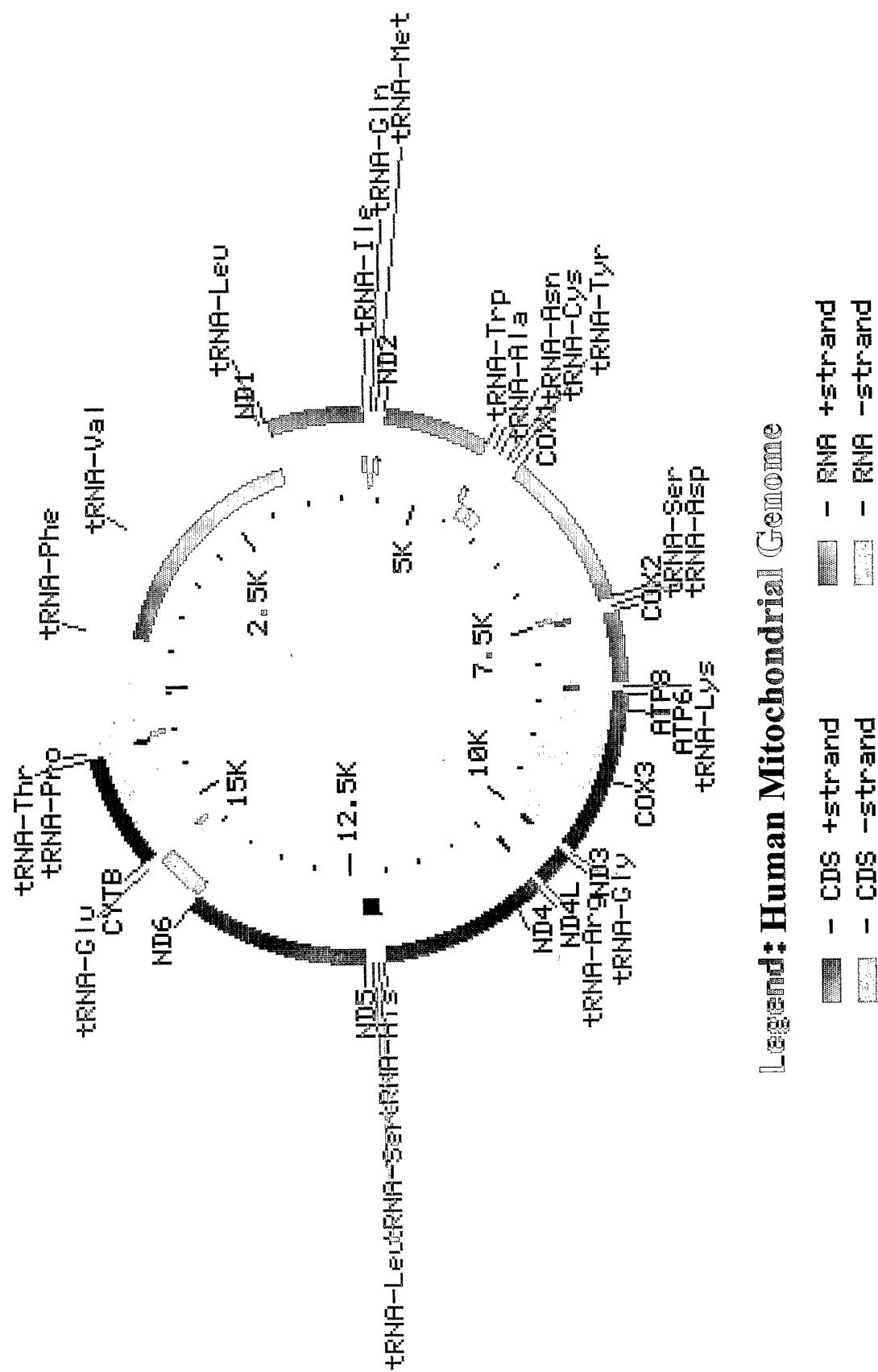
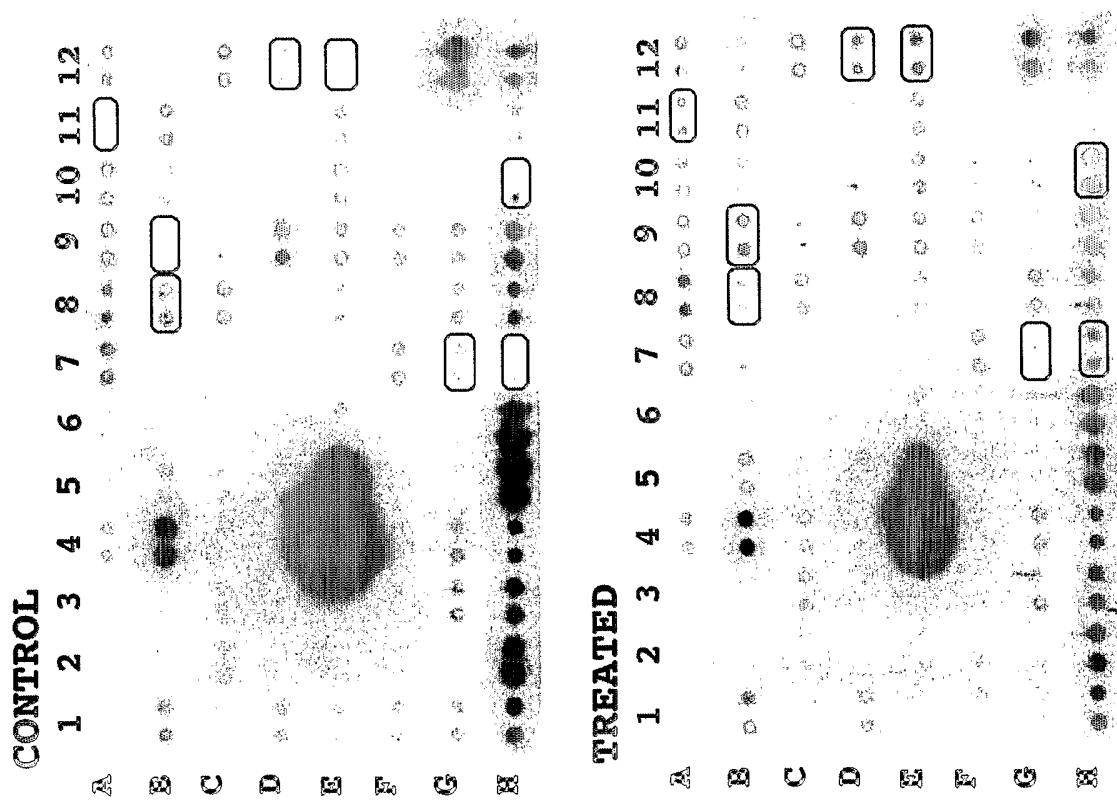


FIG. 3

FIG. 4



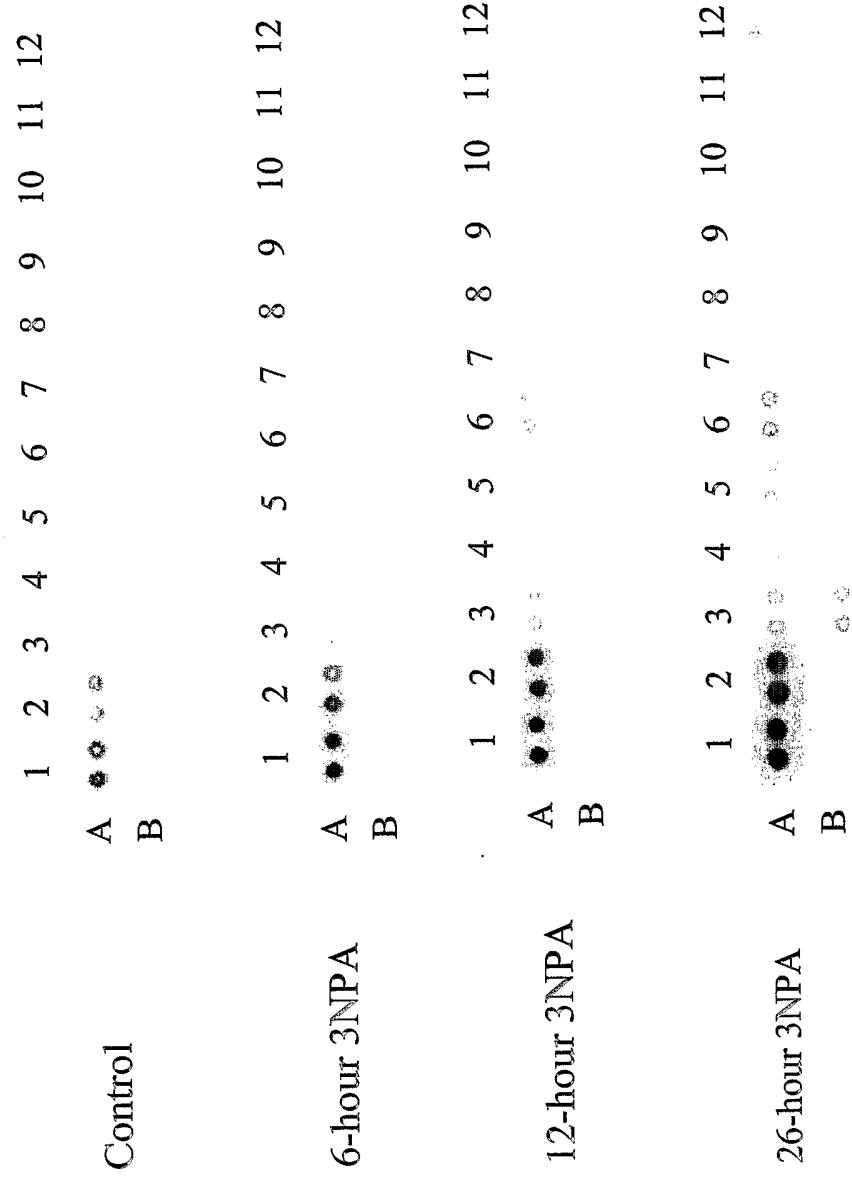


FIG. 5A

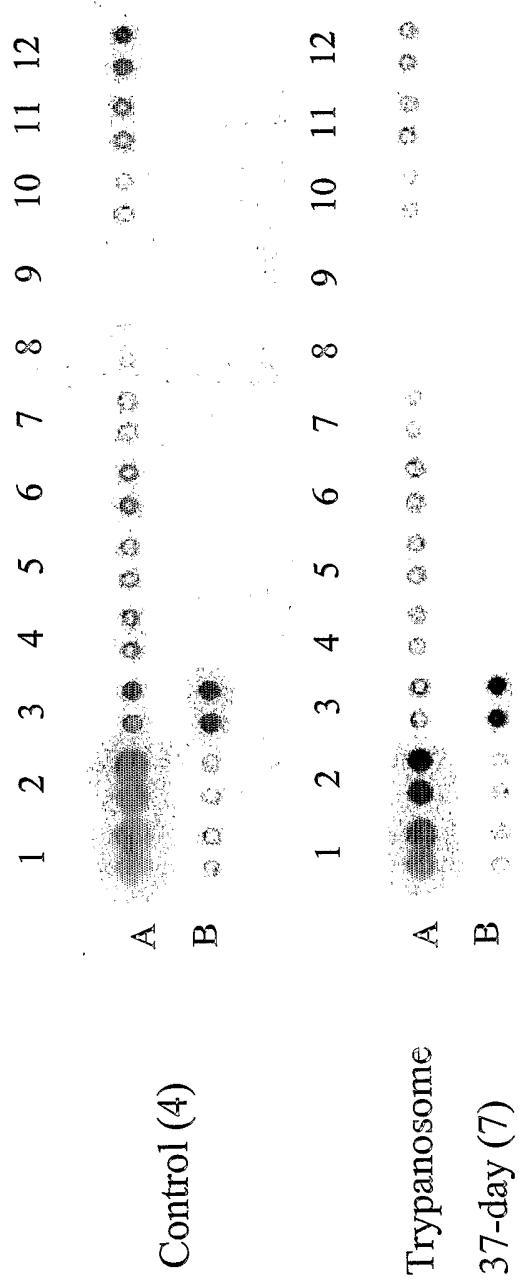
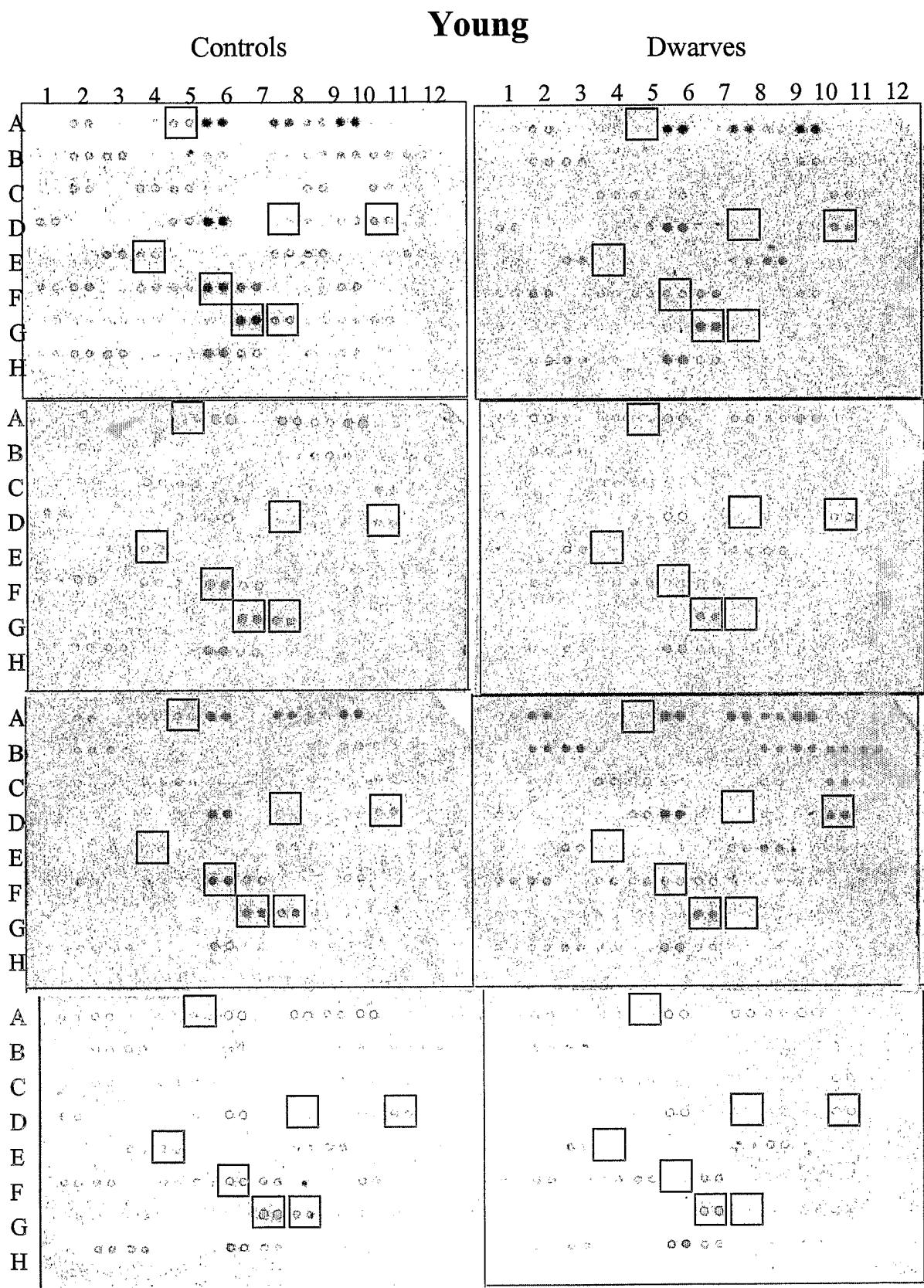


FIG. 5B

**FIG. 6A**

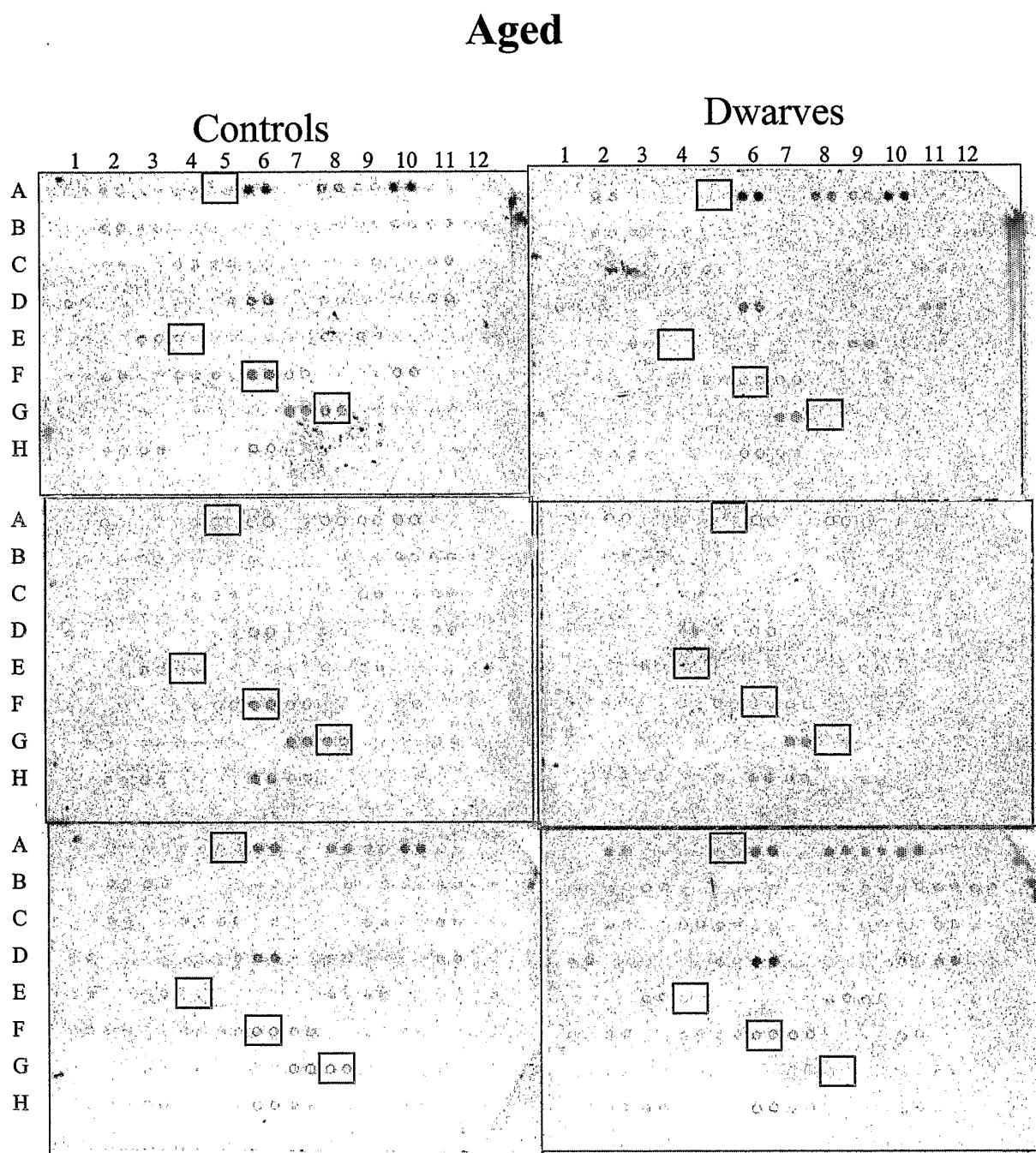


FIG. 6B

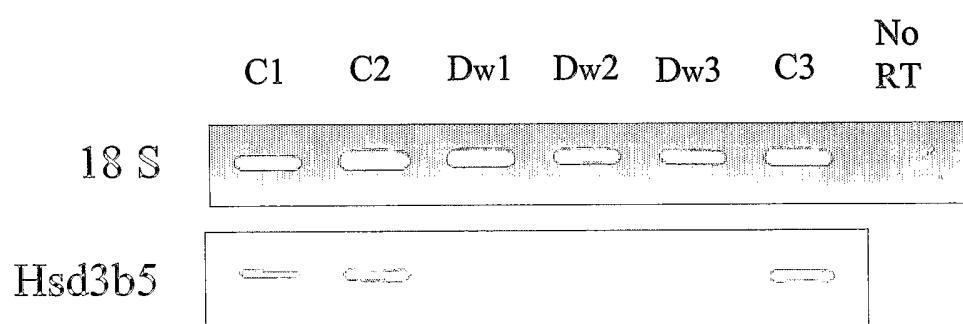
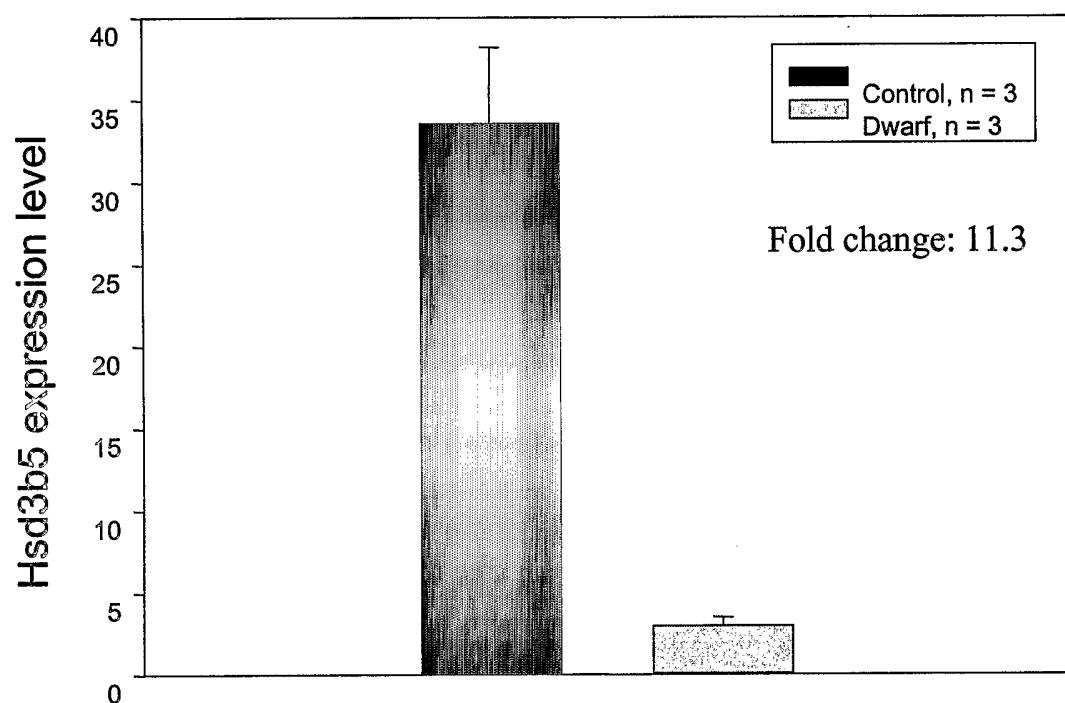
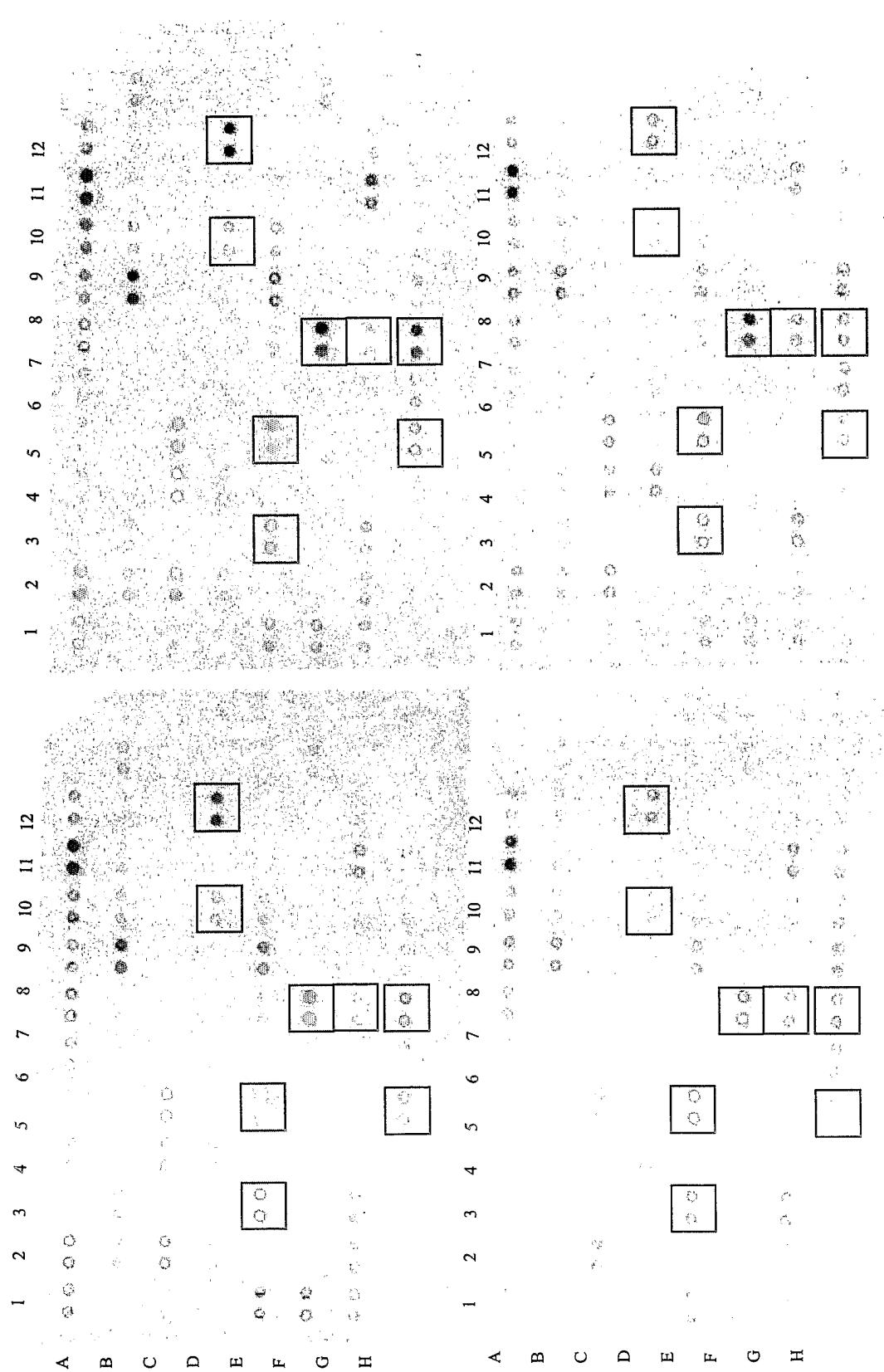
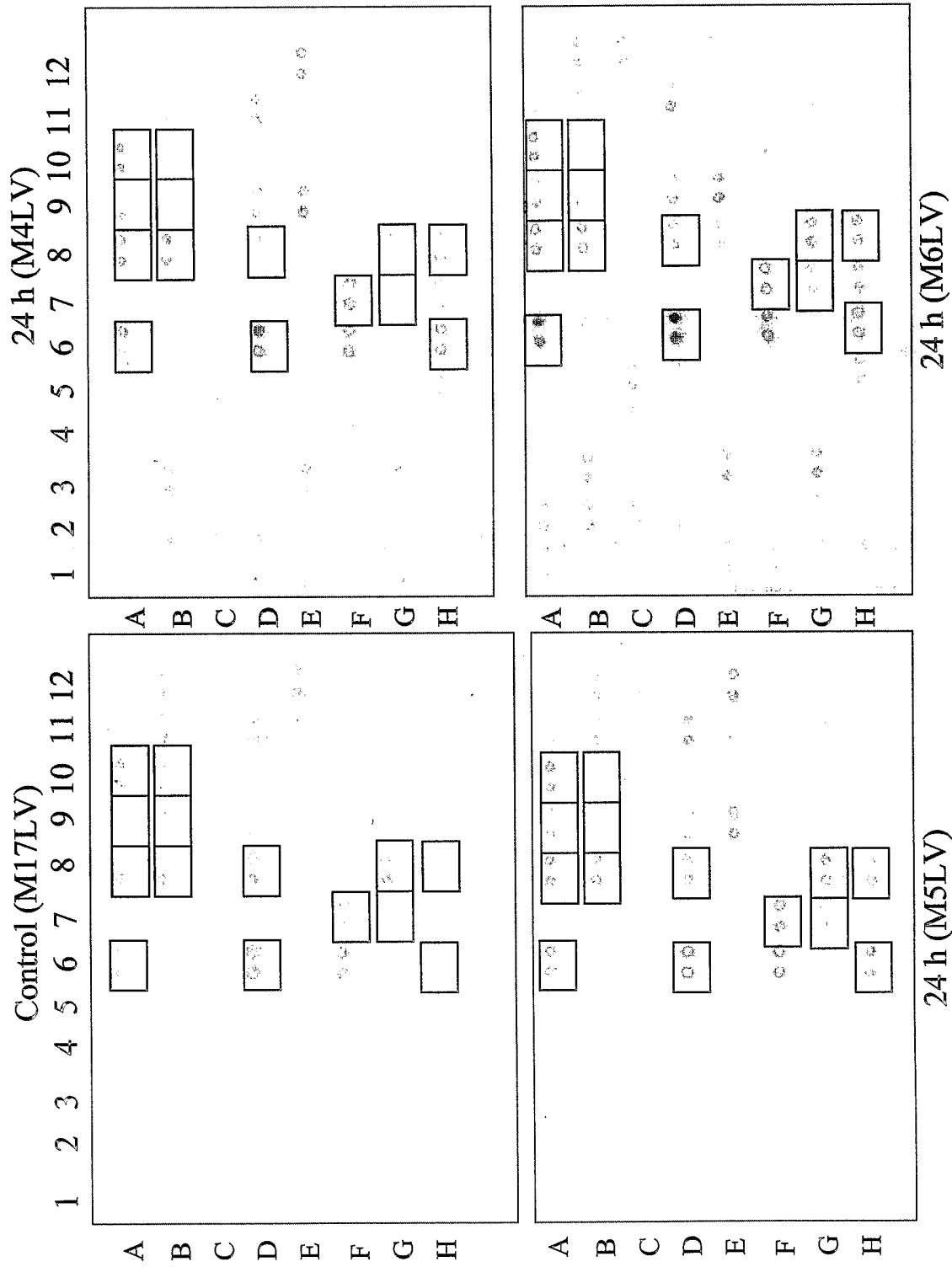


FIG. 6C

**FIG. 7A-7D**

**FIG. 8A-8D**

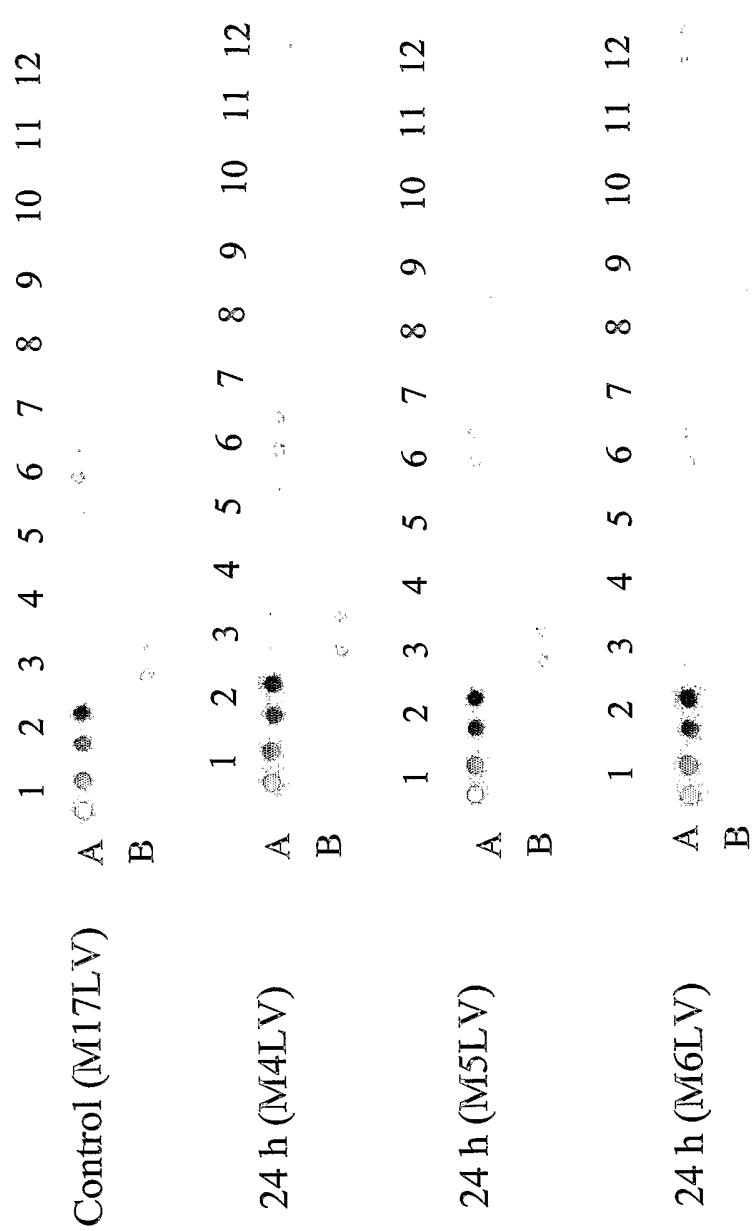


FIG. 9

SEQUENCE LISTING

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DEFORD, JAMES
GERSTNER, ARPAD

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<211> 1038
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<213> Mus musculus

<400> 2
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 <213> Mus musculus

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 catgcttttgc ttataatttt cttcatagta ataccaataa taattggagg ctttgaaac 240
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 ggagcaggaa caggatgaac agtctaccca cctctagccg gaaatccagt ccatgcagga 420
 gcatcagtag acctaacaat ttctccctt catttagctg gagtgcatac tatttttaggt 480
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<210> 4
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 <212> DNA
 <213> Mus musculus

<400> 4
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atcatctcgc taatattaac aacaaaacta acacatacaa gcacaataga tgcacaagaa 180
 gttgaaacca tttgaactat tctaccagct gtaatccta tcataattgc tctccctct 240
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 gggcaccaat gatactgaag ctacgaatat actgactatg aagacctatg ctttgattca 360
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 cgagtgcgttc tgccaataga acttccaatc cgtatattaa ttcatctga agacgtcctc 480
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 ggatctaacc atagcttat gcccattgtc ctagaaatgg ttccactaaa atattcgaa 660
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<210> 5
<211> 204
<212> DNA
<213> Mus musculus

<400> 5
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tcaactaaca ccataaaagt aaaaacccct tgagaattaa aatgaacgaa aatctatttg 180
cctcattcat tacccaaaca ataa 204

<210> 6
<211> 681
<212> DNA
<213> Mus musculus

<400> 6
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cgtctccatt ctttccaaaca ctgactagt aaacttatta tcaaacaat aatgctaatc 180
cacacaccaa aaggacgaac atgaacccta ataattgttt ccctaattcat atttatttgg 240
tcaacaaatc tccttaggcct ttaccacat acatttacac ctactaccctt actatccata 300
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attctagaat ttgcagtagc attaattcaa gcctacgtat tcaccctcct agtaagccta 660
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<210> 7
<211> 784
<212> DNA
<213> Mus musculus

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acactattaa cccttggcct actcaccaat atcctcacaat tatataatg atgacgagac 180
gtaattcggt aaggaaccta ccaaggccac cacactccta ttgtacaaaa aggactacga 240
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 cgacaactaa aatttcaatt cacatcaaaa catcaactcg gatttgaagc cgccagcatga 720
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<210> 8
<211> 345
<212> DNA
<213> Mus musculus

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gaccctacaa gctctgcacg tctaccatc tcaataaaaat ttttcttggt agcaattaca 180
tttcttattat ttgacctaga aattgcttctt ctacttccac taccatgagc aattcaaaca 240
attaaaaacct ctactataat aattatagcc tttattctag tcacaattct atctctaggc 300
ctagcatatg aatgaacaca aaaaggatta gaatgaacag agtaa 345

<210> 9
<211> 294
<212> DNA
<213> Mus musculus

<400> 9
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tttattataa cttcagtaac ttccctaaac tccaactcca taagctccat accaatcccc 180
atcaccttag ttttcgcagc ctgcgaagca gctgttaggac tagccctactt agtaaaagtt 240
tcaaacacgt acggaacaga ttacgtccaa aatctcaacc tactacaatg ctaa 294

<210> 10
<211> 1378
<212> DNA
<213> Mus musculus

<400> 10
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cttctatgac aaaccgacga aaattataaa aactttcaa atatattctc ctcagacccc 180
ctatccacac cattaattat ttaaacagcc tgattactgc cactaatattt aatagctagc 240
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<210> 11
 <211> 1824
 <212> DNA
 <213> Mus musculus

<400> 11
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<210> 12
 <211> 519
 <212> DNA
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<400> 12
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<210> 13
<211> 1144
<212> DNA
<213> Mus musculus

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ccat 1144