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

(57) Abrégé/Abstract:
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(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. 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 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, 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 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 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 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 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 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; 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 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 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 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 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, 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 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.

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 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 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 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.

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 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 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 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.

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.

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 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.

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.

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 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.

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 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 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

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 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 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, 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, 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.

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 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 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 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 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 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 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 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 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

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 that will affect mitochondrial biogenesis and assembly (replication) and mitochondrial function under any physiological or pathophysiological conditions.

Table 1

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
-	I48884	-	MITOP_D1	Deficiency of complex I
Abc7	U43892	ABAT	GABT_HUMAN	
Acadl	ACDL_MOUSE	ABC7	ABC7_HUMAN	X-linked sideroblastic anemia and ataxia (XLSA/A)
Acadm	A55724	ACAA2	S43440	
Acads	I49605	ACADL	A40559	LCAD deficiency
Acadvl	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	87978	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	
Atp5a1	JC1473	AKAP1	I39173	
Atp5b	P56480	AKAP84		
Atp5g1	AT91_MOUSE	AKAP84	I39173	
Atp5k	JC1412	AKAP1		
ATP5I		ALAS1	SYHUAL	
Atp7b	U38477	ALAS		

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
Bax	BAXA_MOUSE	ALAS2	SYHUAE	X-linked sideroblastic anemia (XLSA)
Bckdha	S71881	ASB		
Bckdhb	S39807	ALDH2	DEHUE2	Alcohol intolerance, acute
Bcl2	B25960	Hs.1230		
D1Nds7		ALDH4	PUT2_HUMAN	Hyperprolinemia, type II (HPII)
D1Nds7		ALDH5	A40872	
Bzrp	A53405	AMACR	CAB44062	Alpha-methylacyl-CoA racemase deficiency (AMACRD)
COII/ND5	ND5 I76673	AMT	I54192	Non-ketotic hyperglycinemia, type II (NKH2)
Car5	S12579	AOP1	TDXM_HUMAN	
Cbr2	A28053	ARG2	ARG2_HUMAN	
Ckmt1	S24612	ATP5A1	PWHUA	
Cox4	S12142	ATP5A2	NNN10	
Cox5a	S05495	ATP5AL1	NNN08	
Cox5b	A39425	ATP5AL2	NNN09	
Cox6a1	COXD_MOUSE	ATP5B	A33370	
Cox6a2	S52088	ATPSB		
Cox6b	107460	ATP5BL1	NNN06	
Cox6c2	S16083	ATP5BL2	NNN07	
Cox7a2	I48286	ATP5C1	A49108	
Cox7c1	S10303	ATP5C2	NNN03	
Cox7c	COXO_MOUSE	ATP5CL1	NNN04	
Cox8a	COXR_MOUSE	ATP5CL2	NNN05	
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	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
Cycs	CCMS	ATP5I	AB028624	
Cyct	CCMST	ATP5J	JT0563	
Cyp11a	88582	ATP5O	ATPO_HUMAN	
Cyp11b1	A41552	OSCOP		
Cyp11b2	88584	ATP7B	S40525	Wilson disease (WD)
Cyp24	S60033	BAX	BAXA_HUMAN	
Cyp27	88594	BCAT2	BCAM_HUMAN	
Dbt	S65760	BCKDHA	DEHUXA	Maple syrup urine disease (MSUD)
BCKADE2		BCKDHB	A37157	Maple syrup urine disease (MSUD)
Dci	S38770	BCL2	D37332	
Dia1	94893	BCL2L1	BCLX_HUMAN	
Dld	107450	BCLX		
Es9	95448	BCS1L	AF026849	
Etfa	106092	BDH	A42845	
Etfb	106098	BID	BID_HUMAN	
Etfdh	106100	BNIP3L	NIPL_HUMAN	
Fdx1	S53524	BZRP-S	A49361	
Fdxr	S60028	BZRP	I38105	
Fech	A37972	C14ORF2	68MP_HUMAN	
Fpgs	S65755	PLPM		
Frda	S75712	CA5	CRHU5	
Gcdh	GCDH_MOUSE	CACT	Y10319	Carnitine-acylcarnitine translocase deficiency
Gls	95752	CASQ1	A60424	
Glud	S16239	CGI-114	T14770	
Got2	S01174	CKMT1	A30789	
Hadh	JC4210	CKMT2	A35756	
Hccs	CCHL_MOUSE	CLPP	S68421	
Hk1	A35244	CLPX	CLPX_HUMAN	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
Hmgcl	HMGL_MOUSE	COQ7	AF032900	
Hmgcs2	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	
Maoa	I59594	COX7C	OSHU7C	
Maob	96916	COX7RP	O14548	
Mcs	A37199	COX8	OSHU8	
Mimt44	U69898	CPO	I52444	Hereditary coproporphyria (HCP)
Mod2	97045	CPS1	JQ1348	Hyperammonemia, type I
Mor1	DEMSMM	CPT1A	I59351	Carnitine O-palmitoyltransferase I deficiency
Mthfd	A33267	CPT1-L		
Mut	S08680	CPT1B	S70579	
Ndufa4	NUML_MOUSE	CPT2	A39018	Carnitine O-palmitoyltransferase II deficiency
Ndufs6	NUMM_MOUSE	CPT1		
Nnt	S54876	CRAT	A55720	Carnitine O-acetyltransferase deficiency
Oat	XNMSO	CS	AF047042	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
Ogdh	ODO1_MOUSE	CYB5	CBHU5	
Oias1	P11928	CYC1	S00680	
Oias2	P29080	Hs.697		
Otc	OWMS	CYP11A1	S14367	
Pcca	97499	CYP11A	A25922	
Pcx	A47255	CYP11B1	S11338	Adrenal hyperplasia, type IV (AH-IV)
Pdha1	S23506	CYP11B		
Pdhal	S23507	CYP11B2	B34181	Deficiency of corticosterone methyloxidase, type II (CMO)
Pla2g2a	I48342	CYP24	A47436	
Polg	DPOG_MOUSE	CYP27	A39740	Cerebrotendinous xanthomatosis (CTX)
Ppox	S68367	CYP3	A41581	
Rmrp	97937	DBT	A32422	Maple syrup urine disease (MSUD)
Rpl23	1196612	DCI	A55723	
Scp2	A40015	DECR	S53352	Deficiency of 2,4-dienoyl-CoA reductase
Slc1a1	EAT3_MOUSE	DFN1	U66035	Mohr-Tranebjaerg syndrome (MTS)
EAAC1		DGUOK	JC6142	
Sod2	I57023	DHODH	PC1219	
Star	A55455	DIA1	RDHUB5	
Surf	B25394	DLAT	XXHU	Dihydrolipoamide S-acetyltransferase deficiency;Leigh syndrome
Tfam	P97894	DLTA		
Tst	THTR_MOUSE	DLAT_h	S25665	
Ucp	A31106	DLD	DEHULP	Dihydrolipoamide dehydrogenase deficiency;Leigh syndrome
Ung	UNG_MOUSE	DLDH		
UNG1		LAD		
Vdac1	106919	DLST	PN0673	
Vdac2	106915	DMGDH	M2GD_HUMAN	Dimethylglycine dehydrogenase deficiency (DMGDHD)
Vdac3	106922	DUT	DUT_HUMAN	
Ywhaz	JC5384	ECGF1	P19971	Myoneurogastrointestinal encephalopathy syndrome (MNGIE)

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
mt-Atp6	PWMS6	ECHS1	ECHM_HUMAN	
MTATP6		EFE2	TFZ_HUMAN	Barth syndrome
mt-Atp8	PWMS8	EFTS-LSB	I84606	
mt-Co1	ODMS1	ENDOG	NUCG_HUMAN	
mt-Co2	OBMS2	ETFA	A31998	Glutaric aciduria, type IIa (GAIIa)
mt-Co3	OTMS3	ETFB	S32482	Glutaric aciduria, type IIb (GAIIb)
mt-Cytb	CBMS	ETFDH	Q16134	Glutaric aciduria, type IIc (GAIIc)
COB		FACL1	LCFA_HUMAN	
mt-Nd1	QXMS1M	FACL2	JX0202	
mt-Nd2	QXMS2M	FARS1	AF097441	
ND2		FDX1	AXHU	
mt-Nd3	QXMS3M	FDX		
ND3		FDXR	A40487	
mt-Nd4	QXMS4M	FECH	A36403	Erythropoietic protoporphyria (EPP)
ND4		FH	UFHUM	Deficiency of fumarate hydratase
mt-Nd4l	QXMS4L	FPGS	A46281	
mt-Nd5	QXMS5M	FRDA1	U43747	Friedreich ataxia 1
ND5		GAT	AF023466	
mt-Nd6	DEMSN6	GATM	S41734	
ND6		GCDH	GCDH_HUMAN	Glutaric aciduria, type I (GA-I)
mt-Rnr1	12S_rRNA	GCK	A46157	Diabetes mellitus, type II (NIDDM)
mt-Rnr2	16S_rRNA	HK4		
mt-Ta	tAla_1	HK4		
mt-Tc	tCys_1	Hs.1270		
mt-Td	tAsp_1	Hs.1270		
mt-Te	tGlu_1	NIDDM		
mt-Tf	tPhe_1	NIDDM		
mt-Tg	tGly_1	GCSH	GCHUH	Non-ketotic hyperglycinemia, type III (NKH3)

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
mt-Th	tHis_1	GK	GLPK_HUMAN	Glycerol kinase deficiency (GKD)
mt-Ti	tIle_1	GKP2	GKP2_HUMAN	
mt-Tk	tLys_1	GLDC	B39521	Non-ketotic hyperglycinemia, type I (NKH1)
mt-Tl1	tLeu_1	GLUD1	DEHUE	
mt-Tl2	tLeu_2	GLUDP1	A53719	
mt-Tm	tMet_1	GOT2	XNHUDM	
mt-Tn	tAsn_1	GPD2	GPDM_HUMAN	Diabetes mellitus, type II (NIDDM)
mt-Tp	tPro_1	GST12	B28083	
mt-Tq	tGln_1	HADHA	JC2108	Trifunctional enzyme deficiency; Maternal acute fatty liver of pregnancy (AFLP)
mt-Tr	tArg_1	HADHB	JC2109	Trifunctional enzyme deficiency
mt-Ts1	tSer_1	HCCS	G02133	
mt-Ts2	tSer_2	HCS	CCHU	
mt-Tt	tThr_1	HHH	AF112968	Deficiency of ornithine translocase
mt-Tv	tVal_1	HIBADH	D3HI_HUMAN	
mt-Tw	tTrp_1	HK1	A31869	
mt-Ty	tTyr_1	HK2	JC2025	Diabetes mellitus, type II (NIDDM)
		HLCS	BPL1_HUMAN	Biotin-responsive multiple carboxylase deficiency
		Hs.12357		
		HMGCL	A45470	Hydroxymethylglutaricaciduria (HMGCL)
		HMGCS2	S51103	
		HSD3B1	DEHUHS	Severe depletion of steroid formation
		HSDB3		
		HSD3B2	DEHUH2	Congenital adrenal hyperplasia (CAH)
		HSPA1L	B45871	
		HSPA9	B48127	
		GRP75		
		HSPD1	A32800	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		GROEL		
		HSPE1	S47532	
		CPN10		
		HTOM34P	Q15785	
		HTOM	AF026031	
		Hs.3816	A56650	
		IDH2	S57499	
		IDH3A	S55282	
		IDH3B	IDHB_HUMAN	
		IDH3G	IDHG_HUMAN	
		IVD	A37033	Isovaleric 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	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		MIPEP	U80034	
		MIP		
		MLN64	S60682	
		MMSDH	MMSA_HUMAN	Methylmalonate semialdehyde dehydrogenase deficiency (MMSDHD)
		MPO	OPHUM	Myeloperoxidase deficiency (MPOD)
		MRRF	AA085690	
		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 sidereoblastic anemia (AISA)
		MTCO2	OBHU2	Alzheimer disease (AD); Mitochondrial encephalomyopathies
		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	

CA 02519191 2005-07-13

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		MTHFD1	A31903	
		MTHFD		
		MTHFD2	DEHUMT	
		MTHSP1	NNN15	
		MTHSP2	NNN16	
		MTIF2	A55628	
		MTLSP	NNN02	
		MTND1	DNHUN1	Leber's hereditary optic neuropathy (LHON); Alzheimer disease and Parkinson disease (ADPD); Diabetes mellitus, type II (NIDDM)
		MTND2	DNHUN2	Leber's hereditary optic neuropathy (LHON); Alzheimer disease (AD)
		MTND3	DNHUN3	
		MTND4	DNHUN4	Leber's hereditary optic neuropathy (LHON);MELAS; Diabetes mellitus, type II (NIDDM)
		MTND4L	DNHUNL	Leber's hereditary optic neuropathy (LHON)
		MTND5	DNHUN5	Leber's hereditary optic neuropathy (LHON); MELAS
		MTND6	DEHUN6	Leber's hereditary optic neuropathy (LHON); LHON with dystonia (LDYT)
		MTOLR	NNN19	
		MTRF1	RF1M_HUMAN	
		MTTRF1		
		MTRNR1	12s_rRNA	Aminoglycoside-induced deafness; Nonsyndromic deafness
		MTRNR2	16S_rRNA	Chloramphenicol resistance; Alzheimer disease and Parkinson disease (ADPD)
		MTRNR3	NNN17	
		MTTA	TAla	Chronic tubulointerstitial nephropathy
		MTTC	TCys	Mitochondrial myopathy (MM)
		MTTD	TAsp	
		MTTE	TGlu	Myopathy and diabetes mellitus (MDM)
		MTTER	NNN18	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		MTTF	TPhe	MELAS
		MTTFH	NNN13	
		MTTFL	NNN14	
		MTTFX	NNN12	
		MTTFY	NNN11	
		MTTG	TGly	Hypertrophic cardiomyopathy; Progressive encephalopathy (PEM)
		MTTH	THis	
		MTTI	TIle	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)
		MTTL2	tLeu_b	CPEO plus; Mitochondrial myopathy (MM)
		MTTM	TMet	Mitochondrial myopathy (MM)
		MTTN	TAsn	Chronic progressive external ophthalmoplegia (CPEO)
		MTTP	TPro	Mitochondrial myopathy (MM)
		MTTQ	TGln	Alzheimer disease and Parkinson disease (ADPD)
		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 infantile mitochondrial myopathy (LIMM); Mitochondrial myopathy (MM)
		MTTV	TVal	Ataxia, progressive seizures, mental detorioration, and hearing loss
		MTTW	TTrp	Dementia and chorea (DEMCHO)

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		MTTY	TTyr	
		MTX1	MTXN_HUMAN	
		MTX2	AAC25105	
		MUT	S40622	Methylmalonic acidemia (MUT-, MUT0 type)
		MUTYH	U63329	
		NDUFA10	O95299	
		NDUFA1	O15239	
		NDUFA2	O43678	
		NDUFA3	O95167	
		NDUFA4	NUML_HUMAN	
		NDUFA5	NUFM_Human	
		NDUFA6	P56556	
		NDUFA7	AAD05427	
		NDUFA8	NUPM_HUMAN	
		NDUFAB1	T00741	
		NDUFB10	O96000	
		NDUFB1	O75438	
		NDUFB2	AAD05428	
		NDUFB3	O43676	
		NDUFB4	O95168	
		NDUFB5	O43674	
		NDUFB6	O95139	
		NDUFB7	NB8M_HUMAN	
		NDUFB8	JE0382	
		NDUFB9	S82655	
		B22		
		NDUFC1	O43677	
		NDUFC2	O95298	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		NDUFS1	S17854	
		NDUFS2	JE0193	
		NDUFS2L	NUEM_HUMAN	
		NDUFS3	O75489	
		NDUFS4	NUYM_HUMAN	
		NDUFS5	O43920	
		NDUFS6	O75380	
		NDUFS7	O75251	Leigh syndrome
		NDUFS8	NUIM_HUMAN	Leigh syndrome
		NDUFV1	A44362	Alexander disease;Leigh syndrome
		NDUFV2	A30113	
		NDUFV3	NUOM_HUMAN	
		NIFS	AAD09187	
		NME4	NDKM_HUMAN	
		NNT-PEN	G02257	
		NOC4	NP_006058	
		NRF1	A54868	
		NTHL1	AB001575	
		NTH1		
		OAT	XNHUO	Ornithinemia with gyrate atrophy (GA)
		OGDH	A38234	Deficiency of alpha-ketoglutarate dehydrogenase
		OGG1	U96710	
		OIAS	A91013	
		OPA1	T00336	Optic atrophy (OPA1)
		OTC	OWHU	Hyperammonemia, type II
		OXA1L	I38079	
		OXCT	SCOT_HUMAN	Deficiency of Succinyl-CoA:3-oxoacid-CoA transferase
		P43-LSB	I53499	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		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	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		POLG	G02750	
		Hs.1436		
		POLRMT	HSU75370	
		PPOX	PPOX_HUMAN	Porphyria variegata (VP)
		PRAX-1	AF039571	
		PRDX5	AAF03750	
		ACR1		
		AOEB166		
		PMP20		
		PRXV		
		PRSS15	S42366	
		LON-PEN		
		LON		
		PSORT	AAC05748	
		PYCR1	A41770	
		P5C		
		RMRP	HSMRP	
		RPL23L	RL23_HUMAN	
		RPL23		
		RPL3	R5HUL3	
		RPML12	RM12_HUMAN	
		RPML19	RLX1_HUMAN	
		KIAA0104		
		RPML37	AAF36155	
		RPML3	R5HUL3	
		RPMS12	RT12_HUMAN	
		SCHAD	JC4879	
		SCO2	AL021683	Fatal infantile cardioencephalomyopathy due to Cox deficiency

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		SCP2	B40407	
		SDH1	A34045	
		IP		
		SDH		
		SDH2	JX0336	Leigh syndrome;Deficiency of succinate dehydrogenase
		SDHC	D49737	Hereditary paraganglioma, type III (PGL3)
		SDHD	DHSD_HUMAN	Hereditary 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	

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		ANT3		
		SLC9A6	Q92581	
		KIAA0267		
		SMAC	NP_063940	
		SOD2	DSHUN	
		SPG7	Y16610	Hereditary spastic paraplegia (HSP)
		SSBP	JN0568	
		STAR	I38896	Congenital lipoid adrenal hyperplasia
		SUCLA2	AF058953	
		SUCLG1	P53597	
		SUCLG2	T08812	
		SUOX	S55874	Sulfocysteinuria
		SUPV3L1	S63453	
		SURF1	S57749	Leigh syndrome
		SerRSmt	AB029948	
		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	OPHUIT	Iodide peroxidase deficiency (IPD)

Mus musculus Gene List		*Homo sapien* Gene List and Related Diseases		
gene	**Accession**	**gene**	**Accession**	**Related Disease**
		TR	THI2_HUMAN	
		TR3		
		TST	ROHU	
		TUFM	S62767	
		UCP1	A60793	
		UCP2	UCP2_HUMAN	
		UCP3	JC5522	
		UCP4	UCP4_HUMAN	
		UNG	A60472	
		DGU		
		UDG		
		UQCRB	A32450	
		UQBP		
		UQCRC1	A48043	
		UQCRC2	A32629	
		UQCRFS1	UCRI_HUMAN	Mitochondrial myopathy (MM)
		UQCRH	S00219	
		UROS	A40483	
		VDAC1	MMHUP3	
		VDAC2	B44422	
		VDAC3	S59547	
		VDAC4	Q36732	
		WARS2	AA227572	
		WFS	Y18064	DIDMOAD
		YME1L1	AJ132637	
		YWHAE	143E_HUMAN	
		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 H_2O 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 $\Delta\psi$ or ATP tend to activate the mtPTP an 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

Mus musculus			*Homo sapiens*		
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 sulfhydryl-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,™ 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.BIND™ 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 (Tm) - 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 Tms in the laboratory are frequently used due to ease of measurement. Routine comparisons using Tm 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 1μm 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 hybrdization 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.*, flourescein), 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 know 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

A	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	86	87	88	89	90	91	92	93	94	95	96

real number PCR	spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
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

CA 02513191 2005-07-13

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 oxydase 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 acetylase)
28	26	Cycs	CCMS	Cytochrome C, somatic
31	27	Dbt	S65760	Dihydrolipoamide transacylase 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	Ferredoxin--NADP+ reductase precursor
124	32	Nrf1	NM_010938	Nuclear respiratory factor
37	33	Fpgs	S65755	Tetrahydrofolylpolyglutamate synthase precursor
38	34	Frda	S75712	Friedreich ataxia

real number PCR	spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
39	35	Gcdh	GCDH_MOUSE	Glutaryl-CoA dehydrogenase precursor (GCD)
40	36	Glud	S16239	Glutamate dehydrogenase (NAD(P)+) precursor
41	37	Got2	S01174	Glutamate oxaloacetaate 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	Hydroxymethylglutaryl-CoA lyase
46	42	Hmgcs2	B55729	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	U69898	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 precuresor
65	60	Pcx	A47255	Pyruvate carboxylase

real number PCR	spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
66	61	Pdha1	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	Skd3		
93(non-Mitop)	78		L00923	Myosin 1
94	79	GAPDH	M32599	Glyceraldehyde 3-phosphate dehydrogenase (G3PDH)
108(non-Mitop)	80	Hsd3b5	L41519	3-keytosteroid 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 PCR	spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
Mito13 95	84	mt-Nd1	QXMS1M	Protein 1 (NADH dehydrogenase (ubiquinone) chain 1)
" 96	85	mt-Nd2	QXMS2M	Protein 2 (NADH dehydrogenase (ubiquinone) chain 2)
" 97	86	mt-Co1	ODMS1	Cytochrome c oxidase subunit I
" 98	87	mt-Co2	OBMS2	Cytochrome c oxidase subunit II
" 99	88	mt-Atp8	PWMS8	Protein A6l (H+-transporting ATP synthase protein 8)
" 100	89	mt-Atp6	PWMS6	ATPase 6 (H+-transporting ATP synthase protein 6)
" 101	90	mt-Co3	OTMS3	Cytochrome c oxidase subunit III
" 102	91	mt-Nd3	QXMS3M	Protein 3 (NADH dehydrogenase (ubiquinone) chain 3)
" 103	92	mt-Nd4l	QXMS4L	Protein 4L (NADH dehydrogenase (ubiquinone) chain 4L)
" 104	93	mt-Nd4	QXMS4M	Protein 4 (NADH dehydrogenase (ubiquinone) chain 4)
" 105	94	mt-Nd5	QXMS5M	Protein 5 (NADH dehydrogenase (ubiquinone) chain 5)
" 106	95	mt-Nd6	DEMSN6	Protein 6 (NADH dehydrogenase (ubiquinone) chain 6
" 107	96	mt-Cytb	CBMS	Cytochrome b (ubiquinol--cytochrome c reductase subunit III)

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 B	1 13	2 14	3 15	4 16	5 17	6	7	8	9	10	11	12

1	mt-Rnr1	12S_rRNA	12S rRNA
2	mt-Rnr2	16S_rRNA	16S rRNA
3	mt-Nd1	QXMS1M	Protein 1 (NADH dehydrogenase (ubiquinone) chain 1)
4	mt-Nd2	QXMS2M	Protein 2 (NADH dehydrogenase (ubiquinone) chain 2)
5	mt-Co1	ODMS1	cytochrome c oxidase subunit I
6	mt-Co2	OBMS2	cytochrome c oxidase subunit II
7	mt-Atp8	PWMS8	Protein A6l (H+-transporting ATP synthase protein 8)
8	mt-Atp6	PWMS6	ATPase 6 (H+-transporting ATP synthase protein 6)
9	mt-Co3	OTMS3	cytochrome c oxidase subunit III
10	mt-Nd3	QXMS3M	Protein 3 (NADH dehydrogenase (ubiquinone) chain 3)
11	mt-Nd4l	QXMS4L	Protein 4L (NADH dehydrogenase (ubiquinone) chain 4L)
12	mt-Nd4	QXMS4M	Protein 4 (NADH dehydrogenase (ubiquinone) chain 4)
13	mt-Nd5	QXMS5M	Protein 5 (NADH dehydrogenase (ubiquinone) chain 5)
14	mt-Nd6	DEMSN6	Protein 6 (NADH dehydrogenase (ubiquinone) chain 6
15	mt-Cytb	CBMS	Cytochrome b (ubiquinol--cytochrome c reductase subunit III)
16	GAPDH	M32599	Glyceraldehyde 3-phosphate dehydrogenase (G3PDH)
17	ß-actin	X03672	beta-actin

WO 2004/066823 PCT/US2004/002535 CA 02513191 2005-07-13

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). 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

A	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	86	87	88	89	90	91	92	93	94	95	96

spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM 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-aminolevulinate 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	S39807	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 VIII
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	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 oxaloacetaate transaminase-2
38	Hadh	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	carbonyl 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 carbamoyltransferase precursor
60	Pcx	A47255	pyruvate carboxylase
61	Pdha1	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 CII-3
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		

spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM each primer
77	Skd3		
78		L00923	Myosin 1
79	GAPDH	M32599	Glyceraldehyde 3-phosphate dehydrogenase (G3PDH)
80	Hsd3b5	L41519	3-keytosteroid 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	Atp5k	S52977	ATP snythase H+ transporting chain e
92	ß-actin	X03672	beta-actin
93		M10624	Murine ornithine decarboxylase (MOD)
94	Tom40		Mitochondrial outer membrane protein
95	Gpam		Glycerol-3-phosphate acyltransferase
96	sdhd	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.

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

A	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	86	87	88	89	90	91	92	93	94	95	96

Spot #	Gene name	Mitop/genbank	Description 10 ng/spot, 0.1μM 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-aminolevulinate 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	S39807	3-methyl-2-oxobutanoate dehydrogenase (lipoamide)
16	Bcl2	B25960	Transforming protein bcl-2-beta
17	Bzrp	A53405	Peripheral-type benzodiazepine receptor 1
18	Car5	S12579	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	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	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 oxaloacetaate transaminase-2
38	Hadh	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)
47	Hsp74	A48127	Heat shock protein 70 precursor
48	HspE1	A55075	Chaperonin-10

CA 02513191 2005-07-13

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	Carbonyl 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 carbamoyltransferase precursor
60	Pcx	A47255	Pyruvate carboxylase
61	Pdha1	S23506	Pyruvate dehydrogenase (lipoamide)
62	Pdhal	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		
78		L00923	Myosin 1

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-keytosteroid 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	Atp5k	S52977	ATP snythase H+ transporting chain e
92	ß-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 butyrly-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

A	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	β-action	HPRT	MYOSIN	PPLA2

Plate 2

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	β-actin	HPRT	MYOSIN	PPLA2

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	ACAA.1	D16294	3-oxoacyl-CoA thiolase	
2	ACADL	M74096	long-chain-acyl-CoA dehydrogenase (LCAD)	LCAD deficiency
3	ACADM	AF251043	acyl-CoA dehydrogenase precurser, 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 c.elegans)	
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) cytochrome P450e	
60	CYP3	NM_005729	peptidylprolyl isomerase 3 precursor	
61	DBT	X66785	dihydrolipoamide S-(2-methylpropanoyl) transferase precursoror	Maple syrup urine disease (MSUD)
62	DCI	Z25820	dodecenoyl-CoA δ-isomerase precursor	
63	DECR	XM_005309	2,4-dienoyl-CoA reductase precursor	Deficiency of 2,4-dienoyl-CoA reductase
64	DFN1	U66035	deafness dystonia protein	Mohr-Tranebjaerg syndrome (MTS)
65	DIA1	XM_010028	cytochrome-b5 reductase	
66	DLAT_h	X13822	dihydrolipoamide S-acetyltransferase heart	
67	DLD	J03620	dihydrolipoamide dehydrogenase precursor	Dihydrolipoamide dehydrogenase deficiency; Leigh syndrome
68	DLST	XM_012353	dihydrolipoamide S-succinyltransferase	
69	ECGF1	M63193	thymidine phosphorylase precursor (TDRPASE)	Myoneurogastrointestinal encephalopathy syndrome (MNGIE)

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 (GAIIa)
75	ETFDH	NM_004453	electron transfer flavoprotein dehydrogenase	Glutaric aciduria, type IIc (GAIIc)
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)	Glutaric aciduria, type I (GA-I)
81	GCSH	XM_010661	glycine cleavage system protein H precursor	Non-ketotic hyperglycinemia, type III (NKH3)
82	GK	XM_010221	glycerol kinase (ATP: glycerol 3 - phosphotransferase)	Glycerol kinase deficiency (GKD)
83	GLDC	XM_011805	glycine dehydrogenase (decarboxylating) precursor	Non-ketotic hyperglycinemia, type I (NKH1)
84	GLUD1	X07769	glutamate dehydrogenase (NAD(P)+) precursor	
85	GOT2	M22632	aspartate transaminase precursor	
86	GPD2	XM_002442	glycerol-3-phosphate dehydrogenase	Diabetes mellitus, type II (NIDDM)
87	GST12	J03746	glutathione transferase, microsomal	

CA 02513191 2005-07-13

Spot No.	Gene Name	Accession No.	Description	Related Disease
88	HADHA	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) human) human	
91	HK1	X66957	hexokinase I	
92	HK2	NM_000189	hexokinase II	Diabetes mellitus, type II (NIDDM)
93	HLCS	XM_009757	biotin--[methylmalonyl-CoA-carboxyltransferase] ligase	Biotin-responsive multiple carboxylase deficiency
94	HMGCL	L07033	hydroxymethylglutaryl-CoA lyase	Hydroxymethylglutaricaciduria (HMGCL)
95	HSD3B1	M27137	3-beta hydroxy-5-ene steroid dehydrogenase type I	Severe depletion of steroid formation
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 translocase	
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 precursorursor	

Spot No.	Gene Name	Accession No.	Description	Related Disease
104	IDH3B	U49283	isocitrate dehydrogenase (NAD), mitochondrial subunit β	
105	IDH3G	Z68907	isocitrate dehydrogenase (NAD), mitochondrial subunit γ	
106	IVD	M34192	isovaleryl-CoA dehydrogenase precursor	Isovaleric acidemia (IVA)
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	
112	MAOA	M68840	amine oxidase (flavin-containing) A	Brunner's syndrome
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	MTERF	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	translation 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 precursror (MCM)	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	OIAS	X02874	(2'-5') oligoadenylate synthetase E16	
172	OPA1	XM_039926	Optic atrophy 1 protein, KIAA0567	Optic atrophy (OPA1)
173	OTC	K02100	ornithine carbamoyltransferase precursor	Hyperammonemia, type II
174	OXA1L	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	
177	PCCA	X14608	propionyl-CoA carboxylase α chain precursor	Propionic acidemia, type I (PA-1)
178	PCCB	XM_051992	propionyl-CoA carboxylase β chain precursor	Propionic acidemia, type II (PA-2)
179	PCK2	S69546	phosphoenolpyruvate carboxykinase (GTP) precursor	Hypoglycemia and liver impairment
180	PC	U04641	pyruvate carboxylase precursor	Deficiency of pyruvate carboxylase, type I and II
181	PDHA1	J03503	pyruvate dehydrogenase (lipoamide) α chain precursor	Pyruvate dehydrogenase deficiency; Leigh syndrome

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, exonIIIA)	
191	PLA2G2A	M22430	phospholipase A2, group IIA, 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 S. cerevisiae	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)
212	SDHD	AB006202	succinate dehydrogenase (ubiquinone) cytochrome b small subunit	Hereditary paraganglioma, type I (PGL1)
213	SerRSmt	AB029948	seryl-tRNA synthetase	
214	SHMT2	NM_005412	glycine hydroxymethyltransferase precursor	
215	SLC20A3	U25147	tricarboxylate transport protein precursor	
216	SLC25A12	Y14494	mitochondrial carrier protein aralar 1	
217	SLC25A16	M31659	mitochondrial solute carrier protein homolog	
218	SLC25A18	AY008285	solute carrier SLC25A18	
219	SLC9A6	AF030409	sodium/hydrogen exchanger 6 (Na(+)H(+) exchanger	
220	SOD2	X14322	superoxide dismutase (Mn) precursor	
221	SSBP	M94556	single-stranded mitochondrial DNA-binding protein precursor	
222	SUCLA2	XM_012310	succinyl-CoA ligase (ADP_forming), β-chain precursor	
223	SUCLG1	NM_003849	succinyl-CoA ligase (GDP_forming), α-chain precursor	
224	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 (HTID-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	UQCRFS1	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	Aminoglycoside-induced deafness;Nonsyndromic deafness Chloramphenicol resistance;Alzheimer disease and Parkinson disease (ADPD)
15	MTRNR2	J01415	mitochondrial ribosomal RNA, 16S	

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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 by reference.

U.S. Patent 4,683,195

U.S. Patent 4,683,202

U.S. Patent 4,800,159

U.S. Patent 4,883,750

U.S. Patent 5,143,854

U.S. Patent 5,578,832

U.S. Patent 5,837,832

U.S. Patent 5,837,860

U.S. Patent 5,861,242

Ames *et al., Proc. Natl. Acad. Sci. USA* 90: 7915-7922, 1993.

Anderson *et al., Nature,* 290:457-465, 1981.

Ballinger *et al., Cir. Res.,* 86:960-966, 2000.

Beckman and Ames, *Physiol. Rev.,* 78:547-581, 1998.

Beckman and Ames, *Mutat. Res.,* 424:51-58, 1999.

British Appl. GB 2,202,328

Corral-Debrinski *et al., Genomics*, 23:471-476, 1994.

Corral-Debrinski *et al., JAMA*, 266:1812-1816, 1991.

Corral-Debrinski *et al., Mutat. Res.*, 275:169-180, 1992a.

Corral-Debrinski *et al., Nat. Genet.*, 2:324-329, 1992b.

European Appl. 329 822

European Appl. 320,308

Finkel and Holbrook, *Nature,* 408:239-247, 2000.

Fodor *et al., Biochemistry*, 30(33):8102-8108, 1991.

Frohman, In: *PCR Protocols: A Guide To Methods And Applications*, Academic Press, N.Y., 1990.

Golden and Melov, *Mech. Aging Dev.*, 122:1577-1589, 2001.

Hacia *et al.*, *Nature Genet.,* 14:441-449, 1996.

Harmon, *Am. J. Geriatric Soc.*, 20:145-147, 1972.

Horton *et al.*, *Neurology,* 45:1879-188, 1995.

Hsieh *et al.*, *Mech. Age Dev.,* 123:1423-1435, 2002.

Innis *et al.*, *Proc. Natl. Acad. Sci. USA,* 85(24):9436-9440, 1988.

Jun *et al.*, *Mol. Cell. Biol.*, 16:771-777, 1996.

Jun *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:6206-6210, 1994.

Kwoh *et al.*, *Proc. Nat. Acad. Sci. USA*, 86: 1173, 1989.

Mandavilli *et al.*, *Mutation Res.,* 509:127-151, 2002.

Melov *et al.*, *Nucleic Acids Res.*, 23:4122-4126, 1995.

Melov *et al.*, *Nucleic Acids Res.*, 25:974-982, 1997.

Mitochondria and Free radicals In: *Neurodegenerative Diseases*, Beal *et al.* (Eds.), Wiley-Liss, NY, 1997.

Newton *et al.*, *Nucl. Acids Res.*, 21:1155-1162, 1993.

Ohara *et al.*, *Proc. Natl. Acad. Sci.* USA, 86:5673-5677, 1989.

PCT Appl. PCT/US87/00880

PCT Appl. PCT/US89/01025

PCT Appl. WO 88/10315

PCT Appl. WO 89/06700

PCT Appl. WO 89/11548

PCT Appl. WO 90/15070

PCT Appl. WO 92/10092

Pease *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:5022-5026, 1994.

Rasmussen *et al.*, *Anal. Biochem.*, 198:138-142, 1991.

Running *et al.*, *BioTechniques,* 8:276-277, 1990.

Saito *et al.*, *J. Biol. Chem.*, 276:29307-29312, 2001.

Sambrook *et al.*, *In: Molecular cloning,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

Sambrook *et al.*, *In: Molecular cloning,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001.

Santos *et al., Methods Mol. Biol.,* 197:159-176, 2002

Shoemaker *et al., Nature Genetics,* 14:450-456, 1996.

Shoffner *et al., Cell*, 61:931-937, 1990.

Shoffner *et al., Genomics*, 17:171-184, 1993.

Shoffner *et al., Proc. Natl. Acad. Sci. USA*, 86:7952-7956, 1989.

Ventura, B., *et al., Biochim. Biophys. Acta,* 1553:249-260, 2002.

Walker *et al., Proc. Natl. Acad. Sci. USA,* 89:392-396 1992.

Wallace *et al., Cell*, 55:601-610, 1988.

Wallace, *Science,* 283:1482-1488, 1999.

Waterston *et al., Nature,* 420, 520-562, 2002.

Wu *et al., Genomics*, 4:560-569, 1989.

Zhang *et al ., FEBS Lett.,* 297:34-38, 1992.

SEQUENCE LISTING

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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.

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.

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.

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.

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.

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.

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.

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.

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. 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.

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.

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.

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 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 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.

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.

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

b) detecting the amount of mRNA or complements thereof hybridizing to mitochondrial-related nucleic acid sequences or complements thereof.

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.

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:

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.

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.

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:

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;

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.

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.

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:

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;

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.

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.

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.

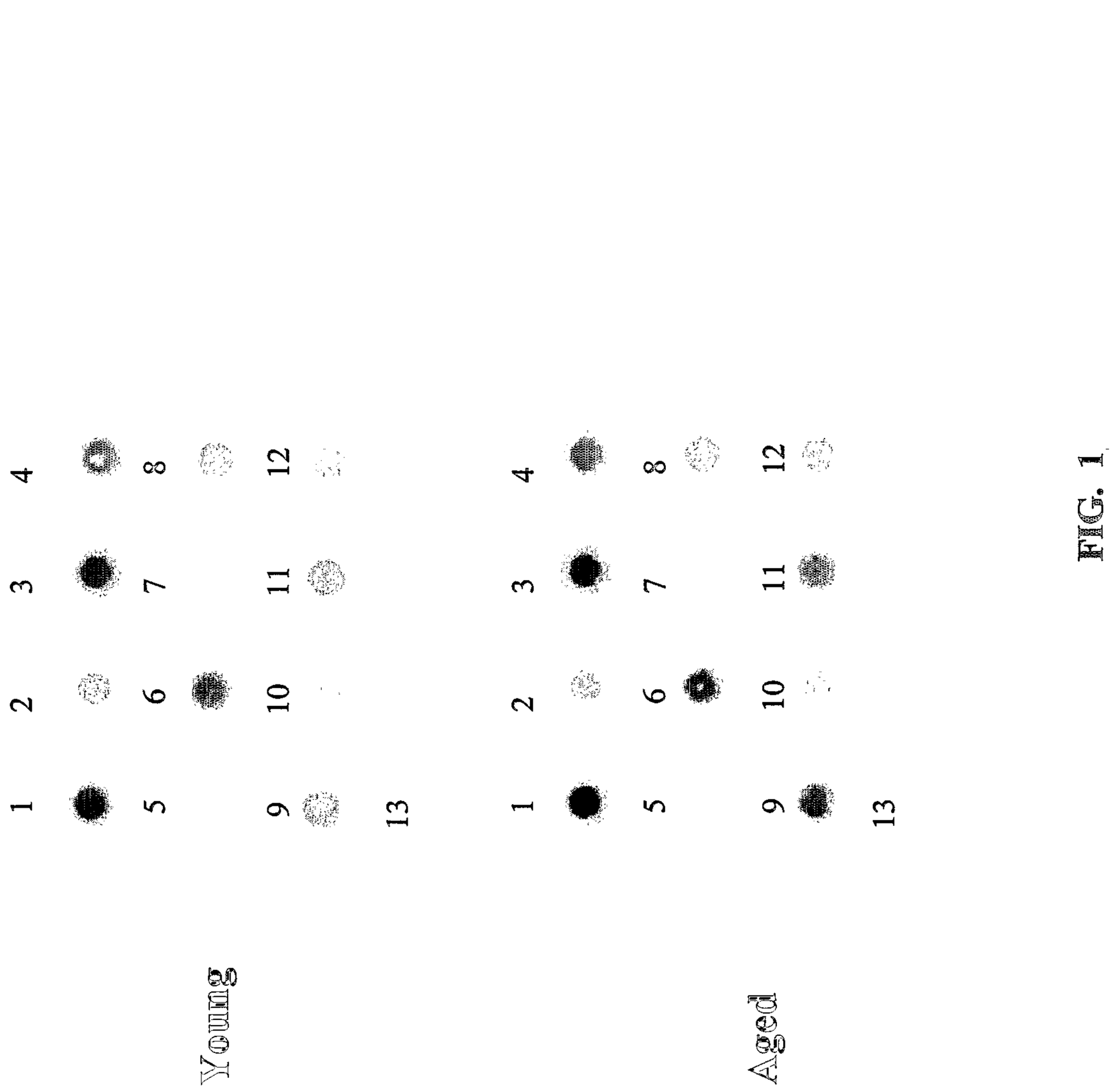


FIG. 1

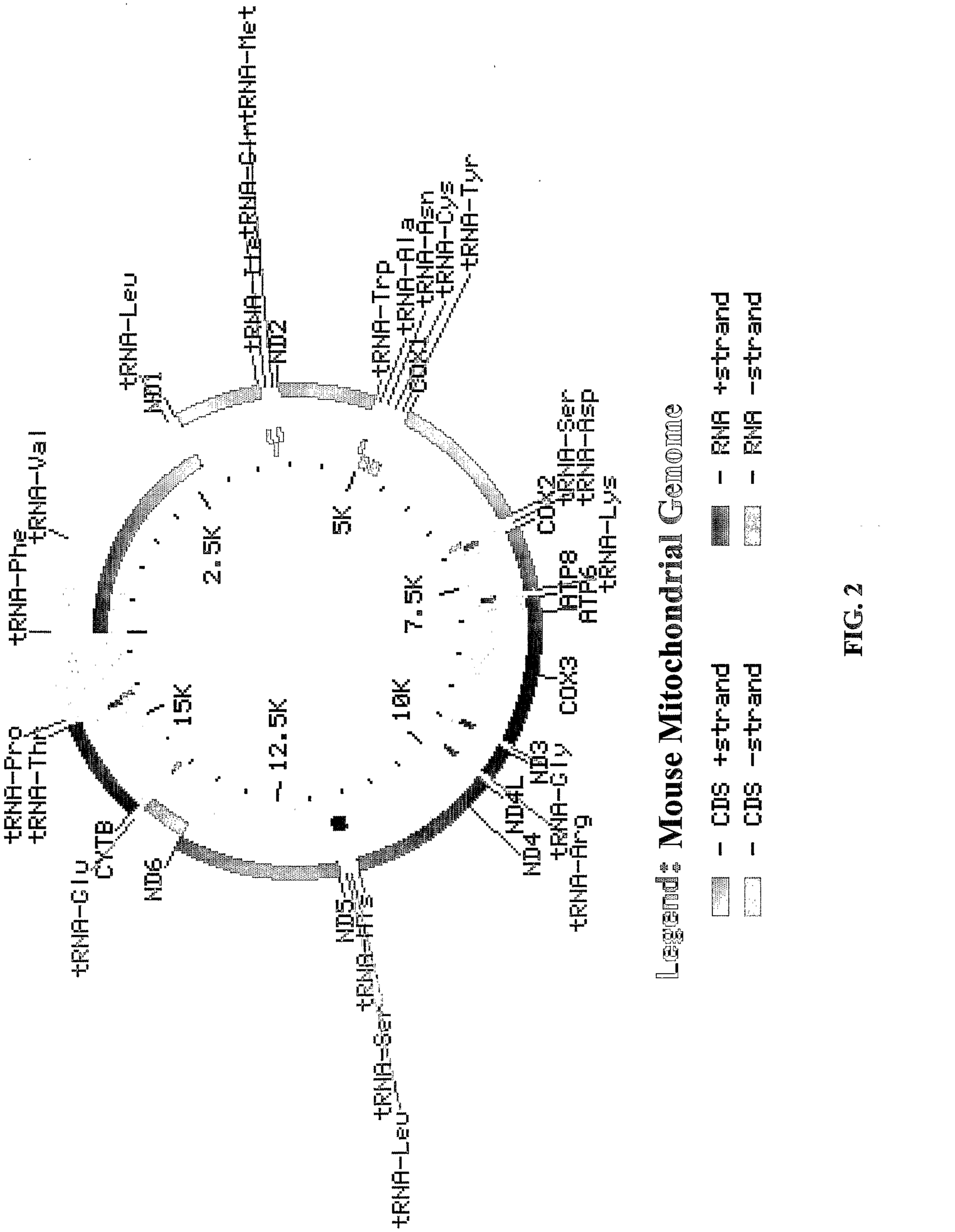

Legend: Mouse Mitochondrial Genome
+ CDS +strand
- CDS -strand
+ RNA +strand
- RNA -strand
2.5K
5K
7.5K
10K
12.5K
15K
tRNA-Phe
tRNA-Val
tRNA-Leu
MD1
tRNA-Ile
tRNA-Gln
tRNA-Met
ND2
tRNA-Trp
tRNA-Ala
tRNA-Asn
tRNA-Cys
tRNA-Tyr
COX1
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tRNA-Asp
ATP8
ATP6
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COX3
tRNA-Gly
ND3
ND4L
ND4
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CYTB
tRNA-Thr
tRNA-Pro

FIG. 2

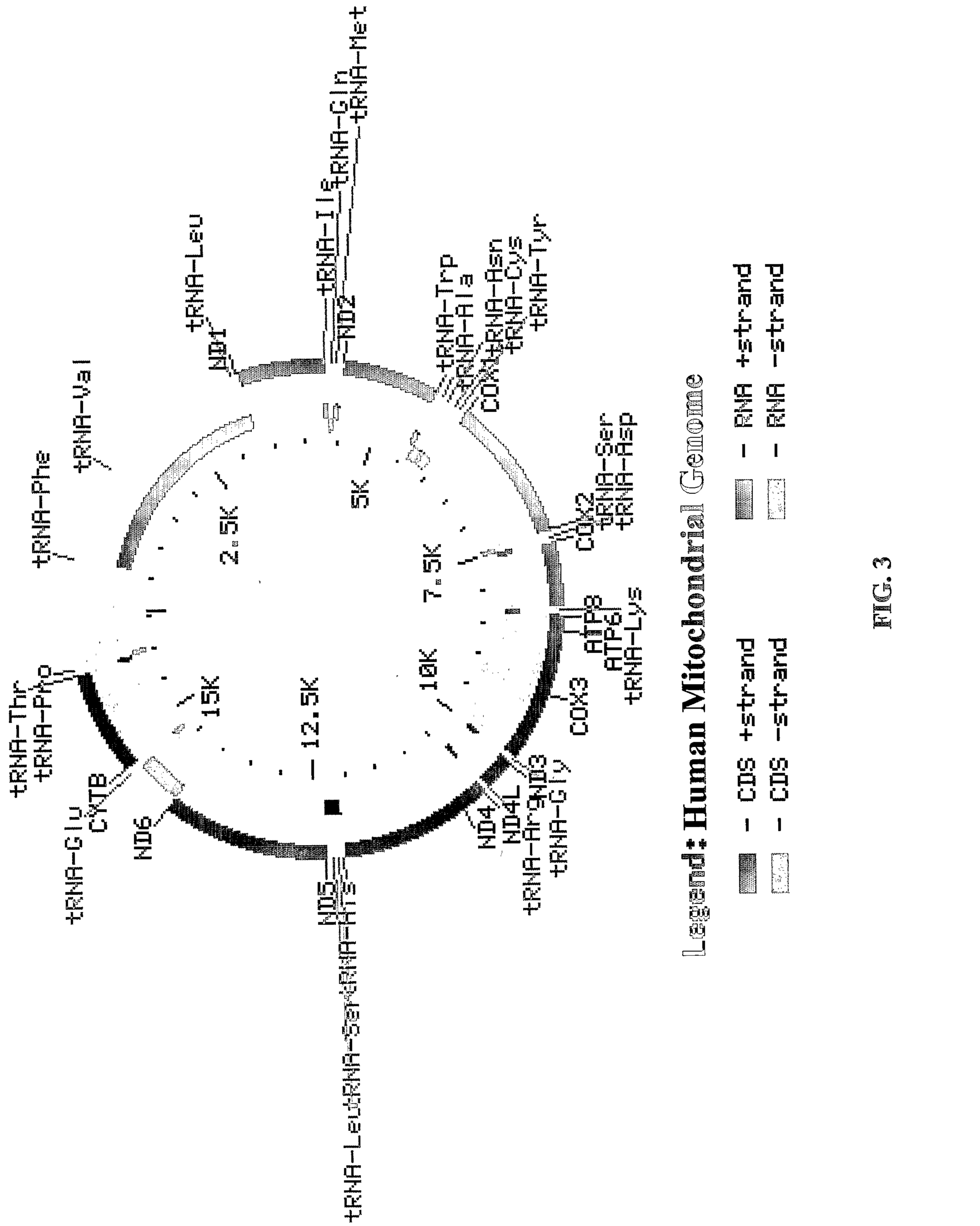
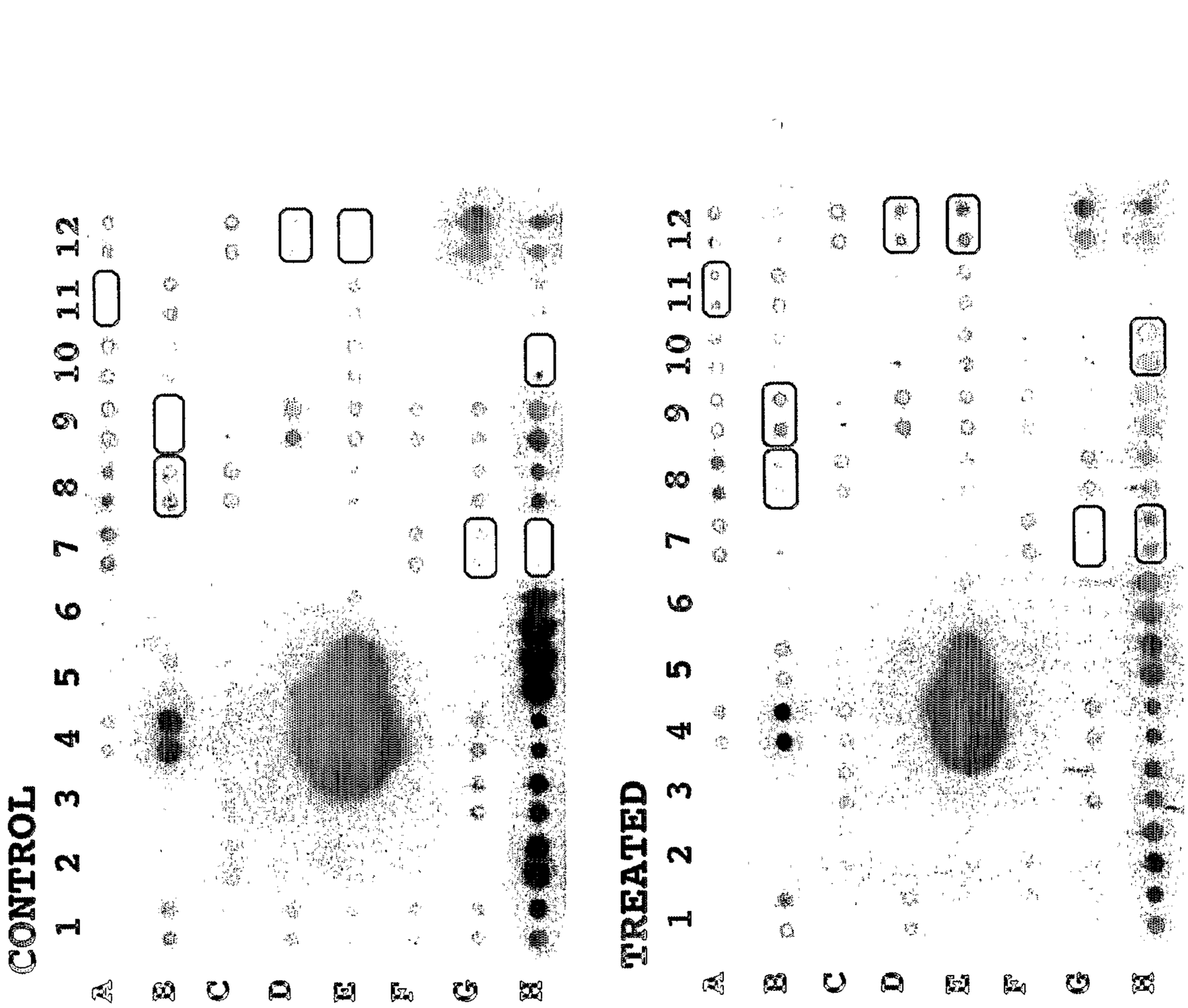

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tRNA-Val
tRNA-Leu
NDI
ND2
tRNA-Ile
tRNA-Gln
tRNA-Met
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tRNA-Asn
tRNA-Cys
tRNA-Tyr
COX1
COX2
tRNA-Ser
tRNA-Asp
2.5K
5K
7.5K
10K
12.5K
15K
tRNA-Thr
tRNA-Pro
tRNA-Glu
CYTB
ND6
ND5
tRNA-His
tRNA-Arg
ND4
ND4L
ND3
tRNA-Gly
COX3
ATP8
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Legend: Human Mitochondrial Genome
+ RNA +strand
+ RNA -strand
+ CDS +strand
+ CDS -strand

FIG. 3

FIG. 4



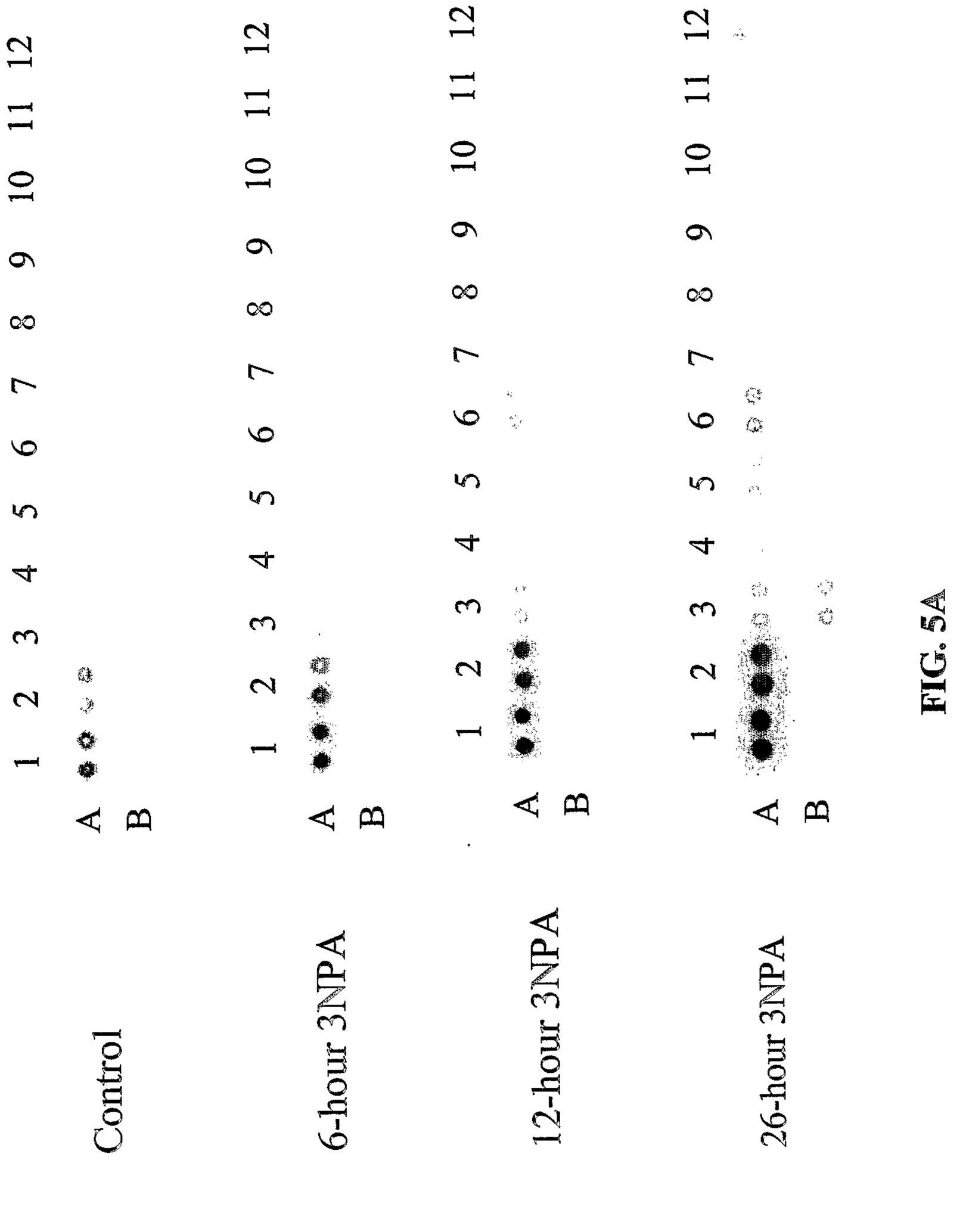

Control
6-hour 3NPA
12-hour 3NPA
26-hour 3NPA
A
B
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 5A

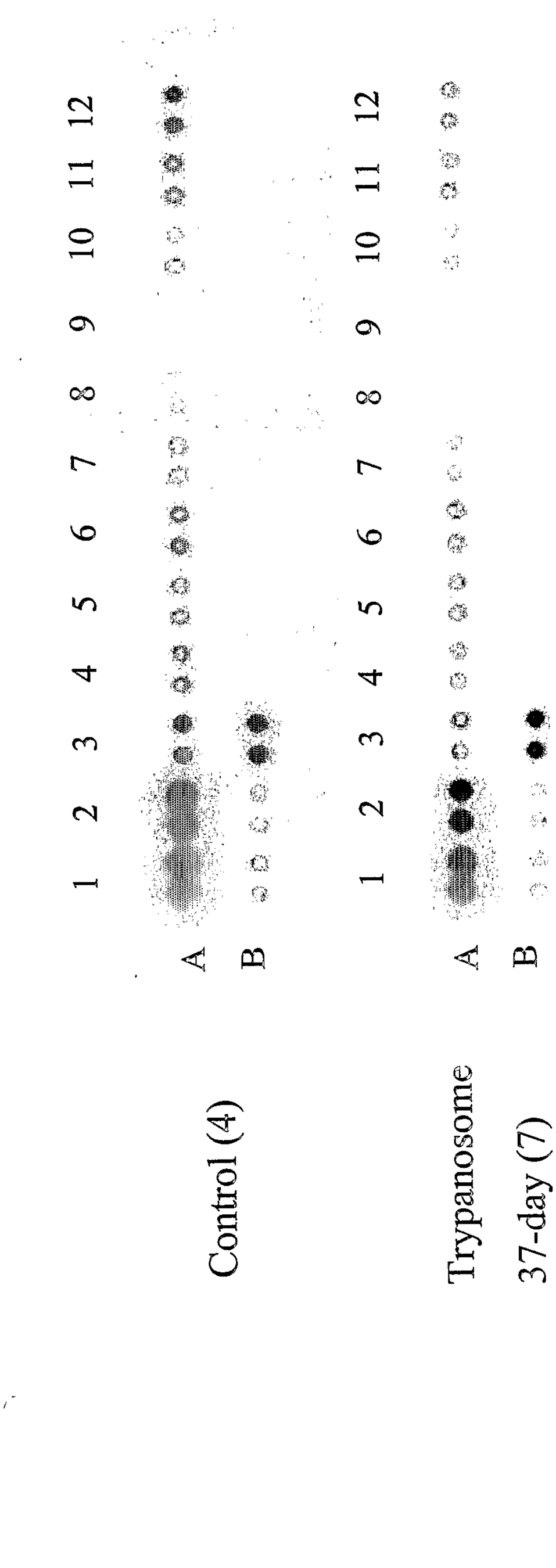


FIG. 5B

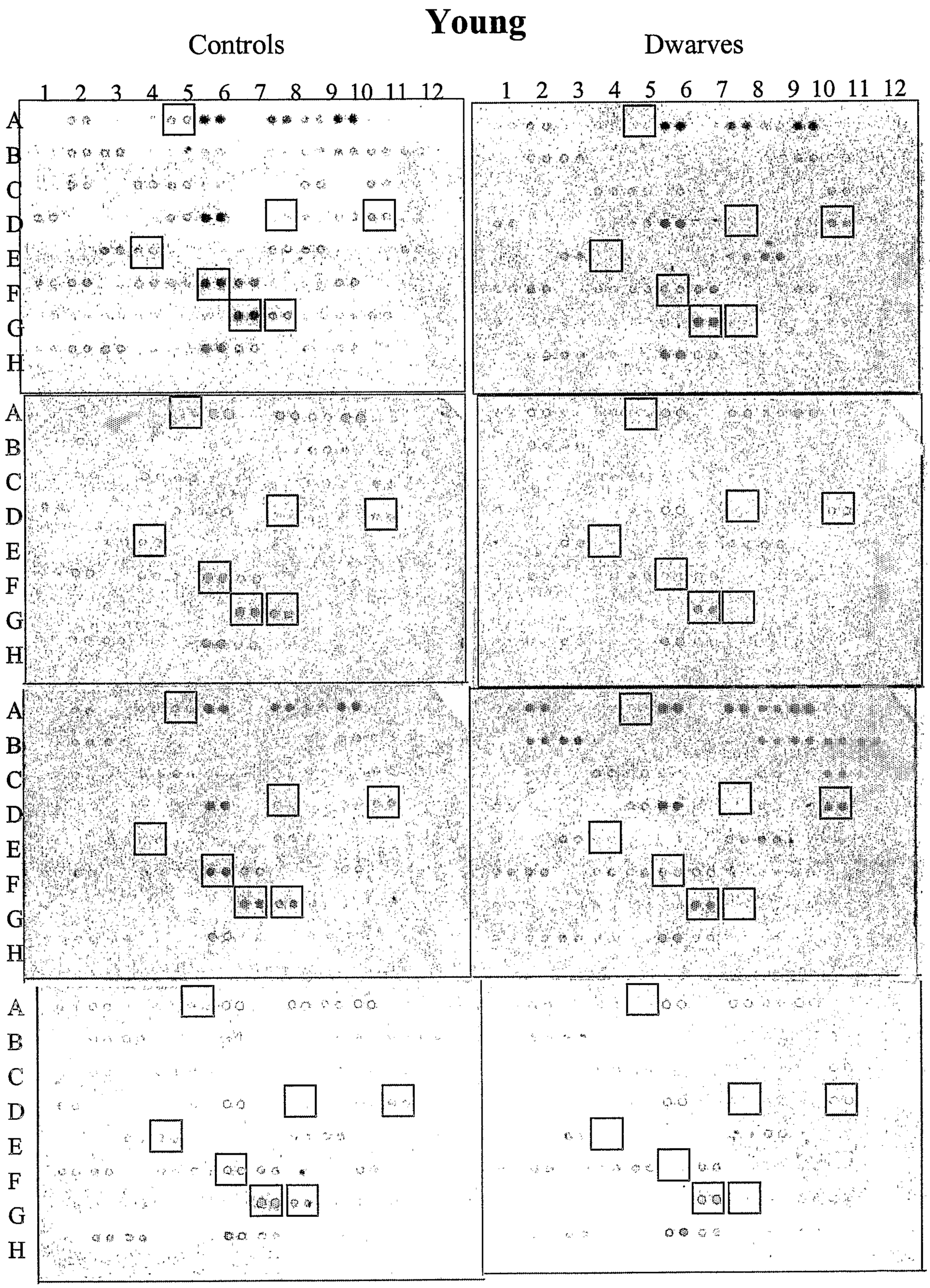


FIG. 6A

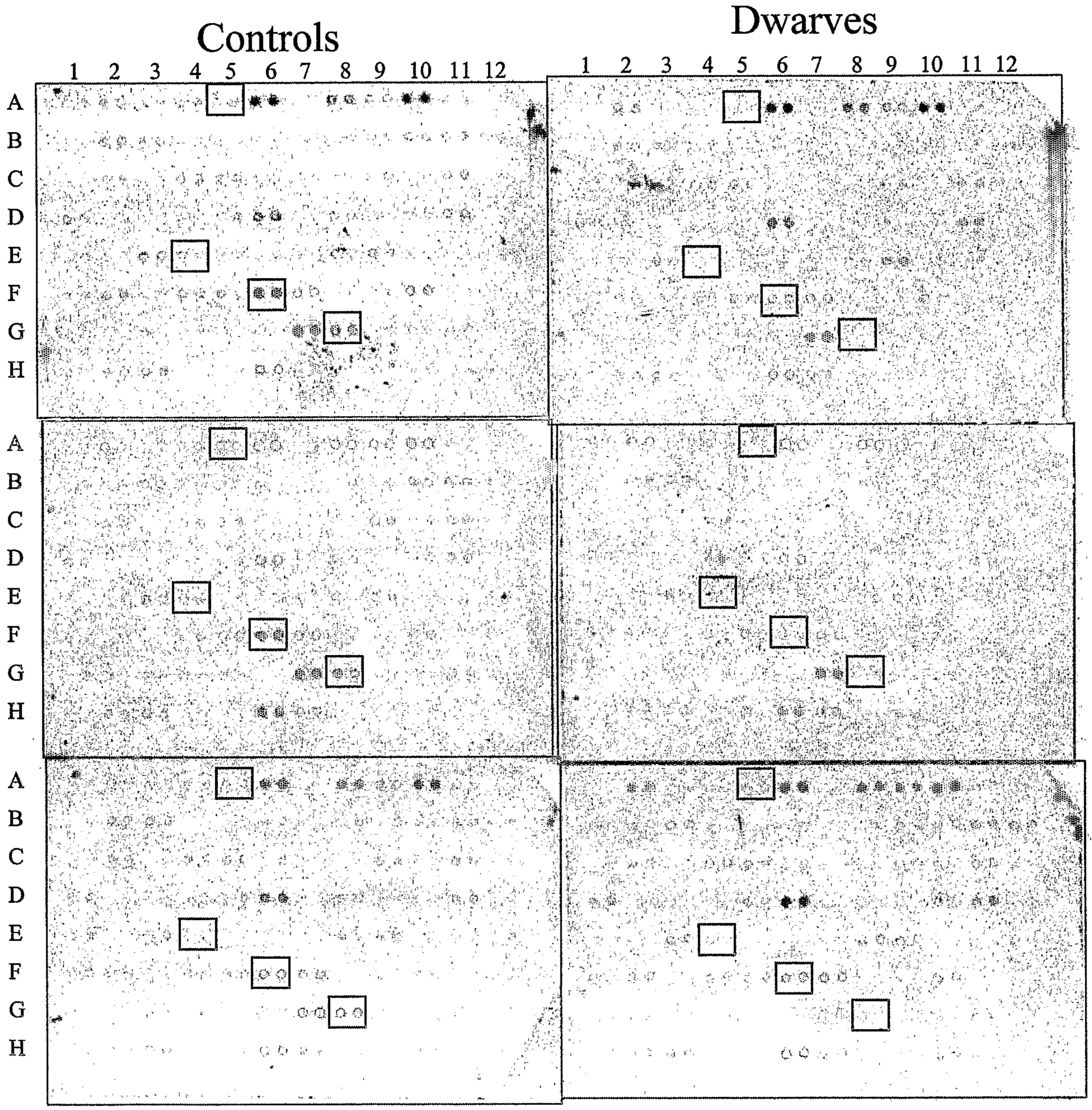


FIG. 6B

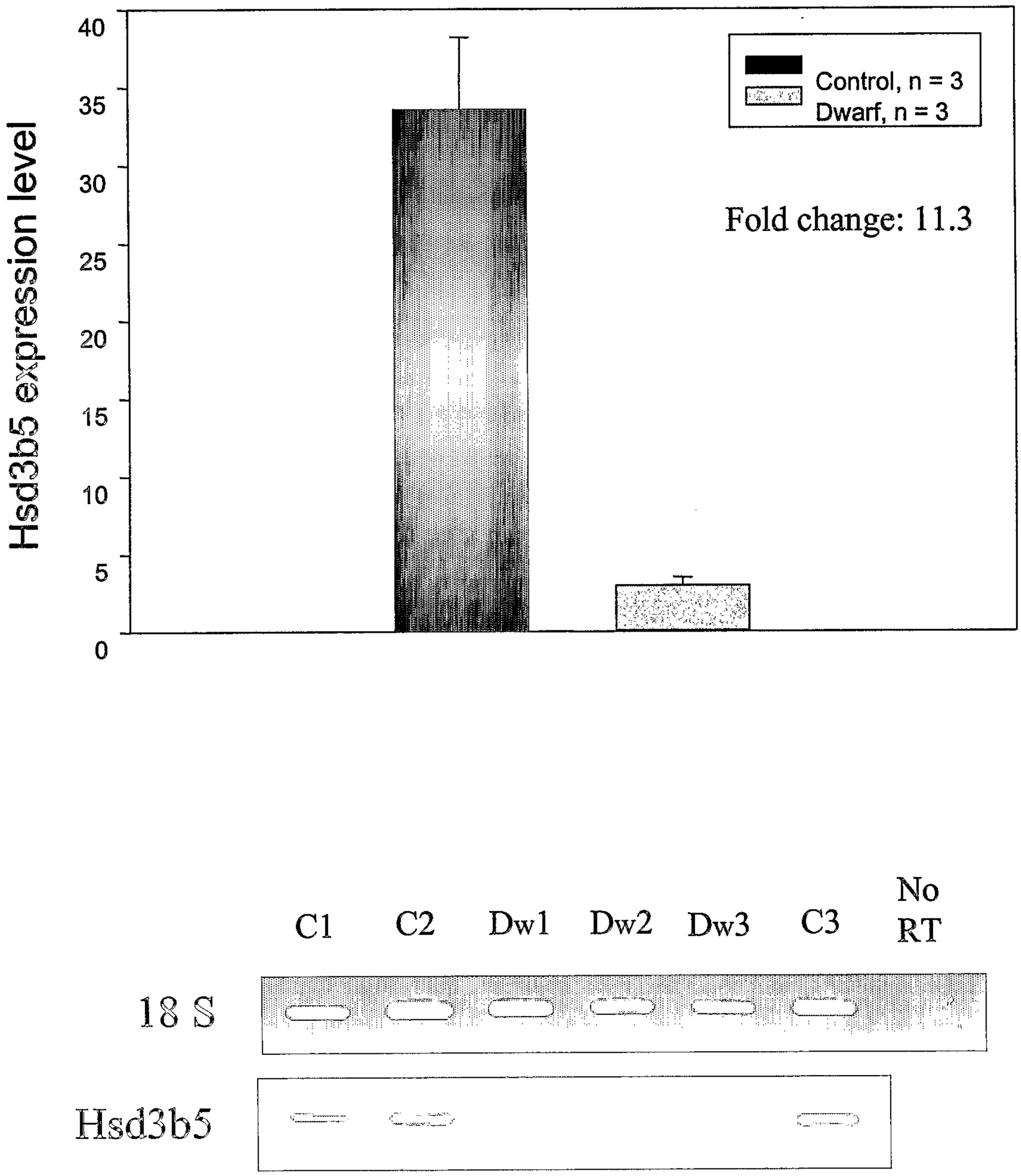

Hsd3b5 expression level
40
35
30
25
20
15
10
5
0
Control, n = 3
Dwarf, n = 3
Fold change: 11.3
C1
C2
Dw1
Dw2
Dw3
C3
No RT
18 S
Hsd3b5

FIG. 6C

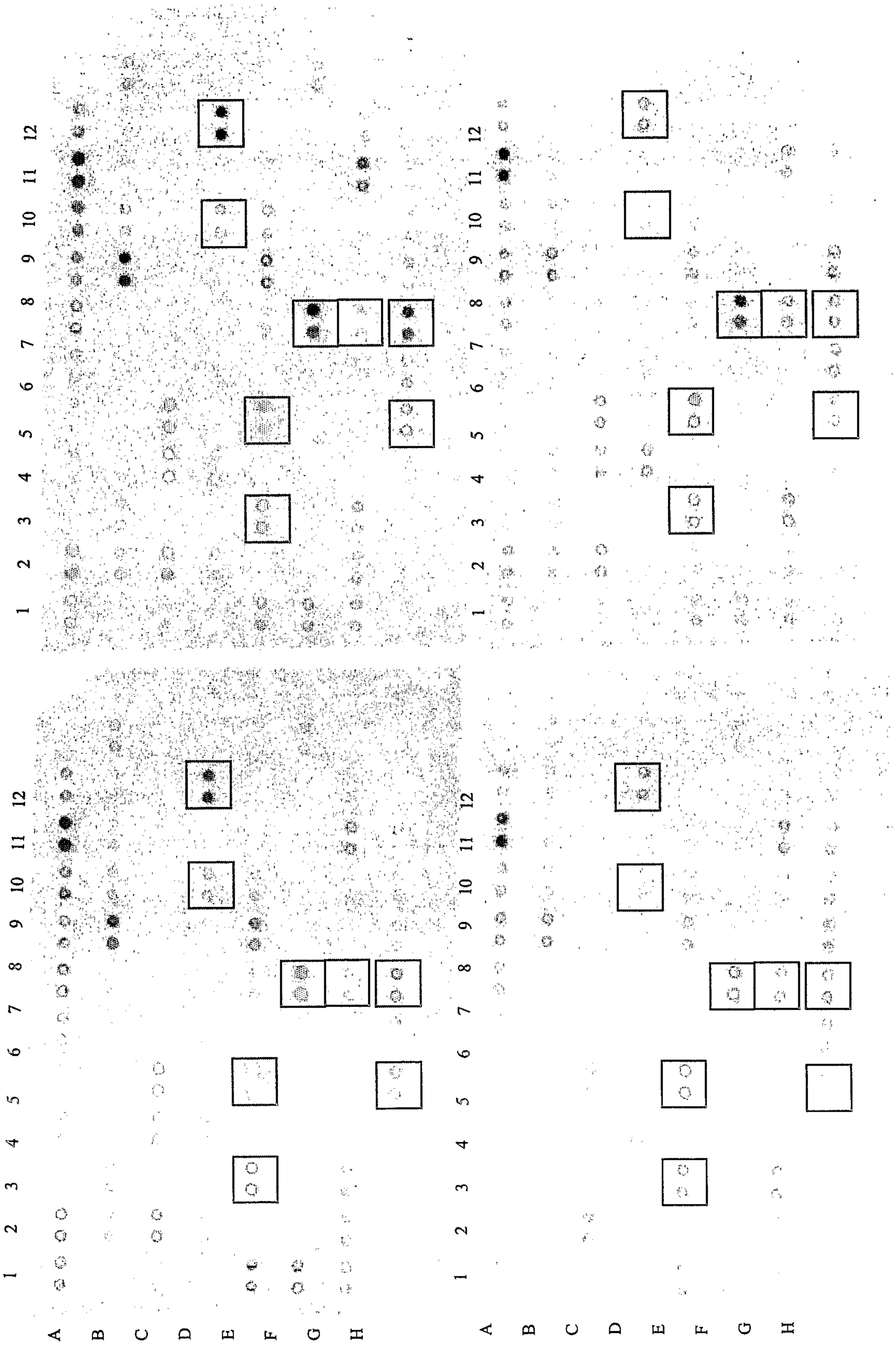


FIG. 7A-7D

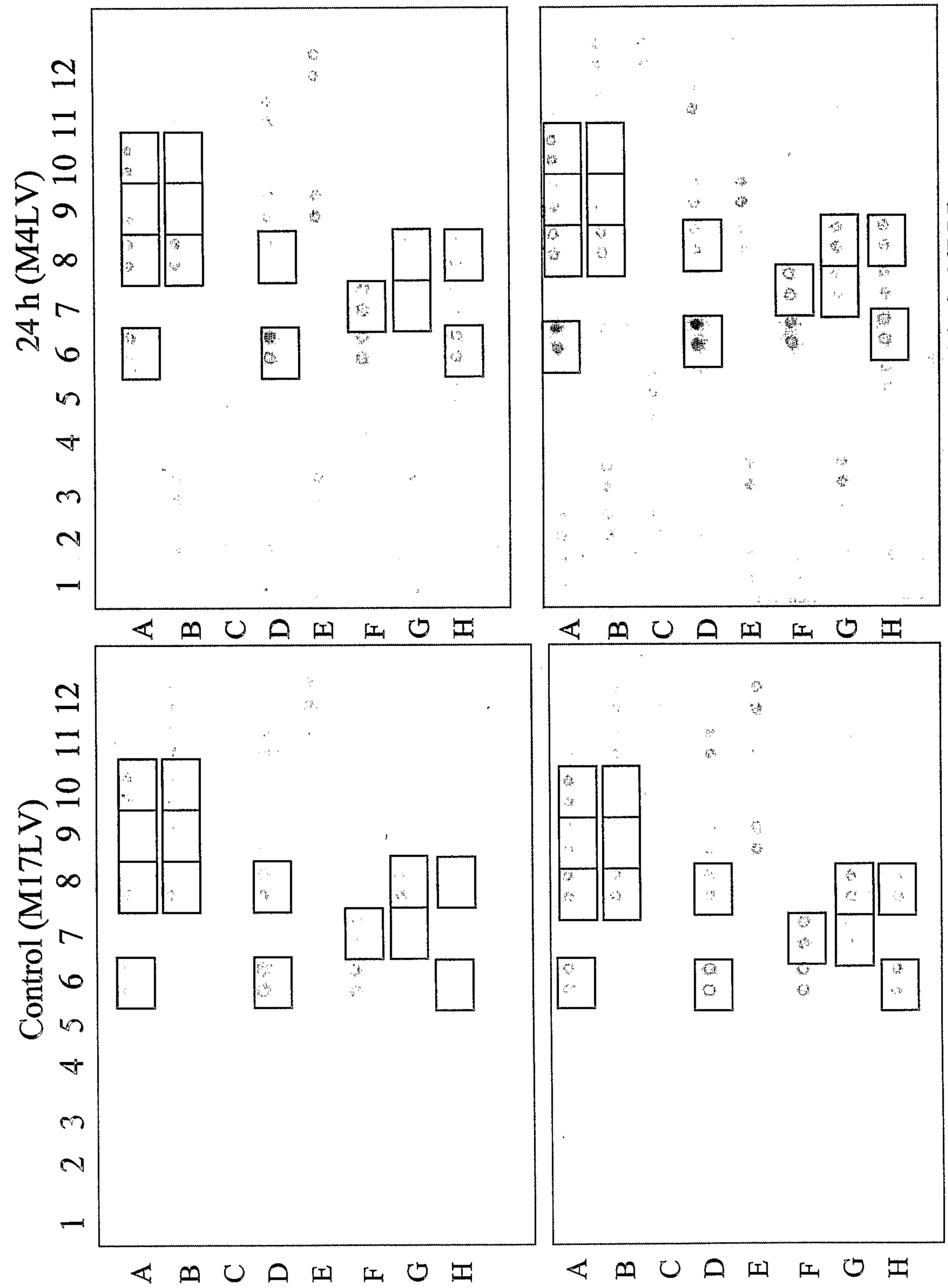

24 h (M4LV)
1 2 3 4 5 6 7 8 9 10 11 12
A B C D E F G H
24 h (M6LV)
Control (M17LV)
24 h (M5LV)

FIG. 8A-8D

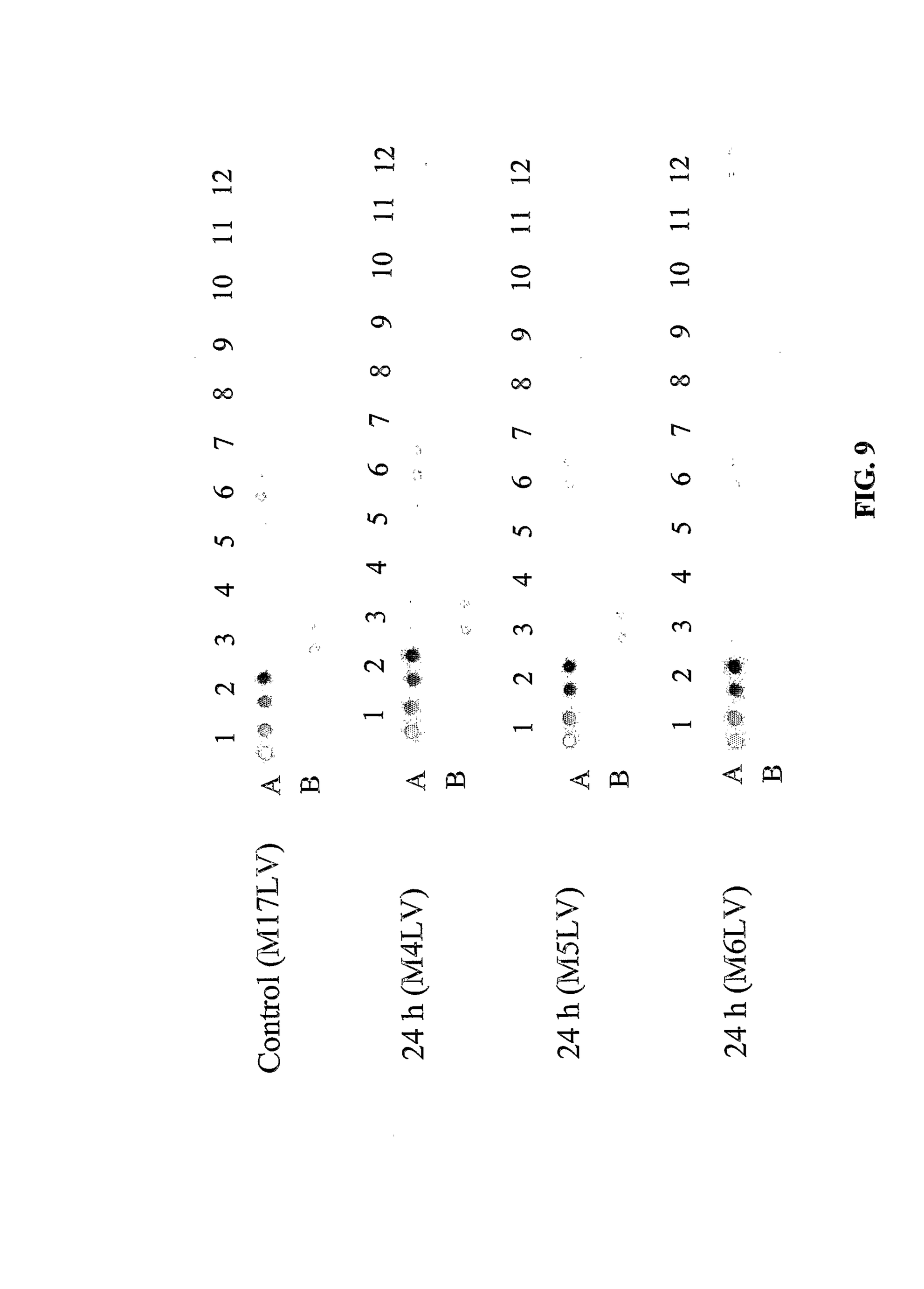

1 2 3 4 5 6 7 8 9 10 11 12
A
B
Control (M17LV)
1 2 3 4 5 6 7 8 9 10 11 12
A
B
24 h (M4LV)
1 2 3 4 5 6 7 8 9 10 11 12
A
B
24 h (M5LV)
1 2 3 4 5 6 7 8 9 10 11 12
A
B
24 h (M6LV)

FIG. 9