

1 **3.4 kb Mitochondrial DNA Deletion for use in the Detection of Cancer**

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3 **CROSS REFERENCE TO RELATED APPLICATIONS**

4 **[0001]** This application is a continuation-in-part of PCT application no.
5 PCT/CA2006/000652 filed on April 18, 2006 which claims priority from U.S. provisional
6 application nos. 60/672,016 filed April 18, 2005, 60/721,522 filed September 29, 2005, and
7 60/789,872 filed April 7, 2006. The entire disclosures of these applications are incorporated
8 herein by reference.

9
10 **FIELD OF THE INVENTION:**
11

12 **[0002]** This invention is related to the field of mitochondrial genomics. In particular it is
13 related to a 3.4 kb deletion in the mitochondrial genome and its utility as an indicator of
14 cancer.

15 **DESCRIPTION OF THE PRIOR ART**

16 **[0003]** *Mitochondrial DNA (MtDNA) as a Diagnostic Tool*

17 **[0004]** MtDNA sequence dynamics are important diagnostic tools. Mutations in mtDNA
18 are often preliminary indicators of developing disease, often associated with nuclear
19 mutations, and act as biomarkers specifically related to: disease, such as but not limited to,
20 tissue damage and cancer from smoking and exposure to second hand tobacco smoke (Lee et
21 al., 1998; Wei, 1998); longevity, based on accumulation of mitochondrial genome mutations
22 beginning around 20 years of age and increasing thereafter (von Wurmb, 1998); metastatic
23 disease caused by mutation or exposure to carcinogens, mutagens, ultraviolet radiation
24 (Birch-Machin, 2000); osteoarthritis; cardiovascular, Alzheimer, Parkinson disease (Shoffner
25 et al., 1993; Sherratt et al., 1997; Zhang et al. 1998); age associated hearing loss (Seidman et
26 al., 1997); optic nerve degeneration and cardiac dysrhythmia (Brown et al., 1997; Wallace et
27 al., 1988); chronic progressive external exophthalmoplegia (Taniike et al., 1992);
28 atherosclerosis (Bogliolo et al., 1999); papillary thyroid carcinomas and thyroid tumours
29 (Yeh et al., 2000); as well as others (e.g. Naviaux, 1997; Chinnery and Turnbull, 1999).

30 **[0005]** Mutations at specific sites of the mitochondrial genome can be associated with
31 certain diseases. For example, mutations at positions 4216, 4217 and 4917 are associated
32 with Leber's Hereditary Optic Neuropathy (LHON) (Mitochondrial Research Society;

Huoponen (2001); MitoMap). A mutation at 15452 was found in 5/5 patients to be associated with ubiquinol cytochrome c reductase (complex III) deficiency (Valnot et al.1999).

[0006] Specifically, these mutations or alterations include point mutations (transitions, transversions), deletions (one base to thousands of bases), inversions, duplications, (one base to thousands of bases), recombinations and insertions (one base to thousands of bases). In addition, specific base pair alterations, deletions, or combinations thereof have been found to be associated with early onset of prostate, skin, and lung cancer, as well as aging (e.g. Polyak et al., 1998), premature aging, exposure to carcinogens (Lee et al., 1998), etc.

[0007] *Prostate Cancer*

[0008] Prostate cancer is a frequently diagnosed solid tumour that most likely originates in the prostate epithelium (Huang et al. 1999). In 1997, nearly 10 million American men were screened for prostate specific antigen (PSA), the presence of which suggests prostate cancer (Woodwell, 1999). Indeed, this indicates an even higher number of men screened by an initial digital rectal exam (DRE). In the same year, 31 million men had a DRE (Woodwell, 1999). Moreover, the annual number of newly diagnosed cases of prostate cancer in the United States is estimated at 179,000 (Landis et al., 1999). It is the second most commonly diagnosed cancer and second leading cause of cancer mortality in Canadian men. In 1997 prostate cancer accounted for 19,800 of newly diagnosed cancers in Canadian men (28%) (National Cancer Institute of Canada). It is estimated that 30% to 40% of all men over the age of forty-nine (49) have some cancerous prostate cells, yet only 20% to 25% of these men have a clinically significant form of prostate cancer (SpringNet – CE Connection, internet, www.springnet.com/ce/j803a.htm). Prostate cancer exhibits a wide variety of histological behaviour involving both endogenous and exogenous factors, i.e. socio-economic situations, diet, geography, hormonal imbalance, family history and genetic constitution (Konishi et al. 1997; Hayward et al. 1998). Although certain mtDNA alterations have been previously associated with prostate cancer, the need exists for further markers for the detection of prostate cancer.

[0009] *3.4kb mtDNA deletion and the detection of prostate cancer.*

[0010] In the applicant's pending PCT application bearing publication no. WO/06/111029 (the entire contents of which are incorporated herein by reference) a deletion of a 3379 bp segment of mtDNA was identified through full mitochondrial genome

1 amplification of prostate tissue. The 3379 bp deletion (referred to as the 3.4 kb deletion) was
2 determined to be located between nucleotides 10744-14124 of the mitochondrial genome. It
3 was determined that the detection of this deletion could be used in the diagnosis of prostate
4 cancer when tissue samples are tested.

5 **[0011]** The 3.4 kb deletion removes all or part of the following genes from the mtDNA
6 genome: (i) NADH dehydrogenase subunit 4L, (ii) NADH dehydrogenase subunit 4, (iii)
7 NADH dehydrogenase subunit 5, (iv) tRNA histidine, (v) tRNA serine2, and (vi) tRNA
8 leucine2.

9 **[0012]** *Breast Cancer*

10 **[0013]** Breast cancer is a cancer of the glandular breast tissue and is the fifth most
11 common cause of cancer death. In 2005, breast cancer caused 502,000 deaths (7% of cancer
12 deaths; almost 1% of all deaths) worldwide (World Health Organization Cancer Fact Sheet
13 No. 297). Among women worldwide, breast cancer is the most common cancer and the most
14 common cause of cancer death (World Health Organization Cancer Fact Sheet No. 297).
15 Although certain mtDNA alterations have been previously associated with breast cancer, for
16 example in Parrella et al. (Cancer Research: 61, 2001), the need exists for further markers for
17 the detection of breast cancer.

18 SUMMARY OF THE INVENTION

19 **[0014]** In one embodiment, the present invention provides a method of detecting a cancer
20 in an individual comprising;

- 21 a) obtaining a biological sample from the individual;
- 22 b) extracting mitochondrial DNA, mtDNA, from the sample;
- 23 c) quantifying the amount of mtDNA in the sample having a deletion in the nucleic
24 acid sequence between residues 10744 and 14124 of the mtDNA genome;
- 25 d) comparing the amount of mtDNA in the sample having the deletion to at least one
26 known reference value.

27 **[0015]** In one embodiment, the present invention provides a method of detecting a cancer
28 in an individual comprising;

- 29 a) obtaining a biological sample from the individual;

1 b) extracting mitochondrial DNA, mtDNA, from the sample;
2 c) quantifying the amount of mtDNA in the sample having a deletion in the nucleic
3 acid sequence between residues 10744 and 14124 of the mtDNA genome;
4 d) comparing the amount of mtDNA in the sample having the deletion to the amount
5 of the deletion in a reference sample of mtDNA from known non-cancerous tissue or body
6 fluid;
7 wherein an elevated amount of the deletion in the biological sample compared to the
8 reference sample is indicative of cancer.

9 **[0016]** In one embodiment, the present invention provides a method of detecting a cancer
10 in an individual comprising;

11 a) obtaining a biological sample from the individual;
12 b) extracting mitochondrial DNA, mtDNA, from the sample;
13 c) quantifying the amount of mtDNA in the sample having a deletion in the nucleic
14 acid sequence between residues 10744 and 14124 of the mtDNA genome;
15 d) comparing the amount of mtDNA in the sample having the deletion to the amount
16 of the deletion in a reference sample of mtDNA from known cancerous tissue or body fluid;
17 wherein a similar level of the deletion in the biological sample compared to the
18 reference sample is indicative of cancer.

19 **[0017]** In one embodiment, the present invention provides a method of monitoring an
20 individual for the development of a cancer comprising;

21 a) obtaining a biological sample;
22 b) extracting mtDNA from the sample;
23 c) quantifying the amount of mtDNA in the sample having a deletion in the nucleic
24 acid sequence between residues 10744 and 14124 of the mtDNA genome;
25 d) repeating steps a) to c) over a duration of time;
26 e) wherein an increasing level of the deletion over the duration of time is indicative of
27 cancer.

28 **[0018]** In one embodiment, the present invention provides a method of detecting a cancer
29 in an individual comprising;

30 a) obtaining a biological sample from the individual;
31 b) extracting mitochondrial DNA, mtDNA, from the sample;

c) quantifying the amount of mtDNA in the sample having a sequence corresponding to the sequence identified in SEQ ID NO: 1;

d) comparing the amount of mtDNA in the sample corresponding to SEQ ID NO: 1 to at least one known reference value.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] An embodiment of the invention will now be described by way of example only with reference to the appended drawings wherein:

[0020] Figure 1 is a schematic diagram showing the design and sequence of a primer useful for the detection of the 3.4 kb deletion.

[0021] Figure 2 is a graph showing a comparison of cycle threshold between malignant and symptomatic benign participants in the 3.4 kb study.

[0022] Figure 3 is a graph showing cycle threshold as related to Example 1.

[0023] Figure 4 shows a ROC curve illustrating the specificity and sensitivity of one embodiment of the present invention.

[0024] Figure 5 shows a ROC curve illustrating the specificity and sensitivity of another embodiment of the present invention.

[0025] Figure 6 shows real-time PCR data relating to 3.4kb mtDNA deletion levels associated with breast cancer.

[0026] Figure 7 shows a ROC curve illustrating the specificity and sensitivity of another embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0027] As used herein, "cycle threshold" (C_T) is the point at which target amplification using real-time PCR rises above background, as indicated by a signal such as a fluorescence signal. The C_T is inversely related to the quantity of the sequence being investigated.

[0028] As defined herein, "sensitivity" refers to the fraction of true positives (true positive rate) results obtained using the method of the present invention.

1 **[0029]** As defined herein, “specificity” refers to the fraction of false positives (false
2 positive rate) results obtained using the method of the present invention.

3 **[0030]** In one embodiment of the present invention, methods are provided for monitoring
4 and diagnosing cancer through the detection and quantification of the aforementioned 3.4 kb
5 mtDNA deletion. For example, the present invention may be used for detecting the presence
6 of pre-neoplasia, neoplasia and progression towards potential malignancy of prostate cancer
7 and breast cancer. In one aspect, the present invention involves the detection and
8 quantification of the 3.4kb mtDNA deletion (SEQ ID NO:1) for the detection, diagnosis,
9 and/or monitoring of cancer. In this method, mtDNA is extracted from a biological sample
10 (for example body tissue, or body fluids such as urine, prostate massage fluid). The extracted
11 mtDNA is then tested in order to determine the levels (ie. quantity) of the 3.4 kb deletion in
12 the sample. In tests conducted by the present inventors, the levels of the deletion were found
13 to be elevated in samples obtained from subjects with cancer when compared to samples
14 obtained from subjects without cancer. Based on the information and data supplied below, the
15 inventors have concluded that elevated levels of the 3.4 kb deletion in the mtDNA is
16 indicative of cancer.

17 **[0031]** As disclosed in PCT WO/06/111029, the 3.4kb deletion spans approximately
18 nucleotides 10744 to 14124 of the mtDNA genome. The mtDNA genome is listed as SEQ ID
19 NO:8 (Genbank accession no. AC_000021). The inventors have determined, as provided by
20 example below, that this deletion is also associated with cancer and in particular prostate and
21 breast cancer. Therefore, such deletion provides an accurate biomarker and, therefore, a
22 valuable tool for the detection, diagnosis, or monitoring of cancer in at least these tissues.

23 **[0032]** The deletion results in the creation of two deletion monomers, one of 3.4kb in size
24 (small sublimon) and one of approximately 12.6kb in size (large sublimon). The occurrence
25 of the deletion may be detected by either identifying the presence of the small sublimon, or
26 by determining that the 3.4 kb sequence has been deleted from the large sublimon.

27 **[0033]** As discussed above, the deletion is approximately 3379 bp, and comprises genes
28 encoding NADH dehydrogenase subunit 4L, NADH dehydrogenase subunit 4, NADH
29 dehydrogenase subunit 5, tRNA histidine, tRNAserine2, and tRNA leucine2.

30 **[0034]** In one embodiment, samples of, for example prostate tissue, prostate massage
31 fluid, urine or breast tissue, are obtained from an individual and tested over a period of time

(eg. years) in order to monitor the genesis or progression of cancer. Increasing levels of the 3.4 kb deletion over time could be indicative of the beginning or progression of cancer.

[0035] Age related accumulation of the 3.4 kb mtDNA deletion may predispose an individual to, for example, prostate cancer or breast cancer, which is prevalent in middle aged and older men, and middle aged and older women, respectively. According to one aspect of the invention, a method is provided wherein regular cancer screening may take place by monitoring over time the amount of the 3.4 kb deletion in body tissues such as breast tissue or body fluids such as prostate massage fluid, or urine.

[0036] The system and method of the present invention may be used to detect cancer at an early stage, and before any histological abnormalities. For example, the system and method of the present invention may be used to detect pre-neoplasia in breast tissue.

[0037] The following primer sequences are preferred for the detection of the 3.4 kb deletion:

3.4 forward (binds to bases 10729-10743/14125-14139 of the mtDNA genome) 5'-TAGACTACGTACATACTAACCCTACTCCTA-3' (SEQ ID NO: 2);

3.4 reverse (binds to bases 14361-14379 of the mtDNA genome) 5'-GAGGTAGGATTGGTGCTGT-3' (SEQ ID NO: 3).

[0038] In one embodiment of the present invention, a pair of amplification primers are used to amplify a target region indicative of the presence of the 3.4 kb deletion. In this embodiment, one of the pair of amplification primers overlaps a spliced region of mtDNA after deletion of the 3.4 kb sequence has occurred (ie. a splice at a position between 10744 and 14124 of the mtDNA genome). Therefore, extension of the overlapping primer can only occur if the 3.4 kb section is deleted.

[0039] In another embodiment of the present invention, a pair of amplification primers are used to amplify a target region associated with the deleted 3.4 kb sequence. The deleted 3.4 kb sequence, upon deletion, may reform as a circular mtDNA molecule. In this embodiment, one of the pair of amplification primers overlaps the rejoining site of the ends of the 3.4 kb sequence. Thus, an increase in the amount of the 3.4 kb molecule detected in a

sample is indicative of cancer. The below primer pair is preferred for the detection of the deleted 3.4 kb nucleic acid.

Forward 14115/10755 5'-CCCACTCATCACCTAAACCTAC-3' (SEQ ID NO: 9)

Reverse 10980R 5'-GGTAGGAGTCAGGTAGTTAG-3' (SEQ ID NO: 10).

[0040] In one aspect of the invention, a kit for diagnosing cancer, for example prostate or breast cancer, comprising means for extraction of mtDNA, primers having the nucleic acid sequences recited in SEQ ID NOS: 2 and 3, or SEQ ID NOS: 9 and 10, reagents and instructions, is provided.

[0041] Another aspect of the invention provides methods for confirming or refuting the presence of a cancer biopsy test from a biopsy sample (eg. prostate or breast cancer), comprising: obtaining non-cancerous tissue from a biopsy sample; and detecting and quantifying the amount of the 3.4 kb mtDNA deletion in the non-diseased tissue.

[0042] In one embodiment the present invention provides a method for screening individuals for prostate or breast cancer from a body fluid sample comprising; obtaining a body fluid sample, and detecting and quantifying the level of the 3.4 kb mtDNA deletion in the body fluid.

[0043] Although real-time quantitative PCR methods, as described in the examples below, represent the preferred means for detecting and quantifying the presence or absence of the 3.4kb deletion, other methods that would be well known to an individual of skill in the art could also be utilized. For example quantification of the deletion could be made using Bio-Rad's Bioplex™ System and Suspension Array technology. Generally, the method requires amplification and quantification of sequences using any known methods.

[0044] The examples provided below illustrate that not only can this deletion be used for the detection of prostate cancer in prostate tissue, but can also be used to detect the presence of cancer in other biological samples, for example prostate massage fluid, urine, and breast tissue. Based on the findings in these examples, the 3.4 kb mtDNA deletion may be used as a biomarker for cancer.

[0045] The various examples provided illustrate a difference in the amount of mtDNA having the 3.4 kb deletion between samples obtained from subjects having cancer, and

1 subjects without cancer. The amount of the 3.4 kb deletion was found to be higher in the
2 samples obtained from subjects having cancer. This determination was made by comparing
3 the amount of the 3.4 kb deletion in the test samples with amounts from known cancer cells
4 and/or known non-cancer cells.

5 **[0046] Example 1: 3.4 kb Deletion in the mtDNA of Prostate Tissue**

6 **[0047]** A deletion of approximately 3.4 kilobases (kb) was identified through full
7 mitochondrial genome amplification of fresh frozen prostate tissue. Using linear regression,
8 the size of the deletion was estimated to be between 3000 base pairs (bp) and 3500 bp. Two
9 possible candidate deletions were identified using MitomapTM (Brandon, M. C., Lott, M. T.,
10 Nguyen, K. C., Spolim, S., Navathe, S. B., Baldi, P. & Wallace, D. C., MITOMAP: a human
11 mitochondrial genome database--2004 update. Nucleic Acids Research 33 (Database
12 Issue):D611-613, 2005; www.mitomap.org), the 3397 bp deletion at 9574-12972, and the
13 3379 bp deletion at 10744-14124. In order to determine which of the two deletions was
14 associated with prostate cancer, if either, a forward primer which bridged the deletion
15 junction was developed for each of the two candidates, ensuring that the primer extended
16 further than the repeat regions that flank the deletions. Figure 1 is a schematic diagram
17 showing the design and sequence of the primer (ie. SEQ ID NO: 2). Positive amplification
18 results for the amplicon corresponding to the 3379 bp deletion (referred to as the 3.4 kb
19 deletion) at 10744-14124 were obtained.

20 **[0048]** As indicated above, the 3.4 kb deletion removes all or part of the following genes:
21 (i) NADH dehydrogenase subunit 4L, (ii) NADH dehydrogenase subunit 4, (iii) NADH
22 dehydrogenase subunit 5, (iv) tRNA histidine, (v) tRNA serine2, and (vi) tRNA leucine2.

23 **[0049]** The 3.4kb deletion was determined to be present in 91% of 33 fresh frozen
24 prostate samples. With the specific deletion primers, formalin fixed tissues were tested in
25 order increase the n value.

26 **[0050]** The present investigators sequenced entire mitochondrial genomes from 32 tissue
27 samples microdissected by laser capture microdissection and 12 needle biopsies from
28 histologically normal prostates. Archived tissue sections from each of these samples were
29 used for the following study. 1-2 serial sections were removed from each sample. DNA was
30 extracted from each sample in its entirety rather than as a microdissection. Thus, each
31 sample consisted of a mixture of glandular prostate tissue as well as stromal prostate tissue.

This extraction was performed using Qiagen's QIAamp™ DNA Mini Kit (Cat # 51304). Following extraction the samples were quantified using a Nano-Drop™ spectrophotometer and the concentrations were subsequently normalized to 2ng/ul. Each sample was amplified using 20ng input DNA and an iQ™ SYBR Green Supermix™ kit (Bio-Rad Laboratories Inc.) Reactions were run on an Opticon® 2 two colour real-time PCR system (MJ Research).

[0051] As shown in Figure 2, a distinct difference was observed in cycle threshold and, by extension, quantity of the deletion between the malignant prostate samples and the symptomatic benign prostate samples. Malignant samples exhibited a consistently earlier cycle threshold than the benign samples.

[0052] Example 2: 3.4kb Deletion Blinded Study – Comparison of Cycle Threshold

[0053] An additional 21 prostate tissue samples were selected, 10 of which were benign and 11 of which were malignant. The pathological status was determined by needle biopsies conducted by a qualified pathologist. The samples were blinded such that the present investigators were unaware of their pathological status when they conducted this test. The present investigators were able to predict pathological status correctly in 81% of the cases by examining the cycle threshold. Of the 4 incorrect calls, two were malignant samples that were determined to be benign and 2 were benign samples that were determined to be malignant. Follow-up clinical information for the 2 individuals in the latter scenario was requested from the physician to determine if they had been diagnosed with prostate cancer subsequent to the needle biopsy results used for this study. One of the individuals who originally produced a benign sample but was predicted by this study to have a malignancy subsequently produced a malignant sample. As a result, one of the false positives became a true positive. Therefore, pathological status was predicted correctly in 86% of the cases examined in this study. The ultimate positive predictive value (PPV, where $PPV = \frac{\text{true positives}}{\text{true positives} + \text{false positives}}$) for this study was 91% and the negative predictive value (NPV, where $NPV = \frac{\text{true negatives}}{\text{true negatives} + \text{false negatives}}$) was 80%.

[0054] Example 3: 3.4kb Deletion Study – Methods (n=76)

[0055] Seventy-six prostate tissue samples were examined for the 3.4 kb deletion in this study. All tissue samples were formalin-fixed, 25 being malignant, 12 being normal, and 39 having benign prostatic disease as shown histologically. Of the latter group more than half

had hyperplasia. All specimens were needle biopsies taken from the investigators' tissue archives.

[0056] Prostate Specimens

[0057] A tapelift was performed on each slide using Prep-Strips (Catalogue Number LCM0207) from Arcturus Bioscience Inc. This allowed the removal of any particulate matter or non-adhering tissue from the slide prior to DNA extraction. With the tissue still on the slides, the slides were rinsed with PBS (Phosphate Buffered Saline Solution) to remove as much fixative as possible. The 1-2 needle biopsy sections on the slides were scraped into sterile microcentrifuge tubes using individually wrapped, sterilized surgical razor blades. DNA was then isolated and purified using a QIAamp[®] DNA Mini Kit (Qiagen, Cat. # 51304) according to manufacturer's specifications. A negative extract control was processed in parallel with the slide extractions as a quality control checkpoint. The total concentration of DNA and purity ratio for each sample was determined by spectrophotometry (Nano-Drop[™] ND-1000) and dilutions of 2ng/ μ l were prepared for the purpose of Quantitative Polymerase Chain Reaction (qPCR).

[0058] Primers (Oligonucleotides)

[0059] Purified oligonucleotide primers were chemically synthesized by Invitrogen (California, USA). The sequences of the primers and the expected sizes of the PCR products amplified are listed in Table 1. In addition, PCR analysis for mtDNA deletions included positive controls (DNA from a source known to carry the mutant mtDNA). Each primer set with the exception of TNF (tumor necrosis factor) were checked against a mitochondria-free rho 0 cell line to confirm the absence of pseudogene coamplification.

[0060] Table 1 Amplification Primers.

Primer Pair	Position Amplified 5'- 3'	Length of amplified product (base pairs)
3.4 Deletion Real-Time	10729-14379 (less 3379bp at 10744-14124)	273
12s mtDNA	708-945	238
TNF	3756-3886	131

3.4 forward (10729-10743 – 14125-14139)
 5'TAGACTACGTACATACTAACCCTACTCCTA-3' SEQ ID NO: 2
 3.4 reverse (14361-14379) 5'-GAGGTAGGATTGGTGCTGT-3' SEQ ID NO: 3
 12s forward (708-728) 5'-CGFTCCAGTGAGTTCACCCTC-3' SEQ ID NO: 4
 12s reverse (923-945) 5'-CACTCTTTACGCCGGCTTCTATT-3' SEQ ID NO: 5
 TNF forward (3756-3775) 5' –CCTGCCCAATCCCTTTATT-3' SEQ ID NO: 6
 TNF reverse (3866-3886) 5'-GGTTTCGAAGTGGTGGTCTTG-3' SEQ ID NO: 7

[0061] Real-Time Polymerase Chain Reaction

[0062] Three separate PCRs were performed on each sample. Each reaction was 25 μ l total volume and included template DNA, one pair of primers (12s or 3.4 Deletion or TNF), an iQTM SYBR Green SupermixTM kit (Catalogue Number 170-8882, Bio-Rad Laboratories Inc.) and distilled deionized water (ddH₂O). The TNF (tumor necrosis factor) comprised single copy nuclear gene primers, and 12s comprised total mitochondrial genome primers. The volume and concentrations for template DNA, primers, and reaction buffer are listed below.

[0063] Table 2 *qPCR Components.*

Reagent	Concentration per Reaction	Volume per Reaction
Reaction Buffer	1X	12.5 μ l
Primer (forward and reverse)	250nM	0.0625 μ l of each 100 umole stock
ddH ₂ O	N/A	2.375. μ l
Template DNA	20ng	10.0 μ l
Total		25 μ l

[0064] The cycling parameters for each amplicon are listed in Table 3.

[0065] Table 3 *Cycling Parameters.*

Step	Temperature (°C)	Duration
1	95	3 min
2	95	30 sec
3	66 (3.4 deletion primers) or 61.5 (12s primers) or	30 sec

	61.5 (TNF primers)	
4	72	30 sec
5	Plate Read	
6	72	10 min
7	Melting Curve 50°C - 110°C reading every 1°C	3 sec
Repeat steps 2-5, 44 times for a total of 45 cycles.		

[0066] Thermal cycling, real-time detection and analysis of the reactions was carried out using a DNA Engine Opticon[®] 2 Continuous Fluorescence Detection System equipped with Intuitive Opticon Monitor[™] software (MJ Research Inc.). The standard curve method was utilized for DNA quantification. A set of serial dilutions (10^6 , 10^5 , 10^4 , 10^3 , 10^2 , 10^1) of three purified PCR generated templates, one product for the 3.4 deletion, one for the 12s primers, and one for TNF. From this, three different standard curves were generated showing the number of copies of total mtDNA (12s amplicon-total mitochondrial genome primers), the amount of mtDNA having the 3.4 kb deletion, or total nuclear DNA (TNF-single copy nuclear gene primers). The C_T values of the samples were then converted to the number of DNA copies by comparing the sample C_T to that of the standards. The 3.4 deletion was considered to be absent or at low levels if the deletion was not detected within 37 cycles.

[0067] The determination of malignancy is based upon the quantity of the 3.4kb deletion present in the normalized sample as indicated by the location of the cycle threshold. This location may be either absolute, as in greater than 25 cycles but less than 35 cycles, or more likely a ratio between the total mitochondrial DNA present as indicated by the 12s amplicon, and the 3.4kb deletion. This may be expressed as a per cent of the total mitochondrial DNA. The number of cells, as represented by the TNF amplicon, may be incorporated to refine the distinction between benign and malignant tissues.

[0068] In order to automate the analyses of these samples, bioinformatics tools were employed. The three variables that were considered for these analyses were the cycle threshold C_T of Tumour Necrosis Factor (TNF), total pecies of mitochondria that contain those specific primer sites, and those mitochondria that harbour the deletion of interest.

[0069] Cluster Analysis

[0070] The clustering was not normalized nor were logarithmic functions used due to the similar and small range of data.

[0071] Figure 3 shows the actual movement and trends of the data. The x-axis is the patient number and the y-axis is the cycle threshold obtained from real time PCR.

[0072] It is important to note that the higher the cycle threshold is, the lower amount of the deletion is present.

[0073] The general trend shown in Figure 3 is based upon the differences/ratios between the variables of Deletion, Total, and TNF. The deletion is low to absent for the benign/normal samples (right side) and increases (toward the left) with abnormal benign and malignant samples. The abnormal benign and malignant samples begin to differentiate themselves from each other based on the cycle threshold ratio of Deletion to TNF.

[0074] Supervised Learning

[0075] Supervised learning is based on the system trying to predict outcomes for known samples. Half of the data was used to train and the other half to test the algorithm.

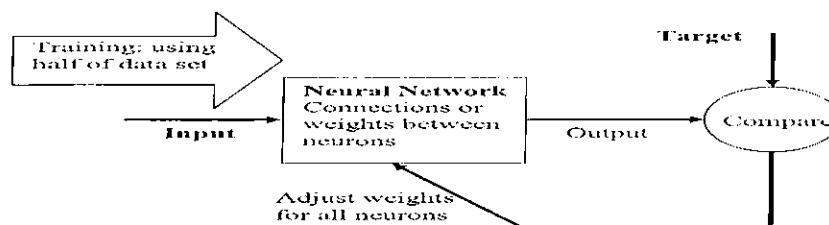
Supervised learning compares its predictions to the target answer and "learns" from its mistakes. But, if the predicted output is higher or lower than the actual outcome in the data, the error is propagated back through the system and the weights are adjusted accordingly.

[0076] Data SET: 5% to 35% - **Benign**
 35% to 65% - **Hyperplasia**
 65% to 95% - **Malignant**

[0077] Artificial Neural Network (ANN) Algorithm (shown schematically below):

Half of Data set used for Training ANN
Other half used to compare the accuracy
Accuracy = Compare expected data set with obtained data set → 86.6%

Artificial Neural Network algorithm



[0078] Supervised Learning of Deletion Data using Artificial Neural Network (ANN)

[0079] Three Classifications:

Benign
Hyperplasia
Malignant

[0080] Three variables for each classification were used based on Real Time PCR Cycle Threshold C_T :

Tumour Necrosis Factor (TNF) – Nuclear copy control.
Total Mitochondria – Mitochondria copy control
Deletion – Mitochondria in the deleted state.

[0081] Results:

[0082] Half of data set is used to train the ANN, and the remaining half is used to compare the accuracy.

Three Classification Accuracy = 86.6%

Positive Predictive Value (PPV);

Benign to Malignant = 88.2%

Negative Predictive Value (NPV)

Benign to Malignant = 76.5%

[0083] **Example 4: 3.4 kb Deletion in mtDNA Associated with Breast Cancer**

[0084] 18 samples were tested from malignant and benign breast tissue, 9 being malignant and 9 being benign, for the presence of the aforementioned 3.4 kb deletion.

Samples were classified as either malignant or benign using conventional histopathological analysis.

[0085] DNA was isolated and purified from the samples using a QIAamp[®] DNA Mini Kit (Qiagen, Cat. # 51304) according to manufacturer's specifications.

[0086] Purified oligonucleotide primers were chemically synthesized by Invitrogen (California, USA). The sequences of the primers and the expected sizes of the PCR products amplified are listed in Table 1 above.

1 **[0087]** Real-Time Polymerase Chain Reaction

2 **[0088]** Three separate PCRs were performed on each sample. Each reaction was 25 μ l
3 total volume and included template DNA, one pair of primers (12s or 3.4 Deletion or TNF),
4 an iQTM SYBR Green Supermix kit (Catalogue Number 170-8882, Bio-Rad Laboratories
5 Inc.) and distilled deionized water (ddH₂O). The TNF (tumor necrosis factor) comprised
6 single copy nuclear gene primers, and 12s comprised total mitochondrial genome primers.
7 The volume and concentrations for template DNA, primers, and reaction buffer are listed
8 below:

9 **[0089]** *Table 4 qPCR Components.*

Reagent	Concentration per Reaction	Volume per Reaction
Reaction Buffer	1X	12.5 μ l
Primer (forward and reverse)	250nM	0.0625 μ l of each 100 μ mole stock
ddH ₂ O	N/A	2.375. μ l
Template DNA	20ng	10.0 μ l
Total		25 μ l

10

11 **[0090]** The cycling parameters for each amplicon are listed in Table 5.

12 **[0091]** *Table 5 Cycling Parameters.*

Step	Temperature (°C)	Duration
1	95	3 min
2	95	30 sec
3	66 (3.4 deletion primers) or 61.5 (12s primers) or 61.5 (TNF primers)	30 sec
4	72	30 sec
5	Plate Read	
6	72	10 min
7	Melting Curve 50°C - 110°C reading every 1°C	3 sec
Repeat steps 2-5, 44 times for a total of 45 cycles.		

[0092] Thermal cycling, real-time detection and analysis of the reactions was carried out using a DNA Engine Opticon[®] 2 Continuous Fluorescence Detection System equipped with Intuitive Opticon Monitor[™] software (MJ Research Inc.). The standard curve method was utilized for DNA quantification. A set of serial dilutions (10^6 , 10^5 , 10^4 , 10^3 , 10^2 , 10^1) of three purified PCR generated templates were performed, one product for the 3.4 deletion, one for the 12s primers, and one for TNF. From this, three different standard curves were generated showing the number of copies of total mtDNA (12s amplicon-total mitochondrial genome primers), 3.4 deletion or total nuclear DNA (TNF-single copy nuclear gene primers). The C_T values of the samples were then converted to the number of DNA copies by comparing the sample C_T to that of the standards.

[0093] The determination of malignancy was based upon the quantity of the 3.4kb deletion present in the normalized sample as indicated by the location of the cycle threshold. This location may be either absolute, as in greater than 25 cycles but less than 30 cycles, or more likely a ratio between the total mitochondrial DNA present as indicated by the 12s amplicon, and the 3.4kb deletion. This may be expressed as a percent of the total mitochondrial DNA.

[0094] In order to automate the analyses of these samples, bioinformatics tools were employed. The three variables that were considered for these analyses were the cycle threshold C_T of Tumour Necrosis Factor (TNF), total species of mitochondria that contain those specific primer sites, and those mitochondria that harbour the deletion of interest.

[0095] Table 6 and figure 7 show the difference in the mean C_T scores for samples from malignant tissue and benign tissue. The mean C_T value for normal tissue was 30.5889, while the mean C_T for malignant tissue was 27.8533 thereby illustrating a difference in the quantity of mtDNA having the 3.4 kb deletion in malignant breast tissue compared to normal breast tissue.

[0096] *Table 6 Mean values for C_T scores*

Group Statistics					
GRP		N	Mean	Std. Deviation	Std. Error Mean
del3.4	normal	9	30.5889	2.53897	.84632
	malignant	9	27.8533	2.52253	.84084

[0097] Figure 8 is an ROC curve illustrating the specificity and sensitivity of the 3.4 kb mtDNA deletion as a marker for breast cancer when testing breast tissue. These results were obtained using a cutoff C_T of 29.1900. The sensitivity of the marker at this C_T was 77.8%, while the specificity was 77.8%.

[0098] Table 7 shows the calculation of the area under the curve for the present example. As a measure of the accuracy of the test.

[0099] *Table 7 Results Showing Area Under the Curve*

Area Under the Curve

Test Result Variable(s): del3.4

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.790	.112	.038	.570	1.010

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

[00100] The determination of the cutoff C_T of 29.1900 is shown in table 8 below. The results listed in table 8 show that a cutoff C_T of 29.1900 provided the highest sensitivity and specificity at 78% and 78% respectively.

[00101] *Table 8: Determination of C_T cutoff.*

Coordinates of the Curve

Test Result Variable(s): del3.4

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
24.6000	.000	.000
25.6800	.111	.000
25.7700	.222	.000
25.9250	.333	.000
26.2050	.444	.000
26.8400	.556	.000
27.4800	.556	.111
28.1600	.556	.222
28.8800	.667	.222
29.1900	.778	.222
29.4600	.778	.333
29.8750	.778	.444
30.5850	.778	.556
31.2200	.778	.667
31.5000	.889	.667
31.7650	.889	.778
32.9900	1.000	.778
34.3350	1.000	.889
35.6400	1.000	1.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

[00102] Example 5: The 3.4kb Deletion in the Prostate Massage Fluid of Individuals with Prostate Cancer as Compared to the Fluid from those without Histological Evidence of Prostate Cancer

[00103] Forty prostate massage fluid samples were collected by urologists from patients who were either subsequently diagnosed with prostate cancer or showed no histological evidence of prostate cancer following a prostate needle biopsy procedure. The sample was deposited on a IsoCode Card™ (Schleicher & Shuell), dried, and then extracted according to the manufacturer's protocol. All DNA extracts were quantified using a NanoDrop™ ND-1000 Spectrophotometer and the DNA concentration normalized to 2ng/ul. Each sample was then amplified according to the following parameters:

1X iQ SYBR Green Supermix™ (Bio-Rad P/N 170-8880)
150nmol forward primer
(5'-TAGACTACGTACATACTAACCCTACTCCTA-3') (SEQ ID NO: 2).
150 nmol reverse primer
(5'-GAGGTAGGATTGGTGCTGT-3') (SEQ ID NO: 3)
20 ng template DNA
in a 25ul reaction.

[00104] Reactions were cycled on an Opticon™ 2 DNA Engine (Bio-Rad Canada) according to the following protocol:

1. 95°C for 3 minutes
2. 95°C for 30 seconds
3. 66°C for 30 seconds
4. 72°C for 30 seconds
5. Plate Read
6. Repeat steps 2-5 44 times
7. 72°C for 10 minutes
8. Melting Curve from 50°C to 105°C, read every 1°C, hold for 3 seconds
9. 10°C Hold

1 [00105] Table 9 Results showing the mean C_T Values for Prostate Massage Fluid Test

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
DEL34 benign	25	37.1869	3.18495	.63609
malignant	15	33.7712	3.98056	1.02778

2 [00106] Tables 9 and 10 show a significant difference between the mean C_T values
3 obtained for the benign sample and the malignant sample groups ($p=0.005$).

4 [00107] Table 10 Results Showing Difference ($p=0.005$) for C_T values of samples.

Independent Samples Test

		Levene's Test for Equality of Variances		t-Test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
DEL34	Equal variances assumed	1251	.270	2989	38	.005	34.1570	1.14283	1.10217	57.2923
	Equal variances not assumed			2825	24.696	.009	34.1570	1.20917	.92382	59.0758

5 [00108] Figure 5 is a Receiver Operating Characteristic (ROC) curve illustrating the
6 specificity and sensitivity of the 3.4 kb mtDNA deletion as a marker for prostate cancer when
7 testing prostate massage fluid. These results were obtained using a cutoff C_T of 37.3683.
8 The sensitivity of the marker at this C_T is 87%, while the specificity is 64%.

9 [00109] The accuracy of the test depends on how well the test separates the group being
10 tested into those with and without the prostate cancer. Accuracy is measured by the area
11 under the ROC curve. Table 11 shows the calculation of the area under the curve for the
12 present example.

1 [00110] Table 11 Results Showing Area Under the ROC Curve

Area Under the Curve

Test Result Variable(s): DEL3_4

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.768	.074	.005	.622	.914

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

2 [00111] Table 12 Determination of Specificity and Sensitivity

Coordinates of the Curve

Test Result Variable(s): DEL3_4

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
26.299 2	.00 0	.00 0
27.378 6	.06 7	.00 0
28.248 4	.13 3	.00 0
29.519 3	.20 0	.00 0
30.175 7	.20 0	.04 0
30.458 0	.20 0	.08 0
30.598 0	.26 7	.08 0
31.570 9	.33 3	.08 0
32.571 2	.33 3	.12 0
32.950 0	.33 3	.16 0
33.331 4	.40 0	.16 0
33.654 7	.46 7	.16 0
33.924 7	.53 3	.16 0
34.355 4	.53 3	.20 0
34.905 6	.53 3	.24 0
35.465 0	.53 3	.28 0
35.917 2	.53 3	.32 0
36.064 8	.60 0	.32 0
36.361 6	.66 7	.32 0
36.642 1	.73 3	.32 0
36.853 1	.73 3	.36 0
37.118 8	.80 0	.36 0
37.368 3	.86 7	.36 0
37.520 0	.86 7	.40 0
37.834 1	.86 7	.44 0
38.253 3	.86 7	.48 0
38.519 8	.93 3	.48 0
38.651 9	.93 3	.52 0
38.855 2	.93 3	.56 0
39.125 8	.93 3	.60 0
39.273 4	.93 3	.64 0
39.495 2	.93 3	.68 0
39.732 3	1.0 00	.68 0
39.895 6	1.0 00	.72 0
41.000 0	1.0 00	1.0 00

3

4 The smallest cutoff value is the minimum observed test value -1, and the largest cutoff value is the maximum

5 observed test value plus 1. All the other cutoff values are the average of two consecutive ordered, observed test

6 values.

[00112] The determination of the cutoff C_T of 37.3683 is shown in table 12 above. The results listed in table 12 illustrate that a cutoff C_T of 37.3683 provided the highest sensitivity and specificity.

[00113] Example 6: The 3.4kb Deletion in the Urine of Individuals with Prostate Cancer as Compared to the fluid from those without Histological Evidence of Prostate Cancer

[00114] Urine samples were collected from 5 patients who were diagnosed with prostate cancer and 5 who have had a needle biopsy procedure which was unable to detect prostate malignancy. These samples were collected following a digital rectal exam (DRE) to facilitate the collection of prostate cells.

[00115] Upon receipt of the samples a 5ml aliquot was removed and then 2mls were centrifuged at 14,000 x g to form a pellet. The supernatant was removed and discarded. Pellets were resuspended in 200ul phosphate buffered saline solution. Both the resuspended pellet and the whole urine sample were subjected to a DNA extraction procedure using the QiaAMP™ DNA Mini Kit (Qiagen P/N 51304) according to the manufacturer's directions. The resulting DNA extracts were then quantified using a NanoDrop™ ND-1000 Spectrophotometer and normalized to a concentration of 0.1ng/ul.

[00116] Samples were analyzed by quantitative real-time PCR with the 3.4kb deletion specific primers according to the following:

- 1X iQ SYBR Green Supermix™ (Bio-Rad P/N 170-8880)
- 100 nmol forward primer (5'-TAGACTACGTACATACTAACCCTACTCCTA-3') (SEQ ID NO: 2)
- 100 nmol reverse primer (5'-GAGGTAGGATTGGTGCTGT-3') (SEQ ID NO: 3)
- 1 ng template DNA
- in a 25ul reaction.

[00117] Reactions were cycled on an Opticon™ 2 DNA Engine (Bio-Rad Canada) according to the following protocol:

1. 95°C for 3 minutes
2. 95°C for 30 seconds
3. 69°C for 30 seconds

4. 72°C for 30 seconds
5. Plate Read
6. Repeat steps 2-5 44 times
7. 72°C for 10 minutes
8. Melting Curve from 50°C to 105°C, read every 1°C, hold for 3 seconds
9. 10°C Hold

[00118] Table 13 Mean values for C_T scores

Group Statistics				
	GREY110138	N	Mean	Std. Deviation
CT	Benign	5	33.2780	1.10900
	Malignant	5	30.6980	2.55767

[00119] Tables 13 and 14 show a significant difference between the mean C_T values obtained for benign sample and the malignant sample groups ($p=0.005$).

[00120] Table 14 Results Showing Difference ($p=0.005$) for C_T values of samples.

Independent Samples Test										
		Levene's Test for Equality of Variances		t Test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
CT	Equal variances assumed	1272	.292	2069	8	.072	258000	124672	-29494	54504
	Equal variances not assumed			2069	5453	.089	258000	124672	-54639	57069

[00121] Figure 6 is a Receiver Operating Characteristic (ROC) curve illustrating the specificity and sensitivity of the 3.4 kb mtDNA deletion as a marker for prostate cancer when testing urine. These results were obtained using a cutoff C_T of 31.575. The sensitivity of the marker at this C_T is 80%, while the specificity is 100%.

[00122] The determination of the cutoff C_T of 31.575 is shown in table 15. The results listed in table 15 show that a cutoff C_T of 31.575 provided the highest sensitivity and specificity.

[00123] Table 15: Determination of C_T cutoff.

Coordinates of the Curve

Test Result Variable(s): C_T		
Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
26.2900	.000	.000
28.4950	.200	.000
30.3850	.400	.000
31.0800	.600	.000
31.5750	.800	.000
32.1400	.800	.200
32.8150	.800	.400
33.8700	.800	.600
34.3350	.800	.800
34.3550	1.000	.800
35.3700	1.000	1.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

[00124] Example 7: Detection of Re-circularized 3.4kb Deleted Sequence in Prostate Malignant and Benign Tissue

In this example, the amount of re-circularized 3.4 kb deleted mtDNA molecules in samples was tested as an indicator for prostate cancer. As mentioned above, the 3.4 kb sequence, upon deletion, may reform as a circular mtDNA molecule. Amplification of a target region from the deleted 3.4 kb mtDNA sublimon was conducted using a primer pair (SEQ ID NOS: 9 and 10). The forward primer (SEQ ID NO: 9), overlaps the rejoining site of the ends of the 3.4 kb sequence.

1 [00125] Prostate tissue was formalin-fixed paraffin embedded prostate tissue needle
2 biopsies.

3 [00126] The reagent setup used for this example was as follows:

4 250nmol each primer

5 12.5ul of 2X reaction mix,

6 20ng (10ul of 2ng/ul) template in 25 ul reaction volume.

7 [00127] The cycling parameters were as follows:

- 8 1. 95 degrees Celsius for 3 minutes
- 9 2. 95 degrees Celsius for 30 seconds
- 10 3. 62 degrees Celsius for 30 seconds
- 11 4. 72 degrees Celsius for 30 seconds
- 12 5. Plate Read
- 13 6. Repeat steps 2-5 44 times
- 14 7. 72 degrees for 10 minutes
- 15 8. Melting Curve from 50-100 degrees, reading every 1 degree for 3 seconds
- 16 9 4 degrees HOLD.

17 [00128] Amplification of a target region from the deleted 3.4 kb mtDNA sublimon was
18 conducted using a primer pair (SEQ ID NOS: 9 and 10).

19 [00129] Table 16 below provides a summary of testing conducted for the detection of the
20 actual 3.4 kb deleted in mtDNA obtained from malignant and benign prostate tissue. Using a
21 C_T score of 30.0, a clear identification of malignant and benign tissue was possible. As such,
22 an increase in the amount of the 3.4 kb molecule present in a sample was indicative of cancer.

23 [00130] *Table 16: C_T scores for Detection of Cancer in Prostate Tissue*

Description	C _T
Benign sample 1	33.75
Malignant sample 1	28.79
Benign sample 2	30.96
Malignant sample 2	28.4
Benign sample 3	32.19
Malignant sample 3	27.38

1 [00131] Although the invention has been described with reference to certain specific
2 embodiments, various modifications thereof will be apparent to those skilled in the art
3 without departing from the spirit and scope of the invention as outlined in the claims
4 appended hereto.

5 [00132] **REFERENCES**

6 [00133] Birch-Machin MA, Online Conference Report (Sunburnt DNA), International
7 Congress of Biochemistry and Molecular Biology, New Scientist, 2000(a)

8 [00134] Birch-Machin MA, Taylor RW, Cochran B, Ackrell BAC, Turnbull DM. *Ann*
9 *Neurol* 48: 330-335, 2000(b)

10 [00135] Birch-Machin, M.A. (2000). Mitochondria and skin disease. *Clin Exp Dermatol*,
11 **25**, 141-6.

12 [00136] Brown, M.D., et al., *Am J. Humn Genet*, 60: 381-387, 1997

13 [00137] Bogliolo, M, et al., *Mutagenesis*, 14: 77-82, 1999

14 [00138] Chinnery PF and Turnbull DM., *Lancet* 354 (supplement 1): 17-21, 1999

15 [00139] Huoponen, Kirsi, *Leber hereditary optic neuropathy: clinical and molecular*
16 *genetic findings*, *Neurogenetics* (2001) 3: 119-125.

17 [00140] Hayward SW, Grossfeld GD, Tlsty TD, Cunha GR., *Int J Oncol* 13:35-47, 1998

18 [00141] Huang GM, Ng WL, Farkas J, He L, Liang HA, Gordon D, Hood R., *Genomics*
19 *59*(2):178-86,1999

20 [00142] Konishi N, Cho M, Yamamoto K, Hiasa Y. *Pathol. Int.* 47:735-747,1997

21 [00143] Landis SH, Murray T, Bolden S, Wingo PA. *Cancer J. Clin.* 49:8-31

22 [00144] Lee HC, Lu CY, Fahn HJ, Wei YHu. *Federation of European Biochemical*
23 *Societies*, 441:292-296,1998

24 [00145] Mitochondrial Research Society <http://www.mitoresearch.org/diseases.html>.

25 [00146] MITOMAP: A human mt genome database (www.gen.emory.edu/mitomap.html)

- 1 [00147] Naviaux, RK., Mitochondrial Disease- Primary Care Physican's Guide. Psy-Ed.
2 Corp D/B/A *Exceptional Parents Guide*: 3-10, 1997
- 3 [00148] Parrella P, Xiao Y, Fliss M, Sanchez-Cespedes M, Mazzarelli P, Rinaldi M, Nicol
4 T, Gabrielson E, Cuomo C, Cohen D, Pandit S, Spencer M, Rabitti C, Fazio VM, Sidransky
5 D: Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle
6 aspirates. *Cancer Res* 2001, 61:7623-7626
- 7 [00149] Polyak Y, et al., *Nature Genet.* 20 (3):291-293, 1998
- 8 [00150] Seidman, M.D. et al., *Arch. Otolaryngol Head Neck Surg.*, 123: 1039-1045, 1997
- 9 [00151] Sherrat EJ, Thomas AW, Alcolado JC., *Clin. Sci.* 92:225-235,1997
- 10 [00152] Shoffner JM, Brown MD, Torroni A, Lott MT, Cabell MF, Mirra SS, Beal MF,
11 Yang C, Gearing M, Salvo R, Watts RL, Juncos JL, Hansen LA, Crain BJ, Fayad M,
12 Reckford CL, and Wallace DC., *Genomics* 17: 171-184, 1993
- 13 [00153] SpringNet – CE Connection: Screening, Diagnosis: Improving Primary Care
14 Outcomes. Website: <http://www.springnet.com/ce/j803a.htm>
- 15 [00154] Taniike, M. et al., *BioChem BioPhys Res Comun*, 186: 47-53, 1992
- 16 [00155] Valnot, Isabelle, et al., A mitochondrial cytochrome b mutation but no mutations
17 of nuclearly encoded subunits in ubiquinol cytochrome c reductase (complex III) deficiency,
18 *Human Genetics* (1999) 104: 460-466
- 19 [00156] von Wurmb, N, Oehmichen, M, Meissner, C., *Mutat Res.* 422:247-254, 1998
- 20 [00157] Wallace et al., Mitochondiral DNA MUTatio Assoicated with Leber's Hereditary
21 Optic Neuropathy, *Science.* 1427-1429
- 22 [00158] Wei YH. Proceedings of the Nat. Sci. Council of the Republic of China April
23 22(2):5567, 1998
- 24 [00159] Woodwell DA. National Ambulatory Medical Care Survey: 1997 Summary.
25 Advance data from vital and health statistics; no. 305. Hyattsville, Maryland: National
26 Center for Health Statistics. 1999

- 1 **[00160]** Yeh, J.J., et al., *Oncogene Journal*, 19: 2060-2066, 2000
- 2 **[00161]** Zhang et al., Multiple mitochondrial DNA deletions in an elderly human
3 individual, *FEBS Lett*, 297, 34-38 1992
- 4 **[00162]** Zhang, C., et al., *BioChem. BioPhys. Res. Comun.*, 195: 1104-1110, 1993

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CLAIMS:

1. A method of detecting a cancer in an individual comprising:
 - a) obtaining a biological sample from the individual;
 - b) extracting mitochondrial DNA, mtDNA, from the sample;
 - c) quantifying the amount of mtDNA in the sample having a deletion in the nucleic acid sequence between residues 10744 and 14124 of the mtDNA genome;
 - d) comparing the amount of mtDNA in the sample having the deletion to at least one known reference value.
2. The method of claim 1 wherein the deletion has a nucleic acid sequence corresponding to the sequence identified in SEQ ID NO: 1.
3. The method of claim 1 wherein the at least one known reference value is the amount of the deletion in a reference sample of mtDNA from known non-cancerous tissue or body fluid.
4. The method of claim 1 wherein the at least one known reference value is the amount of the deletion in a reference sample of mtDNA from known cancerous tissue or body fluid.
5. The method of claim 1 wherein the step of quantifying is conducted using real-time PCR.
6. The method of claim 5 wherein the quantifying of the deletion includes first amplifying a target region of mtDNA that is indicative of the deletion, and quantifying the amount of the amplified target region.
7. The method of claim 5 wherein a PCR primer having a sequence corresponding to SEQ ID NO: 2 is used as part of a pair of amplification primers for amplifying the target region.
8. The method of claim 1 wherein the cancer is prostate cancer.

9. The method of claim 1 wherein the cancer is breast cancer.
10. The method of claim 1 wherein the biological sample is a body tissue or body fluid.
11. The method of claim 10 wherein the biological sample is selected from the group consisting of breast tissue, prostate tissue, prostate massage fluid, and urine.
12. The method of claim 6 wherein the reference value is a cycle threshold.
13. A method of detecting a cancer in an individual comprising;
 - a) obtaining a biological sample from the individual;
 - b) extracting mitochondrial DNA, mtDNA, from the sample;
 - c) quantifying the amount of mtDNA in the sample having a deletion in the nucleic acid sequence between residues 10744 and 14124 of the mtDNA genome;
 - d) comparing the amount of mtDNA in the sample having the deletion to the amount of the deletion in a reference sample of mtDNA from known non-cancerous tissue or body fluid;wherein an elevated amount of the deletion in the biological sample compared to the reference sample is indicative of cancer.
14. The method of claim 13 wherein the deletion has a nucleic acid sequence corresponding to the sequence identified in SEQ ID NO: 1.
15. The method of claim 13 further comprising the step of comparing the amount of mtDNA in the sample having the deletion to the amount of the deletion in a reference sample of mtDNA from known cancerous tissue or body fluid.
16. The method of claim 13 wherein the quantifying of the deletion includes amplifying a target region of mtDNA that is indicative of the deletion, and quantifying the amount of the amplified target region.

17. The method of claim 16 wherein a PCR primer having a sequence corresponding to SEQ ID NO: 2 is used as part of a pair of amplification primers for amplifying the target region.
18. The method of claim 16 wherein the step of quantifying is conducted using real-time PCR.
19. The method of claim 13 wherein the cancer is prostate cancer.
20. The method of claim 13 wherein the cancer is breast cancer.
21. The method of claim 13 wherein the biological sample is a body tissue or body fluid.
22. The method of claim 21 wherein the biological sample is selected from the group consisting of breast tissue, prostate tissue, prostate massage fluid, and urine.
23. A method of detecting a cancer in an individual comprising;
 - a) obtaining a biological sample from the individual;
 - b) extracting mitochondrial DNA, mtDNA, from the sample;
 - c) quantifying the amount of mtDNA in the sample having a deletion in the nucleic acid sequence between residues 10744 and 14124 of the mtDNA genome;
 - d) comparing the amount of mtDNA in the sample having the deletion to the amount of the deletion in a reference sample of mtDNA from known cancerous tissue or body fluid;wherein a similar level of the deletion in the biological sample compared to the reference sample is indicative of cancer.
24. The method of claim 23 wherein the deletion has a nucleic acid sequence corresponding to the sequence identified in SEQ ID NO: 1.
25. The method of claim 23 further comprising the step of comparing the amount of mtDNA in the sample having the deletion to the amount of the deletion in a reference sample of mtDNA from known non-cancerous tissue or body fluid.

26. The method of claim 23 wherein the quantifying of the deletion includes amplifying a target region of mtDNA that is indicative of the deletion, and quantifying the amount of the amplified target region.
27. The method of claim 26 wherein a PCR primer having a sequence corresponding to SEQ ID NO: 2 is used as part of a pair of amplification primers for amplifying the target region.
28. The method of claim 26 wherein the step of quantifying is conducted using real-time PCR.
29. The method of claim 23 wherein the cancer is prostate cancer.
30. The method of claim 23 wherein the cancer is breast cancer.
31. The method of claim 23 wherein the biological sample is a body tissue or body fluid.
32. The method of claim 31 wherein the biological sample is selected from the group consisting of breast tissue, prostate tissue, prostate massage fluid, and urine.
33. A method of monitoring an individual for the development of a cancer comprising;
- a) obtaining a biological sample;
 - b) extracting mtDNA from the sample;
 - c) quantifying the amount of mtDNA in the sample having a deletion in the nucleic acid sequence between residues 10744 and 14124 of the mtDNA genome;
 - d) repeating steps a) to c) over a duration of time;
 - e) wherein an increasing level of the deletion over the duration of time is indicative of cancer.

34. The method of claim 33 wherein the deletion has a nucleic acid sequence corresponding to the sequence identified in SEQ ID NO: 1.
35. The method of claim 33 further comprising at least one step selected from the group consisting of: (a) comparing the amount of mtDNA in the sample having the deletion to the amount of the deletion in a reference sample of mtDNA from known non-cancerous tissue or body fluid; and (b) comparing the amount of mtDNA in the sample having the deletion to the amount of the deletion in a reference sample of mtDNA from known cancerous tissue or body fluid.
36. The method of claim 33 wherein the quantifying of the deletion includes amplifying a target region of mtDNA that is indicative of the deletion, and quantifying the amount of the amplified target region.
37. The method of claim 36 wherein the step of quantifying is conducted using real-time PCR.
38. The method of claim 36 wherein a PCR primer having a sequence corresponding to SEQ ID NO: 2 is used as part of a pair of amplification primers for amplifying the target region.
39. The method of claim 33 wherein the cancer is prostate cancer.
40. The method of claim 33 wherein the cancer is breast cancer.
41. The method of claim 33 wherein the biological sample is a body tissue or body fluid.
42. The method of claim 41 wherein the biological sample is selected from the group consisting of breast tissue, prostate tissue, prostate massage fluid, and urine.

43. The method according to any one of claims 6, 16 or 26 wherein the amplifying of the target region is conducted using a pair of amplification primers, one of the pair of amplification primers overlapping a splice joining regions on opposite ends of the deletion.
44. A method of detecting a cancer in an individual comprising:
- a) obtaining a biological sample from the individual;
 - b) extracting mitochondrial DNA, mtDNA, from the sample;
 - c) quantifying the amount of mtDNA in the sample having a sequence corresponding to the sequence identified in SEQ ID NO: 1;
 - d) comparing the amount of mtDNA in the sample corresponding to SEQ ID NO: 1 to at least one known reference value.
45. The method of claim 44 wherein the at least one known reference value is the amount of the sequence corresponding to SEQ ID NO: 1 in a reference sample of mtDNA from known non-cancerous tissue or body fluid.
46. The method of claim 44 wherein the at least one known reference value is the amount of the sequence corresponding to SEQ ID NO: 1 in a reference sample of mtDNA from known cancerous tissue or body fluid.
47. The method of claim 44 wherein the step of quantifying is conducted using real-time PCR.
48. The method of claim 47 wherein the quantifying of the deletion includes first amplifying a target region of mtDNA that is indicative of the deletion, and quantifying the amount of the amplified target region.
49. The method of claim 44 wherein one of a pair of PCR primers used in the amplifying of the target region overlaps a rejoining site of the sequence corresponding to SEQ ID NO: 1, after the sequence has re-circularized.

50. The method of claim 47 wherein a PCR primer having a sequence corresponding to SEQ ID NO: 9 is used as part of a pair of amplification primers for amplifying the target region.
51. The method of claim 44 wherein the cancer is prostate cancer.
52. The method of claim 44 wherein the cancer is breast cancer.
53. The method of claim 44 wherein the biological sample is a body tissue or body fluid.
54. The method of claim 53 wherein the biological sample is selected from the group consisting of breast tissue, prostate tissue, prostate massage fluid, and urine.
55. The method of claim 47 wherein the reference value is a cycle threshold.