

CANCER MARKERS AND DETECTION METHODS

FIELD OF THE INVENTION

The present invention relates to methods of detecting cancer markers in the blood of a subject, such as a human suspected of having cancer. The invention more particularly relates to methods of detecting metastatic cancer or other cancers that release markers into the blood. It may be used for initial diagnosis and prognosis, treatment direction, and treatment or disease monitoring. Detection may be accomplished using cancer detection reagents corresponding to the cancer markers.

BACKGROUND

Cancer results when a cell in the body malfunctions and begins to grow uncontrollably. These malfunctions result from mutations in the cell's DNA blueprint. Thus, while early cancer diagnosis focused on the growth properties and the physical appearance of suspected cancer cells, more modern techniques have begun to examine the cell's inner workings.

Not all cancers are caused by the same mutation. Some treatments that work well for particular cancer-causing mutations are ineffective against cancer having other types of mutations and may actually cause more harm than good if inappropriately prescribed. Thus, it is imperative that cancer diagnostics' ability to distinguish different types of cancer keep pace with the ability to treat different types of cancers appropriately. Current diagnostic methods are struggling to match the speed at which new treatments are developed.

Another problem with current cancer diagnostic methods lies in the need for tissue samples to analyze.

All presently successful cancer diagnostic methods, other than pure imaging, require cancer cells to be removed from the patient's body. These cells are most commonly obtained from a tissue biopsy. While effective, tissue
5 biopsies are expensive, time-consuming, and painful for the patient. Additionally, the time required to schedule and obtain a tissue biopsy then analyze it causes a delay in treatment and the biopsy process itself may release cancer cells into the blood stream, resulting in
10 increased metastasis.

Even worse, in some cases a tissue biopsy is not possible due to the location of a tumor. In those instances, the exact nature of the cancer cannot be determined until after surgery has been performed and the
15 tumor removed. While these post-operative tests are still useful in directing further treatment of the patient, if the nature of the tumor could be determined in advance, it might be much more feasible to try non-invasive treatments, such as chemotherapy, before putting
20 a patient through the rigors of surgery. Even if surgery were required, the patient might still benefit from a more detailed pre-operative diagnosis. Such a diagnosis might, for example, allow pre-operative treatment with drugs designed to minimize the chance of metastatic
25 spread of cancer cells dislodged from the tumor during surgery. It might also provide greater direction for surgical techniques, such as how much tissue surrounding the tumor to remove.

Currently, some of the most successful cell-based
30 diagnostic methods utilize non-biopsy samples. For example, PAP smears look for cellular irregularities, but utilize cells normally sloughed off by the body. PAP

smears continue to save thousands of lives each year by allowing easy and very early detection of cells in the process of becoming cervical cancer.

Because of problems associated with biopsies and the
5 success of simpler methods, such as PAP smears, the medical community has spent years searching for cancer diagnostics using another readily available sample, blood, particularly peripheral blood. Their efforts have met with some success. For example, the progress or
10 recurrence of prostate cancer is readily monitored using a blood test. However, current blood-based cancer diagnostics, like the prostate cancer test, still remain focused on particular types of cancer. The need remains for a cancer diagnostic able to use blood to diagnose a
15 wide variety of cancers or cancer in general.

Outside of tissue-based cancer diagnostics, most diagnostic methods rely on imaging techniques ranging from simple X-rays to MRIs and nuclear imaging, often using cancer- or tissue-targeted contrast agents to
20 produce better images. However, even the most powerful imaging techniques cannot detect tumors smaller than about 2-5 mm in diameter. By the time a tumor has reached that size, it contains thousands of cells. Further, these sophisticated imaging techniques are too
25 expensive to use during early stages of cancer, when the patient otherwise has no symptoms besides a small tumor that could easily be removed. Rather, complicated imaging diagnostics are most often reserved for patients who have had a large primary tumor and are suspected of
30 having developed metastatic cancer. The small tumors detected are actually metastases produced as the cancer has spread. Thus, unlike primary tumors which often

contain large numbers of benign cells, the small tumors detected contain thousands of malignant, metastatic cells, each of which is able to seed another tumor elsewhere in the body.

5 Clearly, detection of small metastatic tumors through current imaging techniques is really a last-ditch effort to save a critically ill patient. If these metastatic cells could be detected much earlier, such as when they first begin to travel through the blood, then a
10 patient could begin receiving treatment for all of the metastatic tumors he or she would likely have while those tumors were still far too small to be detected by diagnostic imaging or any other current techniques. Thus a need exists for much earlier diagnosis of metastatic
15 tumors, or detection of a greatly increased likelihood of metastatic tumors.

Yet another drawback in modern cancer diagnosis relates to its ability to be coupled to treatment. While some common mutations can be diagnosed through tissue
20 samples and used to direct treatment somewhat specific for the patient's type of cancer, this approach is applicable for only a few types of cancer. Currently no diagnostic method is able to detect a wide range of types of cancer or to provide detailed targets for treatment in
25 numerous types of cancer.

Finally, current cancer diagnostics, particularly those that rely upon tissue biopsies, are very poor at monitoring the progress or effectiveness of treatment. Thousands of dollars and possibly even patients' lives
30 could be saved if treating physicians were able to tell when all or a substantial number of the cancer cells, or of a particular type of cancer cell have been eradicated.

Additionally, by their nature cancer cells are able to change very rapidly. Thus, they may mutate even further during the course of a treatment, causing what was once a helpful drug to become powerless or harmful. In essence, the cancer cells may become resistant to the drug, much as bacteria become resistant to antibiotics. Cancer treatment would benefit greatly from diagnostic methods able to detect these and other changes that show the effectiveness of treatment or any further mutations of the patient's cancer cells.

SUMMARY

The present invention relates to cancer markers, in particular a hyperset of markers for cancer generally and supersets of markers for a specific type of cancer, as well as subsets of this hyperset and supersets.

The invention also relates to methods of screening blood or tissue using cancer detection reagents to detect cancer markers. Cancer detection reagents are short nucleic acids at least 17 bases in length having a specific sequence determined to correlate with the presence of cancer in a subject, but not with healthy tissue. Thus, the present invention relates to pathology-based diagnostics.

When blood is screened, it may be any type of blood, but to facilitate obtaining a sample, in most instances peripheral blood may be used. Although aspects of the present invention may be employed to detect cancer in a tissue, the descriptions here focus on peripheral blood due to the relative ease of obtaining a peripheral blood sample from a subject and its capacity to represent the cancer status of an entire animal, rather than a single tumor. However, it will be apparent to one skilled in

the art how to adapt techniques designed for peripheral blood for use with other blood or tissues.

Cancer markers may include any mutation in the transcribed portions of the cellular DNA of a cell.

5 These mutations may be detected through analysis based on the cancer cell's DNA or its mRNA using cancer detection reagents that correspond to the mutated DNA region, or cancer marker. In specific embodiments, PCR analysis, microarray analysis, or bead-based analysis may be used
10 for cancer marker assays.

The cancer markers and corresponding cancer detection reagents were identified using proprietary software to examine databases of transcribed nucleic acid sequences from known cancers and cancer cell lines and to
15 compare the sequences to the normal human transcriptome. Thus, these nucleic acid sequences represent mutations or abnormalities as compared to the transcriptome of humans without cancer. Specifically, the cancer markers are present in mRNA transcripts from cancer and universally
20 absent in the entire healthy human transcriptome. Because the cancer markers only include transcribed sequences exclusive to cancer cells, they correspond to cancer-related mutations. Such mutations may include somatic mutations resulting in cancer, or they may also
25 include rare abnormal variations present in the subject's genome.

Cancer detection reagents corresponding to these cancer markers, alone or in combination, may be used to determine the cancer marker profile of a subject. The
30 cancer detection reagents may be used to detect cancer and to monitor the process of the cancer or of its treatment. Additionally, testing with the cancer

detection reagents may be used to provide a cancer marker profile showing several mutations or abnormalities present in one or more metastatic cancer cells within the subject. Repeated testing can detect changes in the
5 cancer marker profile of a subject, perhaps indicating the efficacy of treatment or the development of different metastatic cells.

In abundance among the cancer markers are sequences that repetitively occur in different cancer mRNA
10 transcripts, thereby giving the cancer markers a one-to-many genetic association. This means one cancer detection reagent can detect multiple genes, each having the same cancer marker, and the detection is not dependent on the expression level of a single gene. The
15 net result, both in-vitro and in-situ, is an enhanced detection capacity, facilitating detection even in samples having relatively low numbers of metastasized cancer cells.

All of the cancer markers will not be found in every
20 cancer patient's blood or tumors. Instead, each patient will typically have a subset of the cancer markers present in their blood or tumors. Because many cancer markers are each associated with one or more genes, these subsets automatically produce genetic profiles that
25 reflect the individuality of the patient's cancer.

In a specific embodiment, a general cancer diagnostic may be provided. Specifically, it has been determined that, while there are some variations in cancer markers among different types of cancer, some
30 markers are very common in multiple types of cancer. Thus, a general diagnostic assay including these markers is provided. Such an assay may be particularly useful

for routine screening or early diagnosis, when it is not known whether a subject has cancer, or the type of cancer the subject may have.

Additionally, cancer markers specific for certain
5 types of cancer have been determined and ranked based on frequency of occurrence. For example, a subset of 59 markers frequently found in colon cancer have been located and used to create cancer detection reagents. Using these cancer type-specific sets of markers,
10 diagnostic assays for a particular type of cancer are provided. These assays may be particularly useful in monitoring the progress or treatment of existing cancer. They may also be useful for routine diagnosis in subjects known to have a susceptibility to a particular type of
15 cancer.

Finally, most cancer markers have been found in more than one gene. Thus, a diagnostic assay using a cancer detection reagent narrowly tailored to the cancer marker is very powerful in general cancer detection, but less
20 useful in knowing which genes are affected. Knowledge of affected genes may affect the prognosis for or treatment of a patient. Thus, in yet another embodiment of the invention, gene-selective cancer detection reagents are provided. Such reagents are readily developed once a
25 cancer marker has been identified. The cancer marker sequence may be located in a given gene, then flanking sequences found in the wild type gene may be included in a cancer detection reagent. Preferably, the flanking sequences included are of sufficient length to allow
30 identification of the gene or genes having the cancer marker mutation in that subject, while remaining compatible with the type of assay being conducted.

Knowledge of the mutations present in a patient's cancer cells may be used in directing treatment. For example, drugs known to be effective against certain types of cancer or mutations in certain genes only may be prescribed or avoided based on the underlying mutations of a patient's cancer. Additionally, knowledge of patient-specific cancer mutations may be used to develop new classes of cancer drugs, including patient-specific cancer drugs targeted to the diagnosed mutations. These targeted drugs may affect the mutant proteins, particularly cell-surface proteins, or they may act on cellular nucleic acids, such as mRNA.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention may be better understood through reference to the following detailed description taken in conjunction with the FIGURES which illustrate various embodiments of the invention.

FIGURE 1 illustrates several mutant cancer markers of the present invention found in the LTBR gene as compared to the sequence from healthy cell transcriptomes. The location of a single nucleotide polymorphism (SNP) is indicated.

FIGURE 2 illustrates a portion of an alignment between mRNA from four different cancer cell lines and four different cancer types, mapped to the corresponding healthy mRNA from 17 different genes.

FIGURE 3 illustrates a method of detecting a cancer marker.

FIGURE 4 illustrates a sample cancer detection reagent.

FIGURE 5 illustrates disparity in the presence of two common cancer markers between cancer cell lines.

FIGURE 6 illustrates correlation between individual cancer markers and cancer types.

5 FIGURE 7 illustrates a method for PCR Reduction using cancer detection reagents.

FIGURE 8 presents the results of PCR Reduction as analyzed on gels for cDNA from a healthy human and from tumor or blood samples of two cancer subjects.

10 FIGURE 9 illustrates a method of blood testing and cancer marker profiling.

DETAILED DESCRIPTION

The present invention relates to the detection of cancer, particularly metastatic cancer in a subject using
15 an assay to detect cancer markers in samples from the subject. In a particular embodiment, detection may be accomplished using cancer detection reagents corresponding to the cancer markers.

The cancer detection reagents used in the present
20 invention are presented primarily in the form of short DNA or other nucleic acid oligomers which correspond to cancer markers. These cancer markers have all been previously exhibited in cancerous tissue in a human. They may include mutations that imminently gave rise to
25 the cancer, earlier mutations that likely increased the propensity for cancer, or abnormal allelic variants of a gene. Many are located in the transcribed portions of cellular DNA, particularly the exons of genes. However, cancer markers in accordance with the present invention
30 may also correspond to other mutated DNA regions. Additionally, the markers may be detected in a sample

using techniques that detect or amplify the mRNA or DNA in the sample. However, the markers may also be detected through assays for the peptides they encode, which may be predicted from the cancer marker sequences.

5 Cancer detection reagents may include both single-stranded and complementary double-stranded nucleic acids. The appropriate form of nucleic acids to use as a cancer detection reagent to identify a cancer marker will be apparent to one skilled in the art.

10 *Identification of Cancer Markers*

 The cancer markers of the present invention were isolated using proprietary software and information from public databases recording genetic information about cancerous and healthy cells and tissues. Specifically,
15 using proprietary software and supercomputers, random portions of mRNA data from cancer cell lines were compared to all the available mRNA data from all healthy cell lines, as diagramed in FIGURE 3. This process yielded a database of cancer markers such as the two in
20 FIGURE 1 and FIGURE 2.

 The resultant database is referred to as the general cancer marker hyperset, which contains the sequences of hundreds of thousands of cancer markers, which may be embodied in cancer detection reagents of length 17 mer or
25 greater, grouped into supersets according to cancer type. Each cancer marker in a superset must show up at least once in a cancer cell corresponding to the superset's cancer type. There is redundancy among the supersets because the cancer markers usually appear in supersets
30 for many different cancer types.

The total number of cancer markers in the total cancer hyperset is constantly increased. Computer software currently runs non-stop, adding several thousand new cancer markers each month. Further, as new cancers
5 arise, new cancer markers may be created. Based on currently available data, it is known that a superset for a single type of cancer may contain tens of thousands of cancer markers.

Because the cell lines used to isolate the mRNA
10 molecules that contained the cancer markers are known and were derived from human subjects with cancer, it is possible to count these cell lines as past occurrences of the cancer markers in humans, as shown in FIGURE 4. This yields a simple method for ranking the likelihood of
15 occurrence of each cancer marker based on its past rate of occurrence in cancer cell lines.

Cancer markers represent a special kind of cancer mutation - one that has nucleic acid content exclusive to cancer cells. If such exclusivity were not present, the
20 mutation would not be considered a cancer marker, as shown in FIGURE 3. This condition in selecting cancer markers produces cancer detection reagents that detect useful differences in the genetics of cancer cells. This is an important criteria for diagnosing and treating
25 cancer.

Multi-Gene Aspects

The cancer markers and detection reagents of the present invention are generally small and thus unsuitable for genomic mapping. However, the mRNA molecules
30 containing the unisolated cancer markers can be mapped. In this manner, one may determine which genes are associated with each cancer marker.

Many genes may be associated with each cancer marker - the number of genes is normally in direct correlation to the number of unique mRNA molecules containing each cancer marker found in the public databases. Sometimes, 5 hundreds of mRNA molecules in the databases contain a cancer marker, yielding hundreds of mapped genes. This is evident in TABLES 1 and 2.

While many of the cancer markers are located in genes with no currently known relevance to cancer, some 10 are located in genes known to be important in cancer. These cancer markers often represent SNPs, cryptic splicing and other genetic defects. For example, FIGURE 1 illustrates a cancer detection reagent found in the Lymphotoxin Beta Receptor (LTBR) gene.

15 FIGURE 1 shows that the same point mutation occurs in the same gene in different subjects with different types of cancer. Specifically, FIGURE 1 shows a portion of an alignment between LTBR mRNA from eight different cancer cell lines and six different cancer types, mapped 20 to the corresponding healthy LTBR mRNA. As the figure shows, the eight cancer LTBRs vary slightly between each other and the healthy LTBR. However at location 6959 bp, the cancer LTBRs vary identically, each missing a guanine (G) and yielding the same cancer marker, 25 CCTGAGCAAACCTGAGC. This marker's presence in LTBR nucleic acids in a cell is an indicator of cancer's presence. This is a one-to-one genetic association.

FIGURE 2 shows that the same cancer marker can result from different mutations in different genes, in 30 different subjects with different types of cancer. Specifically, FIGURE 2 shows a portion of an alignment between mRNA from four different cancer cell lines and

four different cancer types, mapped to the corresponding healthy mRNA from 17 different genes. The mutations vary from gene to gene, but the net result is that the same cancer marker, CGCATGCGTGGCCACCA, is present in each
5 gene. This marker's presence in each of the 17 genes is an indicator of cancer's presence in the corresponding cell or tissue. This sequence has a one-to-many genetic association.

The cancer markers shown in FIGURE 1 and FIGURE 2
10 are not dependent on any common functionality among the genes in which they appear or in the tissues in which these genes are expressed. Further, neither cancer marker has been found in the healthy human transcriptome. Therefore the presence of these markers in any mRNA
15 transcript, not just those from genes shown in the figures, is an indicator of cancer's presence in the host cell. Because the sequences represent mRNAs exclusive to cancer cells, they reflect cancer-associated mutations. Also, if they are detected, one immediately knows which
20 set of genes may contain them.

Cancer markers may be common to many genes and many cancers. This does not mean that every cancer marker will exist in every cancer cell line or cancer subject. This is demonstrated in FIGURE 5 for two cancer markers
25 and the cancer cell lines in which they occur.

Specific Subsets of Markers

Analysis of the cancer marker hyperset and supersets has revealed that a number of cancer markers are found frequently in a variety of different types of cancer.
30 Thus these cancer markers may be identified as general cancer markers. General cancer markers have been identified and are included in TABLEs 1 and 2. These

cancer markers were first identified as high frequency colon cancer markers and may also be used for that purpose.

TABLEs 1 and 2 lists the highest ranked 59 cancer markers in the colon cancer superset. These 59 cancer markers constitute a high frequency colon cancer marker subset. Associated genes are also indicated. Combined, there are over 1000 genes represented in the table. This means that the 59 colon cancer markers, when used in a detection capacity, can detect mutations in over 1000 genes - a sensitivity made possible by their one-to-many genetic association.

TABLE 1: Cancer Detection Reagents

ID	Candidate Apoptotic Sequence	Affected Cancers
5	+ GCCCAAGGAACCCCTT - AAGGGGGTTCCTTGGGC	ovarian colorectal brain epid testis liver
Targeted Genes		
CHCHD3 (7) EEF1G (11) LOC136337 (X) ABCC3 (17)		

ID	Candidate Apoptotic Sequence	Affected Cancers
8	+ GCTCAGGTTTGCTCAGG - CCTGAGCAAACCTGAGC	ovarian colorectal lung testis liver skin
Targeted Genes		
LTBR (12)		

ID	Candidate Apoptotic Sequence	Affected Cancers
9	+ TGTGCTTCTGGCAGGCC - GGCCTGCCAGAAGCACA	breast colorectal brain adrenal eye
Targeted Genes		
GNB2L1 (5)		

ID	Candidate Apoptotic Sequence	Affected Cancers
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10	+ ACCTGGGATCCAGTTGGAGGACGGC - GCCGTCCTCCAACCTGGATCCCAGGT	colorectal lung brain
Targeted Genes		
ZNF500 (16)		

ID	Candidate Apoptotic Sequence	Affected Cancers
11	+ CGCATGCGTGGCCACCA - TGGTGGCCACGCGATGCG	colorectal brain lymph
Targeted Genes		
LOC388707 (1) LAMR1 (3) LOC389672 (8)		

ID	Candidate Apoptotic Sequence	Affected Cancers
12	+ CCCAGCAGGGACATCCG - CGGATGTCCCTGCTGGG	ovarian colorectal lung cervix uterus skin pancreas testis liver
Targeted Genes		
MOV10 (1)		

ID	Candidate Apoptotic Sequence	Affected Cancers
13	+ GGCTAGGTACGAGGCTGG - CCAGCCTCGTACCTAGCC	ovarian colorectal lung brain uterus skin kidney pancreas muscle lymph eye
Targeted Genes		
AACS (12) AAMP (2) ABCF3 (3) ACTB (7) ACTBP2 (5) ACTG1 (17) ACTN1 (14) ADCK4 (19) ADPRT (1) AES (19) AFG3L2 (18) AHSA1 (14) AIPL1 (17) AKT1 (14) ALDOA (16) ANAPC2 (9) ANKRD19 (9) ANXA11 (10) ANXA7 (10) AP1M1 (19) AP2A1 (19) AP2M1 (3) APCL (19) APOE (19) ARHGDIA (17) ARHGEF1 (19) ARHGEF16 (1) ARL6IP4 (12) ARPC2 (2) ASPH (8) 11ASRGL1 (11) ASS (9) ATF4 (22) ATF5 (19) ATP1A1 (1) ATP5A1 (18) ATP5F1 (1) ATP5O (21) AUTL2 (X) AZ2 (3) bA395L14.12 (2) BAT3 (6) BCAS3 (17) BLP1 (8) BRMS1 (11) BSG (19) BTF3 (5) C10orf45 (10) C14orf126 (14) C20orf41 (20) 2orf17 (2) C3orf4 (3) C4orf9 (4) C5orf6 (5) C6.1A (X) C6orf107 (6) 6orf11 (6) C6orf48 (6) C7orf30 (7) CACNA2D3 (3) CAMKK2 (12) CASP4 (11) CASQ1 (1) CBS (21) CBX7 (22) CBX8 (17) CCND3 (6) CCT3 (1) CCT5 (5) CCT6A (7) CCT7 (2) CD74 (5) CD79A (19) CD79B (17) CDC20 (1) CDC2L2 (1) CDCA5 (11) CDCA8 (1) CDH12 (5)		

CDH24(14) CDIPT(16) CDK4(12) CDW92(9) CEECAM1(9) CENPB(20)
 CGI-96(22) CHCHD3(7) CIDEB(14) CNOT10(3) COMT(22) ORO1A(16)
 CORO2A(9) COTL1(16) CRN(4) CRTAP(3) CRYBB2P1(22) CS(12)
 CTAG3(6) CYB5-M(16) DBH(9) DBI(2) DCLRE1C(10) DCTN2(12)
 DDB1(11) DDX10(11) DDX56(7) DGCR8(22) DGKA(12) DHCR24(1)
 DKFZp434B227(3) DKFZp434C171(5) DKFZp434K046(16)
 DKFZp564D172(5) DKFZp564K142(X) DKFZp586M1819(8) DNAJB1(19)
 DNCH1(14) DNM2(19) DRIM(12) DustyPK(1) E1B-AP5(19) E2F4(16)
 EDARADD(1) EEF1D(8) EEF1G(11) EEF2(19) EIF2B5(3) EIF2S1(14)
 eIF3k(19) EIF3S1(15) EIF3S2(1) EIF3S5(11) EIF3S7(22)
 EIF3S8(16) EIF3S9(7) EIF4G1(3) ELMO2(20) ENDOG(9) ENO1(1)
 ENO1P(1) ENTPD8(17) EPAC(12) ETFDH(4) FAH(15) FAM31B(1)
 FANCA(16) FBL(19) FBXO7(22) FDFT1(8) FECH(18) FGFR4(5)
 FKBP1B(2) FKBP8(19) FKSG17(8) FLI1(11) FLJ00038(9)
 FLJ10241(19) FLJ12750(12) FLJ12875(1) FLJ14800(12)
 FLJ14827(12) FLJ20071(18) FLJ20203(1) FLJ20294(11)
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 FLJ25222(15) FLJ27099(14) FLJ31121(5) FLJ32452(12)
 FLJ35827(11) FLJ38464(9) FLJ44216(5) FMN2(1) FMO5(1)
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 GALK2(15) GAPD(12) GCC1(7) GCDH(19) GDI2(10) GA1(22)
 GGCX(2) GIT1(17) GLUL(1) GNB2L1(5) GOLGB1(3) GPAA1(8)
 GPI(19) GRHRP(9) GRSF1(4) GSPT1(16) GSTM4(1) GYS1(19)
 H3F3B(17) HAND1(5) HARS2(20) HAX1(1) HCA127(X) HCCR1(12)
 HCG4(6) HDAC1(1) HDLBP(2) HLA-B(6) HMGA1(6) HMGA1L3(12)
 HMGNI(21) HMGNI(1) HNRPD(4) HNRPH3(10) HNRPU(1) HPS4(22)
 HRMT1L1(21) HS3ST4(16) HSA9761(5) HSPA9B(5) HSPB1(7)
 HSPC142(19) HSPC242(22) HSPCB(6) HSPCP1(4) HSPD1(2) ID3(1)
 IER3(6) IGFBP4(17) IGHV4-34(14) L1RL1LG(19) ILF2(1)
 ILVBL(19) IMPDH2(3) ITGB4BP(20) JIK(12) JM4(X) K-ALPHA-
 1(12) KCNN2(5) KCTD1(18) KHSRP(19) KIAA0141(5) KIAA0182(16)
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 PPP1R8(1) PPP2R1A(19) PPP4C(16) PRAME(22) PRDX1(1)
 PRKACA(19) PRNPIP(1) PRO1855(17) PRPF31(19) PSAP(10)
 PSMC2(7) PSMD2(3) PSME1(14) PSPC1(13) PTBP1(19) PTPN6(12)
 PTPRCAP(11) PTPRD(9) PTPRG(3) PTTG1IP(21) PYCR1(17)
 RAB32(6) RAE1(20) RALGDS(9) RAN(12) RANP1(6) RARS(5)
 RASAL1(12) RBBP7(X) RDH11(14) REC14(15) RER1(1) RFC2(7)
 RGS16(1) RHEBL1(12) R1OK1(6) RNF10(12) RNF20(9) RNF8(6)
 RoXaN(22) RPL10(X) RPL10P1(21) RPL13(16) RPL14(3) RPL15(3)
 RPL15P2(14) RPL24(3) RPL28(19) RPL3(22) RPL30(8) RPL35(9)
 RPL35A(3) RPL37A(2) RPL37AP1(20) RPL5(1) RPL8(8) RPL9(4)
 RPLP0(12) RPLP0P2(11) RPLP2(11) RPS10(6) RPS14(5) RPS15(19)
 RPS16(19) RPS17(15) RPS17P2(5) RPS19(19) RPS19P1(20)
 RPS2(16) RPS20(8) RPS20P3(5) RPS2L1(20) RPS3(11) RPS6(9)
 RPS9(19) RPS9P2(22) RRP4(9) RRP40(9) RTKN(2) RUVBL1(3)
 RUVBL2(19) S100A16(1) SAFB(19) SARS(1) SART3(12) SATB1(3)
 SBDS(7) SCD(10) SCYL1(11) SEC31L1(4) SFRS2(17) SH2D3A(19)
 SH3BP1(22) SH3BP5(3) SHMT2(12) SIAHBP1(8) SIN3A(15)
 SKB1(14) SLC25A3(12) SLC25A6(X) SLC25A6(Y) SLC7A5(16)
 SMARCA4(19) SMARCB1(22) SNRPA(19) SNRPA1(15) SNRPB(20)
 SNRPC(6) SNX17(2) SNX6(14) SOD1(21) SPINT1(15) SPPL2B(19)
 SRP14(15) ST7(7) STAG3(7) STAMBP(2) STARD7(2) STAT6(12)
 STIM1(11) STK33(11) STMN1(1) STXBP2(19) SUPT16H(14)
 SUPT5H(19) SV2A(1) SV2C(5) TADA2L(17) TADA3L(3) TAF11(6)
 TAGLN2(1) TCEB1(8) TCL1A(14) TD-60(1) TDPX2(9) TIC(2)

Tino(19) TIP120A(12) TK1(17) TMEM4(12) TMSB4X(X) TOR3A(1)
 TPI1(12) TPK1(7) TPM3(1) TRAP1(16) TRAPPC1(17) TRAPPC3(1)
 TRBC2(7) TRIP10(19) TRP14(17) TUBA3(12) TUBA6(12) TUBB2(9)
 TUSC2(3) TXNDC5(6) TXNIP(1) UBAP2(9) UBC(12) UBE2J2(1)
 USP11(X) USP7(16) VAMP8(2) VWF(12) VWFP(22) WAC(10)
 WBSCR1(7) WDR1(4) WDR18(19) WDR34(9) XPNPEP1(10) XPO5(6)
 YAP(1) YKT6(7) YWHAB(20) ZNF212(7) ZNF24(18)
 ZNF41(X) ZNF44(19) ZNF574(19) ZSWIM6(5)

ID	Candidate Apoptotic Sequence	Affected Cancers
14	+ GGCTGGTGTTAATCGGCCGAGG - CCTCGGCCGATTAACACCAGCC	ovarian colorectal lung brain uterus skin kidney pancreas muscle lymph eye
Targeted Genes		
ARHGDI(17) ATP7A(X) BTF3(5) CAD(2) CD59(11) CLNS1A(11) CSNK2B(6) DAP3(1) DHTKD1(10) DNAJB12(10) FBL(19) FLJ22688(19) GPT(8) H2AFX(11) HDLBP(2) HSPB1(7) INSM1(20) JIK(12) LOC129138(22) LOC144483(12) LOC145414(14) LOC158078(9) LOC221838(7) LOC285752(6) LOC286444(X) LOC389912(X) LOC401146(4) LOC51149(5) LOC83468(12) MSH6(2) NFAT5(16) NME2(17) RPL3(22) RPS2L1(20) SDBCAG84(20) SDCCAG3(9) SH3BP1(22) SMARCA4(19) WHSC2(4) XPO5(6) ZSWIM6(5)		

ID	Candidate Apoptotic Sequence	Affected Cancers
15	+ GGGGGTGAATCGGCCGAGG - CCTCGGCCGATTACCCCC	ovarian colorectal lung brain uterus skin kidney pancreas muscle lymph eye
Targeted Genes		

ACTB(7) ANKRD19(9) ASB1(2) ATF4(22) Clorf26(1) CHGB(20)
 COG1(17) CPS1(2) CPT1A(11) CX3CL1(16) CYFIP2(5) ELKS(12)
 FMO5(1) FTL(19) G2AN(11) GFPT1(2) GNB2L1(5) GOT2(16)
 GTF3C5(9) HCA127(X) HSPA4(5) HSPA8(11) HSPCB(6) HSPCP1(4)
 ILVBL(19) KDELR1(19) KIAA1917(17) LAPTM4B(8) LOC116166(15)
 LOC126037(19) LOC138198(9) LOC143920(11) LOC158714(X)
 LOC283820(16) LOC340600(X) LOC388783(20) LOC390730(16)
 LOC391044(1) LOC391634(4) LOC392437(X) LOC401308(7)
 LOC401677(11) LOC402461(7) LOC84549(8) LOC90850(16) LYN(8)
 MAP4(3) NCL(2) NICE-3(1) NICE-4(1) NJMU-R1(17) NONO(X)
 ODC1(2) PHB(17) PKD1P3(16) PKM2(15) PM5(16) PRNPIP(1)
 PTPN11(12) RCN1(11) RGS4(1) RNF8(6) RPL5(1) RPN1(3)
 S100A11(1) SAE1(19) SCAMP3(1) SLC25A3(12) SORD(15) ST7(7)
 TIMM50(19) TM4SF11(16) U5-116KD(17) UBE2G2(21) UCHL1(4)
 VARS2(6) WDR6(3) ZNF160(19)

ID	Candidate Apoptotic Sequence	Affected Cancers
16	+ GCTGGGTGTGAATCGGCCGAGG - CCTCGGCCGATTCACACCCAGC	ovarian colorectal lung brain uterus skin kidney pancreas muscle lymph eye
Targeted Genes		
ABCB6(2) ACTB(7) ARHGEF1(19) ATP5G2(12) AZ2(3) BAT3(6) BCL2L14(12) BID(22) C14orf94(14) C6orf49(6) Cab45(1) CBX7(22) CDK4(12) CHCHD2(7) CHCHD3(7) CNOT7(8) COX5B(2) DKFZP761D0211(16) DMAP1(1) DNPEP(2) EDARADD(1) EML2(19) ENDOG(9) ENO1(1) ENO1P(1) FGFR4(5) FLJ11773(12) FLJ13868(16) FLJ22169(2) FTL(19) FUS(16) G22P1(22) GOLGA3(12) HDLBP(2) HH114(15) HIC2(22) HLA-B(6) HSPCA(14) HSPCB(6) HSPCP1(4) HSRNAFEV(2) ILKAP(2) IMPDH2(3) IRX4(5) ITGA1(5) K-ALPHA-1(12) KIAA0195(17) LDHB(12) LIG1(19) LOC128439(20) LOC130617(2) LOC134147(5) LOC136337(X) LOC220717(2) LOC285741(6) LOC387703(10) LOC388783(20) LOC389169(3) LOC389181(3) LOC389424(6) LOC389787(9) LOC389901(X) LOC391634(4) LOC392437(X) LOC392647(7) LOC399942(11) LOC400006(12) LOC401316(7) LOC402057(22) LOC402579(7) LOC90321(19) LOC90850(16) LYRIC(8) MACF1(1) MAPT(17) MGC13170(19) MGC4549(19) MRPL23(11) MVP(16) NIFIE14(19) OSGEP(14) PA2G4(12) PDIP(16) PELO(5) PEX10(1) PKD1-like(1) PKM2(15) POFUT1(20) PREP(6) PRKAB1(12) PSMD3(17) PTMA(2) RPL13A(19) RPLP0(12) RPLP0P2(11) RPS11(19) RPS17(15) RPS17P2(5) RPS3(11) SH3YL1(2) SLC25A19(17) SNRPA(19) SNRPC(6) SPTAN1(9) SUPT5H(19) SYNGR2(17) TH1L(20) TIMM50(19) TPM3(1) TPT1(13) TRAF4(17) TRIM29(11) TUBA3(12) TUBA6(12) TUFM(16) UPK3B(7) UQCRH(1) WBSCR1(7) WDR18(19) WDR34(9)		

ID	Candidate Apoptotic Sequence	Affected Cancers
17	+ AGGTACGAGGCCGGGTGTT - AACACCCGGCCTCGTACCT	ovarian colorectal lung brain uterus skin kidney pancreas muscle lymph eye
Targeted Genes		
ANXA2(15) ANXA2P1(4) ANXA2P2(9) AP4E1(15) ARF3(12) ATF4(22) ATP1A1(1) ATP5A1(18) AUTL2(X) BANP(16) C20orf43(20) C6orf69(6) CCT3(1) CCT7(2) CDT6(1) CHCHD3(7) CLDN2(X) CLECSF9(12) CTAG3(6) DKC1(X) E2F4(16) EEF1G(11) EIF3S8(16) EST1B(1) FLJ10349(1) FLJ10871(8) FLJ32370(8) FRAP1(1) FSCN1(7) GAPD(12) GNPAT(1) HMOX1(22) HNRPF(10) K-ALPHA- 1(12) KIAA1917(17) KRT18(12) LOC136337(X) LOC145414(14) LOC158345(9) LOC284393(19) LOC285752(6) LOC339395(1) LOC388975(2) LOC389181(3) LOC389342(5) LOC389849(X) LOC399942(11) LOC400966(2) LOC401369(7) LOC92755(8) LOC92755(8) LOC94431(16) M96(1) MAP3K13(3) MGAT4B(5) MRPL48(11) MRPL48P1(6) NFE2L1(17) NIFU(12) NIPSNAP1(22) OK/SW-cl.56(6) P4HB(17) PCDH11X(X) PFKM(12) PITRM1(10) PKM2(15) RNPC4(14) RPL18(19) RPL3(22) RPLP0P2(11) RPS17P2(5) RPS3(11) RPS5(19) RRN3(16) RYK(3) SEC24A(5) SLC25A3(12) SOD1(21) STRN4(19) TINF2(14) TM9SF4(20) TRIM2(4) TUBA3(12) TUBA6(12) TUBB2(9) UQCRC1(3) WBP1(2) YARS(1) YKT6(7) ZFP106(15) ZSWIM6(5)		

ID	Candidate Apoptotic Sequence	Affected Cancers
18	+ GTGTTAATCGGCCGAGG - CCTCGGCCGATTAACAC	ovarian colorectal lung brain uterus skin kidney pancreas muscle lymph eye
Targeted Genes		
ABCF2(7) ABHD3(18) ACOXL(2) ACTB(7) ACTG1(17) ADCY6(12) ADRM1(20) AK2(1) AK3(1) ANP32B(9) ANXA2P2(9) ARF4L(17) ARG2(14) ARHC(1) ARHGDIA(17) ARPC1B(7) ARPC2(2) ARRB2(17) ASPH(8) ATP5B(12) ATP7A(X) BACH(1) BANP(16) BAZ1A(14) BGN(X) BID(22) BLP1(8) BTF3(5) C14orf94(14) C20orf35(20) C22orf5(22) CAD(2) CAP1(1) CAPNS1(19) CARM1(19) CASP4(11) CASQ1(1) CCT3(1) CD59(11) CDK2(12) CHCHD3(7) CLDN2(X) CLECSF9(12) CLNS1A(11) CNOT7(8) COMT(22) COQ6(14) CPE(4) CSNK2B(6) CTSB(8) CYB5-M(16) DAP3(1) DAXX(6) DBH(9) DCI(16) DDOST(1) DDR1(6) DDX42(17) DHCR24(1) DHTKD1(10) DJ159A19.3(1) DKFZp434B227(3) DKFZP586J0619(7) DNAJA1(9) DNAJB12(10) DND1(5) E2F1(20) EDARADD(1) EEF1D(8) EEF1G(11)		

EI24(11) EIF2B5(3) EIF3S6IP(22) EIF3S8(16) EMD(X) ENO1(1)
ENO1P(1) ENO2(12) EPLIN(12) ESD(13) EXT2(11) FBL(19)
FBXO7(22) FLJ10597(1) FLJ11822(17) FLJ12541(15)
FLJ12949(19) FLJ21103(11) FLJ22688(19) FLJ22843(X)
FLJ27099(14) FLJ34836(5) FLNA(X) FSCN1(7) FTL(19) FTS(16)
GAPD(12) GBF1(10) GCN5L2(17) GGA2(16) GOLGA3(12) GOSR2(17)
GPR17(2) GPT(8) GUSB(7) GYS1(19) H2AFX(11) H3F3B(17)
HADHA(2) HADHAP(4) HDGF(1) HDLBP(2) HMOX2(16) HNRPAB(5)
HNRPDL(4) HNRPU(1) HOXA9(7) HRB2(12) HRIHFB2122(22)
HS2ST1(1) HSPB1(7) HSPCA(14) HSPCAL2(4) HSPCAL3(11)
IDH3B(20) IFI30(19) IL4I1(19) IMPDH2(3) IMUP(19) INSIG1(7)
INSM1(20) ISYNA1(19) JARID1A(12) JIK(12) JMJD2B(19) JRK(8)
JUNB(19) K-ALPHA-1(12) KHSRP(19) KIAA0182(16) KIAA0582(2)
KIAA0738(7) KIAA1614(1) KIAA1952(9) KPNB1(17) KRT17(17)
KRT19(17) KRT7(12) KRT8(12) LDHB(12) LDHBP(X) LIMR(12)
LIMS2(2) LMNA(1) LOC113444(1) LOC115509(16) LOC129138(22)
LOC136337(X) LOC144483(12) LOC145414(14) LOC145767(15)
LOC146053(15) LOC149501(1) LOC153027(4) LOC158078(9)
LOC158473(9) LOC192133(14) LOC220433(13) LOC221838(7)
LOC256000(4) LOC283820(16) LOC285741(6) LOC285752(6)
LOC286444(X) LOC339395(1) LOC339736(2) LOC341056(11)
LOC387851(12) LOC388076(15) LOC388524(19) LOC388642(1)
LOC388707(1) LOC388783(20) LOC388907(22) LOC388975(2)
LOC389912(X) LOC390819(17) LOC392437(X) LOC392647(7)
LOC399942(11) LOC399994(12) LOC400397(15) LOC400631(17)
LOC400879(22) LOC400966(2) LOC401146(4) LOC401308(7)
LOC401316(7) LOC401426(7) LOC401504(9) LOC401972(1)
LOC401987(1) LOC402461(7) LOC402618(7) LOC51149(5)
LOC83468(12) LOC90313(17) LOC92755(8) LSM4(19) LTBP3(11)
LYPLA2(1) MAGED1(X) MAP1LC3B(16) MAP2K1(15) MBD3(19)
MCM5(22) MCM6(2) MESDC2(15) MGC11335(16) MGC19595(19)
MGC20446(11) MGC2714(11) MGC35182(9) MIR16(16) MRPL12(17)
MRPL41(9) MRPL45(17) MRPS26(20) MSH6(2) MYBL2(20)
NAP1L1(12) NCSTN(1) NDUFA9(12) NF1(17) NFAT5(16)
NIPSNAP1(22) NME1(17) NME2(17) NONO(X) NPEPPS(17) NUDT5(10)
NUP62(19) OK/SW-cl.56(6) ORC6L(16) P2RY6(11) PDLIM1(10)
PEA15(1) PEF(1) PFKM(12) PFKP(10) PGK1(X) PGK1P2(19)
PIK4CA(22) PITRM1(10) PKM2(15) PM5(16) PMM2(16) POLR3D(8)
PPAP2C(19) PPM1G(2) PPP1CA(11) PPT1(1) PQLC1(18) PRDX4(X)
PRO1855(17) PROCR(20) PRSS15(19) PSMC3(11) PSMC3P(9)
PSMC4(19) PTOV1(19) QDPR(4) RAB8A(19) RABEP1(17) RAC1(7)
RAC4(X) RAE1(20) RARS(5) REC14(15) RELA(11) RNF10(12)
RNF26(11) RNPS1(16) RPL22(1) RPL3(22) RPL35A(3) RPL5(1)
RPL8(8) RPLP2(11) RPN2(20) Rpp25(15) RPS2(16) RPS2L1(20)
RPS3A(4) RPS4X(X) RPS5(19) RPS6KB2(11) RRM2(2) RRM2P3(X)
RSHL1(19) S100A16(1) SAE1(19) SARS(1) SDBCAG84(20)
SDCCAG3(9) SDHB(1) SF3B3(16) SF4(19) SH3BP1(22) SIN3A(15)
SLC25A6(X) SLC25A6(Y) SLC41A3(3) SLC43A1(11) SMARCA4(19)
SNRPN(15) SOX10(22) SPARC(5) SPINT1(15) SRPRB(3) STRN4(19)

SUPT5H(19) TAGLN2(1) TCOF1(5) TEAD2(19) THOC3(5)
 TIMELESS(12) TM4SF8(15) TM9SF4(20) TMEM4(12) TNIP1(5)
 TPI1(12) TPT1(13) TRAP1(16) TUBA1(2) TUBA3(12) TUBA6(12)
 U5-116KD(17) UBA2(19) UBE1(X) UCHL1(4) UPK3B(7) UQCRC1(3)
 VASP(19) VCP(9) VIP32(10) WBP1(2) WBSCR1(7) WDR1(4)
 WHSC2(4) XPO5(6) YARS(1) ZDHHC12(9) ZDHHC16(10) ZNF313(20)
 ZNF559(19) ZNF584(19) ZSWIM6(5)

ID	Candidate Apoptotic Sequence	Affected Cancers
19	+ AGATGGGTACCAACTGT - ACAGTTGGTACCCATCT	ovarian colorectal lung brain pancreas muscle testis eye
Targeted Genes		
LOC220717(2) RPLP0P2(11) RPLP0(12)		

ID	Candidate Apoptotic Sequence	Affected Cancers
20	+ CGGCTAGGTACGAGGCTGGGGT - ACCCCAGCCTCGTACCTAGCCG	ovarian colorectal lung brain uterus skin kidney muscle lymph eye
Targeted Genes		
C5orf6(5) CASQ1(1) CCT3(1) CORO2A(9) CTAG3(6) ENTPD8(17) FLNA(X) FOSL1(11) GAPD(12) HSPC171(16) HSPCB(6) HSPCP1(4) KIAA0296(16) LOC388556(19) LOC389849(X) LOC391634(4) MBTPS1(16) NARF(17) NONO(X) PEA15(1) RER1(1) RIOK1(6) RPS3(11) RPS9(19) RPS9P2(22) SATB1(3) SLC12A4(16) TADA3L(3) ZNF44(19)		

ID	Candidate Apoptotic Sequence	Affected Cancers
21	+ GAGGCGGGTGTGAATCGGCCGAGG - CCTCGGCCGATTACACCCGCCTC	ovarian colorectal brain uterus skin pancreas muscle lymph eye
Targeted Genes		
ACTG1(17) ATP5G3(2) CCT6A(7) CN2(18) CORO1A(16) FTL(19) HMGA1(6) HSPCB(6) HSPCP1(4) LMAN2(5) LOC257200(2) LOC388783(20) LOC391634(4) LOC392437(X) MGC16824(16) MGC5178(16) NASP(1) NASPP1(8) PFDN5(12) PME-1(11) RAB5C(17) SPTAN1(9) TERF2IP(16) UBB(17) UBBP4(17) UQCR(19)		

ID	Candidate Apoptotic Sequence	Affected Cancers
22	+ AGGTACGAGGCCGGTGT - ACACCGGCCTCGTACCT	ovarian colorectal brain uterus skin kidney pancreas muscle lymph
Targeted Genes		
ALDH1A1(9) ARPC2(2) ATP5A1(18) BST2(19) CD79B(17) DBH(9) DDB1(11) EIF2B5(3) EIF3S6IP(22) EIF3S6IPP(14) ELF3(1) ENO1(1) FLJ27099(14) G22P1(22) G6PD(X) GAPD(12) GTF3C1(16) KIAA1068(7) KIAA1068(7b) KIAA1952(9) LOC145414(14) LOC192133(14) LOC285741(6) LOC346085(6) LOC387703(10) LOC387922(13) LOC388076(15) LOC389849(X) LOC389901(X) LOC92755(8) MCM7(7) MCSC(9) MRPL45(17) NASP(1) NASPP1(8) NDST2(10) OAZ1(19) OK/SW-cl.56(6) RPL18(19) RPS8(1) TAGLN2(1) TPT1(13) XRCC1(19) ZNF271(18) ZSWIM6(5)		

ID	Candidate Apoptotic Sequence	Affected Cancers
23	+ GTTAATCGGCCGAGGCGC - GCGCCTCGGCCGATTAAC	ovarian colorectal lung brain uterus skin kidney pancreas muscle lymph
Targeted Genes		
CSNK2B(6) EIF3S6IP(22) INSIG1(7) KIAA1115(19) KRT7(12) LOC401658(11) LOC402057(22) LOC89958(9) LOC92755(8) MGC3047(1) OK/SW-cl.56(6) PROCR(20) RAN(12) RPS17(15) RPS17P2(5) SMT3H1(21) UPP1(7) WHSC2(4)		

ID	Candidate Apoptotic Sequence	Affected Cancers
24	+ AGACCAACAGAGTTCGG - CCGAACTCTGTTGGTCT	ovarian colorectal lung skin kidney pancreas
Targeted Genes		
novel mapping		

ID	Candidate Apoptotic Sequence	Affected Cancers
25	+ TGGCTTCGTGTCCCATGCA - TGCATGGGACACGAAGCCA	breast ovarian colorectal lung skin muscle liver
Targeted Genes		

GAPD(12) GAPDL4(4) KIAA0295(15) KLHL8(4) LOC389849(X)

ID	Candidate Apoptotic Sequence	Affected Cancers
26	+ CCGGGTGTAAATCGGCCGA - TCGGCCGATTTACACCCGG	ovarian colorectal brain uterus skin pancreas muscle lymph
Targeted Genes		
C19orf13(19) EIF3S6P1(6) EIF3S6(8) GNB2L1(5) GTF2H3(12) HDAC1(1) HSPCA(14) KRT5(12) PAK1IP1(6) PD2(19) QARS(3) SFRS10(3)		

ID	Candidate Apoptotic Sequence	Affected Cancers
27	+ GCCGGTGTGAATCGGCCGA - TCGGCCGATTCACACCCGGC	colorectal lung brain uterus skin kidney pancreas muscle
Targeted Genes		
ARHC(1) ATP7B(13) BCAP31(X) C20orf35(20) CTDSP2(12) EBNA1BP2(1) FLJ10737(1) FLJ20254(2) G22P1(22) HDLBP(2) HMG2(1) HS3ST4(16) HSA272196(17) HSPC117(22) LCP1(13) LOC339395(1) LOC387703(10) LOC389901(X) MGC11242(17) MRPL51(12) NAP1L1(12) NDUFV1(11) POLDIP2(17) PSMB1(6) SIRT2(19) SQSTM1(5) SRPR(11) STK25(2) SV2C(5) TAGLN2(1) TJP1(15) XRCC1(19)		

ID	Candidate Apoptotic Sequence	Affected Cancers
28	+ TCATGATGGTGTATCGATGA - TCATCGATACACCATCATGA	ovarian colorectal lung brain skin bone
Targeted Genes		
JIK(12) LOC400963(2) LOC91561(11) LOC286444(X)		

ID	Candidate Apoptotic Sequence	Affected Cancers
29	+ GCTCGGTGTTAATCGGCCGA - TCGGCCGATTAACACCGAGC	ovarian colorectal brain uterus skin pancreas lymph eye
Targeted Genes		

CASP4(11) GGA2(16) HRIHFB2122(22) INSIG1(7) KHSRP(19)
 LOC388642(1) LOC400879(22) PRDX4(X) RPS2(16) SDHB(1)
 SLC25A6(X) SLC25A6(Y) TPI1(12) TRAP1(16) VIP32(10)

ID	Candidate Apoptotic Sequence	Affected Cancers
30	+ TGGGGTTAATCGGCCGAGG - CCTCGGCCGATTAAACCCA	ovarian colorectal lung uterus skin pancreas lymph eye
Targeted Genes		
ADRBK1(11) BCKDK(16) LOC220717(2) MGC3329(17) MRPL15(8) QARS(3) RPLP0(12) RPLP0P2(11) RPS9(19) RPS9P2(22) SPATA11(19) SRM(1) TADA3L(3) TUFM(16)		

ID	Candidate Apoptotic Sequence	Affected Cancers
31	+ AGGCCGGTGTTAATCGGCCGA - TCGGCCGATTAAACACCGGCCT	ovarian colorectal lung brain uterus skin kidney pancreas lymph
Targeted Genes		
ACTG1(17) AK3(1) ANXA2P2(9) ARPC2(2) ATP5B(12) CPE(4) DBH(9) DCI(16) DHCR24(1) DJ159A19.3(1) EEF1D(8) ENO1(1) GOLGA3(12) HADHA(2) HADHAP(4) HIP-55(7) HNRPU(1) JMJD2B(19) K-ALPHA-1(12) KIAA1952(9) LOC145414(14) LOC158473(9) LOC285741(6) LOC387851(12) LOC388524(19) LOC388707(1) LOC392647(7b) LOC399942(11) LOC399994(12) LOC401316(7) LOC401504(9) LOC401987(1) MRPL45(17) NF1(17) NME1(17) PRSS15(19) RABEP1(17) SOX10(22) SRPRB(3) TAGLN2(1) TPT1(13) TUBA3(12) TUBA6(12) VCP(9) WBSCR1(7) ZSWIM6(5)		

ID	Candidate Apoptotic Sequence	Affected Cancers
32	+ TGGTGAATCGGCCGAGGGT - ACCCTCGGCCGATTACCA	ovarian colorectal brain uterus skin kidney pancreas lymph
Targeted Genes		
ACADS(12) C20orf149(20) DCTN3(9) DPYSL3(5) EIF3S1(15) IPO4(14) KIAA0152(12) LOC388556(19) LOC401092(3) PRDX5(11) PSMF1(20) RAB11A(15) RPL10(X) RPS9(19) RPS9P2(22) STXBP2(19) ZNF3(7) ZNF-U69274(3)		

ID	Candidate Apoptotic Sequence	Affected Cancers
33	+ AGCAAGTATGACAACAGC - GCTGTTGTCATACTTGCT	colorectal lung cervix skin pancreas muscle
Targeted Genes		
GAPD (12) LOC389849 (X)		

ID	Candidate Apoptotic Sequence	Affected Cancers
34	+ CTTAAACCAAGCTAGCC - GGCTAGCTTGGTTTAAG	colorectal prostate brain skin bone testis eye
Targeted Genes		
LOC143371 (10) LOC150554 (2) LOC158383 (9) YWHAZ (8)		

ID	Candidate Apoptotic Sequence	Affected Cancers
35	+ CAGTCTACATCACGTGG - CCACGTGATGTAGACTG	colorectal lung cervix brain kidney lymph liver eye
Targeted Genes		
LOC359792 (Y) LOC400039 (12) PCDH11X (X) PCDH11Y (Y)		

ID	Candidate Apoptotic Sequence	Affected Cancers
36	+ AATCTCCTGTTACACTCA - TGAGTGTAACAGGAGATT	ovarian colorectal brain epid testis
Targeted Genes		
LOC146909 (17)		

ID	Candidate Apoptotic Sequence	Affected Cancers
37	+ GCCCAAGGAACCCCTT - AAGGGGGTTCCTTGGGC	ovarian colorectal lung skin testis liver eye
Targeted Genes		
ABCC3 (17) CHCHD3 (7) EEF1G (11) LOC136337 (X)		

ID	Candidate Apoptotic Sequence	Affected Cancers
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38	+ GGCTAGGACGAGGCCGGG - CCCGGCCTCGTCCTAGCC	colorectal brain skin kidney pancreas muscle lymph
Targeted Genes		
ATP6V1E1 (22) CCT4 (2) CHGB (20) DHX9 (1) EIF3S8 (16) LOC343515 (1) MAP2K2 (19) NDUFA9 (12) NDUFA9P1 (22) SCARB1 (12)		

ID	Candidate Apoptotic Sequence	Affected Cancers
39	+ GAGAAGGTTCCCGGGAA - TTCCCGGGAACCTTCTC	colorectal lung pancreas lymph liver eye
Targeted Genes		
CHCHD3 (7) EEF1G (11) LOC136337 (X) MGC10471 (19)		

ID	Candidate Apoptotic Sequence	Affected Cancers
40	+ GTGTTACTCGGCCGAGG - CCTCGGCCGAGTAACAC	colorectal lung brain uterus skin kidney pancreas muscle
Targeted Genes		
ACLY (17) ADAR (1) ALDH1A1 (9) C12orf10 (12) GNAI2 (3) K-ALPHA-1 (12) LMNB2 (19) LOC400671 (19) PPIE (1) RYK (3) TTYH3 (7) TUBA3 (12) TUBA6 (12)		

ID	Candidate Apoptotic Sequence	Affected Cancers
41	+ TTGAATCGGCCGAGGGTG - CACCCTCGGCCGATTCAA	ovarian colorectal lung brain pancreas muscle eye
Targeted Genes		
CINP (14) COTL1 (16) FLJ39075 (16) GNB2L1 (5) KRT19 (17) KRT4 (12) LOC92305 (4) MCSC (9) PCNT1 (17) PH-4 (3) RPL8 (8) ZNF337 (20)		

ID	Candidate Apoptotic Sequence	Affected Cancers
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42	+ GCCGGGTGGTGAATCGG - CCGATTCACCACCCGGC	ovarian colorectal brain uterus skin kidney muscle
Targeted Genes		
ACTG1(17) CHCHD3(7) DFFA(1) DPYSL3(5) PRDX5(11) SYMPK(19) TSPAN-1(1) ZDHHC16(10)		

ID	Candidate Apoptotic Sequence	Affected Cancers
43	+ GCCGGTGGTTAATCGGC - GCCGATTAACCACCGGC	colorectal lung brain uterus skin kidney pancreas
Targeted Genes		
C6orf109(6) CFL1(11) FLJ30934(11) GALNT2(1) K-ALPHA-1(12) LOC145414(14) LOC285752(6) LOC399942(11) LOC56931(19) PCDH18(4) PSMC3(11) RPL3(22) SARS(1) STK19(6) TCF7L1(2) TETRA(4) TUBA3(12) TUBA6(12)		

ID	Candidate Apoptotic Sequence	Affected Cancers
44	+ GGGCGCAGCGACATCAG - CTGATGTCGCTGCGCCC	colorectal prostate lung adrenal pancreas lymph eye
Targeted Genes		
TREN2(X)		

ID	Candidate Apoptotic Sequence	Affected Cancers
45	+ GCTATTAGCAGATTGTGT - ACACAATCTGCTAATAGC	colorectal lung kidney muscle testis eye
Targeted Genes		
LOC399942(11) K-ALPHA-1(12) TUBA3(12) TUBA6(12)		

ID	Candidate Apoptotic Sequence	Affected Cancers
46	+ TGTTAATCTCCTGTTACTCA - TGAGTGTAACAGGAGATTACA	ovarian colorectal brain epid testis liver
Targeted Genes		

LOC146909 (17)

ID	Candidate Apoptotic Sequence	Affected Cancers
47	+ CCACCGCACCGTTGGCC - GGCCAACGGTGCGGTGG	ovarian colorectal cervix skin kidney testis
Targeted Genes		
FBXW5 (9)		

ID	Candidate Apoptotic Sequence	Affected Cancers
48	+ ACCTGGAGCCCTCTGAT - ATCAGAGGGCTCCAGGT	colorectal lung skin kidney muscle liver
Targeted Genes		
LOC399942 (11) K-ALPHA-1 (12) TUBA3 (12) TUBA6 (12)		

ID	Candidate Apoptotic Sequence	Affected Cancers
49	+ TCAGACAAACACAGATCG - CGATCTGTGTTTGTCTGA	colorectal prostate lung brain muscle
Targeted Genes		
LOC285900 (7) DGKI (7) LOC402525 (7b) LOC388460 (18) RPL6 (12)		

ID	Candidate Apoptotic Sequence	Affected Cancers
50	+ GAGAATACTGATTGAGACCTA - TAGGTCTCAATCAGTATTCTC	ovarian colorectal skin kidney lymph testis
Targeted Genes		
LOC92755 (8) OK/SW-cl.56 (6)		

5

ID	Candidate Apoptotic Sequence	Affected Cancers
51	+ CCAGCCAGCACCCAGGC - GCCTGGGTGCTGGCTGG	colorectal gall skin pancreas lymph
Targeted Genes		

ATP5A1(18) FLJ10101(9) IL9R(X) IL9R(Y) LOC392325(9)
LOC400481(16) RELA(11)

ID	Candidate Apoptotic Sequence	Affected Cancers
52	+ TAGACCAACAGAGTTCC - GGAACCTCTGTTGGTCTA	colorectal lung skin kidney muscle liver
Targeted Genes		
novel mapping		

ID	Candidate Apoptotic Sequence	Affected Cancers
53	+ CTAGGTACGAGGCTGGGTTTT - AAAACCCAGCCTCGTACCTAG	colorectal lung uterus skin muscle lymph
Targeted Genes		
ACTG1(17) LOC81691(16) PSAP(10) SFRS2(17)		

ID	Candidate Apoptotic Sequence	Affected Cancers
54	+ CGAGGCGGGTGTTAATCGGCC - GGCCGATTAACACCCGCCTCG	colorectal lung brain skin pancreas lymph eye
Targeted Genes		
ACTB(7) ADCY6(12) BID(22) EIF3S6IP(22) EIF3S8(16) K-ALPHA-1(12) MRPL12(17) PDLIM1(10) RARS(5) RPN2(20) S100A16(1) TUBA1(2)		

5

ID	Candidate Apoptotic Sequence	Affected Cancers
55	+ AAGGCTAGGTAGAGGCTG - CAGCCTCTACCTAGCCTT	ovarian colorectal brain pancreas muscle eye
Targeted Genes		
ANP32B(9) C20orf14(20) CAD(2) COL14A1(8) CTNNBL1(20) DOK4(16) ENO1(1) FLJ22301(1) HSPCB(6) HSPCP1(4) K-ALPHA-1(12) LOC339395(1) LOC391634(4) LOC400397(15) PKM2(15) RACGAP1(12) STATIP1(18) VASP(19)		

ID	Candidate Apoptotic Sequence	Affected Cancers
56	+ CATGGCCATGCTGTGCA - TGCACAGCATGGCCATG	colorectal uterus skin testis
Targeted Genes		
DNPEP (2) MATP (5)		

ID	Candidate Apoptotic Sequence	Affected Cancers
57	+ AGGTACGAGGCCGGTGTTAATCGGCCGA - TCGGCCGATTAAACACCGGCCTCGTACCT	ovarian colorectal lung brain kidney lymph
Targeted Genes		
ARPC2 (2) DBH (9) ENO1 (1) KIAA1952 (9) LOC145414 (14) LOC285741 (6) MRPL45 (17) TAGLN2 (1) TPT1 (13) ZSWIM6 (5)		

ID	Candidate Apoptotic Sequence	Affected Cancers
59	+ TGCTGCCCTCAATGGTC - GACCATTGAGGGCAGCA	colorectal lung cervix skin muscle eye
Targeted Genes		
novel mapping		

ID	Candidate Apoptotic Sequence	Affected Cancers
60	+ AGGCCGGTGGTTAATCGGCCGAGG - CCTCGGCCGATTAAACCACCGGCCT	colorectal brain uterus skin kidney pancreas
Targeted Genes		
C6orf109 (6) GALNT2 (1) LOC145414 (14) LOC285752 (6) LOC56931 (19) PCDH18 (4) PSMC3 (11) RPL3 (22) STK19 (6) TETRA (4)		

5

ID	Candidate Apoptotic Sequence	Affected Cancers
61	+ GAGGCCGGTGGTTAATCGGCCGAG - CTCGCCGATTAAACCACCGGCCTC	colorectal brain uterus skin kidney pancreas
Targeted Genes		

C6orf109(6) LOC145414(14) LOC285752(6) LOC56931(19)
 PCDH18(4) PSMC3(11) RPL3(22)
 STK19(6) TETRA(4)

ID	Candidate Apoptotic Sequence	Affected Cancers
62	+ GCTAGGTACGAGGCTGGGTTTT - AAAACCCAGCCTCGTACCTAGC	colorectal lung uterus skin muscle lymph
Targeted Genes		
ACTG1(17) PSAP(10) SFRS2(17)		

ID	Candidate Apoptotic Sequence	Affected Cancers
63	+ AACATACGGCTAGGTACGA - TCGTACCTAGCCGTATGTT	ovarian colorectal brain uterus lymph eye
Targeted Genes		
CIZ1(9) FLJ20203(1) FLJ23416(17) MGC3162(12) MSF(17) SWAP70(11) YAP(1)		

ID	Candidate Apoptotic Sequence	Affected Cancers
64	+ GGTGGTAATCGGACGAGG - CCTCGTCCGATTACCACC	colorectal lung brain uterus skin muscle
Targeted Genes		
AKT1(14) CHGA(14) CHRNA3(15) EMS1(11) FLJ20244(19) FLJ22169(2) GNB2L1(5) LOC130617(2) LOC284393(19) LOC347422(X) LOC388642(1) LOC389342(5) SLC4A2(7) TIMM17B(X) TPI1(12) YKT6(7)		

ID	Candidate Apoptotic Sequence	Affected Cancers
65	+ GGGTGATCGGACGAGGC - GCCTCGTCCGATCACCC	ovarian colorectal lung brain pancreas eye
Targeted Genes		
ACTG1(17) ANKRD19(9) DNAJB11(3) EEF1D(8) HSPCA(14) HSPCAL2(4) HSPCAL3(11) LOC126037(19) LOC399704(6) RABAC1(19)		

ID	Candidate Apoptotic Sequence	Affected Cancers
66	+ ACATGCCTAGGGTTCAA - TTGAACCCTAGGCATGT	colorectal lung cervix pancreas testis eye
Targeted Genes		
EEF1A1 (6) LOC401146 (4)		

TABLE 2: Colon Cancer Marker Subset and Primers

5	3 Cancer Oligo:	+ CCTCTGTTAATCTCCTGTTACA - TGTAACAGGAGATTAACAGAGG
	Primer Oligo:	+ CCTCTGTTAATCTCCTGTT - AACAGGAGATTAACAGAGG
10	Reference:	Tissue: colon_14528_155 Cancer Temp: 62 C Primer Temp: 54 C
	5 Cancer Oligo:	+ GCCCAAGGAACCCCTT - AAGGGGGTTCCTTGGGC
15	Reference:	Tissue: colon_52909_1157 Cancer Temp: 56 C
	6 Cancer Oligo:	+ GACTGAATGCACCCAATATCCGACCTGGCTGCGTGT - ACACGCAGCCAGGTCGGATATTGGGTGCATTCAAGTC
20	Primer Oligo:	+ GACTGAATGCACCCAATAT - ATATTGGGTGCATTCAAGTC
	Reference:	Tissue: colon_7084_373 Cancer Temp: 112 C Primer Temp: 54 C
25	7 Cancer Oligo:	+ CACCCTCTGTTAATCTCCTGTTACA - TGTAACAGGAGATTAACAGAGGGTG
	Primer Oligo:	+ CACCCTCTGTTAATCTCC - GGAGATTAACAGAGGGTG
30	Reference:	Tissue: colon_14528_154 Cancer Temp: 72 C Primer Temp: 54 C
	8 Cancer Oligo:	+ GCTCAGGTTTGCTCAGG - CCTGAGCAAACCTGAGC
35	Reference:	Tissue: colon_22882_6 Cancer Temp: 54 C
40	9 Cancer Oligo:	+ TGTGCTTCTGGCAGGCC - GGCCTGCCAGAAGCACA
	Reference:	Tissue: colon_46693_132 Cancer Temp: 56 C
45	10 Cancer Oligo:	+ ACCTGGGATCCAGTTGGAGGACGGC - GCCGTCCTCCAAGTGGATCCCAGGT
	Primer Oligo:	+ ACCTGGGATCCAGTTGG - CCAAGTGGATCCCAGGT
50	Reference:	Tissue: colon_38162_1403 Cancer Temp: 82 C Primer Temp: 54 C
	11 Cancer Oligo:	+ CGCATGCGTGGCCACCA

- Reference: - TGGTGGCCACGCATGCG
Tissue: colon_41436_2209
Cancer Temp: 58 C
- 5
12 Cancer Oligo: + CCCAGCAGGGACATCCG
- CGGATGTCCCTGCTGGG
Reference: Tissue: colon_134925_1443
Cancer Temp: 58 C
- 10
13 Cancer Oligo: + GGCTAGGTACGAGGCTGG
- CCAGCCTCGTACCTAGCC
Primer Oligo: + GGCTAGGTACGAGGCTG
- CAGCCTCGTACCTAGCC
15 Reference: Tissue: colon_121812_797
Cancer Temp: 60 C Primer Temp: 56 C
- 20
14 Cancer Oligo: + GGCTGGTGTTAATCGGCCGAGG
- CCTCGGCCGATTAACACCAGCC
Primer Oligo: + GGCTGGTGTTAATCGGC
- GCCGATTAACACCAGCC
Reference: Tissue: colon_122287_1352
25 Cancer Temp: 72 C Primer Temp: 54 C
- 30
15 Cancer Oligo: + GGGGGTGAATCGGCCGAGG
- CCTCGGCCGATTACCCCC
Primer Oligo: + GGGGGTGAATCGGCCG
- CGGCCGATTACCCCC
Reference: Tissue: colon_122308_1392
Cancer Temp: 66 C Primer Temp: 56 C
- 35
16 Cancer Oligo: + GCTGGGTGTGAATCGGCCGAGG
- CCTCGGCCGATTACACCCAGC
Primer Oligo: + GCTGGGTGTGAATCGGC
- GCCGATTACACCCAGC
40 Reference: Tissue: colon_123371_2691
Cancer Temp: 74 C Primer Temp: 56 C
- 45
17 Cancer Oligo: + AGGTACGAGGCCGGGTGTT
- AACACCCGGCCTCGTACCT
Primer Oligo: + AGGTACGAGGCCGGGT
- ACCCGGCCTCGTACCT
Reference: Tissue: colon_124205_4458
Cancer Temp: 62 C Primer Temp: 54 C
- 50
18 Cancer Oligo: + GTGTTAATCGGCCGAGG
- CCTCGGCCGATTAACAC
Reference: Tissue: colon_124503_5628
55 Cancer Temp: 54 C
- 19 Cancer Oligo: + AGATGGGTACCAACTGT

- Reference: - ACAGTTGGTACCCATCT
Tissue: colon_132239_12738
Cancer Temp: 50 C
- 5
- 20 Cancer Oligo: + CGGCTAGGTACGAGGCTGGGGT
- ACCCCAGCCTCGTACCTAGCCG
Primer Oligo: + CGGCTAGGTACGAGGC
- GCCTCGTACCTAGCCG
- 10 Reference: Tissue: colon_124479_5522
Cancer Temp: 74 C Primer Temp: 54 C
- 15 21 Cancer Oligo: + GAGGCGGGTGTGAATCGGCCGAGG
- CCTCGGCCGATTACACCCGCCTC
Primer Oligo: + GAGGCGGGTGTGAATCG
- CGATTACACCCGCCTC
Reference: Tissue: colon_124382_5017
Cancer Temp: 82 C Primer Temp: 56 C
- 20
- 22 Cancer Oligo: + AGGTACGAGGCCGGTGT
- ACACCGGCCTCGTACCT
Reference: Tissue: colon_124545_5835
Cancer Temp: 56 C
- 25
- 23 Cancer Oligo: + GTTAATCGGCCGAGGCGC
- GCGCCTCGGCCGATTAAC
Primer Oligo: + GTTAATCGGCCGAGGCG
- GCGCCTCGGCCGATTAAC
Reference: Tissue: colon_124554_5891
Cancer Temp: 60 C Primer Temp: 56 C
- 30
- 35 24 Cancer Oligo: + AGACCAACAGAGTTCGG
- CCGAACTCTGTTGGTCT
Reference: Tissue: colon_128799_3222
Cancer Temp: 52 C
- 40
- 25 Cancer Oligo: + TGGCTTCGTGTCCCATGCA
- TGCATGGGACACGAAGCCA
Primer Oligo: + TGGCTTCGTGTCCCATG
- CATGGGACACGAAGCCA
Reference: Tissue: colon_128901_3427
Cancer Temp: 60 C Primer Temp: 54 C
- 45
- 50 26 Cancer Oligo: + CCGGGTGTAATCGGCCGA
- TCGGCCGATTTACACCCGG
Primer Oligo: + CCGGGTGTAATCGGCC
- GGCCGATTTACACCCGG
Reference: Tissue: colon_121791_713
Cancer Temp: 62 C Primer Temp: 56 C
- 55
- 27 Cancer Oligo: + GCCGGTGTGAATCGGCCGA

- 5
Primer Oligo: - TCGGCCGATTACACCGGC
+ GCCGGTGTGAATCGGC
- GCCGATTACACCGGC
Reference: Tissue: colon_122271_1321
Cancer Temp: 64 C Primer Temp: 54 C
- 10
28 Cancer Oligo: + TCATGATGGTGTATCGATGA
- TCATCGATACACCATCATGA
Reference: Tissue: colon_122810_2119
Cancer Temp: 56 C
- 15
29 Cancer Oligo: + GCTCGGTGTTAATCGGCCGA
- TCGGCCGATTAAACACCGAGC
Primer Oligo: + GCTCGGTGTTAATCGGC
- GCCGATTAAACACCGAGC
Reference: Tissue: colon_123361_2652
Cancer Temp: 64 C Primer Temp: 54 C
- 20
30 Cancer Oligo: + TGGGGTTAATCGGCCGAGG
- CCTCGGCCGATTAAACCCCA
Primer Oligo: + TGGGGTTAATCGGCCGA
- TCGGCCGATTAAACCCCA
Reference: Tissue: colon_123408_2783
Cancer Temp: 62 C Primer Temp: 54 C
- 25
31 Cancer Oligo: + AGGCCGGTGTTAATCGGCCGA
- TCGGCCGATTAAACACCGGCCT
Primer Oligo: + AGGCCGGTGTTAATCGG
- CCGATTAAACACCGGCCT
Reference: Tissue: colon_124428_5212
Cancer Temp: 68 C Primer Temp: 54 C
- 30
32 Cancer Oligo: + TGGTGAATCGGCCGAGGGT
- ACCCTCGGCCGATTACCA
Primer Oligo: + TGGTGAATCGGCCGAGG
- CCTCGGCCGATTACCA
Reference: Tissue: colon_124548_5844
Cancer Temp: 62 C Primer Temp: 56 C
- 35
40
45
33 Cancer Oligo: + AGCAAGTATGACAACAGC
- GCTGTTGTCATACTTGCT
Reference: Tissue: colon_124841_107
Cancer Temp: 52 C
- 50
55
34 Cancer Oligo: + CTAAACCAAGCTAGCC
- GGCTAGCTTGGTTTAAG
Reference: Tissue: colon_125327_1240
Cancer Temp: 50 C
- 35 Cancer Oligo: + CAGTCTACATCACGTGG

- Reference: - CCACGTGATGTAGACTG
Tissue: colon_131175_9725
Cancer Temp: 52 C
- 5
- 36 Cancer Oligo: + AATCTCCTGTTACACTCA
- TGAGTGTAACAGGAGATT
Reference: Tissue: colon_131332_10159
Cancer Temp: 50 C
- 10
- 37 Cancer Oligo: + GCCCAAGGAACCCCCTT
- AAGGGGGTTCCTTGGGC
Reference: Tissue: colon_52909_1157
Cancer Temp: 56 C
- 15
- 38 Cancer Oligo: + GGCTAGGACGAGGCCGGG
- CCCGGCCTCGTCCTAGCC
20 Primer Oligo: + GGCTAGGACGAGGCCG
- CGGCCTCGTCCTAGCC
Reference: Tissue: colon_121817_833
Cancer Temp: 64 C Primer Temp: 56 C
- 25
- 39 Cancer Oligo: + GAGAAGGTTCCCGGGAA
- TTCCCGGGAACCTTCTC
Reference: Tissue: colon_123283_2553
Cancer Temp: 54 C
- 30
- 40 Cancer Oligo: + GTGTTACTCGGCCGAGG
- CCTCGGCCGAGTAACAC
Reference: Tissue: colon_123389_2740
Cancer Temp: 56 C
- 35
- 41 Cancer Oligo: + TTGAATCGGCCGAGGGTG
- CACCCTCGGCCGATTCAA
40 Reference: Tissue: colon_124408_5119
Cancer Temp: 58 C
- 45
- 42 Cancer Oligo: + GCCGGGTGGTGAATCGG
- CCGATTCAACACCCGGC
Reference: Tissue: colon_124566_5929
Cancer Temp: 58 C
- 50
- 43 Cancer Oligo: + GCCGGTGGTTAATCGGC
- GCCGATTAACACCCGGC
Reference: Tissue: colon_124579_5999
Cancer Temp: 56 C
- 55
- 44 Cancer Oligo: + GGGCGCAGCGACATCAG
- CTGATGTCGCTGCGCCC
Reference: Tissue: colon_128875_3358

Cancer Temp: 58 C

- 5 45 Cancer Oligo: + GCTATTAGCAGATTGTGT
 - ACACAATCTGCTAATAGC
Reference: Tissue: colon_130347_7716
 Cancer Temp: 50 C
- 10 46 Cancer Oligo: + TGTTAATCTCCTGTTACTCA
 - TGAGTGTAACAGGAGATTAACA
Primer Oligo: + TGTTAATCTCCTGTTACT
 - AGTGTAACAGGAGATTAACA
Reference: Tissue: colon_131332_10158
15 Cancer Temp: 60 C Primer Temp: 54 C
- 20 47 Cancer Oligo: + CCACCGCACCGTTGGCC
 - GGCCAACGGTGCGGTGG
Primer Oligo: + CCACCGCACCGTTGGC
 - GGCCAACGGTGCGGTGG
Reference: Tissue: colon_131939_11900
 Cancer Temp: 60 C Primer Temp: 56 C
- 25 48 Cancer Oligo: + ACCTGGAGCCCTCTGAT
 - ATCAGAGGGCTCCAGGT
Reference: Tissue: colon_132839_14455
30 Cancer Temp: 54 C
- 35 49 Cancer Oligo: + TCAGACAAACACAGATCG
 - CGATCTGTGTTTGTCTGA
Reference: Tissue: colon_133990_18461
 Cancer Temp: 52 C Primer Temp: 52 C
- 40 50 Cancer Oligo: + GAGAATACTGATTGAGACCTA
 - TAGGTCTCAATCAGTATTCTC
Reference: Tissue: colon_134014_18566
 Cancer Temp: 58 C
- 45 51 Cancer Oligo: + CCAGCCAGCACCCAGGC
 - GCCTGGGTGCTGGCTGG
Primer Oligo: + CCAGCCAGCACCCAGG
 - CCTGGGTGCTGGCTGG
Reference: Tissue: colon_78026_722
50 Cancer Temp: 60 C Primer Temp: 56 C
- 55 52 Cancer Oligo: + TAGACCAACAGAGTTCC
 - GGA ACTCTGTTGGTCTA
Reference: Tissue: colon_121771_670
 Cancer Temp: 50 C
- 53 Cancer Oligo: + CTAGGTACGAGGCTGGGTTTT

- 5
Primer Oligo: - AAAACCCAGCCTCGTACCTAG
+ CTAGGTACGAGGCTGGG
- CCCAGCCTCGTACCTAG
Reference: Tissue: colon_121801_753
Cancer Temp: 64 C Primer Temp: 56 C
- 10
54 Cancer Oligo: + CGAGGCGGGTGTTAATCGGCC
- GGCCGATTAACACCCGCCTCG
Primer Oligo: + CGAGGCGGGTGTTAATC
- GATTAACACCCGCCTCG
Reference: Tissue: colon_123056_2392
Cancer Temp: 70 C Primer Temp: 54 C
- 15
55 Cancer Oligo: + AAGGCTAGGTAGAGGCTG
- CAGCCTCTACCTAGCCTT
Reference: Tissue: colon_123353_2625
Cancer Temp: 56 C
- 20
56 Cancer Oligo: + CATGGCCATGCTGTGCA
- TGCACAGCATGGCCATG
Reference: Tissue: colon_123371_2693
Cancer Temp: 54 C
- 25
57 Cancer Oligo: + AGGTACGAGGCCGGTGTTAATCGGCCGA
- TCGGCCGATTAACACCGGCCTCGTACCT
Primer Oligo: + AGGTACGAGGCCGGTG
- CACCGGCCTCGTACCT
Reference: Tissue: colon_123372_2695
Cancer Temp: 90 C Primer Temp: 54 C
- 30
58 Cancer Oligo: + TGCACCACAAGCAAACAGGCC
- GGCCTGTTTGCTTGTGGTGCA
Primer Oligo: + TGCACCACAAGCAAACAG
- CTGTTTGCTTGTGGTGCA
Reference: Tissue: colon_123799_3379
Cancer Temp: 66 C Primer Temp: 54 C
- 35
59 Cancer Oligo: + TGCTGCCCTCAATGGTC
- GACCATTGAGGGCAGCA
Reference: Tissue: colon_124226_4533
Cancer Temp: 54 C
- 40
60 Cancer Oligo: + AGGCCGGTGTTAATCGGCCGAGG
- CCTCGGCCGATTAACCACCGGCCT
Primer Oligo: + AGGCCGGTGTTAATCG
- CGATTAACCACCGGCCT
Reference: Tissue: colon_124431_5222
Cancer Temp: 80 C Primer Temp: 54 C
- 45
61 Cancer Oligo: + GAGGCCGGTGTTAATCGGCCGAG

5
 Primer Oligo: - CTCGGCCGATTAACCACCGGCCTC
 + GAGGCCGGTGGTTAATC
 - GATTAACCACCGGCCTC
 Reference: Tissue: colon_124442_5305
 Cancer Temp: 80 C Primer Temp: 54 C

10
 62 Cancer Oligo: + GCTAGGTACGAGGCTGGGTTTT
 - AAAACCCAGCCTCGTACCTAGC
 Primer Oligo: + GCTAGGTACGAGGCTGG
 - CCAGCCTCGTACCTAGC
 Reference: Tissue: colon_124449_5356
 Cancer Temp: 68 C Primer Temp: 56 C

15
 63 Cancer Oligo: + AACATACGGCTAGGTACGA
 - TCGTACCTAGCCGTATGTT
 Reference: Tissue: colon_124461_5420
 Cancer Temp: 56 C

20
 64 Cancer Oligo: + GGTGGTAATCGGACGAGG
 - CCTCGTCCGATTACCACC
 Reference: Tissue: colon_124495_5584
 Cancer Temp: 58 C

30
 65 Cancer Oligo: + GGGTGATCGGACGAGGC
 - GCCTCGTCCGATCACCC
 Reference: Tissue: colon_124565_5924
 Cancer Temp: 58 C

35
 66 Cancer Oligo: + ACATGCCTAGGGTTCAA
 - TTGAACCCTAGGCATGT
 Reference: Tissue: colon_128283_2235
 Cancer Temp: 50

These 59 cancer markers include many SNPs, but they also include longer mutations.

40
 Cancer marker supersets specific for other types of cancers have also been identified. Cancer markers for lung cancer are provided in TABLE 3 and those for lymph cancer in TABLE 4.

45 *TABLE 3: Lung Cancer Marker Subset*

A Cancer Oligo: + TGAGACAGCTCATCACA
 - TGTGATGAGCTGTCTCA
 Reference: Tissue: lung_97380_1525
 Cancer Temp: 50 C Primer Temp: 50 C

	B Cancer Oligo:	+ TCTGGACTGATCTAACA - TGTTAGATCAGTCCAGA
5	Reference:	Tissue: lung_114200_11255 Cancer Temp: 48 C Primer Temp: 48 C
	C Cancer Oligo:	+ CAAGTTCCTATAGGAGT - ACTCCTATAGGAACTTG
10	Reference:	Tissue: lung_116399_15887 Cancer Temp: 48 C Primer Temp: 48 C
	D Cancer Oligo:	+ TGCCATAAACTGGGTTA - TAACCCAGTTTATGGCA
15	Reference:	Tissue: lung_107413_2916 Cancer Temp: 48 C Primer Temp: 48 C
20	E Cancer Oligo:	+ GGCTAGGTACGAGGCTGGGTGTG - CACACCCAGCCTCGTACCTAGCC
	Primer Oligo:	+ GGCTAGGTACGAGGCTG - CAGCCTCGTACCTAGCC
25	Reference:	Tissue: lung_99814_4327 Cancer Temp: 76 C Primer Temp: 56 C
	F Cancer Oligo:	+ AAACCTGCAATATGATG - CATCATATTGCAGGTTT
30	Reference:	Tissue: lung_124202_2868 Cancer Temp: 46 C Primer Temp: 46 C
	G Cancer Oligo:	+ GCGTGATGGCGGGGGGCTCT - AGAGCCCCCGCCATCACGC
35	Primer Oligo:	+ GCGTGATGGCGGGGGG - CCCCCGCCATCACGC
	Reference:	Tissue: lung_98869_3329 Cancer Temp: 70 C Primer Temp: 54 C
40	H Cancer Oligo:	+ GCTTACATCCGTGATGT - ACATCACGGATGTAAGC
45	Reference:	Tissue: lung_108655_5362 Cancer Temp: 50 C Primer Temp: 50 C
	I Cancer Oligo:	+ TTA CTCTCATGTGGCCAA - TTGGCCACATGAGAGTAA
50	Reference:	Tissue: lung_123536_1762 Cancer Temp: 52 C Primer Temp: 52 C
	J Cancer Oligo:	+ TCTGATGAACAGAAGAAG - CTTCTTCTGTTCATCAGA
55	Reference:	Tissue: lung_125101_4407 Cancer Temp: 50 C Primer Temp: 50 C

TABLE 4: Lymph Cancer Marker Subset

5	i Cancer Oligo:	+ GCTGAACCTGCGACTGGTA
		- TACCAGTCGCAGGTTTCAGC
	Primer Oligo:	+ GCTGAACCTGCGACTGG
10		- CCAGTCGCAGGTTTCAGC
	Reference:	Tissue: lymph_67664_6573
		Cancer Temp: 60 C Primer Temp: 56 C
15	ii Cancer Oligo:	+ TAGGTACGAGGCTGGGT
		- ACCCAGCCTCGTACCTA
	Reference:	Tissue: lymph_55415_7578
20		Cancer Temp: 54 C Primer Temp: 54 C
	iii Cancer Oligo:	+ GGCTAGTACGAGGCTGGGT
		- ACCCAGCCTCGTACTAGCC
25	Primer Oligo:	+ GGCTAGTACGAGGCTGG
		- CCAGCCTCGTACTAGCC
	Reference:	Tissue: lymph_55600_7985
30		Cancer Temp: 62 C Primer Temp: 56 C
	iv Cancer Oligo:	+ CTAGGTACGAGGCTGGGTG
		- CACCCAGCCTCGTACCTAG
35	Primer Oligo:	+ CTAGGTACGAGGCTGGG
		- CCCAGCCTCGTACCTAG
	Reference:	Tissue: lymph_60248_7359
40		Cancer Temp: 62 C Primer Temp: 56 C
	v Cancer Oligo:	+ GTACGAGGCTGGGTGTT
		- AACACCCAGCCTCGTAC
45	Reference:	Tissue: lymph_60270_7430
		Cancer Temp: 54 C Primer Temp: 54 C
	vi Cancer Oligo:	+ GAAACTGTTGGCGTGAT
50		- ATCACGCCAACAGTTTC
	Reference:	Tissue: lymph_50077_1076
		Cancer Temp: 50 C Primer Temp: 50 C
55	vii Cancer Oligo:	+ GAGCAGAAACGGGAGACCTG
		- CAGGTCTCCCGTTTCTGCTC
	Primer Oligo:	+ GAGCAGAAACGGGAGAC
50		- GTCTCCCGTTTCTGCTC
	Reference:	Tissue: lymph_69924_10602
		Cancer Temp: 64 C Primer Temp: 54 C
55	viii Cancer Oligo:	+ GGCCTTCGAGCGGGGTGTTGGGG
		- CCCCAACACCCCGCTCGAAGGCC
	Primer Oligo:	+ GGCCTTCGAGCGGGG
55		- CCCCGCTCGAAGGCC
	Reference:	Tissue: lymph_50152_1336
		Cancer Temp: 80 C Primer Temp: 54 C

ix Cancer Oligo: + AGTTTCTTCAAGATCAC
- GTGATCTTGAAGAACT
5 Reference: Tissue: lymph_62939_1828
Cancer Temp: 46 C Primer Temp: 46 C

x Cancer Oligo: + GAGGAAGTAATCTGCCC
- GGGCAGATTACTTCCTC
10 Reference: Tissue: lymph_13680_599
Cancer Temp: 52 C Primer Temp: 52 C

Samples Tested

15 The cancer detection reagents discussed herein may be used on any sample likely to contain the cancer markers. However, in preferred embodiments, the markers are detected in an easily obtainable bodily fluid, such as peripheral blood. Use of peripheral blood may also
20 provide the advantage of allowing markers from several differentiated tumors in the same subject to be detected at once. Yet there may be circumstances, such as when information about only one tumor is desired, in which tissue samples or other samples are examined.

25 Cancer tissue samples and biopsies usually come from a single tumor, even when multiple tumors are present. In the early stages of cancer most cancer cells are daughters of a parent tumor and often have the same mutations as the cells in the tumor. However, metastatic
30 cancer cells often have different mutations. Further, metastatic tumors, even if initially similar, follow different development pathways and may accumulate different additional mutations over time. Finally, it is well known that many cancer treatments cause further
35 mutations in cancer cells. Therefore, cancer cells in later stages of cancer often do not have the same mutations as those in early stages. Variation in

mutations is also often seen among metastatic tumors in the same individual.

Because tumors tend to have individual mutations, it follows that a tissue sample taken from a single tumor will likely not contain all the cancer mutations found throughout a subject's cancer. A profile of all or most mutations in the subject's body using traditional methodologies would thus require samples from multiple tumors. In contrast, in embodiments of the present invention using blood as a sample, all or most of the mutations present in metastatic cancer may be detected in a single sample because it contains cells from multiple tumors. Further a blood sample may even contain cells from small metastatic tumors not detectable using conventional techniques.

Diagnostic Uses

The cancer markers of the present invention and corresponding cancer detection reagents may be used in diagnosis of metastatic cancer, particularly pathology-based diagnosis, including initial diagnosis as well as treatment and disease progression monitoring, and also including monitoring of targeted cancer cell death.

In a preferred embodiment, the present invention is used to detect a plurality of cancer markers to provide a cancer marker profile of the subject. The markers tested may be selected based on a variety of factors. Two factors include overall likelihood of occurrence in any type of cancer, or association with a cancer originating in a particular tissue.

The screening methods of the present invention may be used for a variety of diagnostic purposes. For

purposes of this specification, "diagnostic" refers not only to initial determinations of whether a subject has a disease, but also to any test to examine the nature of a disease. For example, forms of diagnosis in the present specification may include screening in a healthy subject or a subject with symptoms to initially determine whether cancer is present, testing at any point after a subject has been determined to have cancer, testing to help recommend or monitor a course of treatment, prognostic testing, testing to monitor the development of cancer, including the development of any new mutations, and testing to determine the presence or absence or eradication of metastatic cells.

For example, the methods of the present invention may be used to detect the presence of cancer cells, particularly metastatic cancer cells or other cancer cells found in the blood. The methods may be used for initial diagnosis of cancer or metastatic cancer, even when tumors are too small to be detected by imaging or other techniques.

Screening according to the present invention may be used to not only indicate the presence of cancer cells, but also to determine some or all of the mutations or abnormalities present in these cells. Knowledge of the mutations present may be used in directing treatment. For example, drugs known to be effective against certain types of cancer only may be prescribed or avoided based on the underlying mutations of a subject's cancer. Additionally, knowledge of subject-specific cancer mutations may be used to develop new classes of cancer drugs, including subject-specific cancer drugs targeted to the diagnosed mutations. These targeted drugs may

affect the mutant proteins, particularly cell-surface proteins, or they may act on cellular nucleic acids, such as mRNA.

Further, additional testing incorporating regions
5 flanking the cancer marker sites may be used to determine the specific genes affected by a cancer marker in a given cancer patient. As TABLES 1 and 2 clearly show, while some cancer markers are associated with only a few genes, most have been found in a number of genes. The function
10 of some of these genes is known. Accordingly, the ability to determine in which gene a cancer marker lies provides additional information that may be used to direct cancer treatment.

Given the way public data is generated, one would
15 expect much chance and coincidence in any commonality or lack thereof between the cancer markers and cancer cell lines. However, FIGURE 5 suggests that some cancer markers appear in some cell lines while others appear in different cell lines. This suggests that some cancer
20 markers are found in some cancer subjects while others are found in different cancer subjects. Each cancer subject is expected have mRNA containing a subset of cancer markers constituting an individual cancer profile, and identifying which genes may be mutated in that
25 individual. It is possible however, that with a large enough subject pool, the same cancer profile may be observed among different subjects, but nevertheless one does not expect every subject in the pool to have an identical cancer profile.

30 The extent of individualism in cancer is not clearly understood. However, individuality nevertheless appears to correlate with cancer type, as illustrated in FIGURE

6. The cancer marker hyperset may constitute all mRNA molecules of length 17 mer or greater that are exclusive to cancer cells. Each cancer type then has a corresponding cancer marker superset, and each cancer
5 subject has a cancer marker subset, which is synonymous to their individual cancer profile.

Because TABLEs 1 and 2 present a set of cancer markers found in a variety of different cancer cells, one should not expect to find all of them in a single cancer
10 subject, although this is not impossible. Rather, the 59 cancer markers of TABLEs 1 and 2 or subcombinations thereof are useful in generating a cancer profile for a particular subject's cancer. By including a large number of cancer markers in any assay or set of assays, a more
15 complete cancer profile may be developed. Additionally, knowledge of what cancer markers are not present in subject's mRNA may also be very useful for diagnosis, including prognosis, as well as cancer progression and treatment monitoring. It may, for example, be useful in
20 selecting a treatment for the subject.

Cancer profiles may be created for cancer subjects using a blood sample and the methodologies described herein. FIGURE 9 illustrates steps for one such exemplary methodology. In most instances, a cancer
25 profile may be obtained within a few hours to a few days after obtaining a blood sample from a subject.

Because most cancer markers are associated with a group of genes, one may quickly determine which group of genes are mutating in a subject's cancer in a way that is
30 exclusive to cancer cells. Any subsequent therapy can utilize this genetic information for specific cancer cell targeting. Unfortunately, most existing therapies do not

have this kind of targeting capacity. Therefore, the blood-based tests of the present invention may also be precursory tests for new therapeutics that can use the cancer detection reagents for specific cancer cell
5 targeting.

In a specific embodiment of the present invention, three general types of assays are provided. The first type of assay examines a sample for the presence or absence of cancer markers common in multiple types of
10 cancers. In a preferred embodiment, the testing subset of cancer markers is selected based on their frequency of occurrence in cancers represented in the general cancer hyperset. For example, all cancer markers that have been found in more than a certain number of cancers may be
15 selected. Alternatively, the cancer markers may be ranked in frequency of occurrence and a certain number of them may be selected. For example, the top 300 cancer markers may be selected for use in the diagnostic assay.

As new cancer samples are added to the hyperset,
20 this has had little significant effect on the relative frequencies with which cancer markers are found in cancer tissue. This indicates that the hyperset is representative of cancer overall and that there are some cancer markers that are simply far more likely to appear
25 in any type of cancer than others.

A general diagnostic assay that examines cancer markers from the general cancer marker hyperset might be used, for example, as part of routine screening, such as yearly blood tests. It might also be used for individual
30 with symptoms, such as weight loss, consistent with both cancer and many other diseases.

A second type of assay may focus on a particular type of cancer, such as colon cancer. Like the general assay, this assay might look for a subset of cancer markers occurring at above a certain frequency, or it
5 might look for a certain number of top markers in a frequency ranked list. Cancer marker supersets for specific cancers also exhibit little change in the relative frequency of higher frequency markers as new data is added.

10 This second type of assay might be used for a subject known to have a specific type of cancer. It might provide a more detailed indication of the mutations present in that subject's cancer than can be obtained using a general cancer assay. It might also provide a
15 more detailed prognosis or treatment plan.

The third type of assay determine which genes are affected by a subject's cancer mutations. This assay may be used at any point, but for cost and efficiency reasons, may be focused on specific cancer markers, and
20 may be used only for subjects previously shown to have those cancer markers. However, in some embodiments, such as those focusing on common cancer markers, it may be efficient to screen for affected genes concurrently with the cancer marker screen.

25 This third type of assay may detect specific genes by also examining the flanking nucleic regions around the cancer marker. These flanking regions tend to differ from gene to gene. Flanking regions suitable for a given assay method and able to distinguish potentially affected
30 genes from one another will be apparent to one skilled in the art.

Cancer Marker Profiles

Cancer marker profiles may be developed for individual subjects. These subjects are most often a human, such as a human having or suspected of having cancer. However, subjects may also include other
5 mammals. Subjects may include patients. In certain contexts, the subject may be a tumor or suspected tumor.

Cancer marker profiles include the identity of a cancer marker and an indication of whether it was detected in the subject. Cancer marker profiles
10 generally provide this information for more than one cancer marker. Cancer marker profiles may provide results in a simple positive/negative format. They may also indicate an amount of cancer marker found either quantitatively or qualitatively. Finally, cancer marker
15 profiles may include information about the gene or genes in which a cancer marker is found in a subject.

All mammals accumulate somatic mutations as they age. Experiments have shown that healthy tissue is free of cancer markers. However, because blood often contains
20 aberrant cells found anywhere in the body, it is likely that an adult mammal, or even a juvenile, will exhibit some cancer markers in its blood.

The presence of some cancer markers in a subject's blood does not necessarily indicate that the subject has
25 cancer. Rather, the number, type, or combination of cancer markers is likely indicative of whether the subject has cancer. For any given set of cancer markers, routine experimentation comparing blood from healthy individuals with that from patients known to have cancer
30 should readily reveal which cancer marker profiles are indicative of cancer and which are not. Further, long-term studies that track whether healthy subjects develop

cancer, when, and what their cancer marker profiles were over the course of the study should reveal cancer marker profiles that are indicative of an increased propensity to develop cancer. This information may be used to guide preventative measures or early cancer treatment.

Diagnosis Protocols and Examples

Cancer markers in a sample may be identified using any appropriate method. However, in a specific embodiment, cancer markers may be identified by PCR analysis of a peripheral blood sample. PCR analysis may include RT-PCR, in which mRNA from the sample is converted to cDNA. This cDNA is then subject to PCR Reduction. Further, PCR analysis may be very readily tailored to include detection of flanking regions, allowing analysis of which gene is affected by a cancer marker.

PCR Reduction

Traditional PCR amplifies a set region of nucleic acid located between the 5' and 3' primers. Because both 5' and 3' primers are used, the newly created nucleic acid strand becomes available as a template in the next cycle. All primers and PCR conditions are not equally effective at amplification, thus some create new templates at a higher rate than other primers. The effect combined with the ability of new strands to serve as templates results in significant differences in the number of individual nucleic acid strands having the amplified sequence when different primers are used. This difference is related to primer and PCR-condition efficiency rather than the actual number of template strands that were available in the original sample.

A more accurate comparison of the numbers of mRNA molecules containing different cancer markers in a given sample may be obtained using a modified type of PCR herein referred to as "PCR Reduction". Using this methodology, only 5' primers are provided. These primers are able to hybridize with the original template nucleic acid, but not with any strands produced as part of the PCR process because such strands contain sequences identical to, but not complementary to the 5' primer. Because only the original template nucleic acid may serve as a template for the PCR reaction, differences in copy number of different cancer detection reagent sequences due to primer or PCR efficiency are not so pronounced. Copy number has a much closer correlation with actual number of original templates.

In PCR Reduction, polymerization occurs until the polymerase falls off of the template strand. This tends to leave a trailing end after the 5' primer. These trailing ends vary somewhat in length, but normally all terminate within several hundred base pairs of the primer. Thus, all of the PCR reaction products may be resolved via electrophoresis on a gel as a single, but slightly blurry band. One example PCR Reduction methodology is illustrated in FIGURE 7.

Although amplification of the cancer markers alone might be useful in some embodiments of the invention, in the PCR Reduction technique described above the tailing end allows for easy gel-based detection that could not be easily achieved using the small cancer detection reagents alone. If there is no cancer detection reagent sequence present in the sample, then the primers have no template and no band shows up at the expected location after

electrophoresis. On the other hand, if the cancer detection reagent sequences are present, a blurry band is present. The intensity of this band may be analyzed using conventional techniques to estimate the relative abundance of templates in the sample containing each detection reagent sequence.

Although it is difficult to detect which gene contains the particular cancer marker using PCR Reduction and a gel alone, such information can be determined through further analysis of the PCR Reduction product. For example, traditional PCR using primers specific to different genes may be performed on the PCR Reduction product. Because the PCR Reduction primer correlates with the cancer marker, but transcription occurs for up to several hundred base pairs, the trailing end will normally be of sufficient length to allow different genes to be distinguished. It is also possible to sequence the PCR Reduction products to determine which gene or genes contain the cancer marker.

20 *MicroArrays*

In another embodiment, a microarray may be constructed based on cancer markers. Cancer detection reagents including these markers may be placed on the microarray. These cancer detection reagents may be different than those used in PCR methods. However, they should be designed and used in conditions such that only nucleic acids having the cancer marker may hybridize and give a positive result. Microarray-based assays are also very amenable to detection of flanking regions, allowing identification of specific affected genes. Most existing microarrays, such as those provided by Affymetrix (California), may be used with the present

invention. Microarrays specifically able to detect SNPs or small deletions may be particularly useful, as many cancer markers fall in these two categories of abnormalities.

5 In particular embodiments, three types of microarrays may be provided that roughly correspond to the three types of assays described above. Specifically, a general cancer marker microarray may be provided, for example for use in general screening. Another type of
10 microarray, each for a specific type of cancer, may be provided, for example for more detailed diagnosis of a subject known to strongly suspected to have a given type of cancer. Finally, a third type of microarray able to distinguish the gene affected by a cancer marker may be
15 provided. This type of microarray may be tailored to one cancer marker, or it may be able to detect specific affected genes for a number of cancer markers.

Hybrid microarrays able to do multiple types of assays on the same array are also possible. For example,
20 a single microarray may be able to both detect cancer markers and determine the affected genes for those markers.

Other Assays

In additional embodiments, other methods of nucleic acid analysis may be used. For example, FACS bead-based
25 assays, such as those available for nucleic acid analysis through Luminex (Texas) or Becton-Dickinson (New Jersey) may be used to detect cancer markers and gene-identifying flanking sequences.

30 Finally, peptide-based assays are also possible. Because the cancer markers were identified through mRNA

analysis, it is expected that most of them will be expressed as an aberrant protein. These assays may be particularly useful for cancer markers often found in surface proteins, although cells may be readily lysed to
5 allow access to internal proteins as well. Peptide analysis using antibodies may be particularly useful, as such antibodies may have later applications in treatment.

Kits and Services

The cancer markers of the present invention may be
10 detected using kits. These kits may include cancer detection reagents suitable for a particular type of assay. Other reagents useful in the assay may be included in the kit. Use of the kit may result in a cancer marker profile for the subject. Kits may be
15 designed for use in any aspect of medical testing, including laboratory research, commercial diagnostic laboratory testing, hospital or clinic laboratory testing, or physician's office testing. Kits may require specific additional equipment, such as a PCR cycler,
20 microarray reader, or FACS machine.

The present invention may also be supplied commercially as a testing service. For example, a sample may be provided to a commercial testing laboratory which then uses appropriate cancer detection reagents and assay
25 to determine the cancer profile for the sample. The results may then be returned to the entity providing the sample.

Uses of Diagnostic Results

Diagnostic results may be used to direct the
30 treatment of a patient who appears to have cancer or to be likely to develop cancer in a number of manners. The

patient may be given preventative treatment based on the presence of a large number of cancer markers or certain combinations. The patient may also be treated differently depending on the stage of the disease. Treatment may be
5 varied as the disease and cancer markers change.

Treatment itself may include conventional treatments, such as chemotherapy. It may also include antibody or antisense therapy based on the particular cancer profile of the patient. The patient's cancer
10 markers may be used to develop antibodies to a cancer marker specific epitope. They may also be used to develop antisense molecules that will interfere with the cellular mechanisms of cancer cells, but not normal cells.

15 Because the cancer detection reagents of the present invention are absent in the healthy cell transcriptome, they represent cancer-specific targets for inducing cancer cell death. For example, although some cancer detection reagents may be translated into peptides
20 located primarily within the cell, some are embedded in sequences that normally encode extracellular or membrane proteins. Such sequences are readily known to the art and are considered predictive of the likely cellular location of a protein and portions of it. Accordingly,
25 particularly for proteins with extracellular regions, administration of an antibody specific for a peptide encoded by a cancer detection reagent is expected to induce cell death. Because only cancer cells exhibit these peptides, only cancer cells are targeted and killed
30 by the antibodies.

Antibodies used in conjunction with the present invention may include monoclonal and polyclonal

antibodies, non-human, human, and humanized antibodies and any functional fragments thereof.

Although a single cancer detection reagent may be used to target multiple genes or gene products in the methods of inducing cancer cell death of the present invention, in some embodiments multiple cancer detection reagents may be targeted to produce an potent effect. Combined agents targeting more than one cancer detection reagent may also be particularly useful if administered to a subject with multiple tumors. The subject's tumors may have differentiated such that every tumor does not contain any one cancer detection reagent sequence. Incorporating agents targeted to multiple cancer detection reagent sequences may allow these differentiated cancer cells to be killed more effectively. Such combined approaches are particularly powerful against new or small tumors that may not be detected using conventional methods, but nevertheless contain a cancer detection reagent sequence detectable when diagnostic methods of the present invention are used to create a cancer profile.

Thus, targeted cancer cell death may be accomplished according to selected methods of the present invention according to a three-step method. First, a cancer profile may be created for the subject. Second, a targeted cancer cell death agent may be created and tested on the subject's blood or other tissue sample. Third, the agent may be administered to the subject to cause targeted death of cancer cells in that subject. This process may be accomplished in as little as three weeks.

Continued monitoring may allow detection of the disappearance of any cancer detection reagents in the

subject as well as the appearance of any new ones. The agent or combination of agents administered to the subject may then be changed accordingly.

EXAMPLES

5 The following examples are included to demonstrate specific embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to
10 function well in the practice of the invention. 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 that are disclosed and still obtain a like or similar result without departing from
15 the spirit and scope of the invention.

Example 1: Methods, Reagents and Subject Background

To accomplish these tests, two volunteers with phase 4 metastasized colon cancer were selected. These volunteers are herein referred to as subject R and
20 subject H. Subject R is a female. Subject R provided a 9 mm, excised tumor for testing as well as a 60 mL peripheral blood sample. Subject H is a male. Subject H provided a 60 mL peripheral blood sample.

cDNA libraries were constructed from all samples. A
25 cDNA library was also constructed from a pool of random tissue samples from healthy, cancer-free individuals. This cDNA pool represents the normal, non-cancerous sample in these Examples.

Example 2: Cancer Marker Sets

30 By comparing mRNA from cancer cells, as reported in public databases, with normal human mRNA, also as

reported in cancer databases, using a proprietary computer program, a number of cancer markers have been identified. These cancer markers have been frequency ranked. Because generally each sample of cancer cells used for reporting in the public database was obtained from a different patient, each occurrence of a cancer marker in the databases correlates with an occurrence in an actual human subject. Thus, the frequency of occurrence in the databases roughly corresponds with the past and expected future frequency at which a cancer marker appears.

Cancer markers have been ranked based on frequency for each type of cancer examined. Additionally, the present invention reveals that many cancer markers are often found in multiple types of cancer. Thus, markers have been ranked based on their frequency of occurrence overall in all cancer examined.

Some colon cancer markers identified thus far that are also good general cancer markers are provided in TABLEs 1 and 2.

Example 3: Blood Sample Preparation

60 mL of peripheral blood was collected using a standard IV phlebotomy needle in purple top a vacuum tube containing EDTA. Tubes containing heparin may also be suitable. The blood was then stored at 4°C until further processing. Processing was completed as quickly as possible in order to lessen RNA degradation.

Total RNA was isolated using a QIAamp RNA blood mini kit. (Quiagen, California) The total yield of RNA was approximately 60 µg.

Later tests revealed that blood samples were prepared using Trizol reagent (Invitrogen, California) yielded approximately 400 μg . However, these samples were not used in the present examples.

- 5 Blood may also be collected in tubes containing pre-aliquoted stabilization reagents, such as Paxgene Blood RNA tubes (Quiagen, California). Paxgene tubes hold 2.5 ml/blood per tube and the blood normally remains stable at room temperature for 5 days. Paxgene tubes are
10 specifically designed to prevent RNA degradation as well as gene induction that sometimes occurs after blood is collected.

Example 4: Primers for PCR testing

- 15 Typical primer data as provided by the manufacturer is as follows.

Synthesis scale: 200 nmol

Length: 17mer

Molecular Weight (Ammonium Salt): 5383.4

Exact Weight per OD (Ammonium Salt): 32.34

- 20 Nanomoles per OD (Ammonium Salt): 6.12

Millimolar Extinction Coefficient: 163.35

Total ODs in This Tube: 20

Total Amount in μg : 646.76

Total Amount in nmoles: 122.44

- 25 Purification: Desalted

Melting Temperature (Celcius): 56.0

5' End: OH

3' End: OH

For each of the 59 general cancer markers identified in TABLE 2, PCR Primers for the markers identified as well as PCR conditions are provided.

Example 5: cDNA Synthesis

5 Prior to cDNA synthesis, residual DNA was removed from the total RNA by DNAase I digestion. Specifically, a reaction mixture was created having a total volume of 10 μ L and containing 5 μ g of total RNA, 1 μ L of 10X buffer and 1 μ L of DNAase I. This mixture was maintained
10 at room temperature for 15 minutes, then 1 μ L of 25 mM EDTA was added. The EDTA mixture was incubated for 15 minutes at 65 °C, then placed on ice for 1 minute. The reaction was collected by centrifugation.

A SuperScript III kit (Invitrogen, CA) was used for
15 first strand cDNA synthesis from the DNAase I digested total RNA samples. A poly T primer was used. However, a random primer may also be used. Random primers may be particularly desirable if the cancer marker is located far upstream of the polyT tail of an mRNA.

20 Approximately 10 μ L of DNAase I digested RNA was mixed with 1 μ L of 10 mM dNTP and 1 μ L of oligodT (0.5 μ g/ μ L) primer. This RNA/primer mixture was incubated at 65 °C for 5 minutes, then placed on ice for 1 minute.

A reaction mixture was prepared containing 2 μ L of
25 10X RT buffer, 4 μ L of 25 mM MgCl₂, 2 μ L of 0.1 M DTT, and 1 μ L of RNAase Out (Invitrogen, California). 9 μ L of reaction mixture was added to the RNA/primer mixture. The total mixture was collected by centrifugation then incubated at 42 °C for 2 minutes. 1 μ L (50 units) of
30 Superscript III RT (Invitrogen, California) was then added and the resulting mixture was incubated at 42 °C

for 50 minutes. The reaction was terminated by incubation at 70 °C for 15 minutes or at 85 °C for 5 minutes, followed by chilling on ice. The reaction was collected by centrifugation.

5 Finally, 1 μ L of RNAase H was added and the sample was incubated for 20 minutes at 37 °C to degrade the remaining RNA.

Each sample was treated in this manner. The single cDNA sample created was then used as the starting
10 material for each subsequent PCR Reduction reaction.

Example 7: PCR Reduction

PCR Reduction was used to amplify any cancer markers in the cDNA. As explained above, PCR reduction gives a more accurate picture of relative amounts of mRNA
15 carrying a cancer marker in the sample because it does not result in products that can themselves become templates for amplification. Rather, through use of only one primer, only the original templates are available for amplification throughout the reaction.

20 A PCR reaction mixture was created having a total volume of 20 μ L and containing 13.8 of μ L DEPC-treated water, 2 μ L of 10X PCR buffer without Mg, 1 μ L of 25 mM $MgCl_2$, 0.5 μ L of 10 mM dNTP mixture, 1 μ L of 20 μ M antisense primer (cancer detection reagent), 1.5 μ L of
25 cDNA sample, and 0.2 μ L of high fidelity 5 units/ μ L Taq DNA polymerase.

PCR was carried out in 35 cycles. First the PCR reaction mixture was denatured at 94 °C for 5 minutes. Then, each of the 35 cycles include 30 sec of
30 denaturation at 94 °C, 30 seconds of annealing at the annealing temperature for the primer (annealing

temperatures are indicated in TABLE 2), and 1 minute of extension at 72 °C. Upon completion, the reactions were maintained at 4 °C.

Conditions were selected to obtain amplification products in the range of 100-500 bp. Conditions may be altered to obtain different sized products.

PCR analysis of both blood and tumor tissue for two terminally ill subjects was performed using antisense cancer detection reagents corresponding to cancer markers 3 and 5-66 of TABLE 2.

Example 8: PCR Results

To determine whether cancer markers were present in the samples, after PCR was complete 10 μ L of PCR reaction mixture was loaded on a 1% agarose gel and electrophoresis was performed. The gels were then imaged.

PCR Results are provided in TABLE 5. As the table shows, the markers identified are generally not present in normal tissue. (The one that did appear in normal tissue has been excluded from inclusion as a cancer marker, although it remains possible that it is a cancer marker that, due to gradual accumulation of somatic mutations, was present in apparently healthy tissue.)

TABLE 5 shows the results of single priming RT-PCR using the primers with the Apoptotic Sequences from TABLE 1, the three cancer samples, and a vascular wall healthy control sample. A plus sign in TABLE 5 indicates a sequence's presence and a minus sign indicates a sequence's absence. Those sequences found in the healthy control sample were discarded from the candidate

Apoptotic Sequence pool, while the others are available for subsequent cell death tests.

Table 5: Candidate Apoptotic Sequence RT-PCR Detection Tests

Candidate Apoptotic Sequence Number	Healthy cDNA	Human colon cancer tumor cDNA from Subject R	Human colon cancer blood cDNA from Subject R	Human colon cancer blood cDNA from Subject H
1	+	+	+	+
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	+	+	+
6	-	-	-	-
7	-	-	-	-
8	-	+	+	+
9	-	+	+	+
10	-	-	-	-
11	-	+	+	+
12	-	+	+	-
13	-	-	+	-
14	-	+	+	+
15	-	-	-	-
16	-	+	+	+
17	-	-	+	-
18	-	-	-	-
19	-	-	-	+
20	-	-	+	-
21	-	-	-	+
22	-	-	+	-
23	-	+	-	-
24	-	-	-	-
25	-	-	+	+
26	-	+	+	+
27	-	-	-	-
28	-	-	+	-
29	-	-	-	-
30	-	+	+	+
31	-	+	+	-
32	-	-	+	+
33	-	+	+	+
34	-	-	-	-
35	-	+	+	+
36	-	-	-	-
37	-	-	+	+

38	-	-	-	+
39	-	-	-	-
40	-	-	-	-
41	-	-	-	-
42	-	-	+	-
43	-	-	-	-
44	-	-	-	-
45	-	-	-	-
46	-	-	-	-
47	-	-	-	-
48	-	-	+	+
49	-	-	-	-
50	-	-	-	-
51	-	-	+	+
52	-	-	-	-
53	-	+	+	+
54	-	-	-	+
55	-	+	+	+
56	-	-	+	+
57	-	+	+	+
58	+	-	+	-
59	-	-	+	+
60	-	+	+	+
61	-	-	+	+
62	-	-	+	-
63	-	-	-	+
64	-	-	+	-
65	-	-	-	+
66	-	+	+	+

TABLE 5 also indicate that analysis of blood actually identifies more cancer markers than analysis of tumor tissue. This is true when comparing blood and tumors from different subjects and from the same subject. This likely results from the presence of multiple tumors in each subject. Different tumors have likely accumulated different mutations over time. Tumor tissue samples can only reveal the mutations in a single tumor. However, the blood analysis techniques of the present invention can reveal mutations from multiple tumors at the same time so long as their cancer markers are present in the blood.

These human sample tests have been conducted to assess: i) validity of cancer markers; ii) their individuality; and iii) the ability to select them from a superset for a random cancer subject based purely on computational ranking. The latter characteristic is significant because it is not currently practical to test the tens of thousands of cancer markers from each superset corresponding to the cancer type of the human test samples.

TABLE 1 shows both strands of cancer detection reagents used to test these samples (although only the antisense strand was actually used). Cancer markers also affect both strands of DNA in the a subject. As described above, the cancer markers were filtered through the healthy human transcriptome contained in databases and neither stand appeared. This design constraint, and the small size of the cancer detection reagents makes them optimal for in-vitro in cDNA library diagnostics. Consequently, a cancer detection reagent was created for each cancer marker in TABLE 1.

FIGURE 8 shows the results from PCR Reduction using the cancer detection reagents in TABLE 1 and the cDNA from patient R's tumor, patient H's peripheral blood, and random tissue from healthy non-cancerous subjects. The healthy subject results are in lane 1, patient R results are in lane 2, and patient H results are in lane 3.

The blurred bands exhibited in FIGURE 8 are caused by the variations in the trailing end lengths. Because of the absolute, cancer-if-present and healthy-if-absent nature of the cancer detection reagents, the results were interpreted as signal = cancer and no signal = healthy.

As FIGURE 8 shows, the cancer detection reagents in TABLE 1 never yielded positive results from the healthy cDNA in lane 1 of the gels. (Other than cancer detection reagent 58, which, as described above, has now been
5 excluded.)

Patient R and patient H exhibited common markers, as was expected given that both suffered from colon cancer. However, some variation was present in their cancer marker profiles as was also expected between different
10 individuals. This reveals the individuality in the cancer marker profiles of the two subjects.

Finally, TABLE 1 includes only the highest ranked markers from the colon cancer superset. As FIGURE 8 demonstrates, computational occurrences of cancer markers
15 in specific types of cancer cell lines presents a viable ranking method for reducing the amount of in-vitro testing required to establish individual cancer marker profiles for actual human subjects.

FIGURE 8 shows a varied degree of band intensity for
20 different cancer detection reagents. Because PCR Reduction was used to in the assays, this amplitude is a good reflection of the number of mRNA transcripts containing each of the cancer markers present in the relevant samples. This information may be helpful in
25 determining applicable targets for diagnostic and therapeutic purposes.

For clarity, TABLE 5 presents a tabular listing of the results in FIGURE 8. TABLE 5 and FIGURE 8 show that PCR Reduction assays using the 59 cancer detection
30 reagents of TABLE 1 are sensitive enough to detect their representative cancer markers in metastasized cancer cells from blood samples. This sensitivity may results

from the one-to-many genetic association of the cancer markers, and thus in many instances, once a blood sample is provided, there will be no further need for tissue samples or biopsies to facilitate cancer pathology
5 analysis.

Cancer detection reagents of the present invention are generally designed to detect mutations that are exclusive to cancer cells, not specific tumors. It has been shown that the cancer detection reagents can detect
10 cancer markers in cells circulating in the blood. So, one would expect PCR Reduction tests for a tumor tissue sample and a blood sample from the same subject to show an increased number of cancer markers in the blood. In fact, any cancer marker profile from a tissue sample
15 alone will likely be inferior to a blood sample because the tissue sample profile is actually a profile for the single, biopsied tumor, and not the subject's cancer in general. This can be seen somewhat in TABLE 5 which shows an increased number of mutations from the blood
20 sample of patient H versus the tissue sample of patient R.

However, to more clearly show the superiority of blood samples over tissue samples, a side by side PCR Reduction assay was conducted using both types of samples
25 from the same cancer subject. The results of this assay for samples from patient R are shown in TABLE 5. Substantially more cancer detection reagents yielded positive results for the blood sample than for the tissue sample alone for patient R. Further, the tissue sample
30 was obtained in March, 2004 and the blood sample was obtained in December, 2004. In the interim the subject underwent extensive cancer therapies. The high number of

mutations in the December blood sample not only reflects the ineffectiveness of the cancer therapies (as confirmed by standard clinical observations), it also reflects the high level of cancer cell traffic in the blood of patient
5 R. This heavy traffic can most likely be correlated with highly active cancer, as is also indicated by patient R's failure to respond to traditional treatment and her continually deteriorating condition.

Example 9: Microarrays

10 Blood samples may also be analyzed using microarrays containing single stranded DNA molecules having the sequences of cancer markers. These DNA molecules represent yet another type of cancer detection reagent. Such microarrays may be created using known techniques,
15 but incorporating the new cancer markers. For example, a microarray for detecting cancer markers 3 and 5-87 may contain single stranded DNA from either strand of the oligos listed in TABLE 1. Blood samples may then be applied to the microarray and the microarray read using
20 known methods to reveal which cancer markers are exhibited by a particular subject's tumors. To affirm the viability of this approach, blood samples may be compared with tumor samples to see if an increased number of cancer markers are observed in the blood samples, as
25 expected. Additionally, results may be compared with those obtained using PCR. It is expected that the results using a microarray should be identical or nearly identical, with any differences explainable by differing sensitivities of the methods.

30 In particular, microarrays may be created using the standard procedures of microarray manufacturers such as Affymetrix (California).

Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alternations can be made herein without departing from the spirit and
5 scope of the invention as defined by the following claims.

CLAIMS

1. A cancer detection reagent set comprising cancer detection reagents corresponding to at least 10 general cancer markers.
- 5 2. The cancer detection reagent set of Claim 1, comprising cancer detection reagents corresponding to at least 50 general cancer markers.
3. The cancer detection reagent set of Claim 1, comprising cancer detection reagents corresponding to at
10 least 100 general cancer markers.
4. A cancer detection reagent set comprising cancer detection reagents corresponding to at least 10 colon cancer markers.
5. The cancer detection reagent set of Claim 4,
15 comprising cancer detection reagents corresponding to at least 50 colon cancer markers.
6. The cancer detection reagent set of Claim 4, comprising cancer detection reagents corresponding to at least 100 colon cancer markers.
- 20 7. A cancer detection reagent set comprising cancer detection reagents corresponding to at least 10 lung cancer markers.
8. The cancer detection reagent set of Claim 7, comprising cancer detection reagents corresponding to at
25 least 50 lung cancer markers.
9. The cancer detection reagent set of Claim 7, comprising cancer detection reagents corresponding to at least 100 lung cancer markers.

10. A cancer detection reagent set comprising cancer detection reagents corresponding to at least 10 lymph cancer markers.

11. The cancer detection reagent set of Claim 10,
5 comprising cancer detection reagents corresponding to at least 50 lymph cancer markers.

12. The cancer detection reagent set of Claim 10, comprising cancer detection reagents corresponding to at least 100 lymph cancer markers.

10 13. A cancer detection reagent set comprising cancer detection reagents corresponding to at least 10 breast cancer markers.

14. The cancer detection reagent set of Claim 13,
15 comprising cancer detection reagents corresponding to at least 50 breast cancer markers.

15. The cancer detection reagent set of Claim 13, comprising cancer detection reagents corresponding to at least 100 breast cancer markers.

16. A cancer detection reagent set comprising at
20 least 50 of cancer markers 3 and 55-66.

17. A microarray comprising at least 50 cancer detection reagents, each operable to detect the presence of a cancer marker in a sample.

18. The microarray of Claim 17, wherein the cancer
25 markers comprise general cancer markers.

19. The microarray of Claim 17, wherein the cancer markers comprise colon cancer markers.

20. The microarray of Claim 17, wherein the cancer markers comprise lung cancer markers.

21. The microarray of Claim 17, wherein the cancer markers comprise lymph cancer markers.

22. The microarray of Claim 17, wherein the cancer markers comprise breast cancer markers.

5 23. A PCR kit comprising at least 50 antisense primers, each corresponding to a cancer marker and operable to detect the presence of a cancer marker in a sample.

10 24. The PCR kit of Claim 23, wherein the cancer markers comprise general cancer markers.

25. The PCR kit of Claim 23, wherein the cancer markers comprise colon cancer markers.

26. The PCR kit of Claim 23, wherein the cancer markers comprise lung cancer markers.

15 27. The PCR kit of Claim 23, wherein the cancer markers comprise lymph cancer markers.

28. The PCR kit of Claim 23, wherein the cancer markers comprise breast cancer markers.

20 29. A method of detecting a cancer marker in a sample comprising:

extracting mRNA from the sample;

creating cDNA from the mRNA;

performing at least 10 separate PCR reduction reactions using the cDNA as a template and at least 10
25 different single primers, with a different single primer in each separate PCR reduction reaction; and

analyzing the product of each of the 10 separate PCR reduction reactions to determine the presence or absence of primer-amplified DNA molecules, where presence of

primer-amplified DNA molecules in any PCR reduction reaction indicates the presence of a cancer marker;

wherein the 10 different single primers each have an antisense sequence corresponding to a different cancer
5 marker.

30. The method of Claim 29, where the sample comprises peripheral blood.

31. The method of Claim 29, wherein the sample comprises tissue.

10 32. The method of Claim 29, wherein the sample comprises a cancer cell having a cancer marker.

33. The method of Claim 29, further comprising performing at least 50 different PCR reduction reactions using at least 50 different single primers.

15 34. The method of Claim 29, further comprising performing at least 100 different PCR reduction reactions using at least 100 different single primers.

35. The method of Claim 29, wherein the single primers comprise 5' primers.

20 36. The method of Claim 29, wherein the single primers comprise 3' primers.

37. The method of Claim 29, wherein any DNA molecules produced by the PCR reduction reactions have a length of between 100 and 500 base pairs.

25 38. The method of Claim 29, wherein analysis comprises electrophoresis of the samples on an agarose gel followed by visual detection of DNA bands of appropriate lengths to be PCR reduction products.

39. The method of Claim 29, wherein analysis
30 comprises:

purification of DNA molecules in the products
resulting from PCR reduction; and

sequencing the DNA molecules.

40. The method of Claim 29, wherein the sequence of
5 the DNA molecules indicates the gene in which the cancer
marker is located.

41. The method of Claim 29, further comprising:
performing further gene detection PCR reactions on
each PCR reduction product using primers corresponding to
10 at least two different genes in which the cancer marker
may be located; wherein the primers are operable to
allow determination, based on the PCR product, of whether
the cancer marker is located in each of the at least two
genes; and

15 determining whether the cancer marker is located in
each of the at least two genes.

42. The method of Claim 29, wherein the 10
different single primers each have an antisense sequence
corresponding to a different general cancer marker.

20 43. The method of Claim 29, wherein the 10
different single primers each have an antisense sequence
corresponding to a different colon cancer marker.

44. The method of Claim 29, wherein the 10
different single primers each have an antisense sequence
25 corresponding to a different colon cancer marker selected
from the group consisting of markers 3 and 5-66.

45. The method of Claim 29, wherein the 10
different single primers each have an antisense sequence
corresponding to a different lung cancer marker.

46. The method of Claim 29, wherein the 10
different single primers each have an antisense sequence
corresponding to a different lymph cancer marker.

47. The method of Claim 29, wherein the 10
5 different single primers each have an antisense sequence
corresponding to a different breast cancer marker.

48. The method of Claim 29, further comprising
creating a cancer marker profile of the sample, wherein
the cancer marker profile includes information indicating
10 the presence or absence of cancer markers in the sample.

49. A method of detecting cancer markers in a
sample comprising:

isolating sample nucleic acids from the sample;

placing the sample nucleic acids on a microarray
15 having DNA molecules operable to detect and distinguish
at least 10 corresponding cancer markers under conditions
sufficient to allow detectable and specific binding of
sample nucleic acids to complementary DNA molecules of
the microarray; and

20 detecting binding of sample nucleic acids to the
microarray,

wherein binding of sample nucleic acids to a DNA
molecule on the microarray indicates the presence of a
cancer marker corresponding to that DNA molecule in the
25 sample.

50. The method of Claim 49, wherein the sample
nucleic acids comprise mRNA.

51. The method of Claim 49, wherein the sample
nucleic acids comprise cDNA created from mRNA present in
30 the sample.

52. The method of Claim 49, further comprising the microarray having DNA molecules operable to distinguish at least 50 corresponding cancer markers.

53. The method of Claim 49, further comprising the
5 microarray having DNA molecules operable to distinguish at least 100 corresponding cancer markers.

54. The method of Claim 49, further comprising the microarray having DNA molecule operable to distinguish the genes in which the cancer markers are located.

10 55. The method of Claim 49, wherein the cancer markers comprise general cancer markers.

56. The method of Claim 49, wherein the cancer markers comprise colon cancer markers.

57. The method of Claim 49, wherein the cancer
15 markers are selected from the group consisting of cancer markers 3 and 5-66.

58. The method of Claim 49, wherein the cancer markers comprise lung cancer markers.

59. The method of Claim 49, wherein the cancer
20 markers comprise lymph cancer markers.

60. The method of Claim 49, wherein the cancer markers comprise breast cancer markers.

61. The method of Claim 49, wherein the sample comprises peripheral blood.

25 62. The method of Claim 49, wherein the sample comprises tissue.

63. The method of Claim 49, further comprising creating a cancer marker profile of the sample, wherein the cancer marker profile includes information indicating
30 the presence or absence of cancer markers in the sample.

64. A method of diagnosing cancer in a subject comprising:

obtaining a sample from the subject;

5 detecting the presence or absence of at least 10 cancer markers in the sample to create a cancer marker profile, wherein the cancer marker profile includes information indicating the presence or absence of cancer markers in the subject; and

10 determining, based on the cancer marker profile, whether the subject has cancer.

65. The method of Claim 64, wherein the sample comprises peripheral blood.

66. The method of Claim 64, wherein the sample comprises tumor tissue.

15 67. The method of Claim 64, wherein the subject is a human.

68. The method of Claim 64, wherein detecting comprises performing PCR reduction on the sample.

20 69. The method of Claim 64, wherein detecting comprises performing a microarray analysis of the sample.

70. The method of Claim 64, further comprising detecting at least 50 cancer markers.

71. The method of Claim 64, further comprising detecting at least 100 cancer markers.

25 72. The method of Claim 64, wherein the cancer markers comprise general cancer markers.

73. The method of Claim 64, wherein the cancer markers comprise colon cancer markers.

74. The method of Claim 64, wherein the cancer markers are selected from the group consisting of cancer markers 3 and 5-66.

75. The method of Claim 64, wherein the cancer
5 markers comprise lung cancer markers.

76. The method of Claim 64, wherein the cancer markers comprise lymph cancer markers.

77. The method of Claim 64, wherein the cancer markers comprise breast cancer markers.

10 78 The method of Claim 64 wherein determining whether the subject has cancer comprises analyzing the number of cancer markers present in the sample.

79. The method of Claim 64, wherein determining whether the subject has cancer comprises analyzing the
15 identity of cancer markers present in the sample.

80. The method of Claim 64, wherein diagnosis includes initial diagnosis.

81. The method of Claim 64, wherein the diagnosis forms part of routine medical screening.

20 82. The method of Claim 64, wherein the subject is suspected of having a particular type of

83. The method of Claim 64, further comprising providing a prognosis for a patient having cancer based on the cancer marker profile.

25 84. The method of Claim 64, further comprising recommending treatment for a patient having cancer based on the cancer marker profile.

85. The method of Claim 64, further comprising monitoring treatment of a patient having cancer based on
30 the cancer marker profile.

86. The method of Claim 64, further comprising comparing the cancer marker profile with a previous cancer marker profile to detect changes in cancer in the subject.