A Method for Screening Cancer

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

The invention relates to the cancer screening method, cancer screening biomarker, and use therof. In particular, to the cancer screening method using methylated DNA as the biomarker.

2. DESCRIPTION OF THE PRIOR ART

Cervical cancer has been one of the main causes of death in females worldwide and in Taiwan. Based on the statistical survey by the World Health Organization (WHO) in 2002, cervical cancer was the second major disease responsible for the death of women worldwide, second to breast cancer. Regular cervical cancer screening is the best way to prevent cervical cancer. Conventional cervical cancer screening includes two approaches: the most commonly used Pap smear, and human papilloma virus testing (HPV testing). Pap smear consists of sampling secreta from cervix uteri, examining under microscope whether there is cancerous pathological change in the exfoliated epithelial cell, so as to detect cervical cancer early. On the other hand, HPV testing consists of examining whether human papilloma virus (HPV) is present in the specimen by using polymerase chain reaction (PCR) or Hybrid Capture.

There are, however, many undesired properties of Pap smear. For one, it requires sampling by a physician, and analysis by a medical

examiner/pathologist, which is a high cost of manpower that poses difficulty on promoting the test in many developing countries. Also, Pap smear has a high false negative rate which delays diagnosis and proper treatment prior to cancerous pathological change. As for HPV testing, although it is highly sensitive, it tends to create a high false positive rate, which not only leaves patients worry in vain, but also wastes much medical resources in examinations follow up to those false positive patients. Accordingly, one of the important topics in promoting cervical cancer examination relies on increasing the accuracy and convenience of cervical cancer examination method.

Genomic deletions have long been considered to be an important factor in tumorigenesis. For a long time, we have been accustomed to the idea that the coding potential of the genome lies within the arrangement of the four A, T, G, C bases. The two-hit theory proposed as early as in 1970s indicates concomitant mutations or deletions of some homologous tumor suppressor genes may cause or predispose to cancer development. However, additional information that affects phenotype can be stored in the modified base 5-methylcytosine. 5-Methylcytosine is found in mammals in the context of the palindromic sequence 5'-CpG-3'. Most CpG dinucleotide pairs are methylated in mammalian cells except some areas called "CpG island." CpG islands are GC- and CpG-rich areas of approximately 1 kb, usually located in the vicinity of genes and often found near the promoter of widely expressed genes. Cytosine methylation occurs after DNA synthesis, by enzymatic transfer of a methyl group from the methyl donor *S*-adenosylmethionine to the carbon-5 position of cytosine. The enzymatic reaction is performed by DNA

methyltransferases (DNMTs). DNMT1 is the main methyltransferase in mammals, and is responsible for the post-replicative restoration of hemi-methylated sites to full methylation, referred to as maintenance methylation, whereas DNMT3A and DNMT3B are thought to be involved primarily in methylating new sites, a process called *isolated* methylation.

Loss of methylation at CpG dinucleotides, i.e., general hypomethylation, was the first epigenetic abnormalities identified in cancer cells. However, during the past few years, it has become increasing apparent that site-specific hypermethylation, e.g., some tumor suppressor genes, is associated with loss of function which may provide selective advantages during carcinogenesis. Dense methylation of CpG islands at promoter regions can trigger chromatin remodeling through histone modifications with subsequent gene silencing. Therefore, in addition to chromosomal deletions or genetic mutations, epigenetic silencing of tumor suppressor genes by promoter hypermethylation is commonly seen in human cancer.

Epidemiologic studies have recently shown the correlation of serum folate level, a major source of methyl group, with the infection and clearance of HPV. Genetic polymorphisms of enzymes in the metabolism of methyl cycle were also reported to be associated with the development of cervical intraepithelial lesions. As the concept of epigenetics evolves, studies exploring the association between DNA methylation and cervical cancer are also booming. Studies of DNA methylation in cervical cancer are accumulating, which showed the potential of using methylation as markers in cervical screening. With the nature of the interface between genetics and environment, the

prevalence of methylation in tumor suppressor genes varies in different genes and different populations. The concept of methylator phenotypes with different disease behaviors was proposed with controversy. The methylator phenotype of cervical cancer and its interaction with HPV genotypes still remains unknown. What genes are specifically methylated in cervical cancer and how many genes are required to achieve clinical application will remain a blossoming issue in the coming future.

The inventor of this application had filed relative patent applications in Taiwan (TW Pat. Pub. No. 200831900)、China(CN Appl. No. 200810094659.2)、Malaysia(UI20085354) and America (US Pat. Pub. No. 20080311570) (Hereinafter referred as prior applications). Comparing with prior applications, the inventor had found some novel cancer screening biomarker and cancer screening method using the same.

SUMMARY OF THE INVENTION

One object of the invention is to provide a cervical cancer screening method as the first line cervical cancer screening.

Another object of the invention is to provide a cervical cancer screening method, characterized in that said method can be used not only in the first line cervical cancer screening, but also in the second line cervical cancer screening to assist HPV testing or uncertain smear result in order to achieve more accurate cervical cancer screening effect.

Yet another object of the invention is to provide a cancer screening method, characterized in that said method can be used in the

screening of cervical cancer as well as in the screening of other cancer (for example: ovarian cancer, colon cancer and the like) to assist the diagnosis of abnormal specimen.

Another object of the invention is to provide the cancer screening biomarker, or a kit containing said biomarker to screen the risk of having cancer.The cancer screening method that can achieve the above-mentioned objects of the invention comprises of detecting the methylation rate of the target gene (biomarker) of the test specimen and use the testing result as a screening index to indicate the risk of having cancer, and said method comprises following steps:

step 1: providing a isolated test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene within the genomic DNA of said test specimen, wherein said target gene comprising DBC1, PDE8B, PTPRR and ZNF582; and

step 3: determining whether there is cancer or pre-cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using said methylation rate of target gene as prognosis marker;

wherein said test specimen comprising a cervical scraping, a ovarian cancer tissue, ascites, blood, urine, feces, sputum, oral mucous membrane cell, gastric juices, bile, cervical epithelial cell, and post-surgery cancer tissue;

wherein testing methods for the methylation state of CpG sequence in said target gene comprising methylation-specific

polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC);

wherein the target gene DBC1 has the nucleotide sequence as depicted in SEQ ID No: 1;

wherein the target gene PDE8B has the nucleotide sequence as depicted in SEQ ID No: 2;

wherein the target gene PTPRR has the nucleotide sequence as depicted in SEQ ID No: 3; and

wherein the target gene ZNF582 has the nucleotide sequence as depicted in SEQ ID No: 4.

In addition, the above-described screening index and screening method can be used further in the screening of cervical cancer, and colon cancer.

The invention provides further a ovarian cancer screening method, characterized in that said method tests the methylation state of the target gene of test specimen, and uses said methylation state as a screening index to determine the risk of having ovarian cancer, and said method comprises following steps:

step 1: providing a isolated test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene within the genomic DNA of said test specimen, wherein said target gene comprising DBC1, PTPRR and ZNF582; and,

step 3: determining whether there is ovarian cancer or cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using said methylation rate of target gene as prognosis marker;

wherein said test specimen comprising ovarian cancer tissue, ascites, blood, urine, and post-surgery cancer tissue;

wherein testing methods for the methylation state of CpG sequence in said target gene comprising methylation-specific polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

wherein the target gene DBC1 has the nucleotide sequence as depicted in SEQ ID No: 1;

wherein the target gene PTPRR has the nucleotide sequence as depicted in SEQ ID No: 3; and

wherein the target gene ZNF582 has the nucleotide sequence as depicted in SEQ ID No: 4.

Term "test specimen" refers herein to isolated test specimen, and said isolated test specimen comprising the above-described cervical scraping, ascites, blood, urine, feces, sputum, oral mucous membrane cell, gastric juices, bile, cervical epithelial cell, post-surgery cancer tissue, or other suitable specimen. The inventive cancer screening method is useful to test the methylation state of target genes in said isolated test specimens, and uses said methylation state of biomarker as a screening index of various types of cancer. The cancer screening

method and the screening index of biomarker provided by the invention can be employed by the researcher to perform the test in the laboratory.

Wherein, "the risk of having cancer" refers herein to the percentage of being cancer, the risk of getting cancer in future, or the presence or not of cancer.

Wherein when the methylation state of target gene (biomarker) being higher compare with suitable control, the risk of having cancer would be higher.

According said screening method, the invention provides further the cancer screening biomarker or a kit containg said biomarker to screen the methylation state of target gene (biomarker) in isolated test specimen for determining the risk of having cancer.

These features and advantages of the present invention will be fully understood and appreciated from the following detailed description of the accompanying Drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows the result obtained by performing bisulfite sequencing (BS) analysis on various cervical specimens using a target gene PTPRR in the inventive cancer screening method;

Figure 1B shows the result obtained by performing bisulfite sequencing (BS) analysis on various cervical specimens using a target gene ZNF582 in the inventive cancer screening method;

Figure 1C shows the result obtained by performing bisulfite sequencing (BS) analysis on various cervical specimens using a target

gene PDE8B in the inventive cancer screening method; and

Figure 1D shows the result obtained by performing bisulfite sequencing (BS) analysis on various cervical specimens using a target gene DBC1 in the inventive cancer screening method.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The invention will be illustrated more detailed with the following examples, but the invention is not limited thereto.

Example 1 Materials and Methods

1. Materials

Test materials comprises a series of full cervical lesion specimens, including: squamous cell carcinoma (SCC, n = 20), adenocarcinoma (AC, n=20), and normal cervical specimen (n=10). All of these cervical specimen, ovarian specimen, and colon cancer specimen were obtained from Tri-service General Hospital, Taipei, ROC. Each specimen was subjected to genomic DNA extraction by means of QIAamp DNA Kit (QIAGEN), and was used in the comparison and analysis of DNA methylation condition within genome-wide DNA methylation. Prior to the analysis, the quality of genomic DNA was checked by Bioanalyzer (Agilent). In this example, 10μg of fragmented DNA was subjected to MeDIP (Methyl DNA IP) assay.

2. DNA methylation analysis by MeDIP and CpG island-Plus-Promoter arrays

Genomic DNA was fragmented to the size ranging from 300 to 1,000 bp by Bioruptor™ UCS-200 (Diagenode). We immunoprecipitated it for overnight at 4 °C with 30 μl of polyclonal Anti-5'-methyl cytosine antibody (Abcam) in a final volume of 100 μl IP buffer (0.15% SDS, 1% Triton X-100, 150mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10 mM Tris and 0.1% BSA). We incubated the mixture with 120 μl of Protein G Sepharose (Amersham) for 2 h at 4°C and washed it twice with 1 ml low salt, high salt buffer, lithium chloride and TE buffer. We then treated twice the protein G with elution buffer (1% SDS, 0.1M $NaHCO_3$) for 15 min at room temperature and recovered the Methylated DNA by phenol-chloroform extraction followed by ethanol precipitation. The enriched methylated DNA and input DNA were amplified by Whole Genome Amplification Kit (Sigma). Enriched and total DNA was end-labeled with Cy5 and Cy3, respectively, and co-hybridized to the CpG Island-Plus-Promoter Arrays designed and synthesized by NimbleGen Systems, Inc. This array contained 385,000 50-75 bp oligonucleotides tiled every 100 bp across the 24,659 HG18 RefSeq promoters (800 bp upstream to 200 bp down stream) and 28,226 CpG islands, present in duplicate.

Each feature on the array has a corresponding scaled log2-ratio. The log2-ratio is normalization to center the ratio data around zero. From the scaled log2-ratio data, a fixed-length window (500 bp) is placed around each consecutive probe and the one-sided

Kolmogorov-Smirnov (KS) test is applied to determine whether the probes are drawn from a significantly more positive distribution than those in the rest of the array. The resulting score for each probe is the -log10 p-value from the windowed KS test around that probe (Scacheri et al, 2006). We detected enriched peaks by searching for at least 2 probes above a p-value minimum cutoff (-log10) of 2. Peaks within 300 bp of each other are merged. Differential methylation regions between SCC/AC and normal cervix within 2500 bp upperstream of transcription star sites were selected for future validation. Finally, p-value data was viewed using SignalMap (NimbleGen).

3. Bisulfite modification, methylation-specific polymerase chain reaction (MSP) and bisulfite sequencing (BS)

A DNA modification kit (Chemicon, Ternecula, CA) was used according to the manufacturer's recommendations to convert 1 μg aliquots of genomic DNA with sodium bisulfite to preserve the methylated cytosines. The final precipitate was eluted with 70 μl of pre-warmed (55°C) TE buffer for MSP.

Normal DNA of human peripheral blood was taken and subjected to bisulfite modification, which was used as the control group having un-methylation promoter sequence.

MSP was performed according to prior art. In short, 1 μl of modified DNA was amplified using MSP primers (table 1) that specifically recognized the methylated gene sequences present in the bisulfite-converted DNA. Methylation-specific PCR was done in a

total volume of 25 μl containing 1 μl of modified template DNA, 1.5 pmol of each primer, 0.2 mmol/L deoxynucleotide triphosphates, and 1 unit of Gold *Taq* DNA polymerase (Applied Biosystems, Foster City, CA). MSP reactions were subjected to an initial incubation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, and annealing at the appropriate temperature for 30 s and at 72°C for 30 s. The final extension was done at 72°C for 5 min. Amplification products were visualized on 2.5% agarose gel containing ethidium bromide and illuminated under UV light.

Table 1. The sequences of MSP primers

Gene		Primer	Sequence	
DBC1	M	Forward (F')	5' gttttcgtcgtttttcgttcggagatc 3'	(SEQ ID No: 5)
		Reverse (R')	5' gctctcgctctcgctattactcgct 3'	(SEQ ID No: 6)
PDE8B	M	Forward (F')	5' tgtgtatgcgcgtttttttcgttc 3'	(SEQ ID No: 7)
		Reverse (R')	5' acctatatatccgccgctccgtc 3'	(SEQ ID No: 8)
PTPRR	M	Forward (F')	5' cggcgttgggtatgtttagtagtc 3'	(SEQ ID No: 9)
		Reverse (R')	5' aattacgaataaaaaaaaacaaaaacgctc 3'	(SEQ ID No: 10)
ZNF582	M	Forward (F')	5' tgacggtttttttgtttattcggttattc 3'	(SEQ ID No: 11)
		Reverse (R')	5' cgaacgcaaacgtacctacgc 3'	(SEQ ID No: 12)

M: The primers can specifically recognize the methylated gene sequences present in the bisulfite-converted DNA.

All MSP data were derived from at least two independent modifications of DNA. The absence of signal in duplicate experiments was scored as negative methylation. PCR product obtained by using MSP primer (M) that can recognize specifically methylation gene sequence was constructed into a pCR4-TOPO vector (Invitrogen, Carlsbad, CA). 5 independent clones were selected and were subjected

to bisulfite sequencing (BS). Primers used in bisulfite sequencing (BS) were listed in Table 2. Bisulfite sequencing was carried out using 377 Auto-sequencer (Applied Biosystems, Foster City, CA). Sequenced results were shown in Sequence List. Sequence numbers in bisulfite sequencing were: DBC1_BS (SEQ ID No: 21), PDE8B_BS (SEQ ID No: 22), PTPRR_BS (SEQ ID No: 23) and ZNF582_BS (SEQ ID No: 24), respectively.

Table 2. The sequences of bisulfite sequencing primers

Gene	Primer	Sequence	
DBC1	Forward (F')	5'ggttaagttttttttyggygtagtt 3'	(SEQ ID No: 13)
	Reverse (R')	5'tactccctctacctcccractctctc 3'	(SEQ ID No: 14)
PDE8B	Forward (F')	5'ttgtggygtagaggattattagtttggt 3'	(SEQ ID No: 15)
	Reverse (R')	5'ctaaaaacrcaacccatccctc 3'	(SEQ ID No: 16)
PTPRR	Forward (F')	5'ggaattttatttgaaatttttttgtt 3'	(SEQ ID No: 17)
	Reverse (R')	5'ccccacttcaaataaaatactattaaaaaaaac 3'	(SEQ ID No: 18)
ZNF582	Forward (F')	5'tagtgayggttttttgtttattyggttatt 3'	(SEQ ID No: 19)
	Reverse (R')	5'taaacrtaaaaacaacaacccracct 3'	(SEQ ID No: 20)

Example 2 Screening of methylated target gene in cervical cancer

After screened by CpG island-Plus-Promoter arrays, four target genes that might have been highly methylated in the cervical cancer cell were screened, namely, DBC1 (SEQ ID No: 1), PDE8B (SEQ ID No: 2), PTPRR (SEQ ID No: 3) and ZNF582 (SEQ ID No: 4), respectively. Their detailed information was shown in Table 3. It could be seen from Table 3 that, among these four genes, except DBC1 that

was known to be associated with bladder cancer, little study up to date had revealed the relation of those genes to cervical cancer.

Table 3. Characteristics of methylated genes in cervical cancer that were identified using a CpG island-Plus-Promoter microarray.

Gene	UniGene	Chromosomal location	Full name	SEQ ID No
DBC1	NM_014618	9q32-q33	deleted in bladder cancer 1	SEQ ID No: 1
PDE8B	NM_003719	5q14.1	phosphodiesterase 8B	SEQ ID No: 2
PTPRR	NM_002849	12q15	protein tyrosine phosphatase, receptor type, R	SEQ ID No: 3
ZNF582	NM_144690	19q13.43	zinc finger protein 582	SEQ ID No: 4

Example 3 The analysis of methylation state of target gene in cervical lesion specimen by bisulfite sequencing (BS)

The target gene : PTPRR

Test specimen groups:

1. HeLa_0: HeLa cervical cancer cell line;
2. HeLa_10D: HeLa cervical cancer cell line (HeLa_0) treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC);
3. HeLa_DT: HeLa cervical cancer cell line (HeLa_0) treated with both of 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC) and 0.33 μM TSA (Sigma Chemical Co., St. Louis, MO);
4. AC: Cervical adenocarcinoma specimen;
5. SCC: Cervical squamous cell carcinoma specimen;

6. Control group (normal): normal cervical blood DNA as the control group without methylation.

Each of those test specimens described above was subjected to bisulfite modification, and then bisulfite sequencing (BS) to analyze whether the target gene (PTPRR) in each test specimen has been hypermethylated or not. The result was shown in Fig. 1, where black indicated methylation region, and white indicated un-methylation region. The target gene PTPRR had not been methylated in the control group and the adenocarcinoma, while the target gene PTPRR had been hypermethylated in HeLa cervical cancer cell line (HeLa_0) and cervical squamous cell carcinoma specimen (SCC). Consequently, the methylation rate of PTPRR could be used as biomarker to screen whether there was cervical cancer or not.

In addition, in order to ascertain whether methylation rate of the target gene in cervical cancer specimen could be regulated through DNA methylation, HeLa cervical cancer cell line was treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC) (Sigma Chemical Co.). The DNA specimen extracted from said cell was subjected to bisulfite modification and then bisulfite sequencing (BS). Result shown in Fig. 1A indicated that, compared with the cervical squamous cell carcinoma specimen (SCC) and HeLa cervical cancer cell line (HeLa_0), the target gene PTPRR in the AZC-treated HeLa cervical cancer cell line (HeLa_10D) had part of region been de-methylated.

Trichostatin A (TSA) is histone deacetylase (HDAC) inhibitors, and can be used to lower or degrade methylation rate. HeLa cervical cancer cell line (HeLa_DT) has been treated with both AZC and TSA.

The result shown in Fig. 1 indicated that, compared with cervical squamous cell carcinoma specimen (SCC) and HeLa cervical cancer cell line (HeLa_0), the target gene PTPRR in the AZC- and TSA-treated HeLa cervical cancer cell line (HeLa_DT) had been highly de-methylated.

In summary, the above-described results had confirmed that the target gene PTPRR in cervical cancer specimen had been methylated through DNA methylation.

The target gene : ZNF582

Test specimen groups:

1. HeLa_0: HeLa cervical cancer cell line;
2. HeLa_10D: the HeLa cervical cancer cell line (HeLa_0) treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC);
3. HeLa_DT: the HeLa cervical cancer cell line (HeLa_0) treated with both of 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC) and 0.33 μM TSA;
4. SCC 1: Cervical squamous cell carcinoma specimen 1;
5. SCC 2: Cervical squamous cell carcinoma specimen 2;
6. The control group (normal): normal cervical blood DNA as the un-methylation control group;

Wherein cervical squamous cell carcinoma specimen 1 and specimen 2 were specimens obtained from different patients.

Those test specimens mentioned above were subjected to bisulfite sequencing (BS) to analyze whether the target gene (ZNF582) in each test specimen had been hypermethylated or not. The result shown in

Fig. 1B indicated that, compared with the control group, the target gene ZNF582 in cervical squamous cell carcinoma specimen 1 (SCC), cervical squamous cell carcinoma specimen 2 (SCC) and HeLa cervical cancer cell line (HeLa_0) had been hypermethylated. Consequently, the target gene ZNF582 in cervical cancer specimens would be highly methylated.

The target gene : PDE8B

Test specimen groups:

1. SiHa_0: SiHa cervical cancer cell line;
2. SiHa_10D: SiHa cervical cancer cell line (SiHa_0) treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC);
3. SiHa_DT: SiHa cervical cancer cell line (SiHa_0) treated with both of 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine(AZC) and 0.33 μM TSA;
4. SCC 1: Cervical squamous cell carcinoma specimen 1;
5. SCC 2: Cervical squamous cell carcinoma specimen 2;
6. The control group (normal): normal cervical blood DNA used as un-methylation control group;

wherein cervical squamous cell carcinoma specimen 1 and specimen 2 were specimens obtained from different patients.

Those test specimens described above were subjected to bisulfite sequencing (BS) to analyze whether the target gene (PDE8B) in each test specimen had been hypermethylated or not. The result shown in Fig. 1C indicated that, compared with the control group, the target gene PDE8B in cervical squamous cell carcinoma specimen 1 (SCC 1),

cervical squamous cell carcinoma specimen 2 (SCC 2) and SiHa cervical cancer cell line (SiHa_0) had been hypermethylated. Consequently, the target gene PDE8B in cervical cancer specimens would be highly methylated.

The target gene : DBC1

Test specimen groups:

1. SiHa_0: SiHa cervical cancer cell line;
2. SiHa_10D: SiHa cervical cancer cell line (SiHa_0) treated with 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC);
3. SiHa_DT: SiHa cervical cancer cell line (SiHa_0) treated with both of 10 μM DNA methyltransferase inhibitor 5'-aza-2'-deoxycytidine (AZC) and 0.33 μM TSA;
4. SCC 1: Cervical squamous cell carcinoma specimen 1;
5. SCC 2: Cervical squamous cell carcinoma specimen 2;
6. The control group (normal): normal cervical blood DNA as the un-methylation control group;

wherein cervical squamous cell carcinoma specimen 1 及 specimen 2 were specimens obtained from different patients.

Those test specimens described above were subjected to bisulfite sequencing (BS) to analyze whether the target gene (DBC1) in each test specimen had been hypermethylated. The result shown in Fig. 1D indicated that, compared with the control group, the target gene DBC1 in cervical squamous cell carcinoma specimen 1 (SCC 1), cervical squamous cell carcinoma specimen 2 (SCC 2) and SiHa cervical cancer cell line (SiHa_0) had been hypermethylated. Consequently,

the target gene DBC1 in cervical cancer specimens would be highly methylated.

Example 4 Methylation analysis of the target gene in cervical cancer specimens

Methylation-specific PCR (MSP) was used to analyze the methylation state of said four target genes in cervical squamous cell carcinoma (SCC) specimens. The methylation state analysis result shown in Table 4 indicated that, in normal cervical specimen, methylation frequency of DBC1, PDE8B, PTPRR and ZNF582 was 11%, 0%, 9% and 6%, respectively; in cervical squamous cell carcinoma specimen, the methylation frequency of DBC1, PDE8B, PTPRR and ZNF582 was 100%, 47%, 100% and 97%, respectively. It could be known from these results that, in cervical squamous cell carcinoma specimens, said four genes were highly methylated. Consequently, methylation rate of DBC1, PDE8B, PTPRR and ZNF582 could be used indeed as the screening index in screening cervical cancer.

Table 4 Methylation state analysis of the target gene in cervical squamous cell carcinoma specimens

	Target Genes CGI methylation rate (%)	
	Normal (n=54)	SCC tissue (n=30)
DBC1	11%	100%
PDE8B	0%	47%
PTPRR	9%	100%

ZNF582	6%	97%

Example 5 Methylation analysis of the target gene in ovarian tumor specimen

Methylation-specific PCR (MSP) was used to analyze the methylation state of the target gene in ovarian tumor specimens. Methylation state analysis results in Table 5 reported the analysis of methylation states of three genes DBC1, PTPRR and ZNF582 in ovarian malignant tumor specimen and ovarian benign tumor specimen. The results indicated that, the methylation frequency of DBC1, PTPRR and ZNF582 in ovarian malignant tumor specimen was 50.3%, 50.0% and 56.3%, respectively; the methylation frequency of DBC1, PTPRR and ZNF582 in ovarian benign tumor specimen was 2.5%, 0.0% and 12.5%, respectively. The differential methylation level was 53.8%, 50.0% and 43.8%, respectively. Consequently, compared with ovarian benign tumor specimen, methylation levels of said three genes in ovarian malignant tumor specimen were remarkably higher. Therefore, methylation rate of DBC1, PTPRR and ZNF582 could be used indeed as the screening index in screening ovarian cancer.

Table 5 Methylation state analysis of the target gene in ovarian tumor

specimens

	Malignance_Ov (n=28)	Benign_Ov (n=28)	Differential Methylation_level
DBC1	56.3%	2.5%	53.8%
PTPRR	50.0%	0.0%	50.0%
ZNF582	56.3%	12.5%	43.8%

Example 6 Methylation analysis of the target gene in colon cancer specimen

Methylation-specific PCR (MSP) was used to analyze the methylation state of the target gene in colon cancer specimen. The methylation state analysis result in Table 6 reported the methylation state of four genes, DBC1, PDE8B, PTPRR and ZNF582, in colon cancer specimen. The result indicated that, methylation frequencies of DBC1, PDE8B, PTPRR and ZNF582 in colon cancer specimen was 100.0%, 100.0, 100.0% and 100.0%, respectively; while methylation frequencies of DBC1, PDE8B, PTPRR and ZNF582 in normal colon tissue specimen were 25.0%, 25.0%, 25.0% and 25.0%, respectively. Consequently, compared with normal colon tissue specimen, methylation levels of said four genes in colon cancer specimen were remarkably higher. Therefore, methylation rates of DBC1, PDE8B, PTPRR and ZNF582 could be used indeed as the screening index in

screening colon cancer.

Table 6 Methylation state analysis of the target gene in colon cancer specimen

	Target Genes CGI methylation rate (%)	
	Normal colon tissue specimen (n=24)	Colon cancer specimen (n=20)
DBC1	25%	100%
PDE8B	25%	100%
PTPRR	25%	100%
ZNF582	25%	100%

The cancer screening method provided by the invention has following advantages over the above-described conventional techniques:

1. The cancer screening method, biomarker and use therof, provided by the invention uses the methylation rate of a particular gene in a isolated specimen as the biomarker to determine the risk of having cancer, and as compared with conventional methods such as cervical scraping and human papilloma virus testing (HPV testing), both of sensitivity and specificity of the inventive cancer screening method are higher than those of the two above-described methods.

2. The cancer screening method, biomarker and use therof, provided by the invention can be used not only as the first line cervical cancer screening, but also in combination with or assisting human papilloma virus testing (HPV testing) as the second line

cervical cancer screening, so as to achieve more accurate cervical cancer screening effect.

3. The cancer screening method, biomarker and use therof, provided by the invention can be applied on not only the screening of cervical cancer, but also on the screening of other cancer (for example: ovarian cancer, colon cancer and the like) to assist the diagnosis of abnormal specimen.

Many changes and modifications in the above described embodiment of the invention can, of course, be carried out without departing from the scope thereof. Accordingly, to promote the progress in science and the useful arts, the invention is disclosed and is intended to be limited only by the scope of the appended claims.

SEQUENCE LISTING

<110> Hung-Cheng Lai

<120> Cancer Screening Method

<130>
<140>
<141>

<160> 24

<210> 1
<211> 5000
<212> DNA
<213> Homo sapiens

<400> 1

ggtcttcacc tgaagttgcc tgactacgtt gtctcagatt gatttcagaa ctgcattatc 60
agcagggcag ctgttgccta ctaacagtca tggaagtaga tctcagctgg gggatgatgg 120
ggaaacaaag agaaagatag ggacgaataa aggaaggaat ggggcacaga cagaaagaga 180
gagagagaaa agaaagaaag gaggagaggc aagagatagt gacctggggt ttcataccgc 240
ggtgggtggg agaaggagtg tgtgtggtgg gcgtatttct tcgtggtcag aatcaaagtc 300
agaactgacc tggggaacct gtgttcaaga cctgaacctg gaccagaagc caagggaaag 360
agaaagttgc ccaagaaaaa agggagaaat ttccaccagg cgaaaataga aatcgctgac 420
tgggtttgtg gctggagagc tgtccgtggt gctgactcct cctcattggg attccagtga 480
aggctgagaa gctctgcact ggctgctcct cctctccctt tcctctcccc tcagtcctga 540
ccttctgaga cccaggatca ttctggctag ctcgatgtct tctcctctct ttctcccttt 600
ctctttgtcg attaaataat ttttcccgag ttcccagttc atcactcaaa catttctctt 660
ccattttgtt tcaagactcc tcttcctcca attagttcac tggctcctag attgttctag 720
gcctccctgg tggtcacatc tctctcttat catcctttga attaaaaaac aaacaaaaaa 780
catacaatgg caatcataaa agcagacaac atgcactgag cactcactag gtgtcaggga 840
gcagtctaag tgcctttgca tacattaact catgagcagt ctaagtgcct ttgcatatat 900
taactcatta caattccaca tcaaccctat tagatgggta gatatattat tattctatta 960
aacagacaag gaattgaggc ttaaggggta agtgatttca ccaagaccag acagctacag 1020
gactccaacc tctctggagc ctgcacttct actgaatctg gctcttttac atattccaac 1080
acctttttcct atgacttcct gcccctccat ctggtagcct taatgctttc tattcttgcc 1140
taatcttcag gggactaggg atctgcattg ctgatttggt ttaaatcaga agggaagcaa 1200
agtaaacaaa cataccaaat gtgcgctgtg tgggaattat catacgaggg ctttattttc 1260
tgcttcagga agaggcccta tgttagcagc cccagcctgc attcaggctg attgcagagt 1320
attttgcttt ttattttcat gtcttagtcc ctgtaccctc gccccttccc cgcctctggt 1380
ggtctccaga gaacttcgtg tcccctcagc ttctccctcc tacatcctgc ctacgtagag 1440
aagctcttgc ttcattctgg gaggttacgt gggctctcgc ctacacaccg agagaaacaa 1500
acagtgtcaa acactcacag agagacgcgc agacacaaac ggacccacac gggcaactcc 1560

cgagacaaaa cccacactcg atggatccac gcggccgtgg aaacacctgc cgccccagaa 1620
acactcaggt actcgcgaca cacacagtac agtcacgctt aagggcacca ggattccggg 1680
tttgcgcgta tgcgcggtcc ctttggatgc tcgtgcgcat agacacaaca ccctacacgc 1740
cccagaccca cgaaactccc tacggctcag ccccagccca cccgggccgc ccttccctcg 1800
aggcggcctc ccgtctctcc tcctctcgct tctcctcctc ctccgcctaa agatgtacaa 1860
aacactcctc ggaagcaacc ccggcgttca gctcctcccc ccccgccccc cggccgccgc 1920
tcccccattc attttcggcc gtcgccggct aagtccctcc cccggcgtag cccggcctcc 1980
gccgctcccc gcccggagac cgcggcgcac ttggacttcc ctctccattc gccagccgcc 2040
tcgctcccgg accccacggc tgcaaactga tctggcgcgc ggggaggagg agagcgcagg 2100
cgagcgaacc cgcgagagag ggagagagcg agcgagcaac agcgagagcg agagcgagag 2160
agccgggagg cagagggagt agtgaccgcc ttccggagcc gggattcatg cctgtcctcg 2220
ggaccagcga aggggacttt acggctgagt atgagccagg ctgctaggag ccaggtaccc 2280
ccacgcctgc agtccccgcg ccgtgcccgg aatgcgagct gcacgcaggg ctctcccaag 2340
ttcccaccga gccgaataaa aagcgtcctc ccgcagctct ccgccaaaga cggacattga 2400
ctccaggtaa ggcggcgccg ggtgcagcgc cccgcagccc cgctgccctt ggacccggcc 2460
ccgggccgca ttcggggcgt ccccgcgctc ctctgccccc ccccctaccg gcacccttgt 2520
ccgctcttca cctggccgcc ccgccgcctc caagtcttct ccagttctag ggaggggggtt 2580
cctgtgcctg gggctcaaag ggctaattgc gggtttgagt gagtggcgtg tgtgtccccg 2640
cgcgctcccg acgtgtgcac catggtggga acttgatgtg gtgctagtgt gtttgcgtgt 2700
gcggcgtcgt ttgcgtttga tgcgtgtgtt cgtggtgtgt gtgtgtgtcc gtgtgtgtaa 2760
gggaggggtg aagagagaga ggtcctataa cctacttacg gcgcgatgtg tgtgtgcatg 2820
tttgtacgta tgtgtttgta tgtgtgccct cgtgtgtctt tttaattagg tctctccagc 2880
ttacacggaa tgggaccctt actataggat cacgtagtca ccgggaaacc cgctgtggac 2940
ttcctcttgg ggctctgggc ttggggtttg gggaggatta tggggctgta gatggcacct 3000
tatttagccc aatgttggta cgcttgaagg aaaattcctc caaacggtgg aatcctgcta 3060
cactgggacc cacagcttaa tatgaaagag acatggccaa cccccgaggc aaatgagacg 3120
ctgtcacttt aaattctaca ctgggcagac tccaagattc tgatgggaat ttggagacac 3180
tggatagtgg gtgacagaga aaggggaag tcagcggtgg gctccttatc tggggcttgg 3240
aaagtctggg atagggattt accctgcatc cccgtttgca atcaacagag cccctccggc 3300
ttctgttggt tttggggagg atctggcaag tttgcatgga tccccctcag gggaaaggag 3360
aaagcgtcct cggggacttg ctcatccatc acagttgcaa agggtctcag aggaaatttc 3420
atctggggcg gctgtggatg atatggaagg agatggatgg ggcactttcc taagacagat 3480
tcgtctttct ttccccattt caggcgggga agcccccaga gagtctctcc ctaaatatgc 3540
ctctccatgg ctcccctgga gttaggggga tattgagaga aggcagagag gtggagaggg 3600
agagagagag agcaagagcg agagagaggg agacagagag agagagtgtt tcagtactga 3660
gggagatcta caatttgaaa aggggctgtg agtgtggaac ccatgacaga atgtggcagt 3720
aattgactta aactgctgtc ggtttgcacc tcgcgtctct cacttggctc caaaatactg 3780
ccaagggcag gggggcggtg gaggaatctc agccaggaag gtagtttggg tcaaggccct 3840
ctttcttctt tcctccatct cctgggaggc tctttaggta ggtcccttgg ccaccctggc 3900
ttggtactgt tgggcttggg ctctggggcc agccattgat ttgattccca gctgcctctt 3960
aaaggcttgc cctactcagc aaaaatgctg agttttactg ctgttagcat ggcttccaga 4020
ctcggcagct gttactttct caaggtaagc ggcagccgtt ctgctctcag gcagggaggc 4080
taggagaaga caagggctgt gacgctggga cacagcctct tgtctagctc agcacggagg 4140
ggccgctgaa aagccctctt aggggaaaaa agtagaaata tatttggcct aaaaaatgta 4200

cacatatatt ctaggaagat atatatatat atatatatat caatcacaca cacacacaca 4260
cacacacatg tgttcatatt ctggggagag ataaaagcaa acatgtattt gtaggaacct 4320
gccctagtgg gtgggttcta cctgcaggca cttgcagaca taccattctc tgtccagagg 4380
ctgatacctc aggctgaagc cttcacatag ccacagagaa tctccttcag gaaactcatg 4440
tcagggcaga gctccctgga ttatcttggg taggggtac tagggccaag gaggagaccc 4500
atttttggga actgcctcat tttctctctt tcctaattca cagacgatcg aaactggaag 4560
gaatattaaa gagcagaaag gcaggactct gtggcctaaa gagggaatga gaattcccca 4620
acattcagta gtgggttagt ggcatgccca ggactagaat ctaggcttat caatcagcta 4680
gatcaatacc ctttccactc ctcccagcaa tatacactct aggactgtat attgctccca 4740
cccctatctt gccaacctgc caccatgacc cctaccaaga caatcgcttg tcttccctca 4800
tcaaacttca cattttcaga gatgcatttc agggtttttc tatctggaaa catggccctg 4860
aagagaaaag atccaatgcc tcaatcacta ttcccgaaag acacacacac acacacacac 4920
acacacacac acacacacac acacacacac acacacacgg tggtgggggg aggactgaga 4980
gagagacaga gagagaacat 5000

<210> 2
<211> 5000
<212> DNA
<213> Homo sapiens

<400> 2
agaacaactc tgaagcatca tcccaccttc agaatcctcc ctgcagggat gcctgaggct 60
tggtcaattt ctccctctgc tcaagcttgc ctccctccat tccctccctt tatttcctcc 120
cacaaatgtt gactacaaga gcacttccta aaaagcctcc ctctgcctgc taaacaccat 180
ctcagaatct gcttctagga ggacccaacc tgtgcagaaa ttttatgagc aatttgcctg 240
tccattaaaa aatacagcat agacttttc caggttagct ggagatggag ttttttgta 300
tcaaaaaaag agagctaaac tgagaagaga agcaaaggta gaagaataag gttagactct 360
ccctgtataa ctgaatttgg gattgggaat gatatcttgc ttctctatga aatttagaaa 420
ctgtcttttg aagtcttcat ccagtcacat ccaagagtgg tccaggaaga aaaaggaaaa 480
taagaatgaa aaaaaaacaa aaaacaaacc ttaaaattgg atacttcttt gtgccttgca 540
ttaagcctct atgttactgc ccttgtccgt ataagcaact ctagataaaa tcctttctta 600
gaccatctaa tttatctccc cgtgccattg tgaaaatcgg gcaggaatta ctgcgggaac 660
cagtgactga atgggcagtg gtgtctcgca gctgtaactc agagtttaca agggaaggat 720
gaacagcagc catccttcta aaggcattct tcctctcctc cagcacagct cagtctgtgc 780
atgcctcacc aaggagaatg agggaaggcc ttgttgactg cccagcccag agtgtgagga 840
acagcagttg acatgggcgg ctggacggga tagaaaccag catgaacatt tagtgacacc 900
taaggaagag gtgaaaatgg gggagaaagc caagaaccat cagacagctt ctttctgttc 960
ttgccttgga tcttagggga acgtatgtgt tcaagggtgc cttcctccgc agggcctccc 1020
atcccttggc acaaacttcc aaacgatgag tagacatgga atgggcttct tctgaagact 1080
gggtcaccat ccaactcagc cttcggagta ggcggtgggg ctgggggaag aggacggaac 1140
catgaggtct ctccatcact tcccgggtgt gttgagaggc aagcaaatgc ggtgggtggg 1200
cctgggctgt agggcccctg ccatttcggg aggggcctcc tggtgtttag ctctgggatg 1260
tgtgaaaatg tgttggtgaa aagcgagagg cttacacagc ccttcagggg aagaggggct 1320

ggggcgctgg gggcggcgtc gggatgagtg cagaagagac gaggcctctg gacagcggag 1380
gaggagggga gggcgccgag gcgcggtgcc agctgccgcg cacaggggcc ccgcggcgga 1440
gccgagccgc gggcacgctc tgccctgtcg ggagagctcc gggagcggcg ggaggggcgg 1500
aggggcgcag tggggcccgg gcggctgcgg ccgcggagcc ggggcacctg aggaggaagg 1560
agggtgggag cgagggaggg aggggacggg cgcagaccga aagtggggaa agaaggtgca 1620
ggcaggcggg caggcgggcg ggcgccctgg cccagggccg cgggtgcggg agcccggcga 1680
ggtcgagctg ggcggcggcg ggggccgcgc cgagggagga ggggaaggcg gaggcgcggg 1740
gagcgtgttt ggggcgccgc ggcggggagg gtggcggccg ctggtgcgcg cggggcgctg 1800
tgtatgcgcg ctccccccgct cggggaggaa gatggcccaa aagggaaagt tggggtgacg 1860
cgcgcggtcc ccggaggctc ggcgggggcc accgcggcca gcccgacgga gcggcggaca 1920
cacaggccgg ggggcgcgca gtccgggcgc cgccgcggcc gccccctcac tgcaggtggc 1980
agcgggtgcg ctgggtcccg gcggccgcgg gcgcgggcgg gcgcgcgggg gagcccggcc 2040
gagggatggg ctgcgccccc agcatccatg tctcgcagag cggcgtgatc tactgccggg 2100
actcggacga gtccagctcg ccccgccaga ccaccagcgt gtcgcagggc ccggcggcac 2160
ccctgccccgg cctcttcgtc cagaccgacg ccgccgacgc catccccccg agccgcgcgt 2220
cgggaccccc cagcgtagcc cgcgtccgca gggcccgcac cgagctgggc agcggtagca 2280
gcgcgggttc cgcagccccc gccgcgacca ccagcagggg ccggaggcgc cactgctgca 2340
gcagcgccga ggccgagact cagacctgct acaccagcgt gaaggtaaat gccccgcgct 2400
ggcacacgcc gtgggggccg tccgccccgt cggcggggct cgcacgggta gggggctccg 2460
gcggagttgg gtgaccgtga ggcggttggt ttggagaggt tgtcactaag gaggagttta 2520
cttttcattt gtggagatga tgggagccca ggaaatgtgg tcagaaaaag gcccctggag 2580
gggtcctgga agcgtcctta gctggtcctg ggggactggg cggggaaggg agcgcagaag 2640
gaagcaggtg ggctggcctg ttcctccttg agggcaggaa ggctgtggct tggtttatgc 2700
aggaagaggg gtggggacca ttgagagcat tcggtggcca gtcctgttga atgaaatctg 2760
agcactgagc tggatttgcg tgccttgtag gtgactggtg cagttgcagc accaggatag 2820
atagtgcccc atattccgat ttttacctgg gattaccagc caggctggag tctcagcaca 2880
ggaaccgagc gtagggattt gtgaatgaat gagtgttcgt gttttaagag atgtgggaac 2940
ggagcagagt ggaacctgtt gtttgtcact gtaacgtttc tctgggttgg ctgcatccta 3000
gacagaattg agagaaccgg gccatgagtt cggagtgtca gcagagccac cgtgagggga 3060
cgtggtttcc agtgcagtac agctgtctga ggatgattct gcacatacaa ctgatcttcc 3120
cagagagtgg gattttgagg aagtggaaaa aattgtttag atggttaaag cagtcgctaa 3180
atatttattt cttaaagaca gaagaaaaat aattatttaa atagtgtcct cccgtatgtt 3240
ctcaaagtac cgttaaatcc aagaggcttt tcattgtgta aatctgggca ctgggtcttt 3300
tttcctttca gcaaacaaga taacaatggc atatcctatt gtacagagag aaaaaaatac 3360
tcctaatgtc agatagaatc acagccttta cctggtcaat aggttaccaa gaaccattcc 3420
atggattatt tgtcaaagac acatttgtat ttttaatagt taaaaacttg gtcttcattg 3480
gatgaaggca gcccacagtg gaggagtgag gagaaggata acatgttttg gcttcaatct 3540
ggaagtccaa aagtttttta atgaacctat ttttttttta acagcatctg attgtttaac 3600
atgaagcttg actgatgttt gcttgtggtt gcagtgtttc tctggcagta atcataatca 3660
ccattatagt aaaaacatcg tttattgagc acctactatg tgccaggagt aagctttctc 3720
tcctaactat tgaatgttaa cagtgtgatc ttagcatagc atgtctgaag actagagtct 3780
aggatttatg acagtacaaa cctaccagtt gcccattcgt tacagaggca tgtgctgaac 3840
gctatacttt gcttttgtca agctctctct ctctcccccct ccctgttcac acaaagttag 3900
ctttaggagc aattttcagg gatgaaaatg ttaaatttgg tgaaaatatt aagttgggat 3960

attatcagta aagaccatcc ttttgcctga cggttgaaga cctgaagcag tagttcaaaa 4020
ttgtgacagc tcatgaactt tgtgatactt tttttggttg ctccagagaa attgtgaatt 4080
tttgttttct agaatatcta taatctcagt ttagtacttg gcaactctag ctactccatg 4140
tttgttcagt agaagaaaga atactgaatg aacccattct gtacattttt aatacttccc 4200
tcaagaggct gcttagagac caaatgaagt aaaaactgaa gggacaatga gccccaatta 4260
ttagcagctt gtataaactt gtattaatct cattgctact tggcttaagt attaaaaggc 4320
ttgacctggg taggagttta actcattttg ctttatcaag aagattccca aataagagct 4380
taattcactc taacccatta ctgggaatgt tggaaatgaa acccctggtt cattgactta 4440
acactacttt gcattgtgtg gatctcagaa agtatatatt tgaagtgaaa tttgagacca 4500
gagttattta tgcaaatctt acagaattat tatcacagaa ctgccctaat atatttaata 4560
gtatcctttt aggcctgtga ctcggtgttg tattgtcttt tctcaatcgt aaagttcaca 4620
tgttctgtgc ccttttgttg ctgagataat tttataaaaa ctttacattc atttttttgt 4680
agactaagca ttctttttt gccgtaagag tttgagatag ttgctaggca cgtggtggtg 4740
ccccacccct ttccaaagca cacaggtgga aaaataaaat ctttaccatt tggtcctgtt 4800
tacagtggtg gaaggttgta atagtttcca ccccctcccc ctggccattt tacatcaatt 4860
atgaagtgta tcctcattat ctacttggcc tctggtattc cccagagtgc aaaaatgtgt 4920
gcttgatctc tctctctctc ttttctagca ataattcatc tcattaagct tttagaatga 4980
tgaggcatta tgatgtcaaa 5000

<210> 3
<211> 5000
<212> DNA
<213> Homo sapiens

<400> 3
aagaaaaata gacctattgt aagtgggata caattatttt tccctaacct ccagccctgc 60
agcatttcta gtaacagatg ttagatgaga agagttattc ttctaagctt tacagaatgc 120
taaataaatt atctcaaagg ttgactcagt aagaatttca aagagttaat tgtcagtatt 180
gaactttgtc aactatcatt ttcatttatt cattttgag tatctatttt gcatgaggca 240
ttgctctagg gagttgggac atatcaataa agtcatcaaa gttcttcaac attggagctc 300
aaattctgtt ttgcgggggg gagtagggaa atagatacta aacaatagat tttataagta 360
attattttgg tttgttagaa agtgatacgg gaaaagcaaa ctggaggaca gtgaatcagg 420
aataggagaa gaggttgcag cttagagtgg tttgggatga aatgatagtg ccacagaaat 480
ggagtgtgcc acacgcatgg gggagtaggg ggtggagcac gttgctagag aggggctaga 540
catggagata tgtctctatt ttcagcagag aaggcctgtc aagatattaa aagtacatct 600
cattgtcccc ttctcccact tgttggagtg cttcctggcc ttgtctctca gagctgaaga 660
tgacataaat cataaaagaa ataaatggta ccgcctgggc tctctgatct tataaggtcc 720
cacctaattt aagctctggt ggatagccta tttcccatta gttgtctcaa ttcacagagg 780
atggagaaag aaagcactga atgagatgtg cagctcaact caatgggcac ttgttagccc 840
caattatttg gctaggacaa ctttattcaa gttaatggaa tacaaaactc agcagttctt 900
atggagactt caatgtgctg tagaacaaac cacagataaa tgaactgtct gaaagaataa 960
tatgaggcac ggctaaacaa gtgttgtgga agagaaagat cactgtaatc tagagttagt 1020
tagagttagg gctttctgga aaagtgagac caacattaca ccttcgaaaa ctagtgtggt 1080

ggtggtgcaa gtggaggaaa atgggaaaga ggcaacagtg tccataaaga cacagaggca 1140
gaaagggcag aggccctggg atagttaaaa gagaagtcta gctgagggac ttcttgactt 1200
gactagcagc aattagccat gactaataag gctttccaca ctccaaagac ttagatggag 1260
ggataaaaaa ccatctaatg gcagactgtg gtagcctccc tagagacaca gagctgggcc 1320
ggatgagtcc aggcactgac gtgatccatt atctttcacc ttaaagagta aaagggaaac 1380
taaagttaat tacctccacg aaacaaaaag gtgccttctt gtgcttcaat tacatggata 1440
tattctacta gtctaaaagt atcttctcac ttctttctgt cactgtgagg acttgagtca 1500
gaagaaagtt taaatacagt cattgagctg gaaagagtgg aaagagaagc aaagaggggg 1560
aagctgtagg aaggacgaag tcacccccaa gatacatggt tactgcttac accaagcaag 1620
ctgccttggg aacgcttccc ccgagcagcc agaatgctca gcagtggaag acacctctat 1680
tcctgtaggc gagtcctggg aagctggtca atctgcaaat gccaattccc agcagtgagc 1740
tcggtccacg tgtaaatcaa gatttgggga aagagtaggg tgggtggcat ggttgacaat 1800
gtcatcagct ccctcctctg actcctgtgg tcgtgccccc atctactctc actcagctac 1860
accccacctt cggatttgtg atggacgctg ggtccctagt aaccacagca agtgtctccc 1920
ccgcacttcc cccttcccca cccccacccc caccccccaac caccacccca gcgatggagc 1980
ctactctgct ccaagccgcc gctaagaccc ggagaagcgg aatttcactt tgaaattccc 2040
ttgcctcgtg agggccggcg ctgggcatgc tcagtagccg cggcgctgct gctgggctgc 2100
tgggctggcg cggagtccac cctgccgtct ccgccttggc ttctgggcgt ccagaaggcc 2160
aggcatttgc cgcctctgag cgcttctgtt ccccttaccc gcaacctcct actgctcttc 2220
ctctctccct ctcttaggga ggttgaagct ggtgctggtt tctgtcggcg ccacagactg 2280
actgctctgc aaaccccagc cgaggacctg aatcccggag actagaagac ccttggcggt 2340
ggctctttct aatagcactt tacctgaagt ggggtcgtgg tggagtttct cctccacctc 2400
tcaatgcaaa cactatgcgg agagcagtct gcttccctgc gctgtgcctg ctccttaatc 2460
ttcacgctgc aggtaagggg ttgccaggct tagagccgga gctgtgcatg agatgggaaa 2520
ctgcacatgc ttaaggactc taggaaaagc ttgccttgcg aagaaagcct ttctaaaaag 2580
gtaaaacagg acagtacctg acagggaagg ggtgagggag tcgtgcacct gttggaaaac 2640
tgaggccgaa aacttaacct aaaattagct cttgattttt ctttacttta tacgaaaata 2700
gtgaacattt tcaatatgaa tacaggtttg ctccccccct cgcctcccct ccgtctagat 2760
tccctgctct tgtttctagg ctatgcactt aactgtcaat ttttcaggag agggataaga 2820
catcctgcta gatgtaacct tttctactgc agcggctact acattcataa ggtctcttgg 2880
tctagcgagc gctcatagga aggcattggc tgtaacctga tggaccacat ctccgcccaa 2940
aagatcgaaa tacatggttc acattagtga tgctgcacca ggctcctttg gcccctgctc 3000
ttgtcactcg aattttctaa gccaataaga ggaagaaaag gtttcaaata gaatcctctt 3060
gtctctttca gcaccgtggc tagcgtccgc tggcatatta aaaaaaaaaa aaaaaaaaaa 3120
aaaaaaaaaa acaattacag ttccaaagct gacaaccctg ggttaggcac tgtccctcgg 3180
acagatttga taaggtgtaa ccaagagtaa cagtgagact gctcccaaat ataaatagat 3240
ttgaggcacc agaatcaata tcaagtctcg tgaggaagca aaccttggga agtacctgag 3300
agatattata tgtggtgtcc ctgctttttg catacatagc acctctattt aatgcagaca 3360
gacttgctag gctgaattcc ttatcattgt cacacacaca tgaaccaaaa taaaatatgg 3420
cagggagatt ttagaaaccc tactgtgaca gcagttgtcc atgcagtagt cctgtaacaa 3480
ccagagacga ggcattttcc ctgagtgtgc ttctcatcca atcacacata tccattcatt 3540
ccttcaagca acatttgctg agtgtatact atgttctagg ggtaatgagt aaaaataatt 3600
gttctgaaat tacaatcctt gcagtatctt ttttaaaaga gtgggtgaat tttattctct 3660
gtgtacaata gtaggagtga aatatggtac tttcttattc cagctaacgc tattatttaa 3720

atgtatcatg aatcttttga gcaaataatg cataggatta ttaaagctat tattaaatac 3780
acatgttaat tgttataatt atgatgttat attgtggagt tttcaatagt tctttggcac 3840
attttgagaa gtagaaaata gctatgactt gattattaaa ttatttgcaa acagctgtag 3900
tgaattattt cgttaccaca tacaattctt caatgctgaa atgtgggatg aaaacataca 3960
cattgggttg agttttattt atagtattgc ttaataatag actgacacaa acattcatta 4020
cagaaaaatc tcaattctta ctaaggtagc aatgatttag ctggtctgcc aacaatatca 4080
gtgtagtcat tttttcaaaa ctaacatata aaaaagttta aagatcacat ttgatacatc 4140
ttgagtaatt atatcttaaa agttataaat aaactgaagc atgtgaaaaa catgtcttga 4200
tttactgtag atgaatattc gggatgagaa aaatgaggac ttttgctcat cagatgagaa 4260
acctagaagc ctgtagccct agattttacc tggagttctg ccaaagggta tattaccaaa 4320
gccaatccaa tccatctcta cttcaacttt ttaatttgca aaacaaagat aaaaagtaag 4380
attaatcaac caattttaat ttaataagaa aatgcattta aggacatttt tatatttcaa 4440
ttgcaagata ctattcattg tattcatatt taatatagta aaatacattg gtgttttatc 4500
acagcagatt gtaagtttaa tattatagtt ctcttgccaa tttcatttta aatgcatttt 4560
aattttaaac agcaatttgt gggtcacaag ctgtcatcag attaaaattt atcacagtta 4620
tctaataaca ttgttagggc ttttcatcag aactaaaaca aacacagctc cttggtctaa 4680
gcttctatac atttggagat taagaaaata agtggatggg atgacaaaga tcaagaaatc 4740
aagatattaa tctctacttg ttgaaggtgt gagtttgtta cttttatgga agacttttct 4800
ctcagaacag aggctgactt cataaagaag ggaaatgaat tctatacatt caggcctctt 4860
taactgtaaa gctaattgat ccattttggt tacgcccagg gtatcagcaa ctgctttatg 4920
ctggtggaac tgatgacacc ttataatgct ttagagatgg gaggaatgtg ctaacgggat 4980
gtaagtgttc tttttagcta 5000

<210> 4
<211> 5000
<212> DNA
<213> Homo sapiens

<400> 4
gacgaccctg aatgatccct gcctcctagt acttatactc ttgtgtagcc tccacctact 60
tagactcaca gctgattttg tgaccaagag tataatggtg tatgacttca gaggcttggt 120
tcagagtcag aagtggccat ttcatcatcc tctaaatttc tctttggcag attaatgaat 180
gtgagaaagt aaaaatggta tagaagaaag ttagcctgag agatggctgg ctttgtacct 240
gtttggtctt tttccttcct ttcctccata agcaaatact gacgcatgta catatagaca 300
cactcaacac agtgggatgt taaagggaaa ctagagccac tcgccccttc ccaagatcag 360
agaacatagg aaagatgatg acttttttgca tctaccctct cctcttgaac tcgcaacact 420
gtcttgaacc acaaataagg tcaagtagga taaatctaca actgtctcct caactgtccg 480
ctcacaagtg tgaaattcaa ccatccttgc tgaagttgtc ttttaatatc agtaatcagc 540
aataaccaac ctctgagctt tgcttatatt attaaatcat tttatttgac cctcaggaca 600
aagctgcaag cccagcatta cattcttcct tttatggaca aaaaaaaaaa aacaaaacaa 660
aaccgagata ggcacgaaga aactaataaa tggctgaaat gctatttgaa tccacatctg 720
tctaattcta aaagtgtgct ttccaccaca tcaccgcacc tacaaggaaa atgcagcctc 780
taccctctcc tgaagaatgt gtaaagaggc aatatggtgt gttttggaaa aagcatcttt 840

gcagaaagaa agcattagct tcagtcatat tagcatcctt atccagcagc agtagcatta 900
ttttacatat cagttactcc tccccaacaa ctcaatgaat tagatgccac aatcatcctc 960
actttcaaat gtggagaccg aggcacatct ctaagactgg agttcaggca ttctggctcc 1020
agtcttagag atggttaagg gttcacactc ttaaccattt attacaccat agagctcacc 1080
aggtttgagg gaaacaggat caaatcaaaa gagtcactca ggactccagt cctcactcaa 1140
ggacaaactg ttccacctcg gacagggaga gtttccgcat tctgagaccc agcataacag 1200
gtcctgaccg gcatctggca ctcggactcc caatcatact ggatcacact ggctcgggat 1260
gtgtaaagtc cagggcttct cacatttgat gacaccaaag ccgcctaaaa acaagagaga 1320
attaacaact acctacggcg gtctgatatt tgcccaagag atgccgcccc ataaaactcc 1380
tttacatctt tataacgttt ttattttgcg ttctccttca taacccacat ttaactcacc 1440
atagatgtaa tgtttaaaat tagttaccag ataaactctt acgcttccaa actttaaggt 1500
tccttcgaaa ccttctggta aaactgttgt tccacggaaa tgggaacgta acggatgagg 1560
caatcttcca cagccgcaca cagttgtgta tccaccgcta aacggtccca gtcatacatt 1620
caacgaccca cgcggagtca gaagctacca ccacacactg tcaaaatcac gcacacacag 1680
tgacggcccc ttgcccactc ggtcactcgc ccacaatctc tcgctagaga atcacacgca 1740
gatagcacac ccagcaccac agaccccagg aagcaaccca gggactcgaa cacacgaaca 1800
gcactcctcc gcgcactgcg caggcacgcc tgcgtccggc tcaccctgaa acatcgcgag 1860
atccggcttc aaggccgggc tgctgccttt acgcctaaag actatgtttc ccggaagaca 1920
ctgcggcgcc ggccctatca tggcgcagca tcggtgtgct ttgtgcgtct gcgccatctt 1980
ccggctgcgc acggcgaatc caccggtacc gtggtggaag cgcgccctgg gctgccgggg 2040
gcgcggccgc ggtggcactt ggacccgagg aggcggcagg tgagaggttc cggagctttc 2100
caggcgctct ggggtccagc aggagctggt gcccgggccg gttgggtctc aggcctgaga 2160
agaacgcaga cgtctcgcct catcgtcgct ctgtggcttt accggcgtga gactacattt 2220
cccgccggcc ctcgcggcgt gcgctttctg gcgccccctt tctgcttcca gcctcatagc 2280
ccaggtgcat ggacccctta ggtgggtgcg caggggtctc cgacccccctg aaattgcgga 2340
tgttttgcat tcactgttat gcgtgccttt ttttttttt aatctgagag gaaatcttgt 2400
ttcatgaggt tctcagagag ttacaagacc ccagaagact tagaaccgct agtttagaaa 2460
aacttatact tgggaacatt ttatatgttc aaattctatg tccagcaatg gggaaacact 2520
tgagtggatc acaggccatc ctttgtaaat aggatatcgt gcagttaata caaataatgc 2580
ttataaaagt ggtcataata aggaaaagct gcctattaca tcatattaag caacattcat 2640
aattatttca cttacacgtt acttagaacc aaatgtttgt aatgcagtag gcaccttata 2700
tactccttta cagagtactt cttgtttaac agtgagctca agaactggtt aaatgacaac 2760
actgcgaata cttgggtgtt ttttcttcca atttttttgt tttgttttgt ttttagcctg 2820
tgtgttagtg agtttgactt ctttttgctg aatgttctca aaggattcag tagttaatat 2880
ctttgcttgt cagtttttga tagtcatttt tgatagtcac ttggcatccc attatatggc 2940
tgtgccaaaa atgattcaac ctcttggcta cttgtggact tgcctactta cgtactaatt 3000
ttcagttctc ctatttagca aatattattg gacatgcagc tttgtgcact gctgtgtctc 3060
tatggaagga cacatttatg tgctgataat ggagcactgg gcgtgctgtg tgtaatttgt 3120
ctcttactgt tccttccaac tctatcccat ttgttgatgc aaggcatgct ccatgaagtg 3180
gcatgcacaa agcctcagtt tgggctgtag agctttttgt atatttggcg gttgggaaaa 3240
gagccaggta gactttcaaa ggtgttatat ttgctgcagg agagagttaa atggcctagt 3300
cctggaaagg ctttattcta ggctggggct atgcaaatga tgcaggcctt ctatgctccc 3360
tggcttgagg gaagccctcc ttgtccttga ggtaacatgt gggtctcagt cactagaaca 3420
gccctcagca tccctgtctt agacttgcat ttgcagcaaa acagaaccac tggatgttta 3480

ggggaaatgg gagggaaagg accactggtt ccccttgcc ttgcagcagc taaagctggt 3540
ttcctggggt tgggtgagga tcccttgcc tccccctcct tctcattttc ttggtgggca 3600
gagggcaagc aggtggggac ttgtaacttg gcattctagg aactgtgagg caagcctagc 3660
ctctcatgtt tgctctttcc tcccccagct ctaccgtcgc aggactctgc ccttccccaa 3720
gagaggaacc agaaggagga cctatcagcc cttgaaattc taaaagtcat gtcccttgtg 3780
agtttttaaa tcccataaat atttgctttc cttatcttga aactttccac ctgggttcca 3840
tccagaaatt tggttctcag actttcccat tttagggaat aaaaggacca ggcaggagtc 3900
tcctctccca ttgtcctctc ccctctgaca aatgcccaca gatttattct ctatagcatt 3960
ctgcatctcc tgatggtttt cgtctgctag gaggagaaac tcatttccct gtatgaaatg 4020
agagcatcct ttgtaagaac tgaagtttga agagatggaa tgaatgctta ggagcaatca 4080
catatagaaa agcaggggag gtggtgttgg ttggtttcag tttcagcgca tggtacagtg 4140
tcctgctttg ggaatgatta atgattggat atgtaattga ggtttggctg ggaatccatc 4200
aaaaatttga tgccagaatt aaagtctagt ttgacgttgg acagagcatt tgaatctggg 4260
acatcctcca ttagtttctt tagcttaaga ggaagaaaag tacagagctg aggatggtga 4320
gcgatgtagc aagtgagatg atcatctagg tgggtgagtg gtcagggctt ggaaaagcct 4380
tagagatctg atccaacctc gggatccaaa ataagaggat gtttgagtca gttgtttcac 4440
ttgatagaaa attgtgcact caataaaagt tatctccatg gtttttccca tgaagaaaaa 4500
tgcttgtgtt atgttccaac ataatgatga actttcatct ccaagaaatt cttgacttac 4560
atgaaaatcc tgtatctaag atagaaaaca tgtttccata attttaaaaa ataaaatatt 4620
tggggaaaat ccatcagaaa tatttttgt tgttattgtt attccaaaag atagaagtga 4680
taggtctcac cttgagaatt tattgtatgc caagtataga gtgggcagtg tgctttatta 4740
cactgtttct taacaataag gaaaactcca tttagcttga ttttaatttt catcccagac 4800
ctgtacacag agcattgatt tgcagttaaa aggaatgttt gagaacaatt tgatcattct 4860
gttttactca tccccatttc ttctgtcacc tttcacattc agtcccaccc ttcttgttca 4920
acaaaacaac ccccctcccc gcaaagacct gccccatctc ctttcatccc actgtgaacc 4980
attgaaatac atatatatca 5000

<210> 5
<211> 27
<212> DNA
<213> artificial sequence
<220>
<223> forward primer

<400> 5
gttttcgtcg tttttcgttc ggagatc 27

<210> 6
<211> 25
<212> DNA
<213> artificial sequence
<220>

<223> reverse primer

<400> 6
gctctcgctc tcgctattac tcgct 25

<210> 7
<211> 23
<212> DNA
<213> artificial sequence
<220>
<223> forward primer

<400> 7
tgtgtatgcg cgtttttcg ttc 23

<210> 8
<211> 23
<212> DNA
<213> artificial sequence
<220>
<223> reverse primer

<400> 8
acctatatat ccgccgctcc gtc 23

<210> 9
<211> 24
<212> DNA
<213> artificial sequence
<220>
<223> forward primer

<400> 9
cggcgttggg tatgtttagt agtc 24

<210> 10
<211> 29
<212> DNA
<213> artificial sequence
<220>

<223> reverse primer

<400> 10
aattacgaat aaaaaaaaca aaaacgctc 29

<210> 11
<211> 28
<212> DNA
<213> artificial sequence
<220>
<223> forward primer

<400> 11
tgacggtttt ttgtttattc ggttattc 28

<210> 12
<211> 21
<212> DNA
<213> artificial sequence
<220>
<223> reverse primer

<400> 12
cgaacgcaaa cgtacctacg c 21

<210> 13
<211> 26
<212> DNA
<213> artificial sequence
<220>
<223> forward primer

<400> 13
ggttaagttt ttttttyggy gtagtt 26

<210> 14
<211> 26
<212> DNA
<213> artificial sequence
<220>

<223> reverse primer

<400> 14
tactccctct acctcccrac tctctc 26

<210> 15
<211> 28
<212> DNA
<213> artificial sequence
<220>
<223> forward primer

<400> 15
ttgtggygta gaggattatt agtttggt 28

<210> 16
<211> 22
<212> DNA
<213> artificial sequence
<220>
<223> reverse primer

<400> 16
ctaaaaacrc aacccatccc tc 22

<210> 17
<211> 27
<212> DNA
<213> artificial sequence
<220>
<223> forward primer

<400> 17
ggaattttat tttgaaattt ttttgtt 27

<210> 18
<211> 33
<212> DNA
<213> artificial sequence
<220>

<223> reverse primer

<400> 18
ccccacttca aataaaatac tattaaaaaa aac 33

<210> 19
<211> 30
<212> DNA
<213> artificial sequence
<220>
<223> forward primer

<400> 19
tagtgayggt tttttgttta ttyggttatt 30

<210> 20
<211> 26
<212> DNA
<213> artificial sequence
<220>
<223> reverse primer

<400> 20
taaacrtaaa aacaacaacc cracct 26

<210> 21
<211> 5000
<212> DNA
<213> unknown
<220>
<223> bisulfite sequencing of DBC1

<400> 21
ggtttttatt tgaagttgtt tgattaygtt gttttagatt gattttagaa ttgtattatt 60
agtagggtag ttgttgttta ttaatagtta tggaagtaga ttttagttgg gggatgatgg 120
ggaaataaag agaaagatag ggaygaataa aggaaggaat ggggtataga tagaaagaga 180
gagagagaaa agaaagaaag gaggagaggt aagagatagt gatttggggt tttatatygy 240
ggtgggtggg agaaggagtg tgtgtggtgg gygtattttt tygtggttag aattaaagtt 300
agaattgatt tggggaattt gtgtttaaga tttgaatttg gattagaagt taagggaaag 360
agaaagttgt ttaagaaaaa agggagaaat ttttattagg ygaaaataga aatygttgat 420
tgggtttgtg gttggagagt tgttygtggt gttgattttt ttttattggg attttagtga 480

aggttgagaa gttttgtatt ggttgttttt ttttttttt ttttttttt ttagttttga 540
ttttttgaga tttaggatta ttttggttag ttygatgttt ttttttttt ttttttttt 600
ttttttgtyg attaaataat ttttttygag tttttagttt attatttaaa tatttttttt 660
ttatttttgtt ttaagatttt ttttttttta attagtttat tggtttttag attgttttag 720
gttttttttgg tggttatatt ttttttttat tatttttga attaaaaaat aaataaaaaa 780
tatataatgg taattataaa agtagataat atgtattgag tatttattag gtgttaggga 840
gtagtttaag tgtttttgta tatattaatt tatgagtagt ttaagtgttt ttgtatatat 900
taatttatta taatttata ttaattttat tagatgggta gatatattat tattttatta 960
aatagataag gaattgaggt ttaaggggta agtgatttta ttaagattag atagttatag 1020
gattttaatt tttttggagt ttgtattttt attgaatttg gttttttttat atattttaat 1080
attttttttt atgatttttt gttttttttat ttggtagttt taatgttttt tatttttgtt 1140
taatttttag gggattaggg atttgtattg ttgatttggt ttaaattaga agggaagtaa 1200
agtaaataaa tatattaaat gtgygttgtg tgggaattat tataygaggg ttttatttttt 1260
tgttttagga agaggtttta tgttagtagt tttagtttgt atttaggttg attgtagagt 1320
attttgtttt ttatttttat gttttagttt ttgtatttty gttttttttt ygtttttggt 1380
ggtttttaga gaatttygtg ttttttttagt ttttttttttt tatattttgt ttaygtagag 1440
aagtttttgt tttattttgg gaggttaygt gggttttygt ttatatatyg agagaaataa 1500
atagtgttaa atatttatag agagaygygt agatataaay ggatttatay gggtaatttt 1560
ygagataaaa tttatattyg atggatttay gyggtygtgg aaatatttgt ygttttagaa 1620
atatttaggt attygygata tatatagtat agttaygttt aagggtatta ggatttyggg 1680
tttgygygta tgygyggttt ttttggatgt tygtgygtat agatataata ttttataygt 1740
tttagattta ygaaattttt tayggtttag ttttagttta ttygggtygt tttttttyg 1800
aggyggtttt tygttttttt ttttttygtt ttttttttttt tttygtttaa agatgtataa 1860
aatatttttty ggaagtaatt tyggygttta gttttttttt tttygttttt yggtygtygt 1920
ttttttattt attttyggty gtygtyggtt aagttttttt ttyggygtag ttyggttttty 1980
gtygtttttty gttyggagat ygyggygtat ttggattttt ttttttatty gttagtygtt 2040
tygttttygg attttayggt tgtaaattga tttggygygy ggggaggagg agagygtagg 2100
ygagygaatt ygygagagag ggagagagyg agygagtaat agygagagyg agagygagag 2160
agtygggagg tagagggagt agtgatygtt tttyggagty gggatttatg tttgttttyg 2220
ggattagyga aggggatttt ayggttgagt atgagttagg ttgttaggag ttaggtattt 2280
ttaygtttgt agttttygyg tygtgttygg aatgygagtt gtaygtaggg ttttttaag 2340
tttttatyga gtygaataaa aagygttttt tygtagtttt tygttaaaga yggatattga 2400
ttttaggtaa ggyggygtyg ggtgtagygt ttygtagttt ygttgttttt ggattyggtt 2460
tygggtygta ttyggggygt tttygygttt ttttgttttt ttttttatyg gtatttttgt 2520
tygtttttta tttggtygtt tygtygtttt taagtttttt ttagttttag ggagggggtt 2580
tttgtgtttg gggtttaaag ggttaattgy gggtttgagt gagtggygtg tgtgttttyg 2640
ygygttttyg aygtgtgtat tatggtggga atttgatgtg gtgttagtgt gtttgygtgt 2700
gyggygtygt ttgygtttga tgygtgtgtt ygtggtgtgt gtgtgtgtty gtgtgtgtaa 2760
gggaggggtg aagagagaga ggttttataa tttatttayg gygygatgtg tgtgtgtatg 2820
tttgtaygta tgtgtttgta tgtgtgtttt ygtgtgtttt tttaattagg ttttttttagt 2880
ttatayggaa tgggattttt attataggat taygtagtta tygggaaatt ygttgtggat 2940
tttttttttgg ggttttgggt ttggggtttg gggaggatta tggggttgta gatggtattt 3000
tatttagttt aatgttggta ygtttgaagg aaaatttttt taaayggtgg aattttgtta 3060
tattgggatt tatagtttaa tatgaaagag atatggttaa ttttygaggt aaatgagayg 3120

ttgttatttt aaattttata ttgggtagat tttaagattt tgatgggaat ttggagatat 3180
tggatagtgg gtgatagaga aaggggaag ttagyggtgg gttttttatt tggggtttgg 3240
aaagtttggg atagggattt attttgtatt ttygtttgta attaatagag ttttttyggt 3300
ttttgttggt tttggggagg atttggtaag tttgtatgga tttttttag gggaaaggag 3360
aaagygtttt yggggatttg tttatttatt atagttgtaa agggttttag aggaaatttt 3420
atttgggyg gttgtggatg atatggaagg agatggatgg ggtatttttt taagatagat 3480
tygttttttt ttttttattt taggygggga agttttaga gagttttttt ttaaatatgt 3540
ttttttatgg ttttttgga gttaggggga tattgagaga aggtagagag gtggagaggg 3600
agagagagag agtaagagyg agagagaggg agatagagag agagagtgtt ttagtattga 3660
gggagattta taatttgaaa aggggttgtg agtgtggaat ttatgataga atgtggtagt 3720
aattgattta aattgttgty ggtttgtatt tygygttttt tatttggttt taaaatattg 3780
ttaagggtag gggggyggtg gaggaatttt agttaggaag gtagtttggg ttaaggtttt 3840
ttttttttt ttttttattt tttgggaggt tttttaggta ggttttttgg ttattttggt 3900
ttggtattgt tgggtttggg ttttggggtt agttattgat ttgattttta gttgtttttt 3960
aaaggtttgt tttatttagt aaaaatgttg agttttattg ttgttagtat ggtttttaga 4020
ttyggtagtt gttatttttt taaggtaagy ggtagtygtt ttgtttttag gtagggaggt 4080
taggagaaga taagggttgt gaygttggga tatagttttt tgtttagttt agtayggagg 4140
ggtygttgaa aagttttttt aggggaaaaa agtagaaata tatttggttt aaaaaatgta 4200
tatatatatt ttaggaagat atatatatat atatatatat taattatata tatatatata 4260
tatatatatg tgtttatatt ttggggagag ataaaagtaa atatgtattt gtaggaattt 4320
gttttagtgg gtgggtttta tttgtaggta tttgtagata tattattttt tgtttagagg 4380
ttgatatttt aggttgaagt ttttatatag ttatagagaa tttttttag gaaatttatg 4440
ttagggtaga gttttttgga ttatttgggg taggggtat tagggttaag gaggagattt 4500
atttttggga attgttttat ttttttttt ttttaattta tagaygatyg aaattggaag 4560
gaatattaaa gagtagaaag gtaggatttt gtggtttaaa gagggaatga gaatttttta 4620
atatttagta gtgggttagt ggtatgttta ggattagaat ttaggtttat taattagtta 4680
gattaatatt ttttttattt tttttagtaa tatatatttt aggattgtat attgttttta 4740
tttttatttt gttaatttgt tattatgatt tttattaaga taatygtttg ttttttttta 4800
ttaaatttta tatttttaga gatgtatttt agggtttttt tatttggaaa tatggttttg 4860
aagagaaaag atttaatgtt ttaattatta ttttygaaag atatatatat atatatatat 4920
atatatatat atatatatat atatatatat atatataygg tggtgggggg aggattgaga 4980
gagagataga gagagaatat 5000

<210> 22
<211> 5000
<212> DNA
<213> unknown
<220>
<223> bisulfite sequencing of PDE8B

<400> 22
agaataattt tgaagtatta ttttatttt agaatttttt ttgtagggat gtttgaggtt 60
tggttaattt ttttttttgt ttaagtttgt ttttttttat ttttttttt tatttttttt 120

tataaatgtt gattataaga gtatttttta aaaagttttt ttttgtttgt taaatattat 180
tttagaattt gtttttagga ggatttaatt tgtgtagaaa ttttatgagt aatttgtttg 240
tttattaaaa aatatagtat agatttttt taggttagtt ggagatggag ttttttgta 300
ttaaaaaaag agagttaaat tgagaagaga agtaaaggta gaagaataag gttagatttt 360
ttttgtataa ttgaatttgg gattgggaat gatattttgt ttttttatga aatttagaaa 420
ttgttttttg aagtttttat ttagttatat ttaagagtgg tttaggaaga aaaaggaaaa 480
taagaatgaa aaaaaaataa aaaataaatt ttaaaattgg atatttttt gtgttttgta 540
ttaagttttt atgttattgt ttttgttygt ataagtaatt ttagataaaa tttttttta 600
gattatttaa tttatttttt ygtgttattg tgaaaatygg gtaggaatta ttgygggaat 660
tagtgattga atgggtagtg gtgtttygta gttgtaattt agagtttata agggaaggat 720
gaatagtagt tatttttta aaggtatttt ttttttttt tagtatagtt tagtttgtgt 780
atgttttatt aaggagaatg agggaaggtt ttgttgattg tttagtttag agtgtgagga 840
atagtagttg atatgggygg ttggaygga tagaaattag tatgaatatt tagtgatatt 900
taaggaagag gtgaaaatgg gggagaaagt taagaattat tagatagttt ttttttgttt 960
ttgttttgga ttttagggga aygtatgtgt ttaagggtgt tttttttygt agggtttttt 1020
atttttggt ataaattttt aaaygatgag tagatatgga atgggttttt tttgaagatt 1080
gggttattat ttaatttagt tttyggagta ggyggtgggg ttgggggaag aggayggaat 1140
tatgaggttt ttttattatt tttygggtgt gttgagaggt aagtaaatgy ggtgggtggg 1200
tttgggttgt agggtttttg ttatttyggg aggggttttt tggtgtttag ttttgggatg 1260
tgtgaaaatg tgttggtgaa aagygagagg tttatatagt tttttagggg aagaggggtt 1320
ggggygttgg gggyggygty gggatgagtg tagaagagay gaggtttttg gatagyggag 1380
gaggagggga gggygtygag gygyggtgtt agttgtygyg tataggggtt tygyggygga 1440
gtygagtygy gggtaygttt tgttttgtyg ggagagttty gggagyggyg ggaggggygg 1500
aggggygtag tggggttygg gyggttgygg tygyggagty ggggtatttg aggaggaagg 1560
agggtgggag ygagggaggg aggggayggg ygtagatyga aagtggggaa agaaggtgta 1620
ggtaggyggg taggygggyg ggygttttgg tttagggtyg ygggtgyggg agttyggyga 1680
ggtygagttg ggyggyggyg ggggtygygt ygagggagga ggggaaggyg gaggygyggg 1740
gagygtgttt ggggygtygy ggyggggagg gtggyggtyg ttggtgygyg yggggygttg 1800
tgtatgygyg ttttttygtt yggggaggaa gatggtttaa aagggaaagt tggggtgayg 1860
ygygyggttt tyggaggtty ggyggggggt atygyggtta gttygaygga gyggyggata 1920
tataggtygg ggggygygta gttygggygt ygtygyggty gttttttttat tgtaggtggt 1980
agyggggtgyg ttgggtttyg gyggtygygg gygyggggygg gygygygggg gagttyggty 2040
gagggatggg ttgygttttt agtatttatg tttygtagag yggygtgatt tattgtyggg 2100
attyggayga gtttagttyg tttygttaga ttattagygt gtygtagggt tyggyggtat 2160
ttttgttygg ttttttygtt tagatygayg tygtygaygt tatttttyg agtygygygt 2220
ygggatttttt tagygtagtt ygygttygta gggttygtat ygagttgggt agyggtagta 2280
gygygggttt ygtagttttty gtygygatta ttagtagggg tyggaggygt tattgttgta 2340
gtagygtyga ggtygagatt tagatttgtt atattagygt gaaggtaaat gtttygygtt 2400
ggtataygty gtgggggtyg ttygtttygt yggyggggtt ygtaygggta gggggtttyg 2460
gyggagttgg gtgatygtga ggyggttggt ttggagaggt tgttattaag gaggagttta 2520
ttttttattt gtggagatga tgggagttta ggaaatgtgg ttagaaaaag gtttttggag 2580
gggttttgga agygttttta gttggttttg ggggattggg yggggaaggg agygtagaag 2640
gaagtaggtg ggttggtttg ttttttttttg agggtaggaa ggttgtggtt tggtttatgt 2700
aggaagaggg gtggggatta ttgagagtat tyggtggtta gttttgttga atgaaatttg 2760

agtattgagt tggatttgyg tgttttgtag gtgattggtg tagttgtagt attaggatag 2820
atagtgtttt atatttygat ttttatttgg gattattagt taggttggag ttttagtata 2880
ggaatygagy gtagggattt gtgaatgaat gagtgttygt gttttaagag atgtgggaay 2940
ggagtagagt ggaatttgtt gtttgttatt gtaaygtttt tttgggttgg ttgtatttta 3000
gatagaattg agagaatygg gttatgagtt yggagtgtta gtagagttat ygtgagggga 3060
ygtggttttt agtgtagtat agttgtttga ggatgatttt gtatatataa ttgatttttt 3120
tagagagtgg gattttgagg aagtggaaaa aattgtttag atggttaaag tagtygttaa 3180
atatttattt tttaaagata gaagaaaaat aattatttaa atagtgtttt ttygtatgtt 3240
tttaaagtat ygttaaattt aagaggtttt ttattgtgta aatttgggta ttgggttttt 3300
ttttttttta gtaaataaga taataatggt atattttatt gtatagagag aaaaaaatat 3360
ttttaatgtt agatagaatt atagttttta tttggttaat aggttattaa gaattatttt 3420
atggattatt tgttaaagat atatttgtat ttttaatagt taaaaatttg gtttttattg 3480
gatgaaggta gtttatagtg gaggagtgag gagaaggata atatgttttg gttttaattt 3540
ggaagtttaa aagtttttta atgaatttat tttttttta atagtatttg attgtttaat 3600
atgaagtttg attgatgttt gtttgtggtt gtagtgtttt tttggtagta attataatta 3660
ttattatagt aaaaatatyg tttattgagt atttattatg tgttaggagt aagttttttt 3720
ttttaattat tgaatgttaa tagtgtgatt ttagtatagt atgtttgaag attagagttt 3780
aggatttatg atagtataaa tttattagtt gtttattygt tatagaggta tgtgttgaay 3840
gttatatttt gtttttgtta agtttttttt ttttttttt ttttgtttat ataaagttag 3900
ttttaggagt aatttttagg gatgaaaatg ttaaatttgg tgaaaatatt aagttgggat 3960
attattagta aagattattt ttttgtttga yggttgaaga tttgaagtag tagtttaaaa 4020
ttgtgatagt ttatgaattt tgtgatattt tttttggttg ttttagagaa attgtgaatt 4080
tttgtttttt agaatattta taattttagt ttagtatttg gtaattttag ttattttatg 4140
tttgtttagt agaagaaaga atattgaatg aatttatttt gtatattttt aatatttttt 4200
ttaagaggtt gtttagagat taaatgaagt aaaaattgaa gggataatga gttttaatta 4260
ttagtagttt gtataaattt gtattaattt tattgttatt tggtttaagt attaaaaggt 4320
ttgatttggg taggagttta atttattttg ttttattaag aagattttta aataagagtt 4380
taatttattt taatttatta ttgggaatgt tggaaatgaa atttttggtt tattgattta 4440
atattatttt gtattgtgtg gattttagaa agtatatatt tgaagtgaaa tttgagatta 4500
gagttattta tgtaaatttt atagaattat tattatagaa ttgttttaat atatttaata 4560
gtatttttt aggtttgtga ttyggtgttg tattgttttt ttttaatygt aaagtttata 4620
tgttttgtgt ttttttgttg ttgagataat tttataaaaa ttttatattt atttttttgt 4680
agattaagta ttttttttt gtygtaagag tttgagatag ttgttaggta ygtggtggtg 4740
ttttattttt ttttaaagta tataggtgga aaaataaaat ttttattatt tggttttgtt 4800
tatagtggtg gaaggttgta atagttttta ttttttttt ttggttattt tatattaatt 4860
atgaagtgta tttttattat ttatttggtt tttggtattt tttagagtgt aaaaatgtgt 4920
gtttgatttt ttttttttt ttttttagta ataatttatt ttattaagtt tttagaatga 4980
tgaggtatta tgatgttaaa 5000

<210> 23
<211> 5000
<212> DNA
<213> unknown

<220>

<223> bisulfite sequencing of PTPRR

<400> 23

aagaaaaata gatttattgt aagtgggata taattatttt tttttaattt ttagttttgt 60
agtattttta gtaatagatg ttagatgaga agagttattt ttttaagttt tatagaatgt 120
taaataaatt attttaaagg ttgatttagt aagaatttta aagagttaat tgttagtatt 180
gaattttgtt aattattatt tttatttatt tatttttgag tatttatttt gtatgaggta 240
ttgttttagg gagttgggat atattaataa agttattaaa gttttttaat attggagttt 300
aaatttttgtt ttgyggggggg gagtagggaa atagatatta aataatagat tttataagta 360
attattttgg tttgttagaa agtgataygg gaaaagtaaa ttggaggata gtgaattagg 420
aataggagaa gaggttgtag tttagagtgg tttgggatga aatgatagtg ttatagaaat 480
ggagtgtgtt ataygtatgg gggagtaggg ggtggagtay gttgttagag aggggttaga 540
tatggagata tgtttttatt tttagtagag aaggtttgtt aagatattaa aagtatattt 600
tattgttttt ttttttttatt tgttggagtg ttttttggtt ttgtttttta gagttgaaga 660
tgatataaat tataaaagaa ataaatggta tygtttgggt tttttgattt tataaggttt 720
tatttaattt aagttttggt ggatagttta ttttttatta gttgttttaa tttatagagg 780
atggagaaag aaagtattga atgagatgtg tagtttaatt taatgggtat ttgttagttt 840
taattatttg gttaggataa ttttatttaa gttaatggaa tataaaattt agtagttttt 900
atggagattt taatgtgttg tagaataaat tatagataaa tgaattgttt gaaagaataa 960
tatgaggtay ggttaaataa gtgttgtgga agagaaagat tattgtaatt tagagttagt 1020
tagagttagg gttttttgga aaagtgagat taatattata ttttygaaaa ttagtgtggt 1080
ggtggtgtaa gtggaggaaa atgggaaaga ggtaatagtg tttataaaga tatagaggta 1140
gaaagggtag aggttttggg atagttaaaa gagaagttta gttgagggat tttttgattt 1200
gattagtagt aattagttat gattaataag gttttttata ttttaaagat ttagatggag 1260
ggataaaaaa ttatttaatg gtagattgtg gtagtttttt tagagatata gagttgggty 1320
ggatgagttt aggtattgay gtgatttatt atttttttatt ttaaagagta aaagggaaat 1380
taaagttaat tatttttayg aaataaaaag gtgtttttttt gtgttttaat tatatggata 1440
tattttatta gtttaaaagt atttttttat ttttttttgt tattgtgagg atttgagtta 1500
gaagaaagtt taaatatagt tattgagttg gaaagagtgg aaagagaagt aaagaggggg 1560
aagttgtagg aaggaygaag ttatttttaa gatatatggt tattgtttat attaagtaag 1620
ttgttttggg aaygtttttt tygagtagtt agaatgttta gtagtggaag atatttttat 1680
ttttgtaggy gagttttggg aagttggtta atttgtaaat gttaattttt agtagtgagt 1740
tyggtttayg tgtaaattaa gatttgggga aagagtaggg tgggtggtat ggttgataat 1800
gttattagtt tttttttttg atttttgtgg tygtgttttt atttatttttt atttagttat 1860
attttatttt yggatttgtg atggaygttg ggtttttagt aattatagta agtgtttttt 1920
tygtattttt tttttttttа ttttattttt tatttttaat tattatttta gygatggagt 1980
ttattttgtt ttaagtygty gttaagatty ggagaagygg aattttattt tgaaattttt 2040
ttgtttygtg agggtyggyg ttgggtatgt ttagtagtyg yggygttgtt gttgggttgt 2100
tgggttggyg yggagtttat tttgtygttt tygttttggt ttttgggygt ttagaaggtt 2160
aggtatttgt ygtttttgag ygtttttgtt ttttttatty gtaatttttt attgtttttt 2220
ttttttttttt tttttaggga ggttgaagtt ggtgttggtt tttgtyggyg ttatagattg 2280
attgttttgt aaattttagt ygaggatttg aatttyggag attagaagat ttttggyggt 2340
ggtttttttt aatagtattt tatttgaagt ggggtygtgg tggagttttt tttttatttt 2400

ttaatgtaaa tattatgygg agagtagttt gttttttgy gttgtgtttg ttttttaatt 2460
tttaygttgt aggtaagggg ttgttaggtt tagagtygga gttgtgtatg agatgggaaa 2520
ttgtatatgt ttaaggattt taggaaaagt ttgttttgyg aagaaagttt ttttaaaaag 2580
gtaaaatagg atagtatttg atagggaagg ggtgagggag tygtgtattt gttggaaaat 2640
tgaggtygaa aatttaattt aaaattagtt tttgattttt ttttatttta taygaaaata 2700
gtgaatattt ttaatatgaa tataggtttg ttttttttt ygttttttt tygtttagat 2760
tttttgtttt tgtttttagg ttatgtattt aattgttaat tttttaggag agggataaga 2820
tattttgtta gatgtaattt tttttattgt agyggttatt atatttataa ggttttttgg 2880
tttagygagy gtttatagga aggtattggt tgtaatttga tggattatat tttygtttaa 2940
aagatygaaa tatatggttt atattagtga tgttgtatta ggttttttg gttttgttt 3000
ttgttattyg aatttttaa gttaataaga ggaagaaaag gttttaaata gaatttttt 3060
gttttttta gtatygtggt tagygttygt tggtatatta aaaaaaaaaa aaaaaaaaaa 3120
aaaaaaaaaa ataattatag ttttaaagtt gataattttg ggttaggtat tgtttttygg 3180
atagatttga taaggtgtaa ttaagagtaa tagtgagatt gtttttaaat ataaatagat 3240
ttgaggtatt agaattaata ttaagtttyg tgaggaagta aattttggga agtatttgag 3300
agatattata tgtggtgttt ttgttttttg tatatatagt atttttattt aatgtagata 3360
gatttgttag gttgaatttt ttattattgt tatatatata tgaattaaaa taaaatatgg 3420
tagggagatt ttagaaattt tattgtgata gtagttgttt atgtagtagt tttgtaataa 3480
ttagagayga ggtatttttt ttgagtgtgt tttttattta attatatata tttatttatt 3540
tttttaagta atatttgttg agtgtatatt atgttttagg ggtaatgagt aaaaataatt 3600
gttttgaaat tataattttt gtagtatttt ttttaaaaga gtgggtgaat tttatttttt 3660
gtgtataata gtaggagtga aatatggtat ttttttattt tagttaaygt tattatttaa 3720
atgtattatg aatttttga gtaaataatg tataggatta ttaaagttat tattaaatat 3780
atatgttaat tgttataatt atgatgttat attgtggagt ttttaatagt tttttggtat 3840
attttgagaa gtagaaaata gttatgattt gattattaaa ttatttgtaa atagttgtag 3900
tgaattattt ygttattata tataattttt taatgttgaa atgtgggatg aaaatatata 3960
tattgggttg agttttattt atagtattgt ttaataatag attgatataa atatttatta 4020
tagaaaaatt ttaattttta ttaaggtagt aatgatttag ttggtttgtt aataatatta 4080
gtgtagttat ttttttaaaa ttaatatata aaaaagttta aagattatat ttgatatatt 4140
ttgagtaatt atattttaaa agttataaat aaattgaagt atgtgaaaaa tatgttttga 4200
tttattgtag atgaatatty gggatgagaa aaatgaggat ttttgtttat tagatgagaa 4260
atttagaagt ttgtagtttt agattttatt tggagttttg ttaaagggta tattattaaa 4320
gttaatttaa tttatttta ttttaatttt ttaatttgta aaataaagat aaaaagtaag 4380
attaattaat taattttaat ttaataagaa aatgtattta aggatatttt tatattttaa 4440
ttgtaagata ttatttattg tatttatatt taatatagta aaatatattg gtgttttatt 4500
atagtagatt gtaagtttaa tattatagtt tttttgttaa ttttatttta aatgtatttt 4560
aattttaaat agtaatttgt gggttataag ttgttattag attaaaattt attatagtta 4620
tttaataata ttgttagggt tttttattag aattaaaata aatatagttt tttggtttaa 4680
gtttttatat atttggagat taagaaaata agtggatggg atgataaaga ttaagaaatt 4740
aagatattaa tttttatttg ttgaaggtgt gagtttgtta tttttatgga agatttttt 4800
tttagaatag aggttgattt tataaagaag ggaaatgaat tttatatatt taggtttttt 4860
taattgtaaa gttaattgat ttatttttggt taygtttagg gtattagtaa ttgttttatg 4920
ttggtggaat tgatgatatt ttataatgtt ttagagatgg gaggaatgtg ttaaygggat 4980
gtaagtgttt tttttagtta 5000

<210> 24
<211> 5000
<212> DNA
<213> unknown
<220>
<223> bisulfite sequencing of ZNF582

<400> 24
gaygattttg aatgattttt gttttttagt atttatattt ttgtgtagtt tttatttatt 60
tagatttata gttgattttg tgattaagag tataatggtg tatgatttta gaggtttggt 120
ttagagttag aagtggttat tttattattt tttaaatttt tttttggtag attaatgaat 180
gtgagaaagt aaaaatggta tagaagaaag ttagtttgag agatggttgg ttttgtattt 240
gtttggtttt ttttttttt ttttttata agtaaatatt gaygtatgta tatatagata 300
tatttaatat agtgggatgt taaagggaaa ttagagttat tygtttttt ttaagattag 360
agaatatagg aaagatgatg attttttgta tttatttttt ttttttgaat tygtaatatt 420
gttttgaatt ataaataagg ttaagtagga taaatttata attgttttt taattgttyg 480
tttataagtg tgaaatttaa ttatttttgt tgaagttgtt ttttaatatt agtaattagt 540
aataattaat ttttgagttt tgtttatatt attaaattat tttatttgat ttttaggata 600
aagttgtaag tttagtatta tattttttt tttatggata aaaaaaaaaa aataaaataa 660
aatygagata ggtaygaaga aattaataaa tggttgaaat gttatttgaa tttatatttg 720
tttaatttta aaagtgtgtt ttttattata ttatygtatt tataaggaaa atgtagtttt 780
tattttttt tgaagaatgt gtaaagaggt aatatggtgt gttttggaaa aagtattttt 840
gtagaaagaa agtattagtt ttagttatat tagtattttt atttagtagt agtagtatta 900
ttttatatat tagttatttt tttttaataa tttaatgaat tagatgttat aattatttt 960
atttttaaat gtggagatyg aggtatattt ttaagattgg agtttaggta ttttggtttt 1020
agttttagag atggttaagg gtttatattt ttaattattt attatattat agagtttatt 1080
aggtttgagg gaaataggat taaattaaaa gagttattta ggattttagt ttttatttaa 1140
ggataaattg ttttatttyg gatagggaga gttttygtat tttgagattt agtataatag 1200
gttttgatyg gtatttggta ttyggatttt taattatatt ggattatatt ggttygggat 1260
gtgtaaagtt tagggttttt tatatttgat gatattaaag tygtttaaaa ataagagaga 1320
attaataatt atttayggyg gtttgatatt tgtttaagag atgtygtttt ataaaatttt 1380
tttatatttt tataaygttt ttatttygyg ttttttttta taatttatat ttaatttatt 1440
atagatgtaa tgtttaaaat tagttattag ataaattttt aygtttttaa attttaaggt 1500
tttttygaaa ttttttggta aaattgttgt tttayggaaa tgggaaygta ayggatgagg 1560
taatttttta tagtygtata tagttgtgta tttatygtta aayggtttta gttatatatt 1620
taaygattta ygyggagtta gaagttatta ttatatattg ttaaaattay gtatatatag 1680
tgayggtttt ttgtttatty ggttattygt ttataatttt tygttagaga attataygta 1740
gatagtatat ttagtattat agattttagg aagtaattta gggattygaa tataygaata 1800
gtatttttty gygtattgyg taggtaygtt tgygttyggt ttattttgaa atatygygag 1860
attyggtttt aaggtygggt tgttgttttt aygtttaaag attatgtttt tyggaagata 1920
ttgyggygty ggttttatta tggygtagta tyggtgtgtt ttgtgygttt gygttatttt 1980
tyggttgygt ayggygaatt tatyggtaty gtggtggaag ygygttttgg gttgtygggg 2040

gygyggtygy ggtggtattt ggattygagg aggyggtagg tgagaggttt yggagttttt 2100
taggygtttt ggggtttagt aggagttggt gttygggtyg gttgggtttt aggtttgaga 2160
agaaygtaga ygtttygttt tatygtygtt ttgtggtttt atyggygtga gattatattt 2220
ttygtyggtt ttygyggygt gygtttttttg gygtttttttt tttgttttta gttttatagt 2280
ttaggtgtat ggatttttta ggtgggtgyg taggggtttt ygatttttttg aaattgygga 2340
tgttttgtat ttattgttat gygtgttttt ttttttttttt aatttgagag gaaatttttgt 2400
tttatgaggt ttttagagag ttataagatt ttagaagatt tagaatygtt agtttagaaa 2460
aatttatatt tgggaatatt ttatatgttt aaattttatg tttagtaatg gggaaatatt 2520
tgagtggatt ataggttatt ttttgtaaat aggatatygt gtagttaata taaataatgt 2580
ttataaaagt ggttataata aggaaaagtt gtttattata ttatattaag taatatttat 2640
aattatttta tttataygtt atttagaatt aaatgtttgt aatgtagtag gtattttata 2700
tatttttta tagagtattt tttgtttaat agtgagttta agaattggtt aaatgataat 2760
attgygaata tttgggtgtt tttttttta attttttttgt tttgttttgt ttttagtttg 2820
tgtgttagtg agtttgattt ttttttgttg aatgttttta aaggatttag tagttaatat 2880
ttttgtttgt tagtttttga tagttatttt tgatagttat ttggtatttt attatatggt 2940
tgtgttaaaa atgatttaat tttttggtta tttgtggatt tgtttattta ygtattaatt 3000
tttagttttt ttatttagta aatattattg gatatgtagt tttgtgtatt gttgtgtttt 3060
tatggaagga tatatttatg tgttgataat ggagtattgg gygtgttgtg tgtaatttgt 3120
tttttattgt ttttttaat tttattttat ttgttgatgt aaggtatgtt ttatgaagtg 3180
gtatgtataa agttttagtt tgggttgtag agtttttttgt atatttggyg gttgggaaaa 3240
gagttaggta gattttaaa ggtgttatat ttgttgtagg agagagttaa atggtttagt 3300
tttggaaagg ttttatttta ggttggggtt atgtaaatga tgtaggtttt ttatgttttt 3360
tggtttgagg gaagtttttt ttgtttttga ggtaatatgt gggttttagt tattagaata 3420
gtttttagta tttttgtttt agatttgtat ttgtagtaaa atagaattat tggatgttta 3480
ggggaaatgg gagggaaagg attattggtt tttttttgtt ttgtagtagt taaagttggt 3540
tttttggggt tgggtgagga tttttttgtt ttttttttttt ttttattttt ttggtgggta 3600
gagggtaagt aggtggggat ttgtaatttg gtattttagg aattgtgagg taagtttagt 3660
tttttatgtt tgttttttttt ttttttagtt ttatygtygt aggattttgt tttttttaa 3720
gagaggaatt agaaggagga tttattagtt tttgaaattt taaaagttat gttttttgtg 3780
agtttttaaa ttttataaat atttgttttt tttatttga aatttttttat ttgggtttta 3840
tttagaaatt tggtttttag attttttttat tttagggaat aaaaggatta ggtaggagtt 3900
ttttttttta ttgttttttt ttttttgata aatgtttata gatttatttt ttatagtatt 3960
ttgtattttt tgatggtttt ygtttgttag gaggagaaat ttatttttttt gtatgaaatg 4020
agagtatttt ttgtaagaat tgaagtttga agagatggaa tgaatgttta ggagtaatta 4080
tatatagaaa agtaggggag gtggtgttgg ttggttttag ttttagygta tggtatagtg 4140
ttttgttttg ggaatgatta atgattggat atgtaattga ggtttggttg ggaatttatt 4200
aaaaatttga tgttagaatt aaagtttagt ttgaygttgg atagagtatt tgaatttggg 4260
atatttttta ttagtttttt tagtttaaga ggaagaaaag tatagagttg aggatggtga 4320
gygatgtagt aagtgagatg attatttagg tgggtgagtg gttagggttt ggaaaagttt 4380
tagagatttg atttaattty gggatttaaa ataagaggat gtttgagtta gttgttttat 4440
ttgatagaaa attgtgtatt taataaaagt tatttttatg gttttttttа tgaagaaaaa 4500
tgtttgtgtt atgttttaat ataatgatga atttttattt ttaagaaatt tttgatttat 4560
atgaaaattt tgtatttaag atagaaaata tgtttttata attttaaaaa ataaaatatt 4620
tggggaaaat ttattagaaa tatttttttgt tgttattgtt attttaaaag atagaagtga 4680

taggttttat tttgagaatt tattgtatgt taagtataga gtgggtagtg tgttttatta 4740
tattgttttt taataataag gaaaatttta tttagtttga ttttaatttt tattttagat 4800
ttgtatatag agtattgatt tgtagttaaa aggaatgttt gagaataatt tgattatttt 4860
gttttattta tttttatttt ttttgttatt ttttatattt agttttattt tttttgttta 4920
ataaaataat tttttttty gtaaagattt gttttatttt tttttatttt attgtgaatt 4980
attgaaatat atatatatta 5000

What is claimed is:

1. A cancer screening method, based on detecting the methylation rate of target gene in test specimen, and using the methylation rate as the screening index to determine the risk of having cancer, the method comprising following steps:

step 1: providing a test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene of said test specimen, wherein the target gene comprising DBC1, PDE8B, PTPRR and ZNF582; and

step 3: determining whether there is cancer or pre-cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using the methylation rate of target gene as prognosis marker.

2. A cancer screening method as recited in claim 1, wherein the test specimen is an isolated specimen selected from the group consisting of cervical smear, ascites, blood, urine, feces, sputum, oral mucous membrane cell, gastric juices, bile, cervical epithelial cell, and post-surgery cancer tissue.

3. A cancer screening method as recited in claim 1, wherein the testing method for the methylation state of CpG sequence of the target gene comprising methylation-specific polymerase chain

reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

4. A cancer screening method as recited in claim 1, wherein the target gene DBC1 has nucleotide sequence as depicted in SEQ ID No: 1, the target gene PDE8B has nucleotide sequence as depicted in SEQ ID No: 2, the target gene PTPRR has nucleotide sequence as depicted in SEQ ID No: 3, and the target gene ZNF582 has nucleotide sequence as depicted in SEQ ID No: 4.

5. A cervical cancer screening method, based on detecting the methylation rate of target gene in test specimen, and using the methylation rate as the screening index to determine the risk of having cervical cancer, the method comprising following steps:

step 1: providing a test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene of the test specimen, wherein the target gene comprising DBC1, PDE8B, PTPRR and ZNF582; and

step 3: determining whether there is cervical cancer or cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using the

methylation rate of target gene as prognosis marker.

6. A cervical cancer screening method as recited in claim 5, wherein the test specimen is an isolated specimen selected from the group consisting of cervical smear, blood, urine, cervical epithelial cell, and post-surgery cancer tissue.

7. A cervical cancer screening method as recited in claim 5, wherein the test specimen is the abnormal cervical smear.

8. A cervival cancer screening method as recited in claim 5, wherein the test specimen is the HPV(human papilloma virus)-positive cervical cell specimen.

9. A cervical cancer screening method as recited in claim 5, wherein the testing method for methylation state of CpG sequence of the target gene comprising methylation-specific polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

10. A cervical cancer screening method as recited in claim 5, wherein the target gene DBC1 has nucleotide sequence as depicted in SEQ ID No: 1, the target gene PDE8B has nucleotide sequence as depicted in SEQ ID No: 2, the target gene PTPRR has nucleotide sequence as depicted in SEQ ID No: 3, and the target gene

ZNF582 has nucleotide sequence as depicted in SEQ ID No: 4.

11. An ovarian cancer screening method, based on detecting the methylation rate of target gene of test specimen, and using the methylation rate as the screening index to determine the risk of having ovarian cancer, the method comprising following steps:

step 1: providing a test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene of the test specimen, wherein the target gene comprising DBC1, PTPRR and ZNF582; and

step 3: determining whether there is ovarian cancer or cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using the methylation rate of target gene as prognosis marker.

12. An ovarian cancer screening method as recited in claim 11, wherein the test specimen is an isolated specimen selected from the group consisting of ovarian cancer tissue, ascites, blood, urine, and post-surgery cancer tissue.

13. An ovarian cancer screening method as recited in claim 11, wherein the testing method for the methylation state of CpG sequence of said target gene comprising methylation-specific polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite

sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

14. An ovarian cancer screening method as recited in claim 11, wherein the target gene DBC1 has nucleotide sequence as depicted in SEQ ID No: 1, the target gene PTPRR has nucleotide sequence as depicted in SEQ ID No: 3, and the target gene ZNF582 has nucleotide sequence as depicted in SEQ ID No: 4.

15. A colon cancer screening method, based on detecting the methylation rate of target gene of test specimen, and using the methylation rate as the screening index to determine the risk of having colon cancer, said method comprising following steps:

step 1: providing a test specimen;

step 2: detecting the methylation rate of CpG sequence in at least one target gene of the test specimen, wherein the target gene comprising DBC1, PDE8B, PTPRR and ZNF582; and

step 3: determining whether there is colon cancer or cancerous pathological change or not in the specimen based on the methylation rate in the target gene, or using the methylation rate of target gene as prognosis marker.

16. A colon cancer screening method as recited in claim 13, wherein

the test specimen is an isolated specimen selected from the group consisting of ascites, feces, blood, urine, and post-surgery cancer tissue.

17. A colon cancer screening method as recited in claim 13, wherein the testing method for the methylation state of CpG sequence of the the target gene comprising methylation-specific polymerase chain reaction (MSP), quantitative methylation-specific polymerase chain reaction (QMSP), bisulfite sequencing (BS), microarrays, mass spectrometry, denaturing high-performance liquid chromatography (DHPLC), and pyrosequencing.

18. A colon cancer screening method as recited in claim 13, wherein the target gene DBC1 has nucleotide sequence as depicted in SEQ ID No: 1, the target gene PDE8B has nucleotide sequence as depicted in SEQ ID No: 2, the target gene PTPRR has nucleotide sequence as depicted in SEQ ID No: 3, and the target gene ZNF582 has nucleotide sequence as depicted in SEQ ID No: 4.