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HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
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TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
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LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
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(54) Title: GENE SIGNATURES PREDICTIVE OF METASTATIC DISEASE

(57) Abstract: Methods for characterising and/or prognosing cancer in a subject comprise determining the expression level of at least one, and preferably 12, genes selected from Table 1 in a sample from the subject wherein the determined expression level is used to provide a characterisation of and/or a prognosis for the cancer. Determined expression levels are used to generate a signature score. The methods permit metastatic disease to be identified and monitored and guide therapeutic interventions.



INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2016/051825

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/68

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/068839 A1 (COMPENDIA BIOSCIENCE INC [US]; RHODES DANIEL R [US]) 9 June 2011 (2011-06-09) paragraphs [0064] - [0065]; claim 1 page 154, column 2, line 7 -----	1-53
X	EP 1 471 154 A2 (VERIDEX LLC [US]) 27 October 2004 (2004-10-27) paragraph [0004]; claim 1; example 3; tables 1, 4-7 sequence 55 ----- -/--	1-53



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

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PCT/GB2016/051825

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RAHUL V. GOPALKRISHNAN ET AL: "Molecular markers and determinants of prostate cancer metastasis", JOURNAL OF CELLULAR PHYSIOLOGY, vol. 189, no. 3, 1 December 2001 (2001-12-01), pages 245-256, XP055297597, US ISSN: 0021-9541, DOI: 10.1002/jcp.10023 the whole document -----	1-53
X	AMRALLAH A. MOHAMMED: "Biomarkers in prostate cancer: new era and prospective", MEDICAL ONCOLOGY, vol. 31, no. 8, 1 August 2014 (2014-08-01), XP055297604, GB ISSN: 1357-0560, DOI: 10.1007/s12032-014-0140-3 the whole document -----	1-53
X	WO 2014/012176 A1 (DIAGNOCURE INC [CA]) 23 January 2014 (2014-01-23) page 61; claims 1-75; example 6; tables 5,6A -----	1-53
X	WO 02/30268 A2 (EOS BIOTECHNOLOGY INC [US]) 18 April 2002 (2002-04-18) page 201; claim 1; table 11 -----	1-53
X	US 2014/018249 A1 (GUYON ISABELLE [US]) 16 January 2014 (2014-01-16) page 26; claim 1; example 7; table 42 -----	1-53
X	WO 2004/085621 A2 (DANA FARBER CANCER INST INC [US]; POLYAK KORNELIA [US]; PORTER DALE [U]) 7 October 2004 (2004-10-07) claims 2, 10,17,22,23; table 15 -----	1-53
X	GORLOV IVAN P ET AL: "Candidate pathways and genes for prostate cancer: a meta-analysis of gene expression data", BMC MEDICAL GENOMICS, BIOMED CENTRAL LTD, LONDON UK, vol. 2, no. 1, 4 August 2009 (2009-08-04), page 48, XP021060675, ISSN: 1755-8794, DOI: 10.1186/1755-8794-2-48 abstract page 4 of 11, column 2 ----- -/--	1-53

INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MCCART REED AMY E ET AL: "Thrombospondin-4 expression is activated during the stromal response to invasive breast cancer", VIRCHOWS ARCHIV, SPRINGER INTERNATIONAL, BERLIN, DE, vol. 463, no. 4, 14 August 2013 (2013-08-14), pages 535-545, XP035373143, ISSN: 0945-6317, DOI: 10.1007/S00428-013-1468-3 [retrieved on 2013-08-14] the whole document -----	1-53

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2016/051825

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-53(partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-53(partially)

Methods for characterising and/or prognosing cancer comprising determining the expression level of CAPN6. Methods of treating or selecting treatment for cancer comprising determining expression of said gene. Systems and devices comprising a computer application configured to carry out the above methods. Probes, primers or primer pairs that specifically hybridize or amplify said gene. Kits comprising said primers or probes.

2-70. claims: 1-53(partially)

Methods for characterising and/or prognosing cancer comprising determining the expression level of THBS4. Methods of treating or selecting treatment for cancer comprising determining expression of said gene. Systems and devices comprising a computer application configured to carry out the above methods. Probes, primers or primer pairs that specifically hybridize or amplify said gene. Kits comprising said primers or probes.

[idem for each one of the genes listed in Table 1]

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011068839 A1	09-06-2011	AU 2010326066 A1 CA 2782620 A1 CN 102858999 A EP 2507397 A1 EP 2692871 A1 JP 2013512000 A US 2012245235 A1 WO 2011068839 A1	21-06-2012 09-06-2011 02-01-2013 10-10-2012 05-02-2014 11-04-2013 27-09-2012 09-06-2011
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DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
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(57) Abstract: Methods for characterising and/or prognosing cancer in a subject comprise determining the expression level of at least one, and preferably 12, genes selected from Table 1 in a sample from the subject wherein the determined expression level is used to provide a characterisation of and/or a prognosis for the cancer. Determined expression levels are used to generate a signature score. The methods permit metastatic disease to be identified and monitored and guide therapeutic interventions.



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GENE SIGNATURES PREDICTIVE OF METASTATIC DISEASE

FIELD OF THE INVENTION

5 The present invention relates to cancer and in particular to prostate cancer and ER positive breast cancer. Provided are methods for characterising and prognosing cancer and in particular prostate cancer and ER positive breast cancer. The methods utilize various biomarkers, specifically in the form of one or more gene signatures. Primers, probes, antibodies, kits, devices and systems useful in the methods are also described.

10

BACKGROUND OF THE INVENTION

Prostate cancer is the most common malignancy in men with a lifetime incidence of 15.3% (Howlader 2012). Based upon data from 1999-2006 approximately 80% of prostate cancer patients present with early disease clinically confined to the prostate (Altekruse et al 2010) of which around 65% are cured by surgical resection or radiotherapy (Kattan et al 1999, Pound et al 1999). 35% will develop PSA recurrence of which approximately 35% will develop local or metastatic recurrence, which is non-curable. At present it is unclear which patients with early prostate cancer are likely to develop recurrence and may benefit from more intensive therapies. Current prognostic factors such as tumour grade as measured by Gleason score have prognostic value but a significant number of those considered lower grade (7 or less) still recur and a proportion of higher-grade tumours do not. Additionally there is significant heterogeneity in the prognosis of Gleason 7 tumours (Makarov et al 2002, Rasiah et al 2003). Furthermore it has become evident that the grading of Gleason score has changed leading to changes in the distribution of Gleason scores over time (Albertsen et al 2005, Smith et al 2002).

It is now clear that most solid tumours originating from the same anatomical site represent a number of distinct entities at a molecular level (Perou et al 2000). DNA microarray platforms allow the analysis of tens of thousands of transcripts simultaneously from archived paraffin embedded tissues and are ideally suited for the identification of molecular subgroups. This kind of approach has identified primary cancers with metastatic potential in solid tumours such as breast (van 't Veer et al 2002) and colon cancer (Bertucci et al 2004).

30

DESCRIPTION OF THE INVENTION

The present invention is based upon the identification and verification of cancer biomarkers, particularly prognostic biomarkers that identify potentially metastatic cancers (such as prostate and ER positive breast cancers).

5

The present inventors have identified a group of primary prostate cancers that are similar to metastatic disease at a molecular level. Primary tumour samples which clustered with metastatic samples define a group with poor (bad) prognosis. These tumours may be defined by down regulation of genes associated with cell adhesion, cell differentiation and cell development. These tumours may be defined by up regulation of androgen related processes and epithelial to mesenchymal transition (EMT). In contrast, benign and primary like benign tumours cluster to define a group with improved (good) prognosis. A series of biomarker/gene signatures that can be used to prospectively identify tumours within either subgroup (i.e. with metastatic or non-metastatic biology) have been generated and validated which have prognostic power. The signatures can thus be used to prospectively assess a tumour's progression, for example to determine whether a tumour is at increased likelihood of recurrence and/or metastatic development. The signatures also display excellent performance in heterogeneity studies as discussed further herein. In particular, a 70 gene signature is described herein. The gene signatures are also shown to be effective in other cancer types including ER positive breast cancer, thus suggesting that the underlying molecular biology may have applicability in defining potentially metastatic primary tumours.

Thus, in a first aspect the invention provides a method for characterising and/or prognosing cancer, such as prostate cancer or ER positive breast cancer, in a subject comprising:
determining the expression level of at least one gene from Table 1 in a sample from the subject wherein the determined expression level is used to provide a characterisation of and/or a prognosis for the cancer.

According to a further aspect of the invention there is provided a method for diagnosing (or identifying or characterizing) a cancer, such as prostate cancer or ER positive breast cancer, with an increased metastatic potential in a subject comprising:
determining the expression level of at least one gene from Table 1 in a sample from the subject wherein the determined expression level is used to identify whether a subject has a

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cancer, such as prostate cancer or ER positive breast cancer, with increased metastatic potential.

The invention also relates to a method for characterising and/or prognosing a cancer, such as prostate cancer or ER positive breast cancer in a subject comprising:

5 determining the expression level of at least one gene from Table 1 in a sample from the subject in order to identify the presence or absence of cells characteristic of an increased likelihood of recurrence and/or metastasis wherein the determined presence or absence of the cells is used to provide a characterisation of and/or a prognosis for the cancer, such as

10 prostate cancer or ER positive breast cancer.

In a further aspect, the present invention relates to a method for characterising and/or prognosing a cancer, such as prostate cancer or ER positive breast cancer in a subject comprising:

- 15 a) obtaining a sample from the subject/ in a sample obtained from the subject
- b) applying a nucleic acid probe that specifically hybridizes with the nucleotide sequence of at least one gene or full sequence or target sequence selected from Table 1 to the sample from the subject
- c) applying a detection agent that detects the nucleic acid probe-gene complex
- 20 d) using the detection agent to determine the level of the at least one gene or full sequence or target sequence
- d) wherein the determined level of the at least one gene (or full sequence or target sequence) is used to provide a characterisation of and/or a prognosis for the cancer, such as prostate cancer or ER positive breast cancer. Suitable probes and probesets are listed in
- 25 Table 1 and further details are provided in Table 1A.

In a further aspect, the present invention relates to a method for characterising and/or prognosing a cancer, such as prostate cancer or ER positive breast cancer in a subject comprising:

- 30 a) obtaining a sample from the subject/ in a sample obtained from the subject
- b) applying a set of nucleic acid primers that specifically hybridize with the nucleotide sequence of at least one gene or full sequence or target sequence selected from Table 1 to the sample from the subject
- c) specifically amplifying the nucleotide sequence using the set of nucleic acid primers

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d) detecting the amplification products using a specific detection agent to determine the level of the at least one gene or full sequence or target sequence
e) wherein the determined level of the at least one gene (or full sequence or target sequence) is used to provide a characterisation of and/or a prognosis for the cancer, such as prostate cancer or ER positive breast cancer. Suitable primers and primer pairs are listed in Table 1B.

The detection agent may comprise a label, such as a fluorescence label or fluorophore/quencher system attached to the nucleic acid probe and/or primer (as appropriate). Suitable systems and methodologies are known in the art and described herein.

The characterization, prognosis or diagnosis of the cancer, such as prostate cancer or ER positive breast cancer can also be used to guide treatment.

Accordingly, in a further aspect, the present invention relates to a method for selecting a treatment for a cancer, such as prostate cancer or ER positive breast cancer in a subject comprising:

(a) determining the expression level of at least one gene selected from Table 1 in a sample from the subject wherein the determined expression level is used to provide a characterisation of and/or a prognosis for the cancer, such as prostate cancer or ER positive breast cancer and

(b) selecting a treatment appropriate to the characterisation of and/or prognosis for the cancer, such as prostate cancer or ER positive breast cancer.

In yet a further aspect, the present invention relates to a method for selecting a treatment for a cancer, such as prostate cancer or ER positive breast cancer in a subject comprising:

(a) determining the expression level of at least one gene selected from Table 1 in a sample from the subject wherein the determined expression level is used to provide a characterisation of and/or a prognosis for the cancer, such as prostate cancer or ER positive breast cancer

(b) selecting a treatment appropriate to the characterisation of and/or prognosis for the cancer, such as prostate cancer or ER positive breast cancer and

(c) treating the subject with the selected treatment.

- 5 -

The invention also relates to a method of treating cancer, such as prostate cancer or ER positive breast cancer comprising administering a chemotherapeutic agent or radiotherapy, optionally extended radiotherapy, preferably extended-field radiotherapy, to a subject or
5 carrying out surgery on a subject wherein the subject is selected for treatment on the basis of a method as described herein.

In a further aspect, the present invention relates to a chemotherapeutic agent for use in treating a cancer, such as prostate cancer or ER positive breast cancer in a subject, wherein
10 the subject is selected for treatment on the basis of a method as described herein.

In yet a further aspect, the present invention relates to method of treating a cancer, such as prostate cancer or ER positive breast cancer comprising administering a chemotherapeutic agent or radiotherapy, optionally extended radiotherapy, preferably extended-field
15 radiotherapy to a subject or carrying out surgery on a subject wherein the subject has an increased expression level of at least one gene with a positive weight selected from Table 1 and/or wherein the subject has a decreased expression level of at least one gene with negative weight selected from Table 1.

20 The invention also relates to a chemotherapeutic agent for use in treating a cancer, such as prostate cancer or ER positive breast cancer in a subject, wherein the subject has an increased expression level of at least one gene with a positive weight selected from Table 1 and/or wherein the subject has a decreased expression level of at least one gene with a negative weight selected from Table 1.

25 In certain embodiments according to all relevant aspects of the invention the chemotherapeutic agent comprises, consists essentially of or consists of
a) an anti-hormone treatment, preferably bicalutamide and/or abiraterone
b) a cytotoxic agent
30 c) a biologic, preferably an antibody and/or a vaccine, more preferably Sipuleucel-T and/or
d) a targeted therapeutic agent

Suitable therapies and therapeutic agents are discussed in further detail herein. The treatment may comprise or be adjuvant therapy in some embodiments.

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According to all aspects of the invention the cancer may be a prostate cancer or ER positive breast cancer. Typically, the cancer is a primary tumor. In some embodiments, the prostate cancer may be a primary prostate cancer.

5

It is shown herein that the gene signatures may have particularly advantageous utility when combined with determination of other prognostic factors. Thus, all aspects of the invention may include other prognostic factors in the characterization, diagnosis or prognosis of the cancer. This may comprise generation of a combined risk score. This is particularly
10 applicable in the context of prostate cancer. Other prognostic factors include prostate specific antigen (PSA) levels and/or Gleason score. MRI scan results may also be taken into account. Thus, according to all aspects of the invention, characterization, prognosis or diagnosis may take into account other prognostic factors such as PSA levels and/or Gleason score. PSA is a well-known serum biomarker and may be used according to the invention, in
15 particular when measured pre-operatively. For example, a PSA value of 4-10 ng/ml may be considered "low risk". A PSA value of 10-20 ng/ml may be considered reflective of "medium risk". A PSA value of 20 ng/ml or more may be considered reflective of "high risk". High risk would correspond to poor prognosis and/or be indicative of aggressive disease. Levels of PSA may contribute towards a final characterization of the cancer in combination with the
20 measured expression levels. Medium risk PSA levels when combined with a positive or high signature score may indicate poor prognosis.

The Gleason system is used to grade prostate tumours with a score from 2 to 10, where a Gleason score of 10 indicates the most abnormalities. Cancers with a higher Gleason score
25 are more aggressive and have a worse prognosis. The system is based on how the prostate cancer tissue appears under a microscope and indicates how likely it is that a tumour will spread. A low Gleason score means the cancer tissue is similar to normal prostate tissue and the tumour is less likely to spread; a high Gleason score means the cancer tissue is very different from normal and the tumour is more likely to spread. Gleason scores are calculated
30 by adding the score of the most common grade (primary grade pattern) and the second most common grade (secondary grade pattern) of the cancer cells. Where more than two grades are observed the primary grade is added to the worst observable grade to arrive at the Gleason score. Grades are assigned using the 2005 (amended in 2009) International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of

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Prostatic Carcinoma. Thus, in some embodiments, a Gleason score of 7 or more contributes to a characterization of poor prognosis. In such embodiments, a Gleason score of less than 7 may contribute to a characterization of good prognosis. In some embodiments, a Gleason score of 7 is classified as an intermediate position between good and poor prognosis. Thus, a Gleason score of 8 or more is classified as poor prognosis. A Gleason score of less than 7 may contribute to a characterization of good prognosis. In some embodiments, a Gleason score of 7 thus contributes less to a characterization of poor prognosis than does a Gleason score of 8 or more, but more than a Gleason score of 6 or less. A Gleason score of 7 when combined with a positive or high signature score may indicate poor prognosis.

Where both Gleason score and PSA levels contribute to the characterization of the cancer, they may be weighted relative to one another. Typically, Gleason score is given greater significance than PSA levels. Thus, for example a Gleason score indicative of poor prognosis in combination with PSA levels associated with low risk, or good prognosis, may still result in a conclusion of poor prognosis (depending upon the measured expression levels of the gene or genes from Table 1). Similar considerations may apply to MRI results, which may be given greater weight than PSA levels in making the final characterization of the cancer.

The genes which may be included in suitable gene signatures and their identifying information are described and defined in further detail in Table 1 below. The genes may also be referred to, interchangeably, as biomarkers. Full sequences, against which suitable expression level determination assays may be designed, are also indicated in the table. Similarly, target sequences, against which suitable expression level determination assays may be designed, are also indicated in the table. Probe sequences interrogating the target sequences are also provided. Each sequence type is useful in the performance of the invention and form a separate aspect thereof.

Table 1

Signature Weight	Signature Bias	Weight (absolute)	Rank by weight	Gene symbol	Probesets	SEQ ID NO of sequence		
						Full	Target	Probe
-0.01089888	4.440873234	0.01089888	1	CAPN6	3Snip.7769-1124a_at	15	247	619-629

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-0.009631509	6.91258636 9	0.00963150 9	2	THBS4	PC3P.12363. C1_s_at	28	260	750-760
-0.008885735	4.38357232 7	0.00888573 5	3	PLP1	PC3P.17142. C1_s_at	64	296	1143- 1153
					PCADA.1273 8_s_at	168	400	2298- 2308
-0.008680747	6.74795697 8	0.00868074 7	4	MT1A	'PCRS3.3951 _at'	231	463	2994- 3003
					'PCRS3.3951 _x_at'	232	464	3004- 3014
-0.008278545	7.21524538 9	0.00827854 5	5	MIR20 5HG	'PC3P.1643.C 1_s_at'	54	286	1033- 1043
					'PC3P.1643.C 4-370a_s_at'	55	287	1044- 1054
					'PC3P.1643.C 6-335a_s_at'	56	288	1055- 1065
					'PCRS2.3147 _x_at'	227	459	2952- 2962
-0.007934619	4.23042262 2	0.00793461 9	6	SEMG1	'3Snip.972- 5a_s_at'	16	248	630-640
-0.007295796	4.29317279 4	0.00729579 6	7	RSPO3	'3Snip.465- 263a_s_at'	8	240	552
					'PCRS2.4412 _s_at'	228	460	2963- 2971
-0.007164357	6.52254777 4	0.00716435 7	8	ANO7	'PC3P.1358.C 1_at'	37	269	849-859
					'PC3P.1358.C 1- 1172a_s_at'	38	270	860-870
					'PC3SNG.174 2-20a_s_at'	125	357	1825- 1835
					'PCHP.560_s_ _at'	205	437	2715- 2724
					'PCHP.564_s_ _at'	206	438	2725- 2735
-0.007138975	7.62175813 8	0.00713897 5	9	PCP4	'PC3P.11557. C1_s_at'	23	255	696-706
-0.006922498	5.92831485	0.00692249 8	10	ANKRD 1	'PC3SNG.154 9-27a_s_at'	124	356	1814- 1824

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-0.006844539	4.574318807	0.006844539	11	MYBPC1	'PC3P.13654.C1_at'	39	271	871-881
					'PC3P.13654.C1_x_at'	40	272	882-892
					'PC3P.3003.C1_s_at'	74	306	1253-1263
					'PC3P.3003.C1_x_at'	75	307	1264-1274
					'PC3P.7685.C1_at'	101	333	1550-1560
					'PC3P.7685.C1_x_at'	102	334	1561-1571
					'PC3P.7685.C1-693a_s_at'	103	335	1572-1582
					'PC3SNGnh.274_x_at'	144	376	2034-2044
-0.00683545	6.756722063	0.00683545	12	MMP7	'PC3P.2763.C1_s_at'	71	303	1220-1230
-0.006830879	5.745461752	0.006830879	13	SERPINA3	'PC3P.104.CB1_s_at'	19	251	663-673
-0.006809804	5.977682143	0.006809804	14	SELE	'PCHP.1458_s_at'	199	431	2639-2649
-0.006402712	6.080493983	0.006402712	15	KRT5	'PC3P.10239.C1_s_at'	17	249	641-651
-0.006400452	6.497259991	0.006400452	16	LTF	'PC3P.167.C1_s_at'	61	293	1110-1120
					'PC3P.9581.C1_x_at'	118	350	1737-1747
					'PC3SNG.1467-30a_s_at'	123	355	1803-1813
-0.006380629	3.55996601	0.006380629	17	KIAA1210	'PC3P.12920.C1_x_at'	34	266	816-826
-0.006312212	8.063421249	0.006312212	18	TMEM158	'PCADA.9364_s_at'	177	409	2397-2407
-0.006271047	9.96082669	0.006271047	19	ZFP36	'PCHP.1147_s_at'	196	428	2606-2407
-0.006108115	6.954936015	0.006108115	20	FOSB	'PC3P.1906.C1_s_at'	65	297	1154-1164
					'PC3P.1906.C	66	298	1165-

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					1-568a_s_at'			1175
					'PCEM.1525_s_at'	192	424	2562-2572
					'PCPD.3244.C1_s_at'	217	449	2845-2853
-0.006101922	5.262341585	0.006101922	21	PCA3	'3Snip.6683-12a_x_at'	12	244	586-596
					'PC3P.11294.C1_s_at'	22	254	685-695
					'PC3P.13143.C1_at'	35	267	827-837
					'PC3P.13143.C1_x_at'	36	268	838-848
					'PC3P.2274.C1_s_at'	67	299	1176-1186
					'PC3P.5053.C1_s_at'	88	320	1407-1417
					'PC3P.5053.C1-490a_s_at'	89	321	1418-1428
					'PC3SNGnh.932_x_at'	163	395	2243-2253
-0.006059944	4.865791397	0.006059944	22	TRPM8	'PC3P.12013.C1_s_at'	24	256	707-717
					'PC3P.12591.C1_x_at'	29	261	761-771
					'PC3P.1261.C1_s_at'	30	262	772-782
					'PC3P.1507.C1_at'	45	277	934-944
					'PC3P.1507.C1_x_at'	46	278	945-955
					'PC3P.3670.C1_s_at'	78	310	1297-1307
					'PC3P.3670.C1-625a_s_at'	79	311	1308-1318
					'PC3P.3670.C2_s_at'	80	312	1319-1329
					'PC3SNGnh.1467_at'	137	369	1957-1967

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					'PC3SNGnh.1 467_x_at'	138	370	1968- 1978
					'PC3SNGnh.2 659_at'	143	375	2023- 2033
					'PC3SNGnh.3 350_at'	145	377	2045- 2055
					'PC3SNGnh.3 350_x_at'	146	378	2056- 2066
					'PC3SNGnh.5 454_at'	159	391	2199- 2209
0.006017344	4.71269280 3	0.00601734 4	23	PTTG1	'PC3P.16730. C1_x_at'	62	294	1121- 1131
					'PCHP.233_x _at'	201	433	2661- 2671
-0.005950381	4.98038094 1	0.00595038 1	24	N/A	'PC3P.12756. C1_x_at'	32	264	794-804
					'PC3P.5784.C 1_at'	96	328	1495- 1505
					'PC3P.5784.C 1_x_at'	97	329	1506- 1516
					'PC3P.8725.C 1_at'	112	344	1671- 1681
					'PC3P.8725.C 1_x_at'	113	345	1682- 1692
					'PC3P.8968.C 1_s_at'	114	346	1693- 1703
					PC3P.9903.C 1_at'	120	352	1759- 1769
					'PC3P.9903.C 1_x_at'	121	353	1770- 1780
					'PC3SNG.638 7-29a_x_at'	132	364	1902- 1912
					'PC3SNGnh.1 48_x_at'	141	373	2001- 2011
					'PC3SNGnh.3 957_at'	149	381	2089- 2099
					'PCADNP.36 40_at'	185	417	2485- 2495
					'PCADNP.36	186	418	2496-

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					40_x_at'			2506
					'PCPD.14169.C1_at'	210	442	2769-2779
					'PCPD.14169.C1_x_at'	211	443	2780-2790
					'PCPD.20005.C1_at'	213	445	2801-2811
					'PCPD.20005.C1_x_at'	214	446	2812-2822
					'PCPD.5961.C1_at'	221	453	2887-2897
-0.005837135	7.07390658	0.005837135	25	PAGE4	'PCHP.651_s_at'	208	440	2747-2757
-0.005684812	8.105295362	0.005684812	26	STEAP4	'3Snip.1577-444a_s_at'	1	233	465-475
					'PC3P.2452.C1_s_at'	68	300	1187-1197
					'PC3P.2452.C1-520a_s_at'	69	301	1198-1208
					'PC3SNG.3670-154a_s_at'	129	361	1869-1879
-0.00564663	7.59452596	0.00564663	27	TMEM178A	'PC3P.2736.C1_at'	70	302	1209-1219
-0.005597719	8.928977514	0.005597719	28	CXCL2	'PCHP.412_x_at'	203	435	2693-2703
-0.005593197	4.232781732	0.005593197	29	HS3ST3A1	'3Snip.377-232a_s_at'	6	238	520-530
					'PCADA.12209_at'	166	398	2276-2286
					'PCADA.12209_x_at'	167	399	2287-2297
-0.005581031	5.504276204	0.005581031	30	EYA1	'3Snip.546-712a_s_at'	10	242	564-574
					'PC3P.4095.C1_at'	82	314	1341-1351
					'PC3P.4095.C1_x_at'	83	315	1352-1362

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					'PC3SNGnh.4 553_s_at'	151	383	2111- 2121
					PCPD.3722.C 1_s_at'	218	450	2854- 2864
-0.005562783	3.92242079 4	0.00556278 3	31	RSPO2	'PC3P.16583. C1_at'	59	291	1088- 1098
					'PC3P.16583. C1_x_at'	60	292	1099- 1109
-0.005553136	5.91218617 1	0.00555313 6	32	PKP1	'3Snip.4433- 2675a_s_at'	7	239	531-541
					'PC3P.6847.C 1_s_at'	98	330	1517- 1527
-0.005522157	6.64003727 4	0.00552215 7	33	MUC6	'PC3P.15628. C1_s_at'	50	282	989-999
-0.005505761	4.51485504 9	0.00550576 1	34	PENK	'PCADNP.90 49_s_at'	190	422	2540- 2550
					'PCRS2.6477 _s_at'	229	461	2972- 2982
-0.005399899	6.82549092 4	0.00539989 9	35	DEFB1	'3Snip.1845- 41a_x_at'	2	234	476-486
					'3Snip.5724- 41a_s_at'	11	243	575-585
-0.005389518	4.64900363	0.00538951 8	36	SLC7A3	'PCADA.1045 9_at'	164	396	2254- 2264
-0.00535523	5.08738932	0.00535523	37	MIR57 8	'PC3SNGnh.4 158_at'	150	382	2100- 2110
-0.005263663	4.85871624 3	0.00526366 3	38	PI15	'3Snip.2873- 1277a_at'	4	236	498-508
					PC3P.7245.C 1_at'	99	331	1528- 1538
					PC3P.7245.C 1_x_at'	100	332	1539- 1549
					'PC3P.8311.C 1_x_at'	110	342	1649- 1659
					'PC3P.8311.C 1-482a_s_at'	111	343	1660- 1670
					'PCADNP.17 332_s_at'	182	414	2452- 2462
-0.005259309	6.06587761	0.00525930	39	UBXN1	'PCPD.39829	219	451	2865-

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	5	9		0-AS1	.C1_s_at'			2875
-0.00524875	4.17409431 2	0.00524875	40	PDK4	'PC3P.16300.C1_at'	52	284	1011-1021
					'PC3P.16300.C1_x_at'	53	285	1022-1032
					'PC3P.16894.C1_x_at'	63	295	1132-1142
					'PC3P.8159.C1_s_at'	108	340	1627-1637
					'PC3P.8159.C1-773a_s_at'	109	341	1638-1648
					'PC3SNGnh.4912_at'	152	384	2122-2132
					'PC3SNGnh.4912_x_at'	153	385	2133-2143
					'PC3SNGnh.5369_at'	157	389	2177-2187
					'PC3SNGnh.5369_x_at'	158	390	2188-2198
					'PCADNP.18913_s_at'	184	416	2474-2484
					'PCEM.2221_at'	194	426	2584-2594
					'PCPD.29484.C1_at'	216	448	2834-2844
-0.0052075	5.18357114 3	0.0052075	41	PHGR1	'3Snip.3288-5a_x_at'	5	237	509-519
-0.005194886	6.69186628 4	0.00519488 6	42	SERPIN E1	'3Snip.7067-10a_s_at'	13	245	597-607
					'3Snip.7068-570a_s_at'	14	246	608-618
					'PC3P.3933.C1_s_at'	81	313	1330-1340
					'PC3P.9147.C1_s_at'	115	347	1704-1714
					'PCADNP.4300_x_at'	187	419	2507-2517

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					'PCHP.1474_s_at'	200	432	2650-2660
-0.005146623	4.75232765 2	0.00514662 3	43	PDZRN 4	'PC3P.15181.C1_at'	47	279	956-966
					'PC3P.15181.C1_s_at'	48	280	967-977
					'PC3P.15181.C1_x_at'	49	281	978-988
					'PC3P.16541.C1_at'	50	290	1077-1087
-0.005105327	6.90054422	0.00510532 7	44	ZNF18 5	'PCHP.120_s_at'	198	430	2628-2638
-0.005054713	7.07837686 4	0.00505471 3	45	ADRA2 C	'PCADA.8850_s_at'	176	408	2385-2396
-0.0050184	8.19117750 1	0.0050184	46	AZGP1	'PC3P.122.CB1_x_at'	26	258	729-739
					'PC3P.122.CB2_at'	27	259	740-749
					'PC3SNG.1055-28a_x_at'	122	354	1792-1802
0.004965887	5.58133457	0.00496588 7	47	TK1	'PCHP.1153_s_at'	197	429	2617-2627
-0.004961473	4.82497632 5	0.00496147 3	48	POTEH	'PC3SNGnh.3389_at'	147	379	2067-2077
					'PC3SNGnh.3389_x_at'	148	380	2078-2088
					'PCPD.5859.C2_at'	220	452	2876-2886
					'PCRS.626_x_at'	224	456	2920-2930
0.004928774	3.91766850 1	0.00492877 4	49	KIF11	'PCADNP.16534_at'	180	412	2430-2440
					'PCADNP.16534_x_at'	181	413	2441-2451

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-0.004924383	4.960282713	0.004924383	50	CLDN1	'PC3P.2825.C1_at'	72	304	1231-1241
					'PC3P.2825.C1_x_at'	73	305	1242-1252
					'PC3SNGnh.7327_x_at'	162	394	2232-2242
					'PCADA.12072_at'	165	397	2265-2275
					'PCADA.7259_at'	172	404	2342-2352
					'PCADA.7259_x_at'	173	405	2353-2363
-0.004907676	10.53645223	0.004907676	51	MIR4530	'PCPD.1539.C1_s_at'	212	444	2791-2800
-0.004901224	8.497945251	0.004901224	52	MAFF	'PC3P.12787.C1_x_at'	33	265	805-815
					'PCADA.13348_at'	169	401	2309-2319
					'PCADA.13348_x_at'	170	402	2320-2330
-0.004861949	3.976333034	0.004861949	53	ZNF765	'PC3P.3163.C1_s_at'	76	308	1275-1285
					'PCRS.812_s_at'	225	457	2931-2941
0.00485589	6.503980715	0.00485589	54	CKS2	'PCHP.43_s_at'	204	436	2704-2714
-0.004855875	4.819327983	0.004855875	55	TCEAL7	'PCADA.8842_at'	174	406	2364-2373
0.004830634	4.629391793	0.004830634	56	PLIN1	'PC3P.12706.C1_s_at'	31	263	783-793
0.004772601	5.503752383	0.004772601	57	SIGLEC1	'PC3SNG.5215-18a_s_at'	131	363	1891-1901
-0.004772585	6.66459522	0.00477258	58	FAM15	'PCRS2.7477	230	462	2983-

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	4	5		0B	_s_at'			2993
-0.004771653	4.12917654 6	0.00477165 3	59	MFAP5	'3Snip.4760- 1950a_s_at'	9	241	553-563
					'PC3SNG.440 7-18a_s_at'	130	362	1880- 1890
-0.004761531	7.90126194 4	0.00476153 1	60	SFRP1	'PC3P.9317.C 1_s_at'	116	348	1715- 1725
					'PC3SNG.195 8- 2386a_s_at'	126	358	1836- 1846
-0.00471806	5.76267783 4	0.00471806	61	DUSP5	'PC3P.1626.C 1_s_at'	51	283	1000- 1010
					'PCPD.2281. C1_at'	215	447	2823- 2833
					'PCRS2.2880 _s_at'	226	458	2942- 2951
0.004675188	5.22345519 2	0.00467518 8	62	VAR52	'PC3P.4347.C 1_s_at'	84	316	1363- 1373
-0.004664227	5.23037674 7	0.00466422 7	63	ABCC4	'PC3P.3552.C 1_s_at'	77	309	1286- 1296
					'PC3P.4471.C 1_s_at'	85	317	1374- 1384
					'PC3P.4471.C 1-536a_s_at'	86	318	1385- 1395
					'PC3P.5711.C 1_at'	92	324	1451- 1461
					'PC3P.5711.C 1_s_at'	93	325	1462- 1472
					'PC3P.5711.C 2_at'	94	326	1473- 1483
					'PC3P.5711.C 2_x_at'	95	327	1484- 1494
					'PC3P.777.C1 _at'	104	336	1583- 1593
					'PC3P.777.C1 _x_at'	105	337	1594- 1564

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					'PC3P.9828.C1_s_at'	119	351	1748-1758
					'PC3SNG.704-22a_s_at'	134	366	1924-1934
					'PC3SNGnh.141_x_at'	136	368	1946-1946
					'PC3SNGnh.1473_at'	139	371	1979-1989
					'PC3SNGnh.1473_x_at'	140	372	1990-2000
					'PC3SNGnh.6624_x_at'	160	392	2210-2220
					'PC3SNGnh.6679_s_at'	161	393	2221-2231
					'PCADA.445_s_at'	171	403	2331-2341
					'PCADNP.1146_s_at'	178	410	2408-2418
					'PCADNP.12255_at'	179	411	2419-2429
					'PCPD.7116.C1_at'	222	454	2898-2908
					'PCPD.7116.C1_x_at'	223	455	2909-2919
-0.004622969	4.882708067	0.004622969	64	SH3BP4	'PC3P.12104.C1_at'	25	257	718-728
					'PC3P.14133.C1_at'	41	273	893-903
					'PC3P.14133.C1_x_at'	42	274	904-914
					'PC3SNGnh.1032_x_at'	135	367	1935-1945
					'PC3SNGnh.1675_x_at'	142	374	2012-2022
					'PC3SNGnh.4946_at'	154	386	2144-2154
					'PC3SNGnh.4946_x_at'	155	387	2155-2165
					'PC3SNGnh.5	156	388	2166-

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					297_x_at'			2176
					'PCADNP.6193_s_at'	189	421	2529-2539
-0.004573155	8.958411069	0.004573155	65	SORD	'PC3P.14629.C1_s_at'	44	276	926-933
					'PC3P.525.CB1_s_at'	90	322	1429-1439
					'PC3P.525.CB1-789a_s_at'	91	323	1440-1450
					'PC3P.9417.C1_s_at'	117	349	1726-1736
0.004522466	5.334198783	0.004522466	66	MTERF D1	'PC3P.14465.C1_s_at'	43	275	915-925
-0.004505906	4.65974831	0.004505906	67	DPP4	'3Snip.2321-634a_s_at'	3	235	487-497
					'PC3P.11025.C1_s_at'	21	253	674-684
					'PC3P.4974.C1_s_at'	87	319	1396-1406
					'PCADNP.9181_at'	191	423	2551-2661
					'PCEM.2151_at'	193	425	2573-2583
					'PCHP.235_s_at'	202	434	2672-2682
0.004502134	4.905312692	0.004502134	68	N/A	'PC3SNG.6626-95a_s_at'	133	365	1913-1923
-0.0044434	7.388071281	0.0044434	69	FAM3B	'PC3P.8122.C1_s_at'	106	338	1605-1615
					'PC3P.8122.C2_s_at'	107	339	1616-1626
					'PCADNP.5263_s_at'	188	420	2518-2528

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-0.00442472	10.22644129	0.00442472	70	KLK3	'PC3P.1038.C2_s_at'	18	250	652-662
					'PCADNP.18829_x_at'	183	415	2463-2473
					'PCEM.799_x_at'	195	427	2595-2605
					'PCHP.604_x_at'	207	439	2736-2746
					'PCHP.785_s_at'	209	441	2758-2768

Further details of the probesets can be found in Table 1A, including orientation information:

Table 1A – Probeset Information

Probeset ID	Orientation	NoPA	ENSEMBL gene no.	Gene Symbol	Entrez Gene ID	HGNC symbol acc no	Strand	Csome no
3Snip.1577-444a_s_at	Fully Exonic	11	ENSG00000127954	STEAP4	79689	21923	Reverse	7
3Snip.1845-41a_x_at	Fully Exonic	11	ENSG00000164825	DEFB1	1672	2766	Reverse	8
3Snip.2321-634a_s_at	Fully Exonic	11	ENSG00000197635	DPP4	1803	3009	Reverse	2
3Snip.2873-1277a_at	Fully Exonic	11	ENSG00000137558	PI15	51050	8946	Forward	8
3Snip.3288-5a_x_at	Fully Exonic	11	ENSG00000233041	PHGR1	644844	37226	Forward	15
3Snip.377-232a_s_at	Fully Exonic	11	ENSG00000153976	HS3ST3A1	9955	5196	Reverse	17
3Snip.4433-2675a_s_at	Fully Exonic	10	ENSG00000081277	PKP1	5317	9023	Forward	1
3Snip.465-263a_s_at	Fully Exonic	11	ENSG00000146374	RSPO3	84870	20866	Forward	6
3Snip.4760-1950a_s_at	Fully Exonic	11	ENSG00000197614	MFAP5	8076	29673	Reverse	12
3Snip.546-712a_s_at	Fully Exonic	11	ENSG00000104313	EYA1	2138	3519	Reverse	8
3Snip.5724-41a_s_at	Fully Exonic	10	ENSG00000164825	DEFB1	1672	2766	Reverse	8

3Snip.6683-12a_x_at	Fully Exonic	11	ENSG00000225937	PCA3	50652	8637	Forward	9
3Snip.7067-10a_s_at	Fully Exonic	11	ENSG00000106366	SERPINE1	5054	8583	Forward	7
3Snip.7068-570a_s_at	Fully Exonic	11	ENSG00000106366	SERPINE1	5054	8583	Forward	7
3Snip.7769-1124a_at	Fully Exonic	11	ENSG00000077274	CAPN6	827	1483	Reverse	X
3Snip.972-5a_s_at	Fully Exonic	11	ENSG00000124233	SEMG1	6406	10742	Forward	20
PC3P.10239.C1_s_at	Fully Exonic	11	ENSG00000186081	KRT5	3852	6442	Reverse	12
PC3P.1038.C2_s_at	Fully Exonic	11	ENSG00000142515	KLK3	354	6364	Forward	19
PC3P.104.CB1_s_at	Fully Exonic	11	ENSG00000196136	SERPINA3	12	16	Forward	14
PC3P.104.CB1_s_at	Fully Exonic	11	ENSG00000273259	N/A	12	NOVEL pc	Forward	14
PC3P.11025.C1_s_at	Fully Exonic	9	ENSG00000197635	DPP4	1803	3009	Reverse	2
PC3P.11294.C1_s_at	Fully Exonic	11	ENSG00000225937	PCA3	50652	8637	Forward	9
PC3P.11557.C1_s_at	Fully Exonic	11	ENSG00000183036	PCP4	5121	8742	Forward	21
PC3P.12013.C1_s_at	Fully Exonic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3P.12104.C1_at	Fully Exonic	11	ENSG00000130147	SH3BP4	23677	10826	Forward	2
PC3P.122.CB1_x_at	Fully Exonic	7	ENSG00000160862	AZGP1	563	910	Reverse	7
PC3P.122.CB2_at	Fully Exonic	10	ENSG00000160862	AZGP1	563	910	Reverse	7
PC3P.12363.C1_s_at	Fully Exonic	11	ENSG00000113296	THBS4	7060	11788	Forward	5
PC3P.12591.C1_x_at	Includes Intronic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3P.1261.C1_s_at	Fully Exonic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2

PC3P.12706 .C1_s_at	Fully Exonic	11	ENSG00000166819	PLIN1	5346	9076	Reverse	15
PC3P.12756 .C1_x_at	Includes Intronic	9	ENSG00000255240	N/A	28319 4	NOVEL as	Reverse	11
PC3P.12787 .C1_x_at	Fully Exonic	11	ENSG00000185022	MAFF	23764	6780	Forward	22
PC3P.12920 .C1_x_at	Fully Exonic	11	ENSG00000250423	KIAA12 10	57481	29218	Reverse	X
PC3P.13143 .C1_at	Includes Intronic	9	ENSG00000225937	PCA3	50652	8637	Forward	9
PC3P.13143 .C1_x_at	Includes Intronic	10	ENSG00000225937	PCA3	50652	8637	Forward	9
PC3P.1358. C1_at	Fully Exonic	11	ENSG00000146205	ANO7	50636	31677	Forward	2
PC3P.1358. C1- 1172a_s_at	Fully Exonic	11	ENSG00000146205	ANO7	50636	31677	Forward	2
PC3P.13654 .C1_at	Includes Intronic	10	ENSG00000196091	MYBPC1	4604	7549	Forward	12
PC3P.13654 .C1_x_at	Includes Intronic	9	ENSG00000196091	MYBPC1	4604	7549	Forward	12
PC3P.14133 .C1_at	Fully Exonic	11	ENSG00000130147	SH3BP4	23677	10826	Forward	2
PC3P.14133 .C1_x_at	Fully Exonic	10	ENSG00000130147	SH3BP4	23677	10826	Forward	2
PC3P.14465 .C1_s_at	Fully Exonic	10	ENSG00000156469	MTERFD 1	51001	24258	Reverse	8
PC3P.14629 .C1_s_at	Fully Exonic	8	ENSG00000140263	SORD	6652	11184	Forward	15
PC3P.1507. C1_at	Fully Exonic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3P.1507. C1_x_at	Fully Exonic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3P.15181 .C1_at	Fully Exonic	11	ENSG00000165966	PDZRN4	29951	30552	Forward	12
PC3P.15181 .C1_s_at	Fully Exonic	11	ENSG00000165966	PDZRN4	29951	30552	Forward	12
PC3P.15181 .C1_x_at	Fully Exonic	11	ENSG00000165966	PDZRN4	29951	30552	Forward	12

PC3P.15628. C1_s_at	Fully Exonic	11	ENSG00000184956	MUC6	4588	7517	Reverse	11
PC3P.1626. C1_s_at	Fully Exonic	11	ENSG00000138166	DUSP5	1847	3071	Forward	10
PC3P.16300. C1_at	Includes Intronic	10	ENSG00000004799	PDK4	5166	8812	Reverse	7
PC3P.16300. C1_x_at	Includes Intronic	10	ENSG00000004799	PDK4	5166	8812	Reverse	7
PC3P.1643. C1_s_at	Fully Exonic	11	ENSG00000230937	MIR205 HG	40698 8	43562	Forward	1
PC3P.1643. C4- 370a_s_at	Fully Exonic	11	ENSG00000230937	MIR205 HG	40698 8	43562	Forward	1
PC3P.1643. C6- 335a_s_at	Fully Exonic	9	ENSG00000230937	MIR205 HG	40698 8	43562	Forward	1
PC3P.16431. C1_at	Fully Exonic	9	ENSG00000196136	SERPIN A3	12	16	Forward	14
PC3P.16541. C1_at	Includes Intronic	11	ENSG00000165966	PDZRN4	29951	30552	Forward	12
PC3P.16583. C1_at	Fully Exonic	11	ENSG00000147655	RSP02	34041 9	28583	Reverse	8
PC3P.16583. C1_x_at	Fully Exonic	11	ENSG00000147655	RSP02	34041 9	28583	Reverse	8
PC3P.167.C 1_s_at	Fully Exonic	11	ENSG00000012223	LTF	4057	6720	Reverse	3
PC3P.16730. C1_x_at	Fully Exonic	8	ENSG00000164611	PTTG1	9232	9690	Forward	5
PC3P.16894. C1_x_at	Fully Exonic	11	ENSG00000004799	PDK4	5166	8812	Reverse	7
PC3P.17142. C1_s_at	Fully Exonic	11	ENSG00000123560	PLP1	5354	9086	Forward	X
PC3P.1906. C1_s_at	Fully Exonic	11	ENSG00000125740	FOSB	2354	3797	Forward	19
PC3P.1906. C1- 568a_s_at	Fully Exonic	11	ENSG00000125740	FOSB	2354	3797	Forward	19
PC3P.2274. C1_s_at	Fully Exonic	11	ENSG00000225937	PCA3	50652	8637	Forward	9

PC3P.2452. C1_s_at	Fully Exonic	11	ENSG00000127954	STEAP4	79689	21923	Reverse	7
PC3P.2452. C1- 520a_s_at	Fully Exonic	11	ENSG00000127954	STEAP4	79689	21923	Reverse	7
PC3P.2736. C1_at	Fully Exonic	9	ENSG00000152154	TMEM1 78A	13073 3	28517	Forward	2
PC3P.2763. C1_s_at	Fully Exonic	11	ENSG00000137673	MMP7	4316	7174	Reverse	11
PC3P.2825. C1_at	Fully Exonic	10	ENSG00000163347	CLDN1	9076	2032	Reverse	3
PC3P.2825. C1_x_at	Fully Exonic	10	ENSG00000163347	CLDN1	9076	2032	Reverse	3
PC3P.3003. C1_s_at	Fully Exonic	11	ENSG00000196091	MYBPC1	4604	7549	Forward	12
PC3P.3003. C1_x_at	Includes Intronic	11	ENSG00000196091	MYBPC1	4604	7549	Forward	12
PC3P.3163. C1_s_at	Fully Exonic	11	ENSG00000196417	ZNF765	91661	25092	Forward	19
PC3P.3552. C1_s_at	Includes Intronic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3P.3670. C1_s_at	Fully Exonic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3P.3670. C1- 625a_s_at	Fully Exonic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3P.3670. C2_s_at	Fully Exonic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3P.3933. C1_s_at	Fully Exonic	11	ENSG00000106366	SERPIN E1	5054	8583	Forward	7
PC3P.4095. C1_at	Fully Exonic	11	ENSG00000104313	EYA1	2138	3519	Reverse	8
PC3P.4095. C1_x_at	Fully Exonic	11	ENSG00000104313	EYA1	2138	3519	Reverse	8
PC3P.4347. C1_s_at	Fully Exonic	11	ENSG00000137411	VAR2	57176	21642	Forward	6
PC3P.4471. C1_s_at	Fully Exonic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13

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PC3P.4471. C1- 536a_s_at	Fully Exonic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3P.4974. C1_s_at	Fully Exonic	11	ENSG00000197635	DPP4	1803	3009	Reverse	2
PC3P.5053. C1_s_at	Fully Exonic	11	ENSG00000225937	PCA3	50652	8637	Forward	9
PC3P.5053. C1- 490a_s_at	Fully Exonic	11	ENSG00000225937	PCA3	50652	8637	Forward	9
PC3P.525.C B1_s_at	Fully Exonic	11	ENSG00000140263	SORD	6652	11184	Forward	15
PC3P.525.C B1- 789a_s_at	Fully Exonic	11	ENSG00000140263	SORD	6652	11184	Forward	15
PC3P.5711. C1_at	Includes Intronic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3P.5711. C1_s_at	Fully Exonic	10	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3P.5711. C2_at	Fully Exonic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3P.5711. C2_x_at	Fully Exonic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3P.5784. C1_at	Includes Intronic	8	ENSG00000255240	N/A	28319 4	NOVEL as	Reverse	11
PC3P.5784. C1_x_at	Includes Intronic	10	ENSG00000255240	N/A	28319 4	NOVEL as	Reverse	11
PC3P.6847. C1_s_at	Fully Exonic	11	ENSG00000081277	PKP1	5317	9023	Forward	1
PC3P.7245. C1_at	Fully Exonic	11	ENSG00000137558	PI15	51050	8946	Forward	8
PC3P.7245. C1_x_at	Fully Exonic	11	ENSG00000137558	PI15	51050	8946	Forward	8
PC3P.7685. C1_at	Fully Exonic	11	ENSG00000196091	MYBPC1	4604	7549	Forward	12
PC3P.7685. C1_x_at	Fully Exonic	11	ENSG00000196091	MYBPC1	4604	7549	Forward	12
PC3P.7685. C1- 693a_s_at	Fully Exonic	11	ENSG00000196091	MYBPC1	4604	7549	Forward	12

PC3P.777.C1_at	Includes Intronic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3P.777.C1_x_at	Includes Intronic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3P.8122.C1_s_at	Fully Exonic	11	ENSG00000183844	FAM3B	54097	1253	Forward	21
PC3P.8122.C2_s_at	Fully Exonic	11	ENSG00000183844	FAM3B	54097	1253	Forward	21
PC3P.8159.C1_s_at	Fully Exonic	11	ENSG00000004799	PDK4	5166	8812	Reverse	7
PC3P.8159.C1-773a_s_at	Fully Exonic	11	ENSG00000004799	PDK4	5166	8812	Reverse	7
PC3P.8311.C1_x_at	Fully Exonic	6	ENSG00000137558	PI15	51050	8946	Forward	8
PC3P.8311.C1-482a_s_at	Fully Exonic	11	ENSG00000137558	PI15	51050	8946	Forward	8
PC3P.8725.C1_at	Includes Intronic	9	ENSG00000255240	N/A	283194	NOVEL as	Reverse	11
PC3P.8725.C1_x_at	Includes Intronic	7	ENSG00000255240	N/A	283194	NOVEL as	Reverse	11
PC3P.8968.C1_s_at	Includes Intronic	11	ENSG00000255240	N/A	283194	NOVEL as	Reverse	11
PC3P.9147.C1_s_at	Fully Exonic	11	ENSG00000106366	SERPINE1	5054	8583	Forward	7
PC3P.9317.C1_s_at	Fully Exonic	11	ENSG00000104332	SFRP1	6422	10776	Reverse	8
PC3P.9417.C1_s_at	Fully Exonic	11	ENSG00000140263	SORD	6652	11184	Forward	15
PC3P.9581.C1_x_at	Fully Exonic	9	ENSG00000012223	LTF	4057	6720	Reverse	3
PC3P.9828.C1_s_at	Fully Exonic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3P.9903.C1_at	Fully Exonic	11	ENSG00000255240	N/A	283194	NOVEL as	Reverse	11
PC3P.9903.C1_x_at	Fully Exonic	11	ENSG00000255240	N/A	283194	NOVEL as	Reverse	11
PC3SNG.1055-28a_x_at	Fully Exonic	11	ENSG00000160862	AZGP1	563	910	Reverse	7

PC3SNG.146 7-30a_s_at	Fully Exonic	11	ENSG00000012223	LTF	4057	6720	Reverse	3
PC3SNG.154 9-27a_s_at	Fully Exonic	11	ENSG000000148677	ANKRD1	27063	15819	Reverse	10
PC3SNG.174 2-20a_s_at	Fully Exonic	11	ENSG000000146205	ANO7	50636	31677	Forward	2
PC3SNG.195 8- 2386a_s_at	Fully Exonic	11	ENSG000000104332	SFRP1	6422	10776	Reverse	8
PC3SNG.366 9-40a_s_at	Fully Exonic	11	ENSG000000196136	SERPIN A3	12	16	Forward	14
PC3SNG.366 9-40a_s_at	Fully Exonic	11	ENSG000000273259	N/A	12	NOVEL pc	Forward	14
PC3SNG.367 0-154a_s_at	Fully Exonic	11	ENSG000000127954	STEAP4	79689	21923	Reverse	7
PC3SNG.440 7-18a_s_at	Fully Exonic	11	ENSG000000197614	MFAP5	8076	29673	Reverse	12
PC3SNG.521 5-18a_s_at	Fully Exonic	11	ENSG000000088827	SIGLEC1	6614	11127	Reverse	20
PC3SNG.638 7-29a_x_at	Includes Intronic	11	ENSG000000255240	N/A	28319 4	NOVEL as	Reverse	11
PC3SNG.662 6-95a_s_at	Fully Exonic	11	ENSG000000215458	N/A	28483 7	NOVEL as	Reverse	21
PC3SNG.704 -22a_s_at	Fully Exonic	11	ENSG000000125257	ABCC4	10257	55	Reverse	13
PC3SNGnh.1 032_x_at	Fully Exonic	6	ENSG000000130147	SH3BP4	23677	10826	Forward	2
PC3SNGnh.1 41_x_at	Includes Intronic	11	ENSG000000125257	ABCC4	10257	55	Reverse	13
PC3SNGnh.1 467_at	Includes Intronic	11	ENSG000000144481	TRPM8	79054	17961	Forward	2
PC3SNGnh.1 467_x_at	Includes Intronic	10	ENSG000000144481	TRPM8	79054	17961	Forward	2
PC3SNGnh.1 473_at	Includes Intronic	7	ENSG000000125257	ABCC4	10257	55	Reverse	13
PC3SNGnh.1 473_x_at	Includes Intronic	6	ENSG000000125257	ABCC4	10257	55	Reverse	13
PC3SNGnh.1 48_x_at	Includes Intronic	9	ENSG000000255240	N/A	28319 4	NOVEL as	Reverse	11

PC3SNGnh.1 675_x_at	Fully Exonic	11	ENSG00000130147	SH3BP4	23677	10826	Forward	2
PC3SNGnh.2 659_at	Includes Intronic	8	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3SNGnh.2 74_x_at	Includes Intronic	11	ENSG00000196091	MYBPC1	4604	7549	Forward	12
PC3SNGnh.3 350_at	Includes Intronic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3SNGnh.3 350_x_at	Includes Intronic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3SNGnh.3 389_at	Includes Intronic	11	ENSG00000198062	POTEH	23784	133	Reverse	22
PC3SNGnh.3 389_x_at	Includes Intronic	11	ENSG00000198062	POTEH	23784	133	Reverse	22
PC3SNGnh.3 957_at	Includes Intronic	11	ENSG00000255240	N/A	28319 4	NOVEL as	Reverse	11
PC3SNGnh.4 158_at	Fully Exonic	10	ENSG00000207559	MIR578	69316 3	32834	Forward	4
PC3SNGnh.4 553_s_at	Includes Intronic	11	ENSG00000104313	EYA1	2138	3519	Reverse	8
PC3SNGnh.4 912_at	Includes Intronic	11	ENSG00000004799	PDK4	5166	8812	Reverse	7
PC3SNGnh.4 912_x_at	Includes Intronic	11	ENSG00000004799	PDK4	5166	8812	Reverse	7
PC3SNGnh.4 946_at	Includes Intronic	9	ENSG00000130147	SH3BP4	23677	10826	Forward	2
PC3SNGnh.4 946_x_at	Includes Intronic	10	ENSG00000130147	SH3BP4	23677	10826	Forward	2
PC3SNGnh.5 297_x_at	Fully Exonic	6	ENSG00000130147	SH3BP4	23677	10826	Forward	2
PC3SNGnh.5 369_at	Includes Intronic	11	ENSG00000004799	PDK4	5166	8812	Reverse	7
PC3SNGnh.5 369_x_at	Includes Intronic	8	ENSG00000004799	PDK4	5166	8812	Reverse	7
PC3SNGnh.5 454_at	Includes Intronic	11	ENSG00000144481	TRPM8	79054	17961	Forward	2
PC3SNGnh.6 624_x_at	Includes Intronic	10	ENSG00000125257	ABCC4	10257	55	Reverse	13

PC3SNGnh.6 679_s_at	Includes Intronic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PC3SNGnh.7 327_x_at	Includes Intronic	11	ENSG00000163347	CLDN1	9076	2032	Reverse	3
PC3SNGnh.9 32_x_at	Includes Intronic	11	ENSG00000225937	PCA3	50652	8637	Forward	9
PCADA.104 59_at	Fully Exonic	11	ENSG00000165349	SLC7A3	84889	11061	Reverse	X
PCADA.120 72_at	Fully Exonic	10	ENSG00000163347	CLDN1	9076	2032	Reverse	3
PCADA.122 09_at	Fully Exonic	11	ENSG00000153976	HS3ST3 A1	9955	5196	Reverse	17
PCADA.122 09_x_at	Fully Exonic	11	ENSG00000153976	HS3ST3 A1	9955	5196	Reverse	17
PCADA.127 38_s_at	Fully Exonic	11	ENSG00000123560	PLP1	5354	9086	Forward	X
PCADA.133 48_at	Fully Exonic	11	ENSG00000185022	MAFF	23764	6780	Forward	22
PCADA.133 48_x_at	Fully Exonic	11	ENSG00000185022	MAFF	23764	6780	Forward	22
PCADA.445_ s_at	Fully Exonic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PCADA.725 9_at	Includes Intronic	11	ENSG00000163347	CLDN1	9076	2032	Reverse	3
PCADA.725 9_x_at	Includes Intronic	11	ENSG00000163347	CLDN1	9076	2032	Reverse	3
PCADA.884 2_at	Fully Exonic	11	ENSG00000182916	TCEAL7	56849	28336	Forward	X
PCADA.884 2_x_at	Fully Exonic	11	ENSG00000182916	TCEAL7	56849	28336	Forward	X
PCADA.885 0_s_at	Fully Exonic	11	ENSG00000184160	ADRA2C	152	283	Forward	4
PCADA.936 4_s_at	Fully Exonic	11	ENSG00000249992	TMEM1 58	25907	30293	Reverse	3
PCADNP.11 46_s_at	Fully Exonic	9	ENSG00000125257	ABCC4	10257	55	Reverse	13
PCADNP.12 255_at	Includes Intronic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13

PCADNP.16 534_at	Fully Exonic	11	ENSG00000138160	KIF11	3832	6388	Forward	10
PCADNP.16 534_x_at	Fully Exonic	11	ENSG00000138160	KIF11	3832	6388	Forward	10
PCADNP.17 332_s_at	Fully Exonic	11	ENSG00000137558	PI15	51050	8946	Forward	8
PCADNP.18 829_x_at	Fully Exonic	11	ENSG00000142515	KLK3	354	6364	Forward	19
PCADNP.18 913_s_at	Fully Exonic	11	ENSG00000004799	PDK4	5166	8812	Reverse	7
PCADNP.36 40_at	Fully Exonic	11	ENSG00000255240	N/A	28319 4	NOVEL as	Reverse	11
PCADNP.36 40_x_at	Fully Exonic	11	ENSG00000255240	N/A	28319 4	NOVEL as	Reverse	11
PCADNP.43 00_x_at	Includes Intronic	11	ENSG00000106366	SERPIN E1	5054	8583	Forward	7
PCADNP.52 63_s_at	Fully Exonic	11	ENSG00000183844	FAM3B	54097	1253	Forward	21
PCADNP.61 93_s_at	Fully Exonic	11	ENSG00000130147	SH3BP4	23677	10826	Forward	2
PCADNP.90 49_s_at	Fully Exonic	11	ENSG00000181195	PENK	5179	8831	Reverse	8
PCADNP.91 81_at	Includes Intronic	10	ENSG00000197635	DPP4	1803	3009	Reverse	2
PCEM.1525_ s_at	Fully Exonic	11	ENSG00000125740	FOSB	2354	3797	Forward	19
PCEM.2151_ at	Includes Intronic	11	ENSG00000197635	DPP4	1803	3009	Reverse	2
PCEM.2221_ at	Fully Exonic	11	ENSG00000004799	PDK4	5166	8812	Reverse	7
PCEM.799_x _at	Fully Exonic	6	ENSG00000142515	KLK3	354	6364	Forward	19
PCHP.1147_ s_at	Fully Exonic	11	ENSG00000128016	ZFP36	7538	12862	Forward	19
PCHP.1153_ s_at	Fully Exonic	11	ENSG00000167900	TK1	7083	11830	Reverse	17
PCHP.120_s _at	Fully Exonic	11	ENSG00000147394	ZNF185	7739	12976	Forward	X

PCHP.1458_s_at	Fully Exonic	11	ENSG00000007908	SELE	6401	10718	Reverse	1
PCHP.1474_s_at	Fully Exonic	11	ENSG00000106366	SERPINE1	5054	8583	Forward	7
PCHP.233_x_at	Fully Exonic	7	ENSG00000164611	PTTG1	9232	9690	Forward	5
PCHP.235_s_at	Fully Exonic	11	ENSG00000197635	DPP4	1803	3009	Reverse	2
PCHP.412_x_at	Fully Exonic	11	ENSG00000081041	CXCL2	2920	4603	Reverse	4
PCHP.43_s_at	Fully Exonic	11	ENSG00000123975	CKS2	1164	2000	Forward	9
PCHP.560_s_at	Fully Exonic	10	ENSG00000146205	ANO7	50636	31677	Forward	2
PCHP.564_s_at	Fully Exonic	11	ENSG00000146205	ANO7	50636	31677	Forward	2
PCHP.604_x_at	Fully Exonic	11	ENSG00000142515	KLK3	354	6364	Forward	19
PCHP.651_s_at	Fully Exonic	11	ENSG00000101951	PAGE4	9506	4108	Forward	X
PCHP.785_s_at	Fully Exonic	11	ENSG00000142515	KLK3	354	6364	Forward	19
PCPD.14169.C1_at	Includes Intronic	11	ENSG00000255240	N/A	283194	NOVEL as	Reverse	11
PCPD.14169.C1_x_at	Includes Intronic	11	ENSG00000255240	N/A	283194	NOVEL as	Reverse	11
PCPD.1539.C1_s_at	Fully Exonic	11	ENSG00000266559	MIR4530	100616163	41764	Reverse	19
PCPD.20005.C1_at	Includes Intronic	11	ENSG00000255240	N/A	283194	NOVEL as	Reverse	11
PCPD.20005.C1_x_at	Includes Intronic	9	ENSG00000255240	N/A	283194	NOVEL as	Reverse	11
PCPD.2281.C1_at	Includes Intronic	6	ENSG00000138166	DUSP5	1847	3071	Forward	10
PCPD.29484.C1_at	Fully Exonic	11	ENSG00000004799	PDK4	5166	8812	Reverse	7
PCPD.3244.C1_s_at	Fully Exonic	11	ENSG00000125740	FOSB	2354	3797	Forward	19

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PCPD.3722. C1_s_at	Fully Exonic	10	ENSG00000104313	EYA1	2138	3519	Reverse	8
PCPD.39829. C1_s_at	Fully Exonic	11	ENSG00000225986	UBXN10 -AS1	10192 8017	41141	Reverse	1
PCPD.5859. C2_at	Includes Intronic	11	ENSG00000198062	POTEH	23784	133	Reverse	22
PCPD.5961. C1_at	Includes Intronic	9	ENSG00000255240	N/A	28319 4	NOVEL as	Reverse	11
PCPD.7116. C1_at	Includes Intronic	11	ENSG00000125257	ABCC4	10257	55	Reverse	13
PCPD.7116. C1_x_at	Includes Intronic	10	ENSG00000125257	ABCC4	10257	55	Reverse	13
PCRS.626_x _at	Fully Exonic	11	ENSG00000198062	POTEH	23784	133	Reverse	22
PCRS.812_s _at	Fully Exonic	11	ENSG00000196417	ZNF765	91661	25092	Forward	19
PCRS2.2880 _s_at	Fully Exonic	10	ENSG00000138166	DUSP5	1847	3071	Forward	10
PCRS2.3147 _x_at	Fully Exonic	8	ENSG00000230937	MIR205 HG	40698 8	43562	Forward	1
PCRS2.4412 _s_at	Fully Exonic	11	ENSG00000146374	RSPO3	84870	20866	Forward	6
PCRS2.6477 _s_at	Fully Exonic	11	ENSG00000181195	PENK	5179	8831	Reverse	8
PCRS2.7477 _s_at	Fully Exonic	11	ENSG00000189292	FAM150 B	28501 6	27683	Reverse	2
PCRS3.3951 _at	Fully Exonic	8	ENSG00000205362	MT1A	4489	7393	Forward	16

NoPA – Number of probes aligned

Csome no – Chromosome number

NOVEL pc – novel protein coding (clone based vega gene)

NOVEL as – novel antisense (clone based vega gene)

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Table 1 lists the sequence identifiers for the full sequences against which gene expression assays may be targeted, more specific target sequences and probes/probesets which hybridize to those target sequences. Suitable primers and/or probes may be designed using known methods to determine gene expression based on the deposited gene sequences, the full sequences and target sequences specified herein. Furthermore, specific nucleic acid

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amplification assays (e.g. PCR, such as qPCR) have also been designed that permit reliable determination of gene expression levels for the genes in table 1. These assays are summarized in Table 1B. The assay target sequence and primers and primer pairs form separate aspects of the invention. For two of the targets, MIR578 and MIR4530, due to the short length of the target sequences, the approach taken by the inventors was not applicable to generate an amplification assay. For those targets, commercial assays are available and the sequences of the primers are provided below. For MIR578, the Life Technologies 4426961 Origene HP300490 assay may be employed. The forward and reverse primers are as follows:

CTTCTTGCTCTAGGAT (SEQ ID NO: 3151)
GAACATGTCTGCGTATCTC (SEQ ID NO: 3152)

For MIR4530, the Life Technologies 4427012 Origene HP301022 assay may be employed.

The forward and reverse primers are as follows:

CCCAGCAGGACGGGAGC (SEQ ID NO: 3153)
GAACATGTCTGCGTATCTC (SEQ ID NO: 3154) seems to be same as above

These specific primers, while useful in performing the methods of the invention, are thus not specifically claimed *per se* as forming part of the invention.

Table 1B – PCR assays designed for each of 70 genes in the signature

Design Template used (Entrez Gene ID)	GeneBank ID	Gene Symbol	Assay ID	Exon spanning	Forward Primer ID	Forward primer SEQ ID NO	Forward Primer ABI TM	Reverse Primer ID	Reverse primer SEQ ID NO	Reverse Primer ABI TM
827	NM_014289.3	CAPN6	CAPN6_A1	Yes	CAPN6_F1	3015	62.30	CAPN6_R1	3083	60.78
7060	NM_001306212.1	THBS4	THBS4_A1	Yes	THBS4_F1	3016	63.34	THBS4_R1	3084	67.66
5354	NM_000533.4	PLP1	PLP_A1	Yes	PLP1_F1	3017	59.72	PLP1_R1	3085	63.75
4489	NM_005946.2	MT1A	MT1A_A1	Yes	MT1A_F1	3018	65.41	MT1A_R1	3086	63.59
406988	NR_029622.1	MIR205HG	MIR205HG_A1	Yes	MIR205HG_F1	3019	63.02	MIR205HG_R1	3087	61.98
6406	NM_003007.3	SEMG1	SEMG1_A1	Yes	SEMG1_F1	3020	63.49	SEMG1_R1	3088	63.59
84870	NM_032784.4	RSP03	RSP03_A1	Yes	RSP03_F1	3021	61.24	RSP03_R1	3089	63.13
50636	NM_001001666.3	ANO7	ANO7_A1	Yes	ANO7_F1	3022	62.34	ANO7_R1	3090	60.93
5121	NM_006198.2	PCP4	PCP4_A1	Yes	PCP4_F1	3023	60.53	PCP4_R1	3091	61.70
27063	NM_014391.2	ANKRD1	ANKRD1_A1	Yes	ANKRD1_F1	3024	64.90	ANKRD1_R1	3092	65.11
4604	NM_001254718.1	MYBPC1	MYBPC1_A1	Yes	MYBPC1_F1	3025	62.31	MYBPC1_R1	3093	62.59
4316	NM_002423.3	MMP7	MMP7_A1	Yes	MMP7_F1	3026	53.80	MMP7_R1	3094	48.86
12	NM_001085.4	SERPINA3	SERPINA3_A1	Yes	SERPINA3_F1	3027	60.39	SERPINA3_R1	3095	62.07
6401	NM_000450.2	SELE	SELE_A1	Yes	SELE_F1	3028	63.62	SELE_R1	3096	62.56

3852	NM_000424.3	KRT5	KRT5_A1	Yes	KRT5_F1	3029	63.40	KRT5_R1	3097	62.30
4057	NM_001199149.1	LTF	LTF_A1	Yes	LTF_F1	3030	62.75	LTF_R1	3098	64.08
57481	NM_020721.1	KIAA1210	KIAA1210_A1	Yes	KIAA1210_F1	3031	60.98	KIAA1210_R1	3099	62.19
25907	NM_015444.2	TMEM158	TMEM158_A1	Yes	TMEM158_F1	3032	58.44	TMEM158_R1	3100	62.20
7538	NM_003407.3	ZFP36	ZFP36_A1	Yes	ZFP36_F1	3033	63.26	ZFP36_R1	3101	35.37
2354	NM_001114171.1	FOSB	FOSB_A1	Yes	FOSB_F1	3034	61.04	FOSB_R1	3102	62.16
50652	NR_015342.1	PCSA3	PCSA3_A1	Yes	PCSA3_F1	3035	62.83	PCSA3_R1	3103	61.36
79054	NM_024080.4	TRPM8	TRPM8_A1	Yes	TRPM8_F1	3036	61.89	TRPM8_R1	3104	63.81
9232	NM_001282382.1	PTTG1	PTTG1_A1	No	PTTG1_F1	3037	60.97	PTTG1_R1	3105	62.25
283194	NR_033853.2	LOC283194	LOC283194_A1	Yes	LOC283194_F1	3038	62.83	LOC283194_R1	3106	61.36
9506	NM_007003.3	PAGE4	PAGE4_A1	Yes	PAGE4_F1	3039	61.09	PAGE4_R1	3107	61.89
79689	NM_001205315.1	STEAP4	STEAP4_A1	Yes	STEAP4_F1	3040	64.22	STEAP4_R1	3108	59.86
130733	NM_001167959.1	TMEM178A	TMEM178A_A1	No	TMEM178A_F1	3041	70.52	TMEM178A_R1	3109	59.86
2920	NM_002089.3	CXCL2	CXCL2_A1	Yes	CXCL2_F1	3042	62.60	CXCL2_R1	3110	64.83
9955	NM_006042.2	HS3ST3A1	HS3ST3A1_A1	Yes	HS3ST3A1_F1	3043	61.52	HS3ST3A1_R1	3111	62.80
2138	NM_000503.5	EYA1	EYA1_A1	Yes	EYA1_F1	3044	32.20	EYA1_R1	3112	60.78
340419	NM_001282863.1	RSPO2	RSPO2_A1	Yes	RSPO2_F1	3045	64.91	RSPO2_R1	3113	63.38
5317	NM_000299.3	PKP1	PKP1_A1	Yes	PKP1_F1	3046	60.55	PKP1_R1	3114	63.39
4588	NM_005961.2	MUC6	MUC6_A1	Yes	MUC6_F1	3047	58.46	MUC6_R1	3115	62.58
5179	~~NM_001135690.1~~	PENK	PENK_A1	Yes	PENK_F1	3048	59	PENK_R1	3116	58
1672	NM_005218.3	DEFB1	DEFB1	Yes	DEFB1_F1	3049	62.3	DEFB1_R1	3117	62.1
84889	NM_001048164.2	SLC7A3	SLC7A3_A1	YES	SLC7A3	3050	60	SLC7A3_R1	3118	59
693163	NR_030304.1	MIR578	MIR578_A1	No	MIR578_F1	N/A	N/A	MIR578_R1	N/A	N/A

51050	NM_015886.3	PI15	PI15_A1	Yes	PI15_F1	3051	61.9	PI15_R1	3119	62.1
101928017	NR_110078.1	UBXB10-AS1	UBXB10-AS1_A1	Yes	UBXB10-AS1_F1	3052	61.55	UBXB10-AS1_R1	3120	61.42
5166	NM_002612.3	PK4	PK4_A1	Yes	PK4_F1	3053	62.00	PK4_R1	3121	61.90
644844	NM_001145643.1	PHGR1	PHGR1_A1	Yes	PHGR1_F1	3054	60.00	PHGR1_R1	3122	59.00
5054	NM_000602.4	SERPINE1	SERPINE1_A1	Yes	SERPINE1_F1	3055	59.00	SERPINE1_R1	3123	59.00
29951	NM_001164595.1	PDZRN4	PDZRN4_A1	Yes	PDZRN4_F1	3056	62	PDZRN4_R1	3124	62.6
7739	NM_001178106.1	ZNF185	ZNF185_A1	Yes	ZNF185_F1	3057	63.92	ZNF185_R1	3125	65.09
152	NM_000683.3	ADRA2C	ADRA2C_A1	No	ADRA2C_F1	3058	61.8	ADRA2C_R1	3126	61.4
563	NM_001185.3	AZGP1	AZGP1_A1	Yes	AZGP1_F1	3059	59.00	AZGP1_R1	3127	59.00
7083	NM_003258.4	TK1	TK1_A1	Yes	TK1_F1	3060	61.8	TK1_R1	3128	61.9
23784	NM_001136213.1	POTEH	POTEH_A1	Yes	POTEH_F1	3061	62.4	POTEH_R1	3129	62
3832	NM_004523.3	KIF11	KIF11_A1	Yes	KIF11_F1	3062	60.00	KIF11_R1	3130	60.00
9076	NM_021101.4	CLDN1	CLDN1_A1	Yes	CLDN1_F1	3063	60.00	CLDN1_R1	3131	59.00
100616163	NR_039755.1	MIR4530	MIR4530_A1	No	MIR4530_F1	N/A	N/A	MIR4530_R1	N/A	N/A
23764	NM_001161572.1	MAFF	MAFF_A1	Yes	MAFF_F1	3064	61.7	MAFF_R1	3132	62.3
91661	NM_001040185.1	ZNF765	ZNF765_A1	Yes	ZNF765_F1	3065	62.1	ZNF765_R1	3133	61.9
1164	NM_001827.2	CKS2	CKS2_A1	Yes	CKS2_F1	3066	59.00	CKS2_R1	3134	59.00
56849	NM_152278.3	TCEAL7	TCEAL7_A1	Yes	TCEAL7_F1	3067	59.00	TCEAL7_R1	3135	60.00
5346	NM_001145311.1	PLIN1	PLIN1_A1	Yes	PLIN1_F1	3068	62.2	PLIN1_R1	3136	62.4
6614	NM_023068.3	SIGLEC1	SIGLEC1_A1	Yes	SIGLEC1_F1	3069	59.00	SIGLEC1_R1	3137	60.00
285016	NM_001002919.2	FAM150B	FAM150B_A1	Yes	FAM150B_F1	3070	60.00	FAM150B_R1	3138	59.00
8076	NM_001297709.1	MFAP5	MFAP5_A1	Yes	MFAP5_F1	3071	61.7	MFAP5_R1	3139	62.2

6422	NM_003012.4	SFRP1	SFRP1_A1	Yes	SFRP1_F1	3072	62	SFRP1_R1	3140	62.1
1847	NM_004419.3	DUSP5	DUSP5_A1	Yes	DUSP5_F1	3073	61.9	DUSP5_R1	3141	61.7
57176	NM_001167733.2	VARS2	VARS2_A1	Yes	VARS2_F1	3074	62.1	VARS2_R1	3142	61.8
10257	NM_001105515.2	ABCC4	ABCC4_A1	Yes	ABCC4_F1	3075	60.00	ABCC4_R1	3143	60.00
23677	NM_014521.2	SH3BP4	SH3BP4_A1	Yes	SH3BP4_F1	3076	58.00	SH3BP4_R1	3144	60.00
6652	NM_003104.5	SORD	SORD_A1	Yes	SORD_F1	3077	60.00	SORD_R1	3145	59.00
51001	NM_001286643.1	MTERFD1	MTERFD1_A1	Yes	MTERFD1_F1	3078	59.00	MTERFD1_R1	3146	60.00
1803	XM_005246371.2	DPP4	DPP4_A1	Yes	DPP4_F1	3079	60.00	DPP4_R1	3147	59.00
284837	NR_026961.1	AATBC	AATBC_A1	Yes	AATBC_F1	3080	61.99	AATBC_R1	3148	62.42
54097	NM_058186.3	FAM3B	FAM3B_A1	Yes	FAM3B_F1	3081	61.8	FAM3B_R1	3149	62.2
354	NM_001030047.1	KLK3	KLK3_A1	Yes	KLK3_F1	3082	59.00	KLK3_R1	3150	59.00

It should be noted that the complement of each sequence described herein may be employed as appropriate (e.g. for designing hybridizing probes and/or primers, including primer pairs).

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In certain embodiments the expression level of at least 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, 67, 68, 69 or 70 of the genes in table 1 is determined. Some analysis reported herein indicates that applying a signature comprising the measured expression levels of 7 or 12 genes can provide acceptable performance. Thus, in some embodiments, the minimum number of genes in the gene signature is 12. They can be any 7 or 12 genes from the 70 genes.

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15 For the avoidance of doubt, additional genes (outside of the 70 genes) can be included in the signatures as would be readily appreciated by one skilled in the art. As is shown in figures 2 to 4, larger gene signatures are also potentially suitable.

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In some embodiments, a signature score is derived from the measured expression levels of the 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, 67, 68, 69 or 70 genes in table 1. Generation of such signature scores is described herein. The signature score may rely upon the weightings attributed to each gene as listed in Table 1, for the 70 gene signature. The weightings would, of course, need to be recalculated where a signature of different composition was utilized, for example including fewer than the total 70 gene signature. Similar considerations apply to the bias and constant offset values, as discussed below.

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Gene signatures may be formulated in rank order in some embodiments, for example a 10 gene signature could be formed from the first 10 ranked genes listed in Table 1. However, the rankings are based on performance in the context of the 70 gene signature. Accordingly, formulation of sub-signatures of the 70 gene signature are not restricted to the same hierarchy and may be formulated using any combination of the 70 genes to form the suitably sized signature.

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Core gene analysis was performed to determine a ranking for the genes based upon their impact on performance when removed from the signature. This analysis involved 10,000 random samplings of 10 signature genes from the original 70 signature gene set. For each iteration, 10 randomly selected signature genes were removed and the performance of the remaining 65 genes was evaluated using the endpoint to determine the impact on HR (Hazard Ratio) performance when these 10 genes were removed.

When this was performed using the FASTMAN Biopsy Validation Cohort of 248 samples, evaluation utilised the biochemical recurrence (BCR) endpoint.

The signature genes were weighted based upon the change in HR performance (Delta HR) based upon their inclusion or exclusion. The gene ranked '1' has the most negative impact on performance when removed and the gene ranked '70' has the least impact on performance when removed. The results are shown in Table 35 below.

Thus, in some embodiments, gene signatures are formulated in rank order. For example a 10 gene signature could comprise the first 10 ranked genes listed in Table 35. Accordingly, in some embodiments, the expression level of at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 of the 10 highest ranked genes in Table 35 is determined.

When this was performed using the Internal Resection Validation Cohort of 322 samples, evaluation utilised the metastatic recurrence (MET) endpoint.

The signature genes were weighted based upon the change in HR performance (Delta HR) based upon their inclusion or exclusion. The gene ranked '1' has the most negative impact on performance when removed and the gene ranked '70' has the least impact on performance when removed. The results are shown in Table 36 below.

Thus, in some embodiments, gene signatures are formulated in rank order. For example a 10 gene signature could comprise the first 10 ranked genes listed in Table 36. Accordingly, in some embodiments, the expression level of at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 of the 10 highest ranked genes in Table 36 is determined.

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The results for combined rankings are shown in Table 38. In some embodiments, gene signatures are formulated in rank order. For example a 10 gene signature could comprise from the first 10 ranked genes listed in Table 38. Accordingly, in some embodiments, the expression level of at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 of the 10 highest ranked genes in Table 38 is determined.

Additional gene signatures representing selections from the genes of Table 1 are described herein and are applicable to all aspects of the invention. These signatures may also provide the basis for larger signatures. The additional signatures are set forth in Tables 2 to 24, together with suitable weight and bias scores that may be adopted when calculating the final signature score (as further described herein). The k value for each signature can be set once the threshold for defining a positive signature score has been determined, as would be readily appreciated by the skilled person. Similarly, the rankings for each gene in the signature can readily be determined by reviewing the weightings attributed to each gene (where a larger weight indicates a higher ranking in the signature – see Table 1 for the rank order in respect of the 70 gene signature).

Thus, in some embodiments, the methods of the invention involve determining expression levels of at least MT1A and PCP4 (two gene signature shown in Table 2). As shown in Figures 2 and 3, signatures as small as the two gene signatures are capable of identifying the relevant biology and predicting metastatic recurrence. Larger signatures can be developed based upon these two genes, examples of which are given in tables 3 to 24, and in Table 1. Suitable probes and probsets to investigate expression of these genes are provided in Table 1 and 1A and primers useful to determine expression are listed in Table 1B.

Table 2 – Two gene signature

Entrez Gene ID	Weight	Bias
4489	-0.0854336	6.74796
5121	-0.0849287	7.62176

Table 3 – Three gene signature

Entrez Gene ID	Weight	Bias

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406988	-0.0584449	7.21525
4489	-0.0594146	6.74796
5121	-0.0590634	7.62176

Table 4 – Four gene signature

Entrez Gene ID	Weight	Bias
406988	-0.0484829	7.21525
4489	-0.0492874	6.74796
5121	-0.0489961	7.62176
827	-0.0438564	4.44087

Table 5 – Five gene signature

Entrez Gene ID	Weight	Bias
406988	-0.0409374	7.21525
4489	-0.0416166	6.74796
5121	-0.0413707	7.62176
6401	-0.0364515	5.97768
827	-0.0370309	4.44087

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Table 6 – Six gene signature

Entrez Gene ID	Weight	Bias
406988	-0.0355221	7.21525
4489	-0.0361114	6.74796
5121	-0.035898	7.62176
5354	-0.0309227	4.38357
6401	-0.0316296	5.97768
827	-0.0321323	4.44087

Table 7 – Seven gene signature

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Entrez Gene ID	Weight	Bias
3852	-0.026477	6.08049
406988	-0.0314283	7.21525
4489	-0.0319498	6.74796
5121	-0.0317609	7.62176
5354	-0.027359	4.38357
6401	-0.0279844	5.97768
827	-0.0284292	4.44087

Table 8 – Eight gene signature

Entrez Gene ID	Weight	Bias
3852	-0.0240174	6.08049
406988	-0.0285088	7.21525
4489	-0.0289818	6.74796
5121	-0.0288105	7.62176
5354	-0.0248175	4.38357
57481	-0.0223493	3.55997
6401	-0.0253848	5.97768
827	-0.0257883	4.44087

Table 9 – Nine gene signature

Entrez Gene ID	Weight	Bias
27063	-0.0189187	5.92831
3852	-0.022443	6.08049
406988	-0.0266399	7.21525
4489	-0.0270819	6.74796
5121	-0.0269218	7.62176
5354	-0.0231906	4.38357
57481	-0.0208842	3.55997

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6401	-0.0237207	5.97768
827	-0.0240977	4.44087

Table 10 – Eleven gene signature

Entrez Gene ID	Weight	Bias
25907	-0.016386	8.06342
27063	-0.0169106	5.92831
3852	-0.0200608	6.08049
406988	-0.0238123	7.21525
4489	-0.0242073	6.74796
5121	-0.0240643	7.62176
5354	-0.0207291	4.38357
57481	-0.0186675	3.55997
6401	-0.0212029	5.97768
827	-0.0215399	4.44087
84870	-0.0157681	4.29317

Table 11 – Thirteen gene signature

Entrez Gene ID	Weight	Bias
25907	-0.0150652	8.06342
27063	-0.0155475	5.92831
3852	-0.0184438	6.08049
406988	-0.0218928	7.21525
4489	-0.0222561	6.74796
5121	-0.0221245	7.62176
5354	-0.0190581	4.38357
57481	-0.0171628	3.55997
6401	-0.0194938	5.97768

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6406	-0.0144896	4.23042
7060	-0.0144516	6.91259
827	-0.0198036	4.44087
84870	-0.0144971	4.29317

Table 12 – Fifteen gene signature

Entrez Gene ID	Weight	Bias
2138	-0.013038	5.50428
25907	-0.0137554	8.06342
27063	-0.0141957	5.92831
340419	-0.0131822	3.92242
3852	-0.0168402	6.08049
406988	-0.0199894	7.21525
4489	-0.020321	6.74796
5121	-0.0202009	7.62176
5354	-0.0174011	4.38357
57481	-0.0156705	3.55997
6401	-0.0177989	5.97768
6406	-0.0132298	4.23042
7060	-0.0131951	6.91259
827	-0.0180818	4.44087
84870	-0.0132366	4.29317

Table 13 – Seventeen gene signature

Entrez Gene ID	Weight	Bias
2138	-0.0122396	5.50428
2354	-0.0114061	6.95494
25907	-0.0129131	8.06342

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27063	-0.0133265	5.92831
340419	-0.012375	3.92242
3852	-0.015809	6.08049
4057	-0.0113308	6.49726
406988	-0.0187653	7.21525
4489	-0.0190767	6.74796
5121	-0.0189639	7.62176
5354	-0.0163356	4.38357
57481	-0.014711	3.55997
6401	-0.0167091	5.97768
6406	-0.0124197	4.23042
7060	-0.0123871	6.91259
827	-0.0169746	4.44087
84870	-0.0124261	4.29317

Table 14 – Nineteen gene signature

Entrez Gene ID	Weight	Bias
12	-0.0105382	5.74546
2138	-0.011593	5.50428
2354	-0.0108034	6.95494
25907	-0.0122308	8.06342
27063	-0.0126224	5.92831
340419	-0.0117212	3.92242
3852	-0.0149737	6.08049
4057	-0.0107322	6.49726
406988	-0.0177739	7.21525
4489	-0.0180688	6.74796
5121	-0.017962	7.62176

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5354	-0.0154725	4.38357
57481	-0.0139337	3.55997
6401	-0.0158262	5.97768
6406	-0.0117635	4.23042
7060	-0.0117327	6.91259
7538	-0.0101011	9.96083
827	-0.0160778	4.44087
84870	-0.0117696	4.29317

Table 15 – Twenty two gene signature

Entrez Gene ID	Weight	Bias
12	-0.0102163	5.74546
2138	-0.0112388	5.50428
2354	-0.0104734	6.95494
25907	-0.0118571	8.06342
27063	-0.0122367	5.92831
340419	-0.0113631	3.92242
3852	-0.0145163	6.08049
4057	-0.0104043	6.49726
406988	-0.0172309	7.21525
4489	-0.0175167	6.74796
4604	-0.0069325	4.57432
50636	-0.0064135	6.52255
5121	-0.0174132	7.62176
5354	-0.0149998	4.38357
57481	-0.013508	3.55997
6401	-0.0153427	5.97768
6406	-0.0114041	4.23042

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7060	-0.0113742	6.91259
7538	-0.0097925	9.96083
827	-0.0155866	4.44087
84870	-0.01141	4.29317
9232	0.00804755	4.71269

Table 16 – Twenty five gene signature

Entrez Gene ID	Weight	Bias
12	-0.0101819	5.74546
2138	-0.011201	5.50428
2354	-0.0104381	6.95494
25907	-0.0118172	8.06342
27063	-0.0121956	5.92831
340419	-0.0113249	3.92242
3852	-0.0144674	6.08049
4057	-0.0103693	6.49726
406988	-0.0171729	7.21525
4489	-0.0174578	6.74796
4604	-0.0069091	4.57432
50636	-0.0063919	6.52255
50652	-0.0035123	5.26234
5121	-0.0173546	7.62176
5354	-0.0149493	4.38357
57481	-0.0134626	3.55997
6401	-0.0152911	5.97768
6406	-0.0113657	4.23042
7060	-0.0113359	6.91259
7538	-0.0097595	9.96083

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79054	-0.0029055	4.86579
79689	-0.0041936	8.1053
827	-0.0155341	4.44087
84870	-0.0113716	4.29317
9232	0.00802047	4.71269

Table 17 – Twenty eight gene signature

Entrez Gene ID	Weight	Bias
12	-0.0113703	5.74546
2138	-0.0102938	5.50428
2354	-0.0091518	6.95494
25907	-0.0112273	8.06342
27063	-0.0109933	5.92831
2920	-0.0080439	8.92898
340419	-0.0103778	3.92242
3852	-0.0118207	6.08049
4057	-0.0105916	6.49726
406988	-0.0163129	7.21525
4489	-0.0148319	6.74796
4604	-0.0117356	4.57432
50636	-0.0122781	6.52255
50652	-0.0100098	5.26234
5121	-0.0131977	7.62176
5354	-0.0145474	4.38357
57481	-0.0112327	3.55997
6401	-0.0109283	5.97768
6406	-0.0125967	4.23042
644844	-0.008567	5.18357

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693163	-0.0087554	5.08739
7060	-0.0156046	6.91259
7538	-0.009639	9.96083
79054	-0.0094113	4.86579
79689	-0.0090982	8.1053
827	-0.0185353	4.44087
84870	-0.0120577	4.29317
9232	0.0102357	4.71269

Table 18 – Thirty two gene signature

Entrez Gene ID	Weight	Bias
12	-0.010156	5.74546
2138	-0.0084546	5.50428
2354	-0.0105369	6.95494
25907	-0.0093177	8.06342
27063	-0.0095296	5.92831
2920	-0.0082867	8.92898
340419	-0.008292	3.92242
3852	-0.0097028	6.08049
4057	-0.0081905	6.49726
406988	-0.0120927	7.21525
4316	-0.0073912	6.75672
4489	-0.012495	6.74796
4604	-0.0121787	4.57432
50636	-0.0122014	6.52255
50652	-0.0102362	5.26234
5121	-0.010326	7.62176
5179	-0.0077226	4.51486

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5354	-0.0133628	4.38357
57481	-0.0095722	3.55997
6401	-0.010634	5.97768
6406	-0.0118163	4.23042
644844	-0.0099334	5.18357
693163	-0.0098705	5.08739
7060	-0.0142594	6.91259
7538	-0.0103042	9.96083
79054	-0.0101624	4.86579
79689	-0.0093796	8.1053
827	-0.0166256	4.44087
84870	-0.010646	4.29317
9232	0.00927419	4.71269
9506	-0.008145	7.07391
9955	-0.007857	4.23278

Table 19 – Thirty six gene signature

Entrez Gene ID	Weight	Bias
12	-0.0093135	5.74546
130733	-0.0075817	7.59453
2138	-0.0084016	5.50428
2354	-0.0099522	6.95494
25907	-0.0091246	8.06342
27063	-0.0096954	5.92831
283194	-0.0076884	4.98038
2920	-0.0082441	8.92898
340419	-0.0081949	3.92242
3852	-0.0098646	6.08049

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4057	-0.0080168	6.49726
406988	-0.0121601	7.21525
4316	-0.008168	6.75672
4489	-0.0123296	6.74796
4604	-0.0103293	4.57432
50636	-0.0106303	6.52255
50652	-0.008396	5.26234
51050	-0.0074885	4.85872
5121	-0.0106667	7.62176
5179	-0.0079247	4.51486
5317	-0.0073104	5.91219
5354	-0.012805	4.38357
57481	-0.0094443	3.55997
6401	-0.0105376	5.97768
6406	-0.0117042	4.23042
644844	-0.007735	5.18357
693163	-0.0085964	5.08739
7060	-0.0129938	6.91259
7538	-0.009653	9.96083
79054	-0.0084699	4.86579
79689	-0.0078376	8.1053
827	-0.0155276	4.44087
84870	-0.0103741	4.29317
9232	0.00860486	4.71269
9506	-0.0083385	7.07391
9955	-0.0078923	4.23278

Table 20 – Forty gene signature

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Entrez Gene ID	Weight	Bias
12	-0.0088635	5.74546
130733	-0.0073773	7.59453
2138	-0.0081002	5.50428
2354	-0.0089276	6.95494
23764	-0.0070488	8.49795
25907	-0.0086677	8.06342
27063	-0.0091158	5.92831
283194	-0.0077222	4.98038
2920	-0.0074337	8.92898
340419	-0.0079644	3.92242
3852	-0.0093986	6.08049
4057	-0.0076408	6.49726
406988	-0.0117445	7.21525
4316	-0.0078189	6.75672
4489	-0.0117016	6.74796
4588	-0.0072195	6.64004
4604	-0.0102513	4.57432
5054	-0.007115	6.69187
50636	-0.0102281	6.52255
50652	-0.0081408	5.26234
51050	-0.007475	4.85872
5121	-0.0102856	7.62176
5179	-0.0076867	4.51486
5317	-0.0072532	5.91219
5354	-0.0124218	4.38357
57481	-0.0091711	3.55997

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6401	-0.0097774	5.97768
6406	-0.0108845	4.23042
644844	-0.0074985	5.18357
693163	-0.0079773	5.08739
7060	-0.012659	6.91259
7083	0.00689113	5.58133
7538	-0.0089554	9.96083
79054	-0.0080402	4.86579
79689	-0.0074587	8.1053
827	-0.0150968	4.44087
84870	-0.0101513	4.29317
9232	0.00824867	4.71269
9506	-0.0081624	7.07391
9955	-0.0075526	4.23278

Table 21 – Forty five gene signature

Entrez Gene ID	Weight	Bias
12	-0.0084719	5.74546
130733	-0.0071653	7.59453
2138	-0.0076354	5.50428
2354	-0.0086978	6.95494
23764	-0.0068137	8.49795
25907	-0.0081883	8.06342
27063	-0.0095258	5.92831
283194	-0.0073756	4.98038
2920	-0.0074016	8.92898
340419	-0.0072676	3.92242

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3852	-0.0086227	6.08049
4057	-0.0076939	6.49726
406988	-0.0109582	7.21525
4316	-0.007433	6.75672
4489	-0.0109596	6.74796
4588	-0.0068952	6.64004
4604	-0.0089751	4.57432
5054	-0.0070642	6.69187
50636	-0.0095383	6.52255
50652	-0.0076953	5.26234
51050	-0.0067347	4.85872
5121	-0.0090383	7.62176
5166	-0.0064467	4.17409
5179	-0.0069808	4.51486
5317	-0.0069448	5.91219
5354	-0.0114369	4.38357
563	-0.0062549	8.19118
57481	-0.008131	3.55997
6401	-0.0090862	5.97768
6406	-0.0097387	4.23042
644844	-0.0069075	5.18357
693163	-0.007503	5.08739
7060	-0.0117799	6.91259
7083	0.00695478	5.58133
7538	-0.008409	9.96083
7739	-0.0062004	6.90054
79054	-0.0076792	4.86579

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79689	-0.0072917	8.1053
827	-0.0138725	4.44087
84870	-0.0094612	4.29317
84889	-0.0067268	4.649
91661	-0.0062403	3.97633
9232	0.00773594	4.71269
9506	-0.0074141	7.07391
9955	-0.0072818	4.23278

Table 22 – Fifty gene signature

Entrez Gene ID	Weight	Bias
100616163	-0.0060146	10.5365
1164	0.00596174	6.50398
12	-0.00788	5.74546
130733	-0.0070582	7.59453
152	-0.005916	7.07838
1672	-0.0057271	6.82549
2138	-0.0069005	5.50428
2354	-0.0074259	6.95494
23764	-0.0060195	8.49795
25907	-0.0076929	8.06342
27063	-0.0084041	5.92831
283194	-0.0075818	4.98038
2920	-0.0062969	8.92898
340419	-0.006979	3.92242
3832	0.00580874	3.91767
3852	-0.0073413	6.08049
4057	-0.0068257	6.49726

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406988	-0.0093852	7.21525
4316	-0.0070704	6.75672
4489	-0.0103164	6.74796
4588	-0.0065059	6.64004
4604	-0.0088755	4.57432
5054	-0.0064482	6.69187
50636	-0.0093967	6.52255
50652	-0.0078998	5.26234
51050	-0.0064943	4.85872
5121	-0.0085839	7.62176
5166	-0.0061711	4.17409
5179	-0.0066949	4.51486
5317	-0.0069413	5.91219
5354	-0.0110133	4.38357
563	-0.0062503	8.19118
57481	-0.0076625	3.55997
6401	-0.0082619	5.97768
6406	-0.0090315	4.23042
644844	-0.0073783	5.18357
693163	-0.0068836	5.08739
7060	-0.012155	6.91259
7083	0.00620598	5.58133
7538	-0.0076694	9.96083
7739	-0.0060281	6.90054
79054	-0.0078154	4.86579
79689	-0.0071002	8.1053
827	-0.0134928	4.44087

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84870	-0.0091115	4.29317
84889	-0.0067284	4.649
91661	-0.0062814	3.97633
9232	0.00694781	4.71269
9506	-0.0070319	7.07391
9955	-0.0067662	4.23278

Table 23 – Fifty six gene signature

Entrez Gene ID	Weight	Bias
100616163	-0.005861	10.5365
10257	-0.0050496	5.23038
1164	0.00569625	6.50398
12	-0.0073822	5.74546
130733	-0.006436	7.59453
152	-0.0058338	7.07838
1672	-0.0055123	6.82549
2138	-0.0068171	5.50428
2354	-0.0071035	6.95494
23764	-0.0056449	8.49795
23784	-0.0055006	4.82498
25907	-0.0075056	8.06342
27063	-0.0082314	5.92831
283194	-0.0066926	4.98038
2920	-0.0062953	8.92898
340419	-0.0068818	3.92242
3832	0.00560094	3.91767
3852	-0.0072034	6.08049
4057	-0.0066854	6.49726

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406988	-0.0090297	7.21525
4316	-0.006866	6.75672
4489	-0.0101527	6.74796
4588	-0.0062002	6.64004
4604	-0.008045	4.57432
5054	-0.0059681	6.69187
50636	-0.008568	6.52255
50652	-0.0069136	5.26234
51050	-0.006074	4.85872
5121	-0.0084668	7.62176
5166	-0.0062193	4.17409
5179	-0.0067401	4.51486
5317	-0.0062775	5.91219
5346	0.00544079	4.62939
5354	-0.0107509	4.38357
563	-0.0057774	8.19118
57176	0.0054321	5.22346
57481	-0.0075962	3.55997
6401	-0.0079086	5.97768
6406	-0.0089768	4.23042
644844	-0.0063947	5.18357
6614	0.00529568	5.50375
693163	-0.0062258	5.08739
7060	-0.0113086	6.91259
7083	0.00606898	5.58133
7538	-0.0073458	9.96083
7739	-0.0059453	6.90054

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79054	-0.0069339	4.86579
79689	-0.0063605	8.1053
827	-0.0130713	4.44087
84870	-0.0092604	4.29317
84889	-0.0064006	4.649
9076	-0.0053751	4.96028
91661	-0.0056536	3.97633
9232	0.00664308	4.71269
9506	-0.0069717	7.07391
9955	-0.0067533	4.23278

Table 24 – Sixty three gene signature

Entrez Gene ID	Weight	Bias
100616163	-0.005042	10.5365
101928017	-0.0048527	6.06588
10257	-0.0056574	5.23038
1164	0.0052823	6.50398
12	-0.0073342	5.74546
130733	-0.0062765	7.59453
152	-0.0051502	7.07838
1672	-0.0052785	6.82549
1847	-0.0048311	5.76268
2138	-0.0056248	5.50428
2354	-0.0064848	6.95494
23764	-0.0051811	8.49795
23784	-0.0058458	4.82498
25907	-0.0062868	8.06342

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27063	-0.0071516	5.92831
283194	-0.0071346	4.98038
285016	-0.0045118	6.6646
2920	-0.0056286	8.92898
29951	-0.0049994	4.75233
340419	-0.0056458	3.92242
3832	0.00505389	3.91767
3852	-0.0064458	6.08049
4057	-0.0063934	6.49726
406988	-0.0083826	7.21525
4316	-0.0069549	6.75672
4489	-0.0087025	6.74796
4588	-0.0062676	6.64004
4604	-0.0080954	4.57432
5054	-0.0056402	6.69187
50636	-0.0080538	6.52255
50652	-0.0072374	5.26234
51050	-0.0056617	4.85872
5121	-0.0071957	7.62176
5166	-0.0052681	4.17409
5179	-0.0052589	4.51486
5317	-0.0062761	5.91219
5346	0.00537235	4.62939
5354	-0.009133	4.38357
563	-0.0057921	8.19118
56849	-0.0048508	4.81933
57176	0.00516736	5.22346

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57481	-0.0063163	3.55997
6401	-0.0069775	5.97768
6406	-0.0081782	4.23042
6422	-0.0048345	7.90126
644844	-0.0064333	5.18357
6614	0.00520155	5.50375
693163	-0.0060983	5.08739
7060	-0.0108538	6.91259
7083	0.00523833	5.58133
7538	-0.0065682	9.96083
7739	-0.0050779	6.90054
79054	-0.0071048	4.86579
79689	-0.0063567	8.1053
8076	-0.0047141	4.12918
827	-0.011285	4.44087
84870	-0.0075344	4.29317
84889	-0.0058044	4.649
9076	-0.0052058	4.96028
91661	-0.0054622	3.97633
9232	0.00626422	4.71269
9506	-0.0058269	7.07391
9955	-0.0055209	4.23278

In some embodiments, applicable to all aspects of the invention, the expression level of PDK4 alone is not measured. PDK4 expression is thus typically measured in combination with at least one further gene up to all 69 further genes from table 1. In some embodiments,

5 PDK4 expression is determined using an assay targeting a sequence within the full sequences of SEQ ID NO: 52, 53, 63, 108, 09, 152, 153, 157, 158, 184, 194 and/or 216 respectively. In some embodiments, PDK4 expression is determined using an assay

targeting a sequence within the target sequences of SEQ ID NO: 284, 285, 295, 340, 341, 384, 385, 389, 390, 416, 426 and/or 448 respectively. In some embodiments PDK4 expression is determined using one or more probes selected from SEQ ID Nos: 1011-1021, 1022-1032, 1132-1142, 1627-1637, 1638-1648, 2122-2132, 2133-2143, 2177-2187, 2188-
5 2198, 2474-2484, 2584-2594 and 2834-2844 or probe sets of SEQ ID Nos: 1011-1021, 1022-1032, 1132-1142, 1627-1637, 1638-1648, 2122-2132, 2133-2143, 2177-2187, 2188-2198, 2474-2484, 2584-2594 and/or 2834-2844. In some embodiments, PDK4 expression is determined using an amplification (PCR, or qPCR) assay employing primers of SEQ ID NO: 3053 and/or 3121 respectively.

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In some embodiments, applicable to all aspects of the invention, the expression level of KIF11, PTTG1 or TK1 alone is not measured. In some embodiments, the expression levels of KIF11, PTTG1 and TK1 may be measured together as a 3 gene signature. In some
15 embodiments, the expression levels of KIF11, PTTG1 and/or TK1 may be measured in combination with at least one further gene from Table 1, including forming the 70 gene signature. In some embodiments, KIF11 expression is determined using an assay targeting a sequence within the full sequences of SEQ ID NO: 180 and/or 181 respectively. In some
20 embodiments, KIF11 expression is determined using an assay targeting a sequence within the target sequences of SEQ ID NO: 412 and/or 413 respectively. In some embodiments KIF11 expression is determined using one or more probes selected from SEQ ID Nos: 2430 - 2440 and 2441- 2451 or probe sets of SEQ ID Nos: 2430 -2440 and/or 2441- 2451. In some
embodiments, KIF11 expression is determined using an amplification (PCR, or qPCR) assay employing primers of SEQ ID NO: 3062 and/or 3130 respectively.

25

In some embodiments, PTTG1 expression is determined using an assay targeting a sequence within the full sequences of SEQ ID NO: 62 and/or 201 respectively. In some
embodiments, PTTG1 expression is determined using an assay targeting a sequence within the target sequences of SEQ ID NO: 294 and/or 433 respectively. In some embodiments
PTTG1 expression is determined using one or more probes selected from SEQ ID Nos: 1121
30 -1131 and 2661- 2671 or probe sets of SEQ ID Nos: 1121-1131 and/or 2661- 2671. In some
embodiments, PTTG1 expression is determined using an amplification (PCR, or qPCR) assay employing primers of SEQ ID NO: 3037 and/or 3105 respectively.

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In some embodiments, TK1 expression is determined using an assay targeting a sequence within the full sequence of SEQ ID NO: 197. In some embodiments, TK1 expression is determined using an assay targeting a sequence within the target sequence of SEQ ID NO: 429. In some embodiments TK1 expression is determined using one or more probes selected from SEQ ID Nos: 2617- 2627 or probe sets of SEQ ID Nos: 2617- 2627. In some
5 embodiments, TK1 expression is determined using an amplification (PCR, or qPCR) assay employing primers of SEQ ID NO: 3060 and/or 3128 respectively.

In some embodiments, applicable to all aspects of the invention, the expression level of
10 ANO7 or MYBPC1 alone is not measured. In some embodiments, the expression levels of ANO7 and MYBPC1 may be measured together as a 2 gene signature. In some embodiments, the expression levels of ANO7 and/or MYBPC1 may be measured in combination with at least one further gene from Table 1, including forming the 70 gene signature.

15 In some embodiments, ANO7 expression is determined using an assay targeting a sequence within the full sequences of SEQ ID NO: 37, 38, 125, 205 and/or 206 respectively. In some embodiments, ANO7 expression is determined using an assay targeting a sequence within the target sequences of SEQ ID NO: 269, 270, 357, 437 and/or 438 respectively. In some
20 embodiments ANO7 expression is determined using one or more probes selected from SEQ ID Nos: 849-859, 860-870, 1825-1835, 2715-2724 and 2725-2735 or probe sets of SEQ ID Nos: 849-859, 860-870, 1825-1835, 2715-2724 and/or 2725-2735. In some embodiments, ANO7 expression is determined using an amplification (PCR, or qPCR) assay employing primers of SEQ ID NO: 3022 and/or 3090 respectively.

25 In some embodiments, MYBPC1 expression is determined using an assay targeting a sequence within the full sequences of SEQ ID NO: 39, 40, 74, 75, 101, 102, 103 and/or 144 respectively. In some embodiments, MYBPC1 expression is determined using an assay targeting a sequence within the target sequences of SEQ ID NO: 271, 272, 306, 307, 333,
30 334, 335 and/or 376 respectively. In some embodiments MYBPC1 expression is determined using one or more probes selected from SEQ ID Nos: 871-881, 882-892, 1253-1263, 1264-1274, 1550-1560, 1561-1571, 1572-1582 and 2034- 2044 or probe sets of SEQ ID Nos: 871-881, 882-892, 1253-1263, 1264-1274, 1550-1560, 1561-1571, 1572-1582 and/or 2034-2044.

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In some embodiments, MYBPC1 expression is determined using an amplification (PCR, or qPCR) assay employing primers of SEQ ID NO: 3025 and/or 3093 respectively.

By "characterization" is meant classification and/or evaluation of the cancer, such as prostate
5 cancer or ER positive breast cancer. Thus, the methods of the invention allow cancers with
high metastatic potential to be identified for example. The methods rely upon determining
whether the cancer is a metastatic biology cancer or a non-metastatic biology cancer. The
methods permit cancers to be identified that are likely to recur. Prognosis refers to predicting
10 the likely outcome of the cancer, such as prostate cancer or ER positive breast cancer for the
subject. A bad or poor prognosis as determined herein, indicates an increased likelihood of
metastases and/or a higher likelihood or recurrence. By diagnosis is meant identifying the
presence of a cancer, of a particular type such as prostate cancer or ER positive breast
cancer with an increased metastatic potential. Thus, it will be readily apparent that there is
15 some overlap between the terms "characterization", "prognosis" and "diagnosis" as adopted
herein. The use of relative terms indicates the position *vis a vis* cancers which do not display
the relevant gene expression characteristics and thus have lower metastatic potential, are
less likely to recur and/or have a good prognosis. The gene signatures described herein may
be useful to stratify (prostate) cancer patients who have been diagnosed, in particular at an
early stage, and identify those at increased risk of developing more aggressive high risk
20 disease. This more aggressive disease may develop within 3-5 years of treatment. The
initial treatment may be radiotherapy and/or surgery (prostatectomy) for example. Upon
identification of the aggressive disease, the methods may require treatments as described
herein to be utilized. In the absence of cancer with high metastatic potential, the subject may
be placed under active surveillance and not further treated, at least initially. Further
25 monitoring, by any suitable means (including use of PSA monitoring or by performing the
methods of the invention) can be used to determine whether further intervention is required.

In some embodiments the characterisation of and/or prognosis for the cancer, such as
prostate cancer or ER positive breast cancer may comprise, consist essentially of or consist
30 of predicting an increased likelihood of recurrence. Cancers with the metastatic biology are
shown herein to be more likely to recur. The characterisation of and/or prognosis for the
cancer, such as prostate cancer or ER positive breast cancer may comprise, consist
essentially of or consist of predicting a reduced time to recurrence. Recurrence may be
considered co-terminus with relapse, as would be understood by the skilled person.

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Recurrence may be clinical recurrence, metastatic recurrence or biochemical recurrence. In the context of prostate cancer biochemical recurrence means a rise in the level of PSA in a subject after treatment for prostate cancer. Biochemical recurrence may indicate that the prostate cancer has not been treated effectively or has recurred. Recurrence may be following surgery, for example radical prostatectomy and/or following radiotherapy.

In some embodiments, the characterisation of and/or prognosis for the cancer, such as prostate cancer or ER positive breast cancer may comprise, consist essentially of or consist of predicting an increased likelihood of metastasis. Metastasis, or metastatic disease, is the spread of a cancer from one organ or part to another non-adjacent organ or part. The new occurrences of disease thus generated are referred to as metastases. In certain embodiments, the methods of the invention are used to facilitate metastases staging of cancer, in particular prostate cancer. Thus, determined expression levels (e.g. determination of a gene signature positive sample) can be used to stage a subject as M1. M1 means that metastases are present (i.e. the cancer has spread to other parts of the body). For gene signature negative samples, that subject may be staged as M0. M0 means that the cancer has not yet spread to other parts of the body. Such methods may be used in conjunction with other measures used to identify metastases e.g. imaging/scanning techniques. Thus, the invention provides a method for metastases staging of a cancer comprising determining the expression level of at least one gene selected from Table 1 in a sample from the subject wherein the determined expression level is used to identify whether a subject has a M1 or M0 cancer. Thus, in some embodiments, the methods may comprise:

- (i) determining the expression level of at least one gene selected from Table 1 in a sample from the subject; and
- (ii) assessing from the expression level of the at least one gene whether the sample from the subject is positive or negative for a gene signature comprising the at least one gene. Suitable gene signatures and derivations of signature scores are discussed in further detail herein.

In some embodiments, characterisation of and/or prognosis for the cancer, such as prostate cancer or ER positive breast cancer may also comprise, consist essentially of or consist of determining whether the cancer has a poor prognosis. A poor prognosis may be a reduced likelihood of cause-specific, i.e. cancer-specific, or long term survival. Cause- or Cancer-

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specific survival is a net survival measure representing cancer survival in the absence of other causes of death. Cancer survival may be for 6, 7, 8, 9, 10, 11, 12 months or 1, 2, 3, 4, 5 etc. years. Long-term survival may be survival for 1 year, 5 years, 10 years or 20 years following diagnosis. A cancer, such as prostate cancer or ER positive breast cancer with a poor prognosis may be aggressive, fast growing, and/or show resistance to treatment.

In certain embodiments an increased expression level of at least one gene selected from Table 1 with a positive weight indicates an increased likelihood of recurrence and/or metastasis and/or a poor prognosis.

10 In further embodiments a decreased expression level of at least one gene selected from Table 1 with a negative weight indicates an increased likelihood of recurrence and/or metastasis and/or a poor prognosis.

Expression levels are weighted accordingly, to account for their contribution to gene signature score as discussed herein. A threshold of expression may be set relative to a median level against which "signature positive" and "signature negative" expression values can be set. Examples of such median threshold expression levels and corresponding signature positive and negative values are set forth in table 25 immediately below. As can be seen, the median values are set individually for each dataset as would be understood by one skilled in the art:

Table 25 – Median threshold expression levels for genes in 70 gene signature

Gene Name	R0185			Taylor			Clinical Validation		
	Up/Down Regulation			Up/Down Regulation			Up/Down Regulation		
	Median Threshold	Sig Pos	Sig Neg	Median Threshold	Sig Pos	Sig Neg	Median Threshold	Sig Pos	Sig Neg
CAPN6	4.42188	2.04472	6.43372	5.5318	5.3482	5.6302	6.315475	4.074	6.569
THBS4	7.06852	5.02893	8.08507	6.09006	5.6854	6.2519	8.91341	8.7469	8.9605
PLP1	4.5448	2.06306	6.49898	4.31333	4.3854	4.2517	3.456275	2.4345	3.7365
MT1A	6.387205	4.06229	8.97844	4.93781	4.5807	5.1455	6.518785	5.6427	6.7175
MIR205HG	8.00701	4.87658	9.24825	7.57876	7.1084	7.8151	8.97736	7.025	9.2159
SEMG1	2.69399	2.3506	4.17395	3.37923	3.5178	3.2859	2.69531	2.6214	2.6953
RSP03	4.82032	2.0699	5.78781	8.8968	8.8373	8.9397	4.2128	2.2819	4.5188
ANO7	6.46441	5.67131	7.44695	8.4678	8.3131	8.5449	8.683835	7.5313	8.7909
PCP4	8.503335	5.4613	9.81501	7.95265	7.4887	8.2149	10.01705	8.9437	10.12
ANKRD1	5.610625	3.90673	7.45987	4.25165	4.0009	4.3893	5.15809	3.17	5.6713

MYBPC1	4.45984	2.87008	5.58119	3.16997	3.027	3.2647	6.173885	5.0181	6.3699
MMP7	7.64552	3.63728	8.81375	2.21155	2.2597	2.1786	8.26743	6.6757	8.4475
SERPINA3	5.8349	4.17103	6.62491	8.08507	5.9558	8.9948	6.869015	5.3198	7.0793
SELE	6.69364	3.42413	7.66659	4.86743	4.5184	5.0608	5.46303	4.3339	5.704
KRT5	6.719415	3.43284	7.9083	8.22671	8.2267	8.2313	7.707815	6.5433	7.9614
LTF	5.83487	5.06191	7.70167	4.45153	4.174	4.5969	7.3738	6.3697	7.7314
KIAA1210	2.74592	1.56824	5.50166	4.76043	4.9023	4.6617	4.578835	2.6833	4.7082
TMEM158	8.40747	6.66104	9.3172	8.39763	8.1777	8.4845	7.655895	6.768	7.7878
ZFP36	10.39315	8.80059	11.1231	9.73981	8.5152	10.592	10.6163	9.1513	10.895
FOSB	7.316875	5.1803	8.05011	8.35888	7.219	9.0206	7.957285	5.6257	8.6746
PCA3	4.782625	4.3872	4.90232	11.4346	10.271	12.114	8.352805	8.0957	8.3847
TRPM8	4.860835	4.0207	5.13583	4.78668	4.5937	4.9832	6.09048	6.1888	6.0901
PTTG1	4.38243	5.40862	3.73654	3.05421	2.9145	3.135	3.73654	4.0886	3.6952
#N/A	4.87794	4.92985	4.85895	6.1573	5.7808	6.5764	6.20071	6.1789	6.2063
PAGE4	7.78752	4.79959	8.60591	5.2044	5.1045	5.3075	7.20806	5.2471	7.3508
STEAP4	8.12307	7.29677	8.41974	3.26122	3.2612	3.2423	10.4898	10.657	10.466
TMEM178A	7.314555	6.61022	7.5254	4.57785	4.5071	4.5939	8.681645	8.4749	8.7561
CXCL2	9.261335	7.34194	10.048	9.24825	9.0011	9.4489	8.75985	7.2543	9.0269
HS3ST3A1	4.45439	2.69531	5.32664	4.82805	4.9046	4.6609	5.18552	4.3254	5.3928
EYA1	6.07141	3.60874	6.91569	4.19606	4.1531	4.2517	5.809395	4.6288	5.9632
RSPO2	3.84235	1.98492	5.30295	2.61807	2.5402	2.6731	2.76883	2.1794	2.9415
PKP1	6.112415	5.26861	6.34026	4.61452	4.3254	4.7781	5.22822	4.7867	5.2662
MUC5	6.01117	5.96861	6.05794	8.69215	8.7469	8.582	6.73111	6.5614	6.7738
PENK	4.0716	2.34573	6.28444	8.74017	8.8199	8.6943	2.810335	2.5609	2.8701
DEFB1	7.25831	4.86935	8.44625	6.346	5.9395	6.5493	6.238925	3.5331	6.7243
SLC7A3	4.517555	3.83265	5.12394	3.06899	2.9415	3.1712	5.131285	4.6388	5.2528
MIR578	5.23268	4.15686	5.74198	3.60874	3.3985	3.7449	3.83251	3.0482	4.0207
PI15	5.175905	3.18336	5.8754	9.11409	7.7045	9.9628	6.06872	4.8925	6.2305
UBXN10-AS1	6.333035	3.50707	7.96714	5.06221	4.6847	5.2619	5.20983	3.7088	5.5369
PKD4	3.907115	2.34383	5.47102	3.16997	3.2654	3.1022	4.05588	3.1565	4.2233
PHGR1	4.83498	4.68471	4.91059	4.07399	4.074	4.068	7.31838	6.8104	7.4198
SERPINE1	6.748165	5.89172	7.29677	4.57785	4.7307	4.3841	6.454425	5.8998	6.6472
PDZRN4	5.065115	2.79653	6.28918	9.92587	10.04	9.8607	4.384745	2.7767	4.6154
ZNF185	7.015235	5.24706	8.18067	5.24706	5.2471	5.2477	6.330095	5.767	6.3871
ADRA2C	7.300155	5.78671	7.99285	7.68072	7.7405	7.6252	6.58485	6.2063	6.6863
AZGP1	8.64502	6.63277	9.1771	7.2166	6.5614	7.6067	8.821125	7.4957	9.031
TK1	5.12958	6.43788	4.55892	7.33302	7.5376	7.2099	4.209675	4.4515	4.1974
POTEH	5.033025	4.68471	5.41636	3.49675	3.3158	3.6403	4.387175	4.3664	4.3872
KIF11	3.77959	5.07156	3.16997	3.38809	3.5827	3.2756	3.0616	3.1386	3.0463
CLDN1	5.175105	4.07399	5.69935	5.25653	5.0154	5.5078	4.69244	4.132	4.7867

MIR4530	10.9443	9.2709	11.6184	11.1975	11.081	11.277	11.3313	10.086	11.504
MAFF	8.49114	7.27831	9.26618	6.22565	6.0525	6.3947	9.6093	8.8522	9.7909
ZNF765	3.602255	3.31232	3.71212	4.82805	4.5185	5.0909	4.70517	4.3841	4.7675
CKS2	6.468755	6.98567	6.19645	2.60809	2.7465	2.3706	4.020185	2.8152	4.2086
TCEAL7	5.114575	3.29422	6.17888	5.42383	5.3301	5.5736	5.06191	2.9046	5.2486
PLIN1	4.436085	5.08342	3.74916	3.48572	3.666	3.2654	3.456275	3.8327	3.3792
SIGLEC1	5.176255	6.12635	4.81258	6.27516	6.4169	6.1338	5.02289	5.1045	5.0181
FAM150B	7.000985	5.10447	8.07842	6.77336	6.8114	6.6669	5.69935	4.4515	5.8569
MFAP5	4.10253	2.34383	5.57364	3.80478	3.7365	3.8325	4.97069	2.9415	5.2471
SFRP1	8.42439	6.87325	8.84832	5.40862	5.4486	5.3868	9.00425	8.4318	9.0461
DUSP5	6.049365	4.07026	6.89159	6.47079	6.5916	6.3697	3.380615	2.609	3.7498
VAR52	5.144165	5.55841	4.68206	3.66826	3.4695	3.8069	3.710975	3.4595	3.7374
ABCC4	5.20667	4.77776	5.43315	5.64272	5.0619	5.9743	6.13912	6.2684	6.1369
SH3BP4	4.840135	4.25165	5.42961	4.57785	4.4515	4.6512	5.320995	4.8281	5.4599
SORD	9.140035	9.07048	9.15822	7.74808	7.2239	8.0572	8.33616	8.2458	8.3401
MTERFD1	5.513935	6.02508	5.22508	4.51834	4.7242	4.3928	3.69208	3.7427	3.6104
DPP4	4.75566	3.70312	5.57364	4.24098	4.3217	4.2055	6.243255	5.4332	6.3479
#N/A	4.890245	5.51612	4.48785	3.49859	3.3219	3.6486	3.538075	3.5905	3.5304
FAM3B	7.73412	7.02686	8.0087	4.82805	4.8423	4.8124	9.0795	7.7829	9.1889
KLK3	10.63635	10.611	10.7045	10.6617	10.395	10.802	12.8215	12.822	12.822

In certain embodiments the methods described herein may comprise determining the expression level of at least one of the genes with a negative weight listed in Table 1 together with at least one gene with a positive weight listed in Table 1. Thus, the methods may rely upon a combination of an up-regulated marker and a down-regulated marker. The combined up and down regulated marker expression levels, as appropriately weighted, may then contribute to, or make up, the final signature score.

10 In certain embodiments the methods described herein comprise comparing the expression level of one or more genes to a reference value or to the expression level in one or more control samples or to the expression level in one or more control cells in the same sample. The control cells may be normal (i.e. cells characterised by an independent method as non-cancerous) cells. The one or more control samples may consist of non-cancerous cells or

15 may include a mixture of cancer cells (prostate, ER positive breast or otherwise) and non-cancerous cells. The expression level may be compared to the expression level of the same gene in one or more control samples or control cells.

- The reference value may be a threshold level of expression of at least one gene set by determining the level or levels in a range of samples from subjects with and without the relevant cancer. The cancer, such as prostate cancer or ER positive breast cancer may be cancer with and/or without an increased likelihood of recurrence and/or metastasis and/or a poor prognosis. Suitable methods for setting a threshold are well known to those skilled in the art. The threshold may be mathematically derived from a training set of patient data. The score threshold thus separates the test samples according to presence or absence of the particular condition. The interpretation of this quantity, i.e. the cut-off threshold may be derived in a development or training phase from a set of patients with known outcome. The threshold may therefore be fixed prior to performance of the claimed methods from training data by methods known to those skilled in the art and as detailed herein in relation to generation of the various gene signatures.
- The reference value may also be a threshold level of expression of at least one gene set by determining the level of expression of the at least one gene in a sample from a subject at a first time point. The determined levels of expression at later time points for the same subject are then compared to the threshold level. Thus, the methods of the invention may be used in order to monitor progress of disease in a subject, namely to provide an ongoing characterization and/or prognosis of disease in the subject. For example, the methods may be used to identify (or "diagnose") a cancer, such as prostate cancer or ER positive breast cancer that has developed into a more aggressive or potentially metastatic form. This may be used to guide treatment decisions as discussed in further detail herein. In some embodiments, such monitoring methods determine whether treatment should be administered or not. If the cancer is identified within the metastatic biology group the cancer should be treated. If the cancer is identified as "non-metastatic" further monitoring can be performed to ensure that the cancer remains stable (i.e. does not evolve into the metastatic form). In such circumstances, no further treatment may be applied.
- For genes whose expression level does not differ between normal cells and cells from a cancer, such as prostate cancer or ER positive breast cancer that does not have an increased likelihood of recurrence and/or metastasis and/or a poor prognosis the expression level of the same gene in normal cells in the same sample can be used as a control.

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Different may be statistically significantly different. By statistically significant is meant unlikely to have occurred by chance alone. A suitable statistical assessment may be performed according to any suitable method.

5 The methods described herein may further comprise determining the expression level of a reference gene. A reference gene may be required if the target gene expression level differs between normal cells and cells from a cancer, such as prostate cancer or ER positive breast cancer that does not have an increased likelihood of recurrence and/or metastasis and/or a poor prognosis.

10

In certain embodiments the expression level of at least one gene selected from Table 1 is compared to the expression level of a reference gene.

15 The reference gene may be any gene with minimal expression variance across all cancer, such as prostate cancer or ER positive breast cancer samples. Thus, the reference gene may be any gene whose expression level does not vary with likelihood of recurrence and/or metastasis and/or a poor prognosis. The skilled person is well able to identify a suitable reference gene based upon these criteria. The expression level of the reference gene may be determined in the same sample as the expression level of at least one gene selected from
20 Table 1.

The expression level of the reference gene may be determined in a different sample. The different sample may be a control sample as described above. The expression level of the reference gene may be determined in normal cells and/or cancer, such as prostate cancer or
25 ER positive breast cancer, cells in a sample.

The expression level of the at least one gene in the sample from the subject may be analysed using a statistical model. In specific embodiments where the expression level of at least 2 genes, up to all 70 genes from Table 1, is measured the genes may be weighted. As
30 used herein, the term "weight" refers to the relative importance of an item in a statistical calculation. The weight of each gene may be determined on a data set of patient samples using analytical methods known in the art. An overall score, termed a "signature score", may be calculated and used to provide a characterisation of and/or prognosis for the cancer, such as prostate cancer or ER positive breast cancer. Typically, the score represents the sum of

the weighted gene expression levels. Suitable weights for calculating the 70 gene signature score are set forth in Table 1 and may be employed according to the methods of the invention. Similarly, suitable weights for exemplary smaller signatures are set forth in Tables 2 to 24.

5

Thus, according to all aspects of the invention, the methods may comprise:

- (i) determining the expression level of at least one gene selected from Table 1 in a sample from the subject; and
 - (ii) assessing from the expression level of the at least one gene whether the sample
- 10 from the subject is positive or negative for a gene signature comprising the at least one gene.

As discussed herein, if the sample is positive for the gene signature this identifies the cancer as of the high metastatic potential type. This may indicate a (relatively) poor prognosis, or any other pertinent associated characterisation, prognosis or diagnosis as described herein.

15 By corollary, a sample negative for the gene signature identifies the cancer as not of the high metastatic potential type. This may indicate a (relatively) good prognosis, or any other pertinent associated characterisation, prognosis or diagnosis as described herein.

Thus, at its simplest, an increased level of expression of one or more genes defines a
 20 sample as positive for the gene signature. For certain genes, a decreased level of expression of one or more gene defines a sample as positive for the gene signature. However, where the expression level of a plurality of genes is measured, the combination of expression levels is typically aggregated in order to determine whether the sample is positive for the gene signature. Thus, some genes may display increased expression and some
 25 genes may display decreased expression. This can be achieved in various ways, as discussed in detail herein.

In specific embodiments, the signature score may be calculated according to the following equation:

30

$$SignatureScore = \sum_i w_i \times (ge_i - b_i) + k$$

Where w_i is a weight for each gene, b_i is a gene-specific bias, ge_i is the gene expression after pre-processing, and k is a constant offset.

Similarly, each gene in the signature may be attributed a bias score. Example bias scores for the 70 gene signature are specified in table 1 and may be adopted according to the performance of the methods of the invention. Of course, where different signatures are utilised, representing a subset of the 70 gene signature, the bias values would be recalculated. Examples are provided in Tables 2 to 24.

As indicated, k is a constant offset. Where the bias and weight values of table 1 are adopted for the 70 gene signature, the constant offset may have a value of 0.4365. Again, where different signatures are utilised, representing a subset of the 70 gene signature, the value of k would be recalculated. The value of k varies dependent upon where the threshold for “signature positive” is set. This threshold may be set dependent upon which considerations are most important, e.g. to maximize sensitivity and/or specificity as against a particular outcome or characterisation. Suitable thresholds may be determined as described above.

In some embodiments, a score above the threshold may indicate a poor prognosis (or other pertinent characterisation, prognosis or diagnosis as described herein). In those embodiments, a score equal to or below threshold may indicate a good prognosis. In other embodiments, a score above or equal to the threshold may indicate a poor prognosis (or other pertinent characterisation, prognosis or diagnosis as described herein). In those embodiments, a score below threshold may indicate a good prognosis. The skilled person would also appreciate that a simple mathematical transformation could be used to invert the score and “above” and “below” should be construed accordingly unless indicated otherwise.

By “signature score” is meant a compound decision score that summarizes the expression levels of the genes. This may be compared to a threshold score that is mathematically derived from a training set of patient data. The threshold score is established with the purpose of maximizing the ability to separate cancers into those that are positive for the biomarker signature and those that are negative. The patient training set data is preferably derived from cancer tissue samples having been characterized by sub-type, prognosis, likelihood of recurrence, long term survival, clinical outcome, treatment response, diagnosis,

cancer classification, or personalized genomics profile. Expression profiles, and corresponding decision scores from patient samples may be correlated with the characteristics of patient samples in the training set that are on the same side of the mathematically derived score decision threshold. In certain example embodiments, the
5 threshold of the (linear) classifier scalar output is optimized to maximize the sum of sensitivity and specificity under cross-validation as observed within the training dataset.

The overall expression data for a given sample may be normalized using methods known to those skilled in the art in order to correct for differing amounts of starting material, varying
10 efficiencies of the extraction and amplification reactions, etc.

In one embodiment, the biomarker expression levels in a sample are evaluated by a (linear) classifier. As used herein, a (linear) classifier refers to a weighted sum of the individual biomarker intensities into a compound decision score ("decision function"). The decision
15 score is then compared to a pre-defined cut-off score threshold, corresponding to a certain set-point in terms of sensitivity and specificity which indicates if a sample is equal to or above the score threshold (decision function positive) or below (decision function negative).

Using a (linear) classifier on the normalized data to make a call (e.g. positive or negative for a biomarker signature) effectively means to split the data space, i.e. all possible
20 combinations of expression values for all genes in the classifier, into two disjoint segments by means of a separating hyperplane. This split is empirically derived on a (large) set of training examples. Without loss of generality, one can assume a certain fixed set of values for all but one biomarker, which would automatically define a threshold value for this remaining
25 biomarker where the decision would change from, for example, positive or negative for the biomarker signature. The precise value of this threshold depends on the actual measured expression profile of all other genes within the classifier, but the general indication of certain genes remains fixed. Therefore, in the context of the overall gene expression classifier, relative expression can indicate if either up- or down-regulation of a certain biomarker is
30 indicative of being positive for the signature or not. In certain example embodiments, a sample expression score above the threshold expression score indicates the sample is positive for the biomarker signature. In certain other example embodiments, a sample expression score above a threshold score indicates the subject has a poor clinical prognosis compared to a subject with a sample expression score below the threshold score.

In certain other example embodiments, the expression signature is derived using a decision tree (Hastie et al. *The Elements of Statistical Learning*, Springer, New York 2001), a random forest (Breiman, 2001 *Random Forests*, *Machine Learning* 45:5), a neural network (Bishop, 5 *Neural Networks for Pattern Recognition*, Clarendon Press, Oxford 1995), discriminant analysis (Duda et al. *Pattern Classification*, 2nd ed., John Wiley, New York 2001), including, but not limited to linear, diagonal linear, quadratic and logistic discriminant analysis, a Prediction Analysis for Microarrays (PAM, (Tibshirani et al., 2002, *Proc. Natl. Acad. Sci. USA* 99:6567-6572)) or a Soft Independent Modeling of Class Analogy analysis. (SIMCA, (Wold, 10 1976, *Pattern Recogn.* 8:127-139)). Classification trees (Breiman, Leo; Friedman, J. H.; Olshen, R. A.; Stone, C. J. (1984). *Classification and regression trees*. Monterey, CA: Wadsworth & Brooks/Cole Advanced Books & Software. ISBN 978-0-412-04841-8) provide a means of predicting outcomes based on logic and rules. A classification tree is built through a process called binary recursive partitioning, which is an iterative procedure of splitting the 15 data into partitions/branches. The goal is to build a tree that distinguishes among pre-defined classes. Each node in the tree corresponds to a variable. To choose the best split at a node, each variable is considered in turn, where every possible split is tried and considered, and the best split is the one which produces the largest decrease in diversity of the classification label within each partition. This is repeated for all variables, and the winner is chosen as the 20 best splitter for that node. The process is continued at the next node and in this manner, a full tree is generated. One of the advantages of classification trees over other supervised learning approaches such as discriminant analysis, is that the variables that are used to build the tree can be either categorical, or numeric, or a mix of both. In this way it is possible to generate a classification tree for predicting outcomes based on say the directionality of gene 25 expression.

Random forest algorithms (Breiman, Leo (2001). "Random Forests". *Machine Learning* 45 (1): 5–32. doi:10.1023/A:1010933404324) provide a further extension to classification trees, whereby a collection of classification trees are randomly generated to form a "forest" and an 30 average of the predicted outcomes from each tree is used to make inference with respect to the outcome.

Biomarker expression values may be defined in combination with corresponding scalar weights on the real scale with varying magnitude, which are further combined through linear

or non-linear, algebraic, trigonometric or correlative means into a single scalar value via an algebraic, statistical learning, Bayesian, regression, or similar algorithms which together with a mathematically derived decision function on the scalar value provide a predictive model by which expression profiles from samples may be resolved into discrete classes of responder
5 or non-responder, resistant or non-resistant, to a specified drug, drug class, molecular subtype, or treatment regimen. Such predictive models, including biomarker membership, are developed by learning weights and the decision threshold, optimized for sensitivity, specificity, negative and positive predictive values, hazard ratio or any combination thereof, under cross-validation, bootstrapping or similar sampling techniques, from a set of
10 representative expression profiles from historical patient samples with known drug response and/or resistance.

In one embodiment, the genes are used to form a weighted sum of their signals, where individual weights can be positive or negative. The resulting sum ("expression score") is
15 compared with a pre-determined reference point or value. The comparison with the reference point or value may be used to diagnose, or predict a clinical condition or outcome.

As described above, one of ordinary skill in the art will appreciate that the genes included in the classifier provided in the various Tables will carry unequal weights in a classifier.
20 Therefore, while as few as one biomarker may be used to diagnose or predict a clinical prognosis or response to a therapeutic agent, the specificity and sensitivity or diagnosis or prediction accuracy may increase using more genes.

In certain example embodiments, the expression signature is defined by a decision function.
25 A decision function is a set of weighted expression values derived using a (linear) classifier. All linear classifiers define the decision function using the following equation:
$$f(x) = w' \cdot x + b = \sum w_i \cdot x_i + b \quad (1)$$

All measurement values, such as the microarray gene expression intensities x_i , for a certain
30 sample are collected in a vector x . Each intensity is then multiplied with a corresponding weight w_i to obtain the value of the decision function $f(x)$ after adding an offset term b . In deriving the decision function, the linear classifier will further define a threshold value that splits the gene expression data space into two disjoint sections. Example (linear) classifiers include but are not limited to partial least squares (PLS), (Nguyen et al., Bioinformatics 18

(2002) 39-50), support vector machines (SVM) (Schölkopf et al., Learning with Kernels, MIT Press, Cambridge 2002), and shrinkage discriminant analysis (SDA) (Ahdesmäki et al., Annals of applied statistics 4, 503-519 (2010)). In one example embodiment, the (linear) classifier is a PLS linear classifier.

5

The decision function is empirically derived on a large set of training samples, for example from patients showing a good or poor clinical prognosis. The threshold separates a patient group based on different characteristics such as, but not limited to, clinical prognosis before or after a given therapeutic treatment. The interpretation of this quantity, i.e. the cut-off
10 threshold, is derived in the development phase ("training") from a set of patients with known outcome. The corresponding weights and the responsiveness/resistance cut-off threshold for the decision score are fixed a priori from training data by methods known to those skilled in the art. In one example embodiment, Partial Least Squares Discriminant Analysis (PLS-DA) is used for determining the weights. (L. Ståhle, S. Wold, J. Chemom. 1 (1987) 185-196; D. V.
15 Nguyen, D.M. Rocke, Bioinformatics 18 (2002) 39-50).

Effectively, this means that the data space, i.e. the set of all possible combinations of biomarker expression values, is split into two mutually exclusive groups corresponding to different clinical classifications or predictions, for example, one corresponding to good clinical
20 prognosis and poor clinical prognosis. In the context of the overall classifier, relative over-expression of a certain biomarker can either increase the decision score (positive weight) or reduce it (negative weight) and thus contribute to an overall decision of, for example, a good clinical prognosis.

25 In certain example embodiments of the invention, the data is transformed non-linearly before applying a weighted sum as described above. This non-linear transformation might include increasing the dimensionality of the data. The non-linear transformation and weighted summation might also be performed implicitly, for example, through the use of a kernel function. (Schölkopf et al. Learning with Kernels, MIT Press, Cambridge 2002).

30

In certain example embodiments, the patient training set data is derived by isolated RNA from a corresponding cancer tissue sample set and determining expression values by hybridizing the (cDNA amplified from) isolated RNA to a microarray. In certain example embodiments, the microarray used in deriving the expression signature is a transcriptome

array. As used herein a “transcriptome array” refers to a microarray containing probe sets that are designed to hybridize to sequences that have been verified as expressed in the diseased tissue of interest. Given alternative splicing and variable poly-A tail processing between tissues and biological contexts, it is possible that probes designed against the same
5 gene sequence derived from another tissue source or biological context will not effectively bind to transcripts expressed in the diseased tissue of interest, leading to a loss of potentially relevant biological information. Accordingly, it is beneficial to verify what sequences are expressed in the disease tissue of interest before deriving a microarray probe set.

Verification of expressed sequences in a particular disease context may be done, for
10 example, by isolating and sequencing total RNA from a diseased tissue sample set and cross-referencing the isolated sequences with known nucleic acid sequence databases to verify that the probe set on the transcriptome array is designed against the sequences actually expressed in the diseased tissue of interest. Methods for making transcriptome arrays are described in United States Patent Application Publication No. 2006/0134663,
15 which is incorporated herein by reference. In certain example embodiments, the probe set of the transcriptome array is designed to bind within 300 nucleotides of the 3’ end of a transcript. Methods for designing transcriptome arrays with probe sets that bind within 300 nucleotides of the 3’ end of target transcripts are disclosed in United States Patent Application Publication No. 2009/0082218, which is incorporated by reference herein. In
20 certain example embodiments, the microarray used in deriving the gene expression profiles of the present invention is the Almac Prostate Cancer DSA™ microarray (Almac Group, Craigavon, United Kingdom).

An optimal (linear) classifier can be selected by evaluating a (linear) classifier’s performance
25 using such diagnostics as “area under the curve” (AUC). AUC refers to the area under the curve of a receiver operating characteristic (ROC) curve, both of which are well known in the art. AUC measures are useful for comparing the accuracy of a classifier across the complete data range. (Linear) classifiers with a higher AUC have a greater capacity to classify unknowns correctly between two groups of interest (e.g., ovarian cancer samples and normal
30 or control samples). ROC curves are useful for plotting the performance of a particular feature (e.g., any of the genes described herein and/or any item of additional biomedical information) in distinguishing between two populations (e.g., individuals responding and not responding to a therapeutic agent). Typically, the feature data across the entire population (e.g., the cases and controls) are sorted in ascending order based on the value of a single

feature. Then, for each value for that feature, the true positive and false positive rates for the data are calculated. The true positive rate is determined by counting the number of cases above the value for that feature and then dividing by the total number of positive cases. The false positive rate is determined by counting the number of controls above the value for that feature and then dividing by the total number of controls. Although this definition refers to scenarios in which a feature is elevated in cases compared to controls, this definition also applies to scenarios in which a feature is lower in cases compared to the controls (in such a scenario, samples below the value for that feature would be counted). ROC curves can be generated for a single feature as well as for other single outputs, for example, a combination of two or more features can be mathematically combined (e.g., added, subtracted, multiplied, etc.) to provide a single sum value, and this single sum value can be plotted in a ROC curve. Additionally, any combination of multiple features, in which the combination derives a single output value, can be plotted in a ROC curve. These combinations of features may comprise a test. The ROC curve is the plot of the true positive rate (sensitivity) of a test against the false positive rate (1-specificity) of the test.

Alternatively, an optimal classifier can be selected by evaluating performance against time-to-event endpoints using methods such as Cox proportional hazards (PH) and measures of performance across all possible thresholds assessed via the concordance-index (C-index) (Harrell, Jr. 2010). The C-Index is analogous to the “area under the curve” (AUC) metric (used for dichotomised endpoints), and it is used to measure performance with respect to association with survival data. Note that the extension of AUC to time-to-event endpoints is the C-index, with threshold selection optimised to maximise the hazard ratio (HR) under cross-validation. In this instance, the partial Cox regression algorithm (Li and Gui, 2004) was chosen for the biomarker discovery analyses. It is analogous to principal components analysis in that the first few latent components explain most of the information in the data. Implementation is as described in Ahdesmaki et al 2013.

C-index values can be generated for a single feature as well as for other single outputs, for example, a combination of two or more features can be mathematically combined (e.g., added, subtracted, multiplied, etc.) to provide a single sum value, and this single sum value can be evaluated for statistical significance. Additionally, any combination of multiple features, in which the combination derives a single output value, can be evaluated as a C-index for assessing utility for time-to-event class separation. These combinations of features

may comprise a test. The C-index (Harrell, Jr. 2010, see Equation 4) of the continuous cross-validation test set risk score predictions was evaluated as the main performance measure.

Methods for determining the expression levels of the at least one gene from Table 1
5 (biomarkers) are described in greater detail herein. Typically, the methods may involve contacting a sample obtained from a subject with a detection agent, such as primers and/or probes, or an antibody or functionally equivalent binding reagent, (as discussed in detail herein) specific for the gene and detecting expression products. The detection agent may be
10 labelled as discussed herein. A comparison may be made against expression levels determined in a control sample to provide a characterization and/or a prognosis for the cancer, such as prostate cancer or ER positive breast cancer.

According to all aspects of the invention the expression level of the gene or genes may be measured by any suitable method. In certain embodiments the expression level is
15 determined at the level of protein, RNA or epigenetic modification. The epigenetic modification may be DNA methylation.

The expression level of any of the genes described herein may be detected by detecting the appropriate RNA. The assays may investigate specific regions of the genes, as described
20 herein. For example, the assays may investigate the regions flanked by specific primer binding sites and/or regions of the gene to which the probe sets described herein hybridize. The assays may investigate, promoter, terminator, exonic and/or intronic regions of the genes as appropriate. The assays may investigate one or more of the full sequences or target sequences, or regions thereof, as specified in Table 1 for the respective genes.

25 In certain embodiments, according to all aspects of the invention, expression of the at least one gene may be determined using one or more probes or primers (primer pairs) designed to hybridize with one or more of the target sequences or full sequences listed in Table 1. The probes and probesets identified in table 1 (and detailed further in Table 1A) may be
30 employed according to all aspects of the invention. The primers and primer pairs listed in Table 1B and identified as SEQ ID NOs 3151-3154 may be employed according to all aspects of the invention.

Accordingly, in specific embodiments the expression level is determined by microarray,

northern blotting, RNA-seq (RNA sequencing), in situ RNA detection or nucleic acid amplification. Nucleic acid amplification includes PCR and all variants thereof such as real-time and end point methods and quantitative PCR (qPCR). Other nucleic acid amplification techniques are well known in the art, and include methods such as NASBA, 3SR and
5 Transcription Mediated Amplification (TMA). Other suitable amplification methods include the ligase chain reaction (LCR), selective amplification of target polynucleotide sequences (US Patent No. 6,410,276), consensus sequence primed polymerase chain reaction (US Patent No 4,437,975), arbitrarily primed polymerase chain reaction (WO 90/06995), invader technology, strand displacement technology, and nick displacement amplification (WO
10 2004/067726). This list is not intended to be exhaustive; any nucleic acid amplification technique may be used provided the appropriate nucleic acid product is specifically amplified. Design of suitable primers and/or probes is within the capability of one skilled in the art. Various primer design tools are freely available to assist in this process such as the NCBI Primer-BLAST tool. Primers and/or probes may be at least 15, 16, 17, 18, 19, 20, 21, 22, 23,
15 24 or 25 (or more) nucleotides in length. mRNA expression levels may be measured by reverse transcription quantitative polymerase chain reaction (RT-PCR followed with qPCR). RT-PCR is used to create a cDNA from the mRNA. The cDNA may be used in a qPCR assay to produce fluorescence as the DNA amplification process progresses. By comparison to a standard curve, qPCR can produce an absolute measurement such as number of copies of
20 mRNA per cell. Northern blots, microarrays, Invader assays, and RT-PCR combined with capillary electrophoresis have all been used to measure expression levels of mRNA in a sample. See Gene Expression Profiling: Methods and Protocols, Richard A. Shinkets, editor, Humana Press, 2004. Many detection technologies are well known and commercially available, such as TAQMAN®, MOLECULAR BEACONS®, AMPLIFLUOR® and
25 SCORPION®, DzyNA®, Plexor™ etc.

Suitable amplification assays (PCR or qPCR) have been designed by the inventors and are described in further detail in Table 1B. The forward and reverse primers listed therein for each gene may be utilized according to all aspects of the invention. Similarly, the primers of
30 SEQ ID NOs 3151-3154 may be used to amplify MIR578 and MIR4530 respectively.

RNA-seq uses next-generation sequencing to measure changes in gene expression. RNA may be converted into cDNA or directly sequenced. Next generation sequencing techniques include pyrosequencing, SOLiD sequencing, Ion Torrent semiconductor sequencing, Illumina

dye sequencing, single-molecule real-time sequencing or DNA nanoball sequencing. RNA-seq allows quantitation of gene expression levels.

In situ RNA detection involves detecting RNA without extraction from tissues and cells. In
5 situ RNA detection includes in situ hybridization (ISH) which uses a labeled (e.g. radio
labelled, antigen labelled or fluorescence labelled) probe (complementary DNA or RNA
strand) to localize a specific RNA sequence in a portion or section of tissue, or in the entire
tissue (whole mount ISH), or in cells. The probe labeled with either radio-, fluorescent- or
antigen-labeled bases (e.g., digoxigenin) may be localized and quantified in the tissue using
10 either autoradiography, fluorescence microscopy or immunohistochemistry, respectively. ISH
can also use two or more probes to simultaneously detect two or more transcripts. A
branched DNA assay can also be used for RNA in situ hybridization assays with single
molecule sensitivity. This approach includes ViewRNA assays. Samples (cells, tissues) are
fixed, then treated to allow RNA target accessibility (RNA un-masking). Target-specific
15 probes hybridize to each target RNA. Subsequent signal amplification is predicated on
specific hybridization of adjacent probes (individual oligonucleotides that bind side by side on
RNA targets). A typical target-specific probe will contain 40 oligonucleotides. Signal
amplification is achieved via a series of sequential hybridization steps. A pre-amplifier
molecule hybridizes to each oligo pair on the target-specific RNA, then multiple amplifier
20 molecules hybridize to each pre-amplifier. Next, multiple label probe oligonucleotides
(conjugated to an enzyme such as alkaline phosphatase or directly to fluorophores) hybridize
to each amplifier molecule. Separate but compatible signal amplification systems enable
multiplex assays. The signal can be visualized by measuring fluorescence or light emitted
depending upon the detection system employed. Detection may involve using a high content
25 imaging system, or a fluorescence or brightfield microscope in some embodiments.

Thus, in a further aspect the present invention relates to use of the kit for characterising
and/or prognosing cancer, such as prostate cancer or ER positive breast cancer. The kit for
(in situ) characterising and/or prognosing prostate cancer in a subject may comprise one or
30 more oligonucleotide probes specific for an RNA product of at least one gene selected from
Table 1. Suitable probes and probesets for each gene are listed in Table 1 and may be
incorporated in the kits of the invention. The probes and probesets also constitute separate
aspects of the invention. By "probeset" is meant the collection of probes designed to target
(by hybridization) a single gene. The groupings are apparent from table 1 (and Table 1A).

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The kit may further comprise one or more of the following components:

- a) A blocking probe
- b) A PreAmplifier
- c) An Amplifier and/or
- 5 d) A Label molecule

The components of the kit may be suitable for conducting a viewRNA assay (<https://www.panomics.com/products/rna-in-situ-analysis/view-rna-overview>).

The components of the kit may be nucleic acid based molecules, optionally DNA (or RNA).

- 10 The blocking probe is a molecule that acts to reduce background signal by binding to sites on the target not bound by the target specific probes (probes specific for the RNA product of the at least one gene of the invention). The PreAmplifier is a molecule capable of binding to a (a pair of) target specific probe(s) when target bound. The Amplifier is a molecule capable of binding to the PreAmplifier. Alternatively, the Amplifier may be capable of binding directly to
- 15 a (a pair of) target specific probe(s) when target bound. The Amplifier has binding sites for multiple label molecules (which may be label probes).

- RNA expression may be determined by hybridization of RNA to a set of probes. The probes may be arranged in an array. Microarray platforms include those manufactured by
- 20 companies such as Affymetrix, Illumina and Agilent. Examples of microarray platforms manufactured by Affymetrix include the U133 Plus2 array, the Almac proprietary Xcel™ array and the Almac proprietary Cancer DSAs®, including the Prostate Cancer DSA®.

- In specific embodiments, according to all aspects of the invention, expression of the at least
- 25 one gene may be determined using one or more probes selected from those listed in Table 1.

- In certain embodiments, according to all aspects of the invention, expression of the at least one gene may be determined using one or more probes or primers designed to hybridize with the target sequences or full sequences listed in Table 1.

- 30 These probes may also be incorporated into the kits of the invention. The probe sequences may also be used in order to design primers for detection of expression, for example by RT-

PCR. Such primers may also be included in the kits of the invention. Suitable primers are listed in Table 1B and SEQ ID NOs 3151-3154.

The corresponding target sequences are listed in Table 1 below for the relevant probesets.

5 The invention may involve use of different probes that target any one or more of these target sequences.

Similarly, the full gene sequences are listed in Table 1 for the relevant probesets. The invention may involve use of different probes that target any one or more of these full gene
10 sequences as target sequences.

Increased rates of DNA methylation at or near promoters have been shown to correlate with reduced gene expression levels. DNA methylation is the main epigenetic modification in humans. It is a chemical modification of DNA performed by enzymes called
15 methyltransferases, in which a methyl group (m) is added to specific cytosine (C) residues in DNA. In mammals, methylation occurs only at cytosine residues adjacent to a guanosine residue, i.e. at the sequence CG or at the CpG dinucleotide.

Accordingly, in yet a further aspect, the present invention relates to a method for
20 characterising and/or prognosing cancer, such as prostate cancer or ER positive breast cancer in a subject comprising:

determining the methylation status of at least one gene selected from Table 1 in a sample from the subject wherein the determined methylation status is used to provide a characterisation of and/or a prognosis for the cancer, such as prostate cancer or ER positive
25 breast cancer.

Methylation typically results in a down regulation of gene expression. Thus, methylation (which may be hypermethylation) of the genes with a negative weighting in table 1 may be determined according to some embodiments in order to indicate a poor prognosis (or related
30 outcome as described herein). Additionally or alternatively, a lack of methylation (which may be hypomethylation) of the genes with a positive weighting in table 1 may be determined according to some embodiments in order to indicate a poor prognosis (or related outcome as described herein).

Determination of the methylation status may be achieved through any suitable means.

Suitable examples include bisulphite genomic sequencing and/or by methylation specific

PCR. Various techniques for assessing methylation status are known in the art and can be used in conjunction with the present invention: sequencing (including NGS), methylation-

5 specific PCR (MS-PCR), melting curve methylation-specific PCR(McMS-PCR), MLPA with or without bisulphite treatment, QAMA (Zeschnigk et al, 2004), MSRE-PCR (Melnikov et al, 2005), MethyLight (Eads et al., 2000), ConLight-MSP (Rand et al., 2002), bisulphite conversion-specific methylation-specific PCR (BS-MSP)(Sasaki et al., 2003), COBRA (which relies upon use of restriction enzymes to reveal methylation dependent sequence differences
10 in PCR products of sodium bisulphite - treated DNA), methylation-sensitive single-nucleotide primer extension conformation(MS-SNuPE), methylation-sensitive single-strand conformation analysis (MS-SSCA), Melting curve combined bisulphite restriction analysis (McCOBRA)(Akey et al., 2002), PyroMethA, HeavyMethyl (Cottrell et al. 2004), MALDI-TOF, MassARRAY, Quantitative analysis of methylated alleles (QAMA), enzymatic regional
15 methylation assay (ERMA), QBSUPT, MethylQuant, Quantitative PCR sequencing and oligonucleotide-based microarray systems, Pyrosequencing, Meth-DOP-PCR. A review of some useful techniques for DNA methylation analysis is provided in Nucleic acids research, 1998, Vol. 26, No. 10, 2255-2264, Nature Reviews, 2003, Vol.3, 253-266; Oral Oncology, 2006, Vol. 42, 5-13.

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Techniques for assessing methylation status are based on distinct approaches. Some include use of endonucleases. Such endonucleases may either preferentially cleave methylated recognition sites relative to non-methylated recognition sites or preferentially cleave non-methylated relative to methylated recognition sites. Some examples of the
25 former are Acc III, Ban I, BstN I, Msp I, and Xma I. Examples of the latter are Acc II, Ava I, BssH II, BstU I, Hpa II, and Not I. Differences in cleavage pattern are indicative for the presence or absence of a methylated CpG dinucleotide. Cleavage patterns can be detected directly, or after a further reaction which creates products which are easily distinguishable. Means which detect altered size and/or charge can be used to detect modified products,
30 including but not limited to electrophoresis, chromatography, and mass spectrometry.

Alternatively, the identification of methylated CpG dinucleotides may utilize the ability of the methyl binding domain (MBD) of the MeCP2 protein to selectively bind to methylated DNA sequences (Cross et al, 1994; Shiraishi et al, 1999). The MBD may also be obtained from

MBP, MBP2, MBP4, poly-MBD (Jorgensen et al., 2006) or from reagents such as antibodies binding to methylated nucleic acid. The MBD may be immobilized to a solid matrix and used for preparative column chromatography to isolate highly methylated DNA sequences.

Variant forms such as expressed His-tagged methyl-CpG binding domain may be used to
5 selectively bind to methylated DNA sequences. Eventually, restriction endonuclease digested genomic DNA is contacted with expressed His-tagged methyl-CpG binding domain. Other methods are well known in the art and include amongst others methylated-CpG island recovery assay (MIRA). Another method, MB-PCR, uses a recombinant, bivalent methyl-CpG-binding polypeptide immobilized on the walls of a PCR vessel to capture methylated
10 DNA and the subsequent detection of bound methylated DNA by PCR.

Further approaches for detecting methylated CpG dinucleotide motifs use chemical reagents that selectively modify either the methylated or non-methylated form of CpG dinucleotide motifs. Suitable chemical reagents include hydrazine and bisulphite ions. The methods of
15 the invention may use bisulphite ions, in certain embodiments. The bisulphite conversion relies on treatment of DNA samples with sodium bisulphite which converts unmethylated cytosine to uracil, while methylated cytosines are maintained (Furuichi et al., 1970). This conversion finally results in a change in the sequence of the original DNA. It is general knowledge that the resulting uracil has the base pairing behaviour of thymidine which differs
20 from cytosine base pairing behaviour. This makes the discrimination between methylated and non-methylated cytosines possible. Useful conventional techniques of molecular biology and nucleic acid chemistry for assessing sequence differences are well known in the art and explained in the literature. See, for example, Sambrook, J., et al., Molecular cloning: A laboratory Manual, (2001) 3rd edition, Cold Spring Harbor, NY; Gait, M.J.(ed.),
25 Oligonucleotide Synthesis, A Practical Approach, IRL Press (1984); Hames B.D., and Higgins, S.J. (eds.), Nucleic Acid Hybridization, A Practical Approach, IRL Press (1985); and the series, Methods in Enzymology, Academic Press, Inc.

Some techniques use primers for assessing the methylation status at CpG dinucleotides.

30 Two approaches to primer design are possible. Firstly, primers may be designed that themselves do not cover any potential sites of DNA methylation. Sequence variations at sites of differential methylation are located between the two primers and visualisation of the sequence variation requires further assay steps. Such primers are used in bisulphite genomic sequencing, COBRA, Ms-SnuPE and several other techniques. Secondly, primers

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may be designed that hybridize specifically with either the methylated or unmethylated version of the initial treated sequence. After hybridization, an amplification reaction can be performed and amplification products assayed using any detection system known in the art. The presence of an amplification product indicates that a sample hybridized to the primer.

5 The specificity of the primer indicates whether the DNA had been modified or not, which in turn indicates whether the DNA had been methylated or not. If there is a sufficient region of complementarity, e.g., 12, 15, 18, or 20 nucleotides, to the target, then the primer may also contain additional nucleotide residues that do not interfere with hybridization but may be useful for other manipulations. Examples of such other residues may be sites for restriction
10 endonuclease cleavage, for ligand binding or for factor binding or linkers or repeats. The oligonucleotide primers may or may not be such that they are specific for modified methylated residues.

A further way to distinguish between modified and unmodified nucleic acid is to use
15 oligonucleotide probes. Such probes may hybridize directly to modified nucleic acid or to further products of modified nucleic acid, such as products obtained by amplification. Probe-based assays exploit the oligonucleotide hybridisation to specific sequences and subsequent detection of the hybrid. There may also be further purification steps before the amplification product is detected e.g. a precipitation step. Oligonucleotide probes may be labeled using
20 any detection system known in the art. These include but are not limited to fluorescent moieties, radioisotope labeled moieties, bioluminescent moieties, luminescent moieties, chemiluminescent moieties, enzymes, substrates, receptors, or ligands.

In the MSP approach, DNA may be amplified using primer pairs designed to distinguish
25 methylated from unmethylated DNA by taking advantage of sequence differences as a result of sodium-bisulphite treatment (WO 97/46705). For example, bisulphite ions modify non-methylated cytosine bases, changing them to uracil bases. Uracil bases hybridize to adenine bases under hybridization conditions. Thus an oligonucleotide primer which comprises adenine bases in place of guanine bases would hybridize to the bisulphite-modified DNA,
30 whereas an oligonucleotide primer containing the guanine bases would hybridize to the non-modified (methylated) cytosine residues in the DNA. Amplification using a DNA polymerase and a second primer yield amplification products which can be readily observed, which in turn indicates whether the DNA had been methylated or not. Whereas PCR is a preferred

amplification method, variants on this basic technique such as nested PCR and multiplex PCR are also included within the scope of the invention.

- As mentioned earlier, one embodiment for assessing the methylation status of the relevant gene requires amplification to yield amplification products. The presence of amplification products may be assessed directly using methods well known in the art, and the ensuing discussion also applies to all other amplification embodiments as described herein.. They simply may be visualized on a suitable gel, such as an agarose or polyacrylamide gel. Detection may involve the binding of specific dyes, such as ethidium bromide, which intercalate into double-stranded DNA and visualisation of the DNA bands under a UV illuminator for example. Another means for detecting amplification products comprises hybridization with oligonucleotide probes. Alternatively, fluorescence or energy transfer can be measured to determine the presence of the methylated DNA.
- A specific example of the MSP technique is designated real-time quantitative MSP (QMSP), and permits reliable quantification of methylated DNA in real time or at end point. Real-time methods are generally based on the continuous optical monitoring of an amplification procedure and utilise fluorescently labelled reagents whose incorporation in a product can be quantified and whose quantification is indicative of copy number of that sequence in the template. One such reagent is a fluorescent dye, called SYBR Green I that preferentially binds double-stranded DNA and whose fluorescence is greatly enhanced by binding of double-stranded DNA. Alternatively, labelled primers and/or labelled probes can be used for quantification. They represent a specific application of the well-known and commercially available real-time amplification techniques such as TAQMAN®, MOLECULAR BEACONS®, AMPLIFLUOR® and SCORPION®, DzyNA®, Plexor™ etc. In the real-time PCR systems, it is possible to monitor the PCR reaction during the exponential phase where the first significant increase in the amount of PCR product correlates to the initial amount of target template.
- Real-Time PCR detects the accumulation of amplicon during the reaction. Real-time methods do not need to be utilised, however. Many applications do not require quantification and Real-Time PCR is used only as a tool to obtain convenient results presentation and storage, and at the same time to avoid post-PCR handling. Thus, analyses can be performed only to

confirm whether the target DNA is present in the sample or not. Such end-point verification is carried out after the amplification reaction has finished.

- The expression level of one or more genes from Table 1 may be determined by
- 5 immunohistochemistry. By Immunohistochemistry is meant the detection of proteins in cells of a tissue sample by using a binding reagent such as an antibody or aptamer that binds specifically to the proteins. Thus, the expression level as determined by immunohistochemistry is a protein level. The sample may be a tissue sample and may comprise cancer (tumour) cells, normal tissue cells and, optionally, infiltrating immune cells.
- 10 In embodiments applicable to prostate cancer, the sample may be a prostate tissue sample and may comprise prostate cancer (tumour) cells, prostatic intraepithelial neoplasia (PIN) cells, normal prostate epithelium, stroma and, optionally, infiltrating immune cells. In some embodiments the expression level of the at least one gene in the cancer (tumour) cells in a sample is compared to the expression level of the same gene (and/or a reference gene) in
- 15 the normal cells in the same sample. In some embodiments the expression level of the at least one gene in the cancer (tumour) cells in a sample is compared to the expression level of the same gene (and/or a reference gene) in the normal cells in a control sample. The normal cells may comprise, consist essentially of or consist of normal (non-cancer) epithelial cells. In certain embodiments the normal cells do not comprise PIN cells and/or stroma cells.
- 20 In certain embodiments the prostate cancer (tumour) cells do not comprise PIN cells and/or stroma cells. In further embodiments the expression level of the at least one gene in the prostate cancer (tumour) cells in a sample is (additionally) compared to the expression level of a reference gene in the same cells or in the prostate cancer cells in a control sample. In yet further embodiments the expression level of the at least one gene in the cancer (tumour)
- 25 cells in a sample is scored using a method based on intensity, proportion and/or localisation of expression in the cancer (tumour) cells (without comparison to normal cells). The scoring method may be derived in a development or training phase from a set of patients with known outcome.
- 30 Accordingly, in a further aspect, the present invention relates to an antibody or aptamer that binds specifically to a protein product of at least one gene selected from Table 1. The epitope to which the antibody or aptomer binds may be derived from the amino acid sequences corresponding to the full sequences or target sequences identified in Table 1.

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The antibody may be of monoclonal or polyclonal origin. Fragments and derivative antibodies may also be utilised, to include without limitation Fab fragments, ScFv, single domain antibodies, nanoantibodies, heavy chain antibodies, aptamers etc. which retain peptide-specific binding function and these are included in the definition of "antibody". Such antibodies are useful in the methods of the invention. They may be used to measure the level of a particular protein, or in some instances one or more specific isoforms of a protein. The skilled person is well able to identify epitopes that permit specific isoforms to be discriminated from one another.

10 Methods for generating specific antibodies are known to those skilled in the art. Antibodies may be of human or non-human origin (e.g. rodent, such as rat or mouse) and be humanized etc. according to known techniques (Jones *et al.*, Nature (1986) May 29-Jun. 4;321(6069):522-5; Roguska *et al.*, Protein Engineering, 1996, 9(10):895-904; and Studnicka *et al.*, Humanizing Mouse Antibody Frameworks While Preserving 3-D Structure. Protein Engineering, 1994, Vol.7, pg 805).

20 In certain embodiments the expression level is determined using an antibody or aptamer conjugated to a label. By label is meant a component that permits detection, directly or indirectly. For example, the label may be an enzyme, optionally a peroxidase, or a fluorophore.

A label is an example of, and may form part of, a detection agent. By detection agent is meant an agent that may be used to assist in the detection of the complex between binding reagent (which may be an antibody, primer or probe for example) and target. The binding agent may form part of the overall detection agent. Where the antibody is conjugated to an enzyme the detection agent may be comprise a chemical composition such that the enzyme catalyses a chemical reaction to produce a detectable product. The products of reactions catalyzed by appropriate enzymes can be, without limitation, fluorescent, luminescent, or radioactive or they may absorb visible or ultraviolet light. Examples of detectors suitable for detecting such detectable labels include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers. In certain embodiments the detection agent may comprise a secondary antibody. The expression level is then determined using an unlabeled primary antibody that

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binds to the target protein and a secondary antibody conjugated to a label, wherein the secondary antibody binds to the primary antibody.

The invention also relates to use of an antibody or aptamer as described above for
5 characterising and/or prognosing a cancer, such as prostate cancer or ER positive breast cancer in a subject.

Additional techniques for determining expression level at the level of protein include, for example, Western blot, immunoprecipitation, immunocytochemistry, mass spectrometry,
10 ELISA and others (see ImmunoAssay: A Practical Guide, edited by Brian Law, published by Taylor & Francis, Ltd., 2005 edition). To improve specificity and sensitivity of an assay method based on immunoreactivity, monoclonal antibodies are often used because of their specific epitope recognition. Polyclonal antibodies have also been successfully used in various immunoassays because of their increased affinity for the target as compared to
15 monoclonal antibodies.

According to all aspects of the invention samples may be of any suitable form. The sample is typically intended to contain nucleic acids (DNA and/or RNA), or protein in some embodiments, from the primary tumour (even if no longer contained within the tumour cells
20 e.g. shed into the circulation). The sample may comprise, consist essentially of or consist of cells, such as prostate or breast cells and often a suitable tissue sample (such as a prostate or breast tissue sample). The sample may comprise or be a primary tumour sample. The cells or tissue may comprise cancer cells, such as prostate cancer cells or ER positive breast cancer cells. In specific embodiments the sample comprises, consists essentially of or
25 consists of a biopsy sample, which may be fixed, such as a formalin-fixed paraffin-embedded biopsy sample. The tissue sample may be obtained by any suitable technique. Examples include a biopsy procedure, optionally a fine needle aspirate biopsy procedure. Body fluid samples may also be utilised. Samples may comprise resection material (e.g. where radical prostatectomy has been performed). Suitable sample types include blood, to encompass
30 whole blood, serum and plasma samples, urine and semen.

The methods described herein may further comprise extracting nucleic acids, DNA and/or RNA from the sample. Suitable methods are known in the art and include use of commercially available kits such as Rneasy and GeneJET RNA purification kit.

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In certain embodiments the methods may further comprise obtaining the sample from the subject. Typically the methods are in vitro methods performed on an isolated sample.

- 5 The methods of the invention may prove useful for determining which patients should undergo a more aggressive therapeutic regime, by identifying high risk cancers (i.e, those within the high metastatic potential group and thus having a poor prognosis).

10 The methods of the invention may comprise selecting a treatment for cancer, such as prostate cancer or ER positive breast cancer in a subject and optionally performing the treatment. In certain embodiments if the characterisation of and/or prognosis for the cancer, such as prostate cancer or ER positive breast cancer is an increased likelihood of recurrence and/or metastasis and/or a poor prognosis the treatment selected may be one or more of

- 15 a) an anti-hormone treatment
 b) a cytotoxic agent
 c) a biologic
 d) radiotherapy
 e) targeted therapy
 20 f) surgery

By anti-hormone treatment (or hormone therapy) is meant a form of treatment which reduces the level and/or activity of selected hormones, in particular testosterone. The hormones may promote tumour growth and/or metastasis. The anti-hormone treatment may comprise a
 25 luteinizing hormone blocker, such as goserelin (also called Zoladex), buserelin, leuporelin (also called Prostag), histrelin (Vantas) and triptorelin (also called Decapeptyl). The anti-hormone treatment may comprise a gonadotrophin release hormone (GnRH) blocker such as degarelix (Firmagon) or an anti-androgen such as flutamide (also called Drogenil) and bicalutamide (also called Casodex). In specific embodiments the anti-hormone treatment
 30 may be bicalutamide and/or abiraterone.

The cytotoxic agent may be administered as an adjuvant therapy. The cytotoxic agent may be a platinum based agent and/or a taxane. In specific embodiments the platinum based agent is selected from cisplatin, carboplatin and oxaliplatin. The taxane may be paclitaxel,

cabazitaxel or docetaxel. The cytotoxic agent may also be a vinca alkaloid, such as vinorelbine or vinblastine. The cytotoxic agent may be a topoisomerase inhibitor such as etoposide or an anthracycline (antibiotic) such as doxorubicin. The cytotoxic agent may be an alkylating agent such as estramustine. Adjuvant taxane and/or topoisomerase inhibitor
5 therapy may be particularly suitable for treatment of ER positive breast cancer.

By biologic is meant a medicinal product that is created by a biological process. A biologic may be, for example, a vaccine, blood or blood component, cells, gene therapy, tissue, or a recombinant therapeutic protein. Optionally the biologic is an antibody and/or a vaccine.
10 The biologic may be Sipuleucel-T. The biologic may be a cancer immunotherapy.

In certain embodiments the radiotherapy is extended radiotherapy, preferably extended-field radiotherapy. In specific embodiments, the radiotherapy comprises or is (pelvic) lymph node irradiation. Adjuvant radiation may be employed.
15

Surgery may comprise radical prostatectomy. By radical prostatectomy is meant removal of the entire prostate gland, the seminal vesicles and the vas deferens. In further embodiments surgery comprises tumour resection i.e. removal of all or part of the tumour. Surgery may comprise or be extended nodal dissection.
20

By targeted therapy is meant treatment using targeted therapeutic agents which are directed towards a specific drug target for the treatment of a cancer, such as prostate cancer or ER positive breast cancer. In specific embodiments this may mean inhibitors directed towards targets such as PARP, AKT, MET, VEGFR etc. PARP inhibitors are a group of
25 pharmacological inhibitors of the enzyme poly ADP ribose polymerase (PARP). Several forms of cancer are more dependent on PARP than regular cells, making PARP an attractive target for cancer therapy. Examples (in clinical trials) include iniparib, olaparib, rucaparib, veliparib, CEP 9722, MK 4827, BMN-673 and 3-aminobenzamide. AKT, also known as Protein Kinase B (PKB), is a serine/threonine-specific protein kinase that plays a key role in
30 multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration. AKT is associated with tumor cell survival, proliferation, and invasiveness. Examples of AKT inhibitors include VQD-002, Perifosine, Miltefosine and AZD5363. MET is a proto-oncogene that encodes hepatocyte growth factor receptor (HGFR). The hepatocyte growth factor receptor protein possesses tyrosine-kinase activity. Examples

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of kinase inhibitors for inhibition of MET include K252a, SU11274, PHA-66752, ARQ197, Foretinib, SGX523 and MP470. MET activity can also be blocked by inhibiting the interaction with HGF. Many suitable antagonists including truncated HGF, anti-HGF antibodies and uncleavable HGF are known. VEGF receptors are receptors for vascular endothelial growth factor (VEGF). Various inhibitors are known such as lenvatinib, motesanib, pazopanib and regorafenib.

If the method identifies the cancer as not within the high metastatic potential group, then different decisions may be taken. If the cancer has already been treated e.g. by radiotherapy or surgery, the decision may be taken not to treat the cancer further. The decision may be taken to continue to monitor the cancer, by any suitable means (e.g. by PSA levels or using the methods of the invention), and not perform any further treatment if the cancer remains in the same state.

The methods of the present invention can guide therapy selection as well as selecting patient groups for enrichment strategies during clinical trial evaluation of novel therapeutics. For example, when evaluating a putative anti-cancer agent or treatment regime, the methods disclosed herein may be used to select individuals for clinical trials that have cancer, such as prostate cancer or ER positive breast cancer, characterized as having an increased likelihood of recurrence and/or metastasis and/or a poor prognosis.

The invention also relates to a system or device or test kit for performing a method as described herein.

In a further aspect, the present invention relates to a system, device or test kit for characterising and/or prognosing cancer, such as prostate cancer or ER positive breast cancer in a subject, comprising:

- a) one or more testing devices that determine the expression level of at least gene selected from Table 1 in a sample from the subject
- b) a processor; and
- c) storage medium comprising a computer application that, when executed by the processor, is configured to:
 - (i) access and/or calculate the determined expression levels of the at least gene selected from Table 1 in the sample on the one or more testing devices

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- (ii) calculate whether there is an increased or decreased level of the at least one gene selected from Table 1 in the sample; and
- (iii) output from the processor the characterisation of and/or prognosis for the cancer, such as prostate cancer or ER positive breast cancer.

5

By testing device is meant a combination of components that allows the expression level of a gene to be determined. The components may include any of those described above with respect to the methods for determining expression level at the level of protein, RNA or epigenetic modification. For example the components may be antibodies, primers, detection agents and so on. Components may also include one or more of the following: microscopes, microscope slides, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers. The discussion of the methods of the invention thus applies mutatis mutandis to these aspects of the invention.

10

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In certain embodiments the system, device or test kit further comprises a(n electronic) display for the output from the processor.

20

The invention also relates to a computer application or storage medium comprising a computer application as defined above.

25

In certain example embodiments, provided is a computer-implemented method, system, and a computer program product for characterising and/or prognosing cancer, such as prostate cancer or ER positive breast cancer in a subject, in accordance with the methods described herein. For example, the computer program product may comprise a non-transitory computer-readable storage device having computer-readable program instructions embodied thereon that, when executed by a computer, cause the computer to characterise and/or prognose cancer, such as prostate cancer or ER positive breast cancer in a subject as described herein. For example, the computer executable instructions may cause the computer to:

30

- (i) access and/or calculate the determined expression levels of the at least one gene selected from Table 1 in a sample on one or more testing devices;
- (ii) calculate whether there is an increased or decreased level of the at least one gene selected from Table 1 in the sample; and,

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(iii) provide an output regarding the characterization of and/or prognosis for the cancer, such as prostate cancer or ER positive breast cancer.

5 In certain example embodiments, the computer-implemented method, system, and computer program product may be embodied in a computer application, for example, that operates and executes on a computing machine and a module. When executed, the application may characterise and/or prognose cancer, such as prostate cancer or ER positive breast cancer in a subject, in accordance with the example embodiments described herein.

10 As used herein, the computing machine may correspond to any computers, servers, embedded systems, or computing systems. The module may comprise one or more hardware or software elements configured to facilitate the computing machine in performing the various methods and processing functions presented herein. The computing machine may include various internal or attached components such as a processor, system bus,
15 system memory, storage media, input/output interface, and a network interface for communicating with a network, for example. The computing machine may be implemented as a conventional computer system, an embedded controller, a laptop, a server, a customized machine, any other hardware platform, such as a laboratory computer or device, for example, or any combination thereof. The computing machine may be a distributed
20 system configured to function using multiple computing machines interconnected via a data network or bus system, for example.

The processor may be configured to execute code or instructions to perform the operations and functionality described herein, manage request flow and address mappings, and to
25 perform calculations and generate commands. The processor may be configured to monitor and control the operation of the components in the computing machine. The processor may be a general purpose processor, a processor core, a multiprocessor, a reconfigurable processor, a microcontroller, a digital signal processor ("DSP"), an application specific integrated circuit ("ASIC"), a graphics processing unit ("GPU"), a field programmable gate
30 array ("FPGA"), a programmable logic device ("PLD"), a controller, a state machine, gated logic, discrete hardware components, any other processing unit, or any combination or multiplicity thereof. The processor may be a single processing unit, multiple processing units, a single processing core, multiple processing cores, special purpose processing cores, co-processors, or any combination thereof. According to certain example embodiments, the

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processor, along with other components of the computing machine, may be a virtualized computing machine executing within one or more other computing machines.

5 The system memory may include non-volatile memories such as read-only memory ("ROM"), programmable read-only memory ("PROM"), erasable programmable read-only memory ("EPROM"), flash memory, or any other device capable of storing program instructions or data with or without applied power. The system memory may also include volatile memories such as random access memory ("RAM"), static random access memory ("SRAM"), dynamic random access memory ("DRAM"), and synchronous dynamic random access memory
10 ("SDRAM"). Other types of RAM also may be used to implement the system memory. The system memory may be implemented using a single memory module or multiple memory modules. While the system memory may be part of the computing machine, one skilled in the art will recognize that the system memory may be separate from the computing machine without departing from the scope of the subject technology. It should also be appreciated
15 that the system memory may include, or operate in conjunction with, a non-volatile storage device such as the storage media.

The storage media may include a hard disk, a floppy disk, a compact disc read only memory ("CD-ROM"), a digital versatile disc ("DVD"), a Blu-ray disc, a magnetic tape, a flash memory,
20 other non-volatile memory device, a solid state drive ("SSD"), any magnetic storage device, any optical storage device, any electrical storage device, any semiconductor storage device, any physical-based storage device, any other data storage device, or any combination or multiplicity thereof. The storage media may store one or more operating systems, application programs and program modules such as module, data, or any other information. The
25 storage media may be part of, or connected to, the computing machine. The storage media may also be part of one or more other computing machines that are in communication with the computing machine, such as servers, database servers, cloud storage, network attached storage, and so forth.

30 The module may comprise one or more hardware or software elements configured to facilitate the computing machine with performing the various methods and processing functions presented herein. The module may include one or more sequences of instructions stored as software or firmware in association with the system memory, the storage media, or both. The storage media may therefore represent examples of machine or computer

readable media on which instructions or code may be stored for execution by the processor. Machine or computer readable media may generally refer to any medium or media used to provide instructions to the processor. Such machine or computer readable media associated with the module may comprise a computer software product. It should be appreciated that a computer software product comprising the module may also be associated with one or more processes or methods for delivering the module to the computing machine via a network, any signal-bearing medium, or any other communication or delivery technology. The module may also comprise hardware circuits or information for configuring hardware circuits such as microcode or configuration information for an FPGA or other PLD.

The input/output ("I/O") interface may be configured to couple to one or more external devices, to receive data from the one or more external devices, and to send data to the one or more external devices. Such external devices along with the various internal devices may also be known as peripheral devices. The I/O interface may include both electrical and physical connections for operably coupling the various peripheral devices to the computing machine or the processor. The I/O interface may be configured to communicate data, addresses, and control signals between the peripheral devices, the computing machine, or the processor. The I/O interface may be configured to implement any standard interface, such as small computer system interface ("SCSI"), serial-attached SCSI ("SAS"), fiber channel, peripheral component interconnect ("PCI"), PCI express (PCIe), serial bus, parallel bus, advanced technology attached ("ATA"), serial ATA ("SATA"), universal serial bus ("USB"), Thunderbolt, FireWire, various video buses, and the like. The I/O interface may be configured to implement only one interface or bus technology.

Alternatively, the I/O interface may be configured to implement multiple interfaces or bus technologies. The I/O interface may be configured as part of, all of, or to operate in conjunction with, the system bus. The I/O interface may include one or more buffers for buffering transmissions between one or more external devices, internal devices, the computing machine, or the processor.

The I/O interface may couple the computing machine to various input devices including mice, touch-screens, scanners, electronic digitizers, sensors, receivers, touchpads, trackballs, cameras, microphones, keyboards, any other pointing devices, or any combinations thereof. The I/O interface may couple the computing machine to various output devices including

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video displays, speakers, printers, projectors, tactile feedback devices, automation control, robotic components, actuators, motors, fans, solenoids, valves, pumps, transmitters, signal emitters, lights, and so forth.

- 5 The computing machine may operate in a networked environment using logical connections through the network interface to one or more other systems or computing machines across the network. The network may include wide area networks (WAN), local area networks (LAN), intranets, the Internet, wireless access networks, wired networks, mobile networks, telephone networks, optical networks, or combinations thereof. The network may be packet
- 10 switched, circuit switched, of any topology, and may use any communication protocol. Communication links within the network may involve various digital or an analog communication media such as fiber optic cables, free-space optics, waveguides, electrical conductors, wireless links, antennas, radio-frequency communications, and so forth. The processor may be connected to the other elements of the computing machine or the
- 15 various peripherals discussed herein through the system bus. It should be appreciated that the system bus may be within the processor, outside the processor, or both. According to some embodiments, any of the processor, the other elements of the computing machine, or the various peripherals discussed herein may be integrated into a single device such as a system on chip ("SOC"), system on package ("SOP"), or ASIC device.
- 20
- Embodiments may comprise a computer program that embodies the functions described and illustrated herein, wherein the computer program is implemented in a computer system that comprises instructions stored in a machine-readable medium and a processor that executes the instructions. However, it should be apparent that there could be many different ways of
- 25 implementing embodiments in computer programming, and the embodiments should not be construed as limited to any one set of computer program instructions. Further, a skilled programmer would be able to write such a computer program to implement one or more of the disclosed embodiments described herein. Therefore, disclosure of a particular set of program code instructions is not considered necessary for an adequate understanding of
- 30 how to make and use embodiments. Further, those skilled in the art will appreciate that one or more aspects of embodiments described herein may be performed by hardware, software, or a combination thereof, as may be embodied in one or more computing systems. Moreover, any reference to an act being performed by a computer should not be construed as being performed by a single computer as more than one computer may perform the act.

The example embodiments described herein can be used with computer hardware and software that perform the methods and processing functions described previously. The systems, methods, and procedures described herein can be embodied in a programmable
5 computer, computer-executable software, or digital circuitry. The software can be stored on computer-readable media. For example, computer-readable media can include a floppy disk, RAM, ROM, hard disk, removable media, flash memory, memory stick, optical media, magneto-optical media, CD-ROM, etc. Digital circuitry can include integrated circuits, gate arrays, building block logic, field programmable gate arrays (FPGA), etc.

10 Reagents, tools, and/or instructions for performing the methods described herein can be provided in a kit. Such a kit can include reagents for collecting a tissue sample from a patient, such as by biopsy, and reagents for processing the tissue. Thus, the kit may include suitable fixatives, such as formalin and embedding reagents, such as paraffin. The kit can
15 also include one or more reagents for performing an expression level analysis, such as reagents for performing nucleic acid amplification, including RT-PCR and qPCR, NGS (RNA-seq), northern blot, proteomic analysis, or immunohistochemistry to determine expression levels of biomarkers in a sample of a patient. For example, primers for performing RT-PCR, probes for performing northern blot analyses or bDNA assays, and/or antibodies or
20 aptamers, as discussed herein, for performing proteomic analysis such as Western blot, immunohistochemistry and ELISA analyses can be included in such kits. Appropriate buffers for the assays can also be included. Detection reagents required for any of these assays can also be included. The kits may be array or PCR based kits for example and may include additional reagents, such as a polymerase and/or dNTPs for example. The kits featured
25 herein can also include an instruction sheet describing how to perform the assays for measuring expression levels.

There is provided a kit for characterising and/or prognosing cancer in a subject comprising one or more primers and/or primer pairs for amplifying and/or which specifically hybridize
30 with at least one gene, full sequence or target sequence selected from Table 1. There is also provided a kit for characterising and/or prognosing cancer in a subject comprising one or more probes that specifically hybridize with at least one gene, full sequence or target sequence selected from Table 1.

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The kit may include one or more primer pairs and/or probes complementary to at least one gene selected from Table 1. In certain embodiments, according to all aspects of the invention, the kits may include one or more probes or primers (primer pairs) designed to hybridize with the target sequences or full sequences listed in Table 1 and thus permit expression levels to be determined. The probes and probesets identified in table 1 and 1A may be employed according to all aspects of the invention. The primers and primer pairs identified in Table 1B may also be employed according to all aspects of the invention.

The kits may include primers/primer pairs/probes/probesets to form any of the gene signatures specified herein (see for example the gene signatures of Tables 1 to 24).

The kits may also include one or more primer pairs complementary to a reference gene.

Such a kit can also include primer pairs complementary to at least 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, 67, 68, 69 or 70 of the genes listed in Table 1.

Thus, in a further aspect the present invention relates to a kit for (in situ) characterising and/or prognosing prostate cancer in a subject comprising one or more oligonucleotide probes specific for an RNA product of at least one gene selected from Table 1. Suitable probes and probesets for each gene are listed in Table 1 and may be incorporated in the kits of the invention. The probes and probesets also constitute separate aspects of the invention. By "probeset" is meant the collection of probes designed to target (by hybridization) a single gene. The groupings are apparent from table 1 (and Table 1A).

The kit may further comprise one or more of the following components:

- a) A blocking probe
- b) A PreAmplifier
- c) An Amplifier and/or
- d) A Label molecule

The components of the kit may be suitable for conducting a viewRNA assay (<https://www.panomics.com/products/rna-in-situ-analysis/view-rna-overview>).

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The components of the kit may be nucleic acid based molecules, optionally DNA (or RNA). The blocking probe is a molecule that acts to reduce background signal by binding to sites on the target not bound by the target specific probes (probes specific for the RNA product of the
5 at least one gene of the invention). The PreAmplifier is a molecule capable of binding to a (a pair of) target specific probe(s) when target bound. The Amplifier is a molecule capable of binding to the PreAmplifier. Alternatively, the Amplifier may be capable of binding directly to a (a pair of) target specific probe(s) when target bound. The Amplifier has binding sites for multiple label molecules (which may be label probes).

- 10 Kits for characterising and/or prognosing cancer, such as prostate cancer or ER positive breast cancer in a subject may permit the methylation status of at least one gene selected from Table 1 to be determined. The determined methylation status, which may be hypermethylation or hypomethylation as appropriate, is used to provide a characterisation of and/or a prognosis for the cancer, such as prostate cancer or ER positive breast cancer.
- 15 Such kits may include primers and/or probes for determining the methylation status of the gene or genes directly. They may thus comprise methylation specific primers and/or probes that discriminate between methylated and unmethylated forms of DNA by hybridization. Such primers and/or probes may include derivatives of the primers and probes described herein, which are adapted to reflect selective modification of the cytosine residues in the
20 target sequence depending upon whether they are methylated or not. Thus, sets of "methylated-specific" and "unmethylated-specific" primers (to include primer pairs) and probes may be designed in order to probe particular cytosine-containing target sequences. Such kits will typically also contain a reagent that selectively modifies either the methylated or non-methylated form of CpG dinucleotide motifs. Suitable chemical reagents comprise
25 hydrazine and bisulphite ions. An example is sodium bisulphite. The kits may, however, contain other reagents as discussed hereinabove to determine methylation status such as restriction endonucleases. Methylation specific PCR primers may be derived from the primer pairs of Table 1B and of SEQ ID NOs 3151-3154, to take account of bisulphite conversion of CpG dinucleotide pairs if present in the unmethylated form (unmethylated-specific) or lack of
30 conversion if the CpG dinucleotide is methylated (methylated-specific).

The invention also relates to a kit for characterising and/or prognosing cancer, such as prostate cancer or ER positive breast cancer in a subject comprising one or more antibodies or aptamers as described above and which are useful in the methods of the invention.

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Informational material included in the kits can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of the reagents for the methods described herein. For example, the informational material of the kit can contain
5 contact information, e.g., a physical address, email address, website, or telephone number, where a user of the kit can obtain substantive information about performing a gene expression analysis and interpreting the results.

10 The kit may further comprise a computer application or storage medium as described above.

The example systems, methods, and acts described in the embodiments presented previously are illustrative, and, in alternative embodiments, certain acts can be performed in a different order, in parallel with one another, omitted entirely, and/or combined between different example embodiments, and/or certain additional acts can be performed, without
15 departing from the scope and spirit of various embodiments. Accordingly, such alternative embodiments are included in the scope of the invention as described herein.

Although specific embodiments have been described above in detail, the description is merely for purposes of illustration. It should be appreciated, therefore, that many aspects
20 described above are not intended as required or essential elements unless explicitly stated otherwise.

Modifications of, and equivalent components or acts corresponding to, the disclosed aspects of the example embodiments, in addition to those described above, can be made by a person
25 of ordinary skill in the art, having the benefit of the present disclosure, without departing from the spirit and scope of embodiments defined in the following claims, the scope of which is to be accorded the broadest interpretation so as to encompass such modifications and equivalent structures.

30

DESCRIPTION OF THE FIGURES

Figure 1: Heat map showing unsupervised hierarchical clustering of gene expression data using the 1000 most variable genes in the 126 prostate FFPE tumour samples. Gene expression across all samples is represented horizontally. Functional processes corresponding to each gene cluster are labeled along the right of the figure.

Figure 2: AUC calculated under cross validation with respect to associating the signature scores with discriminating the molecular subgroups (cluster 1 and 2 V cluster 3 and 4). The number of genes in each signature is depicted along the x-axis and the AUC on the y-axis.

Figure 3: C-index calculated under cross validation with respect to associating the signature scores with time to metastatic recurrence in the Taylor primary tumour samples. The number of genes in each signature is depicted along the x-axis and the C-index on the y-axis.

Figure 4: Standard Deviation (SD) calculated as a percentage of the signature score range under cross validation within the five sections that were profiled to evaluate the impact of biological heterogeneity on signature score. The number of genes in each signature is depicted along the x-axis and the percent SD on the y-axis.

Figure 5: Kaplan Meier generated in the Taylor primary tumour samples using the time to metastatic recurrence endpoint and the Good/Poor prognosis 70 gene signature predictions. Univariate hazard ratio = 0.62 [1.98,20.20]; $p < 0.0001$

Figure 6: Kaplan Meier generated in the Taylor primary tumour samples using the time to biochemical recurrence endpoint and the Good/Poor prognosis 70 gene signature predictions. Univariate hazard ratio = 3.76 [1.70, 8.34]; $p < 0.0001$

Figure 7: Wald test of multivariate Cox analysis of key prognostic factors from Taylor analysis

Figure 8A: ROC curve in the Glinsky data using the 70 gene signature scores and the corresponding biochemical recurrence outcome for each patient. The AUC = 0.69 [0.57, 0.79]; $p = 0.0032$.

Figure 8B: ROC curve in the Erho data using the 70 gene signature scores and the corresponding metastatic recurrence outcome for each patient. The AUC = 0.61 [0.57, 0.65]; $p < 0.0001$.

Figure 9: Kaplan Meier generated in the breast cancer data (GSE2034) ER positive tumour samples using the time to relapse endpoint (time in months) and the Good/Poor prognosis 70 gene signature predictions; signature_call_median 1 (poor prognosis) and signature_call_median 0 (good prognosis). Univariate hazard ratio = 1.24 [0.80, 1.92]

5

Figure 10: ROC curve in the breast cancer data (GSE2034) ER positive tumour samples using the 70 gene signature scores and the corresponding recurrence outcome for each patient. The AUC = 0.62; p = 0.002

10 Figure 11: Kaplan Meier generated in the breast cancer data (GSE7390) ER positive tumour samples using the relapse free survival endpoint (time in days) and the Good/Poor prognosis 70 gene signature predictions; signature_call_median 1 (poor prognosis) and signature_call_median 0 (good prognosis). Univariate hazard ratio = 1.74 [1.04, 2.93]

15 Figure 12: Kaplan Meier generated in the breast cancer data (GSE7390) ER positive tumour samples using the distant metastasis free survival endpoint (time in days) and the Good/Poor prognosis 70 gene signature predictions; signature_call_median 1 (poor prognosis) and signature_call_median 0 (good prognosis). Univariate hazard ratio = 2.01 [1.02, 3.96]

20 Figure 13: Kaplan Meier generated in the breast cancer data (GSE7390) ER positive tumour samples using the overall survival endpoint (time in days) and the Good/Poor prognosis 70 gene signature predictions; signature_call_median 1 (poor prognosis) and signature_call_median 0 (good prognosis). Univariate hazard ratio = 2.54 [1.24, 5.18]

25 Figure 14: Kaplan Meier generated in the breast cancer data (GSE2990) ER positive tumour samples using the relapse free survival endpoint (time in years) and the Good/Poor prognosis 70 gene signature predictions; signature_call_median 1 (poor prognosis) and signature_call_median 0 (good prognosis). Univariate hazard ratio = 1.91 [1.17, 3.09]

30 Figure 15: Kaplan Meier generated in the breast cancer data (GSE2990) ER positive tumour samples using the distant metastasis free survival endpoint (time in years) and the Good/Poor prognosis 70 gene signature predictions; signature_call_median 1 (poor prognosis) and signature_call_median 0 (good prognosis). Univariate hazard ratio = 2.37 [1.26, 4.44]

Figure 16 – Kaplan Meier survival analysis over 10-years showing the association of the 70-gene signature at predicting time to biochemical recurrence in the resection validation cohort following surgery. Survival probability (%) showed reduced progression-free survival (PFS) in months of the 'Met-like' subgroup (blue) of 81 patients when compared to the 'Non Met-like' subgroup (green) of 241 patients (HR = 1.74 [1.18-2.56]; p = 0.0009).

Figure 17 – Kaplan Meier survival analysis over 10-years showing the association of the 70-gene signature at predicting time to metastatic disease progression in the resection validation cohort following surgery. Survival probability (%) showed reduced progression-free survival (PFS) in months of the 'Met-like' subgroup (blue) of 81 patients when compared to the 'Non Met-like' subgroup (green) of 241 patients (HR = 3.60 [1.81-7.13]; p < 0.0001).

Figure 18 – Kaplan Meier survival analysis over 10-years showing the association of the 70-gene signature at predicting time to biochemical recurrence in the FASTMAN biopsy validation cohort following curative radiotherapy. Survival probability (%) showed reduced progression-free survival (PFS) in months of the 'Met-like' subgroup (blue) of 54 patients when compared to the 'Non Met-like' subgroup (green) of 194 patients (HR = 2.18 [1.14-4.17]; p = 0.0042).

Figure 19 – Kaplan Meier survival analysis over 10 years showing the association of the 70-gene signature at predicting time to metastatic disease progression in the FASTMAN biopsy validation cohort following radiotherapy with curative intent. Survival probability (%) showed reduced progression-free survival (PFS) in months of the 'Met-like' subgroup (blue) of 54 patients when compared to the 'Non Met-like' subgroup (green) of 194 patients (HR = 3.50 [1.28-9.56]; p = 0.0017).

Figure 20 - Core set analysis for FASTMAN Biopsy Validation dataset.

Figure 21 – Core set analysis for internal resection validation dataset.

Figure 22 – Minimum gene set analysis for FASTMAN Biopsy Validation dataset.

Figure 23 – Minimum gene set analysis for internal resection validation dataset.

EXAMPLES

The present invention will be further understood by reference to the following experimental examples.

Example 1: Tissue processing, hierarchical clustering and subtype identification**5 *Tumor Material***

70 primary prostate cancers with no known concomitant metastases, 20 primary prostate cancers with known lymph node metastases, 11 lymph nodes containing metastatic prostate cancer, 25 normal prostate samples.

Gene Expression Profiling from FFPE

- 10 Total RNA was extracted from macrodissected FFPE tissue using the High Pure RNA Paraffin Kit (Roche Diagnostics GmbH, Mannheim, Germany). RNA was converted into complementary deoxyribonucleic acid (cDNA), which was subsequently amplified and converted into single-stranded form using the SPIA® technology of the WT-Ovation™ FFPE RNA Amplification System V2 (NuGEN Technologies Inc., San Carlos, CA, USA). The
- 15 amplified single-stranded cDNA was then fragmented and biotin labeled using the FL-Ovation™ cDNA Biotin Module V2 (NuGEN Technologies Inc.). The fragmented and labeled cDNA was then hybridized to the Almac Prostate Cancer DSA™. Almac's Prostate Cancer DSA™ research tool has been optimised for analysis of FFPE tissue samples, enabling the use of valuable archived tissue banks. The Almac Prostate Cancer DSA™ research tool is an
- 20 innovative microarray platform that represents the transcriptome in both normal and cancerous prostate tissues. Consequently, the Prostate Cancer DSA™ provides a comprehensive representation of the transcriptome within prostate disease and tissue setting, not available using generic microarray platforms. Arrays were scanned using the Affymetrix Genechip® Scanner 7G (Affymetrix Inc., Santa Clara, CA).

25 *Data preparation*

- Quality Control (QC) of profiled samples was carried out using MAS5 pre-processing algorithm. Various technical aspects were assessed including: average noise and background homogeneity, percentage of present call (array quality), signal quality, RNA quality and hybridization quality. Distributions and Median Absolute Deviation of
- 30 corresponding parameters were analyzed and used to identify possible outliers.

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Almac's Prostate Cancer DSATM contains probes that primarily target the area within 300 nucleotides from the 3' end. Therefore standard Affymetrix RNA quality measures were adapted – for housekeeping genes intensities of 3' end probe sets with ratios of 3' end probe set intensity to the average background intensity were used in addition to usual 3'/5' ratios.

5 Hybridization controls were checked to ensure that their intensities and present calls conform to the requirements specified by Affymetrix.

Hierarchical Clustering and Functional Analysis

Sample pre-processing was carried out using Robust Multi-Array analysis (RMA) [1]. The data matrix was initially summarised to Entrez gene ID level using Ensemble annotation
10 version 75, specifically utilising the probe set that was least associated to present call for each Entrez gene. Probe sets that 1) did not map to an Entrez gene ID or 2) mapped to multiple Entrez gene IDs were removed. The resulting gene level data matrix was sorted by decreasing variance and intensity and incremental subsets of the data matrix were tested for cluster stability: the GAP statistic [2] was applied to calculate the number of sample and gene
15 clusters while the stability of cluster composition was assessed using partition comparison methods. The final most variable gene list was determined based on the smallest and most stable data matrix for the selected number of sample cluster.

Following standardization of the data matrix to the median gene expression values, agglomerative hierarchical clustering was performed using Euclidean distance and Ward's
20 linkage method [3]. The optimal number of sample and gene clusters was determined using the GAP statistic [2] which compares the change in within-cluster dispersion with that expected under a reference null distribution. The significance of the distribution of clinical parameter factor levels across sample clusters was assessed using ANOVA (continuous factor) or chi-squared analysis (discrete factor) and corrected for false discovery rate (product of p-value
25 and number of tests performed). A corrected p-value threshold of 0.05 was used as criterion for significance.

Functional enrichment analysis was conducted to identify and rank biological entities which were found to be associated with the clustered gene sets using the Gene Ontology biological processes classification [4]. Entities were ranked according to a statistically derived
30 enrichment score [5] and adjusted for multiple testing [6]. A corrected p-value of 0.05 was used as significance threshold. The identified enriched processes were summarised into an overall group function for each gene cluster.

From the hierarchical clustering analysis, primary tumour samples clustering with metastatic samples will be labelled as 'bad whereas primary tumour samples clustering with normal samples will be labelled as 'good'.

5 ***Signature generation***

Following the identification of class labels a gene signature was derived to enable prospective identification of the bad prognosis group within the primary tumour samples. The following steps summarise the procedure for developing the gene signature:

- 10 1. Cross-validation: The samples were randomly split into 5 cross-validation (CV) folds for signature training/testing, and this was repeated 10 times to allow an unbiased estimation of the model performance.
- 15 2. Pre-processing: RMA background correction of the data at the probe intensity level, followed by a median summary of the intensities of probes to probe sets and subsequently probe sets to Entrez gene ID. The Entrez gene level summarised data matrix was log2 transformed and quantile normalised. Note that samples in the CV test set were normalised using a quantile normalisation model from the corresponding CV training set to ensure that all estimates of model performance are based on signature scores pre-processed on a per sample basis.
- 20 3. Filtering: A gene filter was applied before model development to remove 75 percent of genes with low variance and low intensity.
4. Machine Learning: Partial Least Squares (PLS) was used to train the algorithm against the "good/poor prognosis" endpoint.
- 25 5. Feature Selection: A wrapper based method for feature selection was implemented, where genes (those remaining after the initial filter) are ranked using the respective weights defined by the PLS algorithm and 10 percent of genes with the lowest absolute weights are removed. This process is repeated after each round of feature elimination (within cross validation) where the genes are re-ranked in order to determine the genes with the lowest absolute weights and removing 10 percent each time until only 2 genes remained.
- 30 6. Interim validation data set 1: A public data set (Taylor et al) was used for interim evaluation where the primary tumour samples from this data set were predicted (signature scores calculated) alongside each CV test set.

7. Interim validation data set 2: Five sections across an FFPE tumour block were profiled in order to evaluate the impact of biological heterogeneity on the signature score. Signature scores for each of these sections were calculated under CV alongside each CV test set.

5

Model selection included the following steps:

1. Evaluating the Area Under the Receiver Operating Characteristic (ROC) Curve (AUC) in the training data under cross validation.
2. Evaluating the C-index in the interim validation Taylor data under cross validation.
10 The C-index is a measure of performance (analogous to AUC) relating to predicting time-to-event data in absence of the threshold for dichotomising the scores for assigning "good" and "poor" prognosis groups.
3. Evaluating the variability in signature scores across the five sections of an FFPE block which were predicted under CV. The variability was determined by calculating
15 the standard deviation (SD) of the signature scores across the five samples and expressing the SD as a fraction of the signature score range (i.e. calculating a percent SD).

The signature length that yielded a high AUC in training set; a high C-index in the Taylor set; and a low SD in the heterogeneity samples was selected.

20

Multivariate analysis

- Of interest is the time until biochemical recurrence in prostate cancer patients in the Taylor dataset. Multivariable Cox survival modelling was used to test for and describe interactions with the biomarker, understand prognostic factors and model the relative effect of prognostic
25 factors. Based on clinical judgement pre-operative PSA (4 ng/ml), pathology stage ("T2 A/B/C", "T3 A/B/C", "T4"), Gleason (<7, 7, 8-9) and the dichotomised signature score were used as independent predictor variables. A log 2 transformation of pre-operative PSA was applied. Multiple imputation was used to ensure all available events were used in the analysis. The sample size is 168 patients with 46 biochemical recurrence events and the
30 median time until biochemical recurrence approximately 15 years. A formal test of the proportional hazard assumption, assessment of the functional form of the log transformation

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of Pre PSA and the model fit using a graphical plot of the Nelson-Aalen cumulative hazard function all provided no cause for concern. Twelve influential data points defined by a change to the regression coefficient equal to or greater than 2 standard errors on removal from the analysis were identified. These were not removed or investigated further.

5

Following model selection two independent prostate cancer data sets were further evaluated with the final model:

1. 70 publically available primary prostate tumour samples (Glinsky et al) which were profiled on the Affymetrix U133A platform.
 - 10 a. Clinical information included biochemical recurrence (as a binary outcome only)
 2. 545 publically available primary prostate tumour samples (Erho et al 2013) which were profiled on the Affymetrix Human Exon array platform.
 - a. Clinical information included metastatic recurrence (as a binary outcome only)
- 15 Performance of each of these data sets was evaluated using AUC, to establish if the signature could discriminate patients with recurrences from those with no recurrences, under the hypothesis that higher scores are more representative of patients with metastatic-like disease (bad prognosis) therefore more likely to have a recurrence outcome.

Evaluation of the final model in breast cancer data sets

- 20 It was of further interest to evaluate the final signature in other hormone related data sets with respect to predicting prognosis in untreated patients. Three ER positive breast cancer data sets were evaluated:
1. Data set retrieved from Gene Expression Omnibus database, accession number GSE2034
 - 25 a. 209 Node negative ER positive patients
 - b. Endpoint: Time to relapse
 2. Data set retrieved from Gene Expression Omnibus database, accession number GSE7390
 - a. 134 Node negative ER positive patients
 - 30 b. Endpoint 1: relapse free survival (RFS)
 - c. Endpoint 2: distant metastasis free survival (DMFS)

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- d. Endpoint 3: overall survival (OS)
- 3. Data set retrieved from Gene Expression Omnibus database, accession number GSE2990
 - a. 149 ER positive patients
 - 5 b. Endpoint 1: relapse free survival (RFS)
 - c. Endpoint 2: distant metastasis free survival (DMFS)

For each data set a median signature score cut-off was applied to predict patients as either signature positive (metastatic-like) if they scored above the median value, or signature negative (non-metastatic-like) otherwise. Kaplan Meier curve was used to observe the survival differences between the two subgroups of patients. Cox proportional hazard regression analysis of the signature calls against each endpoint was used to calculate a univariate hazard ratio for the signature as a measure of performance against the respective clinical endpoint.

15 **Results**

126 samples passed microarray QC and subsequently underwent unsupervised hierarchical clustering based on 1000 most variable genes. Four sample clusters and four gene clusters were identified (Figure 1). There was a significant association between sample clusters and tumour type: cluster 1 and 2 (highlighted with blue box) comprised mainly metastatic and primary tumours and cluster 3 (highlighted with red box) and 4 (highlighted with yellow box) comprised benign and primary tumours respectively ($p < 0.0001$, Table 1). Functional analysis (Figure 1) revealed that clusters 1 and 2 (metastatic and primary like metastatic tumours) were characterized by down-regulation of genes associated with cell adhesion, cell differentiation and cell development, up-regulation of Androgen related processes and Epithelial to mesenchymal transition (EMT) (cluster 1 and 2 referred to as “bad prognosis” group forthwith). Cluster 3 and cluster 4 (benign and primary like benign tumours) were associated with up-regulation of genes associated with cell adhesion, inflammatory responses and cell development (cluster 3 and cluster 4 referred to as “good prognosis” forthwith). Patients in cluster 1 and cluster 2 were class labelled “bad prognosis” and patients in cluster 3 and cluster 4 were class labelled as “good prognosis” for the purpose of signature development.

The results from signature development at all considered signature lengths are provided in Figure 2, Figure 3 and Figure 4 which respectively show; the AUC in the training set for predicting the endpoint; the C-index in the Taylor data with respect to time to metastatic recurrence; and the percent SD in the heterogeneity samples. A signature length of 70 genes was selected as this was the signature length whereby the AUC remained high (Figure 2); the SD remained low (Figure 4); and is the smallest signature length where the c-index values remained high in the Taylor samples (Figure 3).

The signature content and weightings of the final 70 gene model are listed in Table 1. The 70 gene scores calculated in the Taylor data were dichotomised at a threshold of 0.4241 where patients with a signature score > 0.4241 were classified as "bad prognosis" and patients with a signature score ≤ 0.4241 were classified as "good prognosis". The signature classifications into good and poor prognosis were used to generate a Kaplan Meier curve to show the differences in survival probabilities for the two predicted groups. Figure 5 represents the Kaplan Meier for the time to metastatic recurrence endpoint (univariate hazard ratio = 6.32 [1.98, 20.20]) and Figure 6 represents the Kaplan Meier for the time to biochemical recurrence endpoint (univariate hazard ratio = 3.76 [1.70, 8.34]).

Figure 7 and the associated table present the results of the multivariable analysis. The plot displays the Wald chi squared statistic minus its degrees of freedom for assessing the partial effect of each variable in the model. Gleason is the most important factor followed by the biomarker (i.e gene signature) and pre-operative PSA. These results demonstrate that the biomarker provides additional prognostic information over and above standard pathological factors. Due to the interaction of the biomarker and pre-operative PSA, one potential would be to combine these variables (and/or other prognostic factors) together to generate a combined risk score. The 70 gene signature model was applied to two independent prostate cancer data sets.

Figure 8A and Figure 8B show the ROC curves from assessing the signature scores against the recurrence outcomes for the Glinksy and the Erho data sets respectively. The AUC in the Glinksy data for predicting biochemical recurrence was 0.69 [0.57, 0.79] and the AUC in the Erho data for predicting metastatic recurrence was 0.61 [0.57, 0.65].

Evaluation of the final model in breast cancer data sets

The results of evaluating the 70 gene signature in three breast cancer data sets is described below:

1. Data set retrieved from Gene Expression Omnibus database, accession number GSE2034
 - 5 a. 209 Node negative ER positive patients
 - b. Endpoint: Time to relapse
 - i. Hazard ratio = 1.24 [0.80, 1.92] (Kaplan Meier is shown in Figure 9)
 - ii. AUC for predicting relapse = 0.62; p=0.002 (ROC curve shown in Figure 10)
- 10 2. Data set retrieved from Gene Expression Omnibus database, accession number GSE7390
 - a. 134 Node negative ER positive patients
 - b. Endpoint 1: relapse free survival (RFS)
 - i. Hazard ratio = 1.74 [1.04, 2.93] (Kaplan Meier is shown in Figure 11)
 - 15 c. Endpoint 2: distant metastasis free survival (DMFS)
 - i. Hazard ratio = 2.01 [1.02, 3.96] (Kaplan Meier is shown in Figure 12)
 - d. Endpoint 3: overall survival (OS)
 - i. Hazard ratio = 2.54 [1.24, 5.18] (Kaplan Meier is shown in Figure 13)
- 20 3. Data set retrieved from Gene Expression Omnibus database, accession number GSE2990
 - a. 149 ER positive patients
 - b. Endpoint 1: relapse free survival (RFS)
 - i. Hazard ratio = 1.91 [1.17, 3.09] (Kaplan Meier is shown in Figure 14)
 - 25 c. Endpoint 2: distant metastasis free survival (DMFS)
 - i. Hazard ratio = 2.37 [1.26, 4.44] (Kaplan Meier is shown in Figure 15)

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10 **Example 2 – Confirmation of effectiveness of all probesets**

Purpose:

The purpose of this analysis is to evaluate the performance of the 70 gene signature when a random probeset per gene is selected. This is to provide evidence of the importance of certain probesets associated to the signature genes.

15 **Data:**

Table 26 outlines the number of probesets available per signature gene. The table shows that the number of probesets that can be selected per gene varies from 1 to a maximum of 21 probesets per gene.

Table 26 - Number of probesets available per signature gene

Entrez Gene ID	Signature Weight	Signature Bias	Weight (abs)	Rank by Weight	# Probesets
827	-0.01090	4.44087	0.01090	1	1
7060	-0.00963	6.91259	0.00963	2	1
5354	-0.00889	4.38357	0.00889	3	2
4489	-0.00868	6.74796	0.00868	4	2
406988	-0.00828	7.21525	0.00828	5	4
6406	-0.00793	4.23042	0.00793	6	1

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84870	-0.00730	4.29317	0.00730	7	2
50636	-0.00716	6.52255	0.00716	8	5
5121	-0.00714	7.62176	0.00714	9	1
27063	-0.00692	5.92831	0.00692	10	1
4604	-0.00684	4.57432	0.00684	11	8
4316	-0.00684	6.75672	0.00684	12	1
12	-0.00683	5.74546	0.00683	13	3
6401	-0.00681	5.97768	0.00681	14	1
3852	-0.00640	6.08049	0.00640	15	1
4057	-0.00640	6.49726	0.00640	16	3
57481	-0.00638	3.55997	0.00638	17	1
25907	-0.00631	8.06342	0.00631	18	1
7538	-0.00627	9.96083	0.00627	19	1
2354	-0.00611	6.95494	0.00611	20	4
50652	-0.00610	5.26234	0.00610	21	8
79054	-0.00606	4.86579	0.00606	22	14
9232	0.00602	4.71269	0.00602	23	2
283194	-0.00595	4.98038	0.00595	24	18
9506	-0.00584	7.07391	0.00584	25	1
79689	-0.00568	8.10530	0.00568	26	4
130733	-0.00565	7.59453	0.00565	27	1
2920	-0.00560	8.92898	0.00560	28	1
9955	-0.00559	4.23278	0.00559	29	3
2138	-0.00558	5.50428	0.00558	30	5
340419	-0.00556	3.92242	0.00556	31	2
5317	-0.00555	5.91219	0.00555	32	2
4588	-0.00552	6.64004	0.00552	33	1
5179	-0.00551	4.51486	0.00551	34	2
1672	-0.00540	6.82549	0.00540	35	2
84889	-0.00539	4.64900	0.00539	36	1
693163	-0.00536	5.08739	0.00536	37	1
51050	-0.00526	4.85872	0.00526	38	6
101928017	-0.00526	6.06588	0.00526	39	1

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5166	-0.00525	4.17409	0.00525	40	12
644844	-0.00521	5.18357	0.00521	41	1
5054	-0.00519	6.69187	0.00519	42	6
29951	-0.00515	4.75233	0.00515	43	4
7739	-0.00511	6.90054	0.00511	44	1
152	-0.00505	7.07838	0.00505	45	1
563	-0.00502	8.19118	0.00502	46	3
7083	0.00497	5.58133	0.00497	47	1
23784	-0.00496	4.82498	0.00496	48	4
3832	0.00493	3.91767	0.00493	49	2
9076	-0.00492	4.96028	0.00492	50	6
100616163	-0.00491	10.53645	0.00491	51	1
23764	-0.00490	8.49795	0.00490	52	3
91661	-0.00486	3.97633	0.00486	53	2
1164	0.00486	6.50398	0.00486	54	1
56849	-0.00486	4.81933	0.00486	55	2
5346	0.00483	4.62939	0.00483	56	1
6614	0.00477	5.50375	0.00477	57	1
285016	-0.00477	6.66460	0.00477	58	1
8076	-0.00477	4.12918	0.00477	59	2
6422	-0.00476	7.90126	0.00476	60	2
1847	-0.00472	5.76268	0.00472	61	3
57176	0.00468	5.22346	0.00468	62	1
10257	-0.00466	5.23038	0.00466	63	21
23677	-0.00462	4.88271	0.00462	64	9
6652	-0.00457	8.95841	0.00457	65	4
51001	0.00452	5.33420	0.00452	66	1
1803	-0.00451	4.65975	0.00451	67	6
284837	0.00450	4.90531	0.00450	68	1
54097	-0.00444	7.38807	0.00444	69	3
354	-0.00442	10.22644	0.00442	70	5

Analysis:

The following analysis steps were performed:

- Training data matrix pre-processing (n=126 samples)
 - RMA background correction
 - Quantile normalisation
 - RMA summary
- Generate signature scores for training samples using a random probeset which is annotated to each signature gene, 1000 times
- Calculate AUC performance using the signature scores with respect to the subtype labels
- Min(AUC) = 0.9964 & Max(AUC) = 1.00
- This indicates that all probesets are effective in the signature for identifying the subtype

For completeness, it is noted that the random selection of probeset per signature gene will only be applicable for signature genes with > 1 probeset i.e. 30 of the signature genes have only 1 probeset per gene, so for these genes, the same probeset is being selected each time.

Example 3 – Validation study for 70 gene signature**Introduction**

As outlined in the earlier examples, using the transcriptional profile and hierarchical clustering of the Discovery cohort of prostate cancer samples, we have identified a distinct molecular subgroup of primary prostate cancers that clustered with metastatic disease and prostate cancers known to have concomitant metastases. This subgroup of primary tumour samples clustered with metastatic samples represented a poor prognostic population, whilst the benign like primary tumours defined a good prognostic subgroup. Functional analysis of the subgroup identified biological processes known to be involved in metastasis such as Epithelial Mesenchymal Transition (EMT) and cell migration. This cluster was hence defined as the 'Metastatic-Like' subgroup and for the purposes of this specification will be referred to throughout as 'Met-like'.

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We developed a 70-gene signature to prospectively identify the 'Met-like' subgroup of patients. This 70-gene assay can be used to prospectively assess disease progression from a primary tumour, to determine the likelihood of disease recurrence and/or metastatic progression. We have also previously shown that the 70-gene signature also displays good performance in heterogeneity studies, maintaining subgroup detection and signature score stability.

We have also demonstrated the prognostic significance of this molecular subgroup using the 70-gene signature in three independent in silico datasets with different clinical endpoints. In the Glinksy dataset (79 prostate cancer cases), the signature showed a good discrimination of biochemical recurrence endpoint with a statistically significant AUC =0.69 [0.57-0.79], $p = 0.0032$ (Glinksy et al 2004). Also in the Erho dataset (545 prostate cancer cases), a statistically significant modest discrimination was observed with the signature for classifying patients metastatic recurrence endpoint (AUC 0.612 [0.569-0.653], $p < 0.0001$) (Erho et al 2013). Finally, in the Taylor dataset, the signature had statistically significant association with patients time to metastatic recurrence (HR = 6.32 [1.98-20.20], $p < 0.0001$) and time to biochemical recurrence with HR 3.76 [1.70-8.34], $p < 0.0001$ (Taylor et al 2010). Importantly, the metastatic biology subgroup has also been shown to predict poor outcome as identified by disease recurrence following surgical removal of the prostate independent of known prognostic factors such as Gleason score.

The identification of prostate cancer patients at high risk of recurrence following curative surgery or radiation is a key clinical requirement to identify those men that should receive adjuvant chemotherapy or radiation treatment whilst avoiding unnecessary interventions and side-effects in those who do not require further treatment. Based on this, the ability and performance of our 70-gene assay in identifying this high-risk population of patients required comprehensive clinical validation in independent cohorts of clinical prostate samples, either resections following curative surgery or biopsy specimens following curative radiotherapy.

OBJECTIVES

To further assess the performance of the prostate prognostic 70-gene assay in primary prostate resections.

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To clinically validate the prostate prognostic 70-gene assay in an independent cohort of primary localised prostate cancer resections with the ability to identify a subgroup of prostate cancer patients at increased risk of developing biochemical recurrence and/or metastatic disease progression following surgery with curative intent.

- 5 To assess the performance of the prostate prognostic 70-gene assay in prostate biopsies in comparison to resection specimens.

- To clinically validate the prostate prognostic 70-gene assay in an independent cohort of primary prostate biopsies with the ability to identify a subgroup of prostate cancer patients at increased risk of developing biochemical recurrence and/or metastatic disease
10 progression following radiation treatment.

MATERIALS & METHODS

- Processing and clinical validations of the 70 gene prognostic assay was performed in a blinded and randomised manner to avoid technical or biological confounding in the
15 expression data which could have the potential to compromise data quality, integrity and validation objectives.

Prostate Cancer Tumour Material

- This study performed gene expression analysis of two separate cohort of prostate cancer
20 specimens. The first validation cohort was collected internally by Almac Diagnostics and included 349 prostate resection FFPE tissue samples obtained from four clinical sites; University College Dublin (62 samples), Wales Cancer Bank (100 samples), University of Surrey (41 samples) and University Hospital of Oslo (146 samples). This cohort consisted of samples across three key clinical groups, Non-recurrence patients (189 samples),
25 Biochemical recurrence (also referred to as PSA recurrence) patients (112 samples) and Metastatic progression patients (48 samples). The resection dataset incorporated samples were collected based on the following inclusion criteria:

Clinical T-stage T1a-T3c (NXM0 at diagnosis)

Received radical prostatectomy surgery with curative intent

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Not received neo-adjuvant hormone or therapy treatments

Patients within the non-recurrence group must not have received adjuvant treatment

3-5 years clinical follow up data available

Demographic, clinical and pathological variables utilised for the data analysis of the prostate
5 resection cohort is summarised in Table 27.

The second validation cohort was collected in collaboration with the QUB as part of the
FASTMAN Research Group and included 312 prostate biopsy FFPE tissue samples. This
cohort consisted of 60 patient failures which incorporated 58 Biochemical recurrence, 24
Metastatic progression and 18 Castrate Resistant Prostate Cancer (CRPC). The biopsy
10 dataset incorporated samples were collected based on the following inclusion criteria:

Clinical T-stage T1a-T3c (NXM0 at diagnosis)

Received radiotherapy with curative intent

3-5 years clinical follow up data available

Demographic, clinical and pathological variables utilised for the data analysis of the prostate
15 biopsy cohort is summarised in Table 28.

Ethical approval for the sample acquisition and dataset analysis as validation of the prostate
prognostic assay was obtained from the East of England Research Ethics Committee (Ref:
14/EE/1066).

20 **Gene Expression Profiling of Prostate Cancer samples**

Prior to sample profiling, clinical samples were randomized into RNA extraction batches and
re-randomised into cDNA amplification processing batches using a list of pre-defined factors
i.e. Clinical T-stage, PSA, Gleason, Age and Response. Clinical site factor was also included
for validation 1. A further randomization of reagents, equipment and operators was
25 performed prior to sample processing.

All samples were centrally pathology reviewed (Prof E. Kay RCSI) and marked-up for
macrodissection based on the tumour area with the most dominant Gleason grade. For

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resection samples 2 x 10 µm sections were processed whereas for biopsy samples 4 x 5 µm sections were used for profiling. Total RNA was extracted from macrodissected FFPE tissue using the Roche High Pure RNA Paraffin Kit (Roche Diagnostics GmbH, Mannheim, Germany). RNA was converted into complementary deoxyribonucleic acid (cDNA), which
5 was subsequently amplified and converted into single-stranded form using the SPIA® technology of the WT-Ovation™ FFPE RNA Amplification System V3 (NuGEN Technologies Inc., San Carlos, CA, USA). The amplified single-stranded cDNA was then fragmented and biotin labelled using the FL-Ovation™ cDNA Biotin Module V3 (NuGEN Technologies Inc.). The fragmented and labelled cDNA was then hybridised to the Almac Prostate Cancer
10 DSATM. Almac's Prostate Cancer DSA™ research tool has been optimised for analysis of FFPE tissue samples, enabling the use of valuable archived tissue banks. The Almac Prostate Cancer DSA™ research tool is an innovative microarray platform that represents the transcriptome in both normal and cancerous prostate tissues. Consequently, the Prostate Cancer DSA™ provides a comprehensive representation of the transcriptome within prostate
15 disease and tissue setting, not available using generic microarray platforms. Arrays were scanned using the Affymetrix Genechip® Scanner 7G (Affymetrix Inc., Santa Clara, CA).

Process Controls

Stratagene Universal Human Reference (UHR) samples and ES-2 cell line material were
20 used as process controls within each processing batch as a standard measure during profiling of clinical cohorts. The UHR control is designed to be used as a universal reference RNA for microarray profiling experiments. These controls have been generated from pooling equal quantities of DNase treated cell line RNA to make a control RNA pool. The ES-2 cell line is a human clear cell carcinoma cell line representing ovarian cancer, established from
25 an ovarian surgical tumour. The ES-2 cell line is characterised by a fibroblast morphology and cultures as an adherent cell line. Cells are maintained in McCoy's 5a Medium Modified with 10% Foetal Calf Serum (FCS), with a doubling time of approximately 24 hours. Due to their adherent properties and their fast doubling time these cells are ideal for bulking up as standard cell line controls. Approximately 1×10^6 ES-2 cells were pelleted and fixed
30 overnight prior to processing as a Formalin Fixed Paraffin Embedded (FFPE) tissue block. One 10 µm section of the prepared ES-2 cell line FFPE block was utilised for RNA extraction prior to downstream profiling as a Prostate Metastatic assay specific processing control.

Data Preparation and QC

A continual QC assessment of samples during sample processing was performed. Samples with RNA and cDNA concentrations were taken forward for microarray profiling i.e. minimum
5 of 12.5 ng/ul for RNA concentration and minimum of 140 ng/ul for cDNA concentration.

Microarray data quality was assessed continuously throughout the profiling of these cohorts on a batch by batch basis, and also cumulatively after the completion of profiling to exclude poor quality samples prior to analysis. Samples were pre-processed using the Robust Multi-Array (RMA) average methodology (Irizarry et al. 2003). The QC assessment comprised a
10 combination of the following quality metrics:

Array Image Analysis: Array data was examined to identify any image artefacts

GeneChip QC: Percent present (%P), average signal absent, scale factor, average background and raw Q. Samples with a %P<15% were deemed QC fail

Principal Component Analysis: Hotelling T2 and residual residual Q method was
15 used to identify sample outliers at the expression level

Intensity Distribution Analysis: Kolmogorov-Smirnov statistic (Massey. 1951) used to examine the intensity distribution of the samples and identify outliers

Pre-defined limits of acceptance for Prostate assay specific cell line ES-2 were monitored using statistical process control (SPC) charts.

20

Generation of Signature Scores

Samples were pre-processed on a per sample basis using the refRMA (Irizarry et al. 2003) pre-processing model generated during the development of the 70 gene assay. Ensemble version 75 was used to annotate the probe sets to the corresponding Entrez Gene ID. Probe
25 set expression was summarised to an Entrez Gene ID level using the median value (and excluding anti-sense probe sets). Assay scores were calculated using the following formula from the partial least squares model:

$$\text{Signature score} = \sum_i w_i \times (x_i - b_i) + k$$

Where w_i is the weight of each entrez gene, x_i is the gene expression, b_i is the entrez gene specific bias and $k=0.4365$ (Table 29). Assay calls were assigned based upon predefined cut-off for all samples. Samples with a continuous signature result $>$ cut-off were labelled

5 'assay positive' otherwise 'assay negative'.

Univariate and Multivariate Analysis

Time to event (survival) analysis using time to biochemical recurrence (BCR) and time to metastatic disease was performed to evaluate the prognostic effects of the 70 gene
10 prognostic assay. The survival distributions of patient groups defined by assay status (positive or negative) are visualized using Kaplan-Meier (KM) survival curves.

The Cox proportional hazards regression model was used to assess 70 gene assay status and survival (BCR and Metastatic disease). The hazard ratio (HR) was used to quantify the effect (association) of assay status with survival endpoints. In addition to the univariate
15 (unadjusted) analysis, the multivariable (adjusted) Cox model was used to assess the effect of the assay status (positive or negative) on BCR and Metastatic disease, adjusting for PSA at diagnosis, patient age and Gleason score on survival outcome. All estimated effects are reported with 95% confidence intervals from an analysis in which the assay and these standard prognostic factors were included, regardless of their significance. Interpretation of
20 estimated parameters from Cox proportional hazards test and the level of significance, the goodness of fit of the fitted model was investigated including checking the fulfilment of the proportional hazards assumption (Grambsch & Therneau, 1994).

Multivariable (adjusted) Cox model was also used to assess the effect of the assay status (positive or negative) on BCR and Metastatic disease, adjusting for CAPRA score
25 (Cooperberg et al. 2006). CAPRA scores for each sample were determined using PSA, Biopsy Gleason score, clinical T-stage, percentage of positive biopsy cores and age.

All tests of statistical significance were 2-sided at 5% level of significance. Statistical analysis was performed using MedCalc version 13.

RESULTS

The 70-gene signature predicts time to biochemical recurrence of the 'Met-like' subgroup in the resection validation cohort

Utilising 5-10 year clinical follow up data, univariate survival analysis was performed on the 322 samples which passed microarray data QC to assess the performance of the 70-gene signature at predicting time to biochemical recurrence in the resection dataset following surgery. The Kaplan-Meier survival curve shows a significant association of the 70-gene signature at predicting earlier time to recurrence (months) of the 'Met-like' subgroup (blue) in comparison to the Non Met-like samples (green). This suggests that the samples within the 'Met-like' subgroup have an increased risk of developing biochemical disease recurrence following radical prostatectomy surgery with curative intent (HR = 1.74 [1.18 – 2.56]; p=0.0009) (Figure 16). Multivariate analysis of the dataset was performed to assess the performance of the 70-gene signature at predicting biochemical recurrence, independent of known clinical prognostic factors including age at surgery, PSA levels at diagnosis and combined Gleason score. Considering these prognostic factors, the prostate prognostic 70-gene signature was significantly associated with predicting biochemical recurrence independent of age, PSA and Gleason grade (both <7 and >7) (HR 1.65 [1.16 – 2.34]; p =0.0055) (Table 30a).

20 The 70-gene signature predicts time to metastatic disease progression of the 'Met-like' subgroup in the resection validation cohort

Next using the 5-10 year clinical follow up data, univariate survival analysis was also performed on the 322 samples which passed microarray data QC to assess the performance of the 70-gene signature at predicting time to metastatic progression either local or distant sites, in the resection dataset following surgery. Similarly to biochemical recurrence, the Kaplan-Meier survival curve shows a significant association of the 70-gene signature at predicting metastatic progression of the 'Met-like' subgroup (blue) in comparison to the Non Met-like samples (green). This suggests that the patients within the 'Met-like' subgroup have an increased risk of developing metastatic disease progression following radical prostatectomy surgery with curative intent (HR = 3.60 [1.81 – 7.13]; p <0.0001) (Figure17). Multivariate analysis of the resection dataset was investigated to assess the performance of the 70-gene signature at predicting metastatic progression, independent of known clinical

prognostic factors including age at surgery, PSA levels at diagnosis and combined Gleason score. The prostate prognostic 70-gene signature scores of the 'Met-like' subgroup were shown to be significantly associated with predicting metastatic disease progression independent of age, PSA and Gleason grade (both <7 and >7) (HR 3.50 [1.95-6.27]; p <0.0001), hence supporting that patients within this group are 'high-risk' for progression (Table 30b). Interestingly, the 70-gene signature appears to show better performance as a prognostic factor as opposed to age, PSA and Gleason <7 for predicting metastatic disease (Table 30b).

10 The 70-gene signature predicts time to biochemical recurrence of the 'Met-like' subgroup in the biopsy validation cohort

Univariate survival analysis was performed using the collated 5-10 year follow up clinical data on the 322 samples to assess the performance of the 70-gene signature at predicting time to biochemical recurrence in the biopsy dataset following radiotherapy with curative intent. The Kaplan-Meier survival curve shows a significant association of the 70-gene signature at predicting earlier time to recurrence (months) of the 'Met-like' subgroup (blue) in comparison to the Non Met-like samples (green). As with the resection dataset, this suggests that the patients within the 'Met-like' subgroup have an increased risk of developing biochemical disease recurrence following radical radiotherapy with curative intent (HR = 2.18 [1.14 – 4.17]; p=0.0042) (Figure 18). Multivariate analysis of the dataset was then performed to assess the performance of the 70-gene signature at predicting biochemical recurrence, independent of other commonly used prognostic factors including age at diagnosis, PSA levels at diagnosis and combined Gleason score. The prostate prognostic 70-gene signature of the 'Met-like' group was significantly associated with predicting biochemical recurrence independent of age, PSA and Gleason grade (both <7 and >7) (HR 1.96 [1.11 – 3.48]; p =0.0220), indicating that the patients within this subgroup are at increasing risk of developing biochemical recurrence (Table 31a). Of note, this data suggests that no other variable within the covariate analysis is significantly associated with identifying the increased risk of disease recurrence in the 'Met-like' subgroup (Table 31a).

30 The 70-gene signature predicts time to metastatic disease progression of the 'Met-like' subgroup in the biopsy validation cohort

Following this, univariate survival analysis was also performed on the 248 QC pass samples to determine the performance of the 70-gene signature at predicting time to metastatic progression either local or distant sites, in the biopsy dataset following surgery. As with biochemical recurrence, the Kaplan-Meier survival curve shows a significance of the 70-gene signature at predicting metastatic progression of the 'Met-like' subgroup (blue) in comparison to the Non Met-like samples (green). This suggests that the patients within the 'Met-like' subgroup have an increased risk of developing metastatic disease progression following radical radiotherapy treatment with curative intent (HR = 3.50 [1.28 – 9.56]; p = 0.0017) (Figure 19). Multivariate analysis of the biopsy dataset was performed to further assess the performance of the 70-gene signature at predicting metastatic progression, independent of other known clinical prognostic factors including age at diagnosis, PSA levels at diagnosis and combined Gleason score. The prostate prognostic 70-gene signature was shown to be significantly associated with predicting metastatic disease progression independent of age, PSA and Gleason grade (both <7 and >7) (HR 2.66 [1.10 – 6.40]; p < 0.0304) (Table 31b). Similarly to the assessment of biochemical recurrence in the biopsy cohort, this data suggests that no other variable within the covariate analysis is significantly associated with identifying the increased risk of disease recurrence in the 'Met-like' subgroup (Table 31b).

Collectively, the data for both the resection and biopsy cohorts support the 70-gene signature as a prognostic assay in the field of prostate cancer which could be implemented as a patient stratifier to identify prostate cancer patients from early detection that may be at increased risk of developing more aggressive high-risk disease within 3-5 years of initial treatment.

Performance of the 70-gene signature as a prognostic tool for biochemical and metastatic recurrence in comparison to the CAPRA scoring system

The CAPRA and CAPRA-S scoring system for prostate cancer is a multivariate prognostic tool which has been developed to predict risk of disease recurrence using pre-operative biopsy material (CAPRA) and post-operative resected material (CAPRA-S). The scoring system can provide outcome based on a range of risk levels and is calculated on a points system taking into account PSA levels, patient age, Gleason grade and clinical T-stage whereby the higher the cumulative points the greater the risk of disease recurrence (Cooperberg et al 2005). CAPRA-S used to assess risk and prediction post-surgery also includes scoring for additional clinical factors including seminal vesicle invasion (SVI),

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extracapsular extension (ECE), lymph node invasion (LNI) and surgical margins. The only additional factor utilised in the CAPRA scoring system for biopsy material is the % of positive cores > or < 34%. Firstly, we investigated the prognostic performance of the novel 70-gene signature in comparison to the CAPRA-S scoring system. In multivariate analysis only the CAPRA-S scoring was significantly associated with biochemical recurrence, (HR = 1.36 [1.28-1.45], $p < 0.0001$) however both the metastatic assay and CAPRA-S scoring were significantly associated with the development of metastatic disease (HR 2.53 [1.40 – 4.60]; $p = 0.0024$ and HR = 1.43 [1.28-1.61], $p < 0.0001$ (Table 32a and 32b). These data indicate that the metastatic signature provided additional information to the CAPRA-S scoring system.

Finally we also interrogated the prognostic performance of our 70-gene signature in comparison to the CAPRA scoring system. Only the 70-gene signature was significantly associated with prognostic outcome and identifying the high-risk 'Met-like' subgroup at increased chance of developing biochemical recurrence in the biopsy dataset (HR 2.05 [1.18 – 3.59]; $p = 0.0119$) whilst the CAPRA score showing no significance independent of the prognostic assay (Table 33a). Similarly, in the biopsy validation cohort, only the 70-gene signature was significantly associated with prognostic outcome and identifying the high-risk 'Met-like' subgroup at increased chance of developing metastatic disease progression (HR 3.39 [1.44 – 7.97]; $p = 0.0054$) (Table 33b). In sum, the comparison of the 70-gene signature to the CAPRA scoring system shows better performance in biopsy material and provides further evidence for the use of the 70-gene signature as a prognostic assay within the field of prostate cancer.

DISCUSSION

Approximately 35% of primary localised prostate cancer progress to a more aggressive and recurrent disease state despite radical treatment such as surgery or external beam radiotherapy, whilst a large number of primary cancers will not progress to clinically significant disease. With this in mind, a great clinical question within the field is how to easily distinguish these subgroups of patients to allow patient stratification which could ultimately determine which patients may require further and more intense treatment regimens and which patients could avoid the toxic less tolerated therapies if unnecessary. It is thought that a potential approach to stratification is the development of compound prognostics factors

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which is based on both a combination of single prognosticators and their associations or alternatively gene expression profiles from DNA-microarray profiling (Buhmeida et al 2006).

Utilising this approach, Almac Diagnostics have developed and validated a 70-gene signature as a potential prognostic assay which could promote the identification of a high-risk prostate cancer population at increased risk of developing more aggressive disease, either biochemical or metastatic recurrence. The data within this specification strongly supports the performance of the prostate prognostic assay in both resection and biopsy material. In two independent clinical validation cohorts of primary prostate resections and biopsies, the 70-gene signature can accurately identify a subgroup of patients with a 'Met-like' biology and a greater risk of biochemical disease relapse or metastatic disease within 3-5 years of follow up. The subgroup of patients with a 'Met-like' biology are considered the population who should receive additional treatment post-surgery, such as adjuvant hormone therapy, radiotherapy or treatment with taxanes. Conversely to this, the patients identified within the Non Met-like subgroup should be spared from further treatment and monitored throughout standard clinical follow-up. It is evident this prognostic assay has two clear clinical utilities:

Predicting a subset of a defined prostate cancer cohort from resection material who may progress with high-risk disease (either biochemical recurrence or metastatic progression) following radical prostatectomy surgery with curative intent.

Predicting a subset of a defined prostate cancer cohort from biopsy material who may progress with high-risk disease (with biochemical or metastatic progression) following radical radiotherapy with curative intent.

TABLE LEGENDS

Table 28 – Summary of demographic, clinical and pathological variables considered for analysis of the internal resection cohort. Table outlines total number of patients, the median and range of age at surgery (years), time to recurrence (months), pre-operative PSA levels (ng/ml) and the number (%) of patients from each of the four clinical sites, within each recurrence subgroup, associated with each of the representative Gleason grades, within each pathological T-stage subgroup, with lymph node invasion (LNI), seminal vesicle invasion (SVI), extracapsular extension (ECE) and patients with negative, diffuse or focal surgical margins.

Table 29 – Summary of demographic, clinical and pathological variables considered for analysis of the FASTMAN biopsy cohort. Table outlines total number of patients, the median and range of age at diagnosis (years), time to recurrence (months), PSA levels at diagnosis (ng/ml) and the number (%) of patients, within each recurrence subgroup, associated with each of the representative Gleason grades and within each pathological T-stage subgroup.

Table 30 – Genes, weightings and bias of the 70-gene signature.

Table 31 – A) Multivariate analysis of the 70-gene signature in the internal resection cohort for biochemical recurrence, demonstrating assay performance independent of other prognostic clinical factors including age at surgery, PSA levels and combined Gleason score. P-values, hazard ratios (HR) and 95% confidence intervals (CI) of the HR are outlined within the table. P-values highlighted in red indicate statistical significance. B) Multivariate analysis of the 70-gene signature in the internal resection cohort for metastatic disease progression, demonstrating assay performance independent of other prognostic clinical factors including age at surgery, PSA levels and combined Gleason score. P-values, hazard ratios (HR) and 95% confidence intervals (CI) of the HR are outlined within the table. P-values highlighted in red indicate statistical significance.

Table 32 – A) Multivariate analysis of the 70-gene signature in the FASTMAN biopsy cohort for biochemical recurrence, demonstrating assay performance independent of other prognostic clinical factors including age at diagnosis, PSA levels and combined Gleason score. P-values, hazard ratios (HR) and 95% confidence intervals (CI) of the HR are outlined within the table. P-values highlighted in red indicate statistical significance. B) Multivariate analysis of the 70-gene signature in the FASTMAN biopsy cohort for metastatic disease progression, demonstrating assay performance independent of other prognostic clinical factors including age at diagnosis, PSA levels and combined Gleason score. P-values, hazard ratios (HR) and 95% confidence intervals (CI) of the HR are outlined within the table. P-values highlighted in red indicate statistical significance.

Table 33 – A) Covariate analysis of the 70-gene signature in comparison to the CAPRA-S scoring system within the internal resection cohort for biochemical recurrence, demonstrating assay performance against alternative prognostic scoring assays. P-values, hazard ratios (HR) and 95% confidence intervals (CI) of the HR are outlined for each comparison within the table. P-values highlighted in red indicate statistical significance. B) Covariate analysis of the 70-gene signature in comparison to the CAPRA-S scoring system within the internal

resection cohort for metastatic disease progression, demonstrating assay performance against alternative prognostic scoring assays. P-values, hazard ratios (HR) and 95% confidence intervals (CI) of the HR are outlined for each comparison within the table. P-values highlighted in red indicate statistical significance.

- 5 **Table 34** – A) Covariate analysis of the 70-gene signature in comparison to the CAPRA scoring system within the FASTMAN biopsy cohort for biochemical recurrence, demonstrating assay performance against alternative prognostic scoring assays. P-values, hazard ratios (HR) and 95% confidence intervals (CI) of the HR are outlined for each comparison within the table. P-values highlighted in red indicate statistical significance. B)
- 10 Covariate analysis of the 70-gene signature in comparison to the CAPRA scoring system within the FASTMAN biopsy cohort for metastatic disease progression, demonstrating assay performance against alternative prognostic scoring assays. P-values, hazard ratios (HR) and 95% confidence intervals (CI) of the HR are outlined for each comparison within the table. P-values highlighted in red indicate statistical significance.

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Table 28: Demographic and Clinical variable summary of Resection validation cohort

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Variable		Validation Cohort
Patient Number	No. of Patients	322
Clinical Site - n (%)	UCD	61 (19)
	Oslo	142 (44)
	Surrey	34 (11)
	WCB	85 (26)
Age at Surgery	Median (range), Years	62 (41 - 75)
Recurrence Event - n (%)	Non-recurrence	172 (53)
	Biochemical recurrence	103 (32)
	Metastatic recurrence	47 (15)
Time to Recurrence - Median (range)	Biochemical recurrence	12 (1 - 100)
	Metastatic recurrence	6 (3 - 63)
Pre-operative PSA	Median (range), ng/ml	8.4 (2 - 253)
Gleason score - n (%)	<6	2 (1)
	6	67 (21)
	7	197 (61)
	8 - 10	55 (17)
Pathological T-stage - n (%)	T1	1 (0.5)
	T2	174 (54)
	T3	146 (45)
	T4	1 (0.5)
Lymph Node Invasion - n (%)	Yes	16 (5)
	No	105 (33)
	Unknown	201 (62)
Seminal Vesicle Invasion - n (%)	Yes	62 (19)
	No	260 (81)
Extracapsular Extension - n (%)	Yes	97 (30)
	No	190 (59)
	Unknown	35 (11)
Surgical Margins - n (%)	Negative	132 (41)
	Focal	40 (12)
	Diffuse	65 (20)
	Unknown	85 (27)

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Table 29: Demographic and Clinical variable summary of Biopsy validation cohort

Variable		Validation Cohort
Patient Number	No. of Patients	248
Clinical Site - n (%)	Belfast	248 (100)
Age at Diagnosis	Median (range), Years	68 (48 - 79)
Recurrence Event - n (%)	Non-recurrence	170 (68)
	Biochemical recurrence	56 (23)
	Metastatic recurrence	22 (9)
Time to Recurrence - Median (range)	Biochemical recurrence	82 (10 - 117)
	Metastatic recurrence	86.5 (10 - 128)
PSA at Diagnosis	Median (range), ng/ml	17.95 (3.2 - 222.3)
Gleason Grade - n (%)	6	41 (17)
	7	100 (40)
	8 - 10	107 (43)
Pathological T-stage - n (%)	T1	51 (21)
	T2	76 (31)
	T3	92 (36)
	T4	4 (2)
	Unknown	25 (10)

Table 30: Genes, weightings and bias of the 70-gene signature

Gene Name	Entrez Gene ID	Weight	Bias
CAPN6	827	-0.010898880	4.440873234
THBS4	7060	-0.009631509	6.912586369
PLP1	5354	-0.008885735	4.383572327
MT1A	4489	-0.008680747	6.747956978
MIR205HG	406988	-0.008278545	7.215245389
SEMG1	6406	-0.007934619	4.230422622
RSPO3	84870	-0.007295796	4.293172794
ANO7	50636	-0.007164357	6.522547774
PCP4	5121	-0.007138975	7.621758138
ANKRD1	27063	-0.006922498	5.92831485
MYBPC1	4604	-0.006844539	4.574318807
MMP7	4316	-0.006835450	6.756722063
SERPINA3	12	-0.006830879	5.745461752
SELE	6401	-0.006809804	5.977682143
KRT5	3852	-0.006402712	6.080493983
LTF	4057	-0.006400452	6.497259991
KIAA1210	57481	-0.006380629	3.559966010
TMEM158	25907	-0.006312212	8.063421249
ZFP36	7538	-0.006271047	9.960826690
FO58	2354	-0.006108115	6.954936015
PCA3	50652	-0.006101922	5.262341585
TRPM8	79054	-0.006059944	4.865791397
PTTG1	9232	0.006017344	4.712692803
#N/A	283194	-0.005950381	4.980380941
PAGE4	9506	-0.005837135	7.073906580
STEAP4	79689	-0.005684812	8.105295362
TMEM178A	130733	-0.00564663	7.59452596
CXCL2	2920	-0.005597719	8.928977514
HS3ST3A1	9955	-0.005593197	4.232781732

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EYA1	2138	-0.005581031	5.504276204
RSP02	340419	-0.005562783	3.922420794
PKP1	5317	-0.005553136	5.912186171
MUC6	4588	-0.005522157	6.640037274
PENK	5179	-0.005505761	4.514855049
DEFB1	1672	-0.005399899	6.825490924
SLC7A3	84889	-0.005389518	4.649003630
MIR578	693163	-0.005355230	5.087389320
PI15	51050	-0.005263663	4.858716243
UBXN10-AS1	101928017	-0.005259309	6.065877615
PK4	5166	-0.005248750	4.174094312
PHGR1	644844	-0.005207500	5.183571143
SERPINE1	5054	-0.005194886	6.691866284
POZRN4	29951	-0.005146623	4.752327652
ZNF185	7739	-0.005105327	6.900544220
ADRA2C	152	-0.005054713	7.078376864
AZGP1	563	-0.005018400	8.191177501
TK1	7083	0.004965887	5.581334570
POTEH	23784	-0.004961473	4.824976325
KIF11	3832	0.004928774	3.917668501
CLDN1	9076	-0.004924383	4.960282713
MIR4530	100616163	-0.004907676	10.53645223
MAFF	23764	-0.004901224	8.497945251
ZNF765	91661	-0.004861949	3.976333034
CKS2	1164	0.004855890	6.503980715
TCEAL7	56849	-0.004855875	4.819327983
PLIN1	5346	0.004830634	4.629391793
SIGLEC1	6614	0.004772601	5.503752383
FAM150B	285016	-0.004772585	6.664595224
MFAP5	8076	-0.004771653	4.129176546
SFRP1	6422	-0.004761531	7.901261944
DUSP5	1847	-0.004718060	5.762677834
VAR52	57176	0.004675188	5.223455192
ABCC4	10257	-0.004664227	5.230376747
SH3BP4	23677	-0.004622969	4.882708067
SORD	6652	-0.004573155	8.958411069
MTERFD1	51001	0.004522466	5.334198783
DPP4	1803	-0.004505906	4.65974831
#N/A	284837	0.004502134	4.905312692
FAM3B	54097	-0.004443400	7.388071281
KLK3	354	-0.004424720	10.226441291

Table 31: Multivariate analysis of the 70-gene signature in the internal resection cohort for a) biochemical recurrence and b) metastatic progression.

a) Biochemical Recurrence

Covariate	HR	95% CI	p
Prostate Metastatic Assay : Negative	1.65	1.16 to 2.34	0.0055
Gleason="<7"	0.59	0.36 to 0.97	0.0388
Gleason=">7"	2.10	1.44 to 3.07	0.0001
Age	1.00	0.97 to 1.03	0.9088
PSA	1.00	1.00 to 1.01	0.0089
Abbreviations: HR, hazard ratio Assessment post-surgical.			

5 b) Metastatic Disease

Covariate	HR	95% CI	p
Prostate Metastatic Assay : Negative	3.50	1.95 to 6.27	<0.0001
Gleason="<7"	0.35	0.11 to 1.17	0.0906
Gleason=">7"	3.11	1.67 to 5.77	0.0004
Age	0.98	0.93 to 1.03	0.4039
PSA	1.01	0.99 to 1.02	0.3634

Table 32: Multivariate analysis of the 70-gene signature in FASTMAN biopsy cohort for a) biochemical recurrence and b) metastatic progression.

a) Biochemical Recurrence

Covariate	P-value	HR	95% CI of HR
Prostate 70 Gene Call: Met-Like	0.0220	1.96	1.11 to 3.48
Age at Diagnosis	0.1375	0.97	0.93 to 1.01
PSA at Diagnosis	0.1308	1.01	1.00 to 1.01
Combined Gleason Score ="<7"	0.1510	0.49	0.19 to 1.29
Combined Gleason Score =">7"	0.9409	0.98	0.55 to 1.73

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b) Metastatic Disease

Covariate	P-value	HR	95% CI of HR
Prostate 70 Gene Call: Met-Like	0.0304	2.66	1.10 to 6.40
Age at Diagnosis	0.7628	0.99	0.93 to 1.06
PSA at Diagnosis	0.2517	1.01	1.00 to 1.02
Combined Gleason Score ="<7"	0.3573	0.37	0.05 to 3.03
Combined Gleason Score =">7"	0.5389	1.35	0.52 to 3.46

Table 33: Analysis and comparison of the 70-gene signature to CAPRA scoring system in the internal resection cohort for a) biochemical recurrence and b) metastatic progression.

a) Biochemical Recurrence

Covariate	HR	95% CI	p
Prostate Metastatic Assay : Negative	1.34	0.94 to 1.90	0.1079
CARPA-S	1.36	1.28 to 1.45	<0.0001

Abbreviations: HR, hazard ratio; CAPRA-s, Cancer of the Prostate Risk Assessment post-surgical.

b) Metastatic Disease

Covariate	HR	95% CI	p
Prostate Metastatic Assay : Negative	2.53	1.40 to 4.60	0.0024
CARPA-S	1.43	1.28 to 1.61	<0.0001

Table 34: Analysis and comparison of the 70-gene signature to CAPRA scoring system in the FASTMAN biopsy cohort for a) biochemical recurrence and b) metastatic progression.

a) Biochemical Recurrence

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Covariate	P-value	HR	95% CI of HR
Prostate 70 Gene Call: Met-Like	0.0119	2.05	1.18 to 3.59
CAPRA Score	0.3443	1.11	0.90 to 1.36

b) Metastatic Disease

Covariate	P-value	HR	95% CI of HR
Prostate 70 Gene Call: Met-Like	0.0054	3.39	1.44 to 7.97
CAPRA Score	0.7455	1.06	0.76 to 1.47

5 Example 4 – Core and minimum gene analysis**Samples:**

- Internal training samples (Discovery cohort): This sample set comprised of 126 FFPE prostate resection FFPE tissue samples profiled on the Almac Prostate DSA™ microarray.
- FASTMAN Biopsy Validation Cohort: This sample set was comprised of 248 prostate biopsy FFPE tissue samples collected in collaboration with the FASTMAN Research Group under the Movember Programme.
- Internal Resection Validation Cohort: This sample set comprised of 322 prostate resection FFPE tissue samples collected internally by Almac Diagnostics. Samples were obtained from four clinical sites; University College Dublin (61 samples), Wales Cancer Bank (85 samples), University of Surrey (34 samples) and University Hospital of Oslo (142 samples).

Methods:**Core gene analysis**

The purpose of evaluating the core gene set of the signature is to determine a ranking for the Entrez genes based upon their impact on performance when removed from the signature.

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This analysis involved 10,000 random samplings of 10 signature Entrez genes from the original 70 signature Entrez gene set. At each iteration, 10 randomly selected signature Entrez genes were removed and the performance of the remaining 65 genes was evaluated using the endpoint to determine the impact on HR (Hazard Ratio) performance when these 10 Entrez genes were removed in the following 2 datasets:

- FASTMAN Biopsy Validation Cohort – 248 samples
- Internal Resection Validation Cohort – 322 samples

FASTMAN Biopsy Validation was evaluated using the biochemical recurrence (BCR) endpoint and Internal Resection Validation was evaluated using the metastatic recurrence (MET) endpoint. Within each of the 2 datasets, the signature Entrez genes were weighted based upon the change in HR performance (Delta HR) based upon their inclusion or exclusion. Entrez genes ranked '1' have the most negative impact on performance when removed and those ranked '70' have the least impact on performance when removed.

15 **Minimum gene analysis**

The purpose of evaluating the minimum number of Entrez genes is to determine if significant performance can be achieved within smaller subsets of the original signature.

This analysis involved 10,000 random samplings of the 70 signature Entrez genes starting at 1 Entrez gene/feature, up to a maximum of 30 Entrez genes/features. For each randomly selected feature length, the signature was redeveloped using the PLS machine learning method under CV and model parameters derived. At each feature length, all randomly selected signatures were applied to calculate signature scores for the following 2 datasets:

- FASTMAN Biopsy Validation Cohort – 248 samples
- Internal Resection Validation Cohort – 322 samples

Continuous signature scores were evaluated with outcome to determine the HR effect; FASTMAN Biopsy Validation was evaluated with BCR and Internal Resection Validation was evaluated with MET. The HR for all random signatures at each feature length was summarized and figures generated to visualize the performance over CV.

RESULTS

Core gene analysis

The results for the core gene analysis of the 70 gene signature in the 2 datasets is provided in this section.

- 5 • FASTMAN Biopsy Validation: Delta HR performance measured in this dataset for the 70 signature Entrez genes is shown in Figure 20. This figure highlights the top 10 ranked Entrez genes in the signature which are the most important in retaining a good HR performance within this dataset. This ranking can also be found in Table 35 below:

Entrez Gene	Gene	Total Delta HR	Rank
6401	SELE	4.761124889	1
340419	RSPO2	3.687852175	2
4489	MT1A	3.565744532	3
3852	KRT5	2.45747844	4
563	AZGP1	2.446961746	5
5123	PCP4	2.440528148	6
51050	PI15	2.353758149	7
5179	PENK	1.642705501	8
25907	TMEM158	1.476987515	9
152	ADRA2C	1.4186879	10
50636	ANO7	1.34866117	11
2138	EYA1	1.348354023	12
3832	KIF11	1.291035934	13
23677	SH3BP4	1.224986822	14
5166	PDK4	1.188342205	15
57481	KIAA1210	1.103651804	16
23784	POTEH	1.043547171	17
6614	SIGLEC1	0.855535152	18
4604	MYBPC1	0.819417585	19
2920	CXCL2	0.813780936	20
6406	SEMG1	0.768923782	21
9955	HS3ST3A1	0.749239331	22
4057	LTF	0.71103352	23
7083	TK1	0.677537934	24
57176	VAR2	0.653632853	25
79054	TRPM8	0.506824534	26
29951	PDZRN4	0.420605146	27

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9506	PAGE4	0.340073483	28
50652	PCA3	0.315775741	29
79689	STEAP4	0.266189243	30
1847	DUSP5	0.178110535	31
6422	SFRP1	0.138569985	32
693163	MIR578	0.118486894	33
101928017	UBXN10-AS1	0.068688136	34
6652	SORD	-0.004486521	35
5346	PLIN1	-0.086533897	36
56849	TCEAL7	-0.13067584	37
1803	DPP4	-0.144066233	38
5317	PKP1	-0.164994289	39
354	KLK3	-0.166136293	40
54097	FAM3B	-0.209897076	41
23764	MAFF	-0.214942264	42
9232	PTTG1	-0.256777275	43
2354	FOSB	-0.264910805	44
406988	MIR205HG	-0.303067689	45
91661	ZNF765	-0.423012094	46
284837	#N/A	-0.449656588	47
5054	SERPINE1	-0.476929578	48
10257	ABCC4	-0.490520163	49
644844	PHGR1	-0.539343141	50
283194	#N/A	-0.555242337	51
4588	MUC6	-0.574748909	52
51001	MTERFD1	-0.770988555	53
7538	ZFP36	-0.842688769	54
1672	DEFB1	-1.003111116	55
9076	CLDN1	-1.074445919	56
130733	TMEM178A	-1.134351	57
84889	SLC7A3	-1.153855918	58
7739	ZNF185	-1.20365806	59
12	SERPINA3	-1.443334853	60
827	CAPN6	-1.618228454	61
5354	PLP1	-1.680375803	62
1164	CKS2	-1.700995591	63
8076	MFAP5	-1.724942849	64
84870	RSPO3	-2.50110156	65
100616163	MIR4530	-2.79787323	66

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285016	FAM150B	-3.055488057	67
27063	ANKRD1	-4.50925449	68
7060	THBS4	-4.556568781	69
4316	MMP7	-4.78562355	70

- Internal Resection Validation: Delta HR performance measured in this dataset for the 70 signature Entrez genes is shown in Figure 2. This figure highlights the top 10 ranked Entrez genes in the signature which are the most important in retaining a good HR performance within this dataset. This ranking can also be found in Table 36 below:

Entrez Gene	Gene	Total Delta HR	Rank
3852	KRT5	5.850910136	1
2354	FOSB	5.341991077	2
9232	PTTG1	4.440300792	3
5179	PENK	4.359290179	4
340419	RSPO2	3.715352525	5
563	AZGP1	3.640373688	6
100616163	MIR4530	3.034458226	7
7538	ZFP36	2.900383458	8
4604	MYBPC1	2.60456647	9
23764	MAFF	2.422195244	10
50652	PCA3	2.343241624	11
50636	ANO7	1.922305172	12
1803	DPP4	1.747968953	13
693163	MIR578	1.70934994	14
4057	LTF	1.457636816	15
1847	DUSP5	1.441368066	16
7083	TK1	1.432224235	17
101928017	UBXN10-AS1	1.249812402	18
1164	CKS2	1.152406332	19
23677	SH3BP4	1.116227302	20
5121	PCP4	1.047369238	21
152	ADRA2C	0.891075934	22
12	SERPINA3	0.854606034	23
57481	KIAA1210	0.762370469	24
3832	KIF11	0.713624009	25
4489	MT1A	0.655338791	26
9506	PAGE4	0.430978289	27

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2138	EYA1	0.384089193	28
91661	ZNF765	0.309943842	29
284837	#N/A	0.303352744	30
25907	TMEM158	0.247359339	31
6614	SIGLEC1	0.202684496	32
9076	CLDN1	0.060049481	33
354	KLK3	-0.07704205	34
79054	TRPM8	-0.07716181	35
5054	SERPINE1	-0.083069191	36
84889	SLC7A3	-0.103594879	37
79689	STEAP4	-0.262219935	38
9955	HS3ST3A1	-0.310839602	39
130733	TMEM178A	-0.328948061	40
10257	ABCC4	-0.420421537	41
51001	MTERFD1	-0.427114354	42
5346	PLIN1	-0.445607269	43
4588	MUC6	-0.452261632	44
644844	PHGR1	-0.527656877	45
283194	#N/A	-0.623963891	46
29951	PDZRN4	-0.672143861	47
57176	VAR2	-0.673665413	48
6652	SORD	-0.711615138	49
7739	ZNF185	-0.796601532	50
5317	PKP1	-0.91761911	51
6401	SELE	-0.943930367	52
23784	POTEH	-0.987487576	53
54097	FAM3B	-1.064799882	54
5354	PLP1	-1.065316284	55
6422	SFRP1	-1.370192928	56
5166	PDK4	-1.863810081	57
84870	RSPO3	-2.4018171	58
56849	TCEAL7	-2.455318029	59
51050	PI15	-2.502066289	60
6406	SEMG1	-2.625125175	61
4316	MMP7	-3.015001652	62
2920	CXCL2	-3.051014073	63
406988	MIR205HG	-3.231330366	64
285016	FAM150B	-3.602511107	65
27063	ANKRD1	-3.836256996	66
1672	DEFB1	-4.174807907	67

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8076	MFAP5	-4.187157544	68
827	CAPN6	-4.472033713	69
7060	THBS4	-5.697080094	70

- Delta HR across these 2 datasets was evaluated to obtain a combined Entrez gene ranking for each of the signature Entrez genes. This is summarized in Table 37 below:

Combined		
Entrez Gene	Gene	Delta HR
		-
12	SERPINA3	0.588728819
152	ADRA2C	2.309763834
		-
354	KLK3	0.243178342
563	AZGP1	6.087335434
		-
827	CAPN6	6.090262167
		-
1164	CKS2	0.548589258
		-
1672	DEFB1	5.177919023
1803	DPP4	1.60390272
1847	DUSP5	1.6194786
2138	EYA1	1.732443216
2354	FOSB	5.077080272
		-
2920	CXCL2	2.237233137
3832	KIF11	2.004659943
3852	KRT5	8.308388576
4057	LTF	2.168670336
		-
4316	MMP7	7.800625203
4489	MT1A	4.221083323
4588	MUC6	-1.02701054
4604	MYBPC1	3.423984055
		-
5054	SERPINE1	0.559998768
5121	PCP4	3.487897386
		-
5166	PK4	0.675467876

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5179	PENK	6.001995681
		-
5317	PKP1	1.082613399
		-
5346	PLIN1	0.532141166
		-
5354	PLP1	2.745692087
6401	SELE	3.817194522
		-
6406	SEMG1	1.856201393
		-
6422	SFRP1	1.231622942
6614	SIGLEC1	1.058219648
		-
6652	SORD	0.716101659
		-
7060	THBS4	10.25364888
7083	TK1	2.109762169
7538	ZFP36	2.057694688
		-
7739	ZNF185	2.000259592
		-
8076	MFAP5	5.912100393
		-
9076	CLDN1	1.014396437
9232	PTTG1	4.183523517
9506	PAGE4	0.771051772
9955	HS3ST3A1	0.438399729
10257	ABCC4	-0.9109417
23677	SH3BP4	2.341214123
23764	MAFF	2.20725298
23784	POTEH	0.056059594
25907	TMEM158	1.724346854
		-
27063	ANKRD1	8.345511486
		-
29951	PDZRN4	0.251538716
50636	ANO7	3.270966342
50652	PCA3	2.659017364
		-
51001	MTERFD1	1.198102909
51050	PI15	-0.14830814
54097	FAM3B	-

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		1.274696959
		-
56849	TCEAL7	2.585993869
57176	VAR52	-0.02003256
57481	KIAA1210	1.866022273
79054	TRPM8	0.429662725
79689	STEAP4	0.003969308
84870	RSPO3	-4.90291866
		-
84889	SLC7A3	1.257450797
		-
91661	ZNF765	0.113068252
		-
130733	TMEM178A	1.463299061
		-
283194	#N/A	1.179206229
		-
284837	#N/A	0.146303844
		-
285016	FAM150B	6.657999164
340419	RSPO2	7.4032047
		-
406988	MIR205HG	3.534398055
		-
644844	PHGR1	1.067000018
693163	MIR578	1.827836834
100616163	MIR4530	0.236584996
	UBXN10-	
101928017	AS1	1.318500539

The ranks assigned to the signature Entrez genes based on the combined core set analysis is summarized in Table 38 below:

Entrez Gene	Gene	Total Delta HR	Rank
3852	KRT5	8.308388576	1
340419	RSPO2	7.4032047	2
563	AZGP1	6.087335434	3
5179	PENK	6.001995681	4
2354	FOSB	5.077080272	5
4489	MT1A	4.221083323	6
9232	PTTG1	4.183523517	7

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6401	SELE	3.817194522	8
5121	PCP4	3.487897386	9
4604	MYBPC1	3.423984055	10
50636	ANO7	3.270966342	11
50652	PCA3	2.659017364	12
23677	SH3BP4	2.341214123	13
152	ADRA2C	2.309763834	14
23764	MAFF	2.20725298	15
4057	LTF	2.168670336	16
7083	TK1	2.109762169	17
7538	ZFP36	2.057694688	18
3832	KIF11	2.004659943	19
57481	KIAA1210	1.866022273	20
693163	MIR578	1.827836834	21
2138	EYA1	1.732443216	22
25907	TMEM158	1.724346854	23
1847	DUSP5	1.6194786	24
1803	DPP4	1.60390272	25
101928017	UBXN10- AS1	1.318500539	26
6614	SIGLEC1	1.058219648	27
9506	PAGE4	0.771051772	28
9955	HS3ST3A1	0.438399729	29
79054	TRPM8	0.429662725	30
100616163	MIR4530	0.236584996	31
23784	POTEH	0.056059594	32
79689	STEAP4	0.003969308	33
57176	VAR52	-0.02003256	34
91661	ZNF765	-0.113068252	35
284837	#N/A	-0.146303844	36
51050	PI15	-0.14830814	37
354	KLK3	-0.243178342	38
29951	PDZRN4	-0.251538716	39
5346	PLIN1	-0.532141166	40
1164	CKS2	-0.548589258	41
5054	SERPINE1	-0.559998768	42
12	SERPINA3	-0.588728819	43
5166	PDK4	-0.675467876	44
6652	SORD	-0.716101659	45
10257	ABCC4	-0.9109417	46

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9076	CLDN1	-1.014396437	47
4588	MUC6	-1.02701054	48
644844	PHGR1	-1.067000018	49
5317	PKP1	-1.082613399	50
283194	#N/A	-1.179206229	51
51001	MTERFD1	-1.198102909	52
6422	SFRP1	-1.231622942	53
84889	SLC7A3	-1.257450797	54
54097	FAM3B	-1.274696959	55
130733	TMEM178A	-1.463299061	56
6406	SEMG1	-1.856201393	57
7739	ZNF185	-2.000259592	58
2920	CXCL2	-2.237233137	59
56849	TCEAL7	-2.585993869	60
5354	PLP1	-2.745692087	61
406988	MIR205HG	-3.534398055	62
84870	RSPO3	-4.90291866	63
1672	DEFB1	-5.177919023	64
8076	MFAP5	-5.912100393	65
827	CAPN6	-6.090262167	66
285016	FAM150B	-6.657999164	67
4316	MMP7	-7.800625203	68
27063	ANKRD1	-8.345511486	69
7060	THBS4	-10.25364888	70

Minimum gene analysis

The results for the minimum gene analysis of the 70 gene signature in 2 datasets is provided in this section.

- 5 • FASTMAN Biopsy Validation: The average HR performance measured in this dataset using the random sampling of the signature Entrez genes from a feature length of 1 to 30 is shown in Figure 22. This figure shows that to retain a significant HR performance (i.e. lower CI of HR > 1) a minimum of 12 of the signature Entrez genes must be selected.
- 10 • Internal Resection Validation: The average HR performance measured in this dataset using the random sampling of the signature Entrez genes from a feature length of 1 to 30 is shown in Figure 23. This figure shows that to retain a significant HR performance (i.e. lower CI of HR > 1) a minimum of 7 of the signature Entrez genes must be selected.

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5 The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. Moreover, all embodiments described herein are considered to be broadly applicable and combinable with any and all other consistent embodiments, as appropriate.

10

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

CLAIMS

1. A method for characterising and/or prognosing cancer in a subject comprising:
determining the expression level of at least one, and preferably 12, genes selected from
5 Table 1 in a sample from the subject wherein the determined expression level is used to
provide a characterisation of and/or a prognosis for the cancer.
2. A method for diagnosing a cancer with an increased metastatic potential in a subject
comprising:
10 determining the expression level of at least one, and preferably 12, genes selected from
Table 1 in a sample from the subject wherein the determined expression level is used to
identify whether a subject has a cancer with increased metastatic potential.
3. A method for characterising and/or prognosing cancer in a subject comprising:
15 determining the expression level of at least one, and preferably 12, genes selected from
Table 1 in a sample from the subject in order to identify the presence or absence of cells
characteristic of an increased likelihood of recurrence and/or metastasis wherein the
determined presence or absence of the cells is used to provide a characterisation of and/or a
prognosis for the cancer.
20
4. A method for characterising and/or prognosing cancer in a subject comprising:
a) in a sample from the subject
b) applying a nucleic acid probe that specifically hybridizes with the nucleotide sequence of
at least one, and preferably 12, genes or full sequences or target sequences selected from
25 Table 1 to the sample from the subject
c) applying a detection agent that detects the nucleic acid probe-gene complex
d) using the detection agent to determine the level of the at least one, and preferably 12,
genes
d) wherein the determined level of the at least one, and preferably 12, genes is used to
30 provide a characterisation of and/or a prognosis for the cancer.
5. A method for characterising and/or prognosing a cancer in a subject comprising:
a) in a sample from the subject
b) applying a set of nucleic acid primers that specifically hybridize with the nucleotide

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sequence of at least one, and preferably 12, genes or full sequences or target sequences selected from Table 1 to the sample from the subject

c) amplifying the nucleotide sequence using the set of nucleic acid primers

d) detecting the amplification products using a specific detection agent to determine the level

5 of the at least one, and preferably 12, genes

d) wherein the determined level of the at least one, and preferably 12, genes is used to provide a characterisation of and/or a prognosis for the cancer.

6. A method for selecting a treatment for cancer in a subject comprising:

10 (a) determining the expression level of at least one, and preferably 12, genes selected from Table 1 in a sample from the subject wherein the determined expression level is used to provide a characterisation of and/or a prognosis for the cancer and

(b) selecting a treatment appropriate to the characterisation of and/or prognosis for the cancer.

15

7. A method for selecting a treatment for cancer in a subject comprising:

(a) determining the expression level of at least one, and preferably 12, genes selected from Table 1 in a sample from the subject wherein the determined expression level is used to provide a characterisation of and/or a prognosis for the cancer

20 (b) selecting a treatment appropriate to the characterisation of and/or prognosis for the cancer and

(c) treating the subject with the selected treatment.

8. The method of claim 6 or 7, wherein if the characterisation of and/or prognosis for the

25 cancer is an increased likelihood of recurrence and/or metastasis and/or a poor prognosis the treatment selected is one or more of

a) an anti-hormone treatment, preferably bicalutamide and/or abiraterone

b) a cytotoxic agent

c) a biologic, preferably an antibody and/or a vaccine, more preferably Sipuleucel-T

30 d) radiotherapy, optionally extended radiotherapy, preferably extended-field radiotherapy

e) targeted therapy

f) surgery.

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9. The method of any one of claims 6 to 8 wherein if the characterisation of and/or prognosis for the cancer is no increased likelihood of recurrence and/or metastasis and/or a poor prognosis no treatment, or no further treatment, is selected/administered.
- 5 10. The method of claim 9 wherein the cancer is subsequently monitored to determine whether treatment, or further treatment, is required.
11. The method of any preceding claim comprising determining the expression level of at least 2 or more genes selected from Table 1, up to all 70 genes, optionally wherein the at
10 least two genes comprise MT1A and PCP4, or wherein expression levels of the genes listed in any one of tables 2 to 24 is determined.
12. The method of any preceding claim which further comprises determining PSA levels and/or Gleason score in the subject and using the determined PSA levels and/or Gleason
15 score in combination with the determined expression levels to provide a characterization and/or prognosis for the cancer (including diagnosing whether the cancer has increased metastatic potential).
13. The method of any preceding claim wherein the cancer comprises or is prostate cancer
20 or ER positive breast cancer.
14. The method of any preceding claim wherein determining the expression levels employs:
- 25 (a) primers (primer pairs) and/or probes that hybridize with at least one of the full sequences or target sequences from Table 1; and/or
- (b) at least one probe and/or probeset from Table 1/1A; and/or
- (c) at least one primer and/or primer pair from Table 1B and/or of SEQ ID NOs 3151-3154.
- 30 15. The method of any preceding claim wherein the characterisation of and/or prognosis for, or diagnosis of, the cancer comprises, consists essentially of or consists of predicting an increased likelihood of recurrence and/or predicting an increased likelihood of metastasis.

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16. The method of any preceding claim wherein the characterisation of and/or prognosis for, or diagnosis of, the cancer comprises, consists essentially of or consists of determining whether the cancer has a poor prognosis.

5 17. The method of any preceding claim comprising comparing the expression level to a reference value or to the expression level in one or more control samples.

18. The method of any preceding claim wherein the expression level is compared to the expression level of the same gene in one or more control samples.

10

19. The method of any preceding claim wherein the expression level is determined by microarray, northern blotting, RNA-seq (RNA sequencing), in situ RNA detection or nucleic acid amplification.

15 20. The method of any preceding claim further comprising extracting total RNA from the sample and/or further comprising obtaining the sample from the subject.

21. The method of any preceding claim wherein a signature score is derived from the measured expression levels, optionally according to the formula:

20
$$SignatureScore = \sum_i w_i \times (ge_i - b_i) + k$$

Where w_i is a weight for each gene, b_i is a gene-specific bias, ge_i is the gene expression after pre-processing, and k is a constant offset.

22. The method of any preceding claim wherein the sample comprises, consists essentially of or consists of prostate cells and/or tissue or breast cells and/or tissue.

25

23. The method of any preceding claim wherein the sample comprises, consists essentially of or consists of a formalin-fixed paraffin-embedded biopsy sample or a resection sample.

30 24. The method of any preceding claim wherein an increased expression level of at least one gene selected from Table 1 with a positive weight indicates an increased likelihood of recurrence and/or metastasis and/or a poor prognosis.

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25. The method of any preceding claim wherein a decreased expression level of at least one gene selected from Table 1 with a negative weight indicates an increased likelihood of recurrence and/or metastasis and/or a poor prognosis.

5

26. The method of claim 21 or any claim dependent therefrom wherein a signature score above threshold indicates an increased likelihood of recurrence and/or metastasis and/or a poor prognosis.

10

27. The method of claim 21 or any claim dependent therefrom wherein a signature score equal to or above threshold indicates an increased likelihood of recurrence and/or metastasis and/or a poor prognosis.

15

28. The method of claim 21 or any claim dependent therefrom wherein the signature score is calculated for measured gene expression levels of the genes in a signature selected from the signatures of Tables 1 to 24.

20

29. A method of treating cancer comprising administering a chemotherapeutic agent or radiotherapy, optionally extended radiotherapy, preferably extended-field radiotherapy, to a subject or carrying out surgery on a subject wherein the subject is selected for treatment on the basis of a method as claimed in any of claims 6 to 8 or any claim dependent thereon.

25

30. A chemotherapeutic agent for use in treating cancer in a subject, wherein the subject is selected for treatment on the basis of a method as claimed in any of claims 6 to 8 or any claim dependent thereon.

30

31. The method of claim 29 or chemotherapeutic agent for use of claim 30 wherein the chemotherapeutic agent comprises, consists essentially of or consists of

- a) an anti-hormone treatment, preferably bicalutamide and/or abiraterone
- b) a cytotoxic agent
- c) a biologic, preferably an antibody and/or a vaccine, more preferably Sipuleucel-T and/or
- d) a targeted therapeutic agent

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32. The method of claim 8 or 29 or any claim dependent thereon, wherein the cytotoxic agent is a platinum based agent and/or a taxane.

5 33. The method of claim 32, wherein the platinum based agent is selected from cisplatin, carboplatin and oxaliplatin.

34. The method of claim 32, wherein the taxane is paclitaxel or docetaxel.

10 35. A system, device or test kit for performing the method of any previous claim.

36. A system, device or test kit for characterising and/or prognosing cancer in a subject, comprising:

- 15 a) one or more testing devices for determining the expression level of at least one, and preferably 12, genes selected from Table 1 in a sample from the subject
- 15 b) a processor; and
- c) storage medium comprising a computer application that, when executed by the processor, is configured to:
- 20 (i) access and/or calculate the determined expression levels of the at least one, and preferably 12, genes selected from Table 1 in the sample on the one or more testing devices
- (ii) calculate whether there is an increased or decreased level of the at least one, and preferably 12, genes selected from Table 1 in the sample; and
- (iii) output from the processor the characteristaion of and/or prognosis for the cancer.

25 37. The system, device or test kit of claim 36 wherein the cancer comprises or is prostate cancer or ER positive breast cancer.

38. The system, device or test kit of claim or which is adapted to perform the method of any one of claims 1 to 34.

30

39. The system, device or test kit of anyone of claims 36 to 38 further comprising a display for the output from the processor.

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40. A computer application or storage medium comprising a computer application as defined in any one of claims 36 to 39.

41. A computer program product for characterising and/or prognosing cancer in a subject,
5 comprising a non-transitory computer-readable storage device having computer-readable program instructions embodied thereon that cause the computer to:

(i) access and/or calculate the determined expression levels of at least one, and preferably 12, genes selected from Table 1 in a sample on one or more testing devices;

(ii) calculate whether there is an increased or decreased level of the at least one, and
10 preferably 12, genes selected from Table 1 in the sample; and,

(iii) provide an output regarding the characterization of and/or prognosis for the cancer.

42. The computer program product of claim 41 wherein the cancer comprises or is prostate cancer or ER positive breast cancer.

15

43. A kit for characterising and/or prognosing cancer in a subject comprising one, and preferably 12, or more oligonucleotide probes that specifically hybridizes with an RNA product of at least one, and preferably 12, gene selected from Table 1, or that specifically hybridizes with for a full sequence or target sequence from Table 1 and further comprising

20 one or more of the following components:

a) a blocking probe

b) a PreAmplifier

c) an Amplifier and/or

d) a Label molecule.

25

44. The kit of claim 43 wherein the cancer comprises or is prostate cancer or ER positive breast cancer.

45. The kit of claim of 43 or 44 which comprises a probe and/or probeset comprising,
30 consisting essentially of or consisting of a nucleotide sequence as identified in Table 1.

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46. A kit for characterizing and/or prognosing cancer in a subject comprising one, and preferably 12, or more probes and/or probesets that specifically hybridize with at least one gene, full sequence or target sequence selected from Table 1.

- 5 47. The kit of claim of 46 which comprises a probe and/or probeset comprising, consisting essentially of or consisting of a nucleotide sequence as identified in Table 1.

48. A probe and/or probeset comprising, consisting essentially of or consisting of a nucleotide sequence as identified in Table 1.

10

49. The kit of claim 46 or 47 or probe and/or probeset of claim 48 comprising at least one probe and/or probset hybridizing with each gene from a signature selected from the signatures in Tables 1 to 24.

15

50. A kit for characterising and/or prognosing cancer in a subject comprising one, and preferably 12, or more primers and/or primer pairs for amplifying of at least one, and preferably 12, genes, full sequences or target sequences selected from Table 1.

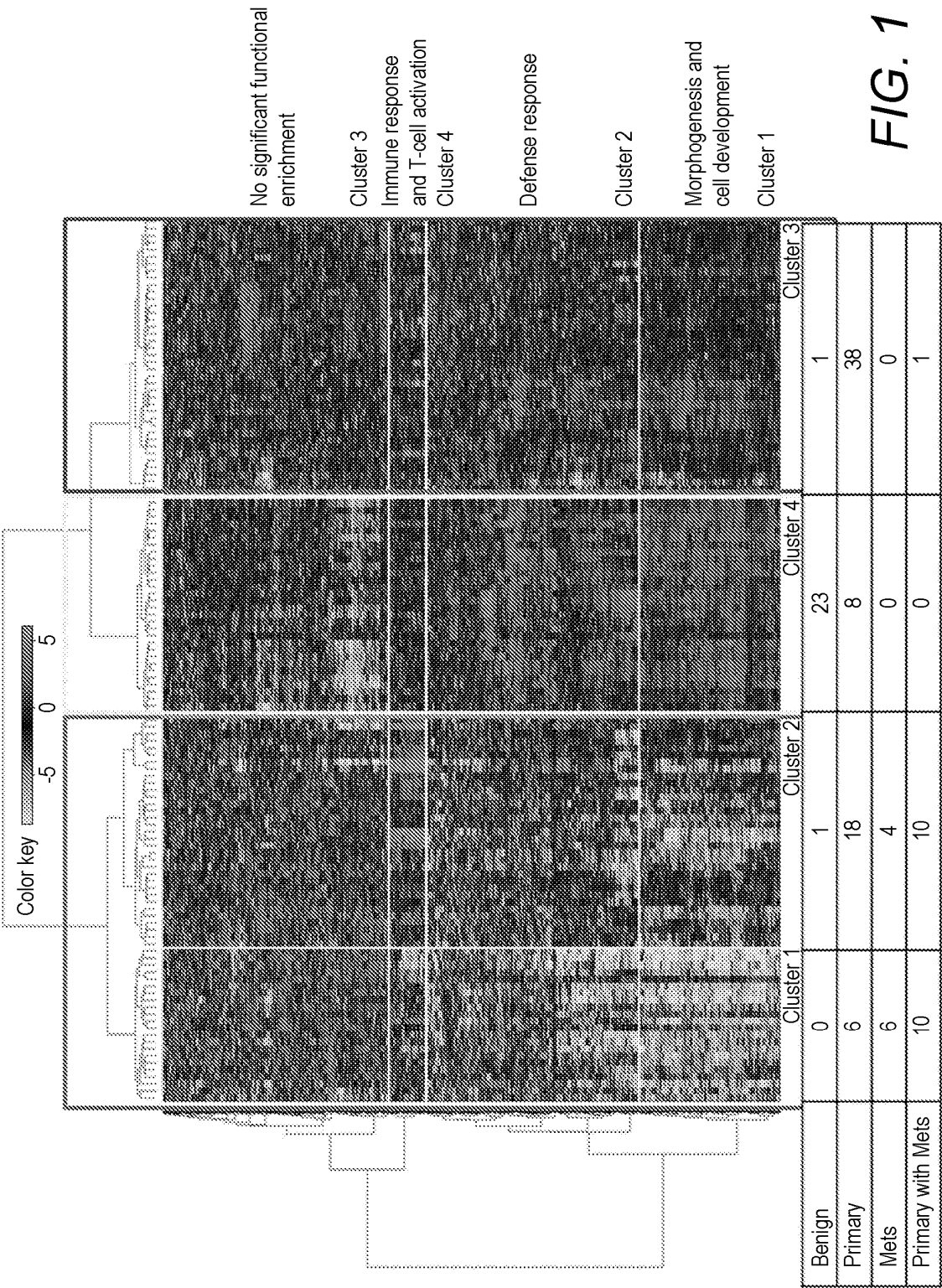
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51. The kit of claim 50 which comprises a primer and/or primer pair comprising, consisting essentially of or consisting of a nucleotide sequence as identified in Table 1B and/or of SEQ ID NOs 3151-3154.

25

52. A primer and/or primer pair comprising, consisting essentially of or consisting of a nucleotide sequence as identified in Table 1B.

53. The kit of claim 50 or 51 or primer and/or primer pair of claim 52 comprising at least one primer and/or primer pair hybridizing with each gene from a signature selected from the signatures in Tables 1 to 24.



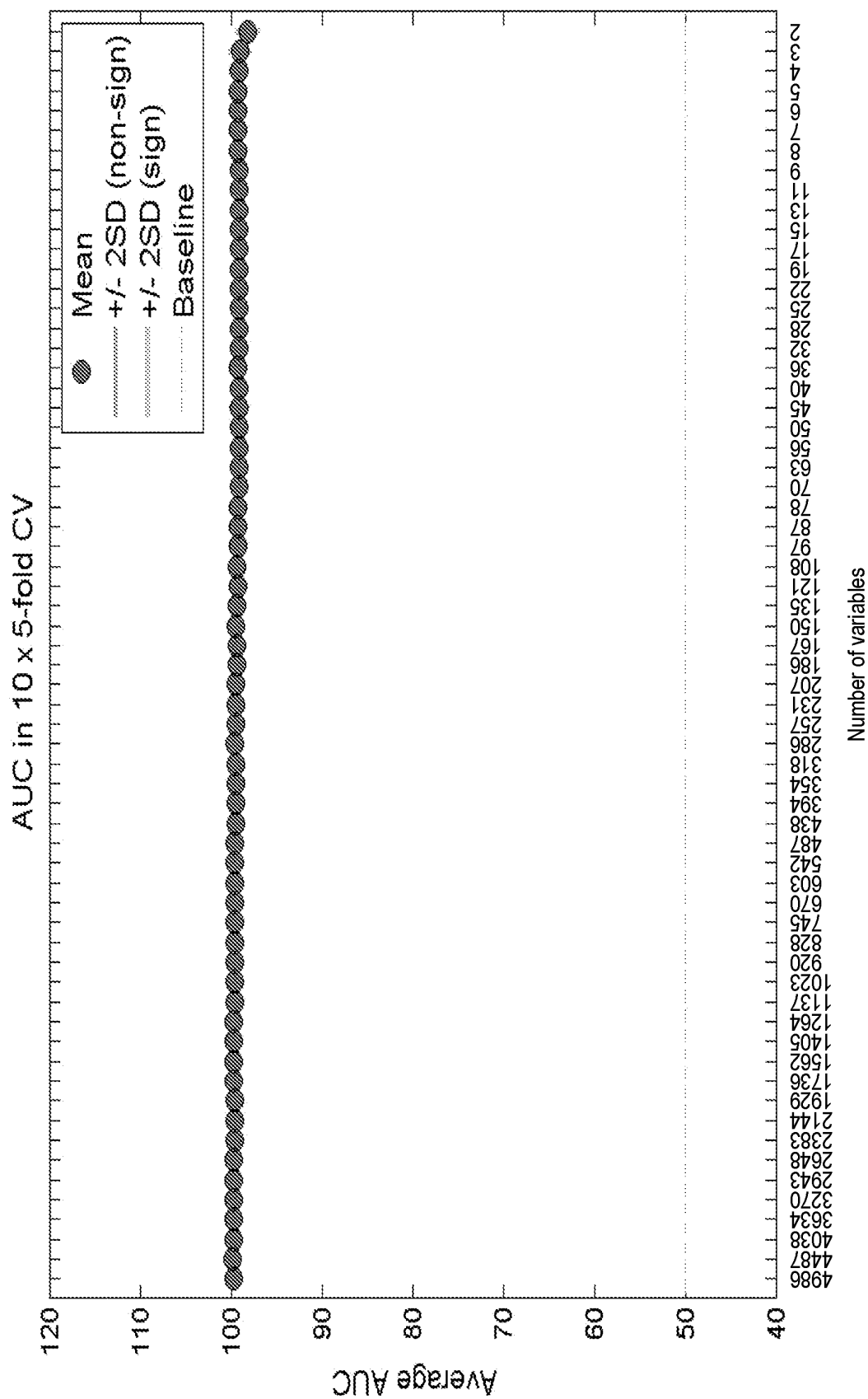
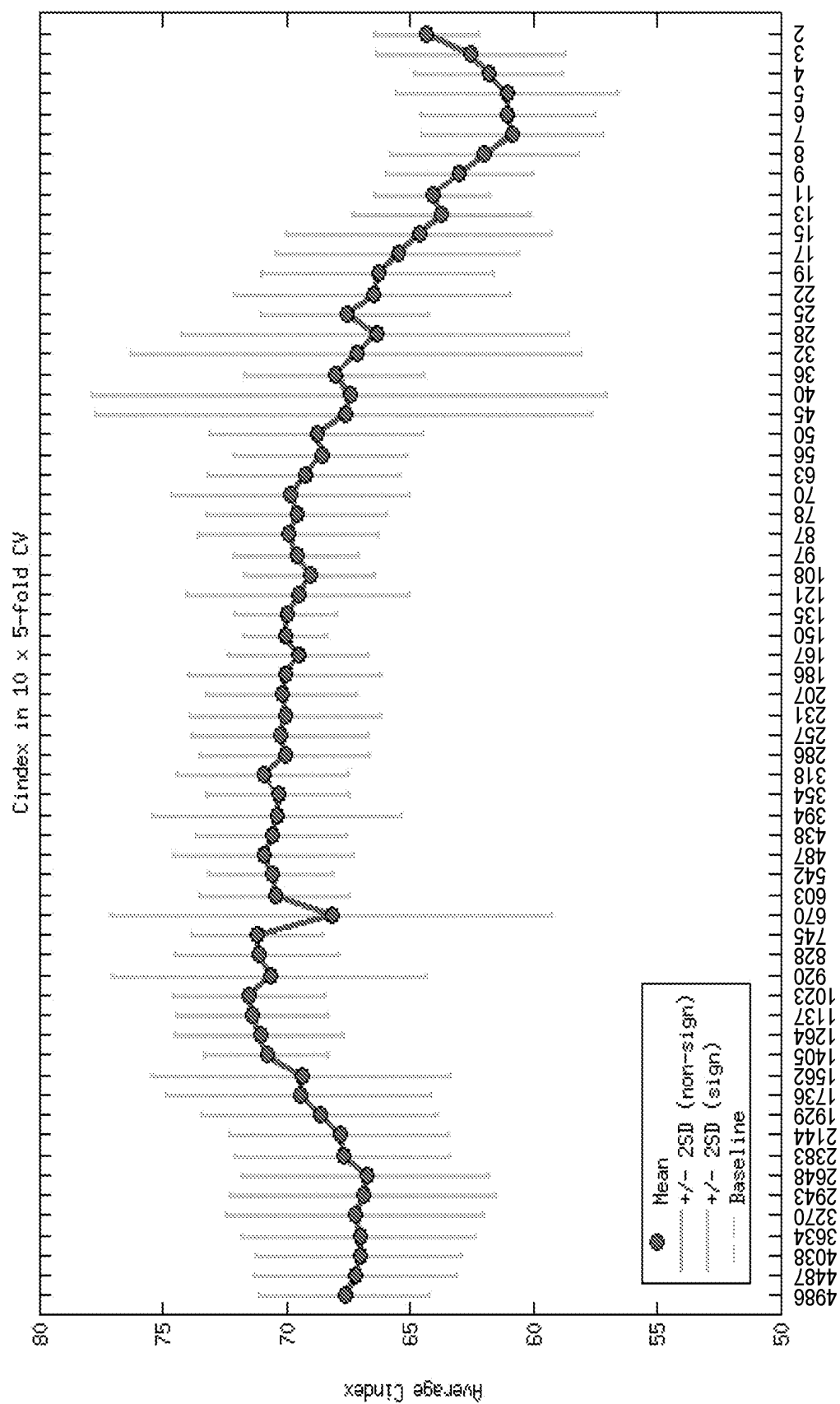


FIG. 2



Number of variables

FIG. 3

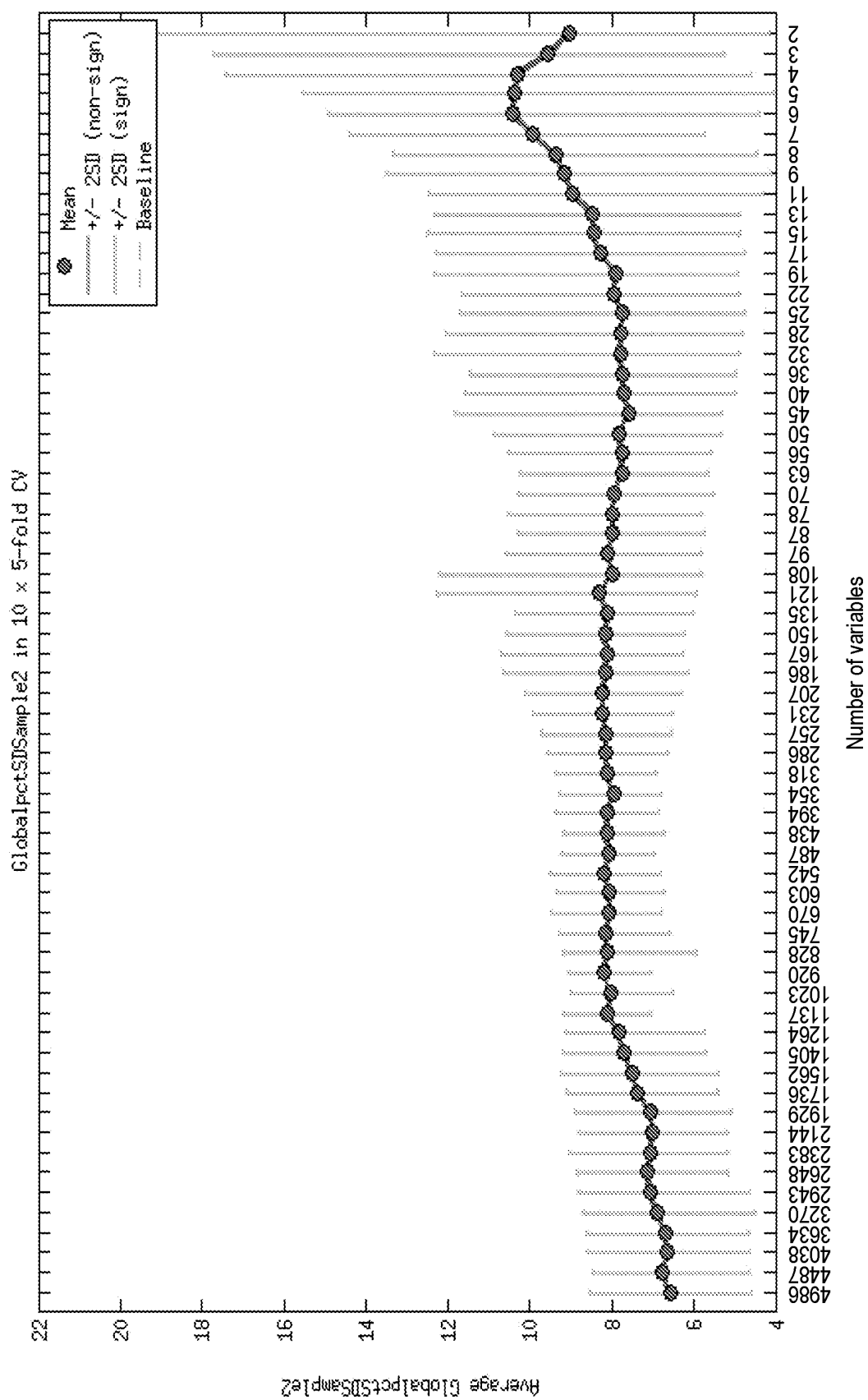
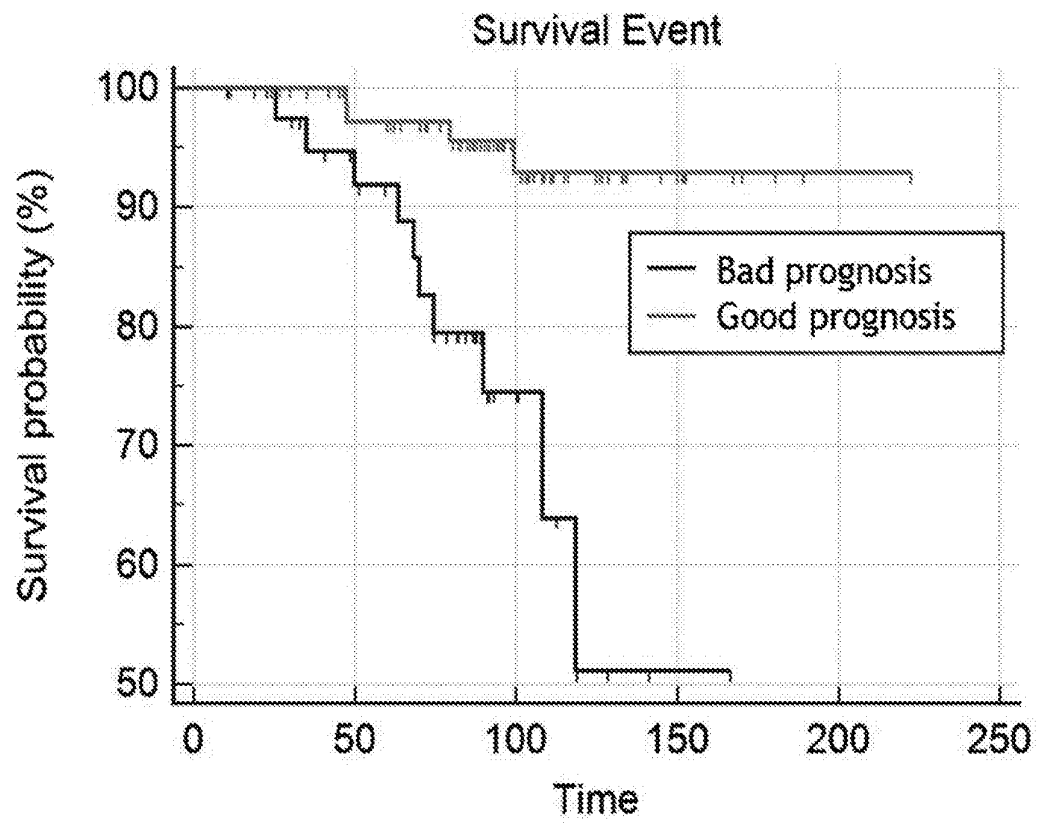
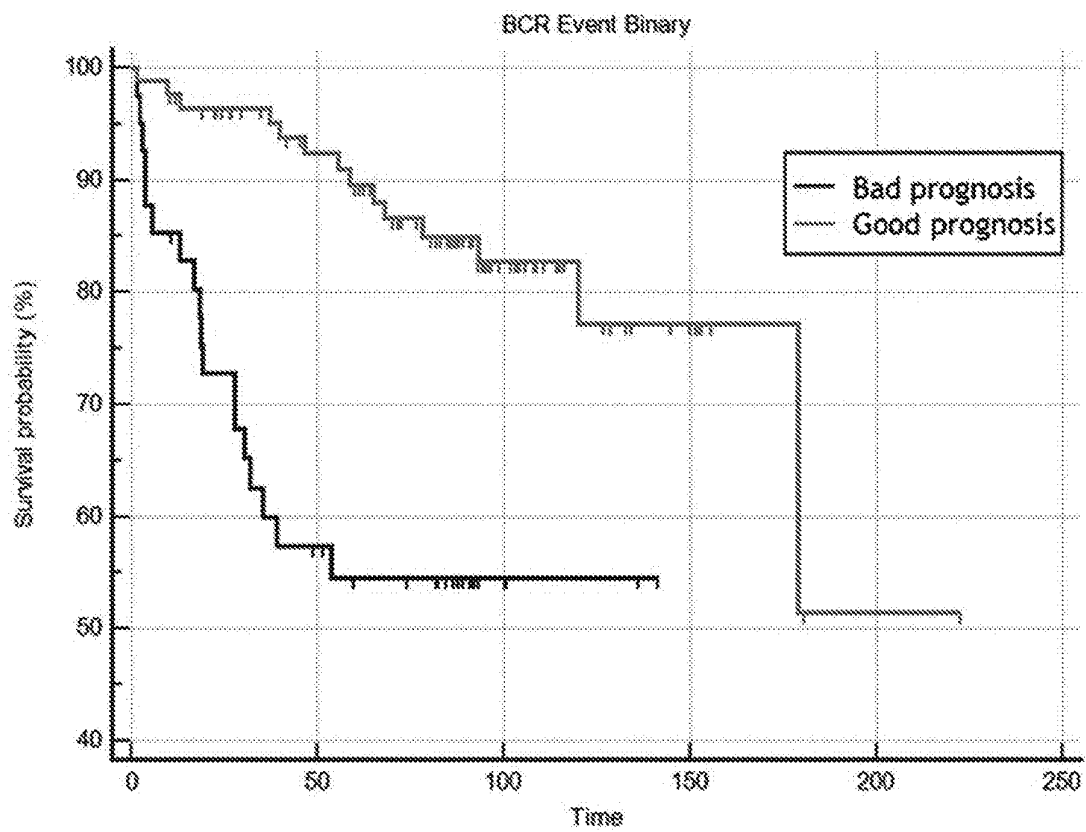
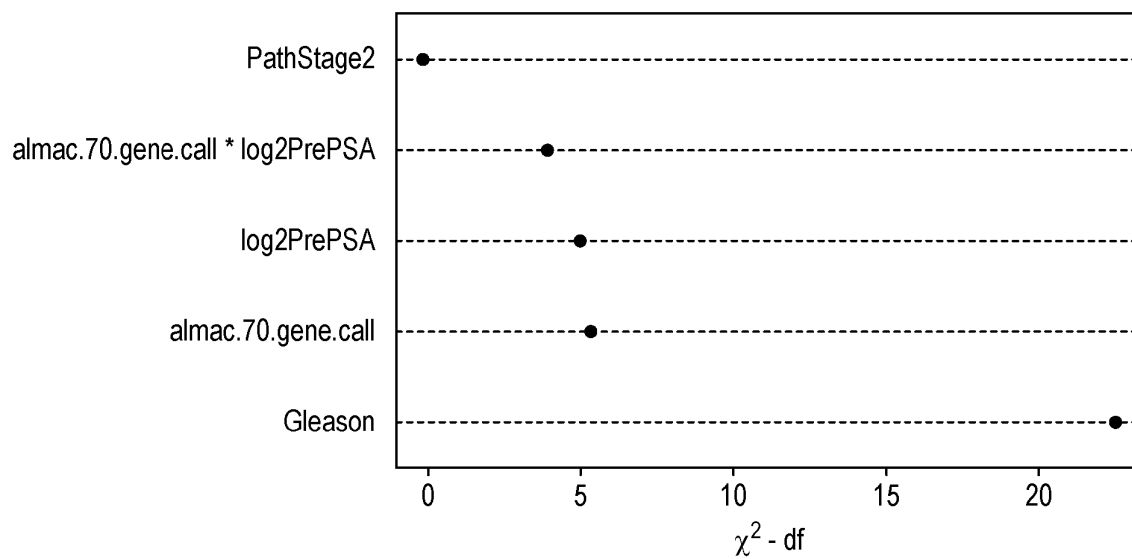


FIG. 4

*FIG. 5*

*FIG. 6*

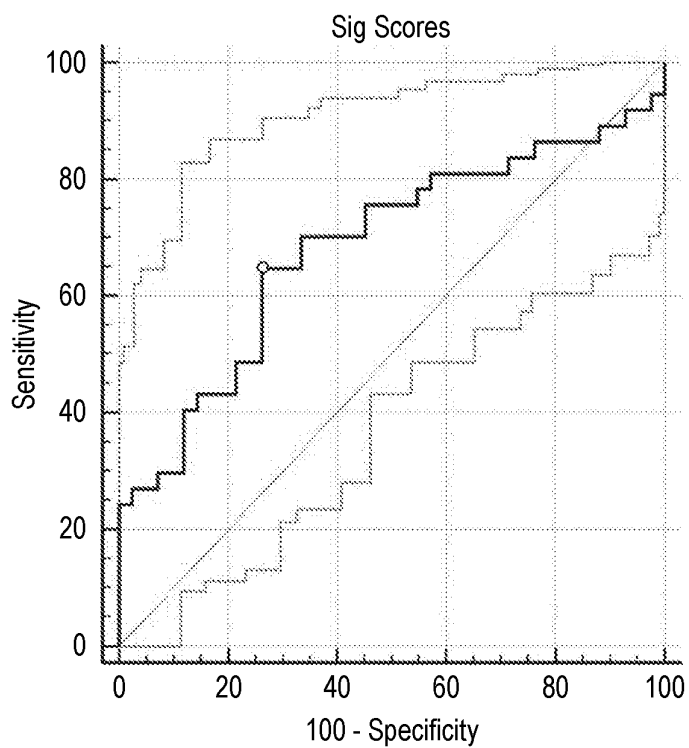
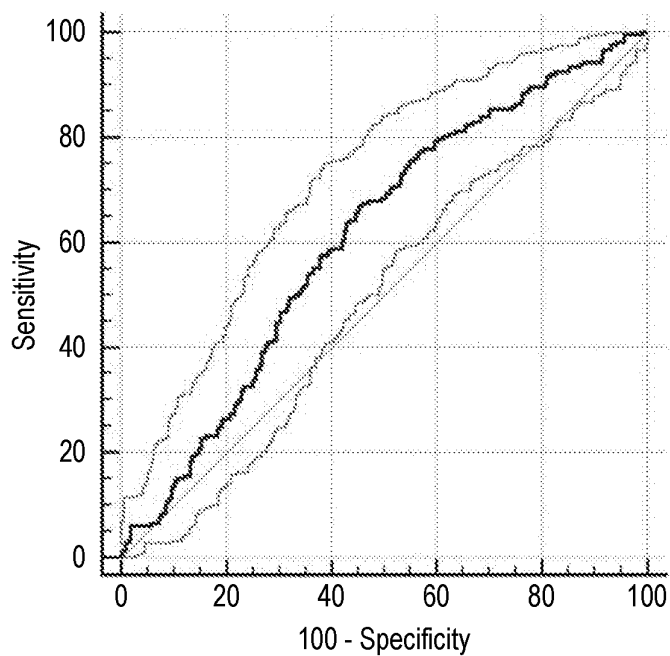
7 / 23

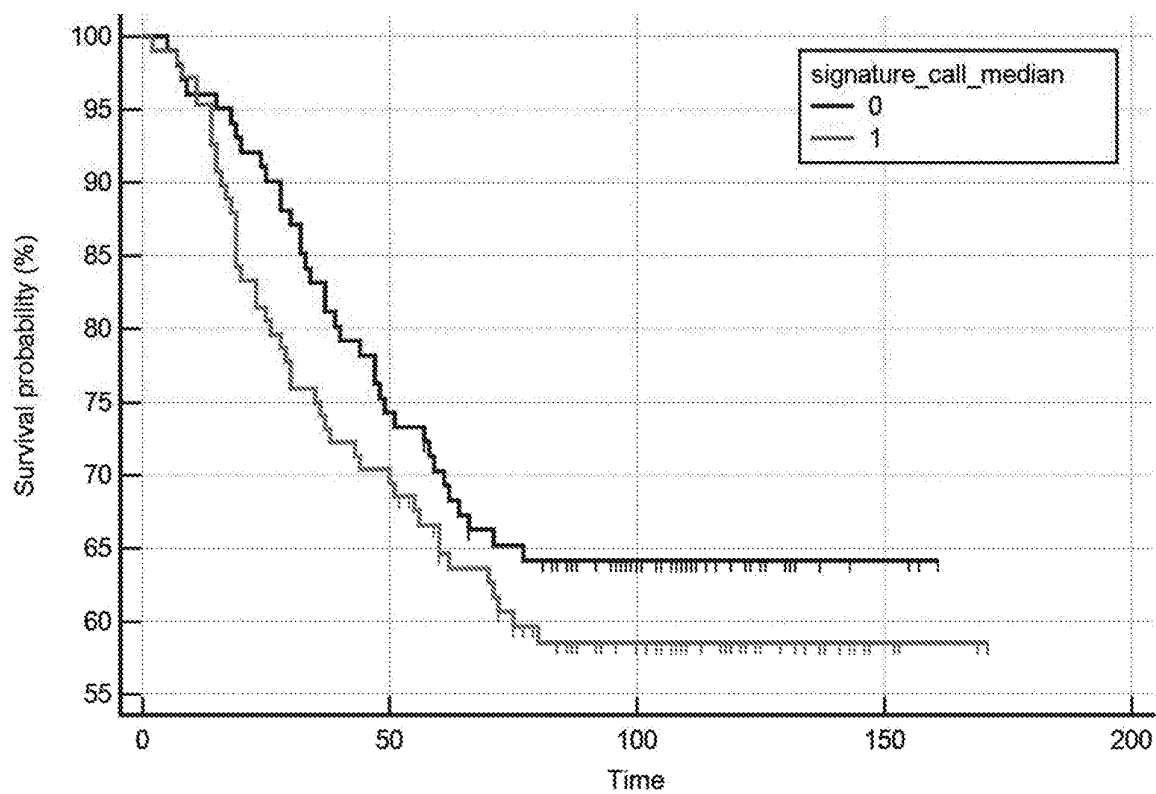


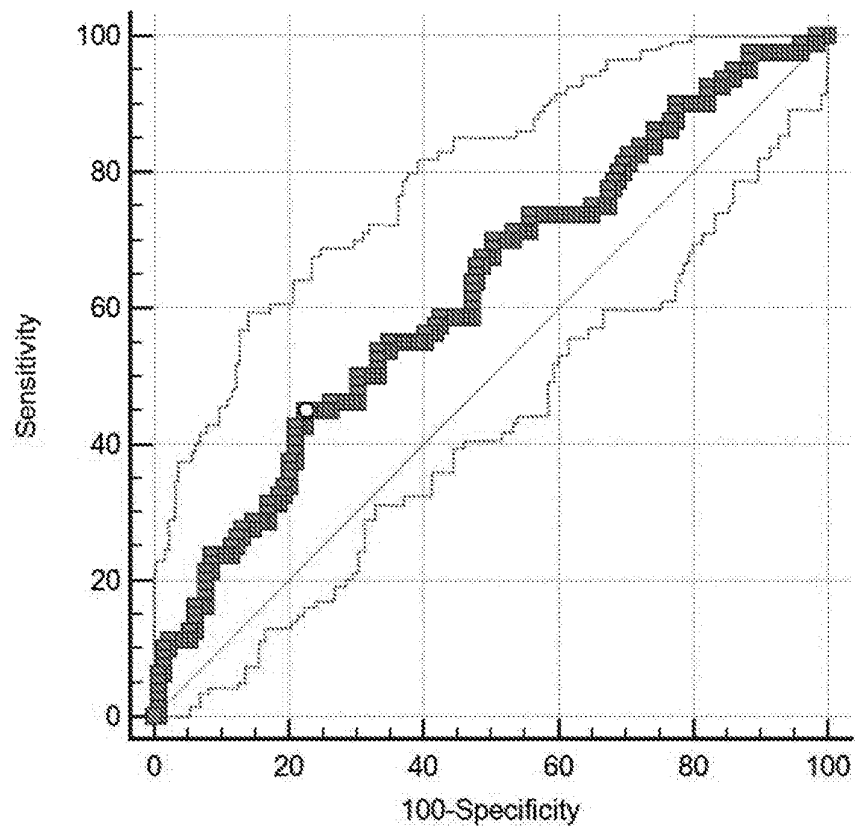
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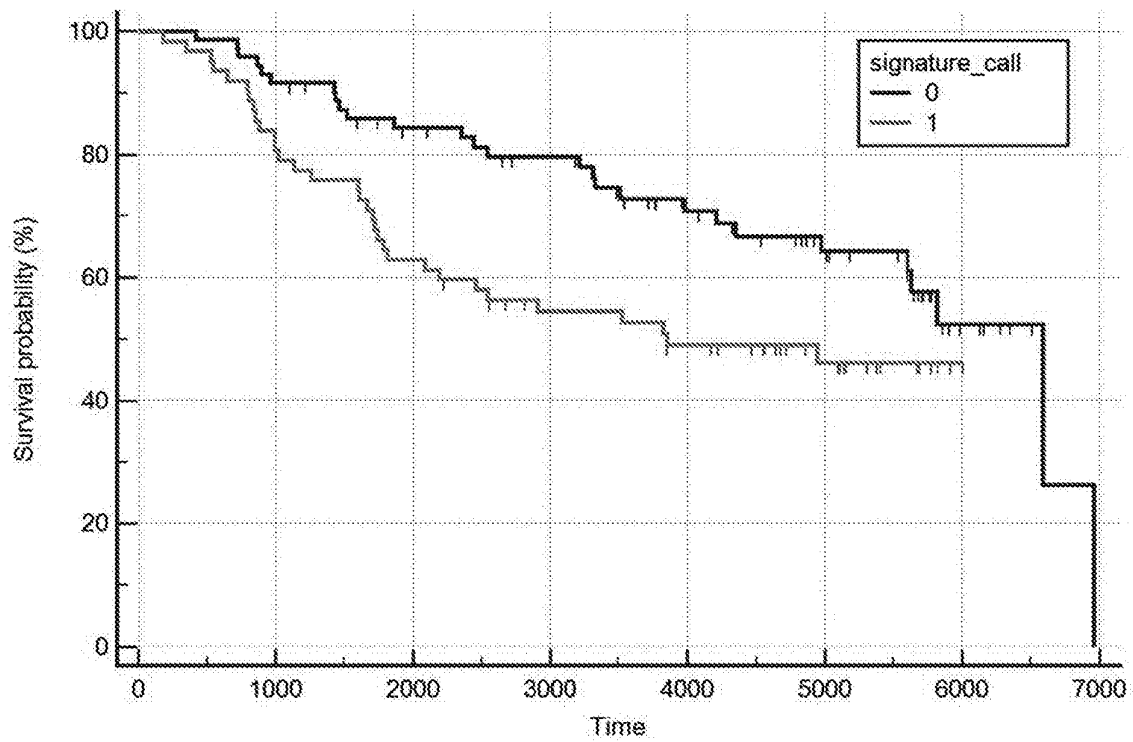
FIG. 7

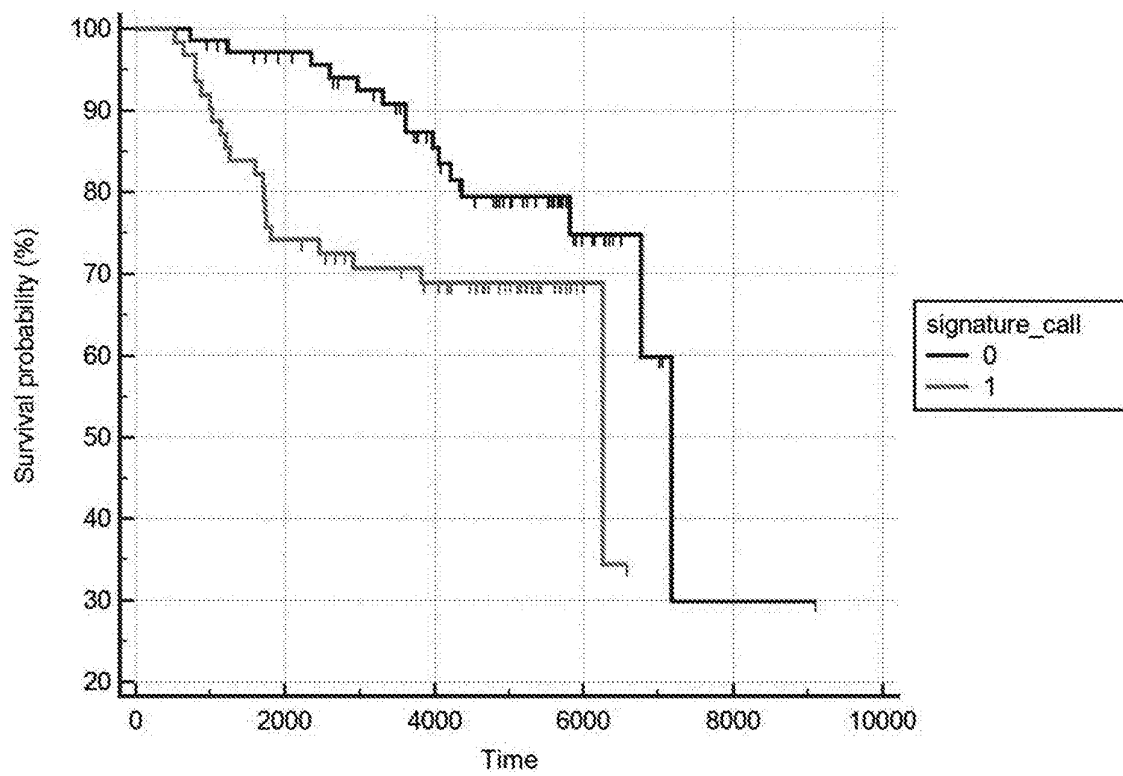
8 / 23

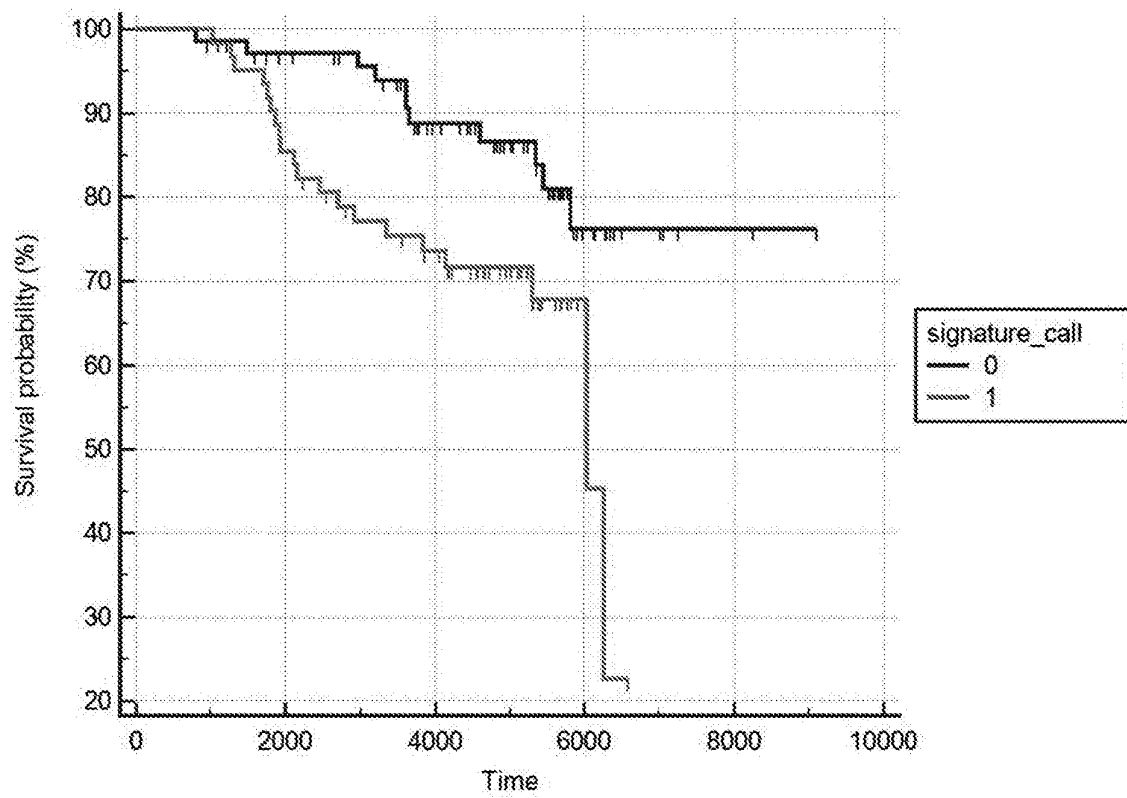
*FIG. 8A**FIG. 8B*

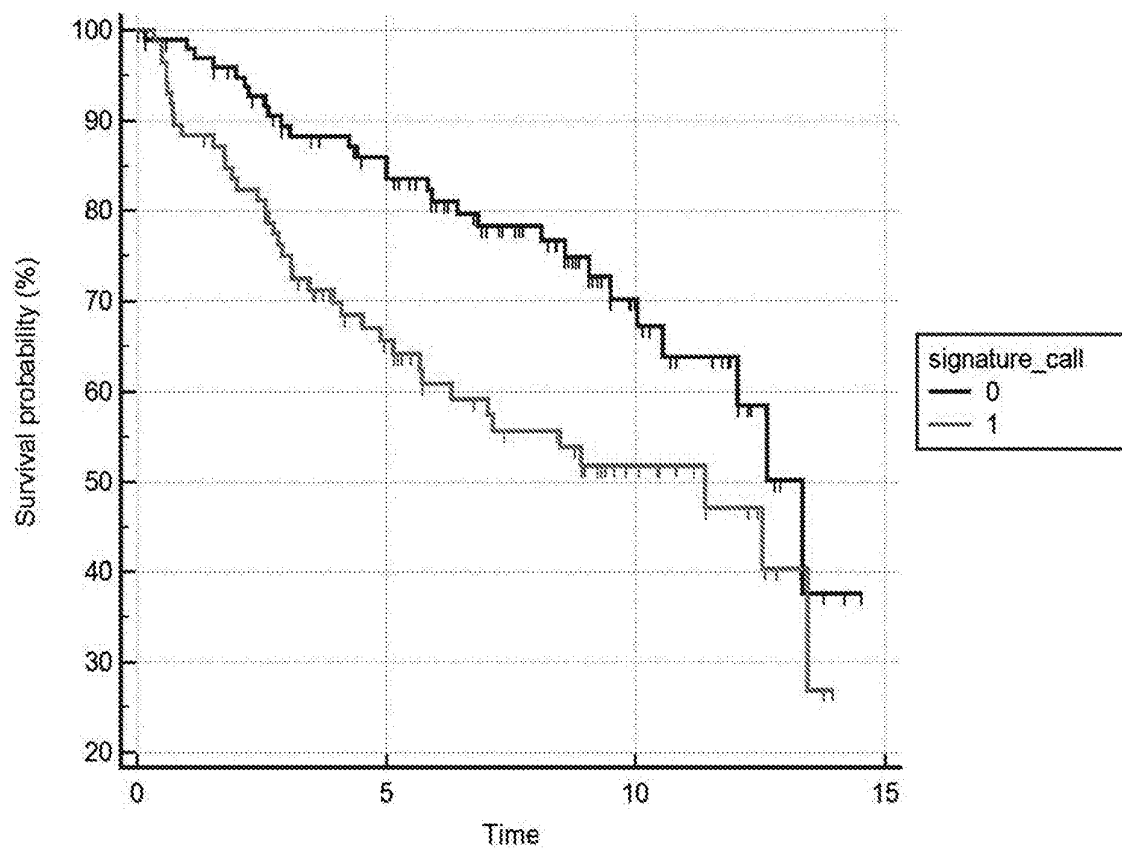
**FIG. 9**

*FIG. 10*

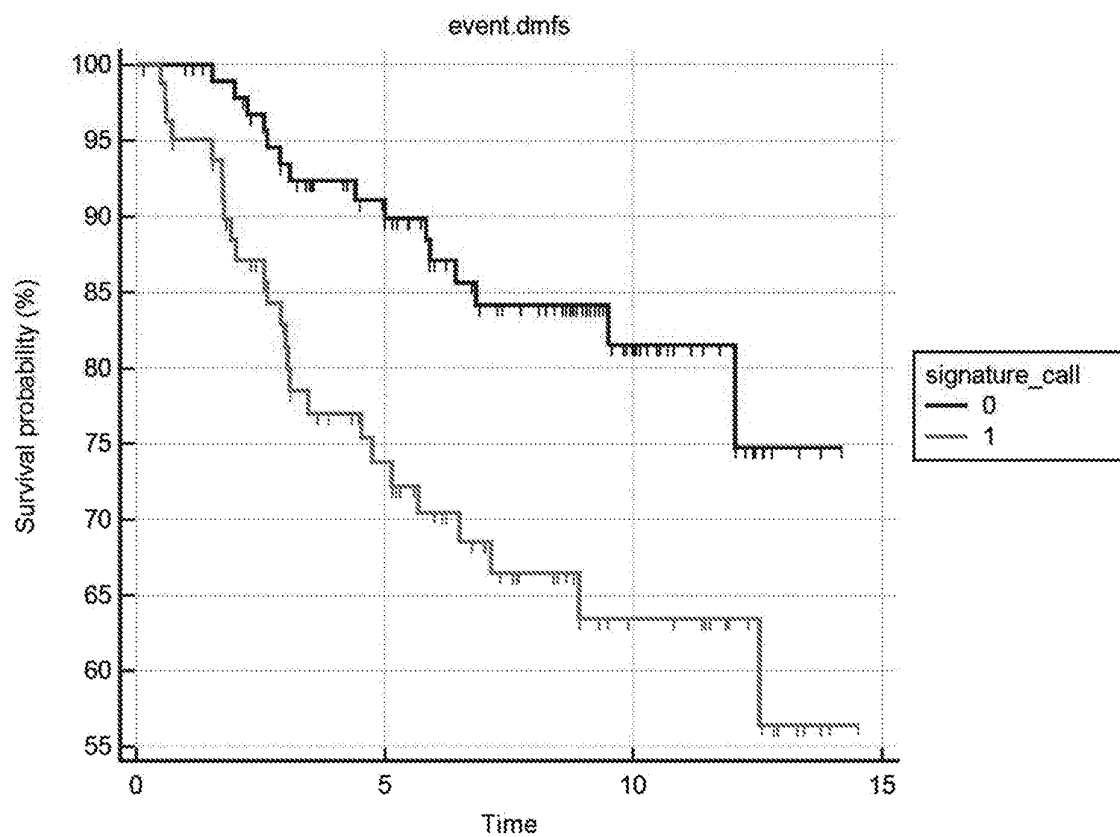
*FIG. 11*

*FIG. 12*

*FIG. 13*

*FIG. 14*

15 / 23

*FIG. 15*

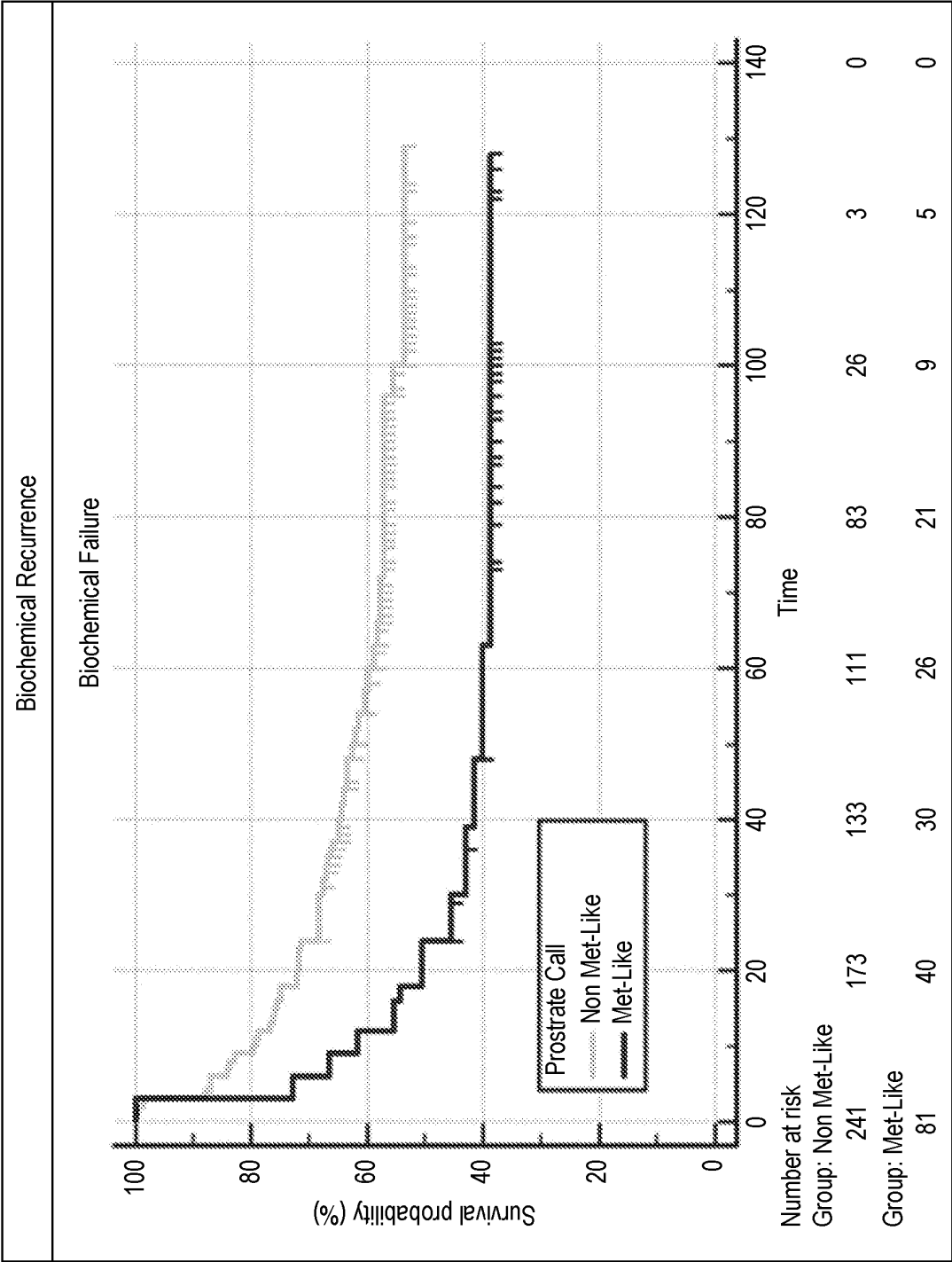


FIG. 16

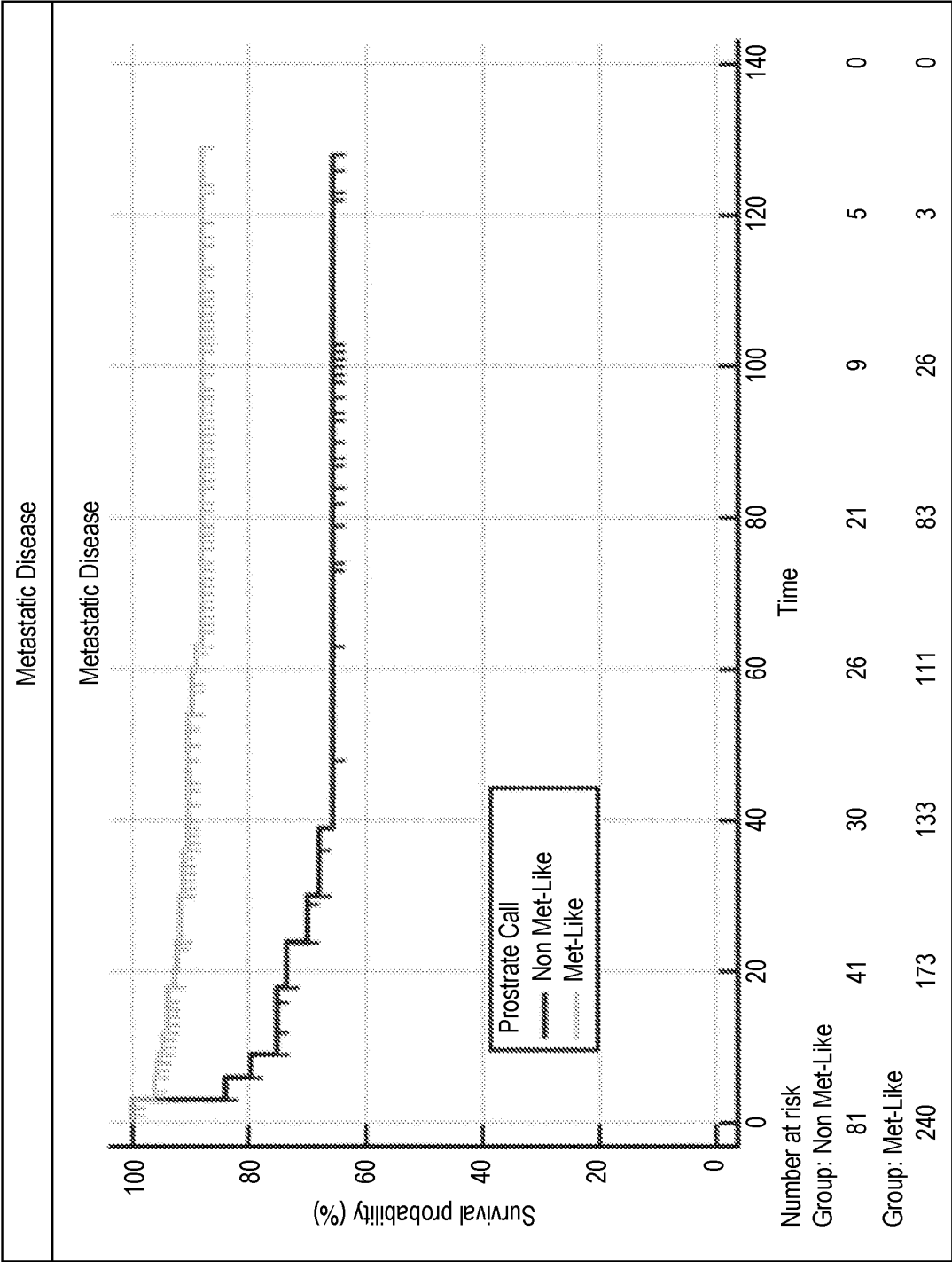


FIG. 17

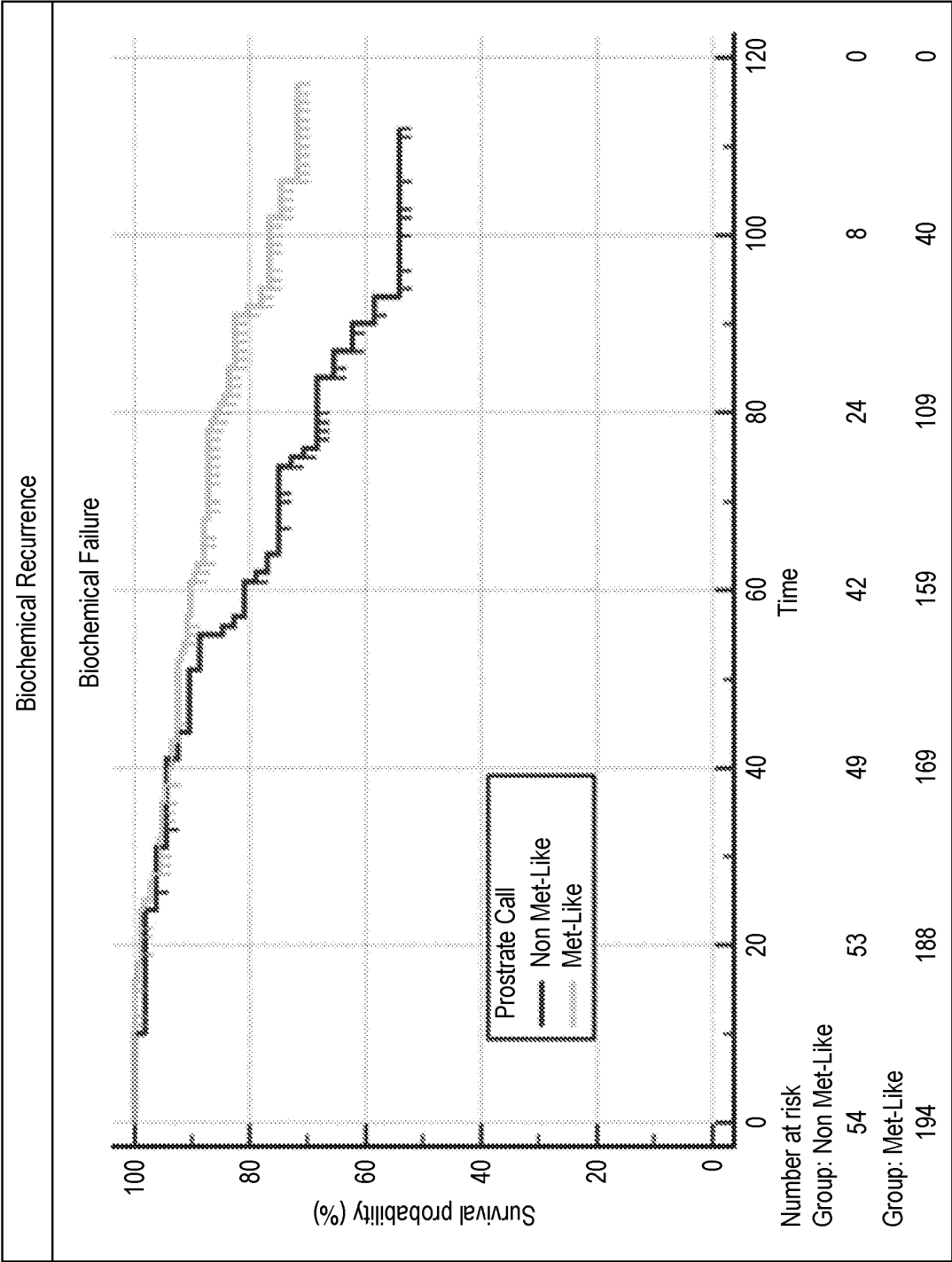


FIG. 18

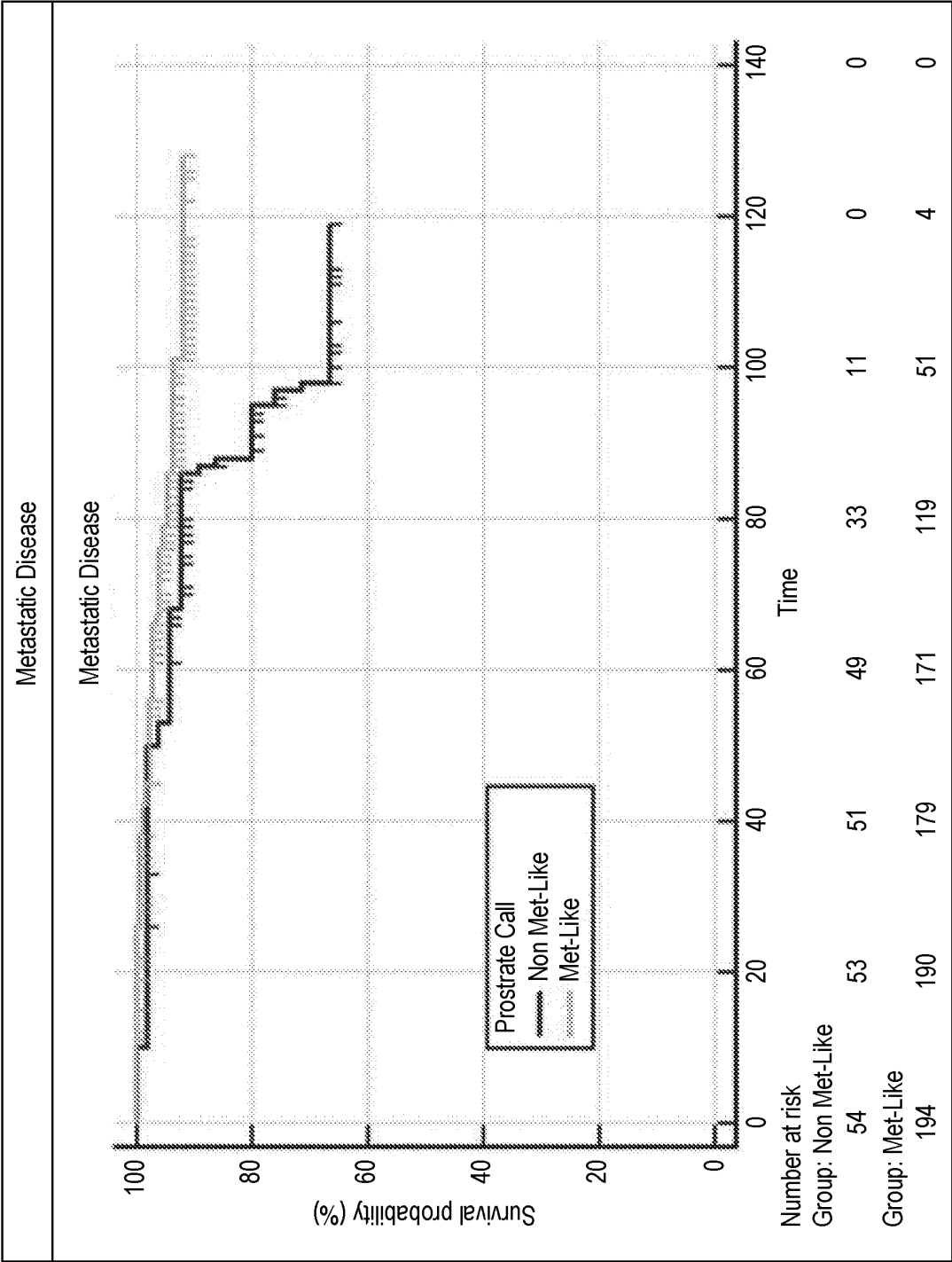
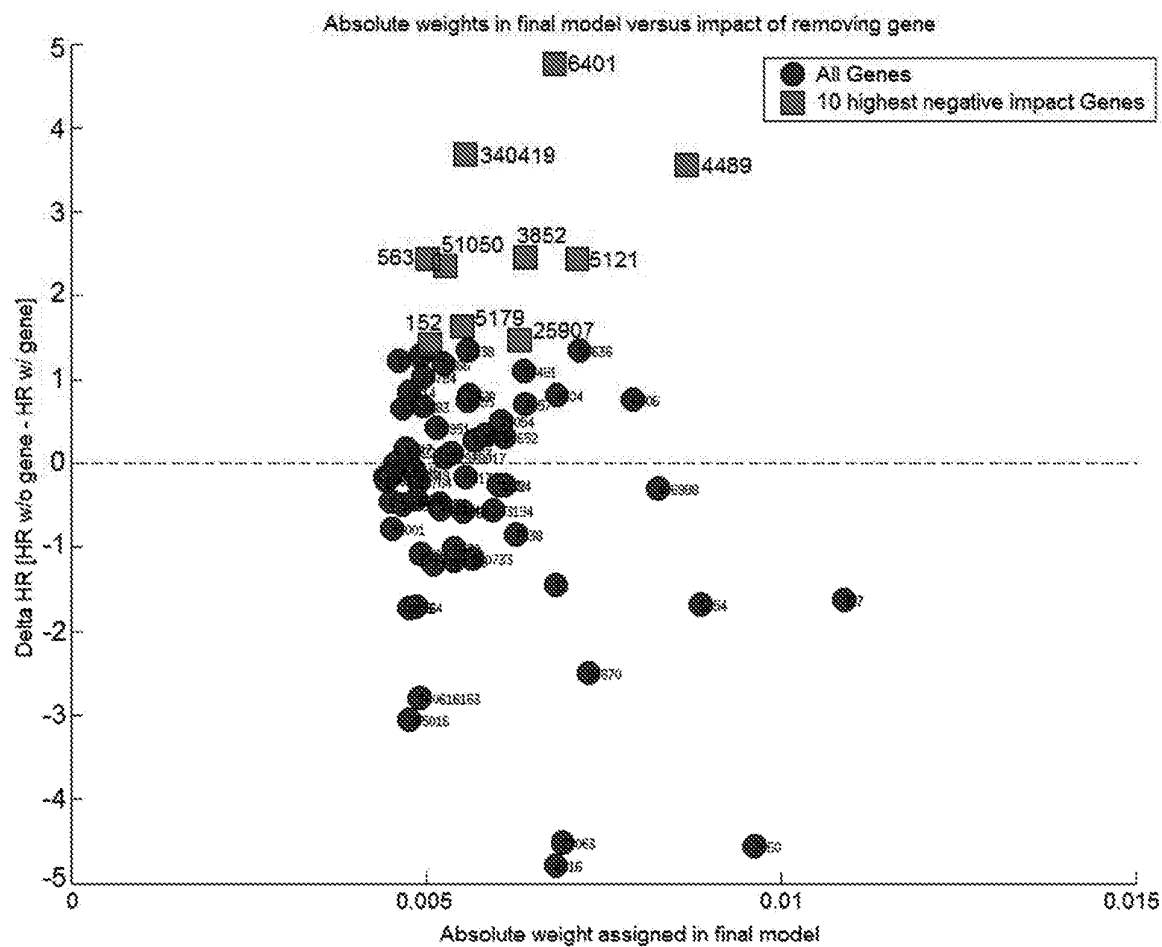
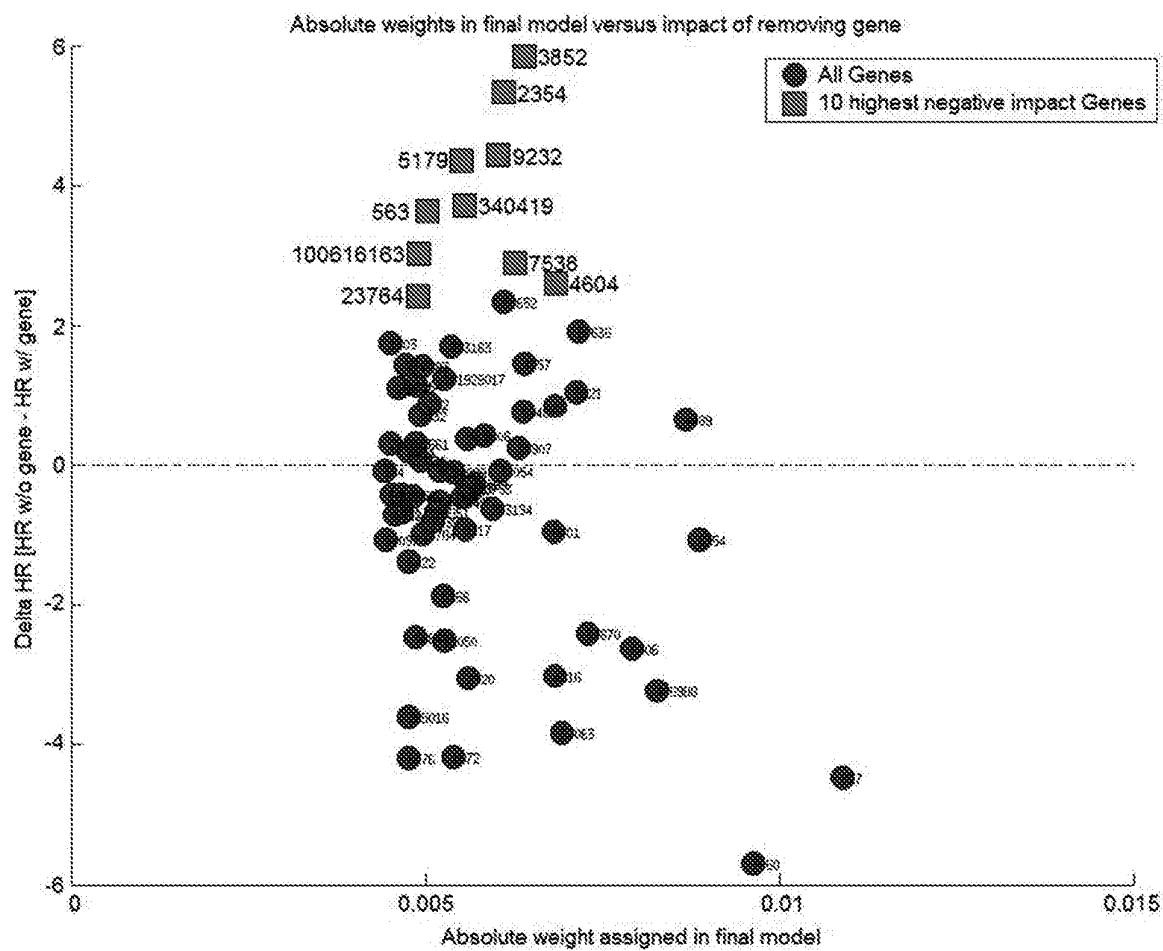


FIG. 19

**FIG. 20**

*FIG. 21*

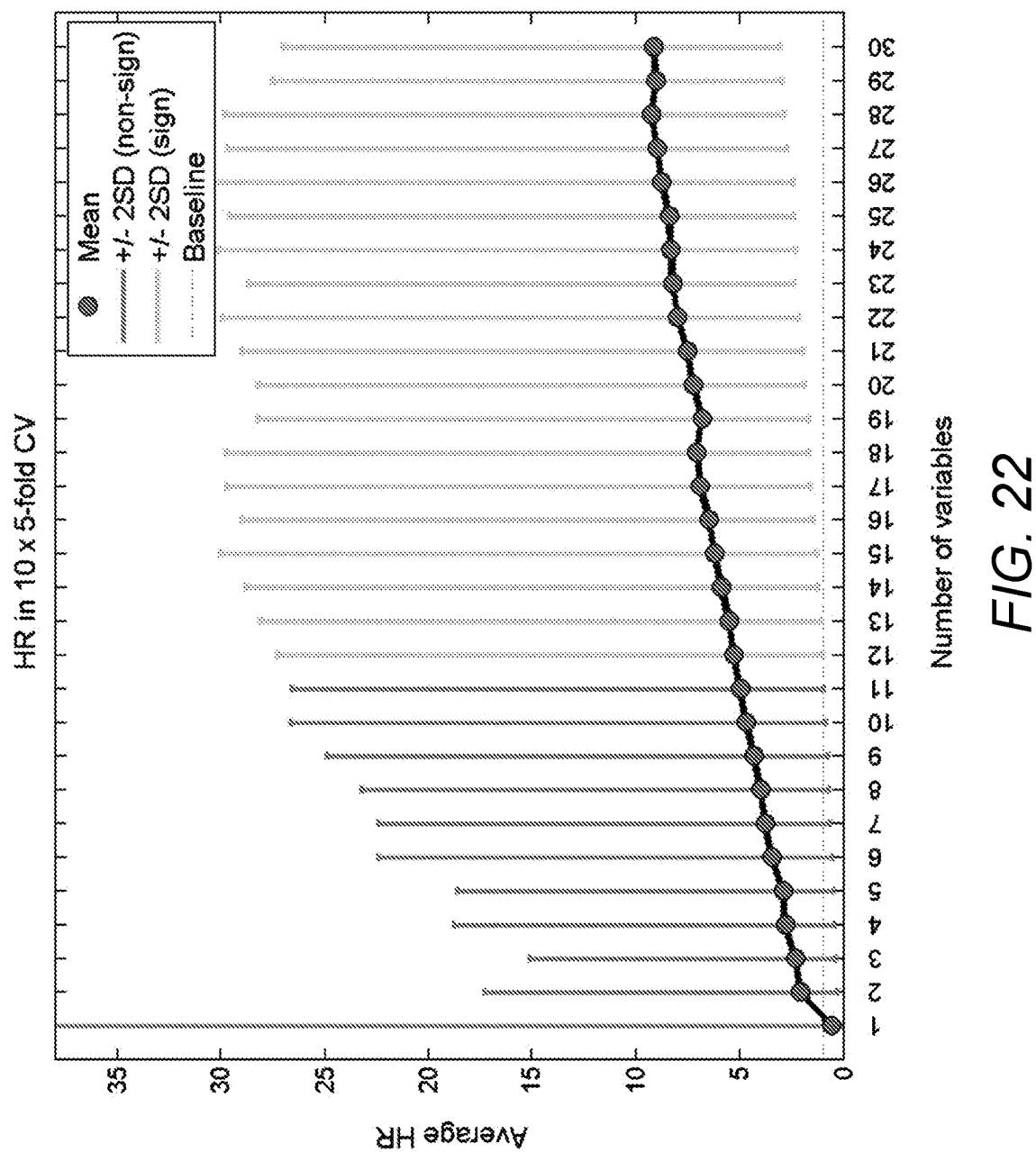


FIG. 22

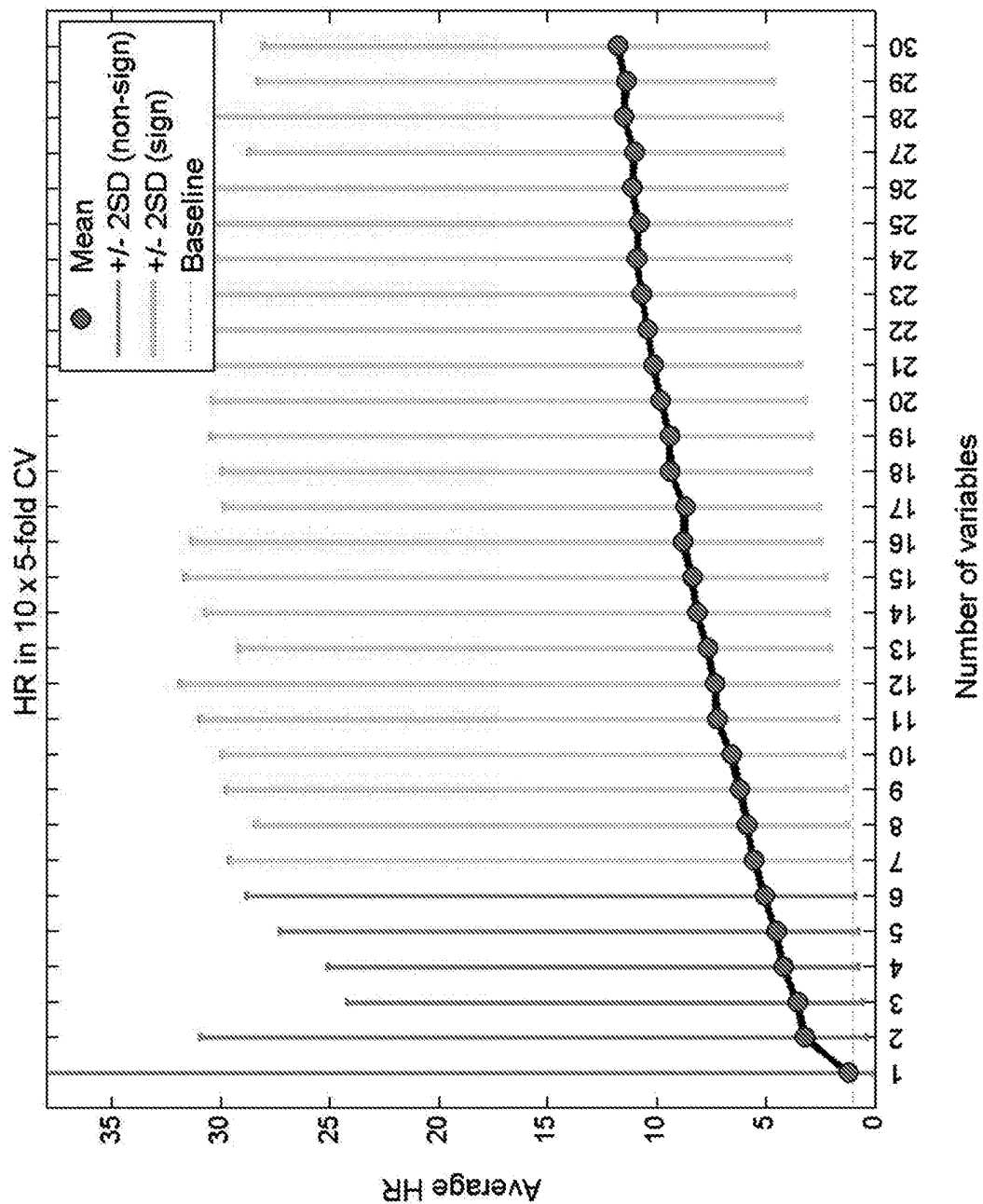


FIG. 23

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 <213> Homo sapiens

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gcctgtaatc ccagcacttt gggaggccga ggcagacgga tcacctaagg acaagagttc	300
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<211> 624

<212> DNA

<213> Homo sapiens

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 <212> DNA
 <213> Homo sapiens

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<210> 37

<211> 1627

<212> DNA

<213> Homo sapiens

<400> 37

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 <212> DNA
 <213> Homo sapiens

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 <212> DNA
 <213> Homo sapiens

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<210> 42

<211> 578

<212> DNA

<213> Homo sapiens

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 <212> DNA
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 <211> 554
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

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 <211> 613
 <212> DNA
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 <212> DNA
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 <212> DNA
 <213> Homo sapiens

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<212> DNA
<213> Homo sapiens
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<212> DNA
<213> Homo sapiens
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<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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<212> DNA
<213> Homo sapiens
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<211> 597
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<213> Homo sapiens
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<223> none

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 <212> DNA
 <213> Homo sapiens

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<211> 913

<212> DNA

<213> Homo sapiens

<400> 71

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 <213> Homo sapiens

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<213> Homo sapiens

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<210> 76

<211> 602

<212> DNA

<213> Homo sapiens

<400> 76

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<211> 586

<212> DNA

<213> Homo sapiens

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<213> Homo sapiens

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<211> 601

<212> DNA

<213> Homo sapiens

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<211> 645

<212> DNA

<213> Homo sapiens

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<210> 82

<211> 576

<212> DNA

<213> Homo sapiens

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<213> Homo sapiens

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<211> 552

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<211> 160

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<213> Homo sapiens

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 <212> DNA
 <213> Homo sapiens

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<210> 116
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<213> Homo sapiens

<400> 116

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<211> 666

<212> DNA

<213> Homo sapiens

<400> 117

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<211> 272
<212> DNA
<213> Homo sapiens

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<212> DNA

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<211> 211

<212> DNA

<213> Homo sapiens

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 <213> Homo sapiens

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acttgacagc tagcagaatt ggtttccatt agacattact cccaggagct atattacata	360
gtaaatgaat attttttcatt atacatccta catgacccat tcataaatgg ctatttacaa	420
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ggaaagcctc ttgtgtgagg atgatgggct tgacgggtggg aagggcgaat tttttttttt	180
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cggctcactg ccacctccgc ctctatgtt caggcgattc tcctgcctca gcctcctgag	300
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ggggttttcac catgttggtc agtctggtct cgagctcctg acctcagggtg atctgcccgc	420
ctcagcctct taaagtgtg gcattacagg tgtgagccac cacgcctggc cagggaaggg	480
tgagttctat tgtcaccact gtttctgctg ctgctacctt ctcaatctag aagggtgtcc	540
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aagattttt	609

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 <212> DNA
 <213> Homo sapiens

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ggaaagcctc ttgtgtgagg atgatgggct tgacgggtggg aagggcgaat tttttttttt      180
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tagctgggat tgcaggcatg caccaccaca cctggctaata tttttacttt tggaggagat      360
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tgagttctat tgtcaccact gtttctgctg ctgctacctt ctcaatctag aaggtgggtcc      540
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<210> 156
<211> 122
<212> DNA
<213> Homo sapiens

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gc                                             122

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<210> 157
<211> 553
<212> DNA
<213> Artificial Sequence

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<220>
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ctttagata aactgctcca actgttggac aaaataaaga ggggattgtg ccagaccttg	300
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<211> 649

<212> DNA

<213> Homo sapiens

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acatggtaat tgggtggcaat taagaaaaat ttaacttgcc aagaagcatc gttttttgtg    360
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tcttttaaca gcattatttt aaaatgttaa atgtcacttt ggggatgtca ttaacgatca    600
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<210> 160

<211> 335

<212> DNA

<213> Homo sapiens

<400> 160

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tacaaaaatt agctgggcat gatggtatgt gcttgtggtc ccagctactt gggaggctga    240
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ggaggattgc ttgaattcag aagtttgaga ctgctgagct ctgacgagat ctgagtgagc 180
tctgatcgct ccactgtact ccacccagcc tgggcaacag agtgagactc catctc 236

<210> 162
<211> 438
<212> DNA
<213> Homo sapiens

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taaatttttt tgataaaaata atattatatt ttccatttaa aataaatacg tgttttttag 180
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agcaacagaa gactgttt 438

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<212> DNA
<213> Homo sapiens

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acgcctgtaa tcccagctac tcgagaggcc gaggcaggag ggtggcttga actcgggagg	360
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 <212> DNA
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<211> 2396
<212> DNA
<213> Homo sapiens

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agtgtgcatg tgtgcccata catgcttatg aggaggtcag tgtgtgaggg gaatcaggaa 240

gtctcttttt tatttttaaa tgaaatcaag taaaacttaa ctttggttc aaaaatatct 300

ctcaaatact gtaaacaata ctttgatac tttttagaaa gagattatgg ccaggcgtag 360

tggctcacgt ctgtaatccc agaactttgg gaggccgagg tgggcagacc acctgaactc 420

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<210> 174

<211> 1176

<212> DNA

<213> Homo sapiens

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acagatagct ccttcctccc cgcccccttc ttttngttng gaggtcccag gatctgtgtt      180
cacagacatc tgggggaaga aaaggagcag gaaactaccc cgcacagagt taagcaggaa      240
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1176

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acctttcaag tgtttatcat cacctcggat ttcacggcct aatgccagtc aatttcatgg	360
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462

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 <223> n is a, c, g, or t

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<212> DNA

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<213> Homo sapiens

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<223> n is a, c, g, or t

<220>

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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

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 <223> n is a, c, g, or t

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 <223> n is a, c, g, or t

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<211> 493

<212> DNA

<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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 <212> DNA
 <213> Homo sapiens

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<223> n is a, c, g, or t

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tcccagagca acgagaaaaac aaacagcagc agaagaagcg aaaagtccaa gataaacaga	1080
aatcggtatc agtcagcact gtacactaga gggttccatg agattattgt agactcatga	1140
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catgtcagtt attgctctgt ctaaacaaca ttcccagtag ttgctatatt cttcatacaa	1260
gcatagttaa caacaaagag ccaaaaagatc aaagaaggga tactttcaga tggttgtctt	1320
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gttttcaagc tgttgtttta agaagcaaaa gatagttctg caaattcaaa gatacagtat	1500
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tgaccgggag atctagagag tgctcagaat tagggcctgg catttggaat cacaggattt	1620
atcatcacag aaacaactgt ttttaagatta gttccatcac tctcatcctg tatttttata	1680
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ggtcacctgg aacaggctct caatctatgt tagcactatg tggctcagcc tctgttacct	1860
cttggtattat atatcaacct gtaaacaatgt gcctgtaact tacttccaaa aacaaaatca	1920
tacttattag aagaaaattc tgattttata gaaaaaaaaat agagcaagga gaatataaca	1980
tgtttgcaaa gtcattgtgt ttctttctca atgagggaaa aacaatttta ttacctgctt	2040
aatggccac ctggaactaa aagggtact attttctaac aaggatatatc tagtagggga	2100
gaaagccacc acaataaata tatttgtaa tagtttttca aaaaaaaaaa aaaaaaaaaa	2160
aaaaa	2165

pctgb2016051825-seq1.txt

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<210> 229
<211> 1239
<212> DNA
<213> Homo sapiens

<400> 229
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ccccgggctc ctggcgaccg tgcgggccga atgcagccag gattgcgcga cgtgcagcta      180
ccgcctagtg cgcccgccg acatcaactt cctggcttgc gtaatggaat gtgaaggtaa      240
actgccttct ctgaaaaattt gggaaacctg caaggagctc ctgcagctgt ccaaaccaga      300
gcttcctcaa gatggcacca gcaccctcag agaaaaatagc aaaccggaag aaagccattt      360
gctagccaaa aggtatgggg gcttcatgaa aaggtatgga ggcttcatga agaaaatgga      420
tgagctttat cccatggagc cagaagaaga ggccaatgga agtgagatcc tcgccaagcg      480
gtatgggggc ttcataaaga aggatgcaga ggaggacgac tcgctggcca attcctcaga      540
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cagtgataat gaggaagaag tgagcaagag atatgggggc ttcataagag gcttaaagag      660
aagcccccaa ctggaagatg aagccaaaga gctgcagaag cgatatgggg gcttcatgag      720
aagagtaggt cgcccagagt ggtggatgga ctaccagaaa cggtatggag gtttcctgaa      780
gcgctttgcc gaggctctgc cctccgacga agaaggcgaa agttactcca aagaagttcc      840
tgaaatggaa aaaagatacg gaggatttat gagattttaa tatttttccc actagtggcc      900
ccaggcccca gcaagcctcc ctccatcctc cagtgggaaa ctgttgatgg tgttttattg      960
tcatgtgttg cttgccttgt atagttgact tcattgtctg gataactata caacctgaaa     1020
actgtcattt caggttctgt gctctttttg gagtctttaa gctcagtatt agtctattgc     1080
agctatctcg ttttcatgct aaaatagttt ttgttatctt gtctcttatt ttgacaaac     1140
atcaataaat gcttacttgt atatagagat aataaaccta ttacccaag tgcaaaaaaa     1200
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa                        1239

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pctgb2016051825-seq1.txt

<210> 230
 <211> 1403
 <212> DNA
 <213> Homo sapiens

<400> 230
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 ggcgggacgc gcctttcggg cgaaagttag gaggggcccgg gctggcgggc gggcgggttc 180
 cgggggctcc tttccgcacc agcggcggtc gctccccgtg ggcgggacct cgggcgtgat 240
 cgtcgaggag accgcggctc cctcacctgc cggcttggtc ctcggggtcc gcggagcgag 300
 cccagctctc ggcgcggtgc ggagtctccc agccccgcgg ccccgagcgc acgatgcgcg 360
 gacccgggca cccctcctc ctggggctgc tgctggtgct gggggcggcg gggcgcggcc 420
 gggggggcgc ggagccccgg gagccggcgg acggacaggc gctgctgcgg ctggtggtgg 480
 aactcgtcca ggagctgcgg aagcaccact cggcggagca caagggcctg cagctcctcg 540
 ggcgggactg cgccctgggc cgcgcgagg cgcgggggct ggggccttcg ccggagcagc 600
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 gccctcttta ttttagtcca aagtgcagca aacacttcca tagactttat cacaacacca 720
 gagactgcac cattcctgca tactataaaa gatgcgccag gcttcttacc cggctggctg 780
 tcagtccagt gtgcatggag gataagcagt gagcagaccg tacaggagca gcacaccagg 840
 agccatgaga agtgccttgg aaaccaacag ggaaacagaa ctatctttat acacatcccc 900
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 tgttgttgac agtttgaga tatatattcg ataatcagt gtacttgaca gtgttatctg 1020
 tcacttattt aaaaaaaaaa caaaaagga atgctccaca tttgacgtgt agtgctataa 1080
 aacacagaat atttcattgt cttcattagg tgaaatcgca aaaaatattt ctttagaaac 1140
 ataagcagaa tcttaaagta tattttcata taacataatt tgatattctg tattactttc 1200
 actgttaa at tctcagagta ttatttggaa cggcatgaaa aattaaaatt tcggtcatgt 1260

pctgb2016051825-seq1.txt

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taaaatagta ttccagctat tgtgcaatat gtaaatagtg taaataaaca caagtaataa	1380
atgaagtgtt tggtataaaa aaa	1403

<210> 231
 <211> 468
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

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accgcggctc gaaatggacc ccaactgctc ctgcgccact ggtggctcct gcacctgcac	120
tggctcctgc aaatgcaaag agtgcaaagtg cacctcctgc aagaagagct gctgctcctg	180
ctgccccatg agctgtgccca agtgtgcccc gggctgcatc tgcaaagggg catcagagaa	240
gtgcagctgc tgtgcctgat gtccggacag ccctgctcga agatatagaa agagtgcct	300
gcacaaactt ggaatTTTTT ttccatacaa ccctgaccca ttactgtat tttttttaat	360
gaaatatgtg aatgataata aaagttgctg acttaaaaaa aaaaaaaaaa aaaaaaaaaa	420
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa	468

<210> 232
 <211> 468
 <212> DNA
 <213> Homo sapiens

<400> 232	
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accgcggctc gaaatggacc ccaactgctc ctgcgccact ggtggctcct gcacctgcac	120
tggctcctgc aaatgcaaag agtgcaaagtg cacctcctgc aagaagagct gctgctcctg	180
ctgccccatg agctgtgccca agtgtgcccc gggctgcatc tgcaaagggg catcagagaa	240

pctgb2016051825-seq1.txt

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gcacaaactt ggaatttttt ttccatacaa ccctgaccca ttactgtat tttttttaat	360
gaaatatgtg aatgataata aaagttgctg acttaaaaaa aaaaaaaaaa aaaaaaaaaa	420
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	468

<210> 233
 <211> 86
 <212> DNA
 <213> Homo sapiens

<400> 233	
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atttcacttg cattgtgcaa taagca	86

<210> 234
 <211> 242
 <212> DNA
 <213> Homo sapiens

<400> 234	
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ggtaatggtc aagggtgtct gtctggagtc tgggagaccc atgtaacgtt ctgtgtacac	120
tgagtcatag tactcccacc gggatacagg cgccacggct attccacact tgaacacgcc	180
acttcccgat cccaggacca ttgaggtagc tacccttcat atgaccagcc ccaaattgca	240
at	242

<210> 235
 <211> 172
 <212> DNA
 <213> Homo sapiens

<400> 235	
ggatgatgat atacctcatc gggtcagtgt gagaattgcg tgagttaaaa tctgttaagt	60
gcatacaact agcactggct cgcaggaagc actaaggaag tttatgctgc tgttattgtc	120
cattgagtgt tttttacatg cagaatgctg ggtcaagccc tgtaaggctg ta	172

pctgb2016051825-seq1.txt

<210> 236
 <211> 213
 <212> DNA
 <213> Homo sapiens

<400> 236
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 acagcaaaaag ccatttctgt accataaaaaa tttgttgtgc aatattagaa ttatcatatg 180
 tttcctacat ctgacagcac ctaaaatggt tga 213

<210> 237
 <211> 144
 <212> DNA
 <213> Homo sapiens

<400> 237
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 ttgcccagct gacctggaat gaggcctaaa ccacaatctt ctcttcctaa taaacagcct 120
 cctagaggcc acattctatt cttt 144

<210> 238
 <211> 239
 <212> DNA
 <213> Homo sapiens

<400> 238
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 tttttcaatg aatgttaaga gattgttctc actcccgccc catcttaatg tataaccaac 120
 accaaacacg tggatcaaca gaaaaggaaa atttcactcg tctaaacact ttcaattttc 180
 agtttttatt ttatgttcta tatacccagt cataaagtat aagcatcagt tgtcattaa 239

<210> 239
 <211> 283
 <212> DNA

<213> Homo sapiens

<400> 239

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ttcacctccc gattctaaga agagactgtc caagcaagtt aggcttgacag gaagatatga      60
cccagctgag aagccctcag gcctcgctgg atgggggttt ctgtccatcc tatgcagtat      120
ttgggaaaagt tcacaagaaa ctgagaagaa acctaaaaac tgtggatagt ggaaagattt      180
ttagatTTTT tttttccttg gggaaaactgg caggcaatgg gggtaggga ggttggggcg      240
gtgggggctt tcttgagtta aaggggctta tatgtgatgt caa                        283

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<210> 240

<211> 253

<212> DNA

<213> Homo sapiens

<400> 240

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cagcctctgt tacccttgg attatatatc aacctgtaaa catgtgcctg taacttactt      60
ccaaaaacaa aatcatactt attagaagaa aattctgatt ttatagaaaa aaaatagagc      120
aaggagaata taacatgttt gcaaagtcac gtgctttctt tctcaatgag ggaaaaacaa      180
ttttattacc tgcttaatgg tccacctgga actaaaaggg atactatTTT ctaacaaggt      240
atatctagta ggg                        253

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<210> 241

<211> 216

<212> DNA

<213> Homo sapiens

<400> 241

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taggagactc cgtcgctcca attacttccg acttcctccc tgtgaaaatg tggatttgca      60
gagacccaat ggtctgtgat cattgaaaaa gaggaaagaa gaaaaaatgt atgggtgaga      120
ggaaggagga tctccttctt ctccaaccat tgacagctaa cccttagaca gtatttctta      180
aaccaatcct tttgcaatgt ccagctttta ccccta                        216

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<210> 242

<211> 297

pctgb2016051825-seq1.txt

<212> DNA

<213> Homo sapiens

<400> 242

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tctaaaattt tttcctgcat cagttcttct gagggccttg atgaaataac acagcagttt 120

cttaaacaat ttgaaacaaa atgagctctc ctaccacctc actttttcat ttccacacta 180

atgtattata tgtaactact tggaaaaaat aattattcaa atgcttcttc ccacaaagaa 240

tatagatgat agtagatata ttttattaat aaaatggttc atgaatcgga gactaac 297

<210> 243

<211> 294

<212> DNA

<213> Artificial Sequence

<220>

<223> none

<220>

<221> misc_feature

<222> (207)..(207)

<223> n is a, c, g, or t

<400> 243

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ctttgcatat ttgatttcct gagactgttt gaatagtcct tctcagaaaa ctgtgccagt 120

ctggctgtga acagctcttc tccgagggga gctgaccagt agcctgctcg tgttggtatg 180

cttggaactgg ggttcccagc atgcccngta atctctctcc caaaccctt tatccgggtcc 240

ctgccccaat gacaatcatg gccaatcttg ccaccctta aagaagagac aaca 294

<210> 244

<211> 103

<212> DNA

<213> Homo sapiens

<400> 244

tttcttttac tactatatta cgttgttatt attttgttct ctatagtatc aattttattg 60

pctgb2016051825-seq1.txt

atttagtttc aatttatattt tattgctgac ttttaaaata agt 103

<210> 245
<211> 157
<212> DNA
<213> Homo sapiens

<400> 245
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ccaactctgt tcatgacact tccttccagc accaaatgct tcccaccct ctactccac 120
tccccattct tcaaaccag ctcaagttcc agttcct 157

<210> 246
<211> 36
<212> DNA
<213> Homo sapiens

<400> 246
caccttctcc atttcacacc ggtggtacaa gaaatc 36

<210> 247
<211> 262
<212> DNA
<213> Homo sapiens

<400> 247
atcttaacct atcaactttc tgcccctcct tcaaggccca gtataaatgc cacctcctcc 60
atgaagcctt ccctaattcc accccaaacc cccaccttca acaatatttc aacgcttctg 120
caatgatgaa aaagaaacat agttgtagta cttagcctac ctagaccagc aagcattcat 180
ttttagctcg ctcatTTTTT accatgtttt ccagtctggt taacttctgc agtgccttca 240
ctacactgcc ttacataaac ca 262

<210> 248
<211> 146
<212> DNA
<213> Homo sapiens

pctgb2016051825-seq1.txt

<400> 248
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 tcagattctt ggtccatgga tgacaccacc tgcccatgct tccttgaatt aggctttcct 120
 aacctgaagc gccttcaaac ttccaa 146

<210> 249
 <211> 252
 <212> DNA
 <213> Homo sapiens

<400> 249
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 caggagagcc ccattcccag cccctggtct cccgtgccgc agttctatat ttgcttcaa 120
 atcagccttc aggtttccca cagcatggcc cctgctgaca cgagaaccca aagttttccc 180
 aaatctaaat catcaaaaaca gaatccccac cccaatccca aattttgttt tggttctaac 240
 tacctccaga at 252

<210> 250
 <211> 191
 <212> DNA
 <213> Homo sapiens

<400> 250
 caaccctatc ccagagacct tgatgcttgg cctcccaatc ttgccctagg ataccagat 60
 gcccaaccaga cacctccttc ttcctagcca ggctatctgg cctgagacaa caaatgggtc 120
 cctcagtctg gcaatgggac tctgagaact cctcattccc tgactcttag ccccagactc 180
 ttcattcagt g 191

<210> 251
 <211> 108
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

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<400> 251
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 ttgcagtgag ccgacatcag ccactgcact caagactctc gatctctc 108

<210> 252
 <211> 62
 <212> DNA
 <213> Homo sapiens

<400> 252
 tgggagtgac cagaagaaat gacgcagaag tgaaatgaac tttttataag cattctttta 60
 at 62

<210> 253
 <211> 240
 <212> DNA
 <213> Homo sapiens

<400> 253
 gaagagctac ttcattcttt tctgaagttg tgtgttgctg ctagaaatag tcattttgtga 60
 attatccaaa ttgtttaaat tcacaattga attagttttt tcttcctttt tgcttgaagc 120
 aaacagttga caatttttaa ctttttcatt ttatgttttt gtactctgca gactgaaaag 180
 acaaagtta tcttggcctt actgtataaa ggtgtgctgt gtccaccgtt gtgtacagaa 240

<210> 254
 <211> 172
 <212> DNA
 <213> Homo sapiens

<400> 254
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 tccctcatct tttaggaatc atttaccagg tttggagagg attcagacag ctcaggtgct 120
 ttcactaatg tctttgaact tctgtccctc tttgtgttca tggatagtcc aa 172

<210> 255
 <211> 206
 <212> DNA

pctgb2016051825-seq1.txt

<213> Homo sapiens

<400> 255

attcaaccat catctgtcaa gaaattaaaa gaacaacacc ctagagagaa gtcattccaca 60
 cacaatccac acacgcatag caaacctcca atgcatgtac agaaacctgt gatattttata 120
 ccctttagtagg aaggatataga caatggaatt gtgagtagct taatctctat gtttctctcc 180
 attttcattc ctcctgcaac tattttt 206

<210> 256

<211> 214

<212> DNA

<213> Homo sapiens

<400> 256

gcgatgctga ggtaccgggtg ggacaggagg aggtctgcta ggtcacatgg aagaaagacc 60
 atggcatggg cctgtggcct gaaccctggg gctctgtgat ggagccagcc agatcatggg 120
 gaagtctgcc tttcaaggag tgcctttggg accttaaagg aattgaaaac aaggatgacg 180
 tacctaatta actgctggga aagagttaac aatg 214

<210> 257

<211> 281

<212> DNA

<213> Homo sapiens

<400> 257

aattcgccaa tcatcccacc atataacctt cgattgtgct tctcaactcc accccataat 60
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 aaaccatgga tatttttttcc tcaggggctt taaatagttt cctatgcaac gtgtcttgta 180
 gcacaaataa aattctacaa aagttgcagt aaattttatt tggatatttt aacctgttaa 240
 gtgtgtgtgt gttttctgta cccaaccaga ctttaaataa a 281

<210> 258

<211> 297

<212> DNA

<213> Homo sapiens

pctgb2016051825-seq1.txt

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 cacaaggcct gaggagcagt gtgggggggac agacaggagg tggatttgga gaccgaagac 180
 tgggatgcct gtcttgagta gacttggacc caaaaaatca tctcaccttg agcccacccc 240
 caccgccattg tttaatctgt agaagctaataaataatcat ccctccttgc ctagcaa 297

<210> 259
 <211> 48
 <212> DNA
 <213> Homo sapiens

<400> 259
 ccttgccctag cataacagag aatccttttt ttaacggtga tgcgctgt 48

<210> 260
 <211> 249
 <212> DNA
 <213> Homo sapiens

<400> 260
 gtcaccatag acaccacaat gcgtggaggc cgacttggcg ttttttgctt ctctcaagaa 60
 aacatcatct ggtccaacct caagtatcgc tgcaatgaca ccatccctga ggacttccaa 120
 gagtttcaaa cccagaattt cgaccgcttc gataattaaa ccaaggaagc aatctgtaac 180
 tgcttttcgg aactactaaa ccatatatat ttttaacttca attttcttta gcttttacca 240
 acccaaata 249

<210> 261
 <211> 292
 <212> DNA
 <213> Homo sapiens

<400> 261
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 ccgaggcaga cggatcacct aaggacaaga gttcaagacc agcctggtca acatggtgaa 120

pctgb2016051825-seq1.txt

accctgtctt tactaaaaat acaaaaaatta gctgggcatg gtggcatgcg cctatagtcc	180
cagctactcg ggaggctgag gcaggagaat cacttgaacc tgggaggtag aggttgagc	240
gagccgagat agtgccactg cactccagcc tcggcatcag agtgagattc tg	292

<210> 262
 <211> 274
 <212> DNA
 <213> Homo sapiens

<400> 262	
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gttttcaacc cagttcatct ggtggatgtt tttgcagggt actctgagaa ttttgctt	120
gaaaaatcat tatttttagt gtagttcaca ataattgtatt gaacatactt ttaatcaa	180
gtgctatgtc cttgtgtatg gtactaaatg tgcctgtgt acttttgcac aactgaga	240
cctgcagctt ggtttaatga gtgtgttcat gaaa	274

<210> 263
 <211> 284
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 263	
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acacaagctg tgggggtgta cgcagtcctg cttttttcac ttaataatac agcttgga	120
gatttttgta tcacattata aatcccactc gctctttttg atggccacat aataacta	180
gcataatatg gatacgctt atttgattta actagttccc taatgatgga cttttaagt	240
gtttcctttt tttttctttt ttgctactgc aaacgatgct ataa	284

<210> 264
 <211> 240
 <212> DNA

<213> Artificial Sequence

<220>

<223> none

<400> 264

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taatacatgt gcatacacat aaacacatct agatgtatat acacacaaag atccaaaagc 120

ttttaccttg gaactctagc catgagatag taatacagtt tcaactgtct gcaaacaatgt 180

ccacatggct gaactttggt agccttgata ggtaatccag ggaaggctgt gaaacaaatt 240

<210> 265

<211> 237

<212> DNA

<213> Homo sapiens

<400> 265

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ggagtccaag gagggggatc gcttgagtcc aggagtttga gaccagcctg gacaacatgg 120

tgaaacccccg tctttacaaa aaatacaaaa attagccatg catggtggct catgcctgta 180

gtcccagcta cttgggaggc tgaggcagga ggatcactta agcccagaag gcagagg 237

<210> 266

<211> 241

<212> DNA

<213> Homo sapiens

<400> 266

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tgttacacct cccttccttt ttttcacagt gaacctgtat tcagctattc tcaactctgag 120

aactctccaa taacaatttc ttttcacag ttaacaacaa agttctgttt ttaaatagaag 180

agattaagtt ctttttaaat gcctaaaggc atattctgac aacttttcta cttctttaac 240

t 241

<210> 267

pctgb2016051825-seq1.txt

<211> 241
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<400> 267
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 ctagtcatct taaataaatg aagttttaac atttctccag tgattttttt atttcacctt 120
 tgaagatact atgttatgtg attaaataaa gaacttgaga agaacagggt tcattaaaca 180
 taaaatcaat gtagacgcaa attttttggga tgggcaatac ttatgttcac aggaaatgct 240
 t 241

<210> 268
 <211> 247
 <212> DNA
 <213> Homo sapiens

<400> 268
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 ttgctagctg catcactagt catcttaaat aaatgaagtt ttaacatttc tccagtgatt 120
 tttttatttc acctttgaag atactatgtt atgtgattaa ataaagaact tgagaagaac 180
 aggtttcatt aaacataaaa tcaatgtaga cgcaaatttt ttggatgggc aatacttatg 240
 ttcacag 247

<210> 269
 <211> 280
 <212> DNA
 <213> Homo sapiens

<400> 269
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 ccggccggag catggcggac agcacacacg gcccggggcg ggaaccttgg aaactttaca 120
 cagatgggga gctcagccat tccacgtgtg ctttcgctca gcacaatgct tactacaaac 180
 ccacgtgtac ttccttccag ctggttgctt tttattgttg ctgtcttaaa ctccaaagtt 240
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pctgb2016051825-seq1.txt

<210> 270
 <211> 287
 <212> DNA
 <213> Homo sapiens

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 gtcccaggcc acagcaaagg tctgttgaac ccctccctcc attcccagtt atctgggtcc 180
 tctggattct tctgtttctt gaatcaggct ctgctttccc cctagccact acaggcagcc 240
 tctgacagtg ccgctttact tgcattctgc agcaattaca tgtgtcc 287

<210> 271
 <211> 287
 <212> DNA
 <213> Homo sapiens

<400> 271
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 tcacatctgg aaatgtgaag aataaacact acttaataga attattgtgc aaattaaatt 120
 aagtgtaaaa agcttgggtc acagtaggtg gcaaataaat atgtacttcc ttttaaggaaa 180
 tgtgtcattt tctttaacat agtttatatt cataaggaga caacacacaa acacatacac 240
 aatcaatcaa caaaaatgtg atacagtaga ttattaagca ccttgct 287

<210> 272
 <211> 299
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 272
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 tcacatctgg aaatgtgaag aataaacact acttaataga attattgtgc aaattaaatt 120

pctgb2016051825-seq1.txt

aagtgtaaaa agcttggttc acagtaggtg gcaaataaat atgtacttcc ttttaaggaaa	180
tgtgtcattt tctttaacat agtttatatt cataaggaga caacacacaa acacatacac	240
aatcaatcaa caaaaatgtg atacagtaga ttattaagca ccttgctttg attaaaaag	299

<210> 273
 <211> 66
 <212> DNA
 <213> Homo sapiens

<400> 273	
gttcatctgt gttgatacct gtagttgtag ttcacatctgtt ttcattacta tgtgcctttc	60
ccctgt	66

<210> 274
 <211> 161
 <212> DNA
 <213> Homo sapiens

<400> 274	
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ctgtttttcat tactatgtgc ctttcccctg taagtagtcc ccccttatt tgcaggggat	120
acagggcccc cagaagggtgc ctgaaacttc agatagtacc a	161

<210> 275
 <211> 233
 <212> DNA
 <213> Homo sapiens

<400> 275	
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cacttgtttc ttacctatatt aggaagagca cagtatgac cagcaaaacc taactacatc	120
tctttggaca aactagtatc tattcctgat gaaatatttt gtgaagagat tgccaaagca	180
tcagtacagg actttgaaaa attccttaaaa acgctttaga tttttatgta tgt	233

<210> 276

pctgb2016051825-seq1.txt

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<211> 32
<212> DNA
<213> Homo sapiens

<400> 276
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<210> 277
<211> 109
<212> DNA
<213> Homo sapiens

<400> 277
aaaccaaggc acataatcgt gtgtgagtgt gtgtgccagt gtgtgtacat gcatccacat 60
atgtgtgctc tcatgtaaat gattaataaag cctggaactt atgatgttt 109

<210> 278
<211> 124
<212> DNA
<213> Homo sapiens

<400> 278
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tacatgcac cacaatgtgt tgctctcatg taaatgatta aaaagcctgg aacttatgat 120
gttt 124

<210> 279
<211> 117
<212> DNA
<213> Homo sapiens

<400> 279
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acgcctaatt gtggattata aaatgtgcac attttctatc acttgtttcc aataaag 117

<210> 280
<211> 94
<212> DNA
<213> Artificial Sequence

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pctgb2016051825-seq1.txt

<220>

<223> none

<400> 280

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agtgacattg gtcattgtagc tagataatta tgggt 94

<210> 281

<211> 278

<212> DNA

<213> Homo sapiens

<400> 281

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gtgacattgg tcatgtagct agataattat ggtaattgtg acaattaaag agaaataaat 120

tattgattat ccttcagaaa aattatcaag gagtggttta ttttccttgt ccacagtgggt 180

tgacagttat aaagtagtta tatgtgtata ctatttagaa gacgcctaata tgtggattat 240

aaaatgtgca cattttctat cacttgtttc caataaag 278

<210> 282

<211> 281

<212> DNA

<213> Homo sapiens

<400> 282

gaagccacag agcaggcgct cgaccagacc catcaggggc gaggagggca cggaaacctg 60

tgccgagatg ggggcaagag gccagggcag ccaccagcac agagaagagg agatccccag 120

agtcaggag ggcagagggt ggcagcgagg gcagggcagc cgccccgct cccagccagg 180

cagaaggccc ccaccagcac cacacccatc cccagcagcc tgccttggg agagggcgctc 240

acccggtcag agactccaaa taaaccggtt cttgtcaagg c 281

<210> 283

<211> 231

<212> DNA

<213> Artificial Sequence

pctgb2016051825-seq1.txt

<220>

<223> none

<400> 283

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cctccctcgc ccctgggtcc tacggagcct gcactttcaa gaggtacagc ggcatcctgt 120

gggggcctgg gcaccgcagg aagactgcac agaaaactttg ccattgttgg aacgggacgt 180

tgctccttcc ccgagcttcc ccggacagcg tactttgagg actcgctcag c 231

<210> 284

<211> 256

<212> DNA

<213> Homo sapiens

<400> 284

ataaacctgt acccttagtga atatitttaga ttagggtaat acagacactg aagattatit 60

gagaactgta cttggcataa actcatttca ttgttataaa aaacaacttc tattccagat 120

gcctttgagt gttcaaggat gctctgtgat cagtattatt tatcatctcc agaattaaag 180

cttacacaag tgaatggtaa gttataagta aaatatatit taacctatgg aaaattctgt 240

gttctcctaa tattat 256

<210> 285

<211> 261

<212> DNA

<213> Homo sapiens

<400> 285

ataaacctgt acccttagtga atatitttaga ttagggtaat acagacactg aagattatit 60

gagaactgta cttggcataa actcatttca ttgttataaa aaacaacttc tattccagat 120

gcctttgagt gttcaaggat gctctgtgat cagtattatt tatcatctcc agaattaaag 180

cttacacaag tgaatggtaa gttataagta aaatatatit taacctatgg aaaattctgt 240

gttctcctaa tattattgct t 261

<210> 286

pctgb2016051825-seq1.txt

<211> 183
<212> DNA
<213> Homo sapiens

<400> 286
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tgcgtgcttt ttcagaggga cgggtgatgg gcagattgga aaaagcaccg cagatgggaa 120
ccttaatctt ttttttttaa aattgatgct atgaaaattt gcgttttctg taacttgtaa 180
aaa 183

<210> 287
<211> 38
<212> DNA
<213> Homo sapiens

<400> 287
gagagaaatc aactattcag gaccggcccc cacctttc 38

<210> 288
<211> 65
<212> DNA
<213> Homo sapiens

<400> 288
gtgctttata taggacactg tcattcttca agtaccatc ttggagggtg cggacccac 60
atgag 65

<210> 289
<211> 290
<212> DNA
<213> Homo sapiens

<400> 289
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ctcgccgcgg cggcgtgaag cgcattctcc gcctcatcta caggagact cgcgggggtg 120
tgaagggtgtt cctggagaac gtgatccggg acgccgtgac ctatacagag cacgccaagc 180
gcaagacggt caccgccatg gatgtgggtt acgcgtcaa gcgccagggc cgcaccctct 240

acggtttcgg tggttgagcg tccttttcta ccaataaaag gcccttttca 290

<210> 290
 <211> 169
 <212> DNA
 <213> Homo sapiens

<400> 290
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 gtgagtcaca gtgctgtaag accacattgc ctaggcagcc atgcccttgg aagtgggtgat 120
 gtctaagtga gaactgatag attagtaaga ggttgctctg gccaggcac 169

<210> 291
 <211> 179
 <212> DNA
 <213> Homo sapiens

<400> 291
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 aatcctgaag tgttacctcc tttcatgtct atttacacta ttttcccatt tactaagtgg 120
 ggaggggggtc tccttatata gtgcttcacg gtaataaagt caatacctgt tgttcctgg 179

<210> 292
 <211> 254
 <212> DNA
 <213> Homo sapiens

<400> 292
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 tcctgaagtg ttacctcctt tcatgtctat ttacactatt ttcccattta ctaagtgggg 180
 aggggggtctc cttatatagt gcttcacgt taataagtca atacctgttg ttcctgggat 240
 gttctttttt gtgc 254

<210> 293
 <211> 103

pctgb2016051825-seq1.txt

<212> DNA

<213> Homo sapiens

<400> 293

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<210> 294

<211> 259

<212> DNA

<213> Homo sapiens

<400> 294

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aaagctgttt cagctgggcc ccccttcacc tgtgaagatg ccctctccac catgggaatc 120

caatctgttg cagtttcctt caagcattct gtcgaccctg gatgttgaat tgccacctgt 180

ttgctgtgac atagatattt aaatttccta gtgcttcaga gtttgtgtgt atttgtatta 240

ataaagcatt cttaaacag 259

<210> 295

<211> 108

<212> DNA

<213> Homo sapiens

<400> 295

ttaaaaactt ggtgcatgtt ggctgggcac agtggctcac gcttgtaatc ccagcacttt 60

gggaggccga ggcaggtgga tcacaagttc agtggttcta gaccagcc 108

<210> 296

<211> 236

<212> DNA

<213> Homo sapiens

<400> 296

gaaaccaatc aagataaagg acagtgaagt aatccgtacc ttgtgttttg ttttgattta 60

ataacataac aaataaccaa cccttccttg aaaacctcac atgcatacat acacatatat 120

acacacacaa agagagttaa tcaactgaaa gtgtttcctt catttctgat atagaattgc 180

pctgb2016051825-seq1.txt

aattttaaca cacataaagg ataaactttt agaaacttat cttacaaagt gtattt 236

<210> 297

<211> 274

<212> DNA

<213> Homo sapiens

<400> 297

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gtagattttt acaatctgta tctttgacaa ttctgggtgc gagtgagaga gtgtgagcag 120

ggcttgctcc tgccaaccac aattcaatga atccccgacc cccctacccc atgctgtact 180

tgtggttctc tttttgtatt ttgcatctga ccccgggggg ctgggacaga ttggcaatgg 240

gccgtcccct ctccccttgg ttctgcactg ttgc 274

<210> 298

<211> 240

<212> DNA

<213> Homo sapiens

<400> 298

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gaggatttct ttatgaggca aatttatatt ttttaatatc ggggggtgga ccacgccgcc 120

ctccatccgt gctgcatgaa aaacattcca cgtgccccct gtcgcgcgct tcccatcctg 180

atcccagacc cattccttag ctatttatcc ctttcttggt ttccgaaagg caattatatc 240

<210> 299

<211> 260

<212> DNA

<213> Homo sapiens

<400> 299

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tccaacaagc ttttcacaga attcatgcag tgcaaatccc caaaggaac ctttatccat 120

ttcatggtga gtgcgcttta gaattttggc aaatcatact ggtcacttat ctcaactttg 180

pctgb2016051825-seq1.txt

agatgtgttt gtcctttag ttaattgaaa gaaatagggc actcttgtga gccactttag 240
 ggttcactcc tggcaataaa 260

<210> 300
 <211> 111
 <212> DNA
 <213> Homo sapiens

<400> 300
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 ttcacattat ttttgtacac tttcccatg ttaagggatg atggctttta t 111

<210> 301
 <211> 156
 <212> DNA
 <213> Homo sapiens

<400> 301
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 accatgctct ttttgttaaa gaagagagta atgtgtttat cctggctcat agtccgtcac 120
 cgaaaataga aaatgccatc cataggtaaa atgctg 156

<210> 302
 <211> 253
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 302
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 cggagaagag ctgttcacag ccagactggc acagttttct gagaaagact attcaaacag 120
 tctcaggaaa tcaaatatgc aaagcactga cttctaagta aaaccacagc agttgaaaag 180
 actccaaaga aatgtaaggg aaactgccag caacgcaggc ccccagggtc cagttatggc 240
 tataggtgct aca 253

pctgb2016051825-seq1.txt

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<210> 303
<211> 79
<212> DNA
<213> Homo sapiens

<400> 303
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catctatact tgcagtggg                                                                79

<210> 304
<211> 219
<212> DNA
<213> Homo sapiens

<400> 304
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catacataga tcttcatgat gtgtgagtgt aattccatgt ggatatcagt taccaaakat      120
tacaacacac ttttatggcc caaaatgacc aacgaaattg ttacaataga atttatccaa      180
ttttgatctt tttatatattt tttaccacac ctggaaaca                                219

<210> 305
<211> 234
<212> DNA
<213> Homo sapiens

<400> 305
gtgccttcca aacctgagaa tatatgcttt tggaagttaa aatttaaattg gcttttgcca      60
catacataga tcttcatgat gtgtgagtgt aattccatgt ggatatcagt taccaaakat      120
tacaacacac ttttatggcc caaaatgacc aacgaaattg ttacaataga atttatccaa      180
ttttgatctt tttatatattt tttaccacac ctggaaacag accaatagac attt          234

<210> 306
<211> 101
<212> DNA
<213> Homo sapiens

<400> 306

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pctgb2016051825-seq1.txt

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actcaagcaa tcctgaggaa tactgaggga gggcctggct a 101

<210> 307
<211> 75
<212> DNA
<213> Homo sapiens

<400> 307
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aggtgggctc tcctt 75

<210> 308
<211> 164
<212> DNA
<213> Homo sapiens

<400> 308
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cgaataacac tgcagcaagc gtgggagtg agatagctct tggaaatacc catttccttc 120
agacgcatac tcagaggtag gattgctgga ttgtttggtc attt 164

<210> 309
<211> 208
<212> DNA
<213> Homo sapiens

<400> 309
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cagcaaactgt tttctgcaaa aggccaccta gtaagtgttt taggcattgt gtgccattat 120
tgtttctgtt gtgatgaatc acctttgcca ttgtagcaca aaagcagatg tcaatacatt 180
ggcaaattggc tgtgactatg tttcaata 208

<210> 310
<211> 66
<212> DNA
<213> Homo sapiens

pctgb2016051825-seq1.txt

<400> 310
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 ttgatc 66

<210> 311
 <211> 283
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 311
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 gcatattgtt aaaagtctct caaattaggc cagattttta aacatgctgc agcaagagga 120
 ccccgctctc ttcaggaaaa gtgttttcat ttctcaggat gcttcttacc tgtcagagga 180
 ggtgacaagg cagtctcttg ctctcttgga ctcaccaggc tcctattgaa ggaaccaccc 240
 ccattcctaa atatgtgaaa agtcgcccaa aatgcaacct tga 283

<210> 312
 <211> 213
 <212> DNA
 <213> Homo sapiens

<400> 312
 cagacccctg ggtacatggt ggatgatttt aaatcacctt agtgtgctga gaccttgaga 60
 ataaagtgtg tgattggttt catacttgaa gacggatata aaggaagaat atttccttta 120
 tgtgtttctc cagaatgggt cctgtttctc tctgtgtctc aatgcctggg actggagggt 180
 gatagtttaa gtgtgttctt accgcctcct ttt 213

<210> 313
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 <212> DNA
 <213> Homo sapiens

<400> 313

pctgb2016051825-seq1.txt

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gtgcatgggt tattttggag tgtaggtgac ttgtttactc attgaagcag atttctgctt    120
ccttttattt ttataggaat agaggaagaa aggtcagatg cgtgcccagc tcttcacccc    180
ccaatctctt ggtggggagg ggtgtaccta aatatttatc atatccttgc ccttgagtgc    240
ttgttagaga gaa                                                         253

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<210> 314
<211> 197
<212> DNA
<213> Homo sapiens

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<400> 314
gtgtgttgct gctagaaata gtcatttgtg aattatccaa attgtttaaa ttcacaattg      60
aattagtttt ttcttccttt ttgcttgaag caaacagttg acaattttta accttttcat    120
tttatgtttt tgtactctgc agactgaaaa gacaaaagttt atcttggcct tactgtataa    180
aggtgtgctg tgtccac                                                         197

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<210> 315
<211> 240
<212> DNA
<213> Homo sapiens

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<400> 315
gaagagctac ttcattcttt tctgaagttg tgtgttgctg ctagaaatag tcatttgtga      60
attatccaaa ttgtttaaat tcacaattga attagttttt tcttcctttt tgcttgaagc    120
aaacagttga caatttttaa ccttttcatt ttatgttttt gtactctgca gactgaaaag    180
acaaaagttta tcttggcctt actgtataaa ggtgtgctgt gtccaccgtt gtgtacagaa    240

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<210> 316
<211> 123
<212> DNA
<213> Homo sapiens

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<400> 316
tttgaggaca aacagatttg tcagctgtca ggggtgcagtg ggacgtcaga gactatgtgg      60

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pctgb2016051825-seq1.txt

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tcc 123

<210> 317

<211> 219

<212> DNA

<213> Homo sapiens

<400> 317

aactcctagt acttagaaat acacgtatgg ttaatgttca gtccaacaaa ccacacacag 60

taaatgttta ttaatagtca tggttcgtat tttagggtgac tgaaattgca acagtgatca 120

taatgagggtt tgttaaaacg atagctatat tcaaaatgtc tatatgttta tttggacttt 180

tgagggttaaa gacagtcata taaacgtcct gtttctgtt 219

<210> 318

<211> 122

<212> DNA

<213> Homo sapiens

<400> 318

gaataggctc tgctaacgtg atgagacaaa ctgaaaatta ttgcgagcat tgactataat 60

tatgcagtac gttctcagga tgcattccagg gggttcatttt catgagcctg tccagggttag 120

tt 122

<210> 319

<211> 152

<212> DNA

<213> Homo sapiens

<400> 319

atgctgataa aaataaccagt gttattaccg tatgtaaatc ttgtgtcatt ttctttcaag 60

tgactgaact ggcttttgct taaacacttg acttctcagg aaacaaaaag ctaactccgg 120

gtgtggggta aattgagaat cctgtggctg gc 152

<210> 320

pctgb2016051825-seq1.txt

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<211> 233
<212> DNA
<213> Homo sapiens

<400> 320
aggctgggaa gcatctcaag atctttccag ggttatactt actagcacac agcatgatca      60
ttacggagtg aattatctaa tcaacatcat cctcagtgtc tttgcccata ctgaaattca      120
tttcccactt ttgtgcccac tctcaagacc tcaaaatgtc attccattaa tatcacagga      180
ttaacttttt tttttaacct ggaagaattc aatgtttacat gcagctatgg gaa          233

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<210> 321
<211> 282
<212> DNA
<213> Homo sapiens

<400> 321
tgaattgatg tgttccttaa aggatgggca ggaaaacaga tcctgttgtg gatattttatt      60
tgaacgggat tacagatttg aaatgaagtc acaaagttag cattaccaat gagaggaaaa      120
cagacgagaa aatcttgatg gcttcacaag acatgcaaca aacaaaatgg aatactgtga      180
tgacatgagg cagccaagct ggggaggaga taaccacggg gcagagggtc aggattctgg      240
ccctgctgcc taaactgtgc gttcataacc aaatcatttc at                          282

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<210> 322
<211> 174
<212> DNA
<213> Homo sapiens

<400> 322
ctaaatcgca tcatgccaac ttgtgacttt gagactattc atcaagaatg aggatatagt      60
agccatgaca tagcttgagc tatagccttt aattccttac tttggctatg ggtggagggt      120
gagtttgaag aggttttgat tttcttgtaa cctgggaaag ccatgacctt gtgc          174

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<210> 323
<211> 179
<212> DNA
<213> Homo sapiens

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pctgb2016051825-seq1.txt

<400> 323
 ggtttcctgg ttccactgct actgacccag aggggaatga gggctgagtt atgaaaagat 60
 aacttcatga agacttaact ggcccagaag ctgattttca tgaaaatctg ccactcaggg 120
 tctgggatga aggcttgtca gcacttccag tttagaacgc aatgtttcta gagacatat 179

<210> 324
 <211> 137
 <212> DNA
 <213> Homo sapiens

<400> 324
 ttagatttca tccaggtaac gttgagagta atgtcaggat ctcaaattga aaacggaagt 60
 tcctatTTTT tcaagccctt ttcattgggg ctgggggtgg gactctcggc ctggctgtgt 120
 gtaatgttaa ctttaata 137

<210> 325
 <211> 118
 <212> DNA
 <213> Homo sapiens

<400> 325
 gaaagctccg gtattattct ttgatagaaa tccaatagga agaattttta atcgtttctc 60
 caaagacatt ggacacttgg atgatttgct gccgctgacg ttttttagatt tcatccag 118

<210> 326
 <211> 149
 <212> DNA
 <213> Homo sapiens

<400> 326
 tttgggtaag cacctattta agtgtcttaa caatttttct attgagtact ctgggttttt 60
 gttttgtttt tcttactgat ttgtagaatt ctttatgtat tttgaattgc agataccttc 120
 cttctgtact aatgcttatc tttttagcc 149

<210> 327
 <211> 213

pctgb2016051825-seq1.txt

<212> DNA

<213> Homo sapiens

<400> 327

tttgggtaag cacctattta agtgtcttaa caatttttct attgagtact ctgggttttt	60
gttttgtttt tcttactgat ttgtagaatt ctttatgtat tttgaattgc agataccttc	120
cttctgtact aatgcttatc tttttagccc tgtaatatg tgttttcata aacatactta	180
tcaatctttt cttttatggg cattgatttt tgt	213

<210> 328

<211> 191

<212> DNA

<213> Artificial Sequence

<220>

<223> none

<400> 328

ttttccactt gtataatcag catatatattat ctttttgatt aaaaaccaag aaagaaaaaa	60
ttaatttttt tctagagaaa ttttcctgca tctcacctta aaaagggtttt tttcaaaatt	120
gcatcgattt tcaatactga aagaaacgtg gtatactatg gaatcagact aggtgaatgt	180
atttccaagt a	191

<210> 329

<211> 300

<212> DNA

<213> Homo sapiens

<400> 329

cactgattac cccaaagggt aggaatggag gagaggaggg caaaggaggt gagaggttat	60
taacttctta aaatcatatt ggatttttcc acttgataa tcagcatata ttatcttttt	120
gattaaaaac caagaaagaa aaaattaatt tttttctaga gaaattttcc tgcattctac	180
cttaaaaaagg tttttttcaa aattgcatcg attttcaata ctgaaagaaa cgtggtatac	240
tatggaatca gactaggtga atgtatttcc aagtaaattg cagcatattt cattctttcc	300

pctgb2016051825-seq1.txt

<210> 330
 <211> 119
 <212> DNA
 <213> Homo sapiens

<400> 330
 gctgctgtga gggatcttgg agcttccctg tagcccacct tccccttgct tcatgtttgt 60
 agaggaacct tgtgccggcc aggcccagtt tccttggtgtg atacactaat gtatttgct 119

<210> 331
 <211> 216
 <212> DNA
 <213> Homo sapiens

<400> 331
 aatatctaaa tatgtctcac atatttatat aatcctcaaa tatactgtac catttttagat 60
 attttttaaa cagattaatt tggagaagtt ttattcatta cctaattctg tggcaaaaat 120
 ggtgcctctg atgttgatgat atagtattgt cagtgtgtac atatataaaa cctgtgtaaa 180
 cctctgtcct tatgaaccat aacaaatgta gctttt 216

<210> 332
 <211> 206
 <212> DNA
 <213> Homo sapiens

<400> 332
 taatcctcaa atatactgta ccattttaga tatttttttaa acagattaat ttggagaagt 60
 ttatttcatt acctaattct gtggcaaaaa tggcgcctct gatgttgatga tatagtattg 120
 tcagtgtgta catatataaa acctgtgtaa acctctgtcc ttatgaacca taacaaatgt 180
 agcttttttaa agtccattgt attggt 206

<210> 333
 <211> 252
 <212> DNA
 <213> Homo sapiens

<400> 333
 gctatactct tcactaggat tccctatata tatactatat tatatgcctg ttatagatat 60

pctgb2016051825-seq1.txt

ctagatattt actatagtc agaagcaaca aaaagaaaca ctttttttct gagattatgt	120
tttatctagc aaatttgtaa ataaacagag taaaactata attttaaatt aattgtatat	180
aatatcagtt ttcatttaac tttccaggct tttaaaatta ctttttagtgc tttctatggt	240
tattaaaaga ct	252

<210> 334
 <211> 291
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 334	
ccattttata agatttctct ccatatatat ttctataact gctatactct tcactaggat	60
tccctatata tatactatat tatatgcctg ttatagatat ctagatattt actatagtc	120
agaagcaaca aaaagaaaca ctttttttct gagattatgt tttatctagc aaatttgtaa	180
ataaacagag taaaactata attttaaatt aattgtatat aatatcagtt ttcatttaac	240
tttccaggct tttaaaatta ctttttagtgc tttctatggt tattaaaaga c	291

<210> 335
 <211> 218
 <212> DNA
 <213> Homo sapiens

<400> 335	
ctcctgaaac gtaggtgaga acaaagtgat ggagtgagac attgtggcct ggaagtcttt	60
cagacaccca ctcagagaag tcaagtttaa agtggatggt cttcagagaa tttttcattt	120
ctgaaaatgt gttttgctta tagaatataa cagagttgac tagaaagaga gaaacaactg	180
cataactaatc ttttaaagcc tttaacagtt gcttttaa	218

<210> 336
 <211> 262
 <212> DNA

<213> Homo sapiens

<400> 336

tttacttgta aacagcatta gttgcagtcc tgaaatttta atattattag gttttcattt 60

ttttttatatt gaaaagtatt taattttatt gtaatttatt tgaaacaggt gttatataga 120

aatatattgc tgaattccta acatttctag ttacattttt aattacattg atttcatgtt 180

aaatccactt atgtcagaat atattctgta taatatctgt ctcttgaagt ttgatgatac 240

ttgctttatg gcataataaa ga 262

<210> 337

<211> 253

<212> DNA

<213> Homo sapiens

<400> 337

tttacttgta aacagcatta gttgcagtcc tgaaatttta atattattag gttttcattt 60

ttttttatatt gaaaagtatt taattttatt gtaatttatt tgaaacaggt gttatataga 120

aatatattgc tgaattccta acatttctag ttacattttt aattacattg atttcatgtt 180

aaatccactt atgtcagaat atattctgta taatatctgt ctcttgaagt ttgatgatac 240

ttgctttatg gca 253

<210> 338

<211> 175

<212> DNA

<213> Homo sapiens

<400> 338

atccagatag aaggctgcat acccaaagaa cgaagctgac actgcagggt cctgagtaaa 60

tgtgttctgt ataaacaaat gcagctggaa tcgctcaaga attttatttt tttaaatcca 120

acagcccata tttgatgagt attttgggtt tgttgtaaac caatgaacat ttgct 175

<210> 339

<211> 205

<212> DNA

<213> Homo sapiens

pctgb2016051825-seq1.txt

<400> 339
aacaacagat attctggctg gcctgcagag atccagatag aaggctgcat acccaaagaa 60
cgaagctgac actgcagggt cctgagtaaa tgtgttctgt ataaacaaat gcagctggaa 120
tcgctcaaga atcttatttt tctaaatcca acagcccata tttgatgagt attttgggtt 180
tgttgtaaac caatgaacat ttgct 205

<210> 340
<211> 198
<212> DNA
<213> Homo sapiens

<400> 340
gaaatgttag tatctcaatt acaccaactg tgcaaatgaa ctttgataaa atagaaataa 60
tttacattgg cctttgtgaa atctgggggaa gagctttagg attttagtag atggatactg 120
aatactcagg ccacttaaaa ttattaatgt atacattgtg tttttgtcct tatgctatgt 180
acagagaaat gtgataat 198

<210> 341
<211> 52
<212> DNA
<213> Homo sapiens

<400> 341
ataatttgaa ggttggcaga ccagttgcgc tgattactct tagagaagaa ga 52

<210> 342
<211> 264
<212> DNA
<213> Homo sapiens

<400> 342
actaagttcc aattttgttg ctgaattgct tctgtgagtt cacttttcag ttttaaggaa 60
gaataatatt tgctacatat ttcacagggg tttttatgaa ggtaaattta ccagattaat 120
aaaaatttat gaatattaaa attatcatta ataataataa acacttattt gagattaaat 180
taaatttttc atgagcccct ctttggcagg aactctgttt aattctttgt atttatccca 240

gcttttttaaa tggtaggctgt aaca 264

<210> 343

<211> 270

<212> DNA

<213> Homo sapiens

<400> 343

aatatgggta gttccagagt gcaaattaca gaaggaagct acttgtttaa aattccatac 60

acgtttgcag ttttttgtac acatttggat actttgaaag atgacagatt gttaaatacca 120

ttcaatggta aagaaactca ccatttggag attgagttta cttgttaatg aatgactagc 180

ccaattatcc ttataaattg aatatgggta ccaaagtctt tgatatcata ctactctgcc 240

tttgtgggca catatgtaga cactactaaa 270

<210> 344

<211> 198

<212> DNA

<213> Homo sapiens

<400> 344

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tttcccattt taacagatga taaaactcac agaagggcct atgggttatat agctagtaaa 120

agtggcagag tccgttgaac ttgaattcag attgggggaat gctttaacag ttggtatcca 180

gcagctacag gacttaat 198

<210> 345

<211> 281

<212> DNA

<213> Homo sapiens

<400> 345

atgtgctatg tactaagtgt ttgacatatg gtatttcatt taaaccttct gtgatccttt 60

gaagagtgtc ctccaatttt tcccatttta acagatgata aaactcacag aagggcctat 120

ggttatatag ctagtaaaag tggcagagtc cgttgaactt gaattcagat tgggggaatgc 180

pctgb2016051825-seq1.txt

tttaacagtt ggtatccagc agctacagga cttaatatTT ttcagtgttg tttcctcatc 240

tttaaattga agatattaaa gctcactttg taaaattgca a 281

<210> 346

<211> 218

<212> DNA

<213> Homo sapiens

<400> 346

tcatgggtgga gtagacacct aggatacaat ttaggcatgg cccactttc aaaaagggtc 60

ccaaatatga gattccagct gtatcaccaa tgatatcacc cattgaaact caggcagaat 120

gaagagaaca cattcaccat cagttacatg ctggcagaag tcaaccctga atcaatgctt 180

tataattcct gtactgatct tgtgagtaga acaacagt 218

<210> 347

<211> 42

<212> DNA

<213> Homo sapiens

<400> 347

tgacgtcagc catgtacagg atccacaggg gtgggtgtcaa at 42

<210> 348

<211> 219

<212> DNA

<213> Homo sapiens

<400> 348

ttcctgtagt actcctcttt tagatcctaa gtctcttaca aaagctttga atactgtgaa 60

aatgttttac attccatttc atttgtgttg tttttttaac tgcattttac cagatgtttt 120

gatgttatcg cttatgttaa tagtaattcc cgtatgtgtt cattttatTT tcatgctttt 180

tcagccatgt atcaatattc acttgactaa aatcactca 219

<210> 349

<211> 213

<212> DNA

<213> Artificial Sequence

pctgb2016051825-seq1.txt

<220>

<223> none

<400> 349

ttaagcctct ttattatagt tccccctaca ttttggttca catgccactg ttatgttgaa 60

gaaactgggt cctttgtctt ctggataccg ttttacaac atacttggtt tcatttaata 120

aatggctaaa tttctttcta tgtcaataat tatacacatc attattttta atcaccacat 180

cccattgtgt ggatagacta tcatttataa agc 213

<210> 350

<211> 299

<212> DNA

<213> Homo sapiens

<400> 350

gagaaatgac aaaaccagcc aggtgtgatg gctcacacct gtcatccac caccttggga 60

agcagagggt ggaggatggc ttgagcccgg gagtttgaga ccaatgtggc caacacagag 120

agacctgtgt ctacaaaaat aattttaaaa attagcctgg tgtggtgtca agcacctcta 180

gtcccagcta ctgagaggct gaaataggag gattgcttga tcccagaagg ttgaagctgc 240

agtgagctat gattgtacca ctacactaca gcctaggtga caccgaaaga cccttcctc 299

<210> 351

<211> 294

<212> DNA

<213> Artificial Sequence

<220>

<223> none

<400> 351

aagagactac agagtcaaag ctcatTTTTA aaggagtagg acagagttgt cacaggTTTT 60

tgTTGTTGTT tttattgccc ccaaaattac atgttaattt ccatttatat cagggaTTCT 120

atttacttga agactgtgaa gttgccattt tgtctcattg ttttctttga cataactagg 180

atccattatt tcccctgaag gcttcttgTT agaaaatagt acagttacaa ccaataggaa 240

caacaaaaag aaaaagtttg tgacattgta gtagggagtg tgtacccctt actc 294

<210> 352

<211> 216

<212> DNA

<213> Homo sapiens

<400> 352

gaagagagtc tgagccctcc tctacacccat gtgagggcac agagagagag cagccatcaa 60

caagctgaga gaggaggact gagaatgaaa cctacctcgc cagaaccttg tcatatccct 120

tcccggtttc caaacgagtg agaaatcaat ttctgttgta taagctactc agtgtatgaa 180

actttgttat ggcagccaga gcagattaat gcatgc 216

<210> 353

<211> 232

<212> DNA

<213> Homo sapiens

<400> 353

gaagagagtc tgagccctcc tctacacccat gtgagggcac agagagagag cagccatcaa 60

caagctgaga gaggaggact gagaatgaaa cctacctcgc cagaaccttg tcatatccct 120

tcccggtttc caaacgagtg agaaatcaat ttctgttgta taagctactc agtgtatgaa 180

actttgttat ggcagccaga gcagattaat gcatgcttct tggactttgt tt 232

<210> 354

<211> 261

<212> DNA

<213> Homo sapiens

<400> 354

tggaggcaat gtgggatctc agaccagta gctgcccttc ctgcctgatg tgggagctga 60

accacagaaa tcacagtcaa tggatccaca aggcctgagg agcagtgtgg ggggacagac 120

aggaggtgga tttggagacc gaagactggg atgcctgtct tgagtagact tggacccaaa 180

aaatcatctc accttgagcc caccaccacc ccattgtcta atctgtagaa gctaataaat 240

aatcatccct ccttgccatg c 261

pctgb2016051825-seq1.txt

<210> 355
<211> 148
<212> DNA
<213> Homo sapiens

<400> 355
tggcccagct cccaagaaa gcctcagcca ttactgccc ccagctcttc tccccagggtg 60
tggtggggcc ttggcctccc ctgctgaagg tggggattgc ccatccatct gcttacaatt 120
ccctgctgtc gtcttagcaa gaagtaaa 148

<210> 356
<211> 182
<212> DNA
<213> Homo sapiens

<400> 356
agtgattctg tgaaaaggac agtgaagcca gctattttac cccagggctg gatttttttt 60
tttttttttt ttttttttac cgagtacaca gagtacccaa gtgaagagaa cgatcatgagt 120
gtaagtgcaa atcagtggaa ggagcggcaa actgggacat gcagaattga atttgctcaa 180
aa 182

<210> 357
<211> 277
<212> DNA
<213> Homo sapiens

<400> 357
cagggcccct cttgctgttt ttacctttgt tccttggggc ctagtaccca gcaagcaccc 60
aaatggggga ggttttggga tgagaggagg aaacgtgtat acctgtaaca tctggtggct 120
cttccccag aagtttgtgt tcatacataa ttgttttcca cgctggatca taatgtgacg 180
tgcagttctg ccctgtgctg gggagccaca tgaagcttcc cctggctaac ttgctacccc 240
gcagcaatcc cagtgtggcc gtctgcttgc taaaaaa 277

<210> 358

pctgb2016051825-seq1.txt

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<211> 203
<212> DNA
<213> Homo sapiens

<400> 358
ctgcccacag tctttccgtg tgattgtctt tgaatctgaa tcagccagtc tcagatgccc      60
caaagtttcg gttcctatga gcccggggca tgatctgac cccaagacat gtggaggggc      120
agcctgtgcc tgcctttgtg tcagaaaaag gaaaccacag tgagcctgag agagacggcg      180
attttcgggc tgagaaggca gta                                              203

<210> 359
<211> 294
<212> DNA
<213> Homo sapiens

<400> 359
caagaaacta caaatcaaga ccacactgag gctgggtgtg gtggctcatg cctgtaatcc      60
cagcactttg ggaggctgaa gtgggcaaat cacttggaca ggagttcaag accagcctgg      120
gcaacatggg gaaaccctgt ctttactaaa aatacaaaaa gttagctggg catggtggta      180
cacacctgta atccctgtta ctggggggct gaggcacaag aatcacttga actctggaga      240
gagaggttgc agtgagccga catcagccac tgcactcaag actctcgatc tctc          294

<210> 360
<211> 217
<212> DNA
<213> Homo sapiens

<400> 360
agtcgccatg agaacttcct accttctgct gtttactctc tgcttacttt tgtctgagat      60
ggcctcaggt ggtaactttc tcacaggcct tggccacaga tctgatcatt acaattgcgt      120
cagcagtgga gggcaatgtc tctattctgc ctgcccgatc ttacaaaaa ttcaaggcac      180
ctgttacaga gggaaggcca agtgctgcaa gtgagct                                217

<210> 361
<211> 249

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pctgb2016051825-seq1.txt

<212> DNA

<213> Artificial Sequence

<220>

<223> none

<400> 361

tggttttggc ataatagctg catttccaga cctgacctt ggccccaacc acaagtgctc	60
caagccccac cagctgacca aagaaagccc aagttctcct tctgtccttc ccacaacctc	120
cctgctccca aaactatgaa attaatttga ccatattaac acagctgact cctccagttt	180
acttaaggta gaaagaatga gtttacaaca gatgaaaata agtgctttgg gcgaactgta	240
ttcctttta	249

<210> 362

<211> 191

<212> DNA

<213> Homo sapiens

<400> 362

ctactcctta cagtctctag aattaaatgt actcatttag acaacatatt aaatgcatat	60
tttagccact ttagagaaac ctcataggca cagagtttcc aagattaatt ttaagaatat	120
cttcacgaac ttgaccctcc tactccacat tgcaacattt ccatcagaca gcatttcaat	180
tccagtatta t	191

<210> 363

<211> 284

<212> DNA

<213> Homo sapiens

<400> 363

gaccaacagg agagagctct tgagtgggct agtgactccc cctgcagcct ggtggagatg	60
gtgtgaggag cgaagagccc tctgctctag gatttgggtt gaaaaacaga gagagaagtg	120
gggagttgcc acaggagcta acacgctggg aggcagttgg gggcgggtga actttgtgta	180
gccgaggccg caccctccct cattccaggc tcattcattt tcatgctcca ttgccagact	240
cttgctggga gcccgtccag aatgtcctcc caataaaact ccat	284

<210> 364
 <211> 269
 <212> DNA
 <213> Homo sapiens

<400> 364
 gaagagtaga tgttttaaaa gacctgattt taaaagaatg accaaaaata gccaaagtaaa 60
 tagtcaagga aatatataaa agaatatcaa agtaaaactag aaaataacaa atggaacaat 120
 ttgtaaaaat gaaagctata aataatgaaa tggagaacaa gacatagtgc aaaggatata 180
 taaagccaaa agttcathtt taaatggcaa aaacagagag agaagaagaa agagagagat 240
 aaacttctgg taaggcattt attaaaaaga 269

<210> 365
 <211> 259
 <212> DNA
 <213> Homo sapiens

<400> 365
 tgaggccggg aggatgcgtg cagggtggcgg ggagacagag gggcgatggc ccagtttctt 60
 ttgggctgaa aatgggtctaa agttgactgt ggtgatggct gtacaacttt gaatatgctg 120
 aaaactcgaa ttacacactt cagttgggcg aactgtactc catgtgaatt acctctcgat 180
 ggttctctcc tgggctcagg cgcttctccc accttggcct cctgagtagc tggaactacc 240
 attcatgtgc ggaaataaa 259

<210> 366
 <211> 233
 <212> DNA
 <213> Homo sapiens

<400> 366
 cgtagtagct gttcaactcc tagtacttag aaatacacgt atggttaatg ttcagtccaa 60
 caaaccacac acagtaaatg tttattaata gtcattgggtc gtatttttagg tgactgaaat 120
 tgcaacagtg atcataatga ggtttgttaa aacgatagct atattcaaaa tgtctatatg 180

tttattttgga cttttgaggt taaagacagt catataaacg tcctgtttct gtt 233

<210> 367
 <211> 113
 <212> DNA
 <213> Homo sapiens

<400> 367
 attttttcct atgtatacat gcctatgaga aagtttagtt tgtaaattag gcccactgag 60

agatgaacaa taatgaataa taaaatagaa caattatagc actatattgt aat 113

<210> 368
 <211> 227
 <212> DNA
 <213> Homo sapiens

<400> 368
 gtttagttgta tgagtctggt ttcacgctgc tgataaagac atacctgaga ctgggcaatt 60

tacaaaagaa gaggttttagt ggacttacag ttccatgtgg ctggggagac ctcacaatca 120

tgacagaagg caaggaggag aaagtcacgt ggatggcagc aggcaaagag agagcttgtg 180

caggcaaact cttattttta aaagcatcag atatcgctg aaccccg 227

<210> 369
 <211> 284
 <212> DNA
 <213> Homo sapiens

<400> 369
 ttgccatatg ttaaataagg tgaacagtaa agaagatctt gaaaacacag cttttctctg 60

gccatgaaca gctatgtata cttaagcttg tattttggaa ggaaaacata ttactttac 120

aaaaatcatt gaggatacaa accatttaaa agaagaccaa tcaatgtatt tatttgctca 180

tttgttcatt cattcaaagg acatttgcta aatgccctga tgtgccagaa acatggggac 240

agagaatata agataaagag tccagctgga agcaaatgct aaca 284

<210> 370
 <211> 296

pctgb2016051825-seq1.txt

<212> DNA

<213> Homo sapiens

<400> 370

atgaatccta cattttgcc	tatgttaa	aat aagg	tgaaca	gtaaaga	aga tctt	gaaaac	60
acagcctttc tctggccat	g aacagc	tatg tata	ctttaag	cttgtat	tttt gga	aggaaaa	120
catatttact ttacaaaa	at cattg	aggat acaa	accatt	taaaaga	aga cca	atcaatg	180
tattttatttg ctcat	ttgtt catt	cattca	aaggac	atgtt gctaa	atgcc	ctgatgtgcc	240
agaaacatgg ggacagag	aa tata	agataa	agagtcc	agc tgga	agcaaa	tgctaa	296

<210> 371

<211> 277

<212> DNA

<213> Homo sapiens

<400> 371

actagcattt gttttcgt	at ctagt	attat tttt	gaagaa	gagtct	ggaa	aatgcactct	60
ggcaaagggt accaaga	att taag	cctttt	tgcggggg	gt ggag	gggggg	cgttgatatg	120
tgcattataa attatc	attt aaga	aaccgt	ccaagaaa	aa tgcc	gcgtcg	ttacatgacc	180
tcgatctttc tttctccc	ct gcaat	cctac acgg	caataa	aataatt	agt gtttt	tagtta	240
agtcaaattt ttgaat	gtct atgt	ttaaact	aagtttt				277

<210> 372

<211> 237

<212> DNA

<213> Homo sapiens

<400> 372

tggagagtca catgtc	acta gcatt	tgttt tcgt	atctag	tattat	ttttt	gaagaagagt	60
ctggaaaatg cactct	ggca aagg	ttacca	agaatt	ttaag	ccttttt	tgcg gggggtggag	120
ggggggcggt gatat	gtgca ttata	aaatta	tcattt	taaga	aaccgt	ccaa gaaaaatgcc	180
gcgtcgttac atgac	ctcga tcttt	ctttt	tccc	ctgcaa	tcctac	acgg caataaa	237

<210> 373

pctgb2016051825-seq1.txt

<211> 282
 <212> DNA
 <213> Homo sapiens

<400> 373
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 agtttttaaaa gacccgattt taaaagaatg ttttaaaaata gccaaagtaaa tattcaaaga 120
 attataaaga gaatatcaaa gtaaaccaga aaataactaa tggaacaatt tgtaaaaata 180
 aaagccataa atactgaaat gtagaaagtt cattattaaa tgggaaaaag agaaagagca 240
 gaagagagag ataaactttt ggtaaggcat ttaaaggaaa ta 282

<210> 374
 <211> 91
 <212> DNA
 <213> Homo sapiens

<400> 374
 ccaaaccctg tatgtactat ttatTTTTTc ctatgtatac atgcctatga gaaagtttag 60
 tttgtaaatt aggcacactg agagatgaac a 91

<210> 375
 <211> 294
 <212> DNA
 <213> Homo sapiens

<400> 375
 cacattcata atctaacttg ctcatTTaac taataataca tcgcaaaatt tttccatatc 60
 cacaaataac catctaactt acatgacaat cttcaatcgt ggagtagtat tccagcctat 120
 ggatgccccca taattaatca gttccttggt aaacatgtag attgttttga tgcttttgct 180
 agcatataat gctatgttaa agagttctat gcattcgttt ctgagcacat gaatactttt 240
 ttcctttgag ttattttctaa agagaaattt ctaaataaag ggtatgcccc tctt 294

<210> 376
 <211> 58
 <212> DNA
 <213> Homo sapiens

pctgb2016051825-seq1.txt

<400> 376
caaagtacac atgagtgtaa agatgacact ccaggctggg gtgggtggcca gcactttg 58

<210> 377
<211> 240
<212> DNA
<213> Homo sapiens

<400> 377
tctaatttcc acctttgtga aagtcccttc tgtcccaacg tcaagggttg atacagtctg 60
gggagagctt ttacatgcat ttcagcctga attgatgtca agacatcaac agtctctccc 120
ttccgaactt ccttttagtt tcctgacaaa agcatcacct gtctttctcc agaaatgaat 180
cgttggggac cctacaggta gtactggctg tgcctgtgaa tttgtgatca ttgaagacaa 240

<210> 378
<211> 224
<212> DNA
<213> Homo sapiens

<400> 378
tttccacctt tgtgaaagtc ctttctgtcc caacgtcaag ggttgataca gtctggggag 60
agctttttaca tgcatttcag cctgaattga tgtcaagaca tcaacagtct ctcccttccg 120
aacttccttt tagtttcctg acaaaaagcat cacctgtctt tctccagaaa tgaatcgttg 180
gggaccctac aggtagtact ggctgtgcct gtgaatttgt gatc 224

<210> 379
<211> 61
<212> DNA
<213> Homo sapiens

<400> 379
taataatagc tctcacggag attttttttt gatacatcgt ctaaccaga agagttctct 60
g 61

<210> 380
<211> 256

pctgb2016051825-seq1.txt

<212> DNA

<213> Homo sapiens

<400> 380

ggttaataat agctctcacg gagatTTTTT tttgatacat cgtcttaacc agaagagttc	60
tctgtataat ttatttctta aaaataattg ttttgtgttt gttttgggta tttttagaag	120
cttttgctca agtcctaaca taatctccag taggagattt tagtctgtca gttcatgtat	180
gtatatgata gtcataattgt catattgtct ttttaaattc ctttttatgt tcactTTTTT	240
ctctgtacaa taatag	256

<210> 381

<211> 181

<212> DNA

<213> Homo sapiens

<400> 381

actggccag gcttatctgc ctgaaagggc aactgtttga ggtagaggga actggcagaa	60
taggaggggg ggaatcttcc tgggagaaaag taaaaagaat tctaggtgga gaggagatca	120
agatgtgaac caaacacatg tcttctaaat tactatagga aatgttggaa aggactttag	180
a	181

<210> 382

<211> 265

<212> DNA

<213> Homo sapiens

<400> 382

gaagatatgt atctccaggt tttcatTTTt cTTTTtctca tgaaatggta ttcaaattcgt	60
gcactttgaa atgccacaga ggaggagggt tatccgagct acgtgatggg tcaaattgaac	120
ttaaatttga aacatgttta aatttgcct tttgtttcct tttcattaat tactggaatt	180
gccgtgagtc cgttgctctg tgaacgtttc tctttctttg aacattcagg atatggtgtt	240
ttgtaagtga tttaacagcc agctt	265

<210> 383

pctgb2016051825-seq1.txt

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<211> 172
<212> DNA
<213> Homo sapiens

<400> 383
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gcatacaact agcactggct cgcaggaagc actaaggaag tttatgctgc tgttattgtc      120
cattgagtgt tttttacatg cagaatgctg ggtcaagccc tgtaaggctg ta              172

<210> 384
<211> 281
<212> DNA
<213> Homo sapiens

<400> 384
gcttggatgt aaaacgtaat agaaagatag aaaagggaga gctaggaaat ggactataaa      60
aaccaatttc tacatttaaa ttaagcttac aaataagaac ttgacagcta gcagaattgg      120
tttccattag acattactcc caggagctat attacatagt aaatgaatat ttttcattat      180
acatcctaca tgacccattc ataaatggct atttacaaag ctgttttact actaacttaa      240
aaaaatagga actttgaatt accctctgtg ctacttttat t                          281

<210> 385
<211> 292
<212> DNA
<213> Artificial Sequence

<220>
<223> none

<400> 385
tagtcttttg tgcttggatg taaaacgtaa tagaaagata gaaaaggag agctaggaaa      60
tggaactataa aaaccaattt ctacatttaa attaagctta caaataagaa cttgacagct      120
agcagaattg gtttccatta gacattactc ccaggagcta tattacatag taaatgaata      180
tttttcatta tacatcctac atgacccatt cataaatggc tatttacaaa gctgttttac      240
tactaactta aaaaaatagg aactttgaat taccctctgt gctactttta tt              292

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pctgb2016051825-seq1.txt

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<210> 386
<211> 132
<212> DNA
<213> Homo sapiens

<400> 386
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gaaggtgggtc cattccattc ctccttttcc agatgagaga tctgaggcca gagttcccaa      120
actatacttt aa                                                                132

<210> 387
<211> 131
<212> DNA
<213> Homo sapiens

<400> 387
ccagggaagg gtgagttcta ttgtcaccac tgtttctgct gctgctacct tctcaatcta      60
gaaggtgggtc cattccattc ctccttttcc agatgagaga tctgaggcca gagttcccaa      120
actatacttt a                                                                131

<210> 388
<211> 91
<212> DNA
<213> Homo sapiens

<400> 388
ccaaaccctg tatgtactat ttatTTTTTtC ctatgtatcc atgcctatga gaaagtttag      60
tttgtaaatt aggcacactg agagatgaac a                                          91

<210> 389
<211> 245
<212> DNA
<213> Homo sapiens

<400> 389
gctccaactg ttggacaaaa taaagagggg attgtgccag accttggtgt ttttgattcg      60
aactttgcca aggcctggaa cgctgaagaa taatactttt atttttcttt tctttcta      120

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pctgb2016051825-seq1.txt

tttattttac tttttatfff tctttccaaa aagcattaag aggtttttgt ttgtctgttt	180
cctttatcct cttggtaaaa ttttagcata tttaacatat aagatttaat gtttgggtga	240
tacaa	245

<210> 390
 <211> 245
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 390	
gctccaactg ttggacaaaa taaagagggg attgtgccag accttgtgtt ttttgattcg	60
aactttgcca aggcctggaa cgctgaagaa taatactttt atttttcttt tctttcta	120
tttattttac tttttatfff tctttccaaa aagcattaag aggtttttgt ttgtctgttt	180
cctttatcct cttggtaaaa ttttagcata tttaacatat aagatttaat gtttgggtga	240
tacaa	245

<210> 391
 <211> 192
 <212> DNA
 <213> Homo sapiens

<400> 391	
gatcataaca tatttctgtg ctctccctat agctgtgagc ccctgaagtc tggctttcag	60
aatataaatg tattcacgac cccgaactcg tgtcatgcct taaagaatca tcatagtcaa	120
ctcttttaac agcattatff taaaatgtta aatgtcactt tggggatgtc attaacgatc	180
acacatatgt aa	192

<210> 392
 <211> 277
 <212> DNA
 <213> Homo sapiens

<400> 392

pctgb2016051825-seq1.txt

ctttaatata ggaattatgg gccagggtgca gtggcttatg cctataatcc cagcacttct 60
 ggaggcagag acagggtggat cgcttgagct caggaattca agaccagctt ggtcaacatg 120
 gcaagaccct gtctctacaa aaaaatacaa aaattagctg ggcatgatgg tatgtgcttg 180
 tgggtcccagc tacttgggag gctgagggtg gaggatcact tgagcccagag aggtcaaggc 240
 tacagtgagc tgtgattgca cagctatact ccagcct 277

<210> 393
 <211> 45
 <212> DNA
 <213> Homo sapiens

<400> 393
 gaagtttgag actgctgagc tctgatcgga tctgagtgag ctctg 45

<210> 394
 <211> 243
 <212> DNA
 <213> Homo sapiens

<400> 394
 aataaatacg tgtttttttag gccagggtgtg gtggttcacg cctgtaatcc cagcactttt 60
 ggaggctgag gagggcggat cacctgaggt cgtgagttca agaccagcct ggccaacatg 120
 gtgaaacccc atctctactg aaaacacaaa aattagctgg gtgtggtggt gcacgcctgt 180
 aatcccatat actccagagg ctgaggcatg agaatcacct caactcggga gccgagatcg 240
 cac 243

<210> 395
 <211> 37
 <212> DNA
 <213> Homo sapiens

<400> 395
 accttttagaa atatctcgtt agaggccaga cgcggtg 37

<210> 396
 <211> 272

pctgb2016051825-seq1.txt

<212> DNA

<213> Homo sapiens

<400> 396

aaccctcacg caagtctaga gccaaaactg tagaccttga tcccggcact ctctatgtcc 60

actcagtttg acatcgtcac acctaaatgc tgtctgggtcc cctgcacaat aatggagagt 120

actcctgacc ccagtgcacg ctagccctcc cctgtgatgg tgggtggtgga tactaataca 180

gttctgtacg atgtgaagga tgtgtctttg ctatttcttg tctatttttaa cccgtctgct 240

tctaaatgat gtctagctgc ttaccaactt ta 272

<210> 397

<211> 248

<212> DNA

<213> Homo sapiens

<400> 397

atggacattg agatactatc attaacatta ggaccttaga attttgggta ttgtaatcta 60

aagtatgtta ttacaaaaca aacaaacaaa caaaaaaccc atgtgttaaa atactcagtg 120

ctaaacatgg cttaatctta ttttatcttc tttcctcaat ataggaggga agatttttcc 180

atttgtatta ctgcttccca ttgagtaatc atactcaaat gggggaaggg gtgctcctta 240

aatatata 248

<210> 398

<211> 147

<212> DNA

<213> Homo sapiens

<400> 398

aaatacaagg ccctgataaa attgatatct atccttatat ttttctccct ttttcccgtg 60

ccactttttc ttaaattatt tcccagttag tattatcata tgtttgtacc cgtcacagtt 120

ttcatagtg c tttcaaatac acctttt 147

<210> 399

<211> 198

<212> DNA

pctgb2016051825-seq1.txt

<213> Homo sapiens

<400> 399

aagtttttcag aaaatcttga ggttaaacad ctctctctct ttttttaaaa tacaaggccc	60
tgataaaaatt gatattctatc cttatatattt tctccctttt tcccgtgccca ctttttctta	120
aattattttcc cagtttagtat tatcatatgt ttgtacccgt cacagttttc atagtgcctt	180
caaatacacc tttttgat	198

<210> 400

<211> 220

<212> DNA

<213> Homo sapiens

<400> 400

ctcaatttga gtaatctgat cggtctttct agctaagga aaatgatttt acttagcaat	60
gttatcttgg tgtgttaaga gttaggttta acataaaggt tattttctcc tgatatagat	120
cacataacag aatgcaccag tcatcagcta ttcagttggt aagcttccag gaaaaaggac	180
aggcagaaaag agtttgagac ctgaatagct cccagatttc	220

<210> 401

<211> 71

<212> DNA

<213> Artificial Sequence

<220>

<223> none

<400> 401

tattgcccgg ctcctagaat ttatttatct cctgacttac agcaagcgag ttatcgtctt	60
ctgtattttg t	71

<210> 402

<211> 84

<212> DNA

<213> Homo sapiens

<400> 402

tattgcccgg ctcctagaat ttatttatct cctgacttac agcaagcgag ttatcgtctt	60

ctgtattttg tagactttct aaat 84

<210> 403

<211> 64

<212> DNA

<213> Homo sapiens

<400> 403

tttctccaaa gacattggac acttgatga ttgctgccg ctgacgtttt tagatttcat 60

ccag 64

<210> 404

<211> 242

<212> DNA

<213> Homo sapiens

<400> 404

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cttttttatt tttaaatgaa atcaagtaaa acttaacctt ggcttcaaaa atatctctca 120

aatactgtaa acaataacctt tgatactttt tagaaagaga ttatggccag gcgtagtggc 180

tcacgtctgt aatcccagaa ctttgggagg ccgagggtggg cagaccacct gaactcagga 240

at 242

<210> 405

<211> 250

<212> DNA

<213> Homo sapiens

<400> 405

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ggaagtctct tttttatatt taaatgaaat caagtaaaac ttaaccttgg cttcaaaaat 120

atctctcaaa tactgtaaac aataaccttg atacttttta gaaagagatt atggccaggc 180

gtagtggctc acgtctgtaa tcccagaact ttgggaggcc gaggtgggca gaccacctga 240

actcaggaat 250

pctgb2016051825-seq1.txt

<210> 406
<211> 218
<212> DNA
<213> Homo sapiens

<400> 406
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cctatctgca tcttctcact tgccaaaaaa tgaatatatg tttatgtgtg tatattactt 120
gtgtcacaaa aaaccctaaa gtagacagta aaagaacttg tcaatcgccct ttggaaggca 180
atgaaacact taataaactc tcaataacag aagcgtaa 218

<210> 407
<211> 252
<212> DNA
<213> Homo sapiens

<400> 407
aagtgtctga ttttattata caccaaagggt taacagtga tctctgtgtg atctcttttt 60
ttttcttttt gcctatctgc atcttctcac ttgccaaaaa atgaatatat gtttatgtgt 120
gtatattact tgtgtcacaa aaaaccctaa agtagacagt aaaagaactt gtcaatcgcc 180
tttgaaggc aatgaaacac ttaataaact ctcaataaca gaagcgtaaa aatgaaatgt 240
aaacctccaa tt 252

<210> 408
<211> 259
<212> DNA
<213> Homo sapiens

<400> 408
tttgactat aataccctcc actgaagtct cactgcaact gcttttaatt atatgtgaag 60
agaaactagc cctttgctaa aatagttgca tgtttcacgg gccagtgagt catttagtca 120
tacacttaag aactttccac ctttcaagtg tttatcatca cctcggattt cacggcctaa 180
tgccagtcaa tttcatgggg catagaagcc ctgtacgtat tagtttgggc cattattgta 240
tcagtcttgt ttattccca 259

pctgb2016051825-seq1.txt

<210> 409
 <211> 228
 <212> DNA
 <213> Homo sapiens

<400> 409
 aaagattaaa ttgctattgc ttagtaaga gaagctcttt gtatctgaac atagttgtat 60
 ttgaaatttg tggtttttta atttatttaa aattgggggg agggcatggg aaggatttaa 120
 caccgatata ttgttaccgc tgaaaatgaa ctttatgaac cttttccaag ttgatctatc 180
 cagtgcgtg gcctgggtgg cgtttcttct tgtacttatg tggttttt 228

<210> 410
 <211> 291
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <222> (259)..(259)
 <223> n is a, c, g, or t

<400> 410
 tcctctcagt gcagtagatg cggaagttag cagacacttg ttcgaactgt gtatttgtca 60
 aattttgcat gagaagatca caattttagt gactcatcag ttgcagtacc tcaaagctgc 120
 aagtcagatt ctgatattga aagatggtaa aatgggtgcag aaggggactt aactgagtt 180
 cctaaaatct ggtatagatt ttggctccct tttaaagaag gataatgagg aaagtgaaca 240
 acctccagtt ccaggaacnt cccacactaa ggaatcgtac cttctcagag t 291

<210> 411
 <211> 216
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature

pctgb2016051825-seq1.txt

<222> (124)..(124)

<223> n is a, c, g, or t

<400> 411

gagtgattta attgtgggat cctcctgaag cagatgaata ggaacagcta tttaaattga 60

tgtaatTTTA ttatgacctc cttcacagtg gttttcaggt aaccctgctt tcttagttaa 120

taangaggTT ctgtgaccca ggtaaccttg atgcacagat gagataatgt ggggattcac 180

tgatgcaaaa tcagaggcaa tcctttcata agtaga 216

<210> 412

<211> 208

<212> DNA

<213> Artificial Sequence

<220>

<223> none

<400> 412

gaataaaattt tctgctcacg atgagtttag tgtgtaaaagt ttagagacat ctgactttga 60

tagctaaatt aaaccaaacc ctattgaaga attgaatata tgctacttca agaaactaaa 120

ttgatctcgt agaattatct taataaaata atggctataa tttctctgca aaatcagatg 180

tcagcataag cgatggataa tacctaatt 208

<210> 413

<211> 280

<212> DNA

<213> Homo sapiens

<400> 413

agatgtttca gcttgaagaa ccaaaacaga aggaatatgt acaaagaata aatTTTctgc 60

tcacgatgag tttagtgtgt aaagtttaga gacatctgac tttgatagct aaattaaacc 120

aaaccctatt gaagaattga atatatgcta cttcaagaaa ctaaattgat ctcgtagaat 180

tatcttaata aaataatggc tataatttct ctgcaaaatc agatgtcagc ataagcgatg 240

gataatacct aataaaactgc ctcagtaaaa tccatggTTA 280

pctgb2016051825-seq1.txt

<210> 414
 <211> 257
 <212> DNA
 <213> Homo sapiens

<400> 414
 cttgtcctcc aagttatggg ggatcttgta ctgacaatct gtgttttcca ggagttacgt 60
 caaactacct gtactgggtt aaataagttt accttttcct ccaggaaata taatgatttc 120
 tgggaacatg ggcatgtata tatatatatg gagagagaat tttgcacata ttatacatat 180
 tttgtgctaa tcttgttttc ctcttagtat tcctttgtat aaattagtgt ttgtctagca 240
 tgtttgttta atccttt 257

<210> 415
 <211> 110
 <212> DNA
 <213> Homo sapiens

<400> 415
 agcaccctta tcaacccctt attgtagtaa acttggaaac ttggaaatga ccaggccaag 60
 actcaagcct cccagttctt actgaccttt gtccttaggt gtgaggcca 110

<210> 416
 <211> 187
 <212> DNA
 <213> Homo sapiens

<400> 416
 ttcctcaaaa acaaacgaca gcaacgaaaa ctccttaatc agaacactga tccaatgagg 60
 aatggagctt gtttctgtga cccaggagaa cttagtcaa gactacagga gttaacagat 120
 ggccagctcc ttatttttta atgtagaata actcctgagt ttatatcaaa tcctgaagaa 180
 ataagcc 187

<210> 417
 <211> 287
 <212> DNA
 <213> Homo sapiens

pctgb2016051825-seq1.txt

<400> 417
aatgaaaaga tccaccccag ttgatccagg cttcaggccc aactctacag acccaggaaa 60
caggcctacc atctgctaac acaggcacca ggccagcctg cctgaggact ttggcaacaa 120
gttcacctgc agaccacact agacacccta caaagagttg ctggatgact aggaaagggt 180
ttttccagat aaagccggta tgcaagaact agaataagtt tcttgTTTTT caaatgtgca 240
gacactaatg cactgcctaa aaatcaagat caagaacaat cagagaa 287

<210> 418
<211> 262
<212> DNA
<213> Homo sapiens

<400> 418
aatgaaaaga tccaccccag ttgatccagg cttcaggccc aactctacag acccaggaaa 60
caggcctacc atctgctaac acaggcacca ggccagcctg cctgaggact ttggcaacaa 120
gttcacctgc agaccacact agacacccta caaagagttg ctggatgact aggaaagggt 180
ttttccagat aaagccggta tgcaagaact agaataagtt tcttgTTTTT caaatgtgca 240
gacactaatg cactgcctaa aa 262

<210> 419
<211> 136
<212> DNA
<213> Homo sapiens

<400> 419
ccctgctctc ccaaaaaaat acaaaaaatta gctgggcgtg ctggtgtgcc tgtattccca 60
gctacttggg aggctgaggt gggaggattg cttcagctgg ggaggcggag gctgcaggga 120
gccaagactg agccat 136

<210> 420
<211> 289
<212> DNA
<213> Homo sapiens

<400> 420

pctgb2016051825-seq1.txt

ggtggagcta tgttatgatc agatcgaagt gtgacccctg tgtgggccag acagccctgc	60
agagagaaaa cctttattcc attatcacca agcacctcct agtttccgac agtcatctcc	120
ttctgctggg agaattagca gcagttcagg gggcttatgt tatgtccttg ttcaactcaa	180
cttgagctct tgaactcctc ctgtgggcct gtgaatgtat tcattcattc cacaactctg	240
ggtgcctccc acgtctggca ccgggggtac agtacacatt ccctgcctt	289

<210> 421
 <211> 217
 <212> DNA
 <213> Homo sapiens

<400> 421	
cagctggccc tgaccgggtg tcgggcgccc agagatgccg cgcgggagga gccgcgggag	60
agccgccgga ctgagcgagc agagggtcgt ggagcctgat ggatcgatga gatctgaaaa	120
agaacactgt gcgagattgt attcctgctt attctttcca agttagtagg cagattggaa	180
ctatagaatt tatccgtgat aataataaaag ttcgcat	217

<210> 422
 <211> 231
 <212> DNA
 <213> Homo sapiens

<400> 422	
caggacttgt gaaagcctgg gacgagggct tggggtttta ttctcaatat gatagaaaat	60
gattcaaggc tgagagcagg agagtggcag gatatactga cttatttaaa tctcactgtc	120
tcccacaaaag aacaagtttt atctctcctc atgctcctga gagaagaatg tactgcttat	180
ctaccatcat ttctgcatcc atcgaaacat ctgggtttac ctgaaaagaa a	231

<210> 423
 <211> 297
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

pctgb2016051825-seq1.txt

<400> 423
 ttttagataat gttgtcaaga ccaaaaagcat ggatgtcaag tgtcagtaag gattttgttt 60
 tctaaaattt tttcctgcat cagttcttct gagggccttg atgaaataac acagcagttt 120
 cttaaacaat ttgaaacaaa atgagctctc ctaccacctc actttttcat ttccacacta 180
 atgtattata tgtaactact tggaaaaaat aattattcaa atgcttcttc ccacaaagaa 240
 tatagatgat agtagatata ttttattaat aaaatggttc atgaatcgga gactaac 297

<210> 424
 <211> 91
 <212> DNA
 <213> Homo sapiens

<400> 424
 agagaaaact cagagattcg tggagactgg aaccacgggt gtggagagag gggttagtag 60
 agaccagatt ctgcaggtag tataatgaca t 91

<210> 425
 <211> 114
 <212> DNA
 <213> Homo sapiens

<400> 425
 tgtccttctg tgtgcttatg tctcttga caattgtttt cctccctgcc cctggagggt 60
 gtcttcaagc tgtggacttc tgggatttgc agattttgca acgtgggtact actt 114

<210> 426
 <211> 277
 <212> DNA
 <213> Homo sapiens

<400> 426
 atggtgaaac aagggatgtc tgattggaag tagaagatat ttatttaggt tctaggacat 60
 tagtatcagt gaggacagta atttcctgct tgtttgatt tcagtgatca catacacttc 120
 ttacctgat aacgtctctc ttctctaggc tggttttgggt tacggcttgc caatttctcg 180
 tctgtatgcc aagtactttc aaggagatct gaatctctac tctttatcag gatatggaac 240

agatgctatc atctacttaa aggtatccct tgaattc 277

<210> 427

<211> 267

<212> DNA

<213> Homo sapiens

<400> 427

ctgtcagagc ctgccgagct cacggatgct gtgaagggtca tggacctgcc caccaggag 60

ccagcactgg ggaccacctg ctacgcctca ggctggggca gcattgaacc agaggagtgc 120

ttgaccccaa agaaacttca gtgtgtggac ctccatgtta tttccaatga cgtgtgtgcg 180

caagttcacc ctcagaaggt gaccaagttc atgtgtgtgt ctggacgctg gacagggggc 240

aaaagcacct gctcgggtga ttctggg 267

<210> 428

<211> 171

<212> DNA

<213> Homo sapiens

<400> 428

tcacaaaagca agtagccaaa gccgttgcca aacccaccc ataaatcaat gggcccttta 60

tttatgacga ctttatttat tctaataatga ttttatagta tttatatata ttgggtcgtc 120

tgcttccctt gtatttttct tccttttttt gtaatatatga aaacgacgat a 171

<210> 429

<211> 80

<212> DNA

<213> Homo sapiens

<400> 429

tggtgatggt ttccacagga acaacagcat ctttcaccaa gatgggtggc accaaccttg 60

ctgggacttg gatcccaggg 80

<210> 430

<211> 226

<212> DNA

pctgb2016051825-seq1.txt

<213> Homo sapiens

<400> 430

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ccccgttcaa ggtatagtct gcctttaagg gagttttaga accaacaatgc aagacattga      60
aagaaatctt gcaagagcca ttattgactt agatccaaaa cagcctctct catgtctaaa      120
aaggcacaga attttgcaga tctgaggaag agggatgcat tacctttttg cttcttttca      180
attgcttagt gtttctaata atacttaata cacactaatg tgcgca                      226
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<210> 431

<211> 249

<212> DNA

<213> Homo sapiens

<400> 431

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aagagagaac aactactgtg atcaggctat gtatggaata cagtgttatt ttctttgaaa      60
ttgtttaagt gttgtaaata tttatgtaaa ctgcattaga aattagctgt gtgaaatacc      120
agtgtggttt gtgtttgagt tttattgaga attttaaatt ataacttaaa atattttata      180
atttttaaag tatatatatta ttttaagctta tgtcagacct atttgacata acactataaa      240
ggttgacaa                                                                249
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<210> 432

<211> 272

<212> DNA

<213> Homo sapiens

<400> 432

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acaggcagct cggattcaac taccttagat aatatttctg aaaacctacc agccagaggg      60
tagggcacaa agatggatgt aatgcacttt gggaggccaa ggcgggagga ttgcttgagc      120
ccaggagttc aagaccagcc tgggcaacat accaagaccc ccgtctcttt aaaaatatat      180
atattttaaa tatacttaaa tatatatatt taatatcttt aaatatatat atatatttta      240
aagaccaatt tatgggagaa ttgcacacag at                                    272
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<210> 433

<211> 261

pctgb2016051825-seq1.txt

<212> DNA

<213> Homo sapiens

<400> 433

tgcgcacctc cccttgagtg gaggccctct catgatcctt gacgaggaga gagagcttga	60
aaagctgttt cagctgggcc ccccttcacc tgtgaagatg ccctctccac catgggaatc	120
caatctgttg cagtctcctt caagcattct gtcgaccctg gatgttgaat tgccacctgt	180
ttgctgtgac atagatattt aaatttctta gtgcttcaga gtttgtgtgt atttgtatta	240
ataaagcatt cttcaacaga a	261

<210> 434

<211> 266

<212> DNA

<213> Homo sapiens

<400> 434

agtagtacca cgttgcaaaa tctgcaaatc ccagaagtcc acagcttgaa gacaacctcc	60
aggggcaggg aggaaaaaca ttgtcacaag agacataagc acacagaagg acaggcagct	120
gaggaaacca gtttgcaccg ccaactgacc aggataccta gcaagtaccc ttctcctcct	180
ggaatctact gctaccacct cgaaggtagt acttttttgg tctatgatct gaagaactct	240
tttcccaaag gaagagttgc tctgag	266

<210> 435

<211> 238

<212> DNA

<213> Homo sapiens

<400> 435

atgtgtgtct attttghtaac tgtaaagatg aatgtcagtt gttatttatt gaaatgattt	60
cacagtgtgt ggtcaacatt tctcatgttg aagctttaag aactaaaatg ttctaaatat	120
cccttggaaca ttttatgtct ttcttgtaag gcatactgcc ttgtttaatg ttaattatgc	180
agtgtttccc tctgtgttag agcagagagg tttcgatatt tattgatgtt ttcacaaa	238

<210> 436

pctgb2016051825-seq1.txt

<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> none

<400> 436
tctttcatcc atacctgtgc atgagctgta ttcttcacag caacagagct cagttaaagt 60
caactgcaa 69

<210> 437
<211> 83
<212> DNA
<213> Artificial Sequence

<220>
<223> none

<400> 437
tgctcatctg tattgatgtc cttggagttc tacaagtgga acttaagtgt caaaaagaat 60
atgtggtttt tagctgagcg tgg 83

<210> 438
<211> 263
<212> DNA
<213> Homo sapiens

<400> 438
gtgacccatg accttgccgc atgaggcctg agggcatggt gtccagagtc ccagagcaga 60
tcaggcccca aagtcctgct ggacccccca gccaccgtga gtcctccgt gtggctaggg 120
agctgctgtc cagaggcgga ggtaaacatt gatccctcct gcacactcag ctctctcatg 180
gaagtcggag ccctcagggt cacctgaaaa ctctgacact acctttgcca ttcacctgtc 240
ccgtctccaa cattaaagct ttt 263

<210> 439
<211> 276
<212> DNA
<213> Homo sapiens

pctgb2016051825-seq1.txt

<400> 439
gaagctggag gcacaacgca ccagacactc acagcaagga tggagctgaa aacataaccc 60
actctgtcct ggaggcactg ggaagcctag agaaggctgt gagccaagga gggaggggtct 120
tcctttggca tgggatgggg atgaagtaag gagagggact ggacccccctg gaagctgatt 180
cactatgggg ggaggtgtat tgaagtcctc cagacaaccc tcagatttga tgatttccta 240
gtagaactca cagaaataaa gagctgttat actgtg 276

<210> 440
<211> 200
<212> DNA
<213> Homo sapiens

<400> 440
ggatattgaa cctggacaag agagagaagg aacacctccg atcgaagaac gtaaagtaga 60
aggtgattgc caggaaatgg atctggaaaa gactcggagt gagcgtggag atggctctga 120
tgtaaaagag aagactccac ctaatcctaa gcatgctaag actaaagaag caggagatgg 180
gcagccataa gttaaaaaga 200

<210> 441
<211> 81
<212> DNA
<213> Homo sapiens

<400> 441
cctctatgtc tctttgtgtc gctttcatta tgtctcttgg taactggctt cggttgtgtc 60
tctccgtgtg actattttgt t 81

<210> 442
<211> 174
<212> DNA
<213> Homo sapiens

<400> 442
attttgctat agctgcaatg ttcacagtaa tccaagttcc aatggcaggg tggcagagtg 60
gaacccaaaag aggccaagtt ttgttgggtga agtataatta ttttttaaga tcagggattc 120

agaattgtag tatcagtgtt cagttaggaa gttcttatga tagtgcattg caca 174

<210> 443
 <211> 198
 <212> DNA
 <213> Homo sapiens

<400> 443
 attttgctat agctgcaatg ttcacagtaa tccaagttcc aatggcaggg tggcagagtg 60
 gaacccaaaag aggccaagtt ttgttggtga agtataatta ttttttaaga tcagggattc 120
 agaattgtag tatcagtgtt cagttaggaa gttcttatga tagtgcattg cacatgtcct 180
 ttccatcatc tgcatttt 198

<210> 444
 <211> 189
 <212> DNA
 <213> Homo sapiens

<400> 444
 aatttgagca ccaggattcc ccctcaggag aggttccagt taccaggaga cactggaacc 60
 tcagggtctat aaaaactgga ggggcagtga aggcctatta ttcacagcac ataagattct 120
 aggagacaga tttgggcagg gaagaggtgg gggagagggt tcattgcctc ccttaaatat 180
 gctacaatt 189

<210> 445
 <211> 41
 <212> DNA
 <213> Homo sapiens

<400> 445
 cttcatttat cttccagata gtagattcta ttaaccaagt t 41

<210> 446
 <211> 80
 <212> DNA
 <213> Homo sapiens

pctgb2016051825-seq1.txt

<400> 446
 aaatccttca tttatcttcc agatagtaga ttctattaac caagttgcat aaagagttct 60
 gattcccaga aggtaaagaa 80

<210> 447
 <211> 152
 <212> DNA
 <213> Homo sapiens

<400> 447
 atgctgataa aaataaccagt gttattaccg tatgtaaatc ttgtgtcatt ttctttcaag 60
 tgactgaact ggcctttgct taaacacttg acttctcagg aaacaaaaag ctaactccgg 120
 gtgtggggta aattgagaat cctgtggctg gc 152

<210> 448
 <211> 232
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <222> (27)..(27)
 <223> n is a, c, g, or t

<400> 448
 gaaatcctat gcctagaaac caattcnaca tgactgctat ttagggaagg cctaattctt 60
 gtaacttaca aagtgattga cagagtgggc tctgggttca aatcctgcct ctgtcacttc 120
 tagttgtgga atccttggca agtgtcttaa actctttatg ccattgagtt attattccca 180
 tctgttatta aagagattaa agtatatgcc tcagagggat gtttgagtgt aa 232

<210> 449
 <211> 231
 <212> DNA
 <213> Homo sapiens

<400> 449
 ccgtagctga agctgattct gtacagcggg acagcgcttt ctgcccctgg gggagcaacc 60

pctgb2016051825-seq1.txt

cctccctcgc ccctgggtcc tacggagcct gcactttcaa gaggtacagc ggcatcctgt	120
gggggcctgg gcaccgcagg aagactgcac agaaactttg ccattgttgg aacgggacgt	180
tgctccttcc ccgagcttcc ccggacagcg tactttgagg actcgctcag c	231

<210> 450
 <211> 289
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <222> (259)..(259)
 <223> n is a, c, g, or t

<400> 450 atctcaagcc agttcagatg ttgctgtttc ctcaagttgc agttaaaaca gagccaatga	60
gcagcagtga aacagcttca acgacagccg acgggtcttt aaacaatttc tcaggttcag	120
caattgggag cagtagtttc agcccacgac caactcacca attctctcca ccacagattt	180
acccttccaa cagaccatac ccacatattc tccctacccc ttcctcacia actatggctg	240
catatgggca aacacagtnt accacaggaa tgcaacaagc tacagccta	289

<210> 451
 <211> 274
 <212> DNA
 <213> Homo sapiens

<400> 451 aagtctgtga cccgtgggag ttgctgggga ggggcctggt ctgggccagg cctcttcttt	60
gggttgcccc ctcacacact ctggatcatcc aggaagcctc tgtggccctc ggaatgtgta	120
aaagatgggtg gttttaggag ctgctgggtt tgcaactgag tgctgacggc ttatttcttg	180
ttaatgattc tgtcaaaaagt gtcttagcga tgctcttcag cccttcatca cccacccaaa	240
aaagattaaa gtggattctt catcagctat tttta	274

<210> 452

pctgb2016051825-seq1.txt

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<211> 37
<212> DNA
<213> Homo sapiens

<400> 452
tcttctgggg actatgtcct agatactcct tgaattt 37

<210> 453
<211> 282
<212> DNA
<213> Homo sapiens

<400> 453
aatctgcggg agtcttcatt ttcttccaac acagagatgt aaaatggaat attcttctga 60
cgcagatatt tcatgtatcg caccatcact cgtgccatgt tgcctgtcct tcggtatttt 120
tccatgctgg aggccattcc tacttcacaa gaagggtcca tggttaccca tgagaccggg 180
actccctctg ggccgagcat acaggctgct ggcaggctctt ctatgcagcg cttgatgtaa 240
tggcagctcc tctcattctt ccctcgcttt cagttgtcat tt 282

<210> 454
<211> 146
<212> DNA
<213> Homo sapiens

<400> 454
gaatgatctg tgaatcaagc agctcctaga accaaaaatag gttcagagcc gctctccttg 60
gttttttttg tcaacacgca gagcaagttg gctacctccc ttcatatgct cgcctcatag 120
tattcaaaga cagctatcat ggccac 146

<210> 455
<211> 165
<212> DNA
<213> Homo sapiens

<400> 455
gaatgatctg tgaatcaagc agctcctaga accaaaaatag gttcagagcc gctctccttg 60
gttttttttg tcaacacgca gagcaagttg gctacctccc ttcatatgct cgcctcatag 120

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tattcaaaga cagctatcat ggccacatta aatcttgggtt tcttt 165

<210> 456

<211> 268

<212> DNA

<213> Homo sapiens

<400> 456

tcatgctaag actggaacta gacataatga aacatcagag ccagctaaga gaaaagaaat 60

atttgaggga aattgaaagt gtggaaaaaa agaatgataa tcttttaaag ggtctacaac 120

tgaatgagct caccatggat gatgatactg ccgtgctcgt cattgacaac ggctctggca 180

tgtgcaaggc cggctttgca ggtgacgatg ccccccgggc tgtcttcctt tccatcgtgg 240

ggtgccccag gcaccagaac atgatggg 268

<210> 457

<211> 137

<212> DNA

<213> Homo sapiens

<400> 457

ttttacactc tctcttcacc tttcaagttt gtttggctct tttgaataat cccagtcagc 60

tggtggtgaa catctctgca atgatcagtg caggtttccc aactgtcagc cgctttcttc 120

tcatgagtgg agactcc 137

<210> 458

<211> 114

<212> DNA

<213> Homo sapiens

<400> 458

tgtccttctg tgtgcttatg tctcttgtga caattgtttt cctccctgcc cctggagggtt 60

gtcttcaagc tgtggacttc tgggatttgc agattttgca acgtggtact actt 114

<210> 459

<211> 224

<212> DNA

<213> Homo sapiens

pctgb2016051825-seq1.txt

<400> 459
caagagtaac tctgacttag gaatagagac agccagagag aaatgtgac aatgaaggag 60
acatctggag tgtgcgtgct tcttcagagg gacgggtgat gggcagattg gaaaaagcac 120
cgcagatggg aaccttaatc tttcttttct aaaattgatg ctatgaaaat ttgcgttttc 180
tgtaacttgt aaaaaactaaa agttgcttgt ctactgaaaa aaaa 224

<210> 460
<211> 232
<212> DNA
<213> Homo sapiens

<400> 460
ttatatatca acctgtaaac atgtgcctgt aacttacttc caaaaacaaa atcataactta 60
ttagaagaaa attctgattt tatagaaaaa aaatagagca aggagaatat aacatgtttg 120
caaagtcatg tgttttcttt ctcaatgagg gaaaaacaat tttattacct gcttaatggg 180
ccacctggaa ctaaaaggga tactattttc taacaaggta tatctagtag gg 232

<210> 461
<211> 156
<212> DNA
<213> Homo sapiens

<400> 461
ttattgtcat gtgttgcttg cttgtatag ttgacttcat tgtctggata actatacaac 60
ctgaaaactg tcatttcagg ttctgtgctc tttttggagt cttaaagctc agtattagtc 120
tattgcagct atctcgtttt catgctaaaa tagttt 156

<210> 462
<211> 249
<212> DNA
<213> Homo sapiens

<400> 462
cattaggtga aatcgcaaaa aatatttctt tagaaacata agcagaatct taaagtatat 60
tttcatataa cataatttga tattctgtat tactttcact gttaaattct cagagtatta 120

pctgb2016051825-seq1.txt

tttggacgg catgaaaaat taaaatttcg gtcattgttt agagacagtg gagggtaaaat	180
ctgtggcctaa ttctgttggc cgtttgtatt ataaatgtaa aatagtattc cagctattgt	240
gcaatatgt	249

<210> 463
 <211> 136
 <212> DNA
 <213> Homo sapiens

<400> 463 cggacagccc tgctcgaaga tatagaaaga gtgacctgca caaacttgga attttttttc	60
catacaaccc tgacccattt actgtatttt ttttaatgaa atatgtgaat gataataaaa	120
gttgctgact taaaaa	136

<210> 464
 <211> 137
 <212> DNA
 <213> Homo sapiens

<400> 464 tgatgtccgg acagccctgc tcgaagatat agaaagagtg acctgcacaa acttggaatt	60
ttttttccat acaaccctga cccatttact gtattttttt taatgaaata tgtgaatgat	120
aataaaaagtt gctgact	137

<210> 465
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 465 gatatcccct tggatttcac ttgca	25

<210> 466
 <211> 25

<212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 466
 agggatatcc ccttggattt cactt 25

<210> 467
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 467
 aaaatggagg gatatcccct tggat 25

<210> 468
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 468
 tttcacttgc attgtgcaat aagca 25

<210> 469
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 469
 ggatatcccc ttggatttca cttgc 25

<210> 470
 <211> 25

<212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 470
 agaataggta taggaaagac tgaat 25

<210> 471
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 471
 atggagggat atccccttgg atttc 25

<210> 472
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 472
 aaatggaggg atatcccctt ggatt 25

<210> 473
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 473
 gaataggtat aggaaagact gaata 25

<210> 474
 <211> 25

<212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 474
 gagggatatc cccttggatt tcact 25

<210> 475
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 475
 ttcctctata aagaataggt atagg 25

<210> 476
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> none

<400> 476
 agtgaccaga agaaatgacg cagaa 25

<210> 477
 <211> 25
 <212> DNA
 <213> Artificial Sequence

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摘要

用于表征和/或预测受试者中癌症的方法包括确定来自受试者的样品中至少一种，优选 12 种选自表 1 的基因的表达水平，其中所述确定的表达水平用于提供癌症的表征和/或预测。确定的表达水平用于生成标记分数。所述方法允许转移性疾病被识别和监测，并指导治疗干预。