

DETECTION OF BIOMARKERS FOR NON-SMALL CELL LUNG CANCER

Field of Invention

The invention relates to the detection of biomarkers for Non-Small Cell Lung Cancer
5 (NSCLC).

Background

Despite technological advances in the area of proteomics research, there are only a handful of biomarkers that have entered the clinic, and 90% of the biomarkers are
10 protein biomarkers. Autoantibody biomarkers as described herein are autoantibodies to antigens, autoantibodies being antibodies which are produced by an individual which are directed against one or more of the individual's own proteins ('self' antigens). Some of the main reasons for failure of biomarkers to make it into clinical practice are:

- 1) Low sensitivity and specificity of diagnosis of cancerous diseases
- 15 2) Low prognostic/predictive value
- 3) Not important for clinical decision making
- 4) Original claims fail validation (false discoveries)

For Non-small cell Lung Cancer (NSCLC), although many individual proteins have
20 been reported to aid diagnosis and prognosis, very few have demonstrated sufficient value to be introduced into clinical use. Furthermore, many protein biomarkers discovered in the serum/plasma samples seem to overlap with other diseases, especially other cancers and inflammatory diseases.

25 An aim of the invention therefore is to provide an improved panel of autoantibody biomarkers for the detection of Non-Small Cell Lung Cancer.

Summary of Invention

In one aspect of the invention, there is provided a method for diagnosing Non-Small
30 Cell Lung Cancer from a sample extracted from a subject, comprising the steps of:

- (i) testing the sample for the presence of autoantibody biomarkers specific for Non-Small Cell Lung Cancer;

- (ii) determining whether the subject has Non-Small Cell Lung Cancer based on the detection of said autoantibody biomarkers;

characterised in that the biomarkers are autoantibodies to antigens comprising XAGE1D, LRRFIP2 and GAGE2C.

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Advantageously the autoantibody biomarkers can be used in the diagnosis of non-small cell lung cancer.

10 In one embodiment the sample is tested using a panel of antigens that correspond to the autoantibody biomarkers. Typically the antigens are biotinylated proteins. Advantageously the biotinylation ensures that the antigens are folded in their correct form to ensure accuracy of detection by the autoantibody biomarkers.

15 In one embodiment the antigens further comprise one or more of DDX53, DDX43, GAGE1, MAGEA10, ZNRD1, MAP2K5, MAGEA4, STAT1, CT47A1, IGF2BP3, CTAG2, RAD23B, FADD, PTPN20A, TPM1, CTAG1A.

20 It should be noted that not all antigens generate an autoantibody response and it is not possible to predict *a priori* which antigens will do so in a given cancer patient cohort – of more than 1600 antigens tested, only autoantibodies against the 19 antigens described above are suitable as biomarkers in NSCLC. Advantageously some of the 19 antigens are recognised by autoantibody biomarkers even when the well-known EGFR test for NSCLC is negative.

25 In one embodiment each biotinylated protein is formed from a Biotin Carboxyl Carrier Protein (BCCP) folding marker which is fused in-frame with the protein.

In one embodiment the biotinylated proteins are bound to a streptavidin-coated substrate.

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Advantageously full-length proteins are expressed as fusions to the BCCP folding marker which itself becomes biotinylated *in vivo* when the fusion partner is correctly folded. By comparison misfolded fusion partners cause the BCCP to remain in the ‘apo’

(i.e. non-biotinylated) form such that it cannot attach to a streptavidin substrate. Thus only correctly folded fusion proteins become attached to the streptavidin substrate via the biotin moiety appended to the BCCP tag.

- 5 In one embodiment the substrate comprises a glass slide, biochip, strip, slide, bead, microtitre plate well, surface plasmon resonance support, microfluidic device, thin film polymer base layer, hydrogel-forming polymer base layer, or any other device or technology suitable for detection of antibody-antigen binding.
- 10 In one embodiment the substrate is exposed to a sample extracted from a person, such that autoantibody biomarkers from the sample may bind to the antigens.

Typically the sample comprises any or any combination of exosomes, blood, serum, plasma, urine, saliva, amniotic fluid, cerebrospinal fluid, breast milk, semen or bile.

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Advantageously as exosomes contain membrane-bound proteins that reflect their originating cell, and in cancer have been shown to be implicated in the crosstalk between tumour cells and normal cells thereby facilitating the malignant process, exosomes have been found to be promising as enriched sources of diagnostic and prognostic markers. The exosomal autoantibody biomarkers detected using the BCCP folding marker technology are therefore potentially superior compared to the majority of serological biomarkers identified using conventional approaches.

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In one embodiment following exposure to the sample, the substrate is exposed to a fluorescently-tagged secondary antibody to allow the amount of any autoantibodies from the sample bound to the antigens on the panel to be determined. Typically the secondary antibody is anti-human IgG, but it will be appreciated that other secondary antibodies could be used, such as anti-IgM, anti-IgG1, anti-IgG2, anti-IgG3, anti-IgG4 or anti-IgA.

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In one embodiment the presence of non-small cell lung cancer corresponds to the relative or absolute amount of autoantibodies from the sample specifically binding to the antigens.

In one embodiment the method is performed *in vitro*.

In a further aspect of the invention, there is provided a method for manufacturing a kit
 5 for diagnosing Non-Small Cell Lung Cancer from a sample extracted from a subject, comprising the steps of:

for each antigen in a panel, cloning a biotin carboxyl carrier protein folding marker in-frame with a gene encoding the antigen and expressing the resulting biotinylated antigen;

10 binding the biotinylated antigens to addressable locations on one or more streptavidin-coated substrates, thereby forming an antigen array;

such that the amount of autoantibodies from the sample binding to the antigens on the panel can be determined by exposing the substrate to the sample and measuring the response;

15 characterised in that the antigens comprise XAGE1D, LRRFIP2 and GAGE2C.

In one embodiment the antigens further comprise one or more of DDX53, DDX43, GAGE1, MAGEA10, ZNRD1, MAP2K5, MAGEA4, STAT1, CT47A1, IGF2BP3, CTAG2, RAD23B, FADD, PTPN20A, TPM1, CTAG1A.

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In one embodiment the method comprises detecting upregulation/downregulation of one or more autoantibody biomarkers. Thus the method can be used for monitoring the response of a subject undergoing chemo/targeted/immuno-therapy for lung cancer and stratifying the subjects based on their autoantibody profile.

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In a further aspect of the invention there is provided a method for detecting non-small cell lung cancer by exposing a composition comprising a panel of antigens as herein described to a sample extracted from a person, and determining the level of autoantibodies from the sample binding to the antigens.

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In a yet further aspect of the invention there is provided a method for diagnosing non-small cell lung cancer by exposing a composition comprising a panel of antigens as

herein described to a sample extracted from a person *in vitro*, and determining the level of autoantibodies from the sample binding to the antigens.

5 In further aspect of the invention, there is provided a composition comprising a panel of antigens for detecting non-small cell lung cancer, characterised in that the antigens comprise XAGE1D, LRRFIP2 and GAGE2C

10 In one embodiment the antigens further comprise one or more of DDX53, DDX43, GAGE1, MAGEA10, ZNRD1, MAP2K5, MAGEA4, STAT1, CT47A1, IGF2BP3, CTAG2, RAD23B, FADD, PTPN20A, TPM1, CTAG1A.

In one embodiment the antigens are biotinylated proteins

15 In one embodiment the amount of one or more exosomal autoantibody biomarkers binding *in vitro* to the antigens in a sample from a patient can be measured to determine the presence of non-small cell lung cancer.

In yet further aspect of the invention, there is provided a composition comprising a panel of exosomal autoantibody biomarkers for detecting non-small cell lung cancer;
20 wherein the level of one or more exosomal autoantibody biomarkers are measured in a sample from NSCLC patient;

characterised in that the one or more exosomal autoantibody biomarkers is selected from autoantibodies specific for one or more of X Antigen Family Member 1D (XAGE1D), LRR Binding FLII Interacting Protein 2 (LRRFIP2) and G Antigen 2C
25 (GAGE2C).

Brief Description of Drawings

30 It will be convenient to further describe the present invention with respect to the accompanying drawings that illustrate possible arrangements of the invention. Other arrangements of the invention are possible, and consequently the particularity of the accompanying drawings is not to be understood as superseding the generality of the preceding description of the invention.

Figure 1 illustrates the structure of the *E. coli* Biotin Carboxyl Carrier Protein domain.

Figure 2 is a graph illustrating variable importance scores across 19 biomarkers
5 identified from the NSCLC study.

Figure 3 illustrates ROC curves for 19 biomarkers.

Figure 4 is a graph illustrating levels of the autoantibody biomarkers at different stages
10 of NSCLC.

Figure 5 illustrates a comparison of the levels of the core biomarker panel (XAGE1D, LRRFIP2 and GAGE2C) with respect to EGFR status in patients.

Figure 6 illustrates the overall autoantibody profiles of the core set of biomarkers in
15 three different stages of NSCLC.

Figure 7 illustrates the distinct molecular signatures for the 19 shortlisted biomarkers
for NSCLC patients.

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Figure 8 illustrates the pPRO9 plasmid used as a vector.

Figure 9 illustrates the ROC curve for the 19 shortlisted biomarkers for NSCLC
(XAGE1D, DDX53, GAGE2C, LRRFIP2, GAGE1, DDX43, MAGEA10, ZNRD1,
25 STAT1, MAP2K5, MAGEA4, IGF2BP3, FADD, RAD23B, CT47A1, CTAG2,
PTPN20A, TPM1, CTAG1A).

Detailed Description

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Materials and Methods

Gene synthesis and cloning. The pPRO9 plasmid (see Figure 8 below) was constructed by standard techniques and consists of a c-myc tag and BCCP protein domain, preceded by a multi-cloning site. A synthetic gene insert was assembled from synthetic oligonucleotides and/or PCR products. The fragment was cloned into pPRO9 using SpeI and NcoI cloning sites. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The final construct was verified by sequencing. The sequence congruence within the used restriction sites was 100%. 5µg of the plasmid preparation was lyophilized for storage.

The recombinant baculoviruses are generated via co-transfection of a bacmid carrying the strong viral polyhedrin promoter together with a transfer vector carrying the coding sequences of protein of interest, into the Sf9 cell line which is a clonal isolate derived from the parental *Spodoptera frugiperda* cell line IPLB-Sf-21-AE. Homologous recombination initiated by the viral system causes the transfected cells to show signs of viral cytopathic effect (CPE) within few days of culture incubation. The most common CPE observed was the significantly enlargement of average cell size, a consequences of viral progeny propagation. These baculoviruses known as P0 were then released into the culture medium, and viral amplification were done to generate a higher titre of P1 viruses.

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Protein Expression. Expressions were carried out in 24 well blocks using 3ml cultures containing 6×10^6 Sf9 cells per well. High titre, low passage, viral stocks of recombinant baculovirus ($>10^7$ pfu/ml) were used to infect sf9 insect cells. The infected cells were then cultured for 72 hours to allow them to produce the recombinant protein of interest.

The cells were washed with PBS, resuspended in buffer, and were frozen in aliquots at -80°C ready for lysis as required. Depending on the transfer vector construct and the nature of the protein itself, recombinant protein lysate can be pelleted either from the cultured cell or the cultured medium. Positive recombinant proteins were then analyzed via SDS-PAGE and Western blot against Streptavidin-HRP antibody. In total, 1630 human antigens were cloned and expressed using this methodology.

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Array fabrication. HS (hydrogel-streptavidin) slides were purchased from Schott and used to print the biotinylated proteins. A total of 9 nanoliters of crude protein lysate was

printed on a HS slide in quadruplicate using non-contact piezo printing technology. Print buffer that have a pH between 7.0 and 7.5 were used. The slides were dried by centrifugation (200 x g for 5 min) before starting the washing and blocking. The printed arrays were blocked with solutions containing BSA or casein (concentration: 0.1 mg/ml) in a phosphate buffer. The pH was adjusted to be between 7.0 and 7.5 and cold solutions were used (4 °C - 20 °C). Slides were not allowed to dry between washes, and were protected from light. In total, each resultant 'Immunome array' comprised 1630 antigens, each printed in quadruplicate.

10 Experimental Procedure. Each critical experimental step of running the Immunome array required a second trained person to thoroughly check, precisely record and cross-check all steps in the protocol, in order to reduce operator bias. Samples were picked, randomised and assigned to assay racks accordingly. These samples were then stored at -20°C until the experimental setup was complete.

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1. Study cohort

A cohort comprising of 66 males and 40 females between the age of 29 and 85 were employed for the study. The subjects were selected across more than 6 ethnicities diagnosed with different types of lung cancer including adenocarcinoma, squamous cell carcinoma, non-small cell lung carcinoma, large cell carcinoma and other types of lung malignancies. A total of 31 patients were diagnosed with early stage lung cancer while 78 patients were diagnosed with late stage lung cancer. A total of 33 subjects were smoker and 44 subjects were non-smoker. EGFR was tested positive in 30 subjects and negative in 39 subjects. A total of 100 samples from age and gender-matched healthy subjects were also collected.

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2. Serum/Plasma Dilution

Samples were then placed in a shaking incubator set at +20°C to allow thawing for 30 minutes. When completely thawed, each sample was vortexed vigorously three times at full speed and spun down for 3 minutes at 13,000 rpm using a microcentrifuge. 22.5 µL of the sample was pipetted into 4.5 mL of Serum Assay Buffer (SAB) containing 0.1%

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v/v Triton, 0.1% w/v BSA in PBS (20°C) and vortexed to mix three times. The tube was tilted during aspiration to ensure that the sera was sampled from below the lipid layer at the top but does not touch the bottom of the tube in case of presence of any sediment. This Serum/Plasma dilution process was carried out in a class II Biological safety cabinet. Batch records were marked accordingly to ensure that the correct samples were added to the correct tubes.

3. Biomarker Assay

The array was removed from the storage buffer using forceps, placed in the slide box and rack containing 200 mL cold SAB and shaken on an orbital shaker at 50 rpm, for 5 minutes. When the slides have completed washing, the slide was placed, array side up, in a slide hybridization chamber with individual sera which had been diluted earlier. All slides were scanned using the barcode scanner into the relevant batch record and incubated on a horizontal shaker at 50 rpm for 2 hours at 20°C.

4. Array Washing After Serum Binding

The protein array slide was then rinsed twice in individual "Pap jars" with 30 mL SAB, followed by 200 mL of SAB buffer in the slide staining box for 20 minutes on the shaker at 50 rpm at room temperature. All slides were transferred sequentially and in the same orientation.

5. Incubation with Cy3-anti IgG

Binding of autoantibodies to the arrayed antigens on replica Immunome arrays was detected by incubation with Cy3-rabbit anti-human IgG (Dako Cytomation) labelled according to the manufacturer's recommended protocols (GE Healthcare). Arrays were immersed in hybridization solution containing a mixture of Cy3- rabbit antihuman IgG solution diluted 1:1000 in SAB buffer for 2 hours at 50 rpm in 20°C.

6. Washing After Incubation with Cy3-anti IgG

After incubation, the slide was dipped in 200 mL of SAB buffer, 3 times for 5 minutes at 50 rpm at room temperature. Excess buffer was removed by immersing the slide in 200 mL of pure water for a few minutes. Slides were then dried for 2 min at 240g at room temperature. Slides were then stored at room temperature until scanning

(preferably the same day). Hybridization signals were measured with a microarray laser scanner (Agilent Scanner) at 10µm resolution. Fluorescence intensities were detected according to the manufacturer's instructions, whereby each spot is plotted using Agilent Feature Extraction software.

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Spot segmentation Semi-automatic QC process was carried out in order to produce a viable result. The output from the microarray scanner is a raw .tiff format image file. Extraction and quantification of each spot on the array were performed using the

10 GenePix Pro 7 software (Molecular Devices). A GAL (GenePix Array List) file for the array was generated to aid with image analysis. GenePix Pro 7 allows for automatic spot gridding and alignment of each spot on the array for data extraction. Following data extraction, a GenePix Results (.GPR) file was generated for each slide which contains numerical information for each spot; Protein ID, protein name, foreground intensities,

15 background intensities etc.

Bioinformatics analysis.

20 Image Analysis: Raw Data Extraction

The aim of an image analysis is to evaluate the amount of autoantibody present in the serum sample by measuring the median intensities of all the pixels within each probed spot. A raw .tiff format image file is generated for each slide, i.e. each sample. Automatic extraction and quantification of each spot on the array are performed using

25 the GenePix Pro 7 software (*Molecular Devices*) which outputs the statistics for each probed spot on the array. This includes the mean and median of the pixel intensities within a spot along with its local background. A GAL (GenePix Array List) file for the array is generated to aid with image analysis. This file contains the information of all probed spots and their positions on the array. Following data extraction, a GenePix

30 Results (.GPR) file is generated for each slide which contains the information for each spot; Protein ID, protein name, foreground intensities, background intensities etc. In the data sheet generated from the experiment, both foreground and background intensities of each spot are represented in relative fluorescence units (RFUs).

Data Handling and Pre-processing

For each slide, proteins and control probes are spotted in quadruplicate - 4 arrays on each slide. The following steps were performed to verify the quality of the protein array data before proceeding with data analysis:

Step 1:

Calculate net intensities for each spot by subtracting background signal intensities from the foreground signal intensities of each spot. For each spot, the background signal intensity was calculated using a circular region with three times the diameter of the spot, centered on the spot.

Step 2:

Remove replica spots with $RFU \leq 0$.

Step 3:

Zero net intensities if only 1 replica spot remaining.

Step 4:

Calculating percentage of coefficient of variant (CV%) of to determine the variations between the replica spots on each slide.

$$CV\% = \frac{S.D.}{Mean} \times 100\% \quad \text{Equation 1}$$

Flag a set of replica spots with only 2 or less replica/s remaining and $CV\% > 20\%$ as “High CV”. The mean RFU of these replica spots (i.e. proteins) will be excluded from the downstream analysis.

For proteins/controls with a $CV\% > 20\%$ and with 3 or more replica spots remaining, the replica spots which result in this high CV% value were filtered out. This was done by calculating the standard deviation between the median value of the net intensities and

individual net intensities for each set of replica spots. The spot with the highest standard deviation was removed. CV% values were re-calculated and the process repeated.

Step 5:

- 5 Calculating the mean of the net intensities for the remaining replica spots.

Step 6:

Inspecting signal intensities of two positive controls: IgG and Cy3-BSA.

10 Step 7:

Composite normalisation of data using both quantile-based and total intensity-based modules. This method assumes that different samples share a common underlying distribution of their control probes while taking into account the potential existence of flagged spots within them. The Immunome array uses Cy3-labelled biotinylated BSA (Cy3-BSA) replicates as the positive control spots across slides. Hence it is considered as a housekeeping probe for normalisation of signal intensities for any given study.

The quantile module adopts the algorithm described by Bolstad *et al.*, 2003. This reorganisation enables the detection and handling of outliers or flagged spots in any of the Cy3BSA control probes. A total intensity-based module was then implemented to obtain a scaling factor for each sample. This method assumes that post-normalisation, the positive controls should have a common total intensity value across all samples. This composite method aims to normalise the protein array data from variations in their measurements whilst preserving the targeted biological activity across samples. The steps are as follows:

Quantile-Based Normalisation of all cy3BSA across all samples

($i = \text{spot number}$ and $j = \text{sample number}$)

1. Load all Cy3-BSA across all samples, j , into an $i \times j$ matrix X
2. Sort spot intensities in each column j of X to get X_{sort}
3. Take the mean across each row i of X_{sort} to get $\langle X_i \rangle$

Intensity-Based Normalisation

1. Calculate sum of the mean across each row i , $\sum < X_i >$
2. For each sample, k , calculate the sum of all Cy3-BSA controls, $\sum X_k$
3. For each sample, k ,

$$\text{Scaling factor (k)} = \frac{\sum < X_i >}{\sum X_k} \quad \text{Equation 2}$$

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Data Analysis

High concentrations of an arrayed protein may occasionally give a “false” positive signal in serology assays because of concentration-driven, non-selective binding of an immunoglobulin to the target. This can arise theoretically due to an avidity effect: weak, non-specific immunoglobulin binding sites on a specific protein becoming coupled across multiple neighbouring protein molecules via an antibody as a result of the high density of immobilized protein, thus making the protein appear to be highly antigenic. Whenever this phenomenon occurs, it would be expected to be observed in the healthy control samples and will give rise to high intensity signals and/or signals that are close to saturation on the arrays. In Sengenics Immunome, proteins such as RBPJ and IGHG1 show consistently high signal intensities across all samples.

For this reason, given a large sample number (i.e. 100 – 200 samples) and availability of sample cohort, a penetrance-based fold change (pFC) analysis method is implemented for the identification of highly expressed proteins in each case sample. This method will remove any false positive signals from the data by setting a protein-specific threshold (i.e. background threshold). This defined per-protein background threshold is calculated based on the signal intensities for each specific protein measured for a given cohort of healthy control samples. A step-by-step description of this method is as follows:

25 Step 1:

Individual fold changes for both case and control are calculated by dividing the RFU value for each protein in each sample, H , by the mean of the RFU values of each protein across all the control samples (i.e. background threshold).

$$\text{Individual FC} = \frac{H_{\text{Case or Control}}}{\mu(H_{\text{Control}})} \quad \text{Equation 3}$$

Step 2:

For proteins with individual fold change of less than 2 fold above the background threshold, their signal intensities (RFU) are replaced with zeroes.

5 Step 3:

Penetrance frequency (number of case and control samples with individual fold changes ≥ 2 fold) for both case ($Frequency_{Case}$) and control ($Frequency_{Control}$) are determined for each protein along with their difference.

$$Frequency_{Case} = n(\text{Individual FC (Case)}) \geq 2 \quad \text{Equation 4}$$

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$$Frequency_{Control} = n(\text{Individual FC (Control)}) \geq 2 \quad \text{Equation 5}$$

$$Frequency_{diff} = Frequency_{Case} - Frequency_{Control} \quad \text{Equation 6}$$

Step 4:

15 Penetrance Fold Changes for both case and control groups are calculated for each protein.

$$Penetrance\ Fold\ Change_{case} = \frac{\mu(H_{Case}[i])}{\mu(H_{Control})} \quad \text{Equation 7}$$

$$Penetrance\ Fold\ Change_{control} = \frac{\mu(H_{Control}[i])}{\mu(H_{Control})} \quad \text{Equation 8}$$

$$H_{Case}[i] = H_{Case} \text{ with FC Case } \geq 2 \text{ fold}$$

$$H_{Control}[i] = H_{Control} \text{ with FC Control } \geq 2 \text{ fold}$$

20 Putative biomarkers are identified and ranked according to the following criteria:

1. $Penetrance\ Fold\ Change_{Case} \geq 2$.

2. $\% Frequency_{Case} \geq 10\%$

$\% Frequency\ differential \geq 10\%$

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The invention utilises the Biotin Carboxyl Carrier Protein (BCCP) folding marker which is cloned in-frame with the gene encoding the protein of interest, as described above and in EP1470229. The structure of the *E. coli* BCCP domain is illustrated in Figure 1, wherein residues 77-156 are drawn (coordinate file 1bdo) showing the N- and C- termini and the single biotin moiety that is attached to lysine 122 *in vivo* by biotin ligase.

BCCP acts not only as a protein folding marker but also as a protein solubility enhancer. BCCP can be fused to either the N- or C-terminal of a protein of interest. Full-length proteins are expressed as fusions to the BCCP folding marker which becomes biotinylated *in vivo*, but only when the protein is correctly folded. Conversely, misfolded proteins drive the misfolding of BCCP such that it is unable to become biotinylated by host biotin ligases. Hence, misfolded proteins are unable to specifically attach to a streptavidin-coated solid support. Therefore only correctly folded proteins become attached to a solid support via the BCCP tag.

The surface chemistry of the support is designed carefully and may use a three-dimensional thin film polymer base layer (polyethylene glycol; PEG), which retains protein spot morphologies and ensures consistent spot sizes across the array. The PEG layer inhibits non-specific binding, therefore reducing the high background observed using other platforms. The solid support used to immobilize the selected biomarkers is thus designed to resist non-specific macromolecule adsorption and give excellent signal-to-noise ratios and low limits of detection (i.e. improved sensitivity) by minimising non-specific background binding. In addition the PEG layer also preserves the folded structure and functionality of arrayed proteins and protein complexes post-immobilisation. This is critical for the accurate diagnosis because human serum antibodies are known in general to bind non-specifically to exposed hydrophobic surfaces on unfolded proteins, thus giving rise to false positives in serological assays on arrays of unfolded proteins, moreover, human autoantibodies typically bind to discontinuous epitopes, so serological assays on arrays of unfolded proteins or misfolded proteins will also give rise to false negatives in autoantibody binding assays.

As biotinylated proteins bound to a streptavidin-coated surface show negligible dissociation, this interaction therefore provides a superior means for tethering proteins to a planar surface and is ideal for applications such as protein arrays, SPR and bead-based assays. The use of a compact, folded, biotinylated, 80 residue domain BCCP
5 affords two significant advantages over for example the AviTag and intein-based tag. First, the BCCP domain is cross-recognised by eukaryotic biotin ligases enabling it to be biotinylated efficiently in yeast, insect, and mammalian cells without the need to co-express the *E. coli* biotin ligase. Second, the N- and C-termini of BCCP are physically separated from the site of biotinylation by 50Å (as shown in Figure 1), so the BCCP
10 domain can be thought of as a stalk which presents the recombinant proteins away from the solid support surface, thus minimising any deleterious effects due to immobilisation.

The success rate of BCCP folding marker mediated expression of even the most complex proteins is in excess of 98%. The technology can therefore be applied in a
15 highly parallelised pipeline resulting in high-throughput, highly consistent production of functionally validated proteins..

The addition of BCCP permits the monitoring of fusion protein folding by measuring the extent of *in vivo* biotinylation. This can be measured by standard blotting
20 procedures, using SDS-PAGE or *in situ* colony lysis and transfer of samples to a membrane, followed by detection of biotinylated proteins using a streptavidin conjugate such as streptavidin-horseradish peroxidase. Additionally, the fact that the BCCP domain is biotinylated *in vivo* is particularly useful when multiplexing protein purification for fabrication of protein arrays since the proteins can be simultaneously
25 purified from cellular lysates and immobilised in a single step via the high affinity and specificity exhibited by a streptavidin surface.

The biomarkers of the present invention can be used in early diagnosis of NSCLC, patient stratification and treatment monitoring. This includes any distinguishable
30 manifestation of the condition, including not having NSCLC. The test can determine the presence or absence of NSCLC in a patient, the risk of developing NSCLC, the stage or severity of NSCLC and the effectiveness or response to treatment of NSCLC. Based on

this status, further medical procedures may be indicated, including additional diagnostic tests or therapeutic procedures or regimens.

- 5 The microarray prototype and final product can be multiplexed far beyond the technical capability of other immunoassay systems and will enable exquisitely sensitive and specific testing of patients and high-risk population for NSCLC.

- 10 The power of a diagnostic test to correctly predict status is commonly measured as the sensitivity and specificity of the assay or the area under a receiver operated characteristic (“ROC”) curve. Sensitivity is the percentage of true positives that are correctly predicted to be positive, while specificity is the percentage of true negatives that are correctly predicted to be negative. The greater the area under the ROC curve, the higher the prediction power of the test.

- 15 Autoantibody biomarkers were determined by Penetrance fold change method where age matched controls are considered as baseline to observe the elevated frequencies (≥ 2 Foldchange) of individual biomarkers in lung cancer patients. The list of the biomarkers identified here for diagnosis of NSCLC is shown in Table 1.

Table 1

Protein	Penetrance Frequency (NSCLC)	Penetrance Frequency % (NSCLC)	Mean Penetrance (NSCLC)	Penetrance Fold Change (NSCLC)	Penetrance Frequency (Control)	Penetrance Frequency % (Control)	Mean Penetrance (Control)	Penetrance Fold Change (Control)	Frequency Differential	Frequency % Differential	Penetrance Fold Change Difference	Mean (Control)
XAGE1D	26	23.85321	24654.94	9.678011	4	4	10338.27	4.058168	22	19.85321	5.619843	2547.522
PTPN20A	17	15.59633	12505.05	3.883074	4	4	12011.96	3.729957	13	11.59633	0.153117	3220.401
TPM1	15	13.76147	16940.47	2.845931	6	6	25399.96	4.267092	9	7.761468	-1.42116	5952.523
CTAG1A	14	12.84404	23341.53	10.43956	4	4	10141.22	4.535687	10	8.844037	5.903878	2235.872
RAD23B	14	12.84404	8193.595	3.231442	5	5	9467.406	3.733816	9	7.844037	-0.50237	2535.585
ZNRD1	14	12.84404	12647.16	2.988961	4	4	12658.28	2.991589	10	8.844037	-0.00263	4231.29
CTAG2	13	11.92661	20739.05	8.484654	5	5	10221.57	4.181798	8	6.926606	4.302857	2444.301
LRRFIP2	13	11.92661	16271.97	3.374414	9	9	11729.15	2.432342	4	2.926606	0.942072	4822.161
MAGEA10	13	11.92661	7393.198	3.156872	5	5	7423.496	3.169809	8	6.926606	-0.01294	2341.938
STAT1	13	11.92661	21774.47	5.037478	9	9	11512.56	2.663407	4	2.926606	2.374071	4322.495
DDX43	12	11.00917	10360.57	4.337159	2	2	8368.541	3.503251	10	9.009174	0.833908	2388.793
GAGE1	12	11.00917	12540.95	5.515388	2	2	7578.361	3.33289	10	9.009174	2.182498	2273.81
GAGE2C	12	11.00917	11572.43	5.342098	3	3	7604.188	3.510266	9	8.009174	1.831832	2166.271
MAGEA4	12	11.00917	9790.552	4.88818	3	3	7559.253	3.774148	9	8.009174	1.114032	2002.903
MAP2K5	11	10.09174	10511.43	3.676394	2	2	6913.192	2.417902	9	8.091743	1.258492	2859.17
FADD	10	9.174312	18237.53	5.423004	6	6	11120.35	3.306683	4	3.174312	2.116321	3362.994
IGF2BP3	9	8.256881	14223.89	6.351528	2	2	6903.872	3.082851	7	6.256881	3.268677	2239.444
CT47A1	8	7.33945	15453.54	6.885753	6	6	9374.437	4.177039	2	1.33945	2.708714	2244.278
DDX53	7	6.422018	30847.36	11.16542	4	4	17135.53	6.202328	3	2.422018	4.963095	2762.758

To evaluate the sensitivity of individual biomarkers towards lung cancer, ROC and area under the curve (AUC), 95% confidence intervals and also likelihood ratios were calculated, as set out in Table 2. Variable ranking was performed by using all combination of 19 biomarkers as separate panels and each panel was subjected to recursive feature elimination by generating random forests. The biomarkers were ranked based on random forest estimated variable importance measure derived from each panel (see Figure 2). Mean variable importance scores determine three core set of biomarkers which are common across all biomarker panels. i.e. XAGE1D, LRRFIP2 and GAGE2C.

10 **Table 2**

Protein	AUC	Confidence Interval (95% CI)	Likelihood Ratio		Variable Importance	Rank based on Importance
			LR-Positive	LR-Negative		
XAGE1D	0.696	[0.622-0.77]	7.75	0.593	7.39	1
LRRFIP2	0.56	[0.48-0.641]	3.068	0.735	4.37	2
GAGE2C	0.612	[0.534-0.691]	13.321	0.713	3.98	3
DDX53	0.635	[0.557-0.714]	3.229	0.614	3.81	4
DDX43	0.641	[0.564-0.719]	2.601	0.588	3.67	5
GAGE1	0.638	[0.562-0.715]	4.239	0.676	3.61	6
MAGEA10	0.651	[0.574-0.728]	3.817	0.598	3.28	7
ZNRD1	0.573	[0.493-0.653]	1.462	0.665	2.9	8
MAP2K5	0.65	[0.573-0.726]	4.037	0.654	2.9	9
MAGEA4	0.654	[0.577-0.73]	3.176	0.553	2.5	10
STAT1	0.554	[0.472-0.635]	2.745	0.776	2.5	11
CT47A1	0.623	[0.544-0.702]	2.382	0.594	2.39	12
IGF2BP3	0.621	[0.542-0.699]	2.295	0.644	2.3	13
CTAG2	0.619	[0.54-0.698]	2.637	0.664	2.04	14
RAD23B	0.59	[0.51-0.67]	2.758	0.722	1.45	15
FADD	0.602	[0.522-0.681]	3.633	0.737	1.38	16
PTPN20A	0.605	[0.525-0.684]	2.327	0.682	1.08	17
TPM1	0.607	[0.527-0.686]	2.22	0.641	0.4	18
CTAG1A	0.625	[0.547-0.703]	2.653	0.687	0.08	19

A total of 19 potential autoantibody biomarkers have been identified for diagnosis of NSCLC; namely XAGE1D, PTPN20A, TPM1, CTAG1A, RAD23B, ZNRD1, LRRFIP2, STAT1, MAGEA10, CTAG2, GAGE1, GAGE2C, DDX43, MAGEA4,

MAP2K5, FADD, IGF2BP3, CT47A1, DDX53. The Uniprot IDs, description, nucleotide sequence and protein sequence are set out in Table 3 below.

5 The Immunome array contains >1630 antigens, presented on the array surface in a folded, functional form, as described above. Notably, it would not have been obvious *a priori* which specific 19 antigens out of the collection of >1630 antigens that were tested would give rise to a measurable autoantibody response that is diagnostic for NSCLC.

10 In this panel of 19 antigens that correspond to the 19 autoantibody biomarkers, CTAG2 is observed in 25-50% of tumor samples of melanomas, non-small-cell lung carcinomas, bladder, prostate and head and neck cancers. CTAG1A is a tumor cell antigen found in various types of cancers, which makes it a good candidate for a cancer vaccine.

15 ZNRD1 contains two potential zinc-binding motifs and may play a role in regulation of cell proliferation. The encoded protein may be involved in cancer and human immunodeficiency virus progression.

20 XAGE1D and MAGEA4 RNA markers have been considered for use in screening of lung neoplasia for detecting presence of lung cancer. In normal tissues, XAGE1D is highly expressed in testis, highly expressed in breast cancer, prostate cancer and many types of lung cancers, including squamous cell carcinoma, small cell carcinoma, non-small cell carcinoma, and adenocarcinoma, as well as in Ewings cell lines, in some Ewings sarcoma patient samples, and in one of one alveolar rhabdomyosarcoma patient
25 sample. MAGEA4 is expressed in many tumors of several types, such as melanoma, head and neck squamous cell carcinoma, lung carcinoma and breast carcinoma, but not in normal tissues except for testes and placenta.

30 LRRFIP2 is involved in the Wnt signalling pathway and aberrant Wnt signalling underlies a wide range of pathologies in humans. It has been suggested that the Wnt signalling pathway has important functions in stem cell biology, cardiac development and differentiation, angiogenesis, cardiac hypertrophy, cardiac failure and ageing (Rao

& Kuhl, 2010). GAGE2C belongs to a family of genes that are expressed in a variety of tumors but not in normal tissues, except for the testis.

PTPN20A is present in many cell lines (at protein level) and is widely expressed. TPM1 is detected in primary breast cancer tissues but undetectable in normal breast tissues in Sudanese patients. Isoform 1 is expressed in adult and fetal skeletal muscle and cardiac tissues, with higher expression levels in the cardiac tissues. Isoform 10 is expressed in adult and fetal cardiac tissues, but not in skeletal muscle.

DDX53, STAT1 and FADD expression levels were elevated in late stage group. DDX53 is a cancer-testis antigen that shows wide expression in many tumours. DDX53 has been reported to interact with EGFR and bind to the promoter sequences of EGFR. Signal transducer and activator of transcription (STAT) 1 is part of the (JAK)/STAT signalling cascade and is best known for its essential role in mediating responses to all types of interferons (IFN). A correlation of STAT1 protein expression levels with poor prognosis, increased invasive and metastatic potential has been reported in three breast cancer studies (Meissl *et al.*, 2017). It was concluded that STAT1 can promote tumour progression, and therefore, it can be a potential marker or indicator of cancer progression (Meissl *et al.*, 2017). Phosphorylation of FADD promotes KRAS induced lung cancer (Bowman *et al.*, 2015). Fas-associated death domain protein (FADD) is the key adaptor molecule transmitting the apoptotic signal delivered by death receptors. It was also reported that the release of FADD by human NSCLC correlates positively with both tumour progression and aggressiveness and could be a new marker of poor prognosis (Cimino *et al.*, 2012).

25

Cancer-testis antigens are a family of >1000 highly developmentally restricted fetal proteins (Wang *et al.*, 2016; Silva *et al.*, 2017) that are silenced in all somatic tissues except the testes and occasionally placenta, but which can be aberrantly expressed in cancerous tissues, thereby driving an autoantibody response. The Immunome array contains 202 cancer-testis antigens, presented on the array surface in a folded, functional form as described above. Notably, the 19 autoantibody biomarkers of non-small cell lung cancer identified here are significantly enriched for cancer-testis antigens, yet it would not have been obvious *a priori* which specific 10 cancer-testis

antigens out of the collection of 202 that were tested would give rise to a measurable autoantibody response that is diagnostic for NSCLC.

5 The best method to verify a lung cancer diagnosis involves a multiple biomarker approach rather than a single biomarker approach.

As illustrated in Figure 3, Receiver operating characteristic (ROC) curves were calculated based on individual fold changes for 19 biomarkers to show sensitivity of biomarkers towards lung cancer patients. Area under curve (AUC), 95% confidence
10 intervals (CI) and Optimal cutoff of individual fold change (Cutoff (IFC) for each were calculated based on the method described in López-Ratón *et al.*, (2014) using “OptimalCutpoints” R package. CI and Optimal cut-off values help to determine diagnostic ability of the biomarkers by showing positive or negative test results with lung cancer patients. The ROC curve for the panel of 19 biomarkers is illustrated in
15 Figure 9.

Figure 4 shows the autoantibody biomarkers determined by Penetrance fold change (pFC) method where age matched controls are considered as baseline to observe the elevated frequencies (≥ 2 Foldchange) of individual biomarkers in lung cancer patients.
20 The data was generated from profiling of Normalised RFU values of 19 biomarkers identified by pFC method across healthy controls (Ctrl), Early stage lung cancer patients (Early) and Late stage lung cancer patients (Late) .

Figure 5 shows a comparison of the core autoantibody biomarker panel (autoantibodies
25 against antigens XAGE1D, LRRFIP2 and GAGE2C) levels with respect to EGFR status in patients. The data was generated from comparing normalised RFU values of the three core set of biomarkers (identified based on variable ranking using random forest) across healthy controls (Control), Early stage lung cancer patients (Earlystg) and Late stage lung cancer patients (Latestg). Patient cohorts were sub-divided based on EGFR
30 mutation status i.e. Positive (patients with EGFR mutation), negative (patients without EGFR mutation), unknown (patients with unknown EGFR mutation status). An overall elevation of the antigen-specific autoantibody levels was observed in late stage NSCLC compared to early stage NSCLC and control.

A similar observation is seen in Figure 6, wherein the data was generated from comparing normalised RFU values of the three core set of biomarkers (identified based on variable ranking using random forest) against healthy controls (Ctrl), Early stage lung cancer patients (Early) and Late stage lung cancer patients (Late).

Furthermore, the elevation of antigen-specific autoantibody levels in late stage NSCLC is independent of EGFR status of patients. In addition, with further reference to Figure 7, distinct differences in autoantibodies against XAGE1D, CTAG1A, CTAG2, GAGE1 and GAGE2C were observed in Late and Early stage NSCLC compared to the control. Unsupervised clustering of individual fold changes across all healthy controls (Control), Early stage lung cancer patients (EarlyStg) and Late stage lung cancer patients (LateStg) for 19 biomarkers identified by pFC method. Clustering was performed for biomarkers based on Ward's method and distance calculated based on Euclidean distance. The shaded bar on the top of the heatmap represents patient cohorts i.e. Control, Early stage and Late Stage samples.

Extracellular vesicles can be divided into three main categories, namely apoptotic bodies, microvesicles, and exosomes. Exosomes are the smallest extracellular vesicles which are naturally secreted by almost every cell type and can be found in almost all biological fluids including blood, serum, urine, saliva, amniotic fluid, cerebrospinal fluid, breast milk, semen and bile. In general, cells release exosomes via two mechanisms. The classic pathway involves the formation of intraluminal vesicles within multivesicular endosomes. In turn, the membrane of multivesicular endosomes fuses with the plasma membrane, resulting in the release of intraluminal vesicles. When secreted, intraluminal vesicles are called exosomes. Alternatively, the direct pathway involves the release of vesicles, indistinguishable from exosomes, directly from the plasma membrane (van der Pol *et al.* 2012). Interestingly, exosomes from cancer cells have been shown to promote angiogenesis, modulate the immune system and remodel the surrounding parenchymal tissue, all factors supporting tumor progression (Hessvik and Llorente, 2018).

Exosome samples from patients with NSCLC and healthy controls were collected and isolated using Invitrogen Total Exosome Isolation (*from plasma*) kit (Thermo Fisher Scientific.) based on the established protocol from the manufacturer.

- 5 The discovery of these autoantibody biomarkers using exosome samples can be more disease-specific and meaningful as they contain membrane-bound proteins that reflect their originating cell. In cancer, exosomes have been shown to be implicated in the crosstalk between tumour cells and normal cells thereby facilitating the malignant process. Several studies have found exosomes to be promising as diagnostic and
10 prognostic markers (Sanfeld-Paulsen *et al.*, 2016).

It will be appreciated by persons skilled in the art that the present invention may also include further additional modifications made to the system which does not affect the overall functioning of the system.

15

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- www.genecards.org

Table 3

Protein Name	UniprotID	Description
XAGE1D	Q9HD64	>P003055_Q211_Q211_tube_XAGE1_9503_0_NM_020411.2_0_Q9HD64_0_ Insert sequence is gene optimized by GeneArt_0_0_0
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GATGGACCCCCCAACCTGAGGAAGTCAAGACCCCGAAGAGGGCGAAGGCCAGTCCCAGTGC

Protein Sequence (Seq ID No. 25):

MSWRGRSTYYWPRPRRYQPPEMIGPMRPEQFSDEVEPATPEEGEPATQRQDPAAQEGEDEGASAGQGPKPE
ADSQEQGHPQTGCECEDGPDGQEMDPPNPEEVKTPPEEMRSHYVAQTGILWLLMNNCFNLSPRKP

MAGEA10 P43363 >P003053_Q211_Q211_tube_MAGEA10_4109_0_NM_001011543.1_0_P43363_0_Insert sequence is gene optimized by GeneArt_0_0_0

Nucleotide Sequence (Seq ID No. 7):

ATGCCCCGTGCTCCCAAGCGTCAGCGTTGCATGCCCGAAGAGGACCTGCAGTCCCAGTCCGAGACTCAGGGC
CTCGAGGGTGCTCAGGCTCCCCCTGGCTGTGGAAGAGGACGCTTCCAGCTCTACCTCTACCTCCTCCAGCTTCC
CCAGCAGCTTCCATCCTCCAGCTCCTCTAGCTCCTCCTGCTACCCCTGATCCCTCCACCCCGAGGA
AGTGTCCGCTGACGACGAGACTCCCAACCCCCCAGTCCGCTCAGATCGCTTGTCTCTCCCTCCGTGGTG
GCTTCCCTGCCTCTGGACCAGTCCGACGAGGGTCCAGCTCCCAGAAGGAAGAGTCCCCCAGCACCTGCAG
GTCCTGCCCCGACTCCGAGTCCCTGCCCCGTTCCGAGATCGACGAGAAGGTTACAGACCTGGTGCAGTTCCTGC
TGTTCAAGTACCAGATGAAGGAACCCATCACCAGGCTGAGATCCTCGAGTCCGTGATCAAGAACTACGAGGA
CCACTTCCCCCTGCTGTTCTCCGAGGCTTCCGAGTGCATGCTGCTGGTGTTCGGTATCGACGTGAAGGAAGTG
GACCTACCGGTCACTCCTTCGTGCTGGTCACTCCTGGGCCTGACCTACGACGGCATGCTGTCCGACGTGC
AGTCCATGCCAAGACCGGTATCTGATCCTCATCTGTCCATCATCTTCATCGAGGGCTACTGCACTCCTGAG
GAAGTATCTGGGAGGCTCTGAACATGATGGCCTGTACGACGGAATGGAACACCTGATCTACGGCGAGCCC
CGCAAGCTGCTGACCCAGGACTGGGTGCAAGAGAACTACCTCGAGTACCGTCAGGTGCCCGGTTCCGACCCC
GCTCGTTACGAGTTCTGTGGGGTCCCCGTGCTCAGCTGAGATCCGCAAGATGTCCCTGCTGAAGTTCCTGG
CTAAGGTCAACGGCTCCGACCCCGTTCCCTCCACTGTGGTACGAGGAAGCTCTGAAGGACGAGGAAGAGA
GGGCTCAGGACCGTATCGCTACCAACGACGACACCAACCGCTATGGCTTCCGCTTCTCTAGCGCTACCGGTTCC
CTTCAGCTACCCCGAG

Protein Sequence (Seq ID No. 26):

MPRAPKRQRCMPPEEDLQSQSETQGLEGAQAPLAVEEDASSSTSTSSSFSSFPSSSSSSSSSCYPLIPSTPEEVSA
DDETPNPPQSAQIACSSPSVVASLPLDQSDEGSSSQKEESPSTLQVLPDSESLPRSEIDEKVTDLVQFLLFKYQMK
PITKAEILESVIRNYEDHFPLLFSEASECMLLVFGIDVKEVDPTGHSFVLVTSGLTYDGMLSDVQSMPKTGILILISIV
IEGYCTPEEVIWEALNMMGLYDGMEHLIYGEPRKLLTQDWVQENYLEYRQVPGSDPARYEFLWGPRAHAEIRKMSL
LKFLAKVNGSDPRSFPLWYEEALKDEEERAQDRIATDDTTAMASASSSATGSFSYPE

ZNRD1 Q9P1U0 >P000875_TRN_TRNp2_ZNRD1_30834_Homo sapiens zinc ribbon domain
containing 1 transcript variant
b_BC010898.1_AAH10898.1_Q9P1U0_0_0_381_0_378

Nucleotide Sequence (Seq ID No. 8):

ATGTCTGTCATGGACCTCGCCAATACTTGCTCCAGCTTTCAGTCGGACCTGGATTTCTGTTTCAGATTGCGGCTC
GGTCTGCCTCTGCCCGGGGCTCAGGATACGGTCACTGTATTGCTGTGGCTTCAACATCAACGTTCCGGGAC
TTTGAGGGGAAGTTGTGAAGACTTCGGTTGTGTTCCACCAACTGGGGACAGCCATGCCTATGTCGGTGGAGG
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ACACCAGACAGATGCGTTAGCCGATGAAGGGCAAAGTGTCTTCTACACCTGTACCAACTGCAAGTTCAGGA
GAAGGAAGACTCT

Protein Sequence (Seq ID No. 27):

MSVMDLANTCSSFQSDLDGSDCGSVLPLPGAQDVTVCIRCGFNINVRDFEGKVVKTSVVFHQLGTAMPMSVEEGP
ECQGPVVDRCPRCGHEGMAYHTRQMRSADEGQTVFYTCTNCKFQEKEDS

MAP2K5 Q13163 >P000081_KIN96_KIN_MAP2K5_5607_Homo sapiens mitogen-activated protein kinase kinase 5, transcript variant A_BC008838.2_AAH08838.1_Q13163_4111679_0_1347_0_1344

Nucleotide Sequence (Seq ID No. 9):

ATGCTGTGGCTAGCCCTTGGCCCTTTCTGCCATGGAGAACCAGGTGCTGGTAATTCGCATCAAGATCCCAAA
TAGTGGCGCGGTGGACTGGACAGTGCACCTCCGGGCCGCAGTTACTCTTCAGGGATGTGCTGGATGTGATAGG
CCAGGTTCTGCCTGAAGCAACAACACAGCATTTGAATATGAAGATGAAGATGGTGATCGAATTACAGTGAGAA
GTGATGAGGAAATGAAGGCAATGCTGTCATATTATTATCCACAGTAATGGAACAGCAAGTAAATGGACAGTTAA
TAGAGCCTCTGCAGATATTTCCAAGAGCCTGCAAGCCTCCTGGGGAACGGAACATACATGGCCCTGAAGGTGAA
TACTCGGGCCGGACCCTCTCAACACAGCAGCCAGCAGTCTCAGATTCACTTCCAAGCAATAGCTTAAAGAAGT
CTTCTGCTGAACTGAAAAAATACTAGCCAATGGCCAGATGAATGAACAAGACATACGATATCGGGACACTCTT
GGTCATGGCAACGGAGGCACAGTCTACAAAGCATATCATGTCCCGAGTGGGAAAAATTAGCTGTAAAGGTCAT
ACTACTAGATATTACACTGGAACCTCAGAAGCAAATTATGTCTGAATTGGAAATCTTTATAAGTGCGATTTCATCA
TATATCATTGGATTTTATGGAGCATTTTTGTAGAAAACAGGATTTCATATGTACAGAATTCATGGATGGGGGAT
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ATTTGTGGAGTTTAAAGATTTTACATAGAGACGTGAAGCCCTCCAATATGCTAGTAAACACAAGAGGACAGGTTA
AGCTGTGTGATTTTGGAGTTAGCACTCAGCTGGTGAATTCTATAGCCAAGACGTATGTTGGAACAAATGCTTATA
TGGCGCTGAAAGGATTTAGGGGAGCAGTATGGAATTCATTCTGATGTCTGGAGCTTAGGAATCTCTTTTATG
GAGCTTGCTCTTGGGAGGTTTCCATATCCTCAGATTGAGAAAAACAGGGATCTTAAATGCCTCTCCAGCTTCTG
CAGTGCATTGTTGATGAGGATTCGCCCGTCTTCCAGTTGGAGAGTTCTCGGAGCCATTTGTACATTTTCATCAC
TCAGTGTATGCGAAAAACAGCCAAAAGAAAGGCCAGCACCTGAAGAATTGATGGGCCACCCGTTTCATCGTGCAG
TTCAATGATGGAAATGCCGCCGTGGTGTCCATGTGGGTGTCCCGGGCGCTGGAGGAGAGGCGGAGCCAGCAG
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Protein Sequence (Seq ID No. 28):

MLWLALGPFAMENQVLVIRIKIPNSGAVDWTVHSGPQLLFRDVLVDVIGQVLPEATTTAFEYEDDGDRTVRSDEEM
KAMLSYYYSTVMEQQVNGQLIEPLQIFPRACKPPGERNIHGLKVNTRAGPSQHSSPAVSDSLPSNSLKKSSAELKKIL
ANGQMNEQDIRYRDLGHGNGGTVYKAYHVPKGILAVKILLDITLQKQIMSEILELYKCDSSYIIGFYGAFFVENR
ISICTEFMDGGSGLDVYRKMPHVLGRIAVAVVKGLTYLWSLKILHRDVKPSNMLVNTRGQVKLCDFGVSTQLVNSIAK
TYVGTNAYMAPERISGEQYGIHSDVWSLGLISFMELALGRFPYPQIQKNQGSMLPLQLLQCIVDEDDSPVLPVGEFSEPF
VHFITQCMRKQPKERPAPPEELMGHPFIVQFNDGNAAVVSMMWVCRALEERRSQGGP

MAGEA4 P43358 >P001216_CAG_CAGp1_MAGEA4_4103_Homo sapiens melanoma antigen family A 4_BC017723.1_AAH17723.1_P43358_0_0_954_0_951

Nucleotide Sequence (Seq ID No. 10):

ATGTCTTCTGAGCAGAAGAGTCAGCACTGCAAGCCTGAGGAAGGCGTTGAGGCCCAAGAAGAGGCCCTGGGC
CTGGTGGGTGCACAGGCTCCTACTACTGAGGAGCAGGAGGCTGCTGTCTCCTCCTCCTCCTCTGGTCCCTG
GCACCCTGGAGGAAGTGCCTGCTGCTGAGTCAGCAGGTCTCCCCAGAGTCTCAGGGAGCCTCTGCCTTAC
CCACTACCATCAGCTTCACTTGTGAGGCAACCAATGAGGGTTCCAGCAGCCAAGAAGAGGAGGGGCCAA
GCACCTCGCCTGACGCAGAGTCCTTGTCCGAGAAGCACTCAGTAACAAGGTGGATGAGTTGGCTCATTTTCTG
CTCCGCAAGTATCGAGCCAAGGAGCTGGTCACAAAGGCAGAAATGCTGGAGAGAGTCATCAAAAATTACAAGC
GCTGCTTTCTGTGATCTTCGGCAAAGCCTCCGAGTCCCTGAAGATGATCTTTGGCATTGACGTGAAGGAAGTG
GACCCACCAGCAACACCTACACCTTGTACCTGCCTGGGCCTTTCTATGATGGCCTGCTGGGTAATAATCA
GATCTTTCCCAAGACAGGCCTTCTGATAATCGTCTGGGCACAATTGCAATGGAGGGCGACAGCGCCTCTGAG
GAGGAAATCTGGGAGGAGCTGGGTGTGATGGGGGTGATGATGGGAGGGAGCACACTGTCTATGGGGAGCCC
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CGCGCTATGAGTTCCTGTGGGGTCCAAGGGCTCTGGCTGAAACCAGCTATGTGAAAGTCTGGAGCATGTGGT
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Protein Sequence (Seq ID No. 29):

MSSEQSKQHCKPEEGVEAQEEALGLVGAQAPTTEEQEA AVSSSSPLVPGTLEEVPAAESAGPPQSPQGASALPTTI
SFTCWRQPNEGSSSQEEEGPSTSPDAESLFREALSNKVDLAHFLLRKYRAKELVTKAEMLERVIKYNKRCFPVIFG
KASESLKMIFGIDVKEVDPASNTYTLVTCGLSYDGLLGNQIFPKTGLLIIVLGTIAMEGDSASEEIIWELGVMGVYD
GREHTVYGEPRKLLTQDWVQENYLEYRQVPGSNPARYEFLWGPRLAETS YVKVLEHVVRVNARVRIAYPSLREAA
LLEEEEGV

STAT1	P42224	>P000068_KIN96_KIN_STAT1_6772_Homo sapiens signal transducer and activator of transcription 1 91kDa transcript varian_BC002704.2_AAH02704.1_P42224_93992.15_0_2139_0_2136
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Nucleotide Sequence (Seq ID No. 11):

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CAGATGTCTATGATCATTTACAGCTGTCTGAAGGAAGAAAGGAAAATTCTGGAAAACGCCAGAGATTTAATCAG
GCTCAGTCGGGGAATATTCAGAGCACAGTGATGTTAGACAAACAGAAAGAGCTTGACAGTAAAGTCAGAAATGT
GAAGGACAAGGTTATGTGTATAGAGCATGAAATCAAGAGCCTGGAAGATTTACAAGATGAATATGACTTCAAATG
CAAAACCTTGCAGAACAGAGAACACGAGACCAATGGTGTGGCAAAGAGTGATCAGAAACAAGAACAGCTGTTA
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ACTGAACCTACCCAGAATGCCCTGATTAATGATGAAGTGTGGAGTGAAGCGGAGACAGCAGAGCGCCTGTA
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ACAAACAAGTGTTATGGGACCGCACCTTCAGTCTTTCCAGCAGCTCATTGAGAGCTCGTTTGTGGTGGAAAGA
CAGCCCTGCATGCCAACGCACCCCTCAGAGGCCGCTGGTCTTGAAGACAGGGGTCCAGTTCACTGTGAAGTTGA
GACTGTTGGTGAAATTGCAAGAGCTGAATTATAATTTGAAAGTCAAAGTCTTATTTGATAAAGATGTGAATGAGA
GAAATACAGTAAAAGGATTTAGGAAGTTCAACATTTTGGGCACGCACACAAAAGTGATGAACATGGAGGAGTCC
ACCAATGGCAGTCTGGCGGCTGAATTTCCGGCACCTGCAATTGAAAGAACAGAAAAATGCTGGCACCAGAACGA
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GTAATTGACCTCGAGACGACCTCTCTGCCCGTTGTGGTGATCTCCAACGTCAGCCAGCTCCCGAGCGGTTGGG
CCTCCATCCTTTGGTACAACATGCTGGTGGCGGAACCCAGGAATCTGTCCTTCTTCTGACTCCACCATGTGCA
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GCTGAACATGTTGGGAGAGAAGCTTCTTGGTCTAACGCCAGCCCCGATGGTCTCATTCCGTGGACGAGGTTT
TGTAAGGAAAATATAAATGATAAAAAATTTCCCTTCTGGCTTTGGATTGAAAGCATCCTAGAACTCATTAAAAAC
ACCTGCTCCCTCTCTGGAATGATGGGTGCATCATGGGCTTCATCAGCAAGGAGCGAGAGCGTGCCTGTTGAA
GGACCAGCAGCCGGGGACCTTCTGCTGCGGTTCAGTGAGAGCTCCCGGGAAGGGGCCATCACATTCACATG
GGTGGAGCGGTCCCAGAACGGAGGCGAACCTGACTTCCATGCGGTTGAACCCTACACGAAGAAAGAACTTCT
GCTGTTACTTTCCCTGACATCATTGCAATTACAAAGTCATGGCTGCTGAGAATATTCCTGAGAATCCCCTGAAG
TATCTGTATCCAAATATTGACAAAGACCATGCCTTTGAAAGTATTACTCCAGGCCAAAGGAAGCACCAGAGCC
AATGGAACCTTGATGGCCCTAAAGGAACTGGATATATCAAGACTGAGTTGATTTCTGTGTCTGAAGTG

Protein Sequence (Seq ID No. 30):

MSQWYELQQLDSKFLEQVHQLYDDSFPMERQYLAQWLEKQDWEHAANDVSFATIRFHDLLSQLDDQYSRFSLEN
NFLQHNIRKSKRNLQDNFQEDPIQMSMIYSCLKEERKILENAQRFNQAQSGNIQSTVMLDKQKELDSKVRNVKDKV
MCIEHEIKSLEDLQDEYDFKCKTLQNREHETNGVAKSDQKQEQQLLLKKMYLMLDNKRKEVVHKIELLNVTELTQNALI
NDELVEWKRRQQSACIGGPPNACLDQLQNWFTIVAESLQQVRQQLKKLEELEQKYTYEHPITKNKQVLWDRTFSL
FQQLIQSSFVVERQPCMPHPQRPLVLKTGVQFTVKLRLLVLKQLNELNYNLKVVLFDKDVNERNTVKGFRKFNILGTH
TKVMNMEESTNGSLAAEFRHLQLKEQKNAGTRTNEGPLIVTEELHLSLSFETQLCQPLVIDLETSLPVVVVISNVSQL
PSGWASILWYNMLVAEPRNLSFFLTTPPCARWAQLSEVLSWQFSSVTKRGLNVDQLNMLGEKLLGPNASPDGLIPWT
RFCKENINDKNFPFWLWIESILELIKHLPLWNDGCMGFISKERERALLKDQPGTFLRFSESSREGAITFTWVER
SQNGGEPDFHAVEPYTKKELSAVTFPDIIRNYKVMAENIPENPLKYLIPNIDKDHAFGKYYSRPKEAPEPEMELDGPK
GTGYIKTELISVSEV

CT47A1 Q5JQC4 >P003099_Q211_Q211_tube_CT47A1_728096_0_NM_001080146.1_0_Q5JQC4_0_Insert sequence is gene optimized by GeneArt_0_0

Nucleotide Sequence (Seq ID No. 12):

ATGTCCGCTACCGGCGACCGTCACCCCTACCCAGGGCGACCAAGAGGCTCCCGTGTCCCAAGAGGGTGTCTCAG
GCTGAGGCTGCTGGTGTCTGGCAACCAGGAAGGTGGCGACTCCGGTCCCGACTCCTCCGACGTGGTGCCTGCT
GCTGAGGTCGTGGGTGTCTGCTGGTCTGTCTGAGGGCCTGGGAGAGGAAGAGGGCGAGCAGGCTGCTGGCCT
GGCTGCTGTGCCTCGTGGTGGTTCCGCTGAAGAGGACTCCGACATCGGTCCCGCTACCGAGGAAGAGGAAGA
AGAAGAGGGCAACGAGGCTGCTAACTTCGACCTGGCTGTGGTGGCTCGTCTTACCCCGCTTCCGGTATCCAC
TTCGTGCTGCTGGACATGGTGCACCTCCCTGCTGCACCGTCTGTCCACAACGACCACATCTGATCGAGAACC
GTCAGCTGTCCCGTCTGATGGTTCGACCCACGCTGCTGCTCGTAACCTGTGGGGCAACCTGCCCCCCTGCTG
TGCTGCCTCAACGCTCTGGGAGCTGGTGTCTGCTGCTAGGGCTGGCGAGGGACTGGGCCTGATCCAAGAGGCTG
CTTCCGTGCCCCGAGCCCGCTGTGCCTGCTGACTTGGCTGAGATGGCCCGCGAGCCTGCTGAGGAAGCTGCTG
AAGAGAAGCTGTCCGAGGAAGCCACCGAGGAACCCGACGCTGAGGAACCAGCTACTGAGGAACCCACCGCTC
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Protein Sequence (Seq ID No. 31):

MSATGDRHPTQGDQEAPVSQEGAQEAAGAGNQEGDSDVVPAAEVVGVAGPVEGLGEEEEGEQAAGLA
AVPRGSAEEDSDIGPATEEEEEEGNEAANFDLAVVARRYPASGIHFVLLDMVHSLHRLSHNDHILINRQLSRLM
VGPHAAARNLWGNLPPLLPRLGAGAAARAGEGLGLIQEAASVPEPAVPADLAEMAREPAEEAAEEKLSEATEE
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IGF2BP3 O00425 >P003224_Q311_Q311_tube_IGF2BP3_10643_0_NM_006547.2_0_O00425_0_Insert sequence is gene optimized by GeneArt_0_0

Nucleotide Sequence (Seq ID No. 13):

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GGCTCTGAAGGCTATCGAGGCTCTGTCCGGCAAGATCGAGCTGCACGGCAAGCCCATCGAGGTCGAGCACTC
CGTGCCCCAAGCGTCAGCGTATCCGCAAGCTGCAGATCCGTAACATCCCCCACACCTCCAGTGGGAGGTGCT
GGACTCCCTGCTGGTGCAGTACGGTGTCTGCGAGTCTGCGAGCAAGTGAACACCGACTCCGAGACTGCTGT
GGTCAACGTGACCTACTCCTCCAAGGACCAGGCTCGTCAGGCTCTGGACAAGCTGAACGGTTTCCAGCTCGAG
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CAGGTGGTGGTCAAGATCACCAGTCACTTCTACGCTTGCCAGGTGGCCAGCGCAAGATCCAAGAGATCTGA
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Protein Sequence (Seq ID No. 32):

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MAAQQNPLQQRGRRLGQQRGSSRQSGPSVSKQKPCDLPLRLVPTQFVGAIGKEGATIRNITKQTSKIDVHRK
ENAGAAEKSITILSTPEGTSAAKSILEIMHKEAQDIKFTTEIPLKILAHNNFVGRIGKEGRNLKKIEQDQDTKITISPLQE
LTLYNPRTITVKGNETCAKAEIEIMKKIRESYENDIASMNLQAHLIPGLNLNALGLFPPTSGMPPPTSGPPSAMTPP
YPQFEQSETETVHLFIPALSVGAIGKQGQHIKQLSRFAGASIKIAPAEAPDAKVRMVIITGPPEAQFKAQGRIYGKIKEE
NFVSPKEEVKLEAHIRVPSFAAGRVIGKGGKTVNELQNLSSAEVVPRDQTPDENDQVVVKITGHFYACQVAQRKIQ
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CTAG2 075638 >P001440_CAG_CAGp2_CTAG2_30848_Homo sapiens cancer/testis antigen
2 transcript variant 2_BC002833.2_AAH02833.1_075638_0_0_633_0_630

Nucleotide Sequence (Seq ID No. 14):

ATGCAGGCCGAAGGCCGGGGCACAGGGGGTTCGACGGGGCGATGCTGATGGCCCAGGAGGCCCTGGCATTCC
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CGCAGGATGGAAGGTGCCCTGCGGGGCCAGGAGGCCGGACAGCCGCTGCTTGAGTTGCACATCACGATG
CCTTTCTCGTCGCCCATGGAAGCGGAGCTGGTCCGCAGGATCCTGTCCCGGGATGCCGCACCGCTCCCCGA
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GATCTCAGAACACCCAAACACAAGGTCTCAGAACAGAGACCTGGTACACCAGGCCCGCCGCCACCCGAGGGA
GCCAGGGAGATGGGTGCAGAGGTGTCGCTTTAATGTGATGTTCTCTGCCCTCACATT

Protein Sequence (Seq ID No. 33):

MQAEGQGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAARASGPRGGAPRGPHGGAASQA
DGRPCGARRPDSRLLQLHITMPFSSPMEAEVRRILSRDAAPLPRPGAVLKDFTVSGNLLFMSVRDQDREGAGRM
RVVGWGLGSASPEGQKARDLRTPKHVSEQRPGTPGPPPEGAQGDGCRGVAFNMFSAPI

RAD23B P54727 >P000309_SIG_SIG1-1_RAD23B_5887_Homo sapiens RAD23 homolog B (S.
cerevisiae)_BC020973.2_AAH20973.1_P54727_54173_0_1230_0_1227

Nucleotide Sequence (Seq ID No. 15):

ATGCAGGTCACCCTGAAGACCCTCCAGCAGCAGACCTTCAAGATAGACATTGACCCCCAGGAGACGGTGAAG
CACTGAAAGAGAAGATTGAATCTGAAAAGGGGAAAGATGCCTTTCCAGTAGCAGGTCAAAAATTAATTTATGCA
GGCAAAATCCTCAATGATGATACTGCTCTCAAAGAATATAAAATTGATGAGAAAACTTTGTGGTGGTTATGGTG
ACCAAACCAAGCAGTGTCCACACCAGCACCAGCTACAACCTCAGCAGTCAGCTCCTGCCAGCACTACAGCAG
TTACTTCTCCACCACCACAACCTGTGGCTCAGGCTCCAACCCCTGTCCCTGCCTTGGCCCCACTTCCACACCT
GCATCCATCACTCCAGCATCAGCGACAGCATCTTCTGAACCTGCACCTGCTAGTGCAGCTAAACAAGAGAAGCC
TGCAGAAAAGCCAGCAGAGACACCAGTGGCTACTAGCCCAACAGCAACTGACAGTACATCGGGTGATTCTTCT
CGGTCAAACCTTTTTGAAGATGCAACGAGTGCATTGTGACGGGTCACTTACGAGAATATGGTAACTGAGAT
CATGTCAATGGGCTATGAACGAGAGCAAGTAATTGCAGCCCTGAGAGCCAGTTTCAACAACCCCTGACAGAGCA
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GTACTGGGGTTCCTCAGTCTTCAGCAGTGGCTGCAGCTGCAGCAACTACGACAGCAACAACCTACAACAACAAG
TTCTGGAGGACATCCCCTTGAATTTTACGGAATCAGCCTCAGTTTCAACAGATGAGACAAATTATTCAGCAGAA
TCCTTCTTGTCTCCAGCGTTACTACAGCAGATAGGTCGAGAGAATCCTCAATTACTTCAGCAAATTAGCCAACA
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Protein Sequence (Seq ID No. 34):

MQVTLKTLQQQTFKIDIDPEETVKALKEKIESEKKGDAFPVAGQKLIYAGKILNDDTALKEYKIDEKNFVVMVTKPKAV
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QAVVDPPQAASTGAPQSSAVAAAAATTTATTTTSSGGHLEFLRNQPFQMRQIIQQNPSSLPALQLQIGRENPD
LLQISQHQEHFIQMLNEPVQEAGGQGGGGGGSGGIAEAGSGHMNYIQVTPQEKEAIERLKALGFPEGLVIQAYFA
CEKNENLAANFLLQQNFDED

FADD Q13158 >P000417_SIG_SIG1-2_FADD_8772_Homo sapiens Fas (TNFRSF6)-
associated via death
domain_BC000334.2_AAH00334.1_Q13158_34265.45_0_627_0_624

Nucleotide Sequence (Seq ID No. 16):

ATGGACCCGTTCCCTGGTGCTGCTGCACTCGGTGTCGTCCAGCCTGTCGAGCAGCGAGCTGACCGAGCTCAAG
TTCCTATGCCCTCGGGCGCGTGGGCAAGCGCAAGCTGGAGCGCGTGCAGAGCGGCCTAGACCTCTTCTCCATG
CTGCTGGAGCAGAACGACCTGGAGCCCGGGCACACCGAGCTCCTGCGCGAGCTGCTCGCCTCCCTGCGGCG
CCACGACCTGCTGCGGCGCGTCGACGACTTCGAGGCGGGGGCGGCGGCCGGGGCCGCGCTGGGGAAGAA
GACCTGTGTGCAGCATTTAACGTCATATGTGATAATGTGGGAAAGATTGGAGAAGGCTGGCTCGTCAGCTCAA
AGTCTCAGACACCAAGATCGACAGCATCGAGGACAGATACCCCGCAACCTGACAGAGCGTGTGCGGGAGTC
ACTGAGAATCTGGAAGAACACAGAGAAGGAGAACGCAACAGTGGGCCACCTGGTGGGGCTCTCAGGTCCTG
CCAGATGAACCTGGTGGCTGACCTGGTACAAGAGGTTACGAGGCCCGTGACCTCCAGAACAGGAGTGGGGC
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Protein Sequence (Seq ID No. 35):

MDPFLVLLHSVSSSLSSSELTELKFLCLGRVGRKRLERVQSGLDLFSMLLEQNDLEPGHTELLRELLASLRRHDLRR
VDDFEAGAAAGAAPGEEDLCAAFNVICDNVKGDWRRRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRWKNTEKEN
ATVAHLVGALRSCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

PTPN20A Q4JDL3 >P003090_Q211_Q211_tube_PTPN20A_653129_0_NM_001042387.1_0_Q4
JDL3_0_Insert sequence is gene optimized by GeneArt_0_0_0

Nucleotide Sequence (Seq ID No. 17):

ATGTCTCCCCCGTGACTTCCGTGCTGAGCCCGTGAACGACTACGAGGGCAACGACTCCGAGGCTGAGGAC
CTGAACCTCCGTGAAACCCTGCCCTCCAGCTCCCAAGAGAACACCCCCGTTCCAAGGTGTTGAGAACAAGG
TCAACTCCGAGAAGGTCAAGCTGTCCCTGCGCAACTTCCCCACAACGATTACGAGGACGTGTTGAGGAACC
CTCCGAGTCCGGTTCGACCCCTCCATGTGGACCGCTCGTGGTCCCTTCCGTCGTGACCGTTGGTCTCCGAG
GACGAGGAAGCTGCTGGACCCTCCAGGCTCTGTCCCCCTGCTGTCCGACACCCGCAAGATCGTGTCCGAG
GGCGAGCTGGACCAGCTGGCTCAGATCCGTCCCCTGATCTTCAACTTCCACGAGCAGACCGCTATCAAGGACT
GCCTGAAGATCCTCGAGGAAAAGACCGCTGCTTACGACATCATGCAAGAGTTCATGGCTCTCGAGCTGAAGAA
CCTGCCCCGGCGAGTTCAACTCCGGCAACCAGCCCTCCAACCGCGAGAAGAACCGTTACCGTGACATCCTGCCT
TTCCAGCACCACGGTTACTCCGGTCCCAACGAGCGTACCACCTTCTGGCACGGTTCCAACGAGGGTGTGTGT
CCCTGCTGCTGCGCTACTGCGCT

Protein Sequence (Seq ID No. 36):

MSSPRDFRAEPVNDYEGNDSEADLNFRETLPSSSQENTPRSKVFENKVNSEKVKLSLRNFPNDYEDVFEPPSES
GSDPSMWTARGPFRRDRWSEDEEAAGPSQALSPLLSDTRKIVSEGELDQLAQIRPLIFNFHEQTAIKDCLKILEKT
AAYDIMQEFMALELKNLPGEFNSGNQPSNREKNRYRDILPYDSTRVPLGKSKDYINASYIRIVNCGEFYIATQGPLL
STIDDFWQMVLNNNSNVIAMITREIEGGIIKCYHYWPISLKKPLELKHFRVFLFENYQILQYFIIRMFQVVEKSTGTSHSVK
QLQFTKWPDHGTPASADSFIKYIRYARKSHLTGPMVVHCSAGIGRTGVFLCVDVVFCAIVKNCSFNIMDIVAQMQREQ
RSGMVQTKEQYHFCYDIVLEVLRLKLLTD

TPM1 P09493 >P001454_CAG_CAGp2_TPM1_7168_Homo sapiens tropomyosin 1
(alpha)_BC007433.2_AAH07433.1_P09493_0_0_855_0_852

Nucleotide Sequence (Seq ID No. 18):

ATGGACGCCATCAAGAAGAAGATGCAGATGCTGAAGCTCGACAAGGAGAACGCCTTGGATCGAGCTGAGCAG
GCGGAGGCCGACAAGAAGGCGGCGGAAGACAGGAGCAAGCAGCTGGAAGATGAGCTGGTGTCACTGCAAAA
GAAACTCAAGGGCACCAGATGAAGTGGACAAATATTCTGAGGCTCTCAAAGATGCCAGGAGAAGCTGGAG
CTGGCAGAGAAAAAGGCCACCGATGCTGAAGCCGACGTAGCTTCTCTGAACAGACGCATCCAGCTGGTTGAGG
AAGAGTTGGATCGTCCCAGGAGCGTCTGGCAACAGCTTTGCAGAAGCTGGAGGAAGCTGAGAAGGCAGCAG
ATGAGAGTGAGAGAGGCATGAAAGTCATTGAGAGTCGAGCCCCAAAAGATGAAGAAAAATGGAAATTCAGGA
GATCCAAGTAAAGAGGCAAAAGCACATTGCTGAAGATGCCGACCGCAAATATGAAGAGGTGGCCCGTAAGCTG
GTCATCATTGAGAGCGACCTGGAACGTGCAGAGGAGCGGGCTGAGCTCTCAGAAGGCCAAGTCCGACAGCTG
GAAGAACAATTAAGAATAATGGATCAGACCTTGAAGCATTAATGGCTGCAGAGGATAAGTACTCGCAGAAGGA
AGACAGATATGAGGAAGAGATCAAGGTCCTTTCCGACAAGCTGAAGGAGGCTGAGACTCGGGCTGAGTTTGCG
GAGAGGTCAGTAACTAAATTGGAGAAAAGCATTGATGACTTAGAAGACGAGCTGTACGCTCAGAACTGAAGTA
CAAAGCCATCAGCGAGGAGCTGGACCACGCTCTCAACGATATGACTTCCATG

Protein Sequence (Seq ID No. 37):

MDAIKKMQMLKLDKENALDRAEQAEADKKAEDRSKQLEDELVSLQKKLKGTELDKYSEALKDAQEKLAEKK
ATDAEADVASLNRRILVEEELDRAQERLATALQKLEEAKEADESERGMKVIESRAQKDEEKMEIQEIQKEAKHIA
EDADRKYEEVARKLVIIESDLERAEEAELSEGKCAEEEEELKVTNNLKSLEAQAEKYSQKEDRYEEIEIKVLSDKLKE
AETRAEFAERSVTKLEKSIDDELYAQKLKYKAISEELDHALNDMTSI

CTAG1A P78358 >P003024_Q211_Q211_tube_CTAG1A_246100_0_NM_139250.1_0_P78358
_0_Insert sequence is gene optimized by GeneArt_0_0_0

Nucleotide Sequence (Seq ID No. 19):

ATGCAGGCTGAGGGTCGTGGCACCGGTGGTTCCACTGGCGACGCTGACGGTCCCGGTGGTCTGGTATCCCC
GACGGTCCTGGTGGCAACGCTGGTGGTCCAGGCGAGGCTGGTGCTACCGGTGGTCTGGTCTCGTGGTGCT
GGTGCTGCTCGTGCTTCCGGTCCAGGTGGTGGTGGTCTCCCGTGGTCTCACGGTGGTGCTGCTCCGGCCTG
AACGGTTGCTGCCGTTGCGGTGCTCGCGGTCCCGAGTCCCGTCTGCTCGAGTTCTACCTGGCTATGCCCTTCG
CTACCCCTATGGAAGCTGAGCTGGCTCGTCTCCCTGGCTCAGGACGCTCCTCTGCCCCGTGCCCGGTGT
CCTGCTGAAGGAGTTCACTGTCTCCGGCAACATCCTGACCATCCGTCTGACCGCTGCTGACCACCGTCAGCTC
CAGCTGTCCATCTCCTCATGCCTGCAGCAGCTGTCCCTGCTGATGTGGATACCCAGTGTTCCTTCCCCGTGTT
CCTGGCTCAGCCCCCTCCGGTCAACGTCGT

Protein Sequence (Seq ID No. 38):

MQAEGRGTTGGSTGDADGPGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAARASGPGGGAPRGPHGGAASGL
NGCCRCGARGPESRLLEFYLA MPFATPMEAE LARRSLAQDAPPLPVPVLLKEFTVSGNILTIRLTAA DHRQLQLSIS
SCLQQLSLLMWITQCFLPVFLAQPPSGQRR

Table 4: Normalised RFU values for the 19 biomarkers

Protein	CT47A1	CTAG1A	CTAG2	DDX43	DDX53	FADD	GAGE1	GAGE2C	IGF2BP3	LRRFIP2	MAGEA10	MAGEA4	MAP2K5	PTPN20A	RAD23B	STAT1	TPM1	XAGE1D	ZNRD1
Control1_021608	1509.358	1665.573	1625.151	2051.253	2063.69	2342.416	1936.961	2921.207	1864.379	10256.51	1707.204	1270.237	2062.856	3315.828	2081.126	2617.833	4049.264	1506.695	2626.203
Control1_021611	744.8538	840.2482	689.2973	1011.785	1008.013	1106.472	1000.557	905.3507	984.5742	1642.046	949.5056	717.2795	980.4752	1986.406	905.2051	977.5851	1796.781	863.7998	1532.123
Control1_021630	2308.622	2320.635	3698.177	2686.878	2394.465	4457.038	2656.298	1767.186	2495.178	3443.966	2016.665	1715.477	2661.723	5203.608	1990.034	3163.671	6107.433	1897.377	3821.562
Control1_021631	1648.979	1653.373	2014.062	1947.228	1745.636	3154.698	1963.353	2408.739	1769.07	2760.66	2086.983	2381.255	2711.591	2212.801	2347.861	2764.548	4874.361	1577.602	2855.739
Control1_021642	1908.076	2463.134	2466.247	2669.508	2731.679	3765.574	2711.856	2790.404	2347.117	5726.215	2239.27	1819.513	2590.68	5952.694	2026.351	6089.905	4518.586	1985.198	4152.455
Control1_021643	903.8178	1280.729	1094.733	1480.856	1737.577	1901.206	1653.898	1536.486	1577.693	2475.029	1392.899	1095.408	1734.76	1687.801	1234.18	2229.621	2248.575	1155.519	2530.437
Control1_021650	2268.797	2064.734	3027.739	1922.742	1862.127	6656.163	1702.062	1538.051	1712.624	3158.91	2166.367	3076.028	3988.011	4640.239	2266.734	9483.072	4720.325	2449.356	5897.559
Control1_021660	1646.455	1641.704	2635.412	2104.055	2228.304	2471.875	2434.567	2913.973	2025.719	10491.47	1699.251	1931.718	2483.095	2033.607	1338.988	3689.077	4882.12	1281.009	3234.591
Control1_021661	1813.748	2232.638	1907.396	3502.799	3281.538	2910.518	3433.232	2851.365	2947.112	4964.015	2260.505	1715.278	2982.163	2494.186	2421.713	3949.958	6032.581	1948.014	3304.639
Control1_021663	7361.666	1747.892	1808.937	3356.196	2994.698	2869.315	2993.111	2762.496	2921.823	3766.776	2208.297	1894.731	2643.505	2964.772	4486.685	3322.445	3789.802	2135.498	4907.796
Control1_021674	1065.267	1391.603	1352.962	2290.515	2156.563	2278.898	2439.543	1912.377	1885.233	5666.105	1571.638	1156.283	2244.439	2457.223	1833.585	2449.947	8639.902	1360.602	4240.082
Control1_021679	1154.795	956.4944	1118.479	1632.494	1530.023	2318.788	1627.435	1436.882	1483.428	2188.354	1499.469	1066.008	2001.052	1444.111	1802.526	1950.754	2527.683	1278.041	2860.415
Control1_021680	3498.123	1930.224	1855.749	2352.687	2406.267	2802.602	2489.483	2315.313	2291.881	7936.889	2166.929	1679.373	2362.385	3149.52	2543.393	15907.4	4196.795	1774.904	3386.469
Control1_021681	8854.106	8411.776	8780.085	4507.529	18451.28	9194.071	4543.1	3534.392	3826.813	10582.86	6322.936	4911.227	5544.572	13939.81	4156.378	9128.958	19809.27	6150.266	9369.373
Control1_021682	1968.383	1557.664	1865.677	2790.045	2426.774	2398.539	2494.837	2171.401	2371.601	4526.223	1991.898	1569.11	4169.351	2907.473	2309.431	2437.564	3893.909	1728.037	3301.781
Control2_021005	19062.75	18286.29	21721.06	7277.07	7509.16	21723.39	8024.274	7810.164	6199.772	11076.7	11112.01	9611.882	6652.302	19237.07	5670.086	4960.157	22784.55	18345.03	20955.56
Control2_021007	3700.532	4218.712	3151.603	4712.313	3869.997	4623.076	2852.438	2929.432	3216.338	5692.778	3194.603	2842.006	4446.303	3910.378	17546.81	7313.667	7748.537	3598.932	4526.242
Control2_021016	2798.772	1907.347	2099.19	2252.848	2179.747	3351.127	2478.487	1937.812	1894.693	3015.911	2324.273	1948.089	2766.241	2710.309	2141.714	3366.335	5555.07	2589.406	3272.007
Control2_021017	1753.571	1402.848	1540.14	1802.829	1780.391	2588.142	1590.338	1678.784	1714.025	3137.241	1811.556	1476.747	2095.534	2572.001	1747.83	3847.813	4092.125	2194.708	2639.944
Control2_021025	1559	2078.736	1612.691	2155.411	2566.253	2697.4	2064.229	1783.645	2301.794	5997.156	1928.071	1764.336	2214.804	2567.013	1995.162	3132.801	4412.822	2386.34	2807.273
Control2_021037	1640.906	1349.049	1760.375	1917.732	2594.986	2383.889	1833.83	1568.244	1786.695	11870.33	1835.236	1526.275	2408.522	3380.793	1679.377	3354.587	4200.855	2184.704	3093.381
Control2_021038	1296.442	1068.401	1352.522	1510.341	1483.363	1730.292	1498.059	1215.978	1468.671	2967.612	1523.531	1207.042	2009.926	1666.915	1384.307	2710.608	3518.954	1880.842	2744.047

Control2_021045 1755.101 1821.33 1657.713 2107.653 2190.138 2525.574 2252.285 1949.365 1833.289 2309.808 2015.164 1736.922 2395.172 2511.068 1973.987 1904.59 3795.172 2411.016 2970.695

Control2_021046 2544.023 3285.646 3830.951 3493.117 4036.784 3754.021 2887.819 2281.996 3026.465 6848.673 2894.598 2433.767 3258.239 5672.938 2410.957 3980.489 5714.518 4737.667 3939.826

Control2_021401 1790.79 2078.056 1554.37 2146.542 2368.271 2731.86 2223.912 2136.282 2153.839 3751.081 2099.173 2967.183 2414.103 2164.446 5811.137 3107.932 7447.278 2357.483 3127.564

Control2_021405 1633.525 1266.149 1722.513 1767.528 1695.027 2204.463 1972.807 1588.648 1577.527 2708.492 1709.801 1549.398 2091.493 2295.796 1536.453 2455.628 3139.008 1753.436 2103.498

Control2_021406 1231.18 1052.587 1221.755 1492.635 1419.205 1617.316 1516.808 1242.477 1377.186 3379.66 1510.359 1138.178 1824.205 1522.514 2339.278 3625.717 3868.642 1370.279 1894.139

Control2_021419 1161.514 969.2148 1340.876 1298.33 1271.956 1791.68 1169.562 1080.648 1327.409 2950.186 1288.873 1206.732 1920.516 3093.032 1303.955 3515.891 2877.05 1374.645 1776.012

Control2_021420 1134.712 1149.875 1308.413 1473.83 1407.462 1798.174 1488.179 1345.406 1427.761 3191.296 1505.299 1258.148 1800.616 1843.103 1192.939 3347.262 3224.197 1403.629 1900.957

Control2_021423 2775.048 2484.158 3319.35 3998.832 3444.215 5014.536 2747.573 2749.234 3570.623 6620.853 3579.061 2918.362 4426.904 4727.215 3432.053 4868.726 11813.17 4171.47 5555.068

Control2_021426 1832.683 3149.643 3941.681 3331.141 3193.393 8540.402 2683.709 2773.037 2939.504 8472.987 2853.974 2939.304 5489.914 2777.067 3783.557 6349.507 6183.704 3560.529 7570.511

Control2_021430 1740.691 1262.074 1439.203 1784.948 1981.945 2212.359 1632.47 1614.165 1712.129 3839.469 1920.69 1343.425 2271.696 2344.028 1525.482 2578.638 70438.51 1758.205 2714.357

Control2_021436 1238.38 1622.209 1339.236 1766.58 1570.416 1970.871 1839.317 6397.377 1492.531 2310.743 1607.355 1279.09 1830.218 1714.501 1358.224 2193.62 2708.096 1785.438 2455.876

Control2_021451 2114.425 1537.93 2366.879 2916.57 2079.048 3845.347 2548.36 2707.548 2780.555 3572.501 2861.352 2283.854 3366.02 3448.814 3060.993 4270.824 6303.973 2949.225 8755.411

Control2_021453 1618.877 1772.711 1704.868 2010.795 1923.692 2306.765 1938.773 1756.808 1955.682 6044.063 2175.041 1564.877 2295.219 2062.084 2084.262 3021.721 6835.281 1951.169 3240.717

Control2_021454 1238.661 1215.827 1245.483 1523.407 1445.537 1949.649 1606.287 1406.954 1577.288 2848.697 1455.822 1306.672 1894.117 1759.484 2367.539 2441.304 4791.178 1549.041 2289.409

Control2_021455 2118.806 2056.799 2135.804 2460.721 2409.344 3278.959 2368.928 2369.251 2564.917 4994.603 2530.726 1924.332 3277.945 2818.599 2358.646 4807.185 6212.127 2661.667 3698.437

Control2_021463 3277.456 2954.683 6329.681 4028.11 3885.942 8013.651 3643.477 3340.58 4073.638 10964.05 4476.259 3557.854 4176.659 5050.335 3590.858 7434.23 9568.385 4946.078 5981.495

Control2_021470 1404.597 1243.895 1509.513 1683.949 1605.746 2041.225 1716.442 1589.304 1751.386 5710.754 1632.83 1402.099 2130.032 2535.66 1462.485 2516.297 10171.16 1667.289 2791.111

Control2_021477 3228.131 2485.288 2693.976 3065.942 3048.999 4125.412 2669.848 2729.497 3498.464 6047.108 2975.548 2798.397 3279.928 6853.659 2609.898 4227.737 7400.694 3379.937 3876.3

Control2_021478 2702.577 2203.357 3179.785 2097.886 1878.866 3515.833 1795.588 1763.884 1975.51 4209.962 3187.603 1924.962 2382.097 3422.74 1919.419 6160.237 4680.497 3213.554 3278.552

Control2_021484 1867.657 1752.908 1825.902 2021.439 1897.227 4221.542 2058.948 1997.38 2030.785 4996.788 2260.441 1635.343 2426.476 2468.278 1856.142 3234.766 4323.386 2336.339 2965.36

Control2_021494 1226.113 1187.936 1371.995 1461.011 1289.884 1730.896 1411.166 1264.948 1358.159 6289.746 1428.486 1155.8 1760.796 3294.846 1606.667 3721.188 2527.812 1406.064 2014.624

Control2_021495 7510.731 6547.905 8569.185 9460.011 24887.84 11979 7132.448 8605.022 7607.972 10760.02 8813.236 8154.649 4858.083 8017.285 10114.45 9327.442 13563.37 10985.96 11552.79

Control2_021497 2103.323 2325.289 2423.39 2140.501 1881.936 2612.635 1915.289 1828.612 2022.71 2381.643 2071.199 1836.147 2880.86 2694.962 8194.55 4910.062 4861.554 2291.933 3220.736

Control2_021801 1684.077 1614.954 1535.561 2231.253 1987.477 1996.131 2130.707 1799.308 2131.438 2938.354 1934.592 1637.284 2040.849 2076.451 2481.315 2854.822 3918.524 2763.663 2642.432

Control2_021802 1196.061 1161.766 1299.801 1585.175 1516.791 1907.229 1876.651 1209.1 1582.688 1721.553 1333.201 1159.959 2289.404 5436_568 1385.581 3183.812 2829.928 1533.262 3402.505
Control2_021804 1813.897 1627.858 1980.221 2420.979 2252.938 2496.24 2142.618 1772.867 2513.429 4985.019 1938.967 1565.209 2898.718 2946.264 2550.711 2801.768 4235.614 2523.631 5712.794
Control2_021805 1823.253 1975.388 1764.159 1876.648 2049.388 2313.581 1586.62 1558.38 1813.176 4710.699 1744.263 1476.581 3112.112 2164.224 2154.696 3056.303 4473.074 1850.957 5102.744
Control2_021806 3227.12 2526.679 3146.424 3098.552 2464.066 3832.353 2866.158 2476.056 2214.155 3592.359 3411.004 2731.398 4373.876 4083.713 2606.994 5057.475 5208.636 2913.279 7700.55
Control2_021809 7768.215 1240.79 1653.211 1662.744 1754.941 2201.225 1664.758 1504.47 1756.475 3069.143 1770.113 1568.689 2315.789 1870.303 3831.109 2834.949 3286.348 1695.697 2539.116
Control2_021810 2441.267 2074.878 2048.36 2517.456 2467.174 2497.116 2520.784 2145.236 2409.725 4595.2 2483.45 2014.932 3161.544 2782.631 2098.649 3753.405 4439.834 2398.99 3046.975
Control2_021811 2362.442 1943.874 2300.76 1417.942 1302.352 2023.503 1786.874 2726.91 1230.763 1702.497 1990.884 1592.71 2056.904 2890.082 1837.785 2695.945 3664.561 1835.566 1736.29
Control2_021812 3601.507 3159.063 3554.258 4238.192 3735.361 4730.045 3681.06 3574.706 3736.236 6074.945 5099.298 3351.37 5101.794 4984.095 3646.176 6592.742 7476.003 4860.687 5299.261
Control2_021818 1212.13 1505.043 1698.016 2248.258 17693.85 3775.838 1669.632 1509.442 1723.422 3067.722 1692.577 2609.891 2401.301 1806.957 1766.938 13912.53 2448.699 1935.279 5124.145
Control2_021822 1783.632 3716.23 1896.898 2328.595 2554.369 4283.265 2294.682 2177.366 2258.871 5106.237 2454.382 1692.475 2726.053 2685.046 1965.292 3332.935 3820.505 2458.486 4966.497
Control2_021823 1099.564 2210.086 1106.176 1579.562 2073.087 2047.938 1886.623 1732.564 1782.433 5978.676 1373.312 1059.723 2141.575 2758.369 1343.488 2322.024 2924.873 1400.678 3526.075
Control2_021824 5689.149 3643.473 5707.856 2441.248 1596.983 7271.611 2337.595 1735.312 1929.38 3387.352 5770.001 3186.82 2379.998 6112.298 1756.883 6932.623 12069.42 5871.825 7241.109
Control2_021825 1927.505 1370.127 1906.145 2845.125 2542.267 3858.186 2619.755 2365.264 2894.506 12133.5 3019.027 2065.98 3470.955 3126.288 2659.892 3660.737 4434.643 3017.324 6072.551
Control2_021826 2170.275 2212.367 2186.7 2585.956 3076.474 2347.794 2926.708 2668.387 2975.528 3619.189 2449.931 1765.577 2665.075 4058.315 1588.61 3918.005 5187.333 2401.518 3481.845
Control2_021829 727.9997 821.4169 966.9506 1252.757 1200.859 1308.101 1360.473 991.0163 1078.297 2541.436 1017.431 829.9832 1492.023 1211.206 974.2268 1802.962 2828.033 1128.866 2941.595
Control2_021831 1082.576 1191.876 1338.483 1541.967 1694.252 1659.618 1599.125 1224.445 1388.789 17426.87 1383.007 1163.831 1803.056 2659.547 1234.548 2592.773 3259.55 1438.736 2054.688
Control2_021834 2115.083 2133.577 2971.31 2478.802 2751.062 4492.736 2664.171 2371.209 2731.65 3946.079 2371.834 2448.149 3712.545 3132.718 2709.399 4566.653 5304.744 2605.529 7395.801
Control2_021835 1800.552 4143.1 2827.079 2165.51 1599.139 3511.991 1954.184 1569.674 1425.636 4211.808 2462.035 1860.317 2387.911 3136.524 1843.991 4033.994 3249.438 2702.522 3695.011
Control2_021836 1245.328 3282.619 1606.868 2021.895 2213.233 2169.312 1826.482 1525.452 2048.427 3871.687 1729.768 1484.16 2294.109 1869.645 2803.469 3491.743 3627.928 1791.521 2439.615
Control2_021837 1232.15 1843.519 1565.995 1737.465 1662.996 3514.607 1634.608 1495.075 1745.759 2669.79 1544.561 1699.246 2605.019 2069.25 1769.233 3720.748 3504.717 1782.507 4207.138
Control2_021839 1520.202 1957.838 1960.711 2279.169 2273.681 3209.642 2471.854 2103.487 2341.041 3813.47 2127.806 1811.276 2915.472 2172.454 2231.778 3304.446 3685.084 2180.862 5415.791
Control2_021840 1006.846 1466.336 1352.943 1436.497 1554.106 2073.867 1482.862 2043.637 1466.736 2785.596 1425.364 1161.345 1872.754 1764.8 1310.607 2112.964 2436.706 1458.616 3747.051
Control2_021844 1527.763 2143.553 2124.143 2583.149 2526.574 3471.964 2444.126 2209.262 2580.429 5380.09 2196.979 1899.78 3372.265 2346.553 2283.847 3591.101 5118.613 2345.115 5396.899
Control2_021845 1708.471 2597.062 3056.738 2764.51 2509.027 3627.698 2486.593 2214.397 2724.959 4277.382 2346.401 1932.341 3312.14 2775.452 2272.65 4054.93 6627.734 2873.661 6528.291

Control2_021848 1511.619 1337.722 1397.268 1647.631 1456.605 2385.061 1408.622 1224.608 1456.744 2689.313 1499.23 1191.049 2159.648 6106.434 1689.162 11687.5 2823.277 1698.773 4285.151
Control2_021849 1866.328 2832.416 4456.51 2224.86 2056.209 3537.863 2201.666 1893.812 1987.449 3206.646 2118.117 2061.679 4011.126 3273.238 3212.107 4313.128 4844.187 1940.267 5146.62
Control2_021850 2220.246 1058.777 1760.467 2172.435 1948.469 2902.667 2243.549 1943.425 2179.94 2594.357 2051.556 1817.563 2913.705 2595.519 1710.077 2985.272 10907.08 2022.729 4563.897
Control2_021851 3033.04 2883.127 3632.569 3669.286 3355.756 4199.051 3220.745 3010.425 3358.091 5593.778 3204.729 3319.564 7174.081 5437.286 2906.247 5124.673 6914.633 3478.561 5951.68
Control2_021852 1652.118 1463.437 1920.526 2158.32 1879.306 2640.924 2086.825 1879.865 1963.275 2311.181 1711.839 1746.169 2653.722 2219.421 1947.486 3140.212 3194.615 2367.34 5520.482
Control2_021853 891.6693 1094.034 1197.545 1478.783 1579.288 1847.533 1463.02 1369.585 1542.191 2405.584 1254.529 2225.92 1840.036 1395.407 1300.163 3414.311 2726.988 1324.848 4586.414
Control2_021855 1430.95 1762.848 1859.345 2189.602 2208.481 2695.143 2471.515 1924.412 2092.828 5193.978 1821.72 1955.116 3112.859 2274.963 4717.033 3128.178 4070.028 2258.982 6357.409
Control2_021861 1597.079 1676.007 1582.567 1887.047 1917.212 2577.912 2051.175 1694.966 1766.345 2249.844 1485.959 1441.116 2357.153 1741.011 1846.351 3192.134 3243.591 1926.237 3090.701
Control2_021862 1113.862 1470.806 1490.145 1769.074 1888.085 2226.298 1665.863 1505.797 1758.152 3119.105 1388.473 1208.254 2264.852 1470.605 1345.939 2384.382 2742.069 1728.213 3670.252
Control2_021864 1668.539 1449.478 1434.442 1329.608 1308.309 1871.11 1584.434 1139.643 1228.498 1617.374 1600.64 1097.203 1773.925 1405.638 1423.723 2487.983 2064.618 1366.074 2795.789
Control2_021866 2106.947 3537.804 2356.46 3515.985 3030.057 4083.057 3325.615 2975.695 3340.706 3692.312 3083.481 2305.219 4003.694 3172.705 3376.394 5048.73 4946.702 3457.549 6293.909
Control2_021869 3801.609 4345.063 4445.315 2569.386 2154.123 5376.007 1874.028 1627.357 2041.283 5513.359 4128.772 3249.421 2908.544 5007.78 2079.015 5426.798 5380.724 4940.874 4144.626
Control2_021870 1405.225 1733.58 1940.332 2278.261 2483.545 3020.657 2064.894 1919.133 2274.004 5381.599 3390.585 1763.338 3202.194 2078.389 2362.266 4841.695 4649.188 2360.201 5403.713
Control2_021872 1286.815 7318.886 2611.127 2152.928 2157.179 2850.372 2268.582 2110.246 2353.007 7394.805 1774.071 1518.602 2707.483 2249.85 1797.54 3300.761 4053.954 2168.691 4660.637
Control2_021874 1724.234 2365.071 2544.429 2717.166 2505.942 3590.685 2480.078 2400.147 2754.599 5223.595 2337.521 1887.844 3652.429 2367.014 2208.745 3585.306 5739.748 2704.132 5517.491
Control2_021875 1417.375 1366.168 1691.543 2298.582 2233.337 2976.039 2081.084 2989.963 2835.615 6109.194 1990.811 1606.504 2701.638 2239.367 1994.916 3145.048 3000.913 2236.848 5457.432
Control2_021876 1537.92 1520.497 1819.945 2074.254 2079.594 2127.166 1807.031 1649.114 2026.153 5614.535 1726.306 1692.19 2859.931 2391.009 3490.562 2938.155 4877.939 1748.16 2883.442
Control2_021877 2169.568 2520.335 2805.955 2901.827 2795.744 3439.995 2824.899 2605.987 2683.601 4044.607 2816.4 2392.499 3991.897 3151.645 2617.009 6545.128 13734.66 3055.266 4792.461
Control2_021882 1853.488 1463.824 2709.683 2630.134 2613.521 4889.535 2481.181 2151.083 2432.832 5635.096 2318.964 3012.98 3779.524 3017.525 2502.86 9005.231 6358.341 2580.803 4540.223
Control2_021884 1966.939 1262.075 1971.59 2222.155 2054.503 2464.76 2088.945 3236.594 2162.308 4175.698 1896.357 1930.513 2802.656 2045.35 2109.724 3084.8 5077.241 2120.757 3006.417
Control2_021885 2316.15 1641.508 2386.34 2210.695 2094.819 2590.088 2073.195 1878.722 2322.551 2360.523 2020.608 2019.363 2912.89 3519.932 3397.924 3343.496 4043.697 2422.662 3089.727
Control2_021887 1531.977 1308.395 1976.089 1951.533 1830.006 2269.807 1804.872 1627.947 1907.668 8062.939 1698.869 1543.445 2905.972 2107.129 1760.354 2829.737 6973.591 1813.83 2448.871
Control2_021888 1816.136 1727.809 2177.399 2355.914 2248.237 3075.7 2270.959 2016.192 2362.468 6705.703 2263.509 2009.607 3116.232 2917.681 2180.773 10092.09 6037.169 2303.328 3242.51
Control2_021889 1191.999 1224.372 1456.478 1671.608 1769.808 1729.559 1738.464 1479.342 1724.205 3193.269 1365.617 1264.229 2102.197 4013.068 1402.45 2661.556 4983.02 1468.28 2082.255

Control2_021893	961.8649	863.8429	1231.316	1339.618	1244.098	1399.984	1578.939	1009.998	1266.354	1532.566	1124.324	1133.104	1673.636	1486.05	1290.029	2445.214	2708.811	1151.813	2311.744
Control2_021896	1175.956	1869.874	1542.43	1767.732	1848.923	2319.642	1848.402	1313.737	1786.878	3973.447	1376.932	1401.714	2109.678	1795.883	1920	2577.86	5293.315	1487.054	2189.525
Control2_021898	1421.408	1274.498	1793.884	2191.324	2017.862	2316.975	2054.023	1780.855	2163.932	4514.717	2123.111	1578.816	2815.736	2178.103	1781.437	15068.83	4513.11	1985.627	2620.897
Control2_021899	1169.115	1018.919	1380.195	1747.41	1623.652	2066.902	1759.251	1608.307	1791.442	2999.335	1525.146	1254.638	2230.895	2104.384	1492.37	2394.735	3072.453	1643.78	2270.203
Control2_021900	1805.134	2272.669	2339.801	2877.322	2870.634	3650.904	3154.306	2943.415	3060.013	3837.566	3456.82	2574.759	3255.242	3745.962	2716.952	4014.755	5178.692	2951.938	7874.77
Control2_021962	1299.376	1469.512	1752.46	2072.822	2154.644	3048.746	1847.226	1801.589	2115.775	5116.496	1706.608	1628.084	2814.555	2084.987	2067.581	5010.089	4188.351	1975.819	3179.895
EarlyStg1_021633	888.7379	1083.956	1139.705	1745.988	1624.386	2126.853	1864.5	2451.019	1599.96	3890.38	1465.2	1037.369	1668.078	1451.583	10190.59	2556.25	3108.472	22871.57	2088.815
EarlyStg1_021651	1718.134	1869.381	2002.153	2829.701	2301.549	3393.112	2494.653	2191.697	2351.123	6750.972	2161.845	1771.912	2854.07	2561.211	2358.632	2967.15	3584.864	1801.215	6011.781
EarlyStg1_021654	1414.691	2009.296	1875.053	3674.002	3193.332	2959.501	3266.428	2816.123	2713.403	3945.555	2002.649	1859.56	2533.283	3189.184	2115.876	2382.979	3204.194	1758.753	5859.363
EarlyStg1_021655	2360.272	2071.366	2048.578	4079.875	3856.399	3233.548	3060.038	2967.291	3210.863	6902.838	2143.019	1764.912	3295.438	3250.834	2346.357	2888.672	5218.852	2056.624	4117.299
EarlyStg1_021662	1046.279	1038.759	1247.205	1608.22	1934.638	1675.178	1641.528	1478.774	1536.208	2295.528	1315.652	1296.914	2313.41	1630.089	1478.589	7011.551	2377.041	18653.11	1784.796
EarlyStg1_021675	2008.526	2211.421	2262.93	3836.686	3311.173	4348.88	3947.239	3216.422	3272.398	5877.409	2722.891	1721.796	3145.656	3171.789	2286.916	3358.434	5073.502	2378.691	6228.465
EarlyStg1_021678	809.411	1196.975	1171.397	1416.018	1513.61	1406.448	3201.611	3765.569	1243.303	5240.118	1195.363	849.612	4169.675	1276.357	1124.136	1342.022	2590.081	2450.418	2513.441
EarlyStg2_021024	1666.611	2348.44	1861.542	1900.167	2175.75	2635.829	2138.762	1641.05	1784.729	2599.311	1926.939	1678.941	2398.034	2452.743	1825.564	3177.2	3875.282	2259.64	2789.643
EarlyStg2_021403	864.2702	908.2656	966.5297	1152.405	1152.189	1208.682	1132.829	999.4889	1193.16	2495.09	1988.684	8670.834	1149.056	1061.351	826.697	1508.257	3280.338	1056.332	1462.285
EarlyStg2_021435	818.7927	946.9137	862.6614	1138.72	1062.796	1348.046	1297.819	1024.028	1020.547	1300.412	1315.539	812.8782	1992.811	12849.7	3039.989	1482.107	1708.522	1111.212	2970.026
EarlyStg2_021440	1115.634	1262.505	1256.625	1676.983	1456.852	1876.273	1804.083	1597.008	1495.375	6394.945	1536.416	1220.193	1741.883	1759.137	1274.902	2116.541	3047.862	1489.374	2207.21
EarlyStg2_021443	2209.834	2323.86	2268.553	4227.051	2746.796	3529.186	2838.406	2627.111	2716.052	3346.902	2821.284	2199.833	4253.328	3857.54	6291.922	3782.145	5357.531	2910.532	4271.11
EarlyStg2_021458	2764.343	2321.842	3096.833	2692.237	2468.356	3620.752	2475.345	2253.773	2492.874	5134.364	2902.898	2525.073	3200.766	7027.378	2891.019	3304.315	5084.472	3052.856	3851.58
EarlyStg2_021462	1168.597	1095.781	1259.984	1473.306	1355.856	1573.764	1735.746	1327.889	1447.512	1962.651	1353.171	1190.796	1692.075	2083.382	1220.256	1848.675	3257.003	1419.641	1844.949
EarlyStg2_021466	2917.855	2471.582	2442.846	3334.242	3444.158	3068.349	2739.576	2864.376	3539.669	3898.782	3162.375	2048.981	7417.409	5946.867	2380.558	3931.357	4237.54	5538.389	4529.409
EarlyStg2_021489	3814.376	64378.83	54002.38	1724.073	1619.1	2201.599	1688.194	1538.798	1833.571	2224.745	1882.389	1361.955	1828.498	2600.137	1708.257	2492.632	3881.379	2120.155	2455.012
EarlyStg2_021496	2140.219	2044.7	2620.907	2860.899	2714.917	3523.238	2716.084	4006.147	2532.979	8436.43	2620.774	2268.858	4002.257	3331.943	3119.467	4494.802	6993.668	2825.078	3852.443
EarlyStg2_021814	1434.233	1436.983	1273.89	1802.932	1544.033	1577.473	1621.802	1248.348	1184.509	2266.998	1189.78	1175.504	1929.441	1631.434	1240.605	2024.513	2875.169	1276.932	1868.431

EarlyStg2_021815 6157.471 3813.734 3852.406 8214.276 10197.87 7104.856 5543.301 6291.762 14434.92 7528.605 5889.771 5140.262 11153.38 23529.81 4652.537 8918.846 13542.18 10916.19 29827.34
 EarlyStg2_021820 1272.23 1459.923 1404.584 2228.886 1796.769 2841.497 3885.665 4033.956 1791.473 2298.301 1780.983 1591.671 2375.364 2462.813 1846.98 2687.747 3147.172 1999.272 4358.43
 EarlyStg2_021827 616.5434 786.2398 791.7878 977.2698 979.6347 1131.393 1511.574 838.4045 862.3957 1000.234 745.288 700.6462 1164.85 862.7676 735.5688 1349.104 1553.147 807.8251 2005.31
 EarlyStg2_021830 1350.571 1322.662 1347.479 1637.868 1574.216 1864.733 1802.094 1318.946 1509.441 2078.313 1378.075 1235.311 1996.329 1743.144 1373.751 2402.751 2745.201 1520.204 1758.649
 EarlyStg2_021832 685.3966 768.7034 771.6687 907.4298 959.8832 881.5877 1484.217 1441.072 940.8926 955.0221 749.3359 680.3148 1144.174 877.5483 693.6878 1215.148 1877.743 821.4908 989.1862
 EarlyStg2_021842 559.1504 728.8939 773.4969 886.8648 874.0025 1128.443 1246.985 812.1934 787.061 1720.34 740.0612 1044.132 1072.525 843.0966 732.0325 1241.119 1438.012 792.5935 1880.585
 EarlyStg2_021843 998.7775 1042.343 1037.048 1316.874 1545.713 1488.783 1464.504 1162.424 1227.167 1199.951 1252.088 932.2355 1420.142 1299.8 1218.456 1565.295 1760.286 1234.466 2293.205
 EarlyStg2_021847 847.6904 834.0404 1065.834 1241.691 984.5962 1537.282 1765.055 1053.372 988.6661 1455.479 1061.089 976.3165 2364.797 1223.133 916.262 1675.788 1953.103 1106.615 2237.163
 EarlyStg2_021858 2884.206 3845.013 4219.314 3940.725 3779.591 4932.521 3850.321 3779.076 3654.264 5782.754 6309.748 4035.687 5097.5 4892.059 3918.399 5739.398 7182.912 4633.46 11607.87
 EarlyStg2_021867 1027.782 3742.673 1421.976 1480.335 1415.318 1834.294 1702.586 1363.897 1471.776 2148.901 1221.715 1187.602 1867.458 2958.356 1508.762 2039.943 2430.543 1407.597 3551.464
 EarlyStg2_021873 1991.314 64387.9 68263.43 2576.418 2450.933 3204.567 61022.98 61141.06 2437.692 3159.13 6431.019 2019.762 3573.626 2866.673 2360.751 3891.723 6348.432 2662.724 6903.598
 EarlyStg2_021895 1309.615 1142.613 1833.176 1552.477 1668.209 2018.585 1748.757 1350.093 1657.107 3322.74 1374.092 1347.937 2303.925 2011.179 1259.669 2291.862 2859.402 1732.711 1940.729
 EarlyStg2_021960 2893.513 3014.18 3981.726 31577.67 4138.143 6326.917 4832.018 7208.038 4473.527 6067.125 2660.867 3715.26 5565.572 4084.967 5344.483 5542.575 7626.605 4567.441 6935.041
 LateStg1_021607 2233.818 33710.4 27509.89 2683.862 2431.354 5415.112 2263.816 2008.929 2639.71 4167.536 21571.06 20747.58 2873.485 7195.459 2548.429 3653.705 11731.3 2238.342 9084.524
 LateStg1_021612 1077.574 1286.721 1463.395 1459.115 1434.265 1880.945 1247.632 1032.569 1283.638 1777.645 1451.691 1189.345 1739.08 1653.534 1226.084 4710.854 27371.76 24821.54 2435.91
 LateStg1_021632 3286.639 1480.678 1291.98 1680.948 1599.405 1828.069 1593.324 1280.285 1799.823 3101.039 2060.273 1602.773 5195.655 1645.745 2239.28 1861.048 3672.684 11641.46 2496.863
 LateStg1_021683 1332.092 920.1105 1136.96 1548.33 1181.713 1415.653 1581.494 1335.489 1143.095 2445.638 1851.534 4298.669 8690.543 1780.728 1150.62 2402.067 3062.754 1141.802 2028.778
 LateStg1_021691 3844.224 3683.971 6199.383 3335.339 3994.33 8536.385 2753.94 2503.818 2924.456 8177.706 4079.96 2844.118 2816.506 6253.56 2437.998 7010.066 4723.274 4053.18 6519.304
 LateStg1_021692 1632.176 7969.794 4384.263 2698.668 2282.849 2693.694 2417.603 2147.67 8336.307 2067.293 2052.88 3534.455 2314.055 2435.415 11902.46 6559.493 12545.8 1963.734 11183.95
 LateStg1_021696 6599.412 5823.924 6186.081 5783.523 4699.548 7686.687 5192.874 4561.238 5254.942 7300.942 5486.125 3686.07 10444.32 10842.8 3874.061 6828.621 7978.743 5397.418 7341.834
 LateStg1_021699 908.5383 943.1396 1345.248 1535.726 1361.538 2004.212 1679.826 1162.804 1463.845 1553.582 1393.758 1065.26 1777.222 1628.989 1197.207 12961.49 3163.428 1263.17 2505.567
 LateStg2_021004 3221.85 4463.517 3620.611 3510.513 3849.087 5065.177 3227.483 3134.377 3447.883 6800.578 3186.653 2627.675 3490.451 4227.487 2755.789 2133.374 7471.008 15774.18 4833.175
 LateStg2_021006 2339.927 2435.977 2422.967 3627.886 4482.165 3510.889 2848.815 2488.987 3785.679 12021 3061.898 3122.281 3726.921 3191.783 3055.728 5022.605 13003.68 3081.717 4111.345
 LateStg2_021028 2707.792 4469.606 3026.737 4088.437 4885.905 4633.554 4514.939 5517.361 4012.385 4351.325 3588.626 3046.919 4898.403 3893.103 4476.661 3874.278 6939.209 15318.87 4612.729

LateStg2_021029	4950.915	4573.694	5365.522	5212.313	5258.751	7136.894	4720.304	4089.176	4817.28	7556.265	4689.437	4079.114	5566.21	8339.843	3731.415	3904.665	18153.11	5939.745	9798.426
LateStg2_021039	3522.816	3382.284	4025.145	4416.734	5475.508	4942.533	4098.692	4279.457	4720.891	10757.38	3931.166	3348.218	5050.079	7727.377	3605.788	4050.392	11286.33	31136.4	10124.7
LateStg2_021040	2002.684	1739.248	2184.697	2733.813	2964.582	2841.992	2364.913	2064.116	2472.303	12349.52	2486.638	2116.17	3101.401	3078.854	2011.853	2894.685	6237.084	12377.03	3488.47
LateStg2_021402	1361.212	1273.69	1436.17	30543.01	1661.542	2105.953	1792.681	1556.671	1665.538	10941.54	2164.561	3789.045	2091.446	23209.96	3864.334	2131.699	16814.28	1855.743	2460.073
LateStg2_021404	1825.759	1561.872	1859.06	2102.176	1937.207	2621.452	1884.312	1886.038	2103.374	10730.8	2301.383	1848.497	2619.036	2114.464	2806.652	3262.728	5923.29	2109.56	2837.274
LateStg2_021409	1821.798	1622.475	1888.661	2005.027	2042.316	2466.845	2172.961	1965.279	2287.658	2560.275	2146.635	1698.201	2258.806	2988.973	6123.263	45207.66	3710.094	2240.805	2672.31
LateStg2_021411	12831.44	2658.677	2981.266	3455.966	66480.3	3872.695	2669.909	2881	3275.688	5937.628	3641.948	2783.715	3631.025	3842.071	2780.356	9740.508	8277.123	86593.27	4647.849
LateStg2_021412	1375.199	1239.54	1325.421	1639.334	3807.727	1852.533	1571.277	1348.274	1421.224	1966.427	1540.838	1344.967	1777.86	1717.123	1367.526	2384.968	2527.974	4301.716	2051.114
LateStg2_021413	1771.981	1487.26	2007.343	1924.228	2049.404	2192.016	1768.964	1748.733	1909.862	8649.381	1902.606	1753.411	2701.412	2172.419	2608.381	3445.016	26526.73	2274.128	2877.102
LateStg2_021418	1312.756	1172.529	1327.484	1582.212	1511.383	2946.084	1518.947	1320.067	1400.382	1756.549	6483.009	12480.7	1712.24	1907.998	1232.774	2193.431	2718.157	1707.805	2045.315
LateStg2_021421	3372.893	1793.388	1865.367	1997.554	2003.966	2556.354	2058.865	1860.537	2016.597	3387.465	1986.933	1592.037	2362.854	2431.391	1757.695	7521.871	6986.991	2146.607	2784.716
LateStg2_021422	2876.725	2164.961	2960.132	2820.349	4247.47	3985.439	2012.289	1996.894	2534.09	9135.84	3163.03	2608.115	3364.623	3781.505	2337.046	6197.789	5642.467	51156.32	3961.097
LateStg2_021424	1510.005	1436.097	1764.94	2312.824	2095.89	2981.925	2034.561	2011.813	2096.701	5080.653	2221.863	1669.388	2880.509	2746.375	2017.996	3606.207	4320.564	81892.36	3396.019
LateStg2_021425	4155.837	3164.881	3549.289	4399.627	3495.884	69262.64	3726.524	3610.125	3821.13	5625.683	5187.55	33888.13	10796.37	22975.41	17817.6	8637.526	10970.58	4581.09	7241.283
LateStg2_021427	6471.712	5562.278	5414.813	2359.317	1931.492	5062.902	2487.975	2015.596	2006.091	4911.945	5642.549	3571.163	2693.277	6688.38	2318.45	6676.587	9097.011	5138.452	3666.584
LateStg2_021428	1315.405	1923.432	1325.323	1540.169	1567.625	1995.128	1862.058	1623.103	1391.924	1660.351	1548.41	1357.469	9092.653	1680.029	1365.524	3474.558	2805.974	1418.446	3555.568
LateStg2_021429	2123.084	2559.612	2236.494	3001.687	2607.627	3412.53	2292.985	2415.871	2844.512	4992.574	2967.538	2066.671	3387.576	2708.596	2401.982	3614.203	5568.374	3001.216	4089.902
LateStg2_021431	1761.144	1820.044	2118.511	2601.028	2288.129	3056.034	2245.269	3071.717	2317.118	4144.749	2499.042	2225.565	3046.623	2595.132	2159.14	4110.286	5855.135	2399.371	4049.696
LateStg2_021432	2360.041	2259.147	2011.696	2624.354	2347.857	3114.266	2267.014	2232.296	2551.755	4449.926	2553.865	1790.208	2791.503	2231.627	2113.099	3429.028	4533.118	2518.522	3669.681
LateStg2_021433	2535.536	2996.462	2903.991	3686.228	3635.17	44392.76	3044.162	3183.385	3632.759	7242.118	3571.259	2868.022	4197.165	4657.339	3288.039	17338.42	7431.575	3735.118	5408.98
LateStg2_021437	2480.932	2964.578	2577.313	3395.92	3330.783	3915.391	2871.597	3034.91	3358.309	36422.07	3309.372	2486.033	4058.445	5346.232	5720.515	51111.89	6558.229	73076.88	5622.161
LateStg2_021441	2178.753	3019.759	2484.399	3276.622	3424.187	4497.703	4627.948	5516.538	3184.235	23989.57	3092.49	2360.327	4081.13	3894.148	7333.667	5608.731	12014.06	3198.293	5385.842
LateStg2_021442	1402.243	1687.07	1595.617	2190.64	2035.534	2949.132	1854.978	1630.612	1953.693	3097.947	2175.145	1574.884	2440.885	1844.334	3378.815	2767.79	3709.88	1951.919	4524.983
LateStg2_021450	72225	4925.92	5586.278	7038.924	6264.657	9305.774	5407.448	6106.828	62465.09	9856.237	6436.29	5774.264	8325.764	7744.436	9800.541	10278.63	11756.66	8106.801	13587.36

LateStg2_021452	2351.882	2117.25	2515.784	2714.059	2709.585	3236.665	2472.468	2335.358	2628.204	7171.939	2770.725	2191.751	3443.649	2911.636	2955.094	3955.263	26551.05	3107.26	4242.407
LateStg2_021456	2281.465	1624.104	2069.136	2084.323	1906.072	2652.478	2118.836	1945.565	2295.275	2391.687	1978.448	1735.68	3055.247	2944.797	7126.781	3647.837	4024.424	2143.845	3360.468
LateStg2_021457	7111.74	5438.97	6081.31	7192.698	6515.937	8094.362	5778.938	6053.032	6740.558	13294.24	7297.811	7370.232	26310.33	10478.36	6932.051	9832.866	19790.06	7344.632	13282.43
LateStg2_021459	1662.911	1488.942	1709.95	5439.639	1756.361	2467.384	2151.735	1685.922	1799.142	3100.266	1814.398	1634.251	2973.406	2348.097	1709.713	2914.202	3567.059	1904.825	2874.884
LateStg2_021467	2871.091	2477.123	2697.42	3051.383	2669.106	4019.018	2084.887	2514.498	3031.402	9162.167	3030.005	3013.527	5899.702	3568.804	2425.005	5735.723	5177.811	3191.262	4545.997
LateStg2_021468	2239.595	2067.363	2455.974	2399.43	2260.627	3172.673	2334.31	2192.644	2555.232	5898.635	2403.385	2061.466	3099.336	3734.919	2250.546	3828.926	9905.679	2752.943	3839.993
LateStg2_021469	1134.769	1041.707	1124.96	1394.012	1423.355	1609.735	1685.225	1274.357	1346.583	2408.616	1284.269	1075.315	1683.193	1550.628	1463.695	2068.044	2449.744	1328.585	2466.248
LateStg2_021471	1883.788	1395.216	2140.146	2416.612	2918.033	2983.943	2138.6	2029.309	2345.696	6284.123	2197.31	3165.057	2970.156	2605.741	2245.675	3627.445	14696.91	2467.378	3167.771
LateStg2_021476	3258.855	2759.33	2993.915	3391.916	2972.729	4209.934	2853.457	3199.763	13932.98	5353.104	4163.6	2583.433	3163.012	14982.35	2189.458	4731.425	5321.727	21050.91	5567.529
LateStg2_021479	2880.929	2591.085	2935.565	3095.02	2751.518	3877.997	2929.471	2761.362	2786.301	3761.414	3220.481	2663.487	3713.563	16733.18	2874.51	4326.346	7389.014	3331.391	4580.993
LateStg2_021485	3236.978	2840.985	3298.95	3514.251	3336.928	4831.199	4070.197	5153.553	3572.308	22588.2	3824.449	3019.581	4860.078	5384.661	7718.838	6508.286	11009.77	3845.124	6364.518
LateStg2_021490	2055.398	2328.332	2304.917	2529.798	2578.518	2827.262	2270.409	2361.421	2700.272	5644.643	2608.352	2090.352	3147.381	3369.221	3426.581	3655.384	9930.515	2640.637	3786.199
LateStg2_021493	2089.283	1969.834	2252.854	2541.756	2554.018	3403.82	2142.646	2208.38	2513.628	4698.848	2510.315	2021.454	2975.269	2943.559	2136.006	54080.8	4759.46	2612.632	3790.428
LateStg2_021498	3994.694	3345.87	3519.226	4221.659	4350.851	4858.934	3506.732	3779.842	4285.45	7355.246	4261.771	3229.588	5198.682	4788.694	3818.572	5490.723	12841.46	5246.952	6432.803
LateStg2_021499	2712.5	2477.536	2535.785	3120.787	3083.258	4619.17	3050.456	3503.044	3477.192	5287.922	3132.654	2428.364	3670.777	4016.163	2945.576	8863.933	5309.148	3196.865	4388.622
LateStg2_021803	2568.571	2524.476	2509.832	2820.704	2609.458	2990.457	2279.461	2172.796	2550.41	9028.317	2471.884	2004.119	3726.039	3126.381	2455.32	3660.465	5247.491	2842.318	5724.04
LateStg2_021817	2184.182	2720.577	2811.529	4809.278	63866.5	4006.276	3240.366	2969.606	3489.376	7562.101	2882.902	2628.133	4314.625	3248.255	5146.921	6176.601	6944.245	3376.811	9550.769
LateStg2_021819	2690.661	2528.864	2868.553	3699.345	4265.994	3856.545	3302.492	2991.057	3685.966	6583.73	3108.269	2545.667	4423.612	3748.161	3673.165	5193.176	6400.219	3449.647	7443.841
LateStg2_021821	3862.728	5570.37	2950.673	3903.449	4548.635	5141.088	3771.425	3412.051	3855.53	7804.9	3294.47	2903.67	4701.069	3552.171	3290.416	5084.206	5759.796	3728.741	8677.661
LateStg2_021828	3612.2	32668.33	13719.52	3451.276	3465.224	5624.391	2798.934	3131.383	3411.682	7526.149	3897.674	3308.141	5139.618	4285.685	3254.309	7498.517	9501.756	4700.525	9935.705
LateStg2_021833	988.1922	1768.821	1152.149	1436.283	1414.762	1567.374	1619.189	1172.344	1279.158	2007.471	1216.094	974.8655	1689.938	1283.639	1469.673	2038.617	2763.421	1149.49	1673.634
LateStg2_021838	1659.286	2605.402	1884.594	2379.837	2659.232	2909.178	2613.869	2226.825	2430.028	2848.576	2289.099	1881.045	2790.638	2913.932	1876.231	3387.326	4041.425	4749.024	5877.246
LateStg2_021841	7280.658	8393.207	6239.35	5096.393	4927.956	7779.298	5001.383	4618.61	4848.952	7746.386	7852.988	5259.368	6938.347	7712.883	4960.22	9289.899	9842.119	12962.39	12360.34
LateStg2_021846	1017.125	1024.531	1290.688	1716.599	2259.379	2301.196	1865.429	1345.855	2159.849	3125.473	1474.7	1227.702	2012.191	1497.391	1289.456	2194.223	3448.56	1486.978	3482.839

LateStg2_021854	1631.081	2256.517	1994.206	2089.055	2115.195	2810.059	2315.125	1818.905	2089.664	2749.929	2461.663	2074.198	2346.469	2996.54	1614.691	2547.062	15433.09	2182.438	2600.972
LateStg2_021856	1757.762	1863.022	1772.417	2268.092	2477.31	2647.954	2176.189	1961.227	2287.684	4127.567	2147.37	1690.916	2545.96	2611.793	1702.953	2811.449	6186.714	2217.469	4138.507
LateStg2_021857	3901.552	3670.615	3626.944	4188.205	4158.46	4751.309	3987.096	3730.004	3967.139	5754.583	3596.755	3645.516	5079.897	5358.171	4070.815	12466.72	8308.568	4216.411	8964.893
LateStg2_021859	1470.294	1885.057	1935.032	2271.902	2294.143	3048.345	2117.187	1776.545	2313.868	3634.111	1929.03	1781.592	2948.952	2406.479	1991.047	3284.598	3241.526	2186.996	4791.754
LateStg2_021860	1377.228	1912.527	1545.966	2029.096	3843.036	2294.62	2148.919	1831.696	1964.879	2275.137	1656.999	1510.77	2197.393	2036.264	1510.892	2753.512	3284.302	4857.857	3644.717
LateStg2_021863	1389.546	1225.311	1220.319	1592.922	1506.453	1743.039	37436.57	20785.09	1770.846	2499.974	1226.013	1140.628	1928.769	1697.19	1274.661	2135.424	5495.126	77817.12	3516.442
LateStg2_021865	847.9526	72243.9	58963.65	1584.339	1561.829	1890.786	1446.563	1301.552	1523.649	2533.475	1172.621	1082.984	2098.228	1465.648	1554.264	2245.101	2560.516	15878.09	2716.509
LateStg2_021868	841.1623	1730.123	1106.992	1520.119	1326.74	1839.049	1526.568	1216.93	1359.645	2195.805	1229.105	1027.971	1746.367	1267.317	1314.046	2054.09	2606.964	1274.319	3338.825
LateStg2_021871	4293.53	11133.89	6076.056	6914.307	6755.31	13075.63	5942.146	5916.069	7184.004	15378.62	6834.217	5741.78	10556.95	7021.684	7260.707	32976.48	12429.49	8715.363	19074.28
LateStg2_021880	1790.208	1980.132	2614.285	2057.024	2096.017	3959.8	1832.259	1487.634	2133.393	4583.188	1977.947	1747.046	2667.728	2526.626	1632.411	3510.768	4512.184	2456.451	3226.212
LateStg2_021881	2384.826	1999.357	2666.056	2852.15	3033.592	3377.481	4271.626	3901.21	2895.45	7062.843	2719.433	2410.467	3115.291	4520.945	2407.219	4258.419	12393.42	2759.778	4259.74
LateStg2_021883	2065.41	1622.946	2569.527	2671.253	4642.282	3220.608	2297.504	2061.914	2484.5	16564.51	2198.001	2264.344	3763.834	17526.91	2545.744	4550.17	6416.215	2650.601	3773.043
LateStg2_021890	3022.65	1988.88	2282.66	2251.566	2248.979	3052.289	2415.519	2128.133	2178.214	16641.9	2523.614	2173.581	3724.101	2897.678	2109.595	7377.704	5154.293	2388.609	3284.202
LateStg2_021891	1124.762	961.2568	1354.482	1361.077	1241.26	1576.201	1462.741	1096.385	1186.718	1491.571	1603.177	1328.941	3133.506	3413.422	1392.966	3067.306	2590.952	1235.433	1900.166
LateStg2_021892	2474.483	4105.839	4501.21	2967.051	2868.365	4078.879	2353.423	2167.423	2937.444	5562.712	3328.625	2585.967	4427.688	3443.103	2566.329	4587.612	6758.511	3181.595	4032.283
LateStg2_021894	2221.702	1767.34	2488.434	1923.35	2652.345	5760.332	4985.451	1684.064	1890.908	3334.574	2396.437	2163.834	2887.473	3018.096	2241.106	4094.685	4474.155	5663.077	2795.796
LateStg2_021897	2094.173	1872.113	2564.54	3134.878	3177.1	3170.251	2812.481	2291.9	3112.535	6338.442	2545.71	2285.715	3852.078	3186.883	2396.901	6763.967	6534.485	2598.865	3976.887
LateStg2_021959	1944.575	1075.842	1370.23	6504.857	55850.97	2370.069	1624.237	1465.625	1477.145	6609.375	1137.616	1249.313	4849.732	1808.159	1777.565	2373.879	3499.995	1359.307	2181.833
LateStg2_021961	2126.008	2601.519	2931.139	3868.475	3742.739	5313.381	3308.218	3188.797	3932.856	8437.228	3262.102	2669.569	4774.873	3491.703	3494.667	5256.041	6366.071	3609.642	5268.444
LateStg2_021499	2712.5	2477.536	2535.785	3120.787	3083.258	4619.17	3050.456	3503.044	3477.192	5287.922	3132.654	2428.364	3670.777	4016.163	2945.576	8863.933	5309.148	3196.865	4388.622

Claims

1. A method for diagnosing Non-Small Cell Lung Cancer from a sample comprising serum and/or plasma extracted from a subject, comprising the steps of:
 - 5 (i) testing the sample for the presence of biomarkers specific for Non-Small Cell Lung Cancer;
 - (ii) determining whether the subject has Non-Small Cell Lung Cancer based on the detection of said biomarkers;characterised in that the biomarkers are autoantibodies to antigens comprising
10 XAGE1D, LRRFIP2 and GAGE2C.
2. The method according to claim 1 wherein the antigens further comprise one or more of DDX53, DDX43, GAGE1, MAGEA10, ZNRD1, MAP2K5, MAGEA4, STAT1, CT47A1, IGF2BP3, CTAG2, RAD23B, FADD, PTPN20A, TPM1, CTAG1A.
15
3. The method according to claim 1 or 2 wherein the antigens are biotinylated proteins.
4. The method according to claim 3 wherein each biotinylated protein is formed from
20 a Biotin Carboxyl Carrier Protein folding marker which is fused in-frame with a protein.
5. The method according to claim 3 or 4 wherein the biotinylated proteins are bound to a streptavidin-coated substrate.

6. The method according to claim 5 wherein the substrate comprises a hydrogel-forming polymer base layer.
- 5 7. The method according to any preceding claim wherein the antigens are exposed to a sample extracted from a person, such that autoantibody biomarkers from the sample may bind to the antigens.
8. The method according to claim 7 wherein the antigens are subsequently exposed to
10 a fluorescently-tagged secondary antibody to allow the amount of any autoantibodies from the sample bound to the antigens to be determined.
9. The method according to claim 8 wherein the presence of non-small cell lung cancer corresponds to the relative or absolute amount of autoantibodies from the
15 sample specifically binding to the antigens.
10. The method according to any preceding claim wherein the steps are performed *in vitro*.
- 20 11. The method according to any preceding claim wherein the method comprises detecting upregulation/downregulation of one or more of said biomarkers.
12. A method for manufacturing a kit for diagnosing Non-Small Cell Lung Cancer from a sample extracted from a subject, comprising the steps of:

for each antigen in a panel, cloning a biotin carboxyl carrier protein folding marker in-frame with a gene encoding the antigen and expressing the resulting biotinylated antigen;

binding the biotinylated proteins to addressable locations on one or more streptavidin-coated substrates, thereby forming an antigen array ;

such that the amount of autoantibodies from the sample binding to the antigens on the panel can be determined by exposing the substrate to the sample and measuring the response;

characterised in that the antigens comprise XAGE1D, LRRFIP2 and GAGE2C.

13. The method according to claim 12 wherein the antigens further comprise one or more of DDX53, DDX43, GAGE1, MAGEA10, ZNRD1, MAP2K5, MAGEA4, STAT1, CT47A1, IGF2BP3, CTAG2, RAD23B, FADD, PTPN20A, TPM1, CTAG1A.

14. A composition comprising a panel of antigens for detecting non-small cell lung cancer, characterised in that the antigens comprise XAGE1D, LRRFIP2 and GAGE2C.

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15. A composition according to claim 14 wherein the antigens further comprise one or more of DDX53, DDX43, GAGE1, MAGEA10, ZNRD1, MAP2K5, MAGEA4, STAT1, CT47A1, IGF2BP3, CTAG2, RAD23B, FADD, PTPN20A, TPM1, CTAG1A.

16. A composition according to claim 14 or 15 wherein the antigens are biotinylated proteins.
- 5 17. A composition according to any of claims 14-16 wherein the amount of one or more exosomal autoantibody biomarkers binding *in vitro* to the antigens in a sample from a patient can be measured to determine the presence of non-small cell lung cancer.
18. A composition comprising a panel of exosomal autoantibody biomarkers for
10 detecting non-small cell lung cancer:
wherein the levels of exosomal autoantibody biomarkers are measured in a sample from a NSCLC patient;
characterised in that the exosomal autoantibody biomarkers are selected from autoantibodies specific for at least X Antigen Family Member 1D (XAGE1D), LRR
15 Binding FLII Interacting Protein 2 (LRRFIP2) and G Antigen 2C (GAGE2C).