(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 April 2015 (23.04.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/057635 A1

(51) **International Patent Classification**:
C07K 14/705 (2006.01) ***C12Q 1/68*** (2006.01)
C07K 14/72 (2006.01) ***G01N 33/53*** (2006.01)
C12N 15/16 (2006.01)

(21) **International Application Number**:
PCT/US2014/060382

(22) **International Filing Date**:
14 October 2014 (14.10.2014)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/892,743 18 October 2013 (18.10.2013) US
61/992,615 13 May 2014 (13.05.2014) US

(71) **Applicant**: **THE REGENTS OF THE UNIVERSITY OF MICHIGAN** [US/US]; 1600 Huron Parkway, 2nd Floor, Ann Arbor, Michigan 48109-2590 (US).

(72) **Inventors**: **CHINNAIYAN, Arul M.**; 51300 Plymouth Valley Drive, Plymouth, Michigan 48170 (US). **ROBINSON, Dan**; 2968 Hunley Drive, Ann Arbor, Michigan 48105 (US). **WU, Yi-Mi**; 2968 Hunley Drive, Ann Arbor, Michigan 48105 (US).

(74) **Agent**: **ARENSON, Tanya A.**; Casimir Jones SC, 2275 Deming Way, Ste 310, Middleton, Wisconsin 53562 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2015/057635 A1

(54) **Title**: SYSTEMS AND METHODS FOR DETERMINING A TREATMENT COURSE OF ACTION

(57) **Abstract**: The present disclosure relates to methods of determining a treatment course of action. In particular, the present disclosure relates to mutations in the gene encoding estrogen receptor and their association with responsiveness to estrogen therapies for cancer.

SYSTEMS AND METHODS FOR DETERMINING A TREATMENT COURSE OF ACTION

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority to United States Provisional Patent Application Serial Number 61/892,743, filed October 18, 2013, and United States Provisional Patent Application Serial Number 61/992,615, filed May 13, 2014, the disclosures of which are herein incorporated by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under CA111275 and HG006508 awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

The present disclosure relates to methods of determining a treatment course of action. In particular, the present disclosure relates to mutations in the gene encoding estrogen receptor and their association with responsiveness to estrogen therapies for cancer.

BACKGROUND OF THE INVENTION

Breast cancer is the second most common form of cancer among women in the U.S., and the second leading cause of cancer deaths among women. While the 1980s saw a sharp rise in the number of new cases of breast cancer, that number now appears to have stabilized. The drop in the death rate from breast cancer is probably due to the fact that more women are having mammograms. When detected early, the chances for successful treatment of breast cancer are much improved.

Breast cancer, which is highly treatable by surgery, radiation therapy, chemotherapy, and hormonal therapy, is most often curable when detected in early stages. Mammography is the most important screening modality for the early detection of breast cancer. Breast cancer is classified into a variety of sub-types, but only a few of these affect prognosis or selection of therapy. Patient management following initial suspicion of breast cancer generally includes confirmation of the diagnosis, evaluation of stage of disease, and selection of

therapy. Diagnosis may be confirmed by aspiration cytology, core needle biopsy with a stereotactic or ultrasound technique for nonpalpable lesions, or incisional or excisional biopsy. At the time the tumor tissue is surgically removed, part of it is processed for determination of ER and PR levels.

Prognosis and selection of therapy are influenced by the age of the patient, stage of the disease, pathologic characteristics of the primary tumor including the presence of tumor necrosis, estrogen-receptor (ER) and progesterone-receptor (PR) levels in the tumor tissue, HER2 overexpression status and measures of proliferative capacity, as well as by menopausal status and general health. Overweight patients may have a poorer prognosis (Bastarrachea *et al.*, Annals of Internal Medicine, 120: 18 [1994]). Prognosis may also vary by race, with blacks, and to a lesser extent Hispanics, having a poorer prognosis than whites (Elledge *et al.*, Journal of the National Cancer Institute 86: 705 [1994]; Edwards *et al.*, Journal of Clinical Oncology 16: 2693 [1998]).

The three major treatments for breast cancer are surgery, radiation, and drug therapy. No treatment fits every patient, and often two or more are required. The choice is determined by many factors, including the age of the patient and her menopausal status, the type of cancer (*e.g.*, ductal vs. lobular), its stage, whether the tumor is hormone-receptive or not, and its level of invasiveness.

Breast cancer treatments are defined as local or systemic. Surgery and radiation are considered local therapies because they directly treat the tumor, breast, lymph nodes, or other specific regions. Drug treatment is called systemic therapy, because its effects are wide spread. Drug therapies include classic chemotherapy drugs, hormone blocking treatment (e.g., aromatase inhibitors, selective estrogen receptor modulators, and estrogen receptor downregulators), and monoclonal antibody treatment (e.g., against HER2). They may be used separately or, most often, in different combinations.

There is a need for additional diagnostic and treatment options, particularly treatments customized to a patient's tumor.

SUMMARY OF THE INVENTION

The present disclosure relates to methods of determining a treatment course of action. In particular, the present disclosure relates to mutations in the gene encoding estrogen receptor and their association with responsiveness to estrogen therapies for cancer.

In some embodiments, the present disclosure provides a method of treating cancer, comprising: assaying a sample from a subject diagnosed with cancer for the presence of a mutation in the estrogen receptor (ESR1) gene (e.g. one or more of p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, or p.Tyr537Asn); and determining a treatment course of action based on the presence of the mutation. In some embodiments, the method further comprises the step of administering the treatment when the mutation is present. In some embodiments, the treatment is an estrogen receptor antagonist (e.g., tamoxifen or fulvestrant). In some embodiments, the sample is, for example, tissue, blood, plasma, serum, endometrial cells, or breast cells. In some embodiments, the cancer is breast cancer or endometrial cancer. In some embodiments, the detecting comprises forming a complex between the ESR1 gene and a nucleic acid primer, probe, or pair of primers that specifically bind to the ESR1 gene. In some embodiments, the nucleic acid primer, probe, or pair of primers bind to the mutation in said ESR1 gene but not the wild type gene. In some embodiments, the ESR1 gene is assayed from circulating tumor nucleic acid. In some embodiments, the detecting comprising forming a complex between the mutant ESR1 polypeptide and an antibody that specifically binds to the variant amino acid sequence.

Further embodiments provide a method of monitoring treatment of cancer, comprising: administering a cancer therapy to a subject; assaying a sample from a subject diagnosed with cancer for the presence of a mutation in the estrogen receptor (ESR1) gene (e.g., p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, or p.Tyr537Asn); and determining a treatment course of action based on the presence of the mutation. In some embodiments, the method further comprises the step of administering the treatment when the mutations are present. In some embodiments, the cancer therapy is an aromatase inhibitor.

Additional embodiments provide a complex comprising a nucleic acid encoding estrogen receptor (ESR1) gene comprising a mutation selected from, for example, p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, or p.Tyr537Asn and a nucleic acid primer or probe that specifically hybridizes to a variant ESR1 nucleic acid endocing the mutant polypeptide but not the wild type nucleic acid. In some embodiments, a reaction mixture comprising a mutant ESR1 polypeptide and an antibody that specifically binds to the variant amino acid sequence is provided. In some embodiments, the present invention provides a multiplex (e.g., microarray) comprising reagents that binds to two or more variant ESR1 amino acid or nucleic acids.

In some embodiments, the present invention provides one or more nucleic acid probes or primers having 8 or more (e.g., 10 or more, 12 or more, 15 or more, 18 or more, etc.) nucleotides and that specifically bind to nucleic acids encoding a variant ESR polypeptide but not the wild type nucleic acid. In some embodiments, the present invention provides an antibody that specifically binds to variant ESR1 polypeptides but not wild type ESR1 polypeptides.

In some embodiments, the present invention provides a system comprising a computer processor and computer software configured to analyze information on the presence and absence of variant ESR1 polypeptides or amino acids encoding the polypeptides; and determine a treatment course of action based on the presence or absence of the variant gene or polypeptide.

Additional embodiments are described herein.

DESCRIPTION OF THE FIGURES

Figure 1 shows clinical timelines of the six index ER-positive metastatic breast cancer patients harboring *ESR1* mutations.

Figure 2 shows a schematic representation of ESR1 mutations identified in the experiments described herein.

Figure 3 shows that acquired ESR1 mutations are constitutively active.

Figure 4 shows that acquired *ESR1* mutations maintain sensitivity to antiestrogen therapies. Steroid hormone-deprived cells were either untreated or treated with increasing doses of antiestrogen drugs tamoxifen (A) or fulvestrant (B) in the presence of 5 nM of β-estradiol (E2) for 24 hrs.

Figure 5 shows that gene copy number landscape of the six index cases as assessed by whole exome sequencing matched to germline.

Figure 6 shows schematic representations of the predicted gene fusions identified by transcriptome sequencing in four breast cancer index cases. a, MO_1031: PLA2G12A-COL15A1. b, MO_1031: IPO9-PM20D1. c, MO_1031: LRP5-FAT3. d, MO_1051:CMASPIK3C2G. e, MO_1051: TBCK-PPA2. f, MO_1051: GPATCH8-MPP2. g, MO_1051: FGFR2-AFF3. h, MO_1069: UBN2-TTC26. i, MO_1069: TBCD-FOXK2. j, MO_1129: DDB1-PAK1. k, MO_1129: VPS35-SLCO2B1.

Figure 7 shows an analysis of transactivational activity of wild type and mutant ESR1 variants by luciferase reporter assay.

Figure 8 shows dose response of the wild type and mutant ESR1 variants to 4-hydroxytamoxifen, in competition with 1 nM estradiol.

Figure 9 shows dose response of the wild type and mutant ESR1 variants to fulvestrant, in competition with 1 nM estradiol.

Figure 10 shows inhibition of transactivation activity of wild type and mutant ESR1 variants by endoxifen.

Figure 11 shows dose response of the wild type and mutant ESR1 variants to estradiol.

DEFINITIONS

To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

As used herein, the terms “detect”, “detecting” or “detection” may describe either the general act of discovering or discerning or the specific observation of a detectably labeled composition.

As used herein, the term “subject” refers to any organisms that are screened using the diagnostic methods described herein. Such organisms preferably include, but are not limited to, mammals (e.g., humans).

The term “diagnosed,” as used herein, refers to the recognition of a disease by its signs and symptoms, or genetic analysis, pathological analysis, histological analysis, and the like.

As used herein, the term "characterizing cancer in a subject" refers to the identification of one or more properties of a cancer sample in a subject, including but not limited to, the presence of benign, pre-cancerous or cancerous tissue, the stage of the cancer, and the subject's prognosis. Cancers may be characterized by the identification of the expression of one or more cancer marker genes, including but not limited to, the ESR1 variants disclosed herein.

As used herein, the term "characterizing cancer in a subject" refers to the identification of one or more properties of a cancer sample (*e.g.*, including but not limited to, the presence of cancerous tissue, the presence or absence of ESR1 mutation, the presence of pre-cancerous tissue that is likely to become cancerous, and the presence of cancerous tissue that is likely to metastasize). In some embodiments, tissues are characterized by the

identification of the expression of one or more cancer marker genes, including but not limited to, the cancer markers disclosed herein.

As used herein, the term "stage of cancer" refers to a qualitative or quantitative assessment of the level of advancement of a cancer. Criteria used to determine the stage of a cancer include, but are not limited to, the size of the tumor and the extent of metastases (*e.g.*, localized or distant).

As used herein, the term "nucleic acid molecule" refers to any nucleic acid containing molecule, including but not limited to, DNA or RNA. The term encompasses sequences that include any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

The term "gene" refers to a nucleic acid (*e.g.*, DNA) sequence that comprises coding sequences necessary for the production of a polypeptide, precursor, or RNA (*e.g.*, rRNA, tRNA). The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (*e.g.*, enzymatic activity, ligand binding, signal transduction, immunogenicity, etc.) of the full-length or fragments are retained. The term also encompasses the coding region of a structural gene and the sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb or more on either end such that the gene corresponds to the length of the full-length mRNA. Sequences located 5' of the coding region and present on the mRNA are referred to as 5' non-translated sequences. Sequences located 3' or downstream of the coding region and present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a

gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene that are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

As used herein, the term "oligonucleotide," refers to a short length of single-stranded polynucleotide chain. Oligonucleotides are typically less than 200 residues long (*e.g.*, between 15 and 100), however, as used herein, the term is also intended to encompass longer polynucleotide chains. Oligonucleotides are often referred to by their length. For example a 24 residue oligonucleotide is referred to as a "24-mer". Oligonucleotides can form secondary and tertiary structures by self-hybridizing or by hybridizing to other polynucleotides. Such structures can include, but are not limited to, duplexes, hairpins, cruciforms, bends, and triplexes.

As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "5'-A-G-T-3'," is complementary to the sequence "3'-T-C-A-5'." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

The term "homology" refers to a degree of complementarity. There may be partial homology or complete homology (*i.e.*, identity). A partially complementary sequence is a nucleic acid molecule that at least partially inhibits a completely complementary nucleic acid molecule from hybridizing to a target nucleic acid is "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (*i.e.*, the hybridization) of a completely homologous nucleic acid molecule to a target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is

permitted; low stringency conditions require that the binding of two sequences to one another be a specific (*i.e.*, selective) interaction. The absence of non-specific binding may be tested by the use of a second target that is substantially non-complementary (*e.g.*, less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids. A single molecule that contains pairing of complementary nucleic acids within its structure is said to be "self-hybridized."

As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. Under "low stringency conditions" a nucleic acid sequence of interest will hybridize to its exact complement, sequences with single base mismatches, closely related sequences (*e.g.*, sequences with 90% or greater homology), and sequences having only partial homology (*e.g.*, sequences with 50-90% homology). Under 'medium stringency conditions," a nucleic acid sequence of interest will hybridize only to its exact complement, sequences with single base mismatches, and closely relation sequences (*e.g.*, 90% or greater homology). Under "high stringency conditions," a nucleic acid sequence of interest will hybridize only to its exact complement, and (depending on conditions such a temperature) sequences with single base mismatches. In other words, under conditions of high stringency the temperature can be raised so as to exclude hybridization to sequences with single base mismatches.

The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" or "isolated polynucleotide" refers to a nucleic acid sequence that is identified and separated from at least one component or contaminant with which it is ordinarily associated in its natural source. Isolated nucleic acid is such present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids as nucleic acids such as DNA and RNA found in the state they exist in nature. For example, a given DNA sequence (*e.g.*, a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence

encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. However, isolated nucleic acid encoding a given protein includes, by way of example, such nucleic acid in cells ordinarily expressing the given protein where the nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid, oligonucleotide, or polynucleotide may be present in single-stranded or double-stranded form. When an isolated nucleic acid, oligonucleotide or polynucleotide is to be utilized to express a protein, the oligonucleotide or polynucleotide will contain at a minimum the sense or coding strand (*i.e.*, the oligonucleotide or polynucleotide may be single-stranded), but may contain both the sense and anti-sense strands (*i.e.*, the oligonucleotide or polynucleotide may be double-stranded).

As used herein, the term "purified" or "to purify" refers to the removal of components (*e.g.*, contaminants) from a sample. For example, antibodies are purified by removal of contaminating non-immunoglobulin proteins; they are also purified by the removal of immunoglobulin that does not bind to the target molecule. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind to the target molecule results in an increase in the percent of target-reactive immunoglobulins in the sample. In another example, recombinant polypeptides are expressed in bacterial host cells and the polypeptides are purified by the removal of host cell proteins; the percent of recombinant polypeptides is thereby increased in the sample.

As used herein, the term "sample" is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, as well as biological and environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues (e.g., biopsy samples), cells, and gases. Biological samples include blood products, such as plasma, serum and the like. Such examples are not however to be construed as limiting the sample types applicable to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure relates to methods of determining a treatment course of action. In particular, the present disclosure relates to mutations in the gene encoding estrogen receptor and their association with responsiveness to estrogen therapies for cancer.

I. Diagnostic and Screening Methods

As described above, embodiments of the present invention provide diagnostic and screening methods that utilize the detection of mutations in ligand binding region of the estrogen receptor (ESR1) gene (e.g., p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, p.Tyr537Asn, and p.Asp538Gly). Exemplary, non-limiting methods are described below.

Any patient sample suspected of containing the ESR1 gene may be tested according to methods of embodiments of the present invention. By way of non-limiting examples, the sample may be tissue (*e.g.*, a breast, endometrial, ovarian, or uterine biopsy sample), blood, urine, or a fraction thereof (*e.g.*, plasma, serum, cells).

In some embodiments, the patient sample is subjected to preliminary processing designed to isolate or enrich the sample for the ESR1 gens or cells that contain the gene. A variety of techniques known to those of ordinary skill in the art may be used for this purpose, including but not limited to: centrifugation; immunocapture; cell lysis; and, nucleic acid target capture (*See*, *e.g.*, EP Pat. No. 1 409 727, herein incorporated by reference in its entirety).

In some embodiments, mutations in the ESR1 gene are monitored in circulating tumor DNA (See e.g., Dawson, S.J. et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 368, 1199-209 (2013); Diehl, F. et al. Nat Med 14, 985-90 (2008)).

In some embodiments, the ESR1 mutations are detected along with other markers in a multiplex or panel format. Markers are selected for their predictive value alone or in combination with the ESR1 mutations. Markers for other cancers, diseases, infections, and metabolic conditions are also contemplated for inclusion in a multiplex or panel format.

i. DNA and RNA Detection

The ESR1 mutation are detected using a variety of nucleic acid techniques known to those of ordinary skill in the art, including but not limited to: nucleic acid sequencing; nucleic acid hybridization; and, nucleic acid amplification.

1. Sequencing

A variety of nucleic acid sequencing methods are contemplated for use in the methods of the present disclosure including, for example, chain terminator (Sanger) sequencing, dye terminator sequencing, and high-throughput sequencing methods. Many of these sequencing

methods are well known in the art. See, e.g., Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467 (1997); Maxam et al., Proc. Natl. Acad. Sci. USA 74:560-564 (1977); Drmanac, et al., Nat. Biotechnol. 16:54-58 (1998); Kato, Int. J. Clin. Exp. Med. 2:193-202 (2009); Ronaghi et al., Anal. Biochem. 242:84-89 (1996); Margulies et al., Nature 437:376-380 (2005); Ruparel et al., Proc. Natl. Acad. Sci. USA 102:5932-5937 (2005), and Harris et al., Science 320:106-109 (2008); Levene et al., Science 299:682-686 (2003); Korlach et al., Proc. Natl. Acad. Sci. USA 105:1176-1181 (2008); Branton et al., Nat. Biotechnol. 26(10):1146-53 (2008); Eid et al., Science 323:133-138 (2009); each of which is herein incorporated by reference in its entirety.

Next-generation sequencing (NGS) methods share the common feature of massively parallel, high-throughput strategies, with the goal of lower costs in comparison to older sequencing methods (see, e.g., Voelkerding *et al.*, *Clinical Chem.*, *55:* 641-658, 2009; MacLean *et al.*, *Nature Rev. Microbiol.*, *7:* 287-296; each herein incorporated by reference in their entirety). NGS methods can be broadly divided into those that typically use template amplification and those that do not. Amplification-requiring methods include pyrosequencing commercialized by Roche as the 454 technology platforms (e.g., GS 20 and GS FLX), the Solexa platform commercialized by Illumina, and the Supported Oligonucleotide Ligation and Detection (SOLiD) platform commercialized by Applied Biosystems. Non-amplification approaches, also known as single-molecule sequencing, are exemplified by the HeliScope platform commercialized by Helicos BioSciences, and emerging platforms commercialized by VisiGen, Oxford Nanopore Technologies Ltd., Life Technologies/Ion Torrent, and Pacific Biosciences, respectively.

In pyrosequencing (Voelkerding *et al.*, *Clinical Chem.*, *55:* 641-658, 2009; MacLean *et al.*, *Nature Rev. Microbiol.*, *7:* 287-296; U.S. Pat. No. 6,210,891; U.S. Pat. No. 6,258,568; each herein incorporated by reference in its entirety), template DNA is fragmented, end-repaired, ligated to adaptors, and clonally amplified in-situ by capturing single template molecules with beads bearing oligonucleotides complementary to the adaptors. Each bead bearing a single template type is compartmentalized into a water-in-oil microvesicle, and the template is clonally amplified using a technique referred to as emulsion PCR. The emulsion is disrupted after amplification and beads are deposited into individual wells of a picotitre plate functioning as a flow cell during the sequencing reactions. Ordered, iterative introduction of each of the four dNTP reagents occurs in the flow cell in the presence of sequencing enzymes and luminescent reporter such as luciferase. In the event that an

appropriate dNTP is added to the 3′ end of the sequencing primer, the resulting production of ATP causes a burst of luminescence within the well, which is recorded using a CCD camera. It is possible to achieve read lengths greater than or equal to 400 bases, and 10^6 sequence reads can be achieved, resulting in up to 500 million base pairs (Mb) of sequence.

In the Solexa/Illumina platform (Voelkerding *et al.*, *Clinical Chem.*, *55:* 641-658, 2009; MacLean *et al.*, *Nature Rev. Microbiol.*, *7:* 287-296; U.S. Pat. No. 6,833,246; U.S. Pat. No. 7,115,400; U.S. Pat. No. 6,969,488; each herein incorporated by reference in its entirety), sequencing data are produced in the form of shorter-length reads. In this method, single-stranded fragmented DNA is end-repaired to generate 5′-phosphorylated blunt ends, followed by Klenow-mediated addition of a single A base to the 3′ end of the fragments. A-addition facilitates addition of T-overhang adaptor oligonucleotides, which are subsequently used to capture the template-adaptor molecules on the surface of a flow cell that is studded with oligonucleotide anchors. The anchor is used as a PCR primer, but because of the length of the template and its proximity to other nearby anchor oligonucleotides, extension by PCR results in the "arching over" of the molecule to hybridize with an adjacent anchor oligonucleotide to form a bridge structure on the surface of the flow cell. These loops of DNA are denatured and cleaved. Forward strands are then sequenced with reversible dye terminators. The sequence of incorporated nucleotides is determined by detection of post-incorporation fluorescence, with each fluor and block removed prior to the next cycle of dNTP addition. Sequence read length ranges from 36 nucleotides to over 250 nucleotides, with overall output exceeding 1 billion nucleotide pairs per analytical run.

Sequencing nucleic acid molecules using SOLiD technology (Voelkerding *et al.*, *Clinical Chem.*, *55:* 641-658, 2009; MacLean *et al.*, *Nature Rev. Microbiol.*, *7:* 287-296; U.S. Pat. No. 5,912,148; U.S. Pat. No. 6,130,073; each herein incorporated by reference in their entirety) also involves fragmentation of the template, ligation to oligonucleotide adaptors, attachment to beads, and clonal amplification by emulsion PCR. Following this, beads bearing template are immobilized on a derivatized surface of a glass flow-cell, and a primer complementary to the adaptor oligonucleotide is annealed. However, rather than utilizing this primer for 3′ extension, it is instead used to provide a 5′ phosphate group for ligation to interrogation probes containing two probe-specific bases followed by 6 degenerate bases and one of four fluorescent labels. In the SOLiD system, interrogation probes have 16 possible combinations of the two bases at the 3′ end of each probe, and one of four fluors at the 5′ end. Fluor color, and thus identity of each probe, corresponds to specified color-space coding

schemes. Multiple rounds (usually 7) of probe annealing, ligation, and fluor detection are followed by denaturation, and then a second round of sequencing using a primer that is offset by one base relative to the initial primer. In this manner, the template sequence can be computationally re-constructed, and template bases are interrogated twice, resulting in increased accuracy. Sequence read length averages 35 nucleotides, and overall output exceeds 4 billion bases per sequencing run.

In certain embodiments, nanopore sequencing (see, e.g., Astier et al., J. Am. Chem. Soc. 2006 Feb 8; 128(5):1705–10, herein incorporated by reference) is utilized. The theory behind nanopore sequencing has to do with what occurs when a nanopore is immersed in a conducting fluid and a potential (voltage) is applied across it. Under these conditions a slight electric current due to conduction of ions through the nanopore can be observed, and the amount of current is exceedingly sensitive to the size of the nanopore. As each base of a nucleic acid passes through the nanopore, this causes a change in the magnitude of the current through the nanopore that is distinct for each of the four bases, thereby allowing the sequence of the DNA molecule to be determined.

In certain embodiments, HeliScope by Helicos BioSciences (Voelkerding *et al.*, *Clinical Chem.*, *55:* 641-658, 2009; MacLean *et al.*, *Nature Rev. Microbiol.*, *7:* 287-296; U.S. Pat. No. 7,169,560; U.S. Pat. No. 7,282,337; U.S. Pat. No. 7,482,120; U.S. Pat. No. 7,501,245; U.S. Pat. No. 6,818,395; U.S. Pat. No. 6,911,345; U.S. Pat. No. 7,501,245; each herein incorporated by reference in their entirety) is utilized. Template DNA is fragmented and polyadenylated at the 3′ end, with the final adenosine bearing a fluorescent label. Denatured polyadenylated template fragments are ligated to poly(dT) oligonucleotides on the surface of a flow cell. Initial physical locations of captured template molecules are recorded by a CCD camera, and then label is cleaved and washed away. Sequencing is achieved by addition of polymerase and serial addition of fluorescently-labeled dNTP reagents. Incorporation events result in fluor signal corresponding to the dNTP, and signal is captured by a CCD camera before each round of dNTP addition. Sequence read length ranges from 25–50 nucleotides, with overall output exceeding 1 billion nucleotide pairs per analytical run.

The Ion Torrent technology is a method of DNA sequencing based on the detection of hydrogen ions that are released during the polymerization of DNA (see, e.g., *Science* 327(5970): 1190 (2010); U.S. Pat. Appl. Pub. Nos. 20090026082, 20090127589, 20100301398, 20100197507, 20100188073, and 20100137143, incorporated by reference in their entireties for all purposes). A microwell contains a template DNA strand to be

sequenced. Beneath the layer of microwells is a hypersensitive ISFET ion sensor. All layers are contained within a CMOS semiconductor chip, similar to that used in the electronics industry. When a dNTP is incorporated into the growing complementary strand a hydrogen ion is released, which triggers a hypersensitive ion sensor. If homopolymer repeats are present in the template sequence, multiple dNTP molecules will be incorporated in a single cycle. This leads to a corresponding number of released hydrogens and a proportionally higher electronic signal. This technology differs from other sequencing technologies in that no modified nucleotides or optics are used. The per-base accuracy of the Ion Torrent sequencer is ~99.6% for 50 base reads, with ~100 Mb to 100Gb generated per run. The read-length is 100-300 base pairs. The accuracy for homopolymer repeats of 5 repeats in length is ~98%. The benefits of ion semiconductor sequencing are rapid sequencing speed and low upfront and operating costs.

Stratos Genomics, Inc. sequencing involves the use of Xpandomers. This sequencing process typically includes providing a daughter strand produced by a template-directed synthesis. The daughter strand generally includes a plurality of subunits coupled in a sequence corresponding to a contiguous nucleotide sequence of all or a portion of a target nucleic acid in which the individual subunits comprise a tether, at least one probe or nucleobase residue, and at least one selectively cleavable bond. The selectively cleavable bond(s) is/are cleaved to yield an Xpandomer of a length longer than the plurality of the subunits of the daughter strand. The Xpandomer typically includes the tethers and reporter elements for parsing genetic information in a sequence corresponding to the contiguous nucleotide sequence of all or a portion of the target nucleic acid. Reporter elements of the Xpandomer are then detected. Additional details relating to Xpandomer-based approaches are described in, for example, U.S. Pat. Pub No. 20090035777, entitled "High Throughput Nucleic Acid Sequencing by Expansion," filed June 19, 2008, which is incorporated herein in its entirety.

Other emerging single molecule sequencing methods include real-time sequencing by synthesis using a VisiGen platform (Voelkerding *et al.*, *Clinical Chem.*, *55:* 641–58, 2009; U.S. Pat. No. 7,329,492; U.S. Pat. App. Ser. No. 11/671956; U.S. Pat. App. Ser. No. 11/781166; each herein incorporated by reference in their entirety) in which immobilized, primed DNA template is subjected to strand extension using a fluorescently-modified polymerase and florescent acceptor molecules, resulting in detectible fluorescence resonance energy transfer (FRET) upon nucleotide addition.

2. Hybridization

Illustrative non-limiting examples of nucleic acid hybridization techniques include, but are not limited to, *in situ* hybridization (ISH), microarray, and Southern or Northern blot. *In situ* hybridization (ISH) is a type of hybridization that uses a labeled complementary DNA or RNA strand as a probe to localize a specific DNA or RNA sequence in a portion or section of tissue (*in situ*), or, if the tissue is small enough, the entire tissue (whole mount ISH). DNA ISH can be used to determine the structure of chromosomes. RNA ISH is used to measure and localize mRNAs and other transcripts within tissue sections or whole mounts. Sample cells and tissues are usually treated to fix the target transcripts in place and to increase access of the probe. The probe hybridizes to the target sequence at elevated temperature, and then the excess probe is washed away. The probe that was labeled with either radio-, fluorescent- or antigen-labeled bases is localized and quantitated in the tissue using either autoradiography, fluorescence microscopy or immunohistochemistry, respectively. ISH can also use two or more probes, labeled with radioactivity or the other non-radioactive labels, to simultaneously detect two or more transcripts.

In some embodiments, ESR1 mutations are detected using fluorescence *in situ* hybridization (FISH). In some embodiments, FISH assays utilize bacterial artificial chromosomes (BACs). These have been used extensively in the human genome sequencing project (see *Nature* 409: 953-958 (2001)) and clones containing specific BACs are available through distributors that can be located through many sources, *e.g.*, NCBI. Each BAC clone from the human genome has been given a reference name that unambiguously identifies it. These names can be used to find a corresponding GenBank sequence and to order copies of the clone from a distributor.

The present invention further provides a method of performing a FISH assay on human cells (e.g., breast or endometrial cells). Specific protocols are well known in the art and can be readily adapted for the present invention. Guidance regarding methodology may be obtained from many references including: *In situ* Hybridization: Medical Applications (eds. G. R. Coulton and J. de Belleroche), Kluwer Academic Publishers, Boston (1992); *In situ* Hybridization: In Neurobiology; Advances in Methodology (eds. J. H. Eberwine, K. L. Valentino, and J. D. Barchas), Oxford University Press Inc., England (1994); *In situ* Hybridization: A Practical Approach (ed. D. G. Wilkinson), Oxford University Press Inc., England (1992)); Kuo, *et al.*, *Am. J. Hum. Genet. 49*:112-119 (1991); Klinger, *et al.*, *Am. J.*

Hum. Genet. 51:55-65 (1992); and Ward, *et al.*, *Am. J. Hum. Genet. 52*:854-865 (1993)). There are also kits that are commercially available and that provide protocols for performing FISH assays (available from *e.g.*, Oncor, Inc., Gaithersburg, MD). Patents providing guidance on methodology include U.S. 5,225,326; 5,545,524; 6,121,489 and 6,573,043. All of these references are hereby incorporated by reference in their entirety and may be used along with similar references in the art and with the information provided in the Examples section herein to establish procedural steps convenient for a particular laboratory.

3. Microarrays

Different kinds of biological assays are called microarrays including, but not limited to: DNA microarrays (*e.g.*, cDNA microarrays and oligonucleotide microarrays); protein microarrays; tissue microarrays; transfection or cell microarrays; chemical compound microarrays; and, antibody microarrays. A DNA microarray, commonly known as gene chip, DNA chip, or biochip, is a collection of microscopic DNA spots attached to a solid surface (*e.g.*, glass, plastic or silicon chip) forming an array for the purpose of expression profiling or monitoring expression levels for thousands of genes simultaneously. The affixed DNA segments are known as probes, thousands of which can be used in a single DNA microarray. Microarrays can be used to identify disease genes or transcripts (e.g., ESR1 mutations) by comparing gene expression in disease and normal cells. Microarrays can be fabricated using a variety of technologies, including but not limiting: printing with fine-pointed pins onto glass slides; photolithography using pre-made masks; photolithography using dynamic micromirror devices; ink-jet printing; or, electrochemistry on microelectrode arrays.

Southern and Northern blotting is used to detect specific DNA or RNA sequences, respectively. DNA or RNA extracted from a sample is fragmented, electrophoretically separated on a matrix gel, and transferred to a membrane filter. The filter bound DNA or RNA is subject to hybridization with a labeled probe complementary to the sequence of interest. Hybridized probe bound to the filter is detected. A variant of the procedure is the reverse Northern blot, in which the substrate nucleic acid that is affixed to the membrane is a collection of isolated DNA fragments and the probe is RNA extracted from a tissue and labeled.

4. Amplification

Nucleic acids may be amplified prior to or simultaneous with detection. Illustrative non-limiting examples of nucleic acid amplification techniques include, but are not limited to, polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), transcription-mediated amplification (TMA), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA). Those of ordinary skill in the art will recognize that certain amplification techniques (*e.g.*, PCR) require that RNA be reversed transcribed to DNA prior to amplification (*e.g.*, RT-PCR), whereas other amplification techniques directly amplify RNA (*e.g.*, TMA and NASBA).

5. Protein Detection

In some embodiments, variant ESR1 polypeptides are detected (e.g., using immunoassays or mass spectrometry).

Illustrative non-limiting examples of immunoassays include, but are not limited to: immunoprecipitation; Western blot; ELISA; immunohistochemistry; immunocytochemistry; flow cytometry; and, immuno-PCR. Polyclonal or monoclonal antibodies detectably labeled using various techniques known to those of ordinary skill in the art (*e.g.*, colorimetric, fluorescent, chemiluminescent or radioactive) are suitable for use in the immunoassays. Immunoprecipitation is the technique of precipitating an antigen out of solution using an antibody specific to that antigen. The process can be used to identify protein complexes present in cell extracts by targeting a protein believed to be in the complex. The complexes are brought out of solution by insoluble antibody-binding proteins isolated initially from bacteria, such as Protein A and Protein G. The antibodies can also be coupled to sepharose beads that can easily be isolated out of solution. After washing, the precipitate can be analyzed using mass spectrometry, Western blotting, or any number of other methods for identifying constituents in the complex.

A Western blot, or immunoblot, is a method to detect protein in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate denatured proteins by mass. The proteins are then transferred out of the gel and onto a membrane, typically polyvinyldiflroride or nitrocellulose, where they are probed using antibodies specific to the protein of interest. As a result, researchers can examine the amount of protein in a given sample and compare levels between several groups.

An ELISA, short for Enzyme-Linked ImmunoSorbent Assay, is a biochemical technique to detect the presence of an antibody or an antigen in a sample. It utilizes a minimum of two antibodies, one of which is specific to the antigen and the other of which is coupled to an enzyme. The second antibody will cause a chromogenic or fluorogenic substrate to produce a signal. Variations of ELISA include sandwich ELISA, competitive ELISA, and ELISPOT. Because the ELISA can be performed to evaluate either the presence of antigen or the presence of antibody in a sample, it is a useful tool both for determining serum antibody concentrations and also for detecting the presence of antigen.

Immuno-polymerase chain reaction (IPCR) utilizes nucleic acid amplification techniques to increase signal generation in antibody-based immunoassays. Because no protein equivalence of PCR exists, that is, proteins cannot be replicated in the same manner that nucleic acid is replicated during PCR, the only way to increase detection sensitivity is by signal amplification. The target proteins are bound to antibodies which are directly or indirectly conjugated to oligonucleotides. Unbound antibodies are washed away and the remaining bound antibodies have their oligonucleotides amplified. Protein detection occurs via detection of amplified oligonucleotides using standard nucleic acid detection methods, including real-time methods.

Mass spectrometry has proven to be a valuable tool for the determination of molecular structures of molecules of many kinds, including biomolecules, and is widely practiced today. Purified proteins are digested with specific proteases (e.g. trypsin) and evaluated using mass spectrometry. Many alternative methods can also be used. For instance, either matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI) mass spectrometric methods can be used. Furthermore, mass spectroscopy can be coupled with the use of two-dimensional gel electrophoretic separation of cellular proteins as an alternative to comprehensive pre-purification. Mass spectrometry can also be coupled with the use of peptide fingerprint database and various searching algorithms. Differences in post-translational modification, such as phosphorylation or glycosylation, can also be probed by coupling mass spectrometry with the use of various pretreatments such as with glycosylases and phosphatases. All of these methods are to be considered as part of this application.

In some embodiments, electrospray ionisation quadrupole mass spectrometry is utilized to detect ESR1 variants (See e.g., U.S. Patent 8,658,396; herein incorporated by reference in its entirety).

6. **Data Analysis**

In some embodiments, a computer-based analysis program is used to translate the raw data generated by the detection assay (*e.g.*, the presence, absence, or amount of a given marker or markers) into data of predictive value for a clinician. The clinician can access the predictive data using any suitable means. Thus, in some preferred embodiments, the present invention provides the further benefit that the clinician, who is not likely to be trained in genetics or molecular biology, need not understand the raw data. The data is presented directly to the clinician in its most useful form. The clinician is then able to immediately utilize the information in order to optimize the care of the subject.

The present invention contemplates any method capable of receiving, processing, and transmitting the information to and from laboratories conducting the assays, information provides, medical personal, and subjects. For example, in some embodiments of the present invention, a sample (*e.g.*, a biopsy or a serum sample) is obtained from a subject and submitted to a profiling service (*e.g.*, clinical lab at a medical facility, genomic profiling business, etc.), located in any part of the world (*e.g.*, in a country different than the country where the subject resides or where the information is ultimately used) to generate raw data. Where the sample comprises a tissue or other biological sample, the subject may visit a medical center to have the sample obtained and sent to the profiling center, or subjects may collect the sample themselves (*e.g.*, a urine sample) and directly send it to a profiling center. Where the sample comprises previously determined biological information, the information may be directly sent to the profiling service by the subject (*e.g.*, an information card containing the information may be scanned by a computer and the data transmitted to a computer of the profiling center using an electronic communication systems). Once received by the profiling service, the sample is processed and a profile is produced (*i.e.*, ESR1 variant data), specific for the diagnostic or prognostic information desired for the subject.

The profile data is then prepared in a format suitable for interpretation by a treating clinician. For example, rather than providing raw expression data, the prepared format may represent a diagnosis or risk assessment (*e.g.*, presence or absence of a mutation in ESR1) for the subject, along with recommendations for particular treatment options. The data may be displayed to the clinician by any suitable method. For example, in some embodiments, the profiling service generates a report that can be printed for the clinician (*e.g.*, at the point of care) or displayed to the clinician on a computer monitor.

In some embodiments, the information is first analyzed at the point of care or at a regional facility. The raw data is then sent to a central processing facility for further analysis and/or to convert the raw data to information useful for a clinician or patient. The central processing facility provides the advantage of privacy (all data is stored in a central facility with uniform security protocols), speed, and uniformity of data analysis. The central processing facility can then control the fate of the data following treatment of the subject. For example, using an electronic communication system, the central facility can provide data to the clinician, the subject, or researchers.

In some embodiments, the subject is able to directly access the data using the electronic communication system. The subject may chose further intervention or counseling based on the results. In some embodiments, the data is used for research use. For example, the data may be used to further optimize the inclusion or elimination of markers as useful indicators of a particular condition or stage of disease or as a companion diagnostic to determine a treatment course of action.

6. Compositions & Kits

Compositions for use in the diagnostic methods described herein include, but are not limited to, probes, amplification oligonucleotides, and the like. In some embodiments, kits include all components necessary, sufficient or useful for detecting the markers described herein (e.g., reagents, controls, instructions, etc.). The kits described herein find use in research, therapeutic, screening, and clinical applications.

The probe and antibody compositions of the present invention may also be provided in the form of an array.

In some embodiments, the present invention provides one or more nucleic acid probes or primers having 8 or more (e.g., 10 or more, 12 or more, 15 or more, 18 or more, etc.) nucleotides, and that specifically bind to nucleic acids encoding a variant ESR polypeptide but not the wild type nucleic acid. In some embodiments, the present invention provides an antibody that specifically binds to variant ESR1 polypeptides but not wild type ESR1 polypeptides.

Embodiments of the present invention provide complexes of ESR1 nucleic acids or polypeptides with nucleic acid primers or probes or antibodies. In some embodiments, the primers, probes, or antibodies bind only to the variant or mutant forms of ESR1 described herein. In some embodiments, a reaction mixture comprising a mutant ESR1 polypeptide and

an antibody that specifically binds to the variant amino acid sequence is provided. In some embodiments, the present invention provides a multiplex (e.g., microarray) comprising reagents that binds to two or more variant ESR1 amino acid or nucleic acids.

III. Treatment Methods

Embodiments of the present disclosure provide methods of determining a treatment course of action and administering an anti-cancer treatment. For example, in some embodiments, subjects diagnosed with cancer (e.g., endometrial cancer or breast cancer) are screened for the presence or absence of one or more of the ESR1 mutations described herein (e.g., p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, or p.Tyr537Asn) and the results are used to determine a treatment course of action. For example, in some embodiments, subjects identified as having one or more of the ESR1 mutations before beginning treatment or that develop during treatment are administered an estrogen receptor antagonist (e.g., tamoxifen or fulvestrant). In some embodiments, subjects not found to have the ESR1 variants are not administered an estrogen receptor antagonist.

In some embodiments, patients currently undergoing cancer treatment (e.g., with an aromatase inhibitor such as, for example, exemestane, anastrozole and letrozole) are screened for the presence or absence of one or more mutations in ESR1. In some embodiments, subjects found to have the mutations are administered an estrogen receptor antagonist in addition to or instead of the aromatase inhibitor.

In some embodiments, assays for ESR1 mutations are repeated (e.g., before, during or after anticancer treatment). In some embodiments, assays are repeated daily, weekly, monthly, annually, or less often.

EXPERIMENTAL

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

Example 1

A. Methods

Clinical Study and specimen collection

Sequencing of clinical samples was performed under Institutional Review Board (IRB)–approved studies at the University of Michigan. Patients were enrolled and consented for integrative tumor sequencing, MI-ONCOSEQ (Michigan Oncology Sequencing Protocol, IRB# HUM00046018). Medically qualified patients 18 years or older with advanced or refractory cancer were eligible for the study. Informed consent details the risks of integrative sequencing and includes up-front genetic counseling. Informed consent was obtained from all subjects included in this study. Biopsies were arranged for safely accessible tumor sites. Needle biopsies were snap frozen in OCT and a longitudinal section was cut. Hematoxylin and eosin (H&E) stained frozen sections were reviewed by pathologists to identify cores with highest tumor content. Remaining portions of each needle biopsy core were retained for nucleic acid extraction.

Extraction of DNA and RNA

Genomic DNA from frozen needle biopsies and blood was isolated using the Qiagen DNeasy Blood & Tissue Kit, according to the manufacturer's instructions. Total RNA was extracted from frozen needle biopsies using the Qiazol reagent with disruption using a 5mm bead on a Tissuelyser II (Qiagen), and purified using a miRNeasy kit (Qiagen) with DNase I digestion, according to the manufacturer's instructions. RNA integrity was verified on an Agilent 2100 Bioanalyzer using RNA Nano reagents (Agilent Technologies).

Preparation of next generation sequencing libraries

Transcriptome libraries were prepared using 1-2 μg of total RNA. Poly(A)+ RNA was isolated using Sera-Mag oligo(dT) beads (Thermo Scientific) and fragmented with the Ambion Fragmentation Reagents kit (Ambion, Austin, TX). cDNA synthesis, end-repair, A-base addition, and ligation of the Illumina indexed adapters were performed according to Illumina's TruSeq RNA protocol (Illumina). Libraries were size-selected for 250-300 bp cDNA fragments on a 3% Nusieve 3:1 (Lonza) agarose gel, recovered using QIAEX II gel extraction reagents (Qiagen), and PCR-amplified using Phusion DNA polymerase (New England Biolabs). The amplified libraries were purified using AMPure XP beads (Beckman Coulter). Library quality was measured on an Agilent 2100 Bioanalyzer for product size and concentration. Paired-end libraries were sequenced with the Illumina HiSeq 2000, (2x100

nucleotide read length). Reads that passed the chastity filter of Illumina BaseCall software were used for subsequent analysis.

Exome libraries of matched pairs of tumor / normal genomic DNAs were generated using the Illumina TruSeq DNA Sample Prep Kit, following the manufacturer's instructions. In brief, 1-3 μg of each genomic DNA was sheared using a Covaris S2 to a peak target size of 250 bp. Fragmented DNA was concentrated using AMPure XP beads, followed by end-repair, A-base addition, and ligation of the Illumina indexed adapters. The adapter-ligated libraries were electrophoresed on 3% Nusieve agarose gels (Lonza) and fragments between 300 to 350 bp were recovered using QIAEX II gel extraction reagents (Qiagen). Recovered DNA was amplified using Illumina index primers for 8 cycles, purified using AMPure XP beads, and the DNA concentration was determined using a Nanodrop spectrophotometer. 1 μg of the library was hybridized to the Agilent SureSelect Human All Exon V4 at 65°C for 60 hr following the manufacturer's protocol (Agilent). The targeted exon fragments were captured on Dynal M-280 streptavidin beads (Invitrogen), and enriched by amplification with the Illumina index primers for 9 additional PCR cycles. PCR products were purified with AMPure XP beads and analyzed for quality and quantity using an Agilent 2100 Bioanalyzer and DNA 1000 reagents.

The publicly available software FastQC was used to assess sequencing quality. For each lane, the per-base quality scores were examined across the length of the reads. Lanes were deemed passing if the per-base quality score boxplot indicated that >85% of the reads had >Q20 for bases 1-100. In addition to the raw sequence quality, alignment quality was assessed using the Picard package. This allows monitoring of duplication rates and chimeric reads that may result from ligation artifacts; crucial statistics for interpreting the results of copy number and structural variant analysis.

Gene Fusion Detection

Paired-end transcriptome sequencing reads were aligned to the human reference genome (GRCh37/hg19) using a RNA-Seq spliced read mapper Tophat2 (Kim, D. & Salzberg, S.L. Genome Biol 12, R72 (2011) (Tophat 2.0.4), with '--fusion-search' option turned on to detect potential gene fusion transcripts. In the initial process, Tophat2 internally deploys an ultrafast short read alignment tool Bowtie (Version 0.12.8) to map the transcriptome data. Potential false positive fusion candidates were filtered out using 'Tophat-Post-Fusion' module. Further, the fusion candidates were manual examined for annotation

and ligation artifacts. Junction reads supporting the fusion candidates were re-aligned using an alignment tool BLAT to reconfirm the fusion breakpoint. Full length sequence of the fusion gene was constructed based on supporting junction reads, and evaluated for potential open reading frames (ORF) using an ORF finder. Further, the gene fusions with robust ORFs, the amino acid sequences of the fused proteins were explored using the Simple Modular Architecture Research Tool (SMART) to examine the gain or loss of known functional domains in the fusion proteins.

Gene Expression

The BAM file 'accepted_hits.bam' which was generated by the Tophat mapping module, was utilized to quantify the expression data, through Cufflinks (Trapnell, C. et al. Nat Protoc 7, 562-78 (2012)) (Version 2.0.2), an isoform assembly and RNA-Seq quantitation package. Structural features of 56,369 transcripts from the Ensemble resource (Ensemble66) was used as an annotation reference for quantifying expression of individual transcripts / isoforms. The 'Max Bundle Length' parameter was set to '10000000' and 'multi-read-correct' is flagged on to perform an initial estimation procedure to more accurately weight reads mapping to multiple locations in the genome.

Mutation analysis

Whole-exome sequencing was performed on Illumina *HiSeq 2000* or *HiSeq 2500* in paired-end mode and the primary base call files were converted into *FASTQ* sequence files using the bcl2fastq converter tool *bcl2fastq-1.8.4* in the *CASAVA 1.8* pipeline. The *FASTQ* sequence files generated were then processed through an in-house pipeline constructed for whole-exome sequence analyses of paired cancer genomes. The sequencing reads were aligned to the reference genome build *hg19*, *GRCh37* using *Novoalign* Multithreaded (*Version2.08.02*) (Novocraft) and converted into BAM files using *SAMtools* (*Version 0.1.18*) (Li, H. et al. Bioinformatics 25, 2078-9 (2009)). Sorting and indexing of BAM files utilized *Novosort* threaded (*Version 1.00.01*) and duplicates reads were removed using *Picard* (*Version 1.74*). Mutation analysis was performed using *VarScan*2 algorithms (*Version2.3.2*) (Koboldt, D.C. et al. Genome Res 22, 568-76 (2012)) utilizing the pileup files created by *SAMtools mpileup* for tumor and matched normal samples, simultaneously performing the pairwise comparisons of base call and normalized sequence depth at each position. For single nucleotide variant detection, filtering parameters including coverage; variant read support,

variant frequency, *P*-value, base quality, homopolymer, and strandedness are applied. For indels analysis *Pindel (Version 0.2.4)* was used on tumor and matched normal samples and indels common in both samples were classified as germline and indels present in tumor but not in normal were classified as somatic. Finally, the list of candidate indels as well as somatic and/or germline mutations was generated by excluding synonymous SNVs. *ANNOVAR* (Wang, K., Li, M. & Hakonarson, H. Nucleic Acids Res 38, e164 (2010)) was used to functionally annotate the detected genetic variants and positions are based on Ensemble66 transcript sequences.

Tumor content for each tumor exome library was estimated from the sequence data by fitting a binomial mixture model with two components to the set of most likely SNV candidates on 2-copy genomic regions. The set of candidates used for estimation consisted of coding variants that (1) exhibited at least 3 variant fragments in the tumor sample, (2) exhibited zero variant fragments in the matched benign sample with at least 16 fragments of coverage, (3) were not present in dbSNP, (4) were within a targeted exon or within 100 base pairs of a targeted exon, (5) were not in homopolymer runs of four or more bases, and (6) exhibited no evidence of amplification or deletion. In order to filter out regions of possible amplification or deletion, we used exon coverage ratios to infer copy number changes, as described below. Resulting SNV candidates were not used for estimation of tumor content if the segmented log-ratio exceeded 0.2 in absolute value. Candidates on the Y chromosome were also eliminated because they were unlikely to exist in 2-copy genomic regions. Using this set of candidates, we fit a binomial mixture model with two components using the R package flexmix, version 2.3-8. One component consisted of SNV candidates with very low variant fractions, presumably resulting from recurrent sequencing errors and other artifacts. The other component, consisting of the likely set of true SNVs, was informative of tumor content in the tumor sample. Specifically, under the assumption that most or all of the observed SNV candidates in this component are heterozygous SNVs, we expect the estimated binomial proportion of this component to represent one-half of the proportion of tumor cells in the sample. Thus, the estimated binomial proportion as obtained from the mixture model was doubled to obtain an estimate of tumor content.

Copy number aberrations were quantified and reported for each gene as the segmented normalized log2-transformed exon coverage ratios between each tumor sample and matched normal sample (Lonigro, R.J. et al. Neoplasia 13, 1019-25 (2011)). To account for observed associations between coverage ratios and variation in GC content across the

genome, lowess normalization was used to correct per-exon coverage ratios prior to segmentation analysis. Specifically, mean GC percentage was computed for each targeted region, and a lowess curve was fit to the scatterplot of log2-coverage ratios vs. mean GC content across the targeted exome using the lowess function in R (version 2.13.1) with smoothing parameter f=0.05.

Partially redundant sequencing of areas of the genome affords the ability for cross validation of findings. We cross-validated exome-based point mutation calls by manually examining the genomic and transcriptomic reads covering the mutation using the UCSC Genome Browser. Likewise, gene fusion calls from the transcriptome data can be further supported by structural variant detection in the genomic sequence data, as well as copy number information derived from the genome and exome sequencing.

Chemicals and reagents

β-Estradiol, (Z)-4-Hydroxytamoxifen, (E/Z)-Endoxifen Hydrochloride Hydrate, and Fulvestrant were purchased from Sigma- Aldrich.

Plasmids and Cloning

cDNA for the wild type *ESR1* was PCR amplified from a breast cell line MCF7 with the introduction of an N-terminal FLAG tag. cDNA encoding the relevant mutations of *ESR1* were generated by site-directed mutagenesis (QuikChange, Agilent) and full-length constructs were fully sequenced. All the *ESR1* variants were placed in the Lentivial vector pCDH (System Biosciences) for eukaryotic expression.

ERE-luciferase reporter assay

For cell transfection experiments, HEK-293T cells were plated at a density of 1–2 × 10^5 per well (24-well plates) in phenol red-free Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and antibiotics. Once cells attached, replaced medium with DMEM containing 10% charcoal-dextran treated FBS (HyClone) and cultured overnight. The next day, cells were transiently co-transfected with ESR1-expression plasmid at 50 ng /well and luciferase reporter constructs at 25 ng per well (SABiosciences) using the FuGene 6 reagent (Promega). The ER-responsive luciferase plasmid encoding the firefly luciferase reporter gene is driven by a minimal CMV promoter and tandem repeats of the estrogen transcriptional response element (ERE). A second plasmid constitutively

expressing Renilla luciferase is served as an internal control for normalizing transfection efficiencies (Cignal ERE Reporter, SABiosciences). After transfection for 18 hrs, cells were serum-starved for a few hours before treatment with β-estradiol or anti-estrogen drugs. Cells were harvested 18 hr post-treatment, and luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega). IC50 values were computed using the GraphPad Prizm application to fit a four parameter doseresponse curve.

Results

Advances in high-throughput sequencing technologies are beginning to establish a molecular taxonomy for a spectrum of human diseases and facilitate a move towards "precision medicine" (Chin, L., et al., Nat Med 17, 297-303 (2011); Meyerson, M., et al., Nat Rev Genet 11, 685-96 (2010)). With regards to oncology, defining the mutational landscape of an individual patient's tumor leads to more precise treatment and management of cancer patients. Comprehensive clinical sequencing programs for cancer patients have been initiated at a variety of medical centers (Roychowdhury, S. et al. Sci Transl Med 3, 111ra121 (2011); Welch, J.S. et al. JAMA 305, 1577-84 (2011)). In addition to the potential of identifying "actionable" therapeutic targets in cancer patients, these clinical sequencing efforts also shed light on acquired resistance mechanisms developed to targeted therapies (Gorre, M.E. et al. Science 293, 876-80 (2001); Korpal, M. et al. Cancer Discov (2013); Joseph, J.D. et al. Cancer Discov (2013)).

ER is the primary therapeutic target in breast cancer and is expressed in 70% of cases (Ariazi, E.A., et al., Curr Top Med Chem 6, 181-202 (2006)). Drugs directly antagonizing ER such as tamoxifen and fulvestrant are a mainstay of breast cancer treatment, however approximately 30% of ER positive breast cancer exhibit *de novo* resistance while 40% acquire resistance to these therapies (Riggins, R.B., et al., Cancer Lett 256, 1-24 (2007)). In addition to anti-estrogen therapies, ER-positive breast cancer patients are also treated with aromatase inhibitors such as letrozole or exemestane (Lonning, P.E. & Eikesdal, H.P. Endocr Relat Cancer 20, R183-201 (2013)). Aromatase inhibitors block peripheral conversion of androgens to estrogen, and in post-menopausal women, lead to over a 98% decrease in circulating levels of estrogen. Like anti-estrogens, patients treated with aromatase inhibitors develop resistance, but presumably due to different mechanisms. Breast cancer patients that develop resistance to aromatase inhibitors, often still respond to anti-estrogen therapies (Ingle, J.N. et al. J Clin Oncol 24, 1052-6 (2006)). The molecular mechanisms of endocrine

resistance in ER positive breast cancer continues to be an active area of research (Osborne, C.K. & Schiff, R. Mechanisms of endocrine resistance in breast cancer. Annu Rev Med 62, 233-47 (2011)).

The clinical sequencing program, called MI-ONCOSEQ (the Michigan Oncology Sequencing Program), enrolls patients with advanced cancer across all histologies (Welch et al., supra). Since April of 2011, it has enrolled over 200 patients by obtaining a current tumor biopsy with matched normal samples (blood and/or buccal swab). The samples are then subjected to integrative sequencing which includes whole exome sequencing of the tumor and matched normal, transcriptome sequencing, and as needed, low pass whole genome sequencing (Welch et al., supra). This combination of DNA and RNA sequencing technologies allows one to be relatively comprehensive with regards to the mutational landscape of coding genes including point mutations, indels, amplifications, deletions, gene fusions/translocations, and outlier gene expression. These results are generated within a 5 to 7 week time frame and are presented at an institutional "precision medicine tumor board" to deliberate upon potentially actionable findings.

As part of the MI-ONCOSEQ program, 11 patients with metastatic ER-positive breast cancer were subjected to sequencing analysis (**Tables 1 and 2**). A diverse array of aberrations were identified in individual patients, including mutations in *PIK3CA* (n=4), *BRCA1* aberrations (n=2), *FGFR2* aberrations (n=2) (Wu, Y.M. et al. Identification of Targetable FGFR Gene Fusions in Diverse Cancers. Cancer Discov 3, 636-647 (2013)), *NOTCH2* frameshift deletion (n=1), cyclins and associated cyclin-dependent kinase aberrations (n=3), and *MDM2* amplification/overexpression (n=1). Aberrations were also found frequently in the tumor suppressor *TP53* (n=6), DNA mismatch repair gene *MSH2* (n=1), and epigenetic regulators (n=2) including *ARID2*, *ARID1A*, *SMARCA4*, among others. The complete spectrum of somatic mutations with associated expression levels and copy number alterations in the index cases are given in **Tables 3 and 4, and Figure 5**. Two of the index cases, MO_1031 and MO_1051, exhibited a high level of mutations consistent with the "Signature B" identified in a whole genome study of mutational processes in breast cancer (Nik-Zainal, S. et al. Cell 149, 979-93 (2012)). There were 39 gene fusions identified in the 6 index cases with 11 encoding in-frame fusion proteins (**Table 5** and **Figure 6**), including an activating fusion of *FGFR2-AFF3* (Wu et al., supra).

Nonsynonymous mutations were identified in the LBD of *ESR1* (n=6). The six index patients MO_1031, MO_1051, MO_1069, MO_1129, MO_1167, and MO_1185 had LBD

mutations in amino acids p.Leu536Gln, p.Tyr537Ser, p.Asp538Gly, p.Tyr537Ser, p.Asp538Gly, and p.Tyr537Ser, respectively. The respective mutation in each case was detected by whole exome sequencing of the tumor relative to matched normal, as well as corroborated with whole transcriptome sequencing since *ESR1* was expressed at moderate to high levels (**Table 3**). The clinical histories of the index patients are depicted as timelines in **Figure 1**. For three of the patients (MO_1051, MO_1069, and MO_1129), primary diagnostic material showed that the *ESR1* mutations were not present at an earlier stage, indicating that they were acquired after endocrine therapy (**Figure 1** and **Table 3**). All of the index patients were treated with anti-estrogens (tamoxifen and/or fulvestrant) and aromatase inhibitors (letrozole, anastrozole, and/or exemestane). Two of the patients also had an oophorectomy. Comparison of the mutations present in each primary versus post-treatment pair showed a significant number of shared mutations in both samples of the pair, including activating mutations in *PIK3CA* in two of the cases. Thus, it is clear that the index patients presented with recurrent disease of the original primary tumor surviving in an estrogen deprived state, and presenting with acquired *ESR1* mutations. Of note, neither *ESR1* amplifications nor gene fusions were observed in these patients.

The 5 novel LBD mutations of *ESR1* identified in this study are depicted in **Figure 2**. Each occur in the vicinity of the synthetic mutations of *ESR1* which are inverted in response to tamoxifen and involve amino acid alterations p.Met543Ala and p.Leu544Ala (Inv-mut-AA2) (Feil, R., et al., Biochem Biophys Res Commun 237, 752-7 (1997)) and served as a positive control for our subsequent *in vitro* studies. It was next assessed whether tumor types other than ER positive metastatic hormone-resistant breast cancer also acquire ligand binding mutations in *ESR1*. The Cancer Genome Atlas Project (TCGA), which has generated whole exome data on 27 tumor types across at least 4000 individual samples was utilized. LBD mutations of *ESR1* were not detected in the 390 ER-positive breast cancers sequenced by TCGA, as these were primary resection samples before hormonal treatment (TCGA. Comprehensive molecular portraits of human breast tumours. Nature 490, 61-70 (2012)), nor have we detected *ESR1* mutations in a cohort of 80 triple negative breast carcinoma transcriptomes (unpublished data).

As the LBD mutations of *ESR1* we identified were somatic and acquired after treatment, we next assessed whether they were dependent on estrogen for activation. We cloned into expression vectors each of the five *ESR1* mutations identified in this study (p.Leu536Gln, p.Tyr537Ser, p.Asp538Gly, p.Tyr537Cys, and p.Tyr573Asn) and subsequently co-transfected

them into HEK-293 cells with an ERE-luciferase reporter system. Steroid hormone deprived cells were then exposed to β-estradiol for 24 hours and ER reporter levels assessed. Unlike wild-type ER which had little ER reporter activity in the absence of ligand, all 5 of the *ESR1* mutations exhibited strong constitutive activation of the ER reporter that was not markedly enhanced with β-estradiol (**Figure 3**). This indicated that each of the mutations developed in the context of evolution during an estrogen deprived state. Consistent with this, a whole genome sequencing study of 46 cases of estrogen receptor positive breast cancer patients on two aromatase inhibitor trials did not identify any of these *ESR1* mutations in the pretreatment samples analyzed (Ellis, M.J. et al. Nature 486, 353-60 (2012)).

Next, it was assessed whether anti-estrogen therapies affected the functional activity of these LBD mutations. As inhibition effects can be influenced by level of ectopic estrogen receptor expression, a dose response study of expression plasmid was performed 50 ng was selected for the following experiments (Huang, H.J., et al., Mol Endocrinol 16, 1778-92 (2002)) (**Figure 7**). Wild-type ER was inhibited in a dose-dependent fashion by the anti-estrogens 4-hydroxytamoxifen, fulvestrant and endoxifen (**Figures 4, 8, 9, and 10**). In addition, the synthetic *ESR1* mutation (Inv-mut-AA2) was activated in a dose-dependent fashion by these anti-estrogens (**Figure 4**), which has been reported previously (Feil, et al., Biochem Biophys Res Commun 237, 752-7 (1997)). Each of the 5 LBD mutations of *ESR1* identified in this study was inhibited by tamoxifen and fulvestrant in a dose-dependent fashion and do not exhibit the inverted response to antiestrogens that the synthetic mutation Inv-mut-AA2 does. It is possible that these mutations did not arise under selective pressure of anti-estrogen treatment, but rather in the context of an estrogen deprivation setting such as treatment with aromatase inhibitors and/or oophorectomy. The IC50s for both 4-hydroxytamoxifen and fulvestrant were 2 to 4 fold higher for all the mutants compared to wild type ESR1. Fulvestrant exhibited greater maximal inhibition than 4-hydroxytamoxifen for all the mutants tested (**Figures 8 and 9**).

The *ESR1* mutations identified in this study cluster near the beginning of helix 12 (**Figure 2**). Structural studies have demonstrated a key role in the position of helix 12 in the response of the estrogen receptor to agonists and antagonists (Shiau, A.K. et al. Cell 95, 927-37 (1998), and p.Tyr537 has been postulated to form a capping motif contributing to activity of the receptor (Skafar, Cell Biochem Biophys 33, 53-62 (2000)). Specifically the p.Tyr537Ser mutant has been reported to have higher affinity for estrogen than wild type and interacts with the SRC1 coactivator in the absence of ligand (Carlson et al., Biochemistry 36,

14897-905 (1997); Weis et al., Mol Endocrinol 10, 1388-98 (1996)). Several studies using experimental mutagenesis have implicated the same three residues identified here as critical determinants of transcriptional activity of the receptor (Carlson et al., supra; Pearce, et al., J Biol Chem 278, 7630-8 (2003); Zhao, C. et al. J Biol Chem 278, 27278-86 (2003)).

As estrogen therapy has been shown to have positive effect in treating aromatase inhibitor resistant advance breast cancers, we tested the effect of low to high dose estrogen on the activity of the mutants in the transient luciferase reporter assay (**Figure 11**) (Ellis, M.J. et al. JAMA 302, 774-80 (2009); Swaby, R.F. & Jordan, Clin Breast Cancer 8, 124-33 (2008)). The results do not suggest the effectiveness of this therapy is via directly influencing the transcriptional activity of these mutants, if present in the responding patients.

The experiments described herein revealed either *de novo* driver mutations and/or potential acquired mutations in breast cancer such as PI3K activation, *PAK1* amplification, and FGFR fusion/amplification which have been described earlier (Wu, Y.M. et al. Cancer Discov 3, 636-647 (2013); Kan, Z. et al. Nature 466, 869-73 (2010); Shrestha, Y. et al. Oncogene 31, 3397-408 (2012). Focal amplification of *MDM2* (a negative regulator of p53 which is targetable) and copy gains of gonadotropin-releasing hormone receptor (GNRHR) were identified.

Since the LBD mutations of *ESR1* identified in this study are constitutively active, they can function in the absence of ligand, and maintain ER signaling. In 1997, an LBD mutation of *ESR1*, p.Tyr537Asn, was detected in a single patient with Stage IV metastatic breast cancer who had been treated with diethylstibesterol--but since then, this mutation has been considered very rare (Barone et al., Clin Cancer Res 16, 2702-8 (2010)). With the advent of widespread aromatase inhibitor therapy, mutation of the *ESR1* LBD is likely a common mechanism of resistance that develops in low estrogen states. LBD mutations of *ESR1* were detected somatically in four out of 373 cases of endometrial cancers (Kandoth, C. et al. Nature 497, 67-73 (2013)).

This example demonstates that LBD mutations do not develop in the context of anti-estrogen treatment, since the mutated *ESR1* variants continue to be responsive to direct ER antagonists such as tamoxifen and fulvestrant. This is consistent with clinical reports showing that patients that develop resistance to aromatase inhibitors still respond to antiestrogen treatment (Ingle, J.N. et al. Fulvestrant in women with advanced breast cancer after progression on prior aromatase inhibitor therapy: North Central Cancer Treatment Group Trial N0032. J Clin Oncol 24, 1052-6 (2006)).

Accession codes.

Sequence data have been deposited at the dbGAP, which is hosted by the National Center for Biotechnology Information (NCBI), under accession dbGAP phs000602.v1.p1, and CSER Clinical Sequencing Exploratory Research Program for the NIH-NHGRI grant (1UM1HG006508).

Table 1. Clinical sequencing of eleven metastatic ER-positive breast cancer cases.

Case	Age	ER/PR/ ERBB2	Treatments[a]	#SNV/ #Fusion	Genetic aberrations[b]
MO_1031	41	+ / + / -	Tamoxifen, Letrozole, Fulvestrant	266 / 18	*ESR1* (p.Leu536Gln), gene copy gains of *FGFR1*, *FGFR2*, *CCND1*, and *GNRHR*
MO_1051	31	+ / - / -	Oophorectomy, Letrozole, Fulvestrant	248 / 5	*ESR1* (p.Tyr537Ser), *PIK3CA* (p.His1047Arg), *TP53* (p.Gly199Glu), *FGFR2-AFF3* fusion
MO_1069	62	+ / + / -	Tamoxifen, Letrozole, Fulvestrant	74 / 9	*ESR1* (D538G), *ARID2* (p.Glu245*), gene copy losses of *TP53*, *BRCA1*, *RB1*, *ARID1A*, and *SMARCA4*
MO_1129	44	+ / + / -	Tamoxifen, oophorectomy, Anastrozole, Fulvestrant, Exemestane	32 / 3	*ESR1* (p.Tyr537Ser), *PIK3CA* (p.Glu542Lys), gene copy gains of *CCND1* and *PAK1*
MO_1030	78	+ / + / -	Tamoxifen (short), Anastrozole, Fulvestrant	26 / 2	*PIK3CA* (p.Glu545Ala), *TP53* copy loss
MO_1068	65	+ / - / -	Tamoxifen, Anastrozole	83 / 10	*PIK3CA* (p.His1047Arg), *TP53* (p.Glu51*), *MSH2* copy loss
MO_1090	52	+ / + / -	Tamoxifen, Anastrozole	28 / 11	No significant drivers identified
MO_1107	46	+ / + / -	Tamoxifen, oophorectomy, Anastrozole, Fulvestrant, Exemestane	63 / 12	*BRCA1* (c.5385_5386insC), frameshift deletions in *TP53*, *SMARCA4*, and *NF1*
MO_1167	60	+ / - / -	Tamoxifen, Letrozole	47 / 3	*ESR1* (p.Asp538Gly)
MO_1185	58	+ / + / -	Tamoxifen, Letrozole, Fulvestrant, Exemestane	88 / 1	*ESR1* (p.Tyr537Ser), *CDH1* (p.Gln641*), *NOTCH2* (frameshift deletion), *TP53* copy loss

TP_2004[c]	52	+ / - / -	Tamoxifen (short)	29 / 22	*MDM2* gene amplification, gene copy losses of *CDKN2A* and *CDKN2B*

Notes:
[a]Only anti-estrogen related treatments are listed in table. Patients also received chemotherapies, radiation, or mastectomy in the interim between diagnosis and MI-ONCOSEQ sequencing.
[b]Amino acid substitutions caused by nonsynonymous somatic mutations are marked in parentheses.
[c]TP_2004 is a male patient.

Table 2

Case ID	Lib ID	Library Type	Sample	Read Length	%PF Clusters	PhiX % Error	% > Q30	# Reads	% Aligned	Gb Aligned
MO_1185	SI_6764	Transcriptome	Tumor Biopsy - 2013	2 X 111	90.6	0.24	84.7	83957912	90.6	8.44
MO_1185	SI_6828	Exome Capture	Tumor Biopsy - 2013	2 X 111	92.0	0.22	91.0	192597898	91.7	19.60
MO_1185	SI_6830	Exome Capture	Normal Blood - 2013	2 X 111	92.0	0.22	91.0	137187312	92.5	14.09
MO_1167	SI_6652	Transcriptome	Tumor Biopsy - 2013	2 X 111	91.2	0.25	89.7	118377118	92.3	11.98
MO_1167	SI_6608	Exome Capture	Tumor Biopsy - 2013	2 X 111	95.0	0.19	95.2	229166770	91.9	24.17
MO_1167	SI_6610	Exome Capture	Tumor Biopsy - 2013	2 X 111	95.0	0.19	95.2	150585064	93.3	15.88
MO_1129	SI_6664	Transcriptome	Tumor Biopsy - 2013	2 X 126	93.2	0.56	90.7	110783125	93.3	13.03
MO_1129	SI_6191	Exome Capture	Tumor Biopsy - 2013	2 X 101	91.7	0.72	89.6	216483626	91.1	19.92
MO_1129	SI_6192	Exome Capture	Normal Blood - 2013	2 X 101	91.7	0.72	89.6	135084370	91.6	12.50
MO_1129	SI_6580	Exome Capture	Tumor FFPE - 2001	2 X 111	92.4	0.58	90.2	113985464	94.4	11.95
MO_1069	SI_5257	Transcriptome	Tumor Biopsy - 2012	2 X 101	92.5	0.63	86.3	108932482	91.2	10.03
MO_1069	SI_5259	Exome Capture	Tumor Biopsy - 2012	2 X 101	93.5	0.54	88.7	228128358	92.1	21.22
MO_1069	SI_5260	Exome Capture	Normal Blood - 2012	2 X 101	93.5	0.54	88.7	143597568	93.0	13.49
MO_1069	SI_6666	Exome Capture	Tumor FFPE - 1994	2 X 126	93.2	0.56	90.7	115997626	96.2	11.28
MO_1051	SI_5091	Transcriptome	Tumor Biopsy - 2012	2 X 101	89.2	0.67	87.4	102882633	91.2	9.47
MO_1051	SI_5121	Exome Capture	Tumor Biopsy - 2012	2 X 101	92.3	0.86	87.4	209297646	92.1	19.47
MO_1051	SI_5080	Exome Capture	Normal Blood - 2012	2 X 101	90.0	0.69	88.4	193338100	89.0	17.38
MO_1051	SI_5447	Exome Capture	Tumor FFPE - 2005	2 X 101	93.3	1.04	83.3	176710228	89.2	15.91
MO_1031	SI_5256	Transcriptome	Tumor Biopsy - 2012	2 X 101	91.8	0.61	84.7	101227958	92.4	9.45
MO_1031	SI_5261	Exome Capture	Tumor Biopsy - 2012	2 X 101	93.7	0.67	86.8	150236180	91.4	13.87
MO_1031	SI_5262	Exome Capture	Normal Blood - 2012	2 X 101	93.7	0.67	86.8	221415120	91.6	20.48

Table 3

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1031	HLA-A	p.A182V	6	29911246	C	T	NA	323.8	0	0.08
MO_1031	ESR1	p.L536Q	6	152419920-1	TC	AG	NA	55.5	0	0
MO_1031	GPS2	p.Q226X	17	7216747	G	A	NA	52.7	0	0
MO_1031	PATZ1	p.R214W	22	31740949	G	A	NA	42.1	0	0
MO_1031	MTOR	p.F319L	1	11308035	G	C	NA	15.6	0	0.65
MO_1031	RNF43	p.E712Q	17	56434860	C	G	NA	14.8	0	0
MO_1031	CRKL	p.S112C	22	21288090	C	G	NA	11.4	0	0.02
MO_1031	BIRC2	p.K102N	11	102220891	G	C	NA	11.4	0	0.02
MO_1031	AKAP9	p.S403F	7	91630403	C	T	NA	10.3	0	0
MO_1031	AKAP9	p.P1393S	7	91652316	C	T	NA	10.3	0	0.51
MO_1031	PSIP1	p.Q384X	9	15469011	G	A	NA	10.1	0	0
MO_1031	GPR124	p.A394V	8	37690611	C	T	NA	8.7	0	0.36
MO_1031	KDM5A	p.R1121T	12	418985	C	G	NA	7.2	0	0.01
MO_1031	NCOA1	p.S1320C	2	24980919	C	G	NA	4.9	0	0
MO_1031	ARID2	p.E1315K	12	46245849	G	A	NA	3.1	0	0.01
MO_1031	BRIP1	p.M1970I	17	59763192	C	T	NA	2.0	0	0
MO_1031	ASXL2	p.E1178Q	2	25965674	C	G	NA	1.7	0	0.02
MO_1031	APC	p.D1558N	5	112175963	G	A	NA	1.1	0	0.09
MO_1031	FAM123B	p.Q1098X	X	63409875	G	A	NA	0.6	0	0.31
MO_1031	BLK	p.M264I	8	11414186	G	A	NA	0.1	0	0.11
MO_1031	IRS4	p.S49F	X	107979429	G	A	NA	0.0	0	0
MO_1031	FN1	p.R1162T	2	216262435	C	G	NA	901.7	0	0.01
MO_1031	KIAA0913	p.R944L	10	75554320	G	T	NA	400.5	0	0.06
MO_1031	DLG5	p.R1685C	10	79565534	G	A	NA	376.4	1	0
MO_1031	FLNA	p.R1951W	X	153581931	G	A	NA	276.3	0	0
MO_1031	ERI3	p.V7L	1	44788522	C	G	NA	231.6	0	0
MO_1031	NUDT5	p.S199C	10	12209765	G	C	NA	215.4	0	0.05
MO_1031	CLTC	p.E33Q	17	57721691	G	C	NA	182.2	0	0.03
MO_1031	ANXA7	p.S301X	10	75143015	G	C	NA	166.1	0	0.38
MO_1031	C14orf166	p.R65K	14	52460448	G	A	NA	156.3	0	1
MO_1031	MRPL38	p.S263C	17	73895678	G	C	NA	148.7	0	0.02
MO_1031	IGLV2-23	p.A27V	22	23040632	C	T	NA	141.3	0	0.01
MO_1031	SCRIB	p.E686K	8	144890838	C	T	NA	107.1	0	0.23
MO_1031	S100A13	p.L71F	1	153591457	G	A	NA	101.3	0	0
MO_1031	CTSZ	p.D72H	20	57581470	C	G	NA	82.9	0	0.01
MO_1031	RAB11FIP1	p.E931K	8	37729529	C	T	NA	80.2	0	0
MO_1031	DLGAP4	p.S225C	20	35060794	C	G	NA	71.7	0	0.01
MO_1031	FAAH	p.R260C	1	46871459	C	T	NA	60.6	0	0
MO_1031	RBM6	p.M1087I	3	50114455	G	A	NA	60.4	0	0.06
MO_1031	DHTKD1	p.V298M	10	12131159	G	A	NA	57.1	0	0.63
MO_1031	PARP12	p.R242T	7	139756691	C	G	NA	56.9	0	0.27
MO_1031	MAN2A2	p.I71M	15	91448561	C	G	NA	55.4	0	0
MO_1031	MAEA	p.E27D	4	1305778	G	C	NA	53.2	0	0
MO_1031	USP34	p.E2101Q	2	61475739	C	G	NA	52.4	0	0
MO_1031	TTC17	p.E329Q	11	43419590	G	C	NA	51.8	0	0.05
MO_1031	MUC19	p.H954D	12	40836890	C	G	NA	49.1	0	0.63
MO_1031	PSMC2	p.E185K	7	103003848	G	A	NA	48.1	0	0.57
MO_1031	POR	p.R554Q	7	75615159	G	A	NA	44.8	0	0.09
MO_1031	CNKSR1	p.A534G	1	26515099	C	G	NA	44.6	0	0.25
MO_1031	MAPKAP1	p.R467T	9	128201227	C	G	NA	43.4	0	0
MO_1031	ADAM9	p.S38L	8	38865420	C	T	NA	41.8	0	0.13
MO_1031	CTNNBL1	p.D274N	20	36405816	G	A	NA	41.7	0	0.34
MO_1031	VPS16	p.E614Q	20	2845214	G	C	NA	39.9	0	0.22
MO_1031	VPS16	p.E684K	20	2845839	G	A	NA	39.9	0	0.01
MO_1031	RTKN	p.G314S	2	74655775	C	T	NA	39.7	0	0
MO_1031	SFSWAP	p.E523Q	12	132241036	G	C	NA	38.8	0	0.16
MO_1031	LONP1	p.I700M	19	5694826	G	C	NA	38.6	0	0.05
MO_1031	FNBP4	p.K938N	11	47741630	C	G	NA	38.2	0	0.02
MO_1031	UPF3A	p.S50F	13	115047263	C	T	NA	33.4	0	0.06
MO_1031	PTPN12	p.I316T	7	77247804	T	C	NA	33.3	0	0.39
MO_1031	CUX1	p.E1492K	7	101892278	G	A	NA	27.3	0	0
MO_1031	NOL11	p.C144S	17	65717611	G	A	NA	27.1	0	0.07
MO_1031	CYP27A1	p.S260F	2	219677467	C	T	NA	27.1	0	0.02
MO_1031	ATP6V1B1	p.Q244H	2	71188770	G	C	NA	25.5	0	0
MO_1031	GMPR2	p.E204K	14	24706513	G	A	NA	24.4	0	0
MO_1031	SPTAN1	p.Q1980K	9	131386727	C	A	NA	24.0	0	0
MO_1031	SPTAN1	p.S2138C	9	131388818	C	G	NA	24.0	0	0
MO_1031	IVNS1ABP	p.D77N	1	185278187	C	T	NA	23.9	0	0.32
MO_1031	LRP1	p.E270K	12	57539240	G	A	NA	23.6	0	0.13
MO_1031	TMEM129	p.S105Y	4	1720245	G	T	NA	23.4	0	0.03

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1031	FARP1	p.D153N	13	99030133	G	A	NA	22.2	0	0.02
MO_1031	KRT10	p.E169K	17	38978333	C	T	NA	22.2	0	0.25
MO_1031	SLC27A4	p.D127N	9	131107651	G	A	NA	21.1	0	0.08
MO_1031	HDAC11	p.P13S	3	13522251	C	T	NA	20.2	0	0
MO_1031	FAM193A	p.K878E	4	2698318	A	G	NA	19.8	0	0
MO_1031	FAM100B	p.S83C	17	74266339	C	G	NA	19.3	0	0
MO_1031	STAU2	p.M429I	8	74439971	C	G	NA	18.9	0	
MO_1031	FAM84B	p.Q129R	8	127569249	T	C	NA	18.6	0	0.34
MO_1031	SIN3A	p.S689C	15	75686626	G	C	NA	18.4	0	0.03
MO_1031	NRP1	p.I121M	10	33559670	G	C	NA	17.6	0	0
MO_1031	PDCD7	p.L3V	15	65426113	G	C	NA	16.4	0	0
MO_1031	FRMD8	p.E462K	11	65178820	G	A	NA	16.2	0	0
MO_1031	SDCCAG8	p.G44A	1	243433470	G	C	NA	16.1	0	0.36
MO_1031	MBD5	p.Q987X	2	149241119	C	T	NA	15.6	0	0
MO_1031	RALGAPB	p.D79H	20	37121621	G	C	NA	15.6	0	0
MO_1031	CAND1	p.E870X	12	67700056	G	T	NA	15.1	0	0
MO_1031	CEP250	p.L682V	20	34065876	C	G	NA	14.9	0	0
MO_1031	HSD17B8	p.E243K	6	33174184	G	A	NA	14.6	0	0.01
MO_1031	BAZ1A	p.E1246Q	14	35233953	C	G	NA	14.6	0	0.3
MO_1031	PUM1	p.E249K	1	31468043	C	T	NA	14.6	0	0.09
MO_1031	RILPL1	p.M134I	12	124008100	C	A	NA	14.0	0	0.31
MO_1031	LACTB2	p.E65K	8	71574062	C	T	NA	13.8	0	0.16
MO_1031	LRIG1	p.T717M	3	66433747	G	A	NA	13.0	1	0.02
MO_1031	RNF6	p.E266K	13	26789223	C	T	NA	12.8	0	0.03
MO_1031	DDX58	p.R6Q	9	32526148	C	T	NA	12.7	0	0.02
MO_1031	UNKL	p.E312D	16	1444133	C	G	NA	12.6	0	0.08
MO_1031	UNKL	p.S246X	16	1448940	C	T	NA	12.6	0	0
MO_1031	LRBA	p.L2000V	4	151520207	G	C	NA	12.4	0	0.14
MO_1031	LRBA	p.F1979L	4	151520268	G	C	NA	12.4	0	0.01
MO_1031	HEATR5A	p.L429F	14	31855668	G	A	NA	12.4	0	0
MO_1031	PRR12	p.L1355V	19	50102913	C	G	NA	12.1	0	0.14
MO_1031	UGGT1	p.I782M	2	128914911	C	G	NA	12.0	0	0.02
MO_1031	SLC10A3	p.Q441E	X	153715959	G	C	NA	12.0	0	0.42
MO_1031	PDLIM2	p.R243W	8	22447218	C	T	NA	11.8	0	0
MO_1031	PVRL4	p.D338H	1	161044152	C	G	NA	11.4	0	0.05
MO_1031	ZNHIT2	p.E111Q	11	64884795	C	G	NA	11.3	0	0.22
MO_1031	CMAHP	p.L152F	6	25109797	G	A	NA	11.0	0	0.02
MO_1031	CNIH2	p.E114K	11	66050747	G	A	NA	10.5	0	0.02
MO_1031	CTAGE5	p.S661C	14	39816944	C	G	NA	10.5	0	0
MO_1031	MMP19	p.S430L	12	56231058	G	A	NA	10.3	0	0.01
MO_1031	MREG	p.R165K	2	216810310	C	T	NA	9.8	0	0
MO_1031	SLCO2A1	p.lice acc.	3	133692670	C	G	NA	9.6	0	
MO_1031	C7orf13	p.A36P	7	156433243	C	G	NA	9.5	0	
MO_1031	NUP160	p.D449Y	11	47840943	C	A	NA	8.6	0	0.02
MO_1031	PNPLA7	p.S555C	9	140395161	G	C	NA	8.5	0	0
MO_1031	GCDH	p.P51R	19	13003198	C	G	NA	8.5	0	0.32
MO_1031	RNF115	p.E238K	1	145687020	G	A	NA	8.3	0	0.1
MO_1031	MFSD11	p.R304C	17	74771114	C	T	NA	8.1	0	0
MO_1031	BZRAP1	p.K1347N	17	56386592	C	A	NA	8.0	0	0
MO_1031	SNX27	,.D283H	1	151634687	G	C	NA	7.8	0	0
MO_1031	ZBTB42	p.M1I	14	105267537	G	A	NA	7.8	0	0
MO_1031	ZBTB42	p.E2K	14	105267538	G	A	NA	7.8	0	0.01
MO_1031	ZBTB42	p.E96K	14	105267820	G	A	NA	7.8	0	0
MO_1031	TTF1	p.K17N	9	135278158	C	A	NA	7.5	0	0
MO_1031	TMEM106B	p.T234R	7	12271477	C	G	NA	7.4	0	0.24
MO_1031	SMARCC1	p.D284N	3	47752241	C	T	NA	7.3	1	0
MO_1031	INTS6	p.I3M	13	52026653	G	C	NA	7.3	0	0
MO_1031	AC008073.6.1	p.Q107X	2	24360929	C	T	NA	7.0	0	0.24
MO_1031	ZNF791	p.R544T	19	12739974	G	C	NA	6.8	0	0
MO_1031	SMG1	p.Q1779E	16	18861397	G	C	NA	6.7	0	0.96
MO_1031	INTS2	p.E759K	17	59958371	C	T	NA	6.5	0	0.26
MO_1031	LRRC1	p.L314F	6	53769212	G	C	NA	6.5	0	0
MO_1031	CEP76	p.C612Y	18	12674541	C	T	NA	6.1	0	
MO_1031	ZCCHC2	p.R214Q	18	60191298	G	A	NA	6.1	0	0
MO_1031	SPICE1	p.L521V	3	113176079	G	C	NA	6.0	0	0.18
MO_1031	LY86	p.I78M	6	6826536	C	G	NA	6.0	0	0.23
MO_1031	CACNB1	p.G169C	17	37343092	G	A	NA	6.0	0	0
MO_1031	ARHGAP29	p.E185K	1	94671197	C	T	NA	6.0	0	0.06
MO_1031	RND1	p.S230Y	12	49251789	G	T	NA	5.7	0	0
MO_1031	ZCCHC3	p.E221K	20	278888	G	A	NA	5.6	0	0.03

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1031	SMOX	p.S64C	20	4158040	C	G	NA	5.5	0	0
MO_1031	LENG9	p.E196Q	19	54974190	C	G	NA	5.4	0	0.23
MO_1031	ZBTB1	p.M653I	14	64990181	G	A	NA	5.0	0	0.03
MO_1031	OBSCN	p.Q1409X	1	228492156	C	T	NA	4.9	0	0.19
MO_1031	ZNF564	p.L237F	19	12638213	G	A	NA	4.8	0	0.7
MO_1031	PAFAH2	p.F278L	1	26301066	G	C	NA	4.6	0	0.47
MO_1031	GBP4	p.M542I	1	89652097	C	T	NA	4.6	0	0.94
MO_1031	ELMO1	p.G559E	7	36927203	C	T	NA	4.5	0	0
MO_1031	RPGR	p.E512K	X	38150250	C	T	NA	4.5	0	0.01
MO_1031	RAPH1	p.P1077T	2	204304684	G	T	NA	4.5	0	0
MO_1031	RGL4	p.R441Q	22	24040460	G	A	NA	4.5	0	0.58
MO_1031	RNF32	p.K94N	7	156447277	G	C	NA	4.3	0	0
MO_1031	KMO	p.S171L	1	241725529	C	T	NA	4.2	0	0
MO_1031	TMOD3	p.S127Y	15	52179882	C	A	NA	4.0	0	0
MO_1031	MEX3A	p.G459S	1	156046553	C	T	NA	4.0	0	
MO_1031	KIF21A	p.S1258L	12	39711971	G	A	NA	3.8	0	0
MO_1031	FAM179B	p.H1193D	14	45497451	C	G	NA	3.7	0	1
MO_1031	DOCK10	p.D744H	2	225714229	C	G	NA	3.7	0	0
MO_1031	FMNL2	p.K217T	2	153431703	A	C	NA	3.4	0	0.03
MO_1031	FUT2	p.S52X	19	49206368	C	G	NA	3.3	0	0.01
MO_1031	ZCCHC14	p.L526P	16	87446339	A	G	NA	3.3	0	0
MO_1031	ZSWIM4	p.E232D	19	13915946	G	C	NA	3.2	0	0.02
MO_1031	POLR2M	p.T126P	15	58001174	A	C	NA	3.2	1	0.01
MO_1031	DET1	p.S169L	15	89074464	G	A	NA	3.1	0	0
MO_1031	SLC35D1	p.F145S	1	67516146	A	G	NA	2.9	0	0.01
MO_1031	CHIC1	p.X218S	X	72900839	G	C	NA	2.9	0	0.86
MO_1031	SEMA3E	p.E764Q	7	82996940	C	G	NA	2.9	1	0.02
MO_1031	ATG2B	p.K11N	14	96829261	C	G	NA	2.8	0	0.04
MO_1031	FAT3	p.E2994X	11	92538402	G	T	NA	2.3	0	0.17
MO_1031	POPDC2	p.I154M	3	119378809	G	C	NA	2.3	0	0
MO_1031	SLIT3	p.T958S	5	168127656	G	C	NA	2.2	0	0.23
MO_1031	SYNPO2	p.R1088H	4	119978566	G	A	NA	2.2	0	0.26
MO_1031	VCPIP1	p.S545C	8	67577560	G	C	NA	2.1	0	0.02
MO_1031	FAM184B	p.E73K	4	17711192	C	T	NA	2.1	0	0.01
MO_1031	RASA2	p.S323L	3	141269858	C	T	NA	2.0	0	0.2
MO_1031	C3orf67	p.S326L	3	58849525	G	A	NA	2.0	0	0.14
MO_1031	C1orf167	p.Q1046E	1	11844289	C	G	NA	2.0	0	0
MO_1031	ANKS1B	p.G573E	12	99793447	C	T	NA	1.9	0	0.17
MO_1031	ZNF837	p.S376L	19	58879573	G	A	NA	1.8	0	0
MO_1031	NCOA7	p.E626K	6	126211082	G	A	NA	1.8	0	0
MO_1031	STXBP5	p.S973L	6	147685247	C	T	NA	1.8	0	0
MO_1031	BCO2	p.N134Y	11	112064303	A	T	NA	1.6	0	0.02
MO_1031	CRLF3	p.I389L	17	29111369	G	A	NA	1.6	0	
MO_1031	TM6SF2	p.F148L	19	19380536	G	T	NA	1.5	0	0.89
MO_1031	ALPK3	p.E1722Q	15	85407731	G	C	NA	1.2	0	0
MO_1031	ZNF717	p.A312S	3	75787840	C	A	NA	1.1	0	0.32
MO_1031	ZNF717	p.V286I	3	75787918	C	T	NA	1.1	0	0.21
MO_1031	ZNF717	p.Y283C	3	75787926	T	C	NA	1.1	0	0.01
MO_1031	TTLL7	p.S487L	1	84385422	G	A	NA	1.1	0	0.07
MO_1031	TET3	p.E913K	2	74320668	G	A	NA	1.1	0	0.11
MO_1031	ACACB	p.I1273F	12	109661644	A	T	NA	1.0	0	0
MO_1031	ADAMTSL3	p.G1502E	15	84694037	G	A	NA	0.8	0	0
MO_1031	FAM161A	p.E114Q	2	62069339	C	G	NA	0.8	0	0
MO_1031	RGS9	p.D65E	17	63154453	C	G	NA	0.8	0	0.03
MO_1031	SCML1	p.S188F	X	17768273	C	T	NA	0.7	0	0.01
MO_1031	CSF2RB	p.P343S	22	37328821	C	T	NA	0.6	0	0
MO_1031	CYP7B1	p.S293F	8	65527762	G	A	NA	0.5	0	0
MO_1031	BTBD11	p.Q163H	12	107713206	G	C	NA	0.5	0	0.19
MO_1031	CCT6B	p.lice donor	17	33269814	C	T	NA	0.5	1	
MO_1031	PRDM5	p.E108K	4	121760408	C	T	NA	0.4	0	0
MO_1031	MYO15A	p.E3242Q	17	18067089	G	C	NA	0.4	0	0.05
MO_1031	TAOK1	p.S826Y	17	27869955	C	A	NA	0.4	0	0
MO_1031	COL28A1	p.I779M	7	7415114	G	C	NA	0.4	0	0
MO_1031	FREM2	p.Q2473K	13	39433625	C	A	NA	0.4	0	0.3
MO_1031	GABRA3	p.N406I	X	151336962	T	A	NA	0.3	0	0.01
MO_1031	RUFY4	p.R422K	2	218940420	G	A	NA	0.3	0	0.42
MO_1031	RP11-6F2.7.1	p.E87Q	3	156570767	G	C	NA	0.3	0	0.25
MO_1031	ICAM5	p.S70L	19	10401874	C	T	NA	0.3	0	0.67
MO_1031	LCTL	p.S111Y	15	66856287	G	T	NA	0.3	0	0
MO_1031	TMEM151A	p.E257K	11	66062486	G	A	NA	0.3	0	0

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1031	RYR1	p.S1172L	19	38959739	C	T	NA	0.2	0	0.41
MO_1031	MACC1	p.Q488E	7	20198522	G	C	NA	0.2	0	0
MO_1031	ARPP21	p.S804R	3	35835423	C	G	NA	0.2	0	0.04
MO_1031	CCIN	p.M409I	9	36170726	G	A	NA	0.2	0	0
MO_1031	MUC16	p.S5280L	19	9071607	G	A	NA	0.2	0	0.02
MO_1031	C11orf41	p.T465I	11	33565394	C	T	NA	0.1	0	0.32
MO_1031	SAMD13	p.L92F	1	84815382	G	C	NA	0.1	0	0
MO_1031	GLT1D1	p.E42Q	12	129360514	G	C	NA	0.1	0	0.05
MO_1031	PKD1L1	p.S2552T	7	47849102	C	G	NA	0.1	0	0.15
MO_1031	C6	p.Q111K	5	41199984	G	T	NA	0.1	0	0
MO_1031	BCL2L14	p.S92C	12	12232514	C	G	NA	0.1	0	0
MO_1031	RYR2	p.L192M	1	237540733	T	A	NA	0.1	0	0
MO_1031	NPAS3	p.L831I	14	34270100	C	A	NA	0.1	3	0.09
MO_1031	UGT8	p.D345E	4	115586905	C	G	NA	0.1	0	0
MO_1031	IGFN1	p.D1266H	1	201177817	G	C	NA	0.1	0	
MO_1031	NCR3LG1	p.E23X	11	17373583	G	T	NA	0.1	0	0.6
MO_1031	GGT2	splice donor	22	21581683	A	G	NA	0.1	0	
MO_1031	SPANXN3	p.D90H	X	142596802	C	G	NA	0.1	0	0.01
MO_1031	CACNA1A	p.D1411N	19	13372295	C	T	NA	0.1	0	0
MO_1031	UNC79	p.S1899X	14	94097168	C	A	NA	0.1	0	0
MO_1031	APOB	p.E3545K	2	21229107	C	T	NA	0.1	0	0.07
MO_1031	CECR2	p.S1005W	22	18028057	C	G	NA	0.1	0	0.02
MO_1031	CACNA1F	p.R402Q	X	49083503	C	T	NA	0.0	0	0.01
MO_1031	DUSP27	p.R551K	1	167096020	G	A	NA	0.0	1	0
MO_1031	ZNF831	p.P659L	20	57768050	C	T	NA	0.0	0	0.01
MO_1031	AC007431.1.1	p.G30A	17	55622545	C	G	NA	0.0	0	
MO_1031	LEKR1	p.E67Q	3	156570767	G	C	NA	0.0	0	0.25
MO_1031	C2orf73	p.A139G	2	54586123	C	G	NA	0.0	0	0.2
MO_1031	CACNA1E	p.A1489T	1	181727218	G	A	NA	0.0	0	0.04
MO_1031	NRAP	p.E1274D	10	115365614	C	A	NA	0.0	0	0.07
MO_1031	SLC10A1	p.S206C	14	70246028	G	C	NA	0.0	0	0.01
MO_1031	PDZD3	p.S356L	11	119059398	C	T	NA	0.0	1	0.08
MO_1031	DMRTA2	splice acc.	1	50885407	C	T	NA	0.0	0	
MO_1031	ALPPL2	p.L273M	2	233273244	C	A	NA	0.0	0	0.3
MO_1031	DDI1	p.E395Q	11	103908733	G	C	NA	0.0	0	0.25
MO_1031	TRDN	p.D275H	6	123818368	C	G	NA	0.0	0	0
MO_1031	C10orf71	p.L960P	10	50533529	T	C	NA	0.0	0	0.23
MO_1031	GPR142	p.G311S	17	72368281	G	A	NA	0.0	1	0.03
MO_1031	GPR142	p.E452Q	17	72368704	G	C	NA	0.0	0	0.13
MO_1031	CCDC27	p.E391K	1	3679886	G	A	NA	0.0	0	
MO_1031	DCDC2C	p.I77M	2	3774595	C	G	NA	0.0	0	0.01
MO_1031	COL6A5	p.R1936W	3	130158438	C	T	NA	0.0	0	
MO_1031	GHSR	p.Q299E	3	172163157	G	C	NA	0.0	0	0.27
MO_1031	HTR1A	p.R297Q	5	63256657	C	T	NA	0.0	0	0.33
MO_1031	GGNBP1	p.E102K	6	33556777	G	A	NA	0.0	0	0
MO_1031	SLC22A2	p.E93Q	6	160679513	C	G	NA	0.0	0	0.06
MO_1031	OR13C5	p.L69M	9	107361490	A	T	NA	0.0	0	0.15
MO_1031	OR52I2	p.S260L	11	4608821	C	T	NA	0.0	0	0
MO_1031	KRT76	p.F269L	12	53169180	G	C	NA	0.0	0	0.1
MO_1031	CYP1A1	p.P82T	15	75015195	G	T	NA	0.0	0	0
MO_1031	FAM46D	p.M368I	X	79699202	G	C	NA	0.0	0	0.04
MO_1031	RBMXL3	p.P321L	X	114424966	C	T	NA	0.0	0	

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1051	CTNNA1	p.D814N	5	138268583	G	A	NO	146.0	0	0.18
MO_1051	TOP1	p.E289K	20	39726867	G	A	NO	45.1	0	0.26
MO_1051	TOP1	p.K321N	20	39726965	G	C	NO	45.1	0	0
MO_1051	MAP4	p.E327Q	3	47933003	C	G	NO	41.3	0	0.03
MO_1051	TP53	p.G199E	17	7578253	C	T	NO	18.3	37	0
MO_1051	ESR1	p.Y537S	6	152419923	A	C	NO	12.3	2	0
MO_1051	PTK2B	p.E474K	8	27294717	G	A	NO	11.9	3	0.02
MO_1051	AR	p.G21A	X	66765050	G	C	NO	9.3	0	0
MO_1051	PTPRT	p.S249L	20	41385215	G	A	NO	8.7	0	0.35
MO_1051	FYN	p.R481Q	6	111983114	C	T	NO	8.6	0	0.17
MO_1051	IGF1R	p.K560N	15	99456363	G	C	NO	4.9	0	0.08
MO_1051	FLT4	p.G723A	5	180048007	C	G	NO	4.4	0	0
MO_1051	KAT6A	p.S378L	8	41834756	G	A	NO	3.3	1	0.01
MO_1051	CD22	p.A483T	19	35831981	G	A	NO	2.6	0	0.46
MO_1051	ETV2	p.S169L	19	36134362	C	T	NO	2.3	0	0
MO_1051	PIK3CA	p.H1047R	3	178952085	A	G	YES	2.1	1928	0.06

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1051	MYBL1	p.E593K	8	67479179	C	T	NO	1.2	0	0.15
MO_1051	BRIP1	p.Q1151K	17	59760956	C	T	NO	0.5	0	0
MO_1051	MAML2	p.Q553X	11	95825538	G	A	NO	0.4	0	1
MO_1051	POU6F2	p.G159K	7	39247183-4	GG	AA	NO	0.0	0	0
MO_1051	ALK	p.E802K	2	29456514	C	T	NO	0.0	0	0.04
MO_1051	RPS25	p.K7N	11	116888746	C	G	NO	407.3	0	0.06
MO_1051	TUBA1B	p.L70F	12	49523299	C	G	NO	327.2	0	0
MO_1051	IL32	p.D172G	16	3119304	A	G	YES	211.6	0	0
MO_1051	QARS	p.F268L	3	49138860	G	C	NO	155.9	0	0
MO_1051	B4GALT3	p.G167E	1	161143829	C	T	NO	155.8	0	1
MO_1051	SF1	p.H415Q	11	64535140	G	C	NO	154.1	0	0
MO_1051	GANAB	p.D434N	11	62398159	C	T	NO	131.0	0	0
MO_1051	PLXNB1	p.E1309K	3	48456626	C	T	NO	121.3	1	0.09
MO_1051	EFHD1	p.A70V	2	233498623	C	T	NO	109.5	0	0.23
MO_1051	DYNC1H1	p.E1284K	14	102466371	G	A	NO	90.4	0	0
MO_1051	PLEKHA6	p.E527K	1	204219688	C	T	NO	84.1	0	0
MO_1051	NTN4	p.V258I	12	96131736	C	T	NO	84.0	0	0.43
MO_1051	A1BG	p.S95L	19	58864350	G	A	YES	83.4	0	0.73
MO_1051	SEC16A	p.Q2332E	9	139338286	G	C	NO	81.6	0	0.01
MO_1051	SDR39U1	p.G13V	14	24909758	C	A	NO	77.5	0	
MO_1051	ZFAND6	p.S97F	15	60414144	C	T	YES	70.5	0	0.01
MO_1051	HDAC7	p.R277W	12	48189511	G	A	NO	68.5	0	0
MO_1051	TMEM214	p.S552F	2	27262930	C	T	NO	62.7	0	0
MO_1051	LUC7L2	p.D85H	7	139083441	G	C	NO	58.9	0	0
MO_1051	PIN1	p.V55I	19	9949216	G	A	NO	51.5	0	0.3
MO_1051	ZNF296	p.K279N	19	45575450	C	G	NO	51.2	0	0.01
MO_1051	ZNF296	p.W170X	19	45575777	C	T	NO	51.2	0	0.42
MO_1051	MGRN1	p.P27Q	16	4675041	C	A	NO	46.3	0	0
MO_1051	NBPF10	p.L92F	1	145295521	C	T	YES	46.3	0	0.02
MO_1051	COMTD1	p.R42Q	10	76995471	C	T	NO	45.4	0	0.65
MO_1051	SLC15A3	p.S358C	11	60709541	G	C	NO	43.2	0	0.01
MO_1051	DAG1	p.F692L	3	49570020	C	G	NO	40.2	0	0.45
MO_1051	DAG1	p.F791L	3	49570317	C	A	NO	40.2	0	0
MO_1051	DAG1	p.L819V	3	49570399	C	G	NO	40.2	0	0.07
MO_1051	DAG1	p.Q864K	3	49570534	C	A	NO	40.2	0	0.12
MO_1051	COPB1	p.D320N	11	14502643	C	T	NO	37.8	0	0.01
MO_1051	HIST2H2BE	p.E114K	1	149857851	C	T	NO	34.4	1	
MO_1051	MSMO1	p.H250Y	4	166262964	C	T	NO	33.7	0	0
MO_1051	SLC38A10	p.E519Q	17	79226385	C	G	NO	33.6	0	0
MO_1051	SLC35B1	p.S321Y	17	47780285	G	T	NO	33.5	0	0
MO_1051	PSMD1	p.G285D	2	231937102	G	A	NO	30.2	0	0.4
MO_1051	NACC1	p.R298W	19	13246913	C	T	YES	30.1	0	0.02
MO_1051	ZFP36L2	p.Q139X	2	43452528	G	A	NO	30.0	0	0.32
MO_1051	THBS2	p.H201Y	6	169648520	G	A	NO	29.8	0	0.05
MO_1051	CHD1	p.Q893E	5	98218833	G	C	NO	29.1	0	0
MO_1051	SKI	p.S515C	1	2235801	C	G	NO	27.0	0	0.3
MO_1051	DHX30	p.E368K	3	47887268	G	A	NO	26.9	1	0.02
MO_1051	FCGR3A	p.F212V	1	161514542	A	C	NO	26.7	0	0.24
MO_1051	GRSF1	p.R42C	4	71705097	G	A	NO	26.7	0	
MO_1051	RAVER1	p.E642K	19	10429021	C	T	NO	25.8	0	0.33
MO_1051	TRIM26	p.E391Q	6	30154102	C	G	NO	25.2	0	0.09
MO_1051	LSS	p.A693S	21	47611140	C	A	NO	25.0	0	0.54
MO_1051	NBPF12	p.E84Q	1	146397433	G	C	NO	24.7	0	0
MO_1051	NBPF12	p.E50Q	1	146398387	G	C	NO	24.7	0	1
MO_1051	BRAT1	p.S274F	7	2582940	G	A	NO	23.9	0	0.7
MO_1051	USP22	p.S307L	17	20916167	G	A	NO	23.3	0	0
MO_1051	FAM208A	p.D824E	3	56675524	G	C	NO	22.7	0	0.63
MO_1051	DYRK1A	p.S258C	21	38862585	C	G	NO	22.6	0	0
MO_1051	CEP104	p.E160K	1	3761864	C	T	NO	22.5	0	0.33
MO_1051	SHROOM3	p.Q331X	4	77660317	C	T	NO	21.8	0	0.36
MO_1051	MAN2A1	p.E1030K	5	109190952	G	A	NO	21.5	0	0.84
MO_1051	LFNG	p.F350L	7	2566532	C	G	NO	21.1	0	0
MO_1051	CC2D1A	p.E772Q	19	14038076	G	C	NO	20.4	0	0.01
MO_1051	ZNF213	p.K355X	16	3191031	C	T	NO	20.2	0	0.04
MO_1051	LRPPRC	p.R799T	2	44170934	C	G	NO	20.1	0	0.02
MO_1051	ANKRD30A	p.E1234K	10	37508508	G	A	NO	20.0	0	0.05
MO_1051	NUP205	p.S1666I	7	135315156	G	T	YES	19.7	0	0.01
MO_1051	RAP1GAP	p.S525C	1	21929351	G	C	NO	19.4	0	0
MO_1051	KLHL17	p.E159K	1	897116	G	A	NO	19.4	0	0
MO_1051	HTATSF1	p.D669H	X	135593909	G	C	NO	19.4	0	0

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1051	GBP2	p.P174A	1	89583365	G	C	NO	19.2	0	0
MO_1051	BAZ1A	p.D639H	14	35253050	C	G	NO	19.0	0	0
MO_1051	ABCG1	p.E191K	21	43697038	G	A	NO	18.8	0	0.31
MO_1051	TRIM41	p.F425L	5	180660747	C	G	NO	18.6	0	0.1
MO_1051	TRAPPC4	p.S132L	11	118890904	C	T	NO	17.5	0	0.01
MO_1051	GAS6	p.E385X	13	114531675	C	A	NO	17.1	0	0
MO_1051	ITSN1	p.E686K	21	35169786	G	A	NO	16.3	0	0.12
MO_1051	CD52	p.G43E	1	26646735	G	A	NO	15.9	0	
MO_1051	HEXDC	p.A413S	17	80399749	C	T	NO	15.3	0	0.73
MO_1051	NT5DC1	p.L21V	6	116422154	C	G	NO	15.1	0	0.01
MO_1051	GATAD2B	p.M107I	1	153800503	C	G	NO	15.1	0	0
MO_1051	USP48	p.D893N	1	22028041	C	T	NO	14.9	0	0.01
MO_1051	USP48	p.E858K	1	22030055	C	T	NO	14.9	0	0.23
MO_1051	SIN3A	p.R1263C	15	75664355	G	A	NO	14.6	0	0
MO_1051	PLEKHG5	p.Q473H	1	6530918	C	G	NO	14.3	0	0.06
MO_1051	CCDC57	p.E754K	17	80086458	C	T	NO	14.1	0	0
MO_1051	POLR2B	p.K497N	4	57876613	A	C	NO	14.0	0	0
MO_1051	HOXB7	p.T163A	17	46685371	T	C	NO	13.2	0	0
MO_1051	NOTCH2NL	p.S67P	1	145273345	T	C	NO	13.2	0	0.4
MO_1051	PCMTD1	p.R335T	8	52732961	C	G	NO	13.1	0	0
MO_1051	MRPS18C	p.P133S	4	84382318	C	T	NO	13.1	0	0
MO_1051	GMNN	p.D204N	6	24786007	G	A	NO	12.9	0	
MO_1051	GTPBP3	p.R14H	19	17446461	G	A	NO	12.8	0	0.1
MO_1051	NOL8	p.E759K	9	95076632	C	T	NO	12.7	0	0.01
MO_1051	IMPAD1	p.S244F	8	57878827	G	A	NO	11.7	0	0
MO_1051	VPS13C	p.E3613K	15	62160884	C	T	NO	11.6	0	0.02
MO_1051	USP36	p.E484K	17	76803676	C	T	NO	11.4	0	0.02
MO_1051	ZNF646	p.D551N	16	31089296	G	A	NO	11.4	1	0.71
MO_1051	ZKSCAN1	p.E320Q	7	99631086	G	C	NO	11.4	0	0.15
MO_1051	MANBA	p.E697Q	4	103557090	C	G	NO	11.2	0	0.01
MO_1051	FAM8A1	p.E94Q	6	17600920	G	C	NO	11.0	1	0.1
MO_1051	SENP3	p.D337H	17	7468329	G	C	NO	10.9	0	0
MO_1051	YLPM1	p.D377H	14	75247126	G	C	NO	10.8	2	0
MO_1051	TBC1D7	p.S292L	6	13305340	G	A	NO	10.7	0	0
MO_1051	CDRT4	p.S60C	17	15341307	G	C	NO	10.0	0	0.07
MO_1051	DDX19A	p.E299X	16	70400639	G	T	NO	9.7	0	0
MO_1051	ZNF747	p.L16V	16	30545955	G	C	NO	9.5	0	0.06
MO_1051	C12orf35	p.M1479I	12	32138326	G	A	NO	9.5	0	0.52
MO_1051	DHX29	p.E1180Q	5	54558748	C	G	NO	9.2	0	0.64
MO_1051	HIVEP1	p.S1864F	6	12125619	C	T	NO	8.4	0	0.05
MO_1051	HDAC5	p.W792L	17	42161001	C	A	NO	8.3	0	0
MO_1051	C5orf51	p.E28K	5	41904551	G	A	NO	7.7	0	0.03
MO_1051	C1orf54	p.D110H	1	150253273	G	C	NO	7.7	0	0.05
MO_1051	AFF4	p.L723F	5	132232153	C	G	NO	7.6	0	0
MO_1051	NUFIP2	p.L644F	17	27613080	C	G	NO	7.6	0	0.26
MO_1051	NUFIP2	p.G331R	17	27614021	C	G	NO	7.6	0	0
MO_1051	NUFIP2	p.A305S	17	27614099	C	A	NO	7.6	0	0
MO_1051	NUFIP2	p.T259P	17	27614237	C	G	NO	7.6	0	0.03
MO_1051	CCDC25	p.E193K	8	27598009	C	T	NO	7.6	0	0.05
MO_1051	MASP1	p.F113L	3	186980407	G	C	NO	7.2	0	0.13
MO_1051	MCM2	p.E235K	3	127324990	G	A	NO	6.8	0	0.79
MO_1051	DNAH14	p.E3150Q	1	225519142	G	C	NO	6.8	0	0.17
MO_1051	DNAH14	p.E3166K	1	225519190	G	A	NO	6.8	0	0.02
MO_1051	GPATCH8	p.D875N	17	42476822	C	T	NO	6.6	0	0.95
MO_1051	PAPD5	p.E547K	16	50259080	G	A	NO	6.6	0	0.08
MO_1051	PCNXL2	p.I1505T	1	233160983	A	G	NO	6.1	0	0
MO_1051	PANX1	p.F15L	11	93862523	C	G	NO	6.1	0	0.25
MO_1051	KIAA1731	p.K2N	11	93399879	G	C	NO	5.7	0	0.01
MO_1051	FAM83D	p.E36K	20	37555101	G	A	NO	5.4	0	0.03
MO_1051	FAM83D	p.D93N	20	37555272	G	A	NO	5.4	0	0.09
MO_1051	MAP4K4	p.R1045Q	2	102493549	G	A	NO	5.4	0	0
MO_1051	LYST	p.L2316V	1	235920694	G	C	NO	5.4	0	0.24
MO_1051	CYP4F2	p.E328Q	19	15997055	C	G	NO	5.2	0	0.07
MO_1051	AVIL	p.E304Q	12	58203409	C	G	NO	5.2	0	0
MO_1051	HSPA13	p.S304C	21	15746443	G	C	NO	5.1	0	0
MO_1051	TRMT12	p.E391K	8	125464339	G	A	NO	5.0	0	0.13
MO_1051	PPP1R12B	p.S516L	1	202411580	C	T	NO	4.9	0	0.02
MO_1051	GTF2E1	p.E389K	3	120500162	G	A	NO	4.8	0	0.07
MO_1051	PGLYRP2	p.R430H	19	15582755	C	T	YES	4.7	0	0
MO_1051	NFATC1	p.E917K	18	77287533	G	A	NO	4.5	0	0

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1051	CD97	p.F645L	19	14517256	C	G	NO	4.3	0	0.04
MO_1051	HELQ	p.D771H	4	84350884	C	G	NO	4.1	0	0.1
MO_1051	RANBP6	p.L818F	9	6013154	C	G	NO	4.1	0	0.03
MO_1051	CCDC99	p.E213K	5	169021254	G	A	NO	3.9	0	0.01
MO_1051	C2orf69	p.G62E	2	200776346	G	A	NO	3.8	0	0
MO_1051	C14orf126	p.E167K	14	31917343	C	T	NO	3.6	0	0.02
MO_1051	TTC30A	p.E518K	2	178481878	C	T	NO	3.5	0	0
MO_1051	FBXL7	p.E314K	5	15936759	G	A	NO	3.3	0	0.07
MO_1051	ZNF770	p.A140T	15	35275218	C	T	YES	3.0	0	0.24
MO_1051	XDH	p.R943W	2	31572694	G	A	YES	2.9	0	0.01
MO_1051	OBSCN	p.E4760K	1	228506731	G	A	NO	2.9	0	0
MO_1051	C2orf67	p.S519L	2	210940475	G	A	NO	2.7	0	0.03
MO_1051	C3orf15	splice acc.	3	119427437	G	C	NO	2.7	0	
MO_1051	PPTC7	p.D78N	12	110989765	C	T	NO	2.7	0	0
MO_1051	DOCK10	p.E1140Q	2	225672795	C	G	NO	2.7	0	0.01
MO_1051	EPDR1	p.D291H	7	37989834	G	C	NO	2.5	0	0.07
MO_1051	SEMA5B	p.E768Q	3	122632250	C	G	NO	2.5	0	0.51
MO_1051	FSIP2	p.E6754K	2	186678437	G	A	NO	2.5	0	0.01
MO_1051	KIAA0753	splice acc.	17	6528182	C	T	NO	2.4	0	
MO_1051	INTS2	p.E368Q	17	59984872	C	G	NO	2.3	0	0.49
MO_1051	KIAA1549	p.P196A	7	138603636	G	C	NO	2.3	0	0
MO_1051	FGD6	p.E1422Q	12	95475325	C	G	NO	2.2	0	0.02
MO_1051	FAM22D	p.H35Y	10	69116125	C	T	NO	2.0	0	0.42
MO_1051	ZNF546	p.S570X	19	40520886	C	G	NO	1.9	0	0.29
MO_1051	MAP1B	p.E678Q	5	71491214	G	C	NO	1.8	1	0.04
MO_1051	SRR	p.G192V	17	2224891	G	T	NO	1.7	0	0
MO_1051	ELOVL2	p.L235H	6	10989997	A	T	NO	1.6	0	0.17
MO_1051	FJX1	p.D291H	11	35641055	G	C	NO	1.6	0	0
MO_1051	FEZ1	p.E190Q	11	125330493	C	G	NO	1.4	0	0.03
MO_1051	P2RX7	p.V475I	12	121622240	G	A	YES	1.4	0	0.17
MO_1051	KIAA1524	p.E785K	3	108272549	C	T	NO	1.2	0	0.18
MO_1051	GRIN2D	p.E815X	19	48945409	G	T	NO	1.2	0	0.1
MO_1051	ATOH8	p.S209L	2	85981938	C	T	NO	1.1	0	0.02
MO_1051	KIF21B	p.L1373F	1	200948667	G	A	NO	0.9	0	
MO_1051	ABCA10	p.G557E	17	67189361	C	T	NO	0.9	0	0
MO_1051	PLXNA4	p.V591I	7	131912321	C	T	YES	0.8	0	0.25
MO_1051	ST8SIA4	p.M134I	5	100222148	C	T	NO	0.7	0	0.01
MO_1051	DNAH7	p.E554K	2	196851884	C	T	NO	0.6	0	0.29
MO_1051	FAM124B	p.S398C	2	225244465	G	C	NO	0.5	0	0.01
MO_1051	LINGO4	p.P524S	1	151773611	G	A	YES	0.5	0	0.02
MO_1051	PNMA3	p.E189K	X	152225977	G	A	NO	0.4	0	0.01
MO_1051	AKR1E2	p.S126X	10	4877919	C	A	NO	0.4	0	0.04
MO_1051	SHANK1	p.S212L	19	51217444	G	A	NO	0.4	0	0
MO_1051	C9orf153	p.R73T	9	88842794	C	G	NO	0.3	0	0.05
MO_1051	FCAMR	p.R18K	1	207140983	C	T	NO	0.3	0	0
MO_1051	CDH7	p.D288N	18	63491948	G	A	NO	0.3	0	0.01
MO_1051	FHOD3	p.K788N	18	34298150	G	C	NO	0.2	0	0.04
MO_1051	CUBN	p.H2474Y	10	16955923	G	A	NO	0.2	0	0.01
MO_1051	PHOSPHO1	p.E117Q	17	47302063	C	G	NO	0.2	0	0.03
MO_1051	FBXO15	p.R297C	18	71790624	G	A	YES	0.2	2	0
MO_1051	CCDC36	p.Q272X	3	49293744	C	T	NO	0.2	0	0
MO_1051	FAT4	p.Q760X	4	126239844	C	T	NO	0.2	0	0.81
MO_1051	FAT4	p.S1870C	4	126329638	C	G	NO	0.2	0	0.06
MO_1051	ADCY10	p.R109Q	1	167871010	C	T	NO	0.2	1	0.42
MO_1051	FBXL13	p.M68I	7	102695601	C	T	NO	0.2	0	0.24
MO_1051	DNAH6	p.D2485Y	2	84921533	G	T	NO	0.2	0	0
MO_1051	PAPPA2	p.R1486C	1	176738881	C	T	NO	0.2	0	0
MO_1051	PI16	splice acc.	6	36926920	G	A	NO	0.1	0	
MO_1051	KIRREL2	p.L684V	19	36357317	C	G	NO	0.1	0	0.16
MO_1051	CR1	p.Q572H	1	207726161	G	T	YES	0.1	0	0.04
MO_1051	C9orf131	p.Q171E	9	35043137	C	G	NO	0.1	0	0
MO_1051	ZPLD1	p.S375F	3	102196290	C	T	NO	0.1	0	0.01
MO_1051	HOXA2	p.Q252X	7	27140722	G	A	NO	0.1	0	0.07
MO_1051	EYS	p.I3056M	6	64430759	G	C	NO	0.1	0	0
MO_1051	BNC1	p.G596A	15	83932216	C	G	NO	0.1	0	0.43
MO_1051	TYRP1	p.E525K	9	12709141	G	A	NO	0.1	0	0.01
MO_1051	GCK	p.E246K	7	44187379	C	T	NO	0.0	0	0.01
MO_1051	FCRLA	p.E156K	1	161681957	G	A	NO	0.0	0	0.02
MO_1051	CNKSR2	p.G368E	X	21549985	G	A	NO	0.0	0	0.05
MO_1051	ODZ1	p.M1531I	X	123554529	C	T	NO	0.0	0	0.34
MO_1051	MYT1L	p.P351S	2	1926490	G	A	NO	0.0	0	0.48

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1051	TTN	p.E4790Q	2	179500735	C	G	NO	0.0	0	
MO_1051	TTN	p.R3402K	2	179621485	C	T	NO	0.0	0	
MO_1051	RPH3A	p.D676H	12	113334526	G	C	NO	0.0	0	0
MO_1051	MUC2	p.G305S	11	1079696	G	A	YES	0.0	0	0.88
MO_1051	FOXI2	p.P14L	10	129535578	C	T	NO	0.0	0	0.11
MO_1051	GABRR1	p.E432K	6	89688635	C	T	NO	0.0	1	0.09
MO_1051	RHAG	p.Q104K	6	49586923	G	T	NO	0.0	0	0.21
MO_1051	LRRTM4	p.D54Y	2	77746835	C	A	NO	0.0	0	0
MO_1051	ADGB	p.L1592F	6	147123105	G	C	NO	0.0	0	0.18
MO_1051	LEKR1	p.E12K	3	156547152	G	A	NO	0.0	0	0.18
MO_1051	A2ML1	p.L1319F	12	9020847	C	T	NO	0.0	0	0.19
MO_1051	ATP12A	p.L898V	13	25283895	C	G	NO	0.0	0	0.01
MO_1051	SI	p.D1389H	3	164727081	C	G	NO	0.0	0	0
MO_1051	CACNA1E	p.R3C	1	181452887	C	T	NO	0.0	0	0
MO_1051	CBLN4	p.H125Y	20	54575822	G	A	NO	0.0	0	0
MO_1051	NKX2-3	p.D234H	10	101295083	G	C	NO	0.0	0	0
MO_1051	CIB4	p.E16Q	2	26864137	C	G	NO	0.0	1	0.02
MO_1051	OR5K3	p.R259X	3	98110284	C	T	NO	0.0	0	1
MO_1051	C9orf135	splice acc.	9	72471470	G	C	NO	0.0	0	
MO_1051	SLC1A6	p.F52L	19	15083567	G	C	NO	0.0	0	0.03
MO_1051	SPINT4	p.R65I	20	44352597	G	T	NO	0.0	0	0.09

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1069	ESR1	p.D538G	6	152419926	A	G	NO	60.5	2	0
MO_1069	ARID1B	p.D2175G	6	157528853	A	G	NO	7.9	0	0.01
MO_1069	MTOR	p.R281H	1	11308150	C	T	YES	5.5	1	0.01
MO_1069	ARID2	p.E245X	12	46230399	G	T	NO	5.4	0	0.04
MO_1069	FANCD2	p.Q1100E	3	10127569	C	G	NO	5.1	0	1
MO_1069	MUC16	p.S2675L	19	9083791	G	A	NO	154.6	0	0.16
MO_1069	UBA1	p.N928I	X	47072525	A	T	NO	140.5	0	0
MO_1069	RPS6KB2	p.V48L	11	67196613	G	T	YES	72.6	0	0.08
MO_1069	C11orf80	p.A582T	11	66605913	G	A	NO	58.7	0	0
MO_1069	MOGS	p.A470D	2	74689507	G	T	YES	38.1	0	0.03
MO_1069	CDR2L	p.E193X	17	72999348	G	T	NO	36.9	0	0.06
MO_1069	TBC1D9B	p.V43M	5	179331804	C	T	NO	34.8	0	0.01
MO_1069	HCFC1R1	p.R46L	16	3073490	C	A	NO	32.1	0	0.35
MO_1069	UBE2A	p.T69I	X	118716605	C	T	YES	29.2	0	0.84
MO_1069	CCDC88C	p.Q1770R	14	91739747	T	C	NO	29.0	0	0.53
MO_1069	CASKIN2	p.S557I	17	73499750	C	A	NO	27.6	0	0
MO_1069	MBD3	p.F138L	19	1562706	G	C	NO	27.0	0	0.01
MO_1069	ACBD3	p.D392Y	1	226340237	C	A	NO	22.1	0	
MO_1069	PDE4DIP	p.K223Q	1	144923791	T	G	NO	20.0	0	0.09
MO_1069	CENPF	p.E1583Q	1	214816428	G	C	NO	18.4	0	
MO_1069	CENPF	p.S1589R	1	214816446	A	C	NO	18.4	0	
MO_1069	TNC	p.V708E	9	117846496	A	T	NO	17.3	0	0
MO_1069	CCDC9	p.G459R	19	47774714	G	A	NO	16.3	1	0.69
MO_1069	NFRKB	p.R184W	11	129755459	G	A	NO	13.7	0	0.02
MO_1069	KIAA1683	p.P36S	19	18378244	G	A	YES	12.1	0	0
MO_1069	CTSC	p.L16F	11	88070795	G	A	YES	12.0	0	0.09
MO_1069	DUSP10	p.Y31S	1	221912995	T	G	NO	10.0	0	0
MO_1069	C15orf39	p.R824X	15	75500859	C	T	NO	8.7	0	0.03
MO_1069	ITGAX	p.C108G	16	31368577	T	G	NO	8.7	0	0
MO_1069	FAM8A1	p.S147F	6	17601080	C	T	YES	8.4	0	0.03
MO_1069	NLGN2	p.R642Q	17	7320535	G	A	NO	6.7	0	
MO_1069	PLS1	p.R274Q	3	142403170	G	A	NO	6.5	0	0.01
MO_1069	TTI2	p.H23Y	8	33370065	G	A	YES	6.1	0	0.15
MO_1069	PHF20L1	p.A95P	8	133807006	G	C	YES	5.5	0	0
MO_1069	ATRN	p.H427Y	20	3541384	C	T	YES	4.6	0	0.13
MO_1069	DIEXF	p.S246L	1	210010231	C	T	YES	4.3	0	0.31
MO_1069	UHRF2	splice donor	9	6493933	G	A	NO	3.9	0	
MO_1069	EFCAB7	p.D269N	1	64011587	G	A	YES	3.3	0	0.02
MO_1069	TTC27	UNKNOWN	2	32991568	G	C	NO	2.9	0	
MO_1069	KLHL24	p.I52V	3	183368298	A	G	NO	2.6	0	0.17
MO_1069	SHPRH	p.E1228A	6	146243847	T	G	NO	2.6	0	0.08
MO_1069	PTPLAD2	p.V119I	9	21015925	C	T	NO	2.2	0	0.76
MO_1069	RFX7	p.Q703H	15	56387526	T	G	NO	1.4	0	0
MO_1069	C7orf60	p.G7D	7	112579786	C	T	YES	1.1	0	0
MO_1069	ADAMTS7	p.V49A	15	79092844	A	G	NO	1.0	0	0.04
MO_1069	FAM227A	p.R369X	22	39003415	G	A	NO	0.9	0	1
MO_1069	CDC14A	p.R236H	1	100928306	G	A	NO	0.7	0	0
MO_1069	CHST2	p.P284A	3	142840508	C	G	NO	0.5	0	0.27

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1069	ADAMTSL1	p.R1093C	9	18777504	C	T	YES	0.5	0	0.01
MO_1069	MCTP2	p.K856Q	15	95020020	A	C	YES	0.5	0	0.02
MO_1069	MAP1A	p.V1155I	15	43817134	G	A	NO	0.5	0	0.06
MO_1069	PRDM8	p.A395T	4	81123799	G	A	YES	0.2	0	0.17
MO_1069	ANKRD1	p.G209A	10	92675953	C	G	NO	0.1	0	0
MO_1069	ZNF469	p.G694V	16	88495959	G	T	NO	0.1	0	0.02
MO_1069	C18orf34	p.L444F	18	30846957	C	G	NO	0.1	0	0.08
MO_1069	PAPPA2	p.P960L	1	176668368	C	T	YES	0.1	0	0
MO_1069	HOXD10	p.E227Q	2	176962240	G	C	NO	0.1	0	0.09
MO_1069	SCN3A	p.F1177V	2	165970466	A	C	NO	0.1	0	0.05
MO_1069	PDIA2	p.W417C	16	336564	G	T	YES	0.0	0	0
MO_1069	SHISA6	p.F128V	17	11145121	T	G	NO	0.0	0	0
MO_1069	KIAA1549L	p.E1244A	11	33612838	A	C	NO	0.0	0	0.01
MO_1069	ATP8A2	splice acc.	13	26043114	G	T	NO	0.0	0	
MO_1069	LRRN4	p.A62V	20	6033201	G	A	NO	0.0	0	0.18
MO_1069	ABCD2	p.D737H	12	39947728	C	G	NO	0.0	0	0.01
MO_1069	FAT3	p.T2716A	11	92534325	A	G	NO	0.0	0	0.18
MO_1069	BTNL8	p.Q426X	5	180377317	C	T	NO	0.0	0	1
MO_1069	LRRC7	p.S1063I	1	70504809	G	T	YES	0.0	1	0
MO_1069	PLA2G1B	p.Y133X	12	120760044	A	T	NO	0.0	0	
MO_1069	HMX1	p.V215D	4	8869822	A	T	NO	0.0	0	0
MO_1069	AKR1B10	p.S305C	7	134225604	C	G	NO	0.0	0	0.05
MO_1069	SLC18A3	p.A180V	10	50819325	C	T	NO	0.0	0	0.38
MO_1069	OGDHL	p.I517F	10	50953470	T	A	NO	0.0	0	0
MO_1069	OR4C16	p.G106R	11	55339919	G	C	NO	0.0	0	0.01
MO_1069	OR10G4	p.A90T	11	123886549	G	A	NO	0.0	0	0.79

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1129	ESR1	p.Y537S	6	152419923	A	C	NO	76.4	2	0
MO_1129	DEK	p.A18T	6	18264167	C	T	YES	22.8	0	0.02
MO_1129	PIK3CA	p.E542K	3	178936082	G	A	YES	4.3	603	0.04
MO_1129	ELK4	p.L412V	1	205585736	G	C	NO	4.2	0	
MO_1129	MLL	p.G1161V	11	118348889	G	T	NO	3.6	0	0
MO_1129	MAP3K5	p.C200Y	6	137026261	C	T	NO	0.7	0	0.05
MO_1129	WDR1	p.A239S	4	10089919	C	A	NO	105.9	0	0.13
MO_1129	TRIO	splice donor	5	14359641	G	A	NO	82.4	0	
MO_1129	SPEG	p.G397S	2	220313069	G	A	NO	37.1	0	0.07
MO_1129	USP5	p.E29Q	12	6961428	G	C	NO	30.2	0	0
MO_1129	HEATR2	p.A666V	7	813750	C	T	YES	20.8	0	0.01
MO_1129	TUBB2A	p.A248V	6	3154692	G	A	NO	10.6	0	0
MO_1129	CMAS	p.V136I	12	22206391	G	A	YES	9.8	0	0.17
MO_1129	DHX8	p.Q317X	17	41570898	C	T	NO	6.5	0	0.01
MO_1129	ZP3	p.M289V	7	76069886	A	G	NO	5.8	0	0.29
MO_1129	ZP3	p.R294T	7	76069902	G	C	YES	5.8	0	0.25
MO_1129	ZCCHC6	p.S1170L	9	88924451	G	A	YES	5.4	0	0
MO_1129	APCS	p.G194D	1	159558407	G	A	NO	4.4	0	
MO_1129	CES1	p.S12A	16	55866934	A	C	NO	3.2	0	1
MO_1129	PRDM11	p.N201Y	11	45226274	A	T	YES	2.2	0	0.02
MO_1129	USP37	p.S968N	2	219319690	C	T	YES	2.1	0	0.41
MO_1129	DMD	p.R1719H	X	32381074	C	T	NO	1.2	0	0.05
MO_1129	HHATL	p.R186H	3	42739770	C	T	NO	1.1	1	0
MO_1129	ODZ4	p.L1229F	11	78433828	G	A	NO	0.5	0	0
MO_1129	FAT4	p.L1005I	4	126240579	C	A	NO	0.5	0	0.19
MO_1129	ZFHX4	p.L2551M	8	77766808	C	A	NO	0.1	0	0.07
MO_1129	PABPC5	p.R169Q	X	90691082	G	A	YES	0.1	1	0.01
MO_1129	ODZ1	p.S1848L	X	123526026	G	A	NO	0.0	1	0.07
MO_1129	TMPRSS11F	splice donor	4	68938039	A	T	NO	0.0	1	
MO_1129	OR2J3	p.R175C	6	29080190	C	T	YES	0.0	0	0.05
MO_1129	OR13C5	p.T160I	9	107361216	G	A	NO	0.0	0	0.05
MO_1129	OR1L4	p.W140R	9	125486686	T	C	NO	0.0	0	1

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1167	ESR1	p.D538G	6	152419926	A	G	NA	657.1	2	0
MO_1167	HLA-A	p.G199R	6	29911296	G	A	NA	277.0	1	0
MO_1167	KIF1B	p.E1506K	1	10425470	G	A	NA	24.5	0	0.08
MO_1167	DNMT3A	p.E408A	2	25469545	T	G	NA	23.9	0	0.02
MO_1167	DNMT3A	p.E426D	2	25469490	T	G	NA	23.9	0	0.13
MO_1167	RBMX	p.R324P	X	135956506	C	G	NA	11.2	0	0.05
MO_1167	MLXIP	p.A518V	12	122618355	C	T	NA	164.9	0	0.24
MO_1167	MACF1	p.V1551G	1	39796897	T	G	NA	141.4	0	

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1167	ATOX1	splicing	5	151138339	G	A	NA	138.2	0	
MO_1167	CTDSP1	p.A69T	2	219266424	G	A	NA	133.1	0	0.9
MO_1167	HIST1H3H	p.Q126X	6	27776227	C	T	NA	61.8	0	0.01
MO_1167	RAPGEF5	p.Y334N	7	22200203	A	T	NA	48.0	0	0.24
MO_1167	MLLT4	p.S1282F	6	168351879	C	T	NA	43.6	0	0
MO_1167	MEF2A	p.Q428P	15	100252738	A	C	NA	34.6	3	0.23
MO_1167	LHFPL2	p.Y154H	5	77784947	A	G	NA	25.2	0	0
MO_1167	EDEM3	p.R253K	1	184692960	C	T	NA	22.7	0	0.08
MO_1167	EIF2C4	p.V154I	1	36291067	G	A	NA	20.5	0	0.59
MO_1167	RP3-402G11.5.1	UNKNOWN	22	50639528	T	C	NA	18.3	0	
MO_1167	ZFP91	p.C435F	11	58384770	G	T	NA	18.1	0	0
MO_1167	NBAS	splicing	2	15613473	T	G	NA	16.8	0	
MO_1167	NBAS	p.T548I	2	15613428	G	A	NA	16.8	0	0.02
MO_1167	NBAS	p.E535D	2	15613466	T	G	NA	16.8	0	0.77
MO_1167	ELL	p.R424H	19	18561481	C	T	NA	12.9	0	0.3
MO_1167	KLHL26	p.D237A	19	18778917	A	C	NA	11.2	0	0.01
MO_1167	FDXR	p.P3L	17	72869062	G	A	NA	9.6	0	0.01
MO_1167	SIRPA	p.G109S	20	1895990	G	A	NA	6.7	0	1
MO_1167	SHROOM4	p.P98S	X	50381286	G	A	NA	6.0	0	0
MO_1167	SLC9A5	p.L836H	16	67304929	G	A	NA	5.6	0	0.19
MO_1167	UNC13D	p.R1065X	17	73824126	G	A	NA	5.0	0	0.98
MO_1167	C22orf39	p.R29G	22	19435238	G	C	NA	2.6	0	0
MO_1167	ADRA1A	p.T391M	8	26627895	G	A	NA	2.5	0	0
MO_1167	PLCE1	p.R435K	10	95892028	G	A	NA	2.5	0	0.18
MO_1167	ZFP91-CNTF	p.C435F	11	58384770	G	T	NA	1.6	0	0
MO_1167	PCDHA10	p.A426V	5	140236910	C	T	NA	1.5	0	0.02
MO_1167	MUC4	p.A3654T	3	195507491	C	T	NA	1.4	0	
MO_1167	IGFN1	p.G2022S	1	201180085	G	A	NA	0.8	0	
MO_1167	ASPM	p.V2717L	1	197070232	C	G	NA	0.8	0	0.04
MO_1167	BMP7	p.D410E	20	55746061	A	T	NA	0.7	0	0
MO_1167	B3GNT3	p.A286T	19	17922668	G	A	NA	0.3	1	0.05
MO_1167	DSG1	p.G535R	18	28919904	G	A	NA	0.1	0	
MO_1167	CTSE	p.R389H	1	206331145	G	A	NA	0.1	0	0
MO_1167	COL17A1	splicing	10	105800822	C	T	NA	0.1	0	
MO_1167	IGHV4-31	p.P28S	14	106805481	G	A	NA	0	0	0.02
MO_1167	IGHV4-31	p.V21L	14	106805502	C	G	NA	0	0	0.42
MO_1167	AC012414.1	p.T40K	15	21071492	G	T	NA	0	0	1
MO_1167	GABRA6	p.R48Q	5	161113340	G	A	NA	0	1	0
MO_1167	ADAM2	p.K349T	8	39627077	T	G	NA	0	0	0
MO_1167	FCER2	p.W167R	19	7755414	A	T	NA	0	0	0

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1185	ESR1	p.Y537S	6	152419923	A	C	NA	46.5	2	0
MO_1185	PTPRT	p.S846F	20	40827900	G	A	NA	13.6	0	1
MO_1185	CDH1	p.S70F	16	68835618	C	T	NA	9.4	0	0
MO_1185	CDH1	p.Q641X	16	68856113	C	T	NA	9.4	0	0.24
MO_1185	LRP1B	p.P3139T	2	141242922	G	T	NA	8.3	0	0.31
MO_1185	FGFR1	p.R840Q	8	38271189	C	T	NA	5.9	0	0.6
MO_1185	TERT	p.V1035I	5	1255456	C	T	NA	0.1	0	0.57
MO_1185	ALK	p.E1299K	2	29430080	C	T	NA	0.0	1	0
MO_1185	MUC5B	p.S1632L	11	1262996	C	T	NA	1131.5	0	
MO_1185	VIM	p.E134K	10	17271821	G	A	NA	603.8	0	0
MO_1185	HNRNPK	p.L68F	9	86591921	G	A	NA	292.6	0	0.03
MO_1185	EIF5A	p.Y157C	17	7214778	A	G	NA	135.4	0	0
MO_1185	HNRNPU	p.S4L	1	245027599	G	A	NA	122.4	0	0
MO_1185	SCCPDH	p.S84L	1	246890254	C	T	NA	98.6	2	0
MO_1185	TXNIP	p.E165K	1	145440059	G	A	NA	79.5	0	0
MO_1185	INF2	p.E58K	14	105167874	G	A	NA	72.3	0	0.07
MO_1185	AHNAK	p.P2833A	11	62293392	G	C	NA	64.9	0	0.07
MO_1185	ALYREF	p.R151C	17	79847145	G	A	NA	50.1	0	0
MO_1185	RERE	p.S1084A	1	8420317	A	C	NA	40.3	0	0.32
MO_1185	MEPCE	p.Q137X	7	100028050	C	T	NA	38.9	0	0.22
MO_1185	SSSCA1	p.S165Y	11	65339099	C	A	NA	32.2	0	0
MO_1185	TBCD	p.P1143T	17	80895956	G	A	NA	30.6	0	0.06
MO_1185	STX16	p.D199N	20	57245606	G	A	NA	30.1	0	0.01
MO_1185	AGPAT6	p.Q278X	8	41470400	C	T	NA	27.2	0	0
MO_1185	RBM25	p.R433Q	14	73572710	G	A	NA	26.6	0	0.42
MO_1185	MUC16	p.R12975W	19	9010995	G	A	NA	24.6	0	0.01
MO_1185	ZFAND2B	p.Q41X	2	220072114	C	T	NA	20.6	0	0.15
MO_1185	PRKAA1	p.R144H	5	40771943	C	T	NA	19.8	0	0

Case ID	Gene	Amino Acid Change	Chr	Coord	Ref	Var	Present in FFPE	Expression (FPKM)	COSMIC @ Pos	AVISIFT Score
MO_1185	ITGAV	p.T76P	2	187466788	A	C	NA	19.7	0	0
MO_1185	NFKBIZ	p.M376I	3	101572498	G	A	NA	19.2	0	0.03
MO_1185	NOP14	splicing	4	2958396	C	A	NA	19.1	0	
MO_1185	AP3B1	p.S31L	5	77590312	G	A	NA	18.2	1	0.03
MO_1185	SNTB1	p.G224D	8	121706049	C	T	NA	17.2	0	0.04
MO_1185	GGPS1	p.E210K	1	235505812	G	A	NA	16.1	0	0
MO_1185	SLC2A11	p.P203L	22	24219643	C	T	NA	14.4	0	0
MO_1185	KIAA0020	p.E353K	9	2824794	C	T	NA	12.7	0	
MO_1185	RBBP6	p.A595T	16	24578657	G	A	NA	12.3	0	0.07
MO_1185	TMEM135	p.R421X	11	87032259	C	T	NA	12.2	1	1
MO_1185	STX18	p.T18M	4	4543639	G	A	NA	11.3	0	0.02
MO_1185	SBNO1	p.M208I	12	123825562	C	T	NA	10.7	0	0.87
MO_1185	NCF2	p.R38L	1	183559352	C	A	NA	10.2	0	0
MO_1185	ZNHIT2	p.Q366P	11	64684029	T	G	NA	9.6	0	0.01
MO_1185	EMR2	p.K154I	19	14877816	T	A	NA	8.4	0	0.22
MO_1185	SIRT6	p.R150X	19	4175924	G	A	NA	7.6	0	1
MO_1185	PASK	p.V1217G	2	242047620	A	C	NA	7.3	0	0
MO_1185	TRIM32	p.P431T	9	119461312	C	A	NA	6.7	0	0
MO_1185	CENPF	p.S1477X	1	214816111	C	A	NA	6.5	0	
MO_1185	PLEKHG2	p.S663F	19	39913682	C	T	NA	5.6	0	0
MO_1185	ATP2B1	p.E1136K	12	89985018	C	T	NA	4.1	0	0.12
MO_1185	FKTN	p.E456K	9	108397525	G	A	NA	3.7	0	0.05
MO_1185	PCDHGB4	p.L212F	5	140768087	G	C	NA	3.2	0	
MO_1185	WDFY4	p.F820I	10	49951565	T	A	NA	2.9	0	0.39
MO_1185	ZNF526	p.S144L	19	52916536	C	T	NA	2.9	0	1
MO_1185	KLF8	p.D65N	X	56291724	G	A	NA	2.2	0	0.43
MO_1185	STX16-NPEPL1	p.D199N	20	57245606	G	A	NA	2.0	0	0.01
MO_1185	EVX1	p.Y317S	7	27285770	A	C	NA	2.0	0	
MO_1185	AMPH	p.E459K	7	38457448	C	T	NA	1.8	0	0.05
MO_1185	CCDC40	p.E991K	17	78064076	G	A	NA	1.4	0	0.04
MO_1185	CCDC40	p.R980Q	17	78064044	G	A	NA	1.4	0	0
MO_1185	SVEP1	p.S1470F	9	113208171	G	A	NA	1.3	0	0
MO_1185	PPFIA3	p.K1132N	19	49652845	G	C	NA	1.1	0	0
MO_1185	HOXD3	p.R422X	2	177036967	C	T	NA	1.0	0	1
MO_1185	DNAH10	p.A1831T	12	124332538	G	A	NA	0.9	0	0
MO_1185	SPTB	p.E171K	14	65268999	C	T	NA	0.9	0	0
MO_1185	ZNF208	p.L34F	19	22171613	T	G	NA	0.5	0	0
MO_1185	KIRREL	p.E460Q	1	158061205	G	C	NA	0.4	0	0.15
MO_1185	RIMS1	p.E1471Q	6	73100344	G	C	NA	0.3	0	0
MO_1185	OR4C3	p.W174X	11	48347014	G	A	NA	0.1	0	0
MO_1185	NR6A1	p.S17L	9	127533349	G	A	NA	0.1	0	0
MO_1185	LAMA1	p.K247N	18	7049104	T	A	NA	0.1	0	
MO_1185	KCNN3	p.C519W	1	154705512	A	C	NA	0.1	0	0
MO_1185	HRNR	p.R2466H	1	152186708	C	T	NA	0.0	0	0.59
MO_1185	KRT6C	p.S227N	12	52865925	C	T	NA	0.0	0	0.26
MO_1185	IRX1	p.G300S	5	3599960	G	A	NA	0.0	0	0.38
MO_1185	PRH2	p.R119C	12	11083515	C	T	NA	0.0	0	0.07
MO_1185	LVRN.1	p.H813Y	5	115350211	C	T	NA	0.0	0	0.05
MO_1185	ALPK2	p.E1444K	18	56203089	C	T	NA	0.0	0	0
MO_1185	UNC79	p.D496N	14	94007139	G	A	NA	0.0	0	0
MO_1185	UNC80	p.Q1837H	2	210791613	G	C	NA	0.0	0	0.34
MO_1185	GRIK3	p.E852Q	1	37270599	C	G	NA	0.0	0	0.58
MO_1185	TGM6	p.E408K	20	2384355	G	A	NA	0.0	0	1
MO_1185	HRG	p.D170N	3	186389528	G	A	NA	0.0	0	0.69
MO_1185	GDA	p.D155N	9	74825681	G	A	NA	0.0	0	0.01
MO_1185	OR10H1	p.E173D	19	15918329	C	A	NA	0.0	0	0.58
MO_1185	ZNF454	p.S398C	5	178392598	C	G	NA	0.0	0	0.04
MO_1185	OR2T27	p.Y120C	1	248813827	T	C	NA	0.0	0	0.02
MO_1185	IGHV4-31	p.V21L	14	106805502	C	G	NA	0.0	0	0.42
MO_1185	IGHV4-31	p.P28S	14	106805481	G	A	NA	0.0	0	0.02

Table 4

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1031	4	68,586,132	68,829,154	24	**10.35**	
MO_1031	10	17,130,256	17,432,516	45	**8.28**	
MO_1031	10	81,316,935	84,498,366	95	**8.26**	*NRG3*
MO_1031	11	92,881,856	92,931,012	11	**7.77**	
MO_1031	4	68,919,654	69,107,468	22	**7.59**	
MO_1031	8	33,230,180	33,251,814	3	**7.50**	
MO_1031	10	74,451,906	75,530,518	186	**7.17**	
MO_1031	10	76,154,036	77,312,230	59	**6.79**	*KAT6B*
MO_1031	11	69,456,232	70,196,120	71	**6.75**	*CCND1, FADD, FGF19, FGF3, FGF4*
MO_1031	8	35,401,916	38,599,900	236	**6.74**	*BAG4, FGFR1, GPR124, LSM1, WHSC1L1, ZNF703*
MO_1031	10	12,802,948	13,568,184	72	**6.69**	
MO_1031	10	11,551,638	12,272,906	88	**6.57**	
MO_1031	18	6,171,882	6,263,988	9	**6.46**	
MO_1031	10	123,233,969	123,629,536	27	**6.32**	*FGFR2*
MO_1031	4	69,111,386	69,215,570	15	**5.81**	
MO_1031	4	66,189,850	67,142,530	19	**5.51**	*EPHA5*
MO_1031	10	75,532,834	76,074,464	88	**5.15**	
MO_1031	10	77,542,720	81,266,457	158	**5.13**	
MO_1031	8	39,442,183	41,164,370	74	**4.72**	
MO_1031	8	38,602,921	39,142,362	94	**4.67**	
MO_1031	11	90,288,976	92,718,066	35	**4.61**	
MO_1031	3	87,275,319	88,205,533	26	**4.11**	
MO_1031	18	2,539,035	6,138,182	201	**4.03**	
MO_1031	10	11,505,120	11,543,156	8	**3.99**	
MO_1031	12	3,982,470	4,481,740	14	**3.94**	*CCND2, FGF23*
MO_1031	18	6,301,996	11,825,000	294	**3.92**	
MO_1031	12	13,093,751	13,154,594	9	**3.86**	
MO_1031	10	17,495,666	17,686,317	7	**3.75**	
MO_1031	18	70,526,211	71,442,070	6	**3.36**	
MO_1031	10	12,277,132	12,767,372	12	**3.23**	
MO_1031	8	33,310,878	35,093,272	29	**3.19**	
MO_1031	18	61,747,550	66,368,940	38	**3.15**	
MO_1031	12	21,919,132	22,837,826	94	**3.09**	
MO_1031	11	70,200,412	71,850,892	187	**3.06**	
MO_1031	11	76,709,811	77,825,721	158	**3.03**	*PAK1*
MO_1031	12	208,418	1,769,452	175	**2.89**	*KDM5A, WNT5B*
MO_1031	12	4,488,584	5,154,919	75	**2.88**	*FGF23, FGF6*
MO_1031	11	78,497,966	79,113,172	14	**2.70**	
MO_1031	18	11,851,800	15,004,215	220	**2.70**	
MO_1031	4	54,853,144	54,967,958	7	**2.67**	*CHIC2*
MO_1031	17	51,901,464	81,083,588	3437	**2.61**	*AATK, AXIN2, BIRC5, BRIP1, CD79B, GRB2, HLF, PPM1D, PRKAR1A, RAD51C, RNF213, RNF43, RPS6KB1, RPTOR, SRSF2, TMC6, TMC8*
MO_1031	18	158,542	1,358,660	93	**2.58**	*YES1*
MO_1031	11	67,864,607	68,031,182	17	**2.54**	
MO_1031	4	73,927,524	74,735,596	110	**2.49**	
MO_1031	1	201,755,699	201,817,549	24	**2.49**	
MO_1031	12	21,201,680	21,807,605	97	**2.42**	
MO_1031	18	61,471,724	61,654,338	25	**2.42**	
MO_1031	1	207,245,672	208,390,788	139	**2.41**	
MO_1031	4	68,202,157	68,547,886	62	**2.38**	

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1031	11	72,945,843	74,209,014	177	**2.37**	
MO_1031	11	28,080,588	36,123,407	436	**2.35**	*EHF, ELF5, LMO2, WT1*
MO_1031	3	85,775,692	87,100,805	15	**2.34**	*CADM2*
MO_1031	8	32,406,234	32,621,669	18	**2.31**	*NRG1*
MO_1031	11	9,983,601	10,875,419	102	**2.25**	
MO_1031	4	75,065,608	75,959,197	24	**2.24**	*AREG, BTC, EPGN, EREG*
MO_1031	11	71,903,301	72,342,154	66	**2.24**	
MO_1031	11	74,907,638	74,915,575	5	**2.24**	
MO_1031	8	146,115,084	146,279,458	14	**2.22**	
MO_1031	11	96,074,868	99,426,930	10	**2.22**	*MAML2*
MO_1031	4	69,313,202	70,826,694	84	**1.99**	
MO_1031	1	190,067,665	193,218,926	72	**1.99**	*CDC73*
MO_1031	12	13,197,380	21,176,141	359	**1.88**	*PIK3C2G*
MO_1031	18	59,894,692	61,468,169	142	**1.87**	*BCL2, KDSR*
MO_1031	18	66,377,286	70,502,474	86	**1.87**	
MO_1031	4	72,607,550	73,923,894	43	**1.85**	
MO_1031	11	22,214,994	28,078,414	156	**1.84**	*BDNF, FANCF*
MO_1031	12	23,686,112	26,985,695	156	**1.82**	*KRAS*
MO_1031	8	66,617,076	141,931,000	2986	**1.81**	*EXT1, HEY1, MTDH, MYBL1, MYC, NBN, NCOA2, PREX2, PTK2, PVT1, RSPO2, RUNX1T1, STK3, TRIB1, YWHAZ, ZNF704*
MO_1031	16	29,820,892	29,842,221	10	**1.81**	
MO_1031	4	52,709,311	54,442,468	99	**1.80**	
MO_1031	1	172,108,064	184,537,648	925	**1.80**	*ABL2, FASLG*
MO_1031	1	206,903,313	207,244,824	72	**1.80**	
MO_1031	1	201,821,210	201,865,775	25	**1.79**	
MO_1031	19	58,904,864	59,083,890	56	**1.77**	
MO_1031	3	27,152,740	32,612,234	204	**1.77**	*TGFBR2*
MO_1031	14	50,044,532	56,150,882	590	**1.75**	*CDKN3*
MO_1031	12	133,728,390	133,770,132	7	**1.75**	
MO_1031	11	75,480,074	76,701,540	86	**1.73**	*C11orf30, WNT11*
MO_1031	10	84,625,118	86,273,618	61	**1.72**	*NRG3*
MO_1031	12	5,541,438	13,068,862	1014	**1.71**	*CDKN1B, ETV6, ING4, LRP6, NTF3, STYK1, ZNF384*
MO_1031	1	209,602,742	210,856,990	100	**1.71**	*IRF6*
MO_1031	4	55,106,349	56,225,649	71	**1.70**	*KDR, KIT, PDGFRA*
MO_1031	16	31,804,064	31,895,792	5	**1.69**	
MO_1031	12	57,111,463	57,118,361	4	**1.68**	
MO_1031	17	38,487,509	40,105,473	356	**1.68**	*RARA, TOP2A*
MO_1031	15	60,715,773	102,389,505	3226	**1.66**	*BCL2A1, BLM, CRTC3, CSK, FANCI, FES, IDH2, IGF1R, IQGAP1, MAP2K1, NRG4, NTRK3, PML, SMAD3, SMAD6*
MO_1031	1	204,915,769	205,760,704	151	**1.62**	*ELK4*
MO_1031	3	12,810,669	19,295,220	467	**1.62**	*WNT7A, XPC*
MO_1031	12	1,863,550	3,949,766	224	**1.62**	*FOXM1*
MO_1031	20	68,356	62,904,843	4648	**1.58**	*ARFRP1, ASXL1, AURKA, BCL2L1, CEBPB, GNAS, HCK, MAFB, MYBL2, NCOA3, NFATC2, PAK7, PLCG1, PTK6, PTPRT, SRC, SRMS, STK4, TOP1, YWHAB, ZMYND8, ZNF217*
MO_1031	7	154,237,588	158,937,252	313	**1.57**	*MNX1, SHH*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1031	14	22,356,506	22,963,836	83	**1.53**	
MO_1031	3	84,962,992	85,156,657	3	**1.49**	*CADM2*
MO_1031	17	25,621,074	25,628,926	3	**1.48**	
MO_1031	16	29,808,376	29,819,978	11	**1.47**	
MO_1031	17	20,639,138	22,023,536	51	**-0.07**	
MO_1031	21	9,825,990	9,826,257	2	**-0.02**	
MO_1031	8	2,796,151	3,611,511	66	**0.00**	
MO_1031	22	39,360,598	39,385,480	6	**0.04**	
MO_1031	14	26,917,625	27,377,958	7	**0.11**	
MO_1031	5	177,166,120	177,302,765	3	**0.20**	
MO_1031	1	211,832,141	211,847,090	6	**0.24**	
MO_1031	10	46,961,903	47,087,312	11	**0.27**	
MO_1031	10	135,077,162	135,123,783	35	**0.30**	
MO_1031	10	133,918,368	135,044,448	183	**0.36**	
MO_1031	11	636,612	1,282,788	251	**0.36**	
MO_1031	2	105,472,456	105,488,778	4	**0.39**	
MO_1031	3	20,218,127	23,312,464	14	**0.42**	
MO_1031	12	132,911,966	133,032,417	5	**0.42**	
MO_1031	11	193,171	534,238	107	**0.43**	*HRAS*
MO_1031	4	7,533,339	10,118,369	180	**0.43**	
MO_1031	16	82,660,648	90,133,268	754	**0.43**	*FANCA, MC1R*
MO_1031	8	183,094	2,148,837	111	**0.44**	
MO_1031	4	3,076,537	3,534,082	114	**0.47**	
MO_1031	11	63,276,172	64,836,076	405	**0.47**	*ESRRA, MEN1, VEGFB*
MO_1031	10	135,125,386	135,381,649	77	**0.48**	
MO_1031	6	31,865,505	31,867,955	3	**0.48**	
MO_1031	5	177,419,839	180,687,459	418	**0.50**	*FLT4, MAML1, NHP2*
MO_1031	11	55,433,136	57,480,180	182	**0.51**	
MO_1031	12	100,451,433	100,463,882	4	**0.52**	
MO_1031	10	255,897	9,450,260	491	**0.53**	*GATA3, KLF6*
MO_1031	18	71,740,798	78,005,236	223	**0.53**	
MO_1031	11	49,059,002	55,340,015	39	**0.53**	
MO_1031	10	123,658,413	133,795,411	542	**0.53**	
MO_1031	17	6,108	20,492,914	2773	**0.53**	*ALOX12B, AURKB, C17orf39, COPS3, CRK, DVL2, FGF11, FLCN, GPS2, MAP2K4, NCOR1, RABEP1, RPA1, TNK1, TP53, USP6*
MO_1031	16	34,257,075	62,055,226	1163	**0.54**	*C16orf57, CYLD, NUP93*
MO_1031	11	2,154,292	9,229,136	832	**0.54**	*CDKN1C, IGF2, LMO1, NUP98, RBMXL2, RRM1*
MO_1031	6	154,727,562	170,893,472	890	**0.55**	*ARID1B, IGF2R, PARK2, TBP*
MO_1031	3	37,308,321	66,550,768	3147	**0.55**	*ALAS1, BAP1, CTNNB1, FHIT, MAP4, MST1, MST1R, MYD88, PBRM1, PTPRG, SETD2, WNT5A*
MO_1031	3	4,817,048	12,791,326	579	**0.55**	*FANCD2, PPARG, RAF1, VHL*
MO_1031	8	41,166,434	43,212,028	260	**0.55**	*IKBKB, KAT6A*
MO_1031	17	26,488,181	34,207,360	973	**0.55**	*NF1, RAD51D, RHOT1, SUZ12, TAF15*
MO_1031	1	205,859,650	206,331,182	33	**0.57**	
MO_1031	10	48,371,062	74,326,404	1228	**0.58**	*NCOA4, PRF1*
MO_1031	9	71,986,384	101,817,485	1617	**0.58**	*FANCC, GALNT12, GNAQ, NTRK2, PTCH1, ROR2, SYK, XPA*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1031	8	3,855,468	31,497,817	1532	**0.58**	*BLK, FGF17, FGF20, NKX3-1, NRG1, PTK2B, TNKS, WRN*
MO_1031	11	13,391,238	21,596,574	669	**0.58**	
MO_1031	1	211,847,711	233,105,699	1448	**0.59**	*H3F3A, PSEN2, WNT3A, WNT9A*
MO_1031	10	13,629,072	17,127,612	236	**0.59**	
MO_1031	11	93,065,442	93,148,321	14	**0.59**	
MO_1031	11	93,428,784	94,597,928	135	**0.59**	*MRE11A*
MO_1031	10	104,809,500	122,668,216	1135	**0.59**	*SHOC2, TCF7L2*
MO_1031	11	99,429,012	134,257,653	2637	**0.59**	*ATM, BIRC2, BIRC3, CBL, CHEK1, DDX6, ETS1, FLI1, GUCY1A2, HMBS, MLL, PDGFD, POU2AF1, SDHD, UBE4A, YAP1, ZBTB16*
MO_1031	15	29,346,458	60,690,024	2861	**0.59**	*BUB1B, FGF7, LTK, NOP10, RAD51, SPRED1, TYRO3*
MO_1031	5	138,611,754	138,860,952	58	**0.59**	
MO_1031	14	56,585,346	82,000,118	1909	**0.59**	*C14orf133, ESR2, FOS, HIF1A, HIF1A, MAX, MLH3, NUMB, PGF, PSEN1, TSHR*
MO_1031	5	135,277,073	136,328,170	48	**0.59**	*SMAD5*
MO_1031	10	88,718,542	104,660,474	1742	**0.60**	*BLNK, CHUK, CYP17A1, FAS, FGF8, GOT1, LDB1, NFKB2, PTEN, SUFU, TLX1, TNKS2, WNT8B*
MO_1031	1	234,350,230	240,656,524	462	**0.60**	
MO_1031	11	77,830,326	78,489,599	57	**0.60**	*GAB2*
MO_1031	4	61,788,424	65,275,026	44	**0.60**	*LPHN3*
MO_1031	X	2,700,167	102,755,742	3800	**0.61**	*AR, ARAF, ATRX, BCOR, BMX, BTK, CCNB3, DDX3X, ELK1, FAM123B, FANCB, FGF16, FIGF, FOXO4, FOXP3, GATA1, KDM5C, KDM6A, MAGED1, MED12, PIM2, SSX1, SSX2, SSX3, SSX4, TBX22, TFE3, USP9X, WAS, ZRSR2*
MO_1031	10	17,702,514	46,222,766	1080	**0.61**	*MLLT10, RET*
MO_1031	5	125,696,010	134,106,614	636	**0.61**	*ACSL6, IL3, RAD50, TCF7*
MO_1031	6	151,144,864	151,939,184	68	**0.61**	
MO_1031	9	3,453,732	26,101,343	768	**0.61**	*CDKN2A, CDKN2B, JAK2, NFIB, PSIP1, PTPRD*
MO_1031	18	18,539,877	58,309,304	1673	**0.61**	*ASXL3, CDH2, GATA6, MALT1, MBD1, PIK3C3, ROCK1, SMAD2, SMAD4, SMAD7, SS18*
MO_1031	3	239,492	4,558,246	119	**0.62**	*CRBN*
MO_1031	4	165,876,302	190,903,876	826	**0.62**	*VEGFC*
MO_1031	3	67,546,367	81,810,649	304	**0.62**	*FOXP1, MITF, ROBO2*
MO_1031	1	211,526,618	211,751,637	16	**0.63**	
MO_1031	6	37,180,450	124,442,998	4130	**0.63**	*BACH2, BAI3, CCND3, EPHA7, FOXO3, FOXP4, FRK, FYN, PKHD1, PNRC1, PRDM1, PTK7, RAB23, ROS1, TFEB, VEGFA*
MO_1031	6	142,399,794	145,823,742	193	**0.63**	*PLAGL1*
MO_1031	14	20,201,656	20,404,300	15	**0.63**	
MO_1031	9	71,080,062	71,152,243	7	**0.64**	
MO_1031	4	25,758,416	43,032,450	578	**0.64**	*PHOX2B, RBPJ, RHOH*
MO_1031	3	32,726,898	37,190,419	251	**0.64**	*MLH1*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1031	6	126,176,264	137,245,469	665	**0.64**	*MAP3K5, MYB, PTPRK, RSPO3*
MO_1031	2	154,334,924	201,305,468	2736	**0.64**	*HNRNPA3, HOXD11, HOXD13, HOXD4, NAB1, NFE2L2, PDK1, PMS1, SF3B1, SP3, STAT1, STAT4*
MO_1031	5	54,415,758	122,522,926	2765	**0.64**	*APC, FER, IQGAP2, MAP3K1, PIK3R1*
MO_1031	11	81,601,827	89,955,936	333	**0.65**	*PICALM*
MO_1031	3	19,322,754	20,216,137	64	**0.65**	
MO_1031	4	74,847,007	75,046,416	16	**0.66**	
MO_1031	4	10,446,372	15,560,868	105	**0.66**	
MO_1031	1	85,869,998	93,913,780	539	**0.66**	*GFI1, RBMXL1*
MO_1031	14	28,733,801	31,056,040	27	**0.66**	
MO_1031	4	81,256,954	110,650,894	1362	**0.66**	*AFF1, FGF5, LEF1, NFKB1, RAP1GDS1, TET2*
MO_1031	14	39,818,203	48,230,312	133	**0.67**	*FANCM*
MO_1031	4	44,682,652	44,719,222	19	**0.68**	
MO_1031	3	23,364,968	26,751,572	97	**0.69**	*TOP2B*
MO_1031	22	23,610,702	23,632,514	10	**0.69**	*BCR*
MO_1031	6	34,741,320	34,840,216	21	**0.70**	

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1051	1	152,484,086	152,816,125	19	**3.31**	
MO_1051	11	6,231,424	6,232,842	3	**2.92**	
MO_1051	20	61,438,941	61,468,452	32	**2.42**	
MO_1051	1	248,604,538	249,211,679	25	**2.30**	
MO_1051	X	34,148,550	34,962,044	10	**2.14**	
MO_1051	X	107,404,842	107,936,036	88	**2.07**	
MO_1051	1	152,857,076	227,843,507	6285	**2.01**	*ABL2, CDC73, CKS1B, DDR2, ELF3, ELK4, ETV3, ETV3L, FASLG, H3F3A, HAX1, IKBKE, INSRR, IQGAP3, IRF6, MDM4, NCSTN, NTRK1, PBX1, PBX1, PRRX1, PSEN2, RAB25, SDHC, SHC1*
MO_1051	X	31,792,194	32,235,103	8	**1.98**	
MO_1051	15	25,416,022	25,496,149	41	**1.95**	
MO_1051	1	144,013,934	152,081,884	754	**1.94**	*APH1A, ARNT, BCL9, CHD1L, MCL1*
MO_1051	12	54,369,140	54,520,236	16	**1.93**	*HOXC11*
MO_1051	1	229,407,014	247,611,745	1149	**1.91**	*AKT3, FH*
MO_1051	17	46,607,082	46,709,920	23	**1.89**	
MO_1051	X	95,940,052	96,639,008	27	**1.81**	
MO_1051	4	53,298	67,754	4	**1.74**	
MO_1051	7	27,140,656	27,282,803	28	**1.71**	*HOXA10, HOXA11, HOXA13, HOXA3, HOXA9*
MO_1051	19	35,803,193	36,018,323	39	**1.70**	*CD22*
MO_1051	19	10,077,158	10,108,796	30	**1.70**	
MO_1051	19	36,054,320	36,556,852	179	**1.65**	*ETV2, PSENEN*
MO_1051	19	45,910,365	46,029,258	32	**1.63**	*ERCC1, FOSB*
MO_1051	1	152,083,634	152,287,808	27	**1.54**	
MO_1051	2	228,135,522	228,163,484	20	**1.53**	
MO_1051	X	106,893,248	107,403,850	45	**1.51**	
MO_1051	2	227,732,024	227,963,521	37	**1.49**	
MO_1051	16	47,462,728	61,687,744	1025	**1.47**	*C16orf57, CYLD, NUP93*
MO_1051	X	107,938,154	117,959,778	339	**1.45**	*IRS4, PAK3*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1051	20	29,652,166	59,197,288	2647	1.43	*ASXL1, AURKA, BCL2L1, CEBPB, GNAS, HCK, MAFB, MYBL2, NCOA3, NFATC2, PLCG1, PTPRT, SRC, STK4, TOP1, YWHAB, ZMYND8, ZNF217*
MO_1051	1	40,756,525	40,947,550	42	1.42	
MO_1051	20	61,470,000	62,904,843	331	1.42	*ARFRP1, PTK6, SRMS*
MO_1051	X	17,095,401	18,606,234	62	1.42	
MO_1051	14	106,303,455	107,283,120	80	1.42	
MO_1051	4	74,286,884	75,719,594	90	1.41	*AREG, BTC, EPGN, EREG*
MO_1051	X	31,462,736	31,747,833	10	1.41	
MO_1051	X	50,147,176	53,560,271	109	1.41	*KDM5C, MAGED1, SSX2*
MO_1051	16	97,456	47,117,398	3513	1.40	*ABCC1, AXIN1, CIITA, CREBBP, ERCC4, FUS, GRIN2A, MKL2, MLST8, MYH11, PALB2, PDPK1, PRSS8, SOCS1, TNFRSF17, TSC2, ZNF668*
MO_1051	7	158,055,758	158,119,500	5	-0.34	
MO_1051	12	50,271,607	50,273,606	3	-0.33	
MO_1051	12	132,911,966	133,032,417	5	-0.30	
MO_1051	6	169,114,856	169,115,787	3	-0.18	
MO_1051	4	88,535,422	88,537,643	3	-0.16	
MO_1051	9	96,424,692	96,425,932	3	-0.11	
MO_1051	7	5,632,990	5,643,110	3	-0.10	
MO_1051	7	141,919,835	141,920,772	3	-0.10	
MO_1051	19	2,524,606	2,576,124	6	-0.03	
MO_1051	17	11,145,038	11,999,005	89	-0.02	*MAP2K4*
MO_1051	9	139,740,311	139,741,391	4	-0.01	
MO_1051	10	134,229,031	134,243,972	5	0.05	
MO_1051	21	9,825,990	10,793,947	7	0.11	
MO_1051	20	59,225,067	59,793,635	6	0.20	
MO_1051	4	30,279,568	31,116,369	12	0.23	
MO_1051	11	1,092,230	1,101,070	13	0.26	
MO_1051	9	125,239,660	125,487,221	15	0.29	
MO_1051	4	25,417,181	25,831,748	34	0.31	
MO_1051	9	139,399,337	139,438,456	22	0.33	*NOTCH1*
MO_1051	7	100,634,044	100,686,182	26	0.36	
MO_1051	11	122,659,980	124,489,588	140	0.36	
MO_1051	5	140,167,837	140,263,912	57	0.37	
MO_1051	22	17,058,814	22,673,386	656	0.41	*CRKL*
MO_1051	6	74,183,252	74,304,854	17	0.42	
MO_1051	3	97,711,728	98,251,403	36	0.42	
MO_1051	22	39,709,356	51,216,322	1353	0.45	*CYP2D6, EP300, MKL1, PIM3, WNT7B*
MO_1051	9	139,992,316	140,083,683	39	0.46	
MO_1051	16	61,689,494	70,884,528	868	0.47	*CBFB, CDH1, CDH5, CTCF, NQO1*
MO_1051	17	12,011,235	21,207,774	755	0.47	*C17orf39, COPS3, FLCN, MAP2K4, NCOR1*
MO_1051	4	337,748	25,416,170	1492	0.48	*FGFR3, WHSC1*
MO_1051	8	183,094	681,251	32	0.48	
MO_1051	17	436,106	7,830,984	1445	0.48	*CRK, DVL2, FGF11, GPS2, RABEP1, RPA1, TNK1, TP53, USP6*
MO_1051	22	23,404,080	39,355,591	1805	0.48	*BCR, CHEK2, CSNK1E, EWSR1, MN1, NF2, PATZ1, RAC2, SMARCB1, SOX10, XBP1*
MO_1051	X	134,986,740	134,993,900	15	0.48	
MO_1051	8	39,521,388	54,730,033	579	0.49	*CEBPD, IKBKB, KAT6A, PRKDC*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1051	17	7,906,986	10,728,695	481	**0.49**	*ALOX12B, AURKB*
MO_1051	11	5,730,622	6,226,929	35	**0.50**	
MO_1051	16	89,300,336	90,142,217	221	**0.51**	*FANCA, MC1R*
MO_1051	16	71,209,628	89,294,050	1193	**0.53**	*MAF, PHLPP2, PLCG2, ZFHX3*
MO_1051	11	110,306,688	122,653,778	1220	**0.53**	*CBL, DDX6, HMBS, MLL, POU2AF1, SDHD, UBE4A, ZBTB16*
MO_1051	12	208,418	18,576,865	1758	**0.54**	*CCND2, CDKN1B, ETV6, FGF23, FGF6, FOXM1, ING4, KDM5A, LRP6, NTF3, PIK3C2G, STYK1, WNT5B, ZNF384*
MO_1051	8	1,497,380	38,879,197	2010	**0.55**	*BAG4, BLK, FGF17, FGF20, FGFR1, GPR124, LSM1, NKX3-1, NRG1, PTK2B, TNKS, WHSC1L1, WRN, ZNF703*
MO_1051	11	124,493,156	134,257,653	661	**0.56**	*CHEK1, ETS1, FLI1*
MO_1051	12	19,282,894	21,168,580	85	**0.57**	
MO_1051	4	31,129,613	69,885,766	1352	**0.57**	*CHIC2, EPHA5, KDR, KIT, LPHN3, PDGFRA, PHOX2B, RHOH, TEC, TXK*
MO_1051	17	41,957,310	42,636,450	157	**0.57**	*G6PC3*
MO_1051	X	63,005,978	79,938,015	771	**0.61**	*AR, ATRX, FAM123B, FGF16, FOXO4, MED12, TBX22*
MO_1051	2	170,019,028	170,101,253	41	**0.62**	
MO_1051	X	118,109,146	118,145,782	9	**0.62**	
MO_1051	22	22,781,917	23,230,282	44	**0.62**	
MO_1051	1	120,480,070	142,540,225	24	**0.63**	*NOTCH2*
MO_1051	12	22,354,800	23,696,160	49	**0.63**	
MO_1051	7	128,457,794	128,527,239	46	**0.64**	
MO_1051	12	57,543,422	57,604,578	56	**0.64**	
MO_1051	4	25,834,611	30,222,415	79	**0.65**	*RBPJ*
MO_1051	20	68,356	29,633,982	1441	**0.65**	*PAK7*
MO_1051	4	106,369,290	107,158,004	67	**0.65**	
MO_1051	16	47,120,256	47,409,815	19	**0.65**	
MO_1051	X	2,947,315	9,621,620	102	**0.66**	
MO_1051	15	20,450,347	23,686,296	87	**0.67**	
MO_1051	3	96,585,718	97,705,619	67	**0.70**	*EPHA6*
MO_1051	4	85,827	331,662	17	**0.70**	
MO_1051	11	4,411,528	5,602,632	88	**0.71**	
MO_1051	19	36,685,981	37,689,941	106	**0.71**	
MO_1051	11	862,652	1,091,424	89	**0.72**	
MO_1051	19	52,497,138	53,716,488	175	**0.72**	*PPP2R1A*
MO_1051	21	45,736,218	46,311,818	115	**0.73**	
MO_1051	15	25,232,160	25,351,768	30	**0.74**	
MO_1051	17	41,168,284	41,603,952	90	**0.74**	*BRCA1*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1069	17	81,006,503	81,083,588	6	**4.47**	
MO_1069	18	641,494	736,954	28	**4.38**	*YES1*
MO_1069	17	67,512,994	68,171,885	13	**4.32**	
MO_1069	17	65,528,925	66,453,492	105	**3.90**	
MO_1069	17	63,746,813	65,214,752	91	**3.83**	
MO_1069	17	71,232,438	73,874,342	538	**3.70**	*GRB2*
MO_1069	12	8,906,702	8,925,873	2	**3.59**	
MO_1069	17	74,846,540	76,100,688	77	**3.36**	
MO_1069	17	46,154,428	46,928,948	72	**3.33**	*HOXB13*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1069	1	211,749,146	211,847,883	10	**3.02**	
MO_1069	18	158,542	633,304	51	**2.95**	
MO_1069	1	248,201,992	248,367,364	9	**2.87**	
MO_1069	18	739,832	1,278,637	14	**2.86**	*YES1*
MO_1069	17	48,423,574	48,721,012	126	**2.54**	
MO_1069	17	60,064,432	63,739,228	442	**2.43**	*AXIN2, CD79B*
MO_1069	17	80,575,285	80,992,948	79	**2.40**	
MO_1069	6	135,778,773	135,839,774	11	**2.35**	
MO_1069	17	65,336,945	65,374,292	11	**2.35**	
MO_1069	17	53,798,298	60,062,226	627	**2.09**	*BRIP1, PPM1D, RAD51C, RNF43, RPS6KB1*
MO_1069	2	44,008,719	44,139,663	44	**2.05**	
MO_1069	17	76,104,558	80,574,524	916	**1.98**	*AATK, BIRC5, RNF213, RPTOR, TMC6, TMC8*
MO_1069	17	73,887,101	74,774,345	247	**1.97**	*SRSF2*
MO_1069	11	65,960,946	67,290,032	367	**1.96**	*AIP, RBM14*
MO_1069	17	48,733,210	51,901,464	138	**1.93**	
MO_1069	17	46,929,932	48,356,715	222	**1.93**	*PHB, SPOP*
MO_1069	17	66,511,554	67,178,942	147	**1.92**	*PRKAR1A*
MO_1069	17	69,334,538	71,228,340	34	**1.91**	
MO_1069	11	67,352,142	134,257,653	4523	**1.90**	*ATM, BIRC2, BIRC3, C11orf30, CBL, CCND1, CHEK1, DDX6, ETS1, FADD, FGF19, FGF3, FGF4, FLI1, GAB2, GUCY1A2, HMBS, LRP5, MAML2, MLL, MRE11A, PAK1, PDGFD, PICALM, POU2AF1, SDHD, UBE4A, WNT11, YAP1, ZBTB16*
MO_1069	1	247,737,584	248,185,858	22	**1.88**	
MO_1069	17	43,718,058	46,153,561	271	**1.81**	*WNT3, WNT9B*
MO_1069	1	248,402,327	249,211,679	37	**1.79**	
MO_1069	2	238,273,091	238,449,050	23	**1.73**	
MO_1069	X	50,055,582	50,167,242	34	**1.65**	*CCNB3*
MO_1069	12	5,915,310	6,062,648	16	**1.64**	
MO_1069	15	101,464,798	101,608,913	34	**1.63**	
MO_1069	20	57,245,562	57,292,974	20	**1.59**	
MO_1069	2	214,727,212	219,146,824	316	**1.55**	*BARD1*
MO_1069	2	43,927,208	44,003,978	23	**1.55**	
MO_1069	12	120,111,766	120,173,122	26	**1.54**	
MO_1069	15	72,338,509	91,769,702	1766	**1.53**	*BCL2A1, BLM, CRTC3, CSK, FANCI, FES, IDH2, IQGAP1, NRG4, NTRK3, PML*
MO_1069	3	195,452,936	195,610,114	40	**1.53**	*TNK2*
MO_1069	3	61,734,674	65,433,782	183	**1.53**	*PTPRG*
MO_1069	3	5,164,069	18,462,351	1018	**1.51**	*FANCD2, PPARG, RAF1, VHL, WNT7A, XPC*
MO_1069	17	67,181,674	67,501,961	56	**1.51**	
MO_1069	18	70,205,454	78,005,236	238	**1.51**	
MO_1069	3	75,790,546	87,100,805	103	**1.51**	*CADM2, ROBO2*
MO_1069	3	97,753,837	98,217,270	20	**1.51**	
MO_1069	19	51,413,897	51,584,944	51	**1.50**	
MO_1069	20	32,441,436	33,012,336	31	**1.49**	
MO_1069	17	42,635,108	42,636,450	3	**1.48**	
MO_1069	2	44,145,334	44,428,753	28	**1.48**	
MO_1069	1	103,480,082	149,899,706	1500	**1.48**	*BCL9, CHD1L, CSF1, FAM46C, NGF, NOTCH2, NRAS, WNT2B*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1069	7	138,400,628	151,433,128	1172	1.48	*BRAF, EPHA1, EPHB6, EZH2, PRSS1, RHEB*
MO_1069	1	150,912,430	211,654,670	5646	1.47	*ABL2, CDC73, CKS1B, DDR2, ELF3, ELK4, ETV3, ETV3L, FASLG, HAX1, IKBKE, INSRR, IQGAP3, IRF6, MDM4, NCSTN, NTRK1, PBX1, PBX1, PRRX1, RAB25, SDHC, SHC1*
MO_1069	19	18,218,476	18,391,780	61	1.46	*JUND, PIK3R2*
MO_1069	8	29,207,626	30,982,114	107	1.46	*WRN*
MO_1069	1	211,923,224	231,155,638	1347	1.46	*H3F3A, PSEN2, WNT3A, WNT9A*
MO_1069	X	48,463,399	48,689,705	44	1.45	*GATA1, WAS*
MO_1069	19	40,514,428	40,589,194	29	1.44	
MO_1069	19	54,632,528	54,659,064	13	1.44	
MO_1069	16	29,001,065	30,393,825	155	1.44	
MO_1069	18	12,008,430	13,105,058	155	1.44	
MO_1069	11	50,003,405	56,237,946	67	1.43	
MO_1069	1	29,385,200	103,385,894	5195	1.42	*ARTN, BCL10, CDKN2C, CMPK1, DPYD, FUBP1, GFI1, JAK1, JUN, LCK, MAST2, MPL, MUTYH, MYCL1, PTCH2, RAD54L, RBMXL1, ROR1, TAL1, TIE1*
MO_1069	19	40,095,937	40,225,610	18	1.42	
MO_1069	12	63,541,308	101,873,515	1849	1.41	*BTG1, DYRK2, ELK3, FRS2, HMGA2, KITLG, MDM2, PTPRR, SPIC, YEATS4*
MO_1069	1	231,299,237	247,729,174	983	1.40	*AKT3, FH*
MO_1069	5	137,734,039	138,118,040	51	1.40	*CTNNA1, KDM3B*
MO_1069	16	31,896,454	34,681,986	29	1.40	
MO_1069	17	33,611,080	33,638,804	4	0.09	
MO_1069	7	100,187,314	100,188,694	4	0.17	
MO_1069	17	32,809,035	32,820,286	5	0.18	
MO_1069	21	41,140,492	41,462,159	3	0.27	
MO_1069	19	11,373,858	11,390,874	8	0.28	
MO_1069	9	113,210,718	113,385,698	37	0.39	
MO_1069	5	70,841,173	70,844,815	6	0.41	
MO_1069	13	109,613,925	109,956,892	79	0.43	
MO_1069	5	137,532,229	137,542,289	8	0.44	
MO_1069	17	40,089,486	40,164,050	18	0.45	
MO_1069	1	39,126,286	39,134,277	3	0.49	
MO_1069	1	25,539,630	29,252,385	552	0.51	*ARID1A, CD52, FGR, MAP3K6, PDIK1L, RPS6KA1*
MO_1069	17	32,822,782	33,606,210	68	0.51	
MO_1069	17	33,708,893	36,332,740	564	0.51	*ERBB2, LASP1, MLLT6, RARA*
MO_1069	17	40,192,184	40,923,970	167	0.51	
MO_1069	19	10,830,006	11,371,818	157	0.51	*SMARCA4*
MO_1069	16	51,432,288	69,401,274	1621	0.52	*CBFB, CDH1, CDH11, CSNK2A2, HERPUD1, PSKH1*
MO_1069	17	6,108	23,543,324	2894	0.52	*AURKB, CAMKK1, FLCN, GAS7, GSG2, GUCY2D, MAP2K3, MAP2K4, MAPK7, PER1, TP53, ULK2, USP6*
MO_1069	17	36,928,482	39,936,174	751	0.52	*BRCA1, ETV4, WNK4*
MO_1069	19	7,512,372	8,392,876	226	0.52	*MAP2K7*
MO_1069	19	11,392,694	18,070,508	1424	0.52	*BRD4, JAK3, LYL1, MAST1, PKN1, PRKACA, TPM4*
MO_1069	3	197,579,348	197,614,259	7	0.54	

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1069	6	68,655,658	126,361,388	2422	**0.54**	*EPHA7, FRK, FYN, GOPC, MAP3K7, PRDM1, ROS1, TTK*
MO_1069	9	126,773,579	130,420,973	558	**0.54**	*CDK9*
MO_1069	16	45,065,780	49,742,682	362	**0.54**	*CYLD*
MO_1069	16	69,767,130	87,910,942	1206	**0.54**	*CBFA2T3, MAF, MLKL*
MO_1069	1	752,046	25,445,741	3061	**0.55**	*CDA, EPHA2, EPHA8, EPHB2, MTHFR, PAX7, PINK1, PRDM16, PRKCZ, RPL22, SDHB, TNFRSF14*
MO_1069	6	36,128,488	63,053,829	1872	**0.55**	*CCND3, ICK, MAPK13, MAPK14, PIM1, PTK7, STK38, TFEB, TTBK1*
MO_1069	9	70,284,154	94,206,698	1056	**0.55**	*DAPK1, GNAQ, NTRK2, PRKACG, ROR2, SYK, TRPM6*
MO_1069	9	94,778,540	112,840,718	1124	**0.55**	*FANCC, NR4A3, TAL2, TGFBR1, XPA*
MO_1069	9	113,388,245	126,671,370	989	**0.55**	*NEK6*
MO_1069	17	23,824,874	32,807,220	1065	**0.55**	*NEK8, NF1, SUZ12, TAF15, TAOK1*
MO_1069	2	102,140,611	157,175,420	2049	**0.56**	*ACVR2A, BUB1, ERCC3, MERTK, PAX8, TTL, YSK4*
MO_1069	9	130,552,829	139,852,678	1690	**0.56**	*ABL1, BRD3, C9orf96, NOTCH1, NUP214, RALGDS, TSC1*
MO_1069	13	18,499,113	109,612,623	3022	**0.56**	*BRCA2, CDK8, CDX2, CSNK1A1L, ERCC5, FLT1, FLT3, LATS2, LCP1, LHFP, NEK5, RB1, STK24*
MO_1069	15	37,793,066	37,855,893	9	**0.56**	
MO_1069	22	15,438,814	49,563,188	3901	**0.56**	*ADRBK2, BCR, CHEK2, CSNK1E, CYP2D6, EP300, EWSR1, LIMK2, MAPK1, MAPK11, MAPK12, MKL1, MN1, MYH9, NF2, PDGFB, PIM3, SMARCB1, TSSK2*
MO_1069	6	160,872,714	170,731,132	449	**0.57**	*FGFR1OP, MAP3K4, MLLT4, RPS6KA2*
MO_1069	19	50,688,434	50,721,098	15	**0.57**	
MO_1069	3	214,492	5,000,018	186	**0.59**	
MO_1069	13	109,958,488	114,108,621	377	**0.59**	
MO_1069	8	144,312,499	146,250,262	372	**0.61**	*ADCK5, MAPK15, NRBP2*
MO_1069	16	88,333,032	88,669,718	118	**0.62**	*FANCA*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1129	11	78,525,452	78,614,830	8	**10.71**	
MO_1129	11	76,507,253	77,734,336	152	**10.44**	*PAK1*
MO_1129	16	53,403,481	53,496,546	18	**8.99**	
MO_1129	11	73,063,928	73,179,552	31	**8.52**	
MO_1129	16	46,702,906	46,725,038	15	**8.27**	
MO_1129	11	74,883,581	75,442,324	74	**8.26**	
MO_1129	16	47,005,363	47,294,464	18	**8.25**	
MO_1129	11	70,118,324	70,858,343	99	**8.05**	
MO_1129	11	75,776,855	75,907,581	8	**7.45**	*WNT11*
MO_1129	11	78,775,826	79,113,172	3	**7.42**	
MO_1129	11	77,820,528	78,523,328	65	**7.34**	*GAB2*
MO_1129	11	77,749,826	77,812,121	6	**7.33**	
MO_1129	11	76,075,526	76,432,738	46	**7.20**	*C11orf30*

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1129	11	75,917,402	76,072,153	4	**5.66**	*WNT11*
MO_1129	11	77,814,044	77,817,892	4	**5.36**	
MO_1129	16	47,345,262	48,643,775	120	**4.92**	
MO_1129	16	49,823,481	50,402,219	76	**4.80**	
MO_1129	16	46,597,978	46,696,978	20	**4.79**	
MO_1129	16	46,726,382	47,001,976	38	**4.58**	
MO_1129	16	52,874,786	53,358,370	42	**4.51**	
MO_1129	16	54,317,524	54,967,290	13	**4.35**	
MO_1129	16	56,672,724	56,839,479	26	**4.22**	*NUP93*
MO_1129	11	73,669,488	74,880,866	172	**3.98**	
MO_1129	11	68,705,664	70,052,410	81	**3.82**	*CCND1, FADD, FGF19, FGF3, FGF4*
MO_1129	16	33,965,608	34,681,986	19	**2.67**	
MO_1129	16	97,456	29,001,065	2902	**2.54**	*ABCC1, AXIN1, CIITA, CREBBP, ERCC4, GRIN2A, MKL2, MLST8, MYH11, PALB2, PDPK1, SOCS1, TNFRSF17, TSC2*
MO_1129	1	145,209,248	147,415,496	171	**2.02**	*BCL9, CHD1L*
MO_1129	1	150,039,962	249,211,679	8247	**2.00**	*ABL2, AKT3, APH1A, ARNT, CDC73, CKS1B, DDR2, ELF3, ELK4, ETV3, ETV3L, FASLG, FH, H3F3A, HAX1, IKDKE, INSRR, IQGAP3, IRF6, MCL1, MDM4, NCSTN, NTRK1, PBX1, PBX1, PRRX1, PSEN2, RAB25, SDHC, SHC1, WNT3A, WNT9A*
MO_1129	14	22,749,583	22,961,931	35	**1.60**	
MO_1129	11	61,091,464	68,704,130	1737	**1.53**	*AIP, ESRRA, FOSL1, LRP5, MEN1, RBM14, SDHAF2, VEGFB*
MO_1129	1	144,864,331	145,115,804	44	**1.51**	
MO_1129	11	30,921,108	46,918,422	805	**1.49**	*CREB3L1, EHF, ELF5, EXT2, LMO2, WT1*
MO_1129	5	140,648	180,687,459	8736	**1.48**	*ACSL6, APC, ARHGAP26, CSF1R, CTNNA1, FER, FGF1, FGF10, FGF18, FGFR4, FLT4, GDNF, HBEGF, IL3, IL7R, IQGAP2, ITK, KDM3B, MAML1, MAP3K1, NHP2, NKX2-5, NPM1, NRG2, NSD1, ODZ2, PDGFRB, PIK3R1, RAD50, RICTOR, SKP2, SMAD5, TCF7, TERT, TLX3, UBE2D2, WNT8A*
MO_1129	16	29,141,009	33,953,903	503	**1.47**	*FUS, PRSS8, ZNF668*
MO_1129	X	2,700,167	94,318,128	3523	**1.47**	*AR, ARAF, ATRX, BCOR, BMX, CCNB3, DDX3X, ELK1, FAM123B, FANCB, FGF16, FIGF, FOXO4, FOXP3, GATA1, KDM5C, KDM6A, MAGED1, MED12, PIM2, SSX1, SSX2, SSX3, SSX4, TBX22, TFE3, USP9X, WAS, ZRSR2*
MO_1129	16	70,863,704	71,127,808	66	**1.44**	
MO_1129	11	48,373,888	50,003,636	30	**1.40**	

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1129	11	75,480,074	75,727,920	20	**0.44**	
MO_1129	11	81,601,827	134,257,653	3268	**0.44**	*ATM, BIRC2, BIRC3, CBL, CHEK1, DDX6, ETS1, FLI1, GUCY1A2, HMBS, MAML2, MLL, MRE11A, PDGFD, PICALM, POU2AF1, SDHD, UBE4A, YAP1, ZBTB16*
MO_1129	X	95,940,052	154,774,783	2605	**0.45**	*BTK, CUL4B, DKC1, ELF4, FGF13, GPC3, IRS4, MAMLD1, MTCP1, PAK3, PHF6, RBMX, SH2D1A, STAG2*
MO_1129	22	17,058,814	51,219,026	3934	**0.45**	*BCR, CHEK2, CRKL, CSNK1E, CYP2D6, EP300, EWSR1, MKL1, MN1, NF2, PATZ1, PDGFB, PIM3, RAC2, SMARCB1, SOX10, WNT7B, XBP1*
MO_1129	11	50,003,984	61,090,461	587	**0.45**	
MO_1129	11	71,139,834	73,057,972	257	**0.46**	
MO_1129	11	73,357,684	73,662,078	40	**0.46**	

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1167	19	281,501	375,803	27	**2.42**	
MO_1167	7	95,906,650	95,926,311	2	**2.38**	
MO_1167	12	34,179,755	40,265,674	91	**1.98**	
MO_1167	2	61,436,070	61,449,714	6	**1.83**	
MO_1167	16	47,622,910	48,643,775	99	**1.82**	
MO_1167	14	50,081,150	51,132,300	195	**1.79**	
MO_1167	17	26,206,406	81,083,588	7129	**1.75**	*AATK, AXIN2, BIRC5, BRCA1, BRIP1, CD79B, CDC6, CDK12, ERBB2, ETV4, G6PC3, GRB2, GRB7, HLF, HOXD13, NF1, PHB, PPM1D, PRKAR1A, RAD51C, RAD51D, RARA, RHOT1, RNF213, RNF43, RPS6KB1, RPTOR, SPOP, SRSF2, STARD3, STAT3, STAT5A, STAT5B, SUZ12, TAF15, TMC6, TMC8, TOP2A, WNT3, WNT9B*
MO_1167	8	67,356,790	69,699,728	230	**1.74**	*MYBL1, PREX2*
MO_1167	20	17,585,297	17,716,465	26	**1.65**	
MO_1167	20	22,563,126	62,904,843	3391	**1.62**	*ARFRP1, ASXL1, AURKA, BCL2L1, CEBPB, GNAS, HCK, MAFB, MYBL2, NCOA3, NFATC2, PLCG1, PTK6, PTPRT, SRC, SRMS, STK4, TOP1, YWHAB, ZMYND8, ZNF217*
MO_1167	2	95,537,503	103,380,773	809	**1.44**	*AFF3, TMEM127, ZAP70*
MO_1167	2	239,974,737	242,946,616	471	**1.39**	
MO_1167	12	25,031,453	34,179,497	529	**1.36**	*KRAS*
MO_1167	9	38,573,205	38,596,389	2	**-0.11**	

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1185	1	196,748,491	196,799,835	7	**3.41**	
MO_1185	7	95,951,322	100,320,578	611	**2.68**	*ARPC1A, LMTK2, SHFM1, SMURF1, TRRAP*
MO_1185	7	63,506,056	95,864,204	1523	**2.66**	*ABCB1, AKAP9, CDK6, GRM3, HGF, MAGI2, SAMD9, SBDS, TYW1*
MO_1185	1	248,802,276	249,211,679	20	**2.65**	
MO_1185	7	193,482	37,072,998	2058	**2.57**	*CARD11, ETV1, FKBP9, HOXA10, HOXA11, HOXA13, HOXA3, HOXA9, JAZF1, PDGFA, PMS2, RAC1*
MO_1185	7	100,344,251	158,937,252	3847	**2.57**	*BRAF, CREB3L2, EPHA1, EPHB4, EPHB6, EZH2, GRM8, MET, MLL3, MNX1, PIK3CG, PRSS1, RHEB, SHH, SMO, WNT16, WNT2*
MO_1185	1	145,415,489	151,547,427	614	**2.40**	*APH1A, ARNT, BCL9, CHD1L, MCL1*
MO_1185	1	196,857,390	248,685,378	3899	**2.38**	*AKT3, ELF3, ELK4, FH, H3F3A, IKBKE, IRF6, MDM4, PSEN2, WNT3A, WNT9A*
MO_1185	1	151,584,804	196,743,924	3920	**2.29**	*ABL2, CDC73, CKS1B, DDR2, ETV3, ETV3L, FASLG, HAX1, INSRR, IQGAP3, NCSTN, NTRK1, PBX1, PBX1, PRRX1, RAB25, SDHC, SHC1*
MO_1185	1	1,221,024	1,231,394	10	**2.13**	
MO_1185	16	97,456	34,681,986	3427	**1.84**	*ABCC1, AXIN1, CIITA, CREBBP, ERCC4, FUS, GRIN2A, MKL2, MLST8, MYH11, PALB2, PDPK1, PRSS8, SOCS1, TNFRSF17, TSC2, ZNF668*
MO_1185	18	158,542	15,004,215	819	**1.80**	*YES1*
MO_1185	1	142,540,225	145,414,742	50	**1.77**	
MO_1185	3	138,724,967	138,739,359	5	**1.76**	
MO_1185	10	225,997	135,381,649	7791	**1.65**	*BLNK, BMPR1A, CHUK, CYP17A1, FAS, FGF8, FGFR2, GATA3, GOT1, KAT6B, KLF6, LDB1, MLLT10, NCOA4, NFKB2, NRG3, PRF1, PTEN, RET, SHOC2, SUFU, TCF7L2, TLX1, TNKS2, WNT8B*
MO_1185	X	33,146,306	33,357,442	3	**-0.73**	
MO_1185	12	115,109,830	117,537,171	80	**-0.71**	*TBX3*
MO_1185	21	9,825,990	9,826,257	2	**-0.55**	
MO_1185	16	55,844,562	55,862,856	8	**-0.25**	
MO_1185	17	7,107,475	7,124,954	11	**-0.09**	
MO_1185	20	32,684,636	32,685,368	2	**-0.07**	

Case ID	Chr	Loc Start	Loc End	# Exon Targets	Adj Copy Num Ratio	MiOncoSeq Panel Genes
MO_1185	X	34,148,030	154,774,783	4899	**0.12**	*AR, ARAF, ATRX, BCOR, BTK, CCNB3, CUL4B, DDX3X, DKC1, ELF4, ELK1, FAM123B, FGF13, FGF16, FOXO4, FOXP3, GATA1, GPC3, IRS4, KDM5C, KDM6A, MAGED1, MAMLD1, MED12, MTCP1, PAK3, PHF6, PIM2, RBMX, SH2D1A, SSX1, SSX2, SSX3, SSX4, STAG2, TBX22, TFE3, USP9X, WAS*
MO_1185	11	74,700,123	76,261,098	146	**0.13**	*C11orf30, WNT11*
MO_1185	16	46,508,278	55,807,285	552	**0.13**	*CYLD*
MO_1185	17	7,125,469	22,023,536	1678	**0.13**	*ALOX12B, AURKB, C17orf39, COPS3, DVL2, FGF11, FLCN, GPS2, MAP2K4, NCOR1, TNK1, TP53*
MO_1185	1	2,700,257	25,350,002	2584	**0.13**	*CAMTA1, CDC42, EPHA2, EPHA8, EPHB2, KIF1B, MDS2, MTOR, PAX7, PLA2G2A, PRDM16, SDHB, SPEN, WNT4*
MO_1185	18	18,539,877	78,005,236	2244	**0.13**	*ASXL3, BCL2, CDH2, CDH20, GATA6, KDSR, MALT1, MBD1, PIK3C3, ROCK1, SMAD2, SMAD4, SMAD7, SS18*
MO_1185	16	55,866,960	70,867,000	1447	**0.13**	*C16orf57, CBFB, CDH1, CDH5, CTCF, NQO1, NUP93*
MO_1185	X	2,700,167	33,038,217	1236	**0.14**	*BMX, FANCB, FIGF, ZRSR2*
MO_1185	16	71,209,628	90,142,217	1419	**0.14**	*FANCA, MAF, MC1R, PHLPP2, PLCG2, ZFHX3*
MO_1185	11	59,540,716	64,883,160	1131	**0.16**	*ESRRA, MEN1, SDHAF2, VEGFB*
MO_1185	17	6,108	7,106,560	1144	**0.16**	*CRK, RABEP1, RPA1, USP6*
MO_1185	11	94,603,981	118,247,352	1413	**0.39**	*ATM, BIRC2, BIRC3, GUCY1A2, MAML2, PDGFD, POU2AF1, SDHD, UBE4A, YAP1, ZBTB16*
MO_1185	7	44,121,933	44,146,309	4	**0.40**	
MO_1185	3	77,526,710	77,595,553	7	**0.40**	*ROBO2*

Table 5

Sample ID	5' Gene	Chr	hg19 position	3' Gene	Chr	hg19 position	# Supporting Reads	Fusion Protein
MO_1031	*CDC123*	10	12272994	*LOC550112*	4	68582640	172	NO
MO_1031	*SFRP1*	8	41161056	*ST8SIA6-AS1*	10	17441200	435	NO
MO_1031	*PLA2G12A*	4	110650757	*COL15A1*	9	101822170	76	YES
MO_1031	*USP6NL*	10	11551593	*UNC5D*	8	35232883	182	NO
MO_1031	*PPIF*	10	81111338	*AL359195.1*	10	82012039	667	NO
MO_1031	*RAB10*	2	26257603	*SPTBN1*	2	54849604	24	NO
MO_1031	*NT5C3L*	17	39991321	*SPATS2L*	2	201324491	21	NO
MO_1031	*RAB11FIP1*	8	37727937	*CCDC3*	10	13006451	45	NO
MO_1031	*LRRC56*	11	540132	*NELL1*	11	21197964	7	NO
MO_1031	*CADM2*	3	85851345	*CCDC3*	10	13021206	8	NO
MO_1031	*IPO9*	1	201817721	*PM20D1*	1	205814684	13	YES
MO_1031	*NLK*	17	26459834	*AC015849.2.1*	17	34211385	40	NO
MO_1031	*STAM*	10	17686377	*CADM2*	3	85288106	72	NO
MO_1031	*ARSJ (AS)*	4	114880675	*TBC1D9*	4	141622767	130	NO
MO_1031	*EVI5*	1	93029198	*PRKACB*	1	84596236	15	NO
MO_1031	*STAM*	10	17686377	*PROSC*	8	37623043	33	NO
MO_1031	*ADIPOR2*	12	1800377	*HEBP1*	12	13142347	258	NO
MO_1031	*LRP5*	11	68080272	*FAT3*	11	92430549	11	YES
MO_1051	*CMAS*	12	22199494	*PIK3C2G*	12	18641380	73	YES
MO_1051	*TBCK*	4	107163626	*PPA2*	4	106367658	81	YES
MO_1051	*ITFG1*	16	47399692	*NETO2*	16	47117706	53	NO
MO_1051	*GPATCH8*	17	42512432	*MPP2*	17	41961522	24	YES
MO_1051	*FGFR2*	10	123243211	*AFF3*	2	100453985	138	YES
MO_1069	*ANKRD11*	16	89484691	*VPS9D1*	16	89783229	46	NO
MO_1069	*ANKRD11*	16	89484691	*ZNF276*	16	89793757	153	NO
MO_1069	*MLPH*	2	238451302	*COL6A3 (AS)*	2	238259785	1476	NO
MO_1069	*UBN2*	7	138936802	*TTC26*	7	138854034	18	YES
MO_1069	*HEXDC*	17	80394613	*OGFOD3 (AS)*	17	80371025	7	NO
MO_1069	*TBCD*	17	80772809	*FOXK2*	17	80544938	24	YES
MO_1069	*CALCOCO2*	17	46928989	*CEP112 (AS)*	17	63755705	43	NO
MO_1069	*CTNNA1*	5	138119060	*KDM3B*	5	137733866	28	NO
MO_1069	*ITCH*	20	32957275	*ASIP*	20	32848170	6	NO
MO_1129	*DDB1*	11	61091450	*PAK1*	11	77066886	208	YES
MO_1129	*VPS35*	16	46702841	*SLCO2B1*	11	74911268	85	YES
MO_1129	*RBL2*	16	53496566	*ANKRD26P1 (AS)*	16	46602603	99	NO
MO_1167	*PFKFB3*	10	6268327	*LOC399715*	10	6368508	10	NO
MO_1167	*STK38L*	12	27450642	*PPFIBP1*	12	27677297	4	NO
MO_1167	*JMJD1C*	10	65140241	*REEP3*	10	65261497	11	NO
MO_1185	*SSH2*	17	28120954	*EFCAB5*	17	28257176	3	NO

Table 6

AF-4 = AF4 domain
C2A = C2 domain
CPSF-A = CPSF A subunit domain
ENSTL = Endostatin-like domain
FH = Forkhead DNA binding domain
FHA = Forkhead associated domain
GMPK = Guanylate kinase domain
GP = G-patch domain
HAD = haloacid dehydrogenase
HRD = Hpc2-related domain
IBN-N = Importin-beta N-terminal domain
Ig = Immunoglobulin domain
Kazal = Kazal type serine protease inhibitor domain
L27 = Lin2 / Lin7 domain
LamG = Laminin G domain
LDL = Low Density Lipoprotein Receptor Class A domain
LY = Low-density lipoprotein-receptor YWTD domain
M20 Dipept = M20 Dipeptidase domain
MFS = Major Facilitator Superfamily domain
MMS1-N = methyl methanesulfonate N-terminal
NeuA = NeuAc synthetase
PBD = p21 binding domain
PDZ = PDZ domain
PIK3a = PIK3 accessory domain
PIK3c = PIK3 catalytic domain
PLA2 = phospholipase A2 domain
PPase = Pyrophosphatase domain
PTKc = Protein Tyrosine kinase catalytic domain
PX = phosphoinositide binding domain
RabGAP = Rab-GTPase activating domain
RHOD = Rhodanese Homology Domain
SH3 = Src homology 3 domain
SP = Signal peptide
STKc = Serine/Threonine kinase catalytic domain
TFCD = Tubulin folding cofactor D C-terminal domain
TM = Transmembrane domain
TPR = Tetratricopeptide repeat domain
TSPN = Thrombospondin N-terminal-like domain
UBN-AB = Ubinuclein conserved middle domain
Zf = Zinc finger domain
14-3-3 = 14-3-3 phosphoserine/threonine-binding domain

Table 7.

Variants of ESR1.

Sample Cancer	hg19 Coord	Reference	Somatic Variant	Reference Transcript	Coding Sequence Change	Reference Protein	Amino Acid Change
Carcinoma, Invasive Ductal	152419923	A	C	NM_000125.3	c.1844A>C	NP_000116.2	p.Y537S
Adenocarcinoma	152419926	A	G	NM_000125.3	c.1847A>G	NP_000116.2	p.D538G
Adenocarcinoma	152419920	T	A	NM_000125.3	c.1841T>A	NP_000116.2	p.L536H
Adenocarcinoma	152419923	A	C	NM_000125.3	c.1844A>C	NP_000116.2	p.Y537S
Carcinoma	152419926	A	G	NM_000125.3	c.1847A>G	NP_000116.2	p.D538G
Carcinoma	152419923	A	C	NM_000125.3	c.1844A>C	NP_000116.2	p.Y537S
Carcinoma, Invasive Ductal	152419926	A	G	NM_000125.3	c.1847A>G	NP_000116.2	p.D538G

All publications, patents, patent applications and accession numbers mentioned in the above specification are herein incorporated by reference in their entirety. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications and variations of the described compositions and methods of the invention will be apparent to those of ordinary skill in the art and are intended to be within the scope of the following claims.

CLAIMS

We claim:

1. A method of treating cancer, comprising:

a) assaying a sample from a subject diagnosed with cancer for the presence of a mutation in the estrogen receptor (ESR1) gene, wherein said mutation is selected from the group consisting of p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, and p.Tyr537Asn; and

b) determining a treatment course of action based on the presence of said mutation.

2. The method of claim 1, further comprising the step of c) administering said treatment when said mutation is present.

3. The method of claim 1 or 2, wherein said treatement is an estrogen receptor antagonist.

4. The method of any one of claims 1 to 3, wherein said estrogen receptor antagonist is tamoxifen or fulvestrant.

5. The method of any one of claims 1 to 4, wherein the sample is selected from the group consisting of tissue, blood, plasma, serum, endometrial cells, and breast cells.

6. The method of any one of claims 1 to 5, wherein said cancer is breast cancer or endometrial cancer.

7. The method of any one of claims 1 to 5, wherein said detecting comprises forming a complex between said ESR1 gene and a nucleic acid primer, probe, or pair of primers that specifically bind to said ESR1 gene.

8. The method of claim 7, wherein said nucleic acid primer, probe, or pair of primers bind to said mutation in said ESR1 gene but not the wild type gene.

9. The method of any one of claims 1 to 8, wherein said ESR1 gene is assayed from circulating tumor nucleic acid.

10. A method of montitoring treatment of cancer, comprising:

a) administering a cancer therapy to a subject;

b) assaying a sample from a subject diagnosed with cancer for the presence of a mutation in the estrogen receptor (ESR1) gene, wherein said mutation is selected from the group consisting of p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, and p.Tyr537Asn; and

c) determining a treatment course of action based on the presence of said mutation.

11. The method of claim 10, further comprising the step of c) administering said treatment when said mutations are present.

12. The method of claim 10 or 11, wherein said treatement is an estrogen receptor antagonist.

13. The method of any one of claims 10 to 12, wherein said estrogen receptor antagonist is tamoxifen or fulvestrant.

14. The method of any one of claims 10 to 13, wherein the sample is selected from the group consisting of tissue, blood, plasma, serum, endometrial cells, and breast cells.

15. The method of any one of claims 10 to 14, wherein said cancer is breast cancer or endometrial cancer.

16. The method of any one of claims 10 to 15, wherein said cancer therapy is an aromatase inhibitor.

17. The method of any one of claims 10 to 16, wherein said detecting comprises forming a complex between said ESR1 gene and a nucleic acid primer, probe, or pair of primers that specifically bind to said ESR1 gene.

18. The method of claim 17, wherein said nucleic acid primer, probe, or pair of primers bind to said mutation in said ESR1 gene but not the wild type gene.

19. The method of any one of claims 10 to 18, wherein said ESR1 gene is assayed from circulating tumor nucleic acid.

20. A complex comprsing a nucleic acid encoding a variant estrogen receptor (ESR1) gene, comprsing a mutation selected from the group consisting of p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, and p.Tyr537Asn and a nucleic acid primer or probe that specifically hybridizes to said variant ESR1 gene but not a wild type ESR1 gene.

21. A complex comprsing a estrogen receptor (ESR1) polypeptide comprsing a mutation selected from the group consisting of p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, and an antibody that specifically binds to said ESR1 polypeptide comprising a mutation but not to wild type ESR1.

22. A kit, comprising: reagents for detecting the presence of one or more variant ESR1 polypeptides or nucleic acids encoding said polypeptides.

23. The kit of claim 22, wherein said reagents are selected from the group consisting of a nucleic acid primer or probe that specifically hybridizes to said variant ESR1 gene but not a wild type ESR1 gene and an antibody that specifically binds to said ESR1 polypeptide comprising a mutation but not to wild type ESR1.

24. The kit of claim 23, wherein said nucliec acid primer or probe is at least 8 nucleic acids in length.

25. The kit of claim 23, wherein said nucliec acid primer or probe is at least 10 nucleic acids in length.

26. The kit of claim 23, wherein said nucliec acid primer or probe is at least 20 nucleic acids in length.

27. A system, comprising:

a) a computer processor; and

b) computer software configured to analyze information on the presence of variant ESR1 polypeptides or amino acids encoding the polypeptides; and determine a treatment course of action based on the presence of the variant gene or polypeptide.

28. The system of claim 27, wherein said treatement is an estrogen receptor antagonist.

29. The system of claim 28, wherein said estrogen receptor antagonist is tamoxifen or fulvestrant.

Figure 1

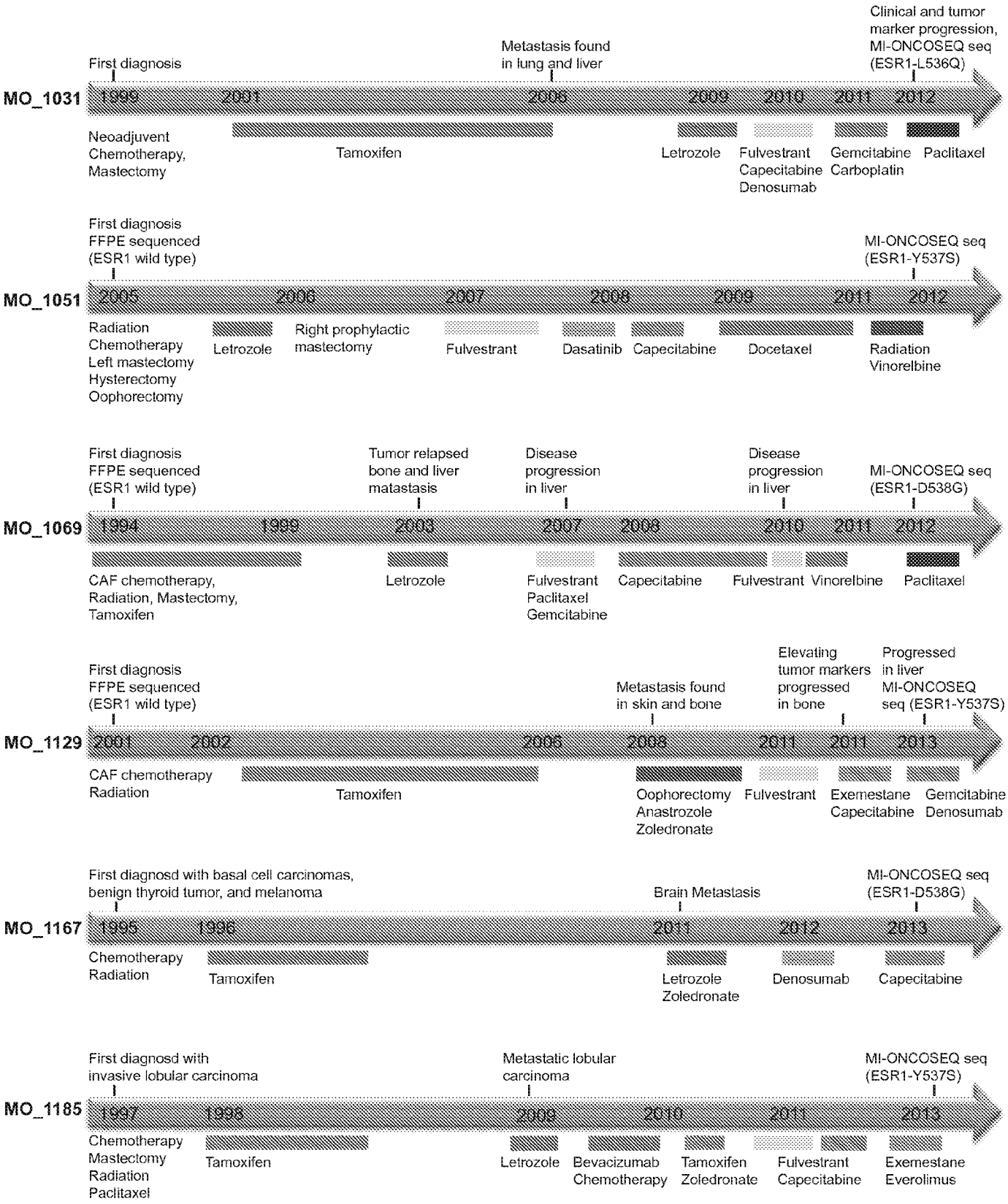

MO_1031
First diagnosis
1999
2001
Metastasis found in lung and liver
2006
2009
2010
2011
Clinical and tumor marker progression, MI-ONCOSEQ seq (ESR1-L536Q)
2012
Neoadjuvent Chemotherapy, Mastectomy
Tamoxifen
Letrozole
Fulvestrant Capecitabine Denosumab
Gemcitabine Carboplatin
Paclitaxel
MO_1051
First diagnosis FFPE sequenced (ESR1 wild type)
2005
2006
2007
2008
2009
2011
MI-ONCOSEQ seq (ESR1-Y537S)
2012
Radiation Chemotherapy Left mastectomy Hysterectomy Oophorectomy
Letrozole
Right prophylactic mastectomy
Fulvestrant
Dasatinib
Capecitabine
Docetaxel
Radiation Vinorelbine
MO_1069
First diagnosis FFPE sequenced (ESR1 wild type)
1994
1999
Tumor relapsed bone and liver matastasis
2003
Disease progression in liver
2007
2008
Disease progression in liver
2010
2011
MI-ONCOSEQ seq (ESR1-D538G)
2012
CAF chemotherapy, Radiation, Mastectomy, Tamoxifen
Letrozole
Fulvestrant Paclitaxel Gemcitabine
Capecitabine
Fulvestrant
Vinorelbine
Paclitaxel
MO_1129
First diagnosis FFPE sequenced (ESR1 wild type)
2001
2002
2006
Metastasis found in skin and bone
2008
Elevating tumor markers progressed in bone
2011
2011
Progressed in liver MI-ONCOSEQ seq (ESR1-Y537S)
2013
CAF chemotherapy Radiation
Tamoxifen
Oophorectomy Anastrozole Zoledronate
Fulvestrant
Exemestane Capecitabine
Gemcitabine Denosumab
MO_1167
First diagnosd with basal cell carcinomas, benign thyroid tumor, and melanoma
1995
1996
Brain Metastasis
2011
2012
MI-ONCOSEQ seq (ESR1-D538G)
2013
Chemotherapy Radiation
Tamoxifen
Letrozole Zoledronate
Denosumab
Capecitabine
MO_1185
First diagnosd with invasive lobular carcinoma
1997
1998
Metastatic lobular carcinoma
2009
2010
2011
MI-ONCOSEQ seq (ESR1-Y537S)
2013
Chemotherapy Mastectomy Radiation Paclitaxel
Tamoxifen
Letrozole
Bevacizumab Chemotherapy
Tamoxifen Zoledronate
Fulvestrant Capecitabine
Exemestane Everolimus

Figure 2

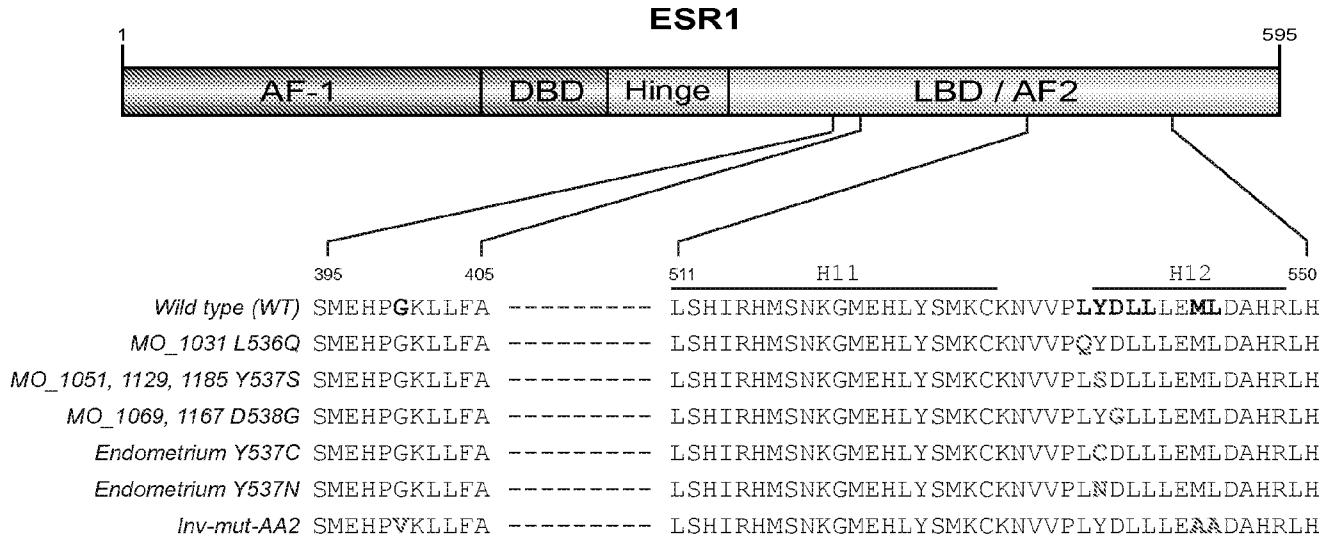

ESR1
1
595
AF-1
DBD
Hinge
LBD / AF2
395
405
511
H11
H12
550
Wild type (WT) SMEHPGKLLFA ---------- LSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEMLDAHRLH
MO_1031 L536Q SMEHPGKLLFA ---------- LSHIRHMSNKGMEHLYSMKCKNVVPQYDLLLEMLDAHRLH
MO_1051, 1129, 1185 Y537S SMEHPGKLLFA ---------- LSHIRHMSNKGMEHLYSMKCKNVVPLSDLLLEMLDAHRLH
MO_1069, 1167 D538G SMEHPGKLLFA ---------- LSHIRHMSNKGMEHLYSMKCKNVVPLYGLLLEMLDAHRLH
Endometrium Y537C SMEHPGKLLFA ---------- LSHIRHMSNKGMEHLYSMKCKNVVPLCDLLLEMLDAHRLH
Endometrium Y537N SMEHPGKLLFA ---------- LSHIRHMSNKGMEHLYSMKCKNVVPLNDLLLEMLDAHRLH
Inv-mut-AA2 SMEHPVKLLFA ---------- LSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEAADAHRLH

Figure 3

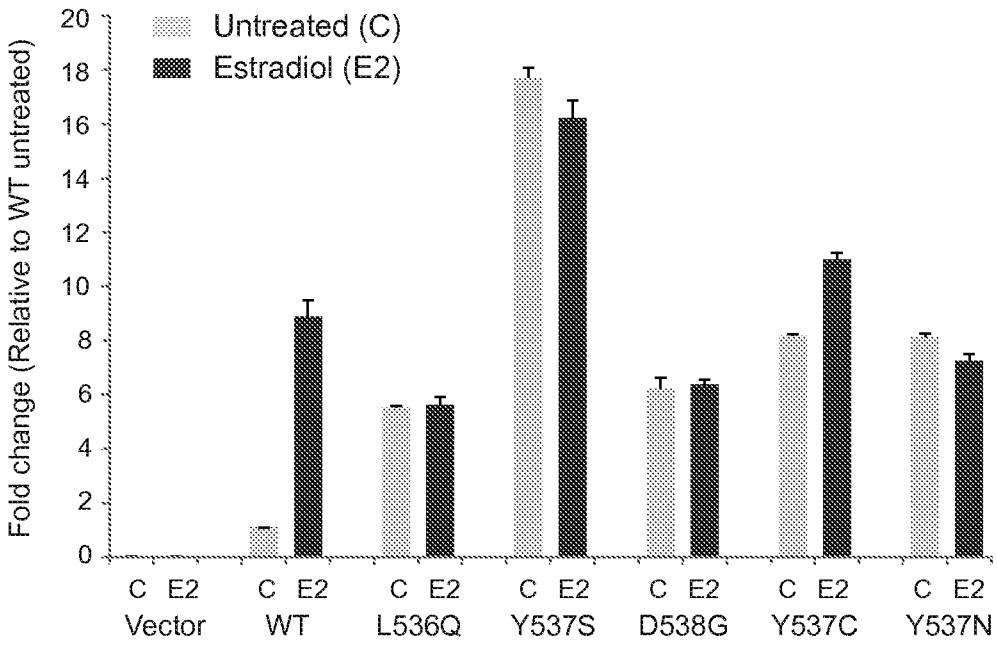

Untreated (C)
Estradiol (E2)
Fold change (Relative to WT untreated)
20
18
16
14
12
10
8
6
4
2
0
C E2
Vector
C E2
WT
C E2
L536Q
C E2
Y537S
C E2
D538G
C E2
Y537C
C E2
Y537N

Figure 4

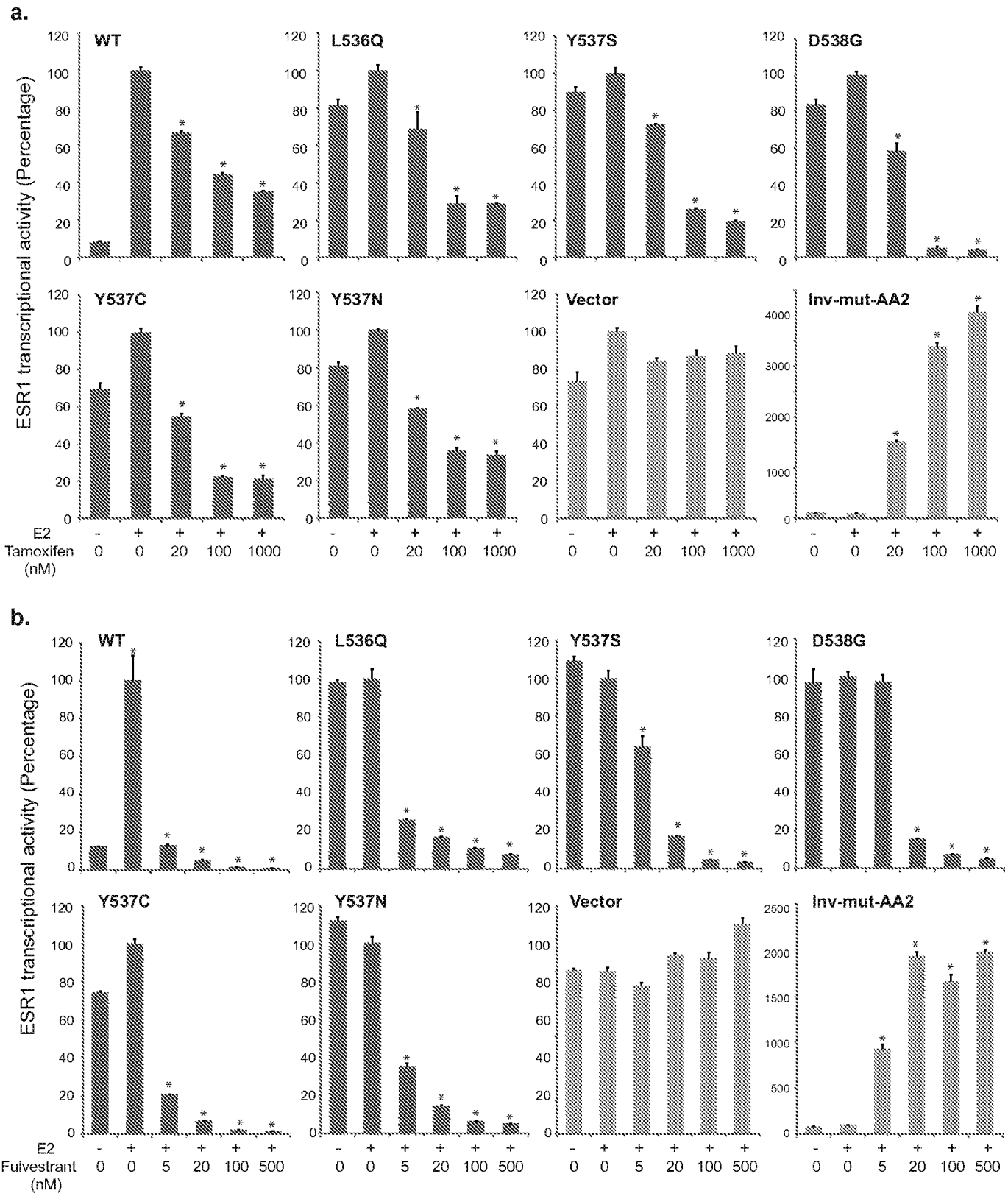

a.
WT
L536Q
Y537S
D538G
Y537C
Y537N
Vector
Inv-mut-AA2
ESR1 transcriptional activity (Percentage)
E2
Tamoxifen (nM)
b.
Fulvestrant (nM)

Figure 5

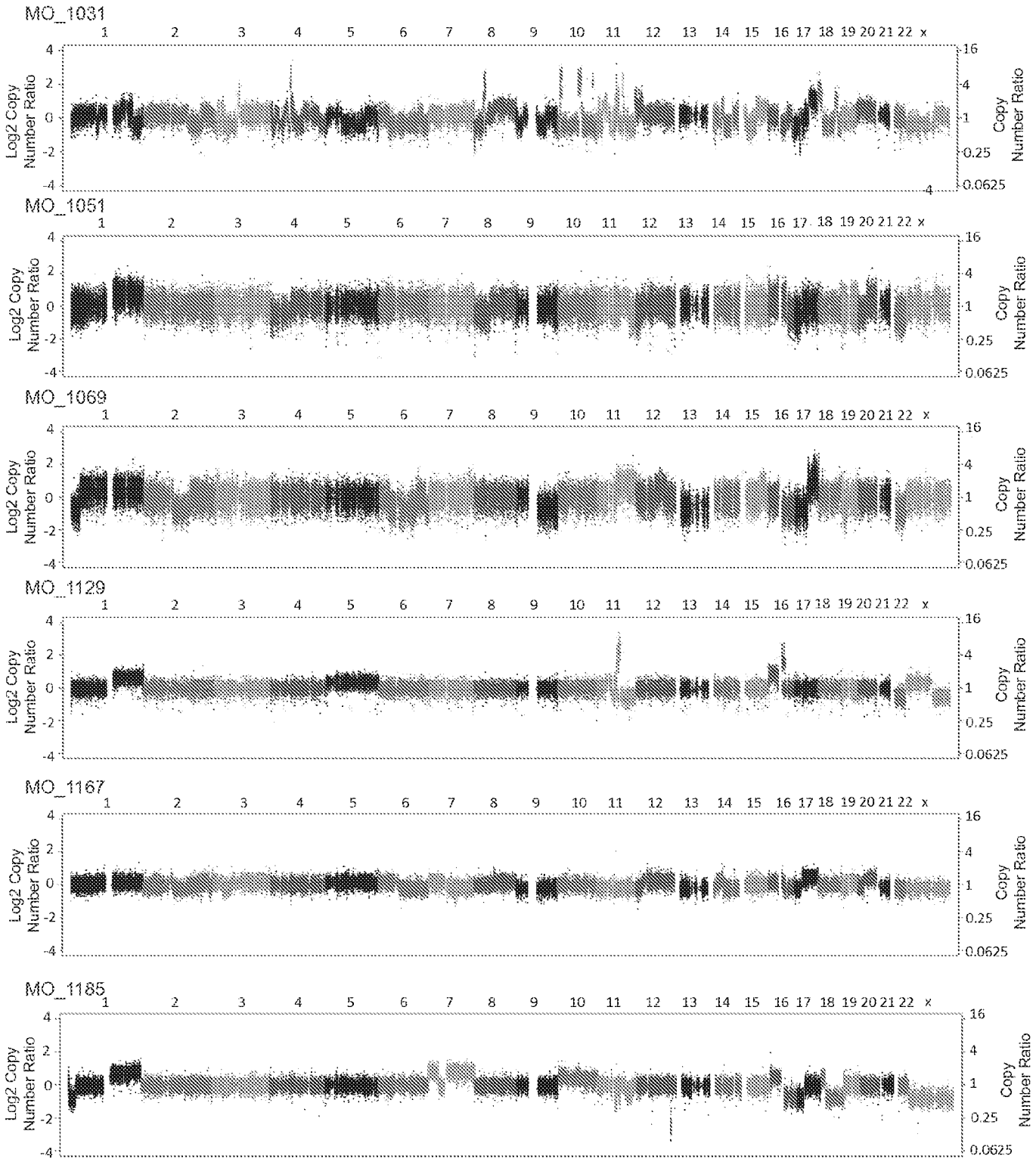

MO_1031
MO_1051
MO_1069
MO_1129
MO_1167
MO_1185
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 x
Log2 Copy Number Ratio
Copy Number Ratio
4
2
0
-2
-4
16
4
1
0.25
0.0625

Figure 6

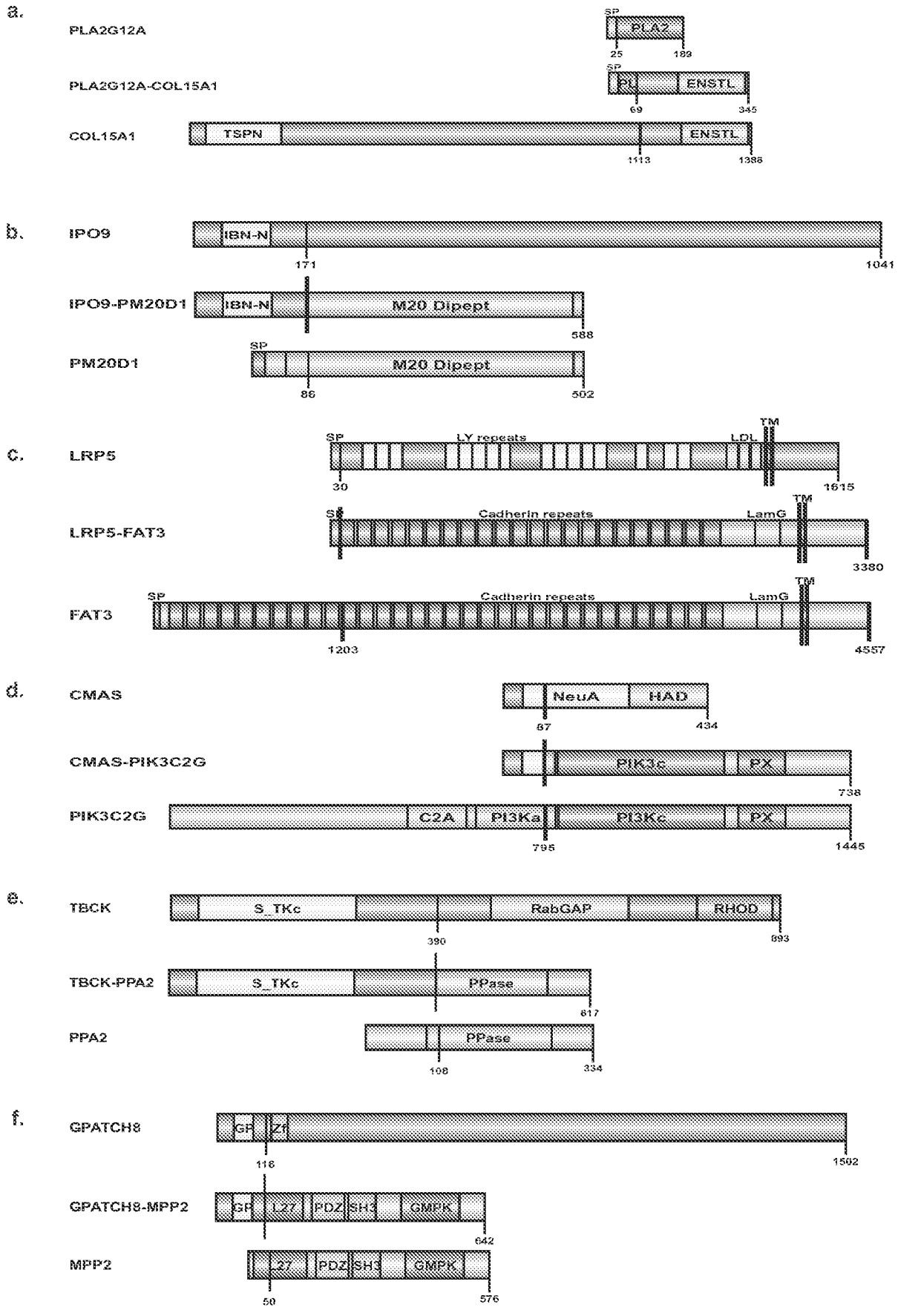

a.
PLA2G12A
SP
PLA2
25
189
PLA2G12A-COL15A1
SP
PLA2
ENSTL
69
345
COL15A1
TSPN
ENSTL
1113
1388
b.
IPO9
IBN-N
171
1041
IPO9-PM20D1
IBN-N
M20 Dipept
588
PM20D1
SP
M20 Dipept
86
502
c.
LRP5
SP
LY repeats
LDL
TM
30
1615
LRP5-FAT3
SP
Cadherin repeats
LamG
TM
3380
FAT3
SP
Cadherin repeats
LamG
TM
1203
4557
d.
CMAS
NeuA
HAD
87
434
CMAS-PIK3C2G
PIK3c
PX
738
PIK3C2G
C2A
PI3Ka
PI3Kc
PX
795
1445
e.
TBCK
S_TKc
RabGAP
RHOD
390
893
TBCK-PPA2
S_TKc
PPase
617
PPA2
PPase
108
334
f.
GPATCH8
GP
Zf
116
1592
GPATCH8-MPP2
GP
L27
PDZ
SH3
GMPK
642
MPP2
L27
PDZ
SH3
GMPK
50
576

Figure 6 (Cont.)

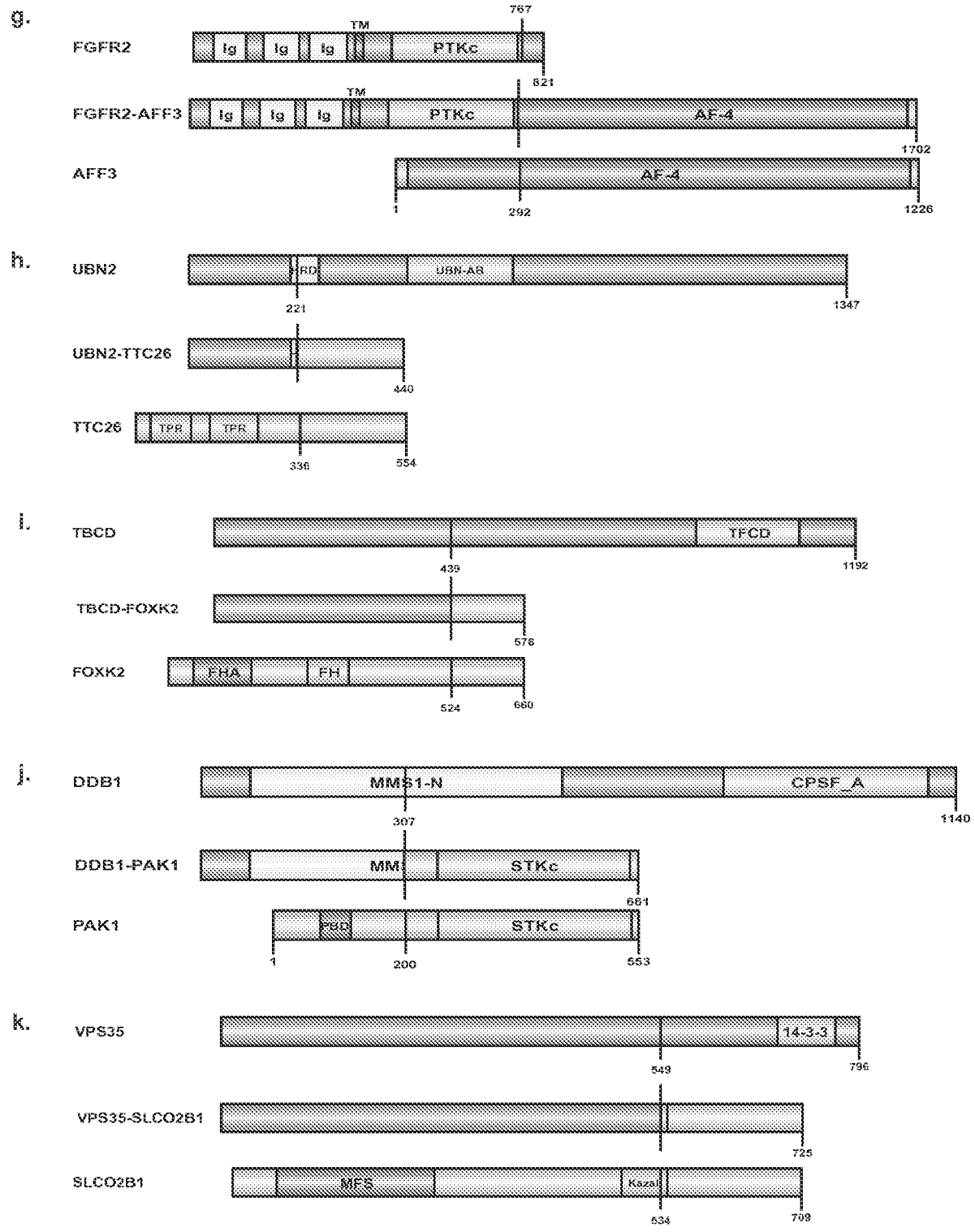


Figure 7

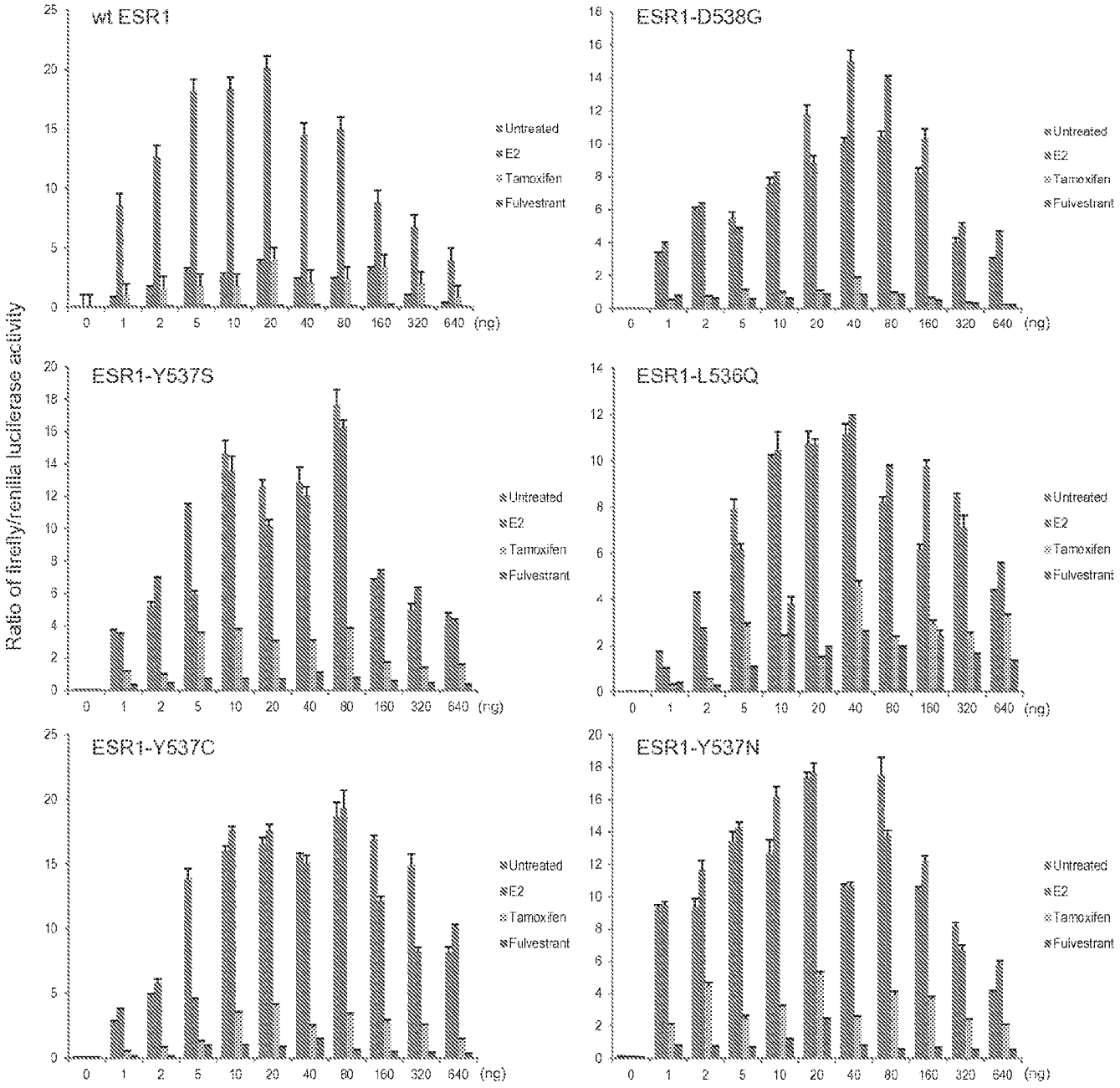

wt ESR1
ESR1-D538G
ESR1-Y537S
ESR1-L536Q
ESR1-Y537C
ESR1-Y537N
Ratio of firefly/renilla luciferase activity
Untreated
E2
Tamoxifen
Fulvestrant
0 1 2 5 10 20 40 80 160 320 640 (ng)

Figure 8

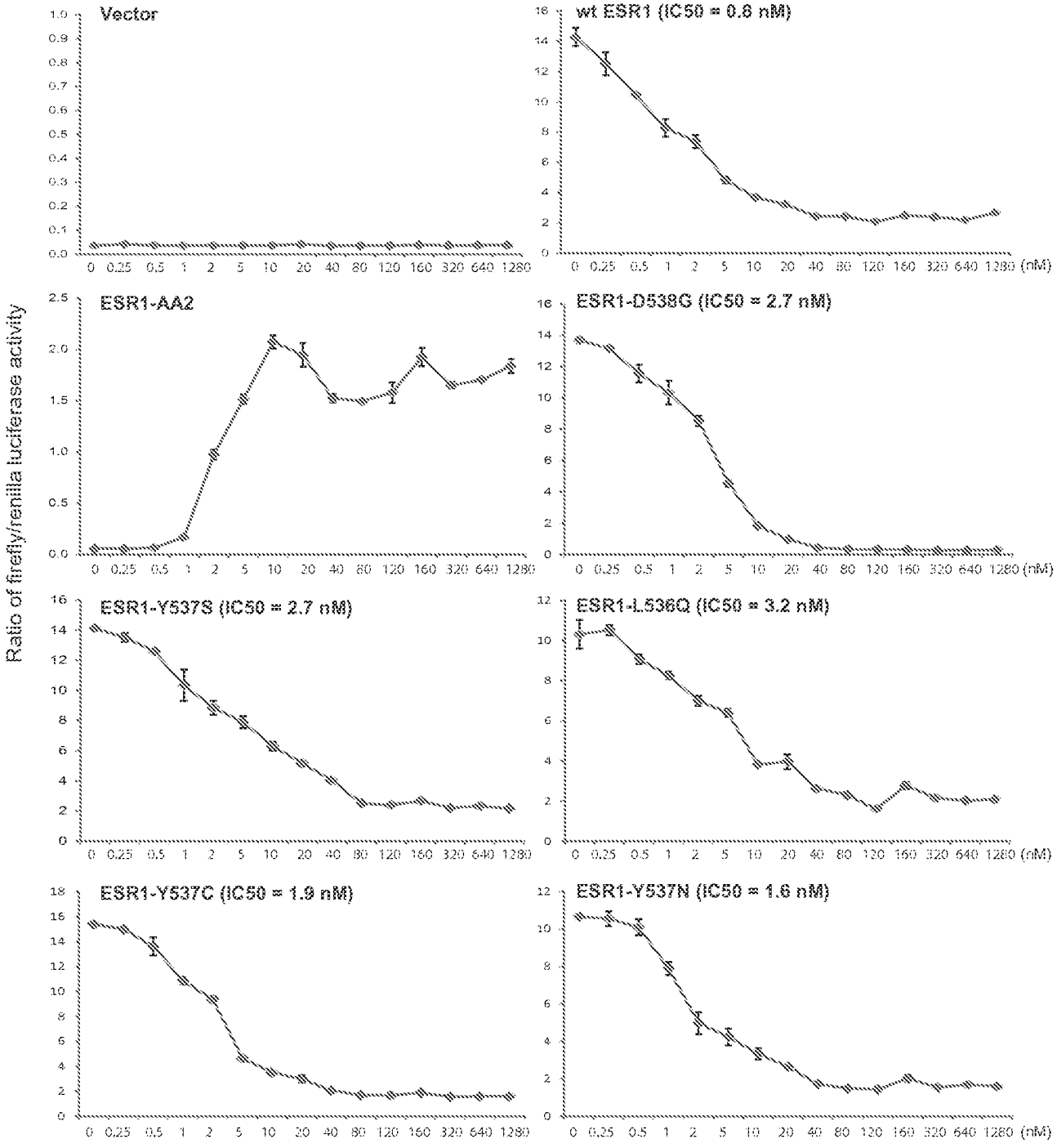

Vector
wt ESR1 (IC50 = 0.8 nM)
ESR1-AA2
ESR1-D538G (IC50 = 2.7 nM)
ESR1-Y537S (IC50 = 2.7 nM)
ESR1-L536Q (IC50 = 3.2 nM)
ESR1-Y537C (IC50 = 1.9 nM)
ESR1-Y537N (IC50 = 1.6 nM)
Ratio of firefly/renilla luciferase activity
0 0.25 0.5 1 2 5 10 20 40 80 120 160 320 640 1280 (nM)

Figure 9

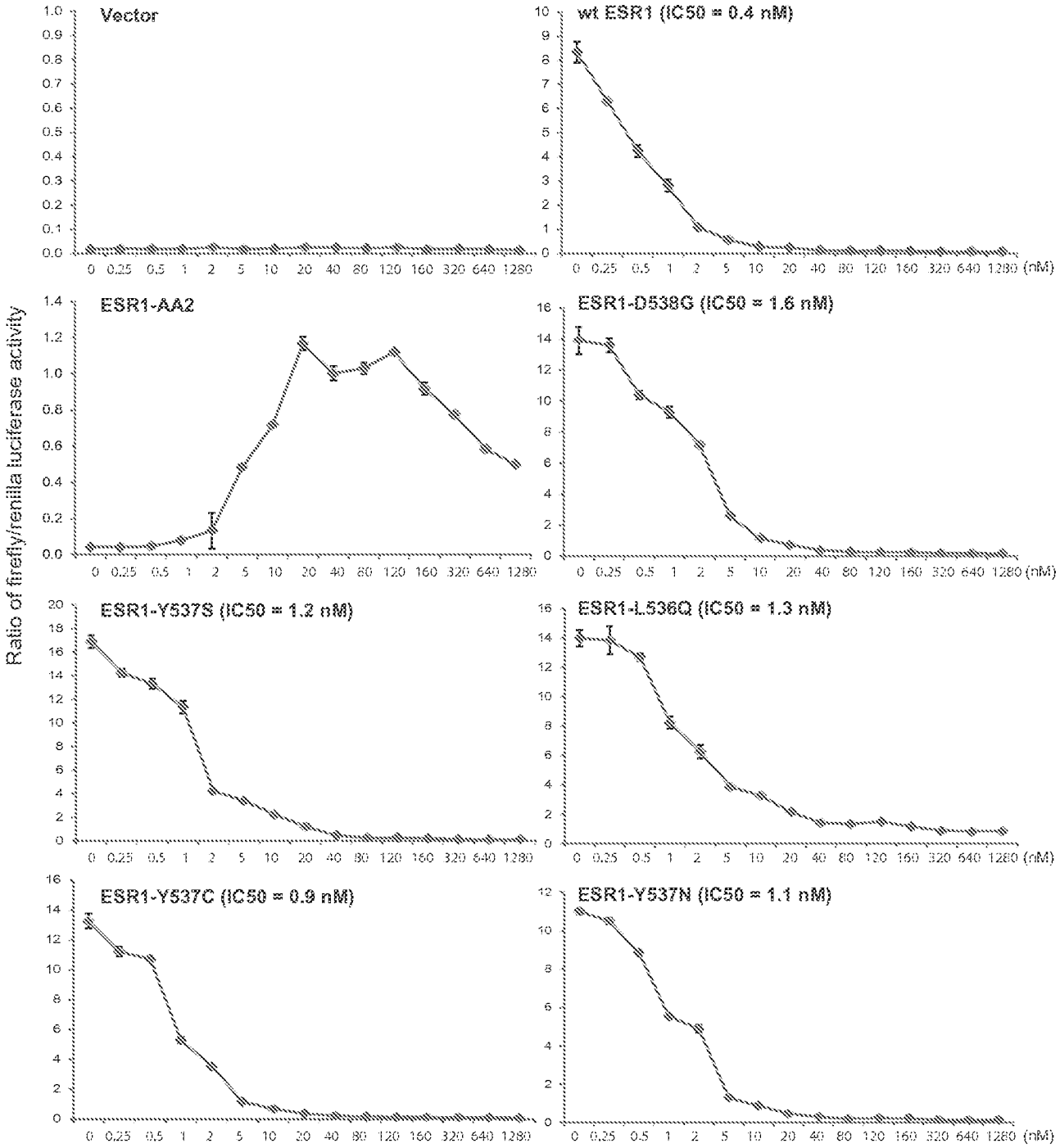

Vector
wt ESR1 (IC50 = 0.4 nM)
ESR1-AA2
ESR1-D538G (IC50 = 1.6 nM)
ESR1-Y537S (IC50 = 1.2 nM)
ESR1-L536Q (IC50 = 1.3 nM)
ESR1-Y537C (IC50 = 0.9 nM)
ESR1-Y537N (IC50 = 1.1 nM)
Ratio of firefly/renilla luciferase activity
0 0.25 0.5 1 2 5 10 20 40 80 120 160 320 640 1280 (nM)

Figure 10

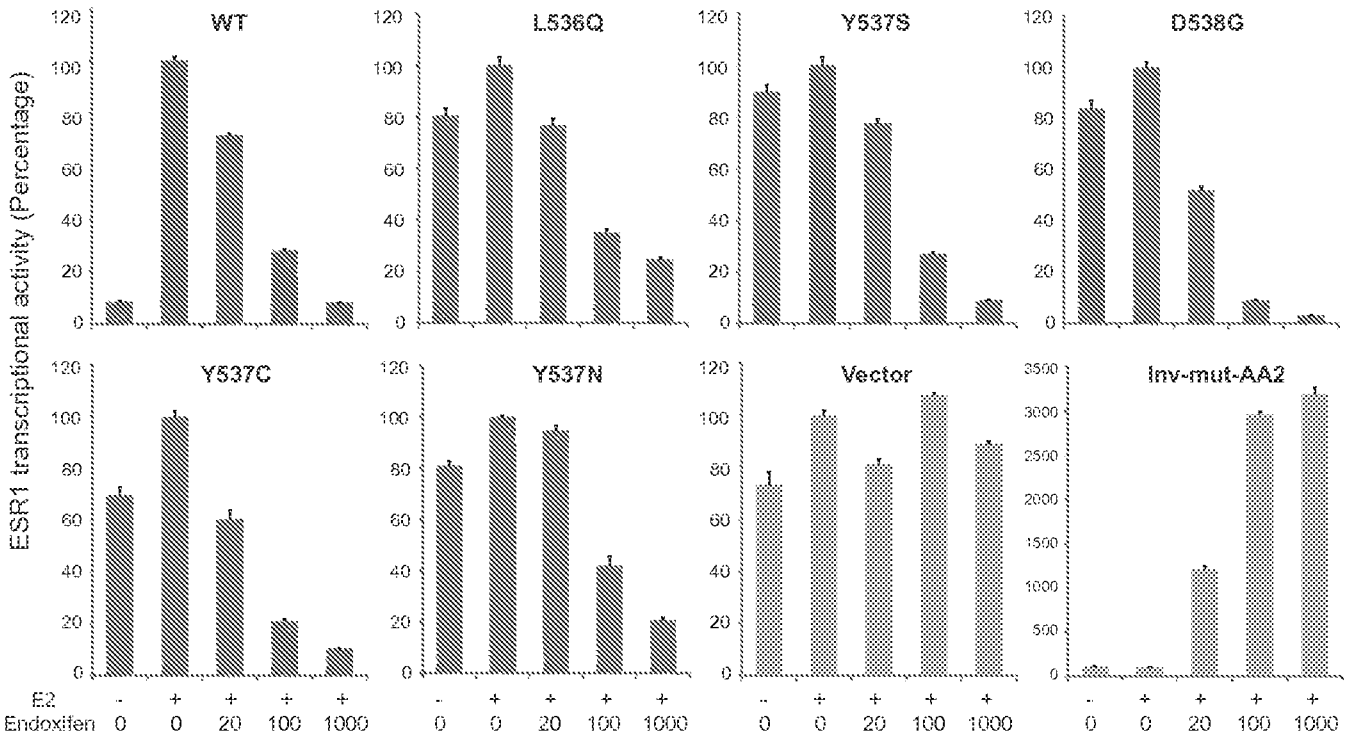
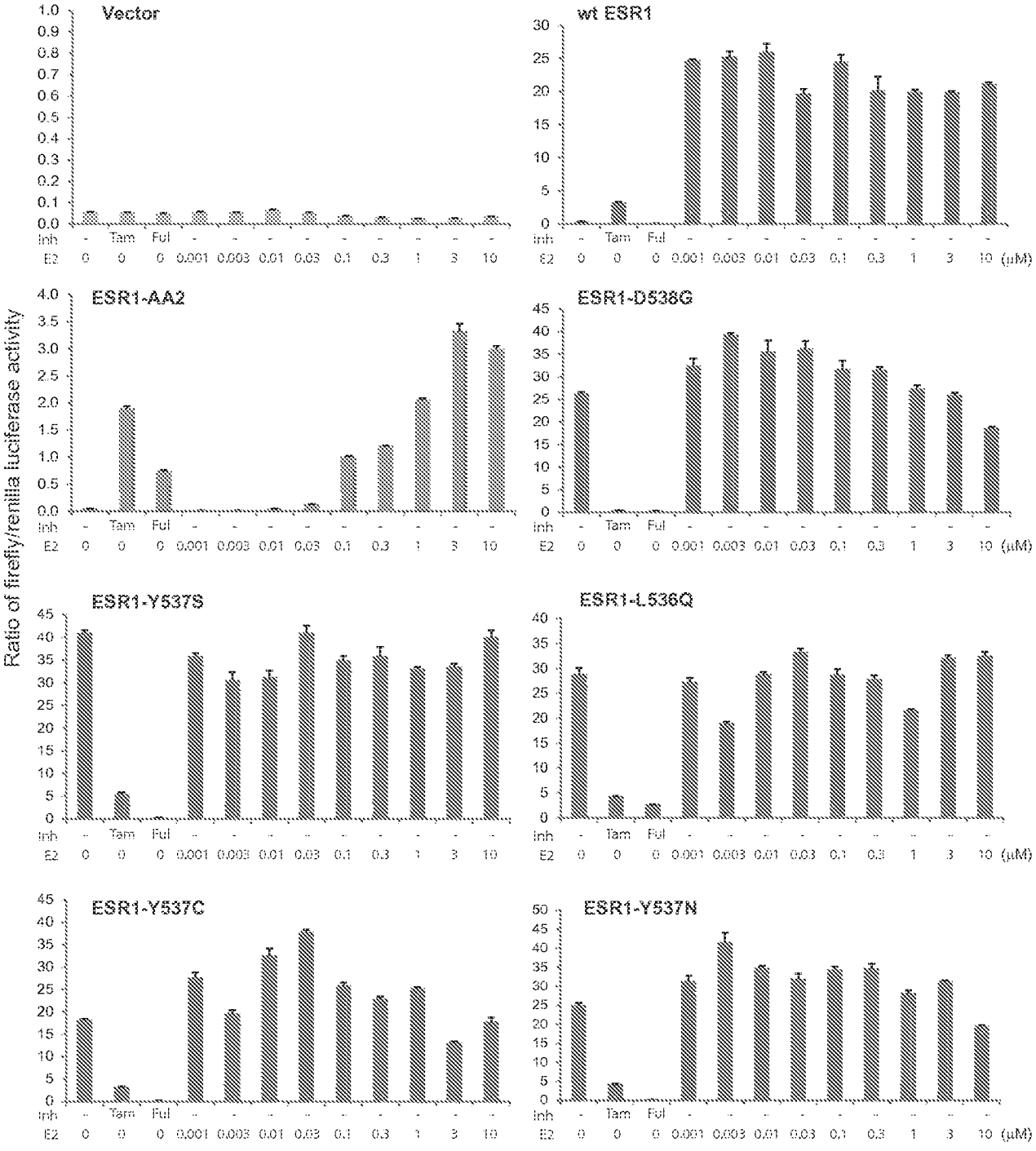

WT
L536Q
Y537S
D538G
Y537C
Y537N
Vector
Inv-mut-AA2
ESR1 transcriptional activity (Percentage)
E2
Endoxifen
0
20
100
1000

Figure 11


Vector
wt ESR1
ESR1-AA2
ESR1-D538G
ESR1-Y537S
ESR1-L536Q
ESR1-Y537C
ESR1-Y537N
Ratio of firefly/renilla luciferase activity
inh - Tam Ful - - - - - - - - -
E2 0 0 0 0.001 0.003 0.01 0.03 0.1 0.3 1 3 10 (μM)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/60382

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/705, C07K 14/72, C12N 15/16, C12Q 1/68, G01N 33/53 (2014.01)
CPC - C12Q 2600/112, C12Q 2600/118, C12Q 1/6813, C12Q 1/6886, G01N 33/53
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07K 14/705, C07K 14/72, C12N 15/16, C12Q 1/68, G01N 33/53 (2014.01)
CPC - C12Q 2600/112, C12Q 2600/118, C12Q 1/6813, C12Q 1/6886, G01N 33/53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12Q 2600/172, C12Q 1/68, C12Q 1/6883
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patent, Google Scholar
Search terms: cancer, tumor, malignancy, estrogen receptor, ESR1, estrogen receptor alpha, ER-alpha, mutation or variant, p.Leu536Gln, p.Tyr537Ser, p.Tyr537Cys, p.Tyr537Asn, estrogen receptor (antagonist or inhibitor) and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/056178 A2 (YELENSKY et al.) 18 April 2013 (18.04.2013) claim 83; pg. 4, ln. 12-13; pg. 5, ln. 10-13; pg. 6, ln. 13-14; pg. 8, ln. 5-30; pg. 16, ln. 21-23; pg. 17, ln. 3, ln. 8-9; pg. 18, ln. 15; pg. 19, ln. 1-2, ln. 16-23; pg. 22, ln. 22-27; pg. 28, ln. 15-17; pg. 30, ln. 24-27; pg. 32, ln. 15, ln. 25; pg. 60, ln. 10-11; pg. 65, ln. 18-19; pg. 66, ln. 5; pg. 76, ln. 6-11; pg. 112, ln. 1; pg. 121, ln. 9-11; pg. 122, ln. 23-27	1-3, 10-12, 20-29

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 2014 (12.12.2014)	08 JAN 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/60382

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

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

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

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

3. ☒ Claims Nos.: 4-9 and 13-19
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

专利名称(译)	用于确定治疗过程的系统和方法		
公开(公告)号	EP3057985A1	公开(公告)日	2016-08-24
申请号	EP2014854474	申请日	2014-10-14
[标]申请(专利权)人(译)	密歇根大学		
申请(专利权)人(译)	密歇根大学董事会		
当前申请(专利权)人(译)	密歇根大学董事会		
[标]发明人	CHINNAIYAN ARUL M ROBINSON DAN WU YI MI		
发明人	CHINNAIYAN, ARUL M. ROBINSON, DAN WU, YI-MI		
IPC分类号	C07K14/705 C07K14/72 C12N15/16 C12Q1/68 G01N33/53		
优先权	61/892743 2013-10-18 US 61/992615 2014-05-13 US		
其他公开文献	EP3057985A4		
外部链接	Espacenet		

摘要(译)

本公开涉及确定治疗过程的方法。特别地，本公开涉及编码雌激素受体的基因中的突变及其与对癌症的雌激素疗法的响应性的关联。