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54 Título: **Método para la detección global de ganancias y/o pérdidas cromosómicas y array de búsqueda de alteraciones genéticas**

57 Resumen:

Método para la detección global de ganancias y/o pérdidas cromosómicas y array de búsqueda de alteraciones genéticas.

La presente invención se refiere a un método in vitro de obtención de datos útiles para la detección y/o cuantificación de ganancias y/o pérdidas cromosómicas en un sujeto con una enfermedad genética. También se refiere a un método in vitro para el diagnóstico o pronóstico de una enfermedad genética o el diseño de tratamiento en un sujeto con una enfermedad genética caracterizado por comprender la detección de cambios en el número de copias de regiones cromosómicas concretas. Además, también se refiere a un array que comprende sondas que detectan cambios en el número de copias de dichas regiones cromosómicas y al uso del array para el diagnóstico o pronóstico de una enfermedad genética o el diseño de tratamiento en un sujeto con una enfermedad genética. También se refiere al uso de determinadas regiones cromosómicas como biomarcador para el mismo fin. La enfermedad genética es cáncer o una enfermedad congénita.

Método para la detección global de ganancias y/o pérdidas cromosómicas y *array* de
búsqueda de alteraciones genéticas

DESCRIPCIÓN

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La presente invención se refiere a un método *in vitro* para el diagnóstico o pronóstico de una enfermedad genética o el diseño de tratamiento en un sujeto con una enfermedad genética caracterizado por comprender la detección de cambios en el número de copias de regiones cromosómicas concretas. Por tanto, la invención se
10 podría encuadrar en el campo del análisis genético.

ANTECEDENTES DE LA INVENCION

La aparición de sistemas de análisis genómico masivo han permitido analizar en un
15 sólo experimento el estado de miles de regiones a lo largo del genoma humano contribuyendo al conocimiento del componente genético de diversas patologías. La miniaturización de los sistemas de hibridación ha convertido a los *arrays* en una herramienta de alto rendimiento para el estudio de enfermedades congénitas y genéticas con afectación de uno o más cromosomas, con alteraciones de tamaños
20 diversos, conteniendo la mayoría de ellas varias decenas, cientos o incluso miles de genes afectados que ponen de manifiesto la necesidad del uso de herramientas de estudio masivo (Fröhling S *et al.*, 2008, N Engl J Med. 359(7):722-34). Los *arrays* identifican cambios en el número de copias a lo largo de todo el genoma tanto ganancias como pérdidas incluyendo delecciones homocigotas y amplificaciones
25 génicas. El conocimiento de las alteraciones en el ácido desoxirribonucleico (ADN, cambios significativos, heterogéneos y recurrentes con posiciones concretas en las regiones cromosómicas implicadas) hace necesario su estudio al establecerse su valor diagnóstico y pronóstico, proporcionando factores objetivos para clasificar enfermedades así como para controlar e indicar tratamientos específicos, al
30 descubrirse genes dianas y terapias específicas (Albertson DG *et al.*, 2003, Hum Mol Genet. 12, 2:R145-52; Zhao X *et al.*, 2004, Cancer Res. 64(9):3060-71). Entre los métodos para el análisis del genoma se encuentran la hibridación genómica comparada (CGH), la hibridación *in situ* (FISH), *arrays* con cromosomas artificiales de levadura ("Bacterial Artificial Chromosome" BAC *arrays*), técnicas basadas en la

reacción en cadena de la polimerasa (PCR) y el cariotipo. El conocimiento genético se ha fundamentado en los hallazgos de análisis citogenéticos convencionales y métodos moleculares, que son técnicas con limitaciones y ventajas, que pueden ser complementarias. El cariotipo tiene una baja resolución, es necesario realizar
 5 previamente cultivos celulares que alarga el tiempo de diagnóstico, selecciona clones con ventaja proliferativa, analiza un bajo número de células y existe subjetividad en la interpretación pero permite el estudio global del genoma. La CGH convencional informa del contenido del ADN de varios miles o millones de células pero presenta inespecificidad en la hibridación por el tamaño de las sondas. La FISH es muy
 10 específica, se puede realizar en interfase y/o metafase pero es parcial, dirigida a zonas del genoma y limitada generalmente a tres fluorocromos y sus combinaciones. Por otra parte, la PCR es económica pero parcial y dirigida a regiones concretas en cada análisis. El uso de *arrays* resuelve algunas de estas limitaciones al proporcionar mayor resolución, mayor número de regiones en cada estudio y objetividad en el análisis
 15 genético.

Existen diversos *arrays* para estudiar globalmente el genoma que comprenden miles de sondas y la tendencia es aumentar las sondas contenidas en los mismos. Así de *arrays* que contenían 77 sondas subteloméricas para identificación de retraso mental,
 20 se pasó a *arrays* de expresión con 20.000 a 50.000 sondas y/o *arrays* que detectan polimorfismos con medio millón ó 1.800.000 sondas. Análisis comparativos de estas plataformas muestran la sensibilidad y especificidad de los distintos *arrays* (Fadista J *et al.*, 2012, PLoSOne. 7,2:e31025). Los *arrays* comerciales disponibles en la actualidad tienen en común el elevado número de secuencias pero características
 25 diferentes como son: el método de producción, distintos tamaños en las secuencias inmovilizadas (entre 25-80 nucleótidos) y el tipo de marcaje para la hibridación (único o doble). La fabricación estandarizada de estos *arrays* ha ido completando y mejorando formatos sucesivos y se han diseñado con fines diferentes: estudio de variaciones génicas, análisis preimplantación de embriones, microdeleciones,
 30 genotipado, estudio de neoplasias hematológicas, análisis de genes supresores y oncogenes y análisis de metilación (Curtis C *et al.*, 2009, BMC Genomics. 10:588-610; Miller DT *et al.*, 2010, Am J HumGenet. 286(5):749-64; Crespo I *et al.*, 2011, J Mol Diagn.13(6):634-47 y Tabernero MD *et al.*, 2012, Genes Chromosomes Cancer. 2012,51(6):606-17).

El alto número de sondas inmovilizadas en los distintos *arrays* diseñados hace imprescindible el uso de herramientas informáticas para obtener la gran cantidad de información contenida. Los análisis matemáticos identifican las variaciones en toda la extensión del genoma, y la dependencia de recursos bioinformáticos tienen un impacto
5 no despreciable en la incorporación al diagnóstico clínico de esta tecnología. Actualmente, los *arrays* contienen millones de sondas de oligonucleótidos con un importante coste de fabricación y de recursos en el análisis de la información que contienen, pero no existe un *array* simple y específico para analizar en muestras de ADN el número de copias de los 24 cromosomas en toda su extensión con utilidad en
10 cáncer, enfermedades genéticas y/o hereditarias u otras patologías. Por lo tanto es necesario un *array* de menor tamaño que los actuales, que siga siendo informativo para detectar el número de copias de todos los cromosomas y las regiones de interés en enfermedades genéticas.

15 DESCRIPCIÓN DE LA INVENCION

La presente invención se refiere a un método *in vitro* para el diagnóstico o pronóstico de una enfermedad genética o para el diseño de un tratamiento personalizado en un sujeto con una enfermedad genética mediante la detección de cambio en el número de
20 copias de regiones cromosómicas concretas que proporcionan información sobre todos los cromosomas. La invención también se refiere a un *array* de búsqueda de alteraciones útil como herramienta diagnóstica y/o pronóstica de alteraciones genéticas y para la individualización de tratamiento en pacientes que proporciona información sobre todos los cromosomas. El método de la invención y el uso del *array*
25 de la invención también permiten orientar los siguientes pasos diagnósticos con otras técnicas complementarias según la muestra analizada para ser una alternativa a las herramientas existentes de diagnóstico convencional y evaluar la muestra con otros métodos complementarios. El método de la presente invención y el *array* de la invención permiten, hibridando con secuencias distribuidas en los 24 cromosomas,
30 identificar las alteraciones del número de copias de regiones cromosómicas y obtener información de estado de genes importantes en enfermedades genéticas de manera más sencilla que los métodos actuales manteniendo la especificidad.

Un primer aspecto de la invención se refiere a un método *in vitro* de obtención de datos útiles para la detección y/o cuantificación de al menos una ganancia y/o pérdida cromosómica en una muestra aislada de un sujeto con una enfermedad genética, donde la detección se realiza en las regiones cromosómicas 1p36, 1p35, 1p34, 1p33, 5 1p32, 1p31, 1p22, 1p21, 1p13, 1p12, 1p11, 1q21, 1q22, 1q23, 1q24, 1q25, 1q31, 1q32, 1q41, 1q42, 1q43, 1q44, 2p25, 2p24, 2p23, 2p22, 2p21, 2p16, 2p15, 2p14, 2p13, 2p12, 2p11, 2q11, 2q12, 2q13, 2q14, 2q21, 2q22, 2q23, 2q24, 2q31, 2q32, 2q33, 2q34, 2q35, 2q36, 2q37, 3p26, 3p25, 3p24, 3p23, 3p22, 3p21, 3p14, 3p13, 3p12, 3p11, 3q11, 3q12, 3q13, 3q21, 3q22, 3q23, 3q24, 3q25, 3q26, 3q27, 3q28, 3q 29, 4p16, 4p15, 4p14, 10 4p13, 4p12, 4q12, 4q13, 4q21, 4q22, 4q23, 4q24, 4q25, 4q26, 4q27, 4q28, 4q31, 4q32, 4q33, 4q34, 4q35, 5p15, 5p14, 5p13, 5p12, 5q11, 5q12, 5q13, 5q14, 5q15, 5q21, 5q22, 5q23, 5q31, 5q32, 5q33, 5q34, 5q35, 6p25, 6p24, 6p23, 6p22, 6p21, 6p12, 6p11, 6q11, 6q12, 6q13, 6q14, 6q15, 6q16, 6q21, 6q22, 6q23, 6q24, 6q25, 6q26, 6q27, 7p22, 7p21, 7p15, 7p14, 7p13, 7p12, 7p11, 7q11, 7q21, 7q22, 7q31, 7q32, 7q33, 7q34, 7q35, 15 7q36, 8p23, 8p22, 8p21, 8p12, 8p11, 8q11, 8q12, 8q13, 8q21, 8q22, 8q23, 8q24, 9p24, 9p23, 9p22, 9p21, 9p13, 9p12, 9p11, 9q12, 9q13, 9q21, 9q22, 9q31, 9q32, 9q33, 9q34, 10p15, 10p14, 10p13, 10p12, 10p11, 10q11, 10q21, 10q22, 10q23, 10q24, 10q25, 10q26, 11p15, 11p14, 11p13, 11p12, 11p11, 11q12, 11q13, 11q14, 11q21, 11q22, 11q23, 11q24, 11q25, 12p13, 12p12, 12p11, 12q12, 12q13, 12q14, 12q15, 12q21, 20 12q22, 12q23, 12q24, 13q12, 13q13, 13q14, 13q21, 13q22, 13q31, 13q32, 13q33, 13q34, 14q11, 14q12, 14q13, 14q21, 14q22, 14q23, 14q24, 14q31, 14q32, 15q11, 15q12, 15q13, 15q14, 15q15, 15q21, 15q22, 15q23, 15q24, 15q25, 15q26, 16p13, 16p12, 16p11, 16q11, 16q12, 16q13, 16q21, 16q22, 16q23, 16q24, 17p13, 17p12, 17p11, 17q11, 17q12, 17q21, 17q22, 17q23, 17q24, 17q25, 18p11, 18q11, 18q12, 25 18q21, 18q22, 18q23, 19p13, 19p12, 19q12, 19q13, 20p13, 20p12, 20p11, 20q11, 20q12, 20q13, 21q11, 21q21, 21q22, 22q11, 22q12, 22q13, Xp22, Xp21, Xp11, Xq11, Xq12, Xq13, Xq21, Xq22, Xq23, Xq24, Xq25, Xq26, Xq27, Xq28, Yp11 y Yq11. En adelante nos referiremos a las regiones cromosómicas aquí descritas como a las “regiones cromosómicas de la invención”.

30

Un segundo aspecto de la invención se refiere a un método *in vitro* para el diagnóstico o pronóstico de una enfermedad genética o para el diseño de un tratamiento personalizado en un sujeto con una enfermedad genética caracterizado por comprender los siguientes pasos: (a) detectar y/o cuantificar al menos una ganancia

y/o pérdida cromosómica en las regiones cromosómicas de la invención en una muestra aislada de un sujeto; (b) comparar el número de copias de las regiones cromosómicas detectadas y/o cuantificadas en el paso (a) con niveles estándar; (c) encontrar al menos una diferencia significativa en la comparación del paso (b); (d) 5 asociar la diferencia significativa del paso (c) a una ganancia o a una pérdida genética; y (e) asociar las ganancias o pérdidas genéticas del paso (d) a una enfermedad genética.

Un tercer aspecto de la invención se refiere al uso *in vitro* del número de copias de las 10 regiones cromosómicas de la invención como marcador diagnóstico o pronóstico de enfermedades genéticas o para el diseño de un tratamiento personalizado en un sujeto con una enfermedad genética.

Un cuarto aspecto de la invención se refiere a un *array* que comprende sondas que 15 detectan ganancias y/o pérdidas de las regiones cromosómicas de la invención donde el número de dichas sondas se sitúa entre 10.000 y 20.000 sondas.

Un quinto aspecto de la invención se refiere al uso del *array* del cuarto aspecto de la invención para el diagnóstico o pronóstico de enfermedades genéticas o para el diseño 20 de un tratamiento personalizado en un sujeto con una enfermedad genética.

DESCRIPCIÓN DETALLADA DE LA INVENCION

La presente invención proporciona un método *in vitro* de diagnóstico y/o pronóstico de 25 alteraciones cromosómicas que comprende la detección de: pérdidas o ganancias de un cromosoma completo, pérdidas o ganancias parciales, regiones de amplificación génica, regiones de delección homocigota o de detección de polimorfismos en todos los cromosomas de un individuo.

30 Por tanto, en un primer aspecto, la presente invención se refiere a un método *in vitro* de obtención de datos útiles para la detección y/o cuantificación de al menos una ganancia y/o pérdida cromosómica en una muestra aislada de un sujeto con una enfermedad genética, donde la detección se realiza en las regiones cromosómicas 1p36, 1p35, 1p34, 1p33, 1p32, 1p31, 1p22, 1p21, 1p13, 1p12, 1p11, 1q21, 1q22, 1q23,

1q24, 1q25, 1q31, 1q32, 1q41, 1q42, 1q43, 1q44, 2p25, 2p24, 2p23, 2p22, 2p21, 2p16, 2p15, 2p14, 2p13, 2p12, 2p11, 2q11, 2q12, 2q13, 2q14, 2q21, 2q22, 2q23, 2q24, 2q31, 2q32, 2q33, 2q34, 2q35, 2q36, 2q37, 3p26, 3p25, 3p24, 3p23, 3p22, 3p21, 3p14, 3p13, 3p12, 3p11, 3q11, 3q12, 3q13, 3q21, 3q22, 3q23, 3q24, 3q25, 3q26, 3q27, 3q28, 3q
5 29, 4p16, 4p15, 4p14, 4p13, 4p12, 4q12, 4q13, 4q21, 4q22, 4q23, 4q24, 4q25, 4q26, 4q27, 4q28, 4q31, 4q32, 4q33, 4q34, 4q35, 5p15, 5p14, 5p13, 5p12,, 5q11, 5q12, 5q13, 5q14, 5q15, 5q21, 5q22, 5q23, 5q31, 5q32, 5q33, 5q34, 5q35, 6p25, 6p24, 6p23, 6p22, 6p21, 6p12, 6p11, 6q11, 6q12, 6q13, 6q14, 6q15, 6q16, 6q21, 6q22, 6q23, 6q24, 6q25, 6q26, 6q27, 7p22, 7p21, 7p15, 7p14, 7p13, 7p12, 7p11, 7q11, 7q21, 7q22,
10 7q31, 7q32, 7q33, 7q34, 7q35, 7q36, 8p23, 8p22, 8p21, 8p12, 8p11, 8q11, 8q12, 8q13, 8q21, 8q22, 8q23, 8q24, 9p24, 9p23, 9p22, 9p21, 9p13, 9p12, 9p11, 9q12, 9q13, 9q21, 9q22, 9q31, 9q32, 9q33, 9q34, 10p15, 10p14, 10p13, 10p12, 10p11, 10q11, 10q21, 10q22, 10q23, 10q24, 10q25, 10q26, 11p15, 11p14, 11p13, 11p12, 11p11, 11q12, 11q13, 11q14, 11q21, 11q22, 11q23, 11q24, 11q25, 12p13, 12p12, 12p11, 12q12, 12q13, 12q14, 12q15, 12q21, 12q22, 12q23, 12q24, 13q12, 13q13, 13q14, 13q21,
15 13q22, 13q31, 13q32, 13q33, 13q34, 14q11, 14q12, 14q13, 14q21, 14q22, 14q23, 14q24, 14q31, 14q32, 15q11, 15q12, 15q13, 15q14, 15q15, 15q21, 15q22, 15q23, 15q24, 15q25, 15q26, 16p13, 16p12, 16p11, 16q11, 16q12, 16q13, 16q21, 16q22, 16q23, 16q24, 17p13, 17p12, 17p11, 17q11, 17q12, 17q21, 17q22, 17q23, 17q24, 17q25, 18p11, 18q11, 18q12, 18q21, 18q22, 18q23, 19p13, 19p12, 19q12, 19q13, 20p13, 20p12, 20p11, 20q11, 20q12, 20q13, 21q11, 21q21, 21q22, 22q11, 22q12, 22q13, Xp22, Xp21, Xp11, Xq11, Xq12, Xq13, Xq21, Xq22, Xq23, Xq24, Xq25, Xq26, Xq27, Xq28, Yp11 y Yq11. En adelante nos referiremos a este método como al
“método primero de la invención”. Tal y como se ha explicado anteriormente, las
25 regiones cromosómicas (loci) aquí descritas se denominan en la presente invención “regiones cromosómicas de la invención”.

Una realización preferida del primer aspecto de la invención se refiere al método donde la enfermedad genética es cáncer o una enfermedad congénita.

30

El término cáncer (o tumor) se refiere en la presente invención a la enfermedad que se asocia a la presencia de células que poseen características de células tumorales conocidas por el experto en la materia, como son la proliferación sin control, inmortalidad, inducción de angiogénesis, resistencia a la muerte celular programada y

potencial metastásico (Hanahan D *et al.* 2011 Cell 144(5):646-74). En la presente invención el término “cáncer” incluye tumores primarios (con células del mismo origen del lugar donde se encuentran) o metastásicos (con células de distinto origen del lugar en el que se encuentran) y también incluye los tumores en cualquiera de sus grados de afectación o estadio.

Una realización aún más preferida del primer aspecto de la invención se refiere al método donde el cáncer es cáncer del sistema nervioso central, cáncer de colon, cáncer de pulmón, cáncer de mama, cáncer de próstata, cáncer de vejiga, leucemias linfáticas crónicas, leucemias linfáticas agudas, mieloma múltiple, síndrome mielodisplásico (por ejemplo, anemia refractaria y síndrome mielodisplásico con delección 5q). Preferentemente, el cáncer del sistema nervioso central es glioma (bajo y alto grado), meningioma benigno, meningioma maligno, oligodendroglioma o glioblastoma multiforme. En la presente invención el término “glioma” incluye tumores de bajo y alto grado.

Se entiende como enfermedad congénita aquella enfermedad que se manifiesta desde el nacimiento, ya sea producida por un trastorno durante el desarrollo embrionario o como consecuencia de un defecto hereditario. Preferentemente la enfermedad congénita es síndrome de Down, síndrome de Edwards o síndrome de Patau.

El término “*in vitro*” se refiere a que el método de la invención se realiza fuera del cuerpo del sujeto.

El término “sujeto” se refiere a un individuo, preferentemente humano, que puede presentar una alteración genética y puede haber sido o no diagnosticado de una enfermedad genética. En la presente invención los términos “sujeto” “individuo” y “paciente” se utilizan indistintamente.

En la presente invención se entiende por “ganancia cromosómica” el aumento en el número de copias de material genético en una región cromosómica comparado con un control. Por “pérdida cromosómica” se entiende la disminución del número de copias de una región cromosómica comparada con un control. En la presente invención se entiende como “alteración génica” o “alteración genética” tanto una ganancia como

una pérdida genética. Como “control” se entiende una muestra de un individuo que no presenta alteración genética.

El término “muestra” se refiere a una “muestra biológica” y se refiere a cualquier muestra que permita obtener ADN del individuo del que se ha obtenido dicha muestra, e incluye, pero sin limitarnos, tejidos y/o fluidos biológicos de un individuo, obtenidos mediante cualquier método conocido por un experto en la materia que sirva para tal fin. La muestra biológica podría ser, por ejemplo, pero sin limitarse, un tejido o una muestra de fluido, como sangre, plasma, suero, mucosa oral, lavado broncoalveolar, linfa, fluido ascítico, líquido pleural u orina. Asimismo, la muestra biológica puede ser, por ejemplo, pero sin limitarse, fresca, congelada, fijada o fijada y embebida en parafina.

De una muestra biológica se extrae el material genético mediante cualquier método conocido por el experto en la materia. En la presente invención como material genético se entiende ADN.

Las “regiones cromosómicas de la invención” son regiones que se sitúan en los cromosomas 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X o Y, en el brazo corto (p) o en el brazo largo (q) del cromosoma.

Se entiende por enfermedad “genética” aquella que está asociada a una alteración en el material genético presente en un sujeto.

Un segundo aspecto de la invención se refiere a un método *in vitro* para el diagnóstico o pronóstico de una enfermedad genética o para el diseño de un tratamiento personalizado en un sujeto con una enfermedad genética caracterizado por comprender los siguientes pasos:

(a). detectar y/o cuantificar al menos una ganancia y/o pérdida cromosómica en las regiones cromosómicas 1p36, 1p35, 1p34, 1p33, 1p32, 1p31, 1p22, 1p21, 1p13, 1p12, 1p11, 1q21, 1q22, 1q23, 1q24, 1q25, 1q31, 1q32, 1q41, 1q42, 1q43, 1q44, 2p25, 2p24, 2p23, 2p22, 2p21, 2p16, 2p15, 2p14, 2p13, 2p12, 2p11, 2q11, 2q12, 2q13, 2q14, 2q21, 2q22, 2q23, 2q24, 2q31, 2q32, 2q33, 2q34, 2q35, 2q36, 2q37, 3p26, 3p25, 3p24, 3p23, 3p22, 3p21, 3p14, 3p13, 3p12, 3p11, 3q11, 3q12, 3q13, 3q21, 3q22, 3q23, 3q24, 3q25,

3q26, 3q27, 3q28, 3q 29, 4p16, 4p15, 4p14, 4p13, 4p12, 4q12, 4q13, 4q21, 4q22, 4q23, 4q24, 4q25, 4q26, 4q27, 4q28, 4q31, 4q32, 4q33, 4q34, 4q35, 5p15, 5p14, 5p13, 5p12, 5q11, 5q12, 5q13, 5q14, 5q15, 5q21, 5q22, 5q23, 5q31, 5q32, 5q33, 5q34, 5q35, 6p25, 6p24, 6p23, 6p22, 6p21, 6p12, 6p11, 6q11, 6q12, 6q13, 6q14, 6q15, 6q16, 6q21, 5 6q22, 6q23, 6q24, 6q25, 6q26, 6q27, 7p22, 7p21, 7p15, 7p14, 7p13, 7p12, 7p11, 7q11, 7q21, 7q22, 7q31, 7q32, 7q33, 7q34, 7q35, 7q36, 8p23, 8p22, 8p21, 8p12, 8p11, 8q11, 8q12, 8q13, 8q21, 8q22, 8q23, 8q24, 9p24, 9p23, 9p22, 9p21, 9p13, 9p12, 9p11, 9q12, 9q13, 9q21, 9q22, 9q31, 9q32, 9q33, 9q34, 10p15, 10p14, 10p13, 10p12, 10p11, 10q11, 10q21, 10q22, 10q23, 10q24, 10q25, 10q26, 11p15, 11p14, 11p13, 11p12, 10 11p11, 11q12, 11q13, 11q14, 11q21, 11q22, 11q23, 11q24, 11q25, 12p13, 12p12, 12p11, 12q12, 12q13, 12q14, 12q15, 12q21, 12q22, 12q23, 12q24, 13q12, 13q13, 13q14, 13q21, 13q22, 13q31, 13q32, 13q33, 13q34, 14q11, 14q12, 14q13, 14q21, 14q22, 14q23, 14q24, 14q31, 14q32, 15q11, 15q12, 15q13, 15q14, 15q15, 15q21, 15q22, 15q23, 15q24, 15q25, 15q26, 16p13, 16p12, 16p11, 16q11, 16q12, 16q13, 15 16q21, 16q22, 16q23, 16q24, 17p13, 17p12, 17p11, 17q11, 17q12, 17q21, 17q22, 17q23, 17q24, 17q25, 18p11, 18q11, 18q12, 18q21, 18q22, 18q23, 19p13, 19p12, 19q12, 19q13, 20p13, 20p12, 20p11, 20q11, 20q12, 20q13, 21q11, 21q21, 21q22, 22q11, 22q12, 22q13, Xp22, Xp21, Xp11, Xq11, Xq12, Xq13, Xq21, Xq22, Xq23, Xq24, Xq25, Xq26, Xq27, Xq28, Yp11 y Yq11 en una muestra aislada de un sujeto; (b) 20 comparar el número de copias de las regiones cromosómicas detectadas y/o cuantificadas en el paso (a) con niveles estándar; (c) encontrar al menos una diferencia significativa en la comparación del paso (b); (d) asociar las diferencias significativas del paso (c) a una ganancia o a una pérdida genética; y (e) asociar las ganancias o pérdidas genéticas del paso (d) a una enfermedad genética. En adelante 25 nos referiremos a éste como al “segundo método de la invención”.

El término “diagnóstico” en la presente invención se refiere a la capacidad de detectar una enfermedad genética en un sujeto.

30 El término “pronóstico” en la presente invención se refiere a la capacidad de detectar una alta o baja probabilidad de desarrollar una enfermedad genética aún cuando en el momento del diagnóstico no presenten dicha patología. También se refiere a la capacidad de predecir la evolución de un paciente con una determinada enfermedad (también denominada patología).

Se entiende por “tratamiento” al conjunto de medios que se emplean para curar o aliviar una enfermedad.

- 5 Con “diseño de un tratamiento personalizado” se entiende la determinación de la necesidad de un individuo de recibir un determinado tratamiento en base a la detección de una ganancia y/o pérdida cromosómica en las regiones cromosómicas de la invención, es decir, con el método de la invención se clasifica al individuo en un grupo de individuos con necesidad de tratamiento específico para una determinada enfermedad o en un grupo de individuos que no presentan necesidad de tratamiento específico para la misma.

Se entiende por niveles “estándar” aquellos que provienen de una muestra control que no contiene alteración genética en la región cromosómica a comparar.

15

Una realización preferida del segundo aspecto de la invención se refiere al método donde en el paso (d) y el paso (e) la ganancia o pérdida genética es una pérdida o ganancia de al menos un cromosoma entero, de al menos uno de los brazos de un cromosoma o de al menos una región cromosómica de al menos uno de los brazos de un cromosoma.

20

Otra realización más preferida se refiere al método del segundo aspecto de la invención donde la detección y/o cuantificación del paso (a) se realiza mediante la detección y/o cuantificación de al menos un gen que se encuentre localizado en las regiones cromosómicas descritas en el paso (a).

25

Una realización aún más preferida del segundo aspecto de la invención se refiere al método donde los genes son los genes de la tabla 1. En adelante nos referiremos a ellos como a los “genes de la invención”. Los genes de la invención se encuentran localizados en las regiones cromosómicas de la invención.

30

Tabla 1. Genes localizados en las regiones cromosómicas de la invención: se proporciona el número de identificación de los 3920 genes “Entrez Gene ID” del “National Center for Biotechnology Information (NCBI)” Bethesda, Maryland, USA.

Cromosoma 1:

10905;4853;200162;6913;10352;79679;829;55917;9860;260425;26191;54665;10390;1
28338;2773;57535;56900;10451;22911;1301;94236;118427;8556;51611;148867;5825;
199857;1806;440594;729970;58155;126969;148534;9411;676;284697;388649;1810;2
5 53152;54874;100131564;79871;56267;2633;2634;2635;163351;2959;23507;84230;55
86;84146;9403;9653;339524;51100;26289;64123;54810;23266;57554;257194;91624;5
567;5733;148418;204962;81849;79739;26009;55635;6121;29929;27329;400757;8544
0;3716;57708;55225;4919;84251;23169;148581;79819;127795;51361;1600;284546;10
31;55268;2060;11124;348378;148979;388633;7804;4898;5865;6342;6512;55706;5106
10 0;23358;54432;23318;9372;84871;79656;51727;284541;1580;199974;388630;54558;8
891;79033;23139;8503;5052;55182;6487;10420;57643;728621;991;114625;440585;22
887;59269;9132;64175;4802;6018;22955;6513;64744;149465;4904;10269;84970;1441
;114784;192669;284656;29889;2899;79932;23499;64769;54933;64121;6421;83931;27
285;9967;9202;5928;127544;64766;8565;7579;1307;149086;8073;347735;9410;51538
15 ;2035;10076;6429;27245;79000;90529;57035;2134;2268;3155;3151;26119;57134;127
294;6006;6007;57190;6548;23585;23038;249;1870;8672;2048;50809;3339;23028;401
944;7798;6920;84196;80818;22977;8659;440574;832;284729;54455;84966;23065;468
1;29943;11240;5081;55920;6390;23013;255104;23352;7709;79814;780789;842;23341
;114827;23254;126755;10630;65121;390999;400735;440561;7799;378708;100526739
20 ;148345;284498;54897;1185;56998;5394;26270;9563;23095;10747;9927;2475;64802;
5195;5226;5293;57540;80176;10277;55187;23261;54206;11315;473;11332;26038;557
35;83715;8514;9903;261734;57449;27237;148870;1677;57212;9731;1953;79258;7990
6;55229;63976;127262;2782;85452;100129534;5590;6339;51150;6497;9906;29101;25
4173;2330;8515;128077;9659;5174;27246;9554;647135;10628;51205;607;2702;2703;
25 440689;54460;56957;51177;11311;405;149428;57530;2029;80308;2312;388698;5745
9;55204;84072;3782;353135;353144;84648;114771;57115;23126;9129;58497;5692;60
97;23248;6282;6278;8991;10500;4184;81609;80222;11022;117145;7286;10485;14909
5;57657;128239;57127;83416;4914;9047;477;1314;50717;23385;26476;114836;22926
;4009;9722;387597;400794;4259;55732;92342;22920;6401;26052;9910;27;84320;460;
30 126859;55103;9462;6646;163589;163590;3918;23057;23179;85397;9213;3080;16348
6;140609;51094;134;493;5129;1118;6900;1465;2005;9641;10765;9928;23046;55220;9
3273;148808;89796;23114;2494;64710;8497;4660;254428;9580;23380;127833;92703;
653659;252839;57172;1604;3756;3914;642587;255928;467;51514;28982;4751;5525;2
6750;7188;10380;2058;2104;55699;3776;25782;51018;7042;7399;127602;3707;142;5

6997;8476;2590;79605;5664;128061;54583;440730;128178;56605;7107;359948;9859;
64388;55127;3964;100130331;23596;6000;6262;10806;128025;10000;163882;339529
;284521;64754;79894.

Cromosoma 2:

5 645784;200523;129293;1768;9451;7844;22934;150572;51652;6936;1496;3099;80059;
9801;72;1639;6242;10505;200424;10574;23233;26056;94097;27332;22848;200558;99
38;400961;5341;10097;23177;25927;400958;200734;130872;84542;7360;9736;11480
0;400954;400957;400955;64895;150962;98;51130;27248;100302652;23198;6711;937
8;27436;63892;130589;92906;6546;5610;54497;6801;5212;57448;84661;51072;55622
10 ;84272;238;92291;79623;81606;253558;6716;7498;9577;388939;79745;165186;2355;
9553;5500;245711;23160;54454;10669;1838;3032;9381;9784;84450;338;114818;4148
;130502;57539;348654;3790;729475;79677;7447;23175;150622;52;285016;339822;26
751;7173;7260;51112;26504;51252;23397;23020;23505;2274;3554;80146;165055;109
13;3987;5903;344558;151011;55289;4867;11159;10096;54520;8886;57628;80255;273
15 6;274;29798;440915;2863;4249;344148;1615;4175;81615;53353;339745;55843;8942;
26122;3800;130576;55777;5000;130574;27249;114805;90134;10213;85461;10010;80
034;6326;129446;1386;1123;1781;254042;79828;140469;8604;285025;4780;91404;15
1126;151242;10203;142679;165215;2487;3685;10787;129401;5136;116093;51454;53
78;6775;84128;57520;9262;57679;151254;65062;316;843;841;130535;58538;60491;5
20 334;151246;26010;8745;391475;130752;117583;389072;389073;2066;4133;200576;5
746;79582;26154;729582;55686;55825;92691;7520;2181;130340;10056;116255;5077;
5270;130367;57590;55619;79843;92737;80309;9320;80210;389084;129563;80303;26
058;55355;3769;4691;25791;5147;5707;3431;93349;79054;54575;54576;116987;2367
7;23192;1293;728294;339768;9855;64090;9759;84289;728323;9208;23178;51540;259
25 92;51078;140739.

Cromosoma 3:

874253559;2632;6091;6092;27086;26018;9223;166336;115286;8618;2272;5793;1322
04;26059;54899;7474;440957;776;55799;25981;55193;1795;51409;7867;80335;72923
0;1232;79443;23395;79442;4134;10180;22908;55129;152206;440952;131375;4336;25
30 924;4820;22906;7433;11280;23122;10491;10015;7342;114884;201595;339896;15211
0;9497;7048;55768;5915;7068;7324;6304;51244;84077;23473;344875;8292;152273;8
0852;64432;7182;9467;6533;79188;348825;7476;64145;132001;6854;80746;10533;49
1;56852;9901;25917;2917;3708;285362;152330;3568;10752;27255;1371;285220;2550
25;10402;25890;55773;55076;91775;50939;131368;56987;25945;523;91653;79669;10

	225;90102;1814;54762;4045;26137;152404;2960;9515;84925;79663;4638;54625;1095 4;54437;57493;3693;8997;114885;84561;7707;10840;348807;55764;54946;644974;84 19;131034;23317;729375;348808;79858;58477;23023;66000;11073;2047;51163;1992 21;83850;22808;347736;64084;55186;92369;253461;205428;5352;285195;116933;11 5 6931;26084;170506;344758;8833;7881;23007;79674;51319;654502;100505385;15174 2;29970;165679;647107;151790;79740;2122;254827;57552;83893;10242;10057;8893; 54800;89857;55486;7066;55689;1608;1962;23355;51726;3273;3827;253573;5648;648 0;55171;152137;9076;3556;55214;4026;8626;285386;84239;344905;152002;1739;842 48;84223;89782;348840;84859;55341;4976;5062;5504;205564;7037.
10	Cromosoma 4: 1259;10699;2557;2560;152518;152519;7006;7294;323;389207;386617;22998;116984; 55584;401124;54502;57728;5099;886;80333;55300;3516;51091;55296;683;114905;57 545;645104;84448;60312;84740;116449;152789;5522;54436;56606;57533;9948;3891 97;132884;55351;118;317648;1487;8603;2868;3064;8602;10336;353497;6047;6452;1 15 0417;10460;100379220;84286;7468;57732;7700;152687;255403;170960;654254;6422 80;132946;9575;23142;55763;81608;57482;3815;84708;644145;132954;5471;5978;15 2579;6443;132671;6731;55858;64854;2044;23284;26228;9407;28983;55236;10941;57 4537;91746;9508;27284;6783;419;3627;25898;55153;57619;84779;153020;84803;834 78;10855;4299;5602;5783;9429;345275;57563;6622;8404;2895;658;285555;10611;56 20 916;8633;124;125;126;127;128;130;131;84103;27071;79982;1977;8649;23173;4547;5 910;93587;10098;6870;84570;9348;11107;2247;11162;166378;80167;132321;55132;2 56471;10424;79960;10733;132320;25819;1047;1998;255520;100129858;55534;4258; 80155;60592;80854;7350;64399;3600;8821;57484;11157;84068;83894;152485;79658; 166614;4306;90826;55751;54798;55294;2244;152503;51802;2982;6999;55016;55319; 25 56884;92345;11199;55601;1182;4750;57630;442117;140458;2823;116966;285501;55 247;133121;3660;79682;8470;2195;2483;285441;4543.
	Cromosoma 5: 2255;2690;348980;1601;133690;3575;92255;6507;79925;133688;167127;29102;4883; 23037;1008;1007;56979;1010;54463;4651;1767;7204;170690;23379;340094;57491;13 30 3957;81037;11336;728613;79888;85409;10016;153478;10723;348932;6531;7015;798 44;3001;3672;3673;5144;91942;375449;55814;153562;64087;64283;64924;8507;2604 9;22987;55255;8546;29958;10184;23440;9522;6902;83734;153339;1462;7518;10085; 84059;10622;153396;831;22936;51752;64167;2745;167410;79772;5122;22836;5066;7 903;83594;1946;4124;9315;814;91137;642987;134430;324;64097;4163;64848;5229;1

176;206338;51397;57556;153443;153241;93166;28966;171019;337876;2201;644873;
6558;28965;27125;645121;9547;10111;153328;4090;7045;57113;64374;23092;57528;
80762;2908;815;22993;3360;5145;133522;5521;6534;84651;643394;202374;2196;206
358;153201;11346;10827;55568;2890;8728;147;26999;1879;84868;6444;7265;57451;
5 5917;662;1794;100131897;8614;92181;10814;253314;23197;3274;57179;285593;643
24;94081;90249;23567;79908;153579;57472;91522;2324;9945;5601;55819.

Cromosoma 6:

5558;653;81578;26268;8521;22858;4172;5314;221395;10231;7021;89845;705;896;98
8;53405;22875;55168;5429;221409;8464;129685;441151;55362;55809;57510;24149;1
10 14781;646888;2739;221458;266727;221468;60685;64771;2914;11165;29993;8831;79
17;135656;170679;7148;387032;7718;7741;6568;6310;84062;9750;401237;9308;5443
8;23408;84830;1906;3096;221692;51256;54898;2651;4739;51522;100113407;654;183
2;9521;63915;266553;6239;7020;2162;9450;285780;154386;4835;63027;56897;56940
;55770;642316;577;346007;56479;22999;90025;51167;22832;4199;112609;167681;98
15 92;1081;9096;23036;57150;206412;2570;84553;51805;84539;85015;10973;2898;5753
1;202;221264;84071;11149;9896;112495;100422737;57107;84816;11231;2444;22255
3;154215;10345;135112;60487;3908;84870;2037;84456;285733;154075;6446;10767;4
217;100131814;167838;3097;9749;8676;7402;389432;9590;2099;25902;57480;23345;
10090;340152;26271;54516;4988;26575;57492;729515;84624;50508;6580;51429;887
20 1;117289;51106;26230;56995;79683;56895;4216;5071;55780;84498;10846;5689;6196
;64094;253769.

Cromosoma 7:

346288;1956;2631;55915;81552;222008;349075;23242;1644;2887;100130988;375567
;11055;154664;136288;80099;3364;168507;256979;64759;54606;23072;4967;5478;83
25 637;9844;2737;5683;23333;23080;27241;168667;222166;100129460;79017;10392;38
7129;51251;5137;84725;9805;9586;221895;8701;9771;9734;7291;1607;2115;4223;34
0267;3382;221981;221806;221937;9907;55698;401303;84433;2768;221935;155185;9
0639;26173;23288;100128374;8379;64940;7697;340252;442319;26053;83698;64409;
9031;57639;2006;3092;54103;5782;222194;781;9863;57157;53616;54677;55972;5218
30 ;219578;55971;799;55610;285987;1780;2792;5445;5446;55607;7979;10165;1523;136
227;222234;222235;402569;222236;5701;5649;375612;55973;10135;5172;1738;9398
6;83943;29969;4897;5506;1811;830;1080;4233;7982;93654;22797;7472;83992;51691;
93664;6561;50833;2918;3952;55131;93979;4899;114960;7328;54927;378805;136259;
5420;1129;64764;9162;5764;346673;50617;27147;84255;8805;254048;56829;26047;1

00302191;27010;11194;10061;653857;2636;155038;285972;51422;1804;285888;5748
8;64327;389602;54892;64434;5799;140545;9690;7434;55112.

Cromosoma 8:

286;10947;7994;5423;7419;8749;2515;2260;203100;6867;157855;84750;23303;3084;
5 11030;1191;51435;286133;10863;51312;55909;9583;4017;4023;10395;23362;137868;
1508;1671;340357;91694;64478;157695;9228;157697;26260;9172;644128;23514;559
1;137902;389658;115294;9821;9705;6917;9760;55636;286183;10987;85479;9650;515
0;10565;116328;57094;1360;80243;100132891;392232;10499;63978;8989;83690;100
192378;79776;51101;3574;759;138046;245972;4325;85481;11059;157657;286148;10
10 0500773;642924;10404;6788;157680;1807;8323;29967;9699;55074;23414;27012;114
788;8667;2131;169026;401474;4982;9897;286053;93594;22882;50807;51571;114;378
6;51105;6482;169044;575;389690;51305;54742;5747;203062;58500;7553.

Cromosoma 9:

441430;441425;253650;647044;79937;9380;9833;5079;64425;152006;138724;971;25
15 853;27019;4799;5646;8434;9853;27290;51271;55833;54926;10497;7415;54840;2683;
3301;158038;4712;80173;79817;7010;286319;1029;100048912;54914;554202;4300;4
507;401494;340485;92949;55667;158297;123;25769;54796;54875;6456;100129385;2
03238;158326;389705;4781;11168;6619;158219;340481;8777;5789;7306;55848;5506
4;2731;90865;23081;2315;115426;169792;5991;6505;1761;58524;81704;23189;6595;
20 320;9413;2395;5239;8395;9414;138255;256691;375743;80036;301;100302287;5125;1
17531;9630;158471;29968;23230;7091;257019;158158;389766;392360;392364;4915;
10927;340515;6850;138649;23299;445577;401541;4920;65268;375748;84909;2176;2
2927;5727;100499483;8555;1306;7464;100499484;57653;4686;9830;19;23446;10592;
11217;652972;23731;158401;8727;54566;8518;23392;1902;100422990;114299;44581
25 5;22949;5774;64420;7357;158399;80709;85301;25861;114991;23245;1620;10012850
5;5069;727;55755;11064;5082;51552;23637;7185;57706;89853;2649;5695;9649;1759;
23048;57720;28989;114789;25;83543;23404;10319;59335;84726;389799;64794;8328;
9329;9442;84628;8021;2889;23064;7248;11094;1757;774;157922;79813;57582;9919;
643224.

30 Cromosoma 10:

94134;3799;6935;387647;1326;6840;10006;2572;57512;56243;84930;53904;23590;56
952;5305;100169750;340895;64215;10529;84898;783;9200;221074;338596;8027;571
18;83643;55691;10133;1787;10659;22944;57713;83860;83592;2665;1316;10276;105;
55853;22982;23185;642394;22884;10771;9790;414194;283033;441666;8187;7582;10

0129482;93550;118738;56624;282966;196740;1103;8031;55753;8505;57705;65217;5
592;288;983;9886;22891;29119;26091;221037;84665;23411;84883;219738;1305;5550
6;5464;8879;23555;83938;23522;100288974;650623;8512;219654;57178;92211;8433
2;27069;2894;4143;10718;387694;81619;657;9060;23063;118932;84896;142913;9023
5 ;9585;3988;643414;643418;5728;55328;54708;9044;22849;100188947;84333;80351;5
5165;1562;1559;118924;338557;26509;64318;51196;29760;100127889;9124;5223;10
580;9849;27291;55118;3257;60495;10613;55719;5076;9033;25956;8945;54805;22978
;79991;9644;9748;120;80217;159686;22986;7511;282996;26033;2674;255082;196047
;118987;6001;196051;55717;26098;64077;100169752;7390;8038;4288;5791;1571;253
10 738;3632;282973;619207;4255;93426;256536.

Cromosoma 11:

441601;221120;60529;9793;1408;2132;2147;51317;56981;55761;51074;341019;4400
34;26298;2001;8050;744;63982;338645;89797;4745;340990;5286;144100;10196;5555
3;55293;10083;84280;797;1315;22800;10418;7003;374378;9645;10335;322;417;5667
15 2;10527;3784;4004;283104;283299;650368;56547;4676;4928;114879;27315;50862;81
846;6764;6786;23075;143425;338440;51272;81532;6150;171389;113746;6181;1500;9
2292;283194;9404;219527;504189;219956;6947;51338;643680;7439;11145;387775;4
296;23649;5526;7536;55867;4041;55690;51111;1374;8500;116985;9828;408;26005;8
1570;9873;81544;387273;220074;4926;283208;5138;283209;10714;51400;254225;22
20 941;9789;55331;28971;1740;92105;65987;79731;26011;2915;4544;7299;84441;14387
2;53942;5241;330;4322;64066;4314;4316;60496;834;2893;84437;120400;4684;7704;2
900;25988;10525;100499227;9099;399959;6653;50937;79607;84623;84881;8538;231
3;29068;112937;22997;83700;50863;4978.

Cromosoma 12:

160518;121512;55297;341350;83857;56938;341346;23012;586;55259;55500;2998;98
25 47;3845;5965;6579;10599;6660;8082;6489;5139;28234;5288;2842;2904;8531;2120;11
8426;50840;2;359787;51348;10219;4074;8076;360030;9918;5858;57494;440077;5710
2;2251;26290;3742;10867;79602;283358;775;93589;196513;23085;144699;440073;57
4538;7004;7289;65125;80070;144402;120892;283463;4753;29951;51535;144165;114
30 134;84216;54934;8909;91523;3906;29843;7421;466;121006;91010;613227;113251;91
012;25875;51474;255411;25840;27289;65244;7009;283471;401720;9119;283404;546
3;6334;7024;9840;3798;4035;4141;5557;5939;65012;6773;116986;10677;1594;33881
1;121227;10956;9958;115749;57460;29110;11260;400046;23426;8091;23592;253827;
144453;10576;1368;10818;27345;56890;5787;5801;117177;8549;83591;29953;7103;1

96441;89795;6857;8499;144448;80184;8411;55591;144193;56899;374467;120935;40
48;4604;27340;121551;50515;83468;253724;4801;51559;6996;160762;23316;160760;
51699;80724;6311;8550;283450;8437;22895;80024;10906;23389;23014;26259;28345
5;4842;160777;11113;84530;51347;55884;140707;9478;10645;84660;80212;6249;196
5 385;8655;338442;27198;2967;9735;5027;353116;9921;65117;55206;949;121665;1444
06;55596;100128554;387895;92293;2802;6433;2054;121256;7556;7699;10795.

Cromosoma 13:

51084;8100;53342;26524;100101938;221143;266695;93492;64328;6445;221178;5550
4;1024;10810;255967;51082;145173;3146;10808;84056;51371;5412;116828;9365;104
10 43;23047;90627;5983;400120;9201;100302239;26960;161003;7223;11215;29103;550
68;94240;2963;26747;23143;10161;5925;81550;79866;11278;10464;4008;1910;64062
;26050;2262;10082;10257;9071;5911;10160;8428;171425;5095;84899;9358;728215;2
3026;8874;23250;8881;8451;55208;79774;23263.

Cromosoma 14:

1603;23428;9472;25831;80224;29966;55837;64067;58533;11177;84312;9692;55012;2
53959;89874;145282;57697;122773;23588;161357;122769;9147;145447;30001;10979
;122786;54331;11183;22795;51199;5706;5836;6815;114088;652;23034;22863;3895;3
87332;317761;5583;51804;646113;27133;6617;23224;83851;2342;2530;4149;87;8816
;55218;57452;9766;5890;161145;8650;9628;64093;53349;55668;2103;399671;122953
20 ;9870;85457;9369;9517;57156;161436;23768;1112;54207;55812;123016;79882;90141
;80017;801;440193;8111;55775;55727;79820;3705;57578;123036;79789;23405;25639
4;866;2009;100129345;283600;7443;1778;23186;9895;4140;7187;122622;113146;233
68;388021;100128927.

Cromosoma 15:

23742;23191;727924;8123;6638;8926;7337;2558;2562;2567;100128714;321;23359;89
24;4948;89839;22909;100288637;1139;342184;51621;161725;9716;89978;57099;845
29;1133;161835;4212;6263;9990;7057;11261;161829;729073;11245;3712;56924;8773
;9333;116179;80021;146057;197131;113201;51496;84978;9836;7158;2200;2585;2625
8;9728;80031;399694;79029;58472;23431;79895;196951;56986;4644;29766;388121;3
30 66;9236;84952;161582;145781;3990;4734;283659;5873;6938;440279;256764;54629;5
4778;79811;4643;6095;83660;53944;84465;79719;4088;10391;22801;5607;10031388
4;4649;79400;8554;79875;80381;4016;5371;1464;26263;730058;145957;192683;4985
5;2184;3419;283688;646892;400410;23102;54469;57188;8120;79631;283726;648809;
6457;388165;123624;11214;57538;5151;11057;176;290;1106;55215;100144604;5428;

56963;28232;8128;56964;374655;145820;400456;91947;22856;3480;204219;55180;79705;5046;123283.

Cromosoma 16:

112869;3683;23214;9726;55114;146395;115584;27327;730094;91949;84516;23062;9
5 956;5347;5579;57478;146177;116285;123876;6296;57020;400506;9738;55567;11247
9;124274;51704;124152;81691;64131;4363;89927;23042;55313;2072;729993;23274;9
2017;2903;8312;254528;146439;23059;84256;114984;9742;83986;54442;64788;1001
28770;55692;64386;79903;4716;197358;9091;9088;9727;54715;9351;23524;79652;10
131;7752;146434;124149;10294;84706;91807;79801;55737;113;123970;1540;81533;8
10 1831;85407;5257;27324;23090;390732;80205;79068;283856;54947;4313;5934;23322;
1071;4489;4496;84166;9688;51090;6559;92922;1009;221188;283847;999;1001;64174
;55282;57610;55512;6645;6483;79613;11060;342371;9785;100130894;342372;83449;
55697;55565;463;10428;11345;2734;55159;79726;162239;84937;124359;83657;1012;
80790;83693;123872;8720;54550;5336;9914;23406;1327;10328;83716;3394;9764;400
15 550;64779;58189;55625;79791;57338;100288525;124245;29123;27132;54849;9780;2
175;2622;6687;22980.

Cromosoma 17:

388341;25979;284124;83450;23164;9611;125144;9487;5636;10743;7732;55244;9252
1;9706;23326;57336;136;9912;284040;1352;60528;201158;284034;9955;9953;93649;
20 5376;64518;54902;57335;245;59344;255877;1107;8522;23135;8735;81565;23533;948
2;7157;124739;146845;489;55421;23125;10749;4927;5023;83394;9135;201305;7442;
162514;29;8447;64359;390756;5306;9501;146760;23293;114826;55275;7531;40;5573
1;51379;26284;8844;399687;4642;4763;51701;57532;57649;6147;6830;23512;31;163;
256957;2064;84961;79893;6928;22806;284100;5469;80179;93210;57125;5892;11758
25 4;146857;342618;162394;11276;8148;146862;22794;284110;9862;339287;7067;47;90
01;317719;162605;125115;284116;81870;6605;6776;6777;1394;80174;1845;23131;28
4058;100128977;4137;4905;113026;6521;7343;124872;124989;996;80279;10642;383
7;100272146;404266;124995;9520;8631;6668;56934;54799;11143;9043;162461;5109
6;162282;339210;124540;22843;252983;1213;51136;51174;54828;84669;10238;1655;
30 2081;81033;4215;64750;92335;26115;55852;201134;8787;23460;10351;10350;2186;9
931;100499466;26207;5578;400619;100499467;5608;201266;283987;23580;642843;6
4446;3396;3993;9400;114804;54549;55808;10610;146664;6397;146712;84733;55036;
284001;9267;8632;388428;284207;146713;5986;57521;6448;124565;125058;57690;5
7602;56270.

Cromosoma 18:

374860;753;55125;63895;2774;4158;65258;5771;8731;56907;23253;8774;4729;9989;
 11031;100192426;5797;79822;9229;23136;91133;284217;339290;642597;56651;2709
 8;81035;84034;23347;9984;7298;9097;7525;85019;80000;3909;57534;5932;80816;37
 5 4864;1828;1829;1830;147409;1837;64762;8715;80206;2589;125476;647946;10982;55
 197;5289;6014;26040;6563;498;147339;54808;9811;57724;494470;220134;4200;6925
 ;5205;23327;9480;147372;89778;5055;8710;79839;643542;220164;29090;1528;64404
 1;125704;201456;10194;55628;22850;374868;284274;9150;284241;100130522;4155;
 84552;7776.

10 Cromosoma 19:

388524;90649;148213;100287226;7643;7644;81931;148198;8907;84167;125972;1052
 3;27151;199786;26017;54815;79709;93323;9441;4650;115861;80714;25796;23025;11
 7286;388512;8529;4051;30817;84658;4218;9524;7171;79173;407018;65249;23370;77
 3;1264;1785;1786;1994;1995;326342;115704;84467;3385;7087;3609;3643;80131;510
 15 73;94025;5141;92960;10908;9545;125950;57139;57153;26526;7297;7730;79230;1481
 56;115950;729648;81616;8943;1820;85300;997;10036;2527;126326;23030;54531;490
 2;23396;5802;8498;84839;5990;6294;9667;8175;6449;360200;9322;115196;83636;87
 25;9745;84079;147991;55094;9710;26065;5184;126248;22847;4099;284406;7705;849
 24;126231;163115;23094;83889;80776;593;90273;1089;8857;23646;57716;5669;6534
 20 92;57731;125931;388550;23474;3783;284344;5329;5672;5673;5676;5678;7515;7771;
 7638;7769;284349;284346;388552;9704;1762;24139;90332;2909;64344;388553;2843
 52;57787;80207;8189;84335;587;374920;22900;29998;259307;3748;3978;79784;2363
 6;8605;54922;23521;728658;114132;400709;57030;28968;79735;55260;54795;7288;5
 1231;25888;3036;5518;8778;946;147660;162966;81856;90321;388558;55769;59283;2
 25 9924;3904;114823;11027;10859;11006;338321;7137;284415;2282;147670;204801;51
 78;100169890;10155;140612;23619;7693;7694;57343;147686;79744;388566;27300;1
 62972;201514;80095;63934.

Cromosoma 20:

84532;57136;1473;164380;140838;63908;22981;5834;79953;22803;55184;26074;513
 30 40;92675;58495;55857;57186;54453;10483;57419;27131;140836;631;11034;51575;55
 614;140733;5126;55304;55617;182;54675;55612;54363;84515;146;8455;25943;11327
 8;57593;2280;100289473;57506;5173;149830;5786;642636;343637;128646;9962;705
 3;343641;22888;64601;79175;598;51654;84701;245929;22919;5326;51297;9777;1643
 95;191;9139;11190;8904;55741;10893;10544;22913;84905;79913;140710;56259;3929

;51230;26051;57148;58490;9675;128434;84181;60625;11122;7150;23051;63916;2139
;26013;79015;8202;11142;10955;55959;84969;90199;7529;9334;3745;284751;57580;
55905;23315;6780;10079;6790;8537;55816;4773;5203;128553;7764;655;140690;5554
4;81030;116154;55370;11047;128826;60437;1002;11083;3785;63930;4661;10014459
5 6;128414;4987;9885;55251;51750;63910;140685;57473.

Cromosoma 21:

391267;54033;388815;140578;4685;5651;29761;54072;9510;11096;351;116159;2897;
58494;54148;29104;193629;10600;54067;56245;59271;114036;54065;3455;3460;358
8;64968;54070;6526;8867;140290;7074;757;54102;9980;3753;23515;1827;54093;235
10 62;84677;3141;3772;6493;10317;25825;54014;1826;2078;150084;5121;9619;104;568
94;54058;85395;80781;23181;29947;11077;8888;114044;378832;54039;5116;5152;53
16;8568;6285;6573;54020;6612;7109;53347;7327;10785.

Cromosoma 22:

23786;128989;8318;27443;54487;84861;5594;51586;57553;5902;7332;29799;7625;27
15 81;96610;9647;27156;140883;613;23523;85379;644165;91316;440822;646074;9609;1
29049;23384;255349;157;89781;23760;23544;23331;253143;4744;4771;23481;5988;1
0740;266629;8542;254240;114904;51493;339669;150297;10369;1439;9681;8664;257
93;11020;91464;9215;4627;23543;10737;6523;8224;7533;8911;1454;27128;150350;9
402;400927;57591;23467;5435;6122;10454;50;27254;1727;64800;150365;27351;2377
20 9;9620;55007;2192;10762;29780;112885;55615;553158;25813;25771;25817;284933;3
48645;644186;386593;1375;54456;85358;85378;9889.

Cromosoma X:

158584;550643;169981;10075;23096;347365;9500;57477;28952;50943;2543;643832;
56850;89801;57232;60506;645090;286464;1756;5638;80231;2710;5165;5251;6611;38
25 9840;5160;6197;6792;4810;6247;8905;2187;4281;8481;3730;57502;416;4267;401577;
207063;8225;55344;28227;142689;55613;1896;1947;27112;9754;6567;9383;7503;340
533;158866;538;546;254065;5230;79983;340562;7552;117154;56062;286499;27328;1
730;695;2717;55998;6173;27286;441509;100302155;51186;1287;1288;2986;26280;18
6;9949;91851;1641;5063;84187;8450;57481;90293;3920;4694;7737;65109;2892;4952;
30 4068;51114;90167;2719;3251;55796;9459;2258;10479;7547;139081;158521;494119;1
39067;441525;79184;1069;2157;65991;139716;2556;100272228;4204;79057;8277;55
217;7411;7739;10838.

Cromosoma Y:

83259;5616;90665;83866;252951;6192;7544;64595;8287;22829;7404;246126;8284;9086;159163;140032;246119;83868.

Una realización aún más preferida del segundo aspecto de la invención se refiere al método donde la detección y/o cuantificación del paso (a) se realiza mediante la detección y/o cuantificación de al menos un polimorfismo genético. Una realización aún más preferida se refiere al método donde la detección y/o cuantificación del paso (a) se realiza mediante la detección y/o cuantificación de los polimorfismos de la tabla 2. En adelante nos referiremos a ellos como a los “polimorfismos de la invención”. Los polimorfismos de la invención se encuentran localizados en las regiones cromosómicas de la invención. Los polimorfismos podrían ser polimorfismos correlativos a los indicados con un intervalo de más menos 200pb.

Tabla 2. Polimorfismos de la invención: polimorfismos de nucleótido único o SNP (n=14933) identificados con las referencias de la base de datos “Single Nucleotide Polymorphism (dbSNP)” del “National Center for Biotechnology (NCBI)”

rs6650104, rs12565286, rs12082473, rs950122, rs4075116, rs9442385, rs10907175, rs2887286, rs6685064, rs1695824, rs7540231, rs3817856, rs2272908, rs12141314, rs7511905, rs2803285, rs7513222, rs3107146, rs1496555, rs2843130, rs10910060, rs2985862, rs10910093, rs6684865, rs10909872, rs729045, rs12063033, rs9782915, rs1338382, rs1109251, rs7525173, rs4648453, rs2376495, rs10492941, rs4648462, rs2455122, rs2500274, rs17399569, rs2487681, rs41495053, rs13375075, rs1128474, rs3903158, rs1181876, rs1891937, rs6663840, rs12734459, rs12079672, rs3935559, rs3856168, rs10492944, rs966321, rs12057139, rs3912752, rs619729, rs13375764, rs780983, rs10489134, rs4475768, rs12724170, rs13376005, rs1418632, rs709184, rs3913319, rs770710, rs1875037, rs17028749, rs4845825, rs806104, rs3789565, rs7531562, rs10864393, rs10489535, rs6682058, rs17361670, rs3789491, rs11801103, rs10864548, rs3007421, rs2986739, rs4908901, rs1556036, rs7549198, rs278020, rs11122119, rs17027008, rs7546903, rs41447147, rs10128022, rs7544004, rs11120896, rs11120902, rs845222, rs10492962, rs9919223, rs227163, rs7516775, rs225100, rs10489450, rs10489451, rs12093176, rs2078288, rs1107284, rs301791, rs172531, rs10492966, rs10492964, rs12024032, rs17032767, rs6577525, rs10864368, rs10489436, rs2268172, rs11121390, rs10492967, rs12405873, rs12561781, rs7413464,

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Otra realización preferida del segundo aspecto de la invención se refiere al método
donde la enfermedad genética es cáncer o una enfermedad congénita.
Preferentemente el cáncer es cáncer del sistema nervioso central, cáncer de colon,
10 cáncer de pulmón, cáncer de mama, cáncer de próstata, cáncer de vejiga, leucemia
linfática crónica, leucemia linfática aguda, mieloma múltiple o un síndrome
mielodisplásico. Más preferentemente el cáncer del sistema nervioso central es glioma
(bajo y alto grado), meningioma benigno, meningioma maligno, oligodendroglioma o
glioblastoma multiforme. Preferentemente la enfermedad congénita es síndrome de
15 Down, síndrome de Edwards o síndrome de Patau.

Una realización preferida del método primero o del método segundo de la invención se
refiere al método donde la detección de la ganancia y/o pérdida cromosómica se
realiza mediante hibridación con sondas de ácido desoxirribonucleico (ADN).
20 Preferentemente el número de sondas se sitúa entre 10.000 y 20.000 sondas
diferentes, más preferentemente el número de sondas se sitúa entre 14.000 y 15.000
sondas diferentes. En otra realización aún más preferida las sondas tienen entre 20 y
200 pares de bases (pb). Las sondas pueden ser sondas polimórficas o sondas no
polimórficas. El término “sondas polimórficas” se refiere a sondas que detectan
25 polimorfismos del tipo “single nucleotide polymorphism” o SNP. Por lo tanto, una
realización aún más preferida del método primero o del método segundo de la
invención se refiere a los métodos donde las sondas detectan al menos un
polimorfismo genético, preferiblemente detectan los polimorfismos señalados en la
tabla 2. Las sondas no polimórficas detectan material genético que no comprende
30 polimorfismos. Las sondas pueden estar repetidas.

El término “polimorfismo genético” se refiere a una variación en la secuencia de
nucleótidos de la cadena de ácido desoxirribonucleico (ADN o DNA,
“desoxirribonucleic acid”) que tiene al menos una frecuencia del 1% en los individuos

de una población. Los polimorfismos genéticos pueden ser variaciones de uno o varios nucleótidos. El polimorfismo de un solo nucleótido, “*single nucleotide polymorphism*” o SNP, generalmente da lugar a dos alelos distintos. Los SNPs o polimorfismos pueden contener información sobre la variación de un solo polimorfismo o la información de un
5 haplotipo si son SNP etiquetas o “tag-SNP”. En la presente invención los términos “polimorfismo” y “SNP” se utilizan de manera indistinta.

Los polimorfismos Tag-SNPs son polimorfismos marcadores de una región genómica que se encuentra dentro de un determinado bloque haplotípico. Cualquier variante
10 genética que se encuentre dentro del mismo bloque haplotípico que los Tag-SNPs se heredarán conjuntamente con éstos con una alta probabilidad. Es decir, los polimorfismos dentro de un bloque haplotípico están en desequilibrio de ligamiento (de Bakker, P.I.W., y cols. 2005. *Nature Genetics*, 37(11):1217-1223).

15 En la presente invención nos referimos a “haplotipo” como un conjunto de polimorfismos (SNPs) que se heredan en bloque.

Los términos “polinucleótido”, “secuencia nucleotídica”, “secuencia de nucleótidos”, “ácido nucleico” y “oligonucleótido” se usan aquí de manera intercambiable, y se
20 refieren a una forma polimérica de nucleótidos de cualquier longitud, que pueden estar, o no, química o bioquímicamente modificados.

Otra realización aún más preferida se refiere a los métodos donde las sondas se sitúan en un soporte sólido. Los soportes sólidos que se pueden utilizar son los
25 conocidos por el experto en la materia, por ejemplo vidrio, nitrocelulosa o nylon.

Con el método de la invención, se puede realizar el análisis de no sólo una única muestra, sino de varias muestras en un mismo soporte sólido debido a la menor densidad (se pueden incluir varias muestras entre 2 a 10 pacientes en un mismo
30 soporte o portaobjetos) pero manteniendo la capacidad de análisis global del genoma, siendo posible el análisis de cada muestra de forma individual. La presente invención constituye por lo tanto una mejora y una simplificación con respecto a los métodos conocidos hasta ahora.

Las sondas se pueden modificar posteriormente a su fijación al soporte sólido mediante la adición de fluorocromos o moléculas quimioluminiscentes por los métodos conocidos por cualquier experto en la materia. Por este motivo, otra realización aún más preferida del método primero y segundo de la invención se refiere a ambos
5 métodos donde las sondas están marcadas con fluorocromos, por ejemplo como biotina, estreptavidina-ficoeritrina.

El término “hibridación”, tal y como se utiliza en la presente descripción, se refiere al apareamiento de dos moléculas de ADN de cadena simple complementarias para dar
10 una molécula de doble cadena. Preferiblemente, la complementariedad es del 100%. Esto es, en la región de complementariedad cada nucleótido de una de las dos moléculas de ácido nucleico puede formar enlaces de hidrógeno con un nucleótido presente en la otra molécula de ácido nucleico. Sin embargo, aquéllos con una experiencia normal en el campo reconocerán que dos moléculas de ácido nucleico que
15 posean una región con complementariedad menor al 100% también pueden hibridar.

El término “nucleótido”, tal y como se utiliza en la presente descripción se refiere a un molécula orgánica formada por la unión covalente de una pentosa, una base nitrogenada y un grupo fosfato. El término nucleótido incluye desoxirribonucleósidos
20 trifosfato como, por ejemplo, pero sin limitarse, dATP, dCTP, dITP, dUTP, dGTP, dTTP, o derivados de los mismos. El término nucleótido incluye también dideoxirribonucleósidos trifosfato (ddNTPs), como por ejemplo, ddATP, ddCTP, ddGTP, ddITP, ddTTP o derivados de los mismos.

25 De acuerdo con la presente invención un “nucleótido” puede ser marcado o etiquetado mediante técnicas bien conocidas en el estado de la técnica. Etiquetas detectables incluyen, por ejemplo, isótopos radiactivos, etiquetas fluorescentes, etiquetas quimioluminiscentes, etiquetas bioluminiscentes o etiquetas enzimáticas.

30 Las sondas deben respetar las posiciones físicas que se muestran la tabla 3 en cada cromosoma, con un intervalo de $\pm$ entre 80 a 200 pares de bases (es decir que la distancia entre una sonda y otra similar es de 100 pares de bases). Las posiciones físicas se señalan a continuación (“Cr” se refiere a “cromosoma”):

Tabla 3. Posiciones físicas que deben cubrir las sondas empleadas en el *array* con un intervalo de más menos 200pb (Cr.: cromosoma).

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7714986,7835840,7891188,7963031,8046731,8214827,8334875,8360818,8502236,8
558969,8667179,8906357,9131385,9170545,9171683,13887941,13939607,13992338
,14001232,14031334,14197867,14197977,14288981,14345705,14416216,14577177,
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15 5472863,15983014,16301305,16578072,16699334,16713318,16773870,17495914,17
559652,17570599,18561042,18578476,18596847,19349615,19372700,19443641,195
63894,20804835,20834703,20837553,21086211,21528257,21604901,21674068,2172
2098,21730257,21872738,22072340,22672031,22725379,22746786,22928344,23473
201,23631629,23749182,23984056,24069943,24069946,24070105,24359931,244019
20 40,24475669,

Otra realización aún más preferida del método primero y segundo de la invención se
refiere a ambos métodos donde la muestra se selecciona de la lista que comprende
tejido, líquido cefalorraquídeo, mucosa oral, sangre, plasma, suero, linfa, líquido
25 pleural y orina.

Otra realización aún más preferida del método primero y segundo de la invención se
refiere a ambos métodos donde el sujeto es un humano.

30 Un tercer aspecto de la invención se refiere al uso *in vitro* del número de copias de las
regiones cromosómicas de la invención como marcador diagnóstico o pronóstico de
enfermedades genéticas o para el diseño de un tratamiento personalizado en un sujeto
con una enfermedad genética. Preferentemente, la enfermedad genética es cáncer o
una enfermedad congénita. Más preferentemente, el cáncer es cáncer del sistema

nervioso central, cáncer de colon, cáncer de pulmón, cáncer de mama, cáncer de próstata, cáncer de vejiga, leucemia linfática crónica, leucemia linfática aguda, mieloma múltiple o un síndrome mielodisplásico. Aún más preferentemente, el cáncer del sistema nerviosos central es glioma (bajo y alto grado), meningioma benigno, 5 meningioma maligno, oligodendroglioma o glioblastoma multiforme. Preferentemente, la enfermedad congénita es síndrome de Down, síndrome de Edwards o síndrome de Patau.

Un cuarto aspecto de la invención se refiere a un *array* que comprende sondas que 10 detectan ganancias y/o pérdidas de las regiones cromosómicas de la invención donde el número de dichas sondas diferentes se sitúa entre 10.000 y 20.000. Preferentemente, el número de las sondas diferentes se sitúa entre 14.000 y 15.000. Una realización más preferida se refiere al *array* donde las sondas se sitúan en un soporte sólido. El soporte sólido se refiere a cualquier soporte sólido conocido por el 15 experto en la materia, por ejemplo de vidrio, nitrocelulosa o nylon; puede referirse, por ejemplo, a un portaobjetos de laboratorio. Las sondas pueden ser sondas polimórficas o no polimórficas. Las sondas polimórficas también pueden ser sondas que detectan polimorfismos que están en el mismo bloque haplotípico. Una realización más preferida del cuarto aspecto de la invención se refiere a un *array* donde las sondas 20 detectan los genes descritos en la tabla 1. Otra realización más preferida del cuarto aspecto de la invención se refiere al *array* donde las sondas detectan los polimorfismos descritos en la tabla 2. Los polimorfismos podrían ser polimorfismos correlativos a los indicados con un intervalo de más menos 200pb. Las sondas pueden estar repetidas en el *array*. El *array* del cuarto aspecto de la invención es un *array* de 25 oligonucleótidos.

Un quinto aspecto de la invención se refiere al uso del *array* del cuarto aspecto de la invención para el diagnóstico o pronóstico de enfermedades genéticas o para el diseño de un tratamiento personalizado en un sujeto con una enfermedad genética. 30 Preferentemente la enfermedad genética es cáncer o una enfermedad congénita. El cáncer, preferentemente es cáncer del sistema nervioso central, cáncer de colon, cáncer de pulmón, cáncer de mama, cáncer de próstata, cáncer de vejiga, leucemia linfática crónica, leucemia linfática aguda, mieloma múltiple o un síndrome mielodisplásico. El cáncer del sistema nervioso central es preferentemente glioma

(bajo o alto grado), meningioma benigno, meningioma maligno, oligodendroglioma o glioblastoma multiforme. La enfermedad congénita es preferentemente síndrome de Down, síndrome de Edwards o síndrome de Patau.

- 5 A lo largo de la descripción y las reivindicaciones la palabra "comprende" y sus variantes no pretenden excluir otras características técnicas, aditivos, componentes o pasos. Para los expertos en la materia, otros objetos, ventajas y características de la invención se desprenderán en parte de la descripción y en parte de la práctica de la invención. Los siguientes ejemplos y figuras se proporcionan a modo de ilustración, y
10 no se pretende que sean limitativos de la presente invención.

DESCRIPCIÓN DE LAS FIGURAS

- Fig. 1.** Ideograma de cada cromosoma con el número de sondas totales de la invención en la parte superior y el número de sondas en cada banda cromosómica.
15

- Fig. 2.** Esquema del proceso de selección de las sondas para el *array* diseñado que incluye sondas que identifican los distintos segmentos afectados: A) con delección homocigota y amplificación génica (densidad elevada de sondas en dichas zonas marcadas con círculos). B) sondas en las zonas de delección heterocigota y/o ganancias recurrentes y C) sondas en las regiones proximales y distales de cada brazo cromosómico. Las flechas indican a modo de ejemplo regiones afectadas por alteraciones en el número de copias en distintos puntos a lo largo de ambos brazos cromosómicos. Las líneas paralelas en los extremos del ideograma de cada cromosoma y en la zona centromérica indican que se excluyeron dichas zonas.
20
25

- Fig. 3.** Detección de alteraciones que afectan a diferentes cromosomas en un meningioma realizado con el método de la invención: pérdidas completas de los cromosomas 11 (A) y 22 (B), pérdidas de un brazo de los cromosomas 1 (C) y 7 (D), y pérdida parcial en 18q (E).
30

- Fig. 4.** Detección de alteraciones heterogéneas del cromosoma 22 en meningiomas. En el panel A, pérdida del cromosoma 22 entero (monosomía 22) que puede aparecer como una pérdida aislada, o en otros meningiomas aparece junto alteraciones de

varios cromosomas. En el Panel B, una pequeña ganancia a nivel de 22q (marcada con un rectángulo), y los paneles C, D y E muestran deleciones de gran tamaño y pequeños segmentos a nivel de 22q con dos copias (marcadas con un círculo).

- 5 **Fig. 5.** Detección de pérdidas homocigotas del gen CDKN2A en glioblastomas multiformes en el cromosoma 9p indicadas con flechas.

Fig. 6. Detección de amplificaciones (AMP) génicas en gliomas. Panel A: muestra AMP de EGFR sin ganancias del cromosoma 7. Panel B: AMP de EGFR junto a ganancia de cromosoma 7. En el Panel C: la AMP de EGFR aparece con ganancia de 7q. Panel D: presenta ganancias intersticiales a lo largo del cr7 junto AMP EGFR. Panel E: varias amplificaciones a nivel de 7p y ganancia del cromosoma 7 y panel F: muestra ganancias del cromosoma 7, sin AMP de EGFR y una AMP a nivel de 7q.

- 15 **Fig. 7.** Detección de patrones típicos de ganancias y pérdidas en los cromosomas 7, 9 y 10 en glioblastoma multiforme. Ganancias del cromosoma 7 sin amplificación del gen EGFR (paneles A, B, C y D) y con amplificación (paneles E y F) junto a deleciones del brazo corto del cromosoma 9 y pérdidas a nivel del cromosoma 10 (observadas en todos los paneles).

20

- Fig. 8.** Detalles de la heterogeneidad de los cromosomas 7 (columna A), 9 (columna B) y 10 (columna C) en glioblastomas, con ganancias a nivel del cromosoma 7 (con dos subgrupos de tumores: los que no amplifican EGFR –subpaneles 1 al 4- y los que si presentan amplificación génica -paneles 5 y 6-), deleciones a nivel de 9p y pérdidas a nivel del cromosoma 10 (generalmente monosomía 10).

25

Fig. 9. Esquema de los pasos seguidos en el diseño de la invención.

- Fig. 10.** Comparación de los perfiles del cromosoma 7 con *arrays* comerciales frente al array de la invención. A, C, array de la invención; B, array comercial GeneChip Human Mapping 500K set de Affymetrix; D, Genome-Wide Human SNP Array 6.0 de Affymetrix. Detección de una deleción parcial del cromosoma 7 (A y B). Detección de una ganancia del cromosoma 7 junto amplificación del gen EGFR (C y D)

30

EJEMPLO

A continuación se ilustrará la invención mediante unos ensayos realizados por el inventor, que demuestran la eficacia del método de la invención la identificación de
 5 alteraciones en el número de copias en los 24 cromosomas

Ejemplo 1: Obtención del método de la invención de detección de cambio de número de copias en todos los cromosomas en tumores del sistema nervioso central.

10

Se estableció el número de copias y los perfiles de cada cromosoma (Figura 1 y 2) en tumores del sistema nervioso central, meningiomas benignos y malignos, y glioblastomas multiformes (GBM) detectando las pérdidas (Figuras 3 y 4) de material genético de distintos tamaños que van desde la afectación de la totalidad de un
 15 cromosoma, uno de sus brazos afectados, o deleciones intersticiales, así como información de genes afectados por deleciones (homocigotas y/o heterocigotas) (Figura 5), amplificaciones génicas (Figura 6) y ganancias (Figuras 7 y 8).

Se fijaron las posiciones físicas para los 24 cromosomas, utilizando las regiones
 20 distales y proximales de ambos brazos de cada cromosoma excluyendo las regiones pericentroméricas y microsatélites que permiten detectar monosomias y poliploidias que afectan a un cromosoma completo o a un brazo entero (brazo p o q del cromosoma) (Figura 2).

25 Para las pérdidas y ganancias intersticiales de gran tamaño (que afectan a la mitad del brazo, a una cuarta parte, o a tres cuartas partes de cada brazo del cromosoma se identifican en cada brazo las zonas afectadas y se utilizan sondas en esas posiciones físicas), así como en las regiones (perdidas y/o ganadas) de tamaño pequeño, regiones comunes y recurrentes detectadas en cada cromosoma que han sido
 30 identificadas en diversos tipos de tumores.

Las regiones de amplificación génica y deleción homocigota llevan una mayor cobertura, aumentando la densidad de las sondas en las posiciones físicas que ocupan y cubriendo los distintos tamaños de estas alteraciones (Figura 2).

El *array* se estabiliza con sondas en todas las bandas cromosómicas (pueden ser varias sondas). Se seleccionaron 14.933 sondas repartidas en los 24 cromosomas, que son suficientes para identificar las alteraciones del número de copias (Tabla 4)

5

Tabla 4. Número de sondas utilizadas en el nuevo *array* objeto de la invención y su distribución en los 24 cromosomas comparado con dos *arrays* comerciales. (--) significa que no hay sondas para ese cromosoma o ADN y MT significa mitocondrial.

Cromosoma	Nuevo <i>array</i>		<i>Arrays</i> comerciales	
	Secuencias	Genes	Secuencias <i>Genome-Wide Human SNP Array 6.0</i>	Secuencias <i>GeneChip Human Mapping 500K</i>
1	1519	459	71.312	40.203
2	890	260	73.937	41.395
3	848	214	60.685	33.792
4	675	204	55.995	32.316
5	611	168	56.416	32.053
6	665	170	56.271	31.466
7	786	175	47.056	25.830
8	494	113	48.608	27.454
9	1045	192	41.442	22.859
10	683	172	48.195	28.497
11	624	150	44.539	26.269
12	857	205	42.555	24.948
13	472	71	34.283	19.184
14	504	106	28.065	15.717
15	510	143	26.074	14.349
16	428	156	27.716	15.309
17	666	207	20.658	11.277
18	518	92	26.529	14.880
19	430	210	11.929	6.396
20	480	137	22.843	12.398
21	290	76	12.579	7.124
22	324	99	11.537	6.206
X	511	123	36.886	10.525
MT	--	--	119	--
TOTAL	14.933	3920	906.487	500.447

10

El *array* se compone de un total de 14933 sondas de las cuales 7201 son secuencias en el interior de 3920 genes.

Las sondas comerciales que se utilizaron se señalan a continuación (tabla 5):

	SNP_A-8575395,	SNP_A-8709646,	SNP_A-8497791,	SNP_A-1677174,
	SNP_A-2116190,	SNP_A-4291020,	SNP_A-1902458,	SNP_A-2131660,
	SNP_A-4277872,	SNP_A-2118217,	SNP_A-1884606,	SNP_A-2169457,
5	SNP_A-2235839,	SNP_A-2218153,	SNP_A-2223642,	SNP_A-1799863,
	SNP_A-1908463,	SNP_A-1955996,	SNP_A-1718890,	SNP_A-2082194,
	SNP_A-2135694,	SNP_A-1789472,	SNP_A-1934233,	SNP_A-4207031,
	SNP_A-2179242,	SNP_A-1783583,	SNP_A-2115409,	SNP_A-2116785,
	SNP_A-1678466,	SNP_A-1681384,	SNP_A-1879486,	SNP_A-1897613,
10	SNP_A-1668776,	SNP_A-1642581,	SNP_A-1723597,	SNP_A-1718237,
	SNP_A-1828771,	SNP_A-1895403,	SNP_A-1930492,	SNP_A-2237566,
	SNP_A-2258506,	SNP_A-1701872,	SNP_A-1930718,	SNP_A-1705537,
	SNP_A-2278622,	SNP_A-1960639,	SNP_A-2022696,	SNP_A-2262266,
	SNP_A-1670878,	SNP_A-2212837,	SNP_A-1646481,	SNP_A-1666738,
15	SNP_A-2181437,	SNP_A-1686722,	SNP_A-1705703,	SNP_A-4282340,
	SNP_A-1749896,	SNP_A-1714480,	SNP_A-1667950,	SNP_A-2129718,
	SNP_A-2175833,	SNP_A-1745466,	SNP_A-1739955,	SNP_A-1743804,
	SNP_A-1952660,	SNP_A-2223437,	SNP_A-2224099,	SNP_A-2170486,
	SNP_A-1743594,	SNP_A-1676349,	SNP_A-2058862,	SNP_A-4224138,
20	SNP_A-1656665,	SNP_A-2050345,	SNP_A-1873575,	SNP_A-1673751,
	SNP_A-1879594,	SNP_A-2100113,	SNP_A-1796045,	SNP_A-2220372,
	SNP_A-4242443,	SNP_A-2243080,	SNP_A-1842009,	SNP_A-1699284,
	SNP_A-1891001,	SNP_A-1924336,	SNP_A-1643131,	SNP_A-4278685,
	SNP_A-8470497,	SNP_A-1818172,	SNP_A-4250097,	SNP_A-2207883,
25	SNP_A-1868600,	SNP_A-1684527,	SNP_A-2165491,	SNP_A-1666985,
	SNP_A-2185717,	SNP_A-2251186,	SNP_A-1652193,	SNP_A-4230374,
	SNP_A-4194252,	SNP_A-1683825,	SNP_A-1691698,	SNP_A-1687843,
	SNP_A-1687400,	SNP_A-1671386,	SNP_A-1671445,	SNP_A-1808066,
	SNP_A-4274938,	SNP_A-2167266,	SNP_A-2095667,	SNP_A-1711211,
30	SNP_A-1711739,	SNP_A-2300181,	SNP_A-1681341,	SNP_A-2113625,
	SNP_A-2111308,	SNP_A-1881321,	SNP_A-2057029,	SNP_A-2042477,
	SNP_A-2314431,	SNP_A-2236812,	SNP_A-1750171,	SNP_A-2188347,
	SNP_A-1661146,	SNP_A-2233462,	SNP_A-2287787,	SNP_A-2187559,
	SNP_A-1718632,	SNP_A-1929772,	SNP_A-1726085,	SNP_A-1948295,

	SNP_A-1654229,	SNP_A-2255111,	SNP_A-2185703,	SNP_A-1686500,
	SNP_A-1717751,	SNP_A-1697953,	SNP_A-1664654,	SNP_A-1756835,
	SNP_A-2298894,	SNP_A-2176916,	SNP_A-2025605,	SNP_A-1852210,
	SNP_A-1945628,	SNP_A-2151539,	SNP_A-2259810,	SNP_A-1813299,
5	SNP_A-1959830,	SNP_A-2263001,	SNP_A-2173869,	SNP_A-2300283,
	SNP_A-1646710,	SNP_A-1782600,	SNP_A-1960638,	SNP_A-1781313,
	SNP_A-2121430,	SNP_A-8298421,	SNP_A-1754728,	SNP_A-2115002,
	SNP_A-1799366,	SNP_A-1706913,	SNP_A-2250990,	SNP_A-1815621,
	SNP_A-4300773,	SNP_A-1909176,	SNP_A-1677240,	SNP_A-4194375,
10	SNP_A-4207490,	SNP_A-8367856,	SNP_A-4196950,	SNP_A-1798804,
	SNP_A-8443077,	SNP_A-2264437,	SNP_A-1845603,	SNP_A-1939799,
	SNP_A-2059135,	SNP_A-1895449,	SNP_A-2218894,	SNP_A-1652320,
	SNP_A-1963782,	SNP_A-1682449,	SNP_A-1723002,	SNP_A-2312971,
	SNP_A-1693483,	SNP_A-1964871,	SNP_A-1999893,	SNP_A-1687849,
15	SNP_A-1937874,	SNP_A-1693165,	SNP_A-1795578,	SNP_A-1841240,
	SNP_A-1683515,	SNP_A-1726304,	SNP_A-1744558,	SNP_A-1705081,
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	SNP_A-1801052,	SNP_A-2048263,	SNP_A-1691431,	SNP_A-1665880,
	SNP_A-1856143,	SNP_A-1872931,	SNP_A-1734347,	SNP_A-1723341,
20	SNP_A-1726821,	SNP_A-1697551,	SNP_A-1726011,	SNP_A-1673750,
	SNP_A-1895297,	SNP_A-4282720,	SNP_A-1741853,	SNP_A-1712561,
	SNP_A-1669999,	SNP_A-1674383,	SNP_A-1673838,	SNP_A-1752247,
	SNP_A-1749256,	SNP_A-1673926,	SNP_A-1709273,	SNP_A-1655289,
	SNP_A-1723021,	SNP_A-1728312,	SNP_A-1666311,	SNP_A-1646335,
25	SNP_A-1703512,	SNP_A-1728267,	SNP_A-1757692,	SNP_A-1650897,
	SNP_A-1678616,	SNP_A-1755243,	SNP_A-1662223,	SNP_A-4236362,
	SNP_A-2235940,	SNP_A-2037804,	SNP_A-2300090,	SNP_A-1915583,
	SNP_A-2121853,	SNP_A-1892255,	SNP_A-4273111,	SNP_A-1653089,
	SNP_A-4211081,	SNP_A-1660258,	SNP_A-1701699,	SNP_A-1715345,
30	SNP_A-1699617,	SNP_A-1745139,	SNP_A-4251587,	SNP_A-8291532,
	SNP_A-1746524,	SNP_A-4202677,	SNP_A-2236414,	SNP_A-4211511,
	SNP_A-1731929,	SNP_A-1792267,	SNP_A-4295032,	SNP_A-1819207,
	SNP_A-1757353,	SNP_A-2186095,	SNP_A-2308314,	SNP_A-1708186,
	SNP_A-2039420,	SNP_A-1652606,	SNP_A-2128216,	SNP_A-2213242,

	SNP_A-2163486,	SNP_A-1704403,	SNP_A-1697051,	SNP_A-1740568,
	SNP_A-1678809,	SNP_A-2069878,	SNP_A-1657323,	SNP_A-1665257,
	SNP_A-2125613,	SNP_A-2282881,	SNP_A-1658232,	SNP_A-1999950,
	SNP_A-1758455,	SNP_A-1718217,	SNP_A-1657166,	SNP_A-1680027,
5	SNP_A-1642665,	SNP_A-1687098,	SNP_A-8659701,	SNP_A-1643473,
	SNP_A-8572253,	SNP_A-8526670,	SNP_A-8531792,	SNP_A-1958834,
	SNP_A-1934733,	SNP_A-2051598,	SNP_A-4238296,	SNP_A-4226678,
	SNP_A-2300840,	SNP_A-1993283,	SNP_A-1652712,	SNP_A-1669337,
	SNP_A-1671442,	SNP_A-2200991,	SNP_A-2255710,	SNP_A-2228635,
10	SNP_A-1920964,	SNP_A-4213904,	SNP_A-2247903,	SNP_A-1999970,
	SNP_A-2212290,	SNP_A-4283138,	SNP_A-1720070,	SNP_A-1722275,
	SNP_A-1725943,	SNP_A-1893286,	SNP_A-1658147,	SNP_A-1997445,
	SNP_A-1666641,	SNP_A-1831518,	SNP_A-2116452,	SNP_A-1698274,
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	SNP_A-1684602,	SNP_A-1659931,	SNP_A-1749045,	SNP_A-4194567,
	SNP_A-1701720,	SNP_A-1752726,	SNP_A-1655991,	SNP_A-2105042,
	SNP_A-2067488,	SNP_A-4224911,	SNP_A-1750225,	SNP_A-1682872,
	SNP_A-1707710,	SNP_A-1751754,	SNP_A-1741706,	SNP_A-1752286,
20	SNP_A-1683393,	SNP_A-1757722,	SNP_A-4249452,	SNP_A-1688881,
	SNP_A-2224354,	SNP_A-2121225,	SNP_A-1744850,	SNP_A-1752120,
	SNP_A-2118379,	SNP_A-2069002,	SNP_A-1704069,	SNP_A-1673699,
	SNP_A-2064092,	SNP_A-1680369,	SNP_A-1706077,	SNP_A-1672650,
	SNP_A-4257891,	SNP_A-4251958,	SNP_A-1680196,	SNP_A-1691785,
25	SNP_A-1661175,	SNP_A-1705109,	SNP_A-1664811,	SNP_A-1668237,
	SNP_A-1724643,	SNP_A-1660594,	SNP_A-1749956,	SNP_A-1735403,
	SNP_A-1743516,	SNP_A-1725807,	SNP_A-2036213,	SNP_A-2003050,
	SNP_A-2260782,	SNP_A-4230632,	SNP_A-2252959,	SNP_A-2163195,
	SNP_A-1846284,	SNP_A-2216152,	SNP_A-1793126,	SNP_A-2299988,
30	SNP_A-1887977,	SNP_A-4214300,	SNP_A-4229541,	SNP_A-2109120,
	SNP_A-1870994,	SNP_A-1729743,	SNP_A-1663743,	SNP_A-1654542,
	SNP_A-1700078,	SNP_A-1753902,	SNP_A-1702410,	SNP_A-1658183,
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	SNP_A-8383167,	SNP_A-8713092,	SNP_A-8566304,	SNP_A-8589367,
	SNP_A-8290007,	SNP_A-8372352,	SNP_A-8611867,	SNP_A-8511719,
	SNP_A-8330416,	SNP_A-8627092,	SNP_A-8290012,	SNP_A-8308145,
	SNP_A-8537348,	SNP_A-8535072,	SNP_A-8333138,	SNP_A-8676343,
15	SNP_A-8581506,	SNP_A-8536948,	SNP_A-8632067,	SNP_A-8531760,
	SNP_A-8293961,	SNP_A-8498269,	SNP_A-8584255,	SNP_A-8640091,
	SNP_A-8396630,	SNP_A-8550103,	SNP_A-8570506,	SNP_A-8634599,
	SNP_A-8290019,	SNP_A-8707909,	SNP_A-8640704,	SNP_A-8507820,
	SNP_A-8487177,	SNP_A-8290264,	SNP_A-8633789,	SNP_A-8290267,
20	SNP_A-8708617,	SNP_A-8642739,	SNP_A-8607809,	SNP_A-8418205,
	SNP_A-8688109,	SNP_A-8290277,	SNP_A-8290278,	SNP_A-8705225,
	SNP_A-8653199,	SNP_A-8417266,	SNP_A-8417375,	SNP_A-8295972,
	SNP_A-8439392,	SNP_A-8372528,	SNP_A-8332143,	SNP_A-8400249,
	SNP_A-8352188,	SNP_A-8486463,	SNP_A-8550815,	SNP_A-8369683,
25	SNP_A-8348747,	SNP_A-8290283,	SNP_A-8521142,	SNP_A-8346444,
	SNP_A-8474444,	SNP_A-8407770,	SNP_A-8290290,	SNP_A-8525311,
	SNP_A-8539904,	SNP_A-8303472,	SNP_A-8390603,	SNP_A-8290291,
	SNP_A-8290292,	SNP_A-8290293,	SNP_A-8371607,	SNP_A-8635714,
	SNP_A-8365189.			

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Se compararon los perfiles obtenidos en cada cromosoma (Figura 1 y 2) con *arrays* comerciales en series amplias de muestras (Tabla 6), tanto de nuestro laboratorio como de *arrays* de otros grupos, observándose la identificación de las alteraciones numéricas detectadas (Figura 3 y 4 número de copias analizadas en muestras de

meningiomas y análisis de muestras de gliomas y perfiles con el número de copias de diversos cromosomas, Figuras 5, 6, 7 y 8).

Tabla 6. Series de *arrays* analizados para establecer el contenido de nuestra invención (donde: ND es no depositado; Glioma (incluye bajo y alto grado), GBM es glioblastoma, SMD es síndrome mielodisplásico, LLC es leucemia linfática crónica, LLA: leucemia linfoblástica aguda y MM: mieloma múltiple).

PATOLOGIA	BASE INTERN.	ARRAY	MUESTRAS	REFERENCIA
1 Meningioma	ND	500K 6.0SNP	50&7	Tabernero <i>et al.</i> Genes Chromosome&Cancer 2012,51:606
2 Meningioma	GSE16583	100K	82	Lee Y <i>et al.</i> Brain Pathol 2010,20(4):751
3 GBM	ND	500K 6.0SNP	35&26	Crespo <i>et al.</i> J Mol Diagn 2011,13,6:634. Plos One 2012,7(9):e46088
4 Glioma	ND	500K	28	Bralten LB <i>et al.</i> Genes Chromosome&Cancer 2010,49:509
5 GBM	GSE19612	250K	24	Chen R <i>et al.</i> Cancer Cell 2010,17:362
6 GBM	GSE19399	250K	19	Beroukhir R <i>et al.</i> Nature 2010,463:899
7 GBM	GSE14804	50K	12	Hodgson JG <i>et al.</i> NeuroOncology 2009,11(5):477
8 GBM	E-MEXP-1330	50K 500K	19&5	Yin D <i>et al.</i> Mol Cancer Res 2009,7:665
9 GBM	GSE10922	50K	14	Kuga D <i>et al.</i> NeuroOncology 2008,10(6):995
10 GBM	GSE13021	250K	30	Solomon DA <i>et al.</i> Cancer Res 2008,68(24):10300
12 Colon	GSE16125	250K	41	Reid JF <i>et al.</i> Genes Chromosome&Cancer 2009,48(11):953
13 Pulmón	GSE 34140	250K	17	Huang Y <i>et al.</i>
14 Mama	GSE7545	500K	15	Haverty PM <i>et al.</i> ChromosomesCancer 2008,47(6):530
15 Próstata	GSE18333	6.0SNP	5	Mao X <i>et al.</i>

				Cancer Res 2010,70(13):5207
16 SMD	GSE19399	250K	6	Kilpivaara O <i>et al.</i> NatGenet 2009,41: 455
17 LLC	GSE16406	250K	164	Gunnarsson R <i>et al.</i> Leukemia 2010,24(1):211
18 LLA	GSE20353/ GSE9611	250K 500k	2/25	Kuiper RP <i>et al.</i> / Paulsson K <i>et al.</i> Leukemia 2010, 24(7): 1258/ProcNatlAcadSci 2008,105(18):6708
19 MM	GSE31339	6.0SNP	34	Sarasquete M <i>et al.</i>

Base Intern.: Base internacional

MATERIALES Y MÉTODOS

- 5 El análisis del número de copias de muestras tumorales para conocer las alteraciones genéticas se realizó con ADN genómico (250ng) hibridado sobre *arrays* comerciales de la casa Affymetrix (Affymetrix Inc, Santa Clara, CA, USA) en dos series de enfermos de nuestro laboratorio: 50 pacientes con meningiomas y 35 glioblastomas con *GeneChip Human Mapping 500K Array Set* y 7 meningiomas y 26 GBM con
- 10 *Genome-Wide Human SNP 6.0 Array*. Además se analizó series de *arrays* de otros autores todos ellos con un porcentaje de llamadas mayor al 92%: 82 meningiomas (GSE 16583, que suman junto a nuestra serie un total de 139 *arrays* analizados), 8 series con gliomas (bajo y alto grado) (212 *arrays*), 78 *arrays* de 4 series de tumores sólidos (colon, pulmón, mama y próstata) y 231 tumores hematológicos: síndrome
- 15 mielodisplásico (SMD), leucemia linfática crónica (LLC), leucemia linfoblástica aguda (LLA) y mieloma múltiple (MM) (Tabla 6), depositados en repositorios públicos [*Gene Expression Omnibus* (GEO) del *National Center of Biotechnology*, USA y *ArrayExpress Archive* del *European Molecular Biology Laboratory* (EMBL) & *European Bioinformatics Institute* (EBI), *Wellcome Trust Genome Campus*, Hinxton, UK].
- 20 Los *arrays* utilizados (Affymetrix) y sus números de catálogo fueron: el *GeneChip® Mapping 100K Set* que contiene 100.000 sondas distribuidas en dos *arrays*, 520043 *GeneChip Human Mapping 50K Array Xba* y el 520044 *GeneChip Human Mapping 50K Array Hind*. El *GeneChip Human Mapping 500K Array Set* con información de
- 25 >500.000 sondas distribuidas en dos chips el *GeneChip Human Mapping 250K Nsp Array* referencia 900767 y 520331 *GeneChip Human Mapping 250K Sty Array* y

Genome-Wide Human SNP Array 6.0 con referencia 901182 que contiene más de 1,8 millones de sondas polimórficas y no polimórficas.

Los *arrays* se realizaron según el protocolo recomendado por el fabricante y conocido por el experto en la materia. Tras la hibridación, se lavaron los chips, se marcaron con biotina (*GeneChip® DNA Labeling Reagent*), se escanearon usando GeneChip Scanner 3000 y 3000 G7 (Affymetrix Inc.) y se generaron los ficheros (.CEL que contienen la intensidad de cada sonda) con los programas *Affymetrix® GeneChip® Operating Software* (GCOS) y *Affymetrix Command Console® Software* (AGCC).

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Los programas informáticos utilizados para el análisis fueron: *Genotyping Console software* (versión 4.1.1.834; Affymetrix Inc.) y *DNA-Chip Analyzer (dChip) software 2010*, del Dana Farber Institute, Harvard, MA, USA para el cálculo de los valores del número de copias y su representación de acuerdo a la localización cromosómica. Los datos para cada sonda fueron extraídos del archivo CEL y convertidos en archivos CHP conocido por el experto en la materia. Se utilizaron los siguientes puntos de corte: para la pérdida de material genético valores $\leq 1,30$ y para las ganancias cromosómicas valores $\geq 2,50$. Las amplificaciones y deleciones homocigotas se establecieron cuando el número de copias era >5 para la amplificación génica y $<0,8$ copias para la deleción homocigota. En la construcción de los perfiles del número de copias y los idiogramas de cada cromosoma también se utilizó la versión 2.13.1 del programa R (software libre y lenguaje de programación para análisis estadístico y gráfico con comandos escritos ejecutados directamente y funciones estadísticas específicas para el análisis de *arrays*, contenidas dichas funciones en las llamadas librerías) y las herramientas de Bioconductor 2.8, ambos software libres y disponibles en la web. Se estableció el número de copias de cada cromosoma en los 139 *arrays* con meningiomas, 212 *arrays* con gliomas, 78 *arrays* de otros tumores sólidos y 231 tumores hematológicos (Tabla 6), detectando el tipo y extensión de las pérdidas y ganancias de material genético que afectaban a la totalidad de un cromosoma, uno de sus brazos, o deleciones intersticiales (homocigotas y/o heterocigotas) así como amplificaciones génicas.

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Para identificar el menor número de sondas necesarias para nuestra invención, se utilizó el programa *Copy Number Analyzer for Genechip (CNAG) 3.3.0.1(Beta)*,

Genome Laboratory, Universidad de Tokio-Japón siguiendo los siguientes pasos (Figura 9):

- 1)- Se localizaron todas las alteraciones y su extensión en cada cromosoma comenzando por las amplificaciones génicas y las deleciones homocigotas, seguidamente las deleciones heterocigotas y cualquier ganancia o pérdida intersticial y sus posiciones concretas en las regiones implicadas en enfermedades diferentes (Figura 2 y Tabla 6). Se seleccionaron las posiciones más distales de las regiones afectadas y los distintos tamaños de cada alteración para cubrir con sondas todas las regiones alteradas, que se localizaban dentro de genes y se evitaron las sondas redundantes. Las herramientas del programa nos muestran las posiciones físicas (inicio y fin) y las sondas implicadas.
- 2)- Se eliminaron sondas sin información incluidas en los *arrays* (sondas sin información de la posición física en ese cromosoma o sin gen asignado).
- 3)- Se eliminaron sondas de las regiones teloméricas y pericentroméricas para evitar hibridación cruzada entre los cromosomas 1, 5 y 19; el cromosoma 13 con el cromosoma 21; y el cromosoma 14 con el cromosoma 22.
- 4)- Se eliminaron las sondas con número de copias 2 (sondas sin alteraciones), como no informativas.
- 5)- Se seleccionaron sondas en todas las bandas cromosómicas para completar el perfil del cromosoma en toda su extensión.
- 6)- Finalmente, se confirmaron los resultados del *array* nuevo diseñado con representaciones gráficas de los ideogramas de cada cromosoma comparando las sondas del nuevo diseño versus los dos *arrays* de alta densidad (más de 500.00 sondas) utilizados. Para ello se utilizó el programa R, versión 2.13.1 y la librería “quantsmooth” (librerías o paquete de funciones para el análisis y visualización de los idiogramas y las copias de cada sonda a lo largo de los cromosomas) disponible en el repositorio de Bioconductor que permite visualizar los datos del número de copias de forma gráfica en los cromosomas.

RESULTADOS

Los resultados mostraron que el método de la invención aún teniendo menor número
 5 de sondas que los *arrays* comerciales de alta densidad, muestra igual resultado
 (detectamos las mismas alteraciones numéricas, ganancias y pérdidas de distintos
 tipos y extensión en los tumores recogidos en la tabla 6). Se identificaron las
 aneuploidías de cada cromosoma (cambios en el número de cromosomas) que
 afectaban a cromosomas completos monosomias y trisomías. Hemos establecido las
 10 alteraciones recurrentes, definido sus características y establecido su impacto en el
 pronóstico tumoral. Encontramos que las monosomias, deleciones y ganancias
 aparecen en tumores benignos y malignos localizándose en los cromosomas 22, 1, 14,
 6, 7 y 18 en los meningiomas y en los gliomas son más frecuentes las ganancias del
 cromosoma 7 con o sin amplificación del gen EGFR (existen 2 grupos de tumores
 15 según presenten o no amplificación de este gen), con pérdidas simultaneas a nivel de
 9p (homocigotas y heterocigotas) y pérdidas de cromosoma 10, alteraciones
 raramente encontradas en gliomas benignos; así como deleciones heterocigotas y
 otras amplificaciones génicas de distintas extensiones y localizaciones cromosómicas
 tanto en tumores sólidos como en neoplasias hematológicas, habiéndose conseguido
 20 así un método más simple y optimizado que mantiene la especificidad y que resulta
 informativo para los 24 cromosomas de forma simultánea. Nuestros resultados avalan
 el método de la invención en diversas enfermedades genéticas: tumores del sistema
 nervioso, así como en otros tumores sólidos y hematológicos. La comparación del
array específico diseñado *versus arrays* de alta densidad muestra todas las
 25 alteraciones encontradas con *arrays* de mayor tamaño (Figura 10).

El análisis de varias series de tumores permitió identificar sus alteraciones genéticas,
 confirmando los grupos de alteraciones, sus posiciones físicas (especialmente
 aquellas que son recurrentes), así como los genes que contienen, por tanto los
 30 cambios numéricos (pérdidas y ganancias) serían útiles en la clasificación de estas
 patologías. El *array* se diseñó con el número mínimo de secuencias sin sondas
 redundantes, evitado las sondas situadas en regiones pericentroméricas y en los
 extremos cromosómicos y se registró la marca "FullChromaArray"® para nuestro
 diseño.

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REIVINDICACIONES

1. Método *in vitro* de obtención de datos útiles para la detección y/o cuantificación de al menos una ganancia y/o pérdida cromosómica en una muestra aislada de un sujeto con una enfermedad genética, donde la detección se realiza en las regiones cromosómicas 1p36, 1p35, 1p34, 1p33, 1p32, 1p31, 1p22, 1p21, 1p13, 1p12, 1p11, 1q21, 1q22, 1q23, 1q24, 1q25, 1q31, 1q32, 1q41, 1q42, 1q43, 1q44, 2p25, 2p24, 2p23, 2p22, 2p21, 2p16, 2p15, 2p14, 2p13, 2p12, 2p11, 2q11, 2q12, 2q13, 2q14, 2q21, 2q22, 2q23, 2q24, 2q31, 2q32, 2q33, 2q34, 2q35, 2q36, 2q37, 3p26, 3p25, 3p24, 3p23, 3p22, 3p21, 3p14, 3p13, 3p12, 3p11, 3q11, 3q12, 3q13, 3q21, 3q22, 3q23, 3q24, 3q25, 3q26, 3q27, 3q28, 3q29, 4p16, 4p15, 4p14, 4p13, 4p12, 4q12, 4q13, 4q21, 4q22, 4q23, 4q24, 4q25, 4q26, 4q27, 4q28, 4q31, 4q32, 4q33, 4q34, 4q35, 5p15, 5p14, 5p13, 5p12, 5q11, 5q12, 5q13, 5q14, 5q15, 5q21, 5q22, 5q23, 5q31, 5q32, 5q33, 5q34, 5q35, 6p25, 6p24, 6p23, 6p22, 6p21, 6p12, 6p11, 6q11, 6q12, 6q13, 6q14, 6q15, 6q16, 6q21, 6q22, 6q23, 6q24, 6q25, 6q26, 6q27, 7p22, 7p21, 7p15, 7p14, 7p13, 7p12, 7p11, 7q11, 7q21, 7q22, 7q31, 7q32, 7q33, 7q34, 7q35, 7q36, 8p23, 8p22, 8p21, 8p12, 8p11, 8q11, 8q12, 8q13, 8q21, 8q22, 8q23, 8q24, 9p24, 9p23, 9p22, 9p21, 9p13, 9p12, 9p11, 9q12, 9q13, 9q21, 9q22, 9q31, 9q32, 9q33, 9q34, 10p15, 10p14, 10p13, 10p12, 10p11, 10q11, 10q21, 10q22, 10q23, 10q24, 10q25, 10q26, 11p15, 11p14, 11p13, 11p12, 11p11, 11q12, 11q13, 11q14, 11q21, 11q22, 11q23, 11q24, 11q25, 12p13, 12p12, 12p11, 12q12, 12q13, 12q14, 12q15, 12q21, 12q22, 12q23, 12q24, 13q12, 13q13, 13q14, 13q21, 13q22, 13q31, 13q32, 13q33, 13q34, 14q11, 14q12, 14q13, 14q21, 14q22, 14q23, 14q24, 14q31, 14q32, 15q11, 15q12, 15q13, 15q14, 15q15, 15q21, 15q22, 15q23, 15q24, 15q25, 15q26, 16p13, 16p12, 16p11, 16q11, 16q12, 16q13, 16q21, 16q22, 16q23, 16q24, 17p13, 17p12, 17p11, 17q11, 17q12, 17q21, 17q22, 17q23, 17q24, 17q25, 18p11, 18q11, 18q12, 18q21, 18q22, 18q23, 19p13, 19p12, 19q12, 19q13, 20p13, 20p12, 20p11, 20q11, 20q12, 20q13, 21q11, 21q21, 21q22, 22q11, 22q12, 22q13, Xp22, Xp21, Xp11, Xq11, Xq12, Xq13, Xq21, Xq22, Xq23, Xq24, Xq25, Xq26, Xq27, Xq28, Yp11 y Yq11.
2. Método según la reivindicación 1 donde la enfermedad genética es cáncer o una enfermedad congénita.

3. Método según la reivindicación 2 donde el cáncer es cáncer del sistema nervioso central, cáncer de colon, cáncer de pulmón, cáncer de mama, cáncer de próstata, cáncer de vejiga, leucemias linfáticas crónicas, leucemias linfáticas agudas, mieloma múltiple o un síndrome mielodisplásico.
- 5
4. Método según la reivindicación 3 donde el cáncer del sistema nervioso central es glioma, meningioma benigno, meningioma maligno, oligodendroglioma o glioblastoma multiforme.
- 10
5. Método según la reivindicación 2 donde la enfermedad congénita es síndrome de Down, síndrome de Edwards o síndrome de Patau.
6. Método *in vitro* para el diagnóstico o pronóstico de una enfermedad genética o para el diseño de un tratamiento personalizado en un sujeto con una enfermedad genética caracterizado por comprender los siguientes pasos:
- 15
- a. detectar y/o cuantificar al menos una ganancia y/o pérdida cromosómica en las regiones cromosómicas 1p36, 1p35, 1p34, 1p33, 1p32, 1p31, 1p22, 1p21, 1p13, 1p12, 1p11, 1q21, 1q22, 1q23, 1q24, 1q25, 1q31, 1q32, 1q41, 1q42, 1q43, 1q44, 2p25, 2p24, 2p23, 2p22, 2p21, 2p16, 2p15, 2p14, 2p13, 2p12, 2p11, 2q11, 2q12, 2q13, 2q14, 2q21, 2q22, 2q23, 2q24, 2q31, 2q32, 2q33, 2q34, 2q35, 2q36, 2q37, 3p26, 3p25, 3p24, 3p23, 3p22, 3p21, 3p14, 3p13, 3p12, 3p11, 3q11, 3q12, 3q13, 3q21, 3q22, 3q23, 3q24, 3q25, 3q26, 3q27, 3q28, 3q 29, 4p16, 4p15, 4p14, 4p13, 4p12, 4q12, 4q13, 4q21, 4q22, 4q23, 4q24, 4q25, 4q26, 4q27, 4q28, 4q31, 4q32, 4q33, 4q34, 4q35, 5p15, 5p14, 5p13, 5p12, 5q11, 5q12, 5q13, 5q14, 5q15, 5q21, 5q22, 5q23, 5q31, 5q32, 5q33, 5q34, 5q35, 6p25, 6p24, 6p23, 6p22, 6p21, 6p12, 6p11, 6q11, 6q12, 6q13, 6q14, 6q15, 6q16, 6q21, 6q22, 6q23, 6q24, 6q25, 6q26, 6q27, 7p22, 7p21, 7p15, 7p14, 7p13, 7p12, 7p11, 7q11, 7q21, 7q22, 7q31, 7q32, 7q33, 7q34, 7q35, 7q36, 8p23, 8p22, 8p21, 8p12, 8p11, 8q11, 8q12, 8q13, 8q21, 8q22, 8q23, 8q24, 9p24, 9p23, 9p22, 9p21, 9p13, 9p12, 9p11, 9q12, 9q13, 9q21, 9q22, 9q31, 9q32, 9q33, 9q34, 10p15, 10p14, 10p13, 10p12, 10p11, 10q11, 10q21, 10q22, 10q23, 10q24, 10q25, 10q26, 11p15, 11p14,
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- 11p13, 11p12, 11p11, 11q12, 11q13, 11q14, 11q21, 11q22, 11q23, 11q24, 11q25, 12p13, 12p12, 12p11, 12q12, 12q13, 12q14, 12q15, 12q21, 12q22, 12q23, 12q24, 13q12, 13q13, 13q14, 13q21, 13q22, 13q31, 13q32, 13q33, 13q34, 14q11, 14q12, 14q13, 14q21, 14q22, 14q23, 14q24, 14q31, 14q32, 15q11, 15q12, 15q13, 15q14, 15q15, 15q21, 15q22, 15q23, 15q24, 15q25, 15q26, 16p13, 16p12, 16p11, 16q11, 16q12, 16q13, 16q21, 16q22, 16q23, 16q24, 17p13, 17p12, 17p11, 17q11, 17q12, 17q21, 17q22, 17q23, 17q24, 17q25, 18p11, 18q11, 18q12, 18q21, 18q22, 18q23, 19p13, 19p12, 19q12, 19q13, 20p13, 20p12, 20p11, 20q11, 20q12, 20q13, 21q11, 21q21, 21q22, 22q11, 22q12, 22q13, Xp22, Xp21, Xp11, Xq11, Xq12, Xq13, Xq21, Xq22, Xq23, Xq24, Xq25, Xq26, Xq27, Xq28, Yp11 y Yq11 en una muestra aislada de un sujeto;
- b. comparar el número de copias de las regiones cromosómicas detectadas y/o cuantificadas en el paso (a) con niveles estándar;
- c. encontrar al menos una diferencia significativa en la comparación del paso (b);
- d. asociar la diferencia significativa del paso (c) a una ganancia o a una pérdida genética; y
- e. asociar las ganancias o pérdidas genéticas del paso (d) a una enfermedad genética.
7. Método según la reivindicación 6 donde en el paso (d) y el paso (e) la ganancia o pérdida genética es una pérdida o ganancia de al menos un cromosoma entero, de al menos uno de los brazos de un cromosoma o de al menos una región cromosómica de al menos uno de los brazos de un cromosoma, donde la región cromosómica comprende al menos un gen.
8. Método según cualquiera de las reivindicaciones 6 o 7 donde la detección y/o cuantificación del paso (a) se realiza mediante la detección y/o cuantificación de al menos un gen que se encuentre localizado en las regiones cromosómicas descritas en el paso (a).
9. Método según la reivindicación 8 donde la detección y/o cuantificación del paso (a) se realiza mediante la detección y/o cuantificación de los genes de la tabla 1.

10. Método según cualquiera de las reivindicaciones 6 a 9 donde la enfermedad genética es cáncer o una enfermedad congénita.
- 5
11. Método según la reivindicación 10 donde el cáncer es cáncer del sistema nervioso central, cáncer de colon, cáncer de pulmón, cáncer de mama, cáncer de próstata, cáncer de vejiga, leucemia linfática crónica, leucemia linfática aguda, mieloma múltiple o un síndrome mielodisplásico.
- 10
12. Método según la reivindicación 11 donde el cáncer del sistema nervioso central es glioma, meningioma benigno, meningioma maligno, oligodendroglioma o glioblastoma multiforme.
- 15
13. Método según la reivindicación 10 donde la enfermedad congénita es síndrome de Down, síndrome de Edwards o síndrome de Patau.
14. Método según cualquiera de las reivindicaciones 1 a 13 donde la detección de la ganancia y/o pérdida cromosómica se realiza mediante hibridación con sondas de
- 20 ácido desoxirribonucleico.
15. Método según la reivindicación 14 donde el número de sondas se sitúa entre 10.000 y 20.000.
- 25
16. Método según la reivindicación 15 donde el número de sondas se sitúa entre 14.000 y 15.000.
17. Método según cualquiera de las reivindicaciones 14 a 16 donde las sondas tienen entre 20 y 200 pares de bases.
- 30

18. Método según cualquiera de las reivindicaciones 14 a 17 donde las sondas se sitúan en un soporte sólido.
19. Método según la reivindicación 18 donde las sondas están marcadas con fluorocromos.
20. Método según cualquiera de las reivindicaciones 1 a 19 donde la muestra se selecciona de la lista que comprende tejido, líquido cefalorraquídeo, mucosa oral, sangre, plasma, suero, linfa, líquido pleural y orina.
21. Método según cualquiera de las reivindicaciones 1 a 20 donde el sujeto es un humano.
22. Uso *in vitro* del número de copias de las regiones cromosómicas 1p36, 1p35, 1p34, 1p33, 1p32, 1p31, 1p22, 1p21, 1p13, 1p12, 1p11, 1q21, 1q22, 1q23, 1q24, 1q25, 1q31, 1q32, 1q41, 1q42, 1q43, 1q44, 2p25, 2p24, 2p23, 2p22, 2p21, 2p16, 2p15, 2p14, 2p13, 2p12, 2p11, 2q11, 2q12, 2q13, 2q14, 2q21, 2q22, 2q23, 2q24, 2q31, 2q32, 2q33, 2q34, 2q35, 2q36, 2q37, 3p26, 3p25, 3p24, 3p23, 3p22, 3p21, 3p14, 3p13, 3p12, 3p11, 3q11, 3q12, 3q13, 3q21, 3q22, 3q23, 3q24, 3q25, 3q26, 3q27, 3q28, 3q29, 4p16, 4p15, 4p14, 4p13, 4p12, 4q12, 4q13, 4q21, 4q22, 4q23, 4q24, 4q25, 4q26, 4q27, 4q28, 4q31, 4q32, 4q33, 4q34, 4q35, 5p15, 5p14, 5p13, 5p12, 5q11, 5q12, 5q13, 5q14, 5q15, 5q21, 5q22, 5q23, 5q31, 5q32, 5q33, 5q34, 5q35, 6p25, 6p24, 6p23, 6p22, 6p21, 6p12, 6p11, 6q11, 6q12, 6q13, 6q14, 6q15, 6q16, 6q21, 6q22, 6q23, 6q24, 6q25, 6q26, 6q27, 7p22, 7p21, 7p15, 7p14, 7p13, 7p12, 7p11, 7q11, 7q21, 7q22, 7q31, 7q32, 7q33, 7q34, 7q35, 7q36, 8p23, 8p22, 8p21, 8p12, 8p11, 8q11, 8q12, 8q13, 8q21, 8q22, 8q23, 8q24, 9p24, 9p23, 9p22, 9p21, 9p13, 9p12, 9p11, 9q12, 9q13, 9q21, 9q22, 9q31, 9q32, 9q33, 9q34, 10p15, 10p14, 10p13, 10p12, 10p11, 10q11, 10q21, 10q22, 10q23, 10q24, 10q25, 10q26, 11p15, 11p14, 11p13, 11p12, 11p11, 11q12, 11q13, 11q14, 11q21, 11q22, 11q23, 11q24, 11q25, 12p13, 12p12, 12p11, 12q12, 12q13, 12q14, 12q15, 12q21, 12q22, 12q23, 12q24, 13q12, 13q13, 13q14, 13q21, 13q22, 13q31, 13q32, 13q33, 13q34, 14q11, 14q12, 14q13, 14q21, 14q22, 14q23, 14q24, 14q31, 14q32, 15q11, 15q12, 15q13, 15q14, 15q15, 15q21, 15q22, 15q23, 15q24, 15q25, 15q26, 16p13, 16p12, 16p11,

16q11, 16q12, 16q13, 16q21, 16q22, 16q23, 16q24, 17p13, 17p12, 17p11, 17q11, 17q12, 17q21, 17q22, 17q23, 17q24, 17q25, 18p11, 18q11, 18q12, 18q21, 18q22, 18q23, 19p13, 19p12, 19q12, 19q13, 20p13, 20p12, 20p11, 20q11, 20q12, 20q13, 21q11, 21q21, 21q22, 22q11, 22q12, 22q13, Xp22, Xp21, Xp11, Xq11, Xq12, Xq13, Xq21, Xq22, Xq23, Xq24, Xq25, Xq26, Xq27, Xq28, Yp11 y Yq11 como
5 marcador diagnóstico o pronóstico de enfermedades genéticas o para el diseño de un tratamiento personalizado en un sujeto con una enfermedad genética.

23. Uso según la reivindicación 22 donde la enfermedad genética es cáncer o una
10 enfermedad congénita.

24. Uso según la reivindicación 23 donde el cáncer es cáncer del sistema nervioso central, cáncer de colon, cáncer de pulmón, cáncer de mama, cáncer de próstata, cáncer de vejiga, leucemia linfática crónica, leucemia linfática aguda, mieloma
15 múltiple o un síndrome mielodisplásico.

25. Uso según la reivindicación 24 donde el cáncer del sistema nervioso central es glioma, meningioma benigno, meningioma maligno, oligodendroglioma o glioblastoma multiforme.
20

26. Uso según la reivindicación 23 donde la enfermedad congénita es síndrome de Down, síndrome de Edwards o síndrome de Patau.

27. *Array* que comprende sondas que detectan ganancias y/o pérdidas de las regiones cromosómicas 1p36, 1p35, 1p34, 1p33, 1p32, 1p31, 1p22, 1p21, 1p13, 1p12, 1p11, 1q21, 1q22, 1q23, 1q24, 1q25, 1q31, 1q32, 1q41, 1q42, 1q43, 1q44, 2p25, 2p24, 2p23, 2p22, 2p21, 2p16, 2p15, 2p14, 2p13, 2p12, 2p11, 2q11, 2q12, 2q13, 2q14, 2q21, 2q22, 2q23, 2q24, 2q31, 2q32, 2q33, 2q34, 2q35, 2q36, 2q37, 3p26, 3p25, 3p24, 3p23, 3p22, 3p21, 3p14, 3p13, 3p12, 3p11, 3q11, 3q12, 3q13, 3q21, 3q22, 3q23, 3q24, 3q25, 3q26, 3q27, 3q28, 3q 29, 4p16, 4p15, 4p14, 4p13, 4p12, 4q12, 4q13, 4q21, 4q22, 4q23, 4q24, 4q25, 4q26, 4q27, 4q28, 4q31, 4q32, 4q33, 4q34, 4q35, 5p15, 5p14, 5p13, 5p12, 5q11, 5q12, 5q13, 5q14, 5q15, 5q21, 5q22, 5q23, 5q31, 5q32, 5q33, 5q34, 5q35, 6p25, 6p24, 6p23, 6p22, 6p21, 6p12, 6p11,
25
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- 6q11, 6q12, 6q13, 6q14, 6q15, 6q16, 6q21, 6q22, 6q23, 6q24, 6q25, 6q26, 6q27, 7p22, 7p21, 7p15, 7p14, 7p13, 7p12, 7p11, 7q11, 7q21, 7q22, 7q31, 7q32, 7q33, 7q34, 7q35, 7q36, 8p23, 8p22, 8p21, 8p12, 8p11, 8q11, 8q12, 8q13, 8q21, 8q22, 8q23, 8q24, 9p24, 9p23, 9p22, 9p21, 9p13, 9p12, 9p11, 9q12, 9q13, 9q21, 9q22, 9q31, 9q32, 9q33, 9q34, 10p15, 10p14, 10p13, 10p12, 10p11, 10q11, 10q21, 10q22, 10q23, 10q24, 10q25, 10q26, 11p15, 11p14, 11p13, 11p12, 11p11, 11q12, 11q13, 11q14, 11q21, 11q22, 11q23, 11q24, 11q25, 12p13, 12p12, 12p11, 12q12, 12q13, 12q14, 12q15, 12q21, 12q22, 12q23, 12q24, 13q12, 13q13, 13q14, 13q21, 13q22, 13q31, 13q32, 13q33, 13q34, 14q11, 14q12, 14q13, 14q21, 14q22, 14q23, 14q24, 14q31, 14q32, 15q11, 15q12, 15q13, 15q14, 15q15, 15q21, 15q22, 15q23, 15q24, 15q25, 15q26, 16p13, 16p12, 16p11, 16q11, 16q12, 16q13, 16q21, 16q22, 16q23, 16q24, 17p13, 17p12, 17p11, 17q11, 17q12, 17q21, 17q22, 17q23, 17q24, 17q25, 18p11, 18q11, 18q12, 18q21, 18q22, 18q23, 19p13, 19p12, 19q12, 19q13, 20p13, 20p12, 20p11, 20q11, 20q12, 20q13, 21q11, 21q21, 21q22, 22q11, 22q12, 22q13, Xp22, Xp21, Xp11, Xq11, Xq12, Xq13, Xq21, Xq22, Xq23, Xq24, Xq25, Xq26, Xq27, Xq28, Yp11 y Yq11 donde el número de dichas sondas se sitúa entre 10.000 y 20.000; donde dichas sondas comprenden las sondas descritas en la tabla 5.
28. *Array* según la reivindicación 27 donde el número de las sondas se sitúa entre 12.000 y 15.000.
29. *Array* según cualquiera de las reivindicaciones 27 a 28 donde las sondas se sitúan en un soporte sólido.
30. *Array* según cualquiera de las reivindicaciones 27 a 29 donde las sondas detectan los genes de la tabla 1.
31. Uso del *array* según cualquiera de las reivindicaciones 27 a 30 para el diagnóstico o pronóstico de enfermedades genéticas o para el diseño de un tratamiento personalizado en un sujeto con una enfermedad genética.

32. Uso según la reivindicación 31 donde la enfermedad genética es cáncer o una enfermedad congénita.
33. Uso según la reivindicación 32 donde el cáncer es cáncer del sistema nervioso central, cáncer de colon, cáncer de pulmón, cáncer de mama, cáncer de próstata, 5 cáncer de vejiga, leucemia linfática crónica, leucemia linfática aguda, mieloma múltiple o un síndrome mielodisplásico.
34. Uso según la reivindicación 33 donde el cáncer del sistema nervioso central es 10 glioma, meningioma benigno, meningioma maligno, oligodendroglioma o glioblastoma multiforme.
35. Uso según la reivindicación 32 donde la enfermedad congénita es síndrome de Down, síndrome de Edwards o síndrome de Patau.

FIGURA 1



FIGURA 1 (continuación)

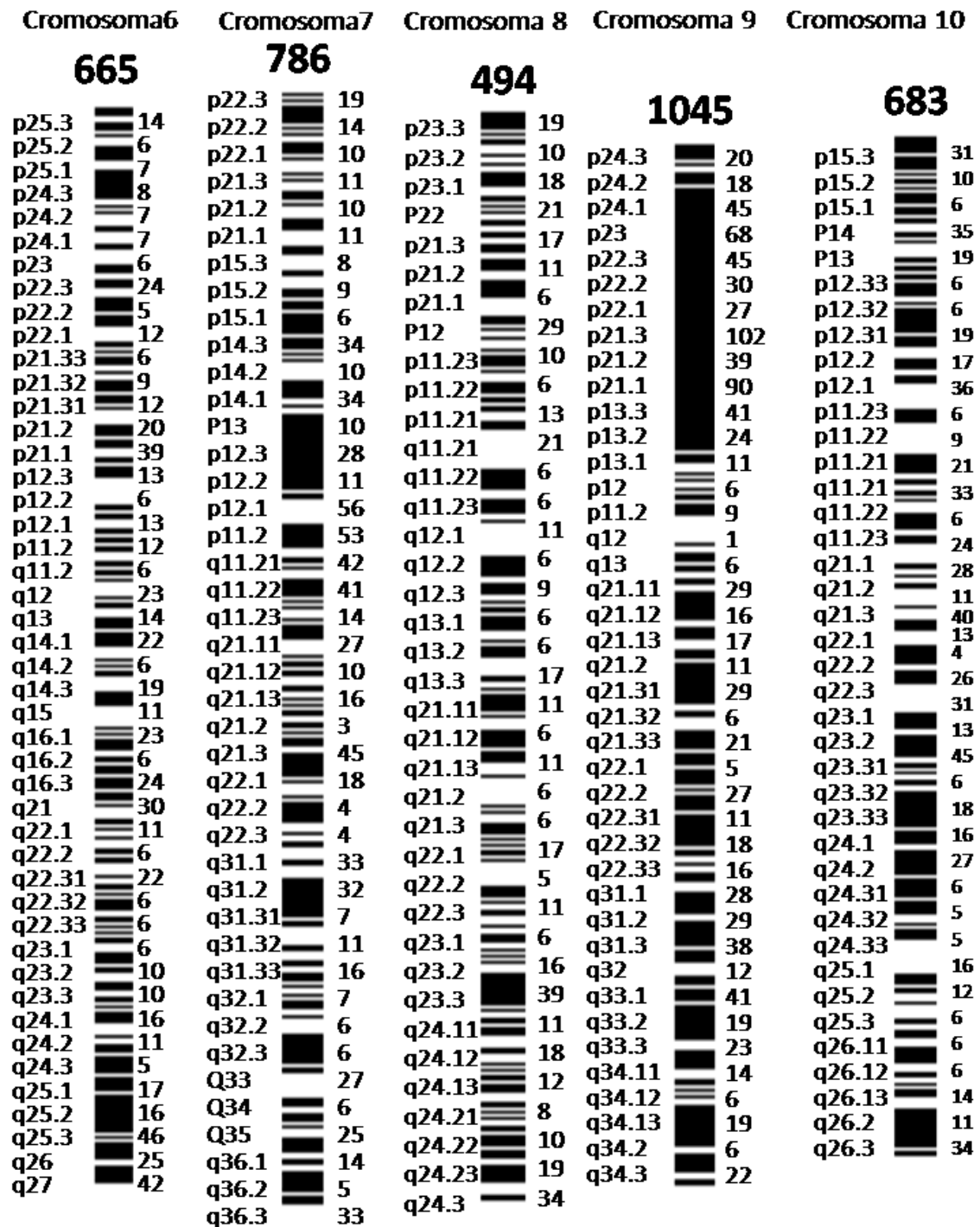


FIGURA 1 (continuación)

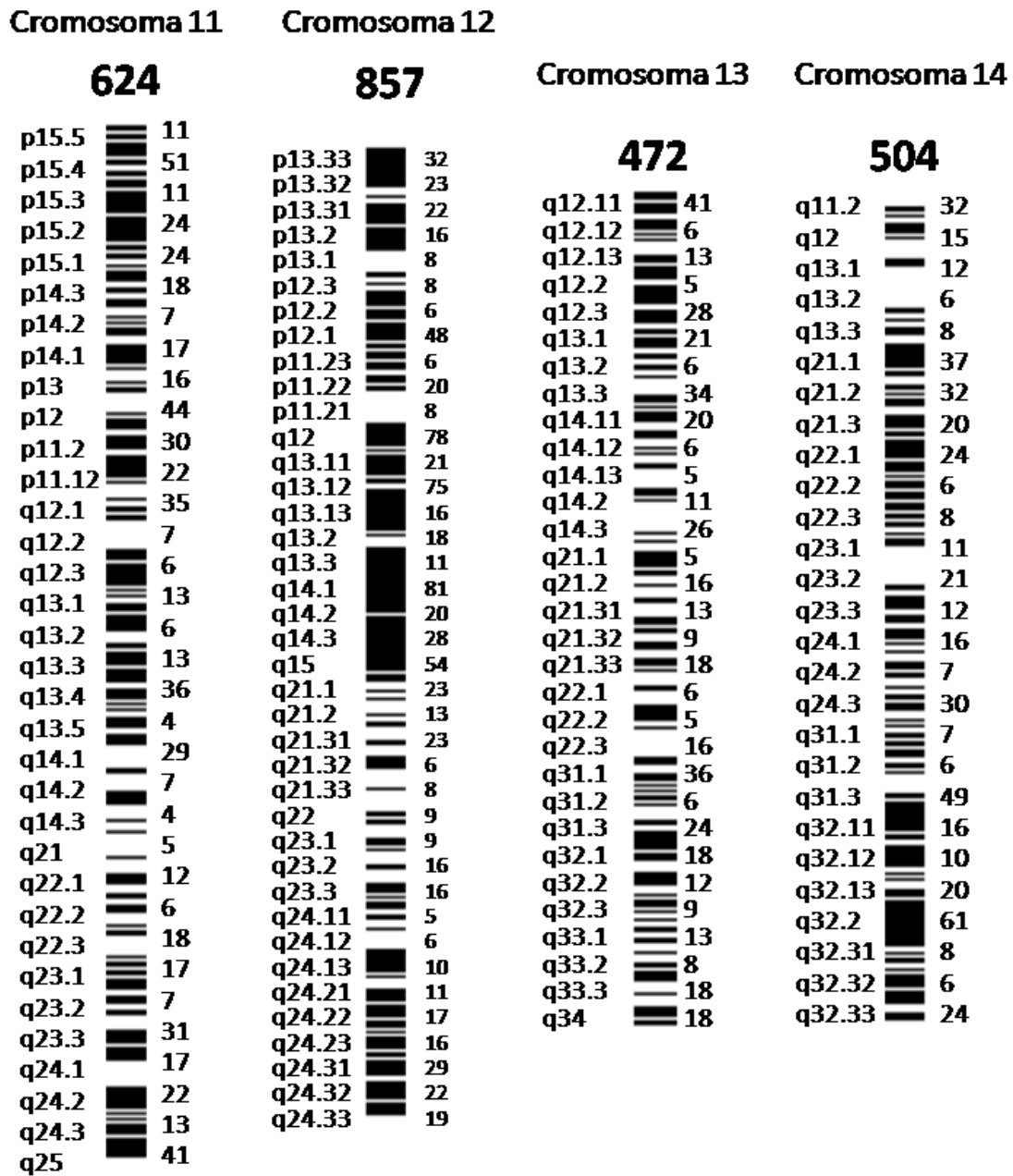


FIGURA 1 (continuación)

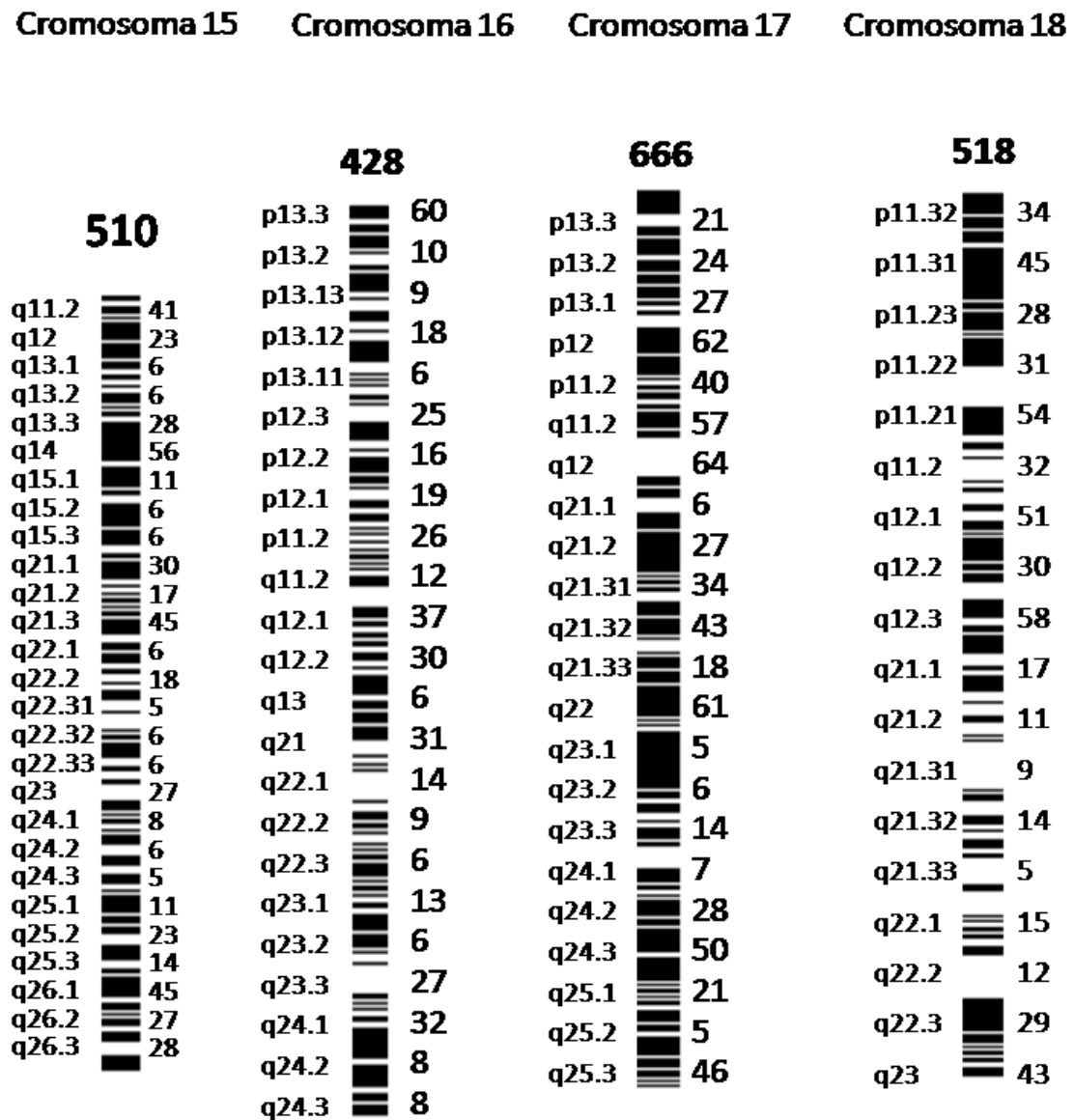


FIGURA 1 (continuación)

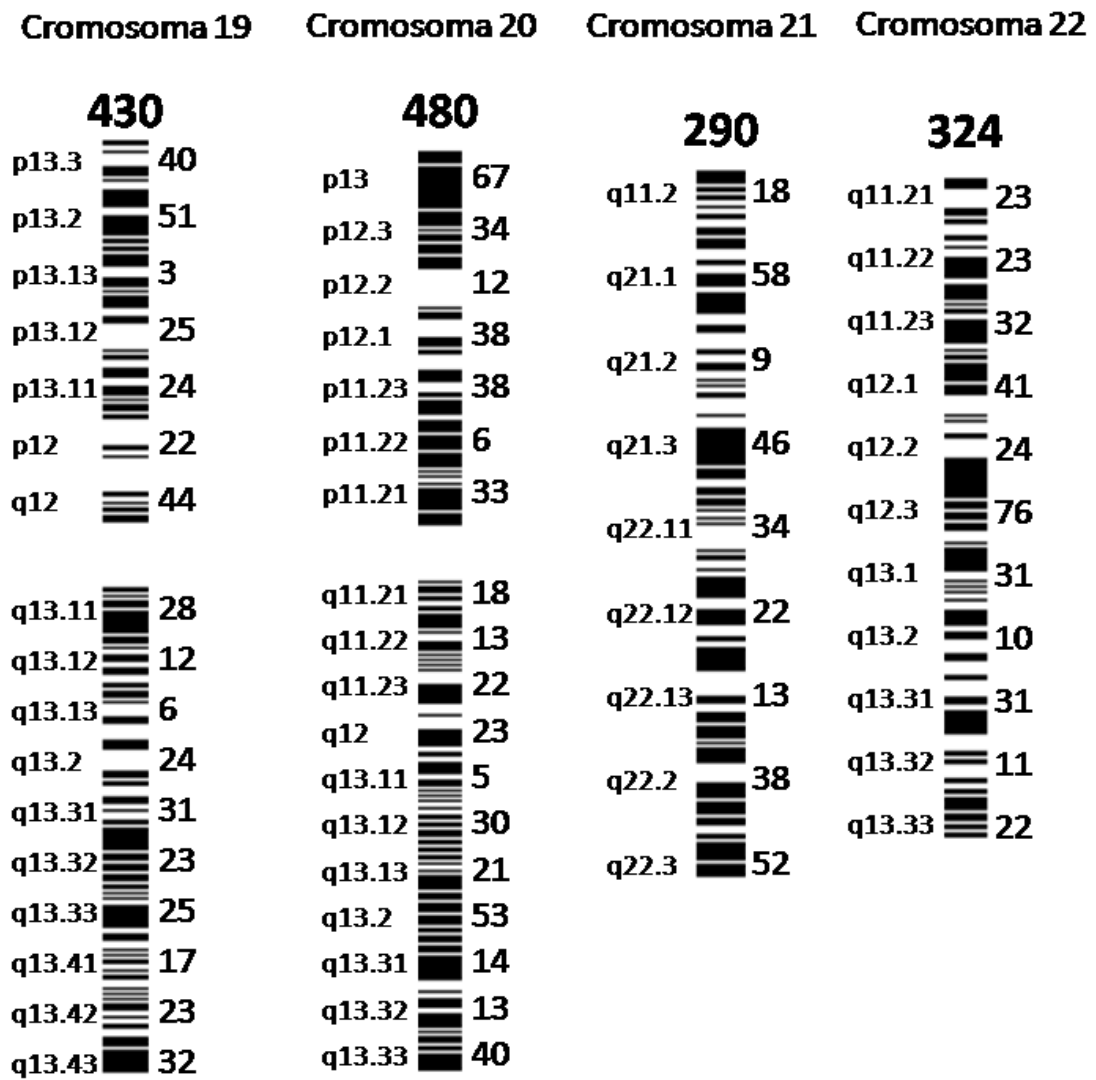
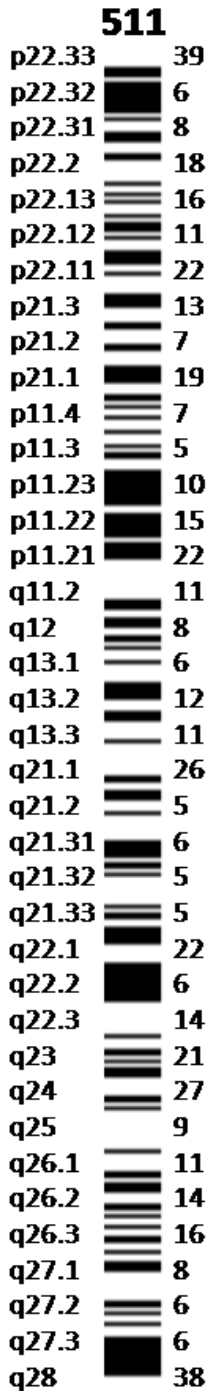


FIGURA 1 (continuación)

Cromosoma X



Cromosoma Y

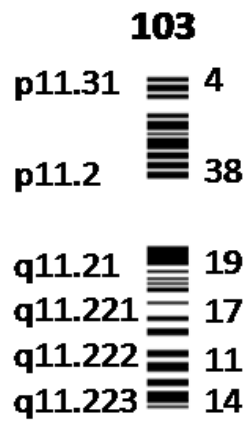


FIGURA 2.

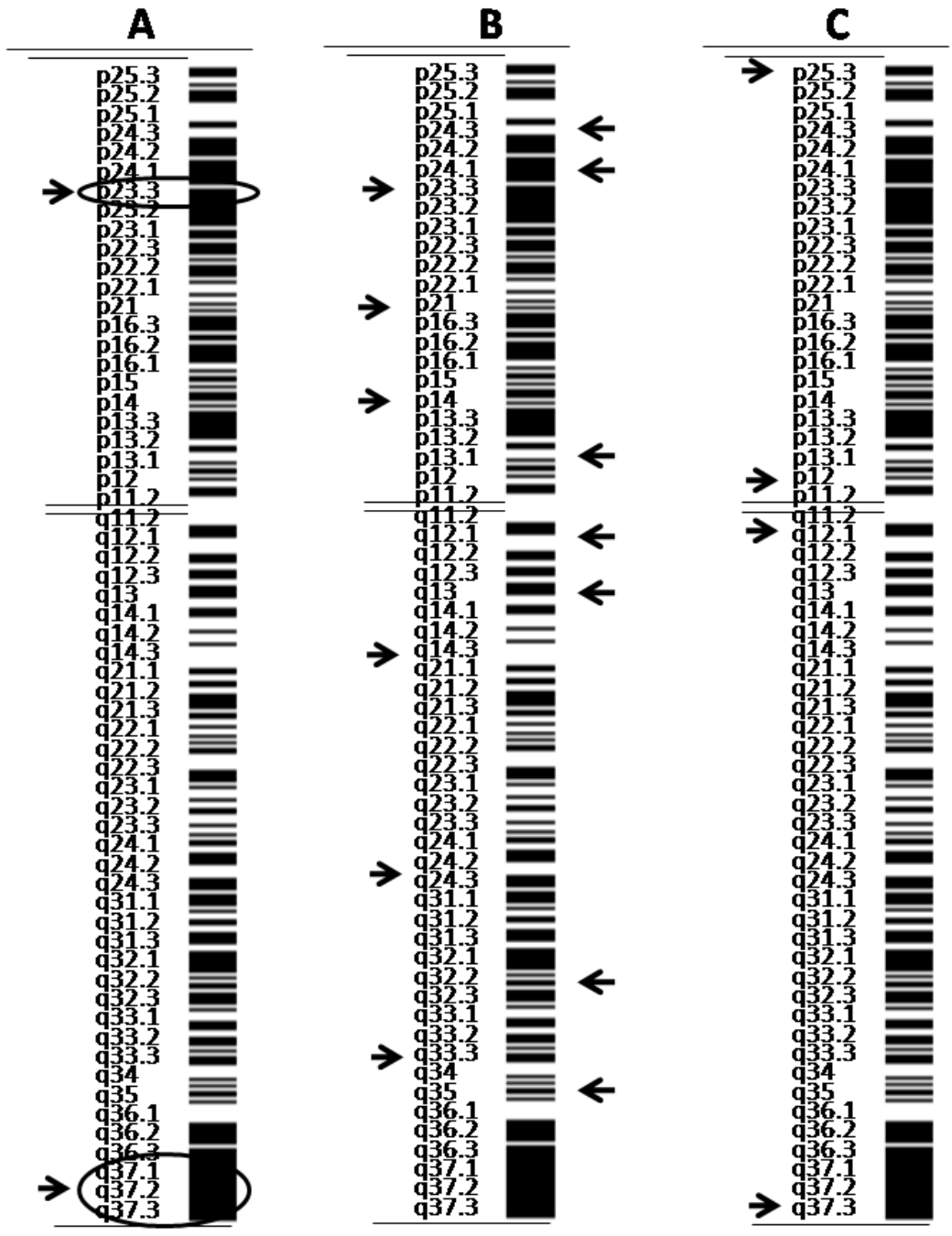
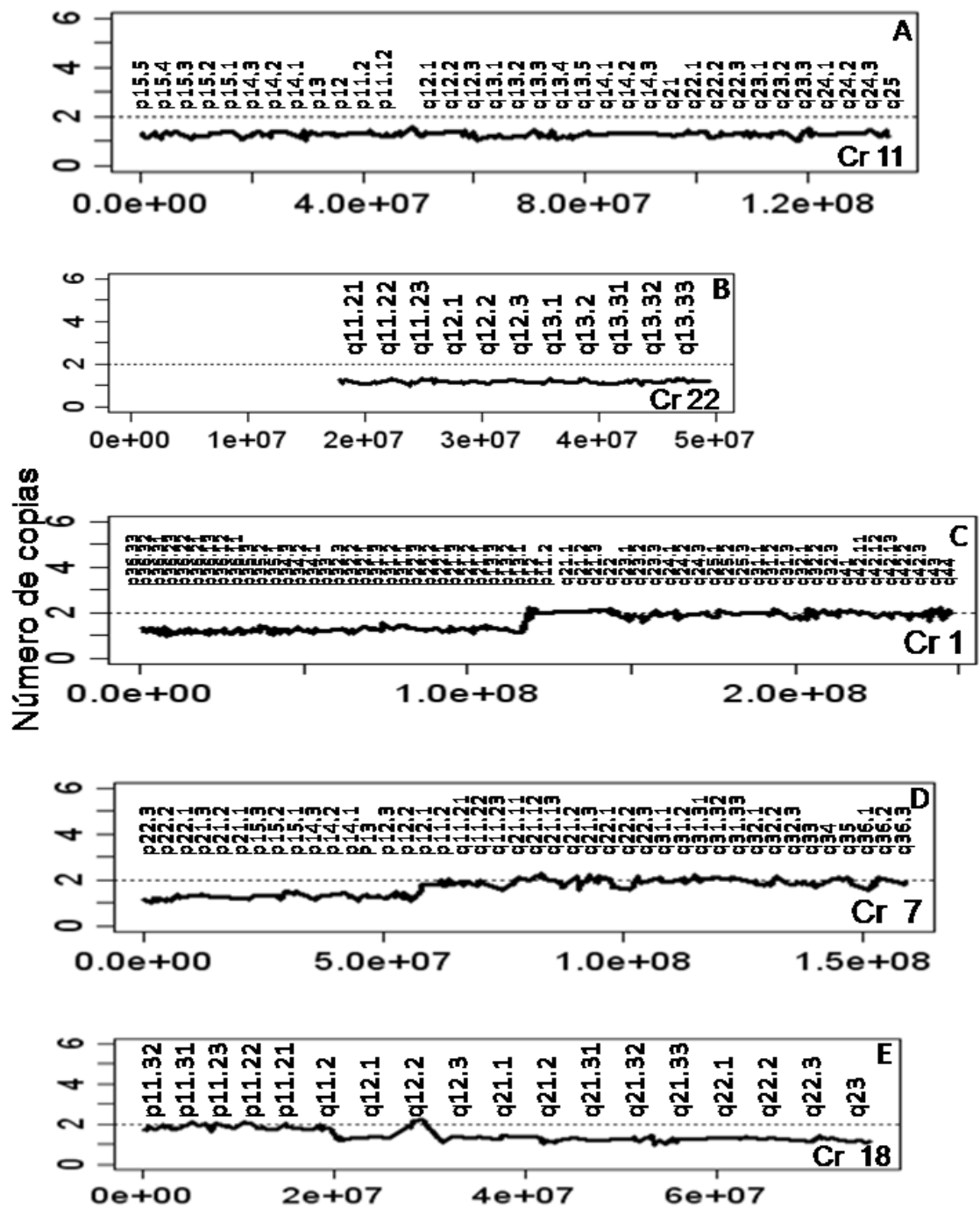


FIGURA 3



Posiciones físicas y bandas cromosómicas

FIGURA 4

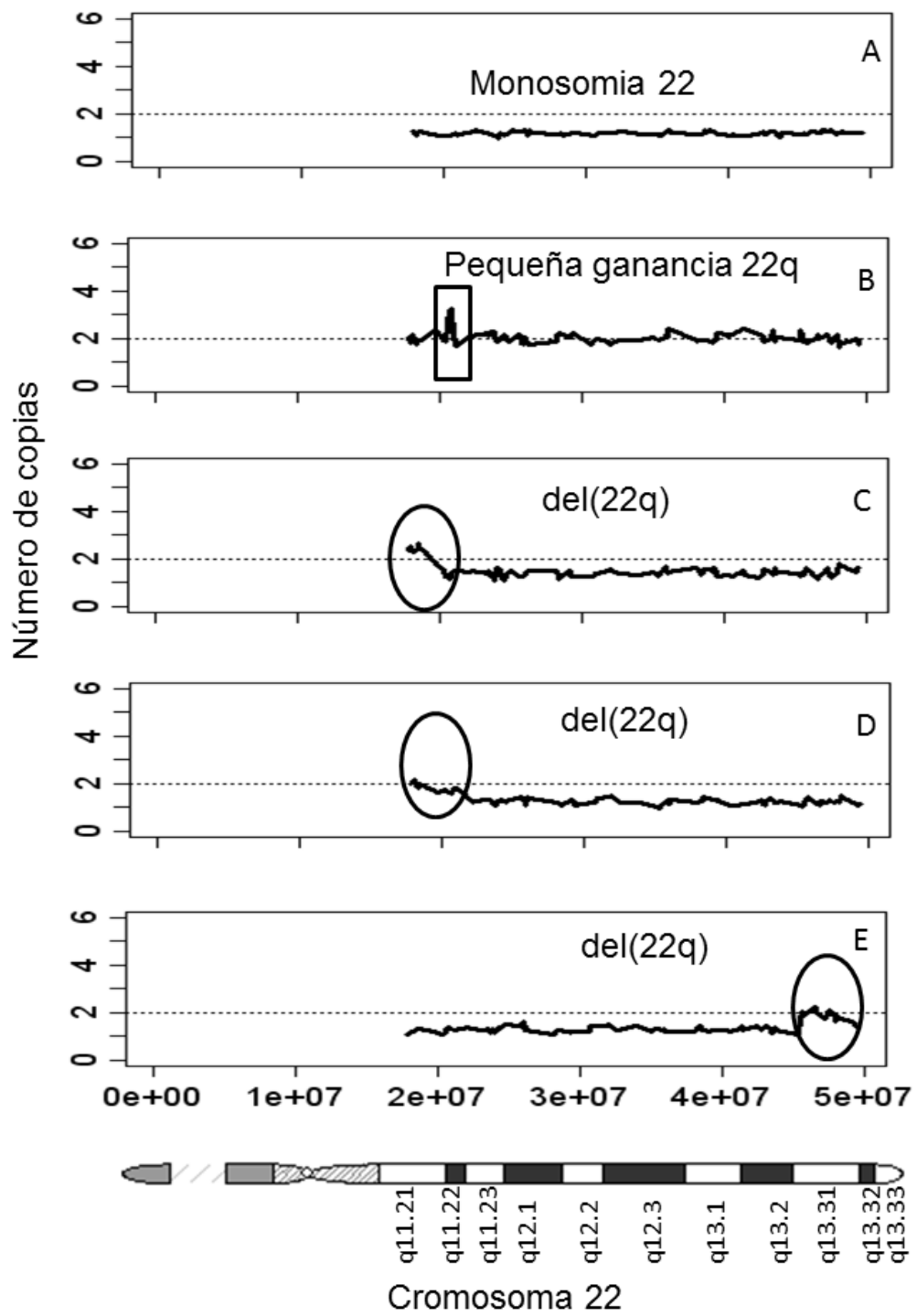


FIGURA 5

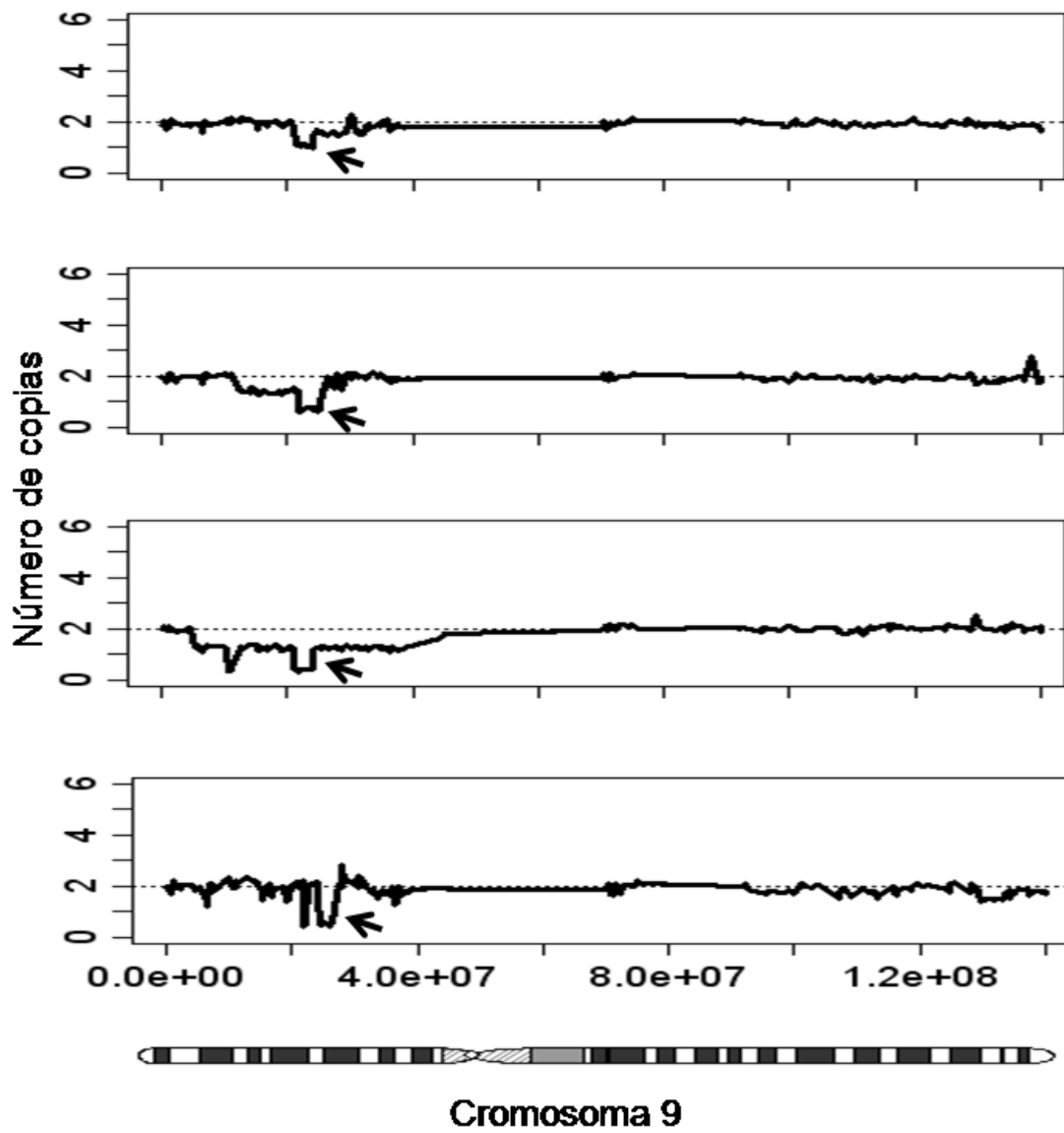


FIGURA 6

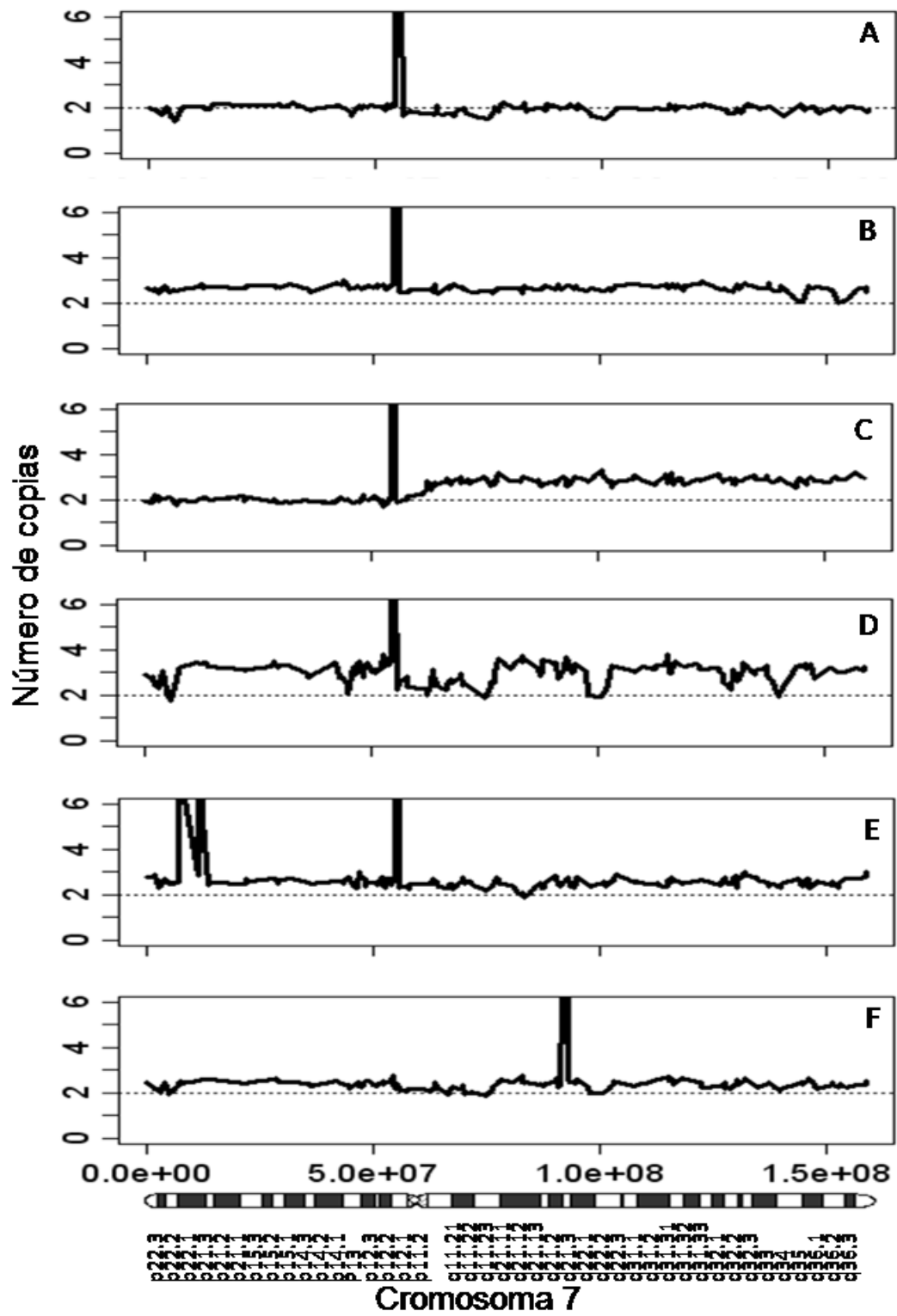


FIGURA 7

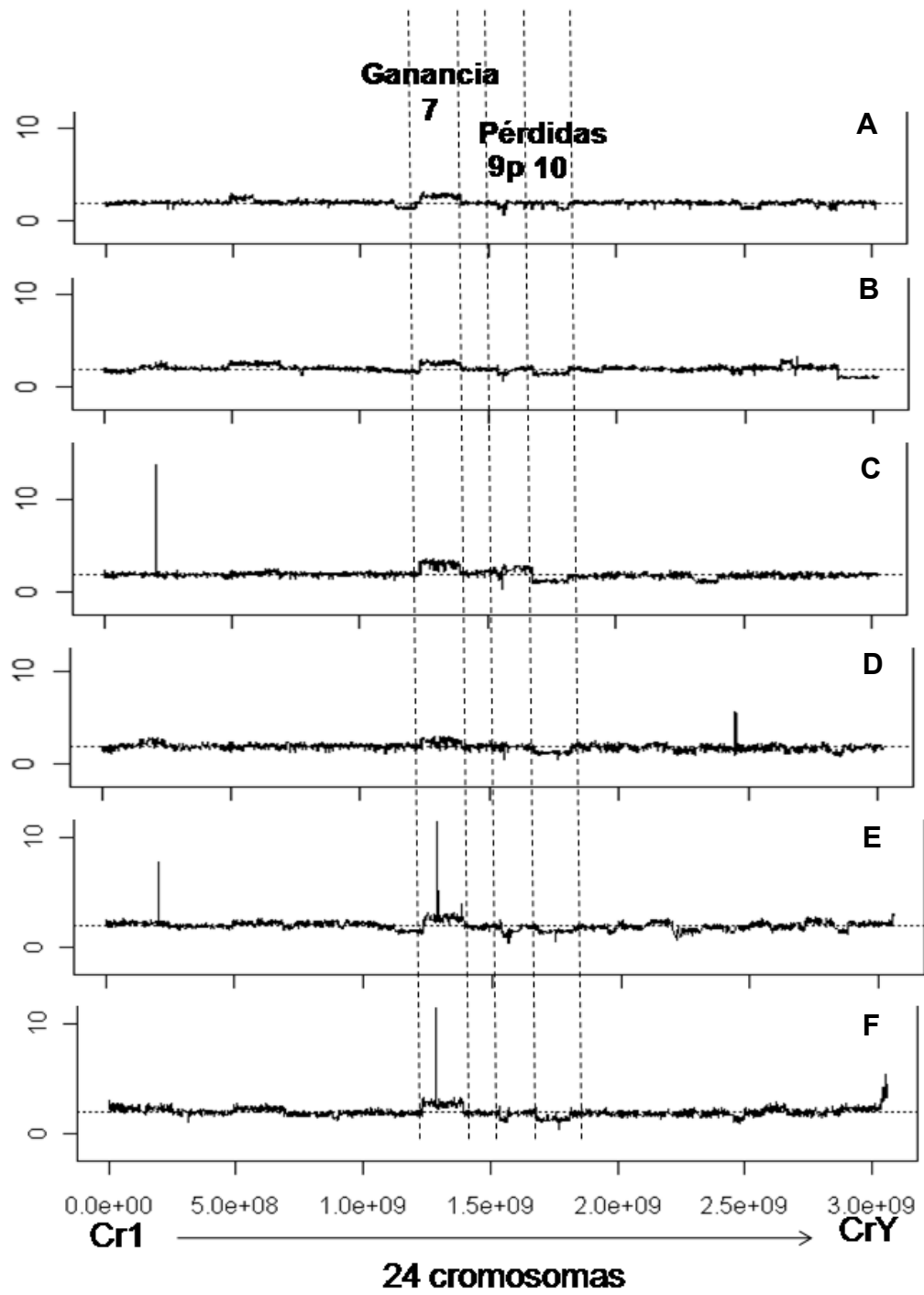


FIGURA 8

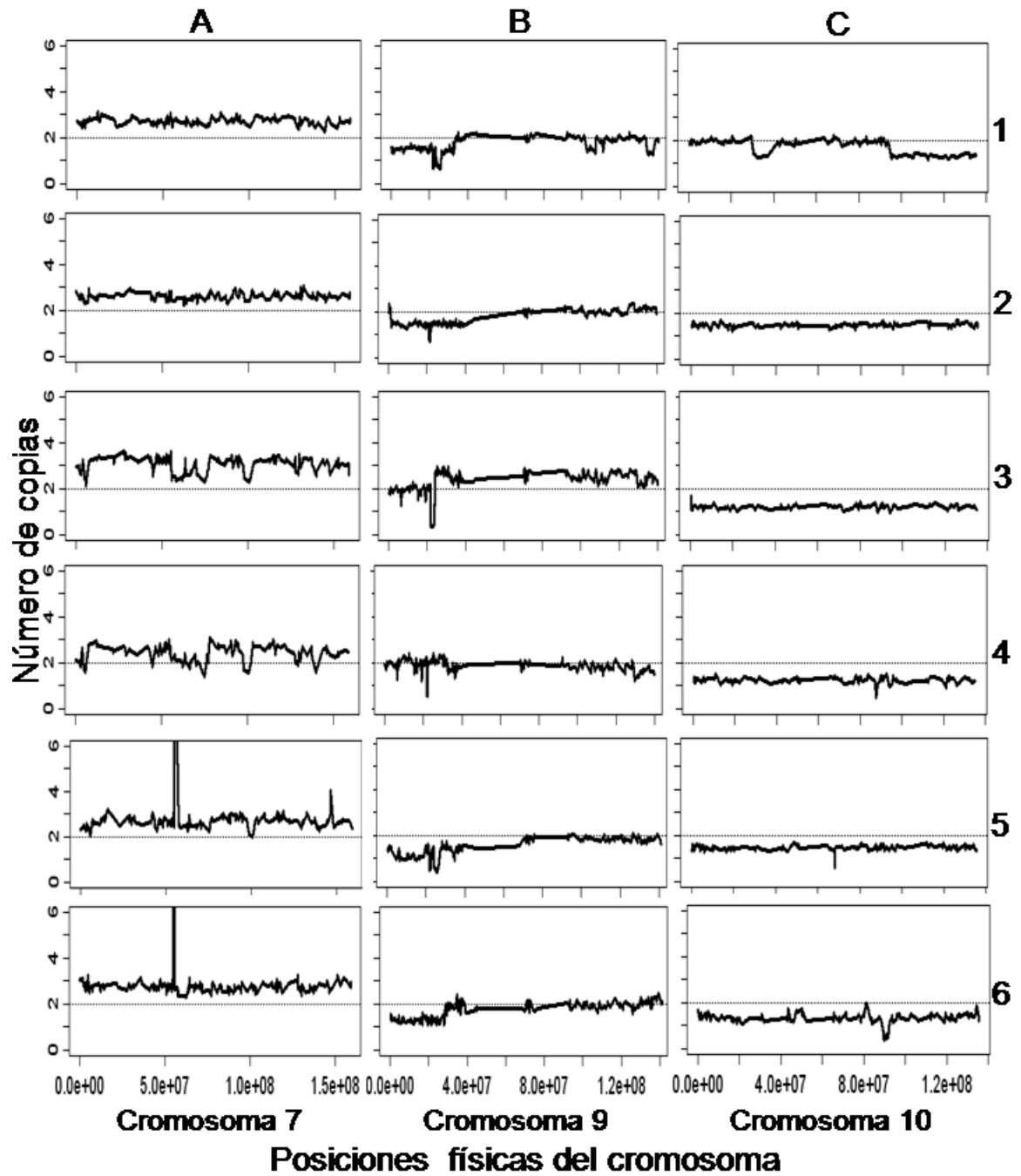


FIGURA 9

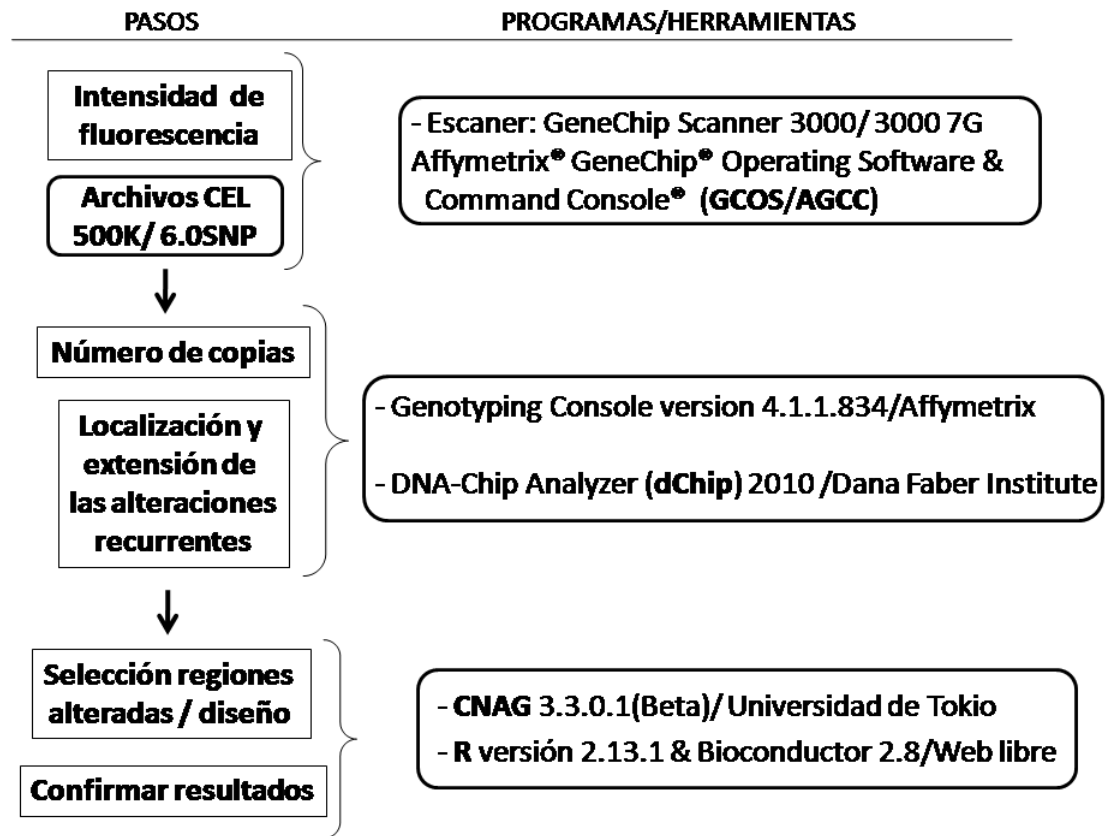
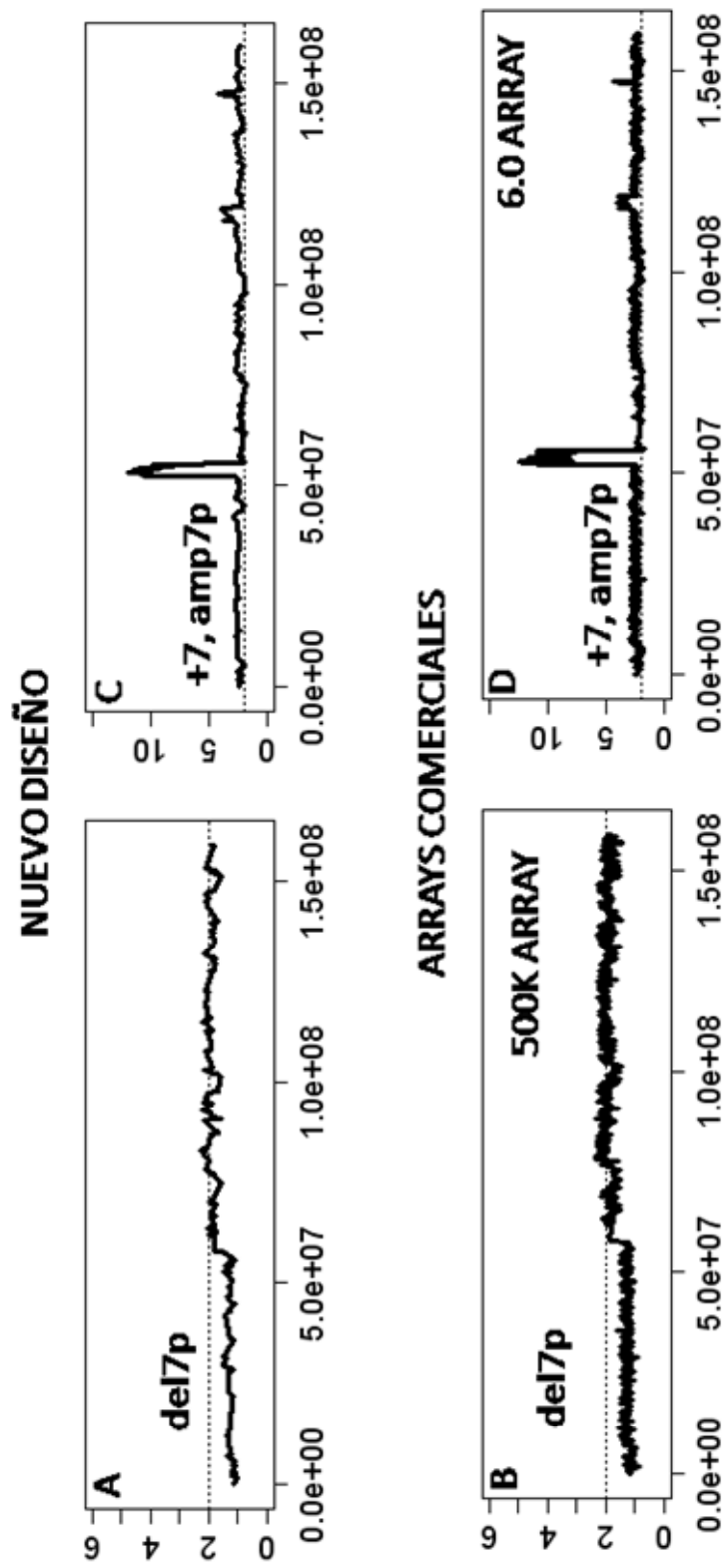


FIGURA 10





- ②① N.º solicitud: 201231829
②② Fecha de presentación de la solicitud: 27.11.2012
③② Fecha de prioridad:

INFORME SOBRE EL ESTADO DE LA TÉCNICA

⑤① Int. Cl.: **C12Q1/68** (2006.01)

DOCUMENTOS RELEVANTES

Categoría	⑤⑥ Documentos citados	Reivindicaciones afectadas
A	US 2005181410 A1 (SHAFFER LISA G et al.) 18.08.2005, especialmente resumen; párrafos 11-12,14-15,29,98-99; tablas 1-2.	1-35
A	<i>Cytosure™. Comparison of gene content of ISCA and Syndrome Plus arrays</i> , 2011 [online], [recuperado el 19.02.2014]. Recuperado de Internet: URL: <http://www.ogt.co.uk/resources/literature/605_cytosure_iscaisca_updsyndrome_plus_gene_list>; <http://www.ogt.co.uk/assets/0000/1739/GeneList_CytoSureISCA_ISCAUPD_SyndromePlus_251110.pdf>, todo el documento.	1-35
A	US 2011160079 A1 (CHAGANTI RAJU S K et al.) 30.06.2011, especialmente resumen; párrafos 11-13; tabla 3.	1-35

Categoría de los documentos citados

X: de particular relevancia

Y: de particular relevancia combinado con otro/s de la misma categoría

A: refleja el estado de la técnica

O: referido a divulgación no escrita

P: publicado entre la fecha de prioridad y la de presentación de la solicitud

E: documento anterior, pero publicado después de la fecha de presentación de la solicitud

El presente informe ha sido realizado

☒ para todas las reivindicaciones

☐ para las reivindicaciones nº:

Fecha de realización del informe
21.02.2014

Examinador
J. Collado Martínez

Página
1/5

Documentación mínima buscada (sistema de clasificación seguido de los símbolos de clasificación)

C12Q

Bases de datos electrónicas consultadas durante la búsqueda (nombre de la base de datos y, si es posible, términos de búsqueda utilizados)

INVENES, EPODOC, WPI, TXTUS, XPESP, NPL, GOOGLE ACADÉMICO

Fecha de Realización de la Opinión Escrita: 21.02.2014

Declaración

Novedad (Art. 6.1 LP 11/1986)	Reivindicaciones 1-35	SI
	Reivindicaciones	NO
Actividad inventiva (Art. 8.1 LP11/1986)	Reivindicaciones 1-35	SI
	Reivindicaciones	NO

Se considera que la solicitud cumple con el requisito de aplicación industrial. Este requisito fue evaluado durante la fase de examen formal y técnico de la solicitud (Artículo 31.2 Ley 11/1986).

Base de la Opinión.-

La presente opinión se ha realizado sobre la base de la solicitud de patente tal y como se publica.

1. Documentos considerados.-

A continuación se relacionan los documentos pertenecientes al estado de la técnica tomados en consideración para la realización de esta opinión.

Documento	Número Publicación o Identificación	Fecha Publicación
D01	US 2005181410 A1 (SHAFFER LISA G et al.)	18.08.2005
D02	<i>Cytosure™. Comparison of gene content of ISCA and Syndrome Plus arrays</i> , 2011 [online]	18.10.2011
D03	US 2011160079 A1 (CHAGANTI RAJU S K et al.)	30.06.2011

2. Declaración motivada según los artículos 29.6 y 29.7 del Reglamento de ejecución de la Ley 11/1986, de 20 de marzo, de Patentes sobre la novedad y la actividad inventiva; citas y explicaciones en apoyo de esta declaración

La presente solicitud tiene por objeto un método para la detección global de ganancias y/o pérdidas cromosómicas, aplicable tanto al diagnóstico o pronóstico de una enfermedad genética como al diseño de un tratamiento personalizado para un sujeto que padezca dicha enfermedad genética. La invención comprende asimismo un *array* para llevar a cabo el método.

El documento D01 divulga un *array* que permite llevar a cabo un método para determinar la existencia de alteraciones genéticas clínicamente relevantes en diferentes partes del genoma (ver resumen; párrafos 11-12, 14-15, 29, 98-99; tablas 1-2).

El documento D02 divulga una lista de las regiones cromosómicas cubiertas por diferentes *arrays* comerciales que incluyen *arrays* de baja densidad (ver todo el documento).

El documento D03 tiene por objeto un *array* útil para el diagnóstico o pronóstico de neoplasias de células B maduras basado en la detección de alteraciones en el número de copias en distintas regiones localizadas en los 22 cromosomas somáticos humanos (ver resumen; párrafos 11-13; tabla 3).

1.- NOVEDAD (Art. 6.1, LP 11/1986).**Reivindicaciones 1-35**

En el estado de la técnica existen numerosos ejemplos de métodos de diagnóstico y/o pronóstico de enfermedades genéticas basados en la detección mediante ensayos de *microarray* de variaciones en el número de copias de diferentes regiones cromosómicas. Sin embargo, ninguno de los documentos D01-D03 considerados divulga métodos de diagnóstico y/o pronóstico de una enfermedad genética, o de diseño de un tratamiento personalizado de un sujeto con dicha enfermedad genética, basados en la detección de ganancias y/o pérdidas cromosómicas en un conjunto de regiones cromosómicas igual al definido en la presente solicitud.

En base a lo anterior, el objeto de las reivindicaciones independientes 1, 6, 22, 27 y 31 y, por extensión el de sus reivindicaciones dependientes 2-5, 7-21, 23-26, 28-30 y 32-35, cumple con el requisito de novedad establecido en el art. 6.1 de la LP 11/1986.

2.- ACTIVIDAD INVENTIVA (Art. 8.1, LP 11/1986).**Reivindicaciones 1-35**

El documento del estado de la técnica que se ha considerado más próximo al objeto de las reivindicaciones independientes 1, 6, 22, 27 y 31 es D01. Dicho documento se refiere a un método de diagnóstico de enfermedades genéticas, incluyendo cáncer. El método se basa en la detección global de variaciones en el número de copias de un conjunto de *loci* localizados en los 24 cromosomas humanos. Estos *loci* han sido seleccionados por proporcionar resultados fiables y clínicamente relevantes en un ensayo de *array* mejorado en el que se han eliminado regiones de bajo valor informativo. Como ya se ha indicado, este conjunto de *loci* de D01 no coincide con el conjunto seleccionado en la invención objeto de la presente solicitud, incluyendo esta última un número notablemente mayor de regiones cromosómicas con valor informativo desde el punto de vista clínico en todos los cromosomas, excepto en el caso de los cromosomas 19 e Y. Por otra parte, al comparar las regiones cromosómicas cubiertas por los *arrays* divulgados en D02 y D03 con las de la presente solicitud se observa también que el número de regiones informativas seleccionadas es claramente menor en D02 y D03, si bien en algún caso, como ocurre en D02, se observa una coincidencia con la presente invención en ciertas regiones seleccionadas, como por ejemplo las correspondientes a los cromosomas 22, X e Y.

El problema técnico que viene a resolver la invención objeto de la presente solicitud es la carencia de un *array* de menor tamaño que los existentes en el estado de la técnica que al mismo tiempo siga siendo de interés para detectar variaciones en el número de copias de todos los cromosomas relevantes en enfermedades genéticas. Para resolver este problema, la inventora ha llevado a cabo un trabajo de investigación que le ha permitido llegar al diseño de un *array* de baja densidad que emplea un número de sondas notablemente menor que el de otros *arrays* existentes de alta densidad, pero que al mismo tiempo demuestra mantener un valor informativo desde el punto de vista clínico similar a éstos últimos. Este resultado no hubiera podido ser alcanzado de forma obvia para un experto en la materia meramente a partir de las enseñanzas de D01-D03, tomados éstos por separado o de forma combinada.

En base a lo anterior se considera que el objeto de las reivindicaciones independientes 1, 6, 22, 27 y 31, y lógicamente el de sus reivindicaciones dependientes 2-5, 7-21, 23-26, 28-30 y 32-35, satisface el requisito de actividad inventiva del art. 8.1, LP 11/1986.