DETECTION OF CHROMOSOMAL INVERSIONS USING NON-REPETITIVE NUCLEIC ACID PROBES

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References Cited
U.S. PATENT DOCUMENTS
6,107,030 A 8/2000 Goodwin et al.
6,140,057 A 10/2000 Lucas
6,270,971 B1 8/2001 Ferguson-Smith et al.

FOREIGN PATENT DOCUMENTS

OTHER PUBLICATIONS


* cited by examiner

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ABSTRACT
A method and a kit for the identification of chromosomal inversions are described. Single-stranded sister chromatids are generated, for example by CO-FISH. A plurality of non-repetitive, labeled probes of relatively small size are hybridized to portions of only one of a pair of single-stranded sister chromatids. If no inversion exists, all of the probes will hybridize to a first chromatid. If an inversion has occurred, these marker probes will be detected on the sister chromatid at the same location as the inversion on the first chromatid.

4 Claims, 1 Drawing Sheet
FIG. 1A

FIG. 1B
DETECTION OF CHROMOSOMAL INVERSIONS USING NON-REPETITIVE NUCLEIC ACID PROBES

CROSS-REFERENCE

This application is a divisional of U.S. patent application Ser. No. 12/375,816, filed Nov 12, 2009, entitled “Detection of Chromosomal Inversions”; which is a 35U.S.C. §371 national phase application of PCT/US2007/079086, filed Sep 20, 2007, entitled “Detection of Chromosomal Inversions” U.S. Pat. No. 8,278,050; which claims benefit from U.S. Provisional Patent Application Serial No. 60/873,149, filed Dec 6, 2006; and from U.S. Provisional Patent Application Serial No. 60/845,885, filed Sep 20, 2006; each of which is incorporated by reference in their entirety.

This invention was made with government support under Grant No. NNX09CE42P awarded by NASA Johnson Space Center. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to detection of chromosomal aberrations and, more particularly, to chromosome-specific chromatid painting for detection of inversions.

BACKGROUND OF THE INVENTION

Analysis of cancer cells has led to the discovery of more than 500 tumor-specific chromosome aberrations. Detailed analysis of the breakpoints involved in these structural chromosomal rearrangements has been instrumental in the discovery of many cancer-related genes. Of all possible types of structural chromosome anomalies, inversions, which represent a reversal of orientation of a DNA segment within a chromosome, are found comparatively rarely among the known tumor-specific aberrations. Inversions can have genetic effects similar to the easily detected translocations between different chromosomes seen in cancer. Both can result in effects such as disrupting regulatory sequences that control gene expression or creating genetic rearrangements like gene fusions. Inversions form through the same mechanism as translocations, the misrepair of DNA double-strand breaks. Thus, it might be expected that translocations and inversions should be found in comparable numbers. One possible explanation for the discrepancy is that standard karyotype analyses are relatively insensitive to the detection of inversions and consequently have largely failed to find many tumor-specific chromosome aberrations of this type.

New approaches for measuring the correct rejoining of radiation-induced DNA double-strand breaks in human cells has led to the conclusion that radiation produces at least ten times the number of chromosomal rearrangements than can now be observed cytogenetically, the vast majority of which are intrachromosomal (that is, small interstitial deletions and inversions). To the extent that radiation is representative of other mechanisms of creating inversions, it appears likely that their significance has been underestimated and underappreciated in many diseases in addition to cancer.

In addition to cancer cytogenetics (the study of chromosomes and how changes in chromosome structure and number can lead to the loss of regulation and control of cell proliferation, and orderly differentiation of cells in tissues), chromosome analysis is widely used in prenatal screening as well as the diagnosis of congenital abnormalities, learning difficulties, impaired fertility, and sexual development problems.
An inversion is an abnormality in chromosome structure that can result from, effectively, two double-stranded breaks occurring at different points along a portion of the chromosome, and rather than the breaks becoming rejoined in their original condition by cellular DNA repair processes, they occasionally rejoin incorrectly in such a way that this interstitial portion of the chromosome becomes effectively rotated through 180° after a "misrejoining" among the broken ends. Importantly, this misrejoining must occur in such a way as to maintain the same 5'→3' polarity of the strands of the chromosome and that of the inverted segment. While the backbone polarity is maintained, the DNA sequence of the nitrogenous bases within the segment is reversed.

Chromosome "paints" are mixtures of fluorescent DNA probes, or other types of molecular markers, highly enriched in sequences unique to a particular chromosome that allow a specific chromosome to be identified based on accepted cytogenetic practices that render the chromosome visible using a fluorescent microscope. Such probes can be purchased from a number of vendors.

The first complete draft of the human genome was made in 2000, and refinements have been made to the database since then. The GenBank database is made available to the public by the National Center for Biotechnology Information (NCBI) of the National Library of Medicine of the National Institutes of Health. Most of the DNA sequences have been ordered into contiguous sequences called contigs.

The CO-FISH technique, developed in the 1990s, permits fluorescent probes to be specifically targeted to sites on either chromatin, but not both. To date, this technique has been used almost entirely for detection of highly repetitive DNA which consists of a series of DNA sequences repeated over and over again, up to thousands of times and which contains few, if any, genes. Such regions are commonly found at sites on a chromosome involved in the mechanics of genome partitioning such as centromeres and telomeres. In "Strand-Specific Fluorescence in situ Hybridization: The CO-FISH Family" by S. M. Bailey et al., Cytogenet. Genome Res. 107: 11-14 (2004), chromosome organization is studied using strand-specific FISH (fluorescent or fluorescence in situ hybridization) [CO-FISH; Chromosome Orientation-FISH] which involves removal of newly replicated strands from the DNA of metaphase (mitotic) chromosomes, resulting in single-stranded target DNA. Each newly replicated double helix contains one parental DNA strand plus a newly synthesized strand, and it is this newly synthesized strand that is removed. When labeled single-stranded probes are hybridized to such targets, the resulting strand-specific hybridization is capable of providing previously unattainable cytogenetic information. Hybridization is a process in which two complementary nucleic acid sequences anneal by base pairing. In the context of FISH, "in situ" refers to hybridization of a nucleic acid sequence probe to the DNA of chromosomes, where the chromosomes are in cells that are attached to a glass microscope slide.

For example, it is known that mammalian telomeric DNA consists of tandem repeats of the (TTAGGG) sequence, oriented 5' to 3' towards the termini of all vertebrate chromosomes. Thus, CO-FISH with a suitable telomere probe reveals the absolute 5' to 3' orientation of DNA sequences relative to the chromosome's qter−pter direction (end of p or short arm of the chromosome to the end of the q or long arm of the chromosome).

The removal of the newly replicated strands using the CO-FISH procedure leaves the original (parental) strands largely intact. Thus, for the purposes of subsequent hybridization reactions, the two sister chromatids of a chromosome are rendered single stranded, and complementary to one another. The ability of CO-FISH to restrict hybridization of single-stranded probes to only one of the two sister chromatids means that it can also be used for inversion detection. Because an inversion reverses the orientation of the DNA sequences within the inversion region, it becomes visible as a jump or switch in probe signal from one chromatid to its sister chromatid. Such a switch can readily be detected when compared to a reference probe outside of the inverted region.

**SUMMARY OF THE INVENTION**

Therefore, it is an object of the present invention to provide a sensitive method for the detection of chromosomal inversions.

Still another object of the invention is to provide a probe kit for the sensitive detection of chromosomal inversions.

Additional objects, advantages and novel features of the invention will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following or may be learned by practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

To achieve the foregoing and other objects, and in accordance with the purposes of the present invention, as embodied and broadly described herein, the method for detecting inversions in a selected mitotic chromosome, hereof, includes the steps of: generating a pair of single-stranded sister chromatids from said selected chromosome, each sister chromatid having a length of DNA and a series of target DNA sequences that span a portion of the length of the DNA of the chromatid; generating a plurality of non-repetitive probes, wherein each of the probes is single-stranded, unique and identical to at least a portion of a target DNA sequence, each of the probes having at least one label, thereby permitting detection thereof; hybridizing the probes to the sister chromatids; and detecting the hybridized probes; whereby if no inversion exists, all of the probes will hybridize to one of the sister chromatids, and whereby if an inversion exists, at least one of the probes will hybridize to the other sister chromatid at the same location as the inversion.

In another aspect of the present invention and in accordance with its objects and purposes, the method for detecting inversions in a selected mitotic chromosome, hereof, includes the steps of: generating a pair of single-stranded sister chromatids from said selected chromosome, each sister chromatid having a length of DNA and a series of target DNA sequences that span a portion of the length of the DNA of the chromatid; generating a plurality of non-repetitive probes, wherein each of the probes is single-stranded, unique and complementary to at least a portion of a target DNA sequence, each of the probes having at least one label, thereby permitting detection thereof; hybridizing the probes to the sister chromatids; and detecting the hybridized probes; whereby if no inversion exists, all of the probes will hybridize to one of the sister chromatids, and whereby if an inversion exists, at least one of the probes will hybridize to the other sister chromatid at the same location as the inversion.

In yet another aspect of the present invention and in accordance with its objects and purposes, the kit for detecting inversions in a selected mitotic chromosome, hereof, includes a plurality of non-repetitive probes, wherein each of the probes in the plurality of probes is single-stranded, unique and identical to at least a portion of a target DNA sequence of
a chromatid of the chromosome, each of the probes having at least one label, thereby permitting detection thereof.

In still another aspect of the present invention and in accordance with its objects and purposes, the kit for detecting inversions in a selected mitotic chromosome, hereof, includes a plurality of non-repetitive probes, wherein each of the probes in the plurality of probes is single-stranded, unique and complementary to at least a portion of a target DNA sequence of a chromatid of the chromosome, each of the probes having at least one label, thereby permitting detection thereof.

Benefits and advantages of the present invention include, but are not limited to, providing a sensitive method for detecting chromosomal inversions.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and form a part of the specification, illustrate an embodiment of the present invention and, together with the description, serve to explain the principles of the invention. In the drawings:

FIG. 1A is a schematic representation of an embodiment of the present method where one DNA strand of each chromatid in a chromosome is removed using the CO-FISH or another procedure, and one of the two resulting single-stranded chromatids is painted, while FIG. 1B is a schematic representation illustrating the same procedure as described in FIG. 1A hereof, but for a chromosome having an inversion.

DETAILED DESCRIPTION OF THE INVENTION

Briefly, the present invention includes a method for detecting inversions in chromosomes using hybridizing probes for painting one chromatid in order to refine the process of chromosome painting to generate additional information; that is, instead of painting an entire chromosome, probes are developed for painting either chromatid. If the 5'→3' base ordering of either of the sister chromatids is known, the DNA sequence for the sister chromatid can be determined since the sequences are complementary and therefore different in terms of annealing (hydrogen bonding process, also known as base pairing) to fluorescent or other markers probes. The probe on one chromatid technique of the present invention uses CO-FISH (See, e.g., S. M. Bailey et al., supra.) to destroy newly replicated strands in both chromatids. This form of the DNA is a packaged DNA strand; that is, there is an inversion present which defines the nucleotide sequence database. Both the actual sequences as input, and their complements are compared to the entire genomic database point-for-point, base-by-base, and matching sequences are returned in order of their percentage homology, from highest to lowest; that is, completely-matched sequences located on alternate chromosomes in the same genome are identified and eliminated.

(3) Analysis of database to determine adequacy of coverage which is defined as the ability of a fluorescent probe set to completely cover a specified chromatid from end to end. Full (100%) coverage would be hybridization to every base, but is clearly not useful for the present method because there are many sequences that are not unique in a chromosome. It is believed by the inventors that the coverage of chromosome-specific unique sequences will allow coverage of unique target sequences where detectable probes are spaced at 1 Mbp intervals along the length of the chromatids, excluding large repetitive regions such as centromeres and telomeres. Coverage of gene-rich regions may be increased in other embodiments of the invention. (4) Synthesizing and labeling suitable probes, as will be described in detail in the EXAMPLES.

(5) Generating single-stranded sister chromatids in accordance with CO-FISH or another suitable procedure (See, e.g., S. M. Bailey et al., supra, and U.S. Pat. No. 6,107,030 for “Determining Orientation And Direction Of DNA Sequences” which issued to Edwin H. Goodwin and Julienne Meyne on Aug. 22, 2000, the teachings of which patent are hereby incorporated by reference herein.).

(6) Hybridizing the single-stranded probes to the single-stranded chromatids, as will be described in the EXAMPLES.

(7) Detecting the single-stranded fluorescent probes hybridized to one chromatid and not the other. Detection of such probes hybridized to chromosome 19p has been achieved. Although high background was observed, probe hybridization appears to be specific.

(8) Developing chromatid paints (mixtures of probes complementary to the same chromatid), as will be described in the EXAMPLES.

Reference will now be made in detail to the present embodiments of the invention, examples of which are illustrated in the accompanying drawings. In the FIGURE, similar structure will be identified using identical reference characters. A metaphase chromosome consists of 2 linear, double-stranded DNA molecules. This form of the DNA is a packaging mechanism that is used to distribute two identical copies of the DNA molecule during cell division. Turning now to FIG. 1A, a schematic representation of an embodiment of the present method is illustrated. Chromosome (i) shows the untouched chromosome after replication, while chromosome (ii) illustrates the newly replicated DNA strand of each chromatid in the chromosome having been removed using CO-FISH or another procedure, leaving the separated parental chromatid strands oriented in opposite directions.

Chromosome (iii) shows the resulting chromatids after having been exposed to the directional probes of the present invention described hereinbelow, and illustrates the situation where there are no detectable inversions present since upon investigation with a fluorescent microscope in the case of fluorescent probes having been used, no probes appear on the second chromatid.

FIG. 1B is a schematic representation illustrating the same procedure as described in FIG. 1A hereof, but for a chromosome having an inversion. It should be mentioned that the identical chromatid probe mixture would be used to generate the results in both FIGS. 1A and 1B. As may be observed from FIG. 1B, the molecules are not identical in the newly replicated DNA strands; that is, there is an inversion present which
is not observed in chromosomes (i) and (ii) until the chroma-
tids are painted using the directional probes of the present
invention and examined using a fluorescent microscope, as an
example, when fluorescent probes have been used [chromo-
some (iii)]. The inverted portion of the first chromatid is not
painted, while only the complementary portion appears on the
second chromatid as a painted section and is portrayed in the
opposite (inverted orientation) by the arrowheads.

Synthetic oligomers (oligonucleotides, or oligos), are
widely used as probes in molecular biology and cytogenetics.
In cytogenetic research, a probe allows the chromosomal
locations of DNA target sequences to be determined. An
oligonomer is a single strand of DNA, RNA or PNA (peptide
nucleic acid). To generate probes, a label must be attached to
the oligomer. For use in chromosome analysis, the label is
often a fluorescent molecule in order that the probe can be
visualized using fluorescence microscopy. A common label-
ing procedure utilizes an enzyme called terminal deoxynu-
nucleotidyl transferase, also known as terminal transferase.
This enzyme is a template-independent DNA polymerase that
does not extend DNA but leaves a 3' hydroxyl group. To
label a probe, a polymerization reaction is prepared with
terminal transferase, the oligomer, and a deoxynucleotide
triphosphate that has a fluorophore (fluorescent molecule)
coupled to it. During the polymerization reaction, terminal
transferase adds one or more fluorescently labeled deox-
nucleotides to the 3' end of DNA oligomers. To
label a probe, a polymerization reaction is prepared with
terminal transferase, the oligomer, and a deoxynucleotide
triphosphate that has a fluorophore (fluorescent molecule)
coupled to it. During the polymerization reaction, terminal
transferase adds one or more fluorescently labeled deox-
nucleotides to the 3' end of the oligomer.

Oligos can also be labeled directly during commercial
synthesis on either their 5' or 3' ends with fluorescent mol-
cules, fluorescent particles or molecules such as avidin
which are readily detectable using secondary reagents such as
avidin to which a fluorescent molecule has been attached.

Having generally described the invention, the following
EXAMPLES provide additional detail:

EXAMPLE 1

Selection of Sequences:
As stated hereinabove, identification of large contiguous
DNA sequences (contigs) that are unique to specific chromo-
somes to be used as targets is achieved by database analysis
of publicly available genomic DNA sequences. Within these
contigs, shorter sequences 40-50 bases long were selected
for use as probes and then checked for uniqueness to one
specific chromatid by performing a blast search. Sequences
20-90 bases long are anticipated to be useful in the practice
of the present invention. Commercially available software pro-
gams such as Array Designer are linked to this database
function.

The database is analyzed to determine the ability of a
fluorescent probe set to cover a specified chromatid. It is
believed by the inventors that coverage of chromosome-spe-
cific unique sequences using detectable probes spaced at 1
Mbp intervals along the length of the chromatids, excluding
large repetitive regions such as centromeres and telomeres
should be adequate to detect a large number of heretofore
undetectable inversions. Increased coverage is expected to
improve detection.

EXAMPLE 2

Preparation of Probes:
1. Generating Sequence Specific DNA:
   (a) By PCR:
   Sequence-specific genomic DNA suitable for locus-spe-
cific probes was obtained by amplifying specific targets in the
gene using PCR. It should be pointed out that DNA
complementary to the specific targets may also be used. The
size of the PCR product was specified from 7.5 to 9.5 kbp.
Primer pairs separated by the desired target size were gener-
at by using 10 kbp DNA sequence segments at approxi-
mately 1 Mbp intervals. As primers were identified they were
verified using blast (GenBank) to assure that the primer
sequence was unique to chromosome 19p. Primer sequences
found to anneal to additional human chromosomal regions
were excluded from further consideration. If no primer pairs
resulted from a 10 kbp sequence, then the next contiguous 10
kbp was screened.

Microgram quantities of pure genomic DNA were prepared
from normal human fibroblasts using Qiagen’s DNAeasy
columns according to manufacturer’s recommendations.
Polymerase Chain Reaction (PCR) was performed using
Stratagene EXL polymerase. The PCR product was examined
by gel electrophoresis, and if multiple bands were observed
PCR was repeated with upward annealing temperature
adjustments until a single band was obtained. PCR products
of the expected size were routinely obtained using EXL poly-
merase, with several micrograms of PCR product being rou-
tinely produced. The PCR products are the size predicted by
gene analysis.

(b) By Oligo Tiling and Commercial Synthesis:

Array designer 4 software from PREMIER Biosoft Inter-
national was used to develop a tiled array covering exon 3 of
the mucin gene located on chromosome 19p. For the present
purpose, a tiled array is defined as a series of ordered, short,
individual DNA sequences that are complementary to a cor-
responding larger contiguous target DNA sequence. The
program provides a number of variable parameters. The exon
DNA sequence was copied from the NCBI database, pasted
into the software, and queried to produce a tiled array. The
array consisted of a series of non-overlapping sequences,
each 48-52 nucleotides long that were separated by 5 inter-
vening bases. The software checked all sequences for homol-
gy elsewhere in the genome and a built-in blast search function. 189 oligos were prepared by a commercial vendor.
The probe regions corresponded to tiled sequences identified
by Array Designer, and were chosen to cover about 10 kbp.

2. Labeling Probes:
   (a) PCR:
   Four of the chromosome 19-specific PCR products were
   chosen for linear DNA amplification. These were at 1 Mbp
   intervals starting at 1 Mbp from the end of the 19p chromo-
some. Approximately 0.5 µg PCR DNA was used for the
   linear DNA amplification template. The templates that will be
   used to cerate probes using the linear amplification process
   are the double-stranded PCR products that were chosen from
   the genomic database to be spaced at 1 Mbp intervals along
   the chromosome. These four products, although separated by
   great distances on the chromosomes can be oriented 5′ → 3′
   based on the information from the genomic database that they
   are all in the same contig. In order to generate a probe to one
   parental strand and not the other, primers were chosen that
   were from the same parental strand. In actual practice this was
done during primer design, those primers designated as for-
ward primers were all on the same strand with respect to a
given contig sequence, and reverse primers were all on the
complementary strand. Only single primers were used in
linear DNA amplification reactions to produce labeled single-
stranded DNA probes. The DNA amplification conditions
were virtually identical to PCR conditions using genomic
DNA in which the PCR template was synthesized except that
the reactions contained AlexaFluor 594-5-dUTP in an
approximately 1:4 molar ratio with dTTP. In side-by-side
comparisons of linear DNA amplification reactions with or
without labeled nucleotides, similar bands were obtained on
electrophoretic gels and similar amounts were determined
spectrophotometrically. The yield of these reactions has repro-
ducibly been several micrograms. It should be mentioned that
other labeled nucleotides may also be used to prepare the
probes of the present invention.

An 8.5 kbp PCR product was used as a template for linear
DNA amplification. Only single primers were used. For
FISH, probe lengths of 300-500 bases have been shown to
produce better results than longer or shorter probes. Commer-
cially available Hae III is a restriction endonuclease having a
four by recognition sequence that on average cuts once every
256 bp, and has been reported to digest both single-stranded
double stranded DNA (See, e.g., Reference 1.). It has been
found that Hae III not only cuts single-stranded DNA,
but reduces it to the desired size for FISH.

CoC1 21 0.1 mM fluorescently labeled nucleotide triphosphate,
and 50 units of terminal transferase, or other suitable enzyme,
where the amounts given are final concentrations and can be
adjusted to give satisfactory results. The reaction mixture is
incubated at 37° C. for a period of time typically 7 to 9 min.
25 mM of EDTA is added to stop the reaction immediately
after 7-9 min. of incubation, and the volume is adjusted to 100
microliters by adding distilled water.

**EXAMPLE 3**

CO-FISH using probes generated by PCR:

CO-FISH has been described in detail previously, and was
used here with some modification. Primary human dermal
fibroblasts (catalog #C-004-5C, sold by Cascade Biologies)
were subcutured into medium containing 5'-bromo-2'-deox-
ynucleoside HCl and 0.1 µg/ml 4',6-diamidino-2-pheny-
lylene diazine (DAPI). It should be mentioned that when probes
are generated by the PCR method described hereinabove,
the presence of repetitive sequences in them. Such
sequences are labeled and bind to multiple places on many
chromosomes which is undesirable. COT 1 DNA is com-
posed of unlabeled DNA from repetitive sequences. During
hybridization, COT 1 DNA competes with and, in effect,
blocks hybridization from the labeled repetitive sequences in
the probe.

**EXAMPLE 5**

Detection:

Individual metaphase spreads are examined with a fluores-
cence microscope and images captured using a CCD camera.
On selected slides, a direct-labeled (FITC), a 19q arm-spe-
cific DNA paint probe was hybridized as per manufacturer’s
instructions (Q-BIOgene) to verify that probes were in fact
hybridizing to chromosome 19.

**EXAMPLE 6**

Results:

Using both strategies to produce probes, PCR and tiled
oligos produced similar results. Red fluorescence from chrom-
osome 19 was observed on single chromatids using an
epifluorescence microscope. Although there was significant
background signal associated with other chromosomes, the
presence of the signal confirmed that the probes had incorpo-
rated at least one molecule of AlexaFluor 594-5 DUTP, and
were capable of being detected. It is expected that non-spe-
cific background staining can be reduced by increasing the
stringency of hybridization and adding subsequent wash
steps. Other fluorescent or non-fluorescent labels, either sin-
gly or in combination, may be incorporated into probes using
the same methodology.

**EXAMPLE 7**

Generation of Points:

Developing points is achieved by performing the same
operation multiple times along the length of a contig at the
stated 1 Mbp interval and then increasing the coverage as
desired. Adjacent contigs have an information gap between
them. In the databases the contigs are presented with a
hypothized orientation. In order to develop a chromatin
paint these orientations must be confirmed. Therefore when
probes have been developed for two adjacent contigs, they
will be labeled with different fluorochromes. Metaphase
chromosomes from multiple normal individuals will be used
for CO-FISH. If the two colors are found on the same chromatid in all individuals, the database is correct and assembly of the paint components can continue. If the colors are found on opposite chromosomes from all individuals, the orientation of the contig is reversed from its published orientation. If this is the situation, a new probe set may be prepared using sequences complementary to the unique sequences chosen previously, and paint assembly can continue again with the correctly oriented probes.

The visual effect of a chromatid paint is to make the two sister chromatids of a mitotic chromosome appear different, and distinguishable, from one another. As an example, the probes of a chromatid paint might be labeled with fluorescein, a green-fluorescing dye, and total DNA stained with propidium iodide, a red-fluorescing DNA-binding dye. In this case, one chromatid would fluoresce red and the other yellow (green plus red appears yellow). If the chromatid paint is applied to a chromosome that has an inversion, label within the inverted region appears on the opposite chromatid producing a distinctive pattern.

The foregoing description of the invention has been presented for purposes of illustration and description and is not intended to be exhaustive or to limit the invention to the precise form disclosed, and obviously many modifications and variations are possible in light of the above teaching. The embodiments were chosen and described in order to best explain the principles of the invention and its practical application to thereby enable others skilled in the art to best utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto.

What is claimed:

1. A kit for detecting inversions in a selected mitotic chromosome, comprising a plurality of non-repetitive probes 20 to 90 bases long, wherein each of the probes in the plurality of probes is single-stranded, unique and identical to at least a portion of a target DNA sequence of one single-stranded chromatid of the chromosome, each of the probes having at least one fluorescent label, thereby permitting detection thereof by fluorescence in situ hybridization, and further comprising 5'-bromo-2'-deoxyuridine, dye and nuclease for performing the CO-FISH process to generate single-stranded chromatids from the selected mitotic chromosome.

2. The kit of claim 1, further comprising reagents suitable for hybridizing the probes to the single-stranded chromatids.

3. A kit for detecting inversions in a selected mitotic chromosome, comprising a plurality of non-repetitive probes 20 to 90 bases long, wherein each of the probes in the plurality of probes is single-stranded, unique and complementary to at least a portion of a target DNA sequence of one chromatid of the chromosome, each of the probes having at least one fluorescent label, thereby permitting detection thereof by fluorescence in situ hybridization, and further comprising 5'-bromo-2'-deoxyuridine, dye and nuclease for performing the CO-FISH process to generate single-stranded chromatids from the selected mitotic chromosome.

4. The kit of claim 3, further comprising reagents suitable for hybridizing the probes to the single-stranded chromatids.

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