DETECTION OF CHROMOSOMAL INVERSIONS USING NON-REPETITIVE NUCLEIC ACID PROBES

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Application Data

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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
DETECTION OF CHROMOSOMAL INVERSIONS USING NON-REPETITIVE NUCLEIC ACID PROBES

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 in measuring incorrect rejoicing 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.

BACKGROUND 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.

NEW APPROACHES TO MEASURING INCORRECT REJOINING OF RADIATION-INDUCED DNA DOUBLE-STRAND BREAKS

The two methods frequently used for detection of gross cytogenetic aberrations such as translocations are whole chromosome painting by fluorescence in situ hybridization (FISH), and G- or R-banding. The sequence does not have to be known for either technique. Both chromatids of a chromosome are indiscriminately targeted by these techniques. Whole-chromosome-specific-FISH painting consists of using DNA, highly enriched for sequences unique to a particular chromosome, labeled with a reporter molecule, such as a fluorochrome, and hybridizing it to metaphase chromosome spreads. At the same time, hybridization of any labeled repetitive sequences (common to all chromosomes) that may be present are blocked by competitive hybridization to unlabeled repetitive DNA. In this manner, stable aberrations such as translocations can be observed. FISH and the combinatorial derivatives of FISH, such as Spectral Karyotyping, are generally limited by their ability to detect only breaks, interchanges and numerical aberrations. G-banding, also known as G-banding, or similar approaches such as R- or Q-banding, is suitable only for detecting changes in banding patterns caused by chromosome inversions when the inversion involves a segment of the chromosome large enough to produce a recognizable change in the pattern of banding. While it may be possible with difficulty to detect an inversion with breakpoints near the midpoints of adjacent dark and light bands, many larger disruptions involving regions containing more than two or three bands might not always produce a recognizable change in these light/dark patterns of banding. Band lengths of fully condensed human mitotic chromosomes average ~10^7 base pairs.

A chromatid is a replicated chromosome consisting of two identical parts that will be divided equally between daughter cells at mitoses when two new cells are created from one as cell populations grow. At mitosis, then, each chromosome consists of two identical chromatids and each of these consists of a linear, double-stranded DNA molecule. A strand of DNA is basically a phosphate deoxyribose polymer, each with one of four purine or pyrimidine base residues (A, T, G, or C) attached. Beginning with the first sugar there is a phosphate group at the 5' position and a hydroxyl group at the 3' position. This hydroxyl group is in turn joined to the next sugar at the 5' position and the alternating chain continues until the other end of the linear strand where there is a 3' hydroxyl group. The strands are associated by hydrogen bonding and are thus not covalently joined. The hydrogen bonding between the two strands occurs only between certain bases; that is, A with T and C with G. This results in what is known as complementary base pairing between the two opposite strands.

The genome of a cell must be replicated prior to the process of cell division in order to provide the same genetic information contained in the parent cell to each of the two new daughter cells. Before this replication, each chromosome consists of one double stranded DNA molecule, with one strand complementary to the other. During replication the complementary single strands of the chromosome are effectively separated, with each one becoming the basic part of a new chromatid. If one of these parental strands is oriented in the 5'→3' direction along its length with respect to some arbitrary reference direction, then the 5'→3' direction of the complementary strand will be oriented in the opposite direction. After replication the new synthesized strands each will likewise be complementary to its respective parental strand. The 5'→3' direction of single strands within a double stranded DNA molecule is sometimes referred to as the polarity of the strand.
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 chromatid, 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 terminii 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 pter−qter 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 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 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 marker probes. The paint 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 packed 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 one 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 hereinafter, 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

DETAILED DESCRIPTION OF THE INVENTION

(1) Large, contiguous DNA sequences (contigs) that are unique to specific chosen chromosomes and therefore the chromatids to be used as targets from which probes are designed, are identified using genomic databases.

(2) These sequences are checked for uniqueness (presence only on one specific chromatid), by performing a blast search, 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 one 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 hereinafter, 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.
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
oligomer 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 labeling
procedure utilizes an enzyme called terminal deoxynucleo-
tid transferase, also known as terminal transferase. This
enzyme is a template-independent DNA polymerase that
adds deoxynucleotides 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 deoxy-
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-
ecules, fluorescent particles or molecules such as biotin
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 chro-
mosomes 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-
grams 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-speak-
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 now 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 reproducibly 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. Commercially 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 and 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.

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 Biologics) were subcultured into medium containing 5'-bromo-2'-deoxyuridine (BrdU). After 24 hours later (one cell cycle). Colcemid (0.1 µg/ml; Gibco) and cells suspended in 75 mM KCl hypotonic solution were subcultured into medium containing 5'-bromo-2'-deox-

EXAMPLE 4

Hybridization:

A probe hybridization mixture containing 50% formamide, 10% 2×SSC and 10% dextran sulfate, 1% COT1 DNA to block repetitive sequences, and labeled, single-stranded pooled probe [—100 ng each] was denatured at 65° C. for 1 min. and applied to slides prepared for CO-FISH (as above). Following an overnight hybridization at 37° C. in a moist chamber, slides were washed at 42° C., three minutes each, in:

1) 50% formamide/2×SSC (two washes); 2) 2×SSC (two washes); 3) PN Buffer (Phosphate NP-40); and 4) PN Buffer at room temperature for 5 min., then mounted in a glycerol solution containing 1 mg/ml of the antifade compound p-phenylenediamine HCl and 0.1 µg/ml 4',6-diamidino-2-phenylindole (DAPI). It should be mentioned that when probes are generated by the PCR method described hereinabove, inevitably there are repetitive sequences in them. Such sequences are labeled and bind to multiple places on many chromosomes which is undesirable. COT 1 DNA is composed 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 fluorescence microscope and images captured using a CCD camera. On selected slides, a direct-labeled (FITC), a 19q arm-specific DNA paint probe was hybridized as per manufacturer’s instructions (Q-HOgene) 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 chromosome 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 incorporated at least one molecule of AlexaFluor 594-5 DUTP, and were capable of being detected. It is expected that non-specific background staining can be reduced by increasing the stringency of hybridization and adding subsequent wash steps. Other fluorescent or non-fluorescent labels, either singly or in combination, may be incorporated into probes using the same methodology.

EXAMPLE 7

Generation of Paints:

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 hypothesized orientation. In order to develop a chromatid 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-standard 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.