Title (en)

METHOD FOR THE QUANTITATIVE ANALYSIS OF THE NUMBER OF COPIES OF A PRE-DETERMINED SEQUENCE IN A CELL

Title (de)

VERFAHREN ZUR QUANTITATIVEN BESTIMMUNG DER KOPIENZAHL EINER VORBESTIMMTEN SEQUENZ IN EINER ZELLE

Title (fr)

PROCEDE D'ANALYSE QUANTITATIVE DU NOMBRE DE COPIES D'UNE SEQUENCE PREDETERMINEE DANS UNE CELLULE

Publication

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Application

EP 06776654 A 20060807

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Abstract (en)

[origin: DE102005045560A1] The quantitative determination of absolute allele copy number per cell by determining count of pre-determined nucleic acid sequence and a biological sample homologous to pre-determined sequence, comprises accomplishing PCR, determining count of different PCR products and comparing the count of PCR products using probability distribution. The PCR-reaction(s) is/are adapted to amplify two non-homologous sequences, which are comprised in the pre-determined sequence and which are short tandem repeats-, variable number of tandem repeats- and single nucleotide polypeptide segments. The quantitative determination of absolute allele copy number per cell by determining count of pre-determined nucleic acid sequence and a biological sample homologous to pre-determined sequence, comprises accomplishing PCR, determining count of different PCR products and comparing the count of PCR products using probability distribution. The PCR-reaction(s) is/are adapted to amplify two non-homologous sequences, which are comprised in the pre-determined sequence and which are short tandem repeats-, variable number of tandem repeats- and single nucleotide polypeptide segments. The pre-determined nucleic acid sequence is a chromosome, a gene or gene segment. A quantity of the biological sample leading to an allelic dropout is provided during the execution of a PCR. The biological sample provides less than 10 pg DNA and less than 1 cell. The probability distribution is composed of the data of the average values of the different PCR-products obtained during the multiple determination procedure with the individual reference samples with a defined and different copy number of the pre-determined sequence respectively. The determination of the probability distribution requires 4-10 reference samples with defined and different copy number of the pre-determined sequence. The PCR reaction(s) is/are accomplished 100 times for each reference sample. The PCR-reaction(s) is/are adapted to amplify two non- homologous, highly polymeric sequences from the non-coding region of the DNA and the sequences occur in the genome of the donor only once per each allele. The PCR-reaction(s) is/are adapted between 5-12 of non-homologous sequences. The number of copies of the pre-determined sequence in the biological sample is 0-5. The execution of the PCR-reaction and the quantity of assigned biological sample are adapted to determine the biological sample that contains the pre-determined sequence in a copy number per cell of 0, 1, 2 or 3. The biological sample is amplified with a nonspecific PCR before execution of the reaction product that is divided into the number of partial quantities. A pair of primers is adapted to amplify two non-homologous sequences that are used in every PCR. The determination of the presence and/or absence of PCR products takes place by gel electrophoresis, hybridizing techniques on a DNA array, bead system or an optical, electrical or electro-chemical measurement. The sequence of the PCR products is determined by DNA sequencing or a hybridizing procedure. The length of the PCR products is determined by capillary electrophoresis. The parameters in the PCR are selected in such a manner that the relative probability for a positive amplification reaction for each of the two non-homologous sequences is 0.5. A pole body is used as biological sample after first meiotic division. Independent claims are included for: (1) kit for the quantitative determination of absolute allele copy number per cell; and (2) a device for the quantitative determination of absolute allele copy number per cell.

IPC 8 full level

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