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(54) **DIGITAL AMPLIFICATION**

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(*) Notice: This patent is subject to a terminal disclaimer.

Related U.S. Application Data

(60) Continuation of application No. 10/828,295, filed on Apr. 21, 2004, now abandoned, which is a division of application No. 09/981,356, filed on Oct. 12, 2001, now Pat. No. 6,753,147, which is a continuation of application No. 09/613,826, filed on Jul. 11, 2000, now Pat. No. 6,440,706.

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(51) **Int. Cl.**
C12P 19/34 (2006.01)
C07H 21/04 (2006.01)

(52) **U.S. Cl.**
USPC **435/91.2**; 536/24.31; 536/24.33

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

To view the complete listing of prior art documents cited during the proceeding for Reexamination Control Number 90/012,895, please refer to the USPTO's public Patent Application Information Retrieval (PAIR) system under the Display References tab.

Primary Examiner — Bruce Campell

(57) **ABSTRACT**

The identification of pre-defined mutations expected to be present in a minor fraction of a cell population is important for a variety of basic research and clinical applications. The exponential, analog nature of the polymerase chain reaction is transformed into a linear, digital signal suitable for this purpose. Single molecules can be isolated by dilution and individually amplified; each product is then separately analyzed for the presence of pre-defined mutations. The process provides a reliable and quantitative measure of the proportion of variant sequences within a DNA sample.

EX PARTE REEXAMINATION CERTIFICATE ISSUED UNDER 35 U.S.C. 307

THE PATENT IS HEREBY AMENDED AS INDICATED BELOW.

Matter enclosed in heavy brackets [ ] appeared in the patent, but has been deleted and is no longer a part of the patent; matter printed in italics indicates additions made to the patent.

AS A RESULT OF REEXAMINATION, IT HAS BEEN DETERMINED THAT:

Claims **10** and **11** are cancelled.

Claims **1**, **8**, **9**, **12-15** and **19-21** are determined to be patentable as amended.

Claims **2-7**, **16-18** and **22**, dependent on an amended claim, are determined to be patentable.

1. A method for determining an allelic imbalance in a biological sample, comprising the steps of:

distributing isolated nucleic acid template molecules to form a set comprising a plurality of assay samples, wherein the nucleic acid template molecules are isolated from the biological sample;

amplifying *the* template molecules within **[**a**]** *the* set **[**comprising a plurality of assay samples**]** to form a population of amplified molecules in **[**each of the**]** *individual* assay samples of the set**[**, wherein the template molecules are obtained from a biological sample**]**;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome, wherein between 0.1 and 0.9 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*;

comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance in the biological sample.

8. The method of claim **1** wherein between 0.1 and 0.6 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*.

9. The method of claim **1** wherein between 0.3 and 0.5 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*.

12. The method of claim **1** wherein between 0.1 and 0.6 of the assay samples yield **[**an**]** *a homogeneous* amplification product **[**as determined by amplification of the selected genetic sequence**]** *of at least one of the selected and the reference genetic sequences*.

13. The method of claim **[1]** *19* wherein between 0.1 and 0.6 of the assay samples yield an amplification **[**product as determined by amplification of the reference genetic sequence**]** *of at least one of the selected and the reference genetic sequences*.

14. The method of claim **1** wherein between 0.3 and 0.5 of the assay samples yield **[**an**]** *a homogeneous* amplification product **[**as determined by amplification of the selected genetic sequence**]** *of at least one of the selected and the reference genetic sequences*.

15. The method of claim **[1]** *19* wherein between 0.3 and 0.5 of the assay samples yield an amplification product **[**as determined by amplification of the reference genetic sequence**]** *of at least one of the selected and the reference genetic sequences*.

19. A method for determining an allelic imbalance in a biological sample, comprising the steps of:

distributing *cell-free* nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome;

comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance between the first chromosome and the second chromosome in the biological sample.

20. The method of claim **19** wherein between 0.1 and 0.9 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*.

21. The method of claim **20** wherein between 0.1 and 0.9 of the assay samples yield a homogeneous amplification product *of at least one of the selected and the reference genetic sequences*.

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