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**Vogelstein et al.**

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- (54) **DIGITAL AMPLIFICATION**
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(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 659 days.

(Continued)

This patent is subject to a terminal disclaimer.

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(65) **Prior Publication Data**  
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Loughlin et al. Association of the interleukin-1 gene cluster on chromosome 2q13 with knee osteoarthritis. *Arthritis & Rheumatism* 46(6):1519-1527, Jun. 2002.\*

**Related U.S. Application Data**

(Continued)

(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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(60) Provisional application No. 60/146,792, filed on Aug. 2, 1999.

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

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

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

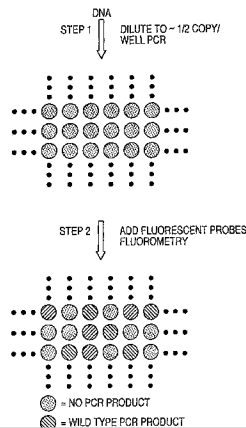
(57) **ABSTRACT**

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.

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**22 Claims, 7 Drawing Sheets**



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FIG. 1A

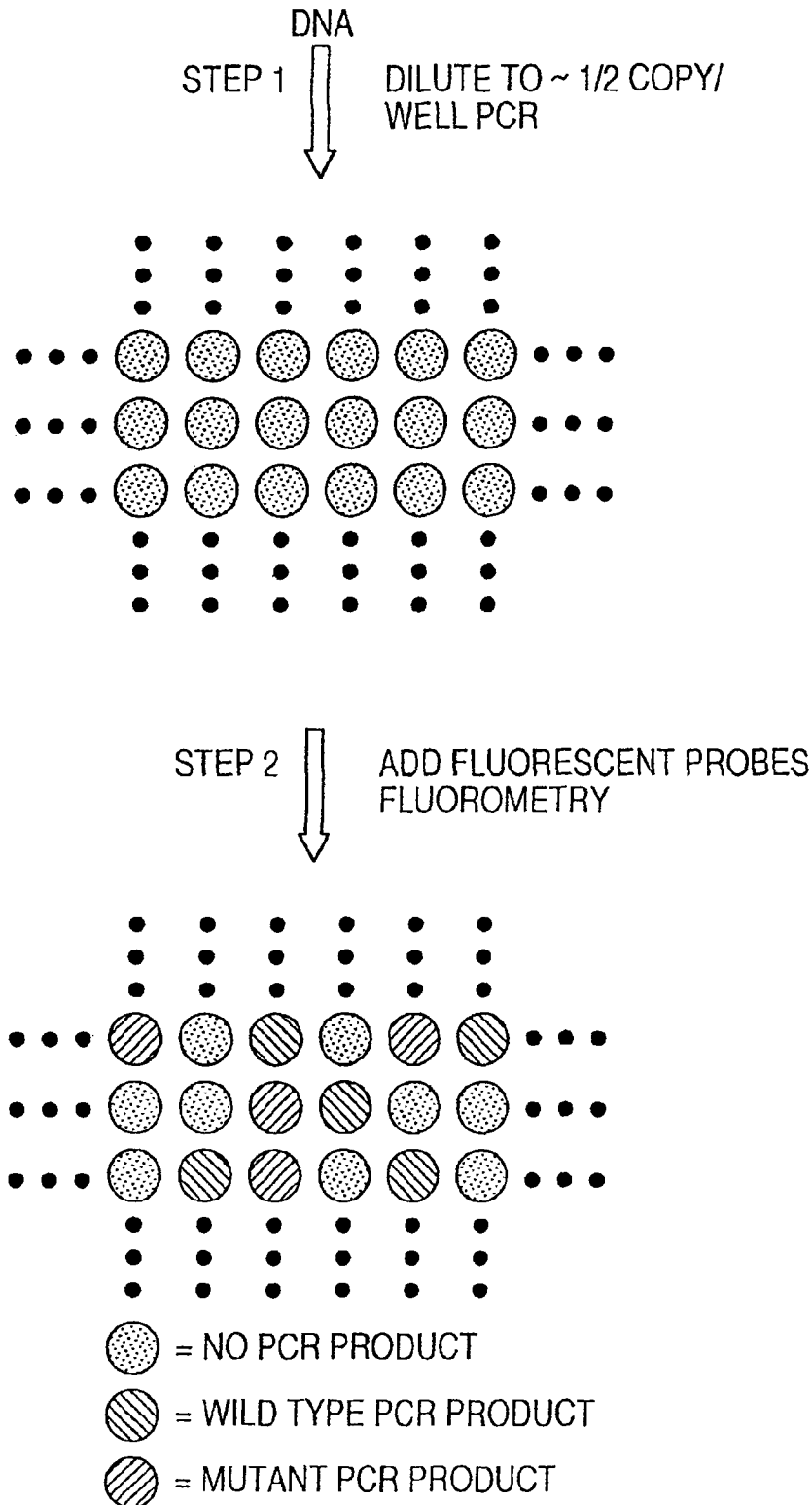


FIG. 1B

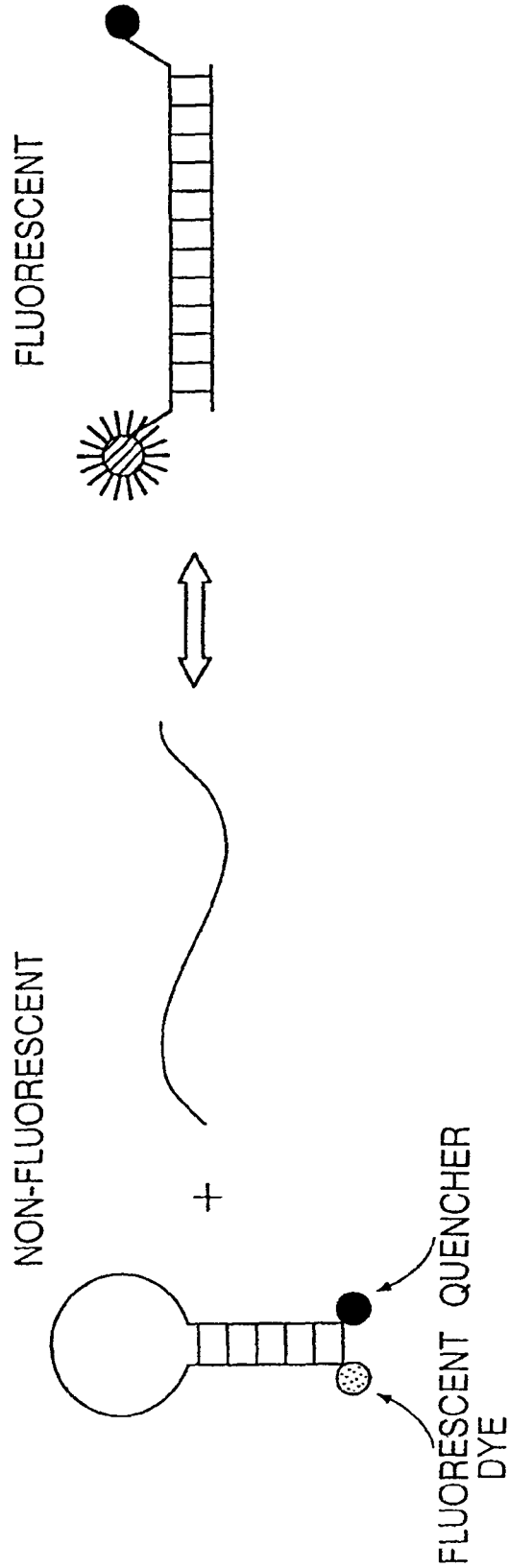
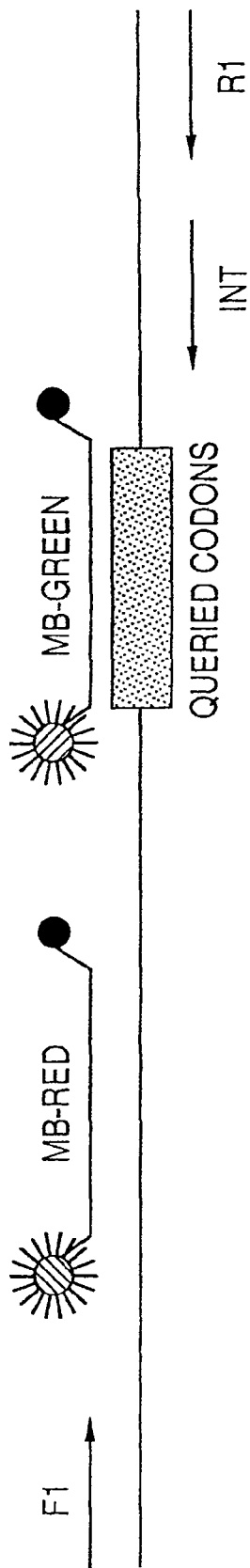


FIG. 1C



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