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Gilchrist et al.

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(54) **METHOD FOR SEQUENCING NUCLEIC ACIDS WITH REDUCED ERRORS**

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(51) Int. Cl.^{7} **G06K 9/00**
(52) U.S. Cl. **382/129**; 382/173; 382/190; 435/6
(58) Field of Search 435/6; 436/86, 436/89; 364/500; 392/129, 173, 190

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,811,218 A		3/1989	Hunkapiller et al.
4,865,968 A		9/1989	Orgel et al.
5,124,247 A		6/1992	Ansorge
5,273,632 A		12/1993	Stockham et al.
5,308,751 A		5/1994	Ohkawa et al.
5,710,628 A		1/1998	Waterhouse et al.
5,712,476 A		1/1998	Renfrew et al.
5,733,729 A	*	3/1998	Lipshutz 435/6
5,776,737 A		7/1998	Dunn
5,786,142 A		7/1998	Renfrew et al.
5,849,542 A	*	12/1998	Reeve et al. 435/91.1
5,853,979 A		12/1998	Green et al.
5,916,747 A		6/1999	Gilchrist et al.
6,083,699 A	*	7/2000	Leushner et al. 435/6
6,195,449 B1	*	2/2001	Bogden et al. 382/129

FOREIGN PATENT DOCUMENTS

EP	86104822.1	10/1986
EP	91118244.2	3/1992
EP	93250264.4	4/1994
GB	8925772.9	11/1989
WO	WO 9741259	11/1997
WO	WO 9800708	1/1998
WO	WO 9811258	3/1998

OTHER PUBLICATIONS

Dear and Staden, A sequence assembly and editing program for efficient management of large projects, Nucleic Acids Research, vol. 19, No. 14, 3907–3911, Oxford University Press, 1991.

Plaschke, Voss, Hahn, Ansorge, and Schackert, Doublex Sequencing in Molecular Diagnosis of Hereditary Diseases, BioTechniques 24:838–841, May 1998.

John M. Bowling, et al., "Neighboring Nucleotide Interationcs During DNA Sequencing Gel Electrophoresis", 1991 Oxford University Press, Nucleic Acids Research, vol. 19, No. 11.

R.L. Miller and T. Ohkawa, "Chain Terminator Sequencing of Double–Stranded DNA With Build–in Error Correction", Genereal Atomics Project 4422, Jul. 1991.

C. Tibbits, et al., "Neural Networks For Automated Base-calling of Gel–based DNA sequencing Ladders".

Allan M. Maxam and Walter Gilbert, "A New Method For Sequencing DNA", Proc. Natl. Acad. Sci. USA, vol. 74, No. 2, pp. 560–564, Feb. 1977, Biochemistry.

Stefan Wiemann, "Simultaneous On–Line DNA Sequencing on Both Strands with Two Fluorescent Dyes", Analytical Biochemistry 224, 117–121(1995).

Ulf Landegren, et al., "DNA Diagnostics—Molecular Techniques and Automation", Science, vol. 242, Oct. 14, 1988.

Lance B. Koutney and Edward S. Yeung, "Automated Image Analysis for Distortion Compensation in Sequencing Gel Electrophoresis", 1369 Applied Spectroscopy, 46(1992) Jan., No. 1, Frederick, M.D. US, vol. 46, No. 1, 1992.

James B. Golden, III, et al., "Pattern Recognition for Automated DNA Sequencing: I. On–line Signal Conditioning and Feature Extraction for Basecalling".

Michael C. Giddings, et al., "An Adaptive, Objective Oriented Strategy For Base Calling In DNA Sequence Analysis", 4530–4540, Nucleic Acids Research, 1993, vol. 21, No. 19. 1993 Oxford University Press.

* cited by examiner

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(57) **ABSTRACT**

Nucleic acid polymers are sequenced by obtaining forward and reverse data sets for forward and reverse strands of a sample nucleic acid polymer. The apparent base sequences of these forward and reverse sets are determined and the apparent sequences are compared to identify any deviations from perfect complementarity. Any such deviation presents a choice between two bases, only one of which is correct. A confidence algorithm is applied to the peaks in the data sets associated with a deviation to arrive at a numerical confidence value for each of the two base choices. These confidence values are compared to each other and to a predetermined threshold, and the base represented by the peak with the better confidence value is assigned as the "correct" base, provided that its confidence value is better than the threshold. The confidence value takes into account at least one, and preferably more than one of several specific characteristics of the peaks in the data set that were not complementary.
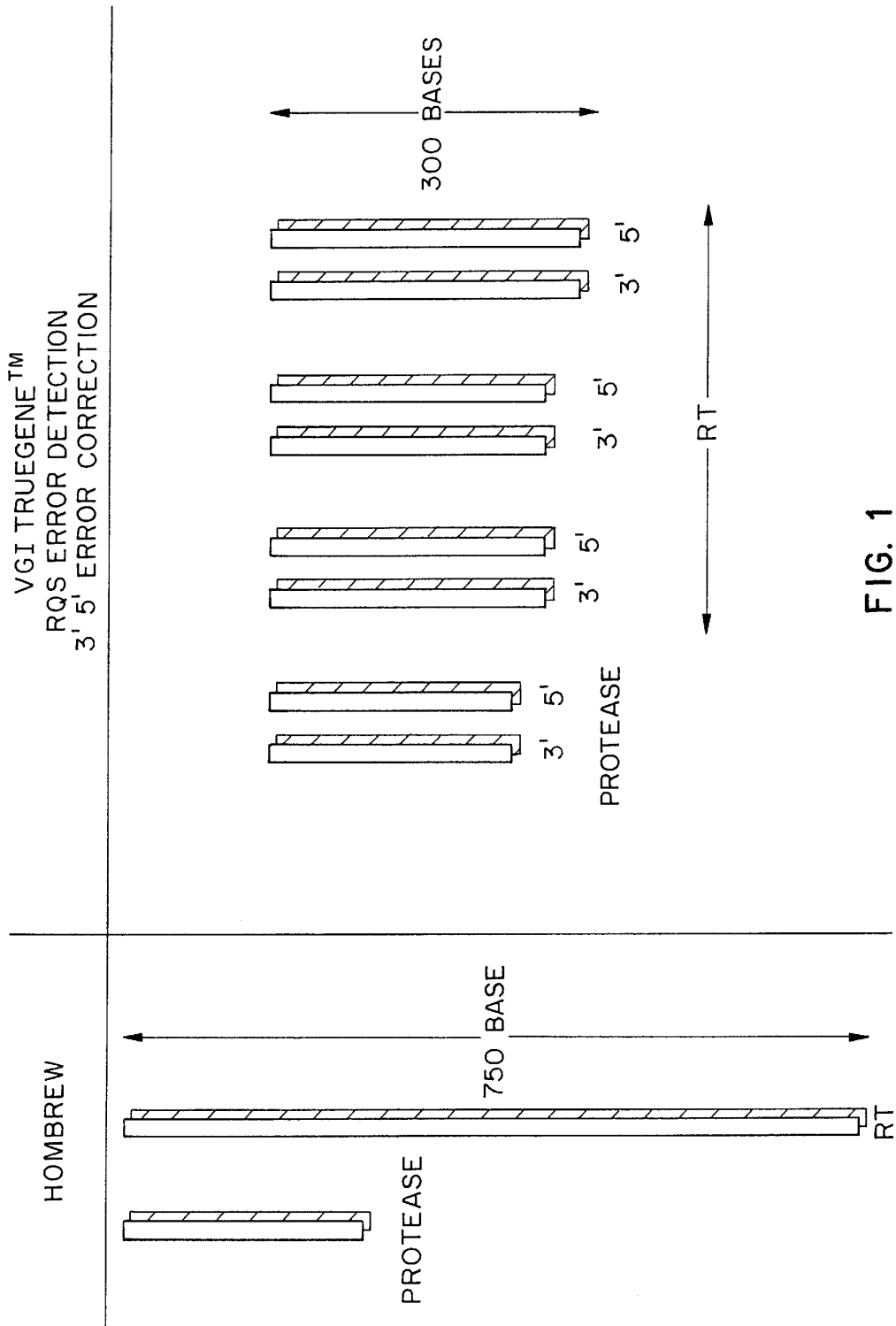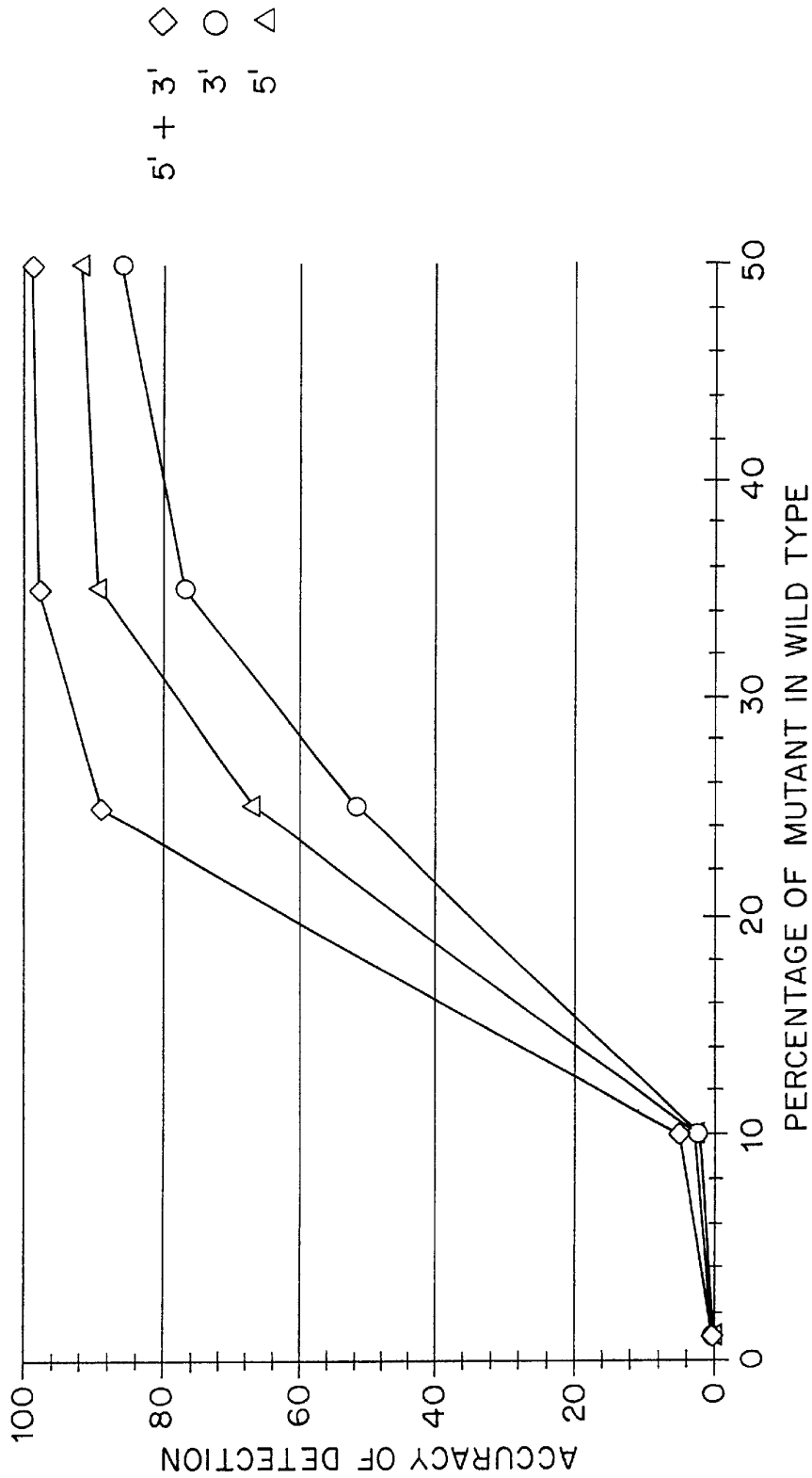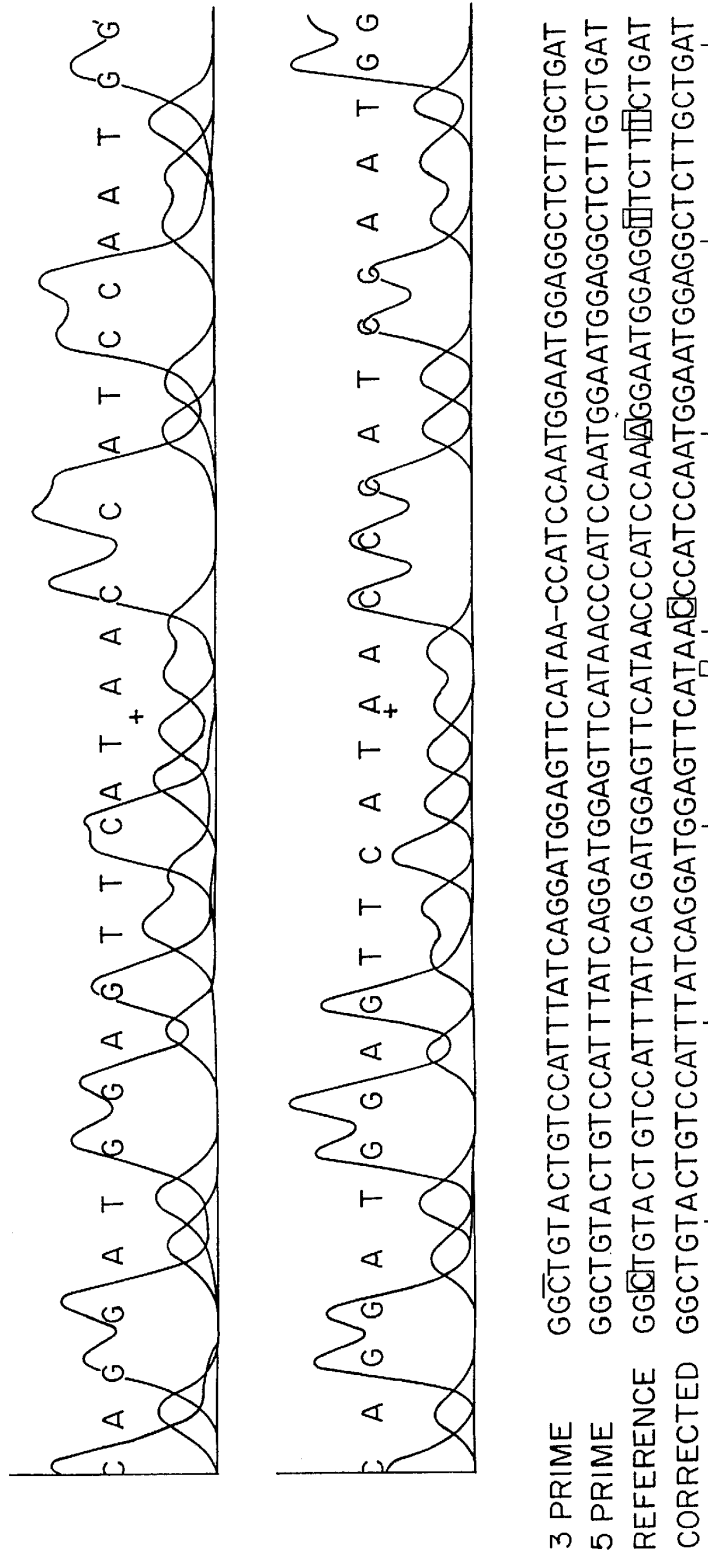
9 Claims, 6 Drawing Sheets

VGI TRUEGENE™
RQS ERROR DETECTION
3' 5' ERROR CORRECTION

300 BASES

3' 5'

3' 5'

3' 5'

3' 5'

3' 5'

3' 5'

RT

PROTEASE

FIG. 1

HOMBREW

750 BASE

RT

PROTEASE

FIG. 2

FIG. 3

3 PRIME   GGCTGTACTGTCCATTTATCAGGATGGAGTTCATAA-CCATCCAATGGAATGGAGGCTCTTGCTGAT

5 PRIME   GGCTGTACTGTCCATTTATCAGGATGGAGTTCATAAACCATCCAATGGAATGGAGGCTCTTGCTGAT

REFERENCE   GGCTGTACTGTCCATTTATCAGGATGGAGTTCATAACCCATCCAATGGAATGGAGGTTCTTCTGAT

CORRECTED   GGCTGTACTGTCCATTTATCAGGATGGAGTTCATAACCCATCCAATGGAATGGAGGCTCTTGCTGAT

FIG. 4

3 PRIME   CTGCTCTGGGAAGTTCAATTAGGAATACCACATCCCGCA-GGTTAAAACAGAGAGAAATCAGTAACAG

5 PRIME   CT--TCTGGGAAGTTCAATTAGGAATACCAACATCCGCAGGGTTAAAACAGAGAAATCAGTA[A]CAG

REFERENCE CT--TCTGGGAAGTTCAATTAGGAATACCA[CAT]CCCGCAGGGTTAAAA[A]AGAA[A]AGAA[A]AAATCAGTAACAG

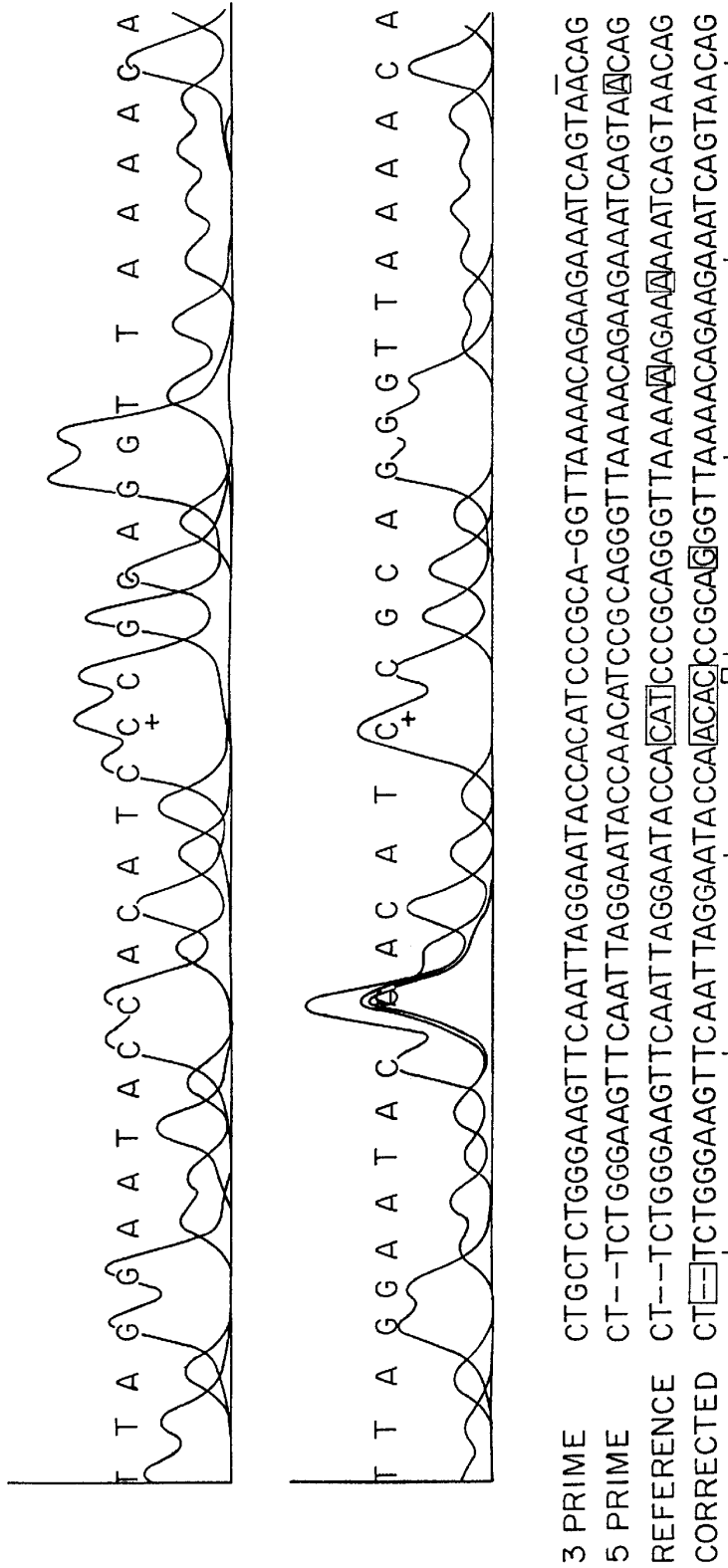CORRECTED CT[__]TCTGGGAAGTTCAATTAGGAATACCA[ACAC]CCGCA[G]GGTTAAAACAGAGAGAAATCAGTAACAG

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