(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 July 2001 (26.07.2001)

PCT

(10) International Publication Number
WO 01/53526 A3

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/IB01/00077

(22) International Filing Date: 23 January 2001 (23.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/490,273 24 January 2000 (24.01.2000) US

(71) Applicant *(for all designated States except US)*: **INGENEUS CORPORATION** [BB/BB]; Trident House, Broad Street, Bridgetown (BB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ERIKSON, Glen, H.** [CA/CA]; 937 Whittier Crescent, Mississauga, Ontario L5H 2X4 (CA). **PICARD, Pierre** [CA/CA]; 119 Braidwood Lake Road, Brampton, Ontario L6Z 4L4 (CA). **DAKSIS, Jasmine, I.** [CA/CA]; 36 Cartier Crescent, Richmond Hill, Ontario L4C 2N2 (CA).

(74) Agent: **TENER, David, M.**; Caesar, Rivise, Bernstein, Cohen & Pokotilow, Ltd., 12th Floor, Seven Penn Center, 1635 Market Street, Philadelphia, PA 19103-2212 (US).

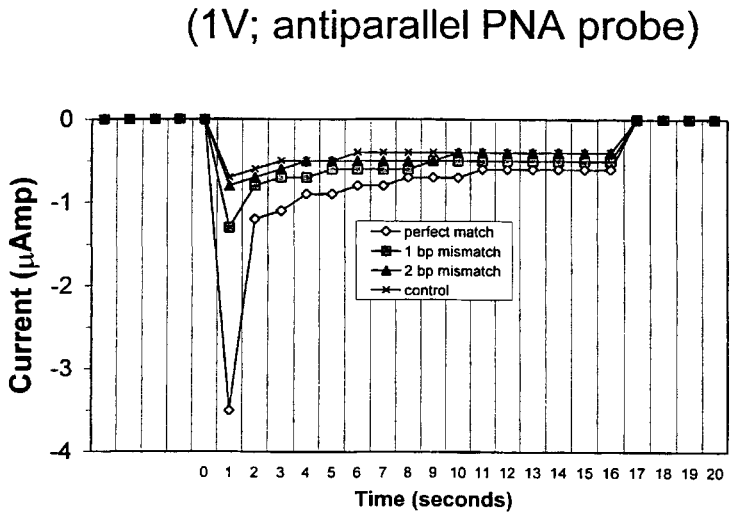
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) Title: HOMOGENOUS ASSAY OF DUPLEX OR TRIPLEX HYBRIDIZATION BY MEANS OF MULTIPLE MEASUREMENTS UNDER VARIED CONDITIONS



(57) Abstract: The invention provides homogeneous assay methods for nucleic acid hybridization, detection and evaluation. The assay includes obtaining signals from a test sample both before and during the application of a voltage to the test sample and correlating the signals, each of which is indicative of the binding affinity of the probe and the target to each other. The assay enables determining an extent of matching between the probe and the target, as the voltage can be calibrated so as to destabilize significantly any hybridization except perfectly complementary hybridization. The signals whose magnitude is correlated with binding affinity can be electrical conductance and/or fluorescent intensity. Preferably, both signal pairs are measured and compared so as to enhance the reliability of the assay. The assay can detect specific hybridization between single-stranded probes and non-denatured double-stranded targets to form triplexes, thus obviating the need to denature the targets. The assay methods can also be applied to duplex hybridization complexes.

WO 01/53526 A3

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
13 June 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.