

Robust SNP Typing Assay by Using ECA Technology

I. Out line of Technology

Exonuclease Cycling Assay (ECA) technology (patent pending: WO 2006/061994) provides you with the following advantages in single-nucleotide polymorphism (SNP) typing:

-Simplicity-

Although PCR products are used as samples, ECA, a microplate-based assay requires no PCR purification step. You need only the following simple procedures: 1) addition of two reagents to each PCR product, 2) incubation of the mixture at 37°C for 10-30 min, and 3) homogeneous detection of fluorescence signals (Fig. 1).

-Rapidity-

The incubation time is 30 min for standard end-point detection. This real-time detection method allows the incubation time to be reduced further.

-Low Cost-

The cost of the assay is currently 10-20 cents per SNP, which could be further reduced to 3-5 cents by introducing an efficient manufacturing system. Only a PCR machine and a fluorescence plate reader are required to carry out the assay manually.

II. Examples of SNP Typing

-NAT2 Genotyping-

N-acetyltransferase 2 (NAT2) is an enzyme participating in the metabolism of the anti-tubercular drug isoniazide. The Japanese population has 4 genotypes, NAT2*4 (WT), *5, *6 and *7, which can be determined by identifying 3 SNPs in the NAT2 gene. Fig. 2 shows the results of genotyping human genomic DNA samples by using the ECA method. These results were perfectly identical with results of a direct sequencing method.

-BRAF Mutation Detection-

BRAF is a proto-oncogene in which a point mutation is frequently found in sporadic colorectal and pancreas cancer. We attempted to detect a small population of mutant BRAF sequences in excess of normal sequences. For this purpose, samples consisting of plasmids with normal and mutant sequences were used. We detected as few as 2% of the mutant sequences (Fig. 3), indicating that the method could be applicable to clinical samples that contain both normal and cancer cells. The results of real-time fluorescence detection during the ECA reaction could further shorten the reaction time to 10-15 min by using a rate assay (Fig. 4).

III. Our Goal

Our goal is to launch a rapid (within 30 min, including amplification step) and high-throughput SNP typing system with high reliability that can be used for clinical diagnosis.

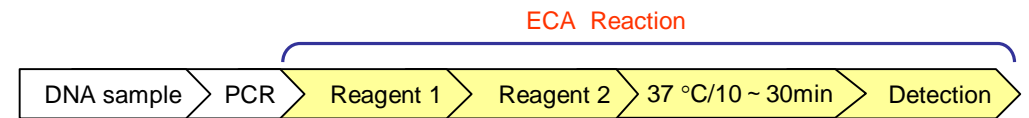


Figure 1. Assay procedure

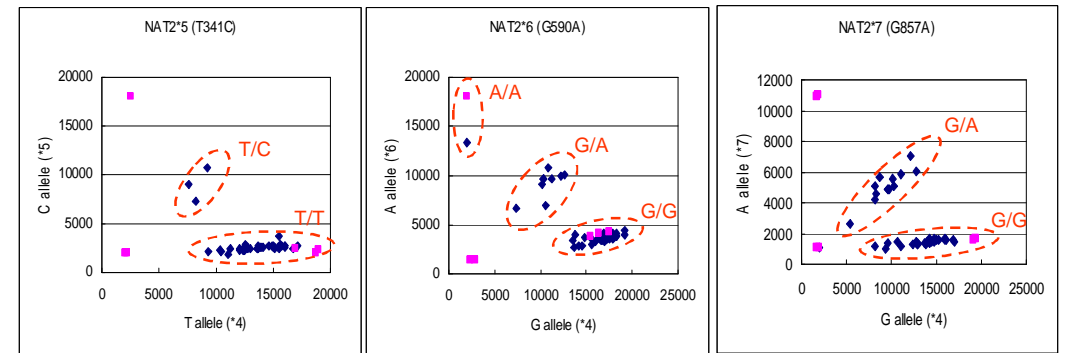


Figure 2. NAT2 genotyping of human samples (:human genomic DNAs, :control DNAs)

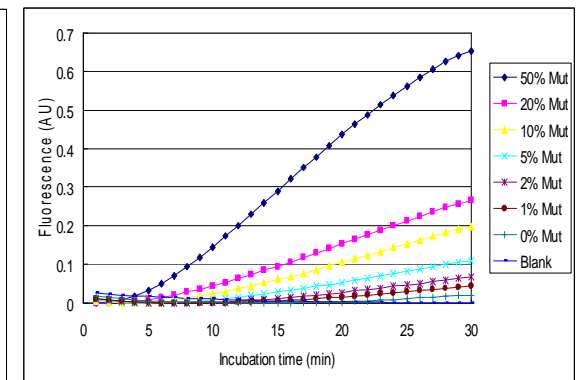
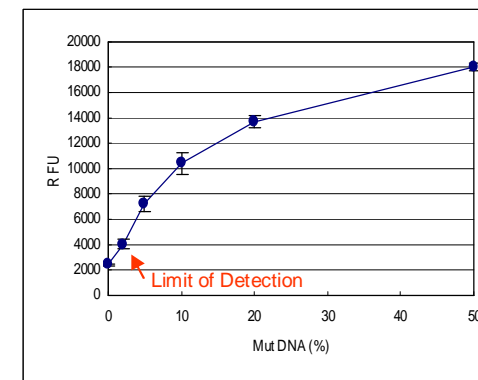


Figure 3. Detection of mutant BRAF sequence in mixed DNA samples (n=4, mean ± SD is indicated).

Figure 4. Detection of mutant BRAF sequence in mixed DNA samples (n=4, mean values are indicated).

Incubation time: 30 min. End-point detection. Incubation time: 30 min. Real-time detection.

Jan. 2007

GeneCare Research Institute Co., Ltd.

TAKESHI YAMAMOTO, Ph.D.

200 Kajiwara, Kamakura, Kanagawa 247-0063 JAPAN

Phone: +81-467-46-9590 Fax: +81-467-45-2871

E-mail: tyamamoto@gene-care.co.jp

URL <http://www.we-care-gene.com>