

Gene Polymorphism Typing Kits Based on ICAN Technology

Takara ICAN® GSTM1 Typing Kit

Remarkable research advances have been made recently on the relationship between genetic polymorphism and constitution. Differences in genetic polymorphism often now serve as the basis for predicting disease risk and selecting appropriate therapy and preventive measures. This has led to an increasing need to develop convenient and rapid methods for typing of genetic polymorphisms. Gene polymorphisms include single nucleotide polymorphisms (SNPs), insertion mutations, and deletion mutations. Various methods for typing of gene polymorphisms have already been developed. Most are two-step methods involving the conversion of base sequence information where the polymorphism is located to some type of signal, followed by detection of the signal.

ICAN and UCAN technology, developed by our company, also permits conversion of genetic polymorphisms to DNA extension or DNA amplification signals. This can be combined with various detection steps for use in typing of genetic polymorphisms.

This article introduces kit for typing of deletion mutations using ICAN technology.

Typing of Deletion Polymorphisms Using ICAN

1) Principles of ICAN Technology and Application in Typing of Deletion Polymorphisms

Genetic polymorphisms include various mutations: deletion, insertion and SNPs. Isothermal and Chimeric primer-initiated Amplification of Nucleic acid (ICAN) technology (Fig. 1) is effective for typing of gene deletion and insertion polymorphisms. ICAN is a method of isothermal amplification of genes using an

ICAN primer (DNA-RNA chimera primer), RNase H, and DNA polymerase. With incubation at a constant temperature (isothermal amplification), the DNA present in the sample can be detected with equal or higher sensitivity than with PCR. More specifically, for typing of deletion or insertion polymorphisms, ICAN primers are designed for part of the region of the deletion or insertion polymorphism to be typed. Then the presence or absence of amplification in the ICAN reaction is confirmed. In determining polymorphisms on the basis of amplification, one must rule out false negatives due to factors such as reaction errors, amplification inhibition, or DNA template preparation errors. False negatives can be distinguished by simultaneous amplification of an internal control such as the β -globin gene by Multiplex ICAN® for confirmation of the presence of a template and amplification reaction.

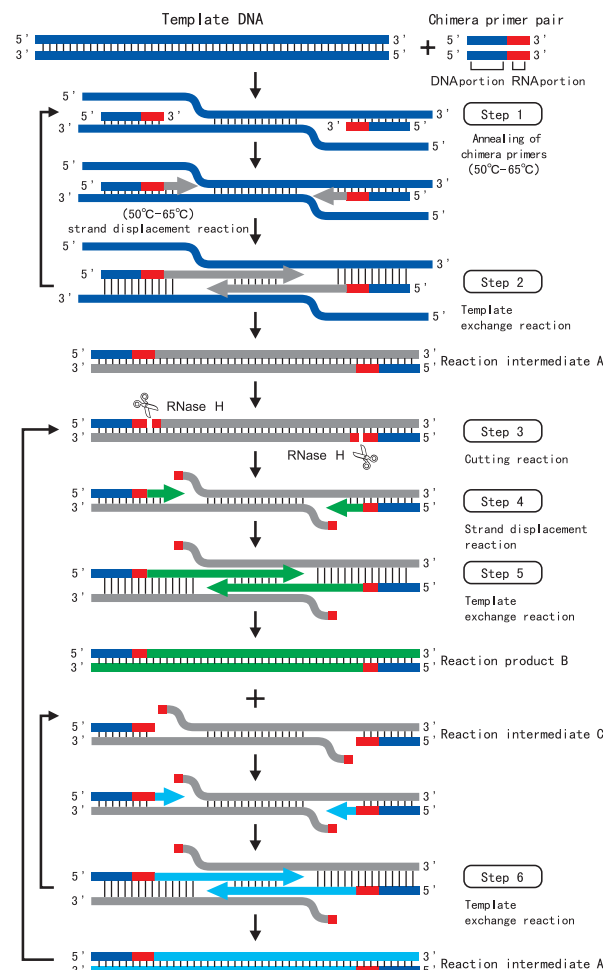


Figure 1. Principles of ICAN technology

2) TaKaRa ICAN® GSTM1 Typing Kit Ver. 1.0

We now introduce the TaKaRa ICAN® GSTM1 Typing Kit that was developed using this technology. Glutathione S-transferase (GST) is a class of detoxifying enzymes that catalyze conjugation reactions between glutathione and xenobiotics. These enzymes inactivate substances such as BP-dihydrodiol epoxide, activated oxygen species, and lipoperoxides. A homozygous deletion of GSTM1, a member of the GST family, is well known. About one half of Japanese people are believed to have some deficiency of GSTM1. Persons with GSTM1 deficiency have impaired detoxification capability. This has also been associated with an increased risk of lung cancer in smokers.

This kit includes ICAN and biotinylated ICAN primers for amplification of the GSTM1 gene by the ICAN reaction. If there is no deletion of the GSTM1 gene, the ICAN primer will anneal to the GSTM1 gene, and amplification will occur during the ICAN reaction. However, if there is deletion of the GSTM1 gene, no amplification will occur. The ICAN amplification products are detected with this kit by the following method (Fig. 2). First, the biotinylated amplification products are captured on an avidin immobilized plate. Then, the captured amplification products are denatured into single strands, hybridized to an FITC labeled probe that is complementary to the internal sequence, reacted with a POD labeled anti-FITC antibody, and colored with TMBZ for detection. If there is deletion of the GSTM1 gene, there will be no ICAN amplification products and thus no color reaction.

This kit also includes a primer for simultaneous amplification by the ICAN method of the β -globin gene (internal control), which is a housekeeping gene in humans. This allows typing for a GSTM1 deletion polymorphism and amplification of the β -globin gene in the same tube. After ICAN amplification, checking for false negatives can be performed by detecting for the presence or absence of β -globin amplification in a separate well from GSTM1 detection.

This kit was used for actual typing of a DNA sample extracted from the blood of volunteer subjects after informed consent. As shown in Fig. 3, GSTM1 deletion polymorphism typing was performed with good reproducibility. This example used a sample of DNA

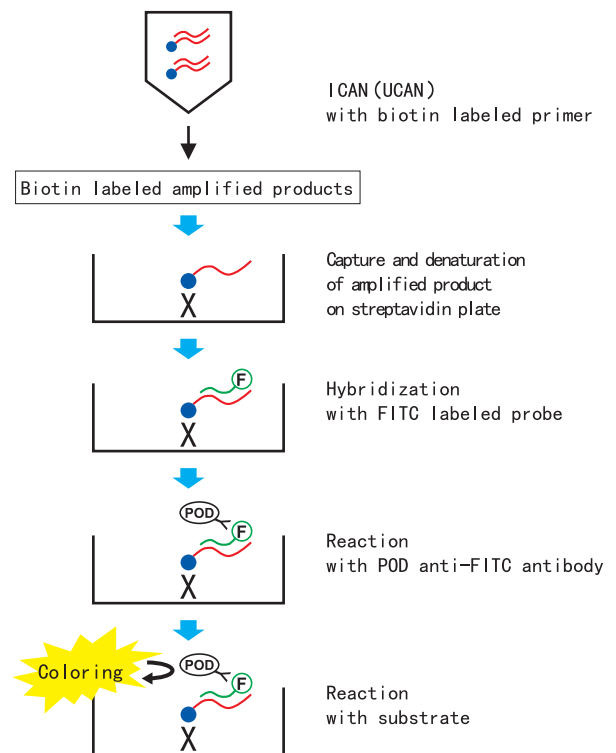


Figure 2. Detection with microtiter plate

extracted from peripheral blood, but the ability to perform similar typing with DNA extracted from the buccal mucosa was also confirmed.

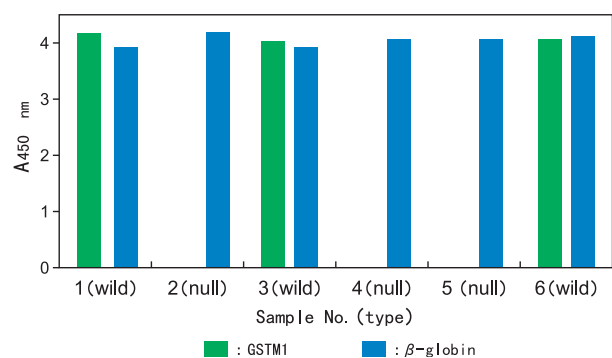


Figure 3. Typing of GSTM1 deletion polymorphism using the TaKaRa ICAN® GSTM1 Typing Kit Ver. 1.0