# Genotyping SNPs

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## Abstract

1) DNA was extracted from blood samples at DNA Chip Research Inc.

2) SNP genotyping: using the DigiTag2 assay.

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## Protocol

### Step 1.

The DNA was anonymously extracted from the blood samples (0.2 mL each) at DNA Chip Research Inc. (QIAamp® series; QIAGEN K.K., Tokyo, Japan).

### Step 2.

All SNP genotyping was performed using the DigiTag2 assay.

Target fragments including target SNP sites were prepared by multiplex PCR from genomic DNA. A multiplexed oligonucleotide ligation assay was performed, and a labeling reaction was achieved with two 5' query probes and one common probe prepared for a single SNP site.

The 5' query probes had a sequence complementary to the 5'-flanking region of the target SNP, and each of the probes had an allele-specific sequence.

Two types of end digit, CCGTGTCCACTCTAGAAAAACCT and ACCACCGCTTGAATACAAAACAT, were attached to each of the 5' query probes.

The 3' query probes were designed to possess a sequence complementary to the 3'-flanking region of the target SNP, and each of the probes had a first digit (D1) on its 3' end.

Next, a hybridization reaction with D1 probes on a DNA microarray (NGK Insulators, Ltd., Nagoya, Japan) was performed with separated areas.