Measuring Gene Quantities

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Deoxyribonucleic acid (DNA) consists of a characteristic double-helix formed by four nucleotides, called bases, which pair specifically with one of the other bases, in bonds called base pairs (Figure 1). The genes, blueprints of all organisms, are written by these four bases.

Measuring Genes by Amplifying Genes

How can we measure gene quantities? At present, for gene quantification, the fastest and most accurate method is a technique called "quantitative PCR". Quantitative PCR combines the polymerase chain reaction (PCR) method with measurement of genes using fluorescent dyes. The PCR method, which is a gene amplification method, amplifies a specific gene with a certain base sequence to twice its size by single reaction. Theoretically, by a continuous reaction (i.e. chain reaction), a single gene can be amplified infinitely (in practice, the reaction will be stopped by lack of available reagent, the heat degradation of enzyme activity, and so on). This technique allows even small quantities of genes, which had originally been impossible to measure, to be quantified with reasonable accuracy.

To measure the amplified gene, several fluorescent dyes are used. Usually, the time needed to reach the threshold fluorescence intensity in the PCR gene amplification is measured. If the original gene quantity before the amplification is small, this interval will be longer; if the original quantity is large, the threshold will be reached more quickly. By creating a graph showing the relationship between the predetermined standard gene quantities and the times to reach the threshold fluorescence intensity, and by measuring the time taken for an unknown quantity of a sample, it is possible to measure the gene quantity in the original sample (Figure 2). The special equipment needed to carry out quantitative PCR is commercially available (see photo).

This measurement technology is used to quantify the amount of genetically modified organisms (soybeans, corn, etc.) in foodstuffs. Future uses may include such medical applications as predicting the time for the onset of AIDS.

Photo
The left black device is a LightCycler®(Roche Diagnostics, GmbH, Germany) ; the right square one is a PRISM®-7900 (Applied Biosystems, USA).

At present, AIST is participating in the development of international protocols to support the reliability of genetic measurement using quantitative PCR, in cooperation with Consultative Committee of Amount of Substances (CCQM) under the Meter Convention.

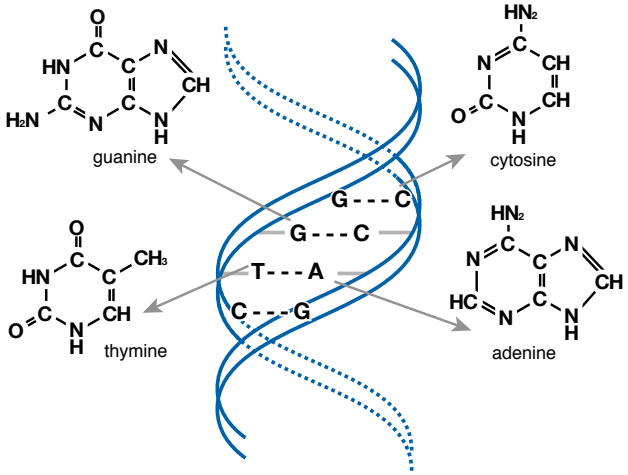

Figure 1 The structure of DNA
Genetic information is written by four bases: adenine, thymine, cytosine and guanine.

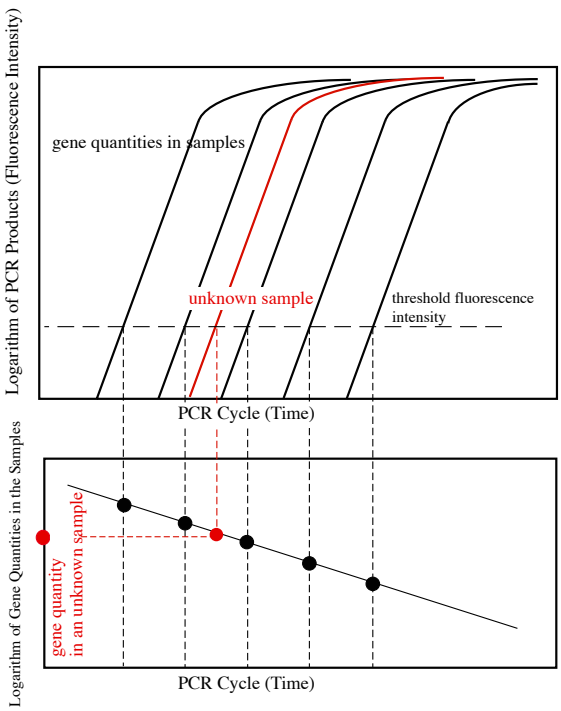

Figure 2 Principle of quantitative PCR
Using a genetic sample whose quantity is already known, the time to reach a threshold level of fluorescence is measured and plotted on a graph. This graph is then used to measure the quantity of the gene in the sample whose genetic quantity was previously unknown.