News Release

June 4, 2018

Ricoh
NARO
Nippon Flour Mills

Bioprinting Technology to Control the Number of DNA Molecules in Units of One

～ Contributing to high genetic testing accuracy using a reference material containing a known number of DNA molecules ～

This news release refers to a joint effort between Ricoh Company, Ltd., the National Agriculture and Food Research Organization (NARO), and FASMAC of the Nippon Flour Mills Group. The three organizations have collaborated using bioprinting technology to make a new reference DNA material in which the absolute number of the DNA molecules is controlled in units of one. The reference material can be used in the quality control of genetic testing instruments and reagents. A reference material contains quantitatively determined ingredients and is used as a measurement standard, but no other reference material contains DNA molecules whose quantity is controlled in units of one. The three organizations have developed a new method to produce reference DNA material, which enables production of reference materials suitable for genetic testings to detect specific DNAs, as in the inspection of GMO foods, cancers, and infections. The new reference DNA materials will increase the reliability of the tests.

These achievements will be announced in the Bio International Convention in Boston, USA (June 4-7th) and Biotech Japan 2018 in Tokyo, Japan (June 27-29th).

Polymerase chain reaction (PCR) is widely used in genetic testing. Reportedly, a PCR-based method can detect even a single DNA molecule by amplification. This high sensitivity is a useful factor and the method is widely used in inspections of GMO (genetically modified organism) foods, cancers, and infections. Some severely strict inspections are not allowed overlook any of the specific DNA sequences (target genes). Thus, it is important for testing laboratories to implement thorough quality control over the testing equipment, the reagents, and the detection method as a whole. Some companies and research institutes have delivered reference material whose DNA types and densities are prescribed, but they are of high densities i.e. the number of DNA molecules is prescribed in mol (one mol is equivalent to 6.02×10^{23} DNA molecules). For use in low-density tests at an accuracy of 100 molecules or less, the reference material generally must be diluted.

Thus, errors can occur in DNA molecule densities during the diluting process. The diluted samples may contain more DNA molecules than prescribed, or conversely no DNA molecules at all, when the required number of DNA molecules is less than ten.

NARO has developed a genetically modified yeast that contains target gene sequences. The genetically modified yeast is injected using Ricoh's bioprinting technology onto wells on a plate, with the number of molecules determined and controlled in units of one. The resulting reference DNA material (reference DNA plate) contains the prescribed number of DNA molecules of a specific gene sequence. It has been impossible to count the number of DNA molecules directly but it can now be indirectly counted by handling cells containing the target gene sequence (yeast, in this example). Using bioprinting technology to handle the cells enables reference DNA material to be produced efficiently.

NARO, FASMAC, and Ricoh have conducted a joint evaluation using real-time PCR; they have demonstrated that materials can be produced with an unprecedented calibration curve (a graph of measurement results based on reference material, which sets the standard for measuring material densities) in the low-density range of 1 to 1,000 molecules.

For the technology component of bioprinting, which uses inkjet technology, Ricoh has been developing an inkjet head to deliver cells stably and a technology to count the number of cells in the delivered droplets.

NARO and FASMAC have long been dealing with technological development regarding genetic testings, development of a single-molecule reference DNA material, and international standardization of test methods, particularly in the area of GMO food inspection.

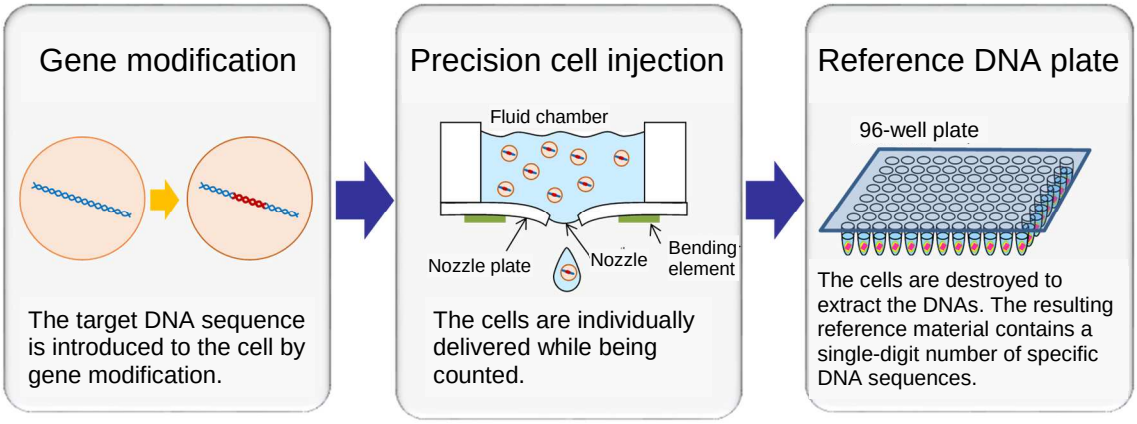
The joint development effort has resulted in a new production method and a new reference DNA material, which will enable stricter quality control of genetic testing instruments, reagents, and genetic testing methods. The material will be useful in improving the accuracy of GMO food inspections and preventing cancers and infections from being overlooked. Furthermore, the technology will contribute to solving larger social issues.

<Technical Highlights of the Reference DNA Material>

1. A newly developed process to manufacture reference DNA material

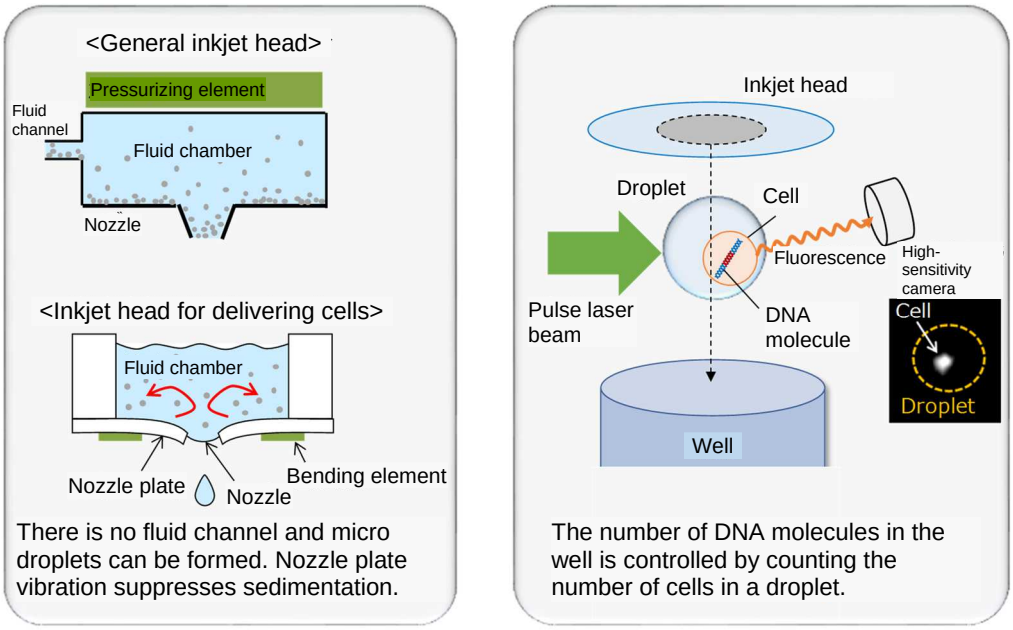
In the newly developed process for the manufacture of reference material, cells are genetically modified to have a target sequence inserted in them enabling the number of DNAs containing the target sequence to be counted. The process uses a 96-well plate, which is the most commonly used in genetic testing. The genetically modified cells are counted and delivered from a special bioprinting inkjet head to each of the 96 wells. In the end, the cells are destroyed to extract the

DNAs. That way, a reference DNA plate is manufactured with a determined number of DNA molecules in each well. The technology exploits the high-speed droplet delivery of an inkjet head and allows reference material to be produced efficiently, with the number of DNA molecules strictly controlled.



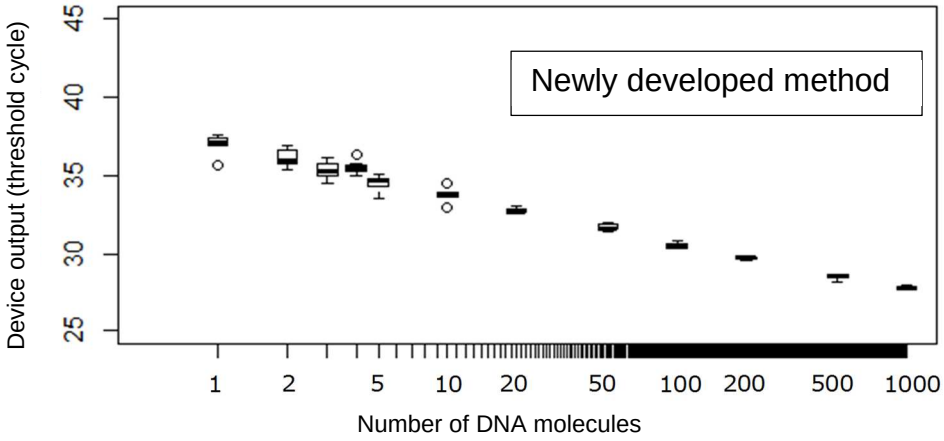
2. Delivering cells from an inkjet head and counting cells in mid-air droplets

Cells are as large as 10 μm and can cause sedimentation and nozzle clogging, so it is not easy to deliver them from a general inkjet head stably. Ricoh has developed a special bioprinting inkjet head for delivering cells. The head has a simple construction without a fluid channel, yet it can deliver fluids in small amounts of solution. Ricoh has also developed a new technology to irradiate the delivered droplets with a pulse laser beam and thus observe the fluorescence of the cells and count the cells in the droplets. The technology enables the droplets to be stably injected into the wells while the cells are counted. Finally, the number of cells can now be strictly controlled.

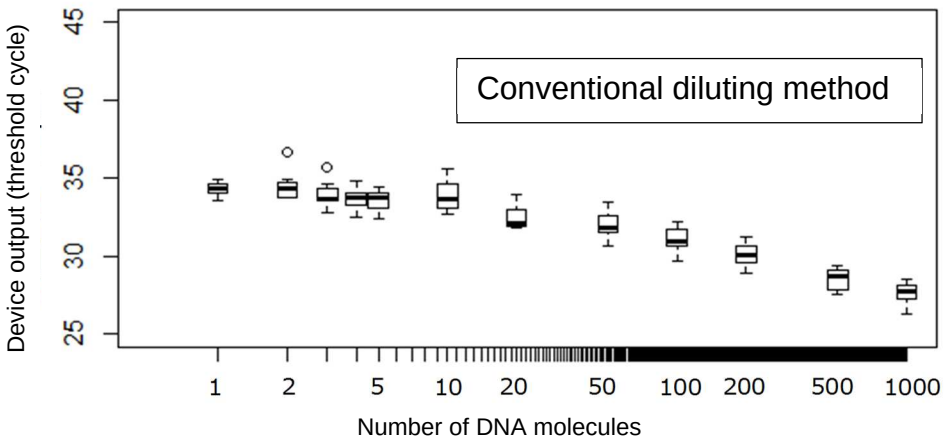


3. Evaluation of the manufactured reference DNA material

A real-time PCR device was used to evaluate the threshold cycle (the output value of the real-time PCR device, indicating how many times of amplification is required for detection) for 1 to 1,000 DNA molecules. In the range of 100 or fewer molecules, the samples manufactured by bioprinting had better properties than those manufactured by the conventional diluting method because they had a better linearity and less dispersion between repeated measurements. The reference materials manufactured by the newly developed method are expected to enable stricter evaluation and accuracy control of real-time PCR, thermal cyclers, and reagents.



Evaluation of reference DNA plate by real-time PCR



Evaluation of diluted sample by real-time PCR

As part of its growth strategy, Ricoh has announced that it will focus its efforts on *applied printing*, a category where the possibilities of printing technologies expand. Ricoh will continue to utilize its bioprinting technology. Expanding on the new technology to strictly control the number of DNA

molecules and arrange the cells, Ricoh will continue to develop element technologies to pattern the cells in two dimensions and to stratify them in three dimensions. Ricoh will create new value in the biomedical field.

Ricoh is committed to providing solutions to healthcare and other social issues by fully using its proprietary technologies through collaboration with its partners.

NARO has been developing and standardizing methods of testing GMO foods in a joint effort with related organizations. In addition to the methods of testing GMO foods, it has been conducting technological development to contribute to the quality control of genetic tests and actively working to globally standardize food test methods using technologies based on molecular biology.

NARO will continue to contribute to society through technological development for the safety and reliability of agricultural products and foods.

FASMAC, since its establishment in 2001, has been developing technologies of Japan's standard method of analyzing GMO foods and food allergens in a joint effort with the Ministry of Agriculture, Forestry and Fisheries; the Ministry of Health, Labour and Welfare; and their related organizations. The analysis technologies are used in the USA and the People's Republic of China as well as in Japan.

FASMAC is also positive in globally standardizing the food testing methods using technologies based on molecular biology.

Food safety and security is a socially important proposition. FASMAC will strive to ensure food safety and security with its world-level analysis technologies.

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