C297-E131

SHIMADZU
Excellence in Science

Technical Report

Utilization of CELL PICKER and MCE-202 MultiNA in the field of genome editing

Toru Kaji[1], Toru Ezure[1], Hidesato Kumagai[1], Takashi Inoue[1]

Abstract:

The use of genome editing technology has spread rapidly since the introduction of CRISPR/Cas9 and is expected to advance research in a broad range of fields, such as animal breeding and healthcare. Genetic modification methods based on genome editing offer dramatically higher modification efficiency than previous methods that depended on natural random recombinations. This article describes an example of the use of Shimadzu CELL PICKER and MCE-202 MultiNA systems within the workflow for cloning a target group of cells modified with gene editing

Keywords: Genome editing, genetic modification, cell, cloning, automation, electrophoresis

1. Introduction

Genome editing refers to a genetic modification technology in which artificial nucleases, designed to break in a particular location in the genome, are used to create mutations by deleting, inserting, or replacing portions of a genome sequence. The main artificial nucleases used as genome editing tools to date are zinc-finger nuclease (ZFN), transcription activator-like effector nuclease (TAL effector nuclease), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9). In particular, CRISPR/Cas9 has spread rapidly due to the ease of designing target sequences. This technology offers dramatically higher modification efficiency than previous methods that depended on natural random recombinations. Given such benefits, it is expected to advance research in a wide range of fields, including breeding and healthcare.

On the other hand, there are many tedious steps involved in introducing intended genetic modifications by genome editing, so a more efficient method is needed. This article therefore describes an example of the use of Shimadzu CELL PICKER and MCE-202 MultiNA systems within the workflow of genome editing to introduce genetic modifications in cells.

2. Workflow for Cloning by Genome Editing

The workflow for establishing clones of target cells with genome editing is shown in Fig. 1. It includes (1) modifying the gene by genome editing, (2A) cloning the gene by colony separation, (2B) cloning the gene by limiting dilution, (3) culturing the clones, (4) confirming that mutations were introduced, (5) verifying the genes, and (6) isolating the clones. In the context of these steps, CELL PICKER can be used for cloning by the colony separation method in step (2A), and MCE-202 MultiNA can be used to confirm that mutations were introduced in step (4).

Though omitted in this article, a cell sorter is also sometimes used for cloning genes by limiting dilution in step (2B). (Refer to Life Sciences Application Note No. 47.)

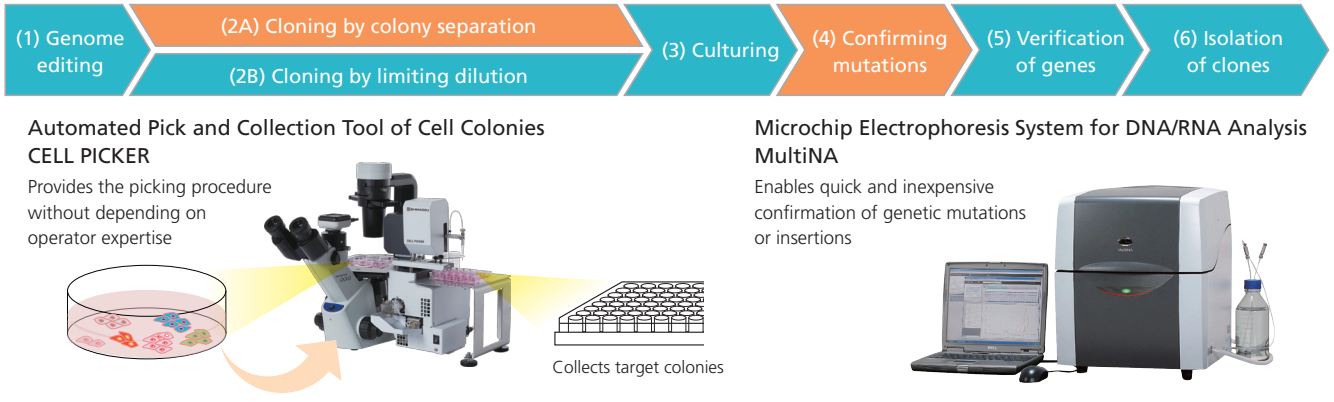


Fig. 1 Summary of the Workflow for Cloning Using Genome Editing

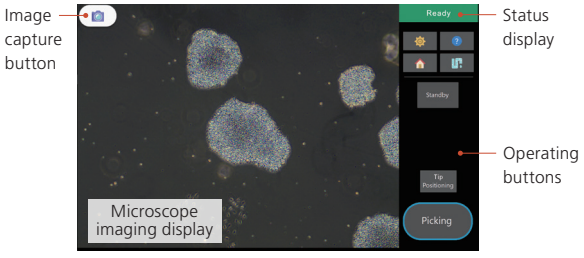
1 Analytical & Measuring Instruments Division

3. CELL PICKER

CELL PICKER can be used to automate the conventionally manual step of picking and removing cell colonies using a pipettor. The CELL PICKER system is shown in Fig. 2. It comprises a picking unit, microscope, and microscope camera.

Fig. 2 CELL PICKER

As shown in Fig. 3, CELL PICKER is operated using a tablet computer while viewing and recording images from the microscope. Operations are easy. Simply identify the target cells and press the [Picking] button. The instrument also automatically records images from the microscope before and after picking, enabling efficient operation regardless of the user's skill level.



Enables picking while viewing/recording cells

Fig. 3 CELL PICKER Operating Screen

Fig. 4 shows the picking time for 48 colonies compared to the time for manual operations. Because CELL PICKER remains consistently efficient throughout the procedure (2 minutes per colony in this verification test) regardless of the elapsed time, it requires only about half the time needed for manual operations. As a result, the efficiency of the entire process is significantly improved.

Whereas manual operations can vary depending on the skill level of individual operators with regard to pipettor sorting force or suction speed, CELL PICKER provides a picking procedure comparable to manual operations but with highly reproducible efficiency. Pipette tips can be positioned at target cell colonies easily, which minimizes operational inconsistencies that can result from inexperienced operators or shaky hands.

Fig. 5 shows an example of the picking of iPS cells. This confirms that the system is able to pick the target colonies. Fig. 6 shows the results from an evaluation of cultures to check the impact from picking. After picking, cells grew steadily without any signs of damage or contamination from picking. The expression of undifferentiated markers at Day 6 also confirms that the undifferentiated state was maintained.

Comparison of Time Required for the Picking of 48 Colonies

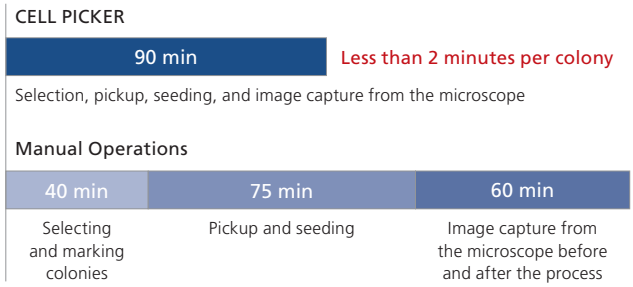


Fig. 4 Comparison of Operational Efficiency

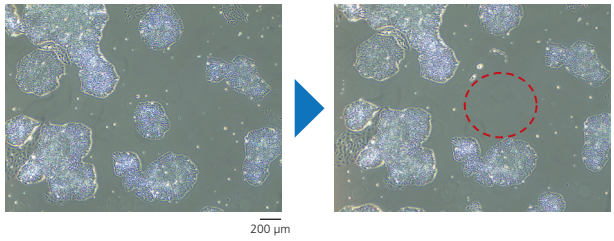
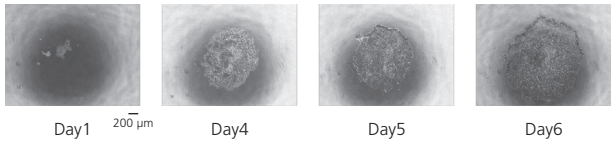


Fig. 5 Picking of iPS Cells

Time-Lapse of Cell Culture after Picking



Immunostaining Results

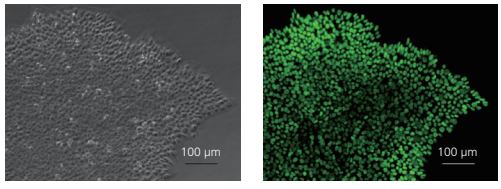


Fig. 6 Confirmation of Cell Culture after Picking

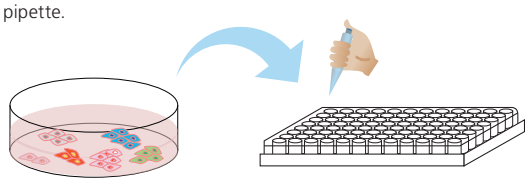
4. Recovery of Target Colonies Using CELL PICKER

In the process of establishing clones of target cells using genome editing shown in Fig. 1, (1) gene modification by genome editing is followed by either (2A) cloning by colony separation or (2B) cloning by limiting dilution. As shown in Fig. 7, the colony separation method clones cells by seeding with a diluted cell concentration, and using a pipette to pick the colonies formed. The issue with this process is that the operations are tedious and require skill. CELL PICKER however replaces these steps, thereby promoting a more efficient overall process.

Similarly, the limiting dilution method involves adjusting the concentration and dispensing cells into each well so that there is one cell per well before cloning. This method is dependent on probability and the steps are tedious and labor-intensive. Consequently, a cell sorter is often used. (Refer to Life Sciences Application Note No. 47.)

Colony Separation Method

Cell concentration is diluted before seeding, and then colonies are picked up with a pipette.



Limiting Dilution Method

Concentration is reduced to one cell per well before dispensing.

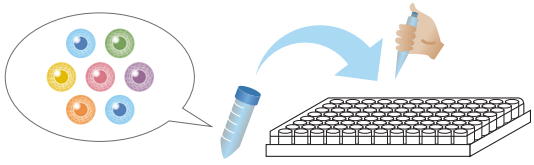


Fig. 7 Colony Separation Method

5. MCE-202 MultiNA

MCE-202 MultiNA (Fig. 8) is a fully automatic electrophoresis system. It features a microchip (Fig. 9) with microchannels that can be used to quickly and easily confirm the size and quantity of DNA/RNA. Given that microchips can be reused, up to 108 samples can be analyzed automatically by configuring the software with the number of samples and then placing specialized reagent and samples in the instrument.

Typically, electrophoresis is dependent on visual determinations, but the MCE-202 MultiNA makes it easy to obtain objective numerical data (Fig. 10). The system also enables high separation and high sensitivity detection, so that samples difficult to detect using conventional methods can be analyzed. Due to its convenience, the system is used in a wide range of applications, not only for genetic research, but also for analyzing foods, microorganisms, and infectious diseases.

Fig. 8 MCE-202 MultiNA

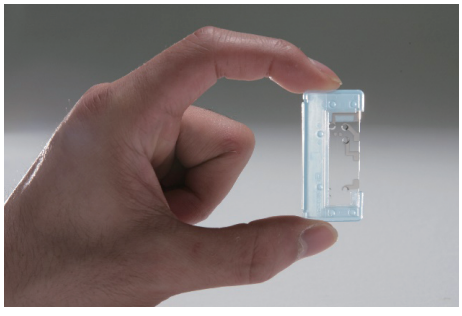


Fig. 9 Microchip

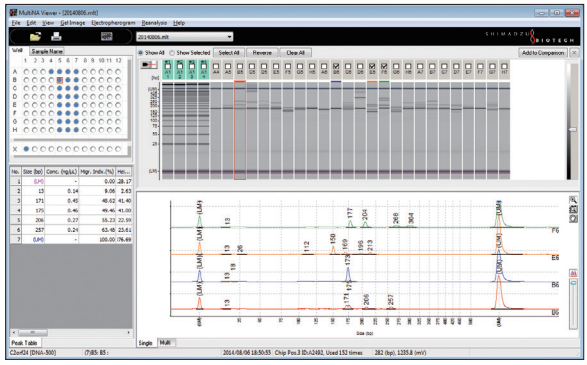


Fig. 10 Analytical Results

6. Using MCE-202 MultiNA to Confirm Mutations

The process of confirming the introduction of mutations in step (4) of the cloning workflow using genome editing, shown in Fig. 1, requires confirmation that the intended genetic mutation was actually introduced in the gene. This section describes how to efficiently confirm whether or not the mutation was introduced by using the MCE-202 MultiNA for heteroduplex mobility assay (HMA).

Mutations created by non-homologous end joining, for the purpose of knocking out a gene, result in tiny deletions or insertions of one to several dozen bases. Confirmation of these mutations requires an analysis of the DNA base sequence of the mutation area. However, due to the expense and effort generally required for DNA base sequencing, it is more efficient to sort specimens in advance. Heteroduplex mobility assay provides a simple and effective sorting method that can be accomplished with the polymerase chain reaction (PCR) method and electrophoresis near the mutation.

If double-stranded DNA is electrophoresed, the migration distance of fully complementary homoduplex DNA depends on the molecular weight. However, with heteroduplex DNA, in which there are mismatches in some areas, the steric structure of the areas with a mismatch differs from that of the homoduplex DNA, and this impedes the speed of migration. The heteroduplex mobility assay process makes use of this phenomenon to detect the presence of mutations.

As shown in Fig. 11, reannealed products corresponding to wild homoduplex DNA, mutant homoduplex DNA, and heteroduplex DNA are obtained by applying PCR, heat denaturation, and reannealing to the amplified products near the target sequence in the sample with mutations introduced. The genotype can then be identified based on the electrophoresis patterns, by analyzing the products of reannealing with electrophoresis in an MCE-202 MultiNA system.

In this way, using the MCE-202 MultiNA system for heteroduplex mobility assay makes it easy to confirm the introduction of mutations by genome editing (Fig. 12).

7. Summary

Due to revolutionary advances in genome editing tools, the process of genetic modification itself is becoming easy. However, the cell cloning process after genetic modification is still tedious and requires expertise. This article has described Shimadzu's genome editing solutions for improving the efficiency of tedious cloning operations.

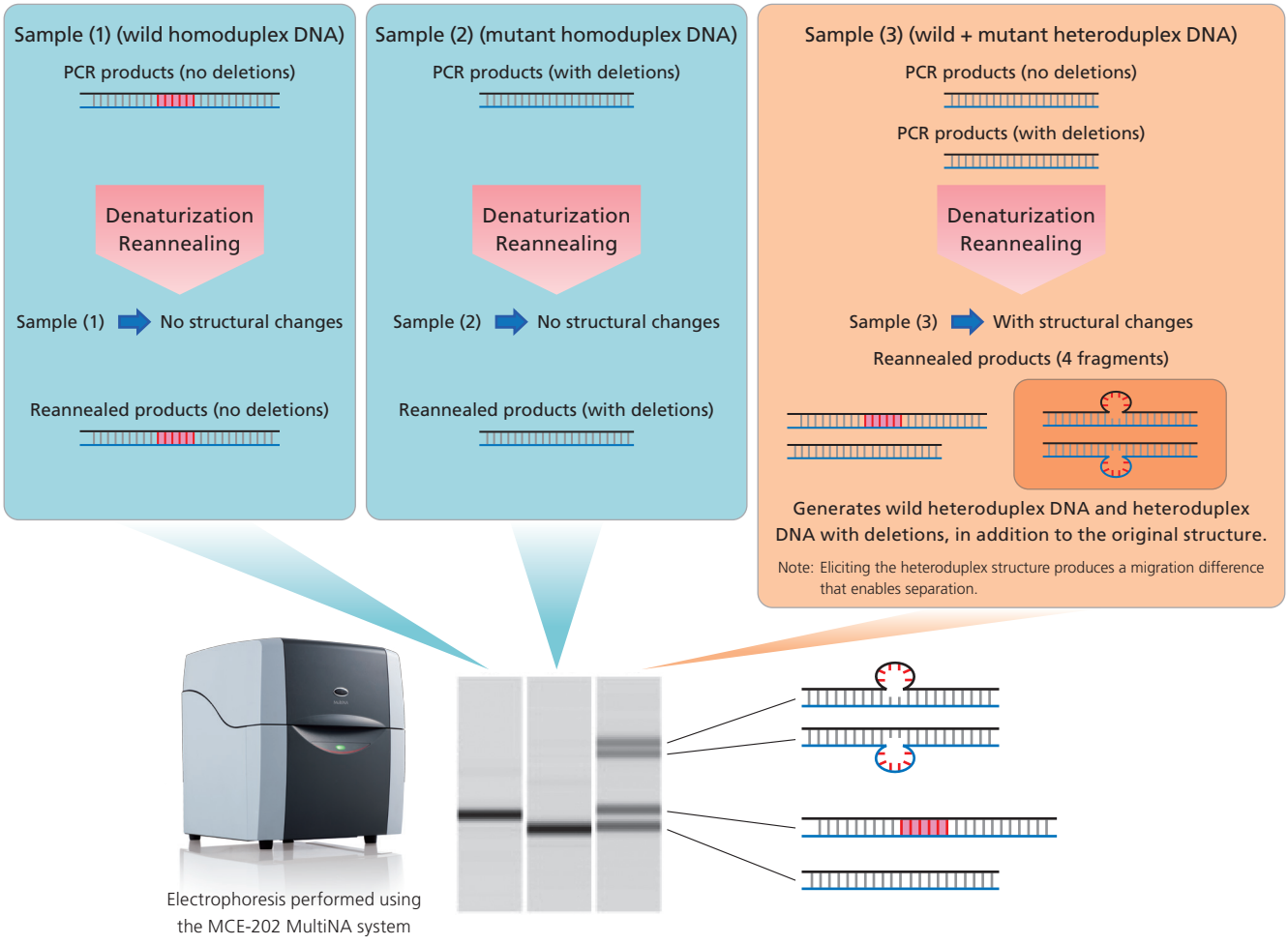


Fig. 11 Heteroduplex Mobility Assay Using MCE-202 MultiNA System

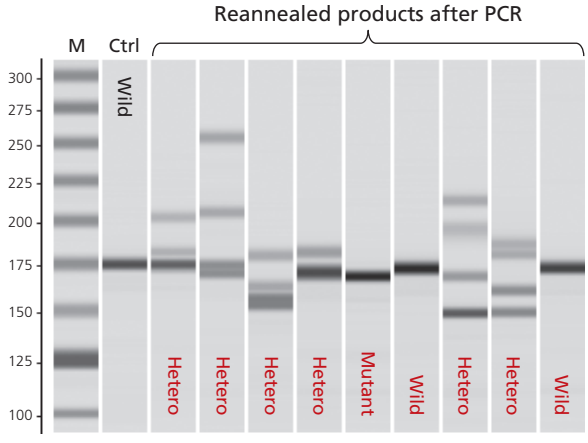


Fig. 12 Example of the Sorting of Samples with Mutations Introduced by Genome Editing

If multiple bands are detected, this indicates heteroduplex DNA. If there is one band, then wild and mutant forms can be differentiated based on the detected band position.

First Edition: November, 2019

Shimadzu Corporation
www.shimadzu.com/an/