TMiMS Profiling Genome Editing Outcomes in Individual Human iPS Cells and Cultured Cells

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Abstract

Grasping outcomes of genome editing can be critical for its applications in therapies and basic science. However, the majority of existing methods to analyze genome editing outcomes are based on cell populations but not on individual cells. Therefore, we have developed a new method utilizing an automated single-cell dispensing device, SPiS, to isolate genome-edited single human cultured cells, and profiled genome editing outcomes in more than 2,600 clones (Takahashi, STAR Protoc 4:102364 2023). We found that genome editing often either happens in all the target alleles or does not happen at all in individual cells (Takahashi, iScience 25:105619 2022).

The same single cell cloning strategy could not be applied to human iPS cells due to their high mortality as single cells. Therefore, we have developed yet another new method to efficiently isolate genome-edited iPS cell clones, where iPS cell clusters derived from single cells are grown in extracellular matrix domes to be robotically transferred into 96-well plates by Cell Handler. With this method, we have been able to analyze genome editing outcomes in more than 1,000 iPS cell clones. We found that the all-or-nothing nature of genome editing was also evident in individual human iPS cells. Furthermore, we found that the same insertions or deletions tend to occur in individual human iPS cells. Our findings lead to a better understanding of the profiles of genome editing outcomes and their applications.

Genome Editing Induction Is Binary in Human Cultured Cells

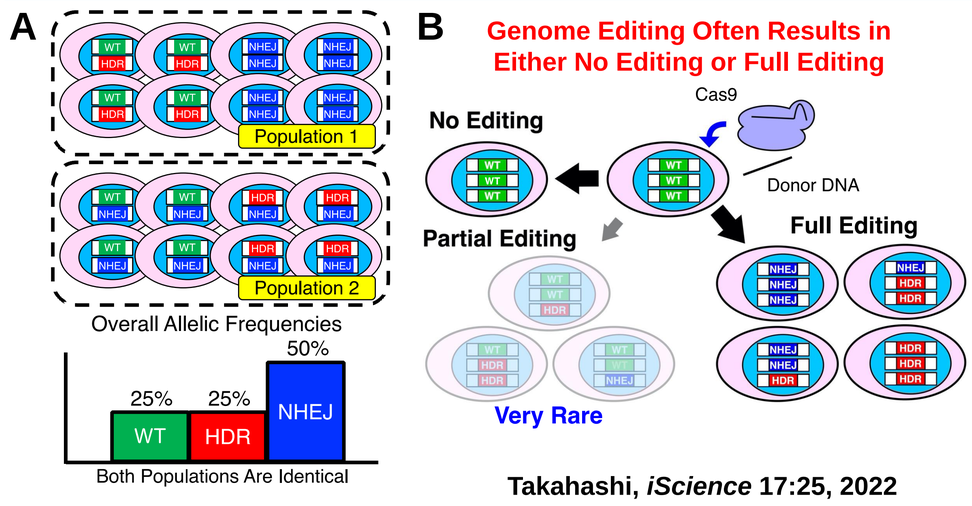
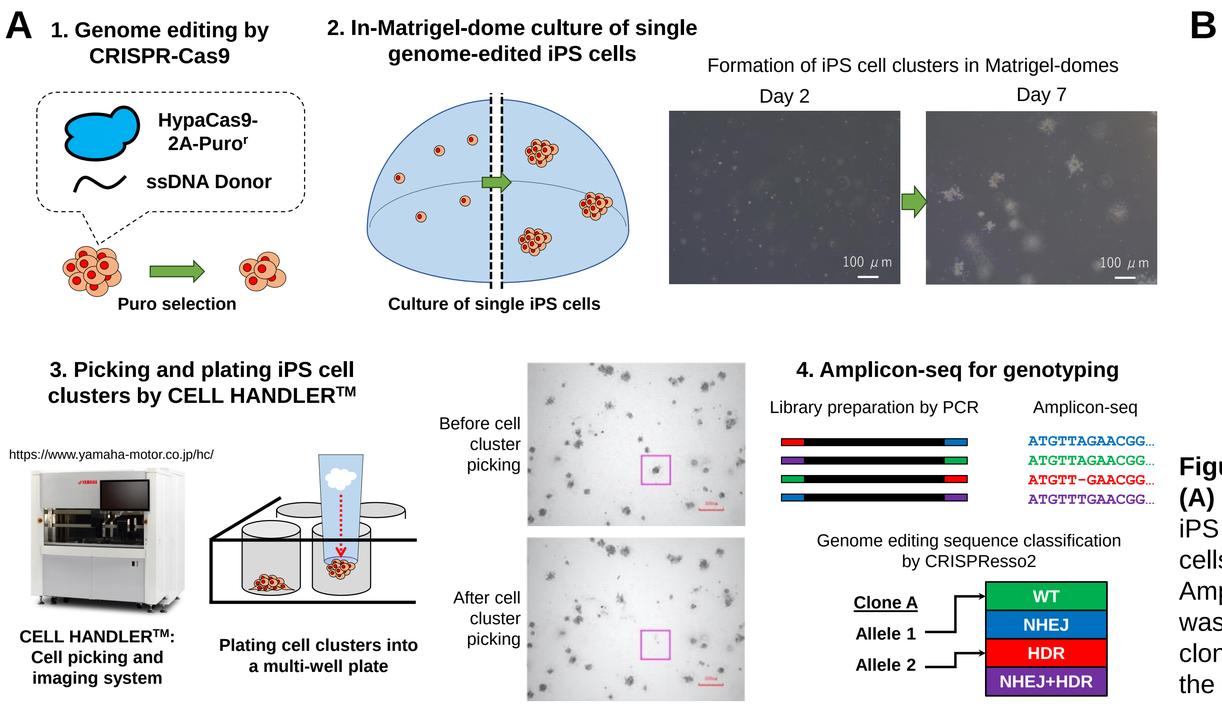


Figure 1. Genome editing outcomes in single cells.
(A) A hypothetical situation that emphasizes the importance of the analysis of genome editing outcomes at the single cell level. Cell populations 1 and 2 consist of cells with totally different genotypes individually. However, the total allelic frequencies of WT, HDR, and NHEJ are exactly the same for both populations. **(B)** Human cultured cells tend to undergo either no genome editing at all or full editing in all the target alleles in single cells (binary manner).

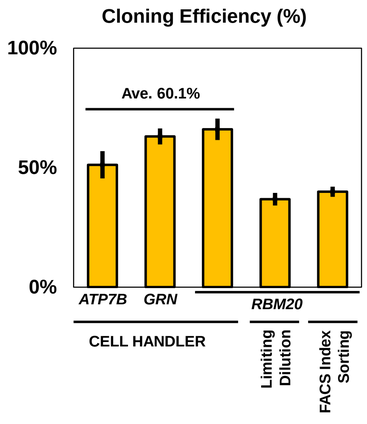
Efficient Robotic Isolation of Genome-Edited iPS Cell Clones Grown in Matrigel Domes



B

iPS cell cloning efficiencies in 96-well plates

Target Edit	Exp.	Wells with clones / Plated wells	Cloning efficiency (%)
ATP7B R778L	N1	181 / 288	62.8
	N2	103 / 233	44.2
	N3	81 / 174	46.6
	Total	**365 / 695**	**53.0**
GRN R493X	N1	174 / 264	65.9
	N2	159 / 240	66.3
	N3	137 / 240	57.1
	Total	**470 / 744**	**62.3**
RBM20 R636S	N1	204 / 288	70.8
	N2	168 / 240	70.0
	N3	165 / 288	57.3
	Total	**537 / 816**	**66.4**



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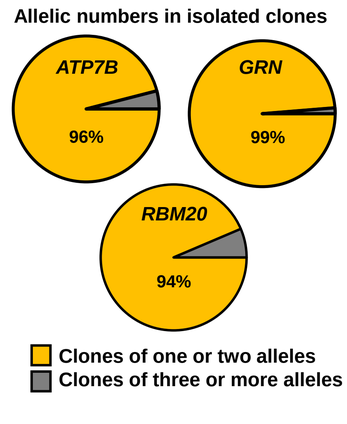


Figure 2. Development of an efficient method to isolate human iPS cell clones.
(A) Method for isolation of a large number of genome-edited iPS cell clones in Matrigel domes. Genome-edited single iPS cells are cultured to form cell clusters in Matrigel domes to compensate for cell death caused by dissociating iPS cells. CELL HANDLER™ was used to recognize and pick iPS cell clusters and plate them into 96-well plates. Amplicon sequencing determined genotypes of isolated clones. (**B**) Efficiency of the Matrigel dome culture method was compared with that of limiting dilution and FACS index sorting. (**C**) Estimated allelic numbers in isolated iPS cell clones. When the number of alleles a clone has is one or two, the clone is likely to be derived from a single cell. On the other hand, when three or more alleles are detected, the clone may be a non-clone derived from multiple cells.

Profiles of Genome Editing Outcomes in Human iPS Cells

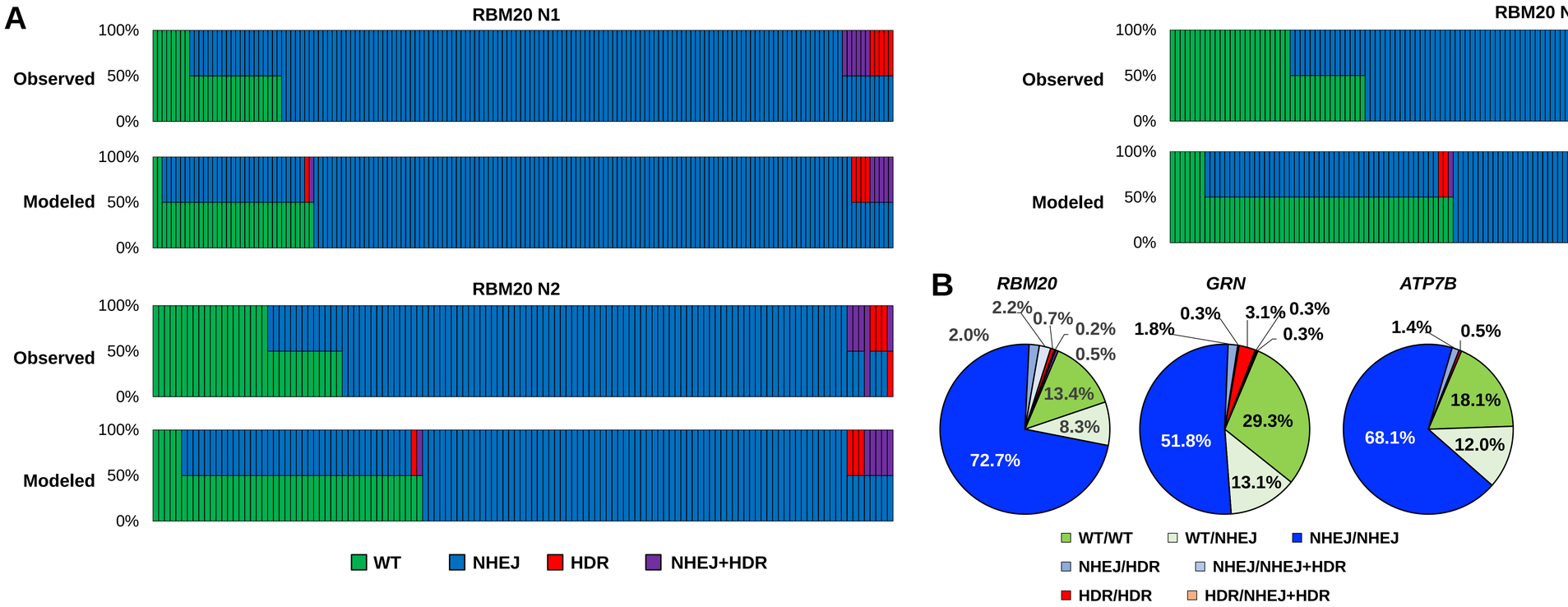
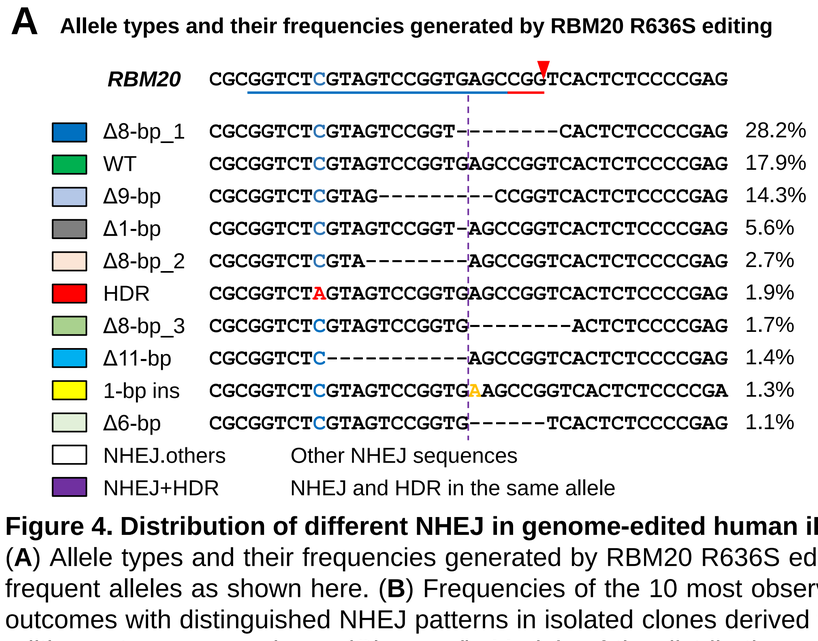


Figure 3. Profiles of genome editing in RBM20 in human iPS cells.
(**A**) Genome editing outcomes in isolated clones derived from single human iPS cells edited by Cas9. RBM20 editing outcomes are shown (Observed). Models of the distributions of clones with different genotypes assuming different alleles are randomly distributed at the observed frequencies are also shown (Modeled). Each bar represents one clone, and the genotypes of WT (green), NHEJ (blue), HDR (red), and HDR + NHEJ (purple) in one clone are also shown in each bar. (**B**) Proportion of genotypes of genome-edited human iPS cell clones. Almost no WT/HDR and WT/NHEJ+HDR clones were isolated.

Profiles of NHEJ in Human iPS Cells



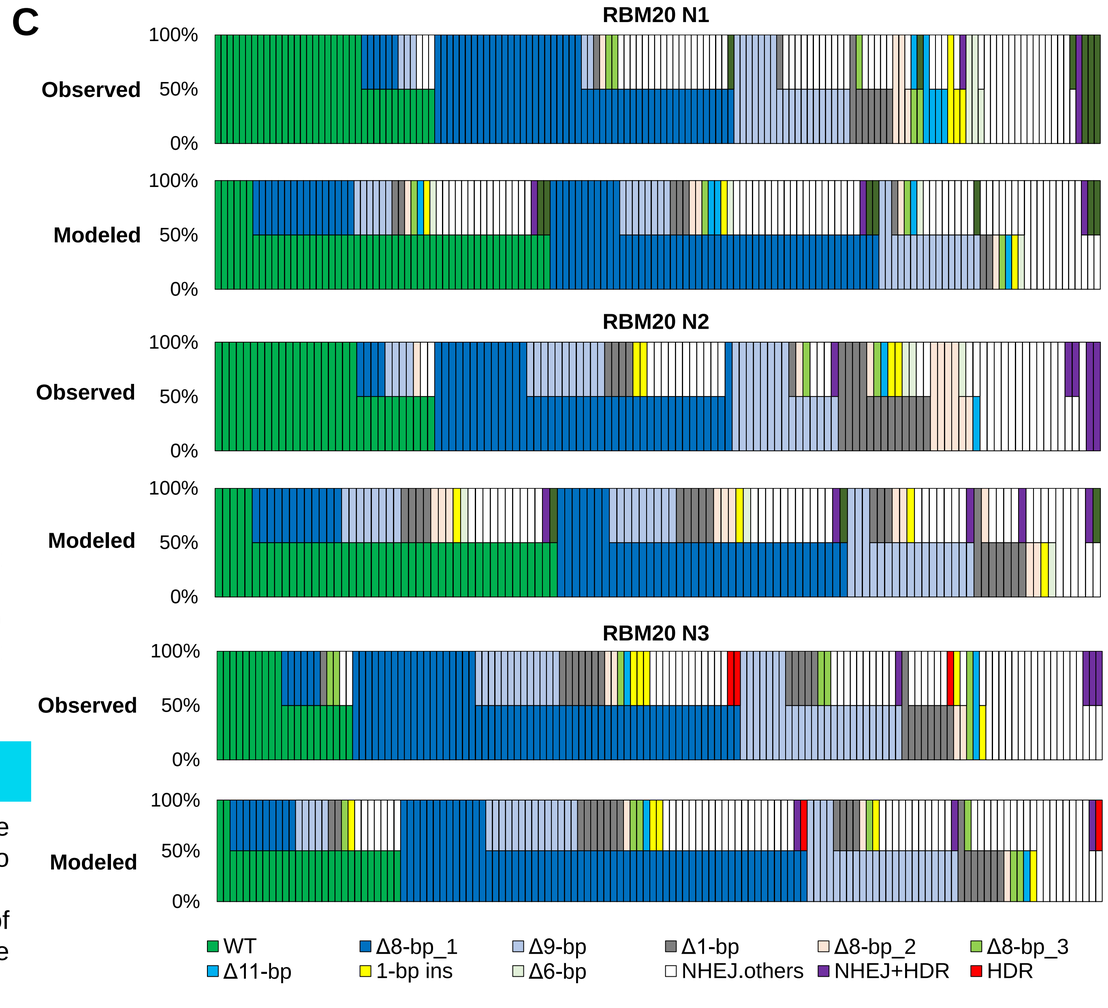


Figure 4. Distribution of different NHEJ in genome-edited human iPS cell clones.
(**A**) Allele types and their frequencies generated by RBM20 R636S editing. After RBM20 editing, we observed the 10 most frequent alleles as shown here. (**B**) Frequencies of the 10 most observed alleles after RBM20 editing. (**C**) Genome editing outcomes with distinguished NHEJ patterns in isolated clones derived from single human iPS cells edited by Cas9. RBM20 editing outcomes are shown (Observed). Models of the distributions of clones with different genotypes assuming different alleles are randomly distributed at the observed frequencies are also shown (Modeled). Each bar represents one clone, and different genotypes are color-coded in each bar.

Conclusions

We successfully developed a method to efficiently isolate genome-edited human iPS cells based on Matrigel-dome culture and robotic handling of the cells. With this method, we were able to isolate more than 1,000 human iPS cell clones to systematically analyze the genome editing results in them.

In human iPS cells, genome editing was also polarized, with many cells showing no editing and others showing editing of all target sequences. Moreover, detailed classification of NHEJ showed that identical insertion/deletion sequences were more likely to occur within a single cell, and this trend was more pronounced in iPS cells than in other human cultured cells.