

Two-step HDR

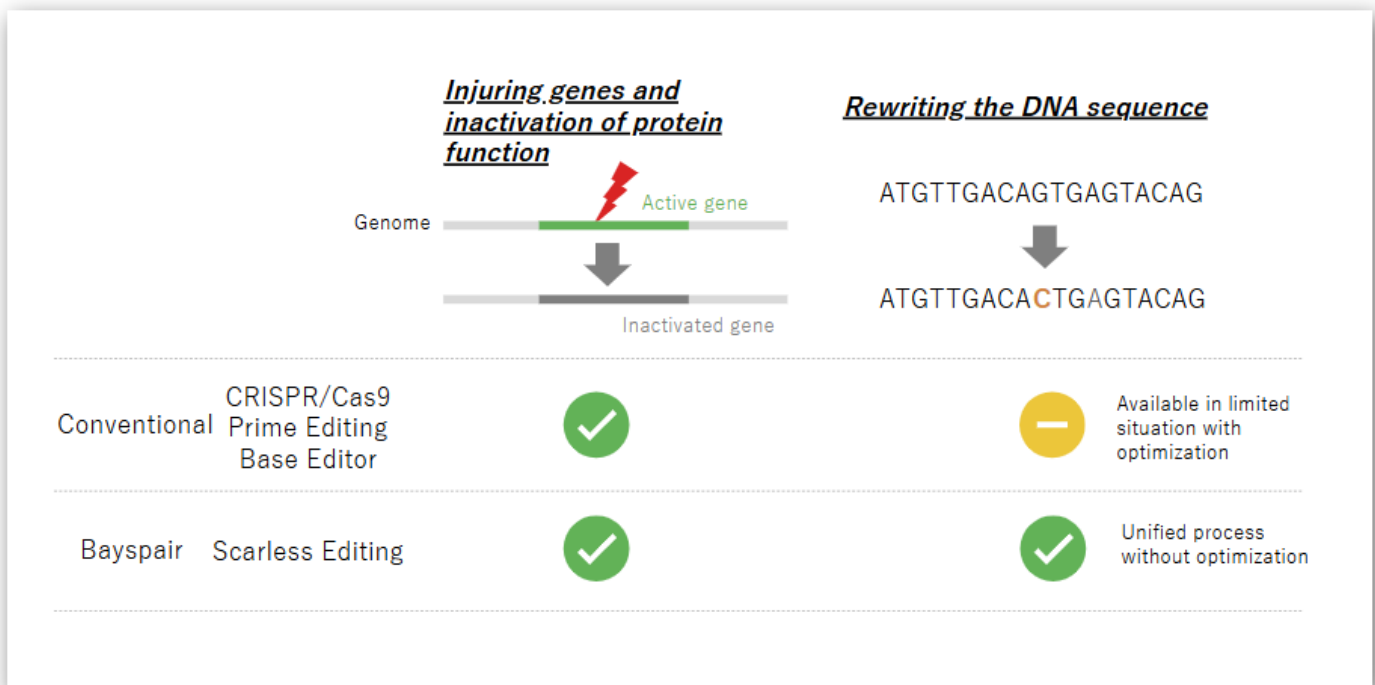
The key technology for Scarless Editing

By maximizing efficiency of homology directed repair (HDR)-based genome editing, it is possible to achieve both high quality and low cost.

Even in cases where it is not possible to design an appropriate guide RNA near the location to be edited, or where the size of the editing is too large and the efficiency is too low to obtain the desired cells, it is possible to proceed with unified genome editing process without the need for detailed optimization and screening of guide RNAs or donor DNA.

What is different?

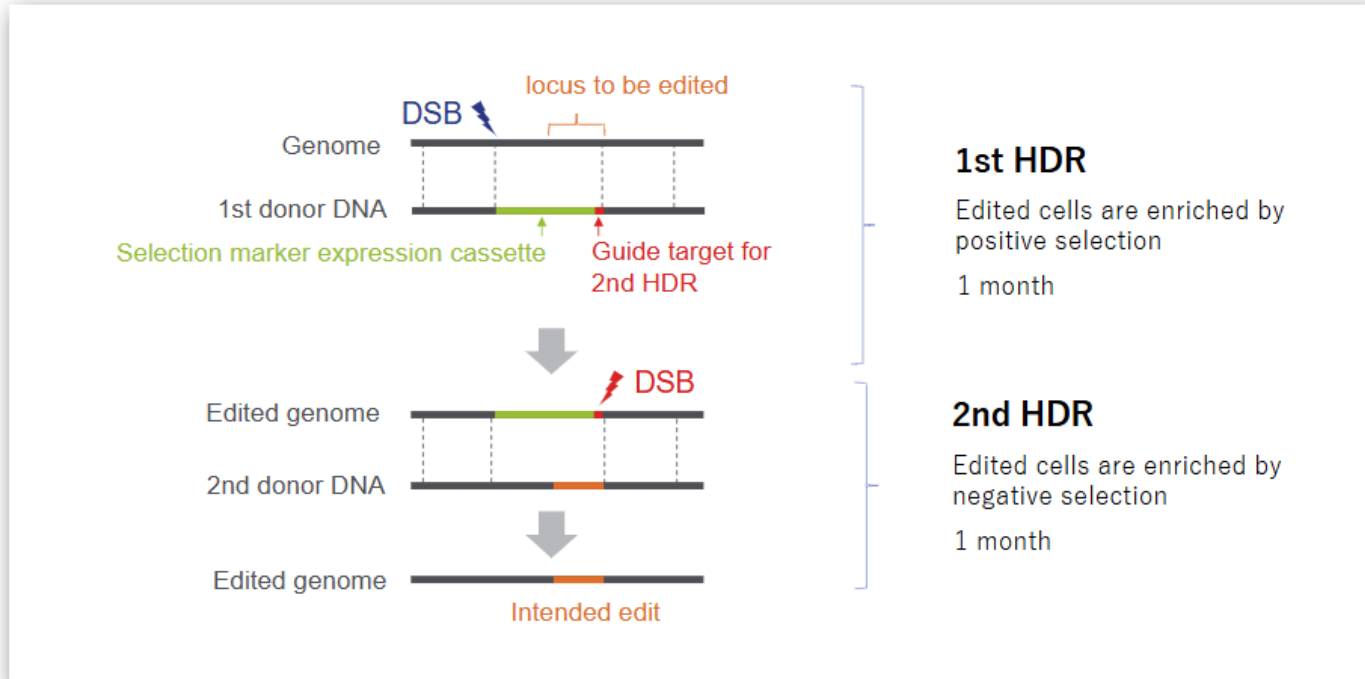
Scarless Editing using Two-step HDR greatly reduces the difficulty of rewriting genome DNA sequences, which is a challenge for conventional genome editing methods. With conventional methods, it is very easy to destroy genes (by pressing the delete key), but in order to input the A, T, C and G letters as desired, it is necessary to design and optimize the guide RNA and donor vector in detail, and also to have some luck.



How the difference is made?

In general, homology-directed repair (HDR) based genome editing is more difficult than simple gene disruption. However, there are not only difficult cases but also easy cases among the genome editing types based on HDR. The easiest HDR based genome editing is the case where a selection marker is inserted and cells with the genome edited as intended can be enriched based on the selection marker.

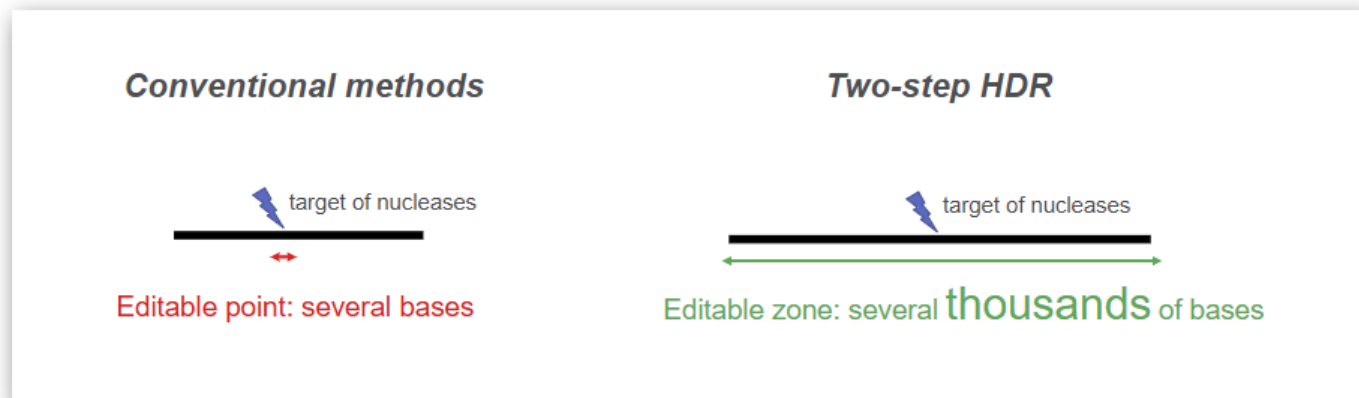
Two-step HDR is a method that achieves the most difficult tasks, such as editing at positions distant from guide RNA targets, single-base substitutions, and insertion of large sequences, without leaving any editing scars, by simply repeating genome editing using the easiest selection marker twice.



Off Guide Editing

Please see the position of the first DSB (double strand break, the target of the guide RNA) indicated in blue and the position that is finally edited in the figure above. They are different. This feature provides unprecedented design flexibility for Scarless Editing based on two-step HDR. With conventional genome editing methods, editing could only occur at the location where the guide RNA was designed.

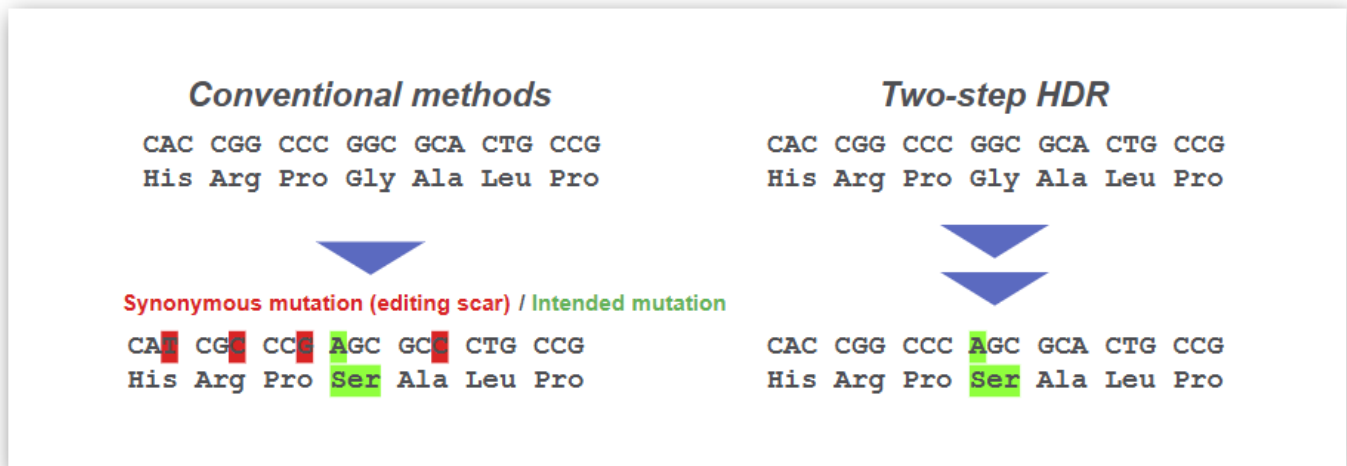
In other words, editing was impossible if there was no appropriate guide RNA target at the position to be edited.



Scarless Single Base Editing

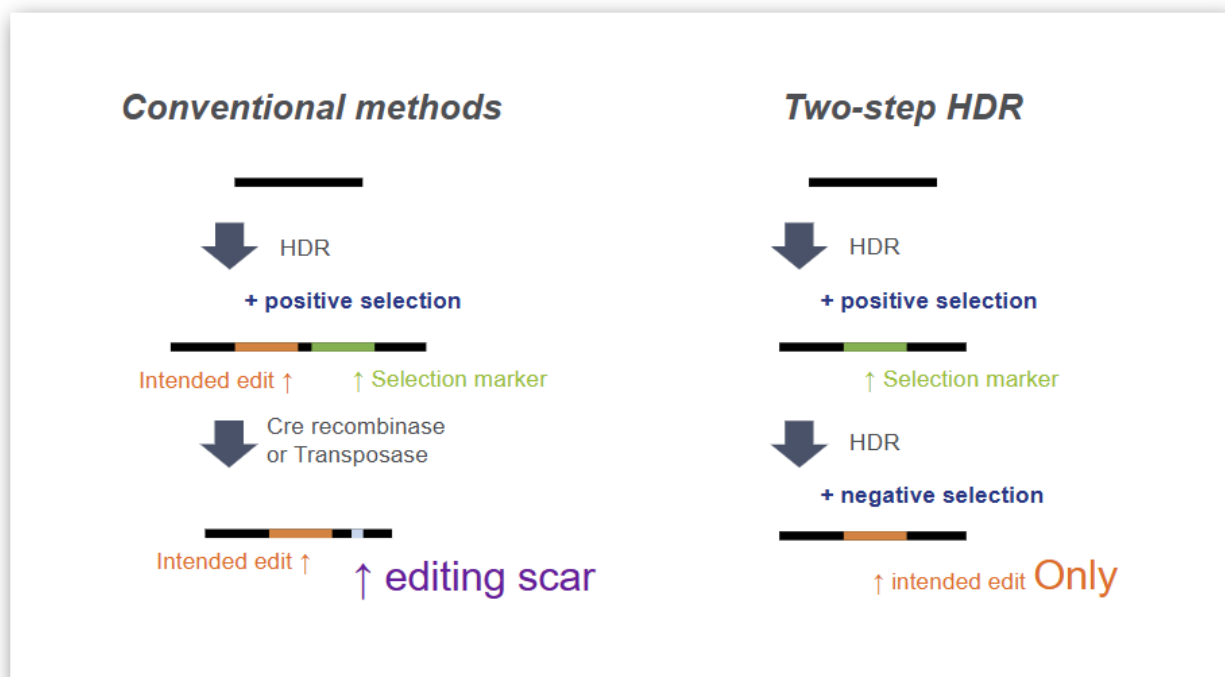
Scarless Editing technology was developed to efficiently perform single-base substitutions, which were difficult with conventional CRISPR/Cas9. With conventional technology, even if a single-base substitution was successful, further cuts could not be suppressed due to the mismatch tolerance of Cas9 sequence recognition, so in many cases it was necessary to add additional synonymous mutations, i.e., editing scars, to increase practicality.

Additionally, in cases where the guide RNA target did not overlap with the editing site, editing was impossible. The two-step HDR method can reliably perform editing even in such cases.



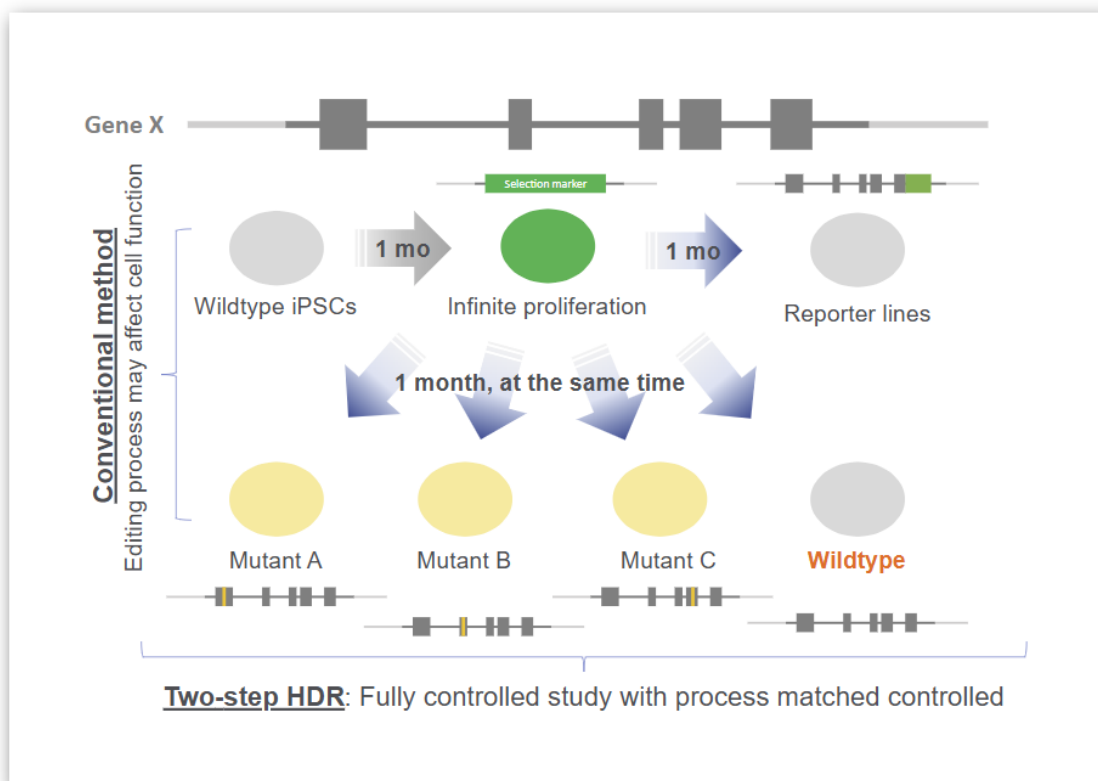
Scarless Large Editing

Two-step HDR is also well suited for editing large genomes of 100 bases or more. Unlike conventional techniques, it leaves no editing scars after the selection markers are removed.



Multigenotype Edit

This feature best illustrates the flexibility of Two-Step HDR. The genome sequence replaced by a selection marker can be freely rewritten. Multiple cell lines with SNPs at different positions, reporter lines and the combinations can be generated in parallel. When the cell line with the selection marker is in stock, the color of the reporter line can be changed immediately, even if a different color reporter is needed later.



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