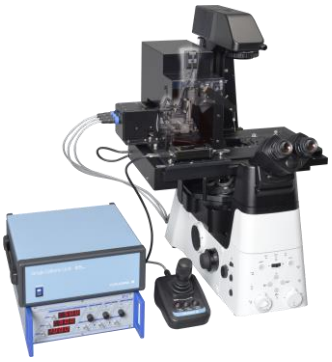
Single Cellome™ Unit SU10
Delivery of Genome Editing Tools to Knockout Target Genes



Genome editing is a technology that enables specific editing of any sequence in an organism's genome (knockout, knock-in, single nucleotide editing, etc.). A diversity of genome editing tools are available such as ZFN, TALEN, and CRISPR/Cas9, and improvements to these technologies are constantly being developed to allow diversification of recognition sequences and improved accuracy of editing, to name a few.

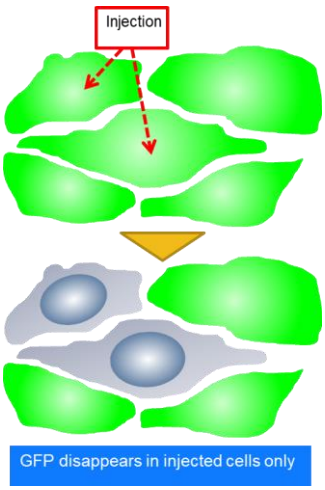
The development of technology that enables reliable delivery of genome editing tools into cells is also an important consideration. Yokogawa has recently introduced the SU10 that is applicable for reagent delivery to a variety of cell types and with most any genome editing tool. The SU10 is a new platform that can deliver genome editing tools directly into the cell (nuclei) using a "nano" pipette (glass capillary) with a tip diameter of substantially less than 100 nanometers.

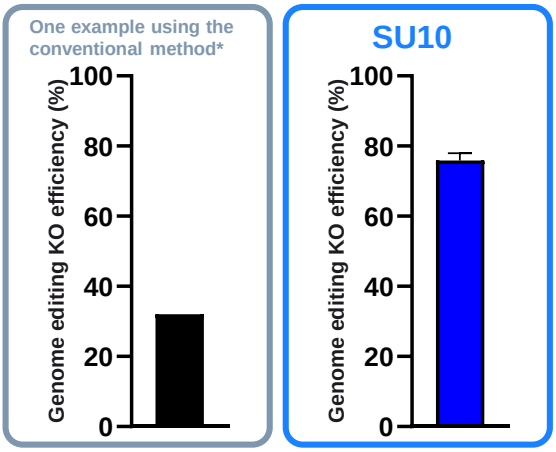
This application note will introduce the features of the SU10 and provide examples demonstrating the delivery of genome editing tools (Cas9 RNP) using the technology.

Knockout Efficiency After Injection of Genome Editing Tools Using SU10

The efficiency of gene knockout (KO) using the SU10 to introduce Cas9-sgRNA ribonucleoprotein (RNP) into cells was evaluated by the Kamakura Research Group, Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science.

Cas9 RNP for targeting the GFP gene was injected into GFP-expressing HeLa cells (222 cells), and the success or failure of gene disruption by genome editing was determined using the loss of the GFP fluorescence signal as an indicator.





*Reference: Appl. Sci. 2019, 9, 965
doi 10.3390/app9050965

- The bar graph on the left is an example of a physical method of for CRISPR delivery, which is from published data that employed a similar experimental strategy for evaluating KO efficiency (Cas9 RNP is physically introduced into EGFP-expressing HeLa cells and KO efficiency is evaluated by the loss of EGFP fluorescence).
- The bar graph on the right shows the KO efficiency in live cells when Cas9 RNP was introduced using the SU10 methodology (average and SD values of the results of injections by three nanopipettes).

[Results]
The results of this verification show the following.

- ◆ Cas9 RNP can be effectively delivered into cells by SU10
- ◆ A knockout efficiency of over 70% is achievable
- ◆ The efficiency of delivery may be greater than conventional physical delivery techniques

With the use of SU10, gene reagent delivery and gene editing can be improved significantly compared to alternative techniques used today. This is especially true for cells that are hard to transfect or otherwise sensitive to toxic reagents or procedures.

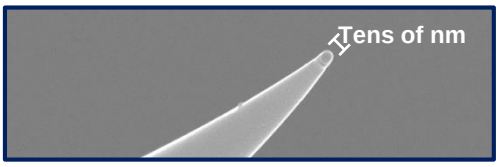
Experimental conditions

Cells used in this study: GFP+ HeLa cells (Cell Biolabs, Inc., AKR-213)
Reagent for injection: 50 ng/μL Cas9 RNP*
* Complex of Cas9 protein (Guide-it ™ Recombinant Cas9, Takara, 632641) and guide RNA (sgRNA)
Transfer method: Injection by SU10 (Note: Contact us for details of the conditions).
Evaluation: Cells were observed and measured after 5 days using the Yokogawa CSU-W1 Confocal Scanner Unit

Features of SU10

■ Minimally invasive intracellular delivery

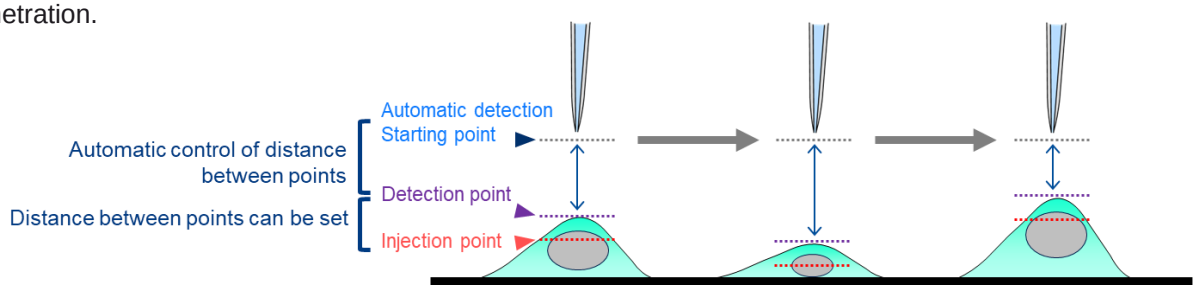
- An ultra-fine glass capillary (nanopipette) with a tip diameter of tens of nanometers is used.
- After loading with the delivery reagent, the tip of the nanopipette is inserted into the cell to introduce the target substance.
  Note: The penetration and injection into the cell are automated by the SU10.
- Due to the very small tip diameter, cell damage during delivery will be minimized.
- It is possible to inject several cells successively using a single nanopipette.



Electron microscope image of the nanopipette tip.

■ SU10 automation of cell surface detection/cell penetration

- The surface of the cell is detected automatically by identifying changes in the ion current.
- The nanopipette vertical movement is precise and automatic regardless of cell-to-cell variation.
- The penetration distance from the cell surface membrane is programmable, and consistent for every cell injected.
  Because of this feature, delivery to the nucleus of cells that vary in thicknesses is achievable.
- The SU10's high injection success rate is mainly driven by automation of cell surface detection and precision cell penetration.



■ Automation of intracellular injection operations

- The flow of solution or solute from the nanopipette during injection is due to an electrochemical mechanism (electro-osmotic flow).
- Injection operations are automated by the SU10.
- The injection volume can be changed in the software settings.
- The injection volume is estimated to be tens of femtoliter (fL) per second (1 fL = $1x10^{-15}$ L).
  Note: The injection volume may vary depending on the solute and solvent.

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