Molecular Physiology Laboratory（2023）
Chief Scientist: Rikiya Watanabe (Ph.D.)

(0) Research field
CPR Subcommittee**:** Biology

Keywords: membrane protein, artificial cell membrane, artificial cell, digital liquid biopsy

(1) Long-term goal of laboratory and research background

Our study aims to understand cellular functions using a bottom-up approach from the single molecule level. To achieve this, we are attempting to elucidate the mechanism by which individual biomolecules or their networks function in a precise manner, by developing novel single-molecule techniques using multidisciplinary approaches, including biophysics, bioMEMS, and chemical biology. In addition, we are developing a methodology to investigate correlations between genetic mutations, dysfunctions, and diseases with single molecule sensitivity, which would provide new insights for biological as well as pharmaceutical studies.

(2) Current research activities (FY2023)

Development of compact testing device for amplification-free genetic test (SATORI)

With the global outbreak of COVID-19, there is an urgent need to establish a versatile diagnostic method for infectious diseases. PCR and antigen tests are widely used for infectious disease diagnosis; however, these methods generally have technical drawbacks in terms of sensitivity, accuracy, and throughput, making it difficult to efficiently analyze a large volume of specimens with high sensitivity and accuracy. To address these issues, since 2020, we have developed an innovative technology using CRISPR-Cas13a (SATORI) that can detect viral RNA from SARS-CoV-2 at the single-molecule level with high sensitivity and throughput (the fastest in the world) (Fig. 1).

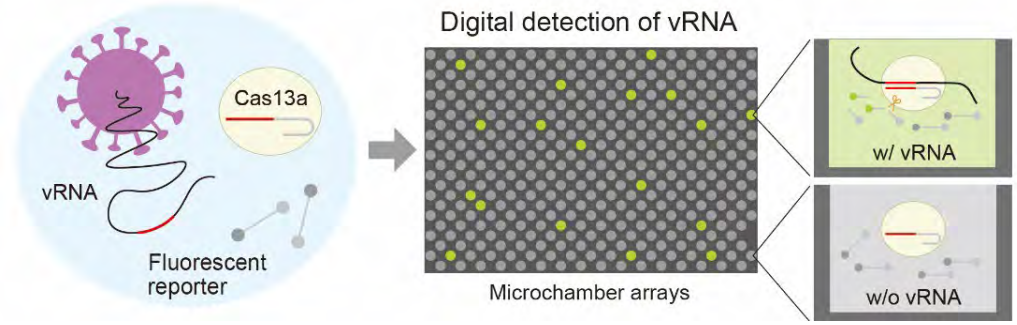

Fig. 1 Amplification-free rapid genetic test (SATORI)

The fluorescence microscope has served as the detection system for SATORI; however, their large size and high cost have hindered their widespread adoption in the medical field. In FY2023, we developed a compact fluorescence detector for SATORI (**Co**mpact **W**ide-field **F**emtoliter-chamber **I**maging **S**ystem for **H**igh-speed Digital Bioanalysis: COWFISH) (Fig. 2). This compact system combines a single-lens reflex (SLR) camera, a telecentric lens, and other commercially available components, resulting in an affordable fluorescence detector capable of capturing wide-field fluorescence images. This advancement significantly accelerates the SATORI process. In addition, to ensure the practicality of our device, we conducted proof-of-concept tests using COVID-19 clinical specimens. The results demonstrated that SATORI achieves nearly identical performance (sensitivity and specificity) compared to conventional fluorescence microscopy.

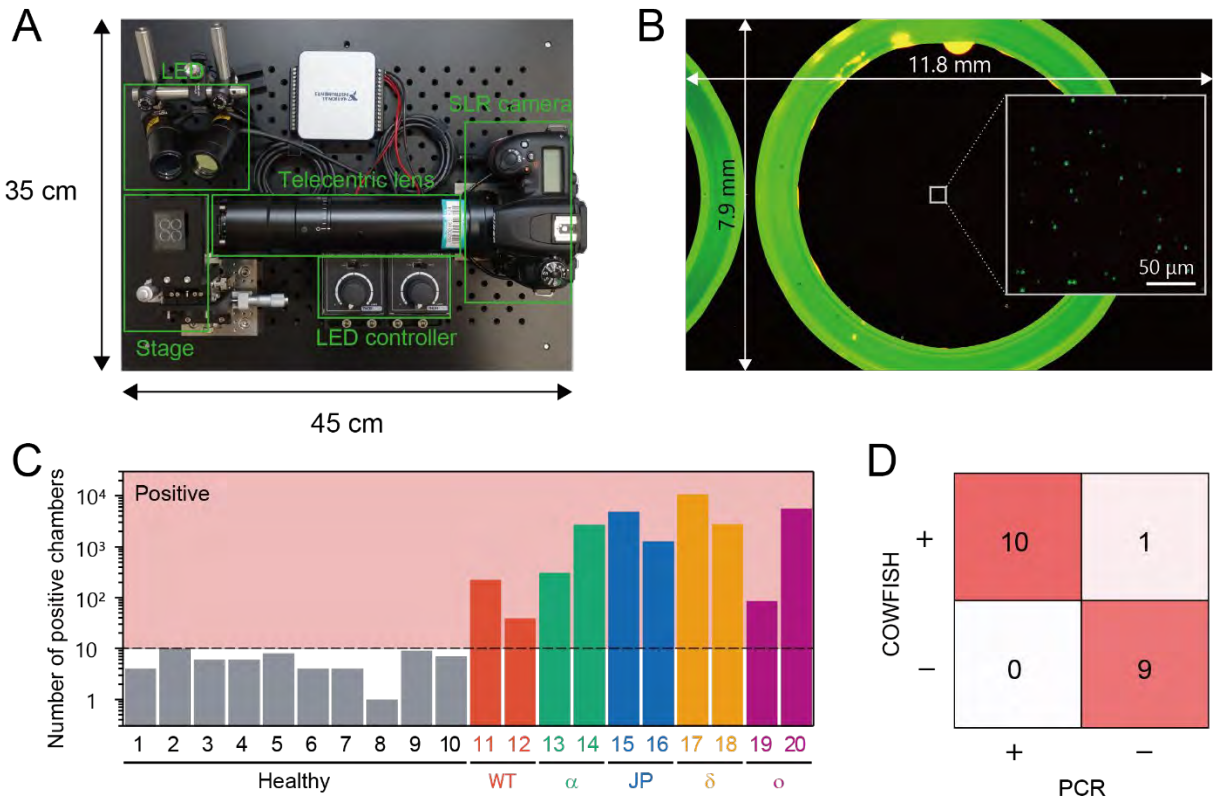

Fig. 2 Genetic testing for COVID-19 by COWFISH
(A) Photograph of COWFISH, (B) Fluorescence image, (C) Number of positive chambers (D) Positive identification (Correct rate: 95%).

Generalization of SATORI: from medicine to agriculture

SATORI, developed in FY2021, stands as the world's fastest amplification-free genetic test for infectious diseases; however, it lacks the ability to directly detect pathogens from biological samples, necessitating prior extraction and purification of pathogen genes using a separate device. Consequently, completing the entire SATORI process, from sample collection at a medical site to result generation, requires at least 30 min. There was a pressing need for simplifying the testing process and reducing testing time. In FY2023, we made significant advancements by developing the "Direct-SATORI". This innovative approach enables direct detection of pathogen genes from biological samples, eliminating the steps of gene extraction, purification, and amplification that were time-consuming in conventional genetic testing. As a result, the Direct-SATORI method achieves a drastic reduction in detection time and enables multiplex detection of various pathogens (Fig. 3).

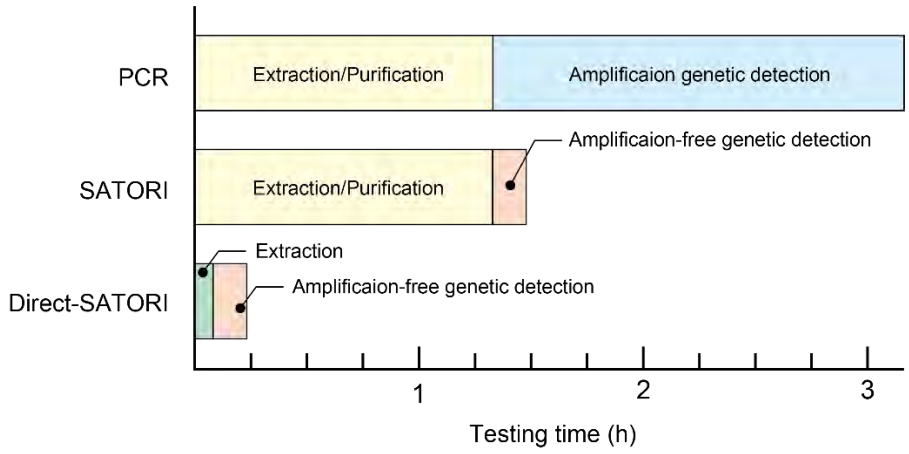

Fig. 3 Comparison of testing time with conventional genetic tests
Breakdown of testing time after a biological sample is collected. As an example, a genetic test using plant leaves is shown.

As a proof-of-concept, Direct-SATORI was demonstrated as genetic testing of eight tomato viruses: Tomato mosaic virus (ToMV), Tomato aspergillosis virus (TAV), Tomato yellowing necrotic virus (TSWV), Cucumber mosaic virus (CMV), Potato X virus (PVX), Potato Y virus (PVY), Tobacco mosaic virus (TMV), and Tobacco necrosis virus (TNV) within 15 minutes (Fig. 4). This represents a significant reduction in detection time compared to the conventional PCR, with Direct-SATORI achieving less than one-tenth of the time required. Moreover, Direct-SATORI demonstrated a sensitivity of 96% and specificity of 99%. These results indicate that Direct-SATORI is highly effective for the rapid detection of multiple genes across a wide range of pathogens.

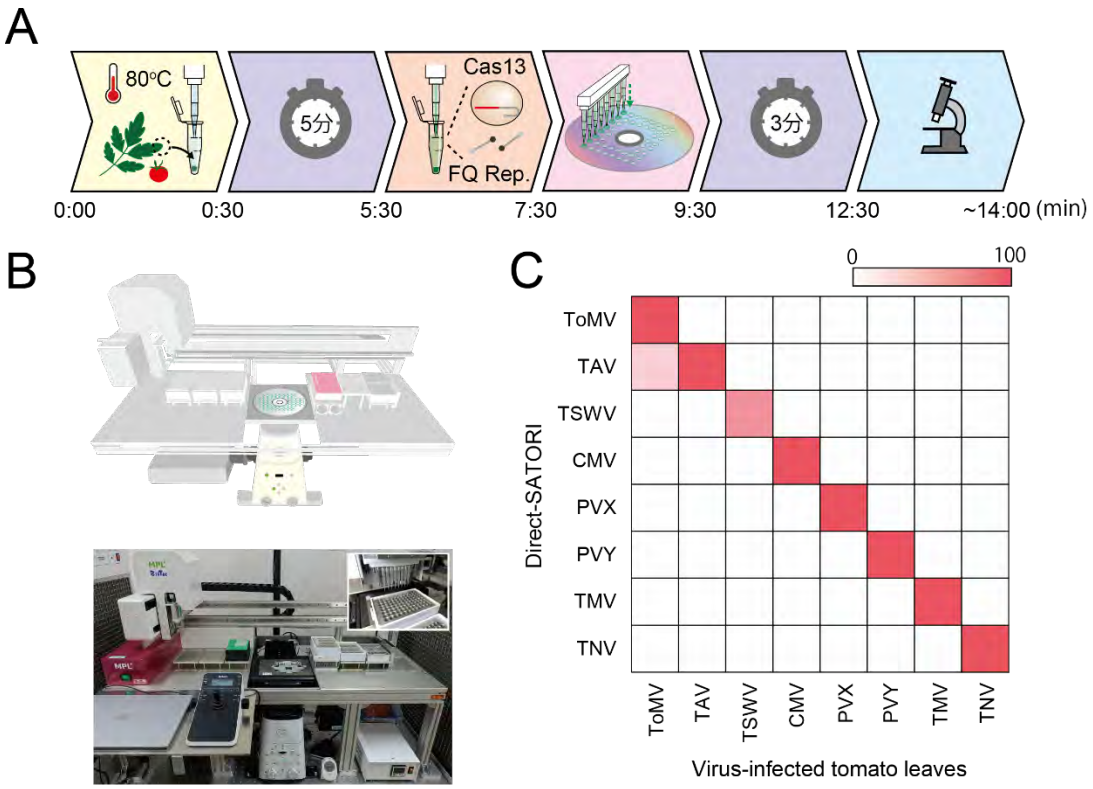

Fig. 4 Multiplex genetic test using Direct-SATORI

(A) Schematic diagram of Direct-SATORI, (B) Automated device optimized for Direct-SATORI (C) Positive identification of 8 pathogenic tomato viruses. Sensitivity 96%, specificity 99%.

(3) Members

(Chief Scientist)
Rikiya Watanabe
(Research Scientist)
Jun Ando, Hajime Shinoda
(Special Postdoctoral Researcher)
Yoshiaki Kinoshita, Tomoya Ueda
(Technical Staff)
Asami Makino, Tatsuya Iida, Mami Yoshimura
(Assistant)
Yuka Takano

(4) Representative research achievements

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Laboratory Homepage

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