Singapore International Water Week

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HOKKAIDO UNIVERSITY

Genomics Methods for Identifying, Tracking and Tracing Pathogens in Irrigation Water

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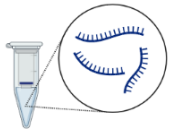
Genomics methods for the detection of pathogens in irrigation water

❑ Amplification-based methods

- qPCR
- LAMP
- NASBA

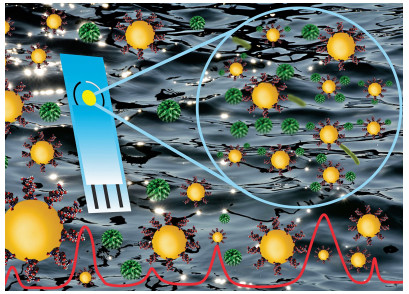
❑ Sequencing

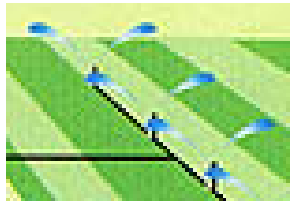
- Amplicon sequencing
- Shotgun metagenomics



❑ Nucleotide-based sensors

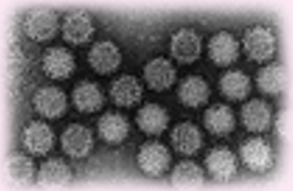
- Aptamer
- CRISPR-Cas





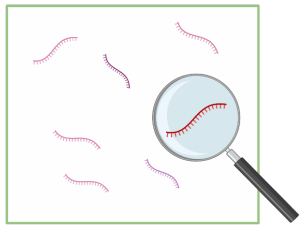
Pathogens of concern in irrigation water and fresh produce

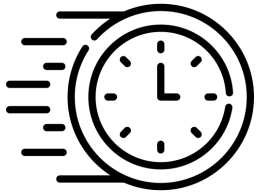
- Viruses (e.g., norovirus, hepatitis A virus)
- Protozoa (e.g., *Cyclospora*)
- Bacteria (e.g., pathogenic *E. coli*, *Campy*)



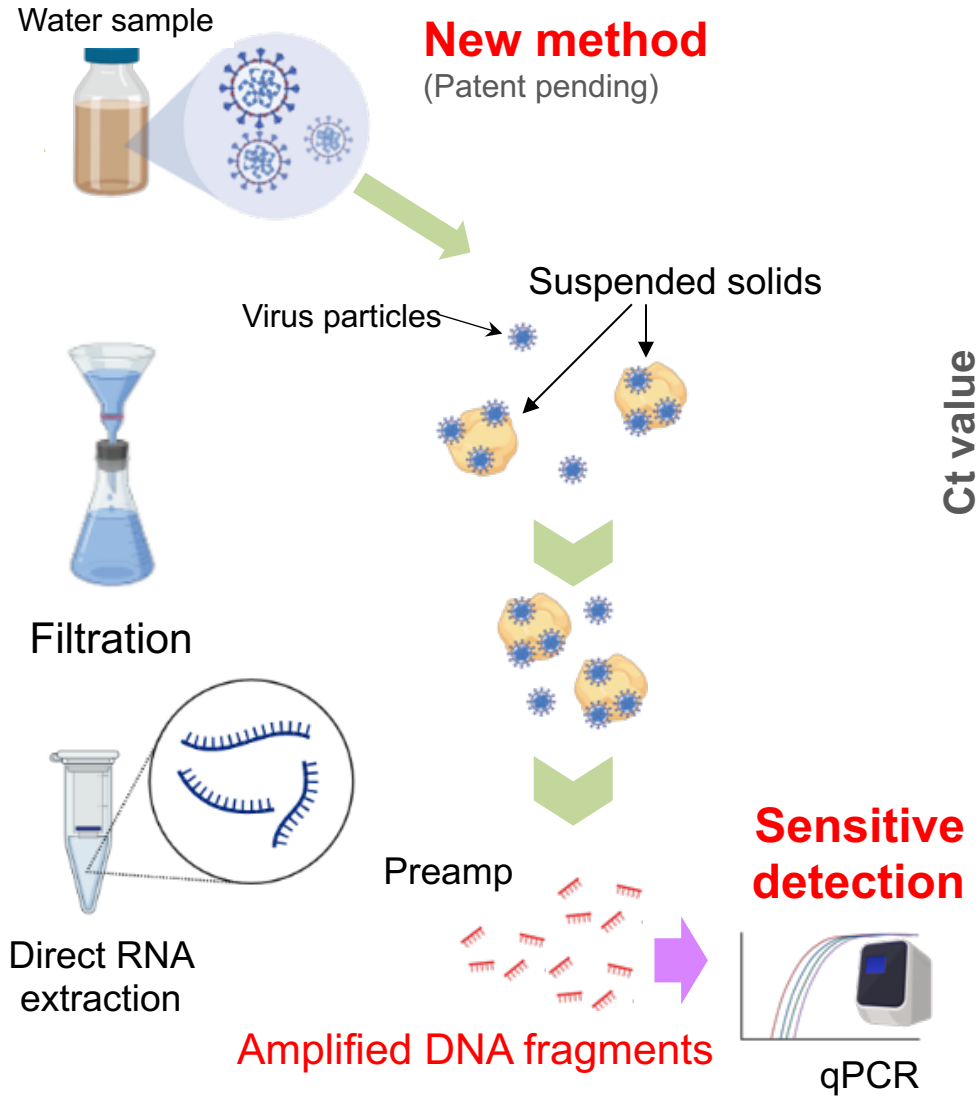
What is expected for the methods for pathogen detection in irrigation water?

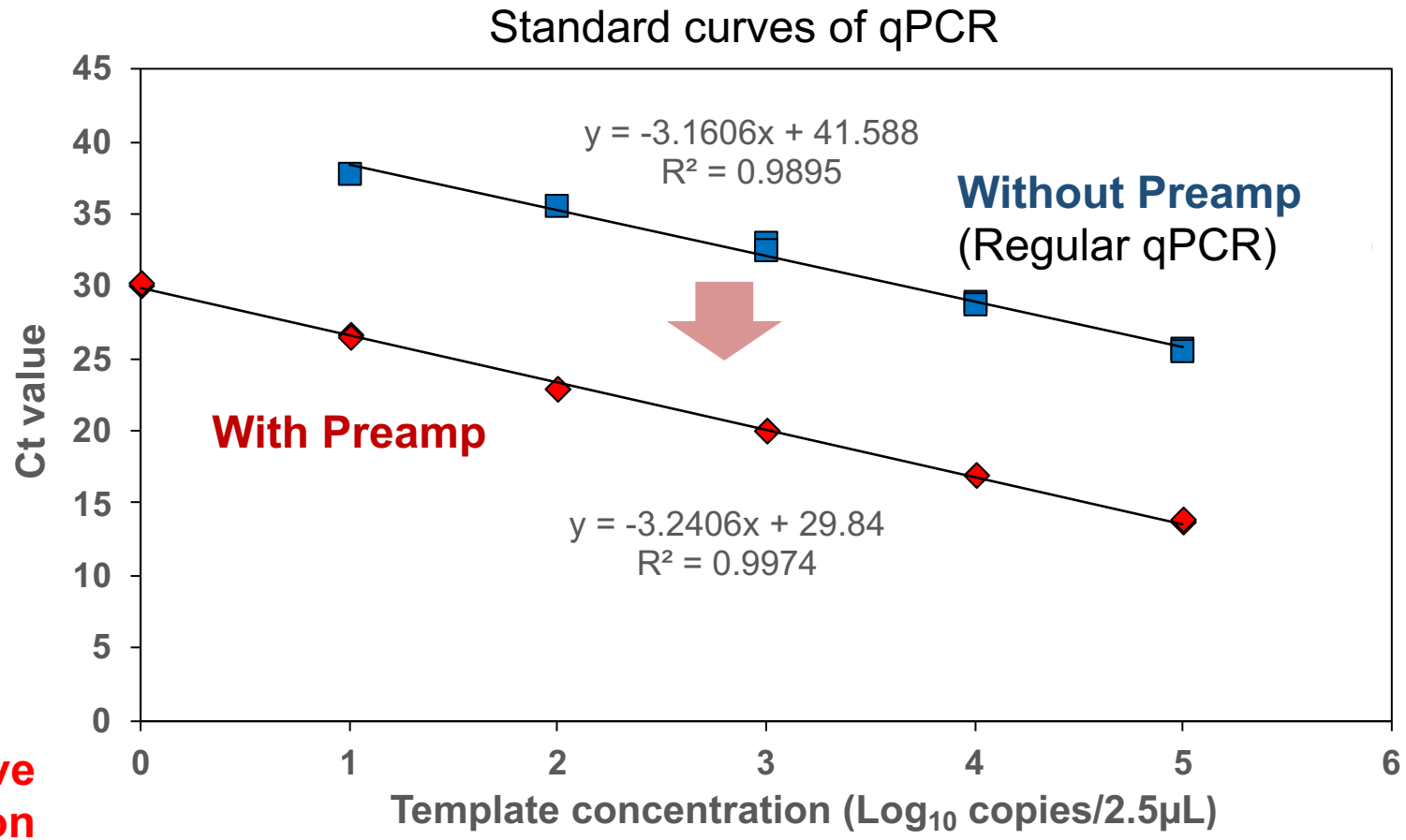
Pathogens in irrigation water		Expectation for detection methods
❑ Present in low concentration	→	High sensitivity
❑ Highly infectious	→	Infectivity assessment
❑ Need timely intervention	→	Short turn-around time





Development of a highly sensitive and practical molecular detection method





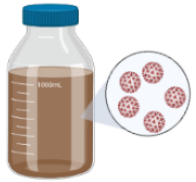
■ **Quantifiability is maintained after Preamp**

- ➢ Enables quantification of low-level viral RNA in water

Ando *et al*. (under review)

Sensitivity and applicability of the new molecular method

Sensitivity evaluation

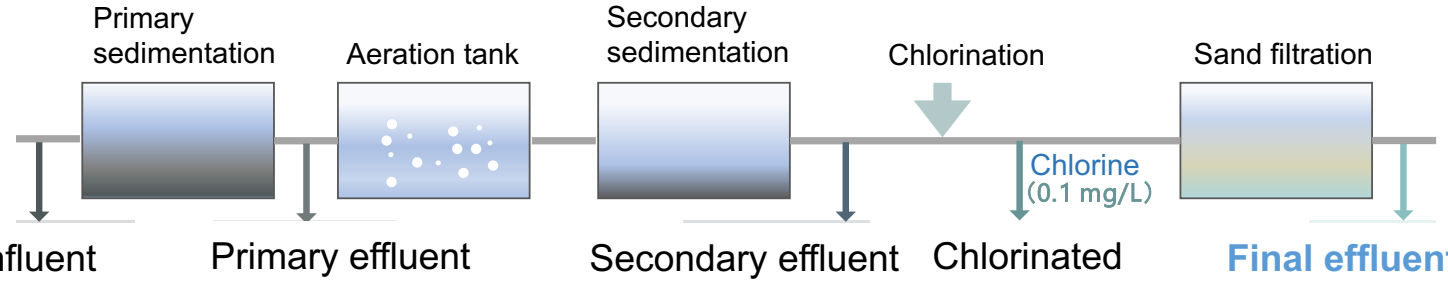


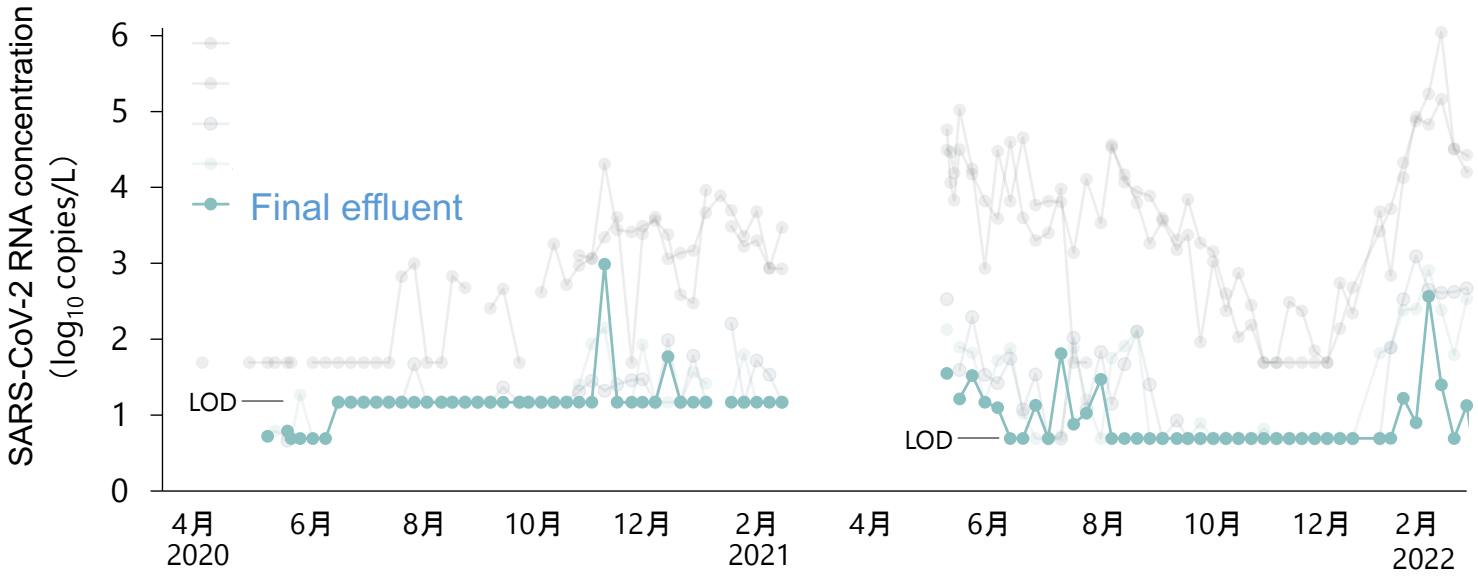
Virus-spiking experiment

Copies/L	New method	Conventional method*
10^4	+	+
10^3	+	-
10^2	+	-
10^1	-	-

❑ **New method is two orders of magnitude more sensitive** (*PEG precipitation-qPCR)

Application to sand-filtered wastewater effluent





❑ **Detection of low-level viral RNA in sand-filtered effluent**

Ando *et al*. (under review)

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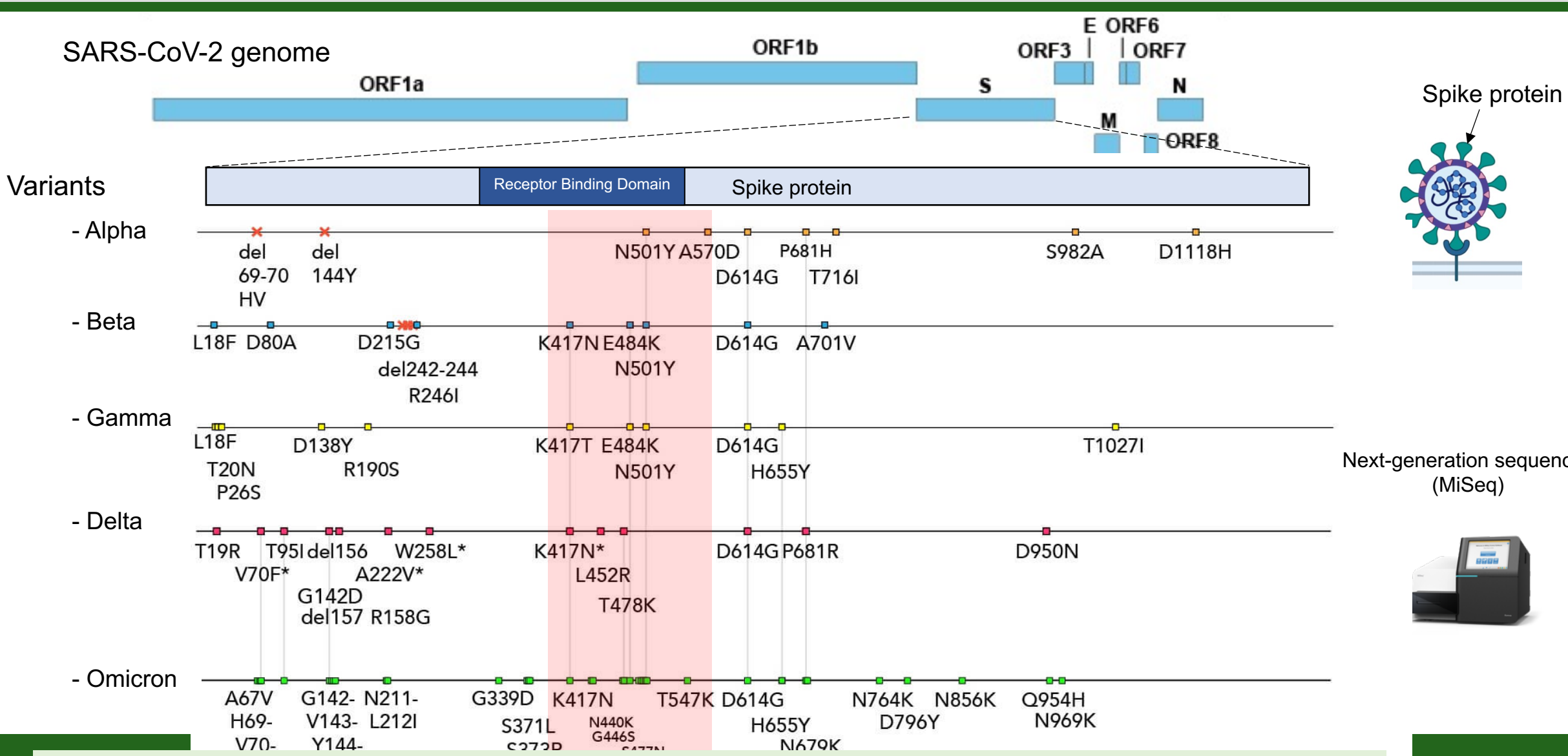
Amplicon sequencing for genomic characterization based on the new method: SARS-CoV-2 experience

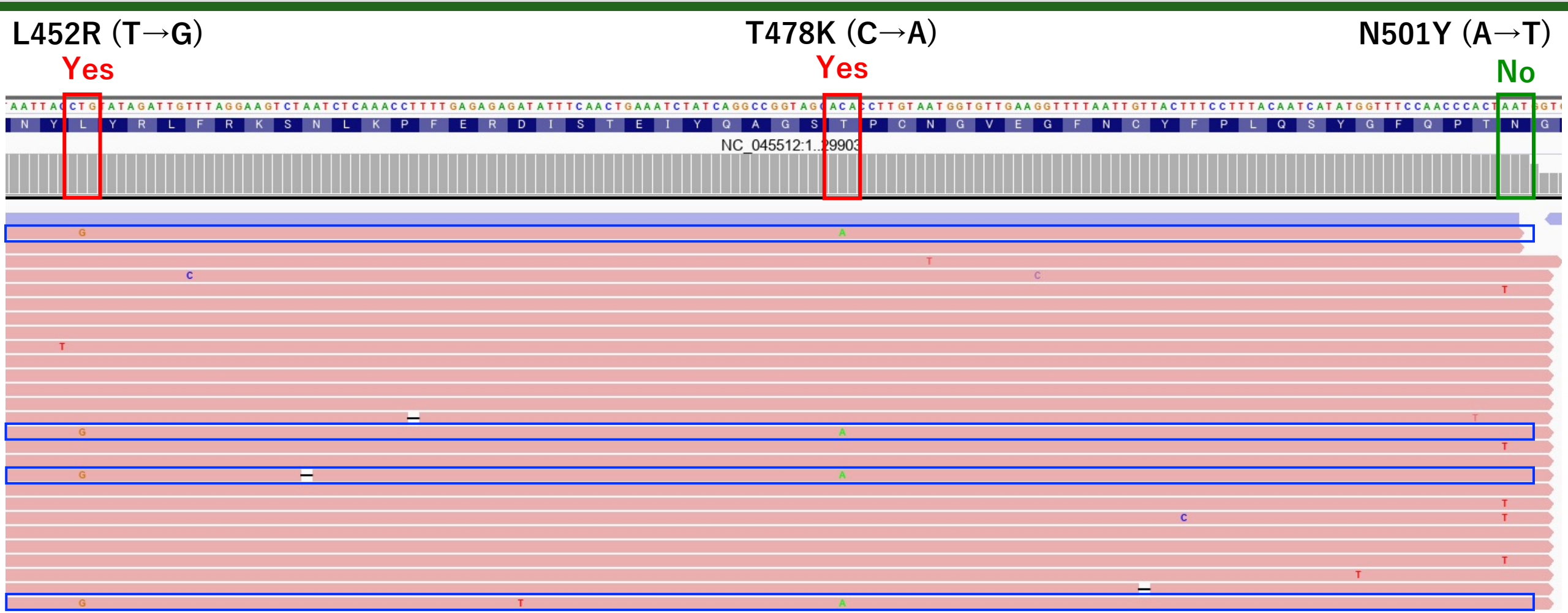


Spike protein

Next-generation sequencing (MiSeq)

- Established a **protocol for the detection of mutations** based on genomic analysis via NGS.

Amplicon sequencing for genomic characterization based on the new method: Example data - Detection of Delta variant（L452R & T478K）in wastewater



This protocol is applicable to **sensitive detection and characterization** of pathogens in irrigation water.

- Useful for pathogenicity evaluation, tracking contamination sources, etc.

（Genetic analysis performed by Prof. Seiya Imoto and Dr. Kiyoshi Yamaguchi from the Institute of Medical Science, The University of Tokyo）

Cyclospora in irrigation water – CycloCORE Consortium Network

CycloCORE
COLLABORATIVE FOR OUTREACH, RESEARCH, AND EDUCATION
Consortium Network

Pillar 1: Extension/Outreach

Lead by Dr. Gerardo Lopez (University of Arizona)

Addressing the Critical Needs of Specialty Crop Industry Stakeholders

Pillar 2: Education and Capacity Building

Pillar 3: Research

International collaboration

- ✓ USA
- ✓ Norway
- ✓ Italy
- ✓ China
- ✓ Japan

Genomics methods for the detection of *Cyclospora cayetanensis*

Development of a qPCR assay for *Cyclospora cayetanensis* (Kitajima et al., 2014)

Contents lists available at ScienceDirect

Science of the Total Environment

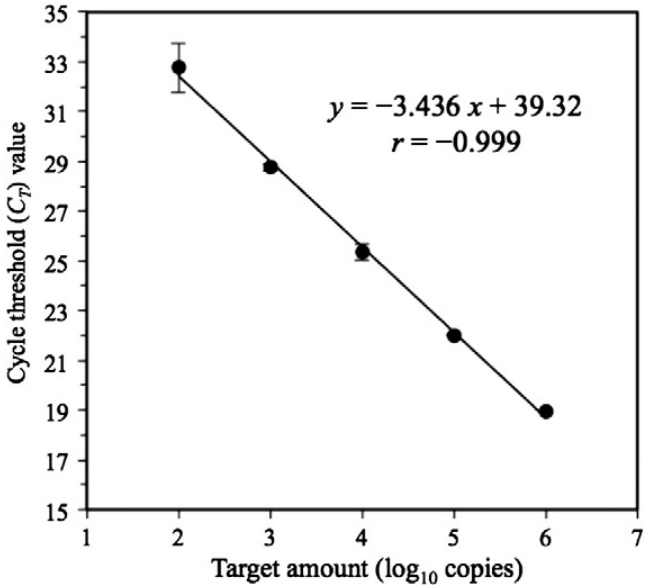
journal homepage: www.elsevier.com/locate/scitotenv

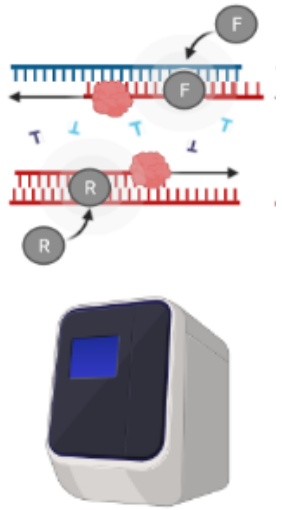
Occurrence of *Cryptosporidium*, *Giardia*, and *Cyclospora* in influent and effluent water at wastewater treatment plants in Arizona

Masaaki Kitajima [a,*], Eiji Haramoto [b], Brandon C. Iker [a], Charles P. Gerba [a]

[a] Department of Soil, Water and Environmental Science, The University of Arizona, Tucson, AZ 85721, USA
[b] International Research Center for River Basin Environment, University of Yamanashi, Yamanashi 400-8511, Japan

C. cayetanensis qPCR standard curve





BAM 19b: Molecular Detection of *Cyclospora cayetanensis* in Fresh Produce Using Real-Time PCR

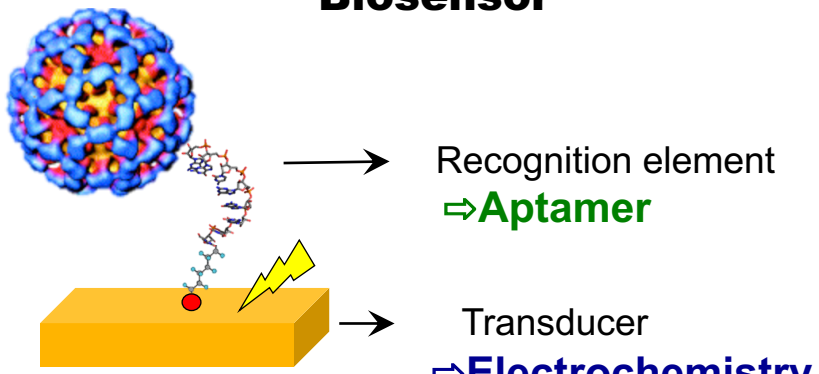
Authors: Helen R. Murphy, Sonia Almeria and Alexandre J. da Silva
Contact: Helen Murphy (mailto:Helen.Murphy@fda.hhs.gov)

- qPCR assays for *C. cayetanensis* have been reported and applicable to its detection from irrigation water.
- Remaining challenge is infectivity evaluation for better risk assessment.

Biosensor for Rapid Detection of Foodborne Virus: An Example - Electrochemical Aptasensor for Norovirus

Aptasensor (aptamer-based biosensor)

Biosensor

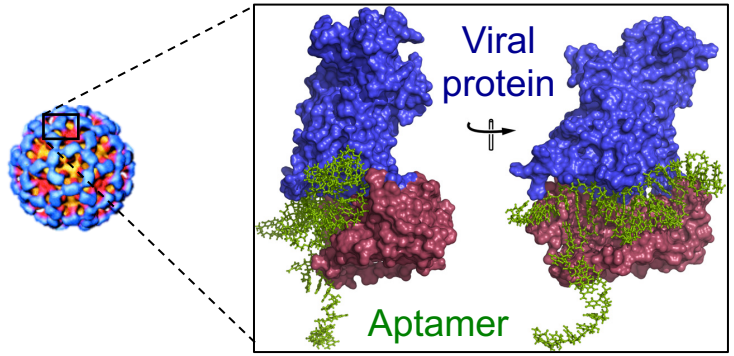


Recognition element
⇨**Aptamer**

Transducer
⇨**Electrochemistry**

Aptamer

- Functional DNA/RNA
- Fold into 3D conformations binding to a specific target



(Modified from Beier et al., 2014)

Electrochemistry
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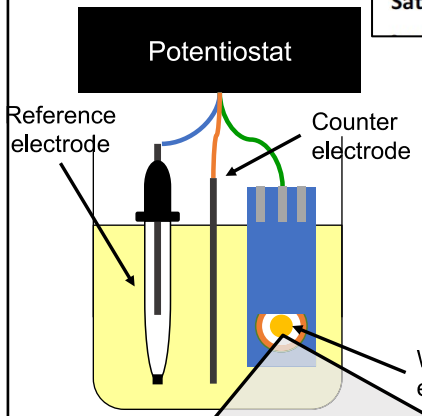
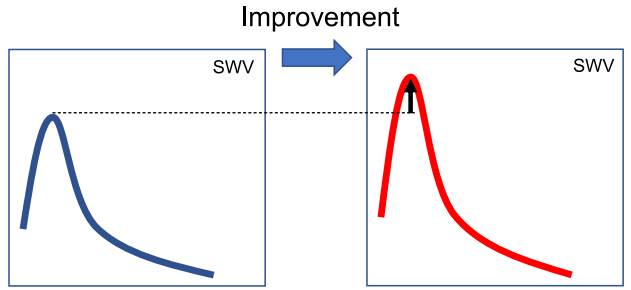
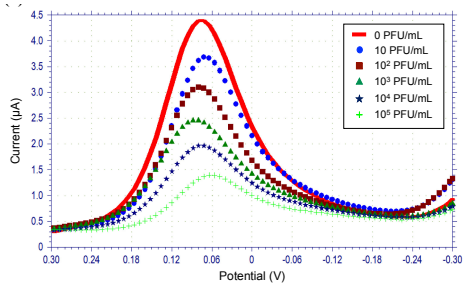
Article
Electrochemistry, **88(3)**, 205–209 (2020)

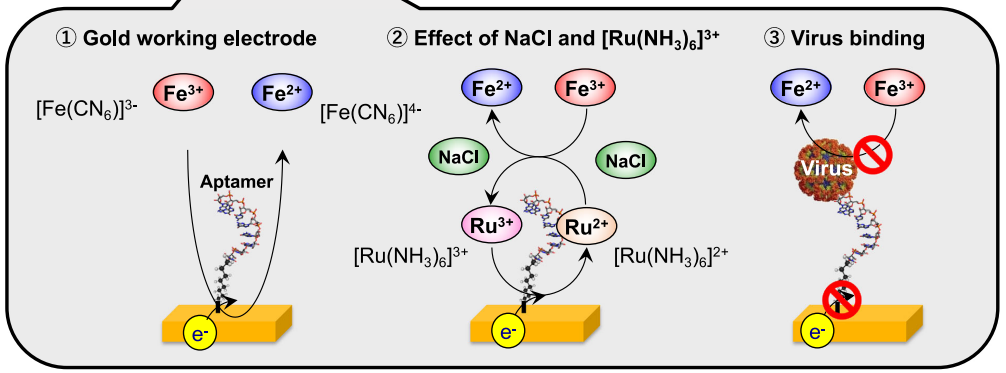
Improvement of Electrochemical Conditions for Detecting Redox Reaction of $K_3[Fe(CN)_6]$ toward the Application in Norovirus Aptasensor

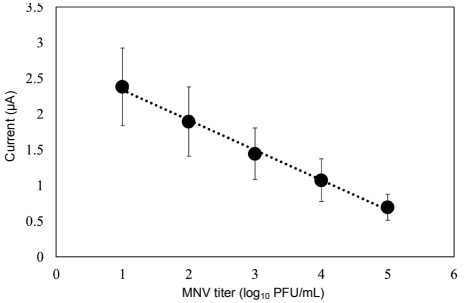
Seiya HIRANO,[a,†] Junki SAITO,[b] Tomoki YUKAWA,[a] Daisuke SANO,[c] Akihiro OKAMOTO,[d,e] Satoshi OKABE,[a] and Masaaki KITAJIMA[a,*]





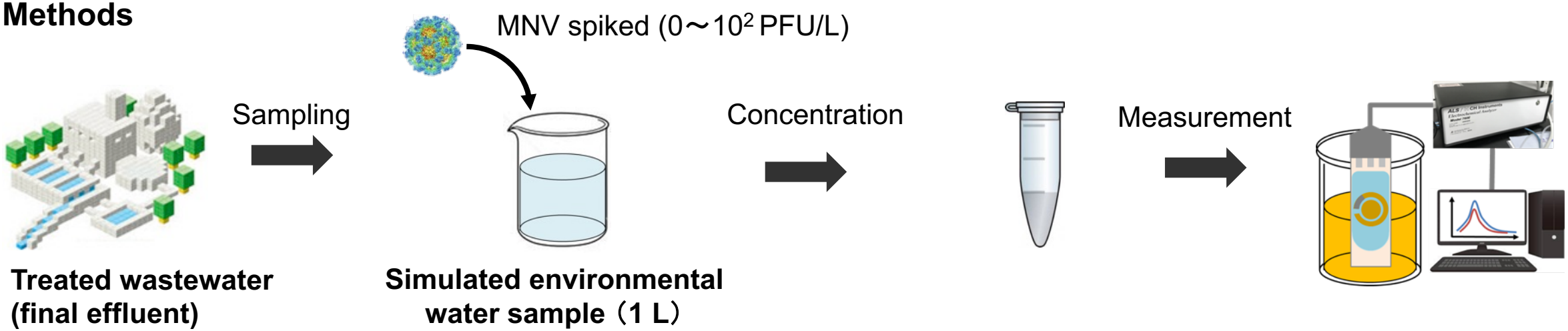




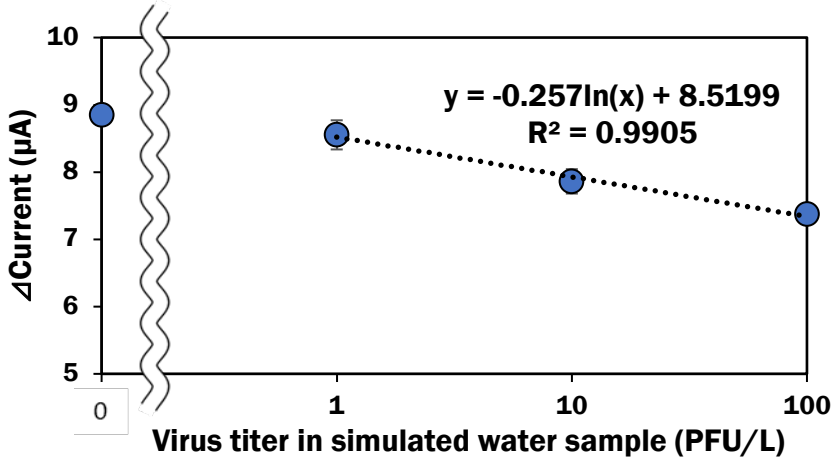


Application of Norovirus Aptasensor to Treated Wastewater

Methods



Results

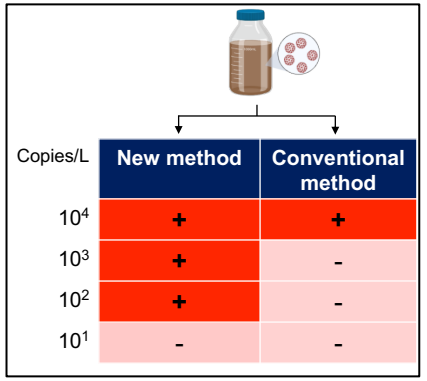


- Difference in SWV peak current intensity between 0 PFU/mL and 1 PFU/mL
- Virus-titer dependent SWV response in 1～10² PFU/L

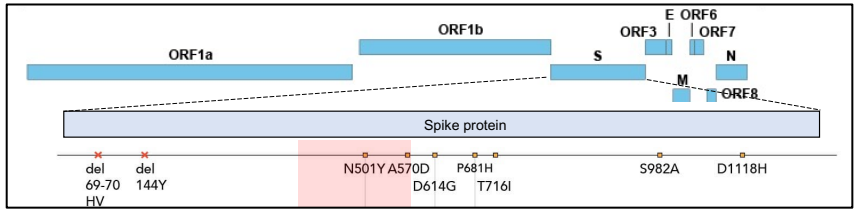
■ Ability to detect low concentration (1 PFU/L) of MNV in treated wastewater within 30 mins

Summary: genomics methods for pathogen detection in irrigation water

- **Development of a highly sensitive and practical method for molecular detection method.**
  - Validated and applicable to any pathogens.



- **Amplicon sequencing for genomic characterization of pathogens.**
  - Useful for pathogenicity evaluation, tracking contamination sources, etc.



- **Potential of biosensors as a rapid pathogen detection method.**
  - Attractive alternative but technical challenges remain:
    - Academic research → commercially viable prototype