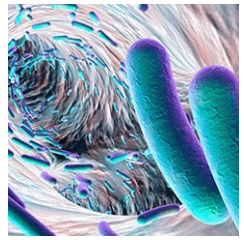






Transform antibiotic resistance surveillance and balance sensitivity with nanoscale high-throughput qPCR technology

Rohit Kadam, PhD

that's GOOD science!®

Outline

- Antimicrobial (AMR) and antibiotic resistance (AR)
- AR bacterial prevalence and incidence
- Limitations in methods for studying AR bacteria
- Challenges for molecular detection of AR bacteria
- SmartChip ND™ Real-Time PCR System
- Case studies & partnerships
- Summary

Antimicrobial resistance (AMR) and antibiotic resistance (AR)

- Microorganisms (bacteria, fungi, viruses, and parasites) can develop resistance to drugs
- AR is one of the largest threats to health and food supply in the world
  - Contributing factors:
    - Improper use (typically overuse) of antibiotics in patients
    - Animal husbandry's reliance on antibiotics for growth and health
    - Lack of well-established networks for surveillance
- AR bacteria are found in people, animals, food, and the environment (e.g., water, soil, and air)

AR bacteria prevalence and incidence

- AR bacteria have been detected in nearly every country in the world

  Prevalence can range anywhere from 0–100%, depending on antibiotic, bacteria, and country

  South Korea: 75% of *Acinetobacter* isolates in blood had some level of AR

  Malawi: nearly 100% of *N. gonorrhoeae* isolates were resistant to ceftriaxone; 15% resistant to azithromycin

- In the United States (since 2013):

  >2,000,000 illnesses caused by AR bacteria

  >23,000 deaths due to AR bacteria

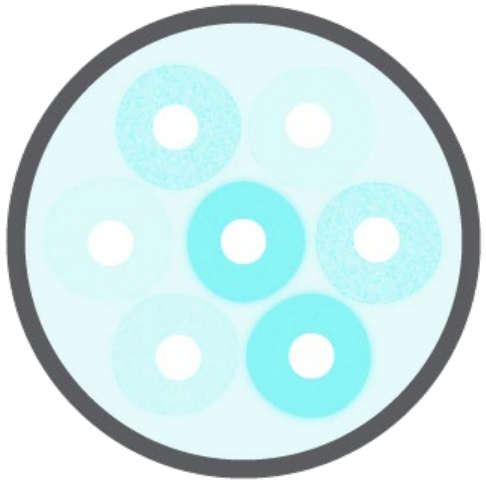
- Prevalence and incidence are increasing every year

Data from WHO, CDC

Limitations in methods for studying AR bacteria

DIFFICULT → ***EASY***

Bacterial Culture



X Long turnaround time (minimum 24–48 hr)

X Labor-intensive

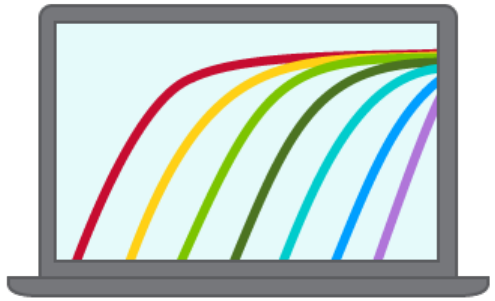
Sequencing

X Technical expertise required

X Expensive

X Complex data analysis

PCR/qPCR



X Difficult to scale up

✓ **Fast turnaround time (3 hr)**

✓ **Easy**

✓ **Cost-effective**

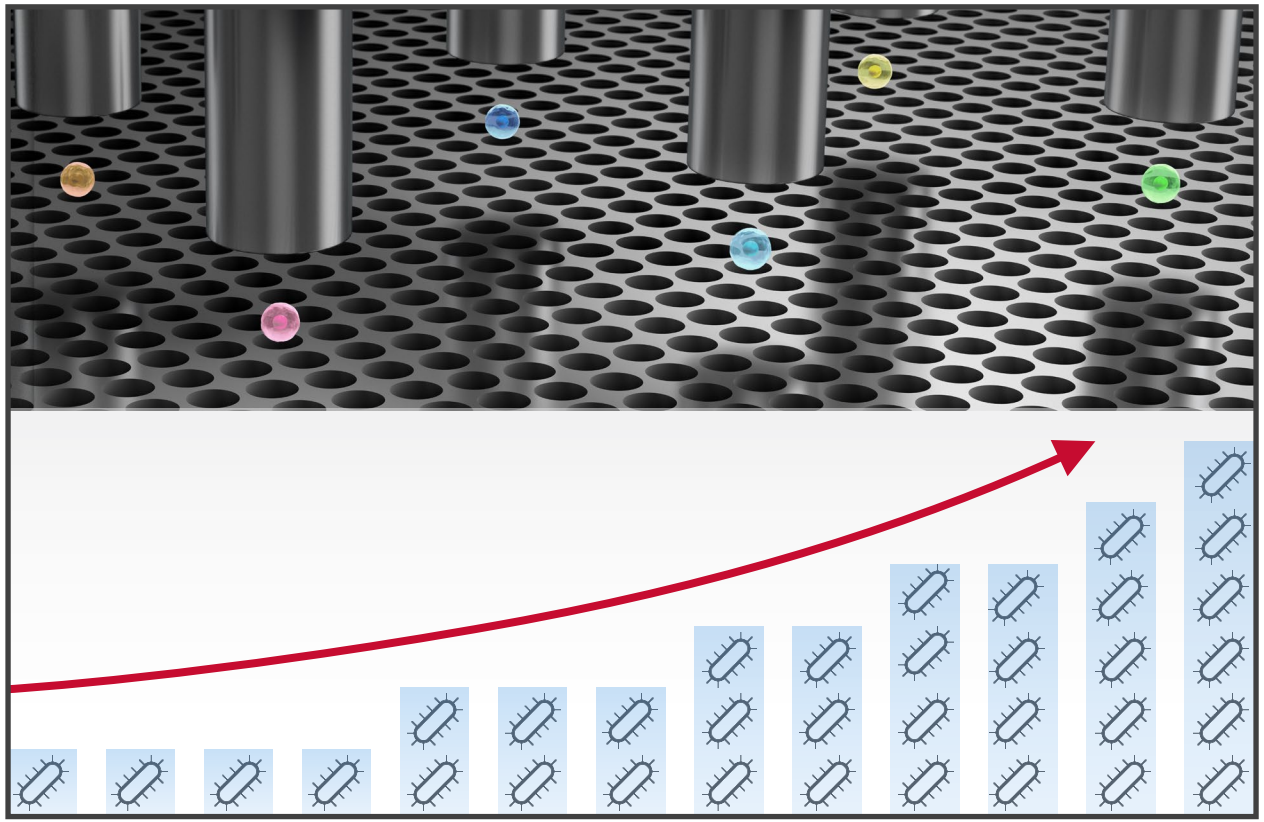
Challenges for molecular detection of AR bacteria

- Conventional, plate-based qPCR can be time-consuming, burdensome, and cost-prohibitive for profiling hundreds of ARGs in multiple samples
- Requires individually testing a high number of antibiotic resistance gene (ARG) targets
  - Databases contain thousands of known targets derived from whole genome sequencing
- Must balance sensitivity with reaction volume
  - **10 µl volume** (384-well plates)—good sensitivity, but limited ARG and sample number
  - **10–50 nl/well volume** (most high-throughput platforms)—better ARG and sample processing power, but poor sensitivity
  - **100 nl volume**—sweet spot that maximizes sensitivity while minimizing reaction costs

A scalable solution for ARG profiling

Automated, miniaturized, high-throughput qPCR can alleviate the obstacles faced by conventional molecular detection

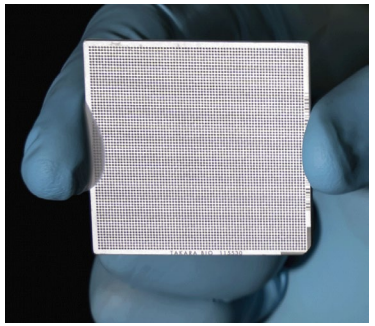
- Less hands-on time
- Increased reproducibility
- Smaller reaction volumes
- Decreased costs
- High-throughput processing of large numbers of ARGs and samples



SmartChip ND™ Real-Time PCR System

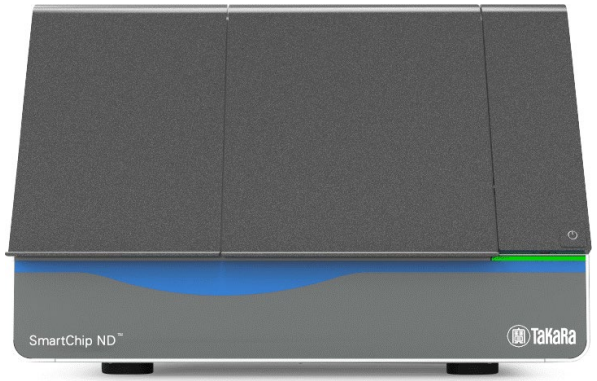
High-throughput genotyping and gene expression analysis

Nanowell chip



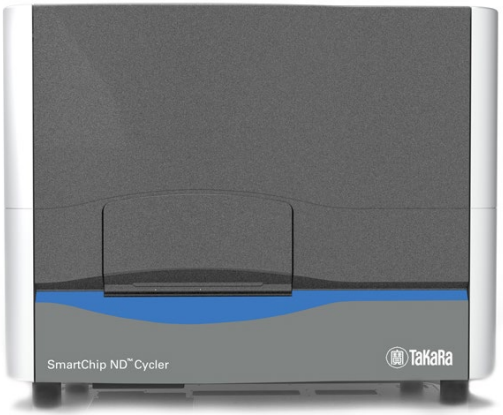
5,184 (100 nl)
reactions/chip

SmartChip ND



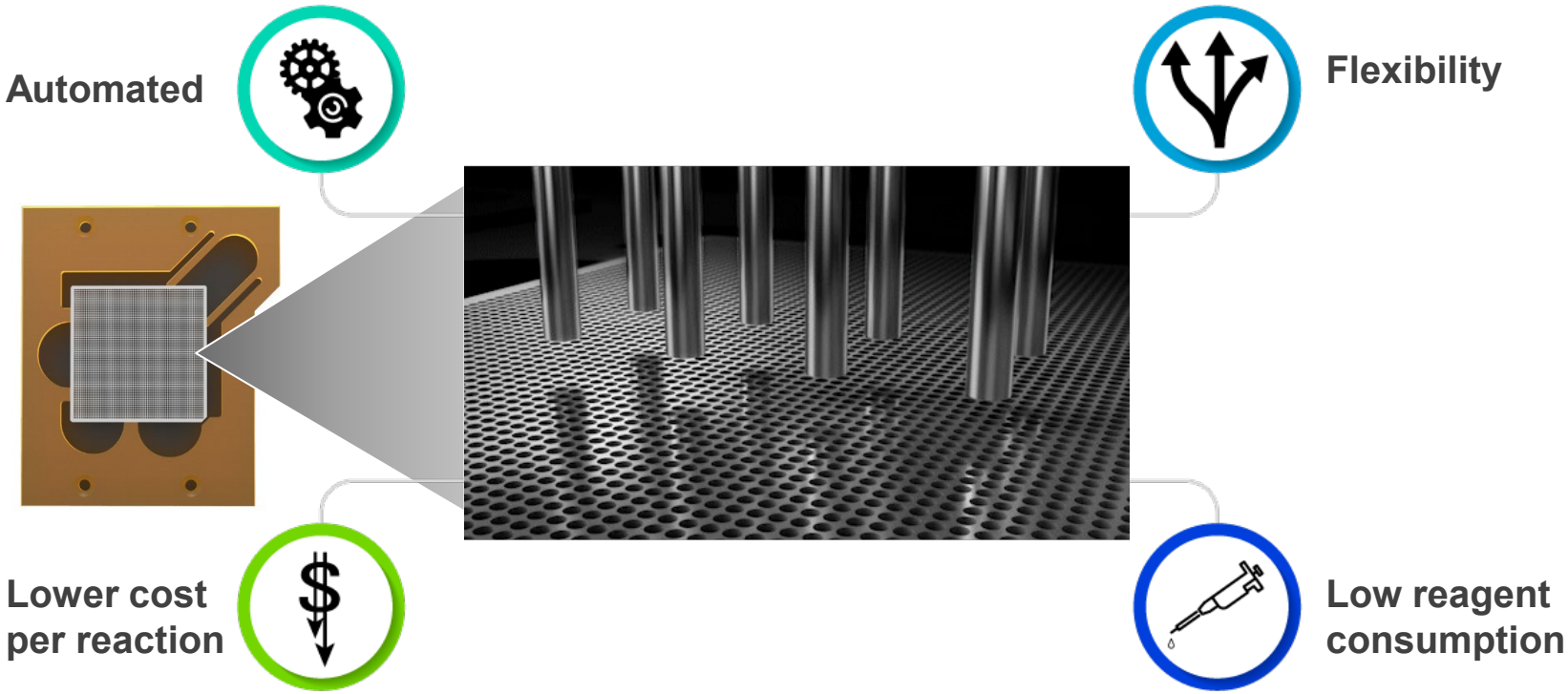
<1 hr sample and
assay dispense

SmartChip ND Cycler



~2 hr qPCR run
to data

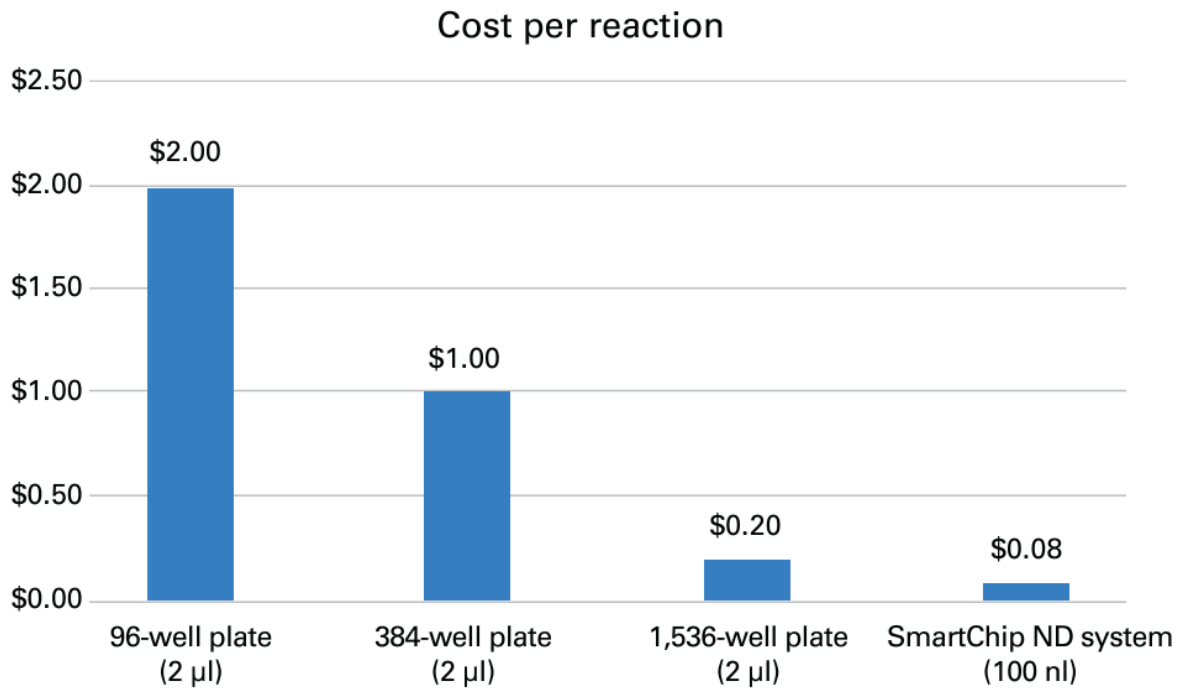
High-efficiency nanodispensing technology



- Low reagent consumption
- Lower cost per reaction than plate-based qPCR systems
- Flexibility to run different assay/sample combinations
- Automated, error-free workflow

Unlock significant savings with the SmartChip ND system

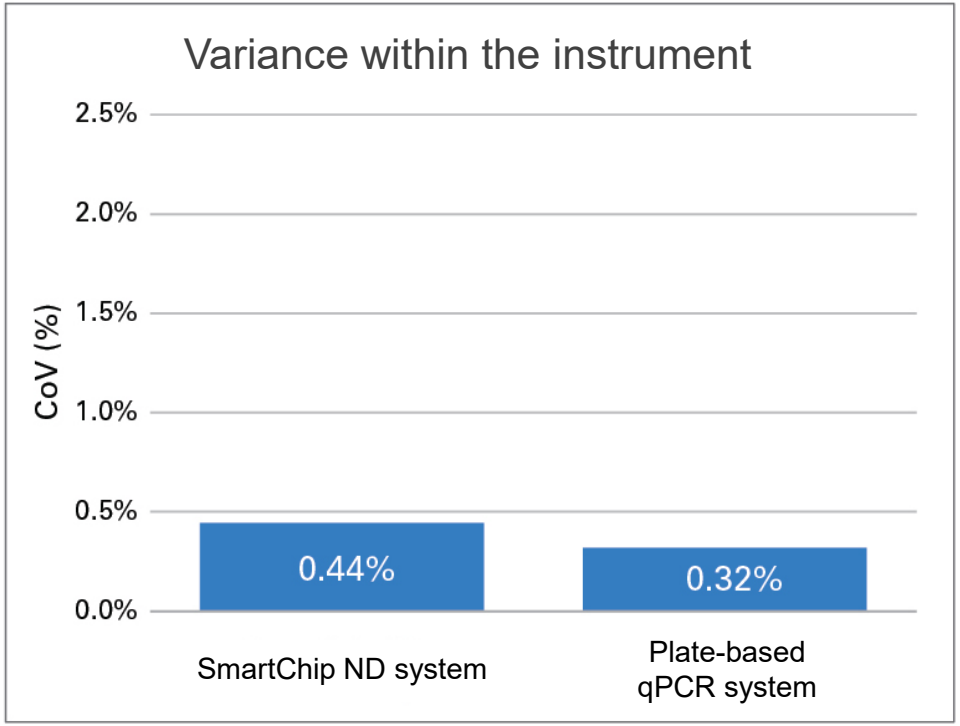
Reduce reaction costs by 200X with the SmartChip ND system



The SmartChip ND system utilizes 100 nl reactions, which offers significant reagent and cost savings over conventional plates.
A typical experiment performed with the SmartChip ND system costs $0.08/reaction, compared to up to $2/reaction for a 96-well plate.

Obtain consistent results at a large scale

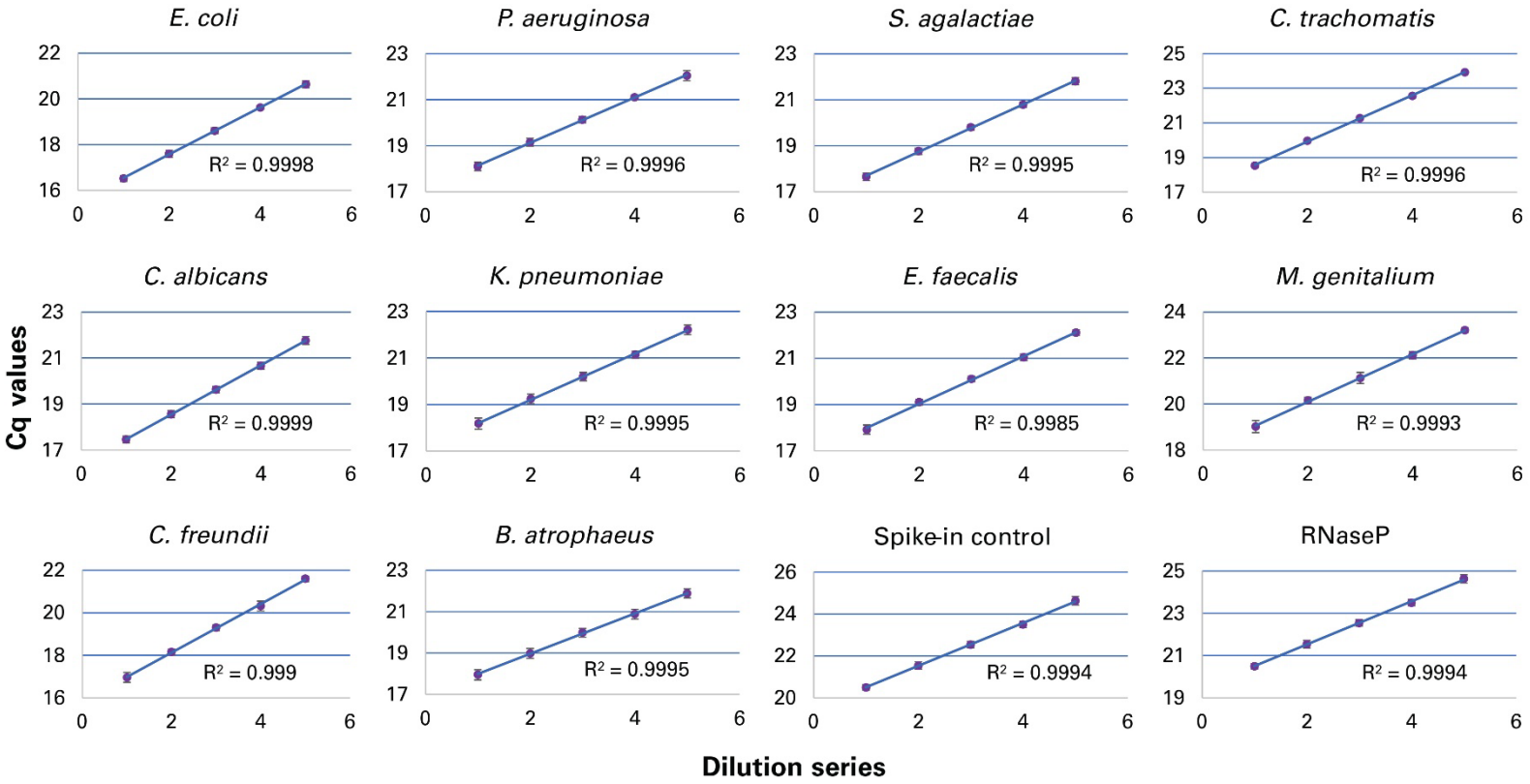
Increase sample throughput without sacrificing reproducibility



The SmartChip ND system exhibits a level of variability that is highly comparable to that of another vendor's 96-well plate-based qPCR system.

Achieve accurate gene expression analysis

Accuracy demonstrated by linear dilution in a variety of samples



Gene expression data using Takara Bio 5X PrimePath™ Probe qPCR Kit, GPR and SmartChip ND system with probe-based assays designed to detect pathogens in urine samples and swabs.

Automate your qPCR workflow with a complete system

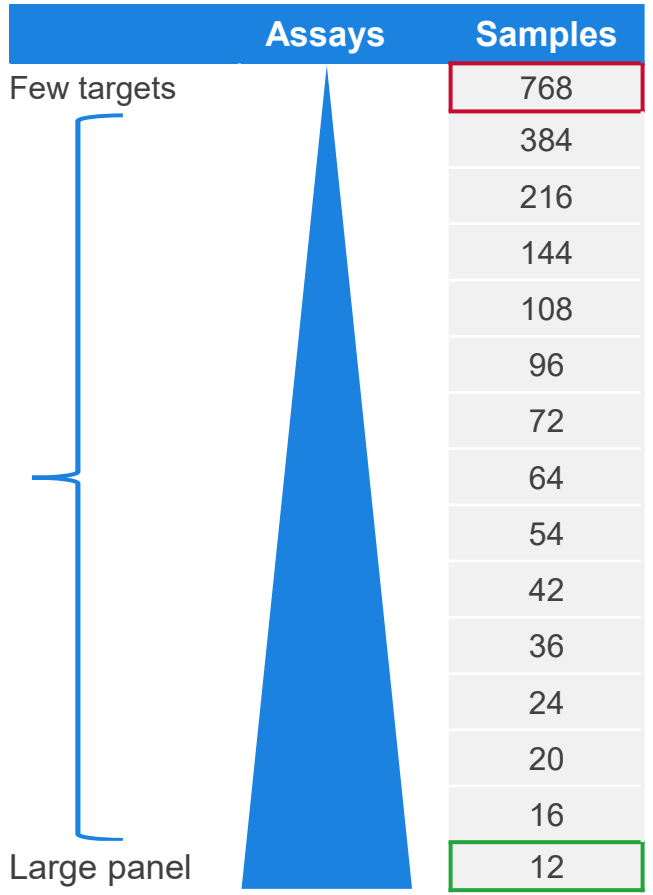
- Prepare source plate
- Load dispense file template
- Dispense chip
- Load qPCR protocol
- Perform qPCR
- Analyze data

Comprehensive software package helps with sample/ assay dispense and real-time PCR protocols. Hand-held barcode scanner tracks reagent and consumable usage.

Flexible panels to fit your application needs

Examples of molecular assays

- High-throughput SARS-CoV-2 testing
  768 different samples
- Nail fungus pathogen panel
  21 targets
- UTI alone/UTI + STI + wound
  up to 72-target pathogen panel
- Highly parallel ARG detection
  384 different targets*



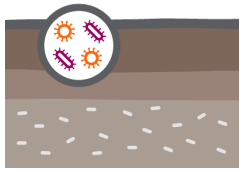
* Stedtfeld, R. D. et al. Primer set 2.0 for highly parallel qPCR array targeting antibiotic resistance genes and mobile genetic elements. *FEMS Microbiol. Ecol.* **94**, 130 (2018)

AMR research enabled by the SmartChip® system

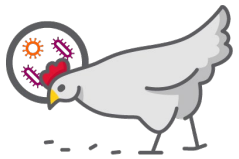
Over 100 publications from multiple research groups

USA, Canada, China, Australia, South America, and Europe

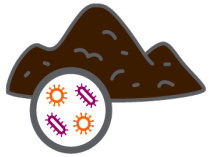
Sample types utilized:

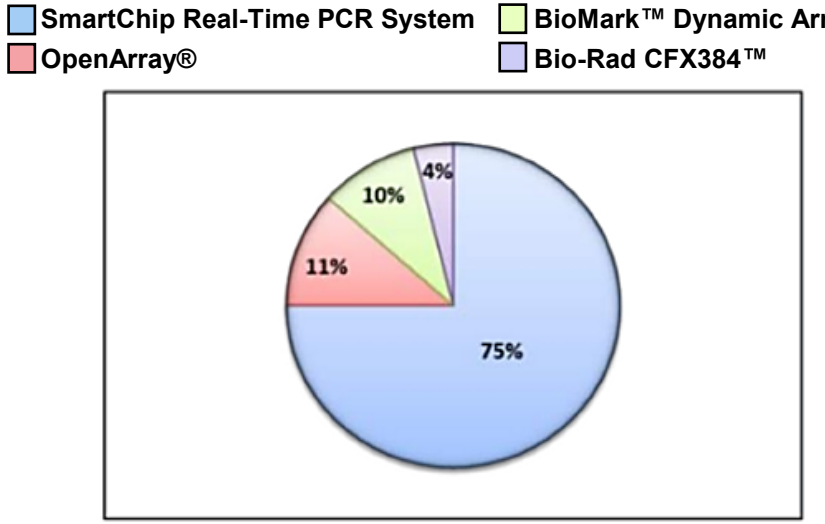

Soil


Sewage


Food


Water


Manure



Overall Percentages of HT-qPCR platforms used for antibiotic resistance over last 7 years

Image reused from “Contributions and challenges of high throughput qPCR for determining antimicrobial resistance in the environment: a critical review.” (Waseem et al. 2019, Molecules) under a CC BY 4.0 license.

Global adoption of the SmartChip system for diverse sample types

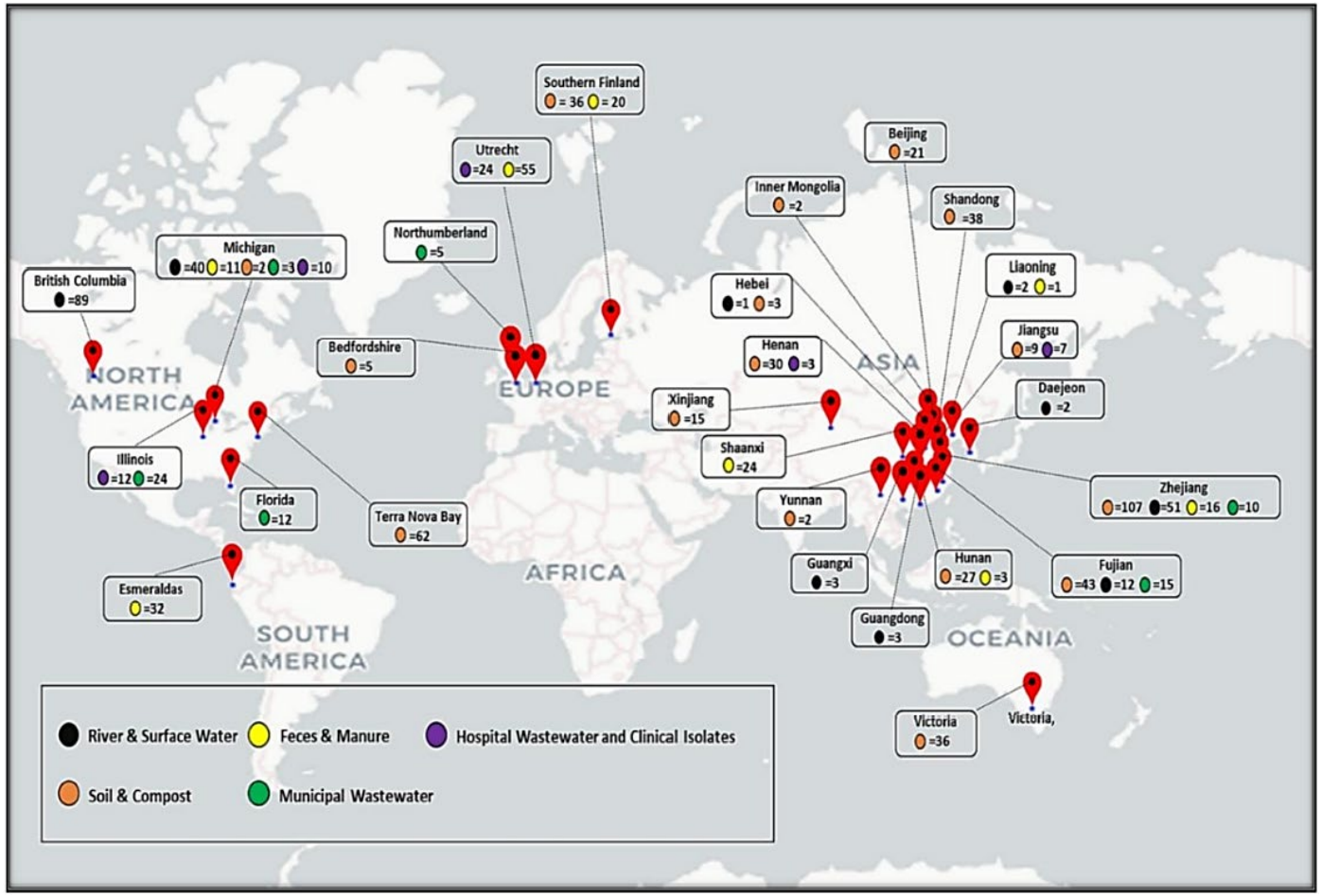


Image reused from “Contributions and challenges of high throughput qPCR for determining antimicrobial resistance in the environment: a critical review.” (Waseem et al. 2019, Molecules) under a CC BY 4.0 license.

Key reasons for using the SmartChip system to research ARGs

Flexibility

Custom panels can be modified to accommodate newly identified ARG targets

Best analytical sensitivity

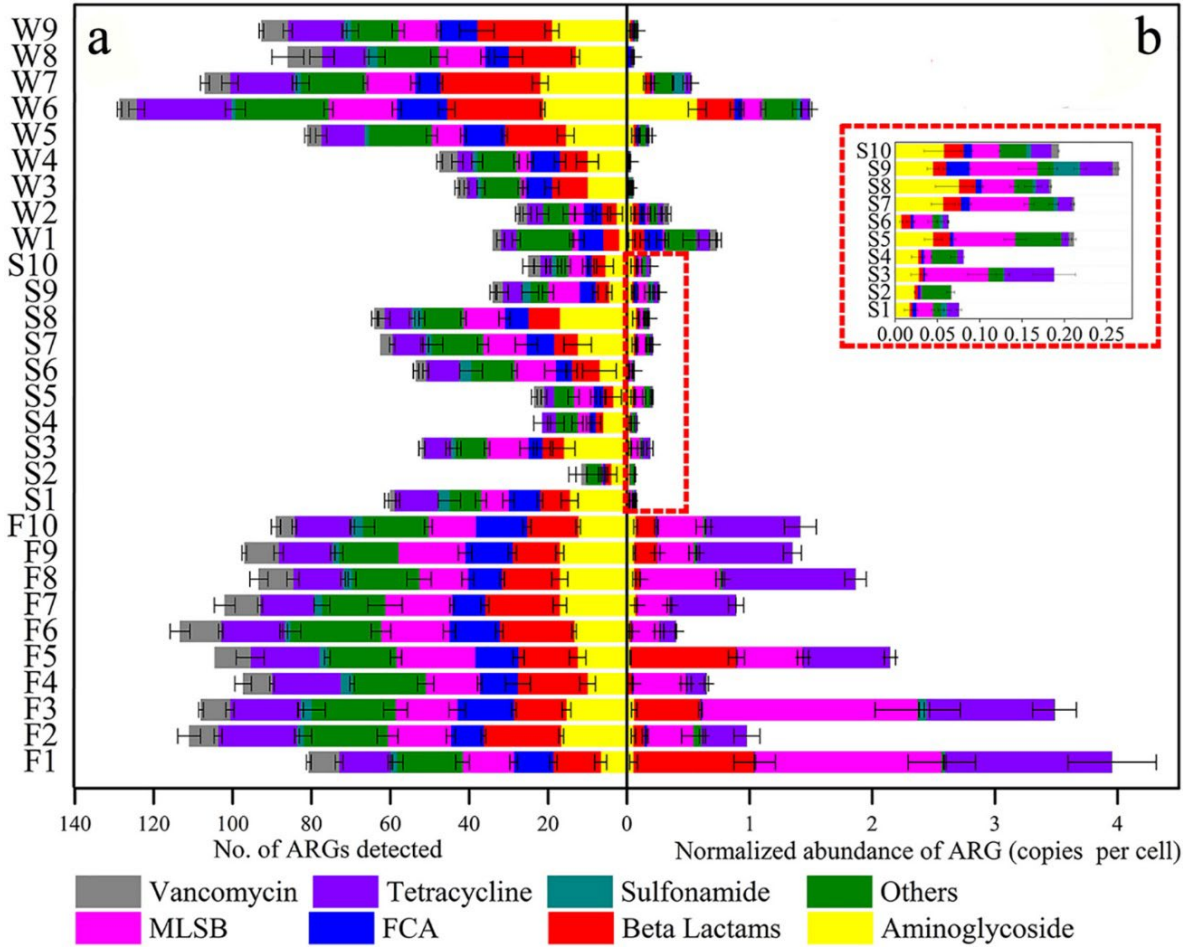
100 nl reaction volume offers advantage over other high-throughput platforms

Maximize savings

Green intercalating dye enables cost-effective chemistry

Case study:
ARGs in water on the SmartChip system

- Studied water from sewage treatment system, water from rivers, and human waste
- Utilized 96 primer pairs
- Detected 234 ARGs in human waste
- Found that ARG abundance in sewage was sevenfold higher than in river samples
- Identified 53 ARGs in human feces that were present in sewage



Case study:
ARGs in hospitals and farms on the SmartChip system

- Studied ARGs in air conditioning filters from hospitals (H), farms (F), cities (C), and villages (V)
- Utilized 296 primer pairs
- Detected 177 ARGs across all samples
- Found hospitals and farms had the most ARGs: 146 and 154, respectively
- Found ARG types were similar across locations

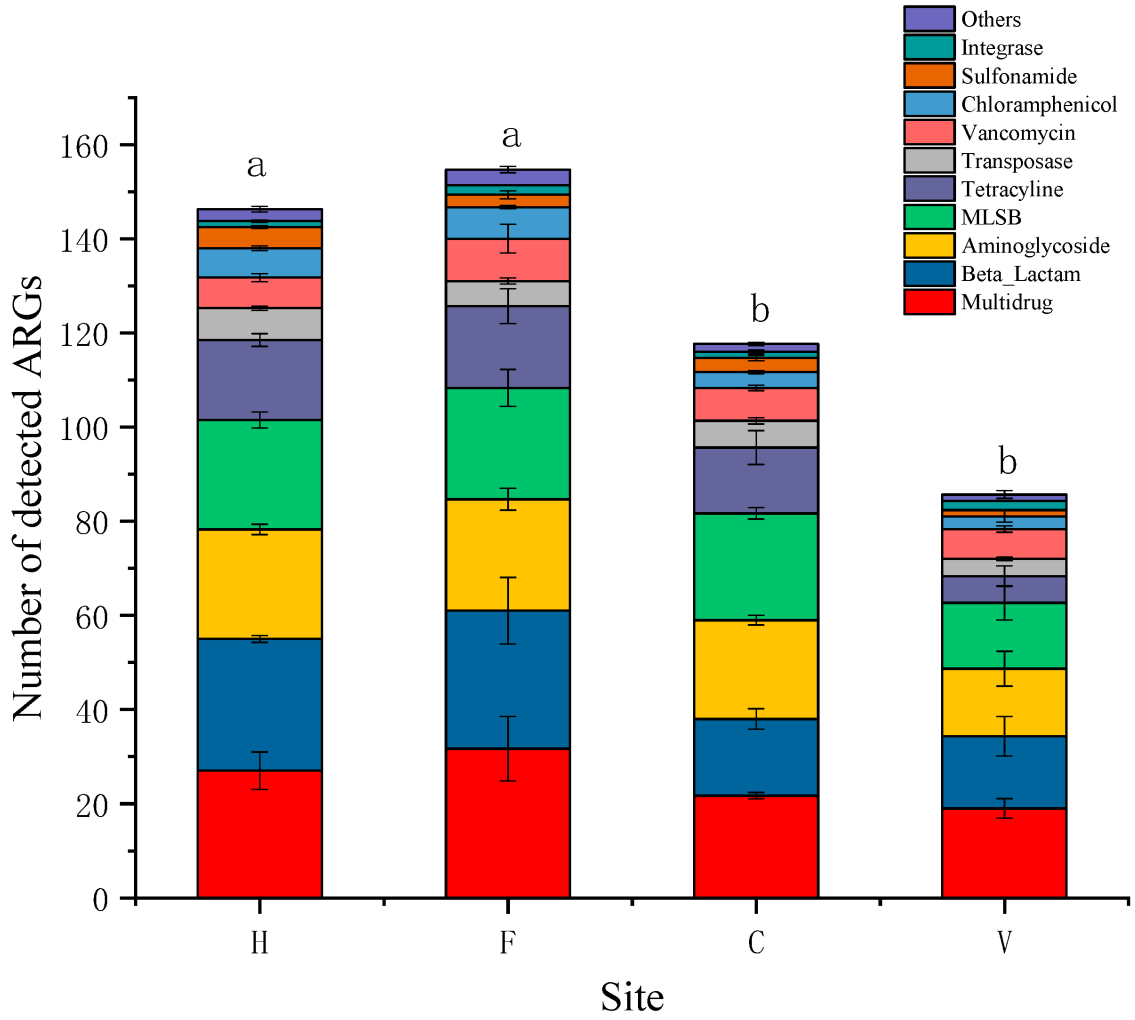
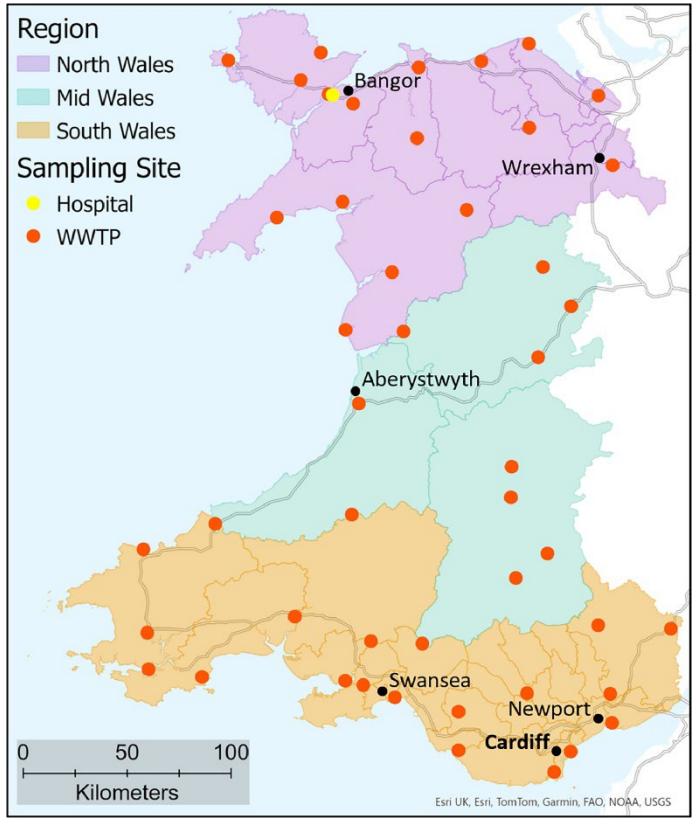
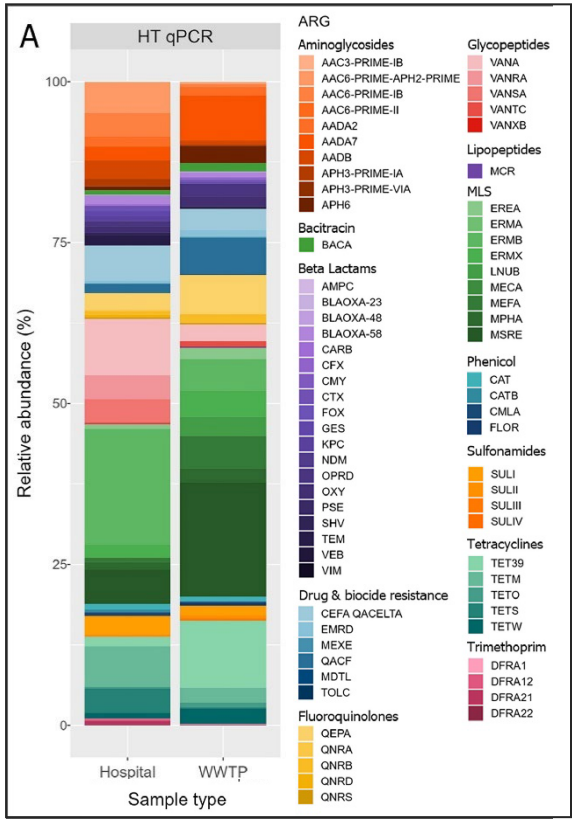


Figure reused from “Prevalence of antibiotic resistance genes in air-conditioning systems in hospitals, farms, and residences” (Li et al. 2019, *Int. J. Environ. Res. Public Health*) under a CC BY 4.0 license.

Case study: Implementing HT qPCR testing on a national scale

- Studied influent from wastewater treatment plants (WWTP) and effluent from major municipal hospital in Wales
- Utilized 96 primer pairs
- Detected 73 genes across samples
- Benefits of HT-qPCR testing
  - High sensitivity for low abundance ARGs
  - Short turnaround time
  - Low cost, data storage, and bioinformatics expertise
- Suited for surveillance programs requiring **precise quantification** (e.g., comparison studies and quantification of clinically relevant AMR genes)





Figures adjusted from “National-scale antimicrobial resistance surveillance in wastewater: A comparative analysis of HT qPCR and metagenomic approaches” (Knight et al. 2024, *Water Research*) under a CC BY 4.0 license.

Partnership with Resistomap Oy to stop the spread of antimicrobial resistance

- Provide comprehensive AMR monitoring solutions utilizing SmartChip technology
- Transform complex genetic data into actionable insights with a cutting-edge biosecurity intelligence platform
- Help customers understand the AMR profiles of their area to prevent resistance hotspots
- Customize ARG panels to identify genes of interest

About Resistomap

- Pioneered commercial environmental AMR (eAMR) gene monitoring
- Built only known quantified eAMR gene database in the world
- Aim to prevent AR spread by unlocking the power of eAMR gene data

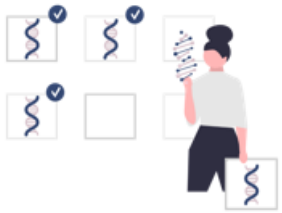
Since 2019:

- **17,400+** samples analyzed, in various forms of environmental samples
- **45 countries** of sample origin, from five continents
- **350+ projects** delivered, with largest coverage in Europe

resistomap

Dr. Windi Muziasari, CEO

Workflow and outcome



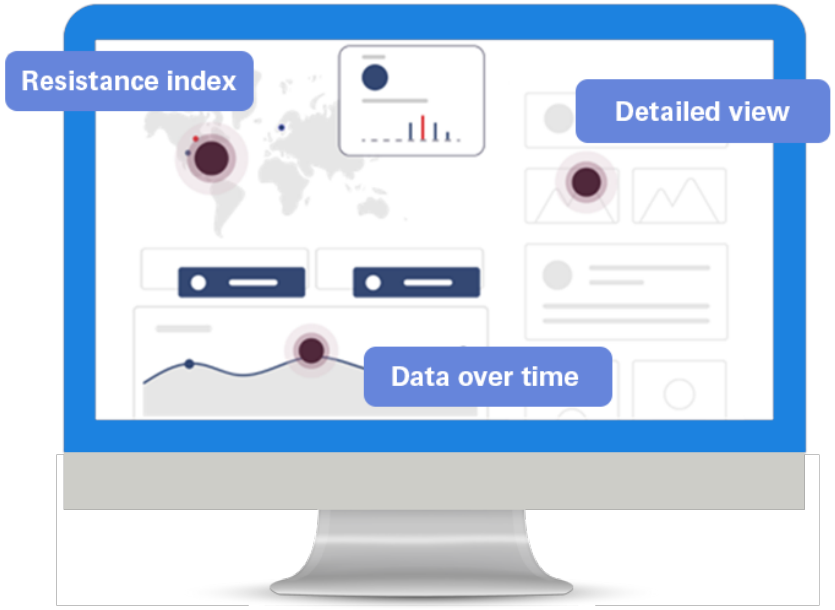
1 Monitoring design and onboarding

2 Sample pickup and shipment to Resistomap's facility in Helsinki

3 Environmental DNA extraction and targeted gene measurement using the **SmartChip ND system**

4 Data analysis and expert reviews

5 Intelligence platform



- **Resistance index** provides a comparable metric for eAMR gene abundance
- **Detailed view** delves into specifics, e.g., antibiotic groups or pathogens of interest
- Timely insights from **data over time** through the subscription model

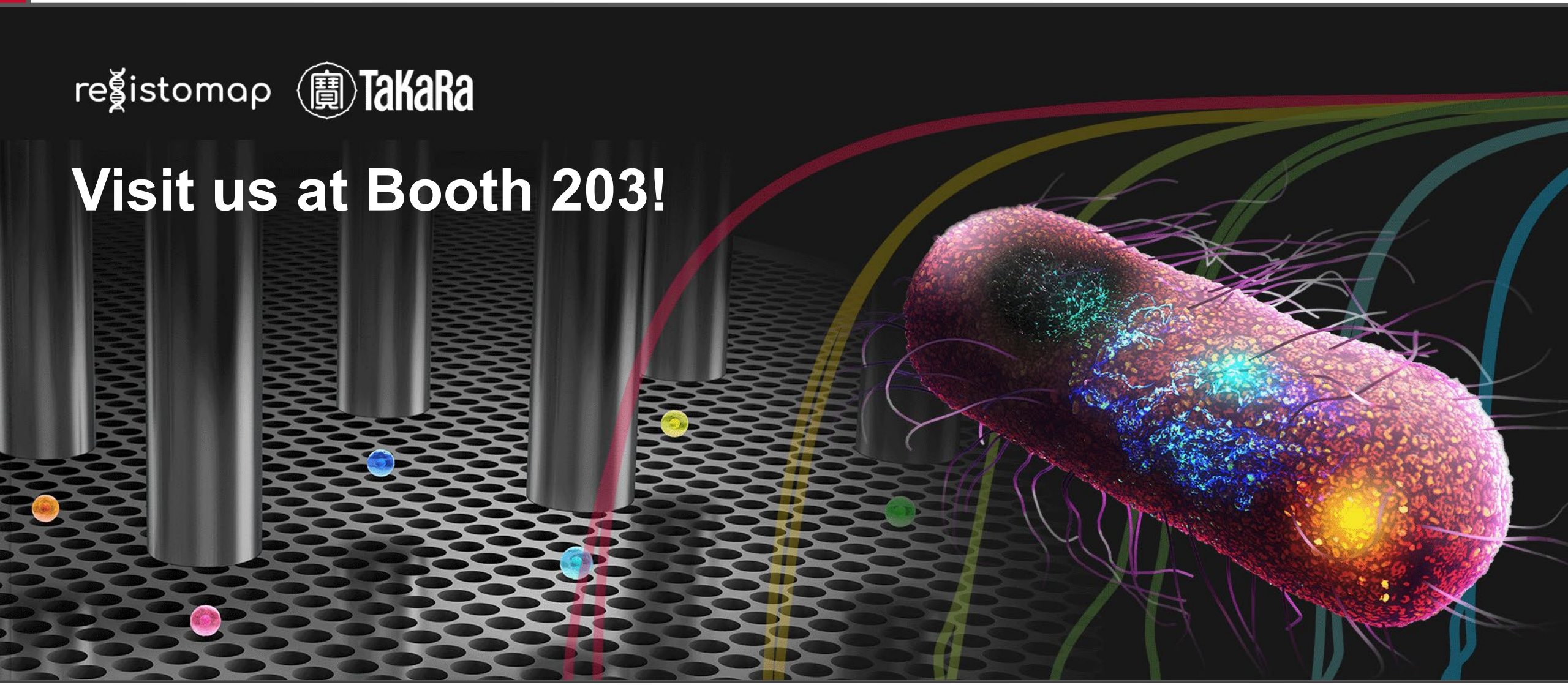
Summary: SmartChip ND system boosts AMR research

- Unmatched high-throughput capability for your large AMR runs
- Balanced sensitivity, even while scaling up
- Significant cost savings with fewer reagents and runs needed
- No significant expertise required to operate the technology and perform downstream data analysis, unlike NGS
- Reduced false positive or negative results compared to conventional, culture-based AMR testing
- Customizable panel design to easily modify ARG targets
- Applicable to a wide range of AMR samples and workflows
- Exceptional published track record of enabling AMR surveillance research

Unlock answers for your AMR surveillance

registomap TaKaRa

Visit us at Booth 203!



TaKaRa

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