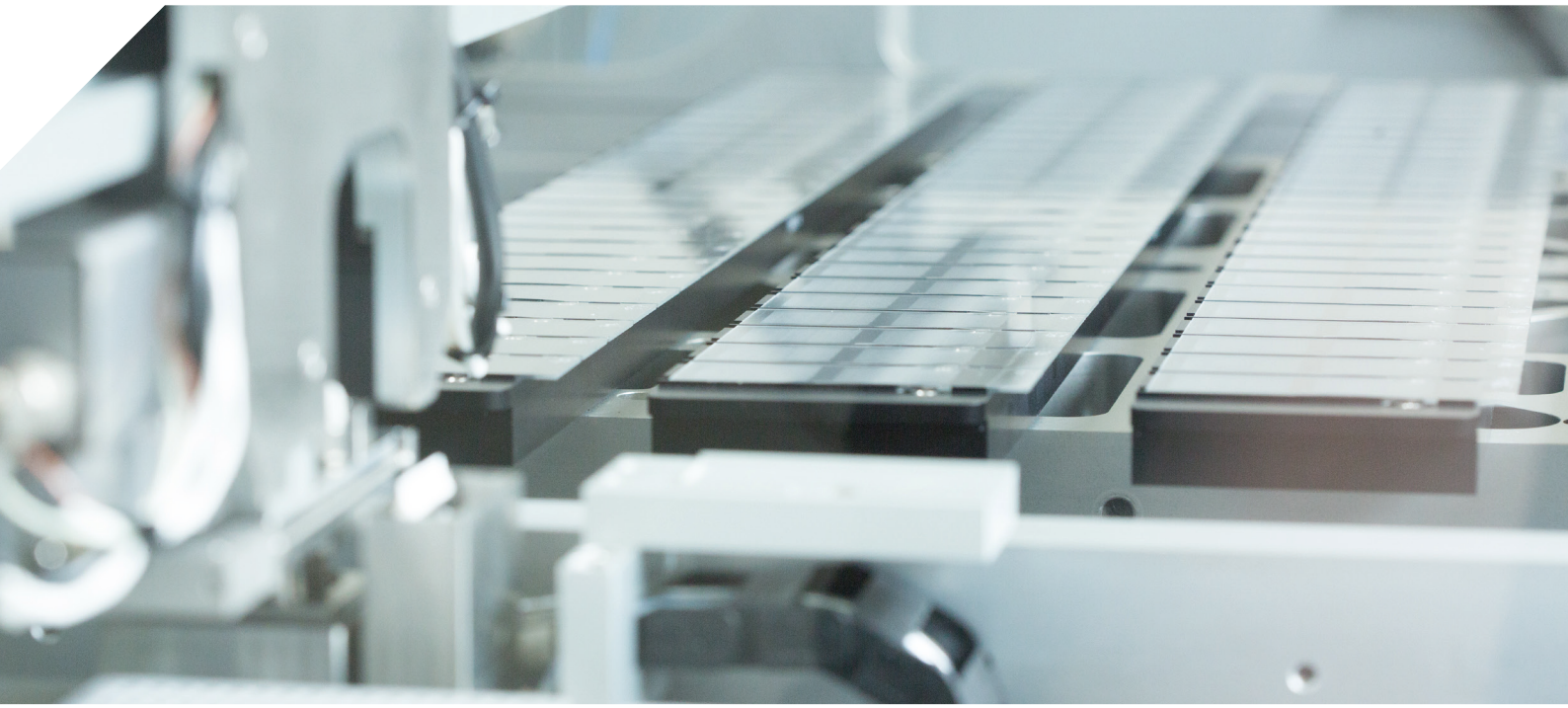
AUSTRIAN INSTITUTE OF TECHNOLOGY



# CUSTOMIZED HIGH-MULTIPLEX ASSAY DEVELOPMENT

## INTRODUCTION

The DNA-based characterization of clinical samples is becoming increasingly popular in many different application areas. An efficient approach to reduce the cost per analysis is to detect multiple targets in a single reaction. However, unintended side-reactions caused by the increased reaction complexity and multiple oligonucleotide interactions significantly reduce the specificity and sensitivity of DNA-based assays. We have developed several novel in silico and in vitro tools to overcome these limitations and to optimize multiplex assays. These technologies were successfully applied during the development of molecular diagnostic tests for human multidrug-resistant pathogens. In our business unit, assays detecting up to 1000 different genetic targets with a single test were designed including their experimental evaluation and pre-clinical validation. Based on this comprehensive know-how, we can offer our customers a wide range of services such as technology consulting, assay development and support for the clinical validation of the final assays.

## COMPLEX DISEASE DIAGNOSTICS

At the beginning of the assay development process, the exact definition of the desired test specifications (cost, limit of detection, specificity, runtime, etc.) is essential for the successful commercialization of the envisioned diagnostic tests. Together with our customers, we discuss the advantages and limitations of each relevant technology and select the most appropriate for the application scenario. Other important issues concern the clinical validation of the multiplex assay which must be considered from the very beginning due to e.g. the availability of a sufficient number of clinical samples comprising the genes of interest.

Based on our customer's requirements, a sequence database comprising all relevant target and non-target sequences can be created. Using our in-house, state-of-the-art software solutions, oligonucleotides are designed and evaluated in silico regarding their sensitivity and specificity. It is also possible to further improve the multiplex assay performance using in vitro high-end techniques capable to identify primer-derived amplification artefacts. In addition to assay implementations on commercial multiplex detection platforms, we have long-lasting experience in developing custom solid-support-based detection systems. Our portfolio is complemented by assay evaluation and validation services.

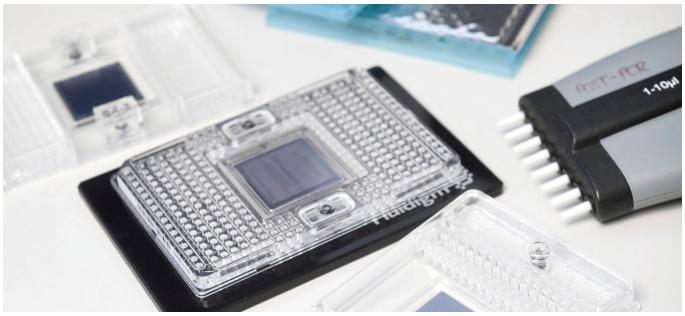
## RESEARCH SERVICES

**Bioinformatics**
- Automated sequence database management
- High-throughput design tools for DNA-based multiplex assays
- Design of complex protein/DNA nanostructures

**Experimental evaluation of primer artefacts**
- Nano-fluidic qPCR for identification of unintended background DNA and primer interactions
- NGS-based primer dimer sequencing
- In silico analysis of oligonucleotide interactions

**Assay performance analysis**
- Evaluation of assay with synthetic DNA templates
- Pre-clinical validation using spiked samples (biobank comprising ca. 100 different bacterial species and all clinically relevant ABR genes is available)
- International clinical network for clinical validation studies

**Assay development**
- Evaluation of appropriate sample preparation and processing
- Selection of general amplification method (PCR vs. isothermal), instruments and customized modifications (asymmetric, crosslinked primers,etc)



**Detection platforms and principles**
- Development of custom micro- and macroarrays with specialized surface structures
- Implementation into existing multiplexing solutions such as Luminex, Fluidigm, NGS platforms, etc.
- Possible detection principles include fluorescence and colorimetric approaches with reporter enzymes, DNAzymes or labeled probes

## SELECTED PUBLICATIONS


- PRIMEval: Optimization and screening of multiplex oligonucleotide assays. Conzemius et al. Scientific Reports (2019)
- Low-cost microarray platform to detect antibiotic resistance genes. Wolff et al. Sensing and Bio-Sensing Research (2019)
- Oli2go: An automated multiplex oligonucleotide design tool. Hendling et al., Nucleic Acids Research (2018)
- Multiplex characterisation of human pathogens including species and antibiotic resistance gene identification. Barisic et al., Journal of Medical Microbiology (2015)
- Fast and highly specific DNA-based multiplex detection on a solid support. Barisic et al., Applied Microbiology and Biotechnology (2014)
- Multiplex detection of antibiotic resistance genes using padlock probes. Barisic et al., Diagnostic Microbiology and Infectious Disease (2013)



**CONTACT: Dr. Ivan Barisic**
AIT Austrian Institute of Technology GmbH
Competence Unit Molecular Diagnostics
Tel +43(0) 50550-4313 // Mobil: +43(0) 664 88390643
Giefinggasse 4 // A-1210 Vienna
Ivan.barisic@ait.ac.at // www.ait.ac.at