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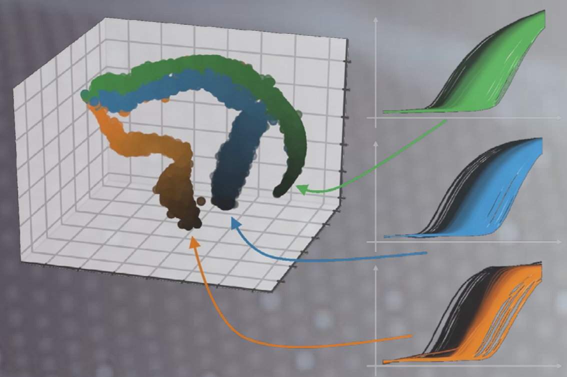

Data-driven Multiplexing for Accurate Gene Detection

Machine learning approach for high-level multiplexing in qPCR and dPCR, up to 21-plex demonstrated

The technology enables accurate multiplexing (up to 21 targets in a single well has been demonstrated). The patented approach enables the recognition of primer-characteristic molecular signatures. This gives rise to truly affordable solutions in established molecular tests, by effectively extracting the kinetic and thermodynamic information from existing real-time data. Importantly, this technology is compatible with conventional qPCR and state-of-the-art dPCR set-ups.

Proposed use

This technology enhances diagnostic performance and increases throughput by identifying multiple nucleic acid targets in a single amplification reaction. It is compatible with a wide range of amplification chemistries (*e.g.*, probe-based, intercalating dyes, and isothermal reactions), and hence, can be seamlessly integrated with various laboratory workflows.

Problem addressed

Efficiency and affordability are paramount for a wide range of diagnostic applications including infectious diseases, genotyping and precision cancer medicine. Multiplexing offers a solution that reduces the requirements in physical space, time-to-result, and volume of reagents and sample. To date, multiplexed assays rely on fluorescent probes (limited by optical instrumentation), post-amplification analysis (lengthy gel-electrophoresis or expensive sequencing approaches) or spatial multiplexing (resource consuming).

Technology overview

The technology leverages machine learning to automatically learn target-specific information encoded in each amplification event (*via* real-time data), to identify the nature of nucleic acid molecules.

Benefits

- Enables a **time and cost-effective** solution to identify multiple nucleic acids in a single chemical reaction
- Provides **extremely reliable and accurate** high-level multiplexing capability
- Applies across real-time PCR platforms and amplification chemistries that are **used in many scientific fields**
- Identifies **millions of single amplification reactions** in seconds

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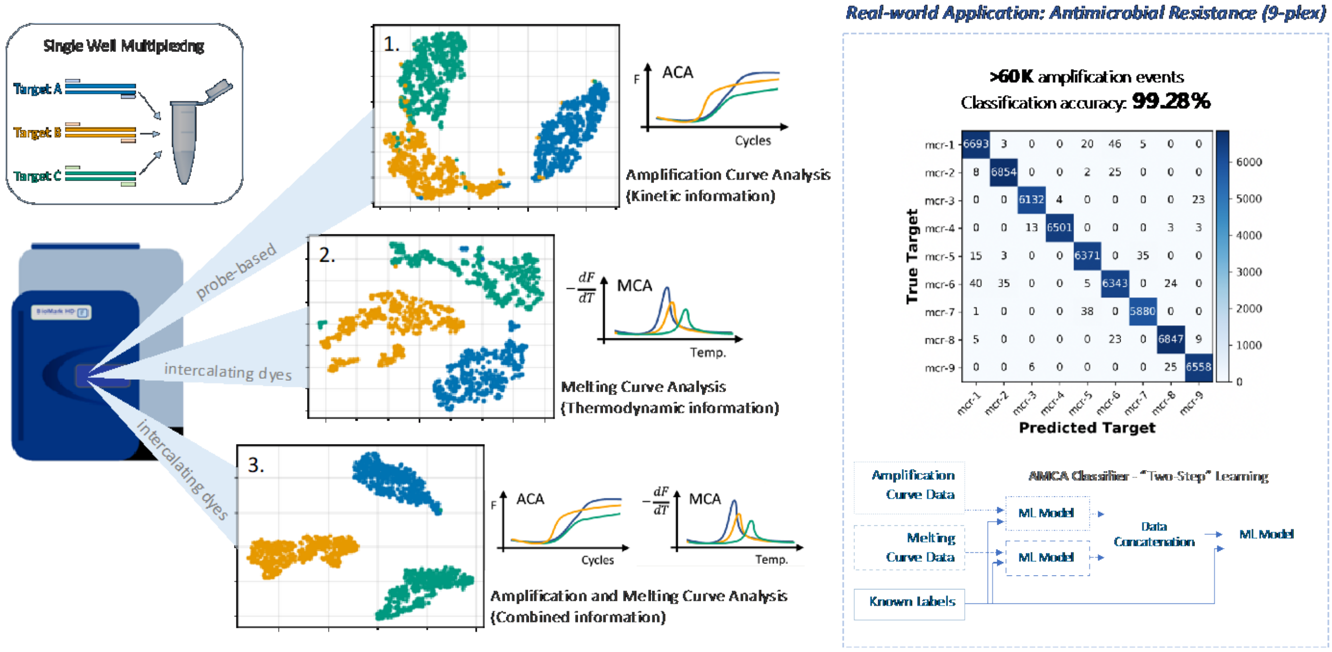

Data-driven Multiplexing can leverage kinetic or thermodynamic information encoded in the amplification event for target detection. If required, performance can be further boosted when multiple data sets are combined together using our proprietary approach. Verified analysis modes include:

1. Amplification Curve Analysis (ACA) consists of training a supervised machine learning model to distinguish targets based on the entire real-time amplification curve. By looking at each curve as a point in multiple dimensions, our algorithms can automatically identify target-specific features for classification

2. Melting Curve Analysis (MCA) consists of training a supervised machine learning model to distinguish targets based on the thermodynamic information of the amplification product

3. Amplification and Melting Curve Analysis (AMCA) combines the probabilistic predictions from ACA and MCA to form a significantly improved identification that is able to increase the classification accuracy and/or number of targets

Intellectual property information

GB 2013035.7 - IDENTIFYING A TARGET NUCLEIC ACID

Link to published paper(s)

Moniri A, Miglietta L, Malpartida-Cardenas K, Pennisi I, Cacho-Soblechero M, Moser N, Holmes A, Georgiou P, Rodriguez-Manzano J. Amplification Curve Analysis: Data-Driven Multiplexing Using Real-Time Digital PCR. Anal Chem. 2020 Oct 6;92(19):13134-13143. doi: 10.1021/acs.analchem.0c02253. Epub 2020 Sep 18. PMID: 32946688.

Moniri A, Miglietta L, Holmes A, Georgiou P, Rodriguez-Manzano J. High-Level Multiplexing in Digital PCR with Intercalating Dyes by Coupling Real-Time Kinetics and Melting Curve Analysis. Anal Chem. 2020 Oct 20;92(20):14181-14188. doi: 10.1021/acs.analchem.0c03298. Epub 2020 Oct 2. PMID: 32954724.

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