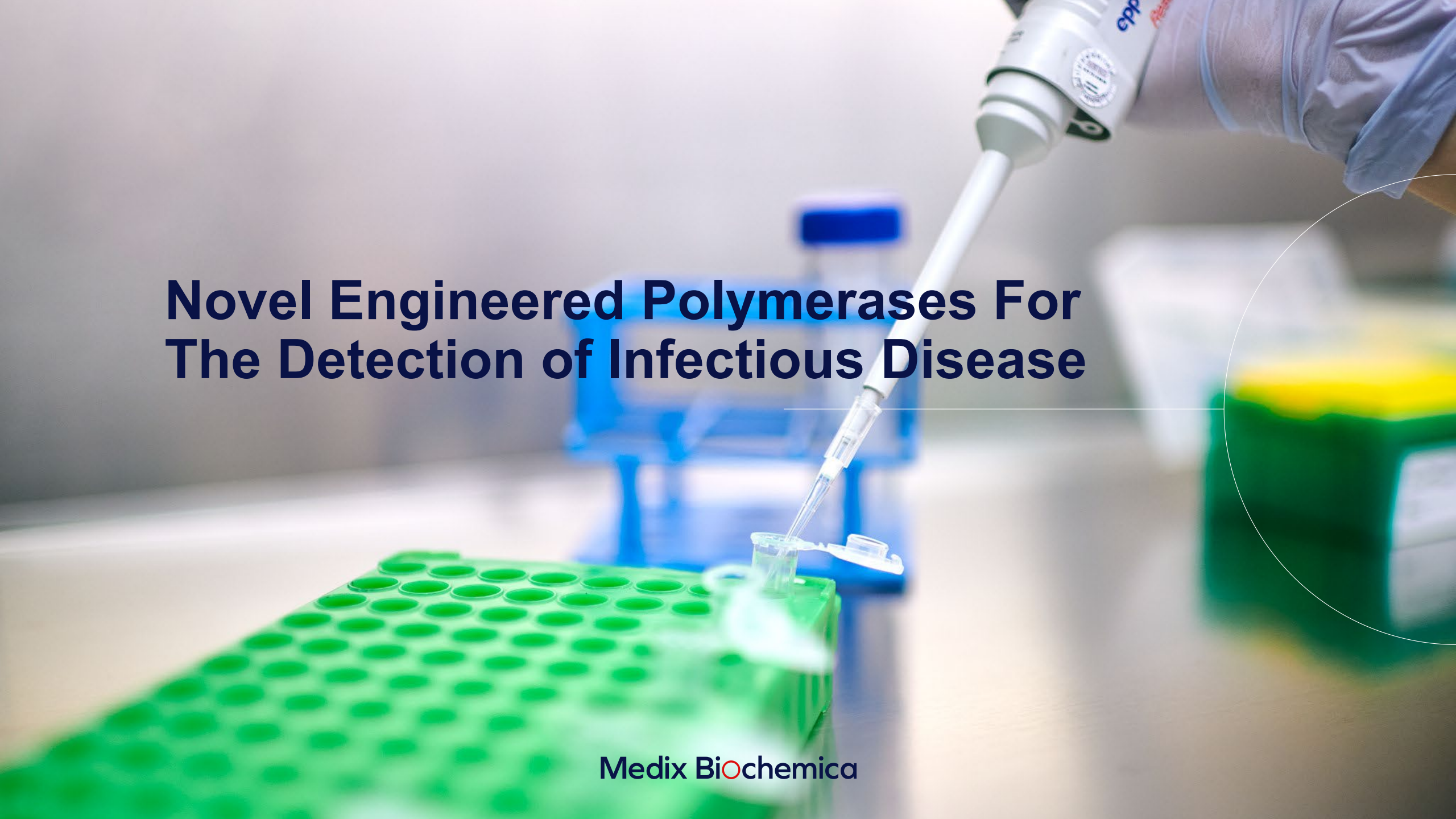


Novel Engineered Polymerases For The Detection of Infectious Disease

Medix Biochemica

Before we get started…

Please use the Q&A button at the bottom of your screen to submit any questions.

There will be a 10-minute Q&A session at the end of this webinar. If we do not have time to answer your questions, we will reach out by email following the webinar.

This webinar is being recorded. The recording as well as on-demand link will be released following the webinar.

Medix Biochemica

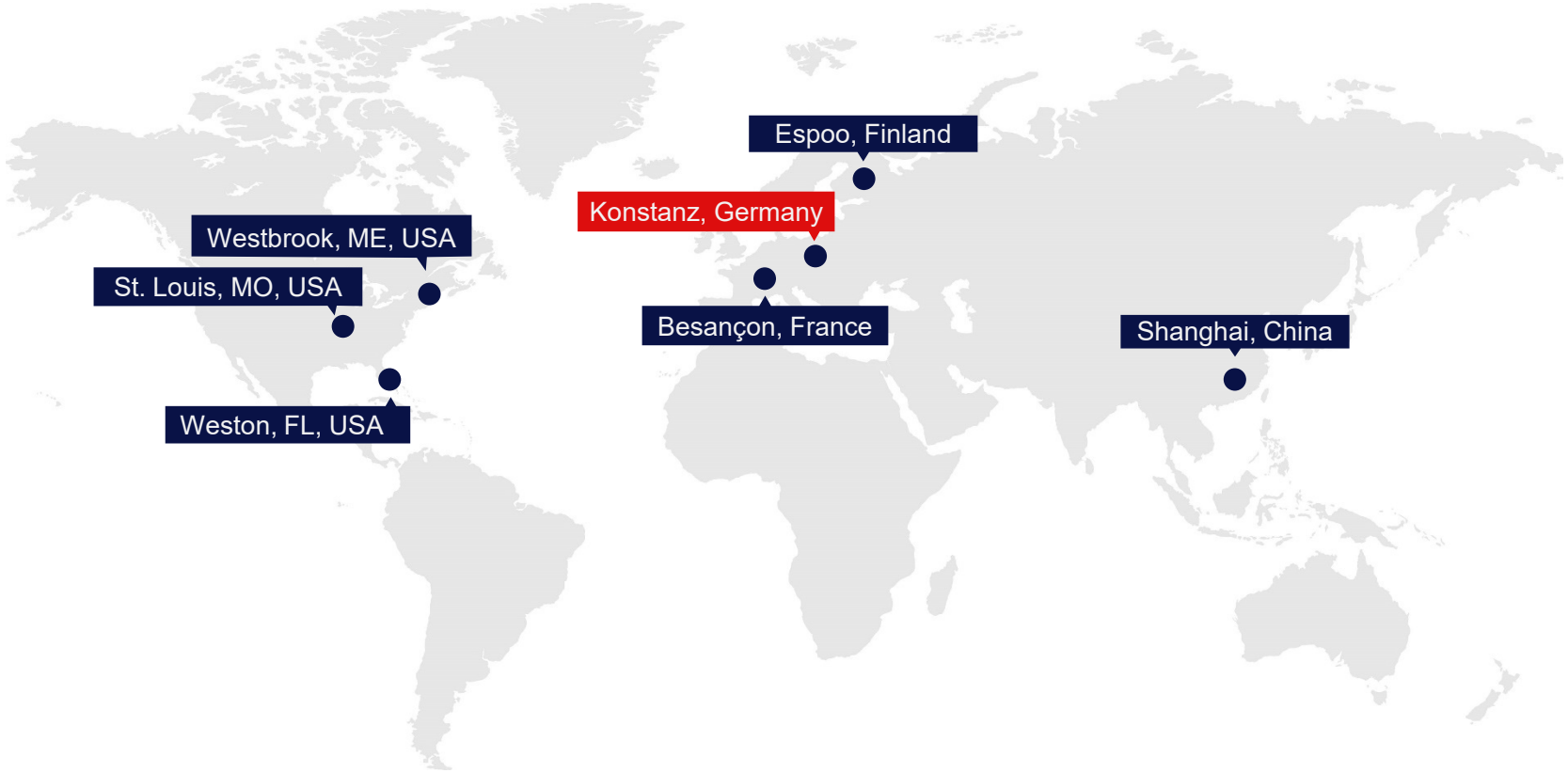
Medix Biochemica

We’re Wherever You Are

- A truly global company with a proudly Finnish background
- Our products are used in over 70 countries
- Offices and manufacturing facilities all around the world

Employee Expertise

- Decades of dedication to developing essential raw materials for diagnostic applications
- Approximately 270 employees, with 1 in 5 people work in R&D



Medix Biochemica

MedixMDx

Engineered Polymerases for Molecular Diagnostics

and more…

- Unique Polymerases for DNA/RNA
- ISO:13485 Conformity
- Assay Development Services
- Lyophilization Services
- Lyo-Ready PCR Products

Medix Biochemica

Today's Speaker:

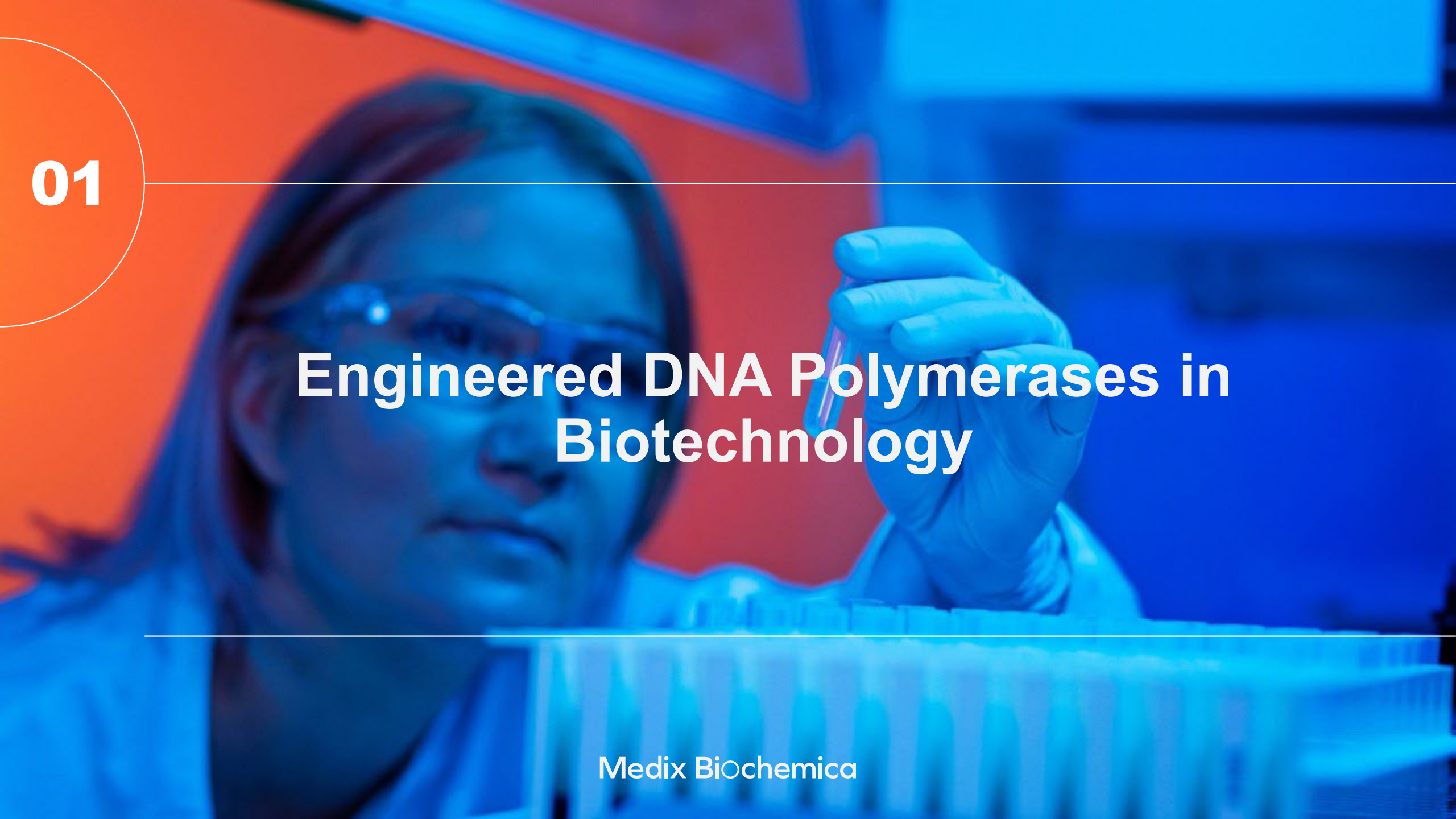
Dr. Maja Studencka-Turski

Scientific Lead R&D

Medix Biochemica

01

Engineered DNA Polymerases in Biotechnology



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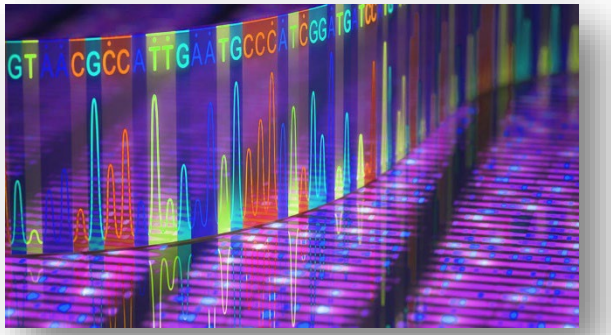
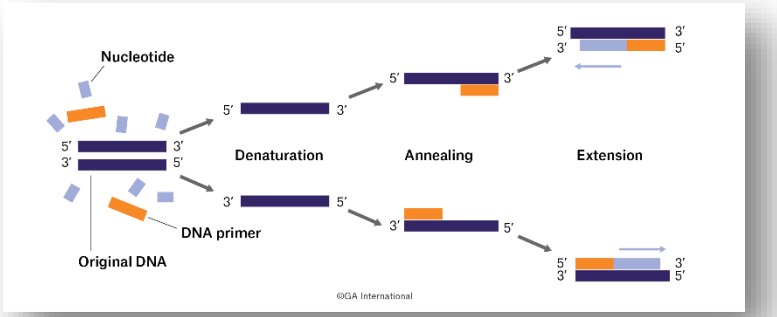
DNA Polymerases Play Central Roles In Modern Molecular Biology And Biotechnology

DNA Cloning

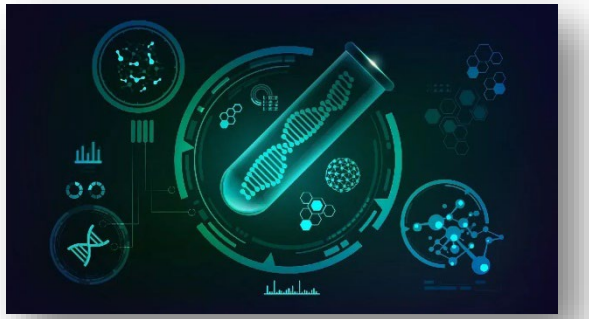


DNA Sequencing

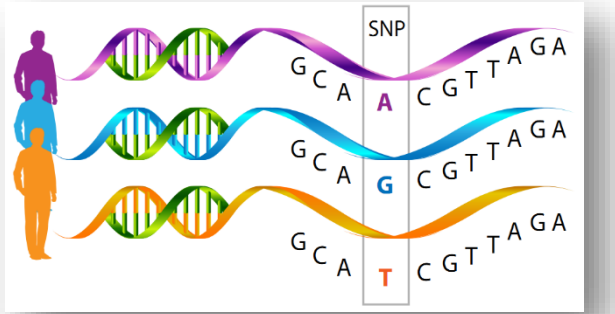
Molecular Diagnostics



Polymerase Chain Reaction (PCR)



Synthetic Biology



SNP Detection

https://www.nutrigeneticsspecialists.com/single-post/2017/03/27/what-is-a-snp
https://blog.labtag.com/a-brief-history-of-pcr-and-its-derivatives
https://www.ck12.org/c/biology/biotechnology/lesson/Biotechnology-BIO/?referrer=concept_details
https://www.jax.org/news-and-insights/jax-blog/2017/January/bridging-the-gaps-in-dna-sequencing
https://www.technologynetworks.com/drug-discovery/blog/how-is-synthetic-biology-shaping-the-future-of-drug-discovery-340290

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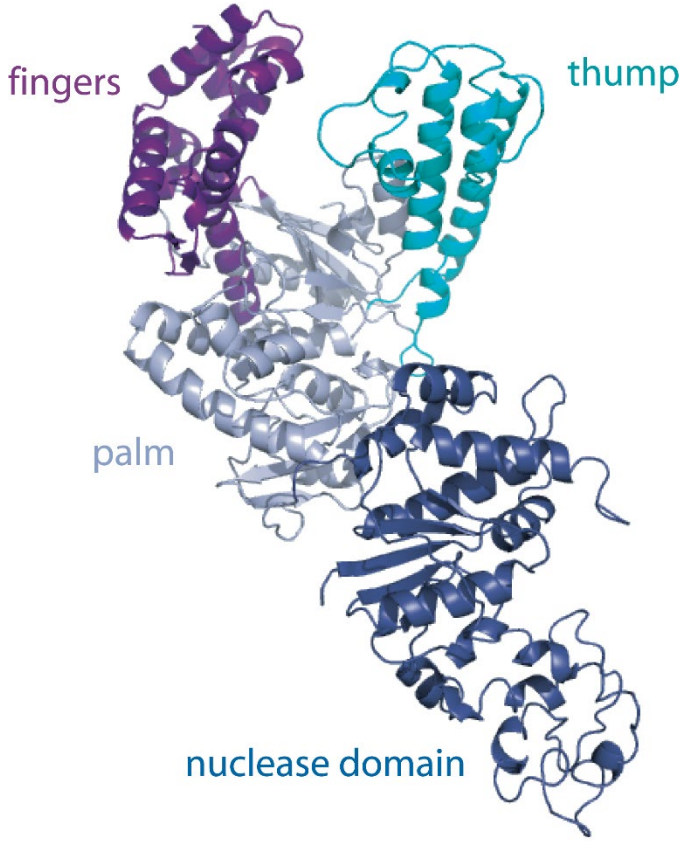
DNA Polymerases – “Nature’s Molecular Machines”

Each thermostable DNA polymerase has its own set of unique characteristics:

- Thermostability
- Extension rate
- Fidelity
- Processivity
- Specificity
- Ability to bypass damage
- Nuclease activity
- Strand displacement activity



“Natural DNA Polymerases Often Do Not Have The ‘Performance Specifications’ Needed For Transformative Technologies”

(Zahra Ouaray, *Et Al.* J Biol Chem. 2020 Dec 11; 295(50): 17046–17059.)

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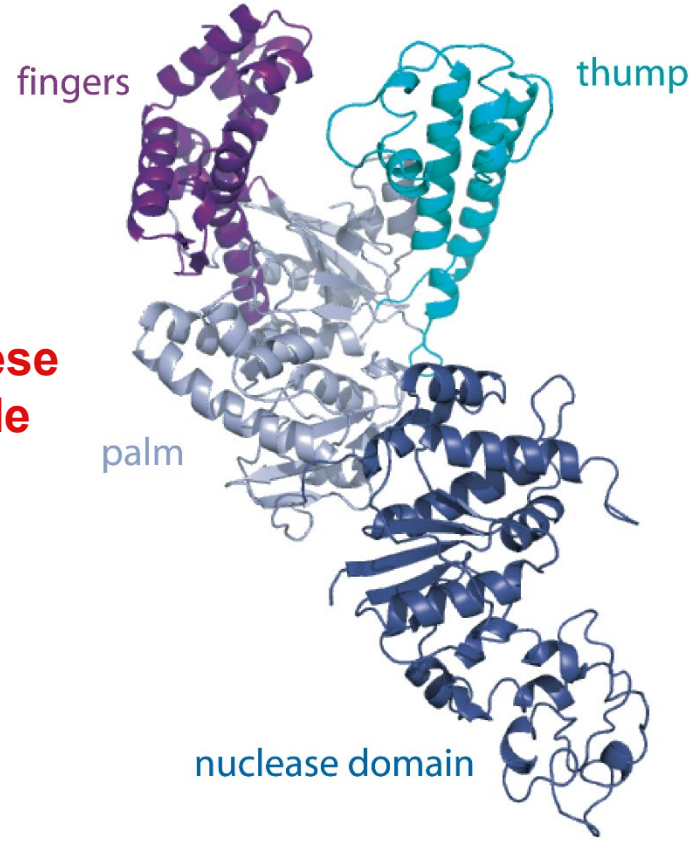
What does Engineering Polymerases Allow Us To Do?

Each thermostable DNA polymerase has its own set of unique characteristics:

- Thermostability
- Extension rate
- Fidelity
- Processivity
- Specificity
- Ability to bypass damage
- Nuclease activity
- Strand displacement activity

Engineering Polymerases allows us to adjust any of these characteristics to solve a wide range of problems



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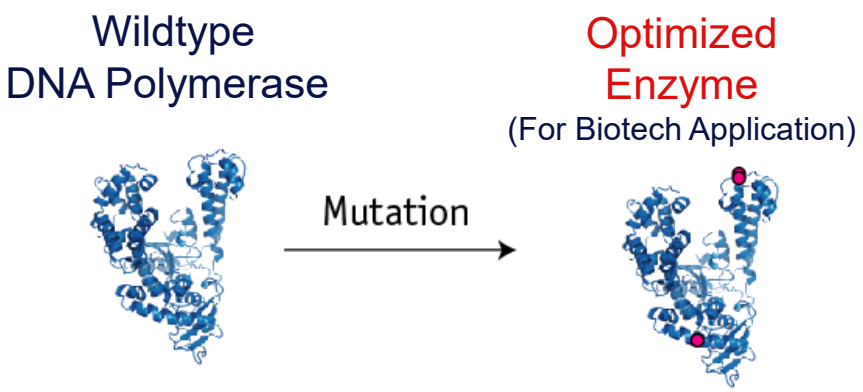
How Do We Genetically Engineer DNA Polymerases?

Tailoring DNA Polymerases By **Random Mutagenesis**



Rational Design Approach

- Sufficient Information About The Enzyme Necessary
  - e.g. Site Directed Mutagenesis
- Example: Klentaq Polymerase → Mutation Of Amino Acids Involved In Substrate Binding

02

Infectious Disease Applications

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Engineered Polymerases And Their Role In Infectious Disease

Engineering polymerases to have unique characteristics could significantly advance the field of infectious disease testing.

Improvements and innovations could include:

Increased Sensitivity and Specificity

- More sensitive detection of pathogens, (i.e. lower limit of detection)
- Enhanced specificity to reduce likelihood of false positives

Faster Reaction Times

- More efficient polymerases to speed up the polymerase chain reaction (PCR) process.
  - Crucial in infectious disease diagnostics where rapid results can lead to quicker treatment decisions and containment measures
  - Reliable results with crude samples without extraction step

Broader Range of Detectable Pathogens

- Engineered polymerases could recognize a wider range of sequences or to function under varying conditions
- Tests could be developed to detect a broader spectrum of pathogens, including emerging or mutating infectious agents.

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Engineered Polymerases And Their Role In Infectious Disease

Improved Stability and Versatility

- Engineered polymerases could be more stable at room temperature, or function effectively in a wider range of conditions
  - Making diagnostic tests **more versatile and easier to distribute**.
  - Particularly important in resource-limited settings or **in-field diagnostics** where laboratory facilities are not available.
  - Key in enabling **Point-of-Care testing**

- As well as enabling the **Integration with Advanced technologies** such as NGS and CRISPR-based technologies

Medix Biochemica

Our Engineered Polymerases

PlexTaq® 5x qPCR
Multiplex Master Mix

RNA Applications
(RT-PCR)

Isothermal Amplification

Medix Biochemica

PlexTaq® 5x qPCR Multiplex Master Mix

PlexTaq® 5x qPCR Multiplex Master Mix

All-In-One - A Unique Engineered Solution

Robust - Up to 30 target multiplexing in real-time

5X Concentration - Maximizing volume available for primers/probes

Fast Time-To-Results - Reliable results with crude samples (High Inhibitor Tolerance)

Highly Specific - No false amplification.

Lyo-ready - Enables RT storage and shipping once dried.

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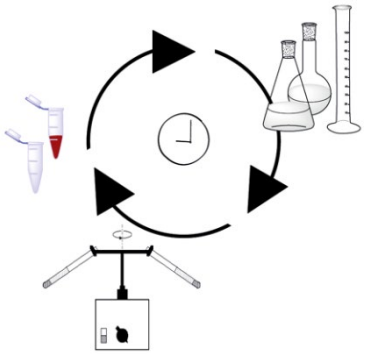
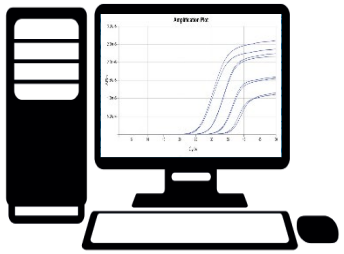
DirectPCR From Blood – PlexTaq® 5x qPCR Multiplex Master Mix





Traditional Approach: Blood Samples → DNA Extraction → qPCR

Direct Approach: Blood Samples → Direct to qPCR → qPCR

- No extraction reagents or equipment required
- Less error-prone
- Higher throughput

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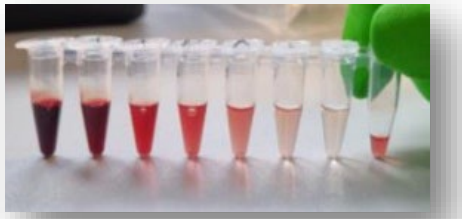
Real-time DirectPCR from blood – PlexTaq® 5x qPCR Multiplex Master Mix

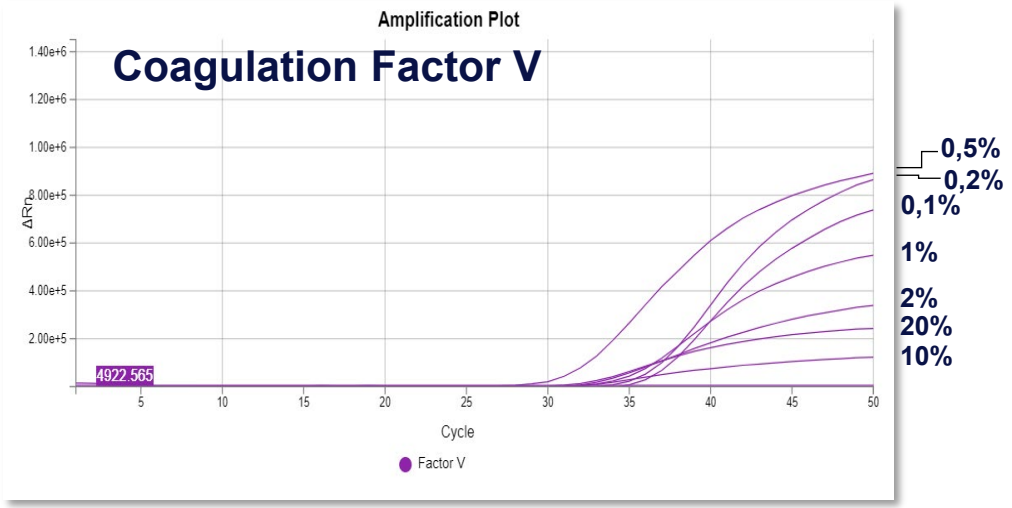
K3 EDTA blood

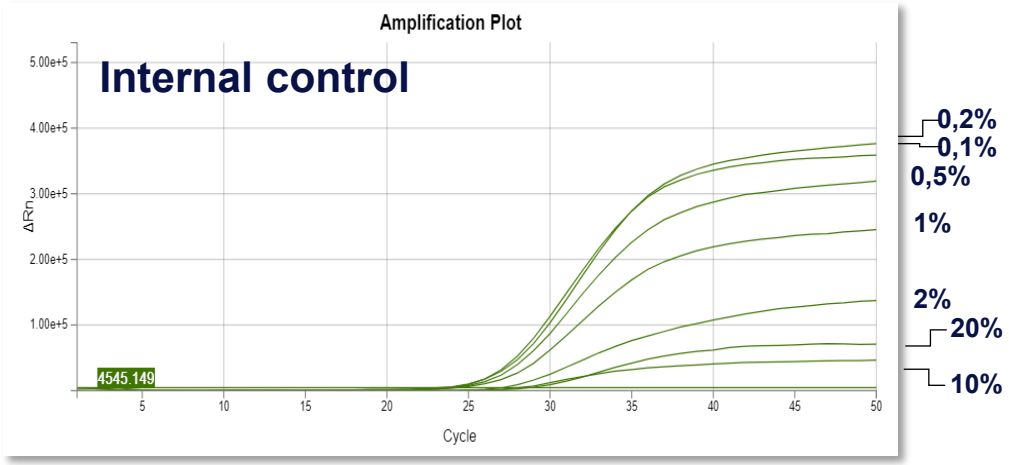


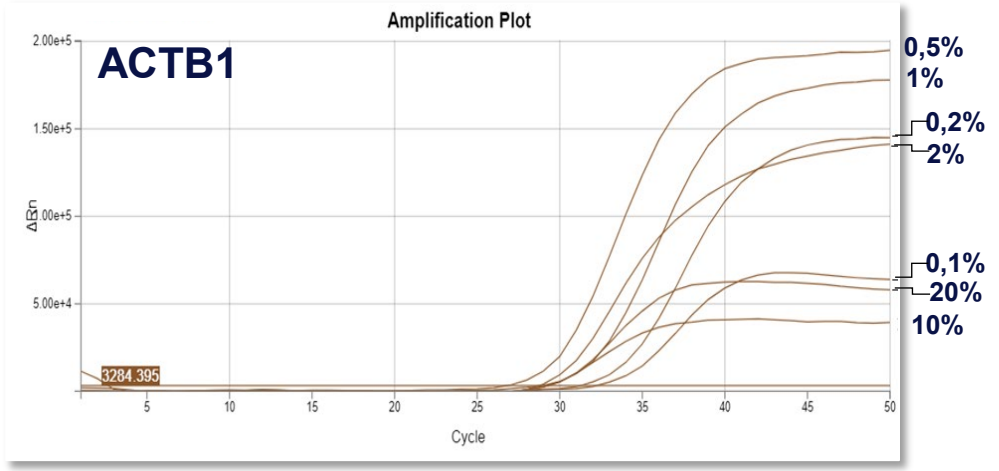
FAM channel: Human coagulation risk factor 5- SNP gene target

HEX channel: spiked internal control target (10^4 c/rxn)

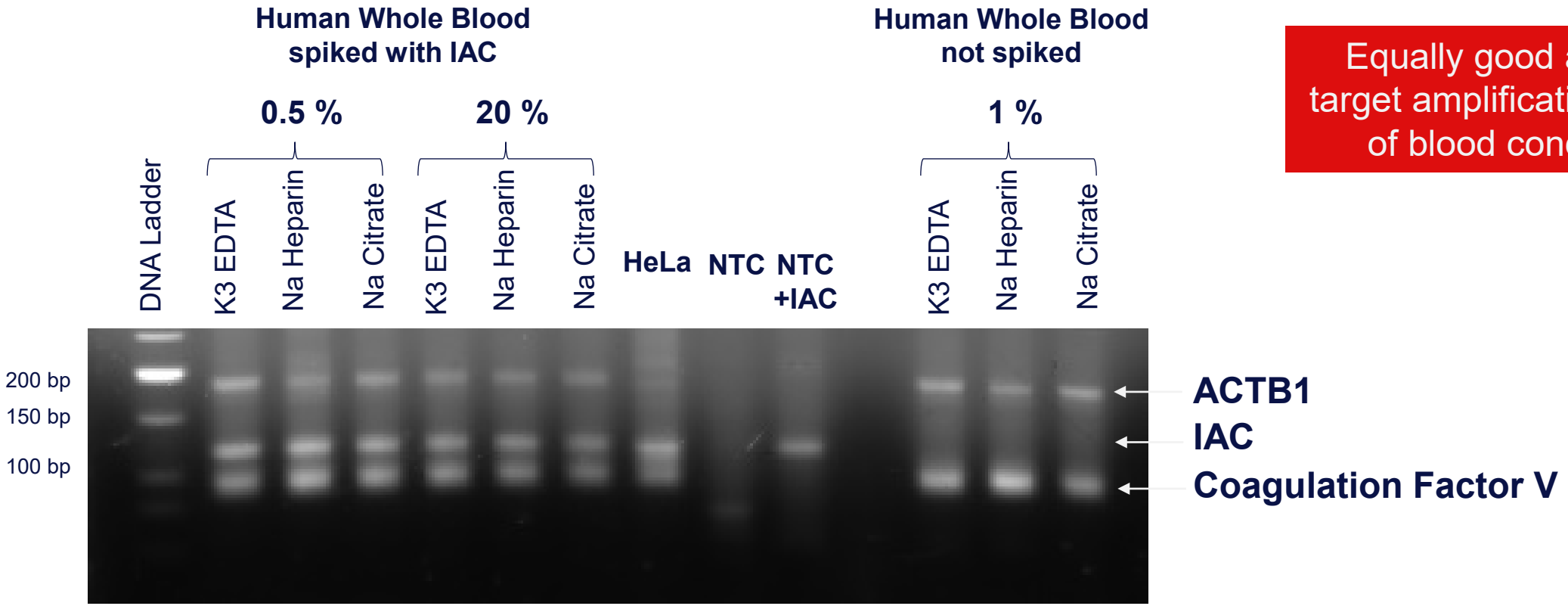
Cy5 channel: ACTB1 target (human gene present in human blood sample)







Agarose gel after DirectqPCR from blood – PlexTaq® 5x qPCR Multiplex Master Mix



Equally good and specific target amplification regardless of blood concentration.

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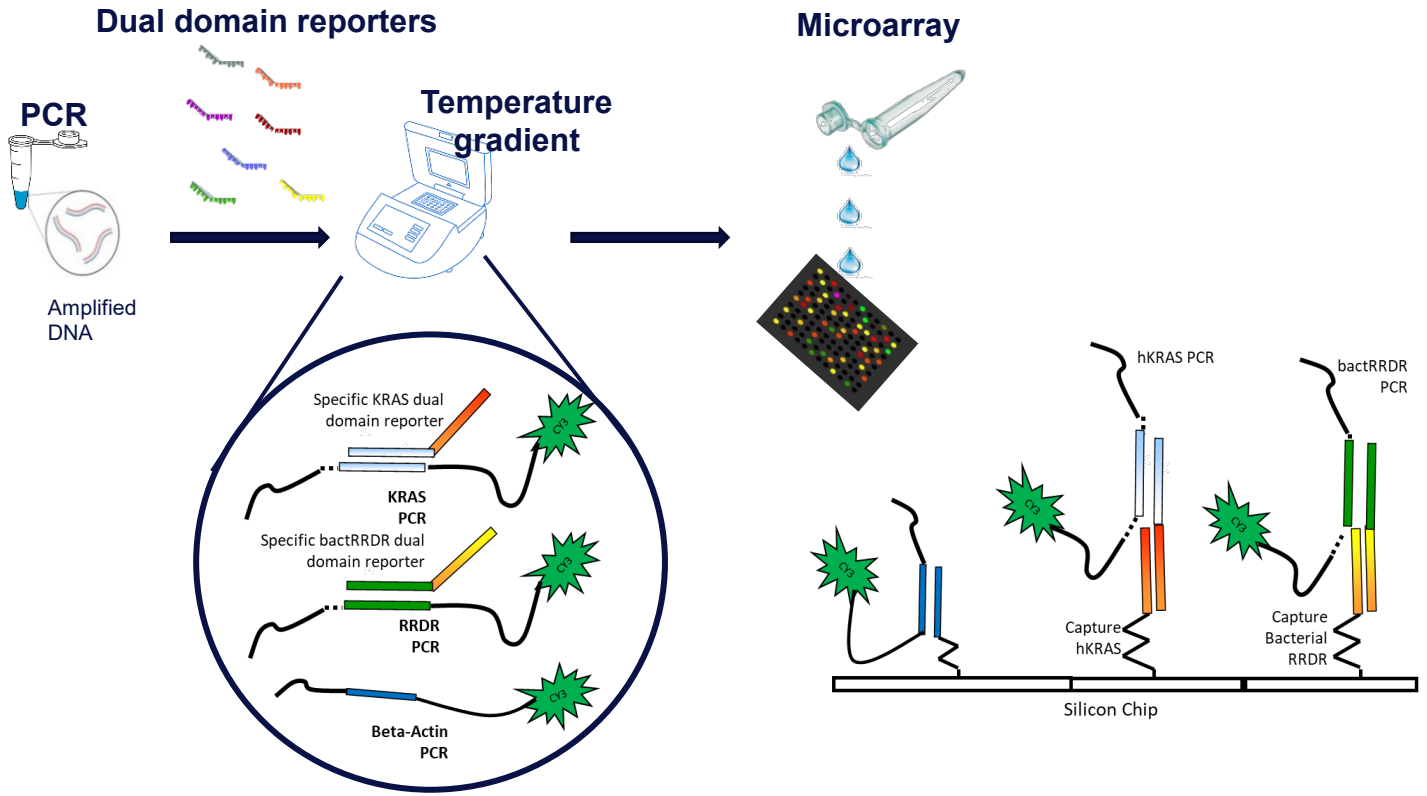
Multiplex Microarray – PlexTaq® 5x qPCR Multiplex Master Mix

Mycobacterium tuberculosis detection by microarray

Spotting scheme of the human β-actin, KRAS and RRDR multiplex



ISTITUTO DI SCIENZE E TECNOLOGIE CHIMICHE GIULIO NATTA

SCITEC National Research Council – CNR, Italy

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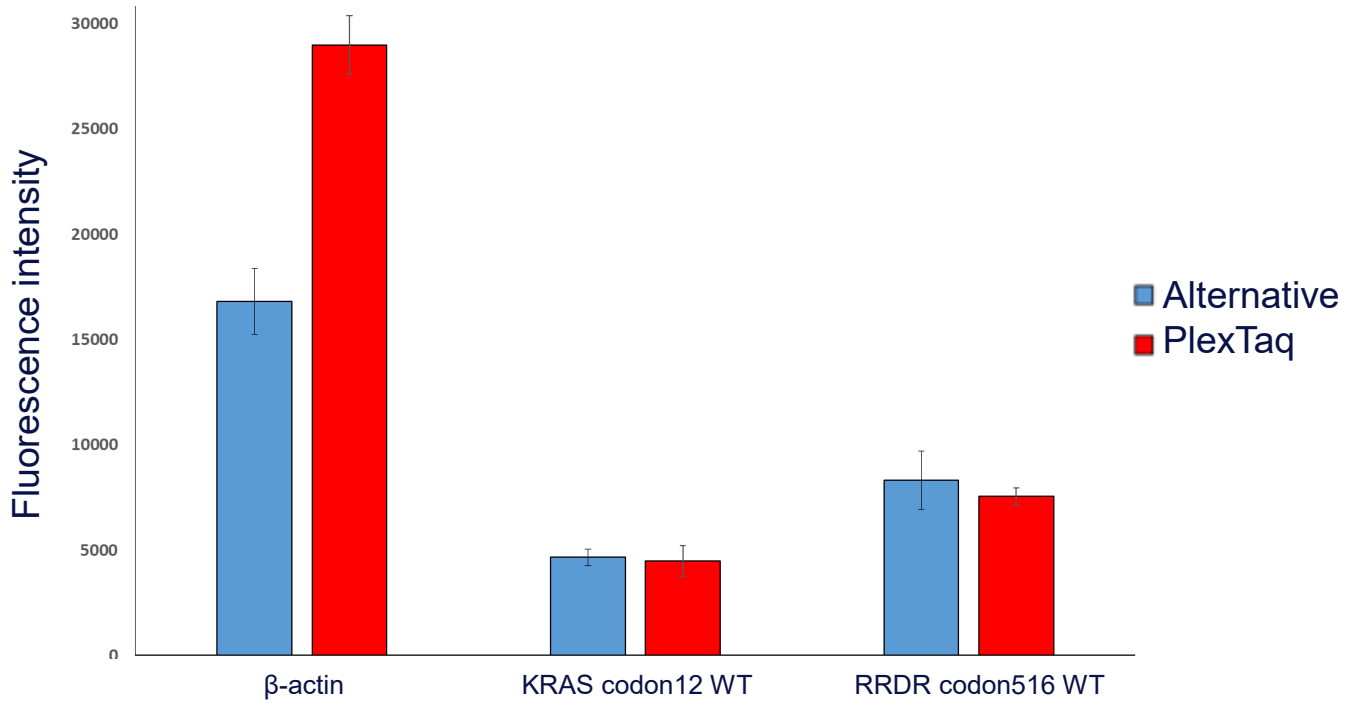
Multiplex Microarray – PlexTaq® 5x qPCR Multiplex

Fluorescence intensities for the three multiplex targets



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PlexTaq® 5x qPCR Multiplex Master Mix

Conclusion

- PlexTaq® 5x qPCR Multiplex Master Mix is a **5X concentration** engineered polymerase, **ideal for multiplexing**.
  - Widely used “workhorse” mix
  - Up to **30 target** multiplexing
  - **Lyo-ready** formulation for lyophilization
  - Highest quality standard - **ISO 13485:2016** conformity
- Direct real-time qPCRs on crude samples – **No Nucleic Acid Extraction**
- Real time multiplex detection qPCRs – **Food Pathogen Testing**

Medix Biochemica

RNA Applications (RT-PCR)

Volcano® RT-PCR Master Mix

Engineered Taq DNA polymerases with reverse transcriptase activity

Unique - Simultaneous reverse transcription and amplification

Ready-To-Use - Simple and sensitive

Fast Time-To-Results - Reliable results with crude samples

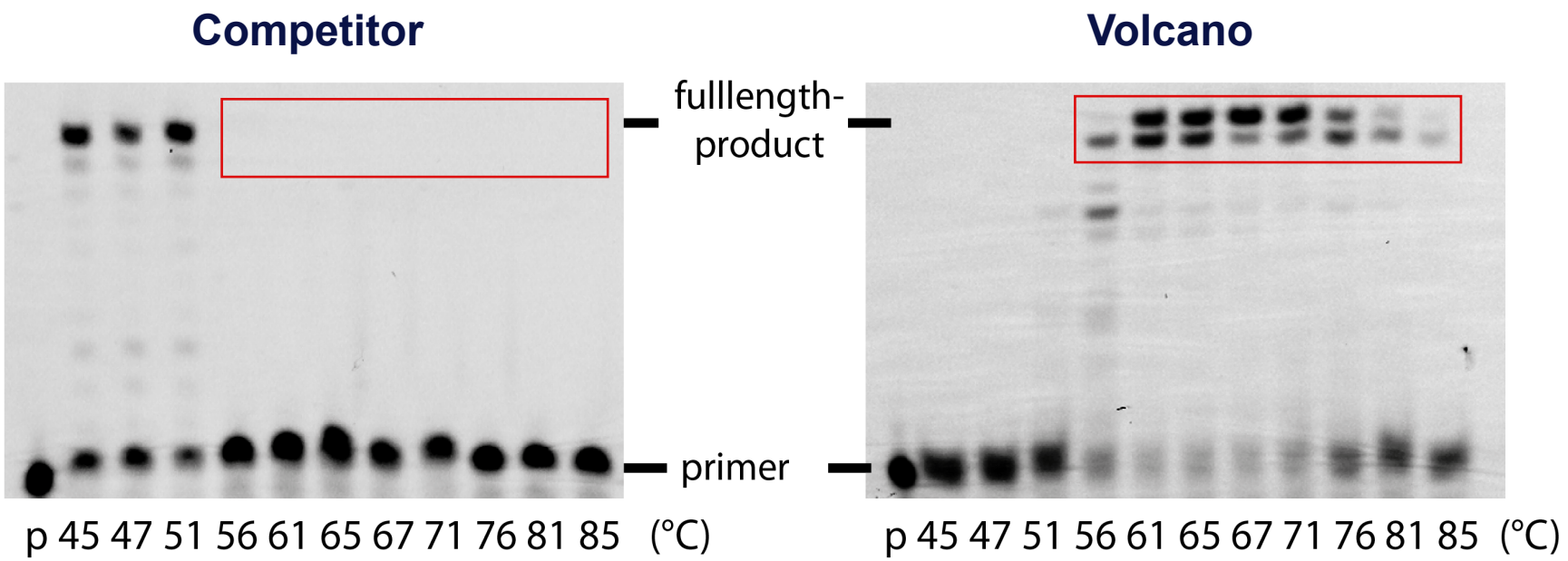
Hot-Start - Aptamer formulation prevents unspecific amplification at lower temperatures

Thermostable – Active at temperatures where complex samples (e.g. secondary RNA structures) are broken down

Medix Biochemica

Volcano® RT-PCR Master Mix – Thermostability

Unique - Truly HOT Reverse Transcription



Performing reverse transcription at a higher temperature can be beneficial for assays dealing with complex samples

Blatter, N., Bergen, K., Nolte, O., Welte, W., Diederichs, K., Mayer, J., Wieland, M. and Marx, A. (2013), Structure and Function of an RNA-Reading Thermostable DNA Polymerase. Angew. Chem. Int. Ed., 52: 11935–11939. doi: 10.1002/anie.201306655

R. Kranaster, M. Drum, N. Engel, M. Weidmann, F. T. Hufert and A. Marx, (2010), One-step RNA pathogen detection with reverse transcriptase activity of a mutated thermostable Thermus aquaticus DNA polymerase, Biotechnol. J., 5(2), 224-31.

Kranaster R, Zeller J, Kühn B, Marx A, (2016) Neues Enzym mit Reverse Transkriptase- und DNA-Polymerase-Funktion, BioSpektrum, 2, 164-165.

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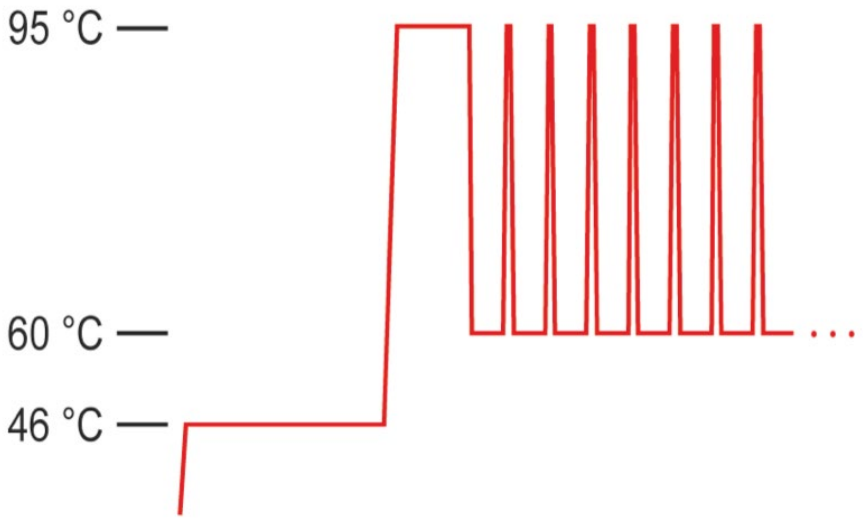
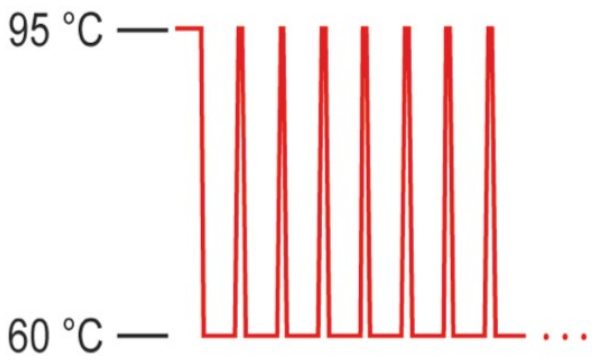
Volcano® RT-PCR Master Mix – 0-Step RT-qPCR

Fast start function - 0-step RT-PCR

Traditional 1-Step Approach



0-Step RT-PCR (Volcano)



- Simplified RT-PCR without isothermal RT-steps
- Compatible with dsDNA-sensitive dyes (e.g. SYBR)
- Additional polymerases can be added

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Kranaster R, Zeller J, Kühn B, Marx A, (2016) Neues Enzym mit Reverse Transkriptase- und DNA-Polymerase-Funktion, BioSpektrum, 2, 164-165.

Volcano® RT-PCR Master Mix – DirectPCR

Wastewater screening of viruses

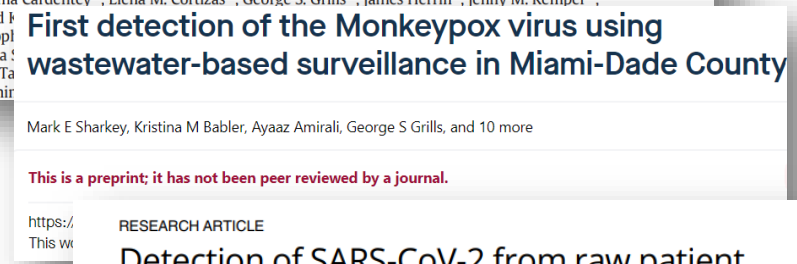
1. SARS-CoV-2
2. HIV
3. Monkeypox

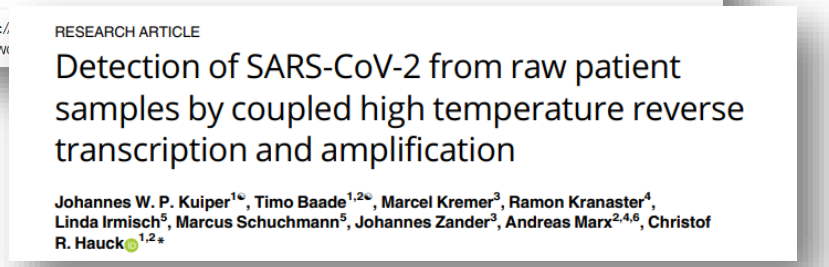
"(…) the entire process was accelerated by a new analytical technique called V2G (for "volcano second generation") quantitative polymerase chain reaction (or qPCR) that can pick up COVID-19 in sewage faster than earlier methods." (after University of Miami))

Qualitative and quantitate detection of viral targets:

1. HIV-1
2. Zika virus

UNIVERSITY OF MIAMI





https://news.miami.edu/stories/2021/08/wastewater-provides-early-detection-of-covid-19.html
https://assets.researchsquare.com/files/rs-2010415/v1/53f42371-a093-469a-b83e-96d5071767ca.pdf?c=1661990654
Lessons learned from SARS-CoV-2 measurements in wastewater - PMC (nih.gov)

MedixMDx qRT-PCR Lyo ready Mix

Sensitive and Versatile RT-PCR mix ready for lyophilization

Lyo-Ready Mix

- Specifically designed for lyophilization. No need for excipients.

Robust And Sensitive

- Optimized for rapid detection and quantification of various RNA templates, including mRNA, viral RNA, and total RNA.
- Effective for detecting multiplexed viral targets directly from human patient samples.

Versatile

- Antibody-regulated hot-start Taq polymerase and reverse transcriptase, along with target-specific primers and probes, and RTase Lyo.

One-Step Probe Mix

- Universal one-step probe mix.
- Directly lyophilized without additional components.

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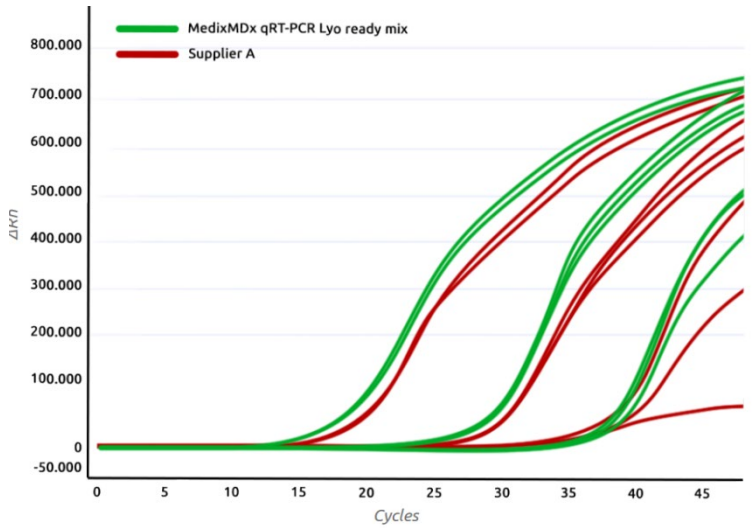
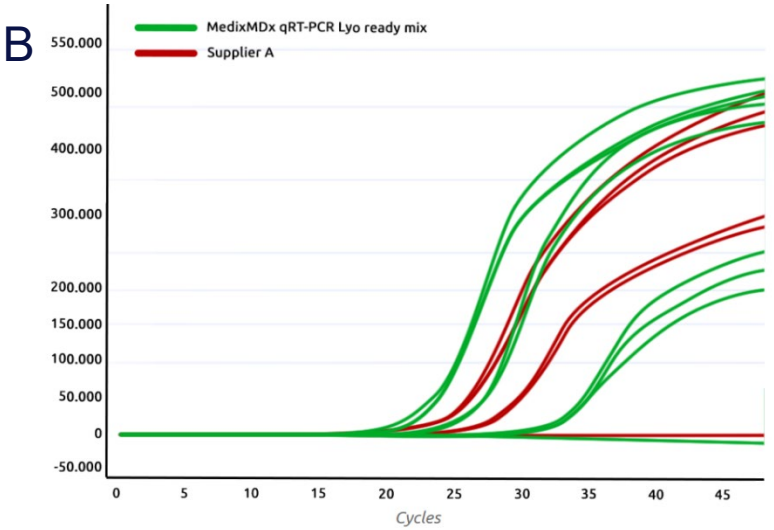
MedixMDx qRT-PCR Lyo Ready Mix

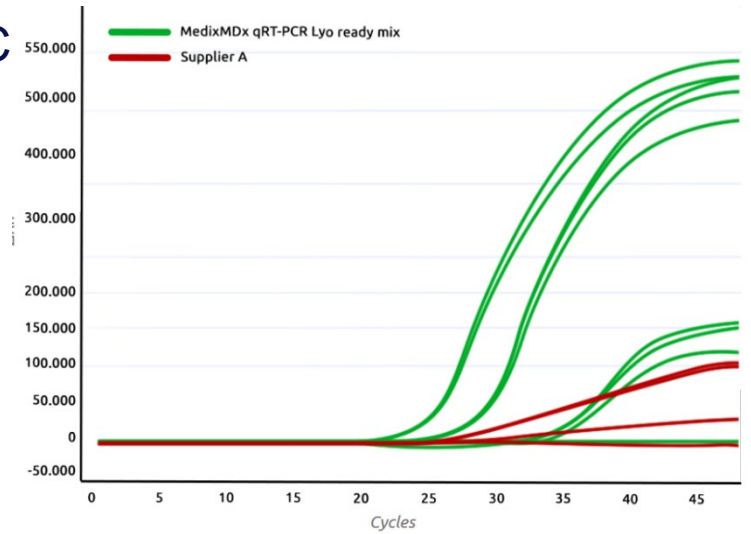
A



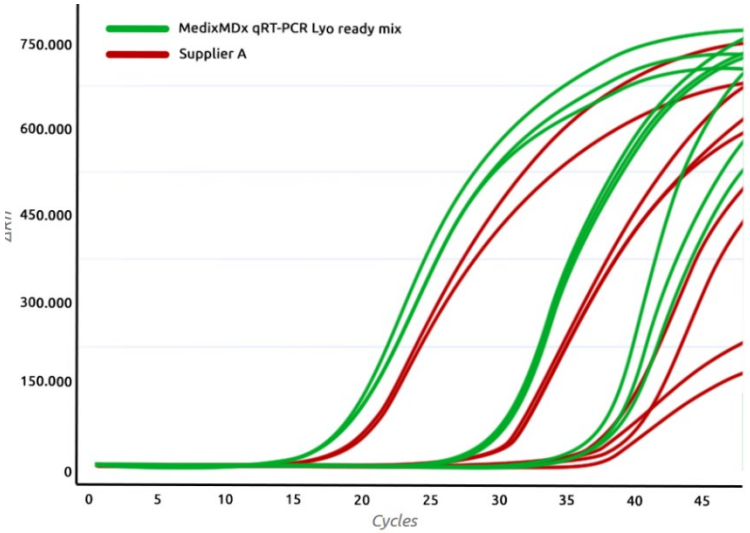
B



C



D



RT-qPCR amplification profiles of multiplexed human viral targets: (A) HAV, (B) HBV, (C) HCV and (D) HEV at three different serum sample quantities.
Green MedixMDx qRT-PCR Lyo ready mix
Red Supplier A

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RNA Applications (RT-PCR)

Conclusion

Volcano3G

- **Simultaneous** reverse transcription and amplification
- **Simple** and **sensitive**
- **Reliable** results with crude samples
- Aptamer-mediated **hot-start -** Active at temperatures where **complex sample structures** are broken down
- Highest quality standard - **ISO 13485:2016** conformity
- **Rapid detection and identification of RNA & DNA targets** (e.g., SARS-CoV-2, RSV and Flu A/B screening)
- **Lyo-ready** formulation for lyophilization available upon request

MedixMDx qRT-PCR Lyo Ready Mix

- **Sensitive**
- **Robust**
- **Lyo-Ready**
- **Multiplex** Capabilities

Medix Biochemica

Isothermal Amplification

Isothermal Amplification

Range of Engineered Bst Polymerases

MedixMDx Fast Bst Product Line

- Perfectly suited for Isothermal Amplifications
- Options for both LAMP and RT-LAMP
- Fast time-to-results
- Available as Master Mixes or separated components

Isotherm3G Product Line

- Single-enzyme LAMP/RT-LAMP
- POC applications

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Perfectly Suited For Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a single-tube technique for the amplification of DNA and a **low-cost** alternative to detect certain diseases.

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) combines LAMP with a reverse transcription step to allow the detection of RNA.

Advantages of LAMP in Molecular Diagnostics

- ✓ No Dedicated Equipment Needed
- ✓ Fast Reaction (~ 15 min)
- ✓ Easy Visualization
- ✓ Simplicity of Operation
- ✓ Ideal for Point-of-Care or At-Home Use

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000). "Loop-mediated isothermal amplification of DNA". Nucleic Acids Res. 28 (12): 63e–63. doi:10.1093/nar/28.12.e63. PMC 102748. PMID 10871386.

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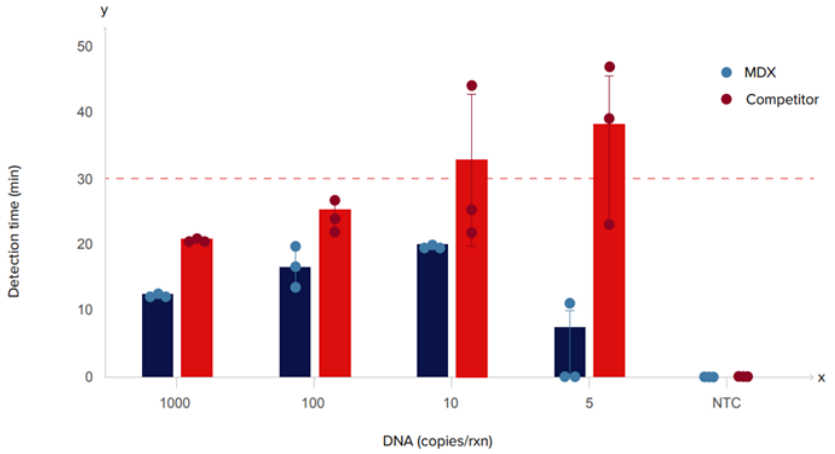
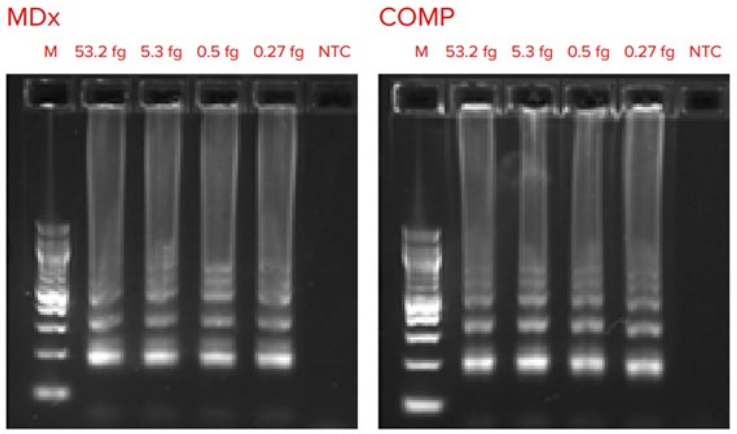
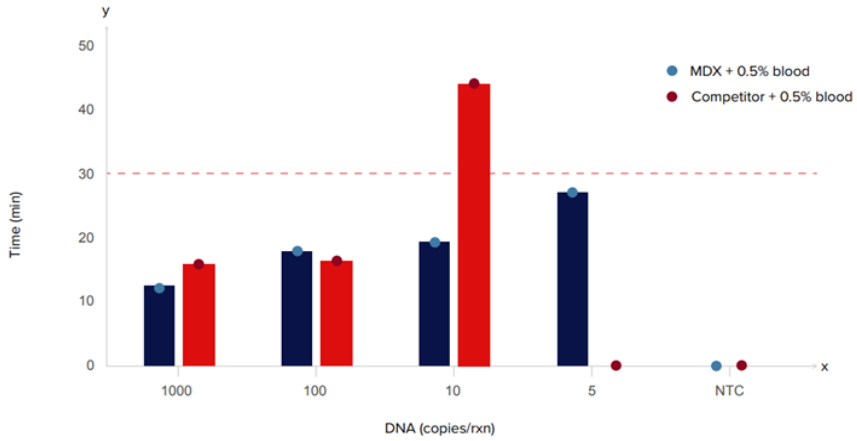
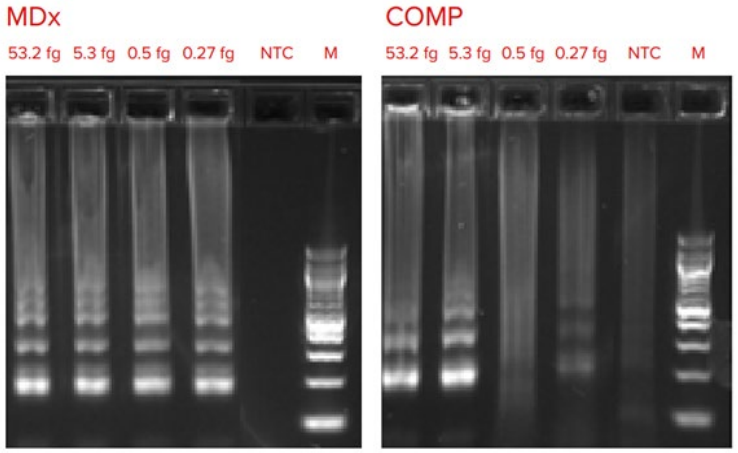
MedixMDX – Fast Bst Family









Faster Time-To-Results without sacrificing performance

High Inhibitor Tolerance

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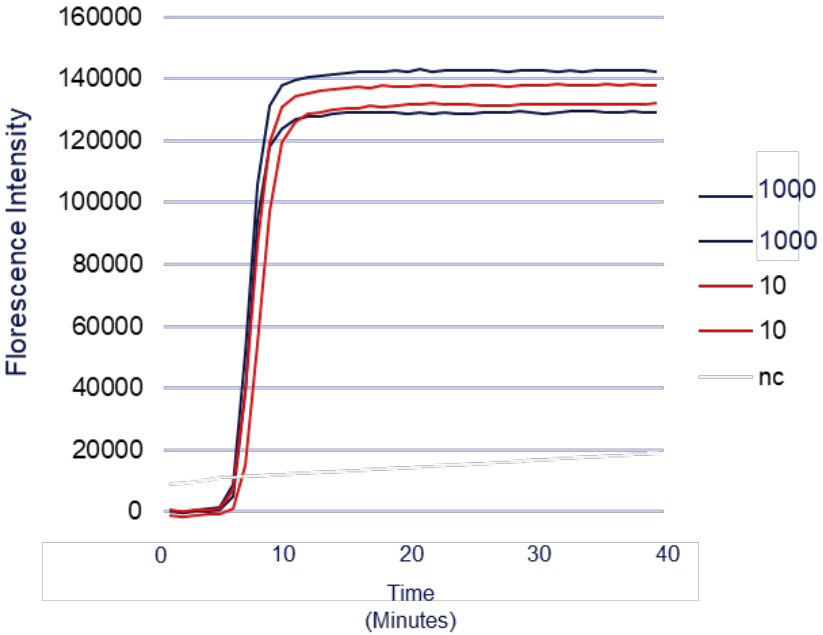
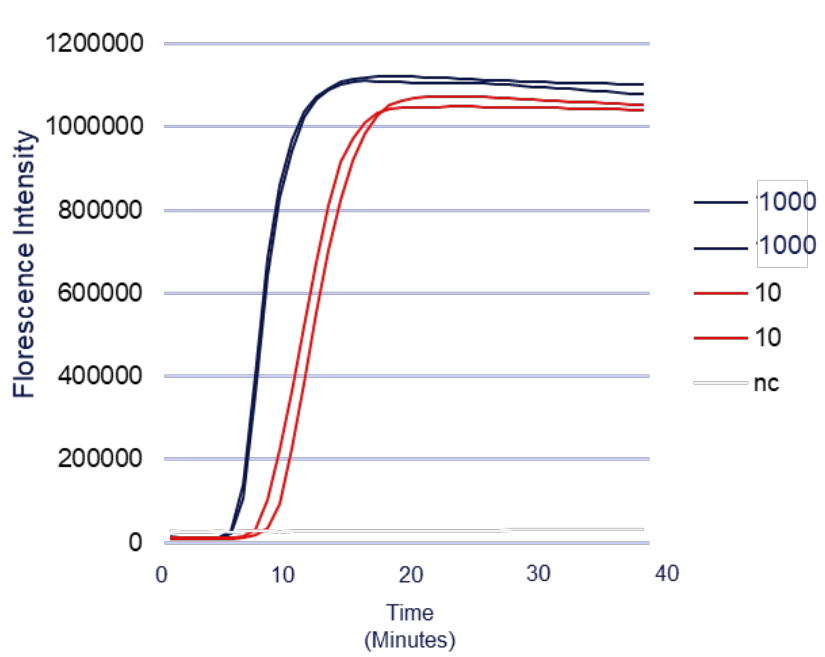
Warm-Start BST + UDG

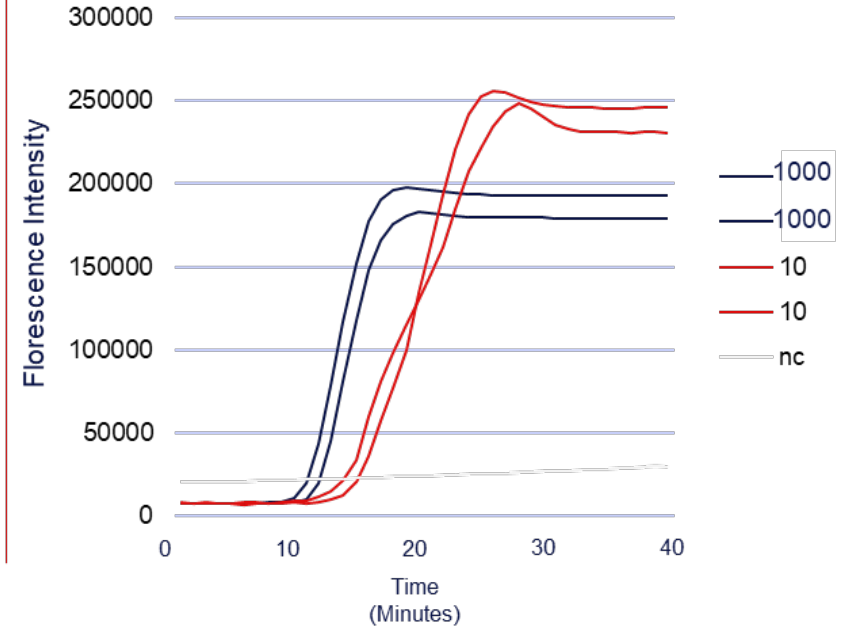
Target: Salmonella enterica



MedixMDx Fast Bst



MedixMDx Warm-Start Bst
+ UDG



Competitor (Warm-Start)
+ UDG

Collaboration with: iit ISTITUTO ITALIANO DI TECNOLOGIA

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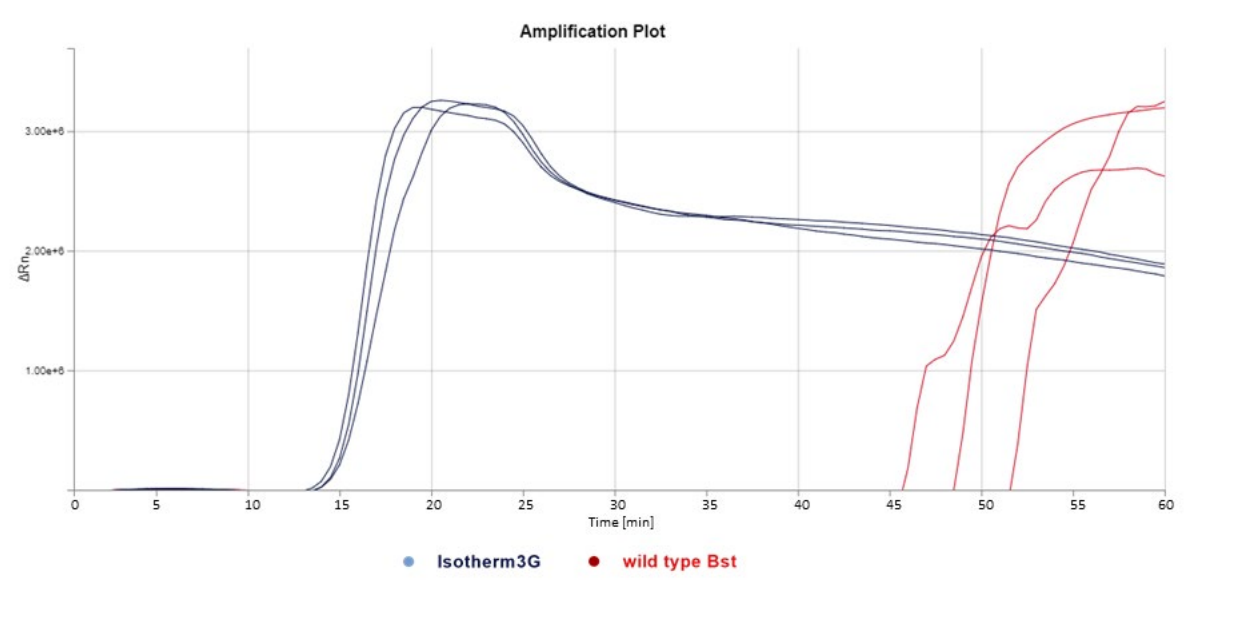
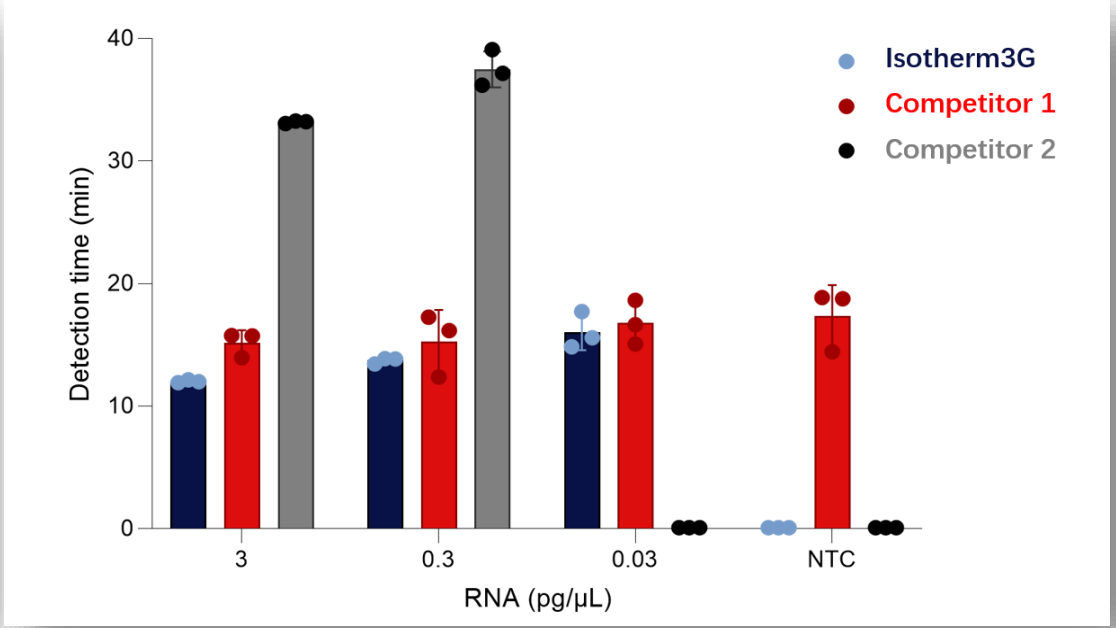
Isotherm3G with Improved RT-Activity

- Fast and specific detection
- No addition of a reverse transcriptase is needed!





Reliable detection of SARS-CoV-2 **N gene**

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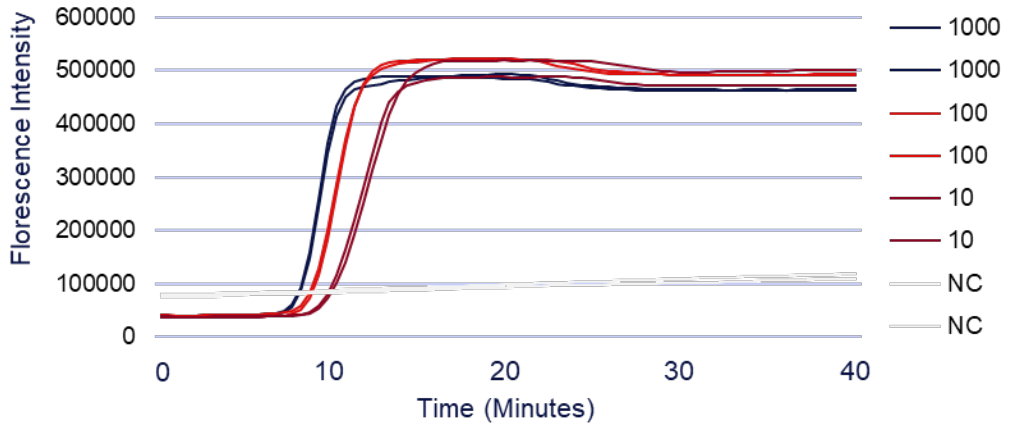
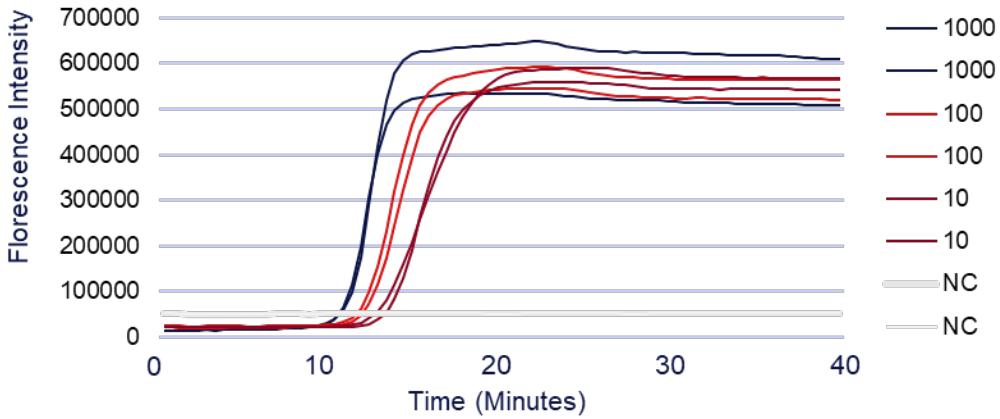
Isotherm3G vs Competitor

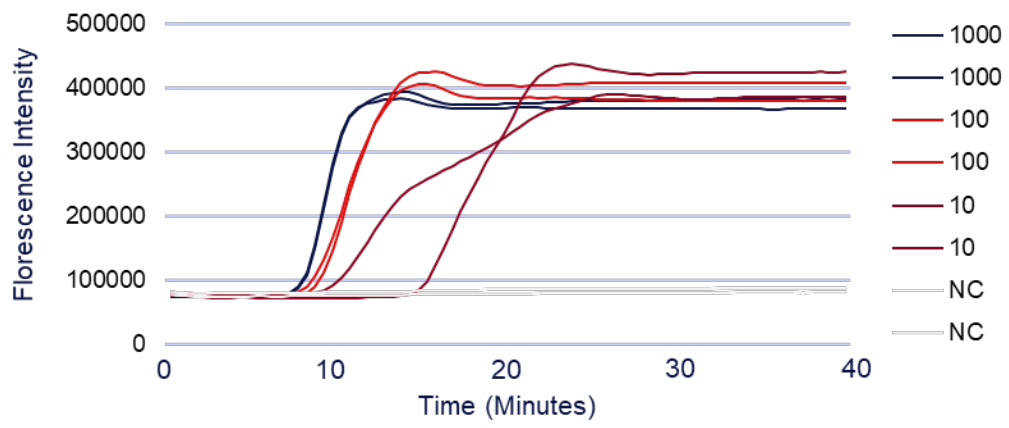
Isotherm3G

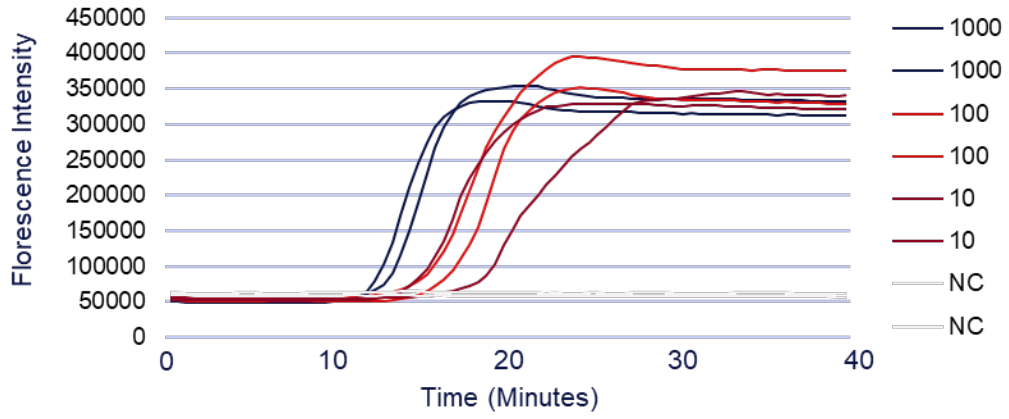
Competitor





Salmonella





Legionella

Similar results when also tested on: Staphylococcus, and Pseudomonas

Collaboration with: ISTITUTO ITALIANO DI TECNOLOGIA

Isothermal Amplification

Conclusion

MedixMDx Fast Bst & Isotherm3G Product Lines

- Ideal for **Point-of-Care** Applications
- Products for both **LAMP** and **RT-LAMP**
- **Fast Time-To-Results**
- **Simultaneous reverse transcription and amplification** with Isotherm3G
- Available as Master Mixes or separated components
- Highest quality standard - **ISO 13485:2016** conformity

Medix Biochemica

Q&A Session

Dr. Maja Studencka-Turski

Dr. Arielle Bryan

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Thank you
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