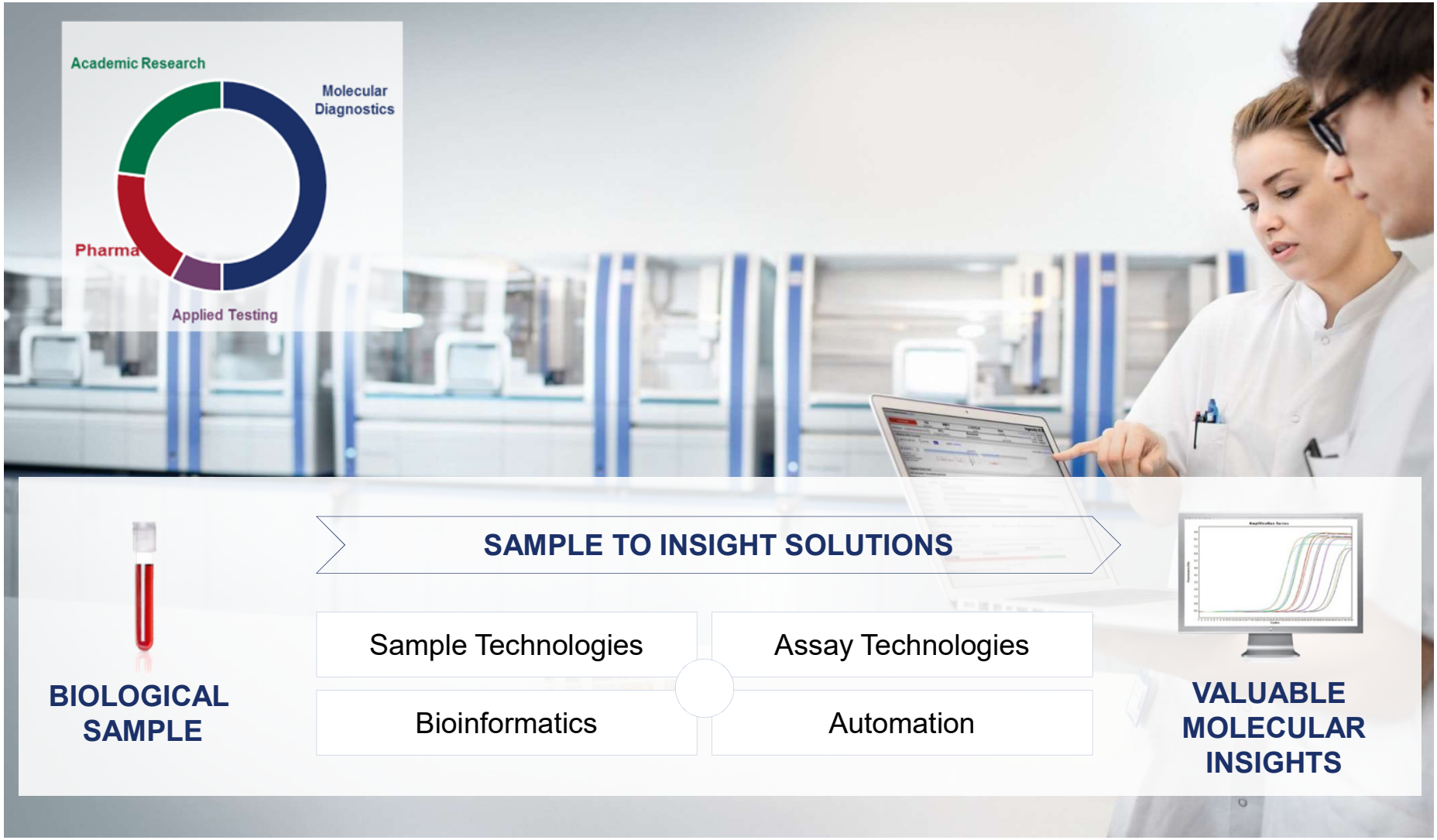
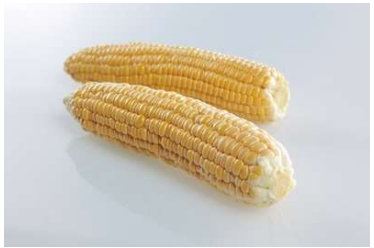
Molecular food testing at QIAGEN: now and the (near?) future
XVII MRAMA 20-23rd November 2018

Leadership in Sample to Insight solutions



Benefits of real-time PCR in food safety testing



Specific

Only detects target sequence

Sensitive

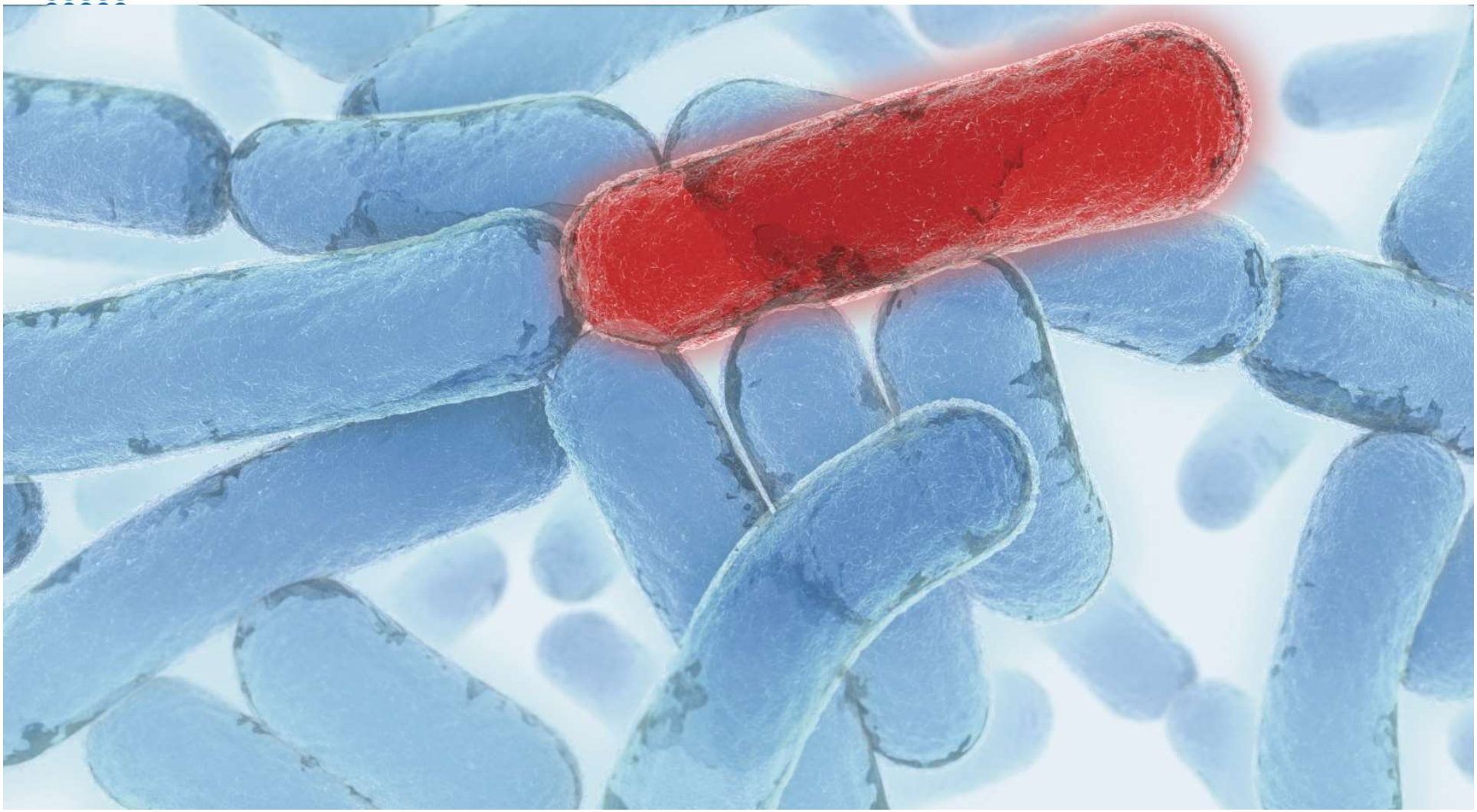
- **Can detect low copy numbers**
- **High inhibitor tolerance**

Rapid

- **Easy to set up**
- **Detection in under 90 minutes**

Standardized

- **Automated protocols**
- **Stable chemical design**



QIAGEN`s *mericon* workflows for pathogen testing

mericon Pathogen workflow

Automation

© Seward

QIAsymphony SP/AS

QIAgility

Rotor-Gene Q

Food sample → Sample homogenization → DNA extraction → PCR assay set up → PCR analysis → Insight

Consumables

© Seward

***mericon* DNA extraction portfolio**

mericon DNA Bacteria Kit

mericon DNA Bacteria Plus Kit

QIAsymphony *mericon* Bacteria Kit

***mericon* pathogen PCR Assays portfolio**

- Salmonella spp.
- Listeria spp.
- L. monocytogenes
- Campylobacter spp.
- Campylobacter triple
- Cronobacter spp.
- Staphylococcus aureus
- Quant Legionella spp.
- Quant L. pneumophila
- Shigella spp.
- Yersinia enterocolitica
- Vibrio triple
- VTEC stx1 / 2
- E. coli O157 screen plus (stx1 /2, eae)
- E. coli STEC O-type

mericon workflows – manual or automated?

Chemically aided thermal lysis at 100 °C
Physical disruption for gram positive bacteria

Chemically aided thermal lysis onboard at 90 °C
Specific inhibitor removal and bind, wash, elute process via magnetic beads

mericon
Pathogen Detection Workflows

mericon DNA Bacteria Kit
mericon DNA Bacteria Plus Kit

Detection of food-borne pathogenic bacteria

QIAsymphony
mericon Bacteria Kit

Manual workflow

mericon Real-Time PCR Kits

Automated workflow

mericon pathogen detection workflow: automated solutions

QIAsymphony SP

DNA extraction

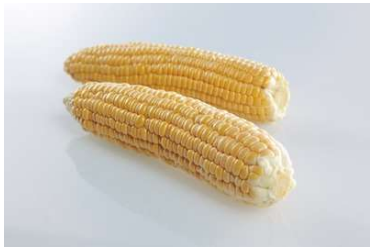
QIAsymphony AS

PCR assay setup

One system for sample preparation and PCR assay setup

QIAGEN`s solutions for ingredient authenticity & GMO testing

Reasons for testing



To assess if foods have been cut with cheaper ingredients or substituted with cheaper equivalents, e.g.:

- ***Horse in beef products***
- Pork in goose or calf liver paté
- Turkey in products labeled as chicken
- Soy or corn as filler in meat products
- Apricot kernel paste labeled as marzipan

As part of a process to check if foods meet religious or other requirements, e.g.,

- No pork in food that is certified halal
- No meat-based products in vegetarian food

To assess animal feed for contamination, e.g.,

- No animal parts in the feed of cattle, sheep, etc.
- Food has appropriate content with no filler

mericon Ingredient Authenticity/Speciation workflow

Automation

Tissue Ruptor

Tissue Lyser II

Tissue Lyser LT

QIAcube

QIAcube HT

QIAgility

Rotor-Gene Q

Food sample → Sample homogenization → DNA extraction → PCR assay set up → PCR analysis → Insight

Consumables

DNeasy *mericon* Food Kit

DNeasy *mericon* 96 QIAcube HT Kit

mericon Ingredient authenticity/speciation portfolio

Cattle
Chicken
Pig
Turkey
Ruminant
Sheep
Goat
Horse
Meat Tracker kit
Apricot Kernels
Soy
Corn

mericon Ingredient Authenticity/Speciation workflow

Automation

Tissue Ruptor

Tissue Lyser II

Tissue Lyser LT

QIAcube

QIAcube HT

QIAgility

Rotor-Gene Q

Food sample → Sample homogenization → DNA extraction → PCR assay set up → PCR analysis → Insight

Consumables

DNeasy *mericon* Food Kit

DNeasy *mericon* 96 QIAcube HT Kit

mericon GMO detection portfolio

- Screen 35S-pat
- Screen bar
- Screen CTP2-CP4EPSPS
- MON 810 Corn
- RR Soy
- Screen 35S
- Screen Nos
- Quant MON 810
- Quant RR Soy

Facilitated DNA extraction for ingredient authenticity testing

DNeasy *mericon* food kit/Dneasy mericon 96 QIAcube HT kit

Rapid

- Optimized version of CTAB protocol
- Takes just 2.5 hours (vs 1-1.5 days)
- 42/73 minutes for 24/96 samples

Convenient

- Suitable for all types of sample material
- One common lysis procedure
- Protocols for 200 mg or 2 g samples
- Automatable bind/wash/elute steps on QIAcube
- silica column purification
- Protocols for 300 mg samples
- Bind/wash/elute steps are automated on QIAcube HT

Reliable

- Efficient removal of inhibitors
- Automation allows for enhanced process safety

The *mericon* food extraction protocol

Tested by QIAGEN

- ✓ Meat
- ✓ Ketchup
- ✓ Cacao
- ✓ Chocolate
- ✓ Cookies
- ✓ Cornflakes
- ✓ Corn chips
- ✓ Soy lecithin
- ✓ Hazelnut flour
- ✓ Potato milk
- ✓ Infant food
- ✓ Vanilla
- ✓ Nutrition supportive
- ✓ Milk
- ✓ Marmalade
- ✓ Bread
- ✓ Olive oil

Fatty foods

Strongly Inhibitory

Highly processed

Low DNA Content

High DNA content

mericon MeatTracker kit

QIAGEN Solution: mericon MeatTracker kit

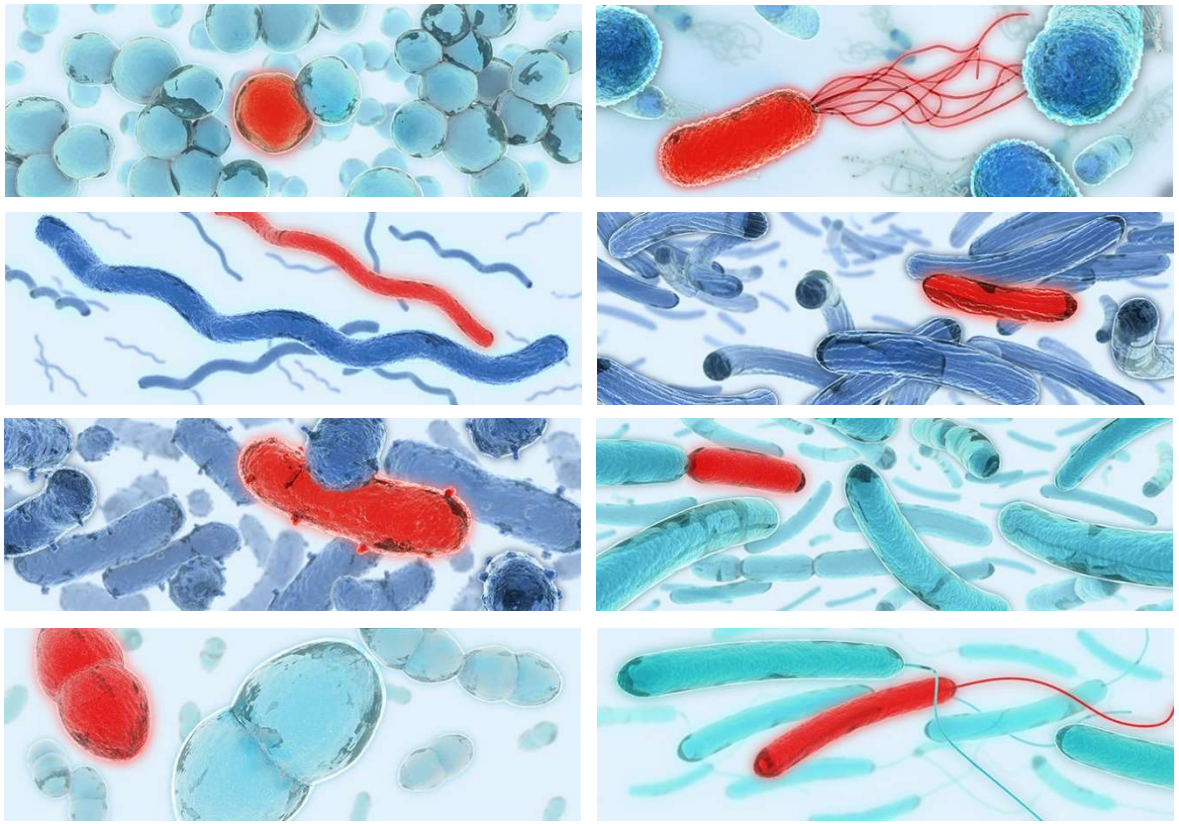
Fast, sensitive & easy detection of even small traces of meat in food products

Features

- Sensitive (10 copies) and specific animal DNA detection (gDNA target), based on standard real-time PCR
- PCR run time of 73 minute on the QIAGEN Rotor-Gene Q
- Same cycling profile as for all other *mericon* animal ID assays. The combination of several targets in one run is easily possible
- Standardized workflow with several options for automation upgrades depending on user needs (QIAcube, QIAcube HT, QIAgility)

Benefits

- Only one assay required to detect any traces of meat – ideal for screening
- User-friendly through easy to perform protocols and „one-fits-all“ workflow principle – just 3 pipetting steps
- Fast result and/or product release



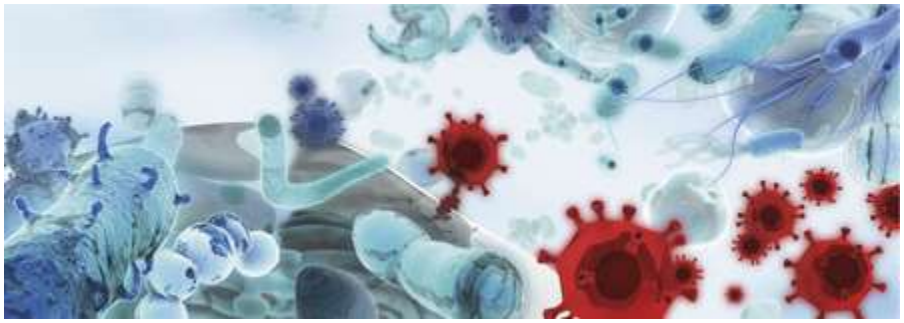
Microbial qPCR Products for food testing

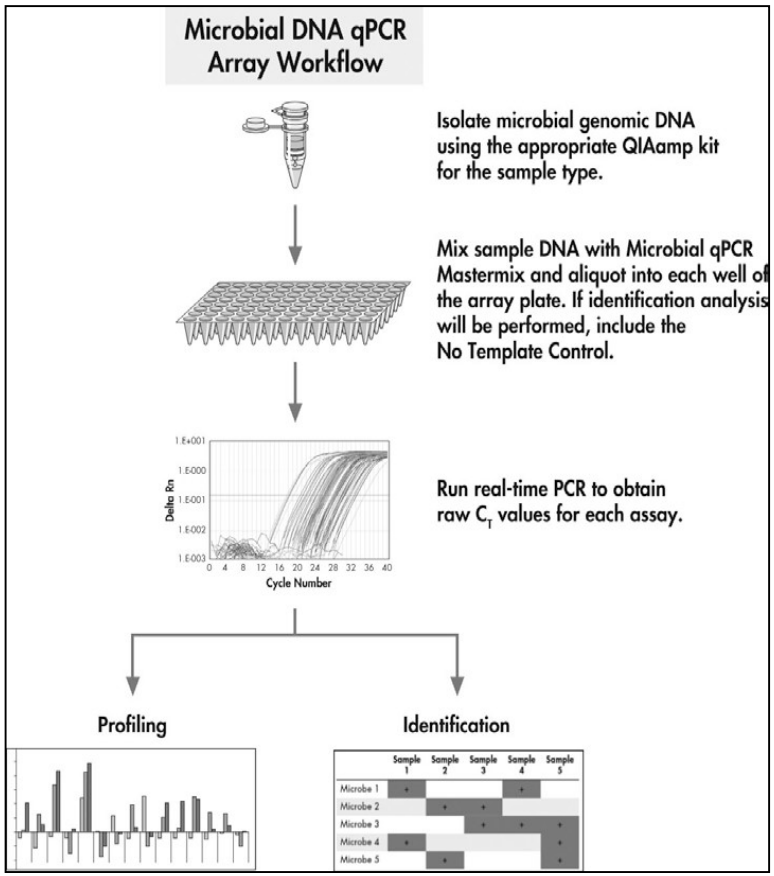
Overview Microbial qPCR arrays and assay portfolio

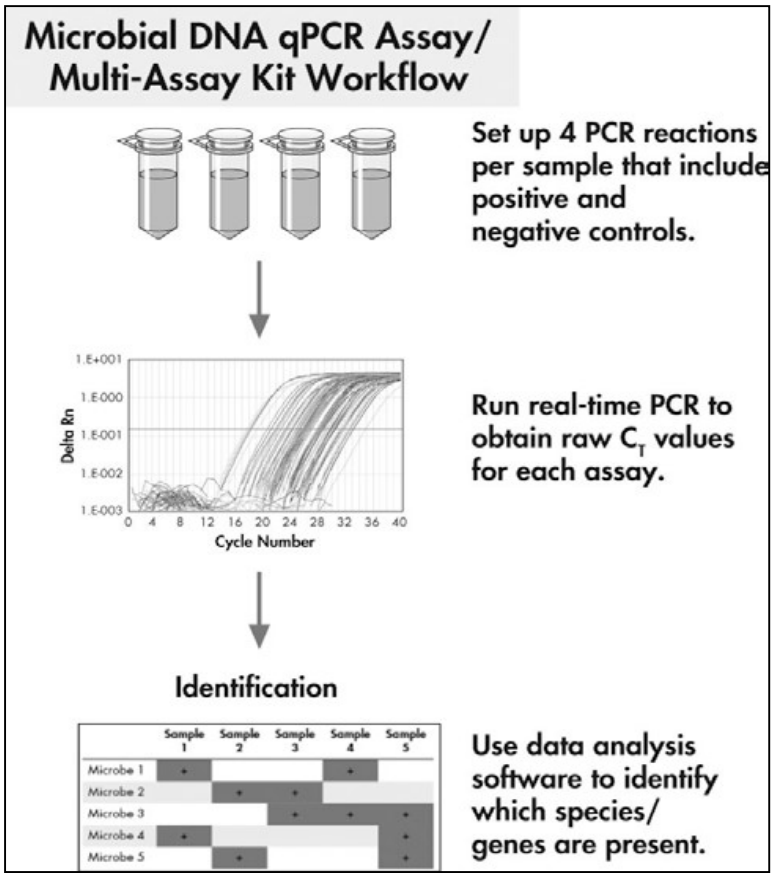
580 Assays

	Species specific Assays	Application based Arrays
Pathogens • Simultaneous detection and profiling for 8-384 pathogens in any instrument • Custom options available	▪ Bacteria- 350 assays ▪ Fungus- 9 assays ▪ Parasites- 10 assays ▪ Virus- 12 assays ▪ Protist – 12 assays ▪ Antibiotic resistance genes- 84 assays ▪ Virulence factors- 84 assays ▪ Control assays- 20 assays	▪ Food Testing- Meat ▪ Food Testing- Seafood ▪ Food Testing- Milk ▪ Food Testing –Vegetable ▪ Food Testing- Poultry ▪ Antibiotic Resistance genes ▪ Virulence Factors ▪ Water Testing ▪ Beer Pathogen Testing
Sample prep	▪ *mericon* DNA Bacteria Kit ▪ *mericon* DNA Bacteria Plus Kit ▪ QIAsymphony *mericon* Bacteria Kit	▪ DNeasy mericon Food Kit ▪ Dneasy mericon 96 QIAcube HT kit
Free data analysis software via the GeneGlobe Data Analysis Center on www.Qiagen.com		

Workflow of qPCR arrays & assays





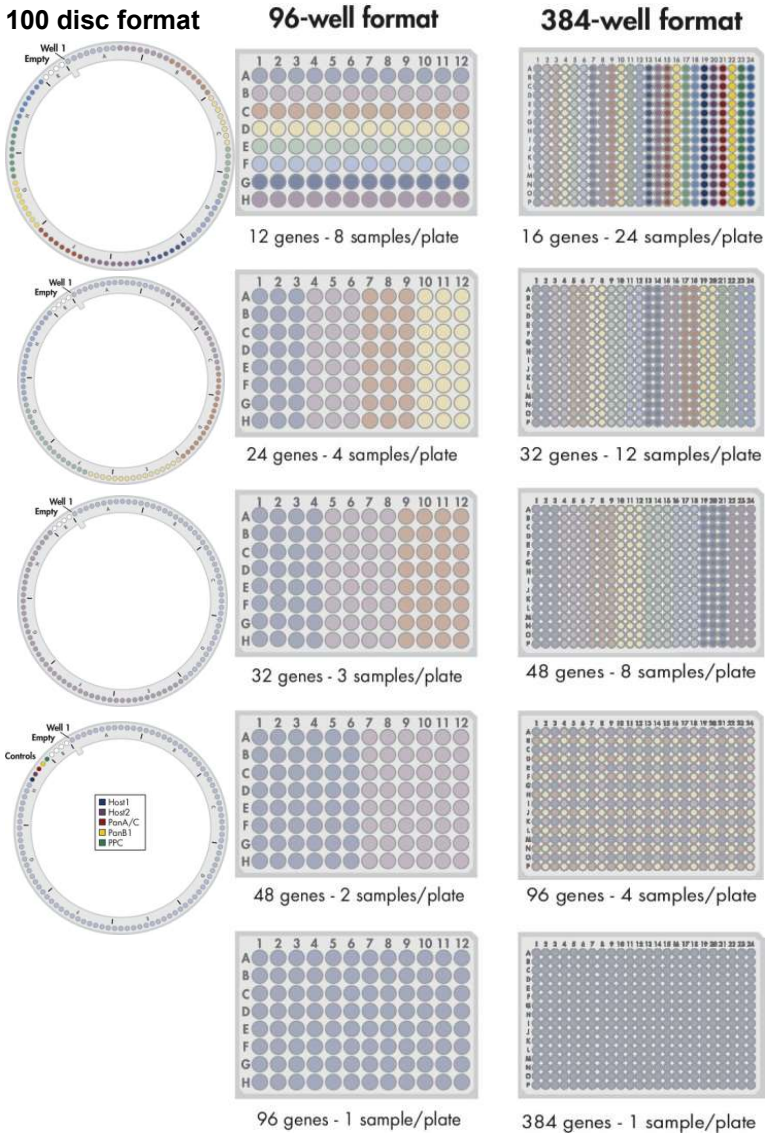


Custom Microbial qPCR DNA Arrays

Complete freedom for the customer to build their own Microbial qPCR Array.

- Choose 8-384 microbial species, antibiotic resistant genes or virulence factors from the 580 assay list and place it on the plate according to your interest along with the controls and pan assays(for normalizing the data).

- Pan-bacteria/ fungal assays that detect a broad range of bacterial species are included to serve as positive controls for bacterial DNA, and the Positive PCR Control assay is included to test for the presence of PCR inhibitors or the efficiency of the polymerase chain reaction.



Example array - Food Testing - Dairy

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bacillus species (2)	Bacillus species (2)	wzt	Campylobacter fetus	Campylobacter speices (5)	Klebsiella & Enterobacter (2)	Enterococcus species (2)	Enterococcus faecalis	Enterococcus faecium	Enterococcus italicus	Escherichia & Shigella (6)	eae
B	stx2A	stxA	Francisella species (2)	Listeria monocytogenes	Salmonella enterica	Shigella dysenteriae	Staphylococcus aureus	Yersinia enterocolitica	Yersinia species (2)	Pan Bacteria 1	Pan Bacteria 3	PPC
C	Bacillus species (2)	Bacillus species (2)	wzt	Campylobacter fetus	Campylobacter speices (5)	Klebsiella & Enterobacter (2)	Enterococcus species (2)	Enterococcus faecalis	Enterococcus faecium	Enterococcus italicus	Escherichia & Shigella (6)	eae
D	stx2A	stxA	Francisella species (2)	Listeria monocytogenes	Salmonella enterica	Shigella dysenteriae	Staphylococcus aureus	Yersinia enterocolitica	Yersinia species (2)	Pan Bacteria 1	Pan Bacteria 3	PPC
E	Bacillus species (2)	Bacillus species (2)	wzt	Campylobacter fetus	Campylobacter speices (5)	Klebsiella & Enterobacter (2)	Enterococcus species (2)	Enterococcus faecalis	Enterococcus faecium	Enterococcus italicus	Escherichia & Shigella (6)	eae
F	stx2A	stxA	Francisella species (2)	Listeria monocytogenes	Salmonella enterica	Shigella dysenteriae	Staphylococcus aureus	Yersinia enterocolitica	Yersinia species (2)	Pan Bacteria 1	Pan Bacteria 3	PPC
G	Bacillus species (2)	Bacillus species (2)	wzt	Campylobacter fetus	Campylobacter speices (5)	Klebsiella & Enterobacter (2)	Enterococcus species (2)	Enterococcus faecalis	Enterococcus faecium	Enterococcus italicus	Escherichia & Shigella (6)	eae
H	stx2A	stxA	Francisella species (2)	Listeria monocytogenes	Salmonella enterica	Shigella dysenteriae	Staphylococcus aureus	Yersinia enterocolitica	Yersinia species (2)	Pan Bacteria 1	Pan Bacteria 3	PPC

Viability PCR

Viability PCR

Standard Real-time PCR

- For detection of different pathogens in a variety of sample types
- Cannot differentiate between **viable** and **non-viable** cells
- Many regulations require **viable cells** rather than **total cell DNA**

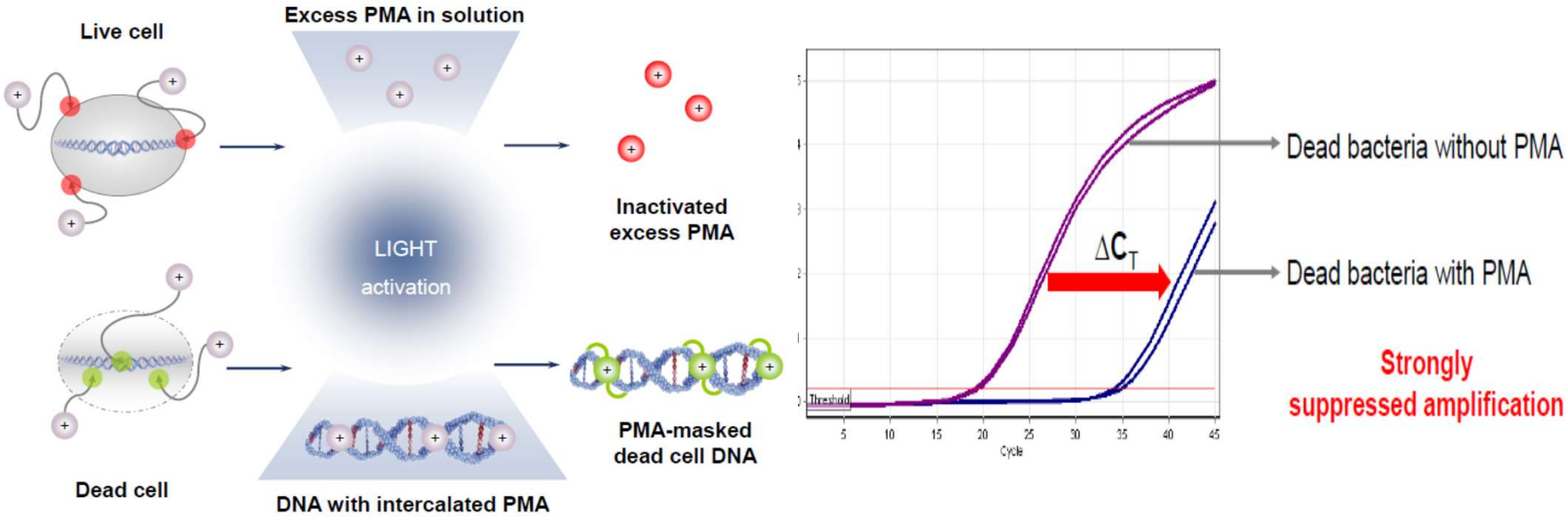
Potential uses

- Food safety testing e.g. microbiological approval before product release – rate limiting step in production
- Industrial hygiene/QC – e.g. testing of disinfection efficiency

Viable cell detection

- Overnight culture (e.g. food samples) allows detection of living cells
- Shorter enrichment times may detect high non-viable cell loads, issues with slow growing organisms

Viability PCR - PMA



Propidium monoazide

Non-toxic for live cells

Membrane impermeable reagent

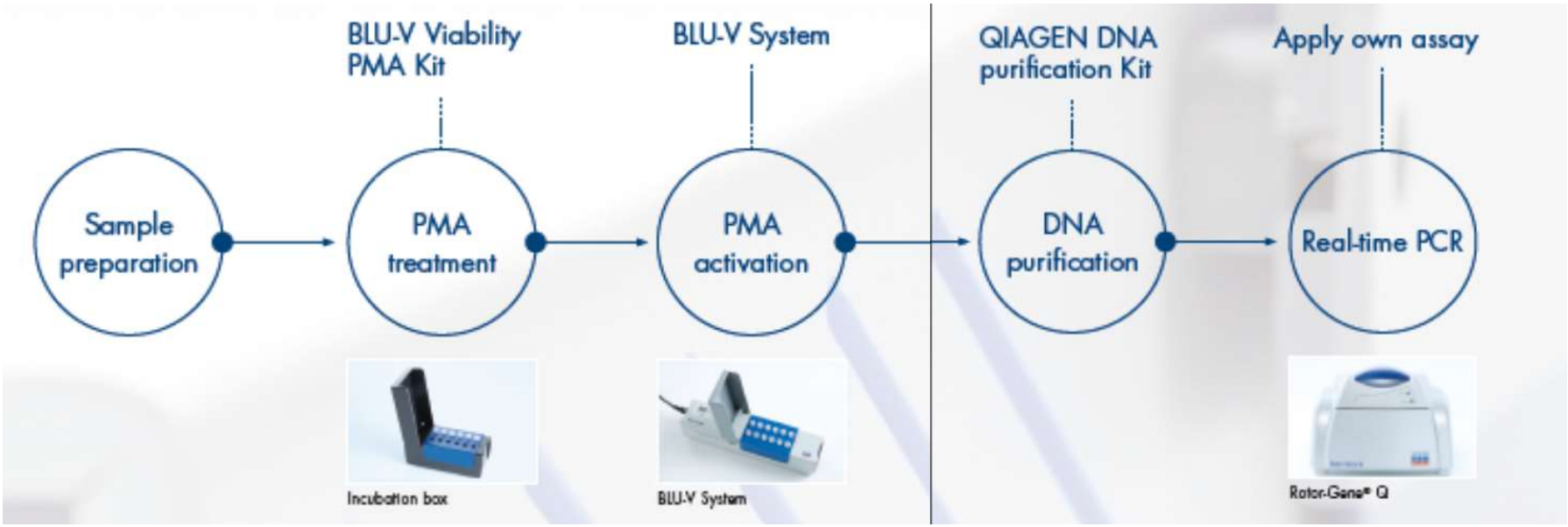
Can cross disrupted membranes of dead cells

Intercalates with DNA in dead cells – prevents PCR amplification

Signal of PMA treated bacteria is significantly shifted to higher Ct values

Difference between both real-time PCR signals: expected range ΔCt 6-15

Blu-V workflow



Microbial Genomics & uNGS at Qiagen – Potential in food safety testing

uNGS @ QIAGEN

Automation

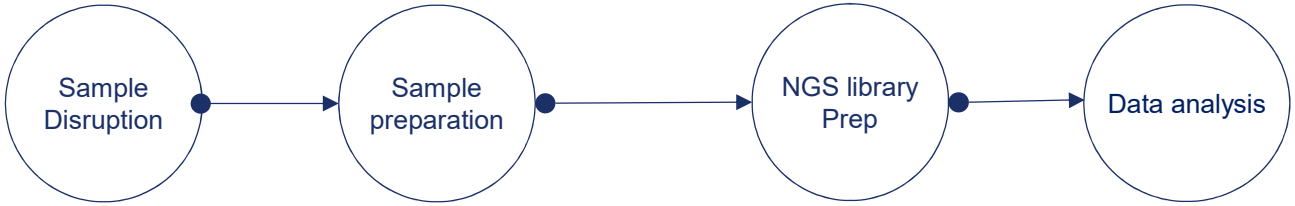
Powerlyzer 24

QIAcube

Tissue Lyser II

QIAcube HT

Tissue Lyser LT



Consumables

QIAamp Powerfecal Pro DNA kit
DNA microbiome kit
96 Powerfecal HT kit
PowerMicobiome kits
Powerfecal DNA/RNA kit
Dnease Powersoil Pro Kits
Dneasy Mericon kits

- Optimized lysis and Inhibitor Removal Technology for pure, high quality DNA

QIAseq 16S/ITS Panels
QIAseq FC DNA library Kit
QIAseq 1 step Amplicon Library Kit
QIAseq Ultralow Input Library Kit
QIAseq FX Singe Cell DNA Library kit

- High-quality libraries for unbiased metagenomic sequencing/WGS/targeted sequencing

CLC Genomics Workbench
Microbial Genomics Pro Suite

- Seamless integration with Illumina®-platforms
- Easy, high-performance analyses spanning taxonomies and biological functions


Thank You