

# Evaluating digital PCR for food monitoring: A case study with *E. coli*

Ronny Kellner, Associate Director R&D

dPCR Applications Development







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## Agenda

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Brief introduction to dPCR

QIAGEN dPCR solutions for monitoring of microbes

Transfer from qPCR to dPCR

Food monitoring: Case study with E. coli

Summary and discussion



## Digital PCR is an excellent tool for monitoring microbes





#### Absolute target quantification

No need for references or standard curves

Fast and easy dPCR workflow **Results within 2 hours** 



#### **Detects very small fold-change differences**

#### **Increased sensitivity**

**Detects low-abundance targets** 

**High reproducibility** 

**Eliminates amplification-efficiency bias** 



#### **Multiplexing**

Parallel quantification of multiple targets

## Digital PCR – from positive partitions to absolute quantification



#### Partitioning and end-point PCR



## **QIAGEN** solutions for microbe monitoring using dPCR

#### >800 wet-lab tested catalog assays including plant-associated microbes

#### **Optimized** mastermixes

for RNA/DNA targets, multiplexing and ultraclean conditions

#### QIAcuity digital PCR advancing food monitoring

#### Custom Assay Design Tool

for custom assay design of bacterial, fungal and viral targets

## **QIAGEN** solutions for microbe monitoring using dPCR

- Probe-based assays for the detection of bacterial, fungal, parasitic, viral, antibiotic resistance and virulence factor genes
- Selectable fluorophores (FAM, HEX, ROX, TAMRA, Cy5, ATTO700) for multiplexing using a mix-and-match approach
- Assays available in GeneGlobe (geneglobe.com)

#### >800 wet-lab tested catalog assays including plant-associated microbes

- QIAcuity Probe PCR Kit for dPCR of DNA targets & QIAcuity UCP Probe PCR Kit for dPCR of DNA targets in ultra clean setups
- QIAcuity OneStep Advanced Probe Kit for one-step RT dPCR of RNA and RNA+DNA targets with increased inhibitor tolerance
- QIAcuity High Multiplex Probe PCR Kit for challenging samples, dPCR and multiplexing beyond five targets

#### **Optimized** mastermixes

for RNA/DNA targets, multiplexing and ultraclean conditions

QIAcuity digital PCR advancing food monitoring

- Target selection based on taxonomy ID or scientific name
- Optimized design algorithm based on 16S (bacteria), ITS (fungi) and predefined genome regions (virus)
- Off-target prediction among closely related species

#### **Custom** Assay Design Tool

for custom assay design of bacterial, fungal and viral targets



## Adaptation of qPCR assays to dPCR is easy

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#### Supplier 1 Supplier 2 Supplier 3 and the second of the second s HDAC3 Histone deacetylase 3 CDKN2A Cyclin dependent kinase inhibitor 2A UBE2D2 Ubiquitin conjugating enzyme E2 D2 10 ng cDNA 0.1 ng cDNA NTC 10 ng cDNA 0.1 ng cDNA NTC 10 ng cDNA 0.1 ng cDNA NTC

#### Probe-based gene expression assays from three alternative suppliers are compatible with QIAcuity dPCR

- 96 probe-based gene expression assays from three suppliers were tested in combination with the QIAcuity Probe PCR Kit
- Included are 10 reference assays for normalization of gene expression
- All 96 assays worked with the supplier-recommended oligo concentrations and the QIAcuity standard cycling protocol for probe assays

### Case study with *E. coli* – Study Design





#### **EXTRACTION**

dPCR

**Template** 

spike-in

Gblock with 3

targets (T1, T2

and QNIC)

+/-

### Case study with *E. coli* – results





Detection of spiked-in *E.coli* after 4 hours of cultivation with potential for earlier detection with targeted assay

## Case study with E. coli – Study Results





#### Linkage analysis of multiple targets

- Calculation using multiple occupancy data output
- Advanced algorithm to account for partitions with multiple separate templates
- Can also be used for determination of template integrity

Accurate calculation of linked target loci for up to 6 targets

## One more thing – direct analysis of *E. coli* cultures/cells in dPCR





#### dPCR directly from E. coli cells



Direct dPCR of *E. coli* cells distributed in partitions of the nanoplate is possible but requires optimization(s)

## Benefits of dPCR for food monitoring using the QIAcuity

#### **Sensitive** Accurate Absolute quantification Can detect low numbers of your target of target molecules **Multiplex** Rapid Up to 12 targets Detection in two hours in one reaction Amenable to routine Combine up to 10 DNA testing with QIAcuity and RNA targets with Four and Eight the QIAcuity OneStep Advanced Probe Kit



## Thank you for your attention. Questions?

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