Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Optimising droplet digital PCR analysis approaches for detection and quantification of bacteria: a case study of fire blight and potato brown rot

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ESM 1: Annealing of primers and probes on the genomes of *R. solanacearum*

Materials and methods

The number of target copies of the *R. solanacearum* assay was examined in the genome sequences of *R. solanacearum* available in NCBI. Using the CLC Main Workbench 6.7.1 suit of programmes, the annealing of the primers and probes was examined. The formation of the product was expected for all of the combinations of primers and probes, in the correct orientation and positioned close enough to allow for efficient amplification.

Results

Both primers and probe had multiple sites of annealing in all of the genome sequences examined (Table S1). Based on the analysis, the target region is expected to be present in three copies per genome.

Table S1 Annealing of the primers and probe for the *R. solanacearum* assay [Weller et al., 2000] on the genome sequences of several *R. solanacearum* genomes

Primer name	Primer sequence[a]	Orientation	Region	Amplicon length (bp)	Mismatches
NC_017559, *Ralstonia solanacearum* CMR15 chromosome, complete genome					
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	376774..376795	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	376812..376834		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(376844..376866)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	568039..568060	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	568077..568099		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(568109..568131)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	2608656..2608677	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	2608694..2608716		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(2608726..2608748)		0
Rs-P	AGCtTGcTaCCtGCCGGCGAGTG	Fwd	1002075..1002097	NA	4

Rs-I-F	gcaTGcCttaCACATGCAAGTC	Rev	complement(2413840..2413861)	NA	7
Rs-P	aGcttgCtAccTGCCGGCGAGTG	Fwd	721031..721053	NA	8
Rs-P	aGcTtGCtacCtgCCGGCGAGTG	Fwd	1858448..1858470	NA	8
Rs-P	agcTTGctaCctGCCGGCGAGTG	Fwd	1912226..1912248	NA	8
Rs-P	aGCttgctACCtgCCGGCGAGTG	Fwd	2008534..2008556	NA	8
Rs-P	AgCTTgctacctGCCGGCGAGTG	Fwd	3238010..3238032	NA	8
Rs-P	AgCttgCtaCctGCCGGCGAGTG	Rev	complement(2239326..2239348)	NA	8
CP004012, *Ralstonia solanacearum* FQY_4, complete genome					
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	1546874..1546895	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	1546912..1546934		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(1546944..1546966)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	2996142..2996163	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	2996180..2996202		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(2996212..2996234)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	3217817..3217838	93	0

Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	3217855..3217877		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(3217887..3217909)		0
Rs-I-F	gcaTGcCttaCACATGCAAGTC	Rev	complement(1340630..1340651)	NA	7
Rs-P	agcTtGcTaCCTgCCGGCGAGTG	Rev	complement(1518554..1518576)	NA	7
NC_003295, *Ralstonia solanacearum* GMI1000 chromosome, complete genome					
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	1532739..1532760	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	1532777..1532799		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(1532809..1532831)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Rev	complement(3100966..3100987)	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Rev	complement(3100927..3100949)		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Fwd	3100895..3100917		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Rev	complement(3279176..3279197)	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Rev	complement(3279137..3279159)		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Fwd	3279105..3279127		0
Rs-I-F	gcaTGcCttaCACATGCAAGTC	Rev	complement(1339866..1339887)	NA	7

Rs-P	agcTtGcTaCCTgCCGGCGAGTG	Rev	complement(1504214..1504236)	NA	7
CP002819, *Ralstonia solanacearum* Po82, complete genome					
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	472875..472896	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	472913..472935		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(472945..472967)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	653230..653251	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	653268..653290		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(653300..653322)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Rev	complement(1997003..1997024)	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Rev	complement(1996964..1996986)		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Fwd	1996932..1996954		0
Rs-P	aGCtTgcTaCCtGCCGGCGAGTG	Rev	complement(2357756..2357778)	NA	6
Rs-P	agcTtGcTaCCTgCCGGCGAGTG	Fwd	2028781..2028803	NA	7
NC_014311, *Ralstonia solanacearum* str. PSI07 chromosome, complete genome					
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	440563..440584	93	0

Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	440601..440623		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(440633..440655)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	620811..620832	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	620849..620871		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(620881..620903)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Rev	complement(2007675..2007696)	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Rev	complement(2007636..2007658)		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Fwd	2007604..2007626		0
Rs-P	agcTtGcTaCCTgCCGGCGAGTG	Fwd	2036393..2036415	NA	7
Rs-I-F	gcaTGcCttaCACATGCAAGTC	Fwd	2202640..2202661	NA	7
Rs-P	agCttGCTacCtgCCGGCGAGTG	Fwd	2716978..2717000	NA	8
NC_014307, *Ralstonia solanacearum* CFBP2957 chromosome complete genome					
Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	419837..419858	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	419875..419897		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(419907..419929)		0

Rs-I-F	GCATGCCTTACACATGCAAGTC	Fwd	601916..601937	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Fwd	601954..601976		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Rev	complement(601986..602008)		0
Rs-I-F	GCATGCCTTACACATGCAAGTC	Rev	complement(1956205..1956226)	93	0
Rs-P	AGCTTGCTACCTGCCGGCGAGTG	Rev	complement(1956166..1956188)		0
Rs-II-R	GGCACGTTCCGATGTATTACTCA	Fwd	1956134..1956156		0
Rs-P	aGCtTgcTaCCtGCCGGCGAGTG	Rev	complement(2298867..2298889)	NA	6
Rs-P	agcTtGcTaCCTgCCGGCGAGTG	Fwd	1984567..1984589	NA	7
Rs-P	AGcTTgctacctGCCGGCGAGTG	Fwd	1315129..1315151	NA	8
Rs-P	agctTgCTacCtGCCGGCGAGTG	Fwd	1331211..1331233	NA	8

[a] nucleotides that are mismatched are written in lower-case letters

ESM 2: R script for ddPCR data analysis

Materials and methods

To provide improved high-throughput analysis of the ddPCR data, an R script was written in the R language and environment [R Development Core Team, 2008]. The script analyses the data as the signals exported from the QuantaSoft software, with its automatic threshold defined individually for each reaction, and its cluster classification or with the application of a selected, manually defined threshold. This incorporates the calculation of the parameters described in Table S2.

The input for the script is (i) signal data: the script reads droplet amplitude data (*.csv files) exported from the proprietary QuantaSoft software (Bio-Rad); (ii) threshold: the script either accepts thresholds and classes assigned to the individual droplets by the Quanta Soft software, or it calculates the parameters based on a manual input threshold (set manually or by the "definetherain" analysis of a reference sample); (iii) a fileList.txt file containing the list of all *.csv files to be analysed; and (iv) choice of the working directory.

Table S2 Description of the parameters calculated in the R script for the ddPCR data analysis

Parameter	Measure	Comment
Basic ddPCR parameters		
PositiveDrop	Number of positive droplets	As classified by the Quanta Soft analysis or based on the selected threshold
AcceptedDrop	Number of accepted droplets	
DefinedThreshold	Number of fluorescence units used as a threshold for positive droplets	If a manual threshold is defined, it is included as part of the results
Lambda	Mean copies per partition	current calculations of lambda make the assumption that all of the (measured) partitions are of equal volume. In this script the volume of the droplets is assumed to be 0.91 nL, consistent with the instrument manufacturer's software. Here, the lambda is calculatedwe calculate it using the number of positive partitions, as described in Huggett et al. (2013): -ln(1-(number of positive partitions)/(number of accepted droplets))
Quantity-Reaction(copies)	Target concentration expressed in copies per ddPCR reaction	Quantity of target per reaction expressed in copies calculated here
P	Percent of positive droplets	(number of positive droplets)/(number of accepted droplets)
EffectiveReactionSize	Total volume of partitions measured (effective reaction size)	Total volume of partitions is calculated by multiplying the number of accepted droplets with the volume of partition. The volume of droplets is assumed to be 0.91 nL, consistent with the instrument manufacturer's software.

PercentRxnAnalyzed	Effective reaction size/ total reaction	The total reaction volume is assumed to be 20,000 nL. calculated by dividing the theoretical volume (20,000 nL)
Signal and rain related ddPCR parameters		
PositiveMean	Mean of the signal in positive droplets	Bassed on the classification of droplets as detemined by Quanta Soft or by a manual threshold
NegativeMean	Mean of the signal in negative droplets	
MeanDifference	(mean of the signal in positive droplets)-mean of the signal in negative droplets	
PositiveSD	Standard deviation of the signal in positive droplets	
NegativeSD	Standard deviation of the signal in negative droplets	
PositiveCV	Covariance of the positive droplets	
NegativeCV	Covariance of the negative droplets	
NegativeLowerBound	Mean signal in negative droplets - 3*standard devaition (lower treshold of negative droplets)	Threshold used for determining the number of "rain" droplets
NegativeUpperBound	Mean signal in negative droplets + 3*standard devaition (upper treshold of negative droplets)	
PositiveLowerBound	mean signal in positive droplets - 3*standard devaition (lower treshold of positive droplets)	
PositiveUpperBound	Mean signal in positive droplets + 3*standard	

	devaition (upper treshold of positive droplets)	
OutlierBelowBound	Number of droplets with signal < (mean signal in negative droplets - 3*standard devaition)	"rain" number
OutlierMiddleNegative	Number of droplets with signal > (mean signal in negative droplets - 3*standard devaition)	
OutlierMiddlePositive	Number of droplets with signal < (mean signal in positive droplets - 3*standard devaition)	
OutlierAboveBound	Number of droplets with signal < (mean signal in negative droplets - 3*standard devaition)	
RainArea	Area between the negative and positive signals distribution (each mean ± 3*SD)	
RainNumber	OutlierMiddleNegative + OutlierMiddlePositive	
RainPerPos	Rain/number of positive droplets	
RainPerAll	Rain/number of accepted droplets	
RainPerArea	Rain/RainArea	
Separability measures		
BhattacharyyaDistance	Bhattacharyya distance	Separability measure
Jeffries-Matusita_(a)	Jeffries-Matusita distance, this formula is bound between 0 and 2.0	

Jeffries-Matusita_(b)	Jeffries-Matusita distance, this formula is bound between 0 and 1414.0	
BH.10RainPercent	BhattacharyyaDistance/10*RainPercent	Used in this study for optimisation of primer concentrations

R script

```
# Measures of separability 1 sample (pos, neg)

#R version 3.0.2 (2013-09-25)
#R Core Team (2013). R: A language and environment for statistical computing. R
Foundation for
#Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

#Script Last Modified: 22.05.2014

# remove other data
rm(list=ls())

# set working directory
setwd("include path to your data folder")

# Read the file containing the list of files to be analysed.
# The script assumes that all files are in a comma separated format, contains three columns
and includes a header line.
# The first contains the amplitude values, the second column is empty.
# The third column contains positive/negative cluster information (as defined by the
QuantaSoft software).
fileList <- read.table("fileList.txt", sep="\t", header=F)
```

```
# Prepare the matrix for the mass file analysis.
drops <- matrix(nrow=dim(fileList)[1], ncol=32)
colnames(drops) <- c('PositiveDrop', 'AcceptedDrop','DefinedThreshold',
                                        'Lambda', 'Quantity-Reaction(copies)', 'P',
'EffectiveReactionSize', 'PercentRxnAnalysed',
                                        'PositiveMean', 'NegativeMean', 'MeanDifference',
'PositiveSD', 'NegativeSD',
                                        'PositiveCV', 'NegativeCV', 'NegativeLowerBound',
'NegativeUpperBound',
                                        'PositiveLowerBound', 'PositiveUpperBound',
'OutlierBelowBound', 'OutlierMiddleNegative',
                                        'OutlierMiddlePositive', 'OutlierAboveBound',
'RainArea', 'RainNumber', 'RainPerPos', 'RainPerAll', 'RainPerArea',
                                        'BhattacharyyaDistance', 'Jeffries-Matusita_(a)', 'Jeffries-
Matusita_(b)','BH.RainPercent')

# Do you want to use your own selected threshold for positive/negative separation [YES/NO].
# If the option is 'YES', then modify the amp.threshold value to your liking.
own.threshold <- 'YES'
amp.threshold <- 7000

for (count in 1:dim(fileList)[1])
{       ## define the positive/negatives
        amp <- read.delim(file=as.character(fileList[count,]), sep=",", header=T) # read from
csv file
```

```
        # Here we assume, that the file contains three columns (colnames(amp)),
        # with the following names: "Assay1.Amplitude" "Assay2.Amplitude" "Cluster".
        # We only keep the Assay1.Amplitude (rename to amplitude) and Cluster
        colnames(amp)[1] <- 'Amplitude'
        amp <- amp[,c('Amplitude', 'Cluster')]

        drops[count,'PositiveDrop'] <- length(which(amp$Cluster == 2))
        drops[count,'AcceptedDrop'] <- length(amp$Cluster)

        if (own.threshold == 'YES')
        {       drops[count, 'DefinedThreshold'] <- amp.threshold
                amp$Cluster[amp$Amplitude <= amp.threshold] <- 1
                amp$Cluster[amp$Amplitude >  amp.threshold] <- 2
                drops[count, 'PositiveDrop'] <- length(which(amp$Cluster == 2))
                drops[count, 'AcceptedDrop'] <- length(amp$Cluster)
        }

        # Separate the list into positive and negative droplets.
        amp.pos <- amp[amp$Cluster == 2,]; amp.neg <- amp[amp$Cluster == 1,]; rm(amp)

        # Calculate the standard values
        drops[count, 'Lambda'] <- -log(1-
drops[count,'PositiveDrop']/drops[count,'AcceptedDrop'])
```

```
        drops[count, 'Quantity-Reaction(copies)'] <- drops[count, 'Lambda']*drops[count,
'AcceptedDrop']
        drops[count, 'P'] <- drops[count, 'PositiveDrop']/drops[count, 'AcceptedDrop']
        drops[count, 'EffectiveReactionSize'] <- (drops[count,'AcceptedDrop']*0.91)/1000
        drops[count, 'PercentRxnAnalysed'] <-
(drops[count,'AcceptedDrop']*0.91/20000)*100

        # Calculate the means, stdev and covariances
        drops[count,'PositiveMean'] <- mean(amp.pos$Amplitude)
        drops[count,'NegativeMean'] <- mean(amp.neg$Amplitude)
        drops[count,'MeanDifference'] <- drops[count,'PositiveMean'] -
drops[count,'NegativeMean']
        drops[count,'PositiveSD'] <- sd(amp.pos$Amplitude)
        drops[count,'NegativeSD'] <- sd(amp.neg$Amplitude)
        drops[count,'PositiveCV'] <- cov(as.matrix(amp.pos$Amplitude))
        drops[count,'NegativeCV'] <- cov(as.matrix(amp.neg$Amplitude))

        # Matrix for boundaries and rain calculations
        drops[count,'PositiveLowerBound'] <- drops[count,'PositiveMean'] -
3*drops[count,'PositiveSD']
        drops[count,'PositiveUpperBound'] <- drops[count,'PositiveMean'] +
3*drops[count,'PositiveSD']
        drops[count,'NegativeLowerBound'] <- drops[count,'NegativeMean'] -
3*drops[count,'NegativeSD']
```

```
        drops[count,'NegativeUpperBound'] <- drops[count,'NegativeMean'] +
3*drops[count,'NegativeSD']
        drops[count,'OutlierBelowBound']     <- dim(amp.neg[amp.neg$Amplitude <
drops[count,'NegativeLowerBound'],])[1]
        drops[count,'OutlierMiddleNegative'] <- dim(amp.neg[amp.neg$Amplitude >
drops[count,'NegativeUpperBound'],])[1]
        drops[count,'OutlierMiddlePositive'] <- dim(amp.pos[amp.pos$Amplitude <
drops[count,'PositiveLowerBound'],])[1]
        drops[count,'OutlierAboveBound']     <- dim(amp.pos[amp.pos$Amplitude >
drops[count,'PositiveUpperBound'],])[1]
        drops[count,'RainArea'] <- drops[count,'PositiveLowerBound'] -
drops[count,'NegativeUpperBound']
        drops[count,'RainNumber'] <- drops[count,'OutlierMiddleNegative'] +
drops[count,'OutlierMiddlePositive']
        drops[count,'RainPerPos'] <- (drops[count,'RainNumber'] /
drops[count,'PositiveDrop'])*100
        drops[count,'RainPerAll'] <- (drops[count,'RainNumber'] /
drops[count,'AcceptedDrop'])*100
        drops[count,'RainPerArea'] <- drops[count,'RainNumber'] / drops[count,'RainArea']

        # Temporarily define the halfsum of cv's as 'p' and multiplication of cv's as q.
        p <- as.matrix((drops[count,'PositiveCV'] + drops[count,'NegativeCV'])/2)
        q1 <- as.matrix(drops[count,'PositiveCV'])
        q2 <- as.matrix(drops[count,'NegativeCV'])
```

```
        # Calculate the Bhattacharyya Distance
        drops[count,'BhattacharyyaDistance'] <-
0.125*(drops[count,'MeanDifference'])^2*p^(-1) + 0.5*log(det(p)/sqrt(det(q1)*det(q2)))
        # Calculate the Jeffries-Matusita (0-2.0): this formula is bound between 0 and 2.0
        drops[count,'Jeffries-Matusita_(a)'] <- 2*(1-exp(-
drops[count,'BhattacharyyaDistance']))
        # Calculate Jeffries-Matusita (0-1414): this formula is bound between 0 and 1414.0
        drops[count,'Jeffries-Matusita_(b)'] <- 1000*sqrt(2*(1-exp(-
drops[count,'BhattacharyyaDistance'])))
        # Calculate the ratio betweeh the Bhattacharyya distance and rain percent
        drops[count,'BH.RainPercent'] <-
drops[count,'BhattacharyyaDistance']/10*drops[count,'RainPerPos']
        rm(p); rm(q1); rm(q2); rm(amp.pos); rm(amp.neg)
}
drops <- cbind(fileList, drops)

if (own.threshold == 'YES')
{       write.table(drops, paste("summarized_results_threshold_", amp.threshold, ".txt",
sep=""), quote=F, row.names=F, sep="\t")
}
if (own.threshold == 'NO')
{       write.table(drops, "summarized_results_threshold.txt", quote=F, row.names=F,
sep="\t")
}
```

```
rm(count); rm(amp.threshold); rm(fileList); rm(own.threshold); rm(drops)
```

ESM 3: Selection of the manual global threshold for the *E. amylovora* ddPCR

Materials and methods

The manual global threshold (MTg) was determined from signals from known negative samples. Different thresholds were defined by adding the mean signal in negative droplets and a number of standard deviations in increments. These thresholds were then applied to the dataset that consisted of the no template controls (NTCs), negative plant material, serial ten-fold dilutions of target DNA, and a set of samples containing low, known amounts of target DNA close to the limit of detection in the plant material. The suitability of the threshold was determined through the resulting diagnostic specificity; i.e., the correctness of the data obtained for the samples with known health status.

Table S3 Concentration (copies/µL of reaction) and the number of positive droplets (in brackets) in the amplification of the *E. amylovora* target DNA using the *AmsC* assay [Pirc *et al*., 2009] in different samples, as influenced by the different manual thresholds. Manual thresholds were chosen through calculation of the average amplitude of droplets that were obtained from the no template controls (a) and (b) both no template controls and negative plant material, and adding a certain number (1-15) of standard deviations. Samples considered positive (two or more positive droplets) are shown in bold. The vertical line defines the upper limit of the negative results. Positive results are shown in gray

a)

Conc. [log(CFU/mL)]	**Times standard deviation used in threshold setting (threshold)**														
	1 SD (1899)	**2 SD (1984)**	**3 SD (2068)**	**4 SD (2153)**	**5 SD (2237)**	**6 SD (2322)**	**7 SD (2407)**	**8 SD (2491)**	**9 SD (2576)**	**10 SD (2660)**	**11 SD (2745)**	**12 SD (2830)**	**13 SD (2914)**	**14 SD (2999)**	**15 SD (3083)**
No template controls															
NA	**55.9 (701)**	**6.55 (84)**	**0.856 (11)**	0.0778 (1)	0.0778 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**34.8 (394)**	**4.79 (55)**	**0.522 (6)**	0.087 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

NA	**3.97 (49)**	**0.324 (4)**	0.0809 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**21.9 (268)**	**2.18 (27)**	**0.242 (3)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**17.6 (204)**	**2.06 (24)**	**0.343 (4)**	**0.172 (2)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**17.5 (223)**	**1.79 (23)**	**0.234 (3)**	0.0779 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**14.5 (195)**	**1.41 (19)**	**0.222 (3)**	0.0741 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**14 (174)**	**1.92 (24)**	**0.399 (5)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Negative plant material															
NA	**99.8 (1239)**	**11.4 (147)**	**0.385 (5)**	**0 (0)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**91.5 (1060)**	**7.81 (94)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**51.1 (617)**	**4.62 (57)**	**0.0809 (1)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**45.4 (640)**	**2.3 (33)**	**0.0695 (1)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

NA	**35.4 (515)**	**3.25 (48)**	**0.406 (6)**	**0.203 (3)**	**0 (0)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**7.42 (105)**	**0.211 (3)**	**0 (0)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**1.09 (12)**	**0 (0)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**0.253 (3)**	**0.0844 (1)**	0.0844 (1)	0.0844 (1)	0.0844 (1)	0.0844 (1)	0.0844 (1)	0.0844 (1)	0.0844 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**0.155 (2)**	**0.0777 (1)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Low amount of target in plant material															
3-4	**5.36 (73)**	**4.55 (62)**	**3.23 (44)**	**2.64 (36)**	**2.35 (32)**	**1.76 (24)**	**1.76 (24)**	**1.47 (20)**	**1.39 (19)**	**1.17 (16)**	**0.953 (13)**	**0.879 (12)**	**0.806 (11)**	**0.733 (10)**	**0.733 (10)**
3-4	**0.801 (10)**	**0.721 (9)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**
3-4	**0.695 (9)**	**0.617 (8)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**
3-4	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**
3-4	**0.5 (6)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**
3-4	**0.477 (6)**	**0.477 (6)**	**0.477 (6)**	**0.477 (6)**	**0.477 (6)**	**0.477 (6)**	**0.397 (5)**	**0.397 (5)**	**0.397 (5)**	**0.318 (4)**	**0.318 (4)**	**0.318 (4)**	**0.318 (4)**	**0.318 (4)**	**0.318 (4)**
3-4	**0.577 (7)**	**0.495 (6)**	**0.412 (5)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**
3-4	**0.59 (8)**	**0.59 (8)**	**0.59 (8)**	**0.516 (7)**	**0.516 (7)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**

3-4	**0.612 (7)**	**0.612 (7)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**
3-4	**0.322 (4)**	**0.322 (4)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**
Range of target concentrations															
8	**5550 (14225)**	**5380 (14210)**	**5300 (14202)**	**5260 (14198)**	**5250 (14197)**	**5230 (14194)**	**5210 (14192)**	**5180 (14189)**	**5150 (14185)**	**5090 (14178)**	**5090 (14178)**	**5070 (14175)**	**5040 (14171)**	**5020 (14169)**	**5020 (14169)**
7	**593 (4829)**	**589 (4804)**	**587 (4794)**	**587 (4792)**	**587 (4791)**	**587 (4791)**	**586 (4790)**	**586 (4790)**	**586 (4790)**	**586 (4789)**	**586 (4789)**	**586 (4789)**	**586 (4787)**	**586 (4787)**	**586 (4787)**
6	**58 (718)**	**57.1 (707)**	**57.1 (707)**	**57.1 (707)**	**57.1 (707)**	**57 (706)**	**57 (706)**	**57 (706)**	**57 (706)**	**57 (706)**	**57 (706)**	**56.9 (705)**	**56.9 (705)**	**56.9 (705)**	**56.9 (705)**
5	**6.04 (73)**	**5.54 (67)**	**5.46 (66)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.29 (64)**	**5.29 (64)**	**5.29 (64)**
4	**0.914 (13)**	**0.703 (10)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**
3	**0.388 (5)**	**0.0777 (1)**	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)
2	**0.236 (3)**	**0.0788 (1)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1	**0.297 (4)**	**0 (0)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Linear range	E4 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8
Slope	0.96	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Intercept	-3.9	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1

b)

Conc. log(CFU/ mL)	**Times standard deviation used in threshold setting (threshold)**														
	1 SD (1904)	**2 SD (2006)**	**3 SD (2108)**	**4 SD (2210)**	**5 SD (2312)**	**6 SD (2414)**	**7 SD (2516)**	**8 SD (2617)**	**9 SD (2719)**	**10 SD (2821)**	**11 SD (2923)**	**12 SD (3025)**	**13 SD (3127)**	**14 SD (3229)**	**15 SD (3330)**
No template controls															
NA	**296 (3338)**	**33.3 (421)**	**2.41 (31)**	**0.156 (2)**	0.0778 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**210 (2195)**	**20.9 (238)**	**1.92 (22)**	**0.174 (2)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**187 (2124)**	**17 (208)**	**1.54 (19)**	**0.162 (2)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

NA	**145 (1680)**	**12.8 (158)**	**0.727 (9)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**134 (1470)**	**9.91 (115)**	**0.858 (10)**	**0.172 (2)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**124 (1506)**	**10.6 (136)**	**0.702 (9)**	**0.0779 (1)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**101 (1298)**	**8.7 (117)**	**0.593 (8)**	**0.0741 (1)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**108 (1285)**	**8.73 (109)**	**0.958 (12)**	**0.0798 (1)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Negative plant material															
NA	**498 (5200)**	**59.3 (750)**	**2.54 (33)**	0.077 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**457 (4517)**	**55.3 (652)**	**1.41 (17)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**308 (3318)**	**28.4 (347)**	**1.13 (14)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**282 (3573)**	**23.8 (339)**	**0.417 (6)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

NA	**233 (3099)**	**19.5 (285)**	**1.35 (20)**	**0.271 (4)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**79.2 (1086)**	**3.31 (47)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**9.13 (100)**	**0.637 (7)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**4.65 (55)**	**0.253 (3)**	0.0844 (1)	0.0844 (1)	0.0844 (1)	0.0844 (1)	0.0844 (1)	0.0844 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**1.48 (19)**	**0.155 (2)**	0.0777 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Low amount of target in plant material															
3-4	**7.06 (96)**	**5.14 (70)**	**3.89 (53)**	**2.79 (38)**	**2.35 (32)**	**1.76 (24)**	**1.69 (23)**	**1.47 (20)**	**1.25 (17)**	**1.03 (14)**	**0.879 (12)**	**0.806 (11)**	**0.659 (9)**	**0.586 (8)**	**0.586 (8)**
3-4	**1.04 (13)**	**0.721 (9)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**	**0.641 (8)**
3-4	**1 (13)**	**0.695 (9)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**	**0.54 (7)**
3-4	**0.916 (11)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**	**0.416 (5)**
3-4	**0.829 (9)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**	**0.461 (5)**
3-4	**0.742 (9)**	**0.577 (7)**	**0.412 (5)**	**0.412 (5)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**	**0.33 (4)**

3-4	**0.635 (8)**	**0.477 (6)**	**0.477 (6)**	**0.477 (6)**	**0.477 (6)**	**0.477 (6)**	**0.397 (5)**	**0.397 (5)**	**0.318 (4)**	**0.318 (4)**	**0.318 (4)**	**0.318 (4)**	**0.318 (4)**	**0.318 (4)**	**0.318 (4)**
3-4	**0.737 (10)**	**0.59 (8)**	**0.59 (8)**	**0.516 (7)**	**0.516 (7)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**	**0.442 (6)**
3-4	**0.7 (8)**	**0.612 (7)**	**0.612 (7)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**	**0.525 (6)**
3-4	**0.645 (8)**	**0.322 (4)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**	**0.161 (2)**
Range of target concentrations															
8	**5830 (14246)**	**5500 (14221)**	**5330 (14205)**	**5270 (14199)**	**5250 (14197)**	**5230 (14194)**	**5210 (14192)**	**5170 (14187)**	**5120 (14181)**	**5090 (14178)**	**5070 (14175)**	**5030 (14170)**	**5010 (14167)**	**4970 (14161)**	**4940 (14157)**
7	**615 (4964)**	**591 (4819)**	**588 (4798)**	**587 (4793)**	**587 (4791)**	**587 (4791)**	**586 (4790)**	**586 (4790)**	**586 (4789)**	**586 (4789)**	**586 (4789)**	**586 (4787)**	**586 (4787)**	**586 (4787)**	**586 (4787)**
6	**63 (779)**	**57.4 (711)**	**57.1 (707)**	**57.1 (707)**	**57.1 (707)**	**57 (706)**	**57 (706)**	**57 (706)**	**57 (706)**	**57 (706)**	**56.9 (705)**	**56.9 (705)**	**56.9 (705)**	**56.8 (704)**	**56.8 (704)**
5	**9.61 (116)**	**5.79 (70)**	**5.46 (66)**	**5.46 (66)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.37 (65)**	**5.29 (64)**	**5.29 (64)**	**5.29 (64)**	**5.29 (64)**
4	**5.85 (83)**	**0.774 (11)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**	**0.633 (9)**
3	**3.42 (44)**	**0.233 (3)**	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)	0.0777 (1)
2	**1.34 (17)**	0.0788 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1	**2.23 (30)**	**0.148 (2)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Linear range	E5 - E8	E4 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8	E3 - E8
Slope	0.93	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Intercept	-3.7	-4.0	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1	-4.1

ESM 4: Selection of manual global threshold for the *R. solanacearum* ddPCR

Materials and methods

The manual global threshold (MTg) was determined from signals from known negative samples. Different thresholds were defined by adding the mean signal in negative droplets and a number of standard deviations in increments. These thresholds were then applied to the dataset consisting of no template controls (NTCs), negative plant material, serial ten-fold dilutions of target DNA, and a set of samples containing low, known amounts of target DNA close to the limit of detection in the plant material. The suitability of the threshold was assessed by the resulting diagnostic specificity; i.e., the correctness of the data obtained on the samples with known health status.

Table S4 Concentration (copies/µL of reaction) and number of positive droplets (in brackets) in the amplification of the *R. solanacearum* target DNA using a broad-range assay [Weller *et al*., 2000] in different samples, as influenced by different manual thresholds. The manual thresholds were chosen by calculating the average amplitude of the droplets obtained from (a) no template controls and (b) both no template controls and negative plant material, and adding a certain number (1-15) of standard deviations. Positive results are shown in gray. A threshold that would allow all negative samples to give negative results with satisfactory sensitivity could not be determined for this assay

a)

Conc. log(CFU/mL)	**Times standard deviation used in threshold setting (threshold)**														
	1 SD (1013)	**2 SD (1120)**	**3 SD (1227)**	**4 SD (1333)**	**5 SD (1440)**	**6 SD (1546)**	**7 SD (1653)**	**8 SD (1760)**	**9 SD (1866)**	**10 SD (1973)**	**11 SD (2080)**	**12 SD (2186)**	**13 SD (2293)**	**14 SD (2400)**	**15 SD (2506)**
No template controls															
NA	**24.2 (251)**	**4.01 (42)**	**2 (21)**	**1.43 (15)**	**0.667 (7)**	**0.476 (5)**	**0.191 (2)**	**0.191 (2)**	**0.191 (2)**	**0.191 (2)**	**0.191 (2)**	**0.191 (2)**	**0.191 (2)**	**0.191 (2)**	0.0953 (1)
NA	**38.6 (465)**	**2.94 (36)**	**1.88 (23)**	**1.22 (15)**	**0.979 (12)**	**0.816 (10)**	**0.653 (8)**	**0.326 (4)**	**0.245 (3)**	**0.245 (3)**	**0.245 (3)**	**0.163 (2)**	**0.163 (2)**	**0.163 (2)**	**0.163 (2)**

NA	**44.1 (566)**	**0.992 (13)**	**0.84 (11)**	**0.763 (10)**	**0.534 (7)**	**0.458 (6)**	**0.382 (5)**	**0.382 (5)**	**0.229 (3)**	0.0763 (1)	0.0763 (1)	0.0763 (1)	0.0763 (1)	0.0763 (1)	0.0763 (1)
NA	**25.6 (333)**	**1.37 (18)**	**0.911 (12)**	**0.683 (9)**	**0.607 (8)**	**0.379 (5)**	**0.228 (3)**	**0.152 (2)**	**0.152 (2)**	**0.152 (2)**	0.152 (2)	0.0759 (1)	0.0759 (1)	0.0759 (1)	0.0759 (1)
NA	**66.8 (803)**	**2.18 (27)**	**1.37 (17)**	**1.13 (14)**	**0.726 (9)**	**0.646 (8)**	0.323 (4)	0.0807 (1)	0.0807 (1)	0.0807 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**28.7 (408)**	**0.765 (11)**	**0.487 (7)**	**0.209 (3)**	**0.139 (2)**	0.0695 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**311 (3322)**	**1950 (11179)**	**13 (158)**	**1.39 (17)**	**1.14 (14)**	**0.98 (12)**	**0.817 (10)**	**0.49 (6)**	**0.163 (2)**	0.0816 (1)	0.0816 (1)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**1.02 (11)**	**0.74 (8)**	**0.74 (8)**	**0.647 (7)**	**0.555 (6)**	**0.555 (6)**	**0.555 (6)**	**0.277 (3)**	**0.185 (2)**	**0.185 (2)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Negative plant material

NA	**117 (1369)**	**75.2 (897)**	**72 (860)**	**68.5 (819)**	**60.2 (722)**	**51.4 (619)**	**47.6 (575)**	**45.9 (555)**	**43.8 (530)**	**42.6 (515)**	**42.1 (510)**	**42.1 (509)**	**41.8 (506)**	**41.6 (504)**	**41.5 (502)**
NA	**179 (2154)**	**104 (1289)**	**95.7 (1195)**	**86.1 (1080)**	**62.4 (791)**	**40.5 (519)**	**30.6 (393)**	**26.4 (340)**	**22.1 (285)**	**19.7 (255)**	**19 (246)**	**18.9 (244)**	**18.7 (242)**	**18.7 (242)**	**18.7 (242)**
NA	**117 (1369)**	**78.2 (932)**	**69.2 (828)**	**61.7 (740)**	**43.7 (529)**	**28.9 (352)**	**23.9 (292)**	**21 (257)**	**18.8 (230)**	**17.8 (218)**	**17.7 (217)**	**17.6 (216)**	**17.5 (214)**	**17.5 (214)**	**17.3 (212)**

NA	**114 (1435)**	**76.5 (975)**	**70.3 (899)**	**63.8 (818)**	**42.2 (547)**	**22.3 (291)**	**15.6 (205)**	**11 (144)**	**6.53 (86)**	**4.78 (63)**	**4.4 (58)**	**4.4 (58)**	**4.4 (58)**	**4.4 (58)**	**4.4 (58)**
NA	**81.7 (1053)**	**41.5 (544)**	**36.3 (478)**	**31.6 (417)**	**20.8 (275)**	**8.71 (116)**	**4.42 (59)**	**2.62 (35)**	**1.35 (18)**	**0.823 (11)**	**0.748 (10)**	**0.748 (10)**	**0.673 (9)**	**0.673 (9)**	**0.673 (9)**
NA	**71.1 (853)**	**47.2 (572)**	**42.6 (518)**	**28.5 (348)**	**7.94 (98)**	**2.42 (30)**	**1.37 (17)**	**0.727 (9)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**
NA	**19.8 (295)**	**6.2 (93)**	**5.74 (86)**	**5 (75)**	**2.46 (37)**	**1.13 (17)**	**0.333 (5)**	**0.2 (3)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**
NA	**75.2 (831)**	**35 (394)**	**32.8 (370)**	**27.9 (315)**	**12.8 (146)**	**2.1 (24)**	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)
NA	**52.2 (653)**	**33.8 (426)**	**31.3 (395)**	**24.6 (312)**	**7.83 (100)**	**1.17 (15)**	0.0781 (1)	0.0781 (1)	0.0781 (1)	0.0781 (1)	0.0781 (1)	0.0781 (1)	0 (0)	0 (0)	0 (0)
NA	**254 (2925)**	**176 (2098)**	**125 (1527)**	**63.8 (799)**	**27 (344)**	**10.1 (130)**	**3.26 (42)**	**0.699 (9)**	**0.155 (2)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Low amount of target in plant material															
3-4	**1780 (11276)**	**1430 (10248)**	**1260 (9602)**	**1040 (8626)**	**743 (6914)**	**456 (4774)**	**329 (3644)**	**297 (3336)**	**287 (3229)**	**277 (3137)**	**266 (3026)**	**256 (2923)**	**245 (2810)**	**234 (2696)**	**220 (2557)**
3-4	**2930**	**2190**	**1770**	**1380**	**886**	**503**	**275**	**196**	**171**	**160**	**147**	**136**	**125**	**114**	**104**

	(12713)	**(11810)**	**(10943)**	**(9783)**	**(7567)**	**(5017)**	**(3022)**	**(2235)**	**(1972)**	**(1849)**	**(1712)**	**(1593)**	**(1465)**	**(1348)**	**(1233)**
3-4	**3640 (14606)**	**2610 (13748)**	**1890 (12442)**	**1300 (10515)**	**768 (7619)**	**435 (4957)**	**270 (3301)**	**200 (2526)**	**167 (2138)**	**146 (1889)**	**127 (1649)**	**109 (1435)**	**92.9 (1229)**	**81.6 (1085)**	**70.2 (938)**
3-4	**3080 (12467)**	**2310 (11645)**	**1770 (10623)**	**1250 (9011)**	**722 (6387)**	**386 (3931)**	**221 (2414)**	**168 (1884)**	**146 (1648)**	**133 (1509)**	**121 (1381)**	**109 (1257)**	**97.1 (1122)**	**85.7 (995)**	**75.9 (885)**
3-4	**870 (7189)**	**530 (5031)**	**461 (4504)**	**402 (4030)**	**304 (3179)**	**173 (1911)**	**65.7 (763)**	**24.6 (291)**	**12.9 (153)**	**8.06 (96)**	**6.46 (77)**	**5.87 (70)**	**5.62 (67)**	**5.62 (67)**	**5.53 (66)**
3-4	**308 (2975)**	**208 (2098)**	**190 (1934)**	**175 (1797)**	**144 (1491)**	**82.1 (877)**	**28.9 (316)**	**12.2 (135)**	**8.15 (90)**	**6.24 (69)**	**5.52 (61)**	**5.52 (61)**	**5.52 (61)**	**5.52 (61)**	**5.52 (61)**
3-4	**11.1 (149)**	**1310 (10357)**	**1800 (11949)**	**2660 (13527)**	**27.7 (370)**	**279 (3332)**	**3.93 (53)**	**4 (54)**	**4 (54)**	**4.08 (55)**	**4.23 (57)**	**4.45 (60)**	**5.79 (78)**	**735 (7240)**	**90.8 (1177)**
3-4	**483 (4286)**	**332 (3141)**	**288 (2782)**	**223 (2210)**	**134 (1382)**	**54.8 (586)**	**14.1 (154)**	**5.85 (64)**	**4.66 (51)**	**4.29 (47)**	**4.2 (46)**	**4.2 (46)**	**4.2 (46)**	**4.11 (45)**	**4.11 (45)**
3-4	**294 (3321)**	**224 (2613)**	**206 (2422)**	**179 (2131)**	**134 (1621)**	**62.9 (787)**	**21.6 (275)**	**9.52 (122)**	**6 (77)**	**4.67 (60)**	**3.89 (50)**	**3.73 (48)**	**3.73 (48)**	**3.66 (47)**	**3.66 (47)**

3-4	**418 (3977)**	**238 (2452)**	**213 (2213)**	**198 (2070)**	**164 (1744)**	**115 (1247)**	**47.4 (531)**	**17.6 (200)**	**8.6 (98)**	**4.82 (55)**	**3.59 (41)**	**3.41 (39)**	**3.33 (38)**	**3.15 (36)**	**3.15 (36)**
3-4	**397 (3955)**	**314 (3245)**	**283 (2961)**	**230 (2468)**	**152 (1690)**	**64 (738)**	**20.6 (242)**	**7.01 (83)**	**4.3 (51)**	**3.37 (40)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**

Range of target concentrations

7	**8750 (14413)**	**7620 (14404)**	**7350 (14400)**	**7290 (14399)**	**7130 (14396)**	**6940 (14392)**	**6710 (14386)**	**6620 (14383)**	**6590 (14382)**	**6440 (14377)**	**6390 (14375)**	**6320 (14372)**	**6250 (14369)**	**6220 (14368)**	**6160 (14365)**
6	**2980 (13825)**	**2890 (13743)**	**2870 (13724)**	**2860 (13712)**	**2850 (13696)**	**2830 (13684)**	**2810 (13663)**	**2800 (13654)**	**2800 (13647)**	**2790 (13634)**	**2780 (13623)**	**2760 (13607)**	**2750 (13596)**	**2740 (13587)**	**2740 (13582)**
5	**328 (3641)**	**304 (3409)**	**302 (3391)**	**301 (3388)**	**301 (3384)**	**301 (3381)**	**300 (3376)**	**300 (3373)**	**300 (3371)**	**299 (3364)**	**299 (3363)**	**299 (3362)**	**299 (3360)**	**299 (3360)**	**298 (3356)**
4	**37.7 (446)**	**32.2 (381)**	**32.1 (380)**	**32 (379)**	**32 (379)**	**31.9 (378)**	**31.8 (377)**	**31.7 (376)**	**31.6 (375)**	**31.6 (374)**	**31.6 (374)**	**31.5 (373)**	**31.3 (371)**	**31.1 (369)**	**31.1 (369)**
3	**7.52 (96)**	**3.75 (48)**	**3.6 (46)**	**3.44 (44)**	**3.44 (44)**	**3.36 (43)**	**3.28 (42)**	**3.2 (41)**	**3.05 (39)**	**2.97 (38)**	**2.97 (38)**	**2.89 (37)**	**2.89 (37)**	**2.73 (35)**	**2.73 (35)**
2	**10.5 (145)**	**0.795 (11)**	**0.795 (11)**	**0.795 (11)**	**0.723 (10)**	**0.65 (9)**	**0.506 (7)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**

1	**10.1 (135)**	**2.39 (32)**	**2.32 (31)**	**2.32 (31)**	**2.32 (31)**	**2.24 (30)**	**2.17 (29)**	**2.02 (27)**	**2.02 (27)**	**1.79 (24)**	**1.72 (23)**	**1.72 (23)**	**1.64 (22)**	**1.49 (20)**	**1.42 (19)**
0	**3.79 (49)**	**0.464 (6)**	**0.464 (6)**	**0.464 (6)**	**0.387 (5)**	**0.387 (5)**	**0.232 (3)**	0.0773 (1)	0.0773 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Linear range	E3 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6
Slope	0.87	0.9	0.9	0.91	0.91	0.92	0.95	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Intercept	-1.8	-2.0	-2.0	-2.0	-2.1	-2.1	-2.3	-2.3	-2.3	-2.3	-2.3	-2.3	-2.3	-2.4	-2.4

b)

Conc. log(CFU/ mL)	**Times standard deviation used in threshold setting (threshold)**														
	1 SD (1209)	**2 SD (1488)**	**3 SD (1767)**	**4 SD (2046)**	**5 SD (2325)**	**6 SD (2604)**	**7 SD (2882)**	**8 SD (3161)**	**9 SD (3440)**	**10 SD (3719)**	**11 SD (3998)**	**12 SD (4277)**	**13 SD (4556)**	**14 SD (4834)**	**15 SD (5113)**
No template controls															

NA	**2.19 (23)**	**0.667 (7)**	**0.191 (2)**	**0.191 (2)**	**0.191 (2)**	0.0953 (1)	0.0953 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**2.04 (25)**	**0.898 (11)**	**0.326 (4)**	**0.245 (3)**	**0.163 (2)**	0.0816 (1)	0.0816 (1)	0.0816 (1)	0.0816 (1)	0.0816 (1)	0.0816 (1)	0.0816 (1)	0.0816 (1)	0.0816 (1)	0.0816 (1)
NA	**0.84 (11)**	**0.458 (6)**	**0.382 (5)**	0.0763 (1)	0.0763 (1)	0.0763 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**1.06 (14)**	**0.379 (5)**	**0.152 (2)**	**0.152 (2)**	0.0759 (1)	0.0759 (1)	0.0759 (1)	0.0759 (1)	0.0759 (1)	0.0759 (1)	0.0759 (1)	0.0759 (1)	0 (0)	0 (0)	0 (0)
NA	**1.45 (18)**	**0.646 (8)**	0.0807 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**0.487 (7)**	**0.139 (2)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**24.3 (295)**	**1.06 (13)**	**0.49 (6)**	0.0816 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**0.74 (8)**	**0.555 (6)**	**0.277 (3)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Negative plant material

NA	**72.4 (864)**	**55.2 (664)**	**45.7 (552)**	**42.2 (511)**	**41.8 (506)**	**41.5 (502)**	**40.6 (492)**	**40.1 (486)**	**39.4 (477)**	**38.4 (466)**	**37.4 (453)**	**35.6 (432)**	**32.1 (390)**	**21.5 (263)**	**7.4 (91)**
NA	**96.8 (1208)**	**50.4 (643)**	**26.1 (337)**	**19 (246)**	**18.7 (242)**	**18.7 (242)**	**18.3 (237)**	**18.2 (235)**	**17.9 (232)**	**17.5 (227)**	**17.2 (222)**	**16.3 (211)**	**15.6 (202)**	**14.4 (187)**	**13.3 (173)**
NA	**70.4 (841)**	**34.8 (423)**	**20.9 (256)**	**17.7 (217)**	**17.5 (214)**	**17.2 (210)**	**16.9 (207)**	**16.2 (199)**	**16 (196)**	**15.4 (189)**	**15.2 (186)**	**14.1 (173)**	**13.2 (162)**	**11.6 (142)**	**6.75 (83)**

NA	**70.7 (904)**	**32.3 (420)**	**10.7 (141)**	**4.4 (58)**	**4.4 (58)**	**4.33 (57)**	**4.25 (56)**	**4.25 (56)**	**4.02 (53)**	**3.95 (52)**	**3.72 (49)**	**3.64 (48)**	**3.34 (44)**	**2.5 (33)**	**1.82 (24)**
NA	**37.1 (488)**	**13.8 (183)**	**2.55 (34)**	**0.748 (10)**	**0.673 (9)**	**0.673 (9)**	**0.673 (9)**	**0.673 (9)**	**0.673 (9)**	**0.673 (9)**	**0.598 (8)**	**0.524 (7)**	**0.449 (6)**	**0.224 (3)**	0 (0)
NA	**43.2 (525)**	**3.64 (45)**	**0.646 (8)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.323 (4)**	**0.161 (2)**	0.0807 (1)	0.0807 (1)
NA	**5.94 (89)**	**1.86 (28)**	**0.2 (3)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**	**0.133 (2)**
NA	**32.9 (371)**	**5.96 (68)**	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)	0.0875 (1)
NA	**32 (404)**	**3.6 (46)**	0.0781 (1)	0.0781 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NA	**137 (1663)**	**18.2 (232)**	**0.699 (9)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Low amount of target in plant material

3-4	**1290 (9714)**	**601 (5923)**	**296 (3326)**	**271 (3071)**	**242 (2783)**	**210 (2442)**	**174 (2064)**	**140 (1683)**	**108 (1315)**	**82.6 (1018)**	**66.5 (826)**	**56.3 (702)**	**49.5 (620)**	**41.1 (516)**	**28 (354)**
3-4	**1840 (11102)**	**683 (6325)**	**194 (2211)**	**151 (1755)**	**122 (1436)**	**95.3 (1135)**	**69.9 (842)**	**51.9 (630)**	**37.6 (460)**	**26.9 (330)**	**20.3 (250)**	**16 (198)**	**13.9 (172)**	**12.3 (152)**	**9.93 (123)**
3-4	**2000**	**596**	**197**	**133**	**88.7**	**59.8 (803)**	**38.4 (521)**	**25.5 (347)**	**17.1 (234)**	**11.8 (162)**	**8.44 (116)**	**6.62 (91)**	**4.94 (68)**	**3.41 (47)**	**2.32 (32)**

	(12702)	**(6346)**	**(2488)**	**(1731)**	**(1175)**										
3-4	**1850 (10814)**	**544 (5180)**	**167 (1867)**	**125 (1428)**	**94 (1088)**	**68 (796)**	**45.2 (535)**	**31.2 (371)**	**21.3 (255)**	**14.5 (174)**	**10.4 (125)**	**8.15 (98)**	**6.4 (77)**	**5.23 (63)**	**3.98 (48)**
3-4	**469 (4564)**	**248 (2658)**	**23.8 (282)**	**6.96 (83)**	**5.62 (67)**	**5.53 (66)**	**5.53 (66)**	**5.36 (64)**	**5.36 (64)**	**5.36 (64)**	**5.28 (63)**	**5.28 (63)**	**5.28 (63)**	**5.28 (63)**	**5.28 (63)**
3-4	**191 (1947)**	**119 (1246)**	**11.4 (126)**	**5.61 (62)**	**5.52 (61)**	**5.52 (61)**	**5.52 (61)**	**5.43 (60)**	**5.43 (60)**	**5.43 (60)**	**5.34 (59)**	**5.25 (58)**	**5.25 (58)**	**5.16 (57)**	**4.97 (55)**
3-4	**1400 (10694)**	**166 (2086)**	**10.6 (142)**	**4.23 (57)**	**4 (54)**	**3.71 (50)**	**3.63 (49)**	**3.48 (47)**	**3.34 (45)**	**3.04 (41)**	**2.89 (39)**	**2.67 (36)**	**2.37 (32)**	**2.3 (31)**	**1.93 (26)**
3-4	**296 (2847)**	**95.8 (1007)**	**5.76 (63)**	**4.2 (46)**	**4.2 (46)**	**4.11 (45)**	**4.11 (45)**	**4.11 (45)**	**4.11 (45)**	**4.11 (45)**	**4.11 (45)**	**4.11 (45)**	**4.11 (45)**	**4.11 (45)**	**4.11 (45)**
3-4	**210 (2458)**	**103 (1261)**	**9.28 (119)**	**4.28 (55)**	**3.66 (47)**	**3.66 (47)**	**3.58 (46)**	**3.58 (46)**	**3.5 (45)**	**3.5 (45)**	**3.5 (45)**	**3.5 (45)**	**3.5 (45)**	**3.5 (45)**	**3.42 (44)**
3-4	**215 (2232)**	**145 (1550)**	**16.3 (185)**	**3.94 (45)**	**3.24 (37)**	**3.15 (36)**	**3.15 (36)**	**3.15 (36)**	**3.15 (36)**	**3.15 (36)**	**3.06 (35)**	**2.98 (34)**	**2.98 (34)**	**2.89 (33)**	**2.71 (31)**

3-4	**287 (2999)**	**111 (1255)**	**6.67 (79)**	**2.95 (35)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**	**2.87 (34)**
Range of target concentrations															
7	**7410 (14401)**	**7080 (14395)**	**6620 (14383)**	**6390 (14375)**	**6220 (14368)**	**6100 (14362)**	**5950 (14354)**	**5870 (14349)**	**5740 (14340)**	**5620 (14331)**	**5530 (14324)**	**5450 (14317)**	**5330 (14305)**	**5250 (14297)**	**5170 (14287)**
6	**2880 (13727)**	**2840 (13691)**	**2800 (13654)**	**2780 (13628)**	**2750 (13593)**	**2730 (13573)**	**2700 (13544)**	**2680 (13514)**	**2660 (13491)**	**2630 (13461)**	**2610 (13428)**	**2580 (13396)**	**2560 (13368)**	**2540 (13338)**	**2520 (13309)**
5	**302 (3391)**	**301 (3383)**	**300 (3373)**	**299 (3363)**	**299 (3360)**	**298 (3353)**	**297 (3345)**	**297 (3340)**	**296 (3336)**	**296 (3333)**	**295 (3327)**	**294 (3318)**	**293 (3306)**	**292 (3299)**	**292 (3293)**
4	**32.1 (380)**	**31.9 (378)**	**31.7 (376)**	**31.6 (374)**	**31.2 (370)**	**31 (367)**	**31 (367)**	**30.7 (364)**	**30.7 (364)**	**30.6 (363)**	**30.5 (362)**	**30.5 (362)**	**30.5 (362)**	**30.4 (361)**	**30.4 (360)**
3	**3.67 (47)**	**3.44 (44)**	**3.2 (41)**	**2.97 (38)**	**2.89 (37)**	**2.73 (35)**	**2.5 (32)**	**2.42 (31)**	**2.42 (31)**	**2.42 (31)**	**2.27 (29)**	**2.27 (29)**	**2.27 (29)**	**2.27 (29)**	**2.27 (29)**
2	**0.795 (11)**	**0.723 (10)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**	**0.434 (6)**
1	**2.32 (31)**	**2.24 (30)**	**2.02 (27)**	**1.72 (23)**	**1.57 (21)**	**1.34 (18)**	**1.2 (16)**	**1.05 (14)**	**0.672 (9)**	**0.597 (8)**	**0.597 (8)**	**0.523 (7)**	**0.448 (6)**	**0.373 (5)**	**0.373 (5)**
0	**0.464 (6)**	**0.387 (5)**	0.0773 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Linear range	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6	E2 - E6
Slope	0.90	0.91	0.96	0.96	0.96	0.96	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.96	0.96
Intercept	-2.0	-2.1	-2.3	-2.3	-2.3	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4

ESM 5: Quantification of *E. amylovora* and *R. solanacearum* using directy ddPCR, in comparison with the analysis of extracted DNA

Direct ddPCR was performed on serial dilutions of the *E. amylovora* and *R. solancaerum* bacteria in 10 mM phosphate buffer, by directly adding bacteria to the ddPCR reaction mixture. The data were assessed by comparing the concentrations determined with the concentrations expected; i.e., with the starting concentrations in CFU/mL and with the ddPCR analysis of the DNA extracted from the same bacterial suspensions. The DNA was first extracted from the highest concentration of bacterial suspensions, and its dilutions were prepared in molecular biology grade water (DNA dilutions). Separately, ten-fold serial dilutions of the bacterial suspensions were prepared in 10 mM phosphate buffer and the DNA was extracted from each dilution separately (DNA extractions). Despite the occurrence of "rain", the estimation of the *E. amylovora* CFU concentrations using direct ddPCR were closer to the true starting values, expressed as CFU/mL, than the quantification following DNA extraction, as seen by the lower concentration bias (Table S5).

Table S5 Concentrations determined by ddPCR and the concentration bias when analysing extracted DNA in comparison with direct ddPCR

Starting concentration		type of material and analysis								
		DNA dilutions			DNA extractions			Direct ddPCR		
Log (CFU/mL)	CFU/mL	Copies/µL ddPCR reaction	Copies/ml	Concentration bias	Copies/µL of ddPCR reaction	Copies/mL	Concentration bias	Copies/µL ddPCR reaction	Copies/mL	Concentration bias
E. amylovora										
8	207600000	5250	31324582	-0.85	5370	32040573	-0.85	6920	34600000	-0.83
7	20760000	587	3502387	-0.83	1680	10023866	-0.52	3850	19250000	-0.07
6	2076000	57.1	340692	-0.84	186	1109785	-0.47	426	2130000	0.03
5	207600	5.37	32041	-0.85	17.2	102625	-0.51	40.9	204500	-0.01
4	20760	0.633	3777	-0.82	1.3	7757	-0.63	4.2	21000	0.01
3	2076	0.0777	464	-0.78	0.236	1408	-0.32	0.25	1250	-0.4
2	207.6	0	0	NA	0	0	NA	0	0	NA
1	20.76	0	0	NA	0	0	NA	0	0	NA

R. solanacearum										
8	130000000	6160	36754177	-0.72	4710	28102625	-0.78	6170	30850000	-0.76
7	13000000	2740	16348449	0.26	3040	18138425	0.4	2020	10100000	-0.22
6	1300000	298	1778043	0.37	306	1825776	0.4	205	1025000	-0.21
5	130000	31.1	185561	0.43	33.6	200477	0.54	18.9	94500	-0.27
4	13000	2.73	16289	0.25	2.23	13305	0.02	1.82	9100	-0.3
3	1300	0.434	2589	0.99	0.216	1289	-0.01	0.0786	393	-0.7
2	130	1.42	8473	64.17	0.223	1331	9.23	0	0	NA
1	13	0	0	NA	0	0	NA	0	0	NA

ESM 6: Results of R script analysis of *E. amylovora* ddPCR

See Table S6 in separate Excel file - 216_2014_8084_MOESM2_ESM.xlsx

ESM 7: Results of R script analysis of *R. solanacearum* ddPCR

See Table S7 in separate Excel file - 216_2014_8084_MOESM3_ESM.xlsx

ESM 8: Use of separability measures and estimation of the "rain" in primer optimisation for R. solanacearum ddPCR

Materials and methods

Due to the high level of droplets with intermediate fluorescence (i.e., the "rain") in the *R. solanacearum* ddPCR assay based on the qPCR described by Weller *et al.* [Weller et al., 2000], we investigated the influence of different primer concentrations on the signal generated and on the quality of the separation between the negative and positive droplets.

The primers were tested at final concentrations of 50 nM, 300 nM and 900 nM, with a constant probe concentration (250 nM). Single reactions were performed with each combination of the forward and reverse primers. We also compared the original primers as described by Weller *et al.* [Weller et al., 2000] with the adapted primers that contained a 5' flap, based on Afonina et al. [Afonina et al., 2007] (see Table S8).

We used a high concentration of the target DNA, of log 4.6 copies/reaction (Cq in qPCR of 23), to allow for a high level of "rain" that could be compared between the samples. At this concentration, the majority of the accepted droplets in ddPCR (94%) were positive.

The primer combinations were assessed according to the signal intensity, distribution of negative and positive droplets (i.e., their respective standard deviations), percentage of "rain" droplets, and calculation of the Bhattacharyya distance. To combine the two separability parameters, the percentage of "rain" and the Bhattacharyya distance, we divided the Bhattacharyya distance with 10-fold the "rain" percentage, and used this ratio as a selection guide in the optimisation of primers. The calculation of the ratio was carried out so as to weight both parameters as similarly important.

Results

The increasing concentrations of the forward or reverse primers led to an increased fluorescence of positive droplets, and to a lesser extent, to fluorescence of the negative droplets. The effect was more pronounced with increased reverse primer concentration than with the forward primer concentration, independent of the type of primers used (original or 5'-flap primers). In general, the fluorescence of both the negative and positive droplets was higher with the 5' flap primers. As seen in the heat map of the ddPCR data, this did not necessarily mean a narrower distribution of the signal. On the contrary, the distribution of positive droplets had the lowest standard deviation with the lowest concentrations of both the forward and reverse primers and with the original primers (Fig. S1). The good separation between the negative and positive droplets at the lowest primer concentrations was reflected in the highest Bhattacharyya distance. The distance was inversely proportional to the "rain", expressed as a percentage of the positive droplets (Fig. S2). The ratio of the two parameters, selected so as to weight both of them as similarly important, confirms the lowest concentrations of primers as optimal.

Based on these data, we can conclude that somewhat counter-intuitively, using lower concentrations of primers improves the performance of the *R. solanacearum* ddPCR under study. However, it is not clear whether the same concentrations would allow for the same analytical sensitivity, or how they would affect the occurrence signals that arise from the cross-reactions with the plant material.

Table S8 Primers used in the primer optimisation. The 5'-flap parts of primers are shown in bold

Assay	Oligonucleotide		Reference
	Name	Sequence (5'-3')[a]	
Rs	RS-I-F	GCATGCCTTACACATGCAAGTC	Weller at al., 2000
	RS-II-R	GGCACGTTCCGATGTATTACTCA	
	RS-P	FAM – AGCTTGCTACCTGCCGGCGAGTG - BHQ1	
Rs flap	RS-I-F flap	**AATAAATCATAACG**GCATGCCTTACACATGCAAGTC	Adapted from Weller at al., 2000 and Afonina et al., 2007
	RS-II-R flap	**AATAAATCATAACG**GGCACGTTCCGATGTATTACTCA	
	RS-P	FAM – AGCTTGCTACCTGCCGGCGAGTG - BHQ1	

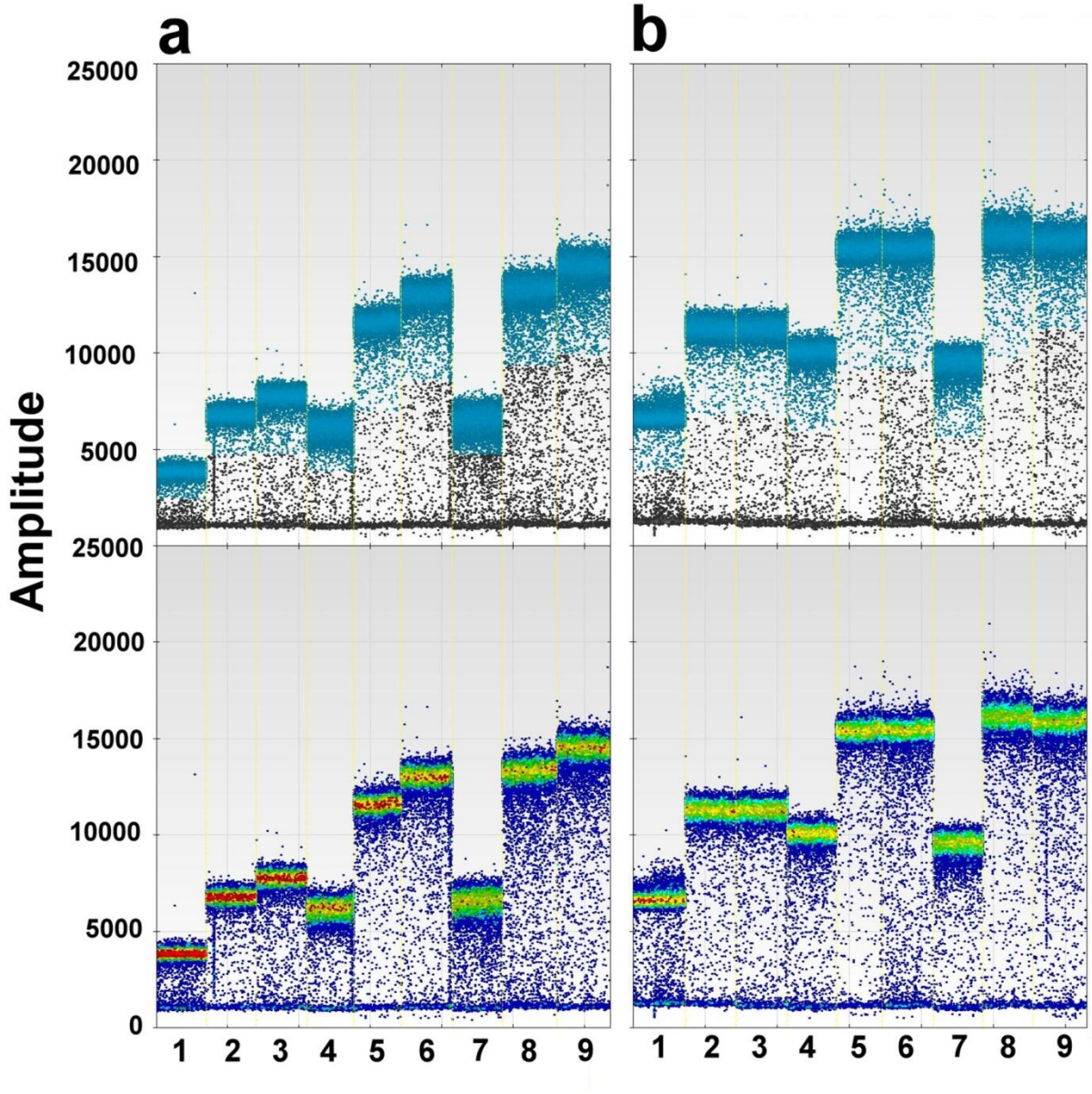

Fig. S1 Comparison of signals generated with the amplification of the *R. solanacearum* target DNA using primers at different concentrations. The data are shown as both negative and positive droplets (as defined by the QuantaSoft analysis of individual reactions; top panels), and as heat maps (bottom panels), and for the original primers as described by Weller et al. [Weller et al., 2000] (a), and for the primers modified by a 5'-flap (b). The primer combinations of forward and reverse were: (1) 50/50, (2) 300/50, (3) 900/50, (4) 50/300, (5) 300/300, (6) 900/300, (7) 50/900, (8) 300/900 and (9) 900/900 (all in nM final concentrations in the ddPCR reaction)

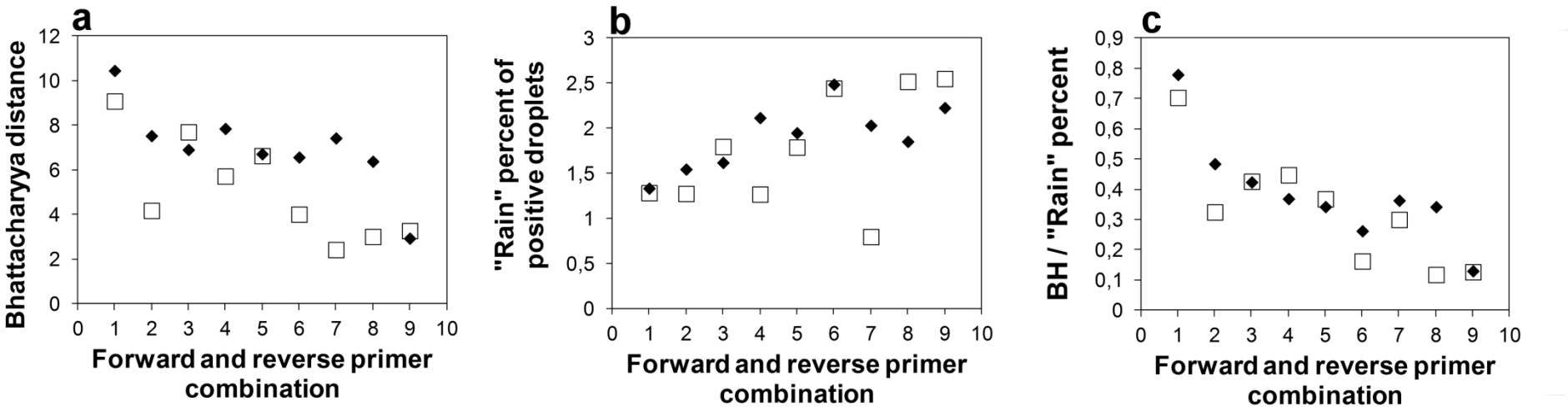

Fig. S2 Bhattacharyya distances, percentages of droplets with intermediate fluorescence ("rain"), and their ratio (as indicated), as influenced by the different primer concentrations. The data are shown for the original primers, as described by Weller *et al.* (2000) [Weller et al., 2000] (squares) and for the primers modified by a 5' flap (diamonds). The primer combinations of forward and reverse were: (1) 50/50, (2) 300/50, (3) 900/50, (4) 50/300, (5) 300/300, (6) 900/300, (7) 50/900, (8) 300/900 and (9) 900/900 (all in nM final concentrations in the ddPCR reaction)