

Supplementary information for “Considerations for droplet digital PCR for environmental samples”

Table S1. Web of Science (Clarivate Analytics 2018) search for environmental studies using ddPCR. For each study, the type of sample used, and the threshold determination method is reported.

Source	Type of sample	Threshold strategy
Kokkoris et al., 2021	Fungal spores	Manual Quantasoft – Based on controls
Fu et al. 2020	Seeds/grains	Auto Quantasoft (1.6.6)
Grudlewska et al. 2020	Sewage/wastewater	Manual Quantasoft – 7000-8000 RFU depending on assay
Suttner et al. 2020	Watershed sediment	Auto Quantasoft (1.7.4)
Rosa et al., 2020	Roots	Manual Quantasoft – Based on controls
Maldonado-González et al., 2020	Soil	Auto Quantasoft - Unspecified
Liu et al., 2020	Soil	Define the rain algorithm
Wen et al., 2020	Soil	Define the rain algorithm
Martínez-Diz et al., 2020	Soil	Auto Quantasoft - Unspecified
Manzari et al., 2020	Bacteria	Manual QuantaSoft - Unspecified
Hansen et al., 2019	Soil	Unspecified
Kokkoris et al., 2019	Soil/plant tissue	Manual Quantasoft – Based on controls
Köppel et al. 2019	Meat tissue	Auto Quantasoft – Unspecified
Cao et al. 2019	Fish muscle tissue	Auto Quantasoft (1.7)
Shehata et al. 2019	Meat	Auto Quantasoft – Unspecified
Cottenet et al. 2019	Plant tissue	Auto Quantasoft (1.7.4)
Deng et al. 2019	Seeds	Manual Quantasoft – Unspecified
Mauvisseau et al. 2019	Water	Manual Quantasoft – 3500 RFU
Giraldo et al. 2019	Plant tissue	Auto Quantasoft – Unspecified
Bahar et al. 2018	Plant tissue	Manual QuantaSoft - High
Cooley et al. 2018	Surface water	Manual – BioRad Rare Mutation Detection guidelines
Gao et al. 2018	Compost	Auto QuantaSoft
Gobert et al. 2018	Faecal	Manual QuantaSoft- Low
Gradoville et al. 2018	Seawater	Three standard deviations above average NTC RFU
Grelewska-Nowotko et al. 2018	Plant tissue	Manual QuantaSoft - Unspecified
Hamaguchi et al. 2018	Coastal sediment	Auto QuantaSoft
Köppel et al. 2018	Plant and animal tissue	Auto QuantaSoft
Naaum et al. 2018	Meat products	Auto QuantaSoft
Paternò et al. 2018	Wheat	Manual QuantaSoft - Unspecified
Paquette et al. 2018	Faecal	Auto QuantaSoft

Steele <i>et al.</i> 2018	Water	Manual QuantaSoft - Low
Selvaraj <i>et al.</i> 2018	Plant tissue	Auto QuantaSoft
Wang <i>et al.</i> 2018	Meat products	Auto QuantaSoft
Zhong <i>et al.</i> 2018	Plant tissue	Auto QuantaSoft
Boynnton <i>et al.</i> 2017	Leaf tissue	Manual QuantaSoft - Unspecified
van Dorssen <i>et al.</i> 2017	Faecal	Auto QuantaSoft
Hunter <i>et al.</i> 2017	Pond water	Manual QuantaSoft - Unspecified
King <i>et al.</i> 2017	Insect tissue	Manual - Low
Košir <i>et al.</i> 2017	Plant tissue	Manual QuantaSoft – 1600-18500 RFU depending on assay
Lee <i>et al.</i> 2017	Seawater	Auto QuantaSoft
Maheshwari <i>et al.</i> 2017	Plant tissue	Auto QuantaSoft
McMahon <i>et al.</i> 2017	Beef, pork, plant tissue	Auto QuantaSoft
Perez-Lopez <i>et al.</i> 2017	Plant tissue	Auto QuantaSoft
Perry and Lee 2017	Wheat	RainDrop Sense
Ren <i>et al.</i> 2017	Meat	Auto QuantaSoft
Shehata <i>et al.</i> 2017	Meat	Auto QuantaSoft
Singh <i>et al.</i> 2017	River sediment	Auto QuantaSoft
Yang <i>et al.</i> 2017b	Wastewater	Auto QuantaSoft
Yang <i>et al.</i> 2017c	Faecal	Auto QuantaSoft
Zink <i>et al.</i> 2017	Insect tissue	definetherain
Bucher and Köppel 2016	Rice	Auto QuantaSoft
Cavé <i>et al.</i> 2016	Soils and organic residues	Auto QuantaSoft
Cremonesi <i>et al.</i> 2016	Cheese	Auto QuantaSoft
Demeke <i>et al.</i> 2016	Plant tissue	RainDrop
Dobnik <i>et al.</i> 2016	Corn	Manual - Mid
Gao <i>et al.</i> 2016	Potato	Auto QuantaSoft
Iwobi <i>et al.</i> 2016	Plant tissue	Auto QuantaSoft
Jerde <i>et al.</i> 2016	Water	Unspecified
Lievens <i>et al.</i> 2016	Plant tissue	Auto QuantaSoft
Palumbo <i>et al.</i> 2016a	Soils	Unspecified
Palumbo <i>et al.</i> 2016b	Grapes	Auto QuantaSoft
Porcellato <i>et al.</i> 2016	Milk	Manual QuantaSoft - 2000-2200 RFU
Simmons <i>et al.</i> 2016	Water	Auto QuantaSoft
Zhao <i>et al.</i> 2016	Plant tissue	Manual - Unspecified
Cao <i>et al.</i> 2015	Water and Faecal	Auto QuantaSoft
Doi <i>et al.</i> 2015a	Water	Manual QuantaSoft - Unspecified
Doi <i>et al.</i> 2015b	Water	Manual QuantaSoft – 1100 RFU
Te <i>et al.</i> 2015	Water	Manual QuantaSoft - 9000 or 5400 RFU depending on assay
Kim <i>et al.</i> 2014	Soil	Auto QuantaSoft

Nathan <i>et al.</i> 2014	Water	Manual QuantaSoft - Unspecified
Racki <i>et al.</i> 201	Plant tissue	Auto QuantaSoft or manual at 7000 RFU
Yang <i>et al.</i> 2014	Faecal	Auto QuantaSoft
Kelley <i>et al.</i> 2013	Nostril swab	Auto QuantaSoft
Morisset <i>et al.</i> 2013	Corn	Manual QuantaSoft- Unclear
Roberts <i>et al.</i> 2013	Eye swabs	Custom Perl and R scripts

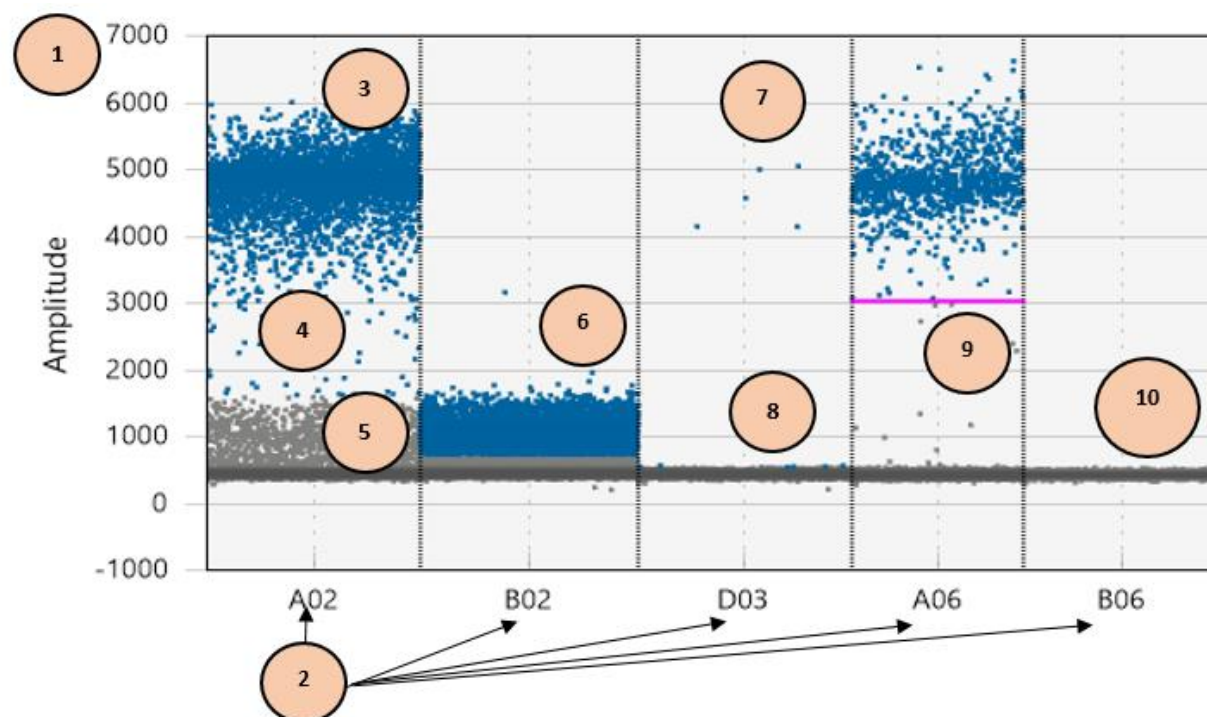


Figure S1. A key to interpreting ddPCR output figures. 1) Y axis represents the number of droplets amplified as a reflection of their fluorescence. Higher amplitude levels can be achieved with optimized cycling PCR protocol and optimized PCR reaction protocol. Higher amplitude levels result in improved cloud separation 2) X axis represents individual samples, in this example five individual samples are presented (A02, B02, D03, A06 and B06) and are named based on their location on the 96 well plate. 3) The cluster of high amplitude droplets (amplitude 4000 – 6000) is called “positive cloud” and represents positive to the target DNA droplets. 4 & 5) droplets between the “negative cloud” (see 10) and the “positive cloud” are called “rain” and may be negative or positive droplets. 6) Blue droplets are recognized as positive droplets from the automatic threshold determination system of the QuantaSoft software. While sample A02 and B02 are from a single ddPCR run, the automatic threshold treats each sample distinctly placing the “rain” as negative or “positive” respectively. 7) Low number of positive to the target DNA droplets. 8) Clean “negative cloud” 9) The pink line represents the manually set threshold. Above the line all droplets are considered as positive while below the line all droplets are considered as negative. 10) This sample represents a non-template control sample where there is no rain and no positive droplets. The negative cloud droplets cluster closely together creating a thick line at low amplitude. In theory, droplets that appear above this cloud in samples other than non-template controls can be potentially positive.

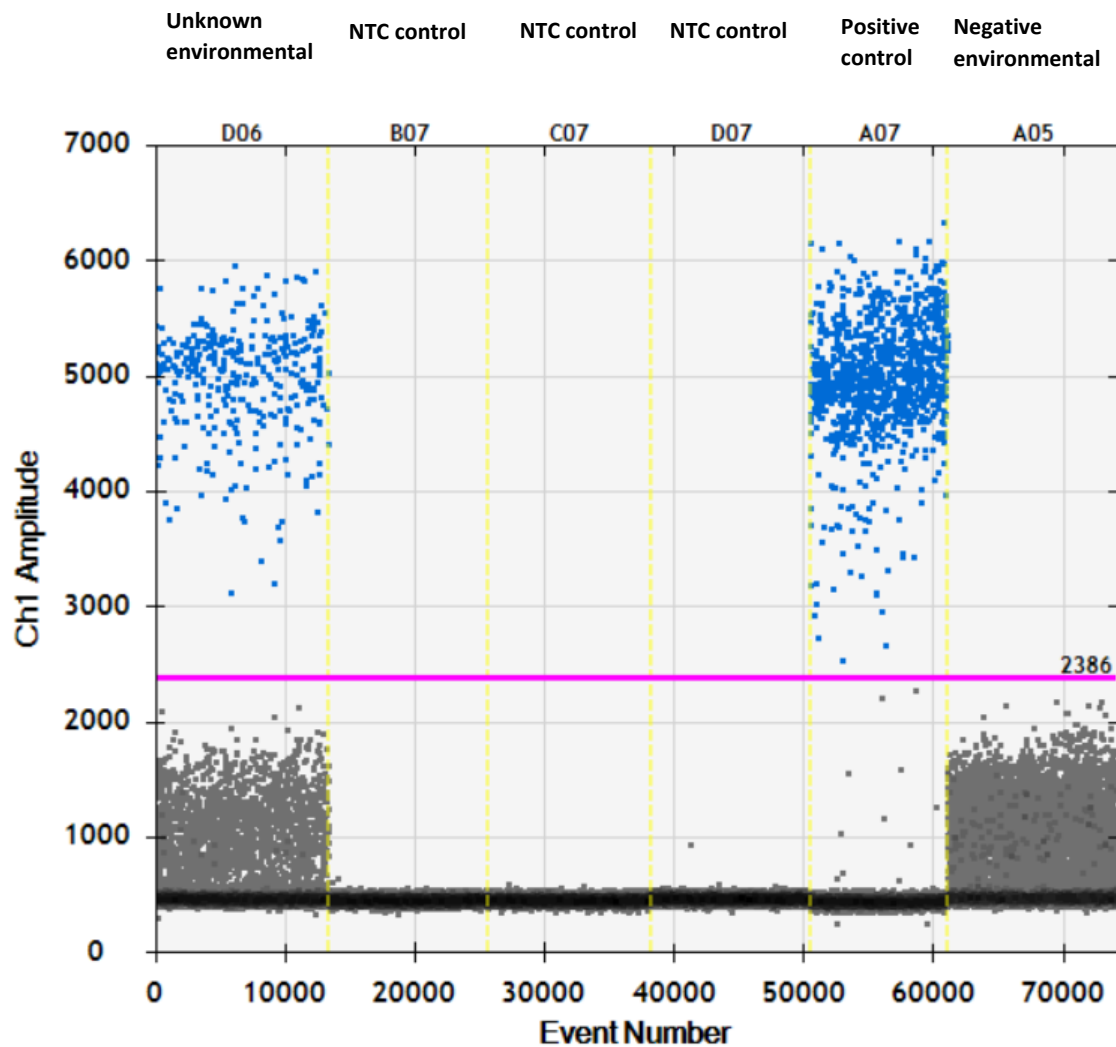


Figure S2. Manual threshold determination. In order to determine the quantity of target DNA in an unknown environmental sample (well D06) we used three NTCs (wells B07, C07, D07), an organismal positive control (well A07) and a negative environmental sample (well A05). The threshold (purple line) was set manually at amplitude of 2386 to include the positive droplet cloud produced by the organismal positive control but to exclude the droplets produced by the

negative environmental samples. Blue droplets represent positive droplets while grey droplets represent negative droplets. Separation of negative from positive cloud was achieved by setting the threshold manually at “2386” amplitude.

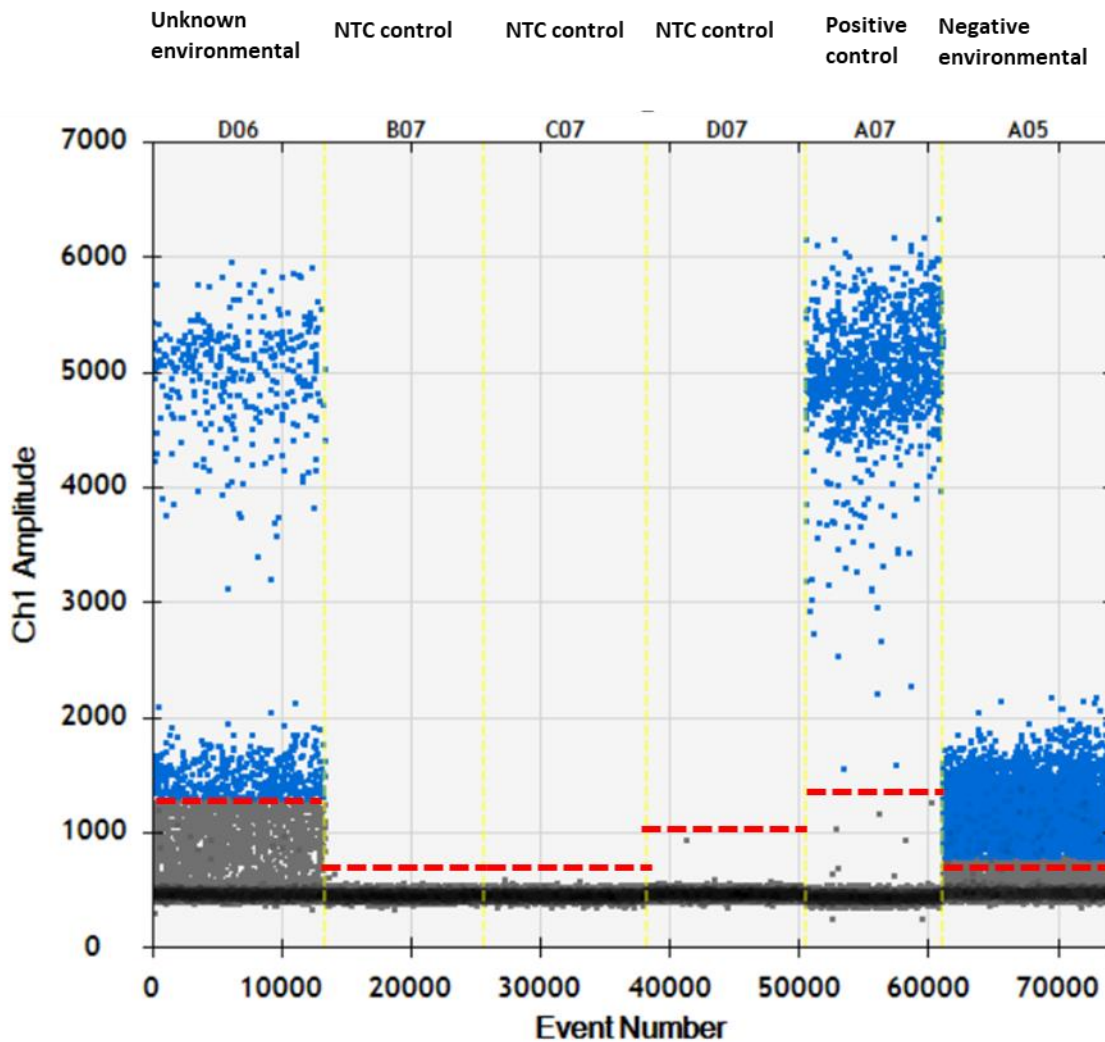
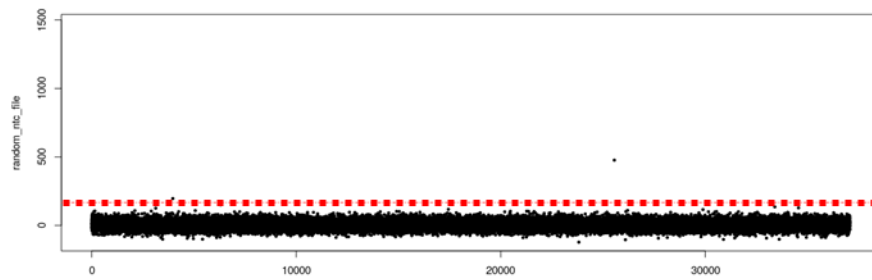
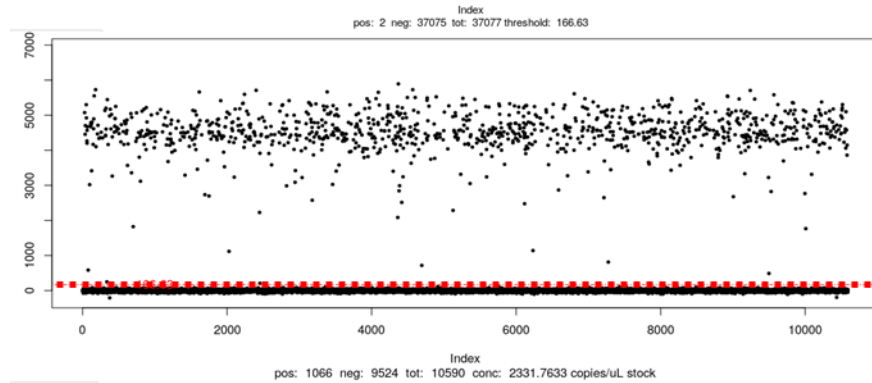


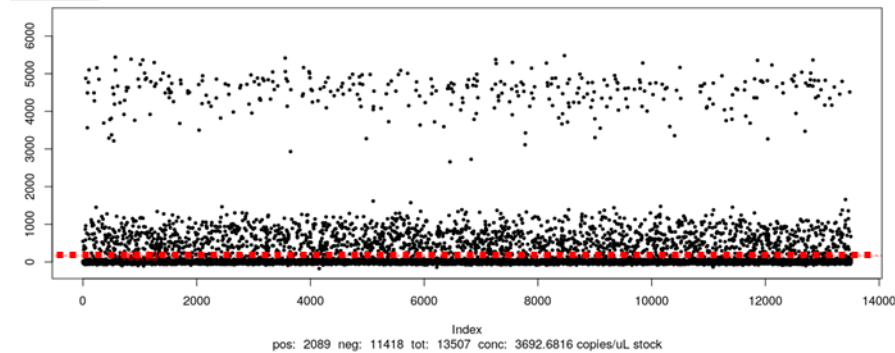
Figure S3. Automatically determined threshold using the QuantaSoft® software. Threshold was variable for each sample. The QuantaSoft® software algorithm failed to discriminate between true positive and negative droplets. Blue droplets represent positive droplets while grey droplets represent negative droplets.



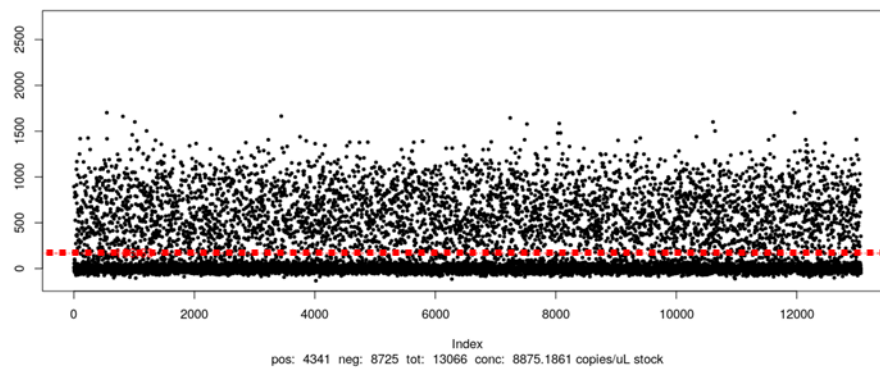
NTC control



Positive control



Unknown
environmental



Negative
environmental

Figure S4. Automatically determined threshold using the 'ddPCRquant' algorithm. The 'ddPCRquant' algorithm merges the three NTC samples and calculates the threshold based on the dense negative droplet cloud. The threshold was set automatically at "166.63" amplitude

(red line in each sample). All samples above this value were considered erroneously as positive, including the negative environmental sample.

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