Supplementary information

Determination of droplet size and coefficient of variation (CV)

In order to assess the droplet size, its CV and the droplet volume, the droplet production was imaged in a LabDisk Player equipped with a stroboscopic setup[1]. With this device, an image of the disk is taken each time it passes the camera (more precisely a mirror that reflects the light sideways into the objective of a camera). Image sequences of droplet generation were taken at high magnification. Afterwards, the images of 30 single free floating droplets were copied and pasted into another image file. Droplets from the beginning, middle and end of the experiments were included. An automated image recognition routine using Matlab 2013 (Mathworks, Natick, USA) was performed. The routine automatically detects and measures circles in the picture. This was done to exclude manual bias. The volume of the droplet was calculated from its diameter assuming a spherical shape (which can be seen at low spinning frequencies up to ~40 Hz). It should be noted, that it is difficult to measure the absolute size of the droplets, as the image recognition might over- or underestimate the diameter of the droplet. The relative droplet size, however, can be determined precisely which is very important to calculate the CV.

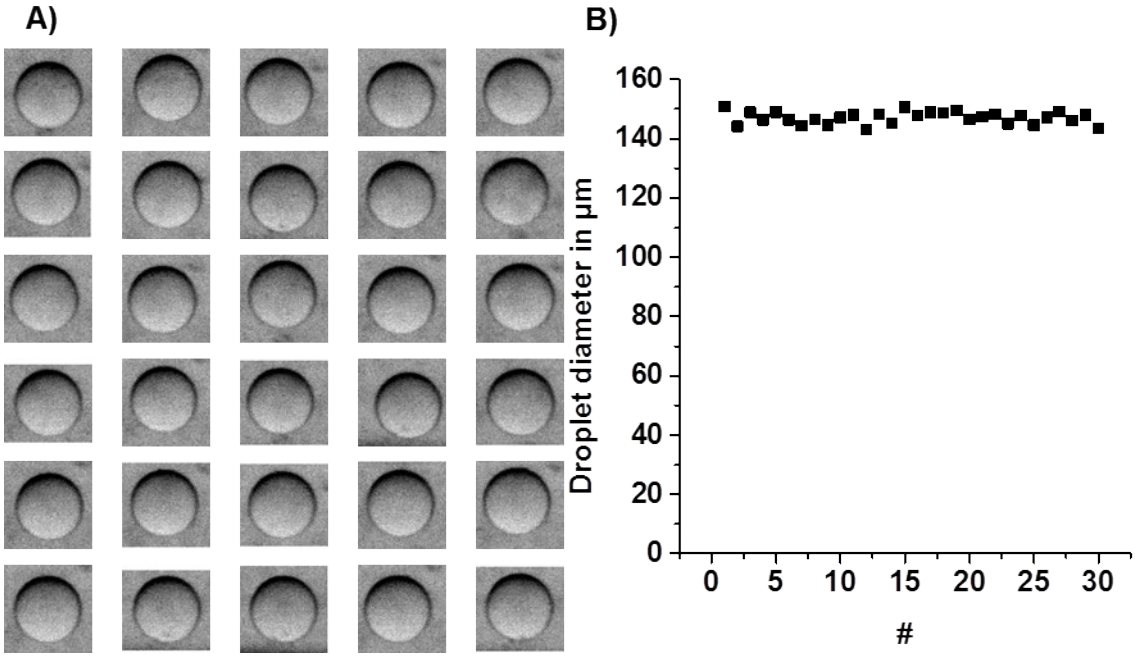

Fig. S 1 A) Cropped images of 30 droplets that were produced with the ddPCR system. B) Diameter of the droplets, average diameter is 147 µm with as CV of 1.4%.

Verification of ddPCR system performance using pTYB1 vector as target DNA

In order to verify the performance of the ddPCR system a ddPCR using pTYB1 as a target was performed.

Sequences of primers, probes and DNA all in 5'→3' direction

Forward primer: AATCCTGGTGTATCCGCTTG (Biomers.net GmbH, Ulm, Germany)

Reverse primer: AGGTGTGGGGCTGCAA (Biomers.net GmbH, Ulm, Germany)

Probe: Atto647N- AGCTTATACTGCGGGACAATTGGTCAC-BBQ650 (IBA GmbH, Goettingen, Germany)

Template DNA was purchased from NEB (#N6701S, 0.2 mg/ml). A dilution series of target DNA was prepared using LoBind tubes (Eppendorf, Hamburg, Germany) and dilution buffer containing 0.2x TE buffer and 10 ng/µl herring sperm DNA. DNAse/RNAse free water (Thermo Fisher Scientific, Waltham, US) was used for the preparation of the buffers. Mastermix, cycling conditions and imaging routine were the same as mentioned in the main paper. The concentration of spiked in DNA was derived from the values given on the tube.

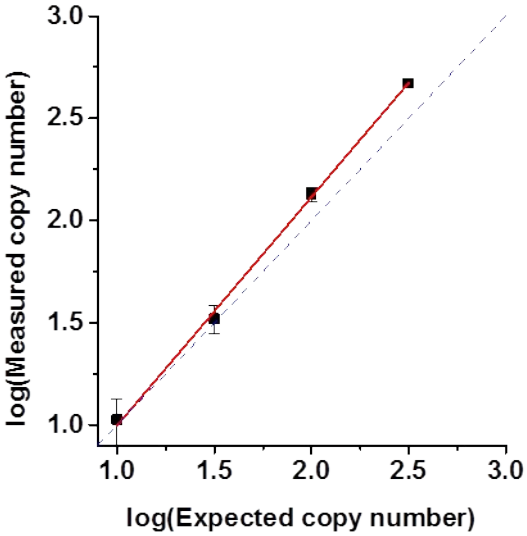

Fig. S 2 ddPCR using pTYB1 vector as a target. Measured copy numbers corrected by Poisson statistics and are depicted on the y-axis. The expected copy numbers (on the x-axis) were taken from the values of DNA upon delivery.

qPCR experiments for estimation of copy numbers of p.Phe508del DNA

A real-time PCR of a dilution series of known standards was performed as a reference for quantification of the DNA sample used for ddPCR. The real-time traces can be seen in Fig. S 3. Another real-time PCR of the DNA sample used for ddPCR was performed. The concentration in the sample was estimated from the C_q-value of the sample that was compared to the C_q values of the calibration curve.

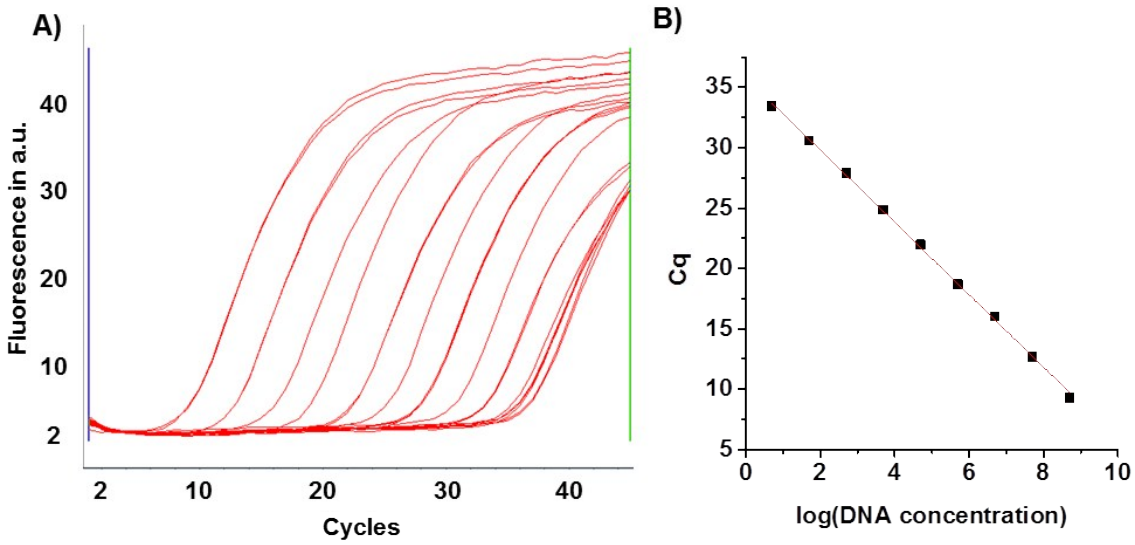

Fig. S 3 A) Real-time traces of calibration run for quantification of the DNA sample that was subsequently used for ddPCR. B) Calibration curve extracted from the real-time data.

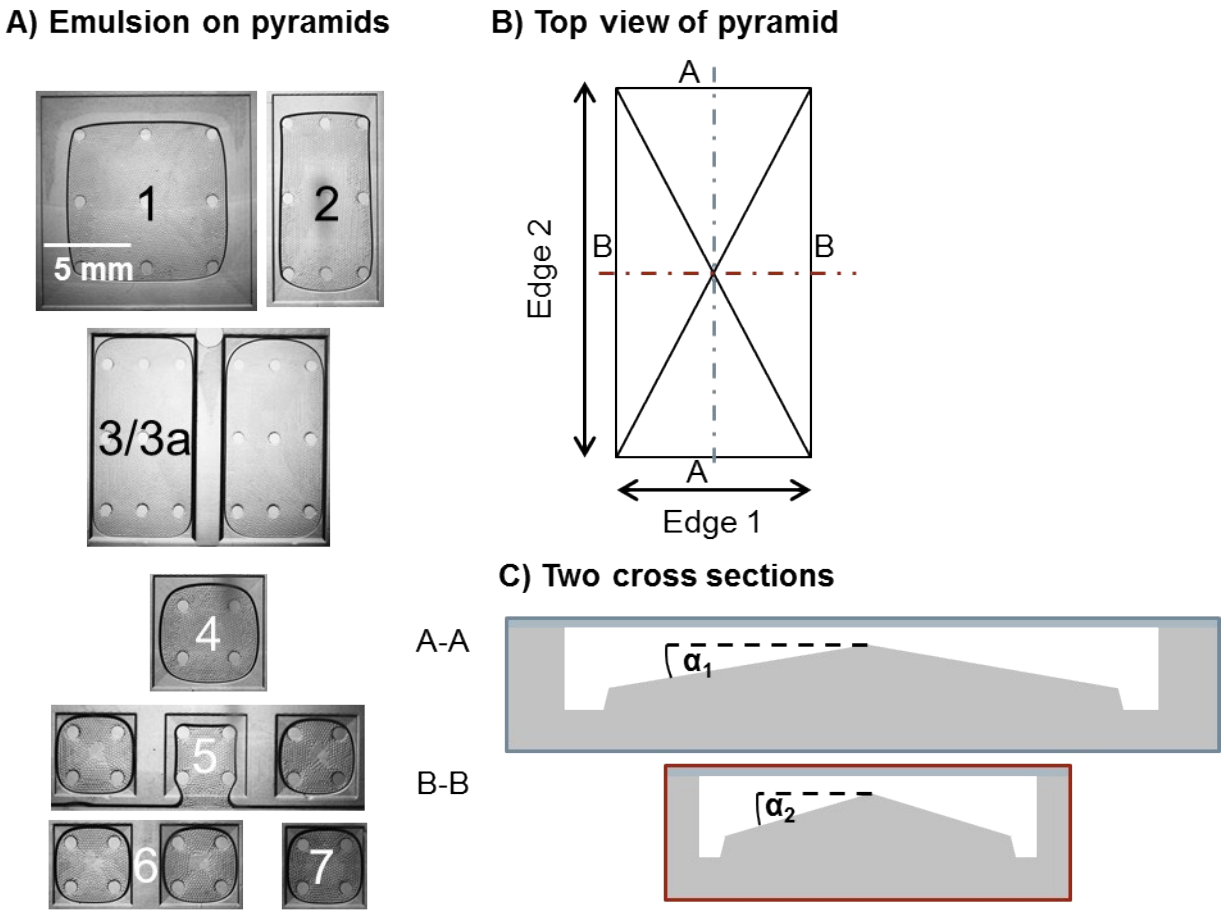

Fig. S 4 A) Microscopic image of different pyramidal structures that were tested. The white or black number indicates the number of the structure, for detailed measurements see Table S1. For all structures emulsion was produced and wicked up to the pyramids. B) Top view of the pyramid only. Dashed lines show position of cross sections. C) Cross sections along two planes of the pyramid. Blue structure on top is sealing foil.

The detailed measurements of the structures depicted in Fig. S 1 are given in Table S 1. For all structures the depth at the tip of the pyramid d_0 (compare Fig 1B), i.e. the distance between sealing foil and tip of the pyramid, was 180 µm. The channel was 60 µm deep and 110 µm wide. The terrace was 100 µm wide.

Table S 1 Measurement of structures. Structure 3 was produced in two different variations, 3 and 3a with varying angles α.

No.	α_1 in °	α_2 in °	Edge 1 in mm	Edge 2 in mm	# of pyramids
1	2	2	12.6	12.6	1
2	2	4	6.3	12.6	1
3	2	4	6.3	12.6	2
3a	1	2	6.3	12.6	2
4	4	4	6.3	6.3	1
5	6	6	4.2	4.2	3
6	6	6	4.2	4.2	2
7	6	6	4.2	4.2	1

Measurements of fluorescence intensity of droplets

As discussed in the main paper, regions of interest were used to measure the fluorescence intensity of the droplets. As can be seen in Fig. S 5, two populations of droplets can be clearly distinguished. However, the fluorescence intensity of very few droplets is in between the two populations. This is a common finding in ddPCR and most likely due to the fact, that in some droplets, the amplification reaction was interrupted in a relatively early stage.

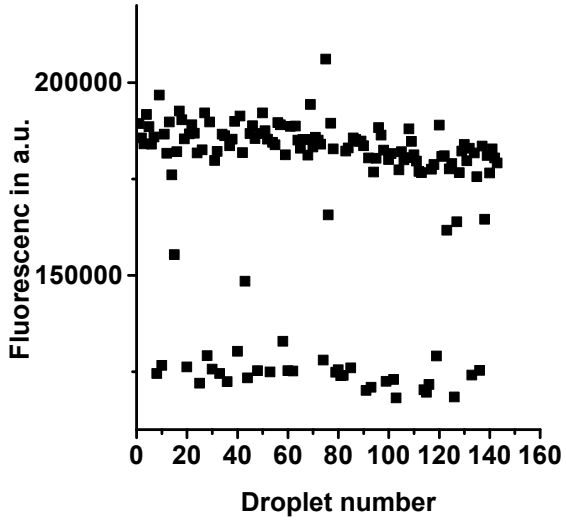

Fig. S 5 Fluorescence intensity of a part of the droplet population.

References

1 M. Grumann, T. Brenner, C. Beer, R. Zengerle and J. Ducrée, *Rev. Sci. Instrum.*, 2005, **76**, 025101.