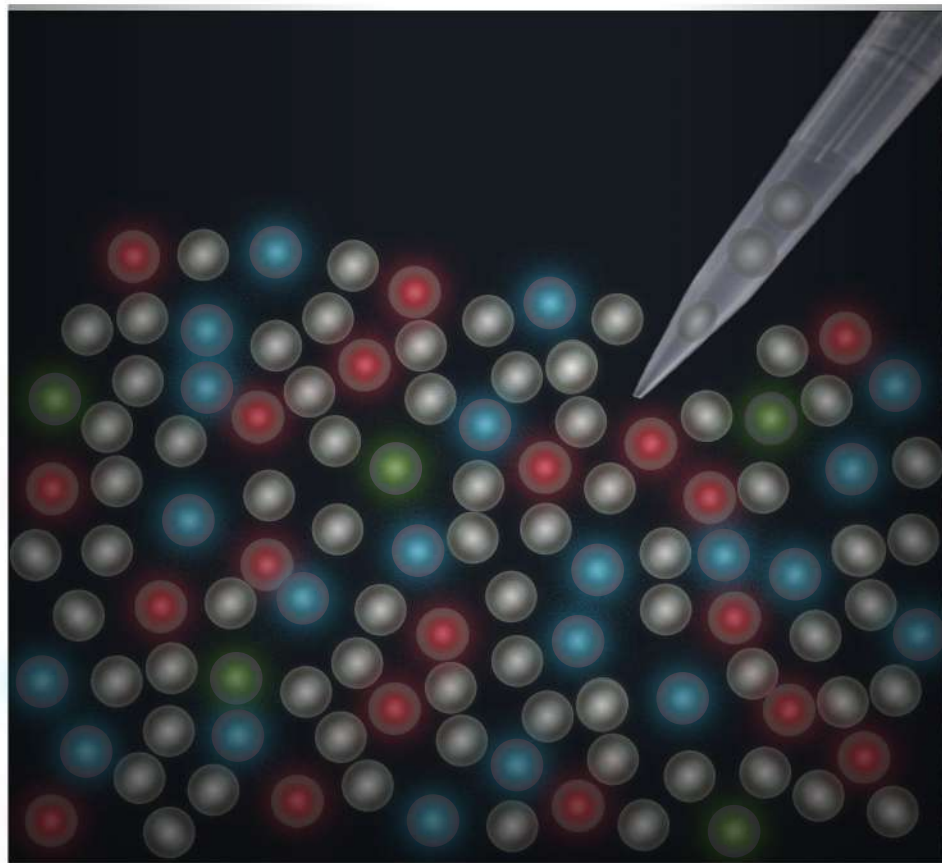




Droplet Recovery

With Crystal Digital™ PCR



Recovering droplets from Sapphire Chips

The Naica System allows the recovery of droplets from the Sapphire chips with a simple procedure that can be performed before or after PCR. DNA contained in the recovered emulsion is extracted using a standard chloroform protocol and is suitable for downstream analysis using NGS, digital or real-time PCR, or gel electrophoresis. The recovery and extraction procedure takes approximately 1h for up to 24 samples (Fig.1).

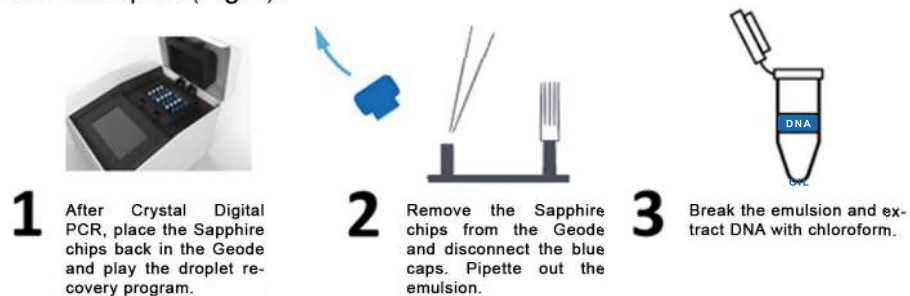


Fig. 1. Overview of protocol for droplet recovery from Sapphire Chips.

DNA Recovery Rate

The recovery rate for this protocol was assessed by generating a droplet crystal containing known amounts of human genomic DNA, counting droplets in the Sapphire Chips before and after recovery, extracting DNA from droplets using the protocol described above, and quantifying the amount of DNA recovered by Crystal Digital PCR. We found that:

- at least 98% of the droplets are recovered from the Sapphire Chips
- on average, 70% of the total DNA present in the initial droplet crystal is recovered (Fig. 2).

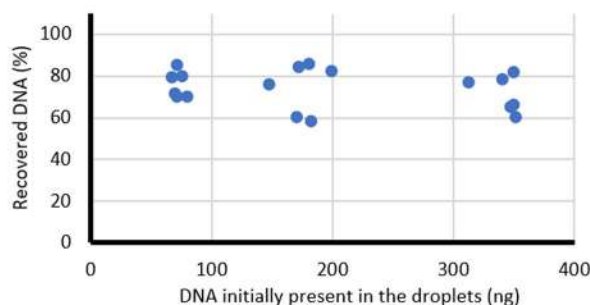


Fig. 2. Graph of the percentage of DNA recovered from droplet crystals vs the amount of total DNA initially present in the droplet crystal (ng). Samples were assayed in duplicate, N=3.

Is it possible to recover the amplicons from a single positive droplet? Yes.

We next evaluated the recovery of amplicons from droplet crystals having a low number of positive droplets. The ALB or BRAF genes were amplified by Crystal Digital PCR using low concentrations of human genomic DNA, in order to generate only a few positive droplets to be recovered. We found that amplicons of the targeted DNA are recovered from all samples where the crystals contained at least 1 positive droplet. No targeted DNA is detected in crystals recovered from NTCs (Table 1).

Number of positive droplets in chamber	0 (NTC)	1	2	3	4
Number of chambers with DNA recovered/Total number of chambers tested	0/3	4/4	9/9	4/4	3/3

Table 1. Amplicon recovery from chambers containing a low number of positive droplets.

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