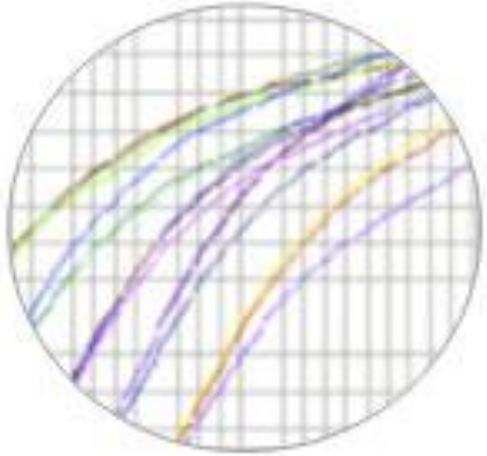
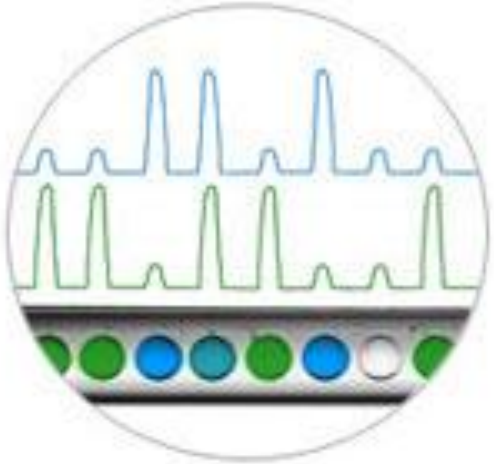
Digital PCR: l'evoluzione della PCR

PCR
Qualitative



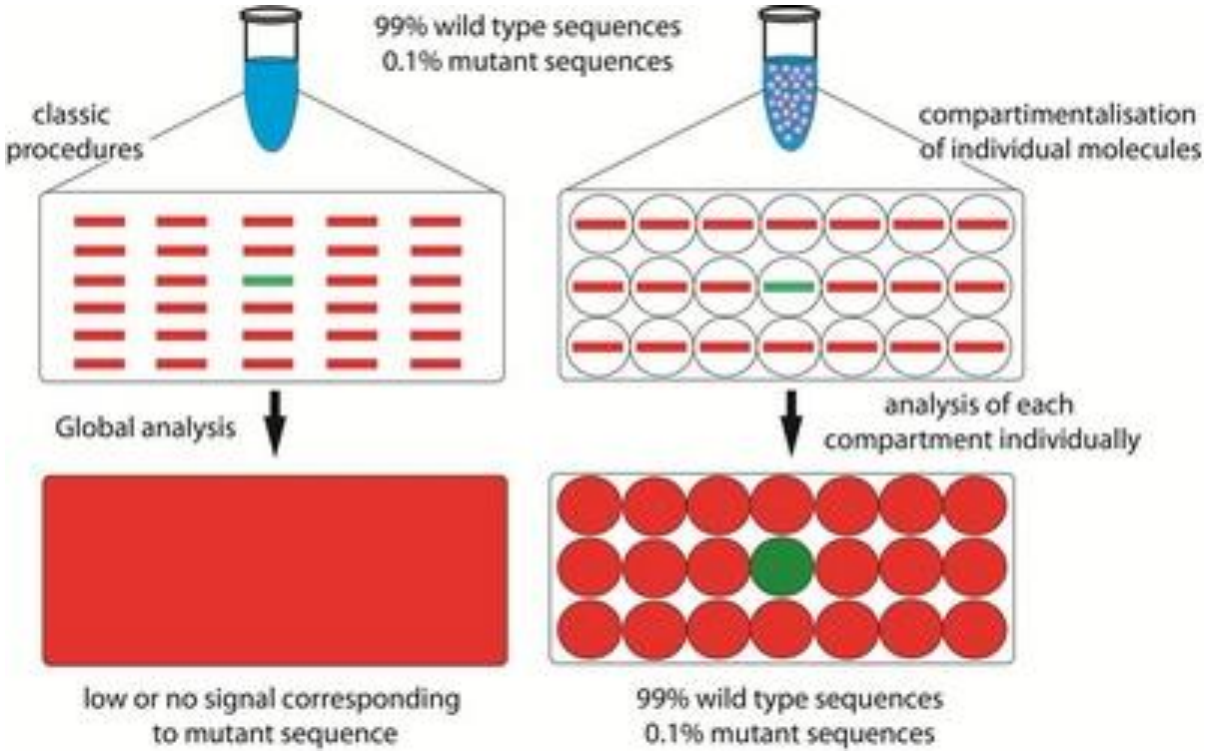
Real-Time PCR
Relative Quantitation



Droplet Digital PCR
Absolute Quantitation

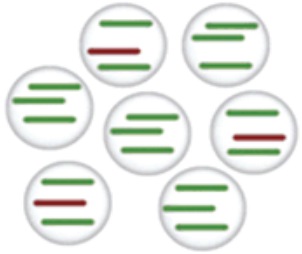
https://www.youtube.com/watch?v=WU3qKhIUc54

Digital PCR: l'evoluzione della PCR



I target rari (es. una variante mutata) non possono essere amplificati mediante PCR a causa della competizione operata del DNA più rappresentato per i reagenti (primers etc…)

Real time PCR *vs*. digital PCR

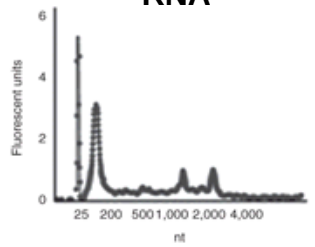


Microvescicole nel siero

1. RNA extraction

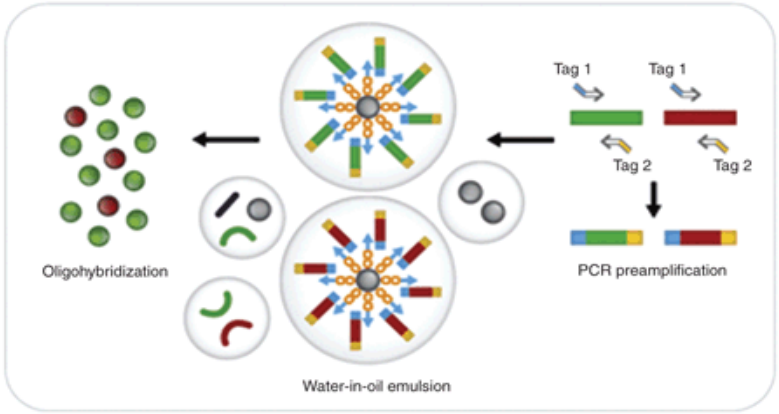
2. RNA analysis

RNA

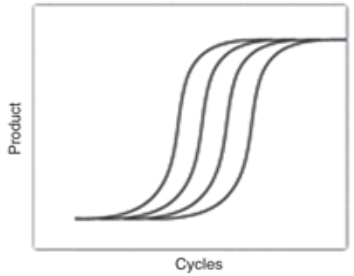


cDNA

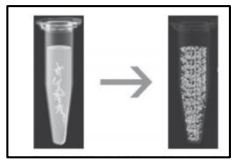
5. BEAMing



qPCR

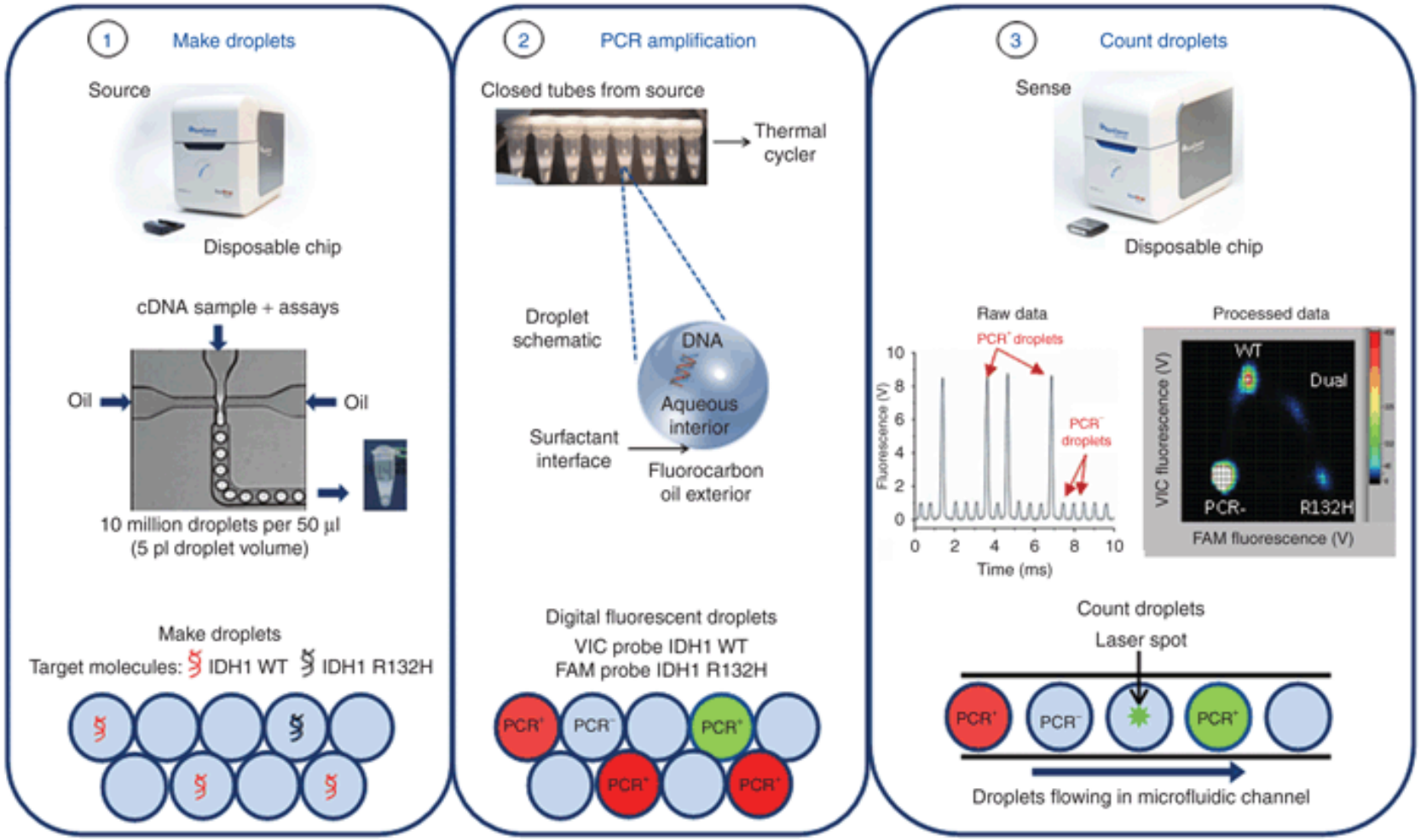


In **ddPCR** (droplet digital PCR) a sigle sample is partitioned in a 20.000 droplets

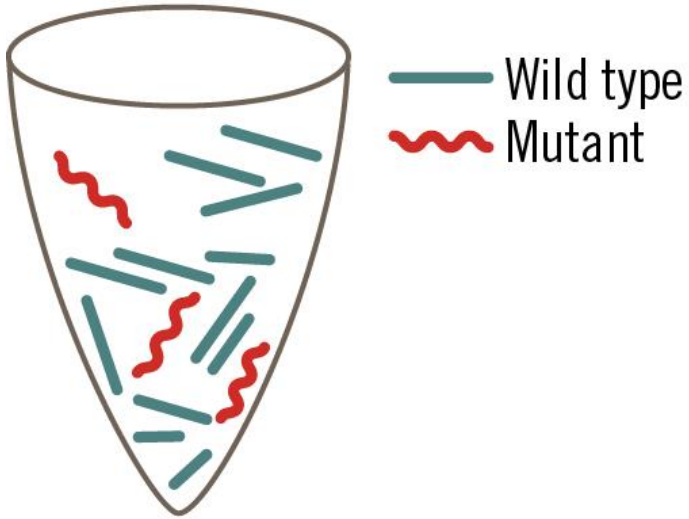


Droplet digital PCR system

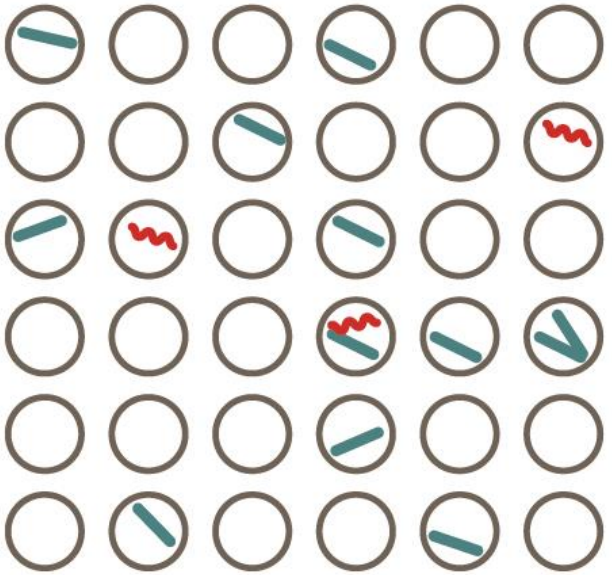
Droplet digital PCR system



Dropled digital PCR: emulsion



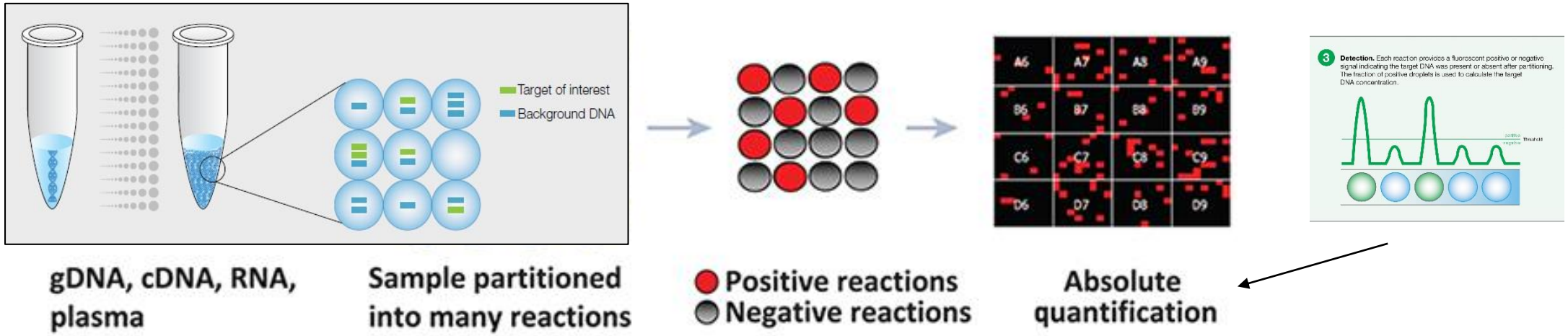
Source: (Sedlaka & Keith, 2013).[10]



Partition reagents and sample into 20.000 droplets

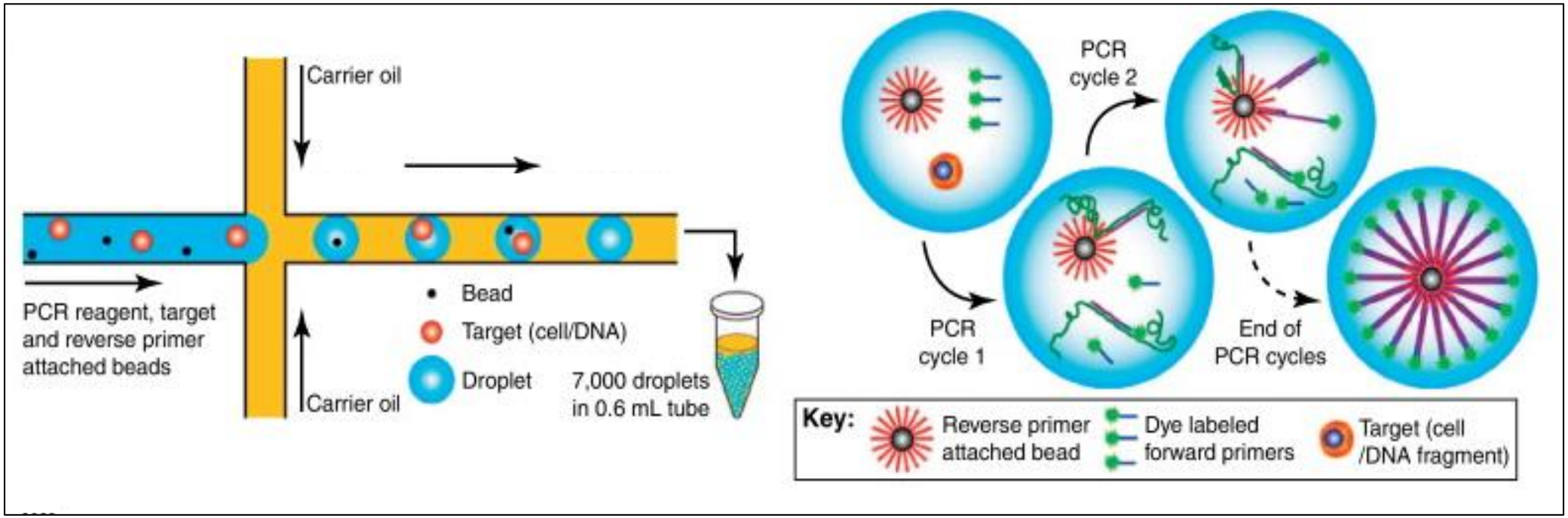
Dropled digital PCR

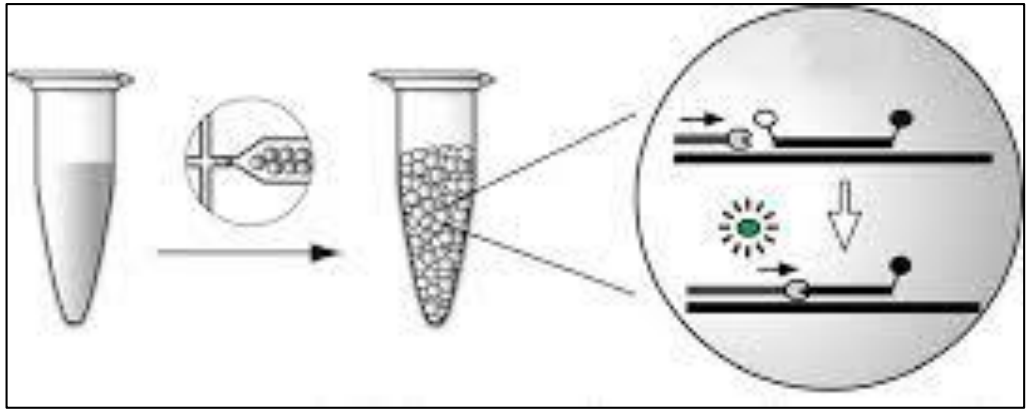
Dropled digital PCR is an analytical technique for quantification of nucleic acid samples based on **PCR amplification of single template molecules**



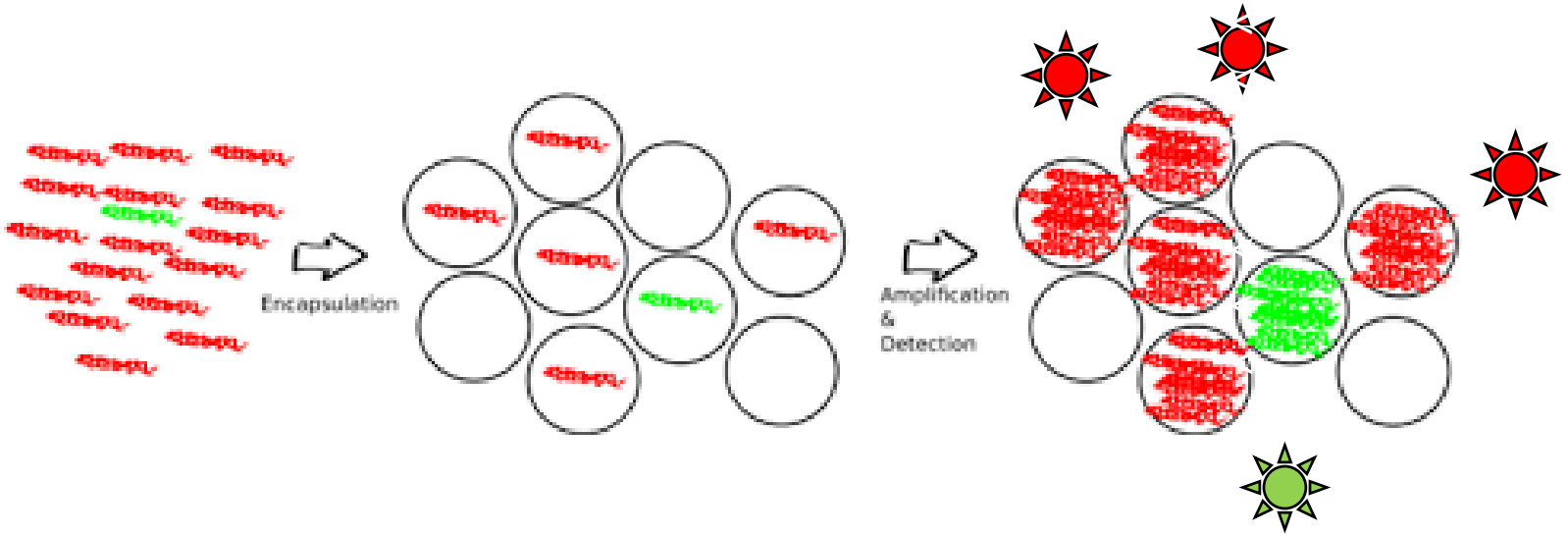
- **Partition reagents and sample into droplets**
- Perform PCR on thermal cycler
- **Count droplets** with a **positive** PCR product (fluorescent) **and a negative** PCR product
- Digital readout provides **concentration of target DNA**

Making droplets

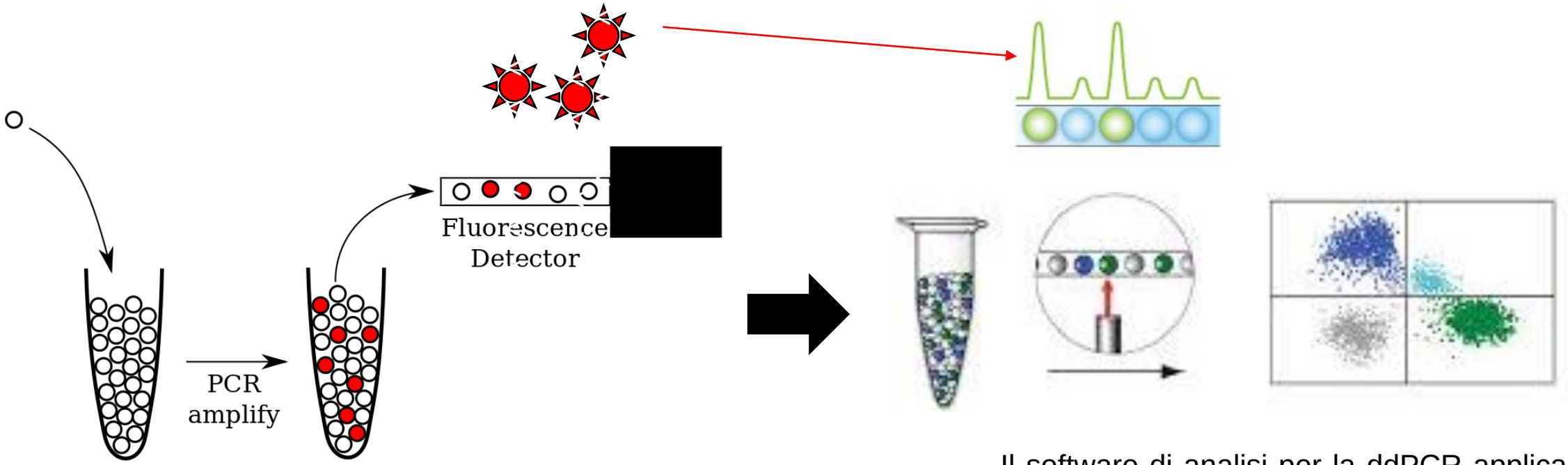




qPCR reaction in the drop



Read droplets



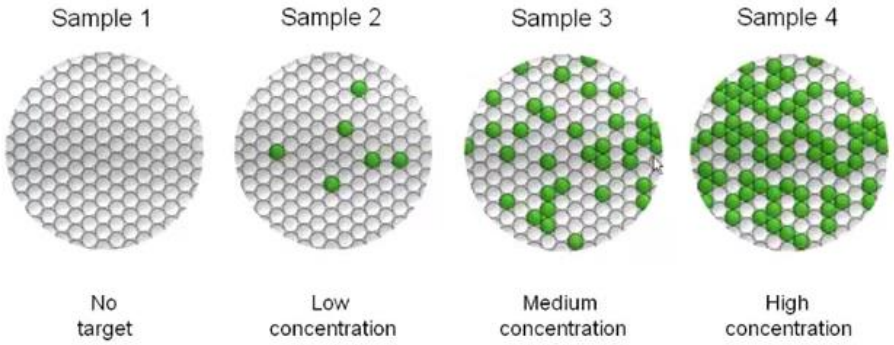
Il software di analisi per la ddPCR applica al dato ottenuto il calcolo statistico della distribuzione di Poisson ed esprime il risultato in **numero di copie di templato per uL di reazione** (copie/uL), considerando che il volume di reazione che è pari a 20 uL

Stima della concentrazione del DNA d'interesse

Vi è una distribuzione casuale (di eventi indipendenti) delle molecole di DNA (da analizzare) nelle gocce, queste vengono ripartite casualmente durante l'emulsione del campione. **Producendo un numero sufficientemente elevato di gocce avremo la probabilità queste conterranno una sola molecola di DNA**.

Il numero di gocce contenenti una molecola di DNA dipenderà dalla **concentrazione di DNA** del campione

Siméon Denis Poisson
(1781-1840)



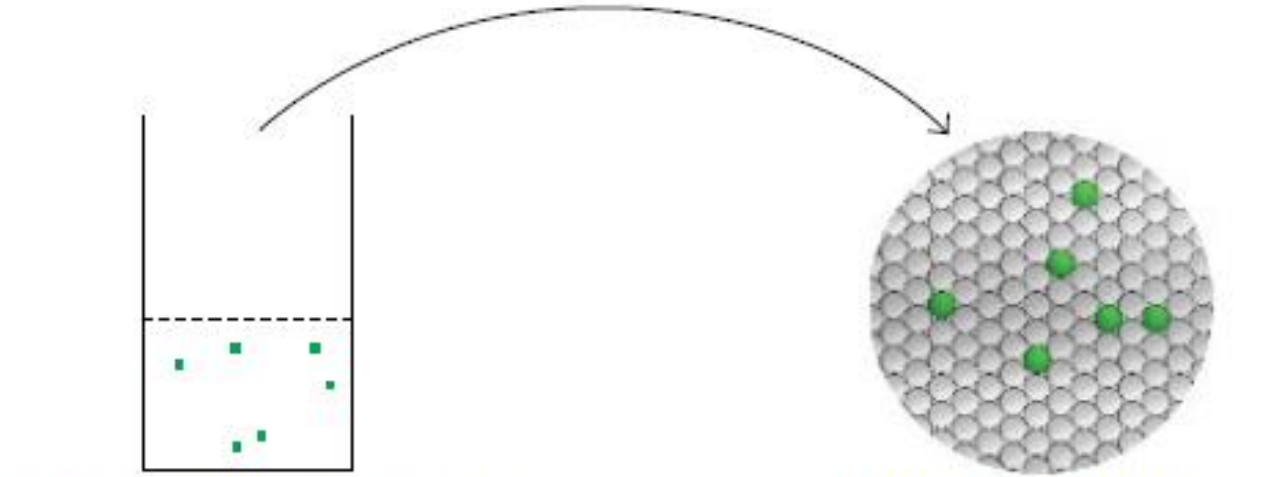
Poisson equation:

$$-\ln\left(1-\frac{\text{positives}}{\text{total counted}}\right)$$

(positives + negatives)

Legge dei piccoli numeri o degli eventi rari

Stima della concentrazione del DNA d'interesse



For 20,000 droplets, the following concentration calculation is used (assumes an approximate droplet volume of 1 nl or 10^{-3} µl).

Volume analyzed = 20,000 x 10^{-3} µl/droplet = 20 µl

$$\text{Concentration} = \frac{\text{Copies of target}}{\text{Volume analyzed}} = \frac{6 \text{ copies}}{20 \text{ µl}} = 0.3 \text{ copies/µl}$$

Stima della concentrazione del DNA d'interesse