Real-Time PCR in food analysis

Introduction to a powerful technology

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Objectives

This presentation will cover the following topics:

- What is real-time PCR used for?
- How does real-time PCR work?
- What instruments are used?
- What does real-time data look like?
- How can real-time data be used to calculate quantities of DNA?

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What is PCR?

Any living being (bacteria, plants, animals, viruses, molds, algae) contains DNA

Food and feed are made of living being

The DNA of any living being can be extracted using appropriate methods.

Thus the DNA contained in food and feed can be extracted and purified from the other components using an appropriate method

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What is PCR?

The Polymerase Chain Reaction (PCR) is a process that allows for copying (tech jargon *amplifing*) of specific fragments of DNA (tech jargon *template*).

Thanks to its specificity the reaction copy only these fragments even when they are dispersed in a plethora of other DNA.

These copies are called *amplicons*

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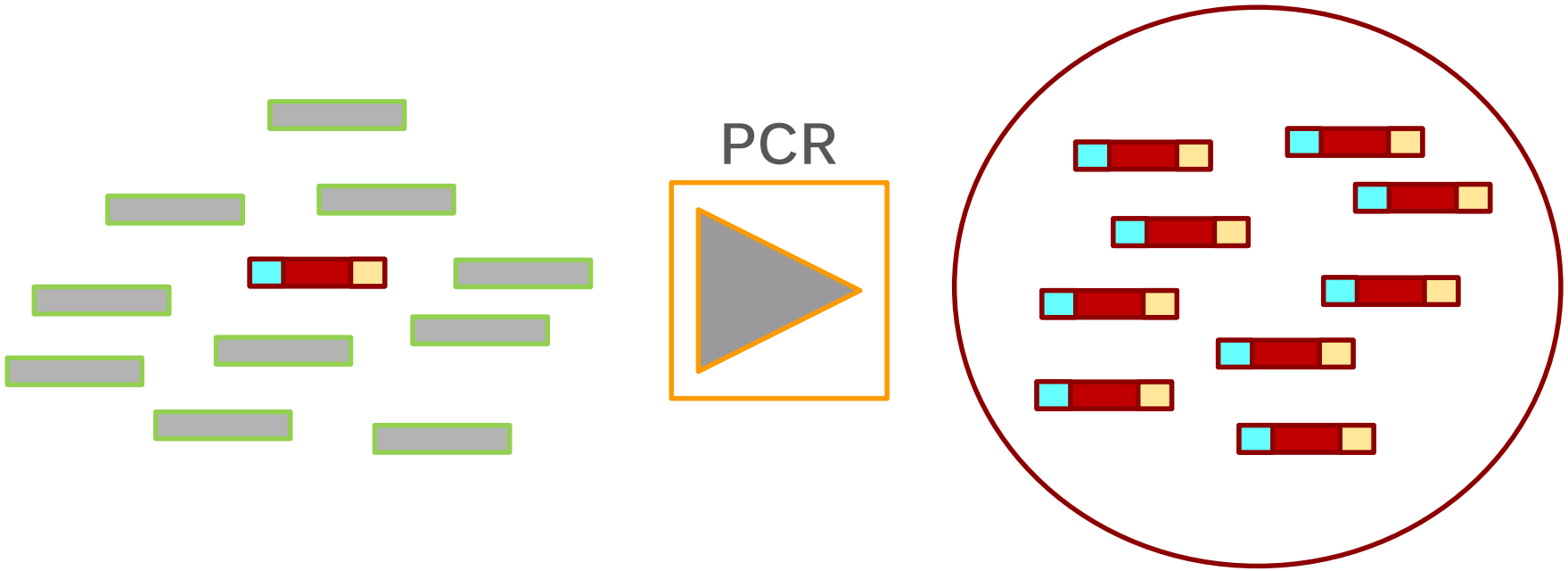
What is PCR?



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What is PCR?

Amplicons can be evidenced through the use of chemical reagents that fluoresce in the presence of DNA

Thus PCR allows for the detection of the DNA of undesired contaminants, even when dispersed in most abundant DNA of a food/feed matrix, through the development of fluorescence detected by an appropriate instrument

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What is Real-Time PCR?

Real-Time PCR (qPCR) is a specialized technique that allows a PCR reaction to be visualized “in real time” as the amplification progresses through an instrument that detects and measure fluorescence

As we will see, Real-Time PCR allows us to measure minute amounts of DNA sequences in a sample

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What is qPCR used for?

qPCR has become a cornerstone of molecular biology in basic research and clinical diagnostics. Just some of the uses include:

- Gene expression analysis (e.g. in cancer and drug research)
- Disease diagnosis and management (e.g. viral load quantification)
- Animal and plant breeding

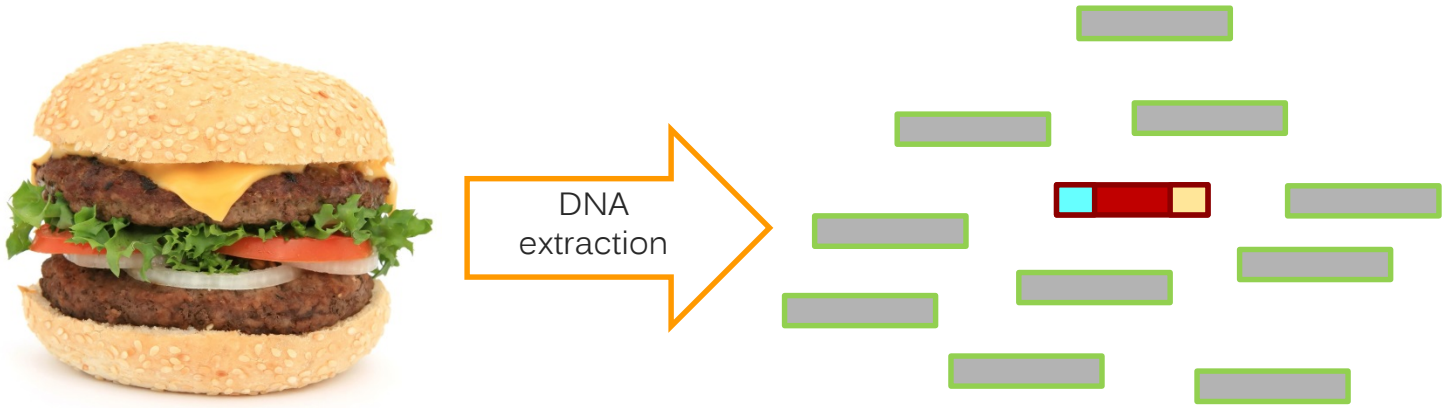
Since a decade qPCR has ben used also in food diagnostics. Just some of the uses include:

- Detection of pathogens and spoilage microorganisms
- Detection and percentage quantification of GMO in food
- Verification of the authenticity of food products
- Detection of the presence of allergens in food products
- Detection of diseases in farmed animals

How does real-time PCR work?

- To best understand what real-time PCR is, let's detail how regular PCR experiment works on a practical case.

Does this hamburger contain soy although non evident?



Soy DNA

Dispersive DNA: wheat, sesame, beef, salad, onion, tomato

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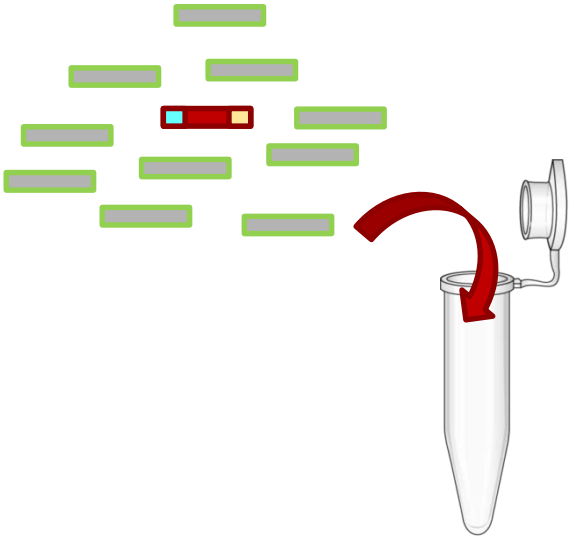
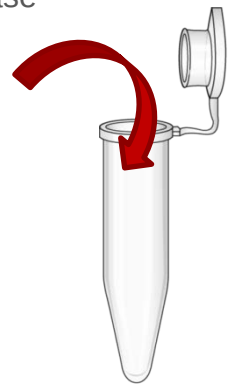
PCR reaction assembly



Add DNA extract
to a sample tube
(*aka PCR tube*)

PCR Reagents:

- Enzyme DNA polymerase
- Primers
- dNTPs
- Buffer



Add PCR
reagents to the
PCR tube

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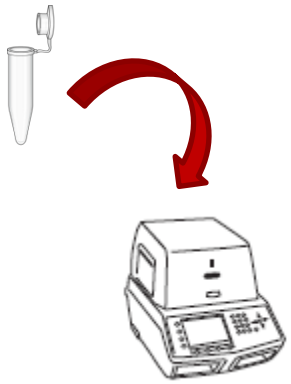
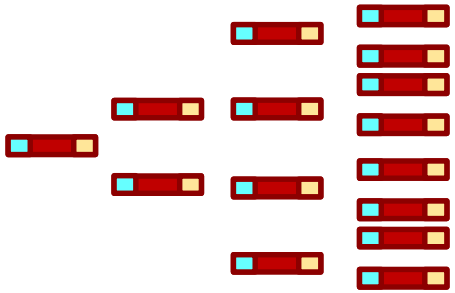
PCR reaction running



Put PCR tube in the PCR machine

Amplification reaction

PCR machine runs **cycles** of heating and cooling of the *reagents in the* PCR tube



During each **cycle** the template is duplicated

At the end of the process (normally 30-40 cycles)
PCR amplified soy DNA sequence

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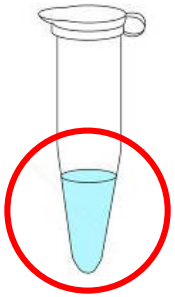
Imagining Real-Time PCR

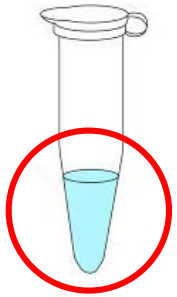
To understand real-time PCR, let's imagine ourselves in a PCR reaction tube at cycles number 23-24-25 when starting from a single copy of the soy template…

Cycle 23



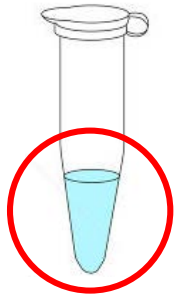
A mix of PCR reagents and 2^{23} copies of the soy template
(8388608 copies)

Cycle 24



A mix of PCR reagents and 2^{24} copies of the soy template
(16777216 copies)

Cycle 25



A mix of PCR reagents and 2^{25} copies of the soy template
(33554432 copies)

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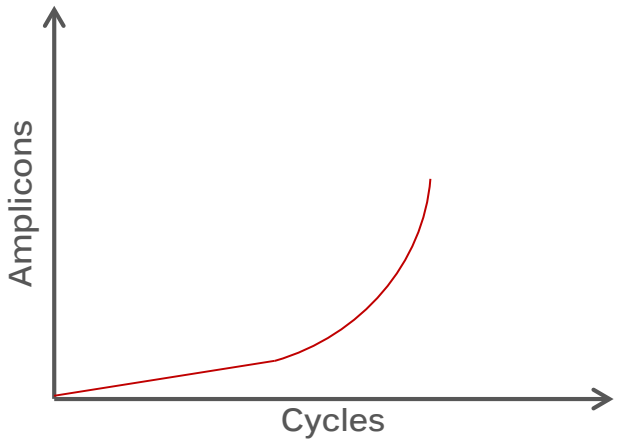
Imagining Real-Time PCR

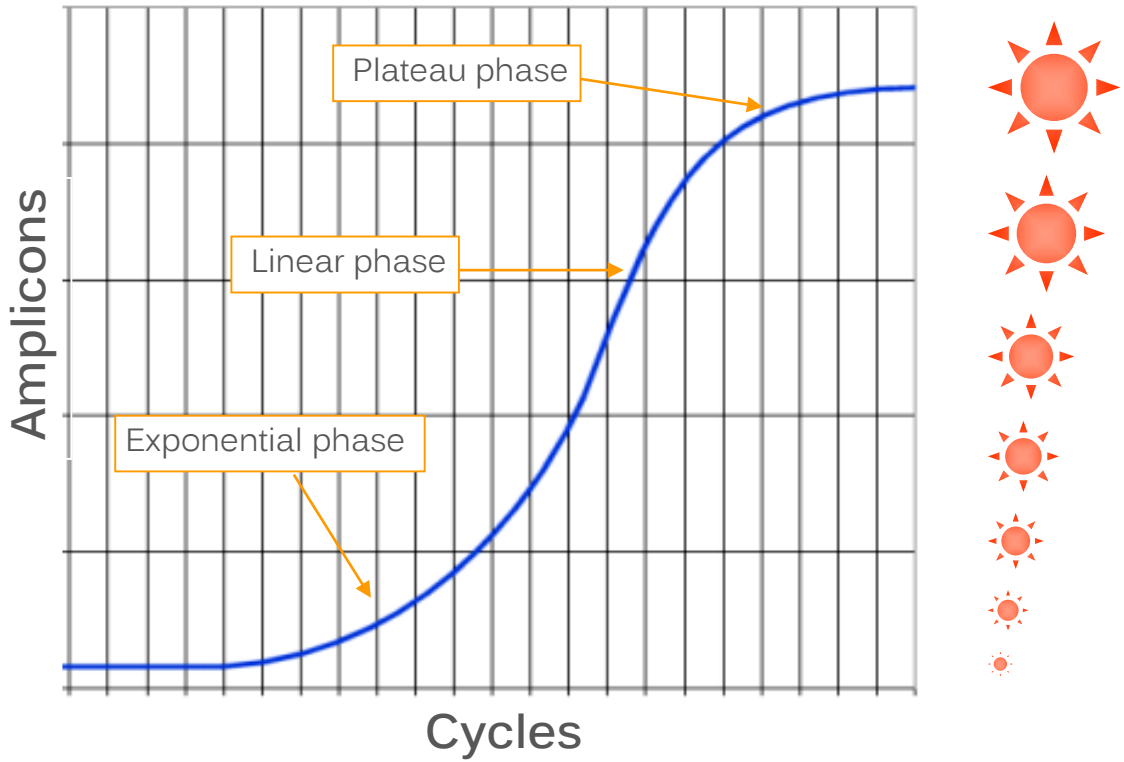
If we were to graph the amount of DNA in our tube, from the start until right now, at cycle 25, the graph would look like this (a logistic curve):



But...as chain reaction progresses, reagents run out so exponential growth does not go on forever!

Imagining Real-Time PCR

The overall kinetic of a PCR reaction is a sigmoidal curve. This kinetic can be monitored using reagents that fluoresce in the presence of amplified DNA!



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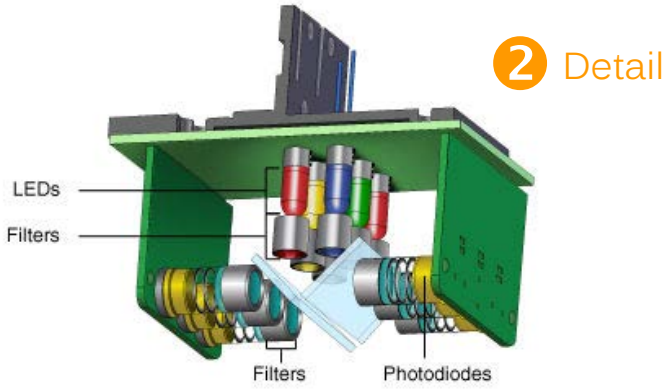
Real-Time PCR instrument

Real-time PCR instruments consist of THREE main components:

1. Thermal Cycler (Heating and cooling PCR tubes)
2. Optical Module (Detecting fluorescence in the PCR tubes)
3. Computer (Translating fluorescence data into meaningful results)



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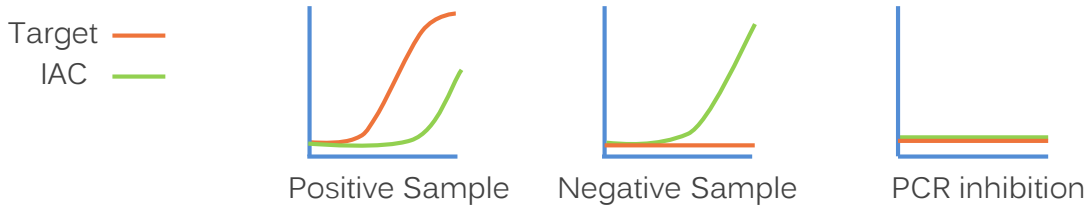
Real-Time PCR instrument

Real-time PCR instruments can detect more than one fluorescence (multiplex) therefore:

1. we can run more then one reaction in the same tube on the same sample and the kinetic of these reactions can be analyzed indipendently using different fluorescent reporters (as an example we can simultaneously detect the presence of soy and celery in the hamburger DNA extract)
2. We can add an internal amplification control (IAC) in each PCR reaction to monitor possible inhibitions thus avoiding false negative results.



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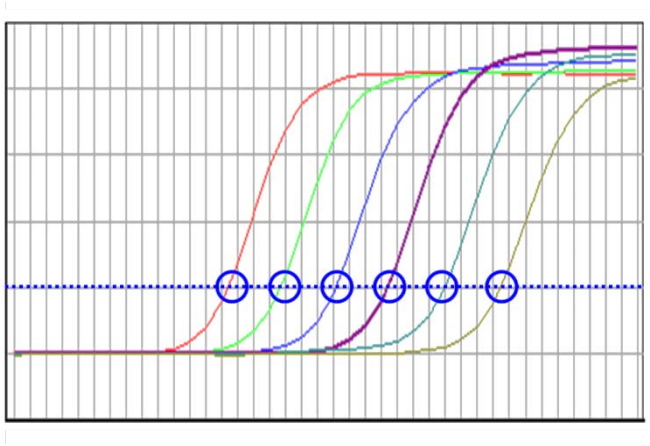
Real-Time PCR measuring quantities

As soon as PCR follows an exact logistic kinetic at least in the exponential phase:

1. We can describe a position of the lines with a value that represents the cycle number where the trace crosses an arbitrary threshold in the exponential phase. This is called the “Ct Value”.
2. Ct values are inversely proportional to the starting quantity of DNA, and each Ct equals to a 2-fold amount of starting amount of DNA, thus a ten-fold dilution series of a DNA extract will show a “3,2 Ct distance” between each sample ($10=2^{3.2}$).



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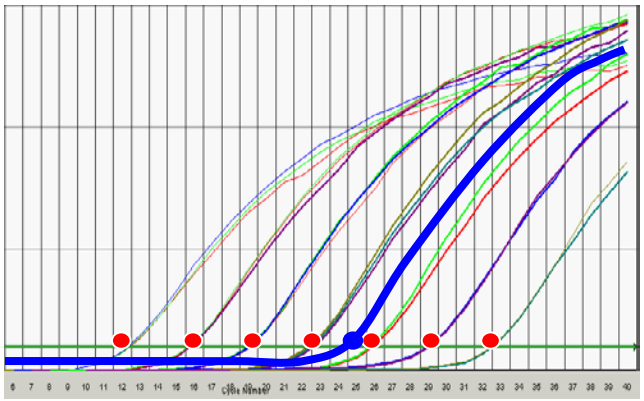
Real-Time PCR measuring quantities

As soon as the fold-quantities have a constant Ct distance then we can provide relative quantity results.

If we have a quantified standard material available we can create a calibration curve thus translating the relative quantification into an absolute quantification and measure unknown samples per interpolation.



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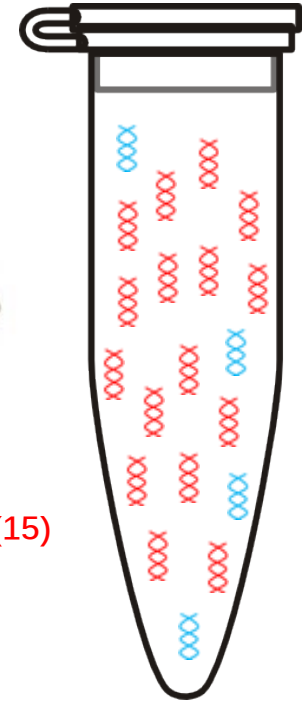
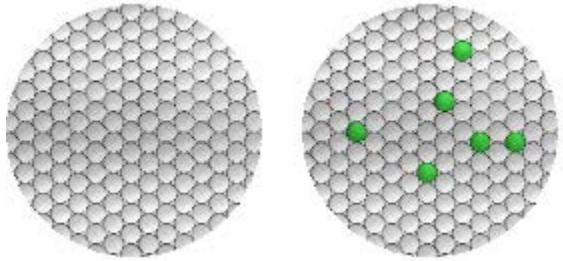
Droplet digital PCR

In droplet digital PCR the DNA extract is segregated into small drops, each drop behaves like a single PCR tube.



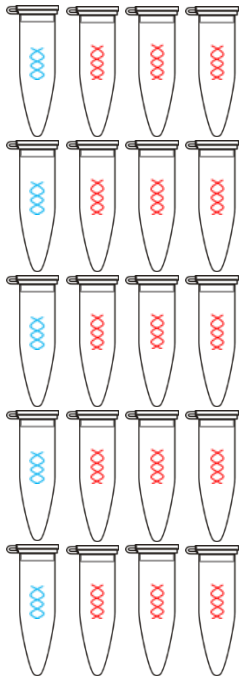
= Dispersive DNA (15)

= Soy DNA (5)



Result

1000 1000 1000 1000 1000



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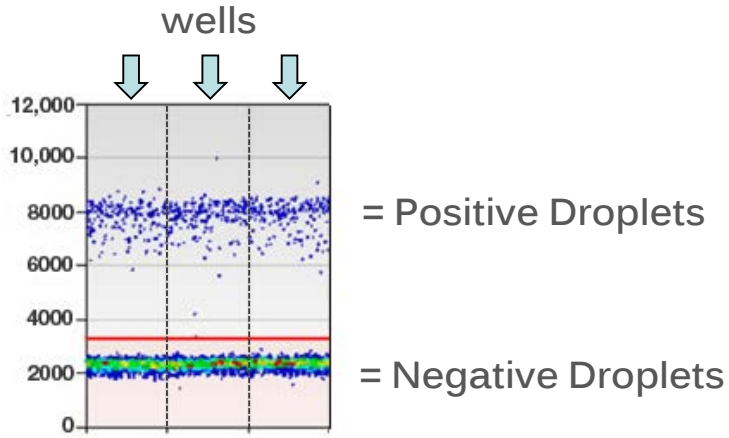
Droplet digital PCR

If the target DNA is present in the drop the PCR reaction works and the drop fluoresce otherwise it is dim.

Counting the number of fluorescent drops we can determine the absolute number of target DNA present in the starting sample, see a typical plot:



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