

Multicolour Digital PCR for ctDNA detection in breast cancer

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Breast cancer: a worldwide health issue

Cases

55,122



New cases of invasive
breast cancer, 2015,
UK

Deaths

11,433



Deaths from breast
cancer, 2014, UK

Prevention

27%



Preventable cases of
breast cancer, UK

Excess bodyweight

9%



Breast cancer cases
linked to excess
bodyweight, UK

Alcohol

6%



Breast cancer cases
linked to alcohol
consumption, UK

Physical inactivity

3%



Breast cancer cases
linked to too little
physical activity, UK

Progress in breast cancer management

Deaths

11,433

Deaths from breast cancer, 2014, UK

Age

90+ YEARS

Peak rate of breast cancer deaths, 2012-2014, UK

Survival

78%



Survive breast cancer for 10 or more years (females only), 2010-11, England and Wales

Age

60-69 YEARS

Age that breast cancer survival is highest, 2009-2013, England

Improvement

40% → 78%



Breast cancer survival in the UK has doubled in the last 40 years

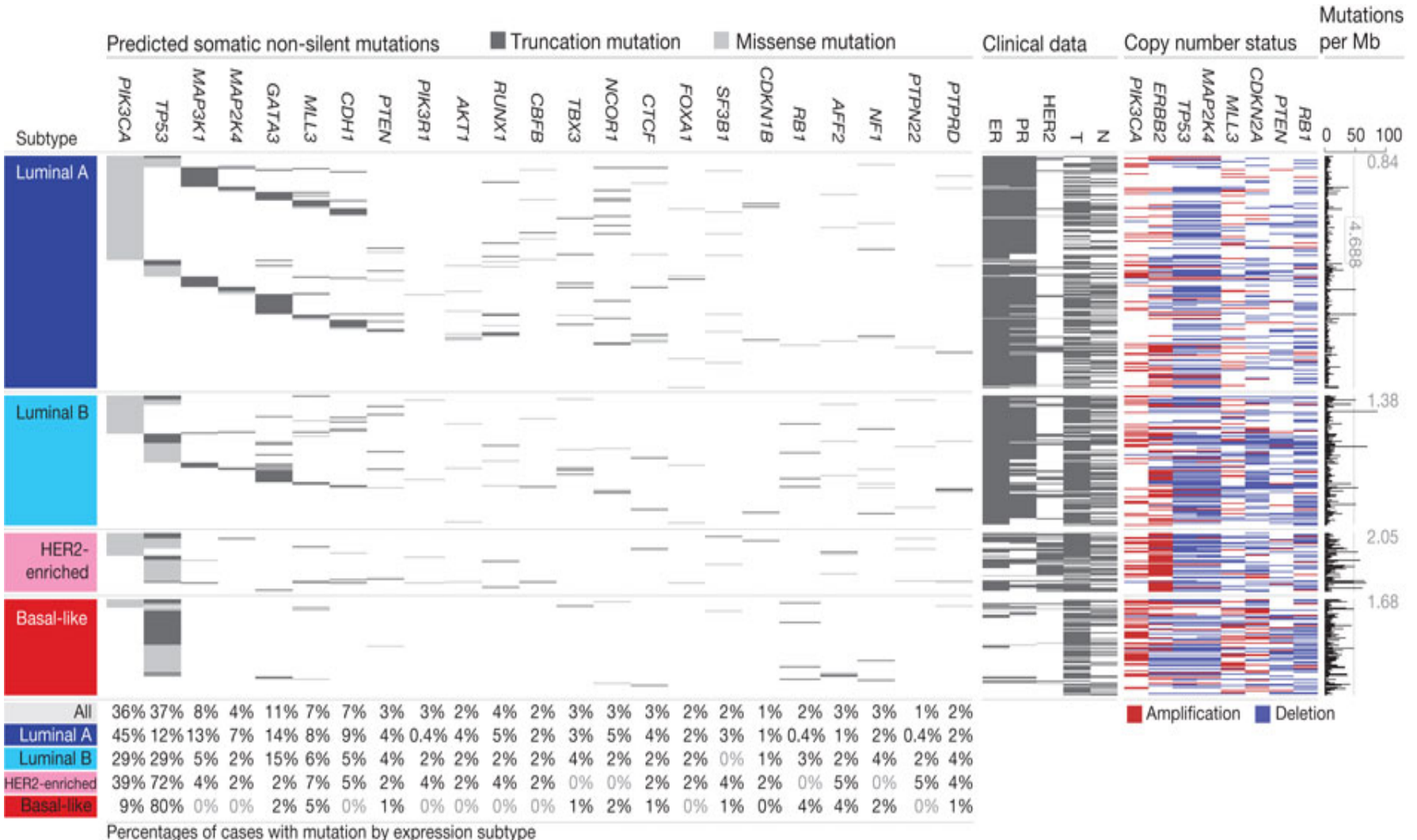
Trend over time



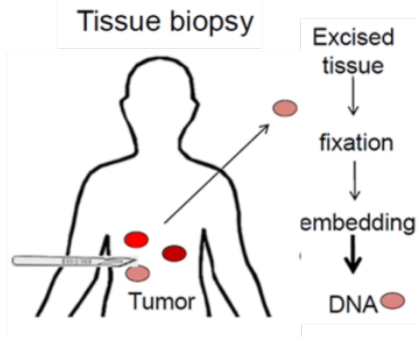
-35%

Breast cancer mortality rates have decreased by 35% since the early 1970s, UK

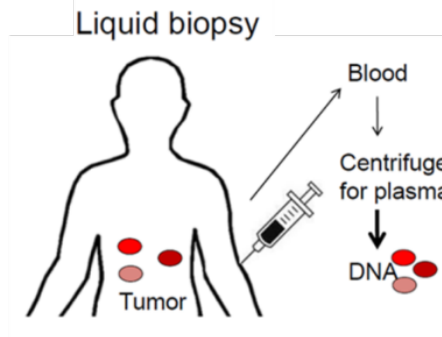
Molecular Analysis of Breast Cancer



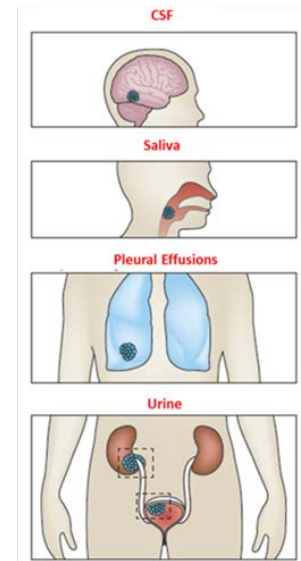
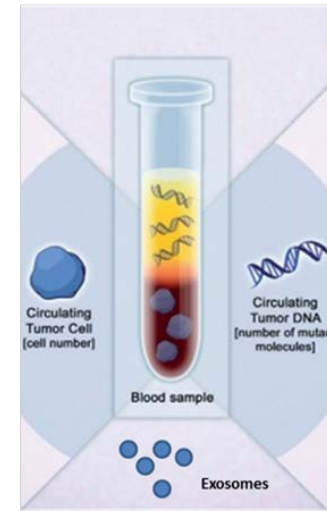
The liquid biopsy spectrum



- Invasive
- Expensive
- Processing takes time

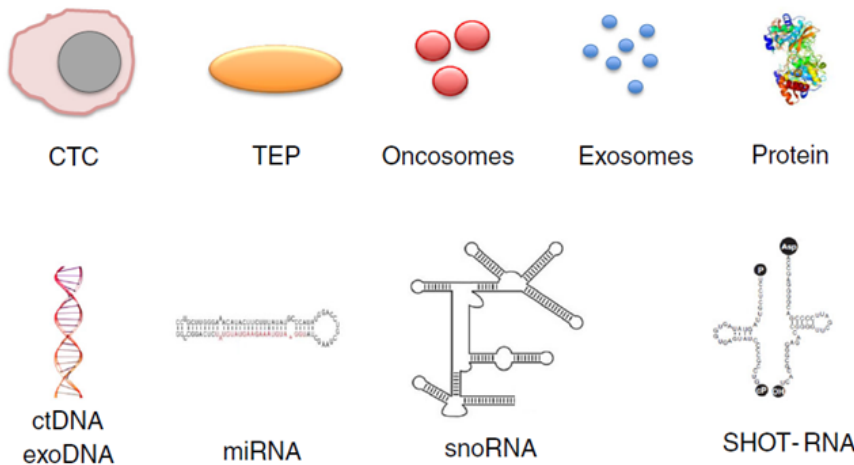


- Non-invasive assessment
- Less expensive
- Rapid purification
- Whole picture
- Surrogate when anatomic biopsies are not feasible



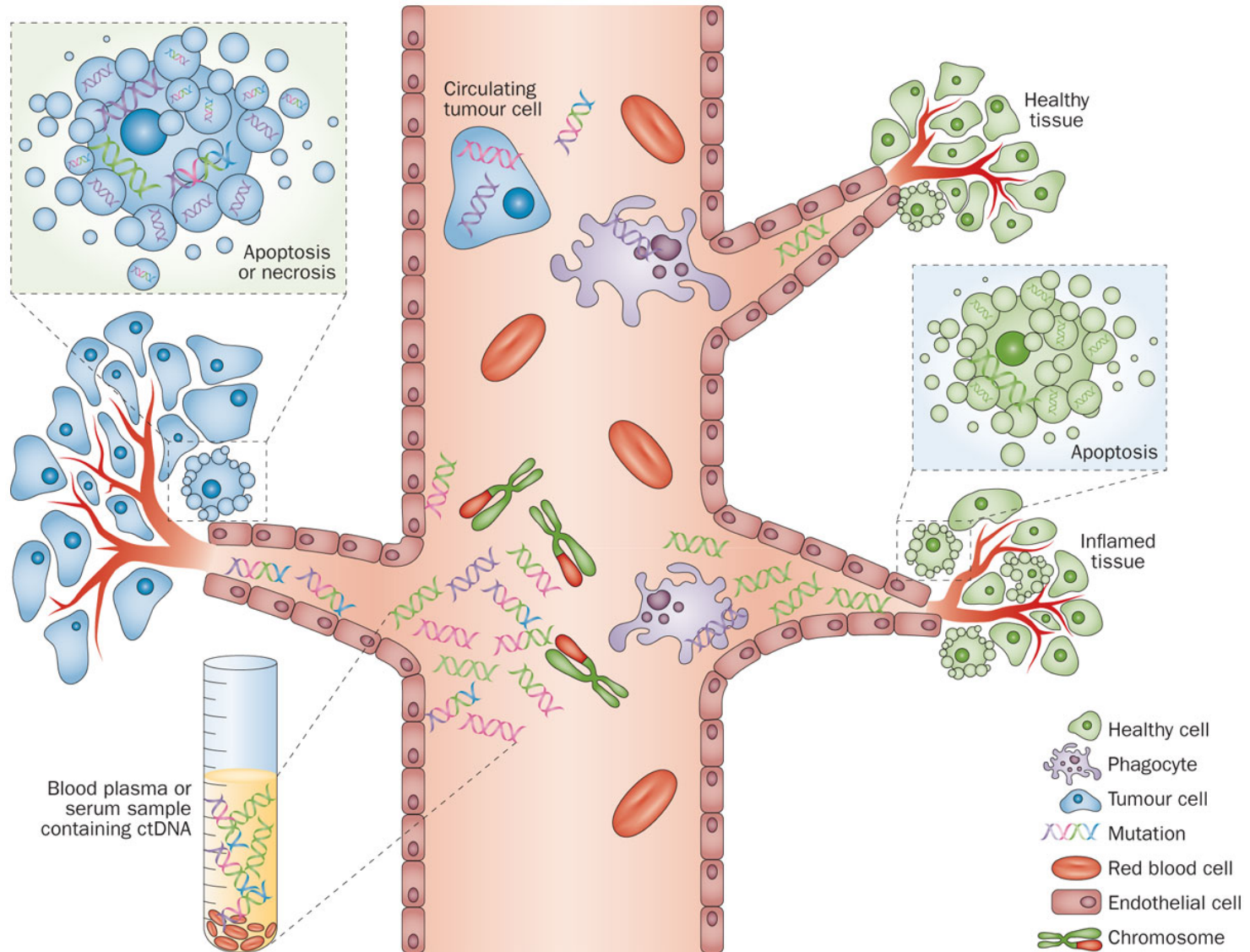
Siravegna et al, Nat Rev Clin Oncol 2017

Blood, urine, saliva, CSF, other body fluids (lavages, effusions...)

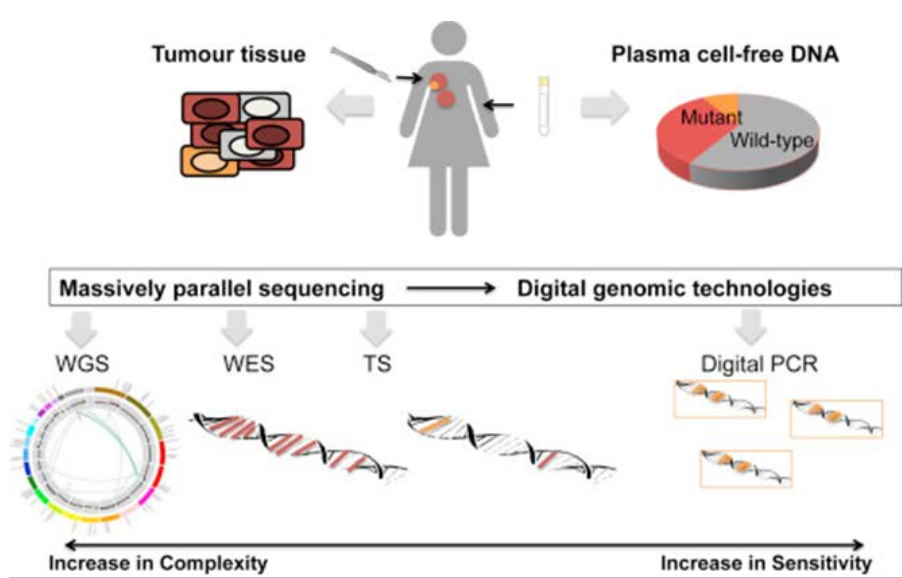


cf/ctDNA, CTCs, exosomes, protein markers, miRNA/RNA, other metabolites

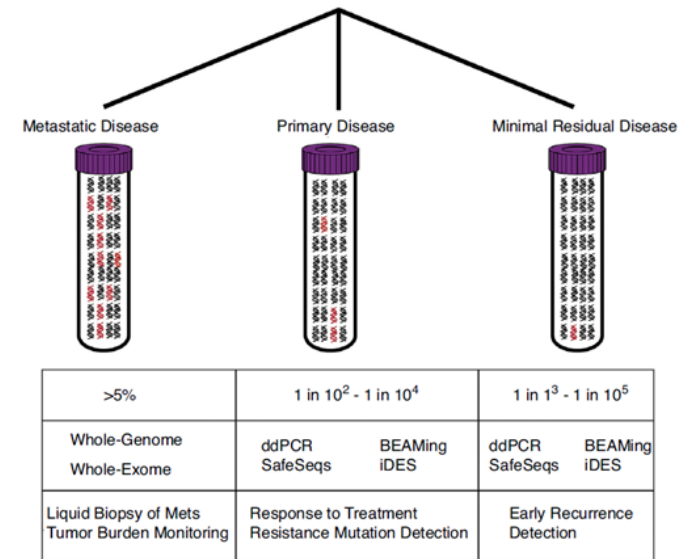
The liquid biopsy spectrum: ctDNA



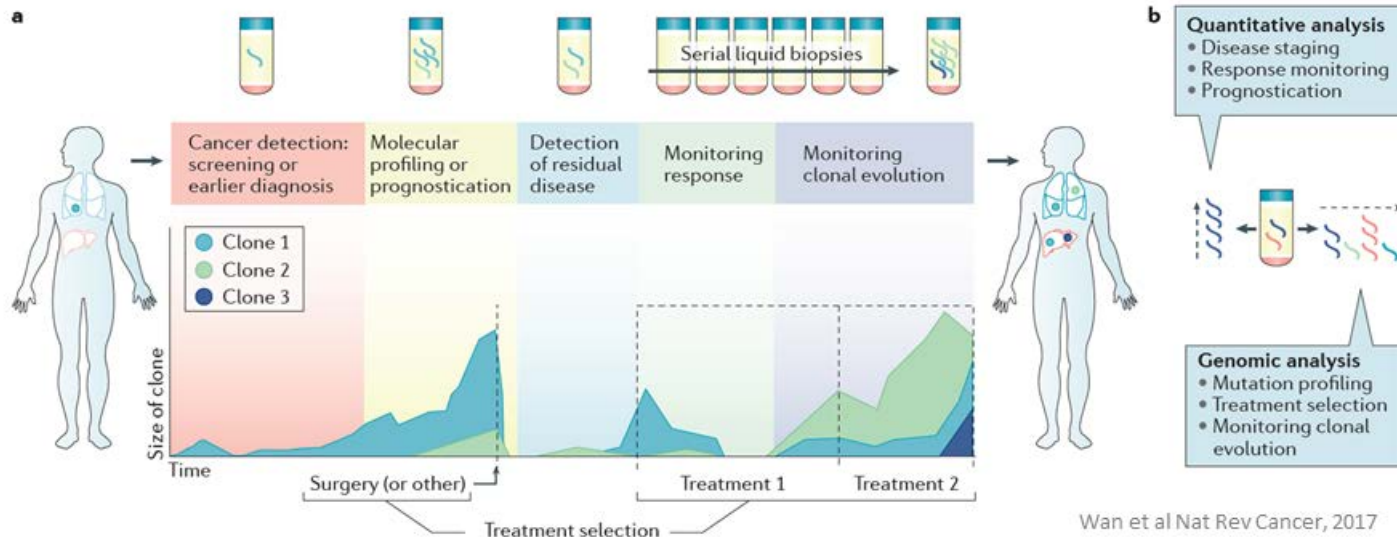
Analysis of ctDNA in breast cancer



De Mattos-Arruda, Mol Oncol 2015



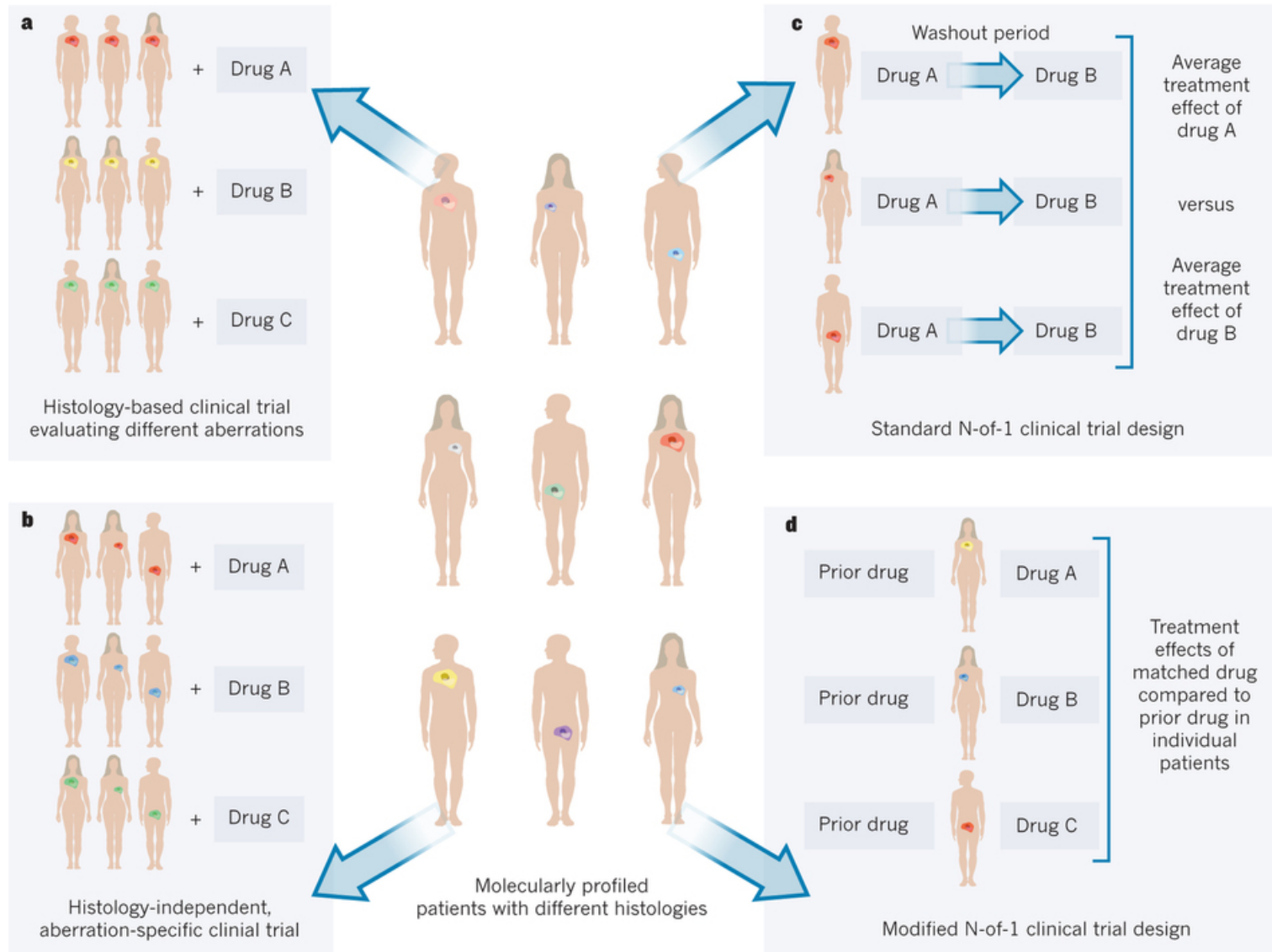
Butler et al, Current Opinion in Genetics & Development 2017



Wan et al Nat Rev Cancer, 2017

Liquid Biopsies in the clinic: Potential strategy for their use

Liquid Biopsy as a tool on clinical trials



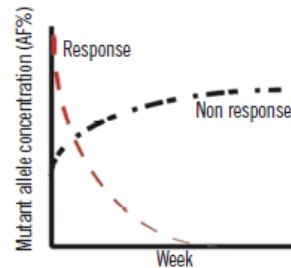
Liquid Biopsy as a tool on clinical trials

Concordance



For assessing the sensitivity and specificity of assays for the detection and quantification tumor variants within plasma. Cancer-related variants detected in cfDNA are compared with those detected in tumor tissue.

Observation

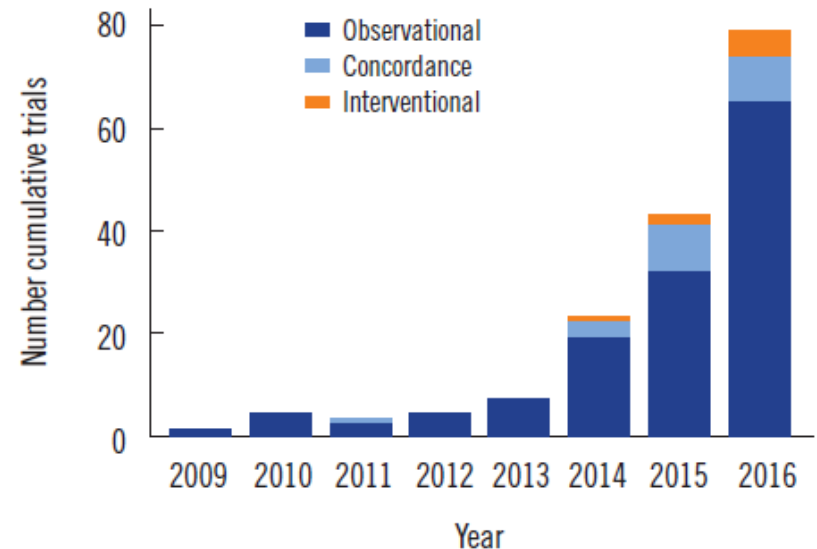


Early diagnosis: does the presence of suspicious variants in cfDNA of healthy individuals predict cancer?
MRD: does reemergence of tumor variants within cfDNA predict relapse due to MRD?
Response/Relapse: does plasma response correlate with tumor response?

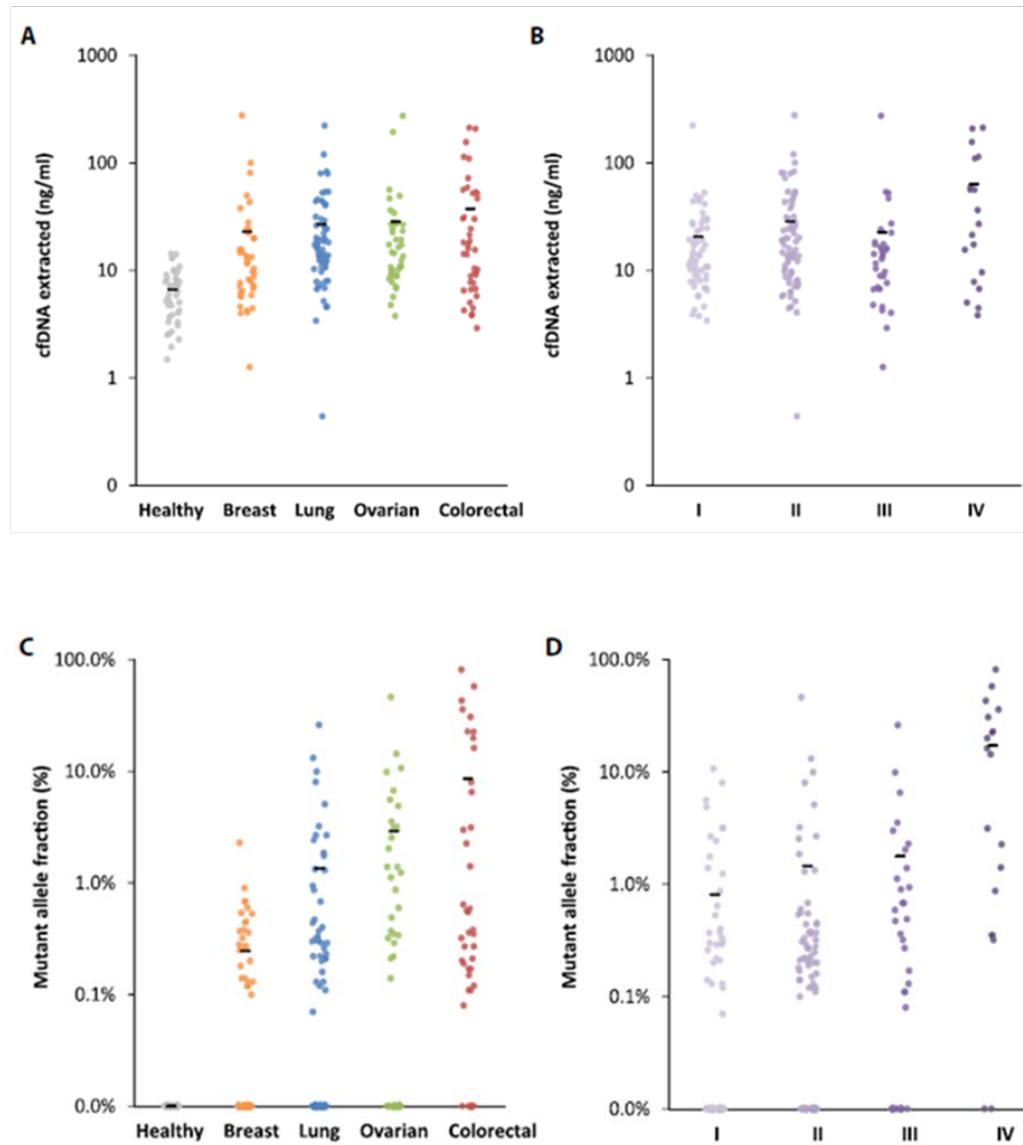
Intervention



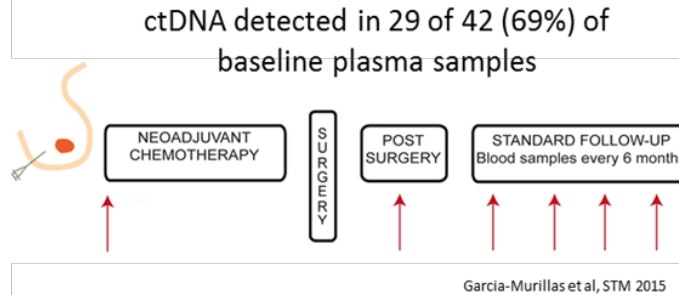
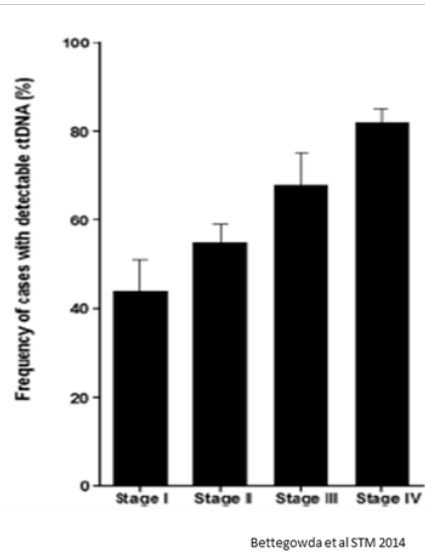
Early diagnosis: initiating therapy upon detection of cancer-related variants in cfDNA.
MRD: initiating adjuvant therapy upon re-emergence of tumor variants in cfDNA.
Response/Relapse: switching treatment upon emergence of resistance mutations in cfDNA.



The challenge of low level mutation detection

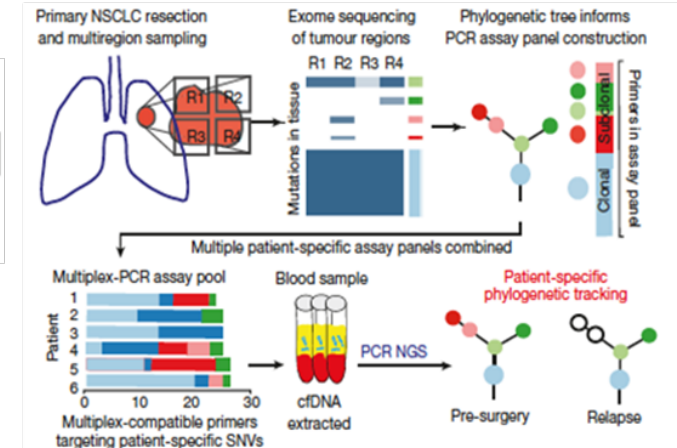


Analytical sensitivity (primary cancer)

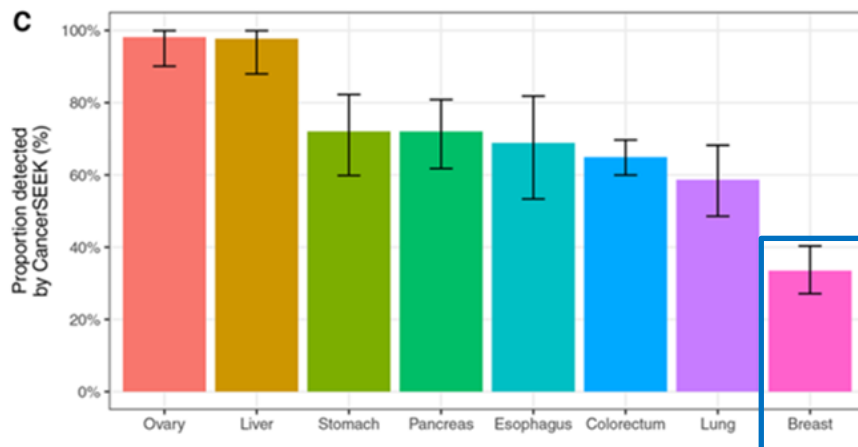
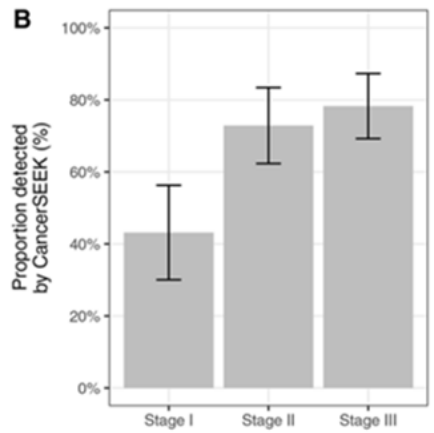


Garcia-Murillas et al, STM 2015

ctDNA detected preoperatively in 46 out of 96 (48%) early-stage NSCLCs



Abbosh et al Nature 2017



Cohen et al Science 2018

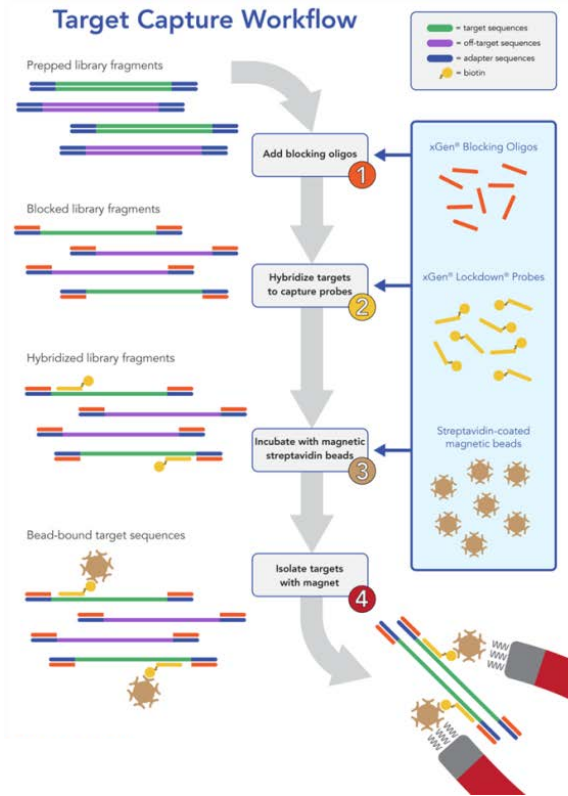
Insufficient sensitivity of current ctDNA assays

Analysing ctDNA: NGS and (Digital) PCR

NGS and ddPCR for Cancer Liquid Biopsies

NGS	ddPCR
Comprehensive detection of known and unknown mutations	Greater sensitivity of a targeted set of known mutations
Costly with longer time to process and analyze results	Cost-effective for rapid genotyping & serial monitoring
Ratios of mutant to WT quantities	Absolute quantification of mutant & WT copies

Next Generation Sequencing on ctDNA

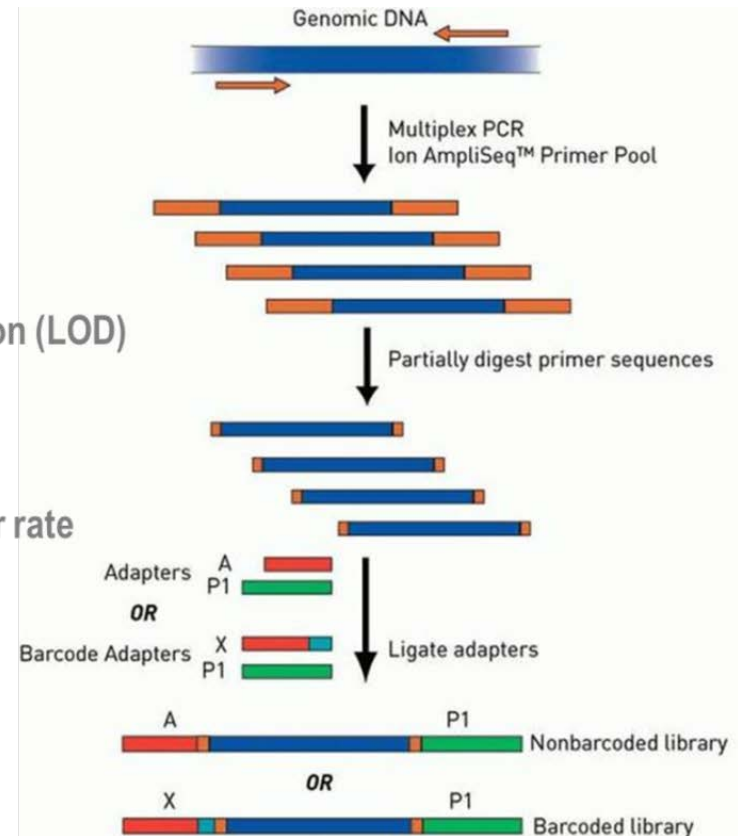


Pros
 Larger Panels
 Low PCR-associated error rate

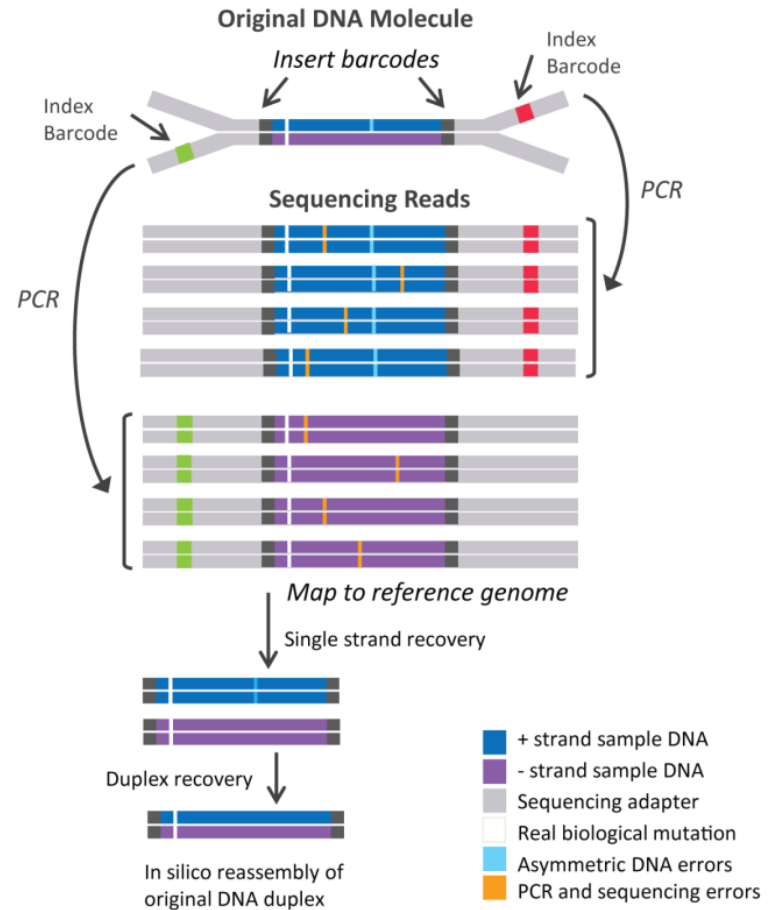
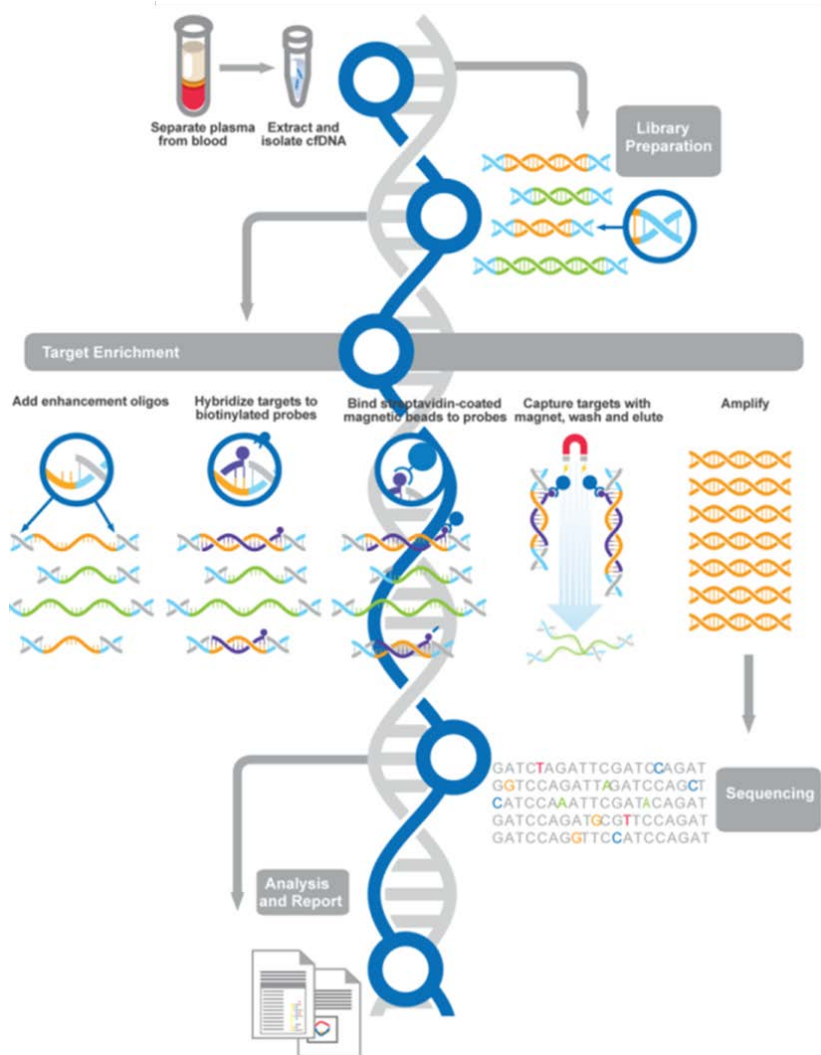
Cons
 Highly inefficient
 Long time to perform
 Expensive

Pros
 Relatively fast
 Relatively inexpensive
 Increased Limit of Detection (LOD)

Cons
 Smaller Panels
 High PCR-associated error rate
 Homopolymeric regions



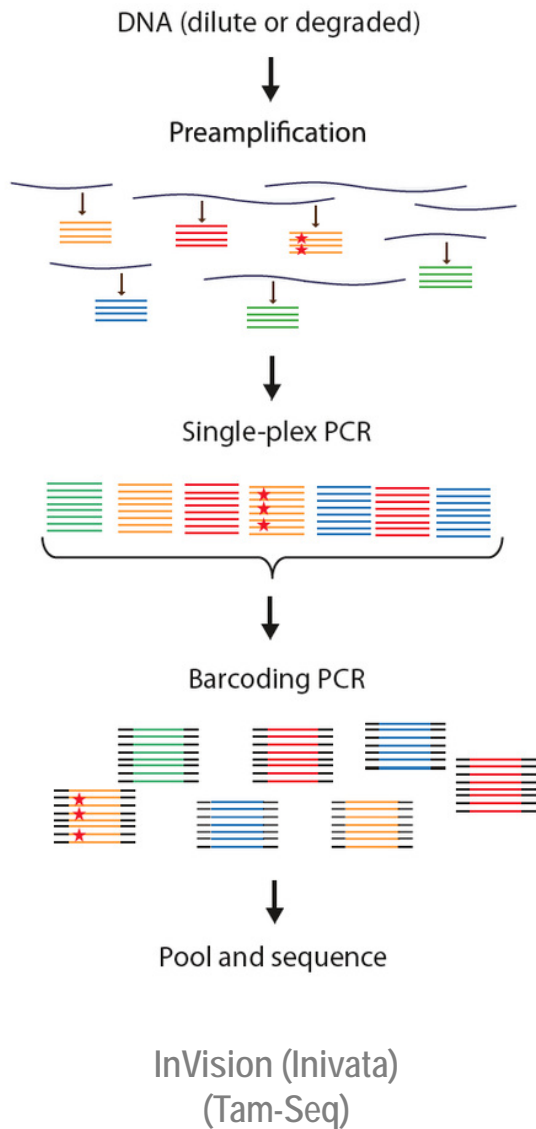
Error corrected NGS for ctDNA



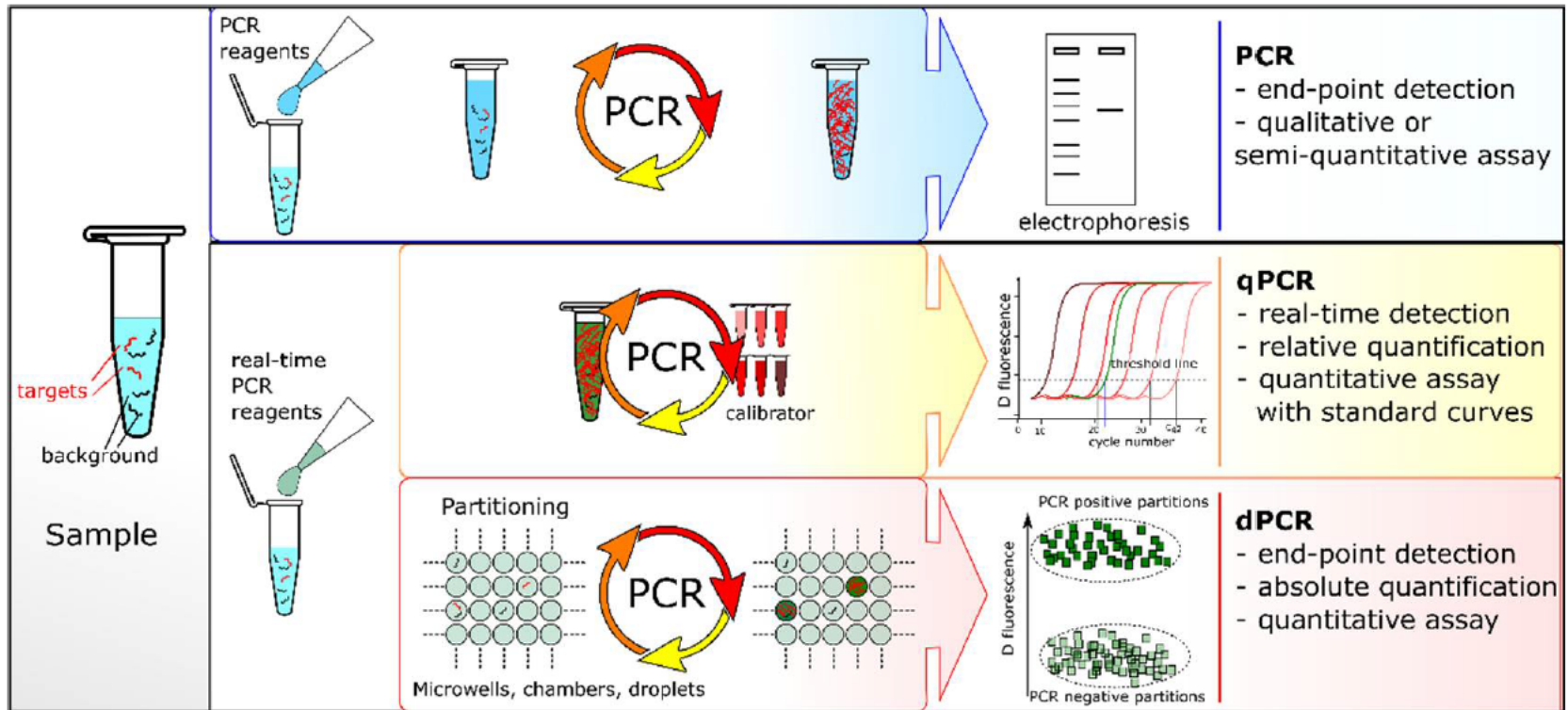
Avenio (Roche)
(CAPP-Seq)

Error corrected NGS for ctDNA

Error corrected NGS for ctDNA

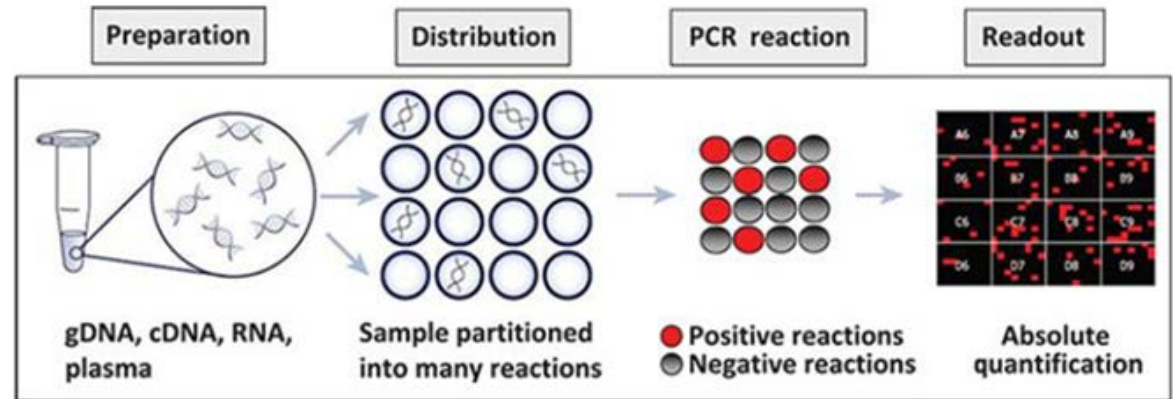
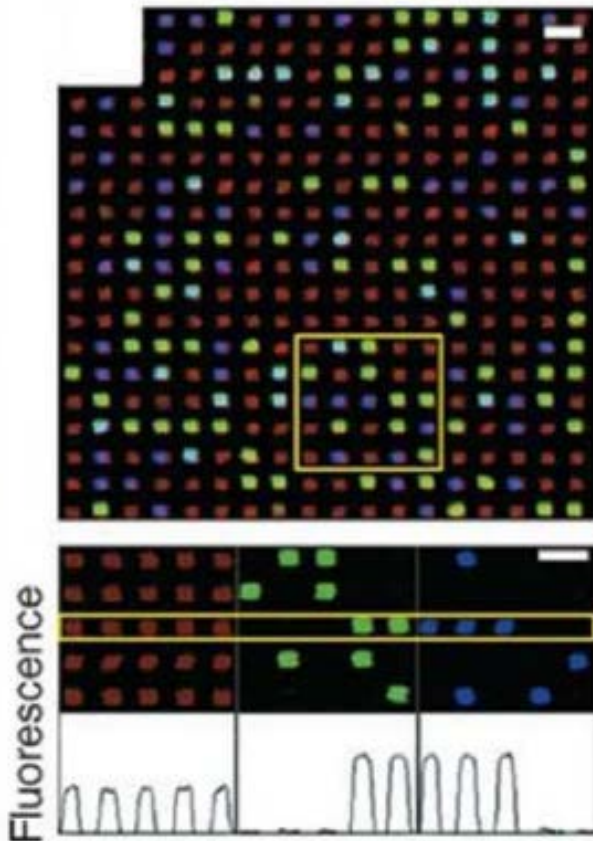


Analysing ctDNA: Different PCR approaches



Analysing ctDNA: Digital PCR

Physical partition-based dPCR

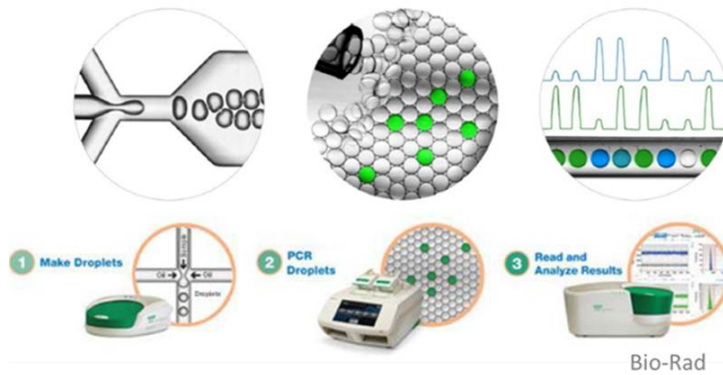
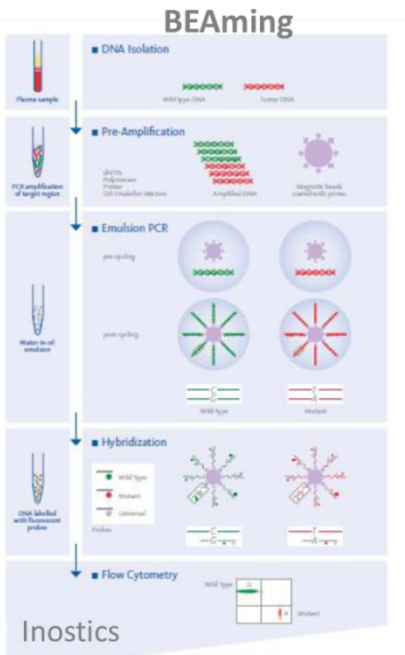
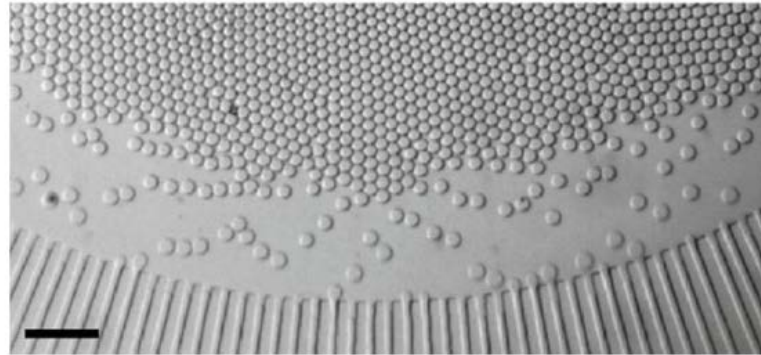


Thermo
Fluidigm

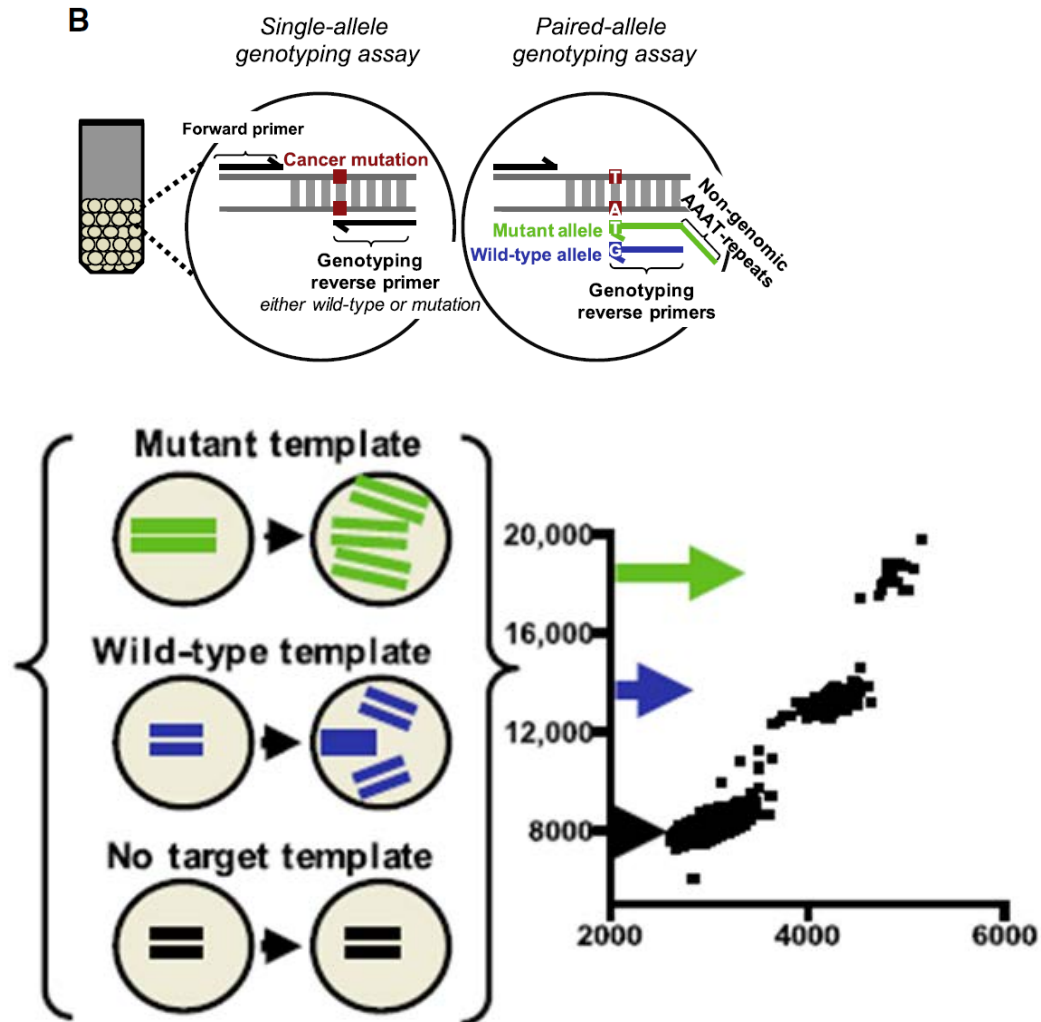


Analysing ctDNA: Digital PCR

Emulsion-based dPCR



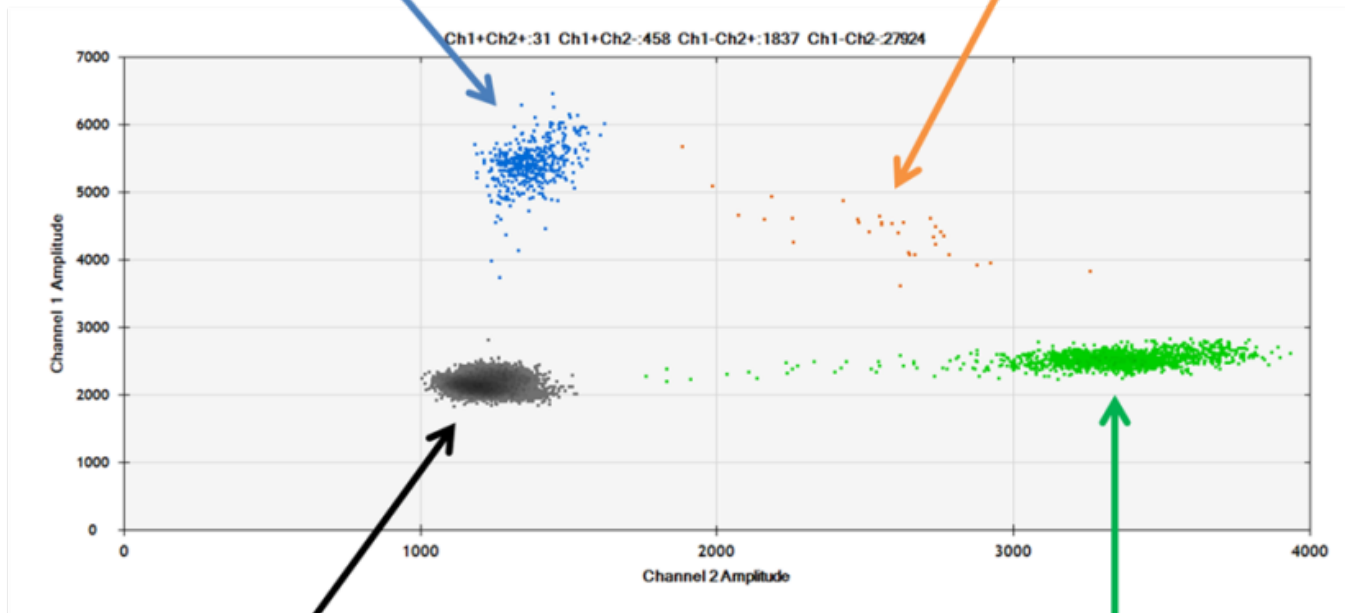
Analysing ctDNA: Single Dye Duplex Digital PCR



Analysing ctDNA: Multiple Dyes Duplex Digital PCR

Droplets containing only mutant DNA
FAM labelled dye

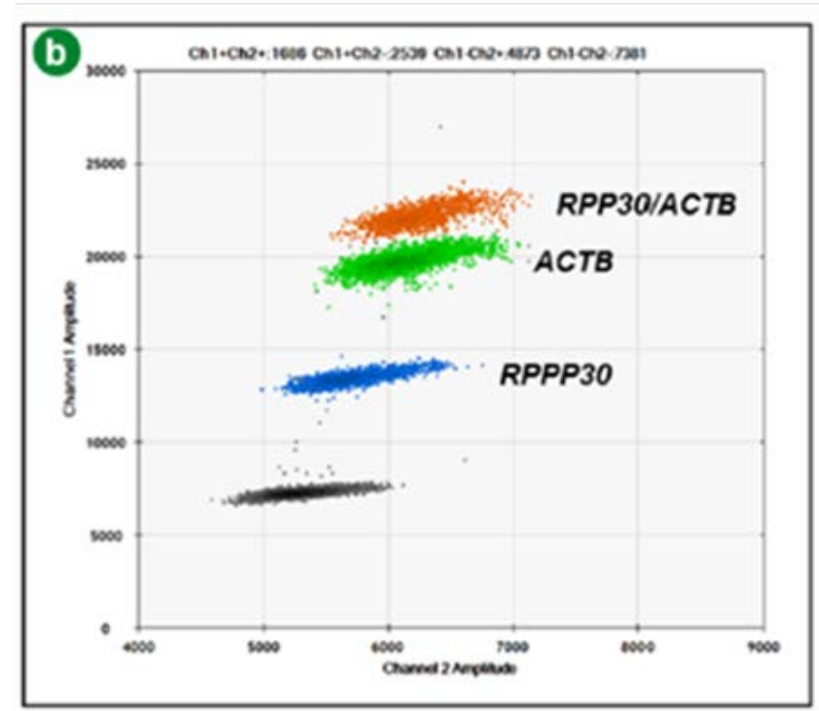
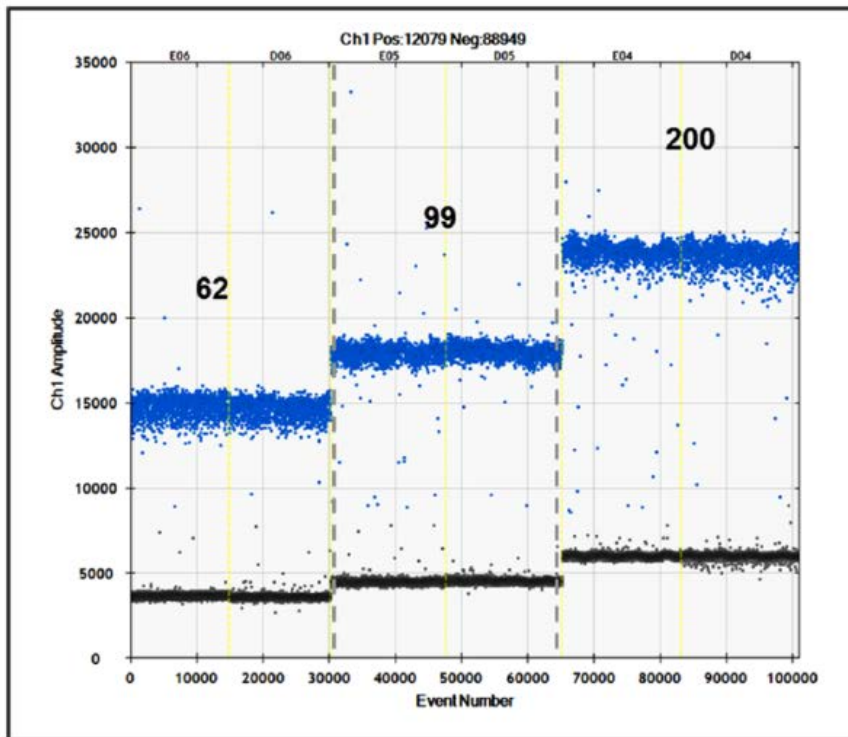
Droplets containing both a WT and mutant DNA copy in the same droplet



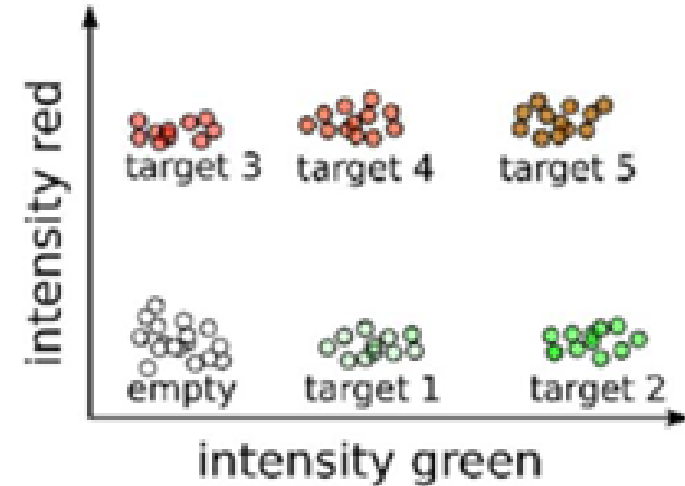
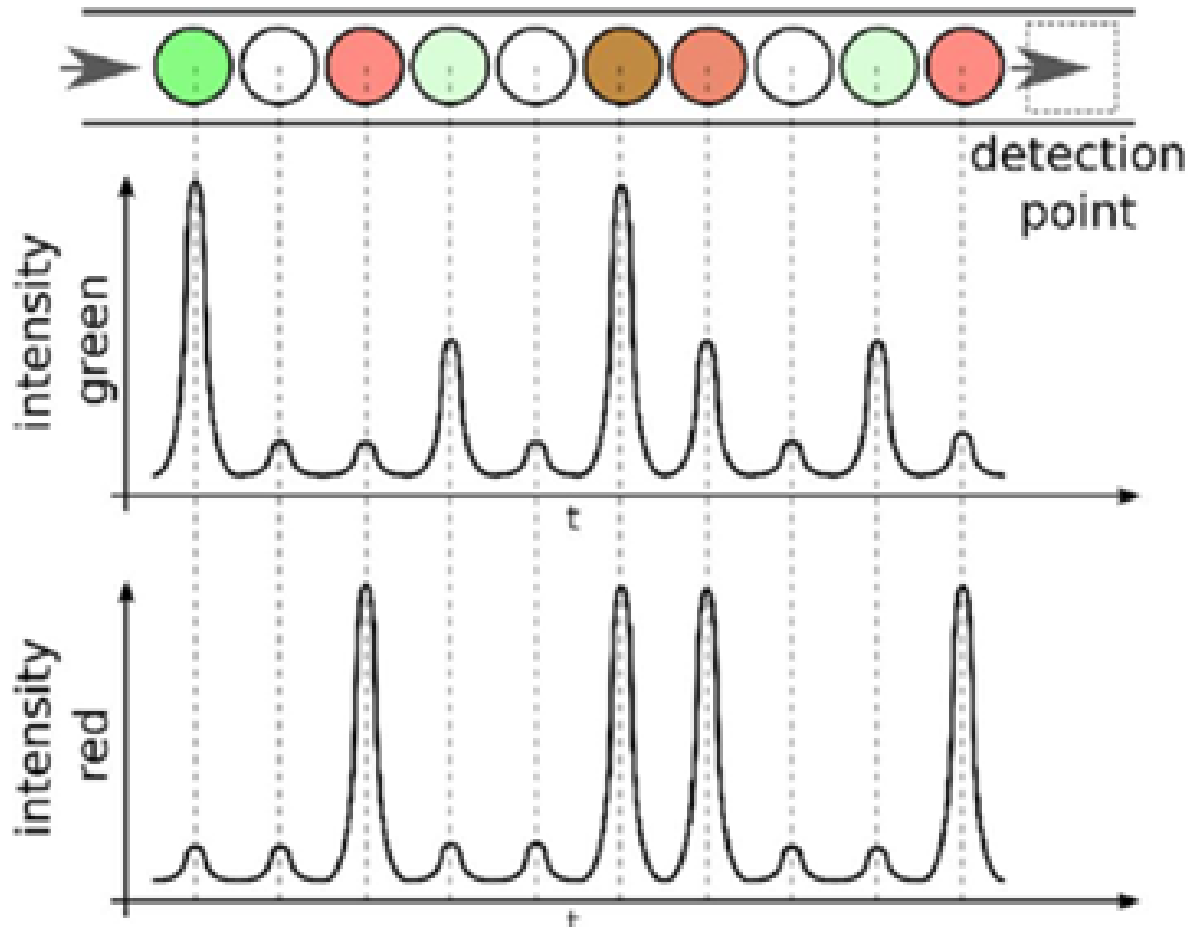
Droplets containing no target DNA
Empty droplets

Droplets containing only WT DNA
VIC labelled dye

Analysing ctDNA: Single Dye Multiplex Digital PCR

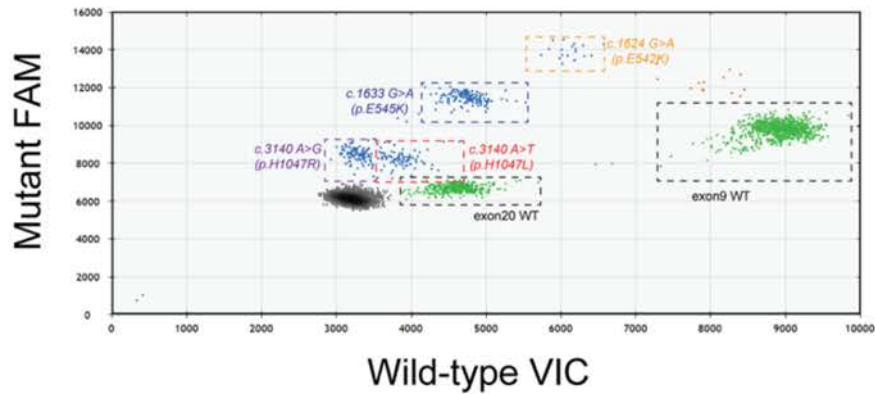


Analysing ctDNA: Multiple Dyes Multiplex Digital PCR

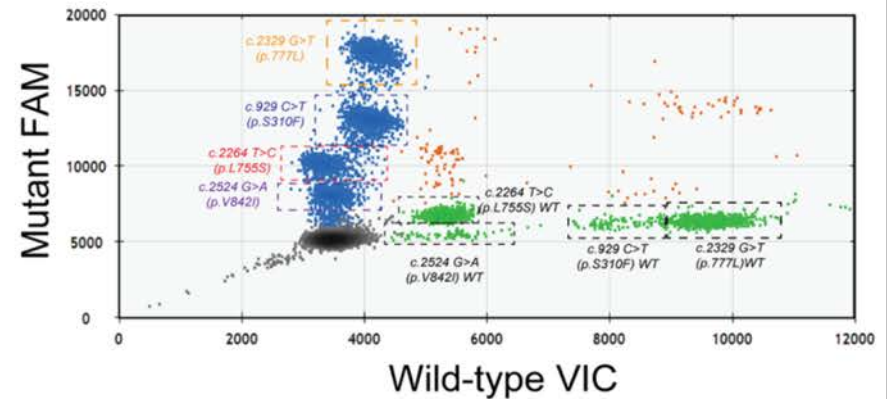


Analysing ctDNA: Multiplex Digital PCR

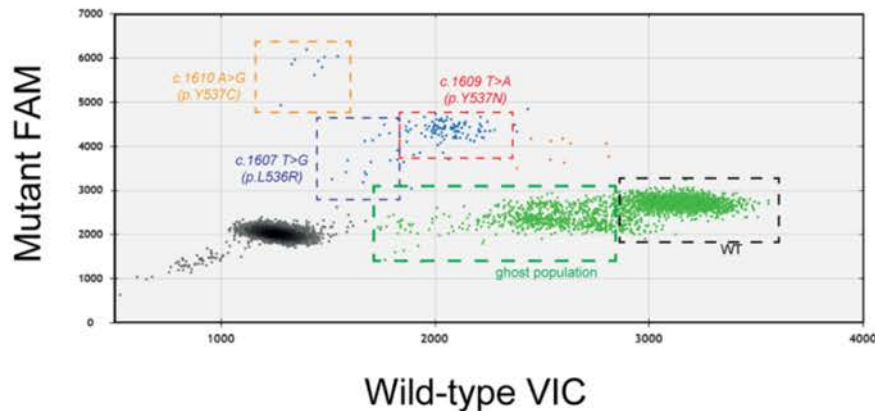
PIK3CA



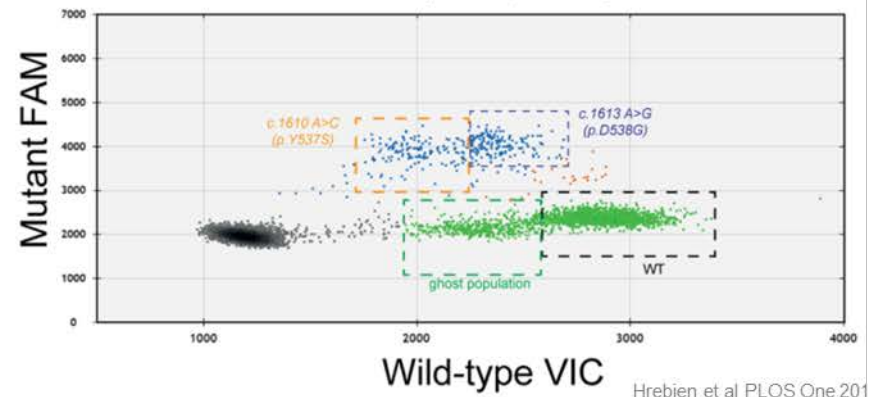
ERBB2



ESR1 (Multiplex 1)



ESR1 (Multiplex 2)



Analysing ctDNA: 3 colours Digital PCR

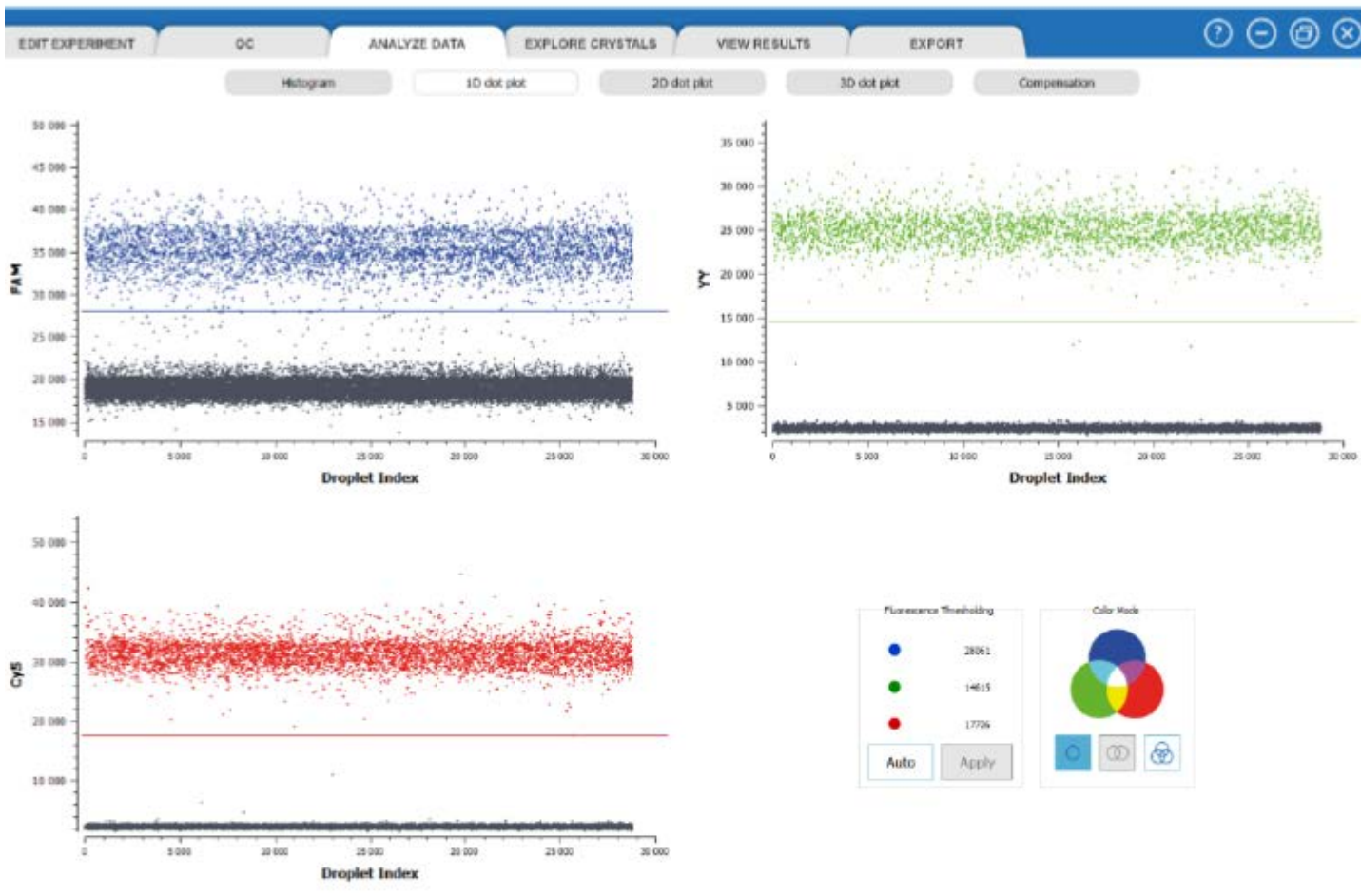


The screenshot shows the software interface for the digital PCR instrument. The interface is divided into several sections:

- Header:** A blue bar at the top contains a "LOGIN" button, a logo, and navigation icons (back, home, refresh).
- Experiment Details:** A text input field contains "20160412_Exp_CR".
- Scanning Parameters:** Three input fields contain "631", "633", and "ChipID3".
- Chamber Details:** A dark sidebar on the left contains a "Reset" button, a "Sample ID" field with "Sample1", and a table for sample configuration:

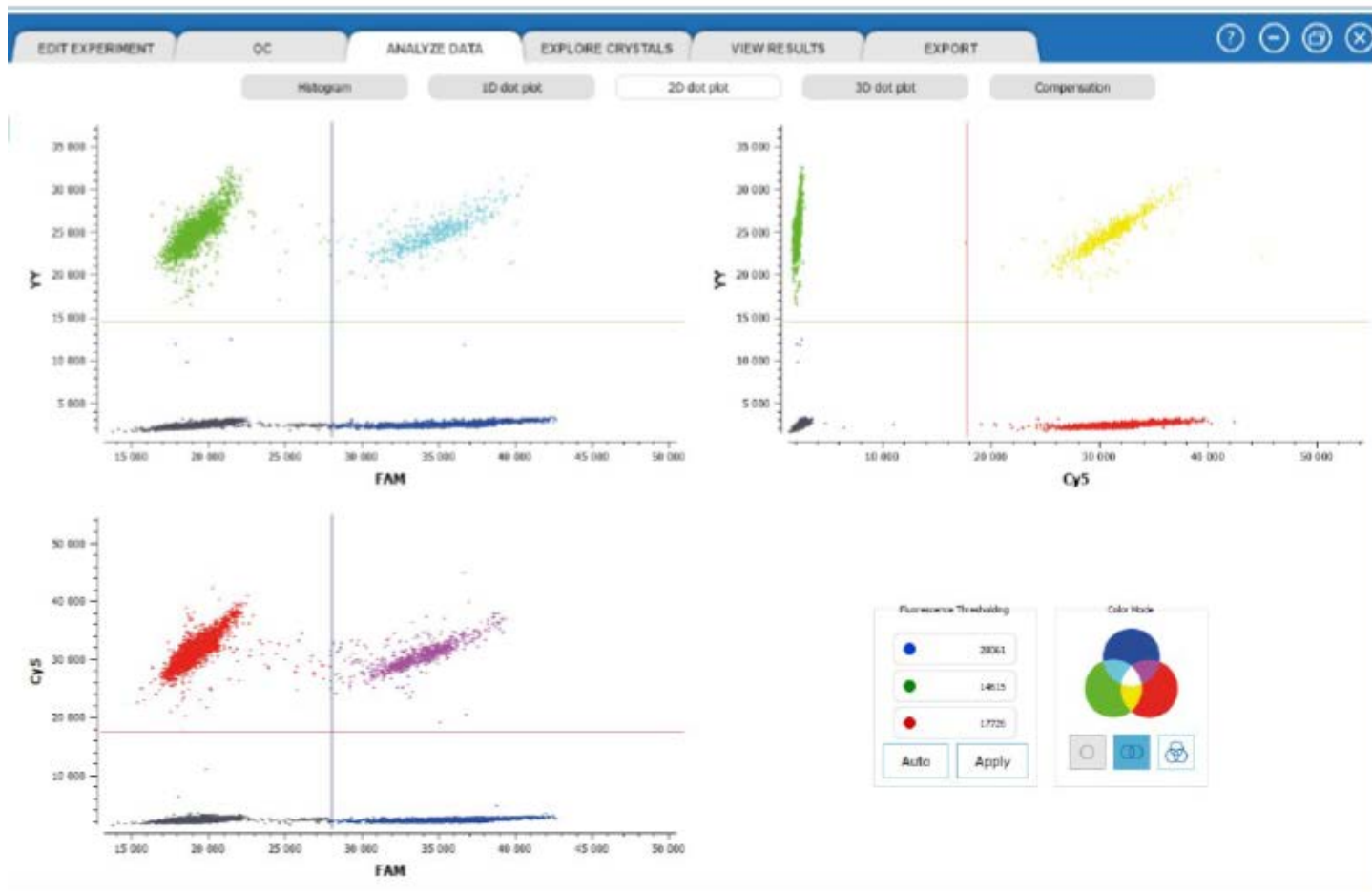
Sample ID	Sample type	Dilution factor
FAM	U	1.0
YY	U	1.0
Cys	U	1.0
- Chamber View:** A central area shows a 3x3 grid of wells. The first column contains three wells labeled "Sample1" with "U" and "U" below them. The second column contains three wells labeled "NNP sample" with "N", "D", and "P" below them. The third column contains three empty wells.
- Bottom Controls:** A dark bar at the bottom contains an "OPEN TRAY" button, a "1/1" page indicator, and a "RESCAN" button.
- Right Side Controls:** Two buttons labeled "SAVE" and "SAVE AS" are positioned on the right side of the interface.

Analysing ctDNA: 3 colours Digital PCR



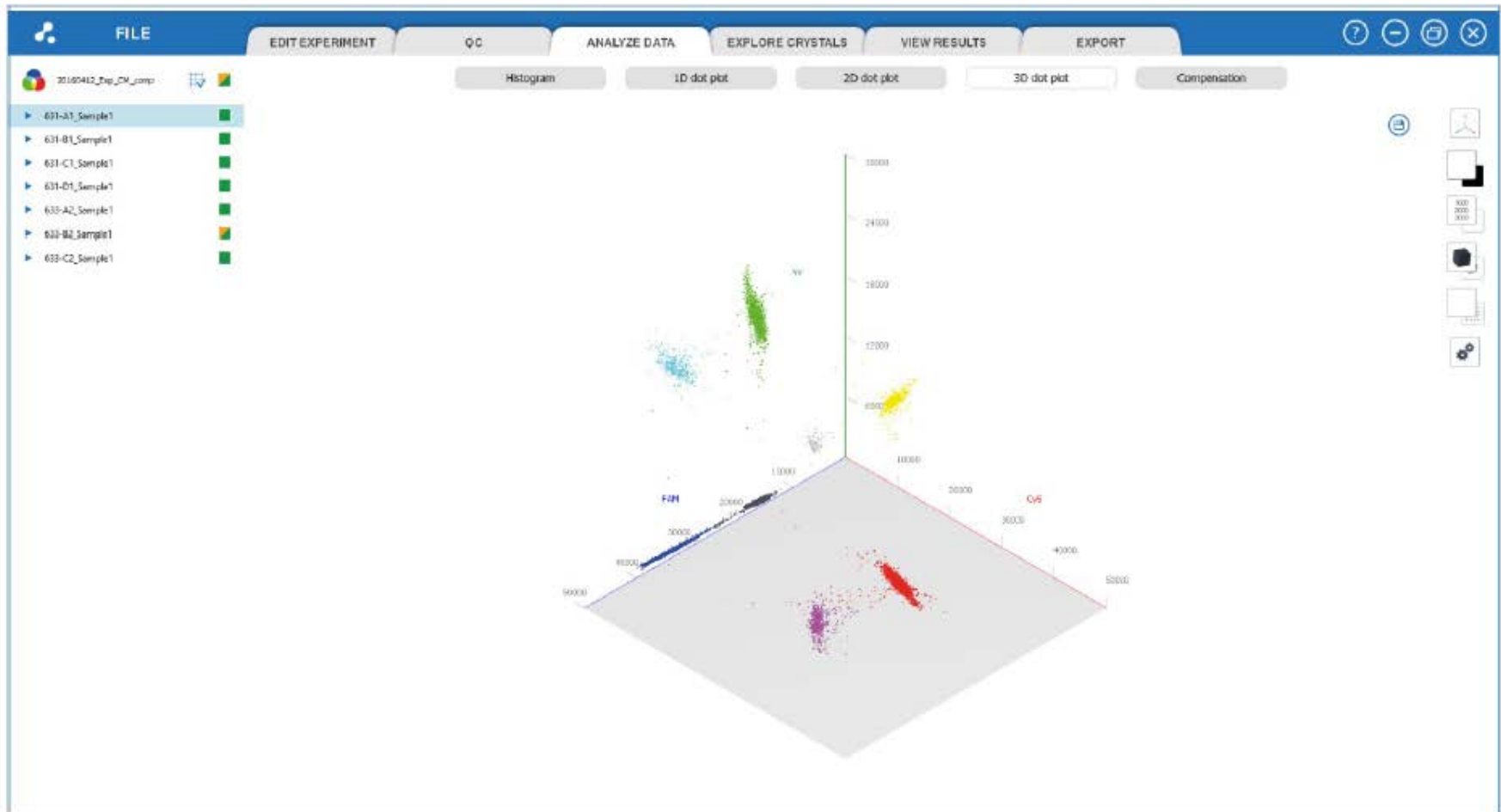
The 2-color diagrams used for 1D data visualization in Crystal Miner

Analysing ctDNA: 3 colours Digital PCR



The 4-color diagrams used for 2D data visualization in Crystal Miner

Analysing ctDNA: 3 colours Digital PCR

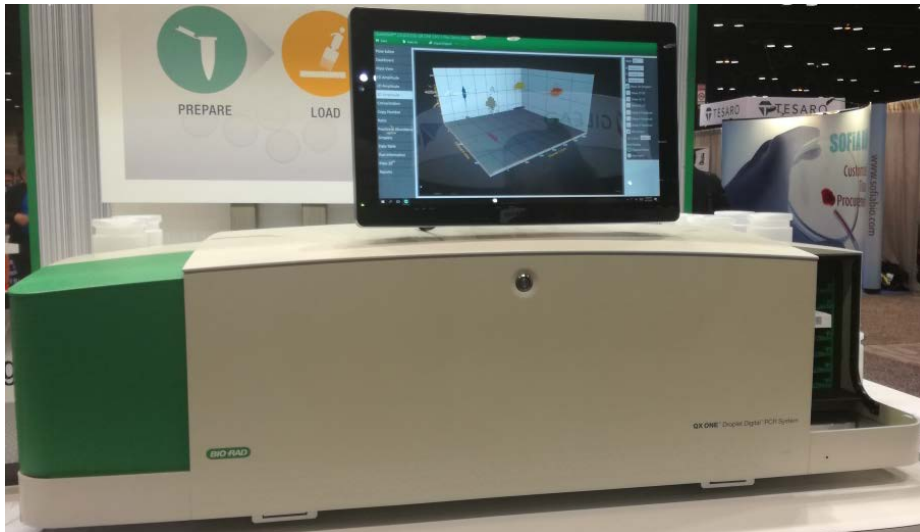


Analysing ctDNA: 2 colours vs. 3 colours Digital PCR

Analysing ctDNA: 2 colours vs. 3 colours Digital PCR

Analysing ctDNA: 2 colours vs. 3 colours Digital PCR

Analysing ctDNA: Digital PCR- The future is multi-coloured

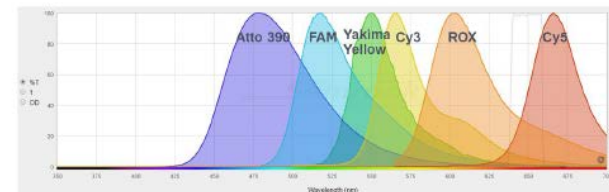


Bio-Rad QX-ONE: 4 colours

Excitation



Emission



Stilla Naica: 6 colours

Final remarks

- Liquid biopsy research has advanced very rapidly in recent years especially the analysis of ctDNA
- ctDNA analysis is currently being integrated as a biomarker in clinical trials with high success
- We are still challenged with several technical limitations that need to be overcome to realise the full potential of the clinical utility of ctDNA
- More sensitive tools are constantly being developed that will help with the problem of low level mutation detection
- Multicolour digital PCR is positioning itself as a powerful tool that will allow more complex analysis of ctDNA

Acknowledgements

The patients that participate on the studies

The Royal Marsden Hospital/The Institute of Cancer Research

Molecular Oncology Lab

The Ralph Lauren Center for Breast Cancer Research

Histopathology Lab

ICR-CTSU

Non-Academic collaborators and Industry partners

