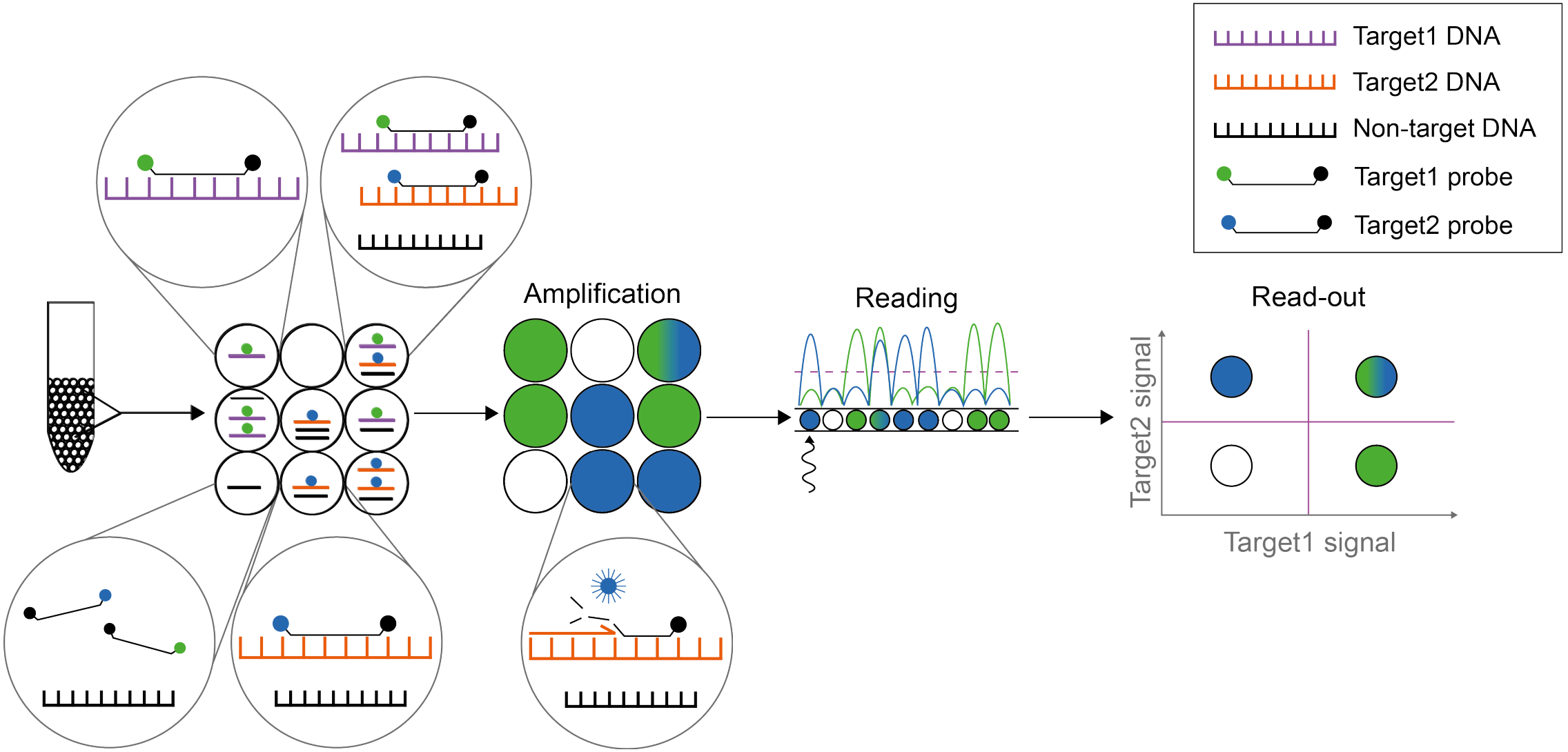
# ctDNA detection – Digital PCR

AARHUS
UNIVERSITY
DEPARTMENT OF CLINICAL MEDICINE

28 MARCH 2022

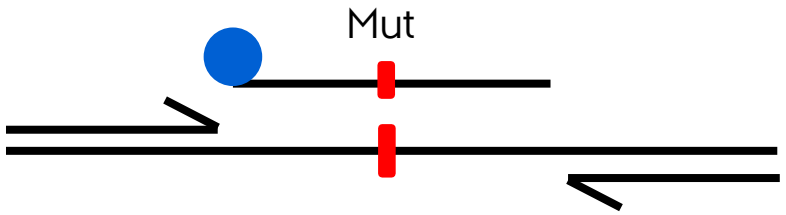
TENNA VESTERMAN HENRIKSEN
PHD STUDENT

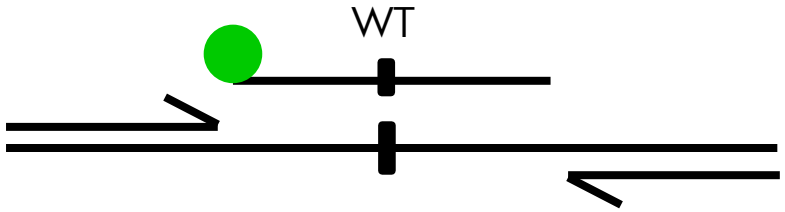

Target1 DNA
Target2 DNA
Non-target DNA
Target1 probe
Target2 probe
Amplification
Reading
Read-out
Target2 signal
Target1 signal

# DIGITAL PCR

| | Bio-Rad | RainDance | Stilla | ThermoFisher | Qiagen |
|---|---|---|---|---|---|
| System | QX200 | RainDrop | Naica | QuanStudio | QiAcuity |
| #Colours | 2 (6) | 2 | 6 | 4 | 5 |
| Partition method | Droplet emulsion | Droplet emulsion | Droplet lattice | Micro-chambers | Micro-chambers |
| #Partitions/reaction | ~20,000 | ~1,000,000 | 15,000-30,000 | 20,480 | 26,000 |

# EXAMPLE 1




Target1: Tumor-specific SNV

Target2: Corresponding WT

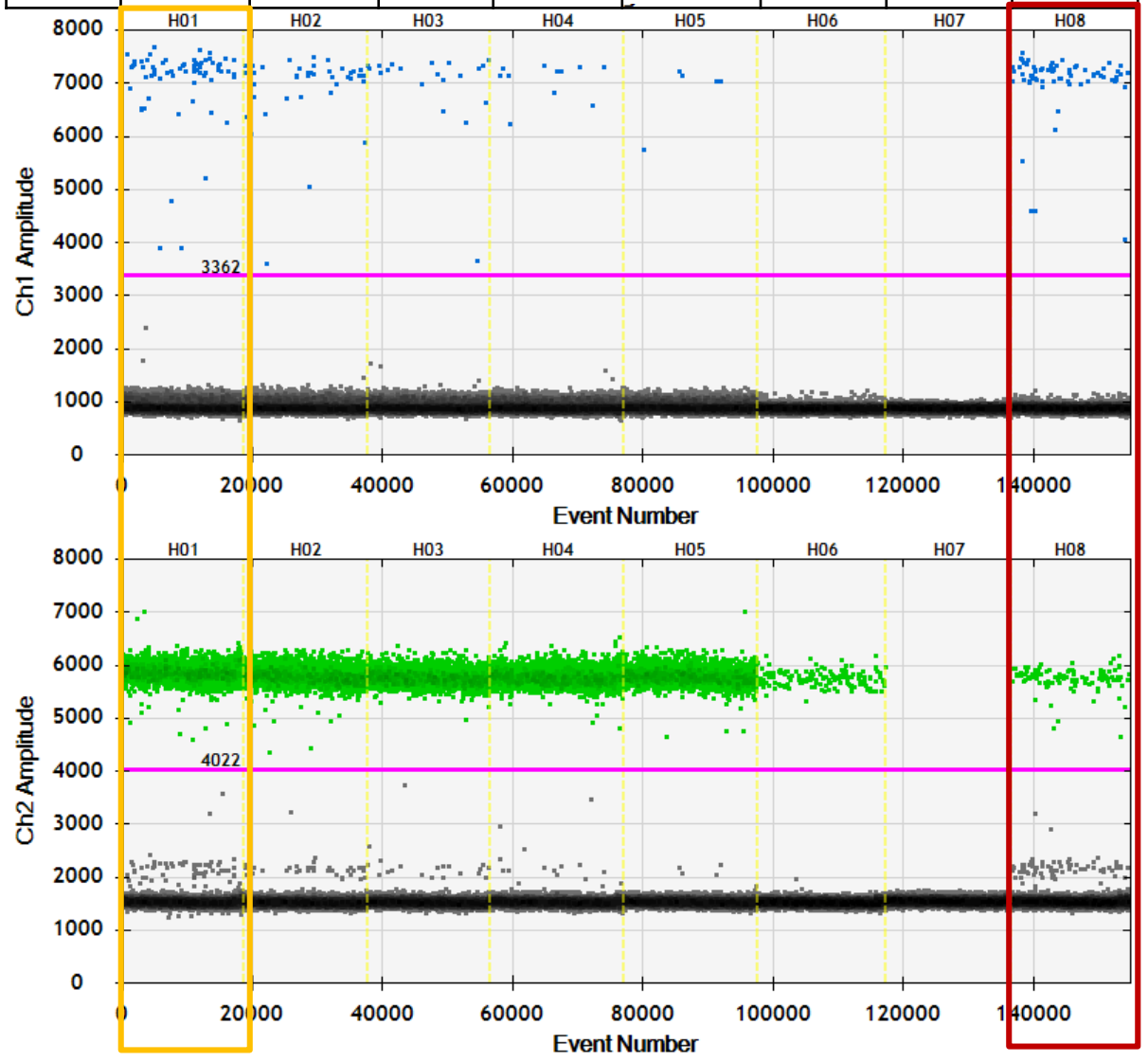
Dilution of tumor DNA in constant WT background

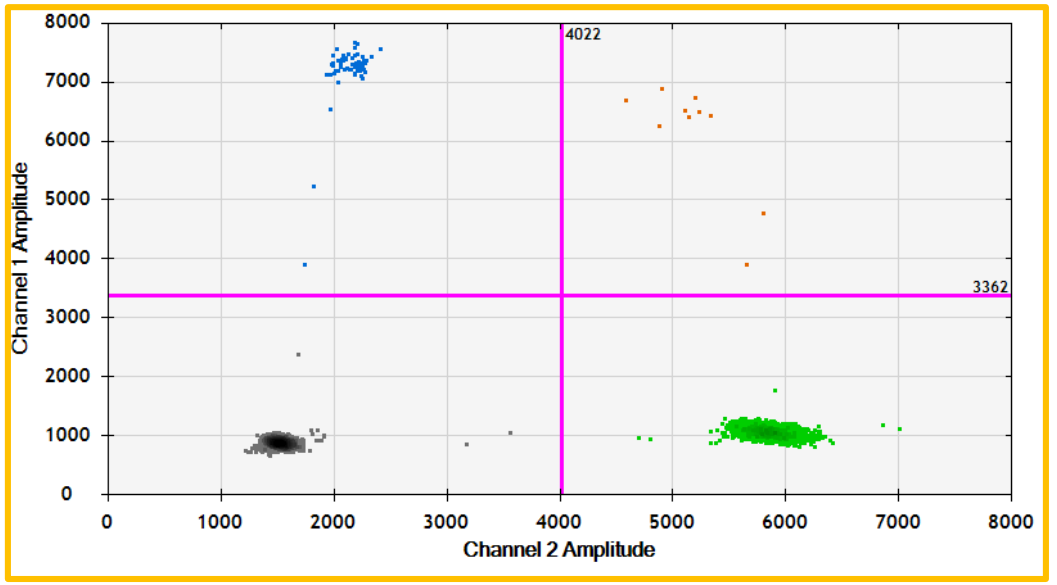
| | Dilutions | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | Neg | NTC | Pos |
| Tumor | 100 | 50 | 25 | 13 | 6 | 0 | 0 | 100 |
| WT | 5000 | 5000 | 5000 | 5000 | 5000 | 150 | 0 | 150 |
| AF(%) | 2% | 1% | 0.5% | 0.25% | 0.13% | 0% | - | 40% |

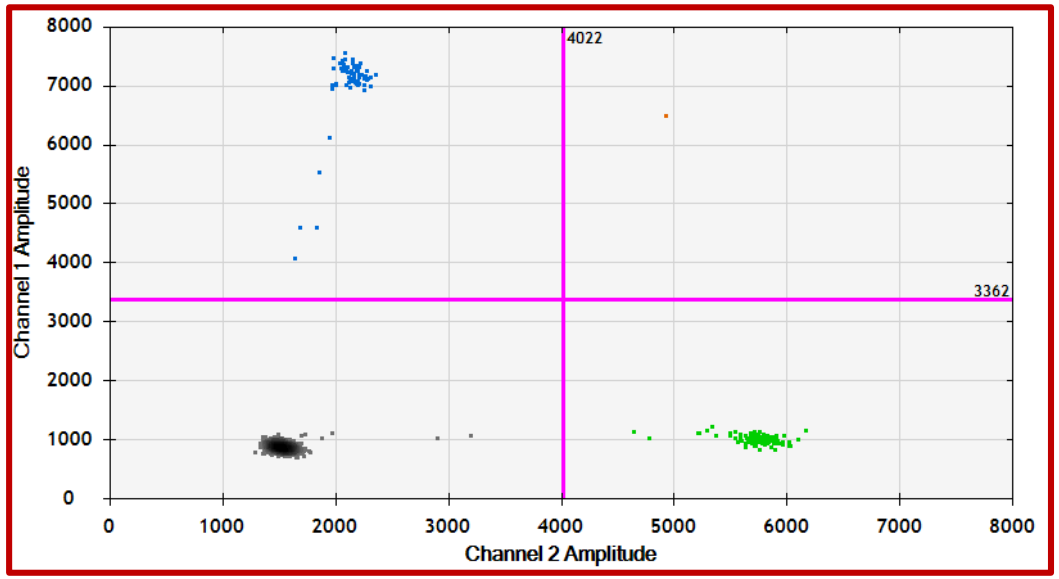
| | Dilutions | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | Neg | NTC | Pos |
| Tumor | 100 | 50 | 25 | 13 | 6 | 0 | 0 | 100 |
| WT | 5000 | 5000 | 5000 | 5000 | 5000 | 150 | 0 | 150 |
| AF(%) | 2% | 1% | 0.5% | 0.25% | 0.13% | 0% | - | 40% |

Mutation

Wildtype



Poisson statistics

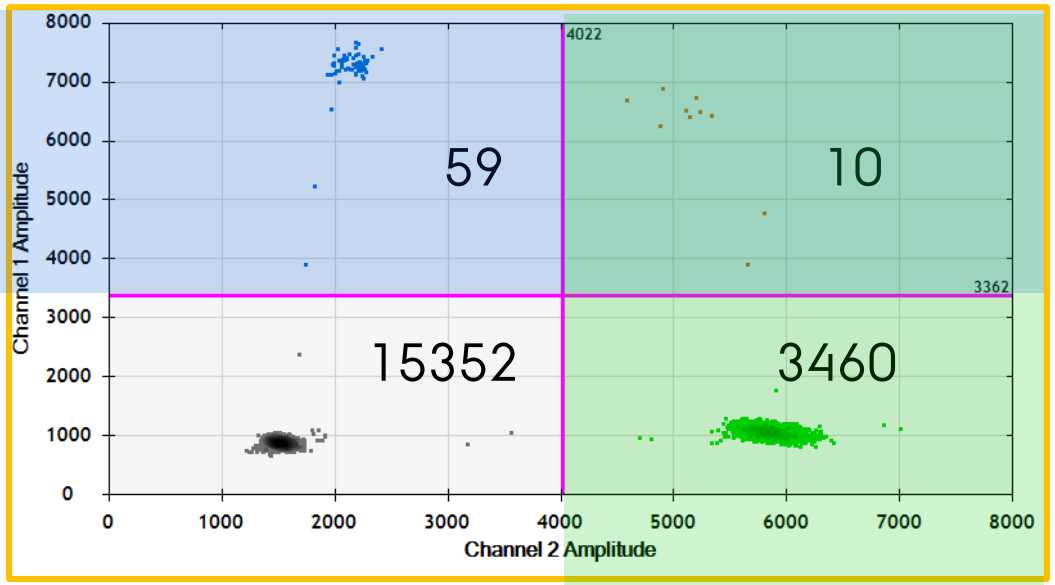
Targets/partition

$$p(k) = \frac{\lambda^k e^{-\lambda}}{k!}$$

#copies

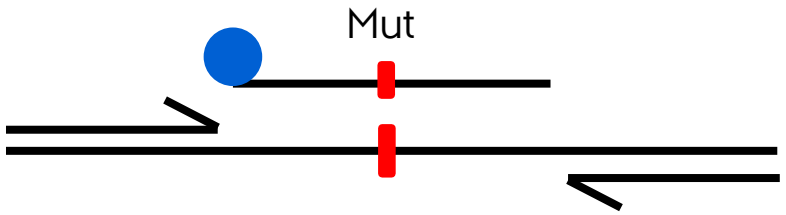
Assumption: λ is small
(large amount of empty droplets)

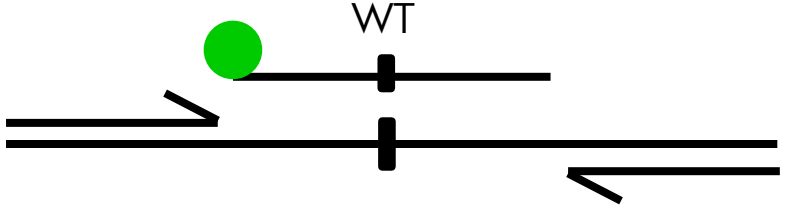
69 droplets
=
86 copies




3470 droplets
=
4780 copies

# EXAMPLE 2




Target1: Tumor-specific SNV

Target2: Corresponding WT

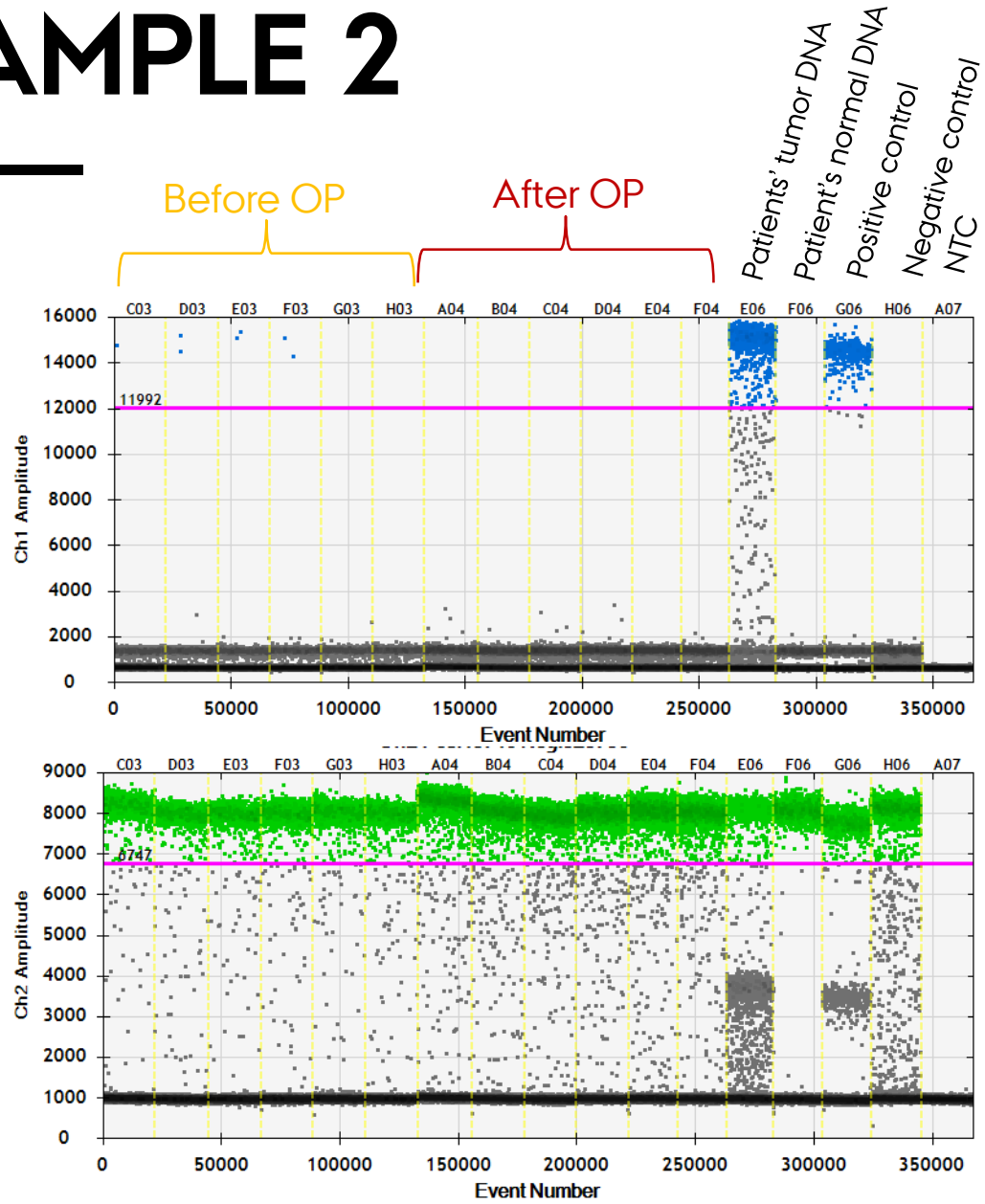
Plasma DNA from colorectal cancer patient

- Before surgery
- After surgery

# EXAMPLE 2

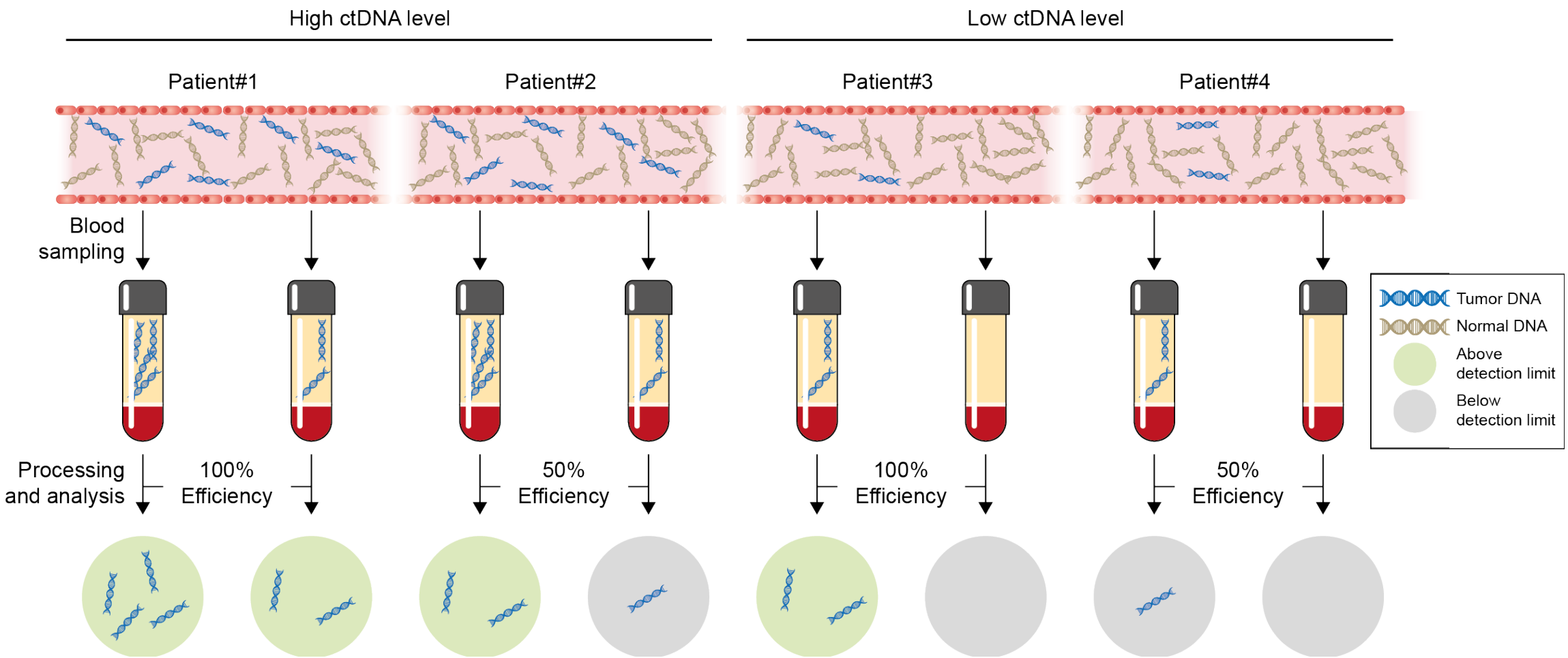
Mutation

Wildtype




| | Before OP | After OP |
|---|---|---|
| #Droplets (Mut/WT) | 7/10,546 | 0/24,938 |
| #Copies (Mut/WT) | 7/11,652 | 0/29,988 |
| AF (%) | 0.064% | 0% |
| ctDNA call | Positive | Negative |

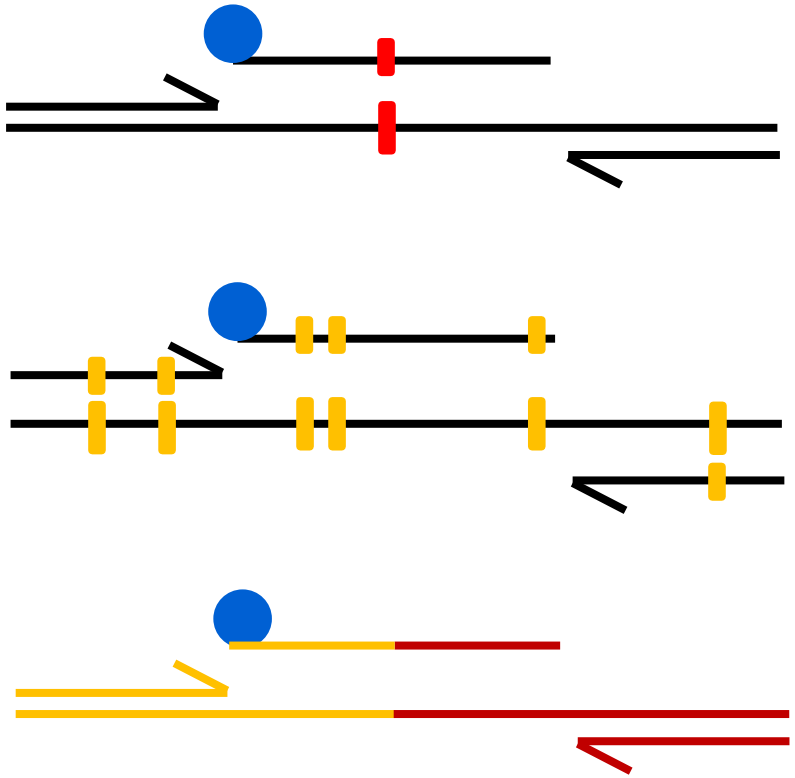
# SUBSAMPLING



# TYPES OF ASSAYS

Targets

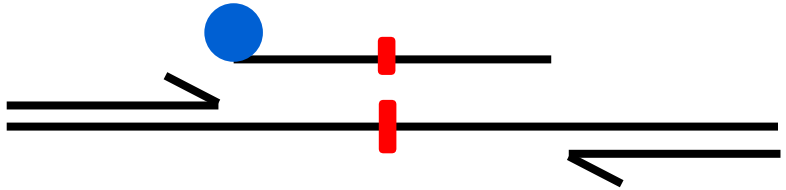
- Single-nucleotide variants (SNVs)
  - Pro: Easy to design
  - Con: Only 1 base to differentiate Mut and WT
- Methylation patterns
  - Pro: Many differentiating positions
  - Con: Requires conversion (bisulfite/enzymatic)
- Large genomic variations (GV)
  - Pro: No PCR amplicon if no GV = Highly specific
  - Con: Difficult to determine breakpoints



# TYPES OF ASSAYS

Assay design

- Single-plex



- Duplex

- Multiplex
  - Multiple colors
  - Different amplitudes

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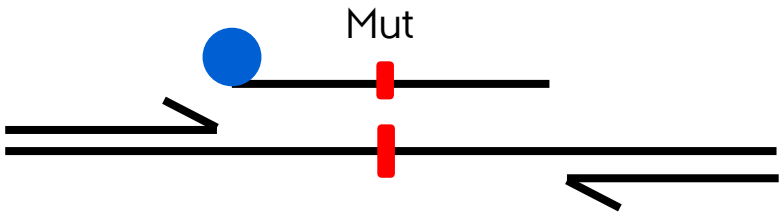


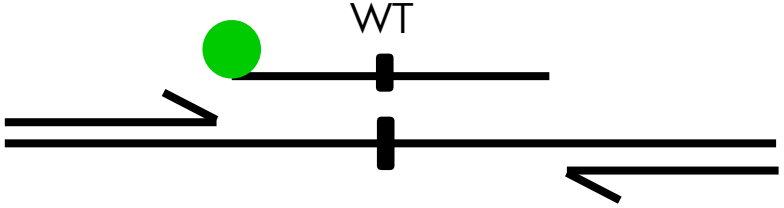


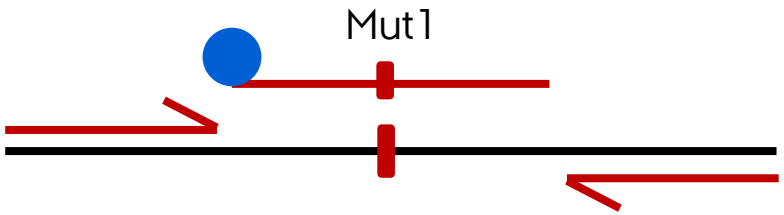


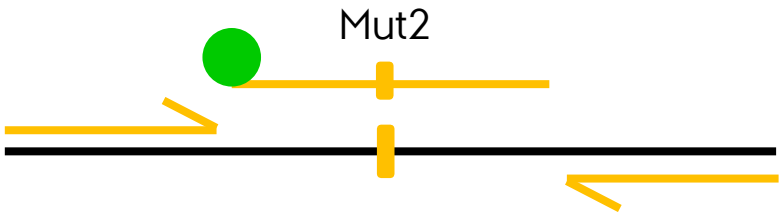

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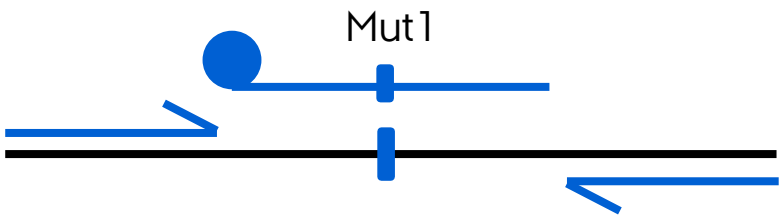


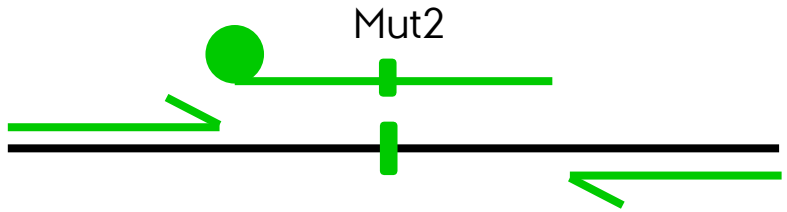


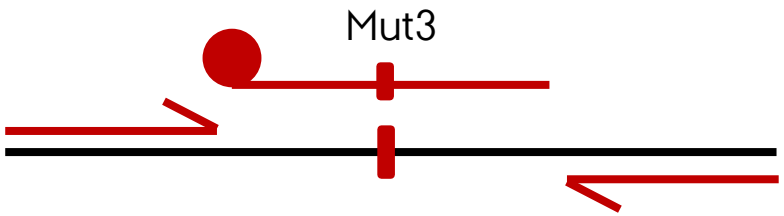


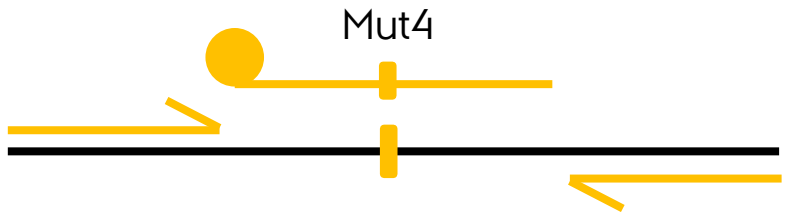

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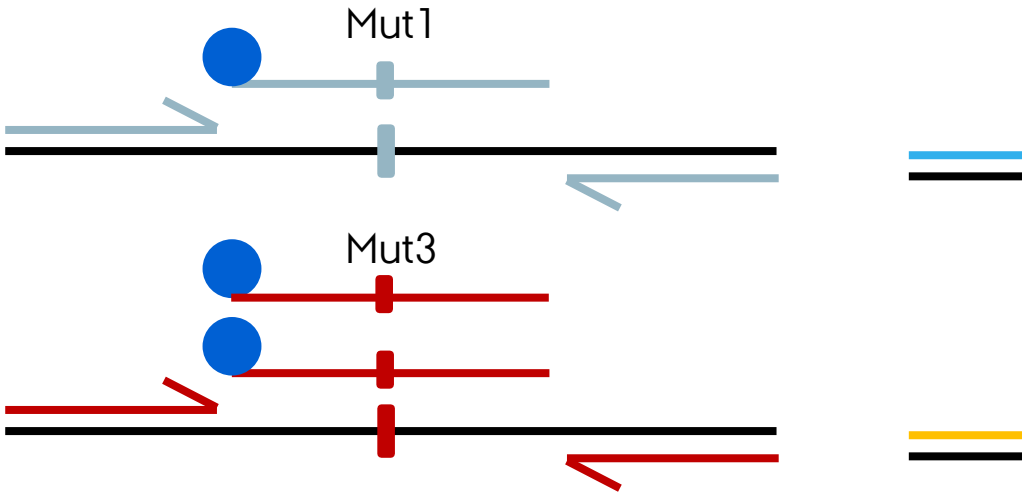


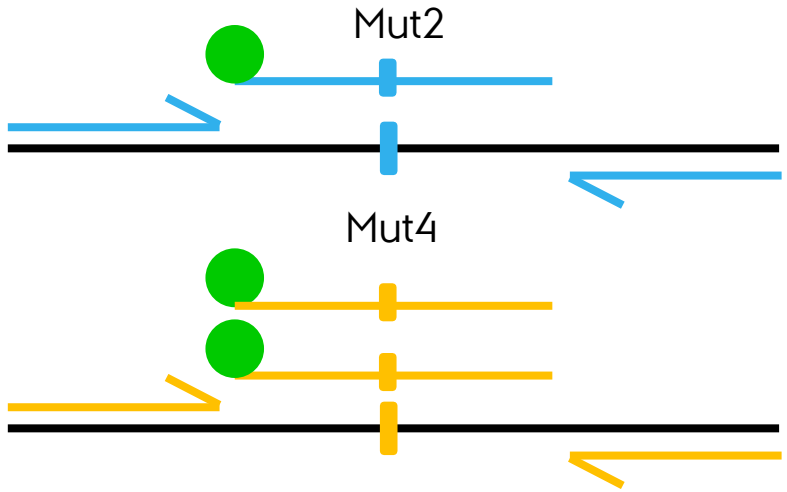


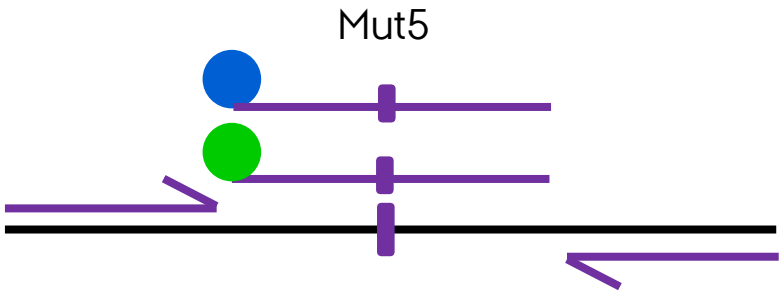


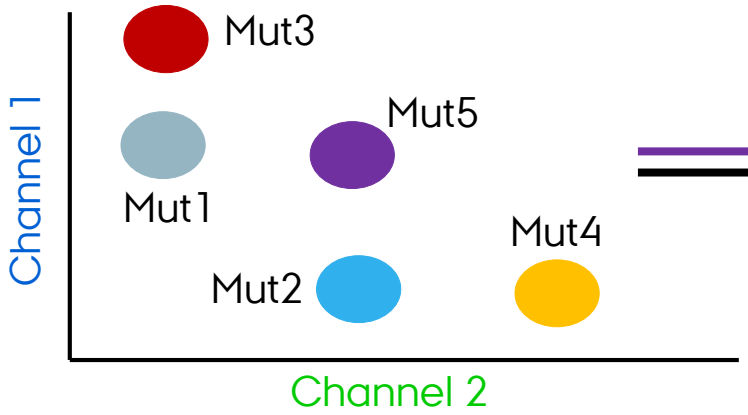

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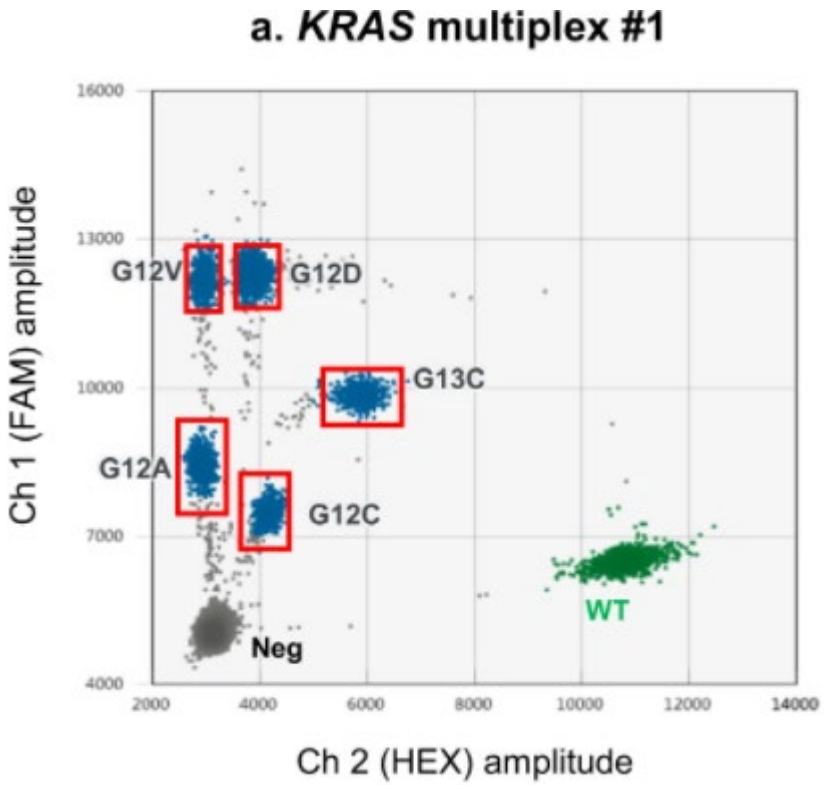
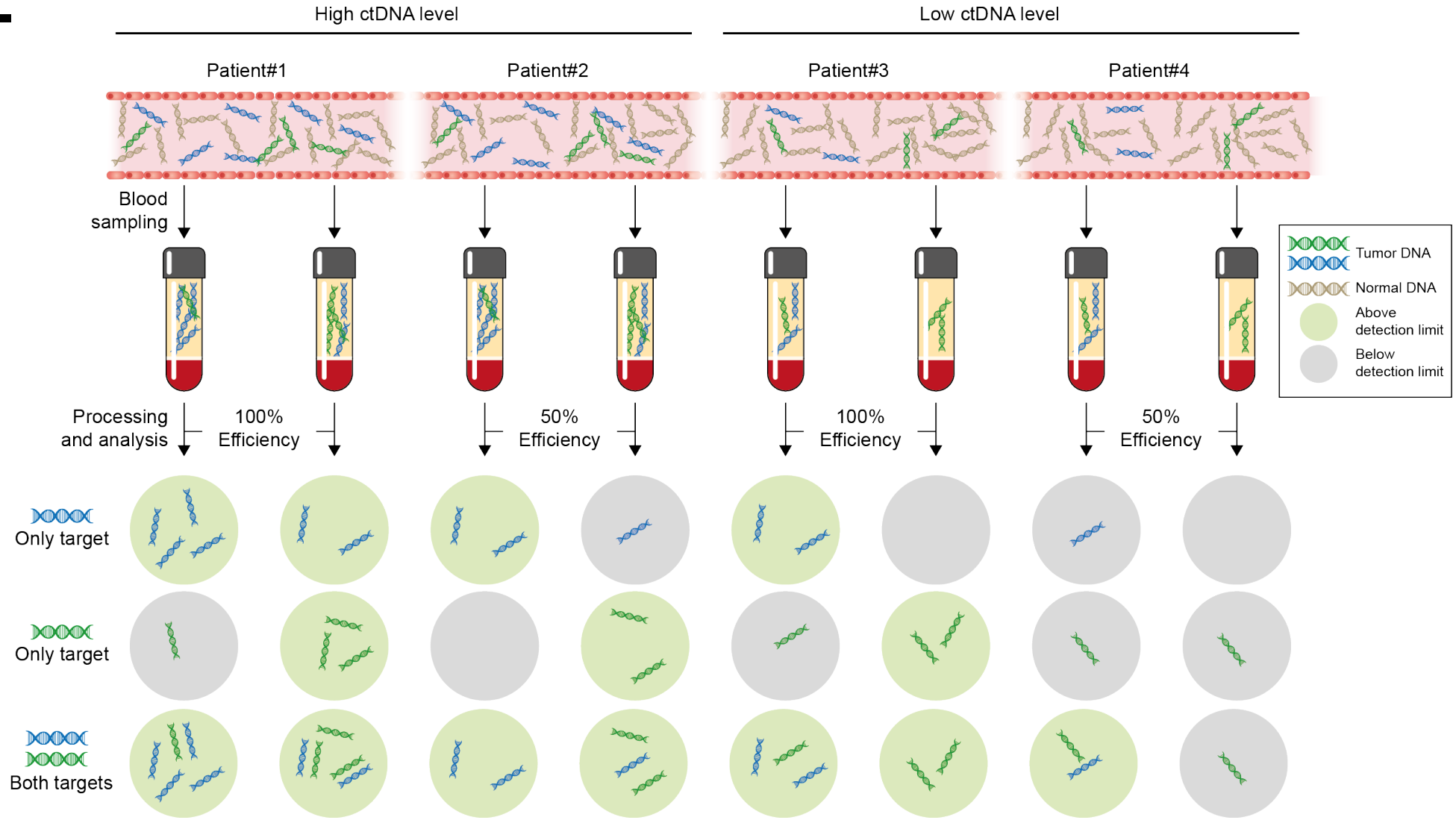



Rowlands et al., *Optimisation of robust singleplex and multiplex droplet digital PCR assays for high confidence mutation detection in circulating tumour DNA*, Scientific Reports, 2019
Figure 9A, Droplet Digital PCR (Bio-Rad)

# WHY MULTIPLEX?



# WORKFLOW

1. Select target

2. Optimize assay
   - Primer/probe concentration
   - PCR conditions

3. Estimate background noise

4. Plasma analysis

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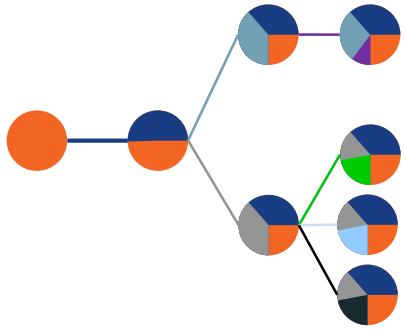
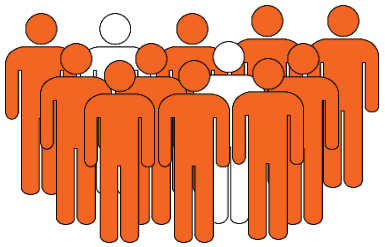


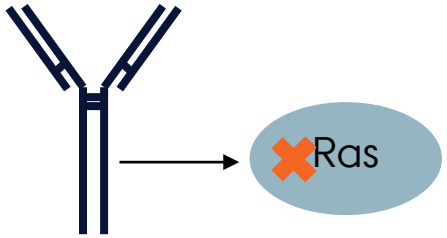
Clonal target

Residual disease detection



Recurrent target

Screening




Functional target

Tracking resistance

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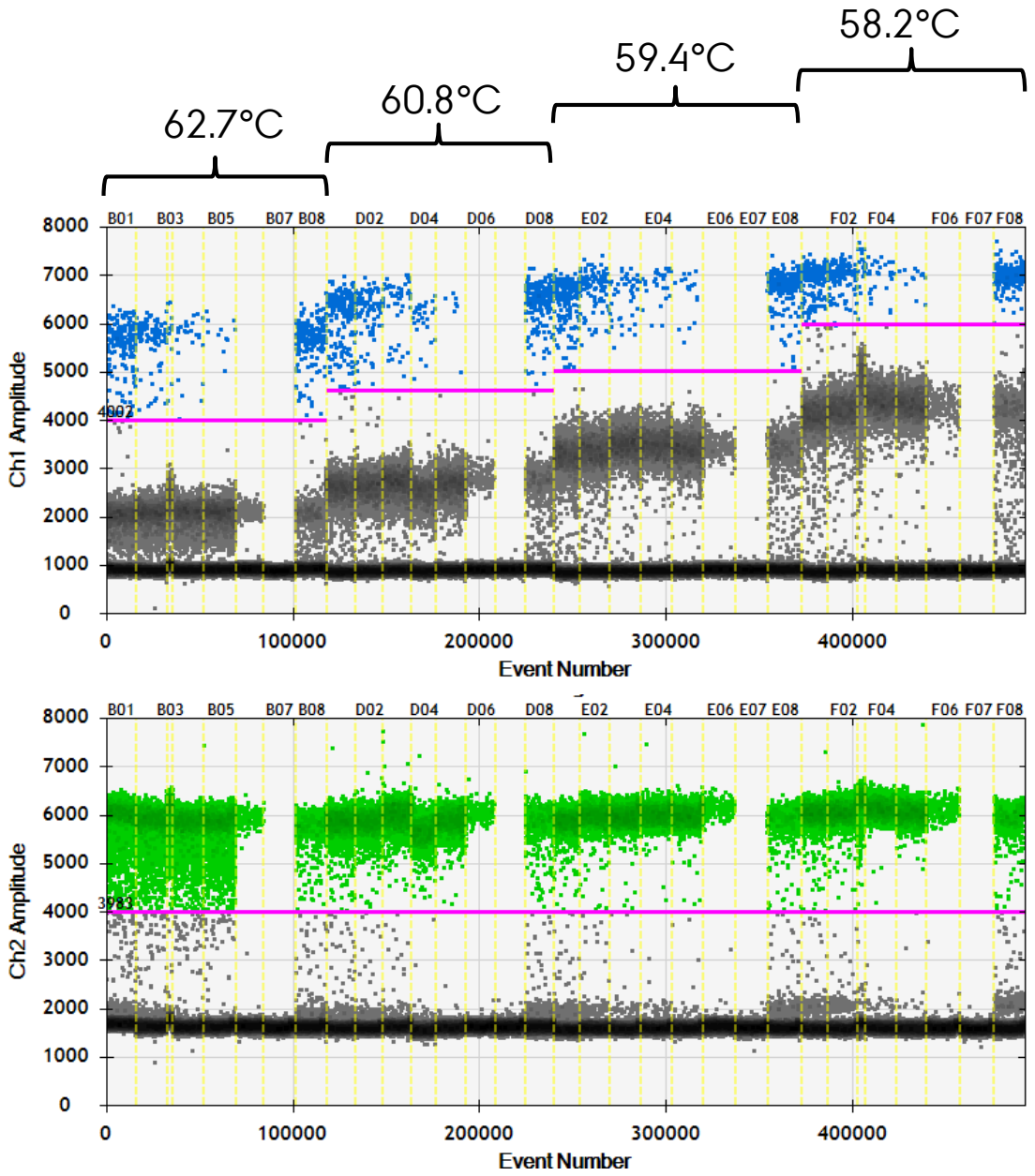

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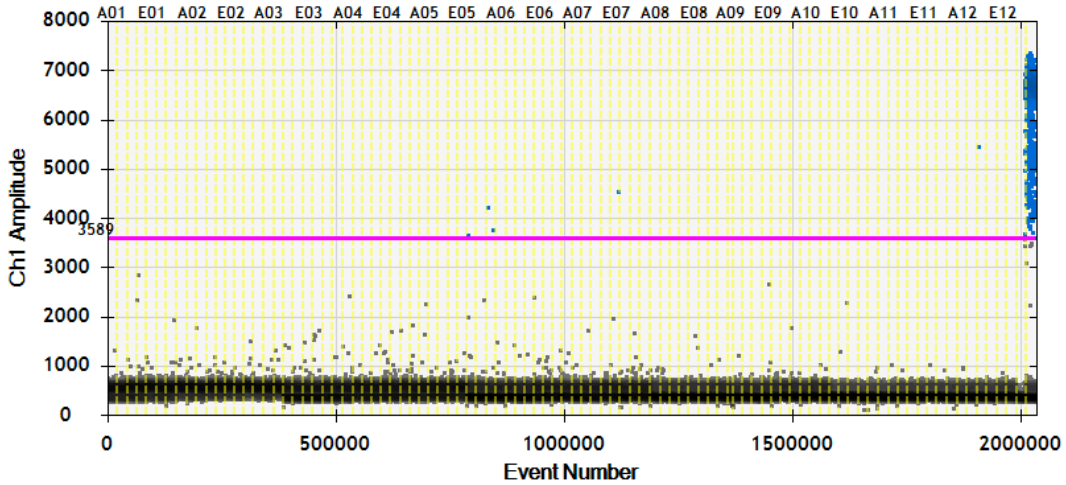
# BACKGROUND NOISE

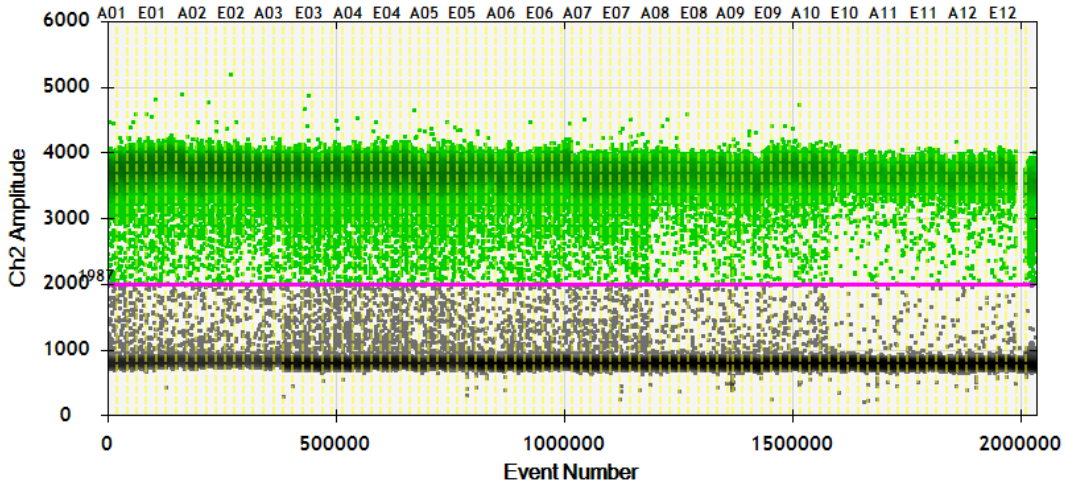
Non-mutated DNA

Assay 1

Mutation

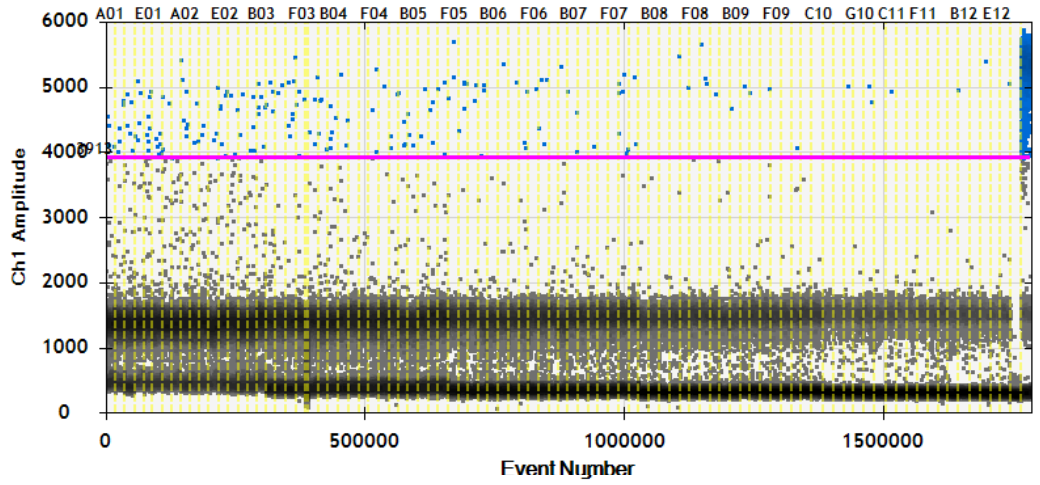


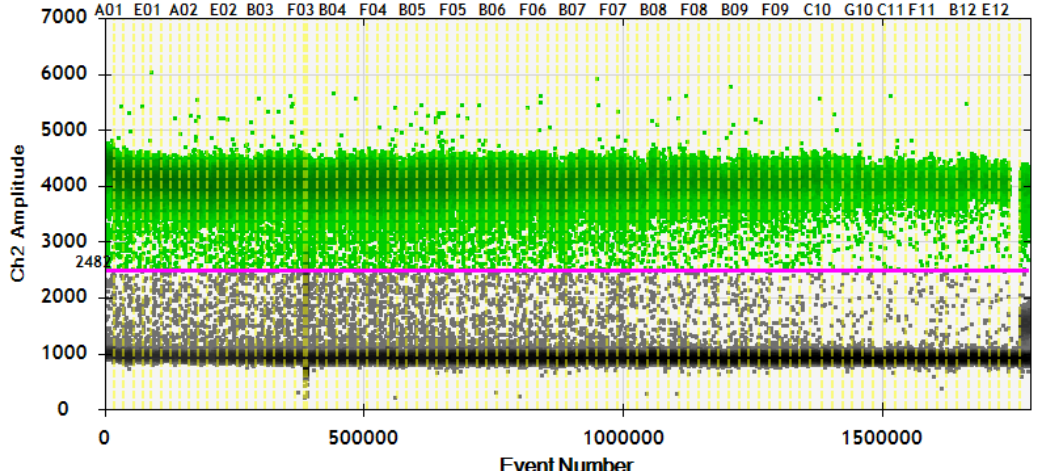

Wildtype



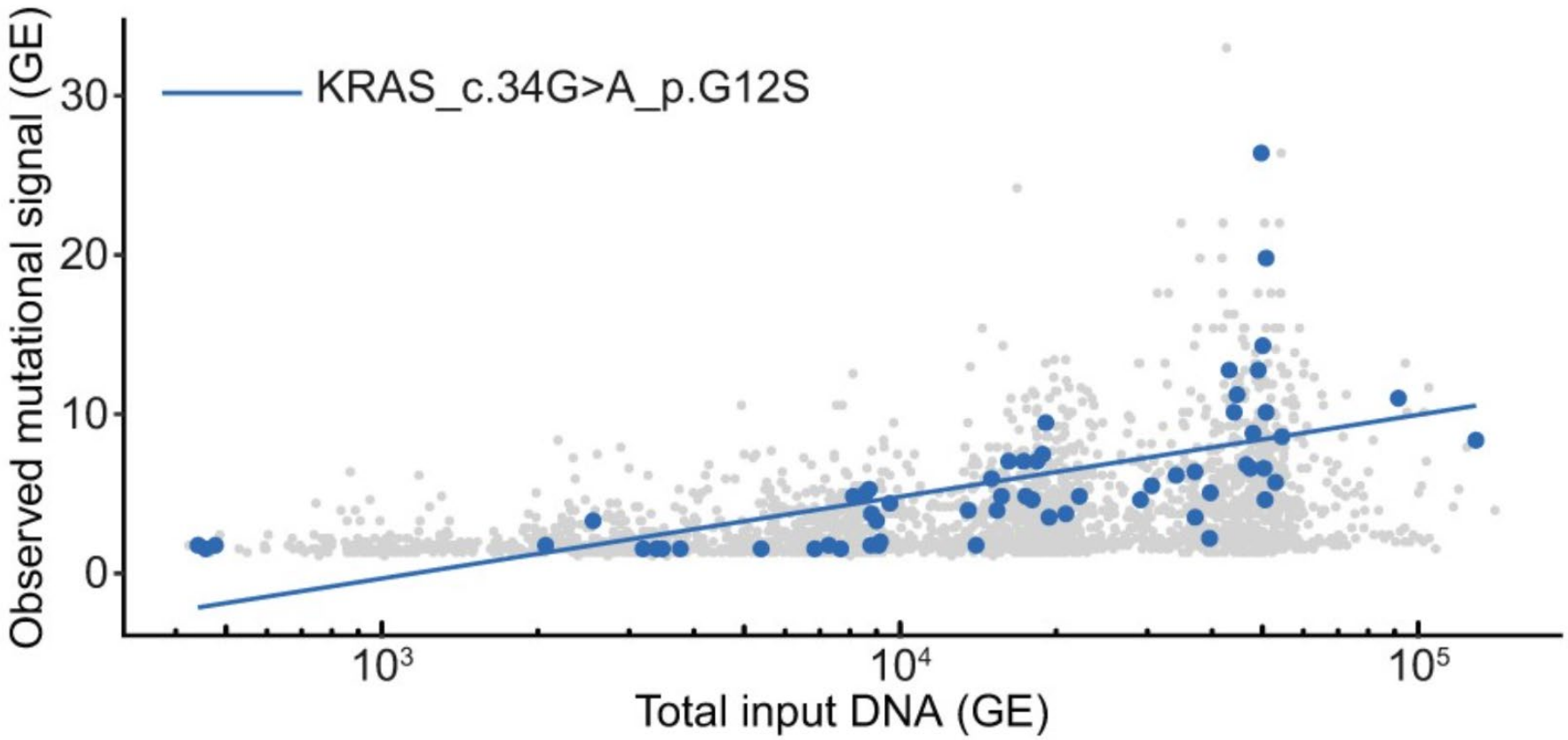

Non-mutated DNA

Assay 2



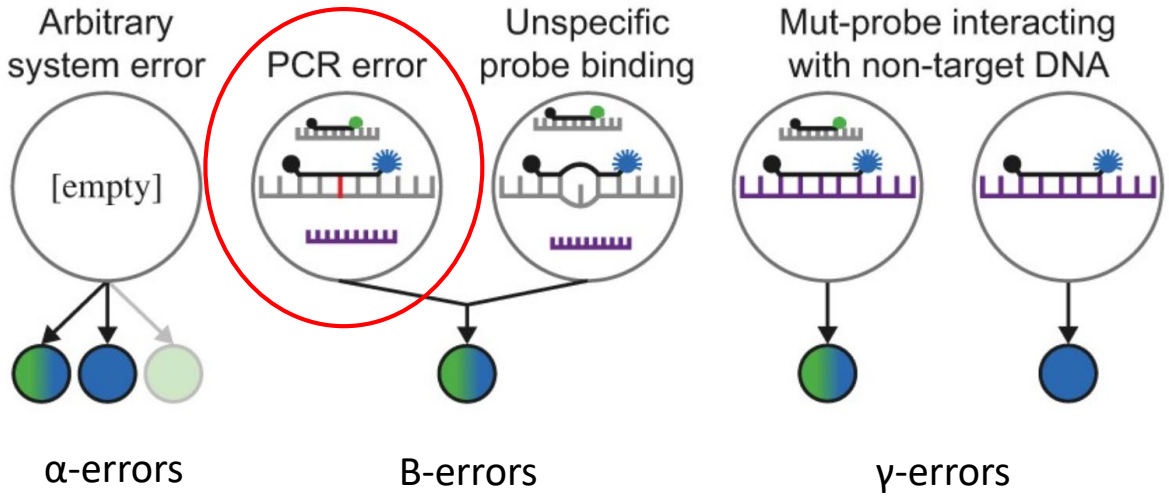



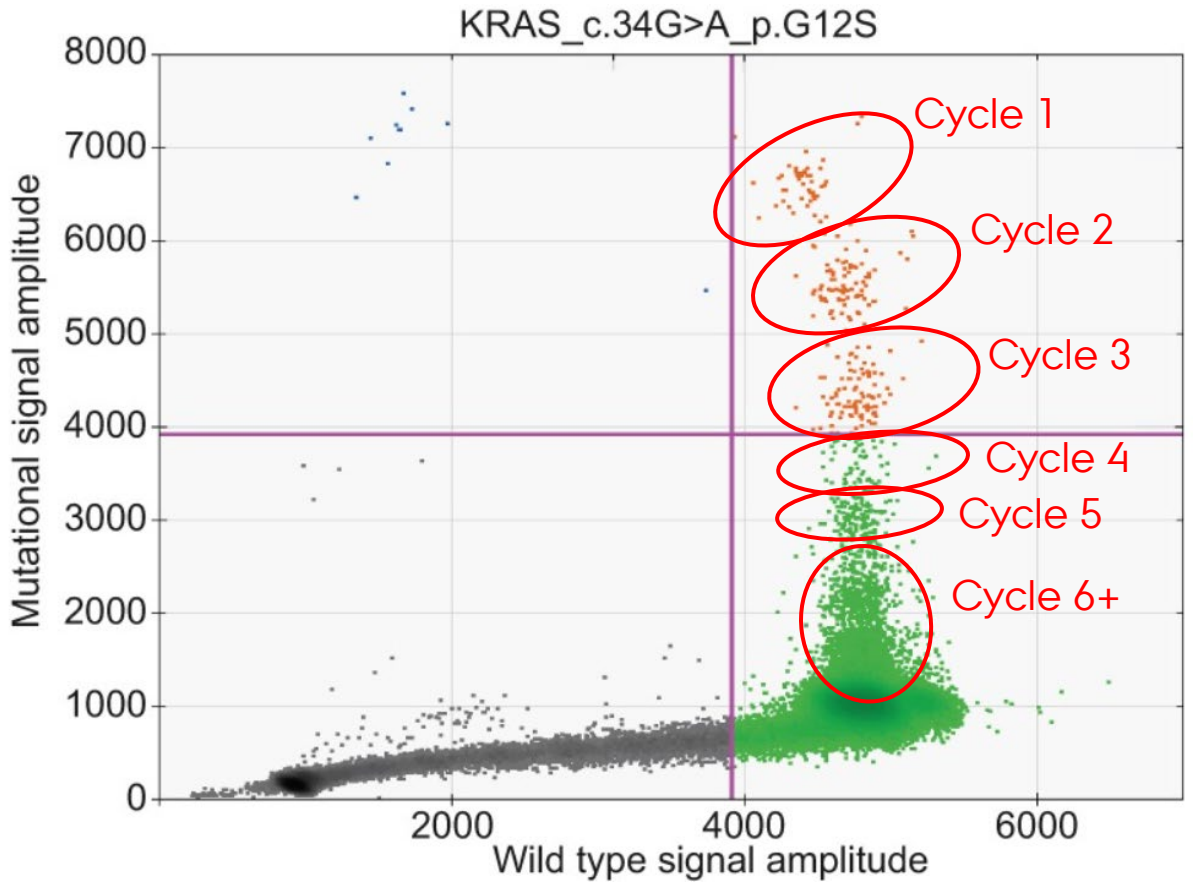
# BACKGROUND NOISE




Henriksen et al., *Error Characterization and Statistical Modeling Improves Circulating Tumor DNA Detection by Droplet Digital PCR*, Clinical Chemistry, 2022

# BACKGROUND NOISE

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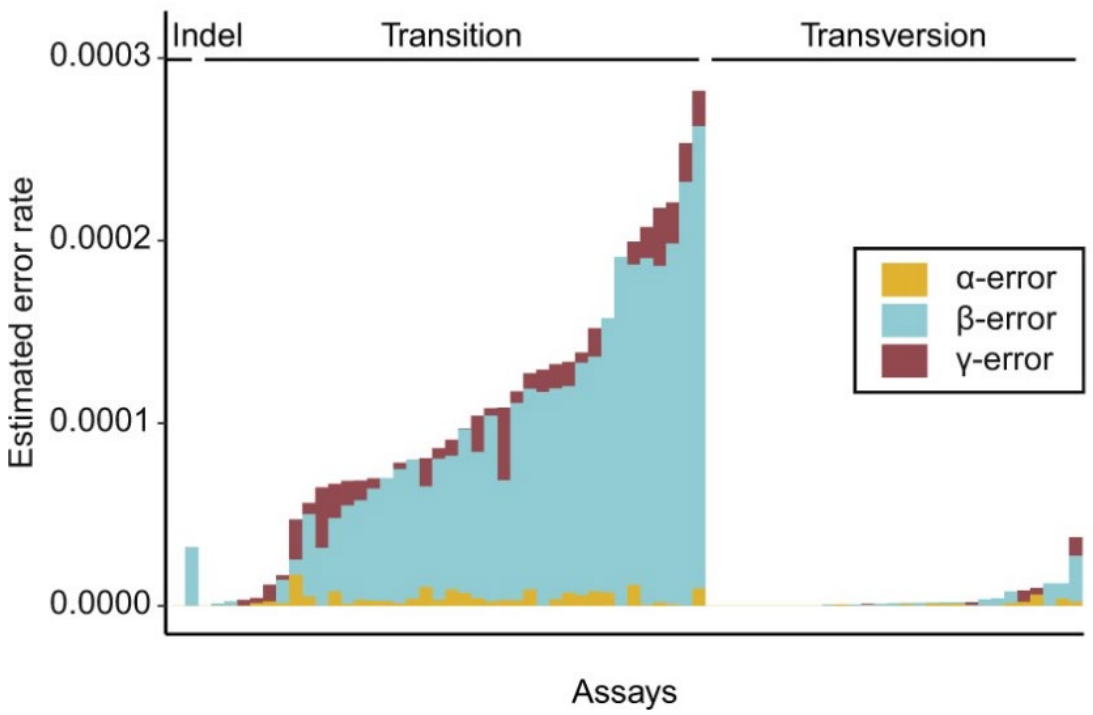


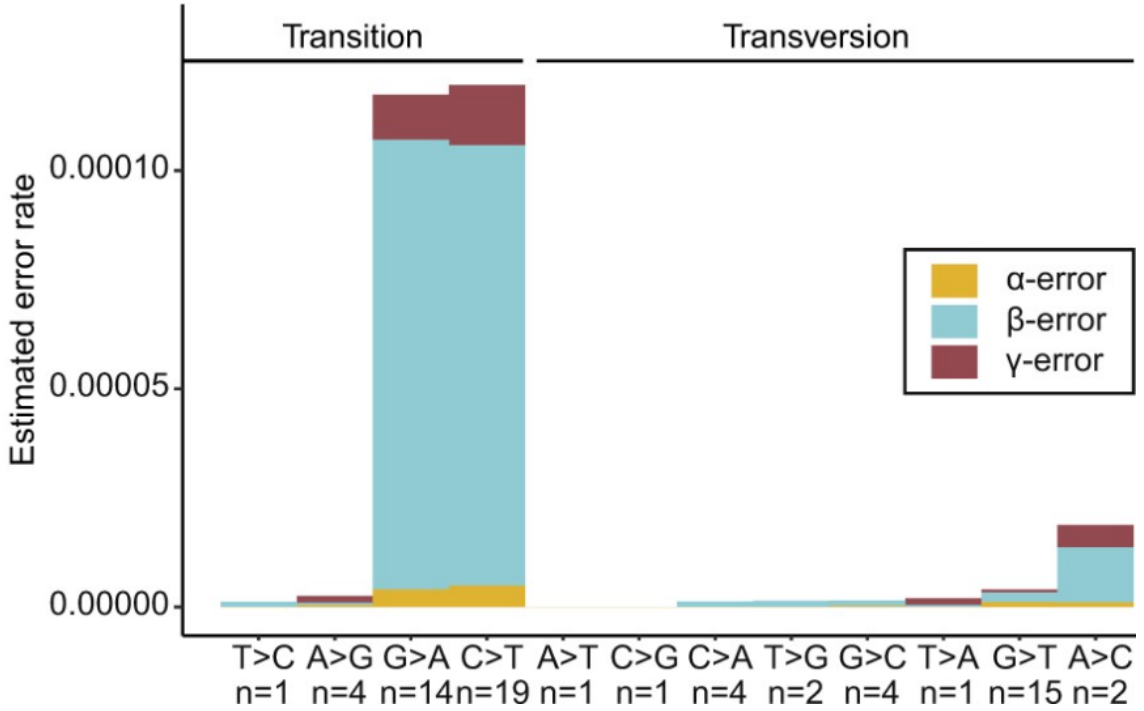


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# SAMPLE CALLING

CASTLE
Poisson
ALPACA
Dynamic LOB
Static LOB

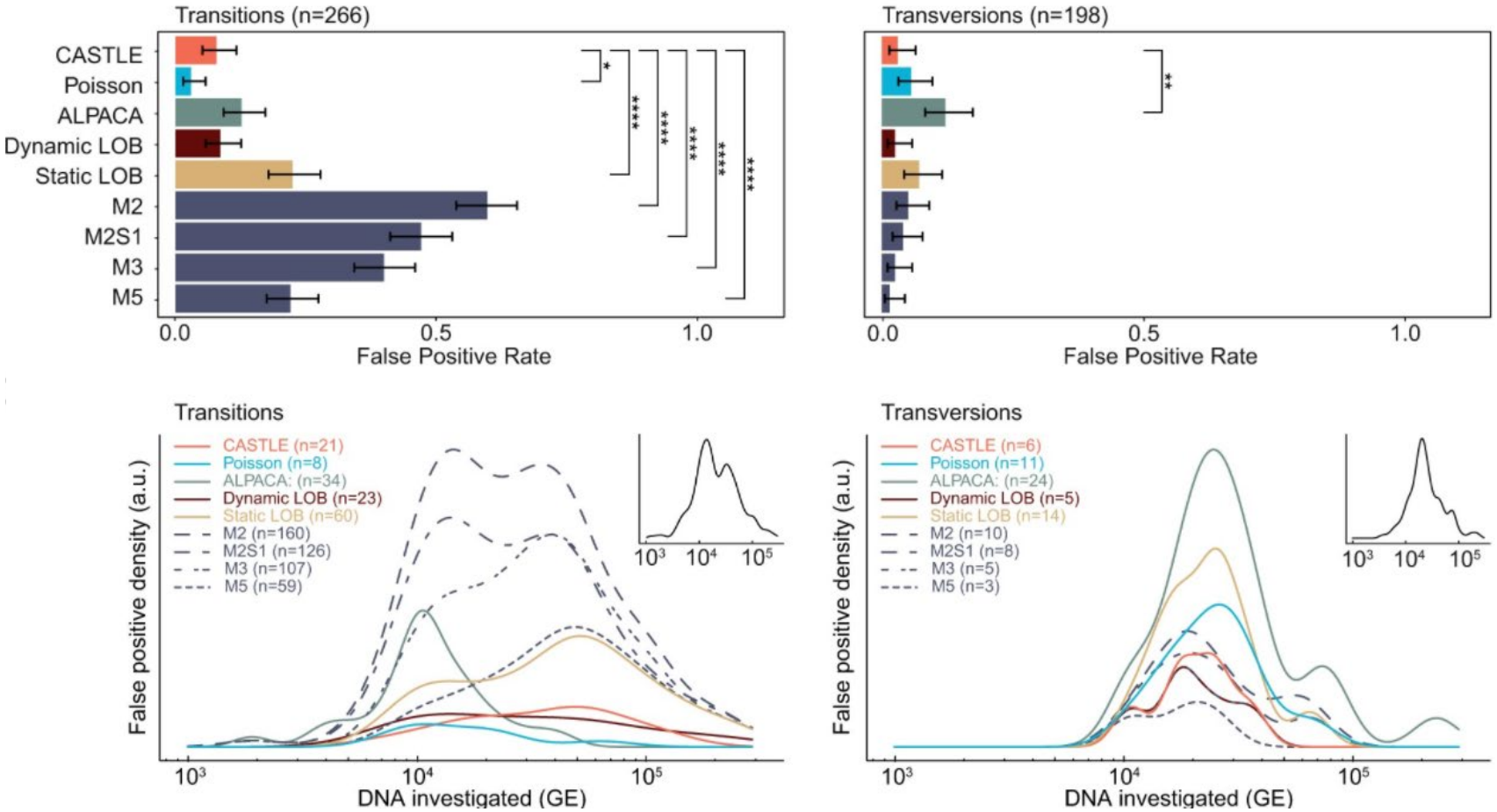
Statistical estimate
Limit Of Blank
Accounts for DNA input
Background-informed

Droplet Cutoffs

- M2 – Min 2 mut droplets
- M2S1 – Min 2 mut droplets (1 singlepos)
- M3 – Min 3 mut droplets
- M5 – Min 5 mut droplets

Un-informed

# SAMPLE CALLING



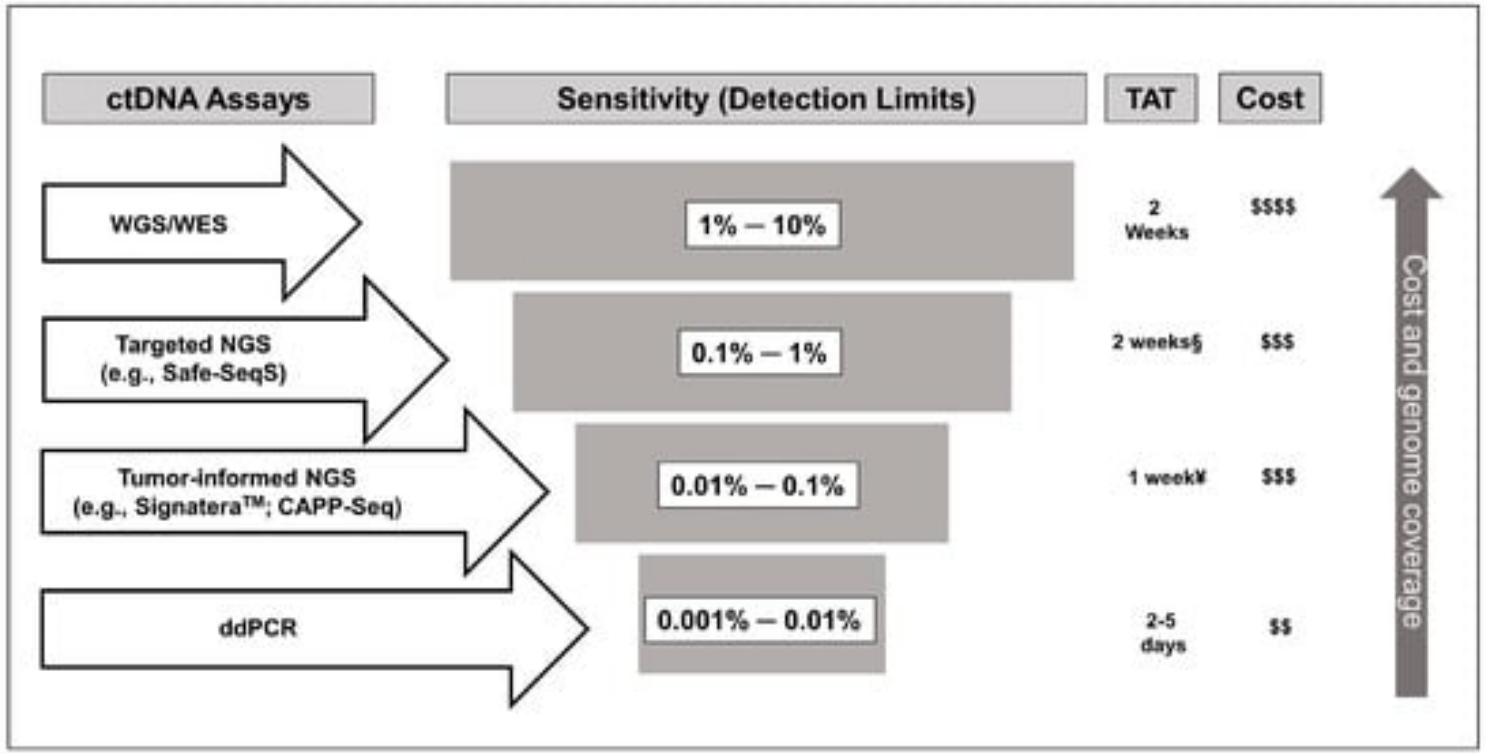

Henriksen et al., *Error Characterization and Statistical Modeling Improves Circulating Tumor DNA Detection by Droplet Digital PCR*, Clinical Chemistry, 2022

# SAMPLE CALLING

Train on non-mutated samples → expected noise profile

Account for DNA-input concentration → high input = more noise

# WHY (NOT) dPCR?




Chakrabarti et al., *The Promise of Circulating Tumor DNA (ctDNA) in the Management of Early-Stage Colon Cancer: A Critical Review*, Cancers, 2020

# WRAP UP

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