Apport de la Next Generation ddPCR dans les analyses génétiques: Exemples des applications Onco-génétique

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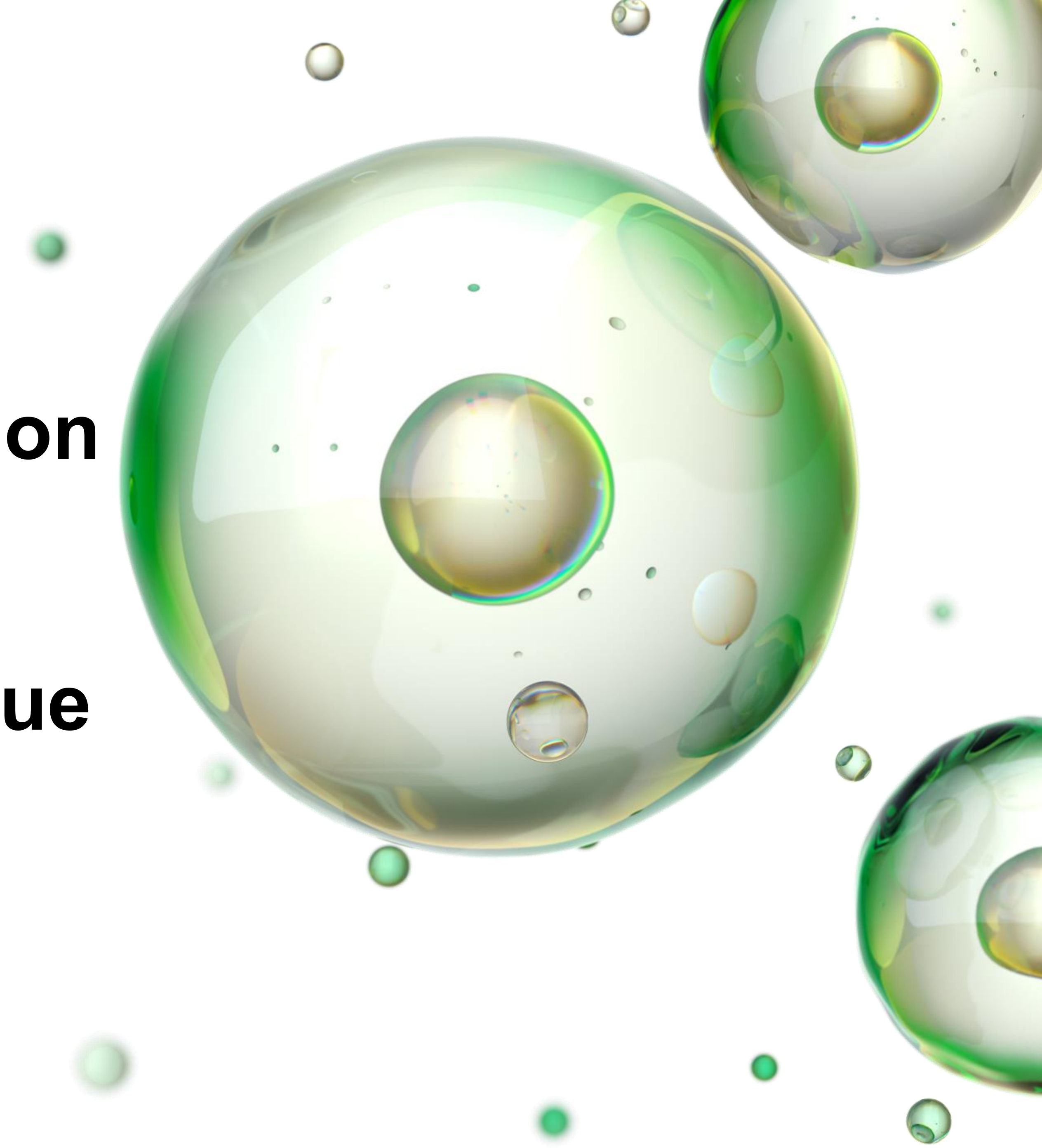
28ème congrès national de cancérologie et de radiothérapie



Overcome FFPE & Liquid Biopsy analysis challenges

with Droplet Digital PCR®

FFPE & Liquid Biopsy samples provides multiple analyte information (Methylation, cfDNA, cfRNA…)

Flexible method to address multiple targets

FFPE could be challenging for some molecular technologies

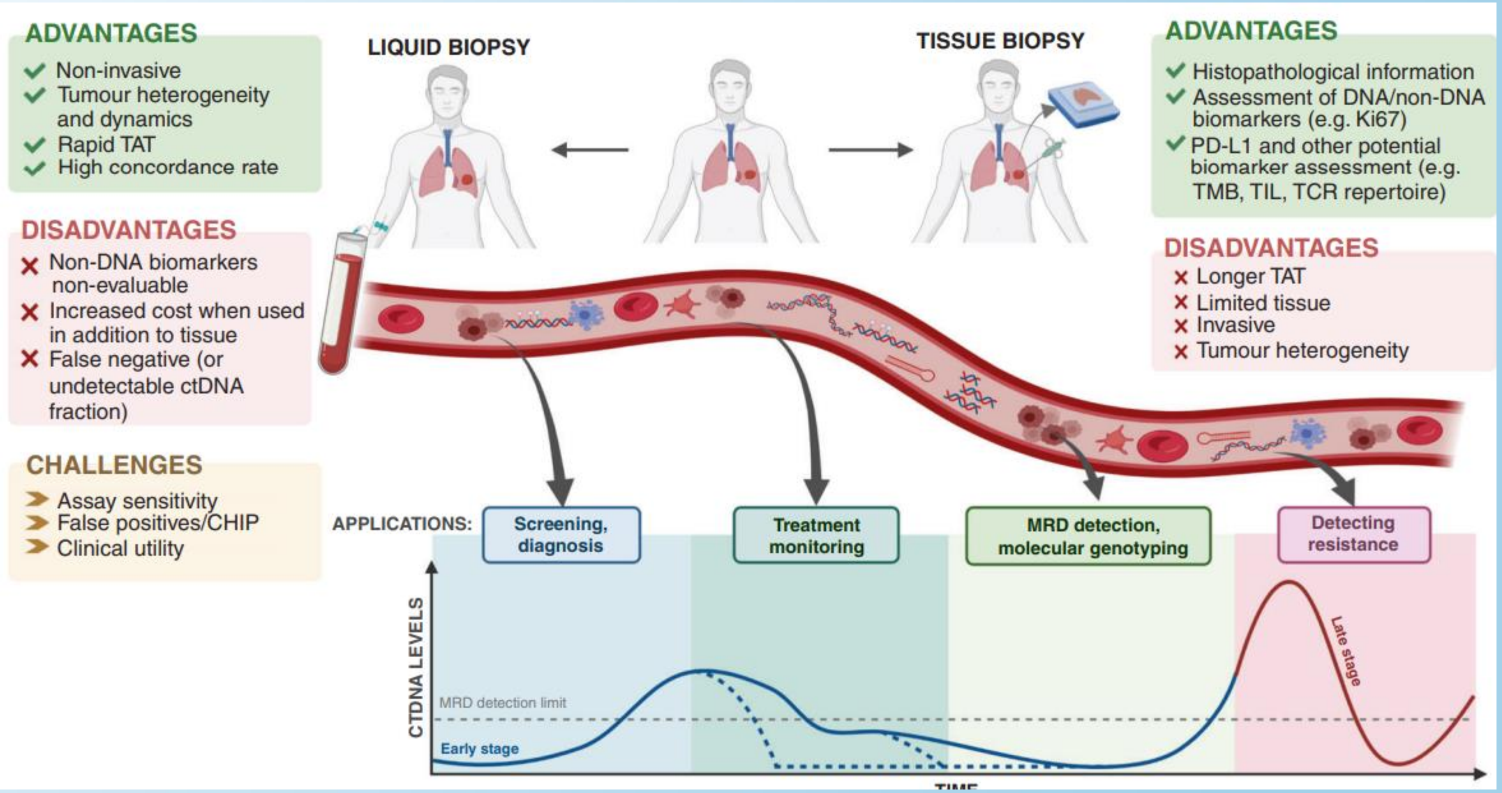
Poor DNA quality & quantity with variability

CtDNA concentration is too variable, and mutation frequency (MAF) is between 0,1-10%

High detection sensitivity required

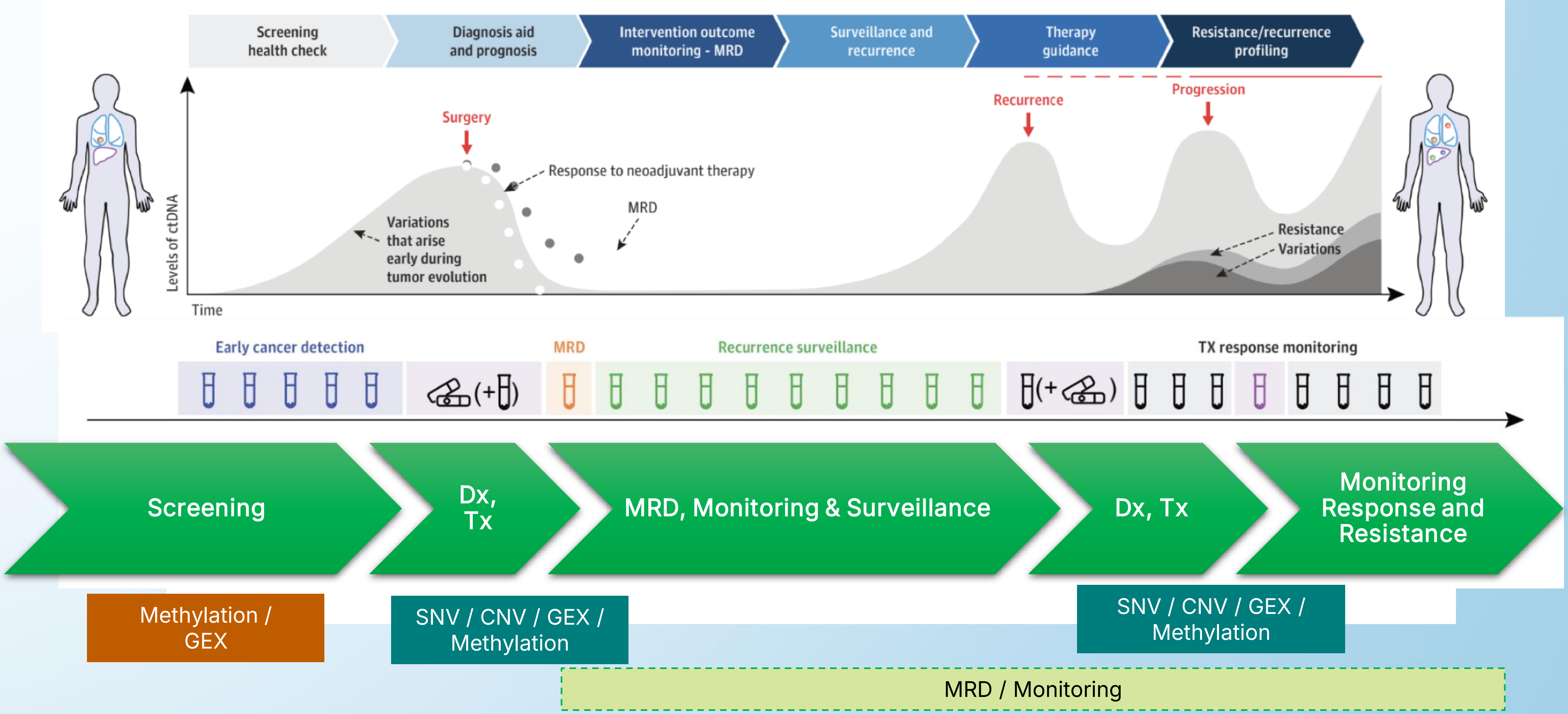
Mutation coverage as high as possible to avoid wasting precious samples

Detection methods needs to screen maximum information with minimum volumes



M. Garcia-Pardo et al.2022

ddPCR Liquid Biopsy Solutions



Screening

Dx, Tx

MRD, Monitoring & Surveillance

Dx, Tx

Monitoring Response and Resistance

Methylation / GEX

SNV / CNV / GEX / Methylation

SNV / CNV / GEX / Methylation

MRD / Monitoring

Available

Partnership/ Enablement

In Development: Internal and/or partnerships

Modified from: Krebs, M., et al. “Practical Considerations for the Use of Circulating Tumor DNA in the Treatment of Patients With Cancer.” JAMA Oncology, 8.12 (2022): 1830-1839.

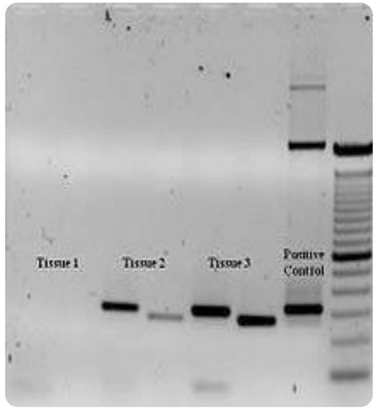
Simplifying Onco-genetics experiments

SAVE TIME

REDUCE COST

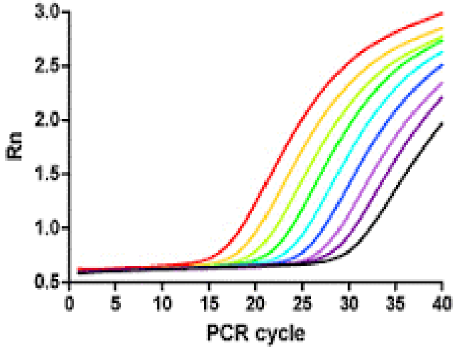
- Detect up to 21 genetic targets in a single reaction, using less reagent.
- One consumable—Ruby chip: Simplifies workflows with a single pipetting step and pre-filled oil chambers, reducing manual effort.
- Minimal data storage needs
- Lower computational requirements – No complex bioinformatics pipelines or alignment processes.

PCR



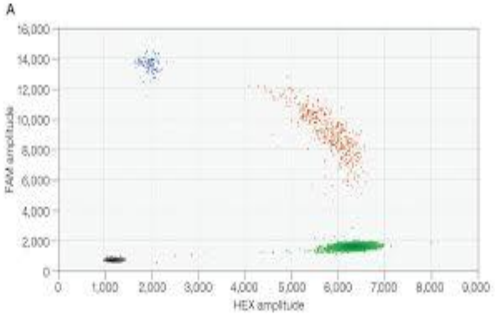
- Amplify Target RNA

QUANTITATIVE PCR



- Relative quantification
- Real-time with standard curves
- Ubiquitously spread method

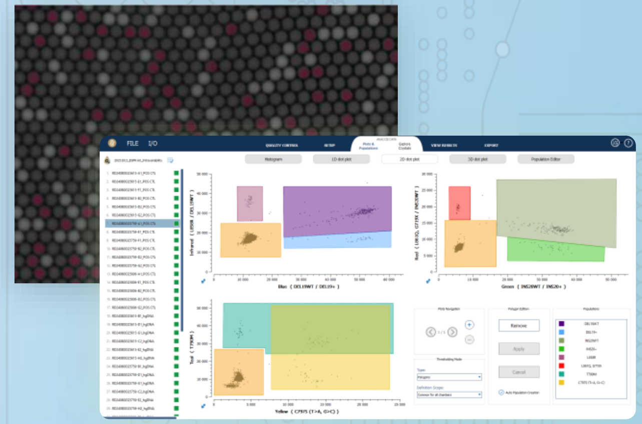
ddPCR



- Absolute quantification
- No standard curve
- Manual workflow & Cost

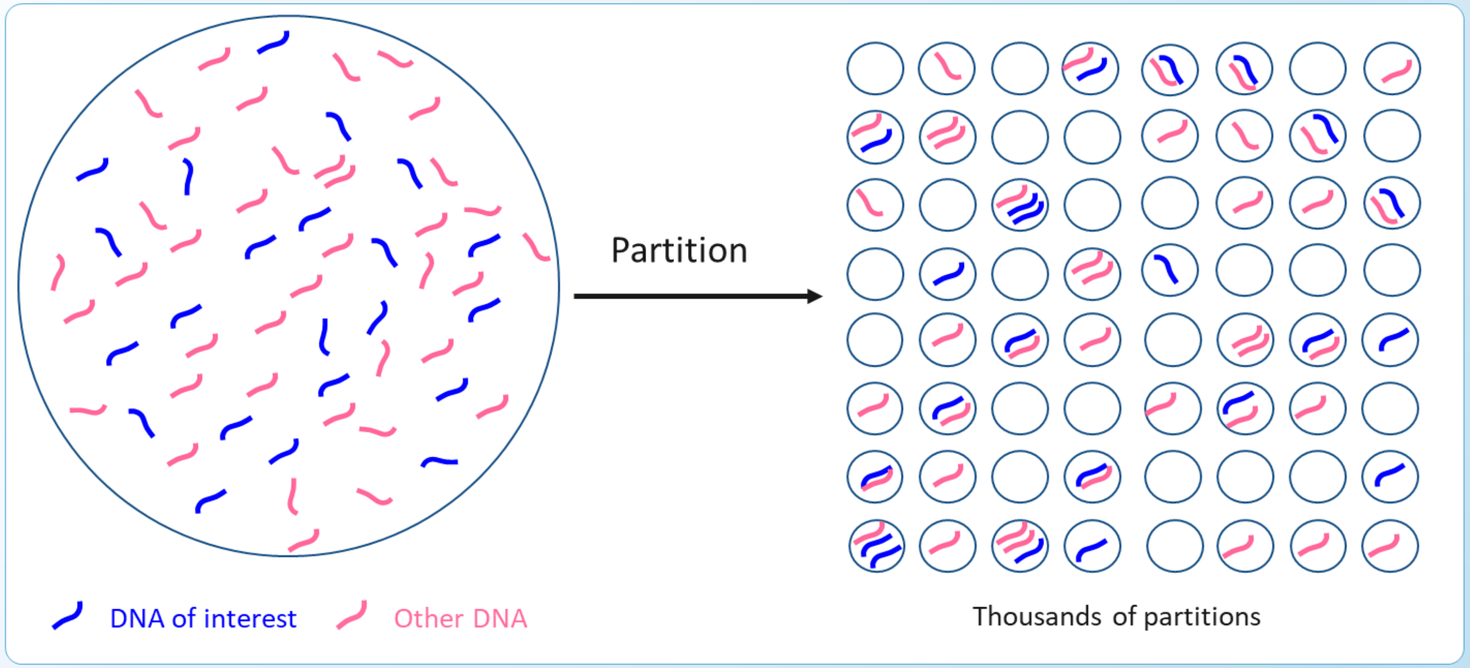
The Next Generation ddPCR

Droplet Digital PCR®



- **Easy to use – fully automated**
- **Continous loading**
- **High Multiplexing – 21 plex**
- Absolute quantification
- No standard curve
- Increased sensitivity

ddPCR basic principle: Limit of dilution



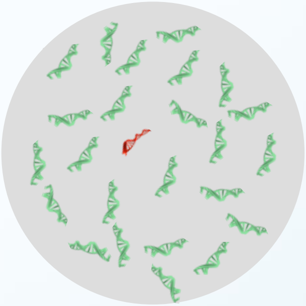
- Limit of dilution means each partition will be positive (1) or negative (0)
- The <u>Limit of dilution</u> is defined as the number of copies in the analyzed volume needed to reach a normal distribution of the results
- Optimal number of partitions and input DNA concentration minimizes number of partitions with 1.6 copy of each gene

<u>Partitioning advantages:</u>

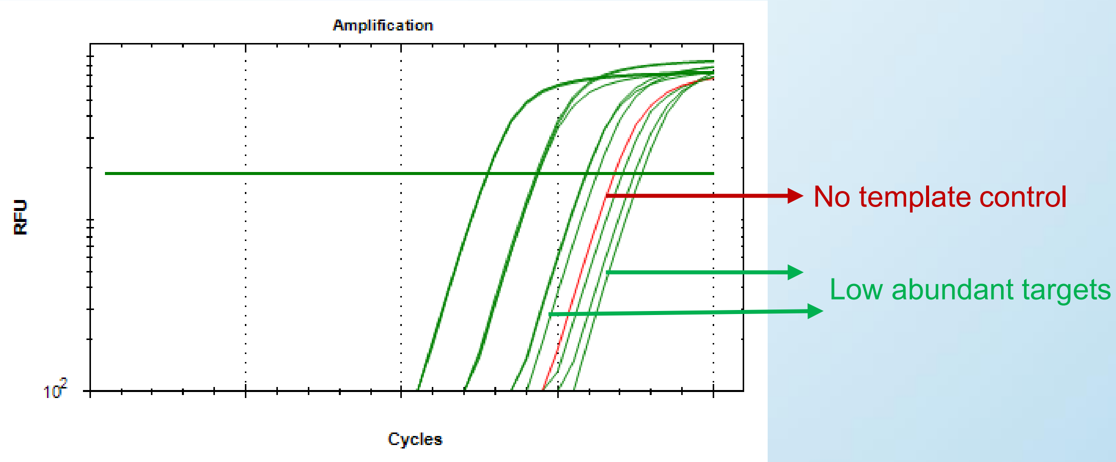
- decreases non-specific amplification and background noise → **improves sensitivity**
- allows for absolute quantification (see next slides) → **improves precision**
- highly dilutes inhibitors → **decreases inhibition effects**

ddPCR can detect and quantify the needle in the haystack!

In traditional PCR and qPCR



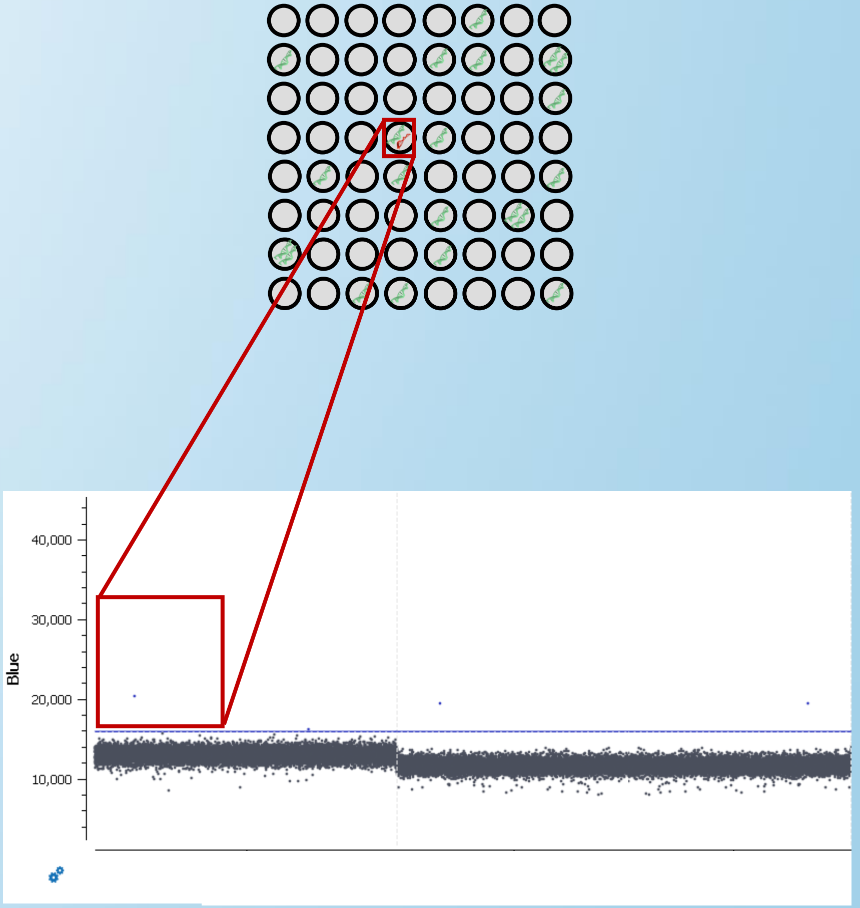
Wild type DNA   Rare mutated DNA



Potential PCR template discrimination

PARTITIONING

In Droplet Digital PCR



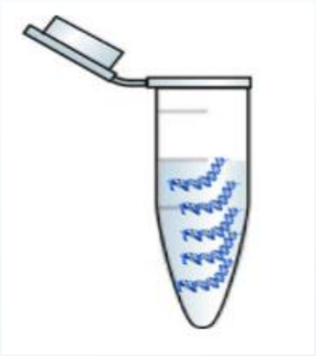
Reliable measurements

Droplet Digital PCR®

Principle

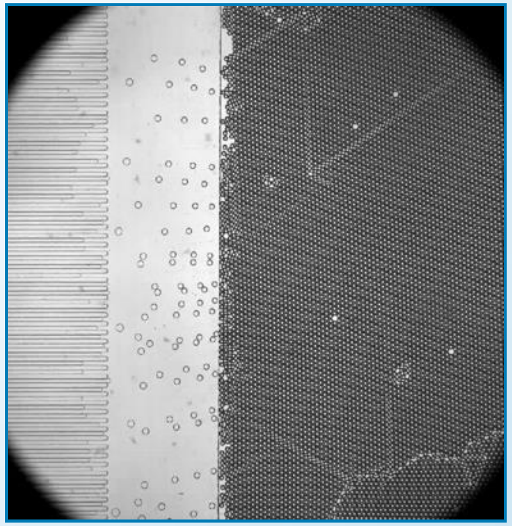
To learn more about ddPCR please visit our learning center by following this link!

Prepare

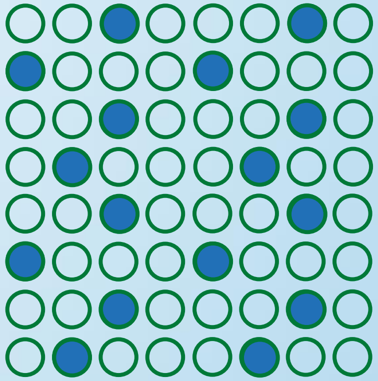


Wild-type DNA

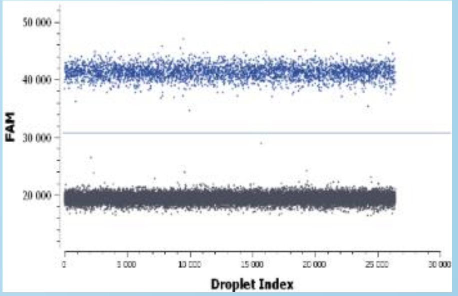
Partition & amplification



Reading



Analysis

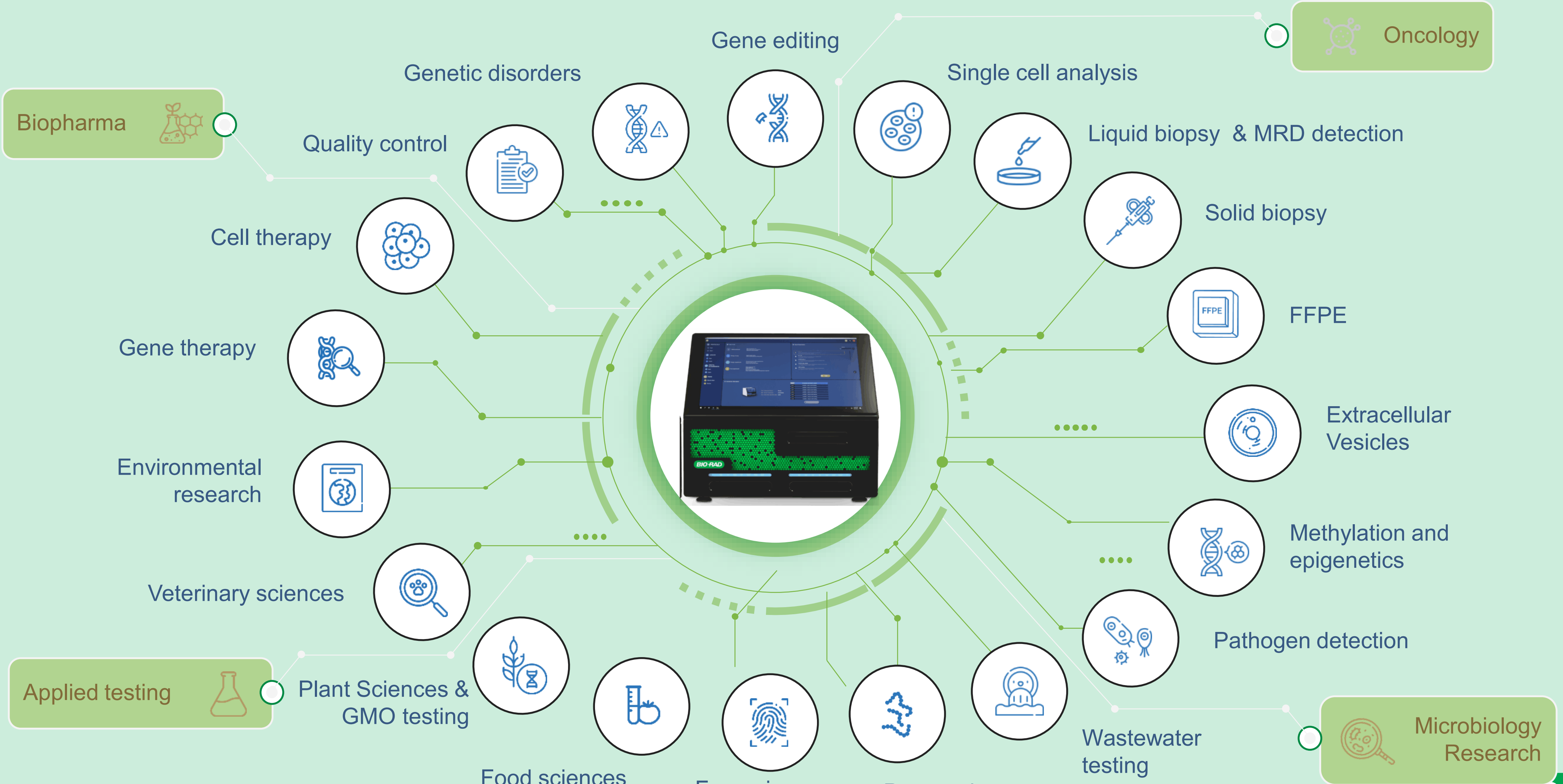


Absolute quantification
Copies/uL

POISSON STATISTICS

$$\frac{N_{pos}}{N_{tot}}$$

Next Generation ddPCR opens new horizons in wide of applications



Biopharma

Quality control

Genetic disorders

Gene editing

Single cell analysis

Oncology

Liquid biopsy & MRD detection

Solid biopsy

FFPE

Extracellular Vesicles

Methylation and epigenetics

Pathogen detection

Microbiology Research

Wastewater testing

Proteomics

Forensics

Food sciences

Plant Sciences & GMO testing

Applied testing

Veterinary sciences

Environmental research

Gene therapy

Cell therapy

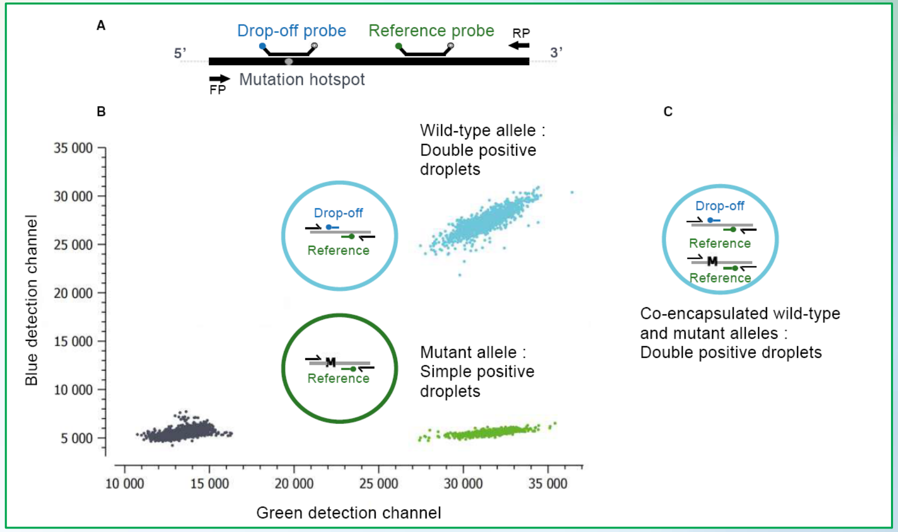
Detection and quantification of BRAF/KRAS/NRAS biomarkers

Context: BRAF, KRAS, and NRAS mutations are associated with different cancer outcomes, such as lung cancer and melanoma

Challenge: Evaluate the robustness and sensitivity of BRAF/KRAS/NRAS mutation detection and quantification for low amounts of samples like liquid biopsy and with low mutation frequency

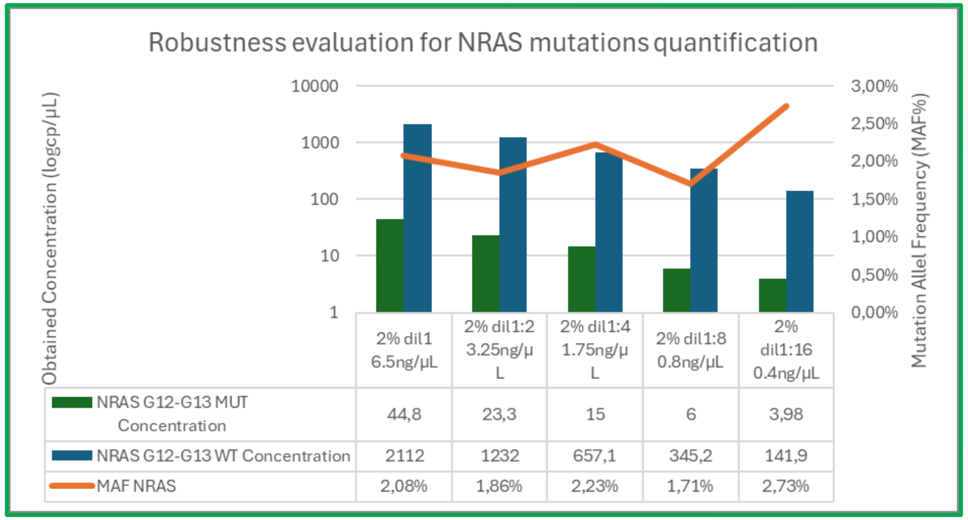
Method: 6-plex assay used on Ruby Chip with different input volumes to increase sensitivity depth for very low concentrated sample:

- Run 1: Sample at 2% of mutations diluted in water
- Run 2: Mutation dilution in WT up to 0.05% of MAF

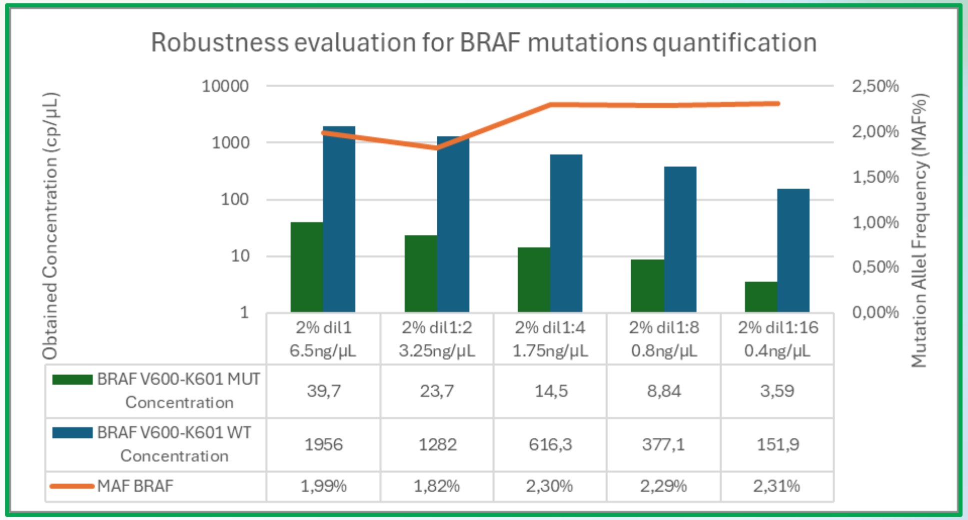


Targets	Blue	Teal	Green	Yellow	Red	Infra-Red
Wild-type (WT) KRAS G12-G13	X	X				
Mutant (MUT) KRAS G12-G13	X					
Wild-type (WT) NRAS G12-G13			X			X
Mutant (MUT) NRAS G12-G13			X			
Wild-type (WT) BRAF V600-K601				X	X	
Mutant (MUT) BRAF V600-K601				X		

Trust in your data Robustness

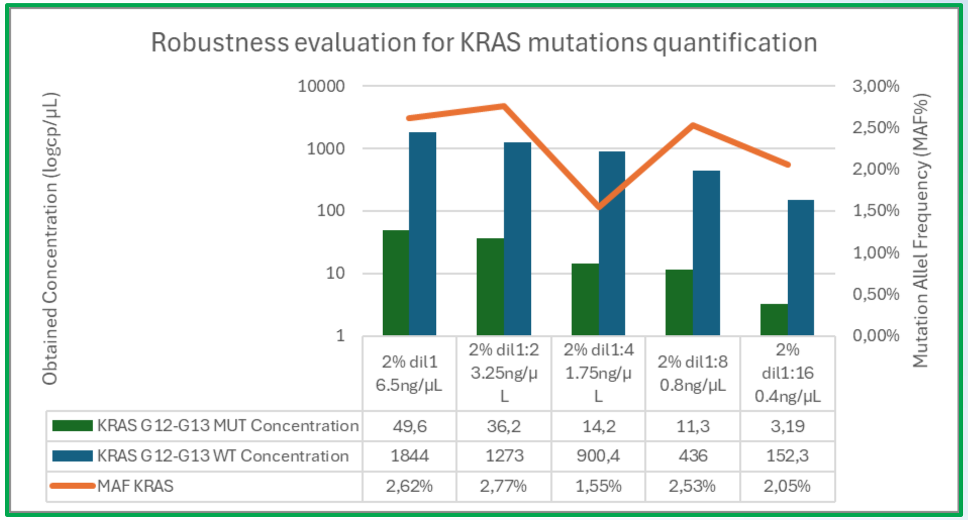

Robustness evaluation for NRAS mutations quantification

	2% dil1 6.5ng/µL	2% dil1:2 3.25ng/µL	2% dil1:4 1.75ng/µL	2% dil1:8 0.8ng/µL	2% dil1:16 0.4ng/µL
🟩 NRAS G12-G13 MUT Concentration	44,8	23,3	15	6	3,98
🟦 NRAS G12-G13 WT Concentration	2112	1232	657,1	345,2	141,9
— MAF NRAS	2,08%	1,86%	2,23%	1,71%	2,73%


Robustness evaluation for BRAF mutations quantification

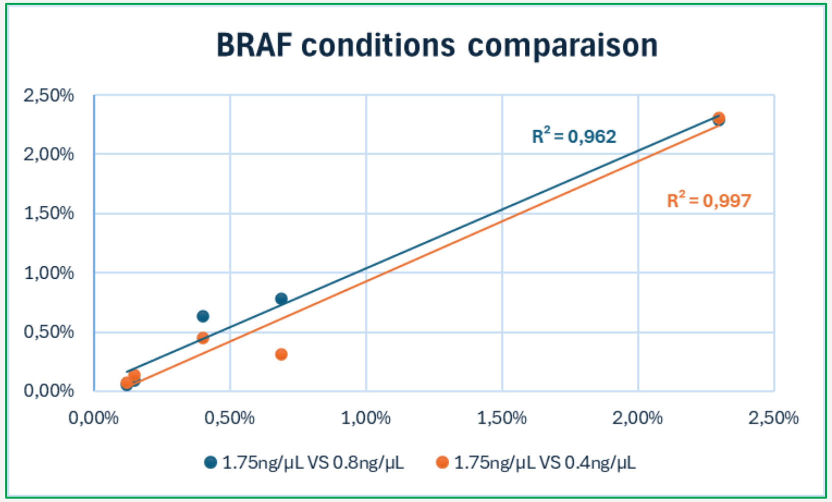
	2% dil1 6.5ng/µL	2% dil1:2 3.25ng/µL	2% dil1:4 1.75ng/µL	2% dil1:8 0.8ng/µL	2% dil1:16 0.4ng/µL
🟩 BRAF V600-K601 MUT Concentration	39,7	23,7	14,5	8,84	3,59
🟦 BRAF V600-K601 WT Concentration	1956	1282	616,3	377,1	151,9
— MAF BRAF	1,99%	1,82%	2,30%	2,29%	2,31%

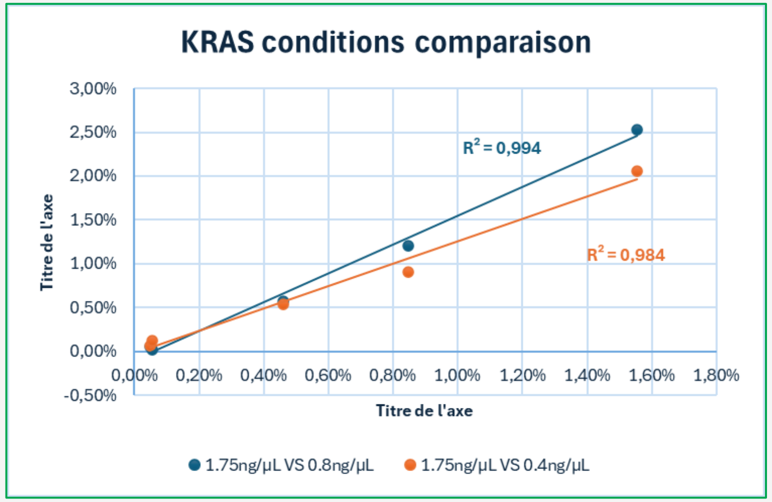
Low concentrated sample dilution shows great linearity in obtained concentrations with good reproducibility in MAF quantification even for low concentration (0.4ng/uL)

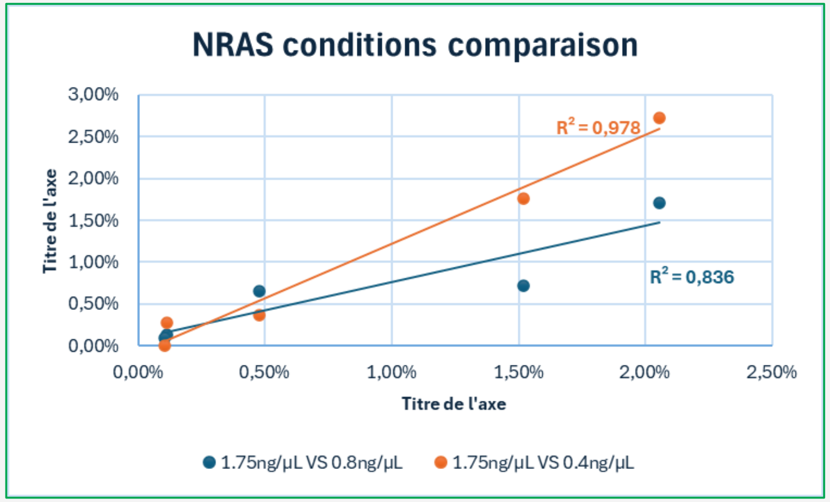

Robustness evaluation for KRAS mutations quantification

	2% dil1 6.5ng/µL	2% dil1:2 3.25ng/µL	2% dil1:4 1.75ng/µL	2% dil1:8 0.8ng/µL	2% dil1:16 0.4ng/µL
🟩 KRAS G12-G13 MUT Concentration	49,6	36,2	14,2	11,3	3,19
🟦 KRAS G12-G13 WT Concentration	1844	1273	900,4	436	152,3
— MAF KRAS	2,62%	2,77%	1,55%	2,53%	2,05%

Get the most from your precious samples


BRAF conditions comparaison


KRAS conditions comparaison


NRAS conditions comparaison

	MAF BRAF		
Sample Name	1.75ng/uL	0.8ng/uL	0.4ng/uL
2% MAF	2,30%	2,29%	2,31%
1% MAF	0,69%	0,78%	0,32%
0.5% MAF	0,40%	0,64%	0,45%
0.1% MAF	0,15%	0,09%	0,14%
0.05% MAF	0,12%	0,05%	0,08%
0% MAF	0,00%	0,10%	0,00%

	MAF KRAS		
Sample Name	1.75ng/uL	0.8ng/uL	0.4ng/uL
2% MAF	1,55%	2,53%	2,05%
1% MAF	0,85%	1,21%	0,90%
0.5% MAF	0,46%	0,57%	0,53%
0.1% MAF	0,05%	0,05%	0,06%
0.05% MAF	0,06%	0,02%	0,12%
0% MAF	0,00%	0,09%	0,00%

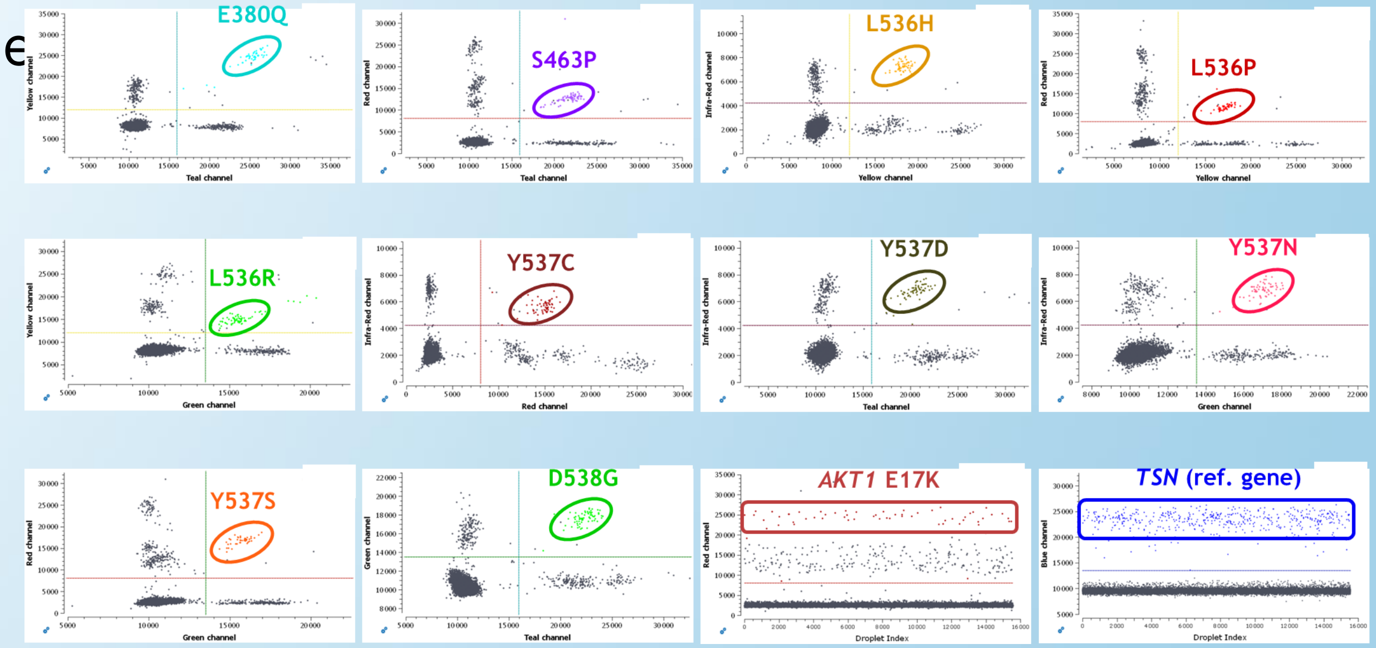
	MAF NRAS		
Sample Name	1.75ng/uL	0.8ng/uL	0.4ng/uL
2% MAF	2,06%	1,71%	2,73%
1% MAF	1,52%	0,71%	1,76%
0.5% MAF	0,48%	0,65%	0,37%
0.1% MAF	0,11%	0,13%	0,28%
0.05% MAF	0,10%	0,09%	0,00%
0% MAF	0,06%	0,00%	0,00%

Whatever the sample concentration, Ruby Chip can achieve great sensitivity up to 0.05% of mutated allele in wt with high reproducibility even in low-concentrated samples.

ESR-1 assay: the power of multiplexing at your fingertips

Assay configuration

Target	Mutation	Exon	Base changes	COSMIC ID	Detection channel 1	Detection channel 2
1	ESR1 exon 8	8	N/A	N/A	LSSD	N/A
2	E380Q	5	c.1138G>C	COSM3829320	Teal	Yellow
3	V422del	6	c.1265_1267del	COSM1074628	Blue	IR
4	S463P	7	c.1387T>C	COSM4771561	Teal	Red
5	D538G	8	c.1613A>G	COSM94250	Green	Yellow
6	D538N	8	c.1612G>A	N/A	Blue	Red
7	L536H	8	c.1607T>A	COSM6843697	Yellow	IR
8	L536P	8	c.1607T>C	COSM6906109	Yellow	Red
9	L536Q	8	c.1607_1608delinsAG	COSM4766050	Blue	Teal
10	L536R	8	c.1607T>G	COSM4774826	Teal	Green
11	Y537C	8	c.1610A>G	COSM1074637	Red	IR
12	Y537D	8	c.1609T>G	COSM6918757	Teal	IR
13	Y537H	8	c.1609T>C	COSM7449624	Blue	Yellow
14	Y537N	8	c.1609T>A	COSM1074635	Green	IR
15	Y537N_delinsTA	8	c.1608_1609delinsTA	N/A	Blue	Green
16	Y537S	8	c.1610A>C	COSM1074639	Green	Red
17	Y537S_delinsAG	8	c.1609_1610delinsAG	COSM6948665	Red	N/A
18	Y537S_delinsCA	8	c.1610_1611delinsCA	COSM6971270	Red	N/A

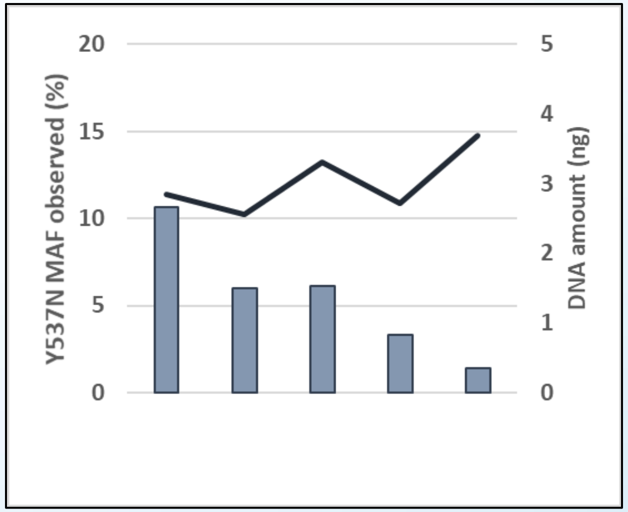


- 17- ESR-1 mutations detected in 2 colors using Droplet Flex Probes and color combination, one reference gene in single color
- Result concordance and MAF correlation with a previous cddPCR study performed with sequential lower multiplexed assays.

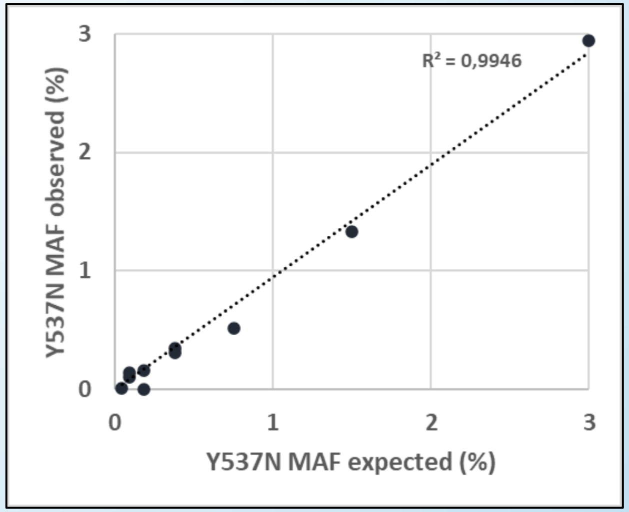
Challenging ESR1 assay in liquid biopsy conditions

Check the Robustness and Ability to detect the Y537N mutation

- Range of diluted 10% mutation sample between 3,3ng and 0,3ng
- Mutation dilution in Wt up to 0.05% of MAF



Mutated sample diluted in H2O

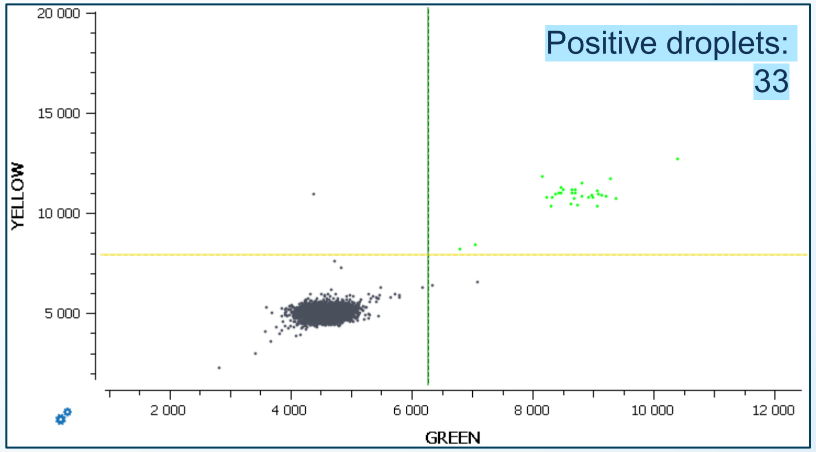


Mutated sample diluted in WT DNA

Mutation Y537N	Obtained MAF	Expected MAF
Sample1 3%	2,75	3
Sample1 1.5%	1,26	1,5
Sample1 0.75%	0,46	0,75
Sample1 0.375%	0,35	0,375
Sample1 0.1%	0,16	0,1
Sample1 0.05%	0,08	0,05

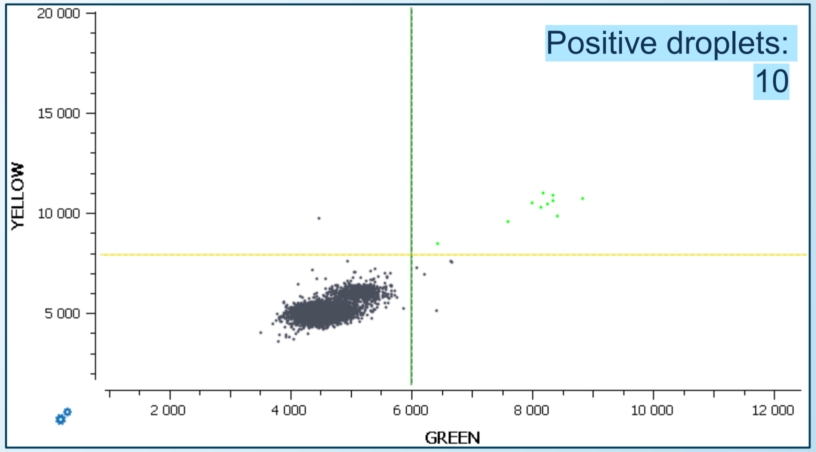
Easy mutations identification and quantification for routine usage

Comparison: NGS vs cddPCR for low MAF samples



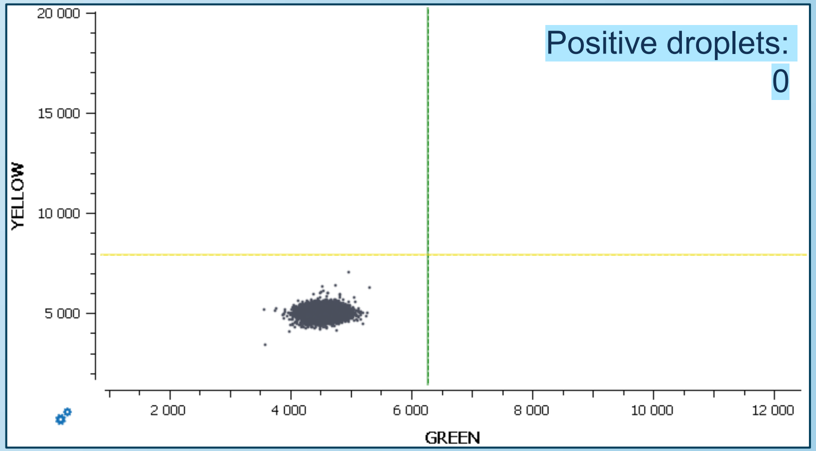
Positive droplets: 33

Sample 1 : D538G (NGS: 8% ddPCR: 10%)



Positive droplets: 10

Sample 2 : D538G (NGS: / ddPCR: 0,5%)



Positive droplets: 0

Sample 3 : D538G (NGS: / ddPCR: /)

absence of background

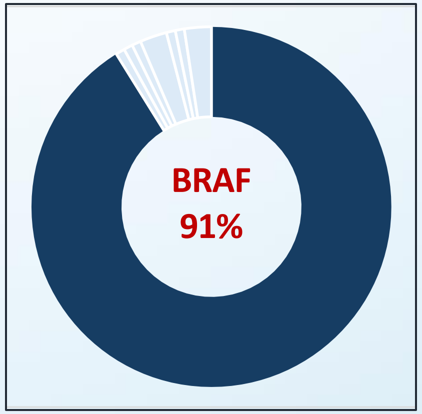
cddPCR shows better sensitivity than NGS for samples with low allele frequency

Multi-Mutations Pan-Cancer Panel

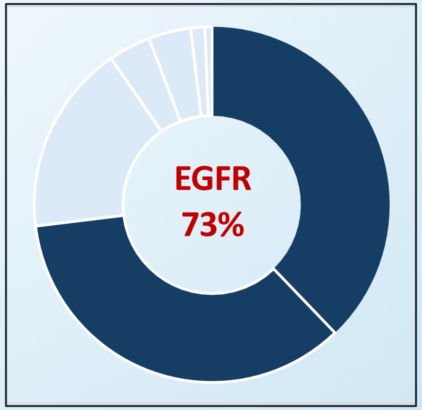
EGFR wt	Blue	Teal		
EGFR Ex19 del	Blue	AA754_759	Teal	AA744_749
EGFR L858R	Green	l-Red		
KRAS G12C	Red	l-Red		

KRAS G12D	Green	Red
BRAF V600E	Yellow	Red
NRAS Q61R	Green	Yellow
IDH1 R132H	Yellow	l-Red

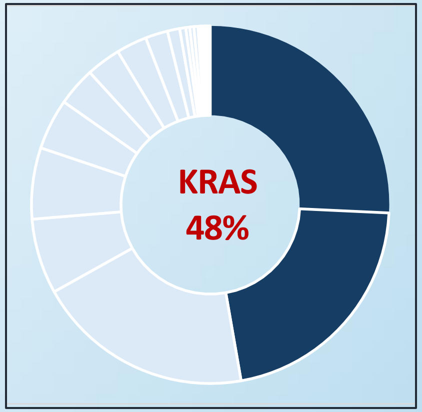
mut ex15



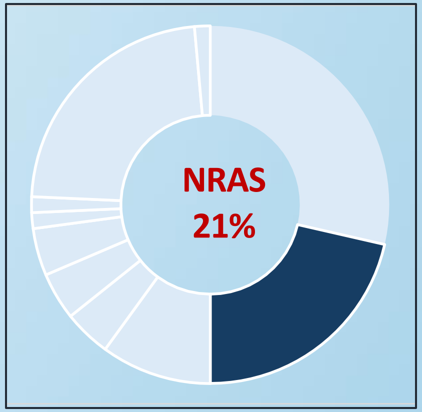
mut egfr thera



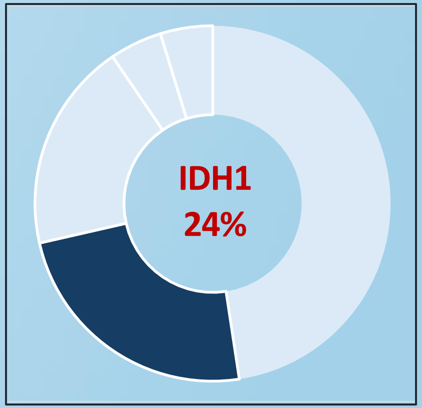
mut kras thera



mut nras thera



mut idh1 thera

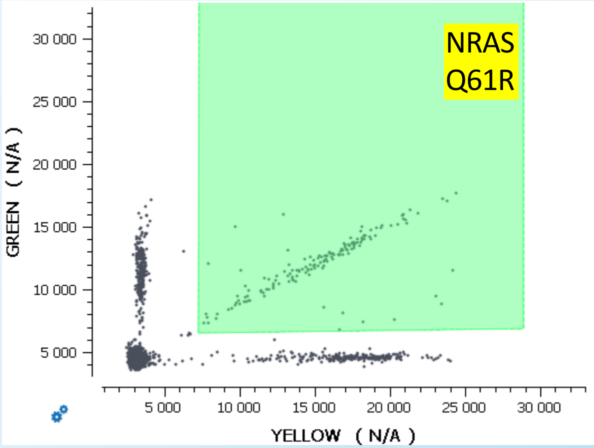


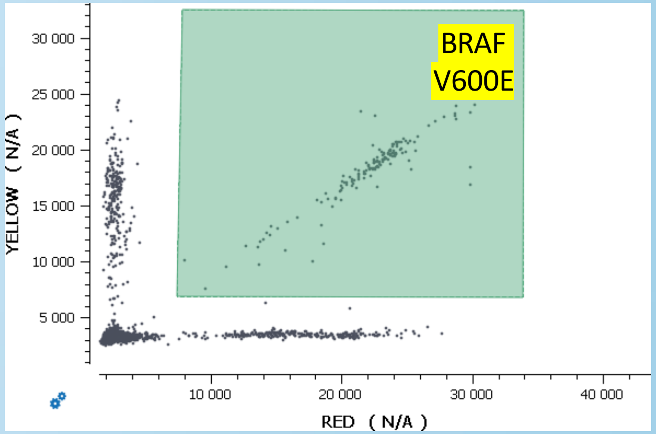
Good coverage for the most commune mutations for diffrent cancers

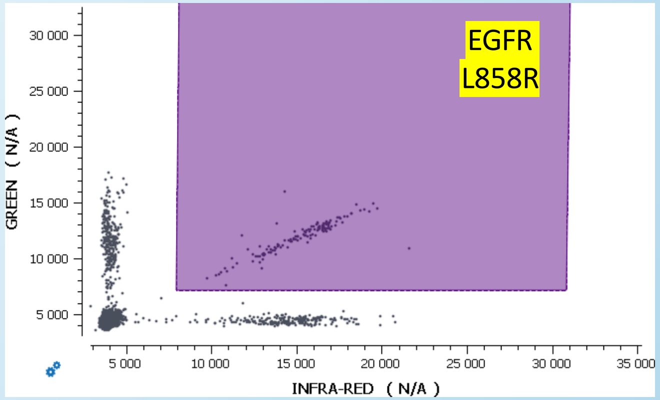
Pan-Cancer mutations analysis

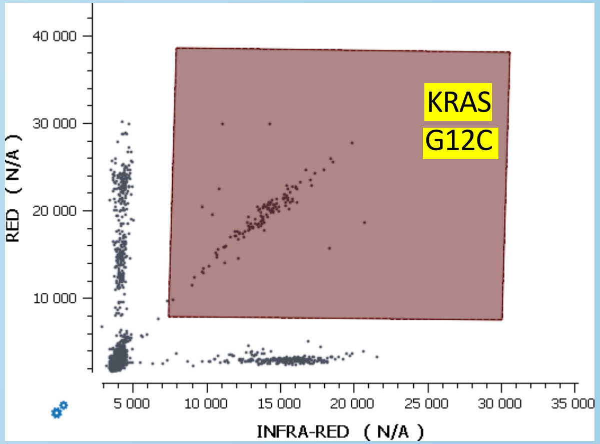
10 - 20 min

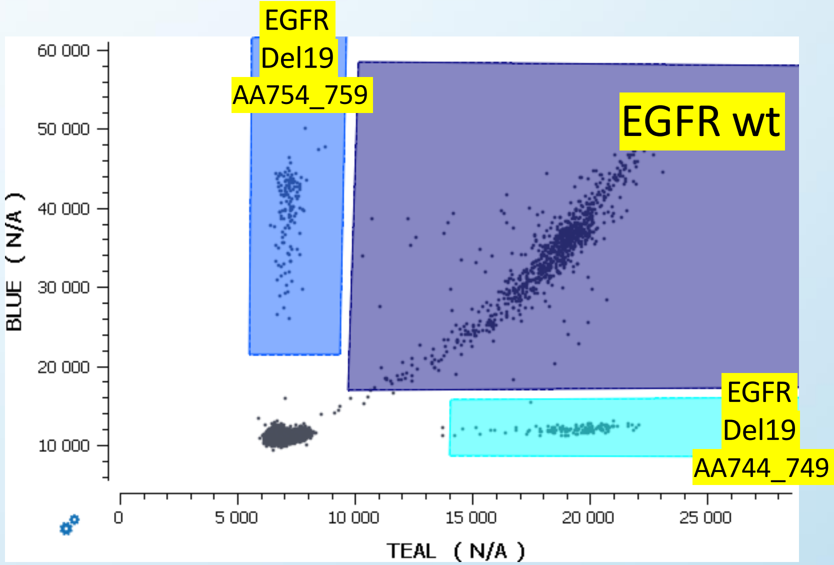
1. Upload template « Plot_config »
2. Upload template « Analysis_config »
3. Visual verification
4. Results Export

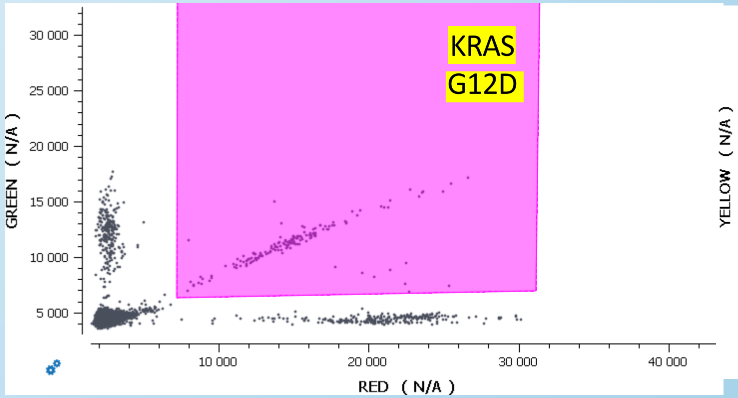


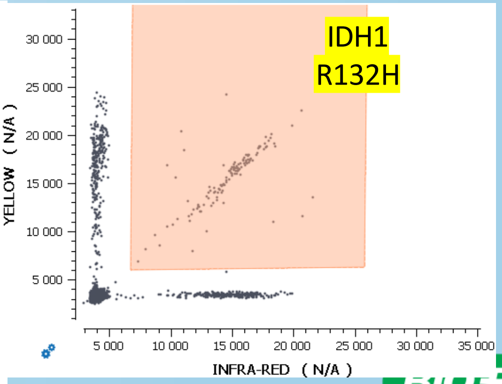








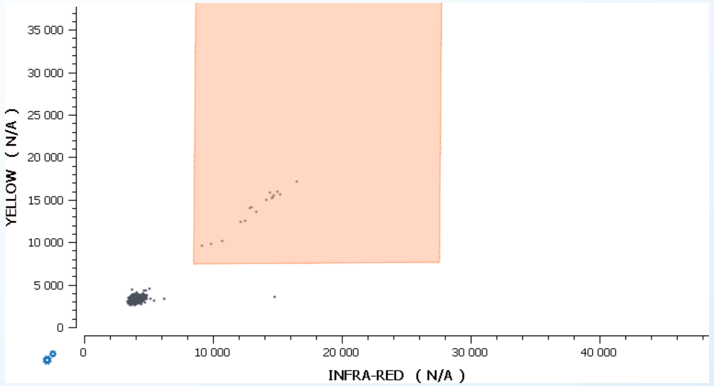


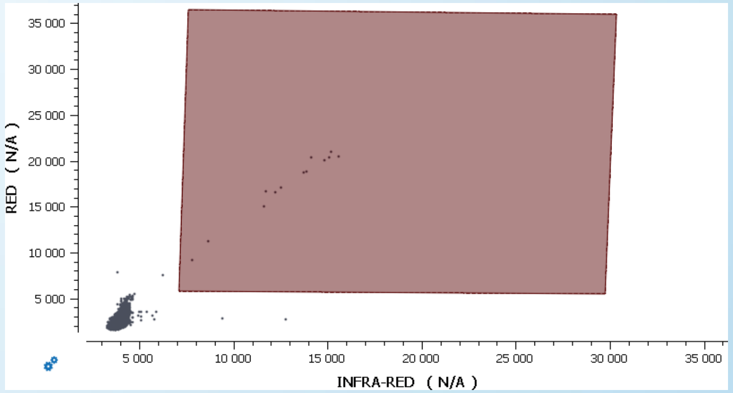


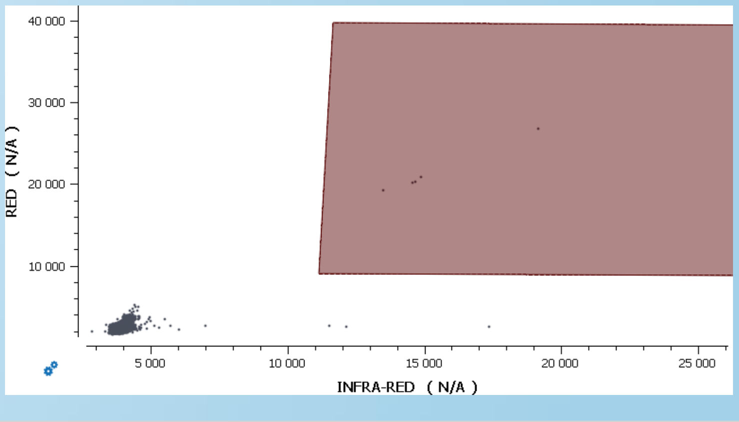
Multi-Mutations Panel specificity

	BRAF_V600E	EGFR_Del19	EGFR_Del19b	EGFR_L858R	IDH1_R132H	KRAS_G12C	KRAS_G12D	NRAS_Q61R
VN	207	197	235	215	223	211	214	214
VP	31	41	3	23	14	25	24	24
FN	0	0	0	0	0	0	0	0
FP	0	0	0	0	0	0	0	0
QUID ??	0	0	0	0	1	2	0	0

sensibilite	100,00%	100,00%	100,00%	100,00%	100,00%	100,00%	100,00%	100,00%
specificite	100,00%	100,00%	100,00%	100,00%	100,00%	100,00%	100,00%	100,00%







sample_id	indication	sample_type	tumor_content	result	vaf	targets_id	repos_drople	centration(cp	Ratio/VAF	Result
23-22130-A-02-00	melanoma	GSCALQDF	> 50 %	NRAS Ex3 c.182A>T, p.(Gln61Leu) (Q61L)	87	IDH1_R132H	16	5,2	0,9	QUID
24-11788-A-03-00	lung	GSCALQDF	25 à 50 %	EGFR Ex19 c.2240_2254del p.(Leu747_Thr751del) (L747_T751del)	25,7	KRAS_G12C	13	3,74	0,8	QUID
24-11788-A-03-00	lung	GSCALQDF	25 à 50 %	EGFR Ex19 c.2240_2254del p.(Leu747_Thr751del) (L747_T751del)	25,7	KRAS_G12C	5	1,48	1,1	QUID

3 true mutations not detected by NGS (reference methodology)

Overview: ddPLEX EGFR KRAS BRAF Kit

NSCLC is the 2nd most common cancer, with high mortality rate

Liquid Biopsy testing preferred (less invasive)

ddPCR has high sensitivity for rare biomarker detection

ddPCR has low run costs vs NGS

Highly multiplexed ddPCR-based kit for simultaneous detection of relevant mutations in three genes related to NSCLC (non-small cell lung cancer) detection. ***Total of 37 variants detected***

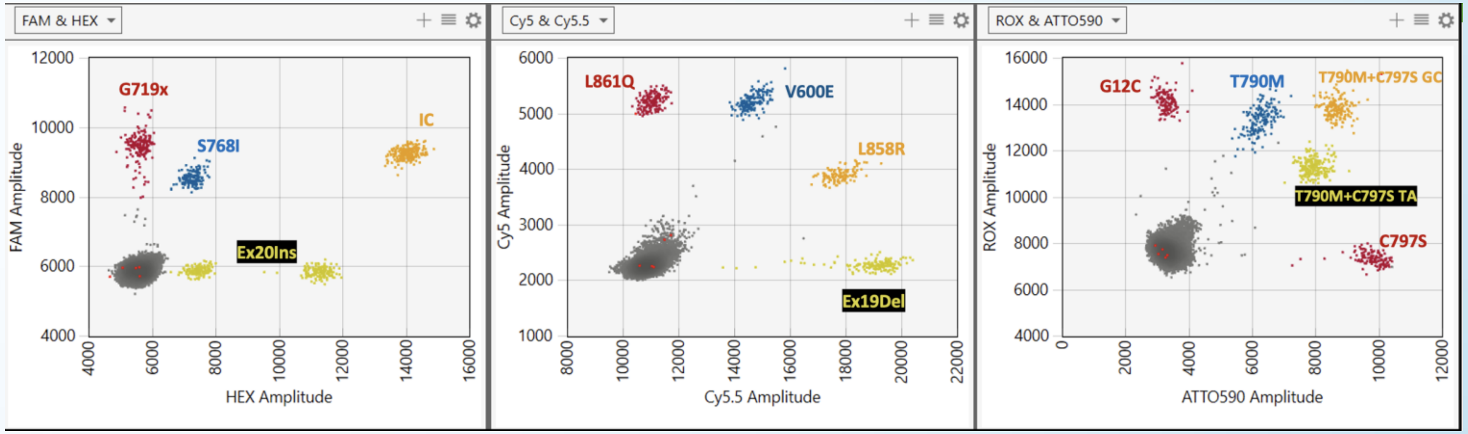
Commonly actionable targets from NCCN, ESMO guidelines

EGFR									KRAS	BRAF
Exon 18	Exon 19	Exon 20				Exon 21			Exon2	Exon15
G719A G719S G719C	Del	Ins	C797S in cis/trans	T790M	S768I	S768I	L858R	L861Q	G12C	V600E

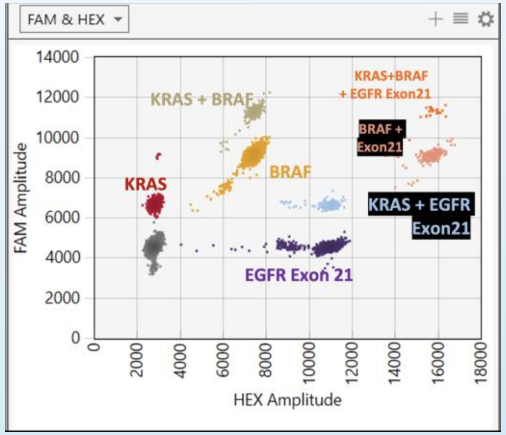
Key differentiator: Distinguishes emerging acquired C797S mutation and its trans and cis position with T790M for further treatment options (third or fourth generation TKI inhibitors)

Highest sensitivity available with ddPLEX

ddPLEX EGFR KRAS BRAF Mutant Assay



ddPLEX EGFR KRAS BRAF Total Quant Assay



Gene	Target	LOD (%VAF)
EGFR	G719X (n=3)	0.05-0.1
EGFR	S768I	0.05
EGFR	EX20INS (n=7)	0.025-0.1
EGFR	L861Q	0.025
BRAF	V600E	0.05
EGFR	L858R	0.05
EGFR	EX19DEL (n=7)	0.025-0.05
KRAS	G12C	0.025
EGFR	T790M	0.1
EGFR	T790MC797SGC	0.025
EGFR	T790MC797STA	0.05
EGFR	C797S (n=2)	0.05

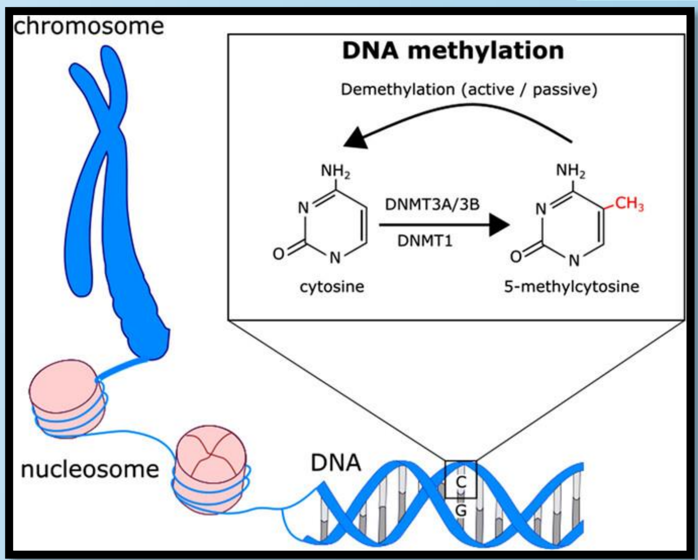
Robust cluster separation with many multiplexed targets
VAFs 0.1% or better (down to 0.025% in some cases)

Epigenetic biomarkers like Methylation for Ovarian cancer

Protocol:

- FFPE samples
- Bisulfite conversion with 50ng DNA (EZ DNA Methylation-Lightning Zymo)
- Elution with 12ul
- Use 2ul of elution for pcr reaction (around 8ng of converted DNA per chamber)

		Digital PCR results			
GIS status	**Meth. status (Institut Curie technique HRM)**	**BRCA1_meth**	**RAD51C_meth**	**BRCA1_unmeth RAD51C_unmeth**	**NC**
10 HRD	BRCA1_meth	7	-	-	-
	RAD51C_meth	-	1	-	-
	BRCA1_&_RAD51C_unmeth	-	-	2	-
25 HRD (deficient)	/	14	3	7	1
25 HRP (efficient)	/	-	-	24	1
10 NC	/	-	-	8	2



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Target	Color 1 (Ref)	Color 2 (Spe)
RAD51C_UnMeth	IR	Red
RAD51C_Meth	IR	Green
BRCA1_UnMeth	Yellow	IR
BRCA1_Meth	Yellow	Red

Satisfactory results compatible with routine activity.
100% concordance with reference technique(s), 5% non- contributive results.

ddPCR Platforms Serving Broad Requirements

			
System	**QX200 (QXDX)**	**QX600**	**QX700 Series**
Colors	2	6	7
Targets[1]	4	12	21
Samples[2]	Up to 96	Up to 96	Up to 384 (depending on model)
Time to data[3]	~6hr (96 wells)	~6hr (96 wells)	~2-6hrs
Automation	Manual	Semi-Automated	Automated
Regulatory Status	US FDA cleared & EU IVDR Dx version	On pathway for FDA clearance in 2025; subsequent IVDR	Research Use Only

+ Software for set-up and analysis (automated in some cases) for all platforms

[1] For discrimination (higher multiplexing if not all targets need to be discriminated)

[2] Actual number of test samples depends on controls used number of wells per assay

[3] Does not include sample extraction, data analysis etc

Example of Droplet Digital PCR in laboratory workflow

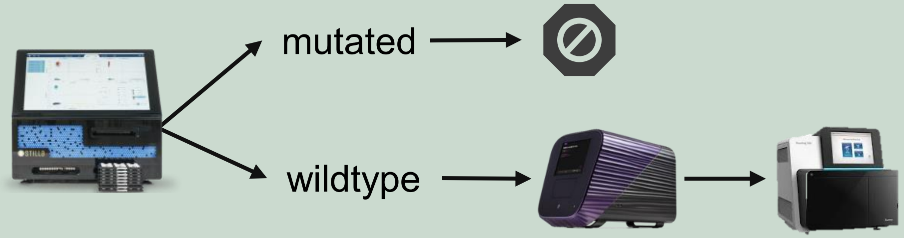
Digital PCR analysis prior to NGS analysis (ffpe and liquid biopsy)

Lung cancer: EGFR$_{\text{T790M}}$, KRAS$_{\text{G12C G12D}}$, BRAF$_{\text{V600E}}$

Melanoma: BRAF$_{\text{V600E}}$

Breast Cancer: ESR1$_{\text{Ex8, Ex5}}$

…



mutated → ⊘

wildtype →

Digital PCR analysis in addition to NGS analysis (ffpe)

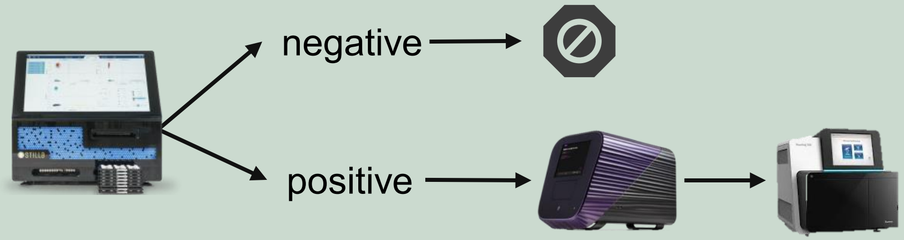
Ovarian cancer: methylation of the promotors: BRCA1, RAD51C + BRCA1/2 by NGS

…

Digital PCR analysis to detect the presence of tumor DNA (liquid biopsy)

Colorectal cancer: methylation of the promotors: WIF1 and NPY

…



negative → ⊘

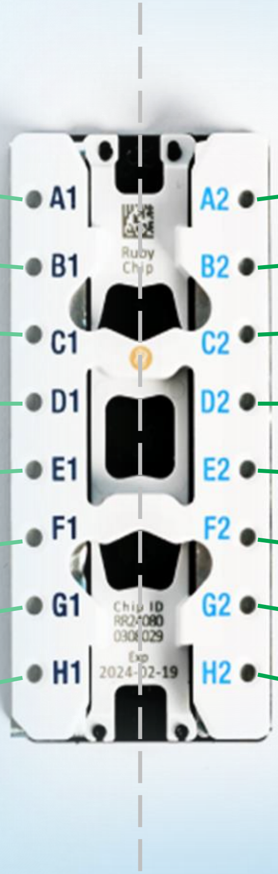
positive →

Next Generation Digital PCR

Roadmap to routine sample testing with comprehensive 100-plex panels

Routine testing with mid-plex assays

Sample 1	ESR1 18-plex assay
Sample 2	ESR1 18-plex assay
Sample 3	ESR1 18-plex assay
Sample 4	ESR1 18-plex assay
Sample 5	ESR1 18-plex assay
Sample 6	ESR1 18-plex assay
Sample 7	ESR1 18-plex assay
Sample 8	ESR1 18-plex assay



Sample profiling with >100-plex panels

PIK3CA 15-plex assay	Sample 1
ESR1 18-plex assay	Sample 1
ERBB2 assay	Sample 1
TP53 20-plex assay #1	Sample 1
TP53 20-plex assay #2	Sample 1
Methylation assay	Sample 1
…	Sample 1
Sample identity assay	Sample 1

Our Industry Leading Solutions Menu

Bio-Rad Maintains a catalog of over 490,000 assays for the research community

High Value Tests

in oncology, cell & gene therapy, and public health

Powerful Design Algorithms

and expert design services to advance scientific discoveries

External Partnerships

to expand the clinical assay menu globally

Oncology Solutions Portfolio

Multiple options covering the broad range of critical biomarkers

Tumor Specific Panels

Mutliplexed solutions, fully kitted for specific set of tumor biomarkers

Developed under strict quality control protocols and with technical validation

Individual Assays

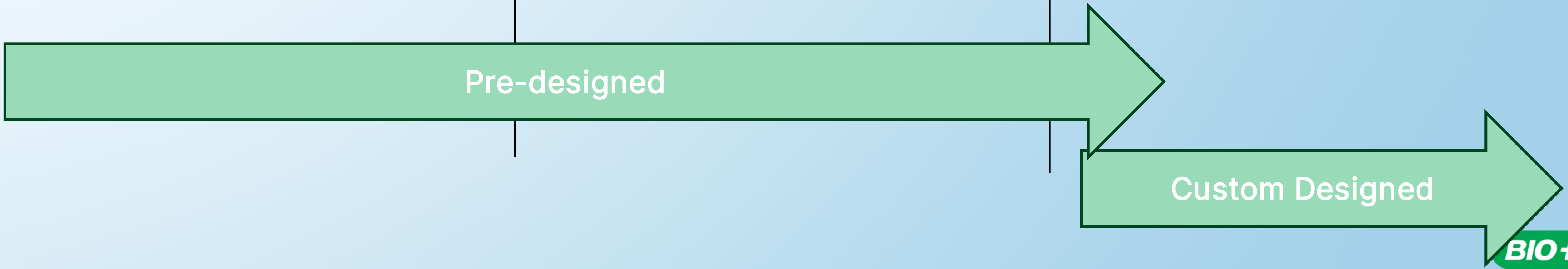
Comprehensive library of assays for variants (SNV, CNV, Gene Expression) relevant as cancer biomarkers

Wet-lab or in-silico validated

Expert & Custom Design

Expert Design Assays (multiplexed or single-plex) developed by Bio-Rad scientists or through collaborations

Custom Design or build your own assay services



Empower you with Next Generation ddPCR

Enable high-sensitivity analysis for low-concentrated samples & targets.

Maximum information analysis from precious samples using high-multiplexing.

Easy workflow with **minimum hands-on time**, reducing experimental variability

Flexible throughput depending on the level of sensitivity and precision expected

Time and cost saving for targeted screening compared to other technologies
or ddPCR systems.

We advance science and save lives,
together.

Thank you for your attention