



BIO-RAD

Advancing discovery
Improving lives
For over 60 years

This is
Bio-Rad.



Clinical Utility of Droplet ddPCR, moving to diagnostics



Koen De Gelas, PhD, CRIG ddPCR mini symposium, 15/05/2018



Disclaimer

- Disclaimer: all consumables, instruments, applications and software covered in this presentation are intended for ***research use only***, unless explicitly stated differently.



Bio-Rad, a true global company





Our Businesses

Bio-Rad is a \$2B IVD company

Life Sciences

- Droplet Digital PCR & Amplification
- Protein Electrophoresis
- Lab-Scale Chromatography
- Process Scale Separation
- Flow Cytometry
- Imaging and Analysis

Clinical Diagnostics

- Diabetes Monitoring
- Quality Controls
- Autoimmune Testing
- Immunohematology
- HIV Testing



Droplet digital PCR

2400+



550+





Droplet digital PCR

2600

More than 2600 published studies have described research breakthroughs using Droplet Digital™ PCR technology.

2657

Publications using Droplet Digital PCR

1105

Publications last year

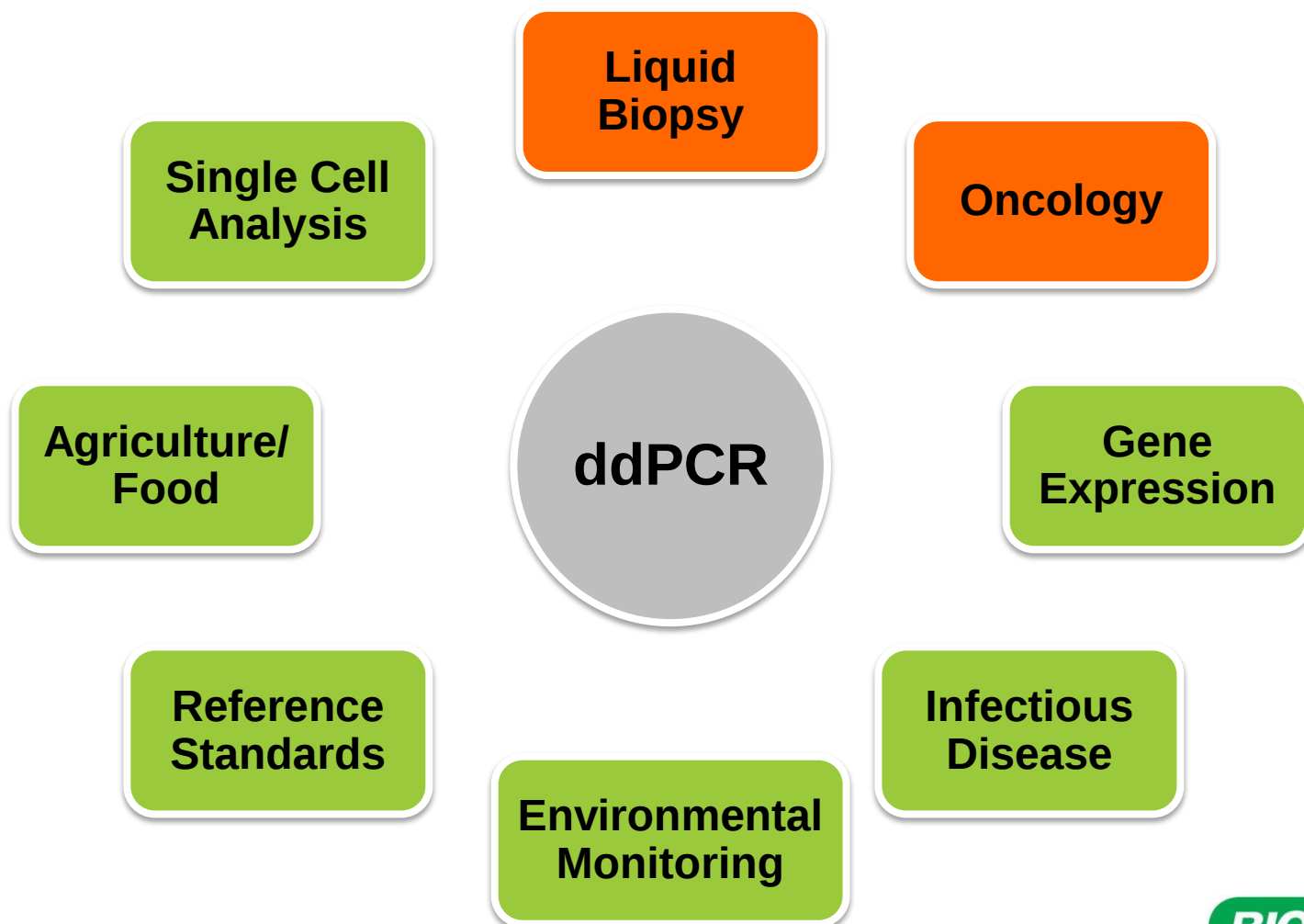
200

Publications this year

<https://www.bio-rad.com/ddpcr/publications/>



Landscape of Digital PCR Applications





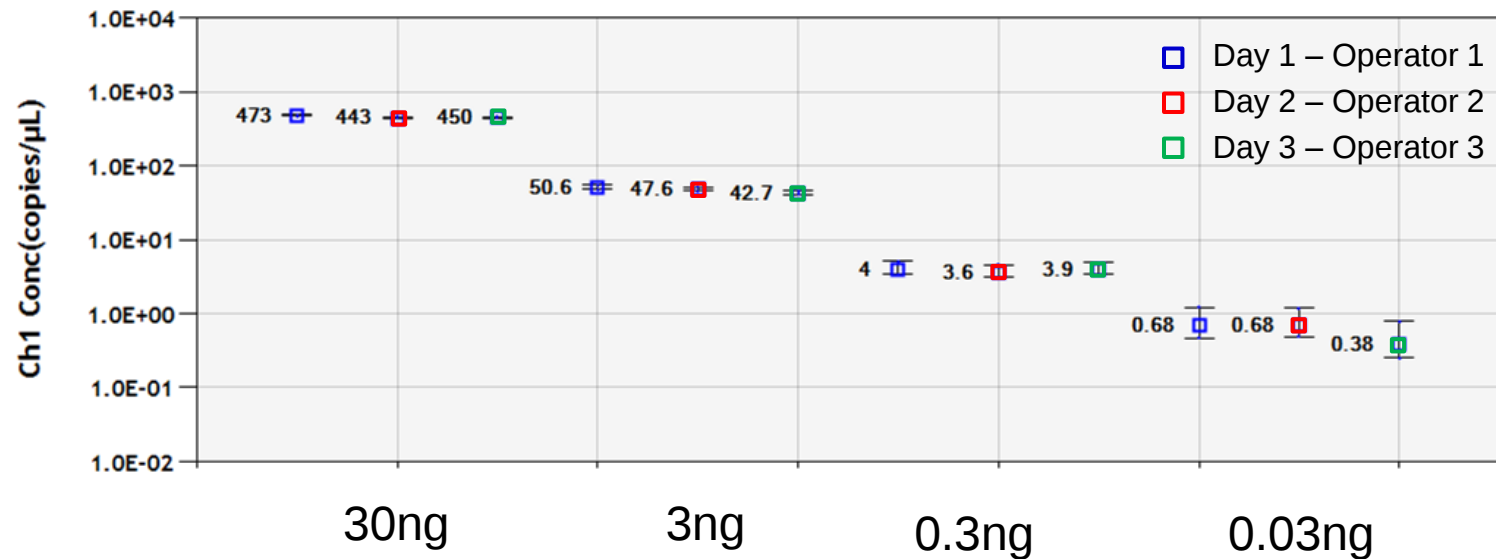
The road to diagnostics: advantages of ddPCR

- Superior precision, sensitivity, ease of use
- Different input matrices, including liquid biopsies
- Fast turn around time and throughput: up to 4x 96 samples / day
- Absolute measurement
- Highly reproducible, robust

High potential to move into clinical diagnostics

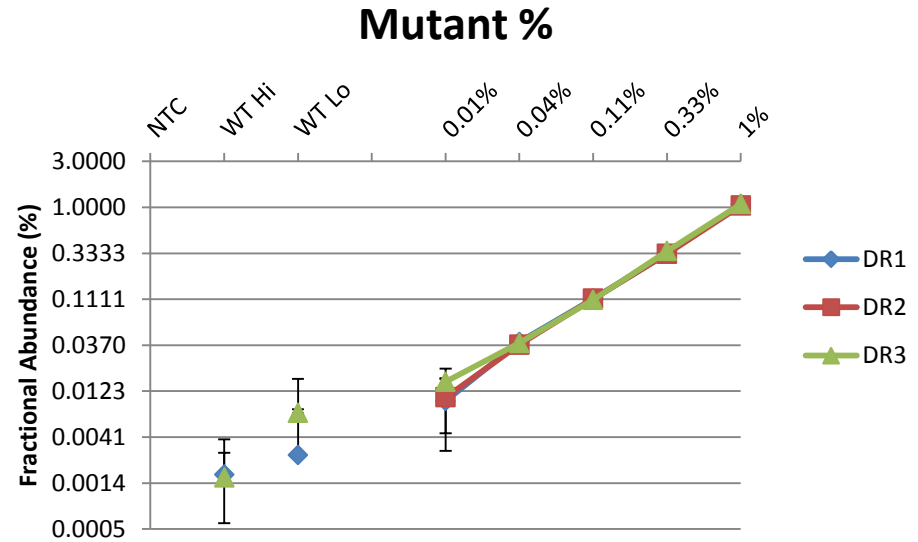
ddPCR reproducibility

- Different days, different operators

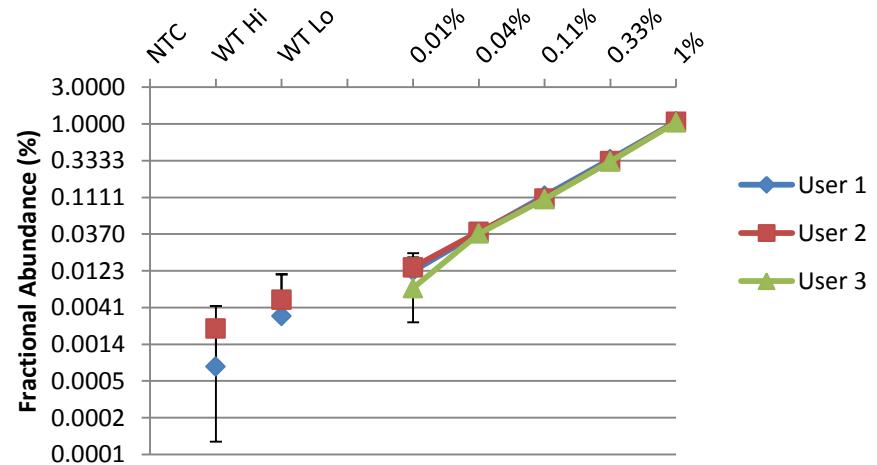


ddPCR reproducibility

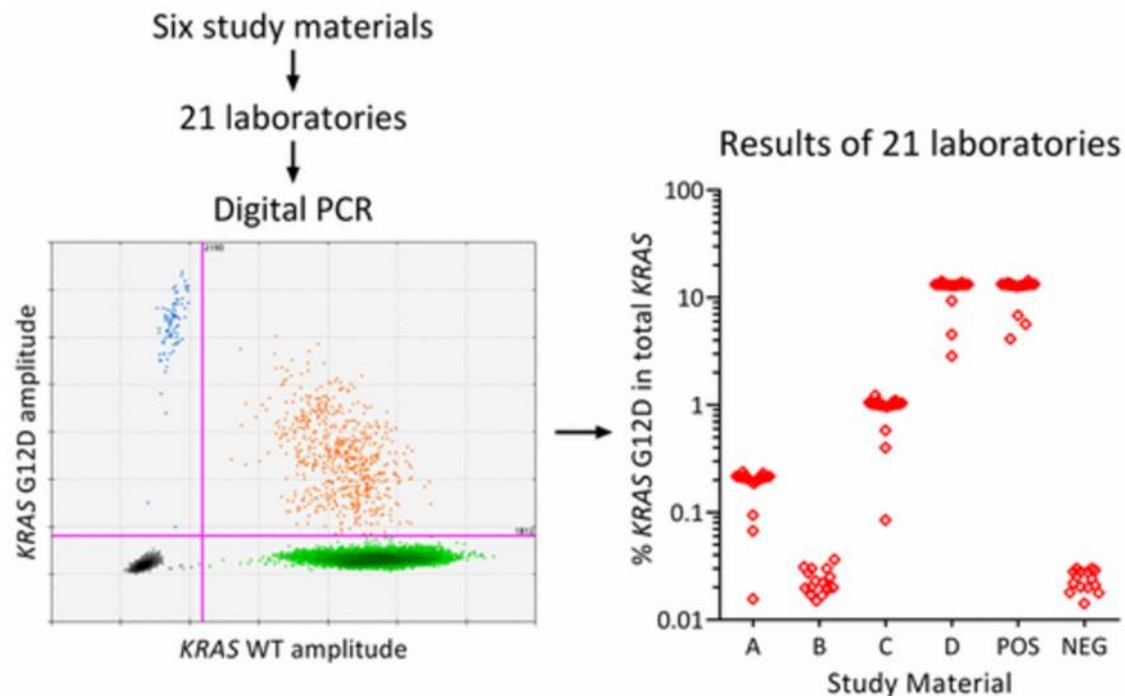
Different instruments



Different users



ddPCR reproducibility



analytical
chemistry

Article

pubs.acs.org/ac

International Interlaboratory Digital PCR Study Demonstrating High Reproducibility for the Measurement of a Rare Sequence Variant

Alexandra S. Whale,[†] Alison S. Devonshire,[†] George Karlin-Neumann,[‡] Jack Regan,[‡]

The road to diagnostics: Introducing QXDx™

QXDx™ Products for In Vitro Diagnostics (IVD)



Instruments

QXDx™ ddPCR™ System

Manual droplet generation

QXDx™ AutoDG™ ddPCR™ System

Automated high-throughput analysis



Kits

QXDx™ Universal Kit for ddPCR™ System

Reagents and consumables for the IVD ddPCR System

QXDx™ Universal Kit for AutoDG™ ddPCR™ System

Reagents and consumables for the automated IVD ddPCR System



Assays

COMING SOON

QXDx™ BCR-ABL %IS Kit

A CE-IVD digital PCR test kit intended to monitor patients with Chronic Myeloid Leukemia (CML) with reported results in both International Scale and MR values



QXDx™ instruments



QXDx™ AutoDG



QXDx™ Manual DG



Open Mode Systems: the system is capable of running both FDA-cleared, CE-IVD and home brew/in house assays

QXDx™ ddPCR Universal Kit

**ddPCR™ Universal Kit
for QX200™ AutoDG ddPCR™ Dx
System**



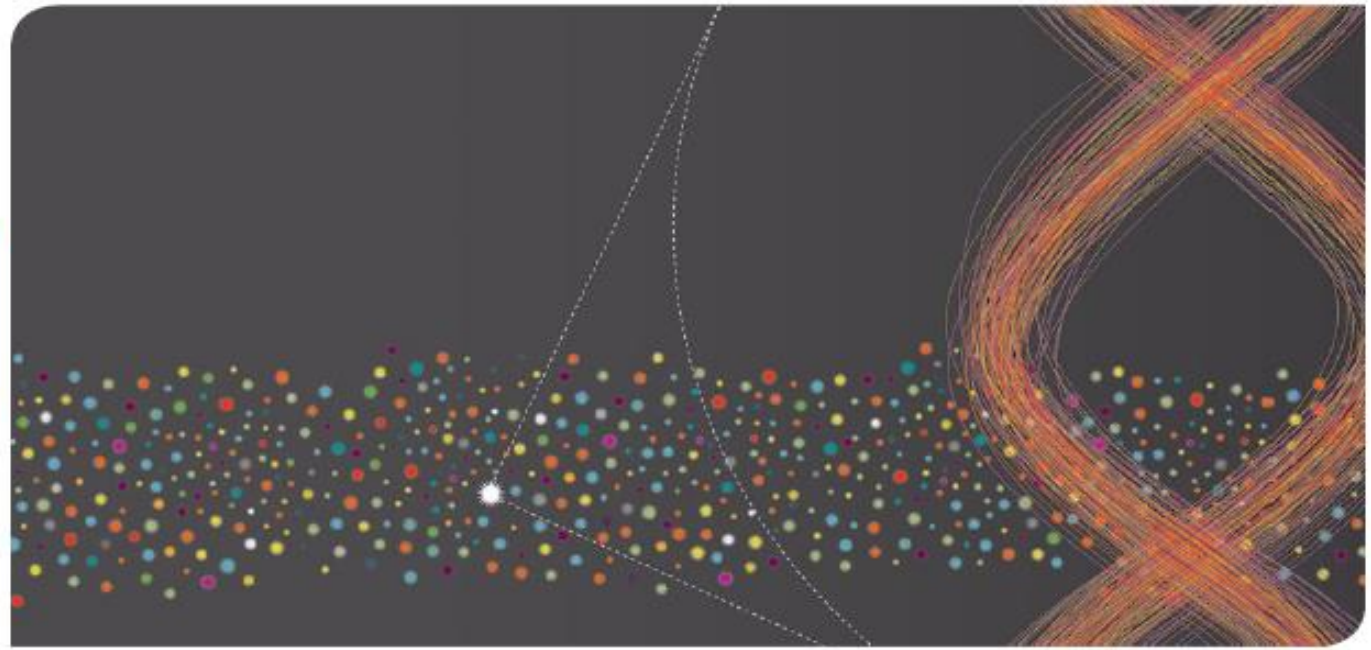
**ddPCR™ Universal Kit
for Qx200™ ddPCR™ Dx System**



QXDx™ ddPCR Universal Kit

- Validated kit
- Verified performance for ddPCR run
- GMP reagents
- Consistent reproducible quality
- Certificate of analysis provided with kit





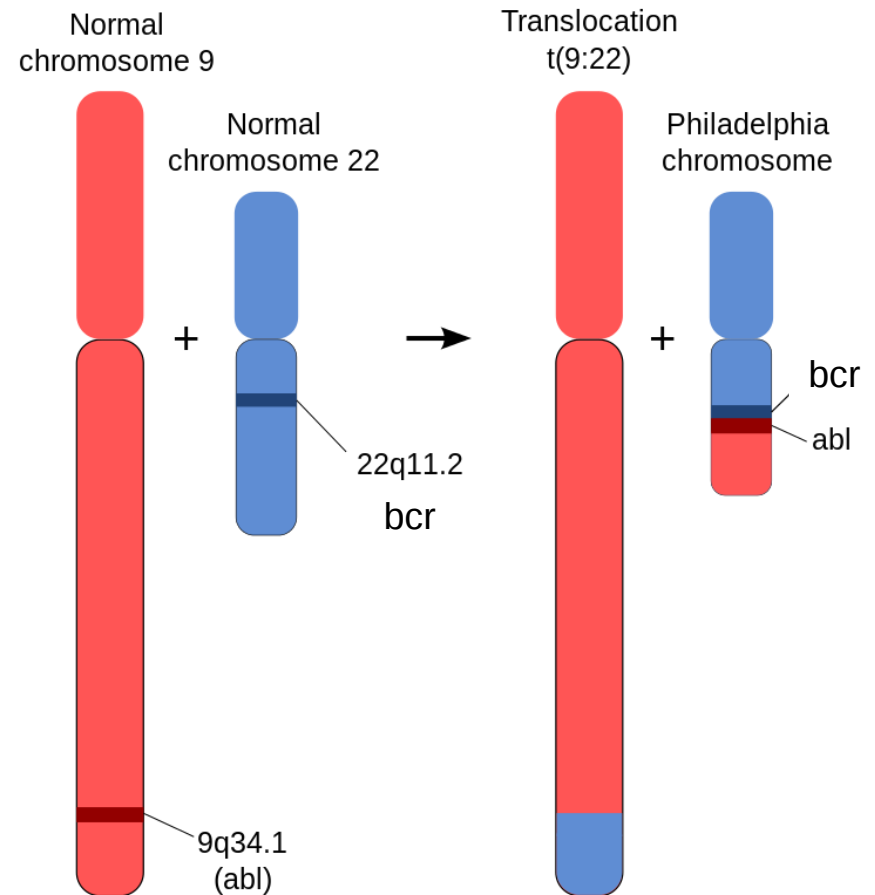
QXDx™ BCR-ABL %IS Kit

Measuring major and deep
molecular response in CML
patients using ddPCR
technology



BCR-ABL or Philadelphia translocation

- Chromosomal abnormality
- Chronic Myeloid Leukemia cells (CML)
- Uncontrolled cell division
- Variants: p190
p210(e13a2)
p210(e14a2)
p230

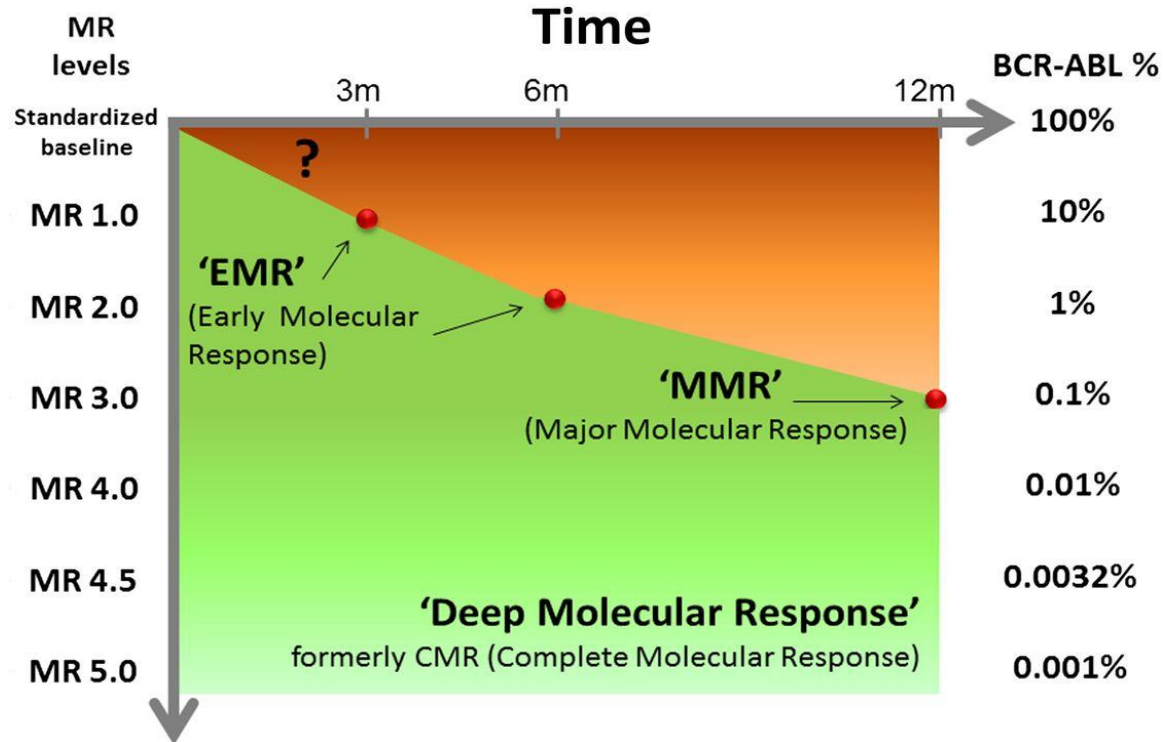


https://commons.wikimedia.org/wiki/File:Schematic_of_the_Philadelphia_Chromosome.svg

CML is treatable but can remain chronic

BCR-ABL tyrosine-kinase inhibitors (TKI)

- 1st generation: Gleevec (Imatinib)
- 2nd generation: Dasatinib, Nilotinib, Radotinib, Bosutinib





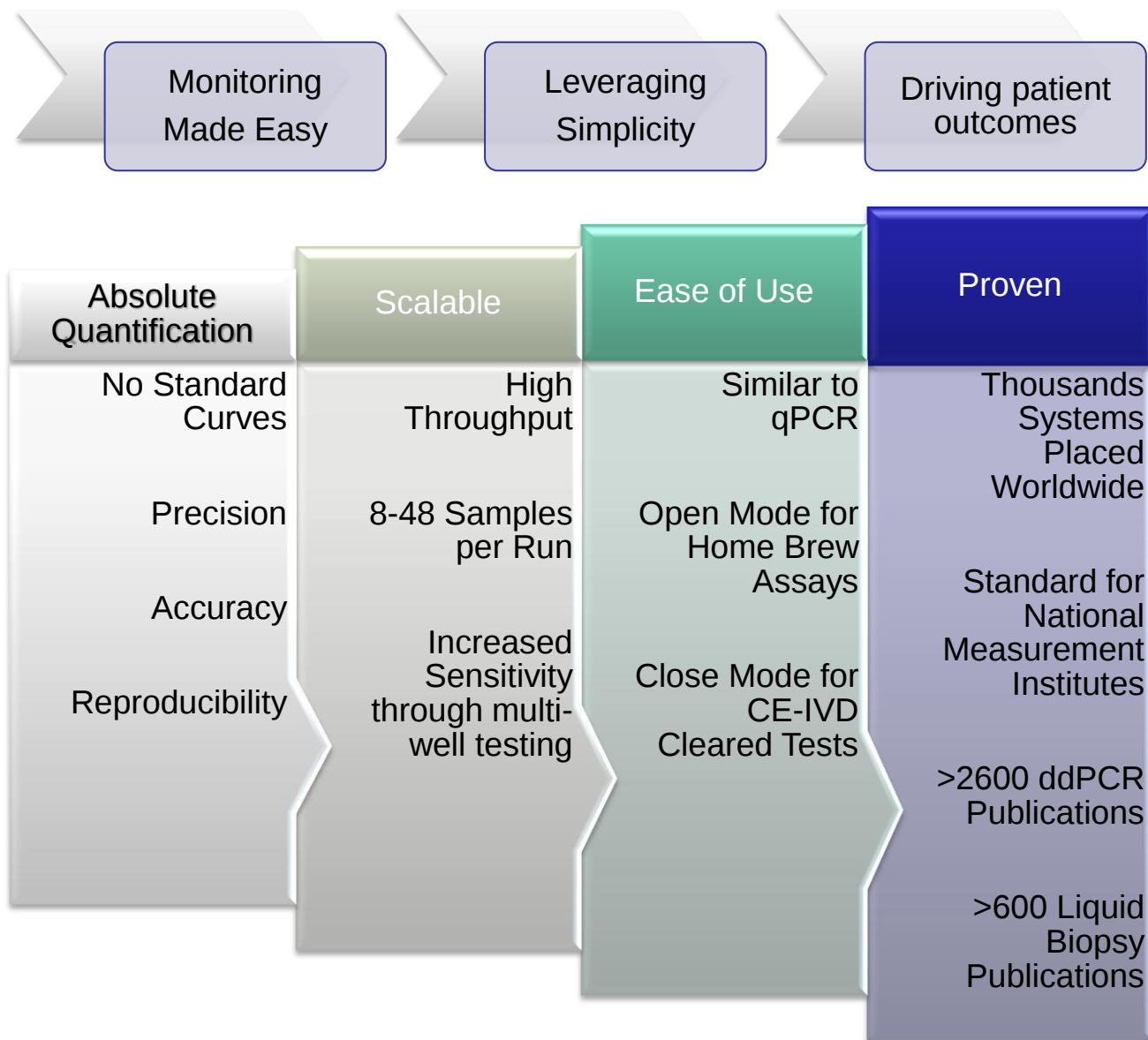
Current Paradigm testing qPCR

- Challenges with qPCR
 - Quantitation dependent on laboratory skill level
 - Most labs have a homebrew assay
 - Dependent on standards (dilution series)
 - High variation among labs
 - Each lab is attributed a correction factor

At low levels, qPCR is not as accurate as most would like.



Delivering a Better Way for BCR-ABL with ddPCR

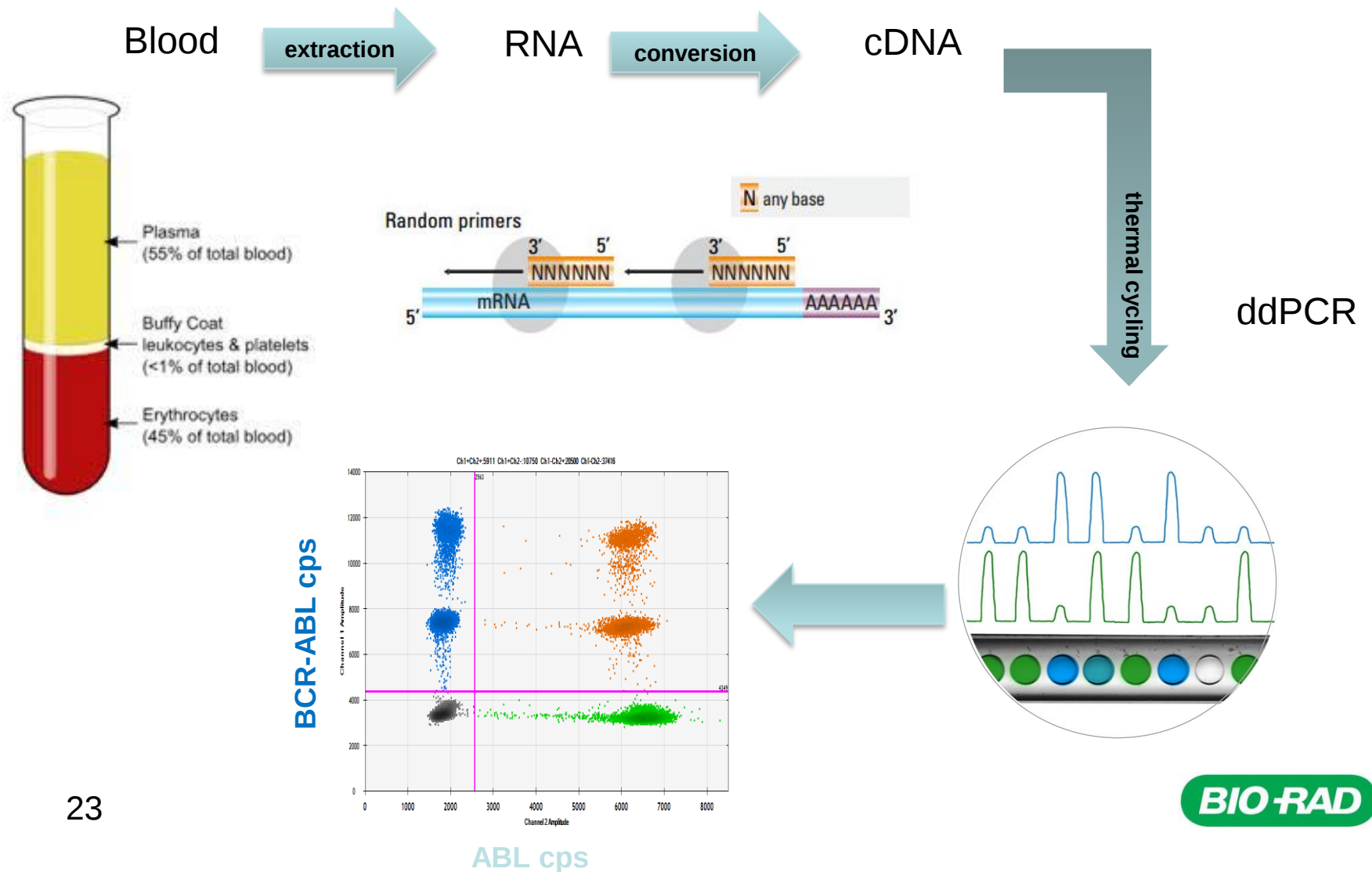




QXDx™ BCR-ABL %IS kit

Multiplex Design	Detects p210 Major transcripts (e13a2, e14a2)
Absolute Quantitation	Ability to count all BCR-ABL fusion gene present in the sample
Accuracy & Precision	Absolute confidence in results at low %IS Ratios
All Inclusive Reagents	Calibrators aligned to WHO standards
Automated Analysis	Automated thresholding, Result interpretation
Controls	Hi, Low %IS controls
Correction factor for WHO-IS	Relate results to WHO-IS (CF = 0.93, kit dependent)

Principle of the Test





Test Signatures

	CE-IVD (2 Wells)	CE-IVD (4 Wells)
Goal	Deep Molecular Response	Deep Molecular Response
LOQ	MR 4.7 (0.002% IS)	MR 5.0 (0.001% IS)
LOD	MR 4.7 (0.002% IS)	MR 5.0 (0.001% IS)



Scalable, Flexible & Reproducible solutions to enable Clinical research/trials and TKI stoppage studies.



QXDx™ BCR-ABL %IS kit

High precision and accuracy

- Increased sensitivity with 2- or 4-well test per patient sample

Absolute quantification

- Eliminates the need for the standard curves required with RT-PCR

Simplified and scalable workflow

- Flexibility to process 8 to 48 samples per run
- Flexible kit design to meet your laboratory's throughput and workflow needs

Standardized interpreted output

- Direct reporting on International Scale (%IS) and molecular response (MR) values





More content for ddPCR (research use only)

- PrimePCR ddPCR assays
- Multiplex screening kits
- Expert design assays
- Hematology assays
- Custom design assays

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PrimePCR ddPCR assay look up tool

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Technical
Support

PrimePCR Real-Time PCR Assay,
Panel, and ddPCR Look Up

Use the PrimePCR Lookup Tool to find assays and panels for your genes of interest. The results will show the available primer and probe assays, PrimePCR Panels, and/or ddPCR assays that match your genes of interest.

1. Select a technology:

☐ qPCR ☒ ddPCR

Note: Digital assays searchable on this site reflect the January 2015 catalog.
Please visit the [Bio-Rad.com Digital Assays](http://Bio-Rad.com/DigitalAssays) site for the most up-to-date assays available.

2. Select an organism:

☒ Human ☐ Mouse ☐ Rat

3. Select an Application (optional):

☐ Copy Number ☐ Mutation Detection ☐ Gene Expression ☒ All

4. Select reference genes (optional):

To additionally search for reference genes, type "reference" below.

Enter a list of genes or mutations: ⓘ

KRAS
EGFR
ESR1

<http://bioradtech.com/data/lookup.php>

Search



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ddPCR™ Multiplex Mutation Screening Kits



ddPCR Multiplex Mutation Screening Kits

ddPCR Multiplex Mutation Screening Kit*	Mutations Detected
<i>BRAF V600</i>	<i>V600E, V600K, V600R</i>
<i>KRAS G12/G13</i>	<i>G12A, G12C, G12D, G12R, G12S, G12V, G13D</i>
<i>KRAS Q61</i>	<i>Q61H (183A>C), Q61H (183A>T), Q61K, Q61L, Q61R</i>
<i>NRAS G12</i>	<i>G12A, G12C, G12D, G12R, G12S, G12V</i>
<i>NRAS G12/G13</i>	<i>G12A, G12C, G12D, G12S, G12V, G13D, G13R, G13V</i>
<i>NRAS Q61</i>	<i>Q61H (183A>C), Q61H (183A>T), Q61K, Q61L, Q61R</i>
<i>EGFR exon 19 deletions</i>	<i>>15 EGFR exon 19 deletions</i>

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[Bio-rad.com/web/MultiplexKits](https://www.bio-rad.com/web/MultiplexKits)

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Droplet Digital™ PCR

ddPCR Assays for Hematologic malignancies

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Assay Name	Unique Assay ID
<i>KIT D816V</i>	<u>dHsaMDV2010023</u>
<i>JAK2 V617F</i>	<u>dHsaMDV2010061</u>
<i>FLT3 D835Y</i>	<u>dHsaMDV2010047</u>
<i>FLT3 D835H</i>	<u>dHsaMDV2510492</u>
<i>FLT3 D835V</i>	<u>dHsaMDV2516838</u>
<i>FLT3 D835E</i>	<u>dHsaMDV2516864</u>
<i>IDH1 R132C</i>	<u>dHsaMDV2010053</u>
<i>IDH1 R132H</i>	<u>dHsaMDV2010055</u>
<i>IDH1 R132G</i>	<u>dHsaMDV2510512</u>
<i>IDH1 R132S</i>	<u>dHsaMDV2516816</u>
<i>IDH1 R132L</i>	<u>dHsaMDV2516818</u>

Droplet Digital™ PCR

ddPCR Assays for Hematologic malignancies

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Assay Name	Unique Assay ID
<i>IDH2 R140Q</i>	<u>dHsaMDV2010057</u>
<i>IDH2 R172K</i>	<u>dHsaMDV2010059</u>
<i>IDH2 R172M</i>	<u>dHsaMDV2516836</u>
<i>IDH2 R172W</i>	<u>dHsaMDV2516938</u>
<i>MPL W515K</i>	<u>dHsaMDS776418812</u>
<i>MPL W515K</i>	<u>dHsaMDS781756785</u>
<i>MPL W515L</i>	<u>dHsaMDV2510572</u>
<i>PML</i>	<u>dHsaCPE5037426</u>
<i>RARA</i>	<u>dHsaCPE5036437</u>

Droplet Digital™ PCR

Expert Design Assays

Available Assays

Assay Name	Unique Assay ID	SKU
<i>EML4-ALK</i> mRNA Fusions Assay	dHsaEXD86850342	12003909
<i>RET</i> mRNA Fusions Assay	dHsaEXD81378442	12003909
<i>ROS1</i> mRNA Fusions Assay	dHsaEXD73338942	12003909
<i>ESR1</i> Multiplex Assay 1	dHsaMDXE91450042	12004118
<i>ESR1</i> Multiplex Assay 2	dHsaMDXE65719815	12003910
<i>TERT</i> C228T_88	dHsaEXD20945488	12003908
<i>TERT</i> C228T_113 (<i>recommended assay</i>)	dHsaEXD72405942	12003908
<i>TERT</i> C228T_163	dHsaEXD59485034	12003908
<i>TERT</i> C250T_88	dHsaEXD85215261	12003908
<i>TERT</i> C250T_113 (<i>recommended assay</i>)	dHsaEXD46675715	12003908
<i>TERT</i> C250T_163	dHsaEXD33754807	12003908

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Assay design tool

Home > Life Science Research > Products > PCR Amplification > Digital PCR > Droplet Digital PCR Assays

Droplet Digital™ PCR Assays [My Assays](#)

Find an assay by ID, e.g., dHsaMDS376568575

Find and Mutation Detection Assay

Get the most out of your assays with Droplet Digital PCR. Choose your assay target, and let our tool design an assay for you.

Enter Target by: ☐ Identifier ☒ Sequence

Assay Target Name:

17 chars. maximum

Sequence ?

e.g.,
GGTCAGATACAGGCAAGCGGTCAGATACAGGCAAGCACAAGG
CAAGCACA[G/A]CTCGGTAAGTGGAGATTCGAGCGGTCAGATA
CAGGCAAGCACAAGGCAAGCAC (minimum length = 61 base
pairs on each side of the mutation)

Genome Edit Detection

[digital-assays/#/](#)

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Conclusion

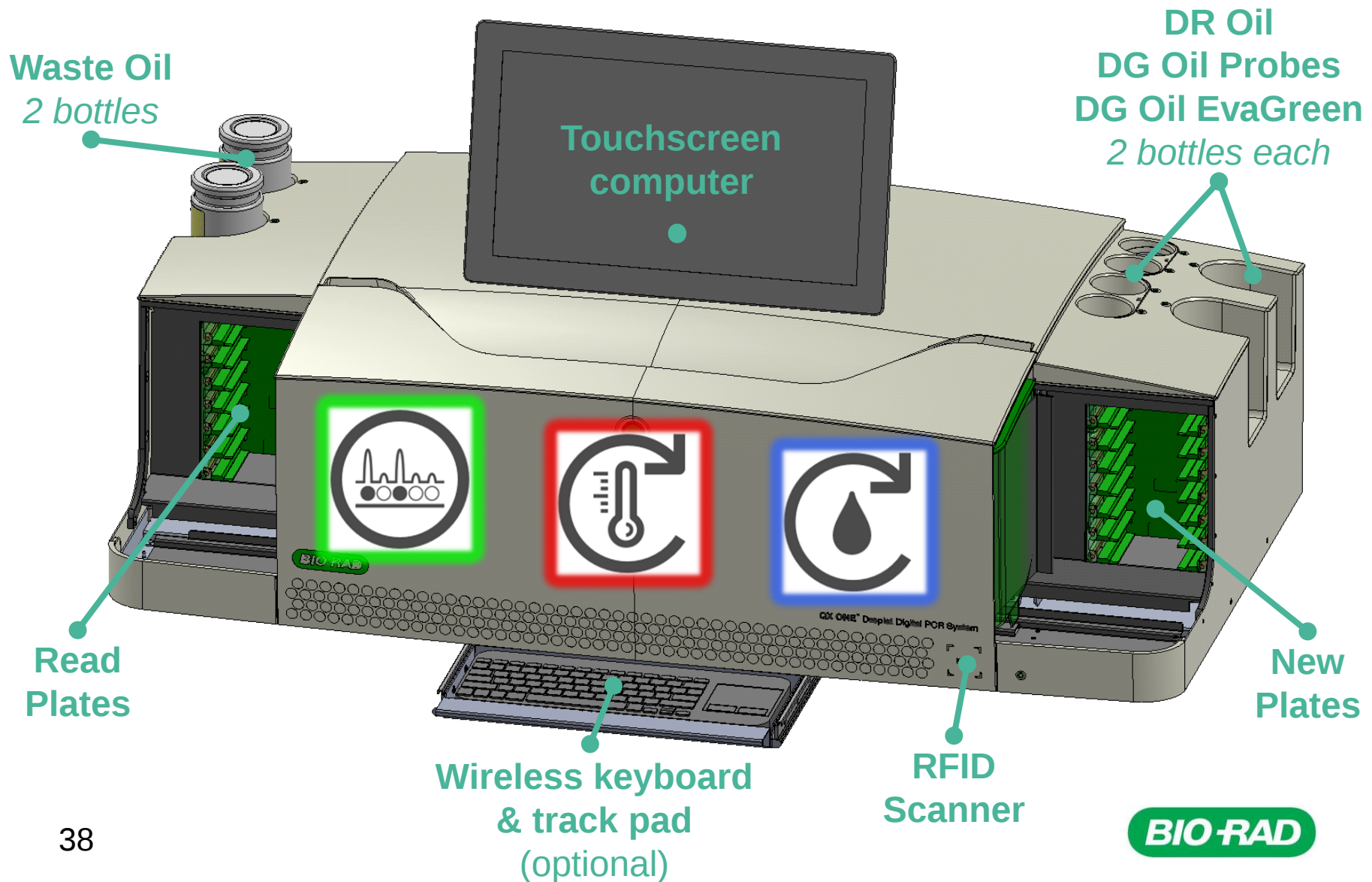
- **Proven Bio-Rad ddPCR technology**
 - Sensitive, reproducible, robust
 - Leverage ddPCR characteristics for entering the clinical diagnostic market
- **Building Bio-Rad QXDx™ product line**
 - CE-IVD instruments, consumables and BCR-ABL %IS kit
- **Bio-Rad PrimePCR assays**
 - Assay content available for own validation / accreditation and lab developed tests

One more thing ...

QX One™ ddPCR system



Instrument Architecture – User Interaction





QX One™ ddPCR system



System integration / High throughput:

automated, walk away, 7 plates, continuous feeding



4 colour multiplexing + gradient run:

advanced analysis capabilities, optimize custom assays



Seamless integration: compatible with existing chemistries, CFR21 part 11



Ease of use: on board touch screen, live data preview, RFID consumables, IQ/OQ/PQ

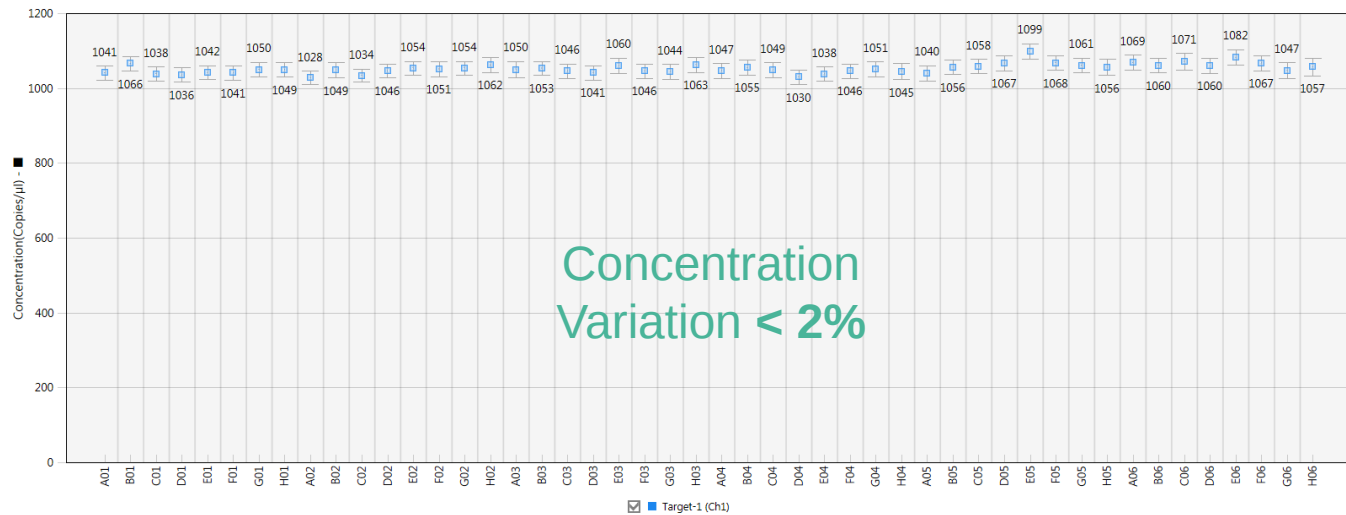
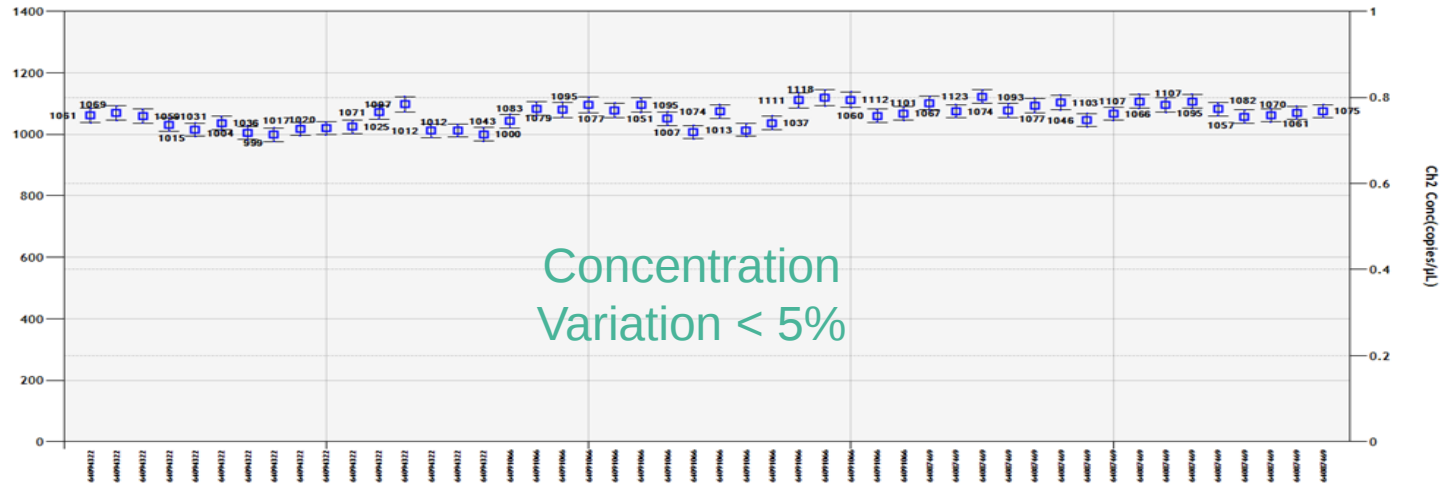




QX One™ ddPCR system

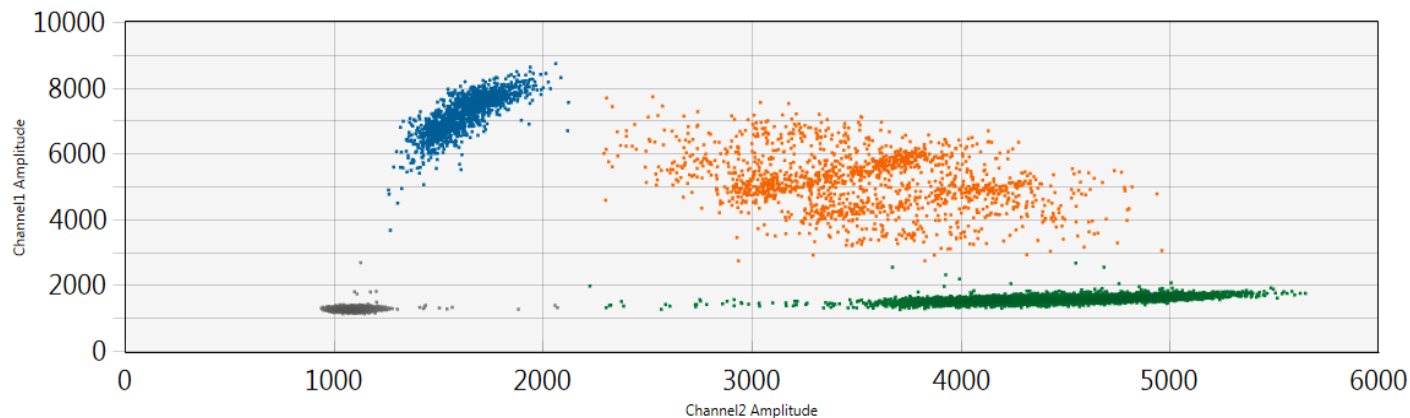
- 4 channel fluorescence detection
 - FAM, HEX, CY5, CY5.5
- Scatter channel
 - Measure droplet size
 - Normalisation per well using instrument calibration curve
 - Increased accuracy & precision

Concentration Uniformity

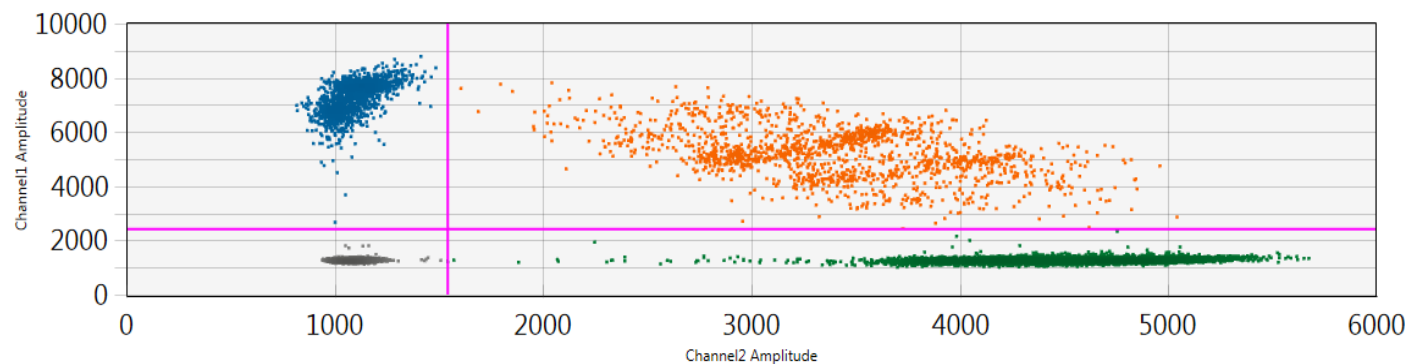


New Analysis Tool – Tilt Correction

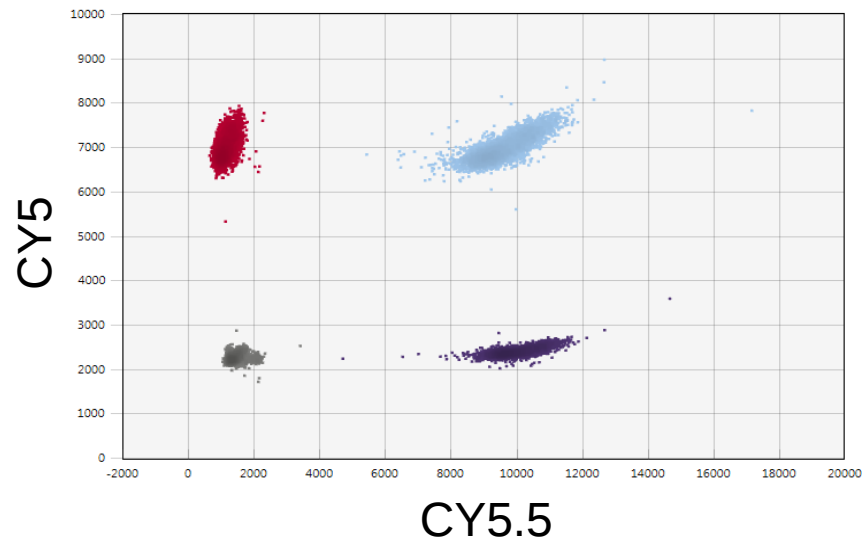
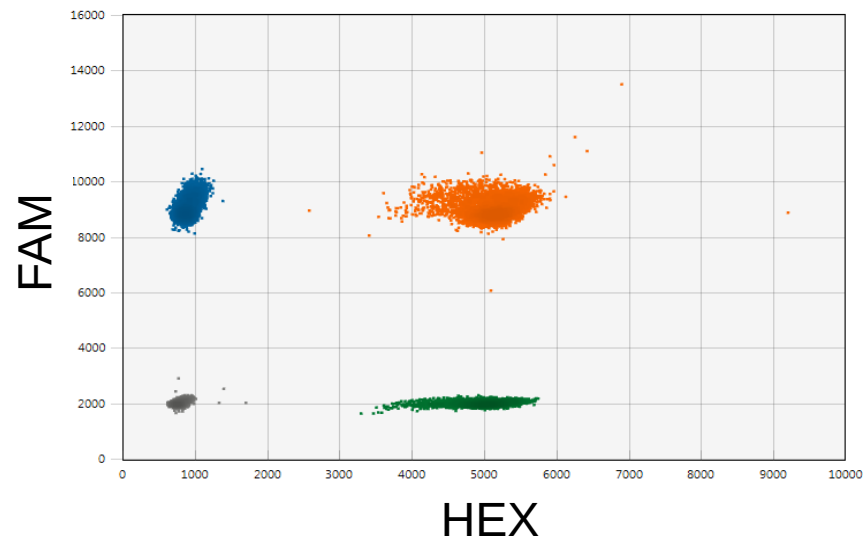
Example of a non-orthogonal 2D amplitude plot (*IDH2 R140Q* mutation detection duplex)



Tilt correction option allows for easier threshold setting



Data Preview – Copy Number 4-Plex

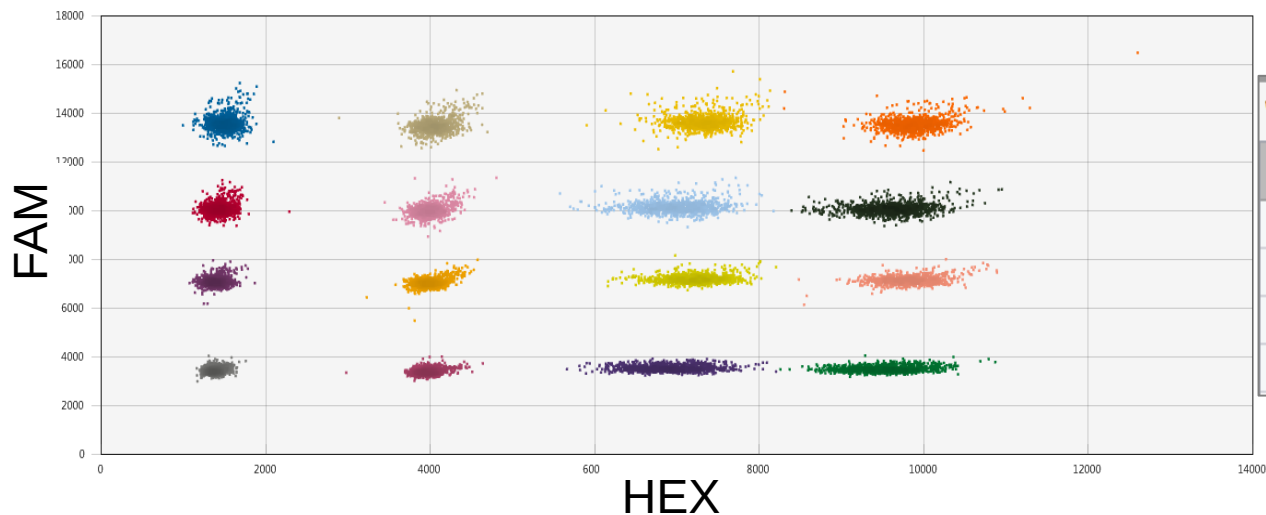


CCND1 (FAM), REN (HEX), PLAU (Cy5), SORL1 (Cy5.5)

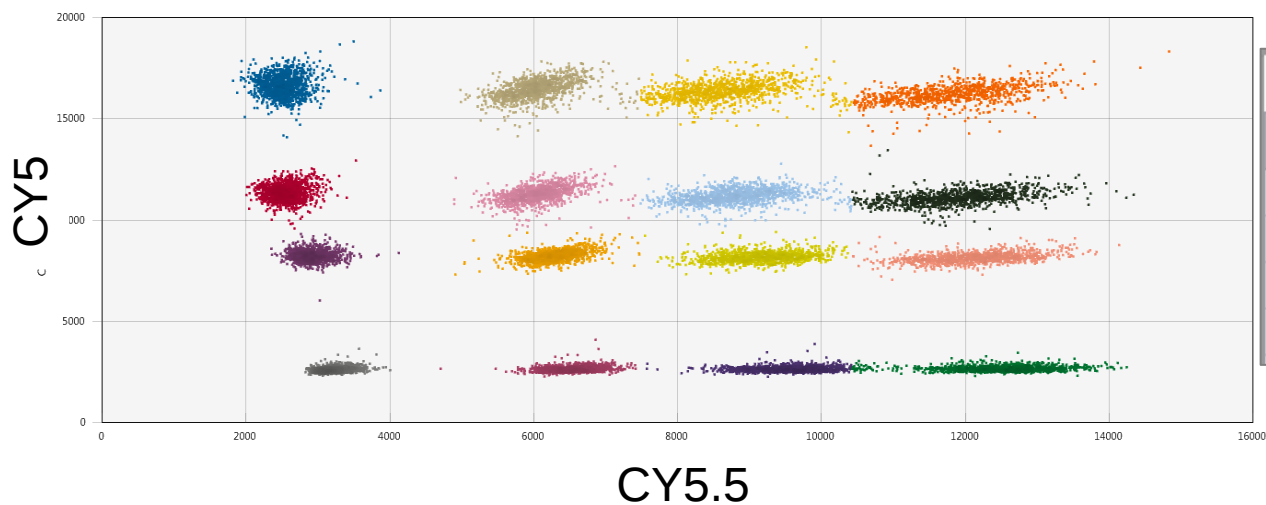
4 targets in one well is the new standard

Data Preview – Copy Number 8-Plex

8 targets in one well is possible



Well Data				
	Well	Sample	Target	Conc(copies/ μ L)
▶	G06	8-plex	Target-1	761
	G06	8-plex	Target-2	753
	G06	8-plex	Target-3	774
	G06	8-plex	Target-4	773

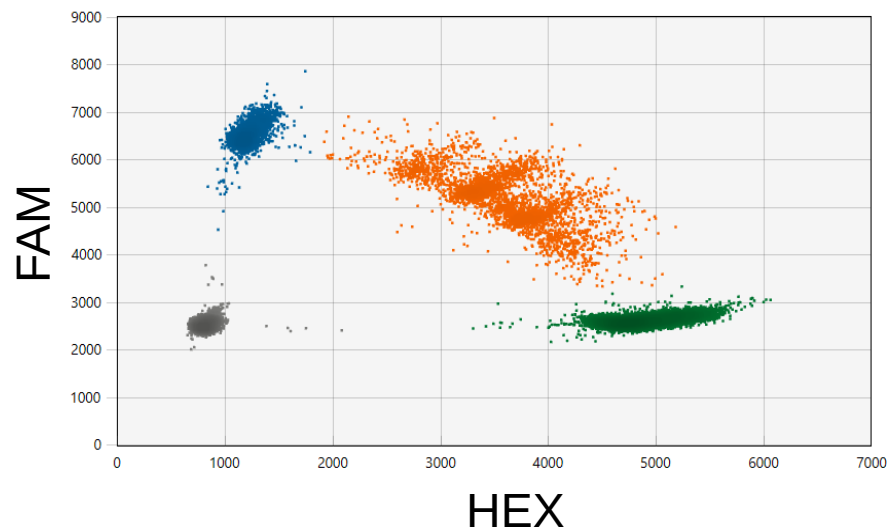


Well Data				
	Well	Sample	Target	Conc(copies/ μ L)
▶	F05	8-plex	Target-1	751
	F05	8-plex	Target-2	780
	F05	8-plex	Target-3	783
	F05	8-plex	Target-4	760



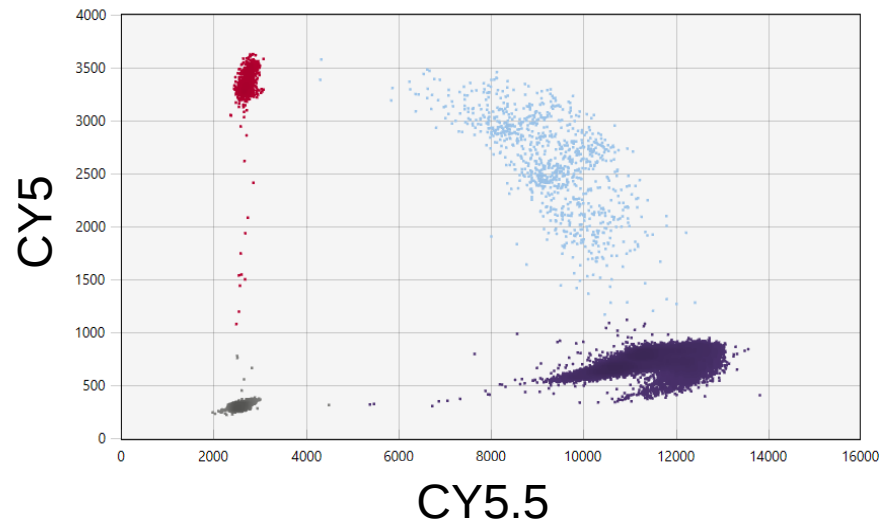
Data Preview – Mutation Screening Multiplex

NRAS G12 (*G12D gBlock*)



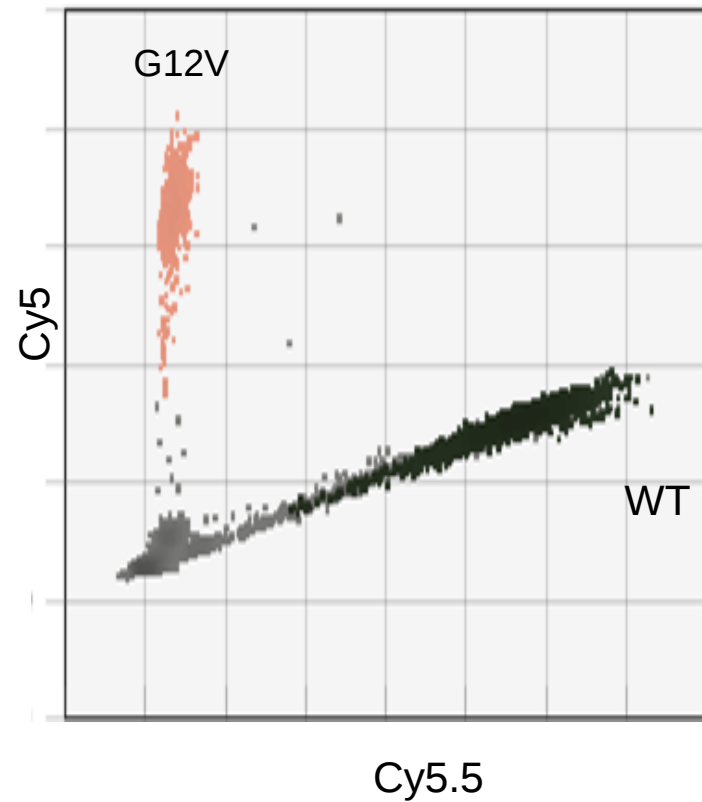
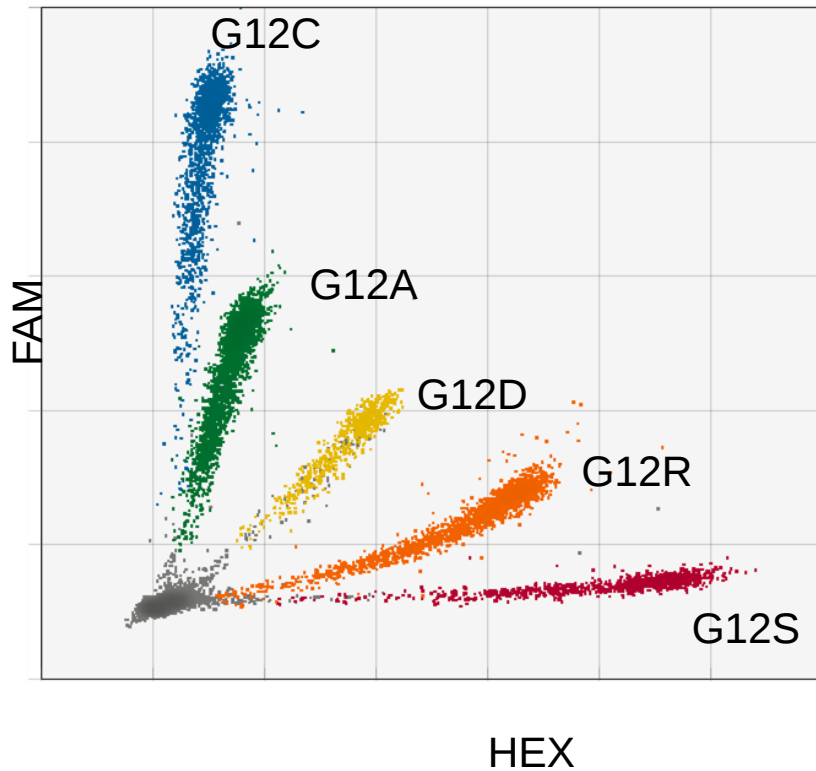
Screening assay detects
G12A, G12C, G12D, G12R, G12S, G12V

EGFR Ex19del (*COSM6223 gBlock*)



Screening assay detects
>15 EGFR exon 19 deletions

R&D Data on a Discriminating KRAS G12/13 Multiplex Kit



QX One™ ddPCR system

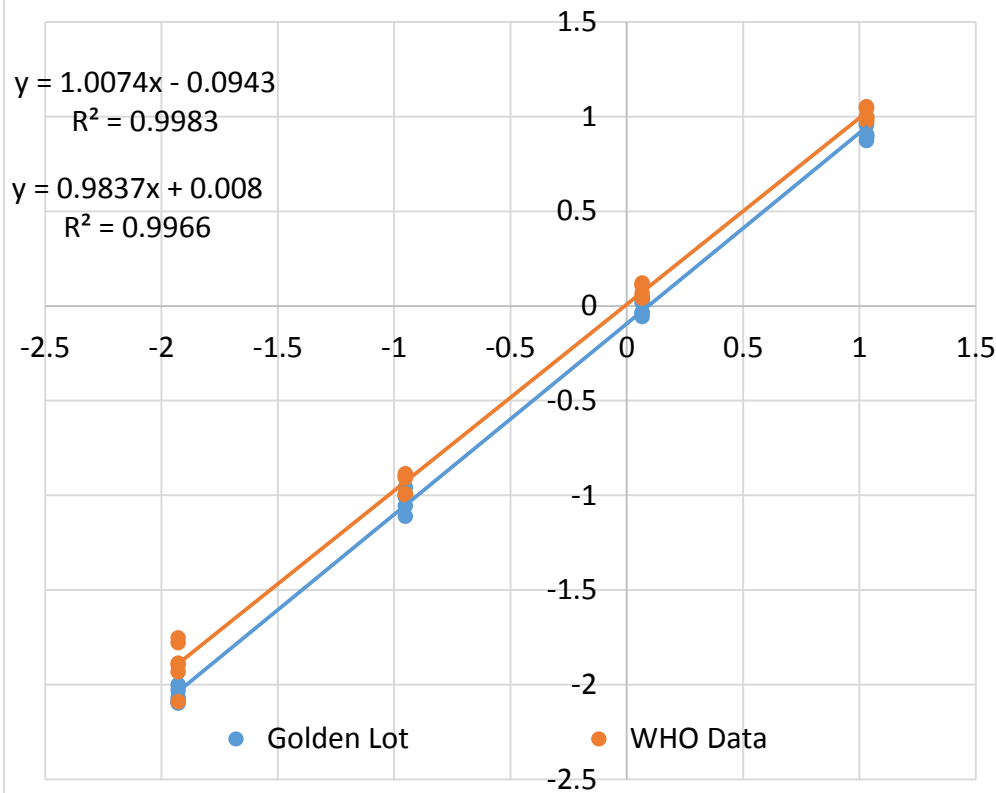
We write the future of ddPCR



<https://www.bio-rad.com/info/qxone>

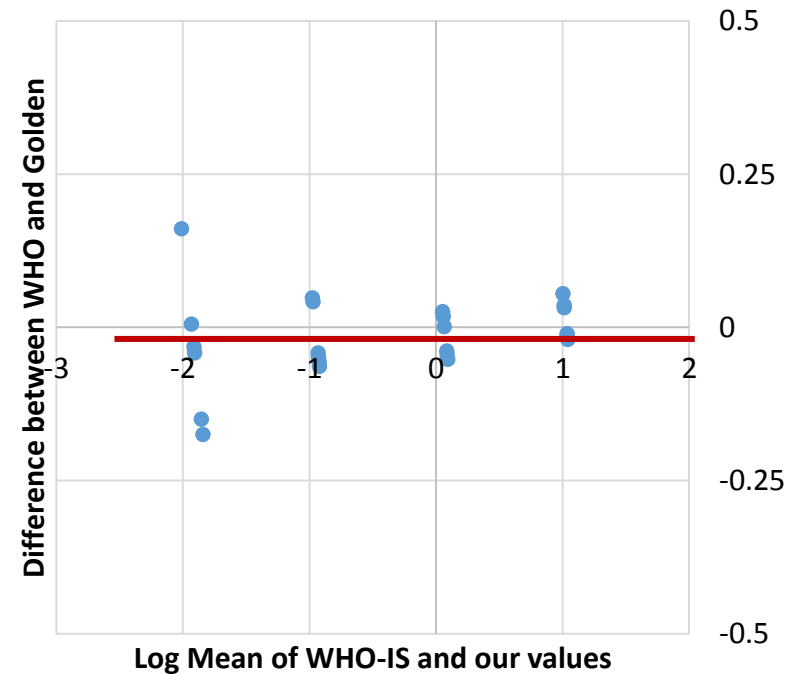
Setting a CF for ddPCR

WHO Actual vs Golden Lot



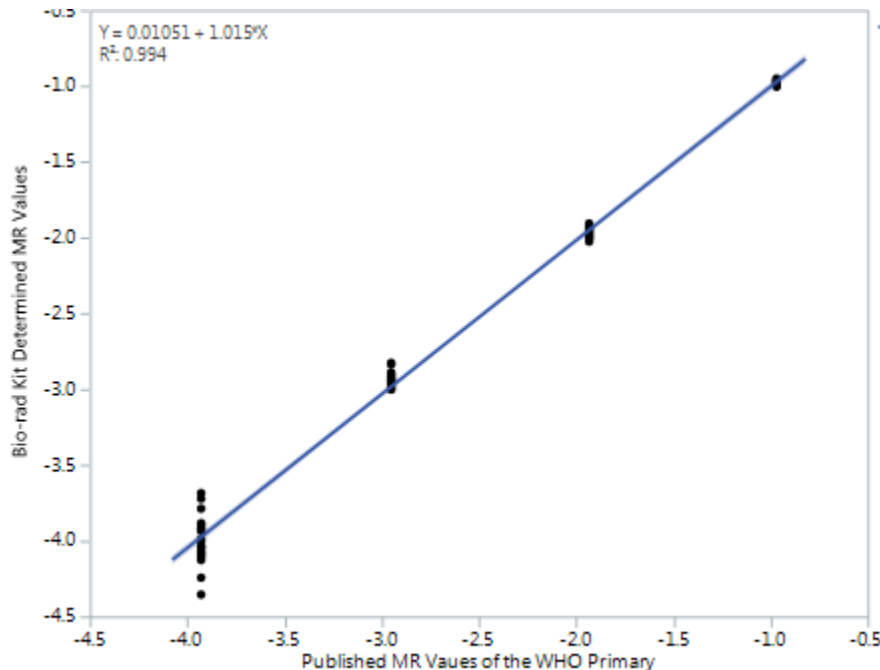
$$CF = 10^{Md} = 0.93$$

WHO Bias Plot



WHO-IS validation

- 5 kit lots tested
- WHO-IS primary materials used as samples
- 3 calibrator and control lots tested
- Regression performed with WHO-IS expected values vs ddPCR IS results



Metric	Minimum	Maximum	Measured
m	0.95	1.05	1.015
r2	0.98	1	0.994
intercept	-0.2	0.2	0.01051

Correction Factor (CF) = 0.93