Droplet Digital™ PCR

Le jeudi 12 octobre 2017

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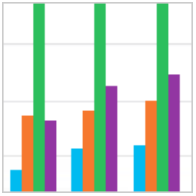
Product specialist qPCR ddPCR

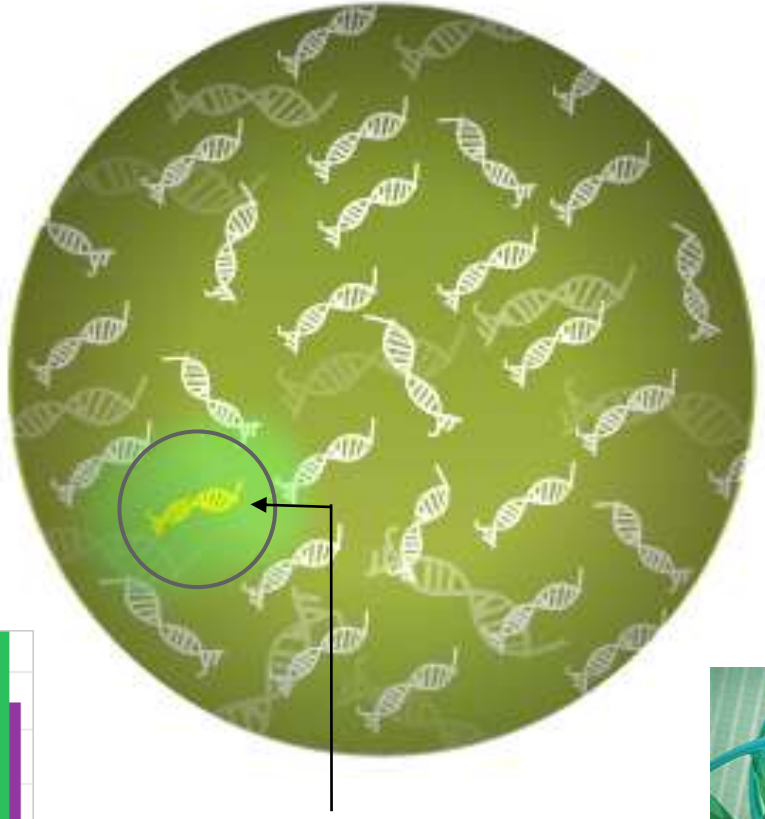
Identifying Target DNA

Cancer SNPs, CNV, Liquid Biopsies

Pathogen Detection



Gene Expression and Single Cell Analysis



Target DNA

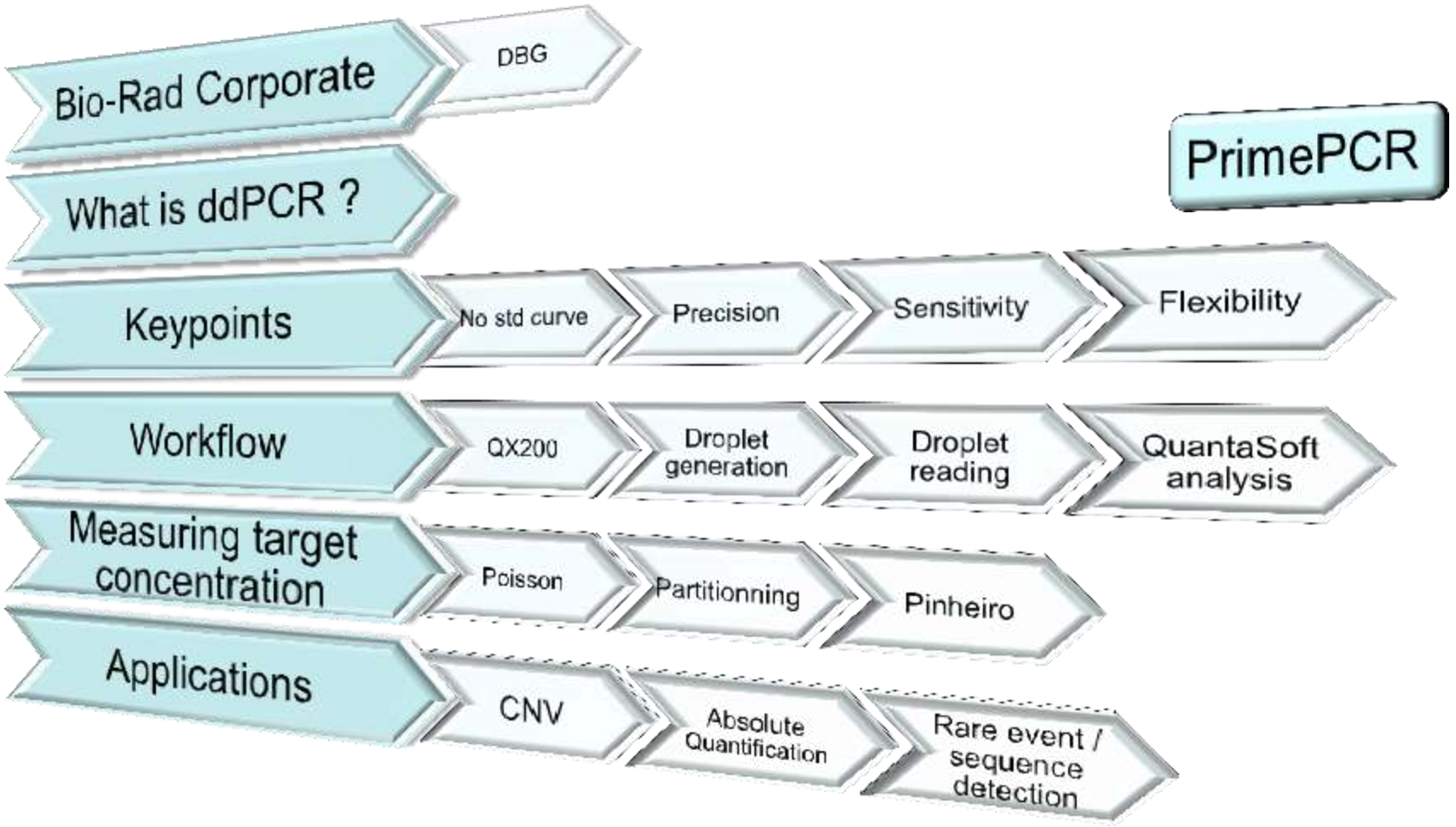
Agriculture, GMOs

Environmental Monitoring

NGS Workflow

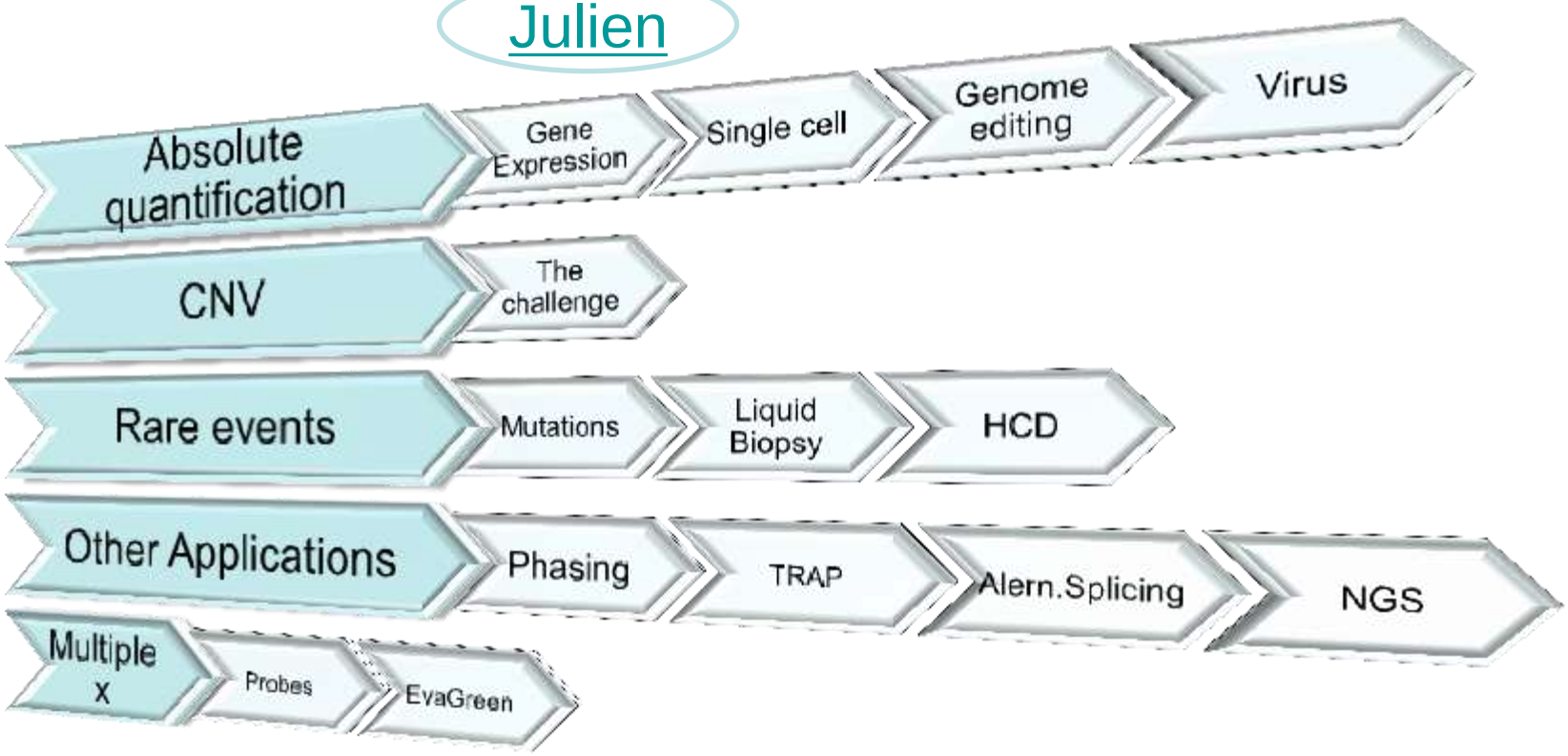
CRISPR Workflow

CONTENT


Bio-Rad Corporate
DBG
What is ddPCR ?
PrimePCR
Keypoints
No std curve
Precision
Sensitivity
Flexibility
Workflow
QX200
Droplet generation
Droplet reading
QuantaSoft analysis
Measuring target concentration
Poisson
Partitionning
Pinheiro
Applications
CNV
Absolute Quantification
Rare event / sequence detection

APPLICATIONS

Julien



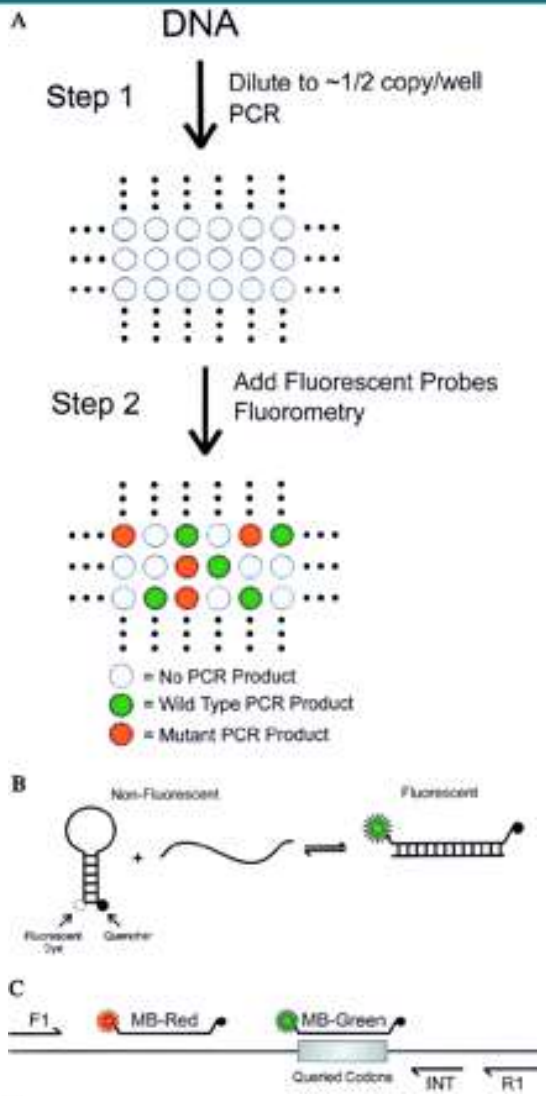
CONCLUSION

What Is Digital PCR?

What is Digital PCR?

PCR reaction that is partitioned

Digital PCR is not new: 1998!



"Here, we describe an approach for transforming the exponential, analog nature of the PCR into a linear, digital signal suitable for this purpose."

Bert Vogelstein, 9236–9241, doi: 10.1073/pnas.96.16.9236

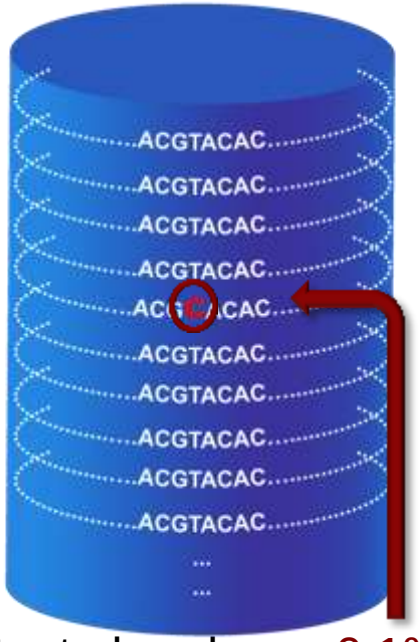
Droplets Enable Thousands of Digital Measurements

Nanodroplet PCR reactions are independent, single amplification events

Partitioning Increases Relative Abundance of Rare Events

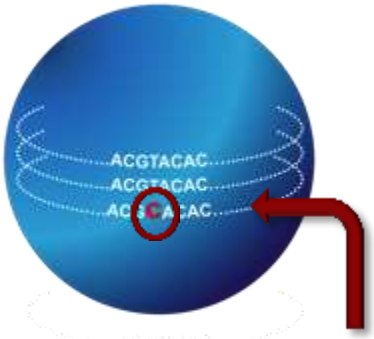
Bulk Sample – 1 X 20 µL

40,000 wildtype molecules
40 mutant molecules



Mutant abundance 0.1%

ddPCR Partitioned Sample – 20,000 × 1nL



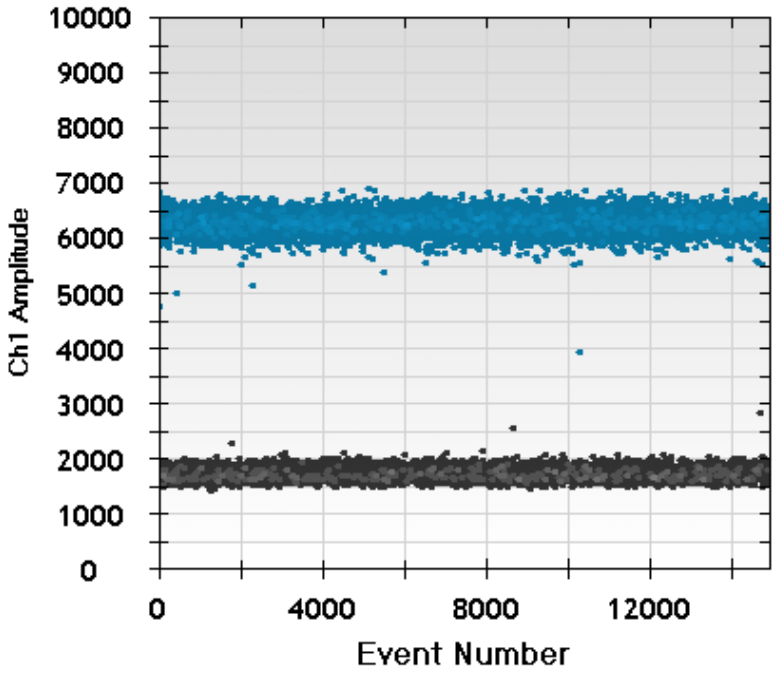
40 droplets w/ mutant

Mutant abundance 33%

19,960 droplets w/o mutant

Droplet Readings Converted to a Digital Signal

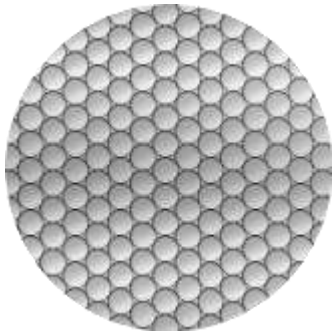
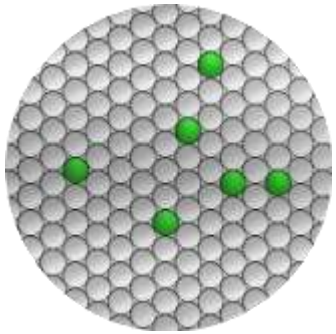
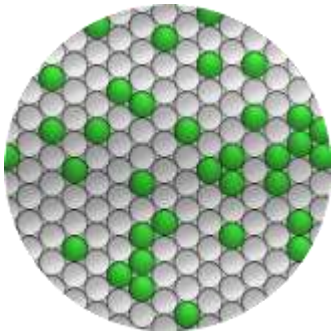
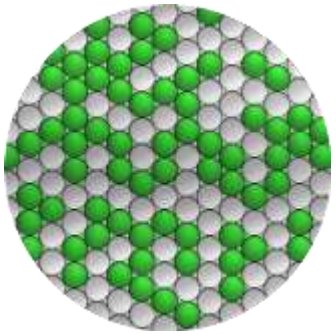
- Positive droplets contain at least 1 copy of target DNA (cDNA)
- Positive droplets have increased fluorescence vs. negatives
- QuantaSoft™ Software measures the number of positive and negative droplets per fluorophore per sample



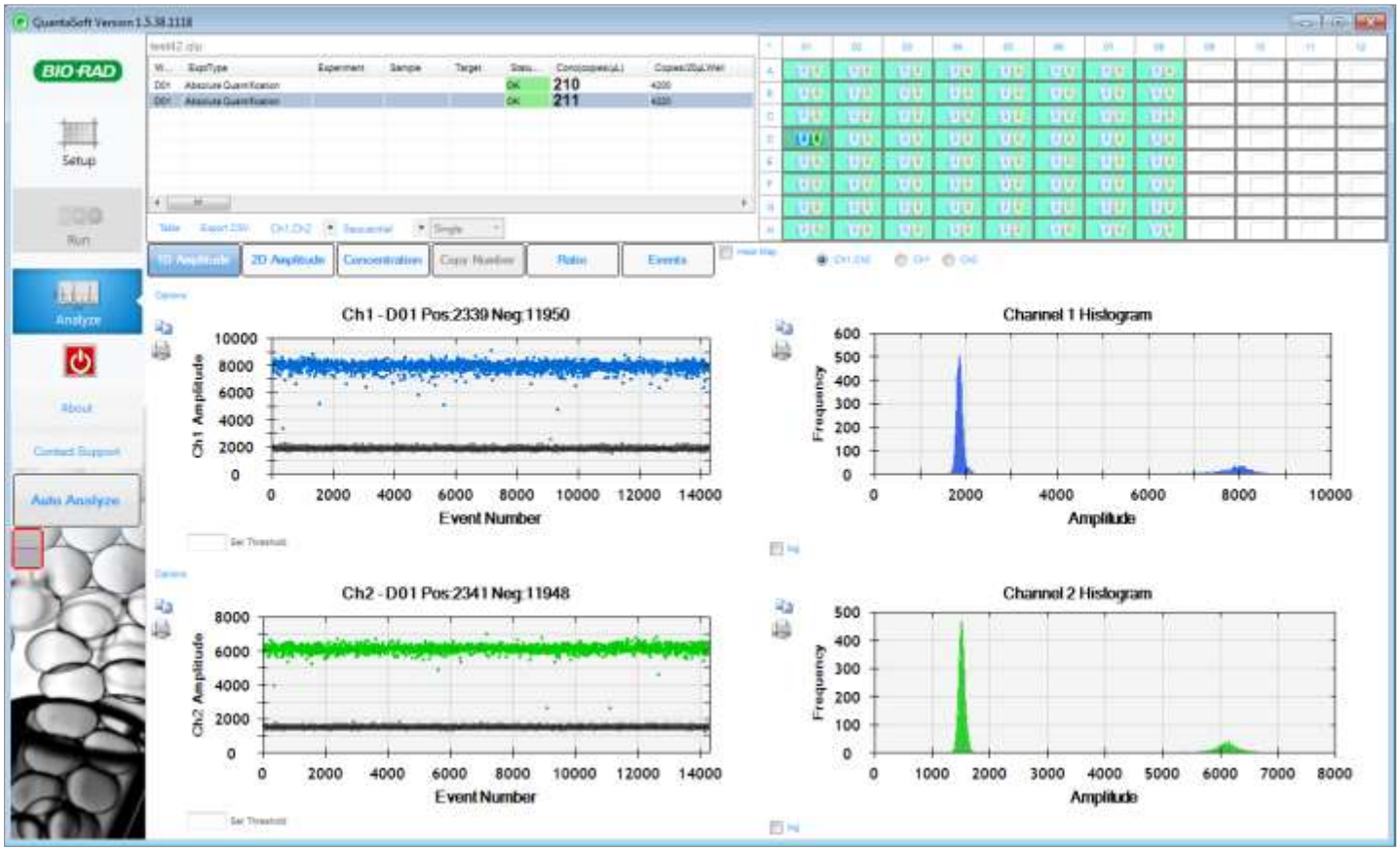
Each positive counted as 1

Each negative counted as 0

Counting Positives to Estimate Target Concentration

Sample 1	Sample 2	Sample 3	Sample 4
			
No targets	Low concentration	Medium concentration	High concentration
P = 0 positive/143 total	*P* = 6/143	*P* = 34/143	*P* = 70/143
	Poisson corrected **6.2**/143	Poisson corrected **38**/143	Poisson corrected **96**/143

Software Calculates Number of Target Molecules



Advantages of Droplet Digital PCR (ddPCR)

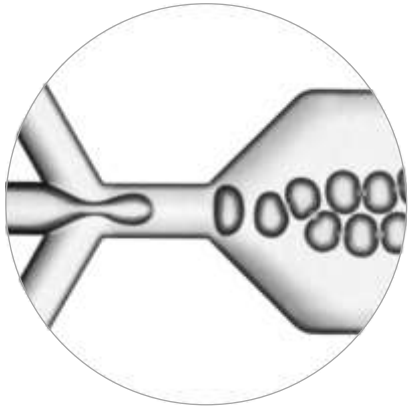
- ddPCR improves precision, sensitivity and reproducibility
  - Endpoint PCR (0's or 1's)
  - Less sensitive to PCR efficiency
  - No standard curve
  - Easy to analyze and interpret

- Used for challenging applications
  - Detect < 2-fold difference of DNA target between samples
  - Quantitate low input concentration of DNA target
  - Quantitate a rare DNA target in a large wild-type background

Workflow

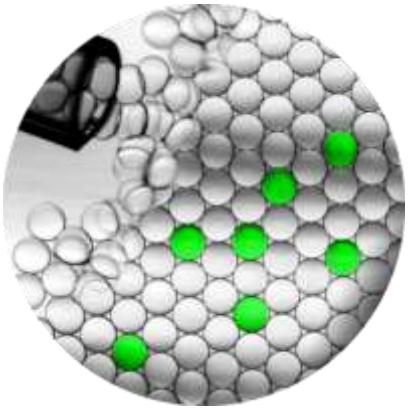
ddPCR Workflow

Partition Samples into Droplets



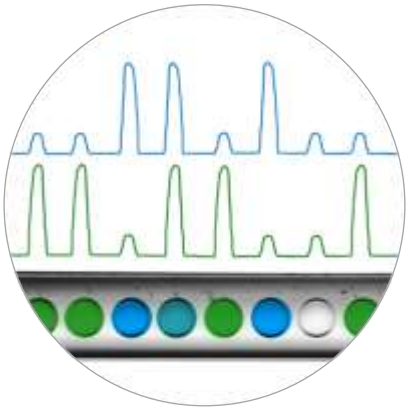
Droplet Generator

Cycle Droplets



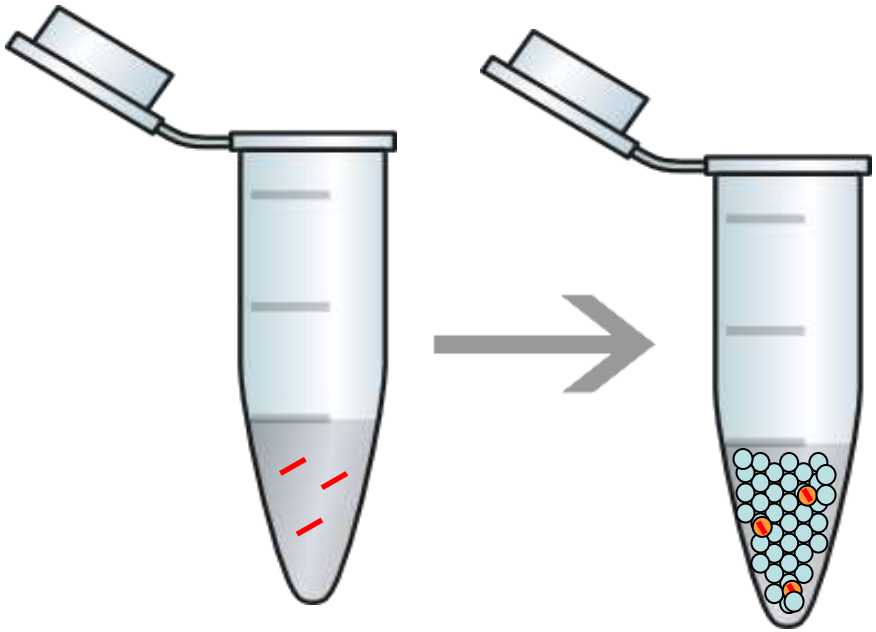
C1000 Touch™ Thermocycler

Read Droplets



QX200™ Droplet Reader

Key Technical Advantages to Droplet Digital PCR : Power In Partitioning



Bulk:
one measurement

Droplets:
many thousands
of independent discrete
measurements

- **Absolute quantification**
  - Input target counting
  - No relative quantification
  - End-point measurement
- **High precision**
  - Reproducibility
  - Discriminability
- **High sensitivity**
  - Rare events

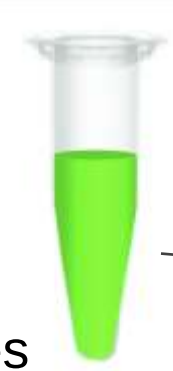
Prepare Sample & manual droplet generation

Prepare samples exactly the same as qPCR or PCR

ddPCR supermix

DNA or RNA sample

Primers/probes

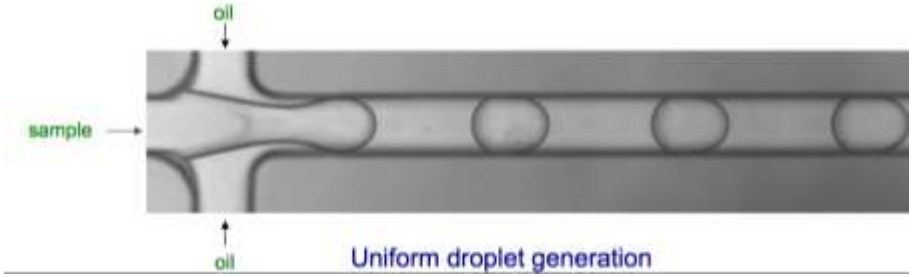


1. sample

2. oil

3. droplets

Compatible with probes (FAM and HEX/VIC) or EvaGreen

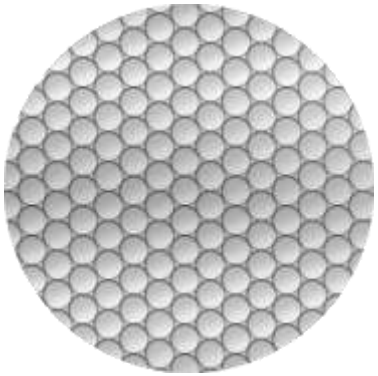


Automated Droplets Generation (optional)

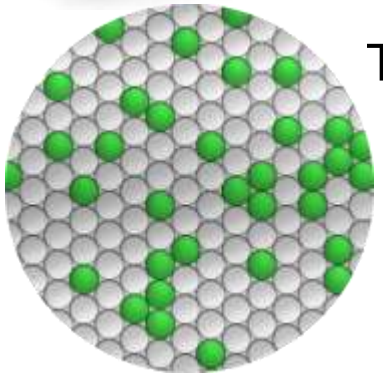
Generate thousands of droplets hands-free in the
Automated Droplet Generator

Amplify Droplets

Thermal cycle droplets to end point

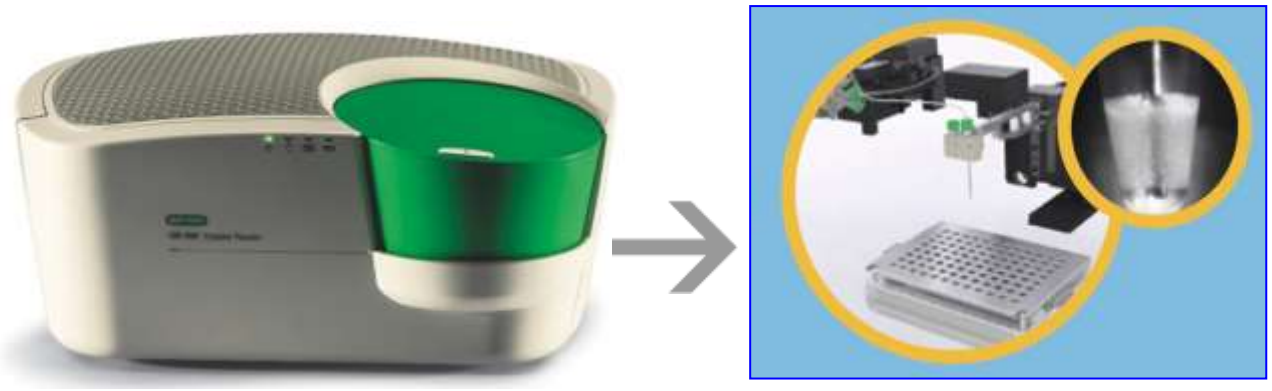


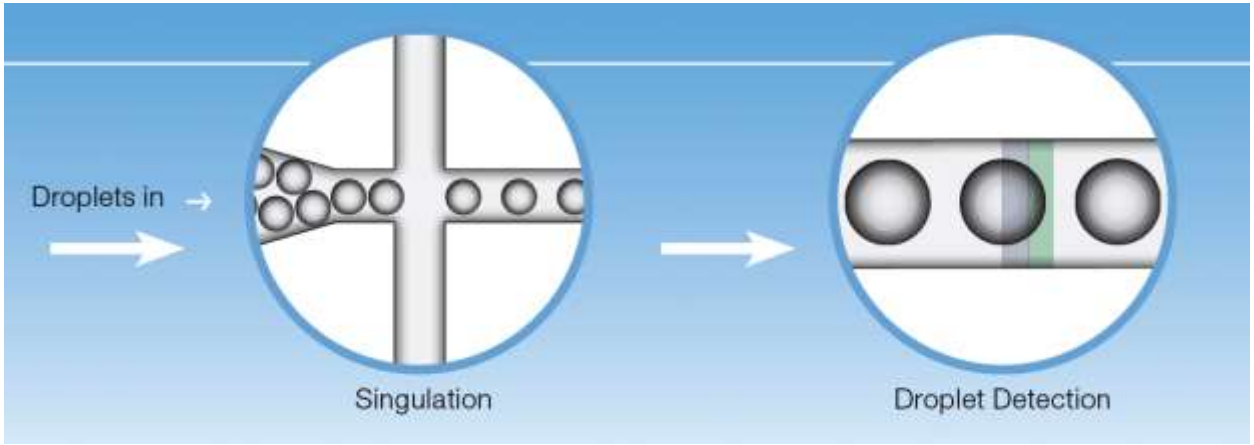
C1000 Touch™ Thermocycler



Read Droplets

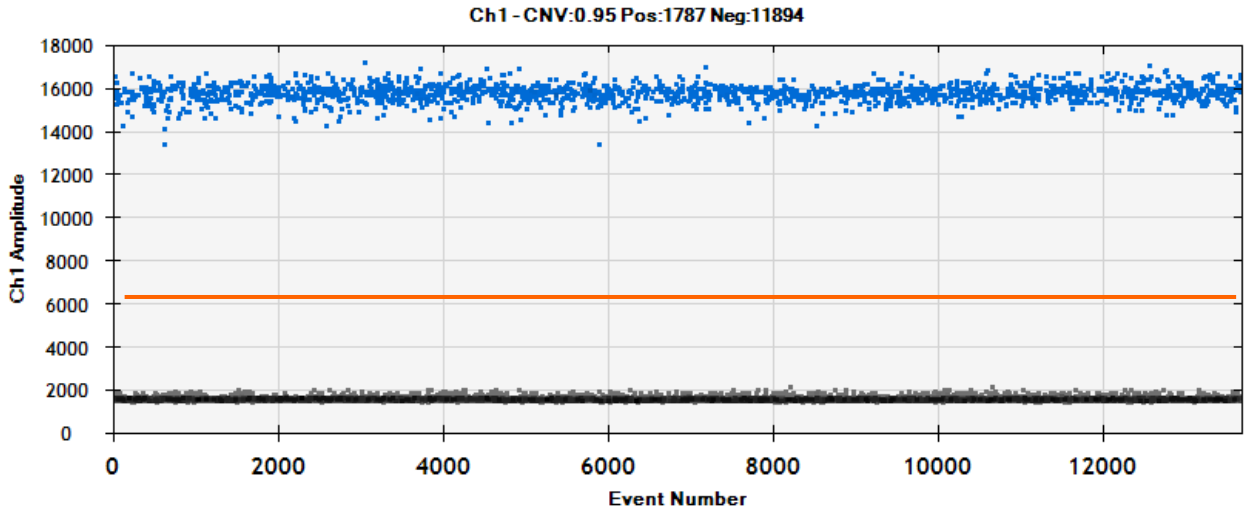
Fluorescence signal detected for each droplet





Automated Data Analysis

QuantaSoft™ plots fluorescence signal of droplets



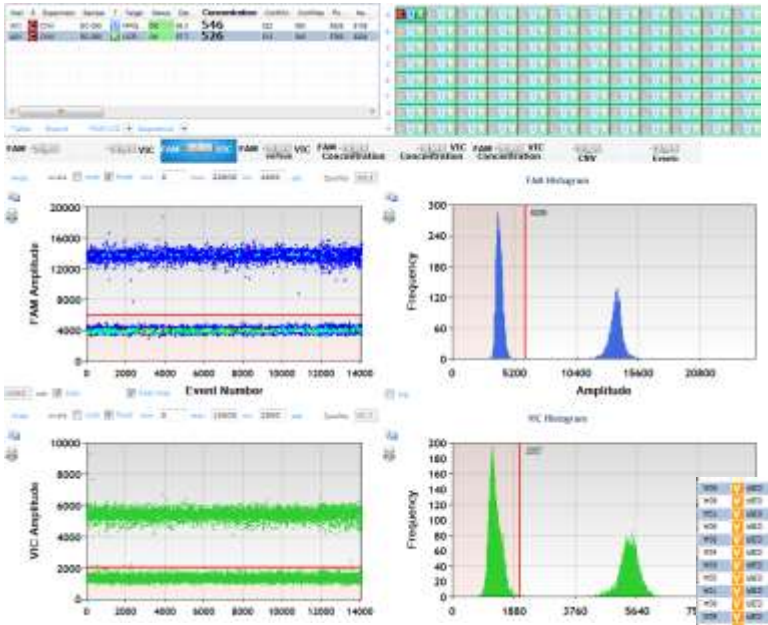
Positive Droplets

Threshold

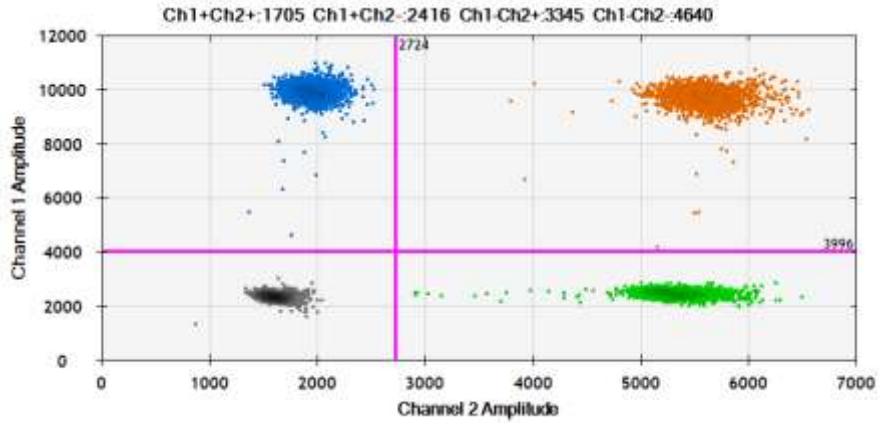
Negative Droplets

QuantaSoft Software: A Rich and Versatile Analysis Suite

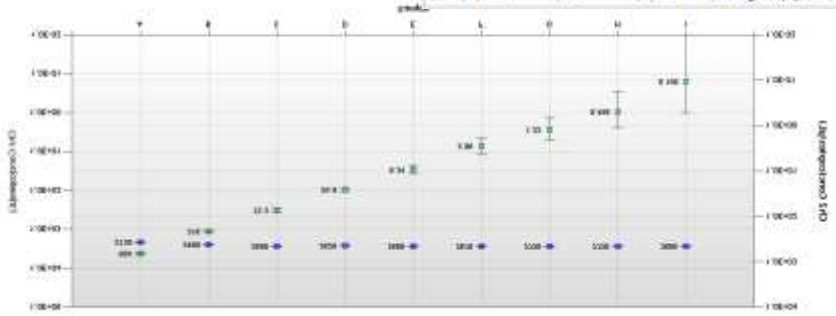
1-D Temporal Plot



2-D Cluster Plot



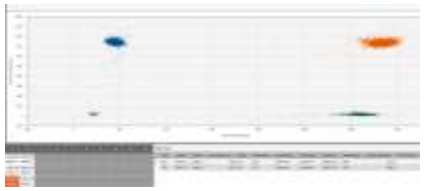
Concentrations Plot



Exportable Results Table

BIO-RAD

8-samples to result time



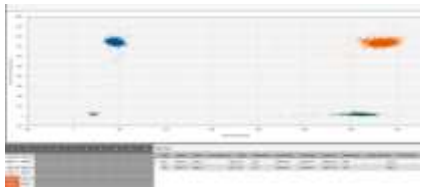
Generate → Amplify → Read → 2 colors analysis

~3 minutes	110 minutes	~12 minutes	~10 minutes

~2h15 from samples to results

~20 mn hands-on time

Manual 96-samples to result time



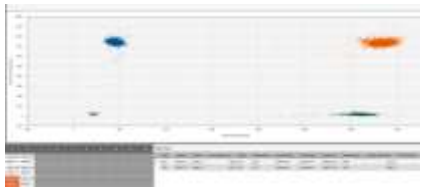
Generate → Amplify → Read → 2 colors analysis

~40 minutes	110 minutes	~150 minutes	~10 minutes

~5h10 from samples to results

~1 h hands-on time

Automated 96-samples to result time



Generate → Amplify → Read → 2 colors analysis

~40 minutes	110 minutes	~150 minutes	~10 minutes

~5h10 from samples to results

~20 mn hands-on time

Bio-Rad's QX200 (IVD) Droplet Digital PCR System

Automated Droplet Generator (ADG)

Droplet Reader (DR)

Droplet Generator (DG)

Thermocycler C1000

Reagents

Consumables

Thermoscelleuse PX1

Bio-Rad solutions for the clinical diagnostic market

Bio-Rad QX200™ CE-IVD Droplet Digital PCR System

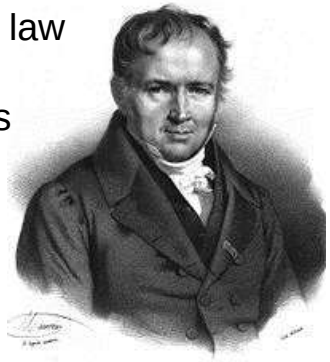
AutoDG System CE-IVD & Universal Reagents CE-IVD kits

Estimating concentration

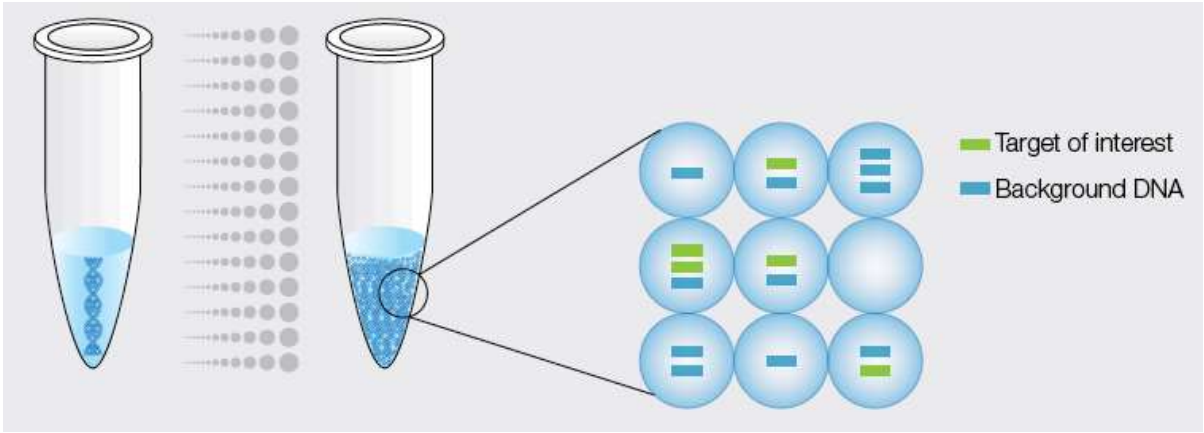
Estimating Target Concentration

- You do not need to dilute your starting sample so that each droplet contains either 0 or 1 copies of target
  - **ddPCR can handle multiple target copies per droplet**
- There is a random distribution of independent events when target copies are partioned into droplets from starting sample
  - No physical link binds the molecules together or pushes them apart from one another

Poisson law of small numbers



Siméon Denis Poisson (1781-1840)



Droplet Digital PCR Applications

Copy Number Variation (CNV)

Rare Event Detection (RED)

Absolute Quantification (ABS)

CNV Applications

Copy Number Variation (CNV)

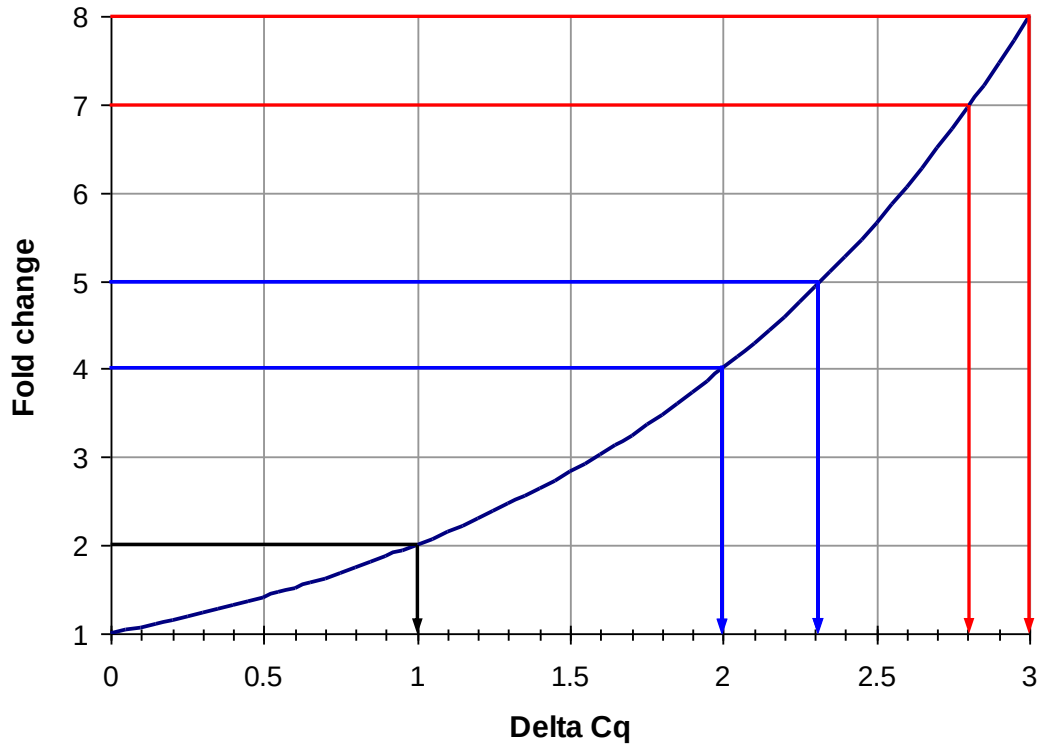
- ddPCR interest
- Applications
- Validated (Peri) Centromeric Reference Assays

Copy Number Variation

- Analysis of the change in ploidy of certain genes, genomic regions or chromosomes
- Can be associated with normal developmental processes or pathological evolution
- Important field of study for cancer, human genetics, crop studies…

CNVs are challenging for Real-Time PCR

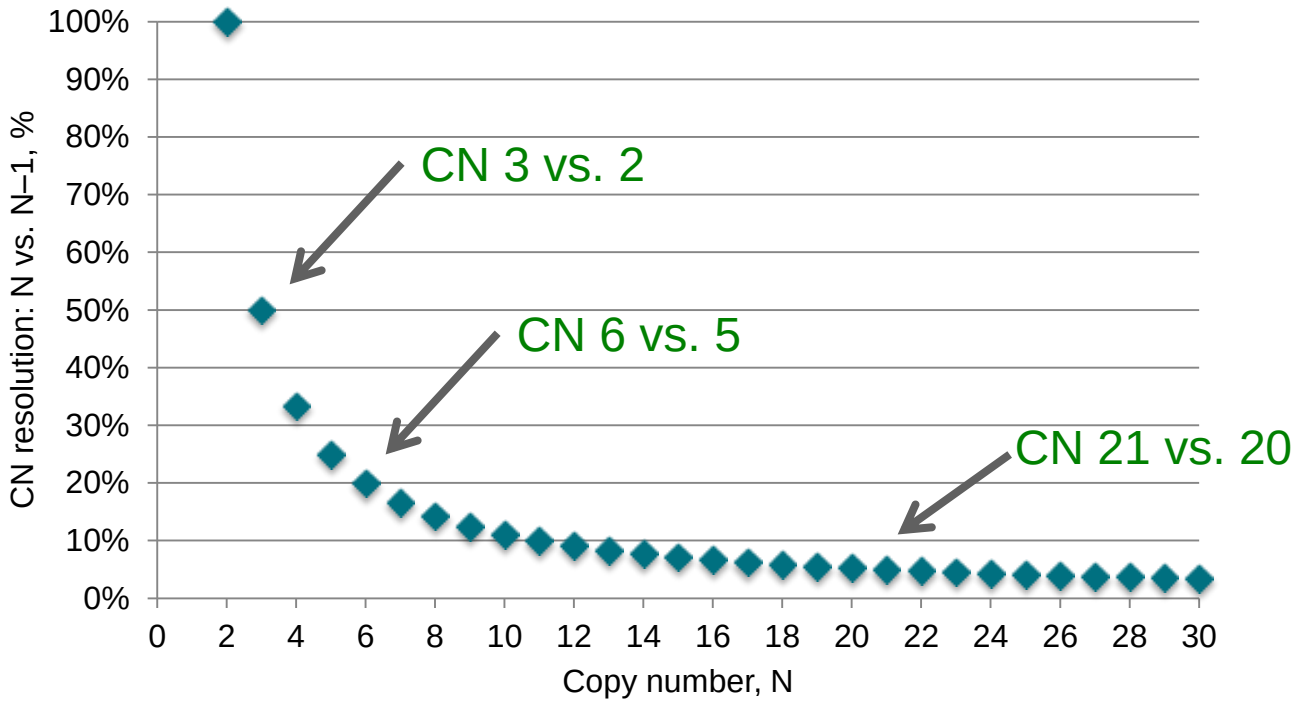
- Real-time PCR results have an **exponential** nature:



- A 2-fold difference in copy number equates to 1 Cq difference.
- A difference of 4 vs 5 copies equates to 0.32 Cq difference.
- A difference of 7 vs 8 copies equates to 0.14 Cq difference.
- Real-time PCR results rely heavily on assay efficiency

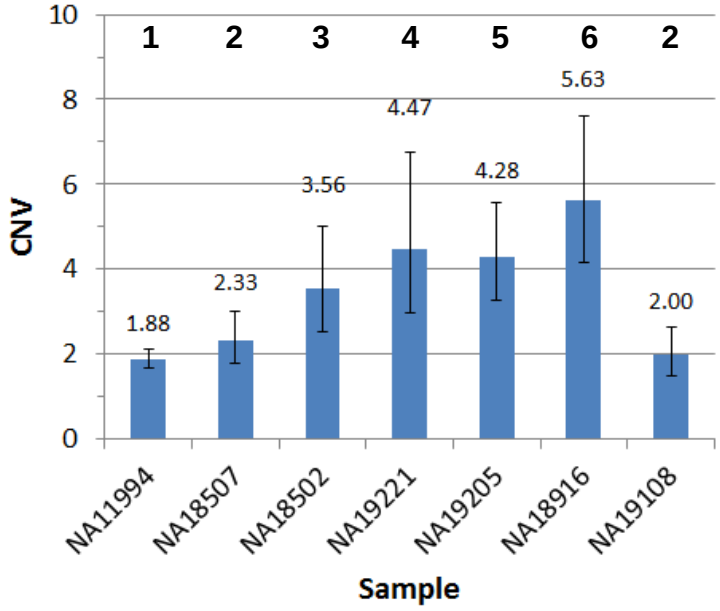
Copy Number Variation: What Is the Challenge?

Homogeneous samples: Discrimination between consecutive copy number states is more difficult at higher order copy number (CN).



Measuring Copy Number for *MRGPRX1* (qPCR)

Real-time PCR
(8 replicates)

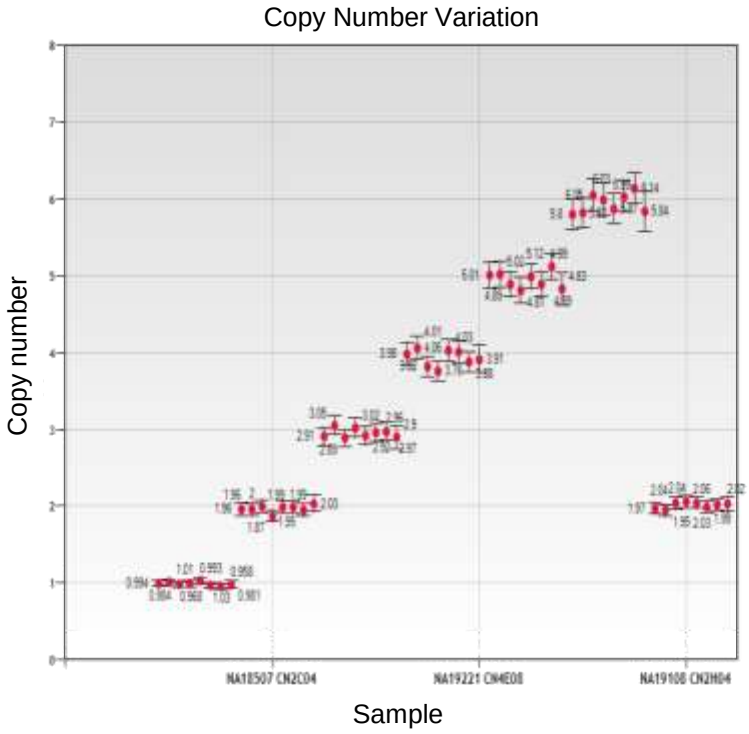


"…it should be possible to distinguish, with at least 95.1% probability, (…) four copies from five copies with 18 replicates"

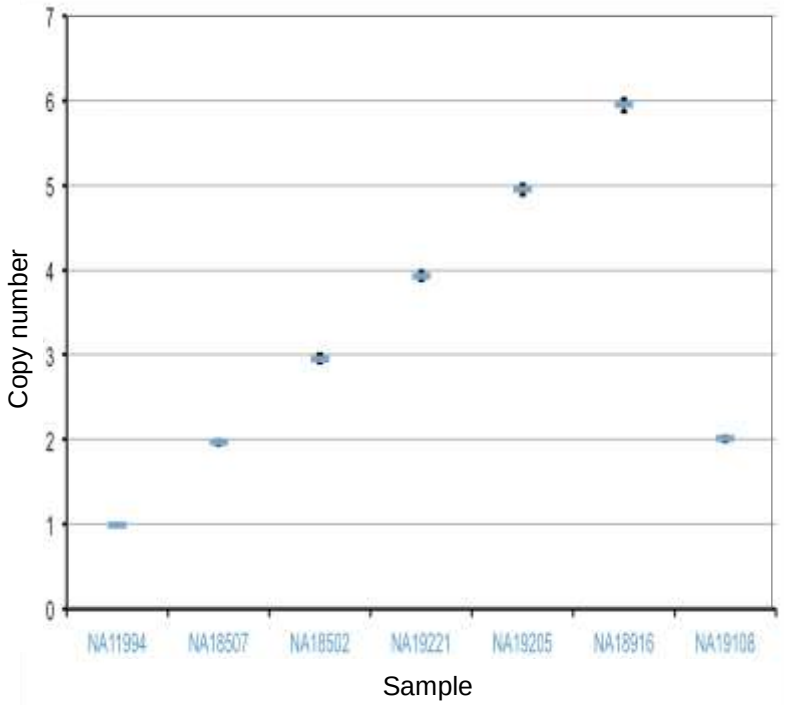
Weaver *et al*. (2010) Methods **50,** 271–276

Measuring Copy Number for *MRGPRX1* (ddPCR)

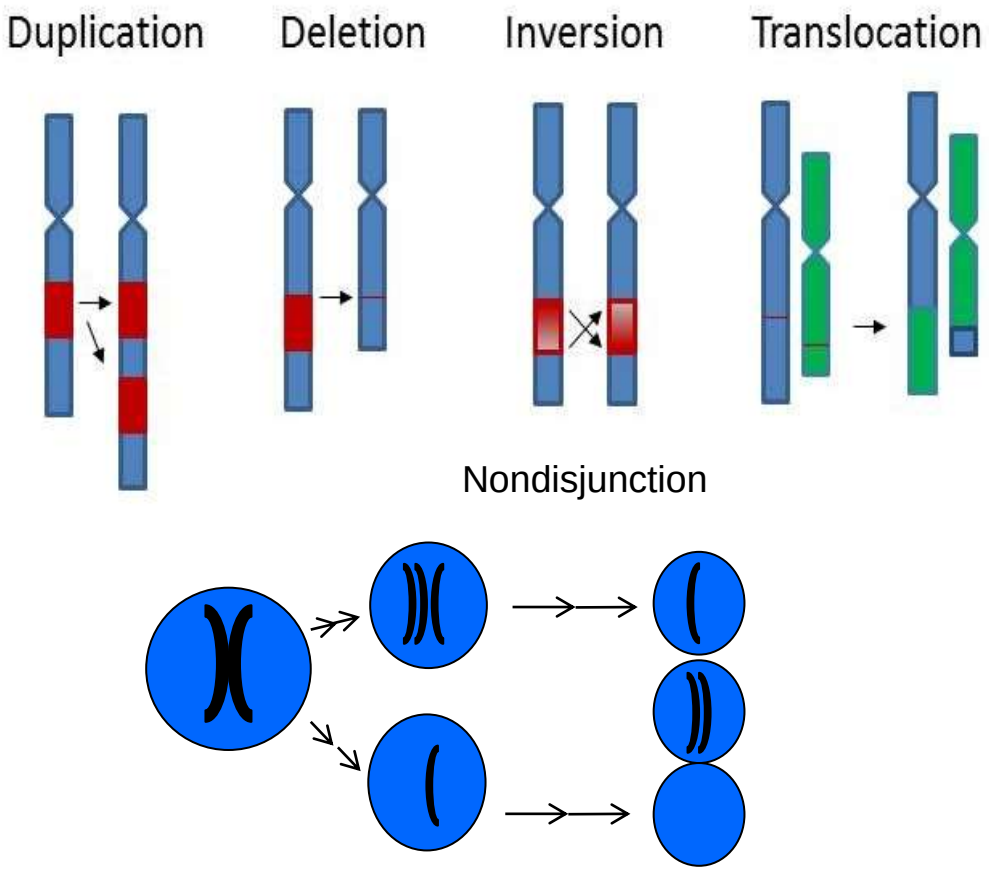
Droplet Digital PCR Individual Wells



Droplet Digital PCR Merged Wells



Copy Number Variation/Alterations in Cancer

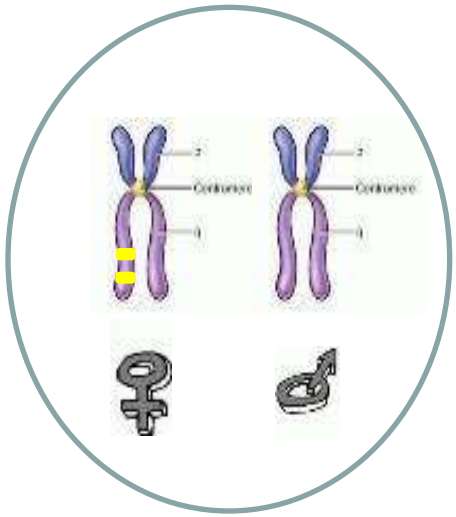


Copy Number Variation

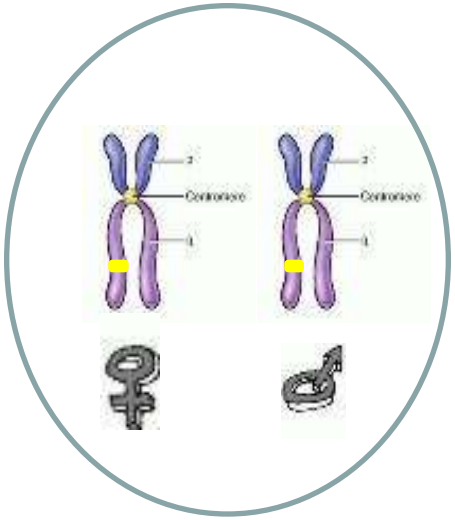
Can ddPCR tell if the copies are on different chromosomes?

For example, determine if a normal-seeming CNV=2 is a deletion carrier

Deletion Carrier



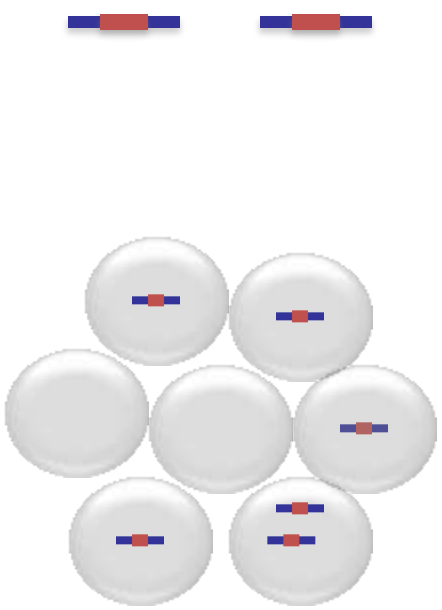
Normal



Let’s compare CNV estimates **with and without** restriction digestion

Can ddPCR tell if copies are on different chromosomes?

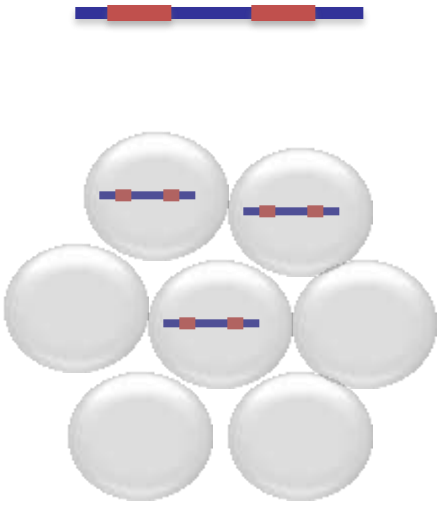
With restriction digestion



CNV estimate = X

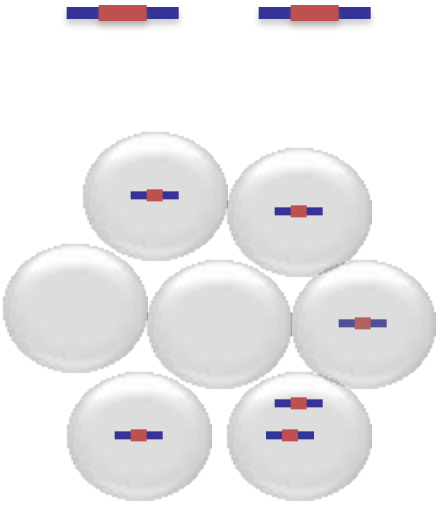
Without restriction digestion

Two tandem copies:



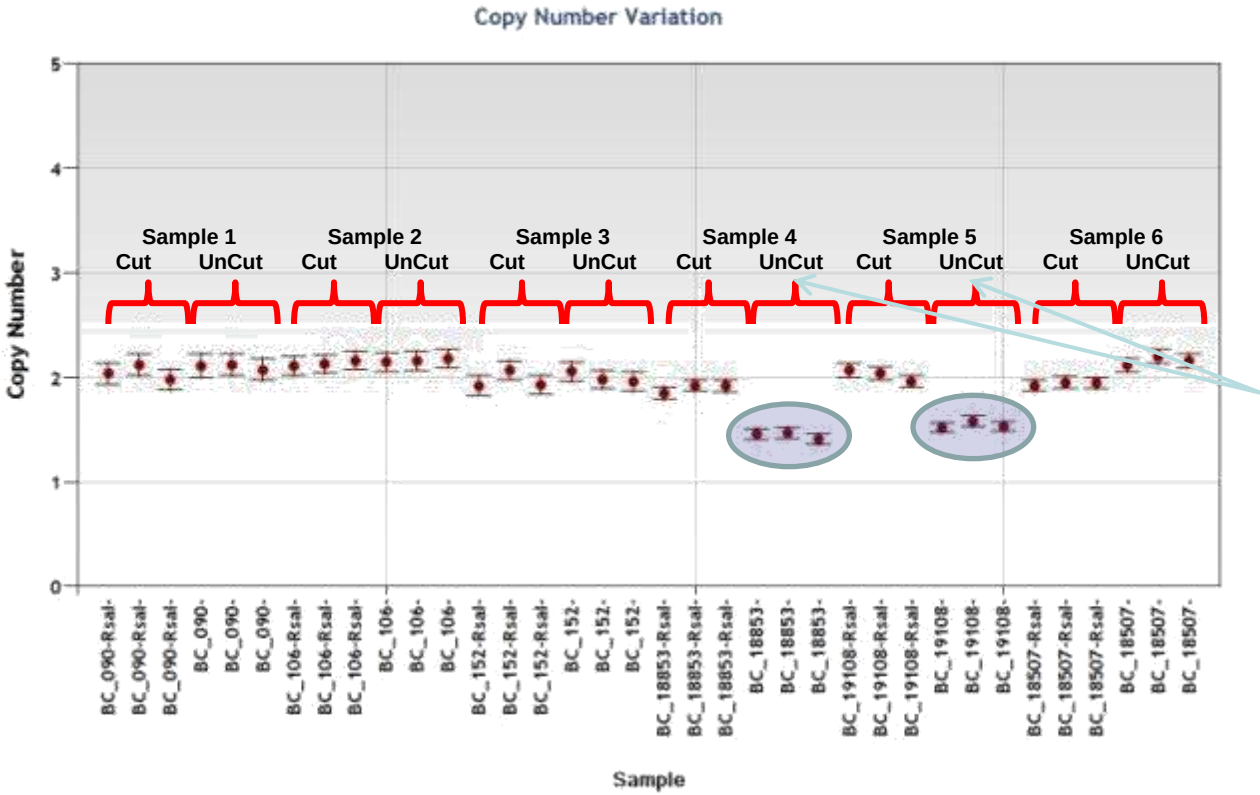
Expect lower
CNV estimate

Two unlinked copies:



Expect similar
CNV estimate

ddPCR precision allows haplotyping of CNV copies



Lower CNV values when sample is not digested suggests that both copies are proximal or on the same chromosome.

* Data for MRGPRX1

ddPCR validates copy number variations (CNVs) discovered by NGS

LETTERS

Nature Genetics 2012

nature genetics

Structural haplotypes and recent evolution of the human 17q21.31 region

Linda M Boettger[1–4], Robert E Handsaker[1,2,4], Michael C Zody[1,2] & Steven A McCarroll[1,2]

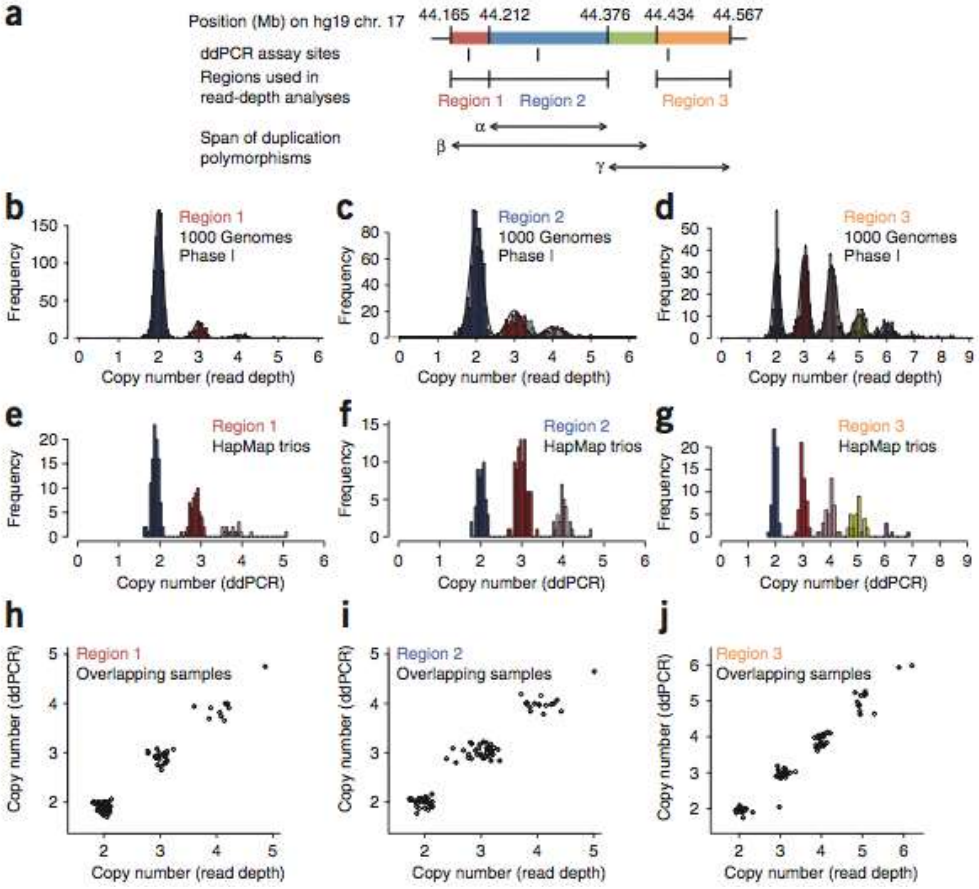
- Significance: Neurological disease and female fertility has been linked to a structurally complex region of chromosome 17 (17q21.31).
- Problem: 17q21.31 has inversions and copy number variations (CNVs) that are difficult to evaluate across populations due to technological limitations. NGS is useful but very expensive.
- Solution: ddPCR enables easy validation & study of CNVs discovered by sequencing from a structurally-complex locus across patient cohorts

ddPCR confirms NGS, screens with sensitivity for CNV

BIO-RAD

ddPCR: an accurate & inexpensive way to validate and study CNVs discovered by NGS

Figure 1



- Copy number analysis of 3 regions of 17q21.31 by whole-genome sequencing (b, c, d), and by ddPCR (e, f, g).
- Copy number determination in 234 samples by NGS and ddPCR >99% concordant (h, i, j)
- ddPCR provides easy, inexpensive, accurate way to validate and further study CNVs discovered by NGS.

ddPCR used to confirm that somatic mosaicism of "normal" parents gives rise to affected progeny

AJHG

Parental Somatic Mosaicism Is Underrecognized and Influences Recurrence Risk of Genomic Disorders

Ian M. Campbell,[1,11] Bo Yuan,[1,11] Caroline Robberecht,[2] Rolph Pfundt,[3] Przemyslaw Szafranski,[1] Meriel E. McEntagart,[4] Sandesh C.S. Nagamani,[1,5] Ayelet Erez,[1,5] Magdalena Bartnik,[6] Barbara Wiśniowiecka-Kowalnik,[6] Katie S. Plunkett,[1] Amber N. Pursley,[1] Sung-Hae L. Kang,[1] Weimin Bi,[1] Seema R. Lalani,[1,5] Carlos A. Bacino,[1,5] Mala Vast,[4] Karen Marks,[4] Michael Patton,[4] Peter Olofsson,[7] Ankita Patel,[1] Joris A. Veltman,[3] Sau Wai Cheung,[1] Chad A. Shaw,[1] Lisenka E.L.M. Vissers,[3] Joris R. Vermeesch,[2] James R. Lupski,[1,5,8,9,*] and Paweł Stankiewicz[1,10,*]

Campbell et al., Parental Somatic Mosaicism Is Underrecognized and Influences Recurrence Risk of Genomic Disorders, The American Journal of Human Genetics (2014), http://dx.doi.org/10.1016/j.ajhg.2014.07.003

Significance: Current clinical tests for carrier status of parents for genomic disorders by aCGH and FISH are not sensitive enough to detect somatic mosaicism, which can lead to affected offspring.

Solution: ddPCR can easily detect somatic mosaicism that clinical tests miss from blood rather than tissue samples.

ddPCR used to confirm that somatic mosaicism of "normal" parents gives rise to affected progeny

Clinically "normal" mother with 2 different fathers gave rise to 3 affected offspring.

ddPCR detected mutation in parent blood sample at 25.1%.

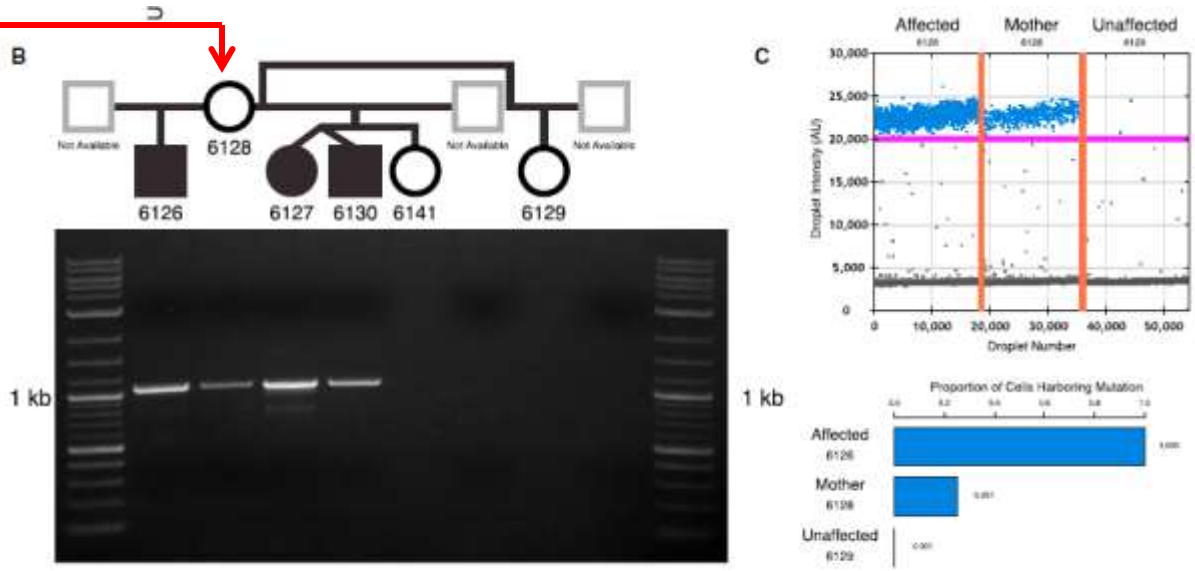


Figure 1. Low-Level Combined Germline and Somatic Mosaicism Inferred from Familial Recurrence of SMS

Table 1. Characteristics of Mosaic CNVs Identified in This Study

Family	Analysis	Coordinates	Size	Inheritance	Gene	MIM	Mutational Signature	Percent Mosaic
1	retrospective	chr17: 17,711,738–217,748,468	36.7 kb	maternal	*RAI1*	607642	39 bp normal intervening sequence, +1 bp identity, +2 bp microhomology	25.1%
2	retrospective	chr1: 242,263,612–244,559,673	2.3 Mb	paternal	*AKT3*[a]	611223	11 bp normal intervening sequence	3.4%
3	prospective	chr12: 23,585,878–23,829,423	244 kb	maternal	*SOX5*	604975	blunt breakpoint	9.0%
4	prospective	chr6: 75,502,925–75,867,029	364 kb	maternal	*COL12A1*	120320	2 bp microhomology	<1%
5	prospective	chr9: 119,474,386–119,587,581	113 kb	paternal	*ASTN2*	612856	3 bp microhomology	3.0%
6	prospective	chr2: 165,659,793–166,267,524	608 kb	paternal	*SCN2A*	182390	*Alu*/*Alu*, 181 bp 100% identity	<1%

All coordinates are according to the GRCh37/hg19 (2009) assembly.
[a]*AKT3* and eight other RefSeq genes.

★ Other families tested for somatic mosaicism detected mutations in carriers from <1% to 25.1%.

ddPCR enables sensitive and quantitative detection of somatic mosaicism

LETTER

Nature 2012
doi:10.1038/nature11629

Somatic copy number mosaicism in human skin revealed by induced pluripotent stem cells

Alexej Abyzov[1,2,3], Jessica Mariani[1,4]*, Dean Palejev[1,4]*, Ying Zhang[1,5]*, Michael Seamus Haney[6,7]*, Livia Tomasini[1,4]*, Anthony F. Ferrandino[1,4], Lior A. Rosenberg Belmaker[1,4], Anna Szekely[1,5,8], Michael Wilson[1,2,4], Arif Kocabas[1,4], Nathaniel E. Calixto[1,4], Elena L. Grigorenko[1,4,9,10], Anita Huttner[1,11], Katarzyna Chawarska[1,4], Sherman Weissman[1,5], Alexander Eckehart Urban[1,6,7], Mark Gerstein[1,2,3,12] & Flora M. Vaccarino[1,4,13]

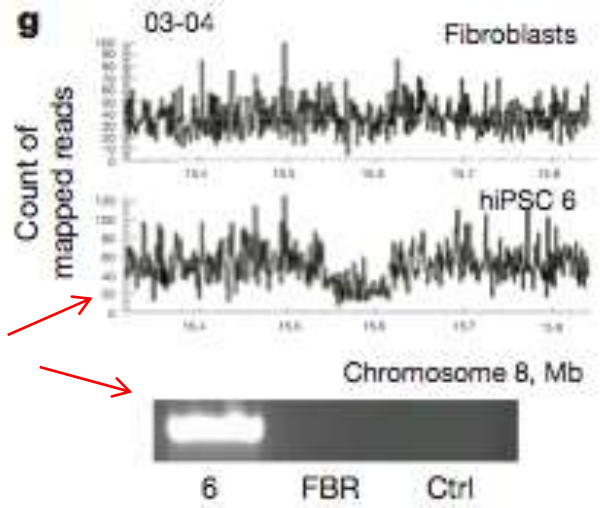
- Significance: Genetic variation challenges development of patient-matched stem cell lines (used for regenerative medicine)
- Problem: Want to detect and quantify Copy Number Variations (somatic mosaicism) that occur at a low frequency in patient cells
- Solution: ddPCR can detect somatic mosaicism at frequencies < 1%. This is more sensitive than other techniques including NGS.

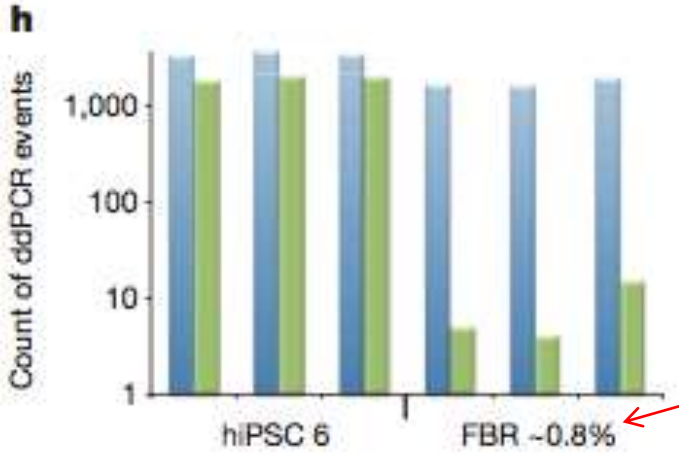
ddPCR sensitivity used to characterize stem cells

BIO-RAD

Somatic mosaicism <1% detected by ddPCR

NGS and PCR cannot detect this rare mosaic event



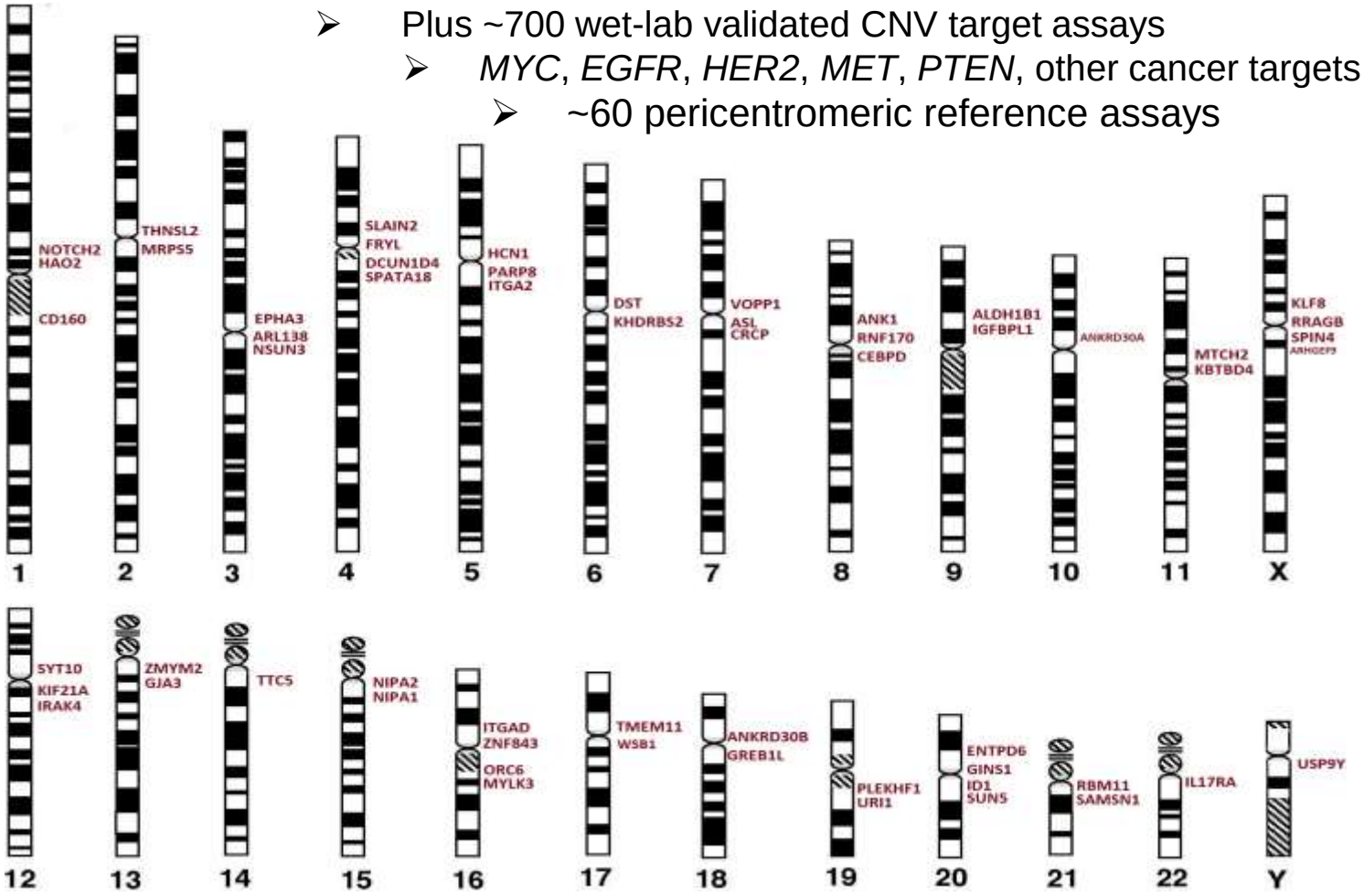


ddPCR detects and quantifies mosaicism at 0.8%

- ddPCR, but not NGS or conventional PCR, able to detect and estimate frequency of a rare somatic mosaicism event (<1%)
- ddPCR enabling better understanding of making patient-matched stem cells for personalized therapies

Validated (Peri) Centromeric Reference Assays

- Plus ~700 wet-lab validated CNV target assays
  - *MYC*, *EGFR*, *HER2*, *MET*, *PTEN*, other cancer targets
    - ~60 pericentromeric reference assays



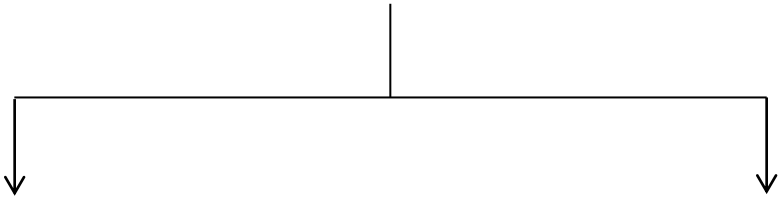
Rare Event Detection (RED)

- ddPCR interest
- Genome Editing
- Multiplexes Kits &
  Validated Bio-Rad assays

The “Needle in a Haystack” questions...

Rare Event Detection

Rare Event Detection (RED)



Rare Sequence Detection (RSD)

→ analysis of sequences with no relation to their background

- Microbial genomes (virus, bacteria, yeast)
- Environmental studies
- GMO

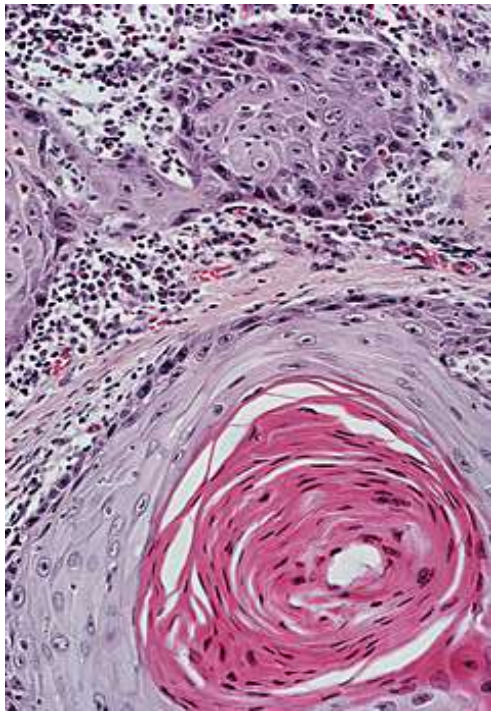
Rare Mutation Detection (RMD)

→ analysis of sequences closely related to the background DNA (wild-type DNA)

- Cancer mutations
- Prenatal diagnosis
- Transplanted organs

Emerging Roles for ddPCR in Cancer

- Low-frequency mutation detection in clinical (degraded) samples
  - Sanger at 20%, next-generation sequencing (NGS) at 5%, **ddPCR at <0.1%**
  - FFPE, cfDNA
- Somatic copy number alterations (SCNAs)
  - Validate or complement array comparative genomic hybridization (aCGH), fluorescence in situ hybridization (FISH), NGS data
- Quantify rare cells
  - Circulating tumor cells (CTCs)
  - Tumor infiltrating leukocytes (TILs)
- Additional applications:
  - microRNA
  - Methylation quantification
  - Telomerase: telomere repeat amplification protocol (TRAP) assay



DBC Webinar:
Applications for the Cancer Field Using Droplet Digital™ PCR (ddPCR™)
bio-rad.com/ddPCRApplicationsVideo

Original Investigation JAMA Oncol. 2016 Aug 1;2(8):1014-22. doi: 10.1001/jamaoncol.2016.0173.

Prospective Validation of Rapid Plasma Genotyping for the Detection of *EGFR* and *KRAS* Mutations in Advanced Lung Cancer

Adrian G. Sacher, MD; Cloud Paweletz, PhD; Suzanne E. Dahlberg, PhD; Ryan S. Alden, BSc; Allison O'Connell, BSc; Nora Feeney, BSc; Stacy L. Mach, BA; Pasi A. Jänne, MD, PhD; Geoffrey R. Oxnard, MD

Questions: ddPCR sensitivity, specificity, turnaround time, and robustness ?

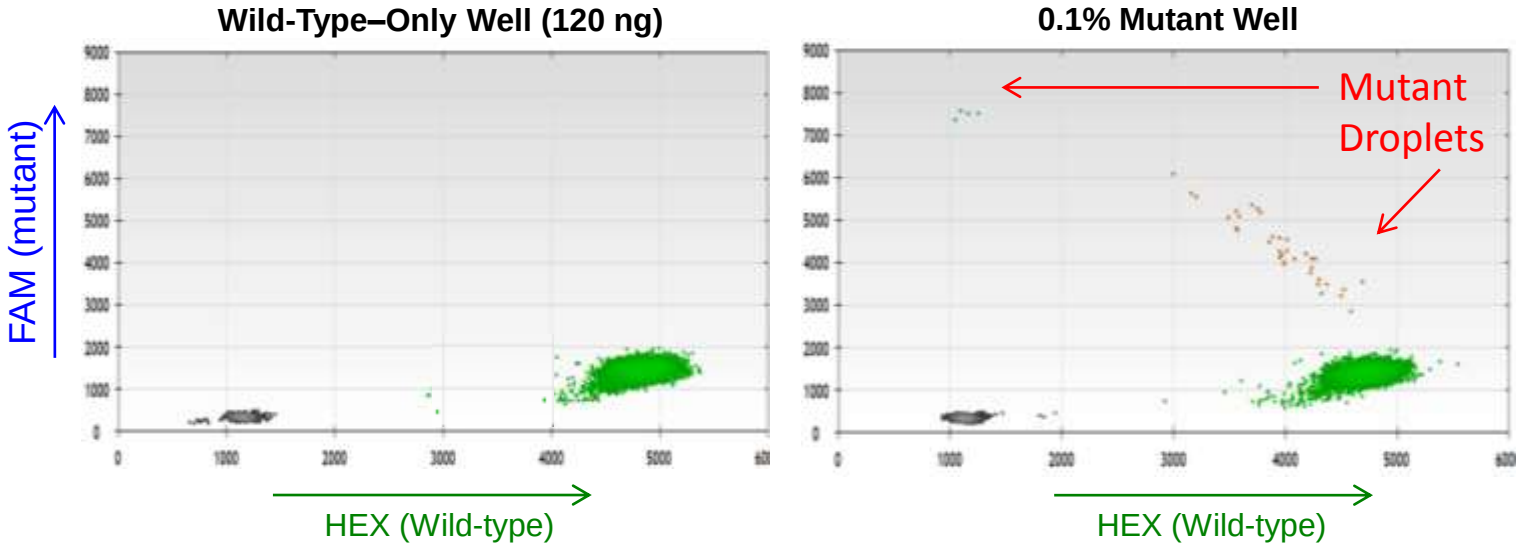
(ddPCR)-based plasma genotyping for the rapid detection of targetable genomic alterations in patients with advanced non–small-cell lung cancer (NSCLC) ?

Findings: In this study of 180 patients with advanced NSCLC (120 newly diagnosed, 60 with acquired resistance to epidermal growth factor receptor [EGFR] kinase inhibitors), plasma genotyping exhibited perfect specificity (100%) and acceptable sensitivity (69%-80%) for the detection of EGFR-sensitizing mutations with rapid turnaround time (3 business days). Specificity was lower for EGFR T790M (63%), presumably secondary to tumor heterogeneity and false-negative tissue genotyping.

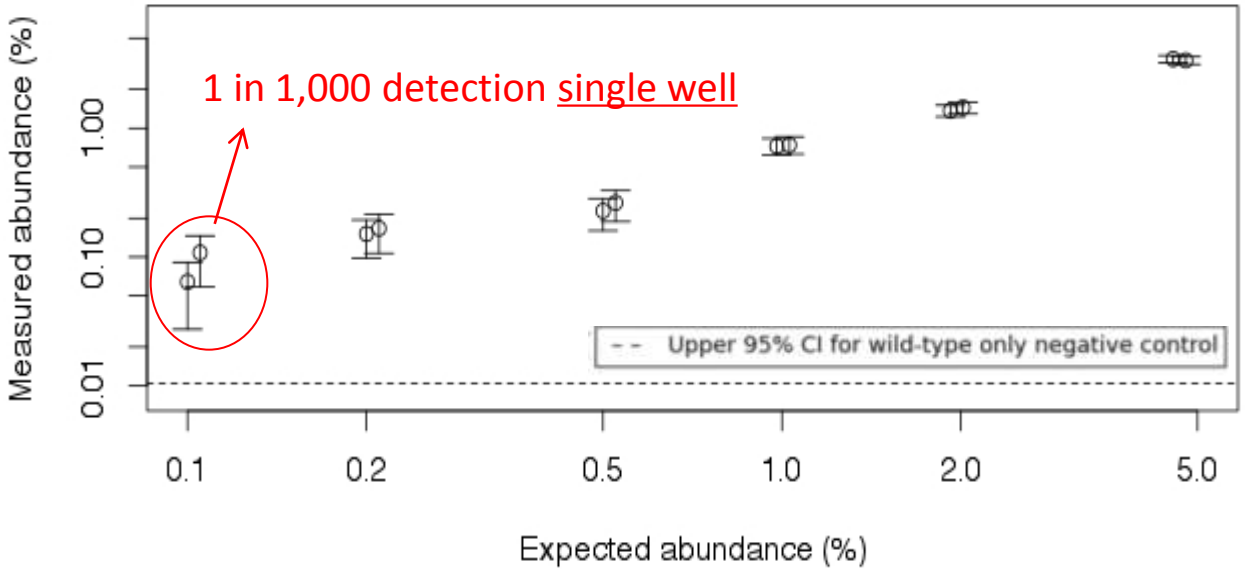
Meaning: The use of ddPCR-based plasma genotyping can detect EGFR mutations with the rigor necessary to direct clinical care. This assay may obviate repeated biopsies in patients with positive plasma genotyping results

Example Validation Data: *BRAF V600E*

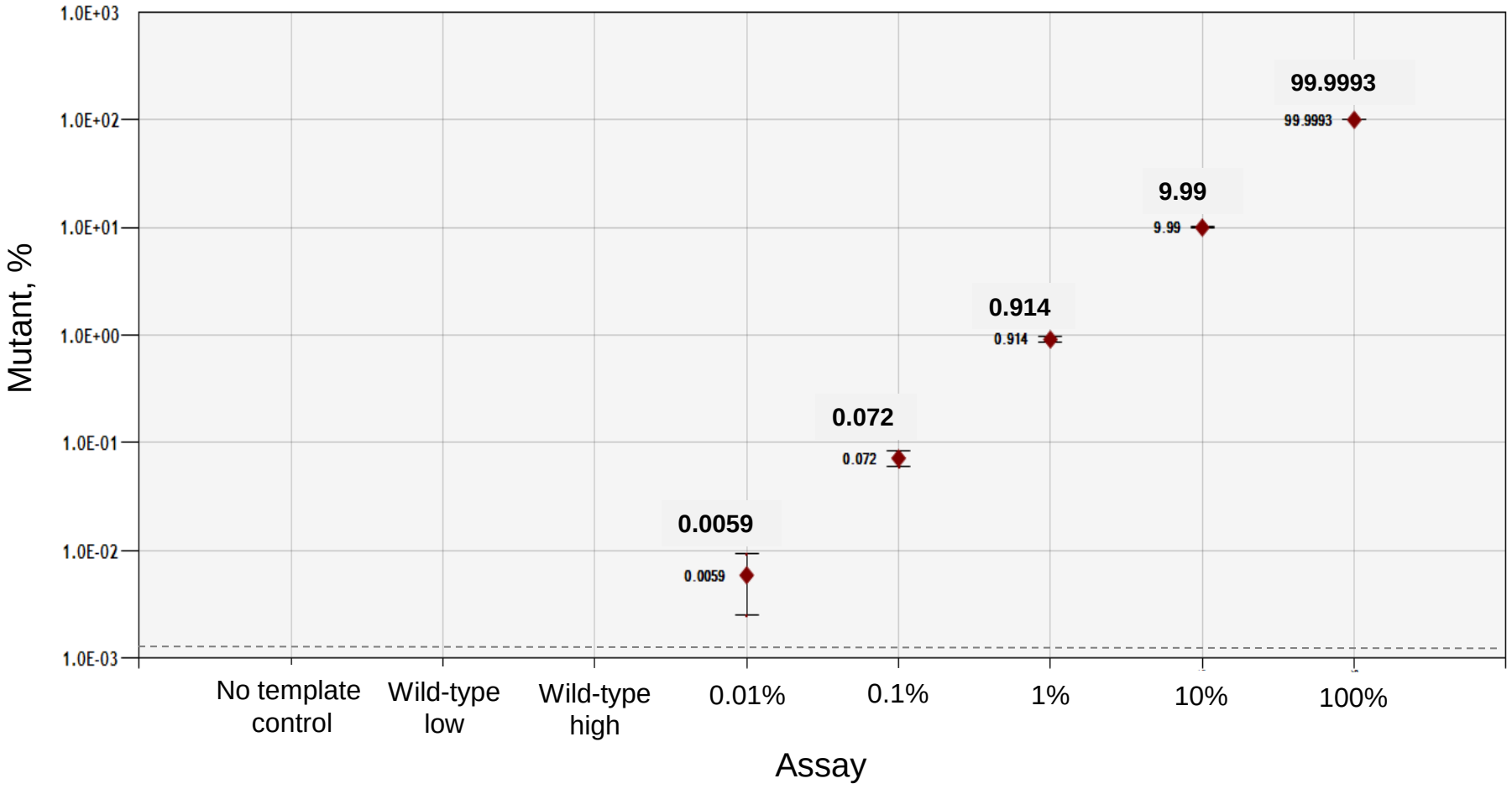
2-D Amplitude Plot



Limit of Detection Plot

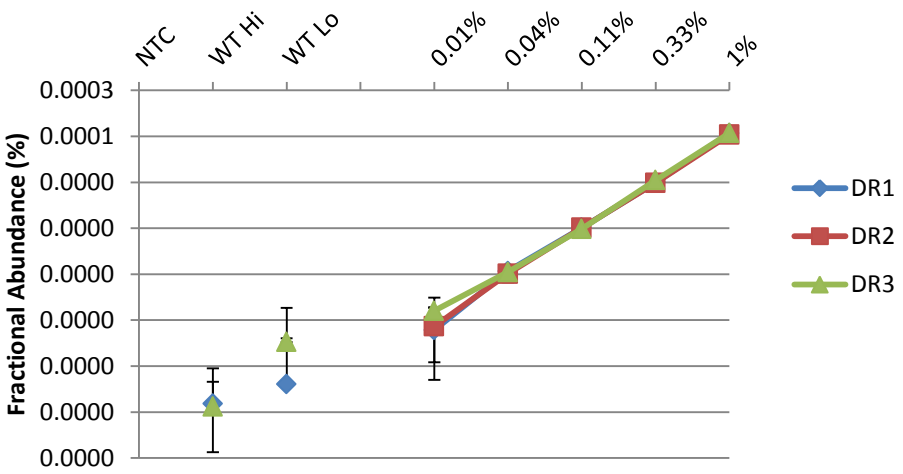


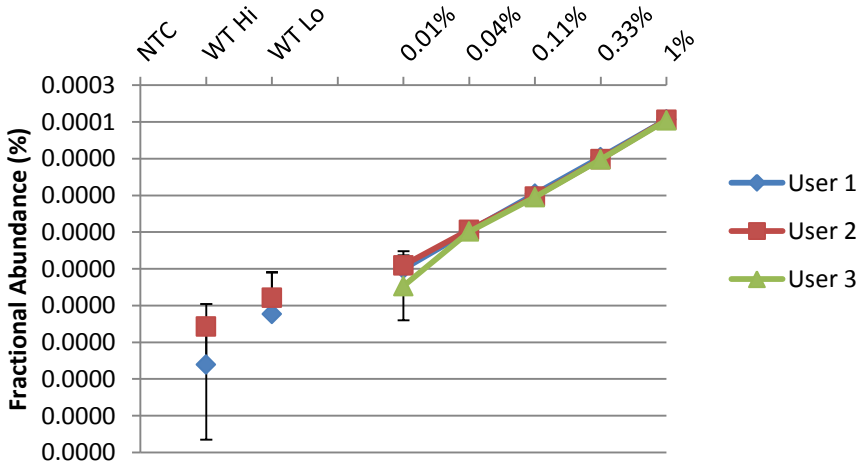
Lower Limit of Detection with *BRAF V600E* in Four Merged Wells



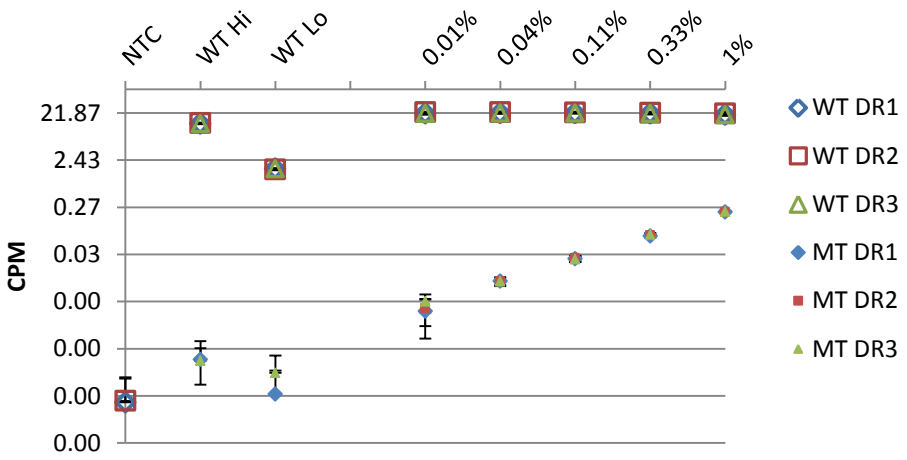
Cross User, Cross Instrument Proficiency Data for *BRAF V600E*

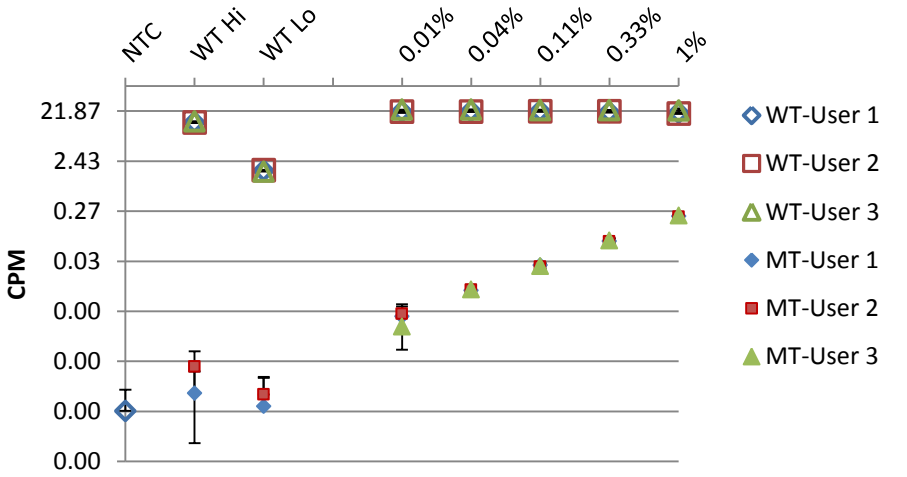
Mutant %





Concentrations





BIO-RAD

Serial and Noninvasive Profiling of EGFR mutations

Predictive Biomarkers and Personalized Medicine

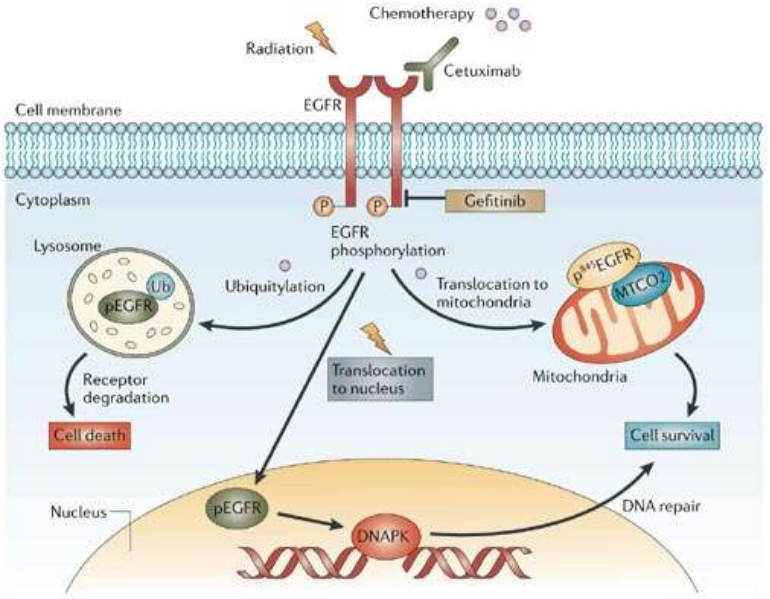
Clinical Cancer Research

Noninvasive Detection of Response and Resistance in *EGFR*-Mutant Lung Cancer Using Quantitative Next-Generation Genotyping of Cell-Free Plasma DNA

Geoffrey R. Oxnard[1,3], Cloud P. Paweletz[1,2], Yanan Kuang[1,2], Stacy L. Mach[1], Allison O'Connell[1,2], Melissa M. Messineo[1,2], Jason J. Luke[1,3], Mohit Butaney[1], Paul Kirschmeier[1,2], David M. Jackman[1,3], and Pasi A. Jänne[1,2,3]

Clin Cancer Res. 2014 Mar 15;20(6):1698-705. doi: 10.1158/1078-0432.CCR-13-2482.

Use of ddPCR to detect and monitor *EGFR* sensitizing (*L858R*) and drug resistance (*T790M*) mutations in cell-free DNA from patients with lung cancer



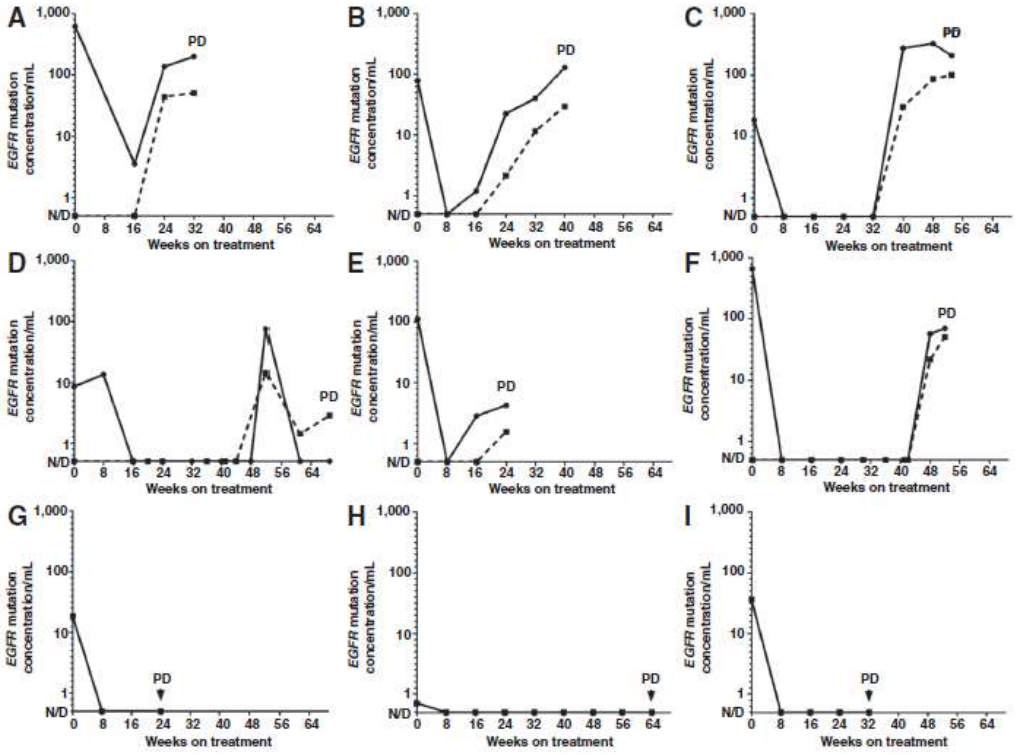
Nyati MK et al., *Nat Rev Cancer*, 2006.

Serial and Noninvasive Profiling of EGFR mutations

ddPCR monitors *EGFR L858R* and *T790M* mutation abundance in 9 patients with lung cancer treated first-line with Erlotinib

Solid line:
EGFR L858R

Dashed line:
EGFR T790M



Oxnard GR et al., *Clin Cancer Res*, 2014.

NIPD - Screening Newborn Blood Spots Using Multiplex Droplet Digital PCR

Clinical Chemistry 61:1
182–190 (2015)

Molecular Diagnostics and Genetics

Screening Newborn Blood Spots for 22q11.2 Deletion Syndrome Using Multiplex Droplet Digital PCR

Dalyir Pretto,[1†] Dianna Maar,[2†] Carolyn M. Yrigollen,[1] Jack Regan,[2] and Flora Tassone[1,3*]

- **RESULTS:** Multiplex ddPCR correctly identified all 22q11DS samples and distinguished between 1.5- and 3-Mb deletions, suggesting the approach is sensitive and specific for the detection of 22q11DS.

- **CONCLUSIONS:** These data demonstrate the utility of multiplex ddPCR for large-scale population-based studies that screen for 22q11DS. The use of samples from blood spot cards suggests that this approach has promise for newborn screening of 22q11DS, and potentially for other microdeletion syndromes, for which early detection can positively impact clinical outcome for those affected.

NIPD - Screening Newborn Blood Spots Using Multiplex Droplet Digital PCR

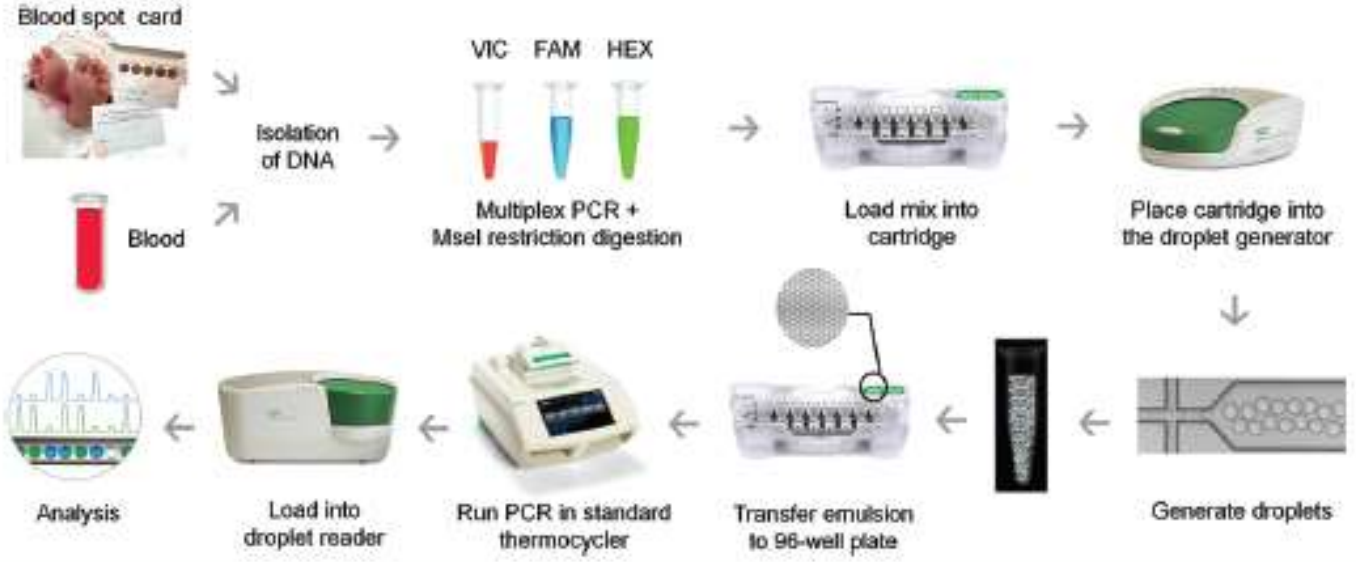
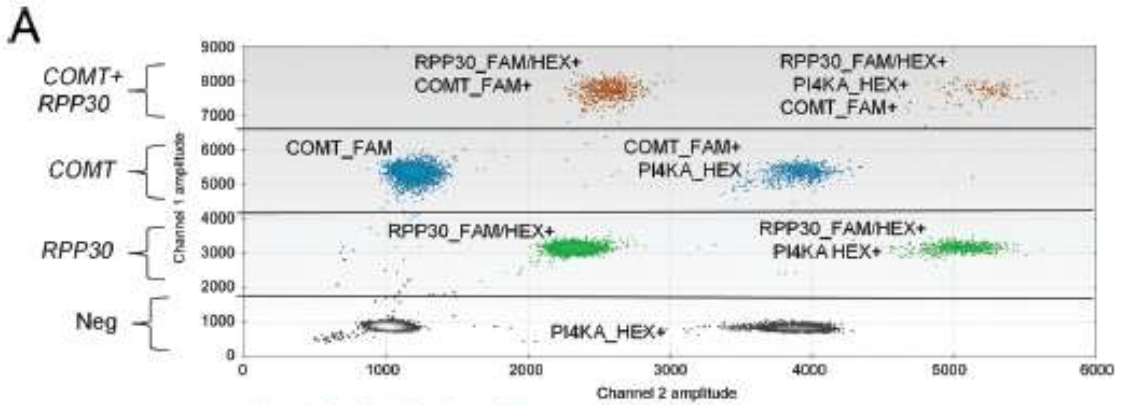


Fig. 1. Work flow chart of ddPCR. gDNA, isolated from either blood spots or whole blood was mixed with *Mse*I restriction enzyme, with Droplet PCR Supermix, and fluorescent tagged probes.

DNA droplets were generated using the droplet generator and transferred in the PCR thermocyder. PCR plates were finally loaded onto the droplet reader for CNV detection analysis.

NIPD - Screening Newborn Blood Spots Using Multiplex Droplet Digital PCR



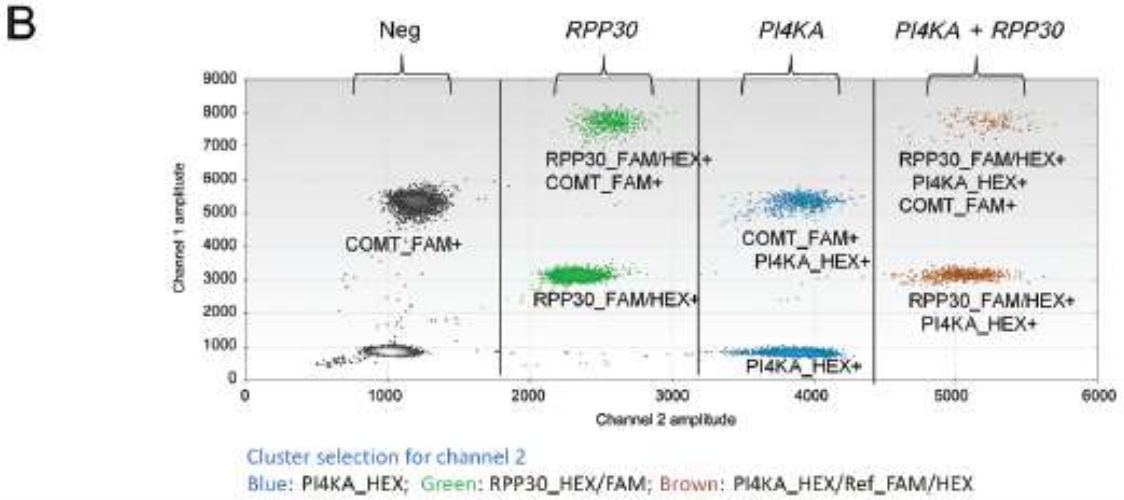


Fig. 2. Clustering of droplets in ddPCR analysis.

NIPD - Screening Newborn Blood Spots Using Multiplex Droplet Digital PCR

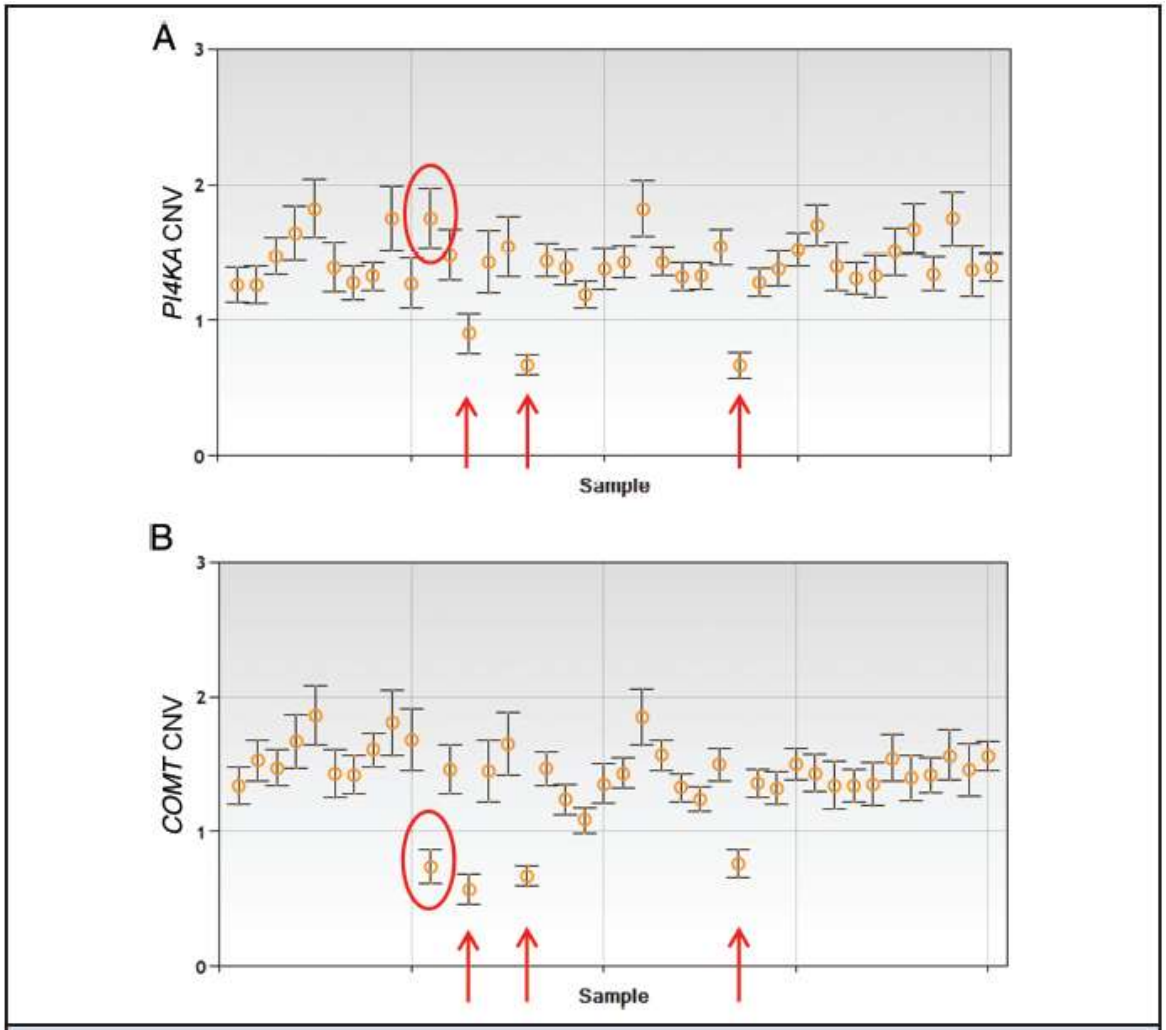


Fig. 5. Diagram of CNV values by Quantasoft.

CNV values for 40 samples showing the presence of an individual with a 1.5-Mb deletion (red cirde) and of 3 individuals with a 3-Mb deletion (arrows). SE bars of 95% CI are shown for each sample.

Multiplexes Kits &
Validated Bio-Rad assays

Multiplex Kit

- The ddPCR *KRAS* Screening Multiplex Kit contains seven *KRAS* mutant assays (*G12A*, *G12C*, *G12D*, *G12V*, *G12R*, *G12S*, *G13D*) and one wild-type assay.
- The kit allows for rapid identification of multiple *KRAS* mutations down to 0.2% mutant in a single well.
- No pre-amplification step is required for use of this kit.

ddPCR™ *KRAS* Screening Multiplex Kit

The ddPCR *KRAS* Screening Multiplex Kit is designed to screen for seven *KRAS* mutations. The kit contains a 20x ddPCR *KRAS* Screening Multiplex Assay and ddPCR Supermix for Probes (No dUTP).

Combined with Bio-Rad's QX100™, QX200™, or QX200™ AutoDG™ Droplet Digital PCR (ddPCR) System, the ddPCR *KRAS* Screening Multiplex Kit allows you to:

- Quantify and screen for multiple *KRAS* mutations in a single well
- Obtain sensitive and precise detection down to 0.2% mutant levels
- Screen multiple samples in a rapid and cost-effective manner

Visit **bio-rad.com/KRASscrmxkit** for more information.

Mutations detected by the ddPCR Multiplex Screening Kits

BRAF	*KRAS Q61*	*NRAS G12*	*NRAS G12/G13*	*NRAS Q61*
V600E	Q61H	G12A	G12A	Q61H
V600K	Q61H	G12C	G12C	Q61H
V600R	Q61K	G12D	G12D	Q61K
	Q61L	G12R	G12S	Q61L
	Q61R	G12S	G12V	Q61R
		G12V	G13D	
			G13R	
			G13V	

Catalog Number	Product Name
12001037	ddPCR™ BRAF V600 Screening Kit
12001094	ddPCR™ NRAS G12 Screening Kit
12001627	ddPCR™ NRAS G12/G13 Screening Kit
12001006	ddPCR™ NRAS Q61 Screening Kit
12001626	ddPCR™ KRAS Q61 Screening Kit

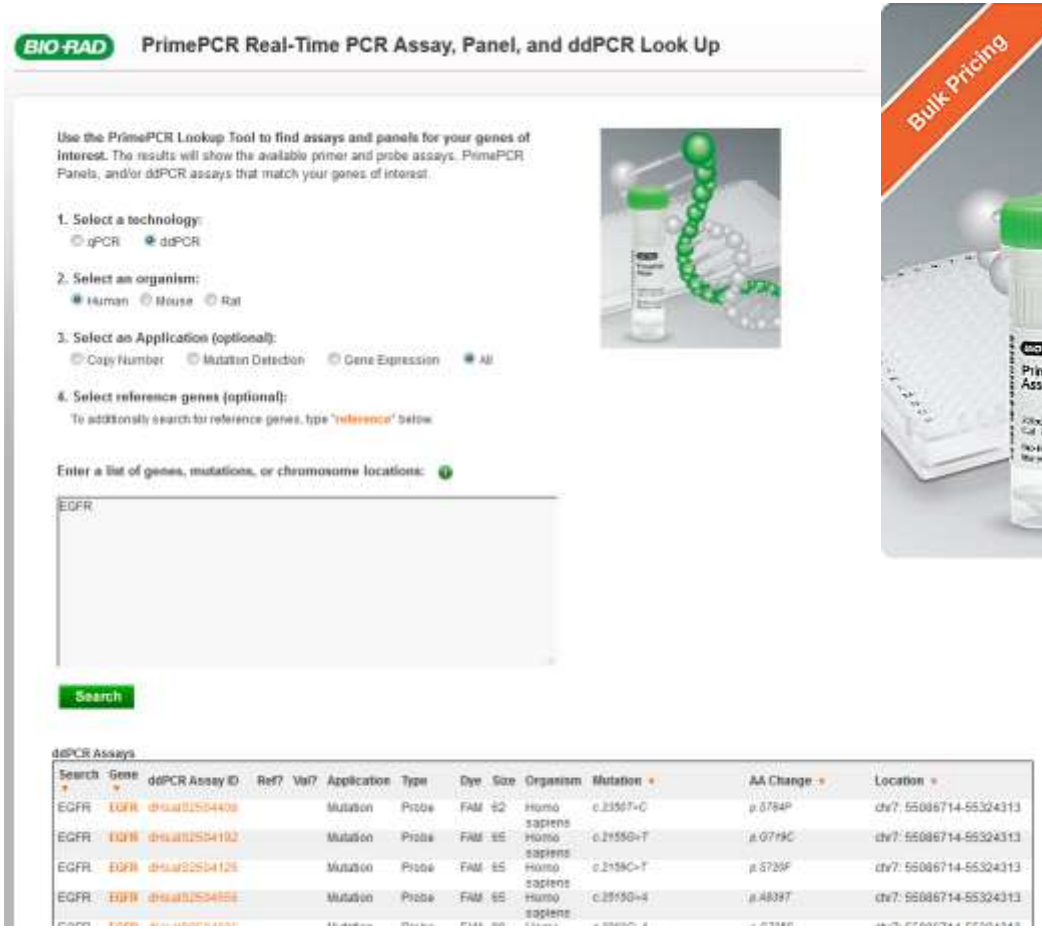
New Mutations detected by ddPCR Multiplex Screening Kits

ddPCR™ ***EGFR*** Exon 19 Deletions screening Kit

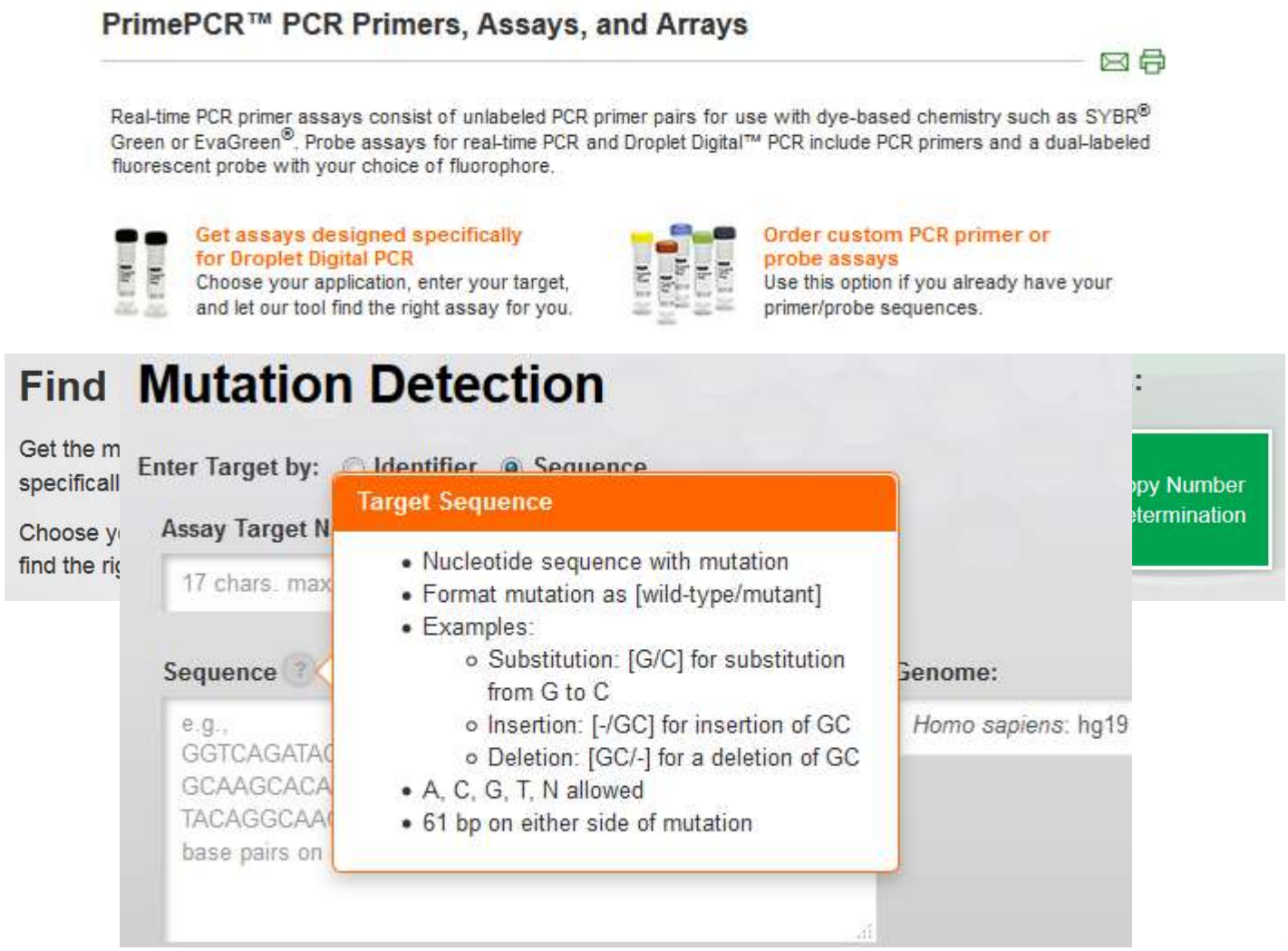
ddPCR™ ***PIK3CA*** *E542/E545/Q546/H1047* Screening Kit

- Yes / No answer
- 0.5% sensitivity
- No pre-amplification
- DNA from FFPE, fresh/frozen tissue, liquid biopsy etc
- Low input DNA

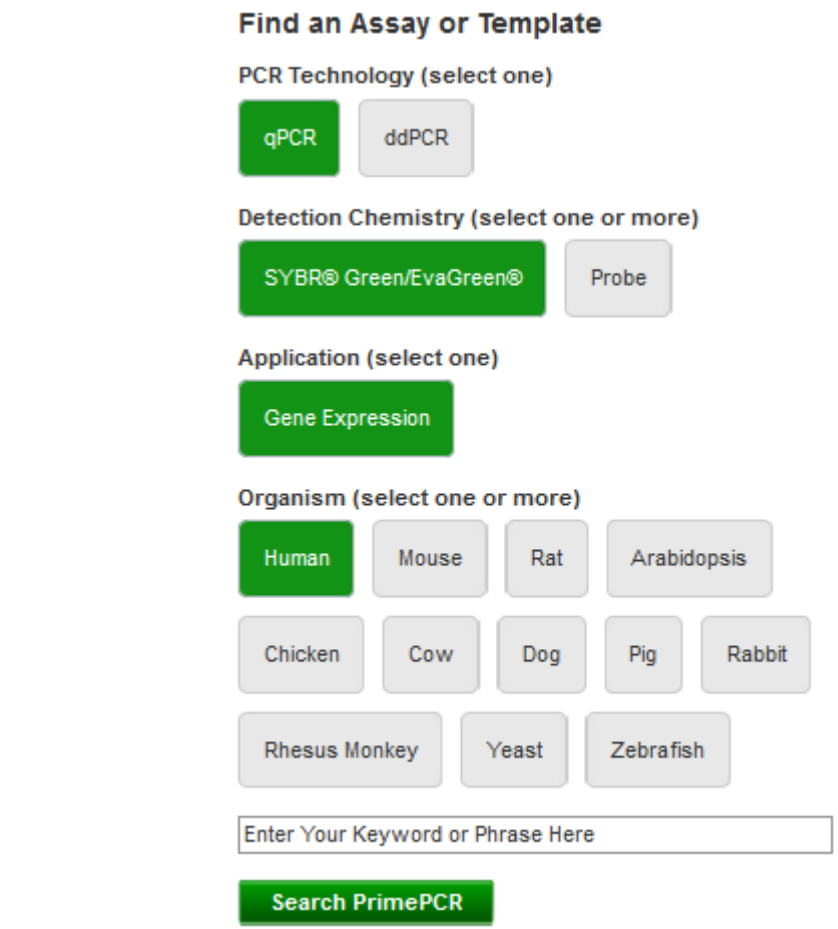
PrimePCR Assays
www.bio-rad.com/fr-fr/product/primepcr-pcr-primers-assays

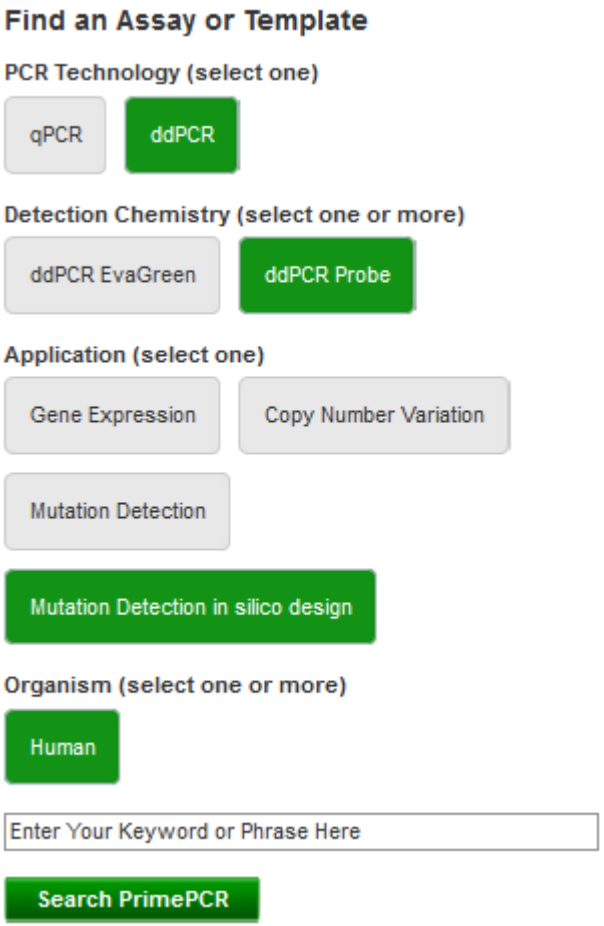


Your PrimePCR own designs (CNV/Mutation/GenomeEditing)



PrimePCR qPCR / ddPCR offer

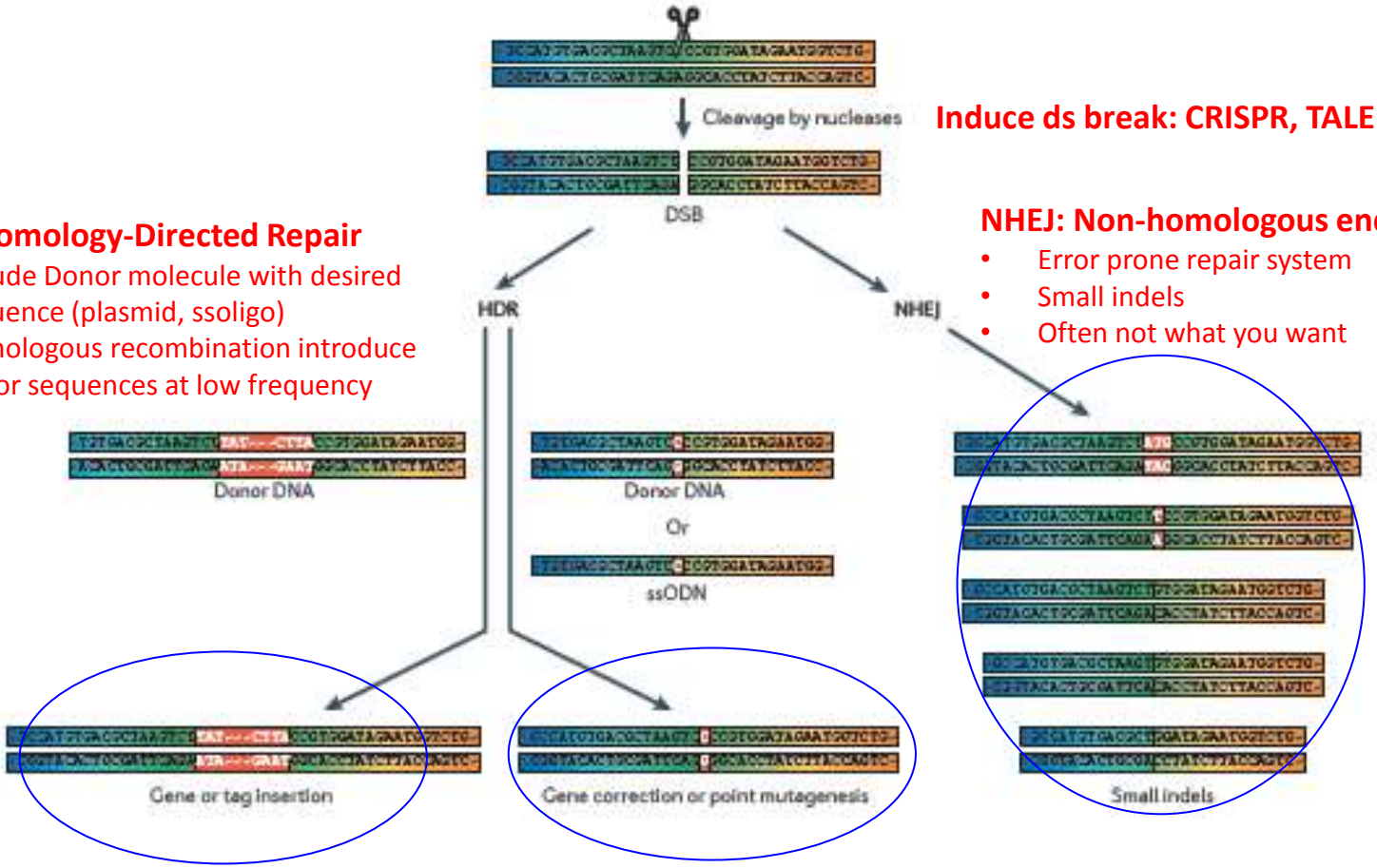




Genome editing

Genome editing: how it works

- Site-specific nucleases target sequences for mutagenesis or editing
- Endogenous DNA repair systems incorporate change at low frequency **(<0.1%)**



Induce ds break: CRISPR, TALEN, ZFN

HDR: Homology-Directed Repair

- Include Donor molecule with desired sequence (plasmid, ssoligo)
- Homologous recombination introduce donor sequences at low frequency

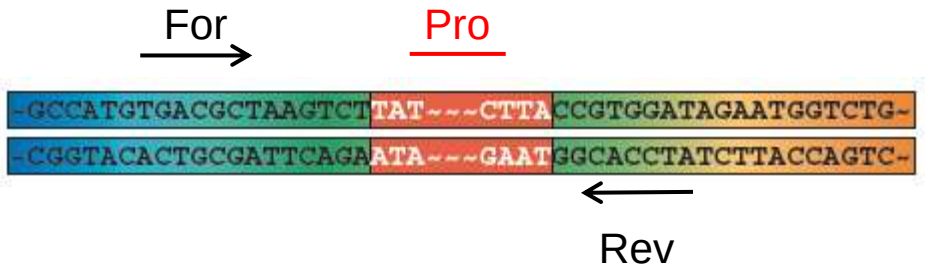
NHEJ: Non-homologous end-joining

- Error prone repair system
- Small indels
- Often not what you want

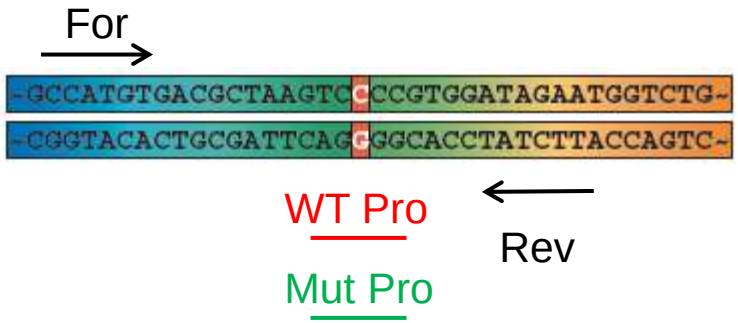
Kim & Kim, Nature Reviews Genetics 2014

What can ddPCR do for HDR quantification?

- HDR (Homologous Directed Repair)
  - –Gene or tag insertion (creation of new sequence)

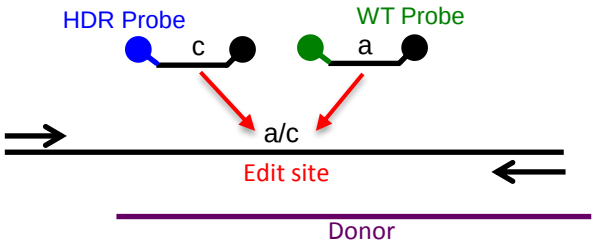


  - –Gene correction or point mutagenesis

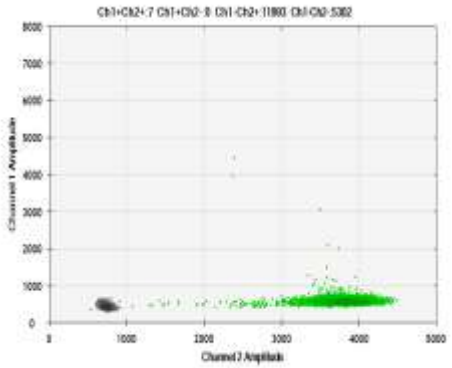


Rare Edit Detection using ddPCR

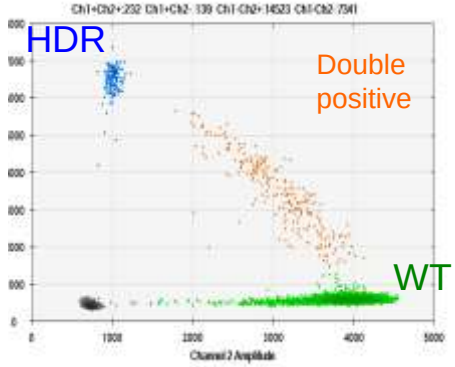
Edit (HDR) Detection Assay



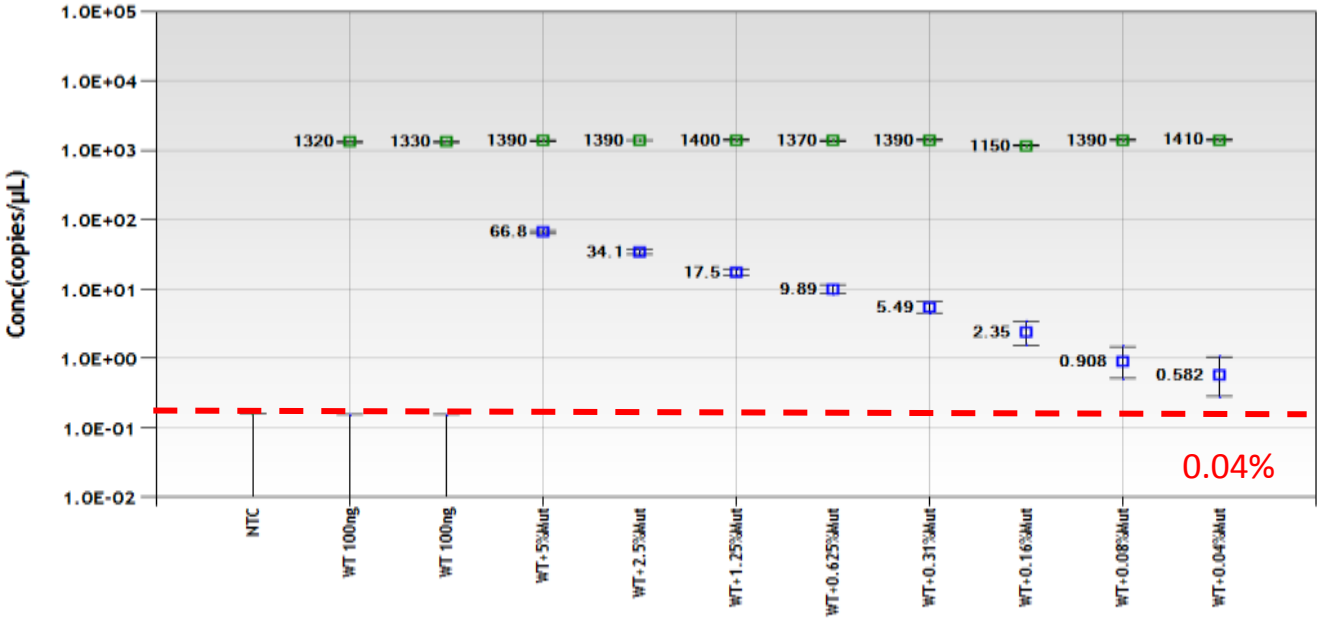
WT-only well



1% HDR Well



RBM20 R636S: sensitive edit detection down to <0.05%

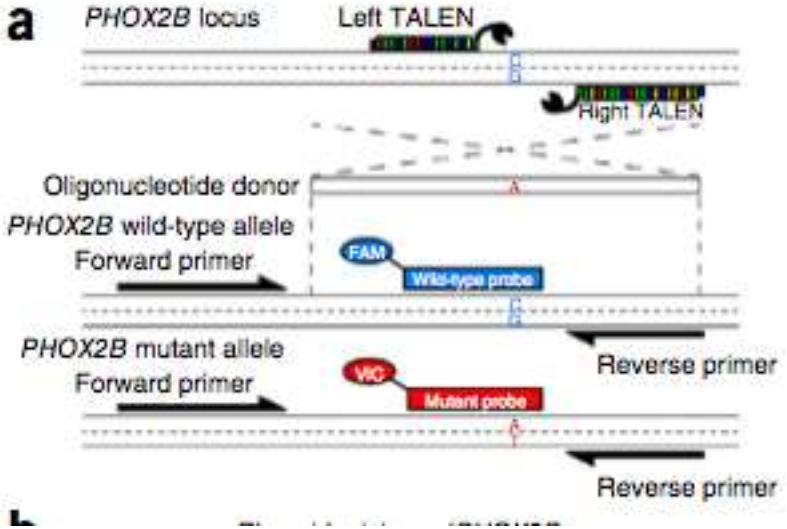


Sensitive detection of iPSC genome editing by ddPCR

Nature Methods, March 2014

Isolation of single-base genome-edited human iPS cells without antibiotic selection

Yuichiro Miyaoka[1], Amanda H Chan[1], Luke M Judge[1,2], Jennie Yoo[1], Miller Huang[3], Trieu D Nguyen[1], Paweena P Lizarraga[1], Po-Lin So[1] & Bruce R Conklin[1,4,5]



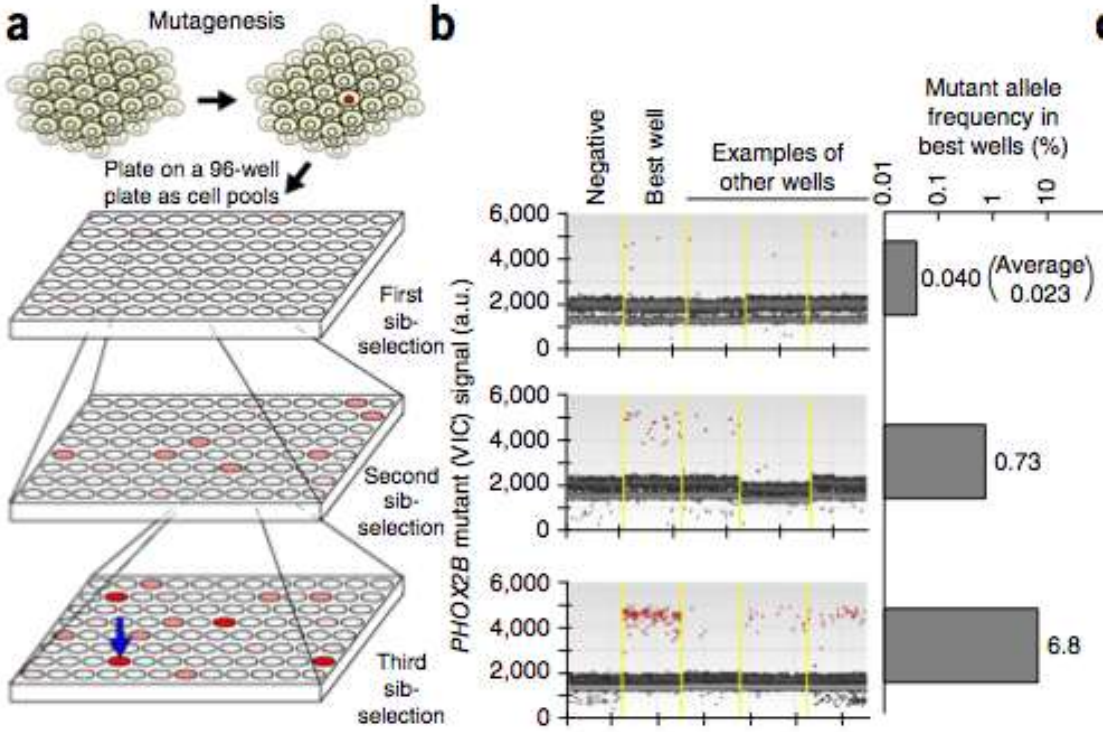
Significance: Use genome editing of induced pluripotent stem cells (iPSCs) to enable patient-specific studies of pathological mutations

Problem: Edited iPSC clones are rare and require laborious screening to detect. Expressing low levels of the site-specific nuclease avoids off-target effects

Solution: ddPCR used to detect mutagenesis events at 100x greater sensitivity than qPCR, significantly speed up protocol

Ultra-sensitive detection of edited human iPSCs

Strategy for mutant detection



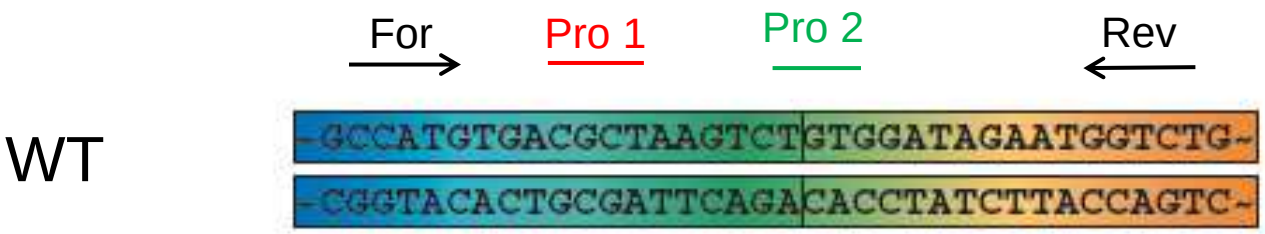
ddPCR detects and quantifies mutant allele freq of 0.02%

Figure 2 | Point mutagenesis in human iPS cells. (**a**) Overview of the approach to is

- ddPCR detected gene editing events as low as 0.02% frequency (1 in 5,000)
- 10-fold decrease in active work time over existing methods (screen 11 clones instead of 2,000!)
- Over 20 independent iPSC clones isolated to date using ddPCR

What can ddPCR do for NHEJ quantification?

- NHEJ (Non Homologous End Joining): loss of signal on one of the 2 WT probes



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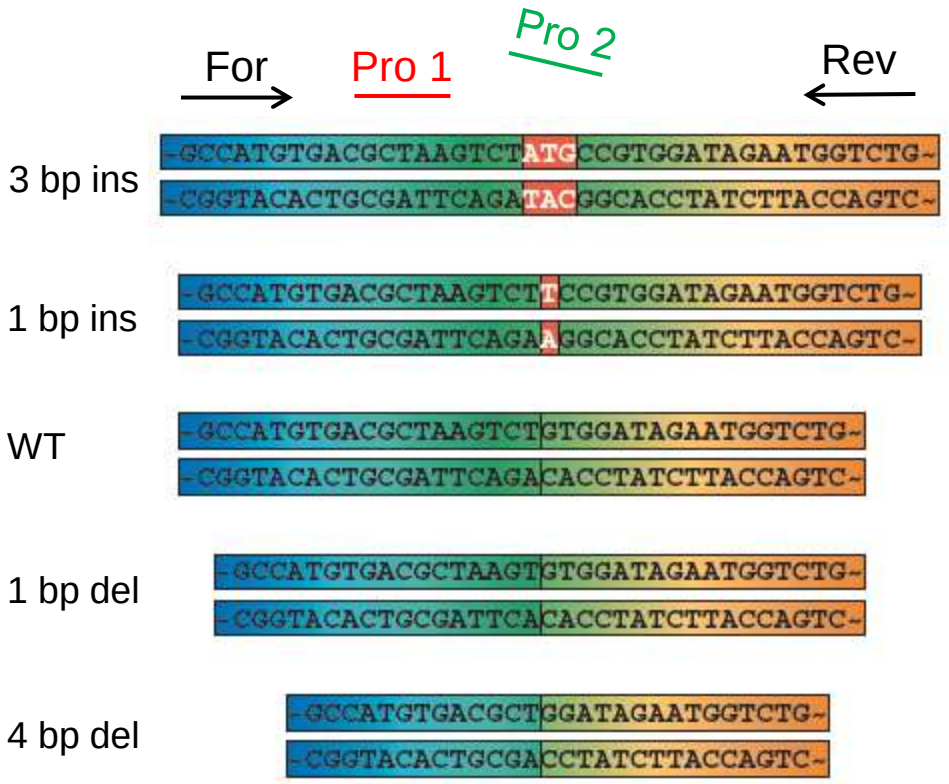

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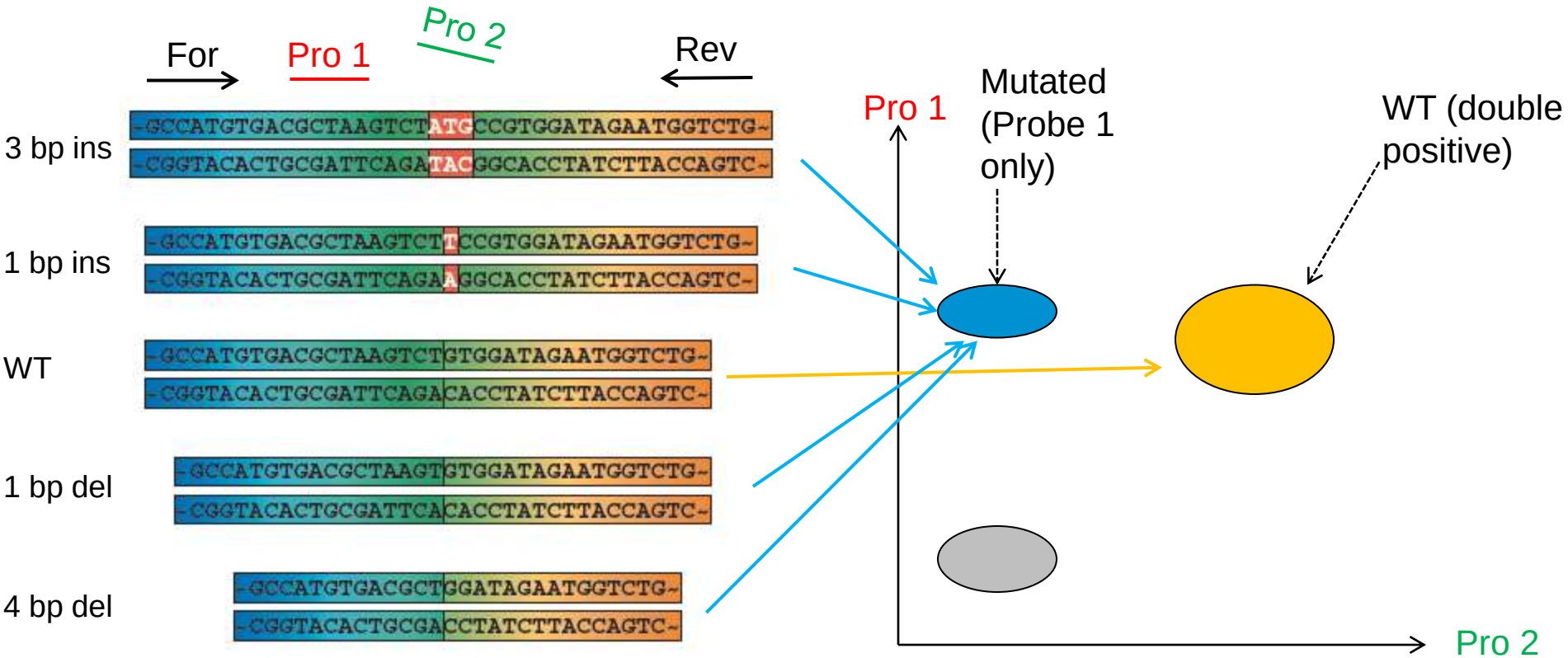


GEF-dPCR (Nature Protocol Feb 2016)

PROTOCOL

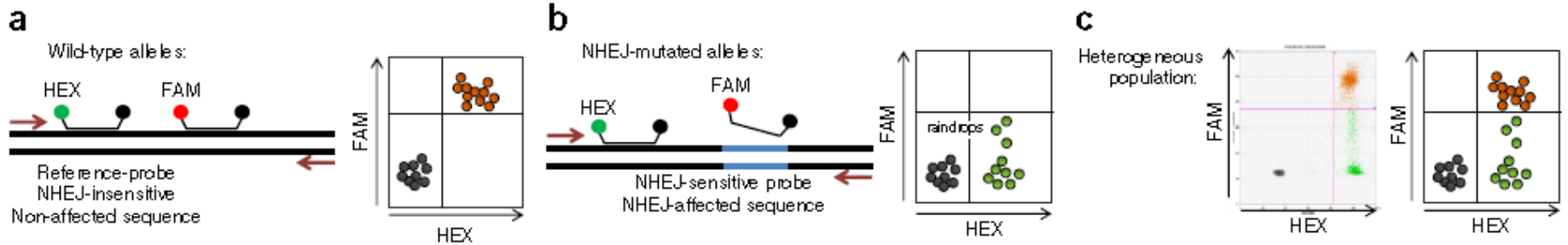
Digital PCR to assess gene-editing frequencies (GEF-dPCR) mediated by designer nucleases

Ulrike Mock[1,3], Ilona Hauber[2] & Boris Fehse[1]

[1]Research Department Cell and Gene Therapy, Department of Stem Cell Transplantation, University Medical Centre, Hamburg-Eppendorf (UKE), Hamburg, Germany. [2]Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany. [3]Present address: Molecular and Cellular Immunology Unit, Institute of Child Health, University College London (UCL), London, UK. Correspondence should be addressed to B.F. (fehse@uke.de) or U.M. (ulrike.mock@ucl.ac.uk).

Published online 25 February 2016; doi:10.1038/nprot.2016.027

PROTOCOL



Sensitive In vivo therapeutic edit detection by ddPCR

Nelson,…Gersbach, Science, Dec 2015

Science

REPORTS

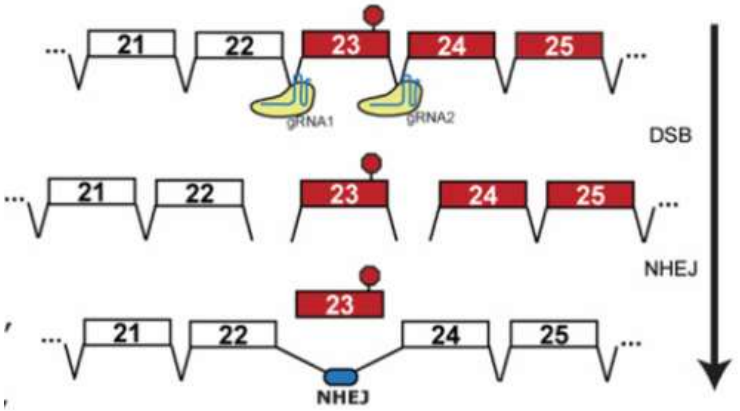
Cite as: C. E. Nelson *et al.*, *Science* 10.1126/science.aad5143 (2015).

In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy

Christopher E. Nelson,[1,2] Chady H. Hakim,[3] David G. Ousterout,[1,2] Pratiksha I. Thakore,[1,2] Eirik A. Moreb,[1,2] Ruth M. Castellanos Rivera,[4] Sarina Madhavan,[1,2] Xiufang Pan,[3] F. Ann Ran,[5,6] Winston X. Yan,[5,7,8] Aravind Asokan,[4] Feng Zhang,[5,9,10,11] Dongsheng Duan,[3,12] Charles A. Gersbach[1,2,13]*

[1]Department of Biomedical Engineering, Duke University, Durham, NC, USA. [2]Center for Genomic and Computational Biology, Duke University, Durham, NC, USA.

Dual- Cas9 excision of exon 23 in dystrophin



Significance: Duchenne's Muscular Dystrophy (DMD) is a devastating, common degenerative muscle disease of genetic origin (dystrophin gene). Accessibility of affected tissue makes DMD a good candidate for therapeutic gene editing.

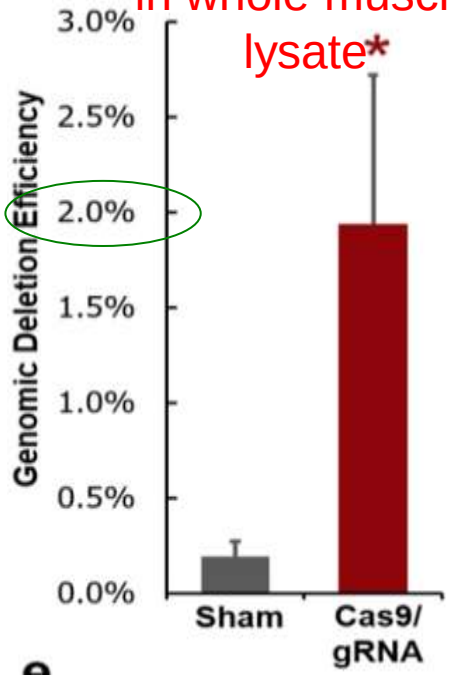
Problem: A spectrum of pathogenic variants requires generalizable correction strategy (pathogenic exon excision) and a sensitive detection method.

Solution: ddPCR used for sensitive (2%) detection of corrected dystrophin DMD alleles

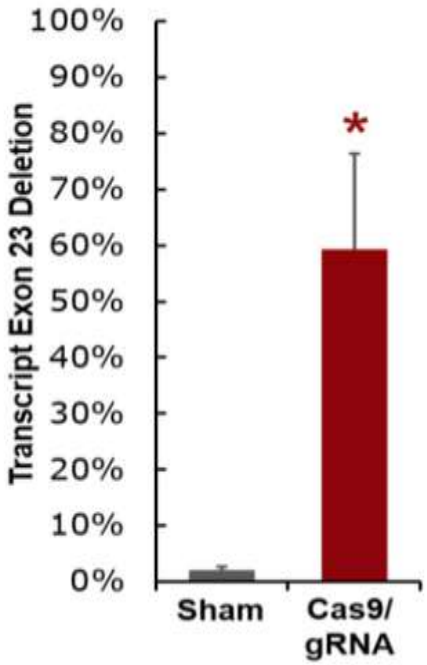
Sensitive In vivo therapeutic edit detection and absoulate quant of edited transcripts by ddPCR

Nelson,…Gersbach, Science, Dec 2015

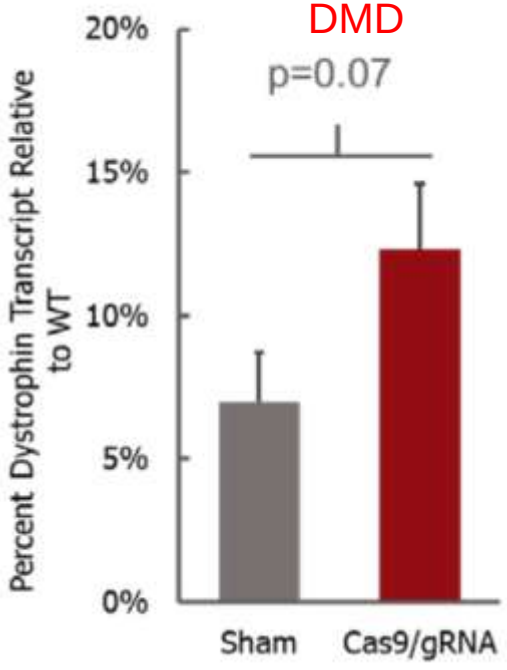
2% Edit Detection in whole muscle lysate*



59% of transcripts have deletion



Dystrophin now 12% of WT transcripts in non-DMD



- ddPCR Evagreen deletion assays quantifies 2% edited alleles in treated mdx mouse muscle
- ddPCR of cDNA shows robust expression of deletion allele, and overall increase in dystrophin transcript
- Characterization of therapeutic editing workflow by ddPCR

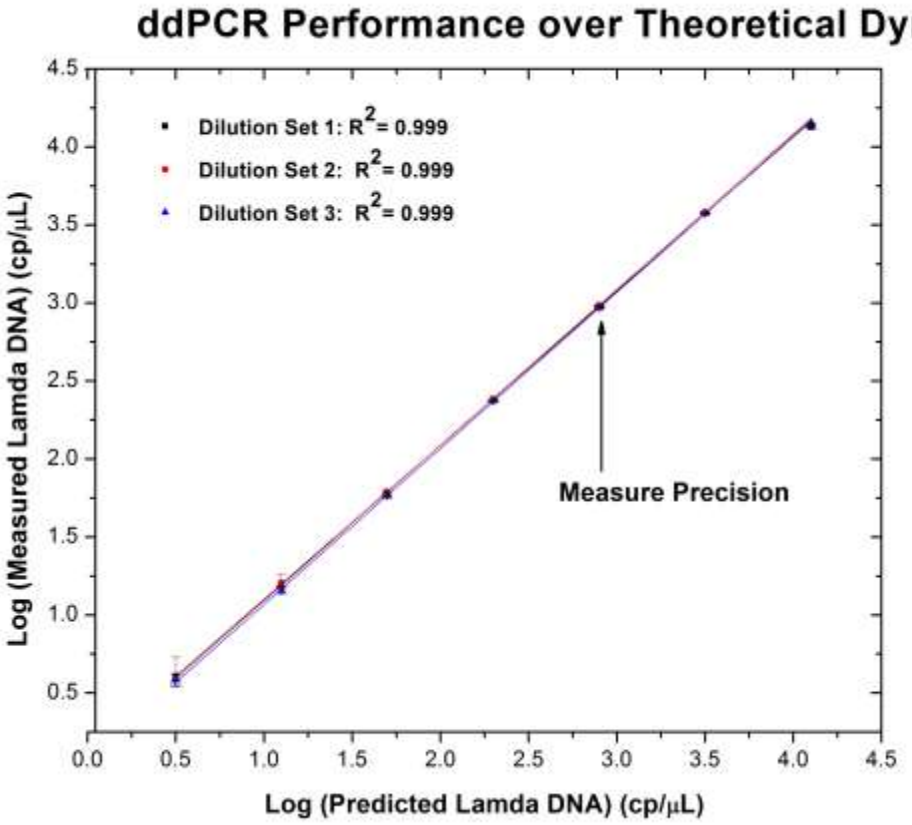
Conclusions

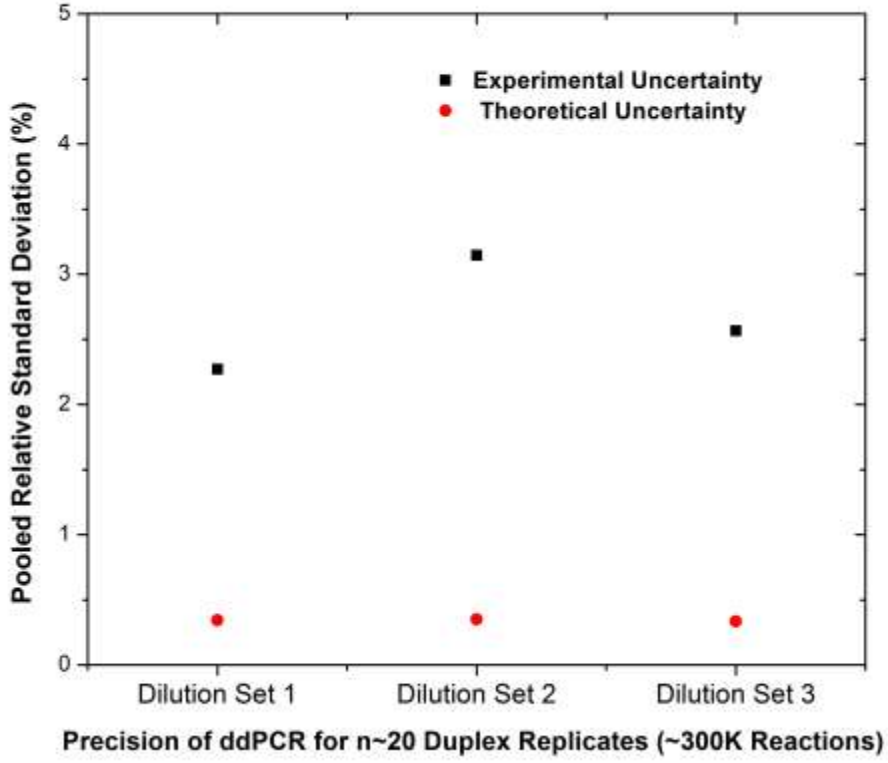
- ddPCR is the ideal companion technology for genome editing studies
- Unrivaled sensitivity (detection of events as rare as 1/10,000 per single well)
- High throughput
- Low cost (in particular compared to NGS)
- High precision (especially for gene regulation applications)

Absolute Quantification (ABS)

- Absolute measurements
- miRNA
- Gene Expression
- Single Cell analysis

System Precision Independently Verified and Observed, +/- 1.5% Uncertainty Over Theoretical Value





Gravimetric Experiments Conducted at [National Measurement Institute, Australia]

Metrology Labs Use ddPCR

Digital Polymerase Chain Reaction Measured pUC19 Marker as Calibrant for HPLC Measurement of DNA Quantity

Daniel G. Burke,*,† Lianhua Dong,‡ Somanath Bhat,† Michael Forbes-Smith,† Shuang Fu,† Leonardo Pinheiro,† Wang Jing,‡ and Kerry R. Emslie†

†National Measurement Institute, Lindfield, Australia 2070
‡National Institute of Metrology, Beijing, China, 100013

National Institute of Metrology
China

Towards standardisation of cell-free DNA measurement in plasma: controls for extraction efficiency, fragment size bias and quantification

Alison S. Devonshire · Alexandra S. Whale · Alice Gutteridge · Gerwyn Jones · Simon Cowen · Carole A. Foy · Jim F. Huggett

Australian Government
National Measurement Institute

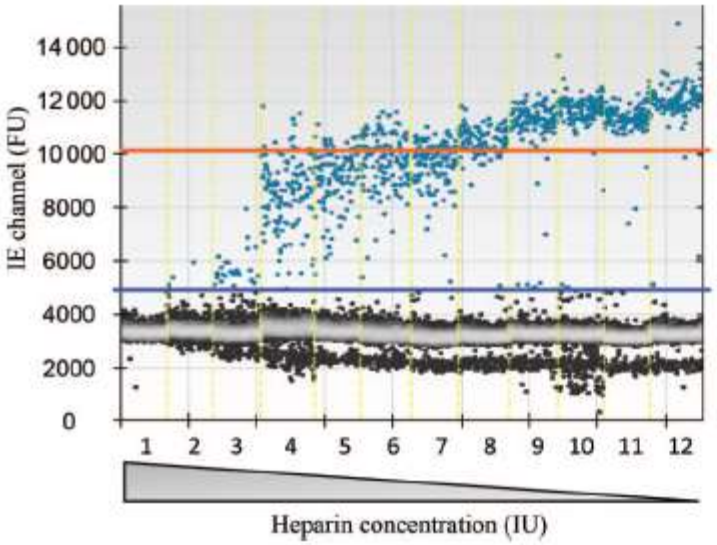
Evaluation of a Droplet Digital Polymerase Chain Reaction Format for DNA Copy Number Quantification

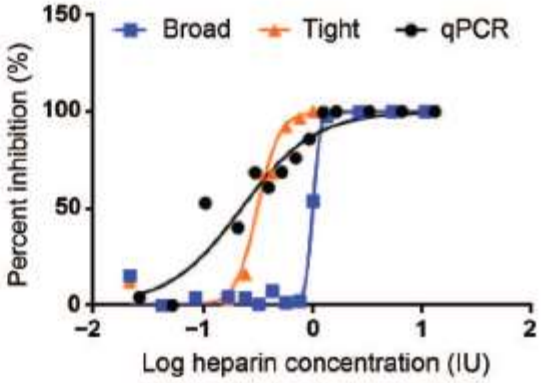
Leonardo B. Pinheiro,*,† Victoria A. Coleman,† Christopher M. Hindson,‡ Jan Herrmann,† Benjamin J. Hindson,‡ Somanath Bhat,† and Kerry R. Emslie†

†National Measurement Institute, Lindfield, New South Wales, Australia
‡Bio-Rad Laboratories, Inc., Pleasanton, California, United States

LGC

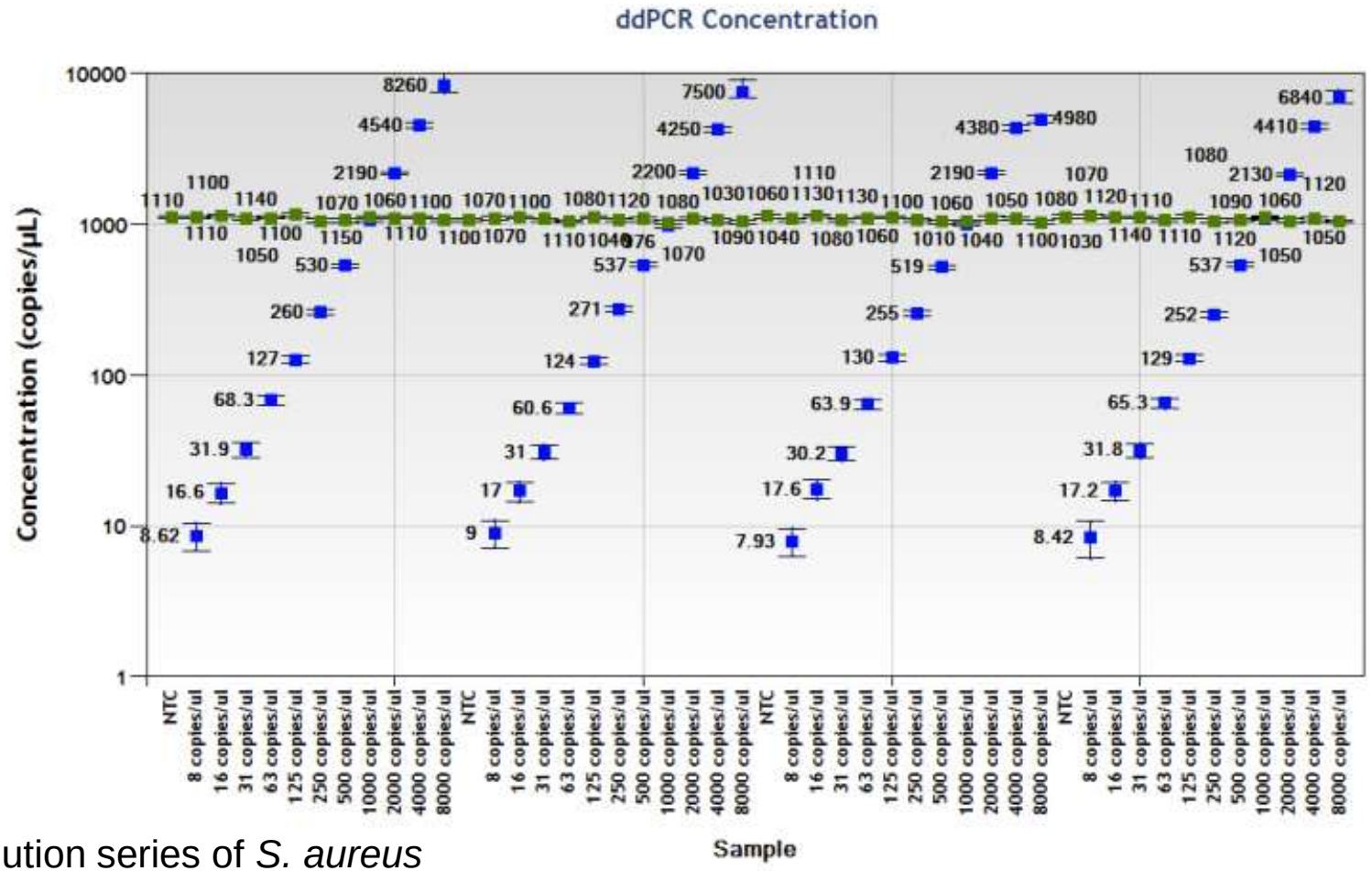
Tolerance of Droplet-Digital PCR vs Real-Time Quantitative PCR to Inhibitory Substances (Dingle et al., Clinical Chemistry 59:11)





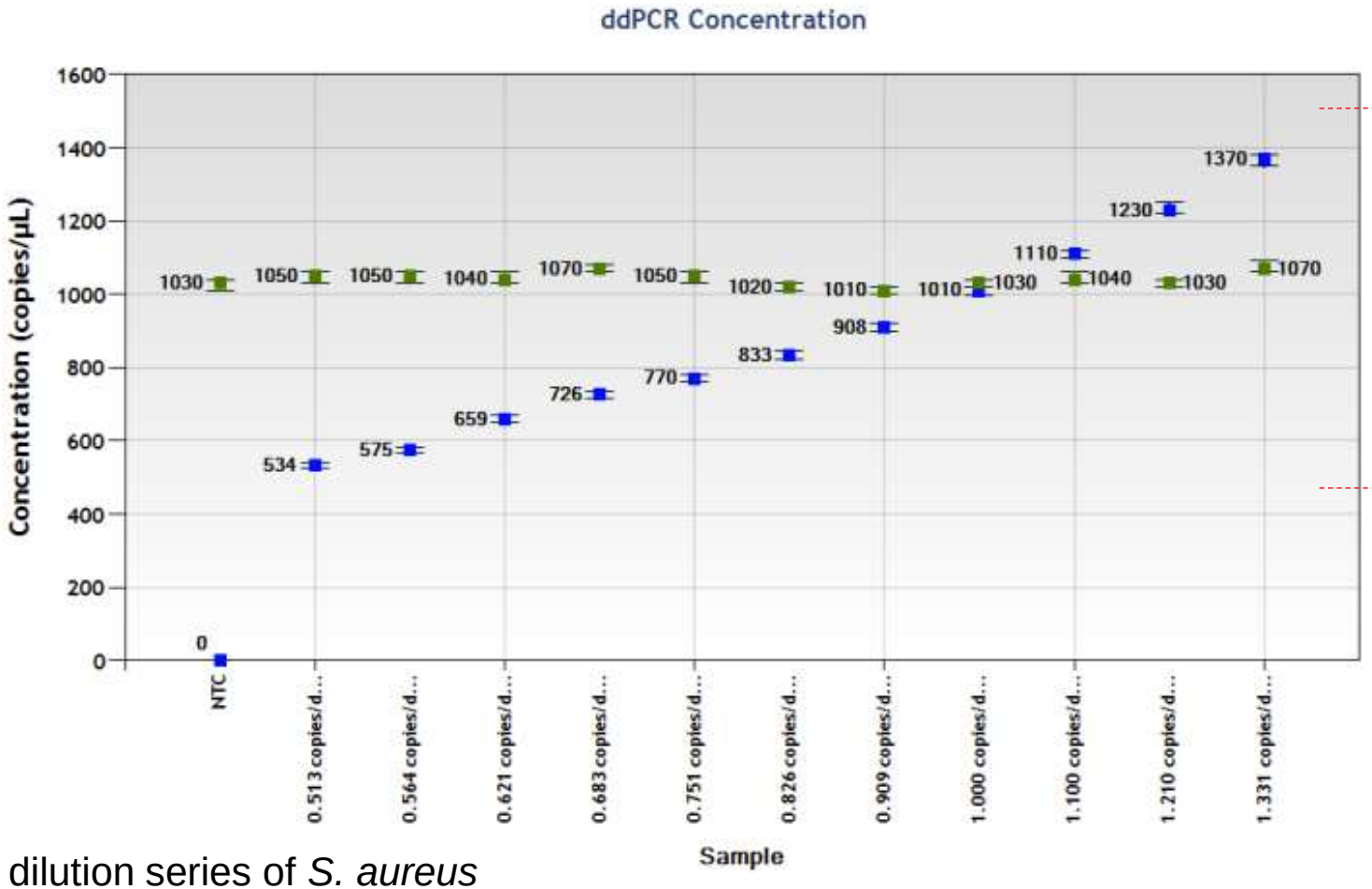
- Analysis of PCR inhibition by heparin, SDS and EDTA
- ddPCR shows higher tolerance to SDS and heparin in comparison to qPCR

Dynamic Range:
2-fold Serial Dilutions With a Constant Background



2-fold dilution series of *S. aureus*
Constant human gDNA

Precision From 10% dilutions



zoomed in

2-fold dilution series of *S. aureus*
Constant human gDNA

6 Merged Wells

Gene Expression Analysis

- Analysis of RNA (transcripts) levels in biological samples
- Usually performed in duplex (in combination with reference gene)
- Includes detection of miRNA
- Growing research on single cell transcriptome

Application miRNA circulant

Julien Faccini

INSERM / Université Paul Sabatier UMR 1048 Institut des Maladies Métaboliques et Cardiovasculaires (I2MC) Equipe 10, Lipides, peroxydation, signalisation et maladies vasculaires

Problématique :
niveau d'expression miRNA circulant (cohorte de patients

SCIENTIFIC REPORTS

OPEN

Circulating miR-155, miR-145 and let-7c as diagnostic biomarkers of the coronary artery disease

Received: 23 August 2016
Accepted: 17 January 2017
Published: 16 February 2017

Julien Faccini[1,2], Jean-Bernard Ruidavets[2,3,4], Pierre Cordelier[5], Frédéric Martins[6], Jean-José Maoret[6], Vanina Bongard[2,3], Jean Ferrières[2,3,4], Jérôme Roncalli[3], Meyer Elbaz[1,2,3] & Cécile Vindis[1,2]

[1]INSERM UMR-1048, Institute of Metabolic and Cardiovascular Diseases, Toulouse, France. [2]Toulouse Paul Sabatier University, Toulouse, France. [3]CHU Toulouse, Department of Cardiology, Toulouse, France. [4]INSERM UMR-1027, Epidémiologie et Analyses en Santé publique, Toulouse, France. [5]INSERM UMR-1037, Cancer Research Center of Toulouse, France. [6]PlateformeGeT, Toulouse, France. Correspondence and requests for materials should be addressed to C.V. (email: cecile.vindis@inserm.fr)

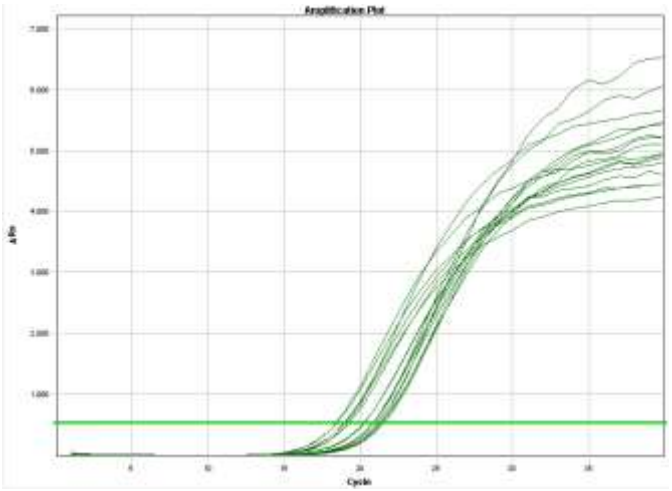
Technique: RTqPCR sonde TaqMan (Applied Biosystems) avec étape de préamplification

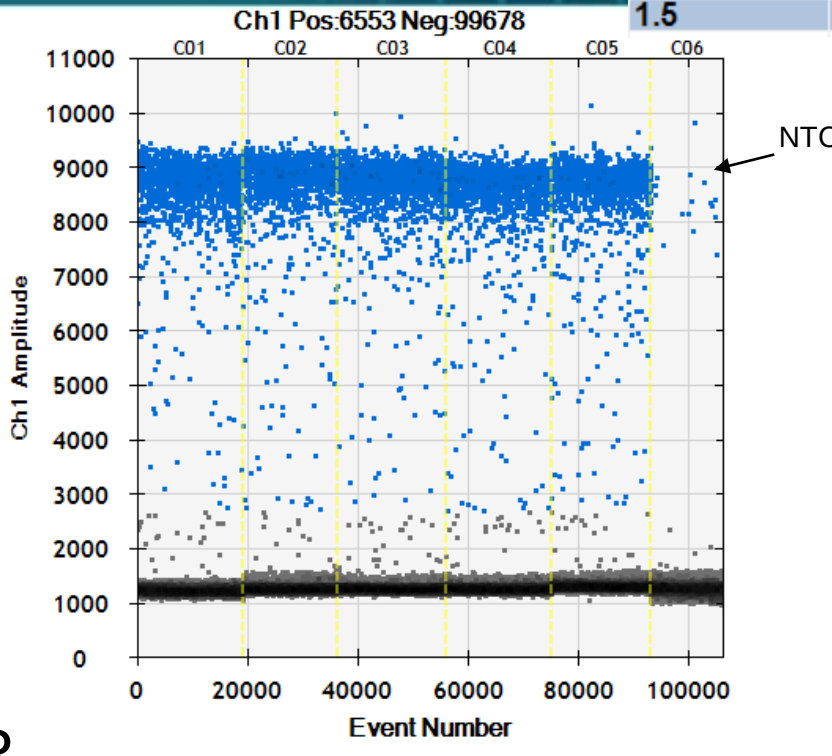
Limitation:

- le choix de la normalisation → ddPCR quantification absolue
- l'utilisation d'une préamplification (coût) → sans préamplification ?

1er cas : échantillon ADNc sans préamplification

Conc(copies/µL)
85.1
87.1
91.8
82.7
80.2
1.5





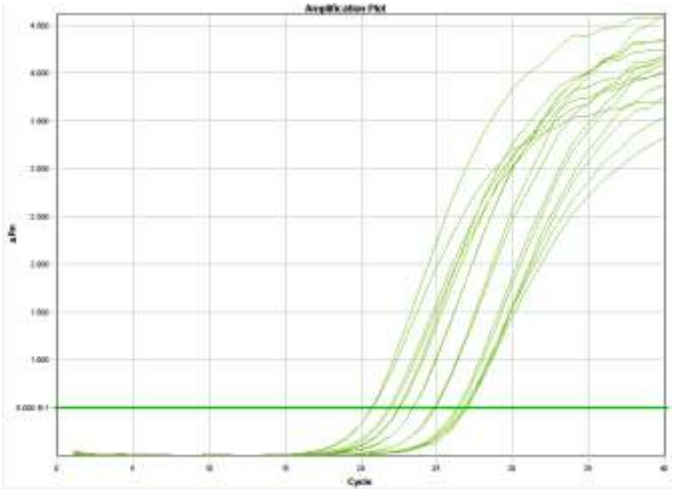
Résultats en TLDA
(TaqMan Low Density Array)
Sorti autour de 15-20 cycles qPCR
après préamplification

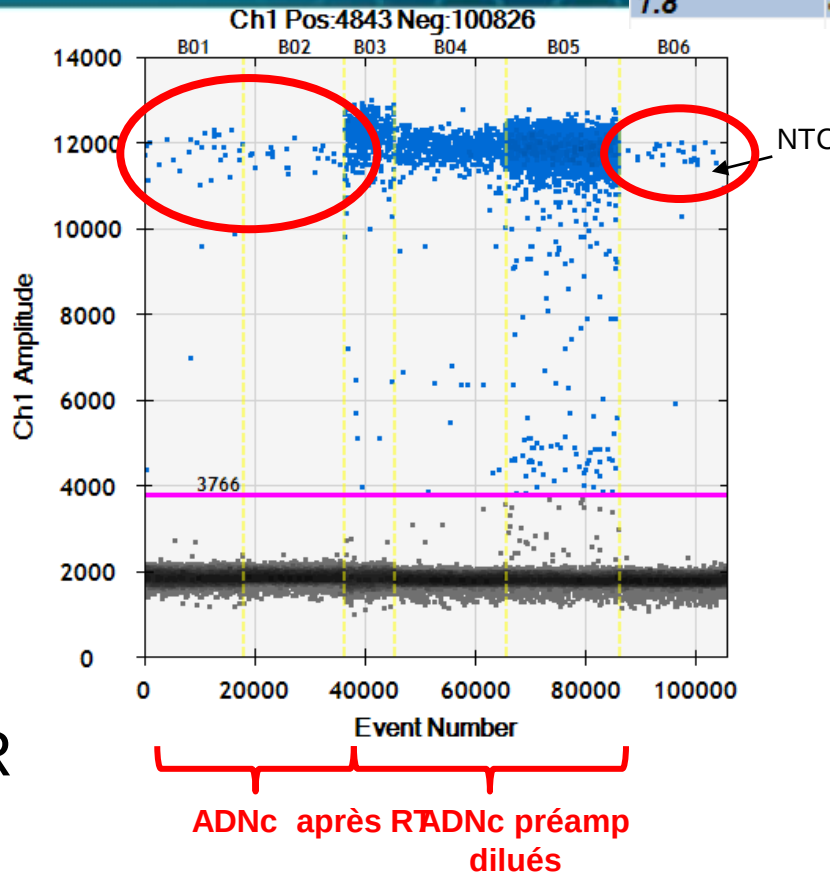
5 réplicats de dépôt en sorti de RT

→ Aucun besoin de préamplification

2eme cas : miRNA exprimé très faiblement

Conc(copies/µL)
2.1
1.4
49
31.3
245
1.8

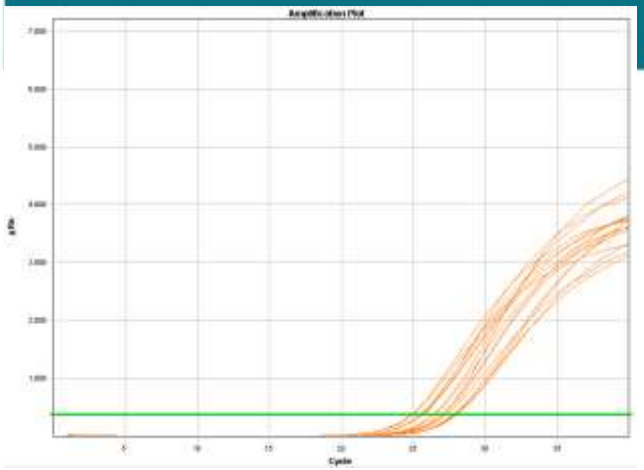


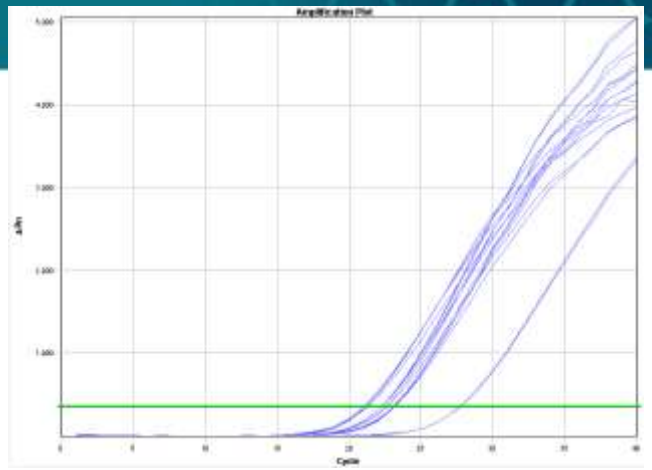


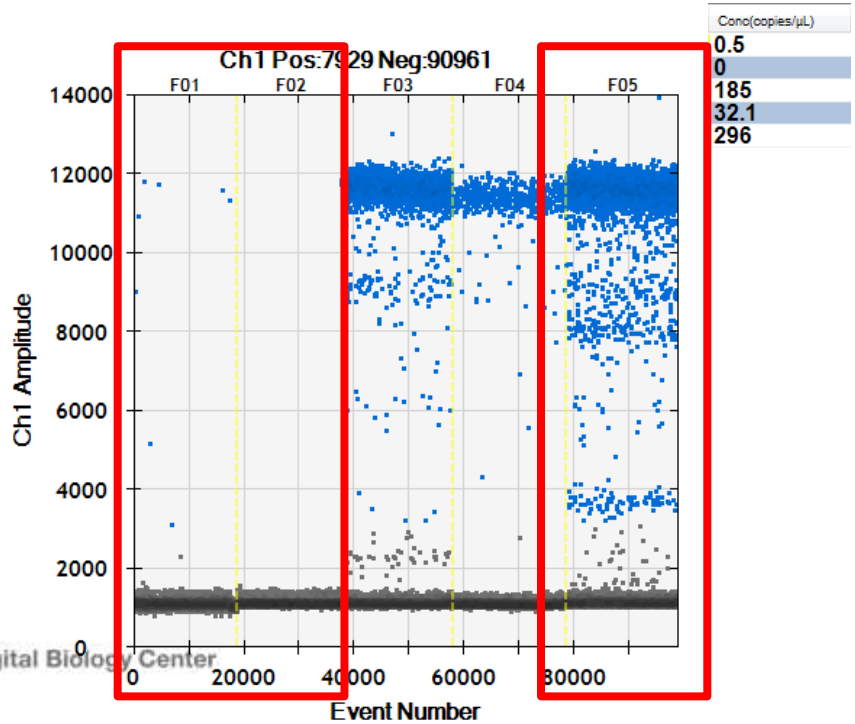
Résultats en TLDA
(TaqMan Low Density Array)
Sorti autour de 20-27 cycles qPCR
après préamplification

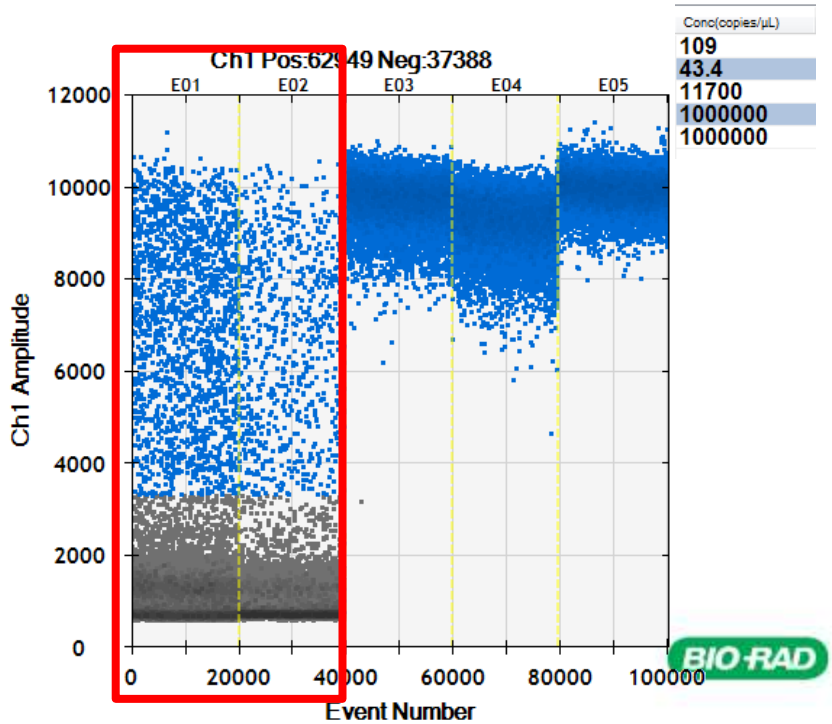
→ besoin de préamplification

3eme cas : problème d'amorces/sondes









Perspectives d'utilisation de la ddPCR

- **Optimisation des conditions** (T° hybridation, quantité d'ADNc …)

- Utilisation de ddPCR en complément de la qPCR pour certaines cibles

  ↳ Possibilité de multiplexage

- Pour les miRNA difficilement observable en QPCR

- Expressions tardives avec différences d'expression faibles

Improved Precision, Reproducibility, and Limit of Quantification of Cancer microRNA Biomarker Quantification in Cell-Free DNA

Nature Methods | VOL.10 NO.10 | OCTOBER 2013 | 1003–1005

Absolute quantification by droplet digital PCR versus analog real-time PCR

Christopher M Hindson[1,6,7], John R Chevillet[2,7], Hilary A Briggs[2], Emily N Gallichotte[2], Ingrid K Ruf[2], Benjamin J Hindson[1,6], Robert L Vessella[3] & Muneesh Tewari[2,4,5]

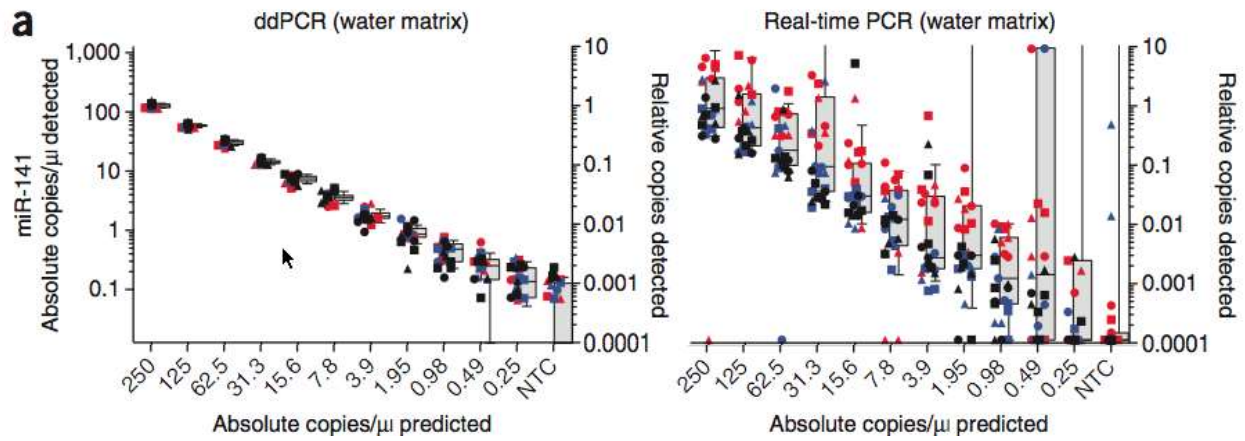
BRIEF COMMUNICATIONS

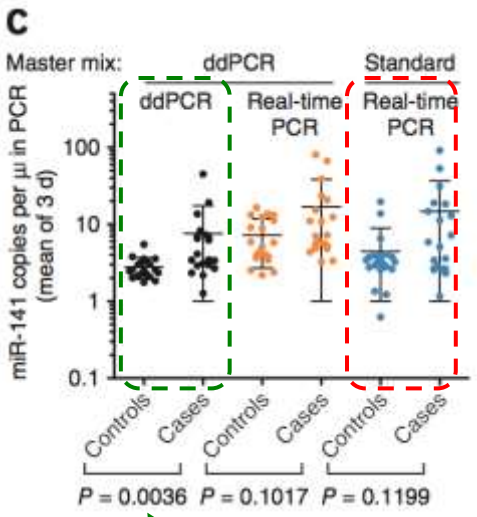
Why: Low-abundance blood biomarkers may be predictive of various cancers.

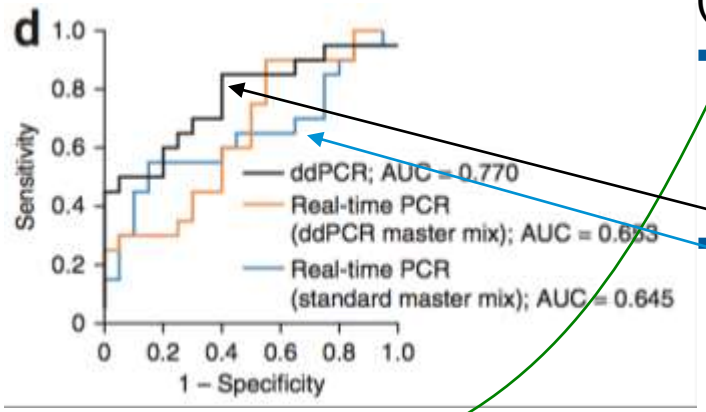
Problem: Standard qPCR method is too variable to effectively score potentially informative low-abundance microRNAs (miRNAs) in plasma and serum.

Solution: ddPCR is more precise and reproducible both within and between experiments and has increased diagnostic sensitivity.

High Precision, Sensitivity & Reproducibility of miRNA Quantification by ddPCR (Hindson et al, 2013)







Synthetic targets (in water & plasma):

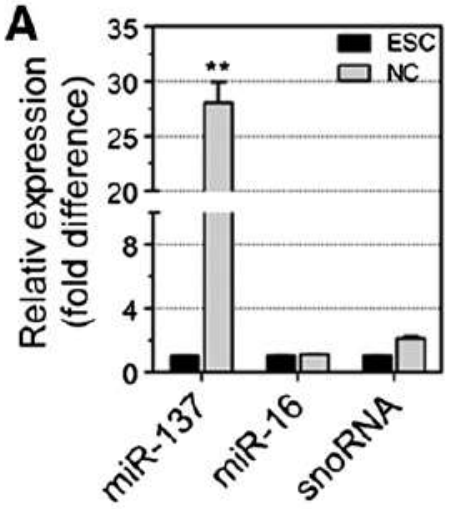
- CV's decreased by 37-86% w/ ddPCR
- Day-to-day reproducibility increased 7-fold
- For miR-141 shown, CV was 86% lower across replicates

Serum samples (prostate cancers):

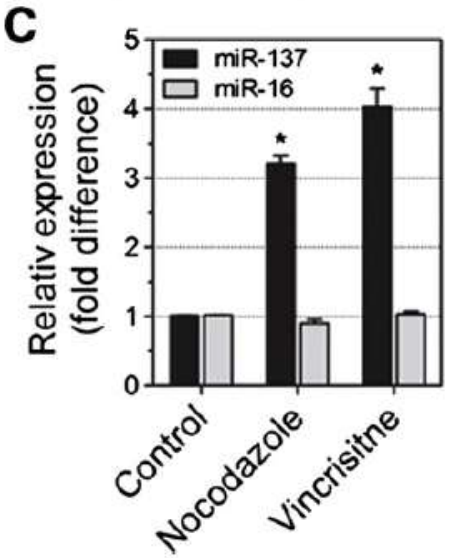
- Only ddPCR showed statistically significant difference between cases and controls
- Greater sensitivity and specificity for ddPCR (AUC=0.77) vs qPCR ((AUC=0.645)

Hindson CM et al. (2013). **Absolute quantification by Droplet Digital PCR versus analog real-time PCR.** Nat Methods 10, 1003–1005.

MicroRNA-137 represses Klf4 and Tbx3 during differentiation of mouse embryonic stem cells (Jiang et al., Stem Cell Research (2013) 11)



qRT-PCR



ddPCR

- Use of ddPCR to quantify miRNA during stem cells differentiation
- While qRT-PCR works well to characterize large changes (Fig A), but ddPCR is needed when the differences are more subtle (Fig C)

Exemple d'une demonstration – But de l'experience

Avec ou sans Pre-Amp ?

Avec une masse attendue de l'ordre de seulement 40 pg, la ddPCR pourrait-elle se passer d'une étape de pré-amplification et conserver les ratios d'expression d'origine ?

Conditions de l'expérience (EvaGreen)

Transposition directe des conditions qPCR :

Amorces 300nM, 60°C t°hybridation.

3 gènes

-> 1 Ref « R1 », 2 GOI « T1 » et « T2 »

8 échantillons

-> C1 à C4 et D1 à D4

3 dilutions

->1/5^{e} 1/50^{e} 1/500^{e}

1 NTC / cible

Masses d'ADNc

Les masses d'ADN mesurées en amont correspondent à :

- 4 ng (dilution RT 1/5^{e})
- 400 pg (dilution RT 1/50^{e})
- 40 pg (dilution RT 1/500^{e})

Cette dernière requière une étape de pré-amplification en qPCR.

Etape dommageable à la conservation des expressions ?

Reproductibilité

Pipettage réalisé par 4 utilisateurs différents dont 3 néophytes.

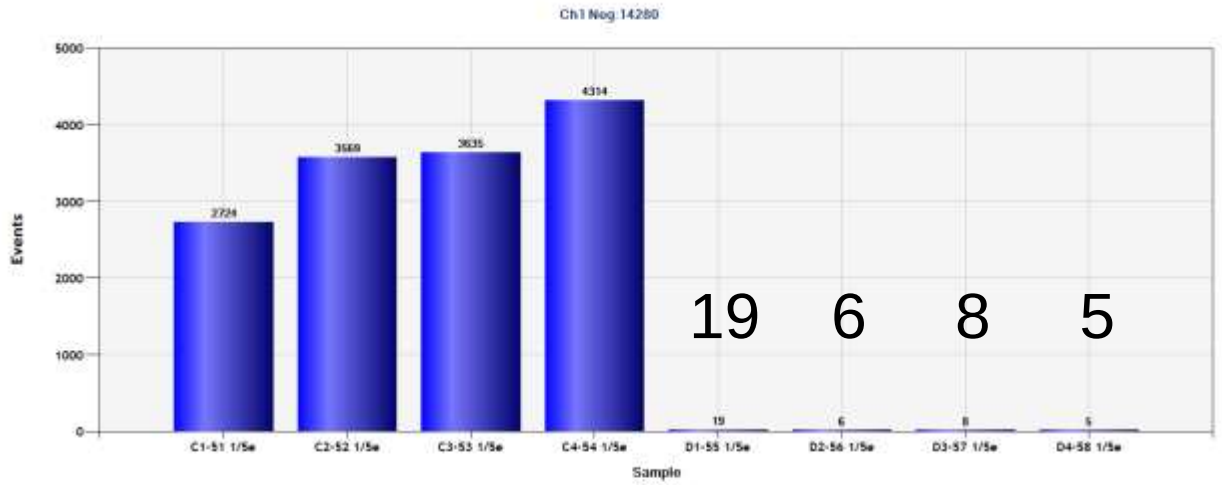
Seuls 2 puits sont en-dessous des recommandations mais restent exploitables.

Grande reproductibilité.

Concentration 1/5e Cible T2

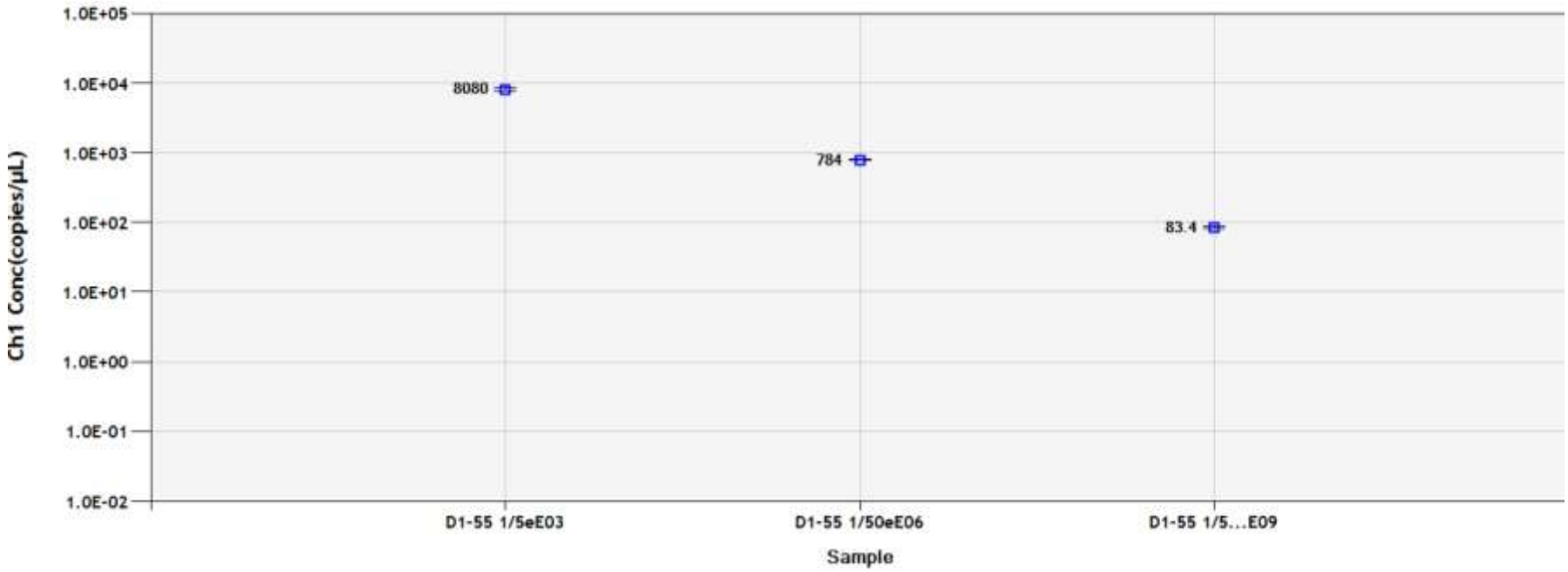
A 1/5e, R1 et T1 saturent (>200 000cp/w)

Mais T2 est mesurable avec un nombre de gouttelettes négatives très faible :

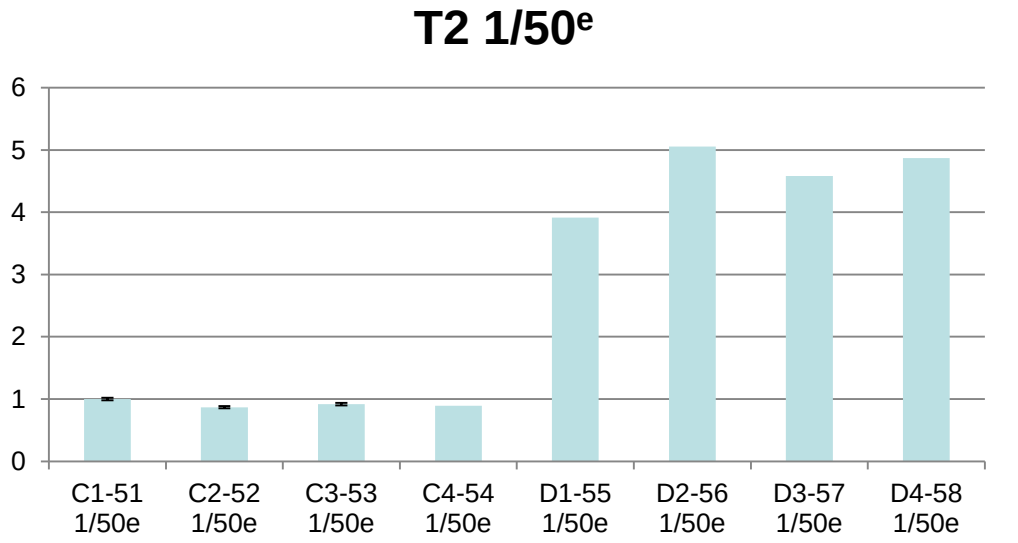


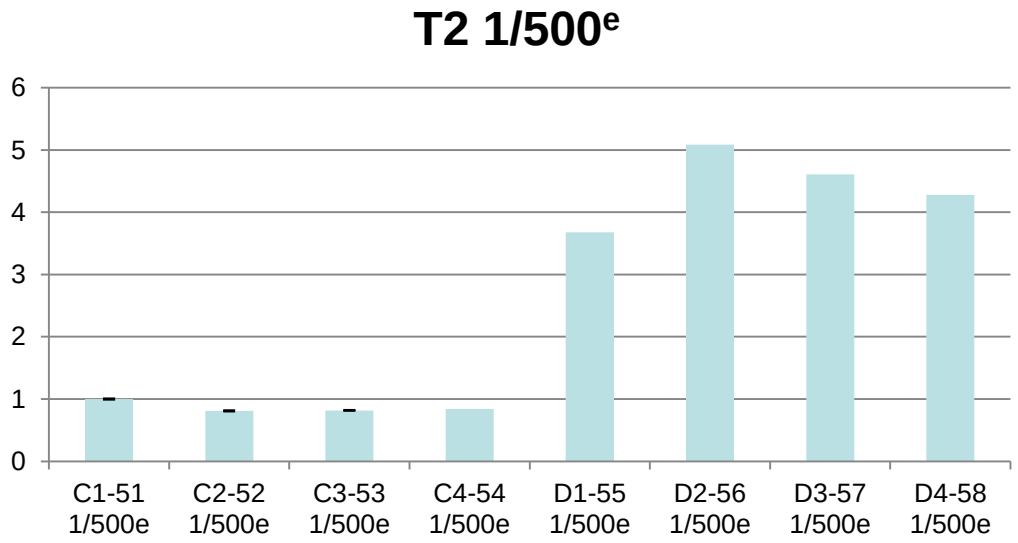
Linéarité et dynamique

Exemple avec D1 (T2). Avec seulement 19 gouttelettes négatives au 1/5^{e} :



Expression et conservation (normalisé par R1)





Conclusion

Prise en main simple avec 3 neophytes

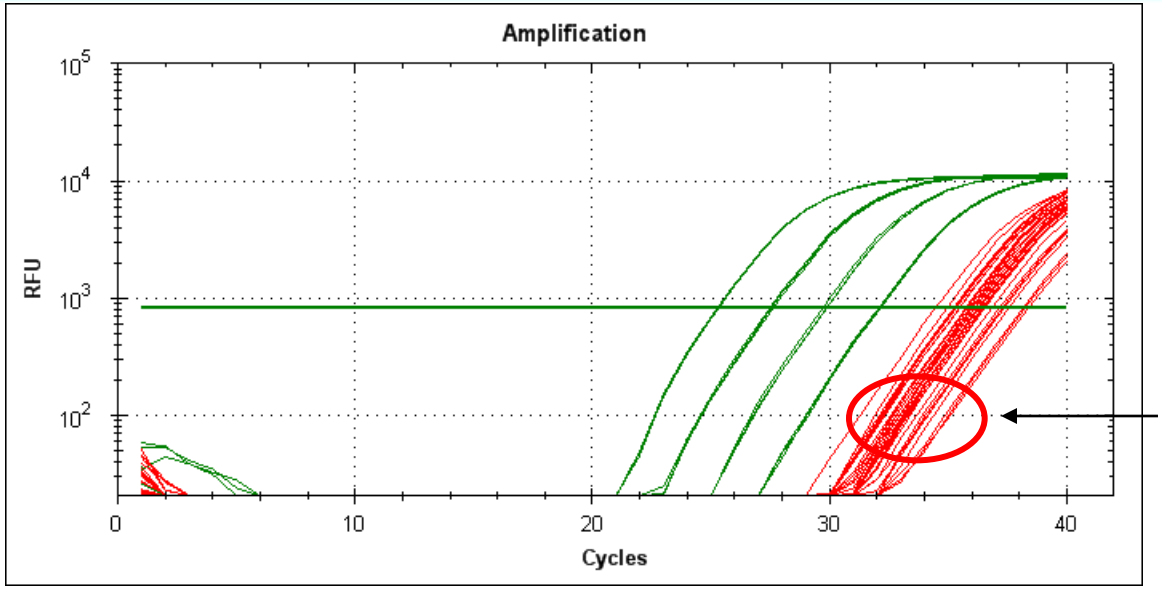
Résultats d'expression en concordance avec ceux obtenu auparavant en qPCR mais plus précis et...

...les inductions (*invisibles en qPCR*) sont conservées malgré les faibles masses (40 pg) utilisées

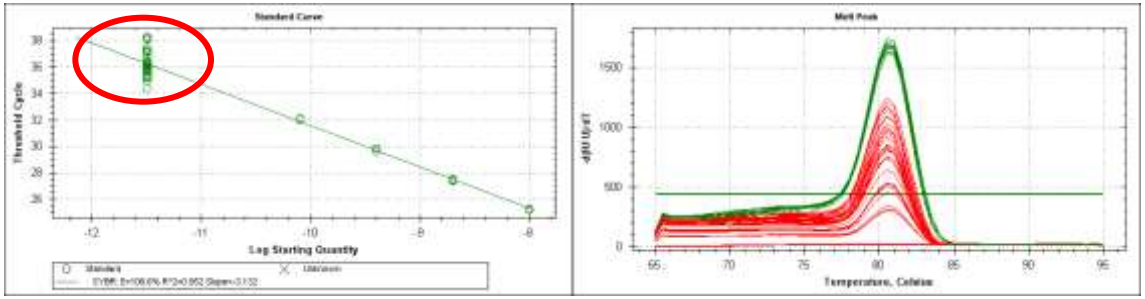
Il est envisageable de quantifier ici des transcrits rares avec encore moins d'ADN (20 pg par ex.)

Single cell using ddPCR

Single-Copy Target Detection



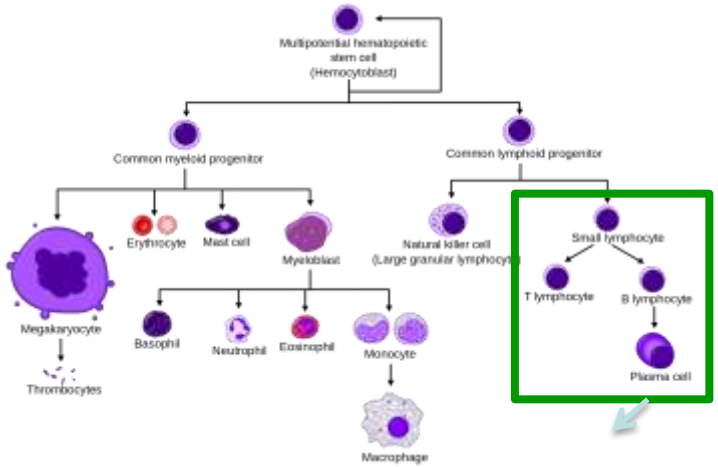
48 replicates; 32 positive
Cq range = 34.88 to 38.78

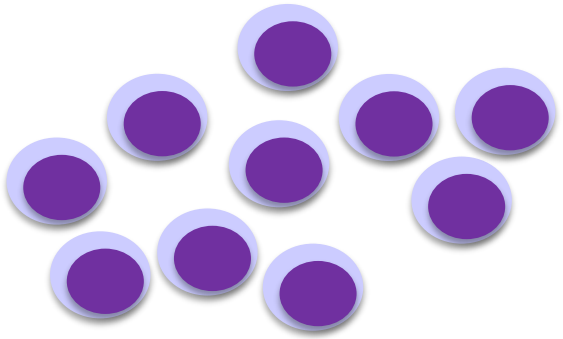


Target: cyclophilin from gDNA
Cycling: 95C/2' + [95C/5" + 60C/10"] x 40
Input: 10ng, 2 ng, 400pg, 80pg, 3.2pg x 48 replicates

Efficiency = 103.0%
R^2 = 1.000

Importance of single cell studies





Bio-Rad workflows for single cell analysis

Cell sorting

Cell lysis

ddPCR

_ high precision
_ allelic discrimination

qPCR

_ high throughput
_ easy multiplexing

Advantages of ddPCR for single-cell gene expression analysis

- No cDNA pre-amplification
  - Simplified workflow
  - Decreased cost
  - Avoid distortion of transcript levels
  - Detection of up to 10 genes per cell (two 5plex ddPCR reactions)
- High precision
  - Absolute quantification of transcripts from a few copies to thousands of copies per cell
  - No standard curve
- High sensitivity
  - Many essential genes are expressed at 1-30 mRNA copies/cell (Zenklusen et al. 2008)

→Simple workflow without preamplification for analysis of 2-10 genes!

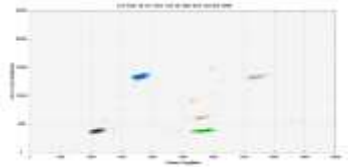
Simplified Workflow, completed in less than 1 workday

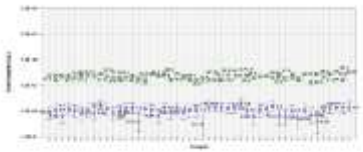
Cell culture

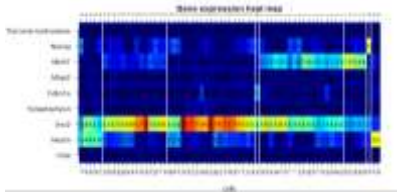
S3 cell sorter

QX200
Droplet Digital PCR

Data
analysis



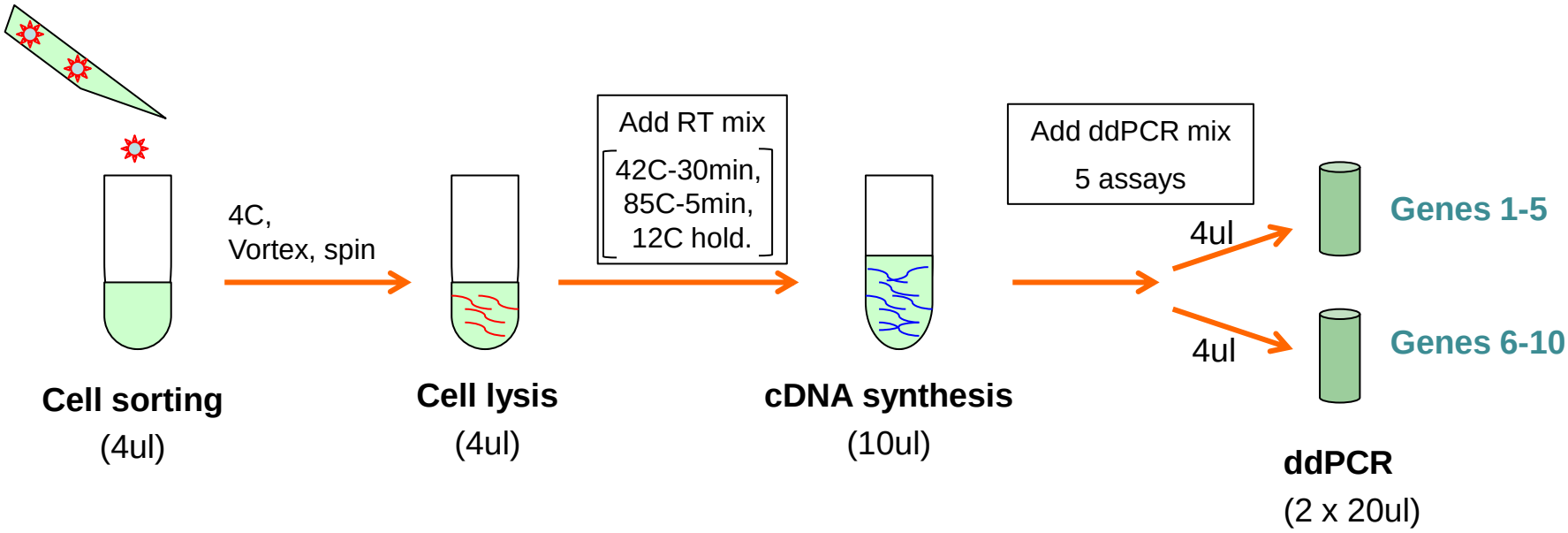




Single cells

PrimePCR Assays

Single-cell gene expression workflow (*no pre-Amp*)

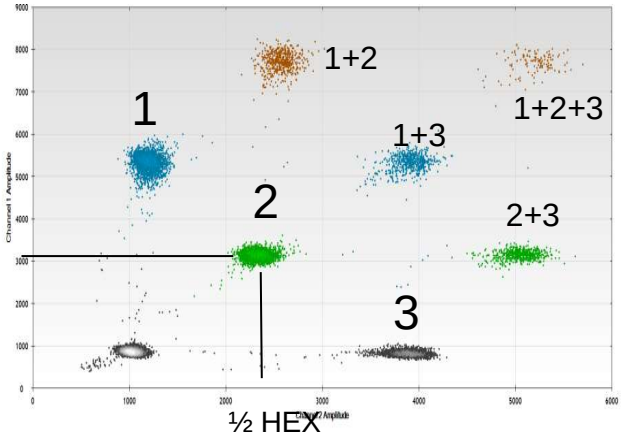


Measure 40% of the cell's cDNA per well (80% total)

5 assays per ddPCR well

Multiplexing in 2 channels overview

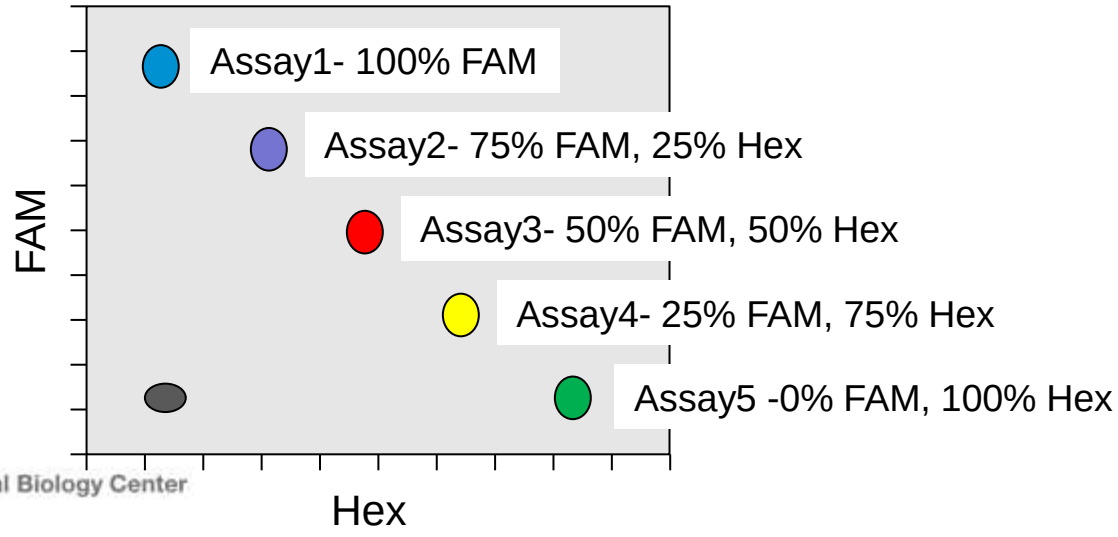
Concept of probe mixing - triplex



Double, Triple positives

3plex	Assay mixing
1	100% FAM, 0% Hex
2	50% FAM, 50% Hex
3	0% FAM, 100% Hex

Theoretical clustering -5plex

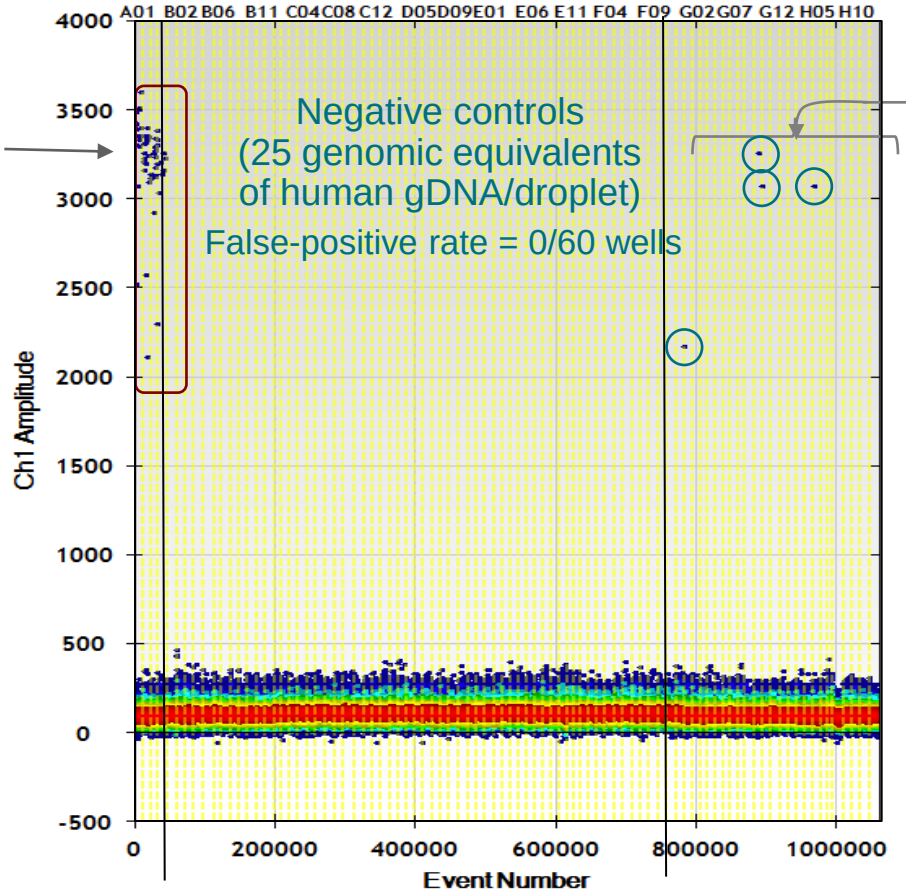


5plex	Assay mixing
1	100% FAM, 0% Hex
2	75% FAM, 25% Hex
3	50% FAM, 50% Hex
4	25% FAM, 75% Hex
5	0% FAM, 100% Hex

Additional Applications

Rare Species Detection (e.g.Virus)

Rare Species Detection: Human Herpesvirus 6 (HHV-6) in Human gDNA (1 in 1,700,000)



Positive controls

Negative controls (25 genomic equivalents of human gDNA/droplet)

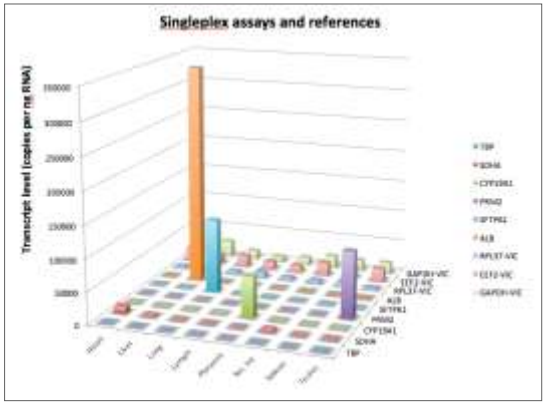
False-positive rate = 0/60 wells

HHV-6 spike-in

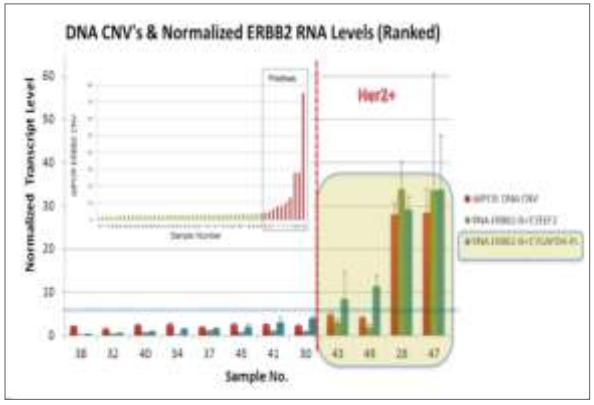
Four HHV-6 positives in 8,000,000 human genomes (4.5 expected)

Gene Expression Applications

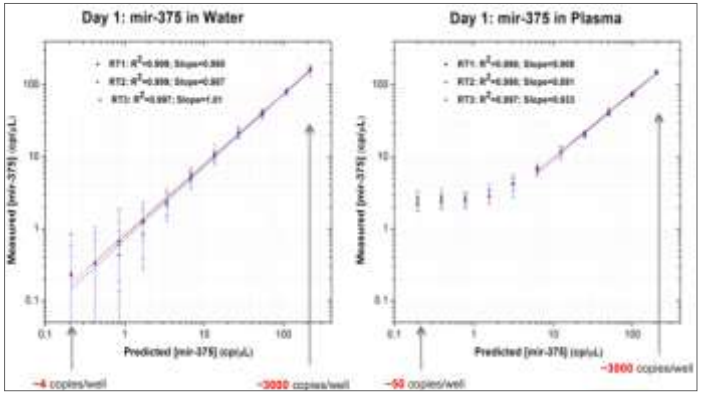
Tissue-Specific Gene Expression



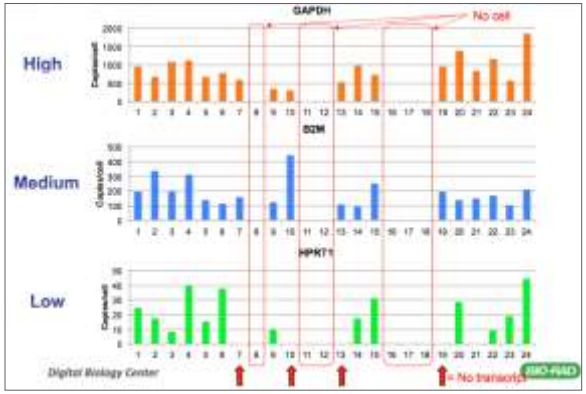
Her2 mRNA in FFPE Samples



microRNAs in Plasma



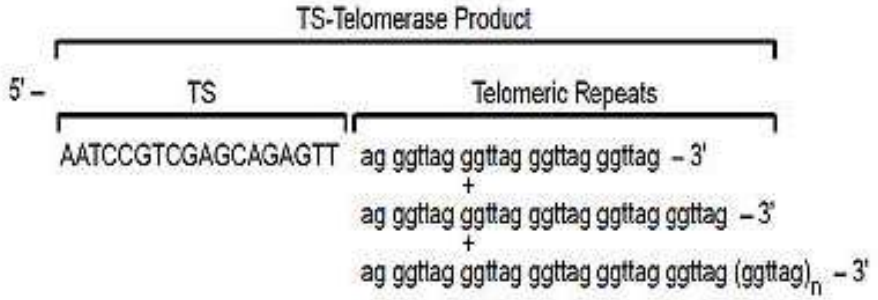
Single-Cell Transcript Detection



Telomerase Repeat Amplification Protocol (TRAP)

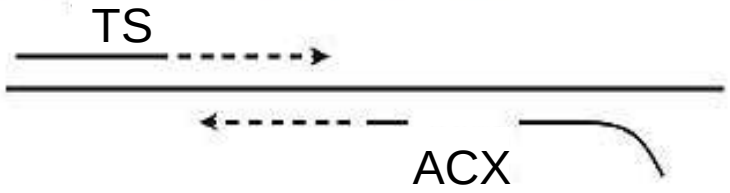
Make cDNA

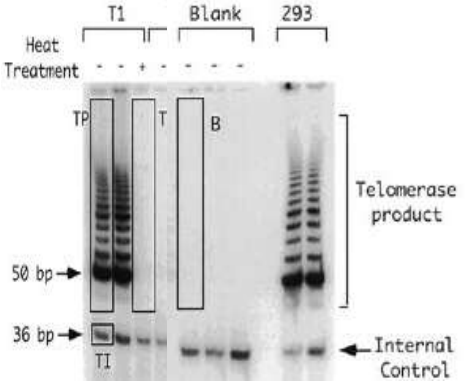
STEP 1. Addition of Telomeric Repeats By Telomerase



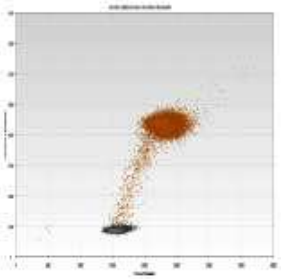
Perform PCR

STEP 2. Amplification of TS-Telomerase Product By PCR





(Kim et al. 1997)



100 cells/reaction

Kim NW and Wu F (1997). **Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP).** Nucleic Acids Res 25, 2,595–2,597.

NGS Library Prep

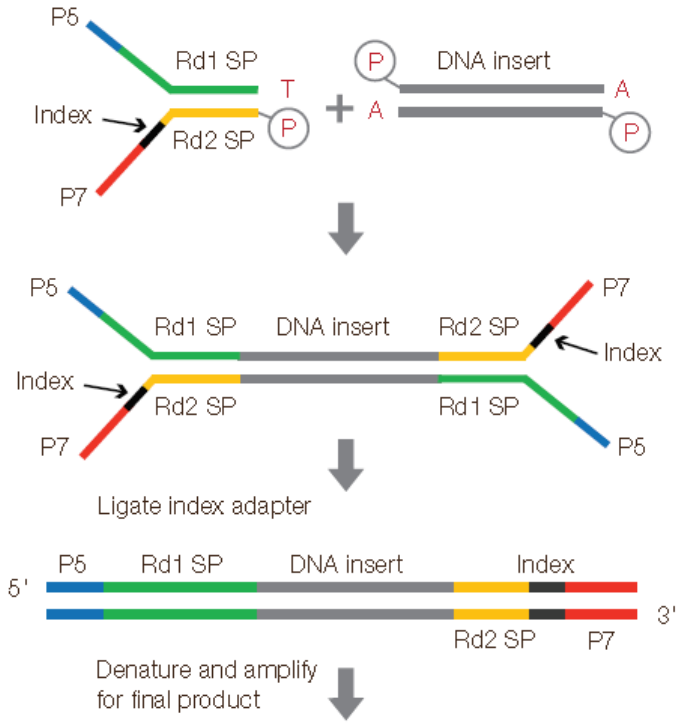


Fig. 7.1. TruSeq v2 library preparation.

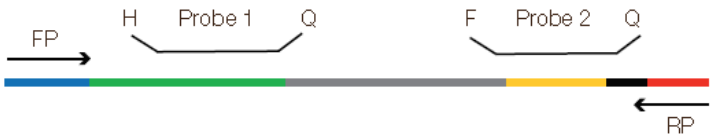
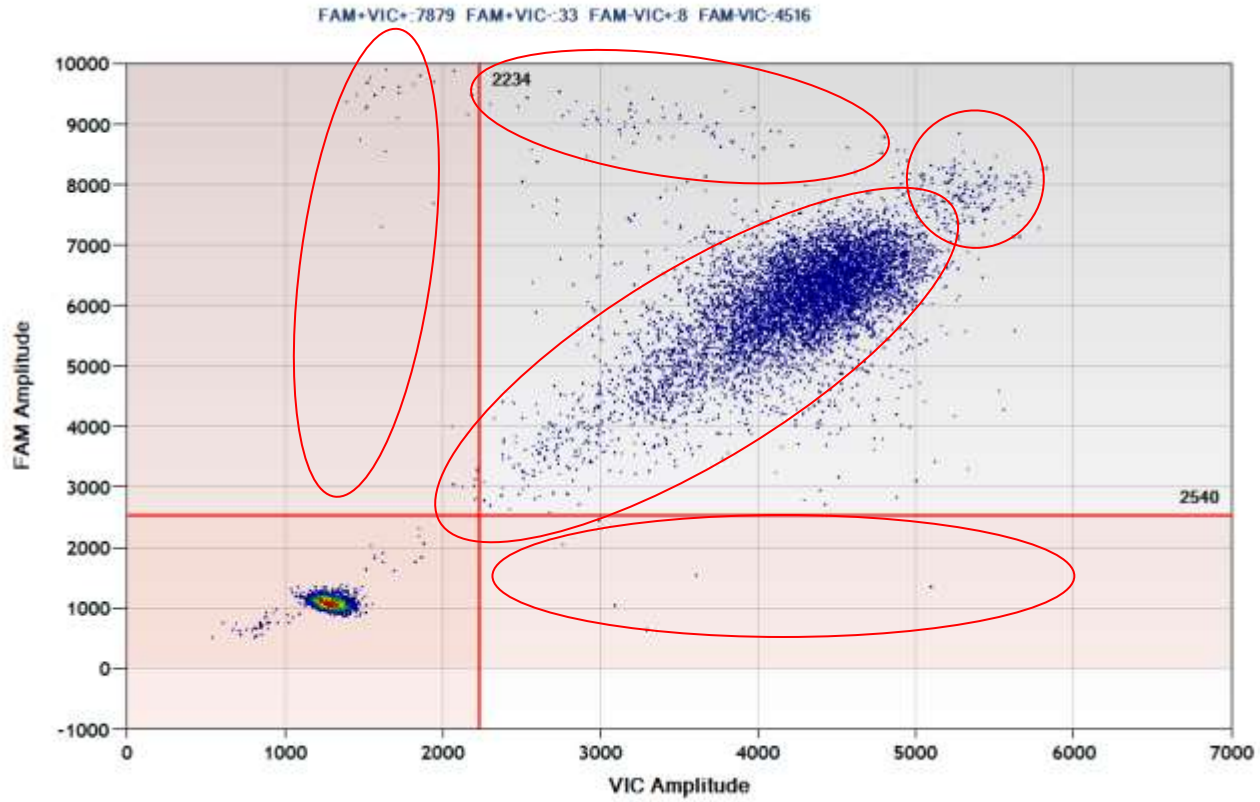


Fig. 7.2. Design of the ddPCR library quantification kit for Illumina TruSeq assay.

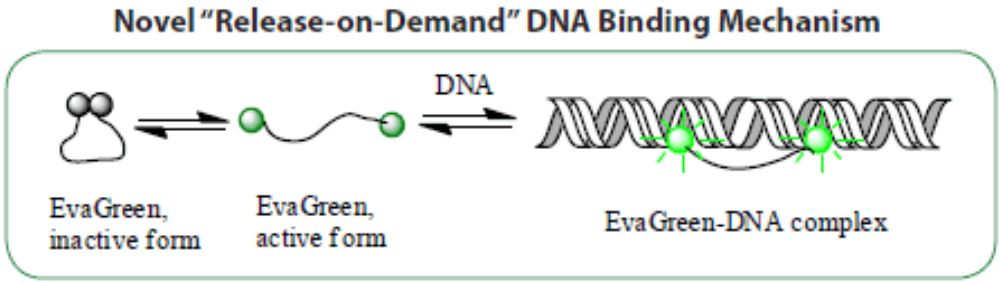
ddPCR quantitation example : libs quant kits



- The abundance of well-formed, as well as, potentially ill-formed library fragments are measured

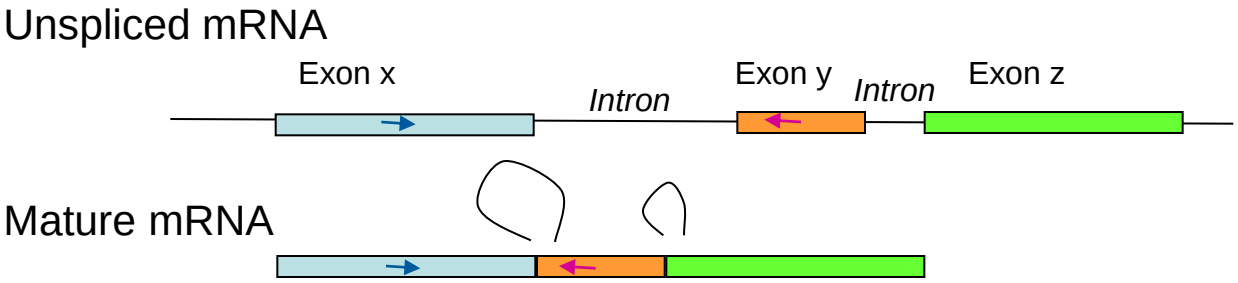
Alternative splicing

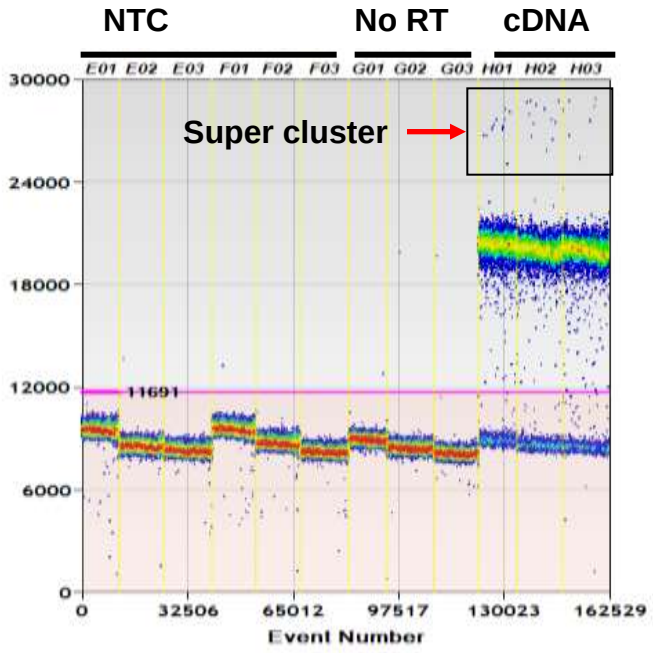
QX200 System Enables dsDNA Detection Capability with EvaGreen (without TaqMan probes)

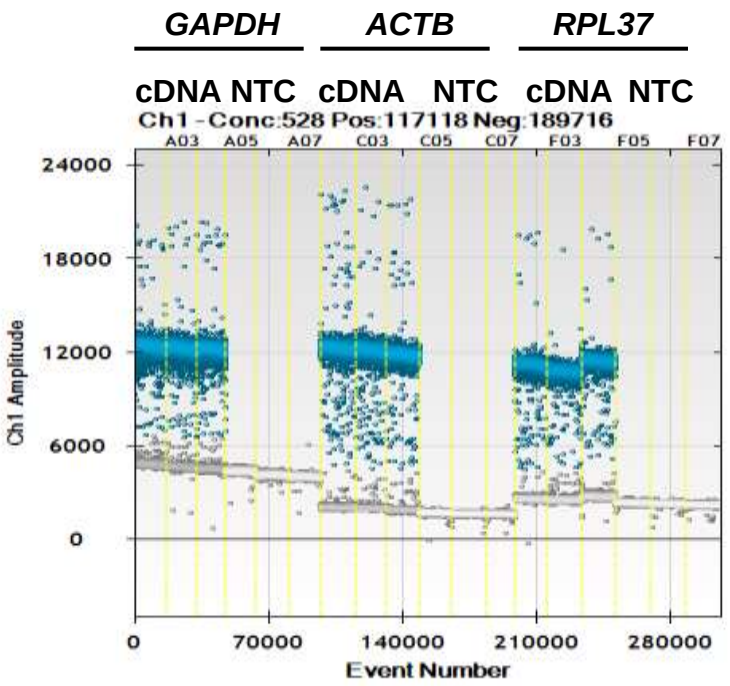


- No preference for GC- or AT-rich sequence
- Less PCR inhibition than SYBR® Green and lower tendency to cause nonspecific amplification
- Tolerated at a higher concentration, which enables a brighter signal
- Good stability
- Safety
  - Dye is impenetrable to both latex gloves and cell membranes
  - Dye is noncytotoxic and nonmutagenic at concentrations used in the laboratory

Detection of Unspliced mRNA Variants Using Intron-Flanking Primers







Haplotyping

What Is Linkage?

Linkage is when two genomic loci are physically connected to one another.

Linked

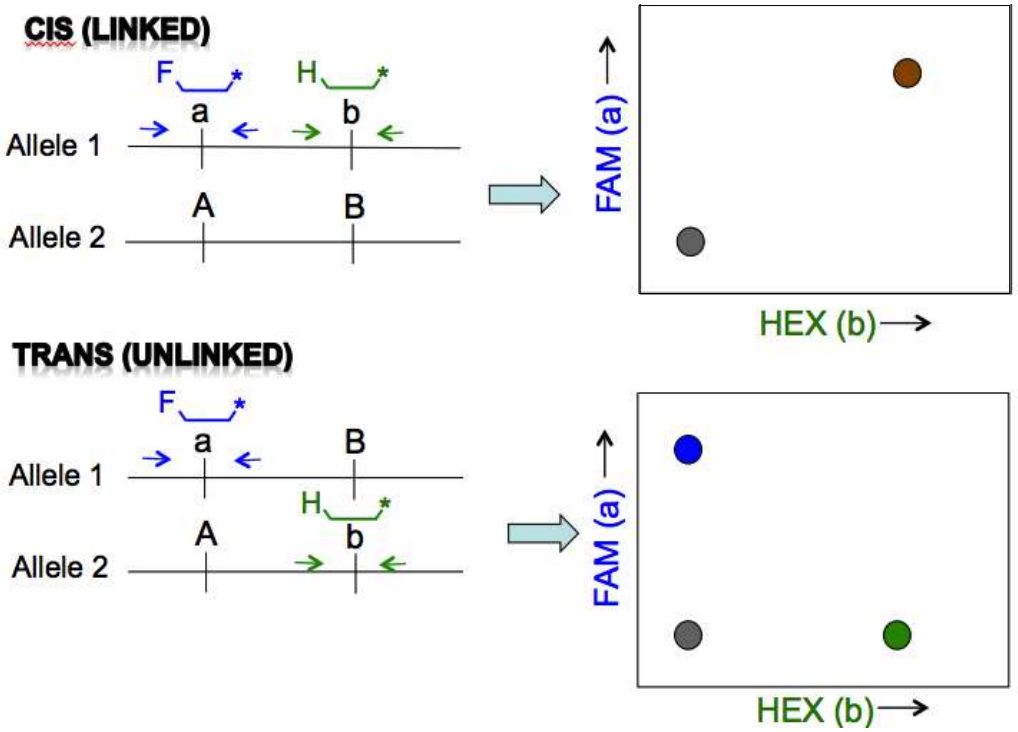
VS.

Not linked

Who Is Interested in Linkage and Why Is It Important?

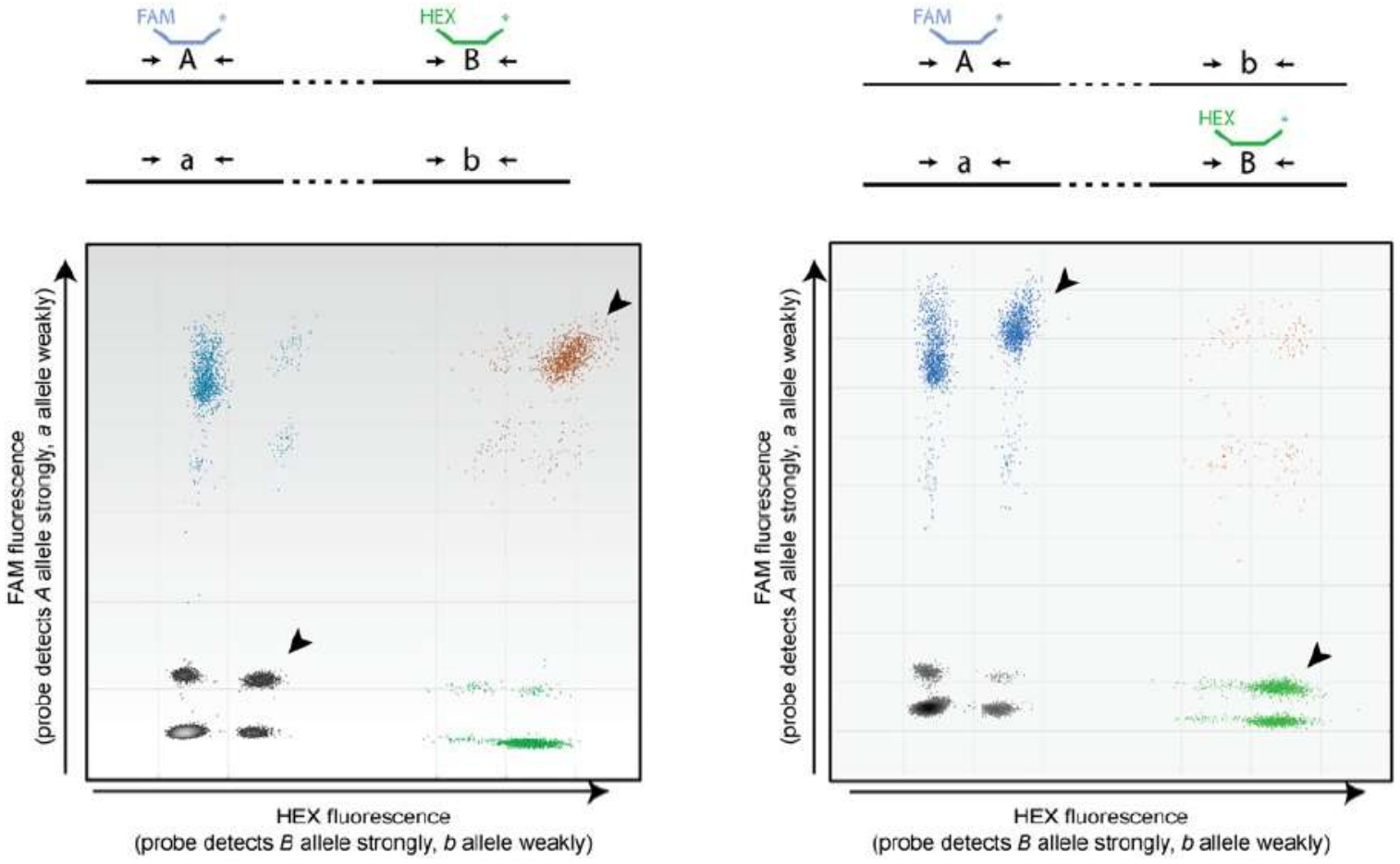
Life science researchers and laboratories that perform molecular diagnostics.

- Cis-/trans-configured genomic variants shown



Example: *CFTR*
(c.350 G>A and *5T* allele)

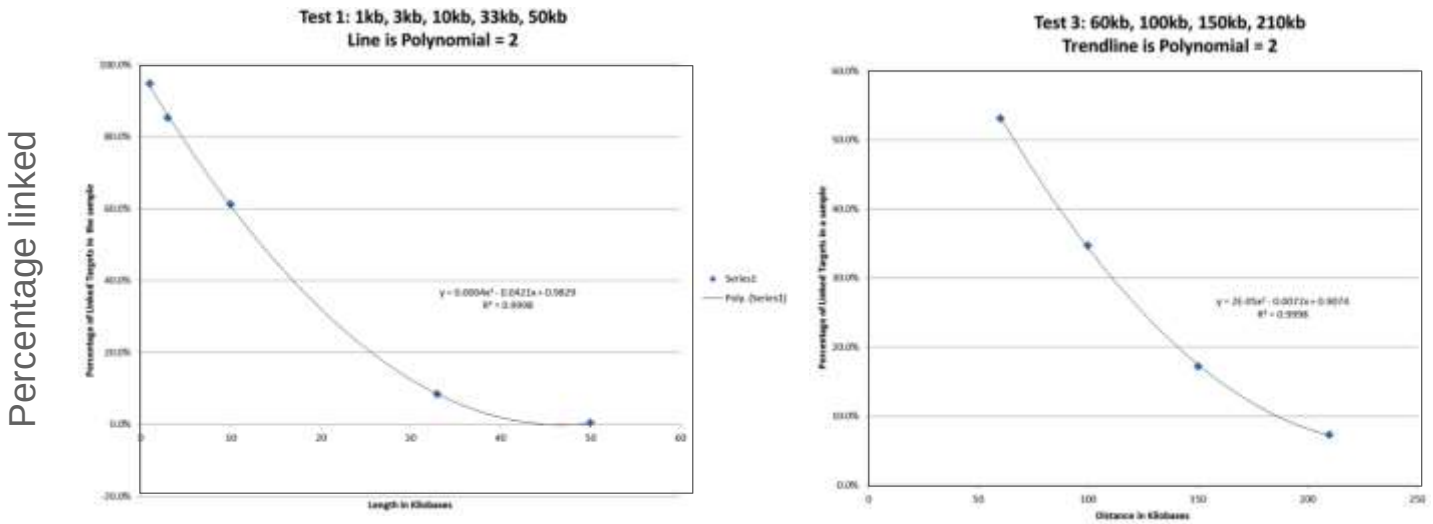
Droplet Digital PCR Cluster Identification



Regan JF et al. (2015). **A rapid molecular approach for chromosomal phasing method.** PLoS One 10, e0118270.

“Anchor” Assay in HEX and “Distance Marker” Assay in FAM (or vice versa) in Duplex

Anchor Assay | 1 kb | 3 kb | 10 kb | 35 kb | 50 kb | 100 kb | 150 kb | 210 kb



Regan JF et al. (2015). **A rapid molecular approach for chromosomal phasing method.** PLoS One 10, e0118270.

Diseases Influenced by Compound Heterozygosity in Single Genes

Studies of these diseases are hampered by the lack of tools to easily determine phase:

- Cystic fibrosis
- Cerebral palsy
- Deafness
- Turcot syndrome
- Chondrodysplasias
- Hyperphenylalaninemia
- Blistering skin
- Charot-Marie-Tooth neuropathy
- Hemochromatosis
- Miller syndrome
- Mediterranean fever
- Paraganglioma
- Ataxia telangiectasia
- Glycogen storage disease type II
- Fructose-1,6-bisphosphatase deficiency

What Are Researchers Doing with Droplet Digital PCR

Mainstream Applications

- Detection and quantification
- Rare mutation detection
- Copy number quantification
- Gene expression
- Next-generation sequencing (NGS) library quantification

Additional Droplet Digital PCR Applications

- Allele-specific gene expression
- microRNA research
- Methylation studies
- Haplotyping
- TRAP assays
- Genome editing

Additional Applications

Testing Applications

- Water treatment testing
- Waterborne viruses and pathogen testing
- Asian carp population studies
- Cow mastitis testing
- Malaria mosquito sexing
- Canine mammary carcinoma
- Cell-free fetal DNA testing

Multiplexing using ddPCR

Multiplexing with ddPCR : Why?

Limited material (clinical samples, degraded material, single cells)

Need for simultaneous analysis of numerous targets (haplotyping, linkage analysis)

Need for multiple references (CNV)

Economy of time and reagents

Approaches to Multiplexing

Using Probes

- Assay concentration
- Probe combination

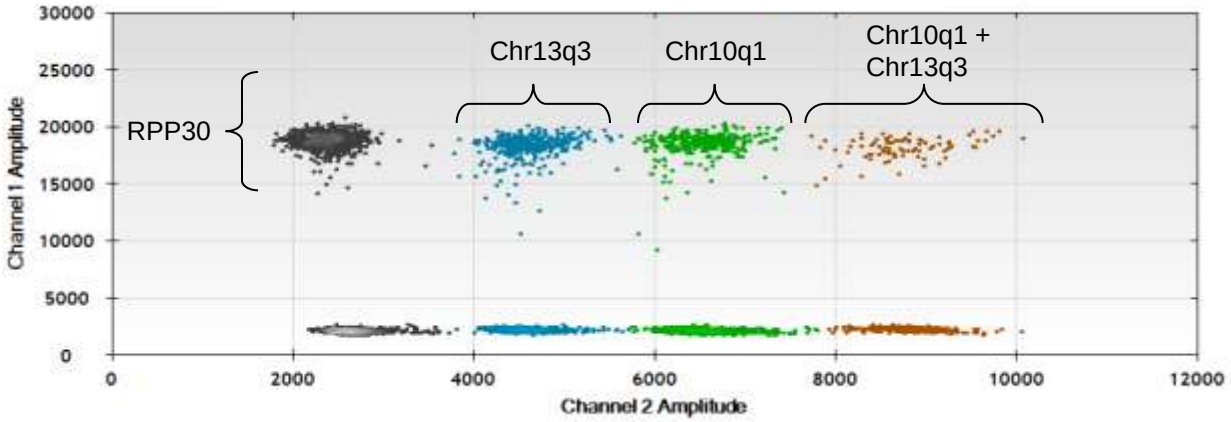
Using EvaGreen

- Amplicon length
- Annealing temperature
- Primer concentration

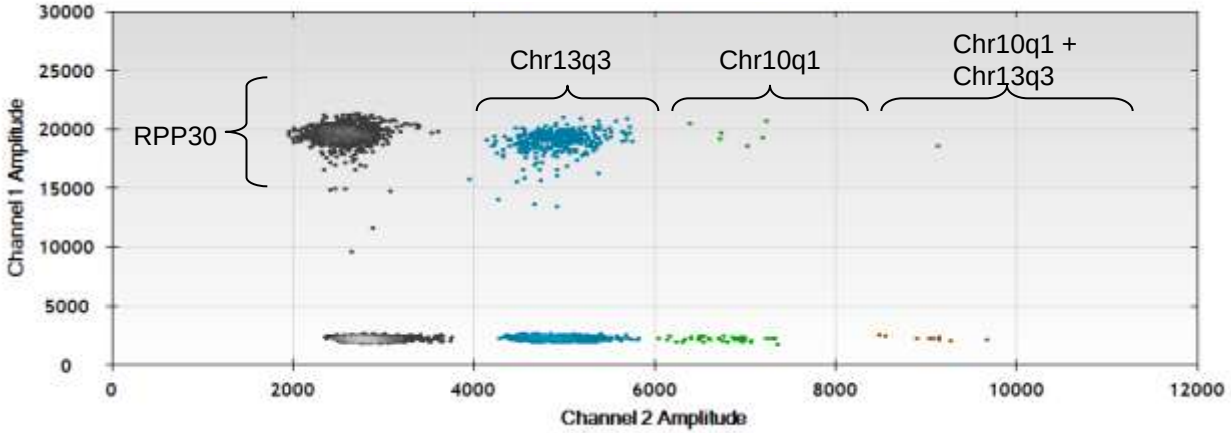
Multiplexing using probe-based assays

Multiplexing with Probes: Assay Concentration
1 X RPP30 (FAM) + 1 X Chr10q1 (VIC) + 0.6 X Chr 13q3 (VIC)

Detection of three targets (8 different clusters)

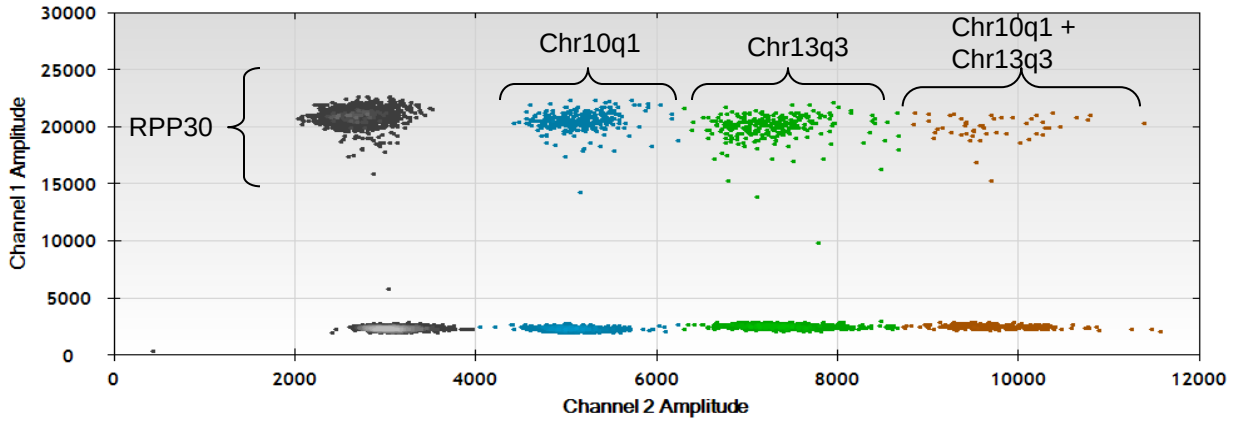


Pre-digestion of one target DNA (Chr10q1). Only 4 clusters are detected.

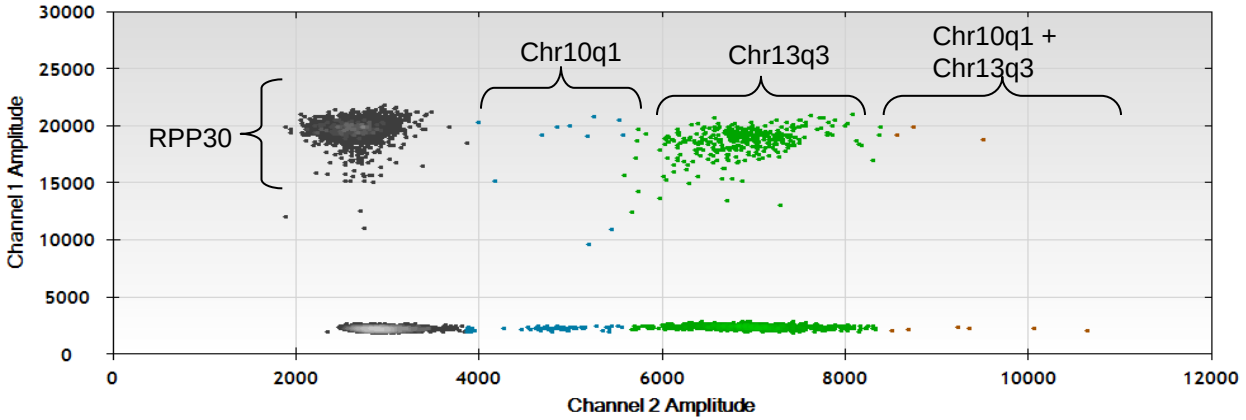


Multiplexing with Probes: Assay Concentration
1 X RPP30 (FAM) + 1 X Chr13q3 (VIC) + 0.6 X Chr 10q1 (VIC)

Detection of three targets (8 different clusters)

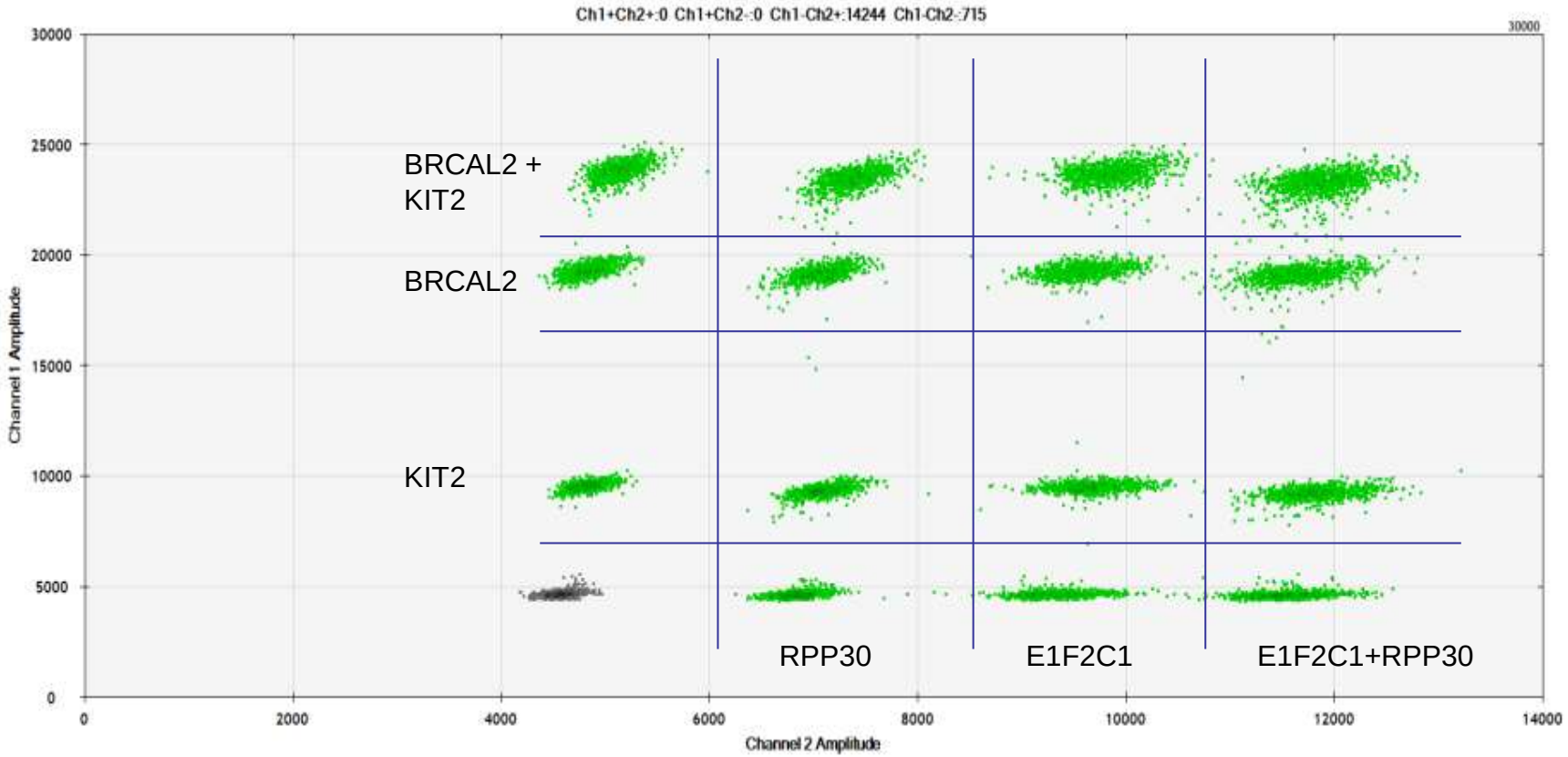


Pre-digestion of one target DNA (Chr10q1). Only 4 clusters are detected.



Multiplexing with Probes: Assay Concentration

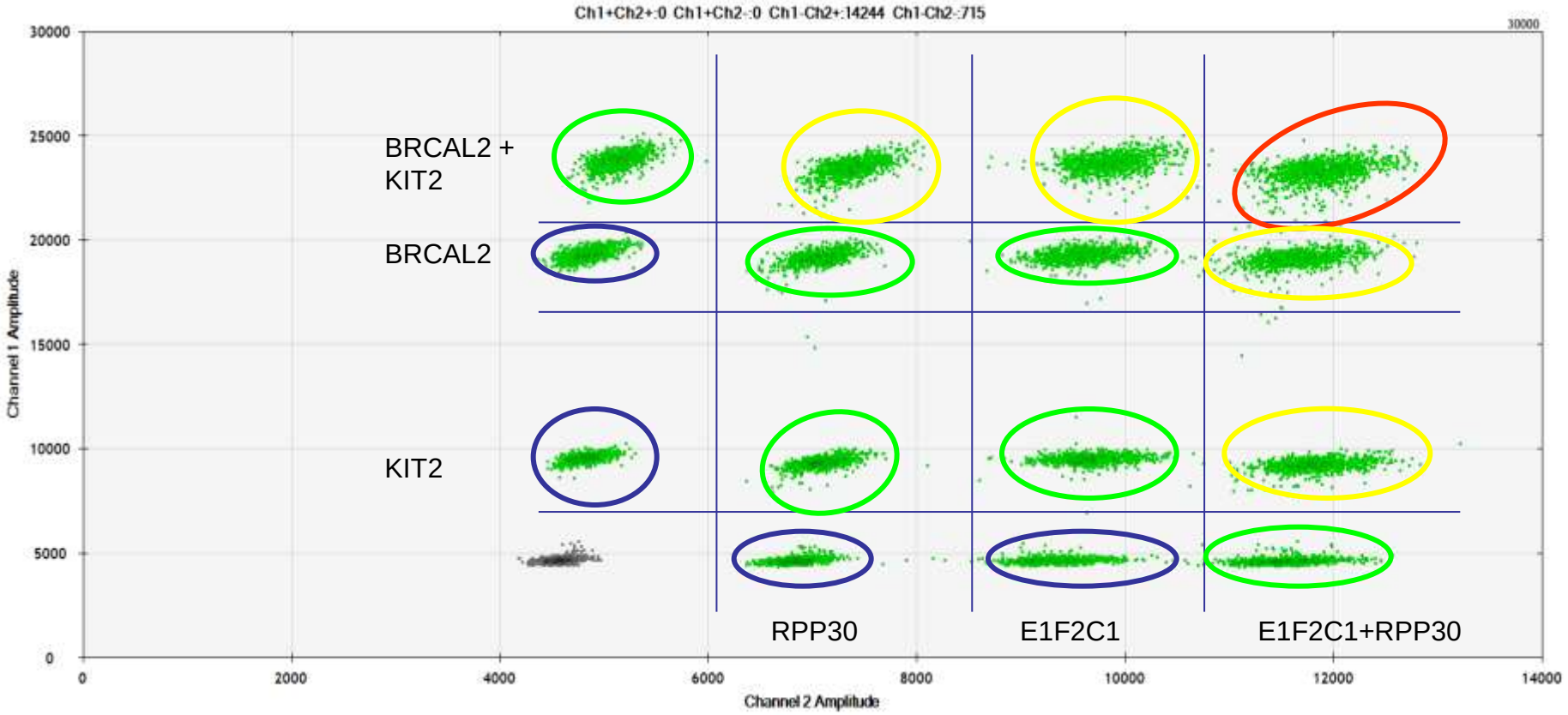
1.4X BRCAL2 (FAM)+ 0.6 X KIT2 (FAM) + 1.4 X E1F2C1 (HEX) + 0.6 X RPP30 (HEX)



Simultaneous detection of 4 different targets by ddPCR. This illustrates the capacity of the QX200 system to separate discrete populations by levels of fluorescence using the same fluorophores

Multiplexing with Probes: Assay Concentration

1.4X BRCAL2 (FAM)+ 0.6 X KIT2 (FAM) + 1.4 X E1F2C1 (HEX) + 0.6 X RPP30 (HEX)



Single positives

Double positives

Triple positives

Quadruple positives

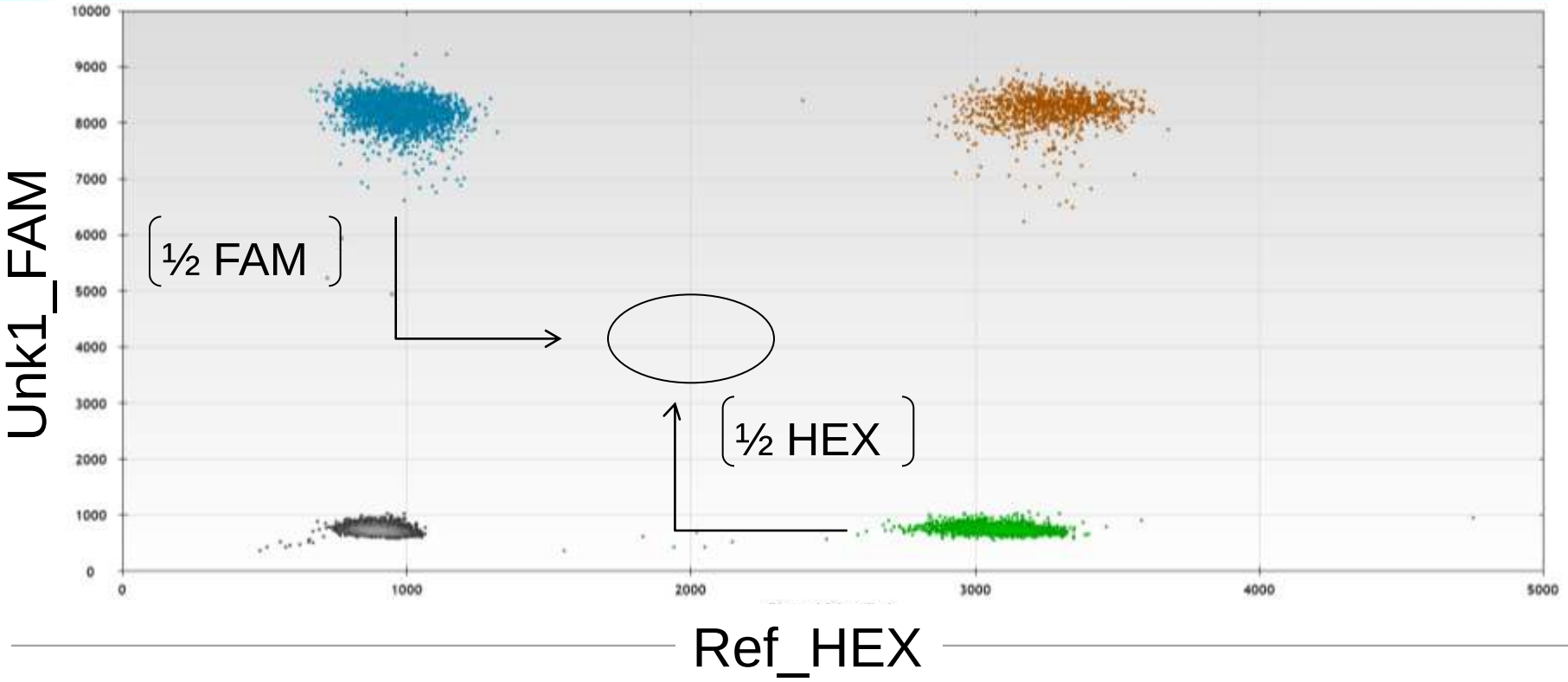
Use of Multiplex ddPCR in the Literature

Coinfection of Human Herpesviruses 6A (HHV-6A) and HHV-6B as Demonstrated by Novel Digital Droplet PCR Assay

Emily C. Leibovitch[1,2], Giovanna S. Brunetto[1], Breanna Caruso[1], Kaylan Fenton[1], Joan Ohayon[1], Daniel S. Reich[1], Steven Jacobson[1]*

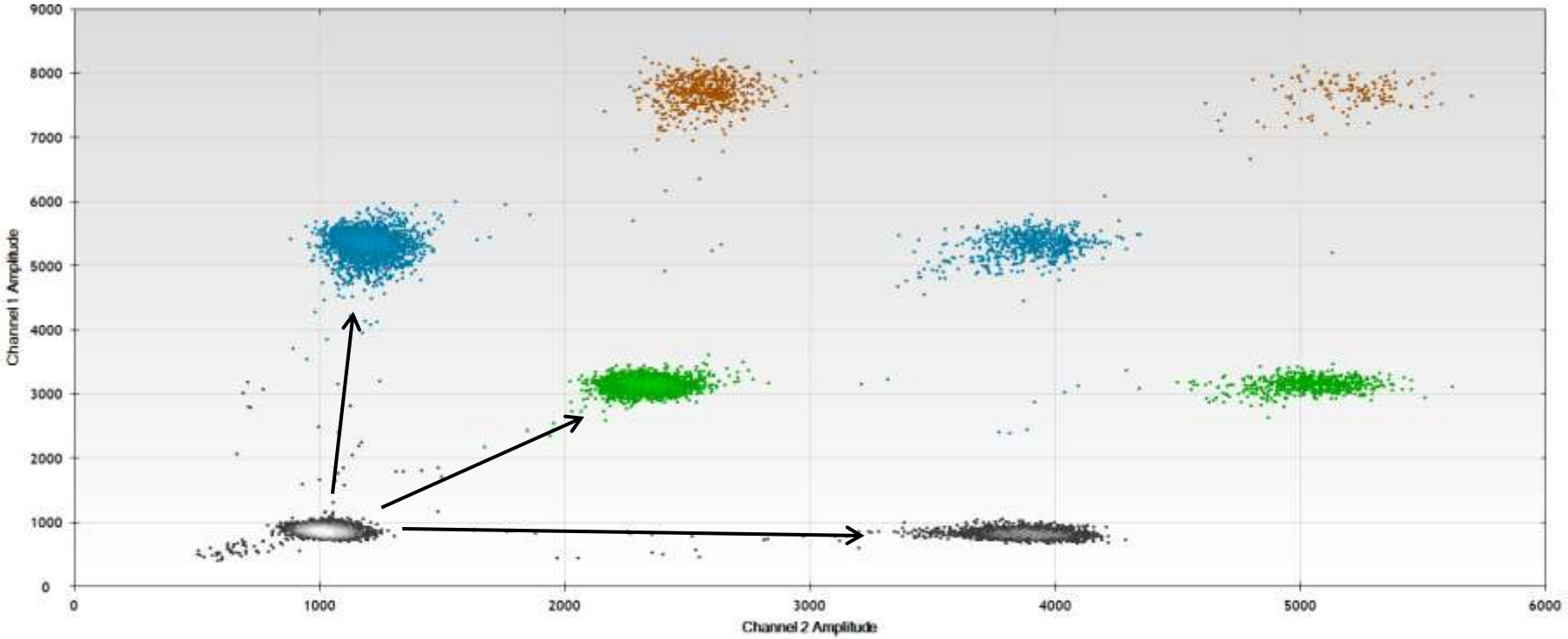
1 Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, United States of America, **2** Institute for Biomedical Sciences, School of Medicine and Health Sciences of The George Washington University, Washington, DC, United States of America

Multiplexing with Probes: Probe Combination



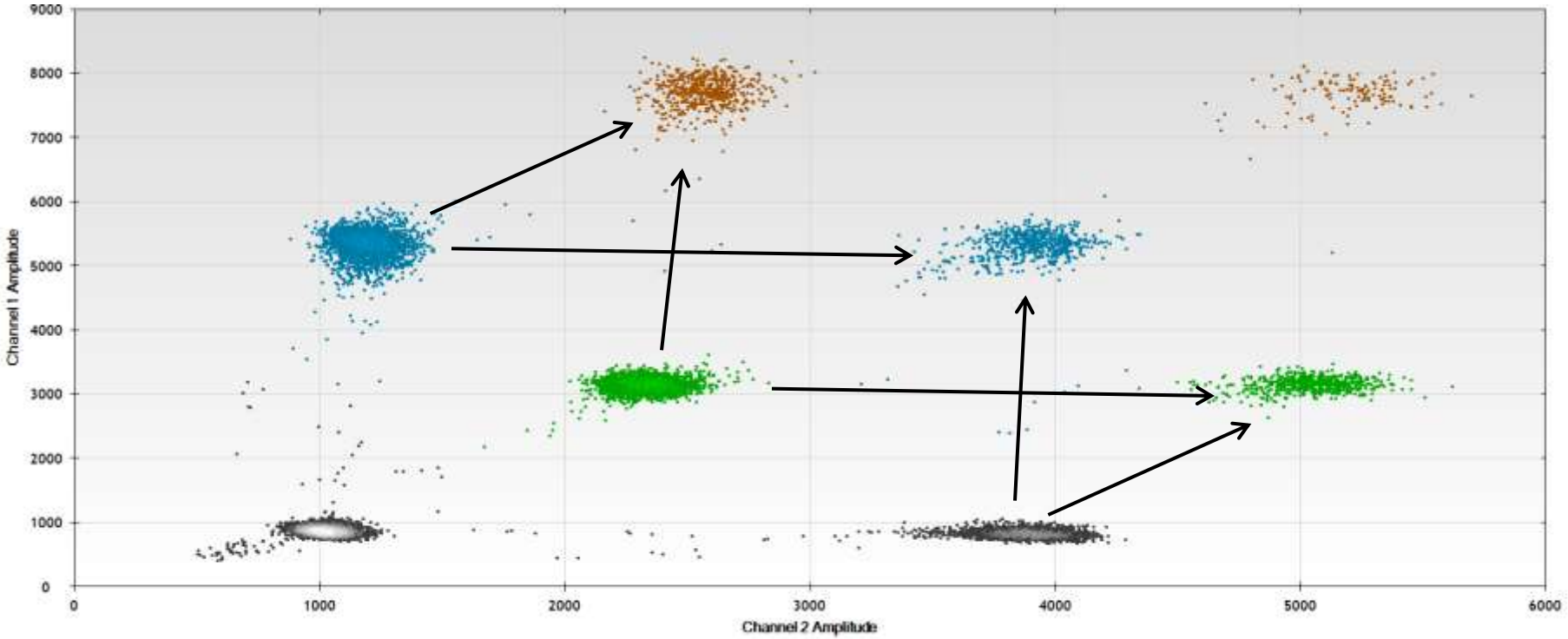
Using the same probe sequence but labeling it with 2 different dyes (FAM and HEX), it should be possible to occupy a different position in the 2D plot

Multiplexing with Probes: Probe Combination



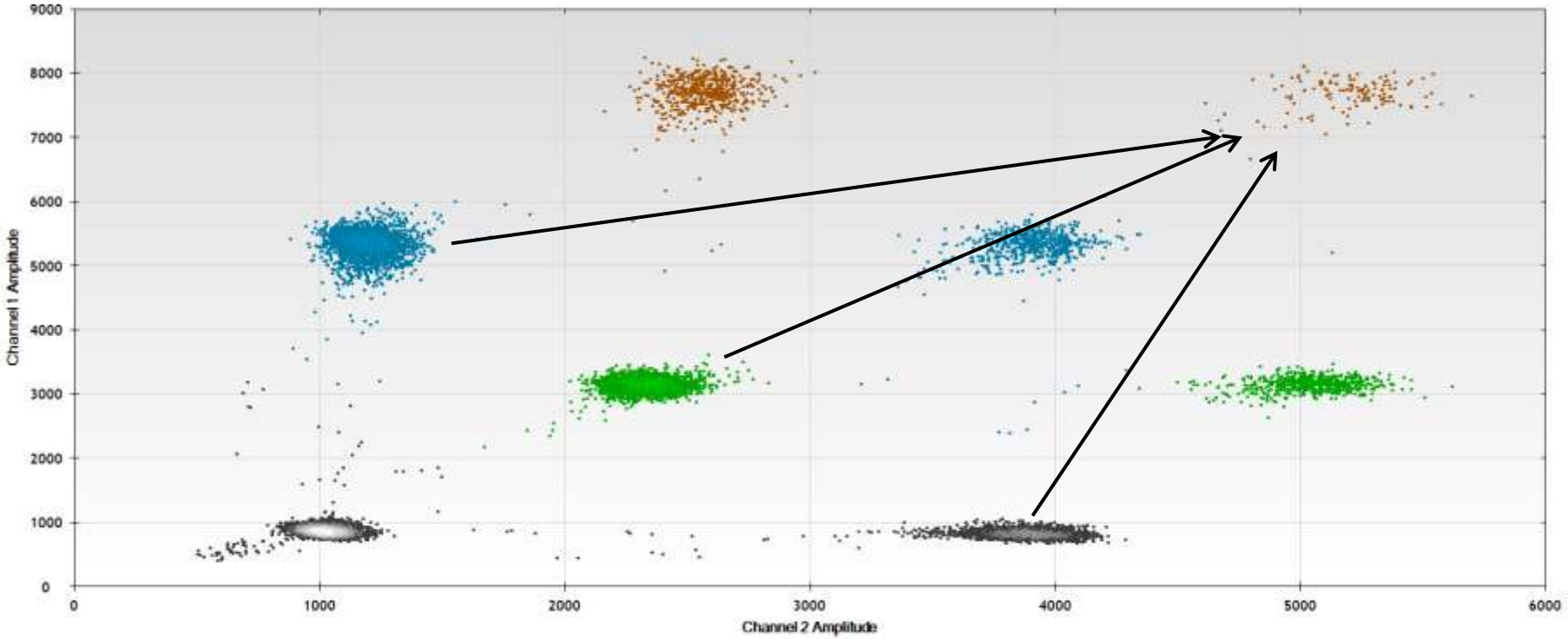
Populations of single positives

Multiplexing with Probes: Probe Combination



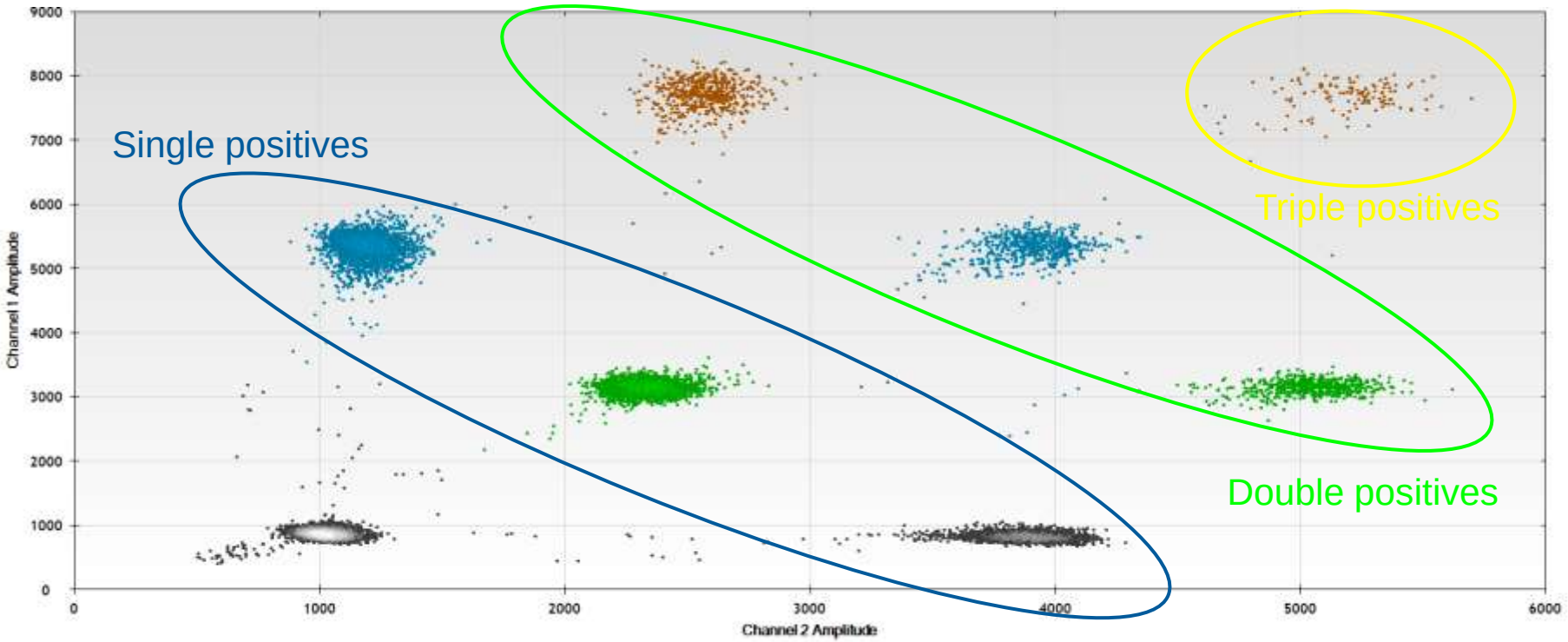
Populations of double positives

Multiplexing with Probes: Probe Combination



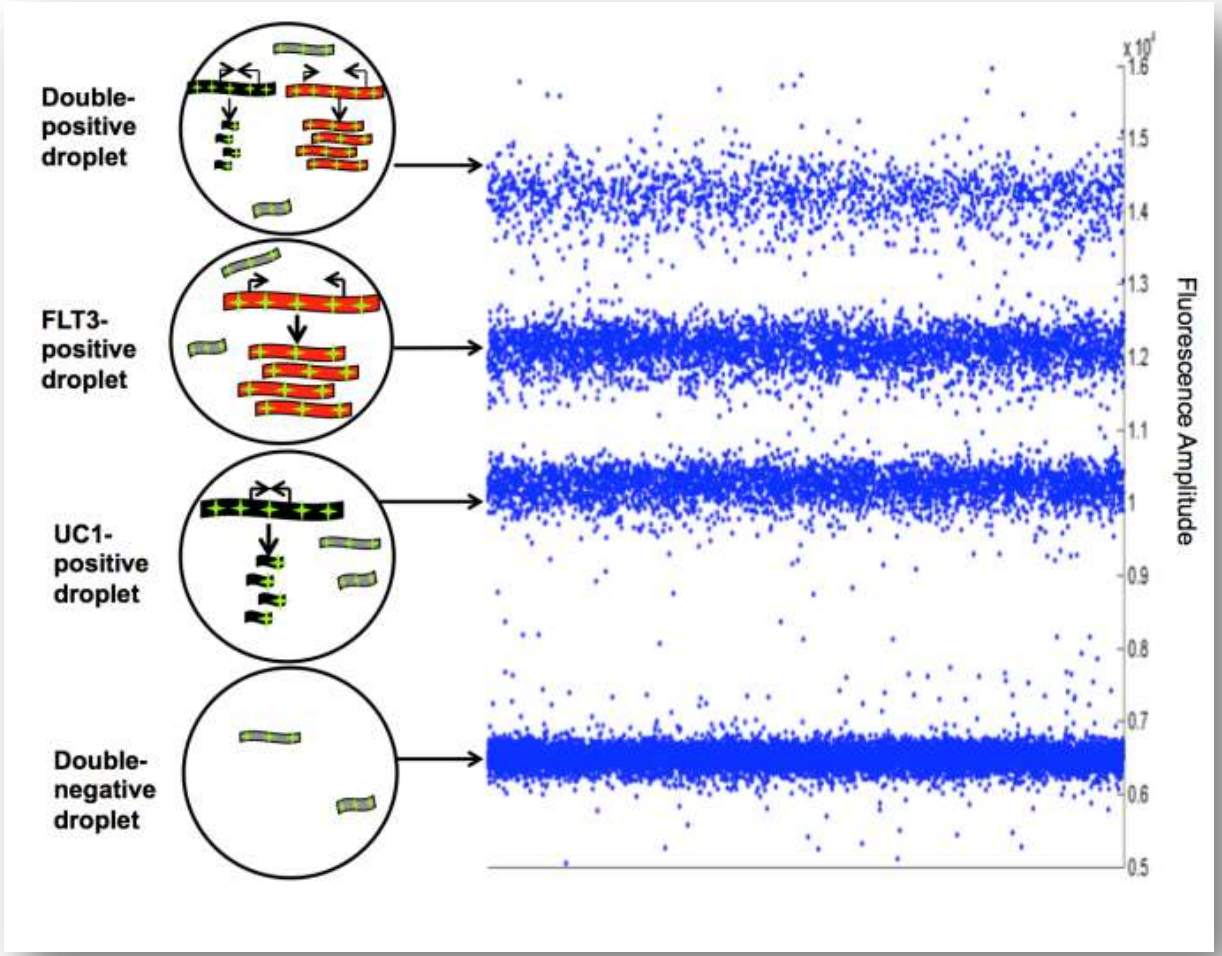
Populations of triple positives

Multiplexing with Probes: Probe Combination



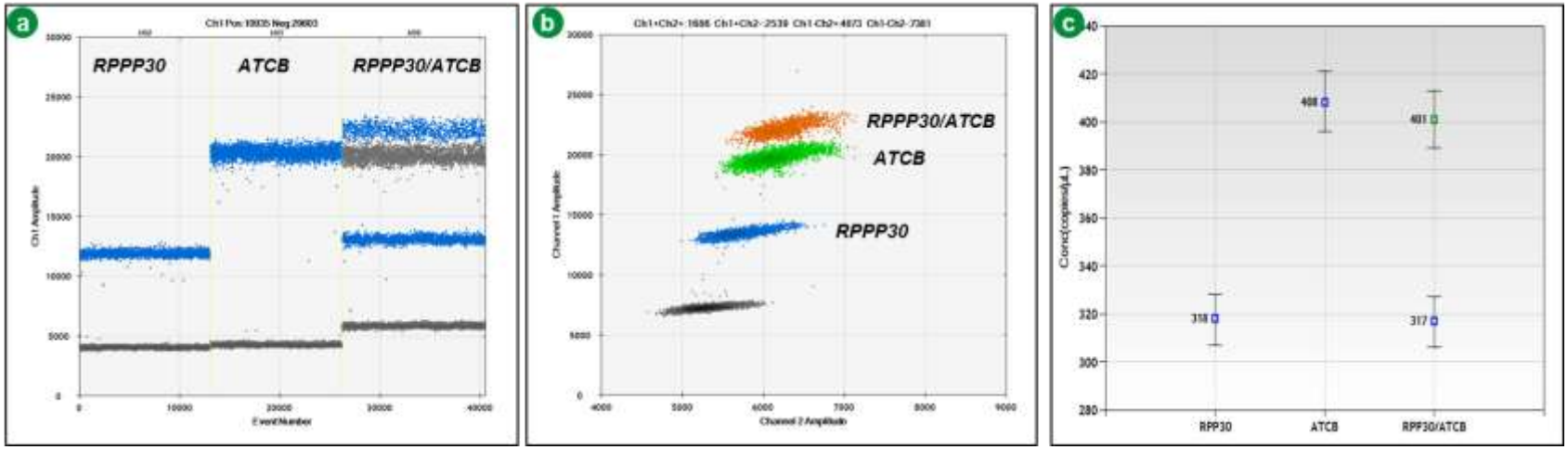
Multiplexing using EvaGreen

EvaGreen Multiplex



Multiplexing with EvaGreen: Amplicon Length

- **Amplicon length:**
  - *RPP30*
    - 62 base-pairs
  - *BetaActin*
    - 137 base-pairs



Conclusion

Best Reasons to use ddPCR

Sensitivity

- Detect rare mutations in complex backgrounds
- Detect rare mutations earlier
- 10-1000x fold improvement over qPCR
- Works with FFPE samples
- Works with blood, tissues, environmental samples…

Absolute Quantification

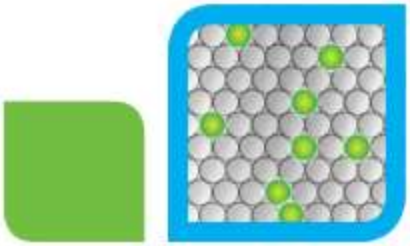
- Answers in absolute numbers of molecules (not Cq)
- Quantify lower levels of targets
- No standard curve

Precision

- Measure more subtle differences in expression or mutation
- Detect structural variants in cancers
- Higher tolerance to PCR inhibitors

Learn More At Our Apps and Techs Pages

Droplet Digital™ PCR Publications [Close]



Droplet Digital™ PCR: Publications List

Droplet Digital PCR Bulletin 6450

- Biomarker Detection
- Cancer
- Copy Number Variation
- Droplet Digital PCR Methods/ Analytical Performance
- Environmental Studies
- Epigenetics
- Food Testing/Food Science
- Gene Expression
- Genome Editing
- Liquid Biopsy
- miRNA Quantification and Expression
- Mutation Detection
- Next-Generation Sequencing Library Quantification/Validation
- Pathogen Detection (microbial)
- Plants
- Rare Event Detection
- Single Cell Detection
- Stem Cells
- Virology

ddPCR Publications

BIO-RAD

2200

Over 2200 published studies have described research breakthroughs using Droplet Digital™ PCR technology.

600

Over 600 published studies have described breakthroughs in the liquid biopsy field using Droplet Digital™ PCR technology.

Droplet Digital PCR is leading the way to discovery

Digital Biology Center

Anything you may need for PCR







Questions?