QIAcuity Digital PCR Applications

A Selection of Breast Cancer-Related Data

October, 2020

Dear researcher,

The COVID-19 pandemic has become a central focus lately, but many other diseases still need our attention. As October is Breast Cancer Awareness Month, we would like to thank you for all your efforts in advancing breast cancer research.

Learn how QIAGEN can further support your research in this area. To this end, we have collected our recent breast cancer-related webinars, white papers, videos and more in one central location.

This presentation discusses two highly sensitive applications using our latest digital PCR technology and presents breast cancer research-relevant data. These are also available in more detail as webinars on-demand at www.qiagen.com.

We hope you find these valuable.

Your QIAGEN Team

Legal disclaimer

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Agenda

Introduction

dPCR LNA Mutation Analysis in Breast Cancer Research

+ Application Data

dPCR Copy Number Variation Analysis in Breast Cancer Research

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Breast cancer – a global burden

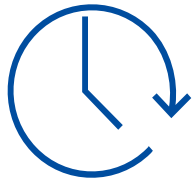
A disease in which malignant cancer cells form in the tissues of the breast, typically resulting in a lump or a mass

Most common cancer among women worldwide

1 in **8** women will be diagnosed with breast cancer in their lifetime

In 2020, an estimated **276,480** new cases of invasive breast cancer will be diagnosed in women in the U.S. alone

64% of breast cancer cases are diagnosed at a localized stage, for which the 5-year survival rate is 99%



On average, every **2 minutes** a woman is diagnosed with breast cancer in the U.S. and every **13 minutes** a woman dies of breast cancer

(1) https://www.nationalbreastcancer.org/breast-cancer-facts

(2) https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/breast-cancer-facts-and-figures/breast-cancer-facts-and-figures-2019-2020.pdf

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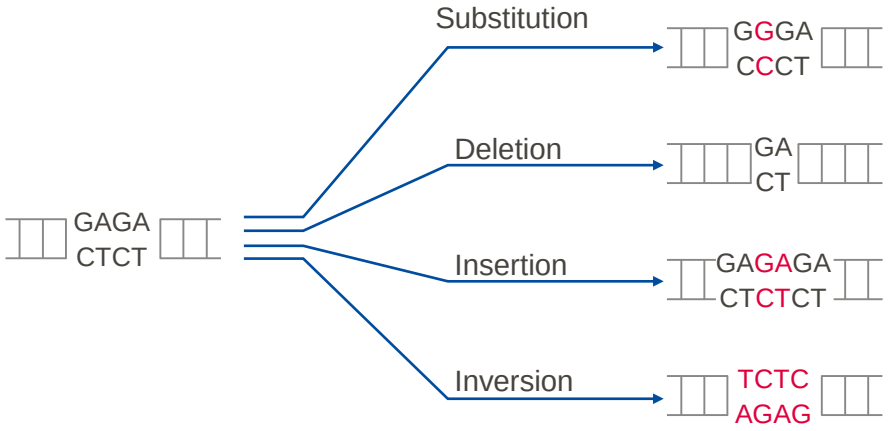
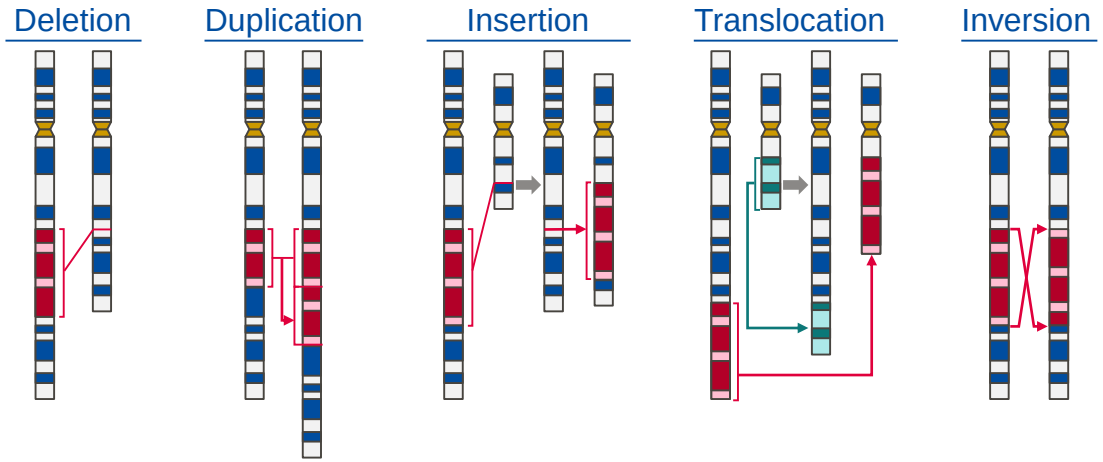
Mutation facts

A collective term for permanent changes in human genome sequence

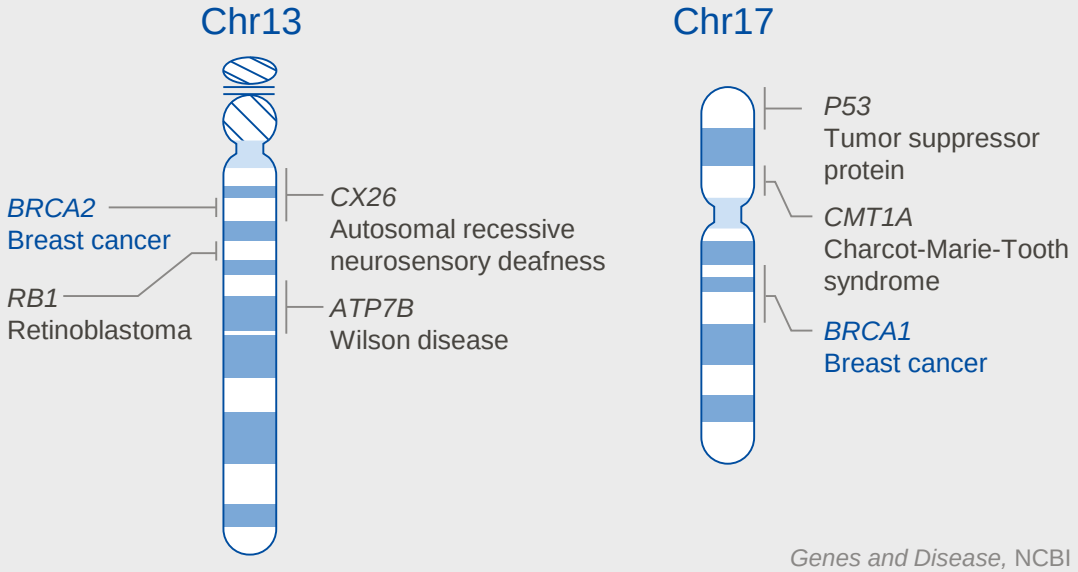
- Can result from DNA replication mistakes, exposure to chemicals, radiation, or infection by viruses
- Deletion, substitution, inversion, translocation, duplication, insertion of genomic regions
- Ranging from single bp to a single gene up to a chromosome segment
- Can be categorized as:
  - Hereditary or *de novo*
  - Autosomal or X/Y-linked
  - Frequent or rare
- Accounts for polymorphisms: hair and eye color, blood type, fingerprint, etc.
- Linked to common and complex diseases and traits





Some common disease-causing mutations in humans

Phenotype	Chromosome	Mutation type	Prevalence
CMT disease	17/1/X	Complex	1:2500
Color blindness	X	Point	1:12 (male)
Cri du chat syndrome	5	Deletion	1:50.000
Cystic fibrosis	7q	Point	1:100.000
Down syndrome	21	Whole chromosome	1:800
Hemophilia	X	Point	25:100.000
Klinefelter syndrome	X	Whole chromosome	1:500 (male)
Polycystic kidney disease	16/4	Point	8:100
Sickle cell disease	11p	Point	112:100.000
Spinal muscular atrophy	5q	Complex	1:10.000
Alzheimer's disease	21/14/1	Complex	1:177
Huntington's disease	4	CAG repeats	1:10.000
Fragile X syndrome	X	CGG repeats	1:4000
Breast cancer	13/17	Complex	1:10000



Genes and Disease, NCBI

Class of phenotype	Phenotype	Gene
Single gene disorders and traits	5618	3908
Susceptibility to complex disease or infection	695	501
"Nondiseases"	150	118

More than 10,000 human diseases are caused by single gene mutations.

Dissected OMIM Morbid Map Scorecard (Updated June 26th, 2020)

Cancer-causing gene mutations

Activation of oncogenes:

Oncogene	Cancer
EGFR	Lung, glioma, colorectal, ovarian, breast
ERBB2	Breast, gastric, ovarian, bladder
BRAF	Melanoma, thyroid, colorectal, ovarian
KRAS	Pancreatic, lung, colorectal, endometrial, ovarian
MYC	Lymphomas, colorectal, breast, prostate, melanoma, ovarian, neuroblastoma
JAK2	Chronic myeloid leukemia, acute lymphocytic leukemia
MET	Kidney, gastric, lung, colorectal

Loss of functions in tumor suppressors:

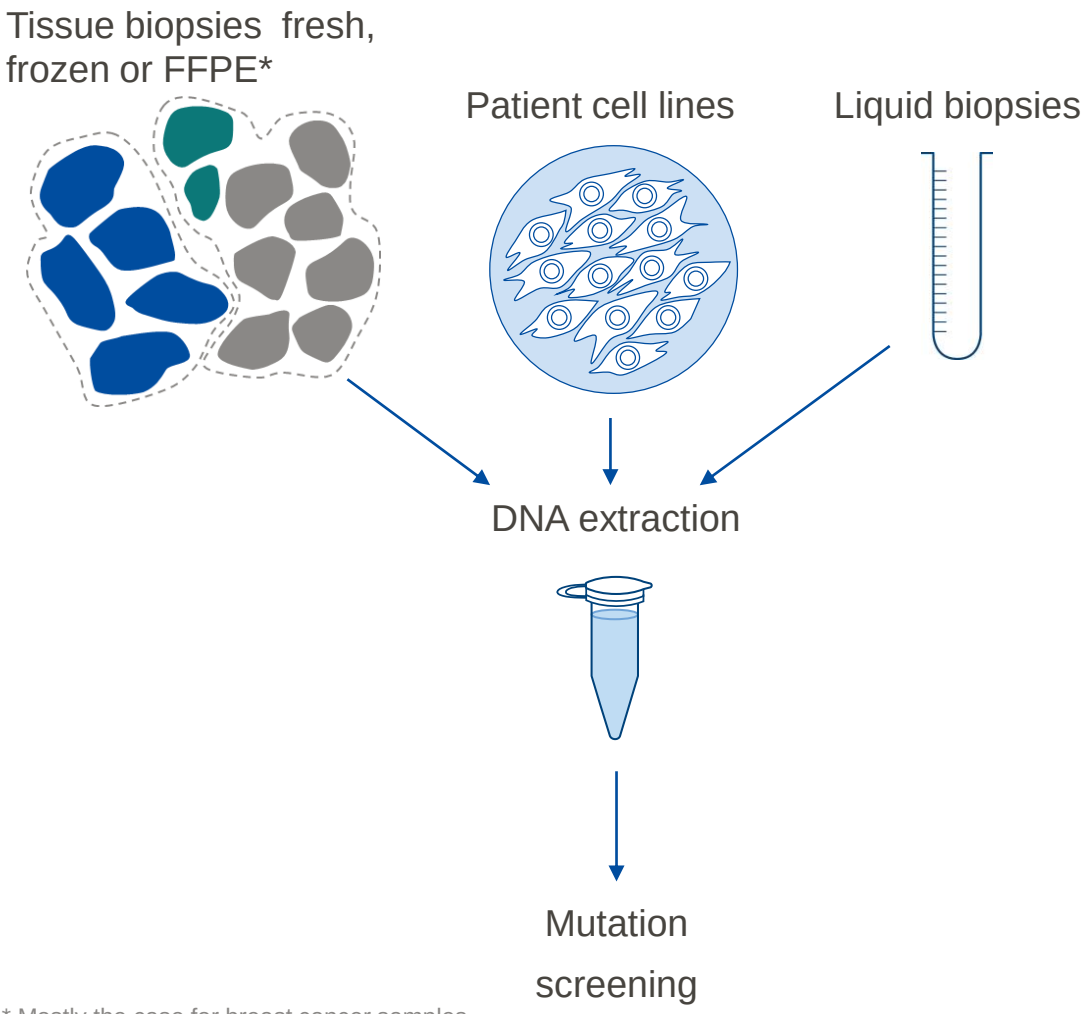
Tumor suppressor	Cancer
P53	Lung, colorectal, bladder, ovarian, breast, prostate, gastric
PTEN	Glioblastoma, melanoma, prostate, breast, thyroid, lung, colorectal
BRCA1/2	Ovarian, breast
VHL	Kidney, adrenal
Rb	Retinoblastoma, lung, bladder, esophageal, glioma, liver, prostate, breast
FBXW7	Acute lymphocytic leukemia, colorectal, gastric, lung, pancreatic, prostate, ovarian

Challenges:

- Identifying residual cancer below current detection levels
- Detecting new mutations in cancer
- Monitoring rare drug-resistance mutations

*Breast cancer genes marked in blue

Limitations of detection

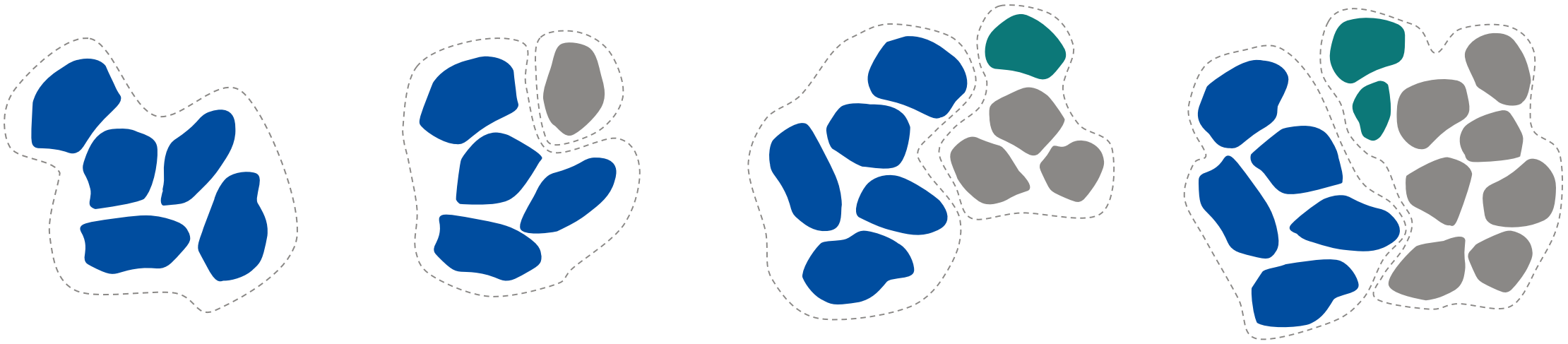


Challenges:

- Highly variable sample quality
- Limited sample amounts
- Suboptimal DNA extraction of samples

* Mostly the case for breast cancer samples

Sample heterogeneity



Multiple tumors
Different types of cells
Multiple mutation events

Frequency of an individual mutation

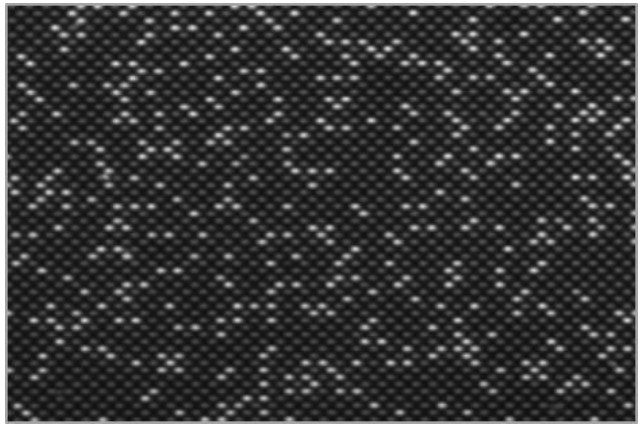
Digital PCR is an advancement in precision and sensitivity

Technique	Sensitivity	Optimal application
Sanger sequencing	>10%	Tumor tissue
Pyrosequencing	10%	Tumor tissue
Next-generation sequencing	2%	Tumor tissue
Quantitative PCR	1%	Tumor tissue
ARMS	0.10%	Tumor tissue
Digital PCR	0.1% or lower	ctDNA, rare variants in tumor tissue

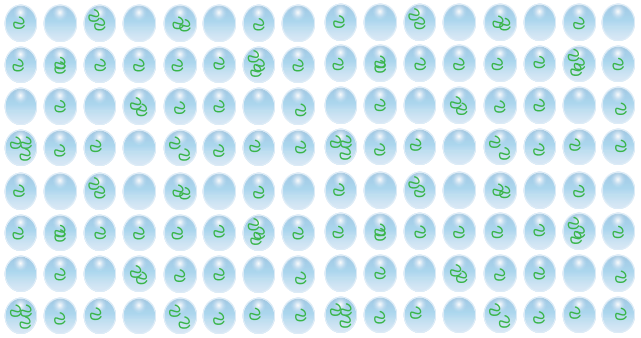
- Absolute quantification of mutated vs. wild type copies
- Digital PCR shows an outstanding precision and sensitivity
- Limit of detection based on DNA input
- Retesting of NGS results for validation of new mutations

Table adapted from: Diaz, L. A., Jr, and Bardelli, A. (2014). Liquid biopsies: genotyping circulating tumor DNA. Journal of clinical oncology, 32(6), 579–586.

Digital PCR is an advancement in precision and sensitivity



Absolute quantification: Copies/µl
calculated with number of partitions in total, number of positive partitions and statistical distribution model

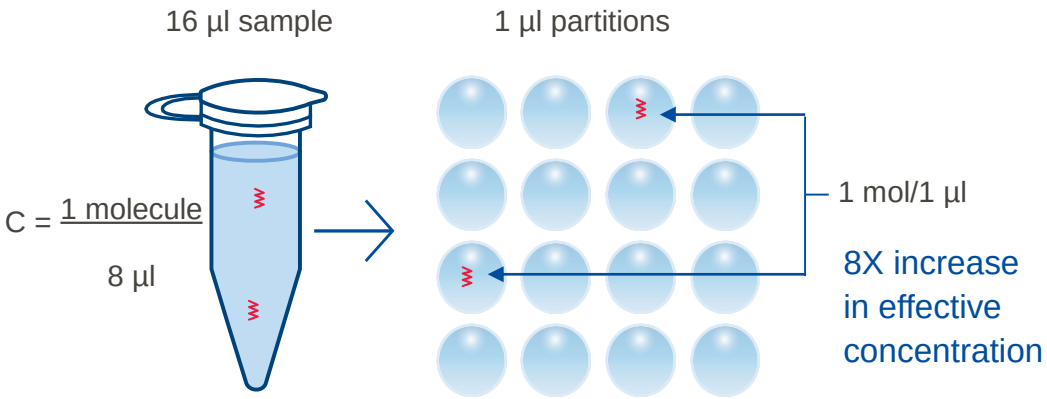


Random distribution of molecules into partitions
creates an increase in effective concentration

- Absolute quantification of mutated vs. wild-type copies
- Digital PCR shows an outstanding precision and sensitivity
- Limit of detection based on DNA input
- Retesting of NGS results for validation of new mutations

Digital PCR for increased accuracy and sensitivity

Increase of effective concentration



Decrease of interfering molecules

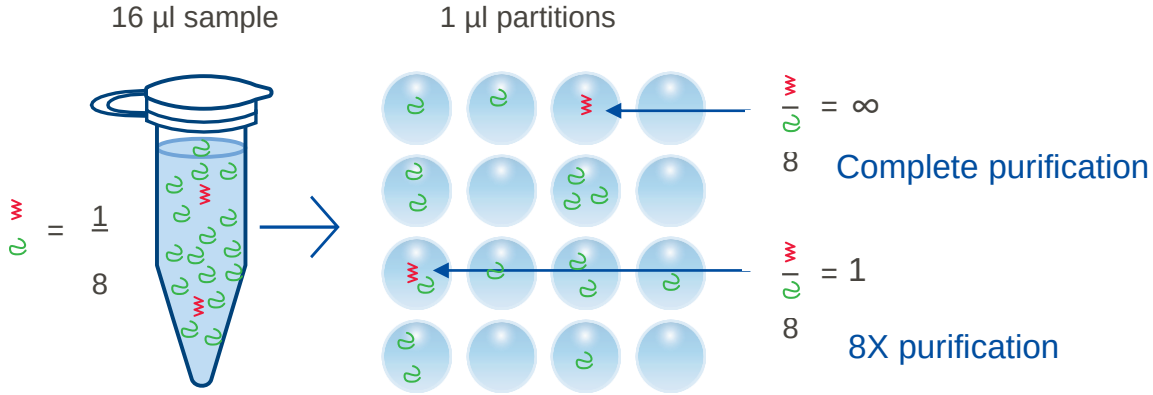
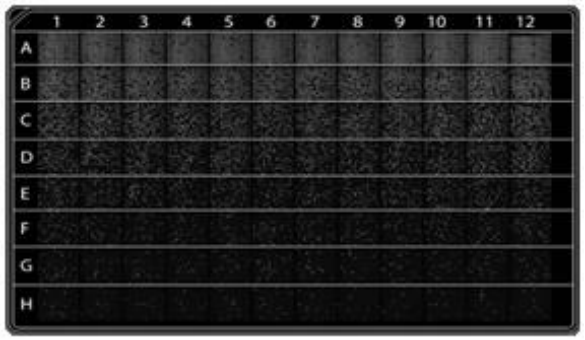


Image adapted from: Basu, A. S. (2017). Digital assays part I: partitioning statistics and digital PCR. SLAS TECHNOLOGY: Translating Life Sciences Innovation, 22(4), 369–386.

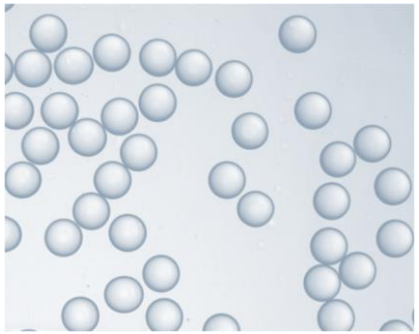
QIAGEN’s new nanoplate digital PCR

Digital PCR nanoplates

Why nanoplates and not droplet?



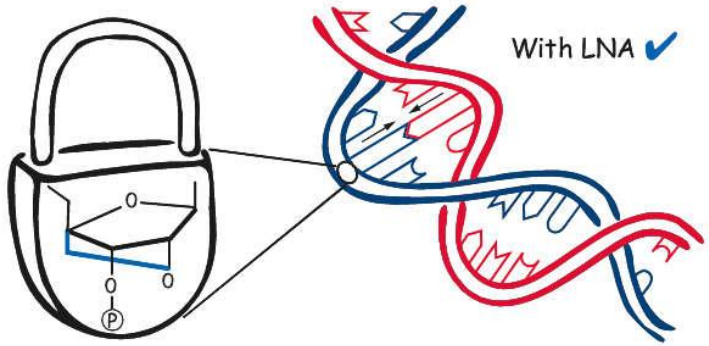
Nanoplate

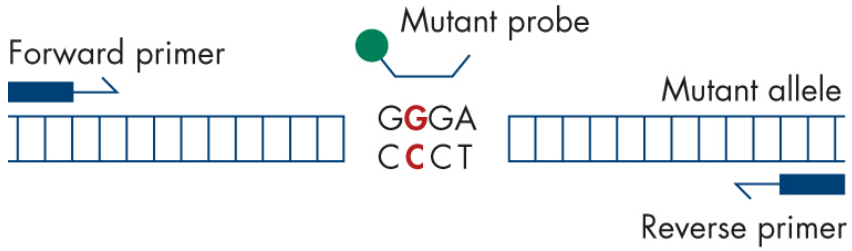


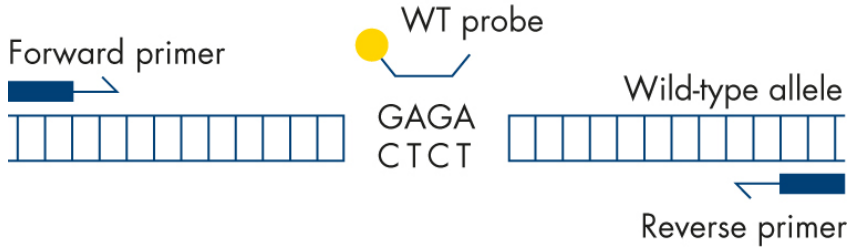
Droplet

- Fixed and sealed partitions prevent variation in size and coalescence
- Sealed nanoplates prevent well-to-well contamination
- Faster readout possible due to simultaneous reading of all partitions of a sample
- Simple workflow and user-friendly handling, just like for qPCR
- Plates are amenable to front-end automation

dPCR LNA Mutation Assays



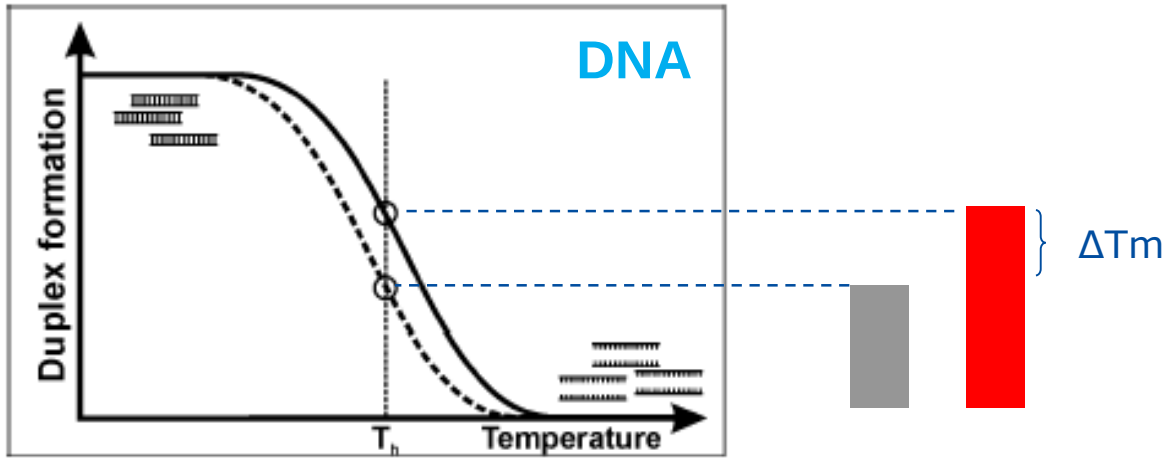


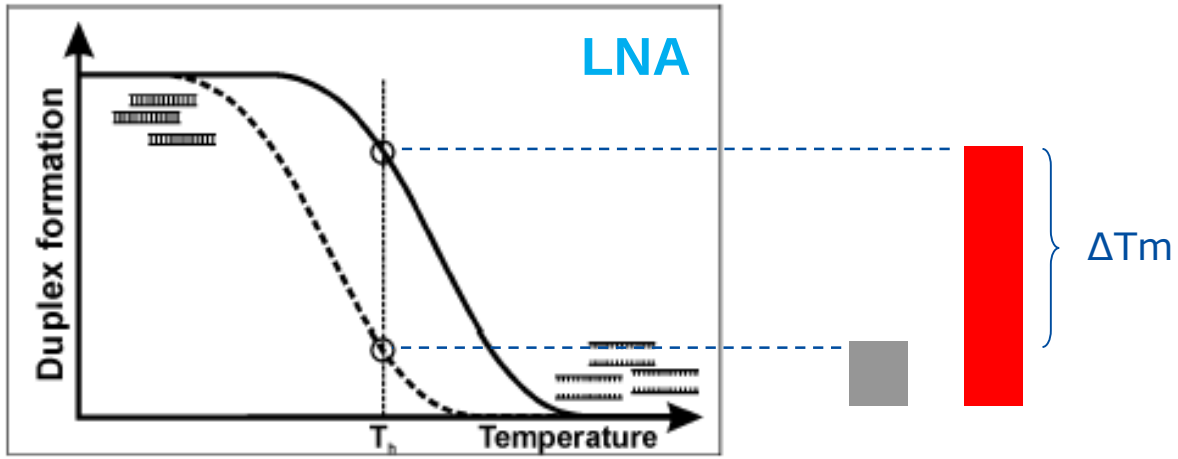


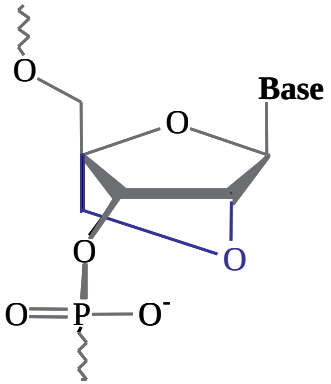
Detection of mutations in a duplex reaction with competing probes

- Single-tube format (containing one primer pair and two probes)
  - 200 or 1000 rxn (Nanoplate 26K)
- LNA-enhanced probes and primers for highest specificity
- FAM/HEX or Atto550/Rox (mutant/WT probe) available
- Wet-lab-tested dPCR with sensitivity down to 0.1%
- Used in combination with QIAcuity Probe PCR Kit

LNA™ technology







LNA

LNA 8-mer TGCTGGTG	DNA 8-mer TGCTGGTG
Perfect match Tm = 71°C	Perfect match Tm = 35°C
Single mismatch Tm = 45°C	Single mismatch Tm = 25°C
ΔTm = 26°C	ΔTm = 10°C

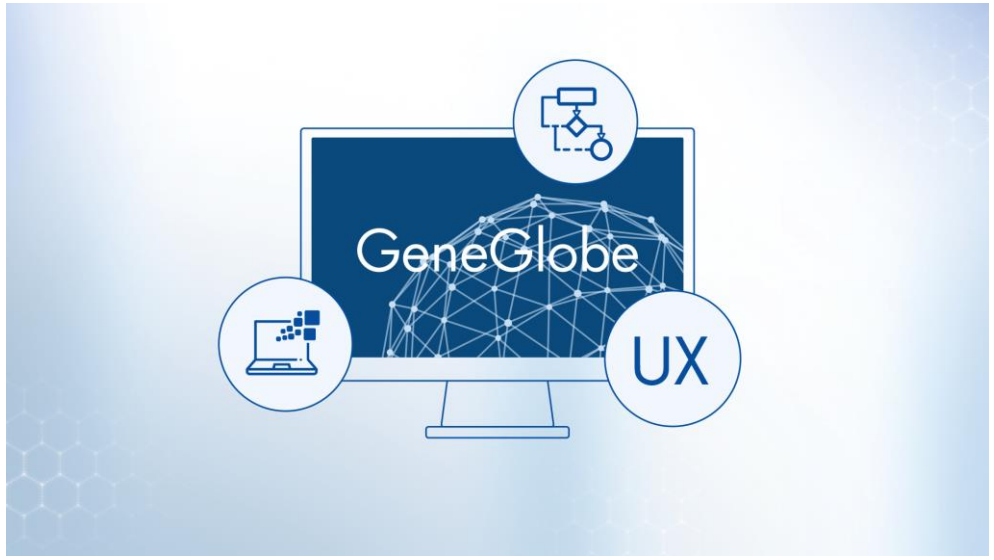
dPCR LNA Mutation Assays

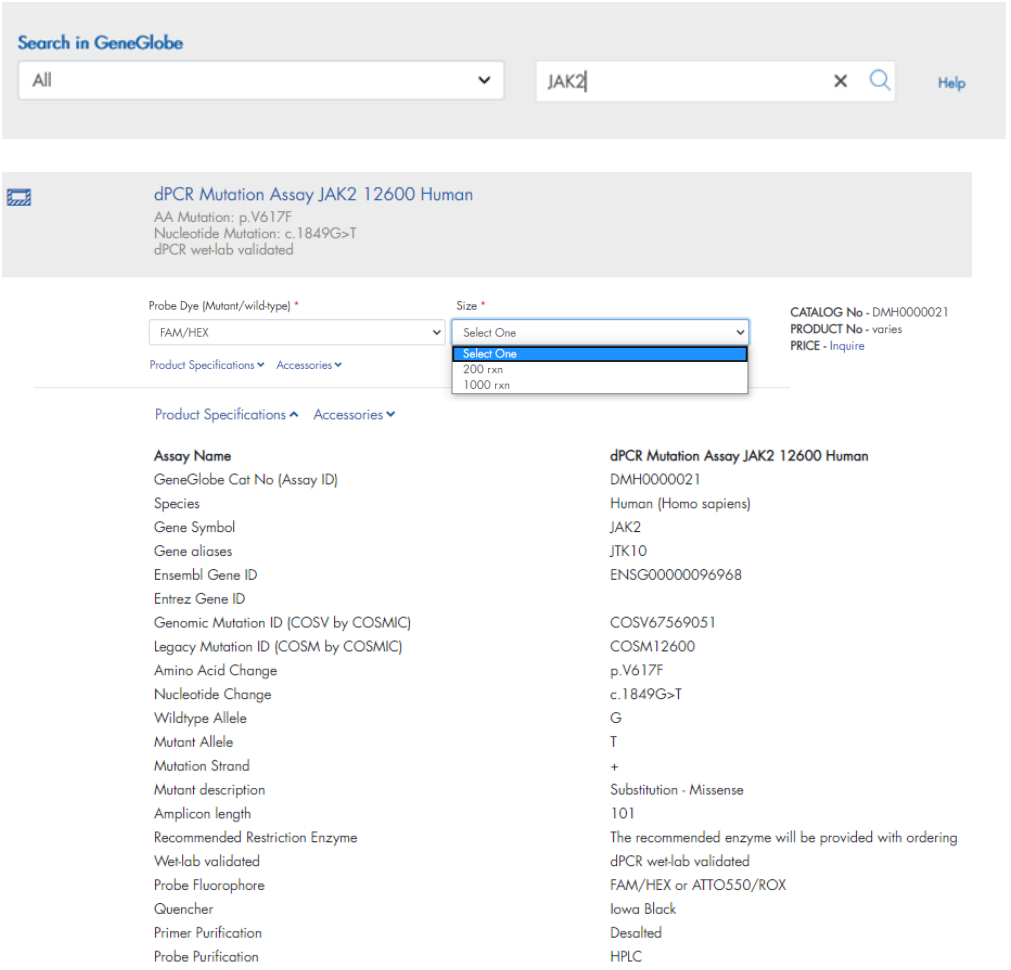
https://geneglobe.qiagen.com/product-groups/dpcr-lna-mutation-assays

Assays available in GeneGlobe

250 wet-lab-validated assays for most studied cancer mutations

- Growing over time





Use on the QIAcuity dPCR instrument – separate products needed

The Master Mix

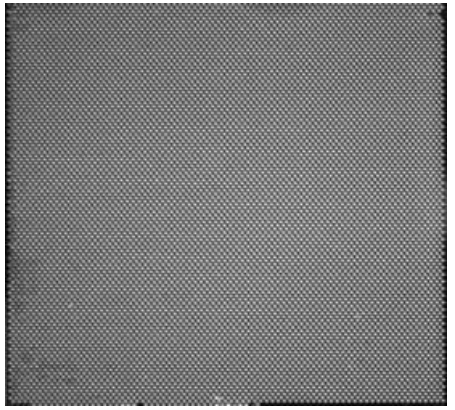
QIAcuity Probe PCR Kit

- For single and multiplex use (up to 5-plex)
- 4x Master Mix
- Optimized for best performance in nanoplate microfluidic
- Includes special reference dye needed for dPCR analysis and counting analyzable partitions

The reference dye – for counting valid partitions

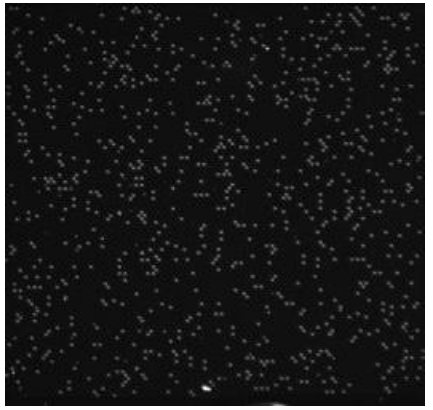
Reference channel

- Counts the number of filled (valid) partitions
- Determines the analyzable volume (µl)



Green (FAM) channel

- Counts the number of positive partitions
- Calculating λ and copies per µl using Poisson statistics



Calculates copies/µl

Use on the QIAcuity dPCR instrument – separate products needed

QIAcuity Nanoplate 26K 24-well

- 26,000 partitions per well
- 24-wells with 40 µl input reaction volume
- Using QIAcuity Probe PCR Kit (4x master mix)
  – you can load up to 28 µl sample
- Nanoplate for best sensitivity and lowest mutation frequencies

QIAcuity Nanoplate 8.5K 24/96-well

- 8,500 partitions per well
- 24- or 96-wells with 12 µl input reaction volume
- Using QIAcuity Probe PCR Kit (4x master mix)
  – you can load up to 7 µl sample
- Nanoplate for great sensitivity and expected mutation frequencies ≥1–5% (assay-dependent)

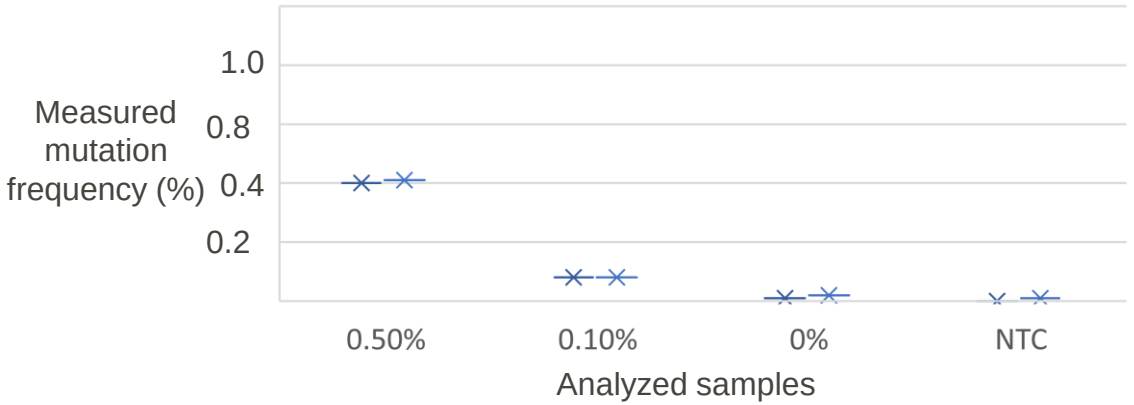
Wet-lab examples on the QIAcuity

EGFR L858R assay

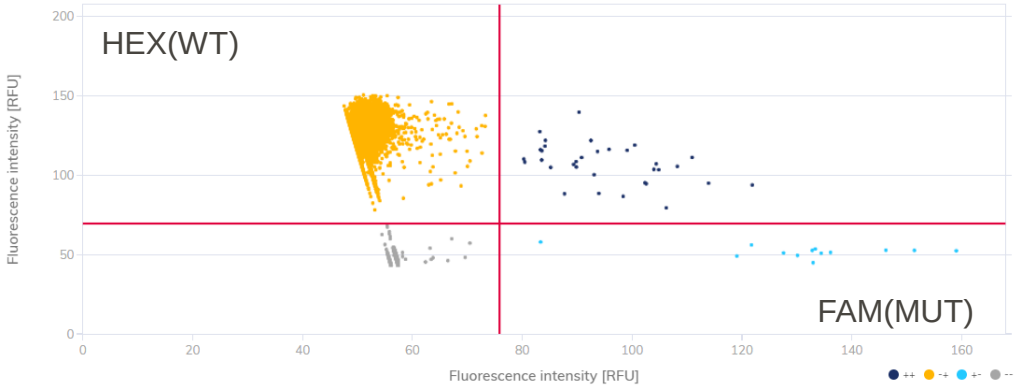
- A point mutation in exon 21, a known ‘hotspot’
- Present in all types of breast cancer
- A predictive marker for lung carcinoma
- The most common mutation detected in patients (40–45% of cases)

- FAM/HEX (mutant/WT probe)
- Sample: Gene blocks
- Nanoplate 26K 24-well

Point diagram showing 0.5%, 0.1%, 0% and NTCs mutation frequency in a wild-type background (48,000 copies)



2D Scatter Plot from a single well showing 0.1% mutation frequency in a wild-type background (48,000 copies)



Sensitivity of LNA Mutation Assays – detection of low-frequency mutations

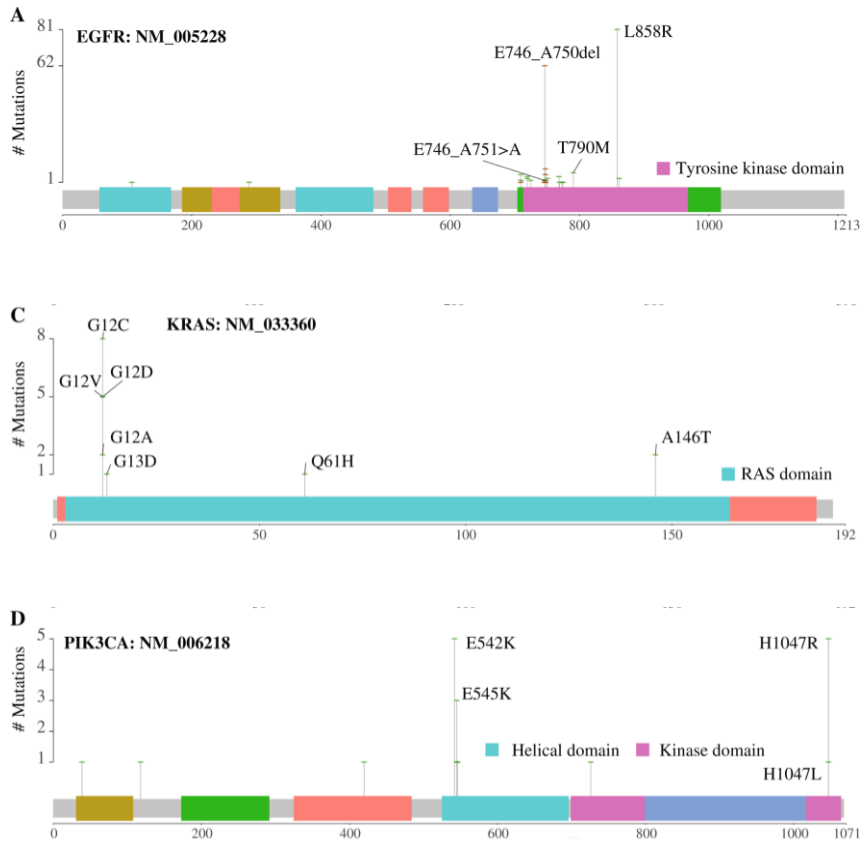
Mutation frequency

	Expected	100.0%	1.00%	0.50%	0.10%	0.00%	NTC
Mutation hotspots	EGFR T790M	99.97%	0.94%	0.49%	0.09%	0.01%	0.00%
	EGFR L858R	99.99%	0.76%	0.40%	0.08%	0.01%	0.00%
	KRAS G13C	99.99%	0.91%	0.47%	0.09%	0.03%	0.00%
	KRAS G12R	100.0%	1.15%	0.52%	0.11%	0.00%	0.00%
	BRAF V600E	99.98%	0.80%	0.40%	0.10%	0.01%	0.01%
	JAK2 V617F	100.0%	1.08%	0.51%	0.11%	0.02%	0.00%

Data showing different mutation frequencies obtained using FAM/HEX probes (mutant/WT probe) and with mutant gBlocks spiked into a wild-type background (48,000 copies)

* Breast cancer genes marked in blue

Representative distribution of mutations and hotspots in selected oncogenes



Jiang, R., Zhang, B., Teng, X. et al. Sci Rep 10, 2070 (2020). https://doi.org/10.1038/s41598-020-58819-5

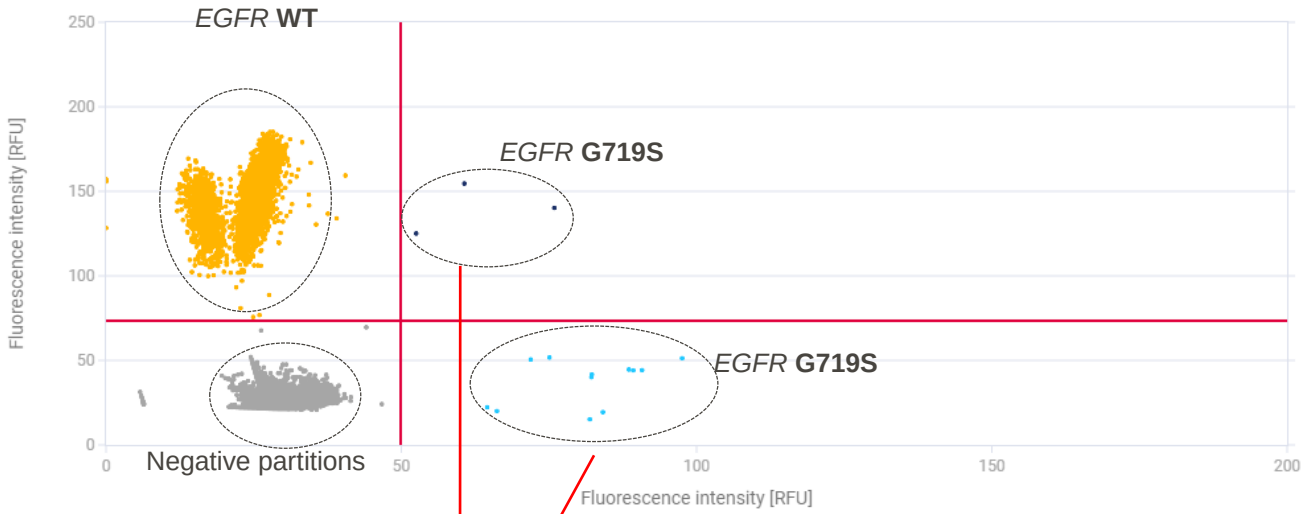
Accurate detection of EGFR G719S mutation in heterogenous FFPE samples

EGFR p. G719S assay

- Horizon FFPE templates: formalin compromised, mild fragmentation
  - Expected mutation frequency: 24.5% resulting
- Spiked into healthy WT gDNA to generate a heterogenous mixture
  - **24.5% to 0.1% mutation frequency**

Heterogenous mix	Horizon FFPE template	30 ng	30 ng	3 ng	1.5 ng	0.3 ng	0 ng
	WT gDNA	0 ng	30 ng	30 ng	30 ng	30 ng	30 ng
Mutation frequency	Expected mut%*	**24.50 %***	**12.25 %**	**2.23 %**	**1.16 %**	**0.24 %**	**0.00 %**
	Measured mut%	**25.50 %**	**11.2%**	**1.67 %**	**1.13 %**	**0.19 %**	**0.04 %**

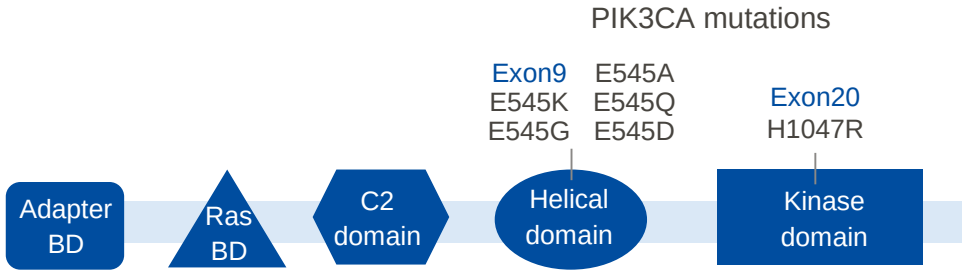
*Expected allelic frequency by sample vendor ≥5 % <20% **± 30%**



2D Scatter Plot showing different populations in one well containing sample with expected mutation frequency of 0.1%.

0.1% mutation frequency = 14 copies in one well

Specificity of LNA Mutation Assays



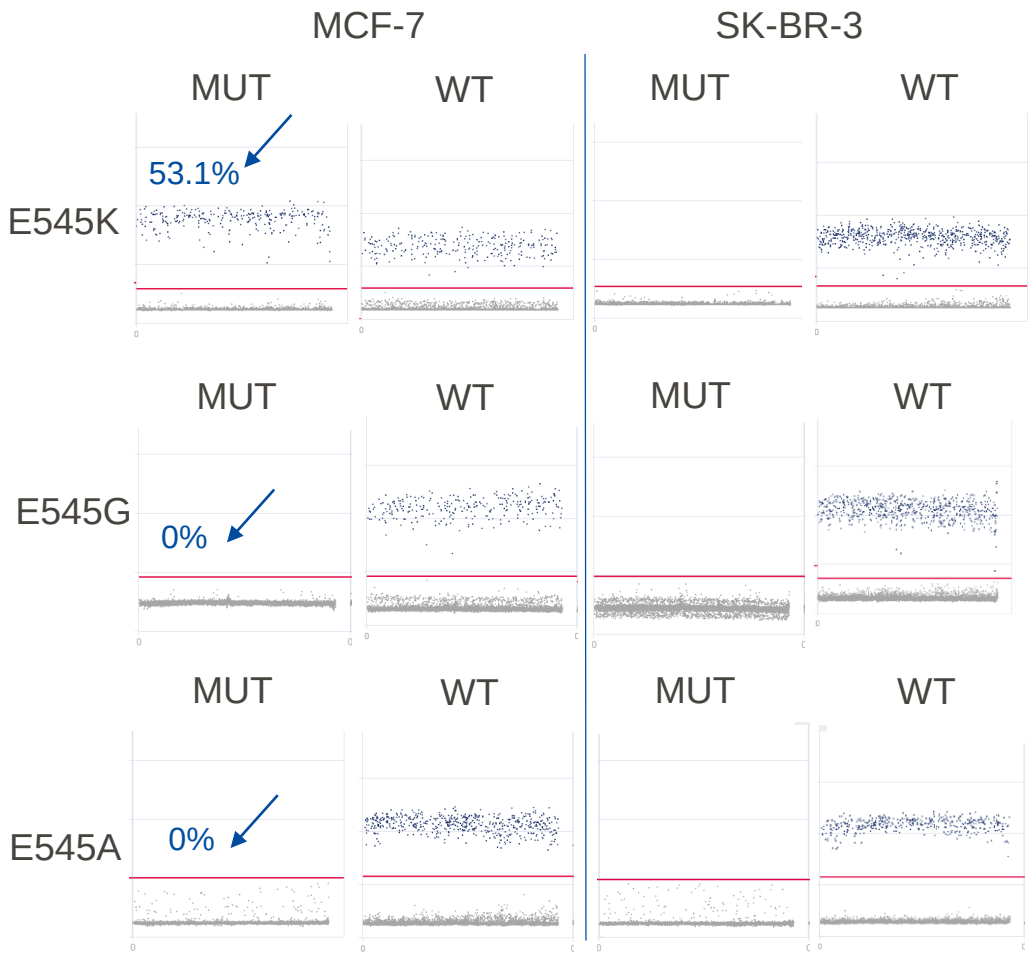
PIK3CA is the most frequently mutated oncogene in breast cancer

- ~45% of all breast cancers harbor PIK3CA mutations
- 90% of all mutations in PIK3CA are in exon9 and exon20

We tested specificity of assays targeting exon9 PIK3CA mutations in breast cancer cell lines MCF-7 and SK-BR3:

- Nanoplate 8.5K 96-well
- 0.2 ng/µl cell line gDNA input

WT	GAG	PIK3CA mutation
E545K	**A**AG	c.1633G>A
E545G	G**G**G	c.1634A>G
E545A	G**C**G	c.1634A>C



- MCF-7 cell line caries only mutation E545K, and only the corresponding assay shows positives. The closely related assays show no positives.
- SK-BR3 carrying no mutation shows as expected no positives for all three mutation assays

Summary

- Mutations not only result in normal variation but also cause genetic diseases and cancer
- QIAcuity Digital PCR provides a flexible throughput, sensitive and accurate absolute quantification of mutations
- dPCR LNA Mutation Assays can detect in a single well ≥0.1% mutation frequencies in variety of samples, such as FFPE samples
- A large dPCR LNA Mutation Assay portfolio allows extensive study of individual cancer genes and related pathways
- dPCR LNA Mutation Assays are optimized using QIAcuity Probe PCR Kit on the QIAcuity Digital PCR instrument

Agenda

Introduction

dPCR LNA Mutation Analysis in Breast Cancer Research

\+ Application Data

dPCR Copy Number Variation Analysis in Breast Cancer Research

\+ Application Data

Copy number variation

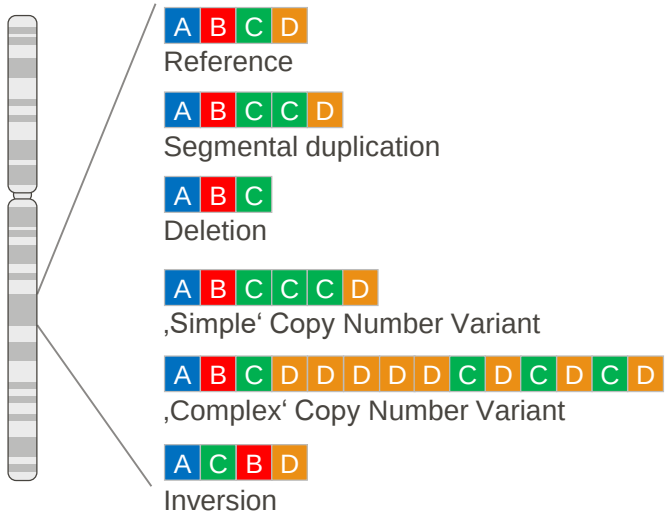
A collective term for structural changes in the human genome

An alteration of the diploid state of genome

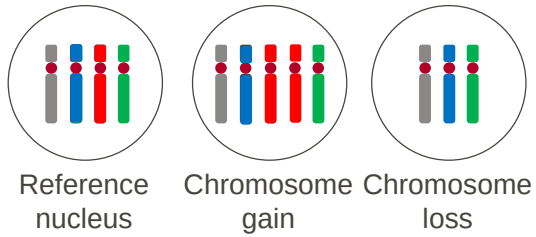
Changes in the copy number of a gene from one individual to another

- Deletion, duplication, inversion, translocation, insertion of genomic regions
- Ranging from 50 bp up to several mega base pairs
- Can be categorized as:
  - Short repeats or long repeats
  - Rare (<1%) or recurrent (>1%) CNVs
- Accounts for up to 20% human genetic variability
- Linked to common and complex diseases and traits

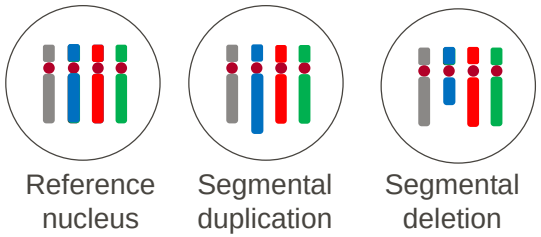
Chromosome



Whole chromosome gain/loss (aneuploidy)



Segmental aneuploidy



Copy number variations in cancer

Gain/amplification of oncogenes and loss/deletion of tumor suppressor genes are major drivers of tumor development:

	Gene	Cancer type
Amplification of oncogenes	*EGFR*	Breast, ovarian, glioma, non-small lung carcinoma
	HER2	Breast, ovarian and lung cancer
	FGFR1	Breast, ovarian and lung cancer
	MYC	Breast cancer, acute myeloid leukemia
	KRAS	Lung, gastric, ovarian and uterine cancer
	NRAS	Lung, gastric, ovarian, melanoma and breast cancer
	MET	Lung, breast and colorectal cancer
	BRAF	Melanoma, non-small lung cancer

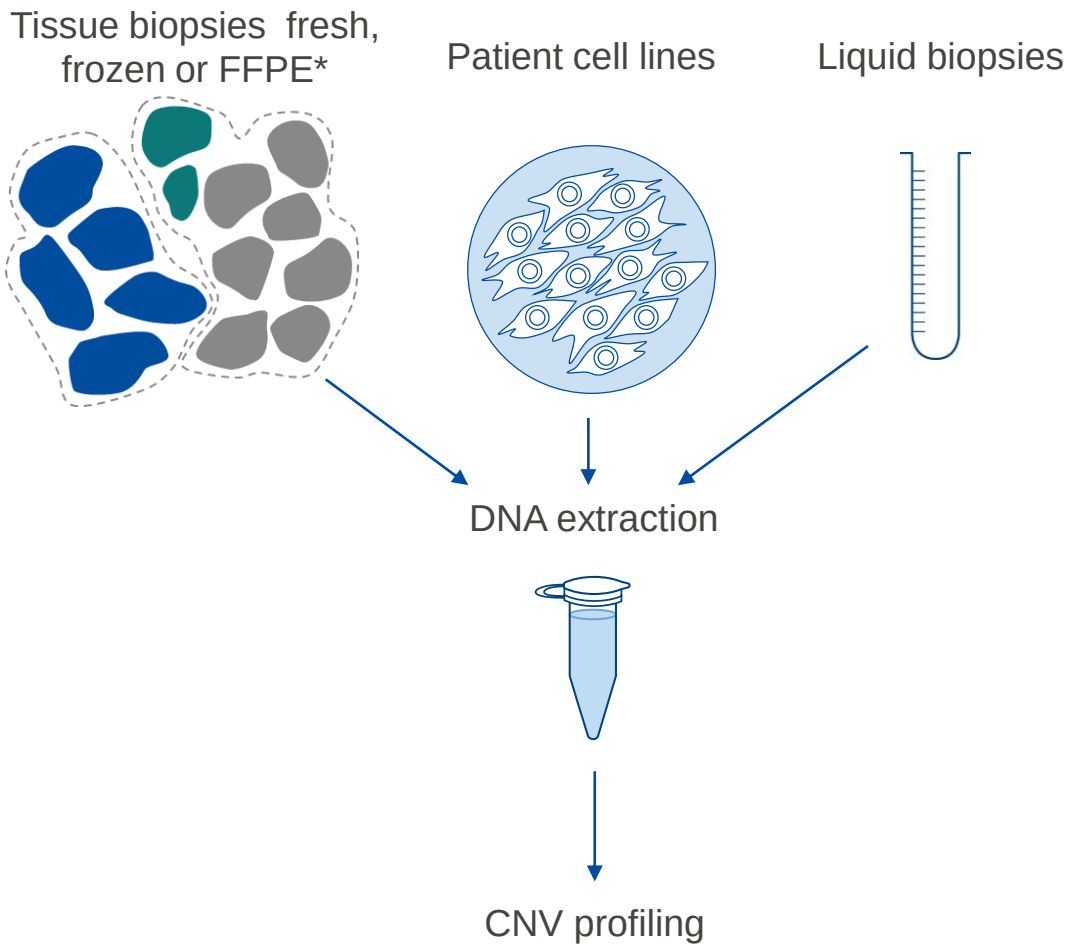
	Gene	Cancer type
Deletion/Loss of tumor suppressors	*TP53*	Breast and gastric cancer, brain tumors
	PTEN	Brain, prostate and breast cancer
	VHL	Renal and pancreatic cancer
	SMAD4	Pancreatic, skin and colorectal cancer

- 26 cancer types, 3131 samples, 76,000 gain and 55,000 loss events
- A typical tumor: 17% amplifications and 16% deletions (0.5% in healthy samples)
- 25% of genome is affected by whole chromosome alterations (in 17 cancer types)

* Breast cancer genes marked in blue

Adapted from *Mol Cell Biol. 2016 Apr 1; 36(7): 1050–1063.*
Nature. 2010 Feb 18; 463(7283): 899–905.
Mol Cell Biol 36:1050–1063. doi:10.1128/MCB.00652-15.

Sample source

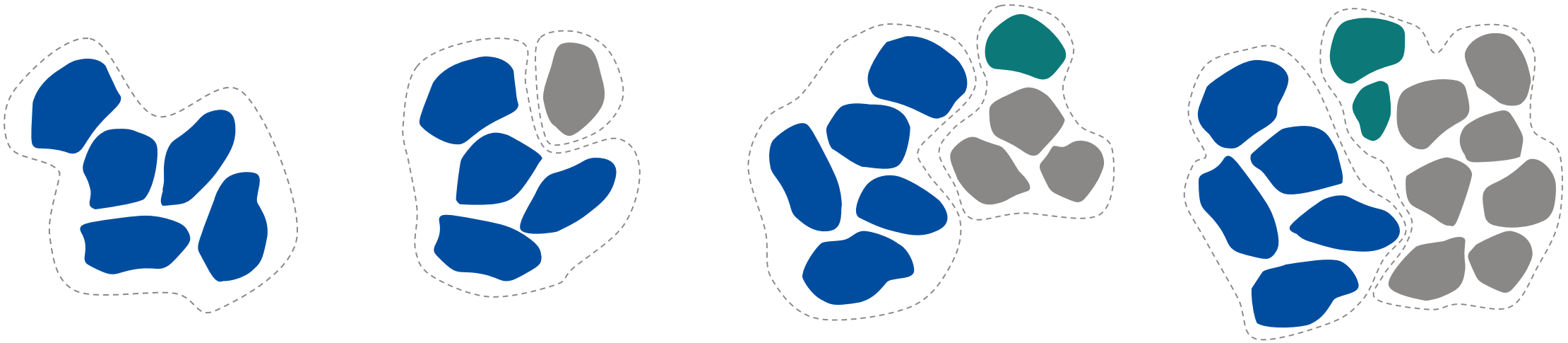


Challenges:

- Highly variable sample quality
- Limited sample amounts
- Suboptimal DNA extraction of samples

* Mostly the case for breast cancer samples

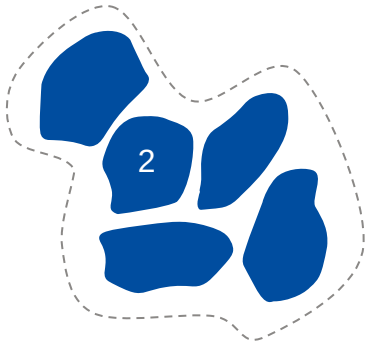
Sample heterogeneity



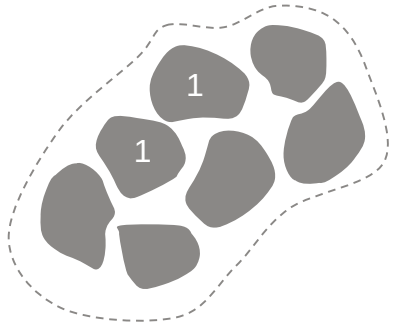
Multiple tumors
Different types of cells
Multiple CNV events

Resolution of copy number change

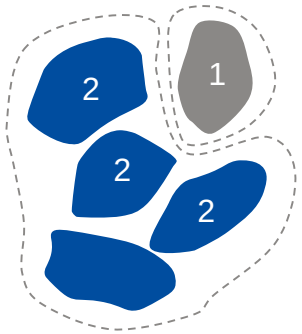
Sample heterogeneity



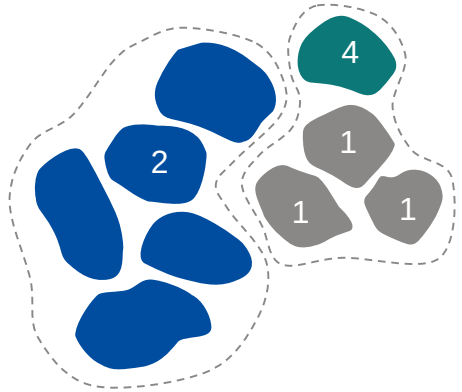
Homogenous sample
'healthy'
CN=2



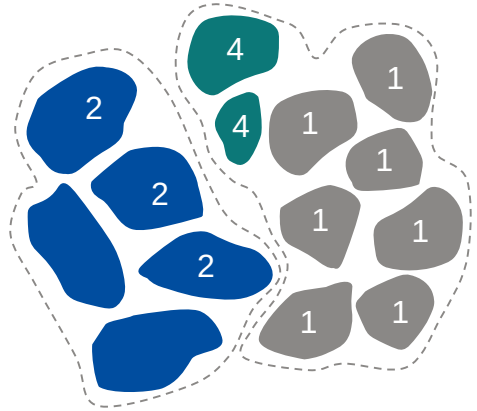
Homogenous sample
CN loss
CN=1



Heterogenous sample
CN loss
CN net=10%loss



Heterogenous sample
CN loss and gain
CN net=No change



Heterogenous sample
CN loss and gain
CN net=10% loss

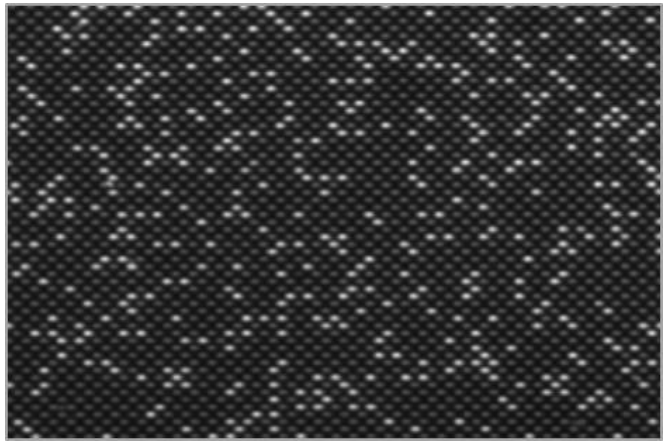
CN loss	Healthy cells, CN=2	Altered cells, CN=1	Resolution needed
% of cells	50%	50%	25%
	80%	20%	10%

CN gain	Healthy cells, CN=2	Altered cells, CN=10	Resolution needed
% of cells	90%	10%	40%
	97.5%	2.5%	10%

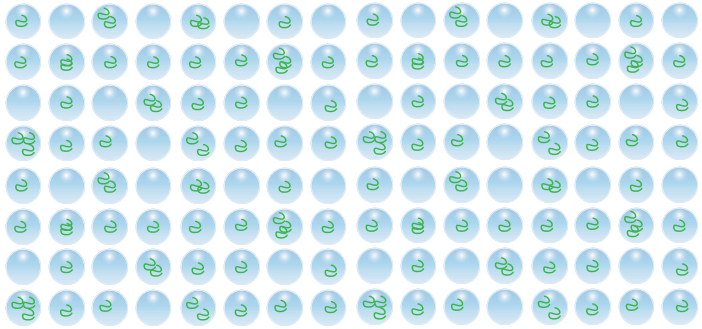
Fine resolution and high quantitative power are needed!

BMC Genomics volume 15, Article number: 84 (2014)

Digital PCR is an advancement in precision and sensitivity



Absolute quantification: Copies/µl
calculated with number of partitions in total, number of positive partitions and statistical distribution model



Random distribution of molecules into partitions
creates an increase in effective concentration

- Absolute quantification of DNA copies
- High sensitivity and precision
- Limit of detection based on DNA input
- High-throughput validation of known mutations
- Retesting of NGS results for validation of new mutations

QIAcuity Digital PCR System

QIAcuity Nanoplates

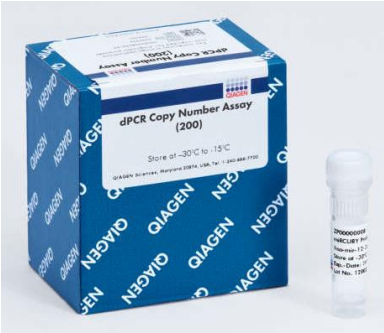
- Nanoplate 26K 24-well
  - 24-well × ~26,000 partitions
- Nanoplate 8.5K 24-well
  - 24-well × ~8,500 partitions
- Nanoplate 8.5K 96-well
  - 96-well × ~8,500 partitions

QIAcuity dPCR instruments

QIAcuity EG PCR Kit

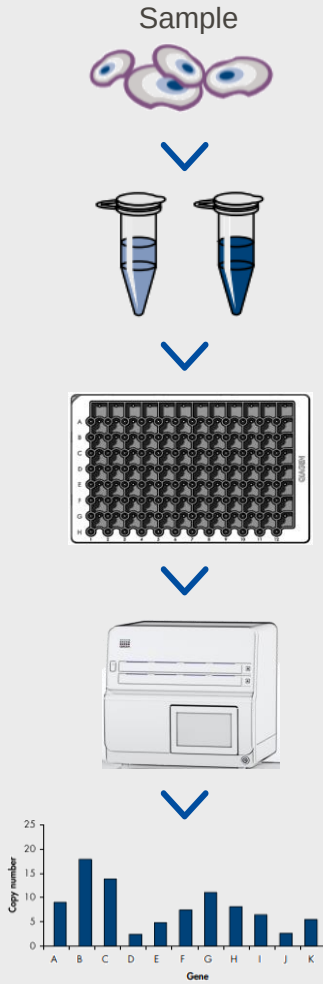
- EvaGreen-based Master Mix
- 3x Master Mix
- Optimized for best performance in nanoplate microfluidic environment
- Includes special reference dye needed for dPCR analysis and counting analyzable partitions

dPCR Copy Number Assays



Focused copy number profiling using EvaGreen

- Assay designs for every human gene
  - 3 assays each gene (**5´-, 3´-prime and middle**)
- 225 dPCR wet-lab tested assays, >100,000 in silico designs
  - Highly studied cancer and cancer-related genes
  - 3 Single-copy Reference Assays: AP3B1, TERT, RPP30
  - 2 Multi-copy Reference Assays available
- Single-tube format, easy handling
- Used in combination with QIAcuity EG PCR Kit



Isolate DNA from research samples

Add genomic DNA to master mix, including **restriction enzyme**

Pipet sample into nanoplate

Run in your dPCR instrument

Analyze your results

The restriction enzyme digest

When to apply?

- For DNA samples with an average length of ≥20 kb and for plasmids. Ensures even distribution of template on the nanoplate and efficient amplification (plasmids)
- Particularly important for copy number variation (CNV) analyses, where multiple copies of a gene might be linked in tandem
- Not required for highly fragmented DNA (FFPE DNA, circulating DNA), cDNA, gBlocks, …

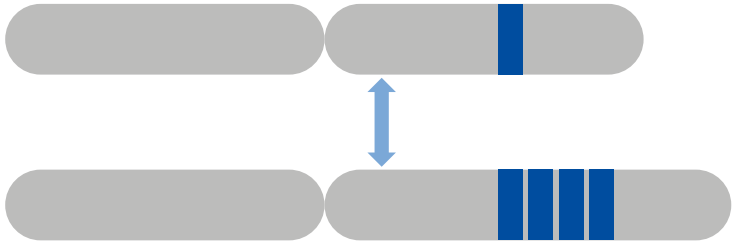
How:

- Compatible restriction enzymes for each assay listed in product specifications
  - Include restriction enzyme in master mix and incubate for 10 min at room temperature after template addition

Important!

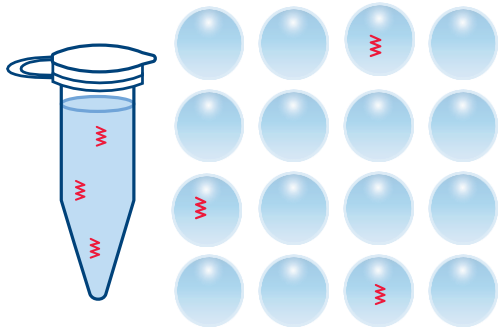
Make sure that restriction enzyme will not cut within your amplicon
For QIAGEN assays targeting genomic DNA (CNV, Mut. detect.), suitable enzymes will be recommended

CNV: duplications, tandem repeats



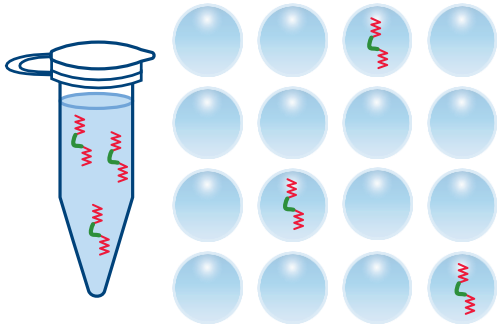
Separation of tandem copies

Single copy variant



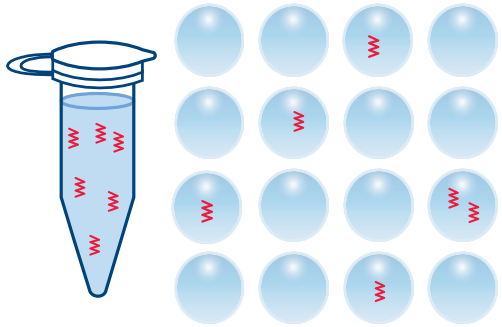
3 positive partitions

Two tandem copies



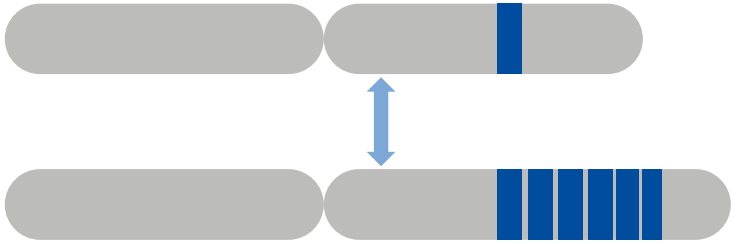
3 positive partitions:
inaccurate CNV estimate

Two unlinked copies



5 positive partitions:
accurate CNV estimate

CNV: duplications, tandem repeats

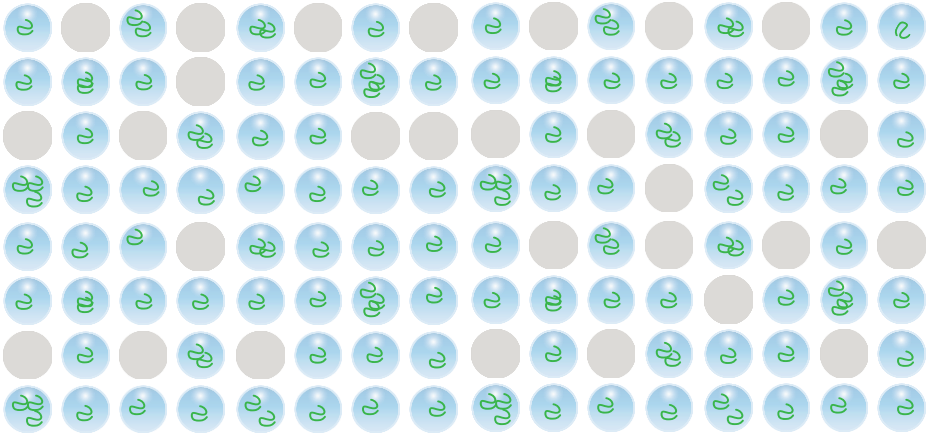


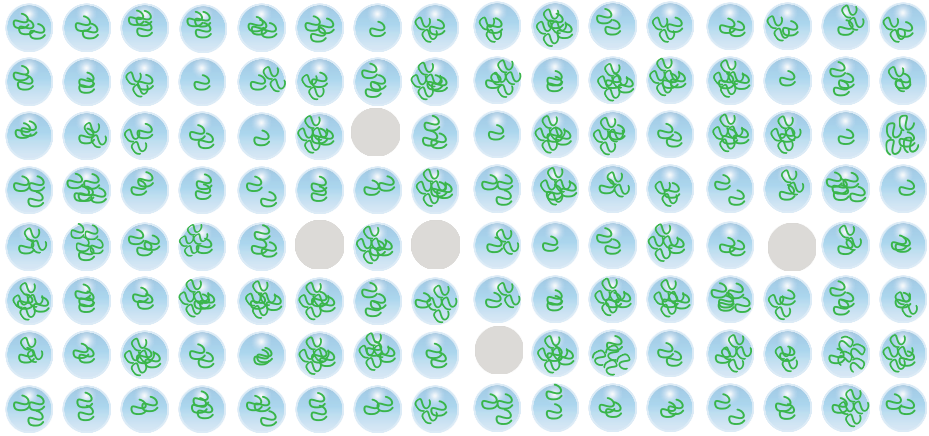
Restriction digestion of genomic DNA:

- Separates tandem repeats or repetitive elements
- Results in higher accessibility of the copy number variant region for detection
- Increases accuracy of copy number estimations and CNV calculations

DNA amount and copy numbers

Poisson distribution statistics





In dPCR, the number of copies/partition must not exceed **5 copies**, ideally in the range of **0.5–2 copies**.

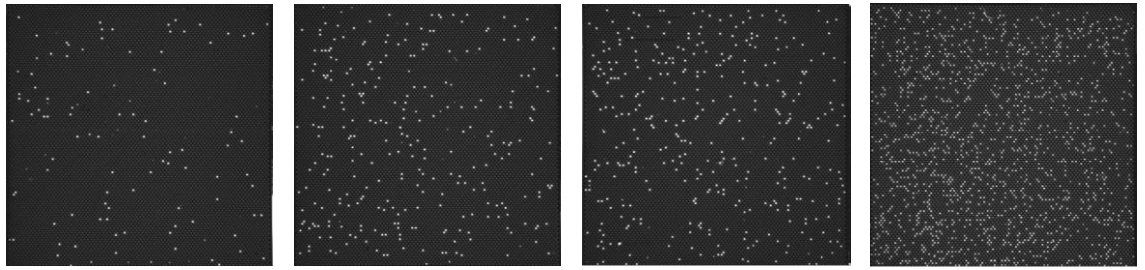
Ideal loading amounts:

Nanoplate	Reaction volume	Copies/reaction	gDNA amount
8.5K	3/12 µl	17,000 – 68,000	ca. 60–250 ng
26K	24.1/40 µl	22,000 – 87,000	ca. 75–300 ng

DNA amount and copy numbers

Single-copy gene vs. Multi-copy gene

Copies/haploid genome	Copies in 10 ng gDNA
1	2,777
5	13,885
10	27,770
20	55,540
50	138,850



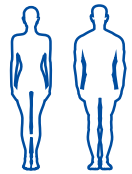
1 copy/genome 2 copy/genome 3 copy/genome 20 copy/genome

Note: Expected copy numbers for targets and reference assays should be taken into consideration when setting up reactions.

DNA amount and copy numbers

Genome size varies among species

Species	Copies in 10 ng gDNA
Human	2,777
Zebrafish	5,368
Yeast	760,466
E.coli	1,983,826
Plasmid (3.5 kb)	2,607,314,286



Homo sapiens: 3.3 x 10E9 bp

Rat: 3.0 x 10E9 bp

Mouse: 3.5 x 10E9 bp

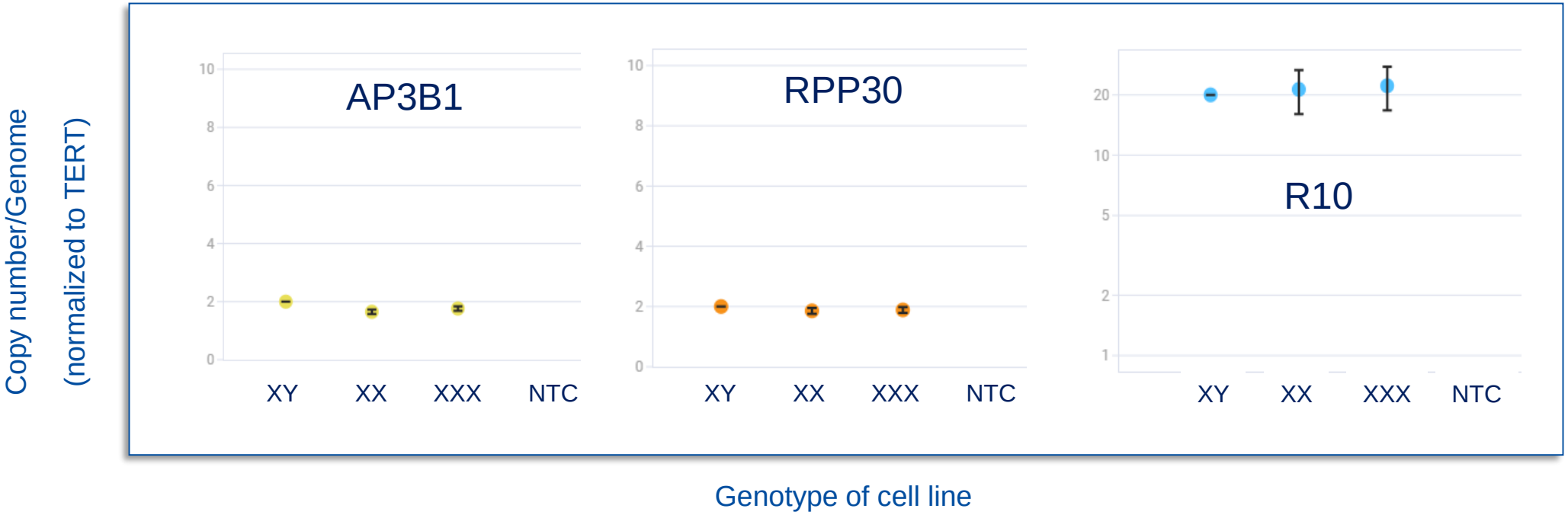
Zebrafish: 1.7 x 10E9 bp

S. cerevisiae: 1.2 x 10E7 bp

E. coli: 4.6 x 10E6 bp

Standard plasmid: 3.5 x 10E3 bp

Reference assay selection

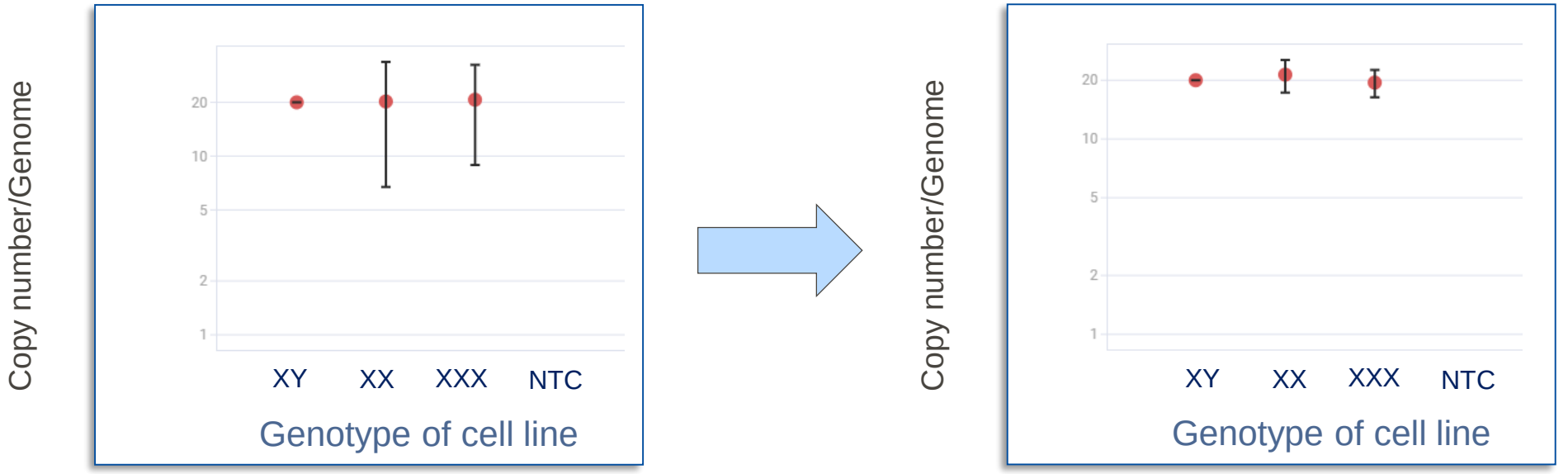


Single-Copy Reference Assays: AP3B1, TERT, RPP30

Multi-Copy Reference Assays: R10, R6

Reference assay selection

Why use Multi-Copy Reference Assays?



(normalized to Single-Copy Reference Assay)

(normalized to Multi-Copy Reference Assay)

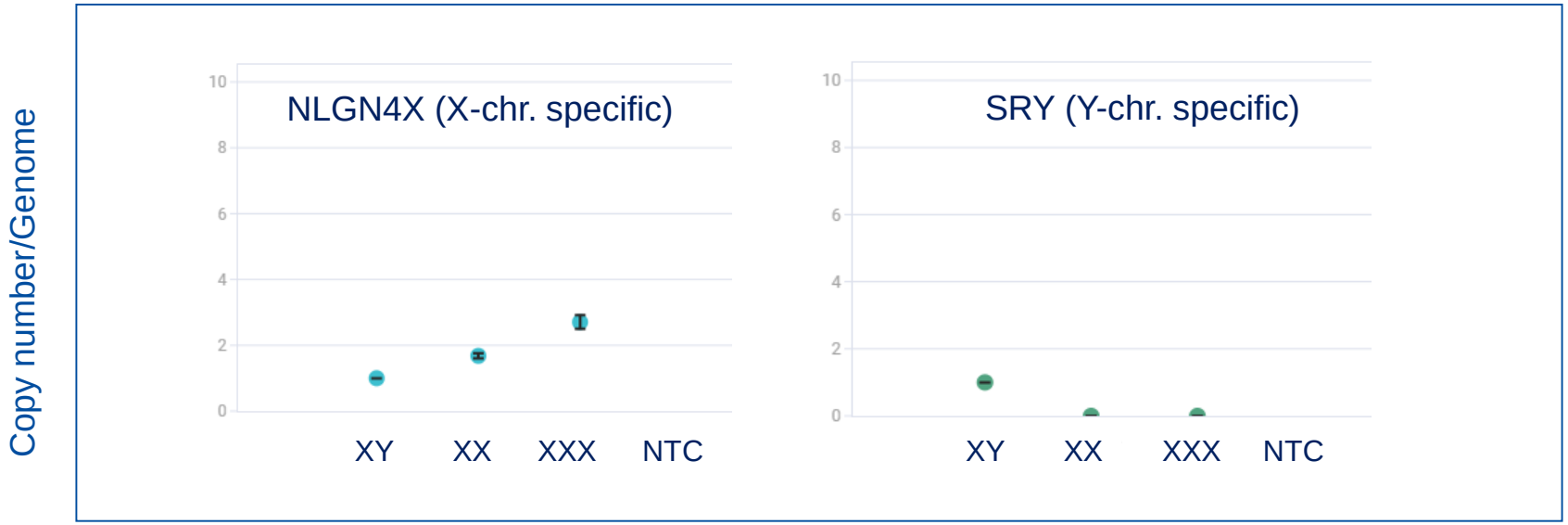
There are ~20 copies of Actin gene in human genome

When high copy no./genome is expected, normalization using multi-copy reference assays provide more accurate results

Two Multi-Copy Reference Assays available: R6, R10

Accurate identification of copy number variations

Aneuploidy testing



Setup:

- QIAcuity Nanoplate 8.5K 96-well
- QIAcuity EvaGreen PCR Kit
- dPCR Copy Number Assays

- dPCR Copy Number Assays targeting NLGN4X and SRY were tested using gDNA from 3 human cell lines that contain 1 copy (XY), 2 copies (XX) and 3 copies (XXX) of X-chromosome
- Human TERT gene was used as a reference to normalize copy numbers

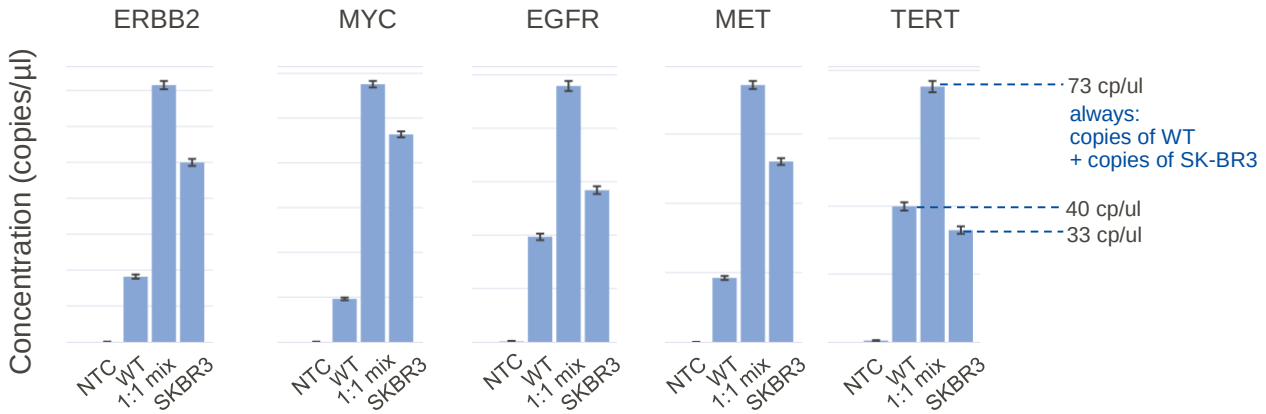
Detection of HER2 copy number status in SK-BR3 cell lines

Breast cancer CNV profiling

- It is the most common type of cancer in women
- Chr8, Chr17 and Chr20 are harboring copy number alterations in breast cancer
- Changes in copy numbers of HER2 (ERBB2), EGFR, MYC and MET are frequently observed
  - TERT as single-copy reference assay
- Multiple cell lines available: MCF-7, T-47D, **SK-BR3**, MDA-MB-231, BT-474, UACC-893 and ZR-75-30

Samples
WT gDNA (4 ng/rxn)
SK-BR3 cell line gDNA (4 ng/rxn) ATCC® HTB-30™
1:1 mix (8 ng/rxn)
96-well nanoplates
5 assays, 4 samples (including NTCs)
4x technical replicates
Sample to results:<2 h

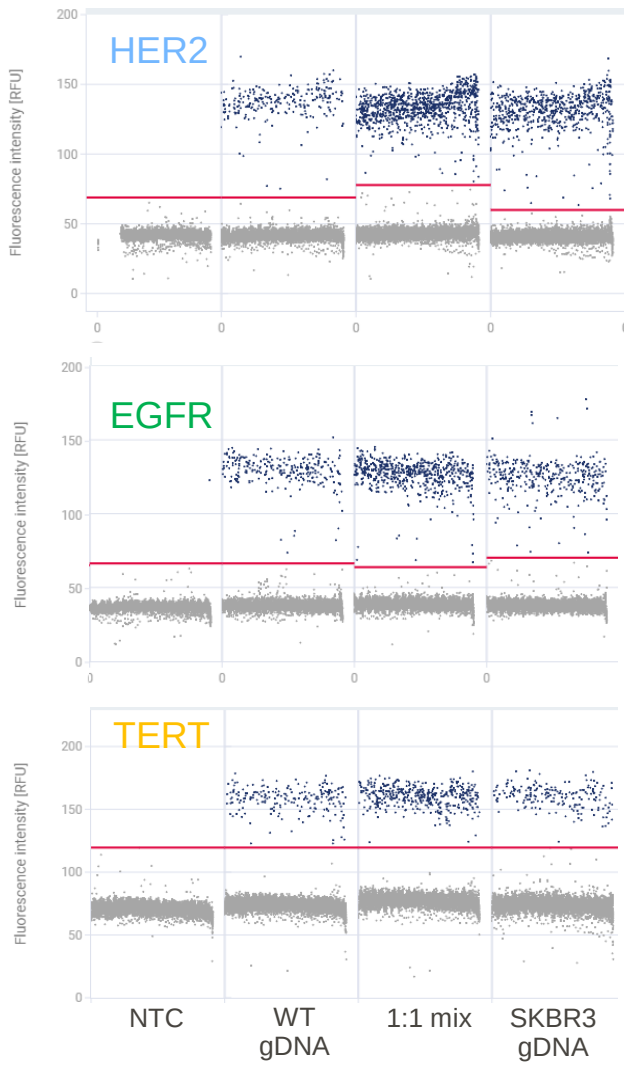
We screened breast cancer cell line SK-BR3 for copy number changes in multiple common breast cancer genes.

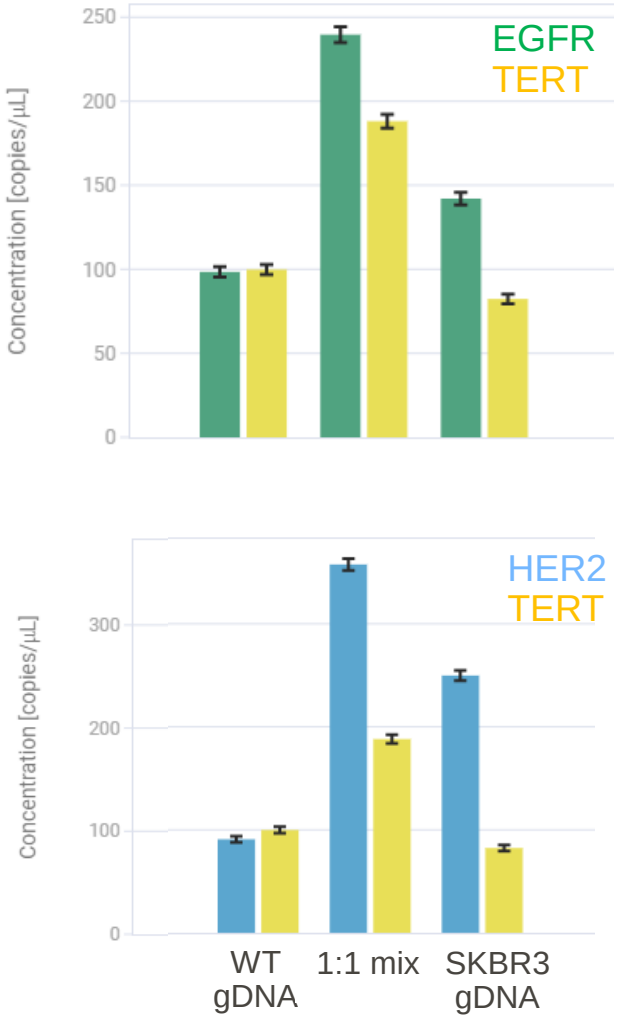


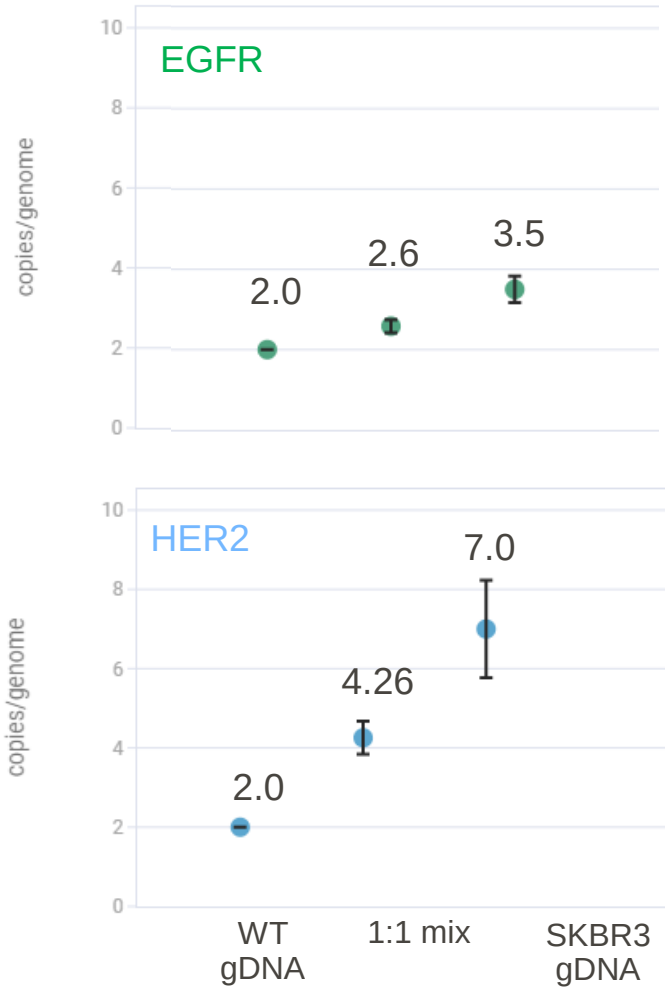
Copy number change in SK-BR3 vs. WT

	CN/genome			
Genes	**ERBB2**	**EGFR**	**MYC**	**MET**
SK-BR3	6.7	3.5	11.7	6.8
WT	2	2	2	2

Copy number changes in HER2 and EGFR in SK-BR3 cell lines







Accurate CNV analysis in heterogenous MCF7 cell lines

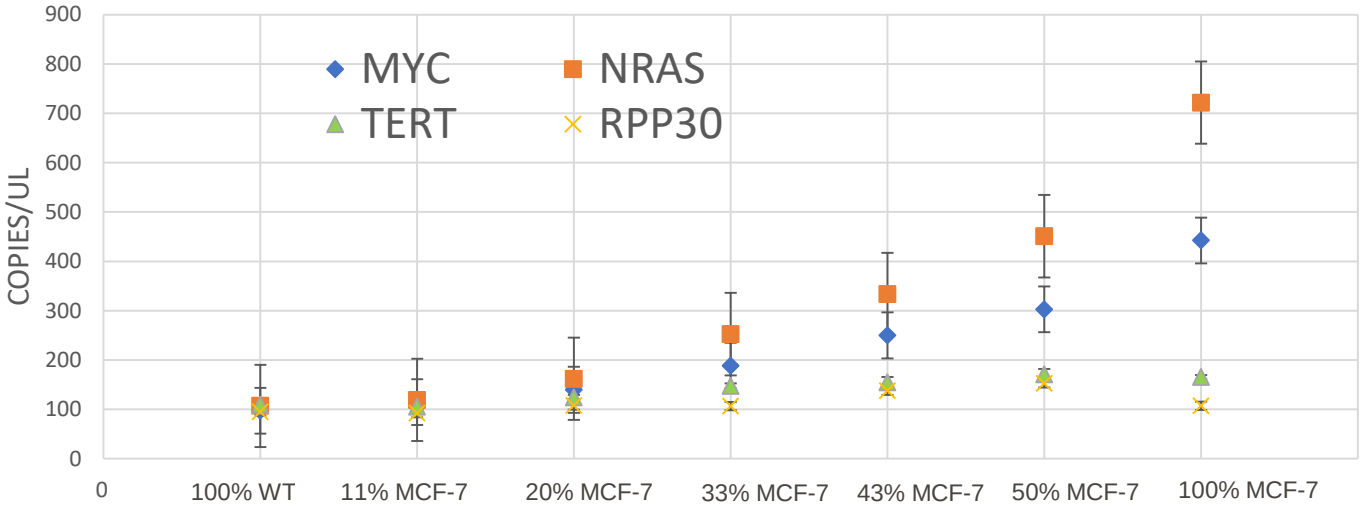
Precise NRAS and MYC copy number determination

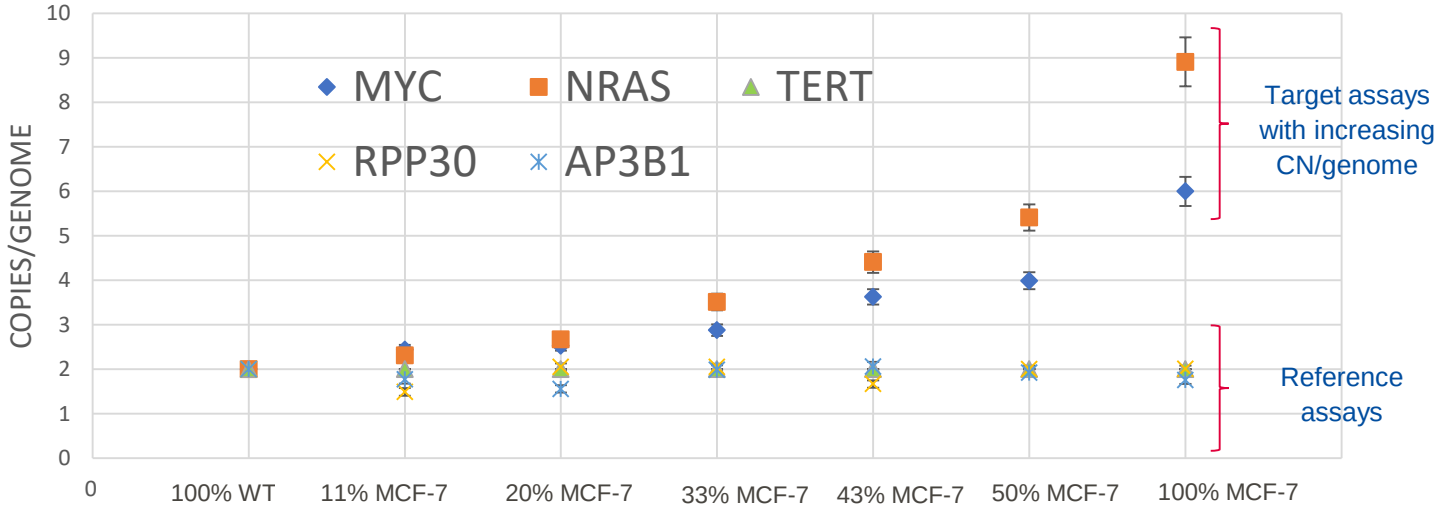
Samples
WT gDNA (4 ng/rxn)
MCF-7 cell line gDNA (4 ng/rxn)
MCF-7 cell line gDNA spiked into WT gDNA: 11%, 20%, 33%, 43%, 50% gDNA mixtures

96-well nanoplates
Assays: MYC, NRAS, TERT, RPP30, AP3B1

Comparable measured and expected values

Target		100% WT	11% MCF7	20% MCF7	33% MCF7	43% MCF7	50% MCF7	100% MCF7
MYC	Expected	2	2.4	2.8	3.3	3.7	4	6
	Measured	2	2.44	2.53	2.88	3.63	3.99	6
NRAS	Expected	2	2.8	3.3	4.3	4.96	5.5	8.9
	Measured	2	2.31	2.67	3.51	4.41	5.41	8.9





Accurate CNV analysis in FFPE samples

- Horizon™ FFPE templates, formalin damaged and fragmented
- Healthy gDNA vs. FFPE samples

Samples
WT & FFPE gDNA (4 ng/rxn)
96-well nanoplates
Assays: BRAF, MET, KRAS, FGFR1, TERT

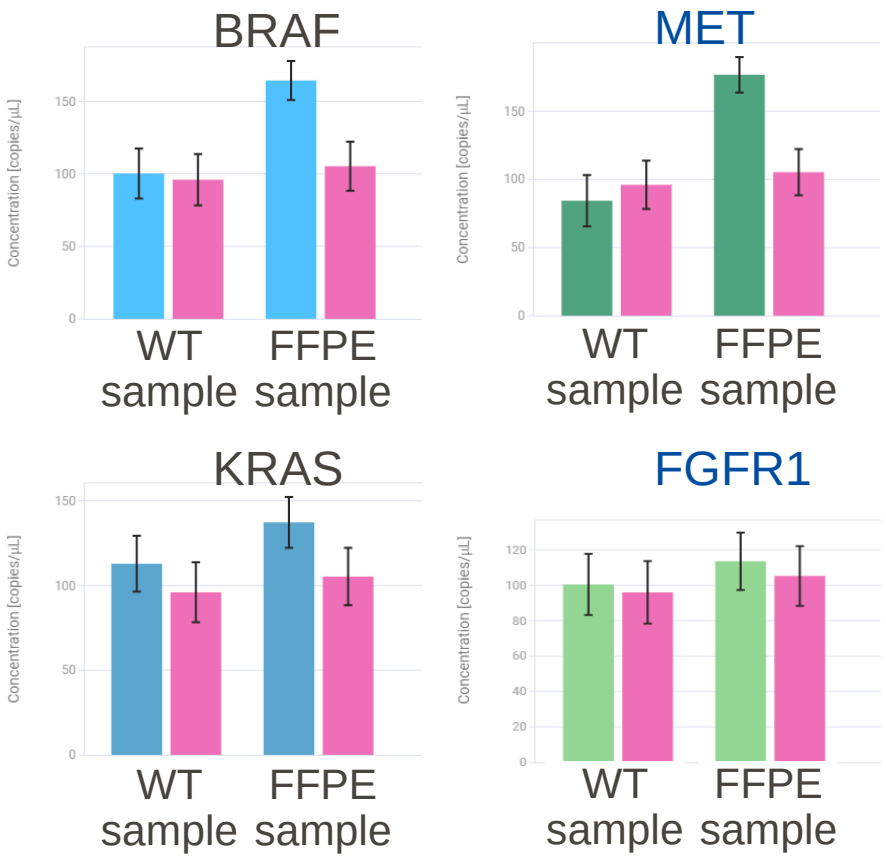
Challenges

FFPE fixation leads to:

- Crosslinking, degradation or deamination
- Fragmentation of DNA molecules

...Inaccurate quantification of CNVs

* Breast cancer genes marked in blue



	CN/genome			
Sample	**BRAF**	**MET**	**KRAS**	**FGFR1**
FFPE	2.99	3.82	2.22	2.06
WT	2	2	2	2

dPCR Copy Number Assays

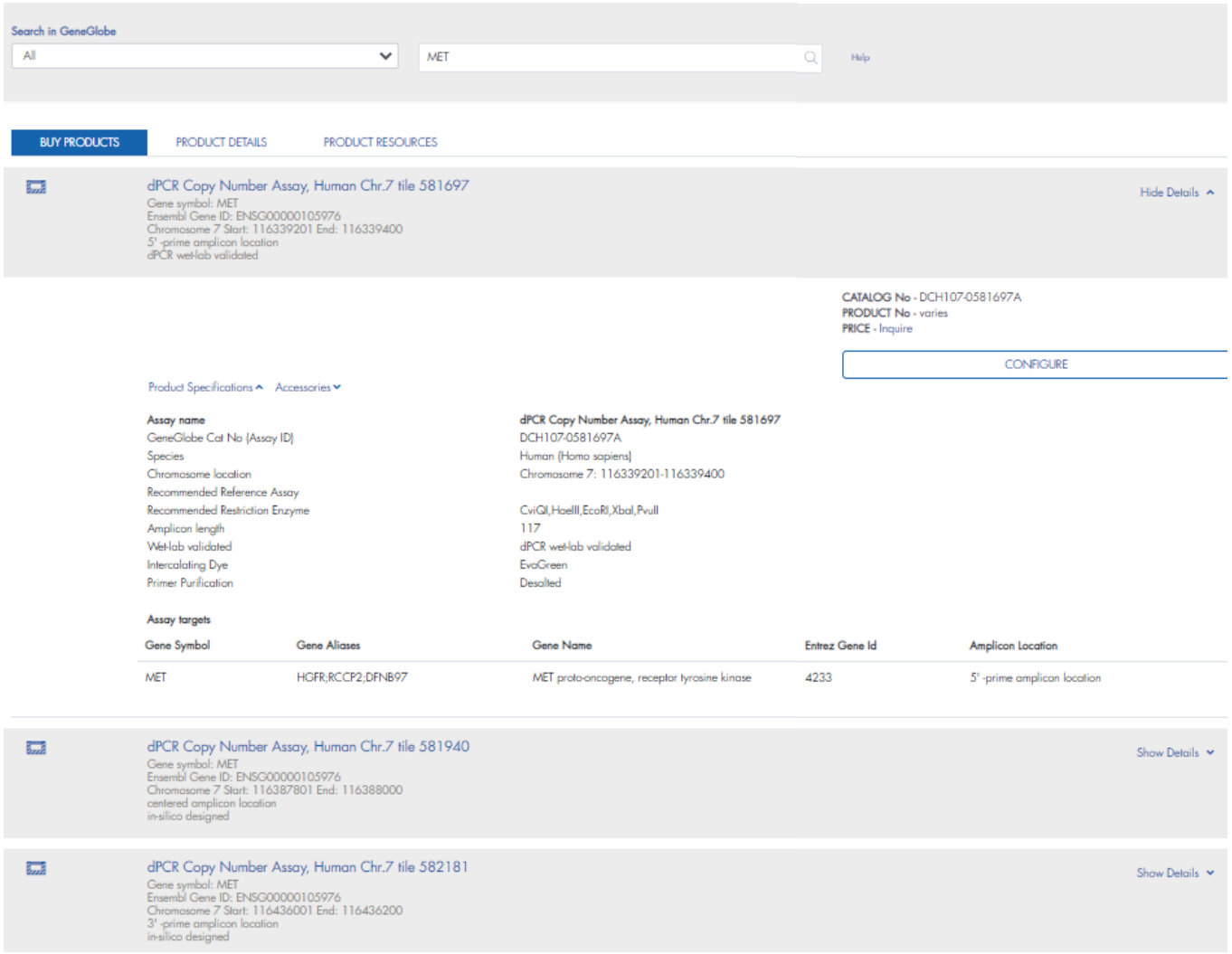
https://geneglobe.qiagen.com/product-groups/dpcr-copy-number-assays

Assays available in GeneGlobe

Wet-lab-validated assays

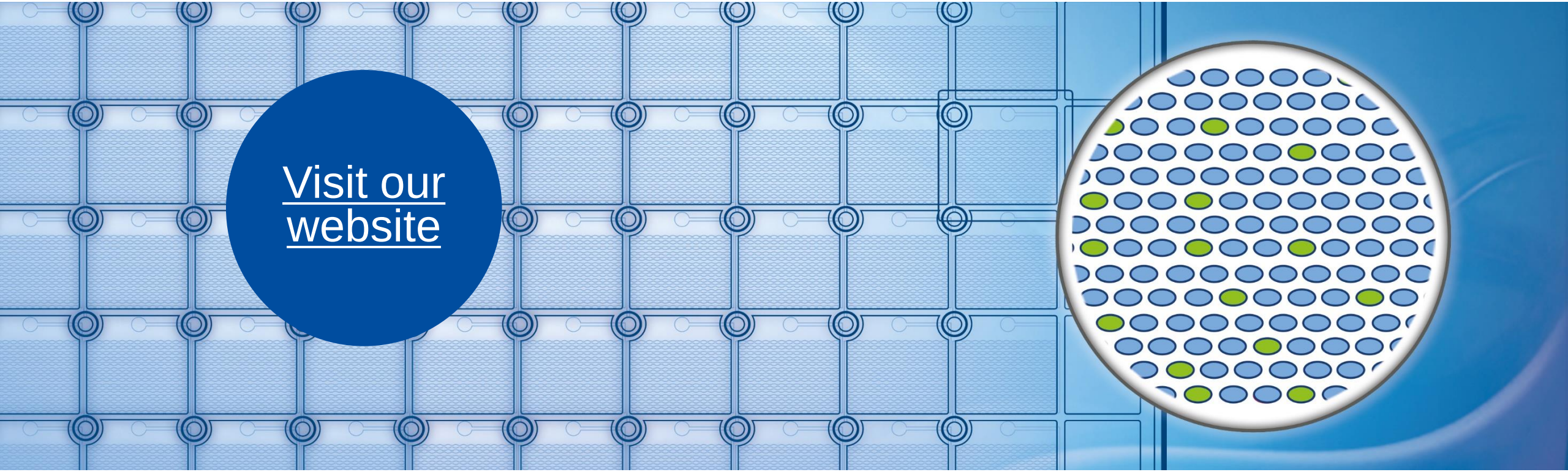
In silico designs

- Growing over time



Summary

- CNVs are important players of cancer, drug metabolism and other common diseases
- Limitations and considerations of CNV measurements
- QIAcuity digital PCR provides a robust and accurate absolute quantification of CNVs
- QIAcuity digital PCR can detect ≥10% changes in copy number variation
- Large dPCR CNV Assay portfolio allows extensive study of individual cancer genes and related pathways
- dPCR CNV Assays are optimized using QIAcuity EG MasterMix on QIAcuity instrument



Digital is the new absolute

www.qiagen.com/dPCRwebinars

New breast cancer resource page

www.go.qiagen.com/BreastCancerResearch

Thank you for your attention. Questions?

Please reach out to us at https://www.qiagen.com/about-qiagen/contact-us