UNIVERSITY OF EASTERN FINLAND

UEF DNA-TUUMA

DNA-analytiikalla uusia ratkaisuja - Terveyttä, luonnon monimuotoisuutta ja elintarviketurvallisuutta kestävästi

Pohjois-Savon liitto

Euroopan unionin osarahoittama

Tarpeesta hankkeeksi

Euroopan unionin osarahoittama

Pohjois-Savon liitto

TARVE

Kasvava analytiikkatarve eri toimialoilla:

1) Terveys- ja hyvinvointi
2) Ympäristö
3) Elintarvike

HAASTE

Teknologia ja osaaminen eivät vielä siirry riittävästi käytäntöön

RATKAISU

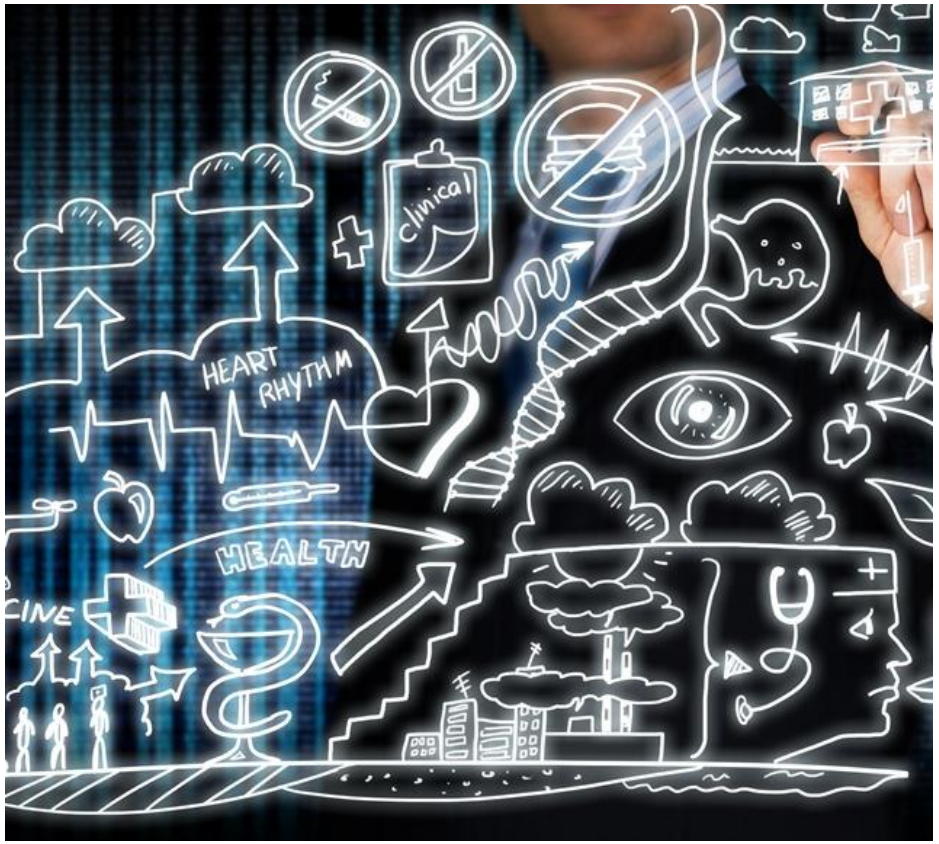
DNA-TUUMA investointi- ja kehittämishankkeen yhdistelmä lisäämään modernin DNA-analytiikan ja siihen liittyvän osaamisen saatavuutta

Kohderyhmät

Euroopan unionin osarahoittama

Pohjois-Savon liitto

- Yritykset (pääkohderyhmä) eri toimialoilta
- Ekosysteemitoimijat ja sidosryhmät
  - Kehitysyhtiöt ja klusterit (esim. Kuopio Health ja Kuopio Water Cluster)
  - Tutkimus- ja asiantuntijaorganisaatiot (esim. THL, Ruokavirasta ja GTK)
  - Hyvinvointialue ja laboratorioratkaisut (esim. PSHVA, ISLAB ja Itä-Suomen biopankki



Analytiikkatarpeita

Euroopan unionin osarahoittama

Pohjois-Savon liitto

- Terveys ja hyvinvointi
  - Syöpädiagnostiikka (esim. tietyn harvinaisen mutaation havaitseminen)
  - Geenieksпressio (esim. terapeuttisen geenin ilmentyminen)
  - Mikrobien tarkan määrän mittaaminen
- Ympäristö
  - eDNA määritys vesistönäytteistä (esim. uhanalaisen lajin havaitseminen)
  - Vesistöjen laadun seuranta (esim. vesihome tai rapurutto)
- Elintarvike
  - Geenimuunneltujen organismien (GMO) havaitseminen
  - Mikrobiologinen tutkimus

Tavoitteet

Euroopan unionin osarahoittama

Pohjois-Savon liitto

1. Vahvistetaan TKI-infrastruktuuria
   - dPCR-laite UEF Kuopion kampukselle
2. Tunnistetaan elinkeinoelämän analytiikkatarpeita ja testataan 2 – 3 sovellusta
3. Lisätään modernien analyysisovellusten ja niihin liittyvän osaamisen saatavuutta
   - Uusia analyysimenetelmiä, palvelupolkuja ja kasvumahdollisuuksia
4. Rakennetaan yhteistyötä ja verkostoja yli toimialarajojen
5. Tuetaan toimijoiden uudistumista
   - Ymmärretään toimintaympäristön muutoksia ja mahdollisuuksia vasta niihin analytiikan keinoin
6. Tiivistetään UEF-yritysyhteistyötä

Hankkeen keskeiset tiedot

Euroopan unionin osarahoittama

Pohjois-Savon liitto

- Hankkeen kesto **1.1.2026 – 30.6.2027**
- Hanke toteutetaan **Itä-Suomen Genomikeskuksella**
- Hankkeessa mukana: RNatives, Orion, Rokote Laboratories Finland, Aurealis Therapeutics, FinVector, SpringDNA ja Vesi-Eko
- Hankkeessa työskentelevät:

Vastuullinen johtaja, erityisasiantuntija Jaana Hartikainen

Projektipäällikkö Hanna Peltonen

Menetelmäasiantuntija Kaisa Luostari

Bioanalyytikko Ulla Lehtoaho

Työpaketit

Euroopan unionin osarahoittama

Pohjois-Savon liitto

TYÖPAKETTI 1
TERVEYDEN EDISTÄMISTÄ TUKEVIEN ANALYTIIKKARATKAISUJEN TUNNISTAMINEN JA KEHITTÄMINEN
- Analytiikkatarpeiden kartoitus kyselyllä
- Virtuaalityöpaja testattavien sovellusten valitsemiseksi
- Laitteen käyttöönotto: 2-3 sovelluksen testaaminen
- Yhteenvetotyöpaja

TYÖPAKETTI 2
EKOSYSTEEMIEN KESTÄVYYDEN JA VESITEKNOLOGIAN EDISTÄMINEN YRITYSYHTEISTYÖLLÄ JA LIIKETOIMINTAPOTENTIAALIN KASVATTAMISELLA
- Selvitys toimintaympäristömuutoksista
- Vesistönäytteenoton hyvät käytänteet
- Yhteenvetotyöpaja

TYÖPAKETTI 3
ELINTARVIKEALAN ANALYTIIKKA- JA TKI-TARPEIDEN KARTOITTAMINEN
- Analytiikkatarpeiden kartoitus kyselyllä
- Yhteiskehittämisen työpaja

TYÖPAKETTI 4
POHJOIS-SAVON HYÖTYJÄVERKOSTON YLEISET TAPAHTUMAT JA HANKEVIESTINTÄ
- Aloitus- ja lopetustapahtumat
- Hankeviestintä

Seuraa viestintää:
Hankkeen verkkosivuilla
DNA-TUUMA Kehittämishanke – UEFConnect
Itä-Suomen Genomikeskuksen LinkedIn-profiilissa
https://www.linkedin.com/company/uef-genome-center-of-eastern-finland-uef-itä-suomen-genomikeskus

Yhteiskehittäminen

Euroopan unionin osarahoittama

Pohjois-Savon liitto

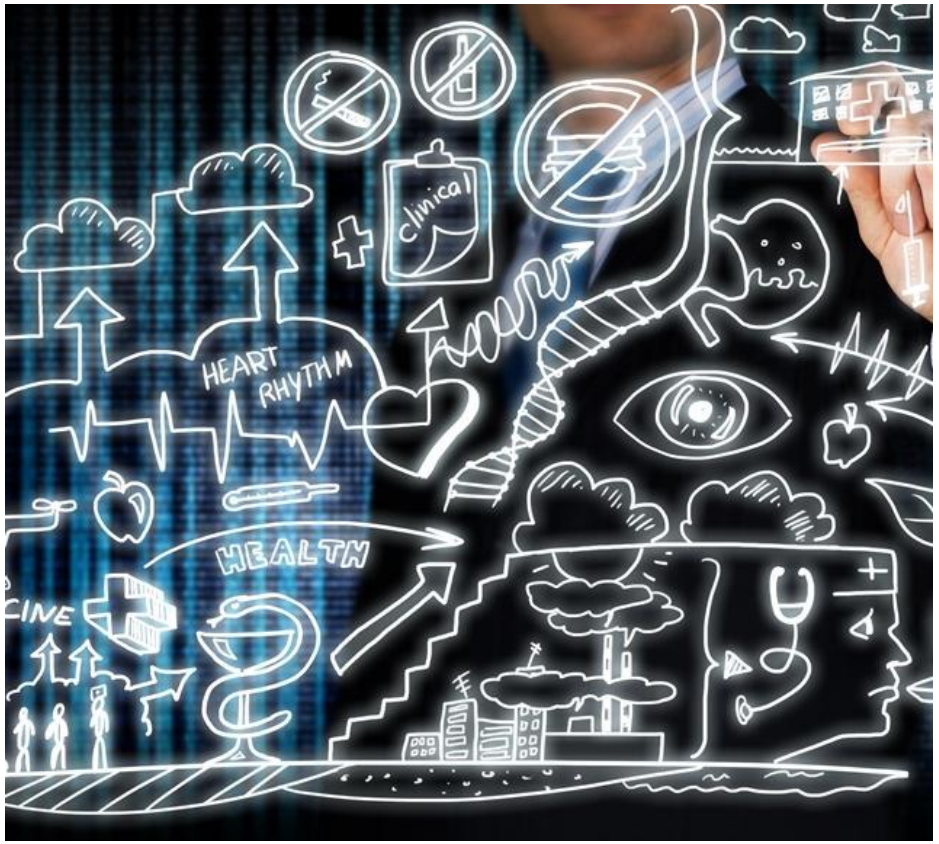
- Hankkeen ydin on yhteiskehittäminen tutkimuksen, yritysten ja sidosryhmien välillä
- Hankkeen eteneminen
  - Tunnistetaan tarpeita:
    kyselyt ja keskustelut yritysten ja sidosryhmien kanssa
  - Valitaan kehityskohteita:
    työpajat ja yhteinen priorisointi
  - Testataan ja arvioidaan ratkaisuja (terveys):
    2-3 sovelluksen kokeilu dPCR-teknologialla
  - Rakennetaan tietoa ja ymmärrystä (ympäristö ja elintarvike):
    selvitykset, hyvät käytännöt, keskustelu
  - Jaetaan tuloksia ja opitaan yhdessä:
    tapahtumat, verkostot, yhteistyö
- Yhteiskehittäminen rakentaa pohjaa uusille sovelluksille, yhteistyölle ja tuleville kehityshankkeille

Hankkeen tilanne

Euroopan unionin osarahoittama

Pohjois-Savon liitto

- Investointi: Laite on hankittu ja saapuu pian kampukselle
- Terveys ja hyvinvointi:
  - Kysely analytiikkatarpeista elinkeinoelämälle tehty
  - Pian avautuu virtuaalityöpaja sovellusten valitsemiseksi
- Ympäristö:
  - Selvitys toimintaympäristömuutoksista alkamassa



Euroopan unionin osarahoittama

Pohjois-Savon liitto

UNIVERSITY OF EASTERN FINLAND

RNATIVES

Vesi·Eko Water·Eco

Kiitos!

FinVector

ROKOTE LABORATORIES

SpringDNA

uef.fi

ORION PHARMA

The future of PCR is digital

QIAcuity digital PCR portfolio

QIAGEN

Legal disclaimer

QIAcuityDx is intended for in vitro diagnostic use. Product availability may differ from country to country based on regulations and approvals. Contact your country representative for further details.

QIAcuity One, QIAcuity Four and QIAcuity Eight are intended for non-clinical applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

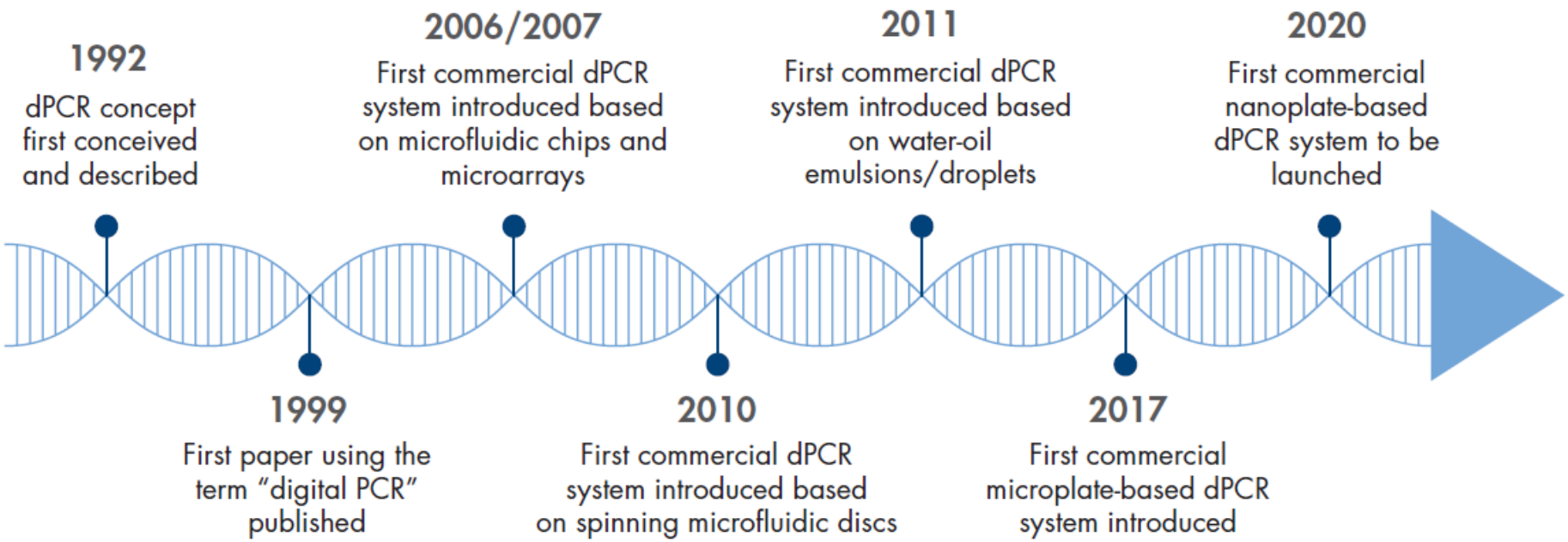
In some cases, data cited pertains to use of a device by another manufacturer.

The future of PCR is digital

- Introduction to digital PCR
- PCR methods – principles and workflow
- QIAcuity – a scalable instrument
- QIAcuity Software Suite
- Multiplexing
- Applications
- Assays and capabilities

The future of PCR is digital

The evolution of digital PCR

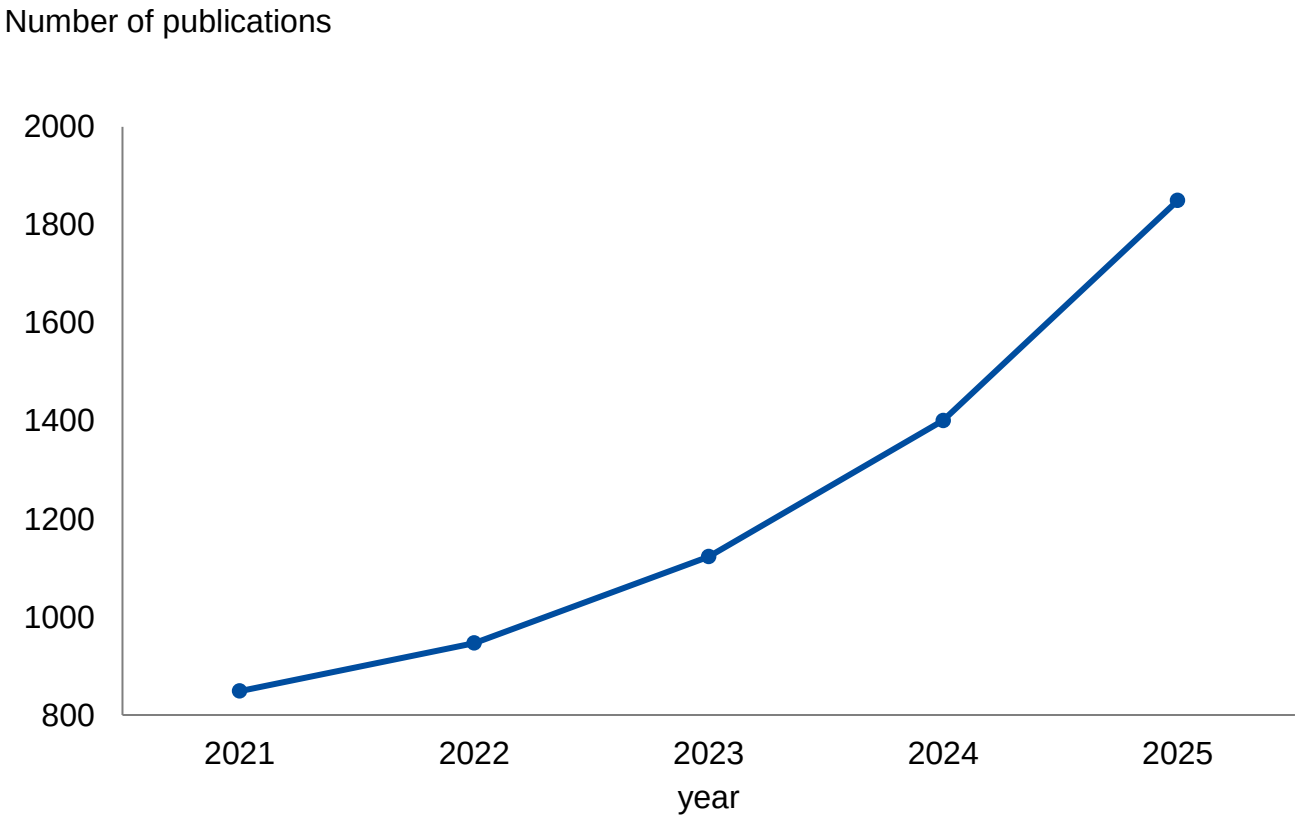


Digital PCR

- Based on real-time PCR chemistry with a workflow very similar to that of real-time PCR
- Absolute quantification at end-point detection as in conventional PCR
- The most advanced PCR technology for specific target detection and absolute quantification of nucleic acids

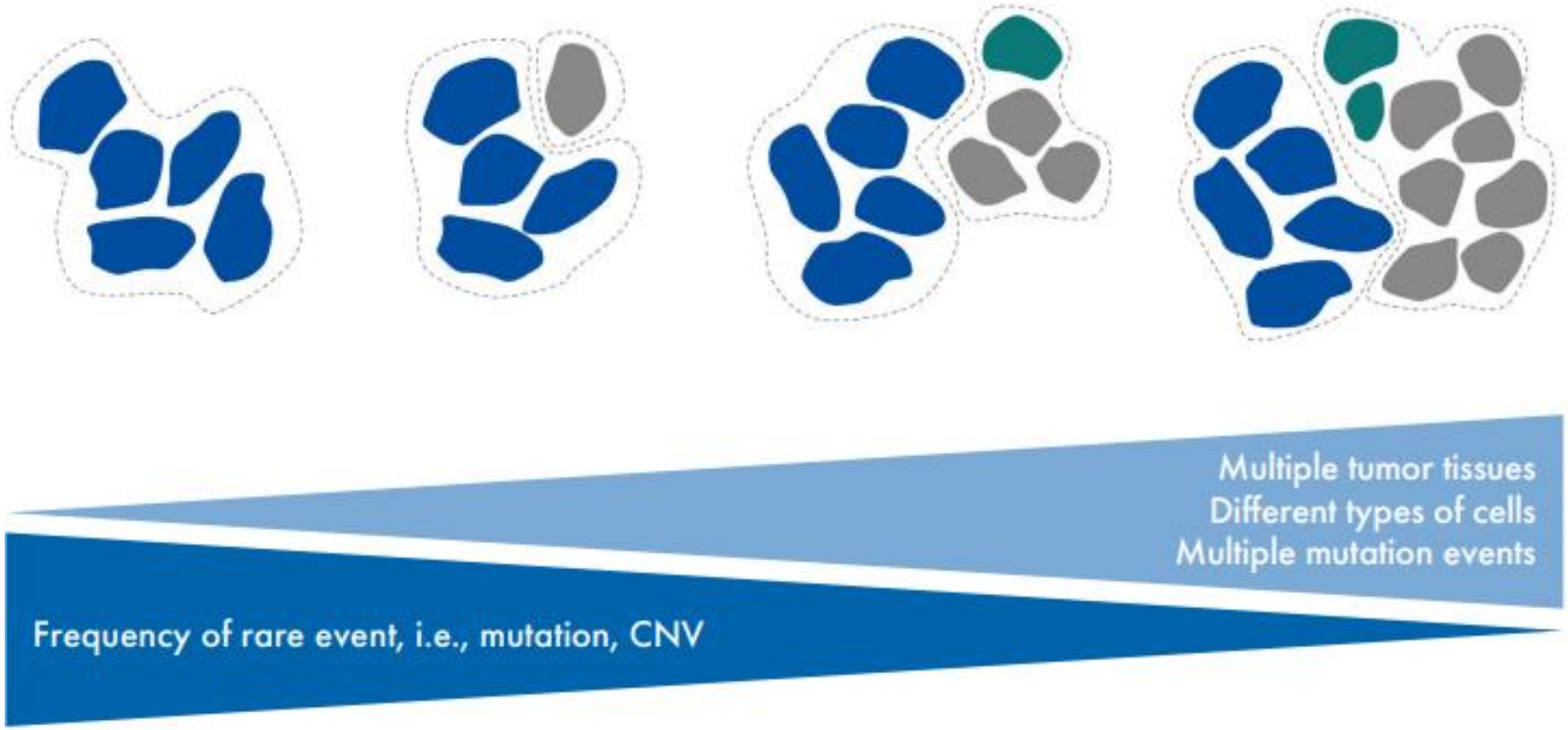
Digital PCR – positive trend in publications

Around 1850 digital PCR scientific publications at the end of 2025



Interrogating rare events in heterogeneous samples is like finding a needle in a haystack

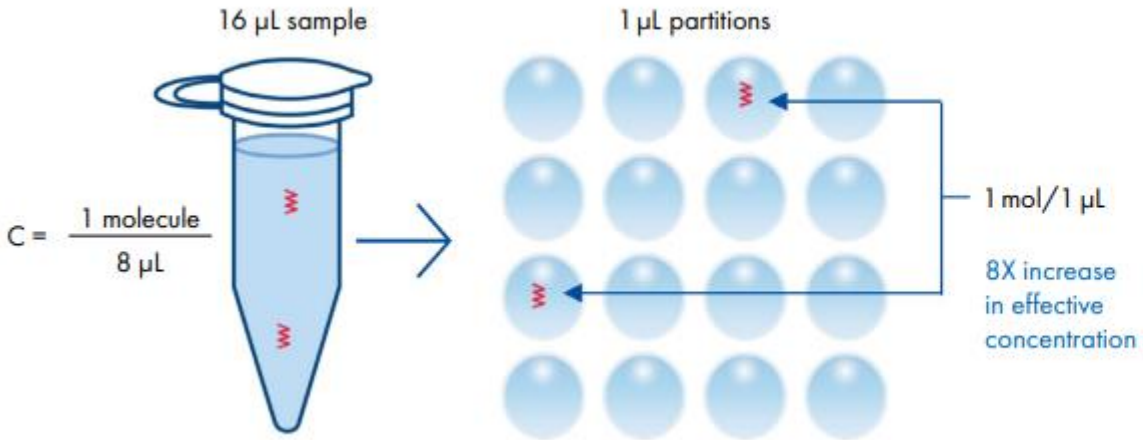
Can you spot it?



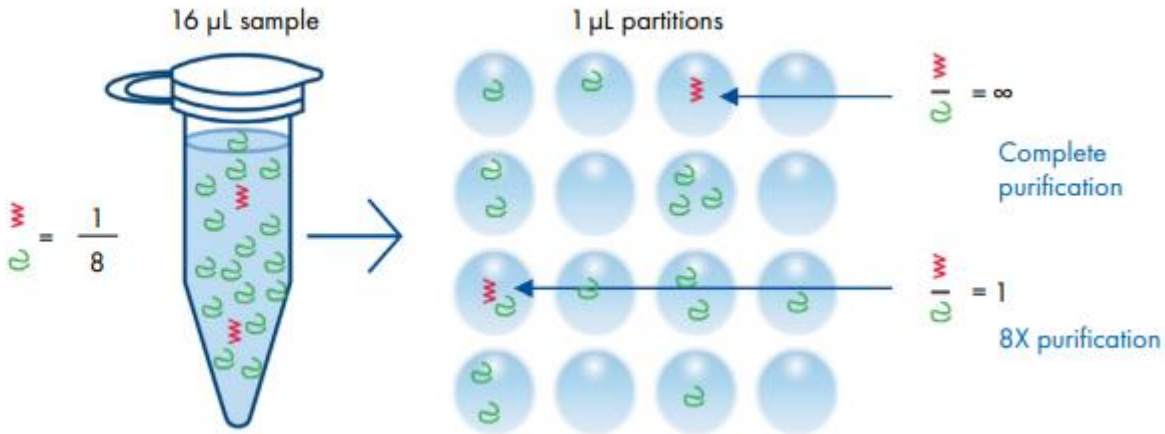
- The frequency of finding a rare event within a sample drastically decreases with an increase in heterogeneity
- The rare event is likely confined to a small fraction of cells, diluted among thousands of wild-type background cells

Partitioning for absolute quantification and increased analytical sensitivity

By partitioning bulk samples, individual target molecules can be effectively isolated from interfering molecules and detected with much higher sensitivity using digital PCR



Increase of effective concentration

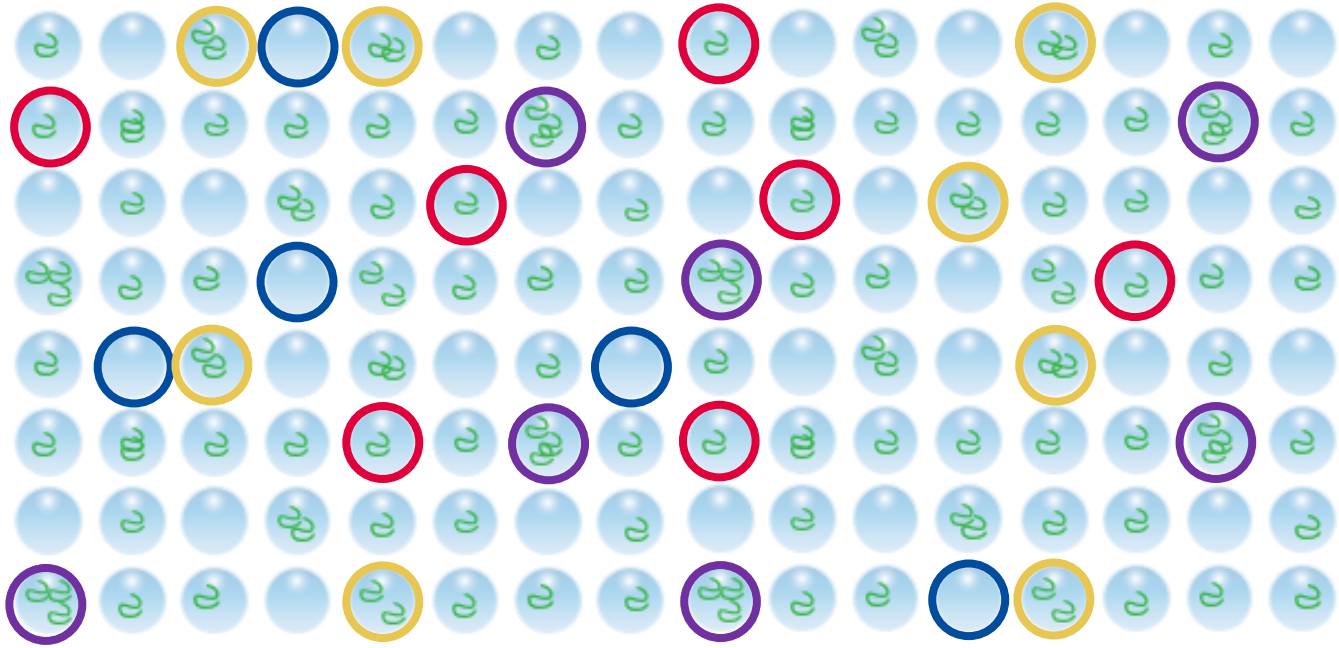


Decrease of effective concentration

Digital PCR increases the accuracy and sensitivity of detection.

Partitioning for absolute quantification and increased analytical sensitivity

dPCR estimates the average number of molecules per partition and calculates copies of the target molecule per positive partition



X number of partitions will have 0 target molecules

X number of partitions will have 1 target molecule

X number of partitions will have 2 target molecules

X number of partitions will have 3 target molecules

… up to a maximum of 12 targets per partition in a multiplex reaction*

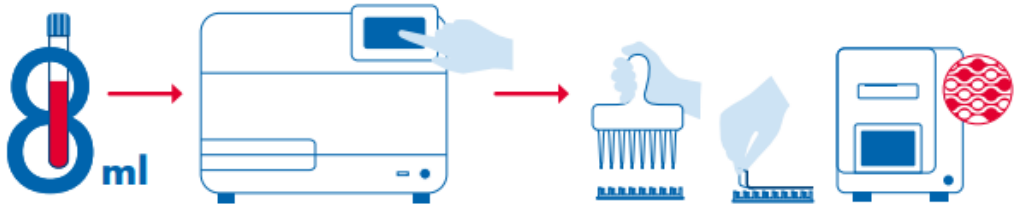
Digital PCR generates a binary signal, where 1 indicates a positive partition and 0 a negative partition.

Information on positive and negative partitions is used for concentration calculation, which is expressed as copies per µL.

***The exact number of molecules in a sample is typically unknown during an experiment. Negative partitions are therefore essential for the calculation, as they represent partitions in which the number of molecules is known to be zero.**

Partitioning for absolute quantification and increased analytical sensitivity

How do you find the needle in the haystack? Load more, see more



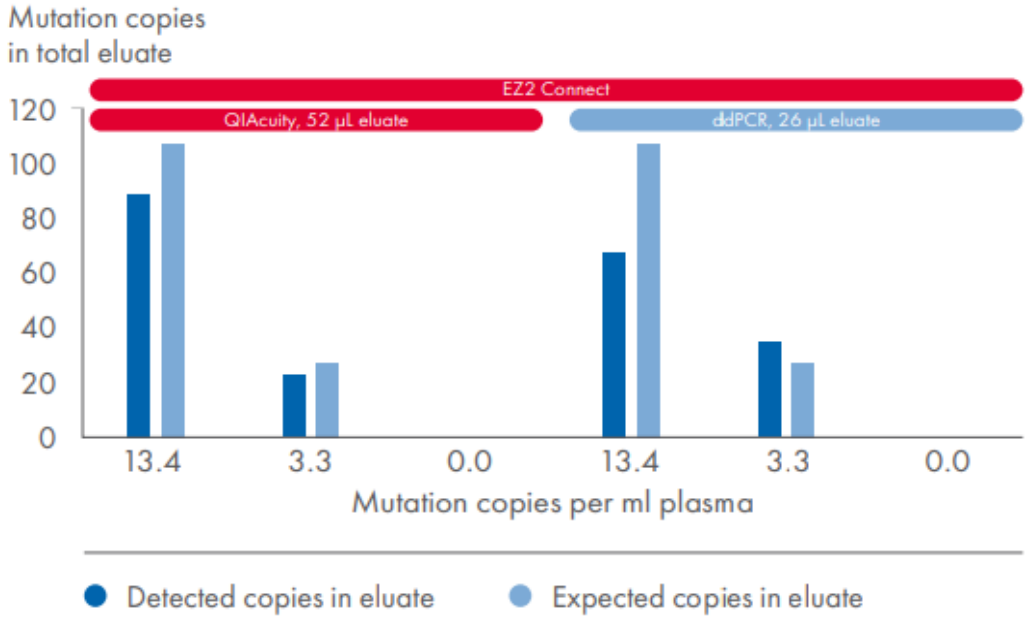
EZ2 Connect QIAcuity workflow. Up to 8 mL plasma can be processed on the EZ2 Connect combined with high eluate loading (up to 26 µL) on the QIAcuity.

Rare event scenario: 10 target copies (cp) in 70 µL eluate

	QIAcuity Nanoplate 26k	Supplier T	Supplier B*
dPCR reaction volume	40 µL	9 µL	20 µL
Maximum eluate volume	26 µL†	5.85 µL	13 µL
Copies analyzed and seen	1.85 cp	0.76 cp	1 cp

* Assuming 16K droplets.
† Based on 4x mastermix and 10x assay.

Data obtained in experiments conducted by QIAGEN R&D, Hilden, Germany.



QIAcuity detects more low-frequency mutation copies than a comparable digital PCR platform when processing high-volume plasma samples on the EZ2 Connect and analyzing them using standardized dPCR workflows

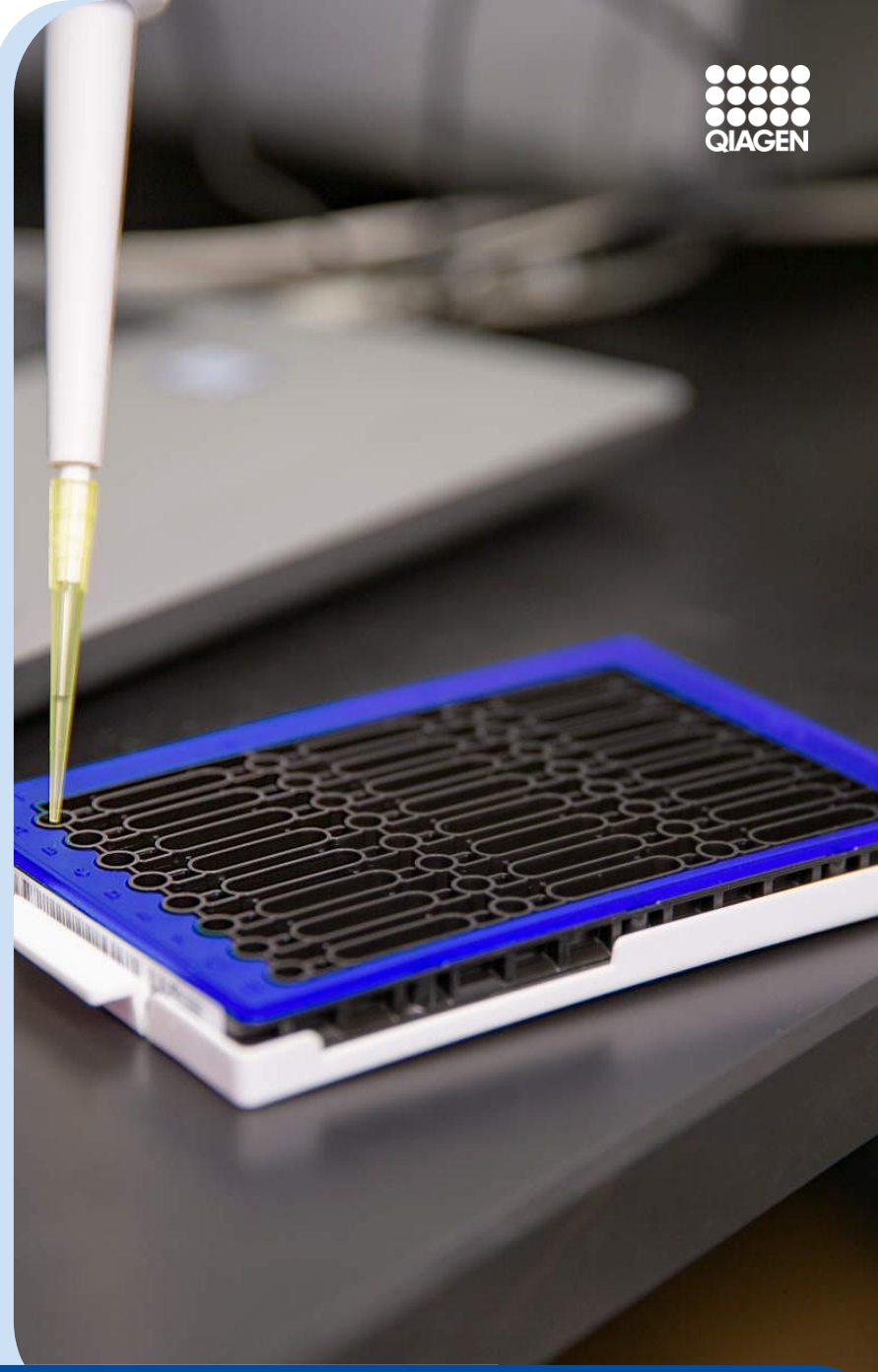
Combine sample processing on the EZ2 Connect with mutational analysis on the QIAcuity to obtain a higher number of mutation copies.

Partitioning for absolute quantification and increased analytical sensitivity

Poisson distribution

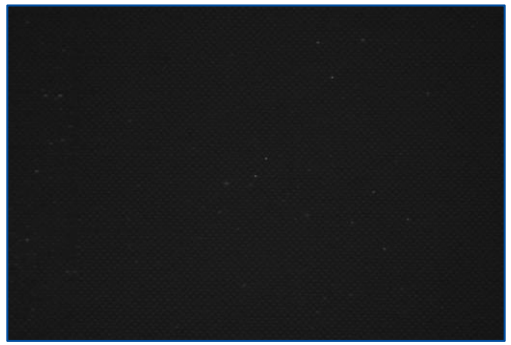
- Gives probabilities for positive, integer, random events
- λ is the expected rate of occurrences
- λ expresses the probability of a given number of events occurring in a fixed interval of time
- Can be used to calculate the copies of the target molecule per positive partition
- In digital PCR, λ depicts the most likely average value of copies/partitions per microliter:

$$\lambda = -\ln\left(\frac{number\ of\ valid\ partitions\ -\ number\ of\ positive\ partitions}{number\ of\ valid\ partitions}\right)$$



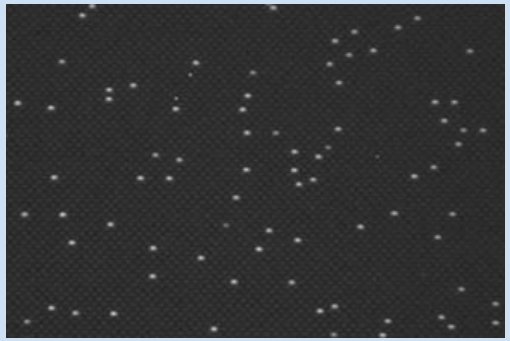
Partitioning for absolute quantification and increased analytical sensitivity

Poisson statistics at 95% confidence intervals – sample concentration estimation



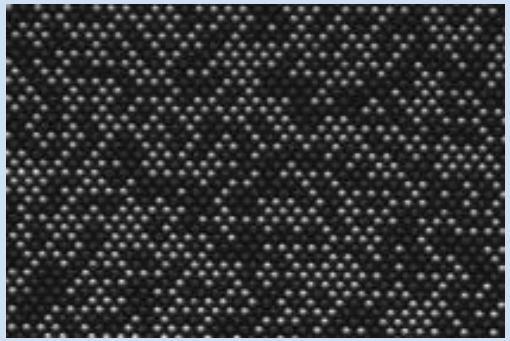
No target

All partitions are negative



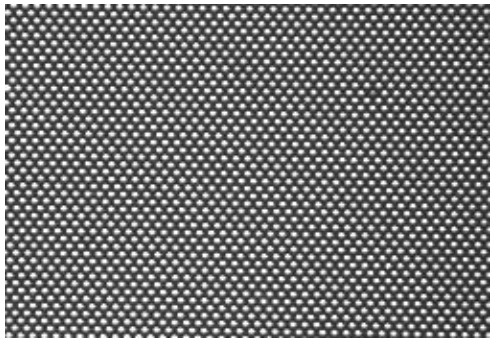
Lower end

Positive and negative partitions



Higher end

Positive and negative partitions



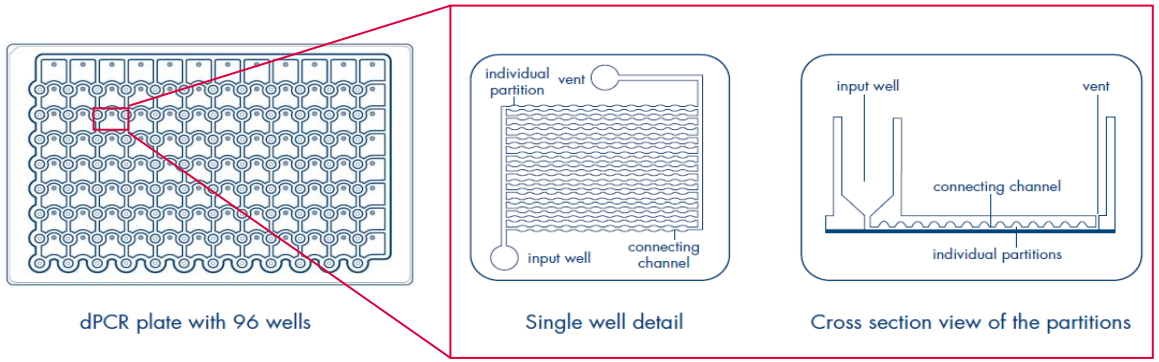
Over saturated

All partitions are positive

Average target copies per partition = -ln(1-p)
p = fraction of positive partitions

Copies of DNA target/microliter

Partitioning for absolute quantification and increased analytical sensitivity

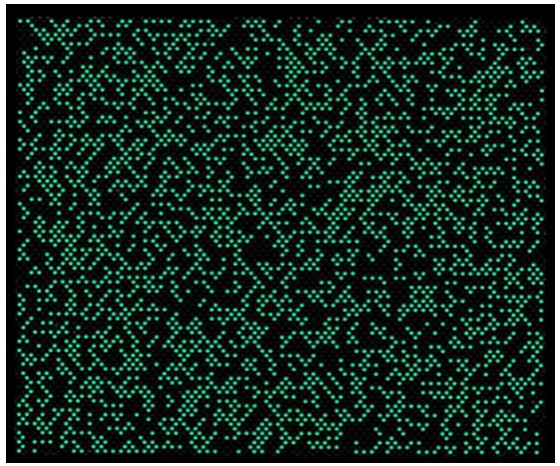


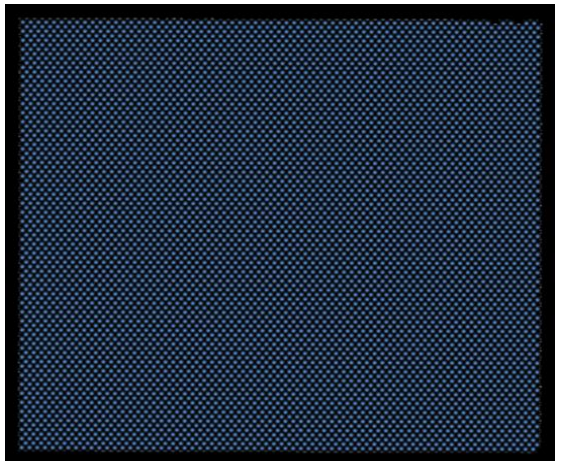
Target signal channel

- Counts the number of positive partitions
- Calculates the number of copies using Poisson statistics

Reference signal channel

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





Calculates copies/µL

The confidence interval (CI)

- Poisson distribution is **a probability distribution** that is used to model the probability that a certain number of events occur (here, copies per partition) when the events are known to occur independently and with a constant mean rate
- Poisson results always come with a defined uncertainty level
- There are different ways to calculate the CI of the Poisson results
- The algorithm used in the QIAcuity software to calculate the CI is based on “normal approximation”

$$\lambda_{range} = -ln\left(1 - p \mp z \cdot \sqrt{\frac{p(1-p)}{n}}\right)$$

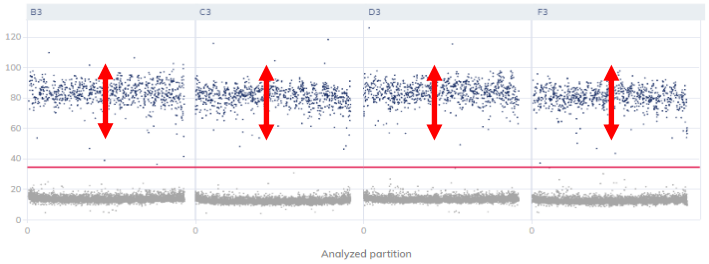
The z used in the formulas is the standard Z-value linked to the normal distribution

For example, to get 95% confidence level, we use $z = 1.96$

$$CV_{relative} = \frac{\lambda_{high} - \lambda_{low}}{2\lambda} * 100\%$$

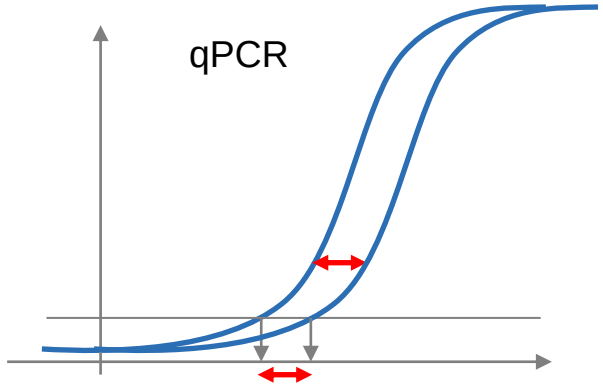
dPCR is highly efficient and unaffected by inhibitors

dPCR



Amplitude has little or no impact on the number of positive and negative partitions and calculated concentration/quantity

qPCR

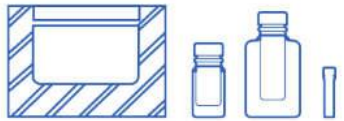


Even a slight difference in PCR efficiency has a big impact on Cq value and calculated quantity

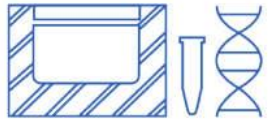
	100 %	95 %	90 %
0	1	1	1
1	2.0	1.95	1.90
2	4.0	3.8	3.6
3	8.0	7.4	6.9
4	16	14	13
5	32	28	25
20	1048576	631964	375900
21	2097152	1232329	714209
22	4194304	2403042	1356998
23	8388608	4685933	2578296
24	16777216	9137569	4898763
25	33554432	17818260	9307650
26	67108864	34745606	17684534
27	134217728	67753932	33600615
28	268435456	132120168	63841168
29	536870912	257634328	121298220

Even a 5% difference in PCR efficiency will shift the Cq value by 1 cycle, resulting in an observed 2x (100%) difference in measured quantity (even though the starting quantity was the same)

Where dPCR fits in your workflow

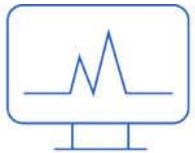


Sample preparation



Nucleic acid isolation

dPCR detection



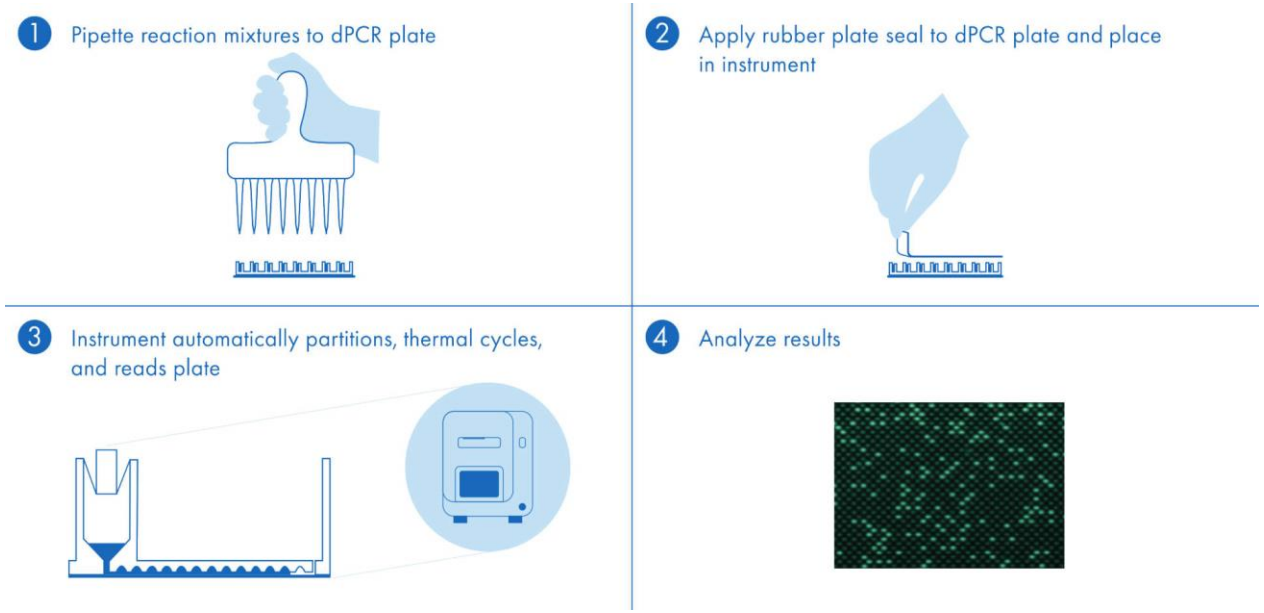
Data analysis and interpretation

Nanoplate-based dPCR

Advantages

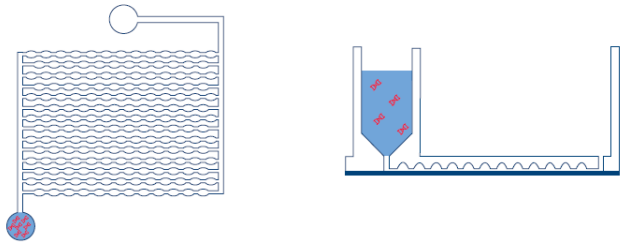
- Fixed and sealed partitions prevent variations in size and coalescence
- Sealed nanoplates prevent well-to-well contamination
- Faster readout due to simultaneous reading of all partitions of a sample
- Simple workflow and user-friendly handling, similar to qPCR
- Plates are not influenced by detergents
- Possibility to re-read plates after first imaging
- Plates are amenable to front-end automation
- New plate types can be easily added
- Under 2-hour time to result

Workflow



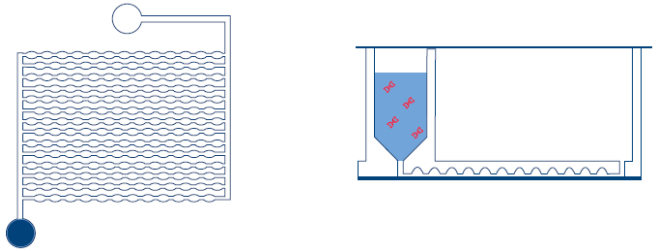
QIAcuity – fast and with minimal hands-on time

1



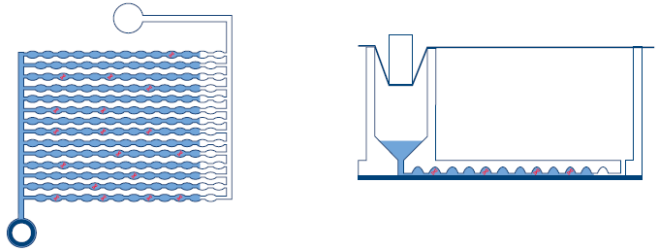
The PCR reaction mixture is pipetted into the input wells

2



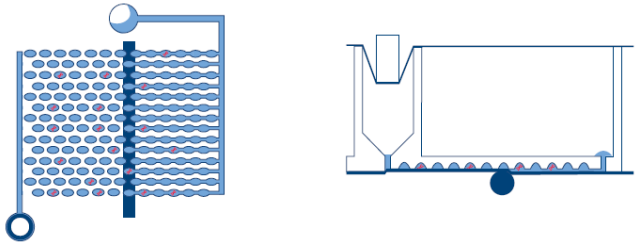
A rubber seal is applied to the top of the plate to seal it

3



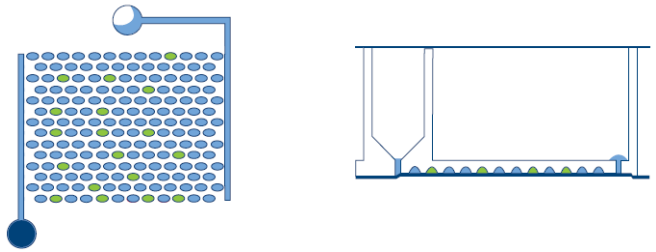
The pistons in the dPCR system push PCR reaction mixture through partitions automatically

4



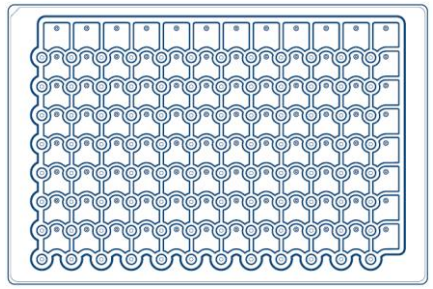
A roller compresses the bottom seal and seals partitions individually, distributing individual copies of target DNA throughout the partitions

5



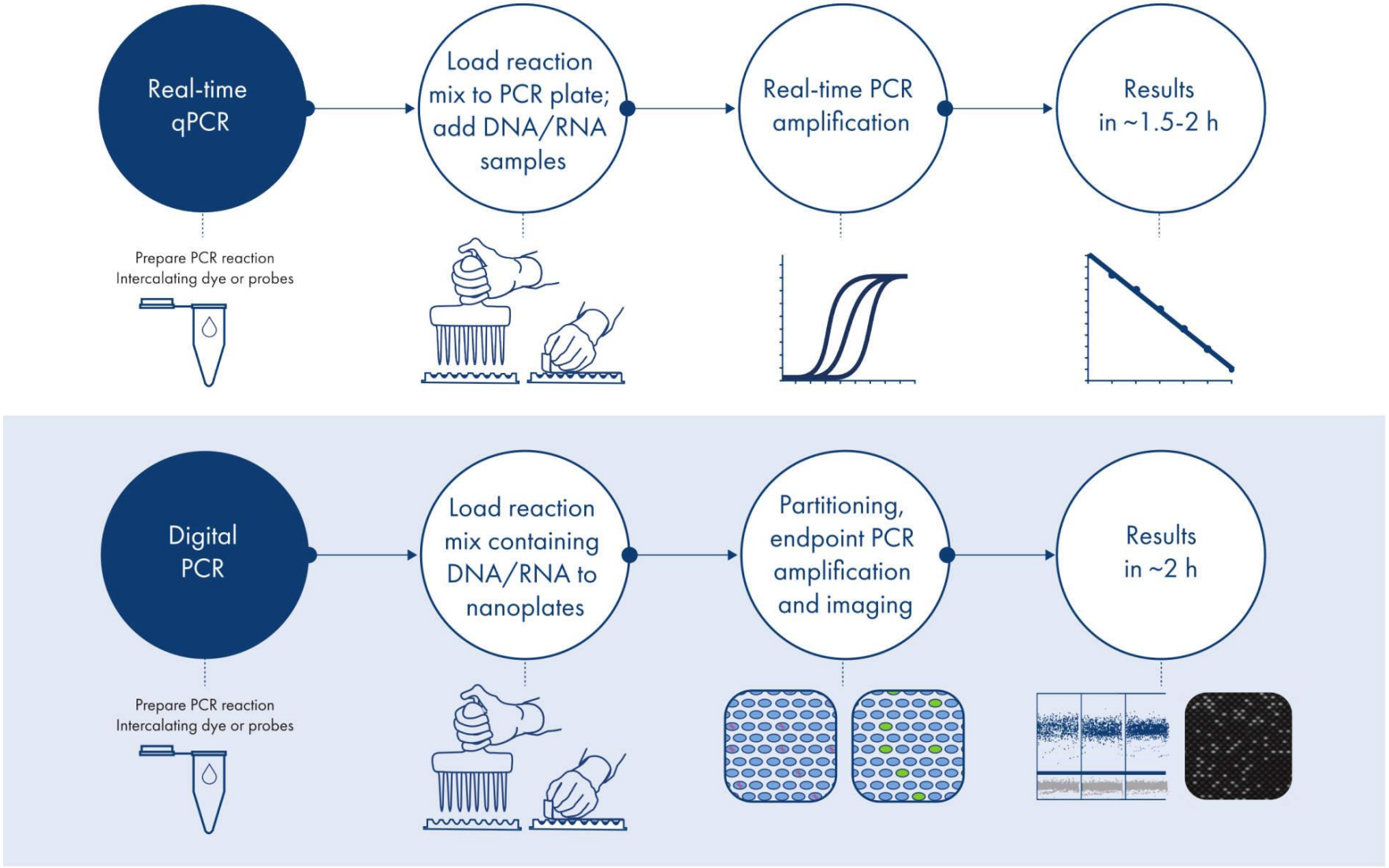
During thermocycling, the target DNA is doubled with each cycle, and partitions containing the target DNA fluoresce

6

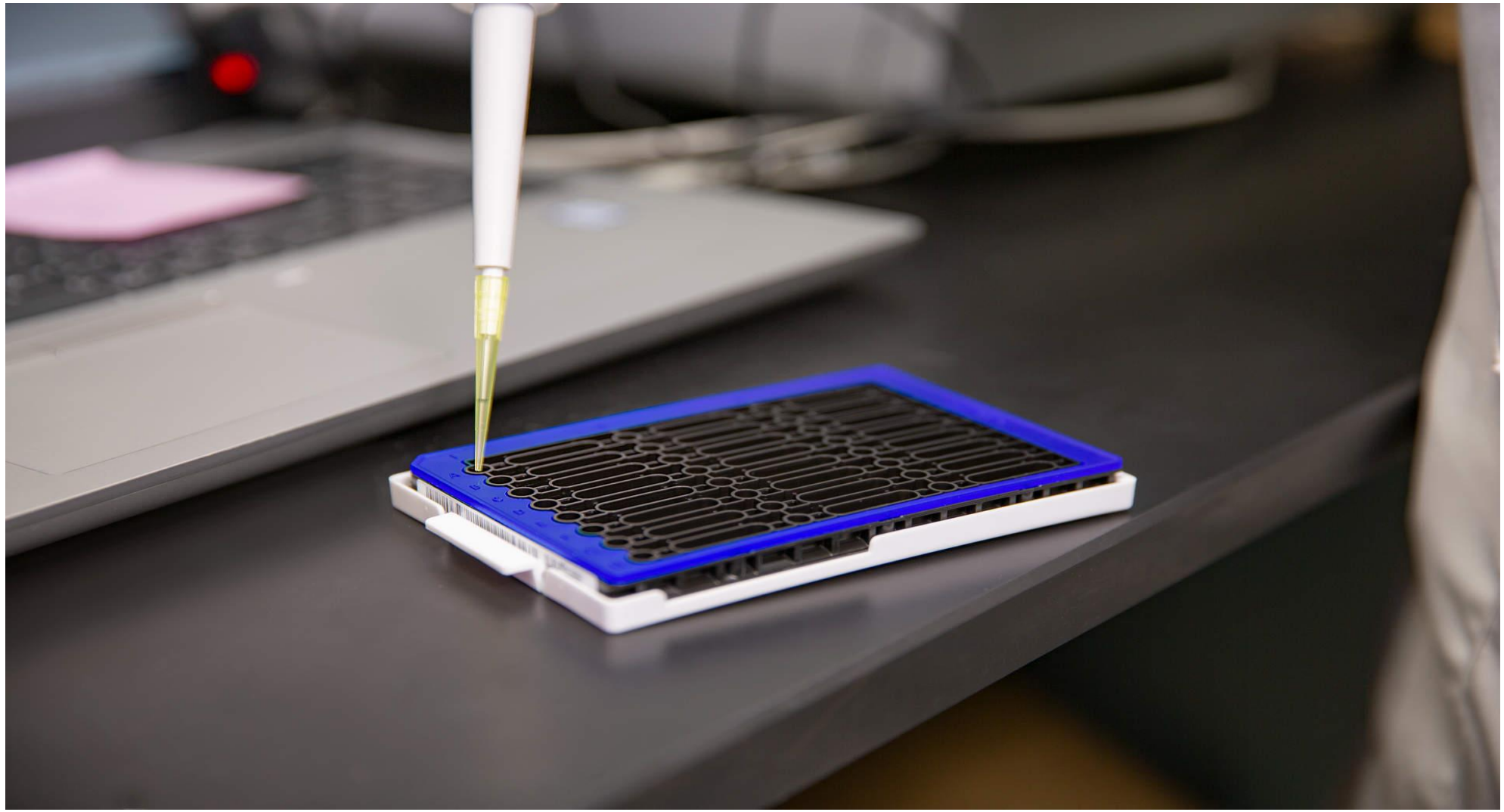


The plate is imaged to count the number of positive/fluorescent partitions

Real time qPCR versus QIAcuity dPCR workflow

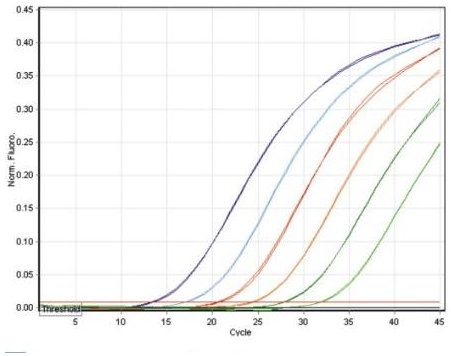


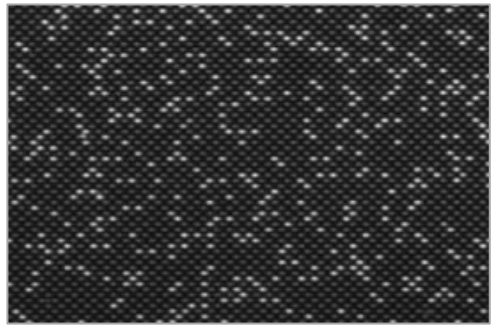
QIAcuity – workflow in action



The future of PCR is digital

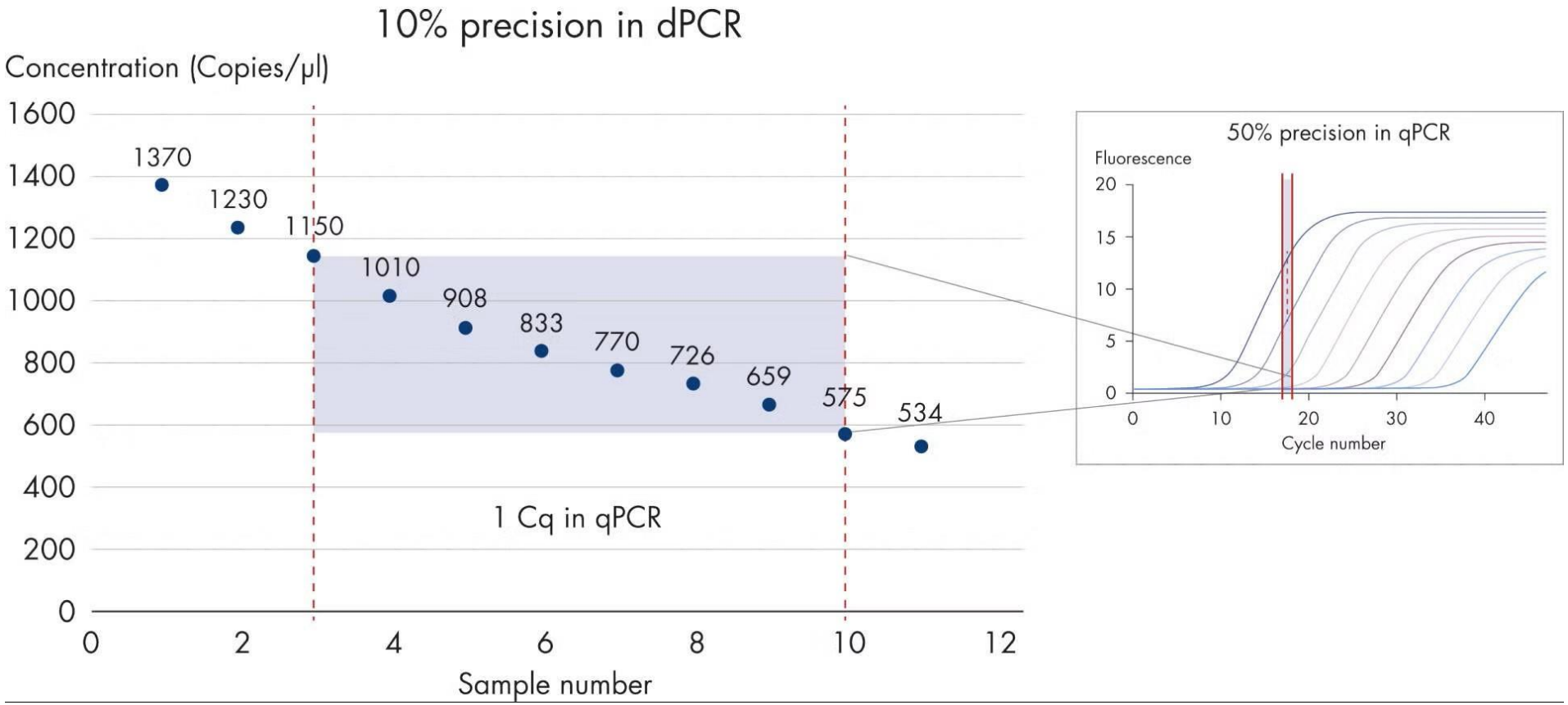
Evaluating strengths of dPCR over qPCR





	qPCR	dPCR
Precision	Low; detects mutation rate at >1%	High; detects mutation rate at ≥0.001%
Reaction	Bulk reaction; flexible volumes	Fixed reaction volume in partitions; higher inhibitor tolerance and statistical power
Detection	Broad dynamic range	Detect small fold changes and rare targets; ±10% precision
Standard curve	Yes; relative quantification	No; absolute quantification
Tolerance to PCR inhibitors	Lower	Higher; robust quantification
Reproducibility	Lower	Higher

Key advantage of dPCR compared to qPCR



When the Cq difference is big enough, qPCR can detect a target of interest with confidence

When the difference in Cq is small, qPCR lacks the precision needed to detect a target, even when it is present

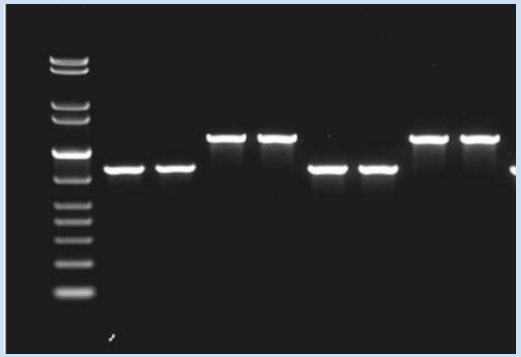
dPCR detects small differences in expression with precision

Comparison of PCR techniques

First generation

Conventional PCR

All molecules in one reaction volume



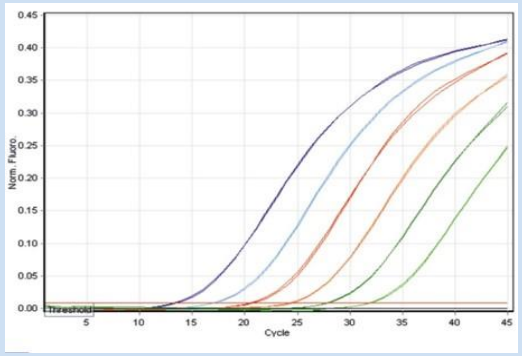
Qualitative

- Technically simple
- Multiplexing capabilities
- End-point detection
- Low cost

Second generation

Real-time PCR

All molecules in one reaction volume



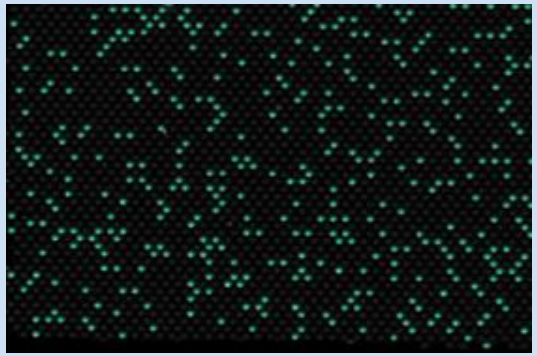
Relative quantification

- High accuracy, sensitivity and specificity
- Rapid cycling and throughput
- Non-specific amplification

Third generation

Digital PCR

Random distribution of molecules into partitions



Absolute quantification

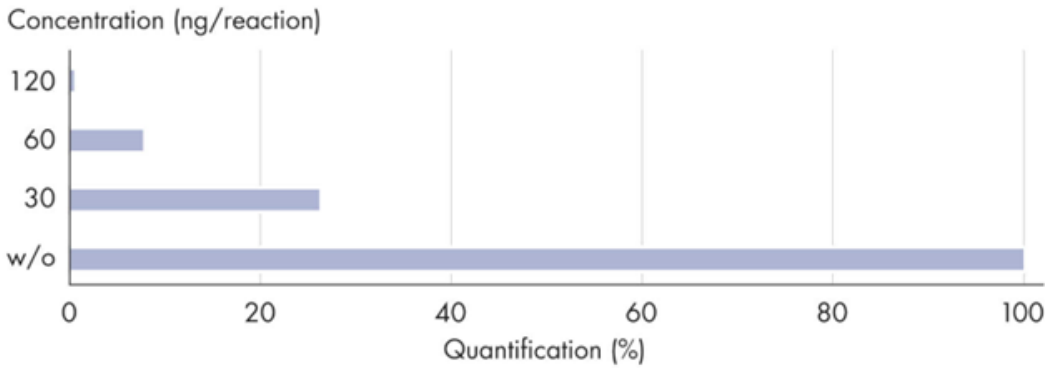
- No standard curves
- Higher precision and reproducibility
- Low sensitivity to inhibitors
- End-point detection

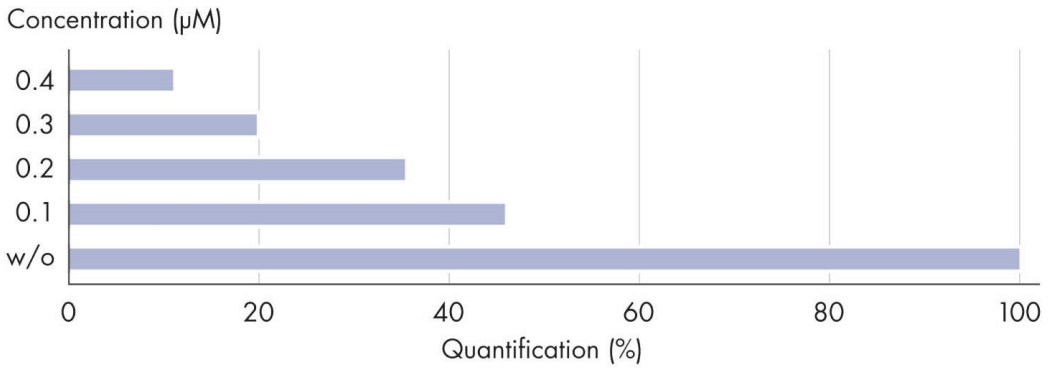
Robustness of a dPCR reaction

Humic acid

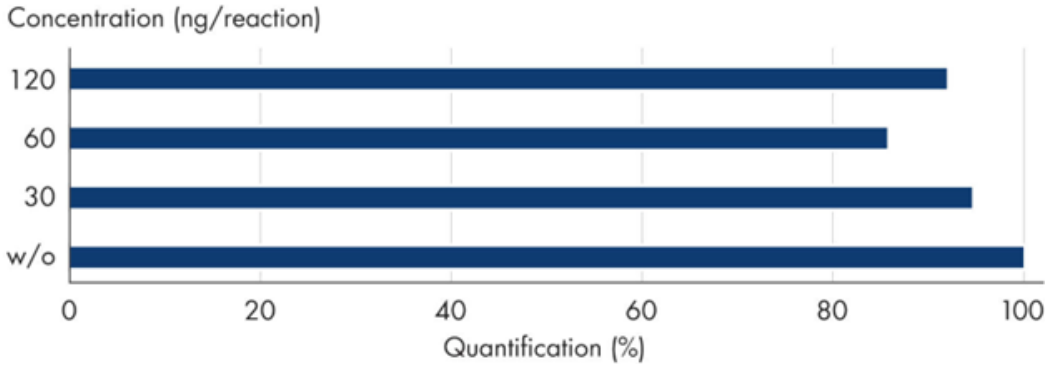
Heparin

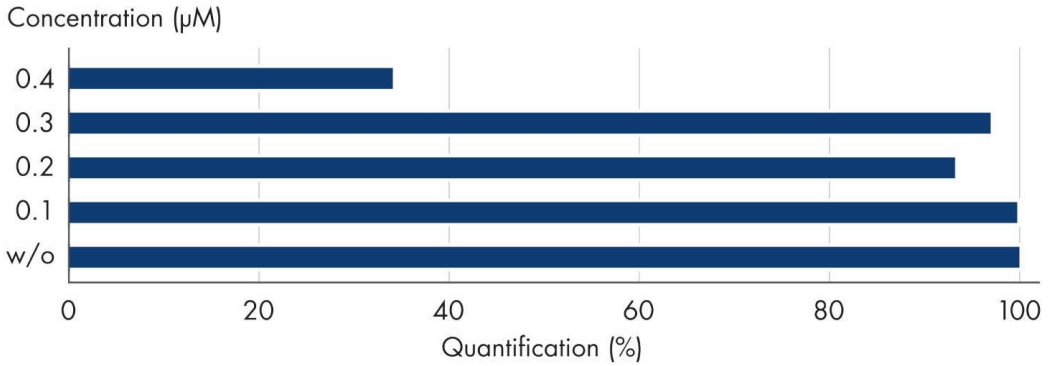
qPCR





dPCR





Highly sensitive *KRAS* mutation detection using dPCR

High sensitivity at 0.05% (Mut:WT) level with 100 ng total sample input

QIAGEN KRAS assay sensitivity with 100 ng total input on the QIAcuity Four instrument

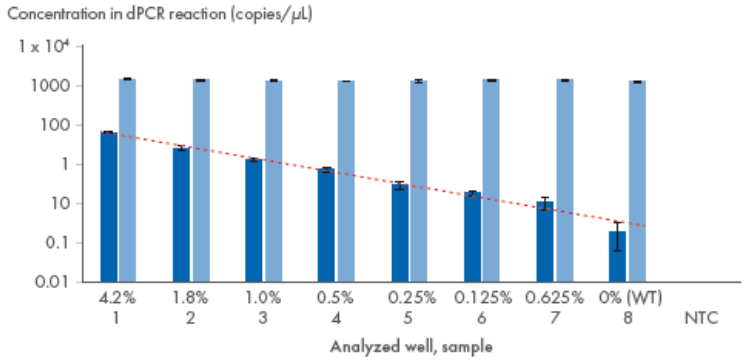
QIAGEN KRAS (G12S; GGT/AGT) LNA Probe 2-plex assay with KRAS G12S reference gDNA from SW48 cell line (Horizon Discovery)

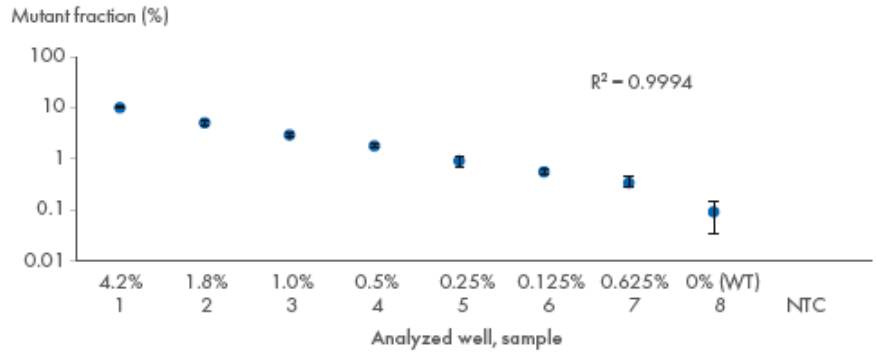
KRAS (G12S) mutation percentage

	1	2	3	
A	4.24%	4.34%	4.08%	4.4% mut
B	1.87%	0.67%	1.96%	2.2% mut
C	1.00%	0.90%	0.89%	1.0% mut
D	0.5%	0.61%	0.52%	0.5% mut
E	0.24%	0.18%	0.25%	0.25% mut
F	0.11%	0.14%	0.13%	0.125% mut
G	0.06%	0.10%	0.07%	0.05% mut
H	0.02%	0.01%	0%	0% (WT)

LNA mutation (FAM) and WT (HEX) 2-plex assay is run against KRAS (G12S) mutant titration with a constant 100 ng of WT background and differing mutant percentages in the mixture (plus NTC in well H3);
3 replicates.

High mutant titration correlation (R2 = 0.9994) with constant 100 ng WT total input





2-plex digital PCR assay conditions: 95°C 2 min; 95°C 15 s/60°C 1 m (40X);
Imaging conditions: 500 ms/Gain 6 (FAM/HEX)

When to choose dPCR vs. NGS

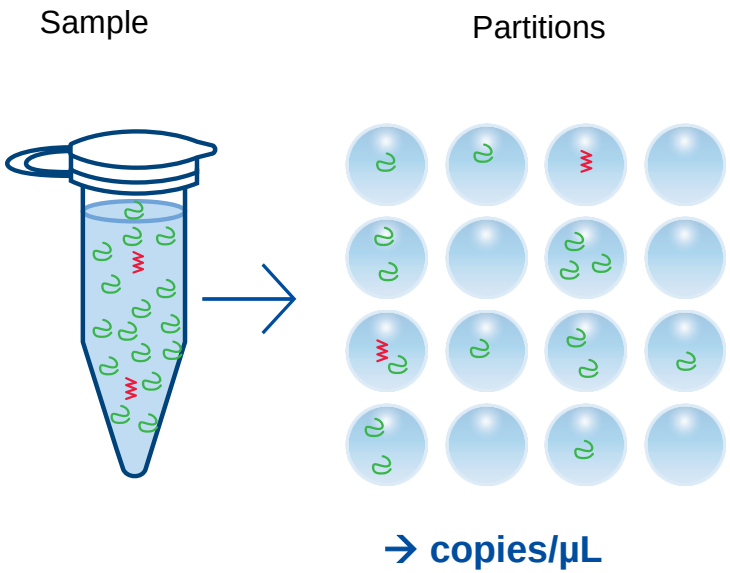
	Choose dPCR when:	Choose NGS when:
Mutation detection	• Detecting **known mutations** with **ultra-high sensitivity**	• Detecting **known and unknown mutations**, fusions, or indels across multiple genes
Low allele frequency variants	• Looking for **rare variants** (down to 0.01%) in cfDNA, FFPE, etc.	• Doing broader profiling
Quantification	• Needing **absolute quantification** (e.g., copy number, allele frequency, viral load)	• Needing **variant calling** or **genomic profiling**, precise copy number quantification more difficult
Speed and cost	• Requiring **faster, cheaper** answers for targeted questions	• Having **more expensive/better** sequencing and bioinformatics pipelines
Target scope	• Having a **small number of targets**	• Looking at **many genes** or **exploring unknowns** (e.g., exome or genome-wide)
Clinical monitoring	• Doing **minimal residual disease (MRD)** or **treatment monitoring**	• Profiling the **tumor landscape** for treatment decisions or research discovery
Regulatory/validation	• Needing a **simple, reproducible, CLIA-validated assay**	• Doing **comprehensive characterization** or **biomarker discovery**

dPCR and NGS complement each other.

Why choose dPCR?

Partitioning and end-point PCR for absolute quantification and increased analytical sensitivity

- The sample is distributed into multiple single partitions
- The target molecules are randomly distributed across all partitions



Absolute target quantification

No need for references or standard curves

Higher tolerance to inhibitors

Due to partitioning and end-point measurement

Precision

Detect very small fold-change differences

Increased analytical sensitivity

Detect rare mutations and low-abundance targets

High reproducibility

Eliminate amplification efficiency bias

Image adapted from: Basu AS. Digital Assays Part I: Partitioning Statistics and Digital PCR. *SLAS Technol.* 2017;22(4):369-386.

The future of PCR is digital

- Introduction to digital PCR
- PCR methods – principles and workflow
- QIAcuity – a scalable instrument
- QIAcuity Software Suite
- Multiplexing
- Applications
- Assays and capabilities

QIAcuity – a nanoplate-based digital PCR system




Feature	QIAcuity One	QIAcuity Four	QIAcuity Eight	QIAcuityDx Four (IVD)
Plates processed	1	4	8	4
Thermocycler(s)	1	1	2	1
Time to result	~2 hours	First plate ~2 hours Every ~ 80 minutes a following plate	First plate ~2 hours Every ~ 40 minutes a following plate	First plate ~2 hours Every ~ 80 minutes a following plate
Detection channels	2/ 6 (up to 12-plex)	6 (up to 12-plex)	6 (up to 12-plex)	5 (5-plex)
Throughput (samples processed in a workday)	Up to 384 samples (96-well) Up to 96 samples (24-well)	Up to 672 samples (96-well) Up to 168 samples (24-well)	Up to 1248 samples (96-well) Up to 312 samples (24-well)	192 samples in Dx mode (Dx 24 well, 26,000 partition plate) 768 samples in Utility Mode (Research Use: 96 well, 8,000 partition plate) (8h working day)

QIAcuity Eight allows analysis of up to 1248 samples in a workday.

QIAcuityDx is intended for in vitro diagnostic use. Product availability may differ from country to country based on regulations and approvals. Contact your country representative for further details. QIAcuity One, QIAcuity Four and QIAcuity Eight are intended for non-clinical applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

QIAcuity – a nanoplate-based digital PCR system

Detect more in a single reaction

- Fully integrated workflow with time to result in ~2 hours
- Distinct nanoplate configurations with flexible sample formats for a wide range of throughput and sensitivity requirements
- Hands-on and qPCR-like workflow
- Flexible throughput and resolution
- High multiplexing (6-plex and beyond)
- Integrated software suite for data analysis

A fully integrated solution

QIAcuity – a nanoplate-based digital PCR system

Detection channels and fluorophores

Channel	Excitation (nm)	Emission (nm)	Example fluorophores
Green	463–503	519–549	FAM™, EvaGreen®, Atto 488, Alexa Fluor® 488
Yellow	513–534	551–565	HEX™, VIC®
Orange	541–563	582–608	TAMRA™, Atto 550
Red	568–594	613–655	ROX™, Texas Red®
Crimson	588–638	656–694	Cy5®, Quasar 680
Far red	651-690	709-759	Cy5.5. Atto 680, Atto700
Green / Yellow	463–503	551–565	DY-482XL (LSS G/Y)*
Orange / Red	541–563	613–655	DY-540XL (LSS O/R)*

* For long stoke shift (LSS) dyes, the software provides generic dye names called "LSS" followed by the abbreviation of the used channel combination denoted by the first channel letters. For example, channel combination Green/Yellow is abbreviated as "LSS G/Y".

QIAcuity Nanoplates – specifications

Distinct nanoplate configurations with flexible sample formats for a wide range of throughput and sensitivity requirements

Instrument	Type	Frame color	Partitions/ well	Input volume	Applications
QIAcuity Digital PCR System	Nanoplate 26K 8-well (# 250031)	Light blue	approx. 26,000	40 µL	Rare mutation detection, liquid biopsy, pathogen detection, etc.
	Nanoplate 26K 24-well (# 250001)	Blue	approx. 26,000	40 µL	
	Nanoplate 8.5K 24-well (# 250011)	White	approx. 8500	12 µL	Copy number variation analysis, gene expression analysis, NGS library quantification, genome editing detection, etc.
	Nanoplate 8.5K 96-well (# 250021)	Gray	approx. 8500	12 µL	
QIAcuityDx	Nanoplate 26k 24-well (# 260001)	Red	approx. 26,000	40 µL	Rare mutation detection, liquid biopsy, pathogen detection etc. Utility- and IVD-mode

Nanoplate 26K 8-well, Nanoplate 26K 24-well, Nanoplate 8.5K 24-well (# 250011) and Nanoplate 8.5K 96-well (# 250021) are intended for non-clinical applications. These products are not intended for the diagnosis, prevention or treatment of a disease. Nanoplate 26k 24-well (# 260001) is intended for in vitro diagnostic use. Product availability may differ from country to country based on regulations and approvals. Contact your country representative for further details.

QIAcuityDx – a nanoplate-based digital PCR system for diagnostic applications

QIAcuityDx Four

Region	Classification
EU-IVDR	**Class A (Rule 5)**
USA (FDA)	**Class II; 510(k) exempt**

QIAcuityDx Software

QIAcuityDx Nanoplate 26k 24-well

Region	Classification
EU-IVDR	**Class A (Rule 5)**
USA (FDA)	**System consumable Class II;510(k) exempt**

QIAcuityDx Universal MasterMix Kit (1 mL and 5 mL)

Region	Classification
EU-IVDR	**Class A (Rule 5)**
USA (FDA)	**General purpose reagent Class I;510(k) exempt**

- Fully integrated workflow with time to result in ~2 hours
- Dual mode software: Utility (open) and IVD (closed) modes
- Integrated software suite for data analysis

Nanoplate 26K 8-well, Nanoplate 26K 24-well, Nanoplate 8.5K 24-well (# 250011) and Nanoplate 8.5K 96-well (# 250021) are intended for non-clinical applications. These products are not intended for the diagnosis, prevention or treatment of a disease. Nanoplate 26k 24-well (# 260001) is intended for in vitro diagnostic use. Product availability may differ from country to country based on regulations and approvals. Contact your country representative for further details.

QIAcuity – detection channels and fluorophores

Detection channels	Recommended dyes
Green	FAM, EvaGreen
Yellow	VIC, HEX
Orange	TAMRA
Red	ROX
Crimson	Cy5
Far Red*	ATTO700*

* Only with software 3.0 and higher and in combination with the High Multiplex Probe PCR Kit

QIAcuity – mastermixes

Master Mix	QIAcuity Probe PCR Kit	QIAcuity EG PCR Kit	QIAcuity UCP Probe PCR Kit	QIAcuity OneStep Advanced Probe Kit	QIAcuity High Multiplex Probe PCR Kit	QIAcuity MasterMix	QIAcuityDx Universal MasterMix Kit
Specification	"Standard Master Mix" for single and multiplex use (1- to 5-plex)	EvaGreen-based master mix	For singleplex and up to 5-plex reactions	For singleplex and multiplex reactions Also available with Eva Green	To ensure best performance as improved specificity vs. background also applies for mutation detection	For singleplex and up to 5-plex reactions	For singleplex and up to 5-plex reactions
Description	Optimized for best performance in nanoplate microfluidic Includes special reference dye needed for dPCR analysis and counting analyzable partitions	Optimized for best performance in nanoplate microfluidic Includes special reference dye needed for dPCR analysis and counting analyzable partitions	Ultra Clean Production (UCP) master mix ideal for applications requiring high purity and accuracy, minimizing contaminating background DNA Also recommended for quality control applications, like residual DNA testing	For one-step RT-PCR on QIAcuity digital PCR instruments, enabling reverse transcription and PCR amplification in a single reaction Advanced robustness against inhibitors	Suitable for challenging samples and dPCR multiplexing beyond five targets	Improved specificity vs. background for mutation detection and lentivirus applications	For use with the QIAcuityDx System and QIAcuityDx Nanoplates for in vitro diagnostic applications Option to tweak binding parameter by titrating $MgCl_2$
Application	Gene expression analysis, mutation detection and copy number variation analysis	Applications requiring EvaGreen dye-based detection, such as gene expression analysis and genotyping	Microbial analysis workflows, such as quantification of bacterial or fungal DNA	Gene expression analysis from RNA templates, viral RNA detection and simultaneous RNA and DNA target detection	Complex genomic analyses requiring detection of multiple targets simultaneously	For use with dPCR LNA Mutation Assays	In vitro diagnostic applications requiring accurate quantification of DNA or cDNA targets

For RNA samples and cDNA synthesis, the QuantiNova Reverse Transcription Kit is recommended upfront.

QIAcuity Probe PCR Kit, QIAcuity EG PCR Kit, QIAcuity UCP Probe PCR Kit, QIAcuity OneStep Advanced Probe Kit, QIAcuity High Multiplex Probe PCR Kit are intended for non-clinical applications. These products are not intended for the diagnosis, prevention or treatment of a disease. QIAcuityDx Universal MasterMix Kit is intended for in vitro diagnostic use. Product availability may differ from country to country based on regulations and approvals. Contact your country representative for further details.

Why dPCR and mass-based measurements may lead to different amounts

dPCR	Mass-based methods
Quantifies absolute DNA copy numbers by partitioning the sample and counting positive partitions	Measure total nucleic acid concentration (ng/µL) based on absorbance or fluorescence (UV-VIS/Qubit)
Resistant to contaminants affecting quantification (detects intact target molecules)	Can be affected by sample impurities or degraded fragments
Measures intact and fragmented DNA/RNA	May overestimate concentration if degraded DNA or RNA fragments are present, as they still absorb light or fluoresce
Unaffected by amplification efficiency	

The future of PCR is digital

- Introduction to digital PCR
- PCR methods – principles and workflow
- QIAcuity – a scalable instrument
- QIAcuity Software Suite
- Multiplexing
- Applications
- Assays and capabilities

Remote analysis using QIAcuity Software Suite

Software concept

Embedded instrument software

dPCR Software Suite installed on a computer or server

dPCR Software Suite (web application)

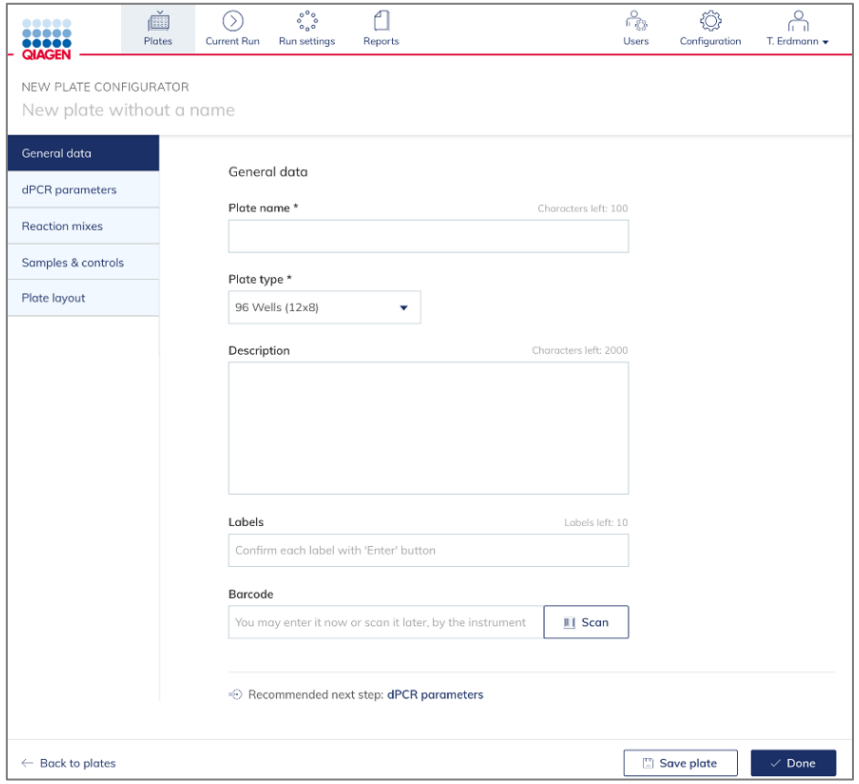
Access, design and analyze from anywhere

- Software updates are free of charge
- License-free software available that is also GMP compliant
- Network connectivity

Designing plates and experiments using QIAcuity Software Suite

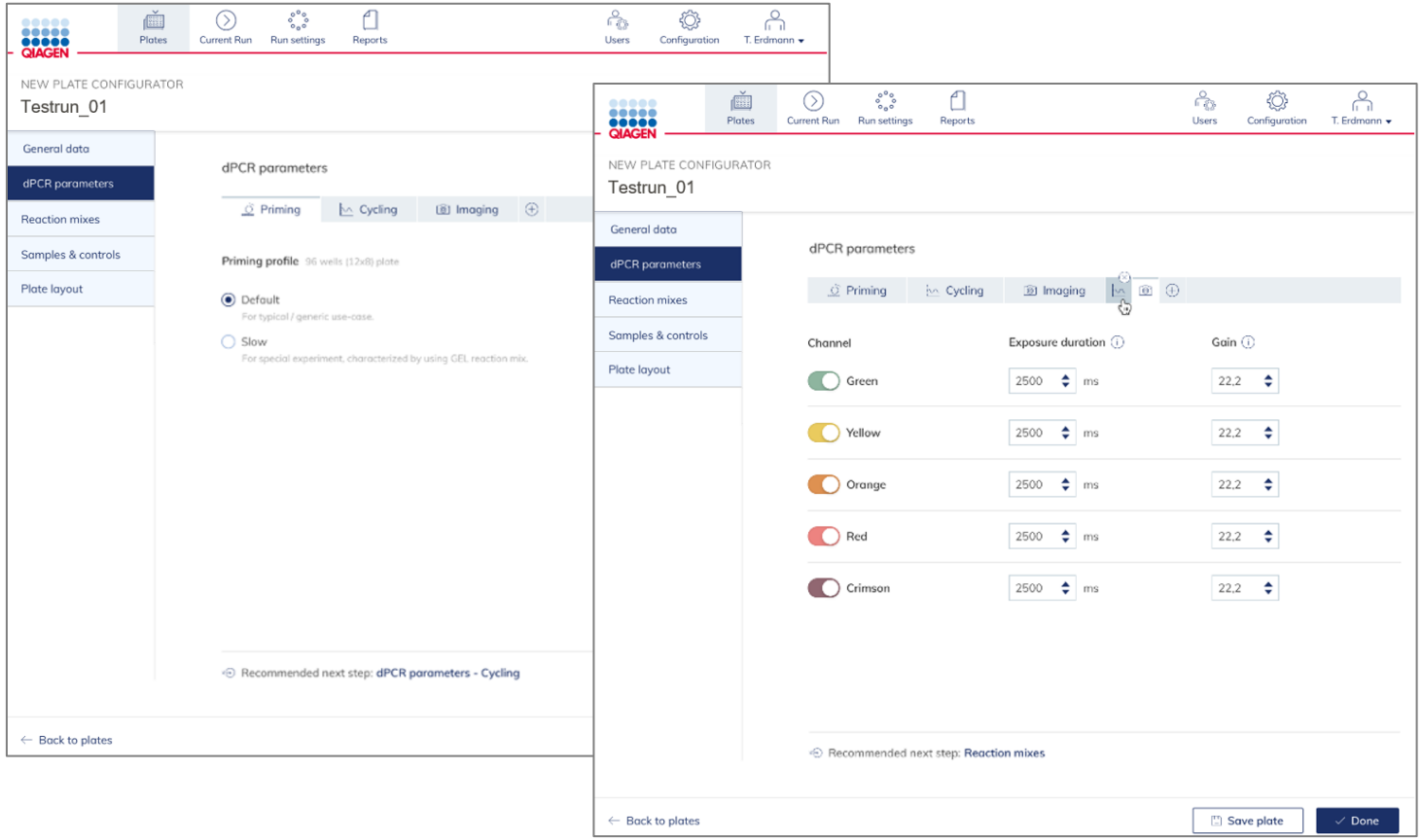
General data

Plate name, type, description, labels, plate ID



dPCR parameters

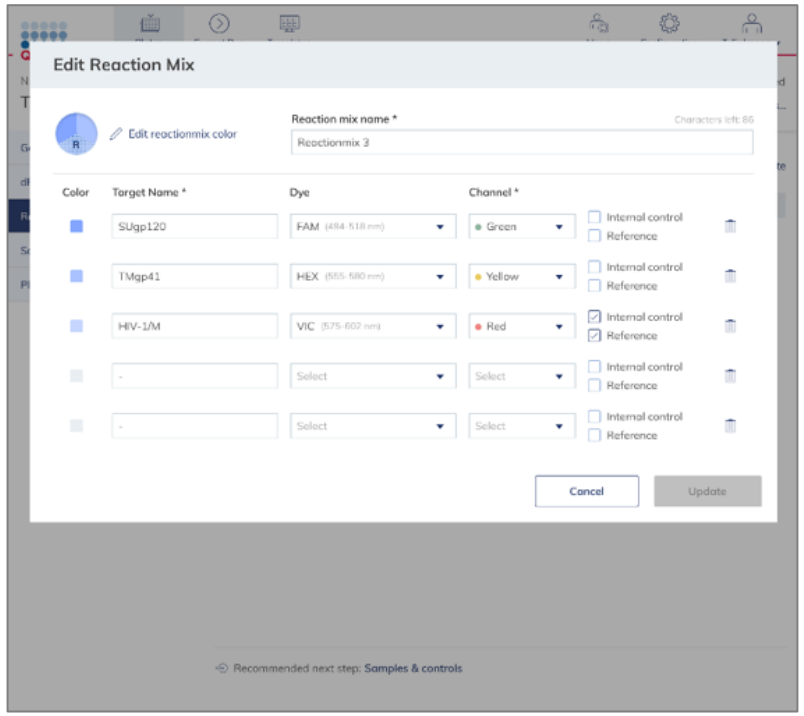
Priming, cycling profile, imaging



Designing plates and experiments using QIAcuity Software Suite

Reaction mix

Targets, dyes and detection channels define internal controls and references



Samples and controls

Define samples, controls and NTC

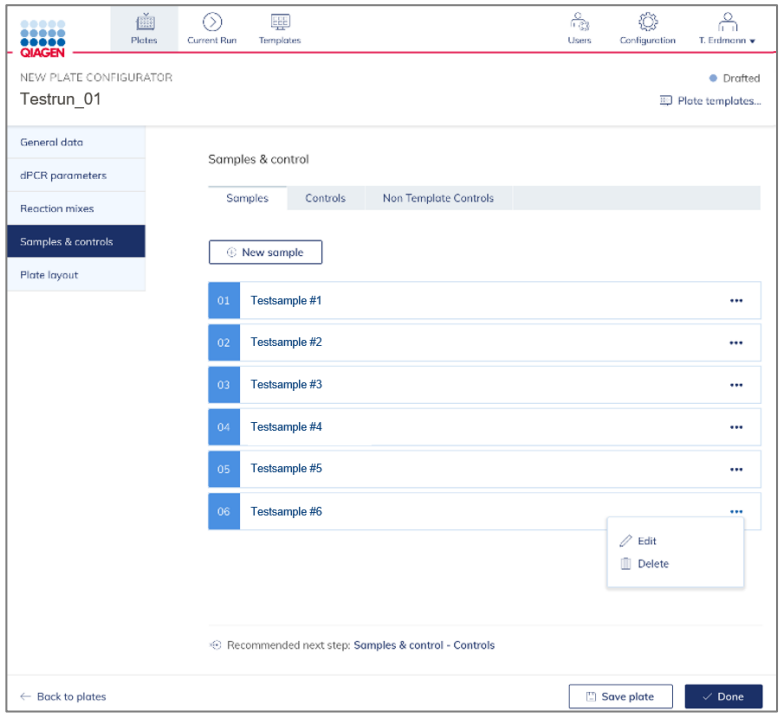
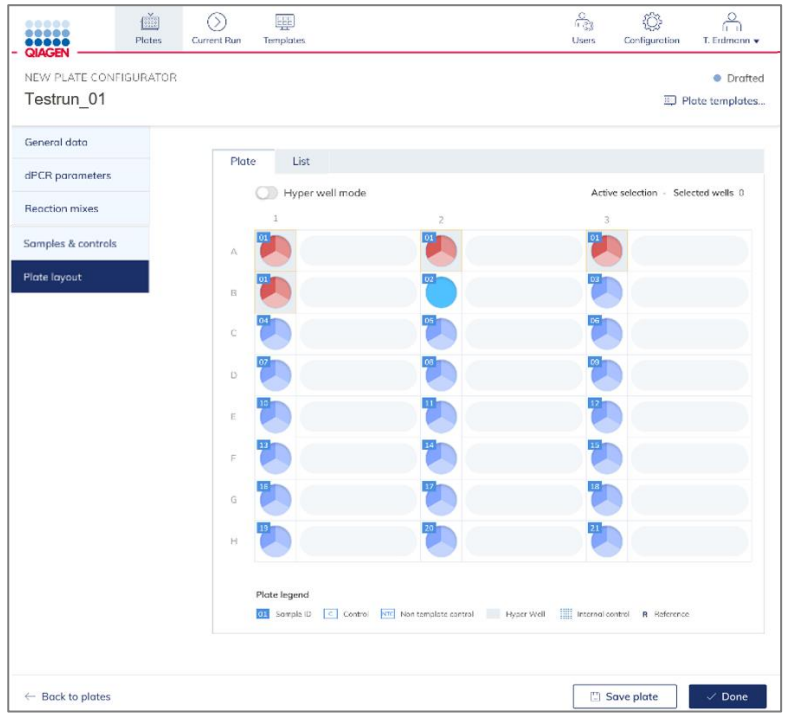
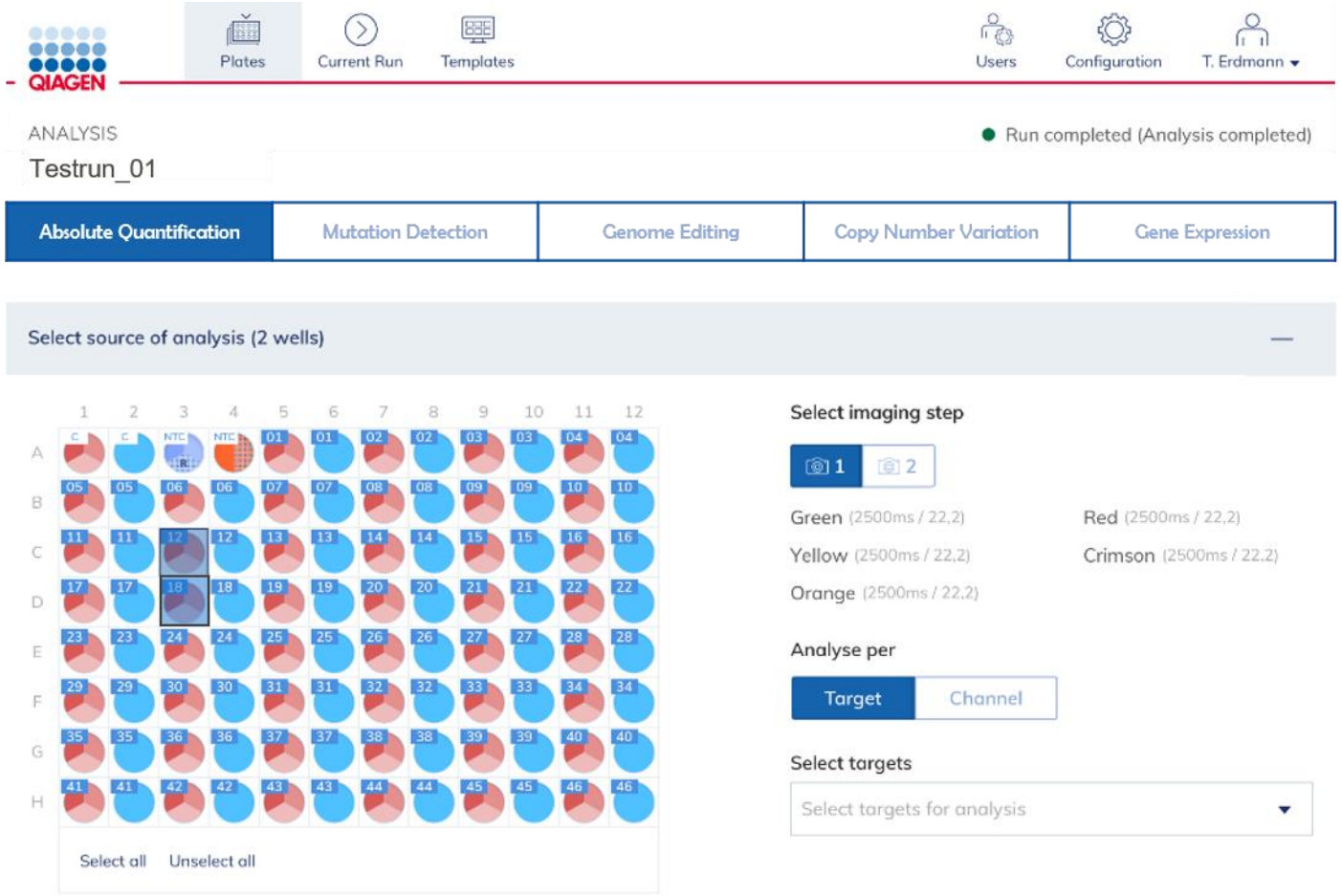


Plate layout

Position of samples and reaction mixes (plate and list overview)



Analysis using QIAcuity Software Suite

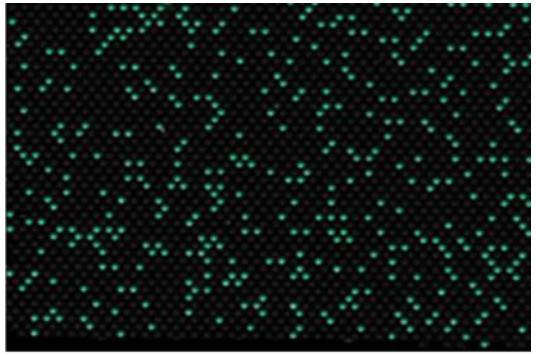


Absolute quantification

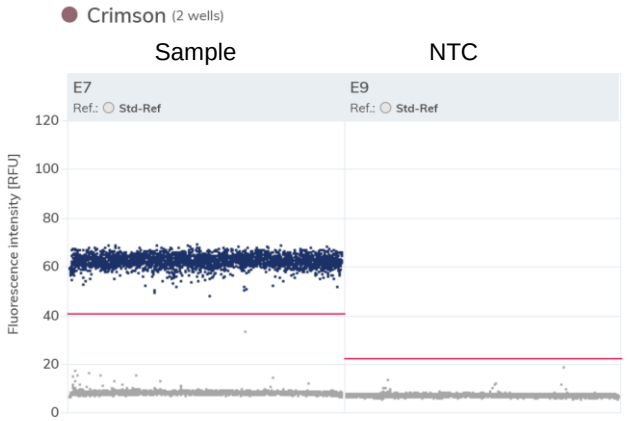
- After selecting the wells to be analyzed, you can view lists, signal maps, heatmaps, histograms, 1D scatterplots, 2D scatterplots, and concentration diagrams in this tab
- Analysis by individual targets, replicates and channels is possible

Output types in QIAcuity Software Suite

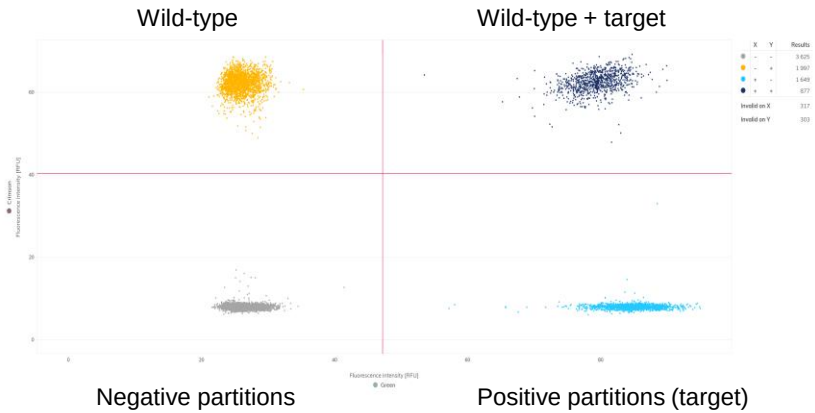
Signal map



1D scatterplot



2D scatterplot



More options:

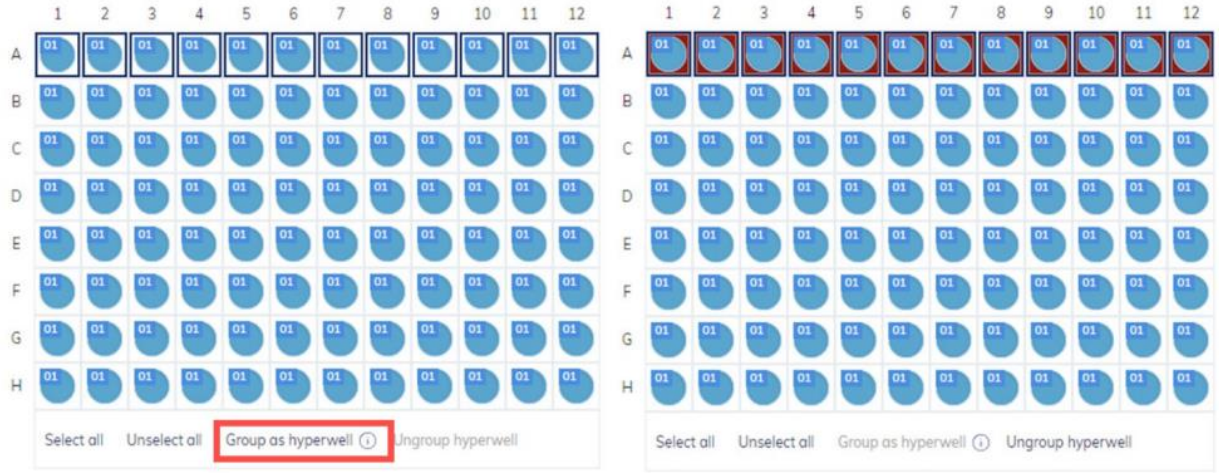
- Heatmap
- Point diagram
- Concentration diagram
- Histogram and more
- Temperature gradient cycler function

Hyperwell analysis using QIAcuity Software Suite

Allows the combination of several wells to one analytic unit

- Provided that the selected wells contain only replicates
- Mainly used for ultra-rare mutation detection, where the same genomic DNA containing a low number of mutation-positive DNA fragments is tested across several wells
- In the analysis view, the 1D plots still display the individual wells; however, in the list view, only one well appears

Example of a 96-well 8.5K plate with the first row in hyperwell view



- Row A contains replicates of the same assay and template
- The selected samples can then be grouped as a hyperwell (left panel)
- The grouped wells then appear with a red background (right panel)

The future of PCR is digital

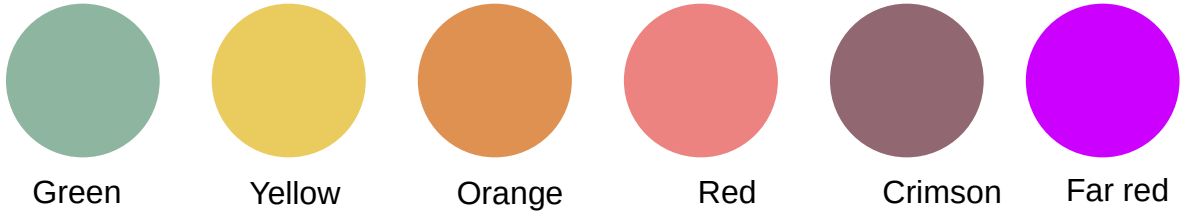
Multiplexing

- Detection of up to 12–14 targets in a single reaction
- Amplitude multiplexing and Long Stokes Shift analysis in combination with SW 3.0/3.1
- Software 3.0 and higher to allow for 6–8 plex
- Software 3.1 to allow for 12-plex
- In combination with QIAcuity High Multiplex Probe PCR Kit (#250133)

Eliminates the need for a new instrument for higher order multiplexing.

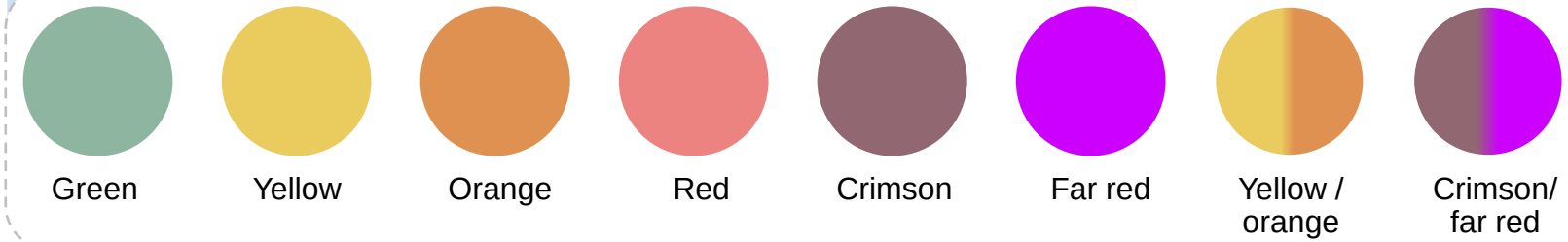
High multiplexing options with QIAcuity Software Suite

6-plex: 6 standard channels — **SW 3.0**

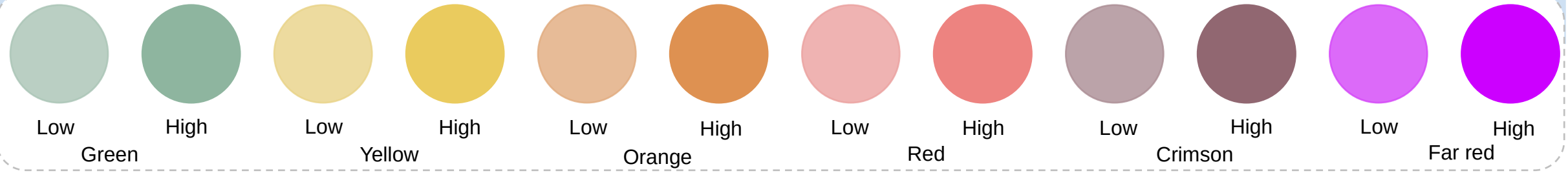


Requires use of QIAcuity High Multiplex Probe PCR Kit

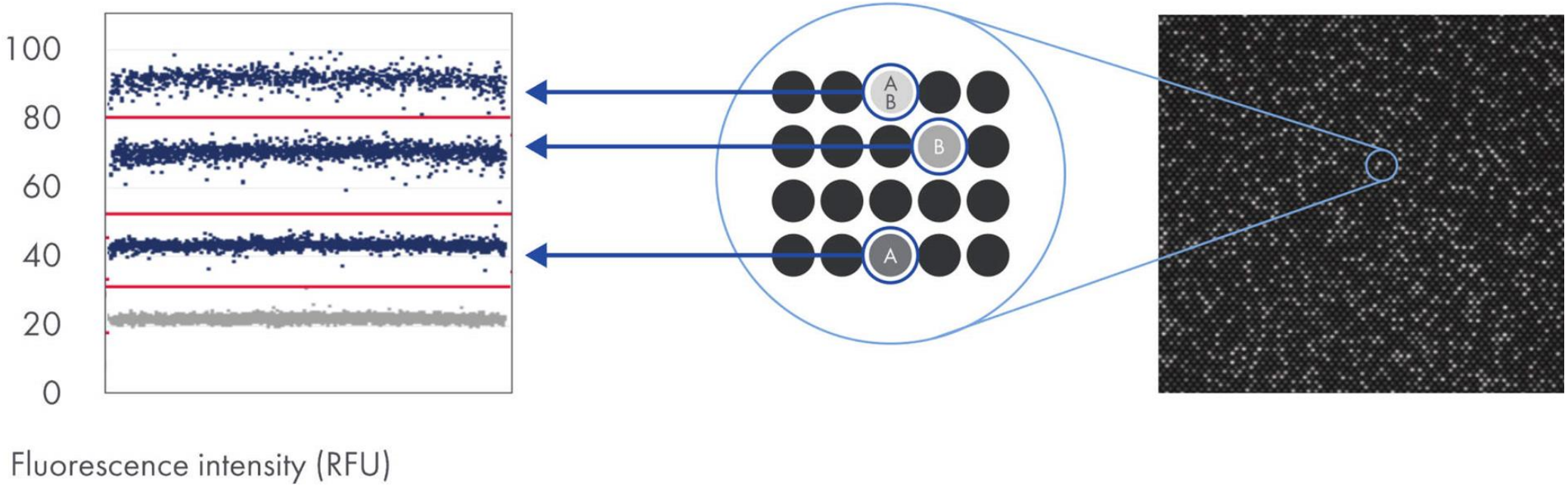
8-plex: 6 standard channels + 2 hybrid channels — **SW 3.0**



12-plex: Amplitude multiplexing using 6 standard channels — **SW 3.1**



Target detection with amplitude multiplexing using QIAcuity Software Suite



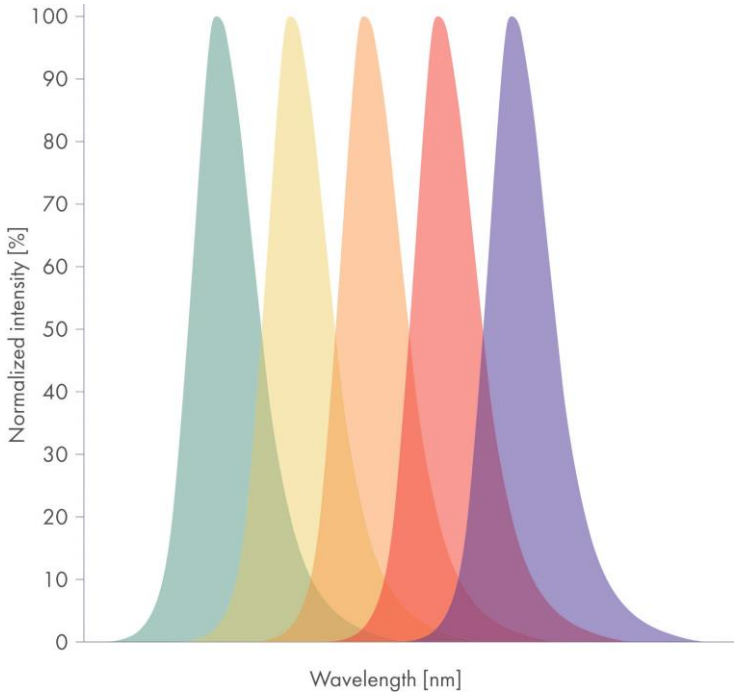
- Detection of 12 targets in parallel using amplitude multiplexing in 6 channels
- Combining hybrid channels for 1-plex LSS dyes with amplitude multiplexing can potentially increase the total number of multiplex assays to 14. However, this approach is not recommended because it requires optimized conditions for all assays in the reaction mix and the associated challenges of crosstalk compensation.

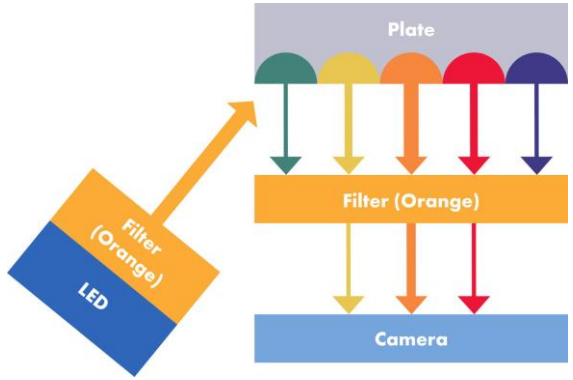
Amplitude multiplexing doubles the number of targets detected in one channel.

Custom cross talk matrix in QIAcuity Software Suite

Cross talk

- Signal overlap between neighboring channels
  - Signal from one channel is visible on the other channel
  - Result of the overlapping bands of the emitted light spectrum and filtered frequencies range
- Can distort the analysis results of multiplex assays





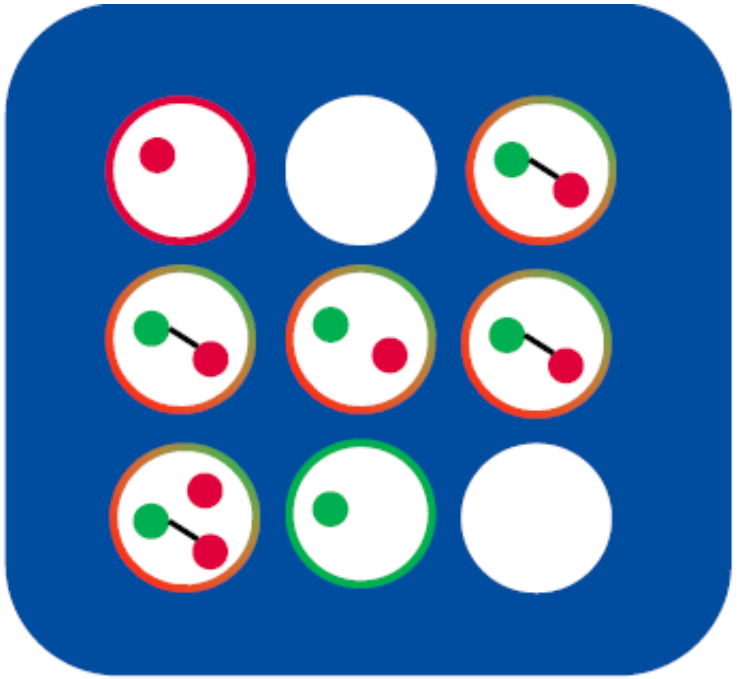
From QIAcuity Software 3.0 on and higher

- Creation of an assay-specific custom cross talk matrix to correct signal overlap between neighboring channels in high multiplexing

Custom cross-talk matrix enables maximum cross-talk compensation, highly recommended for high-multiplexing applications.

Template integrity analysis using QIAcuity Software Suite

AAV production and similar applications typically utilize fully linked template molecules; however, some template molecules are not linked and may be derived from different molecules within a single partition



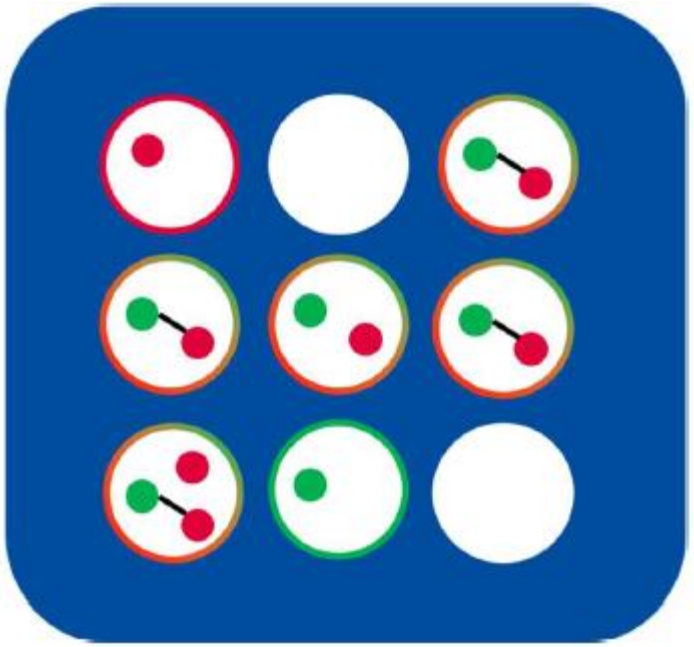
Only red assay template intact and distributed – positive in red channel only

Only green assay template intact and distributed – positive in green channel only

Both single intact green and red assay template randomly distributed – positive in green and red channel only

Template is intact for green and red assay template and co-distributed

Template integrity analysis using QIAcuity Software Suite



Potential ways of integrity calculation

1. % concentration of linked [red-green] (excluding random red/green) in the overall amount of positive template concentration

   Formula: [red-green] / sum of [red] + [green] + [red-green]

2. % concentration of linked [red-green] (excluding random red/green) compared to the amount of “broken” [red-green] into [red] + [green], assuming single [red]/[green] can only occur in a similar amount

   Formula: [red-green] / mean of [red] + [green]

QIAcuity Lab Automation API

The QIAcuity Software Suite provides a (REST) service enabling a third-party lab automation software that is controlling one or multiple robots to interact with the QIAcuity system. The service is ideal for running fully automated experiments without human interaction.

The Suite Lab Automation (REST) API provides functions to control the instruments, define experiments and retrieve experiment results.

QIAcuity platform and software support GMP compliance

21 CFR 11 enabling software

- Data security features/advanced archiving
- Complete audit trail
  - Event detail tracking
  - Advanced search and filter feature
- Centralized user management
  - Assignable user permissions
  - Plate ownership
- Electronic signatures
  - Multiple roles and signatures allowed
- Reporting features
  - SD and CV%
  - Genome integrity
  - Dilution factor

Instrument qualification and support services

- IQ/OQ installation and annual OQ service
- Next-day on-site support available
- Assay design services

Product support

- Quality management systems
  - ISO certificates
  - Certificates of analysis
- Supply management
  - Lot reservations
  - Standing orders
  - Custom fillings

Dual modality software on QIAcuityDx

IVD Mode

Diagnostic assays

Utility Mode

Laboratory Developed Tests (LDTs) and non-clinical laboratory research

QIAcuityDx dual mode software enables you to run IVD assays alongside the provision of tools to support menu expansion beyond approved assays.*

***Laboratories may choose to incorporate QIAcuityDx Utility (open) mode within their workflow to build their own laboratory-developed tests in compliance with the US Clinical Laboratory Improvement Amendments of 1988 and European Union regulatory requirements on "In-House Assays" (Regulation (EU) 2017/746 – IVDR- Art. 5(5))**

QIAcuityDx integration capability

LIS integration IVD: End-to-end bidirectional integration.

LIS integration UTL: Report absolute quantification through HL7 global standards for the transfer of clinical and administrative health data.

Why QIAcuity dPCR?

Scalable instrument and nanoplates

- Low- to high-throughput instrument configurations (1-, 4- and 8-plate)
- Up to 2 integrated thermocyclers

High throughput

- Samples processed in a work day can range from 96 to 1248 samples, depending on the plate (24- and 96-well) and instrument (1-, 4- and 8-plate) configuration used

Faster to result

- Simple and rapid plate-based workflow takes users from sample to result in under 2 hours

Broad product portfolio

- More than 120,000 dPCR assays, many of them wet-lab verified and ready for immediate use
- From DNA to miRNA, from oncology to microbiology, all types of analytes and digital PCR applications are covered

Multiplexing capability

- Up to 8 detection channels (6 standard +2 hybrid channels used with Long Stokes-Shift dyes)
- Detection of 12 targets in parallel possible with amplitude multiplexing and the QIAcuity High Multiplex Probe PCR Kit

The future of PCR is digital

Non-clinical applications of dPCR

Rare mutation detection
dPCR LNA Mutation Assays
IDNAPTEX dPCR Assays

Pathogen detection
dPCR Microbial DNA Detection Assays
QIAcuity UCP Probe PCR Kit

Copy number variation
dPCR Copy Number Assays
dPCR CNV Probe Assays

Gene expression
QuantiNova LNA PCR Assays

Cell and gene therapy
QIAcuity CGT dPCR Assays
QIAcuity RCL Quant Kit
Viral Vector Lysis Kit
QIAcuity Residual DNA Quant Kits
QIAcuity Mycoplasma Quant Kit

miRNA detection
miRCURY LNA miRNA PCR Assays

Wastewater testing
QIAcuity OneStep Advanced Probe Kit

Liquid biopsy
dPCR LNA Mutation Assays
dPCR CNV Probe Assays
dPCR PanCancer Kit

GMO detection
dPCR Copy Number Assays

All dPCR kits and assays are compatible with the QIAGEN sample extraction kits

Clinical applications of dPCR

Infectious diseases

- Viral
- Bacterial
- Fungal
- Parasitic

Oncology

- Cancer biomarker detection
- Monitoring and MRD testing
- Liquid biopsy
- Cell and gene therapy

Genetic testing

- Quantify specific DNA sequences at low analyte concentration with high precision

Transplant rejection

- Molecular monitoring
- Chimerism

The future of PCR is digital

Assays and capabilities

Microbial detection

Multiplexable

Custom tool available

Detection of bacterial, fungal, viral, virulence & resistance targets

Copy number quantification

Multiplexable

Custom tool available

Locus specific analysis of small variations of gene copy numbers

Rare mutation detection

Multiplexable

Sensitive and specific detection of mutations in duplex

Residual host cell DNA quantification and sizing

Sensitive detection of CHO, HEK293 and E.coli host cell DNA

Food testing

Bringing precision of dPCR to food testing

Viral vector quantification and RCL detection

Multiplexable

Precise and robust quantification of vector titer and detection of RCL

Gene expression

EvaGreen

Custom tool available

Extremely sensitive quantification of miRNA, mRNA and lncRNA

Multiplex mutation analysis

Parallel detection of cancer hallmark mutations in a single reaction

Mycoplasma detection

Pharmacopeia-compliant detection of Mycoplasma RNA and DNA

Custom assay design

Multiplexable

Tailored to fit any application

The QIAcuity dPCR assays

Gene expression

- QuantiNova LNA PCR Assays for dPCR
- miRCURY LNA miRNA PCR Assays for dPCR

Oncology

- dPCR LNA Mutation Assay
- dPCR Copy Number Assay
- dPCR CNV Probe Assay
- Custom assay design tool for dPCR CNV Probe assays
- dPCR PanCancer Kit

BioPharma

- QIAcuity Residual DNA Quantification Kits and Standards
- QIAcuity HEK293 resDNA Sizing Kit
- QIAcuity Mycoplasma Quant Kit and Standards
- Viral Vector Lysis Kits
- QIAcuity Cell and Gene Therapy Assays
- QIAcuity RCL Quant Kit

Microbial/Pathogen detection

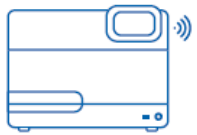
- dPCR Microbial DNA Detection Assays
- Custom assay design tool for dPCR Microbial DNA detection assays

These assays are intended for non-clinical applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

Sample collection and stabilization

Collect and stabilize liquid biopsy sample in a single tube for storage and transport for standardized preanalytical processing with our integrated solutions.

- PAXgene® Blood ccfDNA Tubes
- PAXgene Urine Liquid Biopsy Set



cfDNA preparation

Complete and optimize your portfolio with our manual and low-, medium- and high-throughput automation solutions for cfDNA preparation to achieve high sensitivity in downstream applications.

Manual cfDNA preparation:

- QIAamp® MinElute® ccfDNA Kits
- QIAamp Circulating Nucleic Acid Kits

Automated cfDNA preparation:

- EZ2® Connect
- QIAsymphony® SP
- QIAcube® Connect

NGS analysis

Our NGS library preparation portfolio enables you to achieve variant detection in DNA, RNA and epigenetics with a scalable workflow that fits your research goals.

- QIAseq® Ultralow Input Library Kit
- QIAseq xHYB Exome Panels
- QIAseq xHYB CGP Panels
- QIAseq Targeted cfDNA Ultra Panels
- QIAseq Targeted Methyl Panels

dPCR analysis

Use our dPCR solutions to precisely detect ultra-rare ctDNA mutations down to 0.01% variant allele frequency.

- QIAcuity® dPCR System
- dPCR LNA Mutation Assays
- dPCR Copy Number Variation (CNV) Probe Assays
- Pan-cancer dPCR assays
- Custom assay-tool CNV dPCR

Data analysis

Complete your NGS and ´omics data analysis tools with flexible access. Our platform provides rapid, evidence-powered variant annotation for human exome, genome, and large cohort sequencing data.

- QIAGEN® CLC Genomics Workbench

Interpretation

Generate biological hypotheses. Identify and prioritize variants for your follow-up.

- COSMIC
- QCI® Interpret Translational for QIAseq
- QIAGEN Ingenuity Pathway Analysis

Quality control

Analyze and perform fully automated quality control of your cfDNA samples or your generated NGS libraries.

- QIAxcel® Connect System

Genomic Services and Custom NGS Panel Design

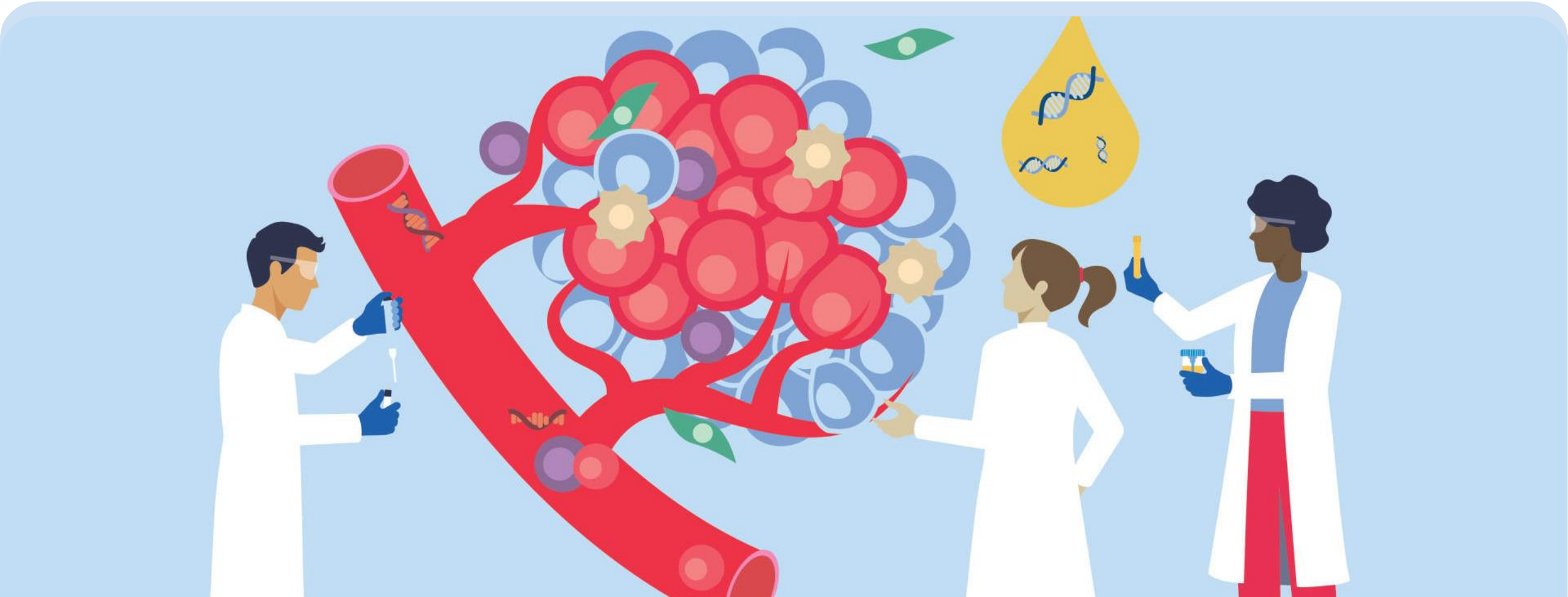
- Benefit from expert consultation for NGS sequencing and custom NGS panel design to make the most of you samples and achieve your goals
- Custom digital PCR assay design for your specific samples
- Extract nucleic acids for DNA, RNA, miRNA and total nucleic acid applications
- Ensure high-quality data for a complete project from sample to report or for just the bottleneck part of your workflow

GeneGlobe® Design & Analysis Hub

- Quickly and easily explore targets in their scientific context
- Find and customize the right products to study those targets
- Analyze the data using complementary, easy-to-use analysis tools

Products shown here maybe intended for non-clinical applications and thus not intended for the diagnosis, prevention or treatment of a disease or for IVD purposes. Refer to product label and instructions for use for details.

dPCR assays for oncology



Oncology: Pre-designed assay portfolio

CNV analysis

- Ready-to-use, comprehensive catalog with over 90,000 dye-based and probe-based dPCR CNV assays
- Detection of ´less than 1.2-fold change in copy number
- High accuracy for multicopy genes and minor CNV shifts

Custom assay design tool for CNVs

- Transition from pre-designed to a custom assay without leaving the platform
- Fully cover disease-relevant CNVs even when newly discovered targets aren’t yet available as pre-designed assays

Mutation analysis

- More than 200 wet-lab tested dPCR LNA Mutation Assays, covering a broad range of clinically relevant mutations drawn from curated mutation databases
- Achieve 0.1% mutant allele detection sensitivity in a wild-type background in one nanoplate well

Assays for all miRNAs

- Over 31,000 unique LNA-enhanced miRNA dPCR assays, covering 271 species with complete miRBase 22 coverage
- Extremely sensitive quantification, detecting miRNAs from as little as 1 pg total RNA

Gene expression analysis

- Over 20 million pre-designed qPCR and dPCR assays, including those for mRNA and lncRNA expression analysis.
- Built for precise low-abundance target detection

dPCR CNV Probe assays (#250210-13)

Sensitive, precise and reproducible copy number variation analysis

- Highly studied cancer and cancer-related genes
- More than **200 dPCR wet-lab tested assays**
- Single/multi-copy reference assays and centromeric assays available
- Multiplex capability
- Complimentary, easy-to-use CNV analysis in dPCR Software Suite
- Used in combination with **QIAcuity Probe PCR Kit**

Specifications and targets

Genes of interest

205 targets (assays)

Examples: *ALK, ARID1A, EGFR, NRAS, TP53, GATA1, GATA2, MYC…*

Reference genes

4 assays

- *AGO1*
- *AP3B1*
- *RPP30*
- *TERT…*

Centromeric genes

24 targets (assays)

- 22 for autosomal chromosomes
- 2 for gonosomal chromosomes...

Visit product page

This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

dPCR CNV Probe assays (#250210-13)

CNV Probe Methylation Assays

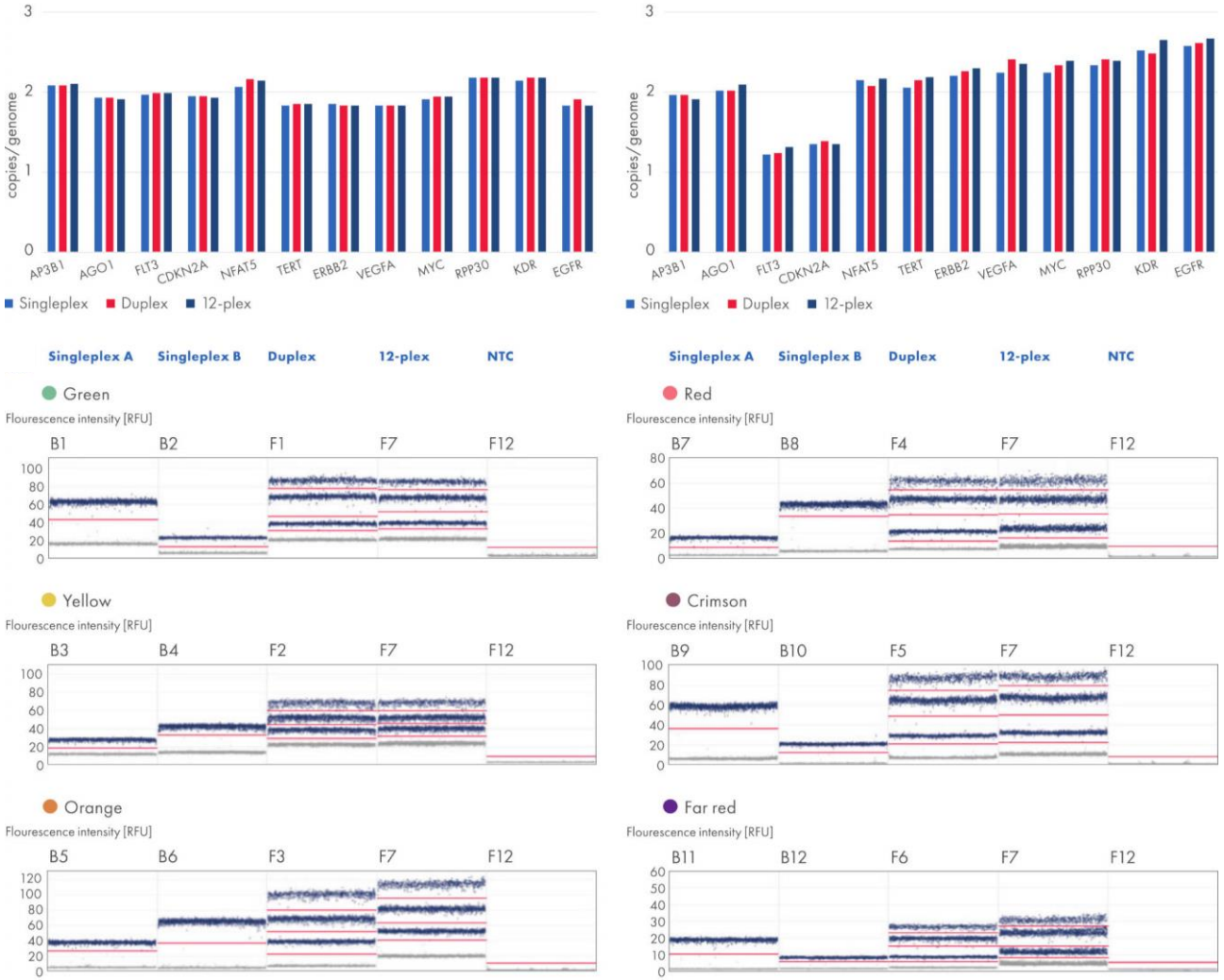
- Targets: MGMT and MLH1
- Available on GeneGlobe
- Wet-lab tested
- Using methylation-sensitive restriction enzymes (MSREs)
- Available with various dyes

Visit product page

This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

Multiplexing dPCR CNV Probe assays

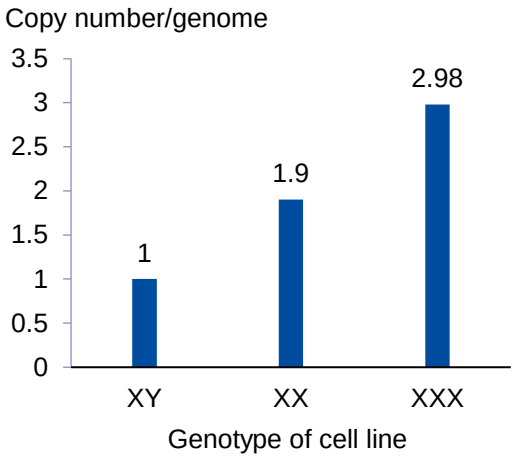
- 12-plex reactions to compare the copy number variations of ten genes of interest and two reference genes
- Healthy human donor was compared to the U-2 OS sarcoma cell line
- Using the QIAcuity High Multiplex Probe PCR Kit and QIAcuity Software Suite 3.1
- Genes of interest: *LT3, CDKN2A, NFAT5, TERT, ERBB2, VEGFA, MYC, RPP30, KDR,* and *EGFR*
- The average copy number of the reference genes *AGO1* and *AP3B1* was used for normalization



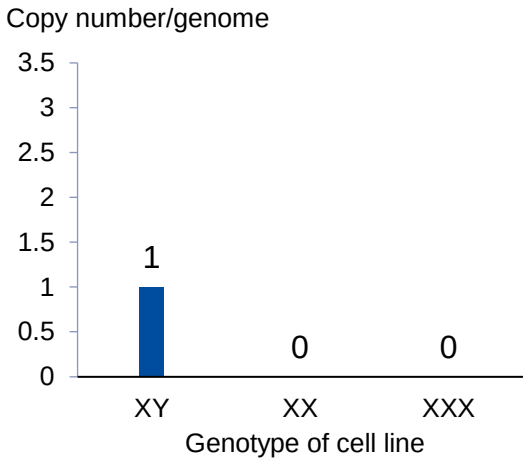
Aneuploidy testing with dPCR CNV assays

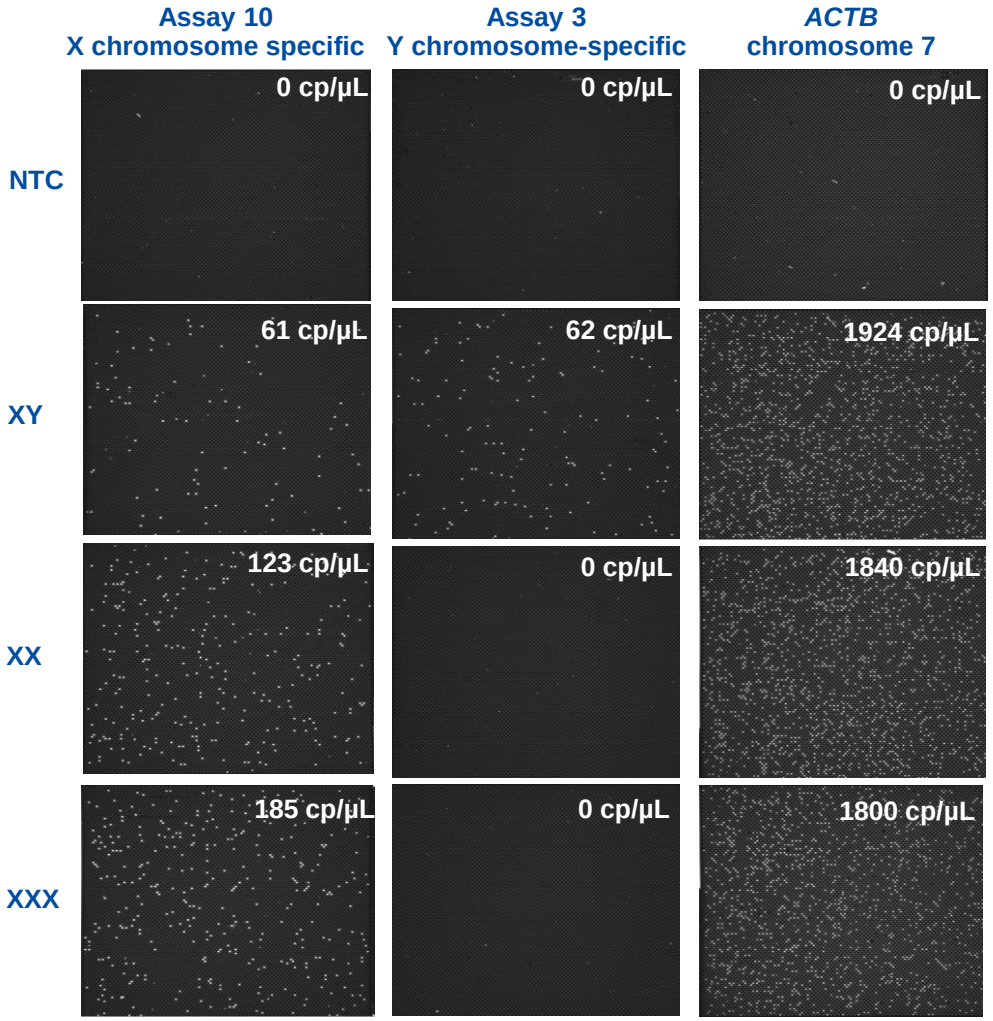
- QIAcuity Nanoplate 8.5K 96-well
- QIAcuity EG PCR Kit/dPCR Copy Number Assays EvaGreen
- Template – 4 ng/rxn human cell lines
- Known CNVs contain 1 copy (XY), 2 copies (XX) or 3 copies (XXX) of the X chromosome
- Human ACTB gene (20 copies/diploid genome) used as a reference to normalize copy numbers

X chromosome specific



Y chromosome specific

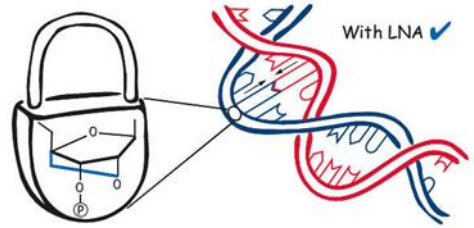


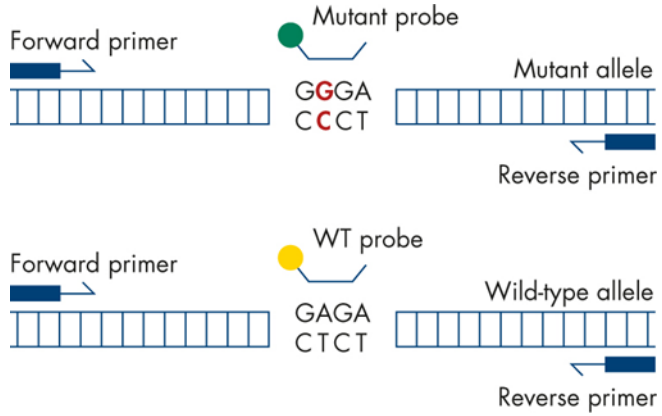


dPCR LNA Mutation assays (#250200)

Detection of mutations in a duplex reaction with competing probes

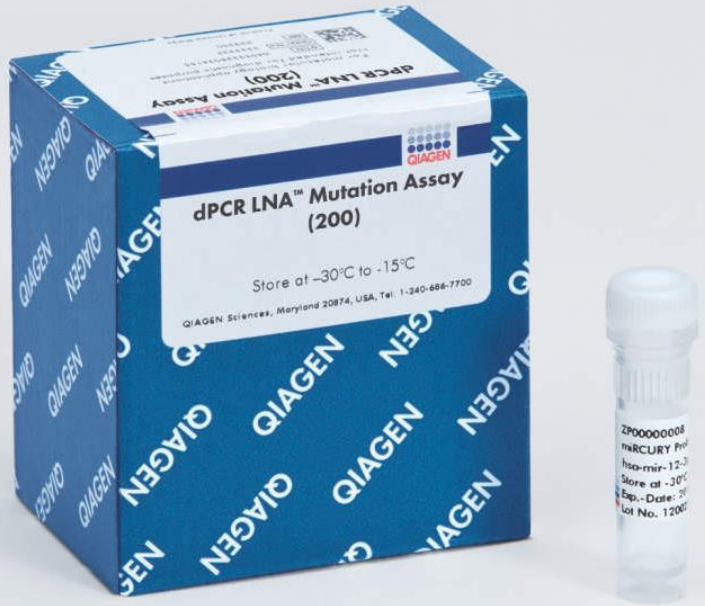
- Single-tube format containing one primer pair and two probes
- LNA-enhanced probes and primers for the highest specificity
- FAM/HEX or Atto550/ROX (mutant/WT probe) available
- Wet-lab-tested dPCR with sensitivity down to 0.1%
- Used in combination with QIAcuity MasterMix or High Multiplex Probe PCR Kit (> 3 assays)
- Over 200 wet-lab-tested assays for the most studied cancer mutations





Visit product page

This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.



Highly sensitive mutation detection using dPCR LNA Mutation assays

- The system can detect as low as 0.05–0.1% of mutation level reliably and consistently from 100–10 ng of total sample input volumes
- Mutation detection to the sub-0.1% level, making dPCR highly suited for use in clinical cancer research with samples that are often scarce or poor quality (e.g., FFPE tumor samples and cell-free DNA)
- Duplex assay design detects mutated and wild-type sequences
- Robust, sensitive and applicable for the detection of cancer targets

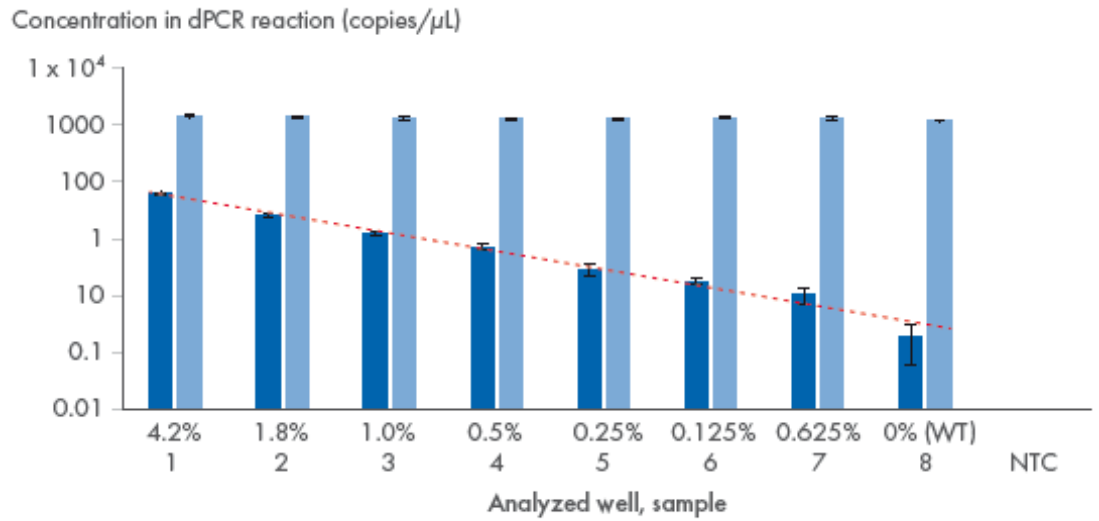
Highly sensitive mutation detection using dPCR LNA Mutation assays

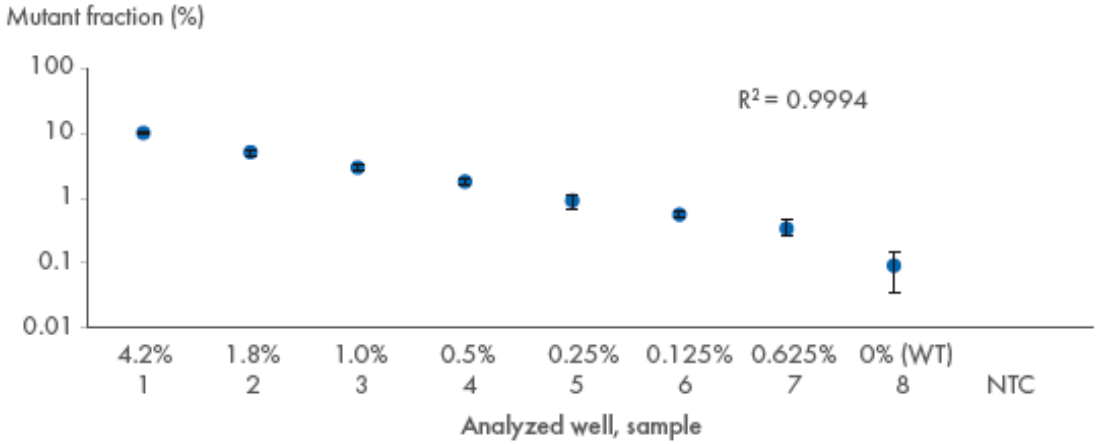
Highly sensitive *KRAS* mutation detection using dPCR

- High sensitivity of 0.05% (Mut:WT) level with 100 ng total sample input on QIAcuity Four instrument
- This assay used QIAGEN KRAS (G12S; GGT/AGT) LNA Probe 2-plex assay, with KRAS G12S reference gDNA from SW48 cell line (Horizon Discovery)

KRAS G12S mutation percentage LNA mutation assay and WT (HEX) 2-plex assay for mutant KRAS (G12S) mutant fraction with constant 100 ng WT background and differing mutant percentages in the mixture (plus NTC in well H3 3 replicates)

	1	2	3	
A	4.24%	4.34%	4.08%	4.4% mut
B	1.87%	0.67%	1.96%	2.2% mut
C	1.00%	0.90%	0.89%	1.0% mut
D	0.5%	0.61%	0.52%	0.5% mut
E	0.24%	0.18%	0.25%	0.25% mut
F	0.11%	0.14%	0.13%	0.125% mut
G	0.06%	0.10%	0.07%	0.05% mut
H	0.02%	0.01%	0%	0% (WT)





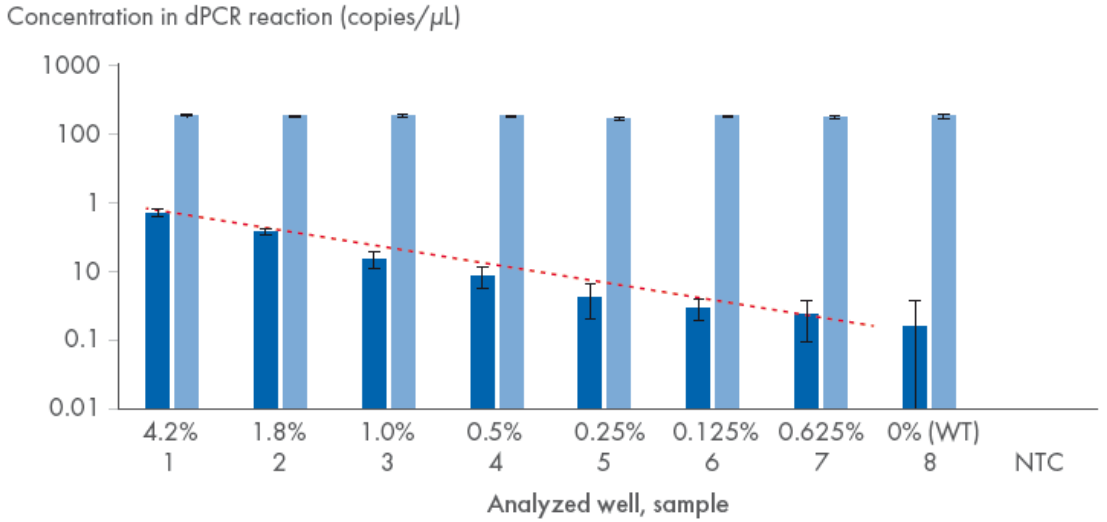
Highly sensitive mutation detection using dPCR LNA Mutation assays

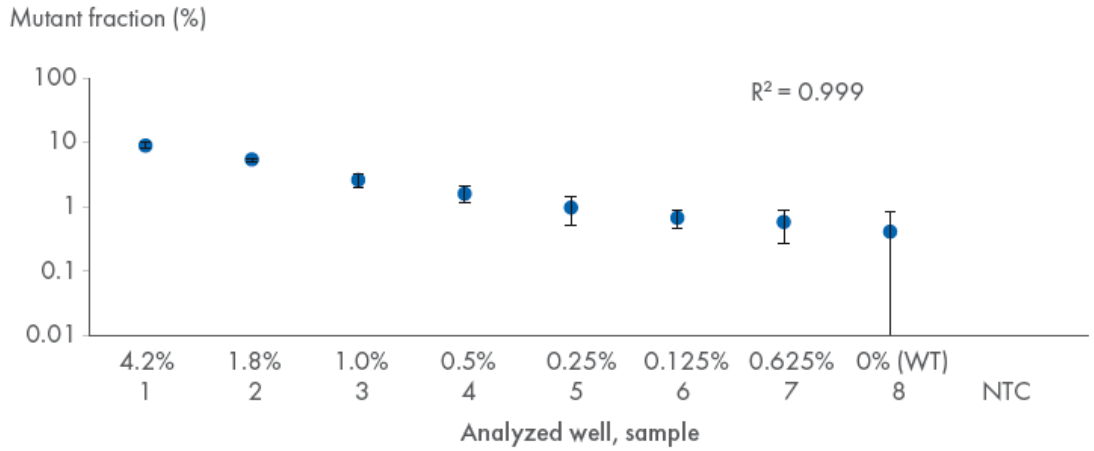
Low-sample input for *KRAS* mutation detection with dPCR

- Moderate-to-low sample input requirement at 10 ng while maintaining excellent mutation detection sensitivity at 0.1% on QIAcuity Four instrument

KRAS (G12S) mutation percentage LNA mutation (FAM) and WT (HEX) 2-plex assay using a human KRAS (G12S) mutant fraction under constant 10 ng WT background with differing mutant percentages in the mixture (plus NTC)

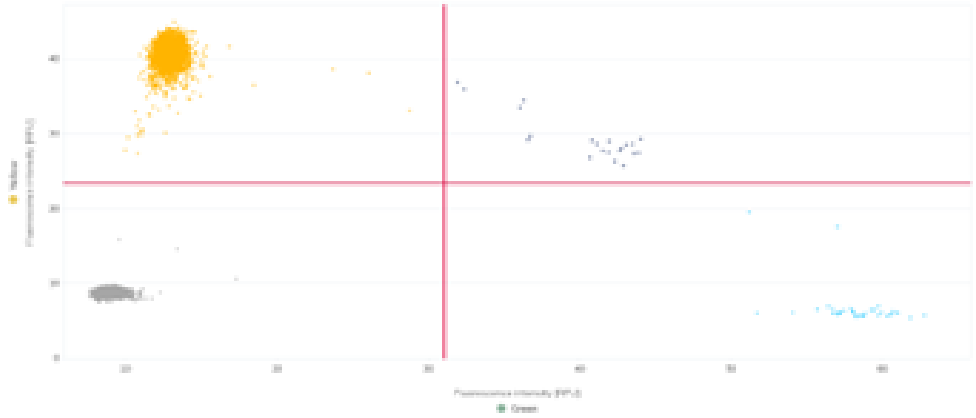
	1	2	3	
A	3.9%	3.29%	3.69%	3.6% mut
B	1.87%	2.18%	2.03%	2.0% mut
C	1.02%	0.69%	0.77%	0.9% mut
D	0.38%	0.41%	0.61%	0.45% mut
E	0.26%	0.14%	0.36%	0.25% mut
F	0.20%	0.15%	0.12%	0.15% mut
G	0.06%	0.13%	0.22%	0.1% mut
H	0.06%	0.12%	0%	0% (WT)

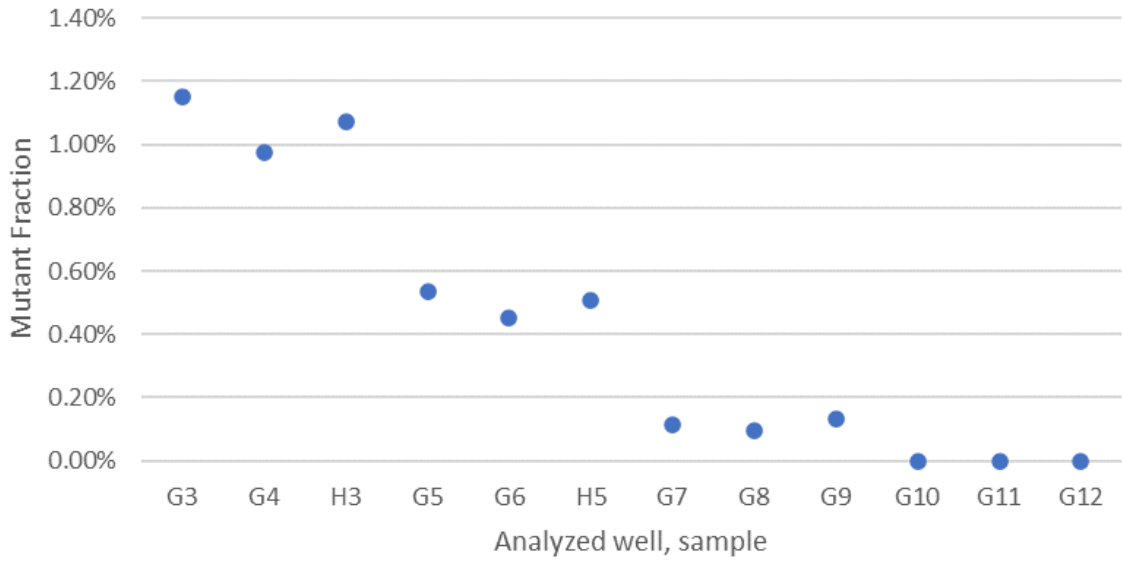




dPCR LNA Mutation assay detecting *ESR1*

- 2D scatter plot showing 1% mutant DNA template (labelled in FAM) spiked into wild-type DNA background (HEX)
- Mutation frequency analysis for various mutation frequencies: G3, G4, H3: 1%, G5, G6, H5: 0,5%, G7-G9: 0.1%, G10-G12: 0%
- Using the QIAcuity MasterMix and the assay for *ESR1*: c.1609T>A (COSV52784978) (# DMH0001107)





IDNAPTEX: Digital PCR assay portfolio from ID Solutions validated on QIAcuity

BRAF-CNS
One-well detection
SNV V600E, KIAA1549-BRAF fusion

FGFR1-CNS
One-well detection
SNV N546K, SNV K656E, TKD duplication

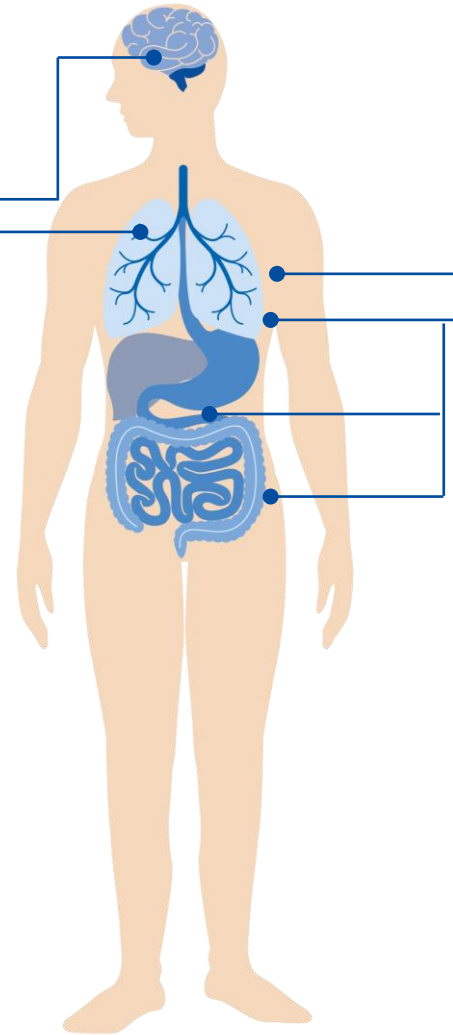
H3F3A
One-well detection
SNV K27M, SNV G34R/V

IDH1-2
One-well detection
SNV R132X, SNV R172X, SNV R140X

TERT
One-well detection
Promotor mutation (-124C/-146C)

EGFR
Two-well detection
1. SNV L858R
   Del Exon 19
2. SNV T790M
   SNV C797S

KRAS-G12C
One-well detection
SNV G12C



PIK3CA
One-well detection
SNV E542/5K, SNV Q546K
SNV H1047R/L, C420R

ESR1
One-well detection
Exons 8 mut, E380Q

KRAS-3X
Two-well detection
1. SNV Q61X
   SNV G12X/G13X
2. SNV A146X/K117N

mMLH1
One-well detection
Methylated MLH1

BRAF
One-well detection
SNV V600X, SNV V600E
SNV L597X, SNV K601E
A598X

NRAS
One-well detection
SNV Q61X, SNV G12X/G13X

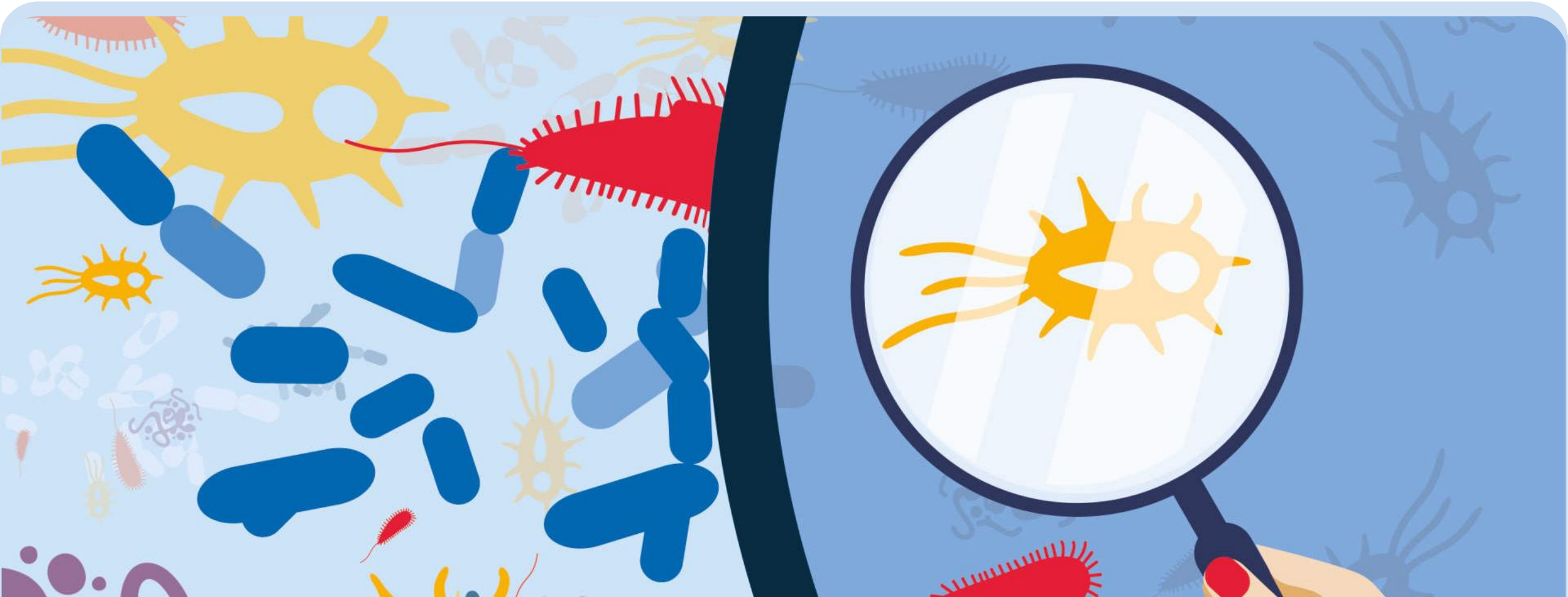
For plasma and FFPE samples

Ready-to-use assays with positive control and ICE*

Visit product page

*Internal Control of Extraction

dPCR assays for microbiology



dPCR Microbial DNA Detection assays: Pre-designed assay portfolio

Bacteria

>400 assays

Fungi/parasites

>40 assays

Viruses

>60 assays

Antibiotic resistance and virulence factor

>200 assays

Reference genes/controls

3 assays

For the complete target list, download the Available Product Catalog on GeneGlobe in the "Resources" section: https://geneglobe.qiagen.com/product-groups/dpcr-microbial-dna-detection-assays

dPCR Microbial DNA Detection assays (#250207)

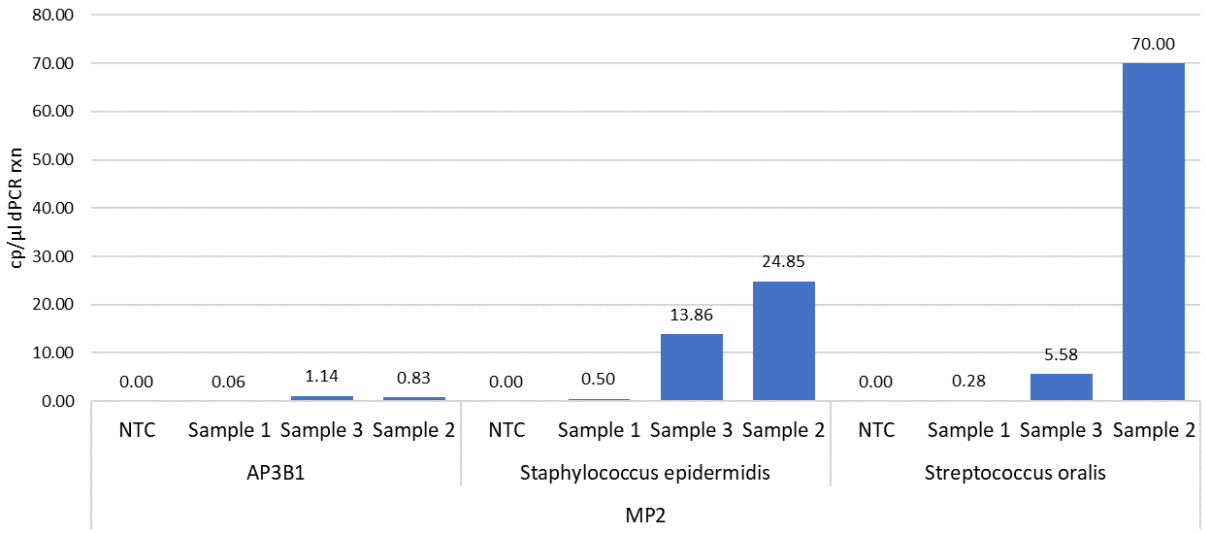
- **Over 900 assays** available for bacteria, fungi and viruses
- For the detection of **microbial species, fungi, viruses, parasites, virulence genes or antibiotic resistance genes**
- Custom assay design tool for bacterial, fungal or viral targets available on GeneGlobe
- Dye selection enables multiplexing of up to 12 targets per reaction
- Wet-lab tested dPCR 5-plex assay bundles for analyzing common targets of interest
- Full target list available online: dPCR Microbial DNA Detection Assays - Assay/target list – QIAGEN

Visit product page

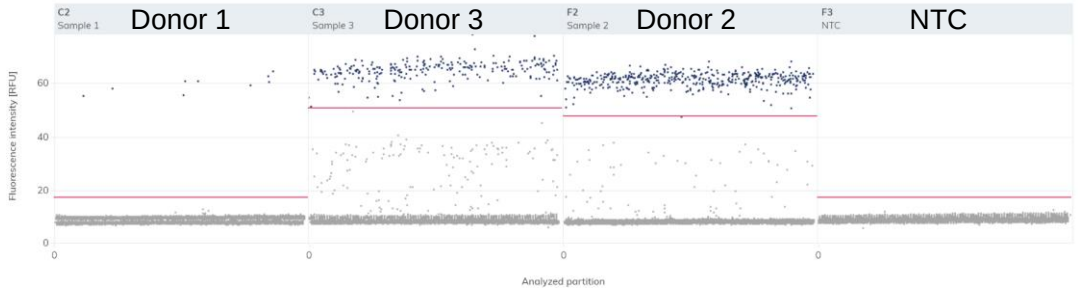
This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

Analysis of the skin microbiome from three healthy donors

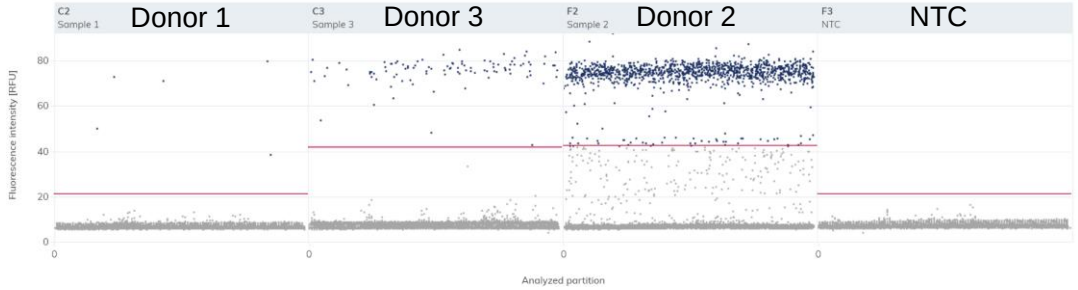
- Pooled swap samples taken from the cheek, antecubital fossa and hypothenar palm
- Manual DNA extraction with DNeasy PowerSoil Pro Kit
- dPCR with QIAcuity Probe PCR Kit
- 96-well 8.5K nanoplate
- Template input: 2 µL (=4.5% of the DNA sample)



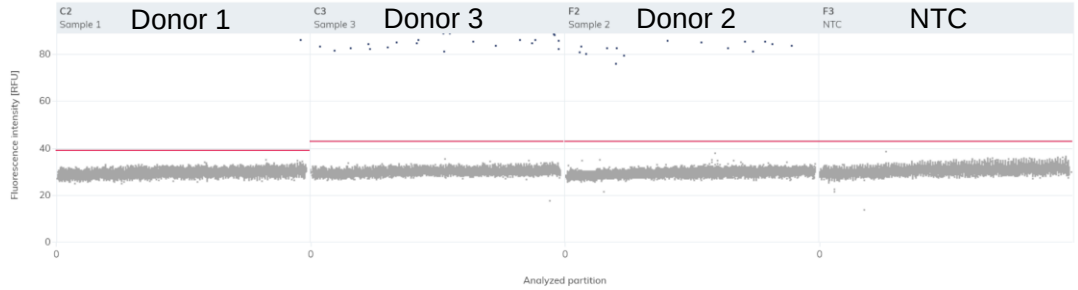
***Staphylococcus epidermidis* (HEX)**



***Streptococcus oralis* (ROX)**

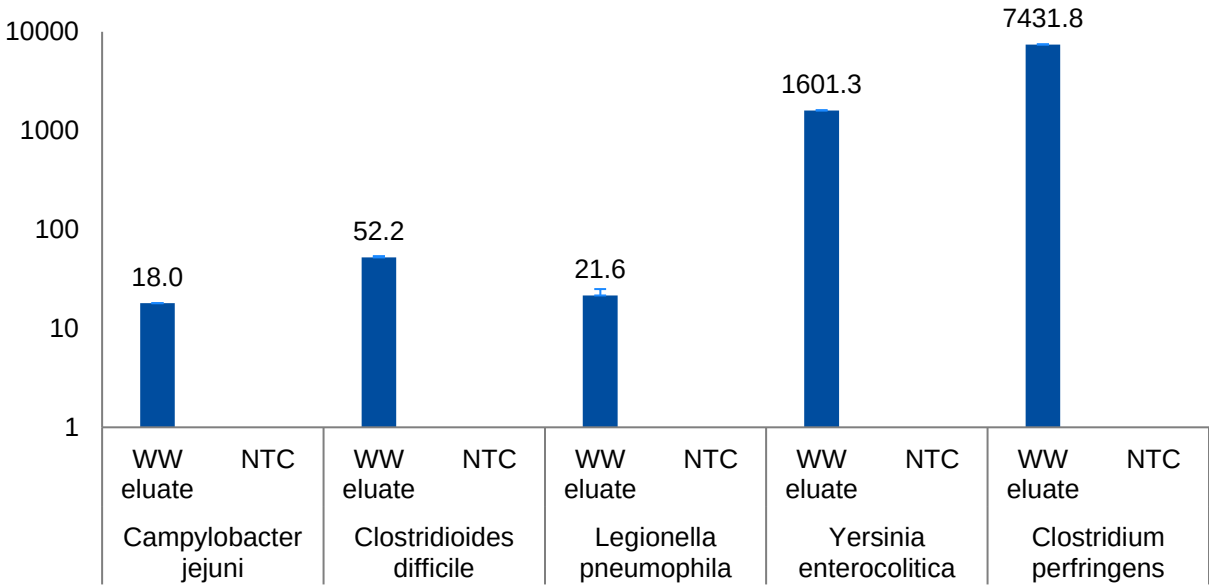


Human ref gene *AP3B1* (FAM)



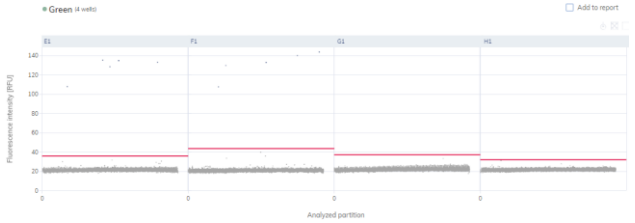
Wastewater surveillance: Five assays targeting bacteria frequently reported from wastewater samples

- Input: wastewater eluate from a German sewage treatment plant
- Three replicates per sample
- 8.5K 96-well Nanoplates and the QIAcuity OneStep Advanced Probe Kit on the QIAcuity Digital PCR System
- 5-plex of five assays targeting bacteria frequently reported from wastewater samples

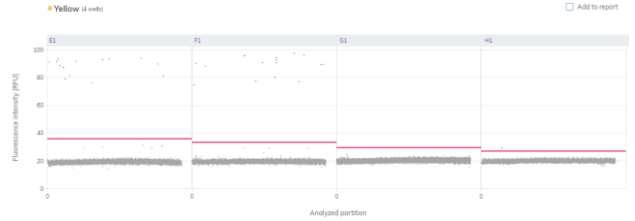


WW = wastewater, NTC= No template control

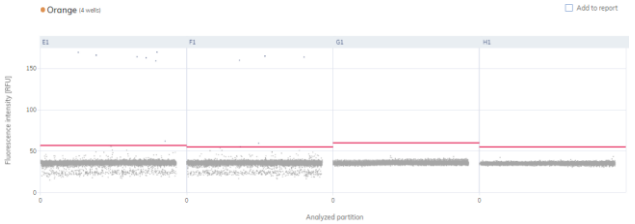
Green channel ***Campylobacter jejuni***



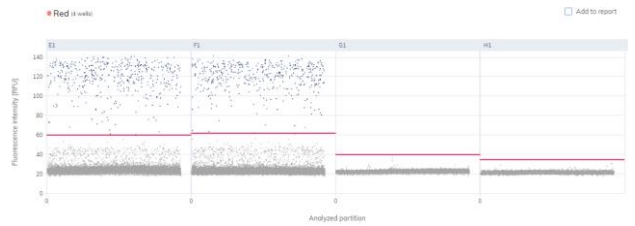
Yellow channel ***Clostridioides difficile***



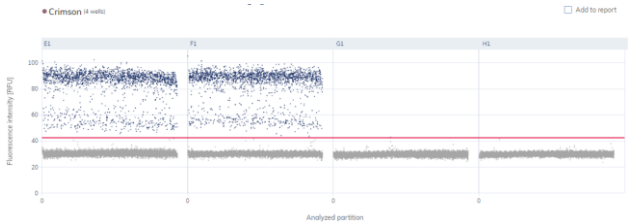
Orange channel ***Legionella pneumophila***



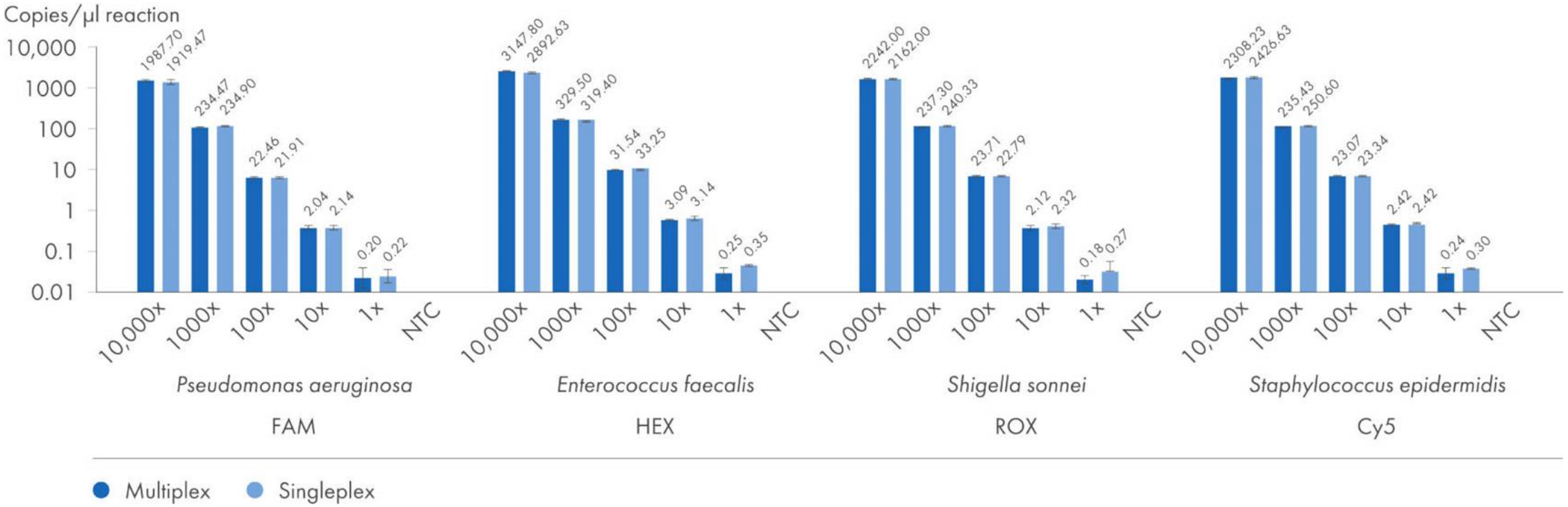
Red channel ***Yersinia enterocolitica***



Crimson channel ***Clostridium perfringens***



Precise quantification in singleplex and multiplex using dPCR Microbial DNA Detection assays

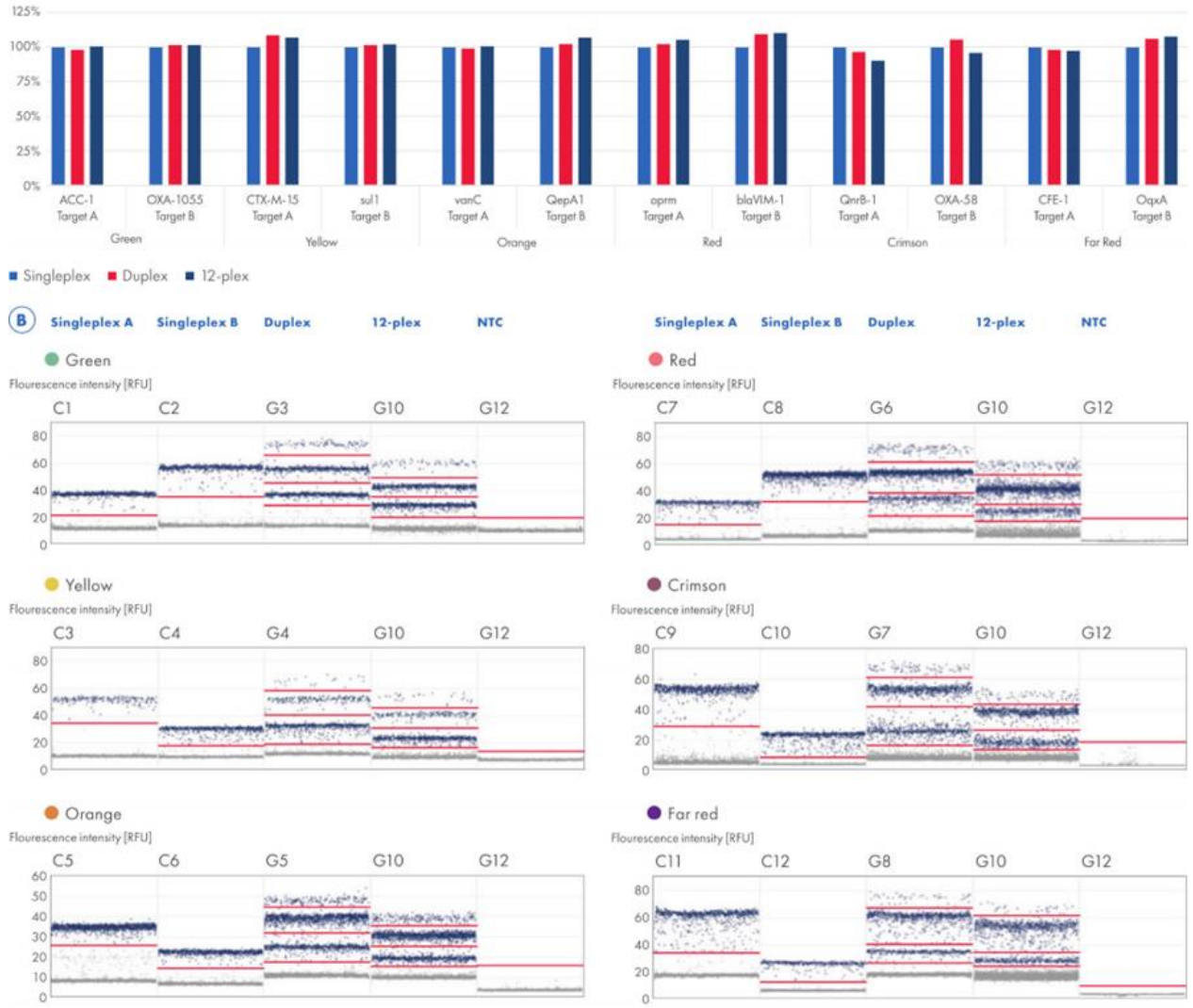


Quantification results were the same, regardless of single- or multiplexing, even over a dynamic range of 4 log levels

- Four assays were run in singleplex and 4-plex reactions using the same template genomic DNA material.
- The same concentrations were observed for concentrations between 0.25 and 2500 copies/µL
- dPCR was performed using 26K 24-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System (n=3)

Multiplexing 12 pathogen targets from the dPCR Microbial DNA Detection Assays: Resistance gene bundles in a single dPCR reaction

- Gene bundles: *ACC-1, OXA-1055, CTX-M-15, sul1, vanC, QepA1, oprm, blaVIM-1, QnrB-1, OXA-58, CFE-1, OqxA*
- Consistent quantification between singleplex and multiplex reactions
- QIAcuity High Multiplex Probe PCR Kit to accurately detect multiple targets simultaneously
- Can be used in a QIAcuity Nanoplate 8.5k and QIAcuity Nanoplate 26k



Custom Assay Design Tools

Custom Assay Design Tools

- Create and order with our online dPCR design tools
- Tailor-made dPCR primer and probe sets for detecting targets where no predesigned products are available
  - Custom dPCR CNV Probe Assays
  - Custom dPCR Microbial DNA detection Assays
- Expert custom assay design service for dPCR available with Genomic Services

Visit product page

Products	Custom dPCR CNV Probe Assays GET STARTED	Custom dPCR Microbial Assays GET STARTED
Supported Species	Human, mouse, rat	Bacteris 16S, fungi ITS, viruses
Detection Chemistry	Hydrolysis probe	Hydrolysis probe
Fluorophores	Cy5, FAM, HEX, ROX, ATTO550, ATTO700	Cy5, FAM, HEX, ROX, TAMRA, ATTO700
Format	Tube containing pre-mixed and lyophilized primers and probe	Tube containing pre-mixed and lyophilized primers and probe
Pack Size	75 or 180 x 40 µl reactions (QIAcuity Nanoplate 26k) or 250 or 600 x 12 µl reactions (QIAcuity Nanoplate 8.5k)	150 or 400 x 40 µl reactions (QIAcuity Nanoplate 26k) or 500 or 1333 x 12 µl reactions (QIAcuity Nanoplate 8.5k)
Design Tool Input	Sequences, gene symbols, genomic coordinates	Organism name, species, taxonomy ID
Reference Database	Human: GRCh38, Mouse: GRCm39, Rat: mRatBN7.2	NCBI
LNA-Enhanced	No	LNA-enhancement for complex designs
Multiplexing	Yes, multiple fluorophores available; no integrated multiplex check	Yes, multiple fluorophores available; no integrated multiplex check
Positive Sequence	Sequence information on amplicon region provided allowing the order of a synthetic positive control	Sequence information on amplicon region provided allowing the order of a synthetic positive control
Other Features	Option to integrate reference assays into the design process	Option to design against customer-defined anchor sequence
Data Analysis	QIAcuity Software Suite	QIAcuity Software Suite

dPCR assays for biopharma applications

Biotherapeutics’ Critical Quality Attributes (CQAs): Examples

Safety

- Viral safety
- Replication-competent virus (RCV)
- Mycoplasma
- Sterility
- Endotoxins

Strength, identity and potency

- Vector genome titer
- Vector copy number (VCN)
- Transgene expression

Purity

- Residual DNA quantity and size
- Residual plasmid
- Residual protein

Product characterization and quality

- Encapsulation efficiency
- Genome integrity
- Stability

dPCR is rapidly emerging as the gold standard in nucleic acid quantification, delivering absolute measurements with exceptional sensitivity and consistency.

dPCR for biopharma analytics: Pre-designed assay portfolio

AAV

- Viral titer:
  - Standardized direct lysis solution
  - dPCR assays
- Viral DNA integrity(software feature)
- Transgene expression

Lentivirus

- Viral titer:
  - Standardized direct lysis solution
  - dPCR assays
- Vector copy number(VCN)
- RCL detection kit
- Transgene integrity
- Transgene expression

Residual DNA

- Residual host cell DNA
  - HEK293, CHO, *E. coli*
  - HEK293 sizing
- Residual plasmid (*AmpR, KanR*)

Mycoplasma

- Pharmacopeia-compliant mycoplasma detection workflow
- Mycoplasma standards for in-house validation

QIAcuity dPCR CGT Assays

Kit features:

- 18 different wet-lab tested dPCR CGT assays, 20x ready-to-use primer-probe mix
- Superior accuracy and precision independent of fluorophores and operators
- Multiplexing – 3-plex capacity (can be extended to 5-plex with customer-chosen GOIs)
- High- to low-throughput applications with different QIAcuity instruments
- End-to-end dPCR workflow comparable to qPCR

Applications:

- Viral vector titer
- Viral vector genome integrity
- Vector copy number (VCN)
- Residual plasmid detection

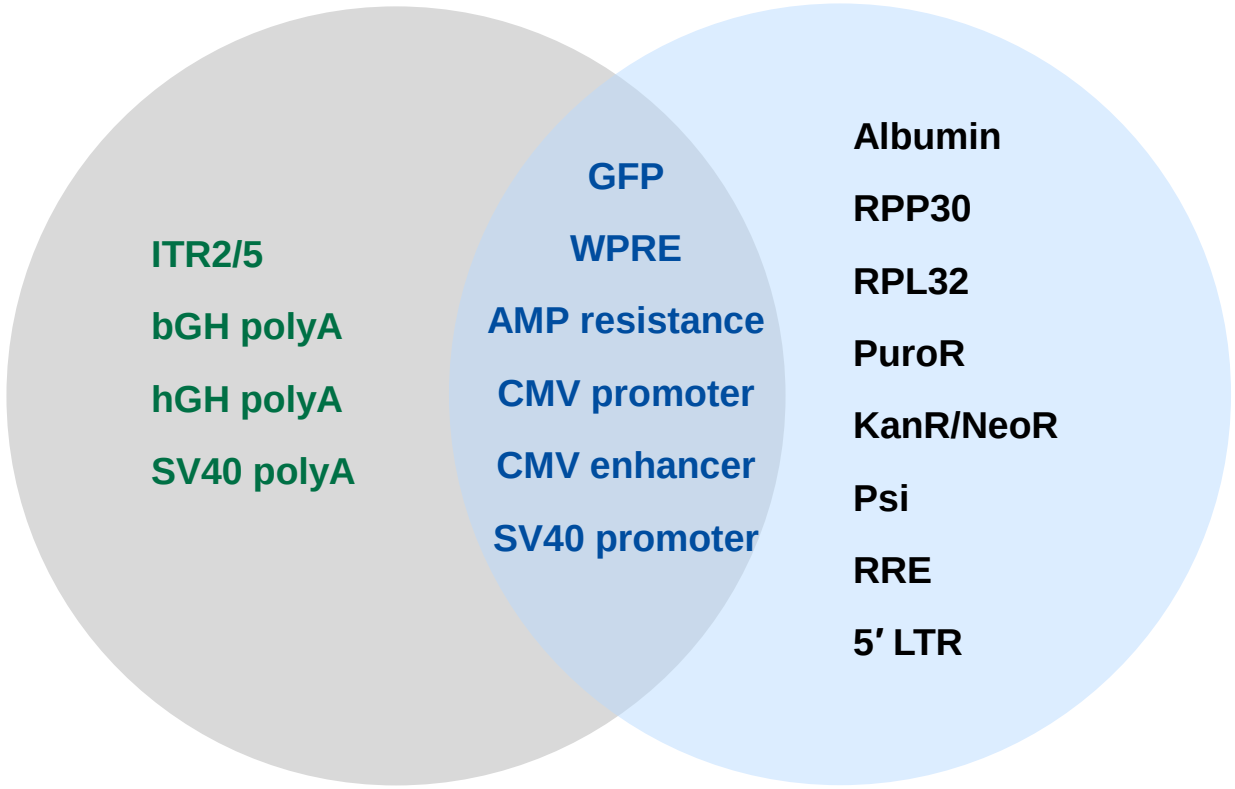
Visit product page

This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

Comprehensive assay portfolio for AAV and Lentiviral workflows

Assays for AAV workflow

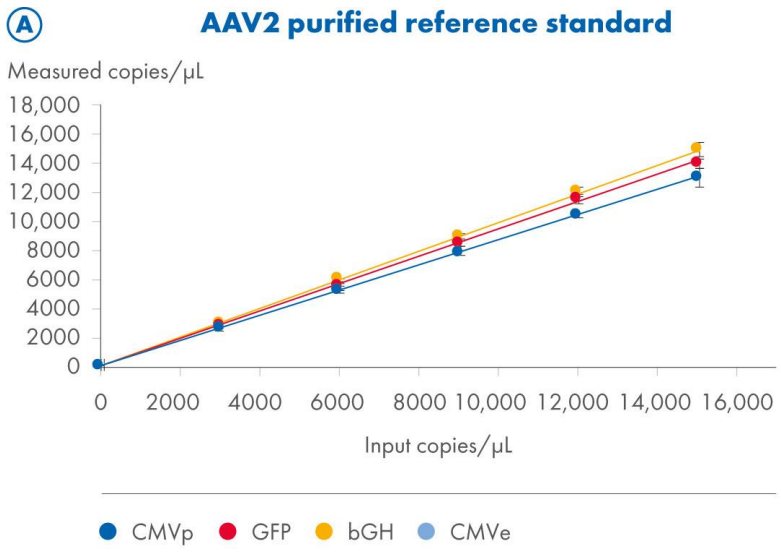
- 10 targets

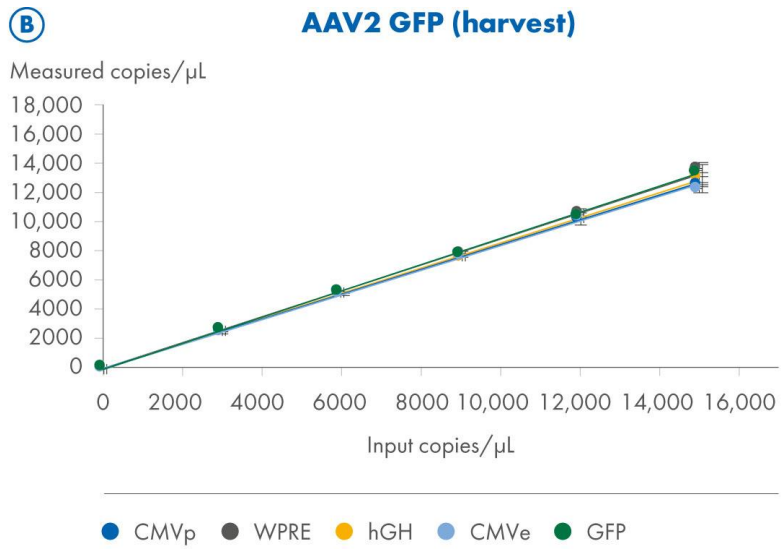


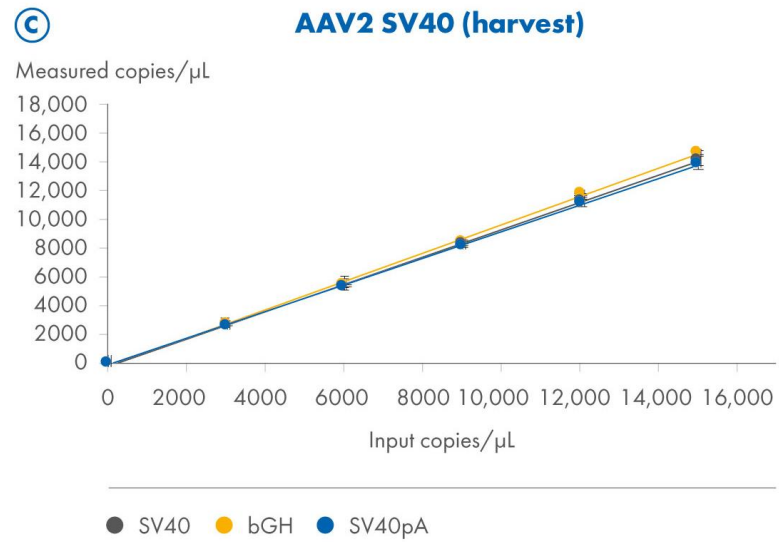
Assays for LV workflow

- 14 targets

High accuracy over a broad dynamic range – CGT Viral Vector Lysis Kit and dedicated CGT assays





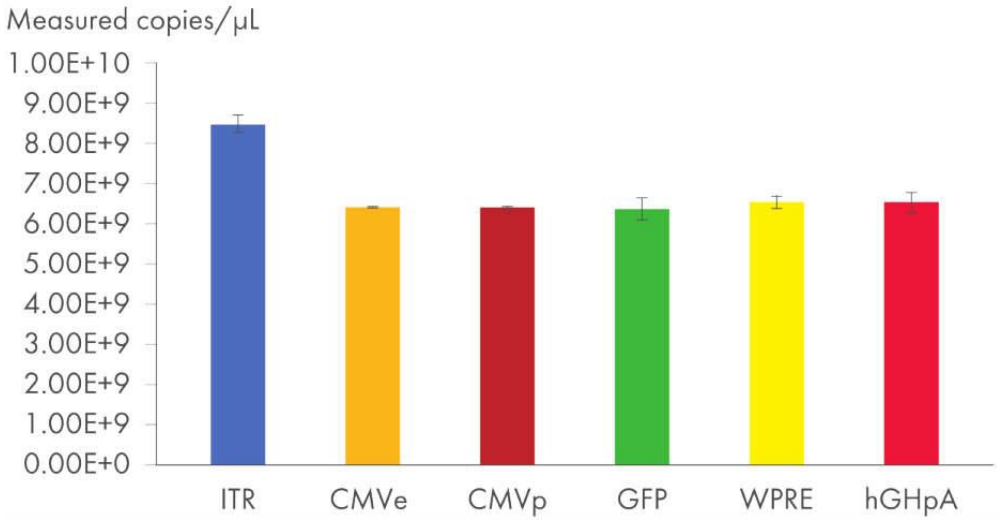


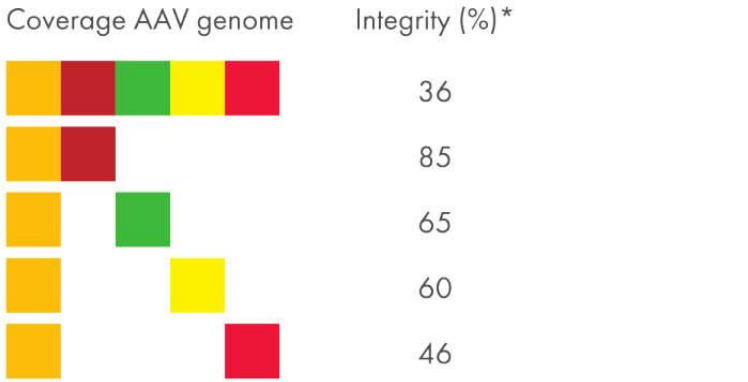
AAV2	CMVe	CMVp	WPRE	hGH pA	GFP	ITR	SV40p	bGH	SV40 pA
Coefficient of variation (8.5k) [%]	2.4	2.7	2.7	3.6	3.4	4.7	4.8	3.4	3.2
Coefficient of variation (26k) [%]	2.9	2.4	2.1	1.7	3.7	5.2	2.2	1.4	1.7
R^2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Deviation (8.5k vs 26k) [%]	1.07	2.49	5.35	4.35	2.6	1.73	0.59	0.07	0.17

For lysis of viral vectors, such as different adeno-associated virus (AAV) serotypes and adenoviruses.

High linearity from 2.5–15,000 copies/µL independent of the targets and purity of the AAV sample.

Integrity determination using QIAcuity dPCR and Software Suite version 2.5 or higher





* AAV2 standard was processed using the CGT Viral Vector Lysis Kit. Processing includes enzymatic removal of ITR secondary structures and takes place outside of the QIAcuity Nanoplate partitions which may lead to reduced integrity scores.

The higher the multiplexing grade of the analysis, the more accurate and precise the integrity calculation.

***AAV2 standard was processed using the CGT Viral Vector Lysis Kit. Processing includes enzymatic removal of ITR secondary structures and takes place outside of the QIAcuity Nanoplate partitions which may lead to reduced integrity scores.**

Standardized viral vector sample processing

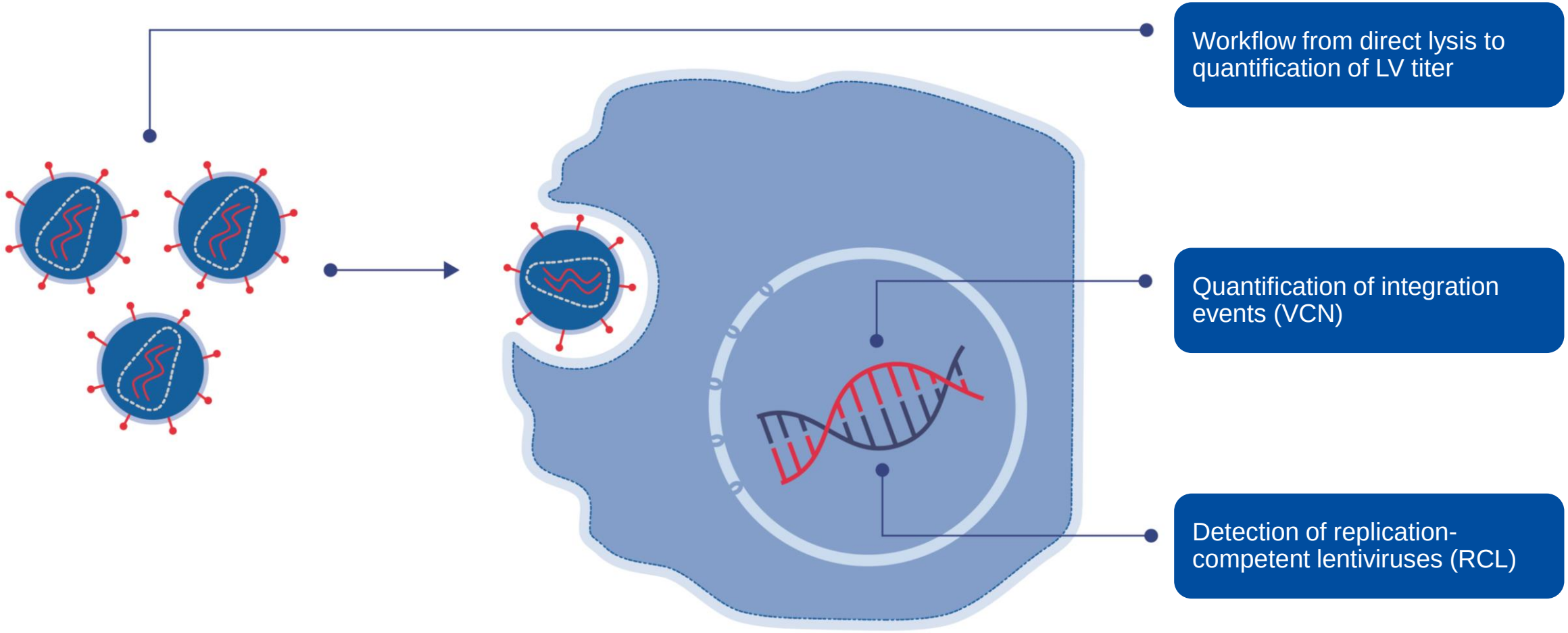
- For the lysis of adeno-associated virus (AAV)/adenovirus or for the lysis of lentivirus
- One kit and one protocol solution for AAV or LV lysis enables standardization and quality control (QC) of current workflows
- Consistent and reproducible measurement of viral titers for multiple serotypes (e.g., AAV serotypes) or of different purities (e.g., from in-process supernatant to highly purified samples)
- Standardized workflow

Product	Description	Cat. No.
CGT Viral Vector Lysis kit	Reagents for 1000 samples	250273
CGT Viral Vector Lysis kit	Reagents for 100 samples	250272
Cell and Gene Therapy Lentivirus Lysis Kit	Reagents for 100 samples	250323
Cell and Gene Therapy Lentivirus Lysis Kit	Reagents for 100 samples	250324

Visit product page

This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

Enhance your LV analytics with fast, reliable and resource-efficient solutions

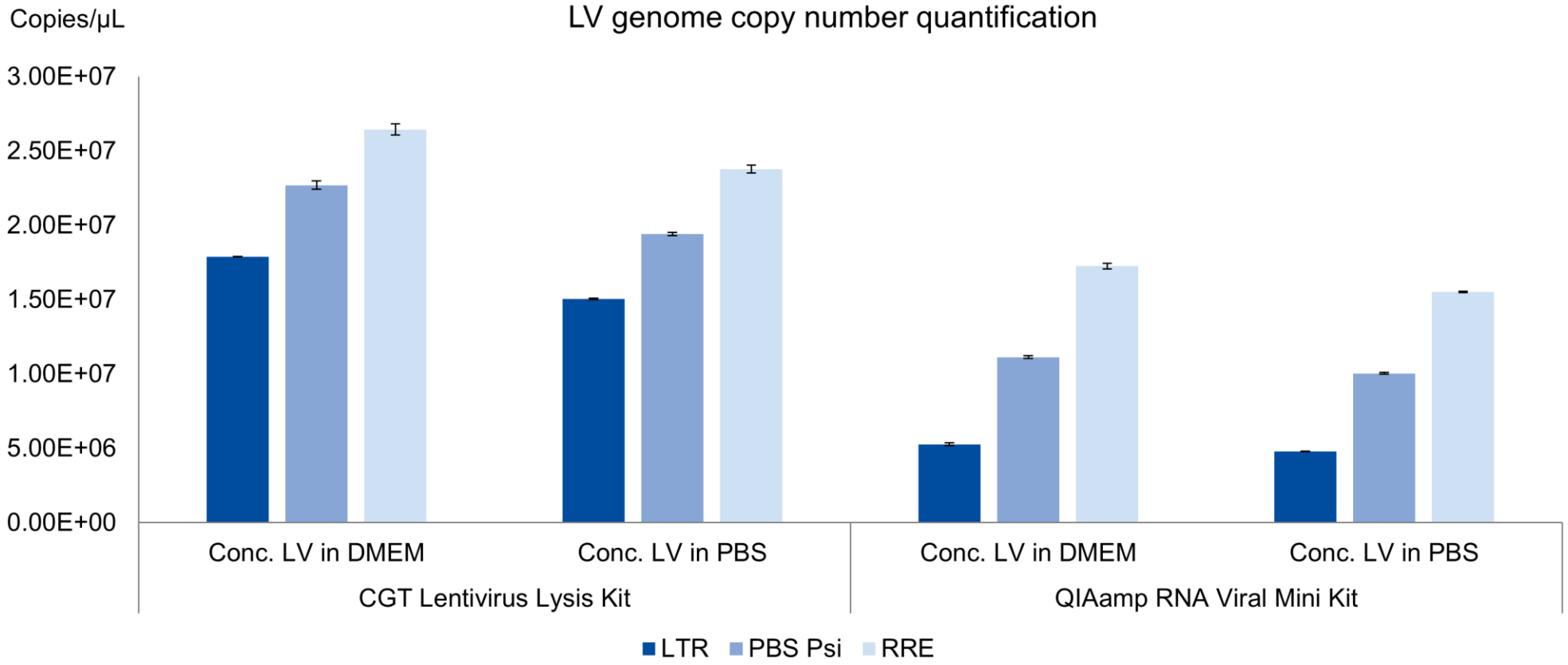


Workflow from direct lysis to quantification of LV titer

Quantification of integration events (VCN)

Detection of replication-competent lentiviruses (RCL)

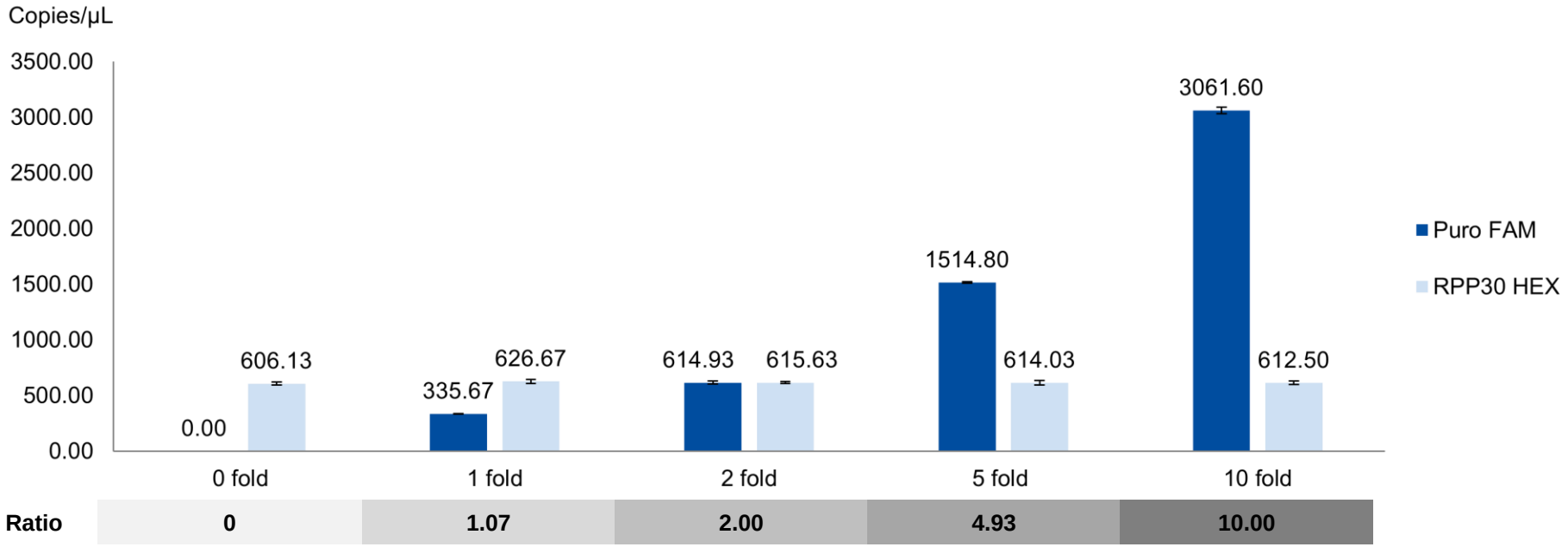
CGT Lentivirus Lysis Kit can be used with LV samples of different purities



Concentrated LV samples can be processed using the CGT Lentivirus Lysis Kit, which yields a higher concentration compared to the QIAamp RNA Viral Mini Kit.

Accuracy for PuroR FAM + RPP30 HEX duplex reaction

Determination of VCN: gDNA with LVV target spiked-in for VCN 1, VCN 2, VCN 5 and VCN 10



$$VCN = 2 \times \frac{vector\ target\ copies}{human\ reference\ target\ copies}$$

Very high accuracy for VCN quantification with PuroR FAM + RPP30 HEX duplex <5%.

QIAcuity RCL Quant Kit

Pack sizes:

96 x 40 µL reaction with positive and internal controls

Restriction enzyme compatibility:

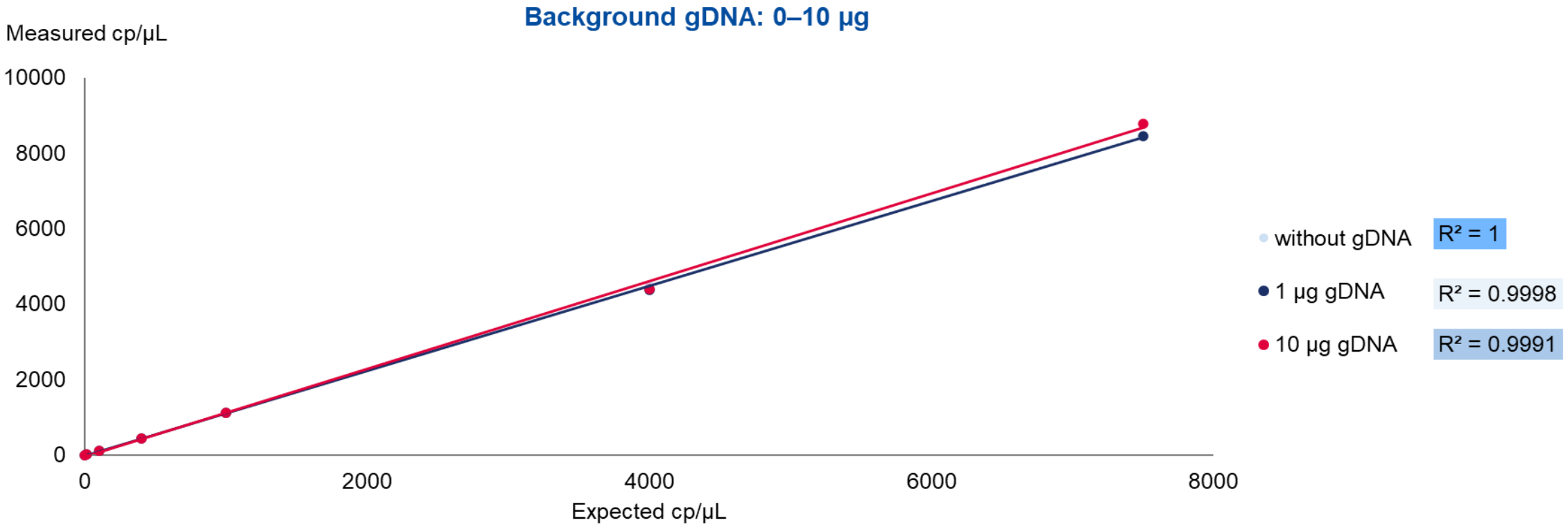
PvuII

Visit product page

Product	Description	Cat. No.
QIAcuity RCL Quant Kit	Detection of the presence of RCL using VSV-G assay	250322

Kit components	Amount	Cap color
QIAcuity MasterMix (1/2)	1	Red
QN Internal Control DNA dPCR	1	Blue
QN IC Probe Assay 10x (200µL)	1	Orange
QIAcuity RCL (VSV-G) Assay	1	Violet
QIAcuity RCL (VSV-G) Assay Positive Control	1	Green
RNase-free Water (1.9 mL/2)	2	Transparent

QIAcuity RCL Quant Kit with high gDNA background



Increasing amounts of human background genomic DNA did not affect the detection of VSV-G target. The assay demonstrated high linearity and a broad dynamic range of 0.35–7500 copies/µL.

QIAcuity Residual DNA Quantification Kits (#250221)

Kit features

- Ready-to-use master mix for easy setup (4x24 reactions)
- Duplex assay: resDNA target and Internal Control
- DNA controls: Positive Control and Internal Control
- High sensitivity with detection down to femtograms
- Multicopy target assays ensuring that the results are not affected by fragmentation of host cell DNA
- Reliable resDNA quantification also in presence of PCR inhibitors
- HEK293/CHO/*E.coli*

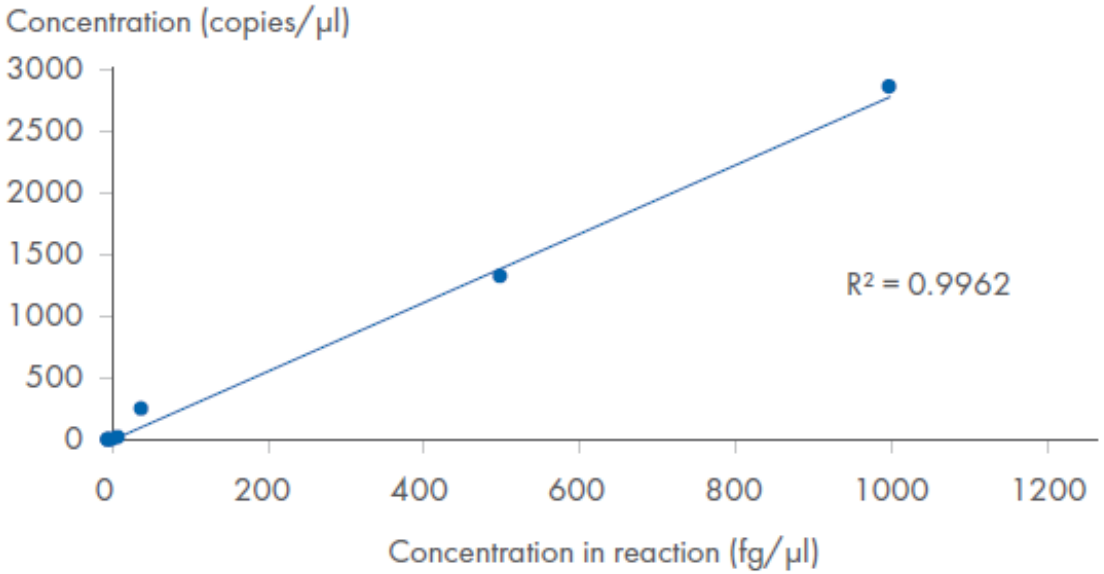
dPCR-verified DNA standards

- resDNA Quant Standards 1000 pg (lyophilized)
- For validation of quantitation accuracy or bridging studies

Visit product page

This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

QIAcuity dPCR offers unmatched accuracy and sensitivity for detection of trace HCD amounts



DNA target (fg)	DNA concentration in reaction (fg/µl)	Mean value (Copies/µl)	STDEV (Copies/µl)	Coefficient of variation (%)
40000	1000	2851.67	105.24	3.69
20000	500	1329.02	39.75	2.99
4000	40	257.83	9.84	3.82
400	10	26.48	1.21	4.58
40	1	2.53	0.14	5.71
10	0.25	0.69	0.12	16.78
4	0.1	0.32	0.09	28.07

Linear detection using the QIAcuity CHO resDNA Quant Kit.

QIAcuity Mycoplasma Quant Kit

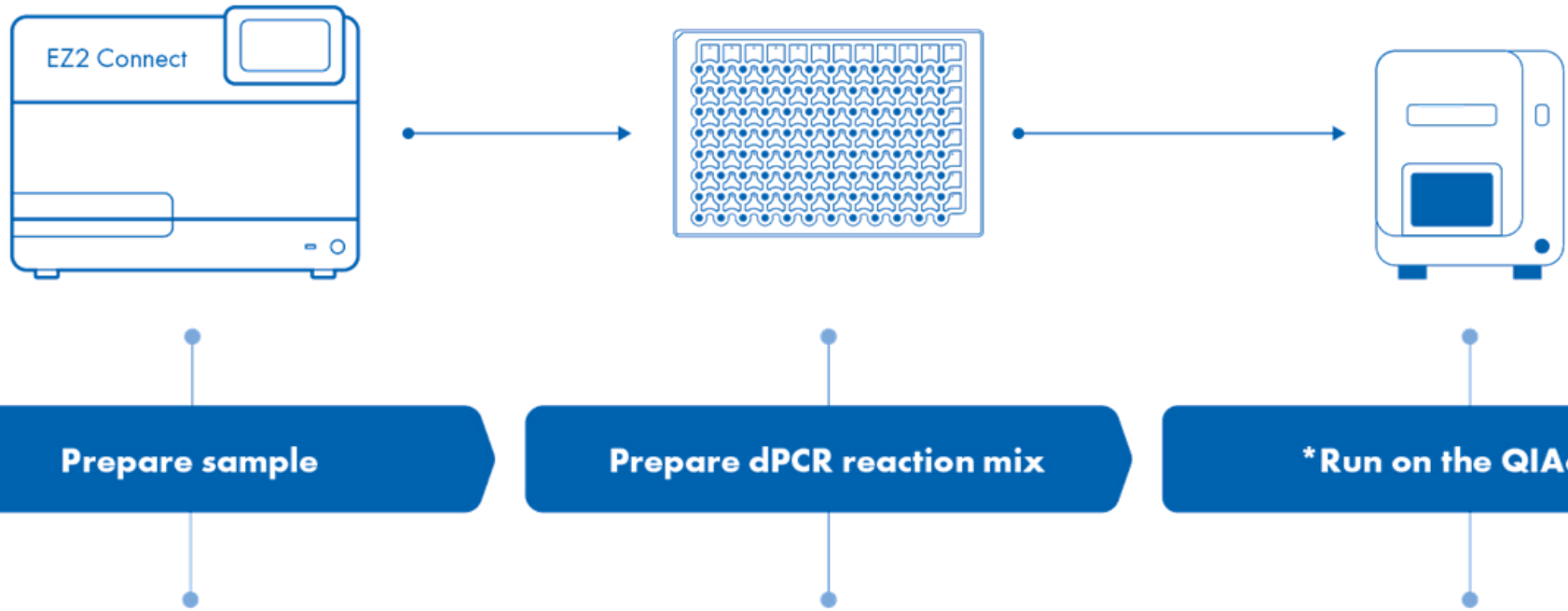
Kit features

- Ready-to-use master mix for easy setup (96 reactions)
- Duplex assay: Mycoplasma – FAM and Internal Control – HEX
- Controls: Positive Control and Internal Control
- Able to detect at least 127 Mollicutes species
- Comprehensive validation report covering all relevant performance aspects according to section 2.6.7 of the European Pharmacopoeia
- CFU standards (optional)

Visit product page

This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

Fast and easy mycoplasma detection workflow using dPCR with same-day results



- Prepare samples using the EZ1& 2 Virus Mini Kit v2.0 on the EZ2 Connect
- Include QIAcuity Mycoplasma Quant Kit Internal Control as spike-in

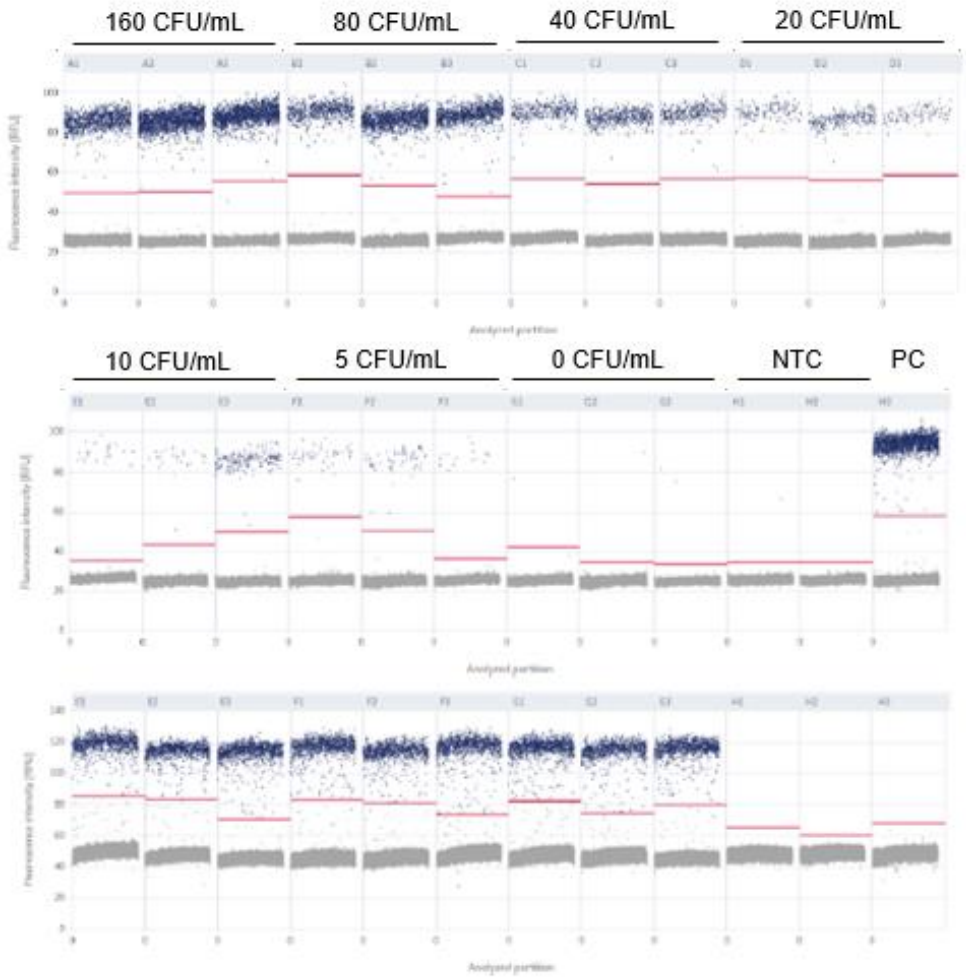
- Add eluate to the QIAcuity Mycoplasma Quant Kit Master Mix
- Pipet samples into nanoplate

- Run in your QIAcuity Digital PCR System and analyze the result

**QIAcuity Digital PCR is available in three formats (One, Four, Eight) for scalable throughput to match testing needs.*

QIAcuity Mycoplasma Quant Kit workflow is validated to meet Pharmacopeia requirements

RT-dPCR 1D scatterplots of *M. orale* in DMEM + 10% FCS and Internal Control Spike-In



Limit of detection of different Mollicutes species

Species/sample	Sensitivity
Acholeplasma laidlawii	5 CFU/mL
Mycoplasma arginini	5 CFU/mL
Mycoplasma fermentans	5 CFU/mL
Mycoplasma gallisepticum	5 CFU/mL
Mycoplasma hyorhinis	5 CFU/mL
Mycoplasma orale	5 CFU/mL
Mycoplasma pneumoniae	5 CFU/mL
Mycoplasma salivarium	10 CFU/mL
Mycoplasma synoviae	10 CFU/mL
Spiroplasma citri	5 CFU/mL
WHO International Standard	10 IU/mL

QIAcuity Mycoplasma CFU Standards

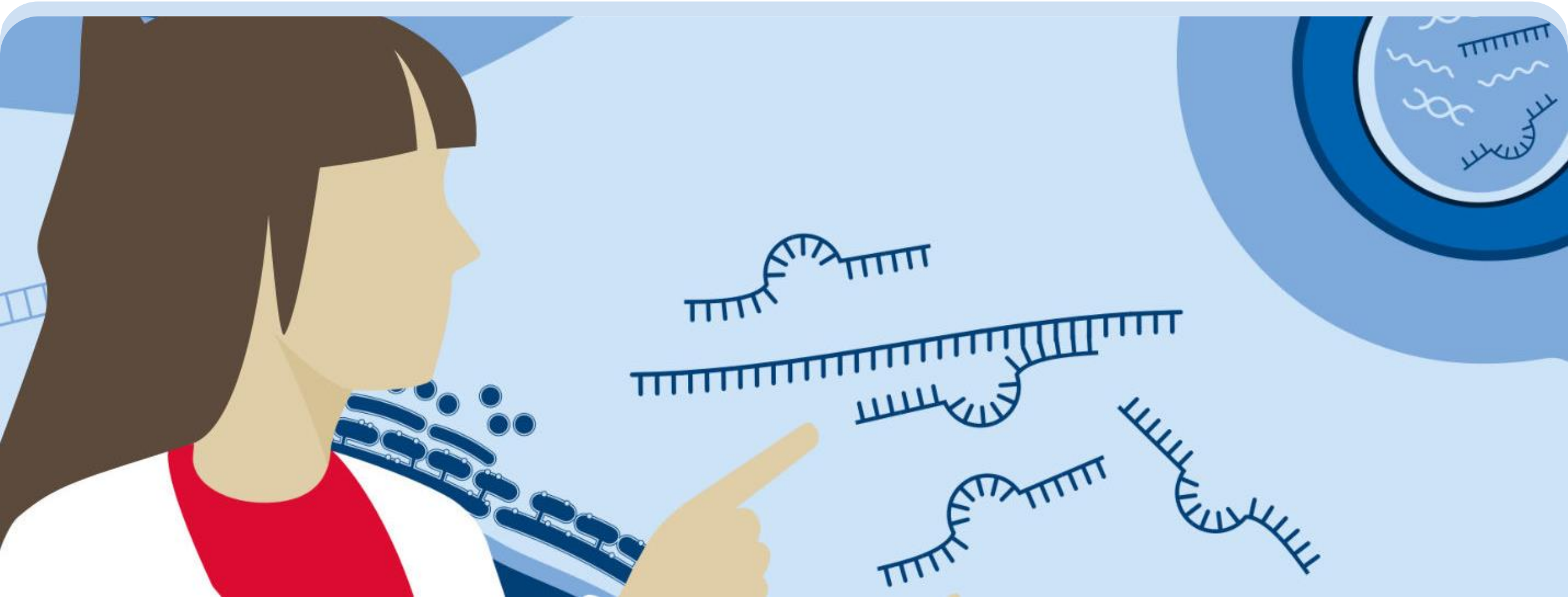
For use as unknown samples in the workflow

- The CFU standards can be used to validate the system at the users' site
- The standards contain inactivated mycoplasma in a pre-defined amount and should be reconstituted with 1 mL matrix to a final concentration of 10 CFU/mL mycoplasma
- The reconstituted standards can then be used as unknown samples and must go through the whole isolation and testing workflow
- We recommend the protocol “Detection of mycoplasma using the QIAcuity Mycoplasma Quant Kit with the use of the Spike-in Internal Control during sample preparation” from the Kit handbook
- Kit content:
  - 3 vials Mycoplasma CFU Standard
  - 1 vial Negative Control

Visit product page

This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

dPCR for gene expression analysis



miRNA and mRNA detection using dPCR: Pre-designed assay portfolio

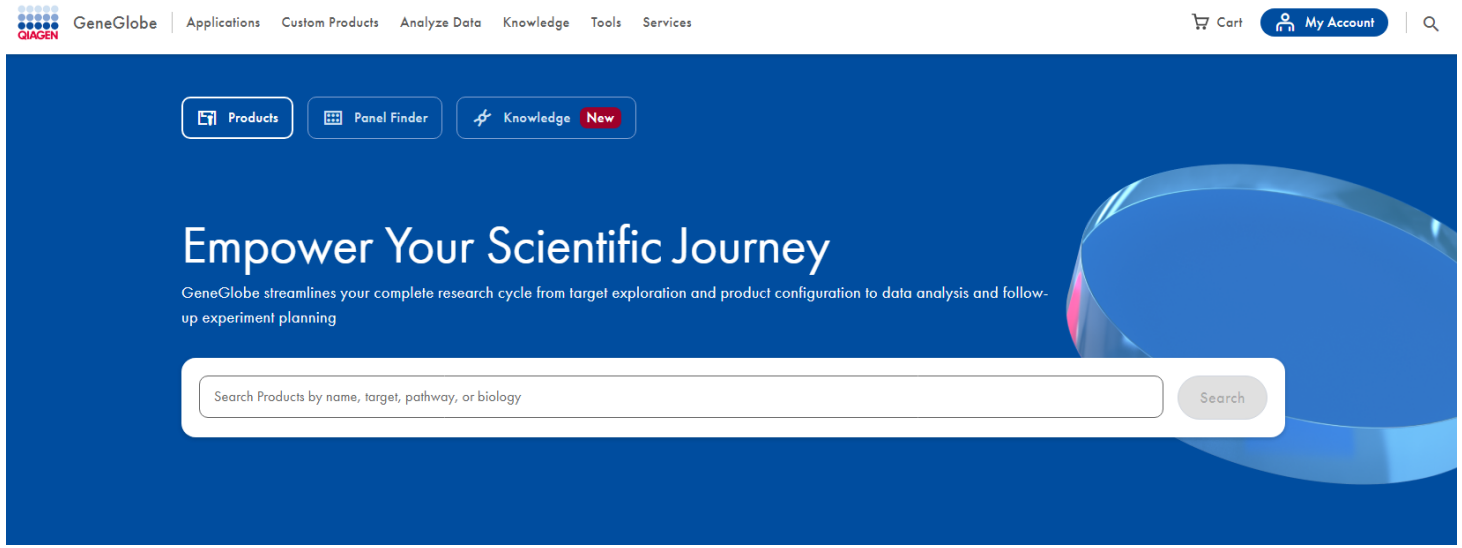
QuantiNova LNA PCR assays

miRCURY LNA miRNA PCR Assays

This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

miRCURY LNA miRNA PCR (#339306)

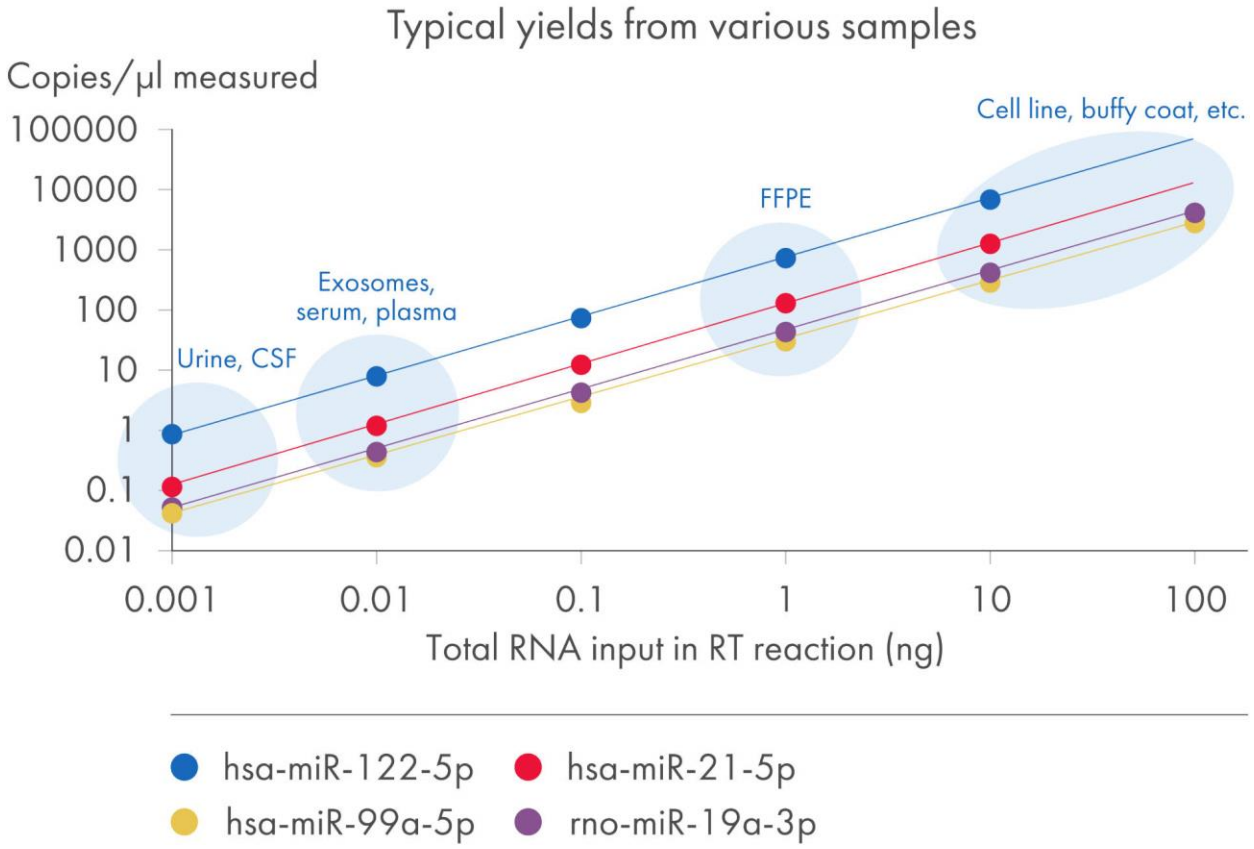
- LNA-enhanced primers for highest specificity
- More than 30,000 pre-designed assays – covering all organisms from miRBase 20
- Around 1400 wet-lab tested PCR assays across various species
- More than 50 focus panels
- Custom assays designable in GeneGlobe



Visit product page

miRCURY LNA miRNA PCR assays for Digital PCR

Enables detection of miRNAs from different samples without pre-amplification – reliable miRNA detection at 1 pg RNA input



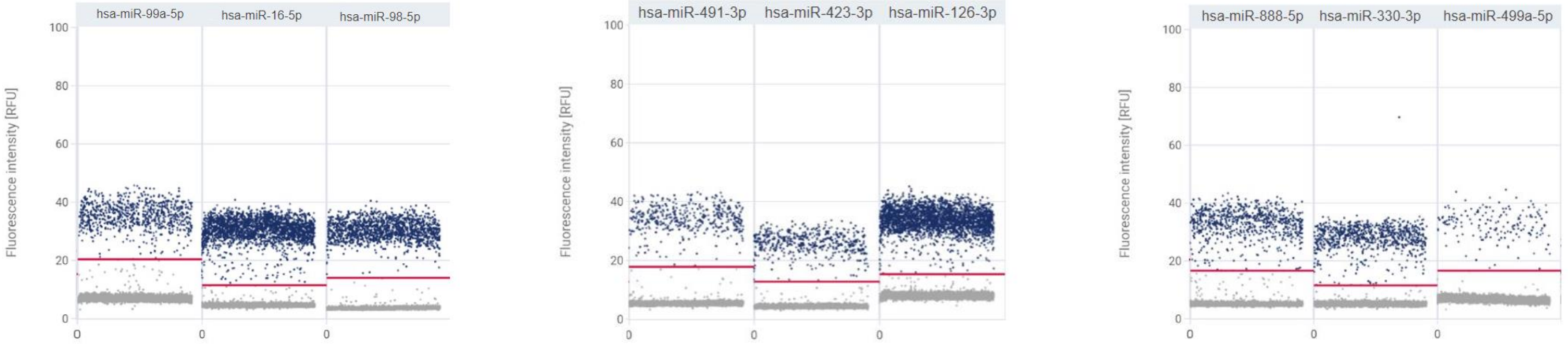
Allows reliable miRNA detection from:

- Wide range of sample types
- Low miRNA content samples (e.g., urine, CSF)
- AU-rich miRNAs

Reliable miRNA detection from a wide range of sample types – even from low-miRNA-containing samples (biofluids).

miRCURY LNA miRNA PCR assays for Digital PCR

1D plots of different miRNAs



Good separation between negative and positive partitions and precise thresholding of the positives.

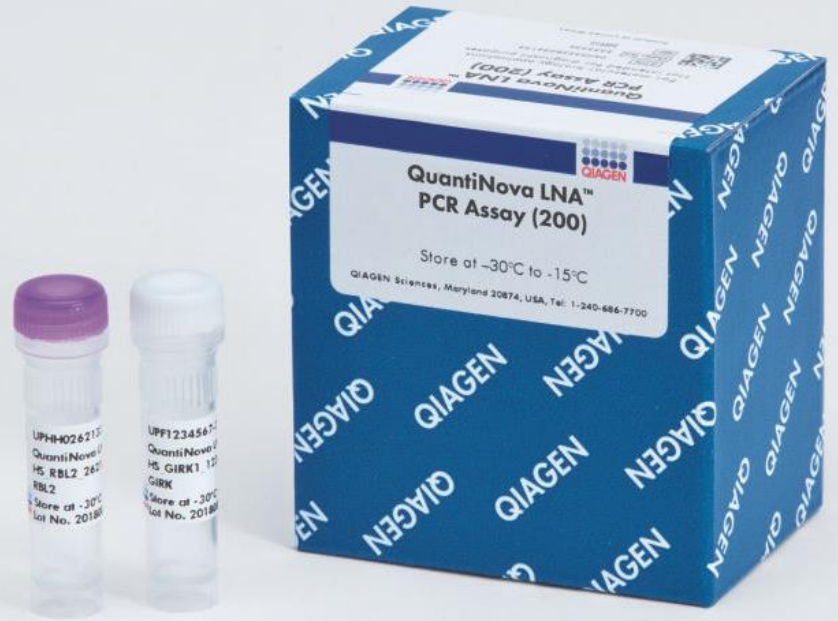
QIAcuity QuantiNova LNA PCR Assays for digital PCR (#249990)

Reliable gene expression analysis using LNA-enhanced EvaGreen dPCR

- EvaGreen detection using QIAcuity EG PCR Kit
- LNA-enhanced primers with substantially higher affinity for complementary nucleic acid strands than traditional DNA oligonucleotides
- Over 1.3 million assays offer the broadest and best coverage of mRNA and lncRNA transcripts
- Human, mouse and rat mRNA and lncRNA transcripts
- Complimentary, easy-to-use QIAcuity Software Suite analysis
- For two-step RT, QuantiTect Reverse Transcription Kit is recommended

Product Name
QuantiNova LNA PCR Assay (200; 750)
QuantiNova LNA PCR Custom Assay (200; 750)
QuantiNova LNA PCR Reference Assay (200; 750)

Visit product page

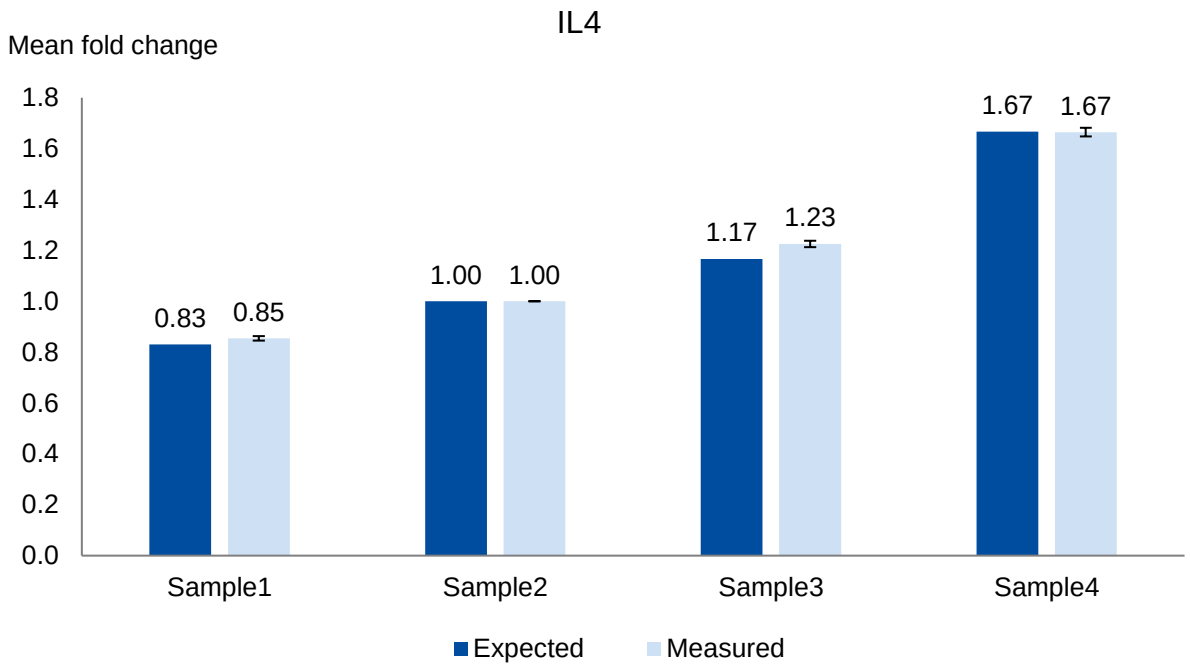


This assay is intended for non-clinical applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

Detection of small fold changes in gene expression

IL4 gene expression analysis, detecting the smallest fold changes with the highest precision using QuantiNova LNA PCR Assays

- Samples: synthetic IL4 RNA spiked into universal human reference RNA, which shows no expression for IL4
- Sample 1: decreased amount of spiked-in synthetic IL4 RNA sample fold changes expected
- Sample 2: reference sample with spiked synthetic IL4 RNA sample, no fold changes expected
- Samples 3 and 4: increased amount of spiked-in synthetic IL4 RNA sample fold changes expected
- *HPRT* and *GAPDH* as reference targets for normalization
- Consumables: Nanoplate 8.5K 96-well, QuantiTect Reverse Transcription Kit, QIAcuity EG PCR Kit



Fold change of IL4 expression. Expected values against measured values show that the smallest expression changes can be reliably detected.

Publications using QIAcuity for gene expression analysis

Molecular Therapy
Methods & Clinical Development
Original Article

Characterization of the function of Adenovirus L4 gene products and their impact on AAV vector production

Yingchao Nie,[1,2] Hao Pan,[1,2] Qingliang Li,[1] Huimin Na,[1] Bruno Figueroa,[1] and Karen Vincent[1]

[1]Genomic Medicine Unit CMC, Global CMC Development, Sanofi R&D, 225 2nd Avenue, Waltham, MA 02451, USA

Molecular Therapy
Methods & Clinical Development
Original Article

CRISPR-Cas9-mediated genome editing delivered by a single AAV9 vector inhibits HSV-1 reactivation in a latent rabbit keratitis model

Nadia Amrani,[1,4] Kevin Luk,[1,4] Pankaj Singh,[2] Mason Shipley,[2] Meltem Isik,[1] Martina Donadoni,[3] Anna Bellizzi,[3] Kamel Khalili,[3] Ilker K. Sariyer,[3] Donna Neumann,[2] Jennifer Gordon,[1] and Guo-Xiang Ruan[1]

[1]Excision BioTherapeutics Inc, Watertown, MA, USA; [2]Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison, Madison, WI, USA; [3]Center for Neurovirology and Gene Editing, Department of Microbiology, Immunology and Inflammation, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA

Received: 5 July 2024 | Revised: 14 August 2024 | Accepted: 20 August 2024

DOI: 10.1002/biot.202400415

Biotechnology Journal

RESEARCH ARTICLE

Cas-CLOVER-mediated knockout of STAT1: A novel approach to engineer packaging HEK-293 cell lines used for rAAV production

Peter Andorfer[1] | Carolin-Isabel Kahlig[1] | Doris Pakusic[1] | Robert Pachlinger[1] | Christiane John[1] | Irene Schrenk[1] | Peter Eisenhut[1] | Johannes Lengler[1] | Bernd Innthaler[1] | Lucia Micutkova[1] | Barbara Kraus[1] | Corey Brizzee[2] | Jack Crawford[2] | Juan A. Hernandez Bort[1,3]

cells

MDPI

Article

Small Extracellular Vesicle-Derived Circular RNA hsa_circ_0007386 as a Biomarker for the Diagnosis of Pleural Mesothelioma

Sareh Zhand [1], Jiayan Liao [2], Alessandro Castorina [3], Man-Lee Yuen [2], Majid Ebrahimi Warkiani [1,2,4,*] and Yuen-Yee Cheng [2,*]

1 School of Biomedical Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia
2 Institute for Biomedical Materials and Devices, Faculty of Science, University of Technology Sydney, Sydney, NSW 2007, Australia
3 Laboratory of Cellular and Molecular Neuroscience (LCMN), School of Life Sciences, Faculty of Science, University of Technology Sydney, Sydney, NSW 2007, Australia
4 Institute of Molecular Medicine, Sechenov First Moscow State University, Moscow 119991, Russia
* Correspondence: majid.warkiani@uts.edu.au (M.E.W.); yuenyee.cheng@uts.edu.au (Y.-Y.C.)

dPCR master mixes

Available QIAcuity master mixes: Choose the best option for your experiment

DNA master mixes

- QIAcuity EG PCR Kit – for dye-based applications
- QIAcuity Probe PCR Kit – for probe-based applications
- QIAcuity UCP Probe PCR Kit – DNA-depleted mix for microbial applications
- QIAcuity MasterMix – for mutation detection and lentivirus applications
- QIAcuity High Multiplex Probe PCR Kit – for multiplexing >5
- QIAcuity DX Universal MasterMix Kit – IVDR compliant for clinical use

RNA Master Mixes

- QIAcuity OneStep Advanced EG Kit – for dye-based applications
- QIAcuity OneStep Advanced Probe Kit – for probe-based applications

Available QIAcuity master mixes: Choose the best option for your experiment

Kit name	Catalog no.	Chemistry type	Plex level	Key features / intended use	Supported applications
QIAcuity EG PCR Kit	250111	EvaGreen (dye-based)	Singleplex	3× EvaGreen Master Mix; optimized for Nanoplates; high stability and specificity.	Dye-based quantification of gDNA/cDNA
QIAcuity Probe PCR Kit	250101–3	Hydrolysis probe	Up to 5-plex	4× probe mix; low nonspecific amplification; optimized for Nanoplates.	Standard probe-based dPCR (CNV, expression, editing, mutation)
QIAcuity UCP Probe PCR Kit	250121–2	Ultra-clean hydrolysis probe	Up to 5-plex	Ultra Clean Production eliminates background DNA; optimized for microbial workflows.	Microbial DNA, low-biomass samples, QC residual DNA
QIAcuity OneStep Advanced Probe Kit	250131–2	One-step RT-probe dPCR	Up to 5-plex	4× PCR + 100× HotStart RT Mix; room-temperature setup; internal RNA control.	RNA quantification, RNA+DNA dual detection, wastewater, viral assays
QIAcuity High Multiplex Probe PCR Kit	250133–4	High-plex hydrolysis probe	Up to 12-plex	Supports amplitude multiplexing + LSS dyes; designed for QIAcuity Software 3.x.	High-order multiplex biomarker panels, microbiome, pathogens
QIAcuityDx Universal MasterMix Kit	260101	IVD probe-based dPCR	Up to 5-plex	4× mix; includes $MgCl_2$ vial; diagnostic-validated for QIAcuityDx.	Clinical dPCR workflows (DNA/cDNA quantification)
QIAcuity MasterMix	1133251	Probe-based (mutation-optimized)	Duplex (with LNA assays)	Designed for LNA Mutation Assays; FAM/HEX or Atto550/ROX; 0.1% allele detection.	Rare mutation detection, ctDNA, FFPE samples
QIAcuity OneStep Advanced EG Kit	250141-2	EvaGreen (dye-based)	Singleplex	For sensitive quantification of RNA or RNA+DNA in one reaction	Dye-based quantification of RNA or RNA+DNA, mutation detection, genome editing, CNV detection, gene expression

QIAcuityDx is intended for in vitro diagnostic use. Product availability may differ from country to country based on regulations and approvals. Contact your country representative for further details. QIAcuity One, QIAcuity Four and QIAcuity Eight are intended for non-clinical applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

Thank you for your attention. Questions?

.

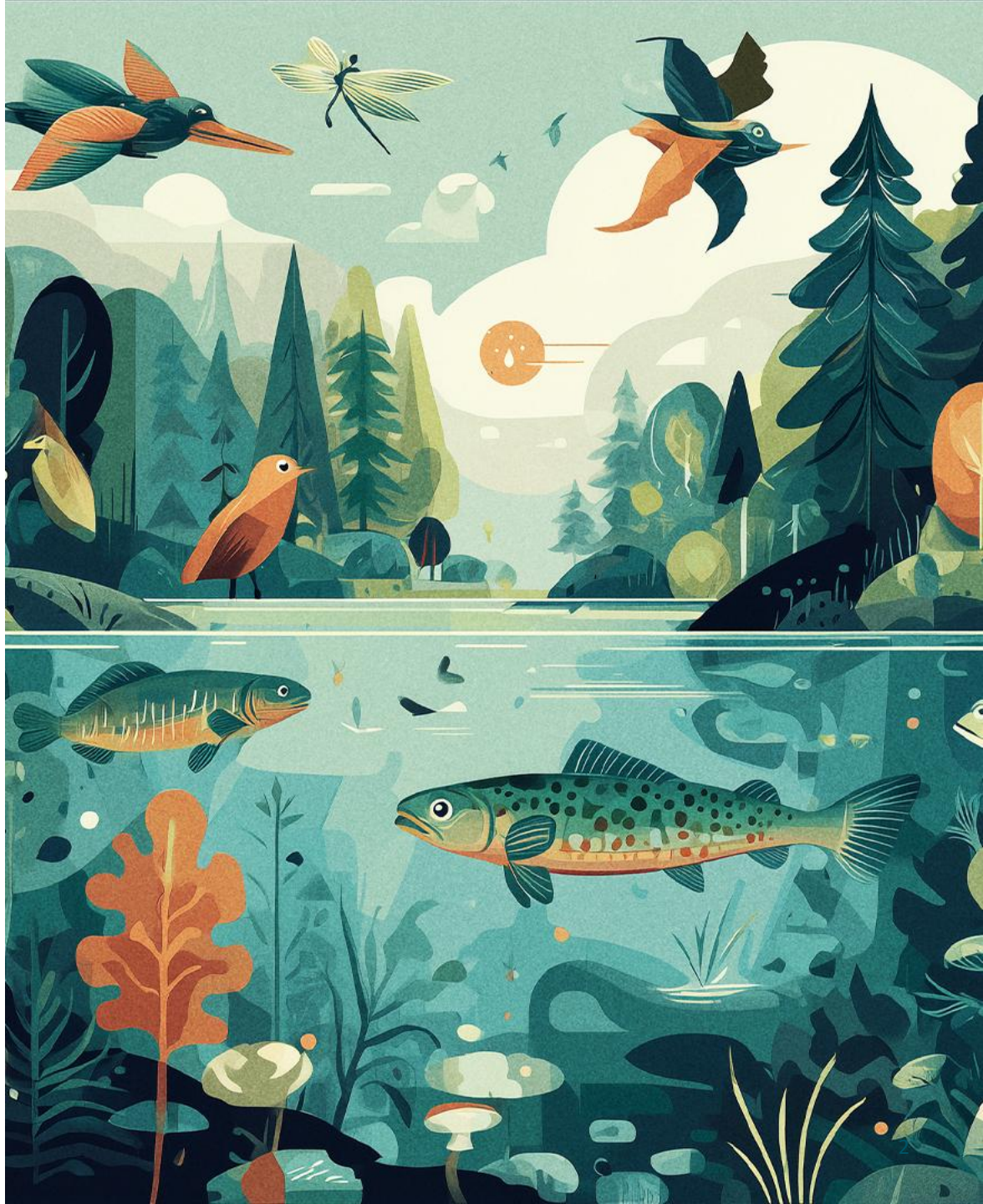
Commonly asked questions

1. **Does the assay work only with QIAGEN primers/probes?**
2. **Which nanoplate type should I use?**
3. **What dyes can I use when multiplexing?**
4. **Can I do gradient dPCR?**
5. **How much DNA can I add to the reaction mix?**
6. **What is the dynamic range of the QIAcuity?**
7. **How long should my amplicon be?**
8. **Do I have to digest my DNA samples?**
9. **How do I know my primers are specific?**
10. **If my primers don’t amplify well in qPCR, will they work in dPCR?**


SpringDNA
Rethink the way you monitor biodiversity

Puheenvuorossa

- Lyhyesti SpringDNA
- Kokemuksia hankkeista vedessä, maalla ja ilmassa
- Ajatuksia jatkoon



Lyhyesti SpringDNA

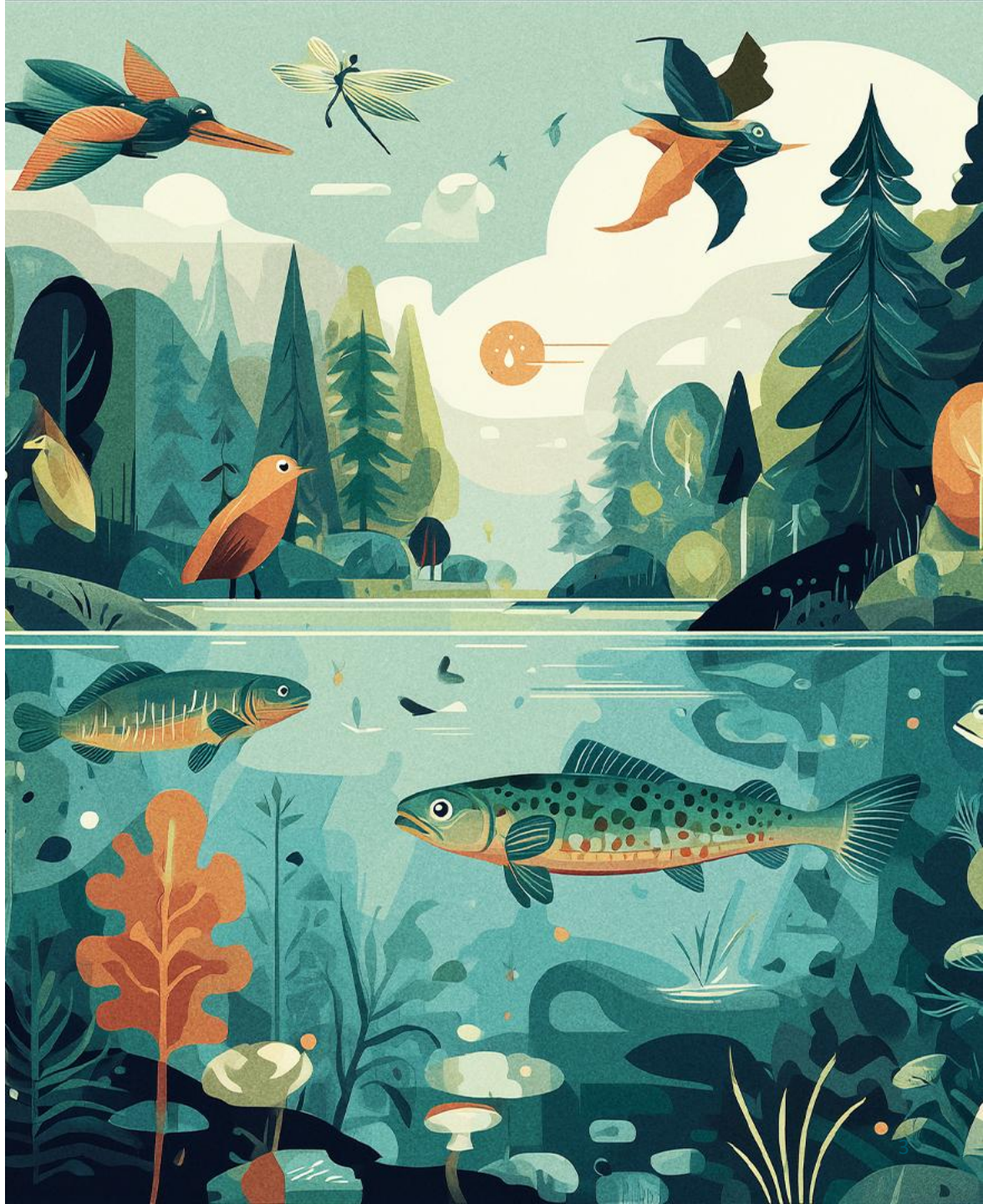
SpringDNA:n eDNA-teknologia mahdollistaa täysin uuden tavan seurata luonnon monimuotoisuutta ja ympäristöä:

- Tuotteet: kohdeanalyysi (kohdelajit) ja yhteisöanalyysi (esim. kalasto, linnusto)
- Indikaattorilajit, ennallistaminen, vieraslajit, laaja-alaiset tason ja suunnan arvioinnit

✓ **Nopeampi kartoitus**

✓ **Parempi data = paremmat päätökset**

✓ **Kustannustehokkuus**



Projekti: Kalatalouden KYMPPI innovaatio-ohjelma 2024-2026
Asiakas: Luonnonvarakeskus

Tarve: Teknologian testaus ja kehittäminen suomalaisiin olosuhteisiin.

Ratkaisu: Eri teknologioiden testaaminen, kontrolloidut olosuhteet, kohdelaji järvilohi.

Hyöty: Luonnonvesiin ja vaelluskalojen monitorointiin tarkoitetun teknologian testaus ja kehittäminen.

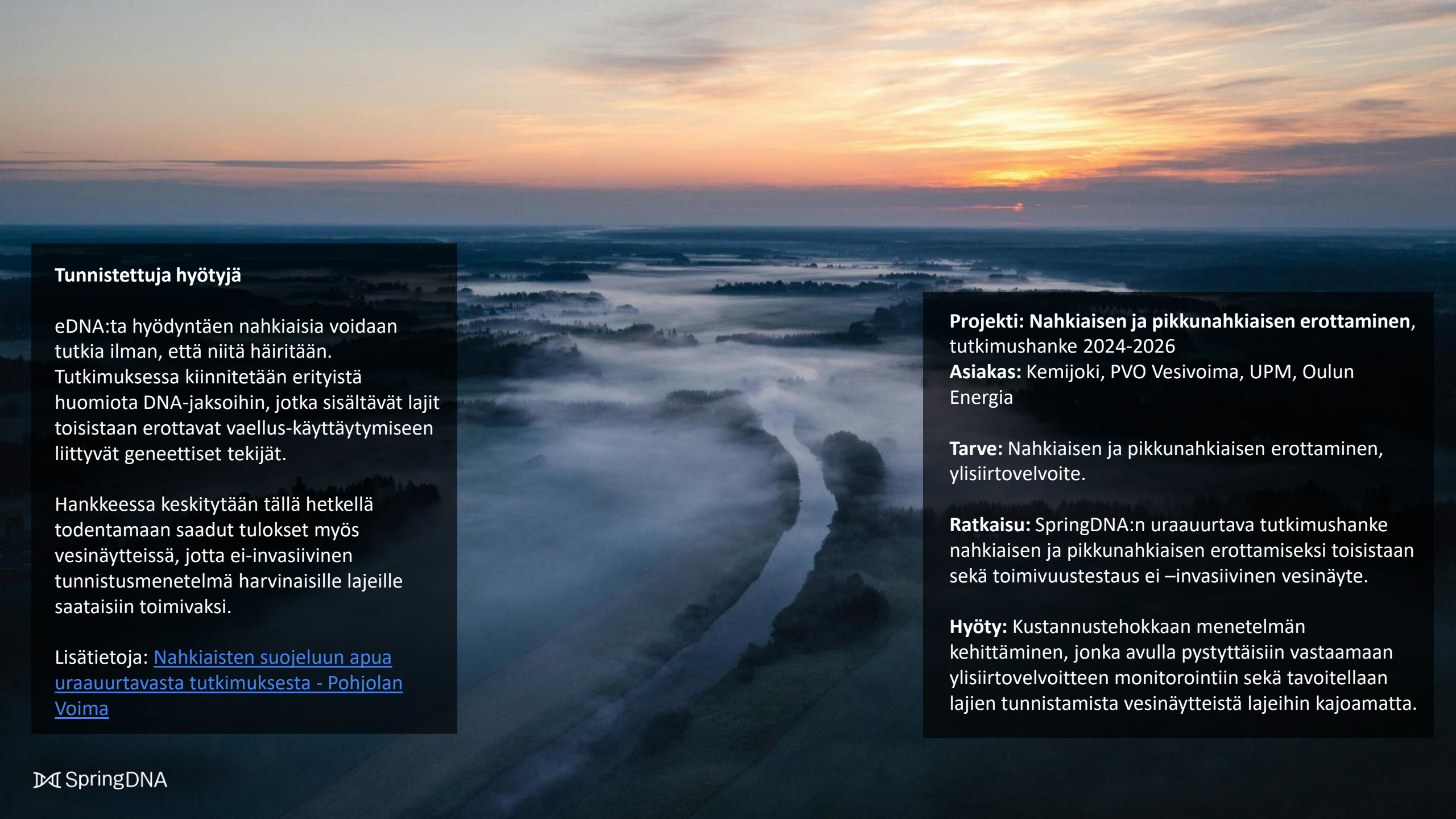
Dataa populaatiokokojen arviointiin

Pinnan alaisia kalastojen populaatiokokoja on ollut työlästä ja vaikeaa arvioida perinteisin menetelmin.

Hankkeessa saatiin teknologian avulla lupaavia tuloksia populaatiokokojen arviointiin, joka simuloi luonnontilaisia virtavesiä sekä alhaisia kalatiheyksiä.

Lisätietoja: ”eDNA avaa uusia mahdollisuuksia sisävesien seurantaan” https://merijakalatalous.fi/kalatalouden-ymparistoohjelma-kymppi/edna-avaa-uusia-mahdollisuuksia-sisavesien-seurantaan

SpringDNA



Tunnistettuja hyötyjä

eDNA:ta hyödyntäen nahkiaisia voidaan tutkia ilman, että niitä häiritään. Tutkimuksessa kiinnitetään erityistä huomiota DNA-jaksoihin, jotka sisältävät lajit toisistaan erottavat vaellus-käyttäytymiseen liittyvät geneettiset tekijät.

Hankkeessa keskitytään tällä hetkellä todentamaan saadut tulokset myös vesinäytteissä, jotta ei-invasiivinen tunnistusmenetelmä harvinaisille lajeille saataisiin toimivaksi.

Lisätietoja: Nahkiaisten suojeluun apua uraauurtavasta tutkimuksesta - Pohjolan Voima

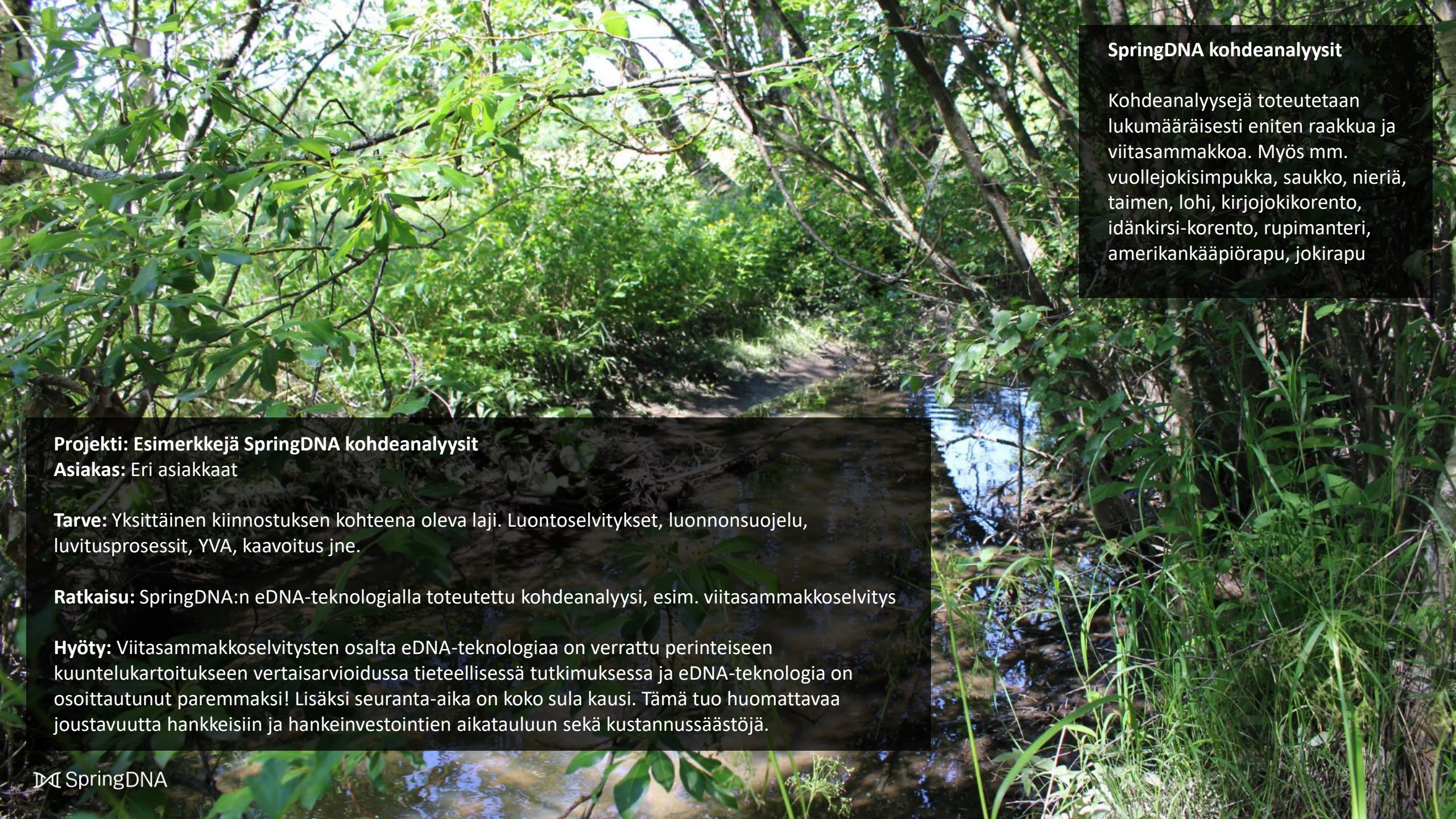
Projekti: Nahkiaisen ja pikkunahkiaisen erottaminen, tutkimushanke 2024-2026
Asiakas: Kemijoki, PVO Vesivoima, UPM, Oulun Energia

Tarve: Nahkiaisen ja pikkunahkiaisen erottaminen, ylisiirtovelvoite.

Ratkaisu: SpringDNA:n uraauurtava tutkimushanke nahkiaisen ja pikkunahkiaisen erottamiseksi toisistaan sekä toimivuustestaus ei –invasiivinen vesinäyte.

Hyöty: Kustannustehokkaan menetelmän kehittäminen, jonka avulla pystyttäisiin vastaamaan ylisiirtovelvoitteen monitorointiin sekä tavoitellaan lajien tunnistamista vesinäytteistä lajeihin kajoamatta.

SpringDNA



SpringDNA kohdeanalyysit

Kohdeanalyysejä toteutetaan lukumääräisesti eniten raakkua ja viitasammakkoa. Myös mm. vuollejokisimpukka, saukko, nieriä, taimen, lohi, kirjojokikorento, idänkirsi-korento, rupimanteri, amerikankääpiörapu, jokirapu

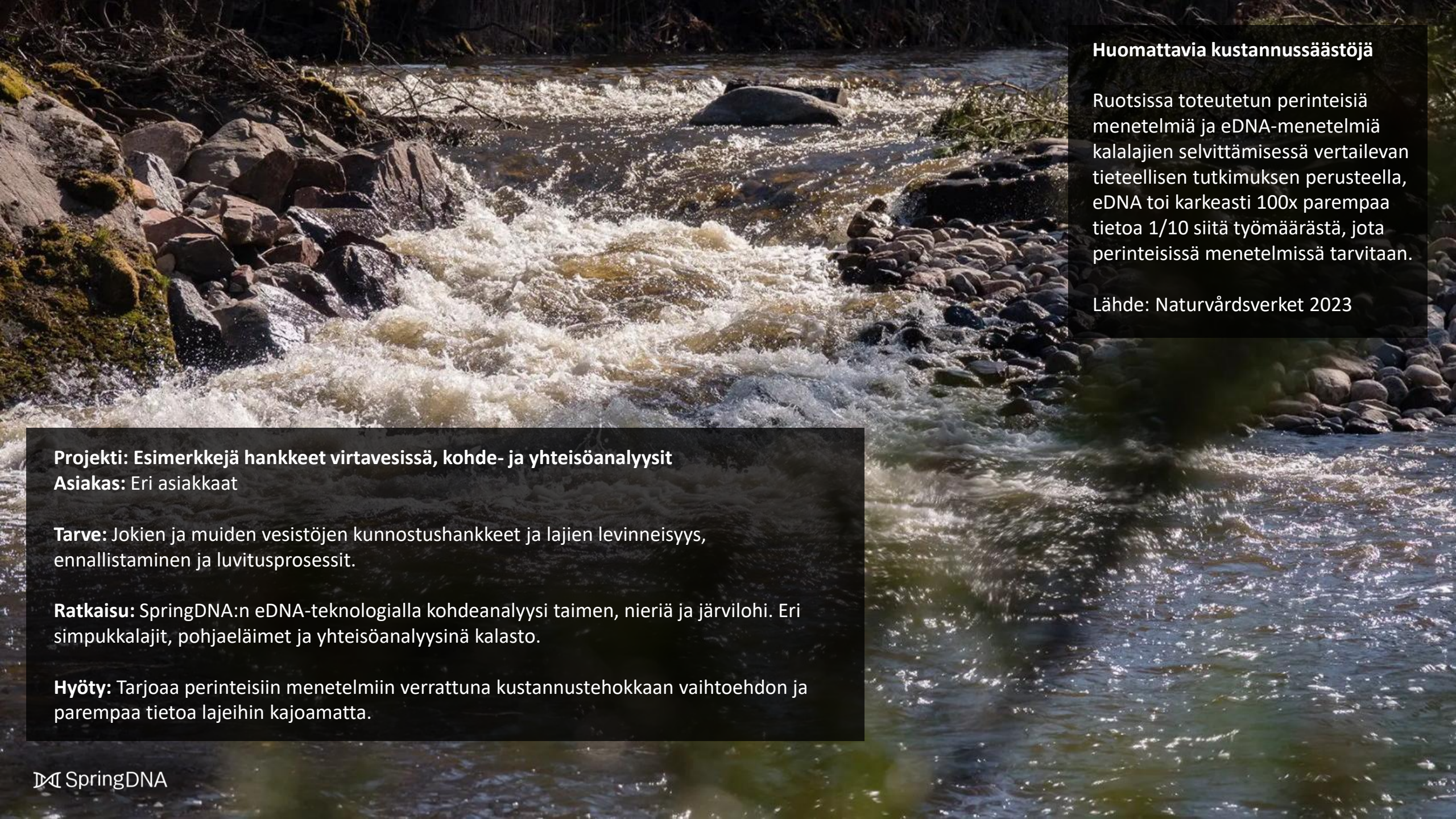
Projekti: Esimerkkejä SpringDNA kohdeanalyysit
Asiakas: Eri asiakkaat

Tarve: Yksittäinen kiinnostuksen kohteena oleva laji. Luontoselvitykset, luonnonsuojelu, luvitusprosessit, YVA, kaavoitus jne.

Ratkaisu: SpringDNA:n eDNA-teknologialla toteutettu kohdeanalyysi, esim. viitasammakkoselvitys

Hyöty: Viitasammakkoselvitysten osalta eDNA-teknologiaa on verrattu perinteiseen kuuntelukartoitukseen vertaisarvioidussa tieteellisessä tutkimuksessa ja eDNA-teknologia on osoittautunut paremmaksi! Lisäksi seuranta-aika on koko sula kausi. Tämä tuo huomattavaa joustavuutta hankkeisiin ja hankeinvestointien aikatauluun sekä kustannussäästöjä.

SpringDNA



Huomattavia kustannussäästöjä

Ruotsissa toteutetun perinteisiä menetelmiä ja eDNA-menetelmiä kalalajien selvittämisessä vertailevan tieteellisen tutkimuksen perusteella, eDNA toi karkeasti 100x parempaa tietoa 1/10 siitä työmäärästä, jota perinteisissä menetelmissä tarvitaan.

Lähde: Naturvårdsverket 2023

Projekti: Esimerkkejä hankkeet virtavesissä, kohde- ja yhteisöanalyysit
Asiakas: Eri asiakkaat

Tarve: Jokien ja muiden vesistöjen kunnostushankkeet ja lajien levinneisyys, ennallistaminen ja luvitusprosessit.

Ratkaisu: SpringDNA:n eDNA-teknologialla kohdeanalyysi taimen, nieriä ja järvilohi. Eri simpukkalajit, pohjaeläimet ja yhteisöanalyysinä kalasto.

Hyöty: Tarjoaa perinteisiin menetelmiin verrattuna kustannustehokkaan vaihtoehdon ja parempaa tietoa lajeihin kajoamatta.

SpringDNA




SpringDNA on toteuttanut kymmeniä uraauurtavia hankkeita maalla, vedessä ja ilmassa eDNA-teknologiansa avulla

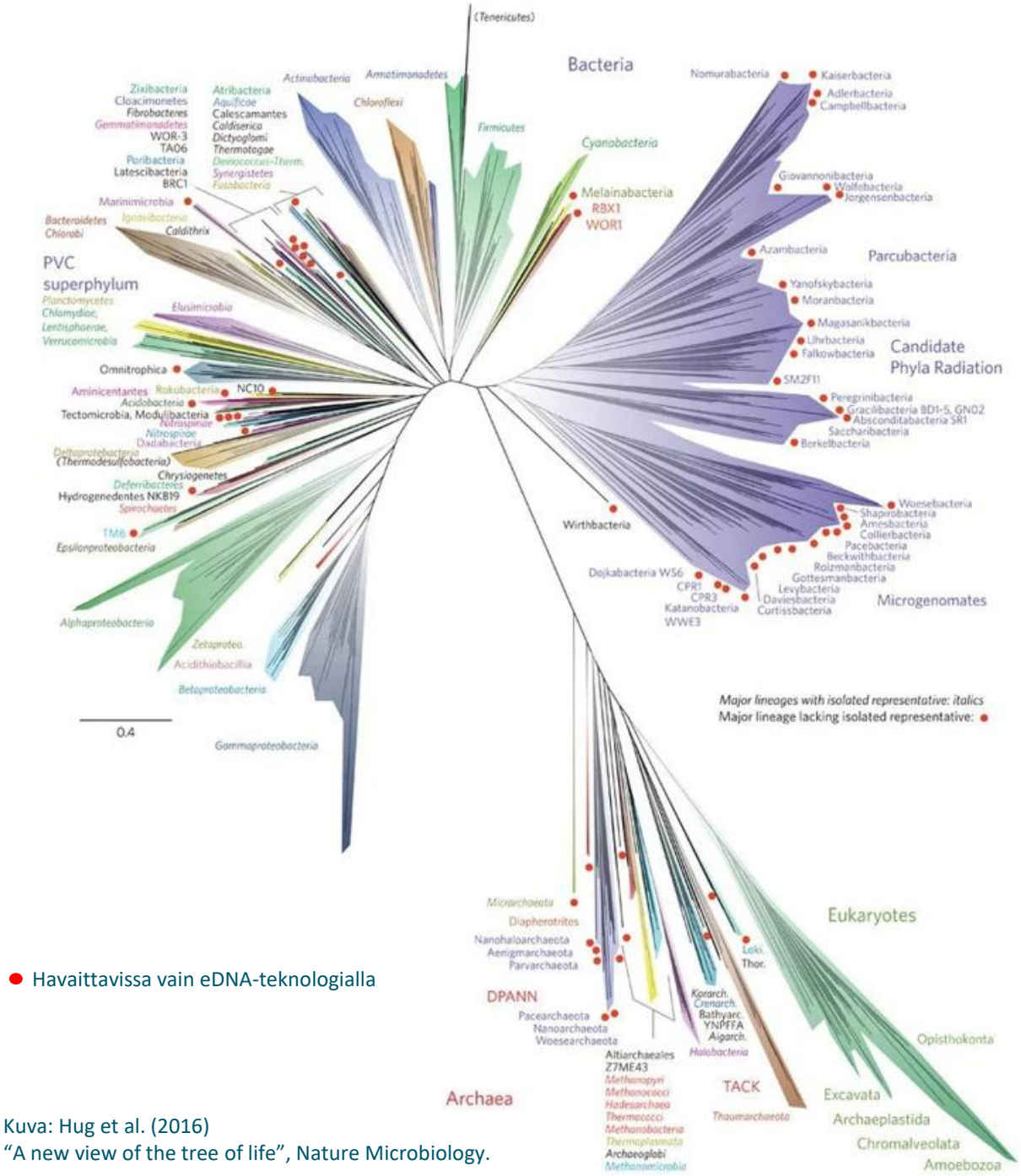







Ajatuksia jatkoon

- Vahva tiedeperusteisuus kun tehdään uutta
- Ongelmien ratkaisu teknologian sijaan keskiössä – ratkaisemme konkreettisia tämän päivän haasteita
- Disruptio näyttää siltä keltä kysyy
- Pilotit ja iterointi – aikasyklin nopeuttaminen, tiede käyttöön
- Uteliaisuus – kysytään avoimia kysymyksiä
- Luonto antaa vastaukset moniin aiheuttamiimme haasteisiin – katsotaan tarkemmin!



● Havaittavissa vain eDNA-teknologialla

Kuva: Hug et al. (2016)
"A new view of the tree of life", Nature Microbiology.

Yhteystiedot SpringDNA

Tarjoamme teknologiaa biologisen monimuotoisuuden seurantaan käyttämällä ympäristö-DNA:ta:

- Nopeampi ja jopa ympärivuotinen selvitys
- Tarkempi data, täysin uudella tiedon tasolla
- Kustannustehokkuus

Lisää meistä: www.SpringDNA.com

Yhteystiedot:

Saara Suurla
CEO SpringDNA
Email. saara.suurla@springdna.com
Mob. +358 40 741 7677

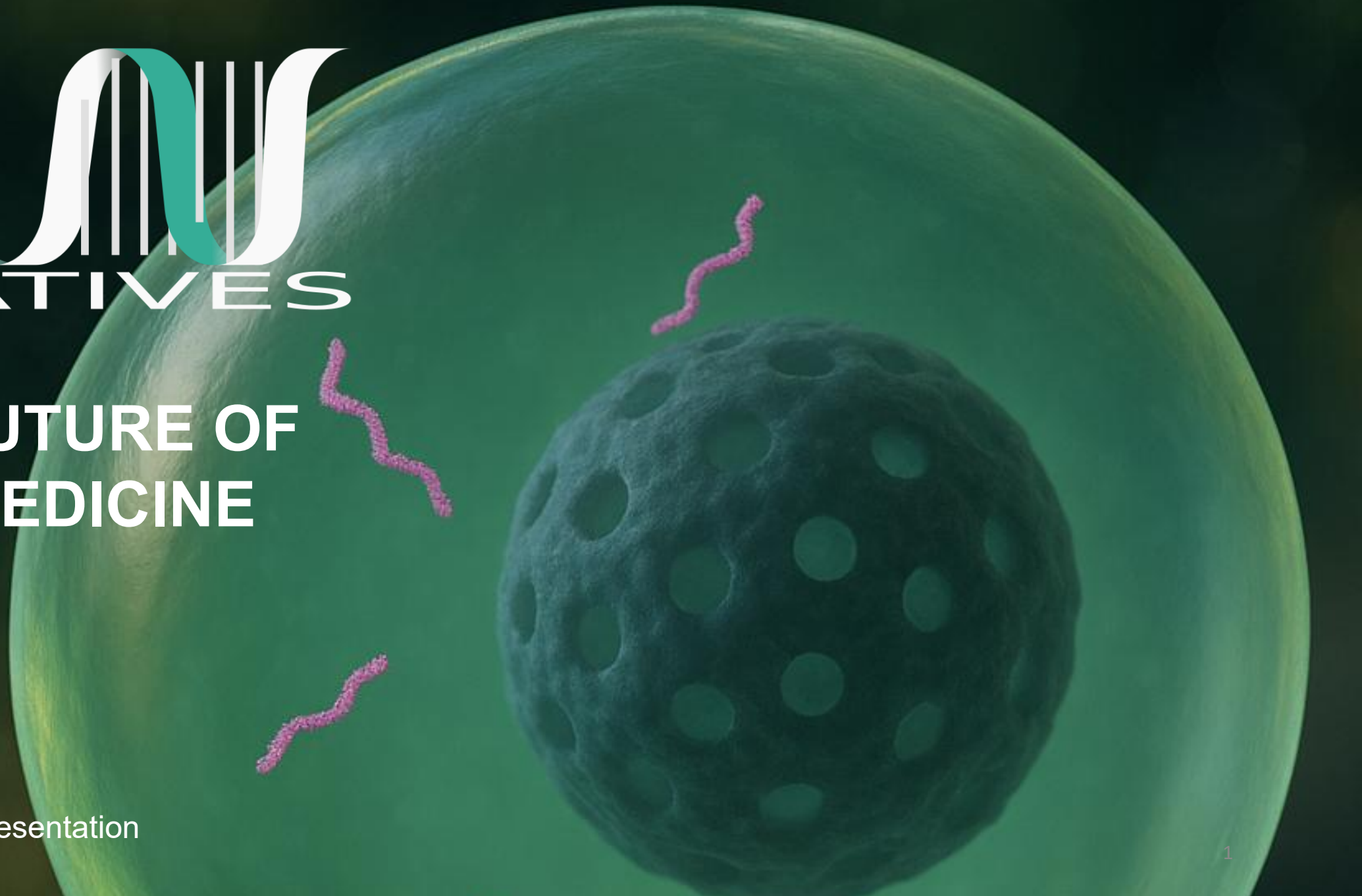
Y-tunnus: 3612073-3

SpringDNA, Lapinlahdenkatu 16, 00180 Helsinki

SpringDNA

RNATIVES

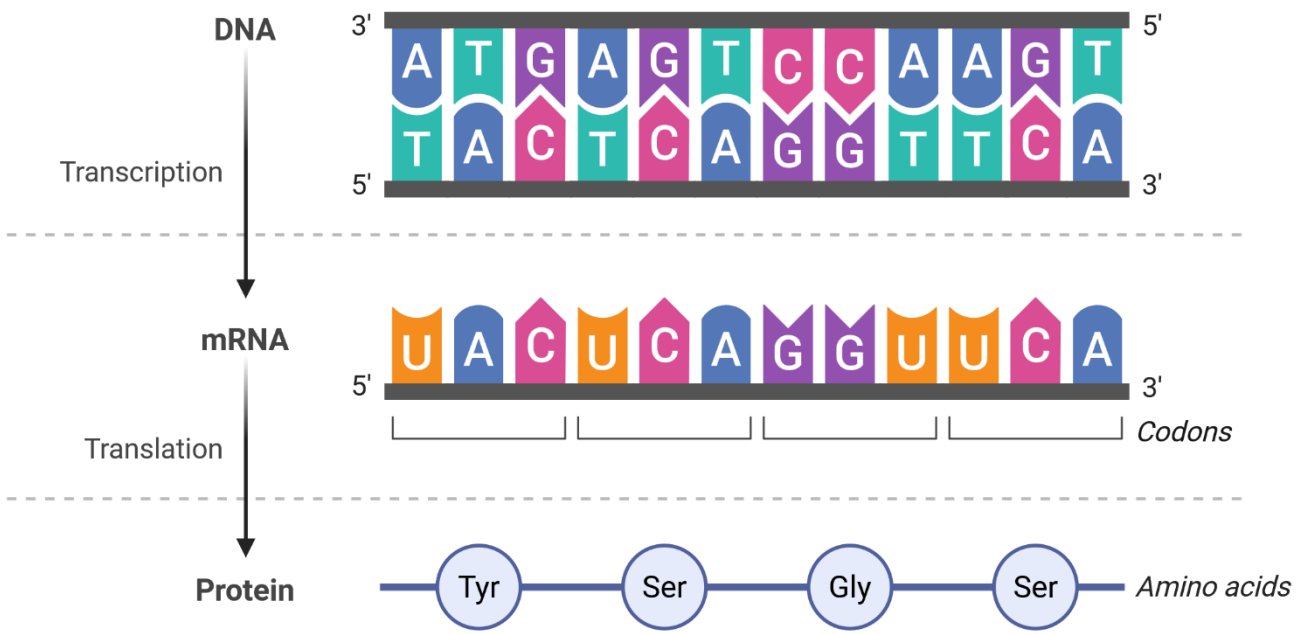
THE FUTURE OF RNA MEDICINE



Non-confidential presentation

Science behind us

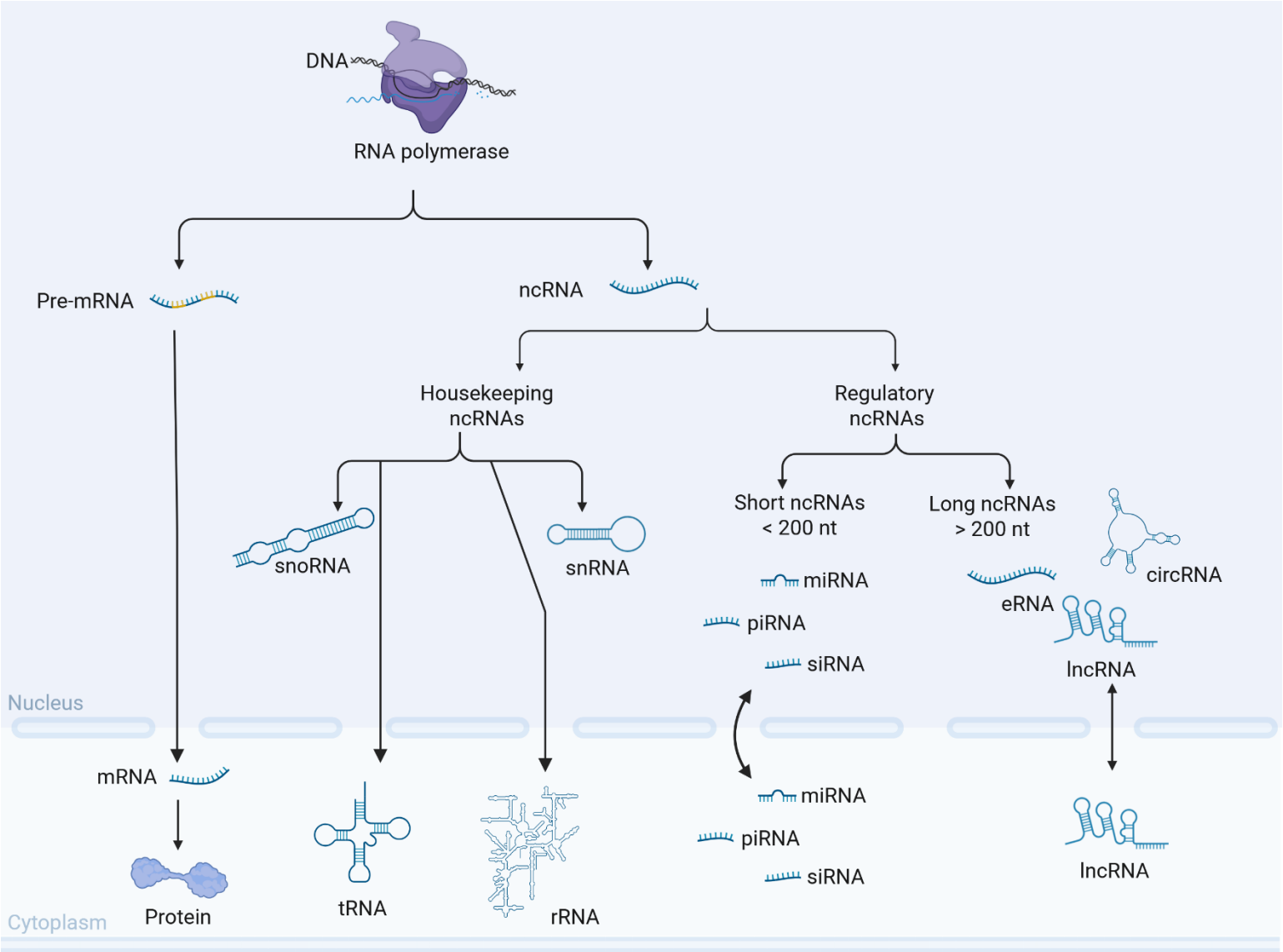
Revision of *central dogma* of molecular biology

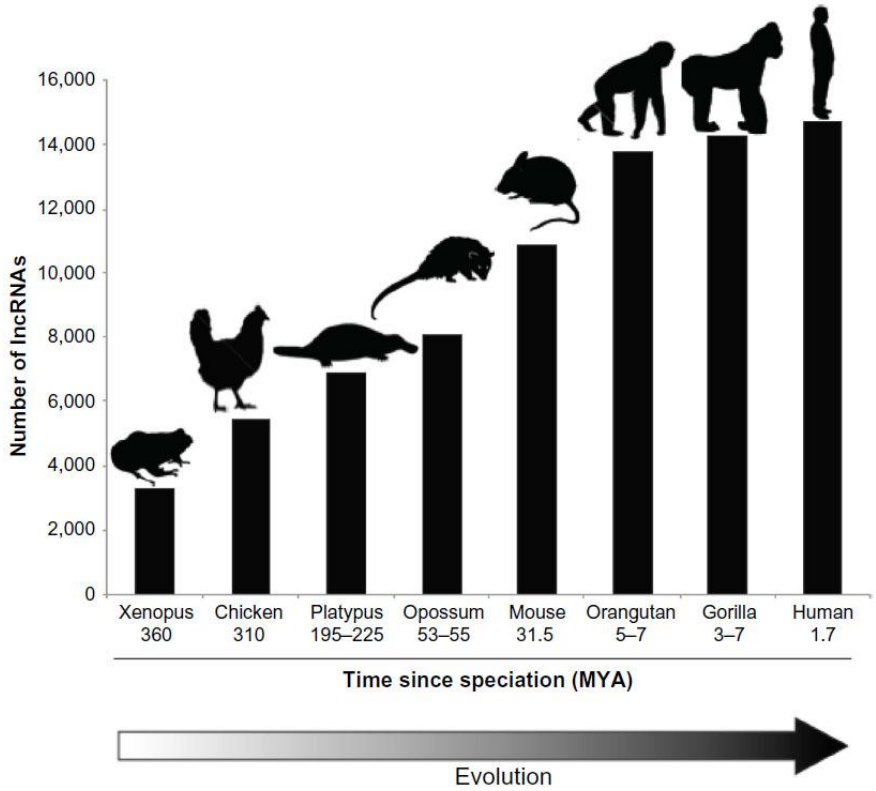


Only 2 % of the genome

Illustrations created in BioRender.com

Non-coding RNAs are key to gene regulation





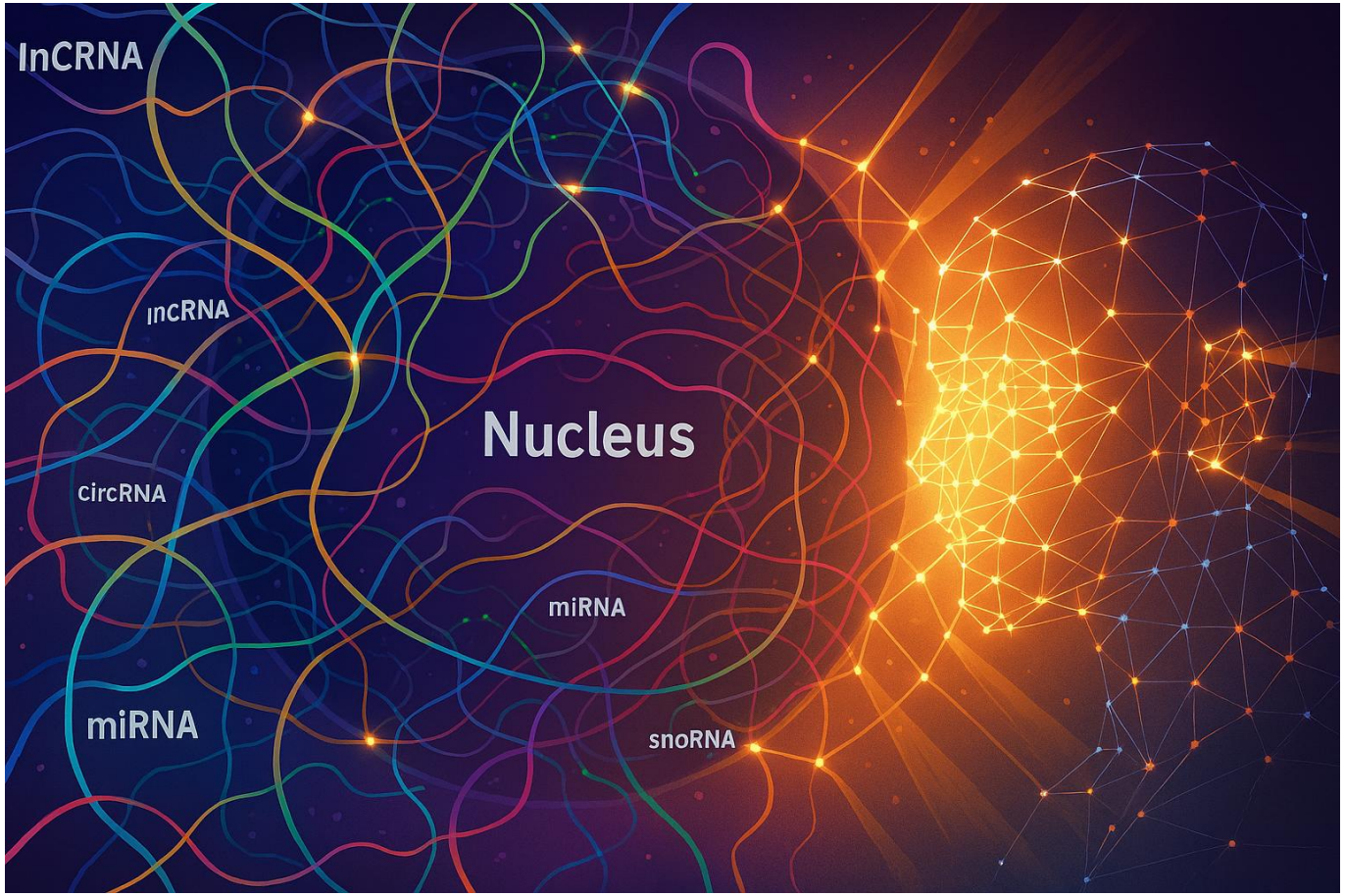
Marín-Béjar & Huarte, 2015. Advances in Genomics and Genetics.

Illustrations created in BioRender.com

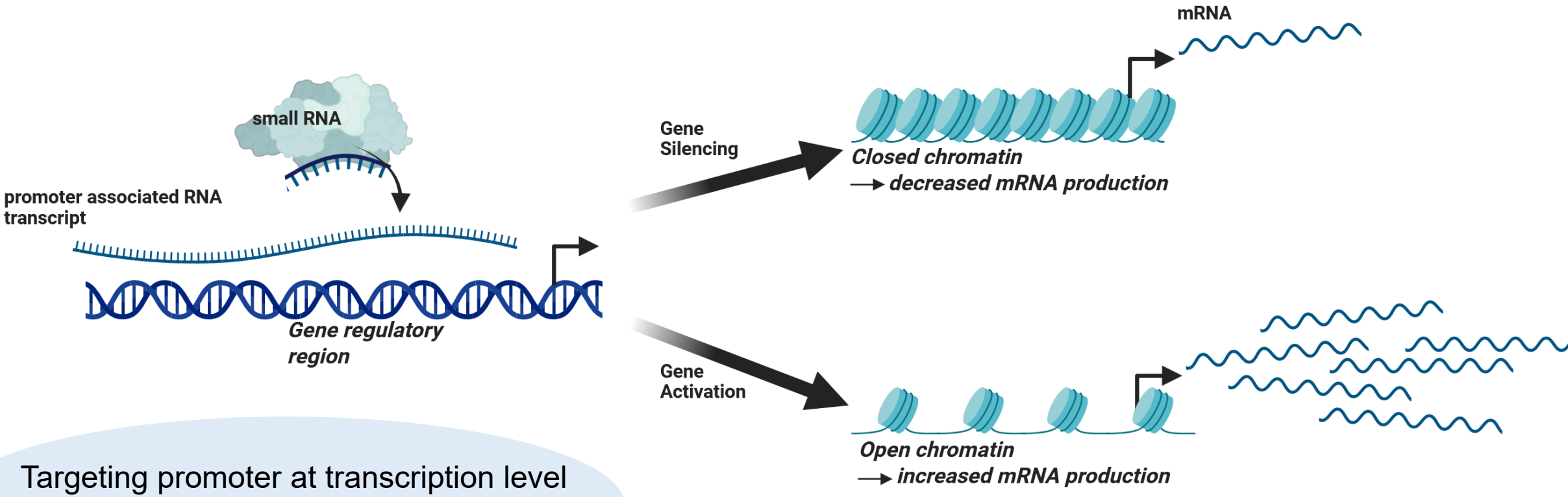
Nuclear non-coding RNAs have been ignored

- The nucleus is rich with non-coding RNAs which regulate chromatin and fine-tune gene expression

- By targeting these nuclear non-coding RNAs, genes can be activated or silenced



Targeting gene transcription in nucleus with small RNA has critical advantages



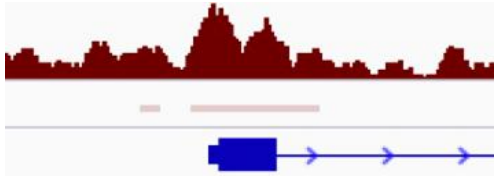
Targeting promoter at transcription level induces heritable **epigenetic changes for long-lasting effect**

Utilizes cell´s own regulatory mechanisms to achieve **natural biological response**

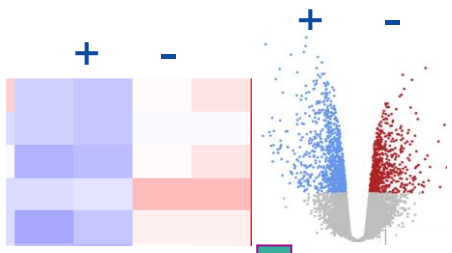
Illustrations created in BioRender.com

Computational platform for therapeutic small RNA discovery

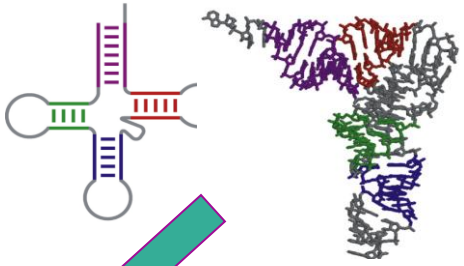
Genomic and Epigenomic Data



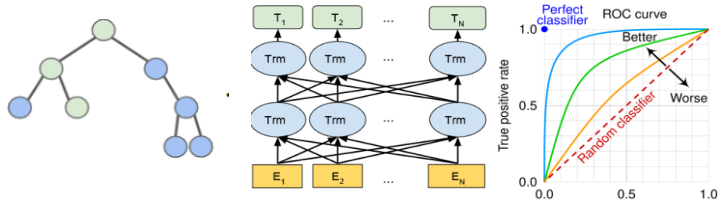
Transcriptomic Data



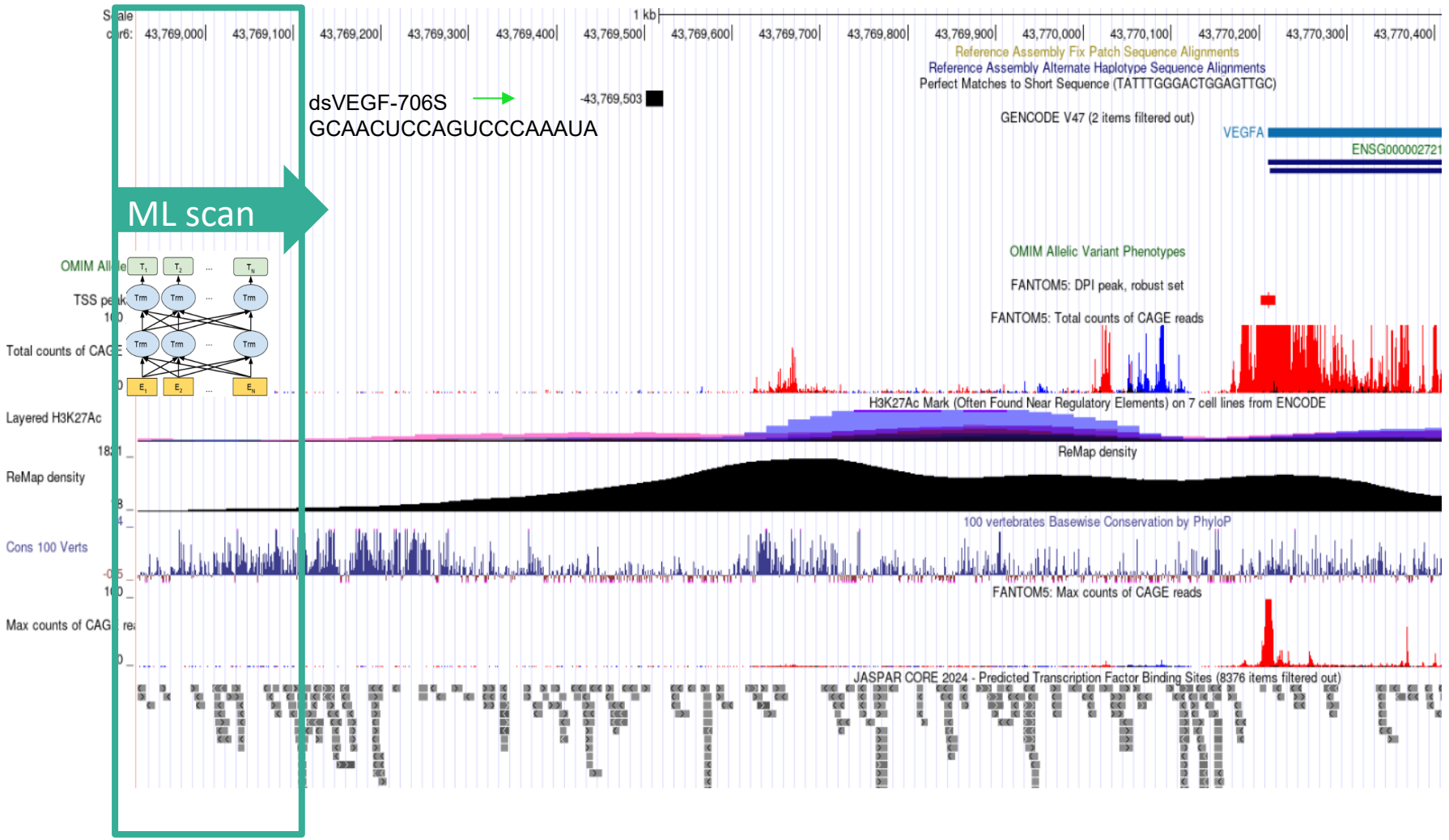
Structural and Thermodynamic Data



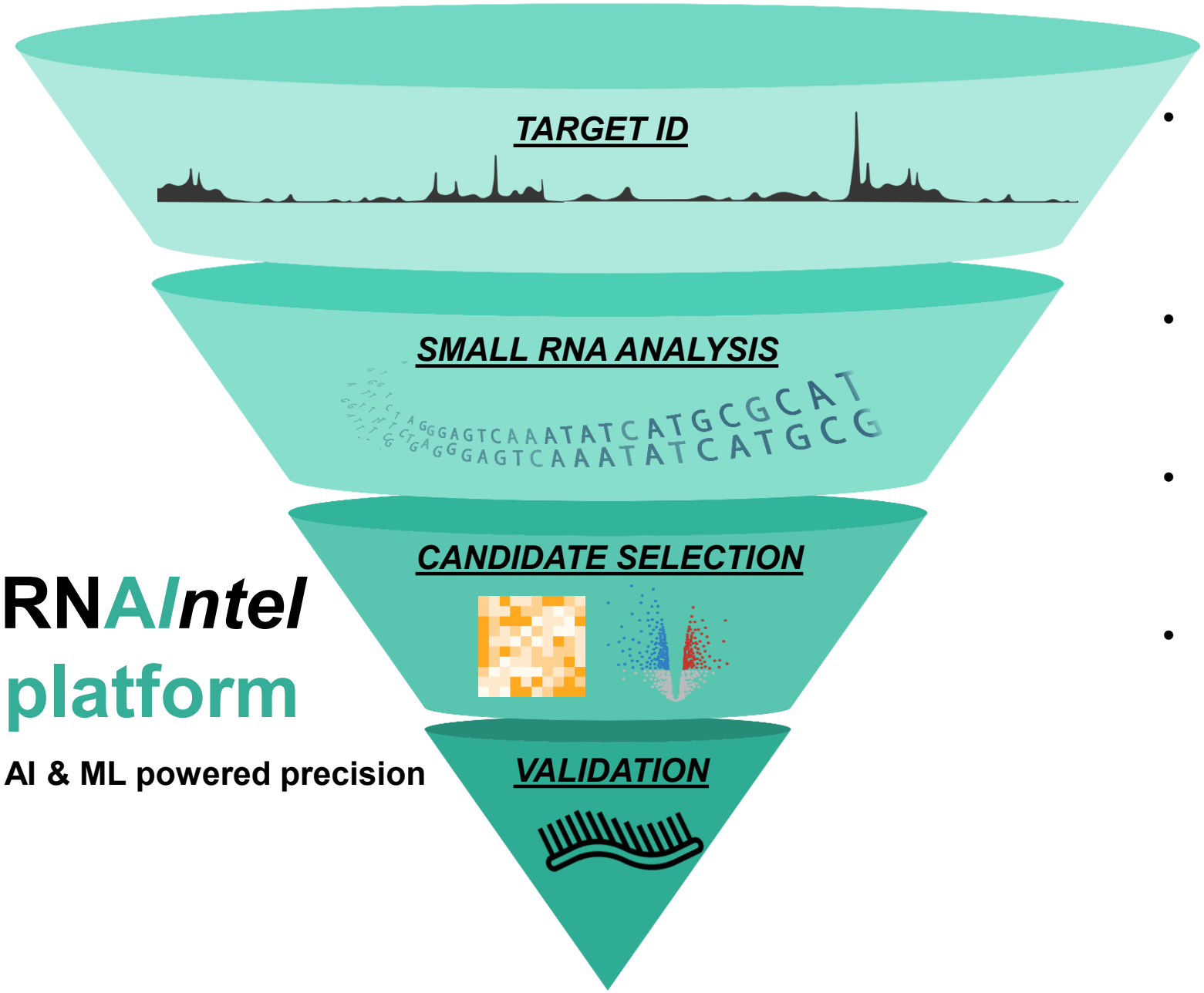
ML/AI Models



ML/AI scans variety of gene regulation/sequence tracks to identify regions of high likelihood of binding



Demonstrates the location of saRNA:VEGF-706S relative to VEGFA promoter, with chromatin, transcription factors and other relevant tracks



RNA*Intel* platform

AI & ML powered precision

- Analyzes 100s of different factors for **all gene promoters in the genome** to determine factors most important for RNA activation
- **Ranks all existing transcripts for every gene** in order of ability to regulate with RNA activation
- Pinpoints w/i 100bp resolution **exact sites in every transcript** targetable by RNA activation
- Identifies unique small RNA targeting transcript, integrating tissue/cell specificity, to **minimize off-target effects**

saRNAs: structure and delivery ensures efficient targeting

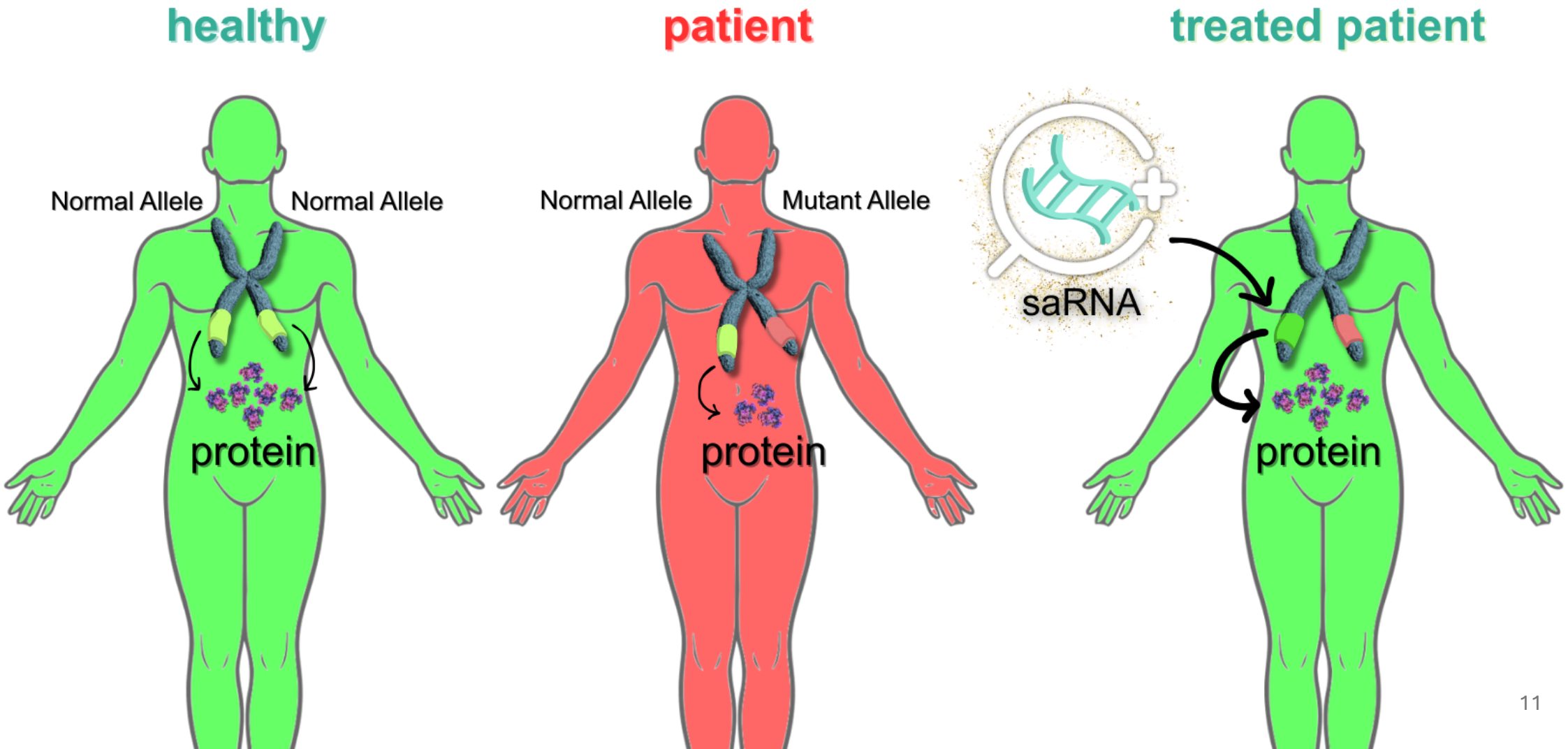
- saRNAs are **fully complementary** to the promoter target
- saRNAs are **structurally** the same as siRNAs: **double-stranded RNA oligos**
- saRNAs can be **conjugated or encapsulated** for delivery
  - LNPs protect saRNA cargo and can be modified to enhance tissue targeting
  - GalNAc-conjugation enriches saRNA in liver
  - Delivery to nucleus substantiated by internal data

```
promoter  TGCCAGACTCCACAGTGCATACGTGGGCTCCAACAGG
                  ||||||||||||||||||||
saRNA             GGUGUCACGUAUGCACCCG
```

saRNA = siRNA in structure

Initial focus on eye, liver to leverage established delivery pathways

saRNAs restore functional gene expression to treat haploinsufficiency-driven diseases



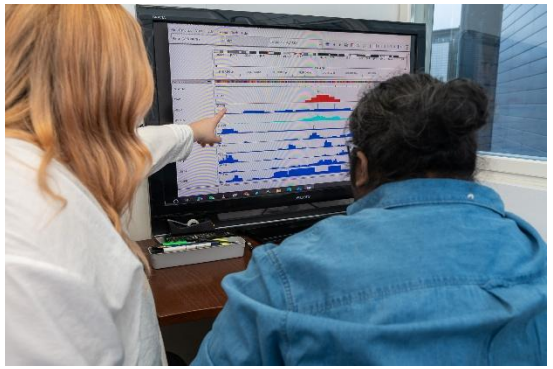
RNatives team and lab

Jeff Abbey, MBA, JD
CEO

Mikko Turunen, PhD
CSO/Founder

Tiia Turunen, PhD
CDO/Founder

Juhani Lahdenperä, MSc
COO/Founder



Key Takeaways

- RNA activation can be used to target diseases by activating the endogenous gene expression in patients' cells
- Delivery of small activating RNA induces epigenetic changes on the promoter, thereby providing transient but potentially long-lasting therapeutic effect
- RNatives' novel RNA*Intel* platform utilizes AI and ML to rapidly identify most efficient candidates for specific targets
- This strategy can be used to treat diseases that have not been treatable before

THANK YOU FOR YOUR TIME

High-resolution biomarker detection using multiplex digital PCR: Evidence from clinical research

Ahmed Elshobaky, PhD
Senior Global Market Development Manager
Molecular Tools & Oncology − dPCR
Ahmed.Elshobaky@qiagen.com

February 5, 2026

Legal disclaimer

QIAGEN products shown here are intended for non-clinical applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit instructions for use or user manual. QIAGEN instructions for use and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services (or your local distributor).

Liquid biopsy enables ongoing monitoring of tumor dynamics through repeated sampling, unlike tissue biopsy, which captures only a single time point.

Changes in circulating tumor DNA (ctDNA) reflect tumor burden and can reveal treatment response or disease progression earlier than imaging-based approaches.[1]

Siravegna G, et al. How liquid biopsies can change clinical practice in oncology. *Ann Oncol.* 2019;30(10):1580−1590.

Agenda

- Rationale for biomarker-driven stratification using liquid biopsy
- ctDNA dynamics during treatment
- Analytical sensitivity is critical: Tumor-informed vs tumor-naïve
- Digital PCR as a high-precision technology for molecular analysis and longitudinal assessment
- Examples from scientific publications

Agenda

Rationale for biomarker-driven stratification using liquid biopsy

ctDNA dynamics during treatment

Analytical sensitivity is critical: Tumor-informed vs tumor-naïve

Digital PCR as a high-precision technology for molecular analysis and longitudinal assessment

Examples from scientific publications

Rationale for biomarker-driven stratification using liquid biopsy

Liquid biopsy

- A minimally invasive test that detects cancer by analyzing tumor-derived materials, such as CTCs and ctDNA/cfDNA in blood or other body fluids
- Real-time information for early detection, treatment decisions and monitoring disease progression or resistance
  - Minimally invasive, repeatable and captures dynamic tumor changes
  - Valuable complement or substitute to traditional tissue biopsies and supports more personalized cancer care
- Biomarker analysis by cfDNA is one of the largest and fastest-growing applications in oncology
  - ctDNA is being used mostly in advanced metastatic disease for therapy selection
  - It is also possible to monitor response to therapy for selected advanced cancer patients

Trends

- Minimal/Measurable residual disease (MRD) monitoring will be the next-term application
- Integration of additional analytes to provide a more comprehensive disease picture
- Interrogation of other features of cfDNA, especially DNA methylation
- Standardization, pre-analytics and QC
- dPCR will play a key role

MRD testing

MRD refers to the small number of cancer cells that remain in a patient's body after treatment, causing no signs or symptoms and eventually leading to recurrence of the disease

MRD in neoadjuvant or adjuvant setting (early solid tumor)

- Almost two-thirds of patients with solid tumors have a locoregional disease and can be treated by curative therapies, e.g., surgery, radiotherapy, systemic therapy or a combination
- Neoadjuvant: Evidence suggests that monitoring ctDNA levels tracks response to neoadjuvant treatment
- Adjuvant: Growing evidence that ctDNA MRD following treatment predicts relapse
- Requires ctDNA detection limits of (LOD95) <0.01% VAF

MRD in later stage/metastatic cancer

- ctDNA is used in the advanced/metastatic setting, where a patient has relapsed, and the physician wants to monitor tumor drug resistance mechanisms (or drug response) and guide therapy
- Single biomarkers (e.g., ESR1 in breast cancer) can be detected with dPCR at levels ~0.1 VAF

MRD in hematologic malignancies

- MRD testing is particularly significant in blood cancers like leukemia, lymphoma and multiple myeloma
- MRD testing is included in the guidelines for a number of hematologic malignancies and is used to guide therapy, i.e., escalation/de-escalation (CML, Ph+ALL, AML)

Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin.* 2021;71(1):7-33.

Today’s approach to MRD testing in AML

Source	Analyte	Technology
Bone marrow	Cells	Multiparameter flow cytometry
Bone marrow	RNA	Real-time quantitative PCR
Peripheral blood	RNA	Real-time quantitative PCR
Bone marrow	DNA	Next-generation sequencing
Peripheral blood	DNA	Next-generation sequencing

Advancing MRD testing in AML will require recognizing it as a predictive biomarker, harmonizing methodologies across laboratories and incorporating new technologies such as digital PCR.

Agenda

Rationale for biomarker-driven stratification using liquid biopsy

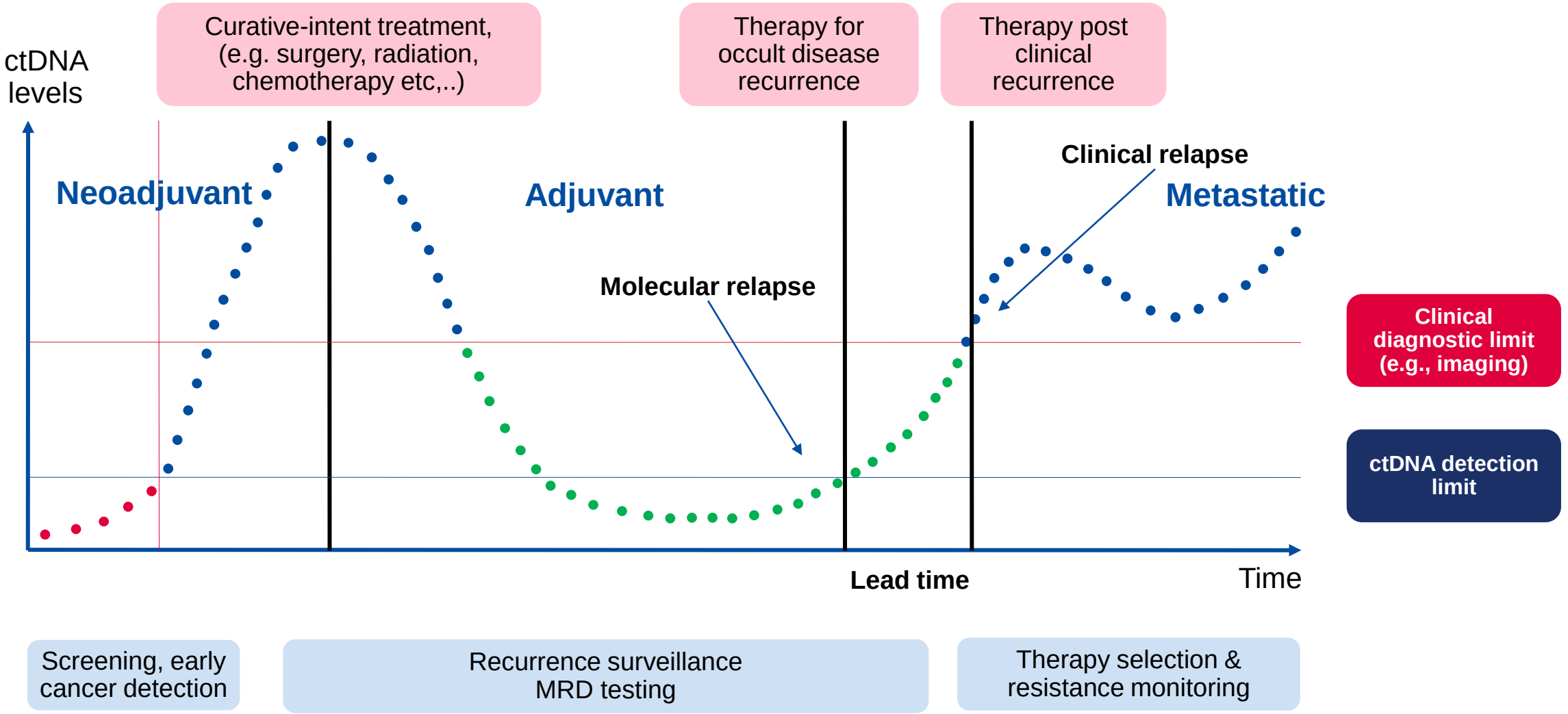
ctDNA dynamics during treatment

Analytical sensitivity is critical: Tumor-informed vs tumor-naïve

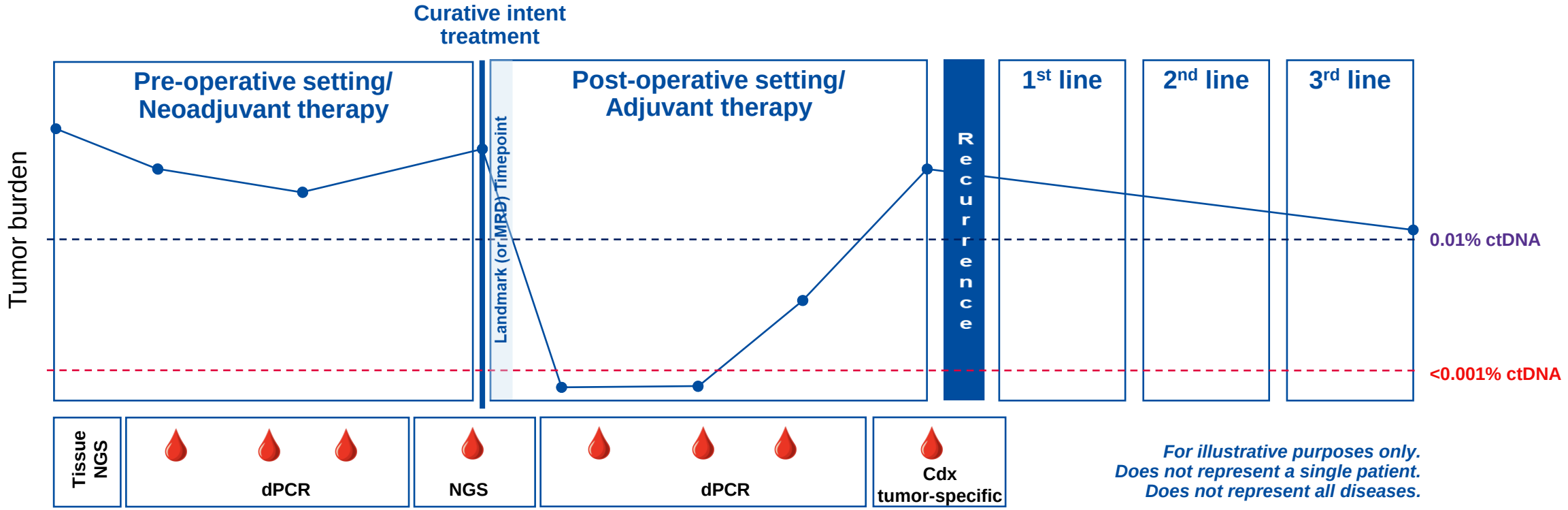
Digital PCR as a high-precision technology for molecular analysis and longitudinal assessment

Examples from scientific publications

ctDNA dynamics during treatment



PPM-level sensitivity can be achieved by a tumor-informed approach



Agenda

Rationale for biomarker-driven stratification using liquid biopsy

ctDNA dynamics during treatment

Analytical sensitivity is critical: Tumor-informed vs tumor-naïve

Digital PCR as a high-precision technology for molecular analysis and longitudinal assessment

Examples from scientific publications

Analytical sensitivity is critical: Tumor-informed vs tumor-naïve

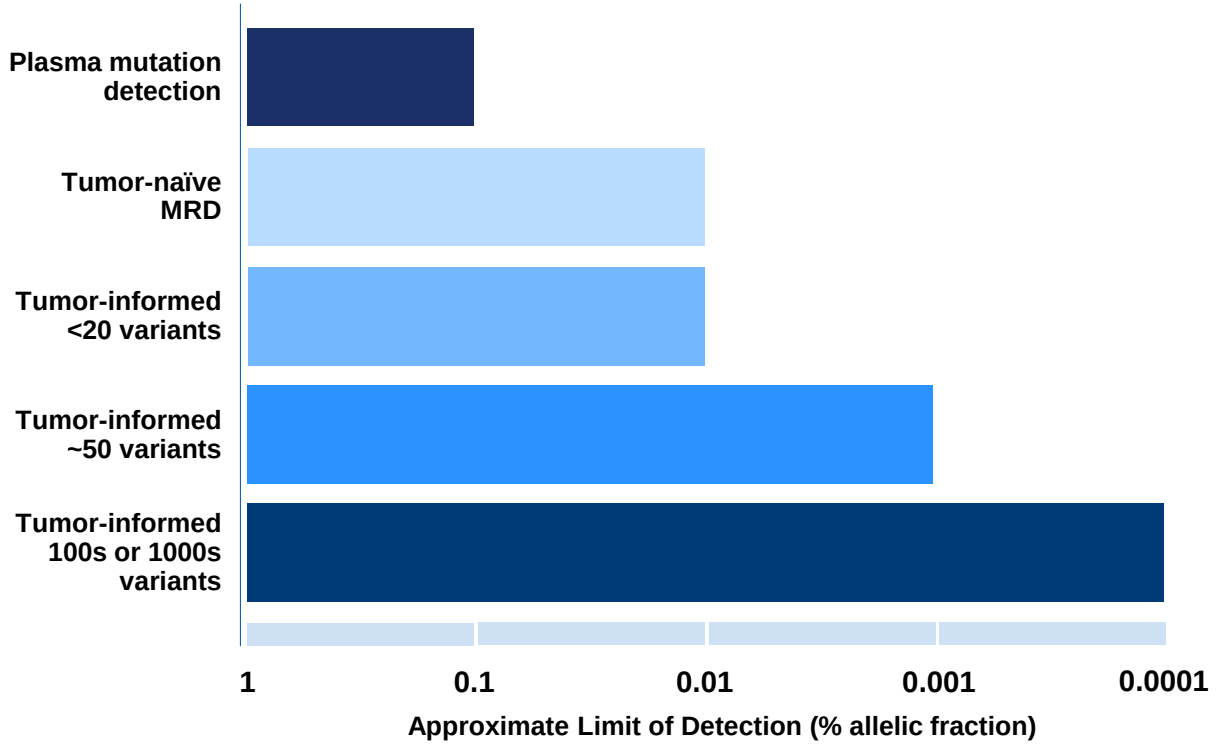
Tumor-informed vs. -naïve MRD in patients with solid tumors

Tumor-informed ctDNA detection for solid tumor MRD

- Requires tumor tissue sample
- Profiling of tumor tissue by WGS, WES or CGP
- Personalization: Selection of variants that are specific to the patient
- This personalized variant panel is used to track ctDNA in the blood of patients
- TAT is generally 3-4 weeks for the initial sample, then 1 week for subsequent blood samples

Tumor-naïve ctDNA detection for solid tumor MRD

- Does not require patient tumor tissue sample
- ctDNA is tracked in plasma through a mixture of SNV detection and methylation signatures (generic panel of mutations without prior knowledge of the patient's tumor)
- TAT is generally 2-3 weeks for all samples (first or subsequent)



Tumor-informed: Lower LOD (<0.01%) will allow identification of more patients with residual disease following curative-intent therapy, especially in Stage I or II disease, but need for tumor tissue may limit use, given long TAT (3-4 weeks initial test)

Tumor-naïve: Useful when no tumor tissue is available and gives analytical sensitivity approximately ~0.01%. Lack of sensitivity may limit use to later-stage disease.

Source: TD Cowen Report, July 2023 www.tdsecurities.com

Agenda

Rationale for biomarker-driven stratification using liquid biopsy

ctDNA dynamics during treatment

Analytical sensitivity is critical: Tumor-informed vs tumor-naïve

Digital PCR as a high-precision technology for molecular analysis and longitudinal assessment

Examples from scientific publications

Digital PCR as a high-precision technology for molecular analysis and longitudinal assessment

Specific

Designed to detect target sequence only

Sensitive

Can detect low copy number targets (LOD)

Rapid & scalable

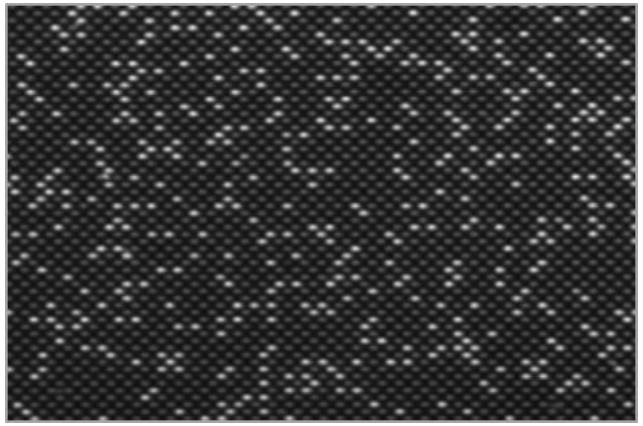
Routine testing

Multiplex

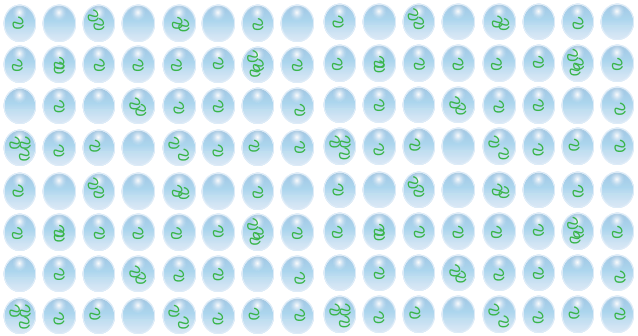
Up to 12 targets in one reaction

Combine different targets in one well

dPCR 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

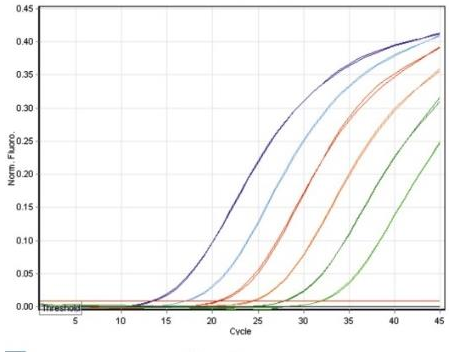


Random distribution of molecules into partitions
creates an increase in effective concentration

Advantages

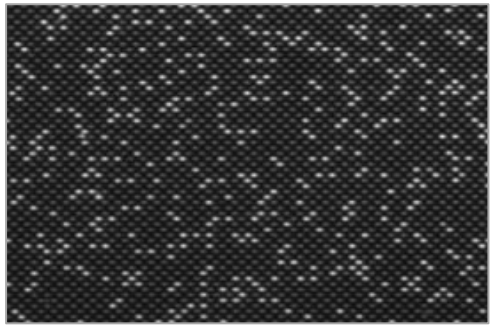
- Absolute and accurate quantification of targets (no need for standard curve)
- dPCR has outstanding precision and sensitivity
- Limit of detection based on DNA input
- High-throughput sample analysis
- Coping with inhibitors contained in heterogeneous samples

qPCR



Standard curve: copies/µL

dPCR



Poisson statistics: copies/µL

Workflow

Agenda

Rationale for biomarker-driven stratification using liquid biopsy

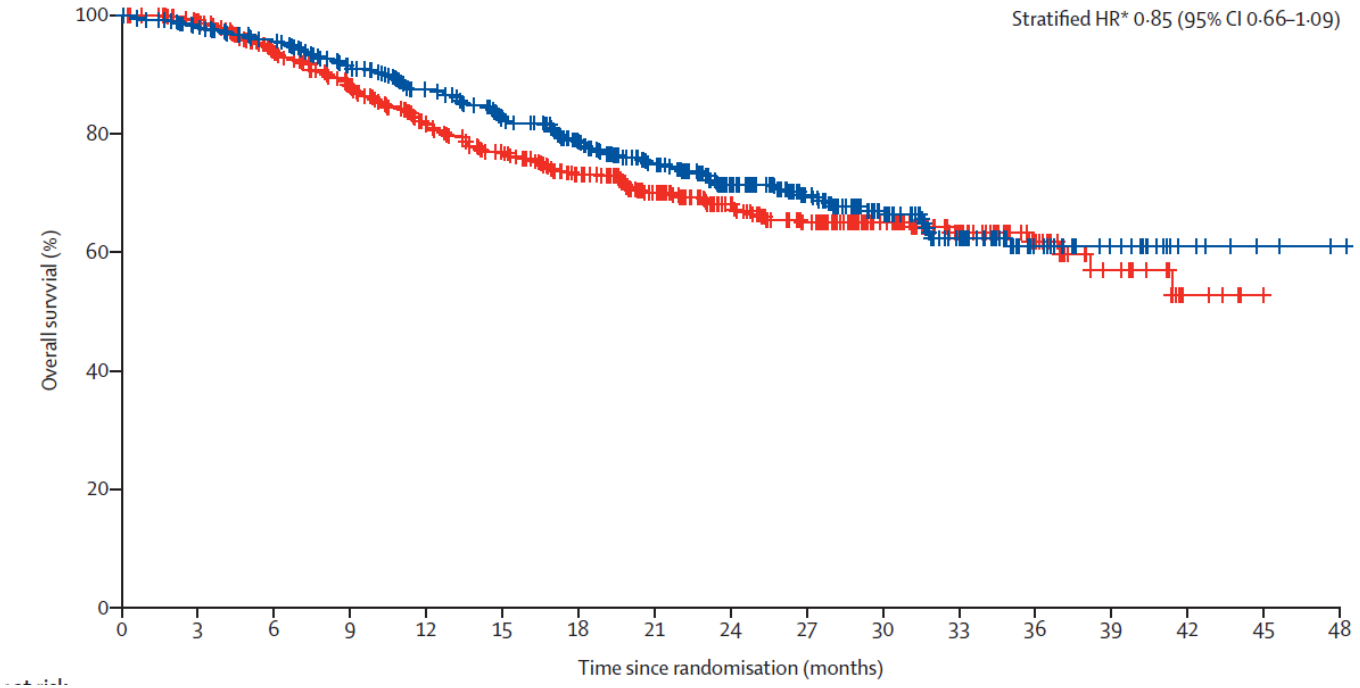
ctDNA dynamics during treatment

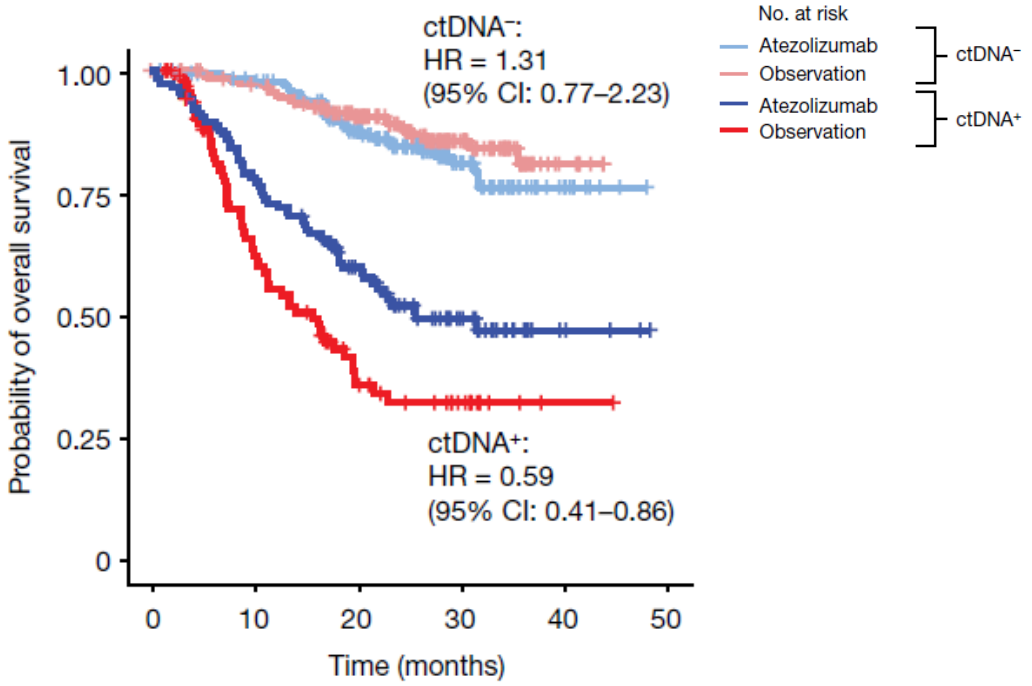
Analytical sensitivity is critical: Tumor-informed vs tumor-naïve

Digital PCR as a high-precision technology for molecular analysis and longitudinal assessment

Examples from scientific publications

Adjuvant atezolizumab in muscle-invasive bladder cancer (IMvigor010) – ctDNA guiding therapy





- In the (red) observation arm of this Phase III study OS was very similar to patients given adjuvant atezolizumab (blue)

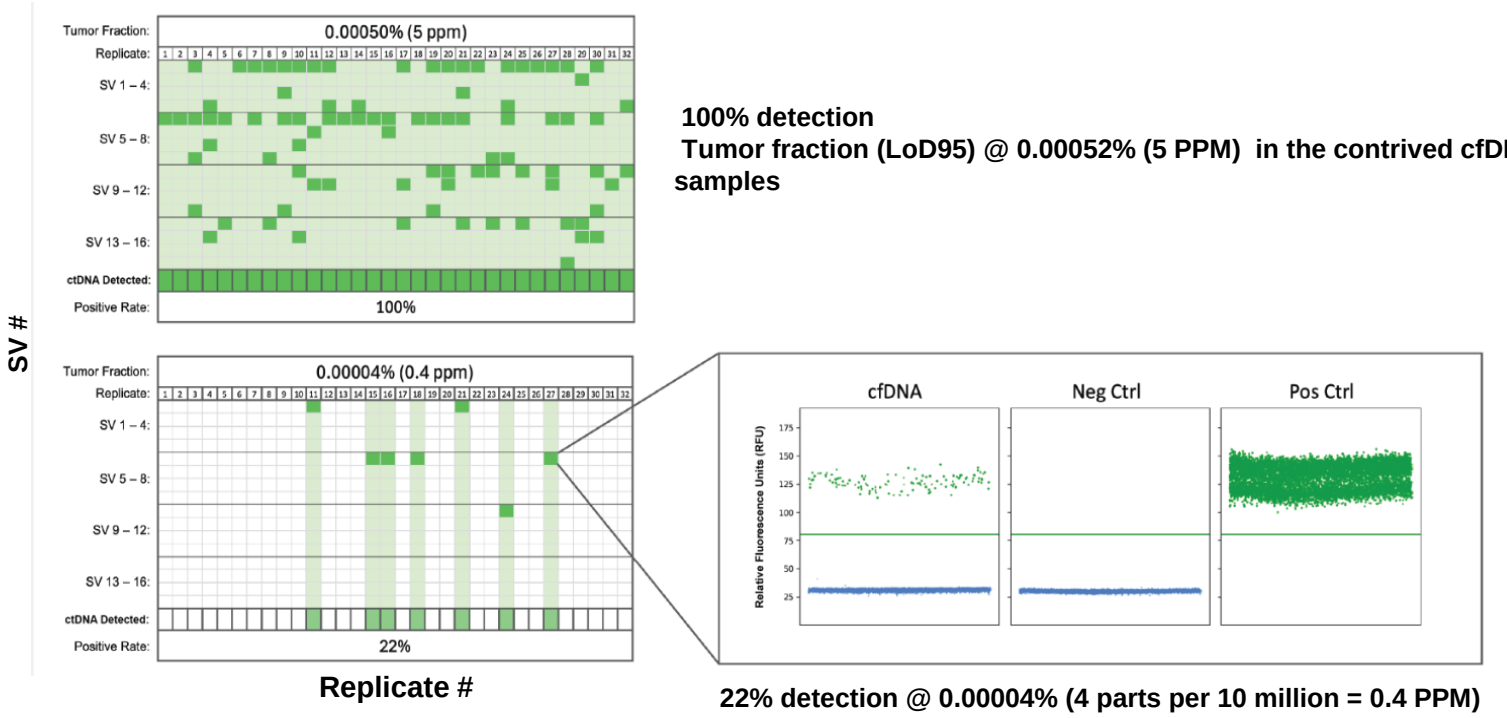
- The study collected plasma samples from all patients after surgery, but before treatment
- Patients who were ctDNA+ did better on atezolizumab

Bellmunt J, et al. Adjuvant atezolizumab versus observation in muscle-invasive urothelial carcinoma (IMvigor010): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol.* 2021;22(4):525-537.

Ultrasensitive detection and monitoring of ctDNA through structural variant analysis in early-stage breast cancer

Rationale & key findings

- MRD monitoring in early breast cancer, retrospective study (100 participants)
- Assay based on structural variants (SVs) instead of SNVs
- Personalized tumor-informed ctDNA assay that uses WGS of tumor tissue and multiplex dPCR analysis of ctDNA
- dPCR MRD detection enables quick and iterative decisions, enabling life-saving treatment decisions to be made

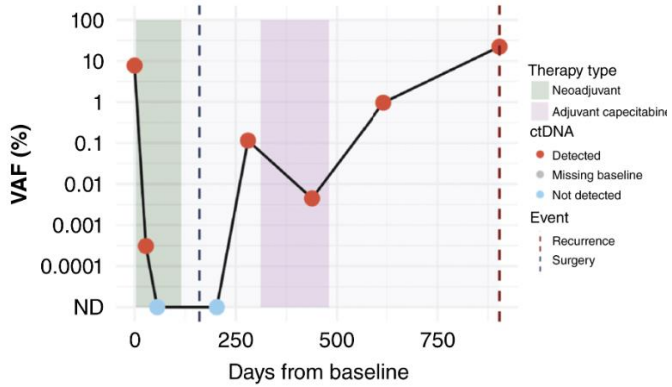


100% detection
Tumor fraction (LoD95) @ 0.00052% (5 PPM) in the contrived cfDNA samples

22% detection @ 0.00004% (4 parts per 10 million = 0.4 PPM)

Pathlight assay

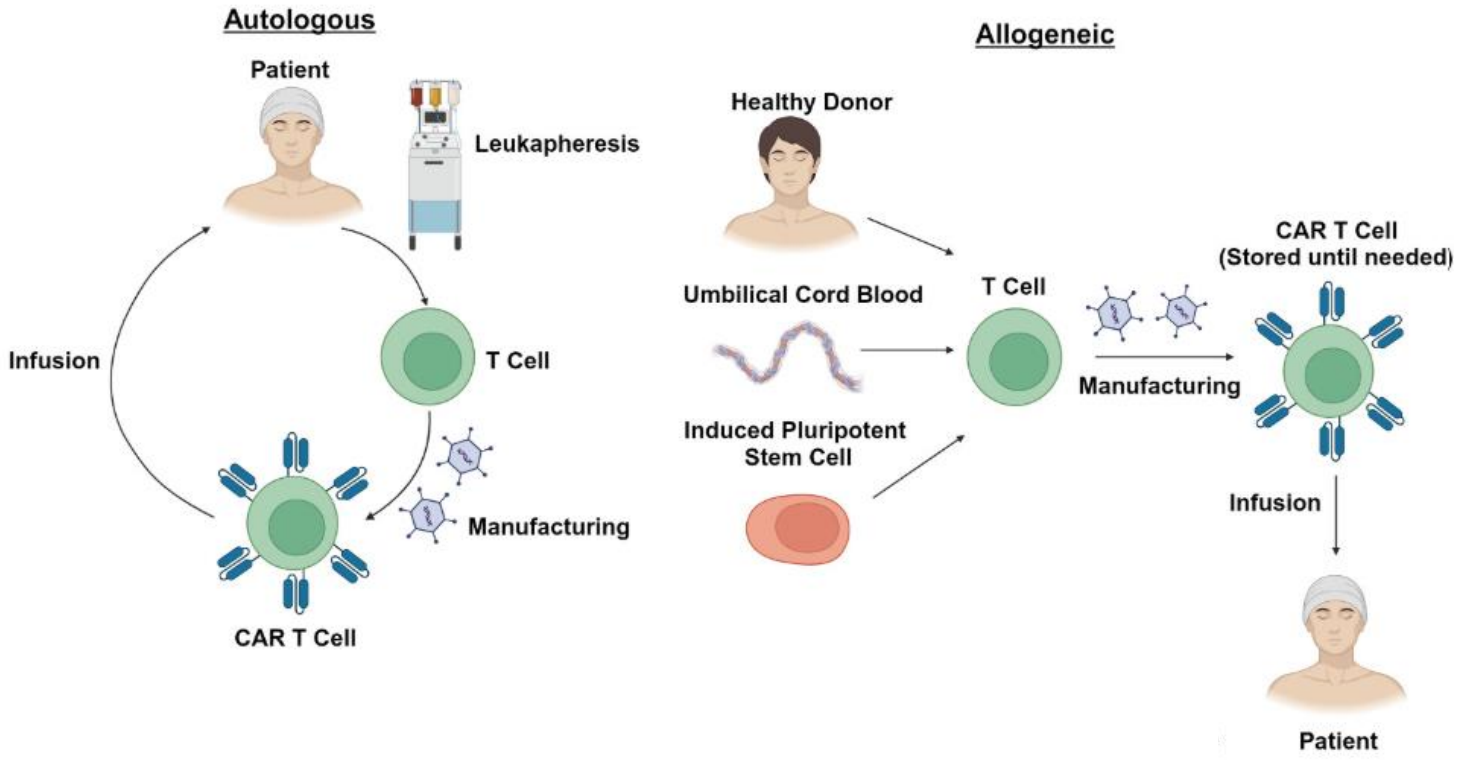
- SV pre-amplification + multiplex dPCR
- Assay targeting 16 SVs per patient
- 4 dPCR reactions per sample
- 4 somatic SVs/reaction



Kinetic plot displaying changes in ctDNA levels vs. treatment timelines

Elliott MJ, et al. Ultrasensitive detection and monitoring of circulating tumor DNA using structural variants in early-stage breast cancer. *Clin Cancer Res.* 2025;31(8):1520−1532.

Monitoring CAR-T therapy response using digital PCR

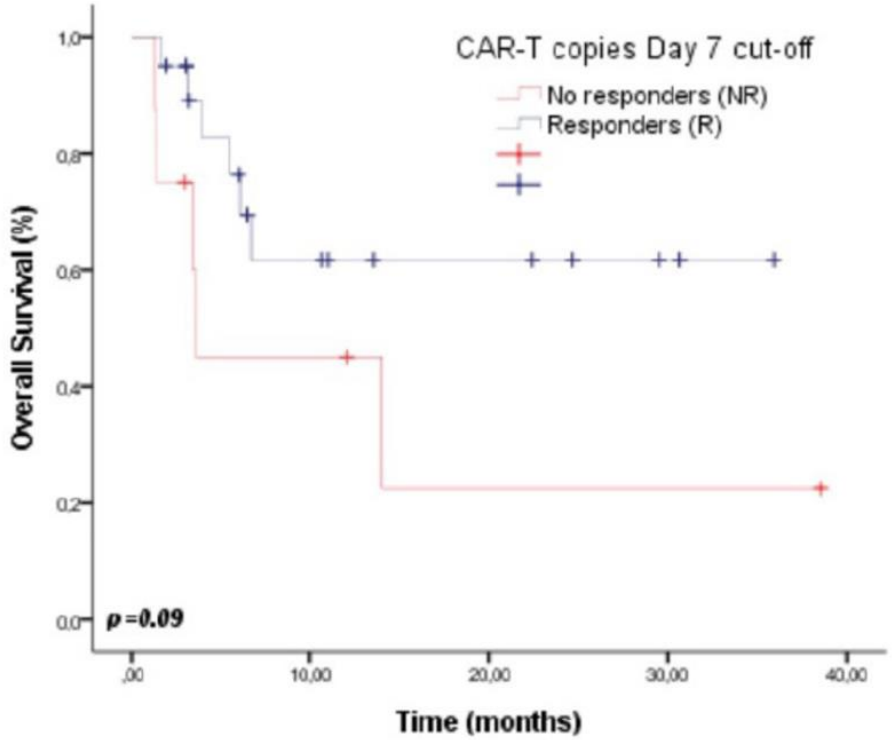


Jallouk AP, Sengsayadeth S, Savani BN, Dholaria B, Oluwole O. Allogeneic and other innovative chimeric antigen receptor platforms. *Clin Hematol Int.* 2024;6(3):61-72.

Assessing in vivo expansion of CD19 CAR-T cells using dPCR: Implications for clinical outcomes in aggressive B-cell lymphoma

Rationale & key findings

- Retrospective analysis of 28 non-Hodgkin lymphoma (NHL) cases treated with Axi-cel CAR-T therapy
- 97 peripheral blood mononuclear cell (PBMC) DNA samples
- Serial measurements of CAR-T-cell levels in blood (7,14, 28 days post-infusion) using a dPCR assay
- Median overall survival (OS) was 23.9 months and 13.2 months for R (responders) and NR (non-responders), respectively, according to CAR-T copies/µL 7 days post-infusion
- Digital PCR for CAR-T evaluation shows that quantitative CAR-T copies/µL could be a potential predictor of short-term response and survival

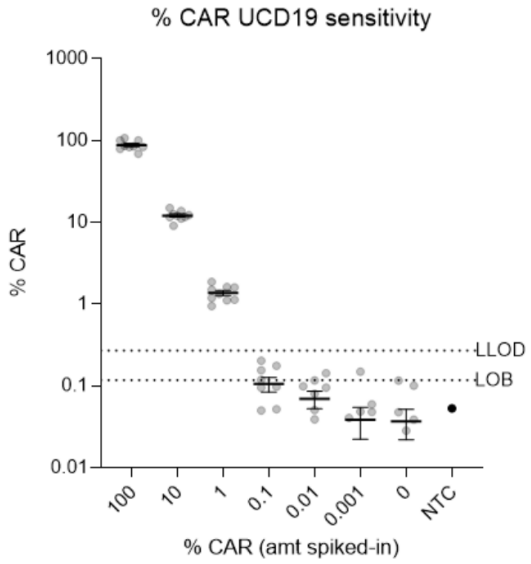


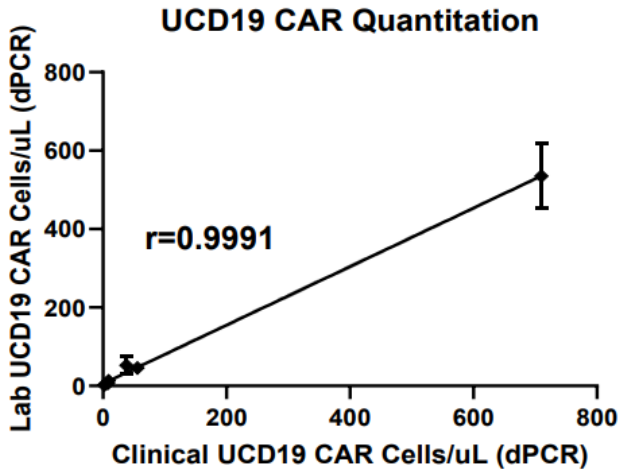
Ventura L, et al. Digital PCR assay for evaluation of CD19-CAR-T cells in vivo expansion in aggressive B-cell lymphoma: Impact on clinical outcome. *Blood.* 2023;142(Suppl 1):7153.

Characterization of a multiplex digital PCR assay to quantify total T cells relative to chimeric antigen receptor-positive T cells

Rationale & key findings

- Peripheral blood samples from 3 subjects enrolled in a clinical trial, collected at 2-3 time points after CAR-T infusion. (8 samples)
- Triplex dPCR assay that can quantify total T cells, CAR-T and total nucleated cells (Control gene)
- dPCR has gained traction as a sensitive and user-friendly method for monitoring CAR-T persistence in patients after infusion and can be adapted to any CAR-T construct
- Eliminates the need for redundant quantitation of T cells by flow cytometry
- Minimal sample needs





CAR-specific assay

TCR assay

Albumin assay

Addition of the TCR assay did NOT skew CAR quantitation.

Murphy LA, et al. Characterization of a multiplex digital PCR assay to quantify total T cells relative to chimeric antigen receptor-positive T cells. *Mol Ther Oncol*. 2025;33(2):200981.

dPCR is a proven technology for biomarker detection and monitoring in liquid biopsies

- Use of MRD testing in tumor patient management is gaining ground
- dPCR has demonstrated clinical utility in accurately assessing treatment response and supporting informed therapeutic decision-making
  - Provides specific, sensitive and rapid detection
  - Up to 12-plex capability conserves precious samples and reduces cost
- Sensitive detection of low-abundance ctDNA can be enhanced by:
  - Tracking multiple variants not associated with tumor growth (non-driver or resistance mutations).
  - Personalizing the plasma assay for each patient.
  - Achieving sensitivity levels down to 0.0001% VAF (1 part per million) using a tumor-informed dPCR approach
- dPCR is a viable option for longitudinal patient monitoring relative to other, more expensive approaches
- dPCR enables quick and iterative decisions, enabling life-saving treatment decisions to be made

Thank you for your attention. Questions?