Некоторые нюансы цифровой ПЦР

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Association of Single Cell Analysis

Digital PCR Association

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Discord server

Telegram channel

Schedule

August 26-27, Mon & Tues (Day 1-2)

Rooms 305, 1 & 2

Room 305 Lectures (Digital PCR)
then Room 1 **Maccura D600 + GenePure Pro**
then Room 2 **Sniper DQ24**

August 28-29, Wed & Thurs (Day 3-4)

Rooms 305, 1 & 2

Room 305 Lectures (Digital PCR)
then Room 1 **Maccura D600**
then Room 2 **Sniper DQ24 + GenePure Pro**

Starting

Master-class on Sample preparation using the GenePure Pro automatic nucleic acid isolation system

GenePure Pro

Digital PCR

maccura

Maccura D600

Master-class on
Maccura D600 digital PCR

SEANA

Digital PCR Association

ASCA Association for Single Cell Analysis

Digital PCR

Master-class on
Sniper DQ24 digital PCR

QVADROS Bio

Sniper DQ24

Association for Single Cell Analysis

Digital PCR Association

qPCR - Gold Standard

PCR > agarose

qPCR

dPCR – New Gold Standard

qPCR

dPCR

Digital PCR – Origins

Biomolecular Detection and Quantification 1 (2014) 1–2

Contents lists available at ScienceDirect

Biomolecular Detection and Quantification

journal

Review Article

Digital PCR: A brief history

Alexander A. Morley

Department of Haematology and Genetic Pathology, Flinders U

The term "digital PCR" was first used in the 1999 paper by Kinzler and Vogelstein [1] in which they described the quantitation of *ras* mutations in a sample by partitioning the sample in order to perform a series of PCRs in 384 well microplates. The term "digital PCR"

ARTICLE INFO

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Received 6 June 2014
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Keywords:
Digital PCR
Limiting dilution
PCR

ABSTRACT

Digital PCR for quantification of a target of interest has been independently developed several times, being described in 1990 and 1991 using the term "limiting dilution PCR" and in 1999 using the term "digital PCR". It came into use in the decade following its first development but its use was cut short by the description of real-time PCR in 1996. However digital PCR has now had a renaissance due to the recent development of new instruments and chemistry which have made it a much simpler and more practical technique.

Digital PCR – Origins

Biomolecular Detection and Quantification 1 (2014) 1–2

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Digital PCR: A brief history

The term "digital PCR" was first used in the 1999 paper by Kinzler and Vogelstein [1] in which they described the quantitation of *ras* mutations in a sample by partitioning the sample in order to per- eries of PCRs in 384 well microplates. The term "digital PCR"

Single molecule PCR

Limiting dilution PCR

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Keywords:
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Digital PC times,
being des term
"digital P short
by the des to the
recent development of new instruments and chemistry which have made it a much simpler and more practical technique.

Digital PCR

цифровая ПЦР

Большинство ПЦР

1 реакция → 1 результат

Один эксперимент – не эксперимент

qPCR и его аналоги

Репликаты (трипликаты, тетрапликаты, пентапликаты)

3-5 повторных реакция → 1 результат

цифровая ПЦР

цифровая ПЦР

цифровая ПЦР

qPCR и его аналоги

Репликаты (трипликаты, тетрапликаты, пентапликаты)

отбор образца ДНК (n= 3-5)

3-5 повторных реакция

1 результат

цифровая ПЦР (digital PCR)

1 образец ДНК

Флуорофоры

qPCR реактивы

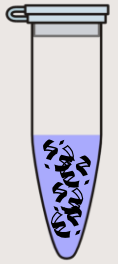
Свыше 20 000 реакций

= 20 000 экспериментов за раз на образец

1 молекула ДНК на реакцию

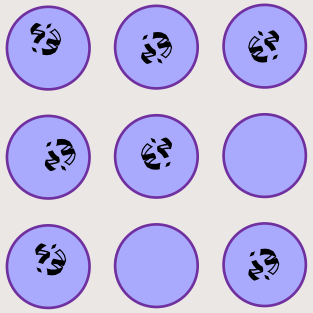
qPCR

Пул молекул ДНК как 1 реакция



dPCR

Независимые ПЦР 1 молекула = 1 реакция



цифровая ПЦР (digital PCR)

1 образец ДНК

Флуорофоры

qPCR реактивы

Свыше 20 000 реакций

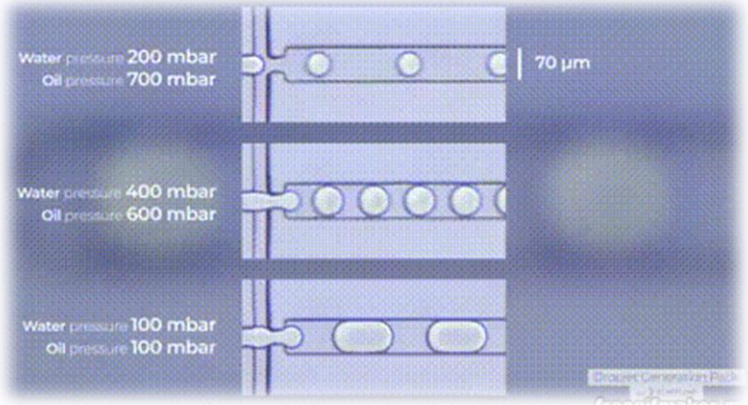
= 20 000 экспериментов за раз на образец

1 молекула ДНК на реакцию

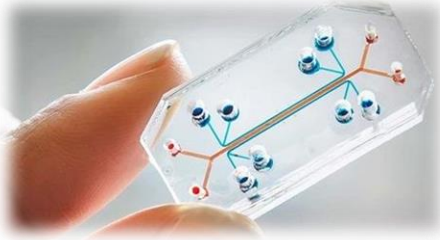
1 точный результат

Абсолютли кванитификация

цифровая ПЦР (digital PCR)



Microfluidics



Digital PCR

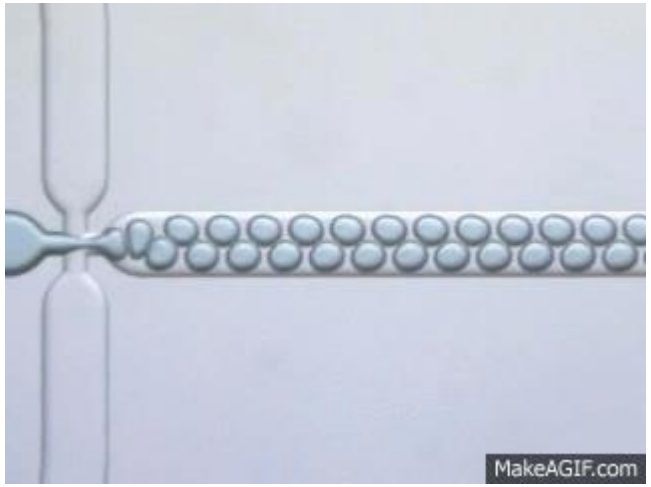
Single Cell Sequencing

Варианты цифровой ПЦР (digital PCR)

Droplet digital PCR

Капельная цифровая ПЦР

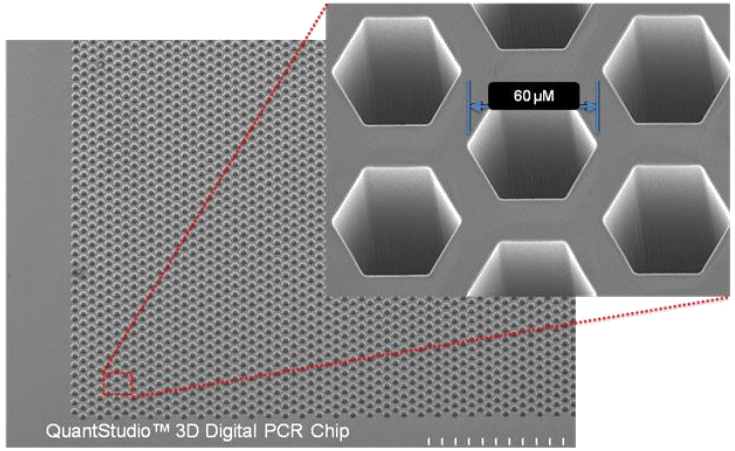
Bio-Rad, RainSure



Digital PCR on array

Цифровая ПЦР на чипе

ThermoFisher, Optolane

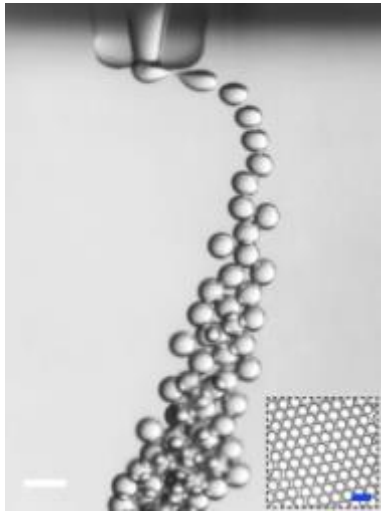


Варианты цифровой ПЦР (digital PCR)

Oscillation
Digital PCR

Генерация капель
осцилляцией

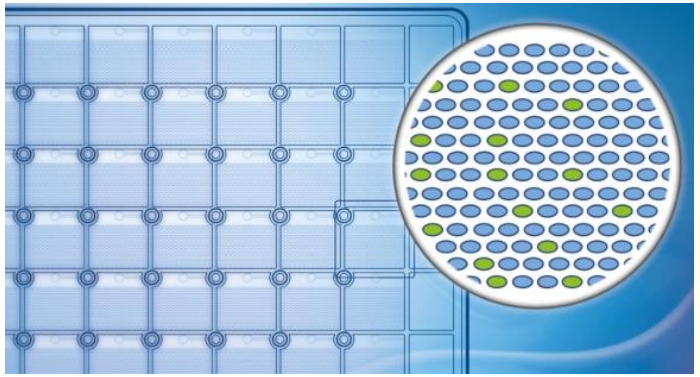
Sniper, Maccura



Digital PCR
on nanoplate

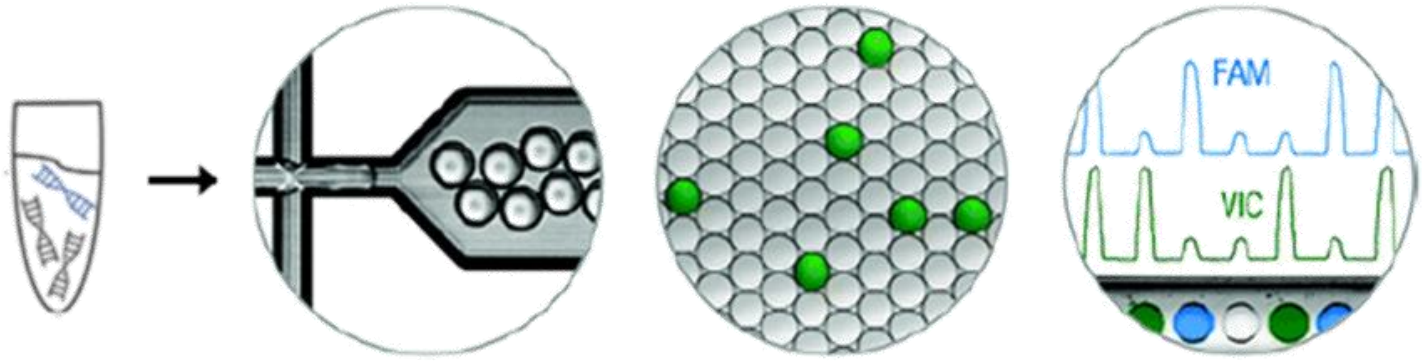
Цифровая ПЦР
на нанопланшете

Qiagen, et.c.



Digital PCR

Droplet digital PCR



1. MAKE

Sample is partitioned into 20,000 droplets

2. CYCLE

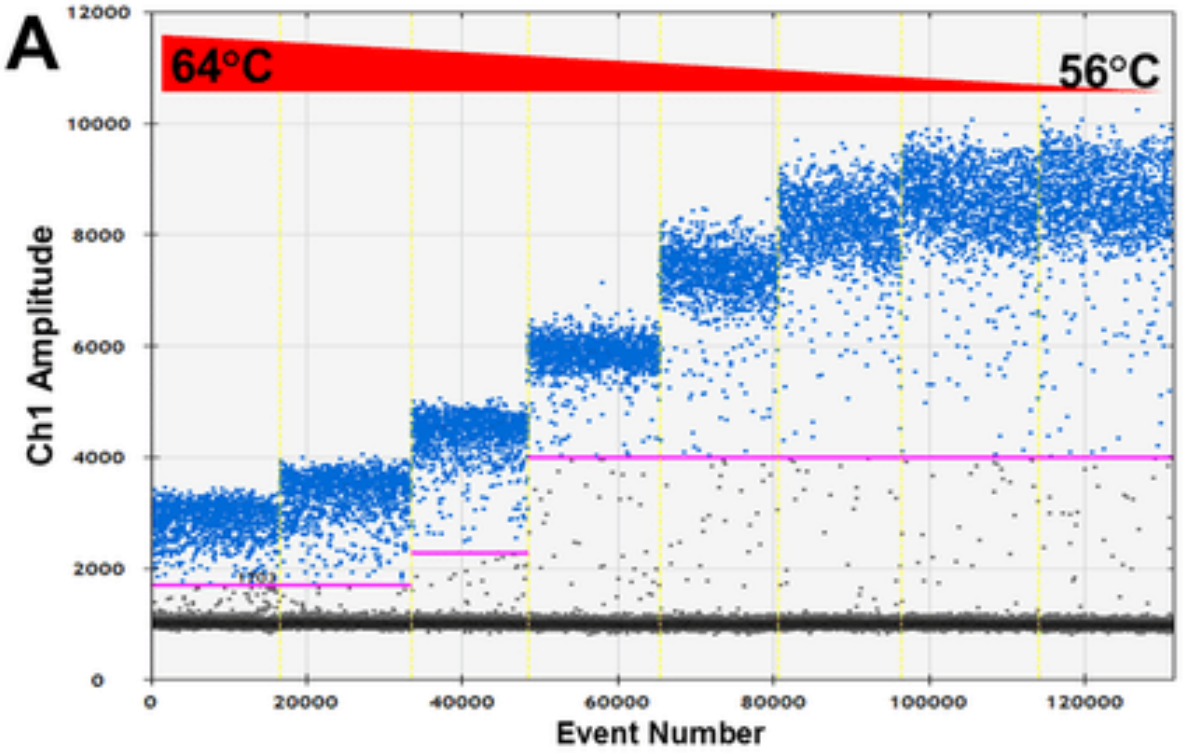
Run PCR cycles in all droplets simultaneously

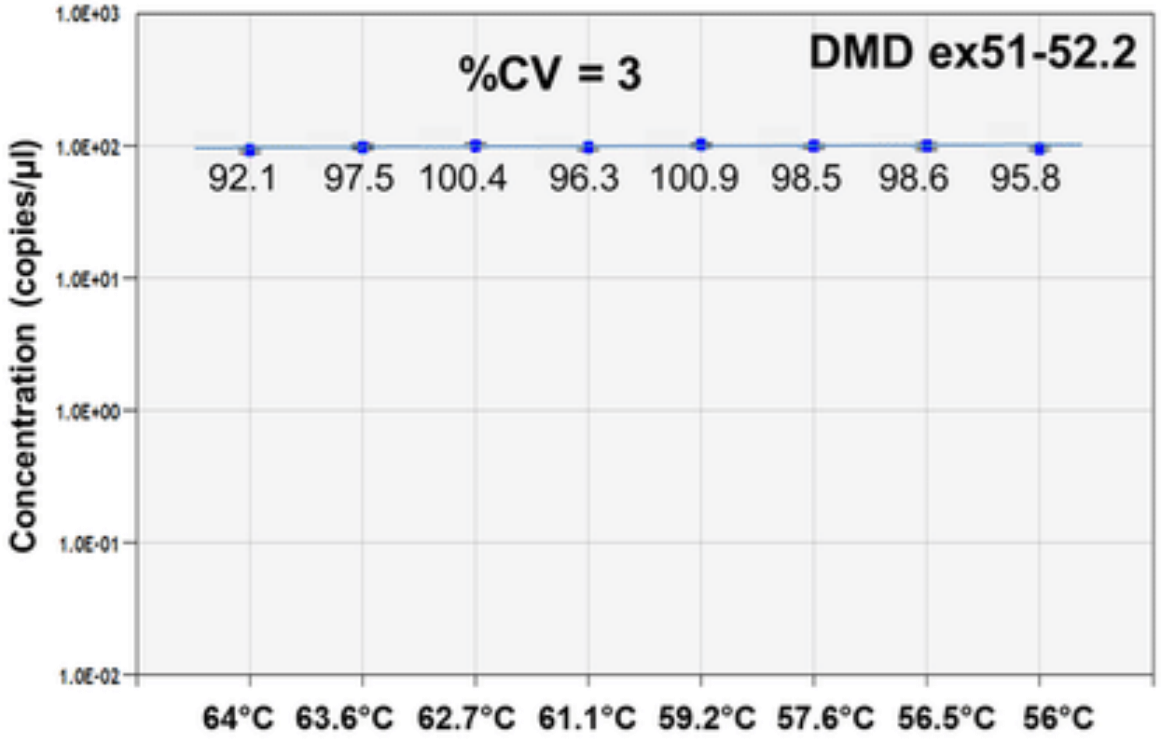
3. READ

Measure fluorescence intensity in each droplet

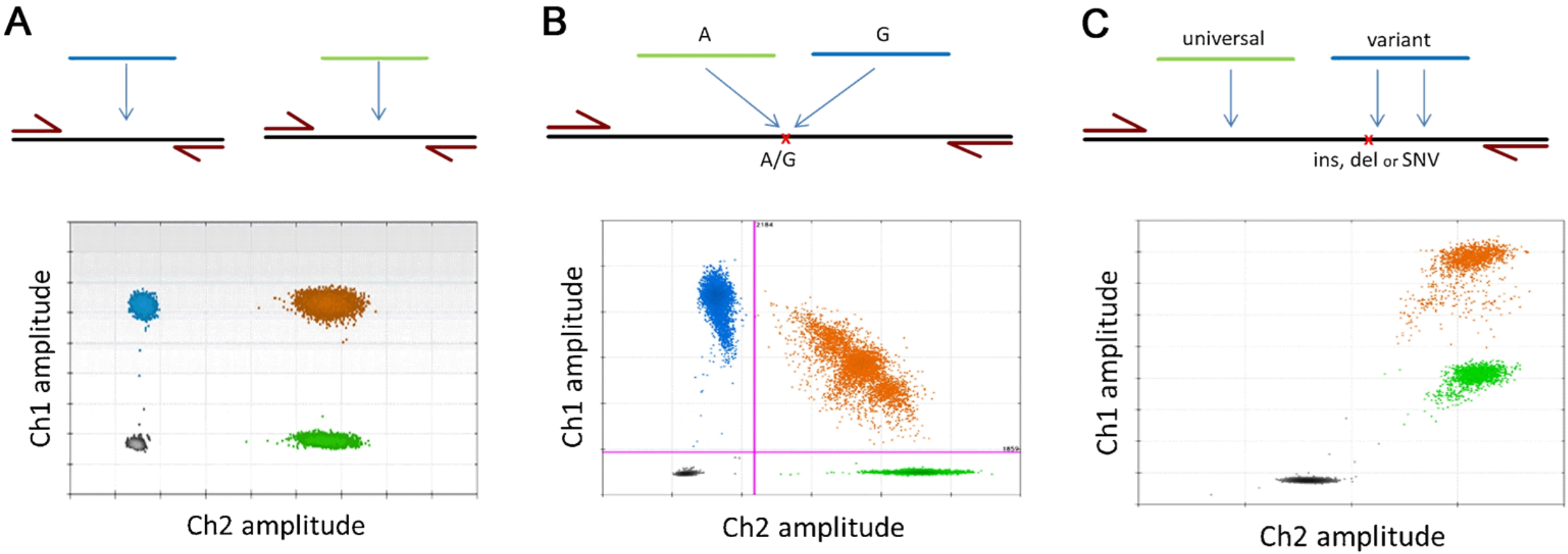
Calculate concentration from number of positive droplets

Digital PCR

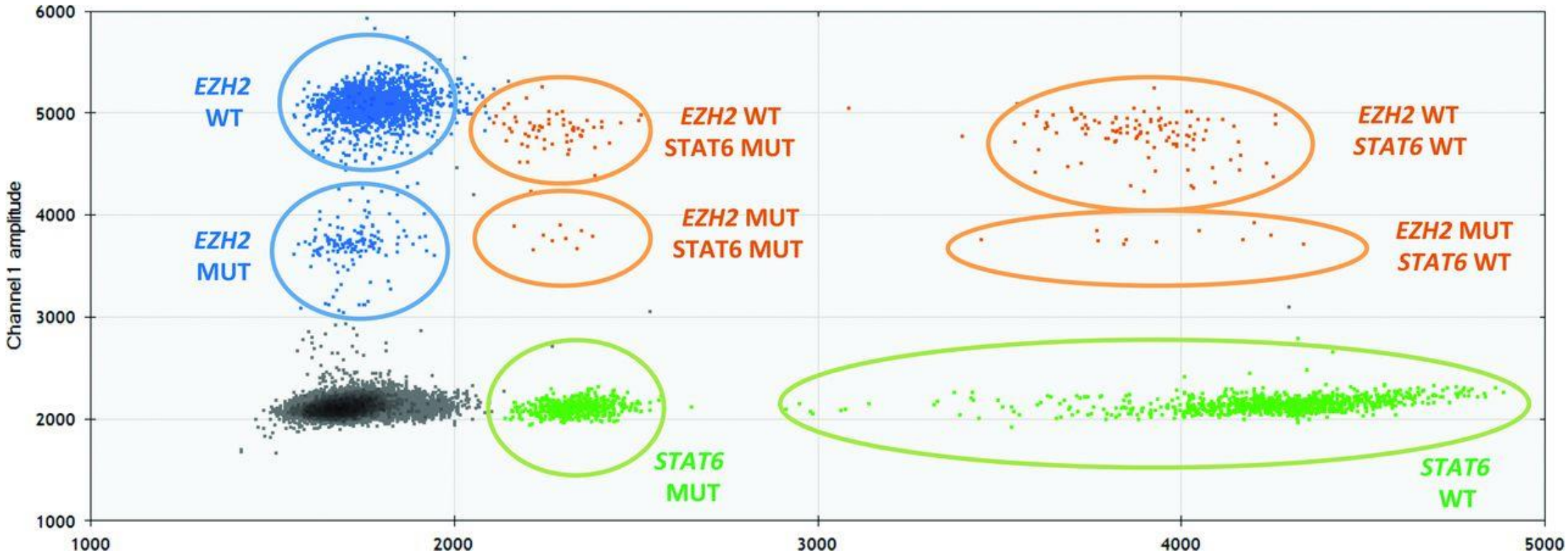

A
64°C
56°C
Ch1 Amplitude
Event Number
0
2000
4000
6000
8000
10000
12000
0
20000
40000
60000
80000
100000
120000


%CV = 3
DMD ex51-52.2
Concentration (copies/µl)
1.0E+03
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
92.1
97.5
100.4
96.3
100.9
98.5
98.6
95.8
64°C
63.6°C
62.7°C
61.1°C
59.2°C
57.6°C
56.5°C
56°C

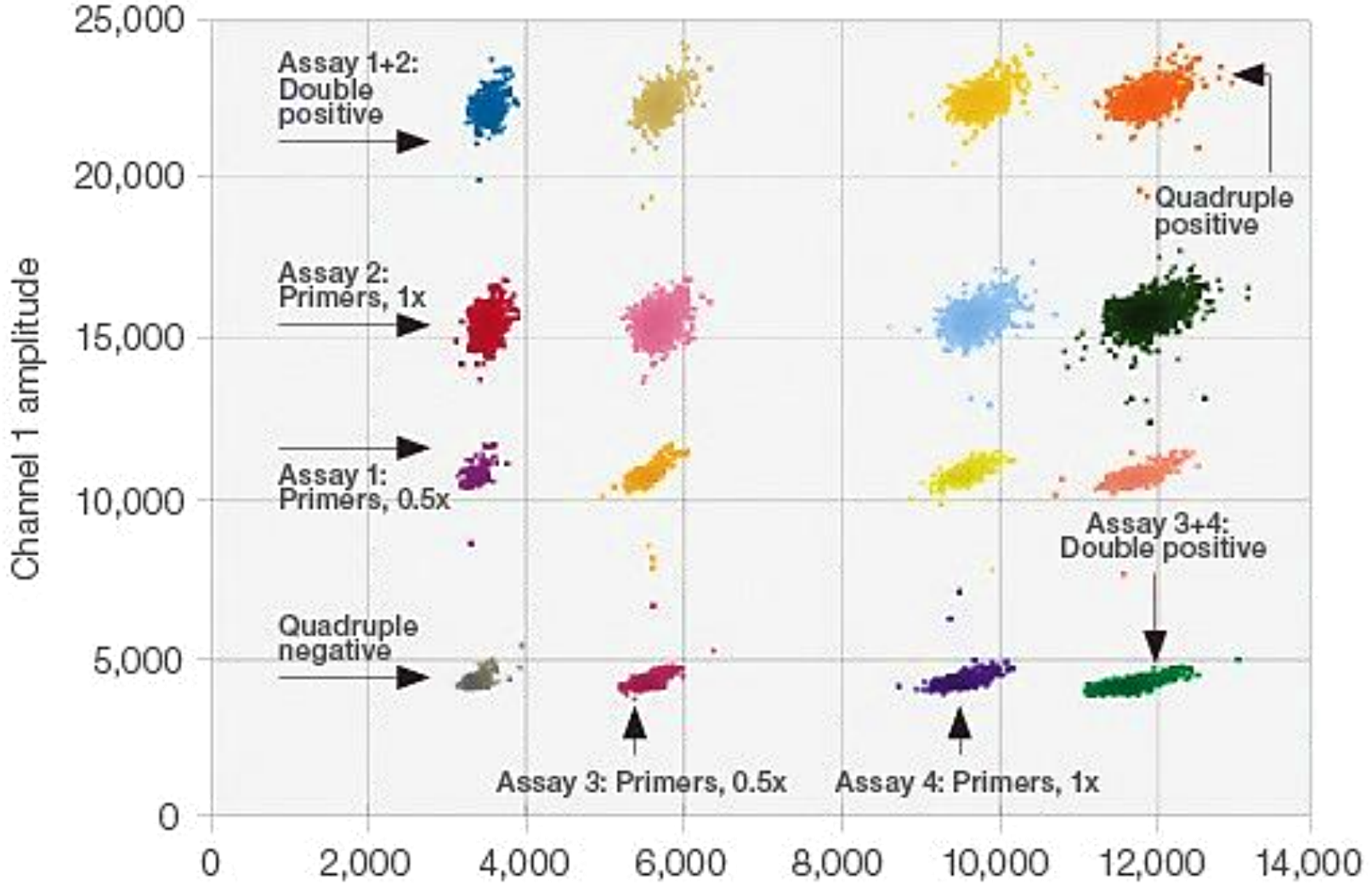
Digital PCR


A
B
A
G
A/G
C
universal
variant
ins, del or SNV
Ch1 amplitude
Ch2 amplitude
Ch1 amplitude
Ch2 amplitude
Ch1 amplitude
Ch2 amplitude

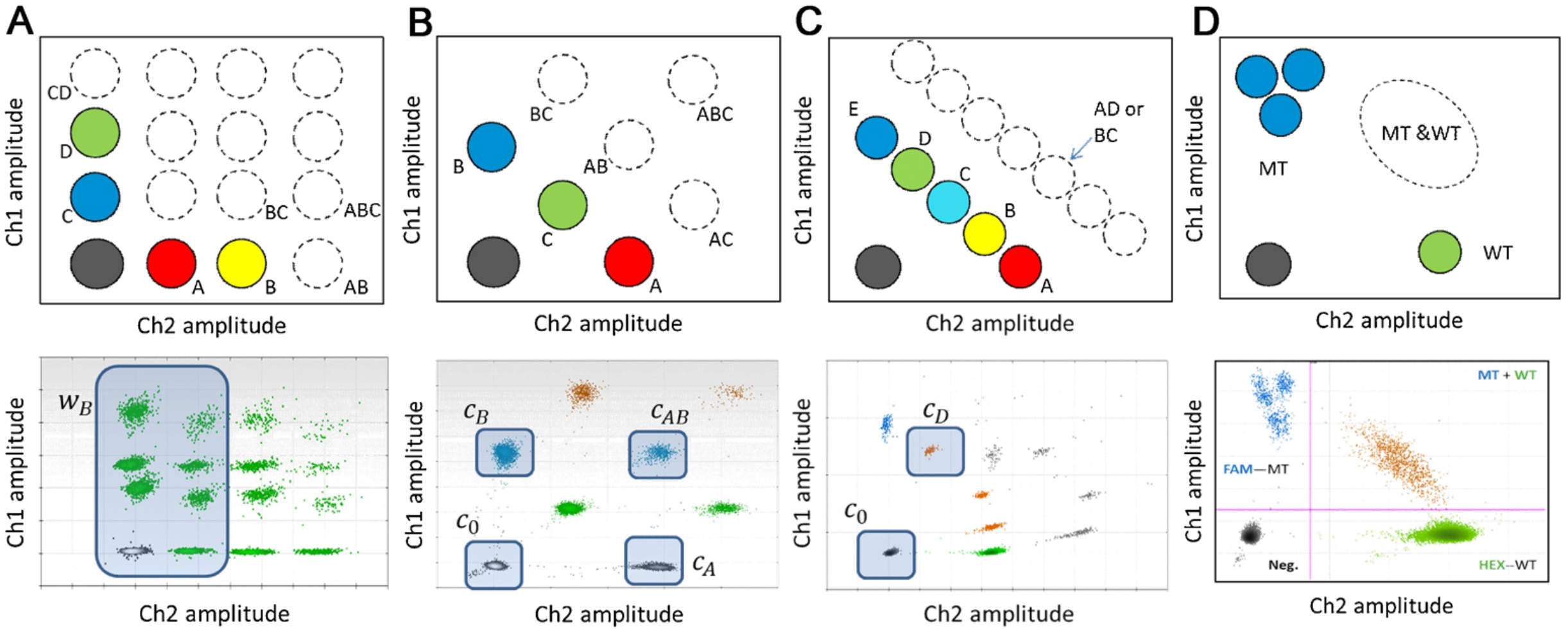
Digital PCR


Channel 1 amplitude
6000
5000
4000
3000
2000
1000
1000
2000
3000
4000
5000
EZH2
WT
EZH2
MUT
EZH2 WT
STAT6 MUT
EZH2 MUT
STAT6 MUT
STAT6
MUT
EZH2 WT
STAT6 WT
EZH2 MUT
STAT6 WT
STAT6
WT

Digital PCR


Channel 1 amplitude
25,000
20,000
15,000
10,000
5,000
0
0
2,000
4,000
6,000
8,000
10,000
12,000
14,000
Assay 1+2: Double positive
Assay 2: Primers, 1x
Assay 1: Primers, 0.5x
Quadruple negative
Quadruple positive
Assay 3+4: Double positive
Assay 3: Primers, 0.5x
Assay 4: Primers, 1x

Digital PCR


A
B
C
D
Ch1 amplitude
Ch2 amplitude
CD
D
C
BC
ABC
A
B
AB
BC
ABC
B
AB
C
AC
A
E
D
C
B
A
AD or
BC
MT
MT &WT
WT
w_B
c_B
c_{AB}
c_0
c_A
c_D
c_0
MT + WT
FAM—MT
Neg.
HEX—WT

Digital PCR

Biomolecular Detection and Quantification 10 (2016) 15–23

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Review Article

Fundamentals of multiplexing with digital PCR

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[b] Digital Biology Centre, Bio-Rad Laboratories Inc., 5731 West Las Positas Boulevard, Pleasanton, CA 94588, United States

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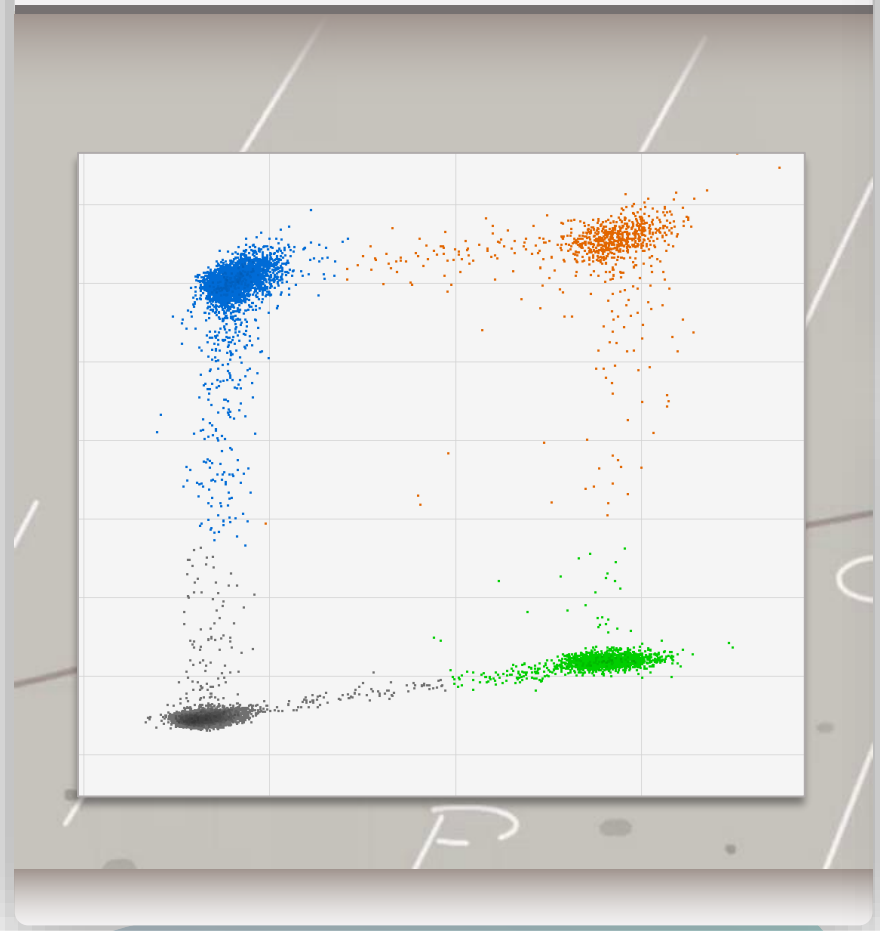
ABSTRACT

Over the past decade numerous publications have demonstrated how digital PCR (dPCR) enables precise and sensitive quantification of nucleic acids in a wide range of applications in both healthcare and environmental analysis. This has occurred in parallel with the advances in partitioning fluidics that enable a reaction to be subdivided into an increasing number of partitions. As the majority of dPCR systems are based on detection in two discrete optical channels, most research to date has focused on quantification of one or two targets within a single reaction. Here we describe 'higher order multiplexing' that is the unique ability of dPCR to precisely measure more than two targets in the same reaction. Using examples, we describe the different types of duplex and multiplex reactions that can be achieved. We also describe

Подводные камни

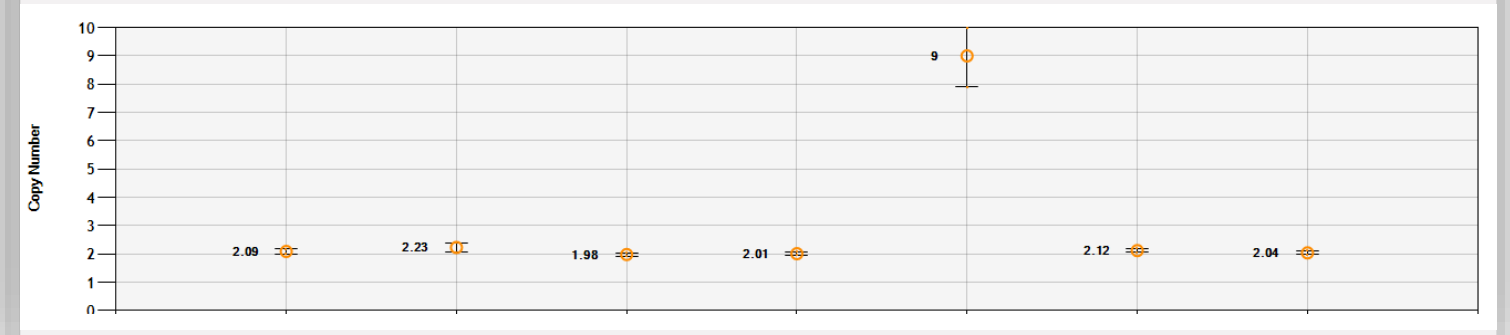
Качество ДНК

"Эффект дождя"

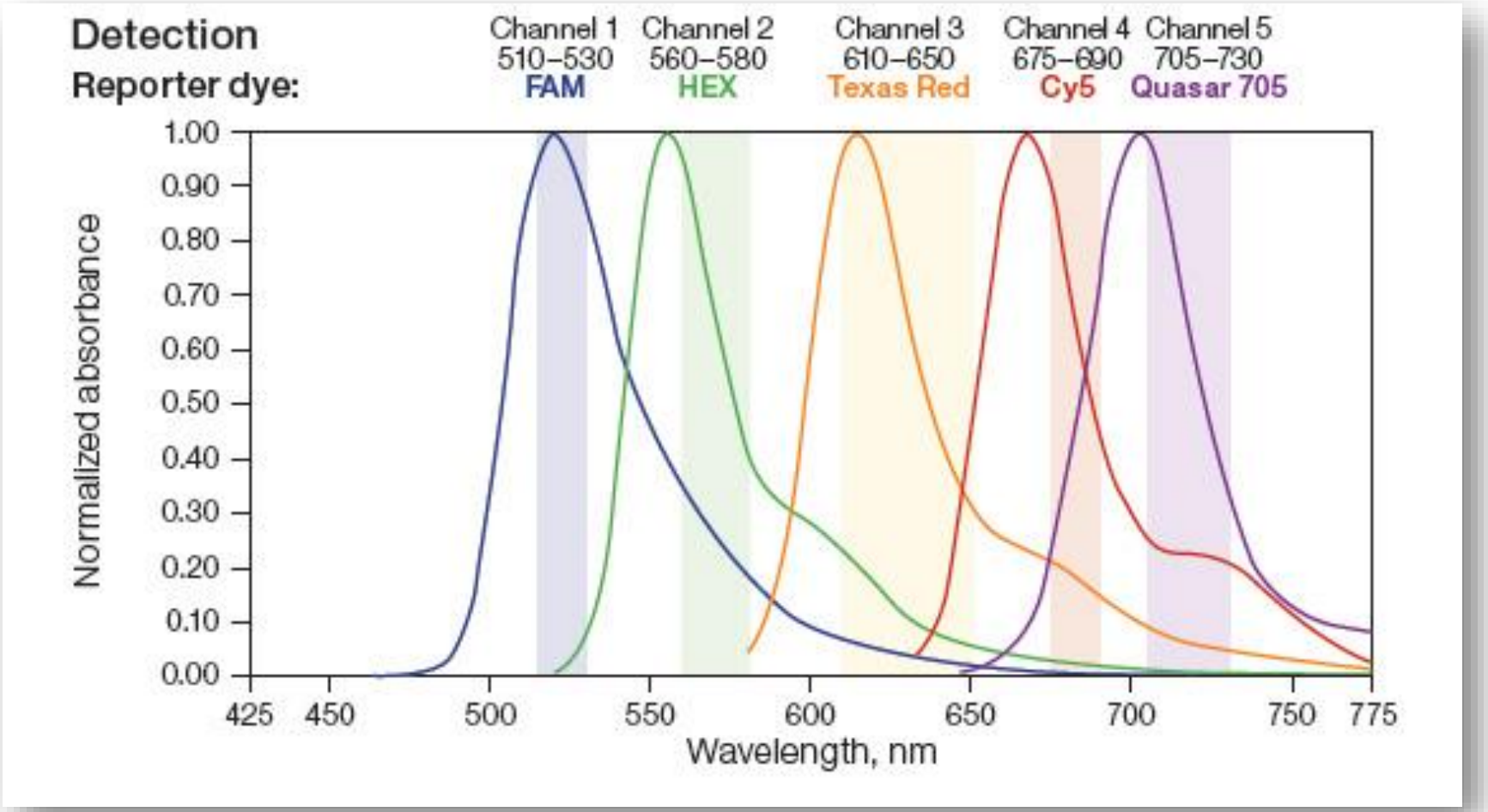


Электрофореграмма образцов геномной ДНК

Результат ddPCR

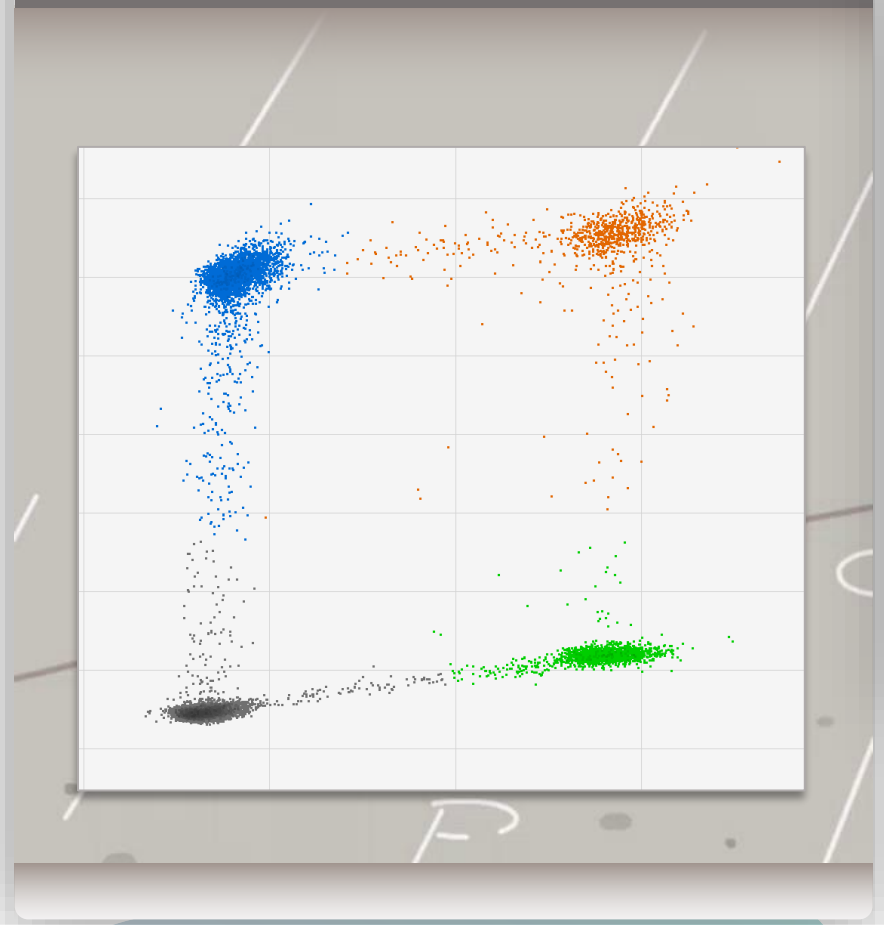


Типы красителей/ гасителей

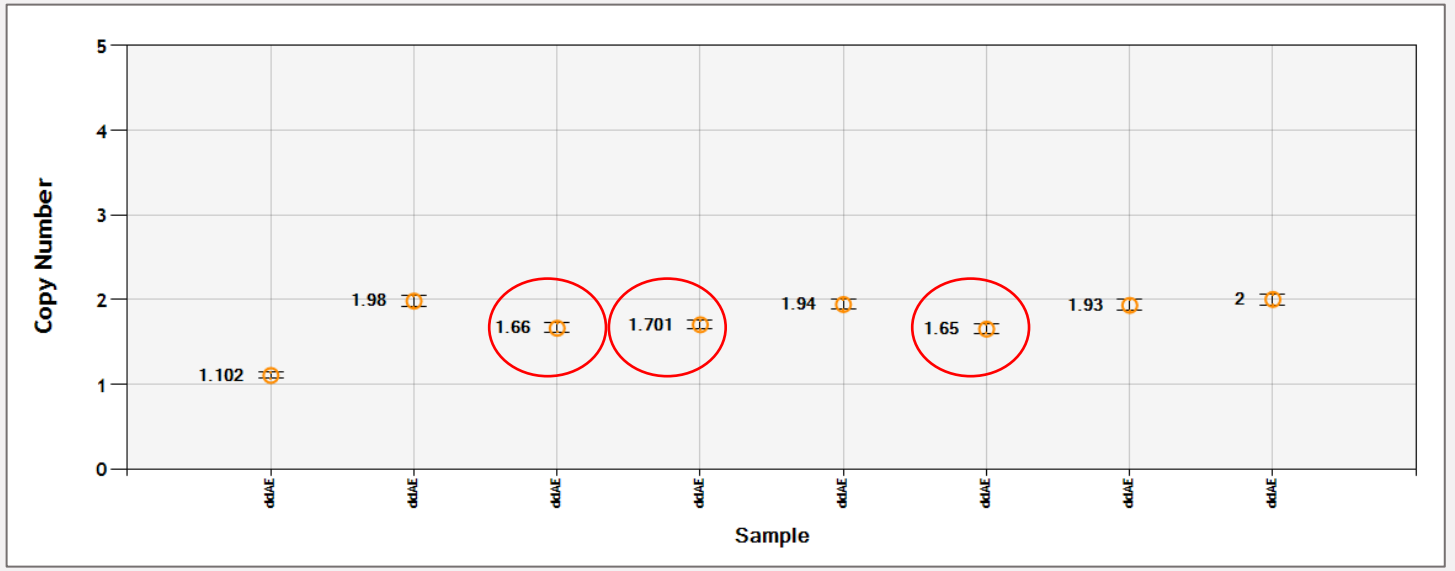


Качество ДНК

"Эффект дождя"

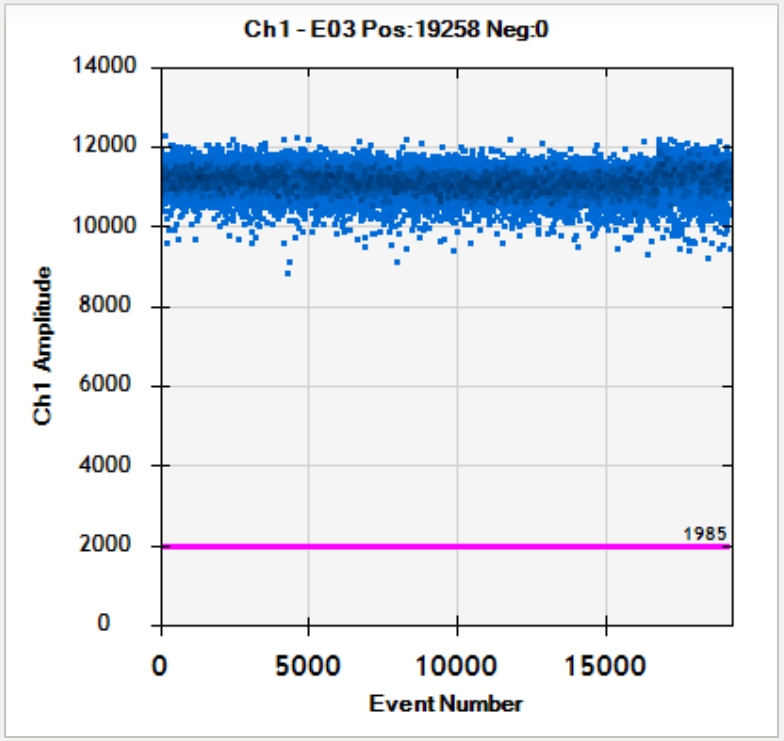


Результат ddPCR при неполной рестрикции



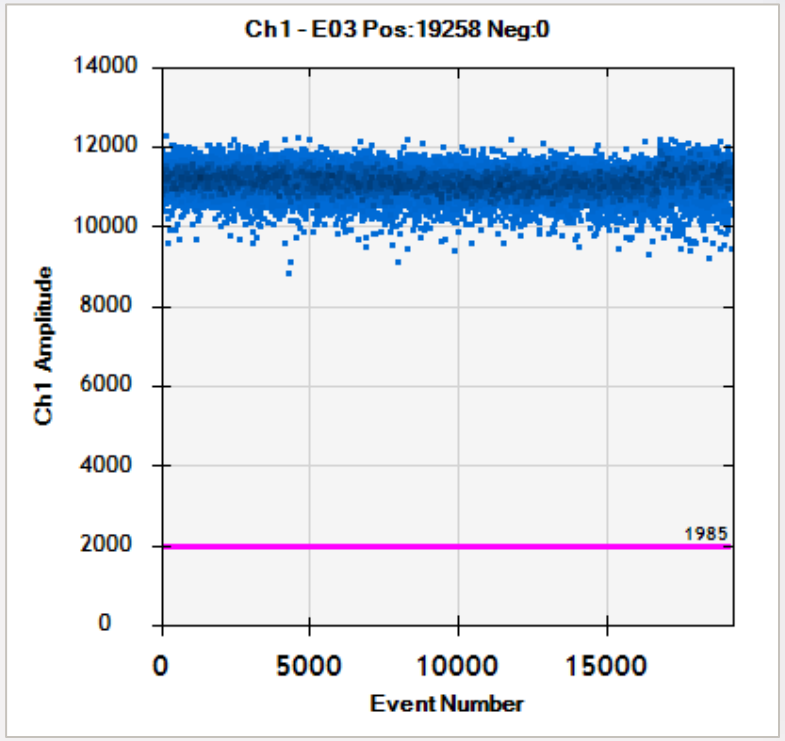
Количество ДНК

"Перегруз"

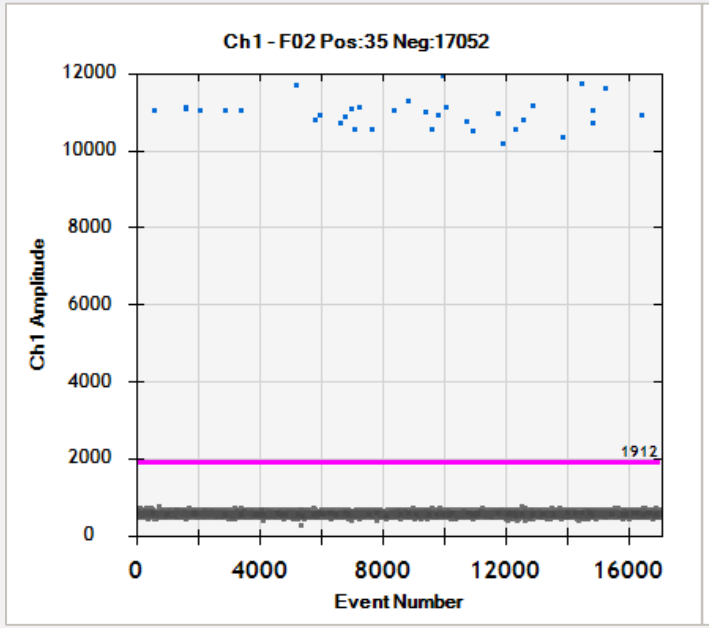


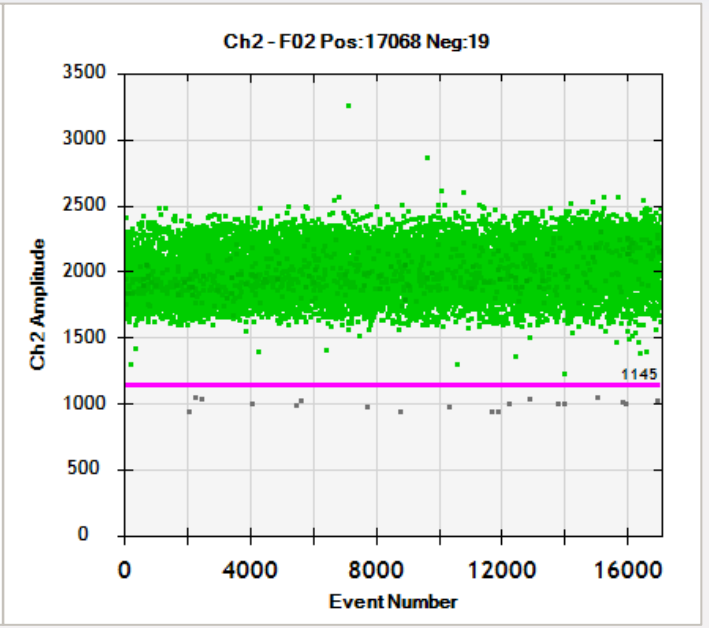
Количество ДНК

"Перегруз"



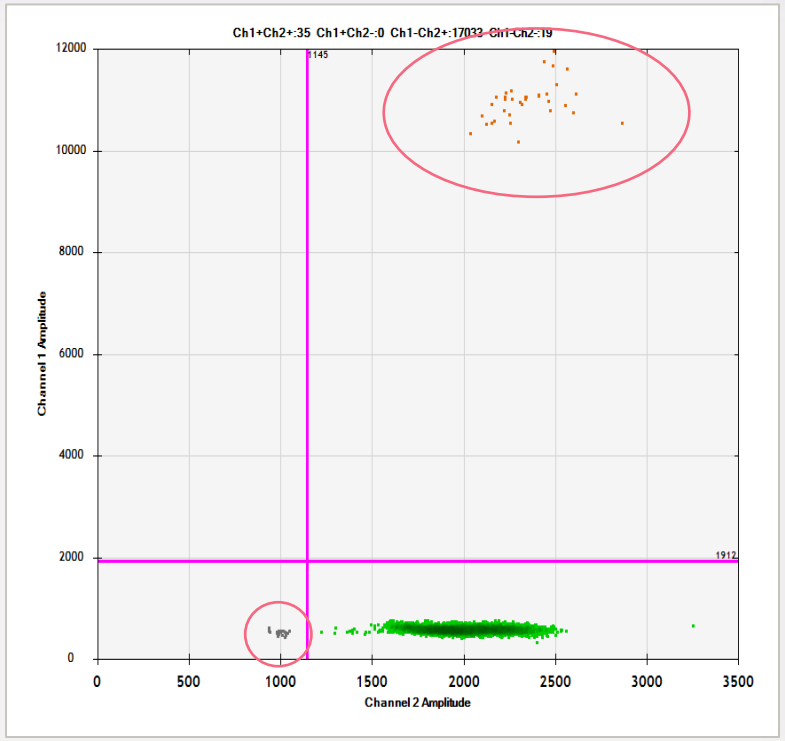
Избыток таргетного региона (кДНК)



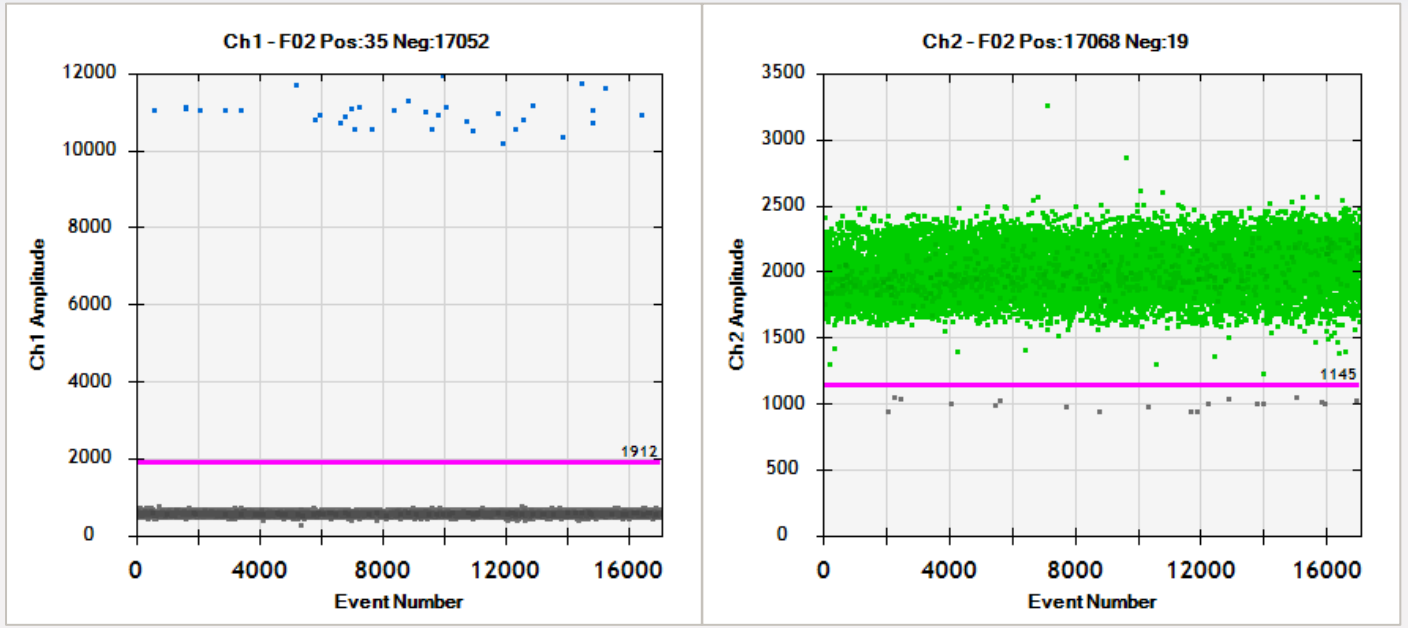


Количество ДНК

"Перегруз"

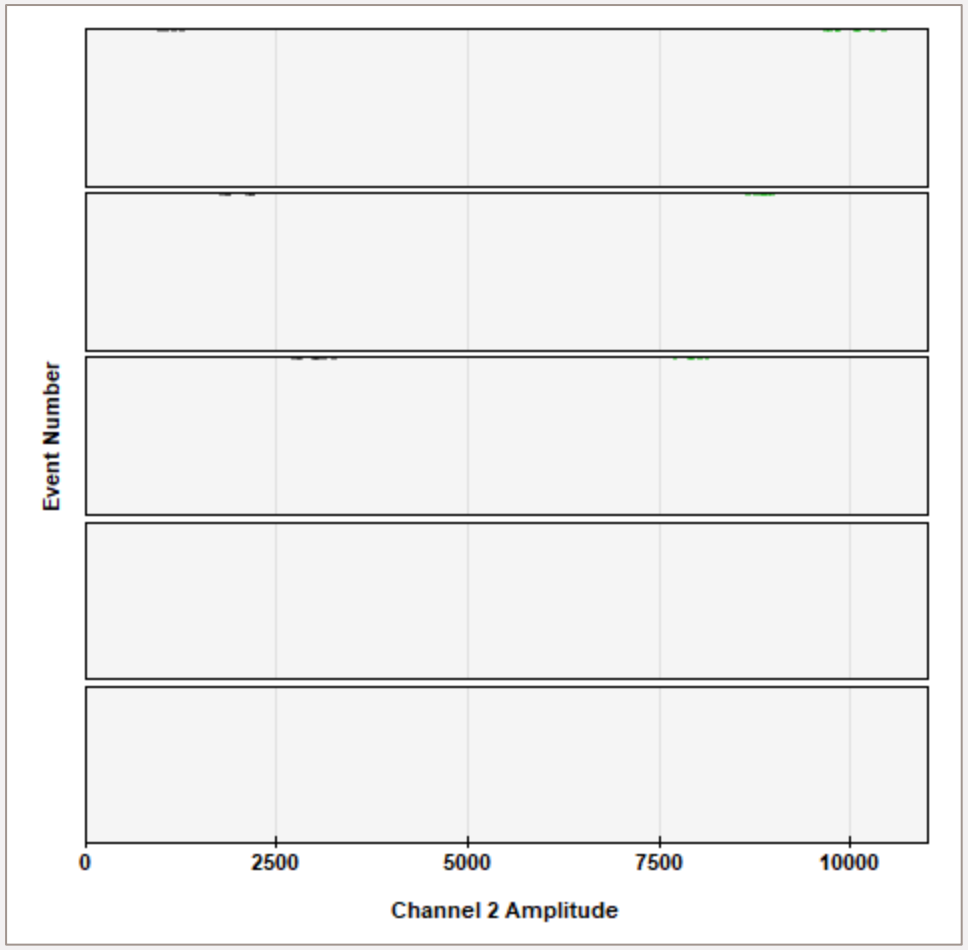


Избыток таргетного региона (кДНК)



Распад зондов

Градиент распада зондов



По времени

1 год

2 года

3 года

4 года

5 год

По циклу заморозка/разморозка

4 з./р.

10 з./р.

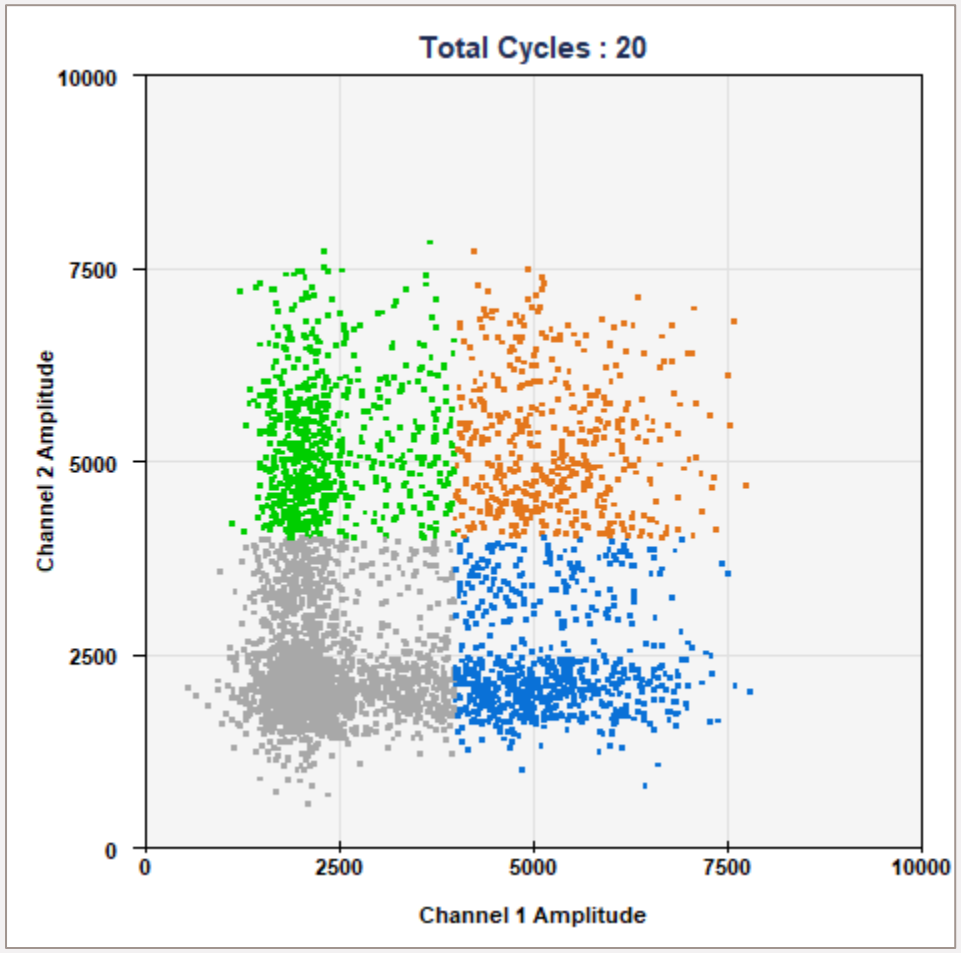
16 з./р.

20 з./р.

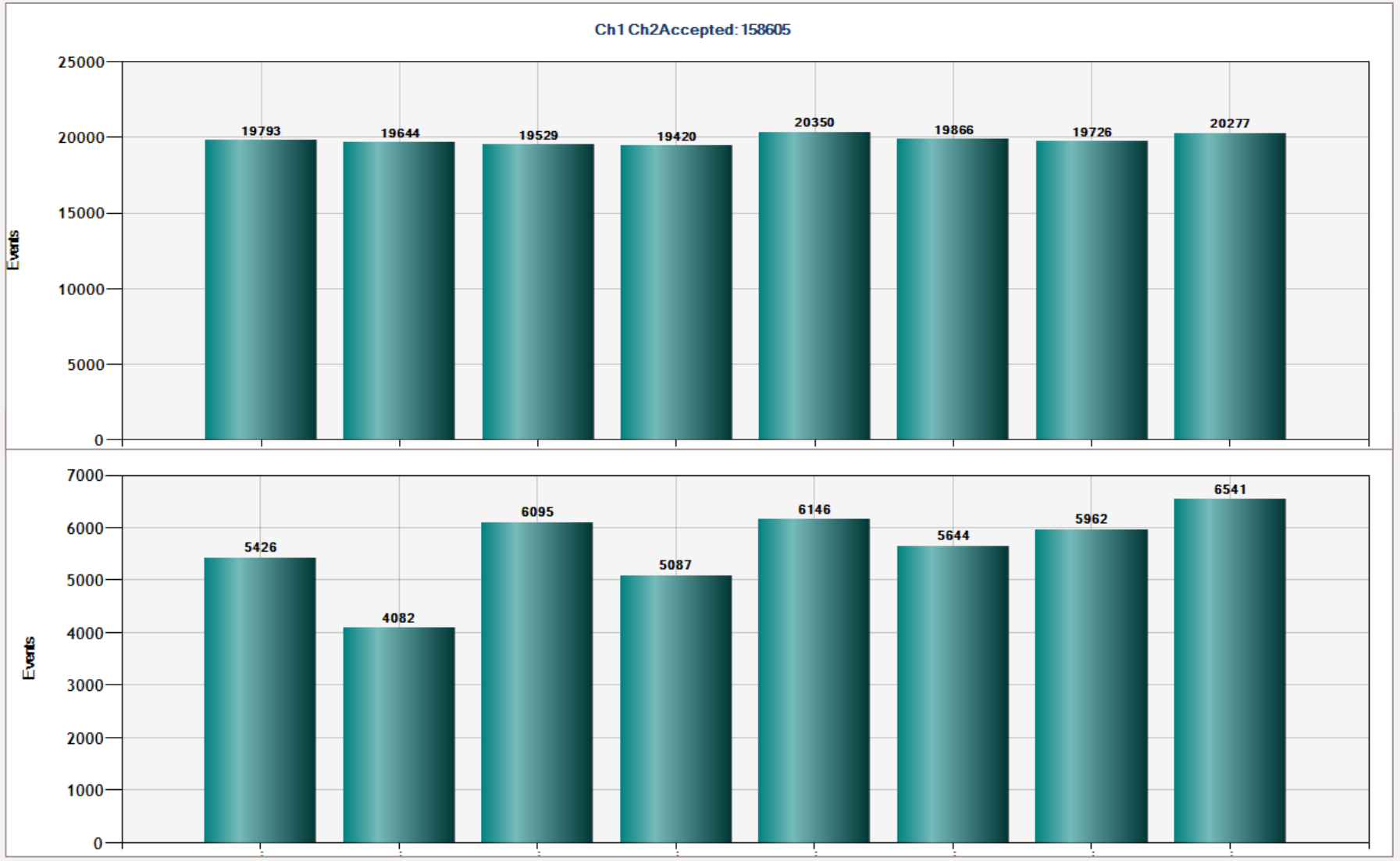
Высвобождение 5'-Reporter

Высвобождение 3'-Quencher

Влияние количество циклов на эффективность ddPCR



Стабилизация капель



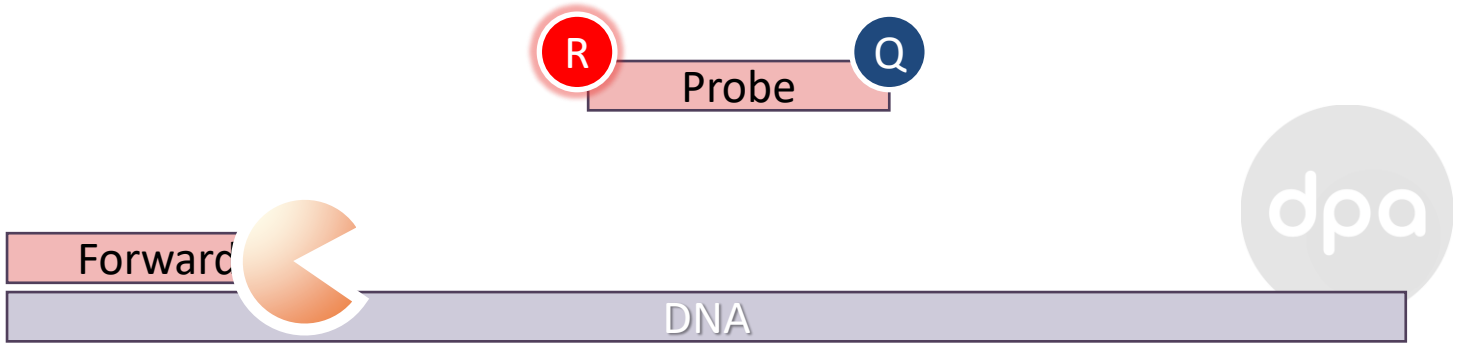
Некоторые решения

qPCR (detection)

TaqMan

Специфические зонды

- TaqMan assay
  - Taq-pol – exonuclease activity

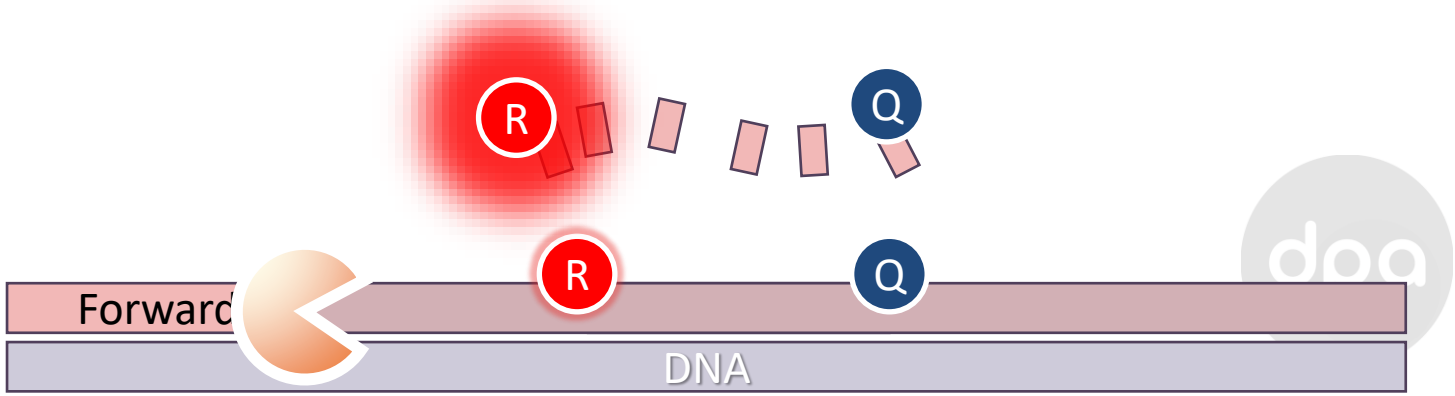


qPCR (detection)

TaqMan

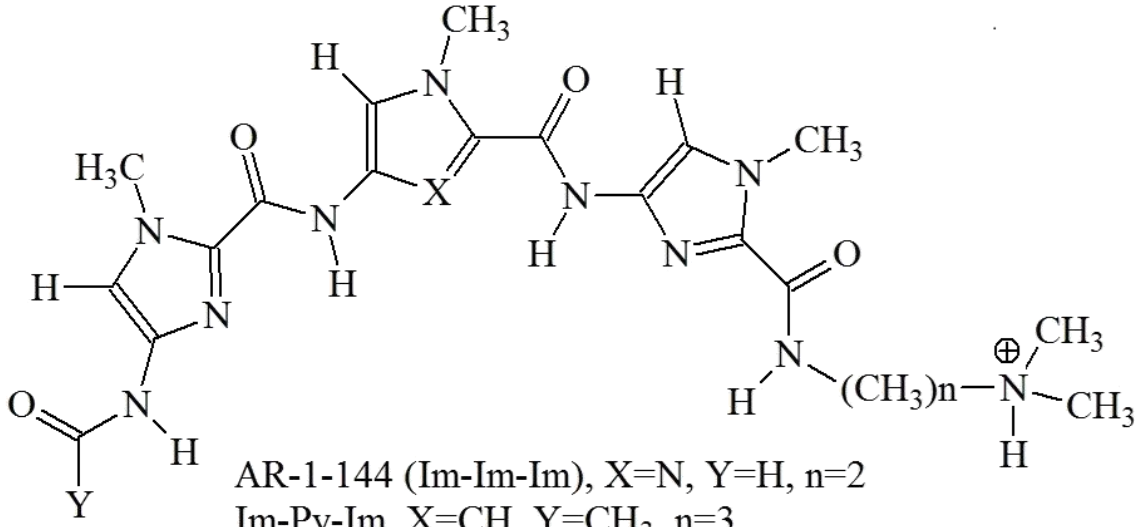
Специфические зонды

- TaqMan assay
  - Taq-pol – exonuclease activity
  - Reporter – флуорофора
  - Quencher - гаситель

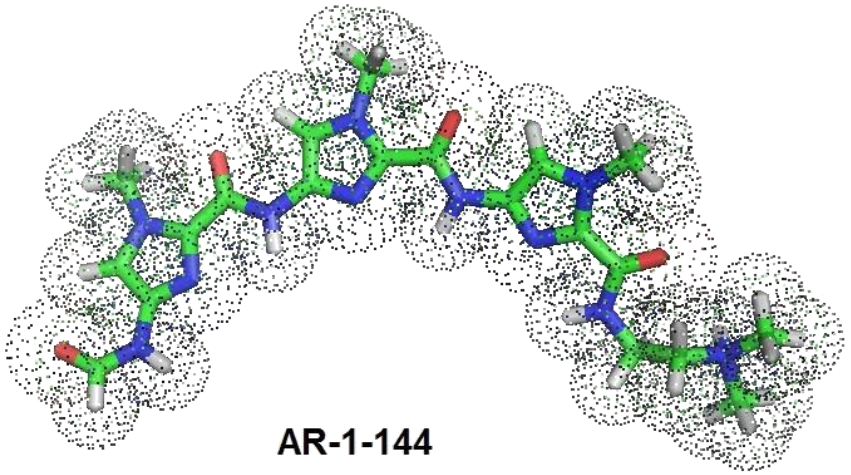


TaqMan

Minor groove binder



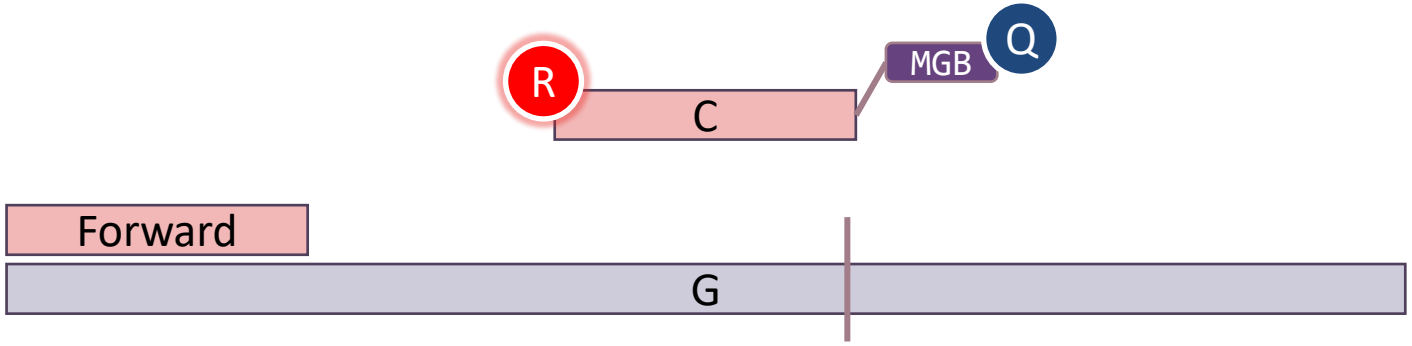
AR-1-144 (Im-Im-Im), X=N, Y=H, n=2
Im-Py-Im, X=CH, Y=CH_3, n=3



AR-1-144

TaqMan

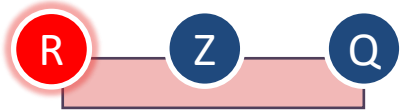
Allele Specific qPCR (AS-qPCR)



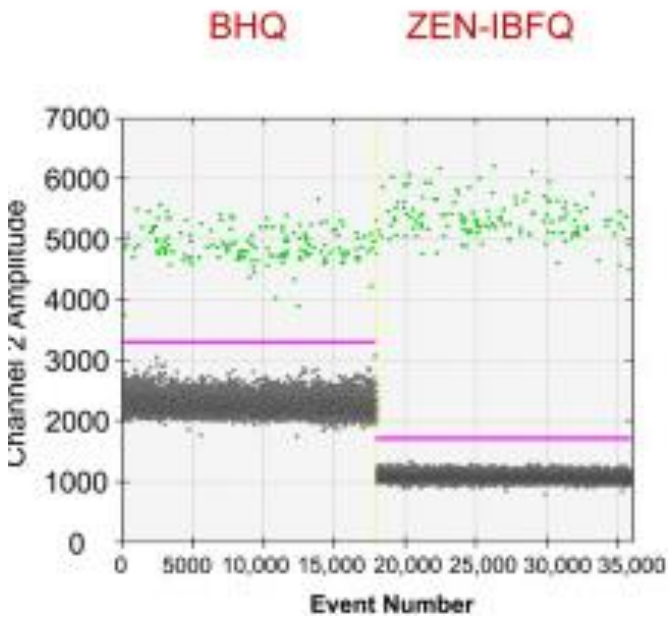
Cycles

TaqMan

Double quenching



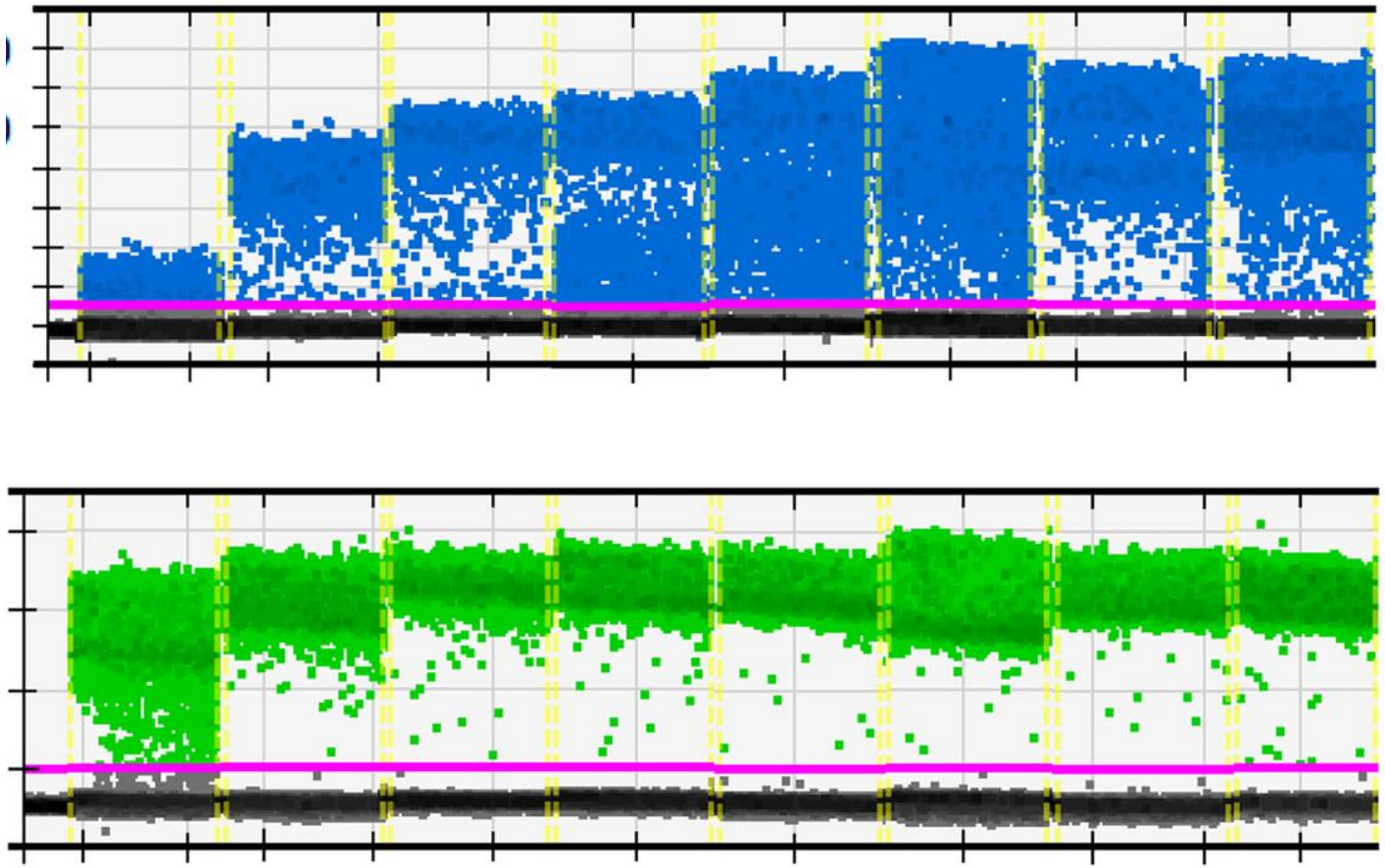
Forward



Maier (2017) JMD

TaqMan

Double quenching



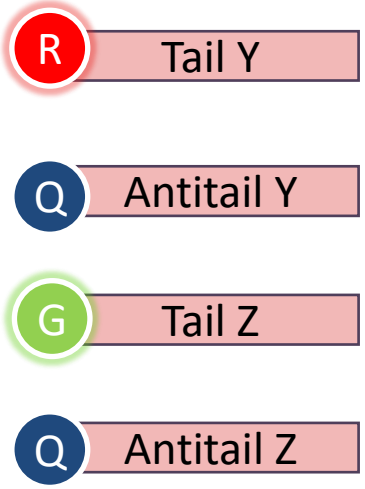
Цифровая ПЦР
это не только TaqMan

KASP

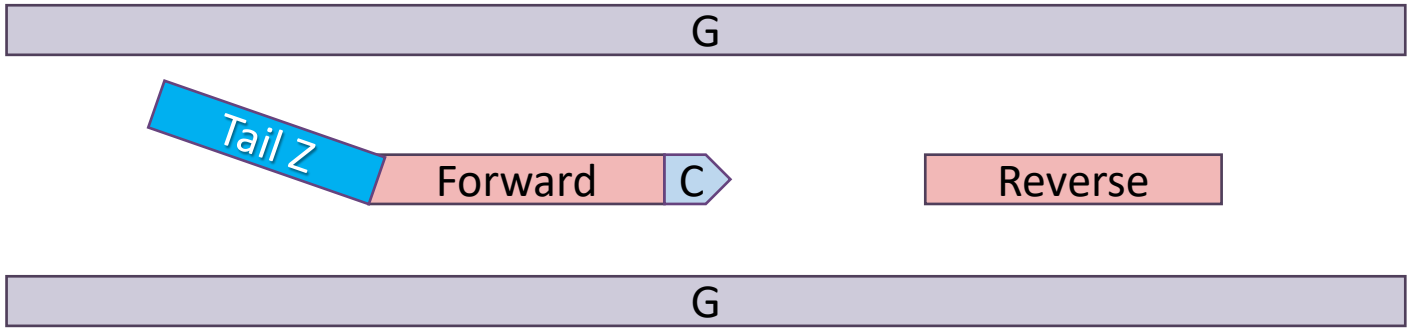
Competitive Allele-Specific PCR

- KASP Assay Mix

- KASP Master Mix



Denaturation Step

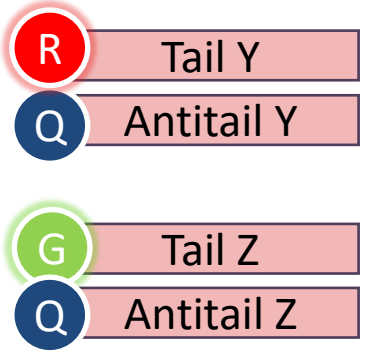


KASP

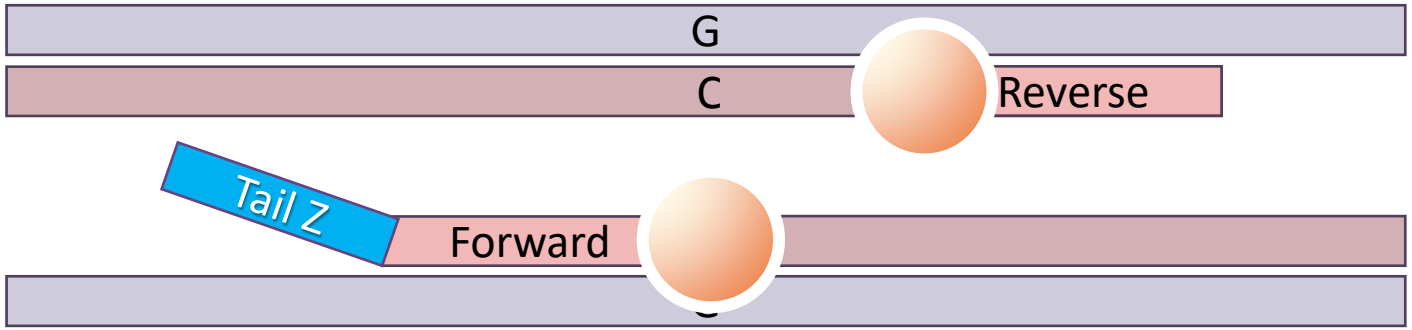
Competitive Allele-Specific PCR

- KASP Assay Mix

- KASP Master Mix



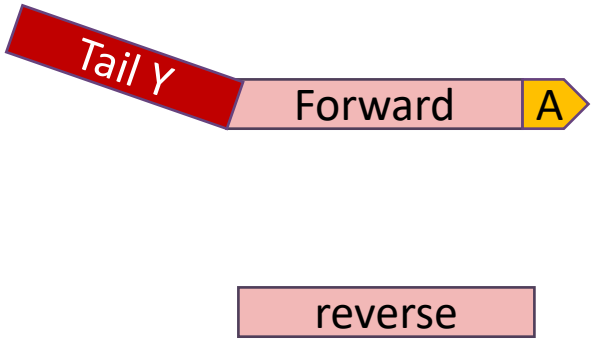
Annealing-Elongation Step



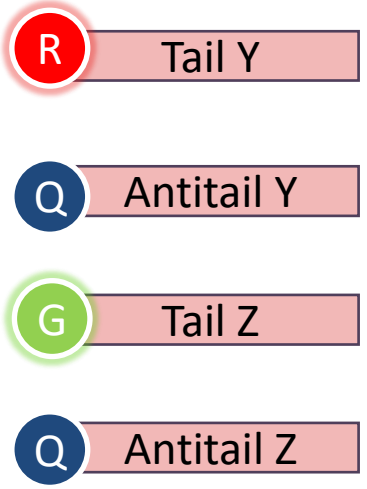
KASP

Competitive Allele-Specific PCR

- KASP Assay Mix



- KASP Master Mix



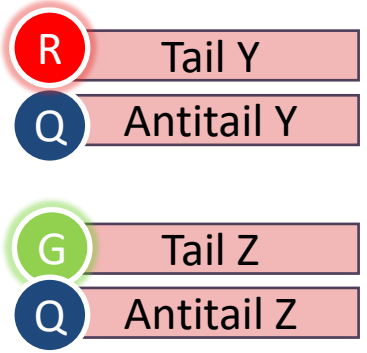
Denaturation Step

KASP

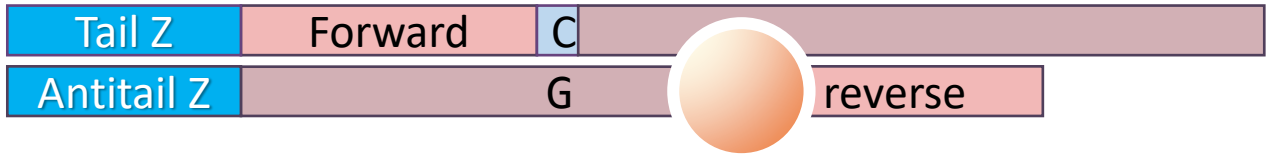
Competitive Allele-Specific PCR

- KASP Assay Mix

- KASP Master Mix



Annealing Step



KASP

Competitive Allele-Specific PCR

- KASP Assay Mix

- KASP Master Mix

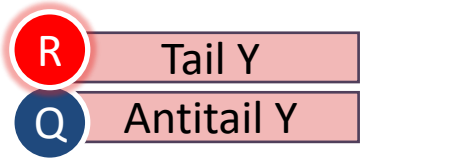
Denaturation Step

KASP

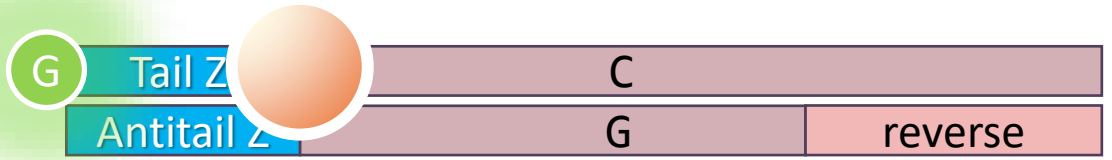
Competitive Allele-Specific PCR

- KASP Assay Mix

- KASP Master Mix



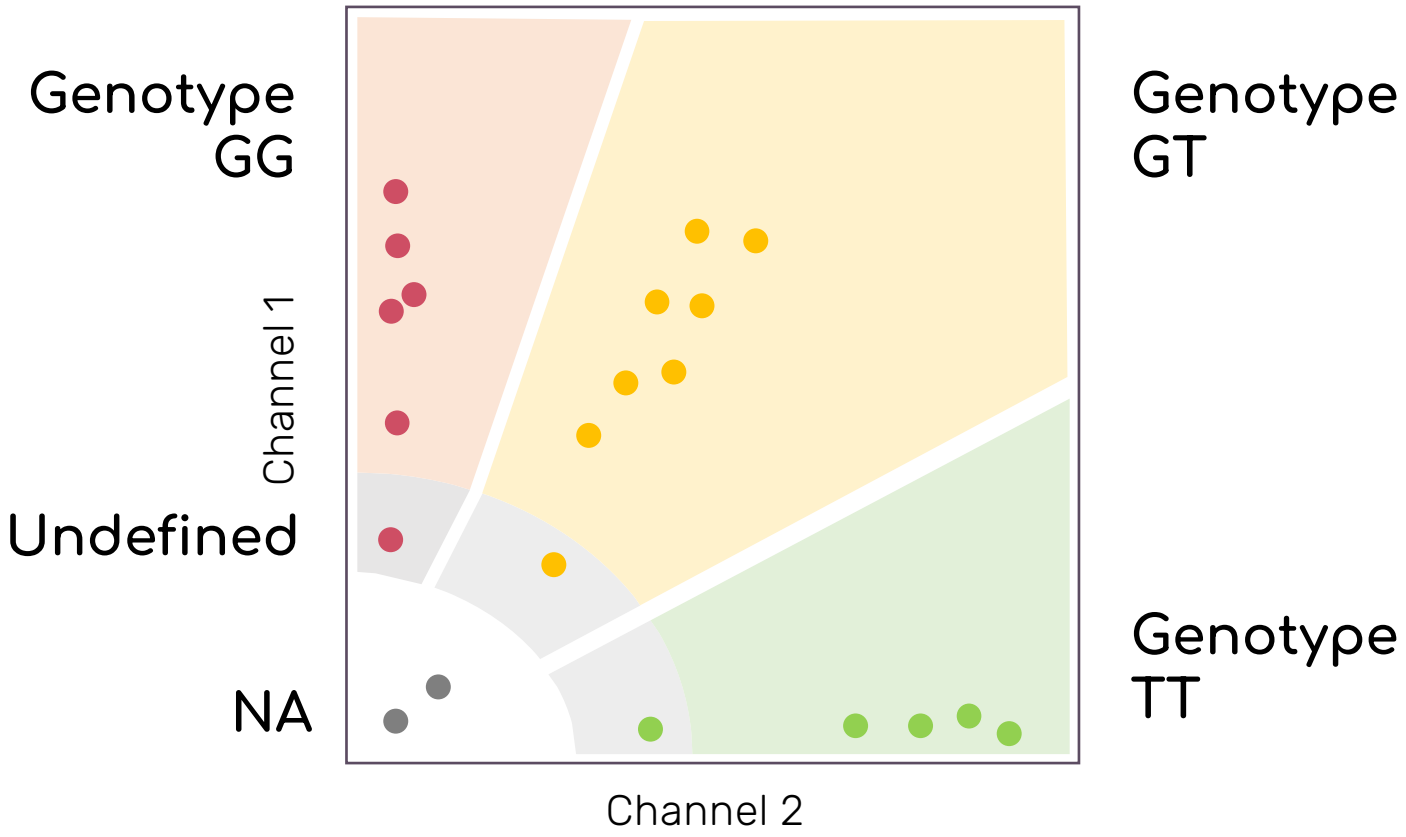
Annealing Step



KASP

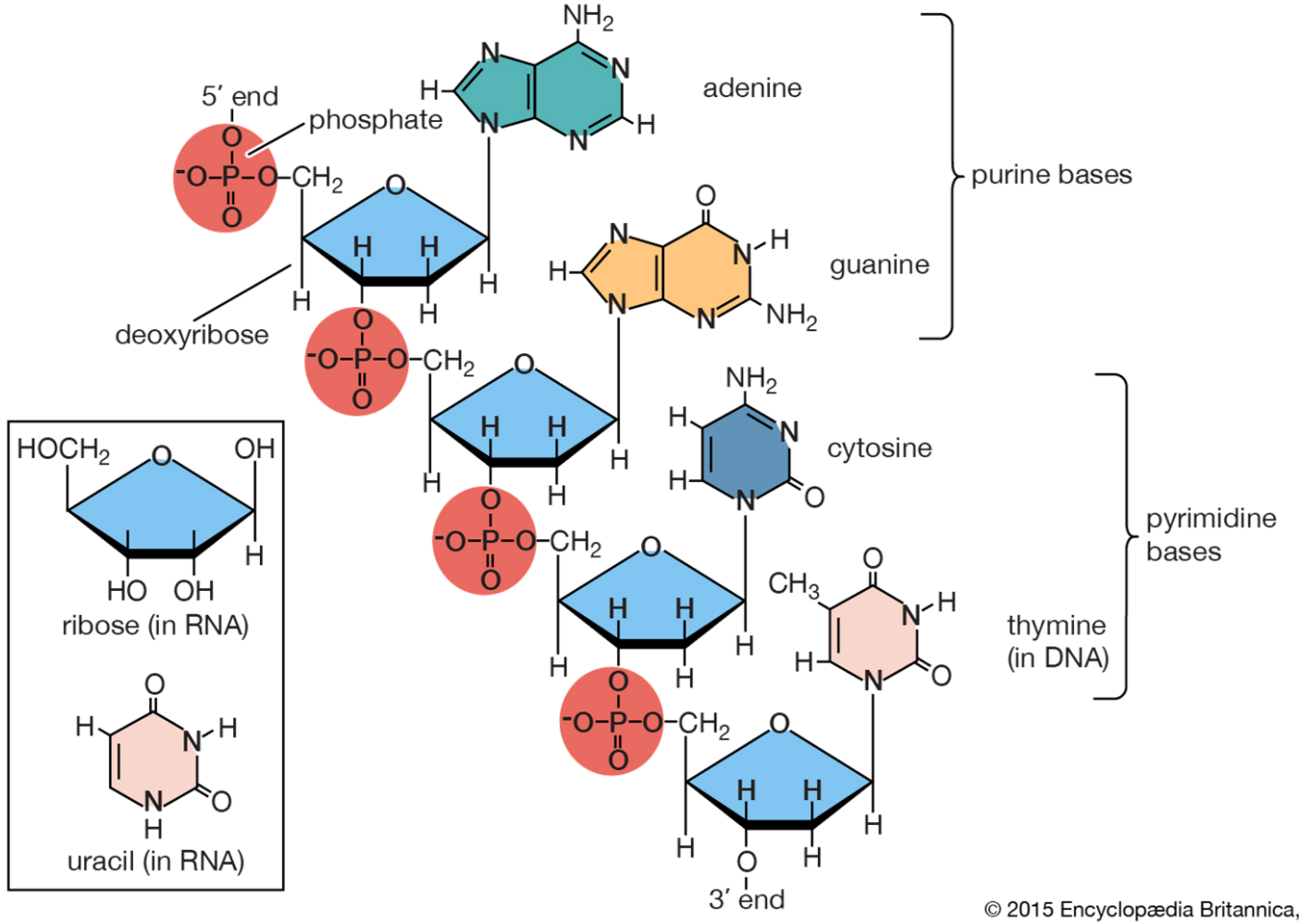
Competitive Allele-Specific PCR

2D plot



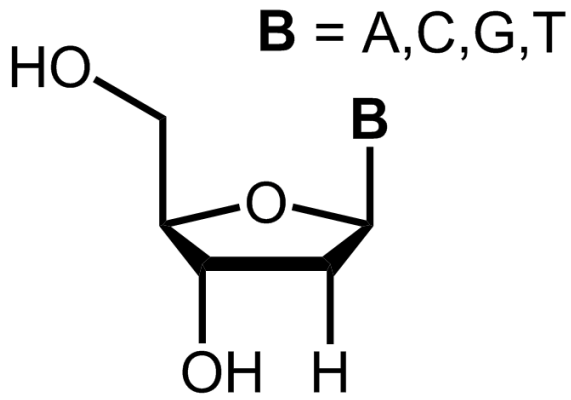
DNA

Formulae

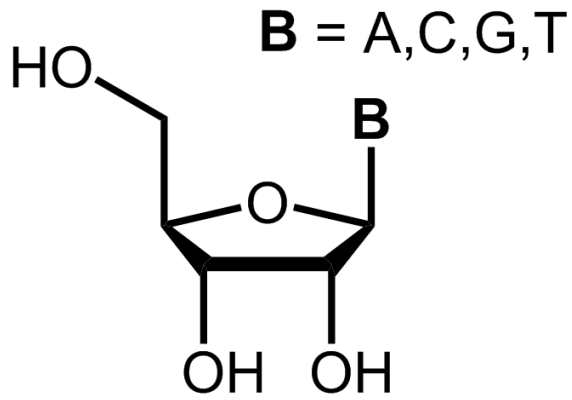


DNA

Formulae



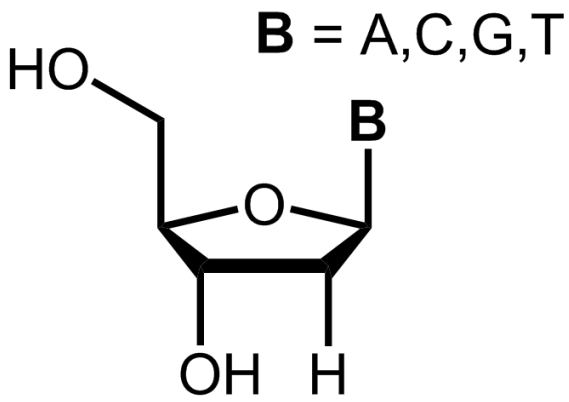
DNA Monomer



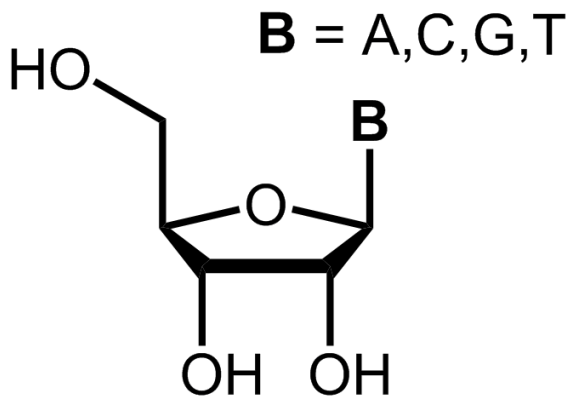
RNA Monomer

LNA

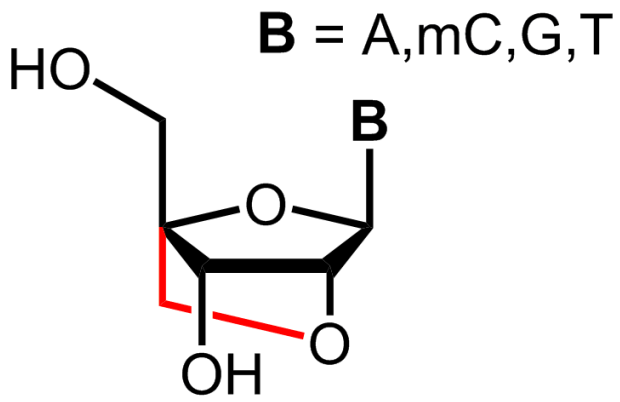
Locked nucleic acid



DNA Monomer



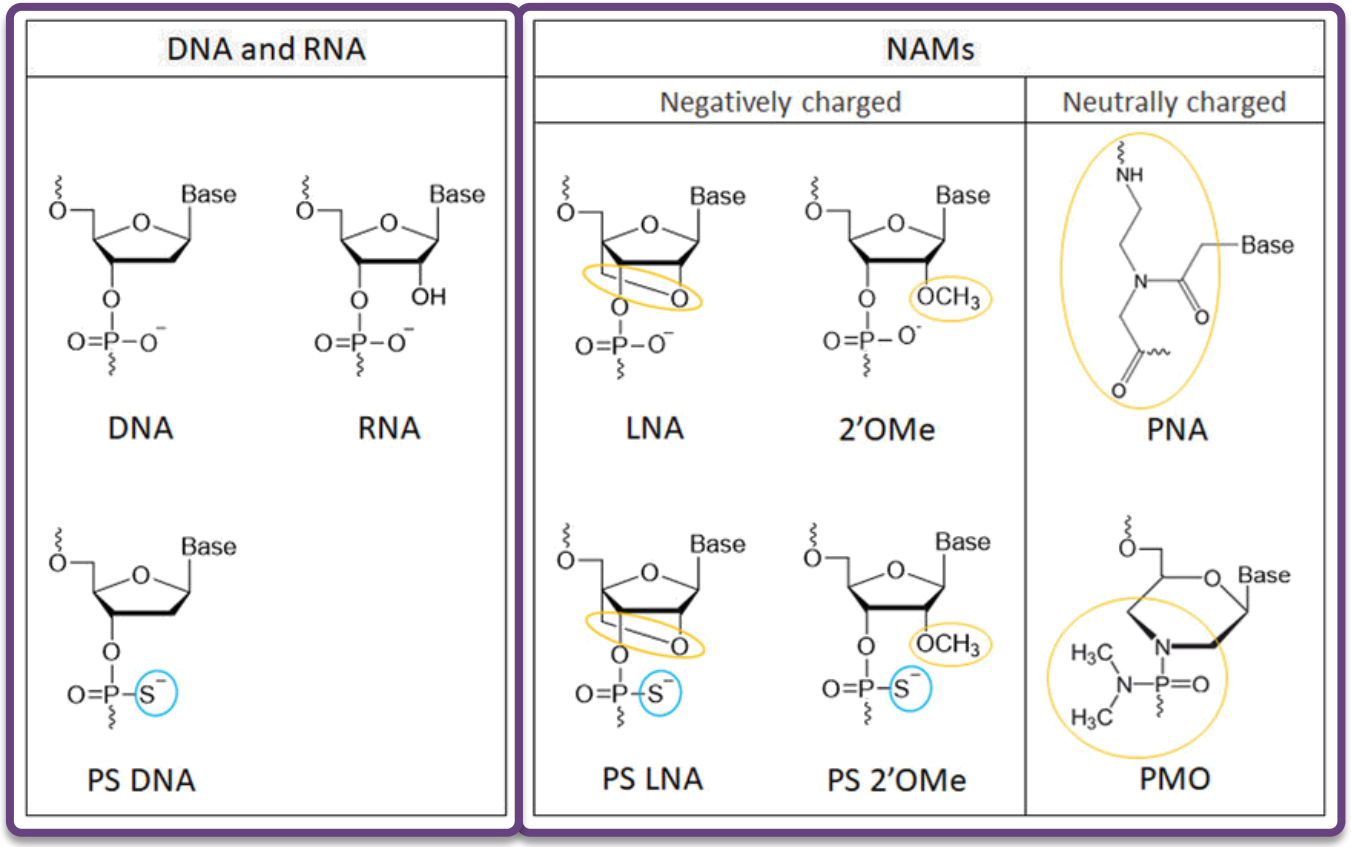
RNA Monomer



LNA Monomer

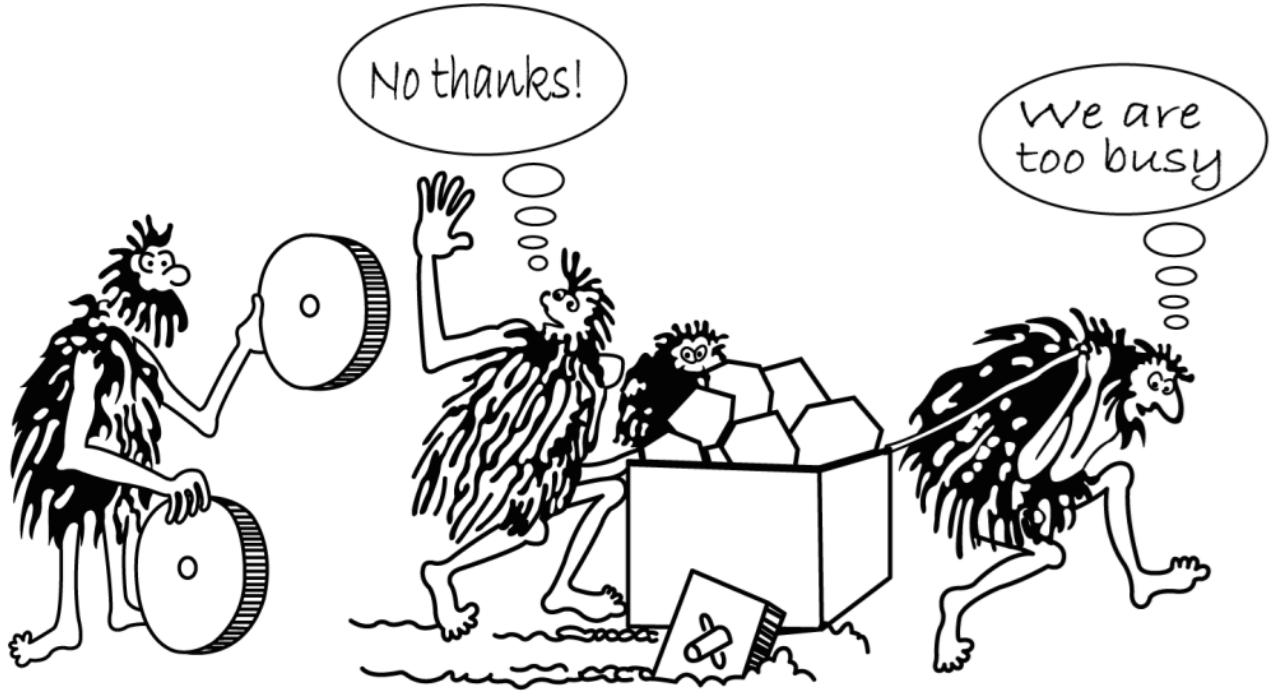
LNA

NAMs – nucleic acid mimics



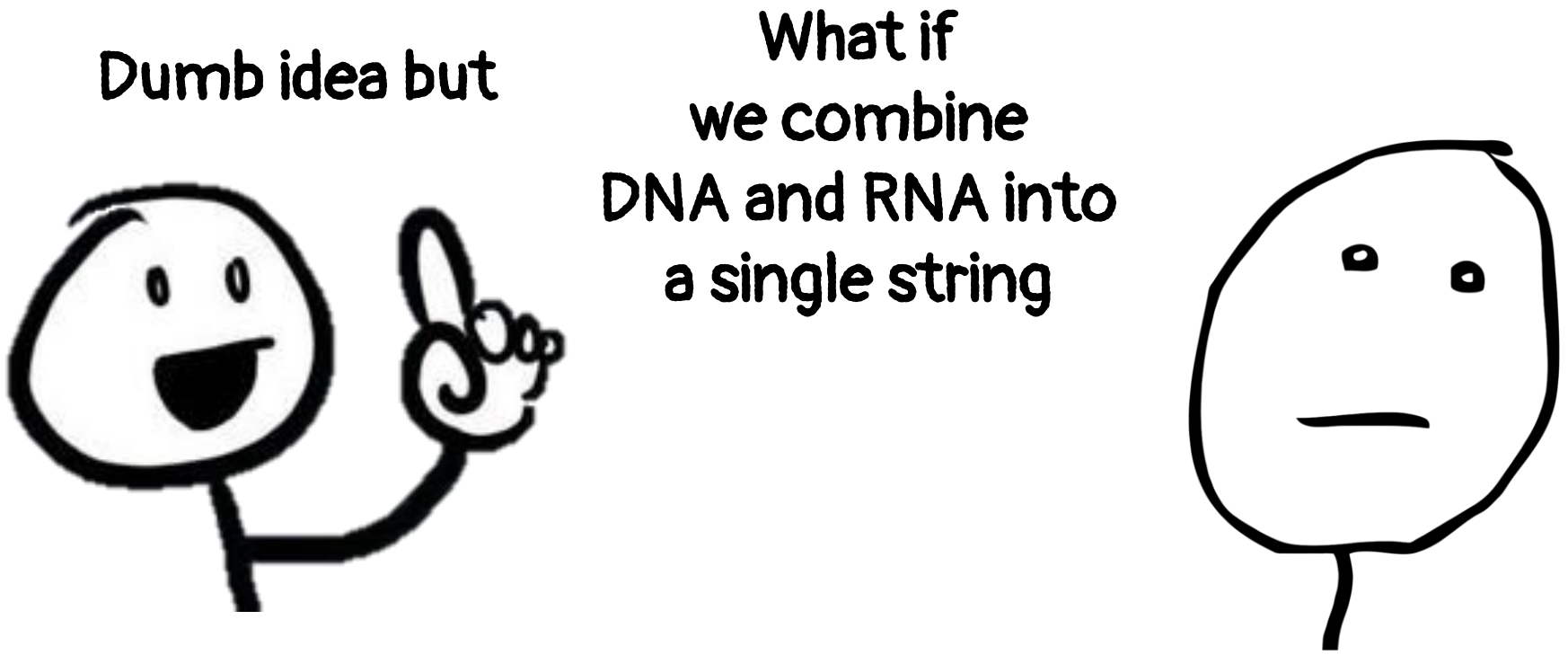
phAmp

RNase H-dependent PCR



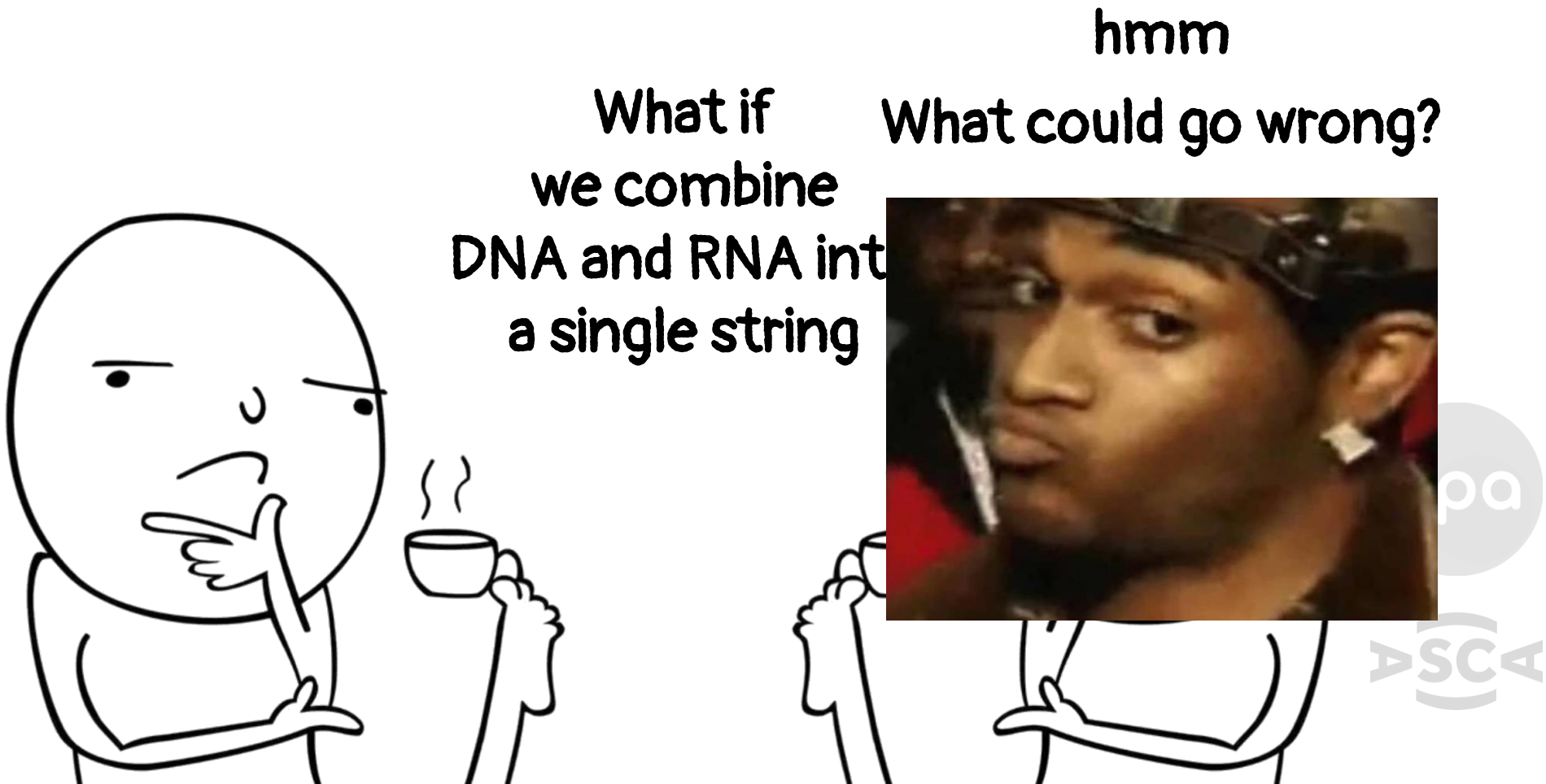
phAmp

RNase H-dependent PCR



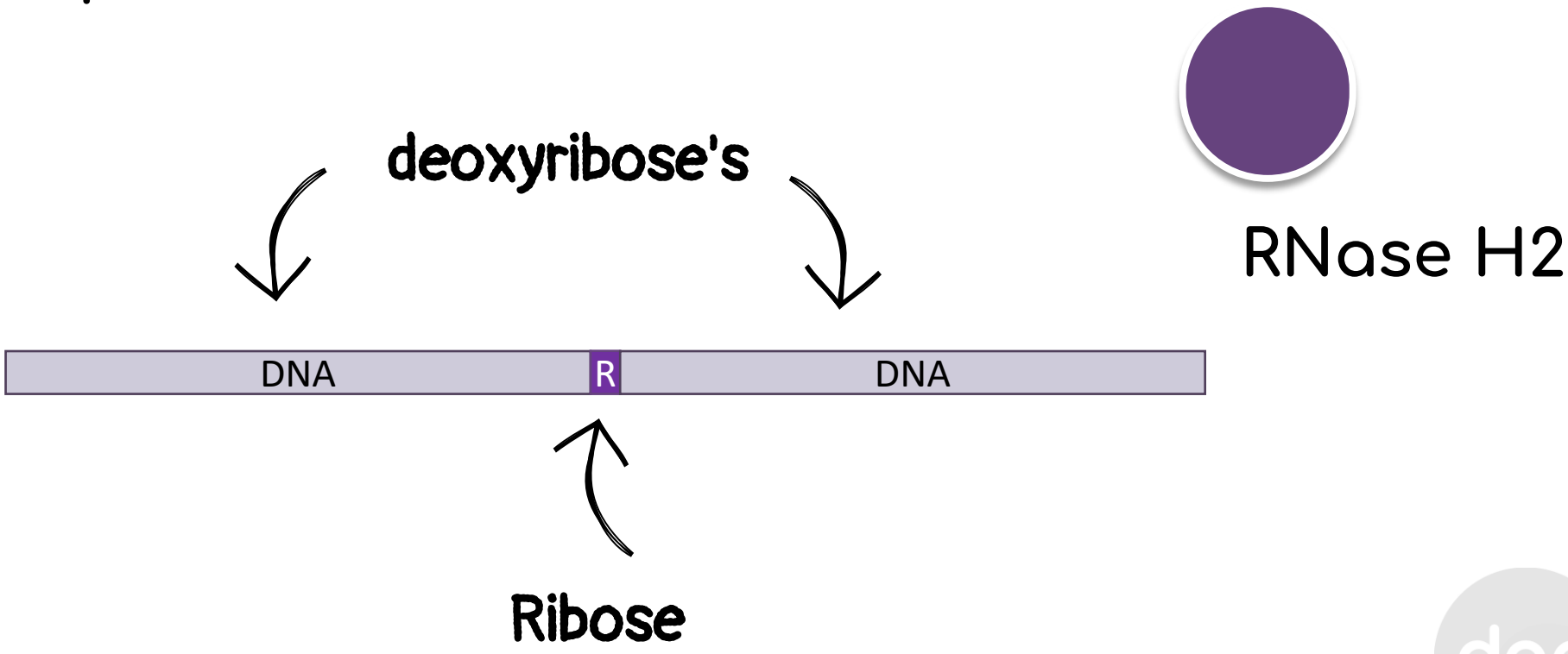
phAmp

RNase H-dependent PCR



phAmp

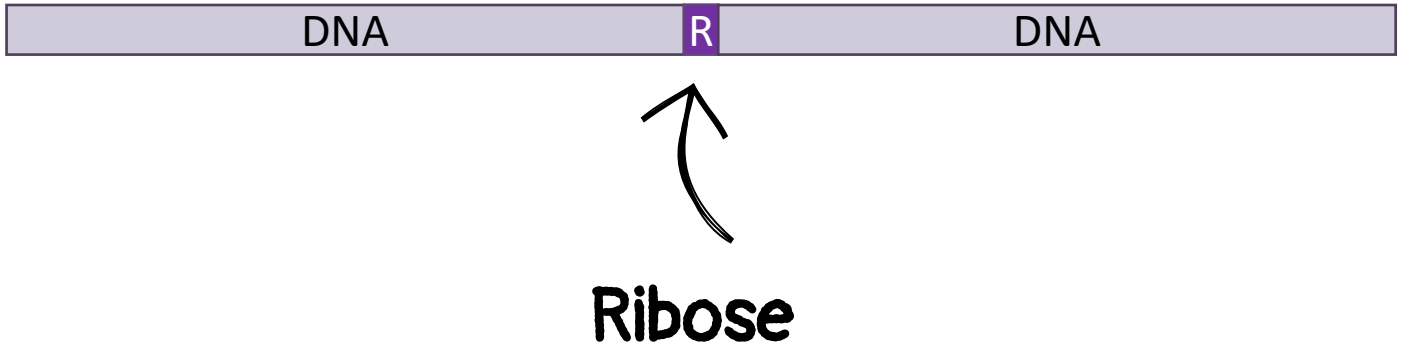
RNase H-dependent PCR



phAmp

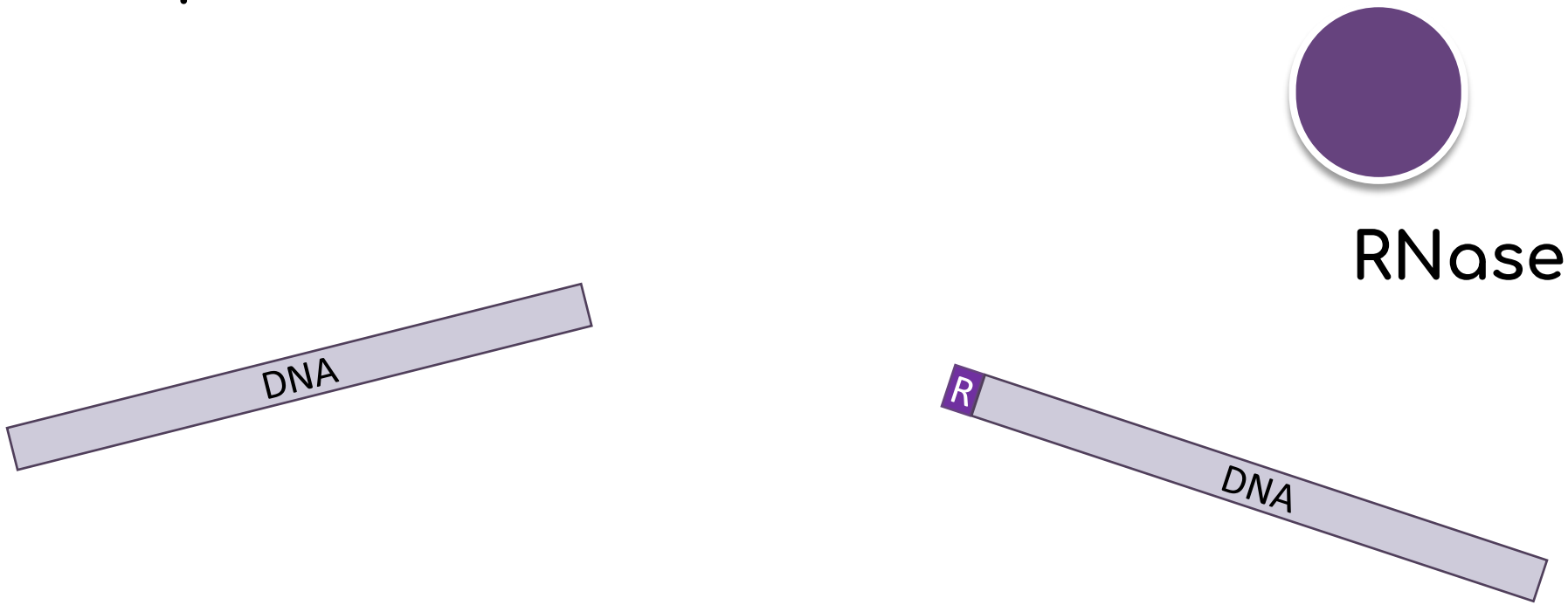
RNase H-dependent PCR

RNase H2



phAmp

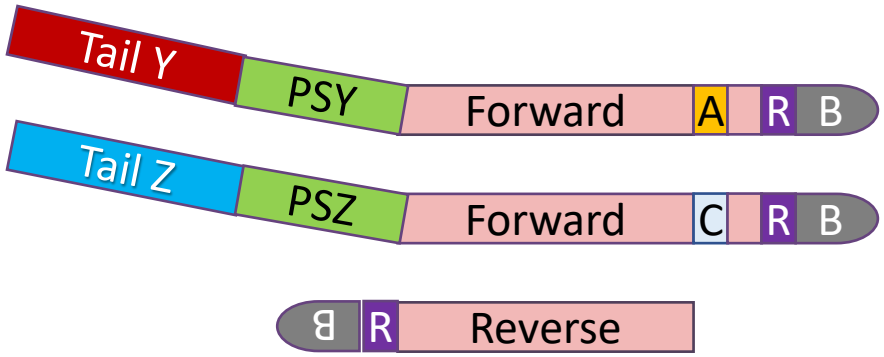
RNase H-dependent PCR



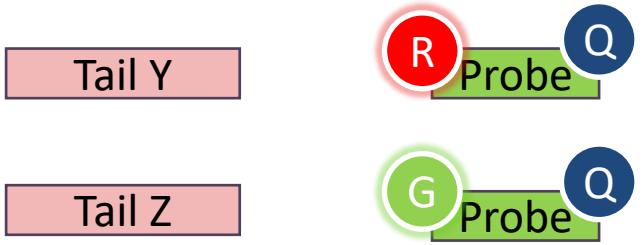
phAmp

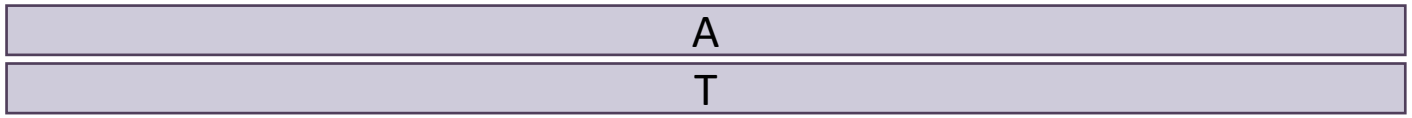
RNase H-dependent PCR

- phAmp Assay Mix



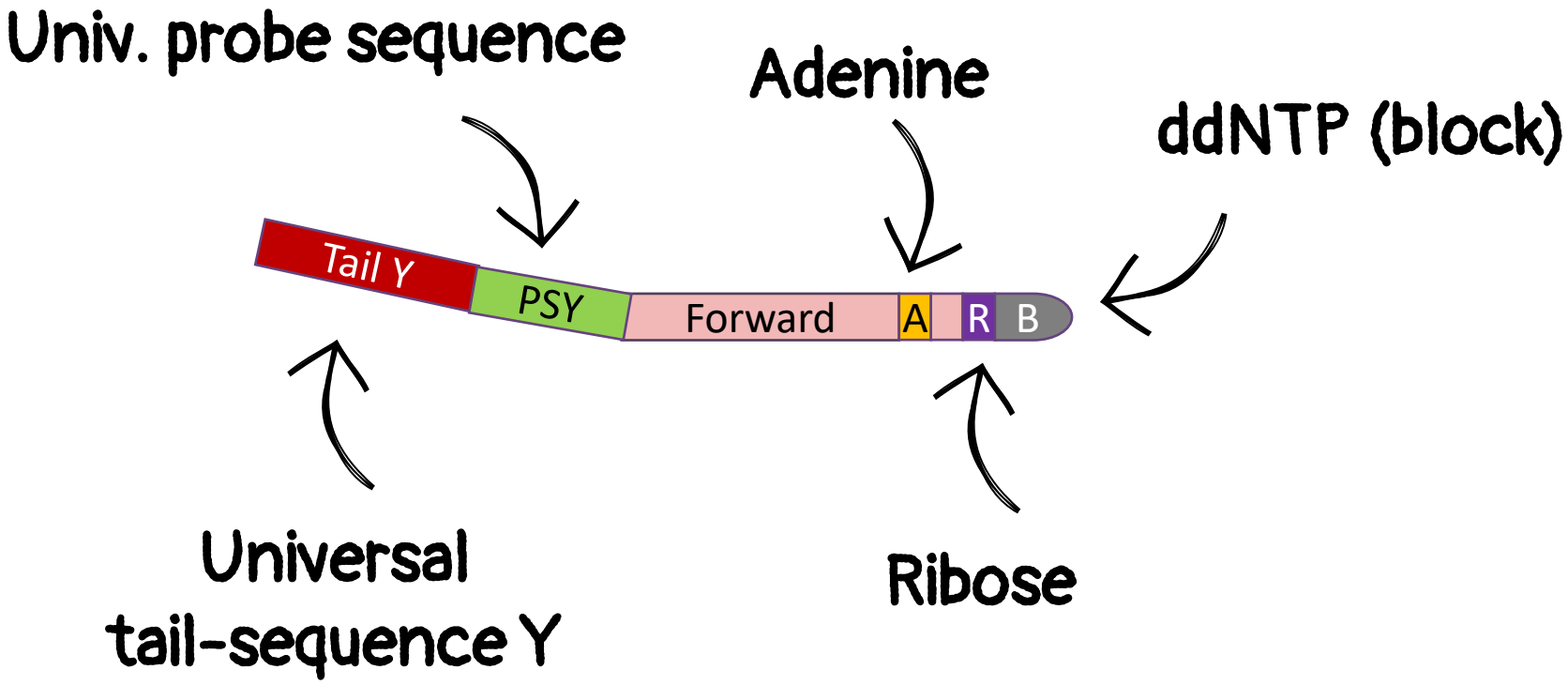
- phAmp Master Mix





phAmp

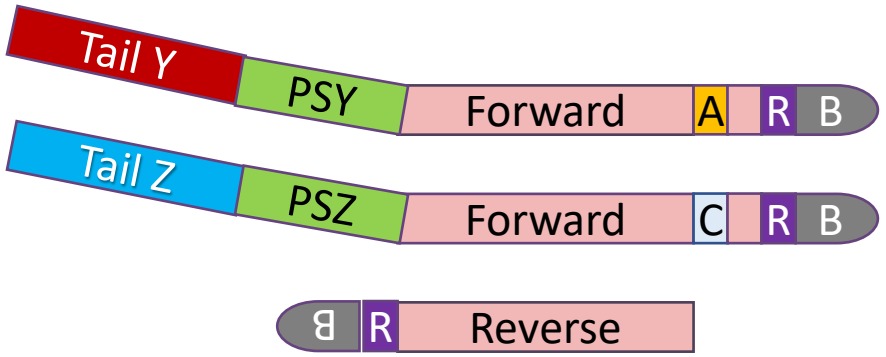
RNase H-dependent PCR



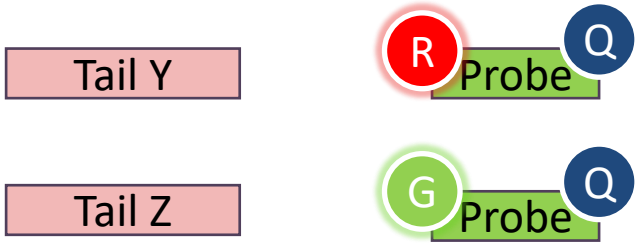
phAmp

RNase H-dependent PCR

- phAmp Assay Mix



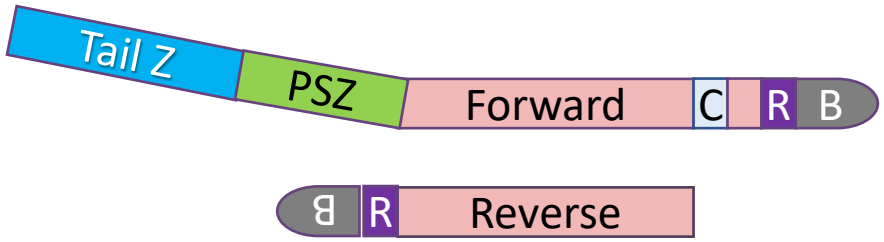
- phAmp Master Mix



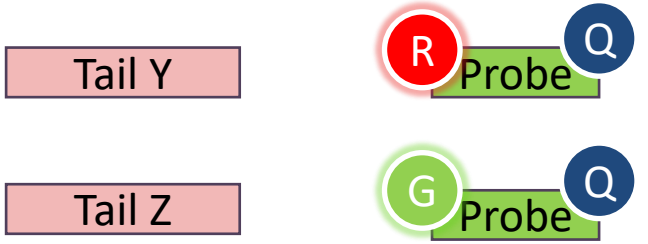
phAmp

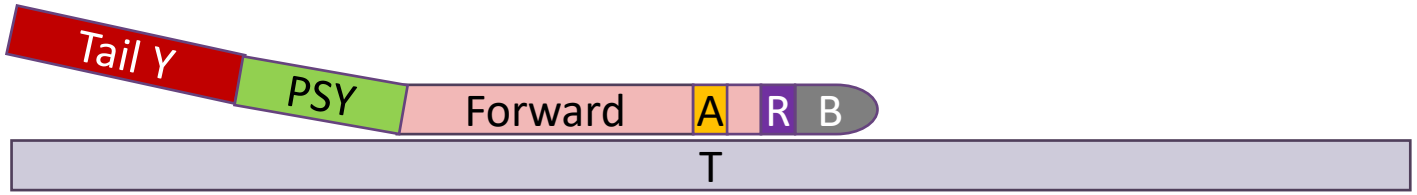
RNase H-dependent PCR

- phAmp Assay Mix



- phAmp Master Mix

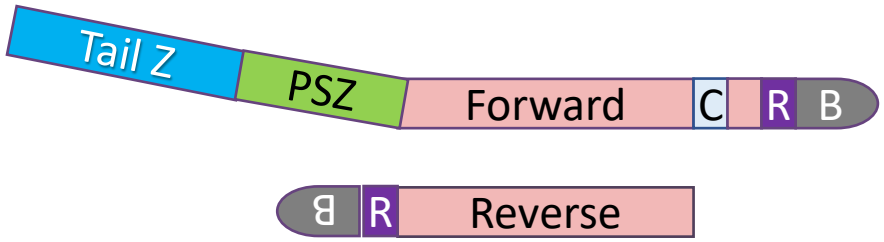




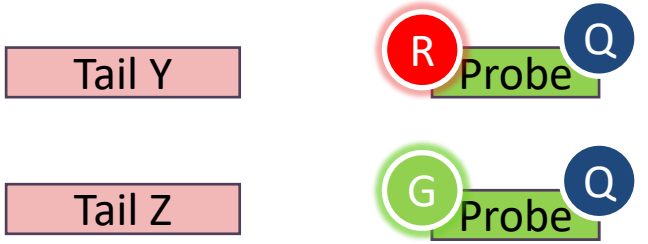
phAmp

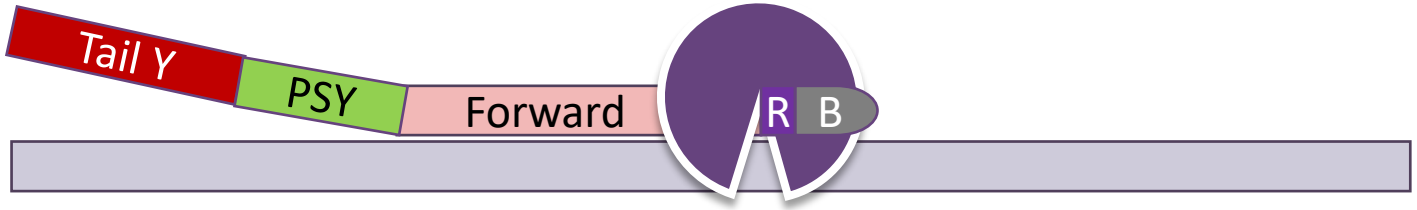
RNase H-dependent PCR

- phAmp Assay Mix



- phAmp Master Mix

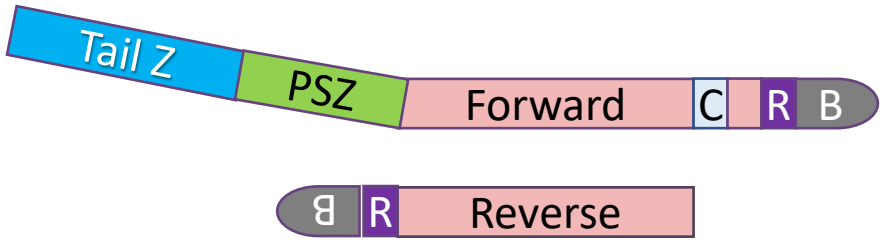




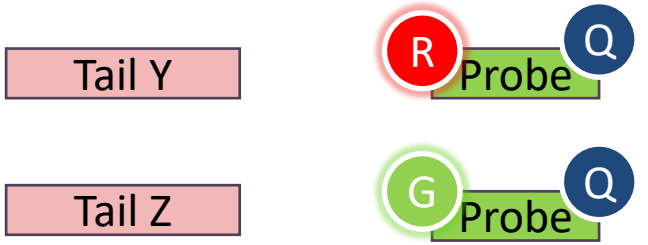
phAmp

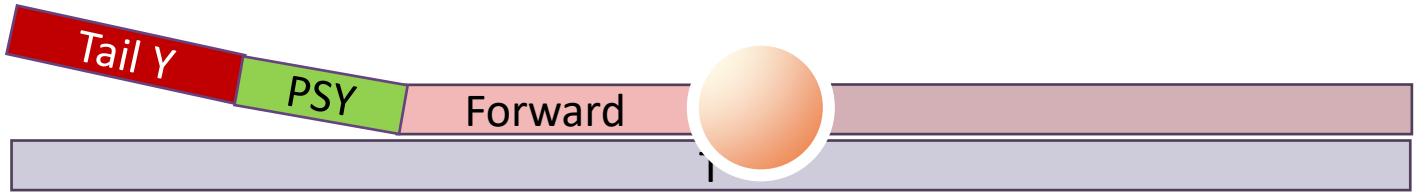
RNase H-dependent PCR

- phAmp Assay Mix



- phAmp Master Mix

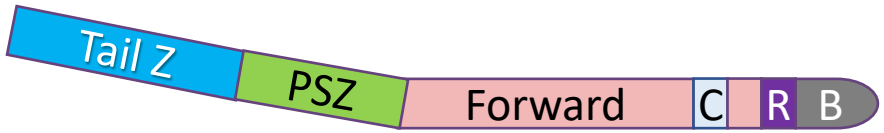




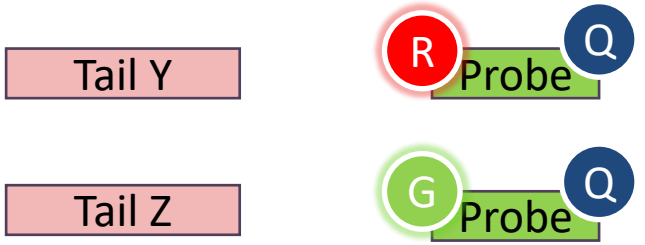
phAmp

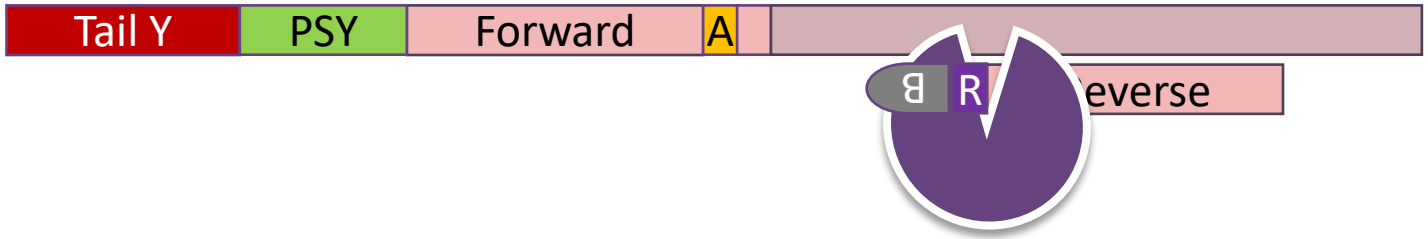
RNase H-dependent PCR

- phAmp Assay Mix



- phAmp Master Mix

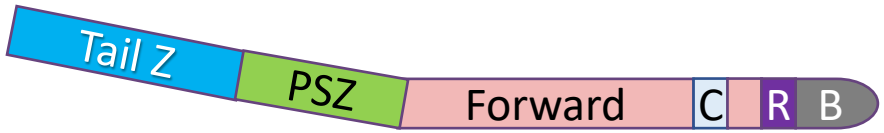




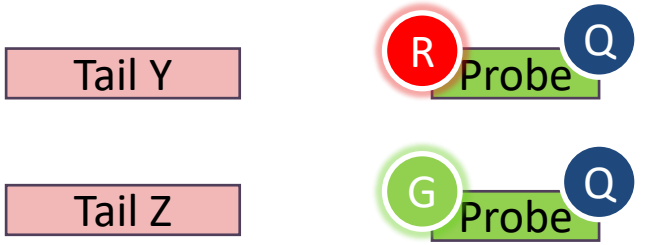
phAmp

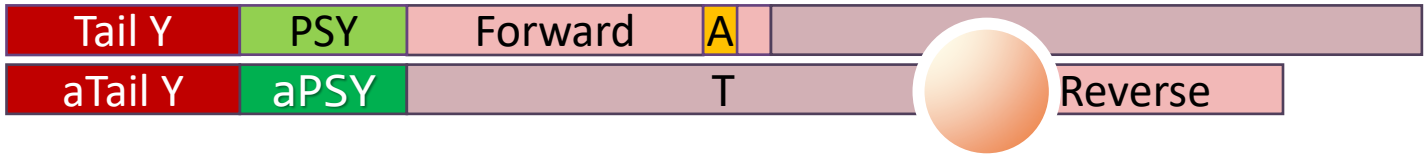
RNase H-dependent PCR

- phAmp Assay Mix



- phAmp Master Mix

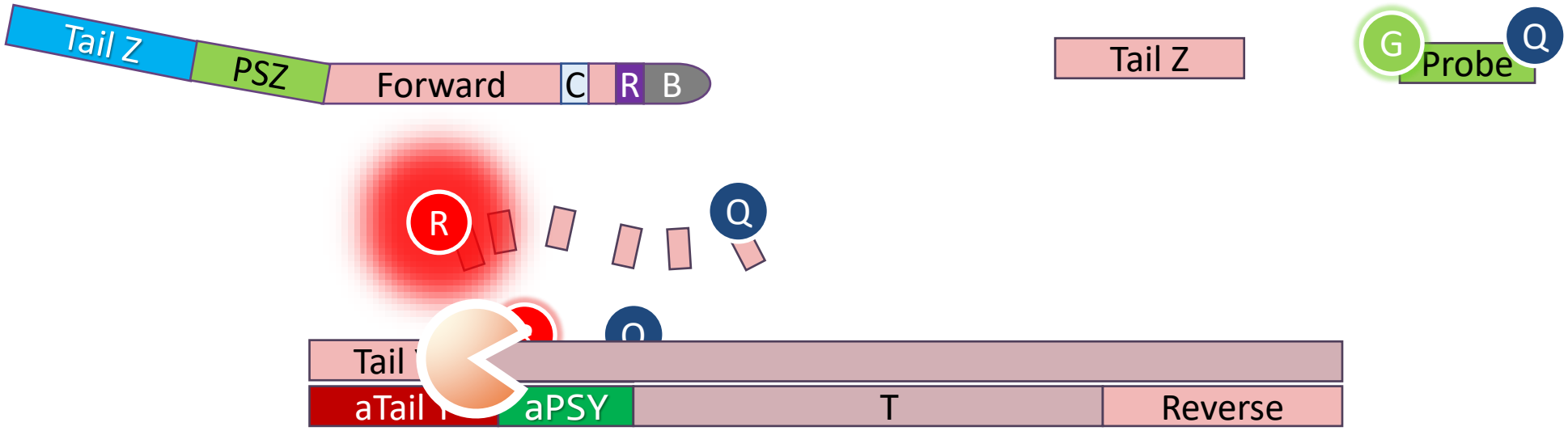




phAmp

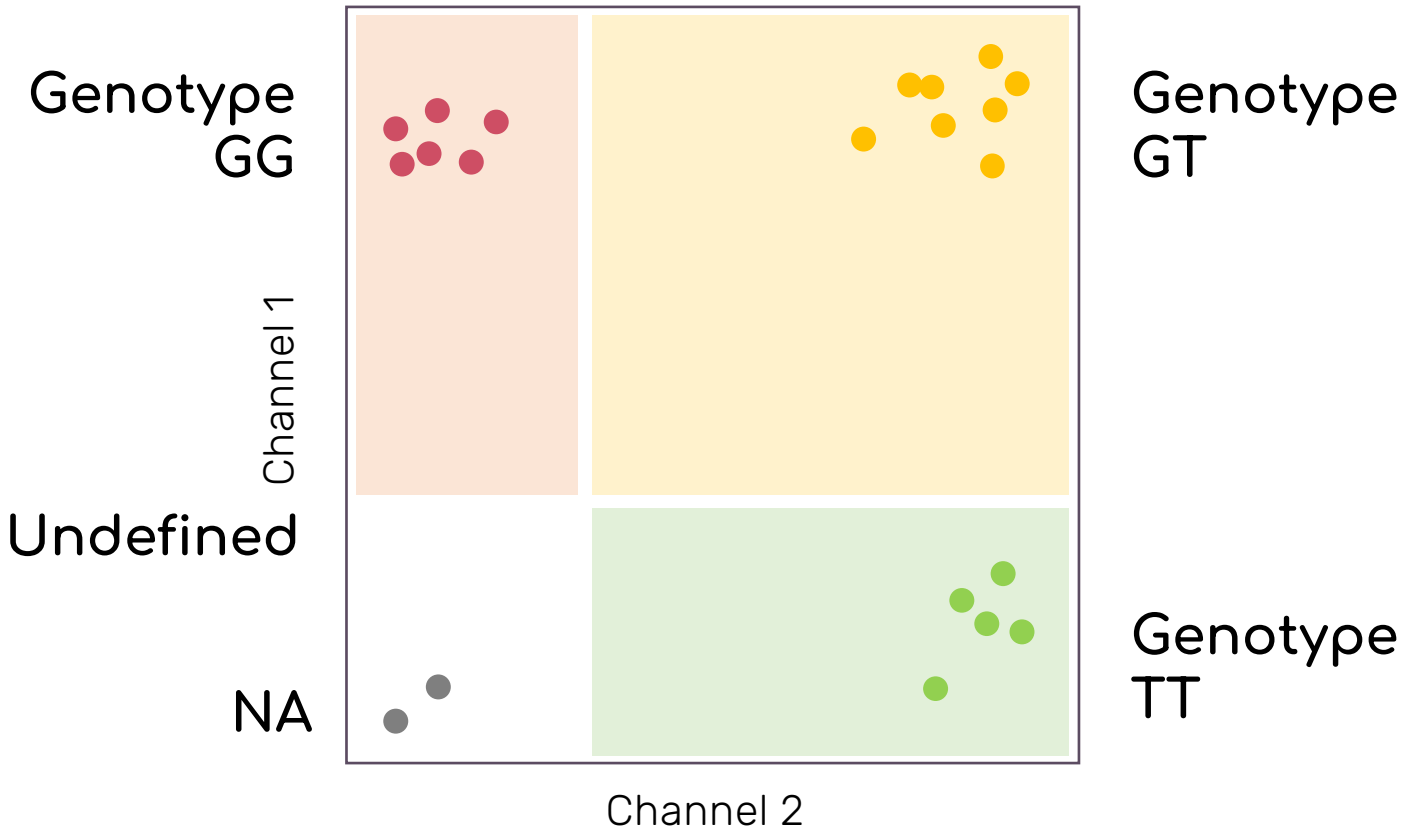
RNase H-dependent PCR

- phAmp Assay Mix
- phAmp Master Mix

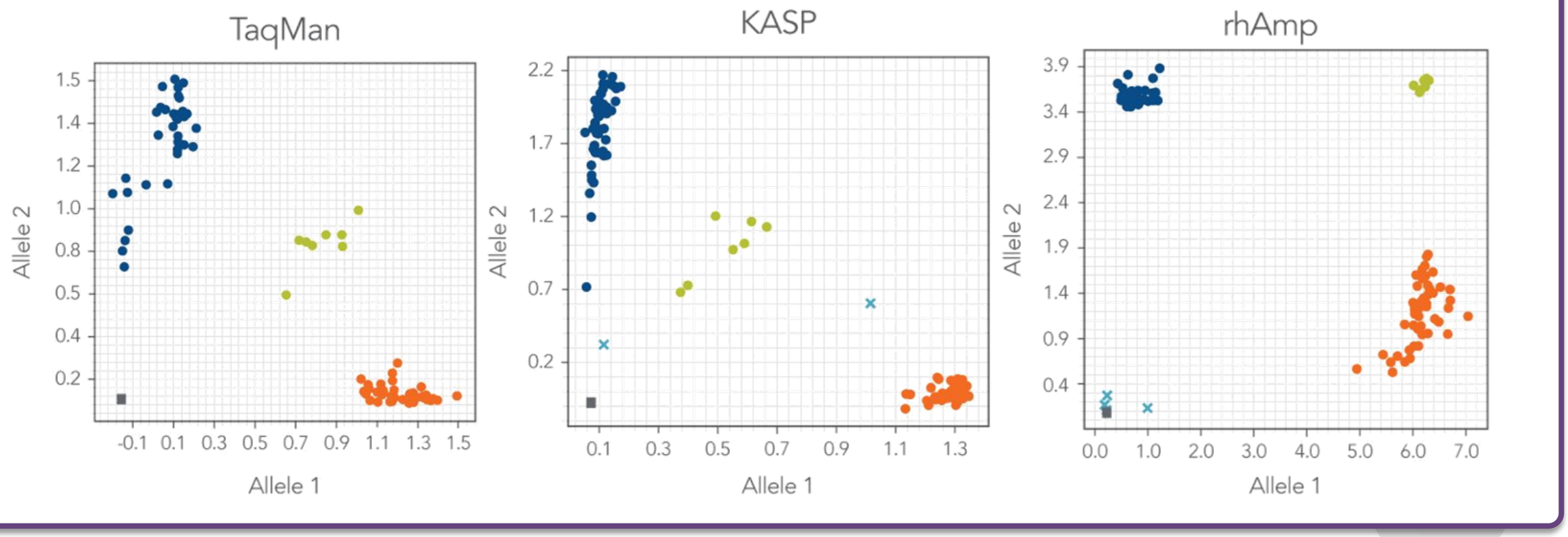


phAmp

RNase H-dependent PCR

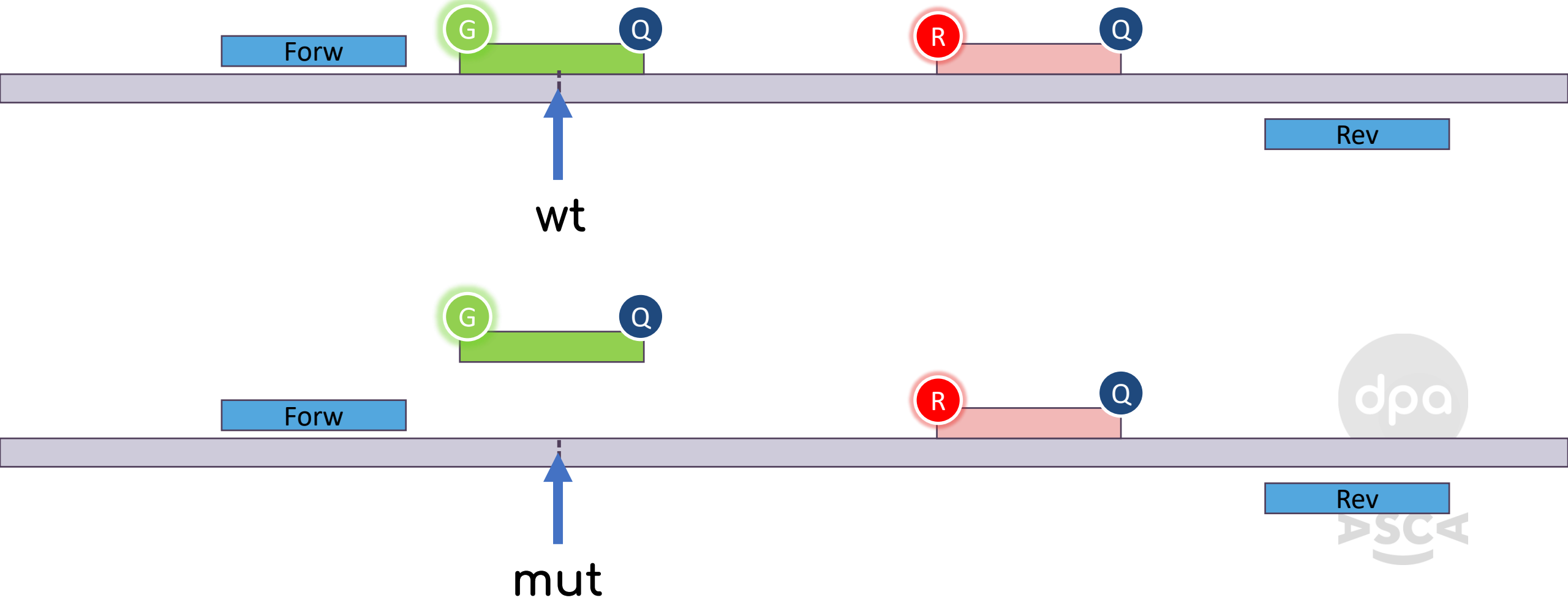


Comparison

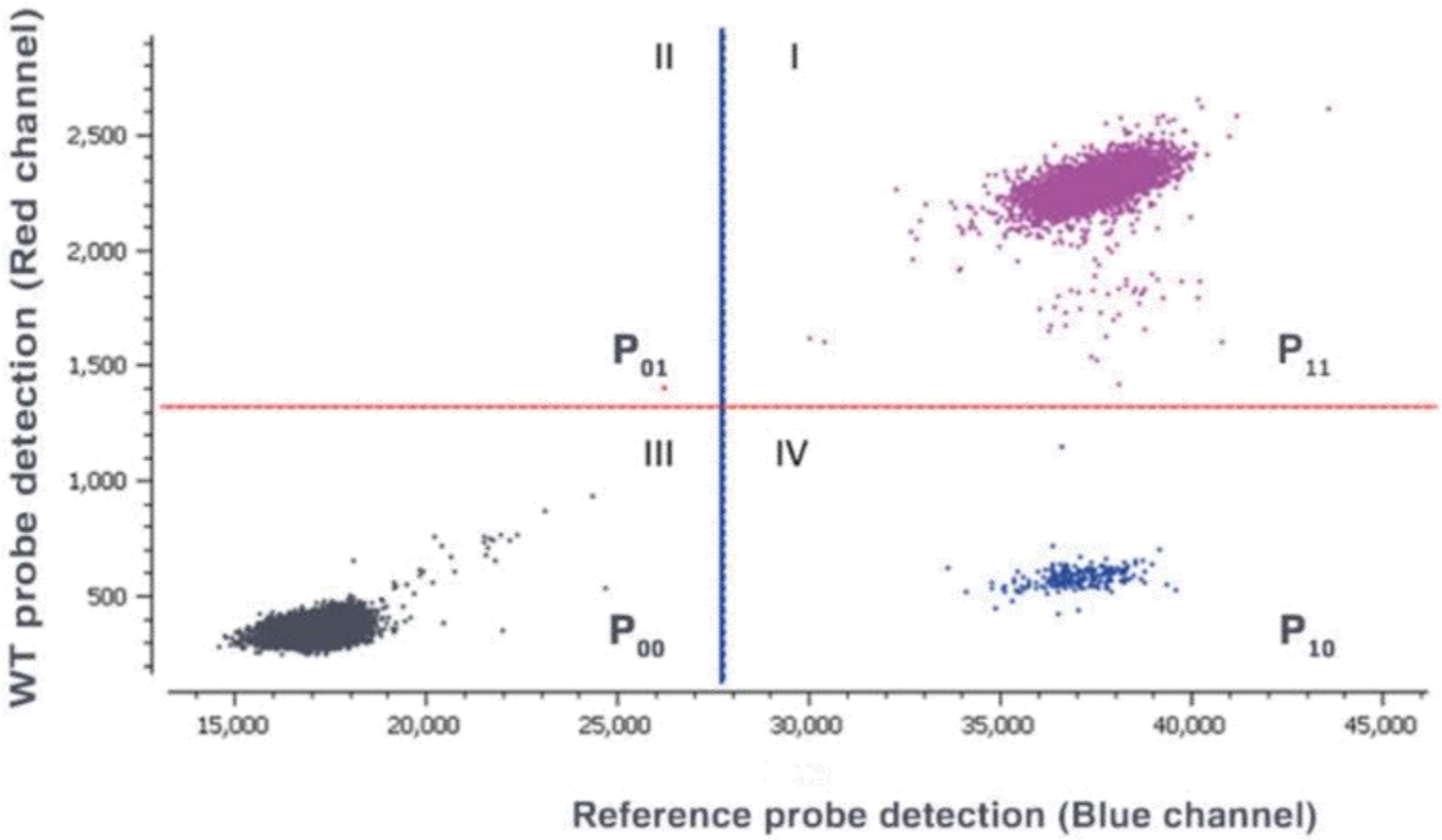

TaqMan
Allele 2
Allele 1
1.5
1.4
1.2
1.0
0.8
0.5
0.4
0.2
-0.1 0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5
KASP
Allele 2
Allele 1
2.2
1.7
1.2
0.7
0.2
0.1 0.3 0.5 0.7 0.9 1.1 1.3
rhAmp
Allele 2
Allele 1
3.9
3.4
2.9
2.4
1.9
1.4
0.9
0.4
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0

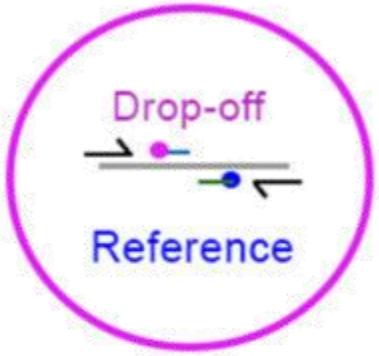

Напоследок

Drop-Off Assays

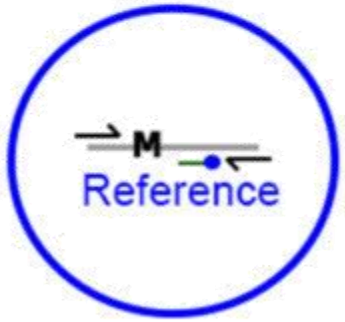


Drop-Off Assays



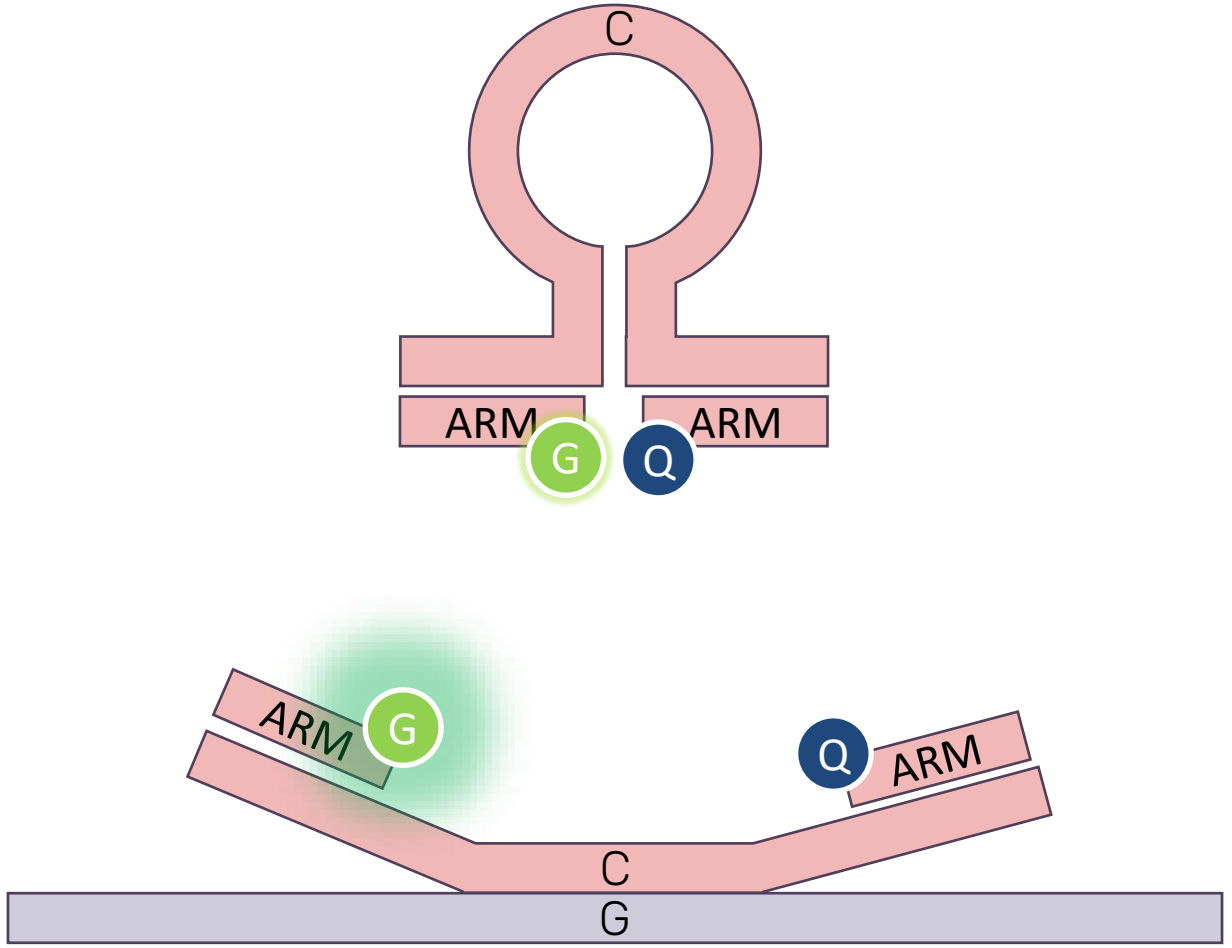


Wild-type allele: Double positive droplets

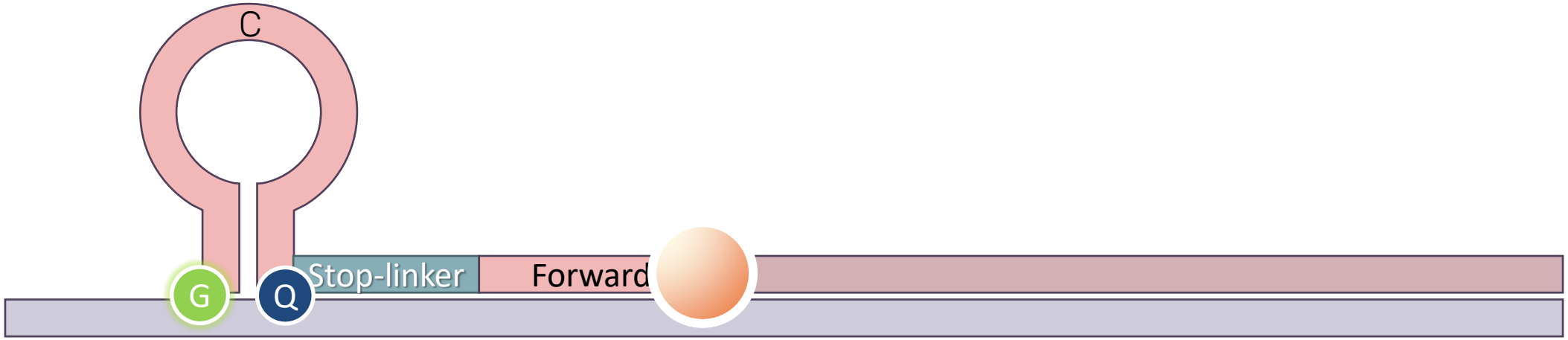


Mutant allele: Simple positive droplets

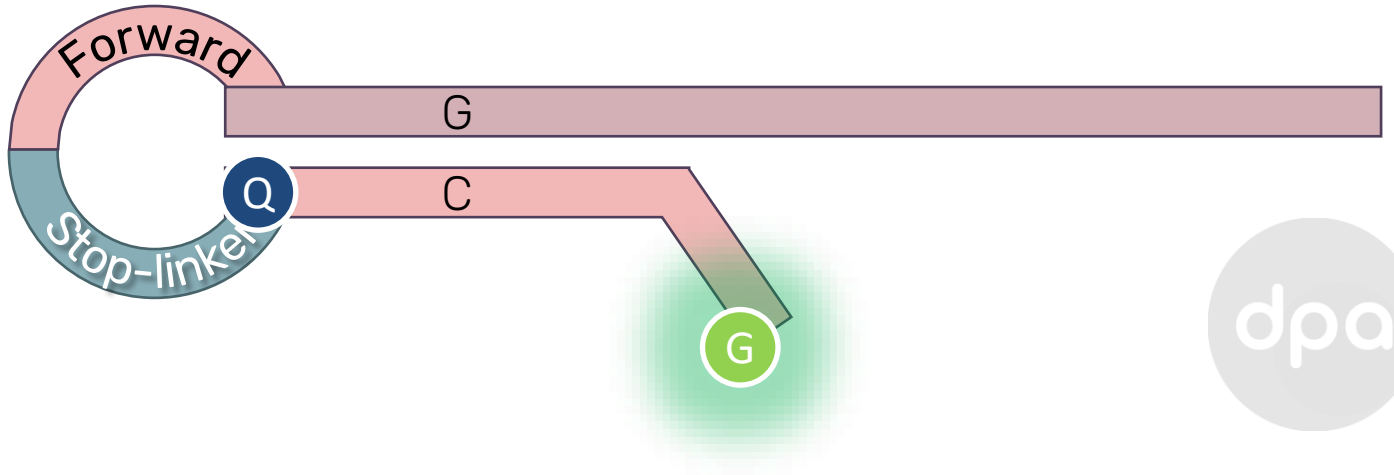
Tripartite molecular beacons



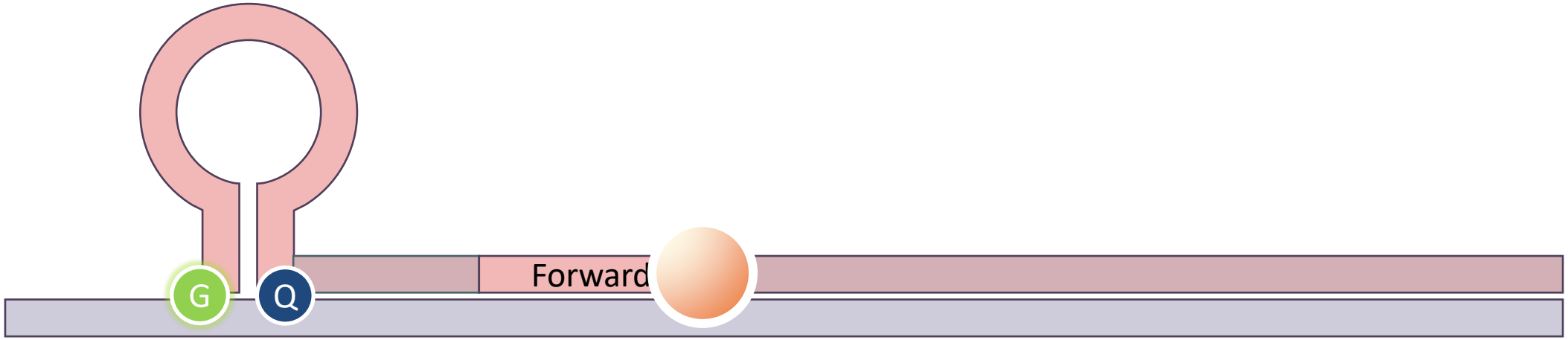
(Beacon-like) Scorpion probe



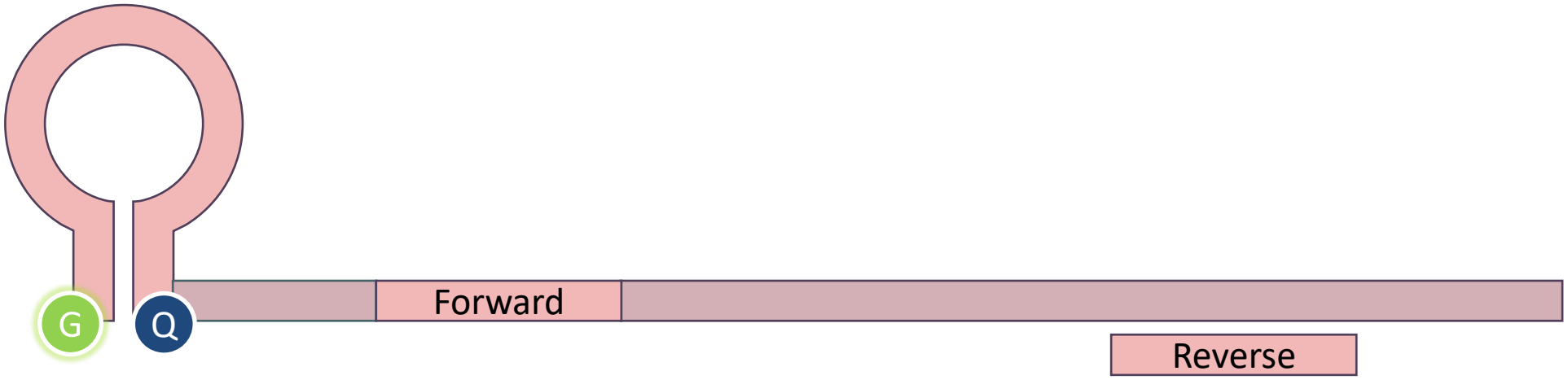
(Beacon-like) Scorpion probe



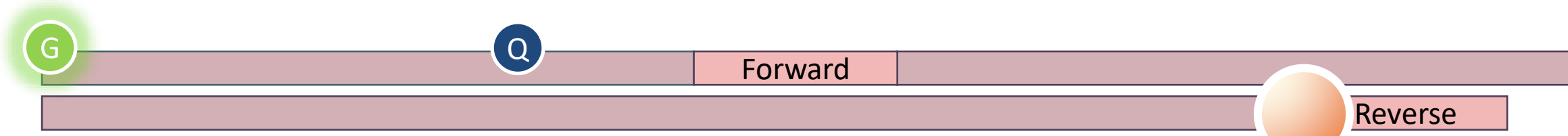
(Beacon-like) Amplifluor assay



(Beacon-like) Amplifluor assay

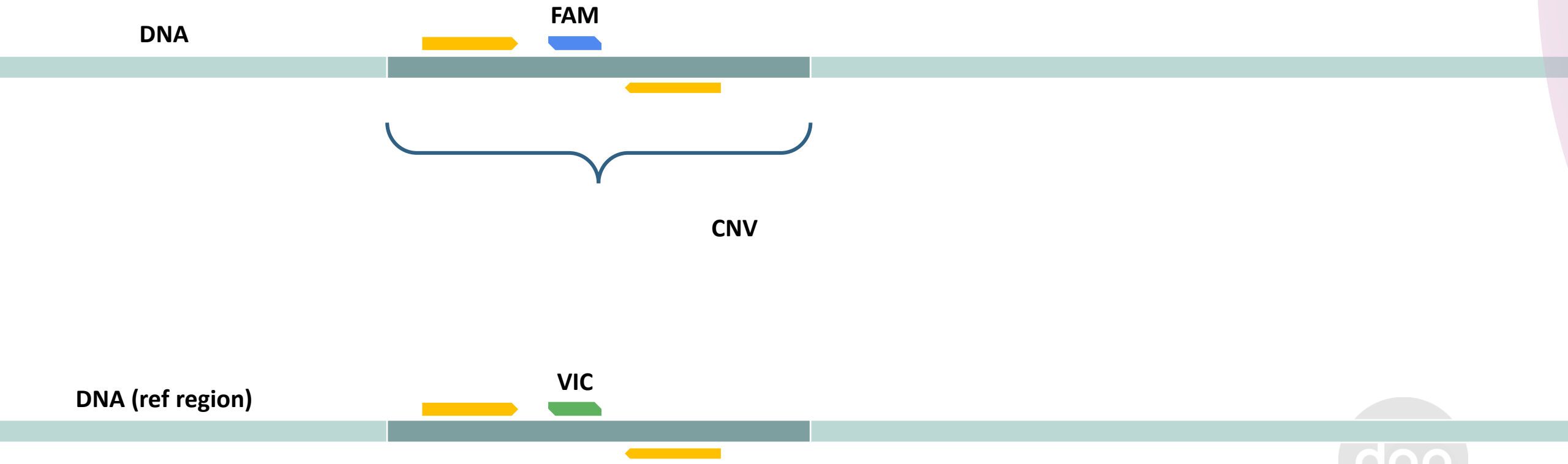


(Beacon-like) Amplifluor assay



Copy number variation

Copy number variation



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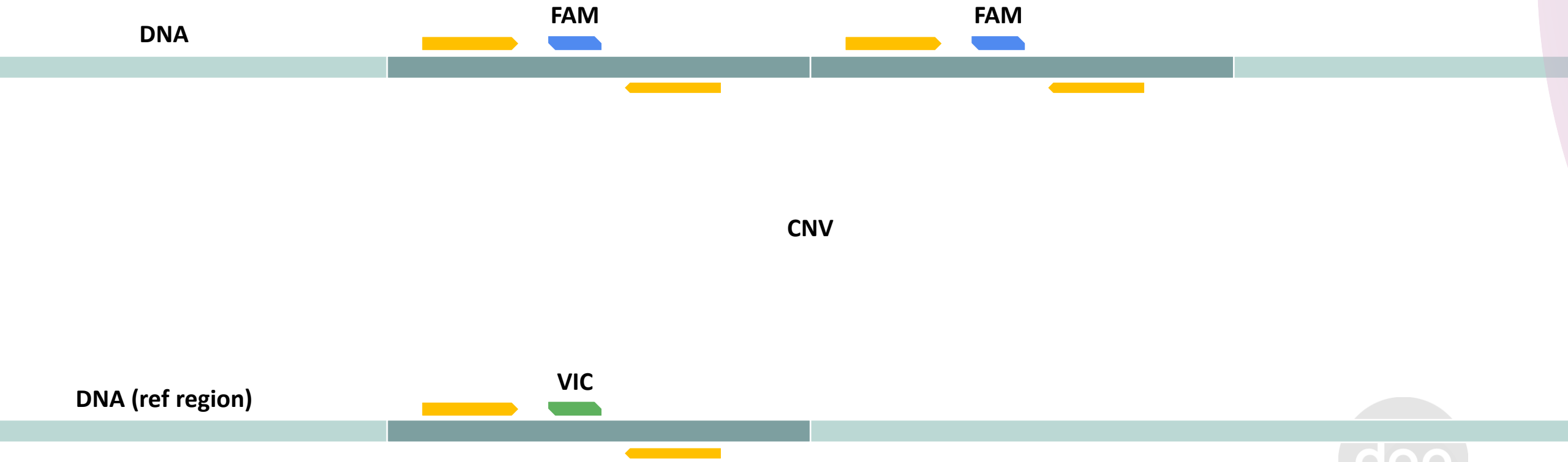

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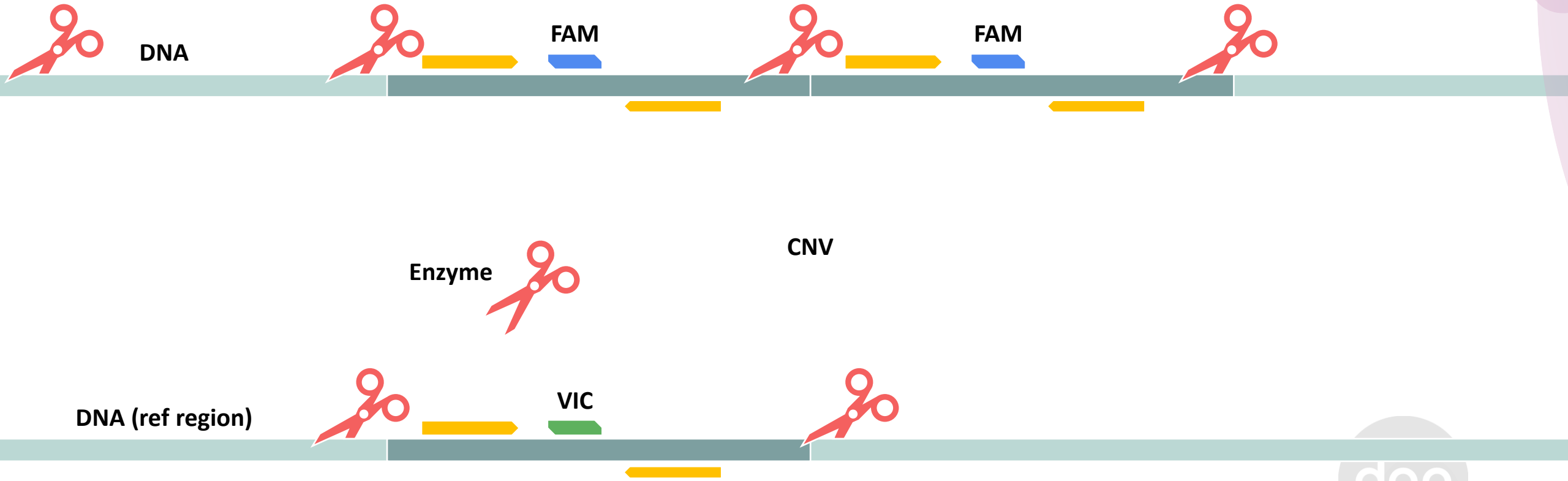

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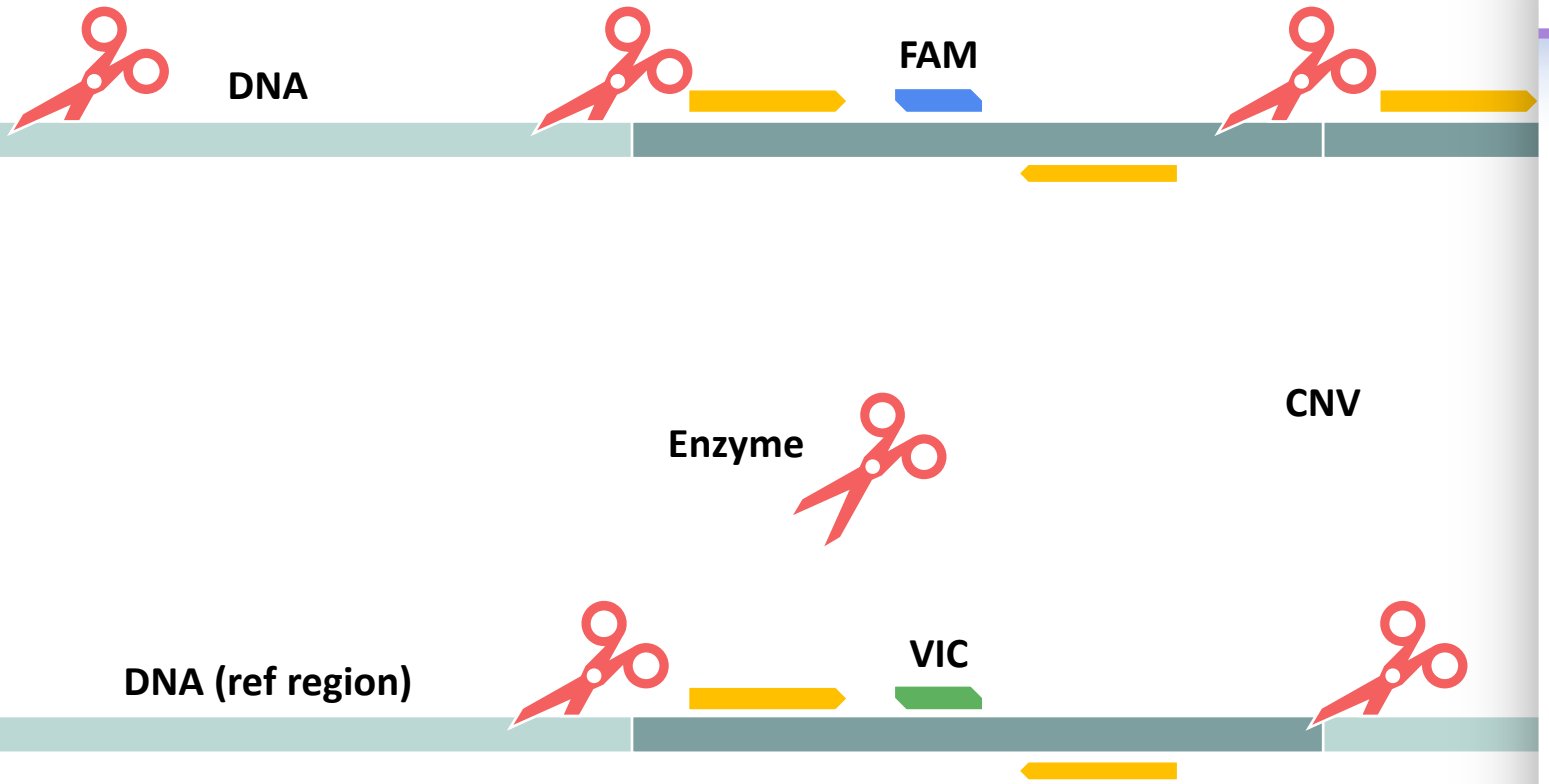
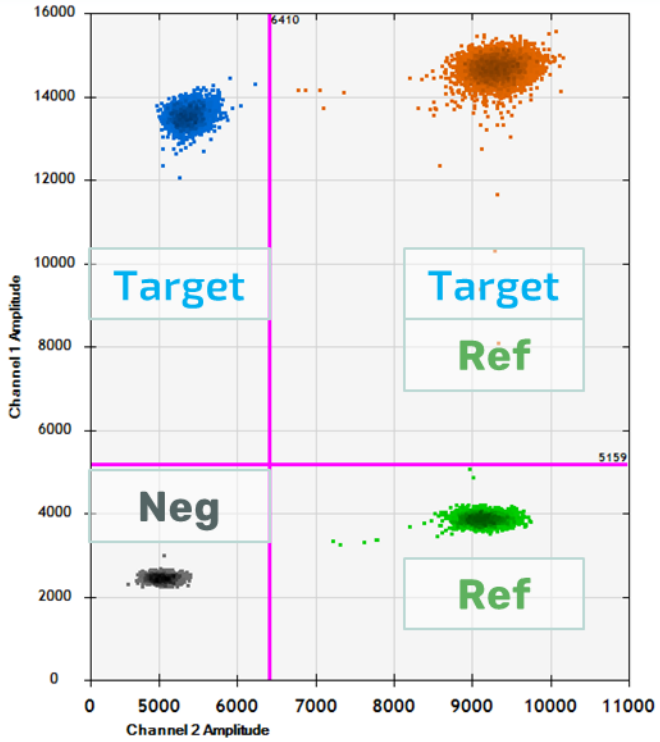
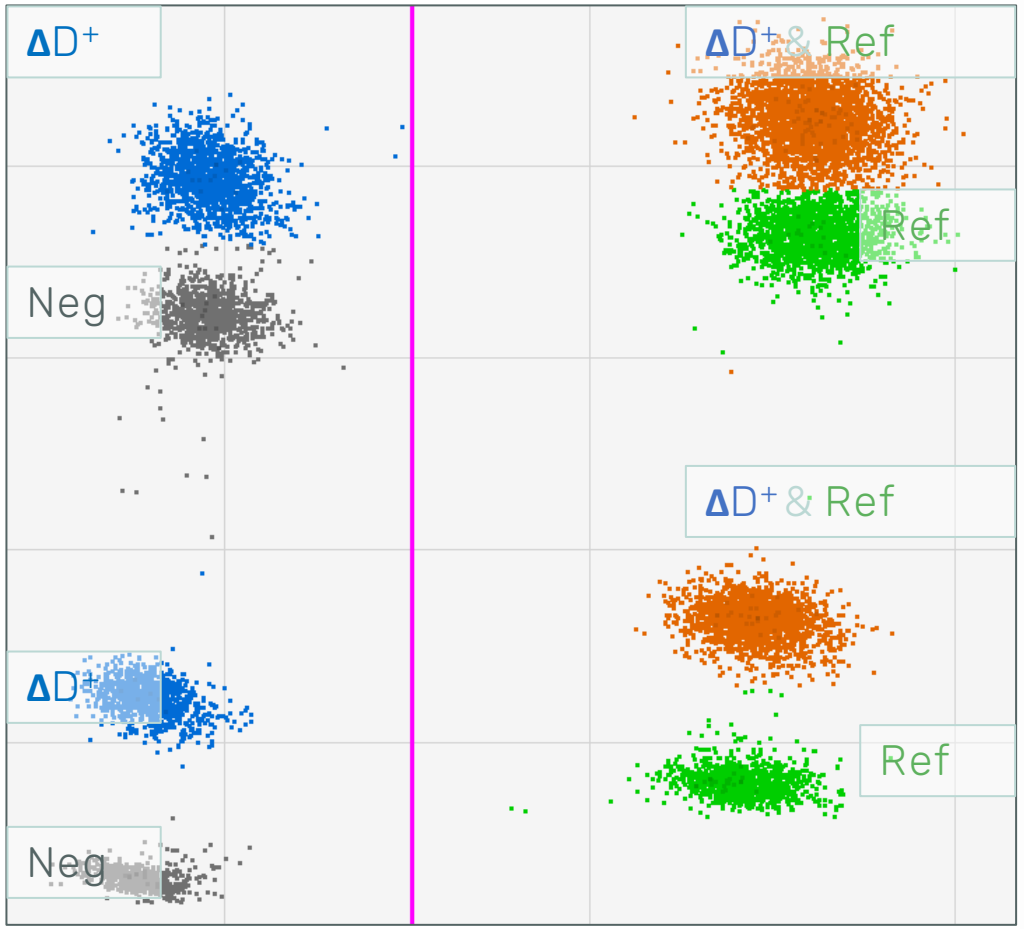


2D ddPCR

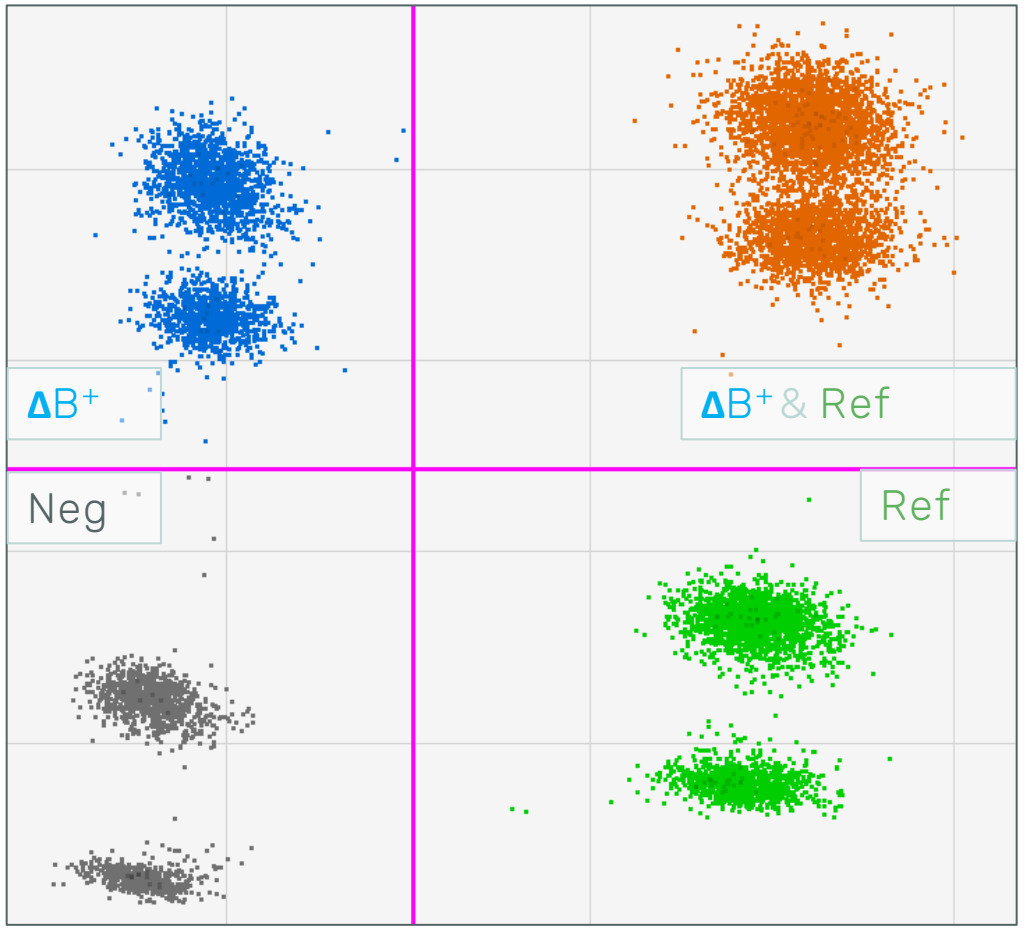


ОЦЕНКА Т-КЛЕТОЧНОГО СОСТАВА В ОБРАЗЦАХ ДНК

Δ Dδ α цепи

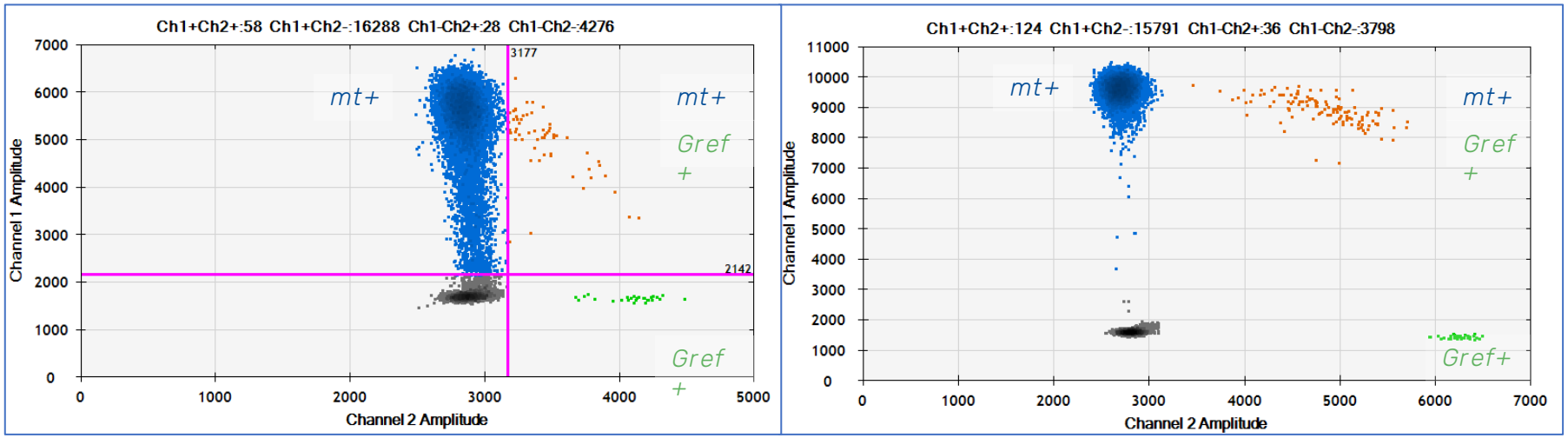


Δ Jβ 1.1 β цепи



ТЕХНОЛОГИЯ ОЦЕНКИ КОЛИЧЕСТВА МТДНК НА КЛЕТКУ

Градиент по времени элонгации

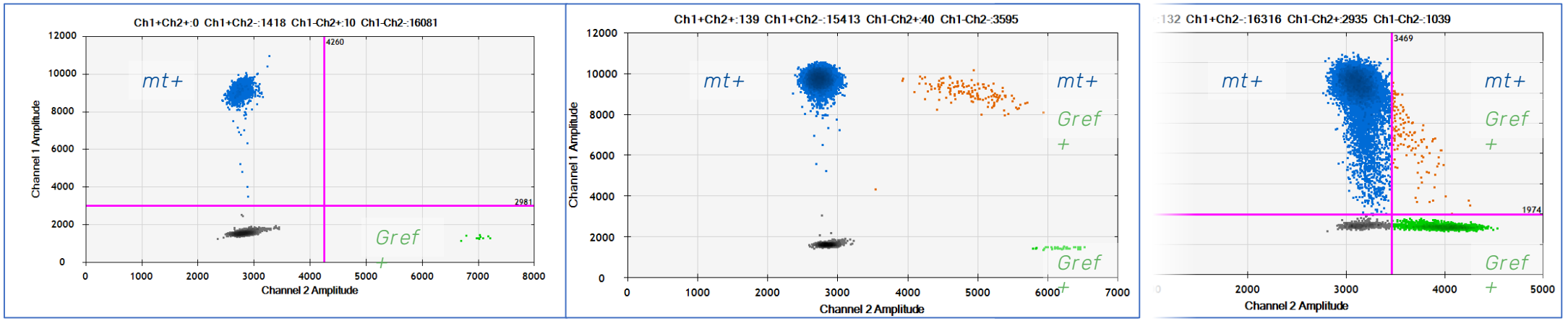


30 сек

Слепцов А.А. и соавт. (2021, Патент № W21020487)

ТЕХНОЛОГИЯ ОЦЕНКИ КОЛИЧЕСТВА МТДНК НА КЛЕТКУ

Градиент по концентрации



0,1 нг — 5 нг

Слепцов А.А. и соавт. (2021, Патент № W21020487)

Association of Single Cell Analysis

Digital PCR Association

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