GMO

29.09.2011

1	USA	66.8	maize, soybean, cotton >70%, canola
2	Brazil	25.4	soybean >75% , maize 56%, cotton 26%
3	Argentina	22.9	soybean, maize, cotton
4	India	9.4	cotton 90%
5	Canada	8.8	canola, maize, soybean, sugarbeet
6	China	3.5	cotton 68%, papaya 99%, poplar, tomato, pepper
7	Paraguay	2.6	soybean
8	Pakistan	2.4	cotton 75%
9	South Africa	2.2	maize, soybean, cotton
10	Urugay	1.1	soybean, maize
11	Bolivia	0.9	soybean
12	Australia	0.7	cotton, canola
13	Phillipines	0.5	maize
14	Myanmar	0.3	cotton
15	Burkina Faso	0.3	cotton 65%
29	Germany	< 0.1	potato

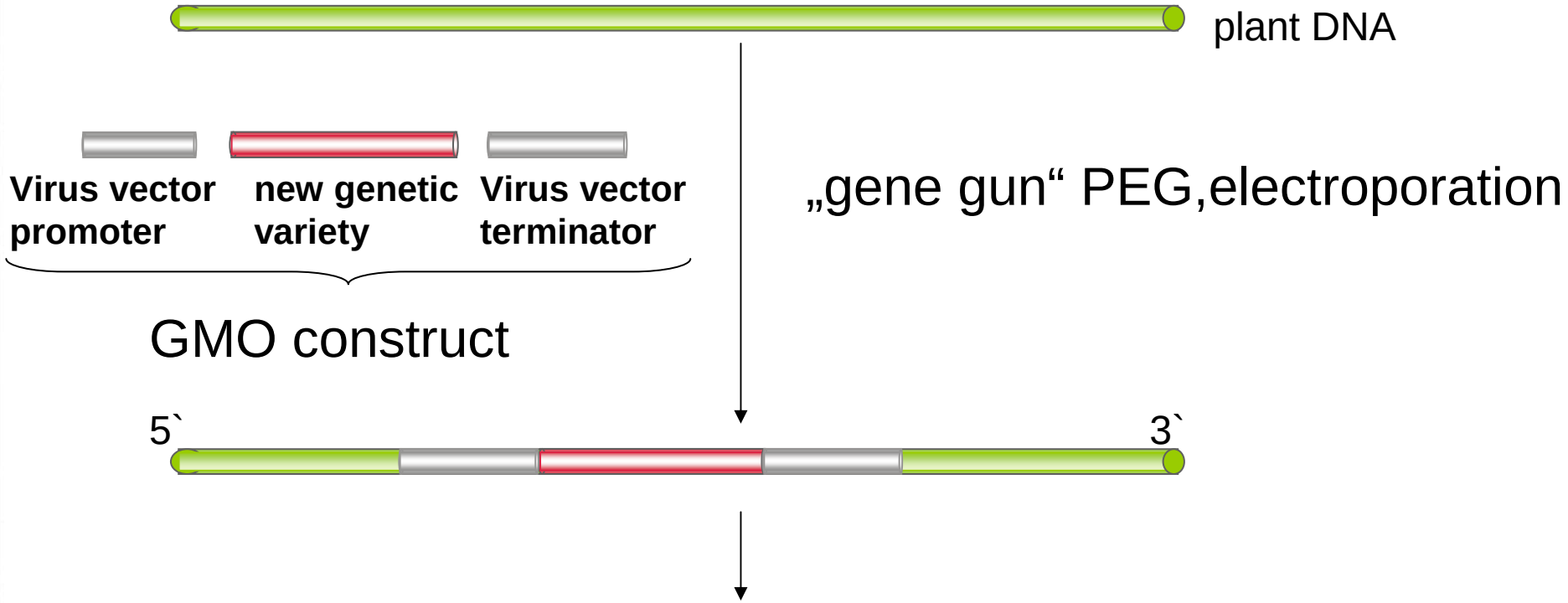
milion hectares

GMO

GMO`s genetically modified organisms are:

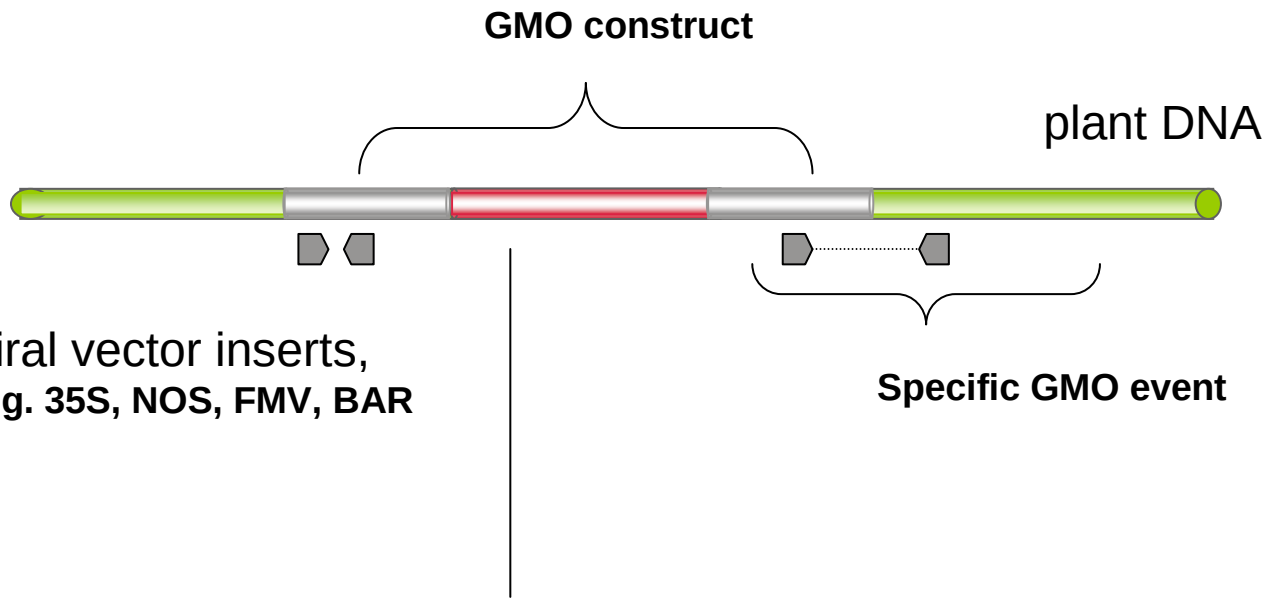
- in practice only **transgenic plants**
- mostly **herbizide, insectizide** resistant stacks (drought resistance, functional food)
- Glyophosphate → Roundup (Monsanto) roundup ready, Bt (bacillus thurengensis) GMO (Monsanto)
- An **economic** issue (patent holders versus farmers)
- An **ecological** issue (low variety, monocultures)
- **No direct health issue** (EFSA and others studies)

GMO production and analytic:



Identification, selection, cultivation: GMO event

GMO


GMO construct
plant DNA
viral vector inserts,
e.g. 35S, NOS, FMV, BAR
Specific GMO event
GMO specific trait (e.g. Bt =bacillus thurengiensis protein)

Food:

EC 1829/2003 /EC 1830/2003 (0.9% labeling rule)

- Any GMO? 35S, NOS, FMV, BAR... **screening**
- Is the GMO approved ? **qualtitative**
- Which quantitiy? **quantitative**

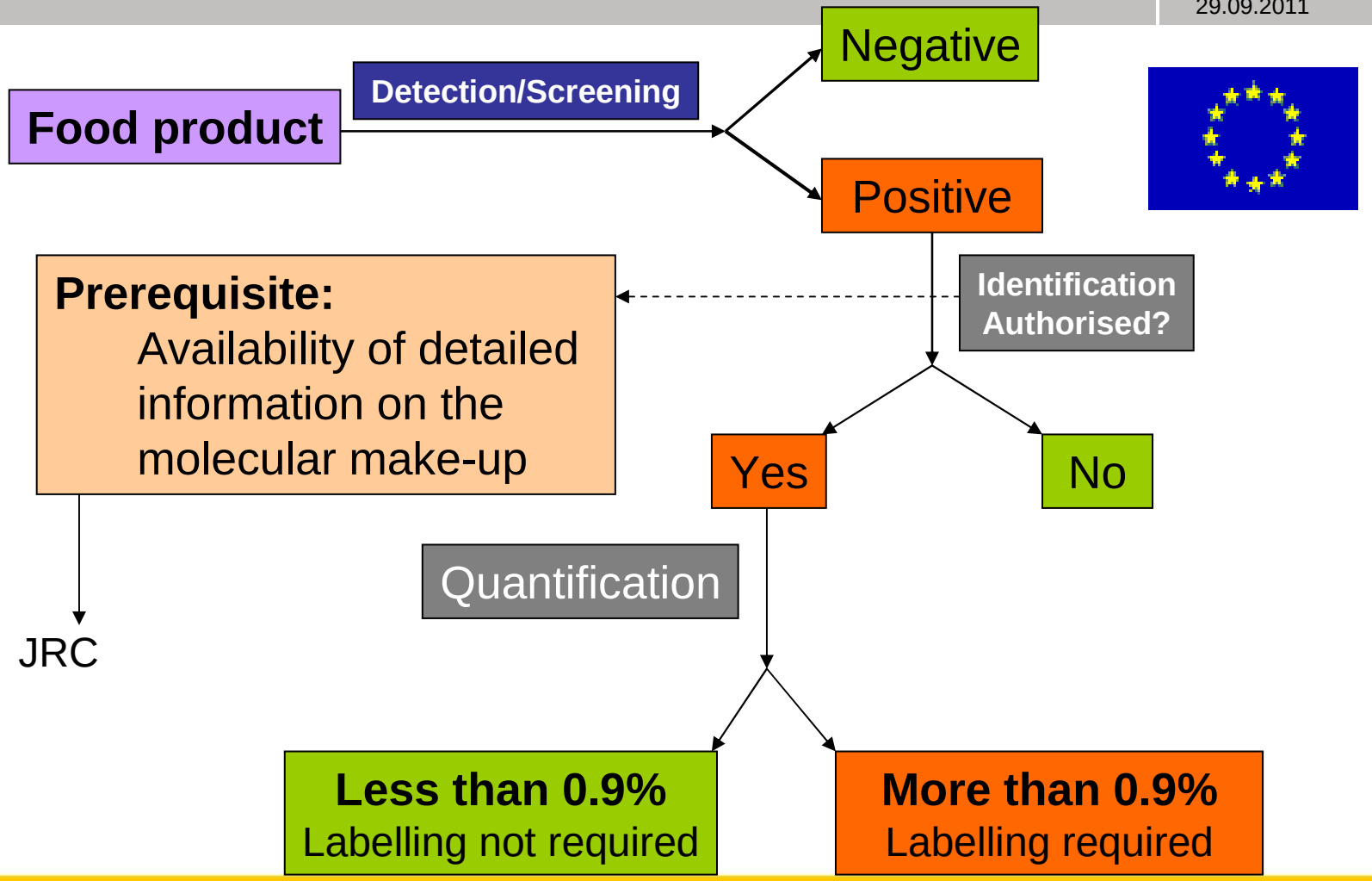
Feed:

EC 619/2011

- Any locally approved GMO > 0.1 %

GMO

29.09.2011



Feed product

all GMO`s may be included up to a technical treshold of 0.1 %

- new EU regulation 619/2011 in force from 15th July 2011

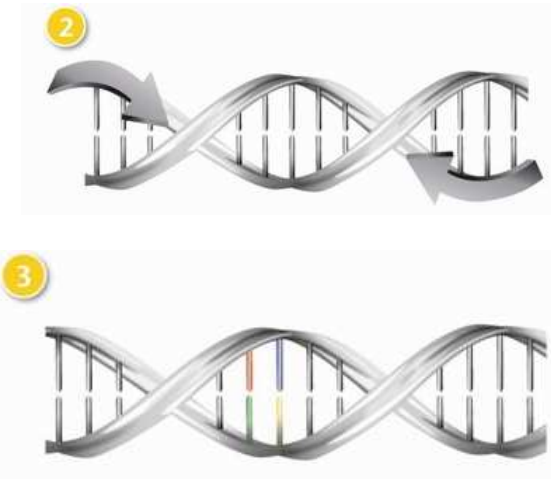
criteria:

- be authorised for commercialisation in a non-EU country
- have a valid EFSA application under Article 17 of Regulation EC 1829/2003 or have an expired authorisation under Regulation EC 1829/2003
- authorisation pending for more than 3 months
- have not been identified by EFSA as susceptible to have adverse effects on health or the environment when present under 0.1%
- quantitative method of analysis published by the EU reference laboratory
- certified reference material must be available to EU-countries and third parties

PCR

PCR (**P**olymerase **C**hain **R**eaction) is the best method for GMO detection, the principle of replication:

primers
nucleotides
Taq polymerase
buffer, Mg^{2+}



Guanine
Adenine
Cytosine
Thymine

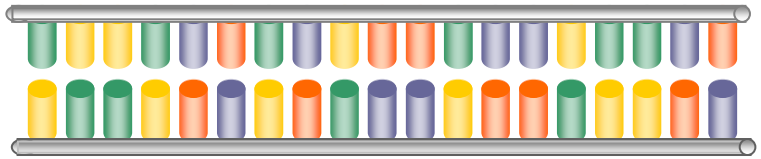
PCR: the principle of replication:

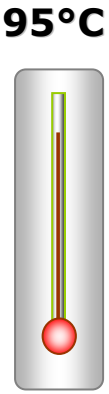
(pure)
sample DNA

primers
nucleotides
Taq polymerase
buffer, Mg^{2+}

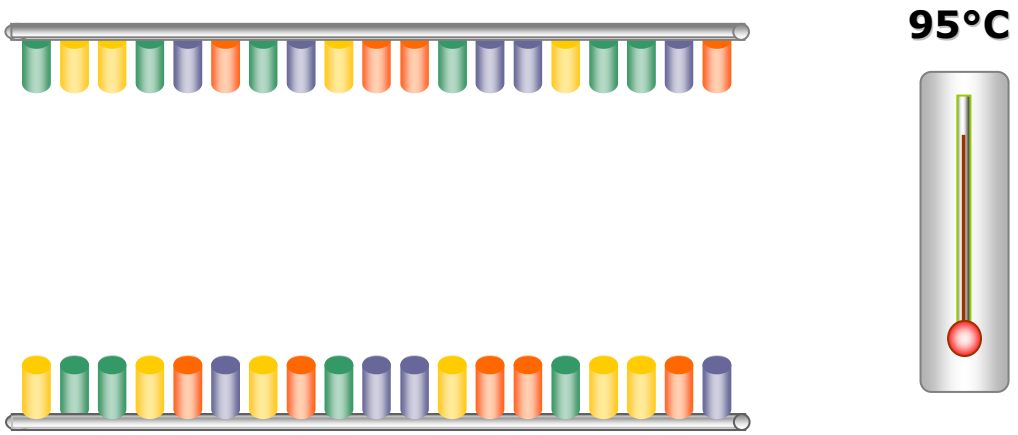
} mastermix

PCR: the principle of replication: denaturation





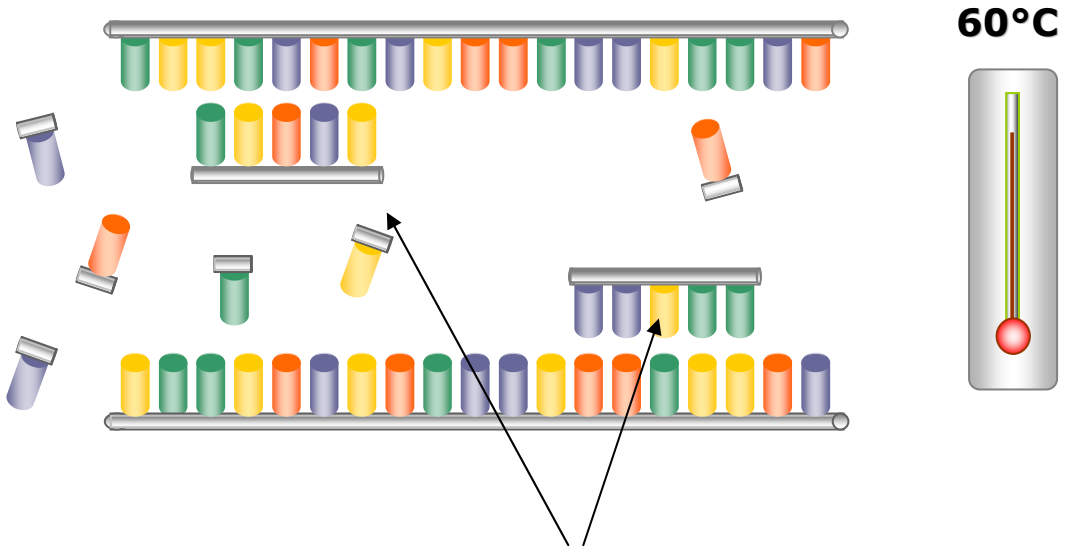
PCR: the principle of replication



PCR: the principle of replication

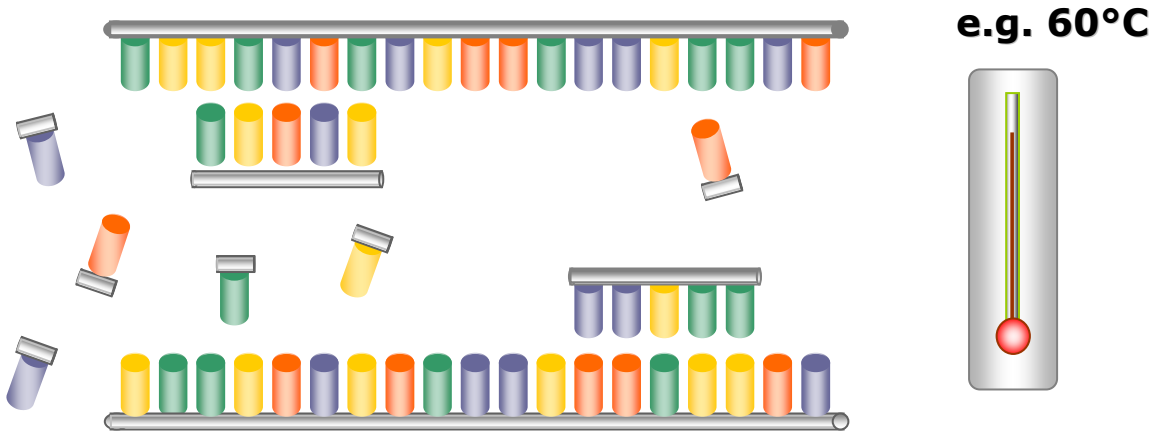
Nucleotides:

Guanine
Adenine
Cytosine
Thymine

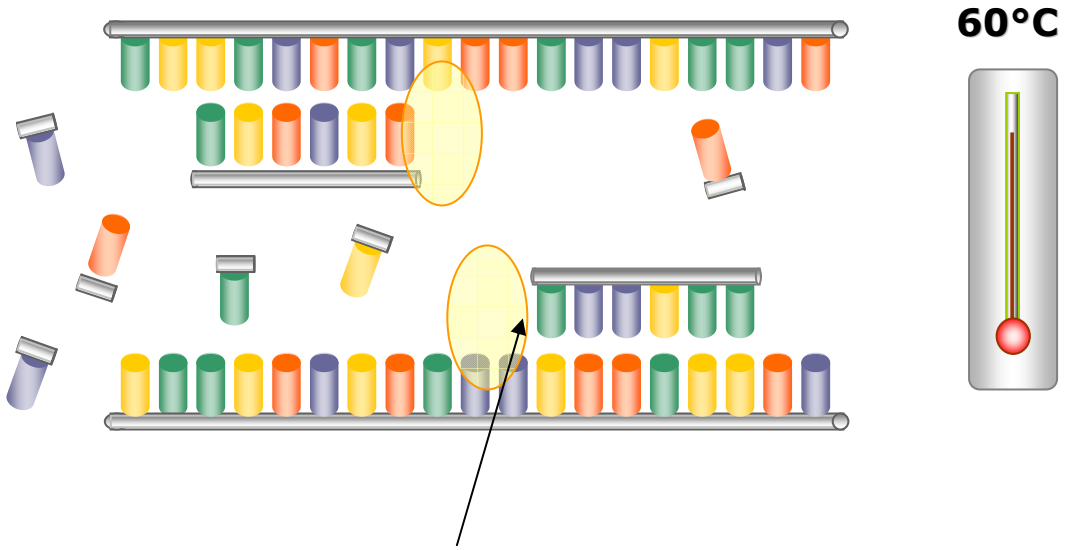


Specific primers: forward and reverse

PCR: the principle of replication: Annealing/Extension

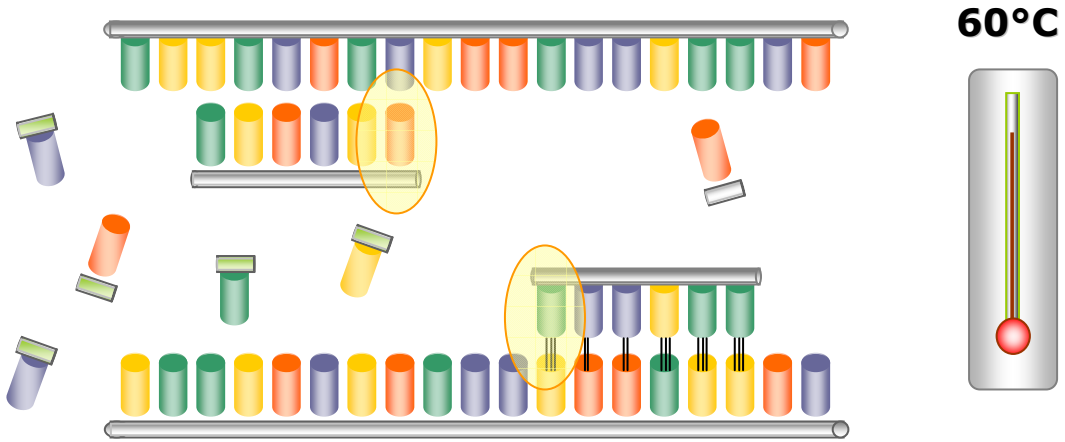


PCR: the principle of replication

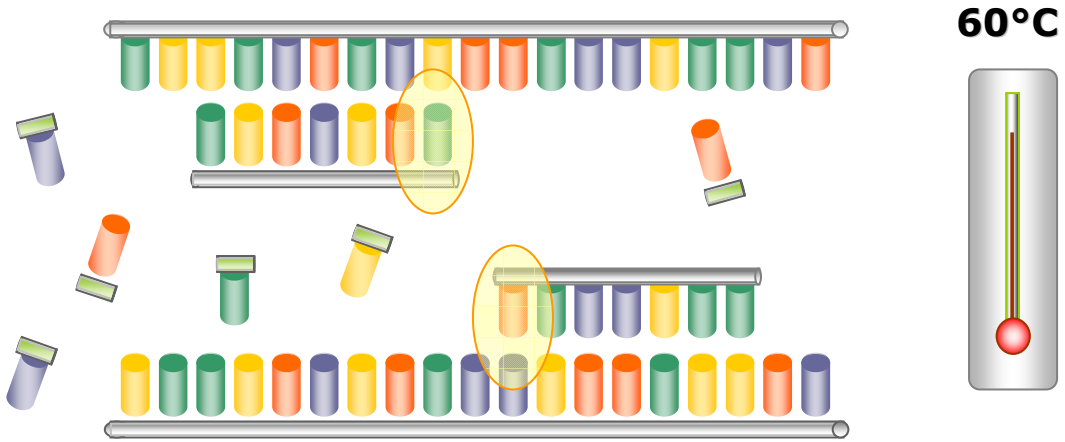


Taq Polymerase: enzyme for replication of DNA

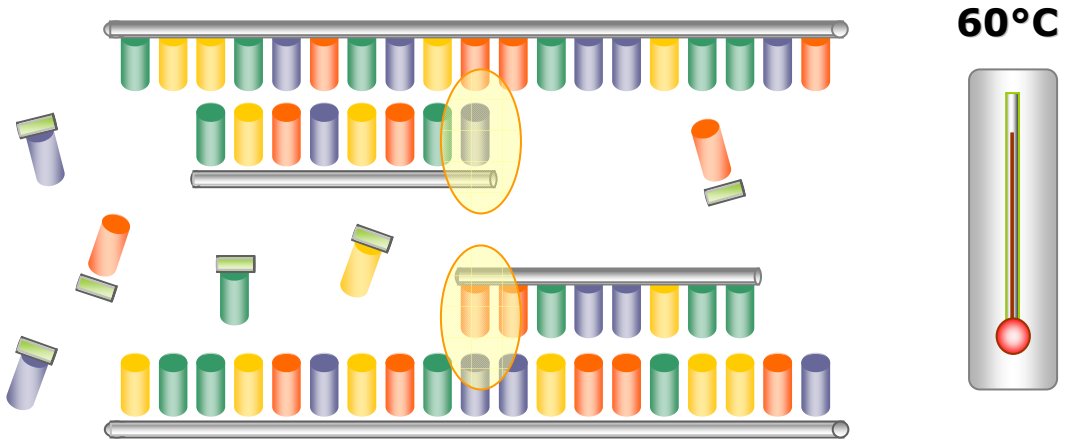
PCR: the principle of replication



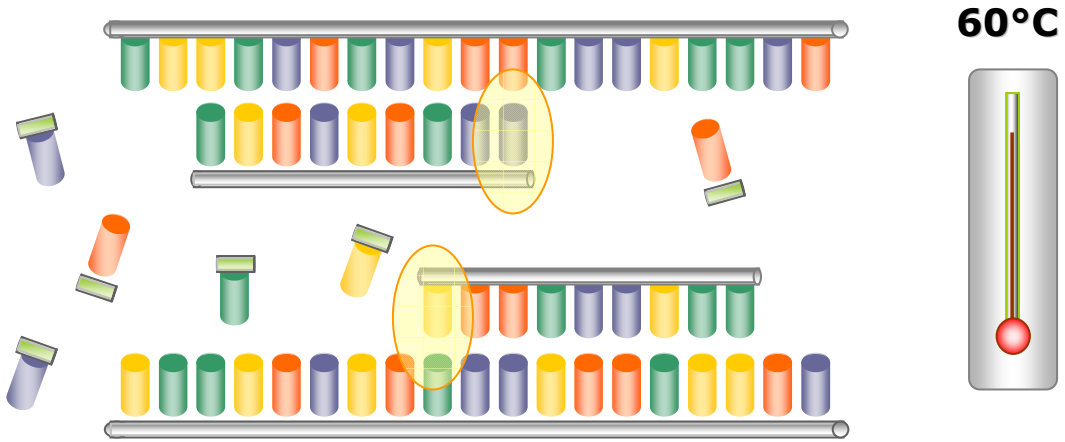
PCR: the principle of replication



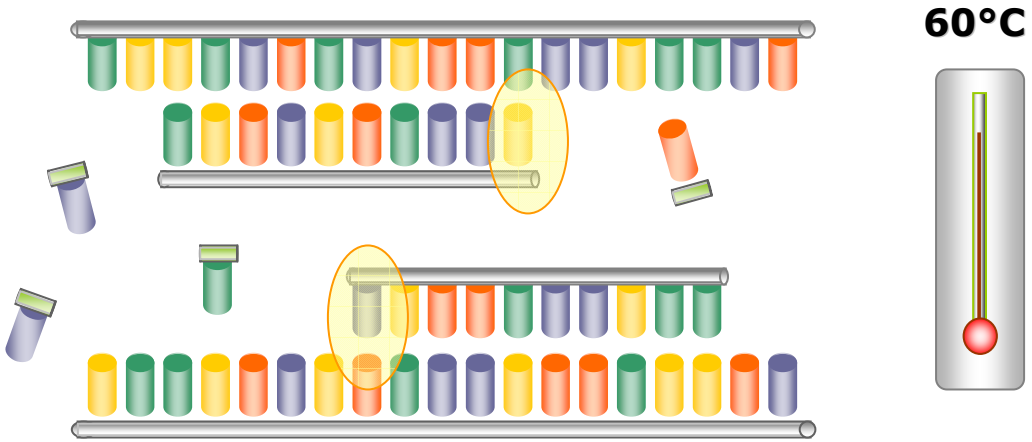
PCR: the principle of replication



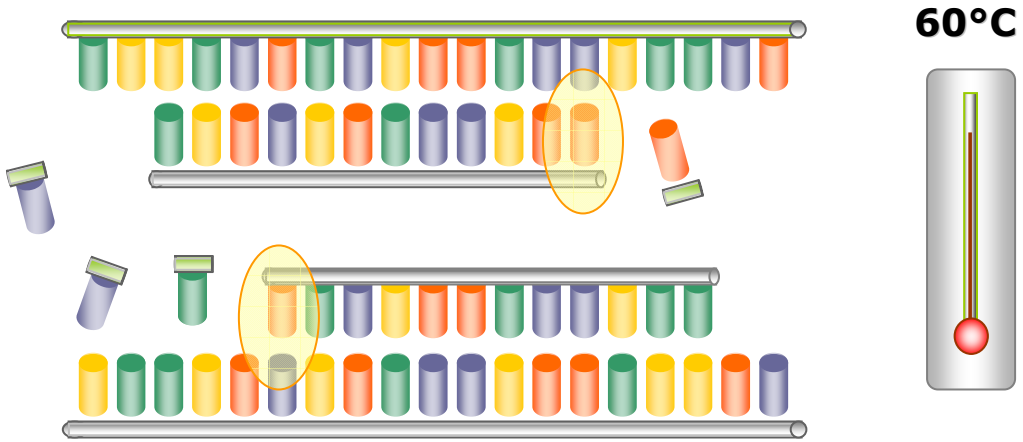
PCR: the principle of replication



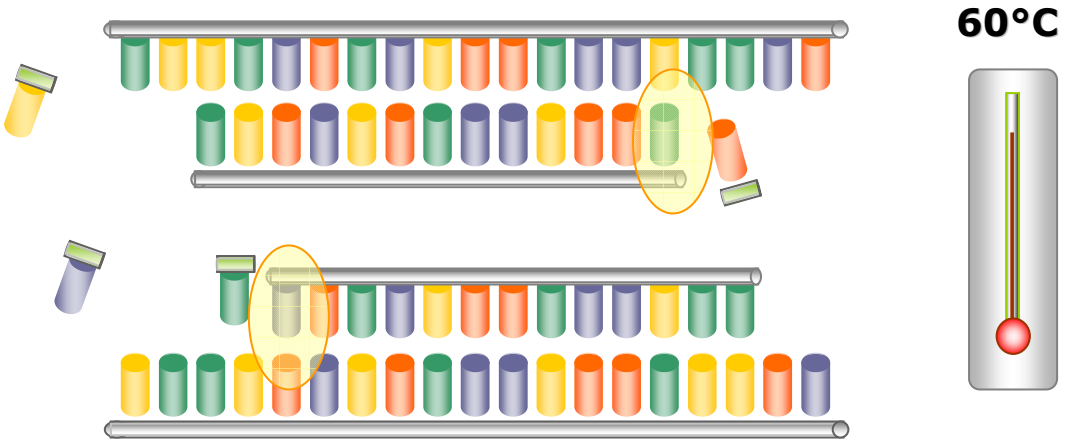
PCR: the principle of replication



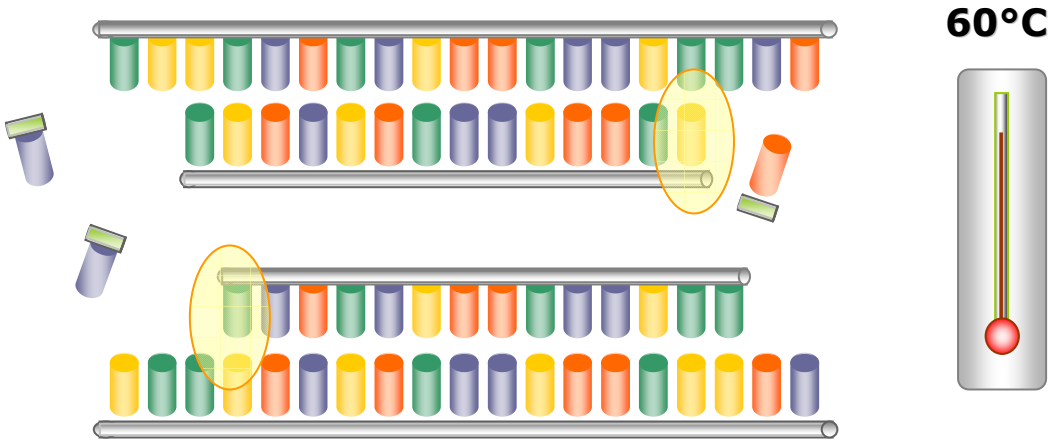
PCR: the principle of replication



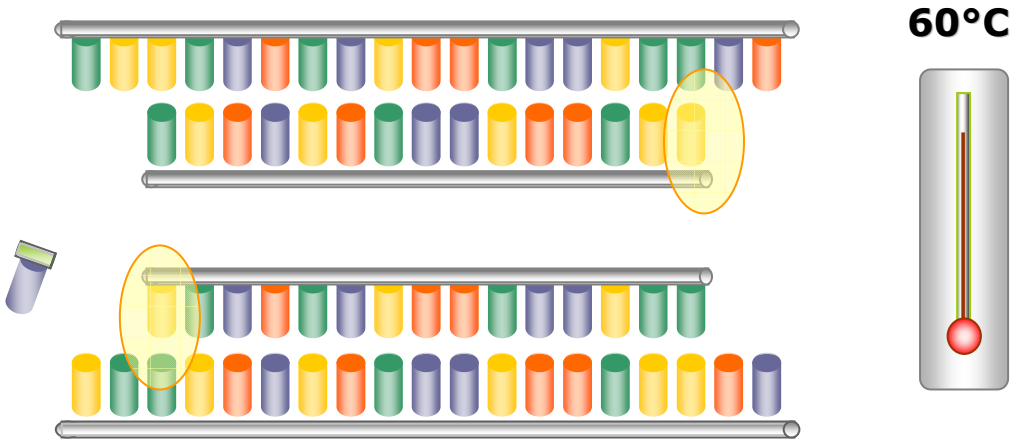
PCR: the principle of replication



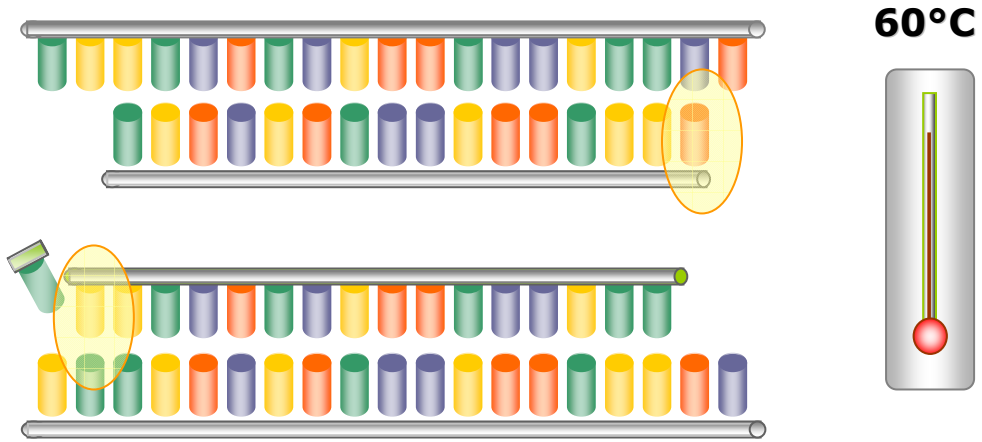
PCR: the principle of replication



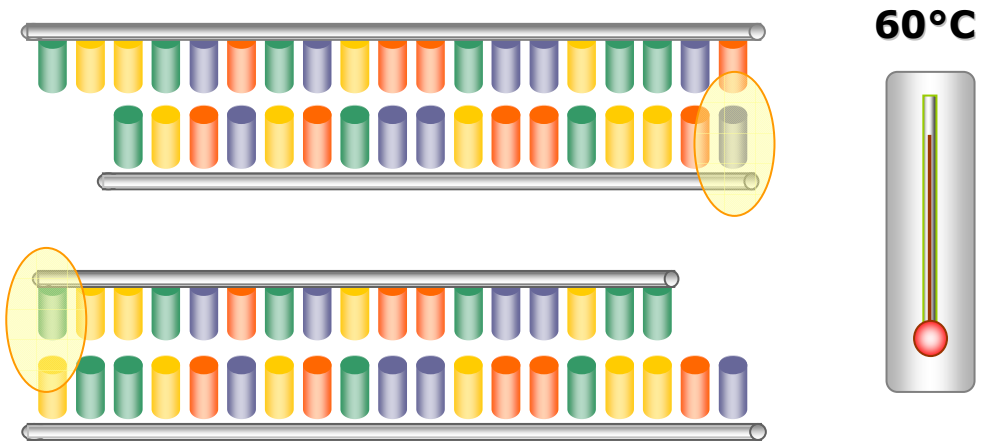
PCR: the principle of replication



PCR: the principle of replication

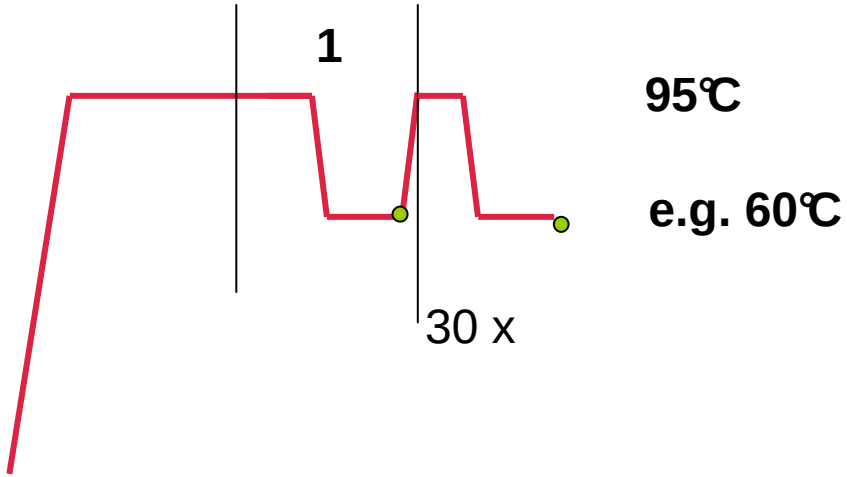


PCR: the principle of replication



PCR

2 cycles:



3 cycles:

4 cycles:

35 cycles: 2^{35} DNA copies = 3.4×10^{10} DNA copies

Detection of the the amplified DNA?

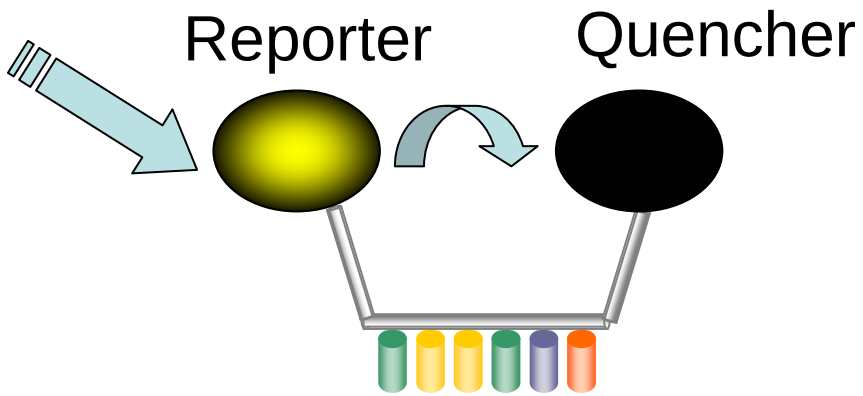
Classical PCR (gel electrophoresis):

- time consuming
- high risk of cross contamination
- quantification, documentation difficult

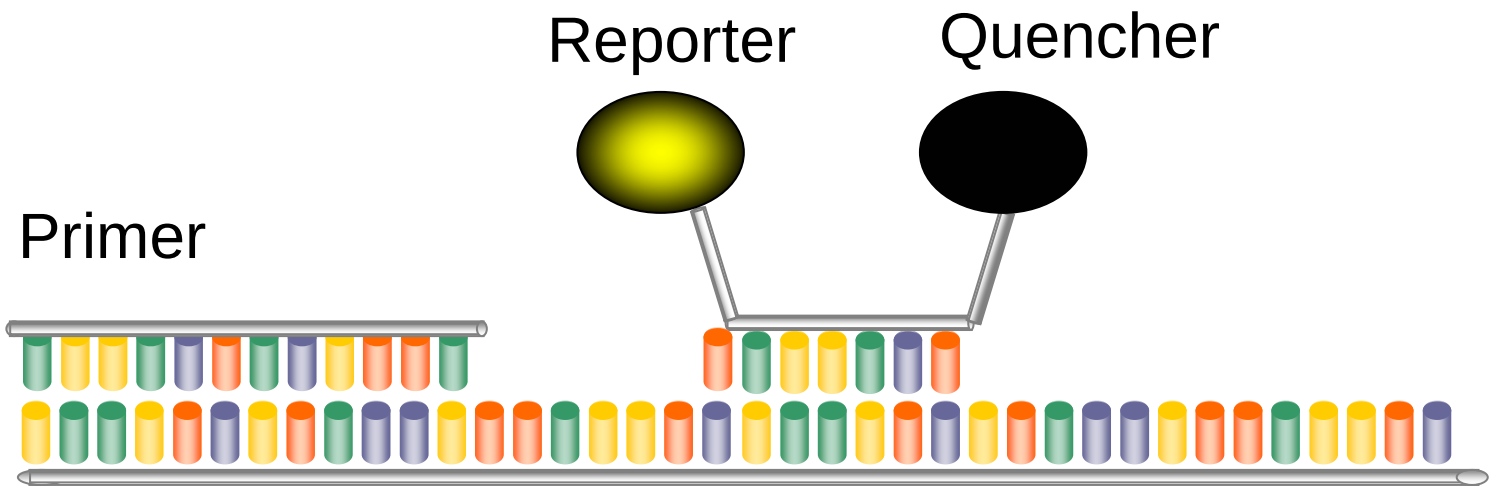
Probe based real-time PCR

- state of the Art technology
- Low risk of cross contamination
- fast, easy to handle
- several real-time PCR thermocyclers available (low price - high throughput)

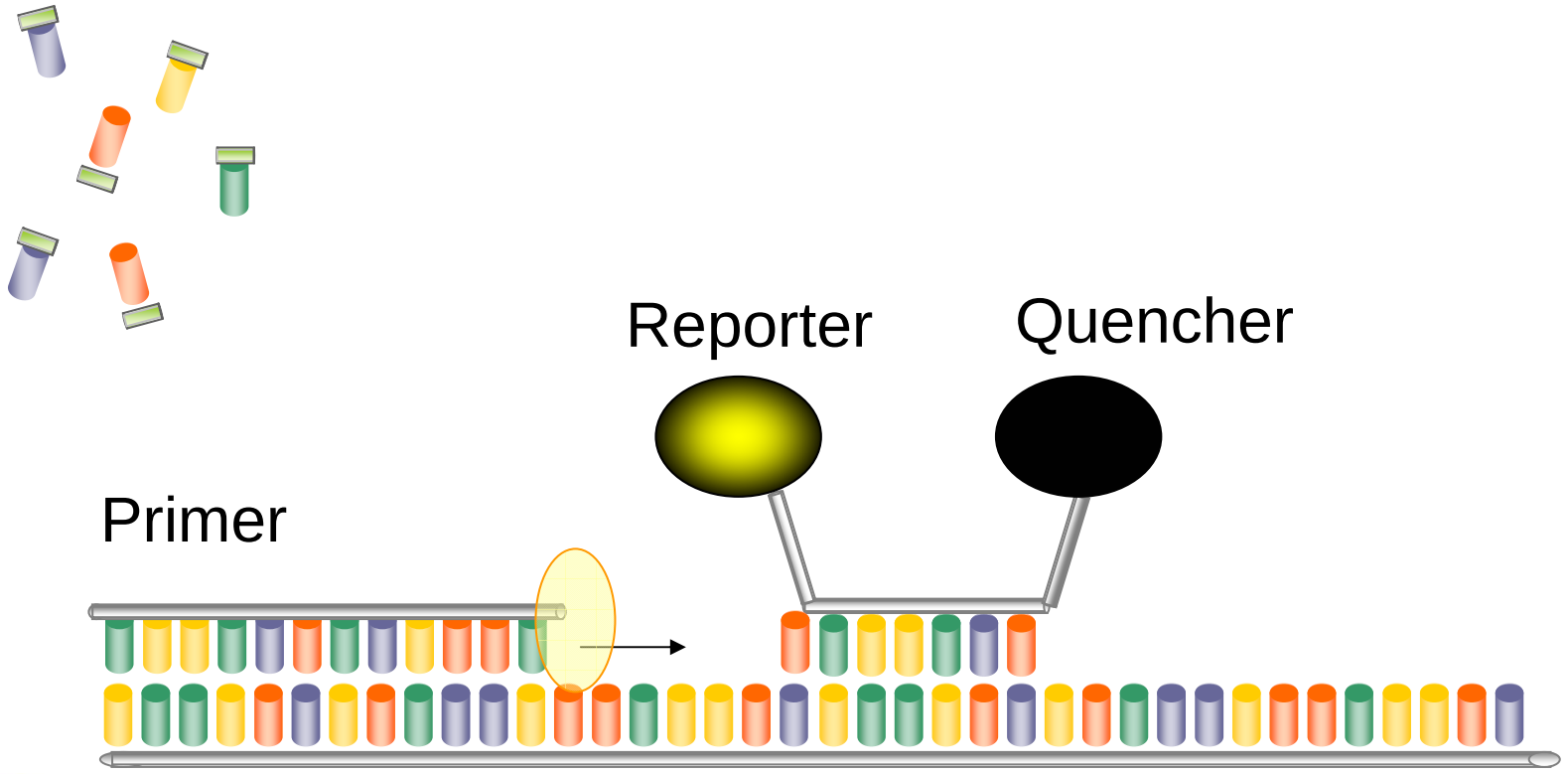
Real-time PCR: the Taqman assay:



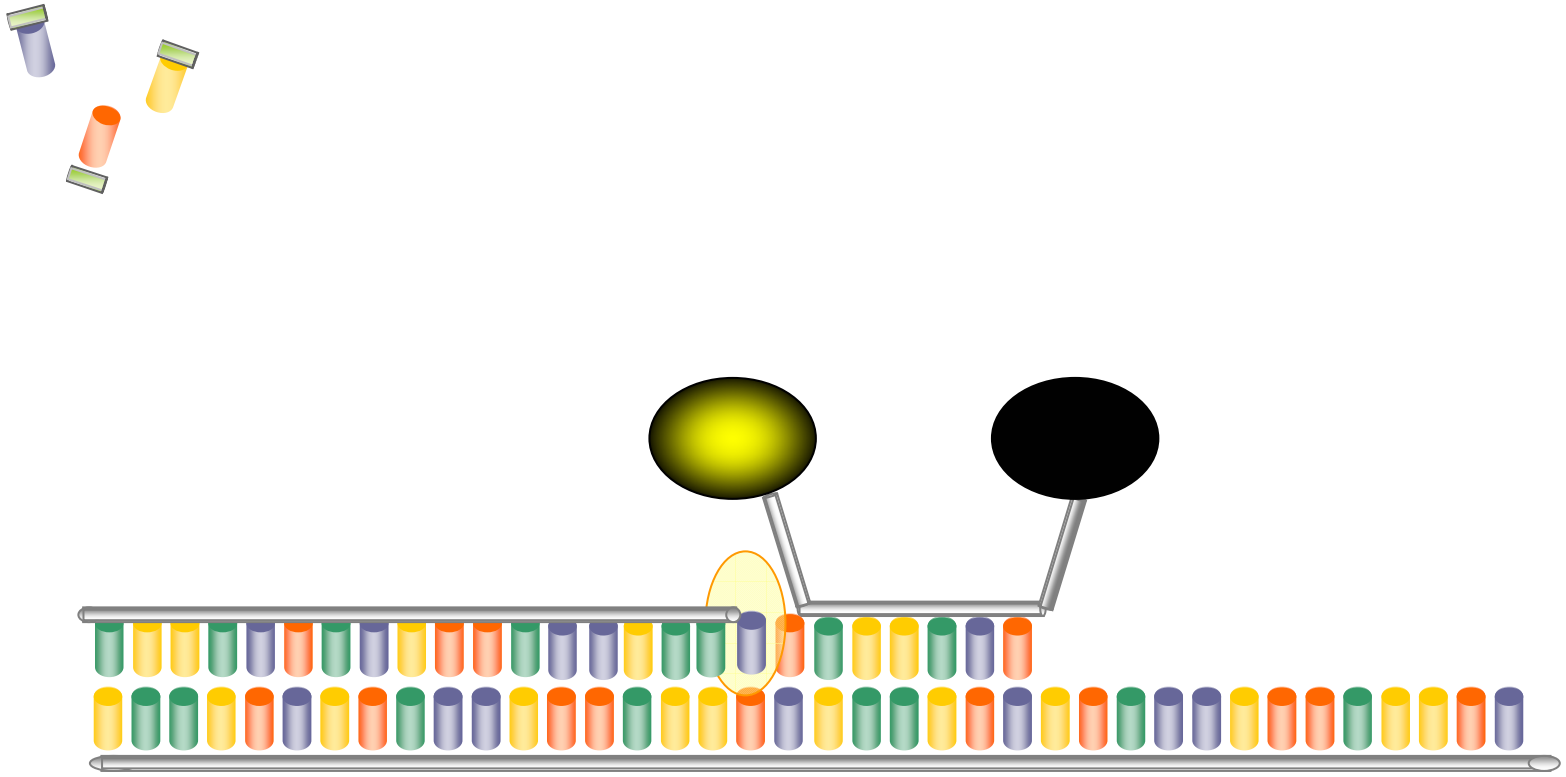
Real-time PCR: the Taqman assay:



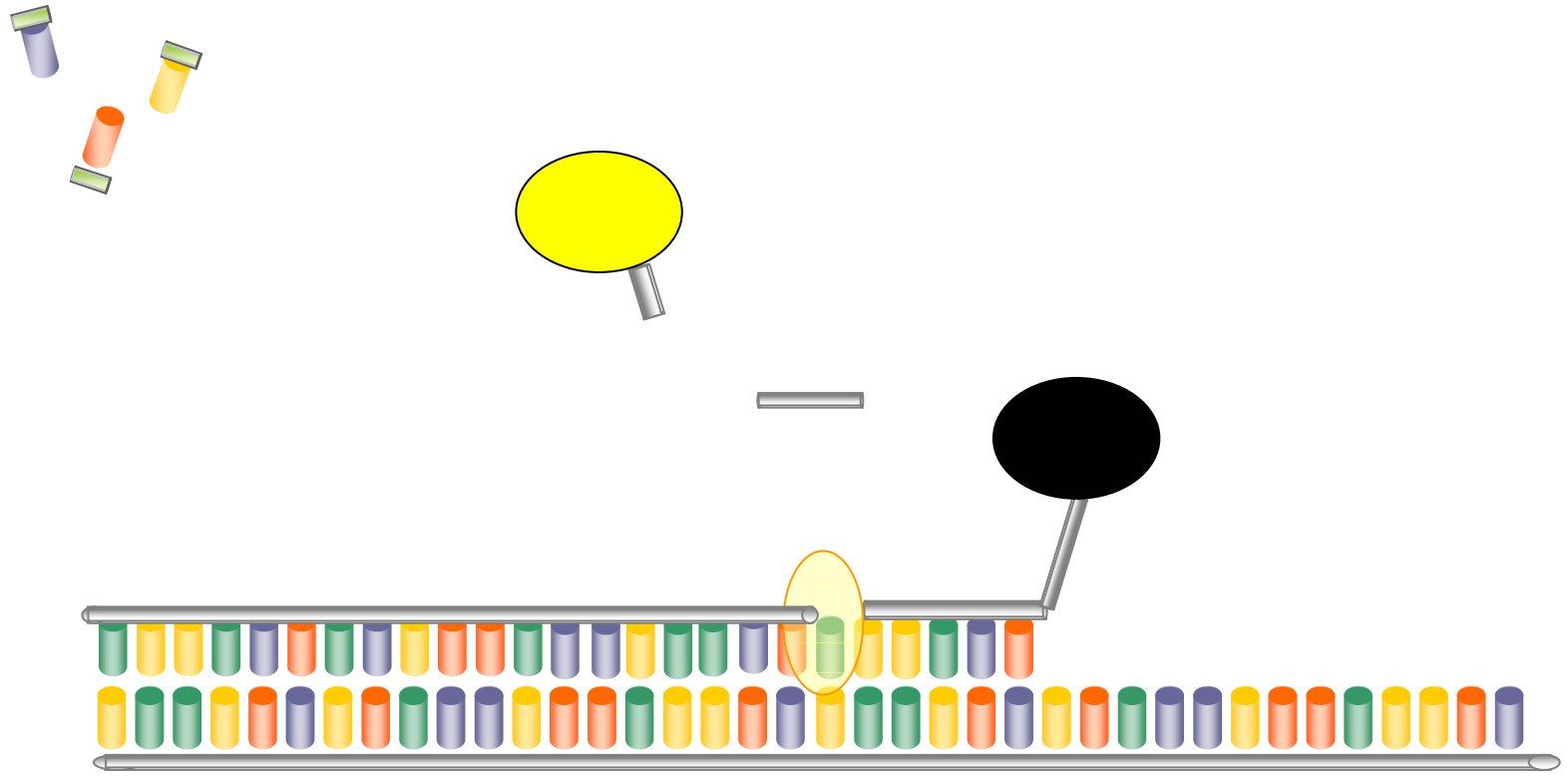
Real-time PCR: the Taqman assay



Real-time PCR: the Taqman assay

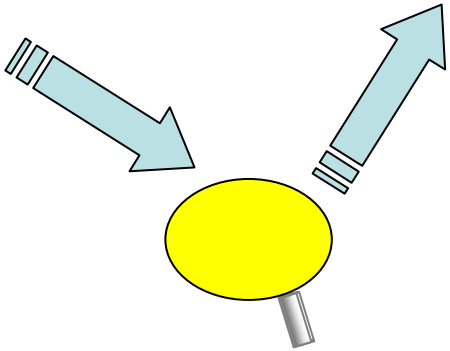


Real-time PCR: the Taqman assay

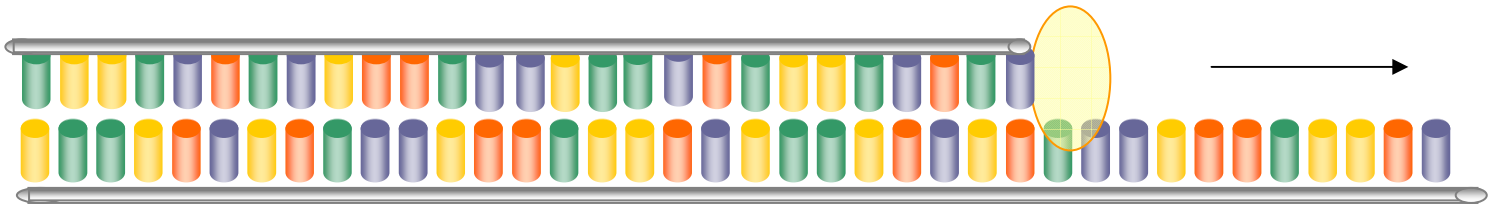


Real-time PCR: the Taqman assay

~488 nm

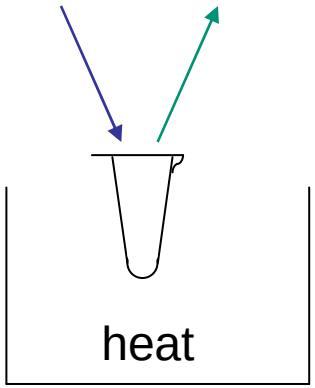


500 nm in real-time, FAM

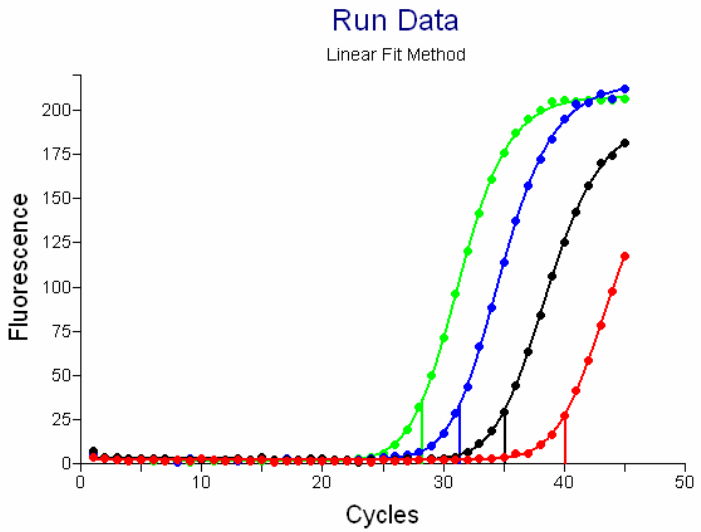


PCR

light sensor

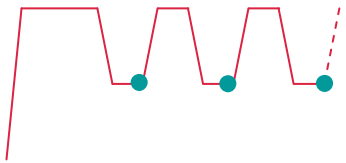


airstream /
metal block



95°

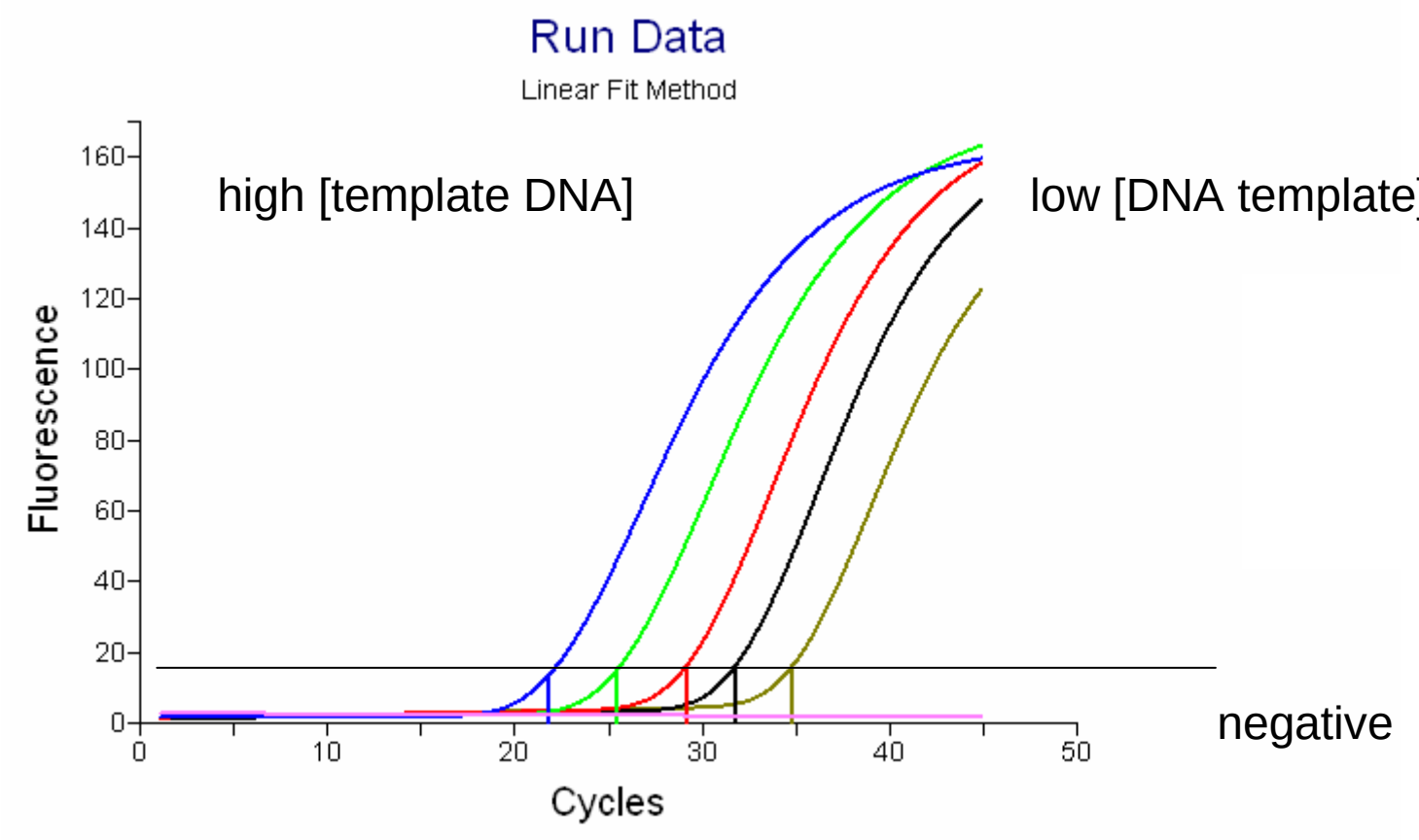
~ 60°



→ computer / software

PCR

29.09.2011



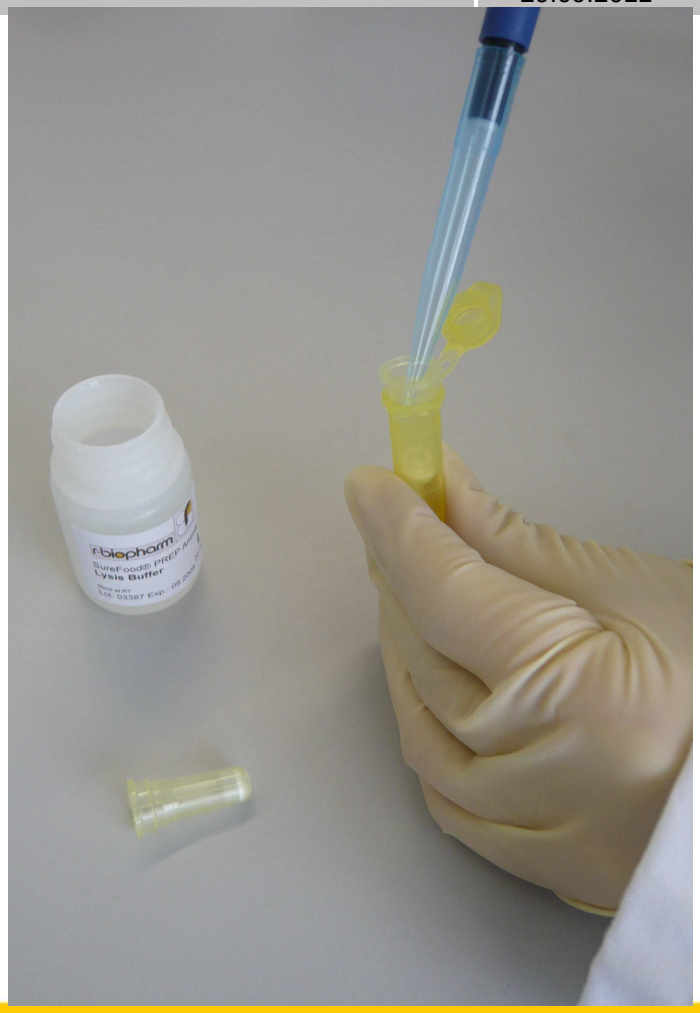
DNA Preparation

Step 1.

- representative sample to be homogenized
- separate place is required

Step 2.:

- DNA preparation:
- Exctraction, lysis
- Purification, removal of inhibiting factors



Step 3.

Preparation of the PCR run

- Sample DNA
- Mastermix
  (Polymerase, primers, nucleotides)
- Controls per run:
- Positive
- Negative
- Inhibition
- Extraction

PCR

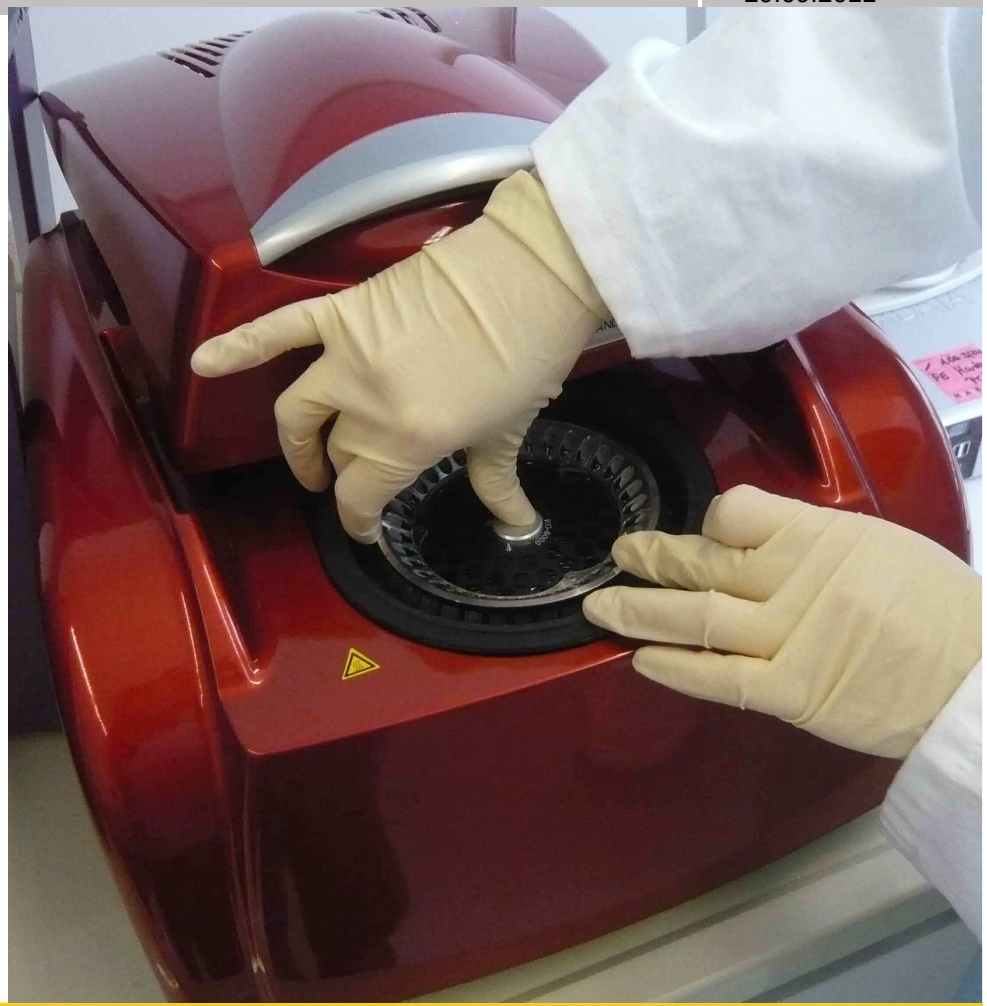
Step 4.

Start of the PCR run

- caroussel based cyclers
- block cyclers

SureFood® is an open system, to
Be used on all commercial available
Real-time thermocyclers,

ABI, Biorad, Eppendorf, Roche...

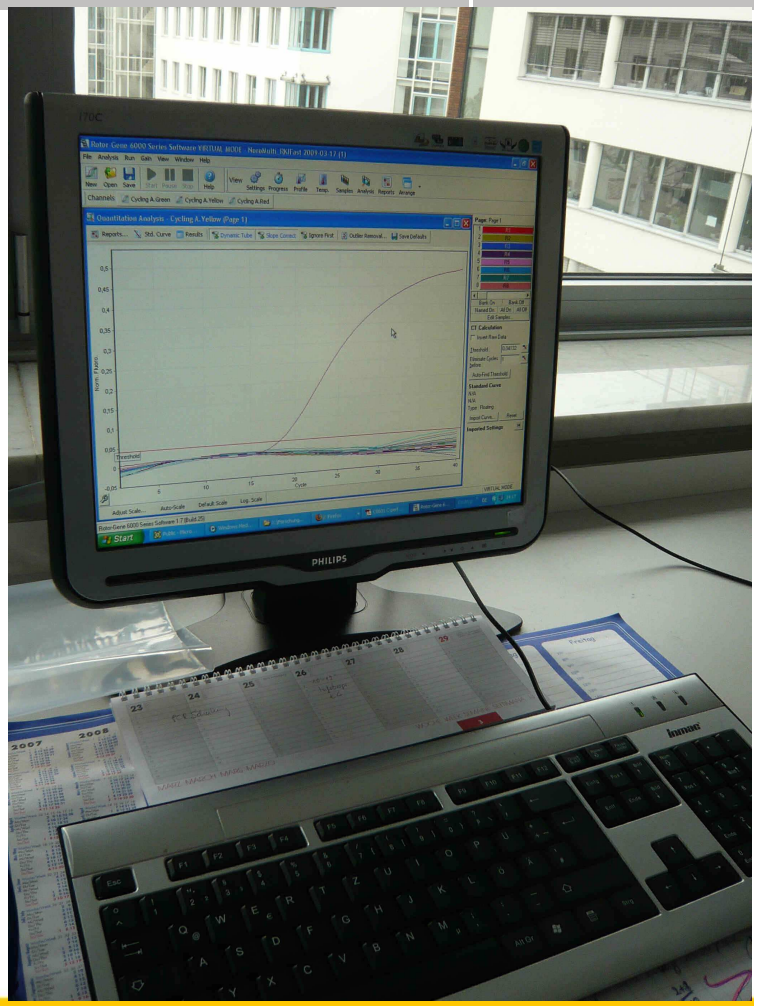


PCR data analysis

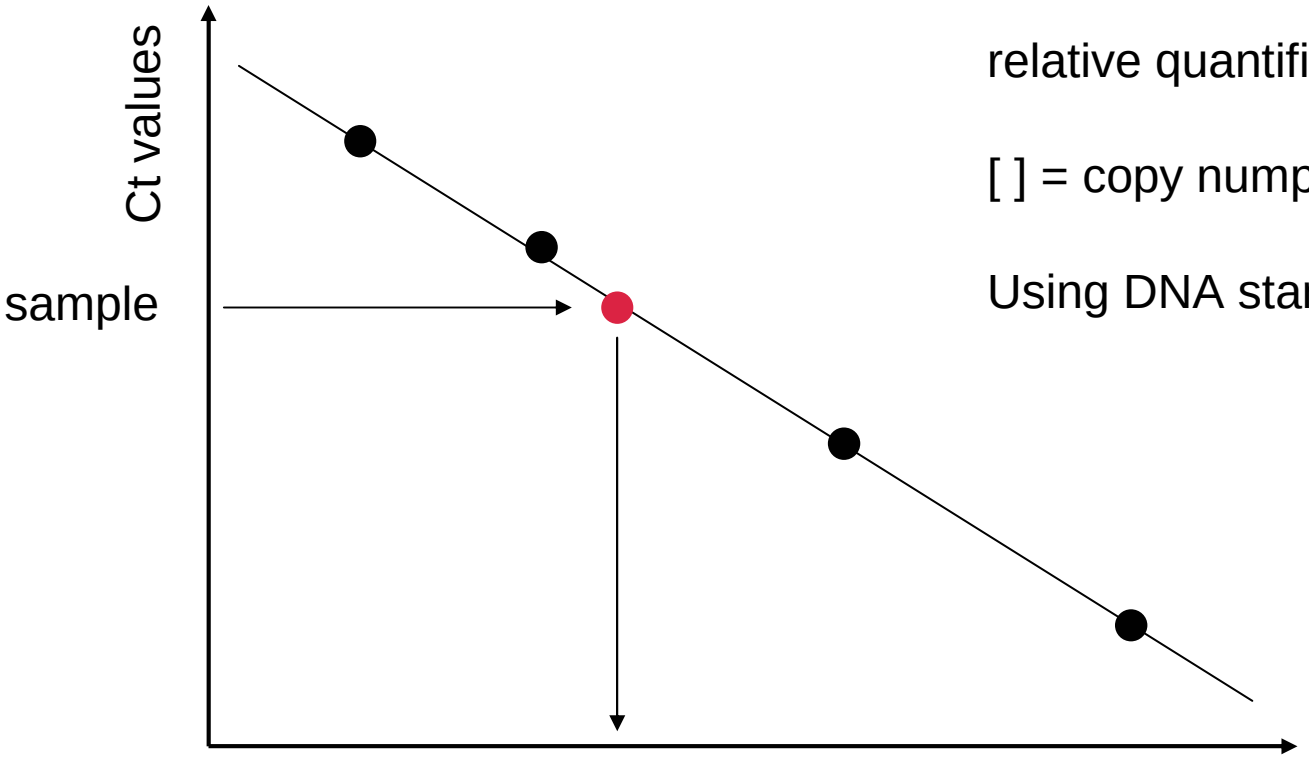
Step 4.

Data interpretation

- positive
- negative
- Ct values
- Data export and storage



Relative quantification by PCR

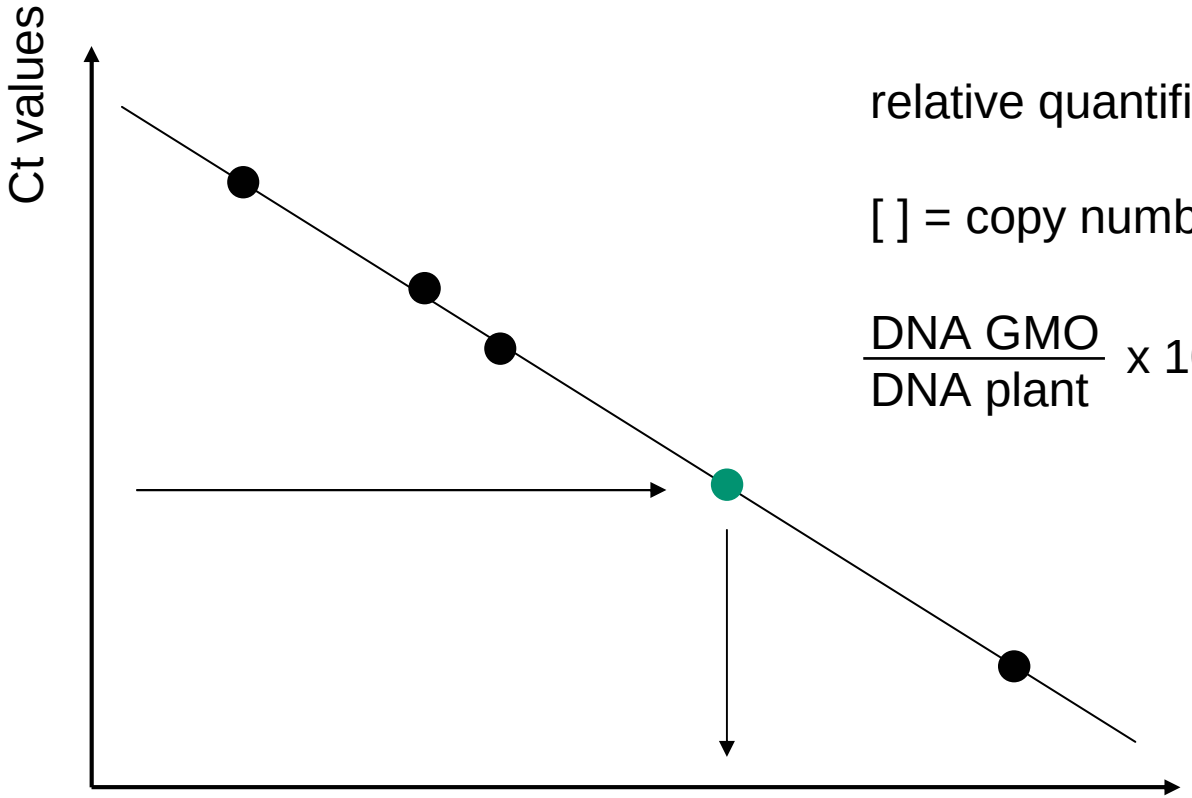


relative quantification

[ ] = copy numpers **GMO**

Using DNA standards

relative quantification



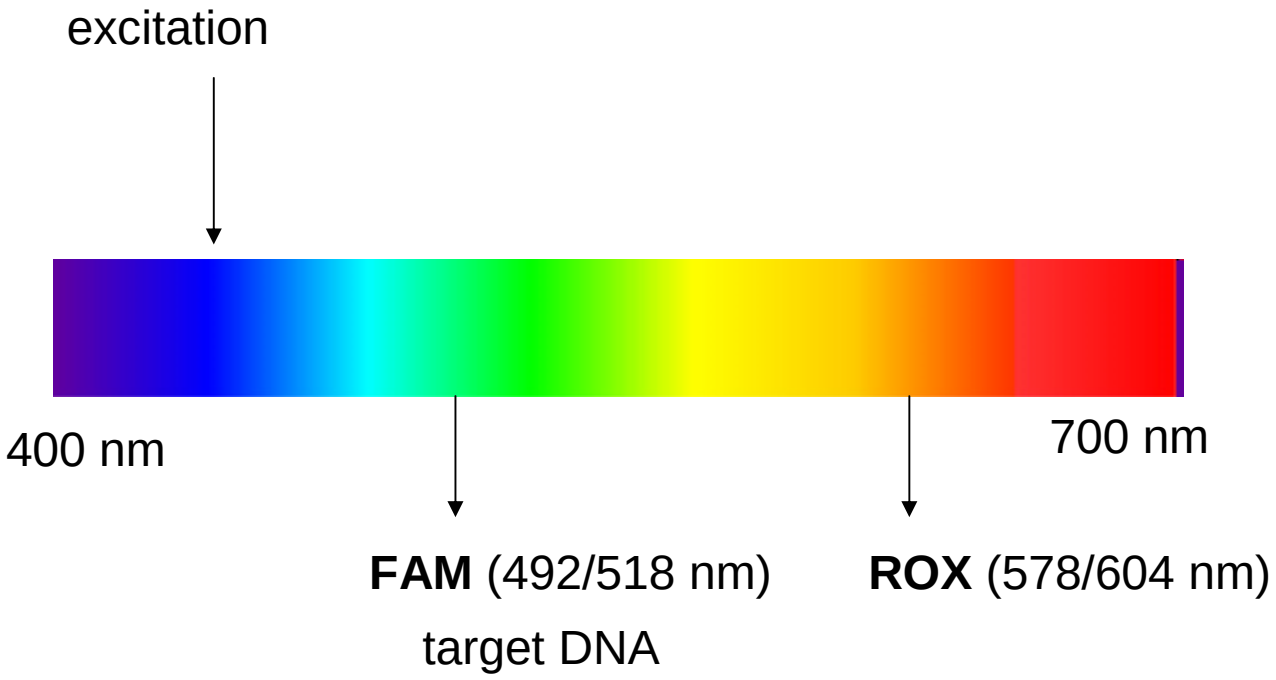
relative quantification

[ ] = copy numbers **plant**

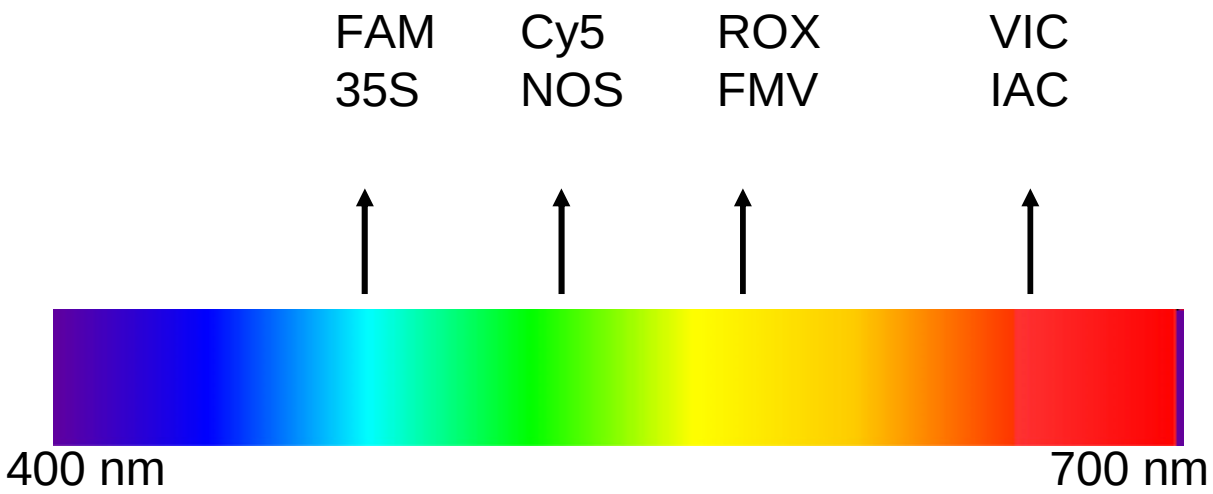
$$\frac{\text{DNA GMO}}{\text{DNA plant}} \times 100 = \%\ \text{GMO in plant}$$

Multiplex PCR

2 channel systems

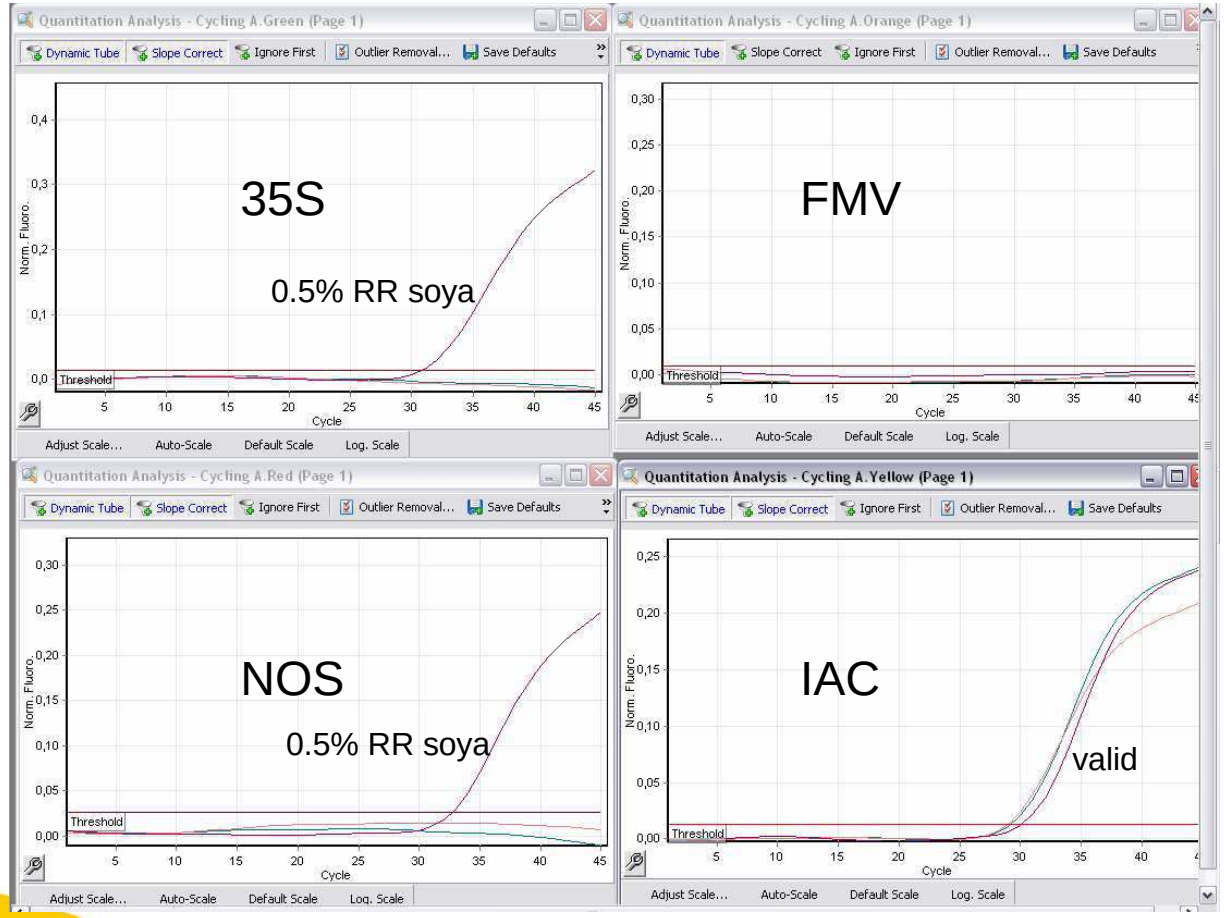


4 channel systems: multiplex systems



GMO

29.09.2011



Example of multiplex PCR:

Roundup ready soya:

35S +
NOS +
FMV -
IAC + valid

GMO

09.08.2011

SureFood® GMO

SureFood PREP Plant S1002

SureFood PREP Plant X S1006

Screen

- **35S + NOS** S2025
- **35S + NOS +FMV** S2026
- **P35S:BAR Rice** S2022
- **35S** S2045
- **NOS** S2046
- **FMV** S2047
- **BAR** S2048
- **CaMV** S2027
- **PLANT PLUS V** S2049 V — **R** S5111R — **LC** S5111LC
- **4-Plex** S2126
- **Plant** S2056

Biorad, LC480, Rotorgene

Qualitative

- **LibertyLink 601** Rice S2023
- **Bt63 Rice** S2024

Quantitative

- **Roundup Ready Soya** S2014
- **Bt176 Corn** S2015
- **Bt11 Corn** S2016
- **T25 Corn** S2017
- **LibertyLink Canola** S2018
- **Mon810 Corn** S2019
- **35S Corn** S2020
- **35S Soya** S2028
- **RR2Y** S2029

1.STEP **2.STEP** **3.STEP**

Real- time PCR thermocyclers:

The SureFood® kits may be used on all commercial available Real-time PCR cyclers, as from ABI, Biorad, Cepheid, Corbett, Eppendorf, Roche etc.