



Trust your case to QIAGEN

Critical quantification considerations in STR analysis of forensic DNA samples

Introducing the Investigator Quantiplex Kit

QIAGEN

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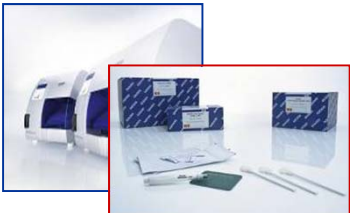
03.10



- Quantification in human identity testing
- Investigator Quantiplex Kit
 - The multi-copy target
 - Sensitivity, accuracy, and specificity
 - Reproducible results between individuals
 - DNA quality assessment
 - Correlation to STR
 - Example of use I – low range DNA
 - Example of use II – casework samples
 - Internal control/Quality Sensor
 - Fast cycling technology
- Data from Field Test
- Summary

Quantification in human identity testing

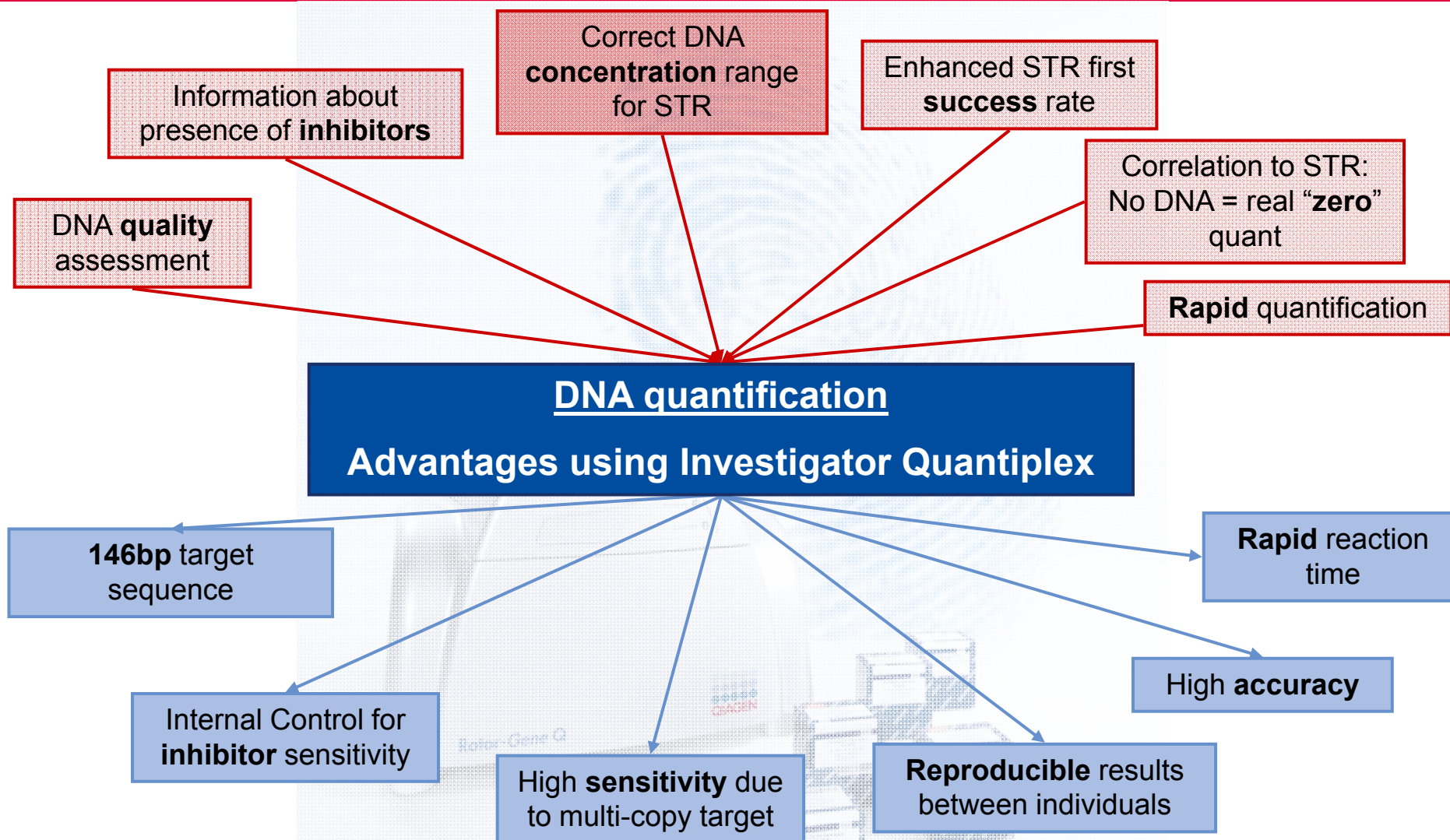
Background — What is it and why is it necessary?

	DNA extraction	Quantification	STR assay	Data analysis
Why?	PCR is sensitive to inhibitors 	STR has a narrow range of optimal DNA concentrations 	Amplification and labeling of forensic markers 	Normalization Allele calling etc 
Technology	Variable	Real-time PCR	Multiplex endpoint PCR	Sequencer / Software
Output	Pure DNA	Pure DNA at a known concentration	A specific set of amplified and labeled genetic markers ready for CE analysis	DNA profile



Quantification in human identification

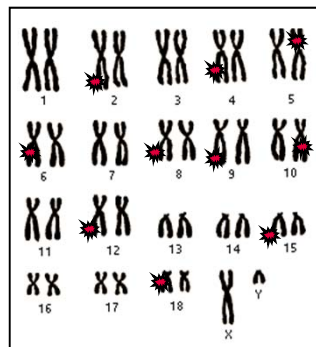
Background – Why is it useful?





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Multi copy target



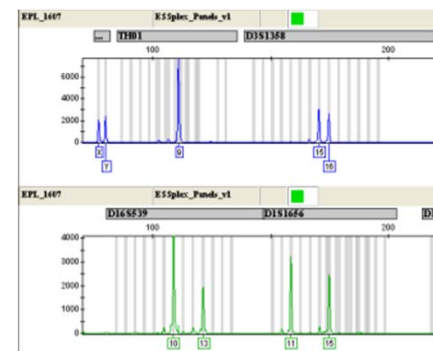
Number of copies per cell is the same in every individual

3 cells/ μ l input for quantification

Quantiplex

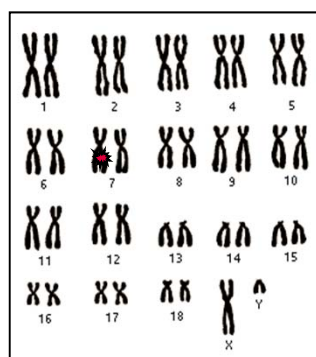
21 pg/ μ l

Sensitivity high enough to detect and Consistent results



STR profile

Single copy target



Always one copy per cell

3 cells/ μ l input for quantification

Supplier A

0 pg/ μ l

Sensitivity too low to detect

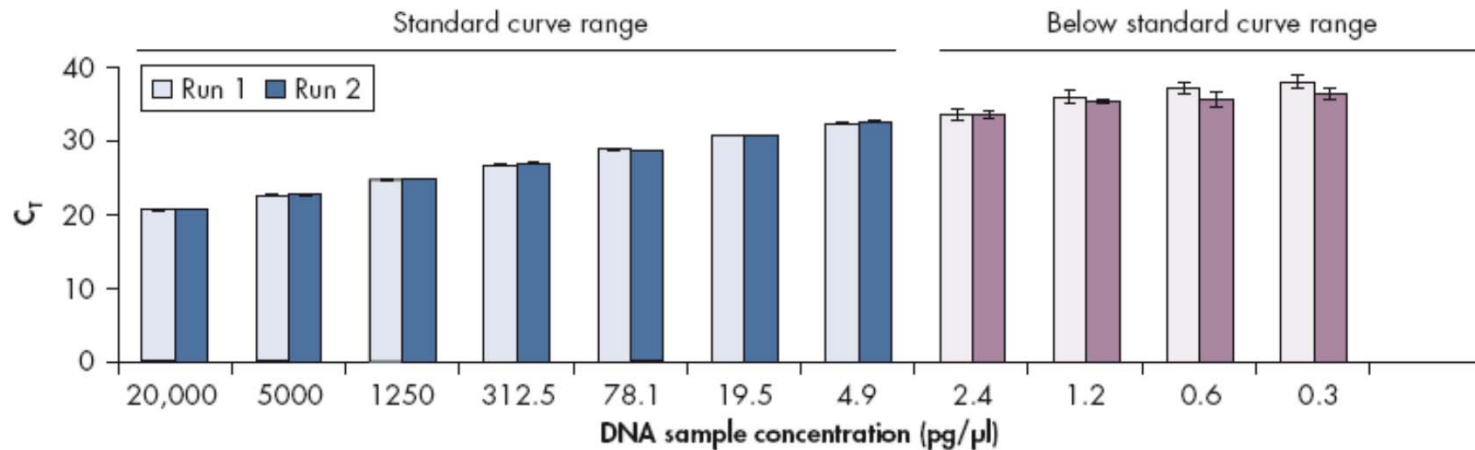


No STR analysis done

Apparent zero quant

Sensitivity

Using the Control DNA Z1

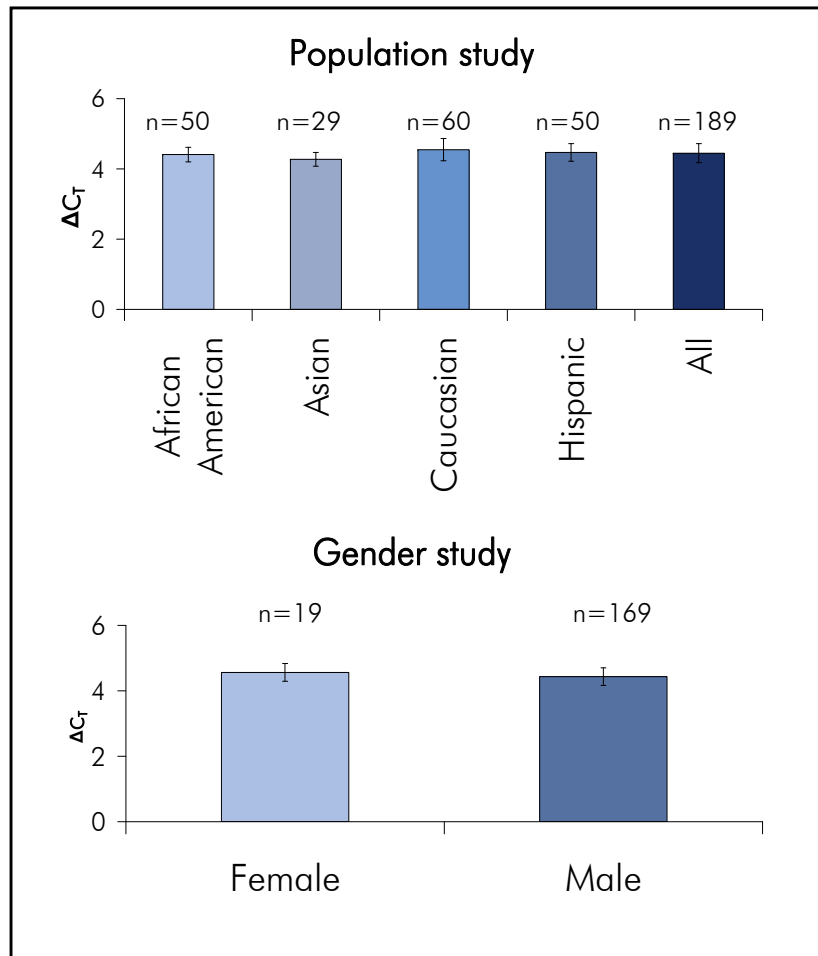


- High linearity of the standard curve down to 4.9 pg/μl. Detection limit: 0.3 pg/μl
- Linear range of detection
 - Quantiplex: < 4.9 pg/μl
 - Supplier A: 23 pg/μl
 - Supplier B: 5 pg/μl
- Good results below this value are still possible with some compromises in linearity
- Stochastic effects may be expected at concentrations below 1 pg/μl. This allows for reliable results by using multiple replicates



Reproducible results across populations and gender

No copy number variation



- Comparison of the C_T value of a human specific single-copy target with the C_T values of the Investigator Quantiplex multi-copy target
- ΔC_T between the two targets is shown for the different gender and different populations
- No copy-number variation of the target between gender
- Target region is present in 19.95 ± 1.20 copies considering a haploid genome

The genetic targets of Quantiplex are highly conserved and do not vary across ethnicity or gender



Investigator Quantiplex is highly specific for humans (and some primates)

Animals		C _T
Cow DNA	2.5 ng DNA/reaction	>40
Pig DNA	2.5 ng DNA/reaction	>40
Horse DNA	2.5 ng DNA/reaction	>40
Sheep DNA	2.5 ng DNA/reaction	>40
Goat DNA	2.5 ng DNA/reaction	>40
Dog DNA	2.5 ng DNA/reaction	>40
Chicken DNA	2.5 ng DNA/reaction	>40
Mouse DNA	2.5 ng DNA/reaction	>40
Rat DNA	2.5 ng DNA/reaction	>40
Hamster DNA	2.5 ng DNA/reaction	>40
Rabbit DNA	2.5 ng DNA/reaction	>40
Cat DNA	2.5 ng DNA/reaction	>40

Microbes		C _T
<i>E. coli</i>	2.5 ng DNA/reaction	>40
<i>Pseudomonas aeruginosa</i>	2.5 ng DNA/reaction	>40
<i>Candida albicans</i>	2.5 ng DNA/reaction	>40
<i>Staphylococcus aureus</i>	2.5 ng DNA/reaction	>40
Primates		C _T
Orangutan	2.5 ng DNA/reaction	>40
Macaque	2.5 ng DNA/reaction	>40
Gorilla	2.5 ng DNA/reaction	27.42
Chimpanzee	2.5 ng DNA/reaction	25.49
Bonobo	2.5 ng DNA/reaction	25.38
Controls		C _T
Control DNA	2.5 ng DNA/reaction	25.78



DNA quality assessment

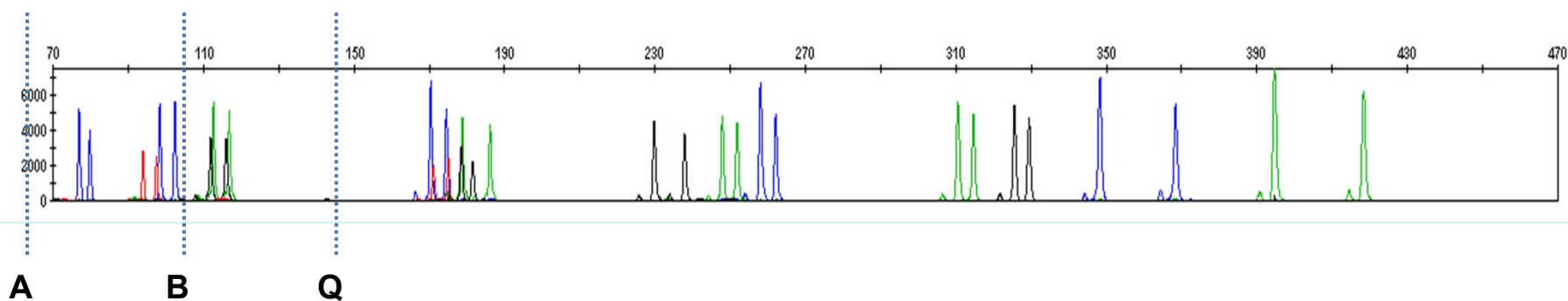
Making quantification results matter



- Increased PCR product size gives meaningful quantification results
- This allows you to better predict whether a degraded DNA sample will still give an STR profile

PCR product length

Quantiplex	146 bp
Supplier B	99 bp
Supplier A	62 bp





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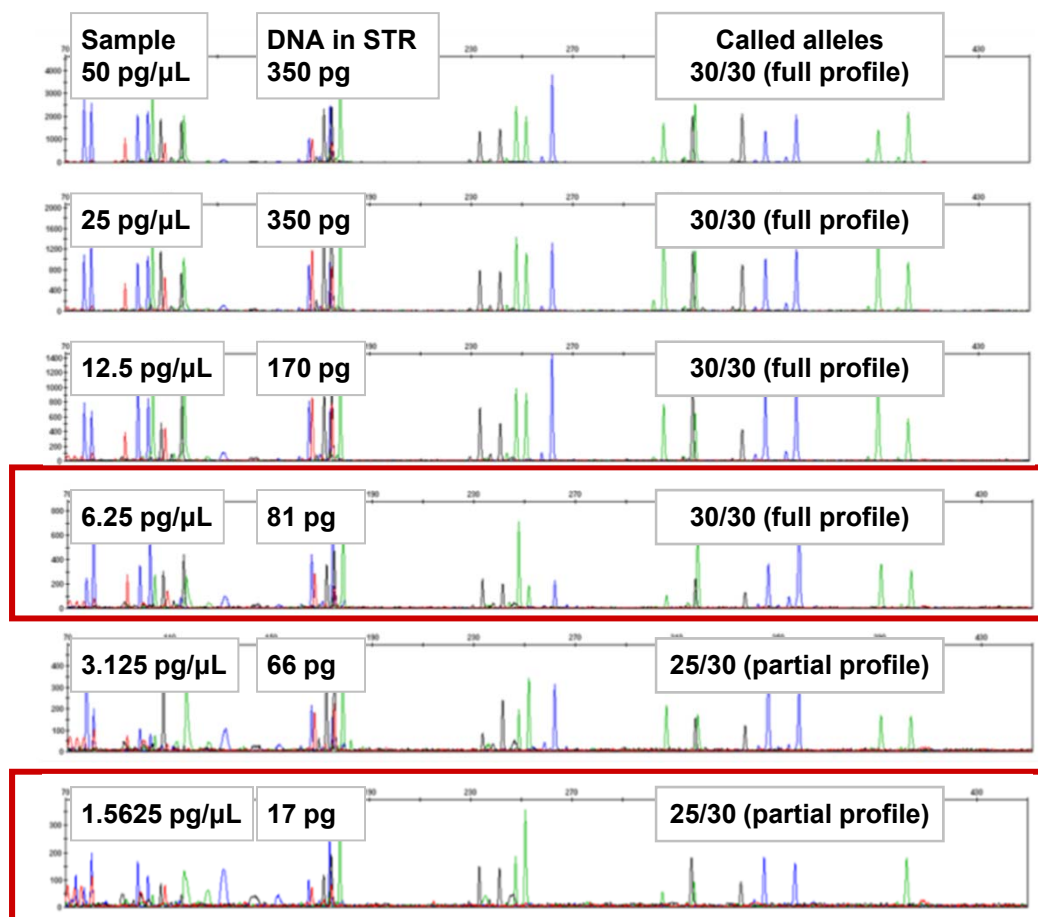
Example of use I – low range DNA

Improved correlation to STR analysis

1. Quant with Quantiplex and two other commercial formats

Theoretical Conc.	Quantiplex	Supplier A	Supplier B
50 pg/μl	40.22	48.35	30.67
25 pg/μl	24.25	23.94	17.56
12.5 pg/μl	9.93	17.50	10.41
6.25 pg/μl	4.72	0.00	9.05
3.125 pg/μl	3.86	5.25	1.16
1.5625 pg/μl	1.02	0.00	0.82
NTC	0.00	0.00	1.64

2. STR analysis with Investigator ESSplex* based on Quantiplex data



Quantiplex data correlate to STR assay results/signal intensity

*ESSplex is distributed worldwide, except in Australia, Canada and USA



Example of use II — casework samples

Improved correlation to STR analysis

Casework sample

DNA extraction

Quantification

STR assay

Data analysis



Workflow

EZ1 DNA Investigator

Investigator Quantiplex

Investigator ESSplex*

Sequencer /
Software



QIAgility automated setup

- Investigator Quantiplex Kit
- Investigator ESSplex Kit
- CE-loading

* ESSplex is distributed worldwide, except in Australia, Canada and USA



Example of use II — casework samples

Improved correlation to STR analysis

Casework sample

DNA extraction

Quantification

STR assay

Data analysis

	Quantification Results		ESSplex* results	
Sample	Measured concentration (pg/μl)	Replicates	DNA amount in STR (using 10μl DNA)	ESSplex* Results (out of 2 replicates)
Cigarette (undiluted)	1,012	2/2	-	-
Cigarette 1:10	106	2/2	1,060 pg	56 / 56 (full profile)
Cigarette 1:100	9.7	2/2	97 pg	56 / 56 (full profile)
Cigarette 1:1,000	0.3	2/2	3 pg	22 / 56 (partial profile)
Cigarette 1:10,000	0.3	1/2	3 pg	5 / 56 (sporadic alleles)
Cigarette 1:100,000	0	0/2	0	0 / 56 (no profile)
NTC	0	0/2	0	0 / 56 (no profile)

* ESSplex is distributed worldwide, except in Australia, Canada and USA



Example of use II — casework samples

Improved correlation to STR analysis

Casework sample

DNA extraction

Quantification

STR assay

Data analysis

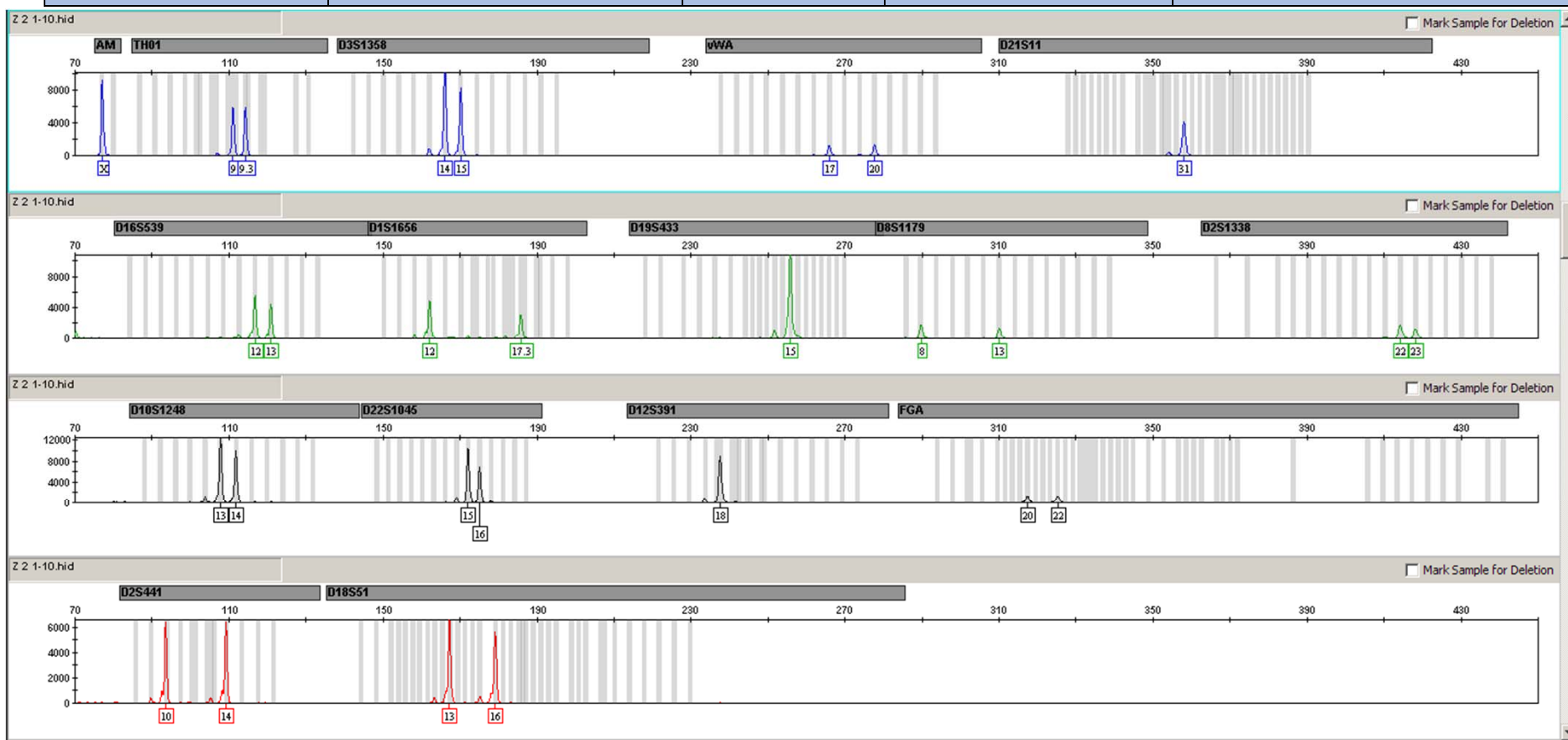
Cigarette 1:10

106 pg/μl

2/2 replicates

1,060 pg/rxn

56 / 56 (full profile)



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Example of use II — casework samples

Improved correlation to STR analysis

Casework sample

DNA extraction

Quantification

STR assay

Data analysis

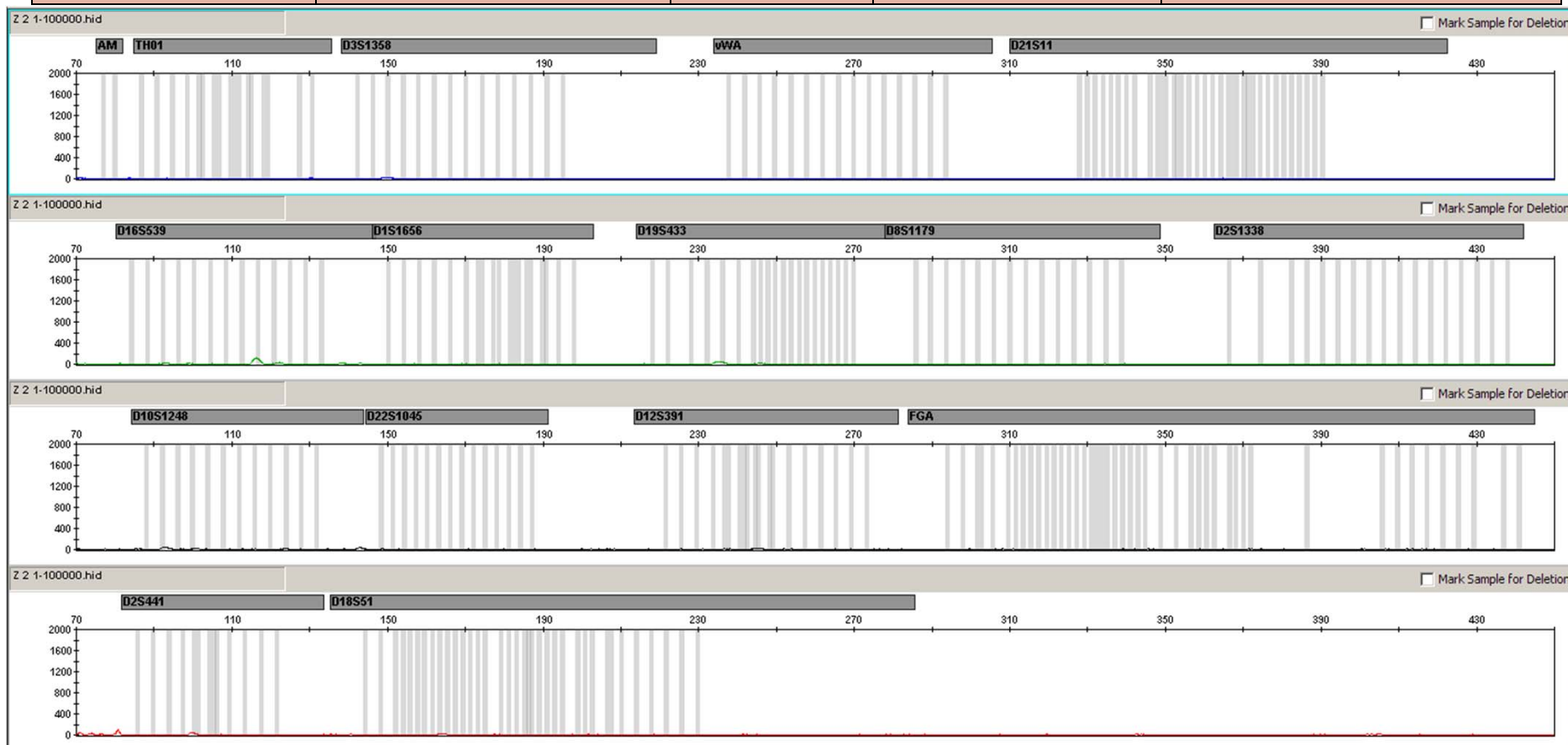
Cigarette 1:100,000

0 pg/μl

0/2 replicates

0 pg/rxn

0 / 56 (no profile)



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Example of use II — casework samples

Improved correlation to STR analysis

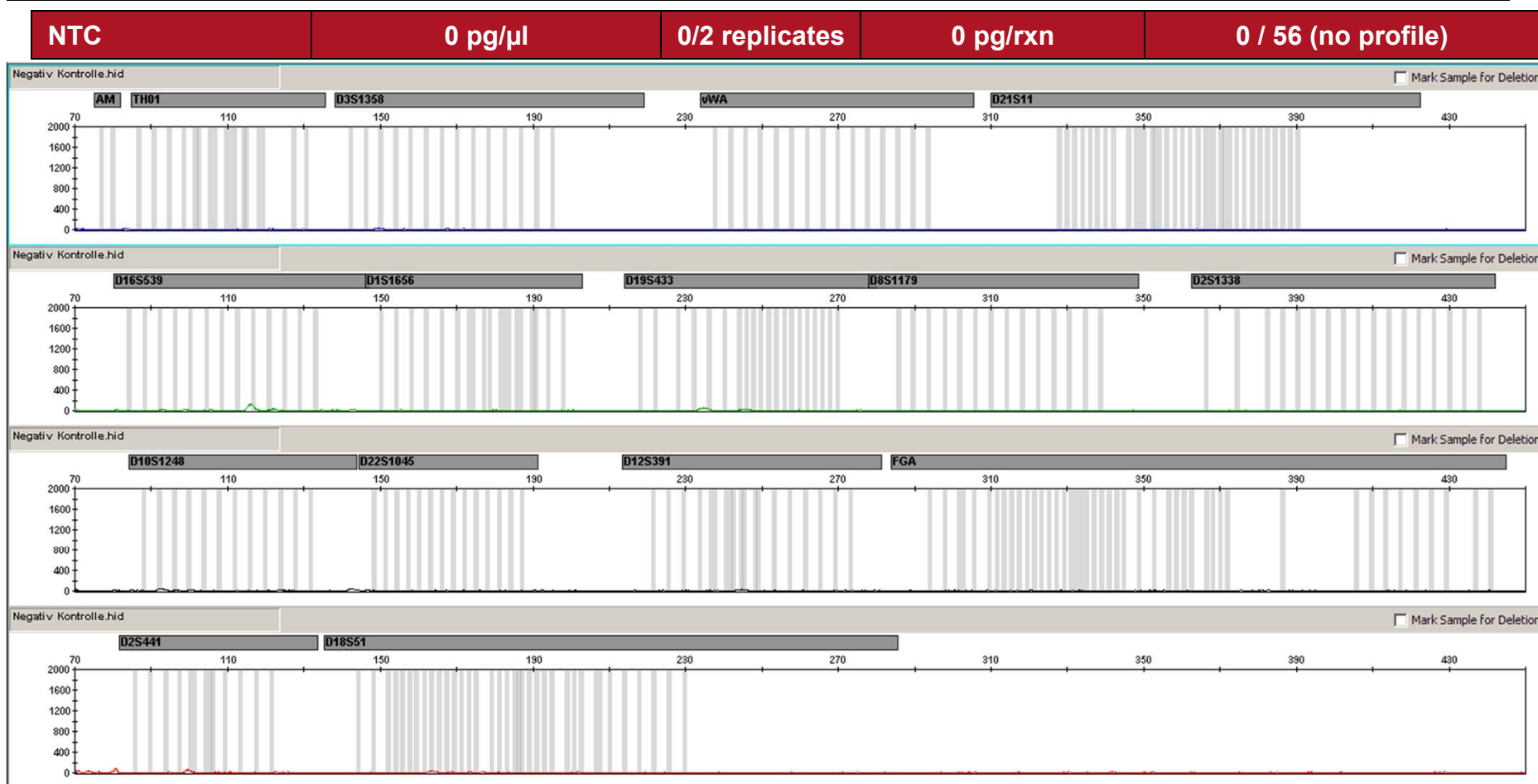
Casework sample

DNA extraction

Quantification

STR assay

Data analysis

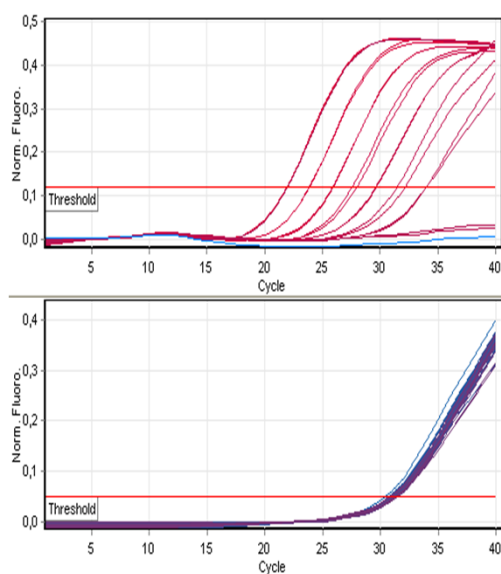


* ESSplex is distributed worldwide, except in Australia, Canada and USA



Example of use II — casework samples

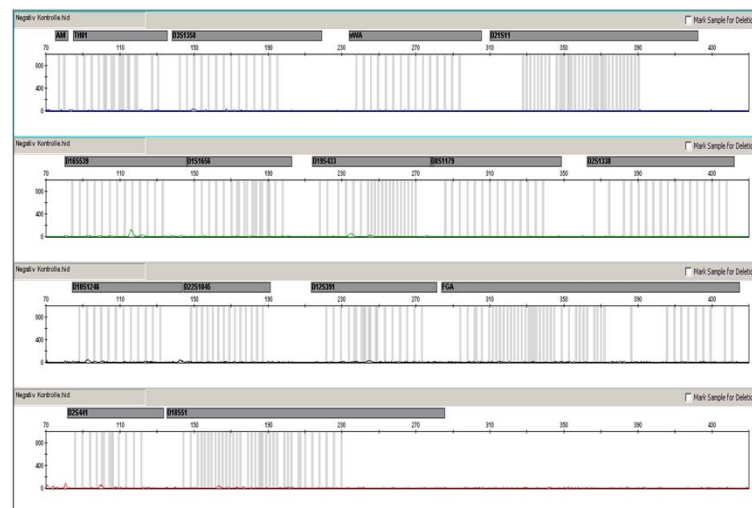
Improved correlation to STR analysis



“0 quant” =



No profile



Quantiplex zero quantification is a “real” no profile

* ESSplex is distributed worldwide, except in Australia, Canada and USA



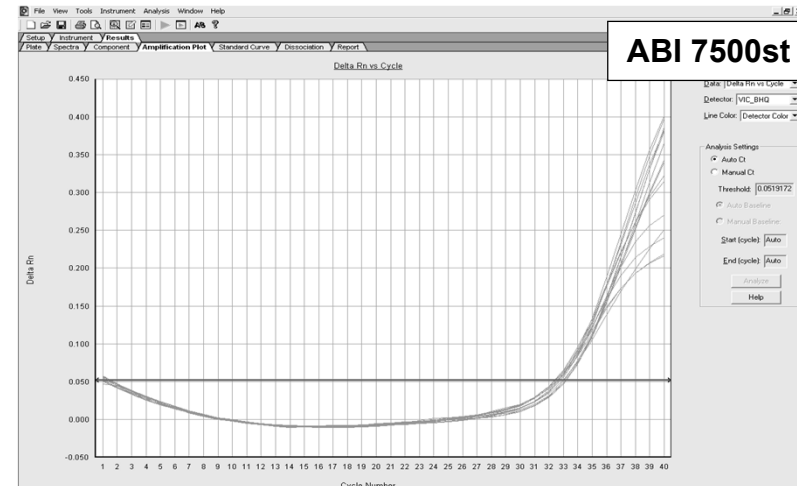
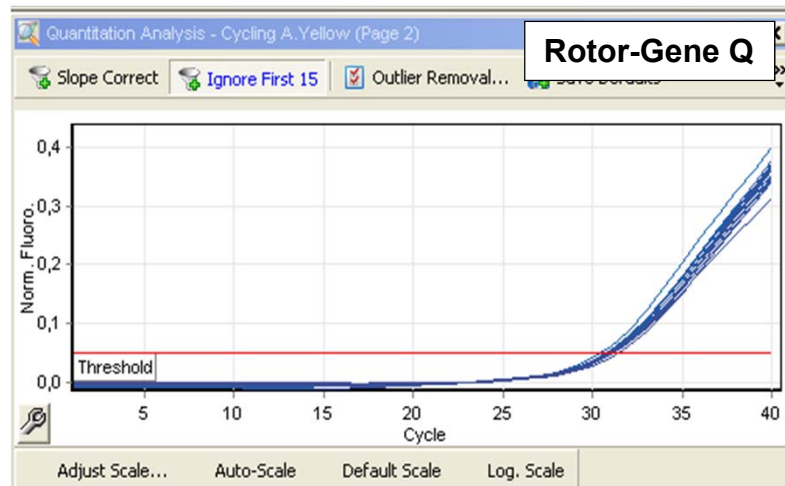
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Internal Control / Quality Sensor

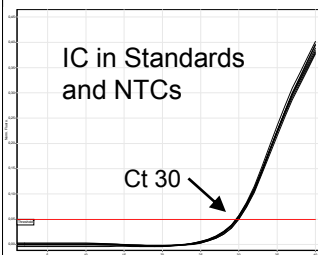
For amplification control and detection of inhibitors

- Detected as a 200 bp internal control
- Dye channel:
 - Yellow channel on the Rotor-Gene Q
 - VIC dye channel on ABI instruments
- Used to test:
 - Successful amplification
 - Detection of PCR inhibitors

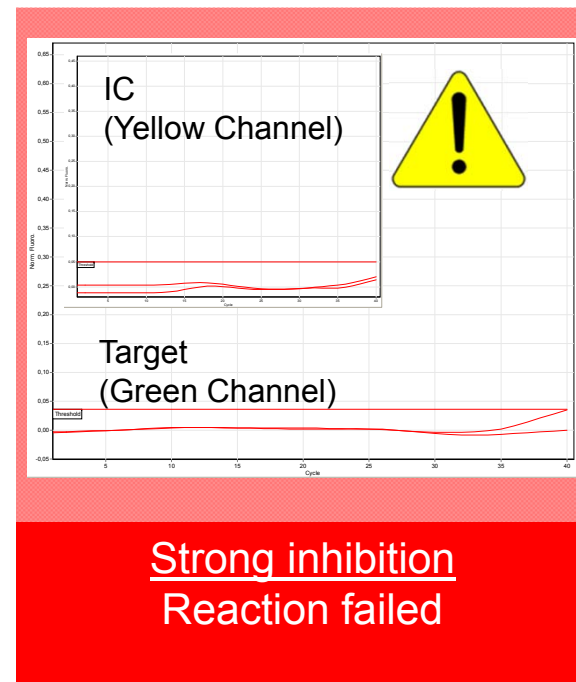
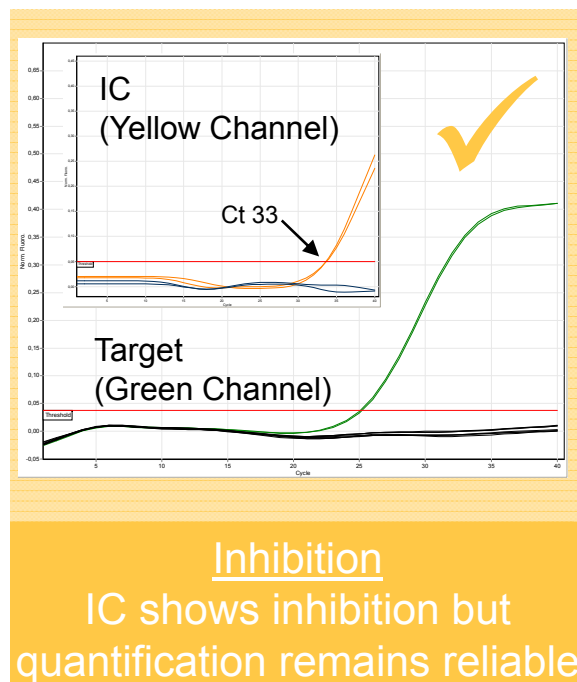
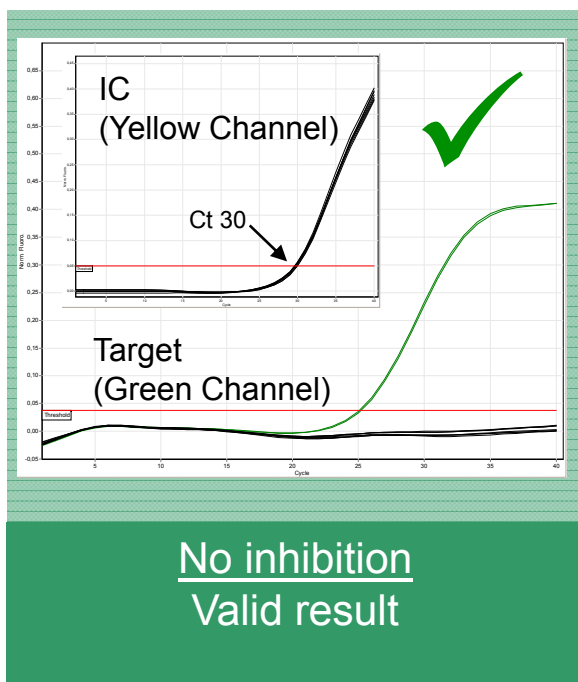


Internal Control / Quality Sensor

Correct interpretation of IC results



- IC signal in Standards and/or NTCs serves as a reference
- C_T shift shows presence of inhibitors



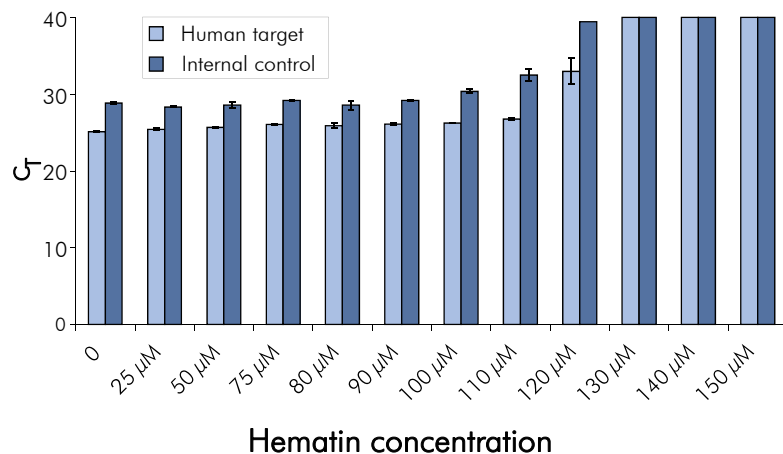
The Internal Control acts like a Quality Sensor



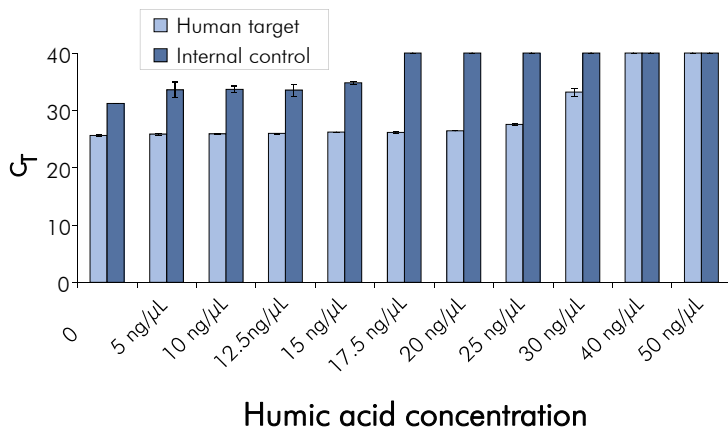
Internal control as quality sensor

Inhibition monitoring

Simulated inhibition with hematin



Simulated inhibition with humic acid



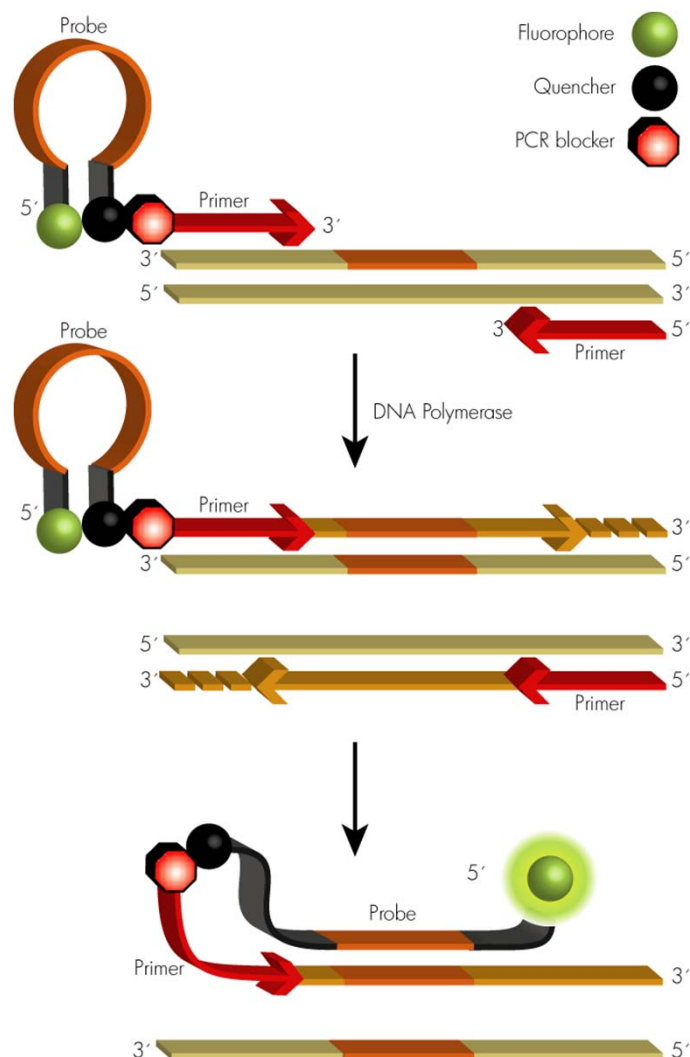
- The IC is designed to be more sensitive to inhibition than the specific target for human DNA
- IC drops off
 - >100 µM hematin
 - >15 ng/uL humic acid (using 1 ng/rxn DNA on ABI 7500)



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Scorpion technology

Fast and sensitive cycling technology



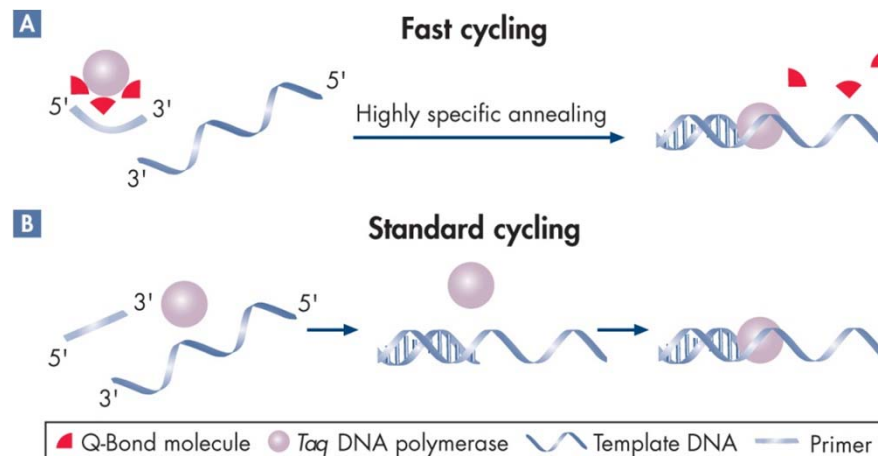
- Scorpion primers
 - Bifunctional molecules containing a PCR primer covalently linked to a probe
- Fluorophore interacts with quencher in probe part of the Scorpion primer
 - Reduced fluorescence during the extension step
- Probe part of the Scorpion primer hybridizes to extended PCR product
 - Increasing fluorescence in annealing step
- Any unextended Scorpions re-anneal and are quenched

Scorpion technology allows for superior reaction speed

Reaction Mix FQ chemistry

Fast and sensitive cycling technology

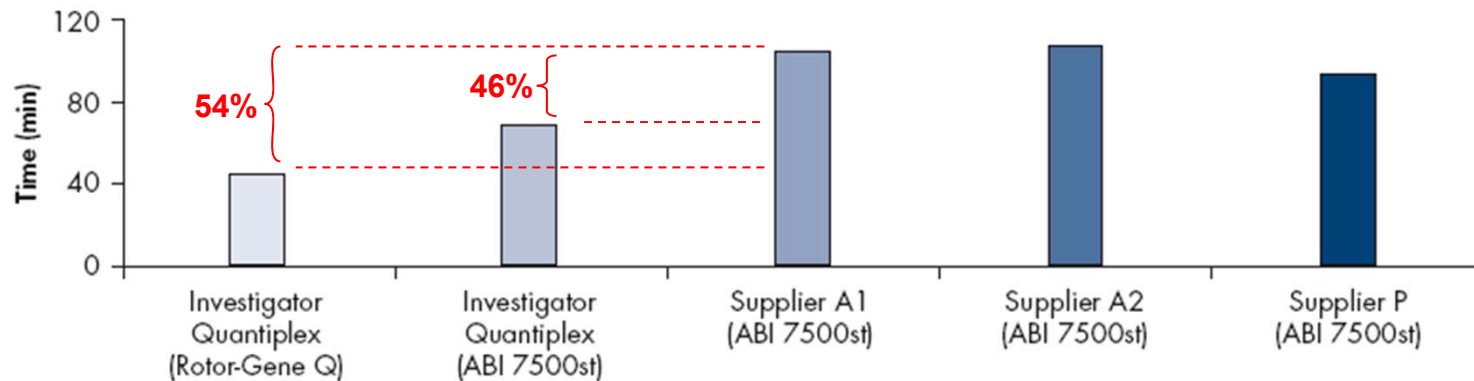
- Contains a uniquely formulated DNA polymerase
- Enables high sensitivity and speed
- Is specifically developed for human identification
- Supports the fast Scorpion primer reaction
- Contains the additive Q-Bond
 - Reduces the time required for primer/probe annealing to a few seconds
- Contains a balanced combination of KCl and NH_4Cl for increased specificity





Reaction speed

Optimized reaction set up for Rotor-Gene Q



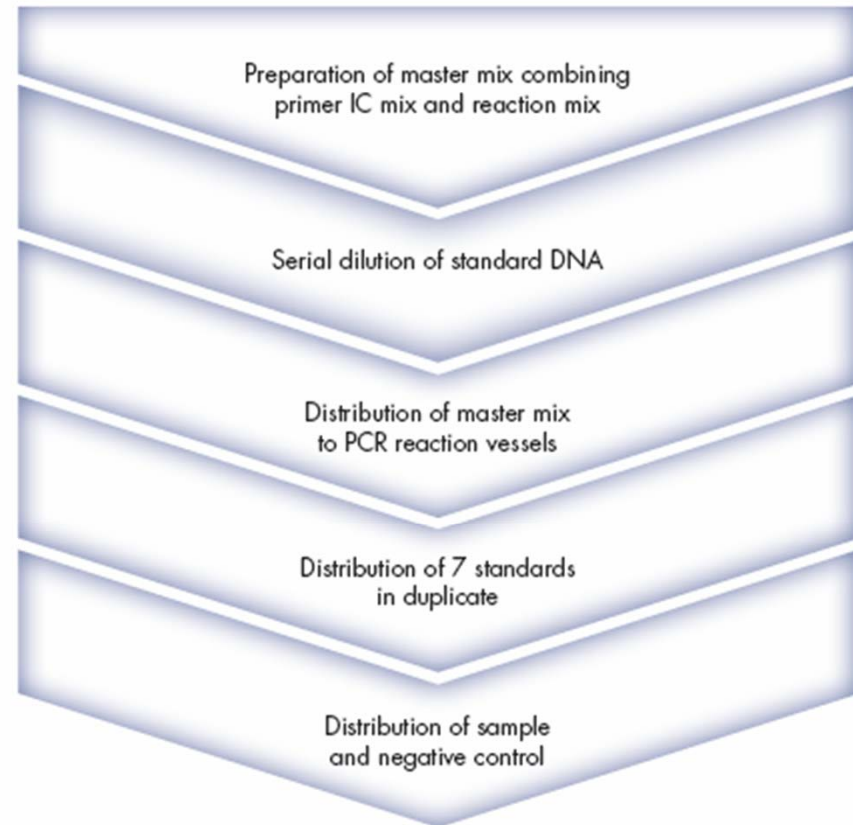
- The Quantiplex allows reduced reaction times to optimize the forensic workflow.
- Rapid results on Rotor-Gene Q – only **48 minutes**. This outperforms any other cyclers and kit combinations with a time-saving of ~ 1h (54%).
- Quantiplex is 46% faster than other kits on the ABI 7500.

Quantiplex allows for time savings of up to 54%



The automated workflow

Quantiplex reaction setup



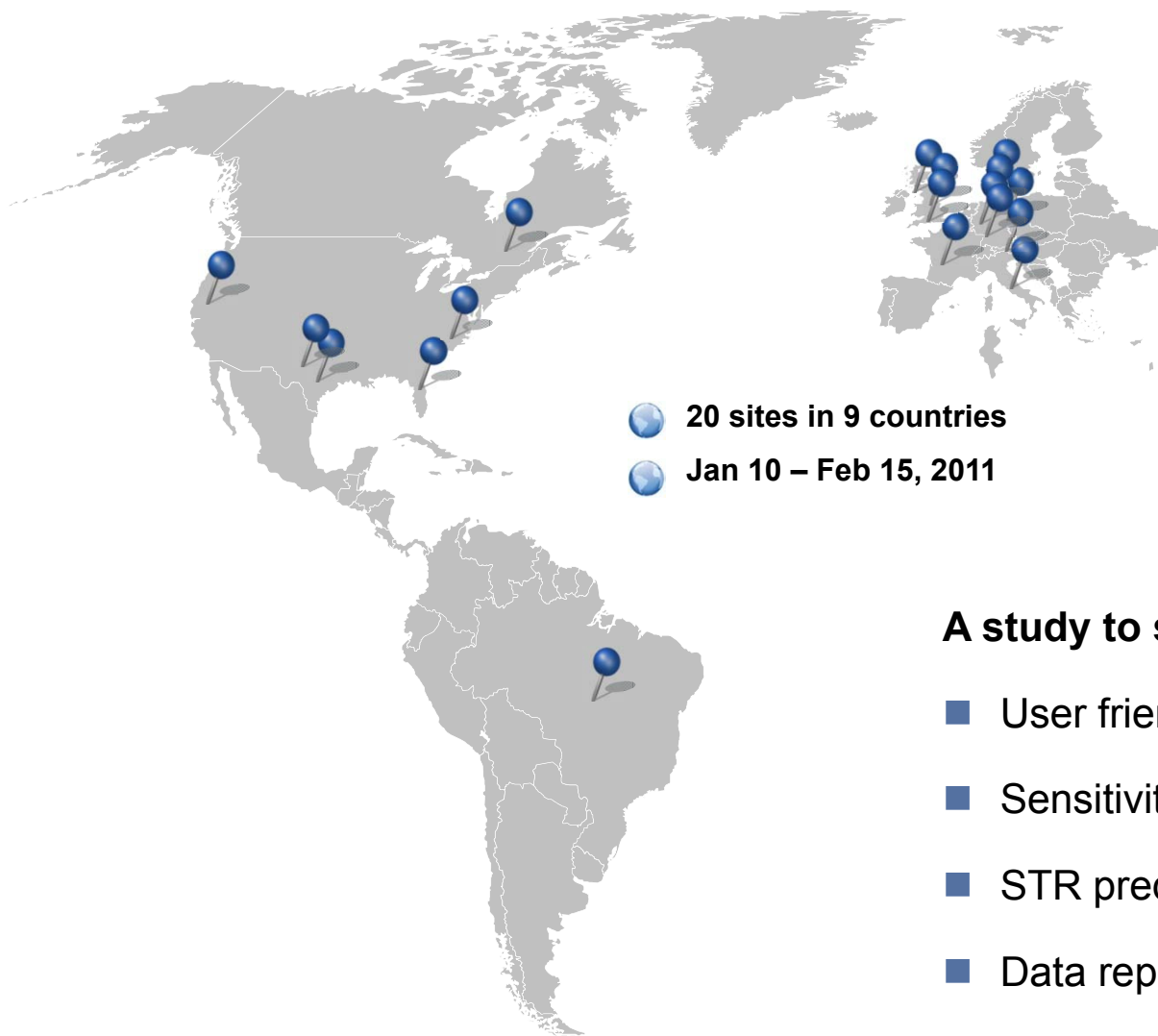
Real-time PCR quantification
(e.g., using Rotor-Gene-Q)

Template
Files



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Quantiplex field test data



A study to survey

- User friendliness
- Sensitivity
- STR predictability
- Data reproducibility



Field Test study

The protocol

Investigator Quantiplex
“DNA box”

Tube 1	DNA 1	1 ng/μl
Tube 2	DNA 2	0.1 ng/μl
Tube 3	Inhibitor 1	weak inhibitor
Tube 4	Inhibitor 2	strong inhibitor
Tube 5	Inhibitor 5	TE buffer

Inhibition studies

1	10μl DNA 1 + 10μl Inhibitor 1
2	10μl DNA 1 + 10μl Inhibitor 2
3	10μl DNA 1 + 10μl Inhibitor 5
4	10μl DNA 2 + 10μl Inhibitor 1
5	10μl DNA 2 + 10μl Inhibitor 2
6	10μl DNA 2 + 10μl Inhibitor 5

Reproducibility studies

1	DNA 2 (undiluted)
2	10μl Sample 1 + 10μl TE-Buffer
3	10μl Sample 2 + 10μl TE-Buffer
4	10μl Sample 3 + 10μl TE-Buffer
5	10μl Sample 4 + 10μl TE-Buffer

The same study was carried out at each site



Field Test study Inhibition studies

Investigator Quantiplex
“DNA box”

Tube 1	DNA 1	1 ng/μl
Tube 2	DNA 2	0.1 ng/μl
Tube 3	Inhibitor 1	weak inhibitor
Tube 4	Inhibitor 2	strong inhibitor
Tube 5	Inhibitor 5	TE buffer

Inhibition studies

Final concentration
in the sample

1	10 μl DNA 1 + 10 μl Inhibitor 1	500 pg/μl
2	10 μl DNA 1 + 10 μl Inhibitor 2	500 pg/μl
3	10 μl DNA 1 + 10 μl Inhibitor 5	500 pg/μl
4	10 μl DNA 2 + 10 μl Inhibitor 1	50 pg/μl
5	10 μl DNA 2 + 10 μl Inhibitor 2	50 pg/μl
6	10 μl DNA 2 + 10 μl Inhibitor 5	50 pg/μl

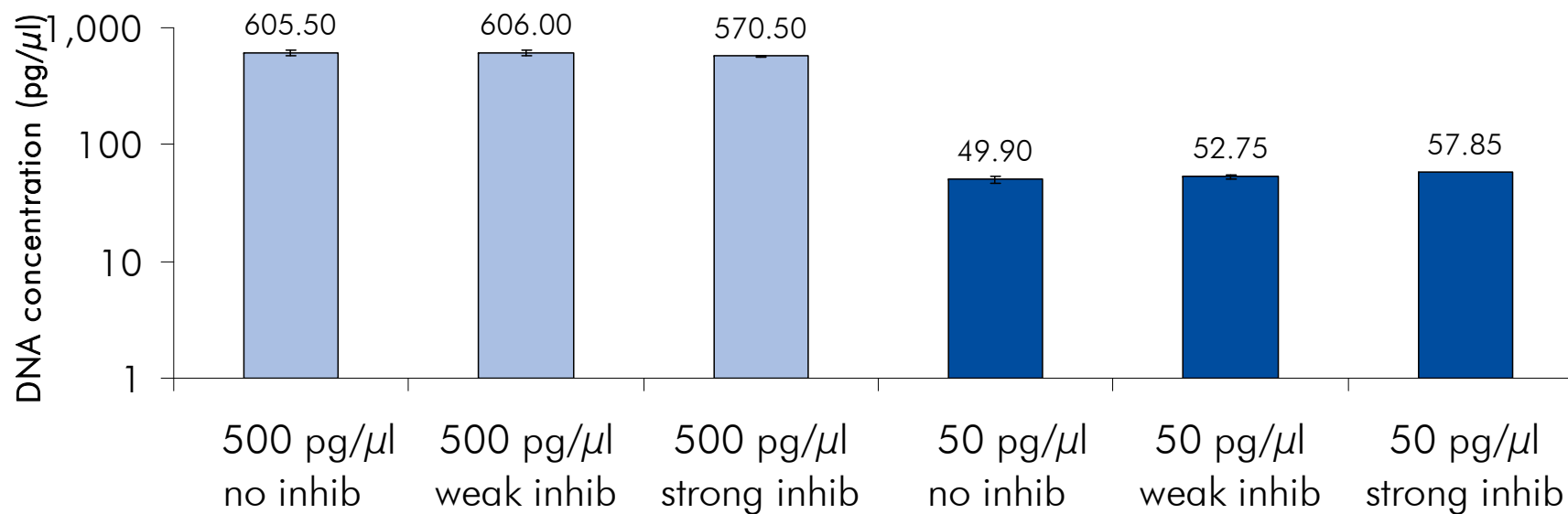
The same study was carried out at each site



Results from the inhibition studies

DNA quantification – Example data from single site

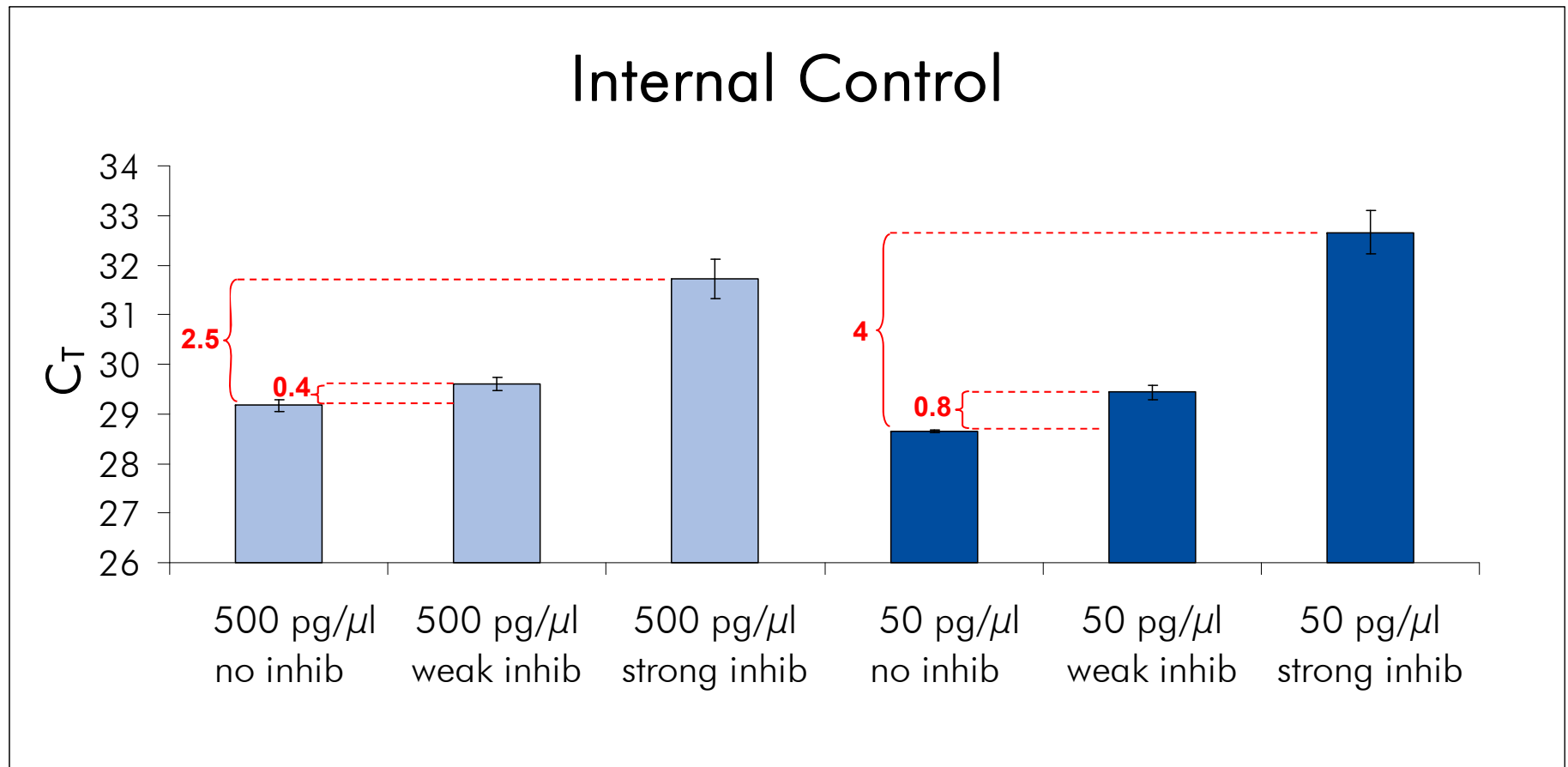
DNA Quantification



The DNA quantification remains reliable even in the presence of inhibitors

Results form the inhibition studies

Internal Control – Example data from single site



The Internal Control shows the presence of inhibitors



Field Test study Reproducibility studies

Investigator Quantiplex
“DNA box”

Tube 1	DNA 1	1 ng/μl
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Tube 3	Inhibitor 1	weak inhibitor
Tube 4	Inhibitor 2	strong inhibitor
Tube 5	Inhibitor 5	TE buffer



Reproducibility studies

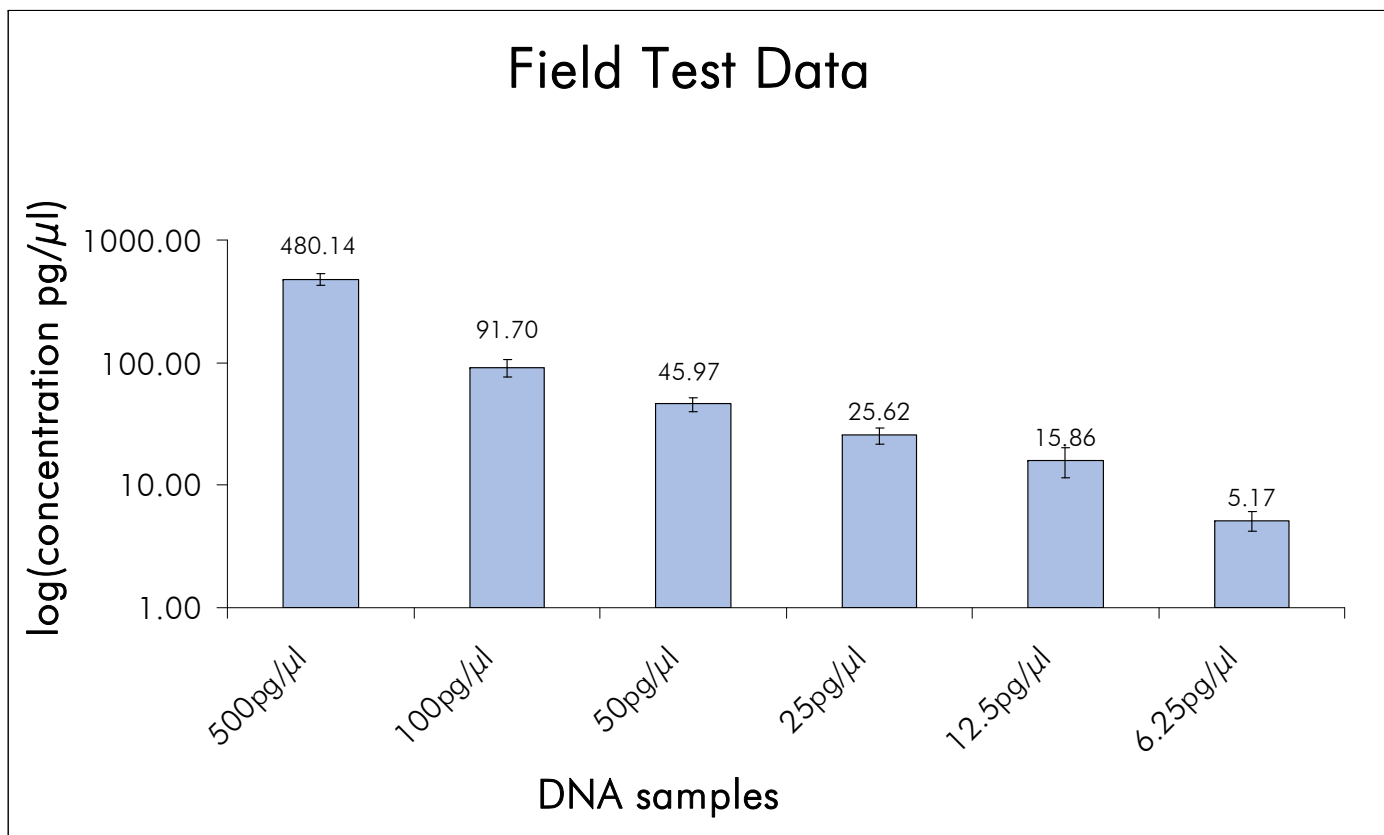
1	DNA 2 (undiluted)	100 pg/μl
2	10 μl Sample 1 + 10 μl TE-Buffer	50 pg/μl
3	10 μl Sample 2 + 10 μl TE-Buffer	25 pg/μl
4	10 μl Sample 3 + 10 μl TE-Buffer	12.5 pg/μl
5	10 μl Sample 4 + 10 μl TE-Buffer	6.25 pg/μl

The same study was carried out at each site



Data from Field Test

Quantification of standard DNAs



Data from 17 forensic labs that did beta testing using the Investigator Quantiplex Kit. Graph shows mean measured concentration $\pm$ standard error

Reliable and consistent DNA quantification within different labs



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Investigator Quantiplex Kit

Summary

Features

- DNA quantification assay based on fast cycling technology

- Validated multi-copy loci marker

- Increased size of PCR product

- Internal control / Quality sensor

- Validated on Rotor-Gene Q and ABI 7500 series

- Automatable in conjunction with the QIAgility

- Normalization Tab

Advantages

- Very fast reaction: only 48 min on Rotor-Gene Q and 57 min on ABI 7500st

- Increased sensitivity
- Concordant results across genders and ethnic populations
- Highly specific for human DNA

- For better predictive value of DNA quality/STR correlation

- Inhibition monitoring

- Protocols for most often used cyclers in human identity and forensics testing
- No calibration necessary

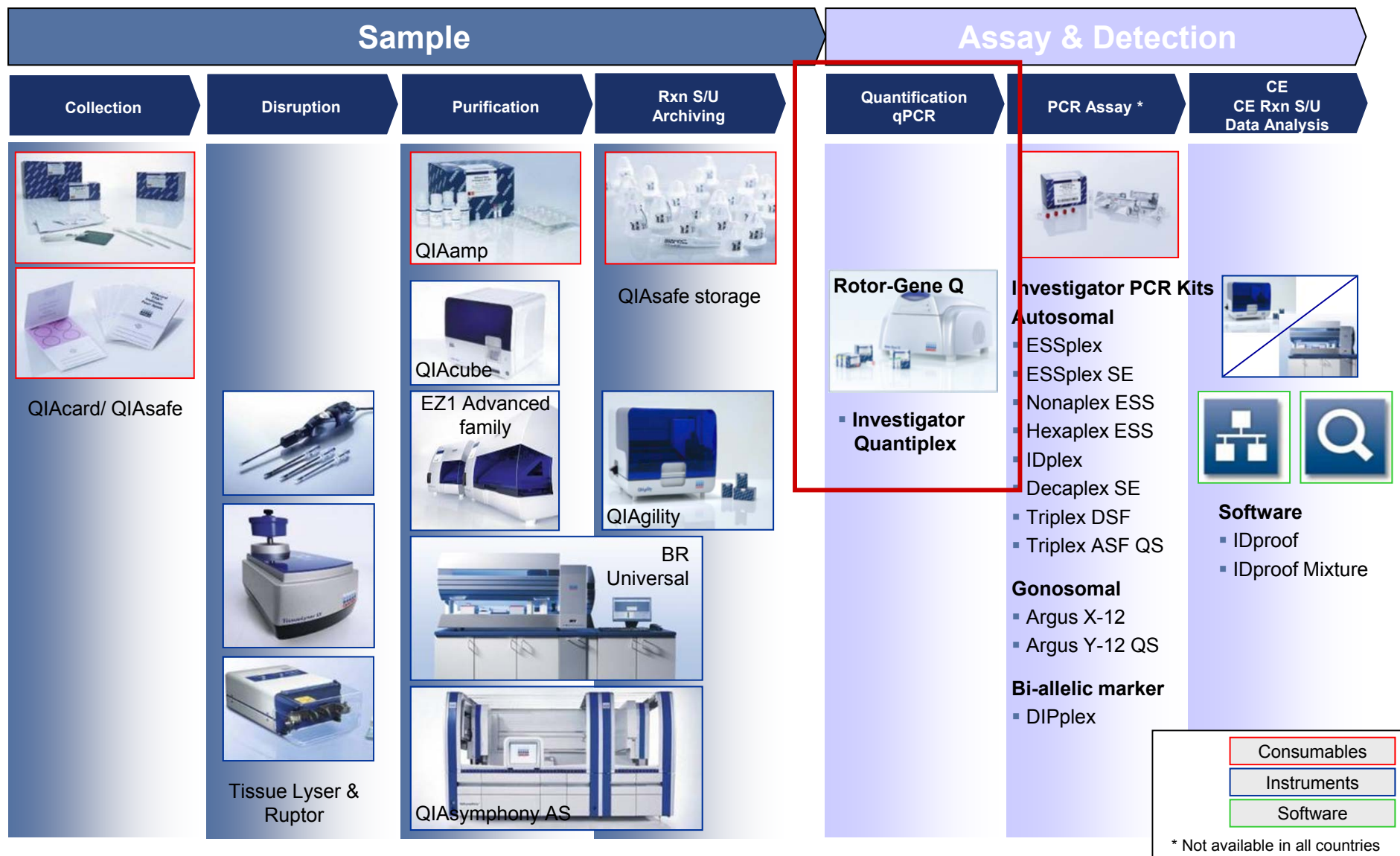
- Accuracy and time saving by offering specific Q-protocols

- Easy setup of downstream STR analysis



Human Identity & Forensics

Portfolio overview





**Accurate and sensitive
real-time PCR-based DNA
quantification
in less than 50 minutes**

Thank you