# Innovations in quantitative and qualitative forensic assessment of human DNA
Streamlining STR & Y-STR profiling workflow

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# Our recent past on DNA Quantification and STR profiling workflow ....

STR & Y-STR profiling workflow

DNA Extraction & Purification

Quantifiler Duo Kit

Human DNA (RPPH1, 140bp)
Male DNA (SRY, 130 bp)
IPC (130 bp)

> 20 pg /ul

LLDNA &
No DNA

IPC
Inhibition

Unbalanced
F/M Mixtures
>40/1

Degraded
DNA

No real cero
By QFduo

Low Tolerance to
PCR Inhibitors

Without
Information

STR
Y-STR

STR
Y-STR

STR
Y-STR

Y-STR

STR
MINI-STR
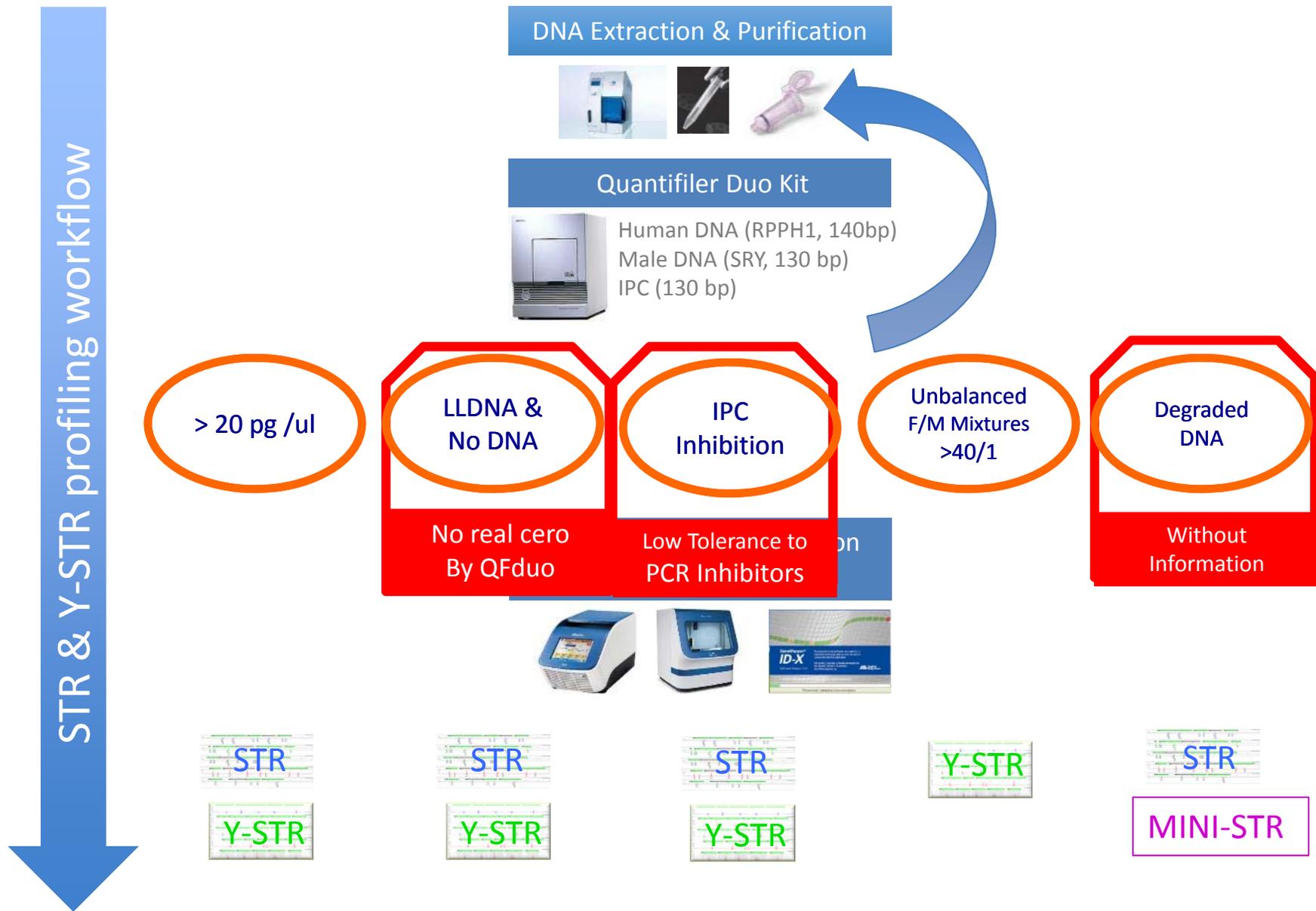
QFTRIO Targets

Target	Amplicon Length	Ploidy	Copy number	Dye/Quencher
Human Target, small autosomal	80 bases	Diploid	Multi-copy	VIC Dye MGB quencher
Human Target, large autosomal	214 bases	Diploid	Multi-copy	ABY Dye QSY quencher
Human Male Target	75 bases	Haploid	Multi-copy	FAM Dye MGB quencher
Internal PCR Control	130 bases	NA	Synthetic DNA	JUN Dye QSY quencher

- **Human DNA**
- **Male DNA**
- **DNA Degradation**
- **PCR Inhibitors**
- **Higher sensitivity and tolerance to PCR inhibitors**

QFTRIO: Sensitivity, Reproducibility, and Specificity Experiments

X2

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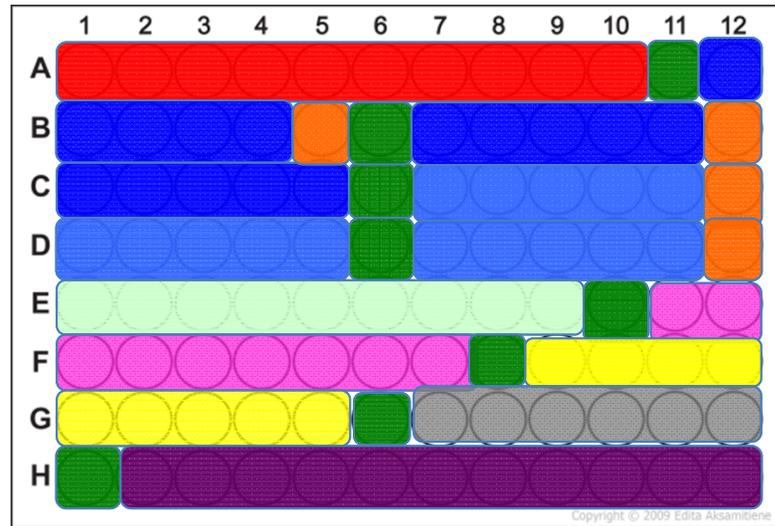
Color		Description
	🟥	**QFTRIO STDs X2** (50,000 5,000 500 50 and 5 pg/µl)
Sensitivity & Reproducibility	🟦	**5 QFDUO STDs X3** (Last 5 dilutions: 1,850 620 210 68 and 23 pg/µl)
	🟦	**5 NIST SRM 2372 Dilution series X3** (5,700 570 57 5.7 and 0.57 pg/µl)
	🟩	**3 Male Genomic DNA dilution series X3** (500, 50 and 5 pg/µl)
	🟪	**3 Female Genomic DNA dilution series X3** (500, 50, and 5 pg(µl)
Reproducibility Dynamic range	🟨	**3 GHEP Proficiency samples X3**
	⬜	**2 High DNA concentrated samples X3** (> 100 ng)
Specificity	🟪	**21 Bacterial DNAs**
	🟩	**8 Negative PCR Controls**
	🟧	**4 Positive PCR Controls** (DNA 007; input 100 pg)
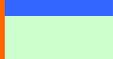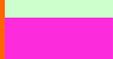
QFTRIO: F/M Mixtures and Forensic samples
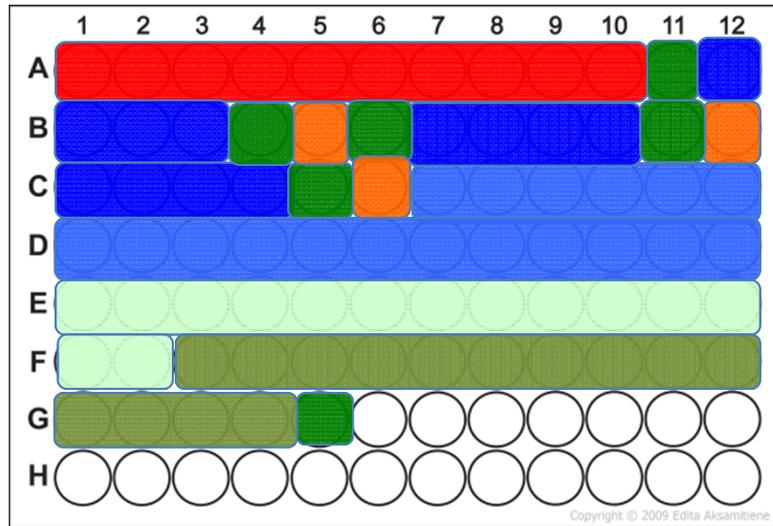
Color	Description
🟥 (red)	**QFTRIO STDs X2** (50,000, 5,000, 500, 50, and 5 pg)
🟦 (blue)	**4 Female/Male DNA Mixtures X3** (100:1 1,000:1 2,000:1 and 4,000:1)
🟦 (light blue)	**9 Body Fluid Mixtures X2** (GHEP & GEDNAP samples) (2 and 3 contributors)
🟩 (light green)	**7 Forensic DNA samples X2:** Low Level Y-DNA content and Inhibition
🟫 (olive green)	**7 Forensic DNA samples X2:** Degraded DNA from bones and FFPE samples
🟩 (dark green)	**6 Negative PCR Controls**
🟧 (orange)	**3 Positive PCR Controls** (DNA 007; input 100 pg)

DNA Mixtures

Forensic samples

QFTRIO: NIST SRM 2372A Quantitative Data

QFTRIO Standard Curves (Small auto, Y, and Large auto)
■■ NIST SRM 2372A,   ■ QFTRIO Standards

QFTRIO: NIST SRM 2372A Quantitative Data

NIST SRM 2372A QF3 log data (6 replications in 2 plates)
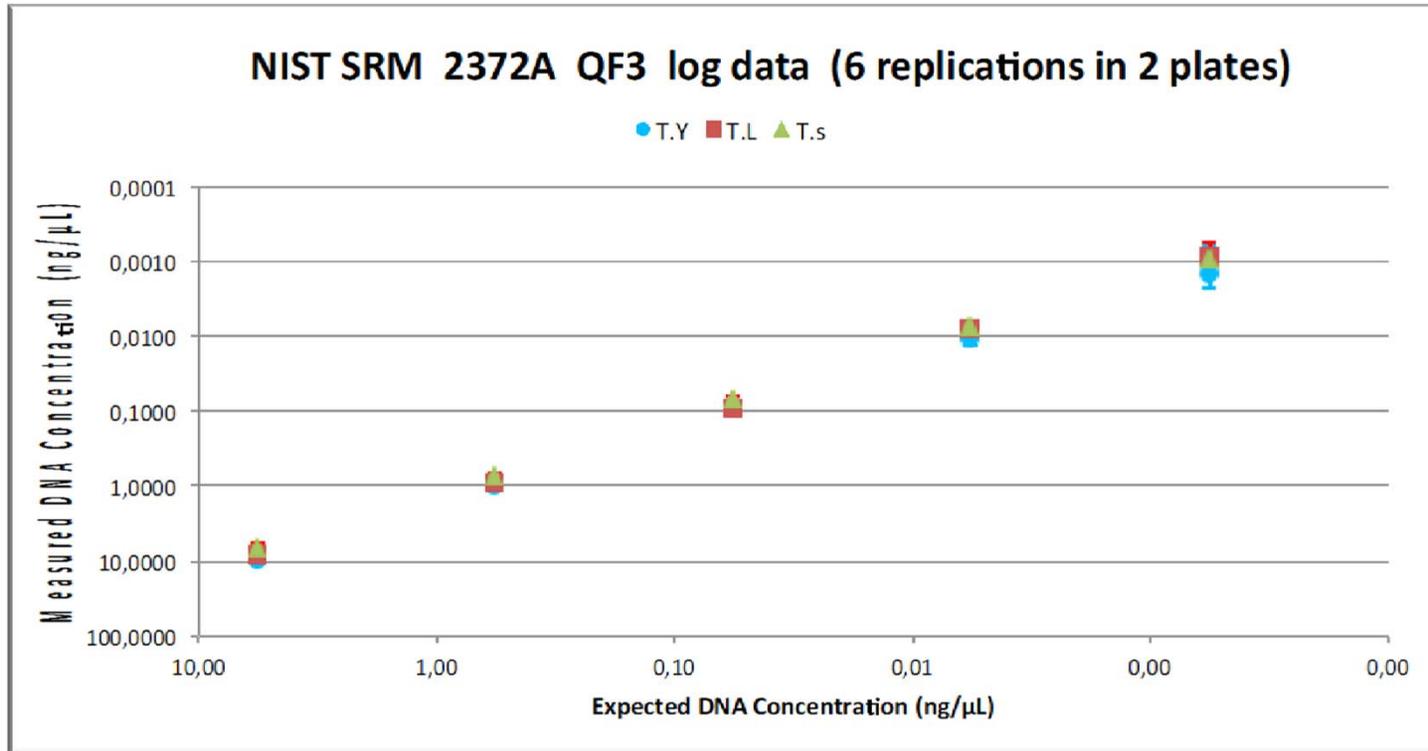
QFTRIO: Sensitivity to detect male DNA from mixtures

| Mixture	F/M ratio	Female DNA (9974)	Male DNA (2800)	Human Male Target results (ng/ul)
MIX_1-1	100:1	5 ul (10 ng/ul)	5 ul (0.1ng/ul)	0.0703
MIX_1-2		5 ul (10 ng/ul)	5 ul (0.1ng/ul)	0.0772
MIX_1-3		5 ul (10 ng/ul)	5 ul (0.1ng/ul)	0.0812
MIX_2-1	1000:1	5 ul (10 ng/ul)	5 ul (0.01ng/ul)	0.0062
MIX_2-2		5 ul (10 ng/ul)	5 ul (0.01ng/ul)	0.0063
MIX_2-3		5 ul (10 ng/ul)	5 ul (0.01ng/ul)	0.0083
MIX_3-1	2000:1	10 ul (10 ng/ul)	5 ul (0.01ng/ul)	0.005
MIX_3-2		10 ul (10 ng/ul)	5 ul (0.01ng/ul)	0.0048
MIX_3-3		10 ul (10 ng/ul)	5 ul (0.01ng/ul)	0.0052
MIX_4-1	4000:1	10 ul (10 ng/ul)	2.5 ul (0.01ng/ul)	0.0004
MIX_4-2		10 ul (10 ng/ul)	2.5 ul (0.01ng/ul)	0.0007
MIX_4-3		10 ul (10 ng/ul)	2.5 ul (0.01ng/ul)	0.0014

Amplification Plots Male DNA Target

100:1

1000:1

2000:1

4000:1

F [DNA]: 8000 pg/ul

M [DNA]: 2 pg/ul
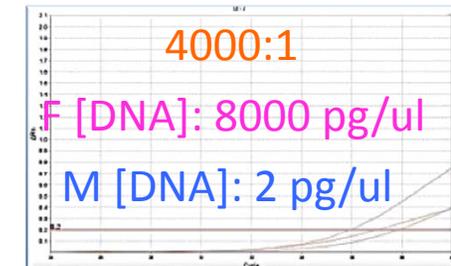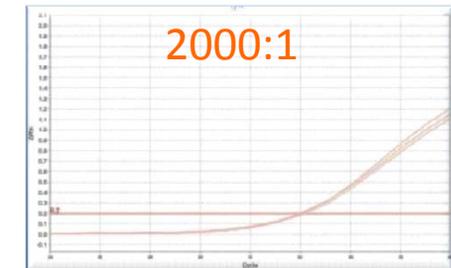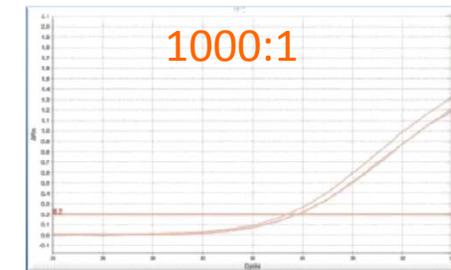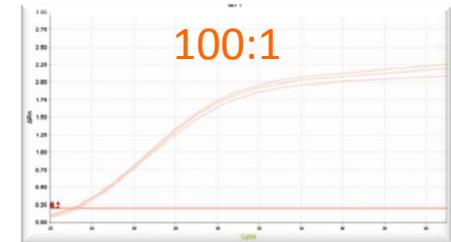
QFTRIO: Specificity against Bacterial DNA

**Amplification Plots
Small Autosomal Target**

Plate 1
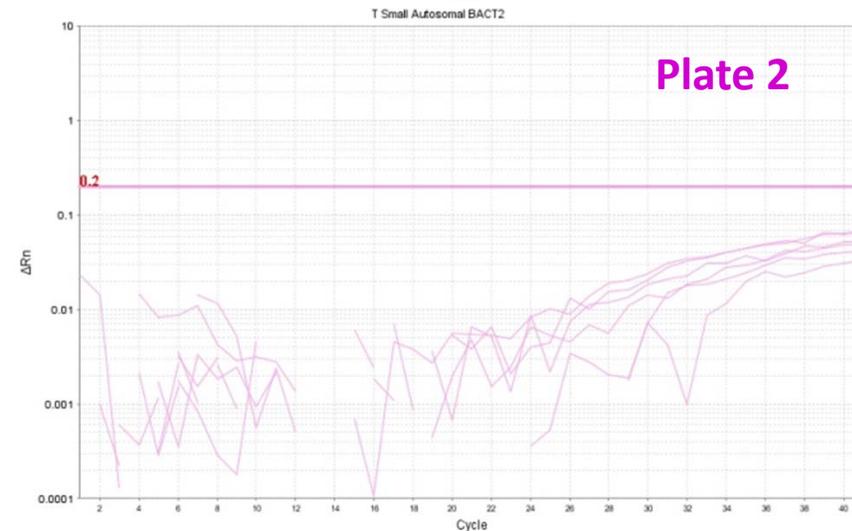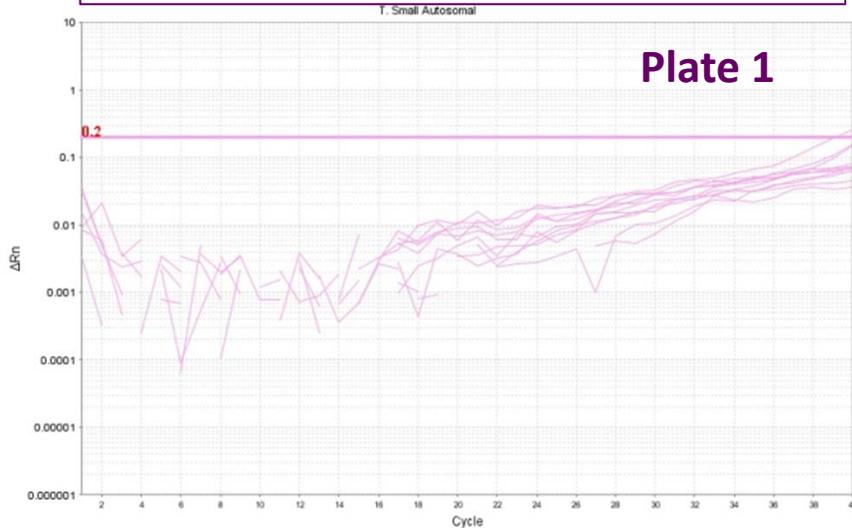
01 *Neisseria meningitidis* *(2 ng/ul)*
02 *Streptococcus pneumoniae* *(2 ng/ul)*
03 *Streptococcus agalactiae (serogrupo B)* *(2 ng/ul)*
04 *Staphylococcus aureus* *(2 ng/ul)*
05 *Escherichia coli,* *(2 ng/ul)*
06 *Bordetella pertussis* *(10 ng/ul)*
07 *Bordetella parapertussis* *(10 ng/ul)*
08 *Alcaligenes faecalis* *(10 ng/ul)*
09 *Acinetobacter calcoaceticus* *(10 ng/ul)*
10 *Flavobacterium odoratum* *(10 ng/ul)*
11 *Morganella morganii* *(10 ng/ul)*

Plate 2

12 *Pseudomonas stutzeri* *(10 ng/ul)*
13 *Providencia stuartii* *(10 ng/ul)*
14 *Proteus mirabilis* *(10 ng/ul)*
15 *Citrobacter freundii* *(10 ng/ul)*
16 *Pseudomonas aeruginosa* *(10 ng/ul)*
17 *Salmonella enteritidis* *(10 ng/ul)*
18 *Neisseria lactamica .* *(10 ng/ul)*
19 *Shigella flexneri* *(10 ng/ul)*
20 *Serratia marcencens* *(10 ng/ul)*
21 *Haemophilus Influenzae (serotipo b)* *(2 ng/ul)*

QFTRIO: Forensic Samples with PCR inhibitors

Bloodstain on leather

QFDUO (ng/ul)	
Human DNA	N.D.
Male DNA	N.D.
IPC	N.D.

QFTRIO (ng/ul)	
Large Auto DNA	N.D.
Small Auto DNA	2.2778
Male DNA	1.6993
IPC	N.D.

Globalfiler Full Profile
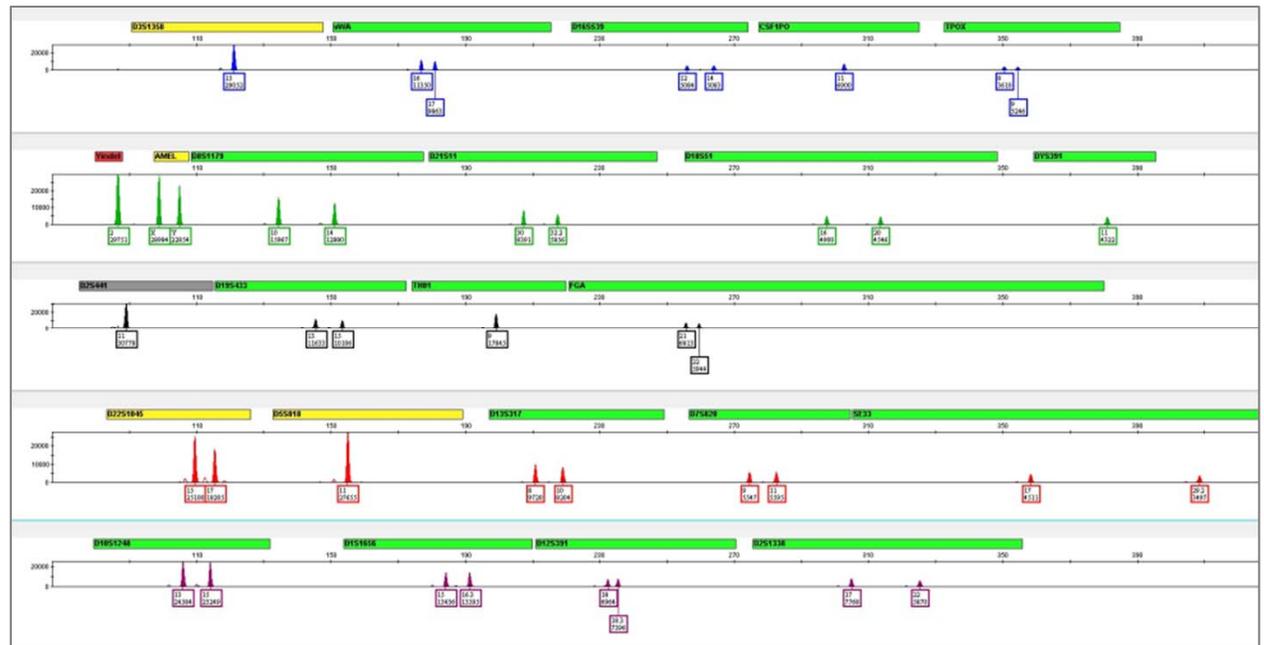
QFTRIO: Forensic Samples with Degraded DNA

QFTRIO (ng/ul)		
Femur	Large Auto DNA	0.0438
	Small Auto DNA	0.3596
	Male DNA	N.D.
	IPC	27.260
	DI	**8.2173**
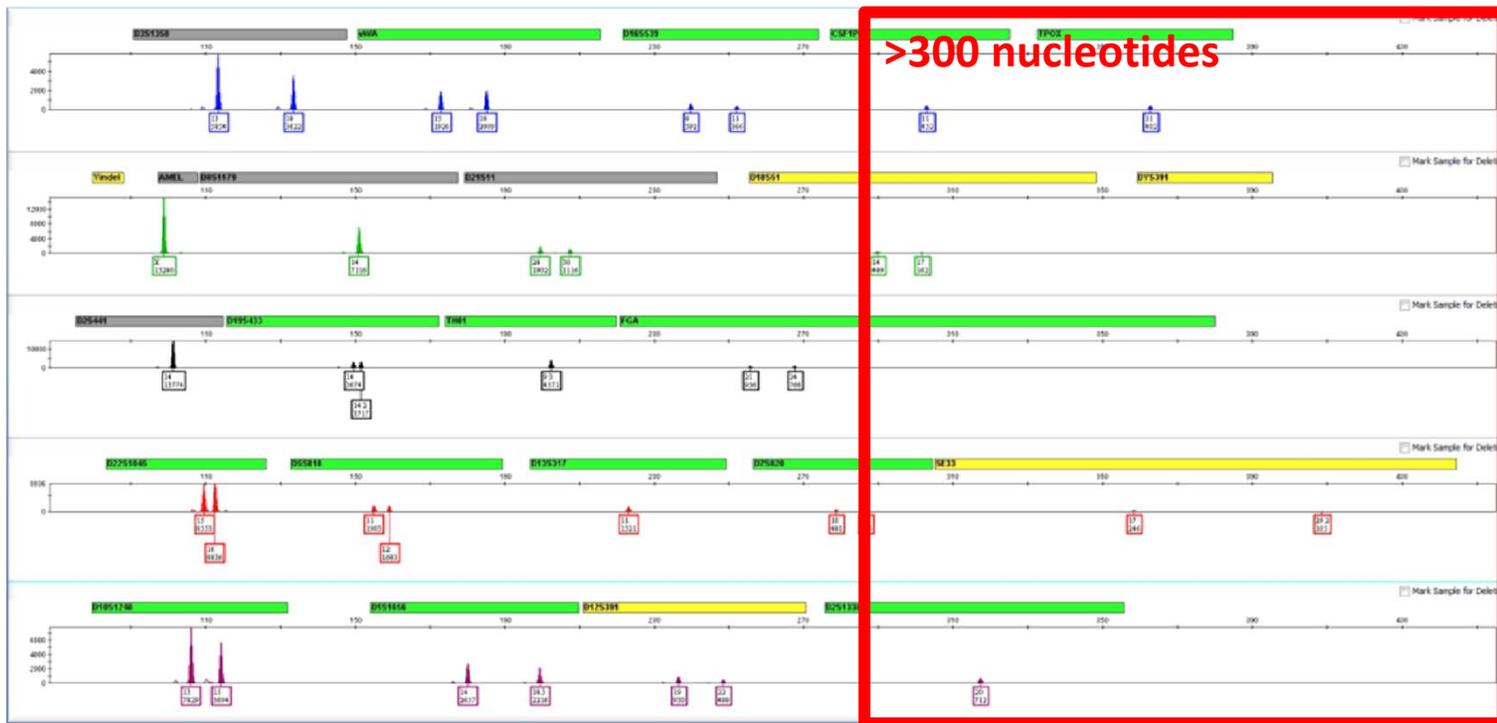
>300 nucleotides

Globalfiler

QFTRIO: Forensic Samples with Degraded DNA

	QFTRIO (ng/ul)	
Femur	Large Auto DNA	0.0061
	Small Auto DNA	0.3011
	Male DNA	N.D.
	IPC	27.333
	DI	**49.098**

>200 nucleotides

Globalfiler

QFTRIO: Forensic Samples with Degraded DNA

QFTRIO (ng/ul)	
Large Auto DNA	**0.0002**
Small Auto DNA	**0.0169**
Male DNA	**0.0141**
IPC	**27.206**
DI	**102.10**

FFPE

>150 nucleotides

Globalfiler

QFTRIO-Y: A Sensitive method for Male DNA screening:
Comparison with Y-STR profiling sensitivity

**245 Forensic DNA samples with male DNA concentration below 10 pg /µl (QFTRIO Y-Target) and also analysed by Y-STR-PCR (YFilerPlus) and CE detection were reviewed to compare and calibrate the detection limits of each method**

The samples belong to criminal cases in which **the investigation of male cell remains** is requested. Body samples (vaginal swabs, pubis swabs, perineal swabs, anal swabs, oral swabs, fingernails,..) were mainly analyzed but also several biological stains on different substrates

DNA samples	Male DNA Concentration	Y-STR Profiling success
164	**< 1 pg/µl**	**N.D. or Inconclusive results**
81	**10 -1 pg/µl**	**59 %** (mainly partial and/or low signal Y-STR profiles)

Samples with Qftrio-Y results under 1 pg/μl rendered Negative (or Inconclusive) Y-STR profiling results

	QFTRIO (ng/ul)	
	Large Auto DNA	1.0476
	Small Auto DNA	1.0871
Vaginal swab	Male DNA	0.0007
	IPC	27.2851
	DI	1.0377
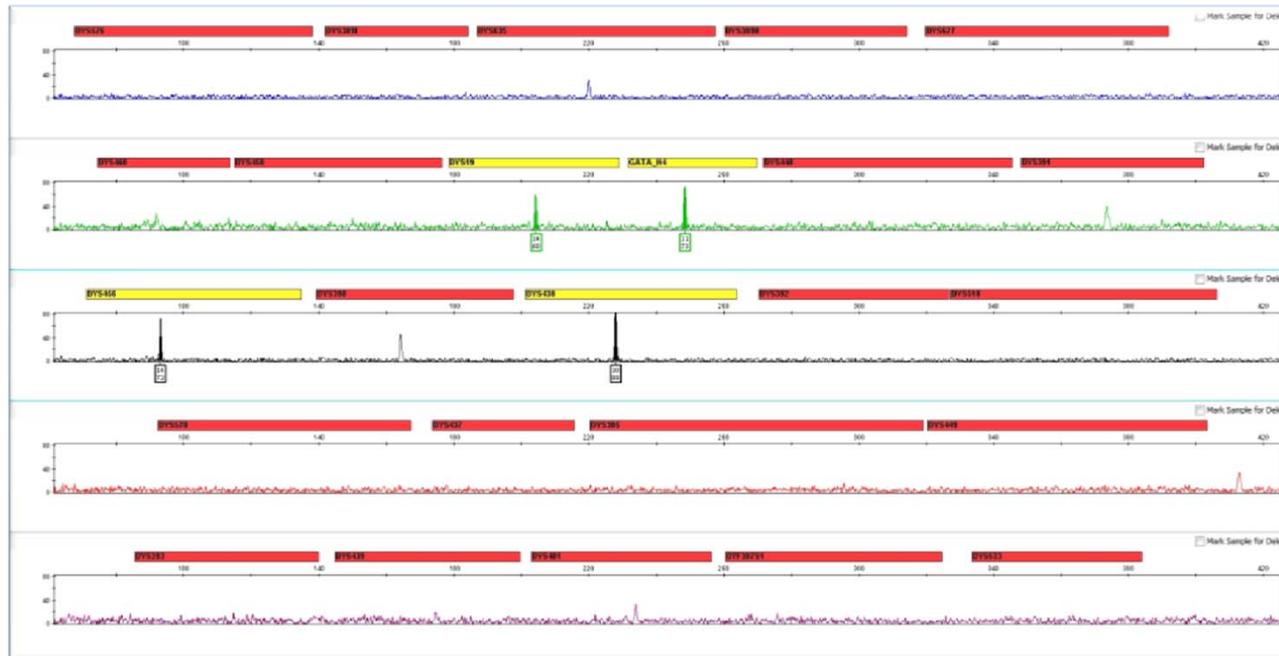
59 % of Samples with Qftrio-Y results between 10- 1 pg/µl rendered Y-STR profiles (mainly partials)

Endocervix Swab
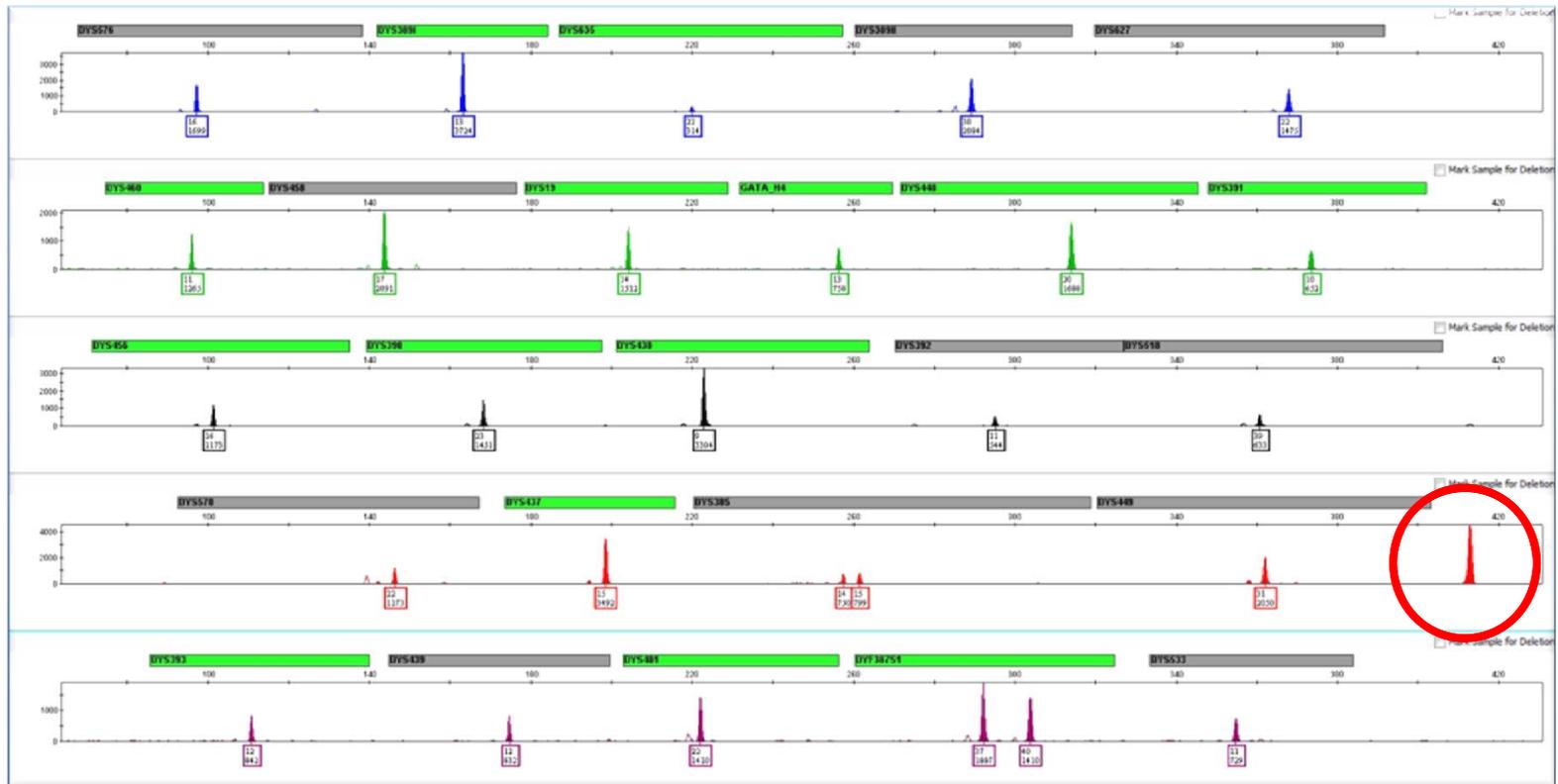
| QFTRIO (ng/ul)	
Large Auto DNA	37.0246
Small Auto DNA	37.4354
Male DNA	0.010
IPC	29.1755
DI	1.0111

F:M DNA ratio: 3700 : 1

Yfiler Plus DNA input (10µl)	
Male DNA	100 pg
Female DNA	**370 ng**

59 % of Samples with Qftrio-Y results between 10- 1 pg/µl rendered Y-STR profiles (mainly partials)
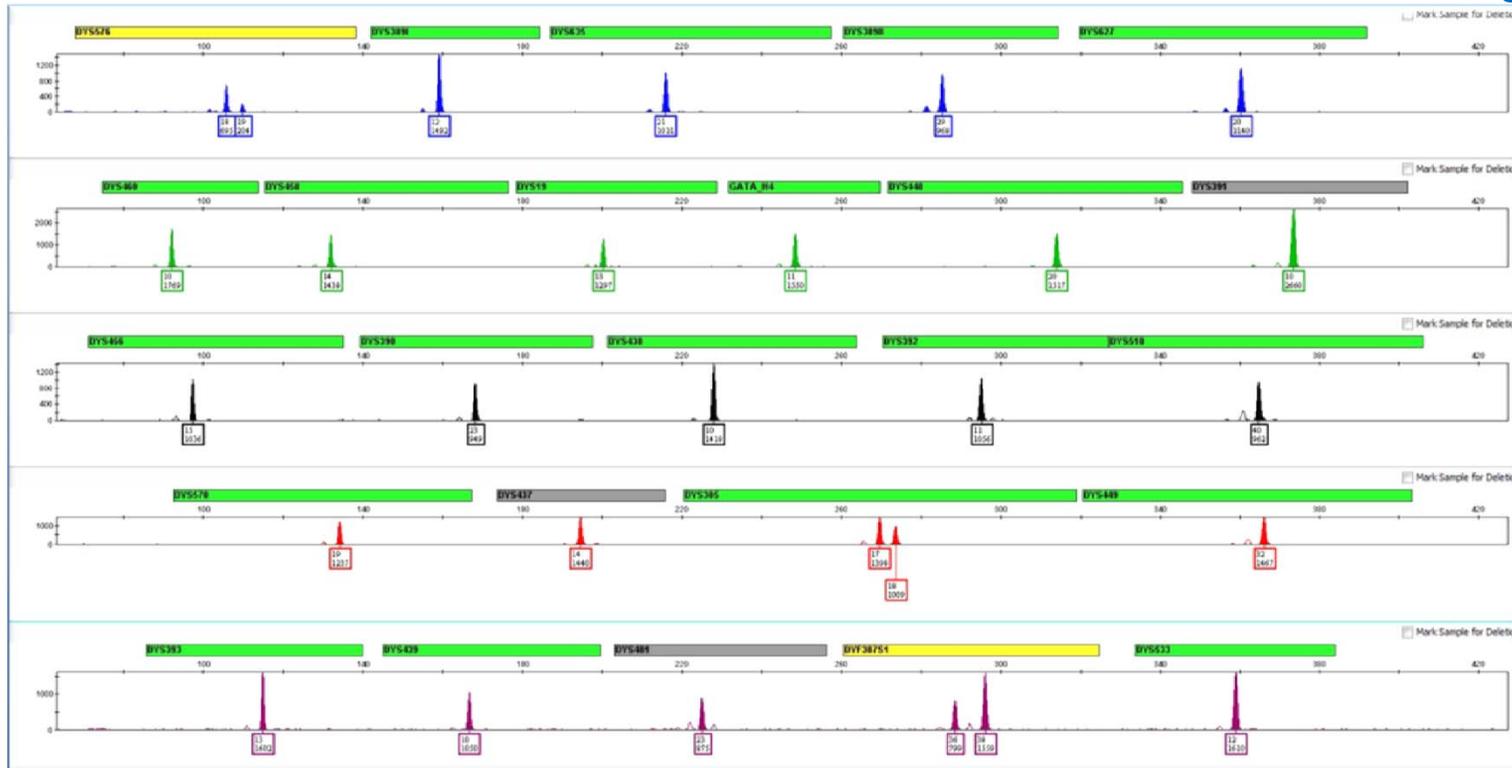
QFTRIO (ng/ul)	
Large Auto DNA	0.0146
Small Auto DNA	0.0208
Male DNA	0.0067
IPC	27.6831
DI	1.4193

Yfiler Plus DNA input (10µl)	
Male DNA	67 pg

Our present on Human DNA Quantification and STR profiling workflow

STR & Y-STR profiling workflow

DNA Extraction & Purification

Quantifiler Trio Kit

2X Male DNA Screening by QFTRIO

| > 1 pg /ul | <1 pg /ul | IPC | Unbalanced | Degraded |
| Any Target | Male-Target | Inhibition (Reduced) | F/M Mixtures >40/1 | DNA DI >10-20 |

Multiplex PCR, CE Detection & Data Analysis

STR
Y-STR

STR

STR
Y-STR

Y-STR

MINI-STR
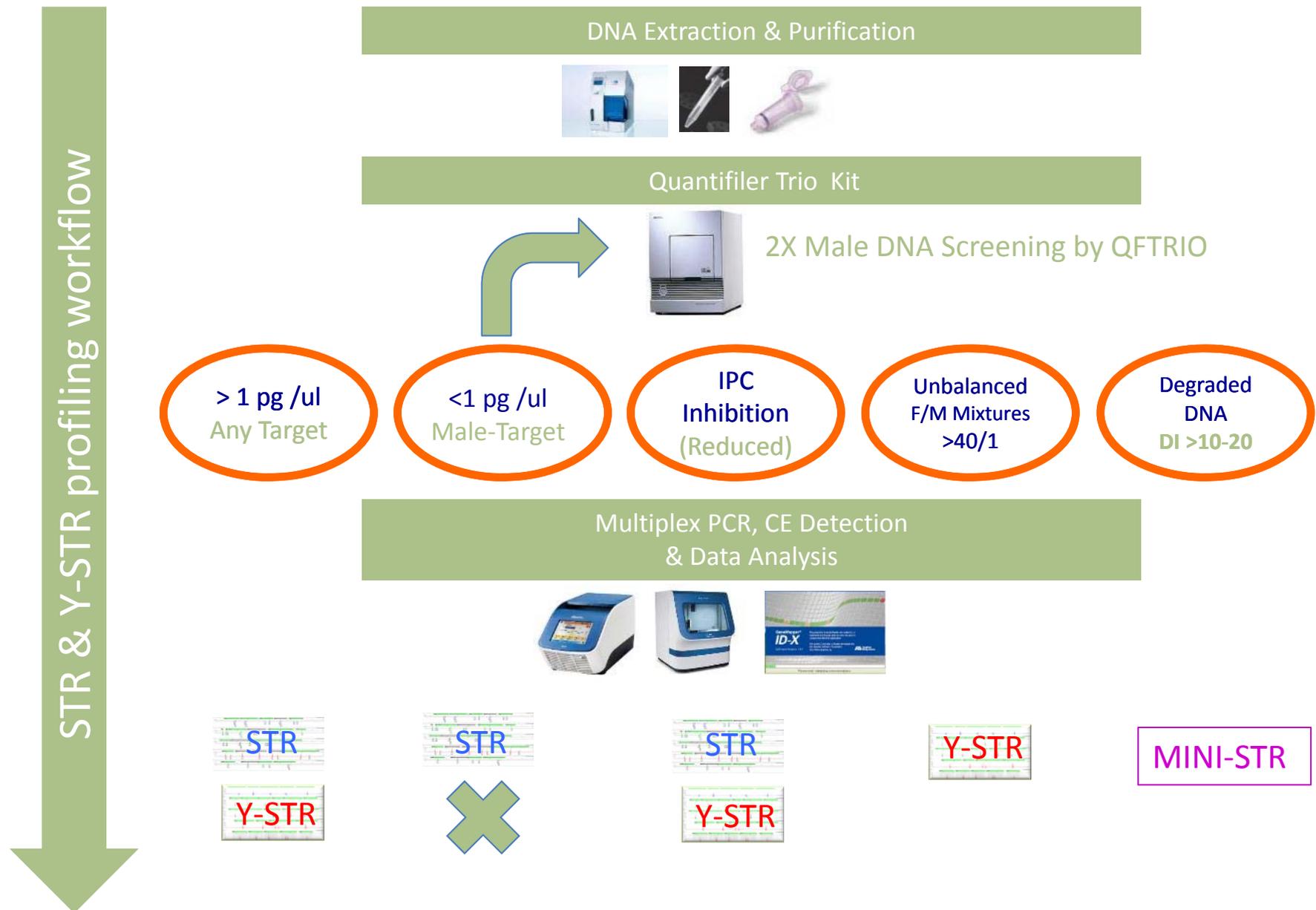
Acknowledgements

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STR and Y-STR EPGs

Pablo Martin

Figure in slide 7

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