

Application of Applied Biosystems™NGM Detect™ PCR Amplification Kit in critical forensic caseworks

Human Identification Solutions (HIDS)



Rome, 3rd and 4th May 2018

Dott.ssa Venusia Cortellini

Università degli Studi di Brescia – Cattedra di Medicina Legale

Applied Biosystems™ NGM Detect™ PCR Amplification Kit



Increased sensitivity for casework samples with low DNA concentrations.

When combined with the NGM SElect™ Kit, it meets the dual amplification requirements.

With complementary marker positions in the two kits, it is possible to gather maximum information from challenging casework samples.

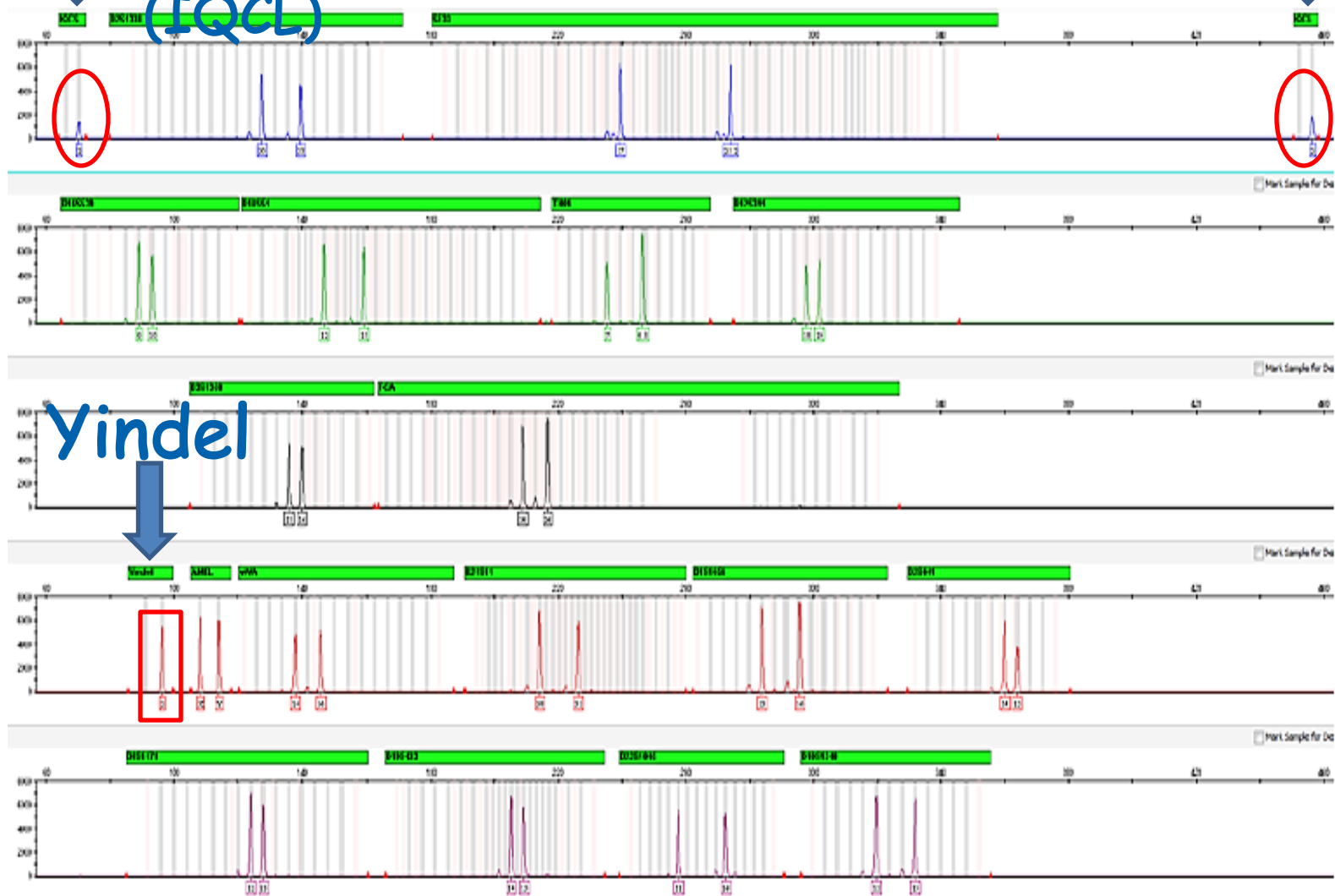
Applied Biosystems™ NGM Detect™ PCR Amplification Kit

This PCR Amplification Kit is a new, powerful forensic DNA analysis solution in casework samples.

Offers new possibilities by using:

- A **six color approach**,
- Newly **designed primers**,
- **Internal quality controls** (IQC),
- a **rearrangement of the loci** compared to the AmpF1STR® NGM SElect™ PCR Amplification Kit,
- An additional **Y-indel**,
- A **higher DNA input volume**

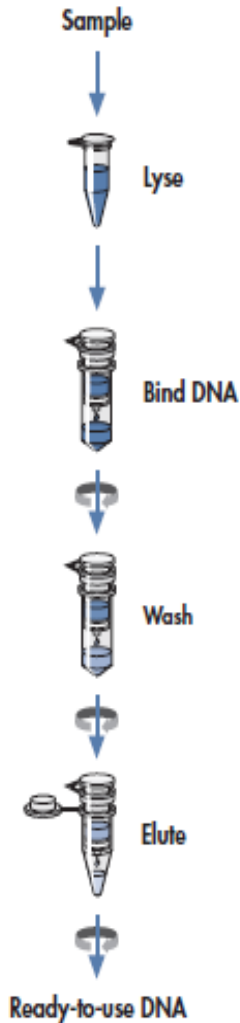
IQCsystem: LMW (IQCS) and HMW (IQCL)



Applied Biosystems™ NGM Detect™ PCR Amplification Kit

Feature	NGM Detect PCR Amplification Kit
PCR cycling time	<60 min
Total number of markers	18 (16 autosomal STRs, Amelogenin and Y indel)
Number of mini-STRs (whole marker range <230 bp)	7
Total reaction volume	25 µL
Sample input volume	15 µL
Size of SE33 amplicon	Minimum: 184, Maximum: 333
Sex identification redundancy	Amelogenin and Y indel
Number of dyes	6
Dual amplification capabilities	Can be partnered with the NGM SElect kit for dual amplification purposes
PCR verification system	IQC-2 markers, one low molecular weight (LMW) and one high molecular weight (HMW) flanking STR and markers for sex confirmation

Extraction:
Chelex[®] 100
QIAamp[®] DNA Micro Kit



Quantification:
Applied Biosystems[®]
7500 Real-Time PCR
Systems

Amplification:
Applied Biosystems[™]
NGM Detect[™]
NGM Select[™]
Identifiler[®] Plus



Electrophoresis:
Applied Biosystems[™]
3500 Genetic Analyzer



Initial incubation step	Cycle (30 cycles)			Final extension	Final hold
	Denature	Anneal	Extend		
HOLD	CYCLE			HOLD	HOLD
95°C, 1 minute	96°C, 5 seconds	59°C, 21 seconds	65°C, 29 seconds	60°C, 5 minutes	4°C ^[1]

Forensic caseworks

- Sexual violence
- Criminal investigation
- Homicide
- Suspicious death
- Paternity
- Exhumation
- Human identification

The sample typologies had referred to: buccal swabs, nail swabs, swabs deriving from sexual violence, human biological tissues and liquids, clothes and objects of common use.

Number of samples analyzed: 71.

Samples

In the table below are reported some samples analyzed in the present study.

Sample type	Forensic casework	Quantification
Buccal swab	Paternity	0.030849
Biological liquid (Blood)	Human identification	0.011017
Human tissue (Spinal cord)	Human identification	0.0064
Fornix swab	Sexual Violence	0.062667
Human tissue (nail)	Exhumation	0.00191
Nail swab	Homicide	0.004151
Biological liquid (Urine)	Human identification	Undetermined
Common objects (Toothbrush)	Suspicious death	0.010698
Histological slide	Sexual violence	0.001057
Clothes (Cap)	Criminal investigation	0.000295
Common objects (Gauze)	Criminal investigation	0.005846
Clothes (Slip)	Sexual Violence	0.000469

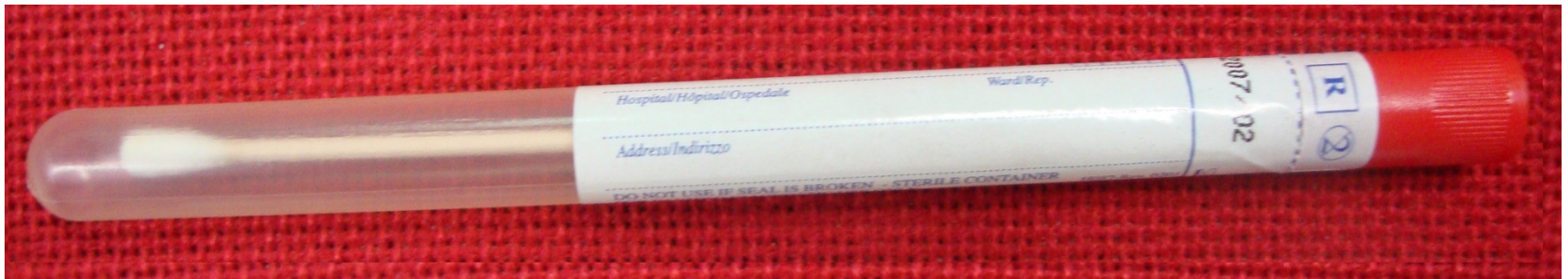
Paternity - Buccal swab

In this paternity case, the subjects involved in the analysis were: father (P), mother (M) and son (F).

The oral mucosa cells of all people were collected by buccal swab.

DNA was extracted with Chelex® 100 resin.

The samples were amplified with the Applied Biosystems™ NGM Detect™ PCR Amplification Kit, AmpFℓSTR® NGM SElect™ PCR Amplification and AmpFℓSTR® Identifiler® Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Buccal swab

Human Identification - Biological liquid (Blood)

Attempted murder-suicide: a man took his own life, throwing himself from the balcony, after stabbing his wife.

During the autopsy, the blood taken.

Subsequently, DNA from this human liquid was extracted with Chelex[®] 100.

The samples were amplified with the Applied Biosystems[™] NGM Detect[™] PCR Amplification Kit, AmpF_lSTR[®] NGM SElect[™] PCR Amplification and AmpF_lSTR[®] Identifiler[®] Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Died`s blood

Human Identification - Human tissue (Spinal cord)

In this case of human identification, the subject was a man found in a lake. Theoretically, the man could have been a diver, who disappeared about 25 years earlier.

Genetic analysis was necessary to determine the true identity.

During the autopsy, the spinal cord was taken.

Subsequently, DNA from this human tissue was extracted with Chelex® 100 and QIAamp® DNA Micro Kit.

The samples were amplified with the Applied Biosystems™ NGM Detect™ PCR Amplification Kit, AmpFℓSTR® NGM SElect™ PCR Amplification and AmpFℓSTR® Identifiler® Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Biocassetta

Sexual violence - Fornix swab

Nigerian woman, reported that she was raped by her boyfriend and two other men.

Genetic investigations were necessary to assess the veracity of the information provided by the victim and to determine any multiple male genetic profile.

One of the samples analyzed was the fornix swab, DNA was extracted by Chelex[®] 100.

The samples were amplified with the Applied Biosystems[™] NGM Detect[™] PCR Amplification Kit, AmpF_lSTR[®] NGM SElect[™] PCR Amplification and AmpF_lSTR[®] Identifiler[®] Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Fornix swabs

Exhumation - Human tissue (finger nail)

Exhumation within a paternity ordered by the judicial authority.

To determine the paternity of a subject whose father died 25 years before.

Genetic analyzes were necessary to determine the filiation.

DNA was extracted from finger nails with Chelex[®] 100 and QIAamp[®] DNA Micro Kit.

The samples were amplified with the Applied Biosystems[™] NGM Detect[™] PCR Amplification Kit, AmpF_lSTR[®] NGM SElect[™] PCR Amplification and AmpF_lSTR[®] Identifiler[®] Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Finger nails

Homicide – Finger nail swab

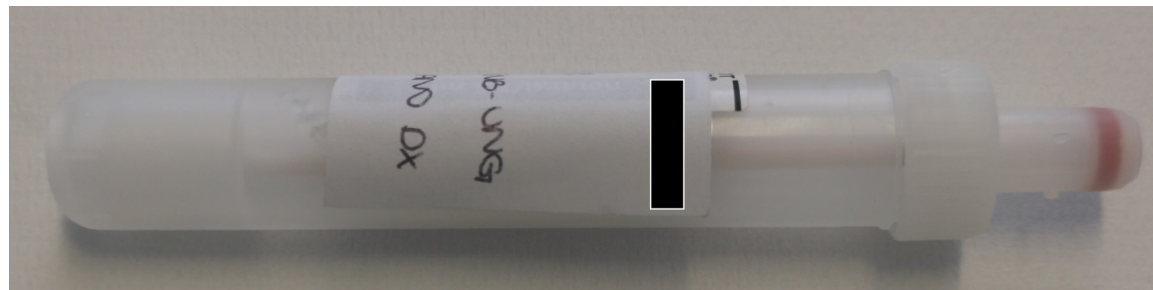
Murder of a woman killed by her husband. They were married to get a residence permit for him.

The man was arrested along with his pregnant lover.

During the autopsy, the victim's nail swabs were taken.

DNA was extracted with Chelex[®] 100 and QIAamp[®] DNA Micro Kit .

The samples were amplified with the Applied Biosystems[™] NGM Detect[™] PCR Amplification Kit, AmpF_lSTR[®] NGM SElect[™] PCR Amplification and AmpF_lSTR[®] Identifiler[®] Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Finger nail swab

Human identification - Biological liquid (Urine)

Determination of the genetic profile of the subject requesting the analysis.

A man undergoing urinalysis to exclude drugs use .

Urine positive results, discordant with other substrates, needed the typing of the genetic profile to identify a possible contamination.

DNA was extracted from urine with Chelex® 100.

The samples were amplified with the Applied Biosystems™ NGM Detect™ PCR Amplification Kit, AmpFlSTR® NGM SElect™ PCR Amplification and AmpFlSTR® Identifiler® Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Urine tube

Suspicious death - Common objects (Toothbrush)

In this case a hand was found in a lake.

No clue about the circumstances and the dynamic.

Genetic analysis was necessary to obtain the genetic profile.

The DNA was extracted from the toothbrush, which belonged to the probable subject, with Chelex® 100.

The samples were amplified with the Applied Biosystems™ NGM Detect™ PCR Amplification Kit, AmpFℓSTR® NGM SElect™ PCR Amplification and AmpFℓSTR® Identifiler® Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Toothbrush

Sexual violence - Histological slide

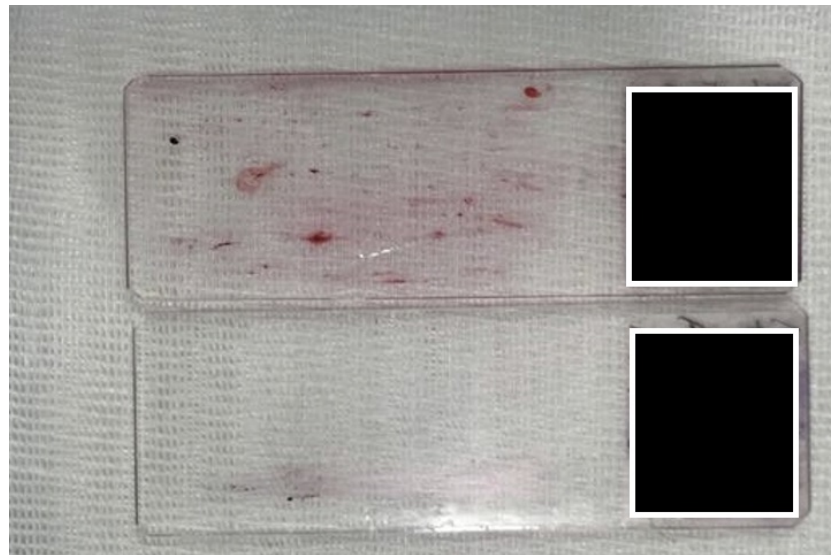
A woman reported sexual violence at her home.

She declared not had a vaginal intercourse but claims to had an oral one.

Genetic analyzes were performed on a series of samples.

DNA was extracted from an oropharyngeal slide the using Chelex[®] 100.

The samples were amplified with the Applied Biosystems[™] NGM Detect[™] PCR Amplification Kit, AmpF_lSTR[®] NGM SElect[™] PCR Amplification and AmpF_lSTR[®] Identifiler[®] Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Histological slides

Criminal investigation - Clothes (Cap)

Case of serial robberies.

A balaclava was found on location of one robbery .

DNA was extracted from the evidence using Chelex® 100.

The samples were amplified with the Applied Biosystems™ NGM Detect™ PCR Amplification Kit, AmpFℓSTR® NGM SElect™ PCR Amplification and AmpFℓSTR® Identifiler® Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Balaclava

Criminal investigation - Common objects (Gauze)

It is a case in which a healthcare assistant was accused of having masturbated during the working hours, inside the hospital.

A gauze had been used to collect the alleged sperm.

DNA was extracted from gauze using Chelex® 100.

The samples were amplified with the Applied Biosystems™ NGM Detect™ PCR Amplification Kit, AmpFℓSTR® NGM SElect™ PCR Amplification and AmpFℓSTR® Identifiler® Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Gauze

Sexual Violence - Clothes (Slip)

A woman, reported she was raped by her boyfriend.

Genetic investigations were necessary to assess the veracity of the information provided by the victim and to determine any male genetic profile.

DNA was extracted from her slip using Chelex® 100.

The samples were amplified with the Applied Biosystems™ NGM Detect™ PCR Amplification Kit, AmpFℓSTR® NGM SElect™ PCR Amplification and AmpFℓSTR® Identifiler® Plus PCR Amplification kits, according to the manuals, using a GeneAmp PCR System 9700 Gold Plate.



Slip

Results

After analyzing the samples with the three cited kits, we had readings the electropherograms.

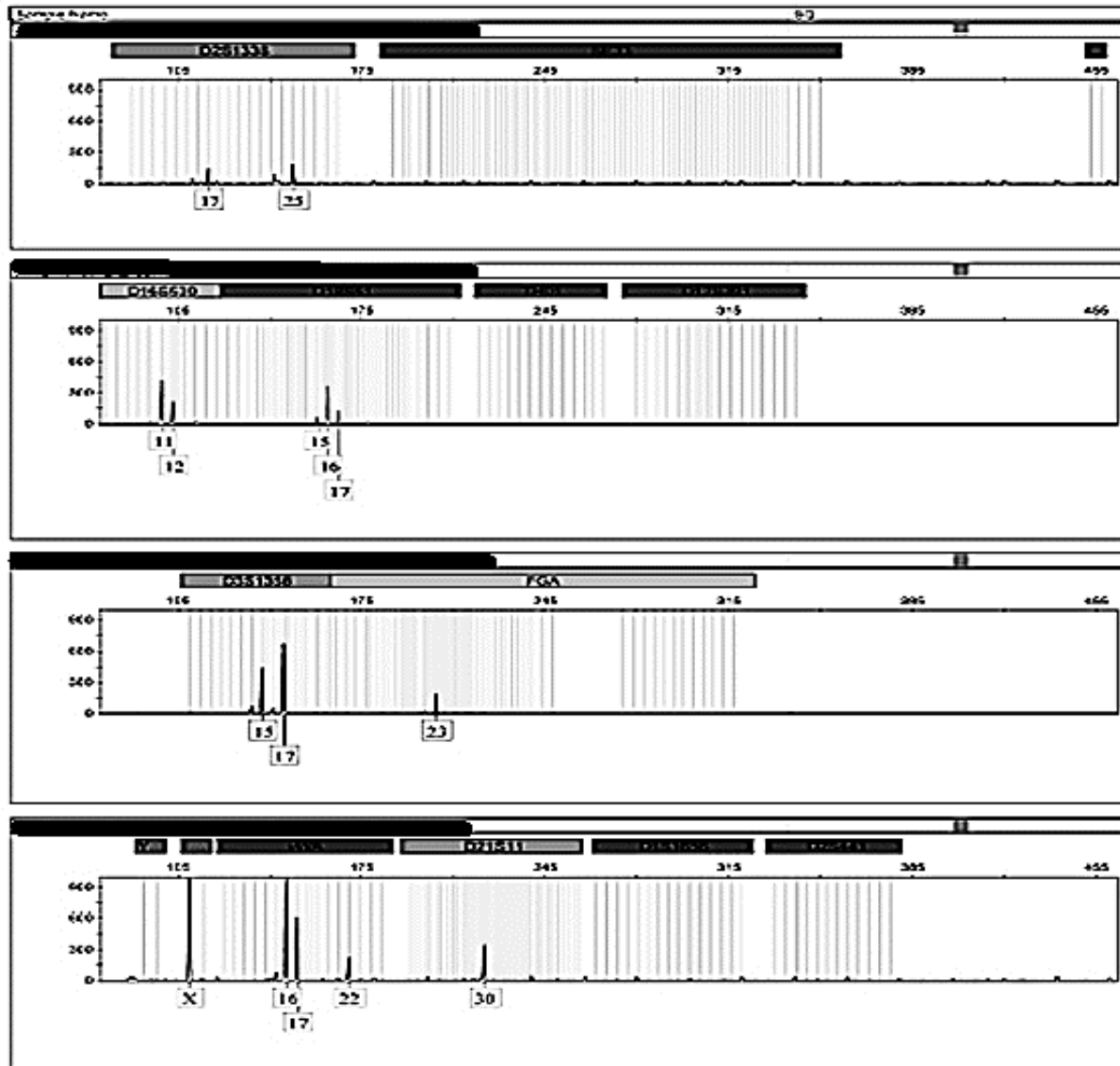
 Presidio Ospedaliero di Brescia Sistema Socio Sanitario  Regione Lombardia ASST Spedali Civili MEDICINA LEGALE LABORATORIO DI GENETICA FORENSE	MODELLO LETTURA MARCATORI <i>NGM Detect</i> <i>NGM Select</i> <i>Identifiler_Plus</i>
--	---

ARCHIVIO N°: _____

CAMPIONE					
<i>Detect</i>		<i>Select</i>		<i>ID</i>	
Data corsa		Data corsa		Data corsa	
Ora corsa		Ora corsa		Ora corsa	
D2S1338		D10S1248		D8S1179	
SE33		vWA		D21S11	
		D16S539		D7S820	
D16S539		D2S1338		CSF1PO	
D18S51					
TH01		Amel		D3S1358	
D12S391		D8S1179		TH01	
		D21S11		D13S317	
D3S1358		D18S51		D16S539	
FGA				D2S1338	
		D22S1045			
Yindel		D19S433		D19S433	
Amel		TH01		vWA	
vWA		FGA		TPOX	
D21S11				D18S51	
D1S1656		D2S441			
D2S441		D3S1358		Amel	
		D1S1656		D5S818	
D8S1179		D12S391		FGA	
D19S433		SE33			
D22S1045					
D10S1248					

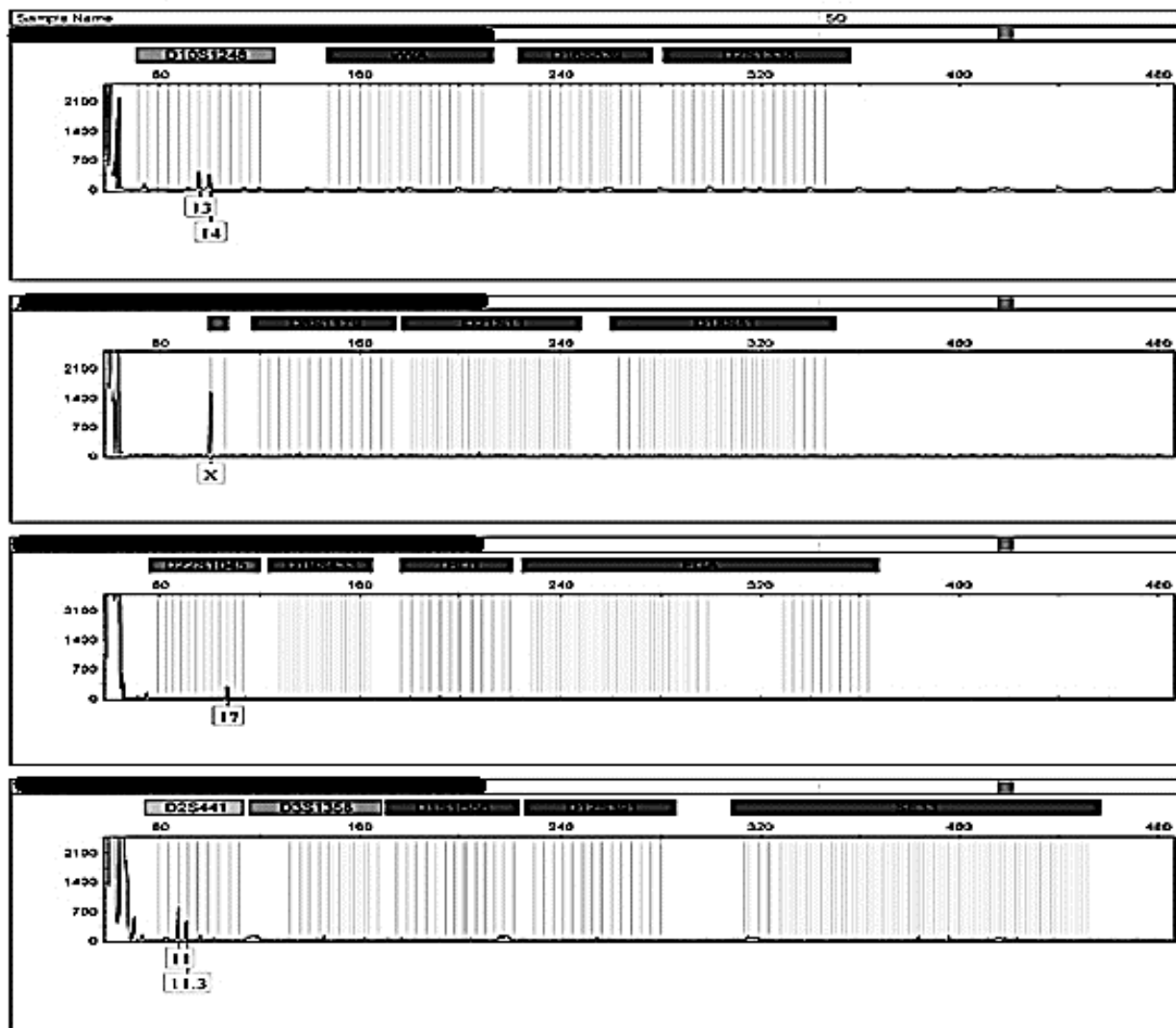
Applied Biosystems™ NGM Detect™ PCR Amplification Kit

AB Applied Biosystems
GenoMapper® 3.10 v. 1.1



AmpFℓSTR® NGM SElect™ PCR Amplification Kit

Applied Biosystems
GeneMapper® ID-X 1.4



AmpF1STR® Identifiler® Plus PCR Amplification kit

AB Applied
Biosystems
GeneMapper® ID-X 1.4

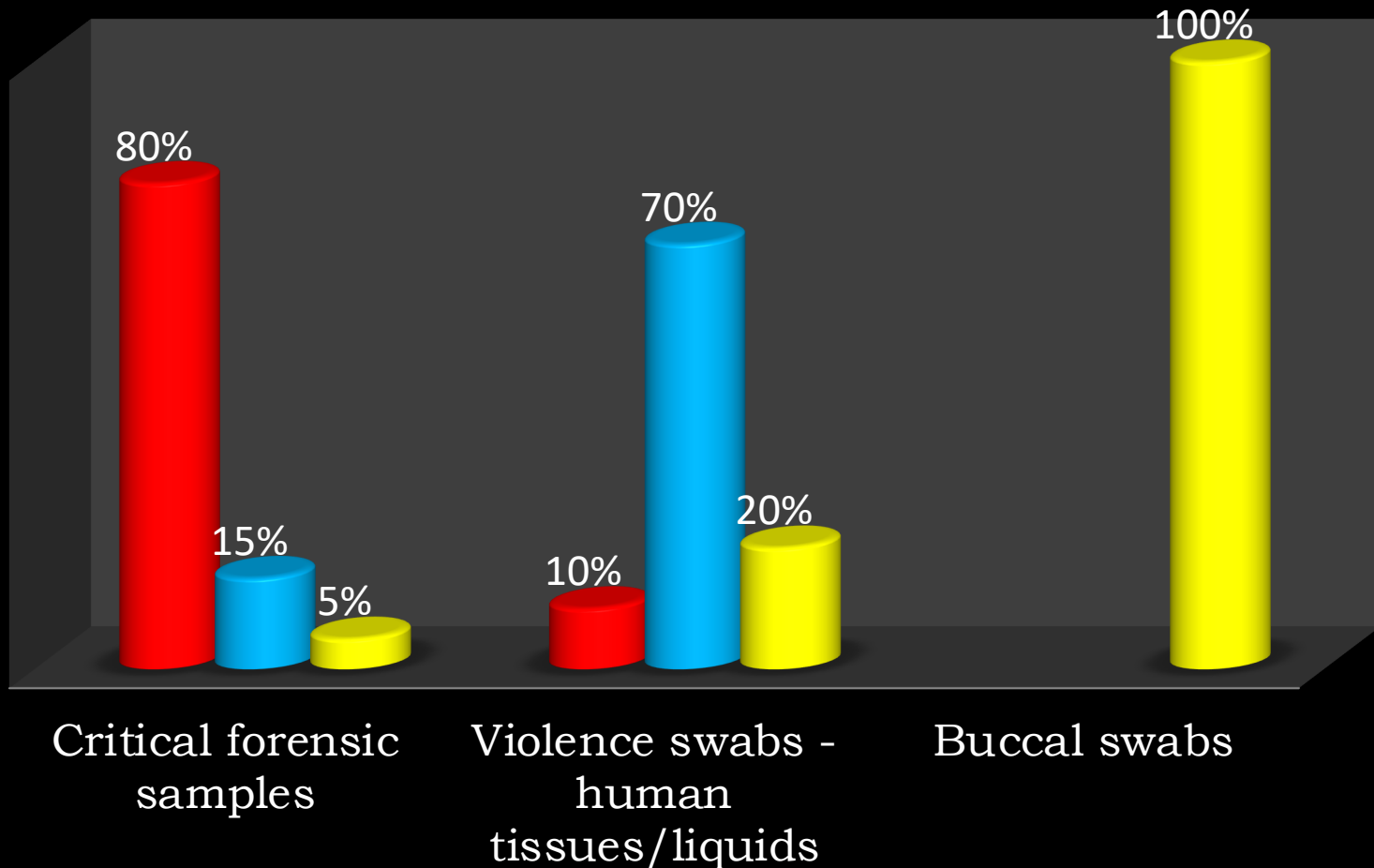


Kit's performance

■ Detect more informative

■ Detect/Select equivalents

■ Detect/Select/ID equivalents



Discussions

- For samples with high DNA concentrations, in particular buccal swabs, the three kits gave the same results.
- The performance of NGM Detect™ is not significantly higher than the other two kits when DNA quantity is good in samples .
- In the case of sexual violence NGM Detect™ is more informative because the positive Y Indel indicates the presence of a male profile, even when there is no perfect mixture.
- For critical forensic samples, NGM Detect™ has a better performance, because the alleles called are greater than other kits.
- For get more information is better using all kits, especially in critical forensic casework.

Thanks for your attention

Speaker was provided travel and hotel support by Thermo Fisher Scientific for this presentation, but no remuneration.

When used for purposes other than Human Identification or Paternity Testing the instruments and software modules cited are for Research Use Only. Not for use in diagnostic procedures.

Thermo Fisher Scientific and its affiliates are not endorsing, recommending, or promoting any use or application of Thermo Fisher Scientific products presented by third parties during this seminar.

Information and materials presented or provided by third parties are provided as-is and without warranty of any kind, including regarding intellectual property rights and reported results.

Parties presenting images, text and material represent they have the rights.