Implementation of a fully automated workflow using MPS technology in a high volume casework laboratory

GCC Forensics Exhibition & Conference 2018

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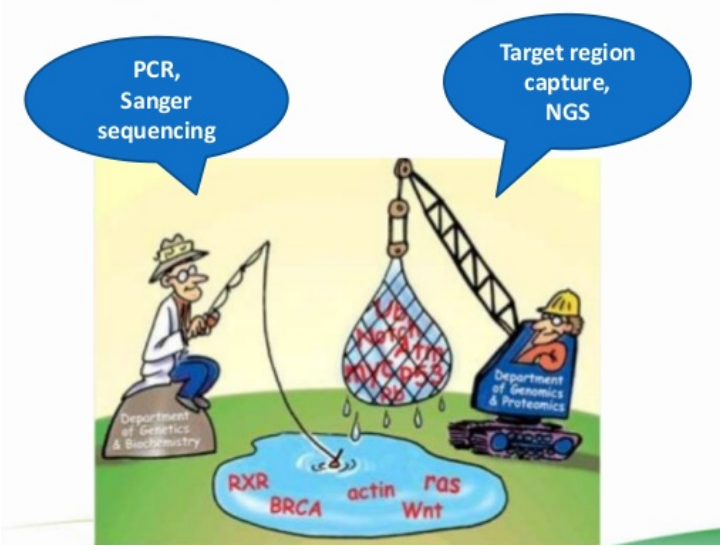
Massively Parallel Sequencing (MPS)

- Also know as **Massive Parallel Sequencing** or **High Throughput Sequencing** (HTS) or **Next-Generation Sequencing** (NGS)
- DNA sequencing using the concept of massively parallel processing
- Commercially available since 2005
- Second and third generation sequencing
- Sequencing of 1 million to 43 billion short DNA fragments (50-400 bases) per instrument run
- Various technologies, engineering configurations and sequencing chemistries
- Whole-genome or amplicon-based sequencing
- 0.5 Gb to 1.8 Tb per run



Coming of age: ten years of next-generation sequencing technologies

Sara Goodwin[1], John D. McPherson[2] and W. Richard McCombie[1]

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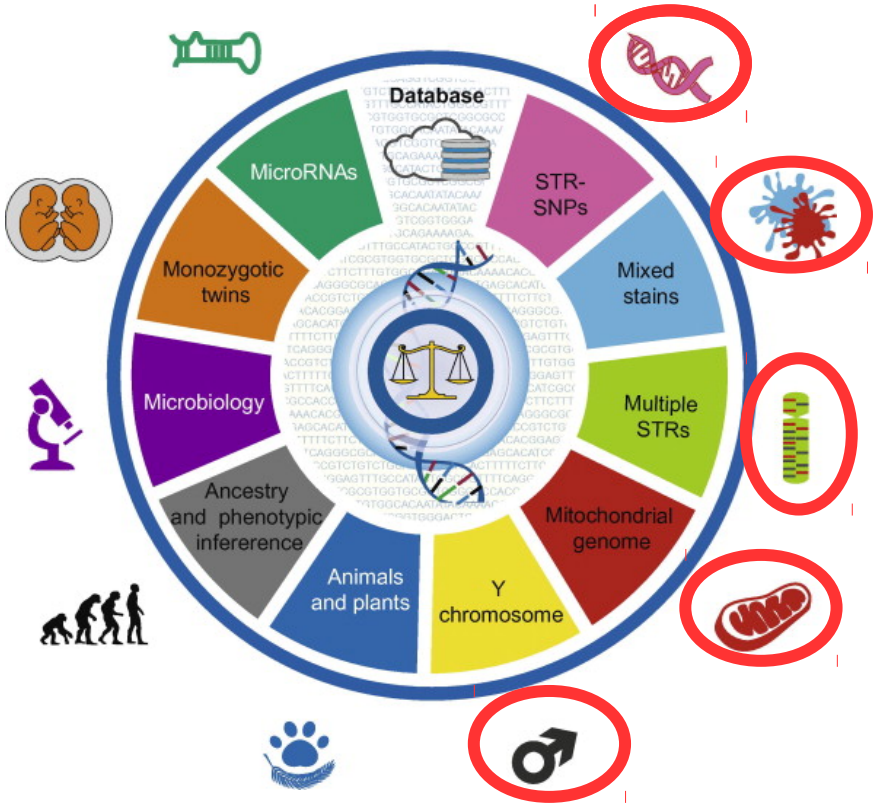
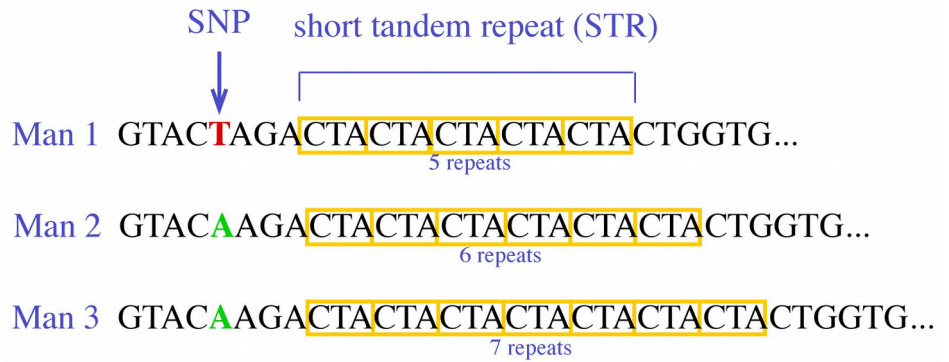
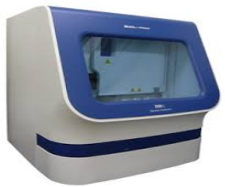
Forensic DNA identification with MPS

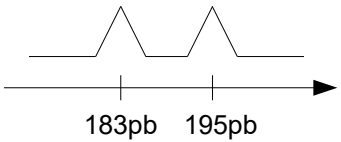


Yang et al.. Application of Next-generation Sequencing Technology in Forensic Science. Genomics, Proteomics & Bioinformatics., 12 (2014), pp. e190-e197



CE





183pb = 6 repeats

195pb = 9 repeats

Amplicon size

TH01
6-9

MPS

AATGAATGAATGAATGAATGAATG = **[AATG]$_6$**

AATGAATGAATGAATGAATGAATGAATGAATGAATG = **[AATG]$_9$**

Amplicon size **+ Amplicon sequence**

European survey on forensic applications of massively parallel sequencing (2017)

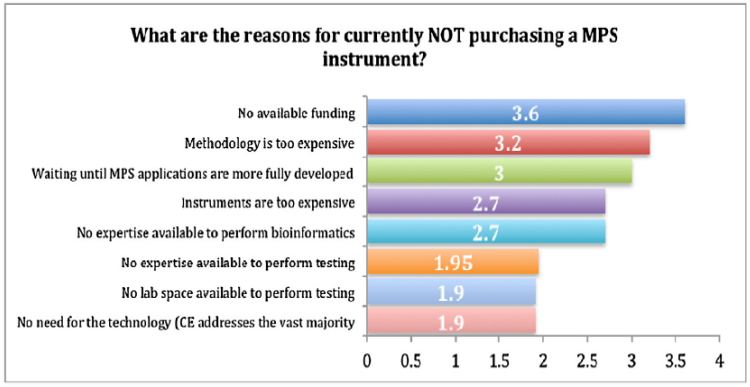


Fig. 1. Reasons for currently not purchasing an MPS instrument. Responses from 16 laboratories ranked in order of priority from 1 (lowest) to 5 (highest).

Conclusions :
Money, complexity, lack of feedback

A. Alonso, P. Muller, L. Roewer, S. Willuweit, B. Budowle, W. Parson. European survey on forensic applications of massively parallel sequencing. Forensic Sci. Int. Genet., 29 (2017), pp. e23-e25

Additionnal questions with regards to massively parallel sequencing implementation

Automation ?

Equipment ?

Training ?

Genetic profile validation and interpretation ?

Developmental validation ?

Sample Tracking ?

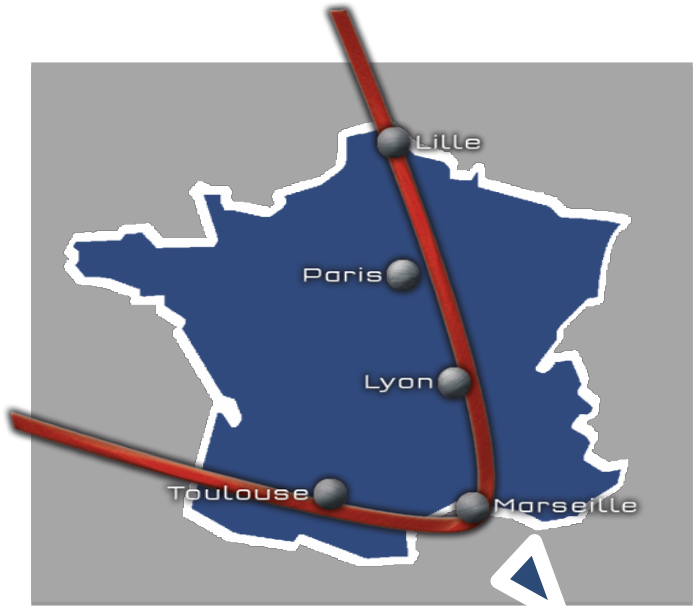
Casework application ?

Accreditation ?

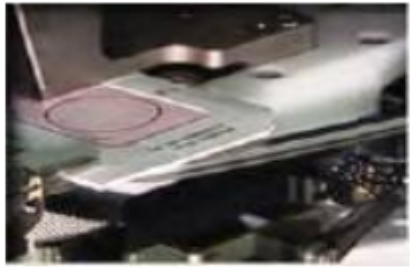
Institut National de Police Scientifique

a.k.a French National Forensic Police Institute

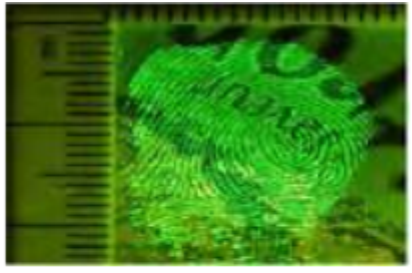
- Biggest forensic institute in France
- Under the administrative supervision of the General Directorate of National Police
- Public establishment officially registered as an expert on the list of Supreme Court
- Network of 5 forensic laboratories (COFRAC accreditation according to the ISO 17025 standards since 2008).
- ENFSI member (since 1994)
- EDNAP/EUROFORGEN



Fields of expertise

Biology / Forensic DNA

Documents and fingerprints

Toxicology

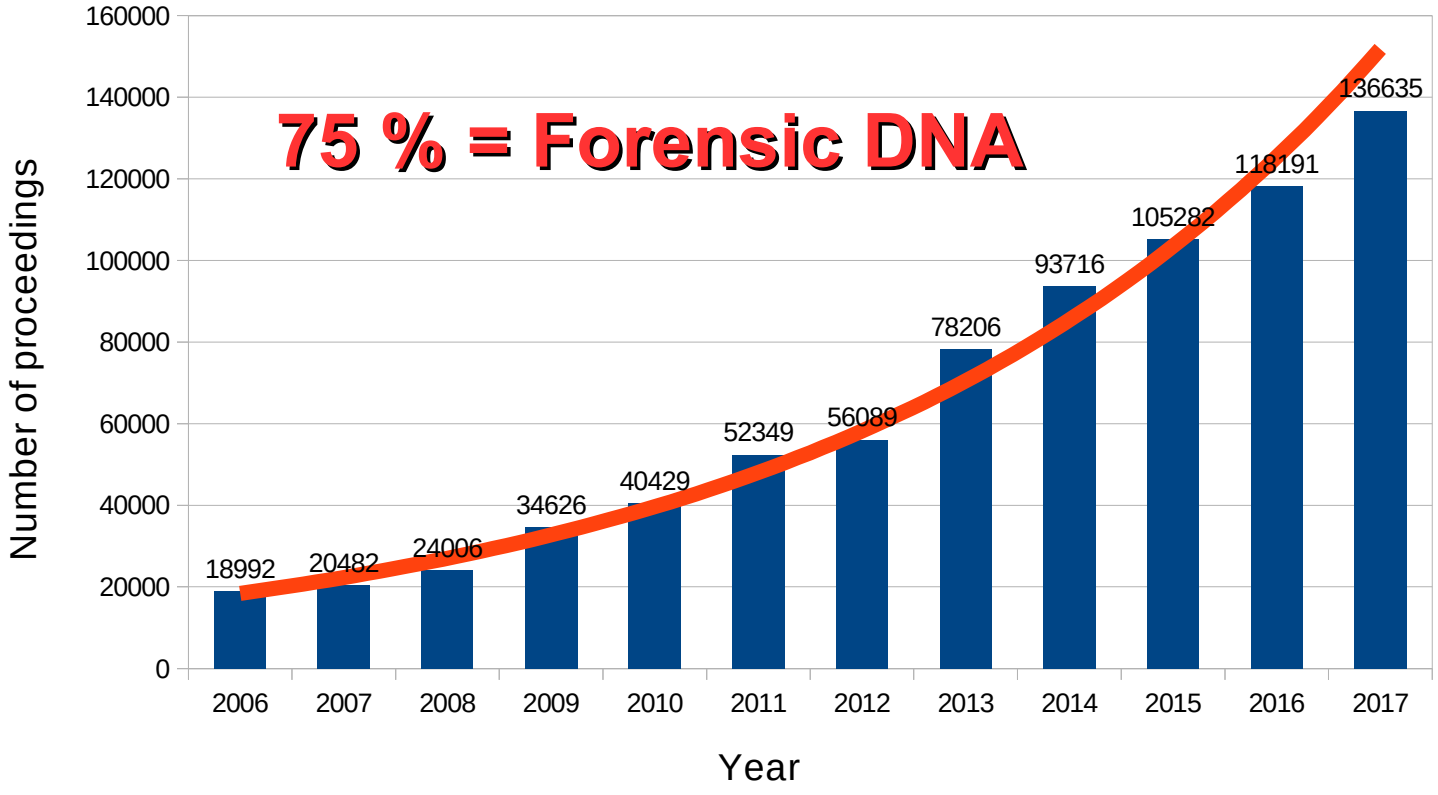
Physics & Chemistry

Illicit drugs

Fire & Explosions

+ ballistics and IT

An increasing activity since 2006...



Forensic DNA analysis

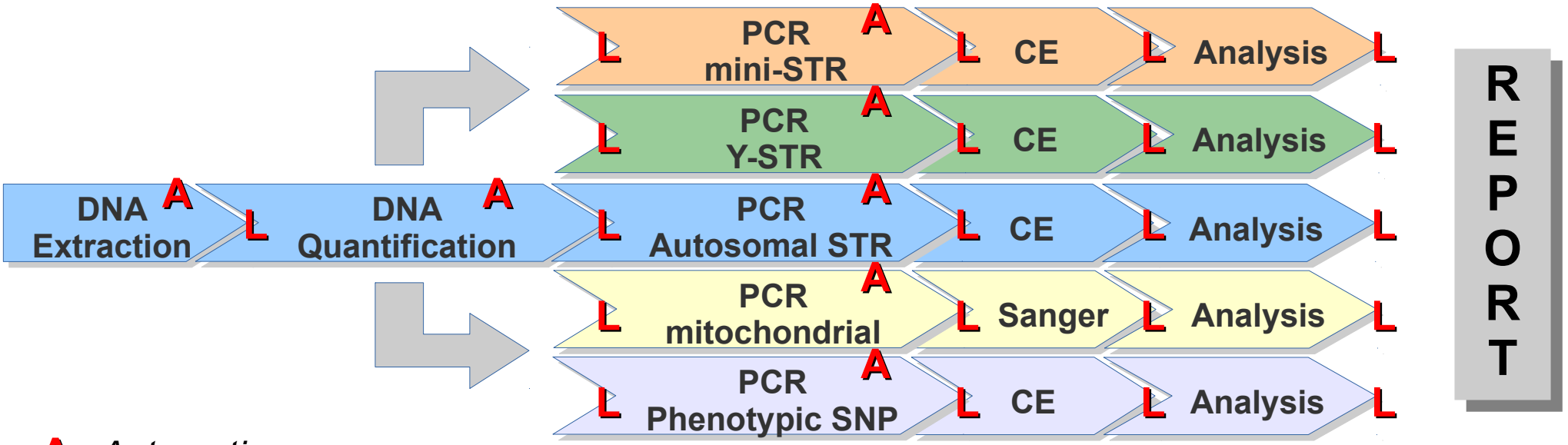
Reference samples (Database)

- Automated Genotyping Unit (Lyon only)
- **220 000 FTA cards / year** (double analysis)

Casework samples

- Volume crime (swabs)
- Criminal cases + Mitochondrial DNA / DNA phenotyping / Synthetic DNA (spray/ink)
- **238 000 DNA samples / year**

Actual DNA workflow at INPS



A : *Automation*

L : *LIMS (Laboratory Integrated Management System)*

Is it worth adding another workflow ?

What we expected… back in 2015 !
Our MPS workflow philosophy

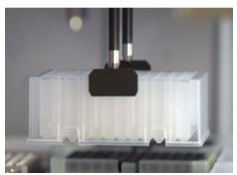
- Premium workflow to allow a deeper analysis of complex DNA samples (degraded, mixtures…)
- Not to fully replace classic workflow (PCR-CE)
- Reserved only for important casework (serious crimes)
- Automation and LIMS
- ISO 17025
- Major roles :
    - Help experts to interpret genetic profiles
    - Bring more caseworks to the lab and become expert in complex analyses
    - Re-open cold cases

Fully automated DNA library preparation

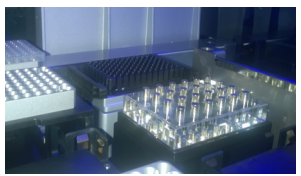
Hamilton ID

NGS-V STARlet

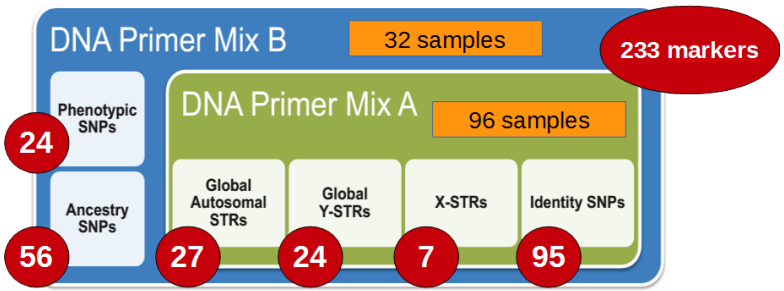
- Automation of Verogen ForenSeq™ Library preparation kit from PCR amplification to library pooling
- 8 to 96 samples
- Hands-on time from reduced from 3 hours to 15 minutes
- DNA analysis to data interpretation in 4 days



Core Gripper



Magnet
+ Heater Shaker

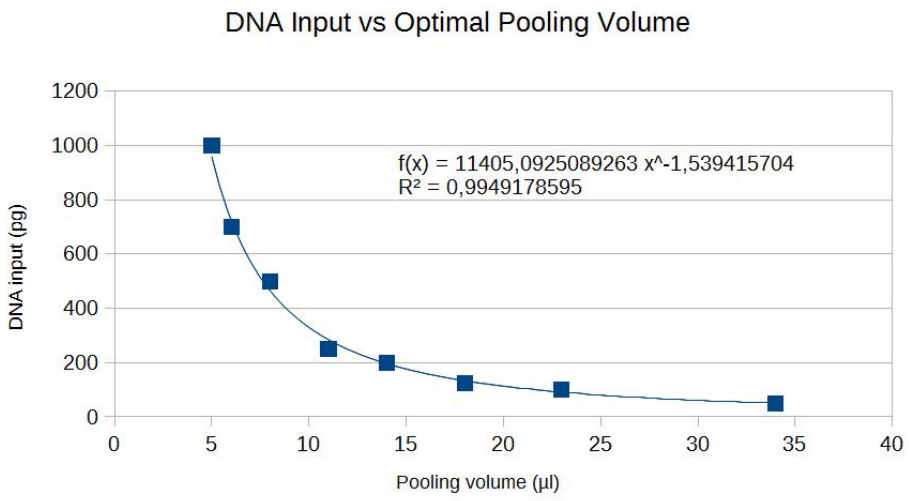


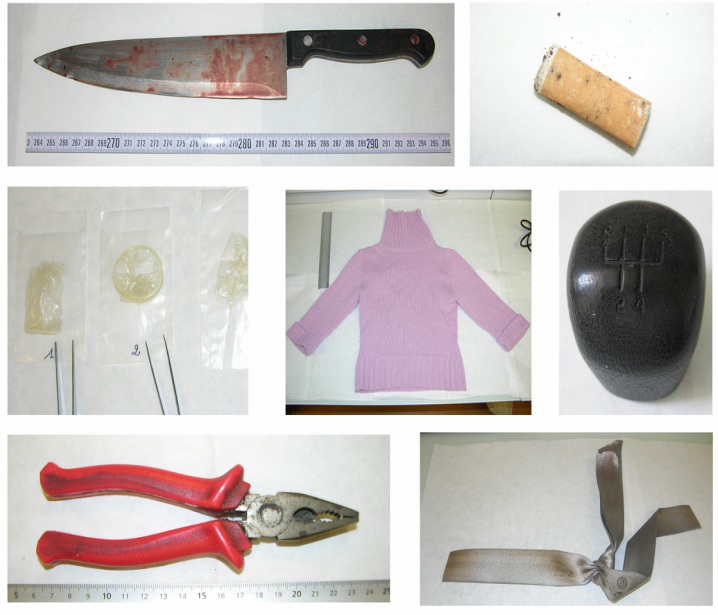
VEROGEN

illumina

Improvements to maximise MPS usage in a forensic laboratory

- Pooling library adjustment



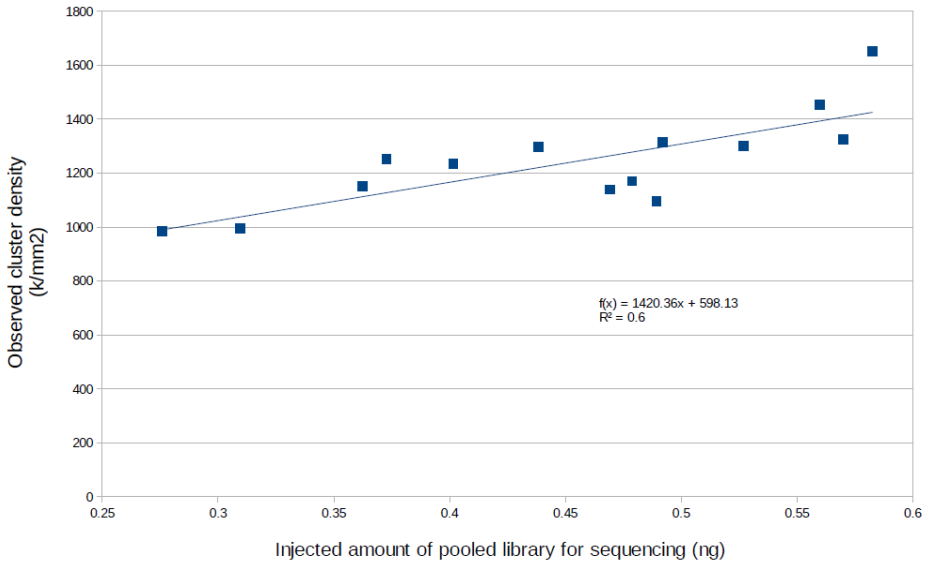


Combined analysis of rich and poor DNA samples in the same run

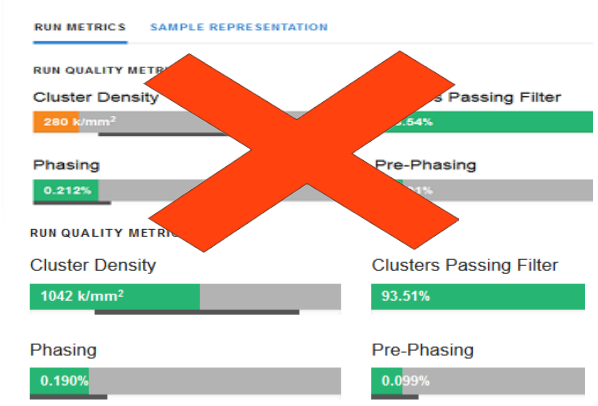
DNA sample concentration > 0.015 ng/µL

Improvements to maximise MPS usage in a forensic laboratory

- Pooling library adjustment
- Pooled library quantification

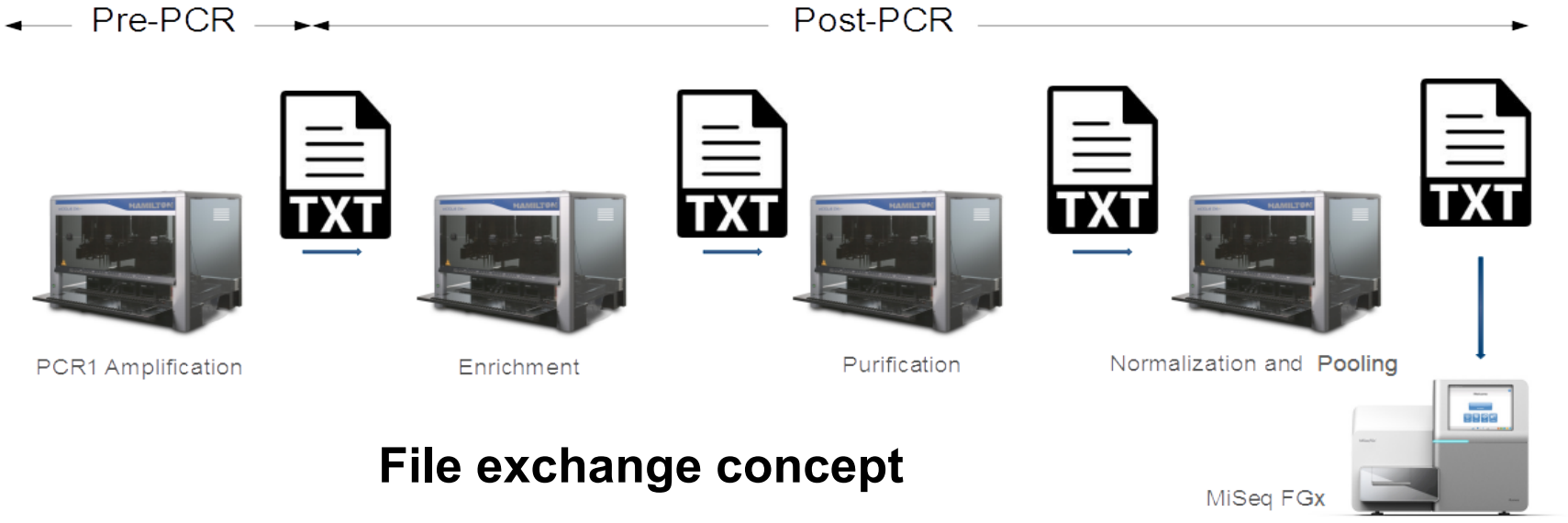


Qubit 3.0
(ThermoFisher Scientific)



Improvements to maximise MPS usage in a forensic laboratory

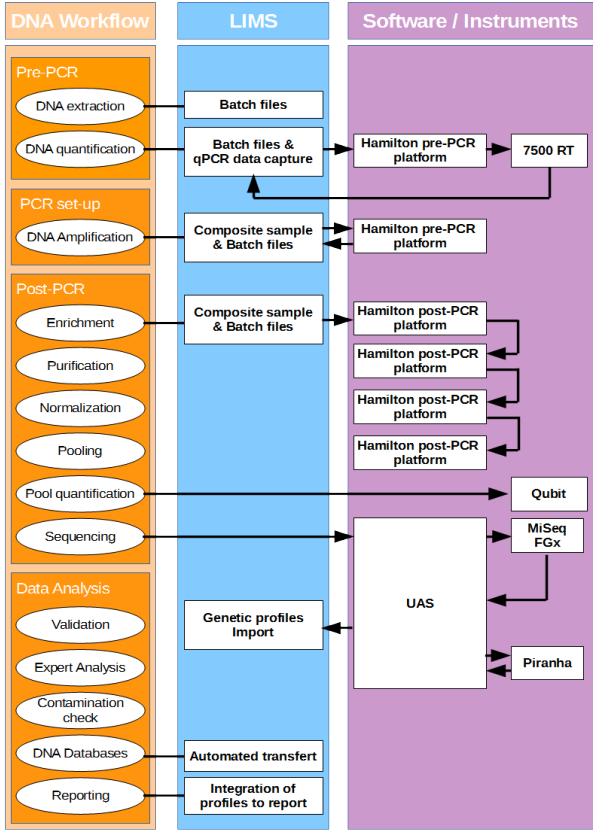
- Pooling library adjustment
- Pooled library quantification
- Sample tracking



File exchange concept

Improvements to maximise MPS usage in a forensic laboratory

- Pooling library adjustment
- Pooled library quantification
- Sample tracking
- LIMS integration



Developmental validation of ForenSeq solution with automated protocol

- Validation guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM) in 2012 – available here : https://www.swgdam.org/publications
- Revised in November 2016 to address Next Generation Sequencing (NGS) technologies
- Parameters tested in our laboratory :
  - Sensitivity (full profile from 250 pg as DNA input)
  - Repeatability (CV < 10%)
  - Reproducibility (CV < 20%)
  - Concordance (**99,54 %** compared to GlobalFiler)
  - Cross-contamination
- Detailled presentation at the workshop yesterday
- Publication under review at FSI Genetics

Caseworks

- More than 150 DNA extracts analyzed (*in parallel to* GlobalFiler analysis)
- DNA concentration: 0,0024 → 128 ng/µL

Why the DNA extracts were analyzed with NGS ?

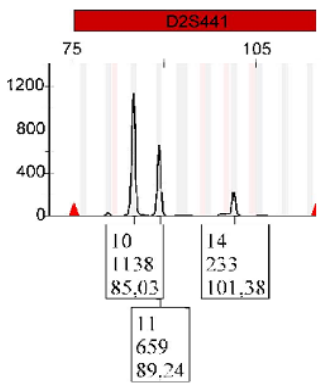
- To complete a genetic profile obtained from a highly degraded DNA

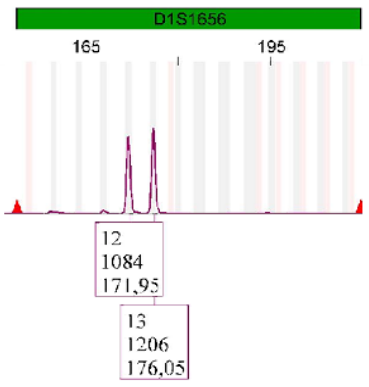
- To identify a very minority contributor

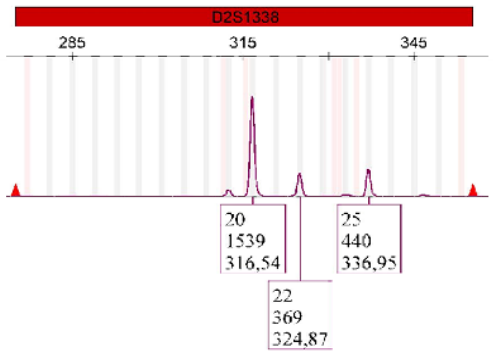
- To deconvoluate mixture with the help of possible isomutations

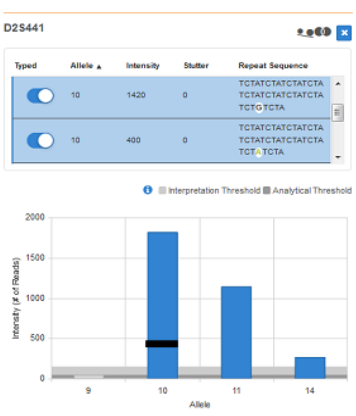
- To have additional markers for complex kinship

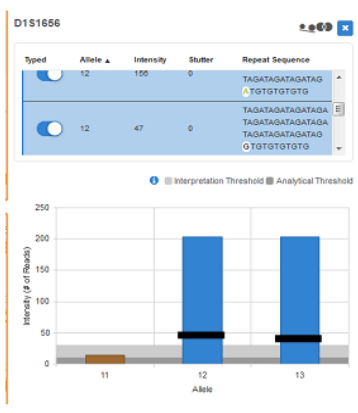
Example 1 : DNA Mixture and isomutations

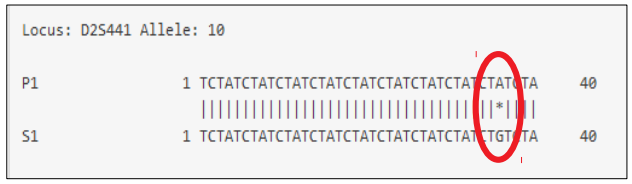








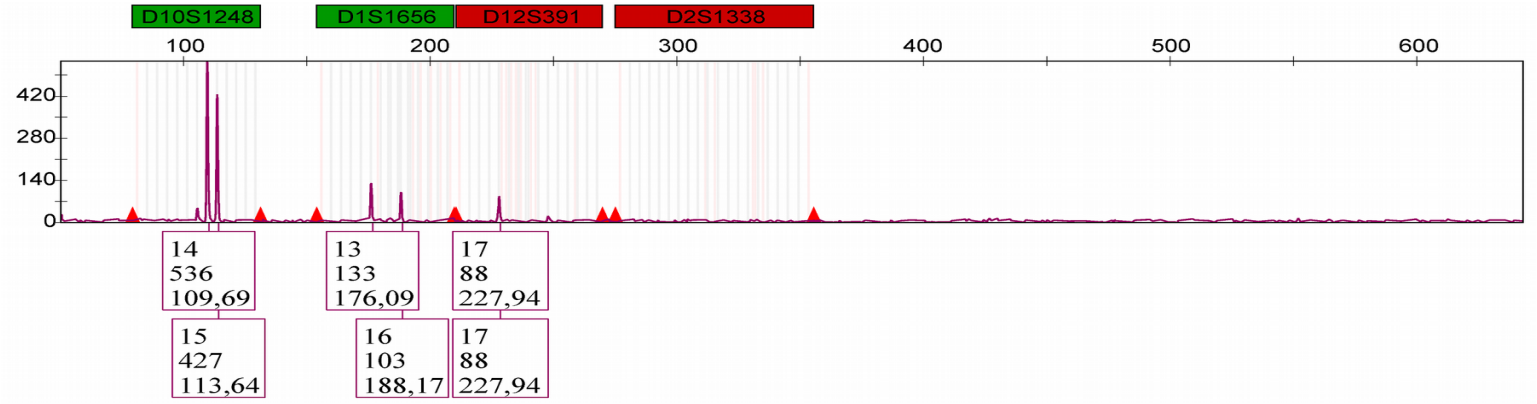




- MPS analysis :
- 6 additional markers validated with confidence by all participants
  - 3 isomutations
  - 3 shorter amplicons

In average : gain of 2 to 6 markers

Example 2 : Degraded DNA



Kit	D3S1358	vWA	D16S539	CSF1PO	TPOX	Yindel	Amelogenin	D8S1179	D21S11	D18S51	DYS391	D2S441	D19S433	TH01	FGA	D22S1045
Forenseq	15-18	16-18	10-10	10-12	8-9		X-X	12-12	-	12-16		10-10	12-13	6-8	21-24	-
MiniFiler			10-10	10-12			X-X		30-31.2	12-16					21-24	
Globalfiler	-	16-18	-	-	-		X-X	12-12	-	-		10-10	12-13	-	-	15-15

	D5S818	D13S317	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338	D4S2408	D6S1043	D9S1122	PentaE	D17S1301	D20S482	PentaD	nb STRa
Forenseq	12-13	12-13	8-11		-	13-16	-	19-24	8-9	-	11-13	-	11-13	14-14	-	20
MiniFiler		12-13	8-11					19-24								8
Globalfiler	-	-	-		14-15	13-16	-	-								7

GlobalFiler : 7 typed loci
Minifiler : + 8 typed loci

ForenSeq : 16 typed loci
+ 4 additional STRs (not in database)

Example 3 : Postmortem Identification

46 SNP with one common allele

0 discordance

Likelihood Ratio (LR) with Familias :

- 20 STR GlobalFiler (1 discordance) : **119**
- 20 STR GlobalFiler (1 discordance) + 4 STR ForenSeq : **555**
- 20 STR GlobalFiler (1 discordance) + 4 STR ForenSeq + 46 SNP ForenSeq : **27357878**

Allelic frequencies obtained from 1000 genomes project

Still questioning if it's statistically ok ?

Independance of SNPs and STRs ?

Père		Fils	
rs1490413	G,A	rs1490413	G,A
rs560681	A,A	rs560681	INC
rs1294331	INC	rs1294331	INC
rs10495407	G,G	rs10495407	G,G
rs891700	G,G	rs891700	INC
rs1413212	G,G	rs1413212	INC
rs876724	C,C	rs876724	INC
rs1109037	G,A	rs1109037	A,A
rs993934	C,C	rs993934	C,C
rs12997453	INC	rs12997453	INC
rs907100	INC	rs907100	INC
rs1357617	INC	rs1357617	INC
rs4364205	T,T	rs4364205	T,G
rs2399332	INC	rs2399332	C,C
rs1355366	A,G	rs1355366	INC
rs6444724	C,C	rs6444724	T,C
rs2046361	INC	rs2046361	INC
rs279844	A,T	rs279844	A,A
rs6811238	G,G	rs6811238	G,G
rs1979255	C,C	rs1979255	C,C
rs717302	INC	rs717302	INC
rs159606	A,A	rs159606	INC
rs13182883	G,G	rs13182883	INC
rs251934	INC	rs251934	INC
rs338882	T,T	rs338882	T,T
rs13218440	A,A	rs13218440	INC
rs1336071	INC	rs1336071	INC
rs214955	INC	rs214955	G,G
rs727811	INC	rs727811	INC
rs6955448	C,C	rs6955448	C,C
rs917118	C,T	rs917118	T,T
rs321198	T,C	rs321198	T,T
rs737681	T,C	rs737681	T,C
rs763869	INC	rs763869	INC
rs10092491	INC	rs10092491	INC
rs2056277	C,C	rs2056277	C,T
rs4606077	C,C	rs4606077	C,C
rs1015250	INC	rs1015250	INC
rs7041158	INC	rs7041158	INC
rs1463729	G,A	rs1463729	A,A
rs1360288	C,C	rs1360288	C,C
rs10776839	INC	rs10776839	G,G
rs826472	INC	rs826472	INC
rs735155	A,A	rs735155	G,A
rs3780962	T,C	rs3780962	INC
rs740598	INC	rs740598	INC
rs964681	T,T	rs964681	T,C

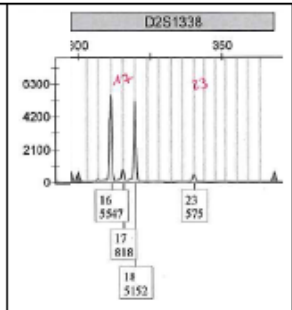
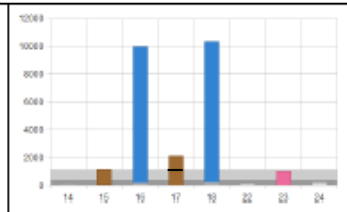
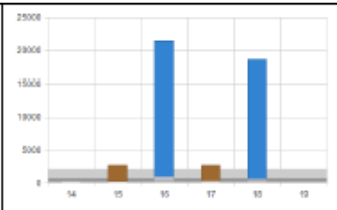
Père		Fils	
rs1498553	C,T	rs1498553	C,C
rs901398	C,T	rs901398	C,T
rs10488710	INC	rs10488710	INC
rs2076848	INC	rs2076848	INC
rs2107612	INC	rs2107612	INC
rs2269355	INC	rs2269355	INC
rs2920816	INC	rs2920816	INC
rs2111980	A,A	rs2111980	A,A
rs10773760	A,G	rs10773760	A,G
rs1335873	A,T	rs1335873	T,T
rs1886510	INC	rs1886510	INC
rs1058083	G,G	rs1058083	A,G
rs354439	A,A	rs354439	INC
rs1454361	A,A	rs1454361	A,A
rs722290	G,G	rs722290	INC
rs873196	T,T	rs873196	T,T
rs4530059	G,G	rs4530059	G,A
rs1821380	G,C	rs1821380	G,C
rs8037429	C,C	rs8037429	C,C
rs1528460	INC	rs1528460	INC
rs729172	A,A	rs729172	INC
rs2342747	INC	rs2342747	INC
rs430046	C,T	rs430046	C,T
rs1382387	T,T	rs1382387	G,T
rs9905977	A,G	rs9905977	A,G
rs740910	INC	rs740910	INC
rs938283	T,T	rs938283	T,T
rs8078417	C,C	rs8078417	C,C
rs1493232	INC	rs1493232	INC
rs9951171	A,A	rs9951171	G,A
rs1736442	INC	rs1736442	INC
rs1024116	G,A	rs1024116	G,A
rs719366	T,T	rs719366	T,T
rs576261	A,A	rs576261	INC
rs1031825	INC	rs1031825	INC
rs445251	C,G	rs445251	C,G
rs1005533	G,A	rs1005533	G,G
rs1523537	T,T	rs1523537	INC
rs722098	INC	rs722098	INC
rs2830795	A,A	rs2830795	INC
rs2831700	INC	rs2831700	INC
rs914165	G,G	rs914165	G,G
rs221956	INC	rs221956	T,T
rs733164	G,G	rs733164	G,G
rs987640	T,A	rs987640	T,A
rs2040411	A,A	rs2040411	A,A
rs1028528	A,A	rs1028528	A,G

First MPS DNA profile sent to the French National DNA Database

Cold Case of 2011 (Homicide)

First analyzes in the laboratory of Paris (Identifiler)

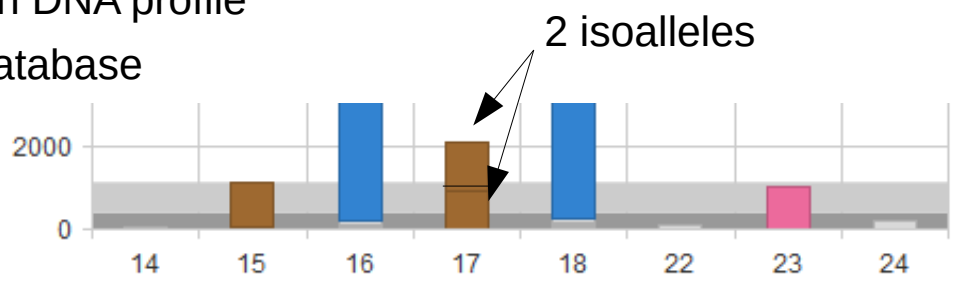
2 DNA extracts (mixture of the victim + a very minor male contributor)

						Minor Contributor		
						CE	**NGS**	
D2S1338		**16**-17-**18**-23 Mixture	? Minor ?	 Mixture	 Victim	?	17-23	Confirmation of the CE results thanks to the two « 17 »

Validation of 4 additional markers and 5 alleles per unknown DNA profile

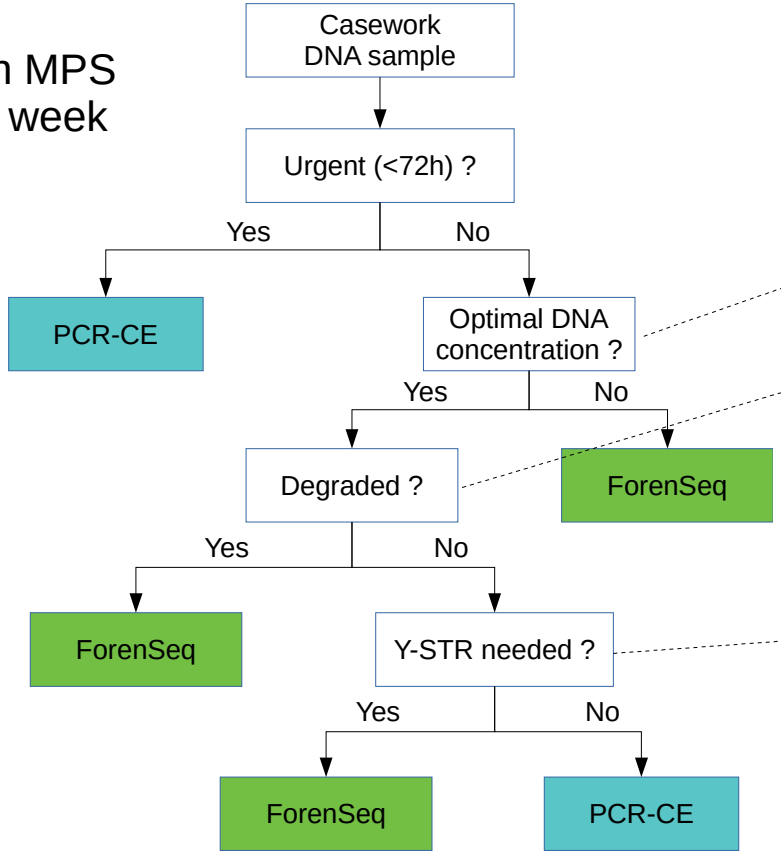
→ One new genetic profile registered to the national DNA database

→ One partial genetic profile completed



Future DNA analysis workflow in Lyon

Goal :
32 samples in MPS workflow per week



	Quantifiler Trio
Quantification targets	Total human & male
Sensitivity	<1 pg
Quality/ degradation index	Yes
Reaction mix and IPC robustness	Excellent
Correlates with latest STR kits	Yes
Accuracy of M:F mixture ratio	Excellent
DNA quantification standard	Excellent
Cycling time	~60 min

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