GRUPO DE HABLA ESPAÑOLA Y PORTUGUESA DE LA ISFG

GRUPO DE LÍNGUAS ESPANHOLA E PORTUGUESA DA ISFG

MINISTERIO DE LA PRESIDENCIA JUSTICIA Y RELACIONES CON LAS CORTES

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INTERCOMPARISON PROGRAM

"ANALYSIS OF DNA POLYMORPHISMS IN BLOODSTAINS AND OTHER BIOLOGICAL SAMPLES" MASSIVE PARALLEL SQUENCING MODULE (MPS)

BASIC LEVEL EXERCISE EIADN –MPS 3 (2025) DEADLINE: 15/05/2025

2025/Kinship Module
M1 to M3: reference items
2025/Forensic module
M4: forensic unknown item
M5: hair sample

Seal number

Approach:

2025/Kinship Module – Basic level

Practical Kinship study

- **M1, M2, M3:** reference items for genetic profiling.

2025/Forensic Module – Basic level

Practical Forensic study

- **M4:** forensic item for genetic profiling.
- **M5:** hair for mitochondrial DNA analysis.

Methodology to be used

The analyses will be performed by using the markers and with the Massive Parallel Sequencing (MPS) methods used by the laboratory: autosomal STRs, Y- STRs, X-STRs and mitochondrial DNA analysis. The items must be processed as real casework and, if possible, as blind samples.

INDEX

1. Methodology *Read carefully the instructions provided before filling in this section*

1.1 Workflow methodology

1.1.1 Library preparation

TABLE 1A

Marker	Kit	Others (specify)	Instrument	Others (specify)
A-STR				
Y-STR				
X-STR				
Mitochondrial DNA				

See Appendix 2025 MPS for codes

1.1.2 Library quantification

TABLE 1B

Marker	Kit	Others (specify)	Instrument	Others (specify)
A-STR				
Y-STR				
X-STR				
Mitochondrial DNA				

See Appendix 2025 MPS for codes

1.1.3 Template preparation

TABLE 1C

Marker	Kit	Others (specify)	Instrument	Others (specify)
A-STR				
Y-STR				
X-STR				
Mitochondrial DNA				

See Appendix 2025 MPS for codes

1.1.4 Sequencing

TABLE 1D

Tipo de marcador	Kit	Others (specify)	Chip/Flow Cell	Others (specify)	Instrument	Others (specify)
A-STR						
Y-STR						
X-STR						
Mitochondrial DNA						

See Appendix 2025 MPS for codes

1.1.5 Reference Sequence used. STR markers

1.1.6 Data analysis

TABLE 1E

Marker	Raw Data	Others (specify)	Allele/Variant Calling	Others (specify)	Analysis	Others (specify)
A-STR						
Y-STR						
X-STR						
Mitochondrial DNA						

See Appendix 2025 MPS for codes

1.2 Other considerations regarding methodology different to reported in the preceding tables

2. Practical studies results:

Read carefully the instructions provided in order to fill in the results tables and the rules of participation in order to know the establishment of assigned values and the evaluation of results https://ghep-isfg.org/en/proficiency/participation/

2.1 Library enrichment and quantification (if relevant)

TABLE 3

	Library amplification volumen (µL)		Individual Library quantification (pM)		Pool Library quantification (pM)	
Items	**STR**	**mtDNA**	**STR**	**mtDNA**	**STR**	**mtDNA**
M1						
M2						
M3						
M4						
M4.1 (if relevant)						
M4.2 (if relevant)						
M5						

2.2 Upload data of chips or flow cells

TABLE 4

	STR	mtDNA
Instrument		
Markers		
Chip / Flow Cell		

	STR	mtDNA
Addressable Wells (Total reads) (1)		
Usable reads (Final library ISPs)*		
Usable reads (%)		
No. Samples per chip / flow cell		
Exercise samples reads		
M1		
M2		
M3		
M4		
M4.1 (if relevant)		
M4.2 (if relevant)		
M5		
Number of markers		-
Chip equalization*		

(1) Total number of wells or cells per chip / flow cell. This is a fixed value per chip / flow cell, and can be obtained through the software, TSS or UAS. In case using more than one chip, indicate the value of each as follows, ex.: 12530194 / 37849615

* For Ion Torrent platforms. Final number of wells used. In case of using more tan one indicate it as follows: chip / flow cell.

2.3 STR results

Fill in the tables using the following recommendations:

-Parson et al. Massively parallel sequencing of forensic STRs: Considerations of the DNA commission of the International Society for Forensic Genetics (ISFG) on minimal nomenclature requirements. Forensic Sci Int Genet. 2016 May;22:54-63.

***-Gettings et al*. Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on short tandem repeat sequence nomenclature Forensic Sci. Int. Genet. Vol. 68, 102946, January 2025.**

-Forensic Sequence STRucture Guide (FSSG) v6 en https://strider.online/nomenclature

2.3.1 Autosomal STR and amelogenine

TABLE 5A-M1

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
AMEL						
D8S1179						
D21S11						
D7S820						
CSF1PO						
D3S1358						
TH01						
D13S317						
D16S539						
D2S1338						
D19S433						

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
vWA						
TPOX						
D18S51						
D5S818						
FGA						
Penta D						
Penta E						
D10S1248						
D22S1045						
D2S441						
D1S1656						
D12S391						
D6S1043						
D1S1677						
D2S1776						
D3S4529						
D4S2408						
D5S2800						
D6S474						
D9S1122						
D12ATA63						
D14S1434						
D17S1301						
D20S482						

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

TABLE 5A-M2

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
AMEL						
D8S1179						
D21S11						
D7S820						
CSF1PO						
D3S1358						
TH01						
D13S317						
D16S539						
D2S1338						
D19S433						
vWA						
TPOX						
D18S51						
D5S818						
FGA						

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
Penta D						
Penta E						
D10S1248						
D22S1045						
D2S441						
D1S1656						
D12S391						
D6S1043						
D1S1677						
D2S1776						
D3S4529						
D4S2408						
D5S2800						
D6S474						
D9S1122						
D12ATA63						
D14S1434						
D17S1301						
D20S482						

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

TABLE 5A-M3

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
AMEL						
D8S1179						
D21S11						
D7S820						
CSF1PO						
D3S1358						
TH01						
D13S317						
D16S539						
D2S1338						
D19S433						
vWA						
TPOX						
D18S51						
D5S818						
FGA						
Penta D						
Penta E						
D10S1248						
D22S1045						
D2S441						

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
D1S1656						
D12S391						
D6S1043						
D1S1677						
D2S1776						
D3S4529						
D4S2408						
D5S2800						
D6S474						
D9S1122						
D12ATA63						
D14S1434						
D17S1301						
D20S482						

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

TABLE 5A-M4

							Nomenclature*	
LOCUS	**Total of alleles detected (CE)**	**1ST fraction (CE)**	**2nd fraction (CE)**	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
AMEL								
D8S1179								
D21S11								
D7S820								
CSF1PO								
D3S1358								
TH01								
D13S317								
D16S539								
D2S1338								
D19S433								
vWA								
TPOX								
D18S51								
D5S818								
FGA								
Penta D								
Penta E								
D10S1248								
D22S1045								
D2S441								
D1S1656								
D12S391								
D6S1043								
D1S1677								
D2S1776								

							Nomenclature*	
LOCUS	Total of alleles detected (CE)	1ST fraction (CE)	2nd fraction (CE)	Coverage	Sequence	Flanking regions variations	Bracketed Repeat Region	ISFG
D3S4529								
D4S2408								
D5S2800								
D6S474								
D9S1122								
D12ATA63								
D14S1434								
D17S1301								
D20S482								

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

2.3.2 Y-STR

TABLE 5B-M1

					Nomenclature*	
LOCUS	Alelle (CE)	Coverage	Sequence	Flanking regions variations	Bracketed Repeat Region	ISFG
DYS505						
DYS570						
DYS576						
DYS522						
DYS481						
DYS19						
DYS391						
DYS635						
DYS437						
DYS439						
DYS389I						
DYS389II						
DYS438						
DYS612						
DYS390						
DYS643						
DYS533						
Y-GATA-H4						
DYS385a-b						
DYS460						
DYS549						
DYS392						
DYS448						
DYF387S1						
DYS456						
DYS458						
DYS393						
DYS461						
GATAA10						
DYS388						

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
DYS627						
DYS518						
DYS449						

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

TABLE 5B-M2

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
DYS505						
DYS570						
DYS576						
DYS522						
DYS481						
DYS19						
DYS391						
DYS635						
DYS437						
DYS439						
DYS389I						
DYS389II						
DYS438						
DYS612						
DYS390						
DYS643						
DYS533						
Y-GATA-H4						
DYS385a-b						
DYS460						
DYS549						
DYS392						
DYS448						
DYF387S1						
DYS456						
DYS458						
DYS393						
DYS461						
GATAA10						
DYS388						
DYS627						
DYS518						
DYS449						

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

TABLE 5B-M3

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
DYS505						
DYS570						
DYS576						
DYS522						
DYS481						
DYS19						
DYS391						
DYS635						
DYS437						
DYS439						
DYS389I						
DYS389II						
DYS438						
DYS612						
DYS390						
DYS643						
DYS533						
Y-GATA-H4						
DYS385a-b						
DYS460						
DYS549						
DYS392						
DYS448						
DYF387S1						
DYS456						
DYS458						
DYS393						
DYS461						
GATAA10						
DYS388						
DYS627						
DYS518						
DYS449						

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

TABLE 5B-M4

							Nomenclature*	
LOCUS	**Total of alleles detected (CE)**	**1ST fraction (CE)**	**2nd fraction (CE)**	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
DYS505								

							Nomenclature*	
LOCUS	**Total of alleles detected (CE)**	**1ST fraction (CE)**	**2nd fraction (CE)**	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
DYS570								
DYS576								
DYS522								
DYS481								
DYS19								
DYS391								
DYS635								
DYS437								
DYS439								
DYS389I								
DYS389II								
DYS438								
DYS612								
DYS390								
DYS643								
DYS533								
Y-GATA-H4								
DYS385a-b								
DYS460								
DYS549								
DYS392								
DYS448								
DYF387S1								
DYS456								
DYS458								
DYS393								
DYS461								
GATAA10								
DYS388								
DYS627								
DYS518								
DYS449								

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2.3.3 X-STR

TABLE 5C-M1

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
DXS8378						
DXS7423						
DXS7132						
DXS10103						
DXS10074						
HPRTB						

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
DXS10135						

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

TABLE 5C-M2

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
DXS8378						
DXS7423						
DXS7132						
DXS10103						
DXS10074						
HPRTB						
DXS10135						

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

TABLE 5C-M3

					Nomenclature*	
LOCUS	**Alelle** (CE)	**Coverage**	**Sequence**	**Flanking regions variations**	**Bracketed Repeat Region**	**ISFG**
DXS8378						
DXS7423						
DXS7132						
DXS10103						
DXS10074						
HPRTB						
DXS10135						

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

TABLE 5C-M4

Nomenclature*

LOCUS	Total of alleles detected (CE)	1ST fraction (CE)	2nd fraction (CE)	Coverage	Sequence	Flanking regions variations	Bracketed Repeat Region	ISFG
DXS8378								
DXS7423								
DXS7132								
DXS10103								
DXS10074								
HPRTB								
DXS10135								

***IT IS COMPULSORY TO FILL ONE OF THESE COLUMNS**. Use preferably the ISFG nomenclature

2.4 Mitochondrial DNA results

In Table 6A, report the initial and final positions of the edited regions and in Table 6B report the haplotypes in the order requested in the instructions**. Remember ONLY to analyze the Control Region.**

TABLE 6A

ITEMS	Ranges of Edited regions	Coverage	Haplogroup
M1			
M2			
M3			
M4			
M5			

TABLE 6B

ITEMS	HAPLOTYPE
M1	
M2	
M3	
M4	
M5	

TABLA 6C

ITEMS	HAPLOGROUPE
M1	
M2	
M3	
M4	
M5	

2.4.1 Specification of the haplogrouping method or software used to obtained the haplogroup

Program	version	Remarks (other software, comments, etc)
EMPOP		
Haplogrep		
Others[1]		

[1]*If your software is not displayed in the table, choose "others" and specify it in the cell "Remarks".*

2.4.2 Phylogeographic assessment of the results.

3.Practical Studies Conclusions

3.1 Massive Parallele Sequencing (MPS) Module

3.1.1 Remarks about items M1, M2, M3, M4 and M5

Indicate any comments or remarks, you consider, about the analyzed items. It is not necessary to investigate a genetic relationship among them.

4. Remarks about this exercise

5. Suggestions for subsequent exercises

6.Compromises to be met by the participant

The analyses, both, the generated results and their statistical evaluation have been performed in the facilities of the participating laboratory and by its own staff, following working protocols used in routine casework together with safety precautions. **In accordance with the donors' consent, these items will be processed anonymously for the Intercomparison Exercise INTCFM/GHEP-ISFG. Additionally they could be used as a reference material and/or quality control for the laboratory either using the techniques required in the Exercise or other forensic techniques but always for the purpose of human identification, analyzing non coding regions or regions that would not provided sensitive information about the donor: illnesses, pathologies or other genetic information which could infringe his/her privacy.**

Name of the person in charge

Date and signature

Note.- In order to receive the certificate of participation you must return this form duly signed.