



GRUPO DE HABLA ESPAÑOLA Y PORTUGUESA DE LA ISFG

GRUPO DE LÍNGUAS ESPANHOLA E PORTUGUESA DA ISFG

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INTERCOMPARISON PROGRAM

“ANALYSIS OF DNA POLYMORPHISMS IN BLOODSTAINS AND OTHER BIOLOGICAL
SAMPLES” MASSIVE PARALLEL SEQUENCING MODULE (MPS)

BASIC LEVEL**EXERCISE EIADN –MPS 2 (2024)****DEADLINE: 15/05/2024**2024/Kinship Module

M1 to M3: reference items

2024/Forensic module

M4: forensic unknown item

M5: hair sample

Seal number

Approach:**2024/Kinship Module – Basic level****Practical Kinship study**

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

2024/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.

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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) |
|--------------------------|-----|------------------|------------|------------------|
| <u>A-STR</u> | | | | |
| <u>Y-STR</u> | | | | |
| <u>X-STR</u> | | | | |
| <u>Mitochondrial DNA</u> | | | | |

See Appendix 2024 MPS for codes

1.1.2 Library quantification

TABLE 1B

| Marker | Kit | Others (specify) | Instrument | Others (specify) |
|--------------------------|-----|------------------|------------|------------------|
| <u>A-STR</u> | | | | |
| <u>Y-STR</u> | | | | |
| <u>X-STR</u> | | | | |
| <u>Mitochondrial DNA</u> | | | | |

See Appendix 2024 MPS for codes

1.1.3 Template preparation

TABLE 1C

| Marker | Kit | Others (specify) | Instrument | Others (specify) |
|--------------------------|-----|------------------|------------|------------------|
| <u>A-STR</u> | | | | |
| <u>Y-STR</u> | | | | |
| <u>X-STR</u> | | | | |
| <u>Mitochondrial DNA</u> | | | | |

See Appendix 2024 MPS for codes

1.1.4 Sequencing

TABLE 1D

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

See Appendix 2024 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) |
|--------------------------|----------|------------------|------------------------|------------------|----------|------------------|
| <u>A-STR</u> | | | | | | |
| <u>Y-STR</u> | | | | | | |
| <u>X-STR</u> | | | | | | |
| <u>Mitochondrial DNA</u> | | | | | | |

See Appendix 2024 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

| Items | Library amplification volumen (µL) | | Individual Library quantification (pM) | | Pool Library quantification (pM) | |
|--------------------|------------------------------------|-------|--|-------|----------------------------------|-------|
| | 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 than 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 2024.

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

2.3.1 Autosomal STR and amelogenine

TABLE 5A-M1

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

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|----------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | 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 can be filled one of them or both.

TABLE 5A-M2

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

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

* It can be filled one of them or both

TABLE 5A-M3

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|----------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | 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 | | | | | | |

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

* It can be filled one of them or both

TABLE 5A-M4

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|----------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | 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 | | | | | | |
| D3S4529 | | | | | | |

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|----------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | Bracketed Repeat Region | ISFG |
| D4S2408 | | | | | | |
| D5S2800 | | | | | | |
| D6S474 | | | | | | |
| D9S1122 | | | | | | |
| D12ATA63 | | | | | | |
| D14S1434 | | | | | | |
| D17S1301 | | | | | | |
| D20S482 | | | | | | |
| | | | | | | |

* It can be filled one of them or both

2.3.2 Y-STR

TABLE 5B-M1

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|-----------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | 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 | | | | | | |

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|--------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | Bracketed Repeat Region | ISFG |
| DYS449 | | | | | | |
| | | | | | | |

It can be filled one of them or both.

TABLE 5B-M2

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|-----------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | 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 can be filled one of them or both

TABLE 5B-M3

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|-----------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | 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 can be filled one of them or both.

TABLE 5B-M4

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|--------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | Bracketed Repeat Region | ISFG |
| DYS505 | | | | | | |
| DYS570 | | | | | | |
| DYS576 | | | | | | |
| DYS522 | | | | | | |
| DYS481 | | | | | | |
| DYS19 | | | | | | |

| LOCUS | Allele (CE) | Coverage | Sequence | Flanking regions variations | Nomenclature* | |
|-----------|-------------|----------|----------|-----------------------------|-------------------------|------|
| | | | | | Bracketed Repeat Region | ISFG |
| 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 can be filled one of them or both

2.3.3 X-STR

TABLE 5C-M1

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

* It can be filled one of them or both

TABLE 5C-M2

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

* It can be filled one of them or both

TABLE 5C-M3

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

* It can be filled one of them or both

TABLE 5C-M4

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

* It can be filled one of them or both

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 | 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 | | |

¹If 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.

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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.

| |
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| |
|--|

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.