

GHEP-ISFG Forensic Advanced Theoretical Challenge 2025

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Instructions

Thank you for participating in the 2025 GHEP-ISFG Forensic Advanced Theoretical Challenge (ATC)! Alike the 2024 edition, this ATC focuses on the interpretation of (complex) DNA mixture profiles and includes interpreting mock case mixture profiles as well as theoretical multiple choice questions.

Before starting with the mock cases, please answer the questions using the questionnaire entitled '*General questions on casework practice*' to provide some general information on your current casework practice. We are aware that the provided DNA profiles (STR typing kit, settings for analysis, etc.) may differ from your casework practice. Therefore, we would like to gain insight in how much these exercises vary from your day to day work, so that this can be taken into account when analyzing the results.

In this ATC, a total of five mock cases are provided. Each mock case includes a scenario, a trace profile and one or more reference profiles of victims and/or suspects. We ask you to interpret the mixture profile, including performing a weight of evidence calculation if regarded suitable, and answering questions with regards to the interpretation of the specific case.

Finally, we ask you to answer multiple choice questions that relate to your theoretical expectations.

Below, you will find details on the DNA profiles and information for performing weight of evidence calculations. Please read this carefully. After this, you can start with the exercises and fill out the corresponding questionnaires.

We are looking forward to receiving your answers and interpretations. If there is anything unclear, or in case you have additional questions, please send these to info@ghep-isfg.org.

Details on the DNA profiles

The DNA profiles in this ATC were generated using either the GlobalFiler kit (with 29 PCR cycles and 15sec CE injection settings on an ABI 3500 Genetic Analyser) or the PowerPlex Fusion 6C kit (with 29 cycles PCR and 1.2kV 24sec CE injection settings on an ABI 3500xL apparatus).

Please note that exercises 1&2 use GlobalFiler data while exercises 3, 4 & 5 use PowerPlex Fusion 6C data.

Details on DNA profile analysis and information for LR calculations are provided in Tables 1-4.

Table 1. DNA profile analysis settings used in the GHEP-ISFG 2025 ATC for GlobalFiler data in exercises 1 & 2.

Filter	Loci	Threshold exercise 1 & 2 (GlobalFiler)
Analytical thresholds	D3S1358, vWA, D16S539, CSF1PO, TPOX	60
	Yindel, AMEL, D8S1179, D21S11, D18S51, DYS391	80
	D2S441, D19S433, TH01, FGA	45
	D22S1045, D5S818, D13S317, D7S820, SE33	75
	D10S1248, D1S1656, D12S391, D2S1338	100
Stutter filters applied	No	

Table 2. DNA profile analysis settings used in the GHEP-ISFG 2025 ATC for PowerPlex Fusion 6C data in exercises 3-5.

Filter	Loci	Threshold exercise 3, 4 & 5 (PowerPlex Fusion 6C)
Analytical thresholds	AMEL, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E	95 RFU
	D16S539, D18S51, D2S1338, CSF1PO, Penta D	140 RFU
	TH01, vWA, D21S11, D7S820, D5S818, TPOX	85 RFU
	D8S1179, D12S391, D19S433, SE33, D22S1045	135 RFU
	FGA and DYS markers	95 RFU
Stutter filters applied	Yes	See Table 3
Minimum heterozygote imbalance (MHI) percentage ^a	All loci	3%
Stochastic threshold (ST) ^b	All loci	800 RFU

^a During profile analysis a MHI was applied, meaning that per marker, every peak that falls within X% of the largest peak is removed by the software.

^b The ST is the threshold below which stochastic effects are likely to have occurred (such as drop-out, drop-in, heterozygote imbalance). For this kit and settings, the ST was set at 98.9%, which means that in 1.1% of the cases, a single peak with a height >800 RFUs (seemingly homozygote) may in fact be a heterozygote with a dropped out allele. The ST is not used in statistical analyses but can provide experts insight into whether stochastic artefacts are to be expected.

Table 3. Stutter filters applied during DNA profile analysis of PowerPlex Fusion 6C profiles, in exercises 3, 4 & 5. Note that although stutter filters are applied, these may not have been removed for 100% of the stutter peaks, specifically at the +/- 1 position.

	-1 Stutter ratio (%)	-0.5 Stutter ratio (%)	+1 Stutter ratio (%)	+0.5 Stutter ratio (%)
Amel	-	-	-	-
D3S1358	13.5	-	2.7	-
D1S1656	14.3	3.6	2.8	-
D2S441	9.0	-	2.1	-
D10S1248	13.0	-	2.9	-
D13S317	10.3	-	3.2	-
PENTA E	7.5	-	1.9	-
D16S539	12.0	-	3.0	-
D18S51	14.6	-	3.0	-
D2S1338	13.6	-	2.2	-
CSF1PO	11.1	-	3.9	-
PENTA D	4.5	-	3.7	-
TH01	4.8	-	1.5	-
vWA	14.4	-	2.7	-
D21S11	12.7	-	2.9	-
D7S820	9.7	-	2.2	-
D5S818	11.0	-	3.3	-
TPOX	5.4	-	1.1	-
D8S1179	11.8	-	3.4	-
D12S391	17.4	-	2.7	-
D19S433	12.1	-	2.6	-
SE-33	17.6	7.4	3.6	2.5
D22S1045	16.8	-	11.2	-
DYS391	14.1	-	2.1	-
FGA	12.4	-	2.8	-
DYS576	18.75	-	3.4	-
DYS570	19.5	-	2.4	-

Information for performing weight of evidence calculations

In this ATC, participants are free to use any system for weight of evidence calculations. For comparison of the results, we do ask you to use the parameters as provided in this document as much as possible, and where applicable to the LR system of use. If your system has any additional, or other, parameter settings that are not mentioned in Table 4, please provide this information in questionnaire '*General questions on casework practice*'.

Table 4. Settings for LR calculations.

Setting (if applicable to the LR system of use)	GlobalFiler (exercise 1 & 2)	PowerPlex Fusion 6C (exercise 3, 4 & 5)
Allele frequency file	NIST 1036-Caucasian	Fusion_6C_Holland2
Rare allele frequency	0.006925208	0.0003
Fst/ theta/ coancestry coefficient	0.03	0.03
Drop-in probability	0.00073	0.05
Drop-in peak height (lambda)	0.03846	0.01
Kit	GlobalFiler	PowerPlex Fusion 6C
Analytical thresholds	See Table 1.	See Table 2.