GEDNAP · c/o Institut für forensische Molekulargenetik GmbH ·
Taubenstr. 51 · 48282 Emsdetten

Emsdetten, September 2025

Sample shipment GEDNAP 70 and/or 71

Dear colleague,

please find enclosed the samples for the GEDNAP Proficiency Tests 70 and/or 71 as well as some important explanations, instructions and conditions. Please read them carefully and forward them also to the person(s) carrying out the practical steps of the analyses.

This information as well as the attached letter concerning the 'Rules for publicising the participation in the GEDNAP Proficiency Tests' and the associated declaration to be signed by the authorised participant will also be available in the „Download service center" section of the GEDNAP Homepage:

https://www.gednap.org/gednap-proficiency-tests/download-servicecenter/

Please return this document stamped and signed by an authorized person until **05 December 2025** (date of postmark). For further details see para V and X.

Kindly note that there is a novel module 14 („BGA", biogeographic ancestry) and we have added an interpretation task in the modules 1, 2, 3, 5 & 6 („Exclusion/inlusion of Person A, B and C as donor of the stains 1 - 4 ").

If you have any queries or problems, please do not hesitate to contact us.

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Sincerely,

Carsten Hohoff, PhD

Director of the
GEDNAP Proficiency Tests

I. Notes on the Samples:

The core of the GEDNAP proficiency tests, i.e. the modules 1, 2, 3, 4, 5, 6 & 8, is composed of three reference samples and four stains:

GEDNAP 70: Person A – C; stain 1 – 4
GEDNAP 71: Person A – C; stain 1 – 4

GEDNAP participants that have registered for the module 7 *(binary biostatistics)* will receive the data for the virtual stain **6** & the 3 virtual persons **Q** - **S** (see III.).

GEDNAP participants that have registered for the module 9 *(extraction efficiency)* will receive in addition stain 5 (see VI.).

GEDNAP participants that have registered for the module 10 *(probabilistic genotyping)* will receive the stain **7** & the 2 persons **N** & **O** (see IV.).

GEDNAP participants that have registered for the module 11 *(pigmentation analysis)* will receive the 3 persons **G – I** (see VII.).

GEDNAP participants that have registered for the module 12 *(age estimation)* will receive the 3 persons **D – F** (see VII.).

GEDNAP participants that have registered for the module 13 *(parentage testing)* will receive the 4 persons **J – M** (see VIII.).

GEDNAP participants that have registered for the module 14 *(BGA)* will receive the 3 persons **T – V** (see IX.).

Please bear in mind that any of the stains could consist of the DNA of a single person ("single source stain") or of the DNA from up to 3 different persons ("mixed stain"), and that the stain material might consist of saliva, blood or semen (as well as mixtures of these materials). In principle, the stains in these Proficiency Tests could simulate any stains encountered in routine casework.

N.B.: Each participating laboratory must retain some material from every stain (except for modules 9 & 10, respectively) to allow a reanalysis if necessary.

II. DNA loci that may be included in the certificate(s) for GEDNAP 70 and 71:

Please note that compared to previous years there might occur changes of the allele ranges due to the launch of new available kits.

Tab. 1: autosomal core STRs and Amelogenin - allelic ranges (Module 1)

locus	TH01	VWA	FGA	D21S11	ACTBP2	D3S1358	D8S1179	D18S51	D16S539
allele range*	2-14.3	9-25	12-34.2, 41.2-52.2	23-39	3.2-43, 48-50	8-21	4-20	6-28	3-17

locus	D2S1338	D19S433	D12S391	D2S441	D10S1248	D22S1045	D1S1656	Amelogenin
allele range *	9-29	4.2-20.2	13-28	7-18	7-20	6-21	8-21.3	X/X; X/Y

Tab. 2: supplementary autosomal STRs - allelic ranges (Module 2)

locus	TPOX	CSF1PO	D5S818	D13S317	D7S820	Penta D	Penta E	D6S1043
allele range *	3-17	4-17	5-19	4-18	4-17	1.2-18	4-25	6-26

Tab 3: Y-STRs - allelic ranges (Module 3)

System	DYS19	DYS385	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS437
allele range *	8-20	5-29	8-18	23-36	16-30	4-17	3-21	6-19	9-19

System	DYS438	DYS439	DYS448	DYS449	DYS456	DYS458	DYS460	DYS481	DYS518
allele range *	5-17	5-18	13-25	21-41	9-25	9-25	6-15	16-33	31-50

System	DYS533	DYS549	DYS570	DYS576	DYS627	DYS635	DYS643	GATAH4	DYF387S1
allele range *	6-18	6-18	9-27	9-26	10-28	14-31	5-18	7-19	29-45

Tab. 4: additional autosomal STRs - allelic ranges (Module 5)

System	D2S1360	D3S1744	D4S2366	D5S2500	D6S474
allele range *	18-33	12-22	8-16	8-19	12-20

System	D7S1517	D8S1132	D10S2325	D21S2055
allele range *	15-29	11.1-28	5-20	15.1-40

Tab. 5: X-STRs - allelic ranges (Module 6)

locus	DXS8378	HPRTB	DXS7423	DXS7132	DXS10134	DXS10074	DXS10101
allele range *	7-16	7-18	11-19	9-18	27-45.3	3-22	20-36

locus	DXS10135	DXS10079	DXS10103	DXS10148	DXS10146
allele range *	12-40.2	13-26	14-23	12.3-34.1, 37.1-39.1	20-48.2

Notes on the tables 1 - 5

*: the numbers indicate the allelic range in which the classification of alleles must be made.
for further explanations see para **X**.

6. mtDNA (Module 4)

The sequence range np 16024 – 576 will be evaluated. If a participating lab uses a differing sequence range (e.g., np 16024-16365, 73 – 340 and/or 438 – 576), we will evaluate the results in these ranges and these ranges will be indicated in the certificate. Please make sure that you provide the sequence range that you have been able to evaluate on both strands.

III. <u>Binary biostatistical calculation</u> (Module 7)

In the course of both GEDNAP Proficiency Tests GEDNAP 70 and/or 71 a paper challenge will be conducted for the biostatistics module. The necessary DNA typing results will be sent by eMail. In case of a mixed stain please use **both** calculation methods that are recommended by P. Schneider et al. (2009) Int J Legal Med 123:1–5), i.e., LR and RMNE. Please use only the allele frequencies that were observed in the course of an ENFSI population study (source: "Europe" in STRidER_frequencies_2024-09-24, https://STRider.online, download on 18 December 2024). They can be downloaded from the GEDNAP website (https://www.gednap.org). For rare alleles please follow the recent recommendations (W. Ulbrich et al. (2016) Rechtsmedizin 26: 291-298):

The frequency of rare alleles (f_{min}) shall be estimated according to the NRC report (1996). If the estimated f_{min} is below 0.001, one shall use the value 0.001.

The calculation steps must be documented by a print-out. The employed software version must be named. Please note that the calculations have to be executed without the correction factor 'theta' (i.e. with a theta value of zero).

<u>IV. Probabilistic genotyping</u> (Module 10)

There will be a particular module for *probabilistic genotyping*. A mixed stain, that is to be evaluated by a software that follows a fully continuous model, and two reference samples will be sent out. The employed software version must be named. The calculation steps must be documented by a print-out. Please note that the calculations have to be executed without the correction factor 'theta' (i.e. with a theta value of zero). For this module a certificate of participation will be issued.

V. Instructions for submitting the results

- To submit your results (e.g., stain characterization, extraction details, genotyping, mixed-person stain calculation), please exclusively use the web forms on the GEDNAP website (https://upload.ifmg-ms.de). Valid submission will end on **05 December 2025 at 23:59 CET**. Detailed information is available on the website. By means of particular checkwords you will be able to submit & save your results in the module that you have booked. Your invoice number (see als the attached delivery note) will be needed upon upload.

- After submitting your results electronically we request you to send a signed and stamped printed copy, which has to be posted to us together with your original laboratory data (e.g., print outs of the relevant electropherograms) as well. The deadline is the **05 December 2025** (date of postmark).

- Also for the submission of your mtDNA results please use the form on the GEDNAP website (https:// upload.ifmg-ms.de). As in previous years, you are requested to score the differences of the sequences of Persons A-C and single source stains to the revised Cambridge Reference Sequence (rCRS) using the nomenclature recommendations of the DNA commission of the ISFG (W. Parson et al. 2014, PMID: 25117402). In the case of a point heteroplasmy the np shall be reported according to the IUPAC code, provided the minor component represents at least 20 %. In the case of a length heteroplasmy, „LHP" shall be reported in the field 'remarks'; the shorter variant shall be named if there are two dominant types. The second dominant variant shall be reported in the field 'remarks' as well.

VI. Extraction Efficiency (Module 9)

In the course of both Proficiency Tests GEDNAP 70 and 71 a fifth stain will be sent out in triplicate for the module "extraction efficiency". The participant shall extract the **three sub-samples (stain 5.1, 5.2 and 5.3) of each proficiency test separately by the same methodology** without prior presumptive testing **and return the three DNA extracts per proficiency tests** at his own expense and on his own responsibility to the IFMG (within 6 weeks after receiving of the samples but no later than **30 November 2025**). The postal address is as follows:

Institut für Forensische Molekulargenetik
GEDNAP XF
Taubenstr. 51
D-48282 Emsdetten
Germany

We will provide labelled Eppendorff tubes for your the DNA extracts. Please do only use our tubes for shipment. The use of different tubes migth hinder the proper evaluation of your extracts.

The choice of transport is left to the participant (on ice, frozen, lyophilized with or without a stabilizer). The IFMG asks for details of the extraction techniques (i.e., analytical platform/hardware & chemistry [if applicable], elution buffer and elution volume) and performs the DNA quantitation by real-time PCR. The individual reporting will be provided in conjunction with the other report forms in March 2026. For this module a certificate of participation will be issued.

VII. Forensic DNA phenotyping (Module 11& 12)

In the course of the GEDNAP Proficiency Tests GEDNAP 70 & 71 there will be two modules entitled „Pigmentation Analysis“ and „Age Estimation“. While for „Pigmentation Analysis“ 41 established markers can be investigated (see https://hirisplex.erasmusmc.nl/), the markers for the module „Age Estimation“ have not been specified. For the module 11 we will issue a simple certificate of participation while for the module 12 („age estimation“) a qualified certificate will be issued.

VIII. Module for parentage testing

In the course of the GEDNAP Proficiency Tests GEDNAP 70 & 71 there will be the novel module entitled „Parentage Testing“. Participants of this module will receive oral swabs of 4 persons (i.e., „child“ (K), „mother“ (**J**), „alleged father 1“ (**L**) & „alleged father 2“ (**M**)), that shall be typed with up to 24 systems (i.e., AMELOGENIN, CSF1PO, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D1S1656, D21S11, D22S1045, D2S1338, D2S441, D3S1358, D5S818, D6S1043, D7S820, D8S1179, FGA, PentaD, PentaE, ACTBP2/SE33, TH01, TPOX and VWA) to answer the question of inclusion/exclusion of the two men. In the case of a non-exclusion a biostatistical evaluation of the findings is expected (W value and/oder paternity index PI) on the basis of the provided allele frequencies for all of the following 17 systems: CSF1PO, D12S391, D13S317, D16S539, D18S51, D19S433, D1S1656, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX and VWA. The calculation steps must be documented by a print-out. The employed software version must be named. Please use only the allele frequencies that were observed in the course of an ENFSI population study

(source: "Europe" in STRidER_frequencies_2024-09-24, https://STRider.online, download on 18 December 2024). They can be downloaded from the GEDNAP website (https://www.gednap.org). For this module a qualified certificate will be issued, both for genotyping and biostatistics.

IX. BGA (biogeographic ancestry, module 14)

In the course of the GEDNAP Proficiency Tests GEDNAP 70 & 71 there will be for the very first time the module „BGA". The samples **T** – **V** are to be analysed. Methods and markers shall be used that have been established in the participating lab. The results form (available at https://upload.ifmg-ms.de) will ask for the markers & methods as well as for a brief verbalization of the results.

X. General Information

- Please enter only numerical allele values in the web-based results' forms; we would consider any other character (e.g., OL, F, ?) as an error, except for < and > (see below).

- 'Off-category' alleles, i.e. those alleles that are smaller than the smallest allele or longer than the longest allele in the STR systems listed in table 1 - 5, can be reported using the "smaller than" (<) or "greater than" (>) signs relative to the shortest or longest allele. Example: allele 18 at TPOX can be given as '**>17**' or as '**18**' – both would be considered correct (i.e., group 1). Please note that allele numbers must be given with a 1bp precision (this does not however mean that the example allele above should be scored as *18.0*). Allele designations not adhering to these instructions will be considered erroneous (i.e., group 4).

- For evaluation and certification it is obligatory to include original laboratory data, i.e., copies of the electropherograms of the samples **and** the corresponding allelic ladders. The allele scoring must be readily visible and unambiguous, amplicon lengths and peak heights must be readable as well. The printed copies must be clearly marked with the Proficiency Test series (GEDNAP 70 or GEDNAP 71, respectively), with the sample name and with its laboratory TAN (i.e., the second part of your invoice number, e.g. „5-**9876**-25-70"). Printed sequence electropherograms must be labelled likewise, and the evaluated range must clearly be indicated by the nucleotide positions (np). The relevant np must be indicated. Furthermore, the steps from the electropherogram to the scoring as deviation(s) from the rCRS must be documented (among other things by

mentioning the software for generating the consensus sequence from sequencing both strands). Examples of a GeneMapper analysis as well as an exemplary print-out of a sequencing electropherogram with proper labels are available upon request.

- If the original laboratory data are not included in the submission, the results will not be evaluated and subsequently a certificate will not be issued.

- If you wish to send your original data in digital form (e.g. USB stick, DVD/CD-ROM, e-mail attachment) please ensure that the files are clearly labelled and comprehensible. (Sequencing) electropherograms have to be sent as PDF files. Please provide your user name (also known as laboratory code)

- Qualified certificates of participation will be issued for the following modules: stain characterisation, autosomal STRs, Y-STRs, X-STRs, sequence analysis of the mtDNA control region, age estimation, parentage testing and binary biostatistics of mixed-person stains. For the other modules (i.e, 9, 10, 11, as well as 14) we will issue a simple certificate of particiation.

- Certificates of participation can be issued only in the name of the institute which has actually undertaken the analysis. An analysis by a third party is not permissible. All participants have to sign a self-declaration stating that their GEDNAP certification may not be used by third parties, for example for advertising purposes. If this self-declaration has been submitted in the previous year it can be omitted this year. If the self-declaration has not been received by us until **05 December 2025**, we will neither evaluate the results nor subsequently issue the certificates.

- The error categories are defined as in the previous years. Details are given on the GEDNAP website (https://www.gednap.org).

XI. Report of the result evaluation

The evaluation of the individual results of the Proficiency Tests GEDNAP 70 and 71 will be presented in written form in March 2026.

XII. Fulfilment of Conditions

The executive of the proficiency tests agrees to provide test samples, evaluate submitted results and to issue certificates if the participating laboratory meets the above conditions and furthermore pays the current participation fee, signs the enclosed self-declaration by an authorized member of the participating laboratory and sends it back to the executive such that it arrives at his address. If any of these requirements is not fulfilled, then the submitted results will not be evaluated and a certificate will not be issued.