Netherlands Forensic Institute
Ministry of Justice

DNAxs

DNAStatistX

A new software suite for data management and probabilistic interpretation of DNA profiles

NFI Division Biological Traces

History

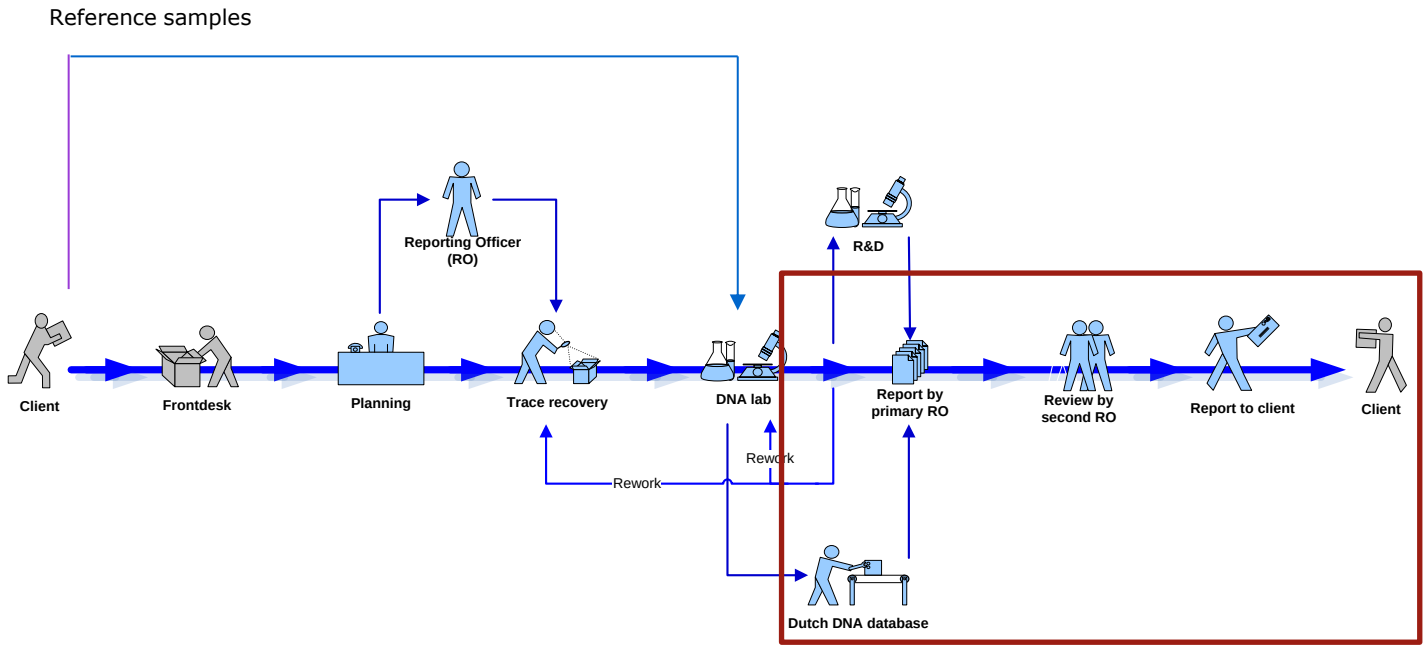
- NFI experience with development of:
  - Automation solution for the laboratory process
  - Automation solution for the storage of samples
  - Software tools used in DNA case work
    - Bonaparte/Napoleon
    - LOCIM tool
    - LRmix/LRmix Studio
    - SmartRank
    - MixCal

Key projects within NFI strategy

Projects to

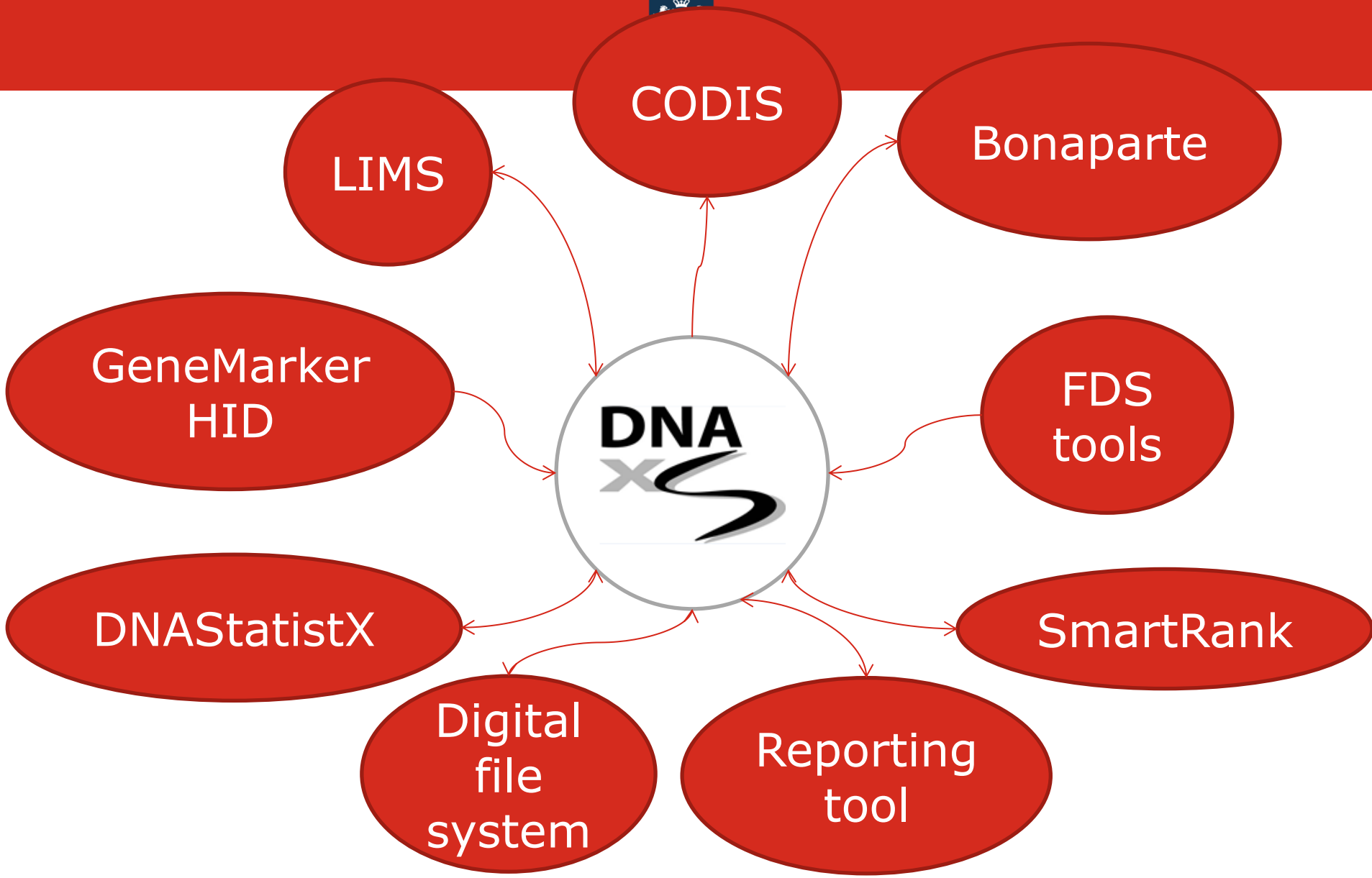
- Allow more capacity for more casework and more traces per casework
- Allow a faster workflow and fast answers in the Police investigation process > investigative leads
- Allow a reduction in costs in DNA profiling
- Enhance evidential value by gathering more information from traces by development and implementation of
  - Molecular tools
  - Analytical and Statistical tools

Workflow: Following a case



Support tools in casework interpretation

- Growing number of markers in profiling systems
  - Global STR marker systems available
  - Standard kit the PPF6C kit (27 loci)
- DNA-profile comparison therefor increasingly
  - complex
  - time-consuming
  - error-prone
- Statistical support integrated in casework workflow


CODIS
LIMS
Bonaparte
GeneMarker HID
DNA xs
FDS tools
DNAStatistX
SmartRank
Digital file system
Reporting tool

What is DNAxs

- NFI developed DNA eXpert System
  - Automatic comparison of sets of DNA-profiles
  - Summary statistics on allele numbers and genotype reproducibility
  - Mixture interpretation
  - Statistical Analysis (March 2019 release)

- In house built (Java)
- Web application (browser)
- Server based
- Validated according to ISO 17025 and NFI standards
- In use since December 2017
- Three releases per year

Functionality of DNAxs

- View profiles
  - Overview of runs and peak heights
  - Bar graphs visualizing alleles/peaks heights/read counts
  - Electropherograms, link to pdf of EPG
- Match profiles
  - Trace vs person
  - Trace vs trace
  - Match matrix
- Derive profiles
  - LoCIM inference of major profile, consensus and composite profile
- Statistics
  - DNAStatistX module
  - Summary statistics (TAC/MAC/type I/II/III loci)
- Supports several NFI profiling workflows (from HVC to complex/severe cases)
- Connectivity to other software LIMS/CODIS/SmartRank/DNAStatistX
- Audit trail

Quality control

- Internal validation according to ISO 17025 standard and internal procedures
  - Validation plans
  - Validation reports
- Q-procedure and software manual
- Internal audit
- External audit

- Integration testing

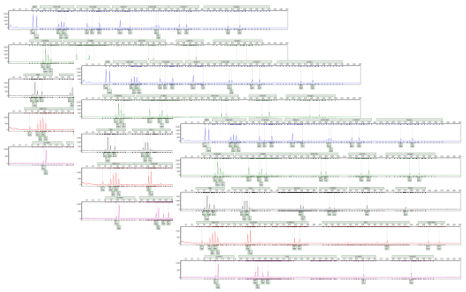
DNAStatistX

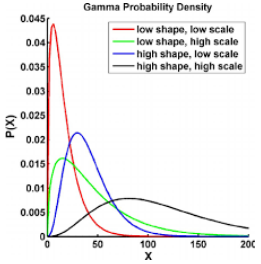
- Based on EuroForMix R code
- As a separate module within DNAxs
- MLE method
- Degradation module included
- Stutter module not included
- Dye Specific detection thresholds
- Tool for number of contributors

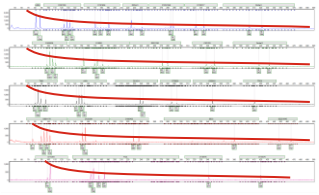
DNAStatistX features

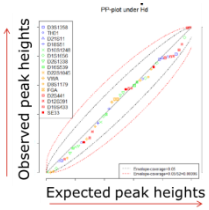
$$LR = \frac{P(E|Hp)}{\boldsymbol{P(E|Hd)}} = \frac{\sum w(E, Gp|\beta p)P(Gp|Hp)}{\sum \boldsymbol{w(E, Gd|\beta d)P(Gd|Hd)}}$$

- MLE method
- Up to four contributors
- Can handle multiple replicates
- Degradation model
- Model validation









- Aim for a maximum run time of 24h for a four-person mixture with three replicates and four unknowns under Hd

From EuroForMix to DNAStatistX: What's the same?

LR calculation using maximum likelihood estimate (MLE)

User:

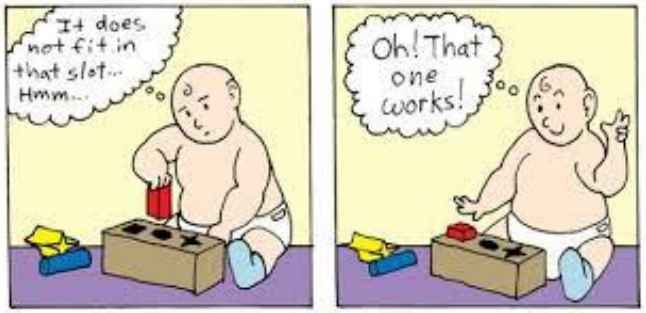
- Define hypotheses

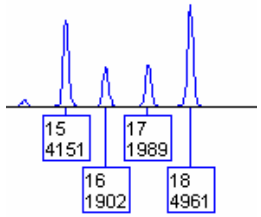
Likelihood computation (under Hp and Hd):

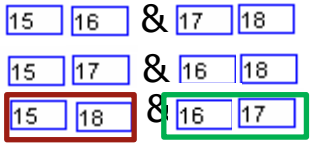
1. Estimate parameters (using optimizer, trial and error)
   - Mixture proportions
   - Peak height expectation
   - Peak height variance
   - Degradation slope
2. Determine possible genotype combinations
3. Calculate genotype probabilities (incl. drop-in)
4. Calculate peak height probabilities (incl. drop-in/-out) for each genotype combination
5. Compute profile likelihood

LR calculation:

- Likelihood Hp / likelihood Hd







From EuroForMix to DNAStatistX: What's different?

- Parts of the EuroForMix code implemented in DNAStatistX
  - Maximum Likelihood Estimate (MLE)

	EuroForMix	DNAStatistX
Code	R and C++	Java
Optimizer	nlm	CMA-ES
Model validation	AdaptIntegrate	TrapezoidIntegrator
Rare allele frequency	Lowest frequency at particular locus	1/(2*size of population)
Detection threshold	Overall	Dye (locus) specific

Which EuroForMix features in DNAStatistX?

➢ Degradation model

- NGM profiles sometimes showed degradation for research samples and often for casework samples
- All PowerPlex Fusion 6C (PPF6C) profiles showed degradation to some extent

Configurable in DNAStatistX, ON by default

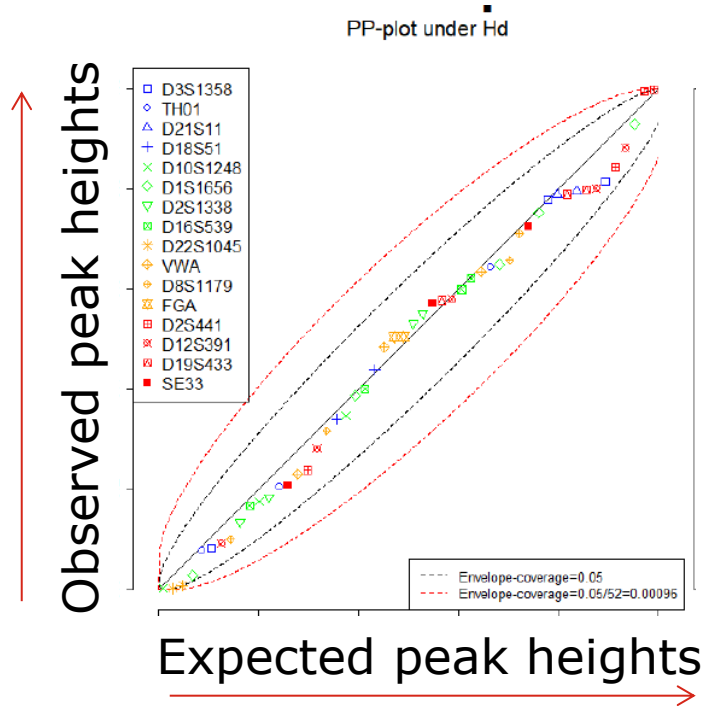
➢ Stutter model

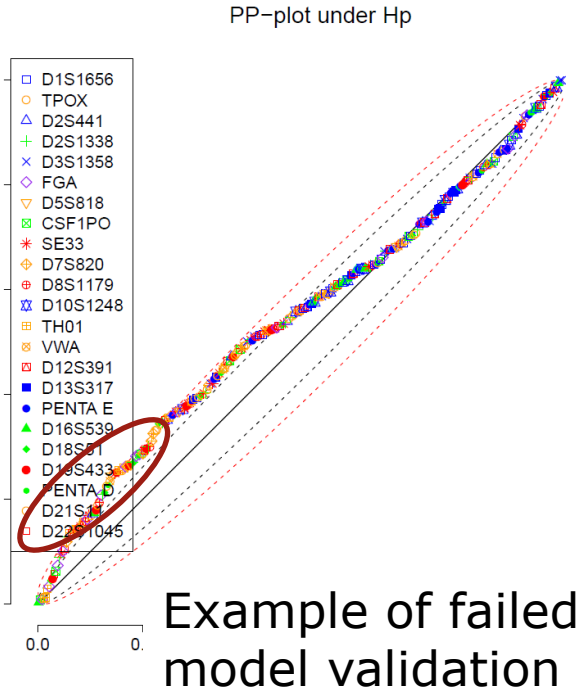
- Types of stutter:
  - GeneMapper/GeneMarker etc: -2, -1, -0,5, +0,5, +1 repeat unit
  - EuroForMix: -1 repeat unit
- Less specific than profile analysis software, very time consuming

NOT (YET) in DNAStatistX

Which EuroForMix features in DNAStatistX?

- Model validation
- Important quality check: Do observed PHs follow model's expected PHs





Example of failed model validation

Implementation in DNAStatistX, for every analysis

Developmental validation of DNAStatistX

- Accuracy:
  - Comparison to analyses using ground truth parameters
  - Comparison to EuroForMix
- Precision:
  - Repeated analyses
  - Optimizer iterations
- Robustness:
  - Analyses that should fail
- Sensitivity:
  - True positives/ false negatives (Type I errors)
- Specificity:
  - True negatives / false positives (Type II errors)

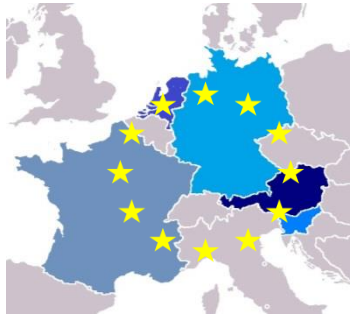
Using a range of case type samples

Collaboration with international partners

- Additional funding for research and development
- Enhance quality of software by incorporating integration testing
- Develop DNAxs in a multi-lab tool for profile comparison/evaluation/interpretation
- Across laboratory validation
- Possibility to disseminate software to other forensic institutes

ISFP-2017-AG-FORENSIC - DNAxs2.0

Partner choice: Different languages, different demands
Excellent network (ISFG, EuroForGen, dna.bases)
Casework laboratories of different size
Law enforcement or academic



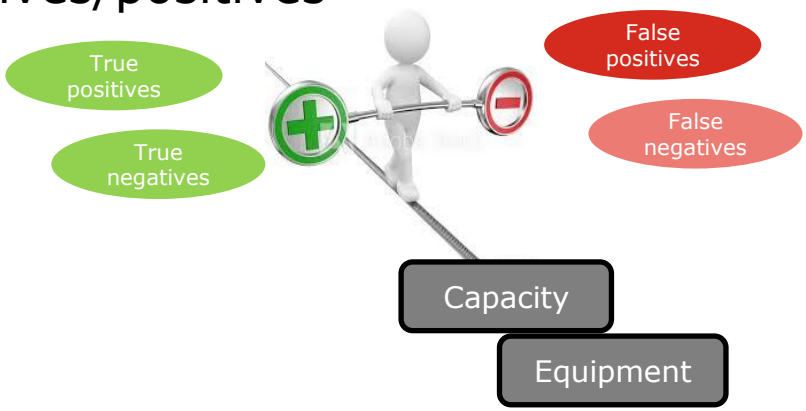
End-users? Forensic DNA caseworkers
High demand for DNAxs-like tools
Partners represent end-users with role in across-Europe validation

Obstacles? Computer requirements: ⇨ Hardware for each partner in project
Expert personnel: ⇨ Software building expertise within NFI
⇨ All partners experienced in validations
⇨ Experienced in organizing workshops

Create guidelines by

➢ Examining:

- True positives/negatives - False negatives/positives
- Effect replicates
- Effect number of contributors
- Effect number of drop-outs
- Etc.



➢ Defining:

- Sample types/hypotheses for which LR calculations can be informative
- LR threshold
- What to consider when examining results
- What to do if model validation fails
- Etc.

Define guidelines for use in forensic DNA casework to:

- Ensure chance of obtaining ‘false-positive’ results is close to zero
- Minimise the number of false-negative results
- Perform LR calculations when regarded useful
- Aim for uniformity among reporting officers

Future functionalities

- MPS data; first module for mtDNA
  - only accessible with mtDNA data
  - Sequential matching
  - Release planned for september 2019
  - EMPOP searches
  - mtDNA matchbox
  - CODIS export
- Followed with STR MPS data
- Under research investigation
  - Stutter model inclusion vs use of laboratory stutter filtered data only
  - Implementation method to estimate number of contributors
  - Deconvolution of all mixed profiles followed by LR computation

Release history DNAxs

Version	Release date	Release theme
1.0	04 December 2017	Initial release
1.0.2		
1.0.3		
1.0.4		
1.0.5		
1.1.0	24 April 2018	HVC release
1.1.1		
1.1.2		
1.1.3		
1.2.0	26 July 2018	Tags/notes/ Bonaparte export
1.3.0	2 April 2019	Initial DNAStatistX release