Complex mixture interpretation

Leuven style

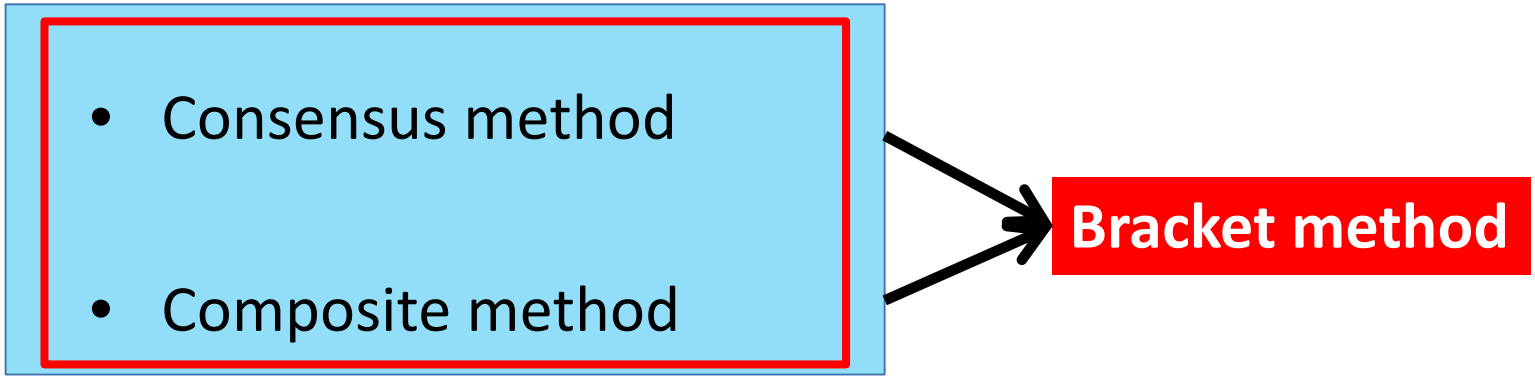
Lab protocol

- Each trace DNA-extract
  - 4 PCRs
    - 2x Powerplex ESI
    - 2x Miniplex-9
- Build consensus profile
  - Perl script (Bracket method)
  - Identify dominant profile

DNA marker	Powerplex ESI	MP-9	Coverage
Amelogenin	√	√	x4
D1S1656	√	√	x4
D1S1677		√	x2
D2S441	√	√	x4
D2S1338	√		x2
D3S1358	√		x2
D8S1179	√		x2
D10S1248	√	√	x4
D12S391	√	√	x4
D16S539	√		x2
D18S51	√	√	x4
D19S433	√		x2
D21S11	√	√	x4
D22S1045	√		x2
FGA	√	√	x4
TH01	√		x2
VWA	√		x2

Interpreting replicate DNA profiles

- Picking the least or most informative profile

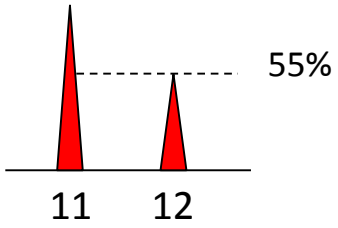


- Semi-continuous methods

- Continuous model (expert software)

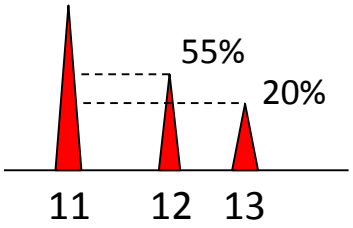
Composite method

Replicate 1



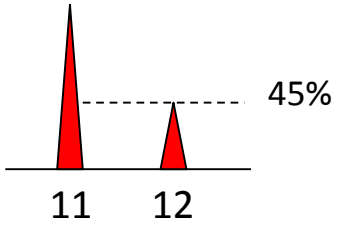
11-12

Replicate 3



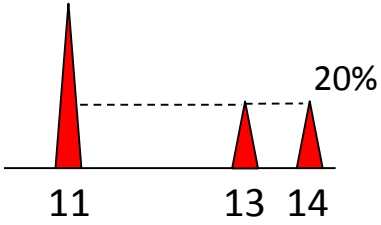
11-12-13

Replicate 2



11-12

Replicate 4

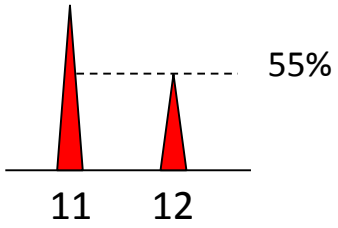


11-13-14

Composite profile 11-12-13-14

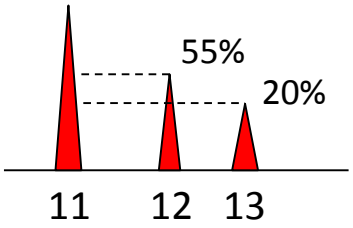
Consensus method

Replicate 1



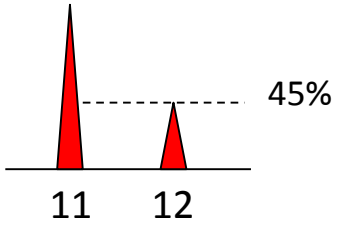
11-12

Replicate 3



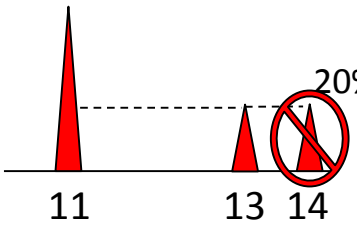
11-12-13

Replicate 2



11-12

Replicate 4

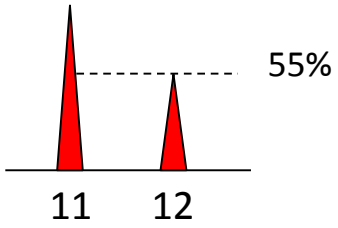


11-13

Consensus profile 11-12-13

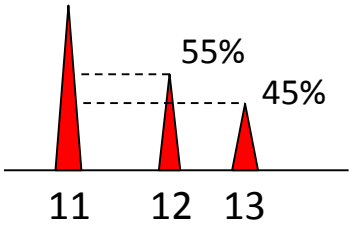
Bracket method

Replicate 1



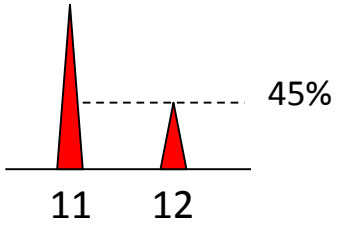
11-12

Replicate 3



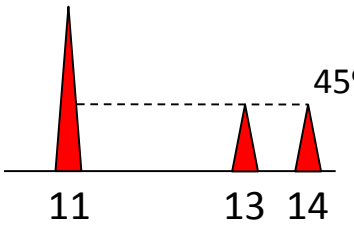
11-12-(13)

Replicate 2



11-(12)

Replicate 4



11-(13-14)

Consensus profile

11-12
(13)
[14]**

AMEL	D10S1248	D12S391	D16S539	D18S51	D19S433	D1S1656	D1S1677	D21S11	D22S1045	D2S1338	D2S441
XY	14	17	**11-12**	13-19	11-14	10-16	14	29-32.2	15-16	24	11-14
		(17.3) [20]**	**(13) [14]****	[14-15]**	[13-15]**	[11-18.3]**		[30-31]**		[16-19]**	

( ) = alleles with weak intensity

[ ]** = not reproducible alleles

“Statistics” (1)

- Useable profiles?
  - 1-person: ≥ 7 reproducible loci (≥4 heterozygotes)
  - 2-person: ≥ 10 reproducible loci
  - 3-person: ≥ 13 reproducible loci
  - 4 or 5-person: only when a dominant profile is present

“Statistics” (2)

- Single person profile or dominant profile which matches reference profile
  - RMP (always >1 billion) (reported as “as close to certainty”)
  - Also partial dominant profiles (at least 7 loci of which at least 4 heterozygotes)

“Statistics” (3)

- Is a dominant profile present?
  - Max 2 alleles per locus

AMEL	D10S1248	D12S391	D16S539	D18S51	D19S433	D1S1656	D1S1677	D21S11	D22S1045	D2S1338	D2S441
XY	14	17	**11-12**	13-19	11-14	10-16	14	29-32.2	15-16	24	11-14
		(17.3) [20]**	**(13) [14]****	[14-15]**	[13-15]**	[11-18.3]**		[30-31]**		[16-19]**	

  - Match with reference profiles = RMP
  - Also partial dominant profiles

“Statistics” (4)

- No dominant profile present?
- Comparison between trace and reference profiles
- Rules before carrying out statistical calculations
  - 2-person mixture
    - 1 difference = inclusion (possible drop-out)
    - 2 differences = not included/excluded unless matches with non-reproducible loci
    - 3 or more differences = excluded
  - 3-person mixture
    - 1 difference = inclusion (possible drop-out)
    - 2 or 3 differences = not included/excluded unless matches with non-reproducible loci
    - 4 or more differences = excluded
  - 4- and 5-person mixtures = only dominant profiles

Example

- 2-person mixture with partial match (consensus from 4 replicates)

Trace

AMEL	D10S1248	D12S391	D16S539	D18S51	D19S433	D1S1656	D1S1677	D21S11	D22S1045	D2S1338	D2S441
XY	14-15-17	17-17.3 [20]**	11-12 (13) [14]**	13-19 [14-15]**	11-14 [13-15]**	10-16 [11-18.3]**	14-16-19	29-32.2 [30-31]**	15-16-17 [14]**	24 [16-19]**	11-14

Reference

AMEL	D10S1248	D12S391	D16S539	D18S51	D19S433	D1S1656	D1S1677	D21S11	D22S1045	D2S1338	D2S441
XY	14	17	11-12	13-19	11-14	10-16	14	29-32.2	15-16	24	11-14

Included

AMEL	D10S1248	D12S391	D16S539	D18S51	D19S433	D1S1656	D1S1677	D21S11	D22S1045	D2S1338	D2S441
XY	14-15-17	17-17.3 [20]**	11-12 (13) [14]**	13-19 [14-15]**	11-14 [13-15]**	10-16 [11-18.3]**	14-16-19	29-32.2 [30-31]**	15-16-17 [14]**	? [16-19]**	11 ?

Included

AMEL	D10S1248	D12S391	D16S539	D18S51	D19S433	D1S1656	D1S1677	D21S11	D22S1045	D2S1338	D2S441
XY	14-15-17	17-17.3 [20]**	11-12 (13) [14]**	13-15 [14-19]**	11-14 [13-15]**	10-16 [11-18.3]**	14-16-19	29-32.2 [30-31]**	15-16-17 [14]**	? [16-19]**	11-14

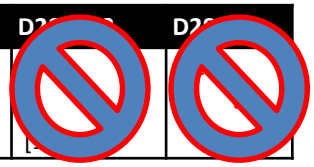
Excluded

AMEL	D10S1248	D12S391	D16S539	D18S51	D19S433	D1S1656	D1S1677	D21S11	D22S1045	D2S1338	D2S441
XY	14-15-17	17-17.3 [20]**	11-12 (13) [14]**	13-15 [14-19]**	11-14 [13-15]**	10-16 [11-18.3]**	14-16-19	29-32.2 [30-31]**	15-16-17 [14]**	? [16-19]**	11 ?

Coping with drop-outs

- Drop the locus

AMEL	D10S1248	D12S391	D16S539	D18S51	D19S433	D1S1656	D1S1677	D21S11	D22S1045	D2	D2
XY	**14**-15-17	**17**-17.3 [20]**	**11-12** (13) [14]**	**13-19** [14-15]**	**11-14** [13-15]**	**10-16** [11-18.3]**	**14**-16-19	**29-32.2** [30-31]**	**15-16**-17 [14]**		



- We want to switch to the semi-continous models but …

Discussion

- How do semi-continuous models deal with drop-outs from replicates in which not all markers are covered to the same frequency?

DNA marker	Powerplex ESI	MP-9	Coverage
Amelogenin	√	√	x4
D1S1656	√	√	x4
D1S1677		√	x2
D2S441	√	√	x4
D2S1338	√		x2
D3S1358	√		x2
D8S1179	√		x2
D10S1248	√	√	x4
D12S391	√	√	x4
D16S539	√		x2
D18S51	√	√	x4
D19S433	√		x2
D21S11	√	√	x4
D22S1045	√		x2
FGA	√	√	x4
TH01	√		x2
VWA	√		x2

UZ LEUVEN

Course on Statistical Methods for Complex Mixture Interpretation

11-12th September 2014 **Department of Forensic Medicine, Campus St-Rafaël, UZ Leuven, Leuven**

Agenda

- **Statistics of complex mixture interpretation**

11th September

09u00 - 12u00: basics on interpreting complex mixtures
13u00 - 17u00: introduction to new software packages, theory on LRmix and the validation of LRmix

12th September

09u00 - 12u00: practical exercises with LRmix
13u00 - 16u00: solving real casework examples from participants
16u00 - 17u00: open discussion

- **Lecturers:**

Prof. Dr. Ronny Decorte: lab director of the Laboratory for Forensic Genetics and Molecular Archaeology

Dr. Charlotte Aelbrecht: DNA-expert NICC

Dr. Guro Dørum: researcher at the Norwegian University of Life Sciences

Dr. Bram Bekaert: assistant lab director of the Laboratory for Forensic Genetics and Molecular Archaeology

Aims

The course is free of charge and is organised as part of the EUROFORGEN (European Forensic Genetics Network of Excellence) project which aims to provide training and education in order to establish common European-wide standards for generating and interpreting genetic data related to biological evidence. The course is therefore aimed at scientists who are actively involved in forensic genetics.

The course, lunch and coffee break are provided free of charge to all participants. Participants will need a laptop with preinstalled LRmix software. Instructions for downloading and installing the software will be provided beforehand. Lectures will be taught in English.

To register send your name, position and lab to bram.bekaert@uzleuven.be before 27th June 2014. Only forensic geneticists can register for the course. There is a limit of 25 participants.

Laboratorium voor Forensische Genetica en Moleculaire Archeologie
Dienst Forensische Geneeskunde
UZ Leuven