Locus allele count : a tool to estimate the number of contributors in a DNA mixture

Marie-Pier Thibault, Forensic biologist, LSJML

Green Mountain DNA Conference – July 2023

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About our lab

- Serves a population of about 8,5 million
- Caseload of >8000/year; 35 000 exhibits
- Team of 84, including 45 forensic biologists

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- Amplification and CE : AmpFLSTR™ Identifiler™ Plus (ID+; 15 sites), 3500 XL, Genemapper ID-X
- STRMix 2.5, validated up to 5C
- Support the inclusion with an LR > 1000 (0.001 to 1000 = inconclusive)

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Plan

- Introduction:
  - Importance of making an informed estimation of the number of contributors (NOC) in a DNA mixture
  - Highlights of our previous work on the Total Allele Count (TAC)
- How can locus allele count (LAC) be used to refine NOC estimation
  - DNA mixtures generation
  - How to use the tool
  - Let's put the tool to the test

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Acknowledgments

Tommy Harding
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Nicolas Tremblay
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Don't shoot the messenger

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DNA evidence

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7

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- DNA evidence is widely used as a circumstantial evidence in crime investigation.
- … but DNA evidence doesn't speak on its own
- Complex DNA mixtures now represent a significant portion of DNA casework results and approaches to mixture **interpretation** are of great interest to the forensic community.

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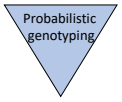
Interpretation is required for probabilistic genotyping



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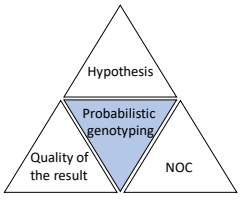
NOC estimation is a core component of the weight of evidence



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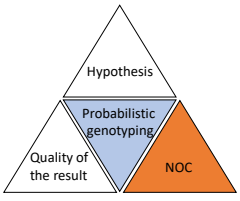

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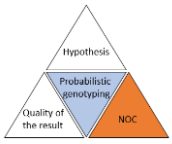

NOC
- the true NOC is never known in forensic samples
- an essential variable of the deconvolution
- uncertainty remains about the NOC even after careful analysis (> 3C)
- Variability between analysts (**DNAmix 2021**)

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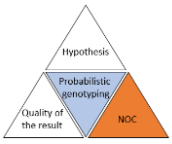
NOC estimation is a core component of the weight of evidence



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- Variability between analysts

What are the consequences of an incorrect NOC estimate?

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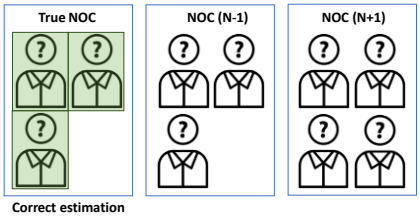
Incorrect NOC estimate can lead to false exclusion



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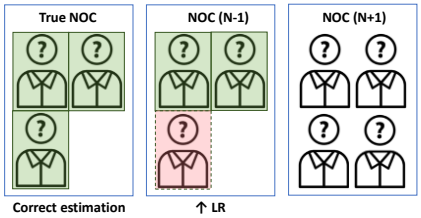

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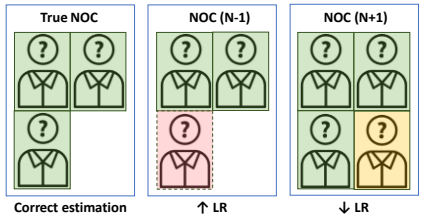

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Tools used to estimate the NOC

Maximum allele count (MAC)
Allelic balance
Inter-loci consistency

18

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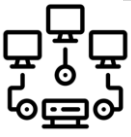

Maximum allele count (MAC)
Allelic balance
Inter-loci consistency

Maximum likelihood estimation (MLE)

NOCit (Bayesian method)
PACE (AI classification)
FaSTR (AI classification)

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Tools used to estimate the NOC

Maximum allele count (MAC)
Allelic balance
Inter-loci consistency

Maximum likelihood estimation (MLE)

NOCit (Bayesian method)
PACE (AI classification)
FaSTR (AI classification)

Need time to learn & validate

20

Total allele count distribution (TAC curves) improves NOC estimation

CANADIAN SOCIETY OF FORENSIC SCIENCE JOURNAL
https://doi.org/10.1080/00085030.2022.2028359
Taylor & Francis
RESEARCH ARTICLE
Check for updates

Total allele count distribution (TAC curves) improves number of contributor estimation for complex DNA mixtures

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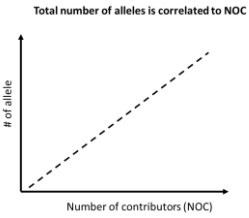
Total allele count (TAC) is proportional to NOC



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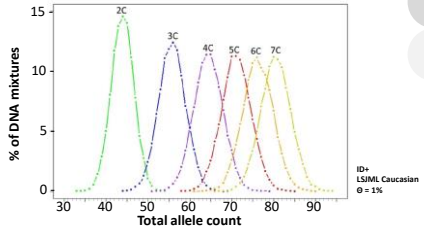

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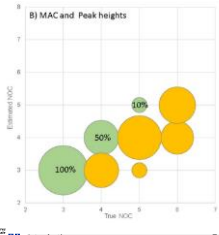

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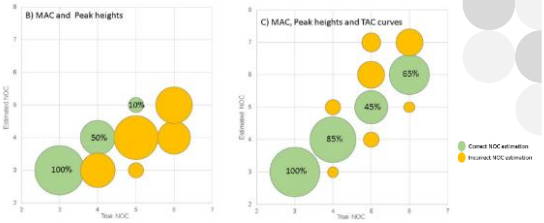

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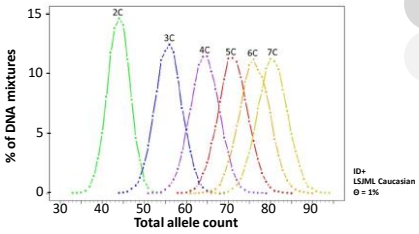
Can we learn more from TAC curves?



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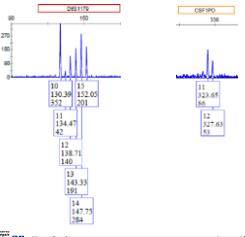

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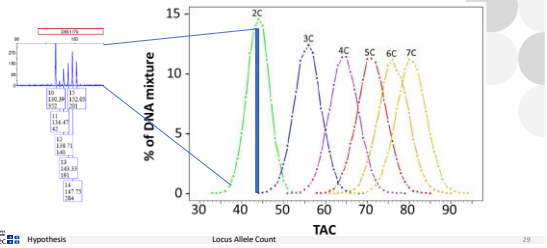

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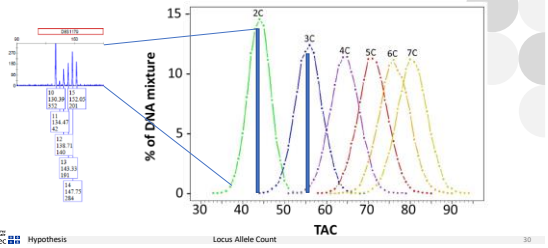

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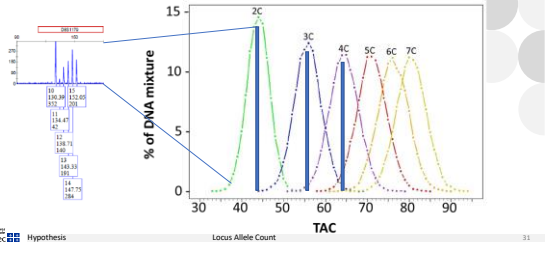

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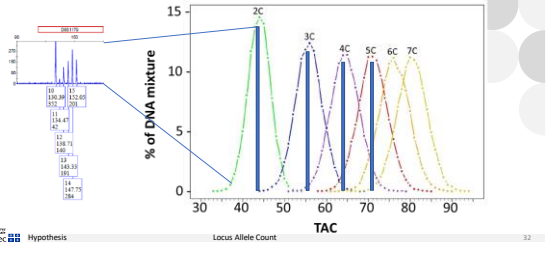

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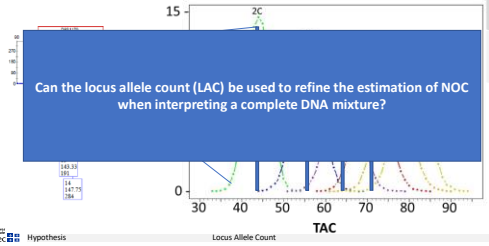

Can the locus allele count (LAC) be used to refine the estimation of NOC when interpreting a complete DNA mixture?

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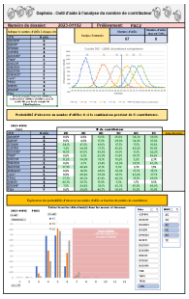

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Generate DNA mixtures

- Dataset generated in Excel
- 2,7 millions profiles were generated
- 100 000 DNA mixtures were generated for each NOC
- LSJML-Caucasian allele frequencies; θ = 1%; ID+

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Step 1 : Draw genotypes

- Example for 2C DNA mixtures

	D8S1179	
	C1	C2
Mix 1	1067	1014
Mix 2	1002	1017
Mix 3	1043	1095
…		
Mix 100 000	1046	1013

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Step 2 : Convert genotypes to alleles

- Example for 2C DNA mixtures

	D8S1179			
	C1	C2	C1	C2
Mix 1	1067	1014	14, 15	8, 10
Mix 2	1002	1017	9, 12	9, 15
Mix 3	1043	1095	12, 11	17, 13
…				
Mix 100 000	1046	1013	9, 12	8, 9

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Step 3 : Count the number of alleles

- Example for 2C DNA mixtures

	D8S1179				
	C1	C2	C1	C2	
Mix 1	1067	1014	14, 15	8, 10	4
Mix 2	1002	1017	9, 12	9, 15	3
Mix 3	1043	1095	12, 11	17, 13	4
…					
Mix 100 000	1046	1013	9, 12	8, 9	3

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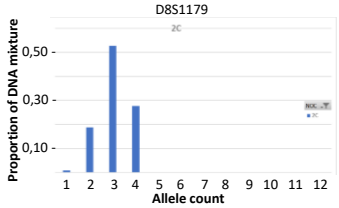
Graphical data analysis

- From the data generated, we can compute:



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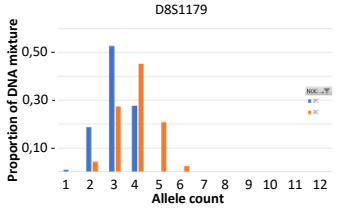

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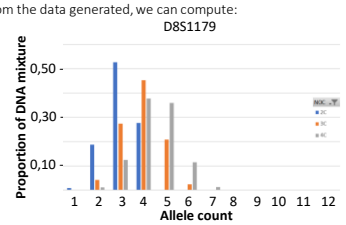

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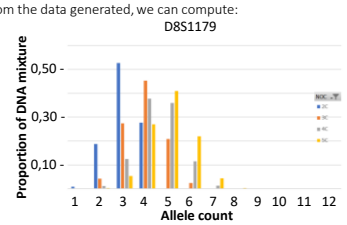

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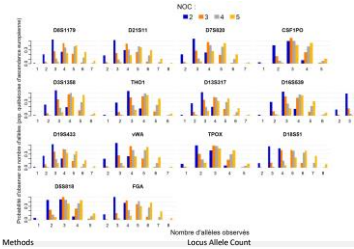

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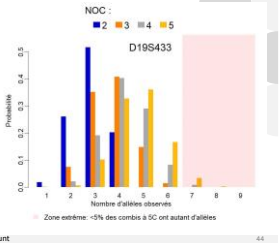
Graphical data analysis

Extreme zone ⚠

Upper region of the probability distribution where <5% of the 5C mixtures have such a high number of alleles



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How to use the tool

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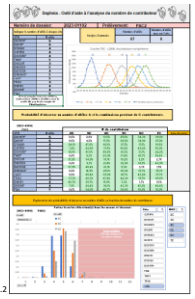

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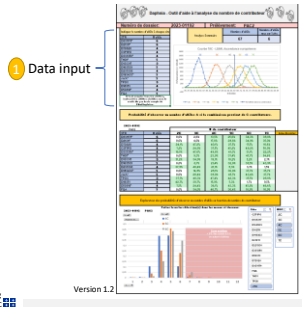

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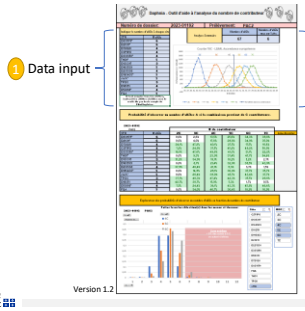

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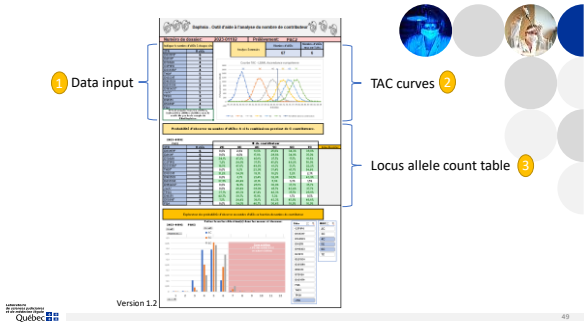

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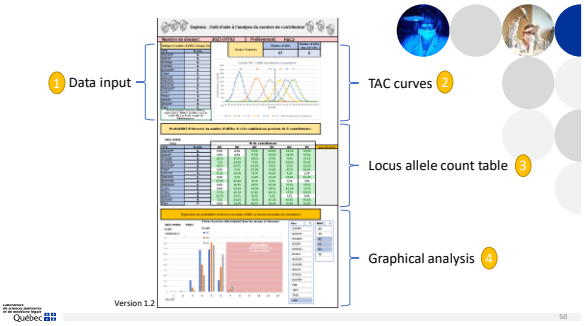

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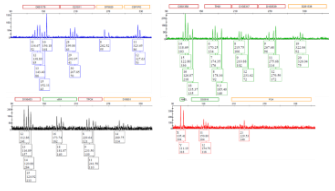
How to use the tool

- Step 1: Count every allele, including those that are visible below the analytical threshold.



Results Locus Allele Count 51

51

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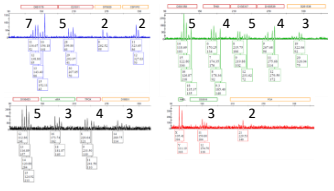

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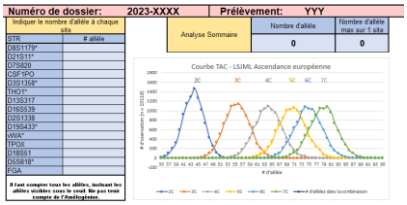
52

How to use the tool

- Step 2: Input the # of allele at each locus



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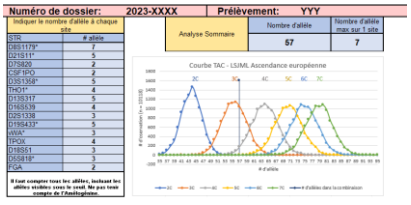

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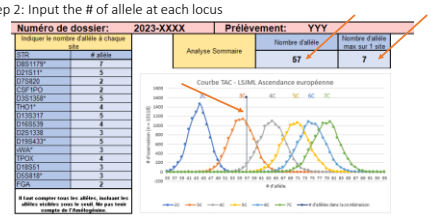

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How to use the tool

- Locus allele count table

2023-XXXX
YYY

STR	# allèle	# de contributeur 2C	3C	4C	5C	6C	7C
D8S1179*	7	0,0%	0,0%	1,6%	6,0%	12,4%	19,2%
D21S11*	5	0,0%	25,6%	38,3%	36,1%	28,2%	21,7%
D7S820	2	20,3%	3,6%	0,7%	0,1%	0,0%	0,0%
CSF1PO	2	40,2%	16,3%	6,3%	2,5%	1,0%	0,4%
D3S1358*	5	0,0%	11,6%	29,8%	45,3%	56,2%	63,2%
THO1*	4	15,8%	42,3%	49,1%	44,9%	37,7%	30,4%
D13S317	5	0,0%	14,5%	29,8%	37,5%	39,4%	37,3%
D16S539	4	14,8%	38,9%	46,5%	44,6%	39,3%	33,0%
D2S1338	3	48,3%	15,8%	4,4%	1,2%	0,3%	0,1%
D19S433*	5	0,0%	14,9%	28,1%	36,0%	37,7%	35,7%
vWA*	3	54,5%	27,3%	10,6%	3,8%	1,4%	0,6%
TPOX	4	4,9%	17,6%	29,3%	38,1%	44,0%	47,3%
D18S51	3	47,2%	11,5%	2,2%	0,4%	0,1%	0,0%
D5S818*	3	44,8%	49,9%	43,9%	35,6%	28,2%	21,8%
FGA	2	12,2%	1,2%	0,1%	0,0%	0,0%	0,0%

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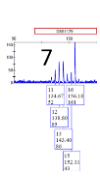
- Locus allele count table



2023-XXXX
YYY

STR	# allèle	# de contributeur					
		2C	3C	4C	5C	6C	7C
D8S1179*	7	0,0%	0,0%	1,6%	6,0%	12,4%	19,2%
D21S11*	5	0,0%	25,6%	38,3%	36,1%	29,2%	21,7%
D7S820	2	20,3%	3,6%	0,7%	0,1%	0,0%	0,0%
CSF1PO	2	40,2%	16,3%	6,3%	2,5%	1,0%	0,4%
D3S1358*	5	0,0%	11,6%	29,8%	45,3%	56,2%	63,2%
THO1*	4	15,8%	42,3%	49,1%	44,9%	37,7%	30,4%
D13S317	5	0,0%	14,5%	29,8%	37,5%	39,4%	37,3%
D16S539	4	14,8%	38,9%	46,5%	44,6%	39,3%	33,0%
D2S1338	3	48,3%	15,8%	4,4%	1,2%	0,3%	0,1%
D19S433*	5	0,0%	14,9%	29,1%	36,0%	37,7%	35,7%
vWA*	3	54,5%	27,3%	10,6%	3,8%	1,4%	0,6%
TPOX	4	4,9%	17,6%	29,3%	38,1%	44,0%	47,3%
D18S51	3	47,2%	11,5%	2,2%	0,4%	0,1%	0,0%
D5S818*	3	44,8%	49,9%	45,9%	35,6%	28,2%	21,8%
FGA	2	12,2%	1,2%	0,1%	0,0%	0,0%	0,0%

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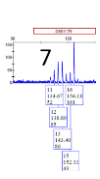
How to use the tool

- Locus allele count table



2023-XXXX
YYY

STR	# allèle	# de contributeur					
		2C	3C	4C	5C	6C	7C
D8S1179*	7	0,0%	0,0%	1,6%	6,0%	12,4%	19,2%
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D7S820	2	20,3%	3,6%	0,7%	0,1%	0,0%	0,0%
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D5S818*	3	44,8%	49,9%	45,9%	35,6%	28,2%	21,8%
FGA	2	12,2%	1,2%	0,1%	0,0%	0,0%	0,0%

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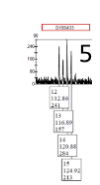
How to use the tool

- Locus allele count table



2023-XXXX
YYY

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D7S820	2	20,3%	3,6%	0,7%	0,1%	0,0%	0,0%
CSF1PO	2	40,2%	16,3%	6,3%	2,5%	1,0%	0,4%
D3S1358*	5	0,0%	11,6%	29,8%	45,3%	56,2%	63,2%
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FGA	2	12,2%	1,2%	0,1%	0,0%	0,0%	0,0%

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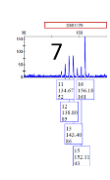
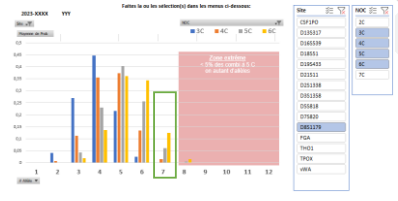
How to use the tool

- Graphical analysis





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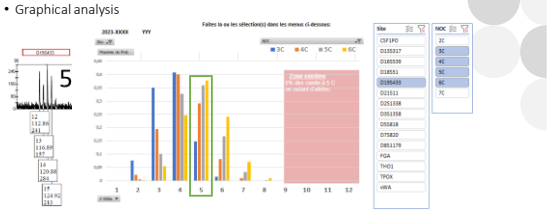
How to use the tool

- Graphical analysis



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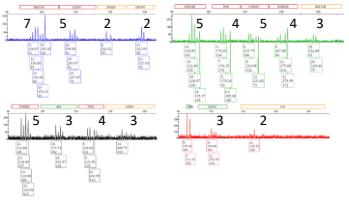
How to use the tool

- MAC: ≥4C
- TAC: 3 or 4C
- LAC: >4C



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Does it help to improve NOC estimation?

- 3 DNA analysts
- 100 DNA mixtures from the Proved-IT degradation data set (> 3C)
- Each analyst:
  1. Used all the tools at his disposal to estimate the NOC
  2. Re-analyzed the DNA mixture, using all the tools **+ LAC curves**

*A single NOC was recorded for each DNA mixture.

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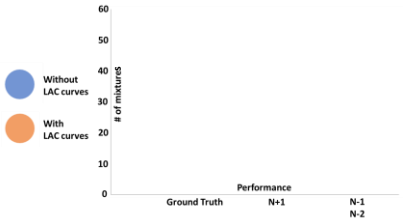
Does it help to improve NOC estimation?



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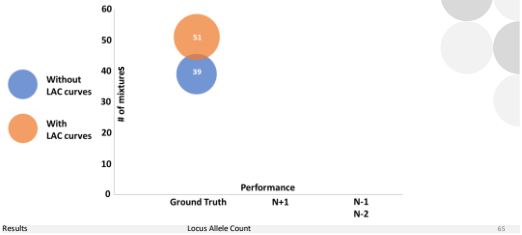
Does it help to improve NOC estimation?



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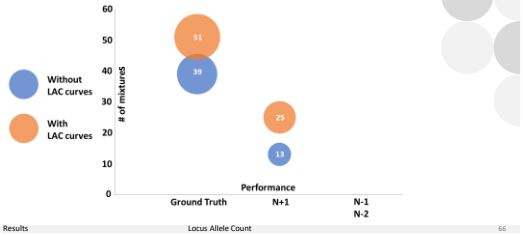
Does it help to improve NOC estimation?



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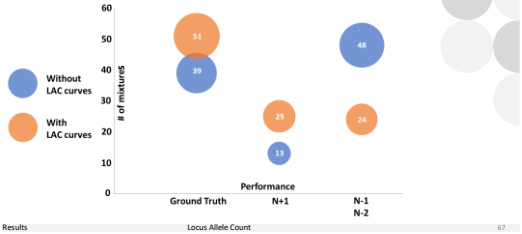
Does it help to improve NOC estimation?



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Limitations

- Ground truth is not known for casework sample
  - Forced to choose a NOC
- Performed well using mixtures from the Proved-IT dataset
  - Compared to our caucasian population
    - Differences in allelic distribution
    - Higher diversity index
  - Tested the tool with ID+ (15 sites)
- We support the inclusion for LR > 1000

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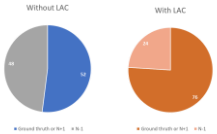
Conclusion

- We have developped a tool that is
  - ✓ Easy to use
  - ✓ Doesn't require any advanced software or coding skills to implement
    - Excel templates are available!
  - ✓ Leverage from the Total Allele Count method
- Version 2.0 coming soon!

Updating...



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Acknowledgements

Tommy Harding

Nicolas Tremblay

Josée Noël

Sarah Noël

Valérie Clermont-Beaudoin

Diane Séguin

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« N'avance rien
que tu ne sois capable de prouver »
Dr Wilfrid Derome

« Don't claim anything
you can't prove» Dr Wilfrid Derome

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Web site …….. www.quebec.ca/lsjml

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