Probabilistic Genotyping: Considerations for Implementation

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Probabilistic Genotyping

"Probabilistic genotyping refers to the use of biological modeling, statistical theory, computer algorithms, and probability distributions to calculate likelihood ratios (LRs) and/or infer genotypes for the DNA typing results of forensic samples ("forensic DNA typing results")." – SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems

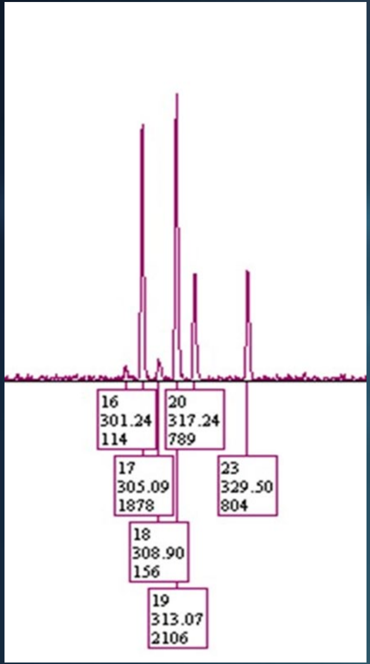
Probabilistic Genotyping

Two Main Functions of Probabilistic Genotyping (Prob Gen):

- *Mixture deconvolution* – based on data in DNA profile, agnostic to race or ethnicity

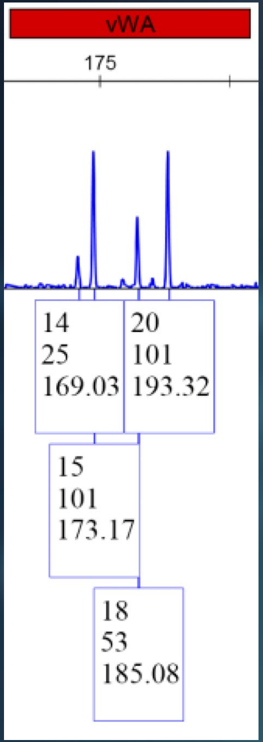
- *Calculation of the statistical weight of a comparison to a Person of Interest (POI)* – based on relevant population databases

Evolution of DNA Mixture Interpretation



Analysis Approach	2:1		
	4 allele		
CPI	17,19; 17,20; 17,23; 19,20; 19,23; 20,23; 17,17; 19,19; 20,20; 23,23		
RMP	17,19; 20,23		
STRmix™	17, 19	20, 23	9.3953E-1
	19, 23	17, 20	1.9485E-2
	17, 23	19, 20	1.6781E-2
	19, 20	17, 23	1.0811E-2
	17, 20	19, 23	8.7925E-3
	20, 23	17, 19	4.6043E-3

Evolution of DNA Mixture Interpretation

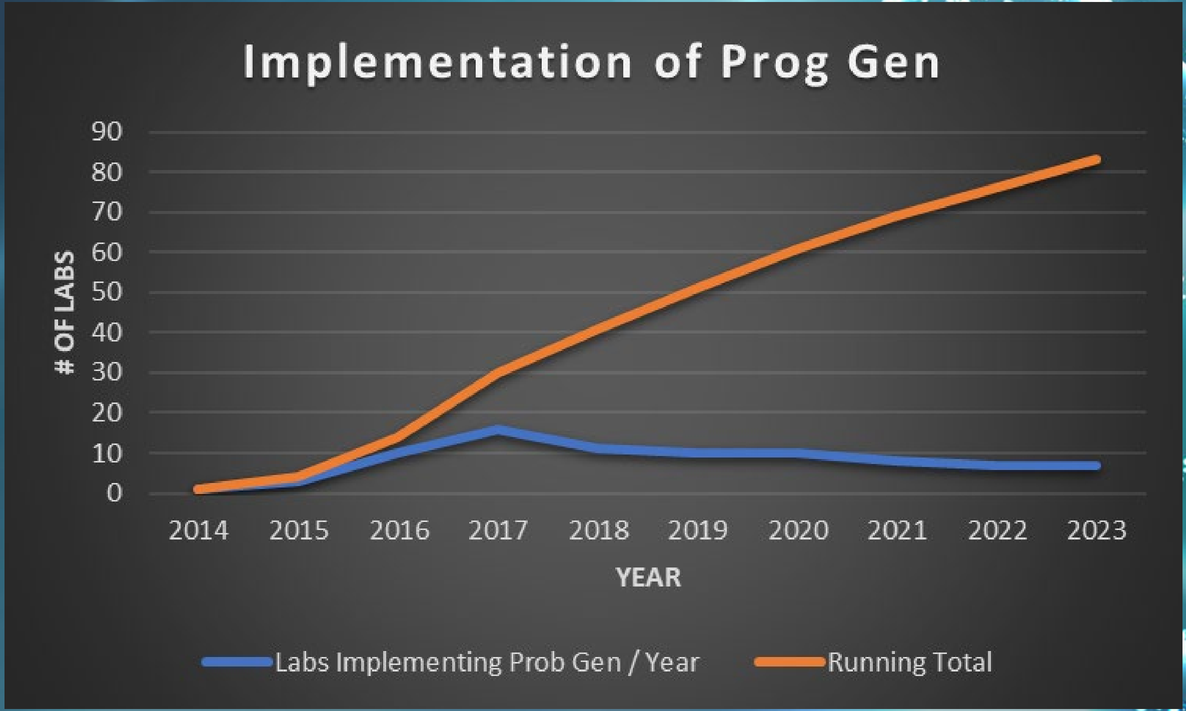


CPI		
14,15	14,18	14,20
15,18	15,20	18,20
14,14	15,15	18,18
	20,20	

Modified RMP		
14,15	14,18	14,20
15,18	15,20	18,20

vWA	15, 20	14, 18	4.8320E-1
	18, 20	14, 15	1.2234E-1
	15, 18	14, 20	1.0884E-1
	14, 18	15, 20	4.6498E-2
	14, 20	15, 18	4.3751E-2
	14, 15	18, 20	3.9500E-2
	15, 20	18, 20	3.2796E-2
	15, 18	20, 20	2.3010E-2
	15, 20	15, 18	2.1751E-2

The Forensic Community is Moving Toward the Use of Prog Gen



Validation Standards / Guidance in the Forensic Community

- Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
- ANSI / ASB
- OSAC
- UK Forensic Science Regulator
- DNA Commission of the International Society of Forensic Genetics

Common Themes of Validation

- Ensure that the software is fit-for-purpose
- Validation performed using the types of evidence samples expected to be encountered in casework (template amounts, mixture ratios, number of contributors, degradation, inhibition, etc.)
- Accuracy
- Precision
- Sensitivity (true contributors, Type I errors)
- Specificity (non-contributors, Type II errors)
- Comparison to previous methods

Common Themes of Validation

- Validation determines the limits of the conditions where the software is expected to produce reliable results – find the breaking points
- The results of the laboratory's validation are used to inform the software use and interpretation protocols

PCAST Report (2016)

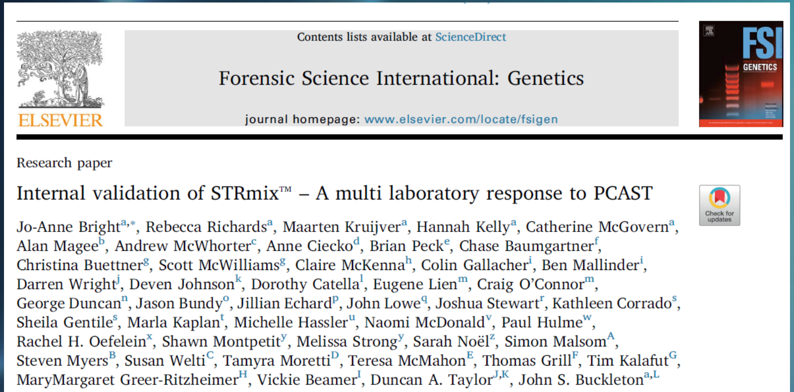
Finding 3: DNA analysis of complex-mixture samples

Foundational validity. PCAST finds that:

(2) Probabilistic genotyping. Objective analysis of complex DNA mixtures with probabilistic genotyping software is relatively new and promising approach. Empirical evidence is required to establish the foundational validity of each such method within specified ranges. At present, published evidence supports the foundational validity of analysis, with some programs, of DNA mixtures of 3 individuals in which the minor contributor constitutes at least 20 percent of the intact DNA in the mixture and in which the DNA amount exceeds the minimum required level for the method. The range in which foundational validity has been established is likely to grow as adequate evidence for more complex mixtures is obtained and published.

Validation

- PubMed search “Probabilistic Genotyping” - > 800 publications listed
- Every US laboratory that has implemented some form of Prob Gen has performed an internal validation (> 80)
- Difficult to publish validations because they are not considered novel by the journals



Contents lists available at ScienceDirect

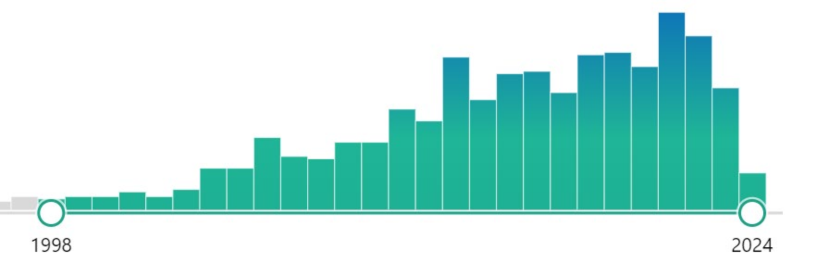
Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Research paper

Internal validation of STRmix™ – A multi laboratory response to PCAST

Jo-Anne Bright[a,*], Rebecca Richards[a], Maarten Kruijver[a], Hannah Kelly[a], Catherine McGovern[a], Alan Magee[b], Andrew McWhorter[c], Anne Ciecko[d], Brian Peck[e], Chase Baumgartner[f], Christina Buettner[g], Scott McWilliams[g], Claire McKenna[h], Colin Gallacher[i], Ben Mallinder[i], Darren Wright[j], Deven Johnson[k], Dorothy Catella[l], Eugene Lien[m], Craig O'Connor[m], George Duncan[n], Jason Bundy[o], Jillian Echard[p], John Lowe[q], Joshua Stewart[r], Kathleen Corrado[s], Sheila Gentile[s], Marla Kaplan[t], Michelle Hassler[u], Naomi McDonald[v], Paul Hulme[w], Rachel H. Oefelein[x], Shawn Montpetit[y], Melissa Strong[y], Sarah Noël[z], Simon Malsom[A], Steven Myers[B], Susan Welti[C], Tamyra Moretti[D], Teresa McMahon[E], Thomas Grill[F], Tim Kalafut[G], MaryMargaret Greer-Ritzheimer[H], Vickie Beamer[I], Duncan A. Taylor[J,K], John S. Buckleton[a,L]



Calibration:

- Logical expectations
- Turing expectations
- Hannig et al.
- Ramos and Gonzalez-Rodriguez

Are reported likelihood ratios well calibrated?

Jan Hannig[a,c,*], Sarah Riman[b], Hari Iyer[a], Peter M. Vallone[b]

[a] *Statistical Design, Analysis, and Modeling Group, ITL/NIST, United States*
[b] *Applied Genetics Group, National Institute of Standards and Technology, United States*
[c] *Department of Statistics and Operations Research, UNC-Chapel Hill, United States*

Forensic population genetics – original research

Testing likelihood ratios produced from complex DNA profiles

Duncan Taylor[a,b,*], John Buckleton[c], Ian Evett[d]

[a] *Forensic Science South Australia, 21 Divett Place, Adelaide, SA 5000, Australia*
[b] *School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia*
[c] *ESR, Private Bag 92021, Auckland 1142, New Zealand*
[d] *Principal Forensic Services Ltd., London, UK*

Applying calibration to *LR*s produced by a DNA interpretation software

Jo-Anne Bright[a], M. Jones Dukes[b], S. N. Pugh[c], I. W. Evett[d] and J. S. Buckleton[a]

[a]Institute of Environmental Science and Research Limited, Auckland, New Zealand; [b]QIAGEN LLC, Germantown, MD, USA; [c]Palo Cedro, CA, USA; [d]Principal Forensic Services Ltd., Bromley, UK

Calibration of STRmix LRs following the method of Hannig *et al.*

John Buckleton[1,2], Maarten Kruijver[2], James Curran[1], and Jo-Anne Bright[2]

1. Department of Statistics, University of Auckland, New Zealand
2. Institute of Environmental Science and Research Limited, Auckland, New Zealand

DNA Analyst Training:

- Basic DNA interpretation and mixture interpretation
- Expected variation in DNA profiles (stochastic effects, stutter, peak height balance, degradation, inhibition, etc.)
- *Manual deconvolution of mixtures is critical*
- Underlying principles of Prob Gen software used
- Limits of the software used based on the validation

D8S11789	Alleles Present	RFU
	11	
	12	
	13	
	Total RFU =	
Possible complete loss of genotype?		N

Potential Genotype Combinations

1 allele

2 alleles

3 alleles				
11	11	y	12	13
12	12	n	11	13
13	13	n	11	12
11	12	y	11	13
12	13		11	13
11	12		12	13
11	X	n	12	13
12	X	n	11	13
13	X	n	11	12

4 alleles

Possible Contributing Genotypes			Caucasian			African American			Hispanic		
			f(Allele 1)	f(Allele 2)	RMP	f(Allele 1)	f(Allele 2)	RMP	f(Allele 1)	f(Allele 2)	RMP
11	11	AA	0.059		0.004	0.036		0.002	0.062		0.004
11	12	AB	0.059	0.145	0.017	0.036	0.108	0.008	0.062	0.121	0.015
11	13	AC	0.059	0.339	0.040	0.036	0.222	0.016	0.062	0.325	0.040
		AD	0	0		0	0		0	0	
		AX	0			0			0		
		BB	0			0			0		
12	13	BC	0.145	0.339	0.099	0.108	0.222	0.048	0.121	0.325	0.078
		BD	0	0		0	0		0	0	
		BX	0			0			0		
		CC	0			0			0		
		CD	0	0		0	0		0	0	
		CX	0			0			0		
		RMP:			0.160			0.074			0.138

DNA Analyst Training:

- Background training then allows the analyst to evaluate the Prob Gen results to ensure they meet logical expectations – not a "black box"

SUMMARY OF CONTRIBUTORS

CONTRIBUTORS	1	2
Template (rfu)	660	178
Mixture Proportion	79%	21%
Degradation starts at 89bp		
Degradation linear approximation (rfu/bp)	0.666	0.196
Degradation exponential curve	1.1280E-3	1.2466E-3

POST BURN-IN SUMMARY

Total iterations	2,563,156	Acceptance rate	1 in 6.41
Effective sample size	7,169.35	log(likelihood)	32
Gelman-Rubin convergence diagnostic	1.06		
Allele variance (mode = 3.574)	5.076	Back Stutter variance (mode = 3.664)	5.865
Forward Stutter variance (mode = 7.081)	10.221	2bp stutter variance (mode = 3.409)	4.462
Double back stutter variance (mode = 14.662)	25.306		

Benefits:

- Better use of the genetic data in the DNA profile
    - Increased discrimination results in higher exclusion of non-contributors
- Reduced subjectivity during interpretation
- Greater consistency over time within the laboratory and between analysts within the laboratory

Weaknesses:

- Conveying the meaning of an LR to a lay person
- Interpretation and review are more complicated and time-consuming
- Validation of software is very time consuming and labor-intensive (6 months to 1 year or more)

THANK YOU