New York Blood Center Enterprises

Copy Number Analysis for Accurate Array-Based Detection of Mi(a+) GYP(B-A-B) Hybrid Alleles

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aabb 2026 ATLANTA OCTOBER 17-19

10/17/2026 AABB 2026

BGC The Blood transfusion Genomics Consortium

Mi^{a} is clinically significant and common in parts of Asia

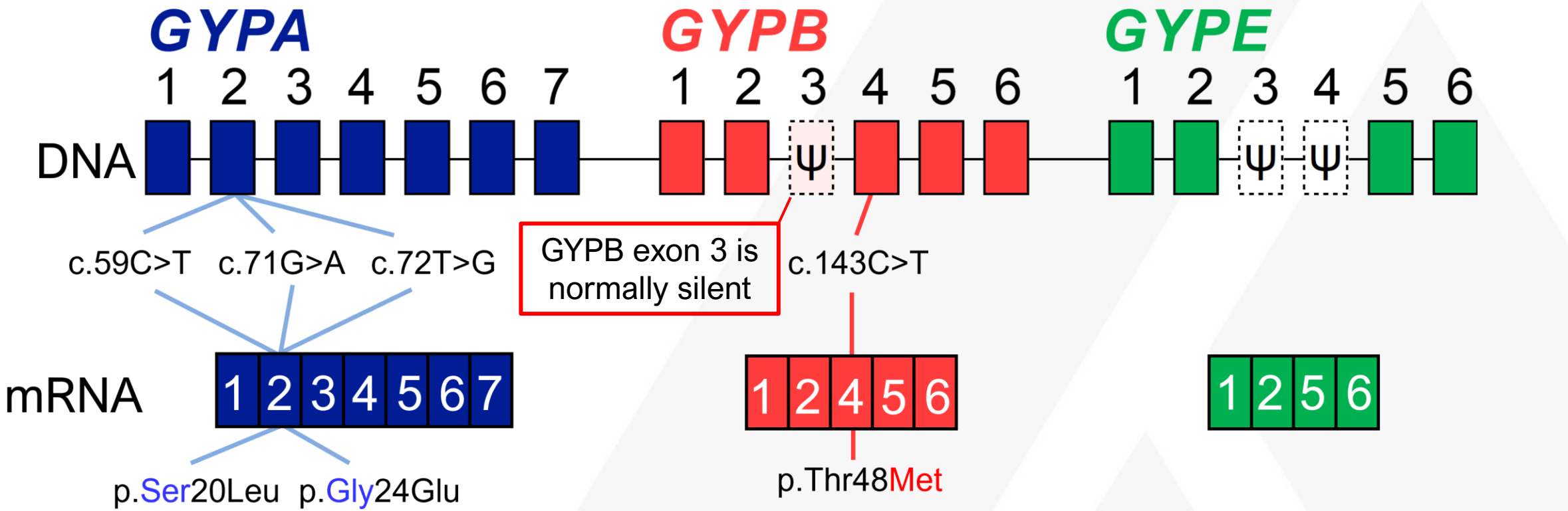


Clinical Relevance

- Mi(a+) occurs at frequencies up to 15% in certain SE Asian populations
- Anti-Mi^{a} can cause HDFN and hemolytic transfusion reactions

High-throughput Mi^{a} prediction could support identification of Mi(a–) blood

The glycophorin locus encodes the major MNS antigens

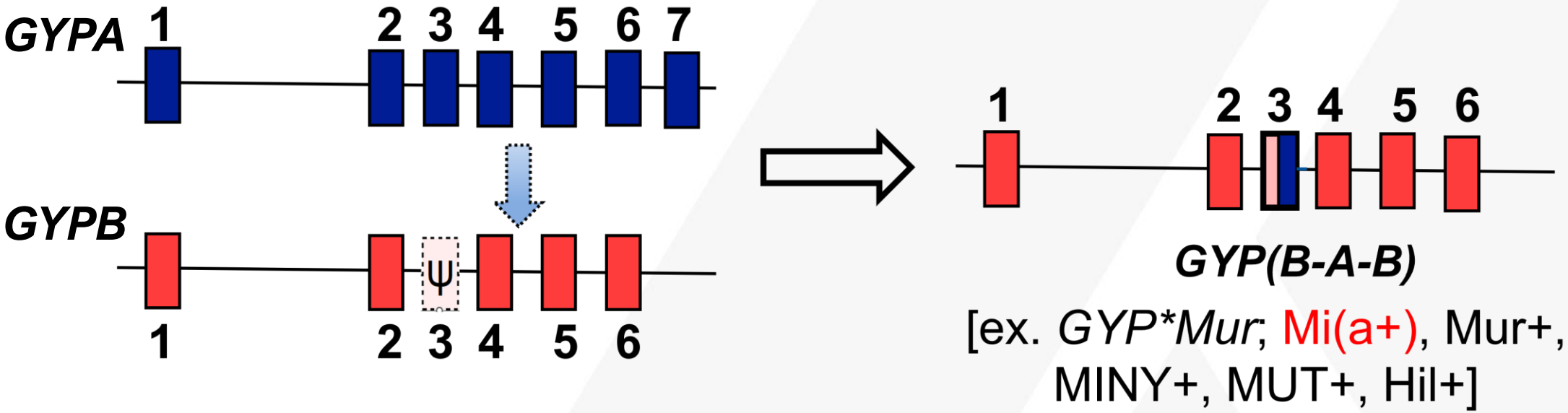


7 exons
M, N antigens (exon 2)

5 exons, 1 pseudo exon
ΨExon 3: altered 3′ splice site
S, s, antigens (exon 4)

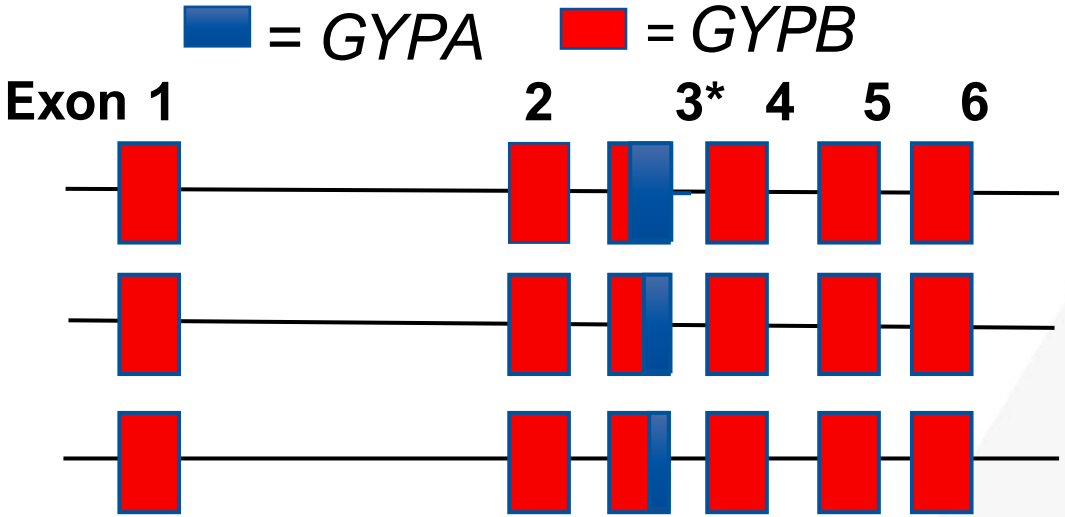
4 exons, 2 pseudo exons
ΨE3 and 4: altered 3′ splice site
No antigens
Involved in hybrid formation

Homology promotes glycophorin hybrid formation



Hybrid alleles combine sequences from homologous glycophorin genes

GYP B-A-B hybrid formation activates GYPB pseudoexon 3



Allele	Shared Phenotype	Additional Antigens
*GYP*Mur*	Mi(a+)	s+, Mur+, MINY+, MUT+, Hil+
*GYP*Bun*	Mi(a+)	s+ Mur+, MINY+, MUT+, Hop+, Hil+
*GYP*Hop*	Mi(a+)	S+ Mur+, MINY+, MUT+, Hop+, TSEN+

****GYPA* sequence restores *GYPB* exon 3 splicing**

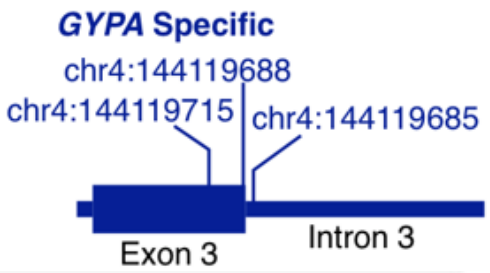
Different breakpoints produce the same key event: expression of an Mi^{a} associated hybrid

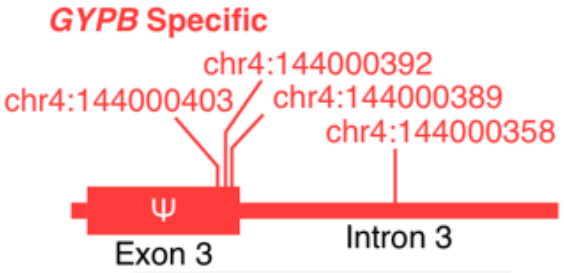
Exon-Specific Copy Number Changes Identify GYP(B-A-B) Hybrids



BloodGenomiX Array

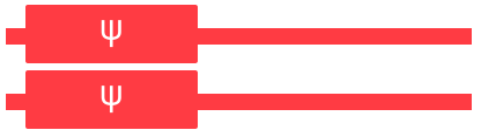
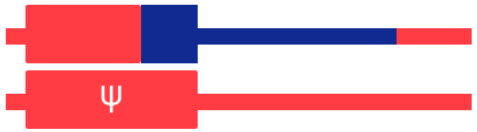
20,000 probes
HEA, HNA, HPA, HLA
384 samples/array





Copy Number Analysis

CN = Copy #

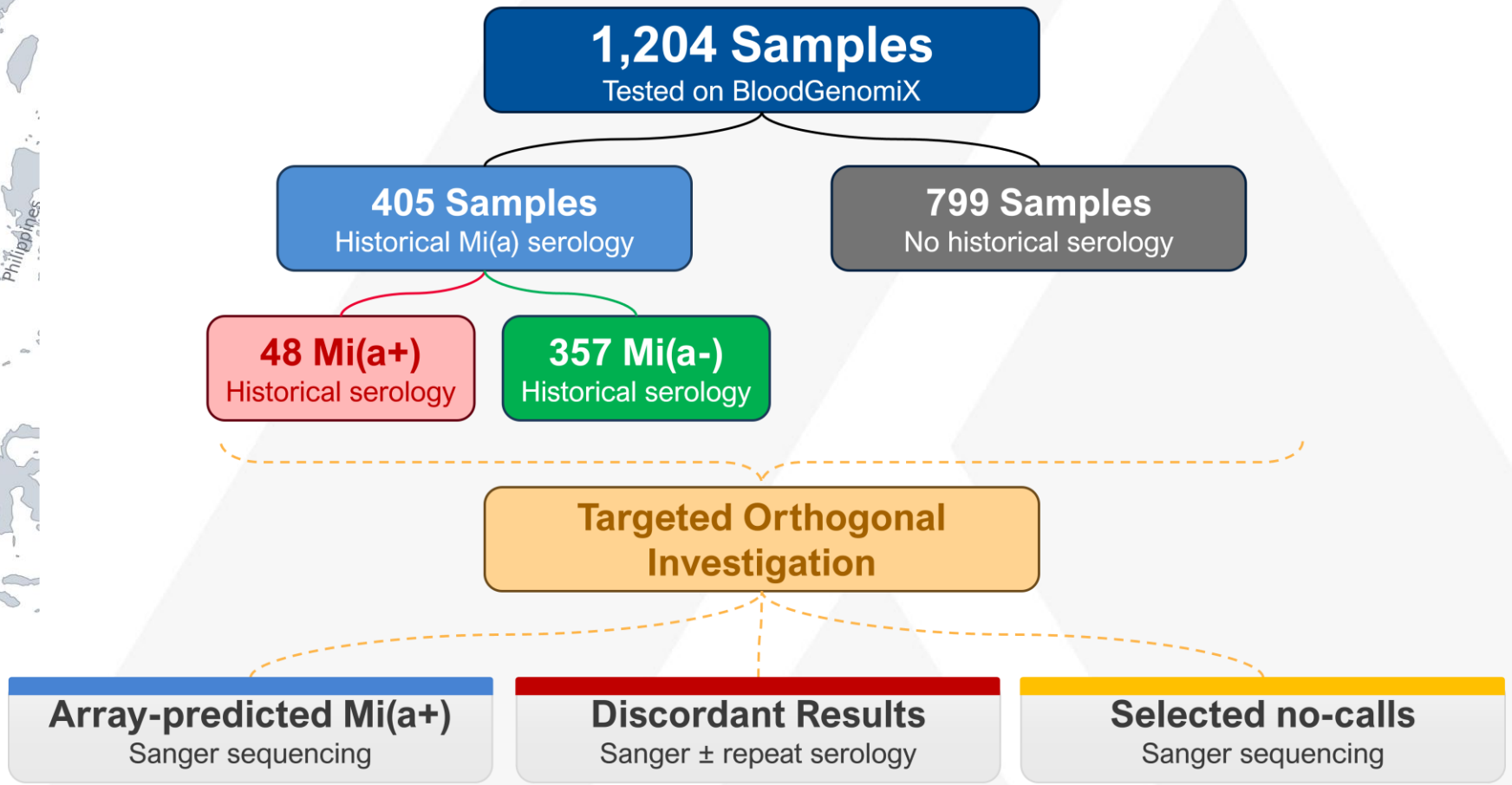
GYPA Exon 3 + Intron	*GYPB Exon 3 + Intron*	Overall *GYPB CN*	Mi(a+) Hybrid CN			
			GYPA	*GYPB*	Hybrid	Mi^a
		2x	2x	2x	none	Mi(a–)
		2x	3x	1x	het	Mi(a+)
		2x	4x	0x	hom	Mi(a+)

Reciprocal *GYPA* gain and *GYPB* loss identify a hybrid (BloodTyper, Bill Lane)

BGC Study cohort and validation strategy

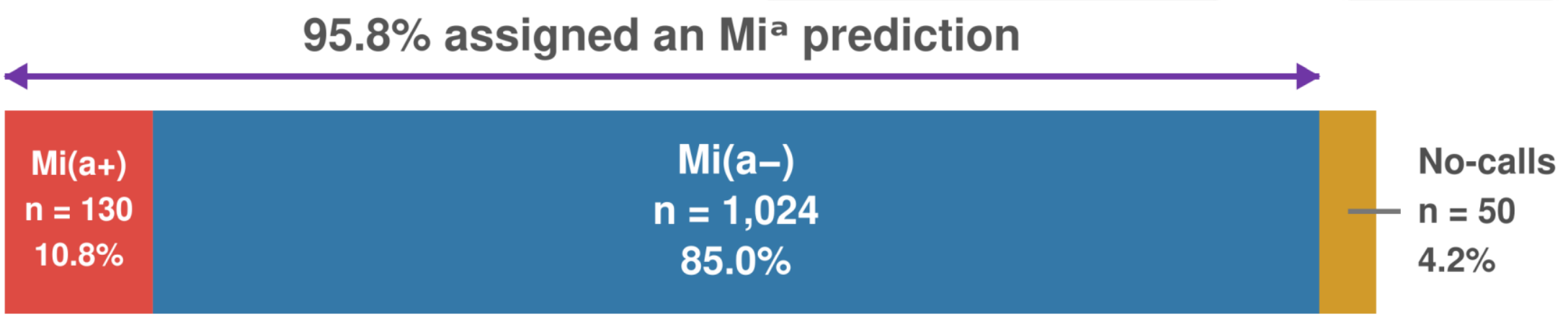


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- 1,204 samples tested on BloodGenomiX array
- 405 historical-serology comparisons

The algorithm assigned Mi^{a} predictions for 95.8% of samples



- 1,154 of 1,204 samples received a prediction
- 130 samples called Mi(a+), 1,024 samples called Mi(a-)
- 50 samples conservatively received no-calls (will discuss later)

Array predictions showed 98.0% initial concordance

		ARRAY PREDICTION		
Historical serology	Total	Array Mi(a+)	Array Mi(a–)	No-call
Mi(a+)	48	42	4	2
Mi(a–)	357	4	338	15
Total	405	46	342	17

- **380/388 callable samples agreed**
- **98.0% concordance (before resolution)**

8 apparent discordances underwent additional testing

Independent testing supported the array in all 8 discordances

# of Discordant Samples	Historical Serology	Array Prediction	Sanger Sequencing	Repeat Serology	Resolution
n = 4	Mi(a+)	Mi(a−)	No *GYPA* or *GYPB* variants detected	2 donors recalled; phenotype confirmed as Mi(a-)	**Sequencing & serology confirm array**
n = 4	Mi(a−)	Mi(a+)	Heterozygous *GYP*Mur* (n = 4)	No successful donor recall	**Sequencing confirms array**

Sequencing confirms 30 Mi(a+) predictions



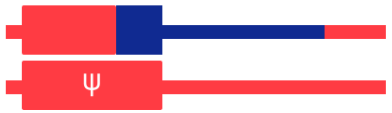
Heterozygous *GYP*Mur* Heterozygous *GYP*Bun/Hop* Homozygous *GYP*Mur*

All predicted Mi(a+) contained a B-A-B hybrid

CN patterns correctly predicted hybrid allele zygosity

Copy Number Analysis

CN = Copy #

GYPA Exon 3 + Intron	*GYPB Exon 3 + Intron*	Overall *GYPB CN*	Mi(a+) Hybrid CN			
			GYPA	*GYPB*	Hybrid	Mi^a
		2x	2x	2x	none	Mi(a–)
		2x	3x	1x	het	Mi(a+)
		2x	4x	0x	hom	Mi(a+)

Copy-number analysis of 17 no-calls identified atypical patterns

Array Copy-Number Finding	Count	Percentage
Non-diploid fixed-region copy number	n = 15	88.2%
Decreased *GYPA* exon 3 copy number	n = 2	11.8%

- 50/1,204 samples received no-calls — 4.2%
- 17 no-calls occurred in the historical-serology subset

Conservative no-calls preserve confidence and flag potential hybrids for follow-up

Sequencing characterized 4 selected no-calls

Sanger Sequencing finding	Count	Historical serology
GYPA hybrid plus heterozygous *GYP*Mur*	n = 1	Mi(a+)
*GYPA*Mta*, c.230C>T	n = 2	One Mi(a+); one Mi(a–)
No *GYPA* or *GYPB* variant identified	n = 1	Mi(a–)

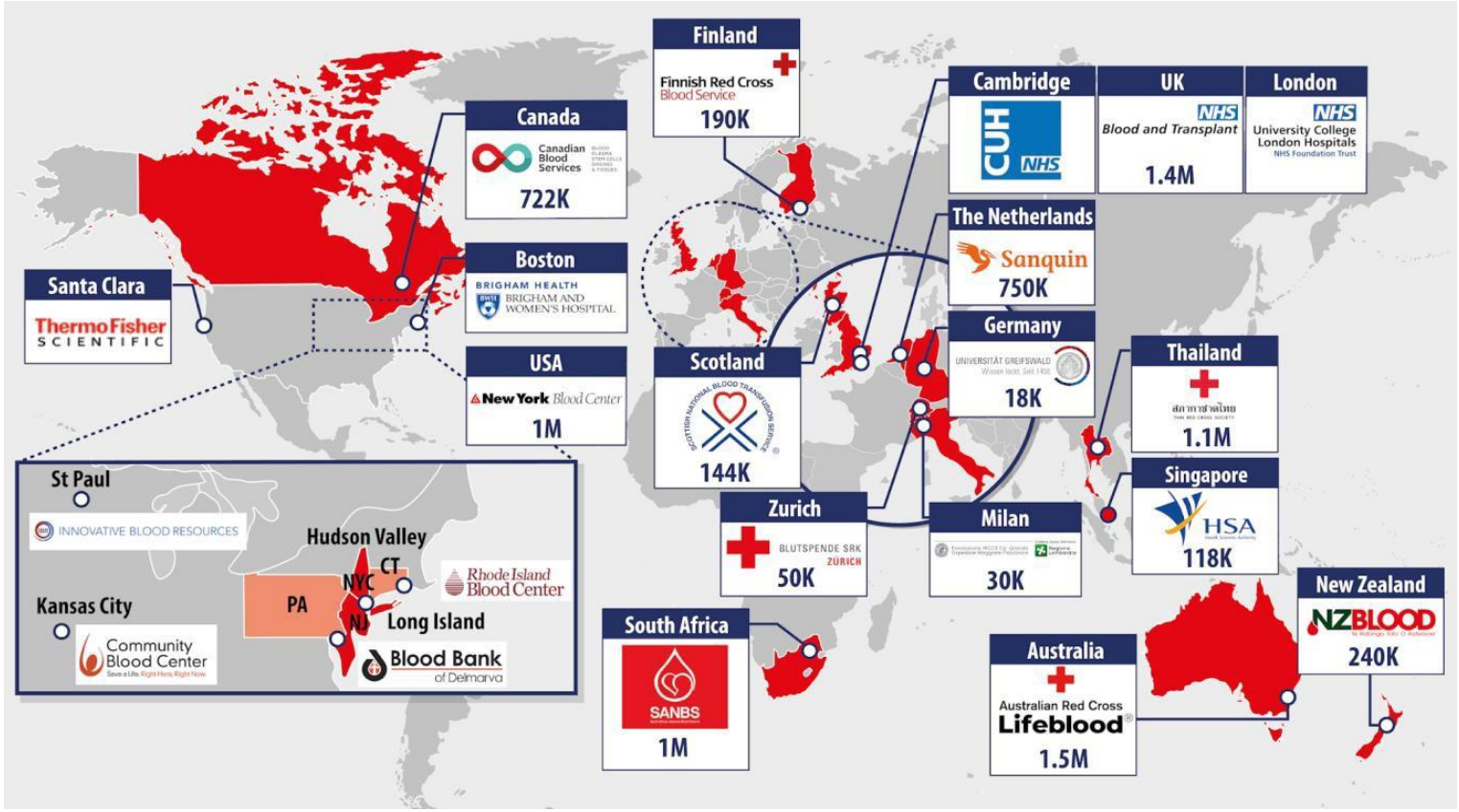
No-calls identified samples outside the expected B-A-B copy-number model

Conclusion: Copy number enables scalable Mi^a prediction

- **95.8%** of samples received an Mi^a prediction
- **98.0% concordance, 8 discordances** resolved in favor of the array
- Sequencing confirmed all 30 Mi(a+) predictions and zygosity
- No-calls preserve confidence and flag potential hybrids for follow-up

BloodGenomiX adds scalable Mi^a prediction to comprehensive donor genotyping

Acknowledgments



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GeneTitan



BloodGenomiX Array

With thanks to our Blood Transfusion Genomics Consortium collaborators and participating institutions.