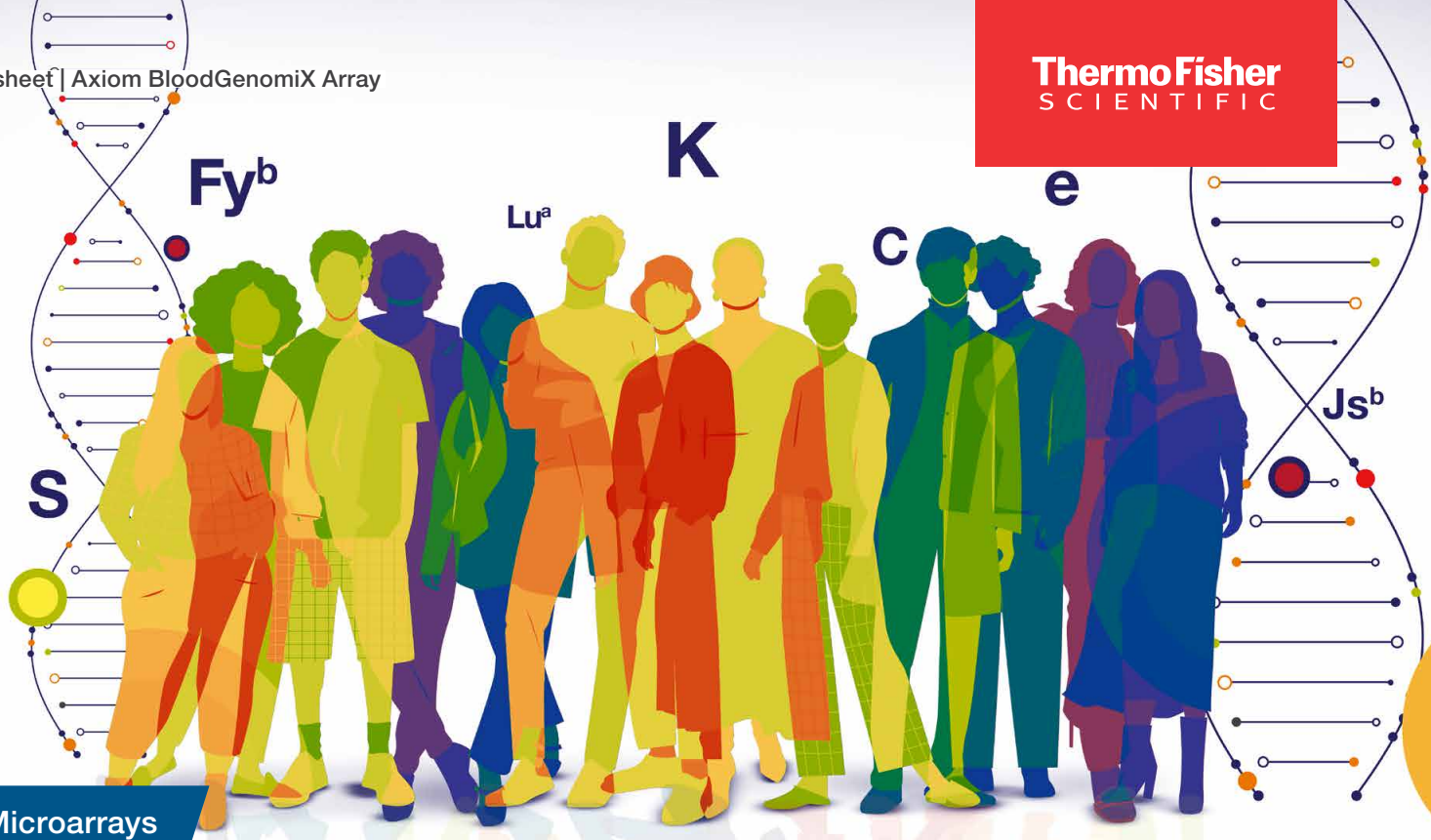

Microarrays

A complete blood typing research solution to enable improved and expanded blood matching

Introduction

Blood transfusion matching practices traditionally have focused on the ABO and Rh antigens, effectively avoiding hemolytic transfusion reactions (HTRs). However, this approach does not avoid the risk of transfusion reactions due to sensitization to other non-self red blood cell (RBC) antigens, particularly affecting patients dependent on frequent transfusions, including those with sickle cell anemia or thalassemia. Avoiding transfusion reactions and the associated increased costs of all future transfusions relies on adoption of “extended antigen matching” by blood services and healthcare providers worldwide.

Unfortunately, the adoption of extended antigen typing is hindered by the lack of a high-throughput, automated, low-cost testing solution. Current extended antigen typing often relies on antibody-based, manual, low-throughput methods, and the challenge is compounded by a lack of appropriate reagents. DNA-based blood typing (genotyping) tests are now available, but do not cover all clinically relevant RBC antigens [1,2].

Developed through collaboration with international blood services and experienced professionals in hematology and transfusion medicine, the Applied Biosystems™ Axiom™ BloodGenomiX™ Array, along with specialized Axiom™ BloodGenomiX™ Reporter Software, offers a comprehensive solution for precise blood group genotyping for blood research. The innovative Axiom BloodGenomiX Array enables research through detection of a wide range of extended and rare blood groups, as well as tissue (HLA) and platelet (HPA) antigen types, in a single, high-throughput assay.

With this DNA array–based blood typing research solution, clinical researchers can support future advancements in patient care using blood products matched for extended antigen phenotypes to mitigate the risk of hemolytic transfusion reactions, contributing to safer transfusions. The Axiom BloodGenomiX Array facilitates research through precise blood genotyping, supporting extended blood matching initiatives across the blood supply chain and positively influencing the future of patient care with the potential to reduce or even eliminate transfusion reactions and sensitization.

Coverage highlights

- Comprehensive coverage with nearly 20,000 probe–variant pairs, meticulously designed for research on genetic variations in blood groups and HLA and HPA classes of genes relevant to transfusion therapy
- Extensive coverage for European, African, East Asian, and South Asian populations
- Variants of human erythrocyte antigens (HEA) in 38 blood group systems, providing valuable screening information for finding rare or uncommon donors and for clinical research
- Human platelet antigens (HPA) and human neutrophil antigens (HNA), targeting the most important antigens identified by experienced professionals in global blood research
- Human leukocyte antigen (HLA) typing, covering the HLA class I genes relevant to transfusion therapy
- Research screening of hemoglobin S and C carriers in donors
- Extensive coverage of iron regulation, specifically addressing hereditary hemochromatosis and providing insights related to iron-deficiency anemia in donors
- Examination of blood product characteristics, including those of restless leg syndrome, enabling researchers to have a holistic understanding of donor health profiles
- Evaluation of variants potentially associated with product quality related to potassium leakage, hemolysis, and storage stability

HEA typing

The Axiom BloodGenomiX Array comprises nearly 400 probe sets meticulously selected to interrogate 261 antigens across 38 blood group systems in addition to multiple HPA and HNA types. The variants underlying HEA types are divided into two categories: core variants, for which samples from at least 5 unrelated individuals have been typed as homozygous for the uncommon or rare allele (Table 1); and research screen variants, which include low-frequency variants where fewer than 5 individuals who are homozygous for the uncommon or rare allele have been typed in the external validation study performed by the Blood transfusion Genomics Consortium (**www.bgc.io**).

Testing for HEA is often conducted through serological (antibody) methods, utilizing the hemagglutination test. However, in sharp contrast to the Axiom BloodGenomiX Array, it is not possible to identify all HEA types by hemagglutination because high-quality typing reagents are not available for all HEA types, and those that are may come at a high cost. Hence, routine testing is often limited to a small set of HEA types. Furthermore, serological testing using a single monoclonal antibody falls short in identifying clinically relevant variation in antigen types (e.g., weak RhD and other RH- variants). The Axiom BloodGenomiX Array is able to identify these, which can strengthen research in these areas, particularly for black populations.

Currently, there are 45 recognized HEA systems encompassing 360 antigens officially acknowledged by the International Society of Blood Transfusion Working Party (ISBT WP) for Red Cell Immunogenetics and Blood Group Terminology [3]. Notably, a subset of relevant HEA types lacks commercial serological typing reagents for testing, as highlighted by experienced blood research professionals [1]. DNA-based methods utilized in the Axiom BloodGenomiX Array and Axiom BloodGenomiX Reporter Software can assist researchers in determining these specific HEA types.

Table 1. Core HEA content of the Axiom BloodGenomiX Array.

System number	Blood group (HEA) system	Gene	Antigen
2	MNS	*GYPA*	M, N
2	MNS	*GYPB*	S, s, U
2	MNS	*GYPB*	*GYPB*05N* (U–)
2	MNS	*GYPB*	*GYPB*NY* (U+^{w})
2	MNS	*GYPB*	*GYPB*P2* (U+^{w})
4	RH	*RHD*	D–, partial C+ [*RHD*03N.01*]
4	RH	*RHCE*	C, c, E, e, C(W), C(X), V, VS
5	LU	*BCAM*	Lu(a), Lu(b)
6	KEL	*KEL*	K, k, Kp(a), Kp(b), Js(a), Js(b)
8	FY	*ACKR1*	Fy(a), Fy(b)
8	FY	*ACKR1*	*FY*02N.01* (Fy^{b} null)
8	FY	*ACKR1*	*FY*02W.01* (Fy(b+^{w}))
9	JK	*SLC14A1*	Jk(a), Jk(b)
9	JK	*SLC14A1*	*JK*01W.01* (Jk(a+^{w}))
9	JK	*SLC14A1*	*JK*02N.01* (Jk^{b} null)
9	JK	*SLC14A1*	*JK*02N.06* (Jk^{b} null)
10	DI	*SLC4A1*	Di(a), Di(b), Wr(a), Wr(b)
11	YT	*ACHE*	Yt(a), Yt(b)
13	SC	*ERMAP*	Sc1, Sc2
14	DO	*ART4*	Do(a), Do(b), Hy, Jo(a)
15	CO	*AQP1*	Co(a), Co(b)
16	LW	*ICAM4*	Lw(a), Lw(b)
21	CROM	*CD55*	Cr(a)
22	KN	*CR1*	KCAM, KDAS, Kn(a), Kn(b), McC(a), McC(b), Yk(a)
34	VEL	*SMIM1*	Vel

Table 2. Research screen HEA content of the Axiom BloodGenomiX Array.

System number	Blood group (HEA) system	Gene	Antigen
2	MNS	*GYPB*	*GYPB**04N.01
3	P1PK	*A4GALT*	P1
4	RH	*RHD*	D, *RHD**01EL.01 (RHD):c.1227G>A, *RHD**08N.01 (RHD):c.807T>G, *RHD**01W.1, *RHD**01W.2, *RHD**01W.3, (RHD):c.602C>G
5	LU	*BCAM*	Au(a), Au(b), Lu13, Lu14, Lu16, Lu17, Lu20, Lu21, Lu4, Lu5, Lu6, Lu7, Lu8, Lu9, LUAC, LUBI, LUGA, LUIT, LUNU, LURA, LURC, LUYA
6	KEL	*KEL*	Kp(c), K11, K12, K13, K14, K17, K18, K19, K22, K23, K24, KALT, KANT, KASH, KEAL, KELP, KETI, KHUL, KTIM, KUCI, KYO, KYOR, RAZ, TOU, Ul(a), VLAN, VONG
7	LE	*FUT3*	Le(a)
		FUT2	Le(b)
9	JK	*SLC14A1*	*JK**01N.06
10	DI	*SLC4A1*	BOW, Bp(a), DISK, ELO, Fr(a), Hg(a), Jn(a), KREP, Mo(a), Rb(a), Sw(a), SW1, Tr(a), Vg(a), WARR, Wd(a), Wu
11	YT	*ACHE*	YTEG, YTLI, YTOT
13	SC	*ERMAP*	Rd, SCAN, SCER, STAR
14	DO	*ART4*	DODE, DOLC, DOLG, DOMR, DOYA
15	CO	*AQP1*	Co4
18	H	*FUT1*	H
19	XK	*XK*	Kx
20	GE	*GYPC*	An(a), Dh(a), Ge2, Ge3, Ge4, GEAT, GEIS, GELP, GETI, Wb
21	CROM	*CD55*	CORS
21	CROM	*CD55*	CRAG, CRAM, CROK, CROV, CROZ, CRUE, Dr(a), Es(a), GUTI, SERF, Tc(a), Tc(b), Tc(c), UMC, WES(a), WES(b), ZENA
22	KN	*CR1*	Sl1, Sl2, Sl3
23	IN	*CD44*	In(a), In(b), INFI, INJA, INRA, INSL
24	OK	*BSG*	Ok(a), OKGV, OKVM
25	RAPH	*CD151*	MER2
26	JMH	*SEMA7A*	JMHG, JMHK, JMHL, JMHM, JMHN, JMHQ
27	I	*GCNT2*	I
28	GLOB	*B3GALNT1*	P
29	GIL	*AQP3*	GIL
30	RHAG	*RHAG*	DSLK, Duclos, Ol(a)
31	FORS	*GBGT1*	FORS
32	JR	*ABCG2*	Jr(a)
33	LAN	*ABCB6*	Lan
35	CD59	*CD59*	59.1
36	AUG	*SLC29A1*	At(a)
36	AUG	*SLC29A1*	ATAM
37	KANNO	*PRNP*	KANNO
38	SID	*B4GALNT2*	Sd(a)
39	CTL2	*SLC44A2*	CTL2.1
40	PEL	*ABCC4*	PEL
41	MAM	*EMP3*	MAM1

HPA and HNA coverage

The Axiom BloodGenomiX Array and Axiom BloodGenomiX Reporter Software provide coverage for 27 HPA types, supporting blood services in improving identification of high-value donors in a research setting. HPA in blood typing is significant for the effectiveness of platelet transfusions for neonates with a low platelet count caused by maternal HPA alloantibodies, which are generally directed against HPA-1a or HPA-5b. Moreover, a portion of subjects who have poor increments after the transfusion of ABO-matched platelet concentrates may have developed HPA antibodies, in addition to HLA class I antibodies. To support these complex subjects effectively with matched platelet concentrates, blood services are required to type a portion of their platelet pheresis donors for HLA class I and HPA types.

HNA in blood typing is primarily associated with the risk of transfusion-related acute lung injury, and on rare occasions, a neonate may have a critically low neutrophil count because the mother has formed HNA antibodies. Matching for HNA is important for patients known to have HNA antibodies and who require a granulocyte concentrate.

Hemoglobinopathy carrier variants

The Axiom BloodGenomiX Array interrogates the HbS (sickle cell) and HbC (hemoglobin C) variants in the *HBB* gene, which encodes β-globin. The array also contains probes for several of the variants known to cause thalassemia.

Donor variant information

The versatility of this platform may extend beyond antigen typing research for extended matching. The predominant cause of hereditary hemochromatosis is DNA variants in the *HFE* gene resulting in alterations in three amino acids. By harnessing the data from the Axiom BloodGenomiX Array, it may be possible in the future to assist blood services in pinpointing donors who are homozygous or compound heterozygous for one or more of these three variants and also help mitigate the future risk of such pathologies in these individuals by encouraging regular donations. Additionally, the Axiom BloodGenomiX Array interrogates other common markers related to iron homeostasis and restless leg syndrome. A randomized study of the length of the interval between blood donations has shown that the latter condition increases significantly in donors who are bled at relative short intervals [4].

HLA genotyping

The *HLA* loci, which are localized on the short (p) arm of chromosome 6, have the highest level of sequence variation in the human genome (over 36,000 alleles) [5]. The HLA class I (A, B, and C) and class II (DR, DP, DQ) genes play a pivotal role in our ability to raise an effective immune response against pathogens. The extremely high level of sequence variation is in part explained by evolutionary selection of HLA types, rendering the human immune system effective in mounting an adequate immune response against pathogens that are prevalent in the environment [6].

However, the consequence of this exuberant polymorphism and immune response capabilities can be problematic when transfusing platelet concentrates or transplanting tissues or organs. Sensitization against HLA types can lead to complications triggered by immune reactions, resulting in platelet destruction or organ rejection [7-10]. The extreme sequence variation in the *HLA* loci, combined with the high level of sequence homology between the HLA class I and II genes, poses a challenge for genotyping. The highest level of resolution is normally achieved by current next-generation sequencing (NGS) techniques, but this approach can be less amenable to high-throughput typing because of cost. The Axiom BloodGenomiX Array comprises a high density of nearly 8,000 markers across the approximately 4 million bases of the *HLA* locus. The marker set has been optimized to define HLA types of individuals of African and other non-European ancestries with great accuracy.

An advanced imputation method is incorporated in Axiom BloodGenomiX Reporter Software for automated inference and research on HLA class I* gene types at high resolution:

- Determination of the HLA class I gene types at first-field (related allele group) resolution with extremely high concordance with HLA types determined by current NGS methodologies
- Determination of the HLA class I types at second-field (coding sequence/individual HLA protein) resolution with high concordance with HLA types determined by NGS
- Research on subjects that are homozygous at HLA class I genes, who are ideally suited to become platelet apheresis donors after confirmation testing
- Integration of HLA typing data with genome-wide genotyping data through genome-wide association studies (GWAS) for gaining insight into associations with common and rare conditions

* HLA class II typing is also available through a separate software workflow, for those requiring full coverage (Figure 1).

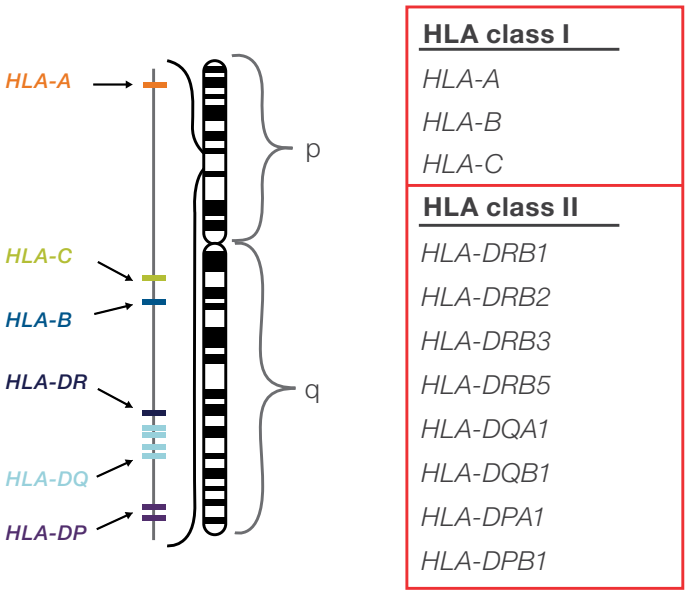

Figure 1. HLA class I and class II gene cluster on chromosome 6p21.3.

Further benefits of array-based typing for HLA by imputation analysis include:

- Utilizing an ancestry-enriched reference panel for populations of interest to improve accuracy of HLA typing; the current reference panel is enriched for European ancestry populations
- Leveraging allele frequency patterns and linkage disequilibrium through imputation of dense genotyping across the extended *HLA* locus to generate accurate *HLA* haplotypes
- Overcoming challenges in multi-ethnic populations for high-accuracy typing of *HLA* loci [11]

Copy number analysis—fixed regions

Copy number variants (CNVs) are structural changes in DNA that include gains or losses, which account for significant variation among human genomes [12]. Therefore, in addition to genotyping SNPs and short insertions or deletions (indels), the Axiom BloodGenomiX Array is designed to perform CNV analysis with integrated workflows using Applied Biosystems™ Automated Axiom™ Analysis software and BloodGenomiX Reporter software. Axiom BloodGenomiX Array utilizes fixed regions for blood typing research (*RHD, RHCE, GYPB, GYPC, ABCC4, ABCG2, EMP3,* and *SLC44A2*).

For more information, see **assets.thermofisher. com/tfs-assets/gsd/technical-notes/axiom-copynumber-analysis-tech-note.pdf**

Copy number–aware genotype calls

Deletions of genes or exons, such as seen with *RHD*, present challenges in accurately calling genotypes of variants in *RHD* and in the related *RHCE*, which has a very similar sequence [13]. For genes with frequent deletion, like *RHD*, the Axiom BloodGenomiX Array uses copy number–aware genotyping (CNAG) to call variants, using a diploid model for samples with two copies of *RHD*, a haploid model for samples with a single copy of *RHD*, and an automatic result of “not present” for samples with no copies. In the closely related *RHCE* gene, probes for *RHCE* variants receive variable amounts of background signal relative to copy number changes in *RHD*. The Axiom BloodGenomiX Array uses remote copy number–aware genotyping (“Remote”) to call *RHCE* variants in samples partitioned according to their number of copies of the *RHD* gene. These algorithms increase accuracy for genes that have been previously difficult to call. Similar approaches are used for calling variants and detecting deletions in the *GYPB* gene, which are causal of the U-negative phenotype if present in homozygosity and mainly found in individuals of African ancestry.

Workflow

Assay

The Axiom BloodGenomiX Array utilizes the Applied Biosystems™ Axiom™ Propel XPRES 2x384HT workflow (Figure 2). The Axiom Propel XPRES 2x384HT workflow, widely used in laboratories across the globe for Axiom genotyping applications, overcomes the bottleneck in scaling up genotyping by eliminating the need for automated liquid handlers. It is an innovative workflow utilizing versatile, easy-to-use Thermo Scientific™ Multidrop™ Combi liquid dispensers and other common laboratory equipment for target preparation without changing the existing steps.

The Axiom Propel XPRES 2x384HT workflow offers a scalable, modular, high-throughput, and cost-effective DNA-based blood genotyping solution for Axiom BloodGenomiX Array research applications.

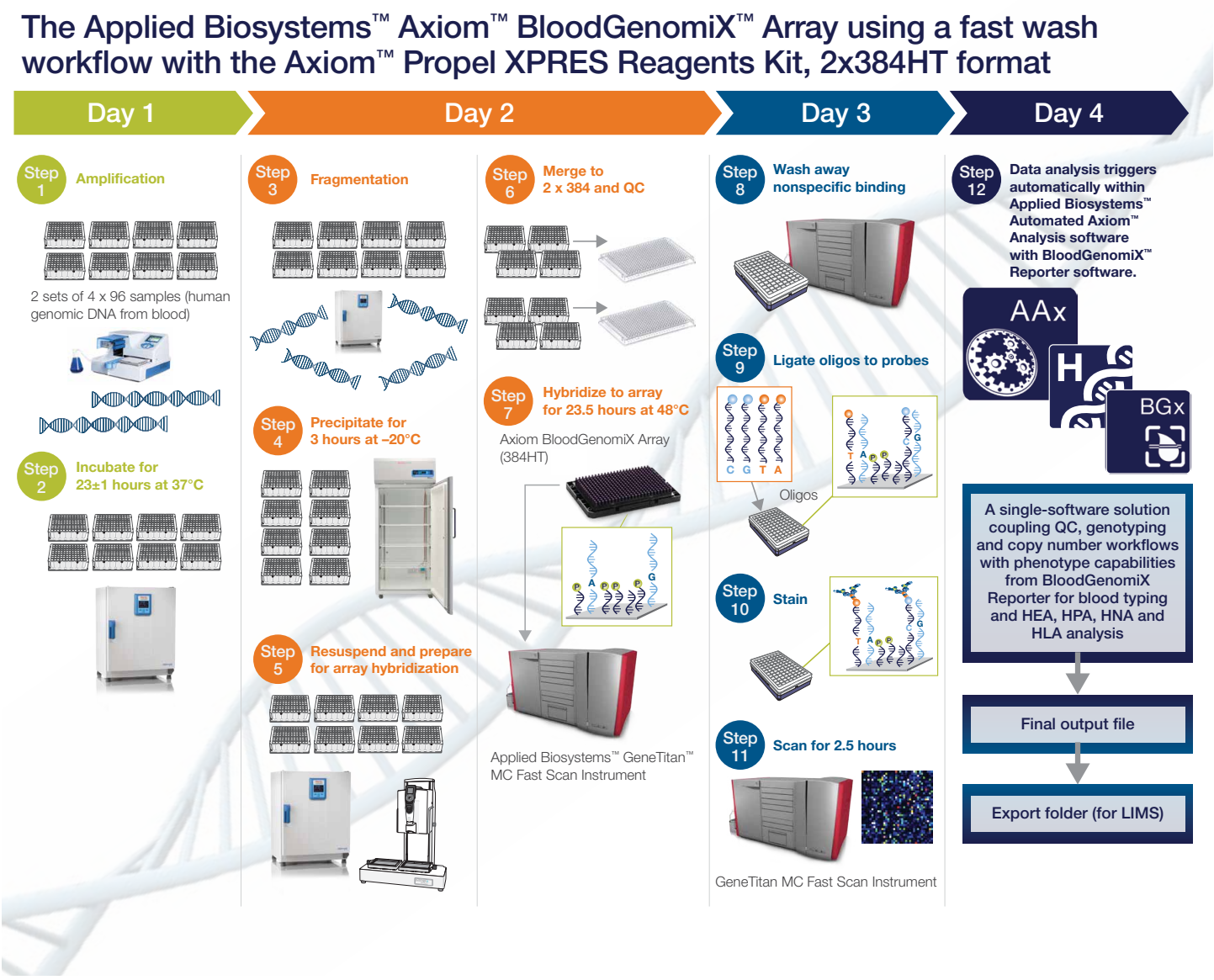

Figure 2. Axiom Propel XPRES 2x384HT workflow for Axiom genotyping applications.

Software

The Axiom BloodGenomiX Array is analyzed using Automated Axiom Analysis software, which allows analysis of .CEL files following microarray scan completion, without user intervention. Multiple workflow and extension options are available, including sample QC, detection of contamination of the DNA sample under investigation, and best-practices workflows with variant call files (.VCF) and AxLE formats. Once the workflow option is set by the user, the software automatically runs the analysis based upon that workflow.

Integrated into the Automated Axiom Analysis software, the Axiom BloodGenomiX Reporter Software transforms genotyping research data output from the Applied Biosystems™ GeneTitan™ MC Fast Scan (FS) Instrument into HEA, HLA, and HPA phenotypes after performing quality control to identify samples with evidence of contamination. For each tested sample, the Axiom BloodGenomiX Reporter Software determines HEA, HPA, and HNA types by directly interrogating relevant DNA variants associated with antigen expression. This is done in accordance with the global standards established by the International Society for Blood Transfusion (ISBT). The embedded HLA software then employs imputation on the genotyping results to predict HLA class I antigen types against a globally maintained database of known reference haplotypes. (HLA class II haplotyping is also available through separate software.)

The final output flat file from Axiom BloodGenomiX Reporter Software has been formatted with direct feedback from blood services for research purposes. This enables easier integration of the output file into electronic records databases utilized by blood service organizations.

Analytical validation performance

The genotyping performance of the Axiom BloodGenomiX Array has been evaluated on 360 DNA samples (Table 3) from the 1000 Genomes Project and 752 unique samples (Table 4) from the Blood transfusion Genomics Consortium (BGC) sample repository using stringent quality control metrics that cover average call rate, concordance, and reproducibility.

Table 3. Analytical performance of the Axiom BloodGenomiX Array for genotyping 360 DNA samples.

Metric	Specification	Performance
Sample pass rate	≥95%	≥98%
Average genotyping call rate	≥98%	≥99.9%
Genotyping reproducibility	≥99.5%	≥99.9%
Average 1000 Genomes Project concordance	≥98%	≥98%

Table 4. Analytical performance of the Axiom BloodGenomiX Array for blood typing 752 unique samples.

Metric	Specification	Performance
Blood typing call rate	NA	≥98.3%
Overall concordance with serology	≥99%	≥99%
Mean concordance with serology of individual antigens	NA	≥97%
95% confidence interval around mean concordance	NA	94–100%

The sample set mentioned in Table 4 provided orthogonal truth through serology for 63 antigens, allowing the analytical validation of 51 of the 58 core antigens and 12 of the research screen antigens (Table 5).

Table 5. Antigens evaluated against orthogonal truth.

System number	Blood group (HEA) system	Antigen	Axiom BloodGenomiX Array category
2	MNS	M, N, S, s, U	Core antigen
3	P1PK	P1	Research screen antigen
4	RH	C, c, C(W), C(x), E, e, V, VS	Core antigen
5	LU	Lu(a), Lu(b)	
6	KEL	K, k, Kp(a), Kp(b), Js(a), Js(b)	
		Ul(a)	Research screen antigen
7	LE	Le(a), Le(b)	
8	FY	Fy(a), Fy(b)	Core antigen
9	JK	Jk(a), Jk(b)	
10	DI	Di(a), Di(b), Wr(a), Wr(b)	
11	YT	YT(a), YT(b)	
13	SC	Sc1, Sc2	
14	DO	Do(a), Do(b), Hy, Jo(a)	
15	CO	Co(a), Co(b)	
16	LW	Lw(a), Lw(b)	
20	GE	Ge2	Research screen antigen
21	CROM	Cr(a)	Core antigen
		Tc(a)	Research screen antigen
22	KN	Kn(a)	Core antigen
23	IN	In(b)	Research screen antigen
27	I	I	
32	JR	Jr(a)	
33	LAN	Lan	
System number	**HPA system**	**Antigen**	**Axiom BloodGenomiX Array category**
1	HPA-1	HPA-1a, HPA-1b	Core antigen
2	HPA-2	HPA-2a, HPA-2b	
4	HPA-4	HPA-4a, HPA-4b	Research screen antigen
5	HPA-5	HPA-5a, HPA-5b	Core antigen
15	HPA-15	HPA-15a, HPA-15b	

Ordering information

Product	Quantity	Cat. No.
Axiom BloodGenomiX Array—combo kits (array, reagents, and consumables)		
Axiom BloodGenomiX Array Kit with Axiom Propel XPRES assay	2 x 384 samples	952536
Axiom BloodGenomiX Array Training Kit with Axiom Propel XPRES assay	4 x 384 samples	952537
Axiom BloodGenomiX Reporter Software		
Axiom BloodGenomiX Reporter Software Report output-1X	1 report	00.1064
Axiom BloodGenomiX Reporter Software Report output-768X	2 x 384 reports	00.1065

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