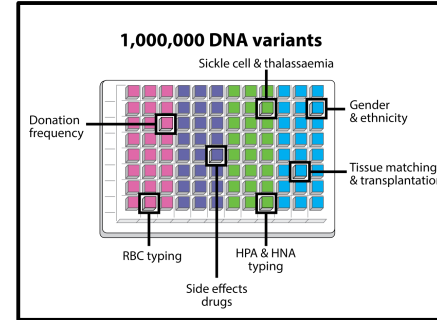
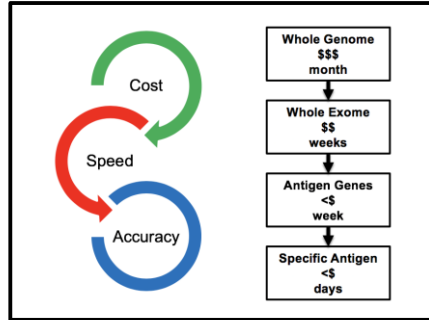
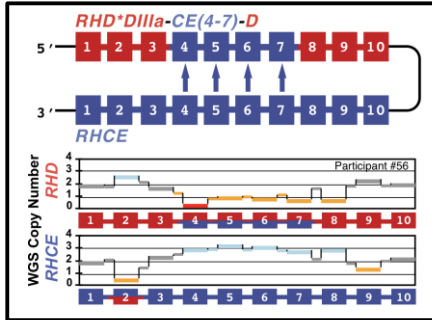
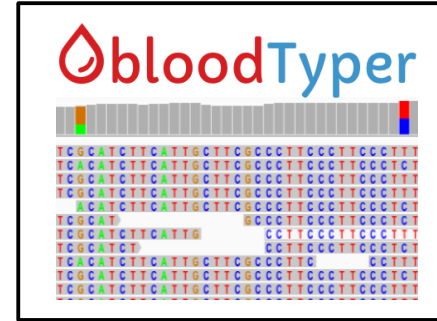
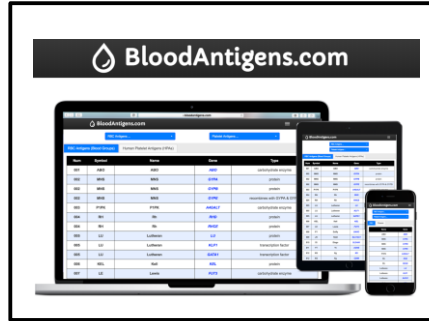
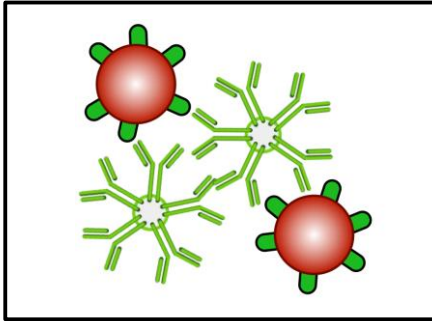


bloodTyper: Blood Group Genotyping from SNP Arrays to Whole Genomes



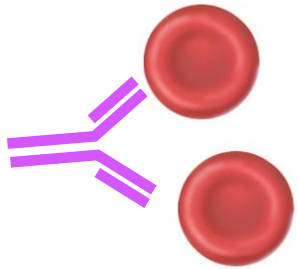
I have no relevant financial relationships to disclose for this presentation.

Talk Outline

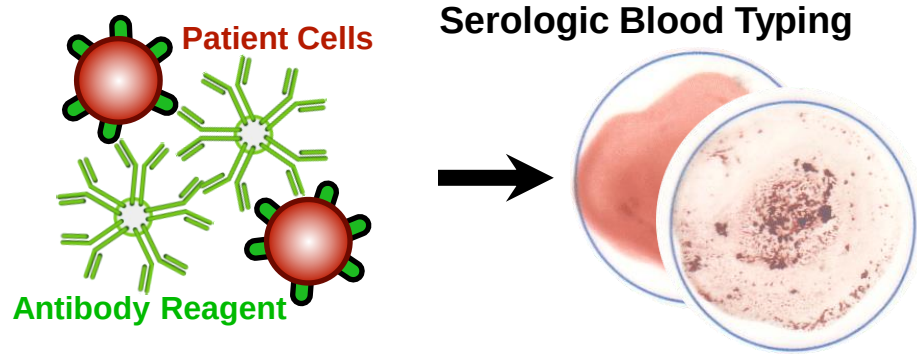
- Blood Group Typing Challenges
- Whole Genome Sequence Based Typing
- High Density Array Based Typing

Importance of Blood Typing

- 112 million units of blood worldwide /year
- Cancer, trauma, pregnancy, sickle cell disease, and stem cell transplant patients



3-30% will form
anti-red blood
cell antibody



300 more blood group antigens

ABO A2, cis-AB, A(B) **Dombrock**

RhD partial D, weak D **Lutheran**

RhCE Kidd Vel Yt **Diego Jr**

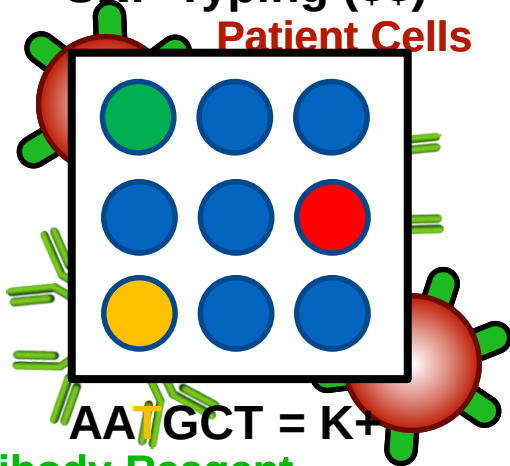
Kel Duffy MNS Colton Lan

Current Typing Methods

Single nucleotide polymorphism (SNP) Typing (\$\$)

SNP Typing (\$\$)

Patient Cells



AATGCT = K+

Antibody Reagent

CTCCTCT = E+

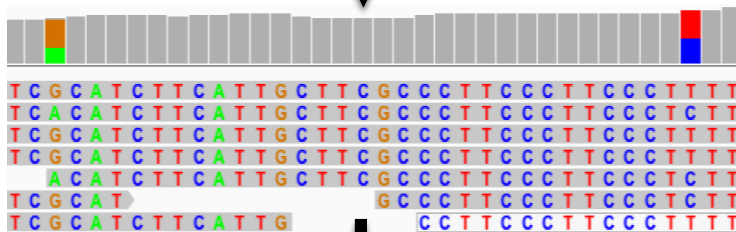
GAAATGG = S+

A1; M+N+, U+, Vr-, Mt(a-), Ri(a-), Ny(a-), Or-, ERIK-, Os(a-), ENEP+, ENEH+, ENAV+, ENEV+, MNTD-; S+s+, He-, M(v-), s(D-), Mit-; P1+/P1-, pk+, NOR-; D+, Tar-; C-c+E+e-, CW-, CX-, EW-, V-, VS-, Rh26+LOC- Be -, Crawford-CELO+, JAL-CEST+, STEM-, JAHK-; Lu(a-b+), LURC+, Lu4+, Lu5+, Lu6+, Lu7+, Lu8+, Lu13+, Lu16+, Lu17+, Au(a+b-), Lu20+, Lu21+; K-k+, Kp(a-b+c-); Js(a-b+), Ul(a-), K11+, K12+, K13+, K14+, K18+, K19+, K22+, K23-, VLAN-VONG-, TOU+, RAZ+, KALT+, KTIM+, KYO-, KUCI+, KANT+, KASH+, KELP+, KETI+, KHUL+; Le(a+b-); Fy(a+b+); Jk(a+b+); Di(a-b+), Wr(a-b+), Wd(a-), Rb(a-), WARR-, ELO-, Bp(a-), Mo(a-), Hg(a-), Vg(a-), Sw(a-), BOW-, NFLD-, Jn(a-), KREP-, Tr(a-), Fr(a-), SW1-, Wu-DISK+; Yt(a+b-); Sc1+Sc2-, Rd-, STAR+, SCER+, SCAN+; Do(a+b+), Jo(a+), DOYA+, Hy+, DOMR+, DOLG+; Co(a+b-), Co4+; LW(a+b-); Ch1+, Ch2+, Ch3+, Ch4+, Ch5+, Ch6+, Rg1-, Rg2-; Ch1-, Ch2-, Ch3-, Ch4-, Ch5-, Ch6-, Rg1+, Rg2+; H+; Kx+; Es(a+), Wb-, An(a-), Dh(a-), GEIS-, GELP+, GEAT+, GETI+; Cr(a+), Tc(a+b-c-), Dr(a+), Es(a+), WES(a-b+), UMC+, GUTI+, SERF+, ZENA+, CROV+, CRAM+, CROZ+; Kn(a+b-), McC(a+b-), Sla+Vil-, Yk(a+), Sl3+, KCAM+/KCAM-, In(a-b+), INFI+, INJA+; Ok(a+), OKGV+, OKVM+; MER2+; JMHK+, JMHL+, JMHG+, JMHM+, JMHQ+; I+; P+; GIL+; Duclos+, Ol(a-), DSLK+, RHAG4-; FORS+; Jra+; Lan+; Vel+; At(a+)

Whole Genome Sequence (WGS) Based Typing



image Courtesy of Illumina



BloodAntigens.com



A1; M+N+, U+, Vr-, Mt(a-), Ri(a-), Ny(a-), Or-, ERIK-, Os(a-), ENEP+, ENEH+, ENAV+, ENEV+, MNTD-; S+s+, He-, M(v-), s(D-), Mit-; P1+/P1-, pk+, NOR-; D+, Tar-; C-c+E+e-, CW-, CX-, EW-, V-, VS-, Rh26+LOC- Be -, Crawford-CELO+, JAL-CEST+, STEM-, JAHK-; Lu(a-b+), LURC+, Lu4+, Lu5+, Lu6+, Lu7+, Lu8+, Lu13+, Lu16+, Lu17+, Au(a+b-), Lu20+, Lu21+; K-k+, Kp(a-b+c-); Js(a-b+), Ul(a-), K11+, K12+, K13+, K14+, K18+, K19+, K22+, K23-, VLAN-VONG-, TOU+, RAZ+, KALT+, KTIM+, KYO-, KUCI+, KANT+, KASH+, KELP+, KETI+, KHUL+; Le(a+b-); Fy(a+b+); Jk(a+b+); Di(a-b+), Wr(a-b+), Wd(a-), Rb(a-), WARR-, ELO-, Bp(a-), Mo(a-), Hg(a-), Vg(a-), Sw(a-), BOW-, NFLD-, Jn(a-), KREP-, Tr(a-), Fr(a-), SW1-, Wu-DISK+; Yt(a+b-); Sc1+Sc2-, Rd-, STAR+, SCER+, SCAN+; Do(a+b+), Jo(a+), DOYA+, Hy+, DOMR+, DOLG+; Co(a+b-), Co4+; LW(a+b-); Ch1+, Ch2+, Ch3+, Ch4+, Ch5+, Ch6+, Rg1-, Rg2-; Ch1-, Ch2-, Ch3-, Ch4-, Ch5-, Ch6-, Rg1+, Rg2+; H+; Kx+; Es(a+), Wb-, An(a-), Dh(a-), GEIS-, GELP+, GEAT+, GETI+; Cr(a+), Tc(a+b-c-), Dr(a+), Es(a+), WES(a-b+), UMC+, GUTI+, SERF+, ZENA+, CROV+, CRAM+, CROZ+; Kn(a+b-), McC(a+b-), Sla+Vil-, Yk(a+), SI3+, KCAM+/KCAM-, In(a-b+), INFI+, INJA+; Ok(a+), OKGV+, OKVM+; MER2+; JMHK+, JMHL+, JMHG+, JMHM+, JMHQ+; I+; P+; GIL+; Duclos+, Ol(a-), DSLK+, RHAG4-; FORS+; Jra+; Lan+; Vel+; At(a+)

FUT1: active, **FUT2:** inactive, **FUT3:** active, **FUT6:** active, **FUT7:** active, **SLC35C1:** active, **ABO:** active **A1**, **B3GALNT1:** active, **GCNT2:** active, **GBGT1:** inactive, **KLF1:** active, **GATA1:** active

001 ABO: **A1**

002 MNS (GYPA): M+N+, Vr-, Mt(a-), Ri(a-), Ny(a-), Or-, ERIK-, Os(a-), ENEP+, ENEH+, ENAV+, ENEV+, MNTD-

002 MNS (GYPB): S+s+, He-, M(v-), s(D-), Mit-

003 P1PK (A4GALT): P1+pk+NOR-/P1-pk+NOR-

004 Rh (RHD): D+, Tar-
004 Rh (RHCE): C-c+E+e-, } R₂R₂ (R₂r'')

C^W-, C^X-, E^W-, V-, VS-, Rh26+LOCR- Be ^a, Crawford-CELO+, JAL-CEST+, STEM-, JAHK-

005 Lutheran (LU): LURC+, Lu(a-b+), Lu4+, Lu5+, Lu6+, Lu7+, Lu8+, Lu13+, Lu16+, Lu17+, Au(a+b-), Lu20+, Lu21+

006 Kell (KEL): K-k+, Kp(a-b+c-), Js(a-b+), Ul(a-), K11+, K12+, K13+, K14+, K18+, K19+, K22+, K23-,
VLAN-VONG-, TOU+, RAZ+, KALT+, KTIM+, KYO-, KUCI+, KANT+, KASH+, KELP+, KETI+, KHUL+

007 Lewis: Le(a+b-)

008 Duffy (DARC): Fy(a+b+)

009 Kidd (SLC14A1): Jk(a+b+)

010 Diego (SLC4A1): Di(a-b+), Wr(a-b+), Wd(a-), Rb(a-), WARR-, ELO-, Bp(a-), Mo(a-), Hg(a-), Vg(a-),
Sw(a-), BOW-, NFLD-, Jn(a-), KREP-, Tr(a-), Fr(a-), SW1-, Wu-DISK+

011 Yt (ACHE): Yt(a+b-)

013 Scianna (ERMAP): Sc1+Sc2-, Rd-, STAR+, SCER+, SCAN+

014 Dombrock (ART4): Jo(a+), DOYA+, Do(a+b+), Hy+, DOMR+, DOLG+

015 Colton (AQP1): Co(a+b-), Co4+

016 Landsteiner-Wiener (ICAM4): LW(a+b-)

017 Chido/Rodgers (C4B): Ch1+, Ch2+, Ch3+, Ch4+, Ch5+, Ch6+, Rg1-, Rg2-

017 Chido/Rodgers (C4A): Ch1-, Ch2-, Ch3-, Ch4-, Ch5-, Ch6-, Rg1+, Rg2+

018 H: H+

019 Kx (XK): Kx+

020 Gerbich (CYPC): Ge2+, Ge3+, Ge4+, Es(a+), Wb-, An(a-), Dh(a-), GEIS-, GELP+, GEAT+, GETI+

021 Cromer (CD55): Cr(a+), Tc(a+b-c-), Dr(a+), Es(a+), WES(a-b+), UMC+, GUTH+, SERF+, ZENA+, CROV+,
CRAM+, CROZ+

022 Knops (CR1): Kn(a+b-), McC(a+b-), Sla+Vil-, Yk(a+), SI3+, KCAM+/KCAM-

023 Indian (CD44): In(a-b+), INFI+, INJA+

024 Ok (BSG): Ok(a+), OKGV+, OKVM+

025 Raph (CD151): MER2+

026 John Milton Hagen (SEMA7A): JMHK+, JMHL+, JMHG+, JMHM+, JMHQ+

027 I: I+

028 Globoside: P+

029 Gill (AQP3): GIL+

030 Rh-associated glycoprotein (RHAG): Duclos+, OI(a-), DSLK+, RHAG4-

031 FORS: FORS1-

032 Jr (ABCG2): Jra+

033 Lan (ABCB6): Lan+

034 Vel (SMIM1): Vel+

- First individual to ever have all blood group antigens determined
- Manual interpretation of WGS variant calling files
- Took 4 months

Comprehensive red blood cell and platelet antigen prediction from whole genome sequencing: proof of principle

*William J. Lane,^{1,2} Connie M. Westhoff,³ Jon Michael Uy,¹ Maria Aguad,¹ Robin Smeland-Wagman,¹ Richard M. Kaufman,¹ Heidi L. Rehm,^{1,2,4,5} Robert C. Green,^{2,5,6} and Leslie E. Silberstein⁷ for the MedSeq Project**

Transfusion. 2016 Mar;56(3):743-54

001 ABO **ABO**:A
002 MNS **GYPA**:M+N+; Vr-; Mt(a-); Ri(a-); Ny(a-); Or-; ERIK-; Os(a-); ENEP+; ENEH+; ENAV+; ENEV+; MNTD-
002 MNS **GYPB**:S+s+; He-; M(v-); s(D-); Mit-; U+
002 MNS **GYPE**:
003 P1PK **A4GALT**:P1+p(k)+NOR-
004 RH **RHD**:D+; Tar-
004 RH **RHCE**:E+e-; C-c+; C^W-; C^X-; E^W-; VS-; Rh26+LOCR-; Be^a-; JAL-CEST+; STEM-; JAHK-; CEAG+; V-
005 LU **LU**:LURC+; Lu(a-b+); Lu4+; Lu5+; Lu6+; Lu7+; Lu8+; Lu13+; Lu16+; Lu17+; Au(a+b-); Lu20+; Lu21+
005 LU **KLF1**:
005 LU **GATA1**:
006 KEL **KEL**:K-k+; Kp(a-b+c-); Js(a-b+); Ul(a-); K11+; K12+; K13+; K14+; K18+; K19+; K22+; K23-; VLAN-VONG-; TOU+; RAZ+; KALT+; KTIM+;
KYO-; KUCI+; KANT+; KASH+; KELP+; KETI+; KHUL+
007 LE **FUT3**:Le(a+b-)
008 FY **DARC**:Fy(a+b+)
009 JK **SLC14A1**:Jk(a+b+)
010 DI **SLC4A1**:Di(a-b+); Wr(a-b+); Wd(a-); Rb(a-); WARR-; ELO-; Bp(a-); Mo(a-); Hg(a-); Vg(a-); Sw(a-); BOW-; NFLD-; Jn(a-); KREP-; Tr(a-);
Fr(a-); SW1-; DISK+
011 YT **ACHE**:Yt(a+b-)
012 XG **XG**:
012 XG **CD99**:
013 SC **ERMAP**:Sc1+Sc2-; Rd-; STAR+; SCER+; SCAN+
014 DO **ART4**:Do(a+b+); Jo(a+); DOYA+; Hy+; DOMR+; DOLG+
015 CO **AQP1**:Co(a+b-b-); Co4+
016 LW **ICAM4**:LW(a+b-)
017 CH **C4B**:Ch1+, Ch2+, Ch3+, Ch4+, Ch5+, Ch6+, Rg1-, Rg2-
017 RG **C4A**:Ch1-, Ch2-, Ch3-, Ch4-, Ch5-, Ch6-, Rg1+, Rg2+
018 H **FUT1**:active
018 H **FUT2**:Found **Homozygous** Null Allele Variant: *FUT2*01N.02* (inactive)
018 H **SLC35C1**:active
019 XK **XK**:Kx+
020 GE **GYPC**:Ge2+, Ge3+, Ge4+; Wb-; An(a-); Dh(a-); GEIS-; GELP+; GEAT+; GETI+
021 CROM **CD55**:Cr(a+); Tc(a+b-c-); Dr(a+); Es(a+); WES(a-b+); UMC+; GUTI+; SERF+; ZENA+; CROV+; CRAM+; CROZ+
022 KN **CR1**:Kn(a+b-); McC(a+b-); SI^a+Vil-; Yk(a+); SI3+; **KCAM+ / KCAM-**
023 IN **CD44**:In(a-b+); INFI+; INJA+
024 OK **BSG**:Ok(a+); OKGV+; OKVM+
025 RAPH **CD151**:MER2+
026 JMH **SEMA7A**:JMHK+; JMHL+; JMHG+; JMHH+; JMHQ+
027 I **GCNT2**:active
028 GLOB **B3GALNT1**:active
029 GIL **AQP3**:GIL+
030 RHAG **RHAG**:Duclos+; Ol(a-); DSLK+; RHAG4-
031 FORS **GBGT1**:Found **Homozygous** Null Allele Variant: *GBGT1*01N.01* (inactive)
032 JR **ABCG2**:Jr^a+
033 LAN **ABCB6**:Lan+
034 VEL **SMIM1**:Vel+
035 CD59 **CD59**:59.1+
036 AT **SLC29A1**:At(a+)

BloodAntigens.com



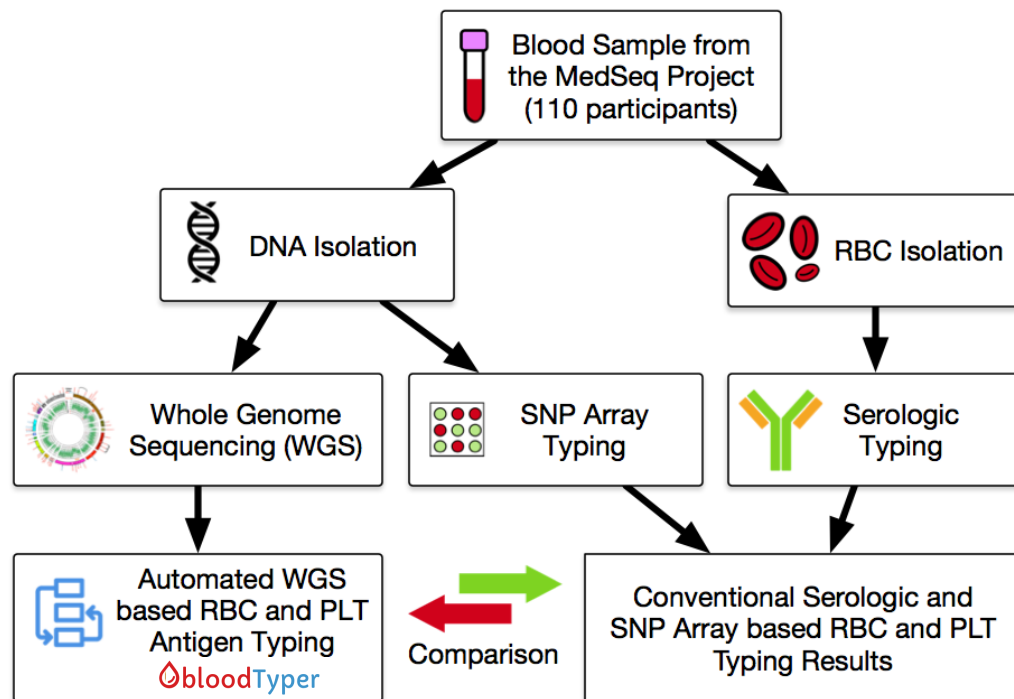
- Same individual
- First automated full blood group typing from WGS data using bloodTyper.
- Took 4 years to develop bloodTyper v3 with 110 genomes
- 10 min to run bloodTyper
- Last 200 genomes 4 days

Automated typing of red blood cell and platelet antigens: a whole-genome sequencing study

William J Lane*, Connie M Westhoff*, Nicholas S Gleadall*, Maria Aguad, Robin Smeland-Wagman, Sunitha Vege, Daimon P Simmons, Helen H Mah, Matthew S Lebo, Klaudia Walter, Nicole Soranzo, Emanuele Di Angelantonio, John Danesh, David J Roberts, Nick A Watkins, Willem H Ouweland, Adam S Butterworth, Richard M Kaufman, Heidi L Rehm, Leslie E Silberstein*, Robert C Green*, on behalf of the MedSeq Project†

Lancet Haematology. 2018. Jun;5(6):e241-e251

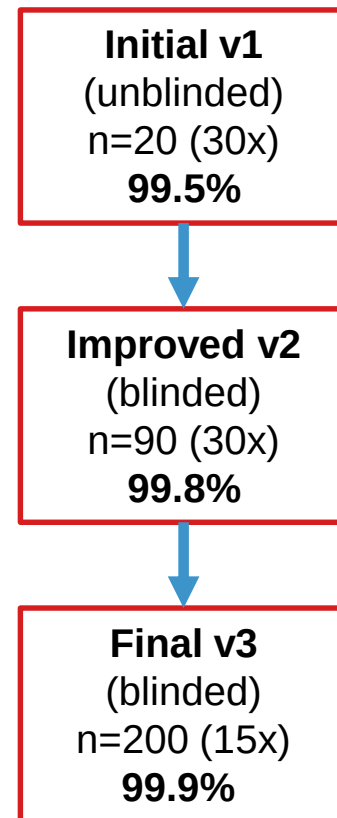
WGS based RBC and PLT Antigen Typing Algorithm Development Data Set



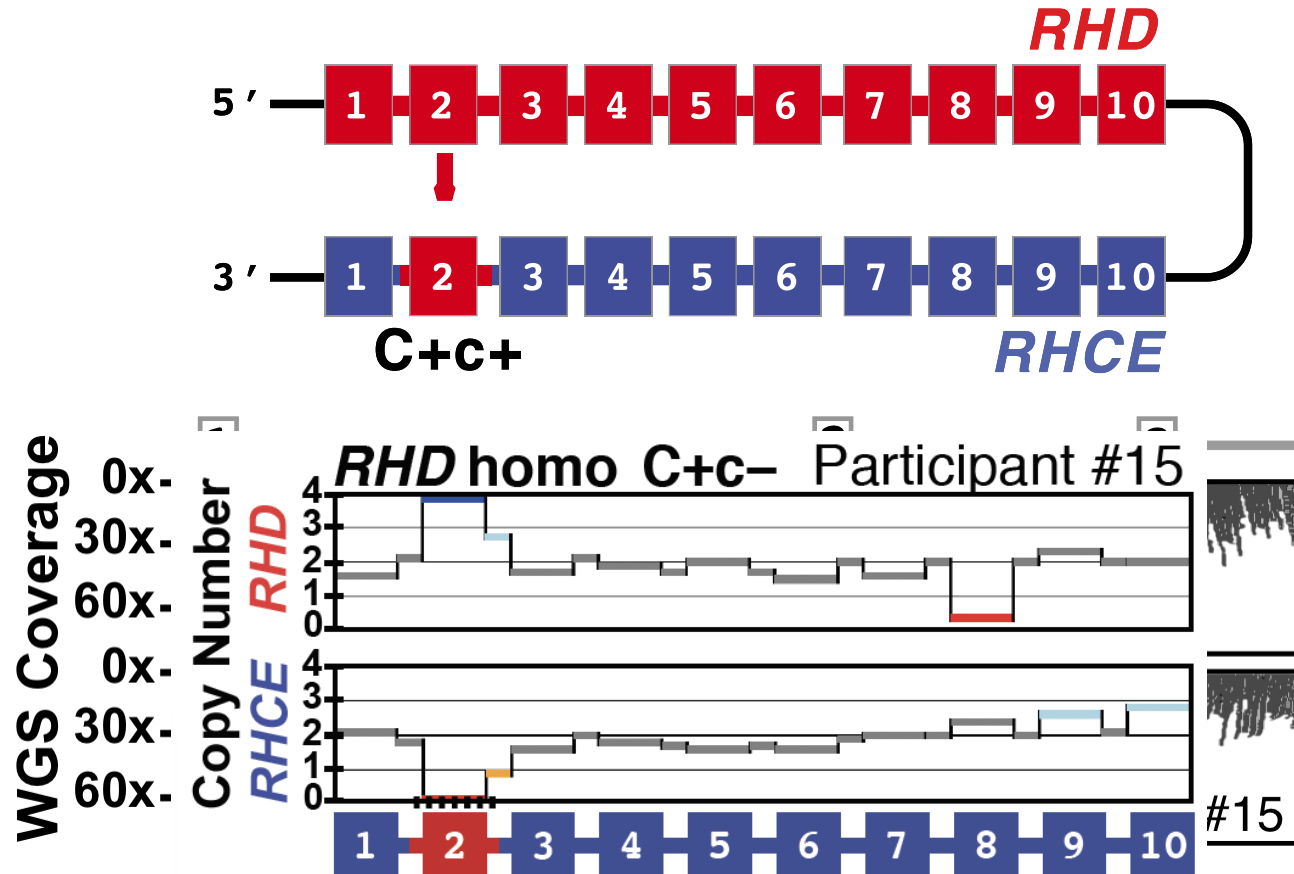
External Validation Data Set



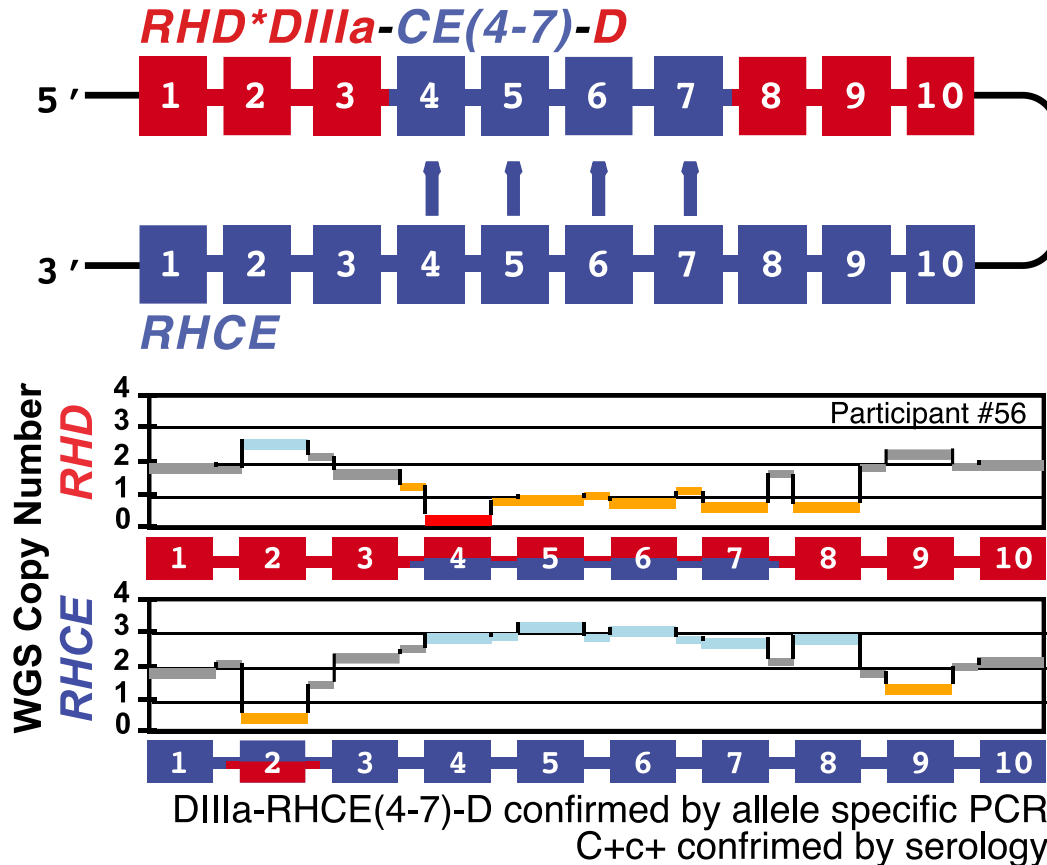
 bloodTyper



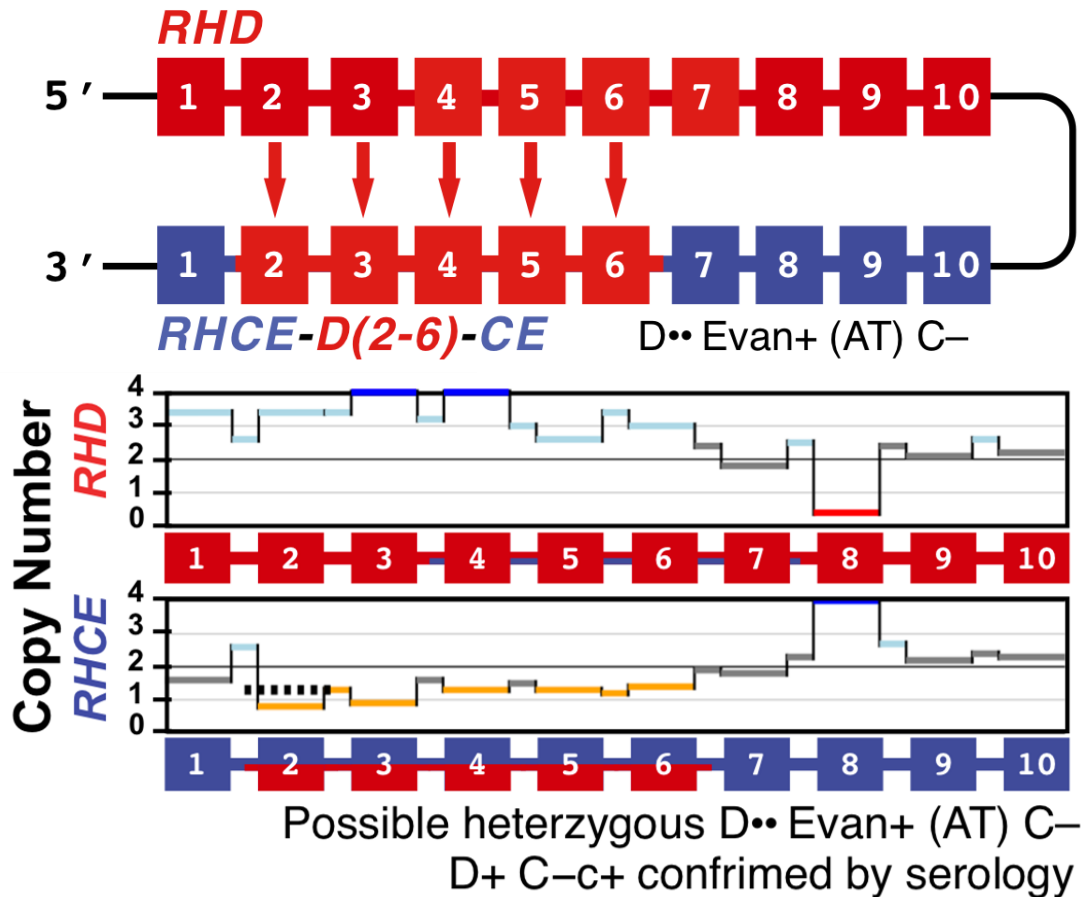
C Antigen Gene Conversion



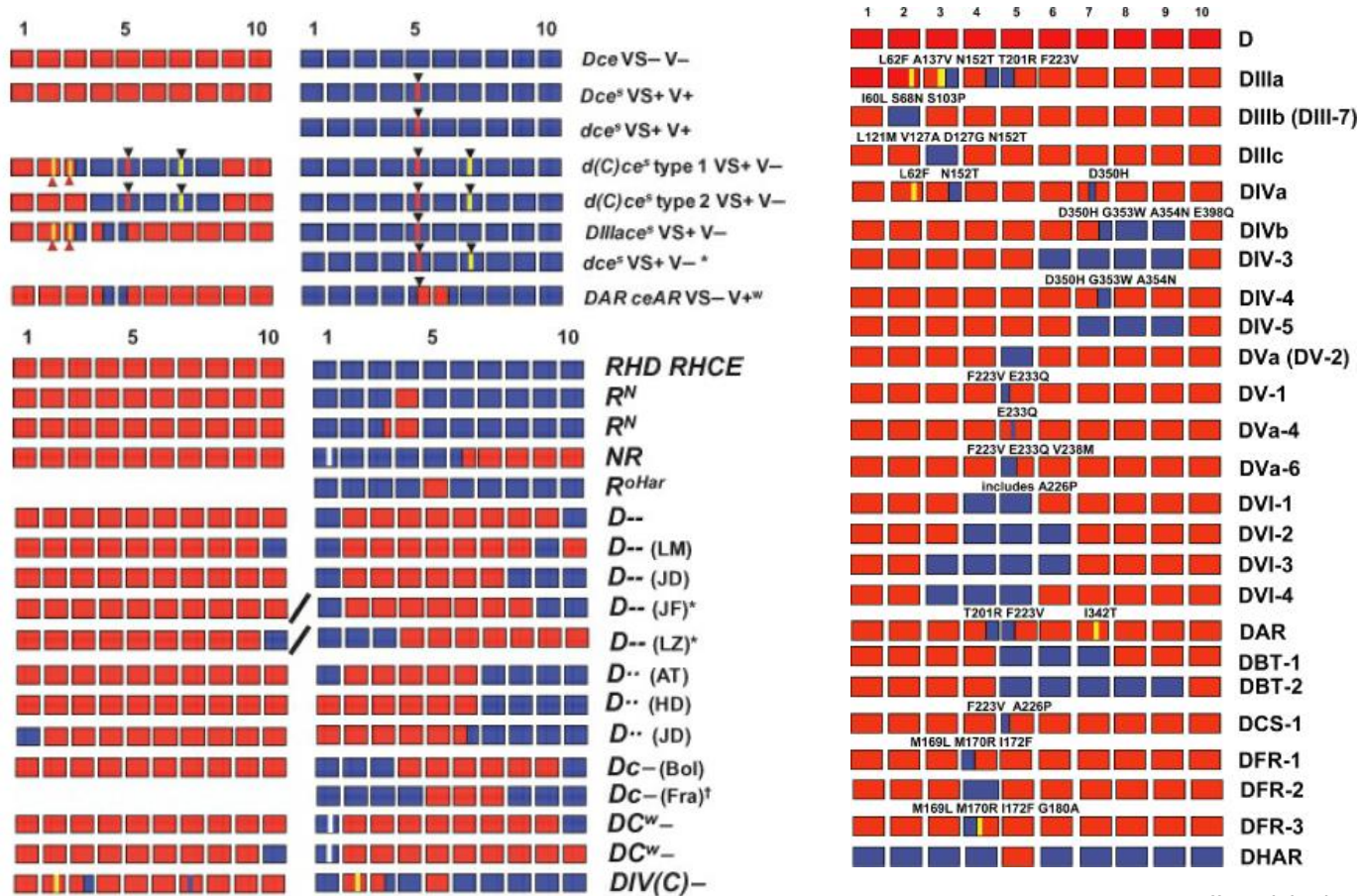
Hybrid *RHD* (Partial D)



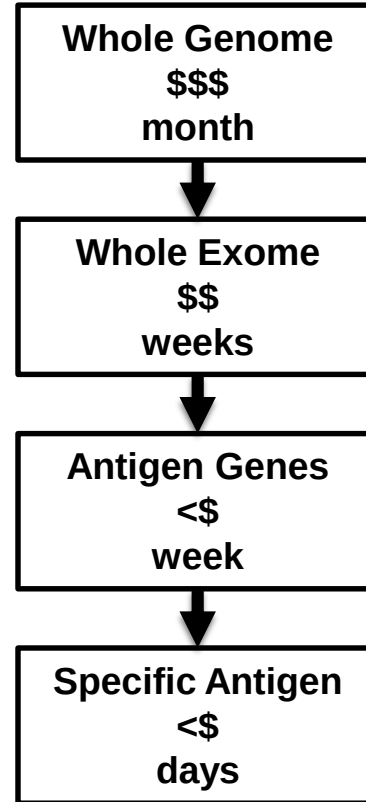
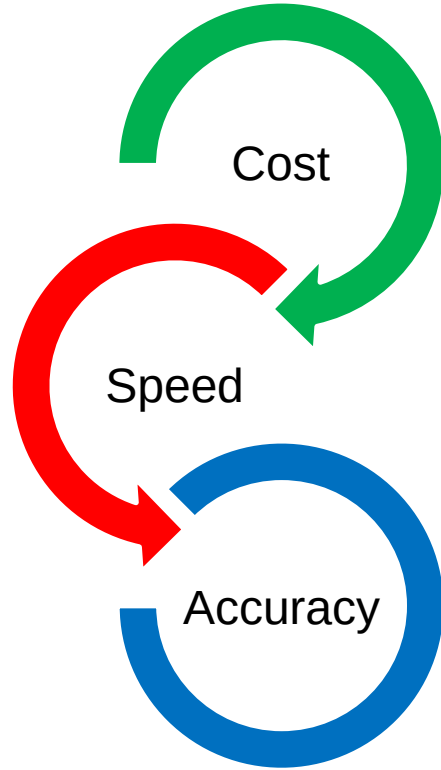
Hybrid *RHCE* (D⁺ Evans+ AT C⁻)



Other *RHD* and *RHCE* Hybrids



NGS Balancing Act



High Density SNP Typing



Cost: \$

Time: week

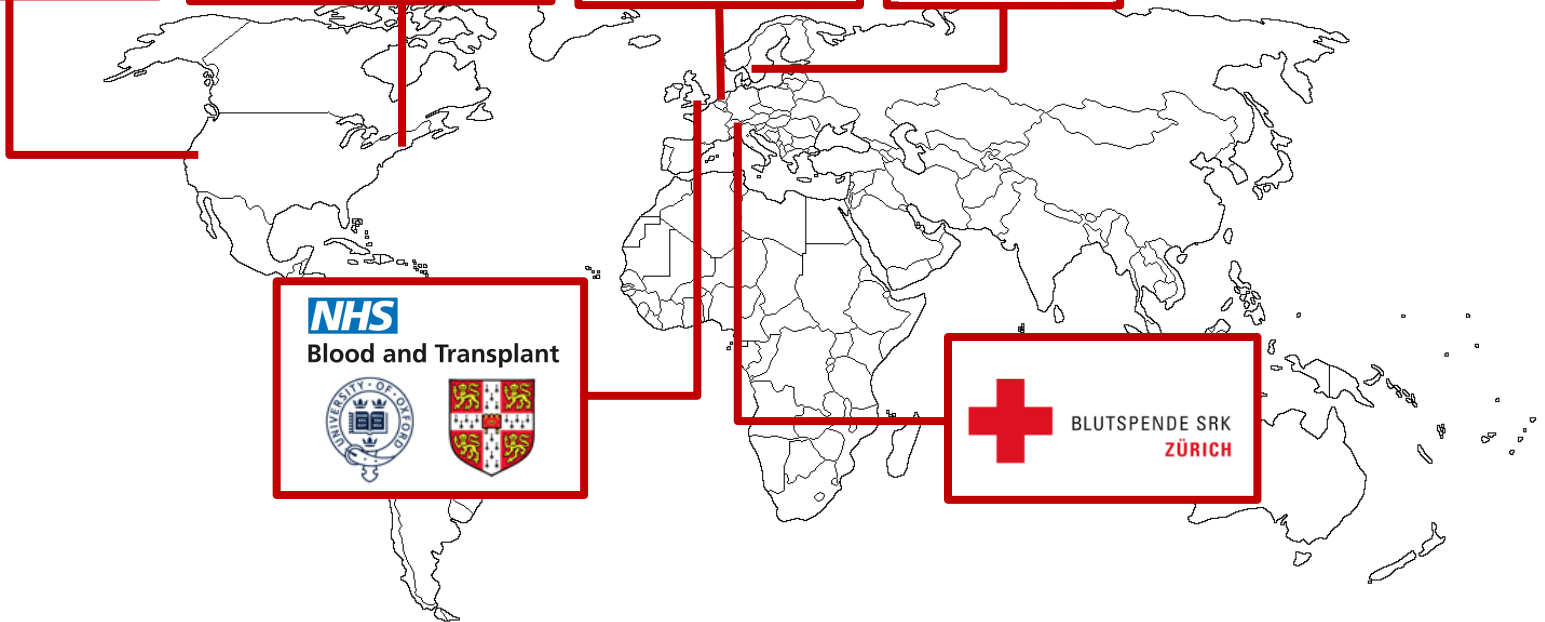
Min Batch Size: 96

Scalability: Very High

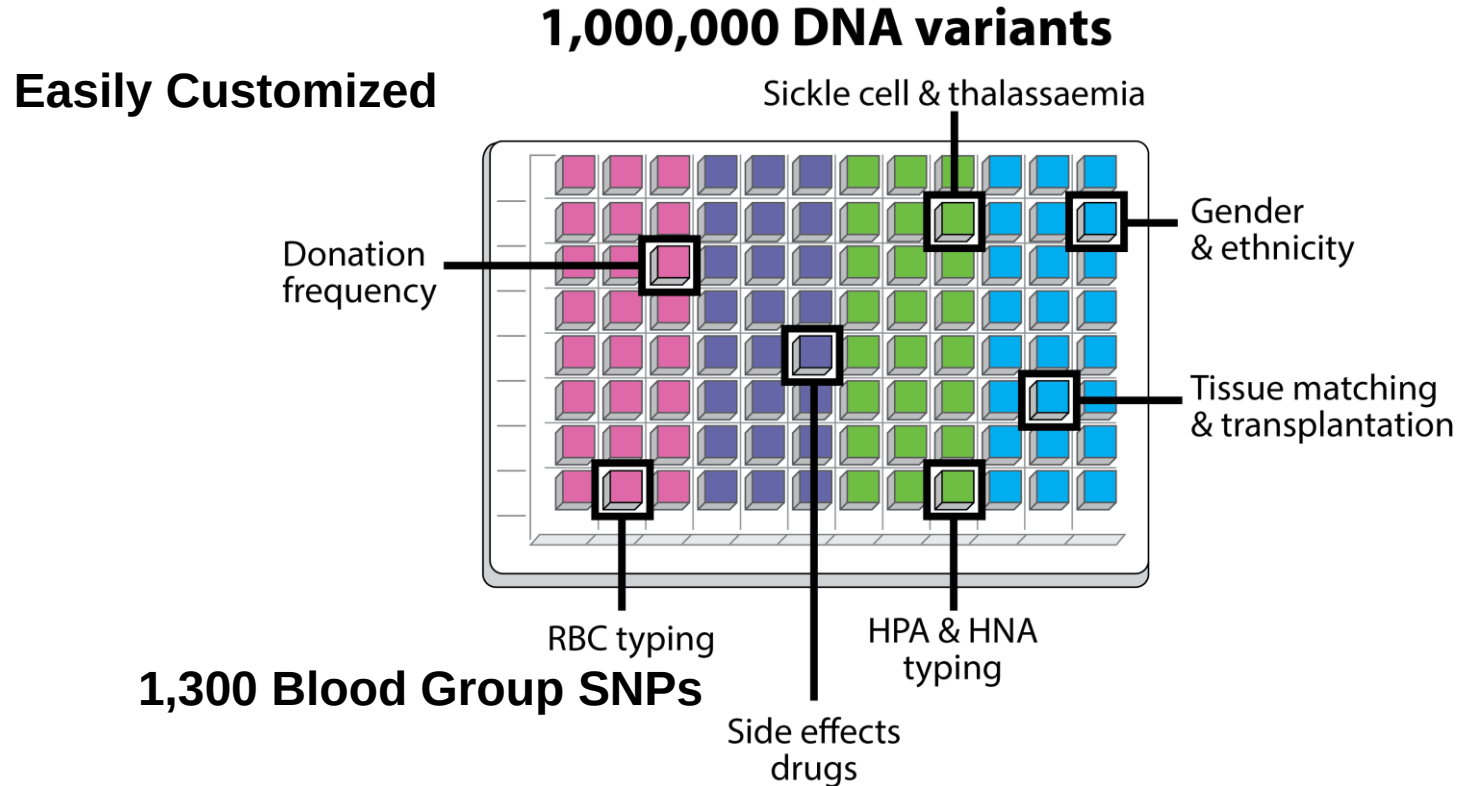
A1; M+N+, U+, Vr-, Mt(a-), Ri(a-), Ny(a-), Or-, ERIK-, Os(a-), ENEP+, ENEH+, ENAV+, ENEV+, MNLD-, S+s+, He-, M(v-), s(D-), Mit-; P1+/P1-, pk+, NOR-; D+, Tar-; C-c+E-e-, CW-, CX-, EW-, V-, VS-, Rh26+LOC- Be -, Crawford-CELO+, JAL-CEST+, STEM-, JAHK-; Lu(a-b+), LURC+, Lu4+, Lu5+, Lu6+, Lu7+, Lu8+, Lu13+, Lu16+, Lu17+, Au(a+b-), Lu20+, Lu21+; K-k+, Kp(a-b+c-); Js(a-b+), Ul(a-), K11+, K12+, K13+, K14+, K18+, K19+, K22+, K23-, VLAN-VONG-, TOU+, RAZ+, KALT+, KTIM+, KYO-, KUCI+, KANT+, KASH+, KELP+, KETI+, KHUL+; Le(a+b-); Fy(a+b+); Jk(a+b+); Di(a-b+), Wr(a-b+), Wd(a-), Rb(a-), WARR-, ELO-, Bp(a-), Mo(a-), Hg(a-), Vg(a-), Sw(a-), BOW-, NFLD-, Jn(a-), KREP-, Tr(a-), Fr(a-), SW1-, Wu-DISK+; Yt(a+b-); Sc1+Sc2-, Rd-, STAR+, SCER+, SCAN+; Do(a+b+), Jo(a+), DOYA+, Hy+, DOMR+, DOLG+; Co(a+b-), Co4+; LW(a+b-); Ch1+, Ch2+, Ch3+, Ch4+, Ch5+, Ch6+, Rg1-, Rg2-; Ch1-, Ch2-, Ch3-, Ch4-, Ch5-, Ch6-, Rg1+, Rg2+; H+; Kx+; Es(a+), Wb-, An(a-), Dh(a-), GEIS-, GELP+, GEAT+, GETI+; Cr(a+), Tc(a+b-c-), Dr(a+), Es(a+), WES(a-b+), UMC+, GUTI+, SERF+, ZENA+, CROV+, CRAM+, CROZ+; Kn(a+b-), McC(a+b-), Sla+Vil-, Yk(a+), SI3+, KCAM+/KCAM-, In(a-b+), INFI+, INJA+, Ok(a+), OKGV+, OKVM+, MER2+; JMHK+, JMHL+, JMHG+, JMHM+, JMHQ+; I+; P+; GIL+; Duclos+, Ol(a-), DSLK+, RHAG4-; FORS+; Jra+; Lan+; Vel+; At(a+)



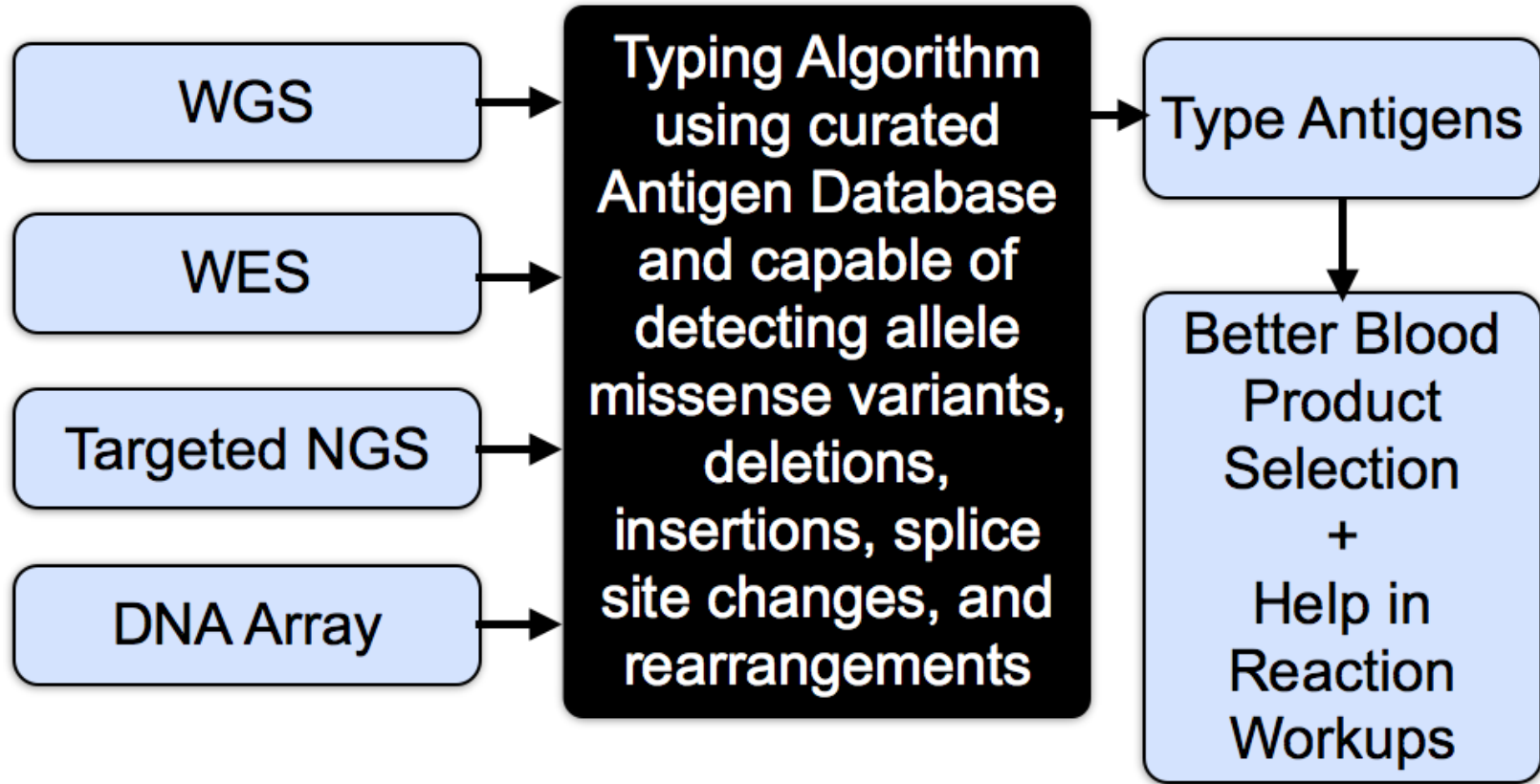
Blood Transfusion Genomics Consortium



High Density Array Design



Blood Typing – The Future





Blood Transfusion Genomics Consortium



Nick Gleadall
Adam Butterworth
Emanuele Di Angelantonio
Karyn Megy
Christopher Penkett
Kathy Stirrups
Ernest Turro
Willem H Ouwehand



David Roberts
Mike Murphy
Gil McVean



Connie Westhoff
Sunitha Vege



Steven Marsh



Blood and Transplant

Nick Watkins
Alan Grey
Shane Grimsley



William Lane
Les Silberstein
Rich Kaufman
Robert Green
Heidi Rehm
Helen Mah
Maria Aguad
Abigail Joseph



Barbera Veldhuisen
Peter Ligthart
Ellen van der Schoot



LUND UNIVERSITY
Mattias Moller
Jill Storry
Martin L Olsson



BLUTSPENDE SRK
ZÜRICH

Christoph Gassner



Will Kruka
Jeremy Gollub



Kim Brügger



Aoife McMahon



saving the lives
of people with
blood cancer



Questions?

