AlloSeq Assign

AlloSeq Assign IMGT/HLA 3.53.0 Reference File Release Notes

For use with ABO and CCR5 spike in probes for[1] Tx17 and Tx9 typing kits

TEC912
Software Version number: 1.0.3+
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[1] ABO + CCR5 are for Evaluation Use Only. Not to be used for diagnostic procedures.

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1. AlloSeq Assign Reference 3.53.0.0 for AlloSeq Tx Version Changes

Changes since AlloSeq Assign Reference 3.51.0.1:

- No changes made to ABO or CCR5 references.

2. IMGT/HLA 3.53.0 Reference Update

See TEC913_ AlloSeq_Assign_3.53.0.0_Reference_Release_Notes_Tx17_Tx9 for details of the IMGT/HLA references provided for AlloSeq Assign.

3. ISBT/ABO 1.1.0 Reference Update[1]

All alleles were validated for consistency with the sequences from ISBT/ABO, which can be found with this link: https://www.erythrogene.com/?q=ABO[2]

With the exception of the following alleles:

- ABO*O.16 is excluded from the reference due to lack of exons 5-7.

A complete list of the alleles from ISBT can be found here:
http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology

Note: the following alleles are identical in the CDS as provided by ISBT and will report as ambiguities. Once genomic sequence is identified for these alleles they will be updated:

- ABO*BA.06 and ABO*cisAB.05
- ABO*O.09.01 and ABO*AW.31.02(-05)
- ABO*O.09.02 and ABO*AW.31.01
- ABO*AW.26 and ABO*A2.09

4. NCBI/CCR5 NG012637.1.0 Reference[1]

There are 2 reference alleles present in the CCR5 reference. These alleles have been validated for consistency with fasta sequences.

Allele	Source
W	NG_012637.1
D	IHW9038/MW002075

Table 1: Alleles included in the CCR5 reference

5. AlloSeq Tx Depth of Coverage

The minimum depth of coverage threshold has been individually set for each locus and may vary depending upon the kit used. Positions below the minimum depth value will be starred out in the sample consensus sequence, base call quality indicated as red and excluded from the analysis.

Locus	Minimum depth (#reads)
A	30
B	30
C	30
DPB1	30
DQB1	30
DRB1	30

Table 2: Minimum Coverage Depth Per Locus

[6] Möller M, Jöud M, Storry JR, Olsson ML. Erythrogene: a database for in-depth analysis of the extensive variation in 36 blood group systems in the 1000 Genomes Project. Blood Adv. 2016 Dec 16;1(3):240-249

Locus	Minimum depth (#reads)
ABO	30
CCR5	30

Table 3: Minimum Coverage Depth Per Locus- ABO & CCR5[1]

6. Regions Included in the Core Analysis

ISBT/ABO Core Layer[1]

- The core layer includes regions important in differentiating specific alleles as listed below.

Allele	Region	Position	Nucleotide change
ABO*AW.28	Intron 1	5055	c.98+2T>C
ABO*O.10	Exon 2	18085	c.66_67insG
ABO*O.04	Exon 2	18107	c.87_88insG
ABO*B3.03	Intron 3	18902	c.155+5G>A
ABO*AW.45	Intron 4	20397	c.203+1delG
ABO*O.01	Exon 6	22694	c.261delG
ABO*O.05	Exon 6	22755	c.322C>T
ABO*AW.44	Intron 6	22811	c.374+4A>G
ABO*AEL.04	Intron 6	22812	c.374+5G>A
ABO*O.13	Exon 7	23937	c.452T>G
ABO*O.11	Exon 7	23991	c.505_507delCAG
ABO.BA, cisAB	Exon 7	24011	c.526C>G
ABO*O.06	Exon 7	24027	c.542G>A
ABO*O.12	Exon 7	24048	c.563G>A
ABO*O.14	Exon 7	24120	c.635T>A
ABO*O.09	Exon 7	24131	c.646T>A
ABO.cisAB.03	Exon 7	24185	c.700C>T
ABO*O.15	Exon 7	24278	c.793T>C
ABO*cisAB	Exon 7	24281	c.796C>A
ABO*BA, cisAB	Exon 7	24288	c.803G>C
ABO*O.03	Exon 7	24290	c.804dupG
ABO*O.07	Exon 7	24378	c.893C>T
ABO*O.08	Exon 7	24412	c.927C>A
ABO*AW.2, 3, 9, 16, 17, 18	Exon 7	24546	c.1061delC

Table 4: Regions Included in The Core Layer

NCBI/CCR5 Core Layer[1]

- The core region includes the Δ32 deletion.

Region	Position
32 bp deletion	8315-8346

Table 5: Regions Included in The Core Layer

7. Base Positions Masked for Variant Calling

The following base position and/or regions have been excluded from analysis due to incomplete coverage of resulting reads or commonly occurring low sequence quality.

Locus	Region
CCR5	5'UTR 1-17425, 3'UTR 24683-42144
ABO	5'UTR 1-17425, 3'UTR 24683-42144

Table 6: Regions Masked Per Locus - ABO & CCR5[1]

8. Assign Matching Validation

ISBT/ABO library version:	**1.1.0 2017-01-20**
Tested using Assign version(s):	4.7.1.988

Notes:

1. **Validation was performed by converting new fasta sequences available from Erythrogene to homozygous fastq files and importing into AlloSeq Assign. Genotyping reports were then generated and compared against the known genotype of the fasta sequence. Any discrepancies are listed in section 2 of this document.**
2. **Some cDNA sequences were validated by importing the homozygous fasta sequences available from Erythrogene for new alleles into Assign SBT.**
3. **Modified and corrected alleles are included in this validation.**

Outputs:	
ABO	
Number of New Alleles:	202
Number of New Alleles Successfully Imported:	202
Number of New alleles Successfully Reported:	202

Table 7: Validation Data for the Assign Matching Algorithm- ISBT/ABO[1]

NCBI/CCR5 library version:	**NG_012637.1.0**
Tested using Assign version(s):	4.7.1.988 and 1.0.2.1236

Notes:

1. **Validation was performed by converting new fasta sequences available from NCBI to homozygous fastq files and importing into AlloSeq Assign. Genotyping reports were then generated and compared against the known genotype of the fasta sequence. Any discrepancies are listed in this document.**
2. **Some cDNA sequences were validated by importing the homozygous fasta sequences available from NCBI for new alleles into Assign SBT.**
3. **Modified and corrected alleles are included in this validation.**

Outputs:	
CCR5	
Number of New Alleles:	2
Number of New Alleles Successfully Imported:	2
Number of New alleles Successfully Reported:	2

Table 8: Validation Data for the Assign Matching Algorithm- NCBI/CCR5[1]

14.Customer Support

Website: https://labproducts.caredx.com/

For Technical Support please email: techsupport-global@caredx.com

For ordering details, please refer to the CareDx website: https://labproducts.caredx.com/

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Revision History

Version	Date	Modification	Reference/ Justification
1.0	02-Oct-23	Updated to 3.53.0.0.	N/A