# AlloSeq Assign IMGT/HLA 3.53.0 Reference File Release Notes

For use with AlloSeq Tx 17 and AlloSeq Tx 9 typing kits

TEC913
Software version number: 1.0.3 +
Document Version No: 1.0
Issue Date: October 2023

REF ASA1.0

CareDx Pty Ltd,
20 Collie Street,
Fremantle, WA 6160,
Australia

CareDx Pty Ltd

Table of Contents

# 1. AlloSeq Assign Reference 3.53.0.0 for AlloSeq Tx 17 and AlloSeq Tx 9 Version Changes

Changes since AlloSeq Assign Reference 3.51.0.1:

1. Updated alleles as per IMGT release 3.53.0.
2. Made improvements to pseudogene references to reduce mis-mapping to targeted genes.
    a. Added UTR sequences to DPB2 alignment sequences. Updated the alleles to include all sequences available in IMGT database.
    b. Reviewed DQA2 reference and removed non-IMGT alleles.
    c. Extended the alignment sequences for DRB2 at the 3'UTR. Added IMGT sequence for DRB2*01:01 and putative sequences for 2 additional DRB2 alleles.
    d. Extended the alignment sequences for DRB8 to include more sequence for intron 2. Added IMGT sequence for DRB8*01:01.
3. Expanded the alignment sequences for HLA-G to include the 14bp indel in the 3'UTR.
    a. Added extended sequences for 8 alleles to facilitate the alignment in the 3'UTR. These sequences are used for alignment purposes only and will not report as genotypes.
    b. Note that there is no impact to saved projects analysed with previous reference versions due to the change to the alignment sequences as the added sequence is 3' of the IMGT reference sequences.
    c. Added the 14bp indel as a motif. See Table 8 for more details.

# 2. IMGT/HLA 3.53.0 Reference Update

All alleles were validated for consistency with the IMGT/HLA [1,2]release database version included in the IMGT/HLA release notes, which can be found with these links:

https://www.ebi.ac.uk/ipd/imgt/hla/docs/version_r3520.html

https://www.ebi.ac.uk/ipd/imgt/hla/docs/version_r3530.html

# 3. AlloSeq Tx Gene Coverage

Consensus sequences have been extended beyond the regions described by IMGT. The sequences used to extend the consensus sequences have been derived from GRCh38[3]

# 4. 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 |
| **DPA1** | 30 |
| **DPB1** | 30 |

[1] Robinson J, Malik A, Parham P, Bodmer JG, Marsh SGE: IMGT/HLA - a sequence database for the human major histocompatibility complex Tissue Antigens (2000), 55:280-287

[2] Robinson J, Barker DJ, Georgiou X, Cooper MA, Flicek P, Marsh SGE: IPD-IMGT/HLA Database. Nucleic Acids Research (2020), 48:D948-55

[3] Schneider, V.A. et al. Evaluation of GRCh38 and de novo haploid genome assemblies demonstrates the enduring quality of the reference assembly. *Genome Res*. 2017 May;27(5):849-864

| | |
|---|---|
| **DQA1** | 30 |
| **DQB1** | 30 |
| **DRB1** | 30 |
| **DRB3** | 30 |
| **DRB4** | 30 |
| **DRB5** | 30 |
| **E** | 30 |
| **F** | 30 |
| **G** | 30 |
| **H** | 30 |
| **MICA** | 30 |
| **MICB** | 30 |

**Table 1. Minimum depth of coverage**

## 5. Regions Included in the Core Analysis

Core layer includes:

- All sequence in Exons 2, 3 and 4 of HLA Class 1, MICA and MICB and exon 2 and 3 of HLA class 2.
- Full sequence of additional exons if these exons include polymorphisms that impact expression.
- Specific non-coding positions that have sequence polymorphisms that impact expression.
- Splice sites as predicted by fruitfly and NetGene2.

| Locus | Region | Position | Known Alleles with impacted expression |
|---|---|---|---|
| **A** | Exon 1, 2, 3, 4, 5, 6, 7, 8 | all | N/A |
| | UTR | 764 | A*02:01:01:02L |
| | Exon 1 splice site | 863-864 | None |
| | Exon 1 donor site | 938-939 | A*33:03:01:21Q, A*26:01:01:52Q |
| | Intron 1 | 1042 | A*31:01:02:03N |
| | Exon 2 acceptor site | 1066-1067 | None |
| | Exon 2 donor site | 1338-1339 | A*02:01:01:134Q, A*24:447Q |
| | Intron 2 | 1342-1345 | A*01:01:01:02N |
| | Intron 2 | 1572 | A*01:301Q, A*24:450Q, A*24:02:01:02L |
| | Exon 3 acceptor site | 1577-1578 | None |
| | Exon 3 donor site | 1855-1856 | None |
| | Exon 4 acceptor site | 2432-2433 | None |
| | Exon 4 donor site | 2710-2711 | A*03:01:01:02N, A*26:01:01:03N, A*29:01:01:02N |
| | Exon 5 acceptor site | 2810-2811 | None |
| | Exon 5 donor site | 2929-2930 | A*31:01:02:51Q |
| | Exon 6 acceptor site | 3369-3370 | None |
| | Exon 6 donor site | 3404-3405 | None |
| | Intron 7 | 3542 | A*24:02:01:02L |
| | Exon 7 acceptor site | 3544-3545 | None |
| | Exon 7 donor site | 3594-3595 | A*31:01:02:30Q |
| | Exon 8 acceptor site | 3761-3762 | A*24:02:01:17Q |
| **B** | Exon 1, 2, 3, 4, 5, 6, **7** | all | N/A |
| | UTR | 691 | B*39:01:01:02L |
| | Exon 1 splice site | 841-842 | None |
| | Exon 1 donor site | 916-917 | None |
| | Exon 2 acceptor site | 1043-1044 | B*15:01:01:39Q |
| | Intron 1 | 1019 | B*15:01:01:02N |
| | Exon 2 donor site | 1315-1316 | B*38:01:01:03Q, B*15:616Q |
| | Exon 3 acceptor site | 1559-1560 | B*18:01:01:42Q, B*27:05:02:04Q, B*18:01:01:12Q |
| | Exon 3 donor site | 1837-1838 | B*44:02:01:13Q, **B*15:01:01:65Q** |
| | Exon 4 acceptor site | 2410-2411 | **B*14:02:01:25Q** |
| | Exon 4 donor site | 2691-2692 | B*40:02:01:32Q, B*08:284Q |
| | Exon 5 acceptor site | 2790-2791 | None |
| | Exon 5 donor site | 2909-2910 | B*44:02:01:02S, B*56:01:01:05S |
| | Exon 6 acceptor site | 3348-3349 | None |
| | Exon 6 donor site | 3383-3384 | None |

| Locus | Region | Position | Known Alleles with impacted expression |
|---|---|---|---|
| | Exon 7 acceptor site | 3487-3488 | B*51:01:01:97Q |
| | Exon 7 donor site | 3537-3538 | none |
| **C** | Exon 1, 2, 3, 4, 5, 7, 8 | All | N/A |
| | Exon 1 splice site | 931-932 | None |
| | Exon 1 donor site | 1006-1007 | C*15:02:01:30Q |
| | Exon 2 acceptor site | 1134-1135 | C*05:01:01:53Q, C*07:01:01:14Q, C*03:03:01:52N, C*07:04:01:16Q |
| | Exon 2 donor site | 1406-1407 | C*15:02:01:35Q |
| | Exon 3 acceptor site | 1655-1656 | C*03:03:01:50N, C*15:02:01:08N, C*07:01:01:100Q, C*07:04:01:15Q, C*17:01:01:16Q, C*02:02:02:74Q, C*03:03:01:55Q, C*04:01:01:170Q, C*05:01:01:80Q, C*07:02:01:137Q, C*07:01:01:127Q |
| | Exon 3 donor site | 1933-1934 | C*02:02:02:34Q, C*03:04:01:35Q, C*07:02:01:17N, C*07:02:01:125Q, C*03:03:01:39Q |
| | Exon 4 acceptor site | 2518-2519 | None |
| | Exon 4 donor site | 2796-2797 | C*04:01:01:47Q |
| | Exon 5 acceptor site | 2915-2916 | None |
| | Exon 5 donor site | 3037-3038 | None |
| | Exon 6 acceptor site | 3477-3478 | C*07:06:01:05Q, C*04:01:01:84Q |
| | Exon 6 donor site | 3512-3513 | None |
| | Exon 7 acceptor site | 3617-3618 | C*07:0201:124Q |
| | Exon 7 donor site | 3667-3668 | C*07:02:01:74Q |
| | Exon 8 acceptor site | 3829-3830 | None |
| **DPA1** | Exon 1, 2, 3, 4 | All | N/A |
| | Exon 1 splice site | 1429-1430 | None |
| | Exon 1 donor site | 1531-1532 | DPA1*01:03:01:18Q |
| | Exon 2 acceptor site | 5113-5114 | None |
| | Exon 2 donor site | 5361-5362 | None |
| | Exon 3 acceptor site | 5699-5700 | None |
| | Exon 3 donor site | 5983-5984 | None |
| | Exon 4 acceptor site | 6195-6196 | None |
| | Exon 4 donor site | 6352-6353 | None |
| **DPB1** | Exon 1, 2, 3, **4** | All | N/A |
| | Exon 1 splice site | 1037-1038 | None |
| | Exon 1 donor site | 1143-1144 | None |
| | Exon 2 acceptor site | 5677-5678 | None |
| | Exon 2 donor site | 5943-5944 | DPB1*04:01:01:24N |
| | Exon 3 acceptor site | 9955-9956 | None |
| | Exon 3 donor site | 10239-10240 | None |
| | Exon 4 acceptor site | 10784-10785 | None |
| | Exon 4 donor site | 10897-10898 | DPB1*02:01:02:46Q |
| | Exon 5 acceptor site | 11224-11225 | None |
| | Exon 5 donor site | 11249-11250 | None |
| **DQA1** | Exon 1, 2, 3, **4** | All | N/A |
| | Exon 1 splice site | 1458-1459 | None |
| | Exon 1 donor site | 1542-1543 | None |
| | Exon 2 acceptor site | 5396-5397 | None |
| | Exon 2 donor site | 5647-5648 | None |
| | Exon 3 acceptor site | 6038-6039 | None |
| | Exon 3 donor site | 6322-6323 | None |
| | Exon 4 acceptor site | 6683-6684 | DQA1*03:03:01:16Q |
| | Exon 4 donor site | 6840-6841 | None |
| **DQB1** | Exon 1, 2, 3, 4 | All | N/A |
| | Exon 1 splice site | 689-690 | None |
| | Exon 1 donor site | 800-801 | None |
| | Exon 2 acceptor site | 2269-2270 | None |
| | Exon 2 donor site | 2541-2542 | None |
| | Exon 3 acceptor site | 5585-5586 | None |
| | Exon 3 donor site | 5869-5870 | DQB1*03:01:01:21N, DQB1*04:02:01:16Q |
| | Exon 4 acceptor site | 6384-6385 | DQB1*02:01:01:16Q |
| | Exon 4 donor site | 6497-6498 | None |
| | Exon 5 acceptor site | 6980-6981 | None |
| | Exon 5 donor site | 7006-7007 | None |
| | Exon 6 acceptor site | 7615-7616 | None |
| | Exon 6 donor site | 7631-7632 | None |
| **DRB1G01** | Exon 2, 3, **6** | All | N/A |
| | Exon 1 splice site | 1204-1205 | None |
| | Exon 1 donor site | 1306-1307 | None |
| | Exon 2 acceptor site | 6593-6594 | None |
| | Exon 2 donor site | 6865-6866 | None |

| Locus | Region | Position | Known Alleles with impacted expression |
|---|---|---|---|
| | Exon 3 acceptor site | 9148-9149 | None |
| | Exon 3 donor site | 9432-9433 | None |
| | Exon 4 acceptor site | 10131-10132 | None |
| | Exon 4 donor site | 10244-10245 | None |
| | Exon 5 acceptor site | 10729-10730 | None |
| | Exon 5 donor site | 10755-10756 | None |
| | Exon 6 acceptor site | 10897-10898 | None |
| | Exon 6 donor site | 11913-11914 | None |
| **DRB1G03** | Exon 2, 3, **6** | All | N/A |
| | Exon 1 acceptor site | 1199-1200 | None |
| | Exon 1 donor site | 1302-1303 | None |
| | Exon 2 acceptor site | 9305-9306 | None |
| | Exon 2 donor site | 9577-9578 | None |
| | Exon 3 acceptor site | 11834-11835 | None |
| | Exon 3 donor site | 12118-12119 | **DRB1*11:01:01:12Q** |
| | Exon 4 acceptor site | 12817-12818 | None |
| | Exon 4 donor site | 12930-12931 | None |
| | Exon 5 acceptor site | 13400-13401 | None |
| | Exon 5 donor site | 13426-13427 | None |
| | Exon 6 acceptor site | 14213-14214 | None |
| **DRB1G04** | Exon 2, 3, **6** | All | N/A |
| | Exon 1 acceptor site | 1199-1200 | None |
| | Exon 1 donor site | 1301-1302 | None |
| | Exon 2 acceptor site | 9554-9555 | None |
| | Exon 2 donor site | 9826-9827 | None |
| | Exon 3 acceptor site | 13288-13289 | None |
| | Exon 3 donor site | 13572-13573 | None |
| | Exon 4 acceptor site | 14267-14268 | None |
| | Exon 4 donor site | 14380-14381 | None |
| | Exon 5 acceptor site | 14850-14851 | None |
| | Exon 5 donor site | 14876-14877 | None |
| | Exon 6 acceptor site | 15502-15503 | None |
| **DRB1G07** | Exon 2, 3, **6** | All | N/A |
| | Exon 1 acceptor site | 1184-1185 | None |
| | Exon 1 donor site | 1287-1288 | None |
| | Exon 2 acceptor site | 11598-11599 | None |
| | Exon 2 donor site | 11870-11871 | None |
| | Exon 3 acceptor site | 14095-14096 | None |
| | Exon 3 donor site | 14379-14380 | None |
| | Exon 4 acceptor site | 15074-15075 | None |
| | Exon 4 donor site | 15187-15188 | None |
| | Exon 5 acceptor site | 15660-15661 | None |
| | Exon 5 donor site | 15686-15687 | None |
| | Exon 6 acceptor site | 16402-16403 | None |
| **DRB3** | Exon 2, 3 | All | N/A |
| | Exon 1 acceptor site | 1199-1200 | None |
| | Exon 1 donor site | 1301-1302 | None |
| | Exon 2 acceptor site | 8972-8973 | None |
| | Exon 2 donor site | 9244-9245 | None |
| | Exon 3 donor site | 11842-11843 | None |
| | Exon 3 donor site | 11842-11843 | None |
| | Exon 4 acceptor site | 12524-12525 | None |
| | Exon 4 donor site | 12637-12638 | None |
| | Exon 5 acceptor site | 13108-13109 | None |
| | Exon 5 donor site | 13134-13135 | None |
| | Exon 6 acceptor site | 13931-13932 | None |
| **DRB4** | Exon 1, 2, 3 | All | N/A |
| | Exon 1 acceptor site | 1232-1233 | none |
| | Exon 1 donor site | 1334-1335 | none |
| | Exon 2 acceptor site | 10896-10897 | DRB4*01:03:01:02N, DRB4*01:14N, DRB4*01:03:01:13N |
| | Exon 2 donor site | 11168-11169 | None |
| | Exon 3 acceptor site | 13909-13910 | None |
| | Exon 3 donor site | 14193-14194 | None |
| | Exon 4 acceptor site | 14895-14896 | None |
| | Exon 4 donor site | 15008-15009 | None |
| | Exon 5 acceptor site | 15480-15481 | None |
| | Exon 5 donor site | 15506-15507 | None |
| | Exon 6 acceptor site | 15806-15807 | None |
| **DRB5** | Exon 2, 3 | All | N/A |
| | Exon 1 acceptor site | 1275-1276 | None |

| Locus | Region | Position | Known Alleles with impacted expression |
|---|---|---|---|
| | Exon 1 donor site | 1377-1378 | None |
| | Exon 2 acceptor site | 9325-9326 | None |
| | Exon 2 donor site | 9597-9598 | None |
| | Exon 3 acceptor site | 11848-11849 | None |
| | Exon 3 donor site | 12132-12133 | None |
| | Exon 4 acceptor site | 12833-12834 | None |
| | Exon 4 donor site | 12946-12947 | None |
| | Exon 5 acceptor site | 13422-13423 | None |
| | Exon 5 donor site | 13448-13449 | None |
| | Exon 6 acceptor site | 13747-13748 | None |
| | Exon 6 donor site | 13763-13764 | None |
| **E** | Exon 1, 2, 3, 4 | All | N/A |
| | Exon 1 donor site | 1597-1598 | None |
| | Exon 2 acceptor site | 1725-1726 | None |
| | Exon 2 donor site | 1997-1998 | None |
| | Exon 3 acceptor site | 2239-2240 | None |
| | Exon 3 donor site | 2517-2518 | None |
| | Exon 4 acceptor site | 3136-3137 | None |
| | Exon 4 donor site | 3414-3415 | None |
| | Exon 5 acceptor site | 3536-3537 | None |
| | Exon 5 donor site | 3655-3656 | None |
| | Exon 6 acceptor site | 4403-4404 | None |
| | Exon 6 donor site | 4438-4439 | None |
| | Exon 7 acceptor site | 4540-4541 | None |
| | Exon 7 donor site | 4585-4586 | None |
| | Exon 8 acceptor site | 4748-4749 | None |
| **F** | Exon 2, 3, 4 | All | N/A |
| | Exon 1 donor site | 1529-1530 | None |
| | Exon 2 acceptor site | 1657-1658 | None |
| | Exon 2 donor site | 1929-1930 | None |
| | Exon 3 acceptor site | 2172-2173 | None |
| | Exon 3 donor site | 2450-2451 | None |
| | Exon 4 acceptor site | 3030-3031 | None |
| | Exon 4 donor site | 3308-3309 | None |
| | Exon 5 acceptor site | 3446-3447 | None |
| | Exon 5 donor site | 3565-3566 | None |
| | Exon 6 acceptor site | 4010-4011 | None |
| | Exon 6 donor site | 4045-4046 | None |
| | Exon 7 acceptor site | 4402-4403 | None |
| **G** | Exon 2, 3, 4 | All | N/A |
| | Exon 1 acceptor site | 1844-1845 | none |
| | Exon 1 donor site | 1919-1920 | none |
| | Exon 2 acceptor site | 2046-2047 | G*01:01:01:14Q |
| | Exon 2 donor site | 2318-2319 | none |
| | Exon 3 acceptor site | 2542-2543 | none |
| | Exon 3 donor site | 2820-2821 | none |
| | Exon 4 acceptor site | 3417-3418 | G*01:01:02:05Q |
| | Exon 4 donor site | 3695-3696 | none |
| | Exon 5 acceptor site | 3815-3816 | G*01:04:01:04Q |
| | Exon 5 donor site | 3934-3935 | None |
| | Exon 6 acceptor site | 4377-4378 | None |
| **H** | Exon 2, 3, 4 | All | N/A |
| | Exon 1 acceptor site | 670-671 | None |
| | Exon 1 donor site | 745-746 | None |
| | Exon 2 acceptor site | 867-868 | None |
| | Exon 2 donor site | 1139-1140 | None |
| | Exon 3 acceptor site | 1379-1380 | None |
| | Exon 3 donor site | 1657-1658 | None |
| | Exon 4 acceptor site | 2240-2241 | None |
| | Exon 4 donor site | 2517-2518 | None |
| | Exon 5 acceptor site | 2617-2618 | None |
| | Exon 5 donor site | 2736-3737 | None |
| | Exon 6 acceptor site | 3174-3175 | None |
| | Exon 6 donor site | 3209-3210 | None |
| | Exon 7 acceptor site | 3349-3350 | None |
| | Exon 7 donor site | 3570-3571 | None |
| **MICA** | Exon 2, 3, 4 | All | N/A |
| | Exon 1 donor site | 805-806 | None |
| | Exon 2 acceptor site | 7744-7745 | None |
| | Exon 2 donor site | 8001-8002 | None |

| Locus | Region | Position | Known Alleles with impacted expression |
|---|---|---|---|
| | Exon 3 acceptor site | 8273-8274 | None |
| | Exon 3 donor site | 8563-8564 | None |
| | Exon 4 acceptor site | 9148-9149 | None |
| | Exon 4 donor site | 9429-9430 | MICA*002:01:13Q |
| | Exon 5 acceptor site | 9527-9528 | None |
| | Exon 5 donor site | 9676-9677 | None |
| | Exon 6 acceptor site | 12224-12225 | None |
| **MICB** | Exon 2, 3, 4 | All | N/A |
| | Exon 1 donor site | 673-674 | None |
| | Exon 2 acceptor site | 8213-8214 | None |
| | Exon 2 donor site | 8470-8471 | MICA*011:01:04Q, MICA*171Q |
| | Exon 3 acceptor site | 8739-8740 | None |
| | Exon 3 donor site | 9029-9030 | None |
| | Exon 4 acceptor site | 9618-9619 | None |
| | Exon 4 donor site | 9899-9900 | None |
| | Exon 5 acceptor site | 9996-9997 | None |
| | Exon 5 donor site | 10130-10131 | None |
| | Exon 6 acceptor site | 12378-12379 | None |

**Table 2. Regions included in Core Analysis**

## 6. 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 |
|---|---|
| **A** | 5'UTR 1-564**,** 3'UTR 4068-5012 |
| **B** | 5'UTR 1-558, Intron 2 1525-1527, 1532, 3'UTR 3568-3591 |
| **C** | 5'UTR 1-429, 3'UTR 4294, 4750-5503 |
| **DPA1** | none |
| **DPB1** | none |
| **DQA1** | 5'UTR 1-507, 1090-1135, Intron 1 2656-4926, Intron 3 6434-6442, UTR 7215-9067 |
| **DQB1** | 5'UTR 1-159, Intron 1 1500-1954, Intron 2 2844-5344, UTR 7837-9867 |
| **DRB1G01** | 5'UTR 1-606, Intron 1 1389-6100, Intron 2 6947-8624, Intron 5 10803-11597, 3'UTR 12000-13004 |
| **DRB1G03** | 5'UTR 1-354, Intron 1 2003-8490, Intron 2 9656-11255, Intron 5 14073-14179, 3'UTR 14239-15315 |
| **DRB1G04** | 5'UTR 1-321, Intron 1 2077-8889, Intron 2 9907-12648, 3'UTR 15762-16609 |
| **DRB1G07** | 5'UTR 1-489, Intron 1 2055-10875, Intron 2 11924-13924, Intron 5 16003-16129, 3'UTR 16741-17509 |
| **DRB3** | 5'UTR 1-596, Intron 1 2140-8211, Intron 2 9310-9386, 10050-10929, Intron 3 12049-12050, 3'UTR 14449-15285 |
| **DRB4** | 5'UTR 1-552, Intron 1 2143-10115, Intron 2 11241-11274, 11705-13267, 3'UTR 16556-17569 |
| **DRB5** | 5'UTR 1-351, Intron 1 2175-8498, Intron 2 9678-9702, 10077-11334, 3'UTR 14648-15558 |
| **E** | 5'UTR 1-1232, Intron 5 3975-3976, 3'UTR 5505-8002 |
| **F** | 5'UTR 1-1164, 3'UTR 4561-4586, 4715-5140 |
| **G** | 5'UTR 1-1545, 31UTR 4684-6503 |
| **H** | 5'UTR 1-371, 3'UTR 3870-4798 |
| **MICA** | Intron 1 1642-7126, Intron 5 10391-11253 / non-coding regions excluded from analysis |
| **MICB** | Intron 1 820-1246, 1435-7555, Intron 5 11136-11422 / non-coding regions excluded from analysis |

**Table 3. Regions masked per locus**

## 7. Base Positions Excluded from The Phasing Calculation

The following base positions have been identified to potentially result in inappropriate phasing and have been excluded from the phasing calculation.

| Locus | Region |
|---|---|
| **HLA-A** | intron 3, positions 2123-2124 |
| **HLA-H** | exon 1, positions 723-728 |
| **DQB1** | intron 1, position 1463 |
| **HLA-C** | 5'UTR position 768-770 |
| **MICA** | Intron 4, position 9525 |

**Table 4. Regions excluded from the phasing calculation per locus**

## 8. Sequence Motifs Reported

For more details of the clinical significance of sequence motifs reports by AlloSeq Assign, refer to TEC747-S.

The following sequence motifs identified as biologically important are reported with IMGT HLA Reference 3.53.0.0:

| Motif | SNP/Details | Assign location, Region, codon | References |
|---|---|---|---|
| **Bw4/Bw6** | Refer to IFU | Refer to IFU | Gumperz, J., Litwin, V., Phillips, J., Lanier, L. and Parham, P. (1995). The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. Journal of Experimental Medicine, 181(3), pp.1133-114 |
| **DPB1** | rs9277534 | 12037, UTR | Effie W. Petersdorf. High HLA-DP Expression and Graft-versus-Host Disease. The Journal of New England Medicine. 2015 August.<br>Bianca Schöne. Predicting an HLA-DPB1 expression marker based on standard DPB1 genotyping: Linkage analysis of over 32,000 samples. Human Immunology. 2018. |
| **MICA 129Met/Val** | rs1051792 | 8403, Exon 3, c.129 | Antje Isernhagen. Impact of the MICA-129Met/Val Dimorphism on NKG2D-Mediated Biological Functions and Disease Risks. frontiers. 2016 Dec.<br>Fuerst D. et.al. Matching for the MICA-129 polymorphism is beneficial in unrelated hematopoietic stem cell transplantation. Blood. 2016 Dec 29;128(26):3169-3176. |
| **MICB 98Met/Ile** | rs3134900 | 8776, Exon 3, c.98 | Kanya Klumkrathok. Allelic MHC Class I Chain Related B (MICB) Molecules Affect the Binding to the Human Cytomegalovirus (HCMV) Unique Long 16 (UL16) Protein: Implications for Immune Surveillance. Journal of Microbiology. 2013.<br>Carapito R. et.al. Compatibility at amino acid position 98 of MICB reduces the incidence of graft-versus-host disease in conjunction with the CMV status. *Bone Marrow Transplant*. 2020 Jul;55(7):1367-1378. |
| **HLA-G +3196** | rs1610696 | 5043, UTR | Adamson.M.B. HLA-G +3196 polymorphism as a risk factor for cell mediated rejection following heart transplant. *Elsevier.* 2020 Jan09. |
| **HLA-B rs1050458C>T dimorphism** | rs1050458 | 853, Exon 1, c.-21 | Petersdorf.E.W. Role of HLA-B exon 1 in graft-versus-host disease after unrelated haemopoietic cell transplantation: a retrospective cohort study. The Lancet. 2020 Jan. |
| **HLA-G 3'UTR 14bp indel** | rs371194629 | 4808, UTR | La Nasa, G. The human leucocyte antigen-G 14-basepair polymorphism correlates with graft-versus-host disease in unrelated bone marrow transplantation for thalassaemia. BJH. 2007 May.<br>Chen, D.P. The association between genetic variants at 3'-UTR and 5'-URR of HLA-G gene and the clinical outcomes of patients with |

| Motif | SNP/Details | Assign location, Region, codon | References |
|---|---|---|---|
| | | | leukemia receiving hematopoietic stem cell transplantation. Frontiers Immunol. 2023 Feb.<br><br>Boukouaci, W. Association of HLA-G low expressor genotype with severe acute graft-versus-host disease after sibling bone marrow transplantation. Frontiers Immunol. 2011 Dec.<br><br>Zhan-Kui Jin. Impact of HLA-G 14-bp polymorphism on acute rejection and cytomegalovirus infection in kidney transplant recipients from north-western China. Transplant Immunology. 2012 Jun. |

**Table 5. Sequence Motifs**

## 9. 3.53.0 CIWD List Update

All alleles in the CIWD list supplied by the Immunogenetics Data Analysis Working Group, version 3.0.0, as well as DPA1 and DQA1 alleles from version 2.0.0 are included in the AlloSeq Assign CWD list. When CIWD alleles have been split into subgroups after the most recent CIWD list, all alleles in the subgroups are included in the AlloSeq Assign CIWD list.

## 10. Reference Compatibility

The IMGT HLA Reference 3.53.0.0 is compatible and validated with AlloSeq Assign v1.0.3 and v1.0.4 when used in conjunction with the AlloSeq Tx 17 or AlloSeq Tx 9 assay.

## 11. Reference Update Instructions

1. Open AlloSeq Assign software.
2. In the Settings menu, select **Tx17 or Tx9** as applicable**.**
3. In the System group, select **Update**.
4. In the Update window, under Import, select **References & CWD.**
5. Browse to the IMGT HLA Reference folder. In the bottom right of the dialogue window, 'Reference Files' is indicated. Select all the files in the folder and select **Open.**
6. Browse to the CWD folder. In the bottom right of the dialogue window, "CWD Files" is indicated. Select the CIWD files and select **Open**.
   The dialogue window indicates files successfully copied.
7. Select 'CIWD 2020 v3.53 cat 1' or 'CIWD 2020 v3.53 cat 1-2' as needed from the 'CWD Set' drop down list on the 'Annotation' panel.
8. Click 'Update' on the 'Settings' panel and choose 'Yes' to confirm the changes.

## 12. Assign Matching Validation

| **IMGT/HLA library version:** | **3.53.0** |
|---|---|
| **Tested using Assign version(s):** | 1.0.3.1337 |

**Notes:**

1. **Validation was performed by converting new fasta sequences available from IMGT 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. **Modified and corrected alleles are included in this validation.**
3. *** The new allele A*24:608N has a large deletion beginning in intron 3 g1001 that removes exons 4-7 thus it gives a "low coverage" result in Assign.**
4. ****The new allele DRB5*01:131N has a large deletion of 366bps from position g8034 in intron 1 to position g8399 intron 2 thus it gives a "low coverage" result in Assign.**

| Outputs: | |
|---|---|
| **HLA-A** | |
| **Number of New Alleles:** | 221 |
| **Number of New Alleles Successfully Imported:** | 221 |
| **Number of New alleles Successfully Reported:** | 220* |
| **HLA-B** | |
| **Number of New Alleles:** | 273 |
| **Number of New Alleles Successfully Imported:** | 273 |
| **Number of New alleles Successfully Reported:** | 273 |
| **HLA-C** | |
| **Number of New Alleles:** | 282 |
| **Number of New Alleles Successfully Imported:** | 282 |
| **Number of New alleles Successfully Reported:** | 282 |
| **HLA-DPA1** | |
| **Number of New Alleles:** | 80 |
| **Number of New Alleles Successfully Imported:** | 80 |
| **Number of New alleles Successfully Reported:** | 80 |
| **HLA-DPB1** | |
| **Number of New Alleles:** | 71 |
| **Number of New Alleles Successfully Imported:** | 71 |
| **Number of New alleles Successfully Reported:** | 71 |
| **HLA-DQA1** | |
| **Number of New Alleles:** | 109 |
| **Number of New Alleles Successfully Imported:** | 109 |
| **Number of New alleles Successfully Reported:** | 109 |
| **HLA-DQB1** | |
| **Number of New Alleles:** | 53 |
| **Number of New Alleles Successfully Imported:** | 53 |
| **Number of New alleles Successfully Reported:** | 53 |
| **HLA-DRB1** | |
| **Number of New Alleles:** | 71 |
| **Number of New Alleles Successfully Imported:** | 71 |
| **Number of New alleles Successfully Reported:** | 71 |
| **HLA-DRB3** | |
| **Number of New Alleles:** | 16 |
| **Number of New Alleles Successfully Imported:** | 16 |
| **Number of New alleles Successfully Reported:** | 16 |
| **HLA-DRB4** | |
| **Number of New Alleles:** | 10 |
| **Number of New Alleles Successfully Imported:** | 10 |
| **Number of New alleles Successfully Reported:** | 10 |
| **HLA-DRB5** | |
| **Number of New Alleles:** | 7 |
| **Number of New Alleles Successfully Imported:** | 7 |
| **Number of New alleles Successfully Reported:** | 6** |
| **HLA-E** | |
| **Number of New Alleles:** | 1 |
| **Number of New Alleles Successfully Imported:** | 1 |
| **Number of New alleles Successfully Reported:** | 1 |
| **HLA-F** | |
| **Number of New Alleles:** | 1 |
| **Number of New Alleles Successfully Imported:** | 1 |
| **Number of New alleles Successfully Reported:** | 1 |
| **HLA-G** | |
| **Number of New Alleles:** | 4 |
| **Number of New Alleles Successfully Imported:** | 4 |
| **Number of New alleles Successfully Reported:** | 4 |

| | |
|---|---|
| **HLA-H** | |
| **Number of New Alleles:** | 4 |
| **Number of New Alleles Successfully Imported:** | 4 |
| **Number of New alleles Successfully Reported:** | 4 |
| **MICA** | |
| **Number of New Alleles:** | 1 |
| **Number of New Alleles Successfully Imported:** | 1 |
| **Number of New alleles Successfully Reported:** | 1 |
| **MICB** | |
| **Number of New Alleles:** | 0 |
| **Number of New Alleles Successfully Imported:** | 0 |
| **Number of New alleles Successfully Reported:** | 0 |

**Table 5: Validation Data for the Assign Matching Algorithm**

## 13.NMDP Nulls

Homozygous fastq sequences were imported into AlloSeq Assign v1.0.3.1337 with the 3.53.0.0 references for null alleles requiring exclusion from the G-group as indicated in the NMDP Policy for HLA Confirmatory Typing Requirements for Unrelated Adult Donors and HLA Typing Requirement for Patients - CIWD Null Allele Exclusion Requirement Table. No allele ambiguities were identified.

| HLA-A | HLA-B | HLA-C | HLA-DRB345 |
|---|---|---|---|
| A*01:04N | B*15:01:01:02N | C*04:09N | DRB4*01:03:01:02N |
| A*02:83N | B*40:155N | | DRB5*01:08N |
| A*03:21N | B*51:11N | | |
| A*11:21N | | | |
| A*24:09N | | | |
| A*24:11N | | | |
| A*31:14N | | | |

**Table 6. CIWD null alleles requiring exclusion from the G-Group.**

Note that due to limitations within the software it is not possible to exclude DRB4*03:01N/DRB4*01:01:01 when the sample is homozygous.

## 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/

**Legal Manufacturer:**
CareDx Pty Ltd,
20 Collie Street,
Fremantle, WA 6160,
Australia

CareDx Pty Ltd

## Revision History

| Version | Date | Modification | Reference/Justification |
|---|---|---|---|
| 1.0 | 02-Oct-23 | Updated to include changes made in 3.53.0.0. | N/A |