Tutorial (November 2019)

HLAMatchmaker ABC antibody analysis (Version 3.0)

Introduction

HLAMatchmaker is an algorithm to predict HLA epitopes by molecular structural modeling and amino acid sequence comparisons between HLA alleles. It considers each HLA allele as a series of small configurations of polymorphic residues referred to as eplets as essential components of HLA epitopes. The website-based International Registry of HLA Epitopes ([http://www.epregistry.com.br](http://www.epregistry.ufpi.br)) describes the repertoires of HLA-ABC, -DRDQDP and -MICA eplets. The registry shows records which eplets correspond to actual epitopes experimentally verified by HLA antibodies. All of them correspond to eplets and there are two patterns. First, a specific antibody reacts with all alleles carrying a given eplet whereas the remaining alleles in the panel are non-reactive. In these cases, an eplet describes the epitope specifically recognized by antibody. Second, an epitope is defined by the combination of an eplet and a critical residue configuration within 15 Angstrom radius and shared between all antibody-reactive alleles.

Version 3.0 of HLAMatchmaker ABC antibody analysis incorporates all antibody verified eplets recorded in the Registry as of November 1, 2019. The remaining eplets have been classified into two groups: eplets with high ElliPro scores and eplets with low ElliPro scores. With the ElliPro epitope predictor program we have found that antibody verified eplets have significantly higher ElliPro scores than eplets that have not been verified experimentally (see Duquesnoy and Marrari: Usefulness of the ElliPro Epitope Predictor Program in Defining the Repertoire of HLA-ABC Eplets. Human Immunology 78:481-488, 2017). We believe that a low ElliPro score <0.250 is associated with low immunogenicity.

Version 3.0 has been designed to analyze HLA antibody reactivity patterns with single allele panels. This tutorial offers stepwise instructions how to identify mismatched immunizer-specific and third-party eplets shared between reactive alleles and identify alleles that are unacceptable mismatches. This program has 60 MB and a smaller version (2.4MB) without determinations of allele mismatch acceptability is also available.

We recommend keeping master copies of this HLAMatchmaker program on your computer and creating working copies to do actual analyses.

This tutorial is accompanied by an Excel document (ABC Analysis Version 3.0 demo) with11 sheets to show how to perform the antibody analysis.

Sheet 1 Panel

This sheet has five columns to describe the composition of the single allele kit. There are 120 rows to enter the panel information. There is information about the kit, lot numbers, bead number, HLA locus and alleles. Version 3.0 has some One Lambda (ThermoFischer) and LifeCodes (Immucor) kits. New lots can be added to the OL and LC sheets but ascertain that the alleles are recorded without any space in front of them.

Please note: Many sheets have cells with formulas important for the calculations; NEVER touch these cells (these sheets are not protected. You can only record on the “Enter” sheet the following information : Panel description and MFI values, the HLA types of antibody producer and Immunizer and the MFI cut-off value.

Sheet 2 Enter

Copy the Panel (rows 2-101, columns A-E) and paste on rows 12-111, column B-F on Enter sheet of the HLAMm program. Columns L, M and N show the antibody-verified eplets, the high ElliPro eplets and the low ElliPro eplets. The eplets are listed sequentially, with no breaks between the names. We must of course know which ones are mismatched for the antibody producer.

Sheet 3 HLAinfo

This sheet has four examples of Luminex data with class I antibodies. All of them have HLA types of antibody producer and immunizer and the cPRA values are very high. Let’s select case #217. For training purposes, you can try out the other cases another time.

Sheet 4EnterpHLA

After recording the HLA type of the antibody producer on the “Enter” sheet, the program automatically determines which eplets in columns L, M and N are mismatched. Note that the number of eplets in each column is reduced. Now enter the MFI values for the Luminex panel.

Please note you must record HLA types at the 4-digit high-resolution level and you can only use alleles that are listed in the “Ep” sheet (N=1893)

Sheet 5 MFIcsv

The easiest way for entering the MFI values is to go to the csv files of the Luminex software programs (you may need some instructions from the manufacturer). Row 22 of this sheet has the trimmed mean values for case #217. Copy the horizontally located values and use the paste-special-transpose command (the shortcut click: alt E, S, E) to enter the MFI values in column J starting on row 12 of the Enter sheet.

Sheet 6 EnterMFI

The next step is the determinations of the cut-off MFI value must for positive and negative reactions. Column G shows the MFI values of self-alleles of the antibody producer; cell G10 shows the mean MFI value. Any other allele in the SAB panel with a MFI with more than three standard deviations above the mean value (cell G8) can statistically be considered as being significantly higher.

You can see in column G that each allele of the antibody producer of Case #217 had an extremely low MFI value. For this analysis and considering that certain negative SAB have somewhat higher MFI values, we chose in cell J10 a cut-off value of 100.

Note you can enter any MFI value as your cut-off and this will change the eplet compositions of reactive alleles.

Sheet 7 Enter Cut-off

Column I has “NEG” annotations for alleles with MFI values below the cut-off value. HLAMatchmaker removes all eplets on the negative alleles and the reactive alleles show only the remaining eplets. You will note fewer eplets in columns L, M and N.

Sheet 8 Enter ImHLA

We now raise the question which eplets are specifically recognized by serum antibodies induced by the immunizer. Information about the immunizing event will offer an answer. This was a post-pregnancy serum and the immunizing paternal haplotype of the child is recorded on Row 5 of the “Entry” sheet.

The “Imm” notations in Column H will provide information about the MFI values of immunizer alleles in the SAB panel. A\*03:01 and B\*07:02 showed high MFI values but C\*07:02 was non-reactive. Now proceed to the protected “Sort Ep” sheet on which you can never enter any data.

Sheet 9 SortBefore

The “Sort Ep” sheet has Columns K, L and M for the immunizer-specific eplets and columns N, O and P have the third-party (TP) eplets. Column Q shows antibody-verified epitopes defined by eplets paired with other residues.

This analysis should focus on antibody-verified eplets, especially immunizer-specific eplets but you might also consider third-party eplets participating in another immunization event. You may see that most reactive alleles have one or few antibody-verified eplets but you have to scroll up and down to see which ones are involved. A dedicated sort command can be used for better visualization of the data.

Highlight Rows 12-111 and under the Data tab click on the Sort Command which uses a certain sequence of sort levels between the columns (A followed by K, L, M, N, O and P).

Sheet 10 SortAfter

You can examine all sorted data or you can filter by HLA locus (HLA-A, HLA-B, HLA-C) with the filter command in cell D12. Let us select HLA-A.

The sorted data for this case demonstrate that the antibodies react with a limited repertoire of immunizer eplets. The immunizing A\*03:01 shows an MFI of 12869 it antibody-verified eplets are 62QE, 79GT and 161D. There lots of reactive alleles with only 79GT. Other reactive alleles have both 62QE and 79GT. A\*32:01 which has only 62QE is weakly reactive (MFI=411) and we can rule out 62QE as an antibody-reactive eplet. The MFI values of 79GT-carrying alleles are all lower than the MFI value with A\*03:01 which has also 161D an eplet unique to HLA-A3 alleles. These findings suggest the presence of antibodies specific for 79GT and 161D.

Now select HLA-B. The immunizing B\*07:02 has the antibody-verified 65QIA, 70IAQ, 163EW and 180E. These eplets are on reactive alleles; B\*08:01 and B\*41:01 are informative for 180E and you can identify five HLA-B alleles that are just mismatched for 163EW. Several weakly reacting alleles share the non-verified 45EE with the immunizing B\*07:02. You will also note that a group of HLA-B alleles with very low MFI values share a third-party 44RT eplet. Although the clinical significance of low MFI values might be considered questionable it is interesting to note that some of them may reflect weakly reacting epitope-specific antibodies.

Now select HLA-C. You will see that the 163EW carrying C\*02:02 and C\*17:01 have very low MFI values suggesting that strong antibody reactivity with the 163EW-defined eplet depends on another amino acid configuration present on HLA-B but absent on HLA-C. Columns T-DQ can suggest the involvement of residue sequence position 66 which is within 15 Angstroms of 163EW. The two HLA-C alleles have 66K, but the HLA-B alleles have 66I thereby suggesting that the epitope may correspond to 163EW+66I. Please note the high MFI value (10509) of the 163EW-carrying A\*66:02 suggests sharing the same configuration (the 163EW-negative A\*66:01 has a much lower MFI value).

This serum reactivity pattern can be completely explained with antibody-verified eplets. A major purpose of HLAMatchmaker program is the determination mismatch acceptability of donor alleles including those not in the SAB panel.

Sheet 11 AccMm

After you have identified the eplets recognized by patient’s antibodies you can proceed to determine which alleles are unacceptable or acceptable mismatches. The program’s “Acc Mm” sheet shows eplet information for 1894 class I alleles. Columns C-I show the numbers and descriptions of antibody verified eplets and high and low ElliPro eplets. Columns J-EA show the individual eplets. Row 5 has a filter command to select alleles in the different HLA loci. Let’s select HLA-A for which two reactive antibody-verified 79GT and 161D were identified on the immunizing A\*03:01. Select these eplets in columns S and AD to determine which alleles are unacceptable mismatches. Alternatively, you can select the blanks and other eplets in these columns to identify the acceptable mismatches.

These selections need also be made for alleles that share the reactive eplets 65QIA, 163EW and 180E with the immunizing B\*07:02 (Columns O, AF and AE) You recall that this 163EW-defined epitope requires the presence of the critical residue 66I. You can identify the 163EW+66I carrying alleles as unacceptable mismatches by selecting 163EW in column AF followed by selecting 66I in column FF

The determination of mismatch acceptability depends on the cut-off MFI value you have chosen. Enter a different the cut-off value and you will see how the unacceptable and acceptable allele repertoires have changed.

Summary

Now try out the analysis of the other three cases listed in this demo. Let us know if you have any questions and suggestions.