**Tutorial (June 2016)**

**HLAMatchmaker DRDQDP antibody analysis (Version 02)**

**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. An important question is which eplets correspond to actual epitopes specifically recognized by HLA antibodies.

Recent publications describe antibody-verified epitopes recorded so far in the HLA Epitope Registry. 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 another polymorphic residue configuration (eplet) uniquely shared between all antibody-reactive alleles. Such epitopes are described by so-called eplet pairs. The repertoires of antibody-verified epitopes must be considered incomplete.

This new Excel program has been designed to analyze HLA antibody reactivity patterns with single allele panels. Reactive alleles carry epitopes which are studied in two steps. First, we determine the presence of antibody-verified epitopes (eplets and eplet pairs) that are mismatched for the antibody producer. Second, we determine if reactive alleles in the panel have other epitopes which have not (yet) been antibody-verified in the HLA Epitope Registry. The updated antibody analysis version has features that permit a quick assessment of epitope specificities and the interpretation of allele mismatch acceptability.

We recommend that you study first the tutorial for the ABC antibody analysis before proceeding with the DRDQDP tutorial.

It applies the same approach as the epitope analysis of HLA-ABC antibodies. This Excel file has 8 sheets that show epitope analysis results for antibodies reacting with three groups of class II alleles: DRB1/3/4/5, DQA/DQB and DPA/DPB.

Panel

This sheet shows the composition of a panel of DRB alleles, DQA-DQB heterodimers and DPA-DPB heterodimers. Each allele has a list of epitopes classified as antibody-verified eplets, antibody-verified pairs and other eplets. The sheet has 250 columns and it should be noted that no antibody-verified pairs are listed for DP and DQ; they will be included after reviewing studies providing experimental evidence for such epitopes.

Enter

This sheet shows the panel, the HLA types of antibody producer and the MFI values that have been entered. The program calculates the mean MFI values for self DRB1/3/4/5, DQB and DQA and the mean+3SD can be used to establish the cut-off MFI value. The MFI value for self DRB is probably the best choice. Some DQ heterodimers may give a high MFI although one of the DQ chains is self (see prep 36: DQB1\*06:01 complex with self DQA1\*01:03, row 53). For this case the cut-off values was determined at 700 and the each reactive allele shows a list of epitopes that are mismatched for the antibody producer. This sheet has lots of information but the program has three sheets that distinguish immunizer-specific and third-party epitopes in each class II group: DRB1/3/4/5, DQB-DQA and DPB-DPA.

SortDR, SortDQ, SortDP

These sheets show reactive alleles sorted by immunizer-specific and third-party epitopes in the order of antibody-verified eplets, antibody-verified pairs (no data) and “other” eplets recorded in the Epitope Registry. These data provide information that permit the determination of mismatch acceptability of class II alleles.

AcMmDR, AcMmDQ, AcMmDP

These sheets show the epitope mismatch information on class II alleles including many that are not in single allele panels. Columns B-E list the immunizer-specific alleles whereas columns F-I list all epitopes shared with reactive alleles in the panel. There is no information about antibody-verified pairs. Row 4 has a filter command to select alleles with certain numbers of mismatched epitopes.

**Please Note:**

The new DRDQDP antibody analysis version has additional features aimed to identify antibodies against new epitopes defined by amino acid residues. The Manual of the programs provides detailed instructions.