**Tutorial (November 2019)**

**HLAMatchmaker DRDQDP antibody analysis (Version 3.0)**

**Introduction**

**Please note: We recommend that you first become familiar with the tutorial for the ABC antibody analysis before proceeding with the DRDQDP tutorial.**

**This new version includes all antibody-verified class II eplets listed in the HLA Epitope Registry ((**[**http://www.epregistry.com.br**](http://www.epregistry.ufpi.br)**) as of November 1, 2019. The non-verified eplets have been annotated using ElliPro scores as possible predictors of immunogenicity: eplets with >0.250 ElliPro scores have upper case letter descriptions and eplets with lower ElliPro scores are displayed with lower case letters. Version 3.0 also incorporates so-called interlocus class II eplets. Many studies have demonstrated the eplet effect for the individual class II loci, but until now little attention has been given to so-called interlocus class II eplets shared between HLA-DR, HLA-DQ and/or HLA-DP alleles. We have recently written a report that summarizes current information about antibody-verified interlocus class II eplets. We have also developed a structural modeling method to determine potentially immunogenic interlocus class II eplets and to identify those that may be non-immunogenic because they are monomorphic at another class II locus. We believe that the inclusion of interlocus class II eplet comparisons will enhance the efficiency of eplet-based HLA-DR,-DQ,-DP matching.**

**The interlocus report was not accepted by *Human Immunology* because the reviewers felt there was insufficient experimental evidence and they were skeptical about our structural model hypothesis. We have uploaded a copy of this manuscript to the list of publications on the HLAMatchmaker website (See Publication #66). The EpB and EpA sheets of the DRDQDP antibody analysis program each have a new category for interlocus class II eplets; their annotations have r, q and/or p to indicate which loci are involved.**

**How to use the DRDQDP analysis demo**

**Open the Version 3.0 DRDQDP analysis DEMO Excel file (0.6 MB). The sheet Raw data shows two cases with HLA information and MFI data with the SAB panel that will need to be copied and pasted. Next open the Version 3.0 DRDQDP analysis program (20 MB) and click on the Enter sheet.**

**Sheet 1 Enter Panel**

**Copy the panel information for Case 1, starting in row 3 of columns F-J in the Raw data sheet of the demo and paste it into columns A-E and starting on row 18 of the Enter sheet of the DRDQDP analysis program. Columns M-R show for each allele in the panel the antibody-verified eplets (VerEp), the Interlocus eplets and the Other, non-verified eplets (those with low ElliPro scores named with lower case letters). For DQ and DP beads, separate lists of eplets appear for B locus and A locus alleles that make up each heterodimer.**

**Sheet 2 Enter Patient HLA and MFI values**

**Copy the HLA type information of the antibody producer in rows 3-5, columns A-D of the Raw data sheet and paste it into the fields for the Patient HLA type (row 3, columns D-K for DR and DQ types; row 8, columns D-G for DP info). Carefully check that the HLA alleles have been entered in the correct locations, as you will not see an Error message if an incorrect locus or allele has been entered. For any unknown alleles (“blanks”), make sure “x” has been entered in the appropriate field.**

**Panel information for specific assay lots can be added to the “OL kits” and “LC kits” sheets in the program. Enter the information as shown for the examples in the sheets, listing the single allele beads in the same order as they appear in the Luminex .csv report. These panels can then be copied and pasted into the Enter sheet for subsequent analyses using the same lots.**

**Copy the MFI values from column L, starting in row 3 of the Raw data sheet and paste them in the Enter sheet of the program, starting in row 18 of column J. Check the sheet in the demo if you have pasted in the correct locations. You will see that the eplets that appeared previously are now absent, and all beads are labeled as Neg in column J.**

**Cells F16, F15 and F14 show the mean MFI values (Average Self count), standard deviation (sd) and the mean + 3 standard deviations (m+3sd), respectively, for the patient’s B locus alleles. This information may guide the selection of a MFI cutoff value. Please note: In some cases, the MFI values with self DP and DQ alleles are higher than expected because there are antibodies reacting with a non-self allele on the alpha chain of the heterodimer. For case 1, this increases the Average Self count value to 2478 which cannot be used to determine the cutoff value. By looking over the MFI values of self alleles and the entire panel you can use your own judgement in determining a cut-off value.**

**An MFI cut-off of 300 might be a reasonable estimate for Case 1 and entering this value in cell J16 will change the appearance of the sheet. Column I will have NEG description and eplets in the corresponding rows have disappeared for beads with MFI values <300. The reactive beads will now have different eplet descriptions as shown in columns L-Q, after the removal of eplets shared with low-MFI alleles.**

**Sheet 3 Enter Im HLA**

**Copy the Immunizer HLA information from rows 8-10 of the Raw data sheet and paste into the appropriate cells on the Enter sheet. Check to make sure the correct information is displayed for each locus. Immunizer alleles are labeled “IM” in columns G and H.**

**Sheet 4 before sort**

**The Sort sheet shows the same information as the Enter sheet, but now displays the distribution of immunizer-specific eplets (VerEp, Interlocus and Other in columns K-P) and Third-party(non-immunizer) eplets (columns Q-V). The next step is to sort the eplets on reactive alleles.**

**Sheet 5 after sort**

**The program includes a custom function that sorts the beads by the eplets in column K followed by those in columns L,M, N, O, P, Q, R, S, T, U and V. The demo sheet shows the results of the sort for DR, DQ and DP in rows 19-136.**

**Click on cell B18 to select results for individual locus. Selecting the DP locus shows that alleles with positive MFI values share one antibody-verified immunizer-specific eplet, 56A. Select DQ and you will see that the high MFI values are associated with two immunizer-specific antibody-verified eplets: 45GV on DQB and 40E on DQA. The 1448 MFI value cannot be explained. Selecting DR shows several immunizer-specific, antibody-verified eplets, including 98E, 70R, 4Q, and 13fe, but additional reactivity (see 13364 and 14405 in column I) suggests reactivity with “other” immunizer-specific eplets such as 31I and 98KN or even possibly reactivity with antibody-verified Third Party eplet 108T.**

**To show all alleles, simply click on cell B18 and check (Select All).**

**Altogether, this analysis reveals DP, DQ and DR eplets associated with the reactivity of this serum, but we cannot rule out that reactive panel alleles have also other eplets responsible for antibody reactivity. Absorption-elution studies with informative alleles may offer more definitive answers. The next two sheets are designed to determine eplet-based mismatch acceptability.**

**Please note: You may wish to do another analysis with a different MFI cutoff value. See how the eplet-specific antibody reactivity pattern will change.**

**Sheet 6 Unacc B**

**This sheet has 667 class II beta chain alleles to identify unacceptable mismatches; they can be selected by the individual loci by using the Filter in cell A3. Select DPB. As described in the previous section, the 56A eplet corresponded with a positive MFI. Identifying the column that shows this eplet (column P) and using the filter in cell P3 to select 56A will identify DPB alleles that share this eplet and may be considered as unacceptable DP mismatches. Selecting any other option in cell P3 (in this case “Blanks”), will identify DP alleles that could be considered as acceptable mismatches. Next, select DQB in cell A3 and determine mismatch acceptability for 45GV in column G. You can also determine mismatch acceptability for a combination of eplets. For instance, DRB eplets 98E, 70R, 4Q and 13fe may be considered as unacceptable mismatches. After selecting DRB in cell A3, apply filters in the respective columns for these eplets (W, Q, E and F, respectively). Uncheck the reactive eplets and this will result in a list of alleles without reactive eplets; they might be considered acceptable mismatches.**

**Changing the MFI cut-off in cell J16 on the Enter sheet will lead to a different list of reactive eplets and this will change the determination about mismatch allele acceptability. Try different cut-off values and see what happens.**

**After you finish, make sure to clear to all filters by checking the Select All box before you start a new search.**

**Sheet 7 Unacc A**

**This sheet has 34 DPA and DQA alleles. For Case 1, 40E was the only DQA eplet associated with serum reactivity, and using the filter in column F can readily identify DQA mismatches that are unacceptable and acceptable based on their sharing of eplet 40E.**

**Try another case**

**The Raw Data sheet has another example, Case 2, that can be used for antibody analysis. In addition to locus-specific reactivity, this serum has antibodies associated with an interlocus class II eplet present on an allele of the immunizing donor. There were no other immunizing events. Can you identify what DRB alleles have become unacceptable mismatches although the antibody producer has never been exposed to these alleles? Do the reactive alleles share an interlocus eplet?**

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