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MPAPASS - Creating an MPAPASS database

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bjnxkmfn

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ABSTRACT

This is collection contains the protocols required for each step in the mpapass software pipeline for performing stitched multiplex analysis. This is one of a number of protocols in the pipeline for using the mpapass software package and is applicable to the latest release of the software.

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PROTOCOL CITATION

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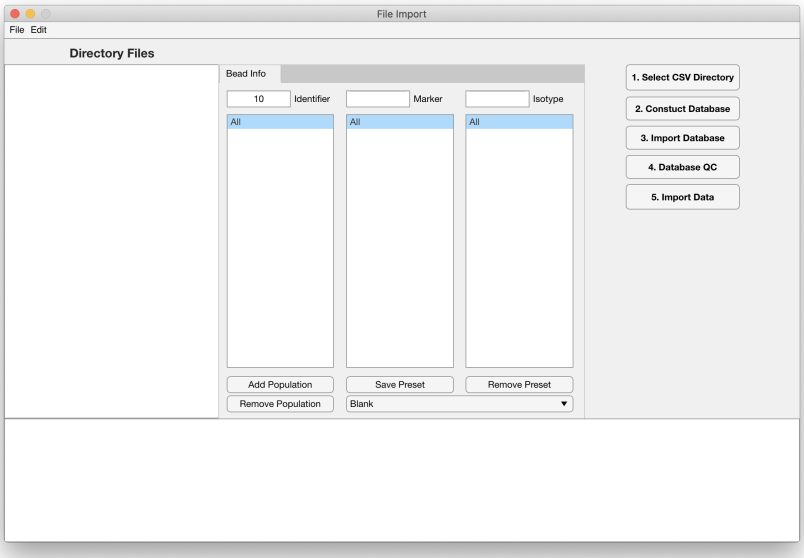
DISCLAIMER:

This protocol summarizes key steps for a specific type of method, which is one of a collection of methods and assays used for EV analysis in the NCI Translational Nanobiology Section at the time of submission of this protocol. Appropriate use of this protocol requires careful, cohesive integration with other methods for EV production, isolation, and characterization.

Open New Dataset Window

1 Open the MPAPASS software and navigate to the Menu tab in the upper left-hand corner. Under the Menu tab, choose the New Dataset option and a new window will pop-up as shown below:



Select CSV Directory

2 To construct the database, follow the steps outlined on the right side of the window.

First, choose a CSV Directory by clicking on the 'Select CSV Directory' button. Simply choose the folder that contains all the desired .csv files for data analysis.

The .csv files in the selected folder should now appear in the 'Directory Files' listbox on the left. Additionally, the command window should display a 'Directory Selected' message.

Bead Info

3 While the 'Bead Info' can be constructed as desired by using the Identifier, Marker, and Isotype textbox, the MACSPlex Human EV dataset has been automatically saved as a preset and is recommended for use with the MACSPlex Human Exosome Kit.

For constructing 'Bead Info', plug the Identifier, Marker, and Isotype into their respective boxes and then click on the 'Add Population' button. To remove a population, highlight the population and press the 'Remove Population' button. To save the new 'Bead Info' for further use, click on the 'Save Preset' button and choose a desired name.

Construct the Database

4 With the choice of desired 'Bead Info', it is now possible to construct the database. Using the MACSPlex Human EV preset, the screen should be similar to the one shown below.

Click on 'Construct Database' on the right and name the .xlsx file.

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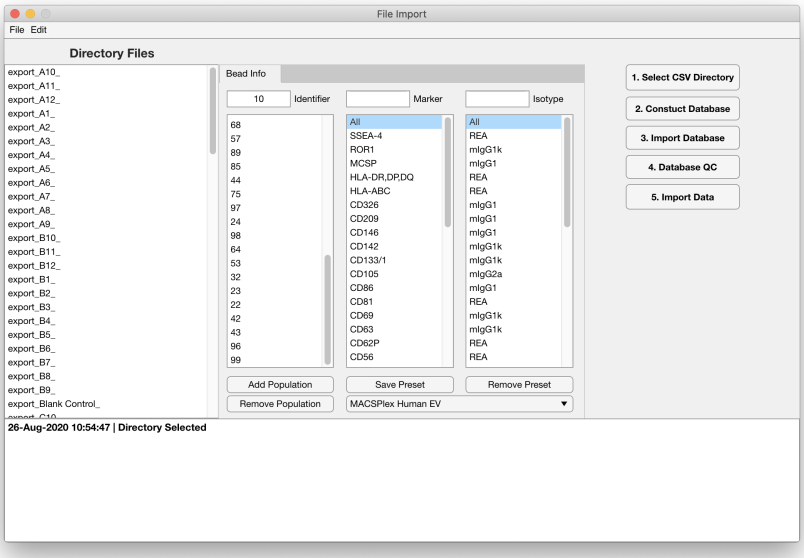
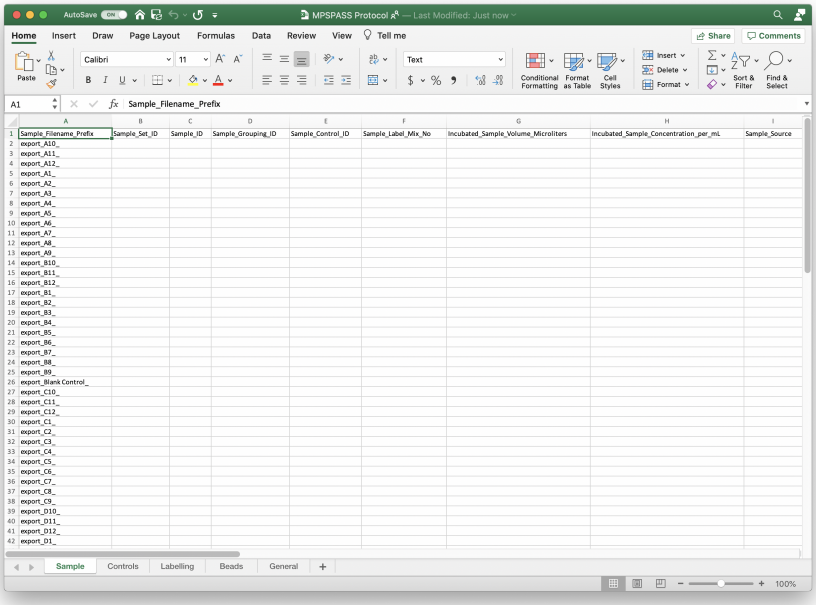


5 Outside of the MPAPASS software, navigate to the newly created .xlsx file and open it in Microsoft Excel. The file should be similar to the one shown below. Note the five worksheet tabs at the bottom of the file.

In this protocol, we will only be competing the bare minimum necessary for the database to be imported.



6 **Sample Worksheet Headings:**

- The first column labeled 'Sample_Filename_Prefix' gives the filename of the exported .csv files. Do not change this column unless the filename of the exported .csv file is changed.
- The second column labeled 'Sample_Set_ID' allows for grouping of files, usually by cell line and concentration.
- The third column labeled 'Sample_ID' allows for the naming of the samples. Filling this out by cell line and

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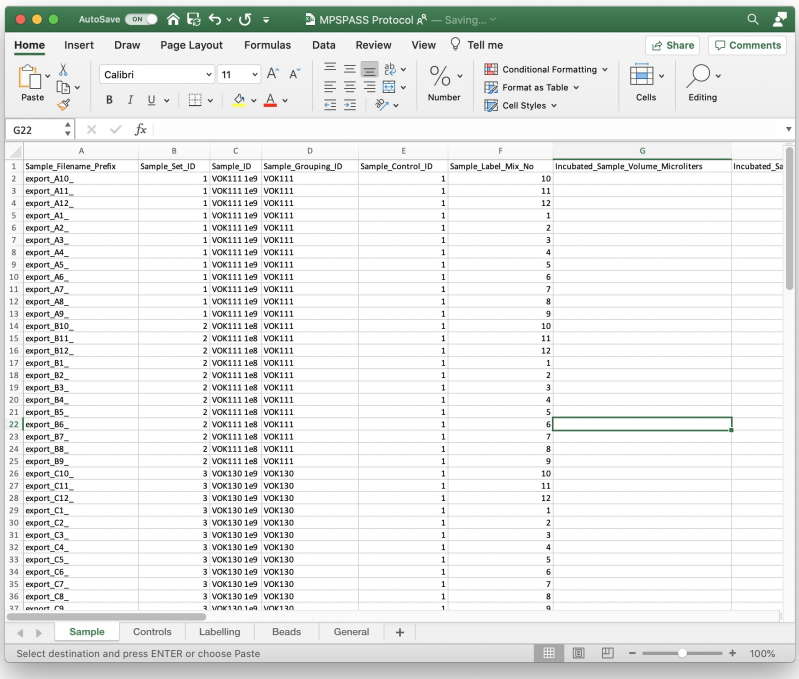
concentration is encouraged as it makes data interpretation easier for the user. Any string of text is allowed. Note that the Sample ID corresponds to the Sample Set ID, thus **samples with the same Sample Set ID cannot have different Sample IDs.**

- The fourth column labeled 'Sample_Grouping_ID' allows for further classification of the sample is is related to the 'Sample_Set_ID'. Thus, in this example the Sample Set IDs of 1 and 2 refer to the Sample Grouping ID of VOK111. Similar to Sample ID, **samples with the same Sample Set ID cannot have different Sample Grouping IDs.** Although this column is recommended for understanding of the data analysis, if you do not want to use it, simply fill it out with ones.

- The fifth column labeled 'Sample_Control_ID' tells the software which file to use as a control for the sample. This is linked with the 'Controls' worksheet and will be discussed later.

- The sixth column labeled 'Sample_Label_Mix_No' tells the software what label mix was used. This is linked with the 'Labelling' worksheet and will be discussed later. **There should be no repeated Label Mix No's within a Sample Set ID as this would indicate a duplicate.**

- The other columns can be filled out as desired, but are not critical for the data analysis.



7 Controls Worksheet Headings:

- The first column labeled 'Control_Filename_Prefix' gives the filename of the exported control .csv file. Find the desired control .csv file and write the filename prefix in this column. Note that the control .csv file will also be somewhere in the 'Sample_Filename_Prefix' column in the Sample worksheet, so copying and pasting that filename may be easier.

- The second column labeled 'Sample_Control_ID' is related to the same column in the Sample worksheet. In the Controls worksheet, this column assigns a number to the control. In the Sample worksheet, this corresponding number in the 'Sample_Control_ID' column assigns the control .csv file to the sample .csv file. In this example, there is only one control .csv file which is why you see only one unique 'Sample_Control_ID'.

- The third column labeled 'Control_Name' assigns a name to the control .csv file.

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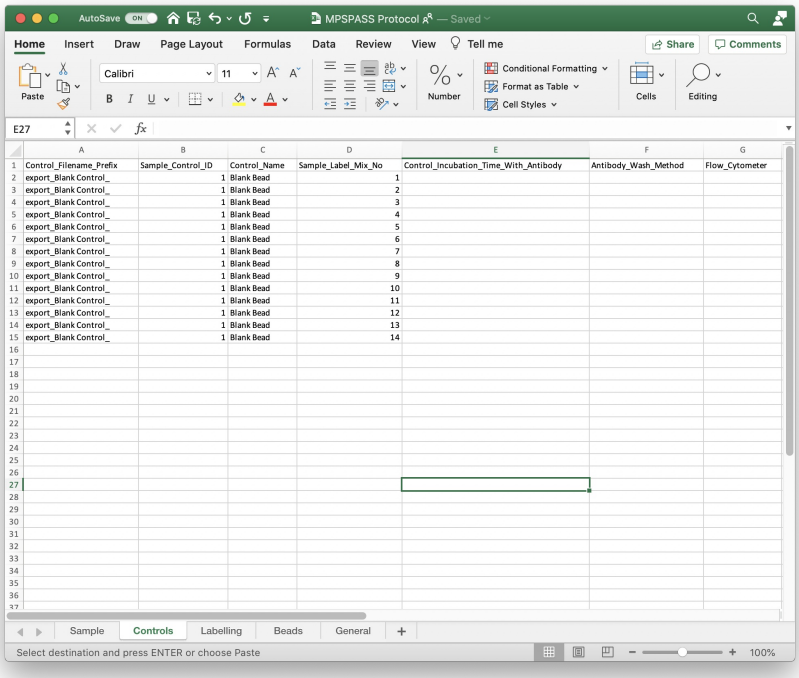
- The fourth column labeled 'Sample_Label_Mix_No' refers to the same information as the same column in the Sample worksheet. **There should be a control for every Label Mix No** which is why there are 14 rows that refer to the same control .csv file in this example.

- The other columns can be filled out as desired, but are not critical for the data analysis.



	A	B	C	D	E	F	G
1	Control_Filename_Prefix	Sample_Control_ID	Control_Name	Sample_Label_Mix_No	Control_Incubation_Time_With_Antibody	Antibody_Wash_Method	Flow_Cytometer
2	export_Blank Control_	1	Blank Bead	1			
3	export_Blank Control_	1	Blank Bead	2			
4	export_Blank Control_	1	Blank Bead	3			
5	export_Blank Control_	1	Blank Bead	4			
6	export_Blank Control_	1	Blank Bead	5			
7	export_Blank Control_	1	Blank Bead	6			
8	export_Blank Control_	1	Blank Bead	7			
9	export_Blank Control_	1	Blank Bead	8			
10	export_Blank Control_	1	Blank Bead	9			
11	export_Blank Control_	1	Blank Bead	10			
12	export_Blank Control_	1	Blank Bead	11			
13	export_Blank Control_	1	Blank Bead	12			
14	export_Blank Control_	1	Blank Bead	13			
15	export_Blank Control_	1	Blank Bead	14			

8 Labelling Worksheet Headings:

- The first column labeled 'Mix_Number' assigns an ID to the different mixes used in the experiment. This ID is used to determine the label mix in both the Sample worksheet and Controls worksheet under the 'Sample_Label_Mix_No' column.

- The second column labeled 'Import_Column_Number' refers to column of the gated and exported .csv data files that will be imported and used for data analysis. **Ideally, only one channel will be exported from FlowJo and this column can then always be filled with ones.**

- The third column labeled 'Label_Target' assigns a target protein to each label mix.

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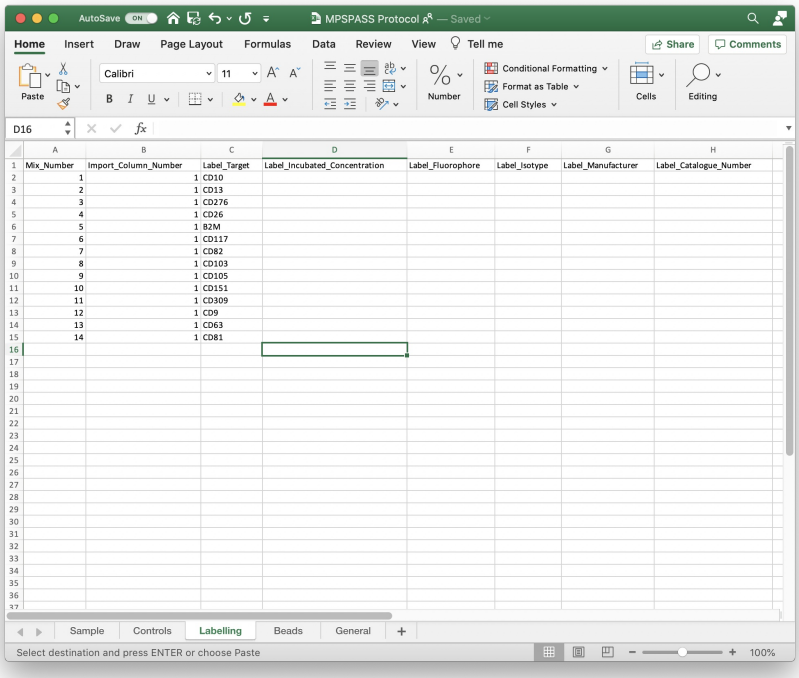

9 **Beads Worksheet and General Worksheet Headings:**

- The Beads worksheet will have the information from the Bead Info Section as discussed in step #3. No changes are necessary for the database to pass QC.
- The General worksheet lists the CSV directory as chosen in step #2. Do not change this worksheet as otherwise the software will not know where the .csv files are.

Import the Database

10 Save the flled out database .xlsx file and return to the MPSPASS software. Click on the 'Import Database' button and choose the database file.

Database QC

11 Once the database has been imported, click on the 'Database QC' button.

If QC is unsucessful, than the associated error will pop-up in the command window below. The error message will offer an explanation for what failed. The database .xlsx file can be opened again and edited to fix the error. Once the database file has been saved again, the database can be once more be imported and subjected to QC.

12 If QC was successful, the software will allow for the import of the data. Click on the 'Import Data' button and the dataset will be constructed.

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