

Analysis of a BD™ CBA Kit using the FCAP Array™ Software

Acquisition of data

Acquire data using the BD CBA Kit as directed in the kit manual. Once the data has been collected, transfer the FCS2.0 files to a single folder for analysis using the FCAP Array Software.

Analysis using the FCAP Array Software

1. Open the FCAP Array Software
2. If this is the first time that data from a particular kit has been analyzed using the FCAP Array Software, please select New Plex in the Selecting Saved Plex view and click Next (Figure 1).

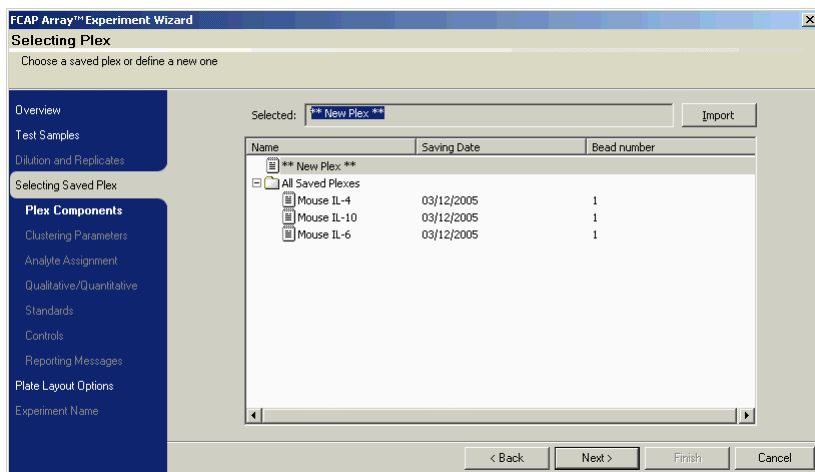


Figure 1. Selecting a Plex.

3. In the Plex Components view, click the Edit... button, opening Bead Library window.
4. Click the Edit Groups... button, opening the Bead groups window.
5. Click the New Group... button, opening the New Bead Group window (Figure 2) and enter the kit name into the New Bead Group window, click Ok.

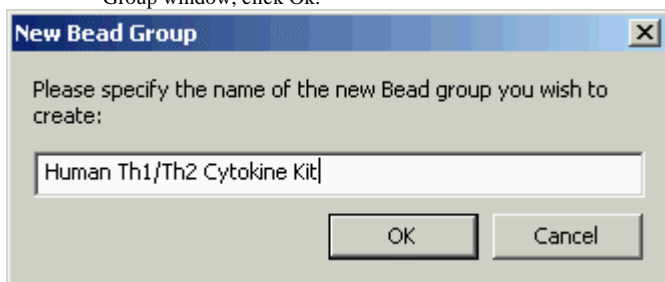


Figure 2. New Bead Group Window.

6. In the Bead Groups window, click Ok to return to the Bead Library window.
7. In the Bead Library window, enter the bead information for the beads in the kit and Add them to the Bead Library (see Figure 3). The bead locations can be entered as 1 → 6 from the brightest bead to the dimmest (such as Human IL-2, bead location 1).

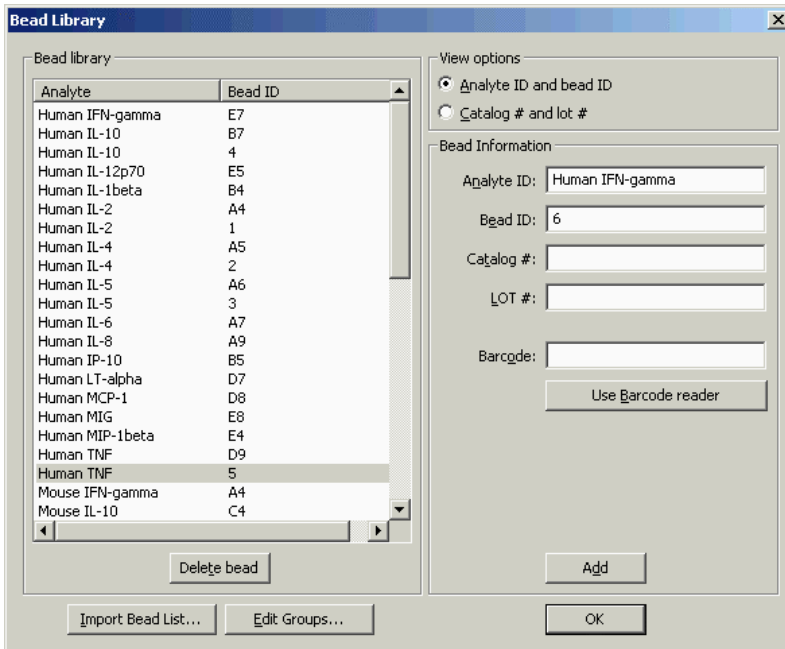


Figure 3. Adding beads to the Bead Library.

8. After all of the beads have been entered into the Bead Library, click the Edit Groups... button to open the Bead Groups window.
9. Select the new kit group and click the Modify Group... button (Figure 4).

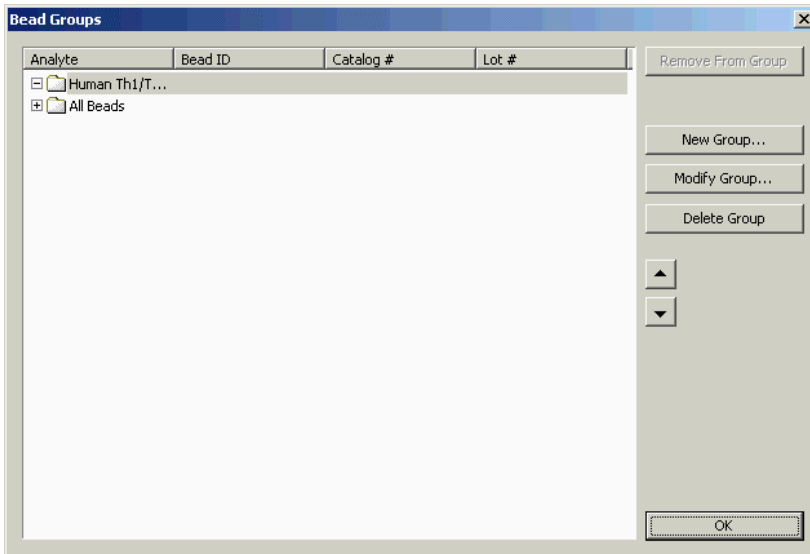


Figure 4. Modifying a group in the Bead Groups window.

10. Select the beads to add to the kit Bead Group (Figure 5) and click Ok. The beads are now added to the kit Bead Group (Figure 6).

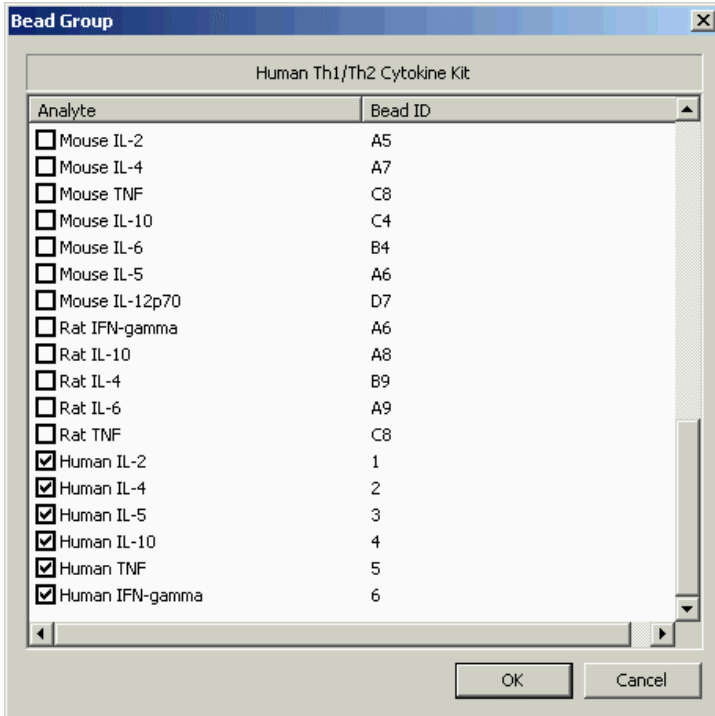


Figure 5. Selecting beads to add to a Bead Group.

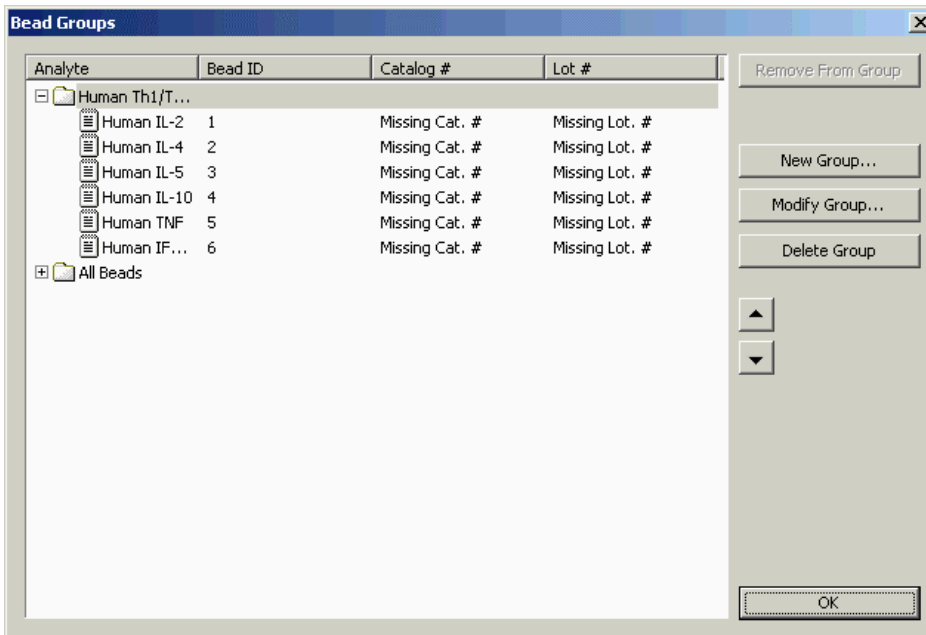


Figure 6. Updated Bead Groups window after beads have been added to a group.

11. Click Ok twice to close the Bead Group and Bead Library windows.
12. Select all of the beads from the kit and add them to the Selected Beads list in the Plex Components view (Figure 7). Click the Next button.

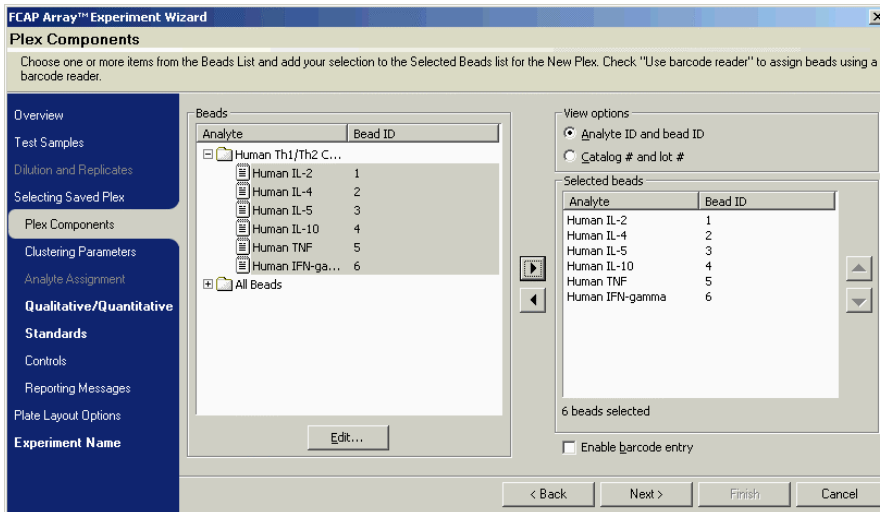


Figure 7. Selecting the beads for the Plex.

13. Load a data file from the experiment. In this case, the data is from the FACSArray. Adjust the Clustering Parameters to have one parameter chosen and the other set to None (Figure 8). The beads should show up as histograms in the data plot. Click the Next button.

NOTE: If you plan to use the FCAP Array Software to analyze BD CBA Flex Set Assays in the future, write down the clustering parameters before changing them in step 13. The FCAP Software will default the parameters to the last configuration chosen, so the parameters will need to be changed back if a BD CBA Flex Set is to be analyzed.

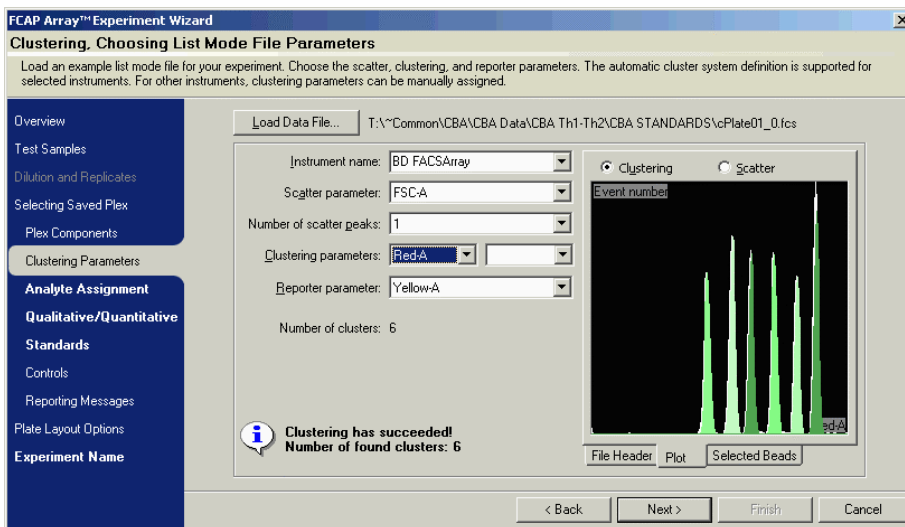


Figure 8. Choosing the clustering parameters for analysis.

14. Assign the beads to the bead populations as described in the FCAP Array Software User's Guide. Bead location 1 will be the brightest bead population (Figure 9).

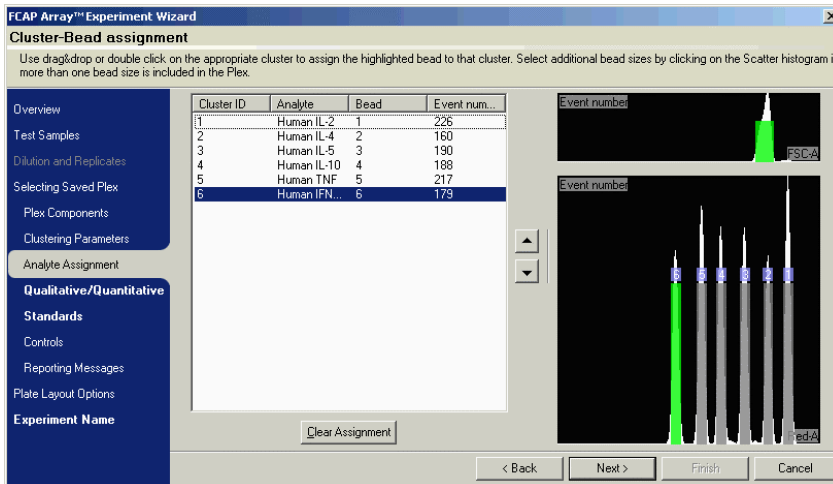


Figure 9. Assigning the beads to the bead populations.

- Proceed with the rest of the experiment setup as described in the FCAP Array Software User's Guide. At the Experiment Name view, be sure to give the plex the name of the kit being analyzed and save the plex so that the plex can be chosen in the future and reduce the steps needed to setup an experiment (Figure 10).

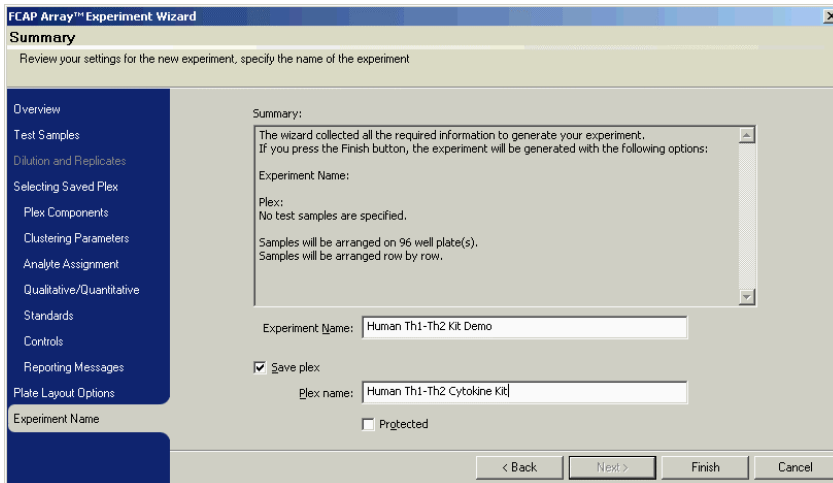


Figure 10. Finishing the experiment wizard and saving the new plex.

- Proceed with analysis as directed in the FCAP Array Software User's Guide. You should be able to assign data files and analyze the experiment normally (Figures 11 and 12).

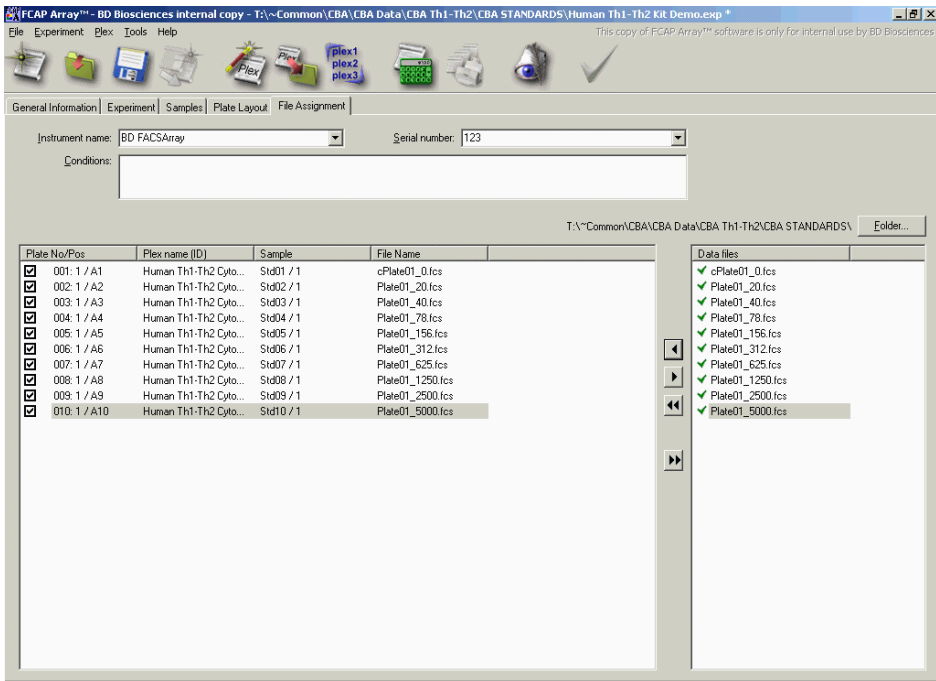


Figure 11. Assigning data files.

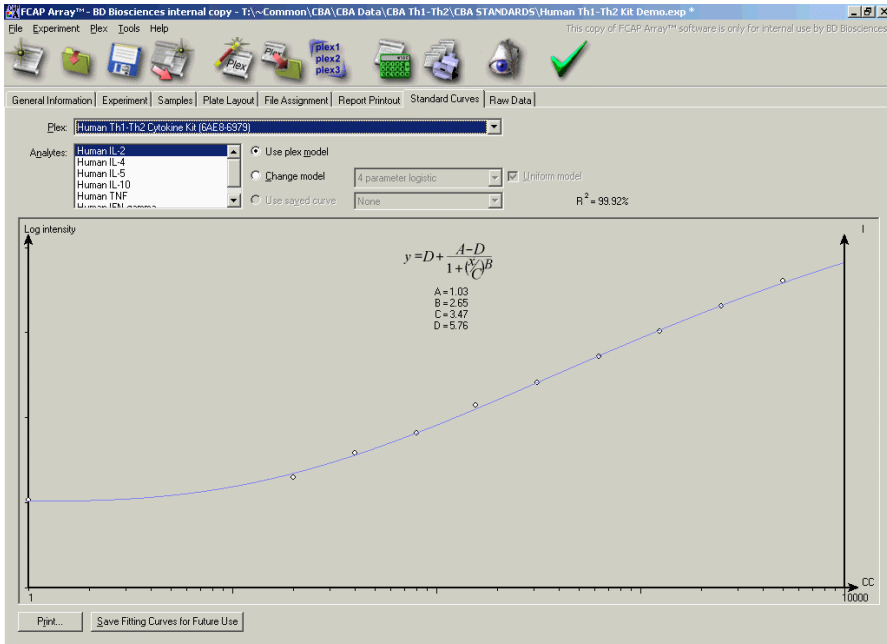


Figure 12. Example standard curve.