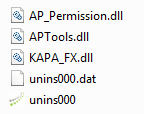
**KAPA Hyper and HyperPlus Library Preparation method for the Biomek FXp – Quick Setup and Installation Guide**

**1. Installation:**

* Make sure you have a copy of the **02C\_Kapa\_HyperPrep\_and HyperPlus\_HTML** folder on a flash drive, CD or DVD.
* Check which version of the **KAPA\_FX installer** is currently running on the computer.
  + If none, skip to the next main step.
  + If it is not the one associated with the method you need to pre-configure, proceed as follows:
    - In **Control Panel**, locate the list of programs installed on your computer. Uninstall the current version of KAPA\_FX. Click “Remove All” if prompted.
    - *For Windows XP:* 
      * Go to **C:\Program Files**. Locate the **Alpaqua** folder and delete it (with its entire content), if it is still there.
    - *For Widows 7:*
      * Go to **C:\Program Files(x86)**. Locate the **Alpaqua** folder and delete it (with its entire content), if it is still there.
      * Go to **C:\Users\Public\Public Documents**. Locate the **Alpaqua** folder and delete it (with its entire content), if it is still there.
    - Close the Control Panel and reboot the computer.
* Go to the **Installers** folder in the **02C\_Kapa\_HyperPrep\_and HyperPlus\_HTML** folder
  + For *Windows XP*, double-click on **Kapa\_FX301.exe** and follow the installation prompts. Don’t change any default options. Select “Yes” if you are prompted about keeping any files.
  + For *Windows 7*, double-click on **Kapa\_FX400\_Setup.exe** and follow the prompts
    - Go to **C:\Program Files(x86)**. Locate the **Alpaqua** folder. Make sure it contains the following files:
* Go to **C:\Users\Public\Public Documents**. Locate the **Alpaqua** folder. It should have one subfolder, **Kapa\_FX**, which in turn contains two subfolders, named **Pictures** and **KFiles**. The content of these folders must correspond to the contents of the folders with the same names in **02C\_Kapa\_HyperPrep\_and HyperPlus\_HTML**. If those folders don’t exist, or the content of either does not correspond to the Master folder, update the folders accordingly.

**2. Method configuration:**

* Open the Biomek software.
* Open the customer’s instrument file.
* Create a new **Project** with a relevant name, e.g. **KAPA Hyper.** 
  + If you are doing the pre-configuration on your computer, include the customer’s name in the Project name.
* Import **KAPA HyperPrepHyperPlusv##\_HTML##.bmf**. Click OK to import all new Labware Classes, Labware Patterns, Liquid Types, Pipetting Templates, Techniques and Tip Classes
* In the main menu, go to **Project, Labware Type Editor.** Alpillo, Incubation, NameTag and the SuperMagnet should have special picture files.
  + *For Windows XP:* the picture files should have been updated automatically
  + *For Windows 7:* you’ll need to change the file path for each item individually, to point to the custom picture. This is done as follows:
    - Double-click on the labware item.
    - With **Basic Information** highlighted, go to the **Bitmap file** window.
    - Use the browse (…) button on the right hand side of that window to change the file path to **C:\Users\Public\ Documents\Alpaqua\Kapa\_FX\Pictures**
    - **Save** and repeat for the next item until all four picture files have been updated.
    - Click **Exit** when all are done.
* In the method window, scroll down to and click on **Modular HTML Prompt**. Simultaneously press the buttons “CTRL + Shift + U”. Select the HTMLheader code section and scroll to the bottom of the code window. Locate the line designated “<IMG SRC = and modify it to include a link to the Kapa logo image:   
   <IMG SRC = "**C:\Program Files\Alpaqua\Kapa\_FX\Pictures\KAPA\_Logo.png** ">
* In the method window, scroll down to and click on **Reagent Calculator**. Simultaneously press the buttons “CTRL + Shift + U”. Select the HTML code section and scroll to the bottom of the code window. Locate the line designated “<IMG SRC = and modify it to include a link to the POGO image:   
   <IMG SRC = "**C:\Program Files\Alpaqua\Kapa\_FX\Pictures\Biomek-POGO-HyperPlus.png**">
* If the customer has a TRobot:
  + In **Method Start**, go to the line **Extend “WithTRobot”**. Change **False** to **True**, if True is not already specified.
* In **Startup Group**, go to **IS1: Instrument Setup – Make changes here**. Map the deck onto the customer’s instrument file, if necessary. The default deck in the instrument file is normally selected by the Biomek software, so you can simply click on the **Map Onto** button.
  + If you had to remap the deck, go to **IS2: Instrument Setup – Don’t change this one** and map the method onto the same deck selected in IS1.
  + Go back to IS1.
* Compare the deck layout in the middle of the screen on the right hand side with the **Deck Layout.JPG.jpg** in **02C\_Kapa\_HyperPrep\_and HyperPlus\_HTML\Pictures**. In the next series of steps you need to ensure that:
  + All labware items in the Standard deck layout are present on the customer’s deck, and are placed in appropriate locations.
  + All labware items are named exactly as in the Standard deck layout. Names are case sensitive.

In this regard:

* + Retain the default deck layout as far as possible.
  + Labware to be accessed by Pod 1 and Pod 2, respectively, must be in locations accessible by the respective pods.
  + **Incubation** must be placed on a Peltier block with a 96-well adapter. It can be a static or shaking Peltier.
  + Make sure the NameTags **LidHome**, **SampleHome** and **TransferHome** are in the appropriate positions (below **Lid, Sample** and **Transfer#**, respectively).
  + If the customer is using a TRobot, the lid on **LidHome** must be replaced with a **P\_plus\_Lid**.
    - If the customer has Nunc lids, use that for the AdapterLid.
    - If the customer does not have Nunc lids, replace the AdapterLid with a P\_plus\_Lid as well.
  + Remember to put the **Alpillo** under the **Adapter** plate if the customer is using an Alpillo.
  + If available, the **Enzymes** chiller block must be placed on a *flat Peltier*. Otherwise, place the chiller block on any passive ALP in the most right-hand column of the instrument.
    - If the customer is not using a *POGO* chiller block, the POGO must be replaced with (the labware definition for) the customer’s chiller block. This may have to be exported from another Project, and imported into the KAPA HTP LPK Project.
    - If the customer doesn’t have a labware definition, or the one you could find doesn’t appear to match their chiller block (check the measurements!), you can start with the labware definition named **Not\_POGO**.
      * To do this, go to **Project**, **Labware Type Editor**.
      * Highlight **Not\_POGO**, right-click and click **Copy**. Rename the copy appropriately, and edit any dimensions as necessary.
    - Once the **Enzymes** block has been configured correctly, double-click on it in the deck layout. In the Labware Properties window, make sure **Unkown** is selected in the first window on the third line. The next window (to the right of **volume**) usually specifies **2000**. You don’t have to change that.
  + If the customer is planning to run HyperPlus there are 3 options for the enzymatic fragmentation incubation:
    - On Deck Incubation: where a second “Incubation” position will be required that is accessible to the 96 channel head. Place an “Incubate” labware on this position named “Incubation2”
    - Off Deck Incubation: In **Method Start**, go to the line **Extend “EnzFragOffDeck”**. Change **False** to **True**, if True is not already specified.
    - TRobot Incubation:
  + Make sure the *tips* with which the method is configured match the customer’s choice (filtered or not). When moving or replacing tip boxes, make sure that:
    - The Span-8 P250 tip box closest to the Span-8 trash is named **TB200**.
    - The Span-8 P50 tip box closest to the Span-8 trash is named **TB50**.
    - The AP250 tip box on the tip loader is named **MC200**.
  + Once everything has been configured, go to **IS2**. All your changes will have updated automatically. However, please note the following:
    - If you had to switch out tip boxes (filtered for non-filtered, or *vice versa*), you need to switch out the AP250 box on the tiploader in IS2 as well. *This is the only item that is changed manually in IS2.*
    - Once you’ve dragged the correct tip box onto the tip loader, double-click on this to name the new tip box MC200.
    - In the window between **Load no more than** and **times,** enter **10** (or use the arrows). Click OK.
    - Finally, make sure every position *except for the tiploader (MC200)* is marked as **As Is** (i.e. hashed out). If any position is not hashed out:
      * Click on **As Is** at the right hand side of the window
      * Click on every position *except for MC200* that is not hashed out.
      * Click on **As Is** again, so it is no longer selected (“indented”).
* The final step of the pre-configuration relates to incubation positions. The method is set up to:
  + - Initialize all Peltiers during the method start.
    - Set Peltier temperatures appropriately.
    - Refer to incubation positions by labware locations (generic), rather than the name of the actual deck positions (specific to each customer’s instrument file). *This has to be changed for shaking Peltiers*.
  + In Startup Group, locate the first line labelled **If NOT SIM**.
    - If **Incubation** is on a *shaking Peltier*:
      * Highlight the first (*Initialize*) line below **Then**. In the window on the right hand side of the screen, select the actual labware name from the first window.
      * Do the same for the second (*Incubate*) line associated with the **Incubation** position. *Untick the block next to* **Shake** (otherwise the Peltier will start shaking, and will not stop).
    - If **Incubation** is on a *static Peltier*, no action is required.
    - If the customer does not have a static or shaking Peltier with a 96-well PCR plate adapter in a position accessible by Pod 1, incubations have to be performed in a TRobot or off-deck. Method customization will be required.
    - If the customer has a *flat, non-shaking Peltier* for the **Enzymes** chiller block, no action is required.
      * If the **Enzymes** chiller block is placed on *a passive ALP*, the two lines associated with initialization and pre-incubation of the **Enzymes** position must be disabled. Highlight each line, right-click and click **Disable step**.
  + There are two additional places in the method where the temperature of the **Incubation** position is set. If a *shaking Peltier* is used for **Incubation**, both positions need to be set up *to not shake* (as outlined above). These lines are found:
    - In the **A-Tailing** step, just below the first **Then**.
    - In the **Adapter Ligation** step, just below the first **Then**.
  + The final actions related to the **Incubation** and **Enzymes** positions are right at the end of the method. The method is configured to:
    - Set the **Incubation** position to 4 oC, as the final libraries are left on that position.
    - Set the **Enzymes** position to 20 oC (room temperature).
    - To access these, scroll right down to the last **If NOT SIM**, just above **Finish**.
      * If a *shaking Peltier* is used for **Incubation**, remap to the actual position name, as outlined before, and make sure shaking is turned off.
      * If the **Enzymes** chiller block is not on a flat Peltier, disable the step related to Enzymes, as outlined before.
* Click File, Save As and save the method with the original name, followed by an underscore and “XP” or “W7”, followed by another underscore and the customer’s name. Delete the Master method.
* Scroll down to and highlight Finish. If you did everything correctly, you will get a specific time in the ETA window right at the bottom of the screen (next to the windows specifying the method name, project name and instrument file name). If not, error messages will appear. These will have to be resolved one by one.

Two final notes:

* The method is configured to provide for additional shaking and resuspension with Pod 1 of the beads in the Ligation Mix. This action consists of three command lines, toward the end of the Adapter Ligation section of the method. In the Master file, these commands are disabled. They should only be enabled and configured if the customer complains about incomplete resuspension of the beads in the Ligation Mix.
* If the customer has a TRobot, methods have to be configured for the TRobot. This is done from Instrument, Device Editor, by selecting the TRobot from the device menu. Once methods are written, make sure they are properly referred to in each TRobot step.