

384-WELL PLATE RESUSPEND WITH MULTIMEK

Before beginning, inspect the checklist at the end of the protocol.

Set Up Turn on the Mek and the computer. Mek switch is the underside of the drive housing, left side, at the back. After the Mek is switched on, it will display "Please Reset" on the screen on the front of the Mek. Press the RESET/INIT button on the panel on the front of the Mek and the Mek will find its home position. Start the software using the MekDaddy shortcut icon on the desktop.

Place a box of tips in the tip loading frame and **center** it. Place black elevator adaptors at Positions 1 and 2. Place a 384-well plate in Position 1 and a upside-down tip box lid in Position 2. When placing plates and reservoirs, **always** push them to the **back-left** corner of the position. If you fail to do this, you will destroy your tips and potentially damage the instrument.

Commands are listed in this protocol as follows:

command	type into the command line
menu > command	select from the menus in the window
COMMAND	is a button on the front of the Mek

Calibration Set the X and Y axis locations for the numbered positions on the deck of the Mek. Begin a run so you can get control of the Mek: **Script > Run**. You do not need to have a method loaded to begin a run.

Open the MultiMek command line: **MultiMek > Show Communications**, then in the new window **Form > Unfold**. You will use this window and the buttons on the the Mek to calibrate the location of the head properly.

NOTE: You have to hit return to get a command to be sent to the Mek from the window; clicking "OK" will simply close the Communications window.

The Mek has two modes of operation. In Remote mode, the Mek listens to the computer; in Manual mode, the Mek listens to the buttons on the front of the Mek. To switch between them, use the MANUAL/REMOTE button. Issuing a command when the Mek is in the wrong mode will throw an error message.

Calibration Steps Please read through the following commands BEFORE you begin.

Mode Command

Remote **tp1d**

This will load the tips from the tip loading frame onto the pipet head. This is essential for accurately determining the alignment of the Mek.

Remote **MultiMek > Goto Frame > Position 1**

The head will move front-leftmost position on the deck, where the plate to be resuspended will be. You will then align this position. You can do other positions by moving to them in the same way.

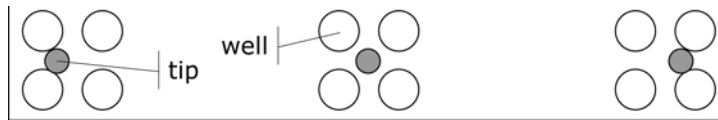
Manual Z DOWN / Z UP

The head will jog down, additional presses INCREASE speed. Press the button for the opposite direction to slow down -- you need to use the same number

of presses to come to a complete stop. Use extreme caution (i.e. a jog speed of 3 or less) when moving the head down!!!
Lower the tips to within a few millimeters of the top of the plate.

Remote `relmovexy #,#`

Adjust head position in the X and Y directions. Align tips to be in the center of each 4 well quad. As shown below, the spacing of the tips and the plates is not equal, so you must average this difference. Example: `relmovexy 50,-50` moves the head 50 steps right on the X axis, and 50 steps back on the Y axis.



Remote **MultiMek > Set Frame > Position 1**

Saves the X and Y values of the current location to memory!! If you don't do this, your changes to the alignment will not be used! Note that the Z axis position is not saved. This value will be set in the method.

Manual Z UP

Get tips clear of the deck for subsequent moves of the head. When moving the head up, the maximum speed can be safely used; the head will stop automatically at the top of its range.

WARNING: When you move from setting Position 1 to Position 2, DO NOT FORGET to return the pipet head to the top of the Z axis first!

Remote **MultiMek > Goto Frame > Position 2**

Move the head over to the reservoir position so that it can be calibrated.

Manual Z DOWN / Z UP

As above, lower the head down so that it is close enough to the top of the reservoir to determine its alignment.

Remote `relmovexy #,#`

Adjust the head X and Y location to be clearly within the reservoir. You will set the height in the method that you will run later.

Remote **MultiMek > Set Frame > Position 2**

Save the position to memory. Make certain to get the correct position!

Manual Z UP

Return the head to the top of its range.

Remote `tpu1`

Unload tips from the print head so that the Mek is ready to begin a method. Be certain to do this: trying to load tips onto the print head when there are already tips loaded can be dangerous to you and damaging to the Mek.

You can use the same system to ensure that the x,y settings are correct for the other Positions on the deck if you are running a different method. You do not need to calibrate the tip loading frame.

Method Load the resuspension method: **File > Open Method** -- choose OneBoxResuspend.

Check the accuracy of the method. This method is set up for P20 tips and labware placed on the black elevators. The Setup screen that opens when you open the method should indicate both of these facts. People will sometimes change this method, but they shouldn't....

Adjust aspirate volume to be appropriate for the set of plates. You will need to change the settings for EACH of the FOUR Aspirate steps in the method. Double click on the step and enter the values into the boxes. Start at 7 μ l for the first print from a set of plates and work down towards 3 μ l as the set is depleted.

Adjust the aspirate height so that the tips are clearly submerged. Depending on the level of water in the reservoir, the aspirate height should be around 30 mm. Keep the reservoir filled close to the top so that the tips are sure to be submerged. Again, each Aspirate step needs to be adjusted individually.

Adjust the Loop step to have the correct number of iterations for the number of plates that you have, or to 2, to do a test run to check your settings.

Run the Method Use **Script > Run** to open the Run-Time Engine and run the method. It is not a bad idea to take a trial run with a test plate and tips and do just 2 loops, especially if you've never run the Mek before. Make sure the first plate, tips, and reservoir are in position and press the "play" button. The method will pause the Mek in between plates so you can change them.

When a plate is finished, switch the filled plate with a fresh one, ensure that it is placed to the back and left of the Frame, and press the "play" button again. Tap the filled plate gently on the countertop to get the drops down to the bottom of the well. Check the plate from the underside to ensure that liquid has been dispensed into every well.

Seal the plates with parafilm, spin the liquid down to the bottom of the well (1000 rpm briefly in a GS-6KR), and shake at 4°C for AT LEAST 24 hours. The duration of shaking is **absolutely essential** to get good resuspension of cDNAs and, thus, good printing. Shorter resuspension times will work for oligos.

Shut Down

Switch off the Mek, quit the program, and shut down the computer.

You Will Need:

- plates to be resuspended
- 1 box of P20 tips (E&K Scientific, 4565-R96, 10/case)
- parafilm for sealing plates
- scissors
- a test plate, if you are using plates that are not the standard Genetix 384-well