

ClonePix 2 Training Guide

Prepare for Pick Run



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The purpose of this chapter is to guide the user through the **Prepare for Pick Run** process on the **ClonePix 2**.

Pick head maintenance & basic pick run setup are <u>not</u> covered in this document. Please consult the dedicated training modules for detailed instructions on these other processes.





Launching Prepare for Pick Run

1. From the Main Menu, under ClonePix Processes select Prepare for Pick Run.

| ClonePix 2 - Unsaved Process | | |
|--|---|------------|
| File View Tools Help | | |
| Molecular Devices | | ClonePix 2 |
| Double click with the left mouse button on a process icon to run it. | | |
| New Process Recent Processes | | |
| ClonePix Processes | | |
| Imaging Run Image a microplate. | Prepare For Pick Run Prepare the instrument for a picking run. Pick Run Image microplate and pick colonies from specified c Review Results Review the results of imaging and picking runs. | |
| Maintenance Processes | | |
| Commissioning Process Configure instrument settings. | Camera Adjustment Determine if any camera adjustment is required. | |
| Utility Processes | | |
| Picking Head Management Select options to prepare the head. | Utility Process Runs a selection of processes to control or diagnos | |





Pin Fire Test

- 2. The **Pin Fire Test** dialog now appears.
 - a) Click and drag the speed slider to select the Fast setting, then click Start.







Pin Fire Test

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 - a) Click and drag the speed slider to select the Fast setting, then click Start.
 - b) You will now see that each **picking pin** in the **head** is fired **(extended)**, then **retracted sequentially**.

Watch carefully for **3-4 full cycles** and ensure that **each** of the 8 picking pins is moving smoothly. If you note any sticking or significant hesitation of the pin movement, contact Molecular Devices Technical support for further assistance (800-635-5577 or support@moldev.com)







Pin Fire Test

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Watch carefully for **3-4 full cycles** and ensure that each of the 8 picking pins is moving smoothly. If you note any sticking or significant hesitation of the pin movement, contact Molecular Devices Technical support for further assistance (800-635-5577 or support@moldev.com)

c) Click the **Stop** button to conclude the test, then click **Next** to proceed.

| 🐻 Clo | | | | | | |
|-----------|----------------------------|-------------------------------|-------------------------------|------------|-----------------|-----------|
| | nePix 2 - | Unsaved | Process | | | |
| File | View | Tools | Help | | | |
| | D e v | <mark>cular</mark> i c e s | | | | |
| Pii | n Fire Te | st | | | | |
| Sta To | art the test adjust the | to see if th speed mo | e pins are m ve the slider | oving free | ly and smoothly | <i>(.</i> |
| | | 1 | | | 9 | |
| | | | | | | |
| 9 | Slow | | | | Fast | |





Select Source Plate Type

3. You will now see a screen where you are prompted to **select your source microplate type**. **Select** your **source microplate type** from the **dropdown list**, then click **Next**.





Interior light is on. 💡 Currently logged in as Molecular Devices



3. The Load Container dialog appears, where you are prompted to **load the source stacker** with your selected **source plate type**.

a) Select Raise Source - the Source Stacker will now be raised within the ClonePix 2 instrument.



Load Container

Please load the SourceStacker with a Nunc Omni Tray WellPlate.











3. The **Load Container** dialog appears, where you are prompted to **load the source stacker** with your selected **source plate type**.

- a) Select Raise Source the Source Stacker will now be raised within the ClonePix 2 instrument.
- b) Place an **empty** (no media, no cells) **source plate** in the **appropriate stacker cassette** (see chart for details), with well A1 facing the lower right hand corner (away from the color-coded lid pusher).



| Source Plate | Lid Pusher Color | Lid Lifter Assembly |
|----------------|------------------|---------------------|
| Greiner 6 well | Green | Red |
| Nunc Omni - | Blue | Silver |
| Nunc – 6 Well | Green | White |





3. The **Load Container** dialog appears, where you are prompted to **load the source stacker** with your selected **source plate type**.

- a) Select Raise Source the Source Stacker will now be raised within the ClonePix 2 instrument.
- b) Place an empty (no media, no cells) source plate in the appropriate stacker cassette (see chart for details), with well A1 facing the lower right hand corner (away from the color-coded lid pusher).
- c) Insert your source plate + stacker cassette into any position within the source stacker with the lid pusher facing the back of the stacker (until you hear the magnet engage), then click Next.

Source Plate + Stacker Cassette





3. The **Load Container** dialog appears, where you are prompted to **load the source stacker** with your selected **source plate type**.

- a) Select Raise Source the Source Stacker will now be raised within the ClonePix 2 instrument.
- b) Place an empty (no media, no cells) source plate in the appropriate stacker cassette (see chart for details), with well A1 facing the lower right hand corner (away from the color-coded lid pusher).
- c) Insert your source plate + stacker cassette into any position within the source stacker with the lid pusher facing the back of the stacker (until you hear the magnet engage), then click Next.
- d) The stacker will now automatically move through the stack and detect the position of your loaded plate (this could take up to a minute). You will see the Loading holders indicator on your computer screen during this process.







3. The **Load Container** dialog appears, where you are prompted to **load the source stacker** with your selected **source plate type**.

e) The **loaded plate** will next be pulled from the **stacker** into the **de-lidding station**, then to the **imaging station** for the next step. You will see the **Loading microplate** indicator on your computer screen during this process.







Adjusting Well Alignment

4. The **ClonePix 2 system** will now capture **white light images** of a **well** of your **source plate** and the software will enable any necessary **adjustment to alignment of the well** as follows:

a) The illumination cover will automatically retract and the system will capture 4 images of your well edges (north, south, east, and west sides). You will see the Acquiring
 Alignment Images indicator appear on your computer screen during this process.









Adjusting Well Alignment

4. The **ClonePix 2 system** will now capture **white light images** of a **well** of your **source plate** and the software will enable any necessary **adjustment to alignment of the well** as follows:

- a) The illumination cover will automatically retract and the system will capture 4 images of your well edges (north, south, east, and west sides). You will see the Acquiring Alignment Images indicator appear on your computer screen during this process.
- b) You will now see the 4 well edge images appear on your screen. The red lines superimposed on the image set should align closely with the well edges. Adjust the line positioning by clicking and dragging – note that the top/bottom (north/south) lines will move together, same for left/right (east/west). If you make an adjustment, click the Reimage button to confirm alignment. Click Next to proceed.



5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

a) The illumination cover will remain retracted and the primary picking pin (#1, closest to you as you face the instrument) will be extended to the center of a well in your loaded source plate. You will see the adjustment dialog appear (screenshot below) – click on Retract Pin before proceeding to the next step.

| ClonePix 2 - Unsaved Process | |
|--|---|
| File View Tools Help | |
| Molecular Devices | |
| Visit Position Goto SourceStacker | The datum can only be changed with the pin extended. |
| Stop Short Above None | Lower the pin until is just touches the bottom of the well. You can use the Air Pressure Meter to find the bottom of the well. Make sure you attach this at the beginning of the datuming process because the process of attaching it can change you datum point outcase diabits. Dear Soft is at the bit of the attached additions the |
| Set Position Click the buttons to jog the specified distance. | as the new Datum point. When this is complete press the 'Next' button. |
| Vertical Lateral | |
| Move Size: 0.01 mm Set | |
| Retract Pin Fire Pin Vertical Position 0.000 | |





10. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

- a) The illumination cover will remain retracted and the primary picking pin (#1, closest to you as you face the instrument) will be extended to the center of a well in your loaded source plate. You will see the adjustment dialog appear (screenshot below) click on Retract Pin before proceeding to the next step.
- b) Disconnect the picking tubing from the primary picking pin, then connect the free end of the air pressure tubing to the primary picking pin as shown below.







10. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

c) Switch on the air pressure gauge by twisting the grey knob counterclockwise so that the handle is vertical as pictured.







5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

d) Click the Goto SourceStacker button - you will now see the primary picking pin extend to the bottom of the well and the air pressure gauge readout value will increase (>0.25).







5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

e) Use the vertical arrow keys to adjust the Z position of the primary picking pin until the air pressure gauge readout color changes to green. Select move size increments from the dropdown to refine the positioning.





5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

f) Once you have refined the position such that the indicator just turns **green** at the *smallest move increment (0.01 mm in the dropdown*), **move the pin up** by selecting the **1 mm move size setting** and clicking the **up key once** (the **air pressure gauge readout** will now change back to **red**).

| ClonePix 2 - Unsaved Process | |
|---|---|
| File View Tools Help | |
| Molecular Devices | |
| Visit Position Goto SourceStacker Stop Short Above None Set Position Click the buttons to jog the specified distance. Vertical Lateral Vertical Lateral Move Size: 0.01 mm Set | The datum can only be changed with the pin extended. Lower the pin until is just touches the bottom of the well. You can use the Air Pressure Meter to find the bottom of the well. Make sure you attach this at the beginning of the datuming process because the process of attaching it can change you datum point ever so slightly. Press 'Set' to set the tip of the extended picking pin as the new Datum point. When this is complete press the 'Next' button. |
| Retract Pin Fire Pin | |
| Vertical Position 0.000 | |





5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

g) Click the **Set** button to save the current **Z datum position**. You will be prompted to confirm your choice to save the position – click **Yes**.







5. The **primary picking pin Z datum position** provides a **reference point** for the instrument to accurately pick your colonies while preventing collision with the plate bottom. This adjustment step ensures proper configuration of this position:

h) Click on Retract Pin. Disconnect the air pressure tubing from the primary picking pin, then reconnect the picking tubing to the pin. Click Next to proceed.



Confirm Plate Type

6. The **Plate Selection** dialog now appears. Click the **Next** button to **confirm** that you want to **continue** to the next step using the **same source plate type** that you have been working with up to this point in the process.

| Pix 2 - Unsaved Process | |
|---|---------------------|
| View Tools Help | |
| Aolecular Devices | ing Head Management |
| e Selection | |
| su wish to continue using the Nunc Omni Tray? | |
| Next for Yes, Cancel/Close for No | |
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Next >

Adjusting Primary Picking Pin XY Alignment

7. This next step allows for **adjustment** of the **primary picking pin XY alignment**. This step is important to ensure accurate colony picking. Follow these steps to adjust the alignment:

 a) Click on the lateral arrow keys to adjust the position of the primary picking pin so that the center of the pin aligns as closely as possible with the intersection of the red crosshairs. Change the Move Size via the dropdown as needed to refine positioning.

NOTE: The **arrow keys** move the **pin** itself (NOT the crosshairs). Please click **once**, then **wait** for the **image** to **refresh before** clicking again.

Adjusting Primary Picking Pin XY Alignment

7. This next step allows for **adjustment** of the **primary picking pin XY alignment**. This step is important to ensure accurate colony picking. Follow these steps to adjust the alignment:

- b) Once the positioning is correct, click the Set button. You will then see a dialog that will ask you to confirm save of the datum point click Yes.
- c) Click the **Next** button to proceed to the next step.

Filling the Ethanol Wash Bath

- 8. The Wash Bath dialog now appears.
 - a) Ensure that your **Wash Bottle** is at least ³/₄ full with **70% Ethanol** (**second** bottle from **left front** side of instrument).
 - b) Ensure that the Auto Replenish Wash Bath checkbox is checked, and keep the default settings (30 seconds for pump duration, 30 minutes for intervals between durations)

Wash Bath

Click the 'Start' button to start filling the Wash Bath.

Click the 'Stop' button to stop filling the Wash Bath.

| Wash Bath Pump | | | |
|-----------------------------------|---|------|---------|
| Start Stop | | | |
| 🔽 Auto Replenish Wash Bath | | | |
| Duration the pump will be on for: | * | 30 s | econds |
| Interval between durations: | * | 30 m | ninutes |

Filling the Ethanol Wash Bath

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 - b) Ensure that the Auto Replenish Wash Bath checkbox is checked, and keep the default settings (30 seconds for pump duration, 30 minutes for intervals between durations) for the Wash Bath Pump.
 - c) Click the Start button to initiate filling of the Ethanol Wash Bath.

Wash Bath

Click the 'Start' button to start filling the Wash Bath.

Click the 'Stop' button to stop filling the Wash Bath.

| - Wash Bath Pump - | | | | | |
|--------------------|---------------------|--------|----|---------|--|
| Waan baan anp | | | | | |
| Start | Stop | | | | |
| 🛛 Auto Replenish | n Wash Bath | | | | |
| Duration the pu | ump will be on for: | * | 30 | seconds | |
| Interval betwee | en durations: | * * | 30 | minutes | |
| | | | | | |

Filling the Ethanol Wash Bath

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 - a) Ensure that your Wash Bottle is at least ³/₄ full with 70% Ethanol (second bottle from left front side of instrument).
 - b) Ensure that the Auto Replenish Wash Bath checkbox is checked, and keep the default settings (30 seconds for pump duration, 30 minutes for intervals between durations) for the Wash Bath Pump.
 - c) Click the Start button to initiate filling of the Ethanol Wash Bath.
 - d) Observe the filling of the ethanol wash bath in the ClonePix 2 allow the fill to continue until the fluid reaches the exit tube at the back of the bath (it is okay if you still see bubbles in the exit tube). Click the Stop button, then click Next to proceed.

Wash Bath

Click the 'Start' button to start filling the Wash Bath.

Click the 'Stop' button to stop filling the Wash Bath.

| Wash Bath Pump | , , | | | |
|----------------|----------------------|--------|----|---------|
| Start | Stop | | | |
| 🔽 Auto Repleni | ish Wash Bath | | | |
| Duration the | pump will be on for: | - | 30 | seconds |
| Interval betw | een durations: | * * | 30 | minutes |

Sanitizing Picking Pins

9. This next step ensures that the **picking pins & fluidics** are **flushed** with **sanitizing solution** (important for maintaining sterility during picking):

a) Ensure that the **5L wash bottle** containing approximately **4L of SporKlenz solution** is attached to the **leftmost fluidics connection** on the ClonePix 2 instrument.

Sanitizing Picking Pins

9. This next step ensures that the **picking pins & fluidics** are **flushed** with **sanitizing solution** (important for maintaining sterility during picking):

- a) Ensure that the **5L wash bottle** containing approximately **4L of SporKlenz solution** is attached to the **leftmost fluidics connection** on the ClonePix 2 instrument.
- b) Leave the **default** settings in the **Sanitise Options** dialog (30/5/10 as pictured).

Sanitise Pins Purge the pump system with sanitising agent Sanitise Options Number of cycles for the head to purge Number of cycles the pins will be scrubbed in the Wash Bath Number of seconds the Dryer will be on for 10 ↓ Start

Sanitizing Picking Pins

915. This next step ensures that the **picking pins & fluidics** are **flushed** with **sanitizing solution** (important for maintaining sterility during picking):

- a) Ensure that the **5L wash bottle** containing approximately **4L of SporKlenz solution** is attached to the **leftmost fluidics connection** on the ClonePix 2 instrument.
- b) Leave the **default** settings in the **Sanitise Options** dialog (30/5/10 as pictured).
- c) Click the Start button in the Sanitise Pins dialog to begin flushing the ClonePix 2 with SporKlenz. You will see the picking head move to the wash station and the pins inserted into the 8 ports within. You will see the Purge Progress dialog appear as well. Once this step is complete, click Next.

| Sanitise F | ins |
|------------|-----|
|------------|-----|

| Purge the pump system with sanitising agent | | |
|---|-----------------|---|
| Sanitise Options | | |
| Number of cycles for the head to purge | <mark>β0</mark> | - |
| Number of cycles the pins will be scrubbed in the Wash Bath | 5 | 1 |
| Number of seconds the Dryer will be on for | 10 | 1 |
| | | |
| | | |

| Molecular Devices |
|----------------------|
| Purge Progress |
| Stop |

UV Sanitizing ClonePix 2 Deck

10. This next step ensures that the **ClonePix 2 instrument deck** is **sterilized** with **UV light** (important for maintaining sterility during picking):

a) The **Ultra Violet Sanitise** dialog appears. Leave the **Duration of Ultra Violet exposure** setting at the **default** (600 seconds).

Ultra Violet Sanitise

1. Check the bed of the instrument is clear of plates.

2. Perform any manual cleaning tasks.

3. Set the number of seconds for the UV light to be on.

4. Click the 'Begin' button to turn the light on. You can stop at any time by clicking the 'Stop' button.

While the UV sanitise is in progress you can check the fluid level in the waste bottles and make sure the feed bottles are full.

| Ultra Violet Sanitise | | |
|------------------------------------|-------------|---------|
| Duration of Ultra Violet exposure: | \$00 | seconds |
| | | |
| | Begin | Stop |

UV Sanitizing ClonePix 2 Deck

10. This next step ensures that the **ClonePix 2 instrument deck** is **sterilized** with **UV light** (important for maintaining sterility during picking):

- a) The **Ultra Violet Sanitise** dialog appears. Leave the **Duration of Ultra Violet exposure** setting at the **default** (600 seconds).
- b) Click Begin to start UV sterilization this step takes 10 minutes. You will see the UV light illuminate the deck, as well as the UV light indicator on the instrument front panel illuminate.

| I | | | | | |
|---|---|--|-----------------------------|-----------------------|------------------------------------|
| Ultra Vio | let Sanitise | | | | |
| 1. Check t 2. Perform 3. Set the 4. Click th | he bed of the instrument any manual cleaning tas number of seconds for th e 'Begin' button to tum th | is clear of plates. ks. e UV light to be or e light on. You car | n. n stop at any time by | r clicking the 'Stop' | button. |
| While the | UV sanitise is in progress | you can check th | e fluid level in the w | aste bottles and ma | ake sure the feed bottles are full |
| - Ultra Vio | let Sanitise | | | | |
| Duration | of Ultra Violet exposure: | \$00 | seconds | | |
| | | | | | |
| | | Begin | Stop | | |
| | | | | | |
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Check Wash & Waste Bottles

11. This next step is a reminder to check that the **wash bottles** are **full** and that the **waste bottles** are **empty.**

- a) Ensure that the **wash bottles** (**SporKlenz 5L, Sterile Water 5L, 70% Ethanol 2L**) are at least **75% full** and that the **waste bottles** (wash & picking waste) are **empty**.
- b) Click the **Next** button to proceed.

| ClonePix 2 - Unsaved I | d Process | |
|------------------------|---|---|
| File View Tools | Help | |
| Molecular Devices | | 🔢 Prepare For Pick Run |
| Pin Fire Test | Check Bottles | |
| Alignment | | |
| Check Wash Bath | Check the fluid level in the wash bottle and make sure the waste bottle is empty. | |
| Wash Bath Utility | | |
| Sanitise Pins In | | |
| Sanitising Agent | | |
| UV Sanitise | _ | |
| Check Bottles | | |
| Samuse Fins | | |
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| Start Time: 9:14:28 AM | | CULA |
| | | CULAI |
| | | < Back Next > Cancel |
| | | 💡 Interior light is on. 🃍 Currently logged in as Molecular Device 🛛 🔱 All devices are connected! |

Rinsing Picking Pins

12. This next step ensures that the **picking pins & fluidics** are **flushed** with **sterile water** (important for removing sanitizing solution & maintaining sterility during picking):

- a) Disconnect the 5L wash bottle containing SporKlenz and then connect the 5L wash bottle containing approximately 4L of Sterile Water is attached to the leftmost fluidics connection on the ClonePix 2 instrument.
- b) Leave the **default** settings in the **Sanitise Options** dialog (30/5/10 as pictured).

Sanitise Pins

Purge the pump system to clear any air bubbles or sanitising agent

Rinsing Picking Pins

12. This next step ensures that the **picking pins & fluidics** are **flushed** with **sterile water** (important for removing sanitizing solution & maintaining sterility during picking):

- a) Disconnect the 5L wash bottle containing SporKlenz and then connect the 5L wash bottle containing approximately 4L of Sterile Water is connected to the leftmost fluidics connection on the ClonePix 2 instrument.
- b) Leave the **default** settings in the **Sanitise Options** dialog (30/5/10 as pictured).
- c) Click the Start button in the Sanitise Pins dialog to begin flushing the ClonePix 2 with Sterile Water. You will see the picking head move to the wash station and the pins inserted into the 8 ports within. You will see the Purge Progress dialog appear as well.

| Molecular Devices | |
|----------------------|------|
| | |
| Purge Progress | |
| | Stop |

Rinsing Picking Pins

12. This next step ensures that the **picking pins & fluidics** are **flushed** with **sterile water** (important for removing sanitizing solution & maintaining sterility during picking):

d) The **picking head** will now **automatically** move to the **ethanol wash bath** and **scrub** the **picking pins**, followed by **drying** of the pins in the **halogen drying station**. You will see the **Washing Pins indicator** followed by the **Drying Pins indicator** on your **computer screen** during these steps. At the conclusion of the drying step, click **Next** to continue.

Ethanol Wash Bath

Conclusion of Process

- 13. This final step will result in **completion** of the **Replace Head** process:
 - a) You will see the **Process Completed** screen appear. Click the **Finish** button to return to the **main menu.** Click the **Finish** button.

Process Completed

Click the Finish button to return to the process properties.

- b) Within the instrument, the following will occur **automatically**:
 - **Re-homing** of the **picking head**
 - Re-lidding and return of the source plate to the source stacker
 - **Closing** of the **illumination cover** (at the imaging station).
- c) Be sure to **remove** your **empty source plate** from the **source stacker** before proceeding with setting up a **Pick Run**.

Support Resources

- Go to the HELP menu within ClonePix 2 Software
- Support and Knowledge Base: <u>http://mdc.custhelp.com/</u>
- Request Support: <u>http://mdc.custhelp.com/app/ask</u> or via email <u>support@moldev.com</u>
- Technical Support can also be reached by telephone:
 - 1 (800) 635-5577
 - Select options for Tech Support → Biotherapeutics Products → ClonePix Instruments

MOLECULAR DEVICES

ADVANCING PROTEIN AND CELL BIOLOGY