Genetix

ClonePix[™] FL

Quick Set-Up Instructions

Software Release: 1.2.15.1032





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What are Quick Set-Up Instructions?

These instructions are designed to enable a new user to undertake a simple picking run on ClonePix[™] FL. The following instructions offer guidelines only. No attempt should be made to use ClonePix FL before the system has been fully installed by a Genetix Approved Engineer.

For full information please read the ClonePix FL **Robot Manual** and **Software Applications Manual**. Please refer to the Genetix website for the latest reagents & supplies, replacement parts and optional extras <u>www.genetix.com</u>

Starting up ClonePix FL

- Ensure robot and compressor are plugged in.
- Ensure compressed air gauge is set to 80 psi (5.5 bar).
- Ensure Emergency Stop button is not pressed in.
- Switch on ClonePix FL. The HEPA filtration system works all the time that ClonePix FL is on.
- Press Reset button at the front of ClonePix FL.
- Initiate ClonePix FL software.
- Wipe out ClonePix FL bed with 70% ethanol or fresh Genetix Sterilizing Agent (K8080) using lint-free cloth.
- Fill the ethanol feed bottle with 70% ethanol and empty the ethanol waste bottle.
- Make sure that the correct Picking Pins are installed for the type of cells to be picked.
 - F1 Picking Pins (400µm internal diameter; X4961) for suspension cell picking from semi-solid medium.
 - F2 Picking Pins (700μm internal diameter; X4962) for adherent cell picking from liquid medium.

Picking Pins should be cleaned by sonication in **aQuClean** (K2505) and autoclaved. It is advisable to autoclave the **Picking Pin Removal Key** (X4948) at the same time. See General Maintenance section of the Robot Manual for instructions.

If the Picking Pins need to be changed remove the **Picking Head** and swap the pins. To do this, click on the **Picking Head Management** icon, then the **Replace Head** icon and follow the on-screen instructions. Refer to the General Maintenance section of the Robot Manual for guidance on how to remove and replace the head and the pins.





Loading plates

It is important to know how to load plates into ClonePix FL correctly.



A ClonePix FL bed layout is as shown below.

The **Source plate stacker system** is located at the rear of the machine, and the **Destination plate stacker system** is at the front. Both the Source and Destination stackers have 1 removable **Feed cassette** (left hand side) and 1 removable **Return cassette** (right hand side).

Each cassette can accommodate up to 10 standard microplates or 12 Genetix microplates. The plates are fed individually from the Feed cassette onto the bed, with automatic lid removal as the plate passes the Return cassette position. On completion of colony picking, the de-lidded plate is then transferred to the Return cassette where the lid is replaced.

Important notes:

- Plates loaded into a Feed cassette must be of the same type and must match the plate type selected by the user in the software. Loading of a plate type different from that selected in the software is likely to damage the plate and the instrument.
- When the Return cassettes are placed in the source and destination stackers, they must be held firmly in place by use of the **Return cassette locking bolt** on the right hand side of the stacker systems. Failure to lock down a Return cassette will cause a malfunction in the return of the microplates.
- Source and destination plates must be loaded into the Feed cassettes with lids on and well A1 in the front right-hand corner as shown below:





Orientation of source plates in ClonePix FL



Orientation of destination plates in ClonePix FL

Preparing for a Pick Run

Prior to carrying out a picking run, it is important to make sure that ClonePix FL is set up correctly. The **Prepare for Pick Run** process is designed for this purpose. This process helps the user to validate that 1) the pins are firing correctly, 2) the camera, pins and microplates are aligned, and 3) the fluid system is sterile and ready for use.

• Click on the Prepare for Pick Run icon and follow the on-screen instructions.

When asked to load a source plate, it is recommended that you use a blank **Genetix PetriWell-6** plate. However, the use of any configured plate (those present in the drop-down list) should work. Once aligned using a configured plate type, ClonePix FL is then ready for use with any configured plate type.

The Prepare for Pick Run process does not need to be carried out prior to every picking run. It must be carried out every time that ClonePix FL is first powered up.

Click Close Process to return to the Main Menu.



The Pick Run

The **Pick Run** process is designed to aid new users through their first picking run. Use of the default parameters provided should facilitate successful imaging, colony selection and picking. The description below assumes that you have plates of cell colonies with fluorescent halos of secreted protein.

• Click on the Pick Run icon to open the following screen:

File Viev	v Tools	Help
Conoti	~	
Genet	X	
Summary	Details	Guide
Pick	Run	
Image mic	roplate and	d pick colonies from specified criteria.
Imaging	n Setting	19
Integrity	Jootang	5
Run Annota Source We	ation: Il plates:	Genetix Petri) Vell 6 Plate
Barcode O	n prates.	Read Barcode: True
Darcodeo	puons.	Auto-assign barcode in case of failure: False
Batch plate	5:	False
Source Plat	e Options:	Prompt for more plates when cassette is empty
Acquisition	Options:	Undefined
		Prime: Undefined
Review Col	ony Selecto	n: First Cycle
Picking	Setting	
Destination	well plates:	Genetix PetriWell-96 Plate
Destination	wells:	All wells will be available
BarcodeO	ptions:	Read Barcode: True Auto-assign barcode in case of failure: False
Deposit Op	tions:	Fill Destination Plates.
Dest. Plate	Options:	Prompt for more plates when cassette is empty
Pick Numbe	er Options:	Organise By: Plate Limit the number of colonies picked: false
Pin Options	c	Aspirate Volume: 5
		Dispense Volume: 7
		Adherent Colonies: True Suspension Colonies: False
		Picking Height Adjustment:-0.1
Dispersal C	Options:	Use Dispersal: False
		Dispersal Volume: 20
		Dispersal Cycles: 10
PICK Progre	ess Options:	Display Image Before Pick: False Display Image After Pick: False
0	D:- 0	4
Sanitis	e Pin Op	uons
Sanitise Pir	n Options:	Purge Cycles: 3
		Daun Cycles: 3 Dry Time: 10
		Start

The settings can be edited by clicking once on the green section headings.



Imaging Settings

• Click on the **Imaging Settings** title to open the following screen:

age microplate and pick colonie	es from specified criteria.
maging Settings	
Run Annotation:	Run 1
Source Well plates:	Genetix 6 well black
Barcode Options:	Read Barcode
	Auto-assign barcode in case of failure
Batch plates:	V
Source Plate Options:	Promot for more plates when casesta is smoth
	 Finish when cassette is empty
Acquisition Options:	
Review Colony Selection:	Batch - Review All

Fill in the following information:

Run Annotation

Enter a name to identify this run.

• Source Well Plates

Enter the source plate type that you will use for this run.

- Barcode Options
 - Select Read Barcode.
 - Select Auto-assign barcode in case of failure.

If you don't have barcodes on your plates, ClonePix FL will automatically assign a code.

Batch plates

Select this option. This assumes that you are loading multiple plates that contain the same sample and that you want them to be processed as a single experiment.

- Source Plate Options Select Finish when cassette is empty.
- Acquisition Options

This option enables you to choose the images that you wish to capture. If you see appropriate Image Acquisition options select them here. If not, the acquisition options can



be created later in Preview (see below). Most users select one white light and one fluorescent option.

- **Prime Probe** The Prime Probe is the acquisition option to be used for colony detection. Select the white light option if available.
- Review Colony Selection Select Batch – Review All.
- Click Apply.

Picking Settings

• Click on the **Picking Settings** title to open the following screen:

Senetix ClonePix FL - Unsaved Process				
File View Tools Help				
Genetix				
Summary Details Guide				
Pick Run				
Image microplate and pick colonies fro	im specified criteria.			
Picking Settings				
Destination Well plates:	Genetix PetriWell-96 Plate			
Destination Wells:	1 2 3 4 5 6 7 8 9 10 11 12			
	A B C D F O F O <td< td=""></td<>			
Barcode Options:	Read Barcode Auto-assign barcode in case of failure			
Deposit Options:	Match Destination plate to Source plate			
Dest. Plate Options:	Prompt for more plates when cassette is empty Finish when cassette is empty			
Pick Number Options:	Organise By Plate Imit Colonies Imit Colonies Imit Colonies			
Pin Options:	Advant.			
	5 Mindale			
	Suspension Suspension Suspension Suspension Suspension Suspension Suspension Suspension			
	Pick Height Adjustment			
	Aspirate Volume (µl) 7			
Disease in the state				
Dispersal Options:				
	Dispersal Volume 20			
Pick Progress Options:	Image Before Pick			
A¢	oply Cancel			

Fill in the following information:

- **Destination Well Plates** Enter the plate type that you will use for this run.
- **Destination Well Plates** Specify the wells into which you want to deposit. Right click on the mouse to select wells, and left click to de-select wells. All destination plates will be filled using this template.
- Barcode Options
 - Select Read Barcode.
 - Select Auto-assign barcode in case of failure.

If you don't have barcodes on your plates, ClonePix FL will automatically assign a code.

- Deposit Options
 - Select **Match Destination Plate to Source Plate** if you want to start a new destination plate each time a new source plate is fed in.
- Destination Plate Options Select Finish when cassette is empty.
- Pick Number Options Leave as default = Organise by Plate.
- Pin Options
 - Select the type of colonies that you wish to pick: suspension or adherent.
 - Pick Height Adjustment: Leave as default.
 - Aspirate Volume: Leave as default = 5µl.
 - **Dispense Volume**: Leave as default = 7µl.
- Dispersal Options
 - Use dispersal: Select if you want to spread out colony cells after picking.
 - Dispersal cycles: Use 3-6 for CHO cells and 6-10 for hybridomas.
 - **Dispersal volume**: Leave as default = 20µl.
- Pick Progress Options
 - Leave as default = unselected.
- Click Apply.

Sanitize Pin Options

Leave as default - no changes required.

Start Pick Run

- Click Start to begin the Process.
- When prompted, load your cell colony plates into the Source Feed cassette.

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Preview

This is where you set up your Image Acquisition and Colony Detection settings. The screen should look like this:

🖒 Genetix ClonePix	FL - Unsaved Process	
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Genetix		\$19 Pick Bun
denetix		
Select Pick Options	Chalinting Coulter Dist Dusking	H () N - Pike Selection
Preview	statistics scatter mot manking	
Select Wells		
Summary		- Wel Map
Route		1 2 3
Select Groups		
Pick Colonies		
Sanitise Pins		
Finish		
		- 30 View
Stud Time: 15:39-03	Palette Export 👌 🚥 🔍 🧊 👘 🗴 🛛	View Acquisition Detection Groups Information
over time: to:act02		(Park Nast Carral
		💡 Andrea Gough 🐰 Connected

Step 1: Set up Image Acquisition settings

- Click on the **Acquisition** tab.
- If the My Configurations box is only populated with Default (see screenshot on right), you will need to set up white light and fluorescent Image Acquisition options.
- Click **New** and then click **Edit**. The following settings should work for most scenarios:

Description	White Light	FITC 1s
Excitation Filter	WHITELIGHT (TRANS)	EGFP/FITC
Exp. Time (ms)	150	1000
LED Intensity	3	128
Camera Focus	2200	2200
Display Color	White	Green

Default > Prime Configuration New Delete Default Sattings Default Edit Description Default Edit Sattings Default Edit Description Default Edit Sattings O Save Emission Filter VHITELIGHT (EPI) Grab Image LED Intensity 0 128 IOI Display Colour 0 + 9170 2200 Display Colour Compatibility 0 128 IOI
Prime Configuration New Delete Description Edul Exochation Filter WHITELIGHT Edul Exotation Filter WHITELIGHT Grab Image LED Intensity 0 128 100 Camera Focus 0 + 9170 2200 Display Colour 0 + 9170 2200
Settings Description Description Description Description Excitation Filter WHITELIGHT (EP) Save Excitation Filter WHITELIGHT Grab Image LED Intensity 0 128 100 Camera Focus 0 + 9170 2200 0 0 + 9170 2200 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Can Can Excitation Filter VHITELIGHT (EP) Save Envision Filter VHITELIGHT Grab Image LED Intensity 0 128 Camera Focus 0 120 Display Colour 0 9
Emission Filer WHITELIGHT Grab Image Exp. Time [me] 200 200 200 LED Intensity 0 128 100 Camera Focus 0 497 2200 Display Colour 0 9170 2200
Exp. Time [me] 200 3 Gaumage LED Intensity 0 128 100 Camera Focus 0 + 9170 2200 Display Colour 0 + 9170 2200
LED Intensity Camera Focus 0 128 100 0 + 9170 2200 0 + 9170 2200 0 ← 9170 2200 0 ← 917 0 ← 917 0 ← 917 0 ← 917 0 ← 91 0 ←
Camera Focus Display Colour Composite Image
Display in composite image
Brightness
Contrast

• Click **Save** to store each option.

 You should now have 3 options under My Configurations: Default, White Light and FITC 1s. Insure that White Light and FITC 1s options are selected and that Default is deselected. Do not delete the Default option – it is required for other ClonePix FL functions.

My Configurations	
📃 Default	
🔽 White Light	
🔽 FITC 1s	

- Set the Prime Configuration to White Light. This is critical for correct colony detection.
- To capture your images either click **Grab Image** which will capture images for the area currently highlighted in the View tab, or go to the View tab and click on the area that you wish to image.
- You should now see an image with two new tabs above it named White Light and FITC 1s. Toggle between the tabs to inspect the images.
- Red pixels on the image indicate that it is overexposed. In this case, lower either the exposure time or LED Intensity and Grab Image again. Adjust until there are no red pixels.

Step 2: Set up Detection settings

- Click on the **Detection** tab.
- Set Algorithm to Local Threshold.
- Using the image under the White Light tab, set the Average Colony Diameter to a size that best detects your colonies. This will probably be between 0.25 and 0.70mm depending on the size of your colonies. You will need to click **Reprocess** after moving the slide bar.
- Leave **Exterior Diameter Multiplier** at the default setting (x3).
- Deselect Use each colony size when calculating exterior statistics.
- Leave Display settings as default (only **Display Detected Colonies** and **Shade Overlap Areas** selected).
- Click Next to proceed.





Select Wells

Selected wells are shown as red.

• Click Next to proceed.

Summary

Click Next to proceed.

Imaging

ClonePix FL will now automatically image the source plates, detect the colonies and generate combined data for all plates.

 While ClonePix FL is imaging, pre-fill one or more 96-well destination plates with liquid medium (150µl recommended) and place in an incubator to equilibrate.

Results

Imaging results are displayed in the **Results** screen:

- Click on the Groups tab.
- This shows which colonies are excluded by which parameter. The **Too Small** group cut-off point can be reduced by clicking on the bottom line (reads Total Area <=0.1 by default) and then dragging the slide bar on the histogram at the bottom of the screen to 0.05.
- Irregular and Proximity group cut-off points can be reduced but this may compromise clonality.
- Colonies in the Accept group are those that have good shape and distance from other colonies.
- Click on All Features to see the total number of detected features and the total number of pickable colonies (shown in the Spot Count box).





- To further isolate only those in the Accept group that have highest associated FITC fluorescence, click New Group. This will bring up a new group at the top.
- Click on the newly-created group (top line) to select it and click **Decrease Priority** multiple times so that it sits just above the Accept group.
- Re-name the group by typing 'High FITC' in the box at the bottom of the pane. Press Enter to save it.
- Click the Colour button and choose an appropriate color.
- Click on the bottom line for this group (reads ID = "" by default) to bring up a blank histogram box at the bottom of the pane.
- In the 'Property' drop-down list, select [FITC] Exterior Mean Intensity. In the Operator drop-down list, select '>' and then use the vertical slide bar to select a group of high FITC colonies.
- Click Next to proceed.



Select Groups

• In the Select Groups window, select only the High FITC group:

<u>Eile V</u> iew <u>T</u> ools I	<u>t</u> elp			
Genetix				
Select Pick Options Preview Select Wells Summary Imaging Results Select Groups Pick Colonies Sanitise Pins Finish	Filter Groups Check Groups To Pick Edg Too Too Irreg Prox V Higt Othe	e Excluded Big Small Ular 1 Ular 2 Ular 2 FITC FITC	Colony Informatic Total: High FITC Edge Excluded Irregular 1 Irregular 1 Too Big Too Small Proximity Other Pick Number:	n 3432 96 53 95 134 34 60 148 2812 96
	Sort Options Organised By Plate Order By [FITC 1s] Exterior Mean Intensit		

- Under Sort Options, select Order By [FITC 1s] Exterior Mean Intensity.
- Click Next to proceed.



Picking

- Before picking can commence, you will be prompted to swap the source cassettes. Remove
 the empty Feed cassette (it can be placed at the back of the ClonePix FL bed). Next, unlock
 the Return cassette and carefully transfer it to the left side of the source stacker. <u>There is no
 need to re-arrange the plates in the cassette</u>. Finally, place the empty cassette into the right
 side of the stacker and lock it in place. Failure to lock down the Return cassette will cause a
 malfunction in the return of the microplates.
- Switch on the ethanol wash pump at the front of ClonePix FL. Check that the ethanol bath has filled with ethanol.
- When prompted, load the destination plate(s) into the Destination Feed cassette.
- Click Next to proceed.
- The picking step will proceed automatically until all colonies in the selected group have been collected.

Finish

- Click **Finish** to return to the Pick Run Process top page.
- Click Close Process to return to the Main Menu. If you have not saved your Process settings, you will be prompted to do so.
- To view the results of the picking run, click on the **Review Results** icon.

Powering down ClonePix FL

- Switch off the ethanol wash pump.
- Shut down ClonePix FL software.
- Shut down the computer and wait for it to switch off.
- Power down ClonePix FL.



Contact Details

Corporate Headquarters

Genetix Ltd

Queensway, New Milton Hampshire BH25 5NN, UK

Tel: +44 (0) 1425 624 600 Fax: +44 (0) 1425 624 700

Visit www.genetix.com for contact details of your nearest Genetix sales and support office.

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