Genetix

ClonePix™ FL

Quick Set-Up Instructions

Software Release: 1.2.15.1032

www.genetix.com

07MAN1055.A3
Effective Date: 27-May-11

Contents

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 www.genetix.com

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.

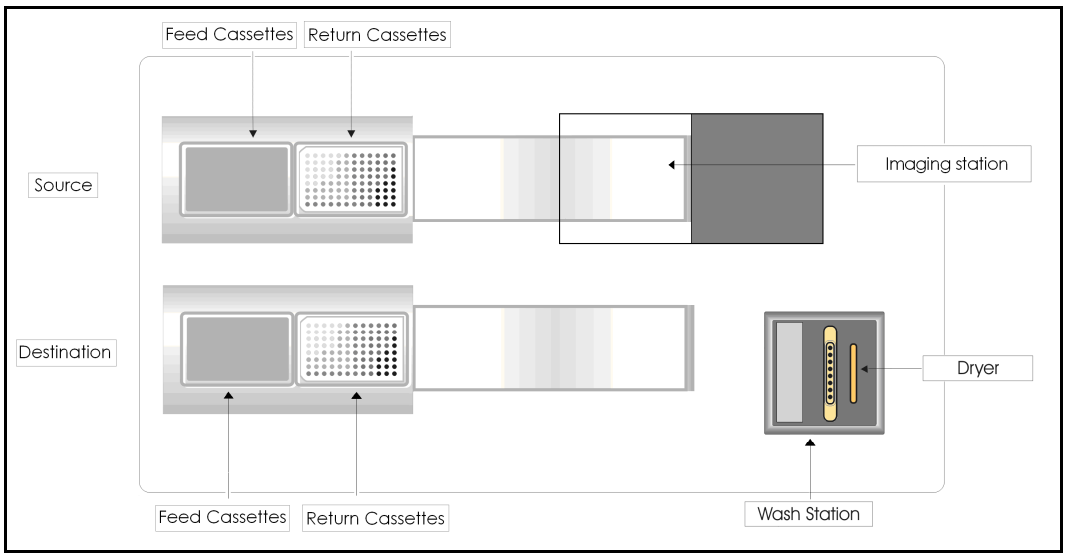
  Picking Pins should be cleaned by sonication in **aQuClean** (K2505) and autoclaved. It is advisable to autoclave the **Picking Pin Removal Key** (X4948) at the same time. See General Maintenance section of the Robot Manual for instructions.

  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).

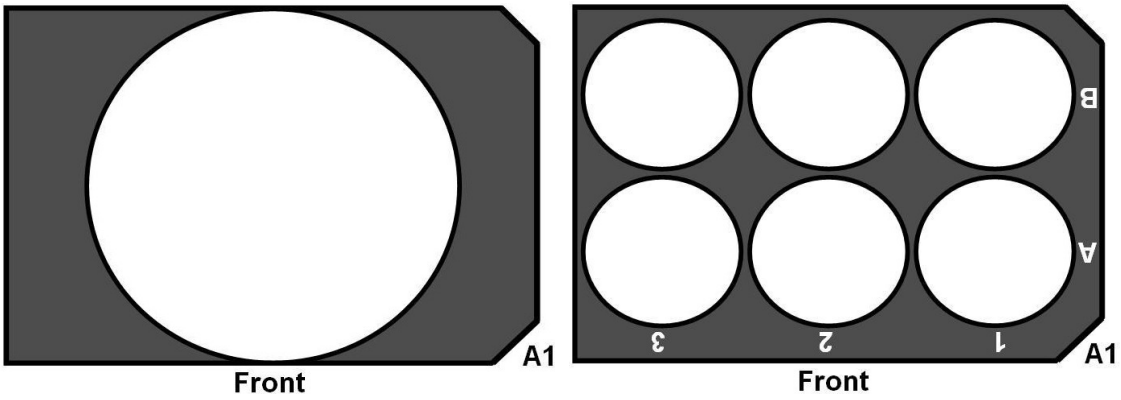
Each cassette can accommodate up to 10 standard microplates or 12 Genetix microplates. The plates are fed individually from the Feed cassette onto the bed, with automatic lid removal as the plate passes the Return cassette position. On completion of colony picking, the de-lidded plate is then transferred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

Important notes:

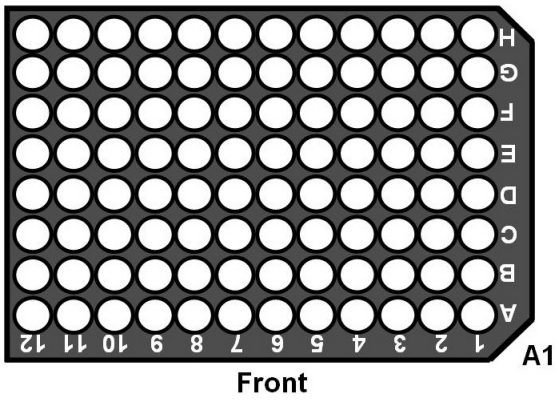
- Plates loaded into a Feed cassette must be of the same type and must match the plate type selected by the user in the software. Loading of a plate type different from that selected in the software is likely to damage the plate and the instrument.

- 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.

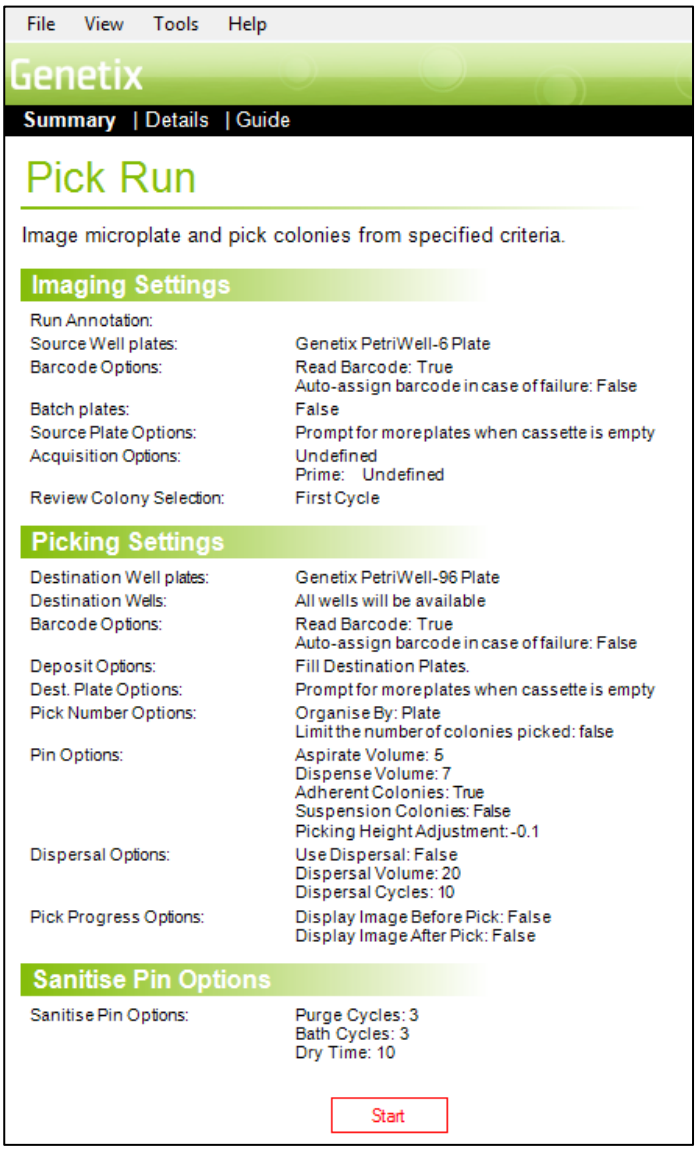
The Prepare for Pick Run process does not need to be carried out prior to every picking run. It must be carried out every time that ClonePix FL is first powered up.

- 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.

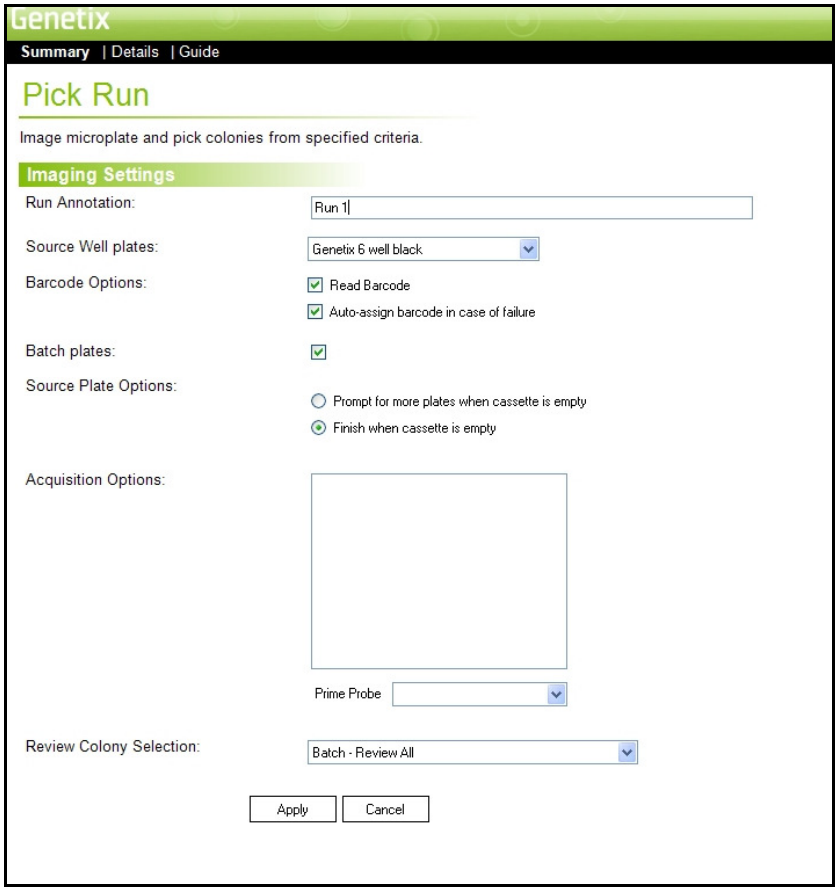
- Click on the **Pick Run** icon to open the following screen:



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:



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**.

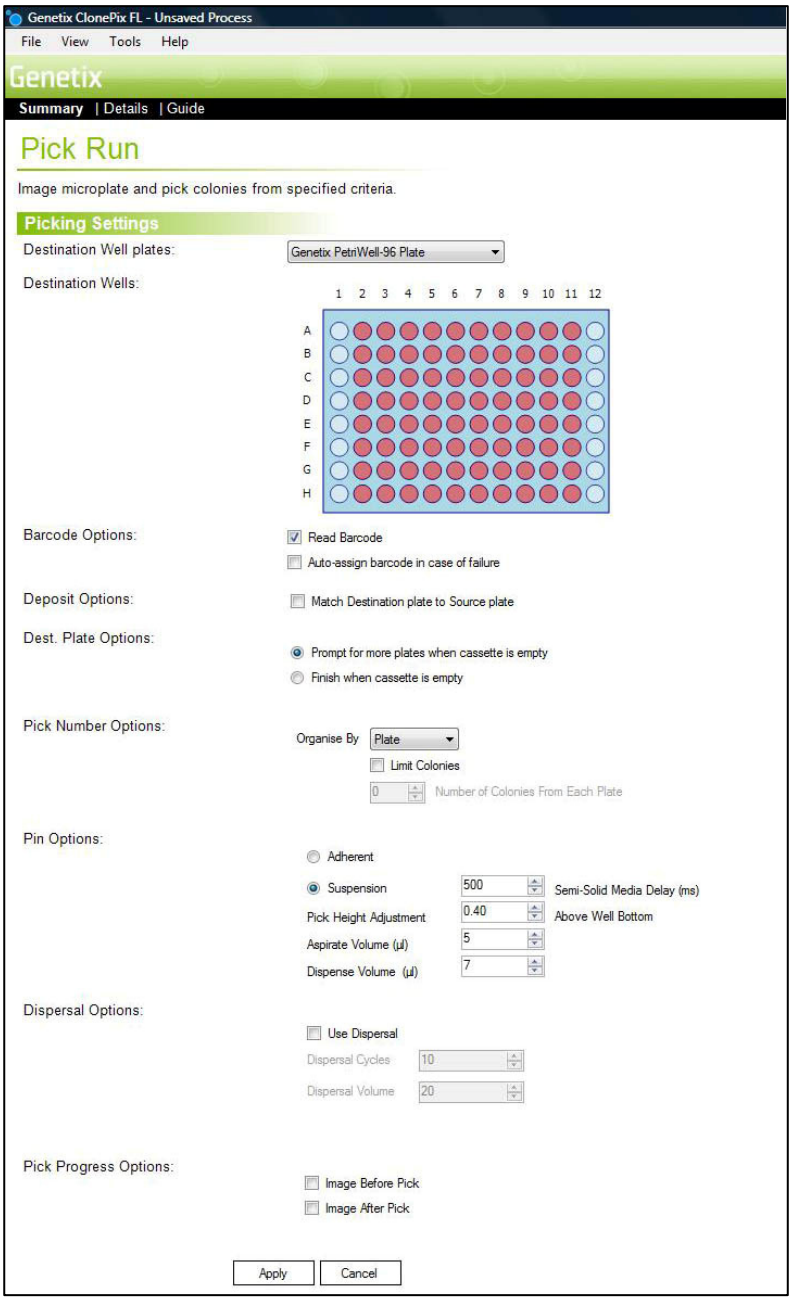
  If you don't have barcodes on your plates, ClonePix FL will automatically assign a code.
- **Batch plates**
  Select this option. This assumes that you are loading multiple plates that contain the same sample and that you want them to be processed as a single experiment.
- **Source Plate Options**
  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:



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

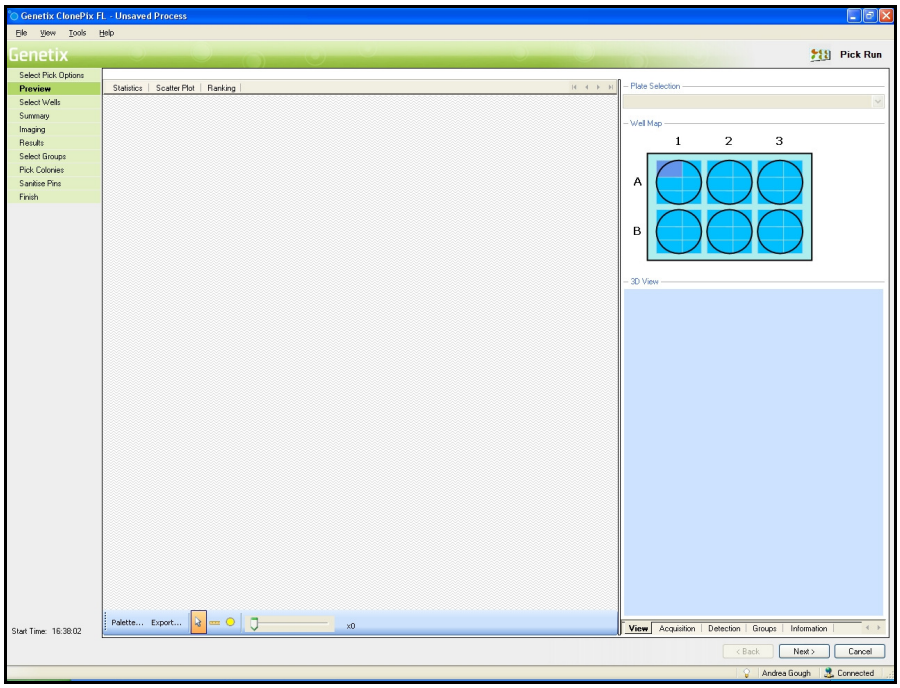
Leave as default – no changes required.

Start Pick Run

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

Preview

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

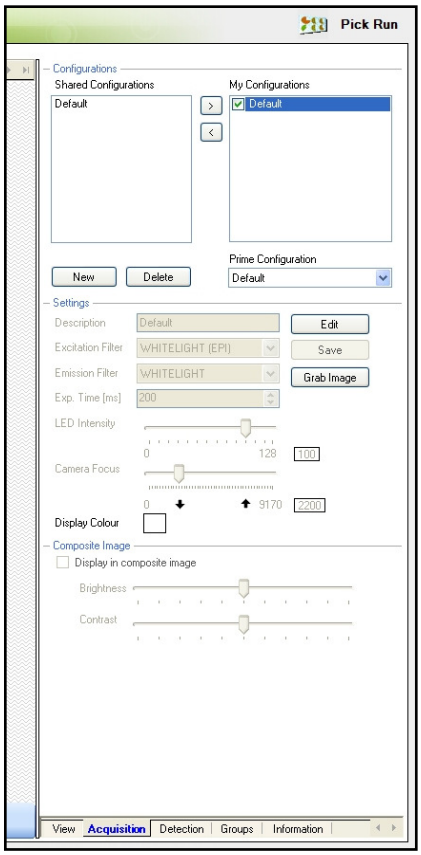


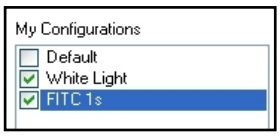
Step 1: Set up Image Acquisition settings

- 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:

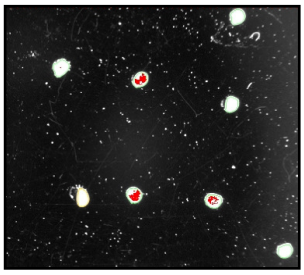
Description	White Light	FITC 1s
Excitation Filter	WHITELIGHT (TRANS)	EGFP/FITC
Exp. Time (ms)	150	1000
LED Intensity	3	128
Camera Focus	2200	2200
Display Color	White	Green

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



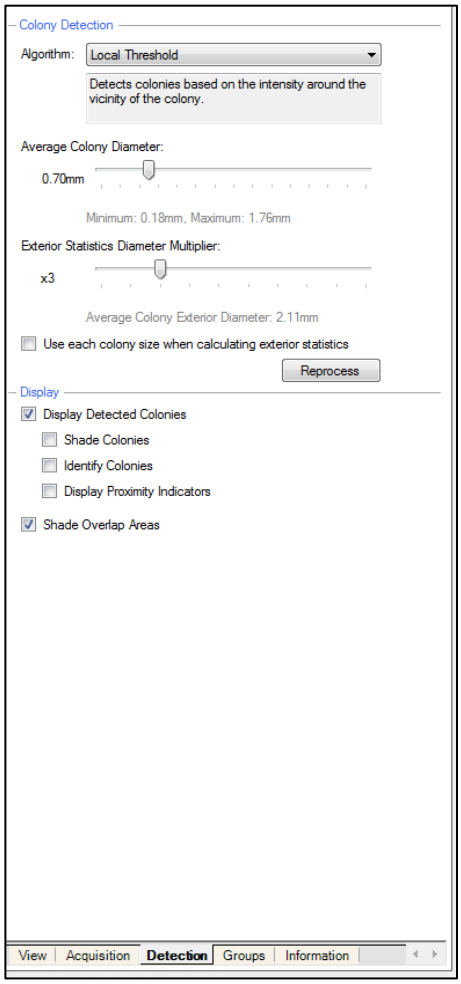


- You should now have 3 options under My Configurations: Default, White Light and FITC 1s. Insure that White Light and FITC 1s options are selected and that Default is deselected. Do not delete the Default option – it is required for other ClonePix FL functions.
- Set the **Prime Configuration** to White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

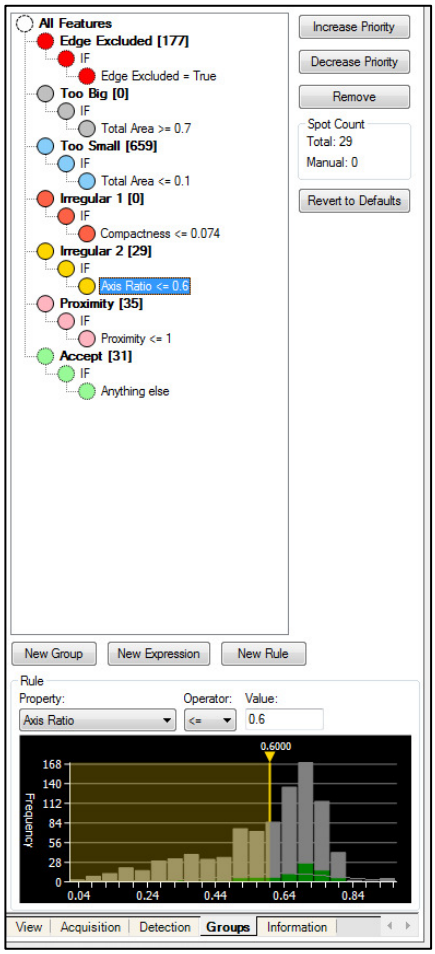
ClonePix FL will now automatically image the source plates, detect the colonies and generate combined data for all plates.

- 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.

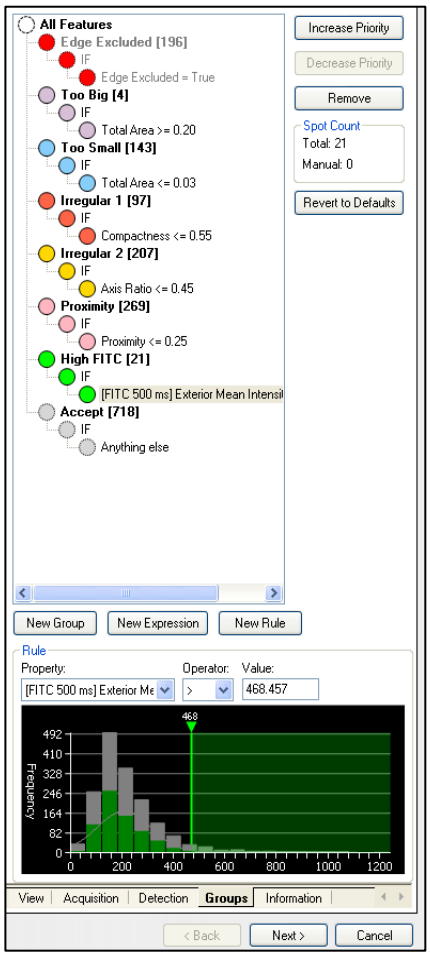
Results

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).

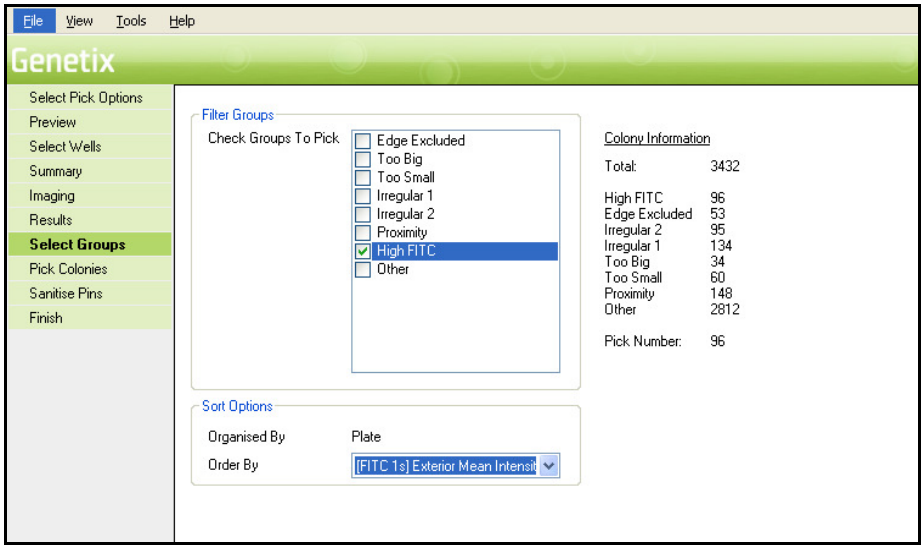


- To further isolate only those in the Accept group that have highest associated FITC fluorescence, click **New Group**. This will bring up a new group at the top.
- Click on the newly-created group (top line) to select it and click **Decrease Priority** multiple times so that it sits just above the 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:



- 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. There is no need to re-arrange the plates in the cassette. 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.

Revised: 6 May 2011