

# ClonePix 2 Training Guide

Preparing For & Setting Up A Pick Run



Date Revised 11/18/2016 Version C

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# Chapter Purpose

The purpose of this chapter is to guide the user through the basics of setting up a pick run on the ClonePix2.

This guide does not include detailed descriptions around sample prep, statistics, etc. Please refer to corresponding chapters for details on these topics.



# System Startup/Instrument Preparation

1. Ensure that the **robot** and **compressor** are plugged in.
2. Turn on the **compressor** and ensure compressed **air gauge** is set to **80 psi (5.5 bar)**.
3. Ensure **Emergency Stop** button on the front right of the instrument is **not** pressed in.
4. Switch **on** ClonePix 2. The **HEPA** filtration system works all the time that ClonePix 2 is on.
5. After approximately **2 minutes**, launch the **ClonePix 2 software** by double clicking on the icon.
6. Wipe out ClonePix 2 bed with **70% ethanol** or fresh **Sterilizing Agent** (SporKlenz [https://us.vwr.com/store/catalog/product.jsp?product\\_id=4621746](https://us.vwr.com/store/catalog/product.jsp?product_id=4621746)) using a lint-free cloth.
7. Fill the **ethanol feed bottle** with **70% ethanol** and empty the **ethanol waste bottle**.
8. Make sure that the correct **Picking Pins** are installed for the type of cells to be picked.
  - **F1 Picking Pins** (400 um internal diameter; X4961) for **suspension cell picking from semi-solid medium**.
  - **F2 Picking Pins** (700 um internal diameter; X4962) for **adherent cell picking from liquid medium**.

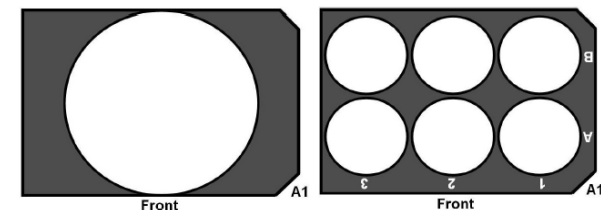
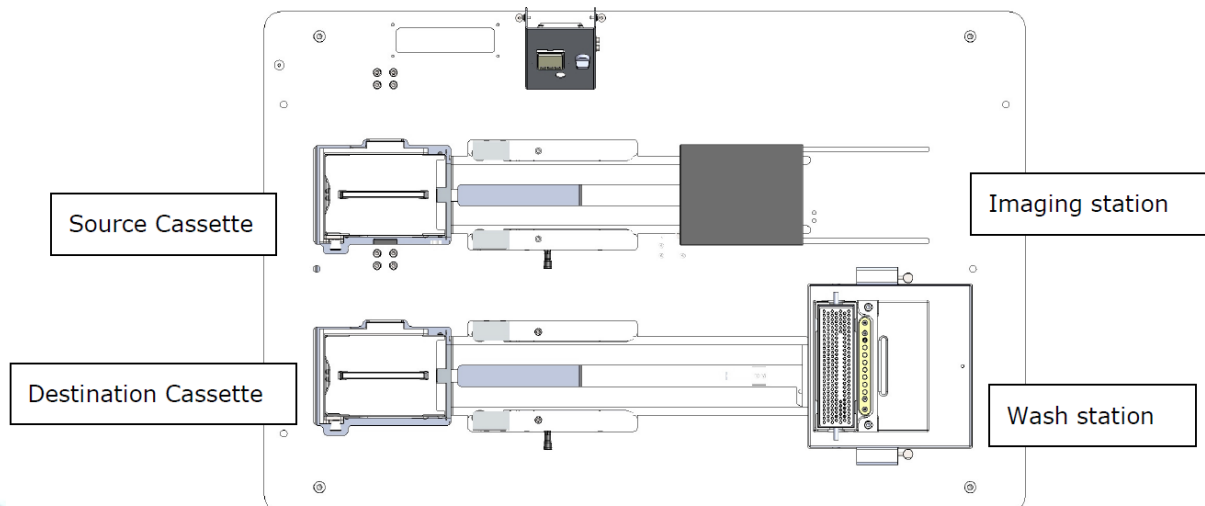
# Picking Pin Preparation

- **Picking Pins** should be cleaned by **sonication in aQuClean (K2505)** and **autoclaved** prior to use.
  - It is advisable to autoclave the **Picking Pin Removal Key (X4948)** at the same time.
  - See training module on **Picking Head Maintenance OR General Maintenance** section of the **Robot Manual** for detailed 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 training module on **Picking Head Maintenance OR General Maintenance section of the Robot Manual** for guidance on how to remove and replace the head and the pins.

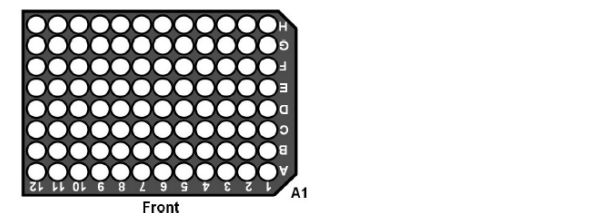


# Loading Plates into Stackers

1. Load plates into the appropriate plate holders. Generally, **source plates** are loaded into **GREEN** holders, **destination plates** into **BLUE** holders.
2. Raise the stacker cassettes either manually or via clicking on the Raise Source or Raise Destination buttons in the software.
  - **Note:** When the cassettes are placed in the source and destination stackers, they must be held firmly in place by the locking bolt on the front left hand side of the stacker systems. Failure to lock a cassette may cause a malfunction of the collection and/or return of the microplates.
3. Place **plates + holders** into the appropriate **stacker cassettes**, with **lids on** and **well A1 in the front right-hand corner** (see below).
  - Each stacker holds a maximum of **10 plates**.
  - Plate holders must be placed **level** into the cassette and **all the way to the back of the cassette** to ensure correct positioning.



Orientation of source plates in ClonePix 2



Orientation of destination plates in ClonePix 2

# Prepare for Pick Run

The **Prepare for Pick Run** process is designed to validate that:

- 1) The **picking pins** are firing correctly,
- 2) the **camera, pins and microplates** are aligned, and
- 3) the **fluid system** is sterile and ready for use.

- 1) From the **Main Navigation Screen**, click on the **Prepare for Pick Run icon** and follow the on-screen instructions
- 2) When prompted to load a **source plate**, it is recommended that a **blank source plate of the type that will be used for the pick run** be used.
- 3) Follow the prompts to carry out the pin firing, alignment, and fluid handling tests. When the tests are all complete, click **Close Process** to return to the **Main Navigation Screen**.



# Carrying Out A Pick Run: Imaging Settings

- 1) From the **Main Navigation Screen**, click on the **Pick Run** icon. You will see the following screen appear. Click on the **Imaging Settings** heading to edit the parameters within:

**Summary** | **Details** | **Guide**

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation:  
Source Microplates: PetriWell-6 Plate  
Barcode Options: Read Barcode: True  
Auto-assign barcode in case of failure: False  
Batch plates: False  
Source Plate Options: Prompt for more plates when cassette is empty  
Acquisition Options: Undefined  
Prime: Undefined  
Review Colony Selection: First Cycle

### Picking Settings

Destination Microplates: PetriWell-96 Plate  
Destination Wells: All wells will be available  
Barcode Options: Read Barcode: True  
Auto-assign barcode in case of failure: False  
Deposit Options: Fill Destination Plates.  
Dest. Plate Options: Prompt for more plates when cassette is empty  
Pick Number Options: Organise By: Plate  
Limit the number of colonies picked: false  
Pin Options: Aspirate Volume: 5  
Dispense Volume: 7  
Adherent Colonies: True  
Suspension Colonies: False  
Picking Height Adjustment: -0.1  
Audit Options: Allow Target and Aspirate images to be acquired but not saved.  
Dispersal Options: Use Dispersal: False  
Dispersal Volume: 20  
Dispersal Cycles: 10

### Sanitise Pin Options

Sanitise Pin Options: Purge Cycles: 3  
Bath Cycles: 3  
Dry Time: 10

**Start**



# Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

a) **Run Annotation** - Enter a name to identify this run.

Summary | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation: Experiment 1023

Source Microplates: PetriWell-6 Plate

Barcode Options:  
 Read Barcode  
 Auto-assign barcode in case of failure

Batch plates:

Source Plate Options:  
 Prompt for more plates when cassette is empty  
 Finish when cassette is empty

Acquisition Options:

- Default
- Trans WL
- FITC 8s
- CFP 8s
- Cy5 1500ms
- FITC 500ms
- Rhod 200ms
- Cy5 500ms
- FITC 200ms
- TransWL - Adherent
- FITC 1000ms

Prime Configuration: Trans WL

Review Colony Selection: Batch - Review All

Apply

Cancel



# Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

- a) **Run Annotation** - Enter a name to identify this run.
- b) **Source Microplates** – Select your source plate type from the dropdown menu.

Summary | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation:

**Source Microplates:**

Barcode Options:  Read Barcode  
 Auto-assign barcode in case of failure

Batch plates:

Source Plate Options:  Prompt for more plates when cassette is empty  
 Finish when cassette is empty

Acquisition Options:

- Default
- Trans WL
- FITC 8s
- CFP 8s
- Cy5 1500ms
- FITC 500ms
- Rhod 200ms
- Cy5 500ms
- FITC 200ms
- TransWL - Adherent
- FITC 1000ms

Prime Configuration:

Review Colony Selection:

# Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

- a) **Run Annotation** - Enter a name to identify this run.
- b) **Source Microplates** – Select your source plate type from the dropdown menu.
- c) **Barcode Options**
  - \* If your source plates are barcoded, select **Read Barcode**.
  - \* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.

Summary | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation:

Source Microplates:

Barcode Options:  Read Barcode  
 Auto-assign barcode in case of failure

Batch plates:

Source Plate Options:  Prompt for more plates when cassette is empty  
 Finish when cassette is empty

Acquisition Options:

- Default
- Trans WL
- FITC 8s
- CFP 8s
- Cy5 1500ms
- FITC 500ms
- Rhod 200ms
- Cy5 500ms
- FITC 200ms
- TransWL - Adherent
- FITC 1000ms

Prime Configuration:

Review Colony Selection:

# Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

- a) **Run Annotation** - Enter a name to identify this run.
- b) **Source Microplates** – Select your source plate type from the dropdown menu.
- c) **Barcode Options**
  - \* If your source plates are barcoded, select **Read Barcode**.
  - \* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.
- d) **Batch plates:** Enable this option. This assumes that multiple plates containing the same sample are being loaded and they will be processed as a single experiment.

**Summary** | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation: Experiment 1023

Source Microplates: PetriWell-6 Plate

Barcode Options:  
 Read Barcode  
 Auto-assign barcode in case of failure

**Batch plates:**

Source Plate Options:  
 Prompt for more plates when cassette is empty  
 Finish when cassette is empty

Acquisition Options:

- Default
- Trans WL
- FITC 8s
- CFP 8s
- Cy5 1500ms
- FITC 500ms
- Rhod 200ms
- Cy5 500ms
- FITC 200ms
- TransWL - Adherent
- FITC 1000ms

Prime Configuration: Trans WL

Review Colony Selection: Batch - Review All

Apply Cancel

# Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

- a) **Run Annotation** - Enter a name to identify this run.
- b) **Source Microplates** – Select your source plate type from the dropdown menu.
- c) **Barcode Options**
  - \* If your source plates are barcoded, select **Read Barcode**.
  - \* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.
- d) **Batch plates:** Enable this option. This assumes that multiple plates containing the same sample are being loaded and they will be processed as a single experiment.
- e) **Source Plate Options:** Select **Finish when cassette is empty**.

Summary | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation:

Source Microplates:

Barcode Options:

- Read Barcode
- Auto-assign barcode in case of failure

Batch plates:

Source Plate Options:

- Prompt for more plates when cassette is empty
- Finish when cassette is empty

Acquisition Options:

- Default
- Trans WL
- FITC 8s
- CFP 8s
- Cy5 1500ms
- FITC 500ms
- Rhod 200ms
- Cy5 500ms
- FITC 200ms
- TransWL - Adherent
- FITC 1000ms

Prime Configuration:

Review Colony Selection:

# Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

- a) **Run Annotation** - Enter a name to identify this run.
- b) **Source Microplates** – Select your source plate type from the dropdown menu.
- c) **Barcode Options**
  - \* If your source plates are barcoded, select **Read Barcode**.
  - \* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.
- d) **Batch plates:** Enable this option. This assumes that multiple plates containing the same sample are being loaded and they will be processed as a single experiment.
- e) **Source Plate Options:** Select **Finish when cassette is empty**.
- f) **Acquisition Options:** This option provides a choice of which images to capture. If there are appropriate Image Acquisition options select them here. ***For instance, Trans WL is generally always selected, plus any fluorescence options.*** Note that specific acquisition options (such as exposure times) can also be created later.

The screenshot shows the 'Pick Run' dialog box with the following settings:

- Summary | Details | Guide** (tabs)
- Pick Run** (title)
- Image microplate and pick colonies from specified criteria.
- Imaging Settings** (section header)
- Run Annotation: Experiment 1023
- Source Microplates: PetriWell-6 Plate
- Barcode Options:  Read Barcode,  Auto-assign barcode in case of failure
- Batch plates:
- Source Plate Options:  Prompt for more plates when cassette is empty,  Finish when cassette is empty
- Acquisition Options:** (highlighted with a red box)
  - Default
  - Trans WL
  - FITC 8s
  - CFP 8s
  - Cy5 1500ms
  - FITC 500ms
  - Rhod 200ms
  - Cy5 500ms
  - FITC 200ms
  - TransWL - Adherent
  - FITC 1000ms
- Prime Configuration: Trans WL
- Review Colony Selection: Batch - Review All
- Buttons: Apply, Cancel

# Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

g) **Prime Configuration:** This is the acquisition option to be used for colony detection. Select the **Trans WL** option from the dropdown.

**Summary** | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation: Experiment 1023

Source Microplates: PetriWell-6 Plate

Barcode Options:  Read Barcode  
 Auto-assign barcode in case of failure

Batch plates:

Source Plate Options:  Prompt for more plates when cassette is empty  
 Finish when cassette is empty

Acquisition Options:

- Default
- Trans WL
- FITC 8s
- CFP 8s
- Cy5 1500ms
- FITC 500ms
- Rhod 200ms
- Cy5 500ms
- FITC 200ms
- TransWL - Adherent
- FITC 1000ms

**Prime Configuration** | Trans WL

Review Colony Selection: Batch - Review All

Apply Cancel



# Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

g) **Prime Configuration:** This is the acquisition option to be used for colony detection. Select the **Trans WL** option from the dropdown.

h) **Review Colony Selection:** Select **Batch – Review All** from the dropdown.

Summary | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation:

Source Microplates:

Barcode Options:

- Read Barcode
- Auto-assign barcode in case of failure

Batch plates:

Source Plate Options:

- Prompt for more plates when cassette is empty
- Finish when cassette is empty

Acquisition Options:

- Default
- Trans WL
- FITC 8s
- CFP 8s
- Cy5 1500ms
- FITC 500ms
- Rhod 200ms
- Cy5 500ms
- FITC 200ms
- TransWL - Adherent
- FITC 1000ms

Prime Configuration:

Review Colony Selection:



# Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

g) **Prime Configuration:** This is the acquisition option to be used for colony detection. Select the **Trans WL** option from the dropdown.

h) **Review Colony Selection:** Select **Batch – Review All** from the dropdown.

i) Click **Apply** to return to the main **Pick Run menu**.

Summary | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation: Experiment 1023

Source Microplates: PetriWell-6 Plate

Barcode Options:

- Read Barcode
- Auto-assign barcode in case of failure

Batch plates:

Source Plate Options:

- Prompt for more plates when cassette is empty
- Finish when cassette is empty

Acquisition Options:

- Default
- Trans WL
- FITC 8s
- CFP 8s
- Cy5 1500ms
- FITC 500ms
- Rhod 200ms
- Cy5 500ms
- FITC 200ms
- TransWL - Adherent
- FITC 1000ms

Prime Configuration: Trans WL

Review Colony Selection: Batch - Review All

**Apply** Cancel

# Carrying Out A Pick Run: Picking Settings

- 1) From the **Pick Run** menu, click on the **Picking Settings** heading to edit the parameters within:

**Summary** | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation:  
Source Microplates: PetriWell-6 Plate  
Barcode Options: Read Barcode: True  
Auto-assign barcode in case of failure: False  
Batch plates: False  
Source Plate Options: Prompt for more plates when cassette is empty  
Acquisition Options: Undefined  
Prime: Undefined  
Review Colony Selection: First Cycle

### Picking Settings

Destination Microplates: PetriWell-96 Plate  
Destination Wells: All wells will be available  
Barcode Options: Read Barcode: True  
Auto-assign barcode in case of failure: False  
Deposit Options: Fill Destination Plates.  
Dest. Plate Options: Prompt for more plates when cassette is empty  
Pick Number Options: Organise By: Plate  
Limit the number of colonies picked: false  
Pin Options: Aspirate Volume: 5  
Dispense Volume: 7  
Adherent Colonies: True  
Suspension Colonies: False  
Picking Height Adjustment: -0.1  
Audit Options: Allow Target and Aspirate images to be acquired but not saved.  
Dispersal Options: Use Dispersal: False  
Dispersal Volume: 20  
Dispersal Cycles: 10

### Sanitise Pin Options

Sanitise Pin Options: Purge Cycles: 3  
Bath Cycles: 3  
Dry Time: 10

**Start**

# Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply**.

- a) **Destination Microplates** – Select your 96-well destination plate type from the dropdown.

Summary | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

**Picking Settings**

Destination Microplates: PetriWell-96 Plate

Destination Wells:

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	●	●	●	●	●	●	●	●	●	●	○
C	○	●	●	●	●	●	●	●	●	●	●	○
D	○	●	●	●	●	●	●	●	●	●	●	○
E	○	●	●	●	●	●	●	●	●	●	●	○
F	○	●	●	●	●	●	●	●	●	●	●	○
G	○	●	●	●	●	●	●	●	●	●	●	○
H	○	○	○	○	○	○	○	○	○	○	○	○

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:

- Collate by Well
- Limit Colonies
- 0 Number of Colonies From Each Plate

Pin Options:

- Adherent
- Suspension
- Semi-Solid Media Delay (ms): 500
- Pick Height Adjustment: 0.40 Above Well Bottom
- Aspirate Volume (µl): 5
- Dispense Volume (µl): 7

Audit Options:

- Save Target and Aspirate Images

Dispersal Options:

- Use Dispersal
- Dispersal Cycles: 10
- Dispersal Volume: 20

Apply Cancel



# Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply**.

- a) **Destination Microplates** – Select your 96-well destination plate type from the dropdown.
- b) **Destination Wells** – Specify the wells into which colonies are going to be deposited. Either **left or right click** on the mouse to select or de-select wells. To deselect entire rows/columns, hold down the right mouse button and drag. All destination plates will be filled using this template.

Summary | Details | Guide

### Pick Run

Image microplate and pick colonies from specified criteria.

**Picking Settings**

Destination Microplates: PetriWell-96 Plate

Destination Wells:

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:  Collate by Well  
 Limit Colonies  
0 Number of Colonies From Each Plate

Pin Options:  Adherent  
 Suspension  
Pick Height Adjustment: 500 Semi-Solid Media Delay (ms)  
Aspirate Volume (µl): 0.40 Above Well Bottom  
Dispense Volume (µl): 5  
Dispense Volume (µl): 7

Audit Options:  Save Target and Aspirate Images

Dispersal Options:  Use Dispersal  
Dispersal Cycles: 10  
Dispersal Volume: 20

Apply Cancel



# Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply**.

- a) **Destination Microplates** – Select your 96-well destination plate type from the dropdown.
- b) **Destination Wells** – Specify the wells into which colonies are going to be deposited. Either left or right click on the mouse to select or de-select wells. To deselect entire rows/columns, hold down the right mouse button and drag. All destination plates will be filled using this template.

### c) Barcode Options

- \* If your destination plates are barcoded, select **Read Barcode**.
- \* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.

Summary | Details | Guide

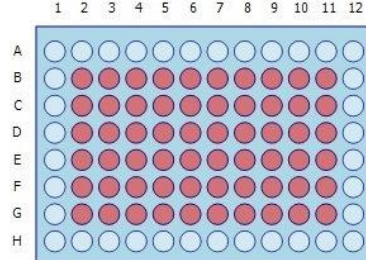
## Pick Run

Image microplate and pick colonies from specified criteria.

### Picking Settings

Destination Microplates: PetriWell-96 Plate

Destination Wells:



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:

- Collate by Well
- Limit Colonies
- 0  Number of Colonies From Each Plate

Pin Options:

- Adherent
- Suspension
- Pick Height Adjustment:  Semi-Solid Media Delay (ms)
- Aspirate Volume ( $\mu$ l):  Above Well Bottom
- Dispense Volume ( $\mu$ l):
- Dispense Volume ( $\mu$ l):

Audit Options:

- Save Target and Aspirate Images

Dispersal Options:

- Use Dispersal
- Dispersal Cycles:
- Dispersal Volume:

Apply Cancel



# Carrying Out A Pick Run: Picking Settings

2) Define your **imaging settings** within this dialog, then click **Apply**.

- a) **Destination Microplates** – Select your 96-well destination plate type from the dropdown.
- b) **Destination Wells** – Specify the wells into which colonies are going to be deposited. Either left or right click on the mouse to select or de-select wells. To deselect entire rows/columns, hold down the right mouse button and drag. All destination plates will be filled using this template.
- c) **Barcode Options**
  - \* If your destination plates are barcoded, select **Read Barcode**.
  - \* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.
- d) **Deposit Options:** Leave unselected.

Summary | Details | Guide

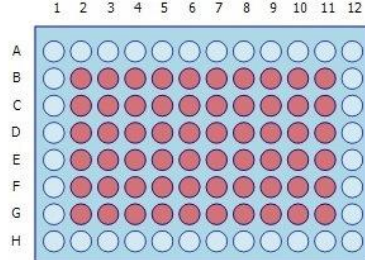
### Pick Run

Image microplate and pick colonies from specified criteria.

**Picking Settings**

Destination Microplates: PetriWell-96 Plate

Destination Wells:



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:

- Collate by Well
- Limit Colonies

Number of Colonies From Each Plate: 0

Pin Options:

- Adherent
- Suspension

Semi-Solid Media Delay (ms): 500

Pick Height Adjustment: 0.40 Above Well Bottom

Aspirate Volume (µl): 5

Dispense Volume (µl): 7

Audit Options:

- Save Target and Aspirate Images

Dispersal Options:

- Use Dispersal

Dispersal Cycles: 10

Dispersal Volume: 20

Apply Cancel



# Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply**.

- a) **Destination Microplates** – Select your 96-well destination plate type from the dropdown.
- b) **Destination Wells** – Specify the wells into which colonies are going to be deposited. Either left or right click on the mouse to select or de-select wells. To deselect entire rows/columns, hold down the right mouse button and drag. All destination plates will be filled using this template.
- c) **Barcode Options**
  - \* If your destination plates are barcoded, select **Read Barcode**.
  - \* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.
- d) **Deposit Options:** Leave unselected.
- e) **Destination Plate Options:** Select **Finish when cassette is empty**.

Summary | Details | Guide

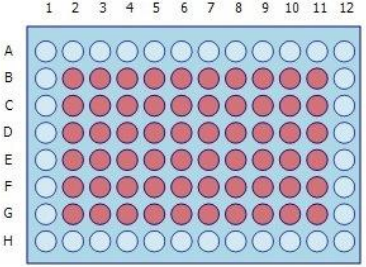
### Pick Run

Image microplate and pick colonies from specified criteria.

**Picking Settings**

Destination Microplates: PetriWell-96 Plate

Destination Wells:



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:

- Collate by Well
- Limit Colonies
- 0  Number of Colonies From Each Plate

Pin Options:

- Adherent
- Suspension

Semi-Solid Media Delay (ms):

Pick Height Adjustment:  Above Well Bottom

Aspirate Volume (µl):

Dispense Volume (µl):

Audit Options:

- Save Target and Aspirate Images

Dispersal Options:

- Use Dispersal
- Dispersal Cycles:
- Dispersal Volume:

Apply Cancel



# Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply**.

- a) **Destination Microplates** – Select your 96-well destination plate type from the dropdown.
- b) **Destination Wells** – Specify the wells into which colonies are going to be deposited. Either left or right click on the mouse to select or de-select wells. To deselect entire rows/columns, hold down the right mouse button and drag. All destination plates will be filled using this template.
- c) **Barcode Options**
  - \* If your destination plates are barcoded, select **Read Barcode**.
  - \* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.
- d) **Deposit Options:** Leave unselected.
- e) **Destination Plate Options:** Select **Finish when cassette is empty**.
- f) **Pick Number Options:** **Do not select any options here.** Leave as default (Collate by Plate).

Summary | Details | Guide

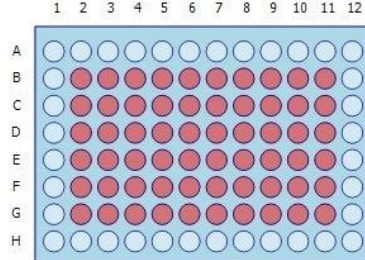
## Pick Run

Image microplate and pick colonies from specified criteria.

### Picking Settings

Destination Microplates: PetriWell-96 Plate

Destination Wells:



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:**

- Collate by Well
- Limit Colonies
- 0  Number of Colonies From Each Plate

Pin Options:

- Adherent
- Suspension

Semi-Solid Media Delay (ms):

Pick Height Adjustment:  Above Well Bottom

Aspirate Volume (µl):

Dispense Volume (µl):

Audit Options:

- Save Target and Aspirate Images

Dispersal Options:

- Use Dispersal
- Dispersal Cycles:
- Dispersal Volume:





# Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply**.

- a) **Destination Microplates** – Select your 96-well destination plate type from the dropdown.
- b) **Destination Wells** – Specify the wells into which colonies are going to be deposited. Either left or right click on the mouse to select or de-select wells. To deselect entire rows/columns, hold down the right mouse button and drag. All destination plates will be filled using this template.
- c) **Barcode Options**
  - \* If your destination plates are barcoded, select **Read Barcode**.
  - \* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.
- d) **Deposit Options:** Leave unselected.
- e) **Destination Plate Options:** Select **Finish when cassette is empty**.
- f) **Pick Number Options:** Do not select any options here. Leave as default (Collate by Plate).
- g) **Pin Options:**
  - Select the **type of colonies** that are going to be picked: **Suspension** or **Adherent**.
  - **Pick Height Adjustment:** Leave as default.
  - **Aspirate Volume:** Leave as default = **5 uL**
  - **Dispense Volume:** Leave as default = **7 uL**

Summary | Details | Guide

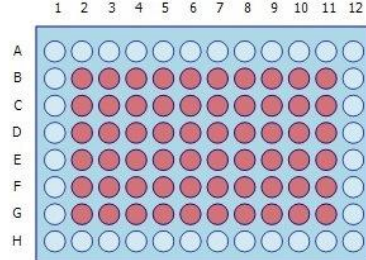
### Pick Run

Image microplate and pick colonies from specified criteria.

**Picking Settings**

Destination Microplates: PetriWell-96 Plate

Destination Wells:



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:

- Collate by Well
- Limit Colonies
- Number of Colonies From Each Plate

**Pin Options:**

- Adherent
- Suspension

Semi-Solid Media Delay (ms):

Pick Height Adjustment:  Above Well Bottom

Aspirate Volume (uL):

Dispense Volume (uL):

Audit Options:

- Save Target and Aspirate Images

Dispersal Options:

- Use Dispersal
- Dispersal Cycles:
- Dispersal Volume:

Apply Cancel

# Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply**.

g) **Audit Options:** Tick this box to save the Target and Aspirate Images. **Note: This will slow down picking.**

Summary | Details | Guide

### Pick Run

Image microplate and pick colonies from specified criteria.

#### Picking Settings

Destination Microplates: PetriWell-96 Plate

Destination Wells:

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	●	●	●	●	●	●	●	●	●	●	○
C	○	●	●	●	●	●	●	●	●	●	●	○
D	○	●	●	●	●	●	●	●	●	●	●	○
E	○	●	●	●	●	●	●	●	●	●	●	○
F	○	●	●	●	●	●	●	●	●	●	●	○
G	○	●	●	●	●	●	●	●	●	●	●	○
H	○	○	○	○	○	○	○	○	○	○	○	○

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:

- Collate by Well
- Limit Colonies
- 0  Number of Colonies From Each Plate

Pin Options:

- Adherent
- Suspension

Semi-Solid Media Delay (ms):

Pick Height Adjustment:  Above Well Bottom

Aspirate Volume (μl):

Dispense Volume (μl):

**Audit Options:**  Save Target and Aspirate Images

Dispersal Options:

- Use Dispersal

Dispersal Cycles:

Dispersal Volume:

Apply Cancel



# Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply**.

- g) **Audit Options:** Tick this box to save the Target and Aspirate Images. **Note: This will slow down picking.**
- h) **Dispersal Options:**
  - **Use Dispersal:** Select to spread out colony cells after picking. *Do not select if intact colonies are required.*
  - **Dispersal cycles:** Use **3-6** for **CHO** cells and **6-10** for **hybridomas**.
  - **Dispersal volume:** Leave as default = **20 uL**.

Summary | Details | Guide

### Pick Run

Image microplate and pick colonies from specified criteria.

**Picking Settings**

Destination Microplates: PetriWell-96 Plate

Destination Wells:

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	●	●	●	●	●	●	●	●	●	●	○
C	○	●	●	●	●	●	●	●	●	●	●	○
D	○	●	●	●	●	●	●	●	●	●	●	○
E	○	●	●	●	●	●	●	●	●	●	●	○
F	○	●	●	●	●	●	●	●	●	●	●	○
G	○	●	●	●	●	●	●	●	●	●	●	○
H	○	○	○	○	○	○	○	○	○	○	○	○

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:  Collate by Well  
 Limit Colonies  
0 Number of Colonies From Each Plate

Pin Options:  Adherent  
 Suspension  
Pick Height Adjustment: 500 Semi-Solid Media Delay (ms)  
Aspirate Volume (µl): 0.40 Above Well Bottom  
Dispense Volume (µl): 5  
7

Audit Options:  Save Target and Aspirate Images

**Dispersal Options:**  Use Dispersal  
Dispersal Cycles: 10  
Dispersal Volume: 20

Apply Cancel



# Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply**.

- g) **Audit Options:** Tick this box to save the Target and Aspirate Images. **Note: This will slow down picking.**
- h) **Dispersal Options:**
  - **Use Dispersal:** Select to spread out colony cells after picking. *Do not select if intact colonies are required.*
  - **Dispersal cycles:** Use 3-6 for **CHO** cells and 6-10 for **hybridomas**.
  - **Dispersal volume:** Leave as default = 20 **uL**.
- i) Click **Apply** to return to the main **Pick Run** menu.

**Summary** | **Details** | **Guide**

### Pick Run

Image microplate and pick colonies from specified criteria.

**Picking Settings**

Destination Microplates: PetriWell-96 Plate

Destination Wells:

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	●	●	●	●	●	●	●	●	●	●	○
C	○	●	●	●	●	●	●	●	●	●	●	○
D	○	●	●	●	●	●	●	●	●	●	●	○
E	○	●	●	●	●	●	●	●	●	●	●	○
F	○	●	●	●	●	●	●	●	●	●	●	○
G	○	●	●	●	●	●	●	●	●	●	●	○
H	○	○	○	○	○	○	○	○	○	○	○	○

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:  Collate by Well  
 Limit Colonies  
0  Number of Colonies From Each Plate

Pin Options:  Adherent  
 Suspension  Semi-Solid Media Delay (ms)  
Pick Height Adjustment  Above Well Bottom  
Aspirate Volume (μl)   
Dispense Volume (μl)

Audit Options:  Save Target and Aspirate Images

Dispersal Options:  Use Dispersal  
Dispersal Cycles   
Dispersal Volume

**Apply** **Cancel**



# Carrying Out A Pick Run: Sanitise Pin Options

- 1) You will see **Sanitise Pin Options** at the bottom of the **Pick Run** main menu. Leave these at the default settings.

**Summary** | Details | Guide

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation:	
Source Microplates:	PetriWell-6 Plate
Barcode Options:	Read Barcode: True Auto-assign barcode in case of failure: False
Batch plates:	False
Source Plate Options:	Prompt for more plates when cassette is empty
Acquisition Options:	Undefined Prime: Undefined
Review Colony Selection:	First Cycle

### Picking Settings

Destination Microplates:	PetriWell-96 Plate
Destination Wells:	All wells will be available
Barcode Options:	Read Barcode: True Auto-assign barcode in case of failure: False
Deposit Options:	Fill Destination Plates.
Dest. Plate Options:	Prompt for more plates when cassette is empty
Pick Number Options:	Organise By: Plate Limit the number of colonies picked: false
Pin Options:	Aspirate Volume: 5 Dispense Volume: 7 Adherent Colonies: True Suspension Colonies: False Picking Height Adjustment: -0.1
Audit Options:	Allow Target and Aspirate images to be acquired but not saved.
Dispersal Options:	Use Dispersal: False Dispersal Volume: 20 Dispersal Cycles: 10

### Sanitise Pin Options

Sanitise Pin Options:	Purge Cycles: 3 Bath Cycles: 3 Dry Time: 10
-----------------------	---

# Carrying Out A Pick Run: Starting A Pick Run

- 1) Click the **Start** button at the bottom of the **Pick Run** main menu to begin your pick run.
- 2) When prompted, load the **cell colony plates** into the **source stacker cassette**.

[Summary](#) | [Details](#) | [Guide](#)

## Pick Run

Image microplate and pick colonies from specified criteria.

### Imaging Settings

Run Annotation:	
Source Microplates:	PetriWell-6 Plate
Barcode Options:	Read Barcode: True Auto-assign barcode in case of failure: False
Batch plates:	False
Source Plate Options:	Prompt for more plates when cassette is empty
Acquisition Options:	Undefined Prime: Undefined
Review Colony Selection:	First Cycle

### Picking Settings

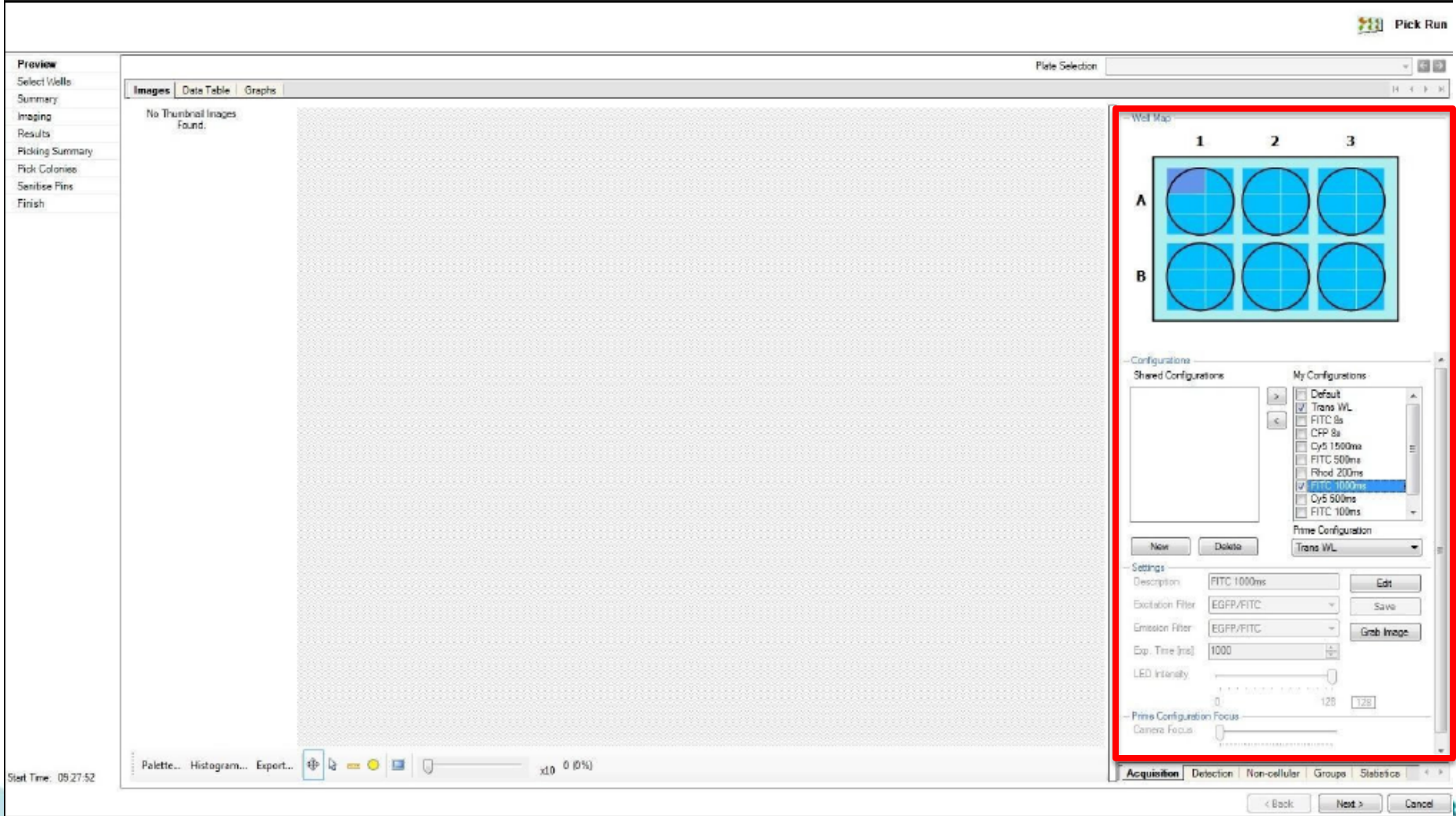
Destination Microplates:	PetriWell-96 Plate
Destination Wells:	All wells will be available
Barcode Options:	Read Barcode: True Auto-assign barcode in case of failure: False
Deposit Options:	Fill Destination Plates.
Dest. Plate Options:	Prompt for more plates when cassette is empty
Pick Number Options:	Organise By: Plate Limit the number of colonies picked: false
Pin Options:	Aspirate Volume: 5 Dispense Volume: 7 Adherent Colonies: True Suspension Colonies: False Picking Height Adjustment: -0.1
Audit Options:	Allow Target and Aspirate images to be acquired but not saved.
Dispersal Options:	Use Dispersal: False Dispersal Volume: 20 Dispersal Cycles: 10

### Sanitise Pin Options

Sanitise Pin Options:	Purge Cycles: 3 Bath Cycles: 3 Dry Time: 10
-----------------------	---

# Carrying Out A Pick Run: Define Image Acquisition Settings

3) The **Preview** screen appears. Here you will first define your **image acquisition, colony detection, and groups to pick settings**.



# Carrying Out A Pick Run: Define Image Acquisition Settings

- 3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
  - a) Click on the **Acquisition** tab.

The screenshot displays the software's configuration interface. At the top, there are two lists: 'Shared Configurations' (empty) and 'My Configurations' (containing various filter and time settings, with 'Config 12' selected). Below these are 'New' and 'Delete' buttons. The 'Settings' section includes:

- Description: FITC 1000ms
- Excitation Filter: EGFP/FITC
- Emission Filter: EGFP/FITC
- Exp. Time [ms]: 1000
- LED Intensity: 128

The 'Prime Configuration Focus' section includes:

- Camera Focus: 2200

At the bottom, a navigation bar has 'Acquisition' highlighted in red, followed by 'Detection', 'Non-cellular', 'Groups', and 'Statistics'.





# Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
- Click on the **Acquisition** tab.
  - If the desired acquisition option is not available in the **My Configurations** box, new Acquisitions options will need to be set up (see example screenshot on right).

The screenshot displays the software's configuration panel. It is divided into several sections:

- Shared Configurations:** An empty box on the left with navigation arrows (> and <).
- My Configurations:** A list on the right containing several options with checkboxes:  Trans WL,  FITC 8s,  CFP 8s,  Cy5 1500ms,  FITC 500ms,  Rhod 200ms,  Cy5 500ms,  FITC 100ms,  TransWL - Adherent, and  Config 12. The 'Config 12' option is highlighted in blue.
- Buttons:** 'New' and 'Delete' buttons are located below the configuration lists.
- Prime Configuration:** A dropdown menu currently set to 'Trans WL'.
- Settings:** A section with various controls:
  - Description:** FITC 1000ms, with an 'Edit' button.
  - Excitation Filter:** EGFP/FITC, with a 'Save' button.
  - Emission Filter:** EGFP/FITC, with a 'Grab Image' button.
  - Exp. Time [ms]:** 1000, with up/down arrows.
  - LED Intensity:** A slider from 0 to 128, with a numerical input field showing 128.
- Prime Configuration Focus:** A section with a 'Camera Focus' slider from 0 to 9332, with a numerical input field showing 2200.

At the bottom, a navigation bar includes tabs for **Acquisition**, Detection, Non-cellular, Groups, and Statistics.

# Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
- a) Click on the **Acquisition** tab.
  - b) If the desired acquisition option is not available in the **My Configurations** box, new Acquisitions options will need to be set up (see example screenshot on right).
  - c) 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)	200	1000
LED Intensity	3	128
Camera Focus	2200	2200

The screenshot displays the software's configuration interface. On the left, 'Shared Configurations' is empty. On the right, 'My Configurations' lists several options, with 'Config 12' selected. Below this, the 'Prime Configuration' is set to 'Trans WL'. The 'Settings' section is expanded, showing various parameters: 'Description' (FITC 1000ms), 'Excitation Filter' (EGFP/FITC), 'Emission Filter' (EGFP/FITC), 'Exp. Time [ms]' (1000), and 'LED Intensity' (128). The 'Prime Configuration Focus' section shows 'Camera Focus' (2200). The 'New' and 'Edit' buttons are highlighted with red boxes.



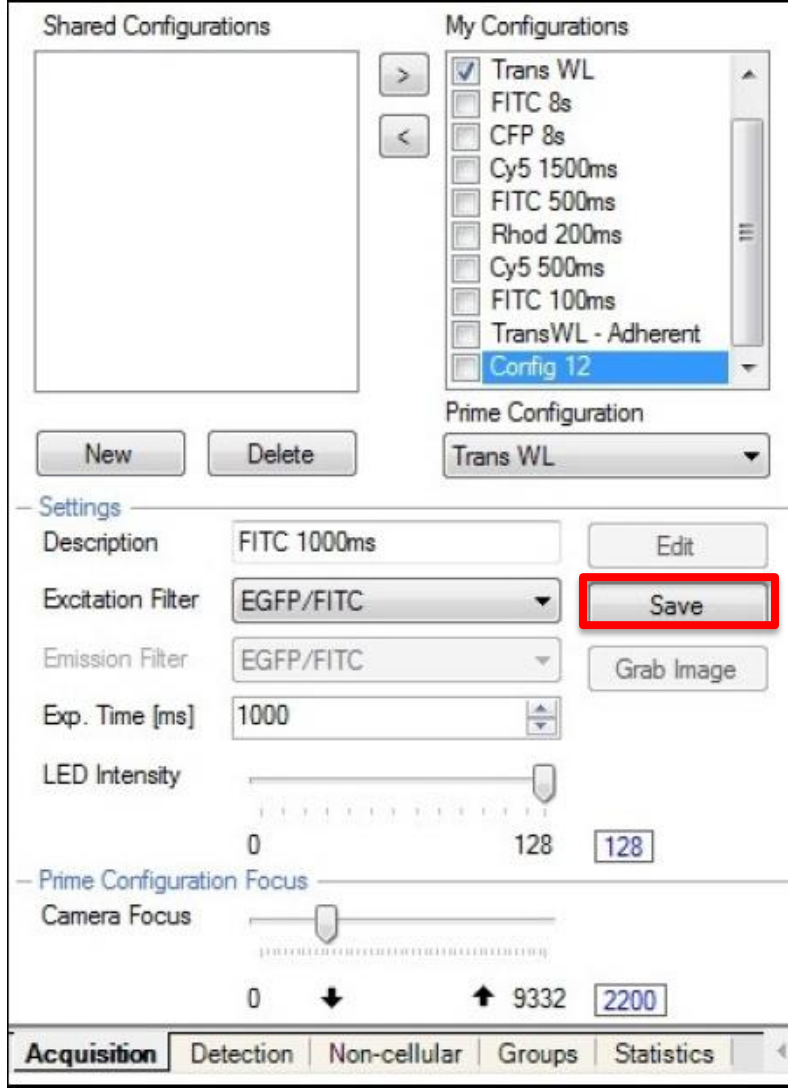
# Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.

- a) Click on the **Acquisition** tab.
- b) If the desired acquisition option is not available in the **My Configurations** box, new Acquisitions options will need to be set up (see example screenshot on right).
- c) 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)	200	1000
LED Intensity	3	128
Camera Focus	2200	2200

d) Click **Save** to store each option.



# Carrying Out A Pick Run: Define Image Acquisition Settings

- 3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
  - e) The new acquisition options will now be displayed under **My Configurations**. Ensure that **TransWL** and **FITC 1000ms** options are selected. *Note: Do not delete the Default option – it is required for other ClonePix 2 functions.*

The screenshot displays the software's configuration interface. At the top, there are two panels: 'Shared Configurations' (empty) and 'My Configurations' (containing a list of options). The 'My Configurations' panel is highlighted with a red border. The list includes:  Trans WL,  FITC 8s,  CFP 8s,  Cy5 1500ms,  FITC 500ms,  Rhod 200ms,  Cy5 500ms,  FITC 100ms,  TransWL - Adherent, and  Config 12. Below this list is a 'Prime Configuration' dropdown menu set to 'Trans WL'. The 'Settings' section includes: Description: FITC 1000ms; Excitation Filter: EGFP/FITC; Emission Filter: EGFP/FITC; Exp. Time [ms]: 1000; LED Intensity: a slider from 0 to 128 with a value of 128; Prime Configuration Focus: Camera Focus slider from 0 to 9332 with a value of 2200. At the bottom, there is a navigation bar with tabs: Acquisition (selected), Detection, Non-cellular, Groups, and Statistics.



# Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
- e) The new acquisition options will now be displayed under **My Configurations**. Ensure that TransWL and FITC 1000ms options are selected. *Note: Do not delete the Default option – it is required for other ClonePix 2 functions.*
- f) Ensure that the **Prime Configuration** is set to **TransWL**. This is critical for correct colony detection.

The screenshot displays the software's configuration window, divided into several sections:

- Shared Configurations:** An empty box on the left.
- My Configurations:** A list of configurations on the right. The 'Config 12' entry is highlighted in blue. Below the list, a dropdown menu is set to 'Trans WL', which is enclosed in a red rectangular box.
- Settings:** A section with various controls:
  - Description:** FITC 1000ms
  - Excitation Filter:** EGFP/FITC
  - Emission Filter:** EGFP/FITC
  - Exp. Time [ms]:** 1000
  - LED Intensity:** A slider ranging from 0 to 128, with the current value set to 128.
- Prime Configuration Focus:** A section with a **Camera Focus** slider ranging from 0 to 9332, with the current value set to 2200.

At the bottom, a navigation bar includes tabs for **Acquisition**, **Detection**, **Non-cellular**, **Groups**, and **Statistics**.

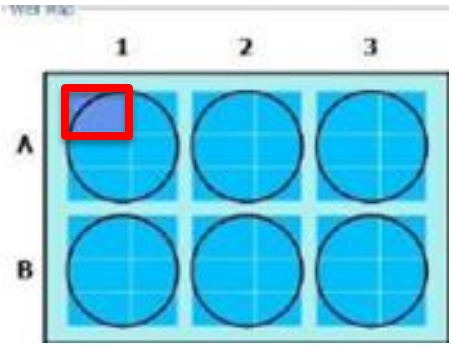
# Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.

e) The new acquisition options will now be displayed under **My Configurations**. Ensure that **TransWL** and **FITC 1000ms** options are selected. *Note: Do not delete the Default option – it is required for other ClonePix 2 functions.*

f) Ensure that the **Prime Configuration** is set to **TransWL**. This is critical for correct colony detection.

g) To test each setting, select it in the **My Configurations** list and click **Grab Image** which will capture images for the area currently highlighted in the **Well Map**, or click on another area on the **Well Map** to display a new image.



Shared Configurations

My Configurations

- Trans WL
- FITC 8s
- CFP 8s
- Cy5 1500ms
- FITC 500ms
- Rhod 200ms
- Cy5 500ms
- FITC 100ms
- TransWL - Adherent
- Config 12

Prime Configuration

Trans WL

Settings

Description: FITC 1000ms [Edit]

Excitation Filter: EGFP/FITC [Save]

Emission Filter: EGFP/FITC [Grab Image]

Exp. Time [ms]: 1000

LED Intensity: 0 to 128 [128]

Prime Configuration Focus

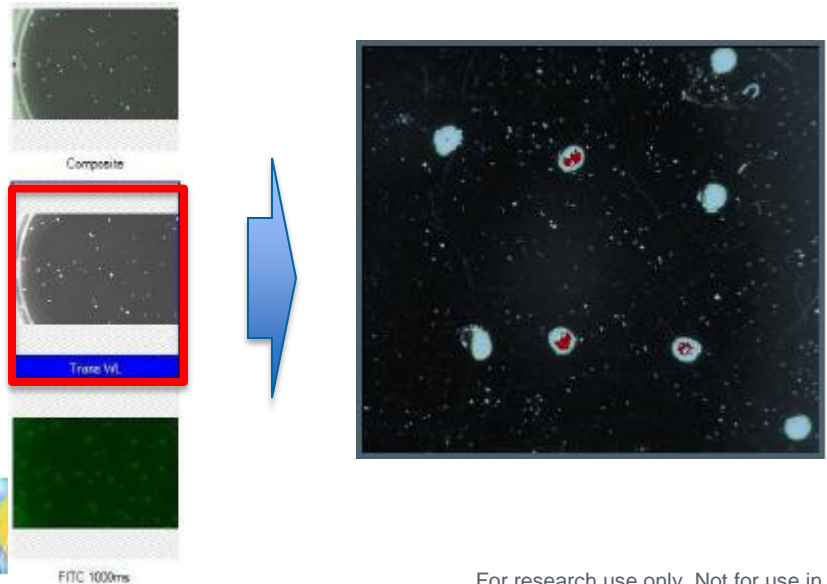
Camera Focus: 0 to 9332 [2200]

Acquisition | Detection | Non-cellular | Groups | Statistics



# Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
- h) There will now be a main image with 3 thumbnails to the left named **Composite**, **TransWL** and **FITC 1000ms**.
- Toggle between the thumbnails 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.



The software interface shows configuration options for image acquisition. It includes sections for 'Shared Configurations' and 'My Configurations'. The 'My Configurations' list includes 'Trans WL', 'FITC 8s', 'CFP 8s', 'Cy5 1500ms', 'FITC 500ms', 'Rhod 200ms', 'Cy5 500ms', 'FITC 100ms', 'TransWL - Adherent', and 'Config 12'. The 'Prime Configuration' is set to 'Trans WL'. The 'Settings' section includes 'Description' (FITC 1000ms), 'Excitation Filter' (EGFP/FITC), and 'Emission Filter' (EGFP/FITC). The 'Exp. Time [ms]' is set to 1000, and 'LED Intensity' is set to 128. The 'Prime Configuration Focus' section includes 'Camera Focus' set to 2200. The 'Acquisition' tab is selected, with other tabs for 'Detection', 'Non-cellular', 'Groups', and 'Statistics'.

# Carrying Out A Pick Run: Define Colony Detection Settings

4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection** settings.

Pick Run

The screenshot displays the software interface for defining colony detection settings. On the left, a sidebar lists navigation options: **Preview**, **Select Wells**, **Summary**, **Imaging**, **Results**, **Picking Summary**, **Pick Colonies**, **Sanitise Pins**, and **Finish**. The main window is titled "Plate Selection" and contains three tabs: **Images**, **Data Table**, and **Graphs**. The **Images** tab is active, showing a large central image of a petri dish with colonies. A red circle highlights a specific colony. To the left of the main image are three smaller image thumbnails: "Composite", "Trans WL", and "FITC 1000ms". The **Detection** settings panel on the right includes a **Well Map** (a 2x3 grid with wells A1, A2, A3 in the top row and B1, B2, B3 in the bottom row), **Colony Detection** settings (Algorithm: Local Threshold, Average Colony Diameter: 0.50mm, Average / Average X: 4 / 2.5, Exterior Statistics Diameter Multiplier: x3), and **Display** options (Display Detected Colonies, Shade Colonies, Identify Colonies, Display Proximity Indicators, Shade Overlap Areas). The **Detection** tab is highlighted in red in the bottom navigation bar.

Start Time: 09:27:52





# Carrying Out A Pick Run: Define Colony Detection Settings

4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection settings**.

- a) Set **Algorithm** to **Local Threshold** from the drop-down menu.

Well Map

1 2 3

A

B

Colony Detection

Algorithm: Local Threshold

Detects colonies based on the intensity around the vicinity of the colony.

Average Colony Diameter: 0.50mm

0.12mm 1.25mm

Average / 4 Average X 2.5

Exterior Statistics Diameter Multiplier: x3

Average Colony Exterior Diameter: 1.50mm

Use each colony size when calculating exterior statistics

Reprocess

Display

Display Detected Colonies

Shade Colonies

Identify Colonies

Display Proximity Indicators

Shade Overlap Areas

Acquisition **Detection** Non-cellular Groups Statistics

# Carrying Out A Pick Run: Define Colony Detection Settings

4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection settings**.

- Set **Algorithm** to **Local Threshold** from the drop-down menu.
- Using the **White Light** image, set the **Average Colony Diameter** to a size that best detects the colonies. This will probably be between **0.25** and **0.70mm** depending on the size of the

Well Map

1 2 3

A

B

Colony Detection

Algorithm: Local Threshold

Detects colonies based on the intensity around the vicinity of the colony.

Average Colony Diameter: 0.50mm

0.12mm 1.25mm

Average / 4 Average X 2.5

Exterior Statistics Diameter Multiplier: x3

Average Colony Exterior Diameter: 1.50mm

Use each colony size when calculating exterior statistics

Reprocess

Display

Display Detected Colonies

Shade Colonies

Identify Colonies

Display Proximity Indicators

Shade Overlap Areas

Acquisition **Detection** Non-cellular Groups Statistics

# Carrying Out A Pick Run: Define Colony Detection Settings

4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection settings**.

- Set **Algorithm** to **Local Threshold** from the drop-down menu.
- Using the **White Light** image, set the **Average Colony Diameter** to a size that best detects the colonies. This will probably be between **0.25** and **0.70mm** depending on the size of the colonies.
- Click **Reprocess** after moving the **slide bar** to apply the changes.

Well Map

1 2 3

A

B

Colony Detection

Algorithm: Local Threshold

Detects colonies based on the intensity around the vicinity of the colony.

Average Colony Diameter: 0.50mm

0.12mm 1.25mm

Average / 4 Average X 2.5

Exterior Statistics Diameter Multiplier: x3

Average Colony Exterior Diameter: 1.50mm

Use each colony size when calculating exterior statistics

Reprocess

Display

Display Detected Colonies

Shade Colonies

Identify Colonies

Display Proximity Indicators

Shade Overlap Areas

Acquisition **Detection** Non-cellular Groups Statistics

# Carrying Out A Pick Run: Define Colony Detection Settings

4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection settings**.

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- Click **Reprocess** after moving the **slide bar** to apply the changes.
- Leave **Exterior Statistics Diameter Multiplier** at the default setting (**x3**).

Well Map

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A

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- Click **Reprocess** after moving the **slide bar** to apply the changes.
- Leave **Exterior Statistics Diameter Multiplier** at the default setting (**x3**).
- Select **Use each colony size when calculating exterior statistics**.
- Leave **Display settings** as **default** (only **Display Detected Colonies** and **Shade Overlap Areas** selected).

Well Map

1 2 3

A

B

Colony Detection

Algorithm: Local Threshold

Detects colonies based on the intensity around the vicinity of the colony.

Average Colony Diameter: 0.50mm

0.12mm 1.25mm

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Use each colony size when calculating exterior statistics

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Display

Display Detected Colonies

Shade Colonies

Identify Colonies

Display Proximity Indicators

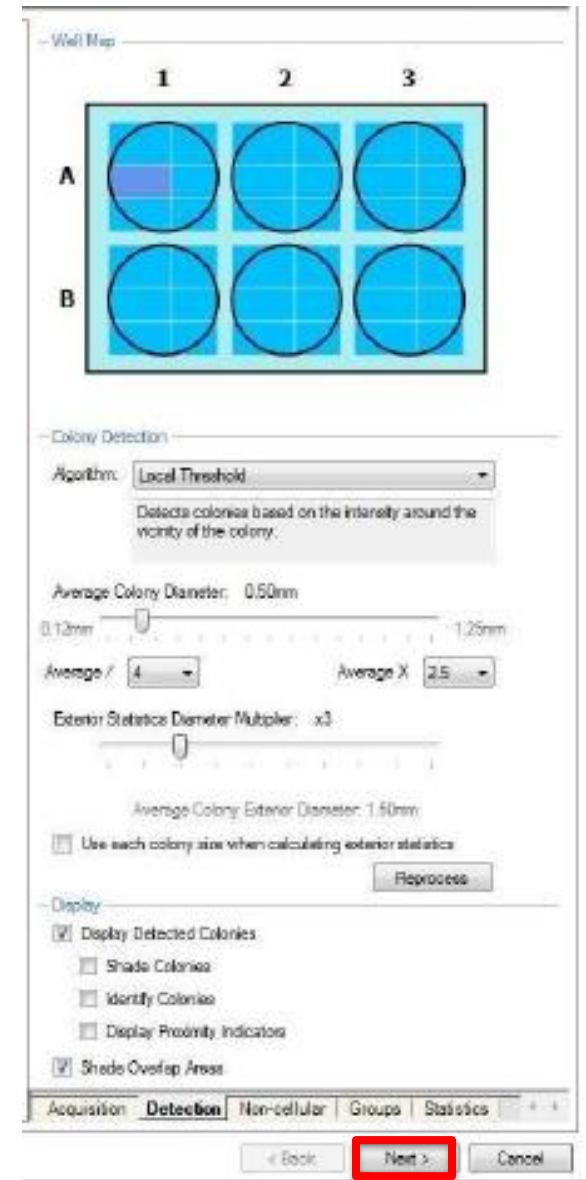
Shade Overlap Areas

Acquisition **Detection** Non-cellular Groups Statistics

# Carrying Out A Pick Run: Define Colony Detection Settings

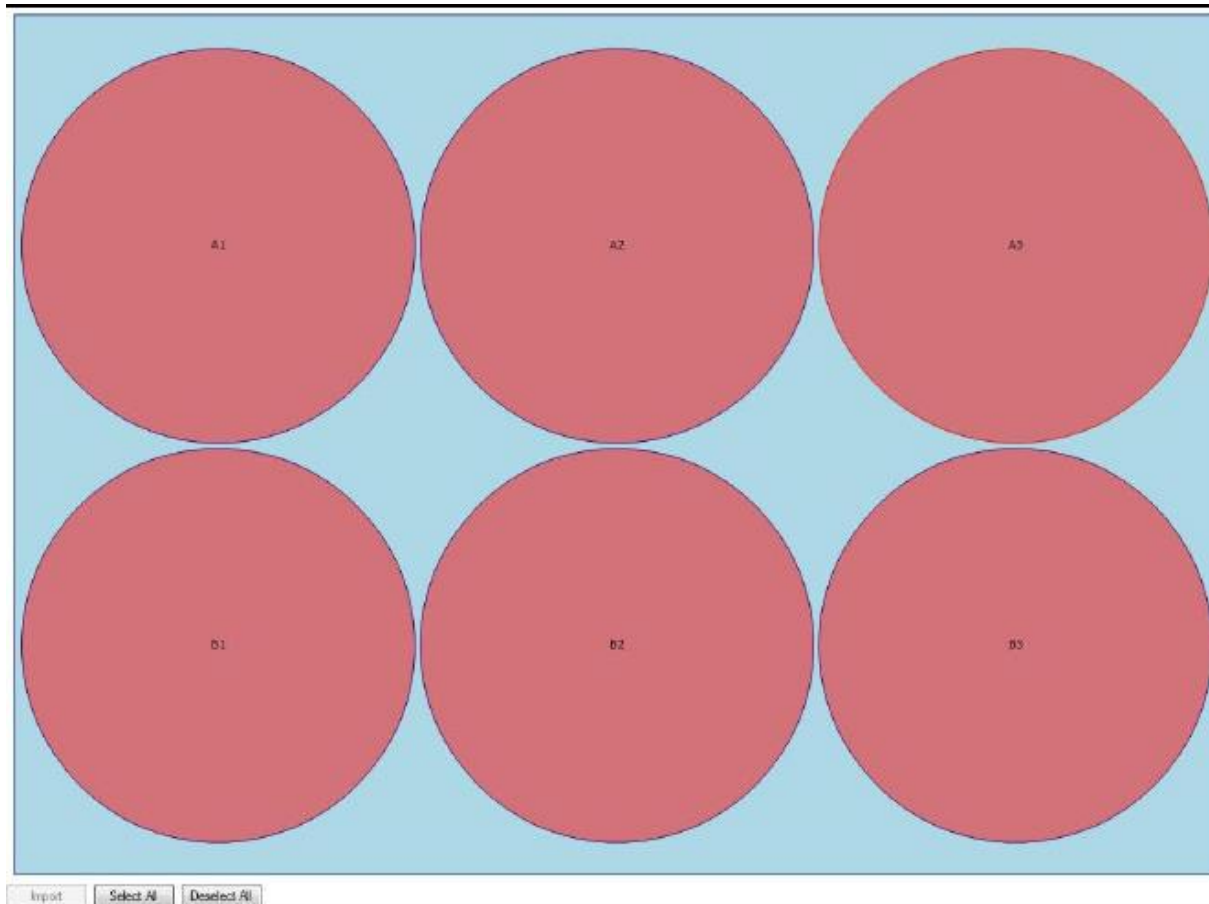
4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection settings**.

- Set **Algorithm** to **Local Threshold** from the drop-down menu.
- Using the **White Light** image, set the **Average Colony Diameter** to a size that best detects the colonies. This will probably be between **0.25 and 0.70mm** depending on the size of the colonies.
- Click **Reprocess** after moving the **slide bar** to apply the changes.
- Leave **Exterior Statistics Diameter Multiplier** at the default setting (**x3**).
- Select **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.



# Carrying Out A Pick Run: Select Wells

5) The **Select Wells** window appears. Click on your source plate wells to select which wells to pick from – selected wells will be highlighted in **pink** as shown below. The **Select All** or **Deselect All** buttons can also be used to add or removed wells to be imaged & picked. Click **Next** to proceed.





# Carrying Out A Pick Run: Summary

6) The **Summary** window appears. This window provides a summary of the imaging and picking parameters that will be used for the run. This is a good point to check all settings are correct before proceeding to imaging. Click **Next** to proceed.

**Summary**

Run Annotation: Experiment 1023  
Microplate: PetriWell-6 Plate  
Read Barcode: True  
Barcode Failure: Auto-Generate  
Source Plate Options: Finish when cassette is empty  
Processing Algorithm: Global  
Average Colony Diameter: 1000µm  
Exterior Statistics Diameter Multiplier: x3  
Use each colony diameter for exterior statistics: False

Discard Groups:  
NC Irregular 1 IF Compactness < 0.00  
NC Irregular 2 IF Axis Ratio < 0.30

Groups:  
Edge Excluded IF Edge Excluded = True  
Too Big IF Total Area > 0.70 mm<sup>2</sup>  
Too Small IF Total Area < 0.10 mm<sup>2</sup>  
Irregular 1 IF Compactness < 0.60  
Irregular 2 IF Axis Ratio < 0.60  
Proximity IF Proximity < 1.00 mm  
Ungeled Anything else

Optical Configurations:  
Description: Trans WL  
Emission Filter: WHITELIGHT  
Excitation Filter: WHITELIGHT (TRANS)  
Exposure: 200  
LED Intensity: 3  
Prime Config: True

Description: FITC 1000ms  
Emission Filter: EGFP/FITC  
Excitation Filter: EGFP/FITC  
Exposure: 1000  
LED Intensity: 128  
Prime Config: False

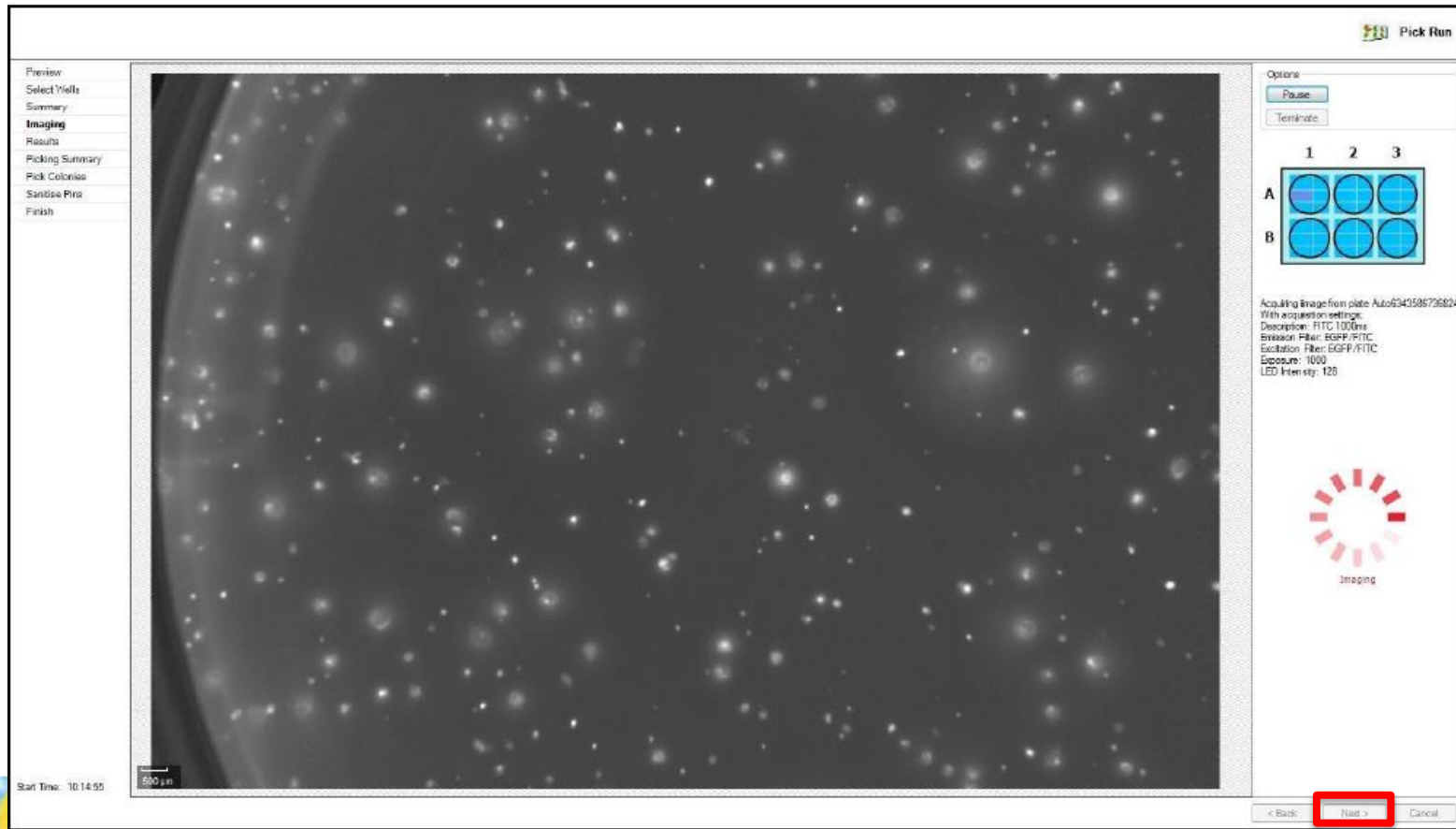
**Picking Summary:**  
Microplate: Genetix PetriWell-96 Plate  
Destination Wells: 60 wells will be available  
Read Barcode: True  
Barcode Failure: Auto-Generate  
Deposit Options: Fill Destination Plates  
Dest. Plate Options: Finish when cassette is empty  
Pick Number Options: Pick all colonies from each plate

Start Time: 10:14:55

Navigation: < Back, **Next >**, Cancel

# Carrying Out A Pick Run: Imaging

7) The **Imaging** window appears. This screen displays the images that are being captured as imaging is taking place. The selected wells will be imaged in sequence for each of the acquisition options selected. The images will then be processed together for colony detection according to the defined settings. Once imaging is complete, click **Next** to proceed.



# Carrying Out A Pick Run: Results Review

8) The **Results** window appears. This screen displays the results of the imaging run/colony analysis. Click on the **Graphs** tab (upper left), then the **Groups** tab (lower right) to proceed with defining colonies to pick.

The screenshot shows the 'Pick Run' software interface. At the top, the title bar reads '16/03/2011 10:22:02 | Auto634358673682485634 Experiment 1023 Aggregated Plate Results'. Below this is a navigation bar with tabs: Overview, Images, Gallery, Data Table, **Graphs** (highlighted with a red box), and Picking Review. The main area is divided into several sections:

- Left Panel:** A vertical menu with options: Preview, Select Wells, Summary, Imaging, **Results** (expanded), Picking Summary, Pick Colonies, Sanitise Pins, and Finish. Under 'Results', there are two images: 'Tiens VL' and 'FTC 2.0s'.
- Top Right:** 'Plate Selection' dropdown menu showing '16/03/2011 10:22:02 | Auto634358673682485634'.
- Center:** A large graph with 'Proximity' on the y-axis (0 to 1,000) and 'Rank' on the x-axis (0 to 400). The data points form a curve that starts at approximately 1,000 for rank 0 and decreases to near 0 by rank 400. Below this is a histogram showing 'Frequency' on the y-axis (0 to 18) and 'Proximity (mm)' on the x-axis (0 to 800). The histogram shows a high frequency of colonies at low proximity values, with a peak near 0.
- Right Panel:** A control panel with 'Scatter' and 'Rank' tabs, and 'Show Graph' (selected) and 'Show Image' radio buttons. It includes a dropdown for 'X Axis: Proximity (mm)' and a 'Display: Stack By Group' dropdown. Below this is a legend titled 'All Undiscarded Features' with a list of categories and counts: Edge Excluded [328], Too Big [0], Too Small [282], Irregular 1 [0], Irregular 2 [4], Proximity [8], and **Ungated [111]** (highlighted with a red box). There are 'Increase Priority' and 'Decrease Priority' buttons. At the bottom of this panel, there is a 'Group' section with 'Name: Ungated', a 'Hidden' checkbox, a 'Colour...' button, and a 'Remove' button.
- Bottom Right:** A navigation bar with 'Non-cellular', **Groups** (highlighted with a red box), and 'Statistics' tabs. Below this are 'Back', 'Next', and 'Cancel' buttons.

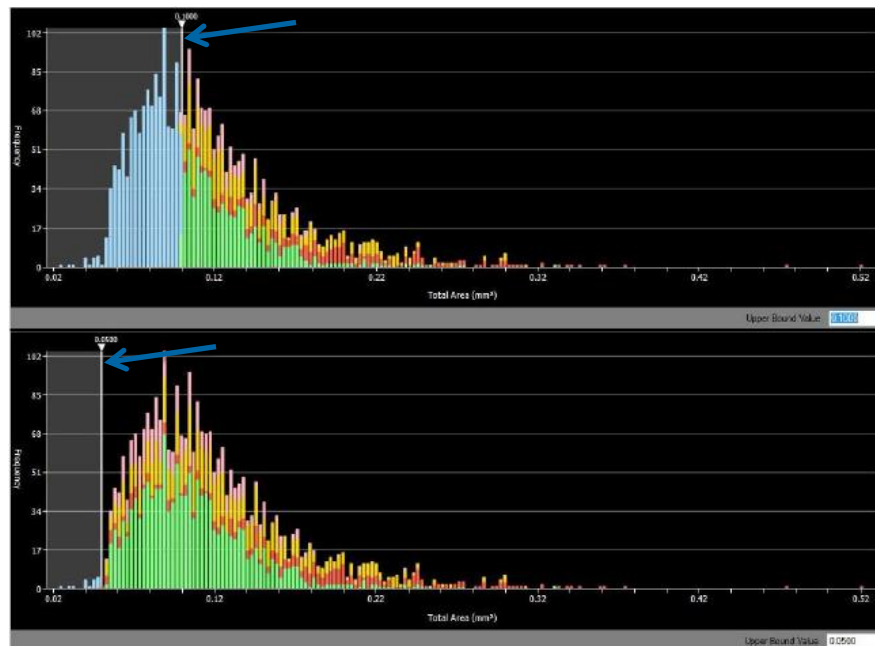
Start Time: 10:14:55



# Carrying Out A Pick Run: Refining Default Groups of Colonies

9) Any default **group** can be altered but this may compromise clonality and viability. Where possible it may be best to leave the groups with the default values (with exception of the **Too Small** cutoff, see below).

- Groups can be edited by **double clicking** on the desired **group** and **moving the default gate**.
- For Example:** the **Too Small** group cut-off point can be reduced by double clicking on the group (which will display the current cut-off value) and then **dragging the slide bar on the histogram** to 0.05 or the desired value.



The screenshot shows the software interface for colony analysis. The 'Groups' panel is active, displaying a list of groups: 'All Undiscarded Features', 'Edge Excluded [328]', 'Too Big [0]', 'Too Small [282]', 'Irregular 1 [0]', 'Irregular 2 [4]', and 'Ungated [111]'. The 'Ungated' group is highlighted in red. Below the list, the 'Group Name' is 'Ungated', and the 'Hidden' checkbox is unchecked. The 'Colour' is set to green. The 'Remove' button is visible. The 'X Axis' is set to 'Proximity (mm)' and 'Display' is set to 'Stack By Group'. The 'Ungated' group is highlighted in red in the legend.

# Carrying Out A Pick Run: Defining Groups of Colonies to Pick

10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

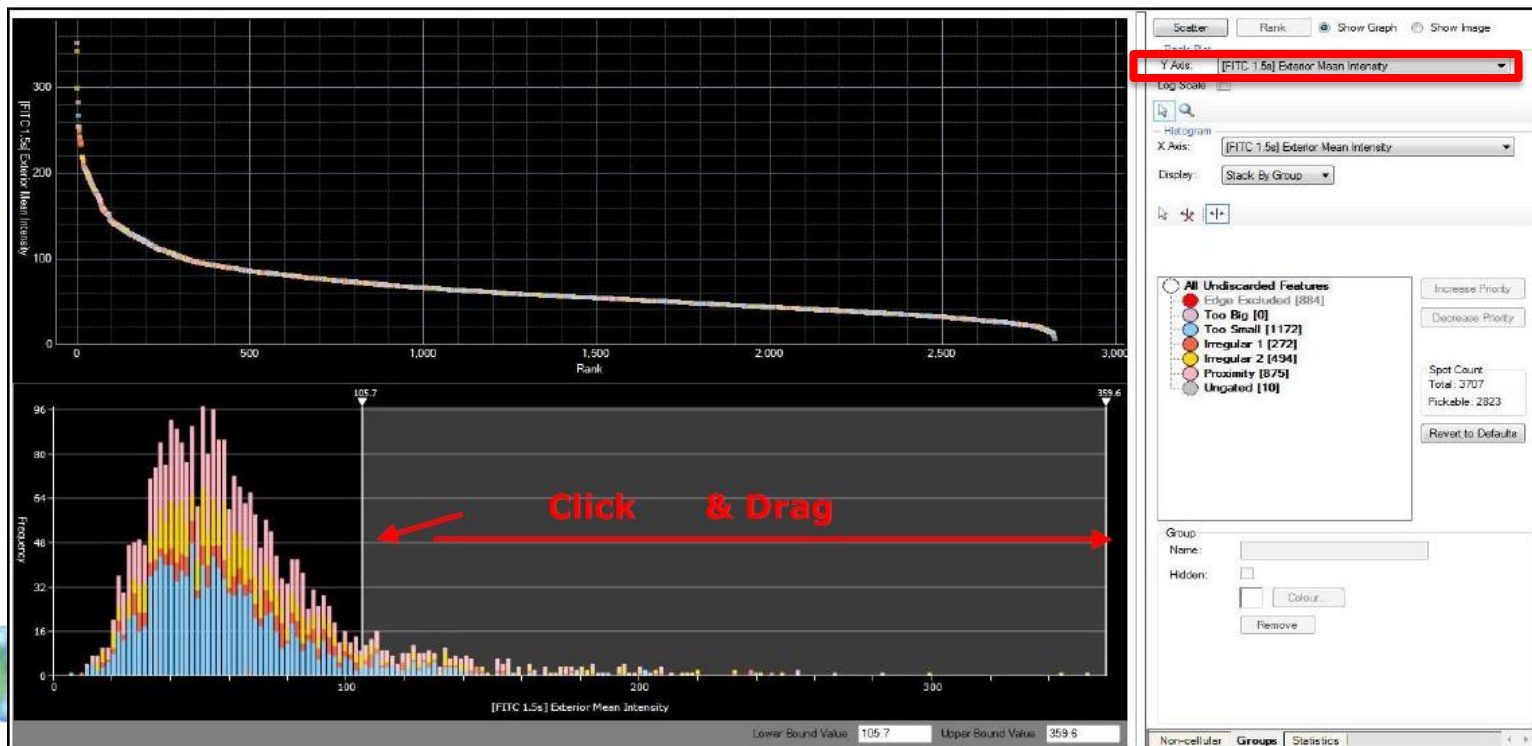
- a) To further isolate only the colonies in the **Ungated** group that have highest associated FITC fluorescence, click **All Undiscarded Features** to display all the colonies on the histogram.



# Carrying Out A Pick Run: Defining Groups of Colonies to Pick

10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

- To further isolate only the colonies in the **Ungated** group that have highest associated FITC fluorescence, click **All Undiscarded Features** to display all the colonies on the histogram.
- Select **[FITC] Exterior Mean Intensity** from the **Histogram drop-down menu**.



# Carrying Out A Pick Run: Defining Groups of Colonies to Pick

10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

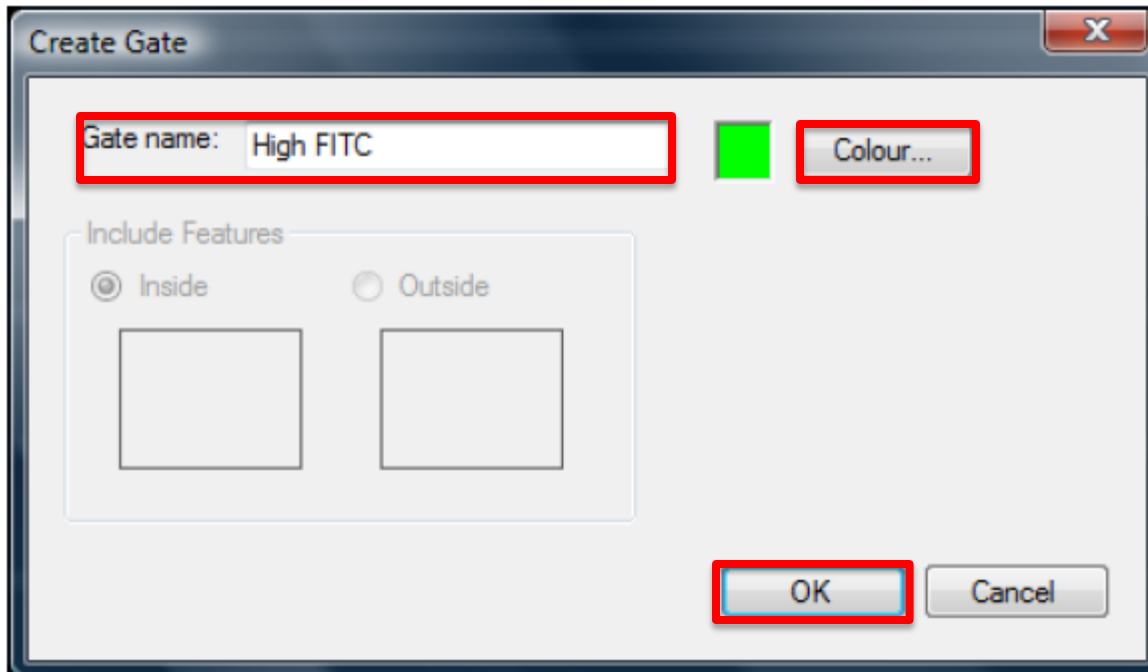
- To further isolate only the colonies in the **Ungated** group that have highest associated FITC fluorescence, click **All Undiscarded Features** to display all the colonies on the histogram.
- Select **[FITC] Exterior Mean Intensity** from the **Histogram drop-down** menu.
- Using the **Add new line gate** tool, draw a gate on the histogram by **clicking on the histogram** where the cut-off point will be and then **drag the gate off the right end of the histogram – Click and Drag**.



# Carrying Out A Pick Run: Defining Groups of Colonies to Pick

10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

d) The **Create Gate** window will appear where it is possible to name the group by typing for example 'High FITC' in the **Gate name** field and assign a group color by clicking on the **Colour** button. Click **OK** to complete this process.





# Carrying Out A Pick Run: Defining Groups of Colonies to Pick

10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

e) You will now be returned to the main graph window. Click on your **newly-created group in the All Undiscarded Features list** and then click on the **Decrease Priority** button until your new group sits just **above the Ungated group** in the list, then click **Next** to proceed.

The screenshot shows a software interface with a 'Rank Plot' section at the top, a 'Histogram' section, and a list of 'All Undiscarded Features'. The list includes:

- Edge Excluded [884]
- Too Big [0]
- Too Small [1172]
- Irregular 1 [272]
- Irregular 2 [494]
- Proximity [875]
- Ungated [10]

The 'Proximity' and 'Ungated' items are highlighted with red boxes. To the right of the list are buttons for 'Increase Priority' and 'Decrease Priority', with the 'Decrease Priority' button also highlighted with a red box. Below the list is a 'Spot Count' section showing 'Total: 3707' and 'Pickable: 2823'. At the bottom, there is a 'Group' section with fields for 'Name', 'Hidden', and 'Colour...', and a 'Remove' button. The bottom status bar shows 'Non-cellular Groups Statistics'.

# Carrying Out A Pick Run: Picking Review

11. The Picking Review window appears.

a) In the **Picking Review** tab, select only the **High FITC** group by checking the box next to it in the **Pick Groups** list.

Overview | Images | Gallery | Data Table | Graphs | **Picking Review**

Colony Images

Trans WL

FITC 2.0s

Pick Groups

Group	Total
<input type="checkbox"/> Too Big	0
<input type="checkbox"/> Too Small	282
<input type="checkbox"/> Irregular 1	0
<input type="checkbox"/> Irregular 2	4
<input type="checkbox"/> Proximity	8
<input checked="" type="checkbox"/> High FITC	45
<input type="checkbox"/> Ungated	66

Sort Options

Match Destination plate to Source plate

Collate by Well

Limit Colonies

0 Number of Colonies From Each Well

Order By: [FITC 1s] Exterior Mean Intensity

Pick Summary

Picked Colonies: 45

Destination Plates Used: 1

Deposit Plates: plate1 | Genetix PetriWell-96 Plate

Deposit Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	X	X	X	X	X	X	X	X	X	X	X	X
B	X	●	●	●	●	●	●	●	●	●	●	X
C	X	●	●	●	●	●	●	●	●	●	●	X
D	X	●	●	●	●	●	●	●	●	●	●	X
E	X	●	●	●	●	●	●	●	●	●	●	X
F	X	●	●	●	●	●	●	●	●	●	●	X
G	X	●	●	●	●	●	●	●	●	●	●	X
H	X	X	X	X	X	X	X	X	X	X	X	X

Source Barcode: 006141 Source Well: A1

Order By Value: 279,568.165



# Carrying Out A Pick Run: Picking Review

11. The Picking Review window appears.

b) Under **Sort Options**, select **Order By [FITC 1s] Exterior Mean Intensity** from the dropdown, then click **Next** to proceed.

Overview | Images | Gallery | Data Table | Graphs | **Picking Review**

Colony Images

Trans WL

FITC 2.0s

Pick Groups

Group	Total
<input type="checkbox"/> Too Big	0
<input type="checkbox"/> Too Small	282
<input type="checkbox"/> Irregular 1	0
<input type="checkbox"/> Irregular 2	4
<input type="checkbox"/> Proximity	8
<input checked="" type="checkbox"/> High FITC	45
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Sort Options

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Order By: **[FITC 1s] Exterior Mean Intensity**

Pick Summary

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Destination Plates Used: 1

Deposit Plates: plate1 | Genetix PetriWell-96 Plate

Deposit Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
B	✗	●	●	●	●	●	●	●	●	●	●	✗
C	✗	●	●	●	●	●	●	●	●	●	●	✗
D	✗	●	●	●	●	●	●	●	●	●	●	✗
E	✗	●	●	●	●	●	●	●	●	●	●	✗
F	✗	●	●	●	●	●	●	●	●	●	●	✗
G	✗	●	●	●	●	●	●	●	●	●	●	✗
H	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗

Source Barcode: 006141

Source Well: A1

Order By Value: 279,568.165



# Carrying Out A Pick Run: Proceed With Picking

- 12) The pick run will now be initiated.
  - a) When prompted, load the **destination plate(s) into the destination stacker cassette** (the number of destination plates required will be displayed in the **Pick Summary** on the **Picking Review** tab).
  - b) Click **Next** to proceed.
  - c) The picking step will proceed automatically until all colonies in the selected group have been collected.



# Finishing the Picking Process

- 12) Once the pick run has completed:
  - a) Click **Finish** to return to the **Pick Run process top page**.
  - b) Click **Close Process** to return to the **Main Navigation Screen**. If settings have not been saved previously a prompt will warn of this and allow settings to be saved.
  - c) To view the results of the picking run, click on the **Review Results** icon.

# ClonePix 2 Instrument Shut Down Procedure

13) Please follow these steps to shut down the ClonePix 2 instrument after a pick run:

- a) Exit from the ClonePix 2 application by selecting **Exit** from the **File** menu on the main setup screen.
- b) Close down Windows – Click the **Start** menu at the bottom of the screen then click **Shut Down**.
- c) Wait for computer to switch off completely.
- d) Turn the instrument off by pressing the **Stop** button on the front of the system.
- e) Turn the power off at the mains.



# Support Resources

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





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