Genetix

Picking

Application Manual

www.genetix.com

08MAN1099.A0
Effective Date: 08-Mar-10

Contents

What is Picking?

Picking is the automated process of imaging source containers such as QTrays or Petri dishes previously plated with microbes, detecting the microbial colonies, and then transferring discrete clones into microplates pre-filled with an appropriate broth media.

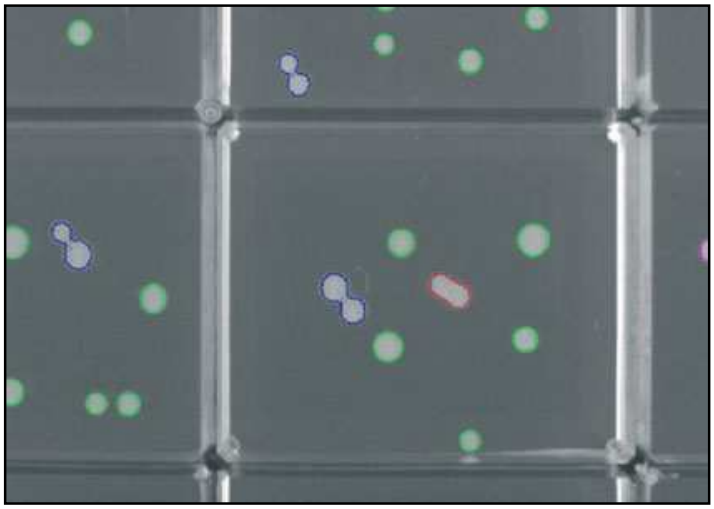

Figure 1: Colony Detection

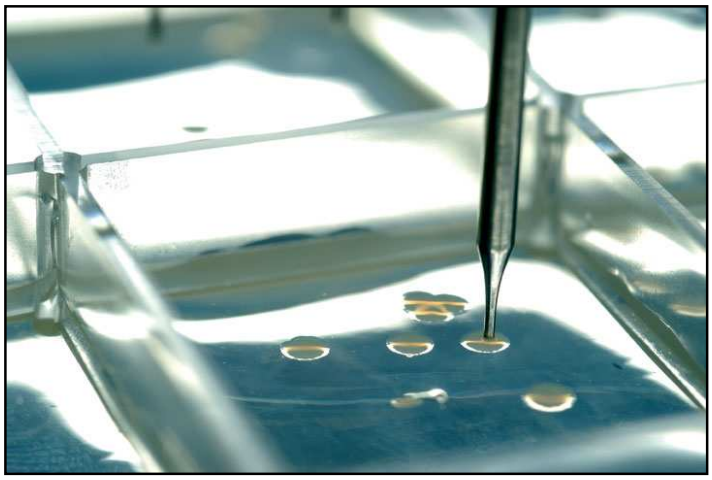

Figure 2: Colony Picking

Preparing for Picking

Preparation of Workbed

Prior to carrying out a Picking Run it is necessary to ensure that:

- The robot has been cleaned
- No extraneous objects have been left on the bed
- The correct head has been fitted
- Source **QTrays** have been prepared
- Destination Plates have been prepared
- The wash bath has clean brushes and ethanol

Fitting the Head

If the robot has been used previously for any other application, the head may need to be changed. The head must be removed and replaced with the correct head (see **General Maintenance** section in the appropriate Robot Manual).

Maintaining a Picking Head

Cleaning the head can be a long process, but it is vital for good results. The head should be cleaned every time a picking routine is completed. Handle all parts with care when cleaning to avoid bending any pins or losing springs.

- Use a flat bladed screwdriver to unscrew the 10 screws. A support is needed that allows for stability of the head but keeps the pins suspended (e.g. a pipette tip box top, or the Robot wash bath).
- Very carefully remove the top plate to expose the springs and the tops of the pins. **Be very careful, the springs are extremely springy and are easily lost!**
- Remove the pins from the main body and place in a container suitable for sonic cleansing. Sonicate the pins, body and springs for 10 minutes in a 1% solution of aQu Clean (K2505).
- Remove the pins, plate and springs from the sonicator and rinse thoroughly in distilled water.
- Blow through the plate with compressed air and dry thoroughly, along with the pins and springs.
- Insert the pins into the holes of the body, followed by the springs (all pins should fall down under their own weight).
- Place the top plate over the pins (aligned with the main body) and screw into place with the 10 screws. **Do not over tighten.**

Replacing Pins

Occasionally colonies may be missed. This is most commonly due to bent or sticking pins.

Bent pins can be easily identified by carefully checking the head before each use. Hold the head so that the tips of the pins are at eye level and look along each row of pins from each side of the head, the problem pin should be clearly identifiable.

Remove and replace the damaged pin as follows:

- Use a flat bladed screwdriver to unscrew the screws. A support is needed that allows for stability of the head but keeps the pins suspended (e.g. a pipette tip box top).
- Very carefully remove the top of the head to expose the springs and the tops of the pins.
- Using the end of an Allen key, push the damaged pin up from the bottom of the head until. Carefully remove the pin.
- Place a new pin into the hole that has been vacated, ensuring that the pin does not stick (as above). Place the top of the head over the pins and tighten the screws. **Do not over tighten**.

Recording Barcodes

Barcodes can be recorded to the log file for both source and destination. To enable barcodes to be recorded, appropriate selections must be made in the barcode options screen. The barcodes are recorded during the program run.

Barcodes can be input either directly from the keyboard or using the in-line barcode reader.

For detailed information about recording barcodes refer to the **Barcode** section.

Loading Microtiter Plate Holders

Once the microtiter destination plates that will receive the picked colonies have been prepared (see Biology Guide in this manual), they need loading on to the robot.

QPix2 uses Destination Plate Holders on the bed. **QPix2 XT** and **QP Expression** use stacker systems.

QPix2

Destination Plate Holders

Place the destination plate holders on the workbed of the robot. Make sure that each holder is flat on the table. The plate holders must to be leveled using the level adjusters – this is preset by Genetix.

QPix2 can accommodate 3 plate holders, each taking up to 5 plates (96 or 384 well):

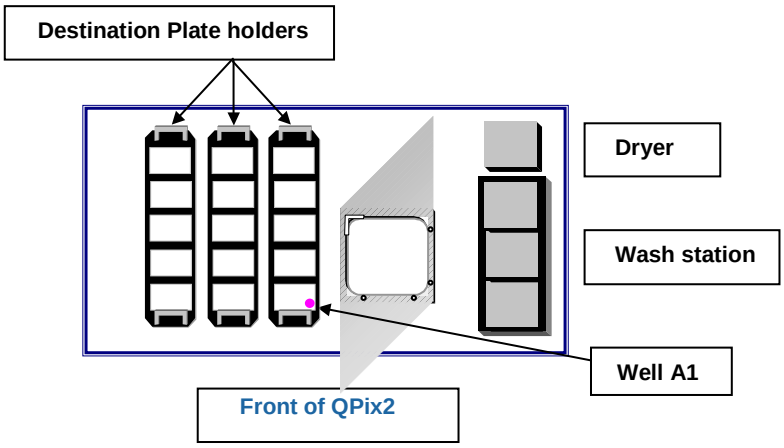

Figure 3: QPix2 Bed Layout showing Destination Plate Holders

If using only one plate holder, it must be set in position 1 on the bed (right side) and the software configured appropriately.

Fit the prepared destination plates into each holder. The destination plates must be put into the holders with lids **off** and well A1 facing front-right as shown in Figure 3: QPix2 Bed Layout showing Destination Plate Holders.

QPix2 XT / QP Expression

QPix2 XT and QP Expression use stacker systems. The microplate stacker usually has 2 cassettes which can each accommodate up to 70 microplates. The destination plates must be put into the cassettes with lids **on** and well A1 facing front-right.

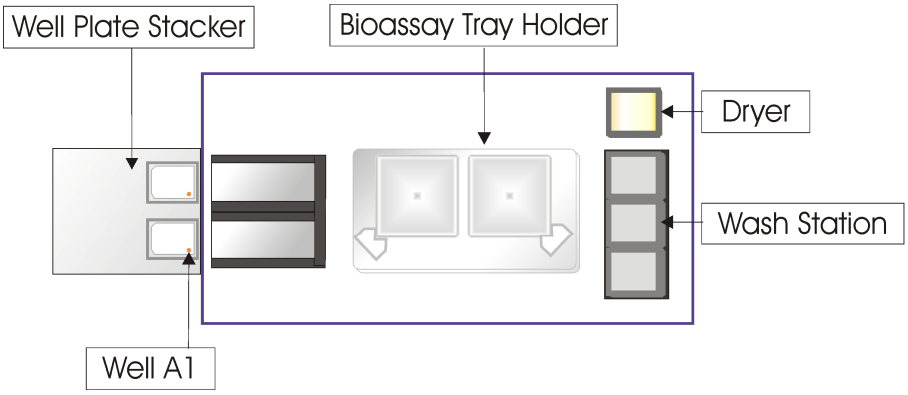

Figure 4: QPix2 XT Bed Layout showing Destination Plates in Stacker Cassettes

Loading Bioassay Tray Holders

It is recommended that colonies should be grown in 22.2 x 22.2cm Genetix **QTrays**, which have been specifically designed for colony picking applications. They offer high clarity for enhanced resolution and discrimination of colonies and use less growth medium. It is essential to have an even thickness of media in the **QTrays** to maximize picking efficiency (see Biology Guide in this manual). Colony size should also be above 0.5mm diameter for efficient picking.

QTrays are located on the robot bed in the Bioassay Tray Holder which is fitted over the light box. The holder should be a good fit and not move around appreciably.

QPix2

The **QPix2** Bioassay Tray Holder accepts one tray which is held in place by 4 locating pins and an adjustable corner bracket. The bracket should be tightened just enough to stop the **QTray** moving around.

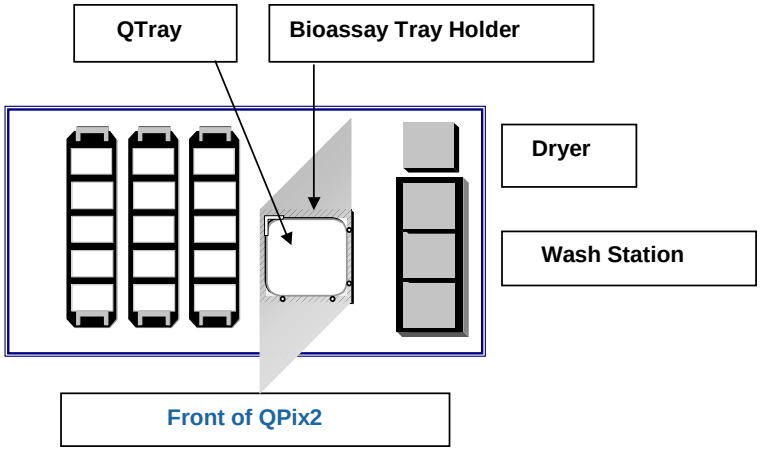

Figure 5: QPix2 Bioassay Tray Holder

It is important that the adjuster is not over tightened, as the **QTrays** will be pushed upwards instead of being held flat.

Once the bed of the robot has been prepared as detailed above, the Picking software can be set up and the Picking routine begun.

QPix2 XT / QP Expression

The **QPix2 XT** / **QP Expression** Bioassay Tray Holder accepts two **QTrays** which are held in place by 4 locating pins and adjustable corner brackets. The brackets should be tightened just enough to stop the **QTray** moving around.

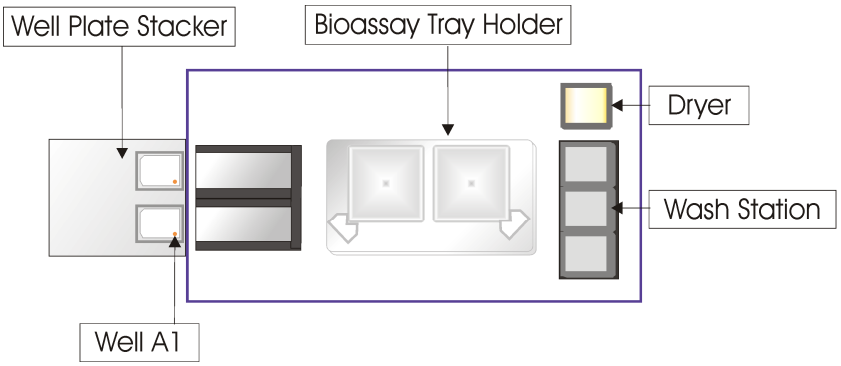

Figure 6: QPix2 XT / QP Expression Bioassay Tray Holder

The Picking Run

Overview

Double click on the **QSoft** Picking Icon on the desktop; the Picking splash screen will appear.

It is possible to create a new routine or load a previously saved routine. Any existing routines will be listed in the lower window.

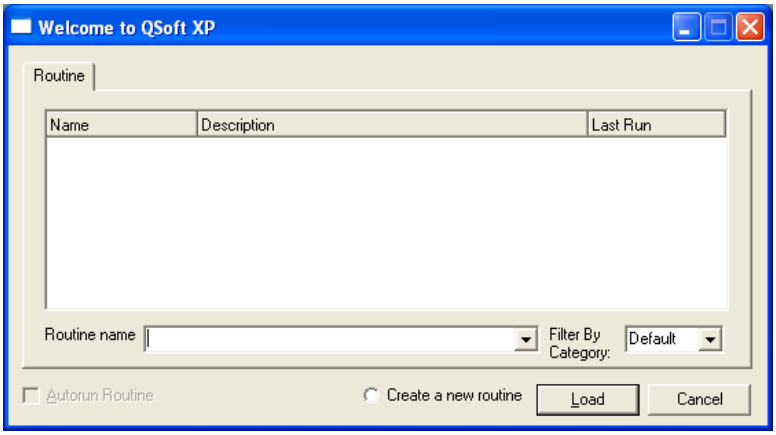

Figure 7: Welcome Prompt

Create a New Routine

Select this option then click OK. The default routine settings will be loaded, this routine can be edited and saved if required.

Load an Existing Routine

Previously saved routines are listed here. Select this option then highlight the required routine name. Click OK to start the application with the routine settings loaded.

Autorun Routine

If this box is checked, the routine is started automatically if OK the selected.

Filter by category

Previously saved routines can be filtered by the category added when the routine was created.

Welcome

Figure 8: Welcome Tab

Description

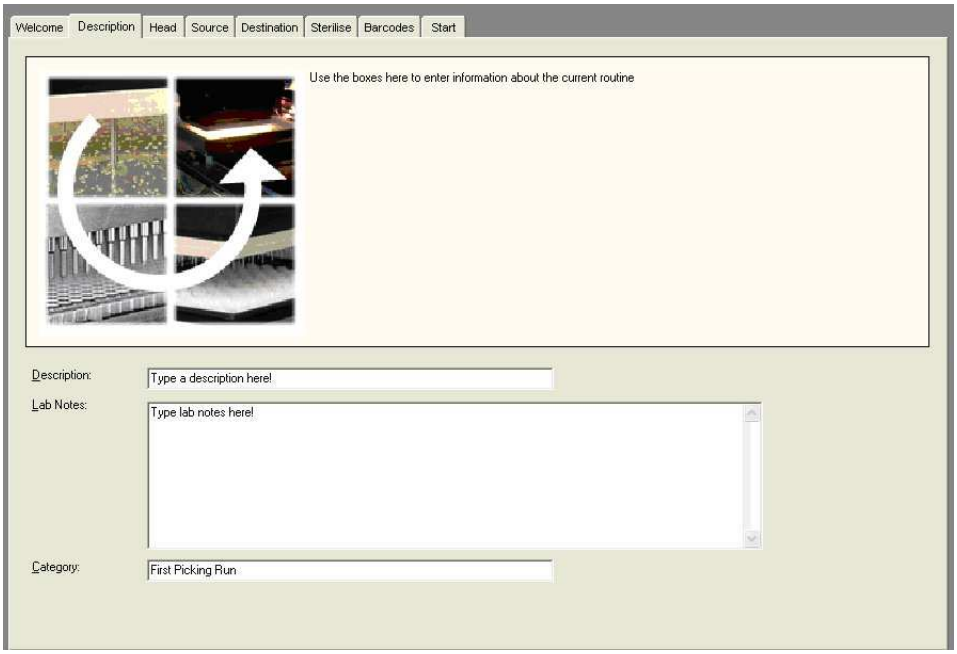

Figure 9: Description Tab

Use the text boxes here to enter any required information about the routine to be created. The category box can be used to group routines together for easy retrieval.

Head

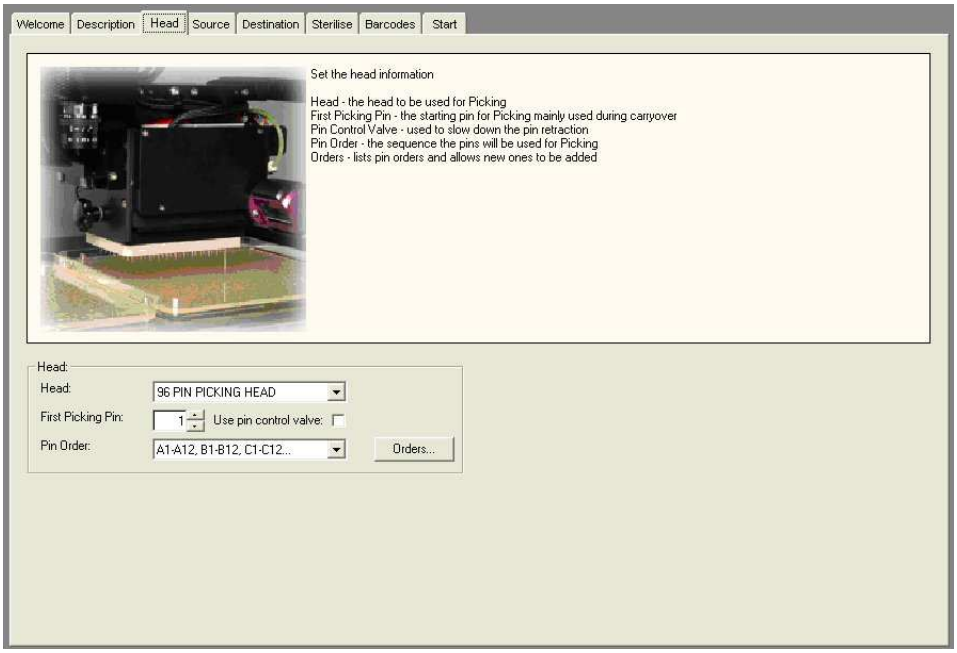

Figure 10: Head Tab

Head – This allows the type of head used to be specified. Currently there is only 1 head option for picking:

- 96 Pin Picking Head

First Picking Pin - If, at the end of a picking run, the Picking Head has picked less than 96 colonies, the remaining pins can be used to pick colonies from the next **QTray**. This option can be achieved by setting the numbered pin at which the picking resumes.

Use pin control valve – Slows down the pin retraction. This is useful for first time use or for demonstration purposes to allowing the observation of the mechanical actions of the pins.

Pin Order – This option enables the order in which the pins in the Picking Head will fire to be chosen. Traditionally the Pins are fired in 1 of 4 orders as follows:

- A1-H1,H2-A2… (snaking)
- A1-A12, B12-B1… (snaking)
- A1-H1, A2-H2, A3-H3…
- A1-A12, B1-B12, C1-C12

Orders - A new custom Pin Order can be defined or an existing customer order can be edited (picking orders defined by Genetix cannot be edited). For example, grouping 6 wells together A1, A2, A3, B1, B2, B3.

Add – Click on this button to create a new pin order. The following screen is displayed:

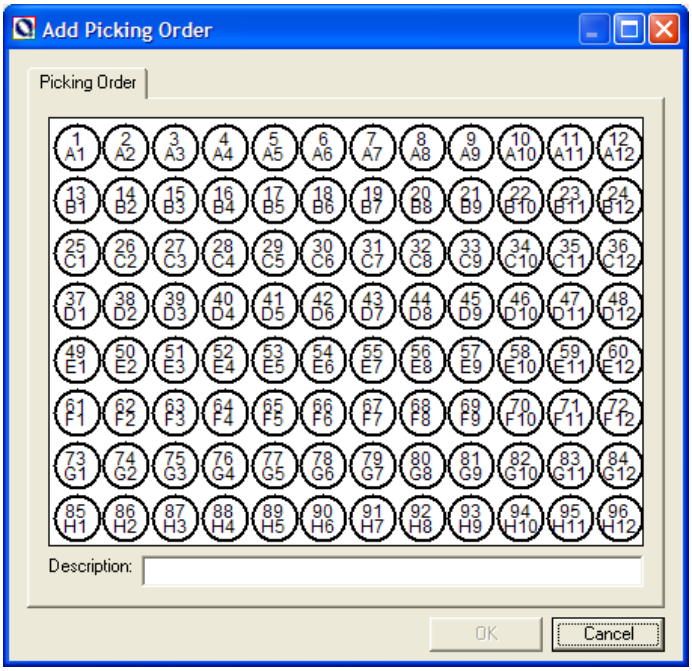

Figure 11: Add Picking Order

Use the mouse to point to the pin references in the desired order and click the right mouse button on each one in turn. When finished defining the custom order, click in the **Description** window and type a name for the new order. Click on **OK** to save the Pin Order.

Edit – Provides the ability to change any custom pin order, ensure that the order is selected in the list and click on **Edit.** The procedure is the same as for **Add.**

Delete – Allows the removal of any custom pin orders (Genetix defined pin orders can not be deleted). Click on **Delete**, a confirm deletion prompt will appear, click **Yes** to remove the pin order or **No** to abort.

OK – Saves the list of pin orders and adds any newly created ones to the drop down list.

Source

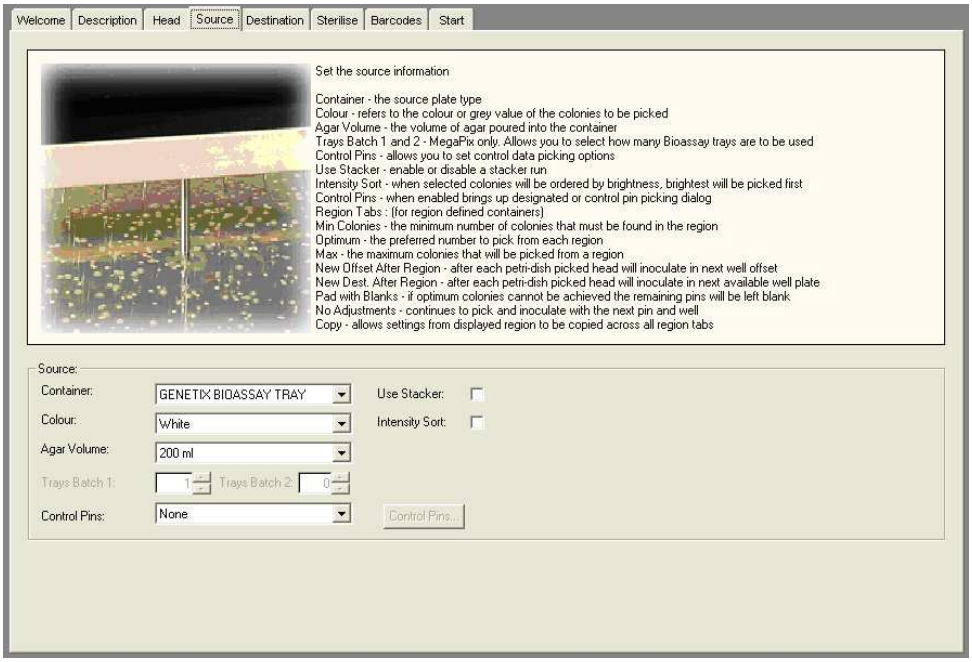

Figure 12: Source Tab

Container - This field defines the type of source plate that is to be picked from. This tray is located above the light box in a special tray holder as described previously. The options available are:

- Genetix Bioassay Tray **–** Used where colonies have been plated out in a standard 22x22cm **QTray**.
- Petri dish holders - (optional extra) If a region bioassay tray has been added to the robot, its name will appear in the drop-down list box. Used when an area within a Bioassay Tray is split into regions. For example, when picking from Petri dishes, square trays, etc. select one of the Petri dish holders.

When one of these objects is selected, the right hand side of the main Picking screen shows the Region dialog boxes which allow the following options to be selected.

- Min Colonies – this represents the desired minimum number of colonies that must be found in the region. A message will be displayed if the minimum number cannot be achieved. The numbers of colonies to be picked can be edited or the run can be cancelled.
- Max Colonies – this represents the maximum colonies that will be picked from a region
- Optimum – this is the *preferred* number to pick from each region given that sufficient colonies are found in each region.

If the Optimum colonies are not found for Picking, then the total will be made up from other regions (providing the Maximum is greater than the Optimum).

For example, if exactly 48 picked colonies are required from each Petri-dish, set the Minimum, Optimum and Maximum to 48 each.

However, if 48 colonies is the ideal number to pick but there may not be 48 in each region, the options can be set as follows:

Minimum: **0**

Optimum: **48**

Maximum: **999**

This basically says, 'Try to Locate & Pick 48 colonies from each region but make the numbers up from other regions if 48 are not found'.

If, for any reason, the Optimum cannot be achieved, adjust the numbers to pick prior to the Picking run in the View/Modify dialog (Figure 70: Picking Data Summary).

- Copy – this option will copy the displayed Minimum, Maximum and Optimum colonies to all the other regions.

The region Bioassay tray can be configured, via the configuration screen, to create up to 100 regions within the footprint of a Bioassay Tray.

Other options in the Regions tab are as follows:

- New Offset After – After each Petri-dish is picked the head will inoculate in the next well offset i.e. A1, B1, A2, B2.
- New Dest After – After each Petri-dish is picked the head will inoculate in the next available Well Plate.
- Pad with Blanks – If the Optimum colonies cannot be achieved the remaining pins will be left blank. For example if the Optimum is 48 and only 25 colonies were found in region #1, the first pin to fire in region #2 would be pin 49 – thereby leaving pins 26 thru 48 blank.
- No Adjustments – Continues to pick and inoculate with the next pin/well reference as per 'normal'.

Use Stacker - If a **QTray** Stacker is installed, you can opt to use it here or, when deselected, use the robot in 'Manual' mode by placing trays on the Bed.

When this option is selected the system will check a valid Stacker object exists in the current configuration and will enable the **No Trays** (number of trays) option.

No Trays – This entry is only available if 'Use Stacker' is opted. It defines the total number of **QTrays** to be processed in this run.

Color - This refers to the color or grey value of the colonies to be picked. Altering the color causes the threshold to be set at a predetermined value. The options are 'White' for normal colonies, 'Blue/White' if White colonies are to be picked on Blue/White plates or 'Phage'.

Intensity Sort – When this box is checked, colonies will be ordered by brightness - the brightest will be picked first. This option is only relevant to fluorescent colonies.

Agar Volume – These settings relate to the volume of agar poured into the bioassay tray. The smaller the amount of agar that can be used the better. The 200ml setting is best but good results can still be achieved using the 250, 300, 350 or 400ml. Each volume setting has a picking height associated with it; the height that the pins are lowered to pick up a sample from the bioassay tray. The volume of agar in each tray must be consistent, and must be poured and set on a level surface. Agar that has not been poured level will degrade picking accuracy.

Use Control Data - Checking this box allows pins to be reserved for Control Data. Control Data is currently defined as either:

- Reserving Pins to pick known controls from a tray.

Or

- Reserving Pins to 'Leave Blank' i.e. not use certain pin(s) for Picking.

Up to 95 Pins can be selected from the 96 Pin Picking Head to be reserved for Control Data.

Control Pins - Enables various pins to be selected as control pins or enables designated pins. See blow for more details. 'None' can also be selected which means no control pins will be used.

Designated Pins

It is possible to designate the Destination Wells for each Picking Region by designating the Pins that will be used to pick from each region.

Each pin can be designated once per Destination plate and Picking will return to each region until all colonies are exhausted, using multiple destination plates during the Picking run.

Using Designated Pins

Select the *Use Designated Pins* option on the Source panel. Selecting this option disables all other Regional settings, i.e. Minimum, Maximum and Optimum Colonies along with Padding options. Click the *Designated Pins* Button and you will be presented with the following screen:

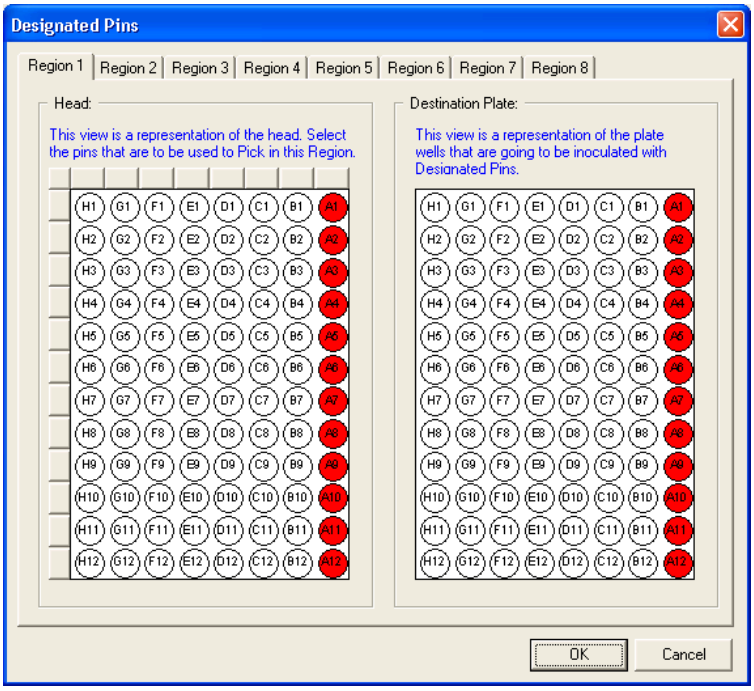

Figure 13: Designated pins screen

Each pin can be designated once per Destination plate and Picking will return to each region until all colonies are exhausted, using multiple destination plates during the Picking run.

Row and Column Selector buttons

To select or deselect a whole row or column of pins, click the row selectors next to the appropriate row or column. To select or deselect the whole head, click the selector button in the top left corner of the head.

Note: Pins that have been selected in other regions will be left blank when using the selector buttons.

Picking by Regions - Examples

1. Optimum colonies can never be less than Min Colonies.
2. The hidden optimum calculation is explained as follows:

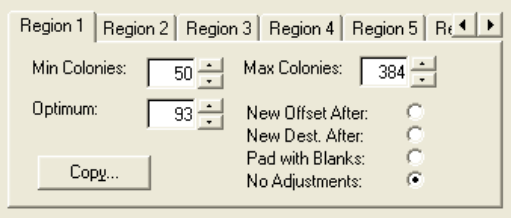

Figure 14: Region Picking Options

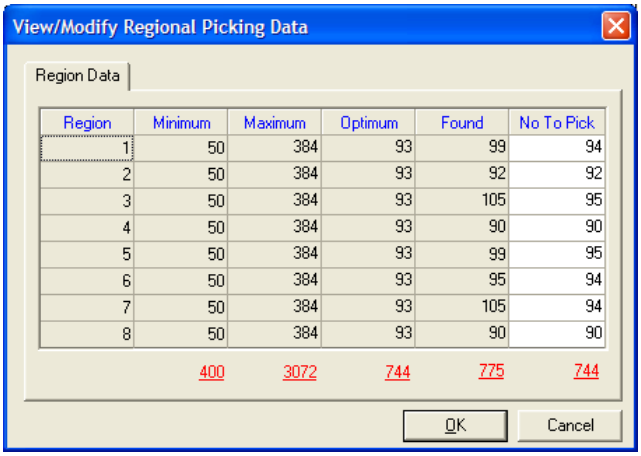

Figure 15: View/Modify Regional Picking Data

The Total optimum is optimum (93) x regions (8) = 744, which is what is going to be picked out of a total colonies found of 775.

The optimum cannot be achieved in regions 2, 4, 8 so the number to pick is the maximum found.

Region	Found	NoToPick
2	92	92
4	90	90
8	90	90
		=272

Total optimum (744) – 272 = 472

So we are looking to achieve 472 colonies from the following 5 regions to make up our total optimum of 744.

This leaves 5 regions 1, 3, 5, 6, 7

Region	Found
1	99
3	105
5	99
6	95
7	105
	= 503

Colonies left (472) ÷ Regions left (5) = 94.5

In this case it will attempt to pick 95 (94.5 rounded up) from each of these 5 regions.

The View/Modify Regional Picking Data dialog will be displayed at this point to allow further adjustments of the numbers. For example in the event that the Total Optimum cannot be achieved because of insufficient numbers of colonies in any of the regions.

Scenarios for Picking Options

Based on: All have regions copied over.

Scenario 1 – No Adjustments

2 QTrays only on QPix2 XT/ QP Expression. 4 x 2 Regions 384 well plate **no adjustments**.

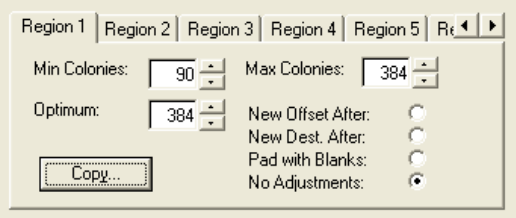

Figure 16

The following screens are displayed because only 1 bioassay tray has been selected for imaging. Therefore, no colonies are found in Regions 5, 6, 7 and 8.

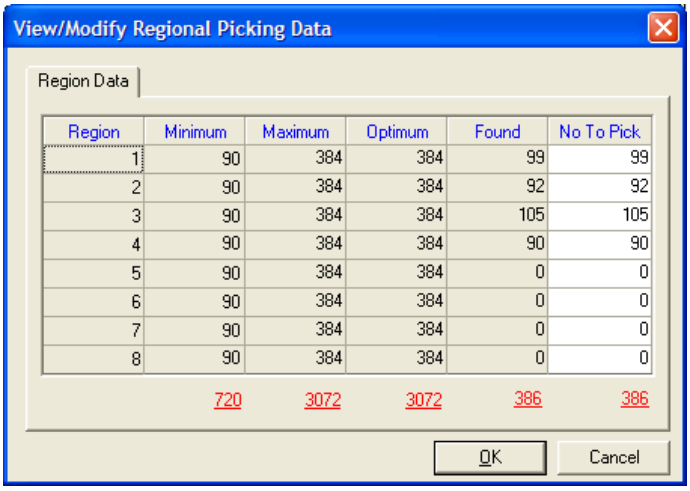

Figure 17

Step	Number to Pick	From Region	To plate-well Offset	Comment
1	96	1	1-A1	Pick first 96 from region 1, deposit to plate 1
2	3	1		Pick remaining 3 from region 1 don't deposit yet
3	92	2		Pick all 92 from region 2
4	1	3	1-A2	Plus 1 from region 3 makes 96, enough to deposit
5	96	3	1-B1	Another head-full from region 3 - deposit
6	8	3		Pick last 8 from region 3
7	88	4	1-B2	Make up a head-full from region 4 - deposit
8	2	4	1-A1	Pick and deposit last 2

Scenario 2 – Pad with Blanks

2 QTrays only on QPix2 XT/ QP Expression. 4 x 2 Regions 384 well plate **Pad with Blanks**.

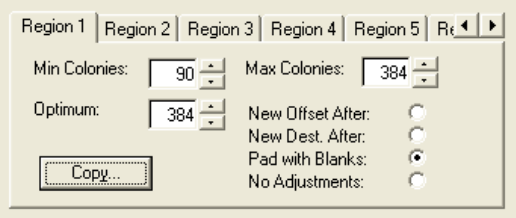

Figure 18

Same message and Options as Scenario 1

Step	Number to Pick	From Region	To plate-well Offset	Comment
1	96	1	1-A1	Pick first 96 from region 1, deposit to plate 1
2	3	1	1-A2	Pick remaining 3 from region 1 and pad with 93 blanks
3	92	2	1-B1	Pick all 92 from region 2 and pad with 4 blanks
4	96	3	1-B2	Pick first 96 from region 3, deposit to plate 1
5	9	3	1-A1	Pick remaining 9 from region 3 and pad with 85 blanks
6	90	4	2-A2	Pick all 90 from region 4 and pad with 6 blanks

Scenario 3 - New Destination After

2 QTrays only on QPix2 XT/ QP Expression. 4 x 2 Regions 384 well plate **New Dest After**.

Note: This means after the optimum have been found and deposited the next regions deposit will be in a new plate.

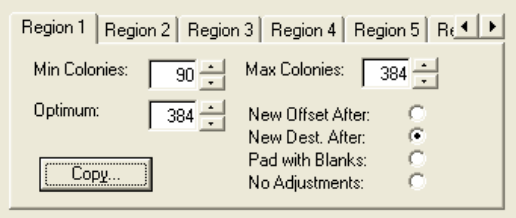

Figure 19

Same message as Scenario 1 & 2

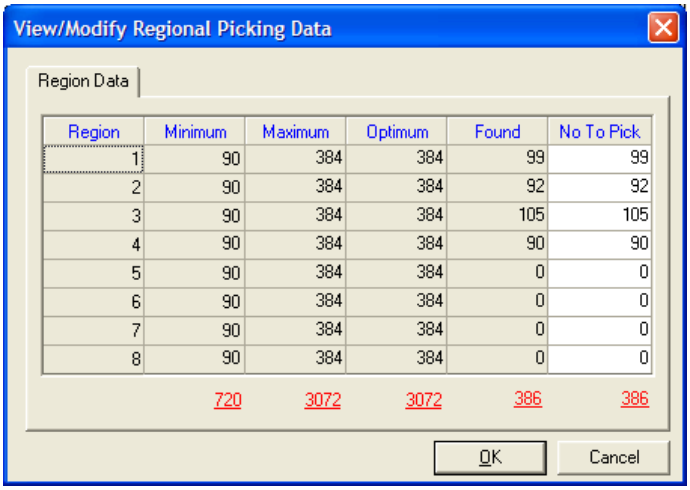

Figure 20

Step	Number to Pick	From Region	To plate-well Offset	Comment
1	96	1	1-A1	Pick first 96 from region 1, deposit to plate 1
2	3	1	1-A2	Pick remaining 3 from region 1, deposit to plate 1
3	92	2	2-A1	Pick all 92 from region 2, deposit to plate 2
4	96	3	3-A1	Pick first 96 from region 3, deposit to plate 3
5	9	3	3-A2	Pick remaining 9 from region 3, deposit to plate 3
6	90	4	4-A1	Pick all 90 from region 4, deposit to plate 4

Scenario 4 - New Offset After

Note: when depositing into a 96 well plate there is no reason to display this option.

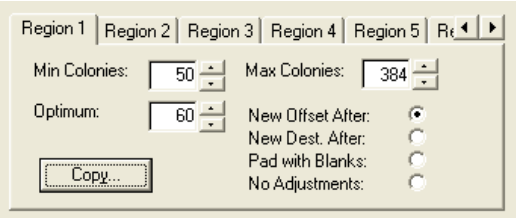

Figure 21

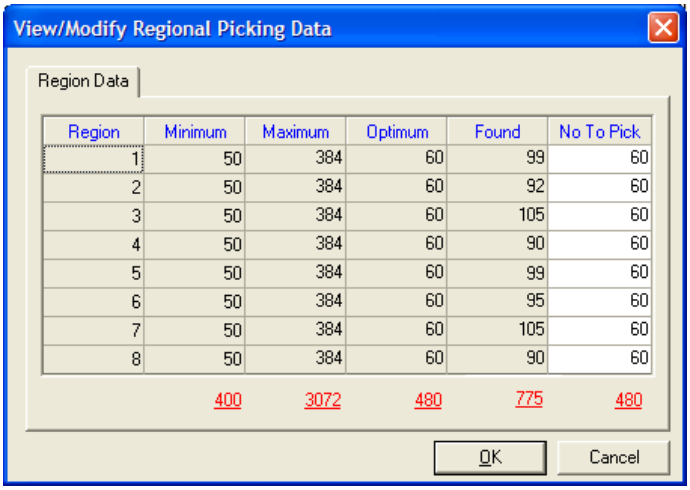

Figure 22

Step	Number to Pick	From Region	To plate-well Offset	Comment
1	60	1	1-A1	Pick 60 from region 1, deposit to plate 1 offset A1
2	60	2	1-A2	Pick 60 from region 2, deposit to plate 1 offset A2
3	60	3	1-B1	Pick 60 from region 3, deposit to plate 1 offset B1
4	60	4	1-B2	Pick 60 from region 4, deposit to plate 1 offset B2
5	60	5	2-A1	Pick 60 from region 5, deposit to plate 2 offset A1
6	60	6	2-A2	Pick 60 from region 6, deposit to plate 2 offset A2
7	60	7	2-B1	Pick 60 from region 7, deposit to plate 2 offset B1
8	60	8	2-B2	Pick 60 from region 8, deposit to plate 2 offset B2

- **Sub-Plate format**

Allows the user to set-up their well plate to directly correspond to their picking regions. This must be done by equal divisions.

Example: 96 destination well plate

Sub-plate format 12

Step	Number to Pick	From Region	To plate-well Offset	Comment
1	12	1		Pick first 12 from region 1
2	12	2		Pick first 12 from region 2
3	12	3		Pick first 12 from region 3
4	12	4		Pick first 12 from region 4
5	12	5		Pick first 12 from region 5
6	12	6		Pick first 12 from region 6
7	12	7		Pick first 12 from region 7
8	12	8	1	Pick first 12 from region 8, deposit all 96 to plate 1
1	12	1		Pick first 12 from region 1
2	12	2		Pick first 12 from region 2
3	12	3		Pick first 12 from region 3
4	12	4		Pick first 12 from region 4
5	12	5		Pick first 12 from region 5
6	12	6		Pick first 12 from region 6
7	12	7		Pick first 12 from region 7
8	12	8	2	Pick next 12 from region 8, deposit all 96 to plate 2

Continue in this way until required numbers have been picked from each region

Destination

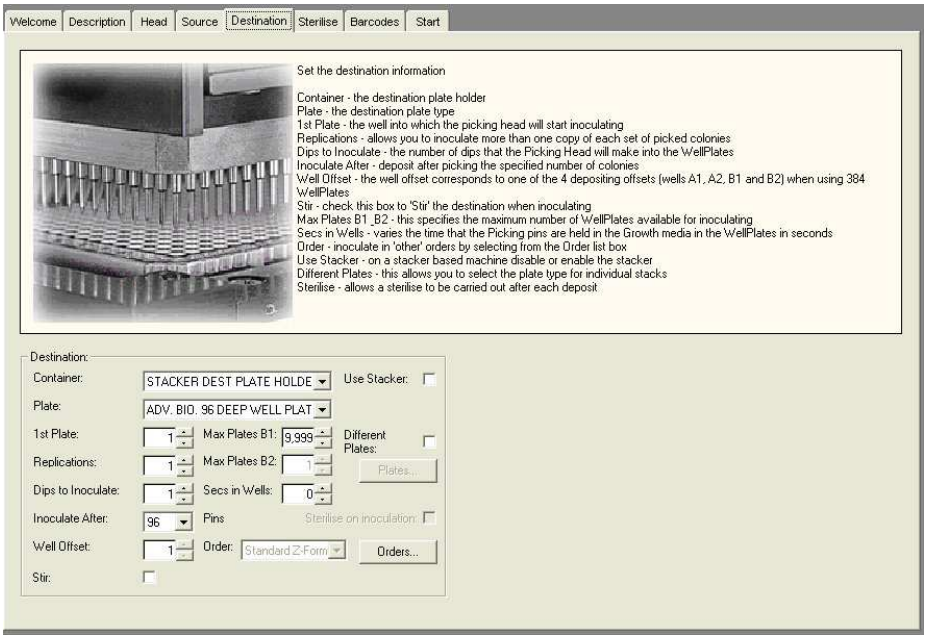

Figure 23: Destination Tab

Container - This refers to the container that is to hold the destination plates.

Use Stacker - If a WellPlate Stacker is installed, click this check box to tell the software to use the stacker.

Plate - This field defines the type of destination plate that is to be picked into. These plates will be located in the container specified above.

For example:

- Genetix 96 well plate
- Genetix 384 well plate

Note: It is essential that the correct type of microplate be selected. Severe damage to the Picking pins or robot can result if incorrectly set.

1st Plate - This determines the microplate into which the picking head will start inoculating. It is normally set at 1 but can be set at any value up to the maximum number of plates available in the destination container(s).

Max Plates - This specifies the maximum number of microplates available for inoculating. If this value is set to a higher number of plates than the total Destination Plate Holder capacity, e.g. 16 for **QPix2**, a 'Pop-up' screen will appear and ask you to 'Change WellPlates' before continuing.

Different Plates – If this box is checked the Plates command will be enabled. This allows you to select the plate type for individual stacks.

Note: This option only applies to stackers that have been custom made for this purpose. Only combinations of different 96-well plates can be used.

Plates – Click to select plate types for each stack.

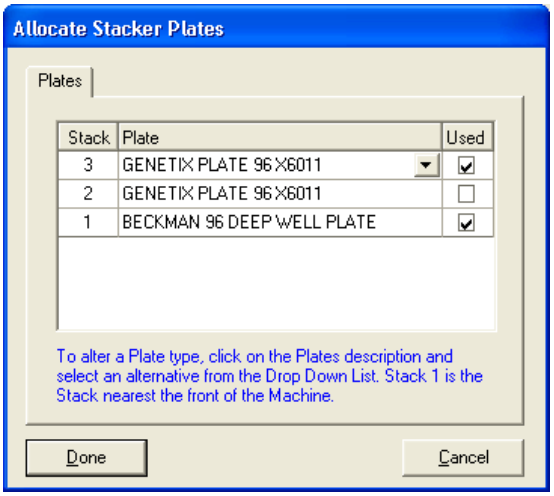

Figure 24: Allocate Stacker Plates screen

Stack **–** Available stack numbers are listed

Plate **–** Select plate type from the drop down list

Used **–** Indicates which stacks are in use

Replications – This option allows inoculation of more than one copy of each set of picked colonies.

Dips to Inoculate - In order to control the inoculation of the destination plates, it is possible to choose the number of dips that the Picking Head will make into the microplates. This setting would normally be in the range of 1 to 3.

Seconds in Wells - Varies the time that the Picking pins are held in the growth media in the microplates in seconds. This parameter is used in conjunction with the number of Dips to Inoculate.

Inoculate After – Will deposit after picking the specified number of colonies. For example, if 16 is selected from the drop down list, 16 colonies will be picked then deposited, another 16 will be picked and deposited and so on up to 96.

Sterilize on Inoculation – When used in conjunction with "Inoculate After", if this box is checked, a sterilize routine will be carried out after each deposit.

Well Offset – The well offset corresponds to one of the 4 depositing offsets (wells A1, A2, B1 and B2) when using 384 well plates. This option is disabled if a 96 well plate is selected.

Order – This option defines the order of inoculation for a 384 well plate. Pre-defined orders are:

- Clockwise - A1, A2, B2, B1
- Counter-Clockwise - A1, B1, B2, A2
- Standard Z-Format - A1, A2, B1, B2 (the current default)

Orders button - A new custom order can be defined or an existing custom order can be edited (orders defined by Genetix cannot be edited).

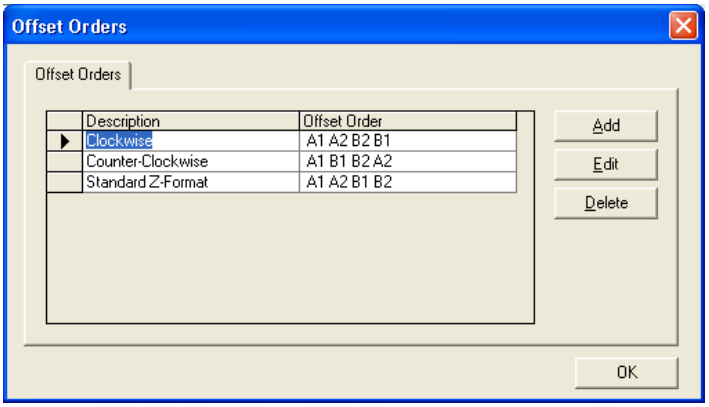

Figure 25: Offset Orders Dialog

Add – Click on this button to create a new order. The following screen is displayed.

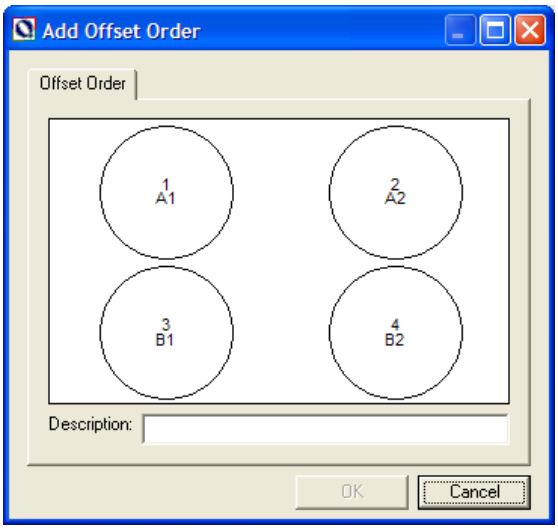

Figure 26: Add Offset Order Dialog

Use the mouse to point to the well references in the desired order and click the right mouse button on each one in turn. When finished defining the custom order, click in the **Description** window and type a name for the new order. Click on **OK** to save the order.

Edit – Provides the ability to change any custom order. Ensure that the order is selected in the list and click on **Edit.** The procedure for editing is the same as for **Add.**

Delete – Allows the removal of any custom orders (Genetix defined orders can not be deleted). Click on **Delete**, a confirm deletion prompt will appear, click **Yes** to remove the order or **No** to abort.

OK – Saves the list of orders and adds any newly created ones to the drop down list.

Stir – Check this box to 'Stir' the destination when inoculating. The Plate:NoStirs property (Configuration – Plate object properties) determines how many 'stirs' will be carried out.

Sterilize

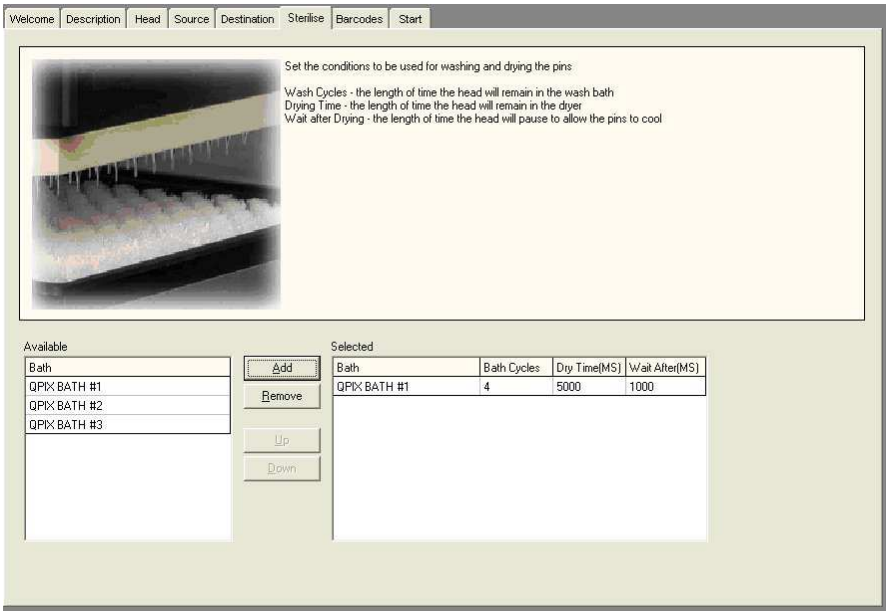

Figure 27: Sterilize Tab

Note: The number of "tabs" displayed on the screen will vary depending on how many baths are installed

Bath Cycles – Defines the number of times the head is scrubbed within the bath. If this is set to 0, the other sterilizing options are not available.

Dry Time – Sets the number of seconds that the halogen heater dries the head. This can be set at zero if drying is not required. If the number of bath cycles is set to zero this box is disabled.

Wait After - A time delay (in milliseconds) can be introduced to allow the head to cool after drying.

Note: QSoft automatically adds wait time in order to allow enough time for the pins to cool properly after the halogen heating.

Wait time is based on the following calculation:

3 Seconds + (1.5 x Dry Time)

Thus even if Wait Time was set to 0 and Dry Time was set to 5000 ms, the head would remain in the dryer for 10.5 seconds after drying.

Wash Solutions - A single wash bath containing 80% ethanol will ensure sterility when handling E. coli. However, some organisms (e.g. yeast) form 'sticky' colonies that can build up on the pins and others are particularly robust (e.g. spore-forming organisms). To ensure sterility in these cases it is recommended to use all three wash baths with the following solutions:

Bath 1 – 1% sodium hypochlorite

Bath 2 – demineralized water

Bath 3 – 80% ethanol

The washes should be performed in the order 1% sodium hypochlorite, water, then 80% ethanol followed by drying and the wait after drying.

Note: Use of sodium hypochlorite at concentrations greater than 1% may cause damage to the instrument.

Barcodes

Clicking this tab allows you to input barcodes for QTrays/Petri-dishes and outputs recorded barcodes through the associated log file. Barcodes can be read automatically, or can be input via the keyboard or an inline barcode reader device available from Genetix.

Barcodes are recorded during the program run.

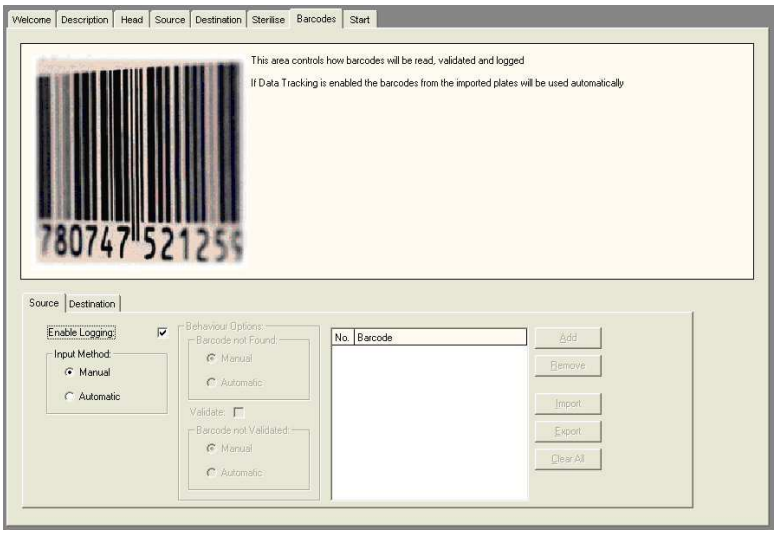

Figure 28: Barcodes Tab

Source

Enable Logging – Check this box to enable barcode reading

Input Method – Choose **Manual** if scanning barcodes is required with a hand held barcode reader or to input barcodes at the keyboard. **Automatic** barcode reading is possible if the robot has a camera installed. If automatic barcode reading is required you will need to contact Genetix Ltd for a license.

The following options are enabled when **Automatic** barcode reading is selected:

Behavior Options – This section determines how QSoft will behave in the specified circumstances.

Barcode Not Found

Set required behavior if a barcode is not present.

- **Manual** will produce a prompt and will wait for the user to input a barcode (either via the keyboard or using a hand held barcode scanner).
- **Automatic** causes QSoft to generate a unique barcode based on the system date and time.

Validate – Check this box to enable barcode validation.

Barcode Not Validated

- **Manual -** will pause to gives the opportunity to either cancel the run or continue the run using the plate barcode just read or the barcode supplied in the validation list.
- **Automatic -** in the event of an invalid barcode the run will continue automatically replacing the *expected* barcode with the *read* barcode. These actions will be recorded in the log.

Add – Allows the input of validation barcodes

Remove – Deletes the highlighted entry

Import – Prompts for the name of the text file that contains the validation barcodes

Export – Creates a text file of the current list

Clear All – Removes all entries from the list

Destination

Barcodes – Checking this box allows you to input barcodes for well plates and will output recorded barcodes through the associated log file. Barcodes can be input via the keyboard or an inline barcode reader device.

Enable Logging – Check this box to enable barcode reading

Input Method – Choose **Manual** if you wish to scan barcodes with a hand held barcode reader or to input barcodes at the keyboard. **Automatic** barcode reading is possible if the robot has a camera installed. If automatic barcode reading is required you will need to contact Genetix for a license.

The following options are enabled when **Automatic** barcode reading is selected.

Behavior Options – This section allows you to determine how QSoft will behave in the specified circumstances.

Barcode Not Found

Set required behavior if a barcode is not present.

- **Manual -** will produce a prompt and will wait for the input of a barcode (either via the keyboard or using a hand held barcode scanner).
- **Automatic -** causes QSoft to generate a unique barcode based on the system date and time.

Validate – Check this box to enable barcode validation.

Validate Mode

- **Exact Barcode –** will only attempt to match the barcode against the one in the corresponding position in the list.
- **Any in List –** will search the whole list in an attempt to match the barcode.

Barcode Not Validated

- **Manual -** will pause to gives the opportunity to either cancel the run or continue the run using the plate barcode just read or the barcode supplied in the validation list.
- **Automatic -** in the event of an invalid barcode the run will continue automatically replacing the *expected* barcode with the *read* barcode. These actions will be recorded in the log.

Add – Allows the input of validation barcodes

Remove – Deletes the highlighted entry

Import – Prompts for the name of the text file that contains the validation barcodes

Export – Creates a text file of the current list

Clear All – Removes all entries from the list

Start

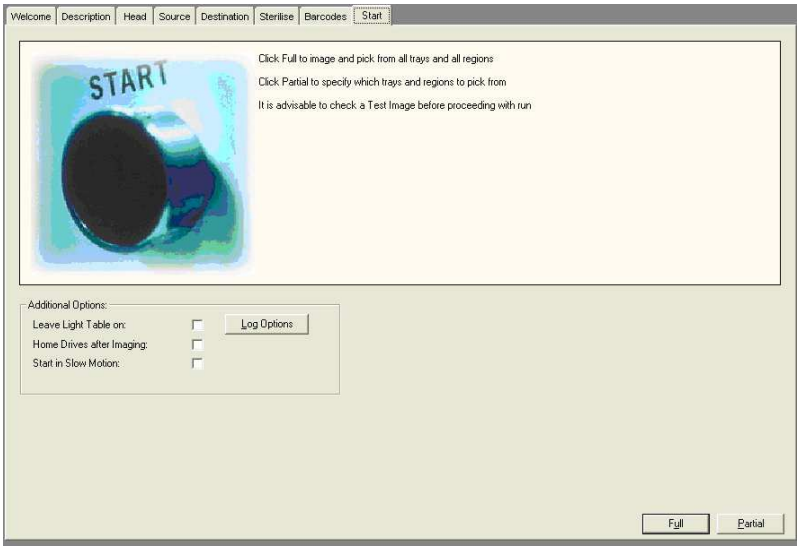

Figure 29: Start Tab

Leave light table on – Check this box to turn on the fluorescent lamps in the light table during picking. It is recommended to enable this option, so that the lamps run at a constant temperature. This option also allows you to monitor accuracy during the picking run.

Home Drives after Imaging – Check this box to home the drives after imaging. This moves the head clear to allow for loading destination plates.

Log Options – Displays the following dialog:

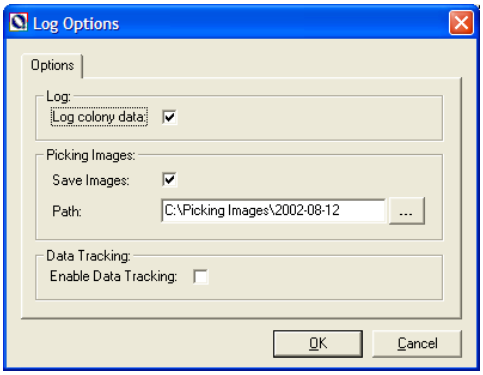

Figure 30: Log Options

Log – Check the Log Colony Data box if you want to record colony information.

The following details are written to the log file:

- Image Region
- Position X
- Position Y
- Area
- Radius
- Picking Region
- QTray No.

Save Images – Checking this box will ensure that the images that are captured are saved to

disk. The default location for saving is a folder called Picking Images which contains a sub-folder named after the current date. Each filename consists of the image number followed by the current time.

If required, change the location for saving images by clicking the browse button alongside the path name.

The Menu Options

File Menu

Switch User – Displays the Login prompt. There are currently 3 levels of user, permissions are as follows:

Operator

- Load routines
- Run routines

Creator

- Create routines
- Load routines
- Run routines
- Save routines
- Save other user's routines with a new name

Admin

- No restrictions on use

Sign On – Allows you to record information about the current run.

Figure 31: Sign on screen

Exit – Closes the application

View Menu

Configuration - entails entering all required objects and then datuming all the co-ordinates for the various objects on the workbed.

The configuration settings can be accessed by selecting the **Configuration** option. These settings are held in a central database (namely **QSoft**.mdb).

This procedure needs to be carried out:

- On first receipt of the machine (to be carried out by Genetix Personnel).
- On the addition or change of any objects to the workbed.
- If the drives have been realigned or subject to a knock.

The procedure for configuring the robot is described in the appropriate Robot Manual.

Diagnostics - The diagnostics option opens the **Diagnostics Screen**, which is useful for observing the various robot movements. Click **Stay on Top** to keep the dialog visible while the application is running.

IO's - To enter the I/O Diagnostics screen select the I/O's option. As this function will allow you to switch various inputs and outputs manually, it will prompt you for a password.

Logs - Displays lists of all available log files. Highlight the log file name and click **Open** to view the log file.

Options Menu

Reset Toolbar - It is possible to rearrange the buttons on the toolbar if necessary. This option will set them back to the default order.

Script Options - Displays a dialog of options that will be displayed during the gridding run. This allows you to choose which screens will appear and which will not.

Note**:** Any option checked here will pause the picking run whilst waiting for user input.

The Toolbar

The buttons on the toolbar represent the most commonly used features of Picking. Most of these have equivalent options in the menus.

If you allow the mouse to hover over any of the buttons, a yellow “tooltip” box appears to identify its function.

Descriptions for some toolbar buttons are given in the section describing Menu Options above.

Other buttons

- UV Light
- White Light
- Fan
- Humidity

These buttons can be used for switching hardware items on and off

Pin Firing Test

Displays a Dialog whilst firing pins continuously A1 through H12 (1 to 96). The purpose of the Pin Firing Test is to verify, by observation and sound, that each pin is firing correctly. Each pin should fire individually, extend and retract fully.

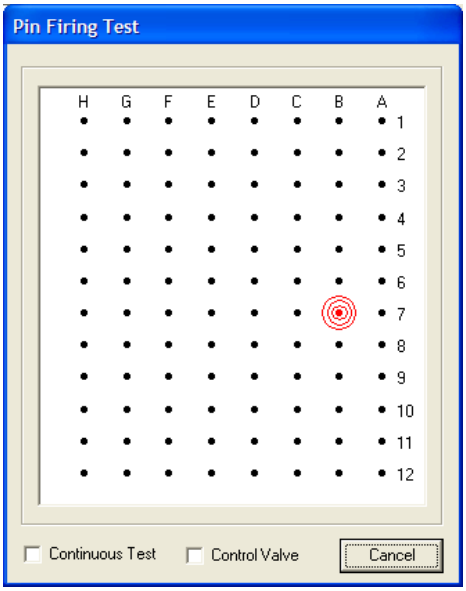

Figure 32: Pin Firing Test

Continuous Test – When checked will continuously fire pins until it is either unchecked or the Cancel button is selected.

Control Valve – When checked, slows down the pin retraction. This is useful for first time use or for demonstration purposes to the observation of the mechanical actions of the pins.

Cancel – Ends the Pin Firing test.

Change Head

Moves the actuator into an accessible position to allow the head to be changed.

Calibrate Camera

By clicking the Calibrate Camera button, the lens calibration routine will start. It is important that the lens is calibrated for each plate to be picked. This will improve picking accuracy.

Note: The lens should be calibrated at the start of every picking day and can only be done after camera alignment has been carried out.

The robot fixes on a single colony and moves the drives in a known pattern around it. This way it calculates the relationship of image pixels to real-world coordinates. At the end of the calibration routine the calibration results will be shown for both the X and Y axis. Values will differ between different robots however the aspect ratio should always be around 0.94. If difficulty in calibrating the robot is experience try using a sparse plate with larger colonies.

Align Camera

The Align Camera option is found either on this Picking main setup screen or by going into the Robot Configuration screen and then clicking on the Datum Points Tab.

Note: The camera should be aligned at the start of every picking day.

A screen similar to the one below will be displayed.

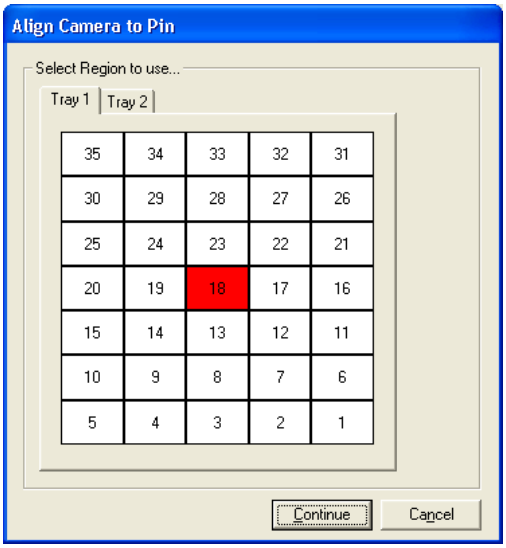

Figure 33: Align Camera to Pin

Select the region to align the camera in then click on continue.

The following screen will appear:

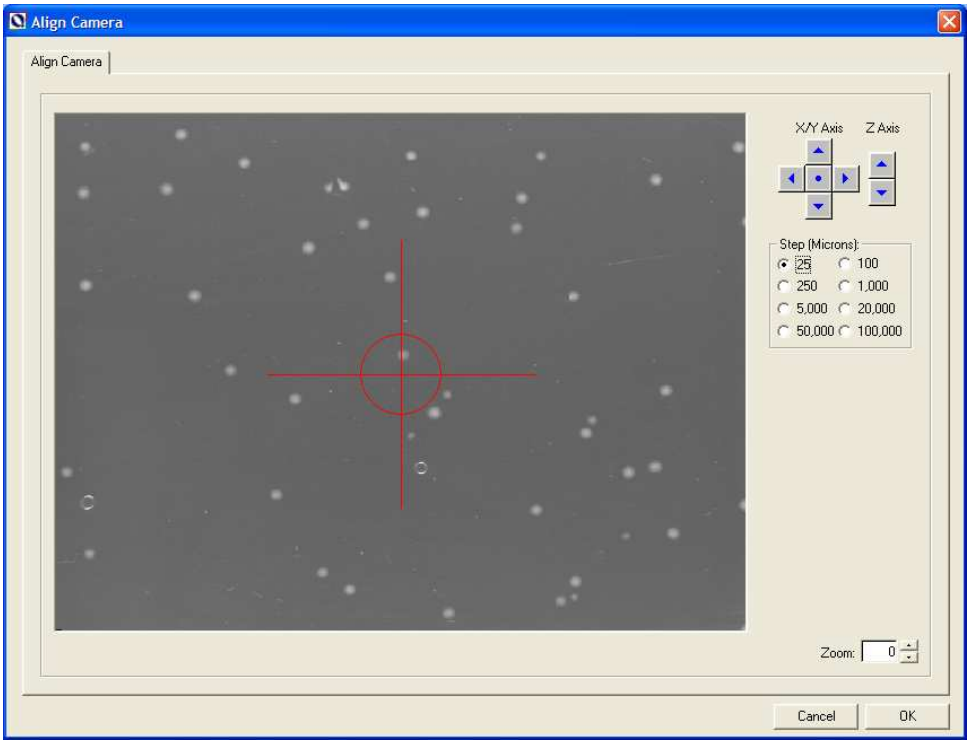

Figure 34: Align Camera screen

Fine adjustments can be made by firing a pin into the agar and then positioning the camera over the "pin-hole". The red crosshair symbol represents the centre of the camera. Jog the camera using the jog buttons and use the **zoom** option to improve accuracy. Accuracy is important here as (combined with Datuming the Bioassay Tray) this affects the picking accuracy.

Set Pin Height

Moves the Head to the centre of the Picking region and displays the **Set Picking Height** dialog for setting the optimum Picking height given the volume of agar selected.

Note: Ensure that a QTray or Petri dish is loaded before this option is used.

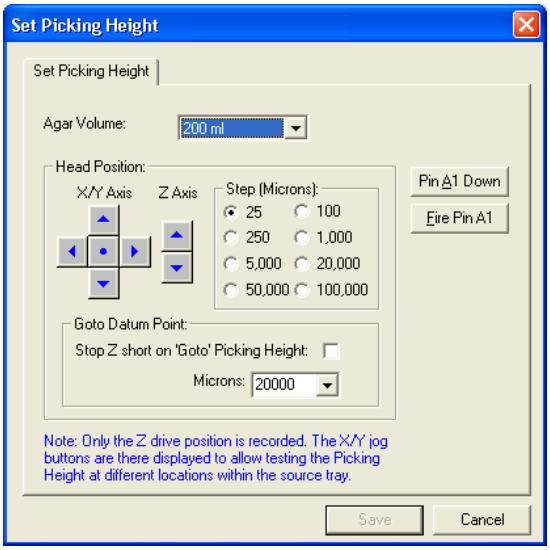

Figure 35: Set Picking Height Dialog

Fire the picking pin by pressing the **Pin A1 Down** button. Then use the Jog buttons to set the optimum picking height for the volume of agar selected. A good way to do this is to jog the pin down and look at the reflection between the tip and the surface of the agar. When the tip meets its reflection it is on the surface of the agar. From there, the robot should be jogged down to the desired penetration depth of 1-2mm.

Finally, test that the correct height has been selected by using the **Fire Pin A1** button, which will fire the picking pin.

Agar Volume – 200, 250, 300, 350 and 400 ml options. The initial volume displayed is the value currently selected on the main Picking Setup screen.

Z Axis – Jog buttons to move the actuator up/down to the optimum height.

Step (microns) – The distance to travel when jogging the Head into position.

Pin A1 Down – Places Pin A1 of the head in the down (fired) position. Useful for jogging the head/pin into the agar. When Pin A1 is in the down position pressing this button again releases the Pin.

Fire Pin A1 – Fires Pin A1 into the agar so the **Picking Height can be tested** before saving.

Save – Saves the new position(s) and returns to **Picking Setup** screen.

Cancel – Abandons any changes made and returns to **Picking Setup** screen.

Test Image

In order to be able to image colonies correctly, it is necessary to set up the various imaging parameters. These parameters will vary with different light levels and colony configurations. To set the imaging parameters click on the **Test Image** button in the main **Picking Setup** dialog.

The Test Image dialog is displayed.

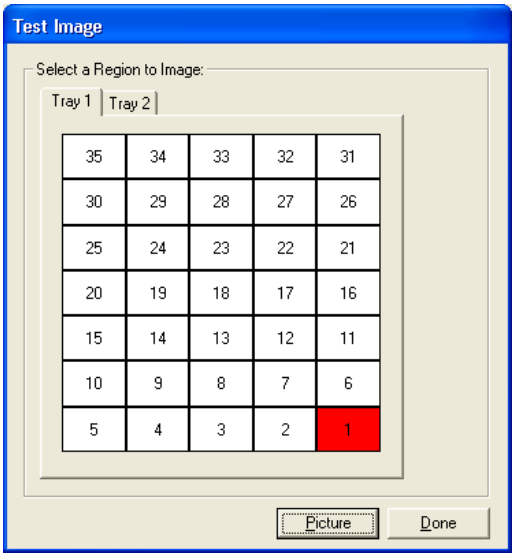

Figure 36: Test Image Dialog

Note: The number of trays is dependent on which model of robot you are using. The layout of the fields shown is for **QPix2**.

Click the tab for the tray that you want to image and select a region then click on **Picture**. The actuator (camera) will move to the selected region and take a picture. The Imaging Window will then appear:

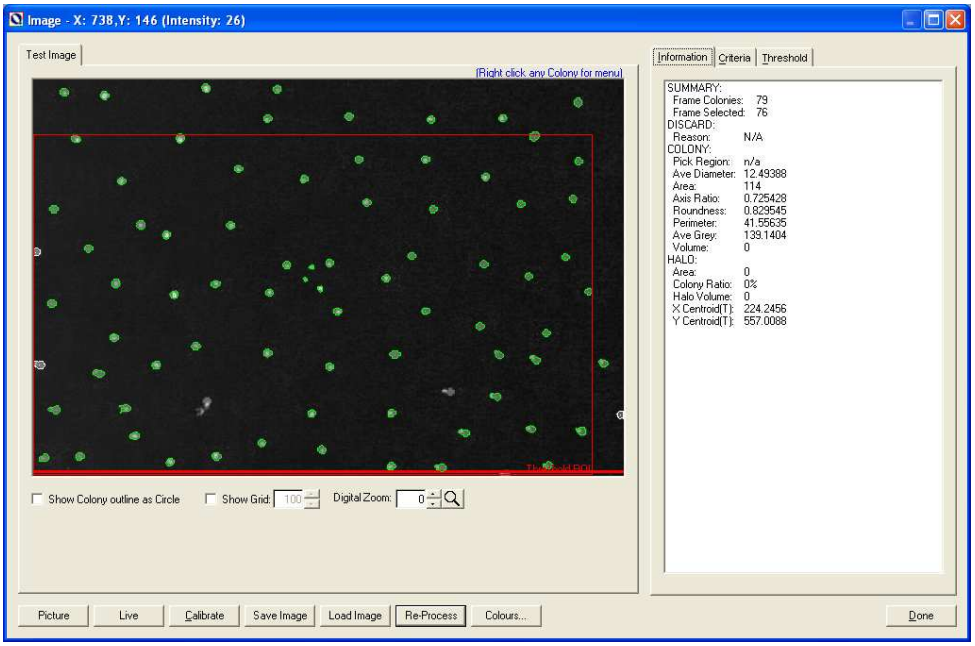

Figure 37: Imaging Window

Imaging Window

The imaging window shows the picture taken of the selected area. **QSoft's** imaging software analyses the image, applies the threshold, locates and then highlights any colonies it finds. Right-clicking with the mouse on any one colony displays a menu which allows the parameters set to be overridden on the **Criteria** tab. This can be carried out to increase the number of colonies to be picked. By looking at the outline color of a colony on the camera image it is possible to see whether it has been marked for picking or not. The aim is to configure the parameters so that the maximum number of "good" colonies are outlined in green (default color) and all others are rejected. A "good" colony is defined as one that fits into **QSoft's** imaging parameters. A rejected colony is one that falls outside the parameters. Options on this dialog are described below.

Figure 38: Command Buttons in the Imaging Window

Show colony outline as circle

This checkbox toggles the display of colonies between a perimeter plot and a circle showing the calculated centroid of a colony and its radius.

Show Grid

Checking this box displays a grid over the image which can be helpful when aligning the camera/CCD in Test Image mode. The grid size can be altered (in steps of 10) as required using the up and down arrows.

Zoom

The zoom control allows digital magnification of the image.

Picture

By clicking the **Picture** button another picture will be taken. The imaging software will then process this. The screen will display any colonies that it has found and will circle good colonies in green and discarded colonies in red. At this stage the selection process will be based on previously stored parameters.

Live

By clicking the **Live** button the camera will be put into live mode. ***LIVE*** will flash in the bottom right-hand corner of the screen. The purpose of Live mode is so that you can adjust the lens aperture and focus.

- The aperture ring adjusts the lens opening. This will increase or decrease the light allowed through the iris with a resultant change in lightness/darkness on the screen.
- Focus is adjusted by turning the focus ring on the camera which is marked with measurements in feet and meters.
- Adjust the focus (and the aperture if necessary) and take another image.
- Repeat the above steps until you have a satisfactory image

Calibrate

See page 33 for calibrate camera.

Save Image

This button allows the current camera image to be saved to disk as a bitmap.

Load Image

This button allows a bitmap image to be loaded for analysis.

Re-process

Clicking on this button results in the following prompt:

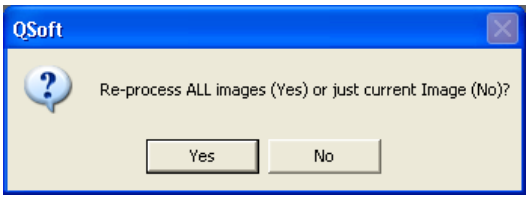

Figure 39: Re-Process Prompt

When making minor adjustments it is advisable to just process the current image to ensure the desired effect is achieved.

When settings are finalized then **All** images should be reprocessed to apply the changes to each image taken.

Colors

Colonies are outlined according to a color code associated with the *discard reason*. The color code is user-customizable (by clicking the **Colors** button) but the default settings are as follows:

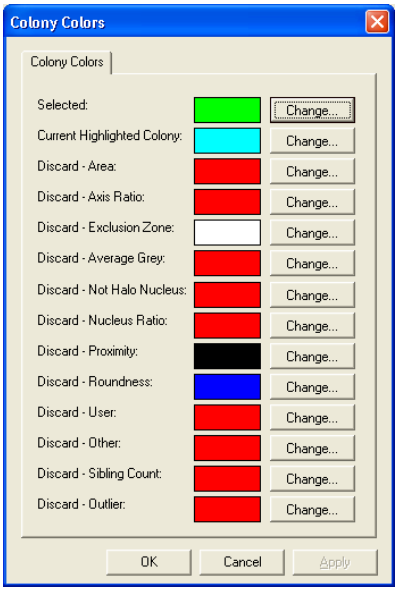

Figure 40: Colony Colors Dialog showing default settings

The “discard” color codes are used to color a colony according to the reason for rejection:

Area – the colony size is outside the specified diameter range.

Axis Ratio – the ratio between the major and minor axes of the colony is below the specified threshold. The colony is too elliptical.

Exclusion Zone – the colony is too close to the edge of the bioassay tray or Petri dish.

Average Grey – the average level of the colony is below the specified threshold. This is used to reject colonies with a dark centre, such as a “Blue” colony.

Not Halo Nucleus – The target is not a halo nucleus.

Nucleus Ratio – The ratio between a halo and its nucleus is less than the specified threshold.

Proximity – The colony is too close to adjacent growth.

Roundness – The ratio of the colony perimeter to its area indicates that the colony edge is too irregular.

User – The colony has been manually marked for rejection by you.

Other – The colony has been rejected for an unspecified reason.

Sibling Count – The halo contains more than one colony.

In the main imaging window, the results of **QSoft’s** image analysis can be seen. Provided the threshold settings have been correctly set up, outlines will appear. **QSoft** checks that a colony is the right size, shape and distance from adjacent colonies before marking it for picking.

Information, Criteria and Threshold

Clicking on the information tab displays a list of statistics that detail the number of colonies detected, and how many of those meet the required criteria. As the mouse pointer is moved over individual colonies in the image, the list is updated with statistics relating to the colony pointed

to. For example roundness, axis ratio, size, discard reason (if applicable).

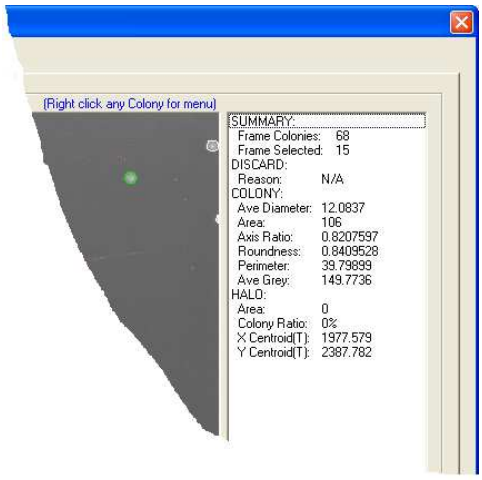

Figure 41: Colony Statistics

Clicking on the remaining tabs enables the selection Criteria and/or threshold values to be refined.

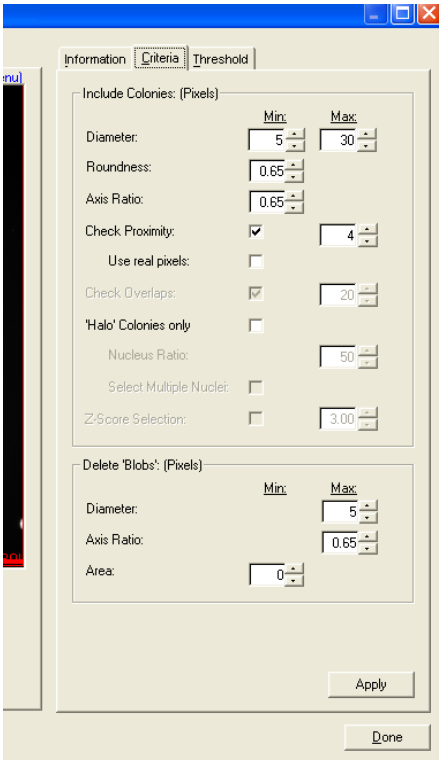

Figure 42: Criteria Tab

Criteria

Include Colonies

These variables define the size, proximity and shape of the colonies that will be picked.

Diameter – Allows the minimum and maximum area of the colony (in Pixels) to be set.

Roundness - Sets the minimum allowed roundness of the colony. It is a ratio of the circumference of a perfect circle (taken from the measured radius of the colony) divided by the

actual circumference of the colony. For a perfect circle the value would be 1.00, the usual range employed is between 0.80 and 0.95.

Axis Ratio - The axis ratio is found by dividing the shortest axis by the longest axis. Again for a perfect circle (or square) the value would be 1.00. Only colonies with an axis ratio equal to or above this value will be picked. Typical values used are 0.60 - 0.90.

Check Proximity - This facility has two settings, the first is a checkbox to turn it on or off, and the second allowing the operator to vary the setting (in pixels), when enabled.

The diagram below illustrates how this is calculated

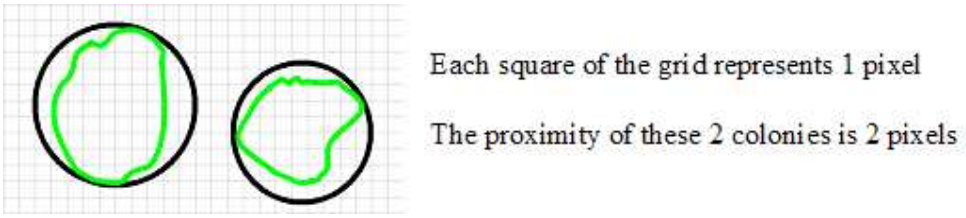

Figure 43: Proximity – standard mode

The irregular outline represents the colony, the black circle is the perimeter used for measuring the proximity. This perimeter is derived from the colony's maximum radius.

The distance between adjacent colonies is measured from the perimeter of each colony.

If the number of pixels between the outer edges of two colonies is less than or equal to the value set for the Proximity detection, they will both be rejected as being too close.

Use Real Pixels – If the proximity of adjacent colonies is less than or equal to the value specified above, selecting this option will check the proximity between adjacent colonies based on the actual perimeter of the colony.

Figure 44: Proximity – Real Pixels mode

Check Overlaps - Because the total image of the plate is made up of 35 frames, in order not to miss any colonies, the frames have to overlap by a small amount. This amount must be greater than the diameter of one colony and in real terms amounts to 4 or 5 mm, in both the X and Y axes.

There may be occasions when an individual colony occupies one of these overlap areas, and so could be identified more than once. If it were present on the very corner of a frame, it might be "seen" in up to three other frames, making a total of four "identified" good colonies. However, the software is designed to recognize instances such as these and Pick multiple identified colonies only once. There are however slight problems with exactly matching a colony "seen" in one frame to the same colony seen on an adjoining frame, so this setting allows for a certain amount of mismatch between the frames. It is normally set to about 20 pixels.

'Halo' Colonies Only - Only halo colonies will be picked if this option is checked.

Nucleus Ratio - If halo picking is enabled, the nucleus ratio may be used to set the percentage ratio between colony and halo area. Below which a colony will be rejected.

Select Multiple Nuclei – If a single halo contains more than one colony, the halo would normally be rejected. Selecting this option ensures that multiple nuclei are included.

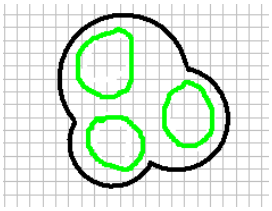

Figure 45: Single halo with multiple nuclei

Z-Score Selection – This works by calculating the mean and standard deviation values for the average intensity property of all the colonies in each picking region. Enter the value to select colonies whose average intensity is less than Z-Score multiples of the standard deviation above the mean.

Note: Intensity Sort (on the Picking setup screen) must be selected in order for Z-Score selection to be enabled.

Delete 'Blobs'

This feature is used to remove speckles from the picture or satellite colonies.

Marks that match its Maximum Diameter, Axis Ratio and Minimum Area parameters are flooded out as background and effectively ignored.

Note: Removing speckles will reduce the time required to process colonies.

Threshold

Threshold Values

A Threshold value is a grey value, between 0 and 255 (0 being the value for black and 255 for White) which enables the software to distinguish between the background and foreground features of an image. To distinguish between agar and colonies, the range of grey levels that represent the agar (background) should be excluded from the image. The range of grey levels that represent the colonies should be higher than the threshold range.

Where the image data is uncomplicated, it is advisable to use the automatic thresholding. This is quicker and simpler to set up.

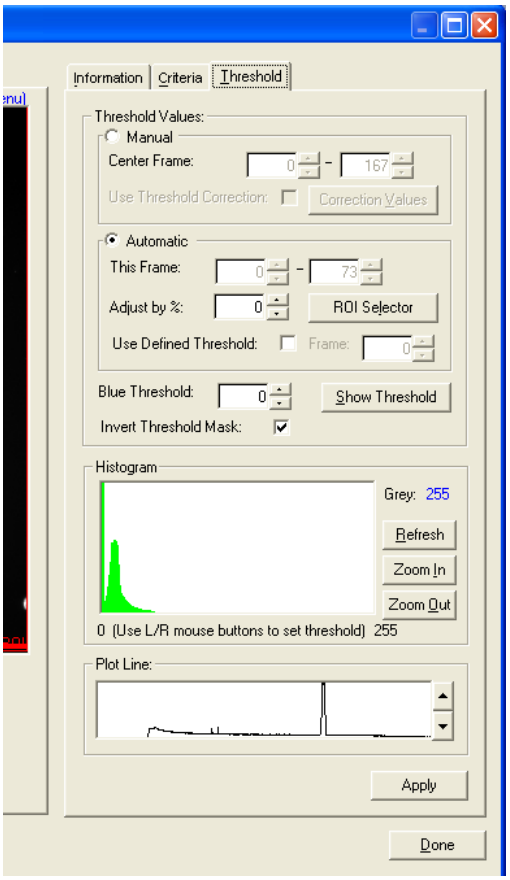

Figure 46: Threshold Tab

Note: When using automatic thresholding, it is not necessary to use threshold correction as this accounted for in the algorithms.

Manual thresholding must be set in the centre frame whereas automatic thresholding can be set in any frame tested.

Manual

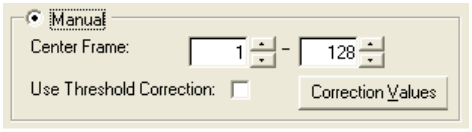

Figure 47: Threshold - Manual

Centre Frame - These two values are used to set the minimum and maximum threshold for the background at the centre of the **QTray**, which is to be picked. The lower and upper limits for centre frame threshold define a range of pixel grey levels that will be considered “background” or agar.

For normal “White” or “Blue” colony picking, the lower threshold must be set to zero, and the upper threshold should be varied to correctly exclude background pixels. Typically, the upper threshold will be a value between 160 and 220. Its limit is 255.

For “phage” picking, the lower centre frame threshold should be varied, and the upper threshold should be set to 255.

Use Threshold Correction - If this checkbox is selected the robot will automatically calibrate for differences in light intensity across the tray. This is especially important if the external light is

very intense or variable, e.g. robot located near to a window.

Correction Values - This shows the current threshold values across the **QTray** determined by the software, these can then be calculated by you and manually adjusted.

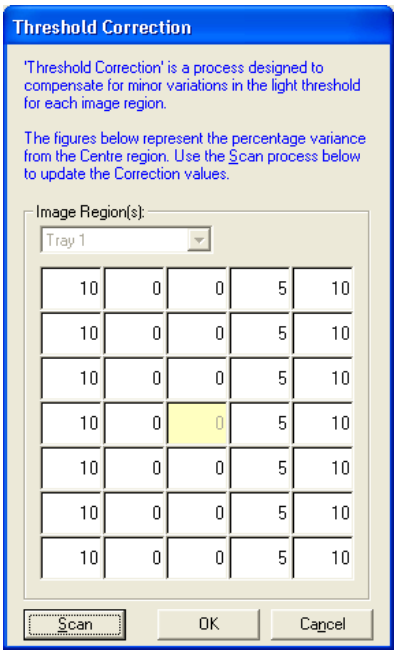

Figure 48: Threshold Correction

Scan - Selecting scan initiates the threshold calibration and the camera will scan each frame in turn and give its value as a percentage of the centre frame.

Automatic

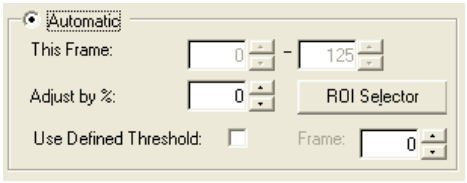

Figure 49: Threshold - Automatic

This Frame – Software-calculated threshold values based on *each* frame.

Adjust by % – A global parameter which allows you to fine-tune the automatic threshold. This parameter allows the upper calculated automatic threshold value to be sharpened or softened, typical range is +10% to –10%.

Use Defined Threshold – When this is selected along with a specific frame, the threshold values will be used for all other frames. On all subsequent test images the specific frame will be imaged first, the threshold calculated and then the test image will be taken and processed.

ROI Selector – Defines an area that represents valid background data. Use this option when an image contains obstacles such as the edge of a Petri dish or bioassay tray. For below for detailed information about Threshold Region of Interest.

Threshold Region of Interest (ROI)

A Threshold value is a grey value, between 0 and 255 (0 being the value for black and 255 for White) which enables the software to distinguish between the background and foreground features of an image. To distinguish between agar and colonies, the range of grey levels that represent the agar (background) should be excluded from the image. The range of grey levels that represent the colonies should be higher than the threshold range.

Previously it was recommended to use Automatic Threshold Control only when the image data was uncomplicated. With the introduction of the ROI control and the new image algorithms it is possible to use Automatic Threshold on very complicated image data to obtain accurate results. **Automatic Threshold using ROI is now the preferred method of Image Thresholding.**

Threshold ROI is a process that allows the user to define an area within each image region that represents valid background data. This area must exclude all superfluous background data such as Bioassay Tray edges and mirrors etc.

Below is an image containing obstacles and a ROI selected over an area that is deemed as valid background for use in image processing.

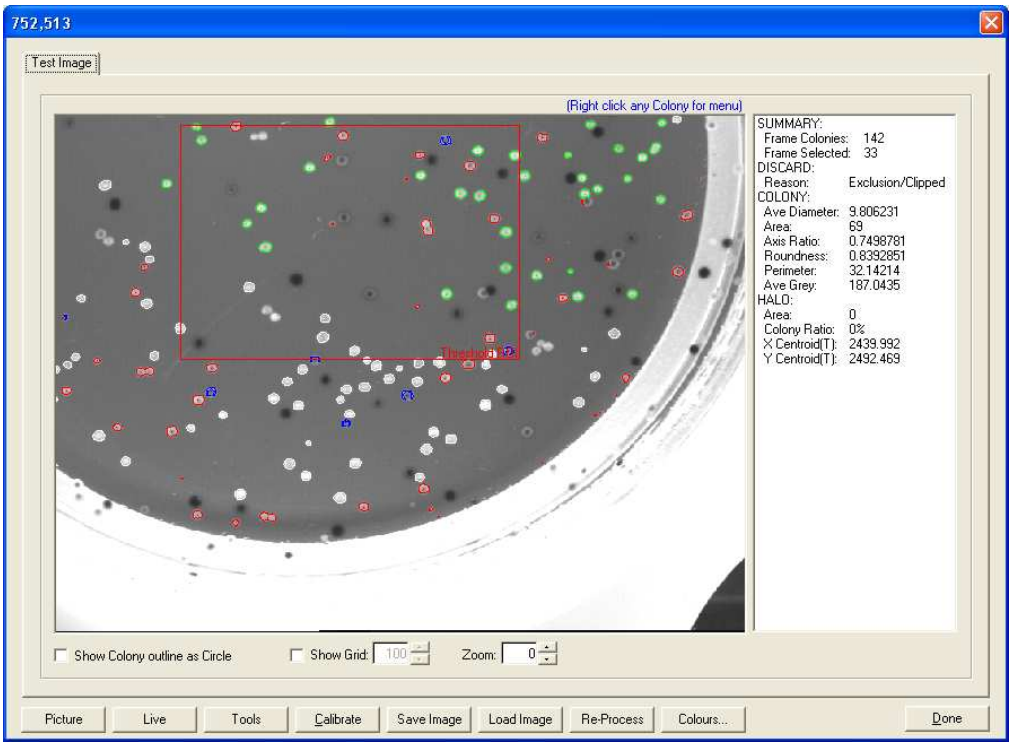

Figure 50: Example of ROI Selection

Selection of an area with a **valid** background enables the software to calculate the threshold more accurately.

ROI operation

Automatic Thresholding must be selected for the **Threshold ROI** to be activated. When activated a red bounding box (the ROI) is displayed on the image. The ROI, set either by the user or, in the first instance, by the **QSoft** database, returns a set of co-ordinates based on the selected region. These coordinates are passed to the image algorithm that calculates the threshold values. These threshold values are used for the entire image. The area chosen must typify the overall agar background of the entire Image and should not include obstacles such as Petri Dishes edges and mirrors etc.

Setting up ROI

3. To access the ROI from the **Threshold** tab click the **ROI Selector** button

A message box is displayed giving instructions about selecting the region of interest.

4. Point with the mouse to the position to start the selection
5. Click the left mouse button
6. Drag the ROI bounding box around the desired region

Release the mouse button
You will notice that the threshold values on this window have changed to reflect the region selected and the coordinates of the region have been saved to the QSoft Database at this time. The user can change the ROI on this image at this point if they so wish. The threshold value can be further "fine-tuned" using the **Adjust by %** parameter.

At this point the image threshold has been successfully set and the process can be repeated for each image of the source container.

During a full picking run as the images are processed the **Threshold ROI** box will be displayed (if Automatic Threshold has already been chosen). If the option **Display Images After Imaging** is selected it will be possible for the user to edit the ROI for all images before picking.

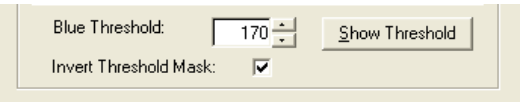

Figure 51: Blue Threshold

Blue Threshold - This facility is only enabled when imaging a set of Blue/White colonies, and provides a secondary check to distinguish the Whites from the Blues. If the average grey value for a colony is below the value set here, then it will be recognized as Blue and will not be picked. For *Blue* picking, the *White* threshold should be first set. Then the Blue threshold should be set to equal the White threshold and increased until dark Blue centered colonies are excluded from picking selection.

Detecting Blue Colonies:

The following information relates to the detection of blue colonies, the rejection of white colonies, and subsequent picking of the blue colonies using the QSoft Picking software on **QPix2**, **QPix2 XT** and **QP Expression**.

It is recommended that this application is carried out by persons familiar with the systems and the QSoft software.

If the intensity of the blue colonies is greater than the background (agar) then:

1. Set the upper threshold to 255.
2. Set the lower threshold to a value that represents an intensity greater than the blue colonies but less than the intensity of the white colonies.
3. Set the blue threshold to a value that represents an intensity above the background intensity but below the intensity of the blue colonies.

If the intensity of the blue colonies is less than the background then:

1. Set the upper threshold to 255.
2. Set the lower threshold to a value that represents an intensity greater than the blue colonies but less than the intensity of the background.
3. Set the blue threshold to a value that represents an intensity less than the intensity of the blue colonies but above any dark areas in the image that might be taken as possible colonies. If the image is clean the blue threshold can be set to zero.

The **Histogram** (on the tools screen) can be used to determine the background intensity and the **Line plot** at the bottom of the tools screen can be used to access the intensities of the blue colonies and the white colonies.

Move the red line to a position on the image that bisects blue colonies.

Move the cursor to the tip on the line plot that represents a blue colony (the line plot represents the intensity levels across the image at the position of the red line on the image).

The number displayed relates to the actual intensity as follows:

Actual Intensity = 255 - displayed value.

Repeat the process to access the intensity of the white colonies.

Having set the thresholds, the levels can be tested by clicking on show thresholds. The image will then be shown as just two intensities white and black.

The black areas define what will be assumed to be background and the white areas define possible colonies. If the white areas are not consistent with the position of the blue colonies then the thresholds need revising.

If the Picture or Reprocess buttons are pressed at the bottom of the Tools screen QSoft Picking will perform colony detection on the image and display the results on the image by coloring the periphery of each of the areas it detects as colonies. The color indicates whether the colony will be picked or not.

The default coloring (which can be customized by the user) is Green for Picking; any other color means the colony has been rejected. No color means the colony has not been recognized at all as a colony. There are a number of reasons why a colony is rejected, e.g. it may be too small, or too big, i.e. it is outside the criteria that have been set (user configurable) for colony size.

Show Threshold – When this button is clicked the screen will differentiate between the thresholds by turning the background to black.

Now, there is the ability to “fine-tune” the threshold values. The image screen now has a horizontal red line running across the centre of the image. By adjusting the values for **Centre Frame**, the threshold values on the screen will change.

When picking **White** or **Blue/White** colonies, the ideal is to have a black background and White colonies, with good definition between the two.

Invert Threshold Mask – For picking colonies, which appear light on a dark background, Invert Threshold Mask should be checked. Phage appear dark on a light background, so for phage picking Invert Threshold Mask should be un-checked, which inverts the image.

Once the thresholds have been set click on the **Picture** button to ensure that the new threshold values are correct. The robot will take a new picture and display the results on the screen. Colonies circled in green are good (if default colors are set – see Figure 40) and those circled in red are bad (assuming default colors are used). Review these results; if they are acceptable continue by clicking the OK button, if not repeat the thresholding process.

Histogram

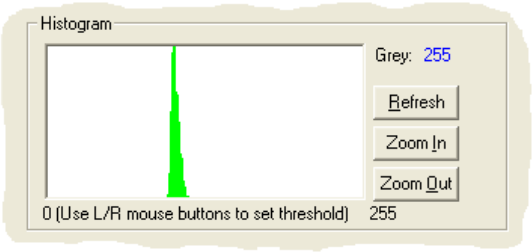

Figure 52: Threshold - Histogram

The Histogram shows the volume of grey pixels in the image by value. The left of the Histogram represents Black pixels whilst the right of the histogram represents White Pixels.

Typically one large peak is expected on the histogram, which represents the grey scale of the agar in the image.

If there is a large population of colonies a second peak may be seen on the histogram, which represents the grey scale of the colony data.

The histogram can be used for setting the Manual Threshold parameters. Click, using the left mouse, in the Histogram to set the Min Threshold level and click, using the right mouse button, to set the Max threshold level.

For typical E-coli plates the Min threshold should be set to zero (0) and the Max threshold can be set by Right clicking just to the right of the first major peak in the Histogram.

Refresh – Occasionally, when re-taking images in the test Image screen the histogram data may not update the Histogram window. In this case clicking the Refresh option button will correct this.

Zoom in/Zoom out – If there is little color contrast between the colonies and the agar the Histogram data will appear very flat and no obvious peaks are displayed. In this instance, use the Zoom In/Out options to alter the scale of the graph to better identify the peaks.

Plot Line

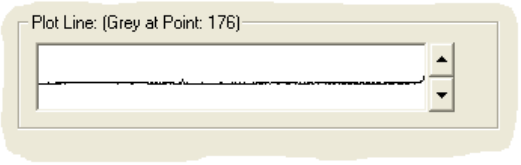

Figure 53: Threshold - Plot Line

The graph in the Plot Line window represents the red line that pans up and down the image window. Click on the scroll buttons to move the red line and as it passes over colonies peaks will appear which describe the intensity of each colony.

Running a Picking Routine

Start the Run

Command buttons

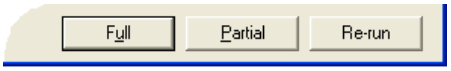

Figure 54: Picking Setup Command Buttons

Having set all the required values and checked them by using **Test Image**, it is now time to start picking. Now the option to pick the full tray or just a certain area of interest is available (partial - **page 53)**.

Full Pick

If picking all colonies from all available trays is required, click the **Full** button on the main Picking Setup screen.

Note: If only colonies from one tray or part of a tray are required to be picked then go to the next section – **Partial Tray (page 53).**

Depending on which script options are selected, there will be a prompt to calibrate the lens and align the camera.

Having clicked the **Full** button, the following screen will appear. To continue click **Yes**.

Figure 55 Prompt to Start the Run

The robot will image each **QTray** and then process the images. As it takes each frame they will be shown on the screen

When it has finished taking all the pictures, it will process them according to the set criteria. A screen will then be displayed showing the number of good colonies found for picking.

Figure 56: Colony Count Dialog

If **Display Images after Imaging** was checked in the Script Options dialog box you will now be presented with the following screen.

Figure 57: Display Image Data Dialog

Click on **Yes** and the Imaging Window is displayed with a list of all images taken.

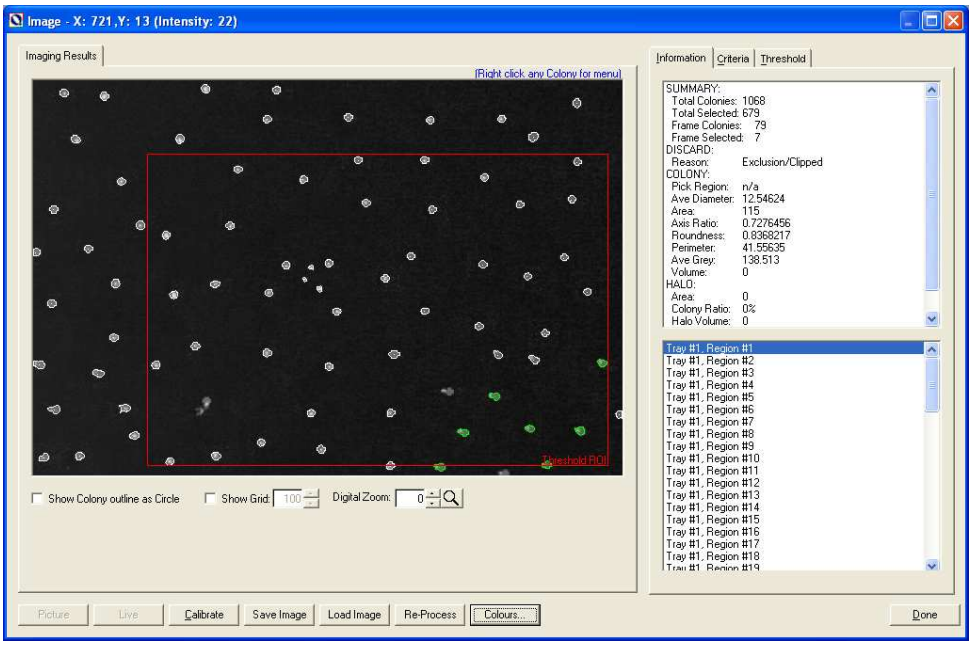

Figure 58: Imaging Window

There are two list boxes on the right hand side of this screen.

Summary Colony Statistics **–** This is similar to the list of stats when viewing test Image, information is displayed about the colony being pointed to with the mouse.

Images Taken **–** A list of the images requested for inclusion in the Picking run. The format in this list is

Tray No – Image region Number.

Any image taken can be viewed by clicking on the image reference in this list. The statistics for the image are shown in the window above.

By right-clicking any colony, a menu will be displayed.

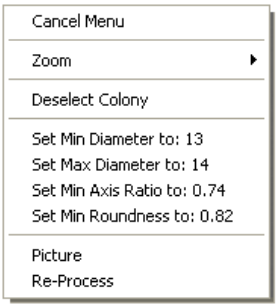

Figure 59: Colony Menu

The menu will show *either* **Select Colony** or **Deselect Colony**. If the Deselect option is shown, this is because the colony has already been selected but this can be overridden. If the colony has been Discarded then the Select option will be available.

Individual colonies can be selected/deselected where necessary from any of the images. This is useful when there is the need to locate a specific number of colonies to populate a given number of Destination well plates.

Note: If Criteria and/or threshold values are modified the individually selected/deselected colonies will be lost when the images are re-processed. It is therefore advisable to refine the Criteria and Threshold values *prior* to any individual selection process.

Click **OK** (on the **"… Good Colonies Found"** message box) and a screen similar to the one below will appear, which shows the results of your imaging.

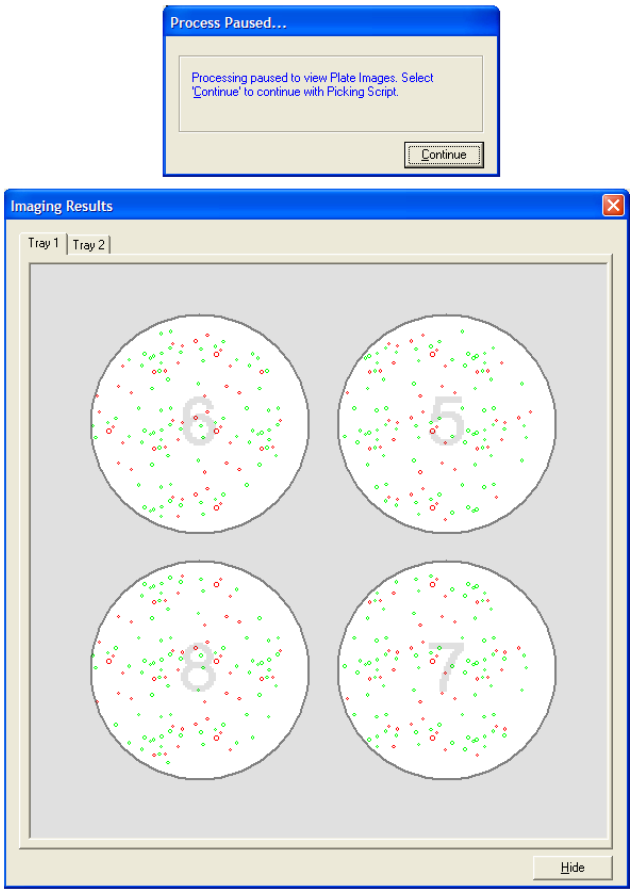

Figure 60: Imaging Results

The software will now create a script, and offer the opportunity to view the script.

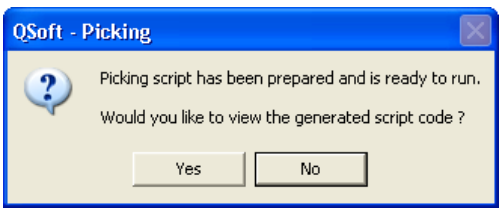

Figure 61: Option to View Script Code

Clicking **Yes** displays the script. **No** skips viewing the script.

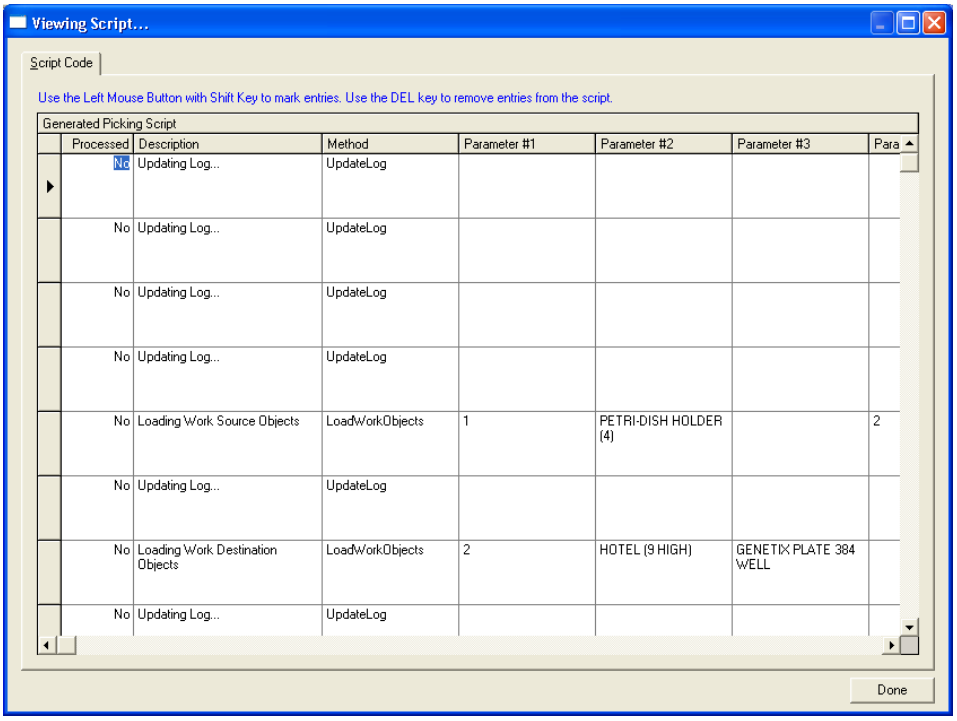

Figure 62: Example Script

Once finished viewing the script, click **Done**.

The script progress screen will then open. This screen displays which process is currently being carried out and the estimated completion time of the run.

A message box will prompt for the head to be checked before continuing.

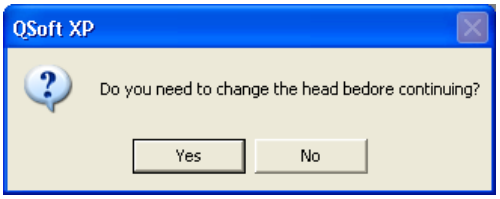

Figure 63: Prompt to Change Head

If Head proceeding to change the Head, press **Yes** and the robot will move to the head change position, at which point the Head can be changed. If the Head is already loaded press **No**.

A message box will be displayed, click on **OK** to continue.

A visual guide showing the loading sequence of the Destination Plates will appear:

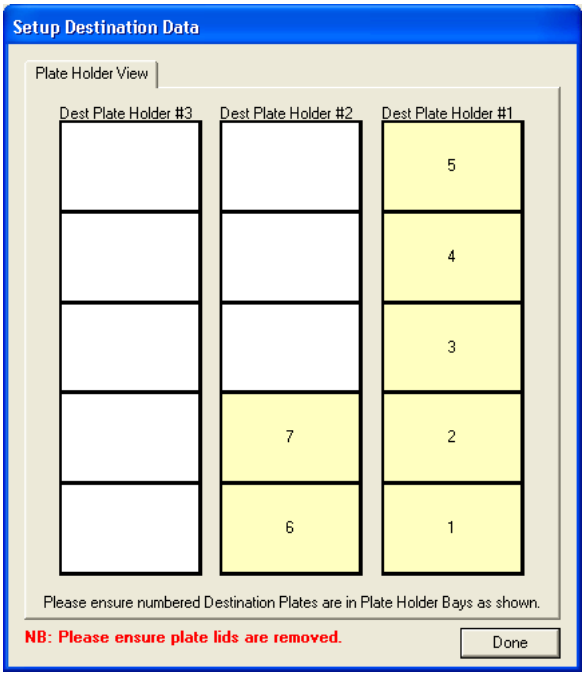

Figure 64: Destination Plate Sequence

Ensure the correct number of plates are loaded and placed on the workbed of the robot. Then click **Done**. The following dialog box will appear:

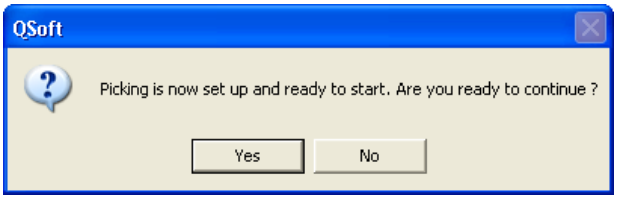

Figure 65: Prompt to start picking

Click **Yes** and the picking routine will commence.

Throughout the routine there will be a script progress box that will display which process is being carried out and displays elapsed time and estimated completion time:

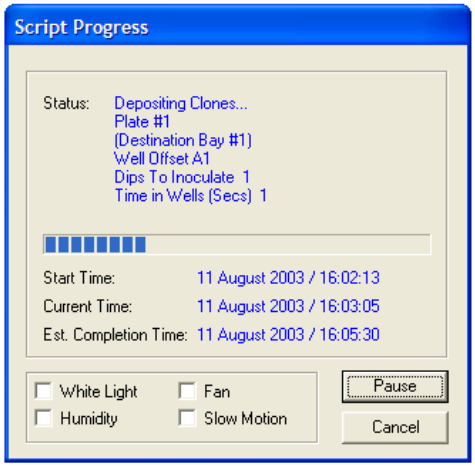

Figure 66: Script Progress Dialog

On completion of the process, the following screen will appear.

Figure 67: End of Run Dialog

Click **OK**. A prompt will appear asking if settings are required to be carried over.

Figure 68: Prompt to Carry over Pin & Well Settings

This screen allows the last well and pin setting to be remembered so that more data can be loaded and further runs can be carried out utilizing any pins on the head that have not been used and any destination plates that have not been filled.

As the system is used most of the above screens will not be required. Under Additional Options in the main setup screen, there is a Script Option, provides the choice of which screens will appear and which will not.

Partial Pick

If only part of the tray is required to be picked, click the **Partial** button on the main Picking setup screen. The following window will appear:

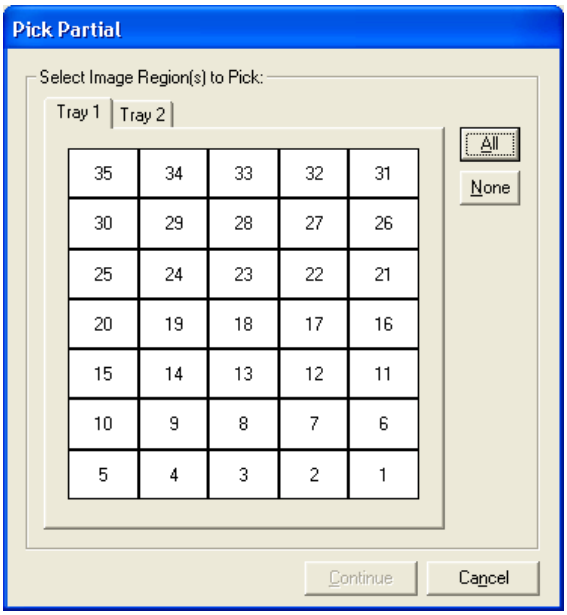

Figure 69: Select Image Regions

The operator is prompted to choose regions within the tray that are to be imaged. To select regions, click on one or more numbers in the grid. If a mistake is made, to clear the screen, click

None. If all regions are required to pick from, then click **All**. Once the required range has been chosen, click **Continue**. The results will now vary, depending on whether the Genetix Bioassay Tray or one of the region divided containers as the Source Container was chosen. See below:

Genetix Bioassay Tray

From now on the procedure is the same as that for the Full pick.

Region divided containers (e.g. Petri dish)

If a region divided container has been selected, the software will still ask the camera to take pictures as normal. However, it will only process colonies that fall within a Region and according to the parameters set for each Region.

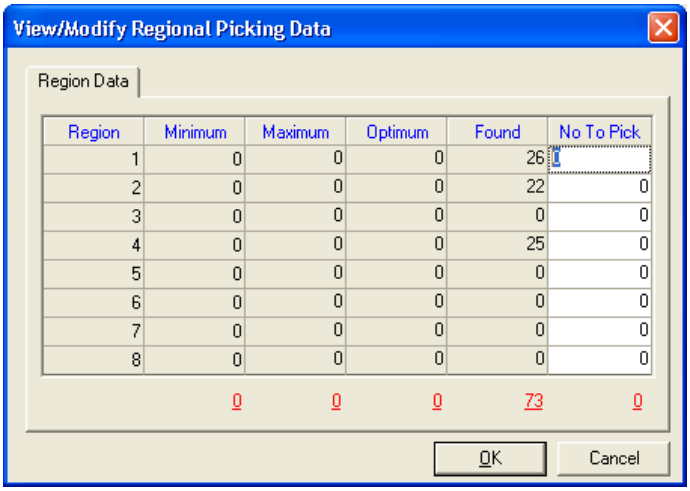

Figure 70: Picking Data Summary

The software now produces results by Region and according to the set criteria.

The Number to Pick can be changed to a value less than the number Found if necessary.

Click on OK to see the Imaging Results:

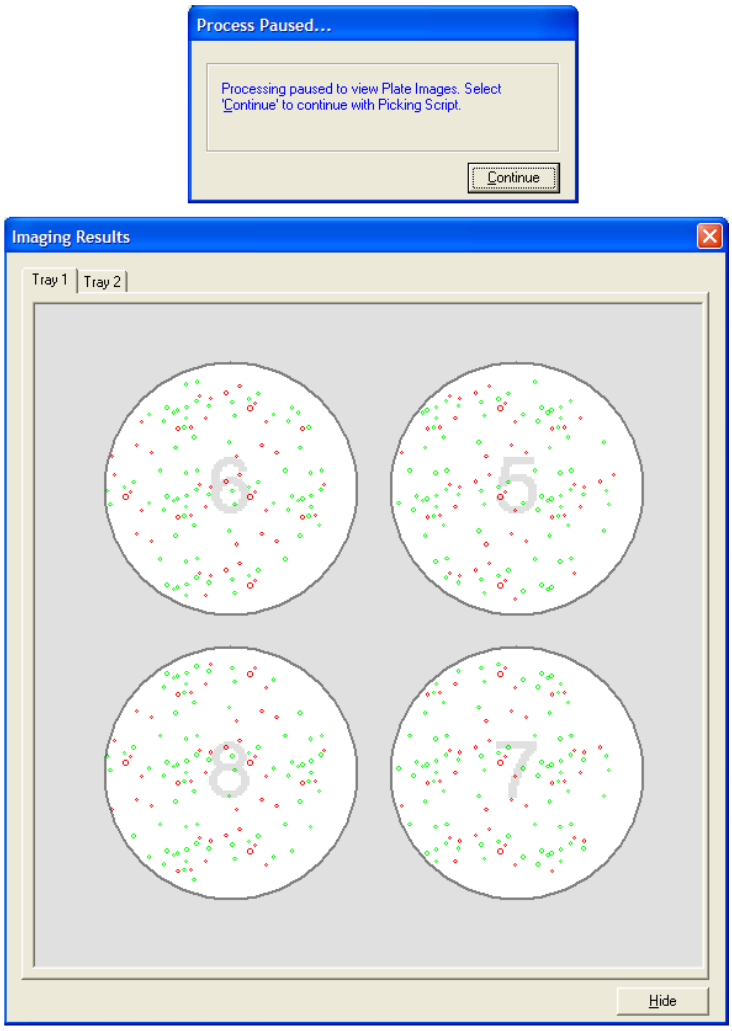

Figure 71: Imaging Results

If the **Process Paused** message box is obscuring the data in the imaging window, it can be moved by clicking on the title bar with the mouse and dragging it.

The Imaging results window now shows the chosen Regions and the colonies to be picked in each Region. Any colonies outside these Regions will not be chosen.

Click on **Continue**. From now on the procedure is the same as that for the Full pick.

Re-run

The Re-run option on the main Picking setup screen allows the previously created script to be run again. This is useful for:

- Creating a second library from the same Colony data without re-imaging (this is the same as picking with replications)
- Repeatability testing

To close the Picking application, open the **File** menu and choose **Exit**

When the picking run is complete and the picked colonies and the source **QTrays** have been removed, the workbed of the robot should be cleaned and UV sterilized and the Head should be cleaned (see General Maintenance section in the appropriate Robot Manual).

Preparing Media

Luria-Bertani medium (LB) – per liter

To 1 liter of de-ionized H_2O add 25g of pre-prepared LB (Sigma, Gibco, LAB3). LB can also be made by adding (per liter) 10g tryptone, 5-10g yeast extract, 5g NaCl (pH 7.2) and stir on a magnetic stirrer until the powder has dissolved. Sterilize by autoclaving at 121°C for 15 minutes.

LB + 8% Glycerol – per liter

As above but replace 80 ml of water with glycerol (80 ml glycerol + 920 ml demonized H_2O). Sterilize by autoclaving at 121°C for 15 minutes.

LB Agar – per liter

To 1 liter of de-ionized H_2O add appropriate amount of pre-prepared LB agar (Sigma, Gibco) and stir until the powder has dissolved. If making your own LB add 16g of agar per liter of LB. Sterilize by autoclaving at 121°C for 15 minutes.

Top Agar

LB + 0.7% (w/v) agar or agarose.

All media should be autoclaved for 15 minutes at 121°C. Make sure that autoclave tape is fixed to the bottle. Bottles of media should have the bottle caps partially screwed on. Under no circumstances screw on tightly. Broth should be removed from the autoclave (using safety gloves), set out to cool and then refrigerated.

Flasks of agar should be covered with foil at the opening. Once sterilized, the agar should be cooled until it is a comfortable temperature to hold (approximately 50°C) at which time the appropriate antibiotic is added and the flask gently swirled. Flame the neck of the flask and pour 200ml of the agar into a sterile glass beaker. Pour into a 22cm x 22cm bioassay tray removing any air bubbles with a flamed needle. Allow the plates to harden in a flow cabinet or other clean environment before refrigerating. Prior to use you may need to dry the agar by placing the trays in a 37°C incubator for approximately 20 minutes. Make sure the plates are clearly marked with the date and the antibiotic used.

Antibiotic Preparation

Ampicillin

Stock solution: Dissolve 1g of ampicillin in 20ml of sterile distilled water. Filter sterilize using a 0.2 µm syringe filter and dispense 1ml aliquots into 1.5ml Eppendorf tubes. Store at -20°C. (50mg/ml stock solution).

Working solution: Add 1ml of stock solution per liter of medium.

Chloramphenicol

Stock solution: Dissolve 1.25g of chloramphenicol in 100ml of ethanol. Store in 1.5ml Eppendorf tubes at -20°C. (12.5 mg/ml stock solution).

Working solution: Add 1ml of stock solution per litre of medium.

Kanamycin

Stock solution: Dissolve 1g of kanamycin in 20ml of sterile distilled water. Sterile filter and store in 1.5ml Eppendorf tubes at -20°C. (50 mg/ml stock solution)

Working solution: Add 1ml of stock solution per litre of medium.

Solutions

Denaturing Solution (Per liter)

87.6g NaCl

20g NaOH

Make up to 1 liter with demonized water

Neutralizing Solution (Per liter)

87.6g NaCl

121.1g Trizma Base

Make up to 900ml with demonized water. Adjust pH to 7.0 with concentrated HCl and then make up to 1 liter

Picking

Important! – Library stock solutions should always be divided into small aliquots and stored at -80ºC. When using stock solutions ALWAYS place them on wet ice and keep them out only for as long as is necessary.

Bioassay Tray Preparation

Pour 200ml of LB agar into a **QTray** and allow to set. When the agar has set completely, invert the trays and store at 4°C until needed. They should be removed from storage and brought up to room temperature before they are used as they will 'sweat' when incubated at 37°C allowing bacterial colonies or bacteriophage plaques to spread across the surface of the plate and increase the chance of cross-contamination. This problem can be avoided by incubating them overnight at 37°C in an inverted position before use. Alternatively if you pour the plates in a laminar flow cabinet leave them open for 30-40 minutes in the air flow.

Determination of Titer

For optimal picking with the robot the **QTrays** should contain about 3000 colonies each.

To determine the volume of bacterial suspension to give approx 3000 colonies/tray:- Make serial dilutions of the stock suspension e.g. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and spread 100 µl aliquots of these on Petri dishes containing LB agar and antibiotic. The spreading can be done using the glass bead method (see next section) or using a sterile spreader. Incubate the Petri dishes at 37° C overnight. Count the colonies on the plates and (taking into account the volume spread and dilution made), calculate the bacterial concentration in the stock. From this calculate the volume of stock required to give approximately 3000 colonies.

Colony Preparation

Remove the fresh bioassay trays, prepared as stated above and bring them to your working area.

Using a pipette aliquot the appropriate volume of stock solution + LB to make 750µl into Eppendorf tubes and vortex briefly (these tubes should be placed on ice). Using the pipette

remove the dilution from the first tube and deposit it onto the agar surface of the first bioassay tray. Shake 10-15 sterile 6mm glass beads (product code X5200) onto the agar and replace the lid of the tray. Gently tilt and shake the tray so that the beads disperse the liquid on the surface. Pour the beads off into a 'used beads' container, invert the bioassay tray and set it aside. Repeat these steps for each tray. The beads can be re-used if autoclaved at 121°C.

When all the samples have been plated out place the inverted trays in a 37°C incubator and incubate overnight. 'Grown' trays can be stored at 4°C for about 72 hours.

Preparation of Destination Plates

Label 96 or 384 well plates either with permanent marker or barcode sprayer. Fill each well with LB + glycerol + antibiotic (150µl for 96 well plates or 50µl for 384 well plates). Load these destination plates into the hotels of the Robot.

A QFill2 can be used for filling plates.

NB some strains of *E.coli* may not grow well in the presence of glycerol and it may be necessary to add glycerol after incubating the plates. For 96 well plates use 100µl LB and add 50µl of LB + 30% glycerol. For 384 well plates reduce the initial volume of LB to 40µl and add 20µl LB + 30% glycerol.

Picking Procedure

Before commencing with picking UV sterilize the bed of the robot for 30 minutes.

Remove the 'grown' bioassay trays from the incubator and fit securely onto the bioassay tray holders of the robot. Commence the picking run.

When the picking routine has reached completion stack the destination plates 6 high, wrap in Clingfilm to prevent evaporation and incubate overnight at 37°C. Add glycerol if not already added. These plates are then stored at -80°C.

Picking Phage

Cultures of bacteria to be infected by bacteriophage lambda should be grown in LB agar containing 2% maltose as the presence of maltose induces the adsorption of phage to the bacteria. Dilutions of phage/bacteria mixture should be prepared so that there will be approximately 2000 plaques per plate.

Plates to be used for picking phage should contain 175ml of LB agar and the trays should be dried thoroughly before use. After the appropriate dilutions of phage/bacteria mixture have been incubated they should be added to separate flasks each containing 25ml molten top agar. Swirl gently for a few seconds then pour the mixture onto the hardened agar plate. Gently tilt the plate to distribute the top agar evenly over the plate taking care to prevent air bubbles forming. Close the lids and allow the plates to stand at room temperature until the top agar has hardened. Invert the plates and incubate at 37°C. Plaques should start forming within 7 hours and should be picked as soon as plaque size is adequate, approximately 10 – 12 hours.

The picked plaques should be deposited into plates filled 2/3 full with SM + 7% DMSO. Plates should be left at room temperature for 1-2 hours, then stored at 4°C.

SM Buffer – per liter

NaCl	5.8g
MgSO4.7H2O	2g
Tris-Cl (pH 7.5)	50ml
Gelatin Solution (2%)	5ml

Make up to 1 liter with de-ionized H_2O. Autoclave and store at room temperature.

Picking Blue/White Colonies

Petri Dishes

To the prepared LB agar Petri dishes add 7.5µl of a stock solution (200mg/ml) of isopropylthio - β - D – galactoside (IPTG) and 75µl of a stock solution of X-gal (20mg/ml in dimethylformamide (DMF)). Spread the IPTG/X-gal over the agar surface using a glass spreader or beads

QTrays

To prepare QTrays with X-gal/IPTG make a stock of 2% X-gal in DMF and a stock of 100mM IPTG in water.

Stocks can be stored at -20°C.

The X-gal and IPTG should be added to molten agar at the same time as the antibiotic. For each 800ml of agar add 300µl of IPTG stock and 3ml of X-gal stock.

Ensure that the plates are dry and inoculate as described in the 'Colony Preparation' section. Invert the trays and place in the 37°C incubator O/N. Remove the trays and store for several hours at 4°C to enable the blue color to develop further.

When checking the colonies be careful in distinguishing the Blues from the Whites. The colonies that have active β- galactosidase have a dense blue centre with a pale blue periphery. White colonies although white at the periphery may also exhibit a pale blue centre.

Non-Radioactive Hybridization and Detection Protocol Using the Roche DIG system

Recipes for all the required solutions are given at the end of the protocols.

Preparation of DIG labeled probe

This is carried out according to the protocols supplied by Roche with their DIG labeling kits e.g. DIG High Prime. The probe is quantified using the protocol supplied by Roche.

Prehybridization Step (all steps normally use about 200ml solution per box e.g. Genetix Hybridization box)

4. Preheat Church Buffer to 65^{o}C.
5. Incubate membrane in buffer for 60-120 minutes.

Hybridization Step

Preheat Church Buffer to 65^{o}C.

6. Denature probe in boiling water for 10 minutes.
7. Cool probe on ice for 2 minutes.
8. Add denatured probe to Church Buffer to a final concentration of 5-25 ng/ml and mix thoroughly.
9. Remove membrane from prehybridization and transfer to Church buffer and probe.
10. Incubate membrane in probe solution over night at 65℃.

Stringency Washes

11. Transfer membrane to 1x washing solution for 20 minutes at room temperature.
12. Preheat second batch of 1x washing solution to 65^{o}C.
13. Transfer membrane to preheated 1x washing solution for 20 minutes.

Detection Steps

14. Prepare blocking solution.
15. Incubate membrane in blocking solution for 45 minutes at room temperature.
16. Centrifuge second batch of blocking solution for 15 minutes at 3000 rpm.
17. Centrifuge stock antibody solution for 1 minute at 14,000 rpm.
18. Dilute antibody solution 1:10,000 in supernatant from centrifuged blocking solution.
19. Incubate membrane in antibody solution at 37ºC for 60 minutes.
20. Incubate membrane in PBS for 30 minutes at room temperature.
21. Repeat incubation for 30 minutes in fresh PBS at room temperature.
22. Incubate membrane in detection buffer for 10 minutes at room temperature.
23. Repeat incubation in fresh detection buffer for 10 minutes at room temperature.

24. Dilute stock AttoPhos solution (5mM in 2.4M DEA buffer) 1:5 with detection buffer.
25. Using motorized spray gun apply 2.5ml of AttoPhos solution to each membrane.
26. Sandwich membrane in between two trimmed hybridization bags ensuring there are no air bubbles.
27. Heat seal the sandwiched membrane into a hybridization bag and cover in aluminum foil.
28. Place membrane in between two filter blocks.
29. Incubate membrane (in the 37^{o}C incubator for 4 hours or leave at room temperature overnight).
30. Image the membrane using a suitable system e.g. the Fuji LAS1000 dark box.
31. Imaged membranes can be stored in 2x SSC.

Stripping Filters

NB If you intend to strip and re-use filters it is very important not to let them dry out before they are stripped, otherwise the stripping process does not work well.

32. Preheat stripping solution one to 65^{o}C.
33. Incubate membrane in stripping solution one for 30 minutes.
34. Incubate membrane in stripping solution two for 45 minutes at room temperature.
35. Preheat filtered Church buffer to 65^{o}C.
36. Incubate membrane in preheated Church buffer over night at 65^{o}C.
37. Membrane can be stored in 2x SSC at 4ºC until required for reprobing.

Solutions Required for Hybridization

Stock Solutions

EDTA (pH 8.0) Autoclave

186.1g + 1 Liter dH_20 = 1litre of 0.5M solution. Adjust pH with HCl.

Na2HPO4 Autoclave

142g + 1 Liter dH_20 = 1 Liter of 1M solution

SDS 10% (Lauryl sulphate)

100g per liter = 10% solution

PBS (pH 7.4) Autoclave

1 x Sachet (order from Sigma) + 1 Liter dH_20 = 1 Liter of solution

NaOH

40g + 1 Liter dH_20 = 1 Liter of 1M solution

20x SSC

175.3g NaCl

88.2g Sodium Citrate

pH 7.0

Make up to 1 Liter with dH_20

5x TBE

270g Trizma base

137.5g Boric Acid

100ml 0.5M EDTA

Make up to 1 Liter with dH_20

Working Solutions

Church Buffer (1 liter)

Na_2HPO_4	250ml
SDS	500ml
EDTA	4ml
dH_2O	246ml

10x Washing solution (1 liter)

Na_2HPO_4	200ml
SDS	100ml
DH_2O	700ml

Detection Buffer (0.5 liter)

Tris pH 9.5	50ml
$MgCl_2$	0.5ml
dH_2O	449.5ml

Stripping Solution One (1 liter)

10% SDS	100ml
1M NaOH	400ml (100ml 4M NaOH + 800ml dH_20)
dH_2O	500ml

Stripping Solution Two (1 liter)

1M Tris (pH 7.4)	100ml
20x SSC	100ml
10% SDS	10ml
dH_2O	790ml

2.4M DEA Buffer

DEA	22.99 ml
1M $MgCl_2$	0.23ml
dH_2O	26.78ml

Attophos Stock Solution

Attophos Powder	36mg
DEA Buffer	12ml

Blocking Solution

5g of Marvel Skimmed Milk Powder per 100ml PBS

PCR direct from cultures in Genetix microplates

After colonies have been picked and grown as liquid cultures in 96 or 384 well plates then it is possible to PCR cloned inserts directly from the cultures without preparing plasmid DNA.

Method 1- using Genetix QReps

38. Prepare PCR master mix in 96 well or 384 well PCR microplates. Master mix can be dispensed using QFill2 or aliQuot dispensers.
39. Dip a Genetix QRep into the cultures in a microplate and then dip it into the PCR master mix. Agitate gently. NB Choose a QRep of appropriate length for the depth of your PCR plates.
40. Repeat the replication process, such that each PCR reaction has had two dips from the culture.
41. Run the PCR reaction as you would for a pure DNA template except that an initial denaturation step of 10 minutes at 95°C is used to lyse the cells. 'Hot start'-type enzymes can be used if preferred.

Genetix supplies the following types of replicator:

QRep:

The QRep disposable replicators are available in 96 pin or 384 pin format, two lengths 10mm or 15mm and are supplied sterile. They are made of polypropylene.

Hand Held Replicators:

Each replicator is comprised of a stainless steel top plate with a handle and an anodized aluminum spacer plate. All parts are fully autoclavable and the pins are easily replaceable and interchangeable.

Method 2 - using pipette

42. Prepare PCR master mix in 96 well or 384 well PCR microplates.
43. Transfer 1µl of culture from the microplate to the PCR plate using the QBot Liquid Handling function or a multichannel pipette.
44. Run the PCR reaction as you would for a pure DNA template except that an initial denaturation step of 10 minutes at 95°C is used to lyse the cells. 'Hot start'-type enzymes can be used if preferred.

The robot replicating function can also be used to inoculate PCRs. We recommend 2 transfers for reliable PCR yield.

PCR reactions can be purified to remove excess primer, nucleotides etc. using Genetix genPURE PCR clean-up kits available in 96 and 384 well formats. PCR yield can be checked by gel electrophoresis e.g. on Genetix MIRAGE gels. These are acrylamide gels on a glass backing with 96 wells plus wells for markers in each row. Well spacing is suitable for loading with multichannel pipettes or robotic pipettors.

Glossary of Terms

Arrayed
Distribution of colonies or samples into known positions from 96 or 384 well plates.

Base Class
Blueprint for the properties of an object.

Bioassay Tray (QTray)
22x22 cm clear plastic tray from which colonies/phage are picked.

Bioassay Tray Holder
Perspex holder fitted to the robot bed for holding two Bioassay trays in place whilst carrying out a Picking routine.

Blue/White
Blue White selection protocol for visualization of colonies expressing β-galactosidase.

CCD Camera
Charge Coupled Device Camera. A cooled digital camera for capturing high-resolution images with a wide dynamic range.

Clone
A particular DNA moiety contained within a DNA vector and propagated in a host cell.

Custom Property
A custom property is a value that you can apply to samples at a given location in order to use later in the Data Tracking search facility.

Datum Point
A series of X, Y, Z co-ordinates that define a set position on the Robot bed.

Destination Plate Holders
Holders for microplates located on the bed of the robot. The number of Plate Holders available depends on which robot is being used.

DMF
Dimethyl formamide.

GFP
Green Fluorescent Protein. Used to monitor subcellular protein localization, analyze differential gene expression. Protein interactions and cell transfection efficiency.

Hybridization intensity
(as judged by pixel intensity) is a measure of gene activity.

I/O
Inputs / Outputs.

IPTG
Isopropyl-thio-β-D-galactoside.

LB
Luria-Bertani Medium.

LIMS
Laboratory Information Management System

Phage
Bacteriophage.

Picking Tray
See Bioassay Tray Holder.

Probe
A labeled DNA or RNA used to hybridize to an array.

Process (Data Tracking)
A routine performed on the data or an import from a file in QSoft Library Manager format.

QSoft Library Manager
This is the predecessor of QSoft Data Tracking.

QSoft.DLL
ActiveX software component housing all the functionality of robot software.

Rearraying
Redistribution of selected colonies into new plates performed with picking head.

Receptacle
Container used in Data Tracking - such as well plate, bioassay tray, slide or filter.

Replicating
To copy, compress or expand 96 or 384 well plates.

Script
Listing of all moves needed to complete a routine.

SDS
Sodium Dodecyl Sulphate.

Spot
Corresponds to a visual hybridization imprint of a clone with a probe.

SSC
Sodium Chloride/Sodium Citrate buffer.

Sub-Grid
(See Block)

X Drive
Axis running from back to front of the QBot bed or right to left on any other bench-top robot.

XML
Extensible Markup Language. A simple text format derived from SGML Originally designed to meet the challenges of large-scale electronic publishing, XML is also used in the exchange of a wide variety of data on the Web.

Y Drive
Axis running from left to right across the QBot bed or back to front on a bench-top robot.

Y2H
Yeast 2-Hybrid. Screening of ‘prey’ proteins encoded by cDNA libraries for interaction with a particular ‘bait’ protein.

Z Drive
Axis running vertically on the Robot bed.

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Revised January 2010