Genetix

Rearraying

Application Guide

www.genetix.com

08MAN1100.A0
Effective Date: 08-Mar-10

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Rearraying

What is Rearraying?

The objective of rearraying is to **REDISTRIBUTE** collected samples into **NEW** well plates. That is, to take user-selected samples from wells on source WellPlates and deposit them into wells on a destination well plate as shown below.

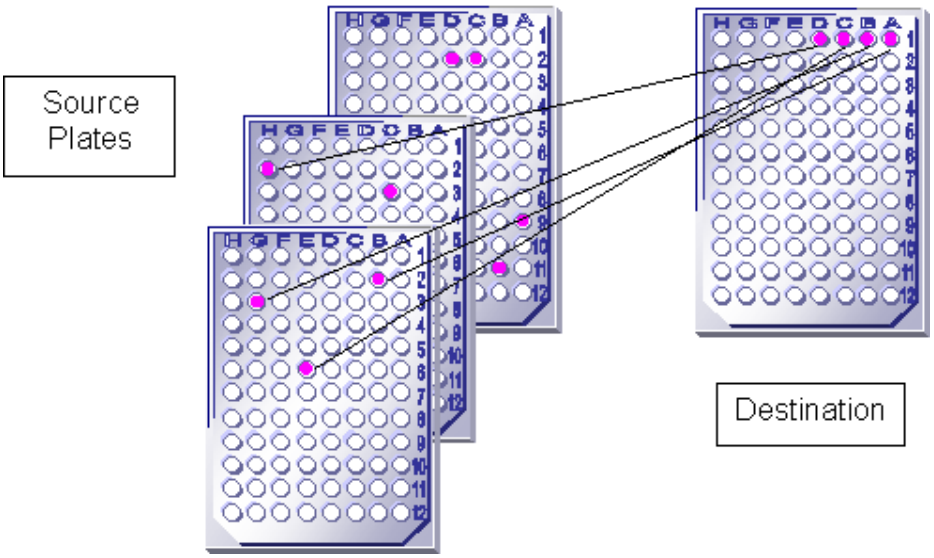


Figure 1: The Principle of Rearraying

Of course the robot can accommodate, simultaneously, many more source well plates and destination well plates in one rearraying sequence than that shown in the diagram.

QPix can accommodate up to 21 well plates. Usually 6 source well plates and 15 destination well plates.

If more destination or source plates are required to complete a rearraying sequence, you will be prompted at the relevant time to replace the well plates.

Preparation

Rearraying uses the following robot accessories:

- Replicating/rearraying table (**QPix**)
- Picking head

Before preparing the robot for Rearraying, check that the workbed area of the base plate is clean and free from debris. The working volume of the machine should also be UV sterilised before setting up the bed for any routine, (see **General Maintenance** section in main robot manual).

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 main 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 not to lose the springs!**
- 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% aQu Clean.
- Remove the pins, plate and springs from the sonicator and rinse thoroughly in distilled water.
- Blow through the plate with an airline 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 sticky 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 easily spotted.

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.**

Loading Source Plates

The source plates may contain the colony libraries that are to be rearrayed, they may also contain just DNA, for example PCR products.

QPix

Traditionally the 2 right-most containers are used for the source plates and the 3 left-most containers are used for the destination plates. For possible variations see the section **"Alternative locations for Source and Destination plates"** (page 10).

On **QPix,** the source plates are loaded on to the source plate holders. The source plate holders are fixed to a removable carrier which fits onto the robot bed.

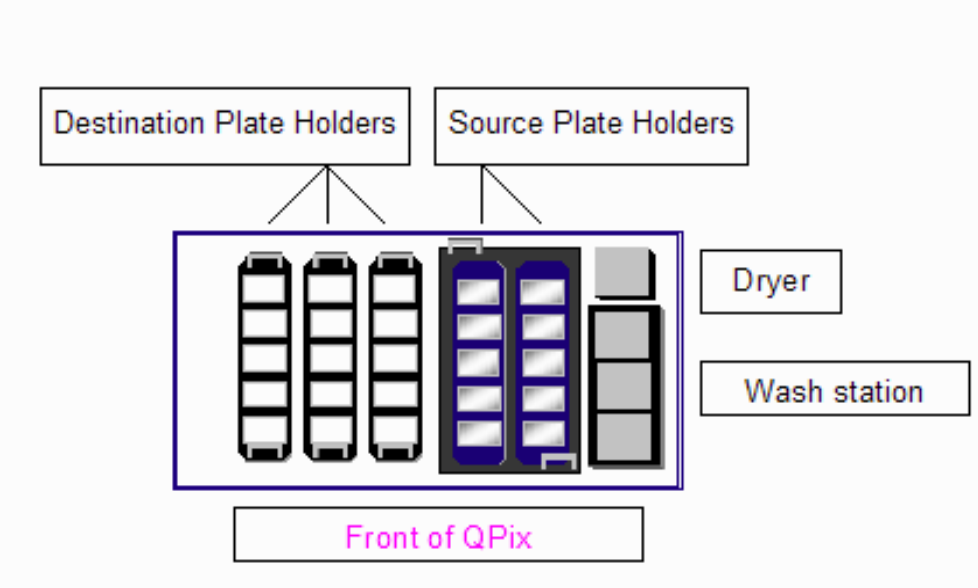


Figure 2: QPix Bed Layout

To fit the source plate carrier, take out the black thumbscrews that hold the gridding table. Remove the bioassay tray locator from the back left corner of the light table, which is held by the white thumbscrew.

Figure 3: Bioassay Tray Locator

Position the source plate carrier so that the four locating lugs are seated over the locating posts on the **QPix** bed and the bioassay tray holder/imaging area is obscured.

Replace the black thumbscrews.

Plates should be loaded **with the lids off** and well A1 should be facing the front right of the machine. Make sure that all plates are correctly inserted into each location.

Loading Destination Plates

QPix

Traditionally the 2 right-most containers are used for the source plates and the 3 left-most containers are used for the destination plates. For possible variations see the section **"Alternative locations for Source and Destination plates"** (page 10).

The destination plates are loaded directly on to plate holders which fit on to the robot bed.

The well plates are loaded without lids and aligned so that well A1 is in position front-right. The well plates are secured in place by the notches in the plate holders.

Deep Well Plates

When using deep well plates you must ensure that the following plate properties are correctly set:

InkingDepth – must be set so that the pins reach no more than 5mm into the media. This is to ensure that media isn't drawn back into the head when the pins are retracted.

UseLowerBedLevel – (QPix only) must be set to TRUE (-1)

QPix

QPix has a section that lifts out to reveal a recess which will accommodate deep well plates if required. To allow access to the lower bed level undo the four holding screws on the removable plate.

Alternative Locations for Source and Destination Plates

Please Note: Although the physical objects are actually referred to as Source **objectname** and Destination **objectname**, it is possible to designate which area is to be defined as the source and destination. For example:

On a QPix….

This is slightly more complicated because there are more permutations.

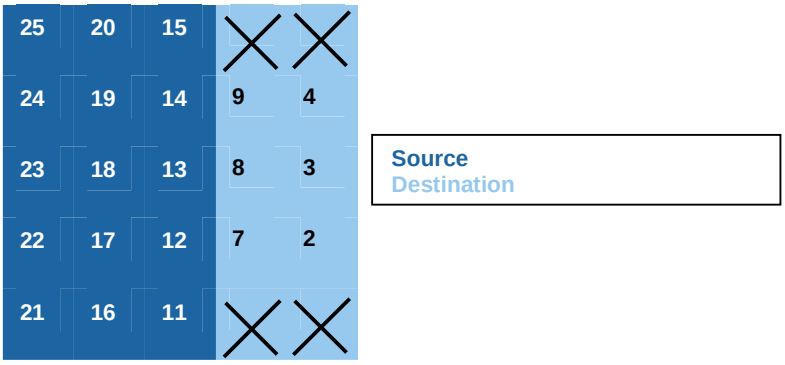
When picking source data in the ReArraying module on a **QPix**, the head cannot address locations on the back row, the front row or the leftmost well plate holder. The unavailable locations are shown in the diagrams with a **X** through them.

When depositing the destination data the head will align directly above well plates and can therefore address any location on the bed.

Standard Well Plates

Option 1

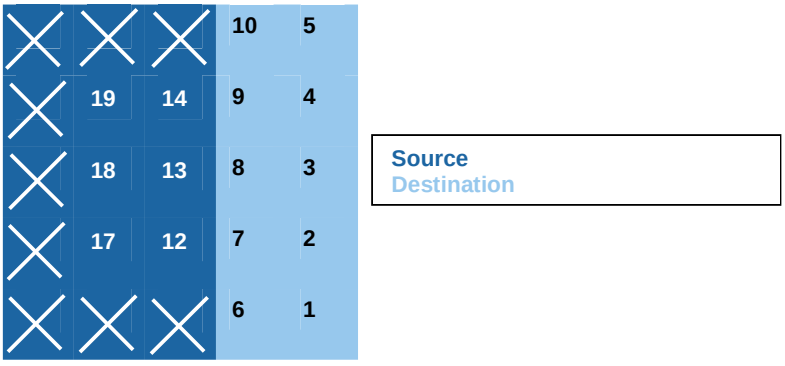
Up to 6 source well plates and up to 15 destination well plates.



Note: Make sure that you select Source Plate Holder (1X3) as the Source container.

Option 2

Up to 6 source well plates and up to 10 destination well plates.



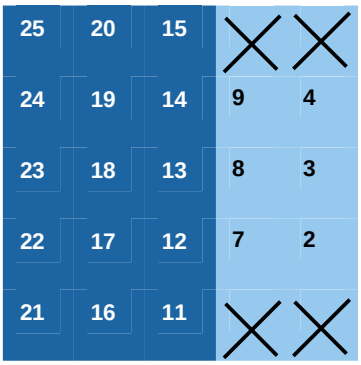
Note: When using locations 1 - 10 as the destination, a **Source Plate Holder (1X5)** as the Destination container and a **Destination Plate Holder (1X3)** as the Source Container must be selected.

Deep well Plates

Note: Deep well plates (indicated by white boxes) can only be used as **either** source **or** destination not as both.

Option 1

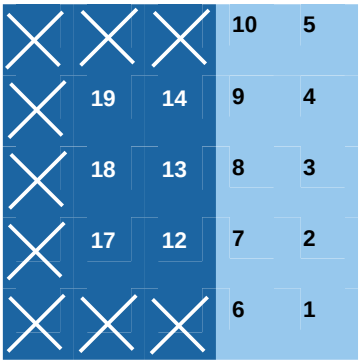
Up to 6 **standard** well plates as source and up to 15 **deep well** plates as destination.



Note: Make sure that you select **Source Plate Holder (1X3)** as the Source container.

Option 2

Up to 6 deep well plates as source and up to 10 standard well plates as destination



Note: When using locations 1 - 10 as the destination, a **Source Plate Holder (1X5)** as the Destination container and a **Destination Plate Holder (1X3)** as the Source Container must be selected.

Defining Well Data

Note

Well data can be either "Source Data Only" or "Source **and** Destination Data".

Define Source Data Only

"Source Data Only" can be defined in one of two ways:

Importing a Source text file.

or

Selecting wells on a plate representation.

Importing a Source Text File

A data file defining the selection of source sample wells could be created *outside* the Rearraying module. This can be done using any software that produces output in text format e.g. Windows® Notepad or Microsoft® Excel®.

Note: If the data file is created using an application other than QSoft, you must ensure that the file is saved as a **comma delimited text** file. It may have the extension TXT or IMP

The data file would then be imported into the rearraying module at run time. You may need to change the "Files of Type" list box option to "All Files (*.*)" in order to locate your file.

Rearraying Import File Format (Source only)

The Rearraying Import File is made up of a list of keywords, well references and plate numbers. All keywords should be uppercase and suffixed with a colon and a space (i.e. ": "). The default import file extension is IMP, however, any ASCII file can be read.

For each plate to be rearrayed, there must be a **PLATE** keyword followed by a list of well references. The import or export file specification is as follows:

PLATE	Required – Source plate number to be rearrayed. The plate numbers must be contiguous.
BARCODE	Optional - The barcode on the plate to be checked when the plate is loaded.
COMMENT	Optional - any comments for/about this plate.

Figure 4:
An Example Import File

```
PLATE: 1
BARCODE: 12345
COMMENT: Some Comments
A3
B3
B9
C1
C5
D5
E7
F5
F11
G9
PLATE: 2
B3
B7
B9
PLATE: 3
COMMENT: Some notes about this plate
B2
C3
D4
E5
```

To import the file - when the Rearraying software is running - select the Source tab and click the Source Data button. The following screen is displayed:

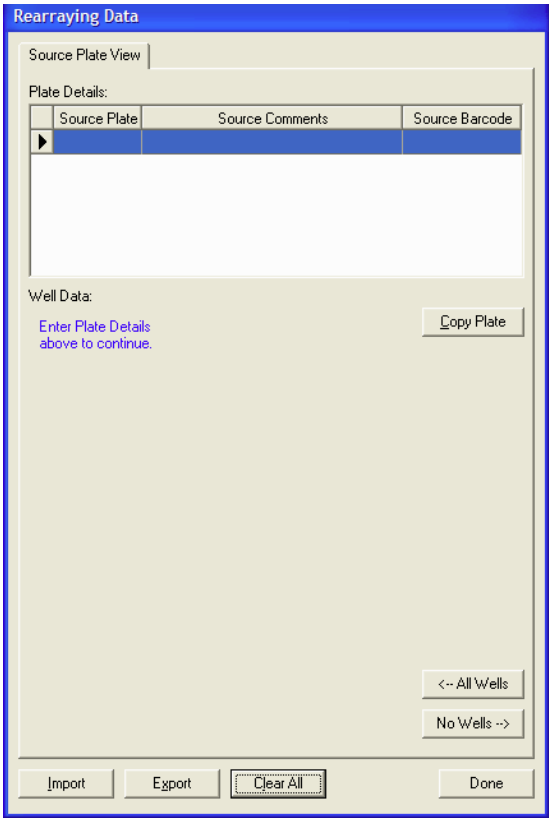


Figure 5: Rearraying Data Selection Dialog

Click the Import button.

If there is rearraying data already defined for the current routine, the following screen will appear.

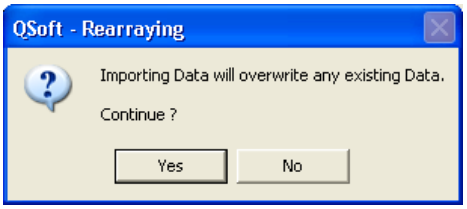


Figure 6: Import Data Message

Click **Yes** to display the **Import ReArraying Data** dialog (Figure 9).

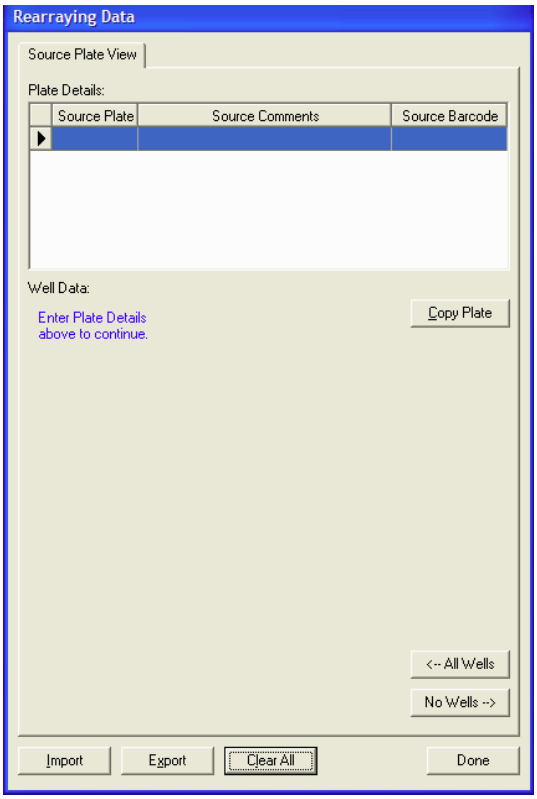


Figure 7: Rearraying Data Selection Dialog

Click the Import button.

If there is rearraying data already defined for the current routine, the following screen will appear.

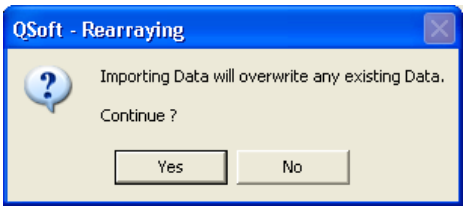


Figure 8: Import Data Message

Click **Yes** to display the **Import ReArraying Data** dialog (Figure 9).

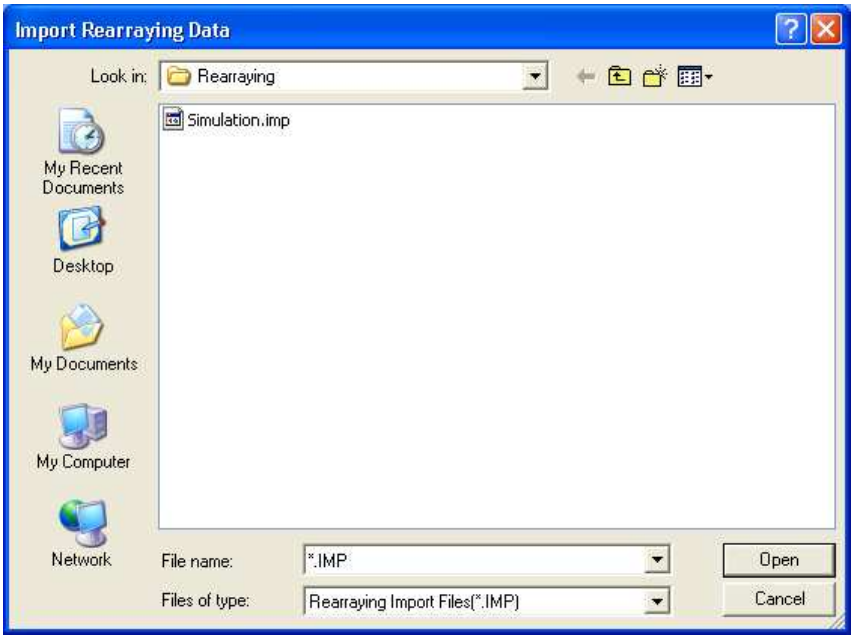


Figure 9: Import Rearraying Data

This is a standard Windows **File Open** dialog and is used to locate your prepared source data file. Highlight the filename and Click on Open.

This will import all the information into the Rearraying Data Input screen. It is then possible to check the data for each plate if necessary - by clicking the plate number in the Plate Details list, every imported plate will be shown and the chosen wells are highlighted in red.

Selecting Wells on a Plate Representation

When the Rearraying software is running, select the Source tab and click the Source Data button.

The screen shown in Figure 7 on page 15 is displayed.

Note: If 'Source and Destination Data' was previously imported, the existing data will need to be cleared using the 'Clear All' button in order to see the Plate Representation.

Enter the plate number (and optional plate barcode and any comments) for the plate to be rearrayed from. The data entry screen for this plate will then appear on screen.

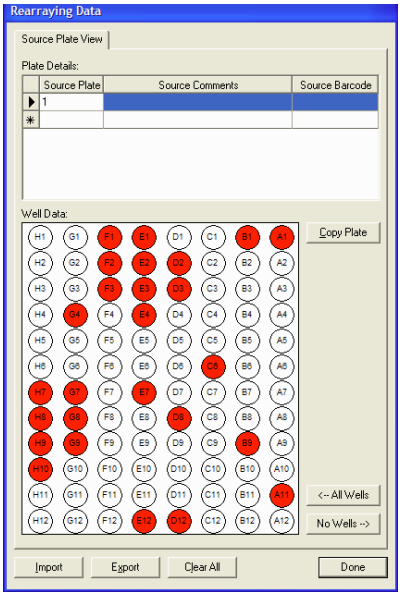


Figure 10: Rearraying Data Plate Representation

To view the wells better, you can expand the "Rearraying Data" window by dragging one of its corners. Individual wells can then be select/deselect by clicking on them. Selected wells are displayed with a red background.

Having chosen the required wells for the first plate, click the next blank row of the **Plate Details** section and the second plate can then be set up. Continue until all required plates have been entered.

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 see the section headed Barcodes later in this manual.

Rearraying Software

Overview

Double click on the **QSoft** Rearraying Icon on the desktop, the application splash screen will appear.

A prompt will appear asking for a new routine to be created or to load a previously saved routine, any existing routines will be listed in the lower window.

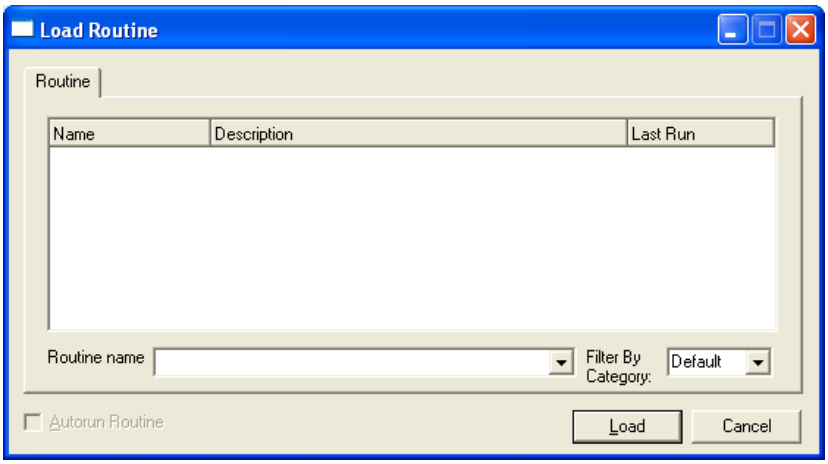


Figure 11: Routine Prompt

Create a New Routine

Select this option then click OK. The default routine settings will be loaded, this routine can be edited then 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 and OK is clicked the selected routine is started automatically.

Filter by category

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

Sign On

If the **Show at Start-up** box has been checked, the **Sign On** screen will be displayed.

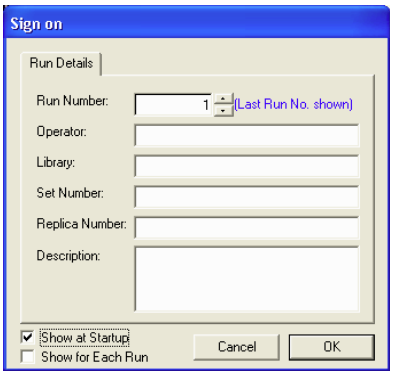


Figure 12: Sign on dialog

Complete the Sign On screen and then click OK. The Rearraying Welcome screen will be displayed.

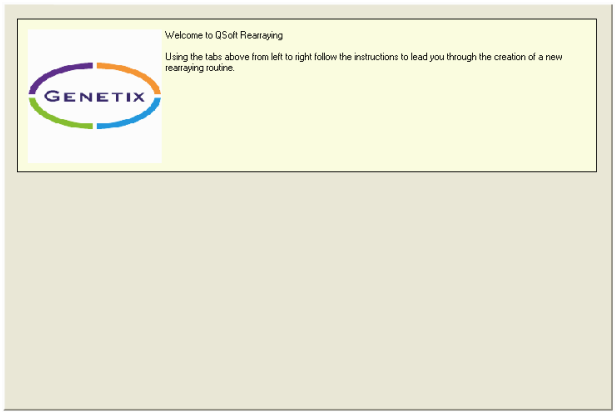


Figure 13: Welcome

The Menu and Toolbar Options

There is a reference to all of the menu items in Appendix A of this manual. More detailed information about the use of some of these can be found in the robot manual.

Rearraying Setup

The Rearraying setup screen is split into tabbed dialogs. Each tab contains instructions to guide you through setting up a routine.

Select each tab in turn from left to right and fill in appropriate fields or select required options.

Description

Use the text boxes here to enter information about the routine you are creating.

Head

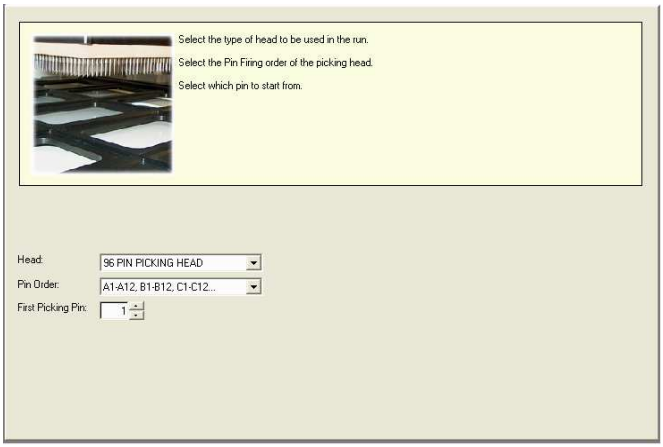


Figure 14: Head

Head - Gives the option to choose between the different heads available. Currently there is only 1 Head option for rearraying: 96-Pin Picking Head.

Pin Order - This option allows the order in which the pins in the Picking Head will fire to be chosen. The options are:

A1-H1, H2-A2… (snaking)

A1-A12, B12-B1… (snaking)

A1-H1, A2-H2, A3-H3…

A1-A12, B1-B12, C1-C12

First Picking Pin - Allows the starting pin to be specified. If the number of wells to be rearrayed from 1 well plate is less 96 then **First Picking Pin** shows which will be the first pin used to rearray from the next well plate.

Source

Source Settings

Figure 15: Source Settings

Container - This refers to the Container that is to hold the source plates – i.e. Well plates that are to be rearrayed from. There are several options available, these vary according to which robot being used. For example…

QPix

Source Plate holder (1 x 3)

Source Plate holder (1 x 5)

Destination Plate holder (1 x 3)

Destination Plate holder (1 x 5)

No. of Containers – Refers to the number of addressable locations depending on the type of Plate selected (below).

Plate - This field allows the type of source plate that is to be rearrayed from to be defined. These plates will be located in the container specified above.

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

Stir Source – This allows the source to be stirred prior to inking. The stir option moves back and forth then side to side within a known region as defined by the following plate properties.

- WellDiameter – Well diameter (microns)
- StirAreaPC – percentage area of the well to stir in (ensure WellDiameter is correct)
- NoStirs – how many times to stir

Source Data - Click on this to open the Rearraying Data dialog (see Figure 10 above).

Copy - Will copy the selected wells to another plate - a prompt to enter a new plate number to copy to will appear.

<--All Wells - Will select all wells on current plate.

No Wells --> - Clears all selected wells on current plate.

Import - Imports predefined source data from a text file.

Export – Allows the plate configuration data in a text file to be saved. The file is automatically given an extension .exp. The file format conforms to the same standards as import data files and can be viewed using text editing software such as Windows Notepad.

Clear All – Clears all information related to all plates.

Done – Click this button when satisfied with the data.

Prior to carrying out a Rearraying run, information relating to the wells that are to be rearrayed must be entered. The methods for doing this are described in detail in the section headed "Defining Well Data" on page 12.

Control Data

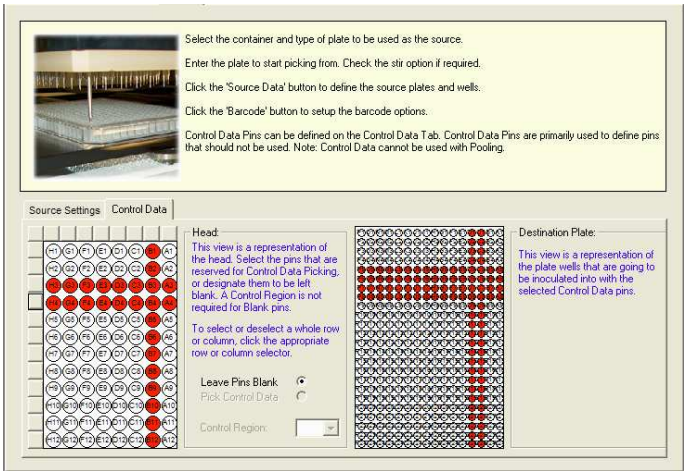


Figure 16: Control Data

Please Note: Control data cannot be used when destination well data has been defined (for example in a Source and Destination data import file).

Head

This diagram is a representation of the head with pin locations labelled. Click to select/deselect pins to be left blank.

Note: A complete row or colum can be selected by clicking the relevant selector button.

Destination Plate

This diagram represents the selected destination plate, as pins to leave blank are selected, the corresponding plate wells are highlighted in red.

Leave Pins Blank

This is the only available option in Rearraying.

Pick Control Data

This is currently not available in Rearraying.

Control Region

Works in conjunction with the **Pick Control Data** option.

Destination

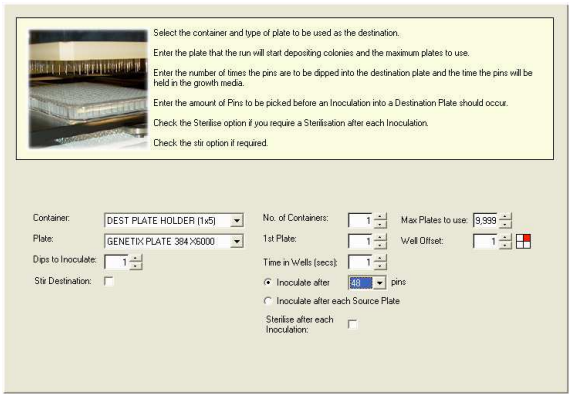


Figure 17: Destination.

Container - This refers to the Container that is to hold the destination plates – i.e. Well plates that are to be picked into. There are several options available. For example…

QPix

Destination Plate holder (1 x 3)

Destination Plate holder (1 x 5)

Source Plate holder (1 x 3)

Source Plate holder (1 x 5)

No. of Containers - Refers to the number of addressable locations depending on the type of Plate selected (below).

Plate - This field allows the type of destination plate that is to be picked into to be selected. These plates will be located in the container specified above. There are several options available, these will vary depending on which robot you are using.

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

1st Plate - This determines the well plate into which the picking head will start depositing colonies. It is normally set at 1 but can be set at any value up to the maximum number of plates.

Max Plates to use - This specifies the maximum number of well plates available. If this value is set to a higher number of plates than the total Destination Holder capacity a **pop-up** screen will appear and prompt for "Change WellPlates" before continuing.

Dips to Inoculate - In order to control the inoculation of the Destination plates, you can choose the number of dips that the Picking Head will make into the well plates canbe chosen. This setting would normally be in the range of 1 to 3.

Time in Wells (secs) - Varies the time that the Picking pins are held in the Growth media in the well plates in seconds. This parameter is used in conjunction with the number of Dips to Inoculate.

Well Offset – The well offset corresponds to one of the 4 inoculation offsets (wells A1, A2, B1

and B2) when using 384 well plates. This is not applicable when using 96 well plates. Default value - 1.

Stir – This allows the source to be stirred prior to inking. The stir option moves back and forth then side to side within a known region as defined by the following plate properties.

- WellDiameter – Well diameter (microns)
- StirAreaPC – percentage area of the well to stir in (ensure WellDiameter is correct)
- NoStirs – how many times to stir

Inoculate After nn pins – Will pick colonies sequentially from all available plates and will only deposit after the set number of pins have been used.

Inoculate After each Source Plate – Will pick colonies sequentially from each plate and will deposit *either* when all pins have been used *or* when all colonies from the current plate have been picked.

Sterilise After Each Inoculation – Check this box if you want to sterilise the head after every deposit.

Sterilize

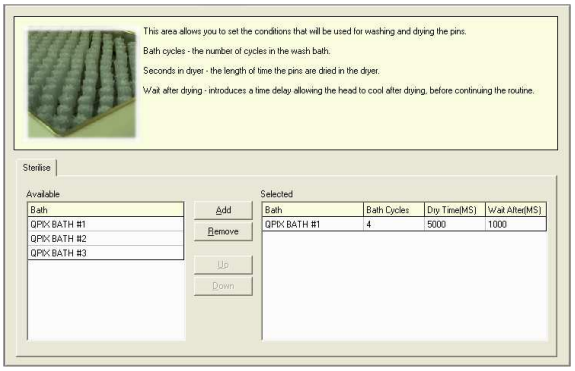


Figure 18: Sterilize

Bath Cycles – The number of cycles in the wash bath. Usually set to 3 or 4.

Dry Time – The length of time the pins are dried in the dryer. If the number of bath cycles is set to zero this box is greyed out.

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

If the robot uses a halogen dryer, QSoft automatically adds to this wait time in order to allow enough time for the pins to cool properly.

This additional 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 – deionised 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

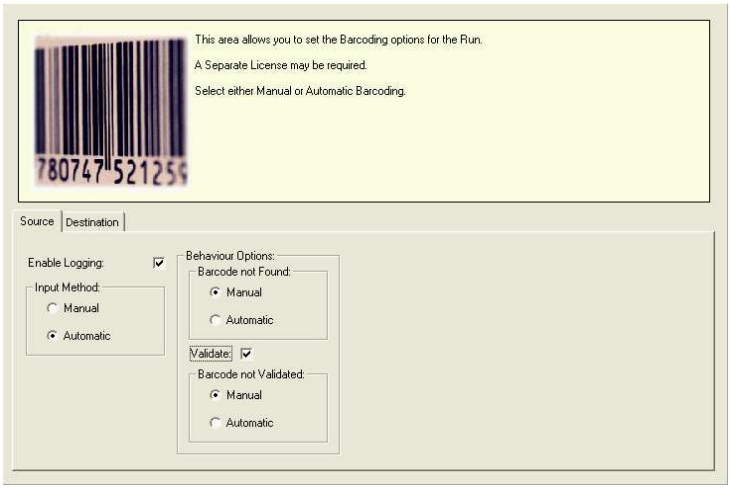


Figure 19: Barcodes

Important Note:

The thickness of some barcode labels can affect the fit of the lid so that it becomes too tight to remove the lid. (Only applies to robots that have Stacker Units and/or Lid Lift mechanisms).

This problem does not arise so much with Genetix plates as they have been designed with special ribs on the lid which do not obstruct the barcode label.

To enable barcodes to be recorded, the appropriate options must be set in the Barcodes setup dialog. Select the Enable Logging option on the Source and/or Destination tabs of the Barcodes dialog.

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

Enable Logging

Check this box to enable barcode reading.

QSoft will automatically generate a unique key in the barcode field for any plate that is used during a run and the logs will display these keys in the barcode field. The 'Enable Logging' barcode reading options will overwrite this key. The keys are generated based on the current date and time and in the format UID-YYMMDDHHNNSSss-X where ss is milliseconds and YY is a two character ascii representation of the year.

Input Method – Choose Manual if scaning barcodes with a hand held barcode reader is required or to input barcodes at the keyboard. Automatic barcode reading is possible if the robot has a barcode reader installed. If automatic barcode reading is required contact Genetix Ltd for a licence.

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 behaviour if a barcode is not present.

- Manual will produce a prompt and will wait for a barcode to be input (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 allowing the order of the plates being processed to be verified before continuing the run.
- 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.

Note: If barcode validation fails a set of options as shown in Figure 26 below will be displayed.

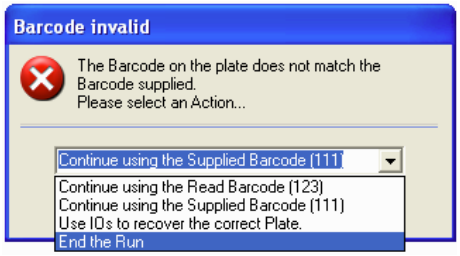


Figure 20: Invalid Barcode Dialog

Add – Creates a new row for entry of the next barcode

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

Disable Barcoding for source or destination

If Datatracking is selected, Barcoding is automatically enabled. Barcoding for a given container can be disabled – for example if barcoding the source bioassay trays is not required – by

changing the **BarcodeReaderType** property this can be achieved. Do this as follows:

- Highlight the container in the **Hardware Configuration** tab
- Click the **Properties** button
- Select **BarcodeReaderType** and click the **Edit** button
- Enter **None-AutoGenerate** and click **OK**

Start

Having set all of the variables, save the routine by clicking the Save or Save As button on the toolbar.

Figure 21: Start

Enable Data Tracking

Selecting this option will generate a Data Tracking XML file, which can then be imported into the Data Tracking database.

This is only applicable if barcoding options have been set for both source and destination plates.

The data tracking file is stored in the following location:

C:\Program Files\Genetix\Logs

To view the xml file, double-click the filename in Windows Explorer.

For more information on Data Tracking, please refer to the **QSoft Data Tracking** manual.

Start in Slow Motion – The robot will run at a slower speed for diagnostic purposes.

Run – Click this button to start the routine.

Running a ReArraying Routine

Once all the parameters have been set and plates have been loaded, the Rearraying Routine can begin.

Click the **Run** button at the bottom of the setup screen.

The following screen appears:

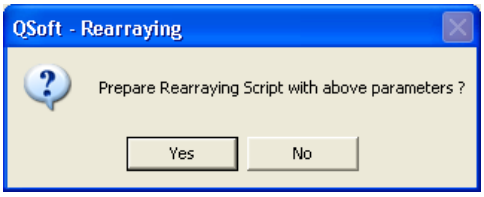


Figure 22: Prepare Script

Click **Yes** to continue **No** to abort.

The following screen will appear if continuing, which displays the total number of wells to rearray.

Figure 23: Wells Found

Click OK. And the following screen will appear.

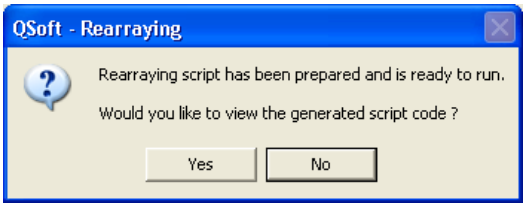


Figure 24: View Script

To view the script code click on **Yes** otherwise click **No**.

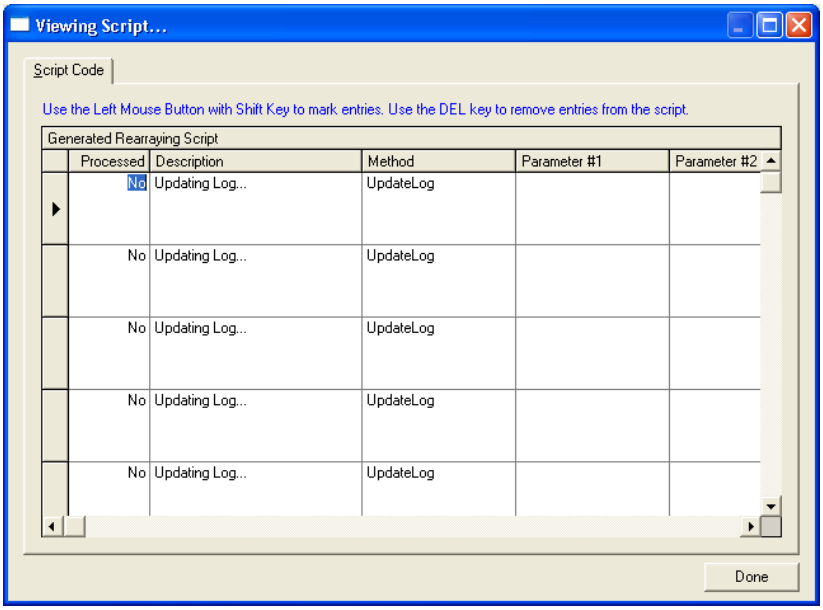


Figure 25: Example Script Code

Click on Done to continue, the following screen will appear:

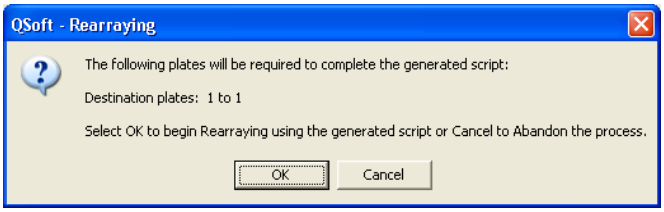


Figure 26: Plates Required

This screen wil indicate how many destination plates are required for the rearraying run. Ensure that these have been loaded. Click OK to carry on.

The screen will now ask to ensure that the correct head has been fitted to the robot.

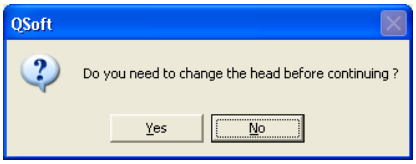


Figure 27: Change Head

Remember that for a rearraying run the Picking Head must be fitted.

Once this has been checked click on **Yes** to continue. The following screen will now appear.

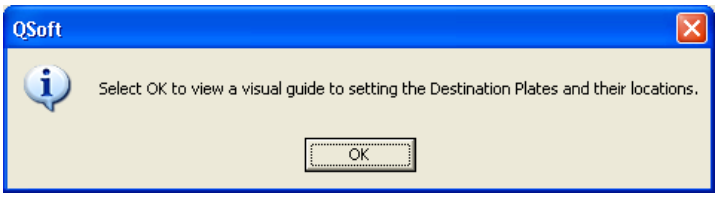


Figure 28: Destination Plate Locations

This prompt is for the destination plate holders to be fitted on the bed of the robot. When you click **OK** you will be presented with a visual display that indicates the positioning of the destination well plates in the holders.

Click **Done** to continue, the following screen appears:

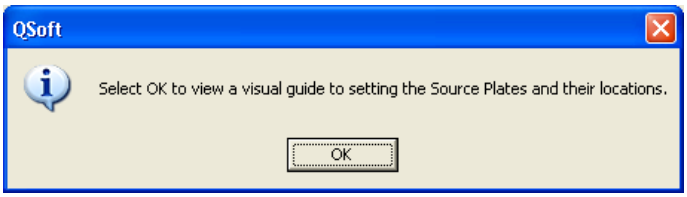


Figure 29: Source Plate Locations

Click **OK** and the screen will show the required source well plates.

Click **Done**.

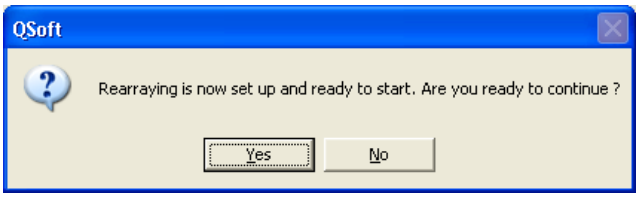


Figure 30: Set Up and Ready

This screen displays that the ReArraying is now ready to start. To commence the run click **Yes.** To abort click **No**.

During the run the progress screen (shown below) will be displayed. This screen displays the status of the robot, the start time of the run, current time and expected time of completion.

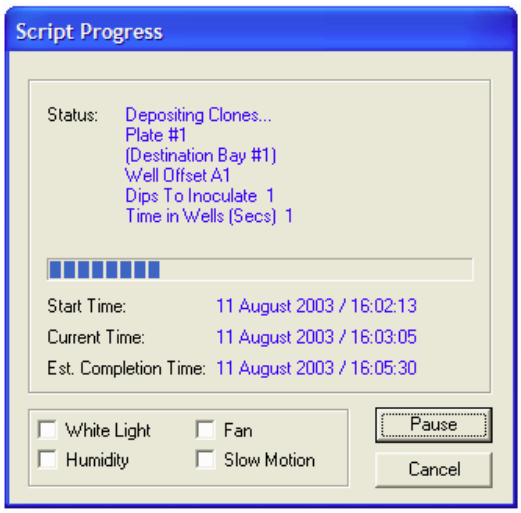


Figure 31: Script Progress

If at any time the run needs to be stopped, the **Pause** button can be clicked.

When the run has finished, the following screen will appear.

Figure 32: Script Complete

Clicking **OK** will generate the following screen.

Figure 33: Carry Over Pin and Well Settings

The last well and pin settings are stored, so that more data can be loaded to carry out a further run, utilising any pins on the head that have not been used and any destination plates that have not been filled.

As you become more familiar with the robot some of the above screens need not be shown. Under **Additional Options** in the ReArraying setup screen, there is a command button – **Script Options**, allows which screens will appear and which will not to be chosen.

Click the **Exit** button when ReArraying is finished.

Biology Guide

Preparing Media

Luria-Bertani medium (LB) – per litre

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

LB + 8% Glycerol – per litre

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

LB Agar – per litre

To 1 litre of de-ionised 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 litre of LB. Sterilise by autoclaving at 121°C for 15 minutes.

Antibiotic Preparation

Ampicillin

Stock solution: Dissolve 1g of ampicillin in 20ml of sterile distilled water. Filter sterilise 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 litre 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.

Glossary of Terms

Arrayed
Distribution of colonies or samples into known positions from 96 or 384 WellPlates

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 that drops into the robot bed for holding two Bioassay trays in place whilst carrying out a Picking routine

Compressing
Converting 4 x 96 well plates into 1 x 384 well plate etc.

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. There are 3 destination plate holders on QPix. They are used for replicating, rearraying and picking.

DMF
Dimethyl formamide

Expanding
Converting 1 x 384 well plate into 4 x 96 well plates etc.

I/O
Inputs / Outputs

IPTG
Isopropyl-thio-□-D-galactoside

LB
Luria-Bertani Medium

Phage
Bacteriophage

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

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

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

SSC
Sodium Chloride/Sodium Citrate buffer

X Drive
Axis running from back to front of the QBot bed or right to left on QPix

Y Drive
Axis running from left to right across the QBot bed or back to front on QPix

Z Drive
Axis running vertically on the Robot bed

Appendix A

Description of Toolbar and Menu Items

	Add Sample to Rearraying Checkout	Add the highlighted sample(s) from the currently selected plate to the Rearraying checkout.	Data Tracking
	Align	Align camera to pin	Picking Excision Excellerate Rearraying
	Analysis path	Allows you to select the Gel Analysis program to be used	Excision
	Calibrate	Calibrate the camera	Picking Excision Excellerate Rearraying
	Change head	Moves the actuator into an accessible position to allow you to change the head	All
	Chiller	Turns the chiller on or off	Robot Specific
	Clear Rearraying Checkout	Will clear all samples from the rearraying checkout.	Data Tracking
	Configuration	Displays the robot configuration dialog which gives you access to all the hardware settings, datum point settings and database facilities	All
	Configure Messaging Server	Messaging Server provides a means for you to remotely monitor your robot. For example: if a robot run is interrupted for any reason, one or more contacts can be notified thus eliminating the need for constant supervision of your robot. Click this button to set messaging server preferences. For detailed information about setting up Messaging Server see Appendix A of this manual.	All
	Create Rearraying file	Write the contents of the Rearraying checkout to a text file.	Data Tracking
	Diagnostics	Displays an animated representation of the robot bed layout for diagnostics purposes or for use when running the software in simulation mode. Click **Stay on Top** to keep the dialog visible while the application is running.	All
	Exit	Exits the application	All
	Fan	Turns the fan on or off	Robot Specific
	Humidity	Turns the humidity system on or off	Robot Specific
	Import Custom Properties	Import properties to use in searches to identify samples of interest	Data Tracking
	Import Process file	Import a Data Tracking process file	Data Tracking

	IO's	Displays the robot Inputs and Outputs to allow you to manually control hardware components	All
	Logs	Displays the QSoft Logs dialog which gives you access to the Text, XML and DataTracking log files. Highlight the log file name and click Open to view it.	All
	Park head	Parks the head in the wash bath	All
	Pin fire test	Displays a dialog whilst continuously firing all pins in the head. The purpose of the Pin Firing Test is to check, by sound, that each pin is firing correctly. **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 allow you to observe the mechanical actions of the pins. **Cancel** – Ends the Pin Firing test.	Picking Excision Excellerate Rearraying
	Purge	Purges an Excision head to clean the pins	Excision Excellerate
	Remove Sample from Rearraying Checkout	This will remove selected samples from the rearraying checkout.	Data Tracking
	Reset toolbars	You can rearrange the buttons on the toolbar if necessary, this option will set them back to the default order.	All
	Routine close	Closes the current routine	All
	Routine delete	Deletes the specified routine	All
	Routine Export	Permits you to save routines as XML files in a user-defined location. This enables the transfer of routines between robots.	All
	Routine Import	Allows externally created routines to be used.	All
	Routine new	Create a new routine	All
	Routine open	Opens a previously saved routine	All
	Save	Saves the current routine	All
	Save as …	Saves the current routine with a new name	All
	Script options	Allows you to select which script options to display during the application run	All
	Select Database	Displays the login dialog to allow you to connect to a different database or login as a different user.	Data Tracking
	Set pin height	Provides the facility for setting the picking height	Robot Specific

	Sign on	Displays the Sign on dialog to allow you to enter specific information about the current run	All
	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	All
	System info	Displays information about your computer system	All
	Test Image	Displays the test image window so that you can set criteria for picking colonies	Robot Specific
	UV light	Turns the UV light on or off	Robot Specific
	Vacuum	Turns the vacuum on or off	Robot Specific
	White light	Turns the white light on or off	All

Appendix B

GTX Messaging Server

Messaging Server provides a means for you to remotely monitor your robot. So that if a robot run is interrupted for any reason, one or more contacts can be notified thus eliminating the need for constant supervision of your robot.

In order to use Messaging Server you need to allocate one or more contacts and ensure that at least one of those contacts is enabled before the run starts.

Once QSoft has started to process the script, Messaging Server will notify the contact(s) whenever the software requires a response. For example any of the standard message boxes that appear during the run and also “Timeout” messages that are displayed when a problem occurs.

The messaging server currently supports dial-up (without voice) and email notification.

Dial-Up Settings

The dial up facility is implemented through the use of a Hayes compatible modem installed in the robot PC. A phone line is also required. Upon receiving a message, the modem will dial the appropriate number, and ring it for a predefined period of time. If the receiving contact's number is a mobile phone, a phone book entry can be made for the robot. Then when the robot rings the number, the caller-id facility will display to the operator which number is called.

Email Settings

The email facility requires that a MAPI compliant mail application (such as Microsoft Outlook® Express) has been configured on the robot PC.

The steps for configuring Outlook Express are as follows:

- Set up the email account that is required to send mail with. If unsure how to do this then look in the Outlook Express help or contact the system administrator.
- Change the security settings to allow the messaging server to automatically send an email unattended:
  - Open the Tools menu, then select Options

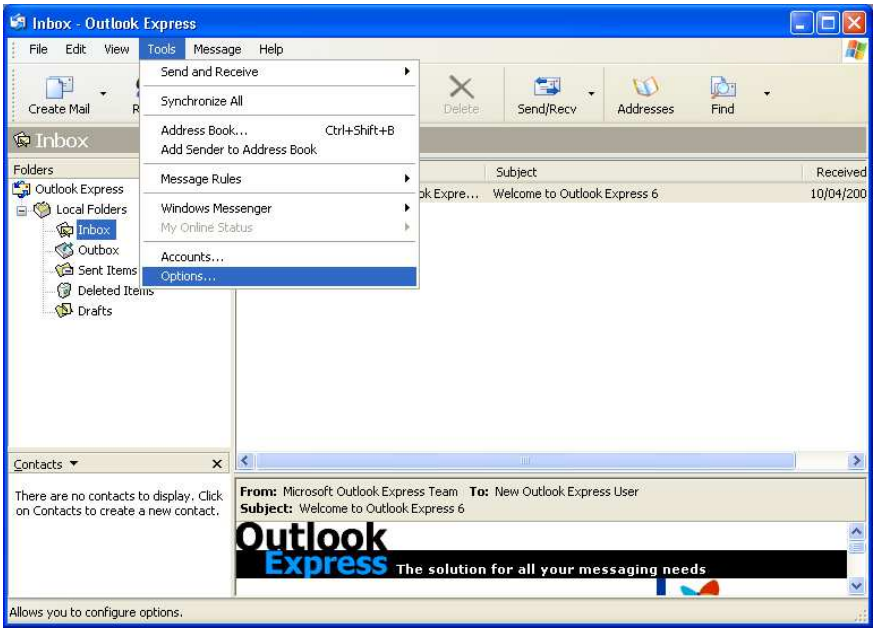


Figure 34: Outlook Express Tools Menu

Uncheck the option that says:

'Warn me when other applications try to send mail as me'

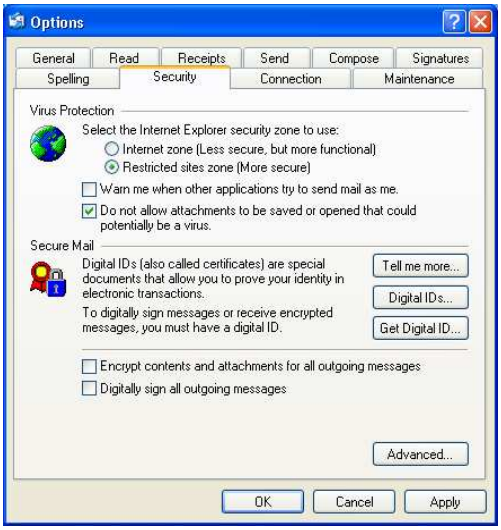


Figure 35: Outlook Express Security Settings

Click **OK**

Configure Messaging Server

Click the **Configure Messaging Server** button on the toolbar.

The following screen will be displayed

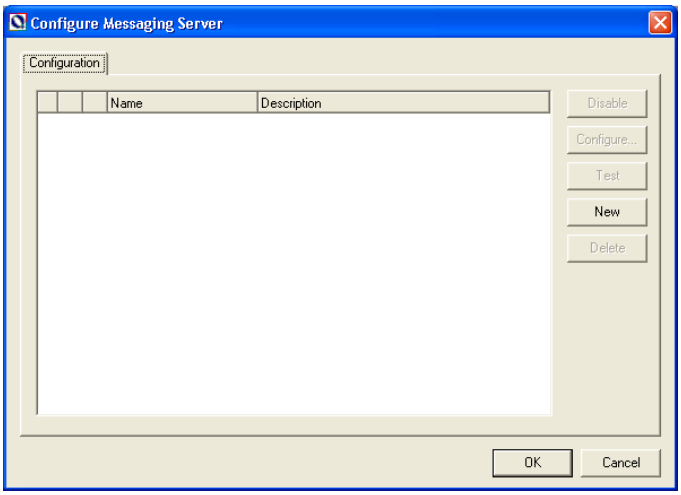


Figure 36: Messaging Server Configuration

Click the **New** button to add a new contact.

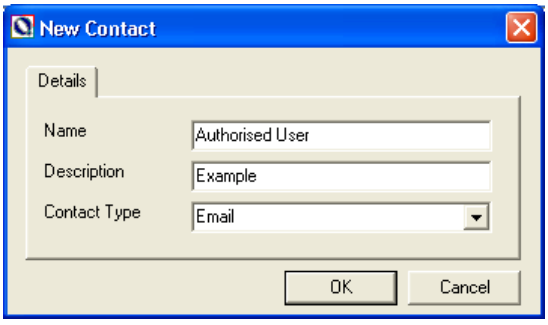


Figure 37: New Contact

All the parameters here are required.

Name

Type in a name for the contact

Description

Type in any descriptive text.

Contact Type

Select the required option from the drop-down list

Click OK when you have entered the required details. The following dialog is displayed.

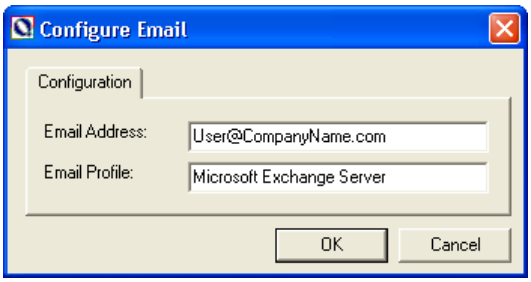


Figure 38: Configure Email

Email Address

Enter the contact's valid email address.

Email Profile

If there is no specific profile set up (such as Microsoft Exchange Server) enter the name of the registered user of the copy of Windows that is installed on the robot PC. To identify the registered user, locate System Properties in Windows Control Panel (see below).

Figure 39: Windows System Properties

Click **OK** to store these settings.

The Configuration screen will show the details of the contact you have just added.

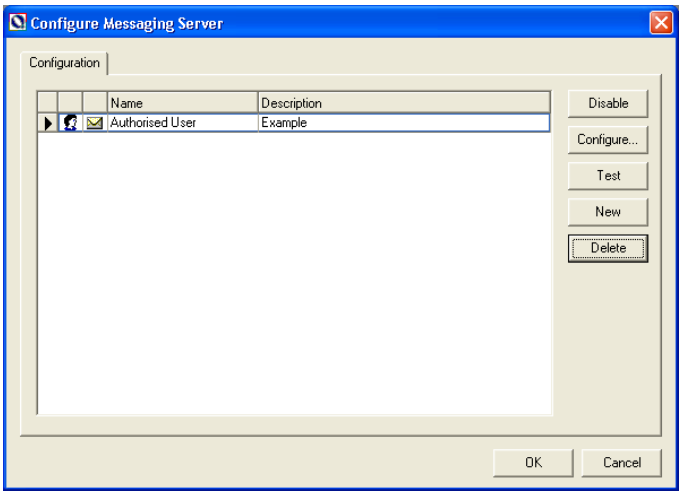


Figure 40: Contact Details

Disable

This allows the selected (highlighted) contact to be disabled. The button's state will change to say **Enable** when a contact with **Disabled** status is highlighted. Operators can be enabled or disabled on a run-by-run basis.

Configure

Allows the configuration settings of the selected contact to be changed.

Test

Use this to check that the email and/or dial-up settings work as expected.

New

Allows you add a new contact and configure their settings.

Delete

Will delete the highlighted contact.

Set the Delay Time

By default the delay time is set in QSoft to 10 minutes. This is the time that will elapse before either a mail message is sent or the dial-up will be activated. If necessary, you can change the delay time as follows:

Click the **Configuration** button on the toolbar.

In the **Hardware** tab select the robot name.

Select the property named **MessagingSendingDelayMinutes.**

Click **Edit** to change the value.

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Contact Details

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Revised January 2010