

QStreak

Applications Guide



Contents

Table of Figures	4
QStreak	5
What is QStreak?	5
Preparation	5
Fitting The Head	5
Maintaining a Streaking Head.....	6
Replacing Pins	6
Loading Work Objects.....	7
Recording Barcodes	7
QStreak Software	8
Overview	8
Sign On	9
The Menu Options	10
File Menu	10
View Menu	12
Options Menu.....	12
The Toolbar.....	12
QStreak Setup	14
Description	14
Head	15
Source.....	15
Source Settings.....	15
Destination	17
Sterilize	20
Barcodes	20
Calibrate.....	23
Start24	
Useful Properties	25
Running a QStreak Routine	25
Biology Guide	29
Preparing Media	29
Luria-Bertani medium (LB) – per liter.....	29
LB + 8% Glycerol – per liter	29
LB Agar – per liter	29
Top Agar	29



Antibiotic Preparation.....	30
Ampicillin.....	30
Chloramphenicol.....	30
Kanamycin.....	30
Appendix A	31
Mapping Regions	31
Glossary of Terms	36
Index.....	39
Contact Details	41



Table of Figures

Figure 1: Well plates loaded showing position of well A1 7

Figure 2: Routine prompt..... 8

Figure 3: Sign on Dialog 9

Figure 4: Welcome..... 9

Figure 5: File menu..... 10

Figure 6: Sign on dialog..... 11

Figure 7: View menu..... 12

Figure 8: Options menu 12

Figure 9: Toolbar 13

Figure 10: Description..... 14

Figure 11: Head..... 15

Figure 12: Source Settings 15

Figure 13: Destination 17

Figure 14: The Set Streaking Height Dialogue..... 18

Figure 15: The Set Pipette Depth Dialogue 19

Figure 16: Sterilize..... 20

Figure 17: Barcodes 20

Figure 18: Barcode tab command buttons 22

Figure 19: Calibration..... 23

Figure 20: Start 24

Figure 21: Create Script 25

Figure 22: Script creation progress..... 25

Figure 23: QTrays Required 26

Figure 24: Example script code 26

Figure 25: Replace Pipettes prompt 26

Figure 26: Please Load Plate Holder(s) prompt 27

Figure 27: Manual Source Plate Dialog 27

Figure 28: Script Progress..... 28

Figure 29: Script complete..... 28

Figure 37: QTray regions layout 31

Figure 38: The positions of the regional QTrays on the QPEXpression. 32

Figure 39: Layout of wells..... 33



QStreak

What is QStreak?

QStreak transfers aliquots of transformation mixes from Well Plates to regional Bioassay trays and spreads the liquid across the agar so that, following incubation, distinct colonies are obtained such that those in each region correspond to the transformation mix from an individual well of the source Well Plate. The colonies can then be picked using the Regional Picking option in Picking.

QPix systems are strictly for the research use only and are not intended or recommended for the diagnosis of disease in humans or animals.

Before using the QPix systems, please refer to the appropriate Robot Manual for important setup, maintenance and safety information.

Preparation

QStreak uses the following robot accessories:

- Pipette Holder
- Well Plate Stacker
- Bioassay Tray Holder
- Streaking Head

Before preparing the robot for QStreak, 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 sterilize before setting up the bed for any routine, (see **General Maintenance** section in 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 Streaking Head (see **General Maintenance** section in Robot Manual).



Maintaining a Streaking Head

Cleaning the head can be a long process, but it is vital for good results. The head should be cleaned every time a Streaking 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 20% degreasing detergent.
- 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 overtighten.

Replacing 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, you should now be able to spot the problem pin.

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, or the QPEXpression wash bath).
- 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. 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 overtighten.**



Loading Work Objects

Depending on the plate configuration you are using either:

- Load the stacker(s) you will be using with the plates.
- Place the Plate Holder onto lane 1 of the Well Plate Stacker (Fig 1), push it as far to the right as possible and turn the locking key to lock it in position. Load the source 96-well plate on the Plate Holder with well A1 facing the front right of the machine (Fig 1).

Place the two destination Regional Bioassay Trays (48 Segment Trays) on the Bioassay Tray Holder, remove the lids and clamp them in position.

Place the pipette tip box in the Pipette Holder (Fig 1) and remove the lid. Fasten a plastic bag to the chute from the Pipette Dump to catch the used pipettes.

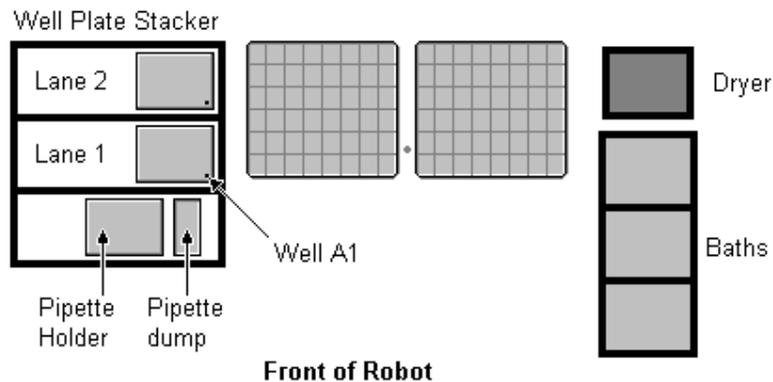


Figure 1: Well plates loaded showing position of well A1

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.

Manual input of barcodes without automatic reading is not available in this version of QStreak, however an option to input a barcode can be enabled if the automatic barcode fails to read the barcode.

For detailed information about recording barcodes see the section headed Barcodes later in this manual.

QStreak Software

Overview

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

You will then be prompted to create a new routine or load a previously saved routine. Any existing routines will be listed in the lower window.

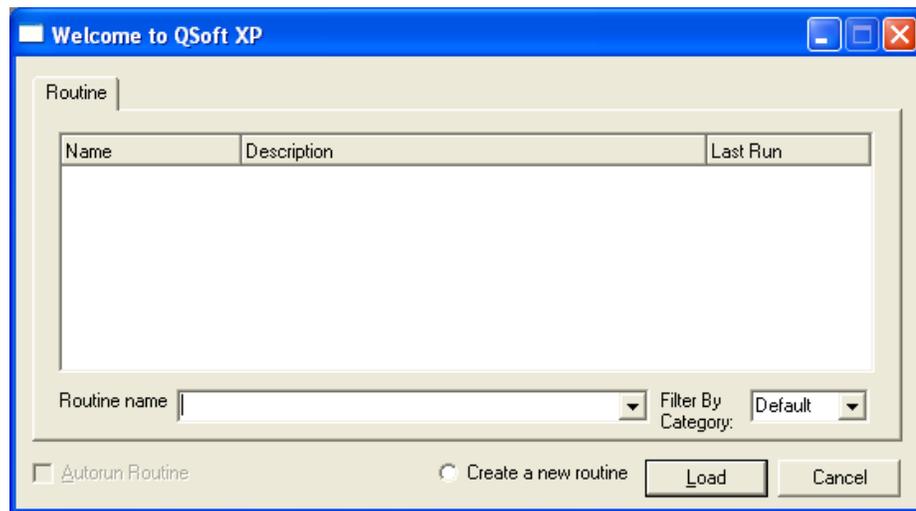


Figure 2: Routine prompt

Create a New Routine

Select this option then click OK. The default routine settings will be loaded, you can edit this routine then save it if required.

Load an Existing Routine

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

Autorun Routine

If this box is checked, when you click OK 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.

The screenshot shows a 'Sign on' dialog box with the following fields and controls:

- Run Number:** A numeric input field containing '2' with a '(Last Run No. shown)' label to its right.
- Operator:** A text input field containing 'Operator Name'.
- Group:** A text input field containing 'Run 1'.
- Set Number:** A text input field containing '1'.
- Replica Number:** A text input field containing '1'.
- Description:** A text area containing 'Run Number 1'.
- Checkboxes:** Two checkboxes at the bottom left: 'Show at Startup' and 'Show for Each Run', both currently unchecked.
- Buttons:** 'Cancel' and 'OK' buttons at the bottom right.

Figure 3: Sign on Dialog

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

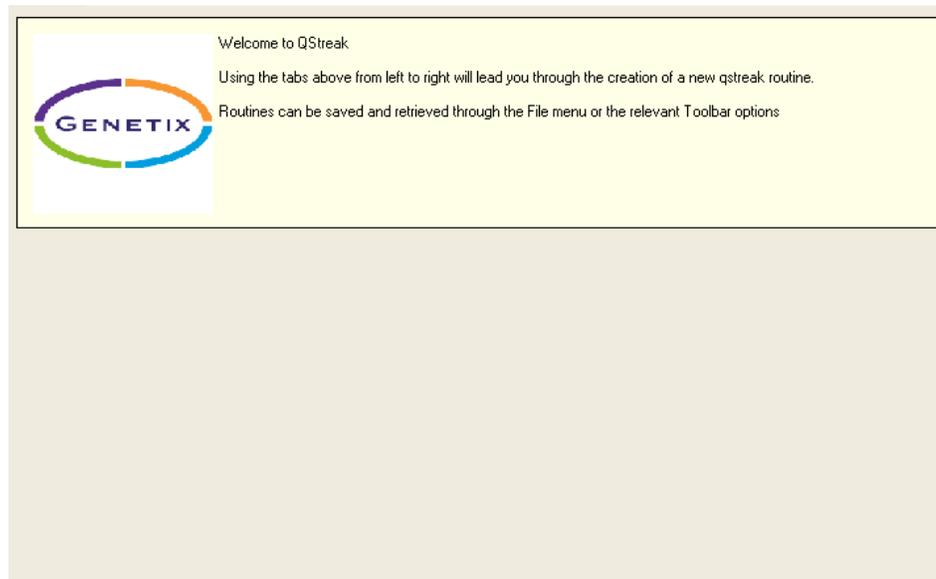


Figure 4: Welcome



The Menu Options

File Menu



Figure 5: File menu

Load Routine – Allows you to open a previously named routine.

New Routine – Allows you to create a new routine.

Close Routine – Will close the current routine.

Delete Routine – Will delete the current routine.

Save Routine – Saves the current routine with the same name.

Save Routine As – Will allow you to save the current routine with a new name.

Import Routine – Allows externally created routines to be used.

Export Routine – Permits you to save routines as XML files in a user-defined location. This enables the transfer of routines between robots.

Switch User – With the appropriate permissions, this menu item 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

User And Groups – With the appropriate permissions, this menu item displays the User and Groups functionality for assigning users to groups of privileges.

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



Sign on

Run Details

Run Number: 2 (Last Run No. shown)

Operator: Operator Name

Group: Run 1

Set Number: 1

Replica Number: 1

Description: Run Number 1

Show at Startup

Show for Each Run

Cancel OK

Figure 6: Sign on dialog

Exit – Closes the application



View Menu

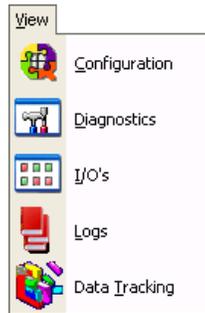


Figure 7: View menu

Configuration - 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.

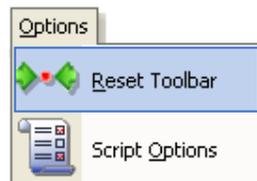
The procedure for configuring the robot is described in the robot manual.

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.

I/O'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



**Figure 8:
Options menu**

Reset Toolbar - You can rearrange the buttons on the toolbar if necessary, this option will set them back to the default order.

Script Options - Currently you can specify if you would like the 'View Script' feature enabled or disabled after generating a script.

The Toolbar

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

The buttons which appear will depend on the configuration of your particular robot.

If you allow the mouse to hover over any of the buttons, a yellow "tooltip" box appears to remind you of the button's

function.

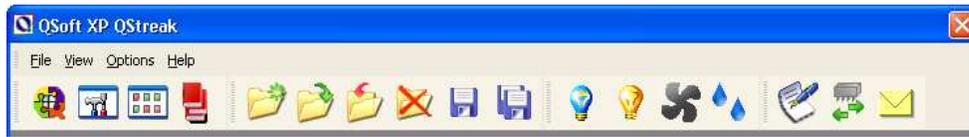


Figure 9: Toolbar

Descriptions for most of these are given in the section describing the menu options.

- UV Light
- White Light
- Fan
- Humidity

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

Change Head

Moves the actuator into an accessible position to allow you to change the head.

Configure 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.

Click this button to set messaging server preferences. For detailed information about setting up Messaging Server see Appendix A of this manual.

QStreak Setup

The QStreak 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

Welcome Description Head Source Destination Source and Destination Order Sterilise Data Tracking Barcodes Start

To start a replicating (stackers) routine either create a new routine or load a previously saved routine.

To run an existing routine - Load the routine and go straight to the Start tab.

You can also edit and delete previously saved routines.

To create a new routine - step through each of the tabs and then save the routine.

To edit an existing Routine - Load the routine, work through the tabs and make the required changes then save the routine or use Save As to save with a different name.

Routine

Description:

Lab Notes:

Category:

Figure 10: Description

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

The category box can be used to group routines together for easy retrieval.



Head



Figure 11: Head

Head - Allows you to choose between the different heads available. The standard QStreak run uses the STREAKING HEAD.

Source

Source Settings



Figure 12: Source Settings



Container - This refers to the Container that is to hold the source plates – i.e. Well plates that are to be aspirated from. Currently there is a single option for Source Container: STACKER DEST PLATE HOLDER.

Use Stacker – This should NOT be checked when using the Plate Holder to hold the source Well Plate.

Plate - This field allows you to define the type of source plate that is to be Aspirated from. These plates will be located in the container specified above. Note that only a 96-Well Plate should be selected.

Source Lane – Use this list to indicate which Stacker Lane is to be used for Source Plates.

Liquid Volume – Use this to indicate the amount of liquid you wish to dispense from the pipette.

Mix Volume – Use this to indicate the amount of liquid the pin should penetrate for mixing.

Mix Steps – Use this to indicate how many mix rotations are required by the pin into the liquid.

Source Data – This feature is disabled as it is non standard and is only available when using a 'Special Streaking Head' and not the regular 'Streaking Head'.

Total Source Wells – This is used combination with 'One To Many' to dictate the length of the run.

Note: Streaking Machines have two Stacker lanes. The Pipette Station is not a Stacker lane. The first Well plate lane is the first Stacker lane.

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



Destination

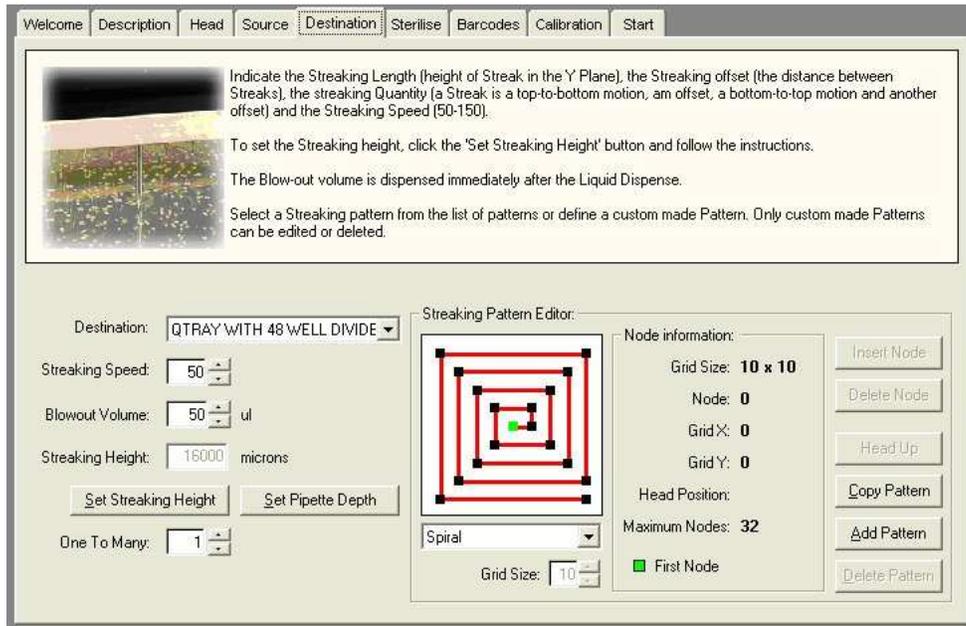


Figure 13: Destination

Note: The Destination Container does not need to be set. Streaking is always performed into a 48 Segment Bioassay Tray.

Note: The number of Destination Containers is dictated by the quantity of Source Plates. There are two 48 Segment Destination Trays per 96 well Plate.

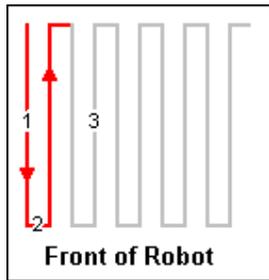


Figure 30: The Streaking Pattern

Streaking Speed – Refers to the speed of the Robot during Streaking. Minimum is 50.

Blow-out Volume – A volume of air that is aspirated prior to aspirating Liquid. This volume is automatically added to the Dispense Volume to ensure that all Liquid is dispensed from the Pipette. In a 'One To Many' run the blow-out volume is used by the last dispense being made from the pipette.

Streaking Height – This figure is the height in Microns that Streaking will occur at. This figure needs to be set before a run by clicking on the Set Streaking Height button. The following Dialogue will be displayed ...



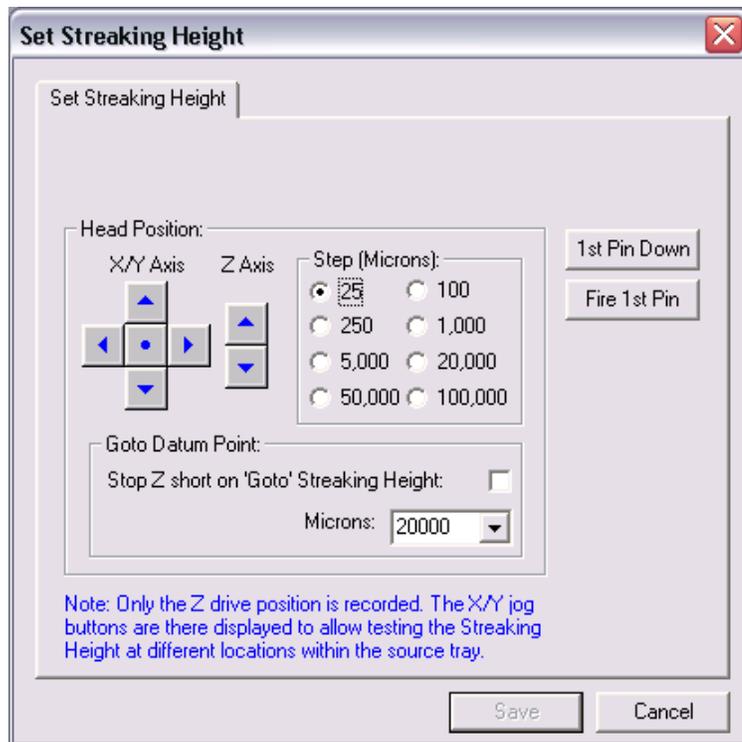


Figure 14: The Set Streaking Height Dialogue

Use the Head Position X/Y Arrows to move the Head over the 48 Segment Tray and click the 1st Pin Down button. Then use the Z Axis arrow buttons to move the head down until the Pin is touching the agar and just being pushed up into the Head. This allows for slight variations in the Agar levels within and between trays.

Click the Save button when complete. Only the Z position will be recorded.

Note: It is essential that the Agar in each Bioassay tray is level and equal in both Trays. Great care should be taken when determining the setting of the pipette depth for the source and destination plates. See 'PipetteDepth' property for these items.

One To Many – This figure is the number of regions to dispense to from an aspiration of a single well. The values must be 1 (default), 2 or 4. Setting the value to 4 will require 24 wells (instead of 96) to be filled with liquid (to populate 96 regions, 2 X 48 Segment Trays). Setting the value to 4 means the wells should occupy the first 3 rows of the plate (3 X 8). The 'Liquid Volume' property should be set to a value which is a multiple of the 'One To Many' figure. The 'Blowout Volume' property should be a high enough value to clear the pipette of any extra liquid. This property is used when the last region is to be dispensed to from a well. The property 'PausePerDispense' should be set to an appropriate value to enable the liquid enough time to dispense from the pipette for each region.

Set Pipette Depth – The pipette depth and the object to pipette into can be selected by clicking 'Set Pipette Depth'.



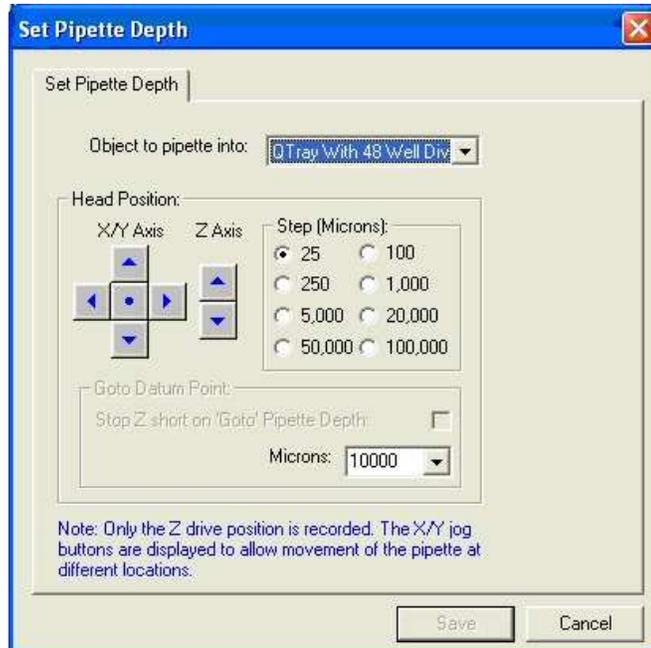


Figure 15: The Set Pipette Depth Dialogue

Select the object to pipette into from the pull down menu. Ensure that you have a pipette placed into the pipette tray at position A1 and then click on the centre button to get the pipette ready for positioning over the selected object.

Use the Head Position X/Y Arrows to move the Head over the object to pipette into and click the 1st Pin Down button. Then use the Z Axis arrow buttons to adjust the pipette depth.

Click the Save button when complete. Only the Z position will be recorded.

Sterilize

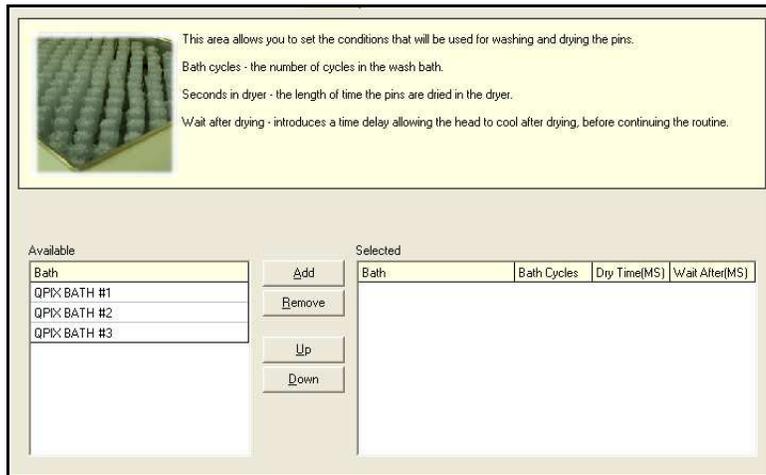


Figure 16: Sterilize

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 grayed 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:

$$3 \text{ Seconds} + (1.5 \times \text{Dry Time})$$

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.

Barcodes

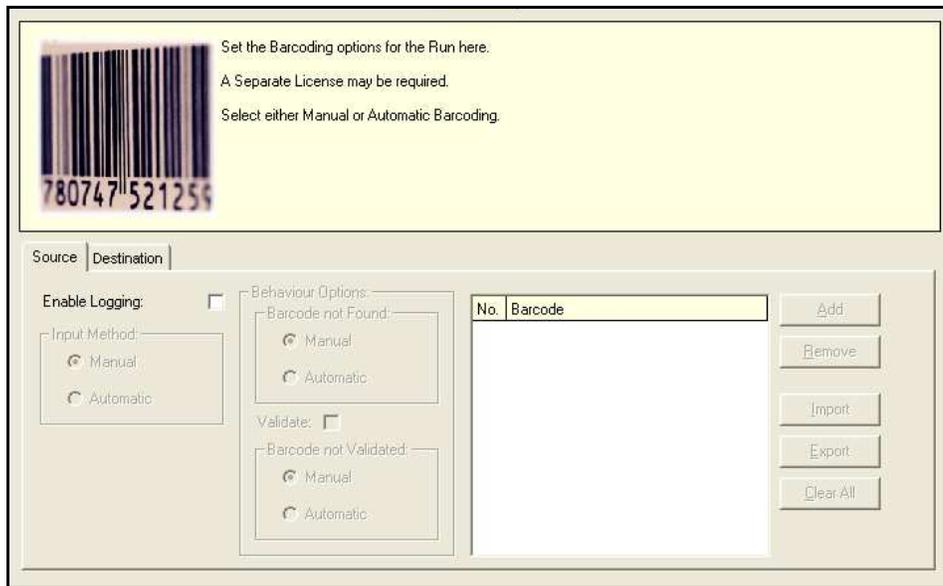


Figure 17: Barcodes



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.

Barcodes 39 and 128 are compatible with the barcode readers.

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-YMMDDHHNNSs-X where ss is milliseconds and YY is a two character ascii representation of the year.

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 barcode reader 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 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 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 give you the opportunity to verify the order of the plates being processed before you decide whether to continue 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 you will be presented with a set of options - Click OK to continue

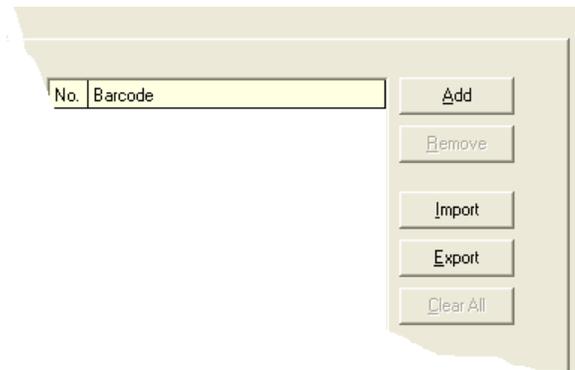


Figure 18: Barcode tab command buttons

Add – Creates a new row for entry of the next barcode – used when manually entering 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.

Disable Barcoding for source or destination

If Data tracking is selected, Barcoding is automatically enabled. You can disable barcoding for a given container – for example if you do not wish to barcode source bioassay trays – by changing the **BarcodeReaderType** property.

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**

Calibrate



Welcome Description Head Source Destination Sterilise Barcodes **Calibration** Start

Indicate a Destination Plate type and the Destination Stacker Lane.
The Destination plate should have the same Well count as the select Source Plate.
Click the Calibrate button to continue. Each Well of the Source plate will be aspirated (with the Source Aspirate Volume) and Dispensed into the corresponding Destination Wells.

Destination Plate: GREINER/COSTAR 96 WELL PLAT Use Stacker

Destination Lane: 2

Calibrate

Figure 19: Calibration

On the Calibration Tab, you can run a basic calibration routine in order to check for correct Aspirate and Dispense Volumes. The Calibration routine will Aspirate a given volume of Liquid from each well of a Well Plate to the corresponding well of another Well Plate (on different stacks), replacing Pipettes after each Dispense.

The Aspirate Volume and Source Stacker Lane from the Source Tab will be used during the calibration run and the Dispense Volume and blowout Volume from the Destination Tab will be used.

Destination Plate – The type of plate to be used for Dispensing into.

Destination Lane – The Lane number of the Destination plate. The Destination Stacker Lane should be different from the Source Stacker Lane or Calibration will not run.

Use Stacker – Check the Use Stacker box if the Stacker is to be used.

When correctly set up, click the Calibrate button to continue. The Calibration run can be cancelled at any stage by clicking the Cancel button on the Calibration progress Dialogue.

Note: The Source Well plate, Destination Well plate and Pipette Box must have the same amount of Wells or Calibration will not run.

Start

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



Figure 20: Start

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

Leave Light Table on - This toggles the light table on and off. The on or off status of the light is recorded with the routine.

Enable Data Tracking - This toggles the data tracking function on and off. The status is recorded with the routine.

Start - Click this button to start the Streaking routine.

Useful Properties

Pipette Depth – This is the aspirate/dispense well depth. The property can be found from selecting the Configuration option and a plate you are using, for instance - select the plate, e.g. 'Genetix 96 F Well Plate X6010' and scroll down for the 'PipetteDepth' option.

Pause Per Dispense ULMS – This is MS pause for each UL dispensed from the Pipette. The property can be found from selecting the Configuration option and the Pipette Actuator you are using and scroll down for the 'PausePerDispenseULMS' option.

Offset Streak Position X (and Y) – These are the offsets from the corner of the region to start streaking. The properties can be found from selecting the Configuration option and the 48 Segment Tray, scroll down for the options. The values can be adjusted here.

Running a QStreak Routine

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

Click the **Start** button at the bottom of the Start tab.

The following screen appears:

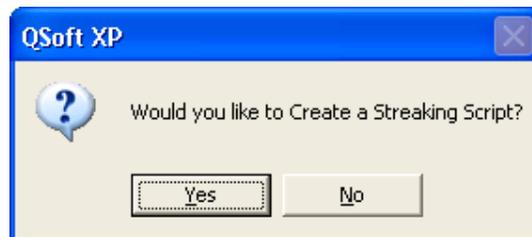


Figure 21: Create Script

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

If you continue, a Script will be created and the following screen will appear to indicate the creation progress. Clicking **Cancel** on this progress dialogue will give you the option of cancelling Script creation.

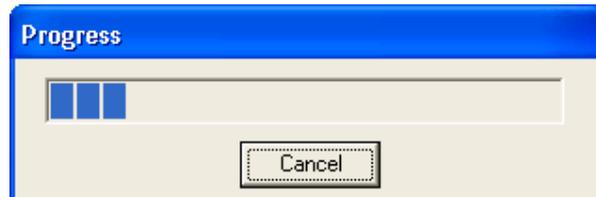


Figure 22: Script creation progress

You will then be prompted with the total quantity of Destination Trays required for the Run. This will typically be twice the quantity of 96 well Source plates.

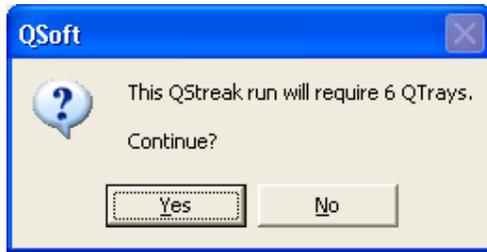


Figure 23: QTrays Required

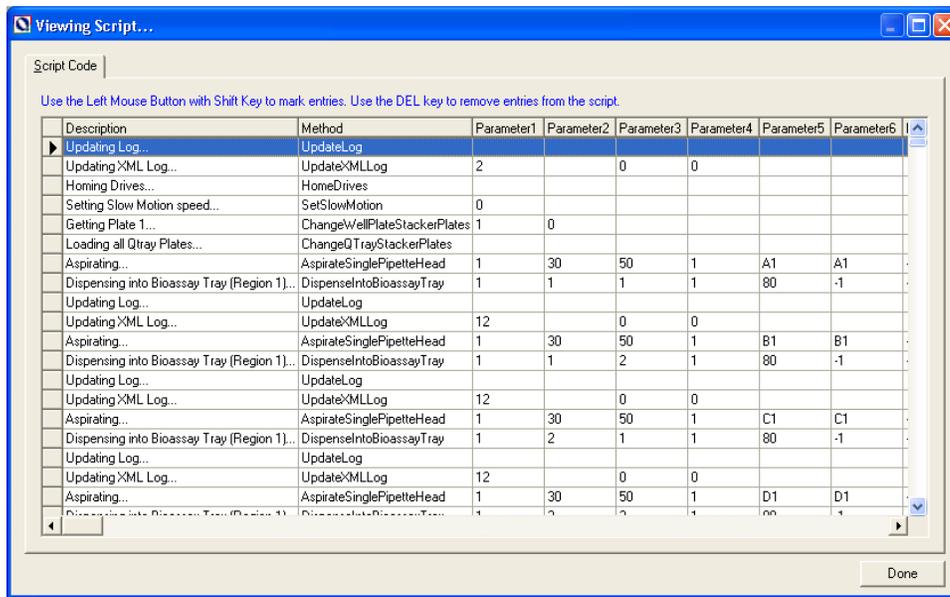


Figure 24: Example script code

Click **Done** to continue. When necessary, you will be prompted to replace the Pipette Holder with a new set of Pipettes:

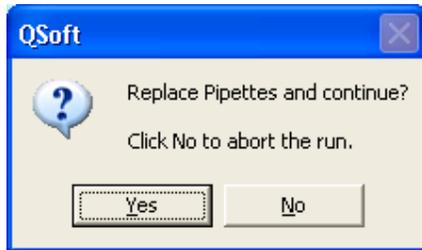


Figure 25: Replace Pipettes prompt

Depending on barcode options if logging is enabled and if you are not using a stacker you may see an additional prompt, click **OK**.



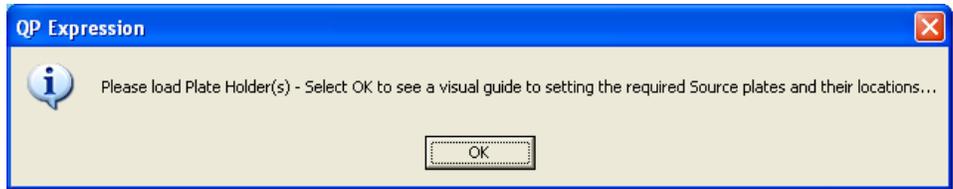


Figure 26: Please Load Plate Holder(s) prompt

After clicking **OK** the following dialog is displayed. This allows a manual barcode to be entered if required or shows the plate in place.

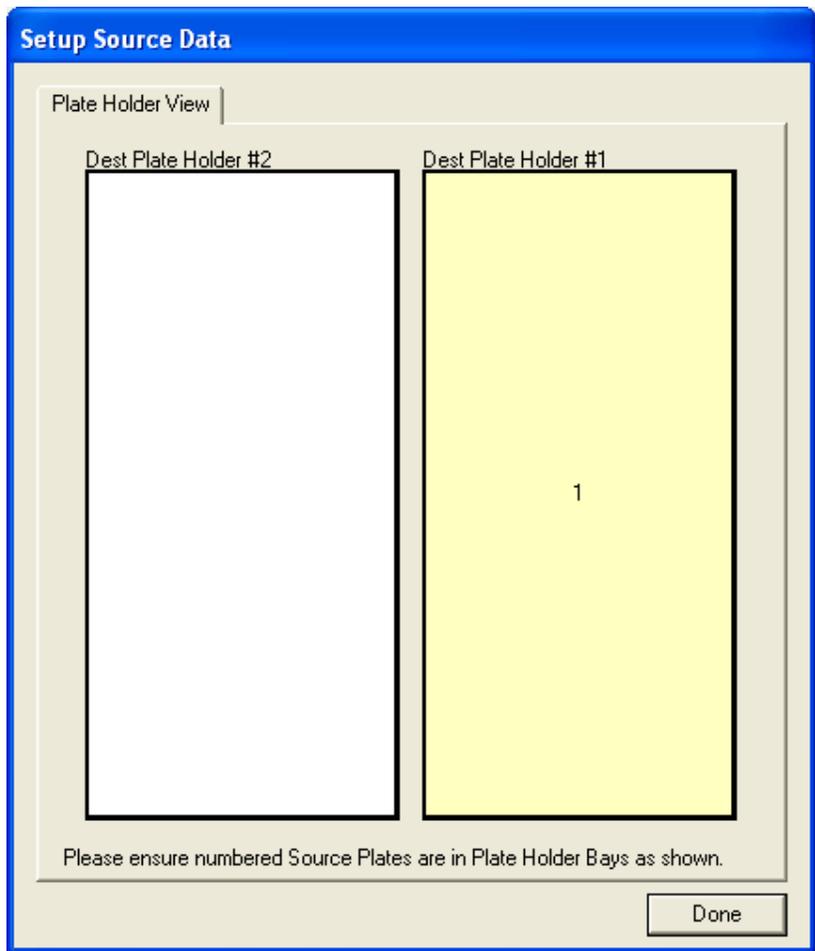


Figure 27: Manual Source Plate Dialog



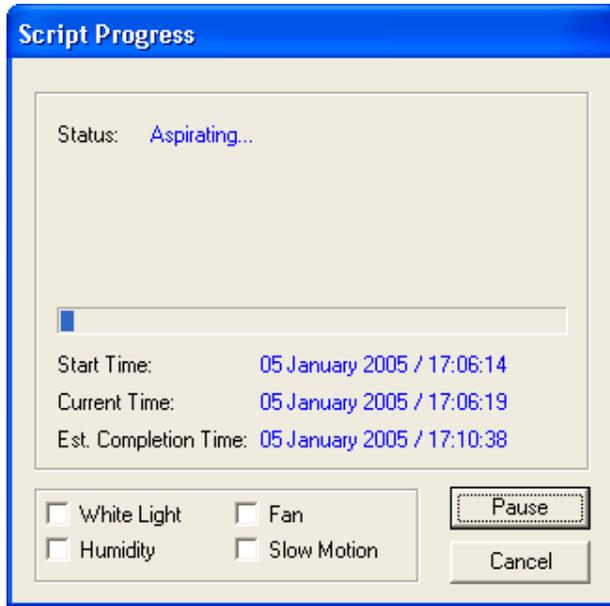


Figure 28: Script Progress

If at any time you need to stop the run, the **Pause** button can be clicked.

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



Figure 29: Script complete



Biology Guide

Preparing Media

Luria-Bertani medium (LB) – per liter

To 1 liter of de-ionized H₂O 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 deionized H₂O). Sterilize by autoclaving at 121°C for 15 minutes.

LB Agar – per liter

To 1 liter of de-ionized H₂O 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 sterilised, 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 μ m 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 liter 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 liter of medium.



Appendix A

Mapping Regions

For each regional QTray the order and orientation of the regions relative to its position on the bed of the QPEXpression is shown in Fig 35.

Figure 30: QTray regions layout

The layout of the regions in a regional QTray for tray 1 (A) and tray 2 (B). Note that the first region in each tray is to the back right of the machine.

BACK

8	7	6	5	4	3	2	1
16	15	14	13	12	11	10	9
24	23	22	21	20	19	18	17
32	31	30	29	28	27	26	25
40	39	38	37	36	35	34	33
48	47	46	45	44	43	42	41

FRONT



Figure 37 (A)

BACK

56	55	54	53	52	51	50	49
64	63	62	61	60	59	58	57
72	71	70	69	68	67	66	65
80	79	78	77	76	75	74	73
88	87	86	85	84	83	82	81
96	95	94	93	92	91	90	89

FRONT

Figure 37 (B)

When running QStreak two regional QTrays are placed on the bed of the machine, as in Fig 38.

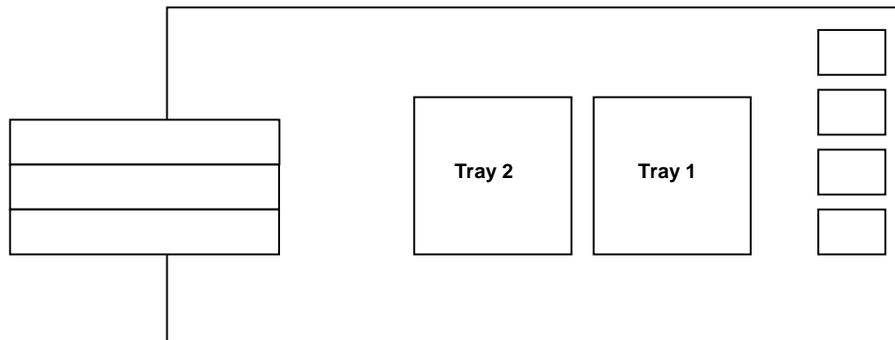


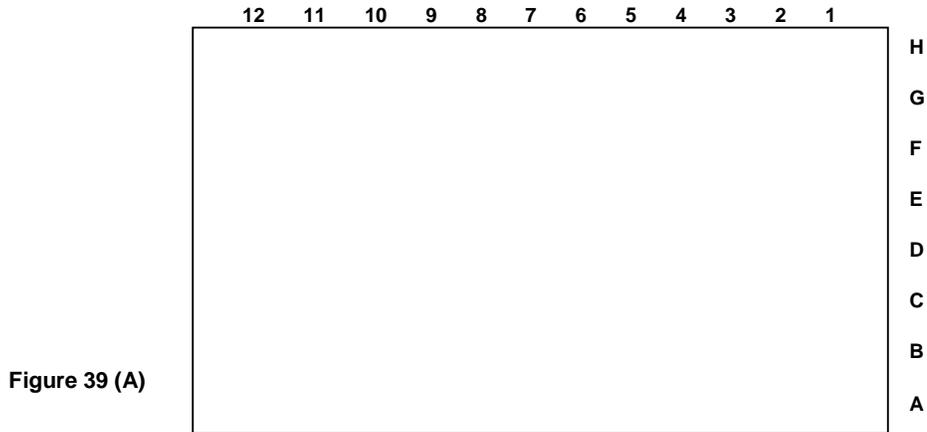
Figure 31: The positions of the regional QTrays on the QPEXpression.



QStreak pipettes cultures from each of the wells in an 8 well column of a 96-well plate to 8 regions in the regional QTray then spreads the regions using the 8 pin spreading head. Hence, each tray is divided into 6 fields of 8 regions in each (Fig 37.) and the field number corresponds to the source well's column co-ordinate and the region letter corresponds to the source well's row co-ordinate in the source well plate.

Figure 32: Layout of wells

The layout of wells in a 96-well plate (A) and the mapping of wells to regions for tray 1 (B) and tray 2 (C). Note that QStreak starts in the front left hand region and finishes in the back right hand region of each tray.



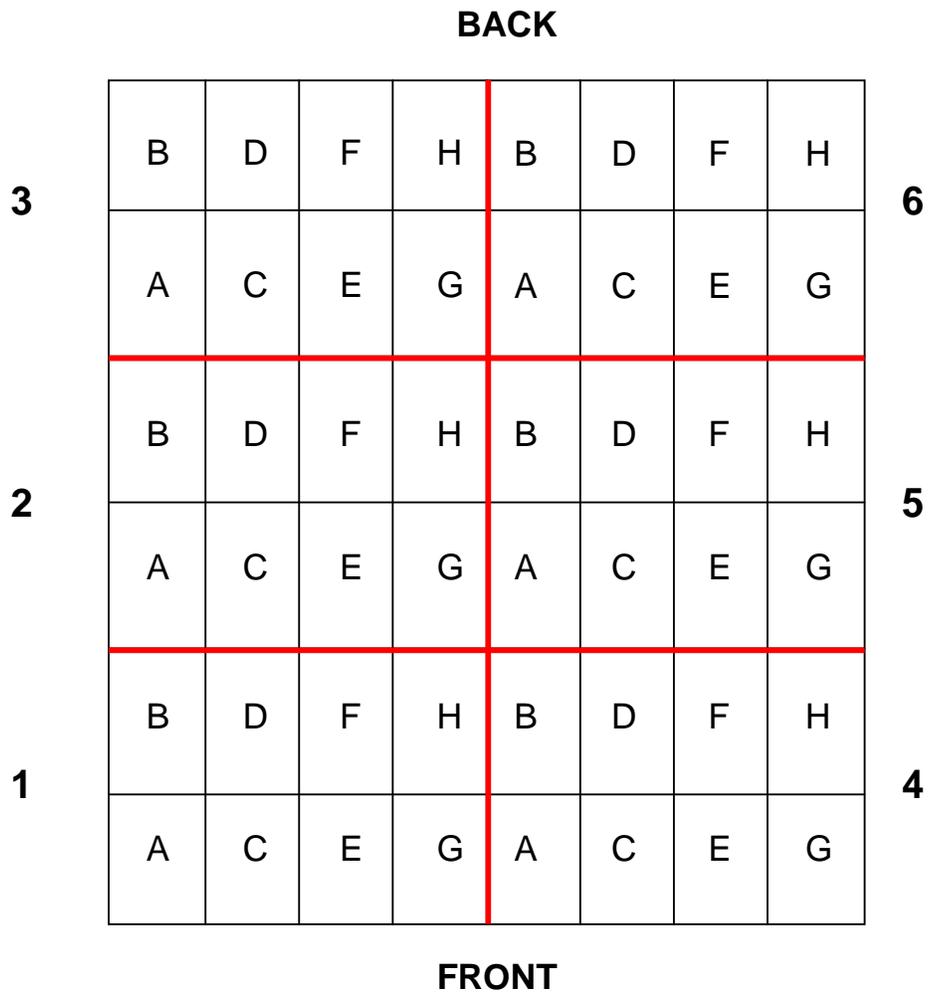


Figure 39 (B)



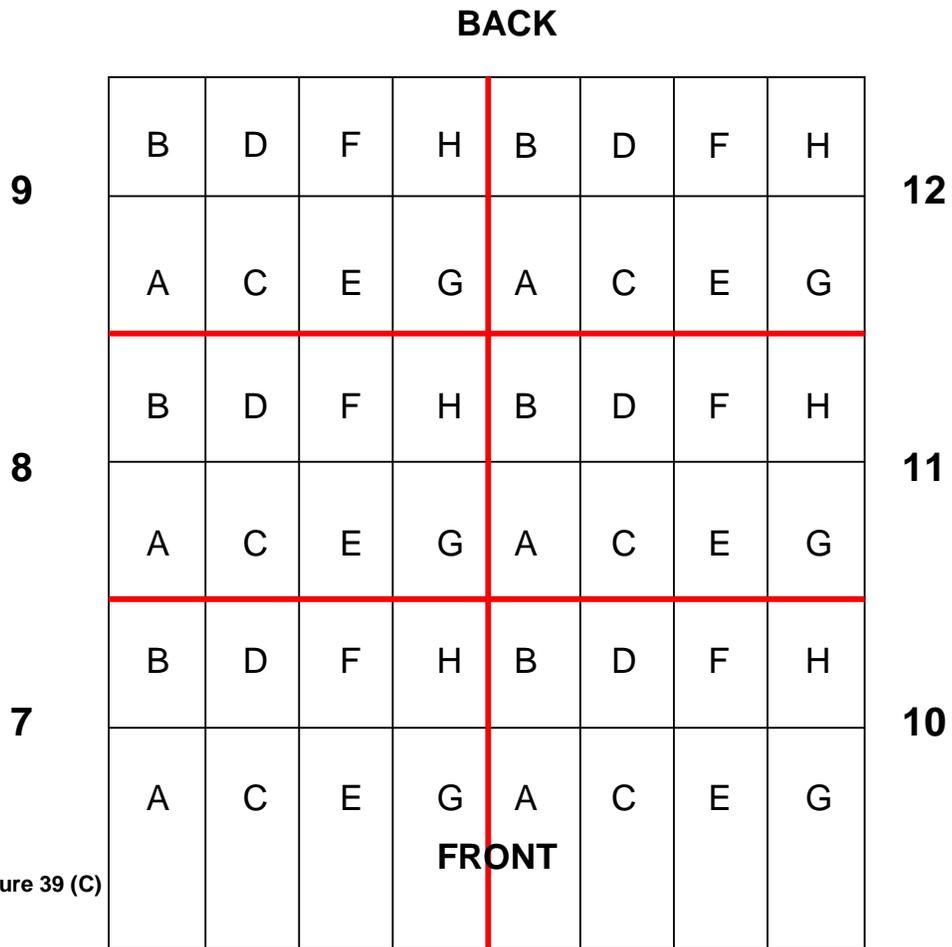


Figure 39 (C)



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

Blue/White

Blue White selection protocol.

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 the **QPix** and 4 on the **QBot** and **QArray**. 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.

Filter Block

Solid blocks onto which gridding membranes are placed (15 per **QBot**, 2 per **QPix**).

Grabber

Set of jaws on **QBot** actuator to pick up microplates.



Gridding head

Head used for gridding and replicating. Available in 96 pin or 384 pin formats, either sprung or gravity.

Guide Spot

A reference spot created by the user, in order to make grids easier to read.

I/O

Inputs / Outputs.

IPTG

Isopropyl-thio-D-galactoside.

LB

Luria-Bertani Medium.

MADGE

Microplate Array Diagonal Gel Electrophoresis.

Multi-spot pin

Microarray pin which is loaded with a volume of sample which is then dispensed in aliquots.

Phage

Bacteriophage.

Picking Tray

See Bioassay Tray Holder.

Plate Hotels

Holders for microplates located at the front of the **QBot**. Available in 2 formats - standard Genetix hold 36 plates each, and non-Genetix hold 24 plates each.

QSoft.DLL

ActiveX software component housing all the functionality of robot software.

Rearranging

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 Sulfate.

SSC

Sodium Chloride/Sodium Citrate buffer.

X Drive

Axis running from back to front of the **QBot** bed or right to left on the **QPix**.

Y Drive

Axis running from left to right across the **QBot** bed or back to front on the **QPix**.

Z Drive

Axis running vertically on the Robot bed.



Index

A

ActiveX	42
Actuator	15, 42
Airline.....	8
Aliquots.....	42
Antibiotic Preparation.....	32

B

Barcode	23, 24
Barcode reader	23
Barcodes	9, 23, 25
Base plate.....	7
Bath	8, 22
Bed	7, 35, 41, 43
Bioassay Tray Preparation	32
Bioassay trays	41

C

Cleaning	8
Colony Preparation.....	33
Configuration	14
Configuring the robot	14
Container	8, 18

D

Destination.....	23
Destination plate holder	41
Determination of Titre	32
Diagnostics	14
Diagnostics screen	14
Diagnostics Screen.....	14
Distilled water	8
Drives	14

E

Export	25
--------------	----

F

File Menu.....	12
Filter block	41
Fitting the head.....	7

G

Galactoside.....	42
Genetix	14, 42
Gridding.....	42
Guide spot	42

H

Head.....	7, 8, 9, 15, 22, 42
-----------	---------------------

I

I/O.....	14
Import	25

L

Licence	23
---------------	----

Log barcodes.....	23
Luria-Bertani.....	42

M

Maintenance.....	7
Menu	15
MicroArraying	7, 15
Microplates	41, 42
Monitor	15

O

Options	14, 24
Options Menu	14

P

Password.....	14
Picking.....	8
Picking Blue/White Colonies.....	34
Picking head.....	9, 17, 43
Picking Phage	33
Pin	8, 42
Pin(s).....	8
Pipette	8
Preparing Media	31
Properties	41

Q

QArray	41
QPix	8, 41, 43
QSoft	10, 14, 24, 42
QSoft.dll.....	42
QTray	41

R

Remove	8, 25
Replacing Pins	9
Replicating	
Preparation.....	7
Run.....	26
Run.....	9, 13, 15, 24, 26, 30

S

Scanner	24
Screen.....	10, 11, 14, 23
Setup	10, 23
Setup screen	23
Sign on	11, 13
Sonic cleansing	8
Sonicate	8
Sonicator	8
Source plates	18

T

Toolbar	14, 15
---------------	--------

V

View	14
View Menu.....	14



W

Wash bath 8, 22
White 15, 41

Workbed7, 14



Contact Details

Corporate Headquarters

Genetix Ltd

Queensway, New Milton
Hampshire BH25 5NN, UK

Tel: +44 (0) 1425 624 600

Fax: +44 (0) 1425 624 700

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

All third party trademarks are the property of their respective owners.

Copyright © 2010 Genetix Ltd

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form by any means, electronic, mechanical, by photocopying, recording, or otherwise, without the prior written permission of Genetix Ltd.

Information furnished by Genetix Ltd is believed to be accurate and reliable; however, no responsibility is assumed by Genetix Ltd, for its use; nor for any infringements of patents or other rights of third parties which may result from its use. No license is granted by implication or otherwise under any patent rights of Genetix Ltd.

