Genetix

Gridding

Application Guide

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Gridding

What is Gridding?

Gridding is the arraying of colonies or DNA (e.g. PCR products) onto nylon filters ready for screening by hybridization. The accurate spot placement achievable by a robotic system allows much higher density arrays to be produced than any manual spotting method. In addition the accurate placement of the grids means that the images produced post-hybridization are amenable to automated analysis by image analysis software. These software packages may find it hard to cope with inaccurately positioned grids of spots and much more user intervention is then required during the analysis.

Gridding can be performed on any of the **QPix systems.** The **QPix2** can take up to 2 filters and 15 source plates at a time. The **QPix2 XT** and can take up to 6 filters and the number of source plates is dependent on the stacker configuration. The samples to be arrayed (e.g. PCR products, bacterial cultures/ phage suspensions from picking) are contained in 96 or 384 well plates and the gridding head has 96 or 384 steel pins, which pick up sample from the wells and transfer it to the filters. Each pin prints a grid of spots to achieve the necessary density of spotting.

QPix systems are strictly for 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

Warning - If you are using QTrays it is advisable to check the datum points before commencing Gridding. Also - if alternating between using QTrays and Filter blocks then re-datuming is required before each run. For details on datuming refer to the "Configuration Guide" in the main Robot Manual.

Prior to carrying out a Gridding run it is necessary to ensure that:-

- The robot has been cleaned and all loose objects removed from the bed.
- The correct gridding head has been fitted.
- Source plates and filters on filter blocks have been prepared.
- The wash bath has been sterilised and contains ethanol.
- The working volume of the machine has been UV sterilized before setting up the bed for any routine (see **Initial Operation** section in the Robot Manual).

Fitting the Head

Important Note - Before using a gravity gridding head for the first time, ensure that the 4 transit screws are removed.

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 Robot Manual).

Note: If any 96 well plates are to be used a 96-pin head must be fitted. If 384 well plates are used as the source, a 96-pin head cannot be used.

Maintaining a Gridding Head

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

- Use an Allen key to unscrew the 4 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 20 minutes in a degreasing detergent.
- Remove the pins, plate and springs from the sonicator and rinse thoroughly in distilled water.
- Quickly blow through the plate with an airline and dry thoroughly, along with the pins and springs.
- Insert the springs into the holes of the body, followed by the pins (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 4 screws.

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 now be clearly identifiable.

Remove and replace the damaged pin as follows:

- Use an Allen key to unscrew the 4 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 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 over tighten.

Loading Source Plates

The source plates may contain the colony libraries that are to be gridded onto the filter membranes; they may also contain just DNA, for example PCR products.

QPix2

Fitting Plate Holders - Hold the plate holder by the handles and push the notched end towards the back of the **QPix2** until the notch is seated against the location pin. Slot the hole at the front of the plate holder over the front location pin and make sure that the plate holder is resting on the level adjustors (these are preset set when the **QPix2** is installed and should not need to be re-adjusted).

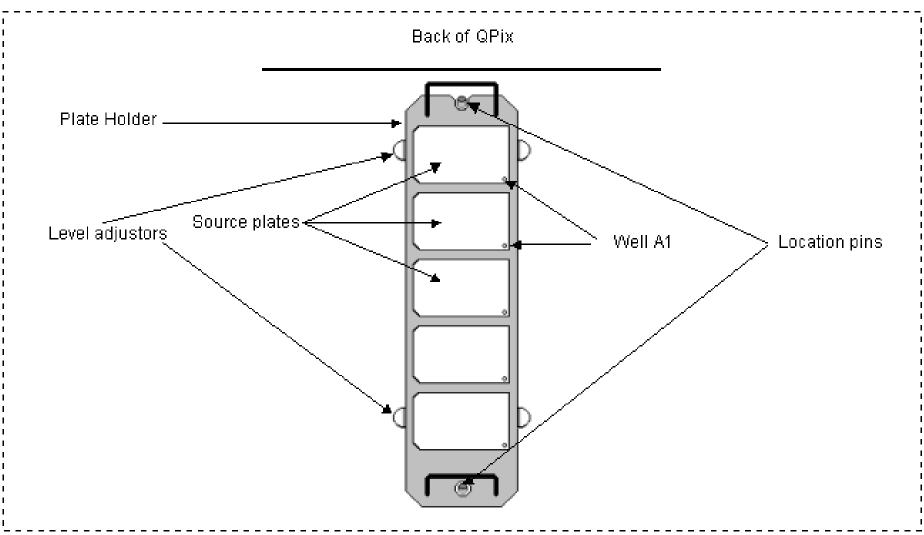

Figure 1: QPix2 Plate Holder

Plates should be inserted into the carrier 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.

QPix2 XT

The QPix2 XT uses a stacker system. The well plate stacker usually has 2 cassettes which can each accommodate up to 70 well plates. The destination plates are put into the cassettes with lids on and well A1 facing front-right.

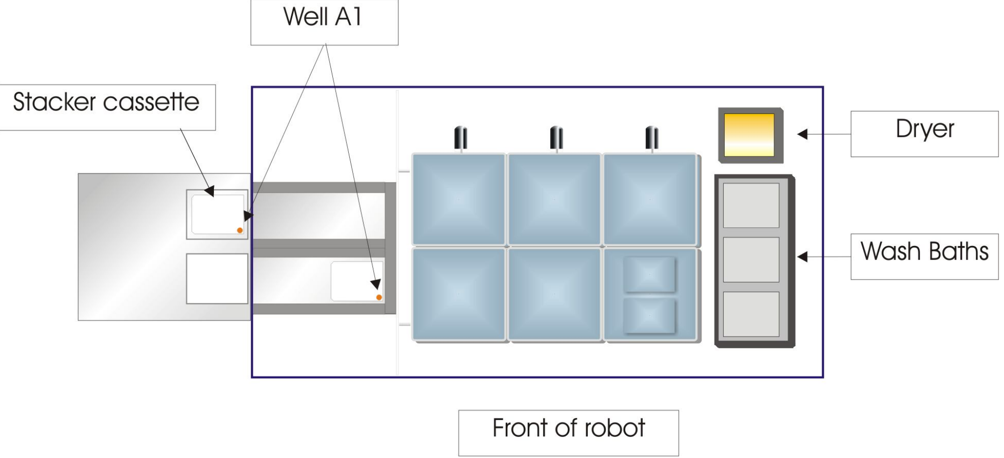

Figure 2: QPix2 XT Bed Layout – Plates in Stacker Cassettes

Loading Filter Blocks

Prepare the required number of filters off station. It is normal practice to lay one or two sheets of wetted filter paper, e.g. Whatman 3M, on to the filter block before placing the filter membrane (or other substrate) on top. Ensure that the membrane is correctly centered on the filter block and is lying flat (no air bubbles - if using wetted filters, and no creasing - if using taped filters).

QPix2

The Gridding software allows you to work with 1 or 2 filter membranes. The filter blocks are fitted on to the gridding table and held in place by the filter clamps and locating posts.

The gridding table is fitted on to the **QPix2** bed to the left of the wash station and on top of the light table. To fit the gridding table, undo the black thumbscrews (one at the front and one at the back of the **QPix2**) and fit the gridding table so that the screw holes sit over the posts. Replace the black thumbscrews and tighten.

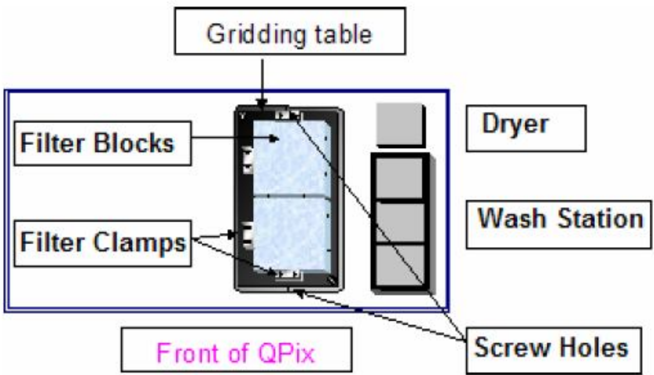

Figure 3: QPix2 Bed Layout

QPix2 XT

The Gridding software allows you to work with up to 6 standard (22x22cm) or 12 small (8x12cm) filter membranes.

The blanking plate is fitted on to the **QPix2 XT** bed to the left of the wash station and on top of the light table.

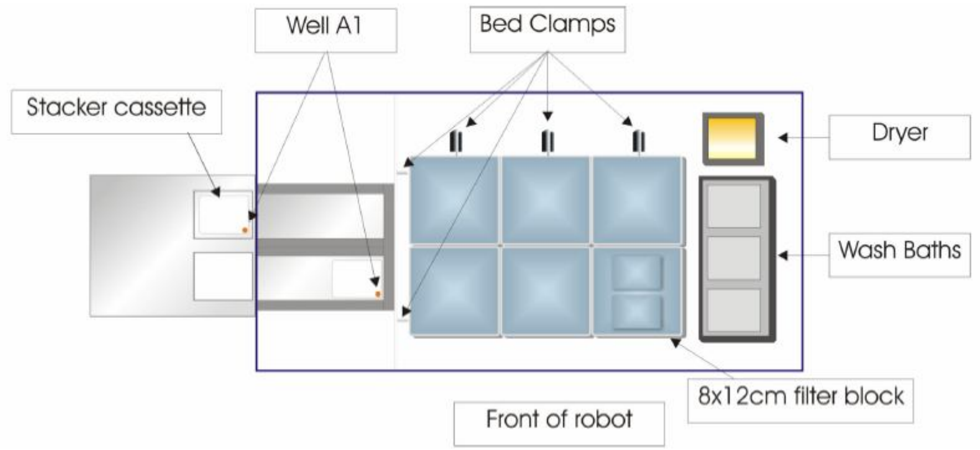

Figure 4: QPix2 XT Bed Layout

The filter blocks are fitted on to the gridding table and held in place by the bed filter clamps. Once all the blocks are in place, the bed clamps are activated by clicking the button on the

toolbar in the Gridding software.

If you are using the 8x12cm membranes you must ensure that they are placed so that the orientation matches the orientation of the head – see Figure 4 above.

Loading QTrays

It is also possible to grid directly on to bioassay trays with the robot.

Prepare the required number of QTrays off station. Ensure that the membrane is correctly centered on the QTray and is lying flat (no air bubbles).

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.

When all the trays have been loaded, check by eye that all of them are properly aligned and have not lifted off the table.

QPix2

The 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.

QPix2 XT

The bioassay tray holder accepts two trays which are held in place by 4 locating pins and adjustable corner brackets. The bracket should be tightened just enough to stop the QTray moving around.

Recording Barcodes

Barcodes can be recorded to the log file for the source plates. To enable barcodes to be recorded, the appropriate boxes must be checked in the main setup screen (see Barcodes on page 22). The barcodes are recorded during the program run.

If you are using a **QPix2 XT** with a barcode reader, source plate barcodes are read automatically.

Otherwise:

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

Gridding Software

Overview

QSoft is the software platform on which the Gridding application is built.

Double click on the QSoft Gridding icon on the desktop, the Gridding 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.

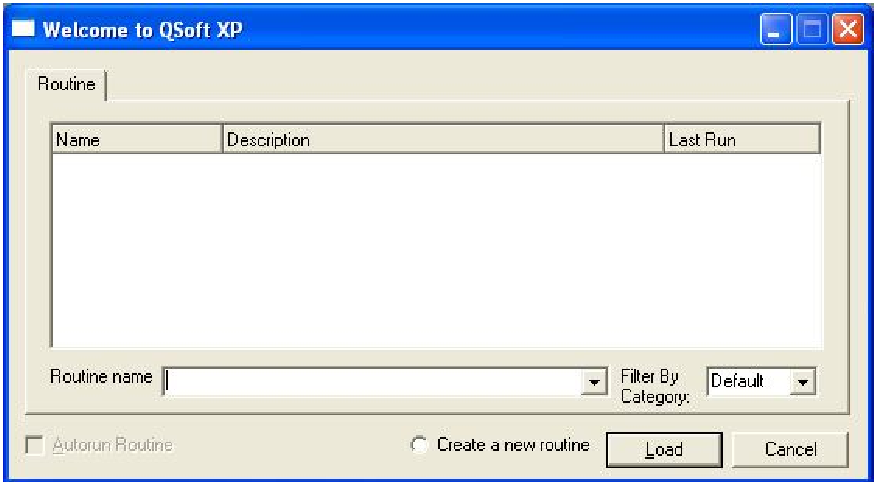

Figure 5: Welcome Prompt

Create a New Routine

Select this option then click OK. The default routine settings will load, 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.

Sign On

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

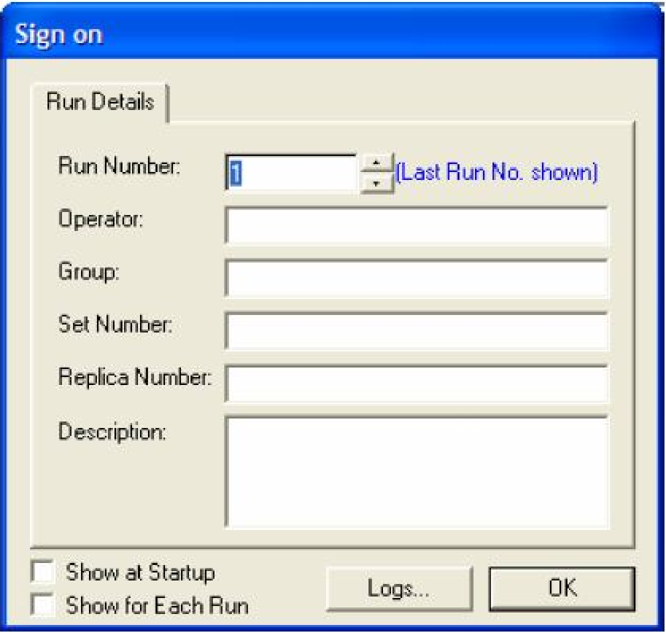

Figure 6: Sign On Dialog

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

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.

Gridding Setup

The Gridding setup screen is split into tabbed dialogs. Each tab contains user 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

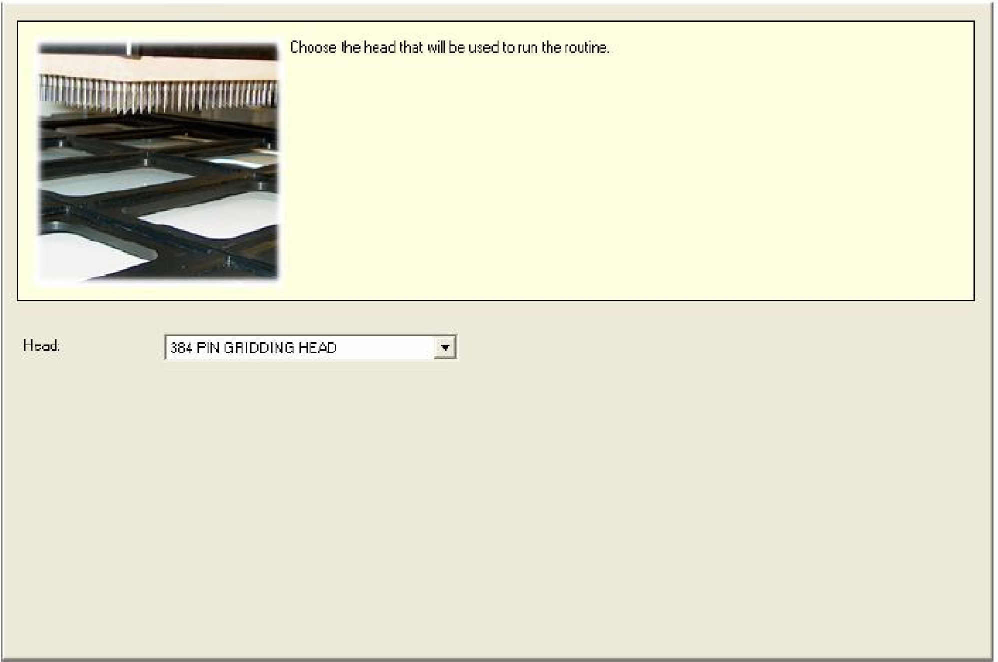

Figure 7: Head

This section is used to set the head that is to be used to carry out the gridding routine.

Head – This allows the type of head used to be specified.

Source

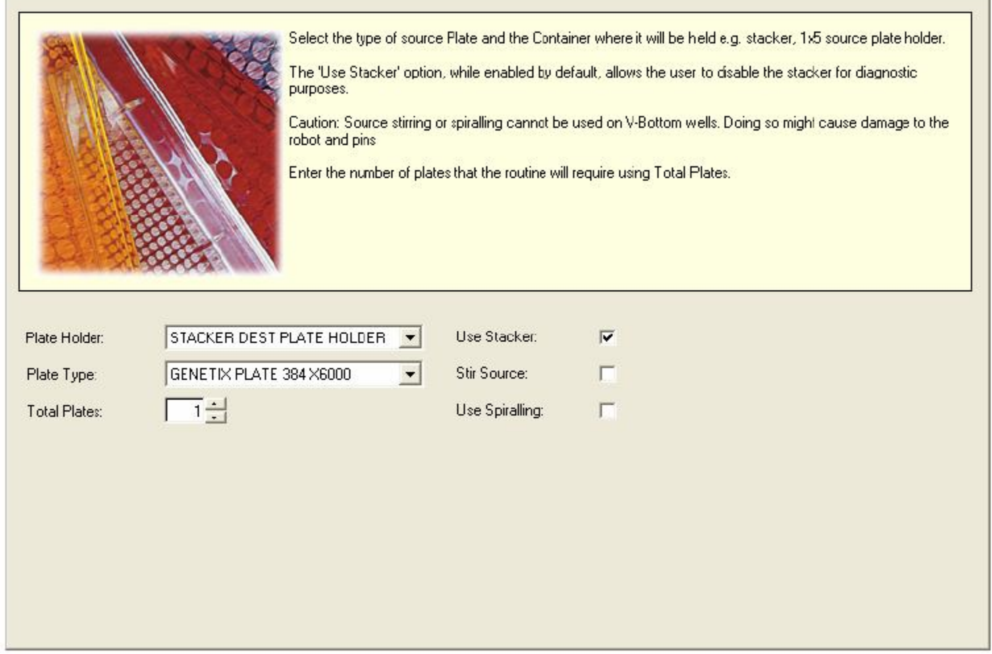

Figure 8: Source

Plate Holder – Depicts where the source plates are to be held e.g. 1x5 source plate holder.
Plate Type – Depicts which source plates are to be used. All plates whose object properties (under configuration) have been set up will be displayed here.

Total Plates – Specifies the maximum number of plates to be used during the run. This is used to restrict the number when it is less than the required amount for the pattern size chosen and will also serve as a check that the pattern created has enough spot positions for the number of samples.

Use Stacker – Allows the stacker to be disable for diagnostics purposes.

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.

Note: If **Stir Source** is selected then **Use spiraling** will be unavailable.

Use Spiraling – This allows a rotational movement to be set when the gridding head enters the source plate. The rotational movement is set for the particular head (object) in the Configuration screen under defined objects.

Filter Design

This group of fields is used to set the actual configuration of the grids that are to be produced on a given number of filters.

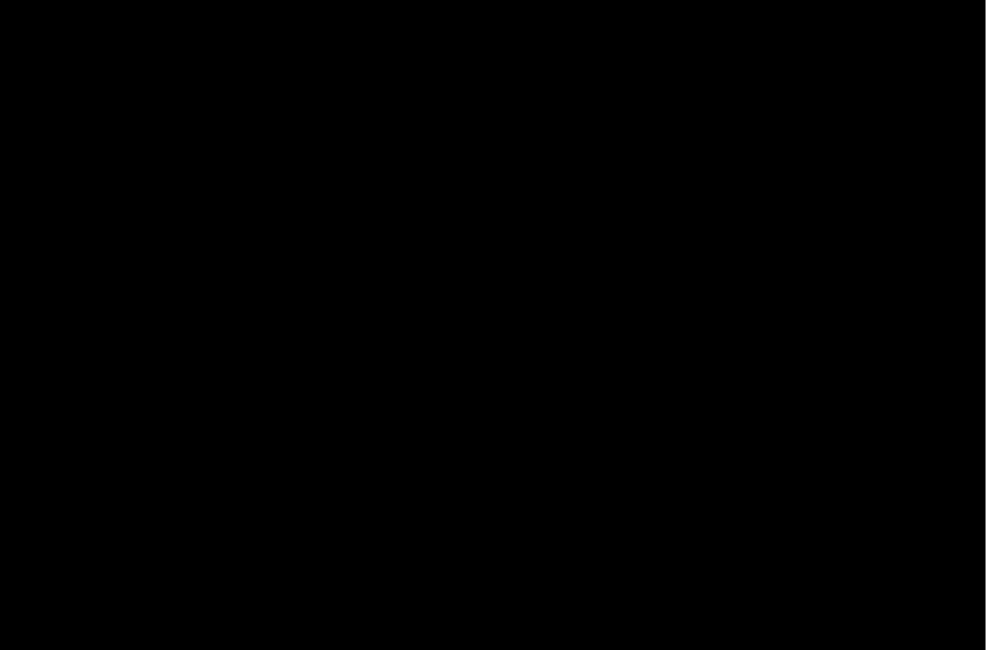

Figure 9: Filter Design

Filter - This provides the option to choose whether the filters are to be put down wetted on Filter Blocks or whether they are they to be put down in QTrays on Agar (note – see "Preparation" on page 5). If QTrays are to be used it is essential to ensure that the holding pegs are inserted on the bed in order to hold them in the correct position. Thus the options here are:

- Filter block
- QTray

Arraying by:

Field Number – This configures the robot to print all spots in a field before moving on to print in the next field (This is used when there is a fixed number of source plates – insufficient to fill every field in the filter. It will ensure that all spots are kept together). If there are any identical fields then all identical fields are completed before moving on to the next unique field.

Spot Position – This configures the robot to print the first spot in each field first, before returning to print the second spot in each field.

Field Layout - This allows required the field pattern to be chosen. The mouse can be used to drag a field and drop it into the required position.

More than one copy of a field in a substrate can be produced, right up to making all fields identical. For example, if quadruplicate spots in a grid are required, then use a duplicate spotting pattern and two identical fields.

Pattern - Click with the mouse on the pattern display to change the current pattern. The pattern can be symmetrical e.g. 2x2, 3x3, 4x4, 5x5 up to 75x75, depending on the density required. Asymmetric patterns can also be created e.g. 3x4, 5x3. The density achievable depends on the spot size printed by the pins being used.

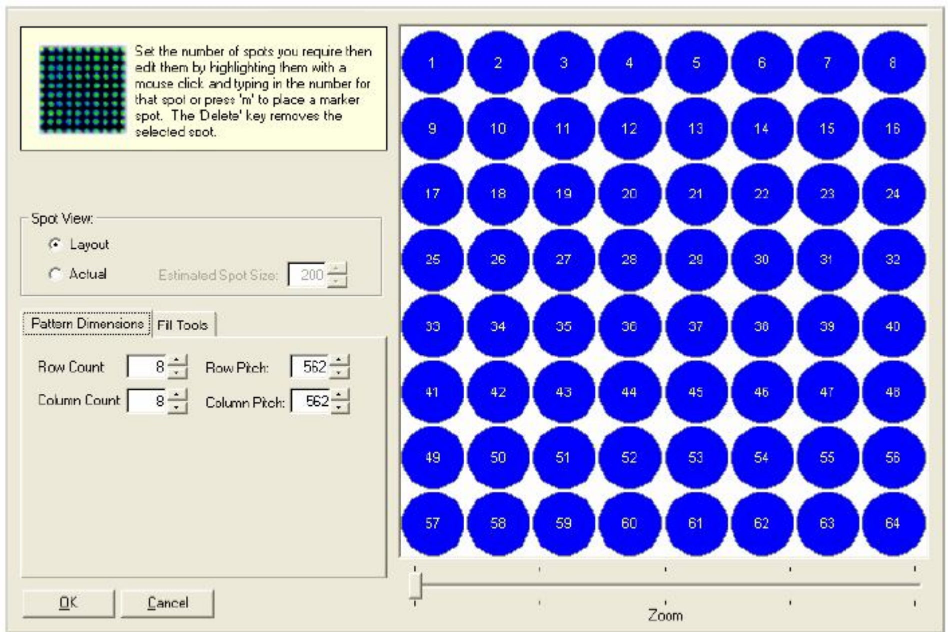

Figure 10: Pattern Dimensions

Pattern Dimensions

Column/Row Count

Use the up/down arrows or type in a value.

Column/Row Pitch

Represents the spacing between spots. The default spot spacing is calculated by the software in order to fit the spots printed by each pin within the 4.5mm x 4.5mm square available (due to the spacing of the pins within the head. Use the up/down arrows or type in a value to change the spacing.

Please remember - Not all pins are recommended for all patterns/densities. As the density is increased then the actual spot size printed must decrease in order to produce a non-overlapping pattern. Different types of gridding pin produce different spot sizes. Spot size is also affected by the composition of the sample and the substrate surface.

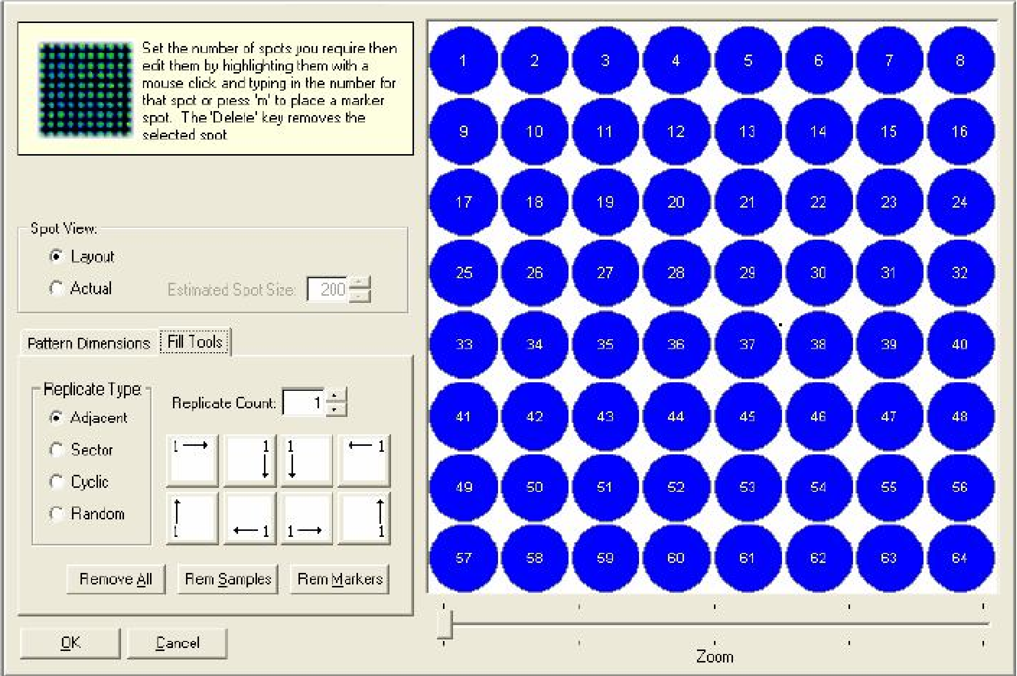

Figure 11: Fill Tools

Fill Tools

Replicate Type

Adjacent - Places replicates of the same sample next to each other.

Sector – If the total number of spot locations in the grid is divisible by the number of replicates, it will divide the grid into symmetrical sectors.

Note: Markers cannot be used with sector replicates, if you wish to create non-adjacent replicates and use markers, use random or cyclic replicates.

Cyclic – Places spots in a progressive sequence and repeats the sequence for the selected number of replicates.

Random – Places the spots in random locations.

Replicate Count

This is the number of replicate spots within each pattern and can be any number up to all spots in the pattern.

Pattern Orientation

The buttons at the bottom left of the dialog allow you to specify the fill direction of spots within the pattern. Click the Clear All button then click the button that represents the required orientation.

Note: The mouse can be used to drag and drop spots to different positions to create custom patterns.

Remove All

Removes spots and markers.

Remove Samples

Will remove the spots but leave the markers in place.

Remove Markers

Will remove markers but leave the spots in place.

Click the button that represents the required orientation.

Filter Layout

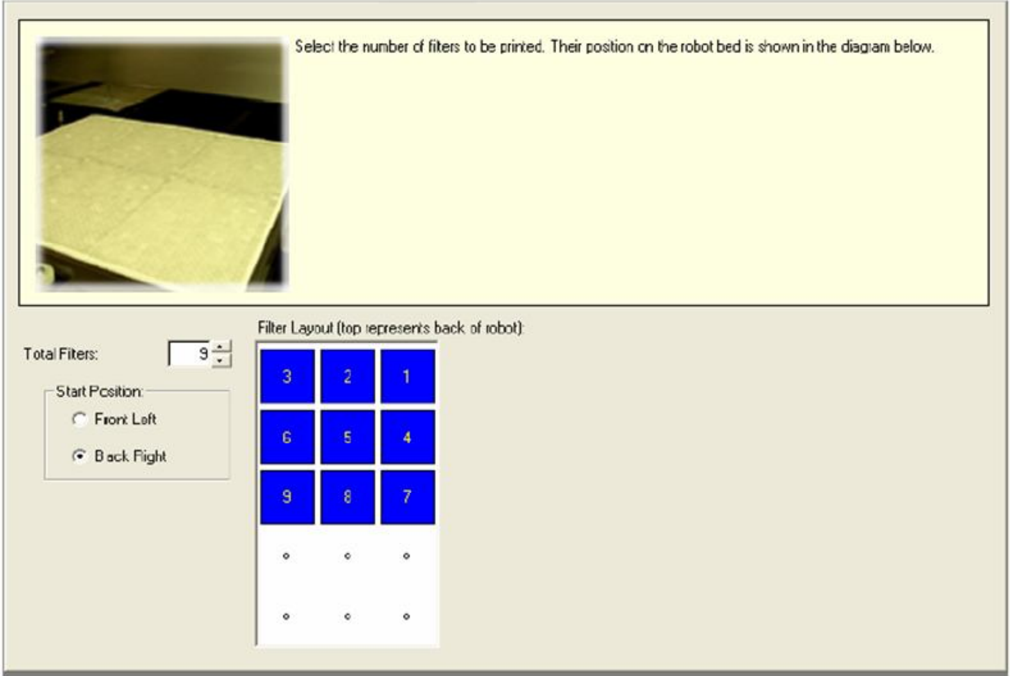

Figure 12: Filter Layout

Total Filters – Use the up/down arrows or type in the number of filters to be used.

Start Position – Determines filter layout. This is reflected in the Filter Layout diagram.

- Front Left – This configures the robot so that the first filter it grids on to is the front left hand filter.
- Back Right – This configures the robot so that the first filter it grids on to is the back right hand filter.

Print

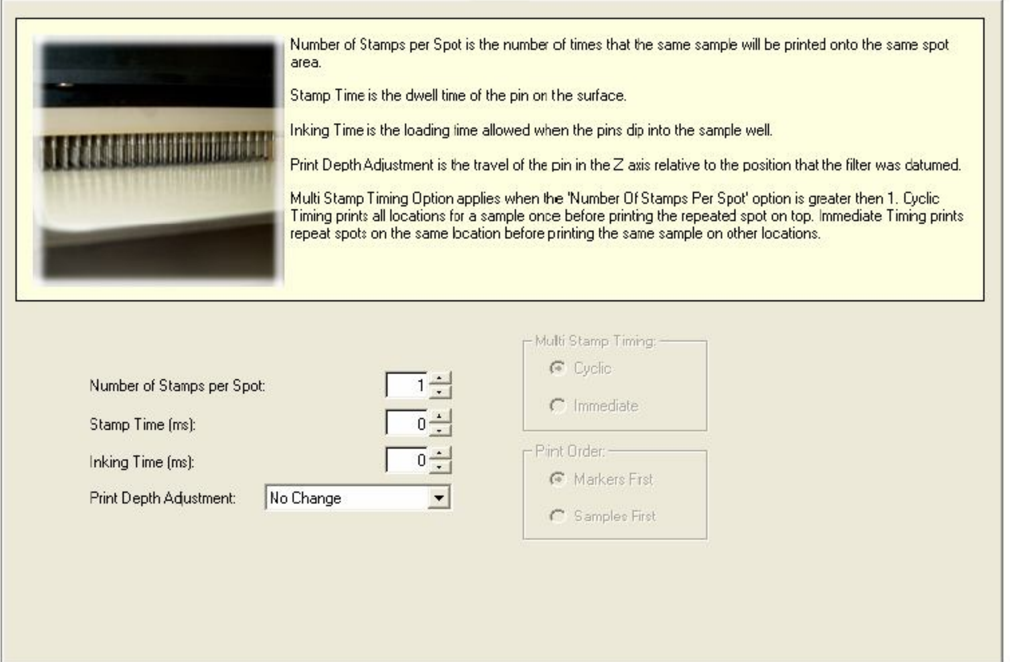

Figure 13: Print

Number of Stamps per Spot - Normally set to 1. This field enables you to set the number of times the machine re-spots onto the same location successively - Useful if larger ‘spots’ within grids are required or if increasing the sample concentration on each spot.

Stamp Time (ms) – Allows the time that the pins are in contact with the filter to be specified.

Inking Time (ms) – Specifies the number of milliseconds that the head will be held in the source plate. For solid pins this can be set to 0.

Ink depth (i.e. how far the pin dips into the well) may need to be changed depending on the sample volume in the wells. The pins should just dip into the sample. Inking depth is changed in the **Configuration** (password required) of the source plate or can be changed at the prompt given when the run is started. Do not set an inking depth greater than the depth of the well.

Note: For Genetix recommendations on inking time and printing depth please see “Printing Parameters” (below).

Print Adjustment – This option allows the contact height between the Head and the substrate to be varied from +0.4mm to –0.4mm in steps of 0.1mm. An adjustment in the positive direction increases the Printing Depth, i.e. the head is brought lower, and the negative adjustment raises the head.

Note: These adjustments are relative to the property "GriddingDepth", which is set in the Configuration the Filter Block. The default Print Adjustment is 0 but if this has been set to 0.1 mm (for example) then the over travel in the Z direction relative to the surface of the membrane is 100 µm.

The use of Print Adjustment means that routines with different grid depths, e.g. for different pins or different filters can be created, without having to change the property in the Configuration each time.

Printing Parameters

Suggested printing parameters are:

Number of Stamps per Spot	= 2 (for slow growing colonies or colonies in Glycerol)
Stamp Time	= 1
Inking Time	= 1
Print Adjustment	(see above)

We recommend that the **Inking Depth** is set so that the pins just dip into the sample (Allow for depletion of the sample during the run). Inking depth can be set in two ways:

- In **Configuration – Hardware**, select the well plate name, click **Properties** and **Edit** the **Inking Depth** property.
- If **Display 'Set Inking Depth'** dialog is checked in the **Script Options**, the robot will pause, at the inking depth set in Configuration, on the first dip and ask you if you want to alter the inking depth. If you select 'yes' you will be able to move the head up or down to the required height.

Multi-Stamp Timing

This option applies when number of stamps per spot is greater than 1.

Cyclic
Prints all locations for a sample once before printing the repeated spot on top.

Immediate
Prints repeat spots on the same location before printing the same sample on other locations.

Print Order – Enabled if marker spots have been defined. Allows you to elect to choose to print Marker Spots first or Samples.

Sterilize

This group of fields is used to select the washing protocols to be used for the particular routine.

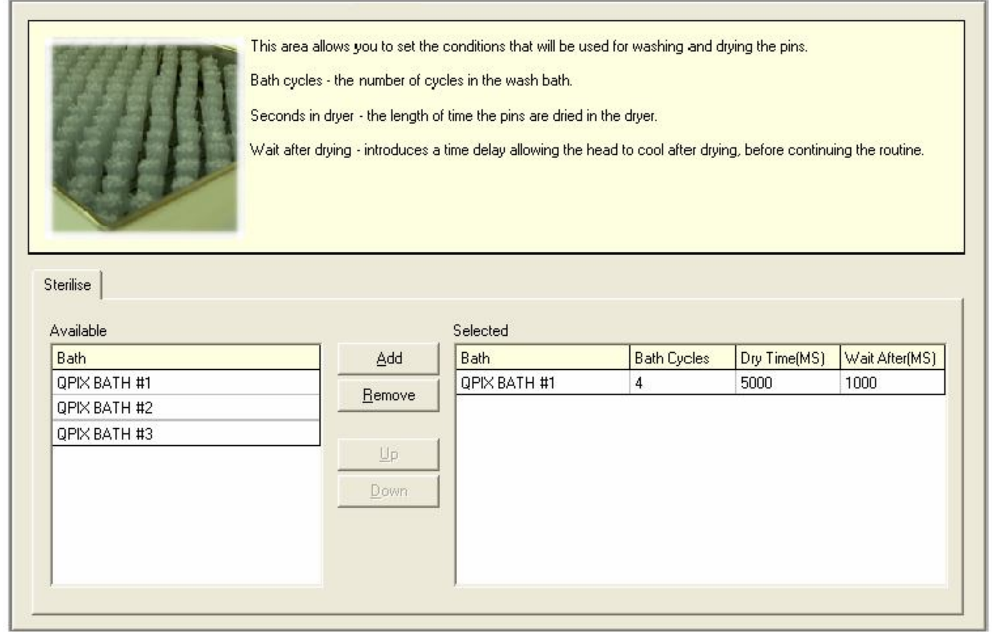

Figure 14: Sterilize

Available

This frame lists the available wash baths.

Selected

This frame displays the selected wash bath and allows the parameters to be changed.

The Add button includes the highlighted wash liquid in the list. To alter the values, click on the relevant field and either type in a new value or use the up-down arrows to increment or decrement the value.

Bath Cycles - Changes can be made to the number of scrubbing cycles that the Gridding Head performs in the sterilization bath.

The wash is performed *n* times clockwise then *n* times anti-clockwise (*n* being the number of bath cycles selected) in a circular movement.

Dry Time - The number of milliseconds that the head will be dried.

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 – demonized 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.

Pin Washing Using Three Bottle Wash System

The following washes are used in the Genetix laboratory:

- Bath Cycles = 3
- Dry Time = 10000
- Wait Time = 7000

If a halogen lamp is used for sterilizing:

- Bath Cycles = 3
- Dry Time = 5000
- Wait Time = 10000

The intense heat of a halogen lamp necessitates the reduction in dry time and the increase in wait time.

Note: These conditions are recommendations only and should be optimized for each particular lab. Temperature, humidity and the pressure that has been set on the dryer will affect the final dry, in particular. It is very important to make sure that the pins are dry before they dip in the next sample.

Data Tracking

Data Tracking enables tracking of printed samples by generating a file of spot locations. The file formats that QSoft currently supports are **.gal** (Axon GenePix®) and **.gsi** (Perkin Elmer ScanArray®, formerly GSI). The file created can be used with automated image analysis tools.

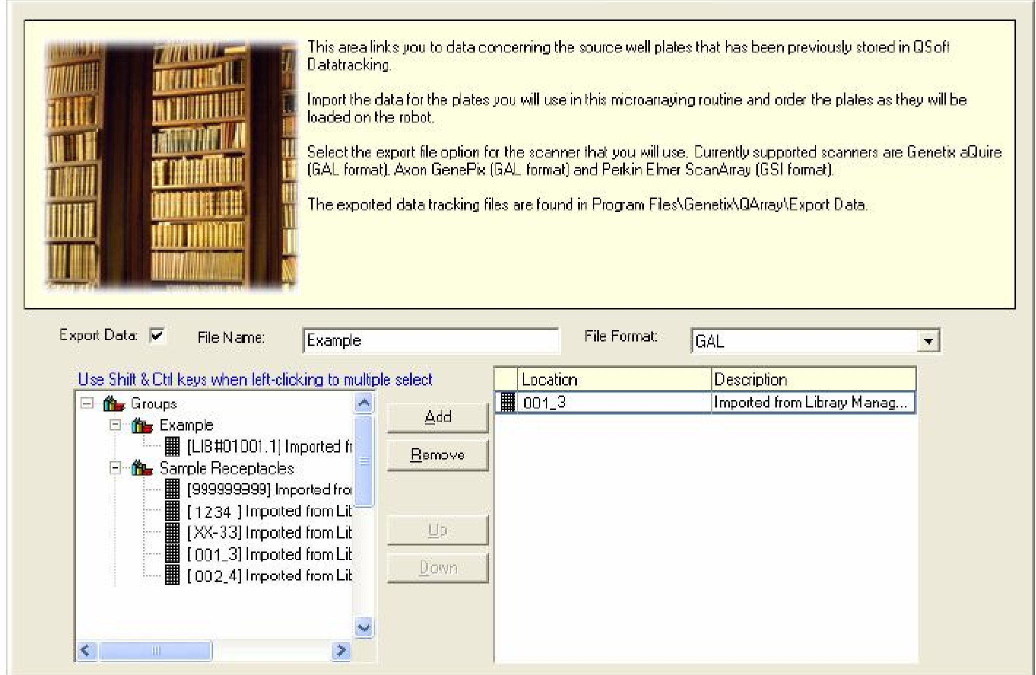

Figure 15: Data Tracking

Make sure the **Export Data** checkbox is ticked.

Enter a **File Name** for the file that is going to be created. If the file already exists on disk a prompt will ask to overwrite it. It is advisable to overwrite existing files to preserve disk space.

Select the format from the **File Format** list.

From the Tree on the left hand side highlight the Plates for use and click the **Add** button.

Note: Make sure that the plates are loaded onto the bed in the same order that they are selected to create the export file.

Select (highlight) plates to be added and click the **Add** button. Plates can be selected individually or in groups by either:

- holding down **Shift** while clicking the plate names (selects contiguous plates)

or

- holding down **Ctrl** while clicking the plate names (selects non-contiguous plates)

The selected Plates will appear in the right hand pane. At this point Plates can be selected and removed or moved up and down the hierarchy by selecting the appropriate button. Plates can also be removed from the list by double-clicking the plate name.

Note: If sufficient plates are not added, an error message will be displayed when you run the program. Click the Data Tracking tab to repeat the above steps, ensuring that the correct number of plates are added.

When running Gridding the file is written to a folder in:

C:\Program Files\Genetix\"robot name"\ExportData

The file can be displayed in a text editor such as Notepad.

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

Barcodes

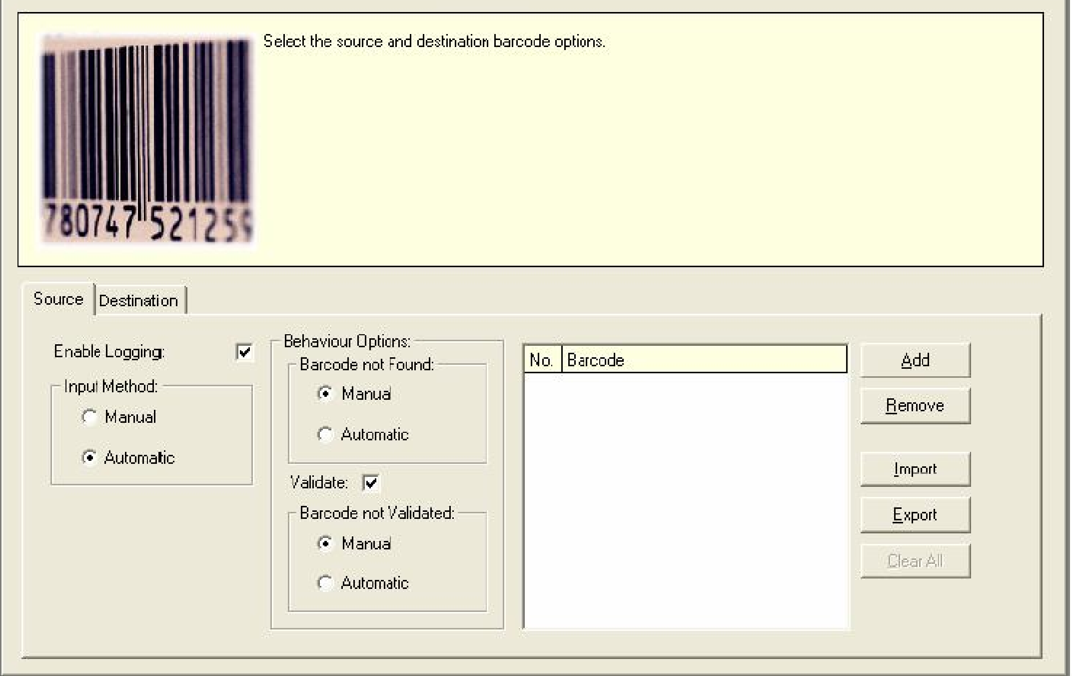

Figure 16: Barcodes

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

Input Method – Choose **Manual** if scanning barcodes with a hand held barcode reader or inputting barcodes at the keyboard is required. **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 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 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 a set of options will be displayed as shown in Figure 17 below

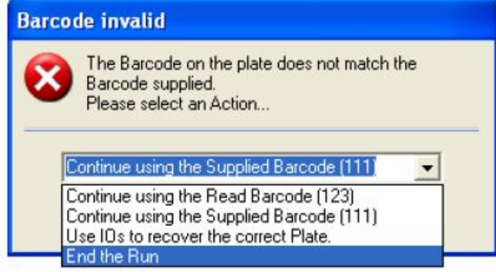

Figure 17: Invalid Barcode Dialog

Click **OK** to continue.

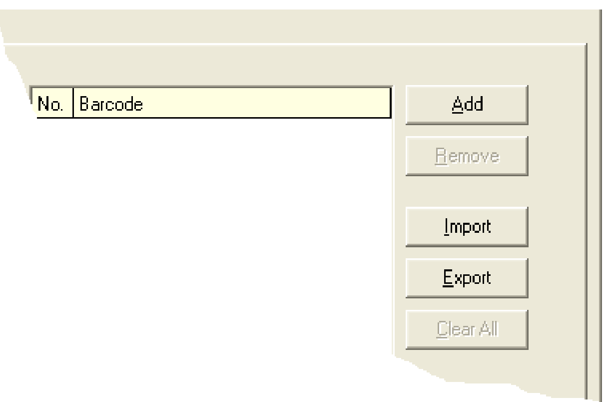

Figure 18: Barcode Tab Command Buttons

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 Datatracking is selected, Barcoding is automatically enabled. Barcoding can be disabled for a given container – for example, if barcodes for a source bioassay tray is not required – 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.**

Start

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

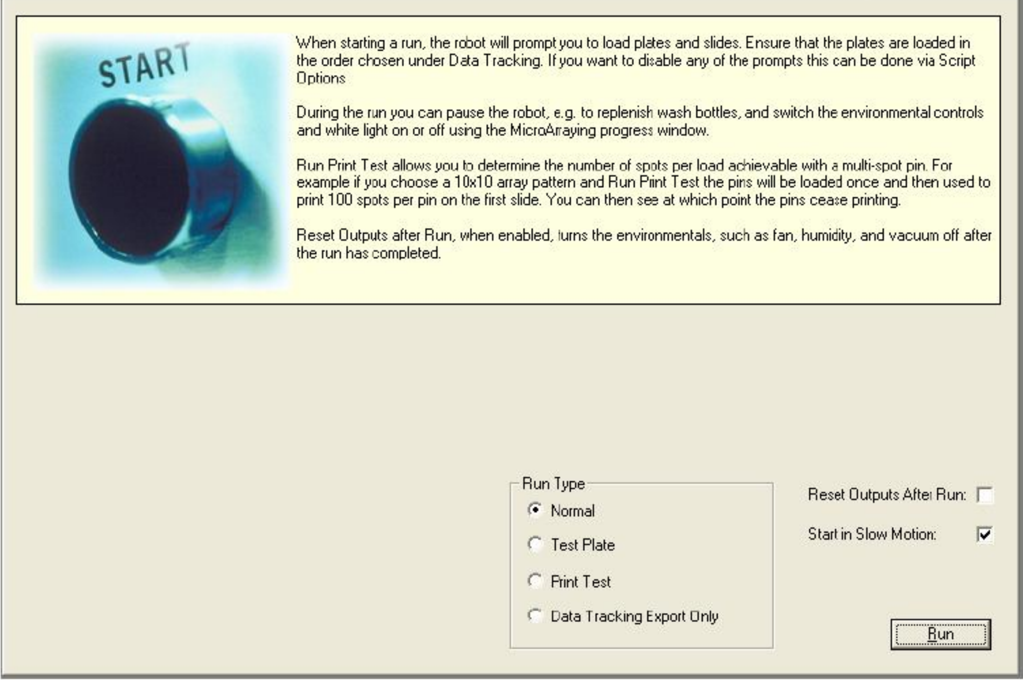

Figure 19: Start

Run Type – Select one of the options:

- Normal – Click this button to start the routine.
- Test Plate - This allows optimization of the printing conditions without wasting precious samples or needing to carry out hybridizations.
- Data Tracking Export Only – Enables the generation of a Data Tracking output file (typically .Gal format) without actually running a routine.
- Reset outputs after run – Turns off the environmental controls such as fan, humidifier and vacuum.

Start in Slow Motion - If this box is checked the routine will be carried out at a low speed. This is useful when you want to carefully monitor the robot movements.

Running a Gridding Routine

Once all the parameters have been set and plates and filters have been loaded, the Gridding routine can begin.

Press the **Normal** button on the **Start** tab.

Figure 20: Prepare Script Message

To continue click **Yes** to abort click **No**.

Note: The prompts that appear on the screen will depend upon which Script Options have been selected (Options menu – Script Options).

The screens that appear may vary depending upon which robot being used.

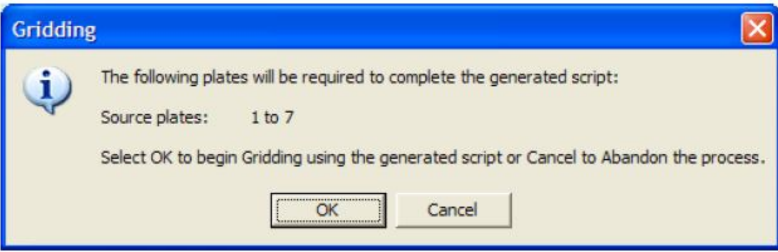

Figure 20: Plates Needed Message

This screen indicates how many source plates will be required for the run. Ensure that these have been loaded. Click **OK** to carry on.

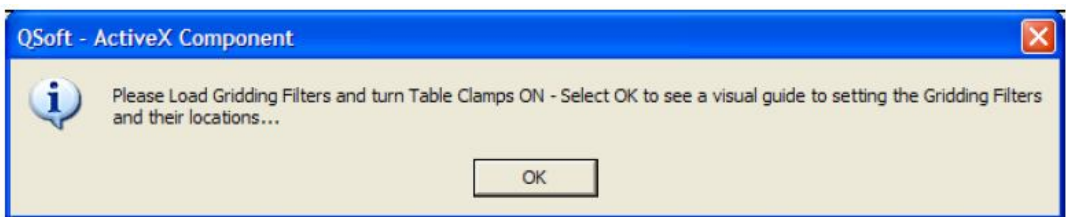

Figure 21: Load Filters Message

Clicking **OK** will present the **Filter View** screen.

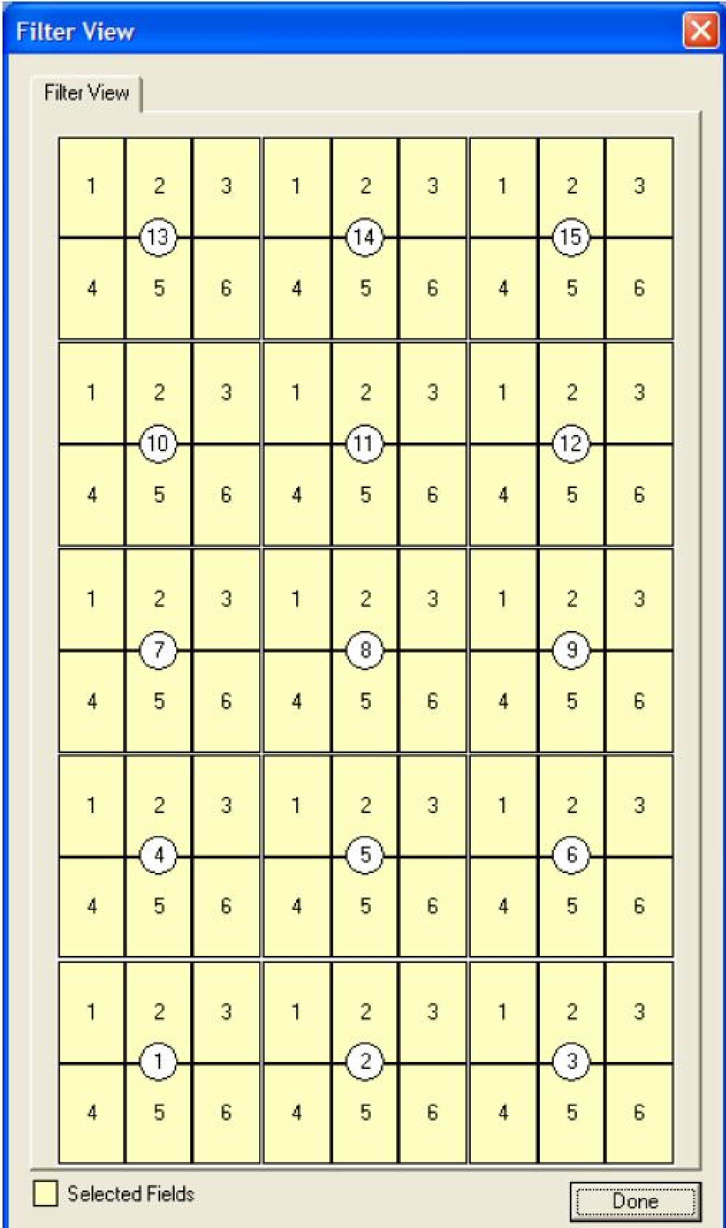

Figure 22: Filter View

Clicking **Done** will generate the following message.

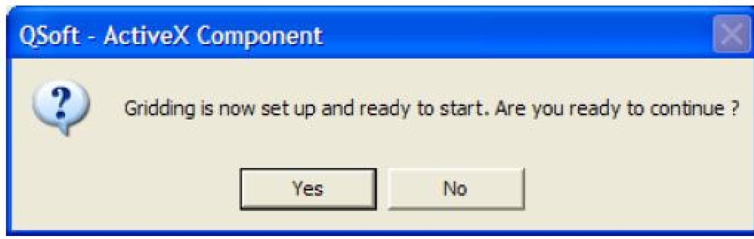

Figure 23: Continue Message

Ensure that the correct gridding head fitted to the robot.

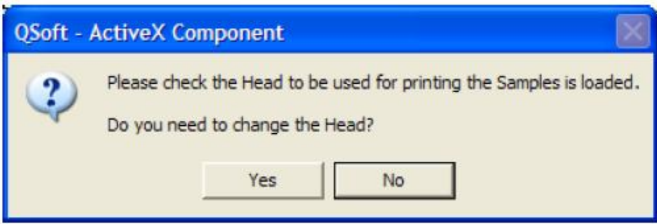

Figure 24: Check Head Message

The next step in the run is a message that will prompt the loading of the source plates, either in hotels or on the robot bed in source plate holders.

The next screen will show the plate locations.

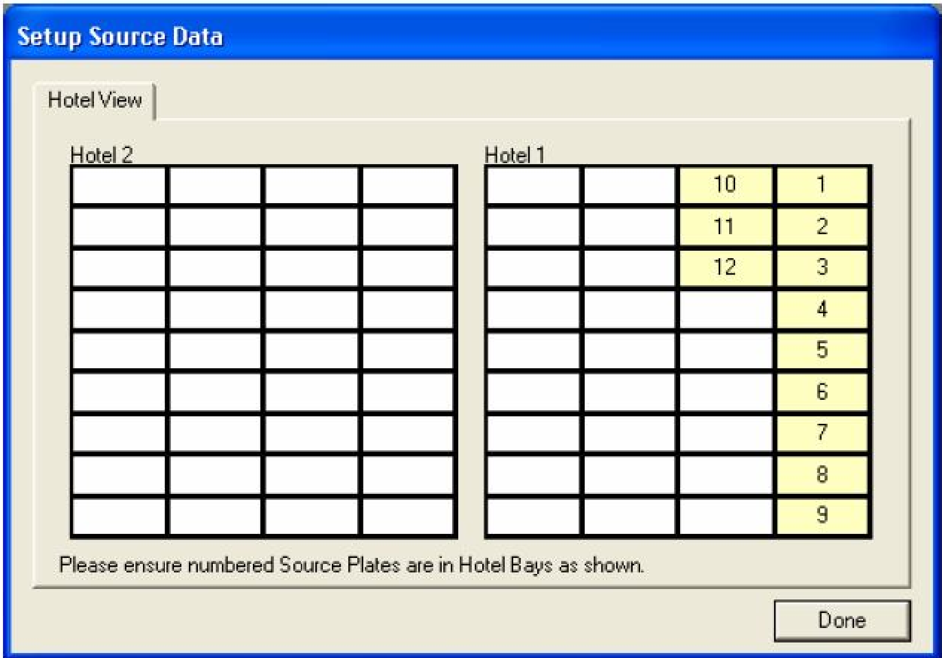

Figure 25: Hotel View

Click **Yes** and the gridding run begins. A progress screen will display which stage of the cycle the robot has reached and also the projected time for completing the run.

Figure 26: Script Progress

If **Start in Slow Motion** was selected as an option in the gridding set-up screen it can be de-selected by un-checking the box in the progress screen. The robot will then run at normal speed.

The process can be paused at any stage, e.g. to fill up the bath. To do this, click on the **Pause** button on the progress screen. The **Pause** button will change to a **Resume** button; click the **Resume** button when ready to continue.

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

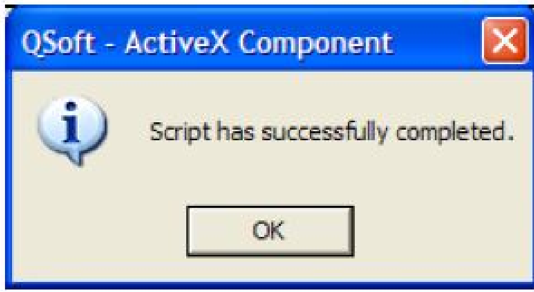

Figure 27: Script Complete Message

After extensive use of the robot, all the above screens may not be required. These can be turned off. In the **Options** menu, select **Script Options** and choose which screens will appear and which will not.

When finished with Gridding application, open the **File** menu and choose **Exit.**

How Many Source Plates will I Need?

The table below (Figure 29) indicates the number of source QPlates required for several standard single-spotting arrays when using different numbers of fields.

Pattern		2x2	3x3	4x4	5x5	6x6	7x7	8x8
No. of Fields	1	4	8	16	24	36	48	64
	2	8	16	32	48	72	96	128
	3	12	24	48	72	108	144	192
	4	16	32	64	96	144	192	256
	5	20	40	80	120	180	240	320
	6	24	48	96	144	216	288	384

Figure 28: Plates Required

For odd-numbered arrays, e.g. 3x3, 5x5 etc. an additional guide spot plate may be required.

Remember
If using a duplicate gridding pattern, only half of the usual number of plates will be required - see table Figure 30 below:

Pattern		2x2	3x3	4x4	5x5	6x6	7x7	8x8
No. of Fields	1	2	4	8	12	18	24	32
	2	4	8	16	24	36	48	64
	3	6	12	24	36	54	72	96
	4	8	16	32	48	72	96	128
	5	10	20	40	60	90	120	160
	6	12	24	48	72	108	144	192

Figure 29: Plates Required for Duplicate Array

Biology Guide

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 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.

Gridding

Gridding onto Filters

Once the library is picked the next stage is to transfer it onto nylon membranes (e.g. 22cm x 22cm Performa) by gridding.

Remove the well plates from the freezer and let them thaw. Remove the filter blocks from the Robot and stack them next to the workstation.

Fill a sterile bioassay tray with LB and appropriate antibiotic. Soak one sheet of sterile blotting paper in the medium let the excess drip off and place on the first filter block. Smooth down by rolling with a sterile 10ml pipette (use the lid of the bioassay tray as a pipette holder. Mark the top right of the membrane with a pencil and, using forceps, soak the membrane in the medium. Carefully position the membrane on top of the blotting paper smoothing it down using the pipette to ensure there are no air bubbles present. Place the block on the bed of the Robot and repeat for the remainder of the membranes. Load the plates into the plate hotels and begin the gridding routine.

Filter Lifting

When the gridding routine is completed remove the plate hotels and return the plates to the -80°C freezer. Remove the first block from the bed of the Robot and bring it to the workstation. Using forceps lift the membrane from the block at opposite corners and carefully place it onto a previously dried agar plate (produced as described in Picking section). Remove any bubbles along the edges with forceps to allow optimum growth. Place a sterile sheet of dry blotting paper in the lid of the tray, invert and incubate overnight at 37°C. Repeat for all membranes.

Gridding Directly in Bioassay Trays

Lay dry membrane onto the surface of a bioassay tray with LB agar and antibiotic. You must use a gravity pin arraying head to array as a sprung head will damage the membrane if it is laid on agar. Place prepared trays on the bed of the robot using the necessary spacers to hold the trays.

After gridding, incubate the trays as described above.

Filter Processing

Remove the plates from the incubator. Using forceps remove the first filter and carefully position it on a piece of blotting paper pre-wetted with denaturing solution. Place the blotting **paper directly** onto a glass plate supported over a boiling water bath for 4 minutes. Remove the membrane from the bath and place on a sheet of blotting paper soaked with neutralizing solution for 4 minutes. Position the membrane on a dry sheet of blotting paper and allow to stand for 1 minute. Invert the membrane (colony side down) into a bioassay tray filled with 100ml of Proteinase K solution and incubate for 45-60 minutes at 37°C. Pour off the Proteinase K, turn the membrane colony side up and place on a dry sheet of blotting paper. Place a sheet of blotting paper on the membrane; roll over twice with a sterile 10ml pipette and leave to dry overnight. (If the cell debris is insufficiently removed then non-specific binding can occur). Immobilize the DNA on the membrane by placing in an UV crosslinker (total exposure should be 120mJ/cm2) or by baking at 80^{0}C for two hours. Store at room temperature and keep dry.

Buffers and Solutions

Denaturing solution

NaCl	87.6g
NaOH	20g

Dissolve in distilled H_2O and bring to a final volume of 1 Liter

Neutralizing Solution

NaCl	87.6g
Trizma	121g

Dissolve in 800ml of distilled H_2O and adjust to pH 7.0 with concentrated HCl. Make up to 1L and autoclave.

Proteinase K solution

Per Liter:

5M NaCl	20ml
1M Tris-Cl (pH 8.0)	50ml
0.5M EDTA (pH 8.0)	100ml
10x Sarkosyl	100ml

Make up to 1 Liter with sterile H_2O and equilibrate at 37°C overnight.

Add Proteinase K (100mg) and mix thoroughly immediately before use. Keep at 37°C

5M NaCl

Dissolve 292.5g of NaCl in 800ml of distilled H_2O. Make up to 1L and autoclave.

1M Tris pH 8.0

Dissolve 121.1g of Tris in 800ml of distilled H_2O and add concentrated HCl to make pH 8.0. Make up to 1L with distilled H_2O and autoclave.

0.5M EDTA pH 8.0

Dissolve 186.6g of EDTA in 800ml of distilled H2O and adjust pH with NaOH. Make up to 1L with H_2O and autoclave.

10x Sarkosyl Solution

Dissolve 100g of N-Lauroylsarcosine in 800ml of distilled H_2O.

Caution: Wear a face mask

Gridding Yeast (containing YACs)

Sterilize the Robot for 20 minutes using the UV light while the sample plates are thawing. Roll the filters out in the same manner as for bacteria but use YPD medium or other suitable medium instead of LB. When the rolled filters are in place sterilize again for 10 minutes. Fill the plate stacker with the sample plates and proceed with the gridding routine. The gridding pins must **gently** touch the bottom of every well or use the **Stir Source** option to mix the well (the inking depth may need to be adjusted). When gridding yeast we recommend using 3 wash baths –one containing 1% sodium hypochlorite (bleach), one of just water and one with 80% ethanol.

After the gridding routine is completed, the filters should be lifted onto plates containing 200ml YPD or AHC agar. This process should be done in the bed of the Robot or the filters can be covered with a sterile **QTray** lid and transferred to a flow cabinet to lift. Invert the plates and incubate at 30°C for at least 18 hours.

Protocol for Gridding PCR products

When performing PCR based arrays it is important that enough DNA is spotted. The optimum concentration of DNA in the sample to be arrayed is in the range of 50-100ng/µl.

Another factor to be considered is the diameter of pin used to transfer the sample*. If either a 0.25mm pin or a 0.4mm pin is used then the product need only be arrayed once onto the membrane. If however the 0.15 pin is used then the sample should be spotted twice.

Before engaging in a gridding routine disinfect the machine by UV radiation.

***Quantification analyses have yet to be carried out to determine volume of liquid transferred. The advice given is based on personal results.**

Gridding Preparation

1. Fill a bioassay tray with denaturing solution and, using forceps, immerse a piece of blotting paper (hold the blotting paper at opposite corners for ease of handling). Remove the blotting paper and quickly drain off any excess denaturing solution. Carefully lay the blotting paper onto a filter block.
2. Using forceps, briefly soak a nylon membrane in denaturing solution, remove any excess denaturing solution and carefully overlay on top of the blotting paper on the filter block. Gently roll a sterile 10ml disposable pipette over the surface of the membrane to remove any air bubbles.
3. Place the filter block on bed of the machine and repeat process for any further membranes.
4. Fill the baths as follows: Bath 1 should contain 1% sodium hypochlorite, bath 2 should contain demonized water and bath 3 should contain 80% ethanol.
5. Refer to the appropriate sections of this manual to determine the field and pattern order.
6. Gridding should now be ready to commence.

Post Gridding Preparation

Remove the filter block from the bed of the machine.

1. Fill a bioassay tray with denaturing solution and, using forceps, immerse a piece of blotting paper. Remove the blotting paper and quickly drain off any excess denaturing solution. Place the blotting paper in the lid of a bioassay tray.
2. Using forceps remove the first nylon membrane from the filter block and place it on the pre-wetted blotting paper.
3. Leave for 10 minutes, then place the nylon membrane onto a second sheet of blotting paper, pre-wetted with neutralizing buffer, in the lid of a bioassay tray.
4. Leave for 5 minutes and then transfer the membrane to a dry piece of blotting paper and

leave to air dry.

5. Place the membrane in a UV crosslinker, DNA side up, and expose at 120,000 microjoules/cm^2. UV crosslinking is recommended for nylon membranes as this leads to covalent attachment of the DNA to the nylon and also allows the membrane to be re-probed several times.

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

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)

1. Preheat Church Buffer to 65°C.
2. Incubate membrane in buffer for 60-120 minutes.

Hybridization Step

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

Stringency Washes

1. Transfer membrane to 1x washing solution for 20 minutes at room temperature.
2. Preheat second batch of 1x washing solution to 65°C.
3. Transfer membrane to preheated 1x washing solution for 20 minutes.

Detection Steps

1. Prepare blocking solution.
2. Incubate membrane in blocking solution for 45 minutes at room temperature.
3. Centrifuge second batch of blocking solution for 15 minutes at 3000 rpm.
4. Centrifuge stock antibody solution for 1 minute at 14,000 rpm.
5. Dilute antibody solution 1:10,000 in supernatant from centrifuged blocking solution.
6. Incubate membrane in antibody solution at 37ºC for 60 minutes.
7. Incubate membrane in PBS for 30 minutes at room temperature.
8. Repeat incubation for 30 minutes in fresh PBS at room temperature.
9. Incubate membrane in detection buffer for 10 minutes at room temperature.
10. Repeat incubation in fresh detection buffer for 10 minutes at room temperature.
11. Dilute stock AttoPhos solution (5mM in 2.4M DEA buffer) 1:5 with detection buffer.
12. Using motorized spray gun apply 2.5ml of AttoPhos solution to each membrane.
13. Sandwich membrane in between two trimmed hybridization bags ensuring there are no air bubbles.
14. Heat seal the sandwiched membrane into a hybridization bag and cover in aluminum foil.
15. Place membrane in between two filter blocks.
16. Incubate membrane (in the 37°C incubator for 4 hours or leave at room temperature overnight).

17. Image the membrane using a suitable system e.g. the Fuji LAS1000 dark box.
18. 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.

1. Preheat stripping solution one to 65°C.
2. Incubate membrane in stripping solution one for 30 minutes.
3. Incubate membrane in stripping solution two for 45 minutes at room temperature.
4. Preheat filtered Church buffer to 65°C.
5. Incubate membrane in preheated Church buffer over night at 65°C.
6. Membrane can be stored in 2x SSC at 4ºC until required for re-probing.

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 sulfate)

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

1. Prepare PCR master mix in 96 well or 384 well PCR microplates. Master mix can be dispensed using QFill2 or aliQuot dispensers.
2. 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.
3. Repeat the replication process, such that each PCR reaction has had two dips from the culture.
4. 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

1. Prepare PCR master mix in 96 well or 384 well PCR microplates.
2. Transfer 1µl of culture from the microplate to the PCR plate using the QBot Liquid Handling function or a multichannel pipette.
3. 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 lyze 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

Array
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.

Block
A pattern of clones or spots immobilized within an array produced by one pin.

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.

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

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.

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

Field
An array is divided into fields. The field is the area covered by the head.

Filter Block
Solid blocks onto which gridding membranes are placed (2 per QPix2; 6 per QPix2 XT).

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

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 (arrays) easier to read.

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

MADGE
Microplate Array Diagonal Gel Electrophoresis.

Marker Spot
A reference spot created by the user, in order to make grids (arrays) easier to read.

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.

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 Sulfate.

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

SSC
Sodium Chloride/Sodium Citrate buffer.

Sub-Grid
(See Block)

White Imaging Light
Illumination for color-stained gels.

X Drive
Axis running from back to front of the 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 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.

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
	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
	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
	Table Clamp	Turns on or off the clamps that secure gridding filter blocks on bed of QPix2 XT.	QPix2 XT
	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

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