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*Bio*Robotics

The *Bio*Grid

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*Bio*Robotics

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WARNING

The *Bio*Grid must be connected to a properly earthed power outlet to protect users from the risk of electric shock. Do not remove any of the fixed covers as there are no user serviceable parts inside. All internal repair work should be referred to *Bio*Robotics approved service personnel.

In the event of liquid spillage or accidental dropping of items into the base cavity of the *Bio*Grid, the power supply should be disconnected at the mains before any attempt is made to retrieve the item or clean up the spillage.

The *Bio*Grid is strictly for research use only and is not intended or recommended for the diagnosis of disease in humans or animals.

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Welcome

Welcome to the *Bio*Grid

A bench top automatic filter preparation and library replication instrument.

Before using the *Bio*Grid, please make sure that you have read this manual and have read all the safety instructions.

Important Note

Prior to using the *Bio*Grid it is **very important** to go through the *Bio*Grid set up procedure. This procedure ensures that all moving parts on the *Bio*Grid are correctly situated in their datum position.

Failure to comply with this procedure could damage the *Bio*Grid.

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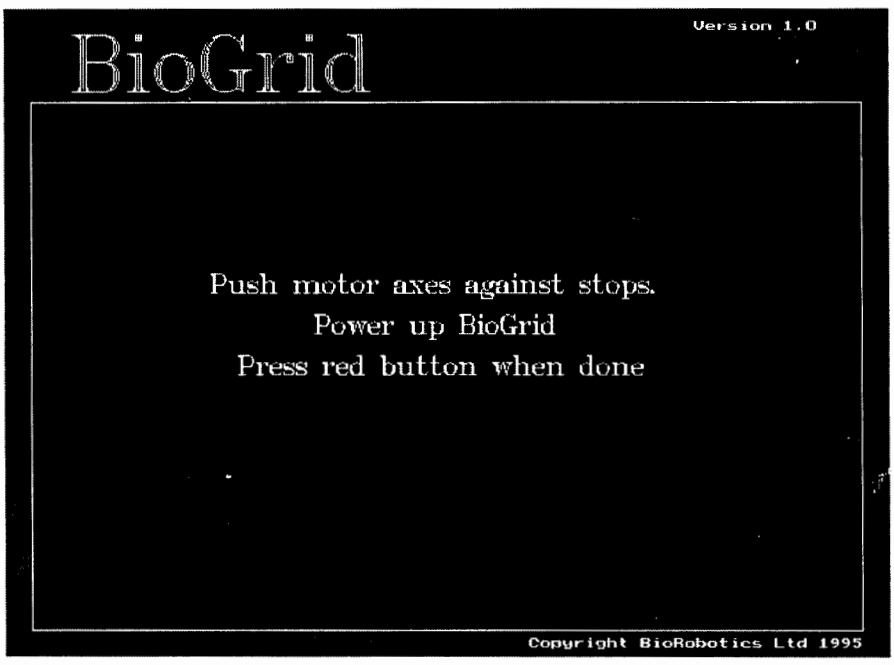
Operating The *Bio*Grid

Start-up

- Ensure that the power connector to the laptop computer is in, and power is on.

The laptop has a battery and will run for a short time off these. However, failure to ensure that mains power is on could result in the batteries running out half way through a run.

- Turn on the laptop, and give it a few moments to boot up.
- After some screen messages, the *Bio*Grid software will automatically start up.
- The following screen will now appear containing instructions as how to proceed :



Firstly ensure that there is no gridding tool on the transfer arm.

Starting up the instrument with a gridding tool on will damage the tool.

Push motor axes against stops

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When the *Bio*Grid is turned off, all nine of the motor axes can be moved by hand. The motor axes should now be pushed to their home positions, against the mechanical stops.

When the *Bio*Grid finishes a run, the axes are moved to their home positions under computer control, so as long as no-one has interfered with the instrument since it was last used, everything should be in the right position. However, it is good to get in the habit of checking.

The home positions are as follows:

Transfer arm in x	- the far right hand side of the machine.
Transfer arm in z	- right up to the highest position.
Filter Trays 1-4	- as far towards the back of the machine as possible.
*Bio*Bank holder	- as far down as possible.
Vacuum lid lifter	- as far down as possible.
Source Plate Hooks	- as far towards the front of the machine as possible.

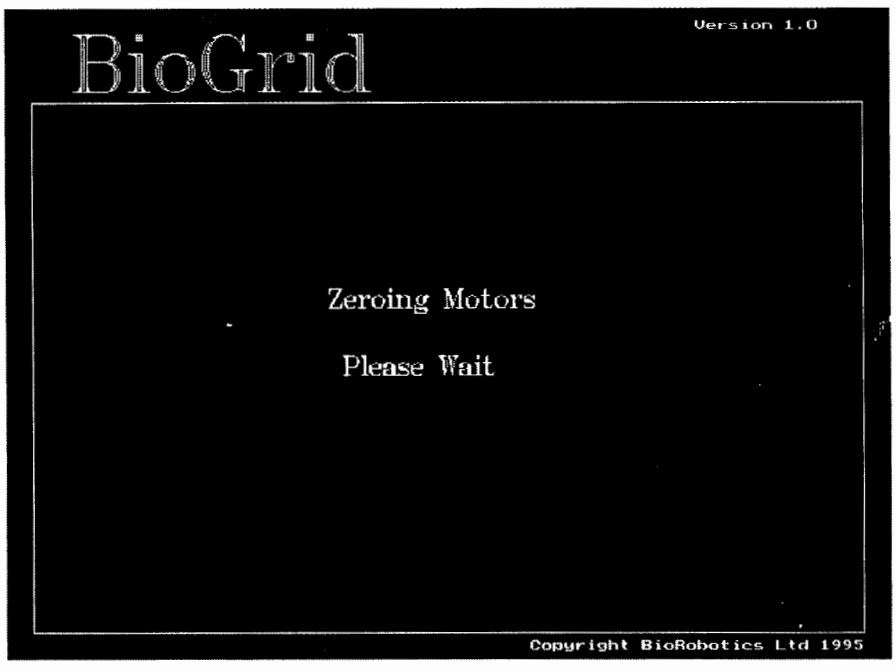
Power up *Bio*Grid

Ensure that the mains supply cable is plugged in at the back right hand side of the instrument and turn the power to the instrument on with the switch next to the power inlet socket.

Press red button when done

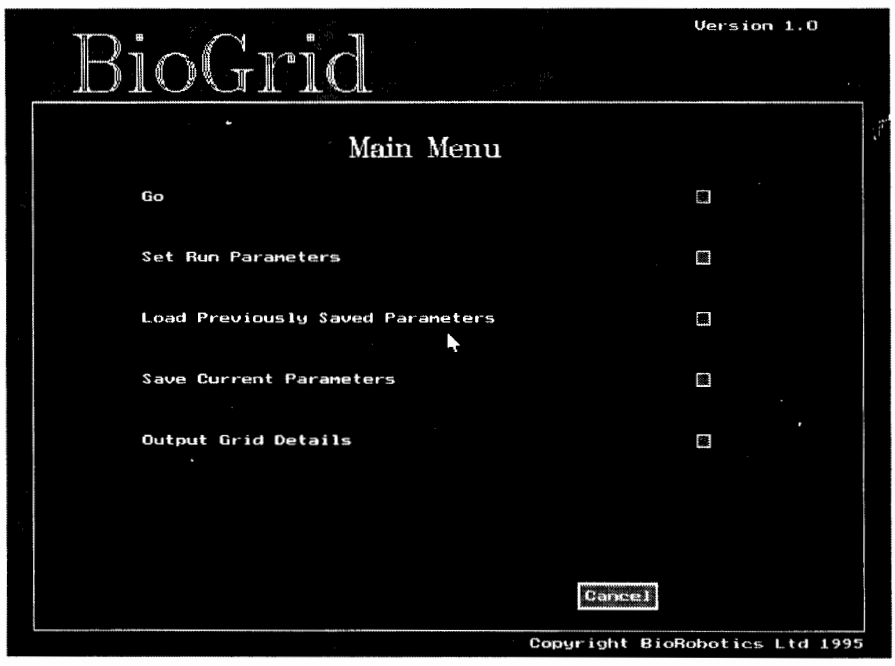
Press the red button at the front right hand side of the instrument once to confirm to the computer that the other instructions have been carried out. The following screen will now appear as the axes move under computer control to accurately locate datums for each axis using sensors on the instrument.

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Once the instrument has finished zeroing the axes, it is ready for use and the Main Menu will appear.

Main Menu



The are five options available from the main menu, plus a cancel button.

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Go Starts a run using the current parameters. Default values will be used if none have been entered.

Set Run Parameters Enables the user to change the current run parameters.

Load Previously Saved Parameters Loads a nominated set of run parameters from the hard disk.

Save Current Run Parameters Saves the current run parameters to the hard disk.

Output Grid Details If the current run parameters describe a gridding run, this option will display on the screen, and output to a printer if required, a description of the gridding pattern.

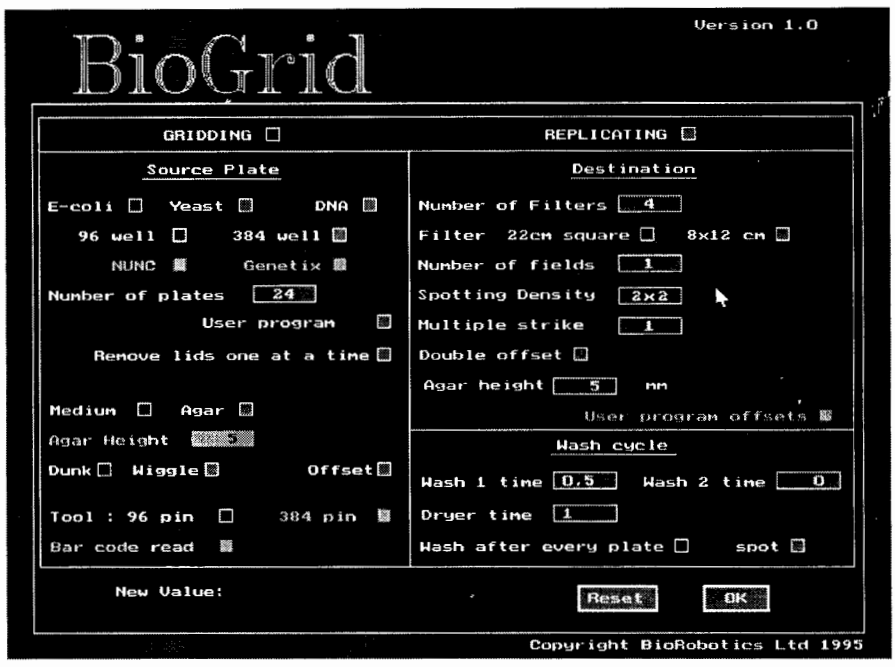
Cancel Quits out of the *Bio*Grid software and into DOS.

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Set Run Parameters

Overview

Clicking on the button adjacent to 'Set Run Parameters' will cause the following screen to appear:



All of the information that defines the gridding or replicating run is contained on this screen. It is recommended that the user works through this screen from top to bottom and from left to right, as this helps to ensure that nothing is overlooked or forgotten.

The screen is divided into three large sections. The left hand side of the screen corresponds to the left hand side of the instrument, and contains all of the options relating to the source plates. The right hand side of the screen corresponds to the right hand side of the instrument, and contains all options relating to the destination membrane or plates, and the sterilisation cycle.

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Information is entered in one of two ways:

1. **Buttons** that are either on (red) or off (blue). Move the mouse pointer over the button and click on it.
2. **Edit Boxes** that contain numerical information. To change the number contained in one of these boxes, click on the box with the mouse. The box will change colour from blue to red, indicating that it is active. Now type in the new number. As you type the number will appear in the bottom left hand corner of the screen, next to the text " New Value :". If you make a mistake, the backspace key works in the normal way. When you are happy that the new value is correct, press the carriage return key on the keyboard, and the value in the edit box will be updated. This edit box will remain active (red) until the mouse is clicked over another edit box.

Any options that are in grey are unavailable, and cannot be changed or set. These may change colour and become available when certain other options are selected.

If at any time you enter a value that would result in an illegal set-up, a warning box will appear upon the screen. The box will contain some text explaining why that selection was not possible. Click on ht OK button within the box to remove it from the screen and continue configuring the run.

When the set-up is complete, clicking on the OK box bottom right will return you to the Main Menu. The Reset button adjacent returns the Run Parameters to the default parameters.

The first option, at the top of the screen is Gridding or Replicating. Click the mouse on the button corresponding to the run you want to configure.

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Gridding

Running from top to bottom, and left to right, the options for gridding are as follows:

Source Plates

E-coli **yeast** **DNA**

select the material that is in the source plate. This selection will affect the recommended wash times.

Number of wells **96** **384**

select the type of microtitre plate to be used. Selecting 384 well plates will cause the options below to change from being unavailable (grey) to being available. If this is the case then the plate manufacturer must be selected from:

NUNC **Genetix**

This is due to the difference in plate thickness between the Genetix plates and all others.

The next selection is **Number of Plates**. This box should contain the number of source plates that are to be gridded onto the membrane. Enter the required number as explained above.

Remove lids one at a time. The source plates are removed from the *Bio*Bank in pairs, and pulled to the lid lifting position. As a default 'Remove lids one at a time' is set to off (blue), and the lids are removed from both lids together, before they are pushed forward to the gridding tool sampling position. However, setting 'Remove lids one at a time' to on (red) would result in only the left hand plate's lid being removed, while that plate is sampled, then the left hand plate's lid would be replaced and the right hand plate's lid removed. Thus each plate's lid is only off for approximately half the time. There is however a slight penalty in that run time is increased by the extra plate manipulations.

Medium **Agar**

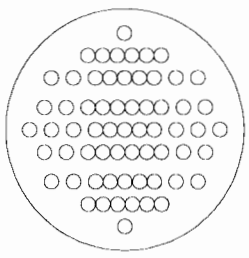
Do the source plates contain medium or agar? If you are sampling from source plates containing agar, then the software needs to know approximately the depth of the agar in the plates, and the box **Agar Height** below will change to an active colour for this information to be entered.

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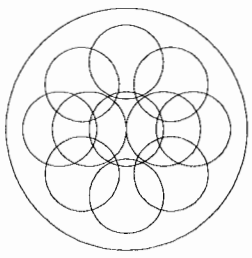
The next selection deals with how the gridding tool samples the source plate:

Dunk Wiggle Offset

The gridding tool can either 'dunk' (straight in and out of the plate) or 'wiggle' (perform a small oscillation from side to side). In either case the pin tips touch the bottom of the well. The 'wiggle' option is recommended for repeat visits to plates containing yeast, where a hole might be formed by the gridding tool pin in a layer of cells that have settled at the bottom of the well. 'Offset' is either set on or off, and can be used in conjunction with dunk or wiggle. Offset is again for repeat visits to a plate. The gridding tool and plate are both moved as follows to ensure that the pin is sampling from a different place in the well each time:



Offset pattern for Thin pin tool into 96 well plate



Offset pattern for Fat pin tool into 96 well plate or thin tool into 384 well plate

It is recommended that offset should **not** be used with round-bottomed microtitre plates .

Tool 96 pin 384 pin

This selection will automatically be set to the most appropriate for the run being configured. It is possible though to set the instrument to grid 384 well plates with a 96 pin tool. This would obviously take nearly four times longer than using a 384 pin tool.

Bar code read

This is either set on or off. On instruments without bar code reading capability this option will remain unavailable.

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Destination Plates

Number of Filters

Using the *Bio*Grid it is possible to make up to four copies of 22cm membranes simultaneously, and up to 24 copies of 11x8cm filters. Set the box to the number of copies you want to make in this run.

Filter Size **22cm square** **8x12cm** ✓

Select the filter size with which you are intending to load the instrument.

Number of Fields

This selection is only available for 22cm square membranes. Since there are six positions available on a 22cm square membrane, it is possible to divide it up , and to make multiple copies on each filter as follows:

A B	A A	A B	A A
C D	B B	A B	A A
E F	C C	A B	A A
Fields = 1	Fields = 2	Fields = 3	Fields = 6

Thus 'Number of Fields' should be thought of as the number of identical copies that are to be made on each membrane.

Spotting Density

The spotting density box shows the density of the gridded array. Whenever an option is entered that affects the density of the pattern (number of plates, filter size, number of fields, and double offset), the **minimum** density that would accommodate those plates on the defined filter is automatically evaluated and the density box is updated.

It may be that on some occasions, the user will want to force the density to a higher density than that evaluated as the minimum required. In this case the edit box is selected with the mouse as for any other, and a figure for the density is entered (e.g. for a 6x6, type 6 and press carriage return).

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Multiple Strike

This option enables the user to increase the transferred sample, by performing repeat spotting to the same point. As a default this is set to one, i.e. the pin will only deposit a sample at each position once. If for example it were set to three, the multi-pin tool would take a samples from the source plate to deposit at the destination three times. Obviously this has implications for cycle time, and another way to increase the transferred volume without such a penalty is to use a tool with a different pin profile.

Double Offset

This option is either on or off. If it is off, each plate is put into the spot pattern once only. If it is set on, each plate is put into the spot pattern twice in different positions, giving a double positive on probing. The positions of these two spots within the arrayed pattern can be arranged such that positives for each plate are individually characterised by the distance and angle between the two spots, thus aiding identification of positives.

Agar Height

The approximate thickness of agar substrate in the destination tray should be entered into this box, this helps to ensure that all the pins of the multi-pin tool make good contact with the membrane. It need only be approximate since the pins in the multi-pin tool have 4mm of travel.

It is recommended that a consistent volume of agar should be used to prepare the destination plates each time. Then the Agar Height option can always be set to the same value.

User Program Offsets

This button enables the user to edit the default double offset patterns.

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Wash Cycle

The *Bio*Grid software gives the user full control over the sterilisation routine. There are two washing baths available, together with a hot air blower.

Wash 1 time

This box contains the time in seconds that the tool spends oscillating in the first washing bath.

Wash 2 time

The time in seconds in the second washing bath. If this is set to zero then the tool will only visit one bath.

Dryer time

Control over the time that the tool dwells over the hot air blower. This time can be as low as 1-2 sec for the thin pin tools, but should be increased to 15-20 sec to ensure that a 2mm pin tool is thoroughly dried.

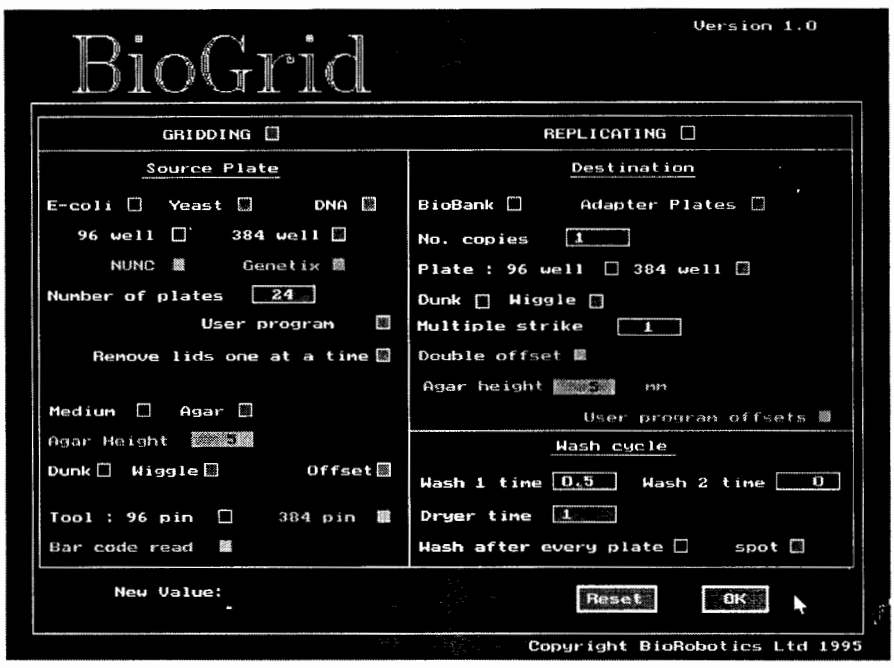
Wash after every plate spot

As a default, the *Bio*Grid will perform the sterilisation routine having finished sampling from one source plate, and before visiting the next. However it is possible to sterilise after each sample-spot cycle by selecting **Wash after every spot** rather then **Wash after every plate**.

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Replicating

Selecting the **Replicating** option at the top of the screen will cause the screen to change :



The **Source Plate** and **Wash Cycle** areas of the screen remain unchanged, and the descriptions in the previous section should be referred to.

The **Destination** area of the screen has changed, to show the different options available for replicating:

BioBank **Adapter Plates**

These are the two basic options for replicating.

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Replicating in the *Bio*Bank makes copies of the plates in the left hand column of the *Bio*Bank by transferring samples into the right hand column. Thus it is a good way of making a single copy of up to 12 source plates at a time.

The Adapter Plates option makes copies on the stack of four destination trays, which have been modified to hold up to 6 microtitre plates on each tray by means of an adapter plate. Thus the capacity of the instrument in this configuration is for up to 24 source plates, and up to 24 destination plates.

Number of Copies

This edit box should contain the number of copies of each source plate that you want to make in this run.

Plate : 96 well 384 well

Select the type of destination plate that you are using. Having selected the Adapter Plate option (above) it is possible to configure the instrument to compress (96 →384) or expand (384 →96) a library. This would not work using the *Bio*Bank option which is only for one to one transfer, and cannot perform one-to-many or many-to-one inoculations.

Dunk Wiggle

As for the dunk and wiggle options in the source plate section, but for the tool dipping into the destination plates.

Multiple Strike

This option works in the same way as when gridding, however it controls repeat visits to a plate, rather than to a spot. Thus the volume of inoculant transferred can be increased at the expense of run time. Another way to increase the inoculant volume is to use a tool with a larger pin diameter. Special tools are available from *Bio*Robotics.

When you have finished the set-up, click on the OK box at the bottom right of the screen, which will return you to the Main Menu.

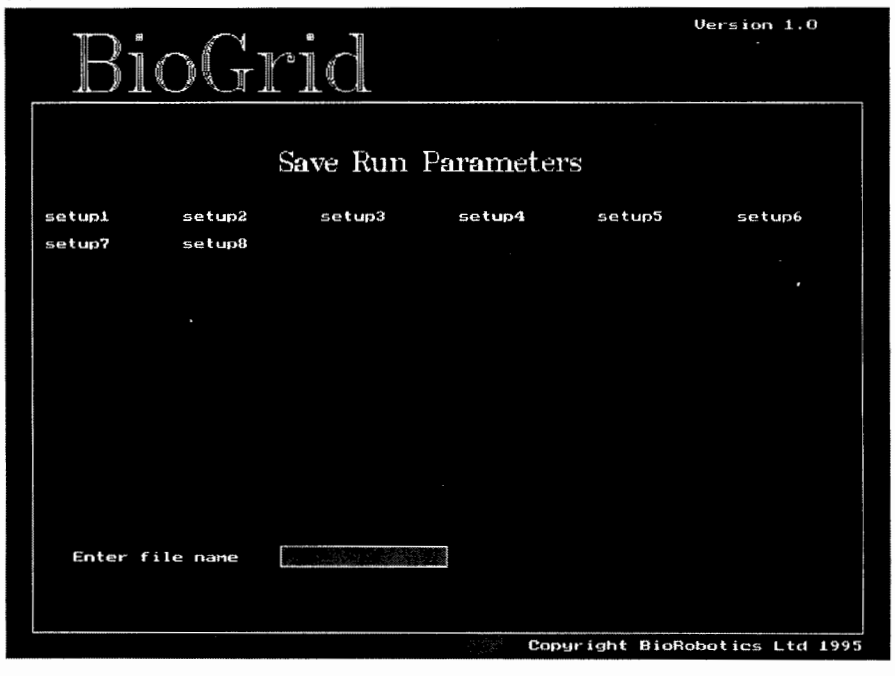
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Run Parameter Files

Once that you have configured a run using the Set Run Parameters option, that set-up can be saved onto the hard disk under a given file name for re-use at a later date.

Saving Run Parameters

From the Main Menu select **Save Current Parameters**. This will bring up a screen like this:



Listed on this page are all of the Parameter files that have been previously saved to the hard disk (up to a maximum of 60). You are asked to enter a name for the file that is to be created containing the current set-up.

Pressing the carriage return key without entering any file name will return the screen to the Main Menu without saving anything.

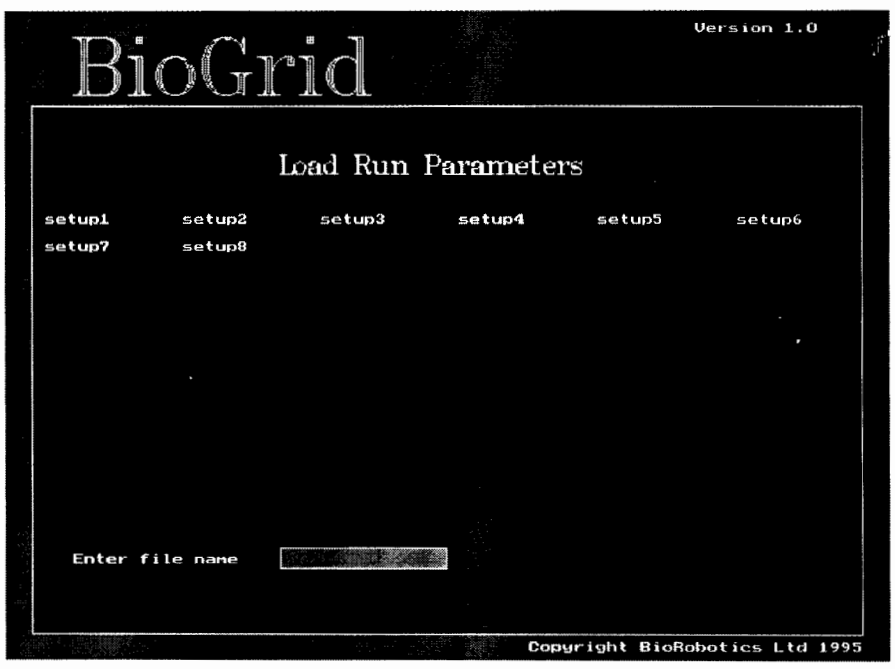
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Type in the name and it will appear in the light blue box at the bottom of the screen. The backspace key works as normal here. When you are happy with the filename, press the return key. The current run parameters will be saved under this name. The screen returns to the main menu.

If you type in a file name that already exists, the software will ask you to confirm that you want to overwrite that file. Pressing the Y key will overwrite and return to the Main Menu. Pressing the N key will allow another name to be entered.

Loading Run Parameters

To load a set-up that has been saved to the hard disk at an earlier occasion, select **Load Previously Saved Parameters** from the Main Menu.



The screen is similar to the Save Run Parameters screen, containing a list of the names of all files that have previously been saved.

As in the previous option, pressing the carriage return key without typing any file name will return to the Main Menu without loading in any parameters.

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To load in a set-up from the disk, type in the required filename and press carriage return. The file is loaded, and the screen returns to the Main Menu.

If you type in a name that is not recognised as being on the list, a warning message will appear. Press any key to make it disappear, and try again.

Other File Manipulations

Other file manipulations must be done in MS-DOS at the moment, using standard DOS commands. Upon quitting out of the *Bio*Grid software the computer is returned to DOS in the root directory. The run parameter files reside in a sub directory to the biogrid directory called setups, so typing:

cd biogrid\setups

will change the directory to the setups directory.

then typing

dir /p

will list all of the run parameter files which should all have a **.prm** extension.

To delete a file that is no longer of any use type

del *filename*.**prm**

To make room for new files in the setups directory (the *Bio*Grid software will allow 60 to be created) without deleting files, it is possible to move some files to another directory. For example, to move old parameter files to a sub directory of the setups file :

If the subdirectory does not yet exist, from the setups directory, type:

md *directoryname*

Next, change the current directory to the destination directory, and move the file:

cd *directoryname*

move . .*filename*.**prm** .

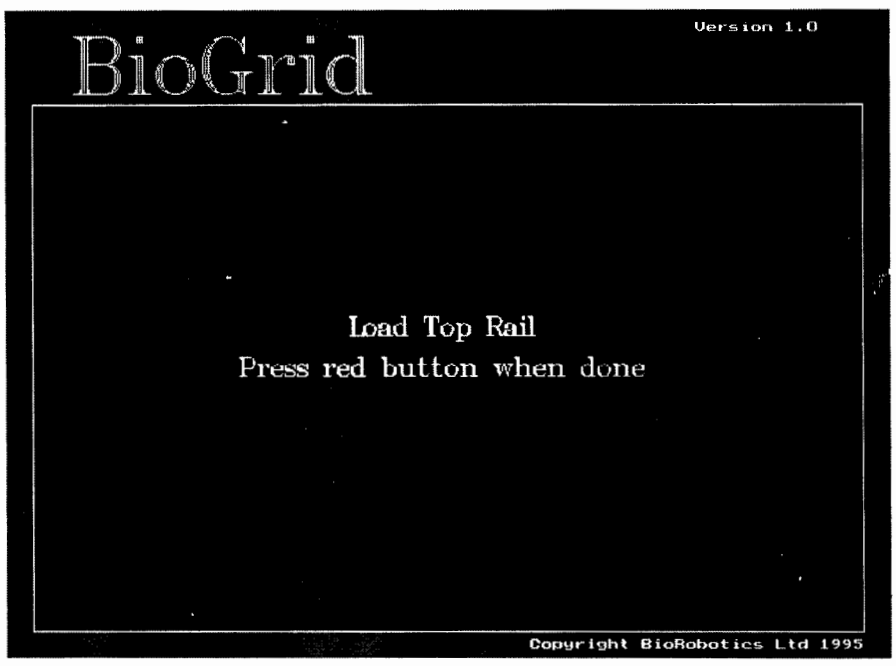
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The Gridding / Replicating Run

When you are happy that the run parameters are correct for the run that you want to perform, select **Go** from the Main Menu to commence the run. Messages on the screen will guide you on how to set up the instrument for the run.

Gridding Run

Upon starting the run, the gridding arm will move to the left side of the instrument, and the correct number of trays in the tray stack will move to the forward position. The following prompt will appear



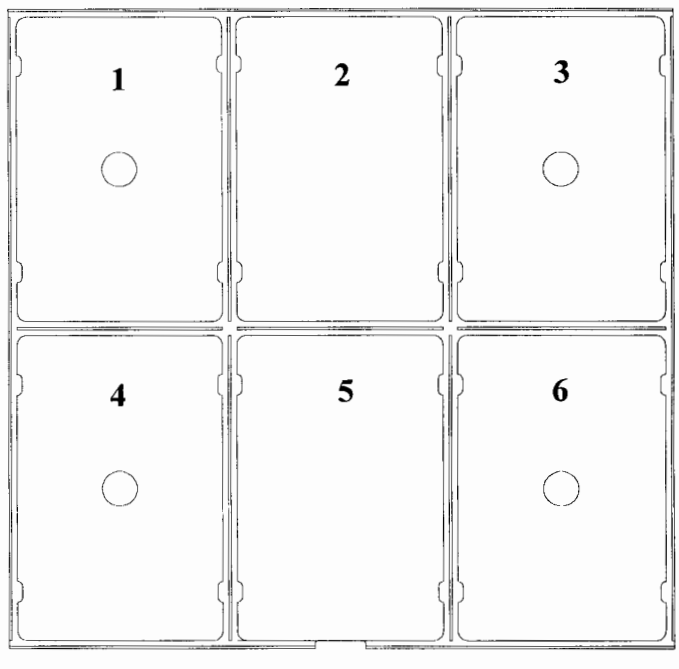
Load Rails

If you are gridding onto a 22cm square filter, load the bioassay plate into the top rail, and tighten the thumbscrew at the front of the tray whilst holding the plate down to prevent it lifting.

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If 8x12 cm filters are to be gridded, place the aluminium adapter plate onto the top rail with the cut-out facing towards the front of the instrument, and load the adapter plate with the required number of Hybaid Colony Picker plates. Care should be taken to ensure that the plates are dropped into the correct slots on the adapter plate, and that the adapter plate has located properly on the top rail with the pins inside the cut-out.

The adapter plate should be loaded as follows:



Front of Instrument

Fill the top rail before continuing on to the second, third and fourth rails as necessary, dependant upon how many filters are to be made. For example if making 8 copies of an 8x12 cm filter there should be 6 on the top rail and two in positions 1 and 2 on the second rail.

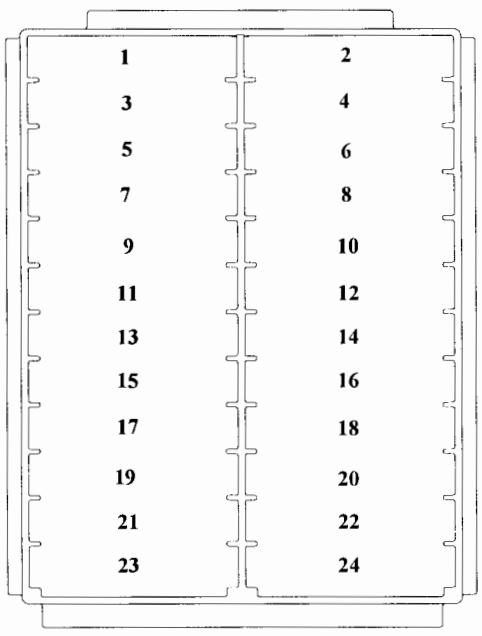
When the top rail has been properly set up, press the large red button at the front right hand side of the instrument. The top rail will move to the back position, and the whole process will be repeated for any remaining rails that are out.

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When the last rail has been loaded, the gridding arm will move to the right hand side of the instrument, and the *Bio*Bank holder will move upwards to the loading position.

Load *Bio*Bank

The screen will prompt you to load the first *Bio*Bank. The *Bio*Bank should be loaded with source plates in the order shown below:



The plates should be thoroughly defrosted, and all condensation should be wiped from the lids. Remember that the plates should be loaded in with a consistent orientation i.e. with the A1 well at either front left or back right for the whole *Bio*Bank , otherwise the results will be hard to interpret.

Lift the *Bio*Bank into the holder, lower it gently to the bottom, and remove the two retaining bars from the front. Press the large red button to indicate to the computer when you are ready to continue.

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Load Gridding Tool

Next the *Bio*Bank moves to the bottom position, the gridding arm moves to a convenient position and a new screen will prompt you to load the correct gridding tool. Check that all the pins are moving freely before loading. Carefully lift the tool up to the dovetail at the end of the gridding arm, slide this into the mating hole in the gridding tool and turn the thumbscrew to tightly clamp the tool onto the arm. Again, press the red button when done.

Load Sterilisation Baths

The tool moves to the top position, and the screen will prompt you to load the sterilisation baths as defined in the set-up.

If the sterilisation is one wash then dry, the bath should be loaded into the right hand position. If the routine is for two washes then a dry, the gridding tool will visit the left hand bath first, then the right hand bath, then the air dryer.

It is easiest to load the baths into position and then to fill them in situ. Decant the washing liquids into the baths up to the level of the step. This ensures that the liquid covers the pins to a height greater than the tallest microtitre plate, but lower than the top of the pin.

Press the red button when this is complete, and the gridding run will commence.

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Replicating Run

There are two types of replicating run available in the set run parameters option, to replicate in the *Bio*Bank or to use the adapter plates.

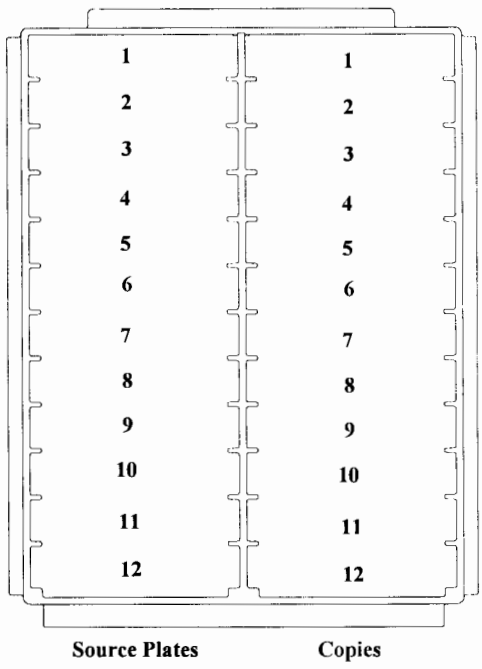
Replicate in *Bio*Bank

This set-up copies the left hand column of the *Bio*Bank into the right hand column, it is convenient for quickly making one copy of a number of plates, but cannot be used for many-to-one or one-to-many copying (i.e. multiple copies of a single plate, library compression, and library expansion).

Upon starting the run, the *Bio*Grid will go through a start up routine similar to that described in the section above, prompting the user to load the *Bio*Bank, the tool and the sterilisation baths, but not the rails since these are not used in this set-up.

The *Bio*Bank should be loaded with defrosted source plates in the left hand column of the *Bio*Bank. The right hand column should be loaded with the required number of destination plates which have been filled with growth media. These plates, as for the source plates, should be loaded complete with their lids.

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Replicate using Adapter Plates

Using this option, the whole *Bio*Bank is available for source plates as in the gridding option. The destination plates are loaded onto the stack of four rails using the adapter plates, in much the same way as the 8x12 cm membranes are loaded (see above).

The *Bio*Grid software will prompt the user to load the rails, the *Bio*Bank, the tool, and the sterilisation baths in the same way as described for the gridding run.

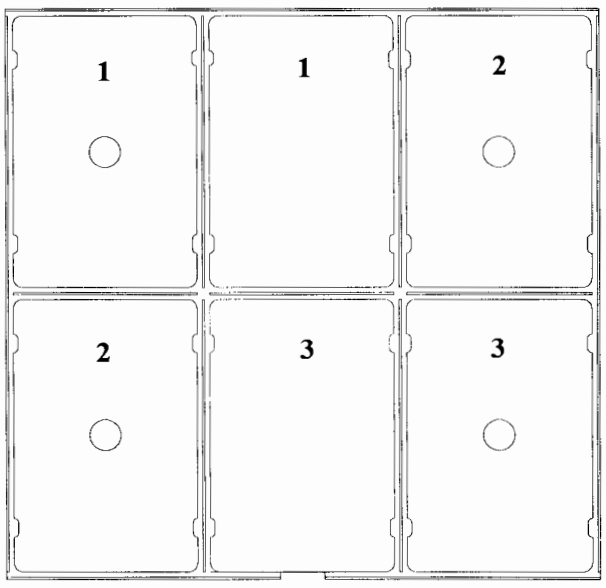
The adapter tray should be loaded with destination plates in the same order as for 8x12 cm membranes, filling from the back left of the instrument. The destination plates should be loaded pre-filled with growth medium, with their lids removed.

For example, when making two copies each of 24 source plates, the copies created on the top rail would be as shown in the following diagram, continuing onto the remaining three rails up to copies of source plate 12. The instrument would then pause at the half way stage and prompt the user to load the four rails with the next 12 destination plates to complete copies of source plates 13-24.

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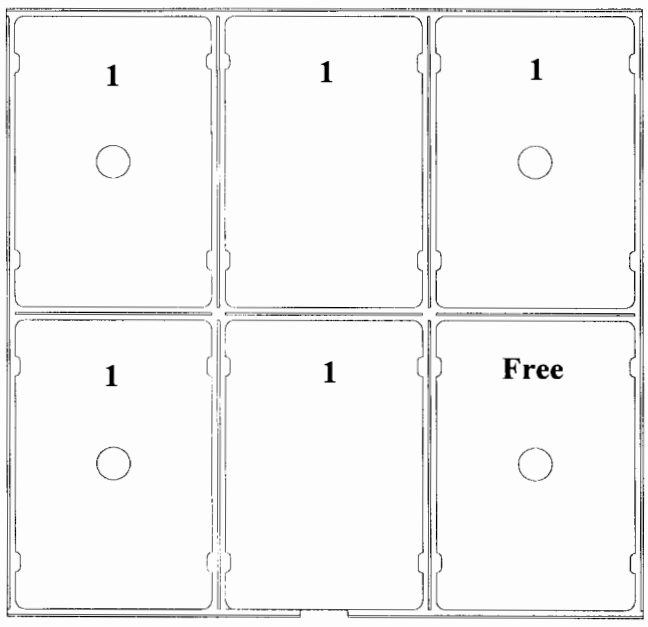
The only exception to this way of loading destination plates is for making 5 copies at a time. In this case the sixth position (front right) is left free, and the copies of the next source plate are put onto the next rail down.

Positions for two copies of each source plate



Front of Instrument

Positions for five copies of each source plate

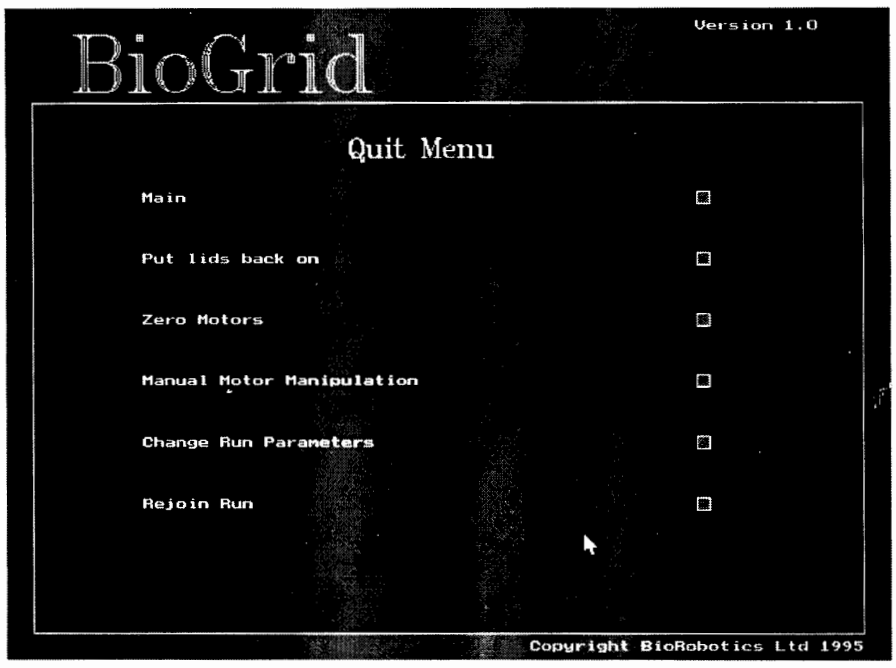


Front of Instrument

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Interrupting A Run

To pause the instrument at any time during a run, press the large red button at the front right hand side of the instrument and **hold it in** until the instrument has finished its current move. The instrument will stop, and the following screen will appear:



The options available here allow you to either tidy up the instrument and return to the main menu (i.e. leave the run), or change certain of the run parameters, and rejoin the run at the point where it was left.

Main

Clicking on the box opposite this option will return the software to the Main Menu.

Put Lids Back On

A 'tidying-up' option before leaving the run and returning to the Main Menu. Selecting this option will cause the hooks to pull the microtitre source plates to the front position, and the vacuum lid lifter will replace the lids onto the plates. The run should **not** be rejoined after using this option.

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Zero Motors

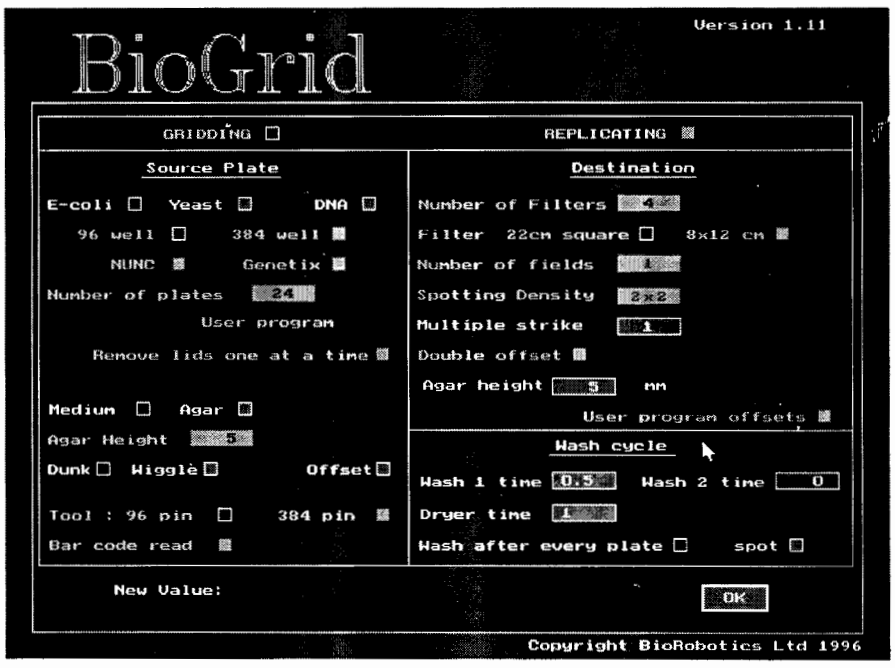
Another 'tidying-up' option. All axes will return to their zero positions. The run should **not** be rejoined after using this option.

Manual Motor Manipulations

Allows individual axes to be moved under user control. This option should be used with great care if the run is to be rejoined.

Change Run Parameters

Upon selection of this option, a screen similar to this appears:



You will recognise this as the Set Run Parameters page, but with many options in grey. These options cannot be changed part-way through a run, as they may cause problems. However, options such as Agar Height, Dunk/Wiggle, Multiple Strike, and washing times can be modified.

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This option is particularly useful when you are not sure about the consistency of the agar height in the plates that have been poured. It is possible to pause the instrument at the point of gridding onto the membrane, and check that all pins are in good contact with the membrane. If they are not in good contact, or are only just touching, then the Agar Height can be adjusted accordingly.

Rejoin Run

Recommences the run from the point at which it was left.

The End of the Run

When the instrument has finished a run, it will prompt the user to unload the tray stack if it was used, and the tool.

If this is the last run using this particular tool then remove it. If you intend to perform further runs using the same tool then leave it on. If you have finished using the *Bio*Grid and intend to turn it off, then it is good practise to remove the tool. You will remember from the Start Up section of this manual that starting the *Bio*Grid software with a tool on the gridding arm will prevent the axis zeroing routine from working, and is likely to damage the tool. Removing the tool when you have finished using the instrument will prevent someone from accidentally starting the instrument with the tool on the gridding arm.

The *Bio*Bank moves to a position that is convenient for unloading, and should be removed if not being used for a subsequent run.

Sterilisation baths can be removed and their contents poured away.

The *Bio*Grid software will return to the Main Menu.

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Gridding Patterns

Selecting the **Output Grid Details** option from the Main Menu when the current run parameters are for a gridding run will put details of which source plate goes where in that grid pattern onto the screen.

This can also be dumped onto a printer by pressing the Y key and following the instructions when given the option:

Output to printer? (press Y/N)

The software will then run through a number of screens showing how the spot pattern is built up.

Two example patterns are included overleaf.

The first illustrates the spot pattern that would be produced with the following set-up:

Number of Plates = 96
Number of Wells = 96
Filter size = 22cm square
Number of fields = 1
Double Offset = off

The labelling A1, A2 etc. assumes that the source plates were loaded into the *Bio*Bank in that orientation (i.e. with the A1 well at the front left).

The second pattern would be produced by the following set-up:

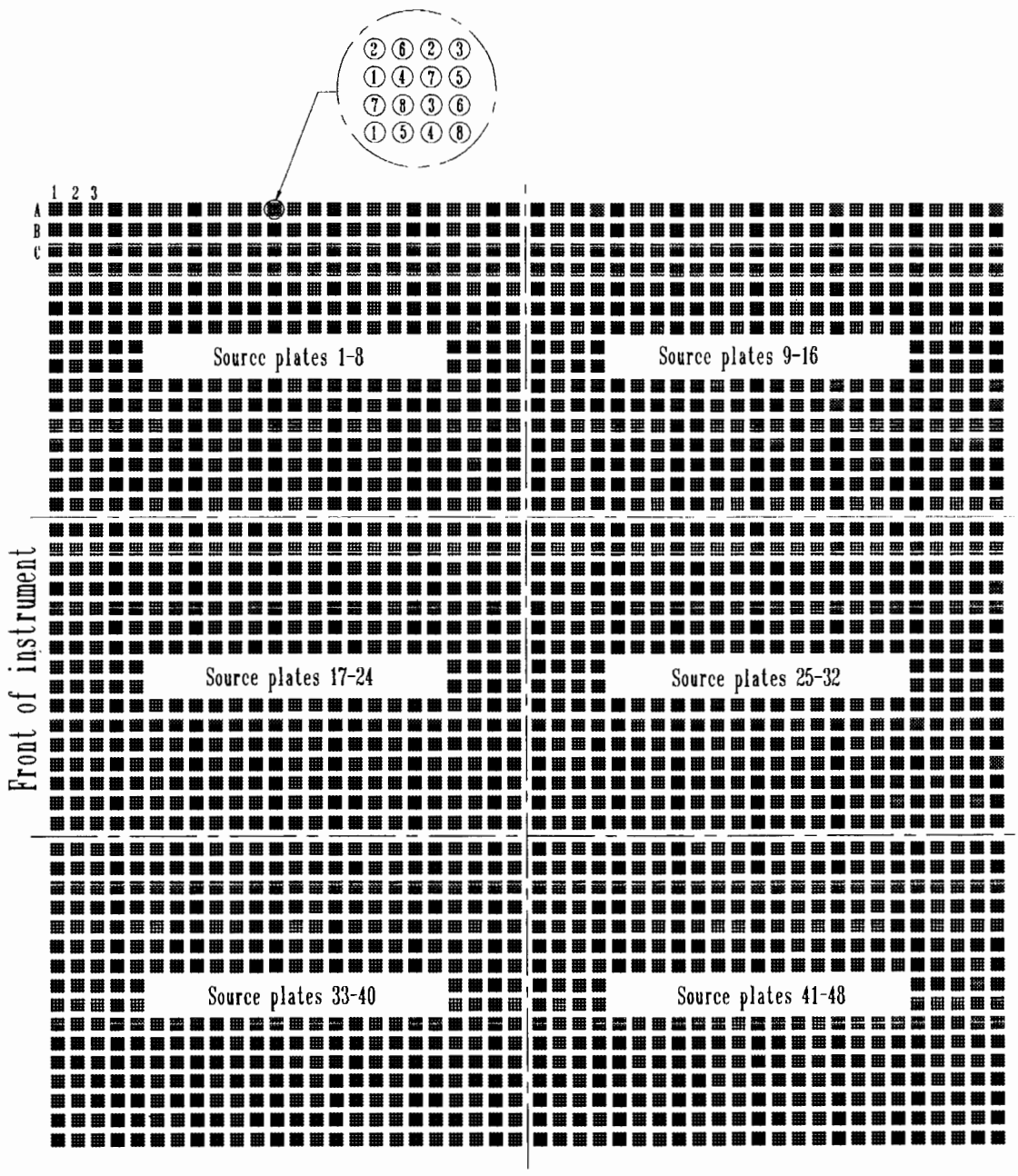
Number of Plates = 48
Number of Wells = 384
Filter size = 22cm square
Number of fields = 1
Double Offset = on

Again, it is assumed that the source plates were loaded into the *Bio*Bank as for the previous example.

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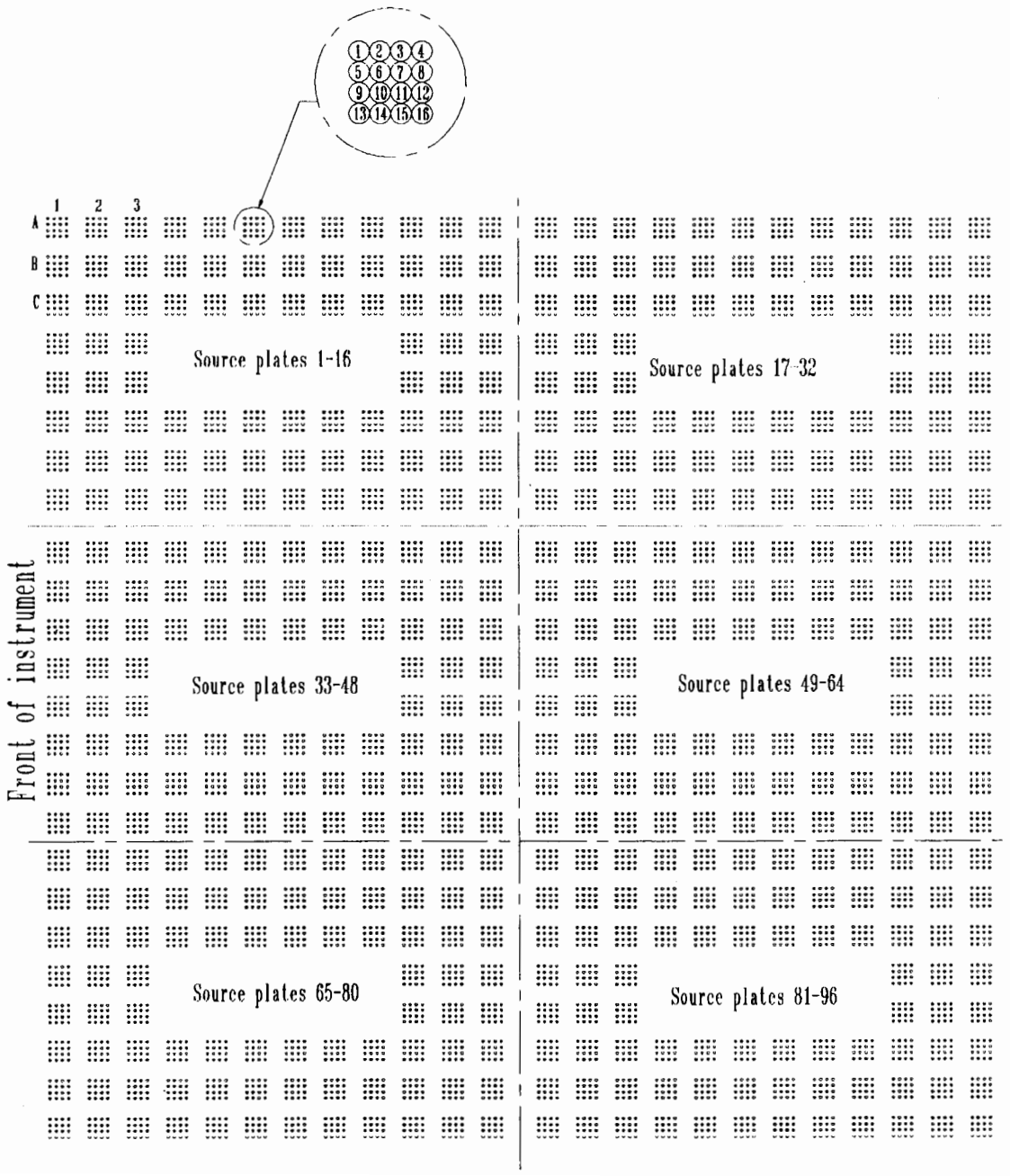
BioRobotics

Default 4x4 Double Offset Pattern



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Example 4x4 Single Offset Pattern



Other set-ups will produce variations on this theme. The six areas available on a 22cm square filter are filled up in this order, unless the filter is divided up using the Number of Fields option (see Set Run Parameters section).

Different grid densities with Double Offset = off are spotted out in the same way as the first example, filling available positions in the grid from left to right, and top to bottom.

Different densities with Double Offset = on will result in different patterns to that in the second example, as defined by the default double offset patterns. It is possible to customise these patterns (see Set Run Parameters section).

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Routine maintenance

IMPORTANT : Always switch the machine off and remove the plug from the electricity supply before attempting any maintenance task.

There is very little in the way of routine maintenance that the *Bio*Grid requires. The components have all been designed with very long mean times between failures, and it is not anticipated that regular replacement of mechanical or electrical parts will be required.

At periodic intervals, swab base plate down with 70% ethanol to minimise the risk of contamination during gridding runs.

Gridding Tool

From time to time the gridding tool can be cleaned using ethanol solution and ultrasonic bath. The tool should be thoroughly dried before being used on the instrument, since liquid on the tool can prevent the pins from dropping.

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*Bio*Robotics

*Bio*Grid version 1.21

Supplement to user manual

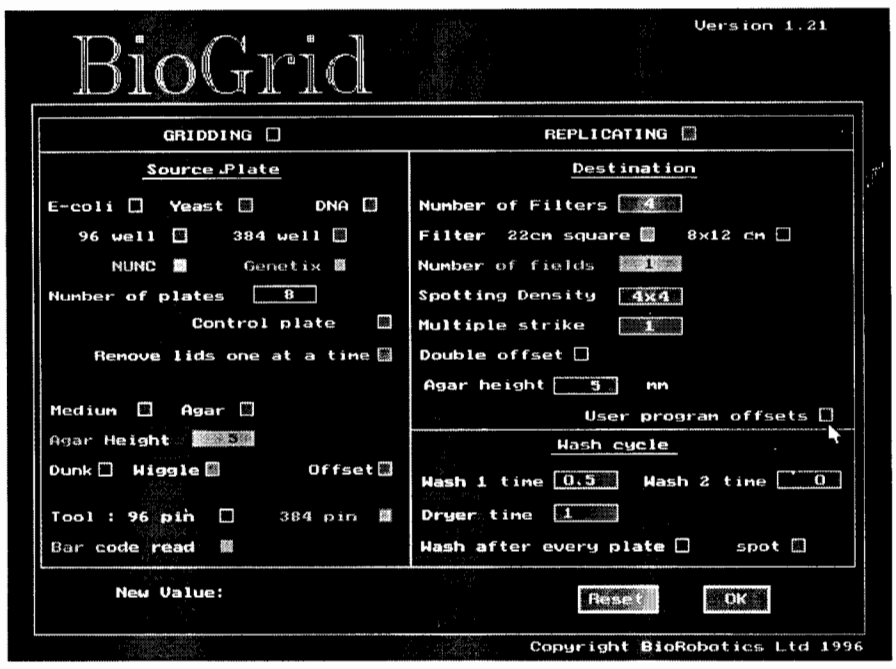
v1.21

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New Functions for v1.21

User Defined Gridding Patterns

Version 1.21 enables the user to edit the default patterns for double offset gridding that are held on the hard disk.



Once the double offset pattern has been modified, then this new spot pattern is applied to all subsequent double offset patterns of that density, not just the current run. You are modifying the **default** pattern.

To change a default double offset pattern :

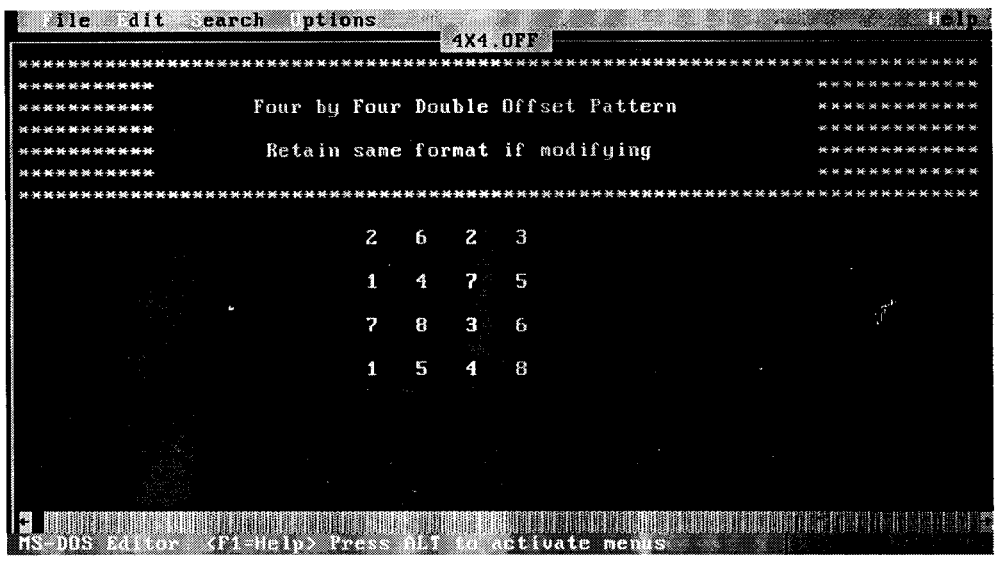
1. From the Set Run Parameters screen, select the required number of microtitre plates, filters, fields and the filter size for the run.

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2. Click on the **double offset** button to turn it from off (blue) to on (red). The text **User Program Offsets** will change from grey to yellow and its associated button from gray to blue to indicate that it is now available for use.

3. Check that Spotting Density is showing the required density. If it is not you can edit the density upwards as discussed in the User Manual.

4. Click on **User Program Offsets.** A screen will appear such as the one shown below, but its content will depend upon the Spotting Density that was showing in the edit box on the Set Run Parameters page when User Program Offsets was selected.



This screen shows the current default double offset pattern for (in this case) a 4x4 spotting density. It shows that plate 1 goes into positions 5 and 13, plate 2 goes into positions 1 and 3 and so on. To change this, move the cursor around the screen and delete the plate numbers, replacing them with your new plate number for that position.

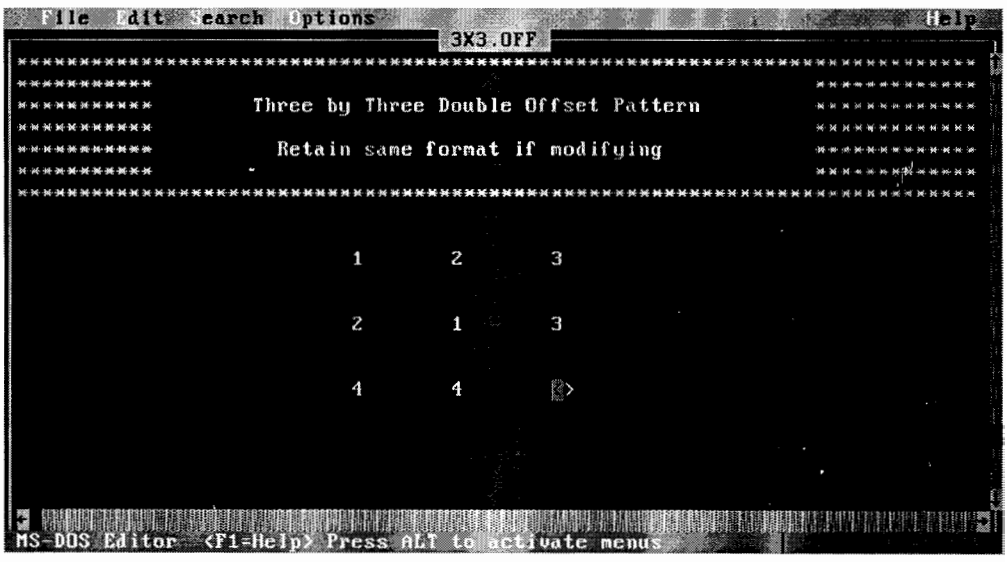
It is very important to keep the same format for the new spot pattern as the old one. Each plate number must be in two positions. For example in the case above, numbers 1 through 8 must appear twice each. **No other numbers are allowed.** Because of the way in which the *Bio*Grid software uses this text file, care must be taken that there are no digits that occur in the file before the spot

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pattern, as these will be recognised as part of the spot pattern, so **do not add text to the file other than changing the spot pattern itself**.

5. When you are happy with the new spot pattern, select **File**, **then Exit**, then answer **Yes** to saving your changes. The computer will then return to the *Bio*Grid software. If the spot pattern that you have entered does not fulfil the criterion mentioned above, then the software will not allow you back into the program, and will return to the text editor. If the new pattern that you have entered is a valid one, then the software will return to the Set Run Parameters page, and the new pattern will be used for all runs that the *Bio*Grid performs using a double offset at that spot density.

For odd number densities (3x3, 5x5, 7x7 etc) a space must be left empty in the pattern:

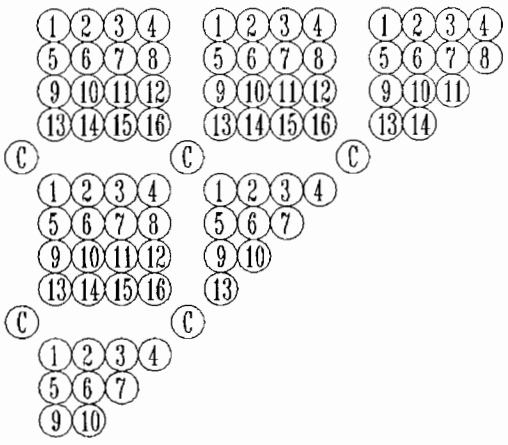


The position of the empty space must be indicated by a less than greater than (<>) symbol, as shown above.

Control Spot

This option puts an extra spot onto the filter into the position shown below (marked C):

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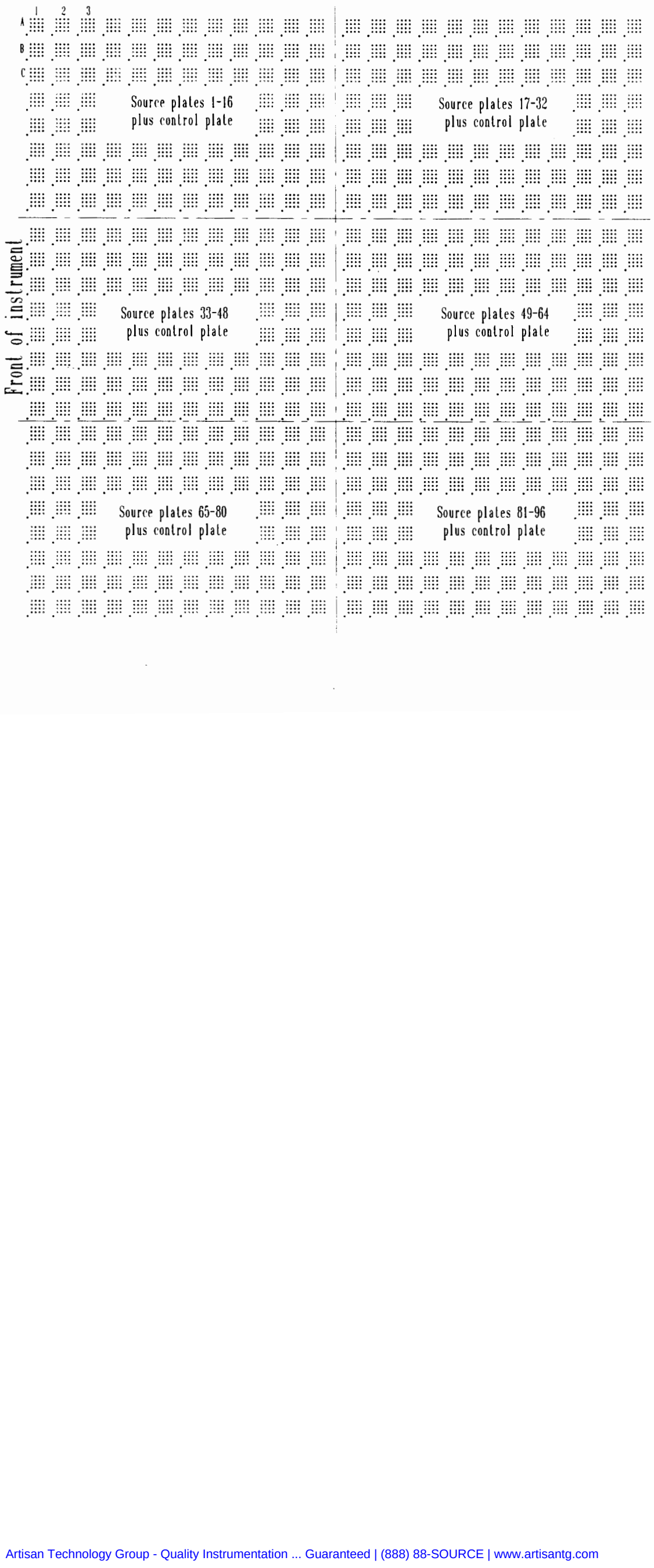


To add a control spot to a spot pattern, click the mouse on the **Control Plate** option within the Set Run Parameters screen, turning the button from blue (off) to red (on). The *Bio*Grid will then expect an additional microtitre plate to be loaded into the *Bio*Bank in the first free slot after the last source plate. The control plate will be pulled out last, after all other plates have been spotted. It will be spotted onto the filter in the position shown (outside of the grid pattern) for all filters. If 22cm filters are being used, the control plate will be spotted into all six positions as shown by the dark spots on the following page.

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Example spot pattern incorporating control



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