X-tractor Gene™

Automated Nucleic Acid Extraction

operation manual

corbett

X-tractor Gene™ Nucleic Acid Extraction System

Operations Manual

Version 4.7.96

X-Tractor Gene™ (CAS-1820)

Operations Manual

by the Corbett Robotics Team

The CAS Series of Robotic Liquid Handling Systems provide a precise and labour saving means to perform a wide range of pipetting tasks. The instruments are highly configurable and can be programmed to set up a variety of tasks.

The software provides a user friendly, easy to follow user interface. The screen layout corresponds to the plates set up on the robot. The status of any component can be examined by clicking it on the screen. Likewise, any alterations are executed by simply pointing to the corresponding hardware on the screen and changing the desired parameters.

This manual is designed to be used as an introduction and quick reference tool to the X-Tractor Gene.

X-tractor Gene™ Nucleic Acid Extraction System

Operations Manual

Version 4.7.96

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X-tractor™ Gene automated nucleic acid extraction

The CAS-1820 X-tractor Gene is designed to automate the extraction of DNA and RNA from a variety of sources with high speed, high throughput and high purity. The system uses an 8-channel pipetting head to extract 96 samples in approximately 60 to 90 minutes depending on sample type. It can accommodate a variety of extraction chemistries, including commercially available kits. The CAS-1820 uses conventional tips for multi-channel pipetting.

The Robotics Software provides a user friendly, easy to follow interface. The Robotics Software automatically detects if the CAS-1820 instrument is connected and switched on. A wizard helps guide the user through the set up process. The screen layout corresponds to the plates set up on the CAS-1820. The status of any component can be examined by selecting the screen image with the mouse pointer. Likewise, any alterations are executed by simply pointing to the corresponding aspect of the hardware on the screen and changing the desired parameters.

The progress of a run can be followed in real time. The software highlights the position of the pipette head on the screen as it moves. The course of the run is tracked on screen via time estimate to run completion and the reaction list, task execution table. Reports are generated before and after a run and are automatically saved for future reference. Laborious calculations are unnecessary as the software calculates the amount of reagents necessary for each protocol.

The Robotics Software can also be run in Virtual Mode. In Virtual Mode, runs can be set up, progress followed and files stored even if the CAS-1820 is switched off or not connected. Therefore, it is possible to configure runs at a remote location. Virtual mode is also a useful tutorial tool. Virtual mode is particularly useful in testing pipetting operations while designing non-standard applications without the use of expensive reagents.

For customer convenience, sample names can be imported from any text-format spreadsheet. The reaction list generated by the robot can also be exported as a spreadsheet and loaded into other programs such as the Corbett Rotor-Gene real time amplification software.

For further information or assistance, please do not hesitate to contact the Corbett Team or your nearest Corbett distributor.

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1 Hardware

Congratulations on your purchase of a CAS-1820 X-tractor Gene. This section covers the unpacking of the CAS-1820, and the accessories that are delivered as standard with your robot. Please refer to Setting Up for details on how to get started with your CAS-1820.

1.1 Unpacking

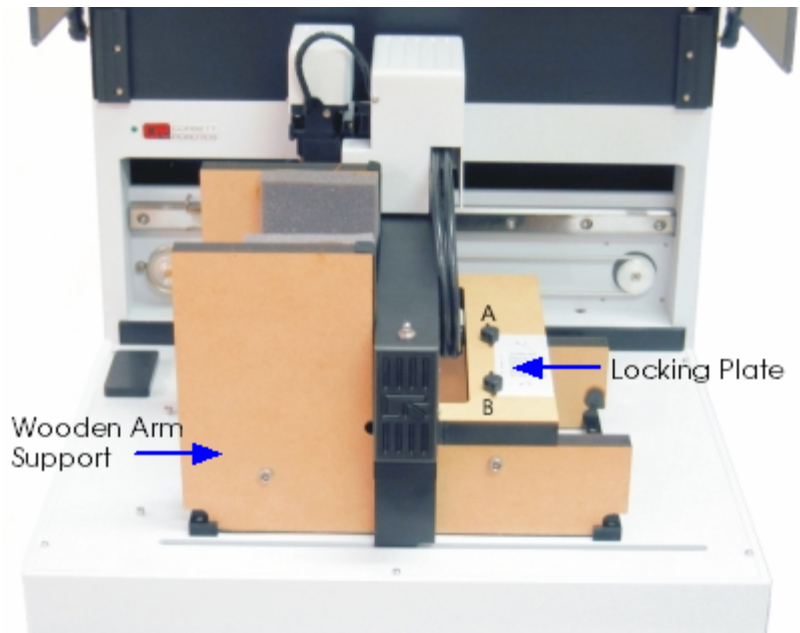
The Robot

Great care and attention have been taken to design the packaging for the CAS-1820 X-tractor Gene to ensure that the instrument arrives in the same condition as it left the factory. Please unpack the instrument following the instructions provided in the lid of the CAS-1820's shipping container. These instructions should also be used when packing the instrument.

If possible please do not discard the packaging. The packaging must be used if the instrument is to be returned for servicing. As you unpack the instrument please ensure that the contents of the box match the packing list included with the CAS-1820.

Once the robot is removed from the shipping container and lifted free of its foam base, the arm support must be removed. To remove the arm support, follow these simple steps:

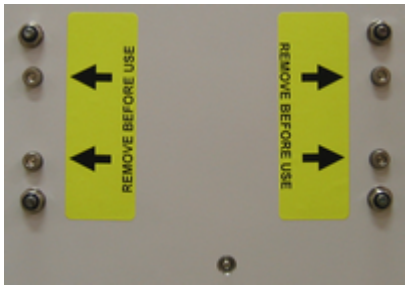
1. Unscrew the screws marked A and B on the locking plate to right of the arm.
2. Slide the arm (and pipetting head) all the way to right.
3. Lift the wooden arm support off the table of the robot.
4. Replace the locking plate and screws A and B into the arm support.



The most important component of the packaging is the wooden arm support. This must be used when ever the CAS-1820 is moved from one location to another. Failure to do so will void warranty.

The Vacuum Pump

For transportation, the vacuum pump motor is locked into place within the vacuum pump station. To release the motor from the locking bolts first turn the vacuum station on to its side. On the underside of the vacuum pump station you will see the following:



Please read the instructions and then remove all four bolts using the provided hex-drive tool provided with the robot.

1.2 Box Contents

The CAS-1820 X-tractor Gene shipping container includes the items listed on the packing slip, typically:

- 1 x CAS-1820
- 1 x Vacuum Control Station (VS-01) (w/ carbon cap/filter/connecting hoses)
- 1 x Pinch Valve (PV-01)
- 1 x Tip Chute
- 1 x IEC Mains Cable (Australia Only)
- 1 x Accessory Power Lead
- 1 x Serial cable 9-pin male to 9-pin female
- 2 x Accessory Cables 15-pin male to 15-pin female
- 1 x Software Installation CD
- 1 x Reagent Tub Plate
- 1 x Auxiliary SBS Plate
- 1 x 270mL (Large) Reagent Tub + Cover
- 3 x 170mL (Medium) Reagent Tub + Cover
- 2 x 70mL (Small) Reagent Tub + Cover
- 1 x Vacuum Chamber
- 1 x Vacuum Sink
- 1 x Low Skirt Transfer Carriage
- 1 x High Skirt Transfer Carriage
- 1 x 17 mm Riser Block
- 1 x 4 Litre Vacuum Waste Bottle (w/ lid and quick connect couplings)
- 1 x Y-hose (w/ couplings)
- 1 x Separator Plate (96-well low skirt)
- 5 x Lysis Block (2mL x 96-well)
- 5 x Elution Plates (Cluster Tubes x 96, grid referenced)
- 5 x Elution Plate Strip Caps (12 x 8 per packet)
- 5 x Capture Plates
- 10 x Racks of fine bore tips 96 x 200uL
- 5 x Tip Disposal Boxes
- 5 x 96-well plastic, plate seals

A computer may or may not be delivered with the instrument; however, a computer is required to operate the CAS-1820 X-tractor Gene.

1.2.1 Power Leads and Cables

IEC Mains Cable

A standard 3-wire IEC mains cable is required to connect the CAS-1820 X-tractor Gene to mains power. Typically, these cables are the same used to provide computers with power. The cable must be a 3-wire cable.

The CAS-1820 is available in a number of power options, please see the Specifications.

Accessory Power Lead

The accessory power lead is required to connect CAS-1820 X-tractor Gene to the vacuum control station. The cable is of the 3-pin male to 3-pin female 'straight through' kind. The accessory power lead is plugged into the rear power port of the CAS-1820 and to the side port of the vacuum control station.

Serial Cable

A 9-pin serial cable is required to connect the CAS-1820 X-tractor Gene to the host computer's RS-232 serial port. The serial cable is of the 9-pin male to 9-pin female 'straight through' kind. The serial cable port is located on the rear of the robot adjacent to the accessory ports.

Accessory Cable

Two accessory cables are required to connect the CAS-1820 X-tractor Gene to the Pinch Valve and to the Vacuum Control Station. The accessory cable is of the 15-pin male to 15-pin female 'straight through' kind. The pinch valve and the Vacuum Control Station can be connected to any two of the three available accessory ports on the rear of the CAS-1820.

1.2.2 Tip Chute

Tip Chute

The tip ejector chute is located to the rear-left of the CAS-1820 X-tractor Gene and is held in place by a locating block. This arrangement facilitates easy placement and removal of the tip ejector chute. The CAS-1820 tip ejector chute is manufactured from high quality aluminium which is Teflon coated to give the block a hard wearing, durable black finish. The non-stick property of Teflon makes it very difficult for tips to stick in the chute. The tip ejector chute can be washed in bleach or water. The tip ejector can be autoclaved if necessary.

1.2.3 Software CD and User Manual

The Software CD contains the latest Robotics Software required to control the CAS-1820 X-tractor Gene. It also contains a multi-media demonstration on the features of the CAS-1820, and the user's manual as a PDF document. The PDF version of the user's manual is generated from the contents of the electronic help files embedded in the software. The latest copy of the manual and software is available from our website at www.corbettrobotics.com.

Also delivered with the CAS-1820 X-tractor Gene is a laminated Quick Start Guide. Please place this guide near your CAS-1820 to ensure frequent and easy access (under the CAS-1820 is often a convenient place to store the guide). The Quick Start Guide is a summary on how to get started with the CAS-1820. For full functionality description, the user's manual or the help files should be consulted.

To ensure you are always using the latest Robotics Software please visit the Corbett Robotics Pty. Ltd. website at www.corbettrobotics.com. Occasional software updates are required to provide new functionality. It is the user's responsibility to update software to ensure the instrument operates to the best of its ability.

The Corbett software team makes all efforts to ensure the software releases are error (bug) free. On occasion, however, software is released to fix bugs that have been discovered in the software. Beta-software will always be released prior to full release. Corbett Robotics encourages all our users to trial beta-software to help us ensure the final release is defect free.

Corbett software updates are free of charge to all users of Corbett instruments. We feel this is the best way to give our users fastest access to new features. This of course means that new versions can be released several times per year.

1.2.4 Reagent Support Plates and Tubs

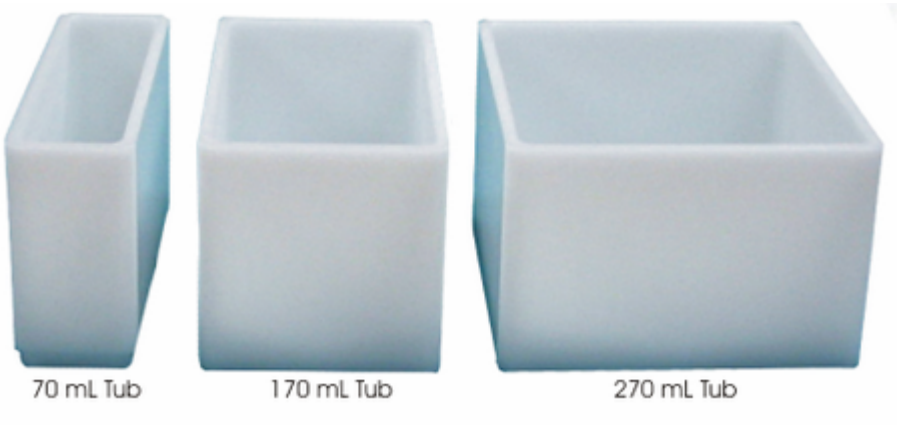
The CAS-1820 X-tractor Gene is delivered with two Reagent Tub Support Plates and six Reagent Tubs. One plate is always positioned in the front-left reagent position of the workspace. This is the default Reagent Support Plate and Tubs. The second plate conforms to the SBS standard footprint for tip racks and can be positioned in any one of the four tip rack positions of the workspace. The default position for this plate is in the front-left SBS position (C1).

1.2.5 Default Reagent Tub Support Plate and Tubs

The Reagent Tub Support Plate is manufactured from high quality aluminium. The white feet are made from high temperature silicone rubber. To keep reagents cold, the plate can chilled in a refrigerator or freezer before placement on the CAS-1820. The support plate can be washed in bleach or water. Please note that some solvents or salts may stain the surface. The support plate can be autoclaved if necessary.

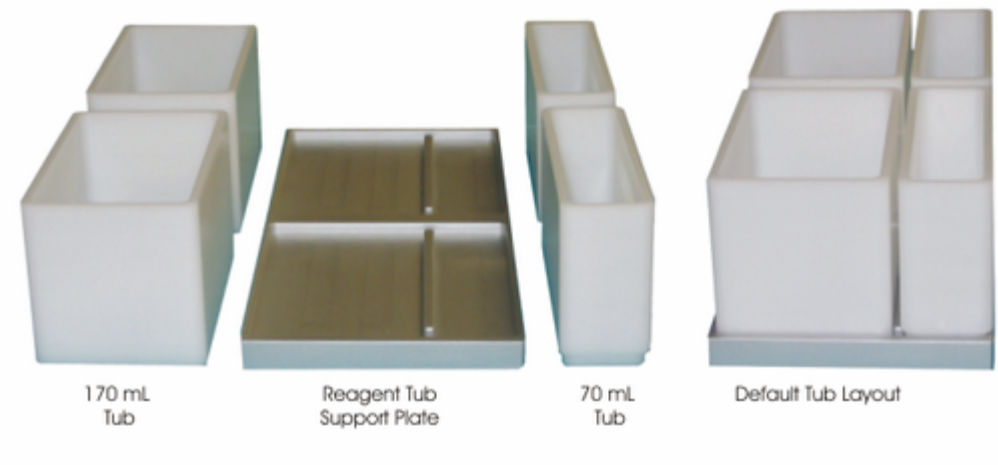
Three Reagent Tubs types are supplied with the CAS-1820. The tubs are disposable, made from high quality polypropylene and differ in their total volume capacity: 70 mL (PN 2137), 170 mL (PN 2136), and 270 mL (PN 2314). Also available as an option are reusable tubs manufactured from Delrin acetal plastic: 70 mL (PN 2365), 170 mL (PN 2364), and 270 mL (PN 2363). Tubs manufactured from Delrin acetal plastic can be washed in bleach or water. DO NOT AUTOCLAVE THESE TUBS!

Reagent Tubs



The default arrangement for the reagent tubs on the reagent support plate is depicted below. In this arrangement, two 70 mL and two 170 mL tubs populate the reagent plate providing tub-space for four reagents.

Reagent Tubs and Default Layout



Alternatively, the 170 mL and 70 mL reagent tubs in the front or back rows can be replaced with a single 270 mL tub to give for example, the following arrangement. This reduces the number of tubs available, but dramatically increases the available volume of one reagent to 270 mL.

Alternative Tub Arrangement

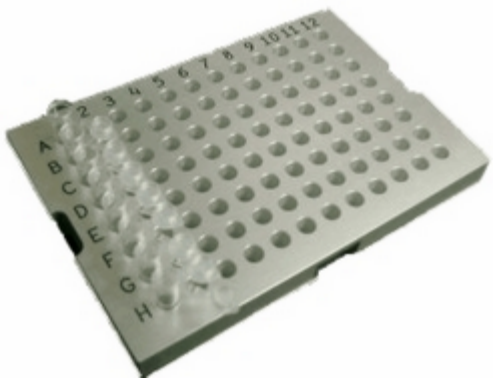
SBS Reagent Tub Support Plate and Tubs

The SBS Support Plate is manufactured from high quality aluminium and anodised. The white feet are made from high temperature silicone rubber. To keep reagents cold, the plate can chilled in a refrigerator or freezer before placement on the CAS-1820. The support plate can be washed in bleach or water. Please note that some solvents or salts may stain the surface. The support plate can be autoclaved if necessary. The default position for this plate is in the front-left SBS position (C1). The picture below is just one example of tub arrangements for this plate. This example shows a combination of the 270 mL and 70 mL tubs. It is possible to place other combinations of tubs on the support plate: 1 x 170 mL and 2 x 70 mL, 2 x 170 mL, and 4 x 70 mL. The maximum number of tubs that can be combined onto the workspace is eight: 2 x 170 mL and 6 x 70 mL. The policy is to place the largest tub to the left as demonstrated in the image of the plate layout screen.

SBS Format Reagent Plate

Optional Standard Footprint Block

In addition to the SBS format reagent support plate, the CAS-1820 will also accept the standard 128 x 86mm footprint block. These blocks hold 96 tubes of dimensions similar to the 200µL PCR tube. These blocks can be used to hold strip tubes, unskirted or half-skirted PCR plates. This optional block can be washed in bleach or water. Please note that some solvents or salts may stain the surface.



200 µL PCR tubes - Single

These tubes are typically used to hold samples and reactions.

200mL PCR tubes - 8 Strip

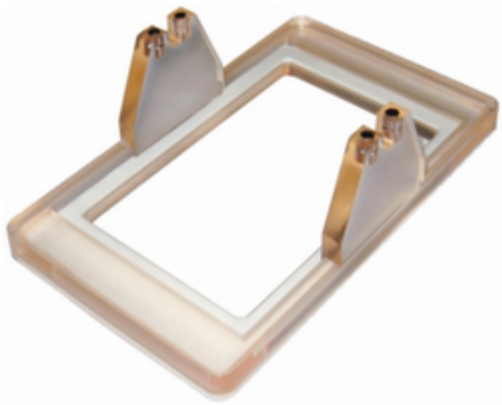
These tubes are typically used to hold samples and reactions.

1.3 Transfer Carriage

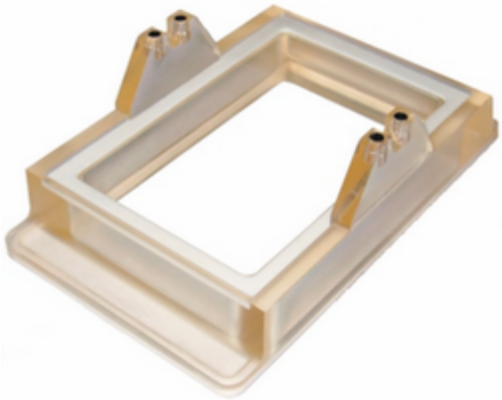
The CAS-1820 X-tractor Gene is supplied with two Transfer Carriages to suit both Low Skirt and High Skirt style Capture Plates (e.g., Whatman, and Invitek). The Low Skirt transfer carriage suits the DNA/RNA capture plates delivered with your CAS-1820. The High Skirt transfer carriage is suitable for capture plates like Macherey-Nagel, Promega, and Corbett High Yield.

The transfer carriages are made from epoxy resin and can be cleaned with warm water and bleach. Under no circumstances should the transfer carriages be autoclaved.

Low Skirt Transfer Carriage



High Skirt Transfer Carriage



1.3.1 Capture Plates

The CAS-1820 X-tractor Gene is supplied with five Low Skirt capture plates. The terms 'Low Skirt' and 'High Skirt' are simply categories to which capture plates have been assigned to accommodate the many commercially available types of plates. The Skirt refers to the plastic shroud extending from the top of the capture plate in a downwards direction towards the filter tips of the capture plate. The shroud on the High Skirt capture plates is approximately 15 mm in length, while on the Low Skirt capture plate the shroud is approximately 30 mm in length. As an open platform, the CAS-1820 accommodates many commercially available capture plates.

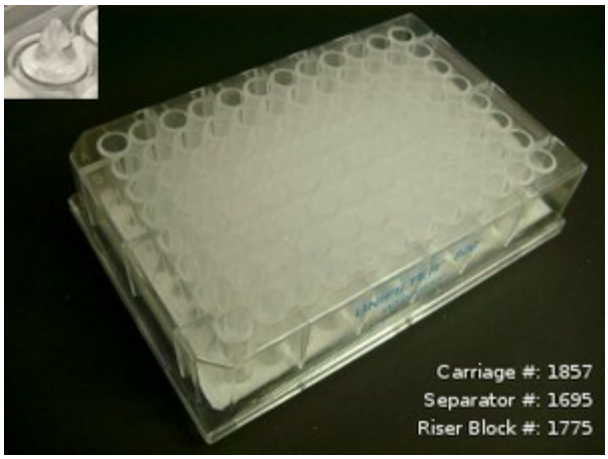
Capture Plate Skirt and Low Skirt Comparison High

Low Skirt (800mL x 96 well) Whatman GF/B

High Skirt (1,400mL x 96 well) Corbett Max Yield

Capture Plate Parts Cross-reference

Capture plates must be used in conjunction with the appropriate Transfer Carriage, Separator Plate, and Riser Block. When the desired Capture Plate is selected in the software, the accompanying picture references the part numbers for the matching Transfer Carriage, Separator Plate and Riser Block. This selector is accessed in the software using the mouse to right click over a reaction or sample plate displayed in the workspace software interface, and then choosing 'Change Plate Type'.



1.3.2 Separator Plate

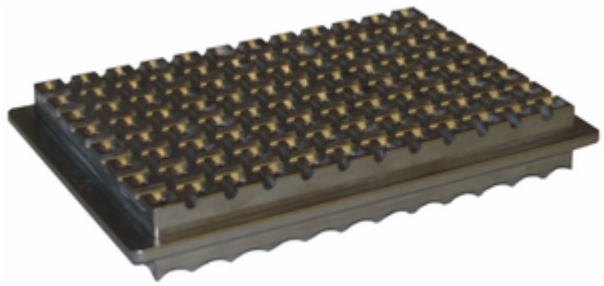
The CAS-1820 X-tractor Gene is supplied with one 96-well separator plate (PN 1695) for use with the Low Skirt transfer carriage and the supplied capture plates. As options, two additional separator plates are available: a 96 well format plate (PN 1696) and a 48 well format (PN 1697) both of which are used in conjunction with the High Skirt Transfer Carriage. These plate formats are suitable for use with plate formats such as the Macherey-Nagel NucleoSpin® 96-well 8-strip systems.

Separator plates are marked with an 'Up Side' for correct insertion into the waste sink. When choosing appropriate capture plates in the Robotics Software, the available photograph of the capture plate also includes the part numbers for the corresponding Transfer Carriage and Riser Block. See Appendix E for the summary of corresponding CAS-1820 parts by part number.

The CAS-1820 separator plate PN 1695 is manufactured from high quality aluminium which is Nickel/Teflon coated to give a hard wearing, durable finish. The separator plates PN 1696 and PN 1697 are manufactured from acetal plastic, a plastic material that is hard wearing and durable. All of the separator plates can be washed in bleach or water, but must be thoroughly washed in

distilled water and rinsed with MilliQ water before use to remove soap or chlorine residue. Please note that some solvents or salts may stain the Nickel-Teflon surface of PN 1695.

Separator Plate



1.3.3 Lysis Block

Lysis Block

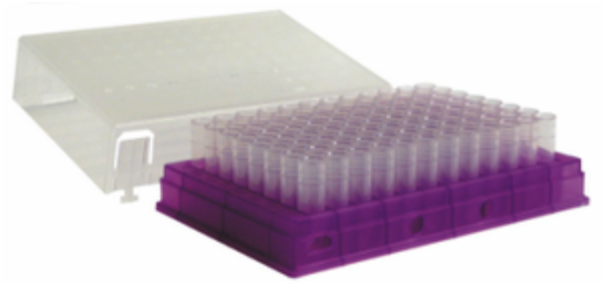
The CAS-1820 X-tractor Gene is supplied with five Lysis Blocks. There are many suitable types of Lysis Blocks available from a range of manufactures. The list of known compatible Lysis Blocks is available through the 'Change Plate Type' selector. This selector is accessed in the software using the mouse to right click over a reaction or sample plate displayed in the workspace software interface, and then choosing 'Change Plate Type'.

Shown left, is an example of a typical Lysis Block. This is an Axygen Deep Well 2 mL lysis block.

1.3.4 Elution Plates

Elution Plate

The CAS-1820 X-tractor Gene is supplied with five elution plain-tube racks. The elution tubes are in a cluster rack format of 96 individual tubes each having a maximum volume of 650 µL. A single lid covers all 96 tubes in elution rack, however 8-well strip caps are also available to seal the individual tubes. There is also an option to use screw cap, non-grid referenced, and 2D cipher tubes compatible with bar-coding applications.



1.3.5 Tips and Tip Racks

Tips and Tip Racks

The CAS-1820 X-tractor Gene is delivered with ten racks of 96 x 200 µL tips. These tips are available as filtered, racked, and sterile or unfiltered, racked, and sterile. We recommend the use of filtered tips to avoid potential cross-contamination. Corbett Robotics Pty. Ltd. recommends using Corbett Robotics 200 µL filtered fine-bore tips as depicted below [lid removed for photograph].

Tips other than those recommended can be used on the CAS-1820 provided the tips have a compatible spacing, pick up height, hub size, and are of the same volume. However, Corbett Robotics Pty. Ltd. does not guarantee the pipetting precision or accuracy if tips other than those recommended are used.

1.3.6 Pipettor Service Kit

All pipetting instruments require regular servicing to maintain precision and accuracy. The CAS-1820 X-tractor Gene pipettes require regular servicing: typically every 12 months when processing 1 plate/day, 6 months when processing 2 to 3 plates/day, and 3 months for 3 or more plates/day (approximately every 300,000 operations).

A pipettor service kit is supplied with the CAS-1820. The kit and its instruction sheet will allow the user (or a trained service technician) to conduct the pipettor service.

1.3.7 Tip Disposal Boxes

Tip Disposal Boxes

The tip disposal boxes are provided as a way of collecting discarded tips on the side of the CAS-1820 X-tractor Gene. These tip boxes will hold approximately 400 used tips. However, due to the way tips fall into the box, occasional shaking of the partially filled tip box may be necessary to avoid tips

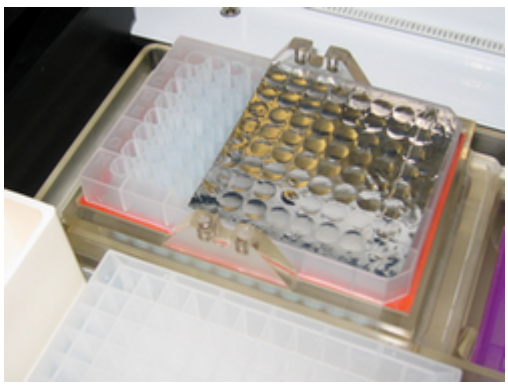
stacking up in the tip ejector chute. Using the supplied tip collection boxes is optional. Alternative boxes can be used at the user's discretion and may better suit the individual situation.

For an alternative tip disposal method, please consider the robot's position. Positioning the robot close to the left hand edge of a laboratory bench or table will allow tips to be readily ejected out of the robot into a biohazard waste bin or bag.

1.3.8 Plastic Plate Seals

Plastic Plate Seals

The CAS-1820 X-tractor Gene relies on vacuum to trap nucleic acids on silica membrane while contaminants pass through the membrane as waste. For this process to achieve optimal outcomes in terms of nucleic acid purity and yields it is imperative that wells not used during the extraction process are sealed. Currently, the best method for sealing unused columns is to cover them with self-adhesive plastic or PCR foil. The picture below depicts an extraction process where only 4 columns of samples are being extracted; the remaining columns are sealed with PCR aluminium foil.

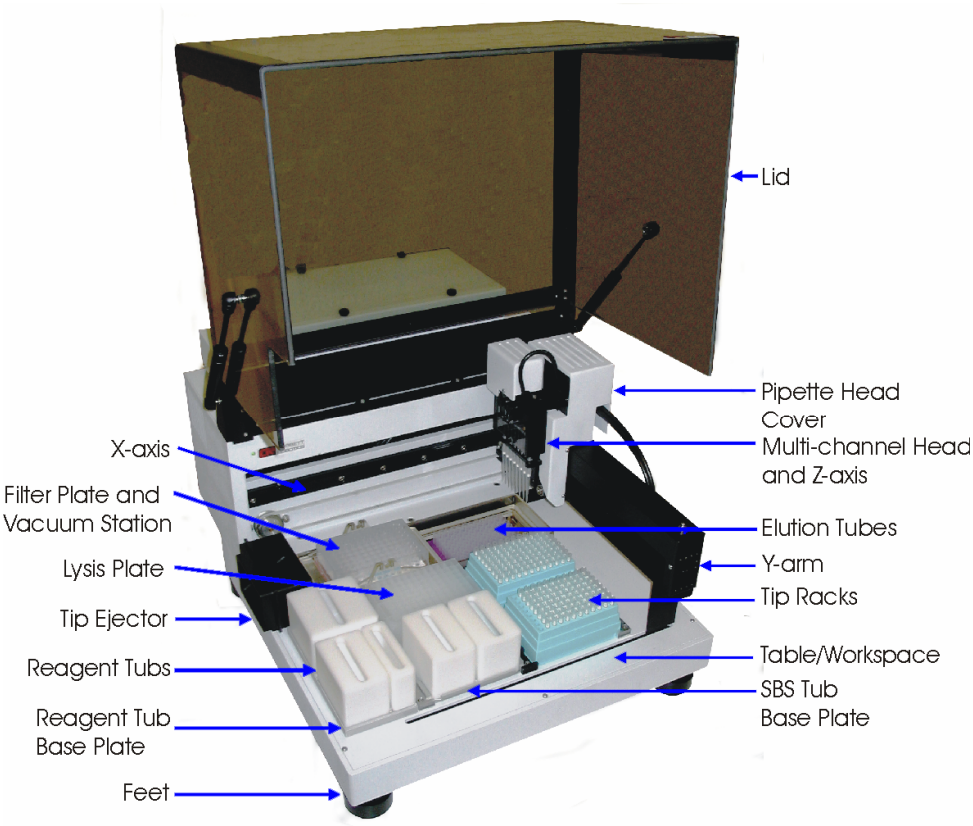


PCR foil can also be used to seal lysis blocks containing samples and lysis/digest buffer while the sample is lysed and the proteins digested. The film covered lysis block can then be placed directly

onto the robot without removing the foil as the pipette tips will penetrate the foil while moving the lysed samples to the capture plate.

1.4 The Instrument

The CAS-1820 X-tractor Gene is a high-precision instrument designed primarily for the extraction and purification of DNA and RNA. The instrument has a number of significant elements which are shown in the figure below.



The paint used on the CAS-1820 is two-part epoxy paint. It provides a very hard wearing and UV resistant finish. The paint may yellow slightly over time. Certain cleaning solvents may also stain the paint. The painted surfaces should be cleaned using a soft cloth with water. The paint finish has been tested to be resistant against bleach, ethanol, methylated spirits and most liquids commonly used in a laboratory environment. However, all spills of any sort should be wiped up immediately to avoid any damage to the paint. Solvents usually associated with painting such as mineral turpentine, acetone or paint thinners should never be used on the instrument. Due to the hard nature of the paint, sharp objects should never be used on the painted finish as chipping of the paint may occur. Similarly, dropping heavy items (such as loading blocks) onto painted surfaces is likely to result in chipping. Damage to the painted surface by solvents and incorrect care is not covered by warranty.

Most black components (such as the table rails) on the CAS-1820 are manufactured from anodised aluminium. The anodizing provides a hard durable finish. The black finish may stain if wiped with certain cleaning products. The tip ejector chute has the added protection of being Teflon coated.

The arm (y-axis) on the robot provides support for the pipetting head. Under no circumstances should the user ever apply any force to the y-arm. This may misalign the arm resulting in pipetting errors.

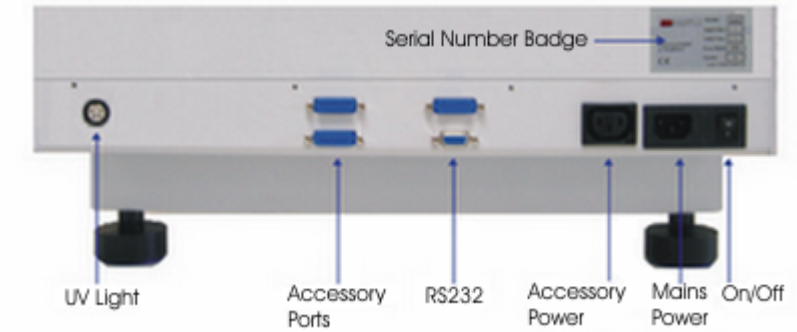
The pipetting head is the black mechanism that can be seen under the pipetting head cover. It consists of a motor driven backlash-compensated pipetting mechanism. This mechanism is similar to most hand pipettes. The pipetting head can be dismantled for the purposes of servicing.

The feet are height adjustable and have a non-slip silicone base to ensure that the CAS-1820 is securely positioned on a laboratory bench. Do not attempt to slide the instrument on a bench without lifting, as damage to the silicone base of the feet may result.

The lid of the CAS-1820 is manufactured from impact resistant polycarbonate. Please refer to the lid section for further details.

All electronics are housed in the rear of the machine or in accessories. The instrument has a number of moving parts. These moving parts are the X, Y, Z-axis as well as the pipetting head. Maintenance of these parts is low. Safety is a primary concern and thus the lid has an electronic interlock. The lid must be closed for the normal operation of the instrument.

On the rear of the CAS-1820 are a number of connectors located on the accessory panel. These connectors are identified below. Connecting the CAS-1820 to the computer and accessories is further described in Setting Up.



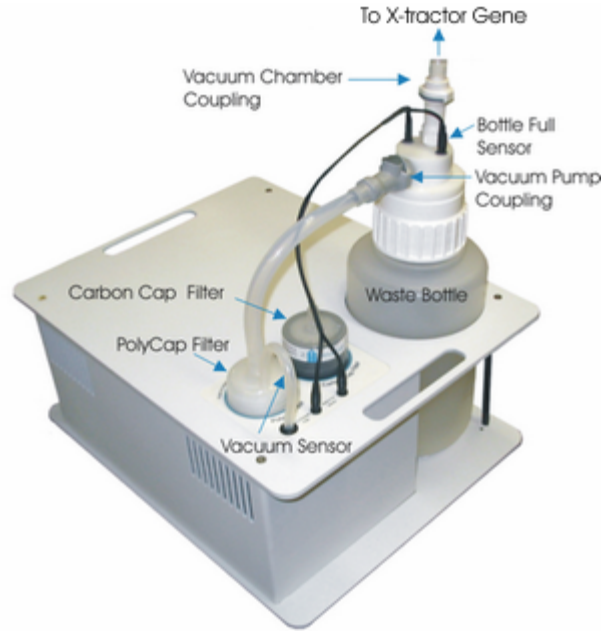
1.5 Vacuum Control Station

The Vacuum Control Station is an integral component of the CAS-1820 X-tractor Gene. The DNA/RNA purification process of the CAS-1820 is vacuum controlled by the Robotics Software. The vacuum pressure is precisely controlled by the user through the Robotics Software. The vacuum control station communicates with the CAS-1820 via an accessory cable connected to a 15-pin port located on the vacuum control station and an accessory port located on the rear of the CAS-1820. Power for the vacuum station is provided through the accessory power lead. The accessory power lead is connected to the accessory power port on the rear of the Instrument.

Vacuum Station Ports

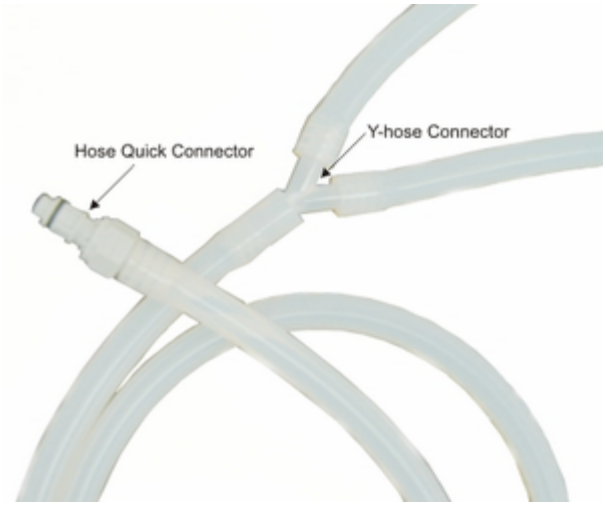
Housed in the Vacuum Control Station is a four litre Waste Bottle. The waste bottle is connected to the washing and elution station by a silicon hose that branches just before passing through the Pinch Valve. Within the waste bottle are two Teflon coated sensor electrodes. Should the bottle fill with waste and this liquid come into simultaneous contact with the two electrodes, the computer will pause the run until the waste bottle is emptied and the waste indicator is reset. The wash bottle has a maximum waste capacity of three litres.

Vacuum Station



Vacuum Bottle Cap

Vacuum Waste Hose and Connectors



1.6 Pinch Valve

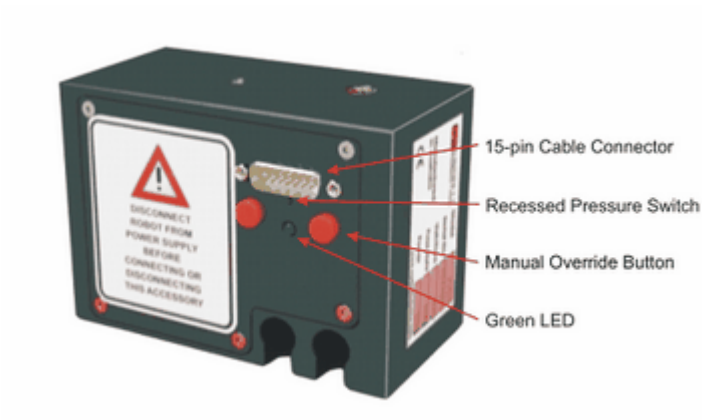
The CAS-1820 X-tractor Gene is equipped with separate Waste and Elution Chambers. The Pinch Valve controls which chamber will be subjected to vacuum at different points during the DNA/RNA purification cycle. The pinch valve can be set to any of three positions: 1) waste chamber open, 2) elution chamber open, 3) both chambers open. Both chambers open is the default position for the pinch valve and is automatically selected at power on. This position protects the silicon hoses controlled by the pinch valve from potential damage from constant pressure when the machine is inactive or turned off. The pinch valve defaults to both chambers open at the end of each run.

The pinch valve is software controlled via an accessory port using a 15-pin accessory cable. It is also connected to one of the accessory ports located on the rear of the CAS-1820. Movement of the valve is in response to specific software commands generated by the vacuum extraction wizard.

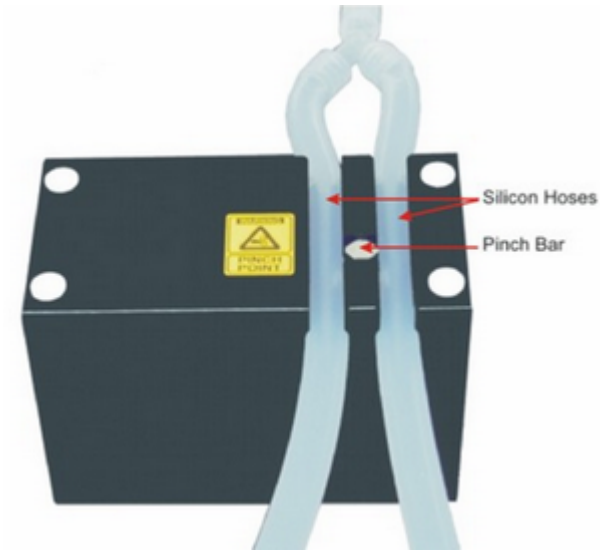
The pinch valve is equipped with manual over-ride buttons located below the 15-pin connector port on the front of the pinch valve. Pressing either button will move the pinch valve in the direction of the button pushed and opens the vacuum to the opposite station. Pressing both buttons simultaneously will move the pinch valve to the centre position and open the vacuum to both stations.

WARNING: The override buttons should not be used during a normal extraction process.

CAS-1820 Pinch Valve Components (Front)



CAS-1820 Pinch Valve Components (Base)



Programming Limits

The Pinch Valve is delivered with predetermined travel limits to constrain the distance the pinch bar moves in either direction to pinch the silicon hose. To adjust the travel limits do the following:

Ensure that the silicon hose is correctly inserted in the Pinch Valve. This is achieved by stretching the silicon hose to reduce its diameter and while stretching the hose, inserting the hose into the guide channel.
A green LED is situated between the two manual override buttons. Directly above the LED is a small recessed pressure switch located within a small hole. Ensure that the pinch valve is connected to the robot via the accessory cable and that the robot is switched on. Now using the tip of a pen a clean 200 µl pipette tip or a similar object depress the pressure switch. The LED will start blinking. Remove the pen tip.

To adjust the left-hand travel limit of the pinch bar, press the left-hand manual override button.
Listen carefully to the noise the pinch valve makes as the pinch bar is moving. The pinch bar has a mechanical limit of travel at which point the motor will stall. You will hear a change in the noise coming from the pinch valve as the motor stalls. The correct travel limit to set is the point immediately before you hear the motor stall. Use both manual override buttons to locate this point. When you are satisfied that the pinch bar is located in the optimum position, press the pressure switch with the pen again. The pinch bar will return to the centre of the pinch valve and the LED will stop blinking.
To adjust the right travel limit, repeat steps (3) and (4) above starting with the right-hand manual override button.

1.7 The Computer

A personal or laptop computer running the Microsoft Windows operating system is required to control the CAS-1820 X-tractor Gene. This computer can be provided by Corbett Robotics Pty. Ltd. or its agent. If a personal computer is supplied by the user, please observe the following minimum specifications to run the Robotics Software for the CAS-1820:

- 1 RS-232 port
- 3.0 GHz Pentium IV CPU
- Windows XP
- 256 MB RAM
- 20 GB HDD
- Monitor with 1024 x 768 pixels screen resolution or better

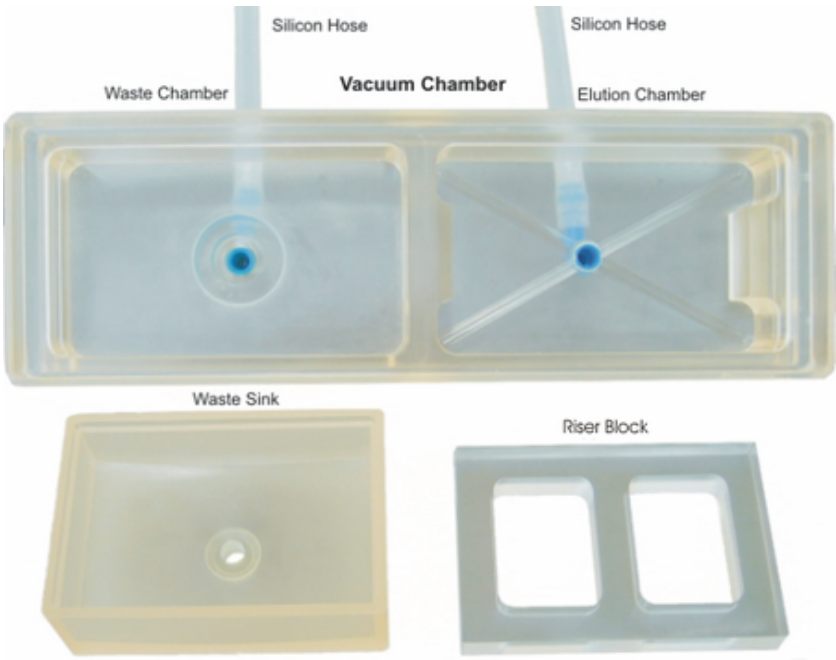
Windows NT, Windows 2000, and Windows 98 are no longer supported. These operating systems may produce unstable or unreliable software operation.

USB port to RS-232 Serial port converters is not supported. No software or hardware support can be given if these devices are used. It has been shown by Corbett engineers that the CAS-1820 may operate on these devices for a certain period of time, eventually followed by unreliable operation.

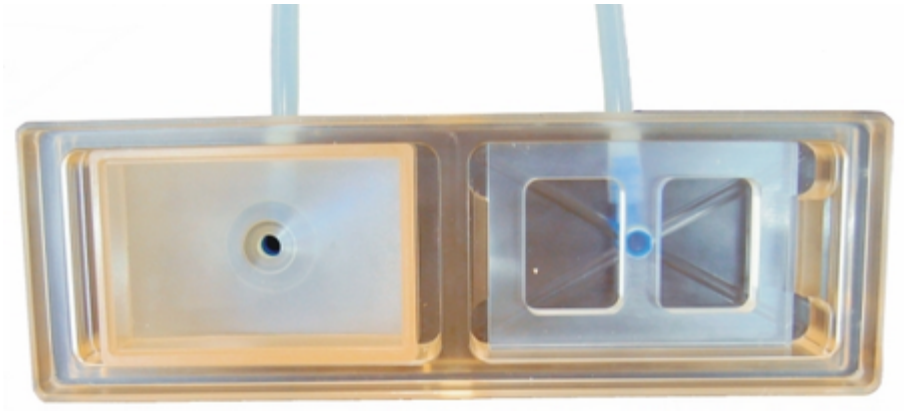
1.8 Vacuum Chamber

The CAS-1820 X-tractor Gene is equipped with separate Waste and Elution Chambers as one of the measures introduced to eliminate potential cross contamination of samples during purification. The Waste Sink is located within the waste chamber. The sink is pushed firmly downwards to engage the waste sink spigot into the well base of the waste chamber. The waste sink is held firmly in place by a rubber O-ring on the Waste Sink Spigot. The Riser Block can also be inserted into the elution chamber at this time or just prior to starting the CAS-1820.

Waste Sink, Riser Block, and Vacuum Chamber



Waste Tub and Riser Block Inserted into the Vacuum Chamber



The waste and elution chambers are connected to the Waste Bottle via silicon hoses that pass through the pinch valve. To insert the silicon hoses into the channels of the pinch valve, first turn the pinch valve upside down. Next, pick up one of the silicon hoses below the Y-piece and stretch it over one of the channels until it slips into the channel. Repeat for the second hose. When both silicon hoses are inserted into the channels of the pinch valve, move the pinch valve along the

silicon hoses until the pinch valve is adjacent to the Y-piece as shown below. Now turn the pinch valve right way up.

Underside of Pinch Valve with Vacuum Hoses Inserted

Connecting the Silicon Hoses to the Vacuum Chamber and the Vacuum Station

Place the pinch valve behind the CAS-1820 and pass the two silicon hoses under the back of the robot passing both hoses up through the cut out in the floor of the robot where the vacuum chamber will be fitted. Place the vacuum chamber on its side in front of the cut out with the barbed silicon hose connectors facing upwards. Now connect the two silicon hoses to the barbed connectors making sure that the hoses don't become twisted; left hose through left side of pinch valve to left barbed connector and so for the right silicon hose.

Now place the right hand side of the vacuum chamber into the cut-out of the deck so that the spring clip just below the cut-out engages the slot on the right hand end of the vacuum chamber. Push the vacuum chamber to the right while at the same time lowering the left hand side of the vacuum chamber into the cut-out. With the vacuum chamber fully inserted allow the chamber to centre and lock into position. To remove the vacuum chamber, follow these steps in reverse order. Use the quick connector on the free end of the silicon hose to connect the vacuum chamber to the Vacuum Control Station.

TIP: When inserting the vacuum chamber into the cut out in the deck, moving the pinch valve body to the rear will make vacuum chamber insertion easier by preventing the silicon tubes bunching under the robot. When finished ensure that the P.V tubes are not kinked or that the airflow is restricted in any way.

1.9 General Specifications

Pipetting Volumes:

- Between 10 µL and 190 µL (wide bore tips)
- Between 10 µL and 190 µL (fine bore tips)

Precision:

- 10 µL to 190 µL, less than 4 % C.V. (fine bore tips)

Accuracy:

- Accuracy can be calibrated in software
- 1% calibrated, volumes > 10 µL

Throughput:

- One 96 well plate of urine or bovine blood, or tissue in 1 - 1 1/2 hours depending on cycle parameters.

Electrical Requirements:

- 220 - 240 Vac 50 Hz, or
- 100 Vac 60 Hz, or
- 110 Vac 60 Hz
- 250 VA
- Good earth connection via mains outlet

Table Capacity:

- 4 standard footprint 128 x 86 mm plates (SBS standard) to accept 96 well plate and pipette tip formats
- Waste Chamber
- Elution Plate
- Reagent plate (4 positions)

Number of Pipetting Channels:

- Eight

Communication:

- RS-232

Lid:

- Polycarbonate lid is standard on all machines
- UV light for work surface sterilisation is available as factory fitted accessory
- HEPA filter unit for positive pressure under lid is available as an optional factory fitted accessory or can be fitted to the robot at a later stage.

Weight:

CAS-1820 Robot
32 kg

CAS-1820 Vacuum Station
19 kg

CAS-1820 Pinch Valve
1.5 kg

Dimensions:

- All dimensions are in millimetres (mm) and exclude any computer equipment.
- CAS-1820 Robot:
- Lid closed, 490 mm (w), 580 mm (d), 390 mm (h)
- Lid open, 490 mm (w), 580 mm (d), 770 mm (h)
- The connection of cables at the rear of the instrument adds 70 mm to its depth
- CAS-1820 Vacuum Station:
- 495 mm (w), 400 mm (d), 520 mm (h)
- CAS-1820 Pinch Valve:
- 130 mm (w), 70 mm (d), 90 mm (h)
- The connection the accessory cable at the front of the Pinch Valve adds 70 mm to its depth
- CAS-1820 HEPA Filter:
- Contact Corbett Robotics for information on this product

Computer Requirements:

- Laptop or Desktop PC with serial port
- Windows XP 2000 or later
- 2.4 GHz Pentium 4 CPU or equivalent
- 512 MB RAM
- 20 GB hard disk
- Monitor with at least 1024 x 768 pixels screen resolution

Operational Temperatures:

- Constant 4°C to 35°C - temperature cycling not permitted
- High temperatures may cause excessive evaporation of some reagents

Operational Humidity:

- 40 - 70% RH
- Lower humidity levels may affect precision due to evaporation

2 Safety and Setting Up

The CAS-1820 X-tractor Gene has a variety of moving parts, with a number of places that present significant pinch points. For your own safety and to prevent damage to the instrument, please observe the following safety requirements:

Always use the packaging provided with the robot when moving the machine:

- Do not lift the CAS-1820 by yourself,
- Do not disable the lid interlock,
- Do not interfere with the robot while it is running,
- Do not attempt to move any plates or tubes while the robot is operating,
- Do not, under any circumstance, place your hand under the pipetting head while it is lowering.

When calibrating plates with the lid up, be sure to always stand clear of the robot. Similarly, do not click on the mouse or keyboard while looking closely at plate calibrations

Use only recommended tips and tip racks.

Keep the lid of the instrument closed whenever possible.

NEVER place any items on the y-arm of the instrument (the pipetting arm).

NEVER apply any force (by hand or otherwise) in the upward or downward direction on the y-arm.

Use the Stop/Pause button located on the tool bar of the software to stop/pause the robot before removing plates or tubes.

The remainder of this section covers the setting up of the CAS-1820 X-tractor Gene. Topics covered include:

- Positioning of the instrument,
- Connecting the instrument to power and the PC,
- Installation of the software,
- Starting the software the first time,
- Virtual Mode,
- Calibrations, and
- The lid.

Please follow these instructions for reliable set up.

2.1 Positioning the Robot

The location/position requirements of the CAS-1820 X-tractor Gene are not very exacting. However, a few points should be observed, as follows:

- The CAS-1820 can be installed on any sturdy laboratory bench or table;
- The CAS-1820 must not be installed near generators of static electricity (e.g. ionisers, large screen televisions etc.);
- The CAS-1820 should be installed in a laboratory environment free of excessive airborne dust;
- The CAS-1820 can operate in temperatures from 4°C to 35°C. Do not subject the robot to repeated temperature cycling. The instrument can be operated in a cool room of consistent temperature;
- Do not subject the CAS-1820 to a relative humidity of > 70% for extended periods of time;
- Ensure that all four of the CAS-1820's feet are firmly located on the table surface (the front feet can be height adjusted using a 10 mm spanner);
- Ensure that the lid can fully open upwards without any obstructions; and
- Ensure that the On/Off switch at the rear of the instrument is easily reached.

A special requirement of the CAS-1820 is the vacuum control station. The vacuum control station is best placed directly under the laboratory table or in the cupboard under the laboratory bench. Placing the CAS-1820 in a cupboard under the laboratory bench will necessitate drilling a 50 mm hole in the laboratory bench for cable and vacuum line connections.

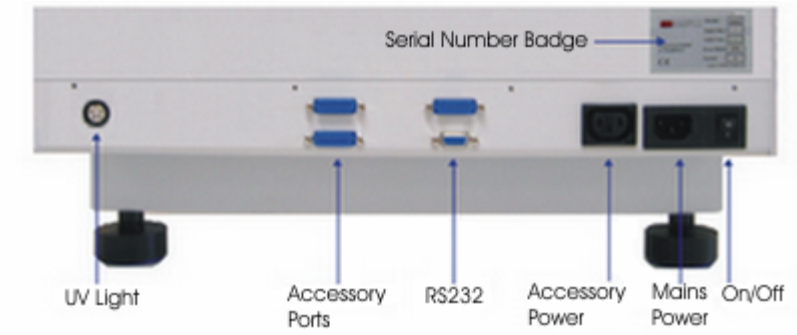
If you have not done so already, please remove the arm support as discussed in the Unpacking Section.

Helpful Hint: Positioning the robot close to the left hand edge of a laboratory bench or table will allow tips to be readily ejected out of the robot into a biohazard waste bin. This negates the need for a tip disposal box.

2.2 Connecting the Robot

The connections for the CAS-1820 X-tractor Gene are very simple. Please set-up the computer according to the manufacturer's instructions and then follow the steps below to connect the CAS-1820 to the computer.

With the host computer and robot both switched off; connect the supplied 9-pin RS-232 serial cable to the instrument. The RS-232 serial cable socket is on the rear of the CAS-1820 as indicated in the diagram below.
Connect the other end of the serial cable to the computer. By default, the software is configured to look for a CAS-1820 on serial port one (COM 1) where possible. Use this serial port. How to change the serial port in the software is described here. Please note that USB to serial converters are not supported.
Plug in the IEC mains cable into the Mains Power socket on the rear of the CAS-1820 and connect it to the mains supply.



All accessory items should also be plugged in at this time. Use the 15-pin accessory cables to connect the vacuum control station and the pinch valve to the CAS-1820. Use the accessory power lead to connect the power socket of the vacuum control station to the Accessory Power supply on the CAS-1820. If your CAS-1820 is fitted with a UV light, ensure that it is firmly connected to the UV light connector on the rear of the robot.

Once all accessories are connected, switch on the CAS-1820 and the computer. The On/Off switch is located at the back of the CAS-1820, next to the power cable socket. When the CAS-1820 is switched on the pipetting head should rise out of view. If this is not the case, please consult the troubleshooting section.

2.3 Software Installation

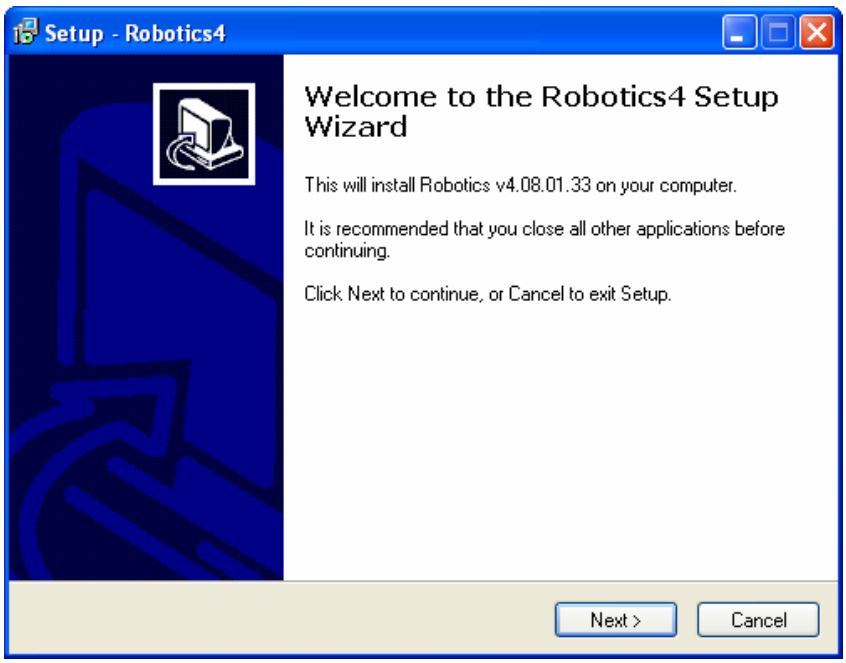
New Installations

1. Ensure that all other software (particularly Corbett software) is shut down.
2. **It is important that all screen savers and power save modes are disabled for error free operation of the CAS-1820 X-tractor Gene. Please consult Microsoft Windows Help on how to disable these. Typically there are two power-save modes and one screen saver option important to the installation. The power-save modes are those of the monitor and the hard disk drive.**
3. **Some virus scanners are known to interfere with software operation. Typically these virus scanners make the software run extremely slowly. Corbett Robotics Pty. Ltd. advises caution when installing the robotics software on computers with virus scanners installed.**
4. Insert the Robotics Software CD into the CD-ROM of the computer.
5. The installer splash screen should be displayed automatically after a few seconds.
6. If the splash screen is not displayed, you can start the installation process by going to the 'Start Menu', selecting 'Run' and typing d:\setup.exe.
7. Select 'Install Operating Software' on the splash screen.
8. The installation process will begin.

You will be presented by a series of screens:

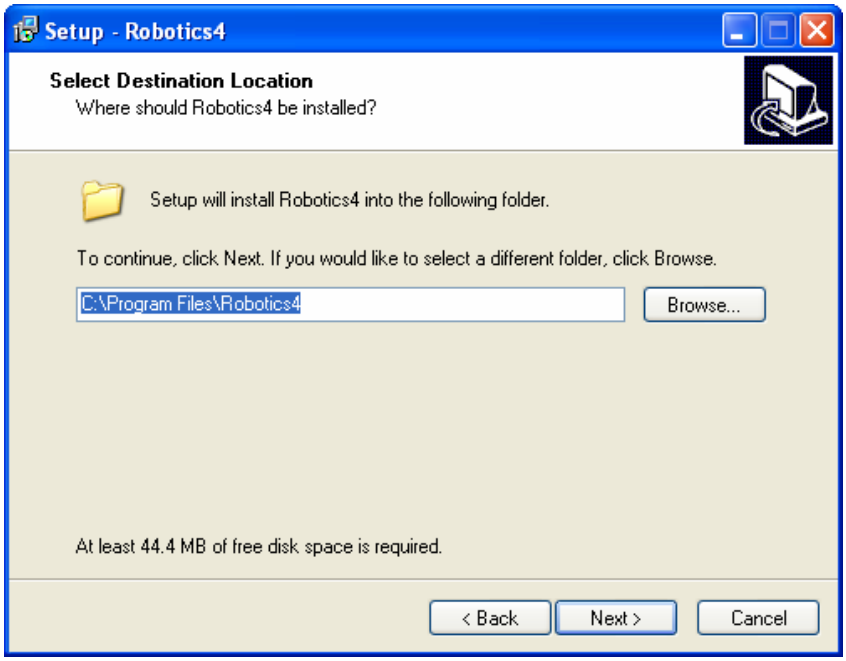
Screen 1 - Welcome to the Robotics4 Setup Wizard

This screen informs the user of the software version and recommends that the user close all other programs before installing the software. Please note that if other Corbett software is not shut down prior to the installation of the Robotics Software it may be necessary to reset the computer. Selecting 'Cancel' allows the user to exit the setup. Selecting 'Next' takes the user to the next screen.



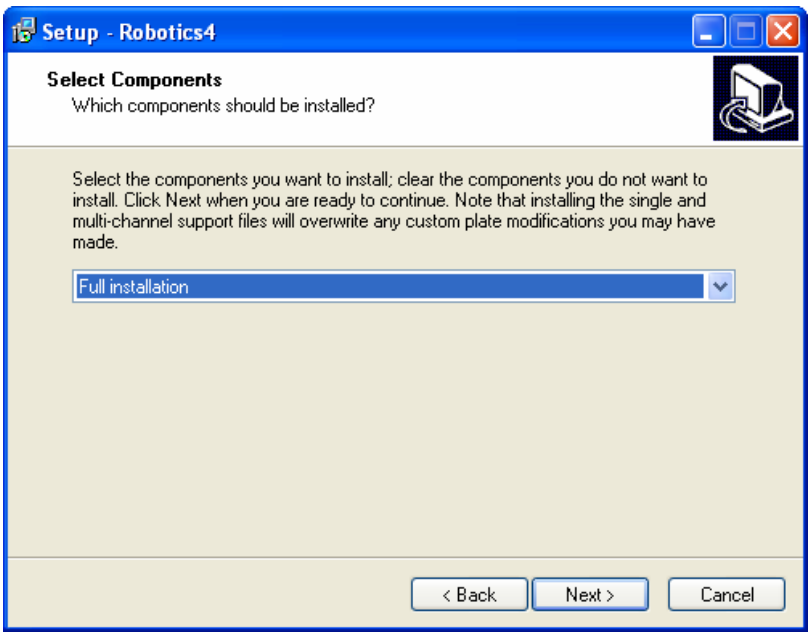
Screen 2 - Select Destination Location

This screen allows the user to select the folder into which they want the software to be installed. The 'Browse' button enables the user to locate specific folders. Selecting 'Back' takes the user back to the previous screen. Selecting 'Cancel' allows the user to exit the setup. Selecting 'Next' takes the user to the next screen.



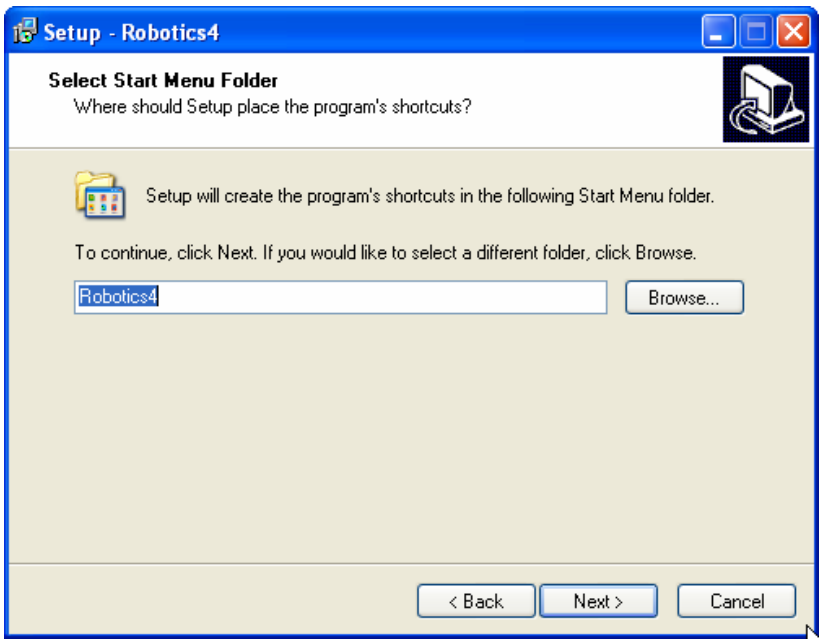
Screen 3 - Select Components

This screen allows the user to select the software components they wish to install. Selecting 'Back' takes the user back to the previous screen. Selecting 'Cancel' allows the user to exit the setup. Selecting 'Next' takes the user to the next screen.



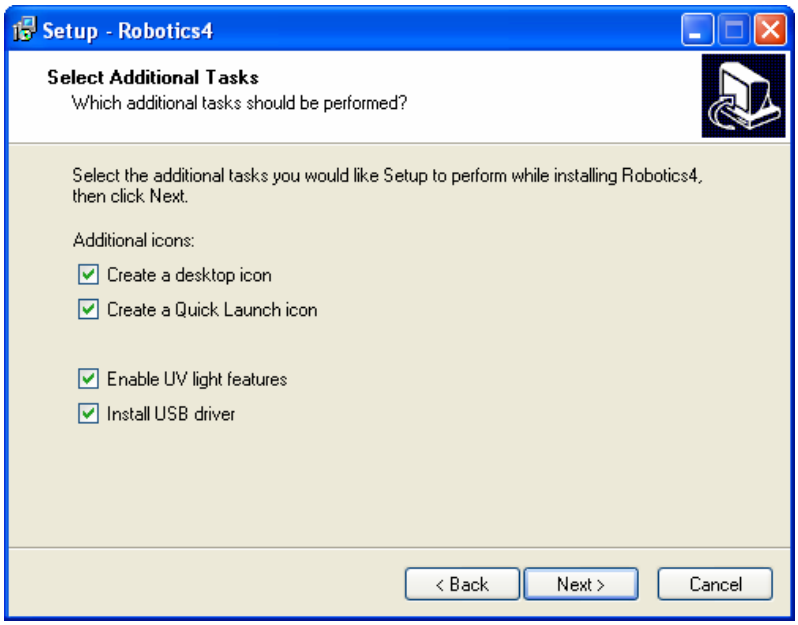
Screen 4 - Select Start Menu Folder

This asks the user which folder location they wish the program to install the program's shortcuts. Selecting 'Back' takes the user back to the previous screen. Selecting 'Cancel' allows the user to exit the setup. Selecting 'Next' takes the user to the next screen.



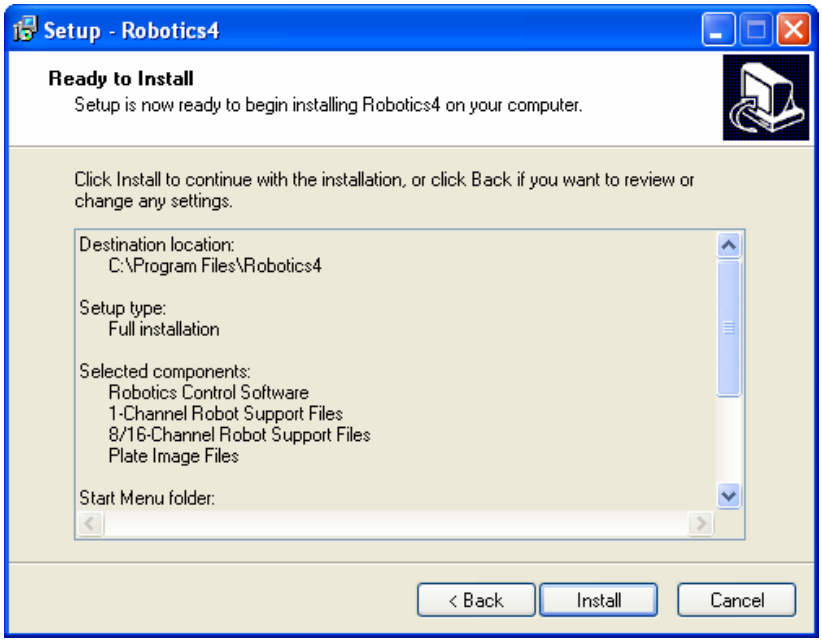
Screen 5 - Select Additional Tasks

This screen asks the user to specify which additional tasks they would like to be performed while the program is installing the software. Selecting 'Back' takes the user back to the previous screen. Selecting 'Cancel' allows the user to exit the setup. Selecting 'Next' takes the user to the next screen.



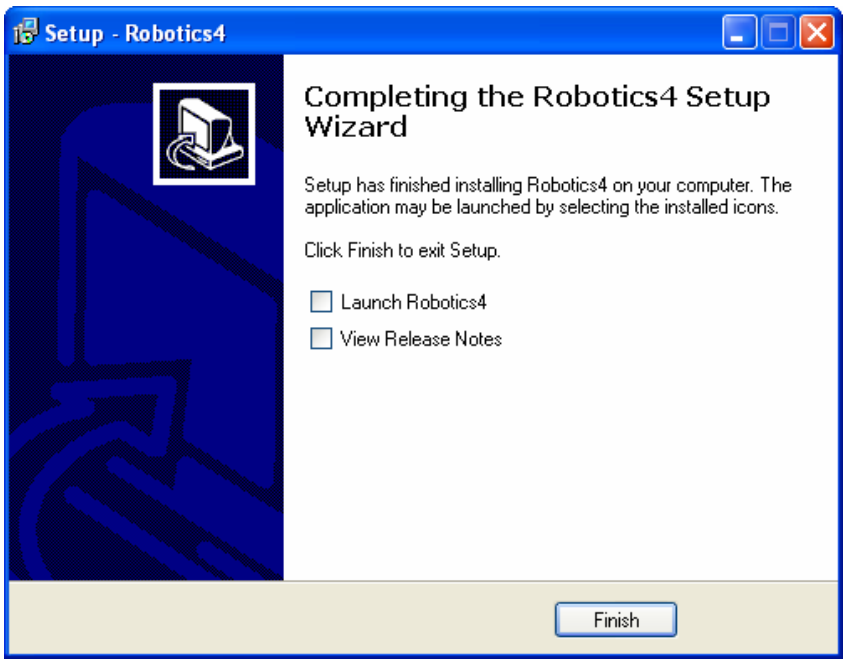
Screen 6 - Ready to Install

This screen allows the user to review details entered and gives the option of selecting 'Back' to alter details, or 'Next' to proceed with the installation. Selecting 'Cancel' allows the user to exit the setup.



Screen 7 - Completing the Robotics4 Setup Wizard

This screen directs the user on how to launch the software.



Un-installing the Software

To completely remove the Robotics Software, you can either select 'Uninstall Robotics4' from the Program Group (accessible via the Start Menu). Alternatively you can select 'Control Panel\Add Remove Programs' and select the Robotics Software from the menu.

You must also remove the c:\Program Files\Robotics4 directory. You can do this using Windows Explorer. Please note that deleting this folder will destroy all calibration data and run files that may have been saved in the c:\Program Files\Robotics4\Data directory.

Other Features on the CD

Other features on the Robotics Software CD include:

- Viewing the CAS-1820, CAS-1200, and Corbett Rotor-Gene Multimedia demo
- Installation of Adobe Acrobat Reader

Multiple robots from one computer

Multiple CAS-1820 and CAS-1200 robots can be operated from one computer. However, multiple installations of the Robotics Software are necessary. Perform the first installation as described above. The second installation will need to be completed in a directory different to the first and so on. To do this, enter an alternate name when prompted for the installation directory.

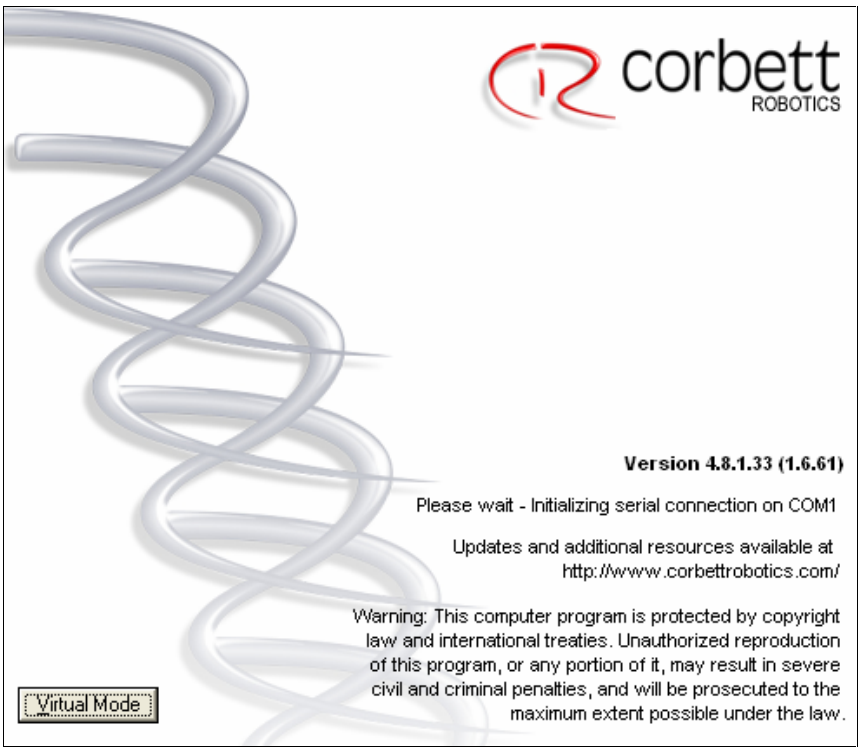
2.4 Starting for the first time

Starting the Robotics Software for the first time is no different than starting the software any other time. However, Virtual Mode is a software feature that the user must be aware of when first starting the software.

Please close the lid of the robot.

Double Click the Robotics Software icon on your desktop. 

You will see the following splash screen when the software starts. The serial port on which the software is trying to communicate is displayed below the Version Number. To change the serial port, see Table Setup.

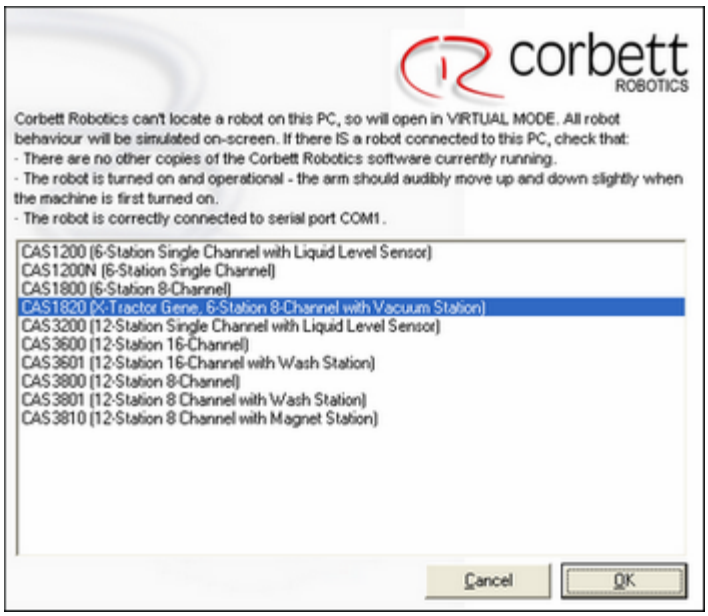


If a robot is correctly connected to the computer via the RS-232 serial cable and then switched on, the software should operate normally. Please wait until the main software window appears (this will take a few seconds).

If, while initialising, the Robotics Software cannot detect the CAS-1820 X-tractor Gene (CAS-1820 turned off or not connected), the Robotics Software will pause, and after 10 seconds it will request that you choose a robot model on a screen similar to the window below (unless only software for one model was installed - see installation). Alternatively, if you know that you want to start in Virtual Mode, you can skip the timeout by clicking on the 'Force Virtual Mode' button on the splash screen.

If you have more than one accessory running off the computer, it is necessary to select the appropriate COM port for the software to communicate with the X-tractor. To do this, start up the software. If the correct COM port is not yet selected, the software will open in Virtual Mode (indicated by the words 'Virtual Mode' displayed at the top of the window). Select the Options

Menu from the toolbar, then from the dropdown menu, select Robot Setup and then Select COM port. A window will open which allows you to select the appropriate port. Once selected, close down the software and restart. The software should then power up the robot, indicating communication is occurring.



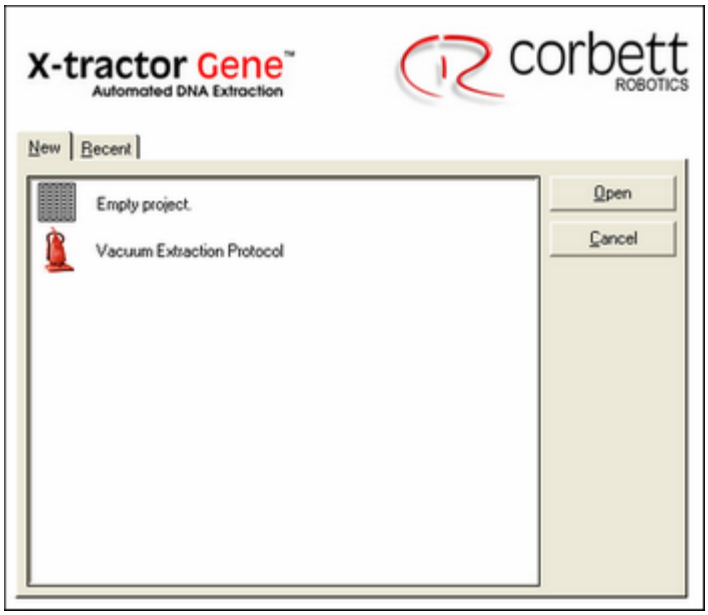
Please select the appropriate robot model. Alternatively, you can choose to cancel and the software will be shut down.

To shut the software down, please consult Shutting Down.

2.4.1 Normal First Time Operation

When the software starts normally, you will see the main window appear. If the robot lid is open, you will be prompted to close the lid. The robot will proceed to home all the axes.

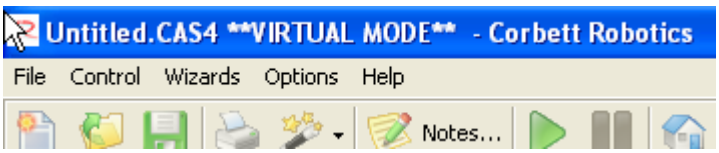
You will be presented with the options screen below (described in Selecting a Run). Select 'Empty Project'.



You are ready to continue with the set up process and select the workspace layout. This involves selecting the types of plates you are using and then performing calibrations for those plates.

2.4.1.1 Virtual Mode

When running in Virtual Mode, the software performs as normal. At the top of the screen you will see a message that reflects that the software is operating in Virtual Mode.



In Virtual Mode, all functions are enabled: parameters can be changed, runs can be set up or modified and virtual runs completed. The software highlights the tubes it is currently pipetting into or out of. Setups can be saved to act as templates for new runs that will be run on a robot at a later stage. This feature enables the user to set up runs on a remote computer and transfer the template onto the PC connected to the robot, reducing the time required on the instrument.

Virtual Mode is also a useful tool for new users to familiarise themselves with Robotics Software.

Your robot has been factory identified with an internal (or electronic) Serial Number. Any modifications made to Calibrations in Virtual Mode will not affect calibrations on the actual instrument. Any software running in Virtual Mode on a PC different to the PC the CAS-1820 X-tractor Gene is connected to, will also not affect any calibration settings.

2.5 Calibrations

The CAS-1820 X-tractor Gene accepts a wide range of plates. Specific plates accompany specific tasks. The CAS-1820 has two locations for plates, two locations for pipette tips, one location for the DNA/RNA capture plate, and two locations for reagent tub sets as described. The CAS-1820 is also equipped with a tip ejector chute that assists in the disposal of tips from the pipetting head. Before the instrument can be used, it needs to know where the locations of these items are, thus the CAS-

1820 must be calibrated. The volume can also be calibrated to ensure the CAS-1820 accurately pipettes the volumes as specified.

There are three calibrations that can be made on the CAS-1820. These are:

- Position Calibration
- Height Calibration
- Volume Calibration

All blocks and plates are removable. For example, the Reagent Tub Support Plate is located by pins which serve to orientate the blocks correctly and subsequently ensure that the four tubs are always positioned correctly. Similarly, the plates are located by table rails. Care must be taken with some plates to ensure that correct orientation is maintained.

Due to these locating mechanisms, once the CAS-1820 has been installed and calibrated, there should be no need to re-calibrate the positions for the tip ejector chute or any given plate. If the instrument has been moved, it is recommended that the position calibrations be checked and re-calibrated if needed.

Similarly, the volume calibration need only be performed following a pipetting mechanism service, as this is found to alter calibration values.

All calibration data is stored as part of the software installation. The calibration data is not saved with the run files. Thus if run files are transferred from one calibrated instrument to another calibrated instrument, the file will execute without any need to perform calibrations. Additionally, the calibrations of an instrument are unique to that instrument and should never be transferred between instruments.

Warning: Usually one instrument is connected to computer. If you change the instruments connected to the computer and the instrument has not been identified with an electronic serial number, it is possible that the wrong calibrations will be used. Serious damage may result (Note: If your instrument has no serial number contact Corbett Robotics).

2.6 Lid

The lid of the instrument serves two primary purposes. The first is to provide an isolated enclosure for setting up reactions; the second is to provide a safety mechanism.

The lid is manufactured from highly impact resistant polycarbonate. The polycarbonate is also 100% UV absorbent. Cleaning the lid should only be performed with water and a clean, soft cloth. Alternatively a cleaning product specifically designed to clean polycarbonates or acrylics can be used. Any cleaning product should be tested on a small inconspicuous section of the lid first. Please note that the black edging of the lid will not resist organic solvents of any kind.

The lid incorporates a magnetic sensor to determine if the lid is closed or not. Under no circumstances should this sensor be bypassed.

The lid must be closed for the software and hardware to initialise upon start-up and for the run to begin.

Opening the lid while the robot is performing a run will cause the robot to pause. This pause will be recorded in the post-run report. Extreme care should always be taken when opening the lid during a run as the pause in the robot's operation may take up to two seconds to register.

The lid need not be closed during position and height calibration of plates. Do not interfere with robot movement during these calibrations and do not put hands or head into the robot's workspace while the robot's head is moving.

For operation in small spaces (such as a laminar flow cabinet), the lid can be removed. Please contact Corbett Robotics Pty. Ltd. for information on the removal of the lid and the disabling of the electronic lid interlock.

3 Software in Detail

Using the Robotics Software is straightforward and the user interface Wizard of the CAS-1820 X-tractor Gene is designed to facilitate the setting up of DNA/RNA extraction protocols. Many options are available on CAS-1820 and can be used to adjust the behaviour of the robot. This section describes all aspects of the software in detail. If you are starting the software for the first time, please refer to Setting Up and Starting for the First Time.

Covered in this section are:

- Software Overview
- Starting the Robotics Software
- Software Workspace (the main screen and its features)
- Toolbar
- File Menu
- Wizards Menu
- Options Menu
- Calibration Management Options
- Robot Setup Options (including calibrations)
- Run Settings Options
- Tip Operations Options
- Plate Operations Options
- Help Menu
- Right-hand Pane (the control area)
- Tips
- Reagents
- Samples
- Reactions
- Warnings
- Starting a Run
- Pre-run Report
- Aborting a Run
- Post-Run report

3.1 Software Overview

The basic functions for setting up a DNA/RNA extraction on the CAS-1820 X-tractor Gene are quite straightforward to use. However, there are many functions in the software that allow the user to set up runs that are quite different from these standard types of runs. When the software is started for the first time, the user is required to perform a number of calibrations. Once these calibrations have been carried out, the software is ready for use.

The software allows a variety of options to be changed that affect the run. These options include tip usage during a run, air volumes, pipetting speeds, and so on. These options are saved with a run file. If the set options are to be the new default options, this can also be set through the options menu.

When the set up of a run has been completed, it is possible for the user to save the run or alternatively, load the run. The options that were set for the run are stored in the run file.

The run can then be loaded. If there are any errors with the run set up, the software will prompt the user to correct these errors. Warnings can be ignored, but must be acknowledged before the run can start. A pre-run report can also be generated that gives a summary of the set up and the contents of the reaction tubs.

A very useful feature of the software is Virtual Mode. If the software is started either without a CAS-1820 connected to the computer or with the robot switched off, it will start in Virtual Mode. In Virtual Mode, all software functions are available to the user and runs can be set up, saved and simulated. This way, the user can create and simulate runs without the use of the actual instrument.

The software main screen, the workspace, visually simulates the robot's table layout. The six main plates/tip racks and reagent blocks are shown on the left and a 'functionality window' known as the right-hand pane is shown on the right. The display in the right-hand pane changes depending on which plate is selected on the left. A plate can be selected by left-clicking on it.

The setting up of a run is performed by a combination of functions in both the left and right areas of the screen.

The Options Menu provides the user with control over a number of functions that are not accessible through other parts of the software.

On the CAS-1820 any pipetting operation is performed on liquids that fall into one of three liquid groups. These four groups are:

- Samples
- Reagents
- Reactions

Each of these liquid groups is treated differently in the Robotics Software. The groups are geared towards simplifying the operations for which these liquid groups are normally used. For example, Samples are the material from which DNA/RNA is to be extracted.

Typically, the liquid groups are used as follows:

- Samples are used to add to the final reaction,
- Reagents typically lysis buffers, wash buffers, or elution buffers are used to process samples, and
- Reactions are a combination of the Sample and Reagents.

With the above in mind, a great variety of runs can be set up to meet many requirements that the user may have.

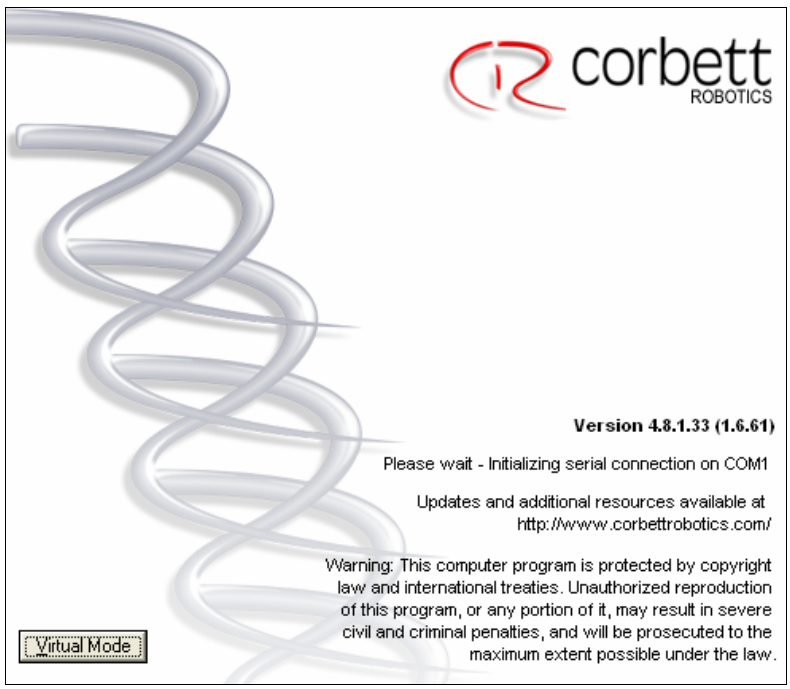
3.2 Starting the Software

To start the software correctly so that it communicates with the CAS-1820 X-tractor Gene, please ensure the instrument is switched on, connected to the PC and the lid is closed.

Double Click the Robotics Software icon on your desktop. 

You will see the following splash screen when the software starts. The serial port on which the software is trying to communicate is displayed immediately under the software version number.

NOTE: To change the serial (COM) port wait until the main workspace screen opens in virtual mode. Select the Options Menu from the toolbar, then from the dropdown menu, select Robot Setup and then Select COM port. A window will open which allows you to select the appropriate port. Once selected, close down the software and restart. The software should then power up the robot, indicating communication is occurring.

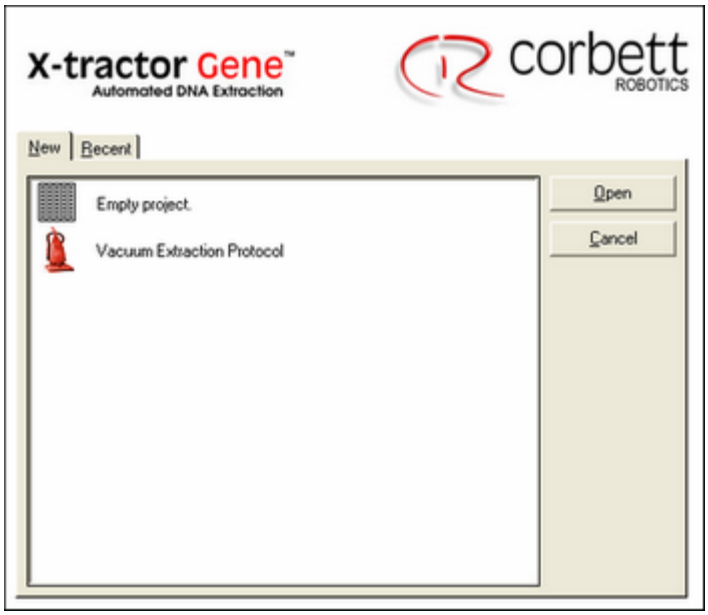


If you wish to run the software in Virtual Mode, click on the 'Force Virtual Mode' button. If the X-tractor Gene is connected or turned off, wait for the time-out (10 seconds) and virtual mode will initialise by default. If you are unfamiliar with Virtual Mode, please refer to the section on Starting for the First Time.

Alternatively, if the CAS-1820 is connected and turned on then within a short period of time, you should see the main Window appear with an options screen; please refer to Selecting a Run.

3.3 Selecting a Run

The option screen below appears when the program is opened.



A run can be created in three ways:

1. If a run had previously been saved, it can be re-opened and utilised or modified as needed;
2. A Wizard, such as the Vacuum Extraction Protocol, can be used to create an entirely new nucleic acid extraction run file;
3. Select 'Empty Project' and create a standard 8 channel pipetting operation/ file.

To open a previously saved run file, click on the 'Recent' tab shown above. From the directory dialogue box, select the file you wish to open and then click on the 'Open' button.

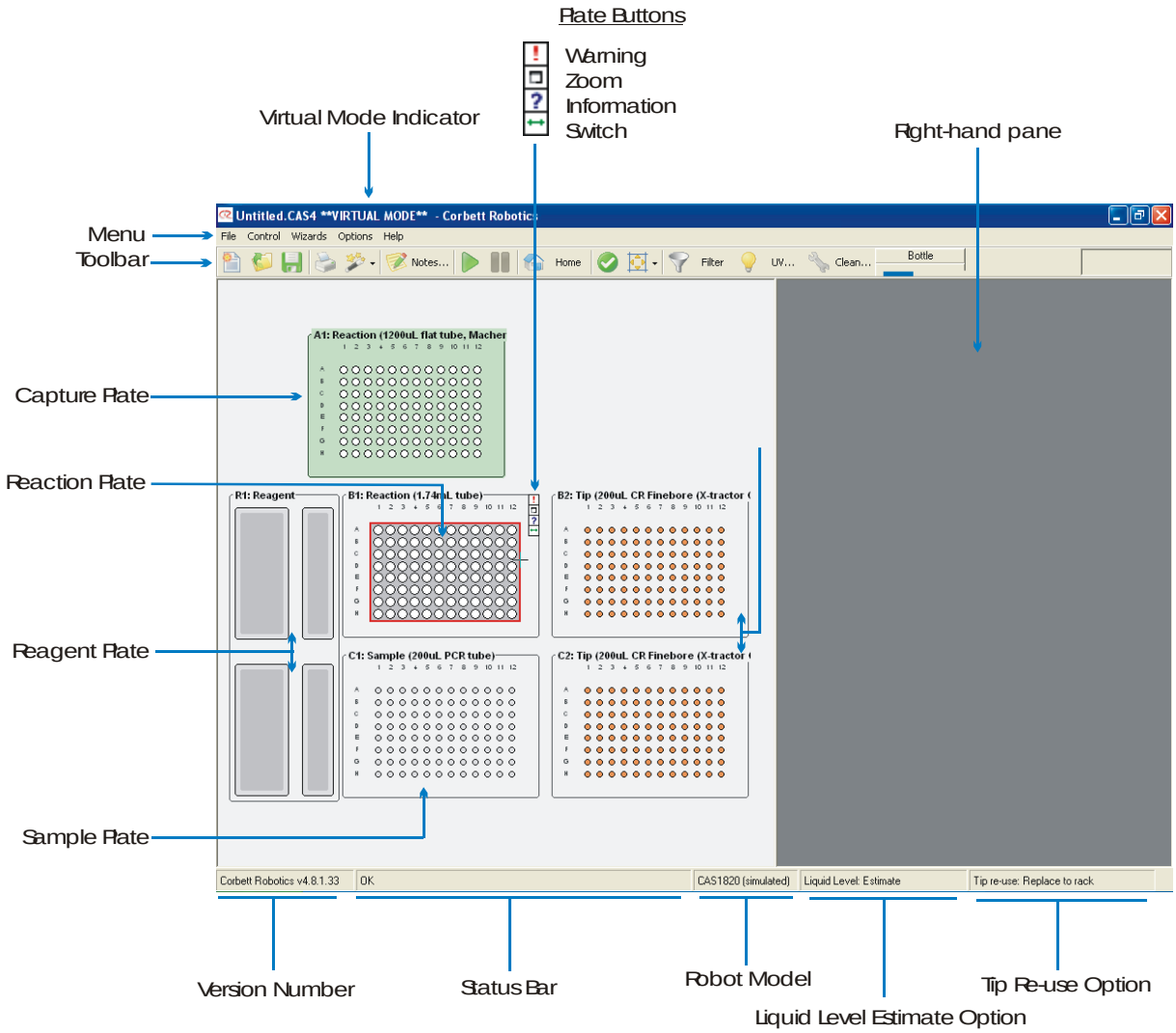
The screens above only show a few Wizards. As the Robotics Software grows, the Corbett Software Team anticipates that more Wizards will be added to the list. To use a specific Wizard, select it from the list and then click on the 'Open' button.

Click on 'Empty Project' if you wish to start an entirely new project. Then click on the 'Open' button.

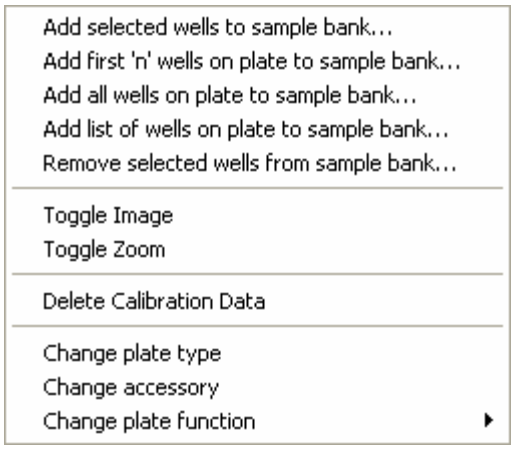
3.4 The Software Workspace

The software workspace is the main user interface. The Robotics Software functions are controlled from here. Important sections of the typical workspace are indicated in the figure below.

CAS-1820 X-tractor Gene Software Workspace:



Choosing pipette tips, plate types, and accessories is achieved by selecting the corresponding region of the workspace with the mouse pointer and clicking the right mouse button to open the pop-up menu depicted below. From this menu you can also manage pipette tip availability for the respective pipette tip racks mounted on the CAS-1820.



The Menu Bar includes the File Menu, Control Menu, Wizards Menu, Options Menu and the Help Menu. From these menus almost all the remaining software functions can be controlled. There is some overlap between the functions that can be controlled directly via either the workspace or the menus.

The Virtual Mode Indicator shows whether or not the software is operating in Virtual Mode.

The Toolbar, which is always visible, allows runs to be opened, saved, started and paused. If there is a problem with a run, this toolbar also shows the appropriate warning message icon.

The 'Plate Buttons' allow for simple plate functions to be accessed directly. If a plate is not calibrated, the calibration screens can also be accessed.

There are tip racks shown in the plate locations on the figure above. The racks are in locations B2 and C2. This is the default configuration. These plate locations can be configured as plates if needed.

One of the major features of the workspace is the Right-Hand Pane; this area changes depending on which plate is currently selected. In this area, all functions relating to setting up reagents, samples, and reactions are accessible.

At the bottom of the screen there are a variety of indicators. The most important indicators are the tips re-use indicator and the liquid level measurement indicator. By double clicking on the tip re-use field, you can change these options without going to the Options Menu. Similarly, double clicking on the Liquid Level Indicator toggles liquid estimation on or off (liquid estimation is not normally used for extraction, so leave as set default).

The robot model and COM port are also shown in the bar at the base of the screen.

The Status Bar displays any errors that may be occurring during a run and individual operations being performed.

The Robotics Software Version Number is displayed in the bottom left-hand corner of the workspace.

By clicking on the Reagent Plate, the user is able to control functions relating to reagents in the Right-Hand Pane.

The Plate Locations shown in the workspace are numbered the same way microtitre plates are numbered. Letters indicate the rows and numbers indicate the columns. Thus the locations are numbered A1, B1, B2, C1 and C2 from the top left across and down towards the lower right. These plate locations can be configured depending on the run that is being set up. The default set up as

shown here has one DNA/RNA capture plate (location A1); two tip racks (locations B2 & C2), one 96-well sample plate (location C1) and one 96-well reaction plate (location B1).

Help is available by accessing the Help Menu or by positioning the mouse over an area of interest and pressing 'F1'.

Plate Buttons:

The plate buttons appear in the top right corner of each of the plates. Normally these buttons only appear when the mouse pointer is hovering over one of the six plates, reagent or master mix blocks. However, the warning button is an exception.

The four plate buttons are:

Warning - this icon appears if the plate is missing either its position or height calibration. The button will remain visible until all the calibrations for the particular plate are complete. By clicking the button, you are given the option to calibrate the plate.

Zoom - by clicking on this icon, you can enlarge the view of the plate. When clicking on this button in the enlarged view, you will return to the normal size.

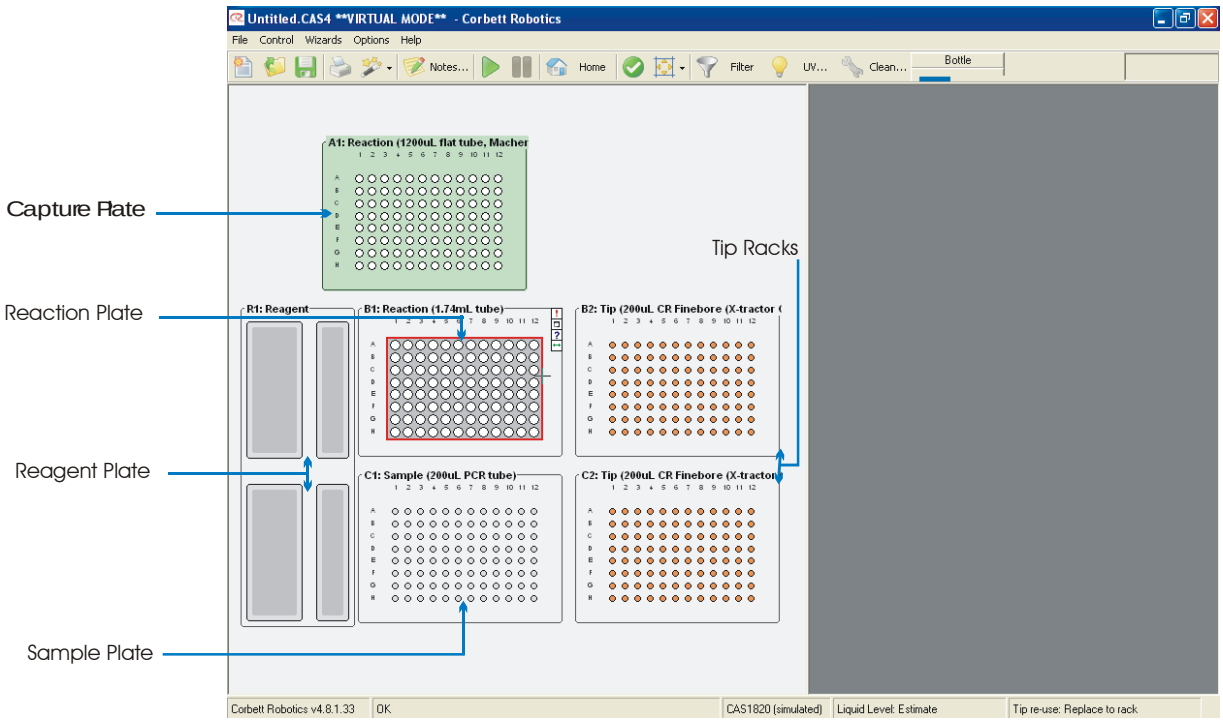
Info - by clicking on this button, details about the current plate are displayed.

Switch - clicking this button toggles between a photo of the current plate and the schematic plate view.

3.4.1 Choosing the Right Pipette Tips

Choosing the right pipette tips for the CAS-1820 X-tractor Gene's table setup is very important and must be performed before calibration of plate heights. Only with the correct pipette tips, will the correct calibration settings for that plate take effect.

CAS-1820 Typical Plate Layout



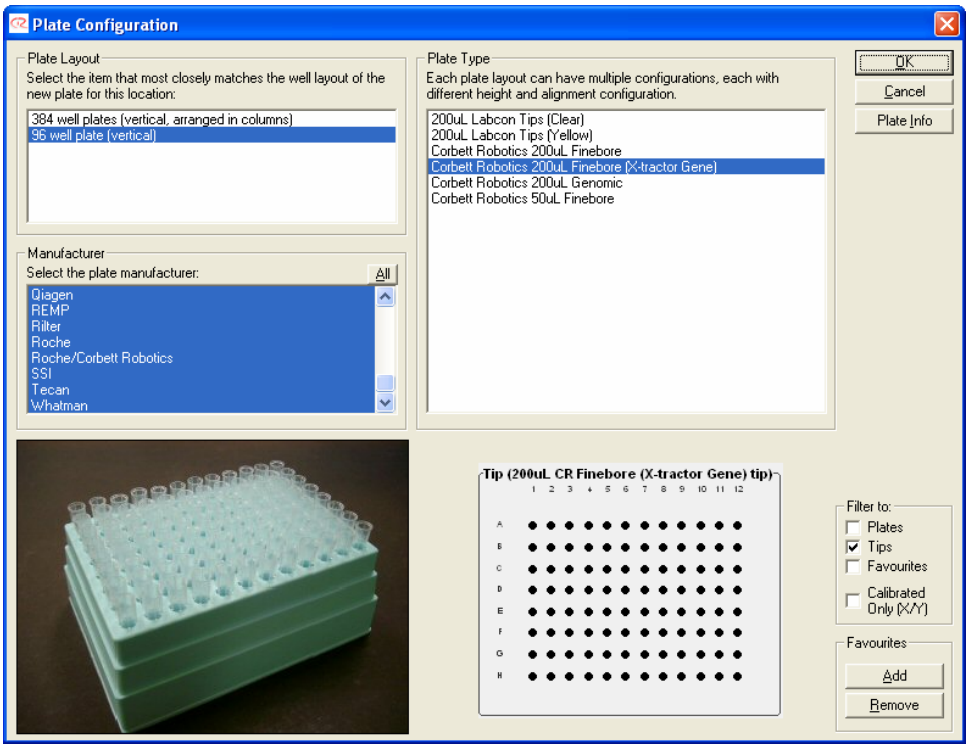
Changing Pipette Tip Type

To accommodate the wide variety of consumables and different types of runs, the type of pipette tips and their locations can be changed in the software. To change the pipette tip type, follow these simple steps:

1. Position the mouse pointer above the plate that is to be changed
2. Right select the plate with the mouse pointer
3. On the menu that appears, select 'Change plate type'
4. The Plate Configuration window will appear

Ensure that only 'Tips' is selected in the 'Filter to' sub window (bottom-right of the window). In the Plate Layout window, the pipette tips will be described as 96-well vertical. From the Plate Type window, choose either Corbett 200 µL or 50 µL fine-bore tips as required. If you cannot find pipette tips that resemble those you are using, contact the Corbett Support Team. Note that pipette tip type is critical to the successful operation of the CAS-1820 and that the user should only use pipette tips recommended by Corbett Robotics.

When you choose pipette tips, you will be presented with the following to facilitate choice:

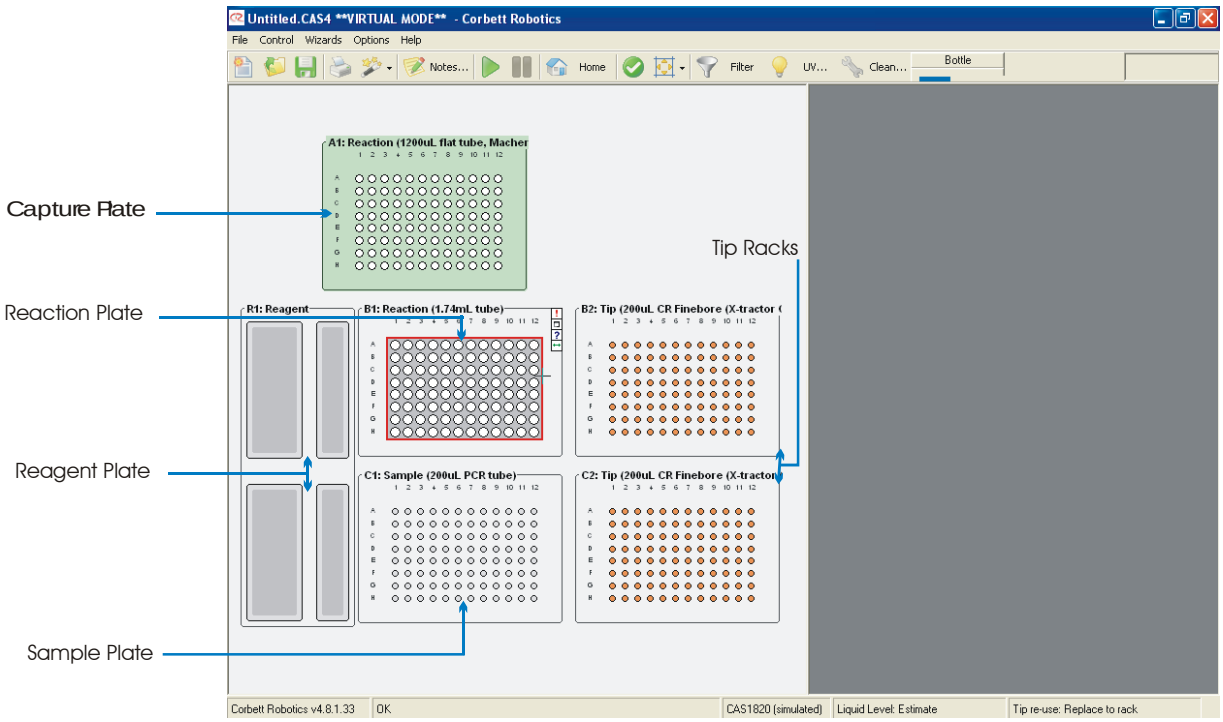


Corbett Robotics pipette tips that you commonly use can also be added to 'Favourites'. When the desired tips are selected, click on 'OK'. The chosen pipette tips will appear on the workspace.

3.4.2 Choosing the Right Plate

Choosing the right plate type and the plate's function for the CAS-1820 X-tractor Gene's table setup is very important. Only with the correct plate type selected, will the correct calibration settings for that plate take effect and overfilling of the wells be avoided. Each robot can support a variety of plates, loading blocks and tip racks.

CAS-1820 Typical Plate Layout



Changing Plate Type

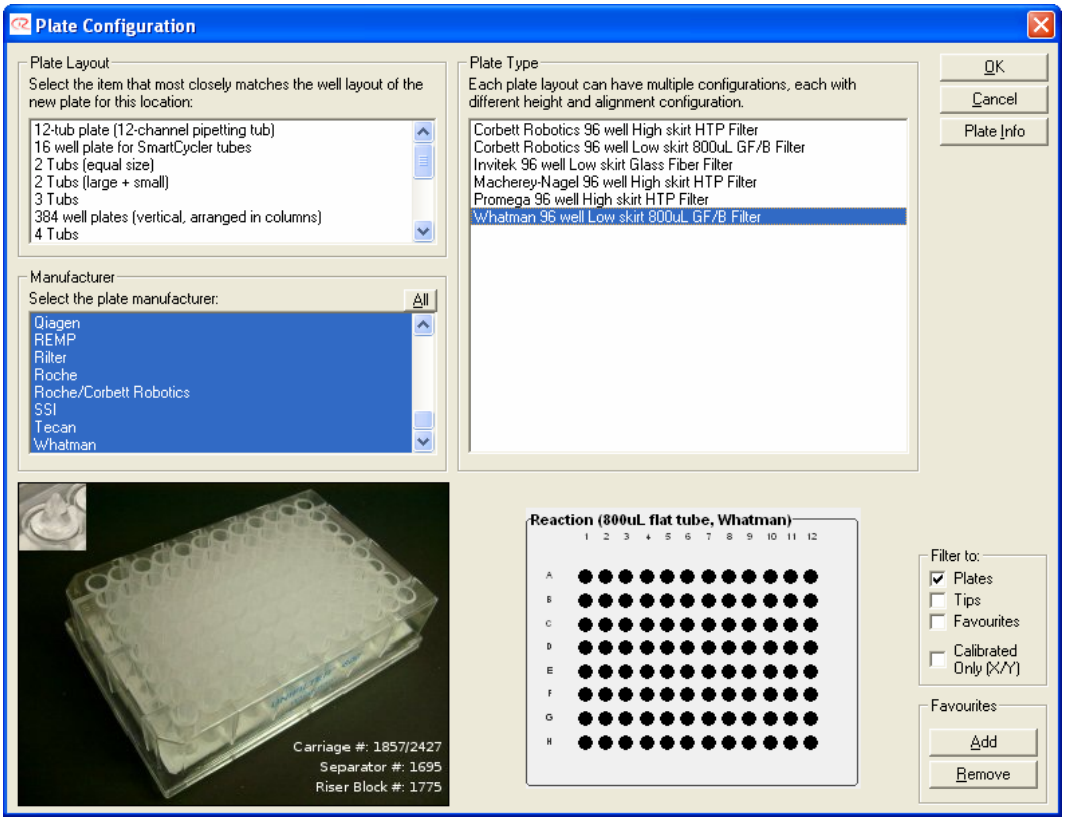
To accommodate the wide variety of consumables and different types of runs, the type of block or plate in these locations can be changed in the software. To change the plate type, follow these simple steps:

1. Position the mouse pointer above the plate that is to be changed,
2. Right click the plate with the mouse pointer,
3. On the menu that appears, select 'Change plate type', and
4. The Plate Configuration window will appear.

In the top-left of the Plate Configuration window, the different plate layouts are listed. These layouts include Capture Plates, 96-well plates, and many others. The top-right window shows the actual plate type, for example a Whatman 96-well Low skirt 800 µL GF/B Filter Plate. To select the plate that matches your plate the closest, scroll through the list until you have found a plate that is either the same or very similar to the plate that you are using. If you cannot find a plate that resembles the plate you are using, contact the Corbett Support Team.

When you choose a plate type, a photograph of the plate will appear in the lower left window to aid the user in plate identification. To assist the user when choosing capture plates, Corbett Robotics

part numbers are displayed in the photograph for the matching Transfer Carriage, Separator Plate and Riser Block. These corresponding numbers are embossed on the actual items for easy identification.



At the bottom right in the Plate Configuration Window are a number of filters. To view all plates that are available in the software, ensure all filters are ticked. If you wish to see tip racks only, ensure that only the tips filter is ticked.

Plates that you commonly use can also be added to 'Favourites'. To add a plate to your favourites, select the 'Add to Favourites' button. Similarly you can remove a plate from the Favourites. To view your list of favourite plates, ensure that only the favourites filter is checked.

When the desired plate is selected, click on 'OK'. The chosen plate will appear on the workspace.

Plate Segments

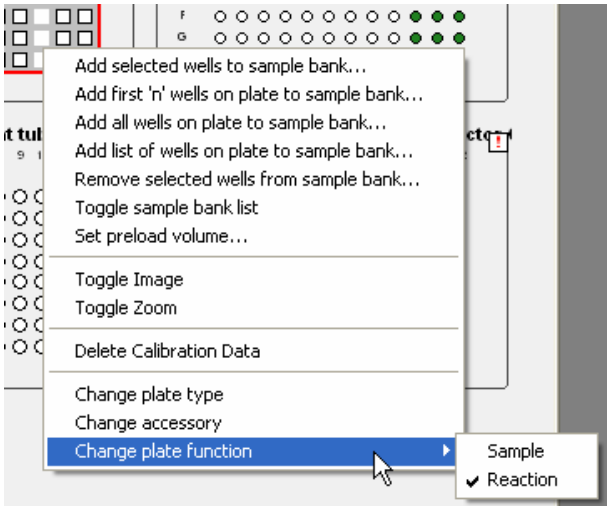
Some plates are broken into several segments, for example the default reagent block can house different combinations of each of the three reagent tub sizes. Other plates may have different segmentations. Most 96-well plates have one segment with all wells belonging to the one segment. Segments are factory defined and cannot be changed. The function of a segment on a plate can be changed independently from all other segments of that plate.

Changing Plate Function

Once the correct plate type is chosen, the plate's function can also be changed. This allows the user to select whether the plate will hold samples, reactions, reagents or some of the other liquid groups. You can change the plate's function as follows:

1. Position the mouse pointer above a well of the plate that is to be changed,

2. Right click the plate with the mouse pointer,
3. On the menu that appears, select 'Change plate function',
4. The available functions will appear as a sub-menu, and
5. From the sub-menu choose the appropriate function.



Please note that on some plates, sub-sections of the plate can have different functions. If the plate is broken into more than one segment, the function of a segment can be changed in the same way. Simply right click in the segment that needs to be changed.

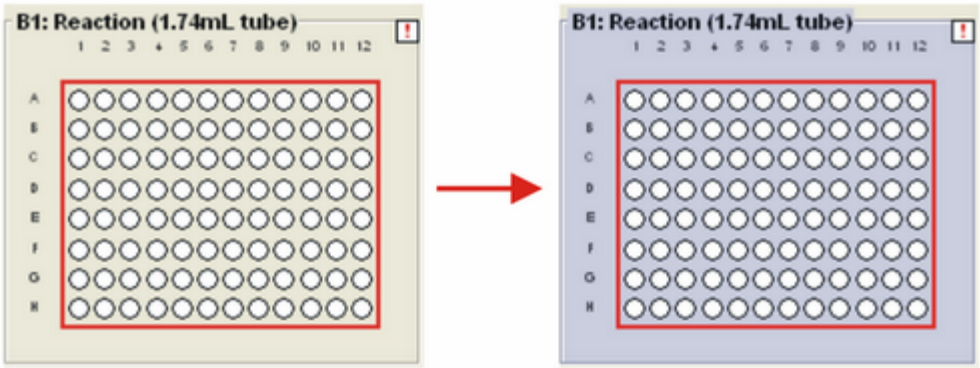
3.4.3 Choosing an Accessory

In some cases it may be necessary to place an accessory under a plate. In most cases this is likely to be a cooling block to keep samples or reactions cool. Placing an accessory under a plate affects the height calibration of that plate.

To add an accessory to a plate, follow these simple steps:

1. Right-click on the plate where the accessory is to be added,
2. Select the 'Change accessory' option,
3. The Select Accessory window will appear,
4. The required accessory can be chosen from the drop-down menu, and
5. Click 'OK'.

Once an accessory is chosen, the background colour at the location of the accessory will change.



Please note that the plate that is on top of that accessory will need to be calibrated regardless of whether or not the plate was previously calibrated. This calibration is separate to the calibration of the plate without the accessory. If the plate was calibrated before the accessory was added, when the accessory is removed through the software, the original calibration will again take effect.

3.5 Menu

The Menu provides access to the main functions in the software. The Menu items are:

- File Menu
- Control Menu
- Wizard Menu
- Options Menu
- Help Menu

3.5.1 File Menu

The CAS-1820 X-tractor Gene run files are those files that contain all the information to construct a run on the robot. These files have the file extension '.CAS4'. As well as containing information on where the liquids are and where these liquids need to be transferred to, the run file also contains all other program settings. These settings are:

- Air volumes
- Pipetting Speed
- Whether or not liquid is present in the target plate
- Sample banks and names
- Sample, reagent names

The File Menu gives access to a number of file related functions. Some of the functions in the File Menu can also be accessed via the Toolbar.

New (Ctrl+N)

This option allows the user to create a new run. Selecting this option will display the Selecting a Run dialog.

Open… (Ctrl+O)

This option opens an existing run file. This file can be modified to create a new run, or it can be used as is. Please note that all options are stored as part of the run file.

Save… (Ctrl+S)

This option saves the current setup to a new run file. All parameters except Tip Availability are saved.

Lab notebook

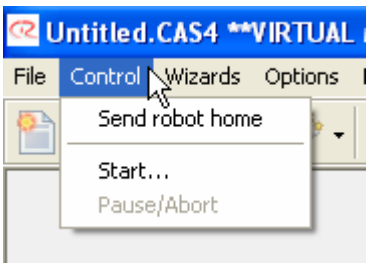
This option allows the user to enter comments for a specific run in plain text. This feature can be used as a type of laboratory notebook to document the purpose of the run.

Exit (Ctrl+Q)

This option closes the Robotics Software that controls the robot.

3.5.2 Control Menu

The Control Menu allows the user to access the CAS-1820 X-tractor Gene Homing, Start and Pause/Abort functions:



Send Robot Home

Occasionally, while using the CAS-1820 the user may wish to force the robot to move the arm and pipette head to the 'Home' position. This is most likely to occur if the user accidentally moves the pipette head of robotic arm while adding or removing consumables. By selecting 'Control' and then 'Send robot home' the robot activates its homing routine for each axis. Upon completion of the homing routine, the robot will return to its resting position at the rear right of the workspace. This is the menu equivalent of selecting the Tool Bar Icon 

for 'homing' the robot arm.

Start

Selecting start begins the run. Please refer to Starting a Run for more details. This is the menu equivalent of selecting the Tool Bar Icon  for starting the extraction run.

Pause/Abort

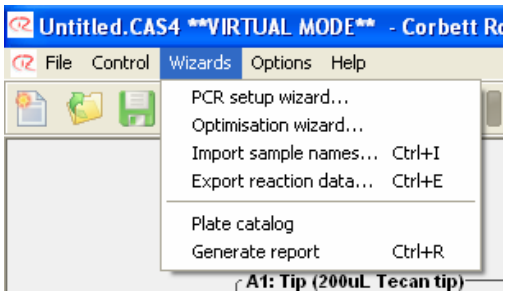
During a run, selecting this menu item while robot is performing a run will cause the robot to pause. This pause will be recorded in the post-run report. To avoid possible injury, extreme care should always be taken when pausing and opening the lid during a run.

When the run is paused, a message box will appear. The run can be continued by selecting 'OK' in the message box. To abort the run click Cancel.

This is the menu equivalent of selecting the Tool Bar Icon  to Stop/Abort the extraction run.

3.5.3 Wizards Menu

The Wizard Menu allows the user to access the CAS-1820 X-tractor Gene Vacuum DNA Extraction Wizard, Import Sample Names, Export Reaction List, and Generate Report functions:



Vacuum DNA Extraction Wizard

By selecting this option, the Vacuum DNA Extraction Wizard is started. This Wizard is designed to make it easy to set up DNA extraction protocols in a stepwise fashion. Please see the DNA Vacuum Extraction Wizard for more details. This is the menu equivalent of selecting the Tool Bar Icon  for opening the Vacuum DNA Extraction Wizard.

Import sample names (Ctrl+I)

The software makes it possible to not only cut and paste sample names but also import these from text based spreadsheets. Please refer to the section on Importing Sample Names.

Export reaction list (Ctrl+E)

The reaction list can also be exported. The components and volume of each reaction can be exported. The reaction list can be exported in a way that the Corbett Rotor-Gene software can import this data. Please see Exporting Reaction Lists for further details.

Generate report (Ctrl+R)

When you have finished setting up a run, the Pre-Run Report is available through the 'Wizard Menu' or by the keyboard combination Ctrl+R. The report is viewed in HTML format but may also be exported to Microsoft Word, e-mailed, printed or saved. A completion of a run the Post-Run Report is also available, again in the same format as the pre-run report.

3.5.4 Options Menu

The options menu controls the bulk of the robot setup. Run settings, calibration settings and pipetting behaviour are configured here. The items in the Options Menu are:

Calibration Management

This sub-menu allows access to functions that are concerned with the transfer of calibration (and other) settings from one PC to another.

Robot Setup

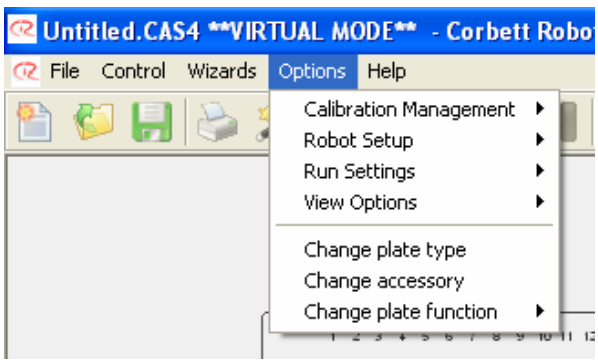
This sub-menu contains options regarding robot setup, such as calibrations.

Run Settings

This menu allows access to the primary options relating to runs. These include options to configure pipette speeds and set standard extra volumes.

3.5.4.1 Calibration Management

This sub-menu allows access to functions that deal with the transfer of calibrations and other settings of the Robotics Software and the Robot from one personal computer to another.



Save calibration on setup disk...

This option saves all calibrations and a variety of other data onto a floppy disk. This disk is called the 'Setup Disk' and is robot specific. Under no circumstances should a Setup Disk created for one robot be used on another machine as damage to the machine may result. The Setup Disk is used to transfer robot-specific calibration information should the CAS-1820 X-tractor Gene be connected to a new computer with newly loaded software. It is not necessary to use the disk when the software is updated to a new version.

To create a Setup Disk follow these simple steps:

1. Click on the 'Options/Calibration Management/Save calibration on setup disk...' option.
2. Locate the folder where you want the set up information to be stored (the a:\ drive is typically the floppy disk drive). A location other than the a:\ drive can be specified.
3. Click on OK.

Load settings from setup disk…

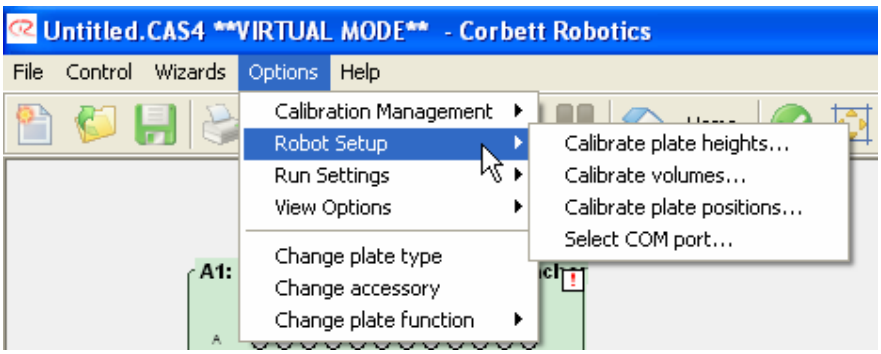
This is the reverse of the above process. Data that was previously saved on a disk can be restored using this function. Follow these steps to retrieve the information:

1. Click on the 'Options/Settings Management/Load calibrations from setup disk...' option,
2. Locate the folder where the set up information is stored (the a:\ drive is typically the floppy disk drive), and
3. Click on OK.

Please Note: While the Setup Disk is robot specific, CAS run files (Name.CAS4) are not robot specific and can be transferred from one CAS-1820 to another.

3.5.4.2 Robot Setup

This section explains how to set up the workspace of your CAS-1820 X-tractor Gene and how to choose the right plates for use with the CAS-1820. Also described are how to perform a variety of calibrations and why these calibrations are important. If you are using an accessory, for example, a passive cooling block, this section explains how to correctly set up your robot to make use of these accessories. Also explained is the use of air during the pipetting operations, pipetting speed and selecting the correct COM port.



Calibrate plate heights….

If this symbol '!' is visible in the top right-hand corner of a plate, the plate may need to be height calibrated. Refer to the section on how to Calibrate Plate Height to perform this task. The height calibration is a very critical calibration and if done incorrectly, it will have the most impact on pipetting results.

Calibrate volumes…

To perform a volume calibration on the CAS-1820, please refer to specific help on Volume Calibration.

Calibrate plate positions…

If this symbol '!' is visible in the top right-hand corner of a plate, the plate may need to be position calibrated. Calibrating plate positions is very straight forward. To perform a position calibration, refer to the specific section on Position Calibration.

Select COM port…

The default setting for connection to the computer is COM port 1. If the robot is connected to a different COM port the software will start in Virtual Mode. In virtual mode, choose the COM port to which the serial cable from the CAS-1820 has been connected. Restart the software to ensure the new COM port setting has taken effect.

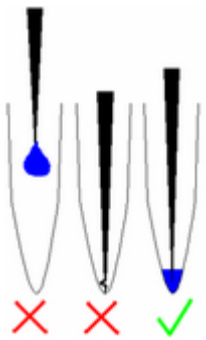
3.5.4.2.1 Plate Height Calibration

A plate height calibration must be carried out for every plate on the robot's table before that plate can be used for the first time. Every plate type in the Robotics Software has its own calibration values. Thus, once a plate has been calibrated, the calibration for that plate will be recalled at a later time even if other plates have been in use in the meantime.

Caution: the robot arm will move during height calibration while the lid is raised. Never click on any buttons while parts of your body are within the robot's workspace.

The height calibration is a very important calibration on the robot. The default height calibration is used as the height when the robot is performing mixing operations.

It is vital that the default height is calibrated correctly at a position neither too high nor too low. If the tip is calibrated too high, as liquid is ejected, a droplet may form at the end of the tip and this droplet may not fall off into the tube due to surface tension. If the default calibration is too low the tip may form a seal at the base of the tube and not eject liquid, alternatively the tip may be damaged. At the correct tip height, the tip is about 1.5 mm the base of the tube and once the liquid is ejected, the liquid is higher than the end of the tip.



There are two ways to perform a tip height calibration: manually and automatic. During the automatic height calibration the robot advances the pipetting head very slowly until the base of the tube is sensed. The calibrated height then becomes the sensed height of the tube base minus a small predetermined offset. The sensing of the tubes base can be automatically repeated to take an average of several values. During manual height calibration, it is up to the user to set the distance between the tip end and the base of the tube.

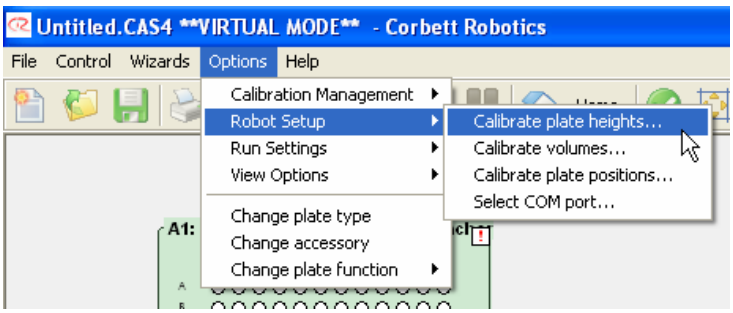
Note: The height calibration for the DNA/RNA capture plate is factory set and does not normally require adjustment by the user. However if height calibration is required, you must use the manual calibration method only. The porous, soft fibre located at the bottom of each well will be damaged if automatic calibration is used, and will also give an incorrect calibration value.

To perform an automatic height calibration, follow these steps:

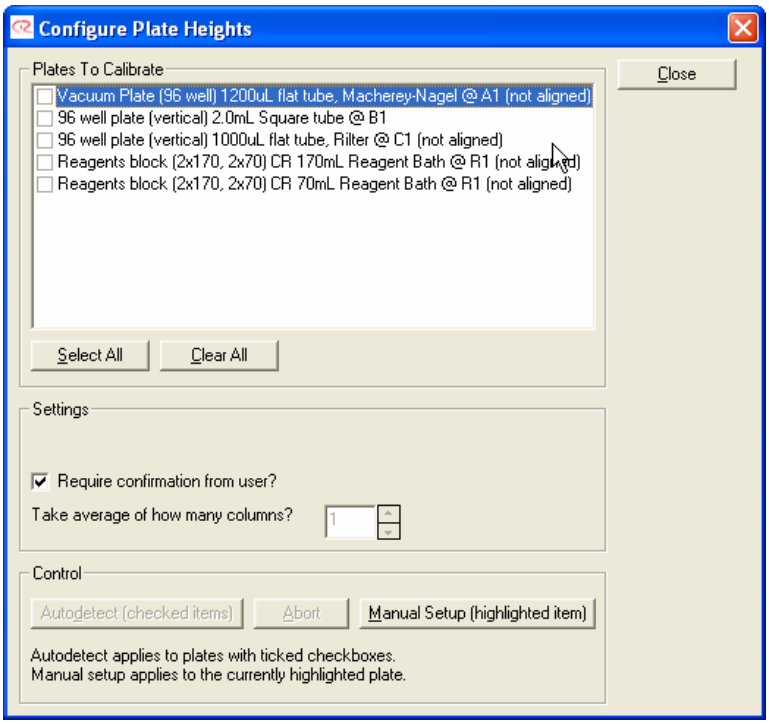
1. Ensure that the plate to be height calibrated is already position calibrated;

2. Ensure that there are 200µL tips available, that the tip rack is calibrated and that the correct tips are set to available;

3. Ensure that each plate has at least the number of tubes in its first locations as the number of averages that will be taken (see below)
4. Select Options/Robot Setup/Calibrate Plate heights...



5. Read the warning about changing these calibrations and select 'Yes'.
6. The Height Calibration Window will appear.

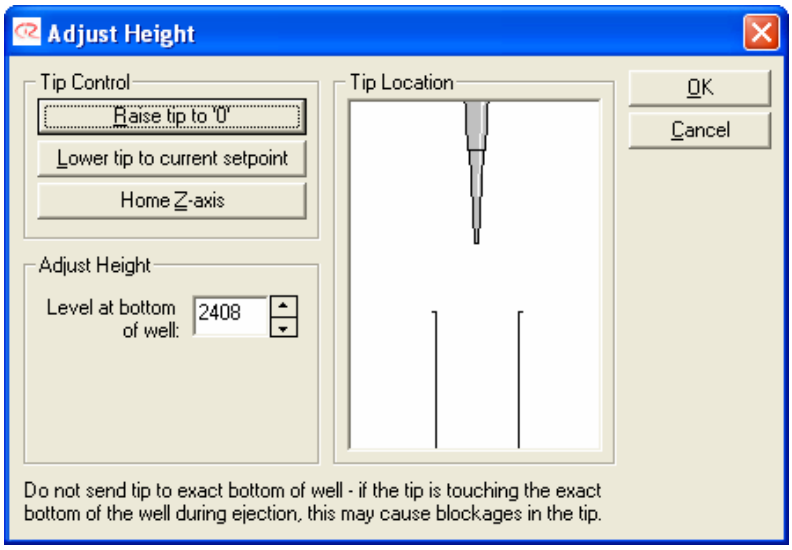


7. Shown at the top of the window, is a list of available plates/tubes. Note that plates which have multiple segments (e.g. reagent plate) appear in the list several times, each time listing the different tube type in each segment.
8. Select the plates that are to be automatically height calibrated. Do this by ticking each box next to the plate in the list. Alternatively you can select all plates by clicking the 'Select All' button.
9. Select how many wells to take the average of by clicking the up/down buttons. Corbett Robotics Pty. Ltd. recommends that an average of two will be taken.

10. The 'Reuse tips during height probe' option allows the user to specify if a new tip should be used for every height probing operation or whether to re-use the same tip. Please note that this only applies to the probing of tubes on the same plate (i.e. the wells that are used in the same average), the re-use tip option does not span plates.
11. Click on the 'Autodetect' button at the base of the window.
12. Read the warning about plate position calibration and select 'Yes'.
13. A final warning will appear regarding the plate type and position that is to be auto height calibrated. Read the warning and select 'Yes'.
14. The robot will proceed to calibrate all the selected plate heights, you are required to accept or reject the sensed numbers. If the averages for one plate are within 10 units of one another, then accept the numbers. If the numbers are not that close, check that all your tubes are sitting correctly and that the tip is not bent. If a calibration is rejected, that calibration will have to be repeated.
15. Click 'Close' when all calibrations are complete.

To perform a manual height calibration, follow these steps:

1. Ensure that the plate to be height calibrated is already position calibrated.
2. Ensure that there are 200 µL tips available, that the tip rack is calibrated and that the correct tips are set to available.
3. Ensure that each plate that is to be calibrated has one tube in its first position.
4. Select 'Options/Robot Setup/Calibrate plate heights'.
5. Read the warning about changing these calibrations and select 'Yes'.
6. The Height Calibration Window will appear.
7. Shown at the top of the window, is a list of available plates/tubes. Note that plates which have multiple segments (eg. reagent plate) appear in the list several times, each time listing the different tubes in each segment.
8. Select the plate that is to be height calibrated, by ensuring the plate to be calibrated is highlighted.
9. Click on the 'Manual Setup' button.
10. Read the warning about changing these calibrations and select 'Yes'.
11. Now the Z-axis pipetting head height warning will appear and ask if the z-axis height is to be set to a safe value. If the current setting is retained and the plate in the position is too high, the tip may jam into the plate and bend. Read the warning and select 'Yes'.
12. Use the up/down arrow buttons in the manual height calibration window to raise and lower the pipette head. To adjust the height correctly, the distance between the tip and the tube base must be adjusted as described above. As in most cases it is not possible to see the distance between the tip and the base, the distance must be 'felt' by manually lifting the tube from its support plate.



13. If the z-axis (the pipetting head) was accidentally moved too far and it bottomed out, ensure that the head has not 'lost its position' by resetting it manually. Do this by clicking the 'Home Z-axis' button.
14. Click 'OK' when the correct height calibration has been set.
15. Click 'Close' when all calibrations are complete.

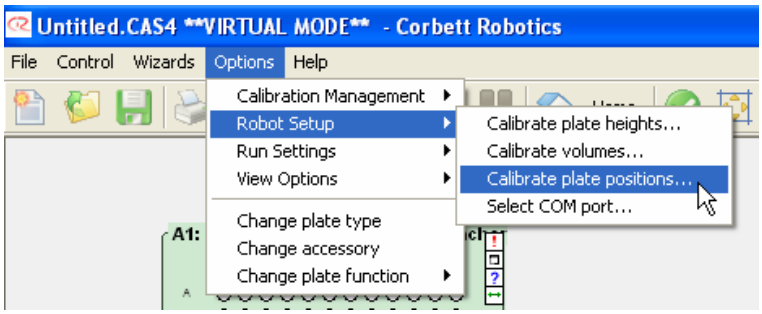
3.5.4.2.2 Plate Position Calibration

A plate position calibration must be carried out for every plate on the robot's table before that plate can be used for the first time. Once a position calibration has been carried out, it does not normally need to be repeated. Every plate type in the Robotics Software has its own calibration values. Thus, once a plate has been calibrated, the calibration for that plate can be recalled at a later time even if other plates have been in use in the meantime.

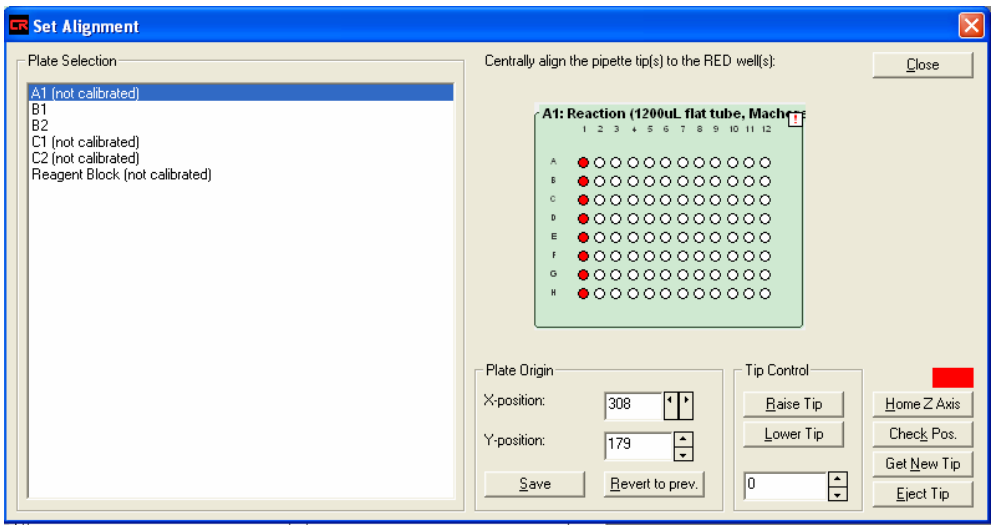
Caution: the robot arm will move during position calibration while the lid is raised. Never click on any buttons while parts of your body are within the robot's workspace.

The position calibration ensures that the robot moves to the correct location for every well on a plate. To perform a position calibration, follow these steps:

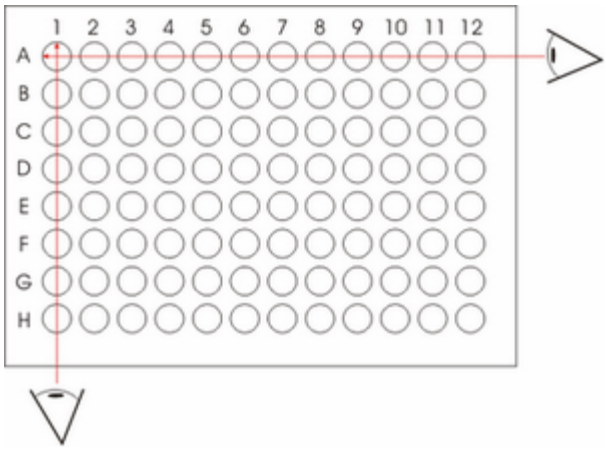
1. Open the robot's lid.
2. Place the plates that are to be calibrated on the robot. Where possible, do not place consumables in the plates. Position calibration should be performed on the 'true' position of a well, not a position that may be skewed by an ill-fitting consumable.
3. Ensure the robot has 200 µL tips available in at least one tip rack.
4. Select 'Options/Robot Setup/Calibrate plate positions'.



5. Read the warning about changing these calibrations and select 'Yes'.
6. The Position Calibration Window will appear.



7. From the list on the left side of the window, select which plate to position calibrate. A position calibration on all plates listed can be performed in turn. Always calibrate any tip racks first. Note that the tip racks are listed as 96-well plates, this is normal. Plates that have not been previously calibrated will display the (NOT calibrated) sign adjacent to them. After calibration and saving of the new settings, 'Not Calibrated' will change to (Updated) to indicate that the changes have been saved.
8. The image on the right of the window indicates which column position is used for the calibration routine. The wells of the column are coloured red.
9. Lower the robot's pipetting head by clicking on the 'Lower Tip' button several times. By lowering the pipetting head to just above the plate to be calibrated, the calibration can be carried out with greater accuracy. Ensure that the tip is not touching any plates or tips before proceeding.
10. Using the X-position and Y-position arrow buttons, move the robot's arm to a location directly above the centre of the well (or tip) indicated by the red well. Look at the robot directly from the front and directly from the side to ensure correct alignment. The pipetting head may be lowered further to facilitate easier alignment.



11. When the correct position has been aligned, click on the 'Save' button, (Updated) will be displayed adjacent to the plate description. Without clicking this button, the current position will not be stored as the calibrated value.
12. If desired, click on the 'Check Position' button to validate that the correct position has been saved.
13. If further position calibrations need to be carried out, click on the next plate in the list and continue from point 7.
14. Once all desired position calibrations have been carried out, click the 'Close' button.

Note: When calibrating plates, it will be necessary to place a tip on the robot's pipetting head. A tip can be placed on the pipetting head manually or, if the tip racks have already been calibrated and the correct tips made available, by clicking the 'Get New Tip' button.

3.5.4.2.3 Volume Calibration

Calibration is the set of operations that establishes the relationship between the actual dispensed volume and the corresponding nominal or selected volume of the pipette.

A simplistic view of this process requires that a pipette delivers a chosen volume of liquid with accuracy, and that in repetitive pipette operations, the chosen volume is delivered with precision. For example, a pipette should deliver 100 µL of liquid exactly, and should do so each time 100 µL is dispensed.

Accuracy and precision are verified by gravimetric measurement. That is, pure water weighs 1 mg/µL and that the volume dispensed by a pipette is validated by its weight; therefore 5 µL weighs 5 mg, 50 µL weighs 50 mg, and so on.

Error

In the process of pipette calibration, there are a number of factors that must be considered in order to minimise error in accuracy and precision. In general terms, these included gravimetric error and pipette error.

Gravimetric error, are those factors that contribute error in weight measurement:

- Accuracy of scales,
- Minimum unit of measurement,
- Barometric pressure,

- Ambient temperature,
- Humidity,
- Vapour pressure of the liquid used, and
- Differences in liquid and air temperatures.

Pipette error, are those factors that contribute to error while dispensing liquid from the pipette:

- Those factors affecting gravimetric measurement,
- Age and condition of the pipette mechanism (O-rings etc),
- The method of operation (individual's technique),
- Fluid retention characteristics of the pipette tip, and
- Viscosity of liquid dispensed.

The factors that affect accuracy and precision are measured as systematic and random error, and that the magnitude of error is inversely proportional to the volume calibrated.

Error in accuracy (Systematic error) - Is the difference between the dispensed volume required and the dispensed volume delivered. The systematic error is determined by taking the mean of 10 measurements.

Error in precision (Random error) - Is the variation of the dispensed volumes around the mean of the dispensed volumes. The random error is determined by taking the repeatability standard deviation of 10 measurements.

Volume calibration

Volume calibration of the robot is performed using a wizard interface (please see screen capture below). The interface is user friendly and straight forward. Two methods of volume calibration are available: manual and gravimetric adjustment. One aspect of the gravimetric adjustment is the ability to use data from a third party certified gravimetric process.

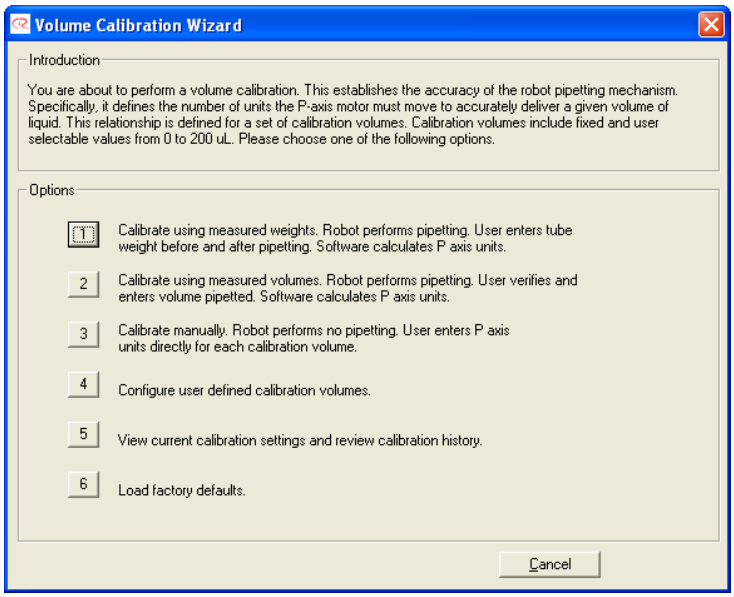
In addition to the default calibration values of 2, 5, 10, 20, 50, 100, 150, and 200 µL, the user can choose four other volumes to calibrate within the default range. Finally, a facility exists to view current and historical calibration settings, and if necessary revert to a historical setting or the factory default settings.

P-axis units

An electronic motor moves the pipette piston up and down in the vertical axis (P-axis). There is a direct relationship between the rotation of the motor shaft and the vertical movement of the pipette's piston. This relationship is known as P-axis units or steps. There are nominally 26 units or steps for each 1 µL of liquid aspirated or dispensed.

To open the 'Volume Calibration Wizard' from the main menu choose Options>Robot Setup>Calibrate Volumes. Read the warning and then select 'Yes'.

Volume Calibration Wizard Interface



Option 1 Calibrate using measured weights. Robot performs pipetting. User enters tube weights before and after pipetting. Software calculates P axis units.

Option 2 Calibrate using measured volumes. Robot performs pipetting. User verifies and enters volume pipetted. Software calculates P axis units.

Option 3 Calibrate manually. Robot performs no pipetting. User enters P axis units directly for each calibration volume.

Option 4 Configure user defined calibration volumes.

Option 5 View current calibration settings and review calibration history.

Option 6 Load factory defaults.

3.5.4.2.4 Option 1

Option 1 Calibrate using measured weights. Robot performs pipetting. User enters tube weights before and after pipetting. Software calculates P axis units.

Option 1 is the recommended method for volume calibrating the robot. The user pre-weighs 200 µL PCR tubes in groups of 8 and enters the average tube weight for each group into the pre-weight table. To do a standard calibration you require 12 x 8 groups of tubes and up to 4 additional groups of 8 tubes for each user selected weight. The robot pipettes the default volumes and any user specified volumes (see Option 4) from the user specified reagent tub containing PCR grade water. The user then re-weighs the tubes and enters the new average tube weights into the post-weight table.

Pre-volume calibration setup:

Place a rack of 200 µL fine-bore pipette tips in SBS position B1 and a rack of 50 µL fine-bore pipette tips in SBS position B2. See 'Choosing the Right Pipette Tips' for further instructions.

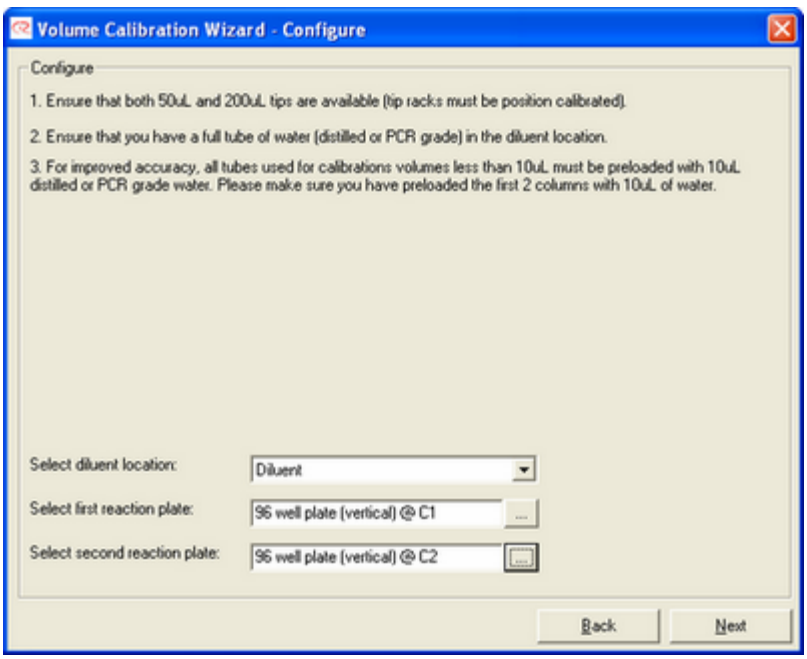
Ensure that SBS positions C1 and C2 are defined with Plate Layout, 96-well Vertical and Plate Type, 96-well column. Also ensure that the function for these plates has been set to 'Reaction'. See the topic 'Choosing the Right Plate' for further instructions. Note: Deselect the 'Favourites Filter' in order to change plate types to correct plate types for volume calibration.

Define the Reagent Tub in the default front-left position to contain PCR Grade water. With the mouse, select the front-left tub in the software workspace to open the right-hand pane. Select the check box for 'Use Reagent?' and enter Diluent in the 'Name' column of the table. Leave the 'Default Volume' set to 0 µL, and 'Viscosity' set to 'No'. Set the 'Anti-drip Pause' to 1 second.

Volume Calibration:

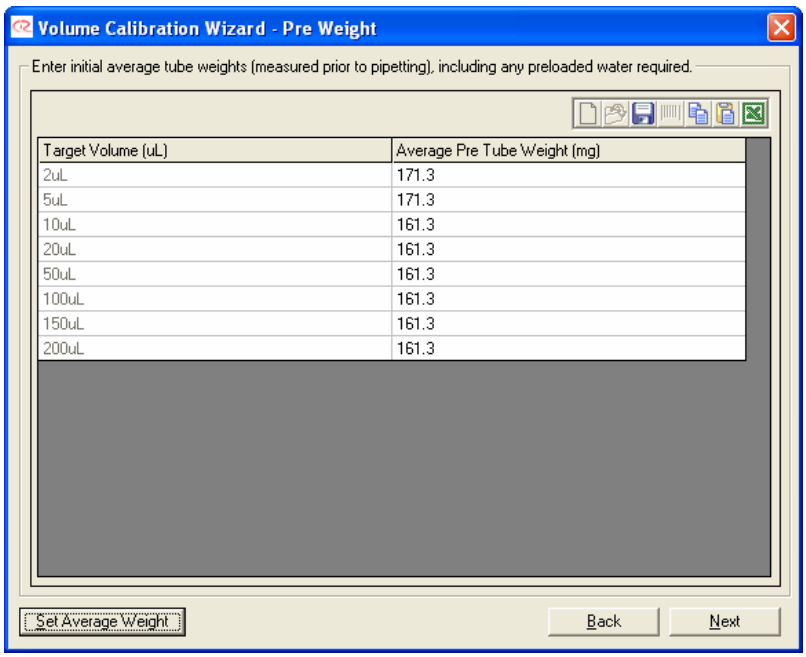
Selecting option 1 opens the 'Volume Calibration Wizard – Configure' window.

Volume Calibration Wizard – Configure



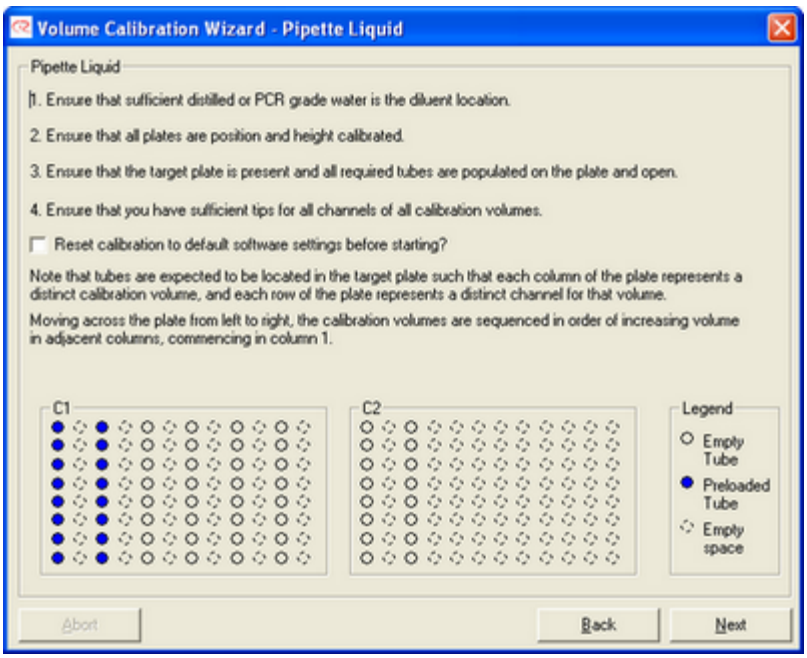
1. Read the instructions in the 'Volume Calibration Wizard – Configure' window. Now set the dilution location and the reaction plate locations as depicted above.
2. Set aside 2 groups of 8 tubes and aliquot 10 µL of PCR grade water into each tube of the 2 groups. One group of 8 tubes will be used to calibrate the 2 µL volume and the other group of 8 the 5 µL volume.
3. Ensure that the first reaction plate is set to C1 and the second to C2 as depicted in the screen shot above.
4. Select 'Next' and the 'Volume Calibration Wizard – Pre-Weight' window opens. Selecting 'Back' returns the user to the main 'Volume Calibration Wizard' window.

Volume Calibration Wizard – Pre Weight



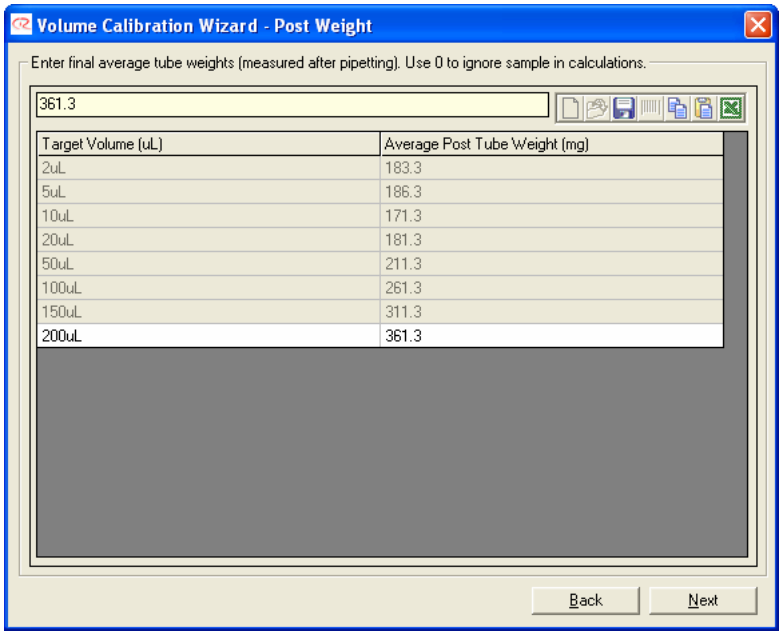
5. Weigh each group of tubes and determine the average weight for each group. Enter the average tube weight in the 'Average Pre Tube Weight' column. If the average weight of the 200 µL PCR tubes is known, the user can select 'Set Average Weight' and enter this value in the pop-up window that appears, the table will be populated with this value automatically. Note that for all volume calibration targets below 10 µL, the average weight will be incremented by 10 mg to allow for the 10 µL of PCR grade water preloaded into these tubes before pipetting starts.
6. Select 'Next' and the 'Volume Calibration Wizard – Pipette Liquid' window opens. Selecting 'Back' returns the user to the 'Volume Calibration Wizard – Configure' window.

Volume Calibration Wizard – Pipette Liquid

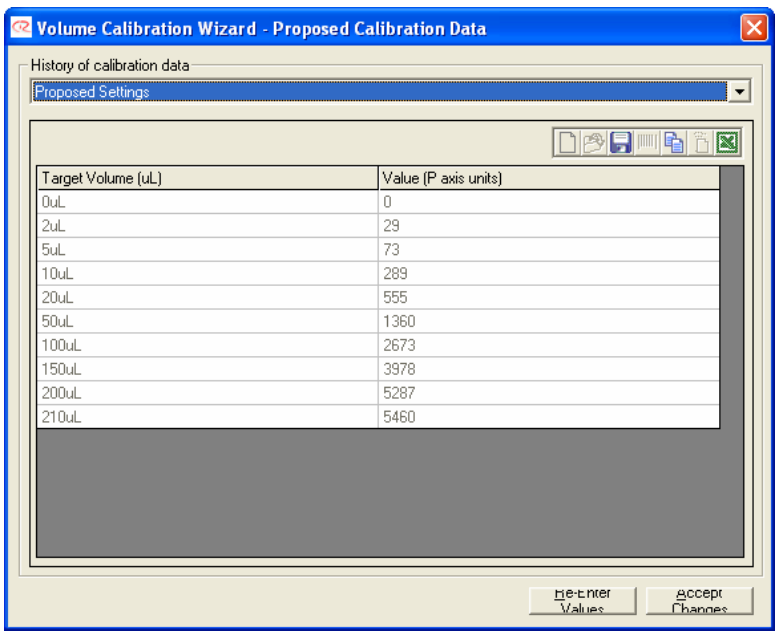


7. Please confirm that the actions indicated in items 1 - 4 depicted above have been completed.
8. Read the 'Note' about the layout of the PCR tubes and the pipetting order.
9. If you are calibrating against the factory defaults volumes, check the 'Reset calibration to default software settings before starting?' box.
10. Starting at Column 1 position C1, insert the groups of pre-loaded tubes into the plate; group 1 in Column 1, Group 2 in Column 3 etc. Then insert groups of dry tubes into the remainder of the plate in position C1 and then in the plate in position C2. Note: An empty column separates each column of tubes.
11. Select 'Next' and the robot will pipette the default volumes and any user specified volumes (see Option 4). Selecting 'Back' returns the user to the 'Volume Calibration Wizard – Pre Weight' window. When the robot has finished the 'Volume Calibration Wizard – Post Weight' window will open.
12. Again, weigh each group of 8 tubes and determine the average weight for each group. Enter these values into the 'Post Weight' table as depicted below.

Volume Calibration Wizard – Post Weight



13. Select 'Next' to open a window that displays the new calculated P-axis values.



14. Select 'Accept Changes' to save the new values and complete the Volume Calibration Wizard. Selecting 'Re-Enter Values' returns the user to the 'Volume Calibration Wizard – Pipette Liquid' window.

3.5.4.2.5 Option 2 Calibrate using measured volumes. Robot performs pipetting. User verifies and enters volume pipetted. Software calculates p-axis units. ..

Option 2 Calibrate using measured volumes. Robot performs pipetting. User verifies and enters volume pipetted. Software calculates P axis units.

Verification is often required by external accrediting organisations. Select option 2 if you need to verify volume calibration for third party certification. The robot pipettes the default volumes and any user specified volumes (see Option 4). The user independently verifies the volume dispensed, usually by a gravimetric method, and enters the verified volume data into the calibration table. The software uses these values to adjust the P-axis values.

Pre-volume calibration setup:

Place a rack of 200 µL fine-bore pipette tips in SBS position B1 and a rack of 50 µL fine-bore pipette tips in SBS position B2. See 'Choosing the Right Pipette Tips' for further instructions.

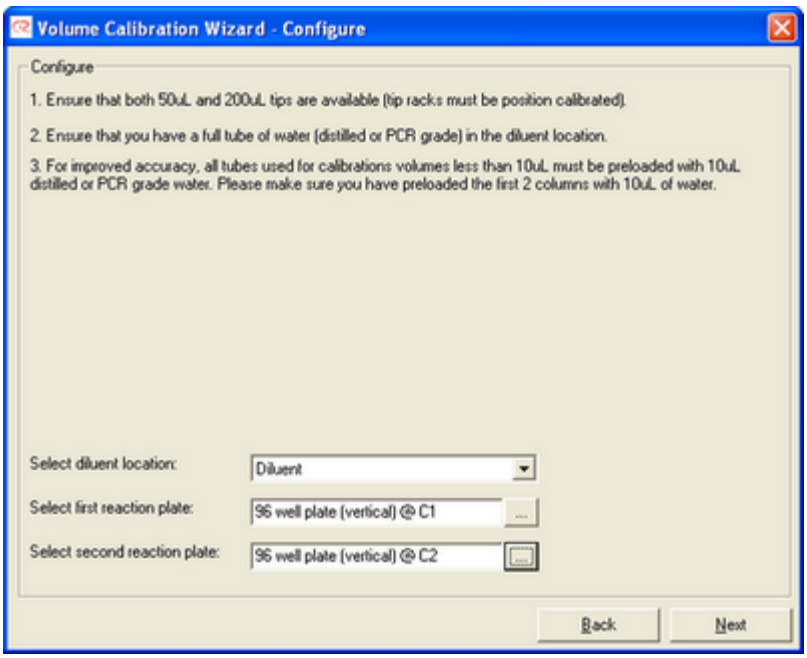
Ensure that SBS positions C1 and C2 are defined with Plate Layout, 96-well Vertical and Plate Type, 96-well column. Also ensure that the function for these plates has been set to 'Reaction'. See the topic 'Choosing the Right Plate' for further instructions. Note: Deselect the 'Favourites Filter' in order to change plate types to correct plate types for volume calibration.

Define the Reagent Tub in the default front-left position to contain PCR Grade water. With the mouse, select the front-left tub in the software workspace to open the right-hand pane. Select the check box for 'Use Reagent?' and enter Diluent in the 'Name' column of the table. Leave the 'Default Volume' set to 0 µL, and 'Viscosity' set to 'No'. Set the 'Anti-drip Pause' to 1 second.

Volume Calibration:

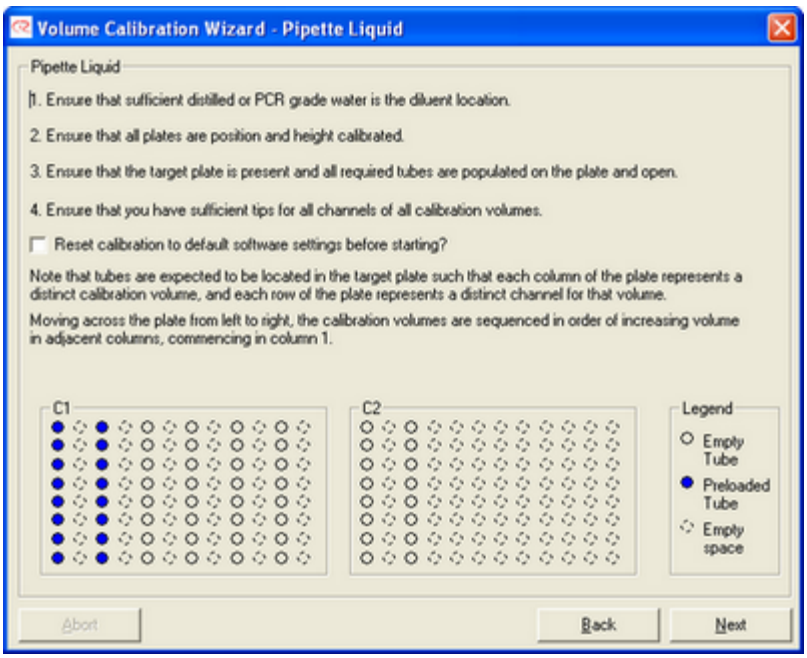
Option 2 opens the 'Volume Calibration Wizard – Configure' window.

Volume Calibration Wizard – Configure



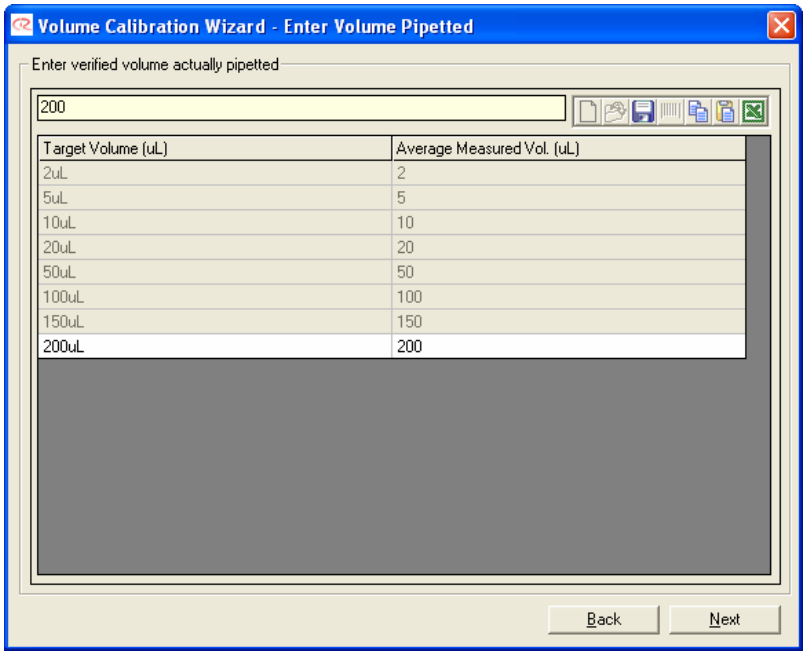
1. Read the instructions in the 'Volume Calibration Wizard – Configure' window. Now set the dilution location and the reaction plate locations as depicted above.
2. Set aside 2 groups of 8 tubes and aliquot 10 µL of PCR grade water into each tube of the 2 groups. One group of 8 tubes will be used to calibrate the 2 µL volume and the other group of 8 the 5 µL volume.
3. Ensure that the first reaction plate is set to C1 and the second to C2 as depicted above.
4. Select 'Next' and the 'Volume Calibration Wizard – Pipette Liquid' window opens. Selecting 'Back' returns the user to the 'Volume Calibration Wizard – Configure' window.

Volume Calibration Wizard – Pipette Liquid

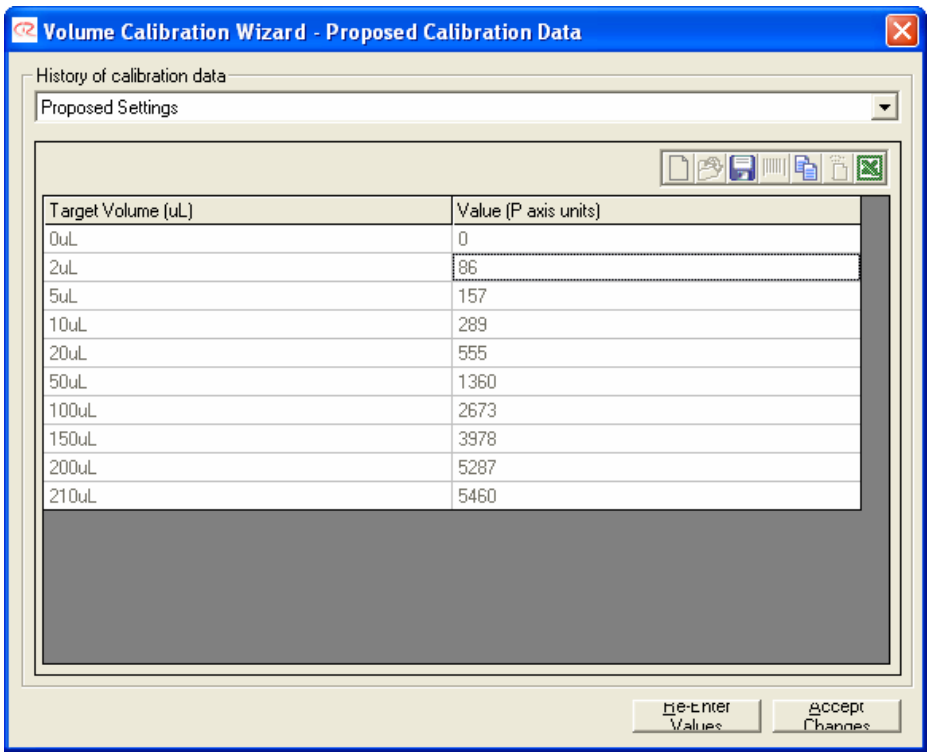


5. Please confirm that the actions indicated in items 1 - 4 depicted above have been completed.
6. Read the 'Note' about the layout of the PCR tubes and the pipetting order.
7. If you are calibrating against the factory defaults volumes, check the 'Reset calibration to default software settings before starting?' box.
8. Starting at Column 1 position C1, insert the groups of pre-loaded tubes into the plate; group 1 in Column 1, Group 2 in Column 3 etc. Then insert groups of dry tubes into the remainder of the plate in position C1 and then in the plate in position C2. Note that an empty column separates each column of tubes.
9. Select 'Next' and the robot will pipette the default volumes and any user specified volumes (see Option 4). Selecting 'Back' returns the user to the 'Volume Calibration Wizard – Configure' window. When the robot has finished the 'Volume Calibration Wizard – 'Enter Volume Pipetted' window will open.
10. Weigh each group of 8 tubes and determine the average tube weight for each group. Calculate the net tube weight for each group and convert this value to an average pipetted volume. Enter the averaged volumes into the 'Enter Volume Pipetted' table as depicted below.

Volume Calibration Wizard – Enter Volume Pipetted



11. Select 'Next' to open a window that displays the new calculated P-axis values.



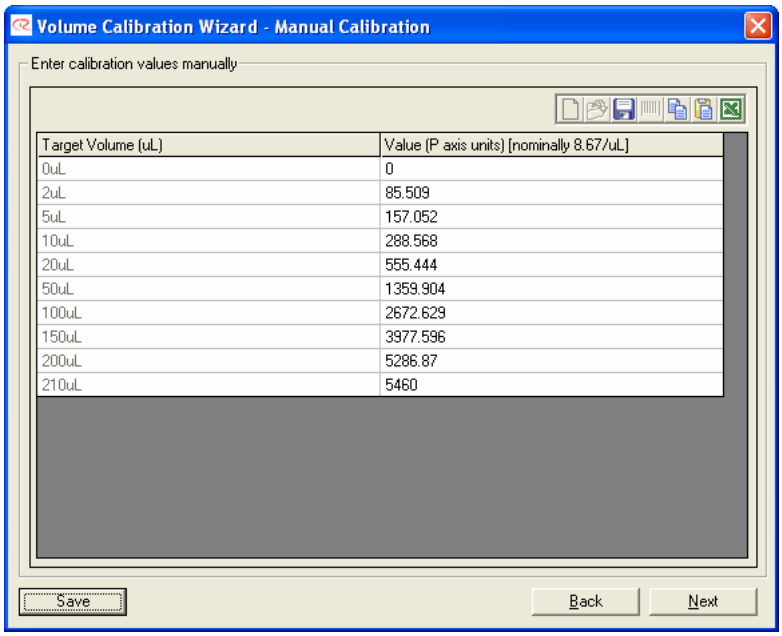
12. Select 'Accept Changes' to save the new values and complete the Volume Calibration Wizard. Selecting 're-Enter Values' returns the user to the 'Volume Calibration Wizard – Pipette Liquid' window.

3.5.4.2.6 Option 3 Calibrate manually. Robot performs no pipetting. User enters p-axis values directly for each calibration volume. ..

Option 3 Calibrate manually. Robot performs no pipetting. User enters P axis values directly for each calibration volume.

Select option 3 if you would like to manually adjust the P-axis unit value. Increasing the P-axis value increases the volume aspirated or dispensed. Conversely, decreasing the P-axis value decreases the volume aspirated or dispensed. Remember that there are approximately 26 P-axis units per 1 µL. Adjust the unit values in the right-hand column of the table as required. When finished, select 'Save' and these will become the new default values. Select 'Back' to return to the Wizards opening window.

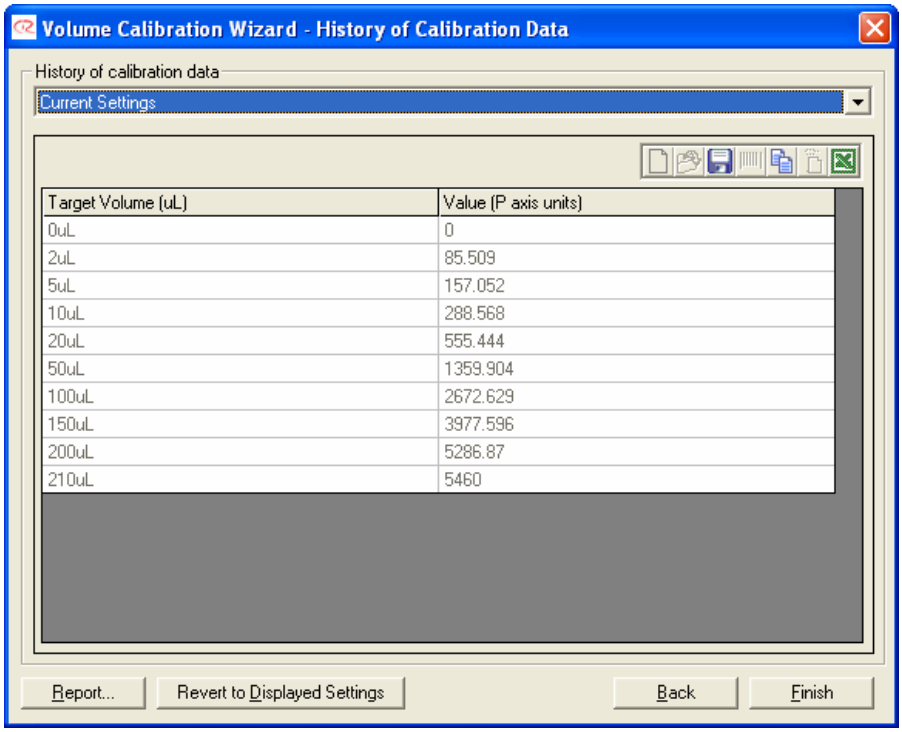
Volume Calibration Wizard – Manual Calibration



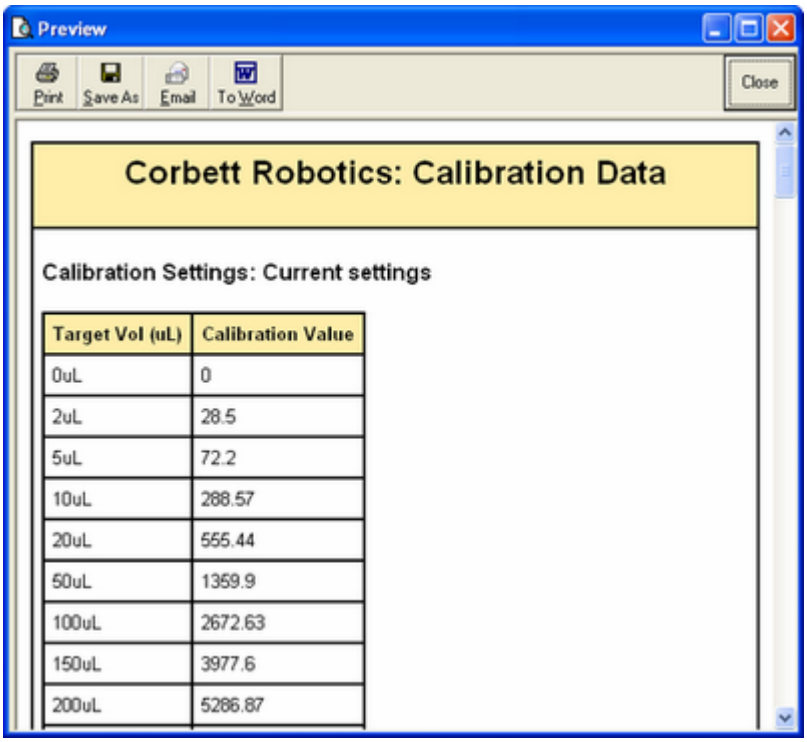
If you have not saved the new values, and proceed to select either 'Next' or 'Back' a pop-up will open and ask if the new values are to be saved. Selecting either the 'Yes' or 'No' option will open the 'History of Calibration Data' window. However, 'No' will result in the historical data being retained and 'Yes' will result in an interim pop-up advising that the new values were saved.

The 'History of Calibration Data' displays the 'Current Settings' and provides the user with several useful options: 'Generate Report', 'Revert to Displayed Settings', 'Back', and 'Finish'.

Volume Calibration Wizard – History of Calibration Data



'Finish' closes the window and advises the user that the wizard is complete, returning the user to the GUI of the robotic workspace. 'Back' returns the user to the 'Volume Calibration Wizard – Manual Calibration' window. 'Generate Report' opens a scrollable window that contains the current and historical data for up to 10 calibrations.



These details can be 'Printed', 'Saved', 'E-mailed', or entered into a Microsoft Word document. When 'Close' is selected, this window closes and returns the user to the 'History of Calibration Data' window. From here selecting 'Finish' closes the window and advises the user that the wizard is complete, returning the user to the GUI of the robotic workspace.

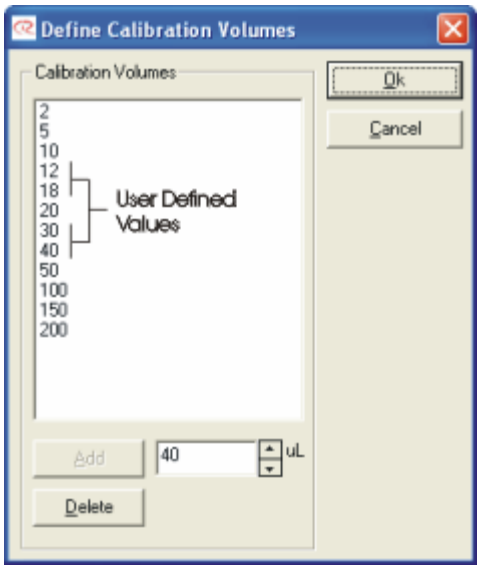
'Revert to Displayed Settings' is used in conjunction with the 'History of Calibration Data' dropdown menu. The 'Current settings' are displayed by default. However, up to nine previous settings are stored by the software, and can be accessed through the dropdown menu. By selecting one of these previous calibrations, the associated calibration data will be displayed. To make this calibration data the new default volume calibration, select 'Revert to Displayed Settings'.

3.5.4.2.7 Option 4 Configure user defined calibration volumes. ..

Option 4 Configure user defined calibration volumes.

Option 4 allows the user to add specific volumes to the volume calibration list. For example, if the user wants to volume calibrate 30 and 40 µL rather then interpolated the P-axis value for 30 and 40 µL from the adjacent default values 20 and 50 µL, the user can add the volumes 30 and 40 µL to the calibration list. Of course, user added volumes can also be removed from the calibration list by selecting the volume in the list and then selecting the 'Delete' button. Values may be chosen using the increment arrows or by typing in a new value in the window next to the 'Add' button.

Note: Default volumes for the reference volumes 0 to 200 µL can not be removed from the list.



Selecting 'Ok' saves the user defined volumes, or 'Cancel' allows the user to exit the calibration setup.

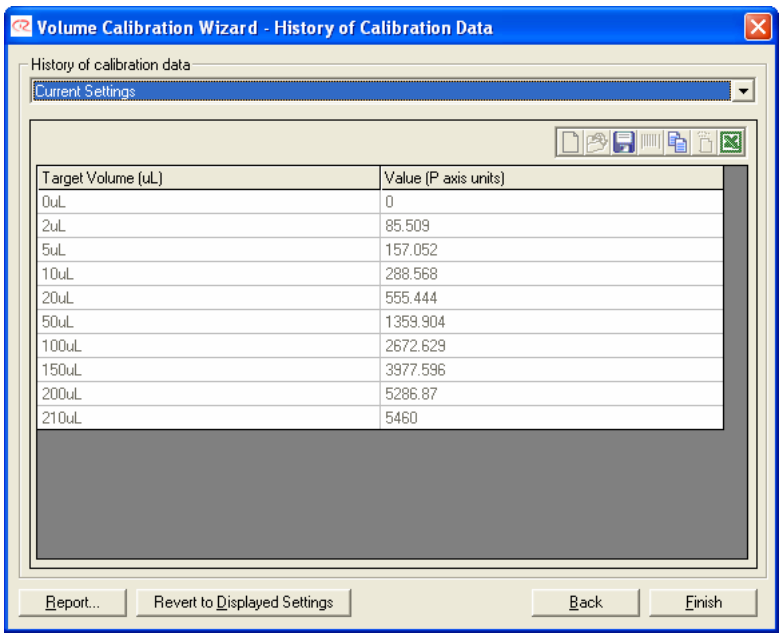
3.5.4.2.8 Option 5 View current calibration settings and review calibration history. ..

Option 5 View current calibration settings and review calibration history.

Select option 5 if you would like to review the current calibration settings and review the calibration history.

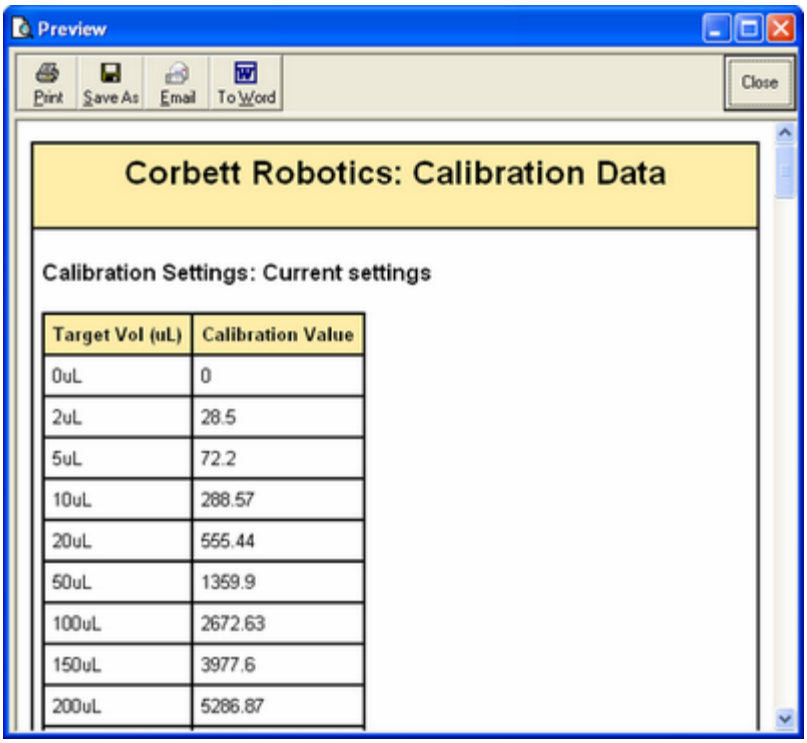
The 'History of Calibration Data' displays the 'Current Settings' and provides the user with several useful options: 'Generate Report', 'Revert to Displayed Settings', 'Back', and 'Finish'.

Volume Calibration Wizard – History of Calibration Data



'Finish' closes the window and advises the user that the wizard is complete, returning the user to the GUI of the robotic workspace. 'Back' returns the user to the main 'Volume Calibration Wizard' window.

'Generate Report' opens a scrollable window that contains the current and historical data for up to 10 calibrations.



These details can be 'Printed', 'Saved', 'E-mailed', or entered into a Microsoft Word document. When 'Close' is selected, this window closes and returns the user to the 'History of Calibration Data' window. From here selecting 'Finish' closes the window and advises the user that the wizard is complete, returning the user to the GUI of the robotic workspace.

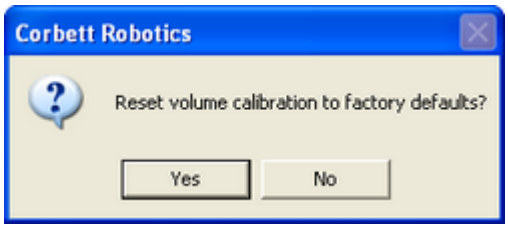
'Revert to Displayed Settings' is used in conjunction with the 'History of Calibration Data' dropdown menu. The 'Current settings' are displayed by default. However, up to 9 previous settings are stored by the software, and can be accessed through the dropdown menu. By selecting one of these previous calibration events, the associated calibration data will be displayed. To make this previous calibration data the new default volume calibration, select 'Revert to Displayed Settings'.

3.5.4.2.9 Option 6 Load factory defaults. ..

Option 6 Load factory defaults.

Select option 6 if you wish to replace the current default calibration settings with the factory default calibration settings. User specified calibration volumes will be retained, however their P-axis values will be recalculated to comply with the factory default settings.

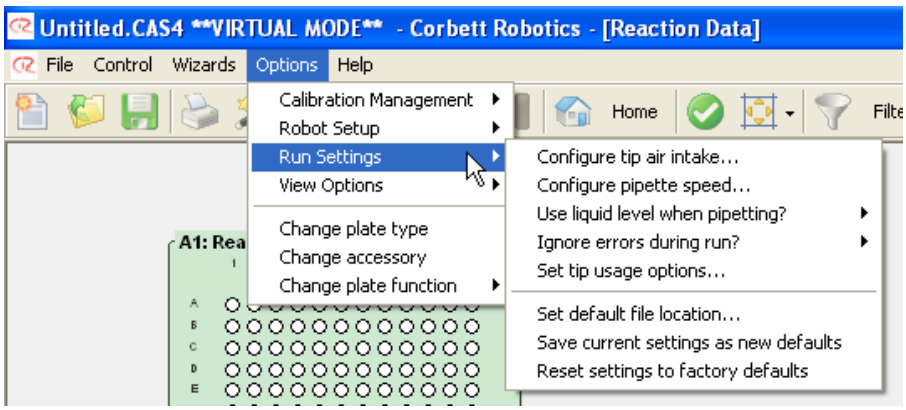
When you select option 6 you will be asked to confirm the restoration of the factory default volume calibration settings to the current settings.



If 'Yes' is selected, the current calibrations will be replaced by the factory default volume calibration settings. Selecting either 'Yes' or 'No' also returns the user to the Volume Calibration window.

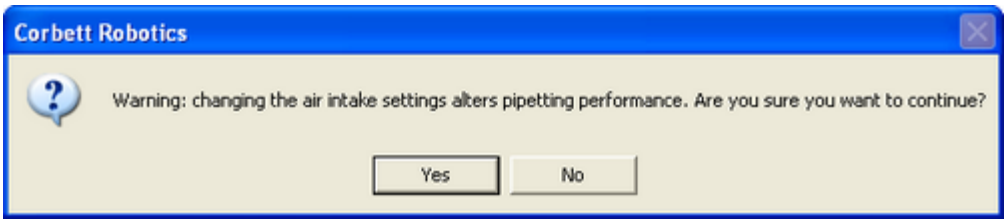
3.5.4.3 Run Settings

The menu options under 'Options/Run Settings' are explained in this section. These settings deal with software functions associated with extraction run-time parameters. New program settings can be set as default or old defaults can be restored.

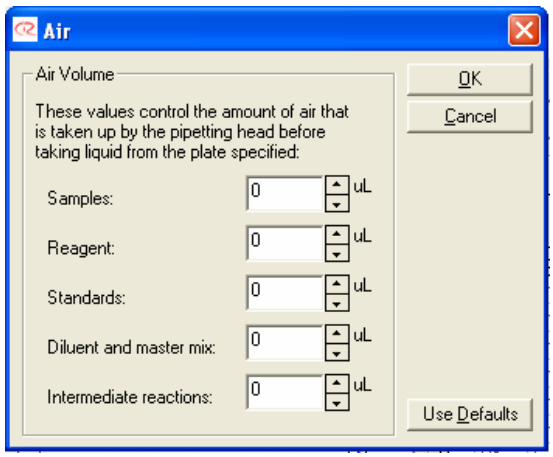


Configure tip air intake

Accessing this sub-menu will present the user with a screen stating that changing these settings may result in reduced robot performance. Click 'Yes' to continue.



This option allows the user to set the volume of air the pipette head takes up before aspirating liquid. The air is then blown out upon ejection. This makes sure all liquid is ejected. Air volumes for Samples, Reagents, Standards and Master Mixes are set independently. Higher air volumes may assist when pipetting viscous liquids. Corbett Robotics Pty. Ltd. recommends 0 µL as the minimum air intake volume.



Configure pipette speed

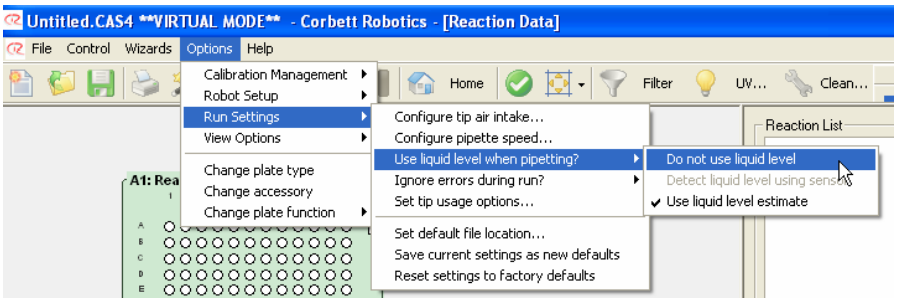
Controls the aspiration and dispense pipetting speed. The default speed of 150 µL/sec should be selected for most purposes. Slower speeds can be chosen if pipetting a viscous liquid. The reagent liquid group has two speed settings, a normal and a viscous setting. The viscous setting is used when the reagent has been selected as viscous, see Reagents for more information.

The mixing speed is the speed that controls all mixing operations. Corbett Robotics Pty. Ltd. recommends setting this speed to as fast as possible to promote good mixing.

The pipette speeds can be reset to their default values through the Run Settings menu.

Use liquid level when pipetting

Gives the user options as to how they want the robot to respond to liquid levels.



<u>*Do not use liquid level*</u>

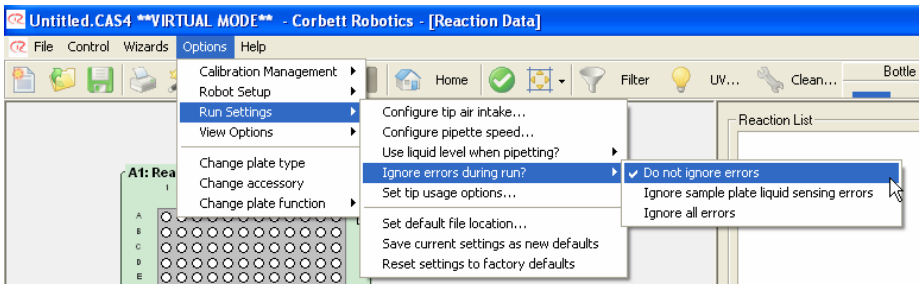
The robot pipettes at a height that was defined during height calibration of the robot. This height is the sensed height of the tube base minus a small predetermined offset.

Use liquid level estimate

The robot pipettes at a height that is estimated to be that of the liquid level. This height estimate is based on details of reagent volumes that were defined during the use of the Vacuum DNA Extraction Wizard.

Ignore errors during run?

All errors reported during the run are ignored. No user intervention is required.



Do not ignore errors...

This is the default in the Robotics Software. All errors are reported during a run, requiring user intervention every time an error occurs.

Ignore sample plate liquid sensing errors...

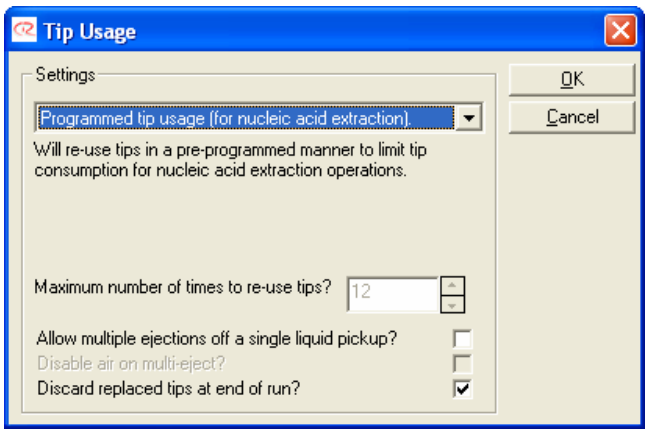
Any level sensor errors that occur in the sample plate are ignored, not requiring user interaction. Errors on any other plates including reagents, master mix or standards are not ignored.

Ignore all errors...

When this option is chosen, all level sensing errors in any plate are ignored.

Regardless of the error level, all errors are reported in the post-run report. This cannot be disabled.

Set tip usage options



A special feature of the CAS-1820 is the ability to configure tip usage. The effect of this feature is generally a dramatic reduction in the number of tips required to complete vacuum extractions. For

example, the DNA can be extracted from 96 samples while only using one and half boxes of fine-bore tips. The following tip usage options are available:

Programmed tip usage (for Extraction)

Will use tips in a programmed fashion that ensures no cross-contamination can occur during reagent addition and sample transfer, but reduces costs by re-using tips during intra-sample mixing operations.

Re-use tips were possible

This option will use simple optimisation strategies to re-use tips where possible. Important: if reaction plates initially contain material, ensure that the 'Reaction plate initially contains liquid' menu option is selected, otherwise contamination will result.

Set default file location

The factory set default file location for all saved files is c:\Program Files\Robotics4\Data. The run files are stored in this directory. This directory also contains two sub-directories, logs and reports, that store the automatically generated log files and post-run reports. The default file location can be changed to suit your particular needs. Please note that the log and report directories will automatically be generated as sub-directories in the new file location.

Save current settings as new defaults

All options that can be changed under the options menu can be altered and set as the new software defaults. By changing any option and setting it as the default, every time the software is started, it will start with these default options. These options and their factor defaults are:

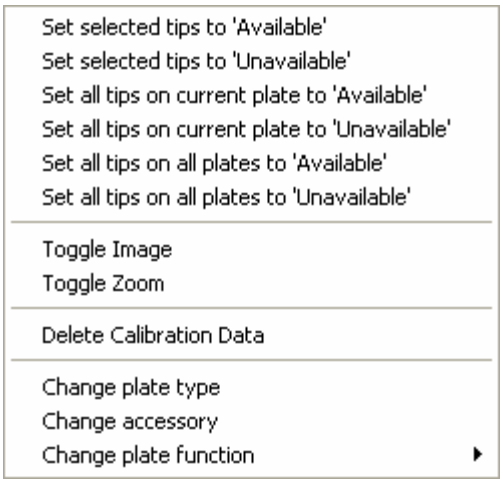
Error Mode: Do not ignore errors
Pipetting Speeds: all 150 µL/sec with the exception of reagent (viscous) 70 µL/sec
Air volumes: all 0 µL
Tip re-use: once only
Please note that the calibration settings are not part of these options. The calibration settings are saved separately and cannot be reset to factory settings.

Reset settings to factory defaults

This option resets all the above options to factory default values. This option does not affect any calibration settings.

3.5.4.4 Tip Operations

The tip operations menu can be accessed by right clicking while the mouse pointer is over a tip rack on the software workspace. These six options are used to set the tip availability.



The software uses this tip availability information to determine where tips are available. If this information is incorrect, then the run will not proceed correctly as the robot may attempt to pick up a tip from a location where no tip is available.

On the software workspace, available tips are shown in dark red or dark blue (depending on the tip type), while unavailable tips are shown as white.

Set selected tip to Available and Set selected tip to Unavailable

These two options can be used to make individual or a group of tips available or unavailable. Before this option can be used, tips must be selected. To select tips, move the mouse pointer to the top left corner of the tips to be selected, press and hold the left mouse button and drag the mouse to the opposite corner of the group that is to be selected. The selected tips will be shown with a red border. Individual tips can be selected by left-clicking them. Once some tips are selected, right-click on the tip rack and select one of the options to make the selected tips either available or unavailable. Only one group of tips can be selected at one time so the process may need to be repeated until the correct tips are set to available.

Set all tips on current plate to Available and Set all tips on current plate to Unavailable

These options can be used to make an entire tip rack unavailable or available without the need for selecting certain tips first.

Set all tips on all plates to Available and Set all tips on all plates to Unavailable

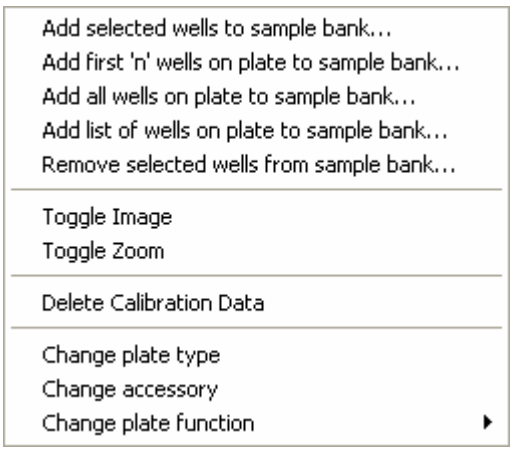
These options can be used to make all tip racks currently on the workspace unavailable or available without the need for selecting certain tips first.

Tip availability shortcut

A convenient shortcut has been added to allow individual tips to be toggled between available and unavailable. To do this, simply hold down the 'Ctrl' button and then click on individual tips. The tip will toggle between available and unavailable.

3.5.4.5 Plate Operations

The Plate Operations options are accessible by 'right-clicking' while the mouse pointer is over either a sample or reaction plate.

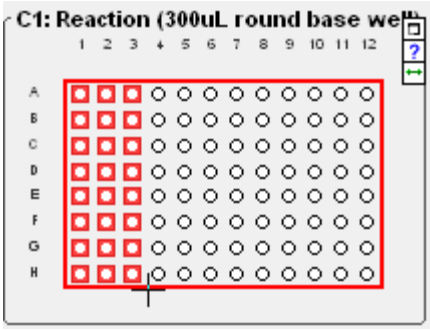


Individual samples (in wells) must first be assigned to sample banks before they are available for use in a reaction set up. This option provides a quick means of assigning columns of wells to sample banks where naming of individual samples is not required by the user. To create sample banks where naming and sample concentration details are required for individual samples please refer to the Samples section. All options with the exception of 'Toggle sample bank list' and 'Set preload volume', deal with adding or removing particular columns of wells from sample banks. Please not that wells with the liquid description of 'reaction' can also be treated like samples and grouped under sample names.

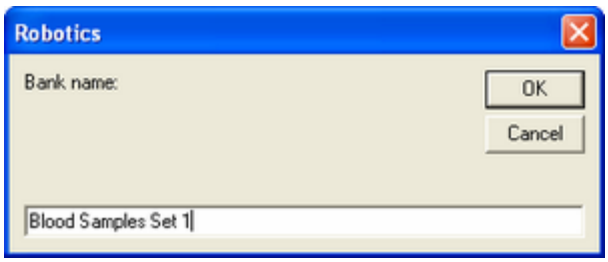
Special Note: The CAS-1820 X-tractor Gene is an eight channel system. Therefore, in the event that not all wells in a column contain samples, place an equivalent sample-volume of water in the unused wells and assign the entire column to the sample bank.

Add selected wells to sample bank

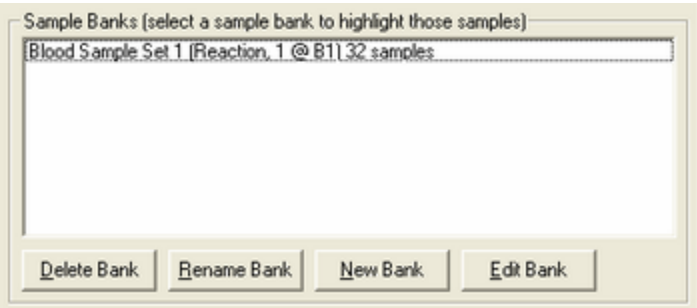
This option adds selected columns of wells to a sample bank. To select, move the mouse pointer to the top left corner of the column of wells to be selected, press and hold the left mouse button and drag the mouse to the opposite corner of the group that is to be selected. The selected wells will be shown with a red border.



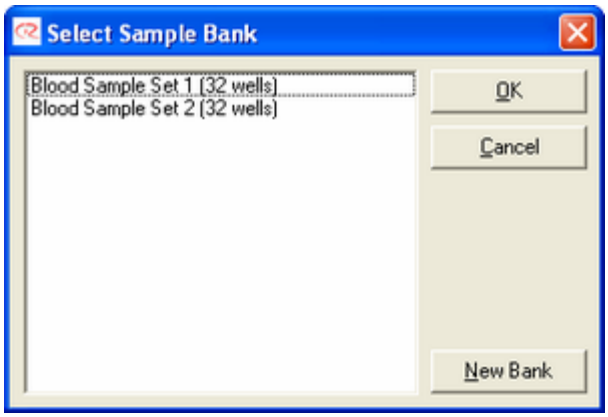
Once the desired columns of wells are selected, 'right-click' on the plate and select 'Add selected wells to sample bank' on the menu that becomes visible. If no sample banks exist at this stage, the user will be prompted to enter a sample bank name.



Enter the sample bank name and click on 'OK' or press enter. The sample bank will be created and the new wells added. The following window should be visible on the right hand pane of the screen.

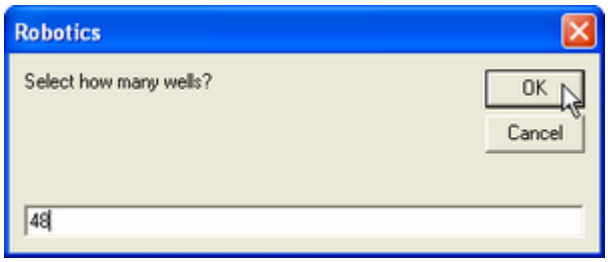


If a sample bank(s) has previously been created, the 'Select Sample Bank' window will appear. To create a new sample bank, click on 'New Bank'. To add samples to an existing sample bank, click on the sample bank name to highlight the sample bank and then click on 'OK'. Your nominated samples will then be assigned to the sample bank of your choice.



Add first 'n' wells on plate to sample bank

As an alternative to 'Add selected wells to a sample bank' for a new sample bank, a specified number of consecutive wells (multiples of eight) can be added to a sample bank. This option always selects wells starting from the first well on the plate (usually position 1 or A1). 'Right click' on the plate that contains the wells to be added and select 'Add first 'n' wells on plate to sample bank'. A prompt will appear for the user to enter the desired number of wells. Type in the number of wells and click 'OK'.



The 'Select Sample Bank' window will appear. To create a new sample bank, click on 'New Bank'. To add samples to an existing sample bank, click on the sample bank name to highlight the sample bank and then click on 'OK'. Your nominated samples will then be assigned to the sample bank of your choice starting at the first available position on the plate.

Add all wells on plate to sample bank

To add all wells on the plate to a sample bank 'right click' on the plate that contains the wells to be added and select 'Add all wells on plate to sample bank'. The user will be prompted to enter a sample bank name. Enter the sample bank name and click on 'OK' or press enter. The sample bank will be created and the new wells added.

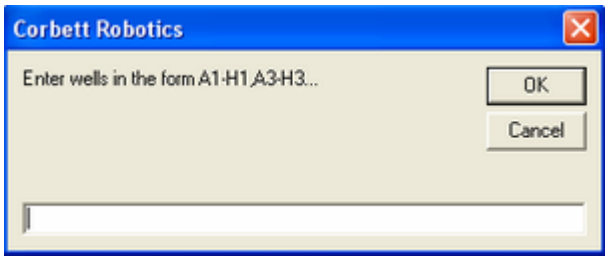
If a sample bank(s) has previously been created, the 'Select Sample Bank' window will appear. To create a new sample bank, click on 'New Bank'. To add samples to an existing sample bank, click on the sample bank name to highlight the sample bank and then click on 'OK'. Your nominated samples will then be assigned to the sample bank of your choice.

Add list of wells on plate to sample bank

This option allows the user to manually specify blocks of wells to add to the sample bank. For example the user might decide to add the odd numbered columns to a sample list. In this case they would enter the following list A1-H1, A3-H3, A5-H5, A7-H7, A9-H9, and A11-H11. Alternatively, a user

might wish to add the first three columns and the fifth column, not using the fourth column. In this case they would enter the following list: A1-H1, A2-H2, A3-H3, and A5-H5

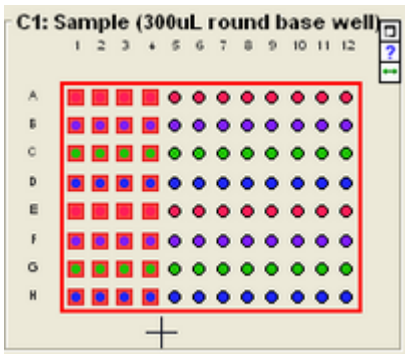
To add a list of wells on the plate to a sample bank 'right click' on the plate that contains the wells to be added and select 'Add a list of wells on plate to sample bank'. The user will be prompted to enter a list of wells.



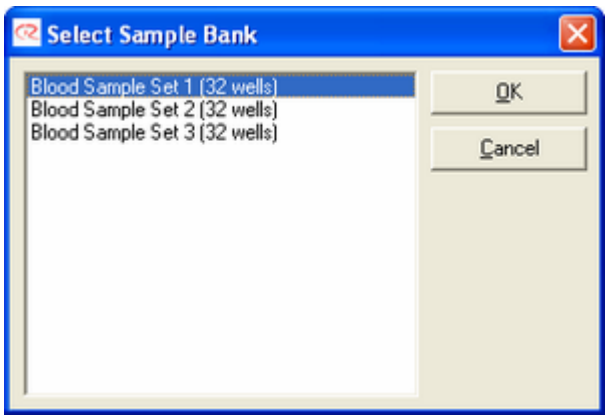
Once the list of wells is entered click on 'Ok' or press enter. Enter the sample bank name and click on 'OK' or press enter. The sample bank will be created and the new wells added.

Remove selected wells sample bank

This allows the removal of wells from a sample bank. Move the mouse pointer to the top left corner of the column of wells to be selected, press and hold the left mouse button and drag the mouse to the opposite corner of the group that is to be selected. The selected wells will be shown with a red border. 'Right click' on the plate and select 'Remove selected wells from sample bank'.



The Sample Bank window will appear. The user must now select the sample bank that contains the wells that are to be removed, once selected, click 'OK'.



If unsure of which bank the wells in question belong to, select 'Cancel'. Sample banks can be highlighted by clicking on the sample banks in the sample bank list in the right-hand pane.

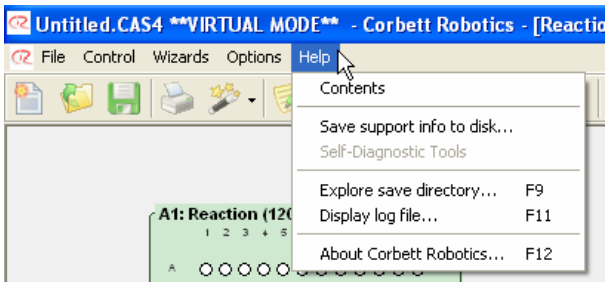
Toggle sample bank list

The sample bank list is part of the sample view in the right hand pane of the workspace. This sample view is only made visible when a sample plate is left-clicked. In some run set ups, it is possible that no plates may be defined as sample plates and only reaction plates are available. However, access to reaction plates may be necessary to modify, delete or highlight sample banks. To make the sample view available in the right-hand pane when only reaction plates are on the workspace, select the 'Toggle sample bank list' option.

For a more detailed description on sample banks please refer to the section on Samples.

3.5.5 Help Menu

The Help Menu is useful when accessing this help file and to create support packages that can help the Corbett Team to solve problems that may have arisen. Please note that context sensitive help is available throughout the software. Position the mouse pointer over an area with which help is required and press 'F1'. This will display the appropriate help section.



Contents

Displays this help file.

Save support info to disk

This option creates a zipped support package. This file can assist the Corbett Team to troubleshoot a run file. Typically, this support file is e-mailed to the nearest Corbett representative or directly to Corbett Support. This support package contains the currently open run file, a movement log file of the last few runs as well as all calibration files of the robot on which the file was created. Thus, it is important when creating the support package that it is created immediately after a suspected error has occurred.

Explore save directory

Launches a file Explorer window with the default file directory where run files are saved.

Display log file

The software logs information about run setup and all commands sent to the robot from the personal computer. The log file is updated as new runs are carried out, the oldest data being removed and newest data added. This log file is part of the support package that can be created.

About Corbett Robotics

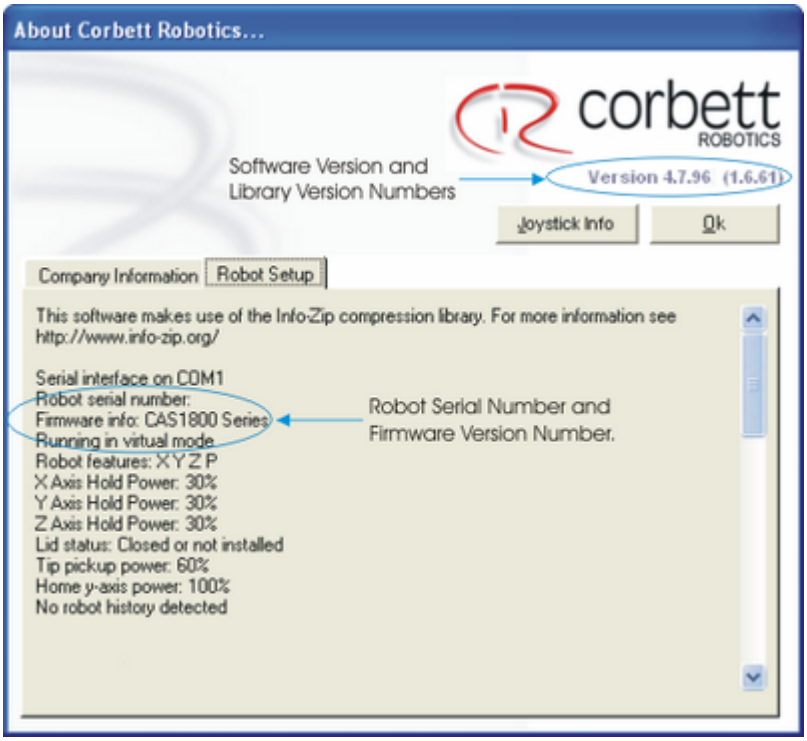
Displays a window which contains information about Corbett Robotics Pty. Ltd. and some other information that you may be asked to supply when support is provided.

The 'About Corbett Robotics' Window shows a variety of information. The most important information that the user may be asked by the Corbett Technical Support Team is the version number of the software, the serial number of the instrument and the firmware version of the instrument.

How to identify the serial number of your CAS-1820 X-tractor Gene

Each CAS-1820 is identified with a serial number on the rear of the robot on the serial number badge. This serial number identifies the instrument. Instruments also have their serial number stored electronically in the robot itself. This electronic serial number, allows the robot to be identified by the software and thus newer software features may be enabled. One example of this feature is the storage of calibration data when running in Virtual Mode.

To identify whether or not your CAS-1820 has an electronic serial number, consult the 'About Corbett Robotics' Window and select the 'Robot Setup' Tab. On this screen you will find the serial number of the instrument - this number should match that on the rear of the machine. If the numbers do not match or the serial number field in the About Window is blank, your CAS-1820 software is running in Virtual Mode.



How to identify the version number of the software you are running

All Robotics Software is identified with a version number consisting of three groups of numbers. In the About Corbett Robotics Window, the version number is found in the upper section. In the figure, the version number is 4.7.96. This software version number can also be identified on the Software Workspace.

How to identify the Firmware version number of your CAS-1820 X-tractor Gene

All robots are identified with a Firmware version number. The Firmware refers to software that is running on the microprocessor computer system inside the robot. The Firmware version can also be identified from the 'Robot Setup' Tab in the About Window as indicated in the figure. If, as shown in the figure, there is no number, your software has not been able to communicate with the robot and is most likely running in Virtual Mode.

3.6 Toolbar

The Toolbar allows easy access to expose some of the main functions in the software. These are described here.

 New file

Begins a new run. See Selecting a Run.

 Open file

Allows you to select and open an existing file.

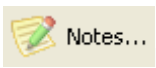
 Save file

Saves the current setup as a *.CAS4 file.

 Print
Prints the current screen.

 DNA Extractor Wizard

Select this icon to open the CAS-1820 X-tractor Gene Vacuum Wizard.



Lab Notebook

Opens the Lab Notebook.T This option allows the user to enter comments for a specific run in plain text. This feature can be used as a type of laboratory notebook to document the purpose of the run. This feature is also accessible via the File Menu.

 Start run

Begins the run. Please refer to Starting a Run for more details.

 Pause the run

During a run, clicking on this icon while robot is performing a run will cause the robot to pause. This pause will be recorded in the post-run report. To avoid possible injury, extreme care should always be taken when pausing and opening the lid during a run.

When the run is paused, a message box will appear. The run can be continued by clicking 'OK' in the message box. To abort the run click Cancel.

Send arm to home position

This option causes the robot to go through its homing routine for each axis. Upon completion of the homing routine, the robot will return to its resting position at the rear right of the workspace. This is the Tool Bar equivalent of selecting the menu item 'Control', and then 'Send robot home'.


Run Status


Change Zoom

Toggle Air Filter

UV Light

If a UV light is installed on the CAS-1820 and the UV functionality is enabled in the software, this will display the UV light dialog. If the UV light has not been enabled or is not installed, this option is greyed out.

Clean Vacuum Chamber

The Vacuum Chamber and vacuum hoses must be cleaned after each use. Selecting this icon will display the Clean Vacuum Chamber dialog.

Bottle Volume Indicator

The Bottle Volume Indicator is an approximate measure of the liquid level in the liquid waste bottle. Volume is calculated based on dispensed volumes of liquids during the run(s). When the bottle indicator (Blue Line) reaches full, the user will be prompted to empty the waste bottle. To reset the Indicator select the bottle icon and select 'Yes' in the sub-window. If the user ignores the Bottle Volume Indicator, the liquid level sensors in the bottle will activate and pause the run, preventing the bottle from becoming overfull.


Warning

If the Warning Icon is shown, it means there is a potential problem with the run. Before a run can commence, these warnings need to be removed or acknowledged.

3.6.1 UV Light Operation

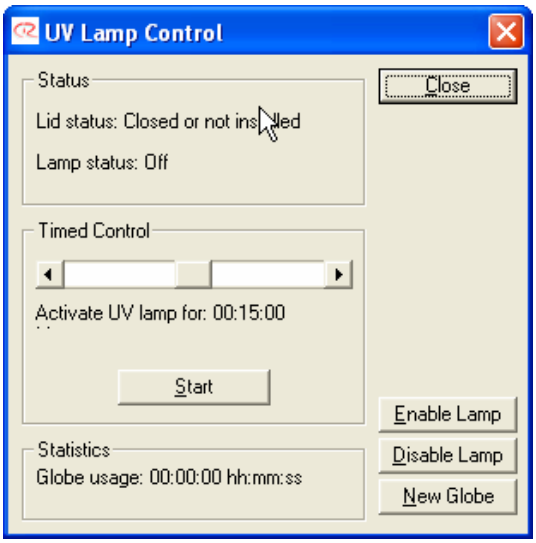
To operate the factory fitted UV light on your CAS-1820 X-tractor Gene; you must ensure that the UV light is connected at the rear of the robot. The UV light feature must also be enabled in the software, please contact the Corbett Support Team to enable this feature.

On the rear of the UV light there is an on/off switch, please ensure that this switch is in the 'On' position.

Due to the nature of UV light, the lid must be closed for the UV light to operate. An electronic interlock ensures that the light can only be operated when the lid is closed. Please ensure that the lid on your CAS-1820 is tightly closed before operating the UV light. Under no circumstances should the interlock be disabled.

During 'UV irradiation and no shadowing', the robot arm moves between two locations in 5 second intervals. Please remove all accessories to ensure the workspace is fully exposed. The UV light does not irradiate certain areas due to the robot's construction. These areas include the pipetting head.

To operate the UV light, click on the  symbol on the toolbar. The UV dialog will appear. There are two ways of enabling the UV light, timed control and manual control. Under manual control the robot arm does not move and shadowing will result. For manual control, click on the 'Enable Lamp' and 'Disable Lamp' buttons.



Under timed operation, the dialog allows exposure times to be set between 1 second and 30 minutes. Please select the desired exposure time by clicking on the slider and dragging it left or right. When the desired time is selected, click the 'Start' button. After the elapsed time, the robot will return to the rear right resting position and the UV light will turn off automatically.

3.6.2 HEPA Filter Operation

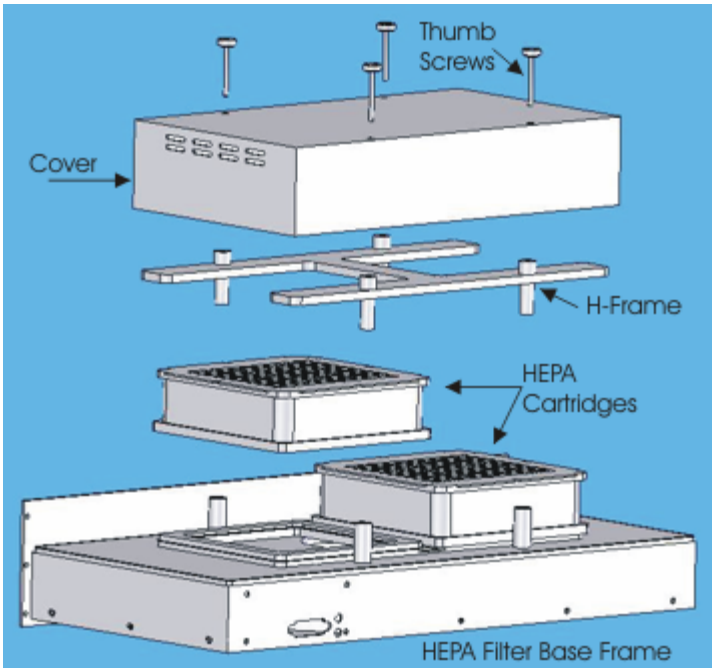
To operate the factory fitted HEPA filter on your CAS-1820 X-tractor Gene, you must ensure that the HEPA filter is connected at the rear of the robot to one of the available accessory ports. The accessory cable supplies power to the HEPA Filter in addition to software commands to turn the HEPA filter on or off.

Please make sure that the HEPA filter is connected to the CAS-1820 via the accessory cable before powering up the robot and initialising the software.

To operate the HEPA filter, select the  symbol on the toolbar.

Servicing the HEPA Filter

Occasionally, the HEPA Filter elements will need replacing. This obviously depends on the frequency of use of the HEPA filter, but should be at least annually. To replace the HEPA filter, remove the four Thumb Screws on the top of the filter; lift the Cover off, lift of the H-shaped HEPA filter holder and then the filters. Clean and remove dust from the components, place the new filters into position and reassemble the HEPA filter in reverse order, finally screw in the four Thumb Screws.



3.6.3 Clean Vacuum Chamber

The CAS-1820 X-tractor Gene must always be kept clean to avoid cross contamination of samples and should be decontaminated at regular intervals depending on level of use. When the user exits the software, the Cleaning Wizard dialog will appear and request that the user clean the vacuum chamber and hoses. Please following the instructions displayed in the Vacuum Wizard.

The user may also choose to initialise the Cleaning Wizard without exiting the software. Reasons for this may be as simple as: the robot software is never closed thus activating the cleaning procedure or as a precaution against the possibility of cross contamination occurring between runs.

To initialise the Cleaning Wizard, select the 

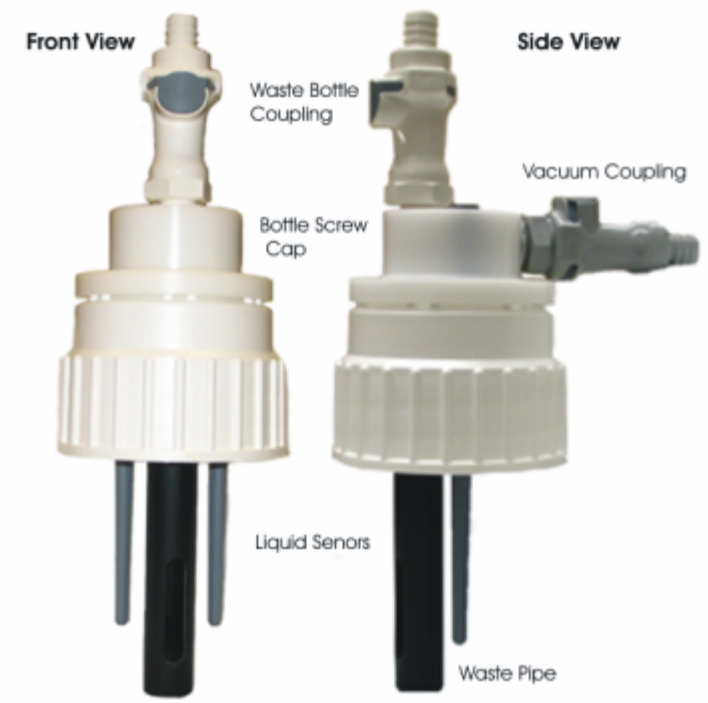
symbol on the toolbar and follow the instructions.

3.6.4 Bottle Full Indicator

The Bottle Volume Indicator 

is an approximate measure of the liquid level of the liquid waste bottle. Volume is calculated based on dispensed volumes of liquids during the run(s). When the bottle indicator (Blue Line) reaches full, the user will be prompted to empty the waste bottle. To reset the Indicator select the bottle icon and select 'Yes' in the sub-window.

Within the waste bottle are two Teflon coated sensor electrodes. If the user ignores the bottle volume Indicator, the liquid level sensor in the bottle will be activated by an overfilled bottle. The computer will pause the run until the waste bottle is emptied and the waste indicator reset.



3.7 Right-hand Pane

The right-hand pane refers to the area on the right hand side of the software workspace. When the software first starts, this area is typically grey in colour and contains no information. Using the mouse pointer to select the various plates displayed in the workspace, information on the plate's function is displayed in the right-hand pane. A plate can have one of four functions, these are linked to the three liquid groups and pipette tips available in the software. The functions and thus the different right-hand panes are:

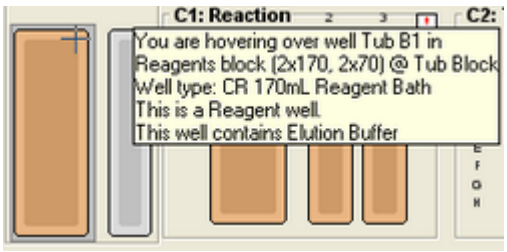
- Reagents
- Samples
- Reactions
- Tips

Each of these four right-hand panes and their associated functionality is described in detail in its own section.

Please note that as with all liquids presented to the CAS-1820 X-tractor Gene, a minimum dead volume in addition to the total reagent required is recommended. The dead volume depends on the plate type chosen, typically 5 µL for 20 µL PCR tubes and 3 to 5 mL for the reagent tubs.

3.8 Reagent Tubs

Information about a Reagent Tub and its contents is displayed when the mouse pointer hovers over the tub of interest displayed on the software workspace.



Setting up the Reagents

The right-hand pane displaying reagent information appears after selecting the Reagent Tub of interest on the software workspace. To configure reagents, select a Reagent Tub to be the source of that reagent. The right-hand pane for reagents will appear on the software's workspace. Initially, the reagent tub position on the software workspace will appear grey as shown in the following diagram. Check the 'Use Reagent?' box then enter the corresponding details in the table: Name, Default Volume in microliters (Def. Vol. (µL)), Viscosity, and Anti-drip Pause. Normally, these values are defined by the user through the extractor wizard not through manual setup as described here:

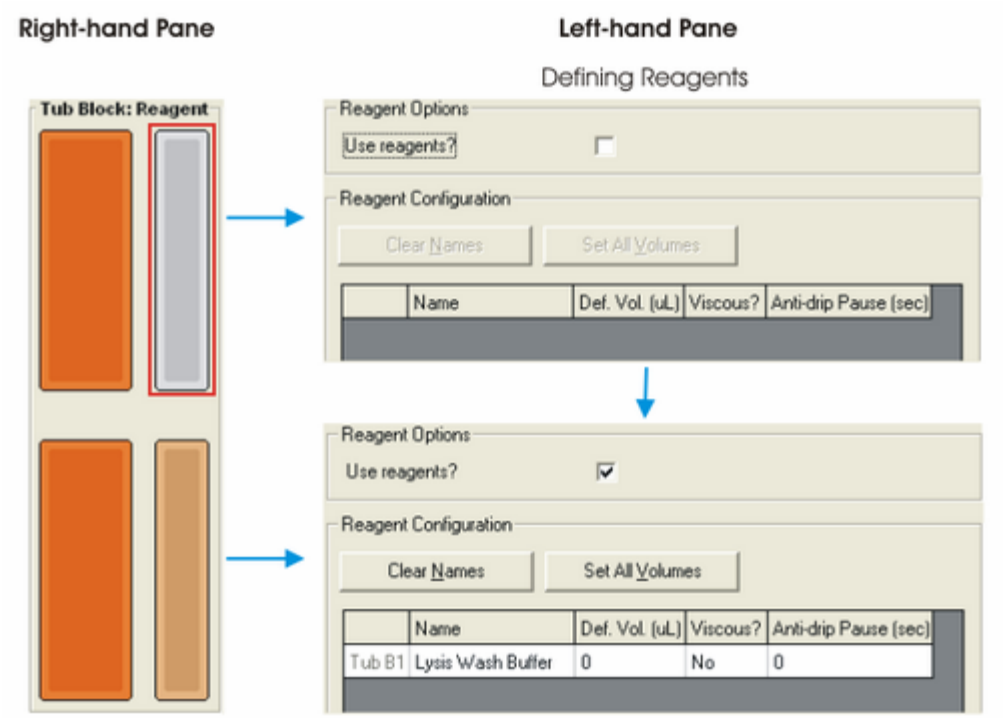
Name: The Reagent Identifier

Def. Vol. (µL): The default volume of reagent to be dispensed into each sample

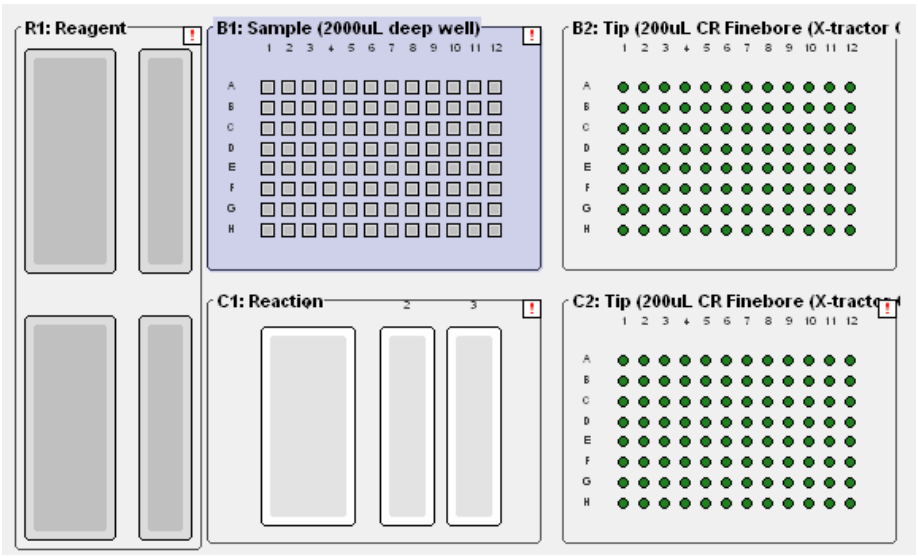
Viscosity: Selecting 'Yes' will decrease the pipetting speed of this reagent by 50% to compensate for correct pipetting

Anti-drip Pause: Set in increments of 0.25 seconds, allows the reagent on the external surface of the pipette tip to drain before the pipette tip moves across the work space.

The reagent tub displayed on the workspace will change colour to orange once the reagent is defined and the right hand pane can be closed by selecting a different region of the workspace.



Reagents are normally located on the reagent support plate in the four tubs, but can also be located on other plates. For example, if the plate function of plate position C1 is defined as reagent and the plate type is defined as a 3 tub reagent plate then the user has an additional 3 reagent tubs available for use. Note that some plates may have several segments and therefore several possibilities for the location of reagents. Refer to the section on 'Choosing the Right Plate' for more details on segments and plate functions.



SPECIAL NOTE: The CAS-1820 X-tractor Gene uses an eight channel pipetting head, therefore, while reagent volumes are per well or sample the total volume pipetted will be eight times this volume and so the number of samples should be interpreted as number of columns.

Reagent Volume Required

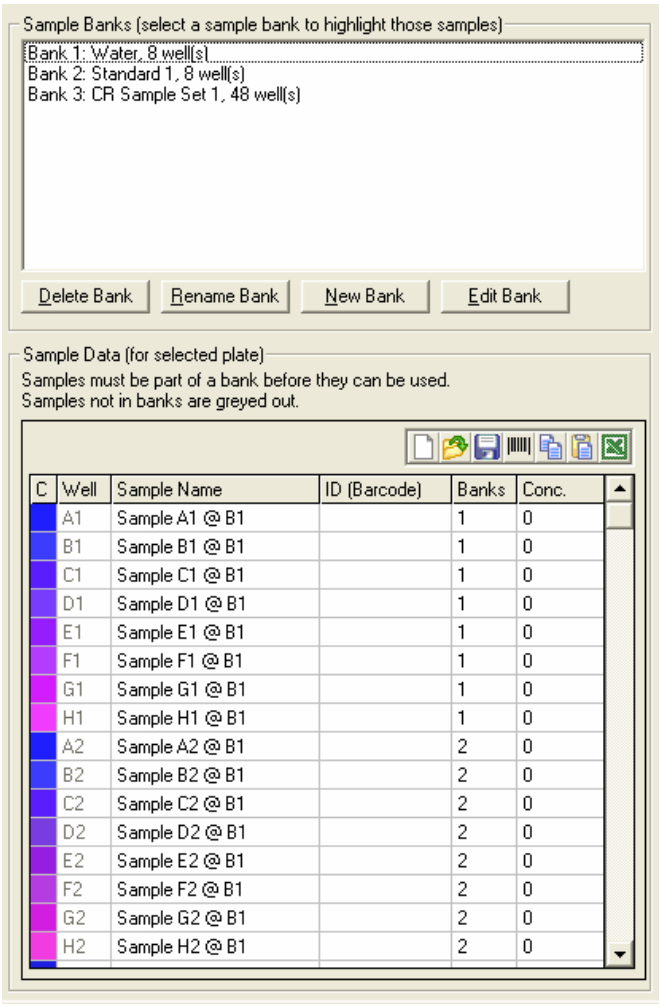
Once reagents have been theoretically added to reactions, during a run setup, the software will calculate the total volume of the reagent that is required prior to the run. The required volume can be checked by positioning the mouse pointer above the reagent tub of interest. The hover box which becomes visible reports the amount needed as shown below.

For reliable pipetting, it is essential to add a dead volume of reagent on top of the actual volume needed. In the above example the dead volume has been determined to be 5 mL for a tub of this capacity.

3.9 Samples

On the CAS-1820 X-tractor Gene, samples are simply groups of wells. These 'sample' groups form the basis for the logic in Robotics Software's behaviour to eliminate cross-contamination by tip re-use. The wells making up the samples also have the advantage that their names, an ID (such as a barcode) and their starting concentration can be defined and imported from an external source such as an Excel spreadsheet.

When a sample plate in the left-hand pane is selected the right-hand pane for samples is shown. At the top of this window, the sample bank list is shown. Under the sample bank list a number of buttons control functions associated with the sample banks. At the bottom of the window, all the samples available on the selected sample plate are shown. Note that the samples in grey are currently not assigned to a sample bank.



Sample Bank List

To delete a sample bank, select the bank to be deleted by left-clicking on the bank in the list. By clicking on the 'Delete Bank' button the sample bank will be deleted. Deleting the sample bank will not affect samples names, IDs or concentration.

To rename a sample bank, select the sample bank by left-clicking on it in the list. Then click on the 'Rename Bank' button. The user will be prompted to enter a new name.

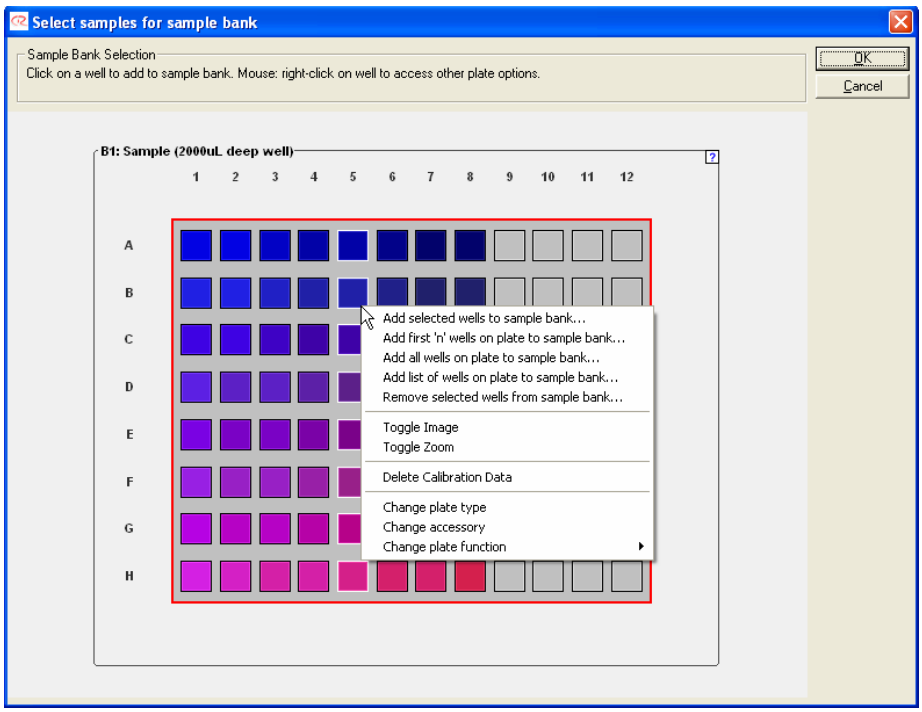
A new bank can be created by clicking on the 'New Bank' button. The user will be prompted to enter a name for the sample bank. Once the new bank is created, this bank will contain no wells. The 'Edit Bank' function is described below.

For the software to be able to use samples, these samples must be grouped together in sample banks. For the purposes of samples, sample banks are groups of wells on a sample plate. The sample banks can be continuous groups of wells. Sample banks cannot span multiple plates.

There are three ways of adding samples to a sample bank, the first method is described under 'Plate Operations' and involves selecting wells on the plate and then adding these to a sample bank. The second and third methods are discussed below.

Adding Wells to Sample Banks using 'Edit Bank'

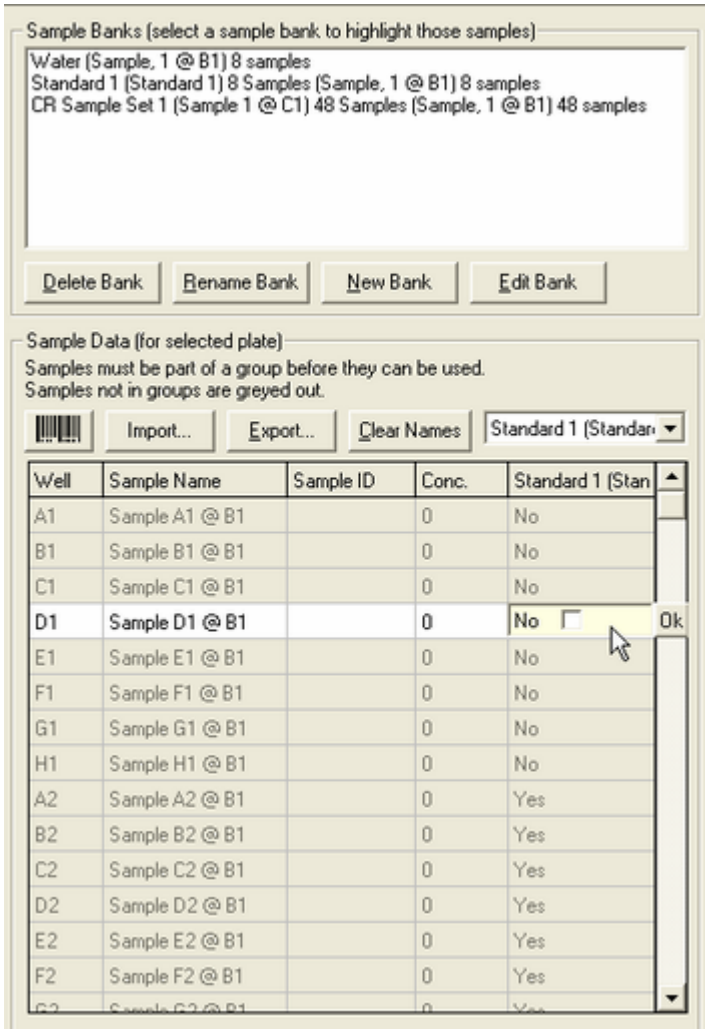
Adding wells to sample banks using the edit function is very straightforward. If a new sample bank is needed, create a bank using the 'New Bank' button. Alternatively, an existing bank can be selected by left clicking it in the sample bank list. With a bank selected, click the 'Edit Bank' button. The Edit Sample Bank window will appear as shown below. Highlight the samples using the mouse left-click drag function to choose the target wells. Now right mouse select the highlighted wells and choose 'Add selected wells to sample bank...' the wells will be added to the sample bank. Select the wells that are to be added to the sample bank. When finished, click on 'OK'.



Note: As the CAS-1820 uses an 8 channel pipetting head, wells must be selected in columns of 8 when added to the sample list.

Adding Wells to Banks using the Sample Table

If a new sample bank is needed, create one first as described above. In the Sample Data area, select the <select sample bank> drop down menu. When the menu appears, click on the sample bank to which the wells are going to be added. Once the sample bank is selected, an additional column will appear in the table. The additional column displays a 'Yes' if these are wells in the samples bank or a 'No' if they are not.



By clicking on the cell with the 'Yes' or 'No', the status of the well can be changed.

Sample Data

Sample data such as a sample name, sample ID and a concentration can be entered in the sample table. Samples are automatically given default names based on their position in the Sample Plate. Sample ID and Concentration can also be entered in separate columns. All sample data can be cut and pasted to and from spreadsheets, including Microsoft Excel. The sample data is used in the post-run report and when exporting reaction data to other systems including the Corbett Rotor-Gene. Alternatively, sample data can be imported from a variety of text-based files. To import sample data, click on the 'Import sample names' button and refer to the section on importing sample names.

Sample Normalisation

The CAS-1820 can dilute the sample into the reaction to normalise the sample to a given concentration. This sample normalisation feature is further discussed under Reactions. To use this feature, all the samples that are to be normalised must have a specified concentration across each column of 8 wells. The sample normalisation works by adding reduced amounts of sample to the reactions and then making up the shortfall by adding diluent. Note that the normalisation is limited by the smallest amount of either sample or diluent that needs to be pipetted - this limitation usually implies that the samples cannot span concentrations of more than one order of magnitude.

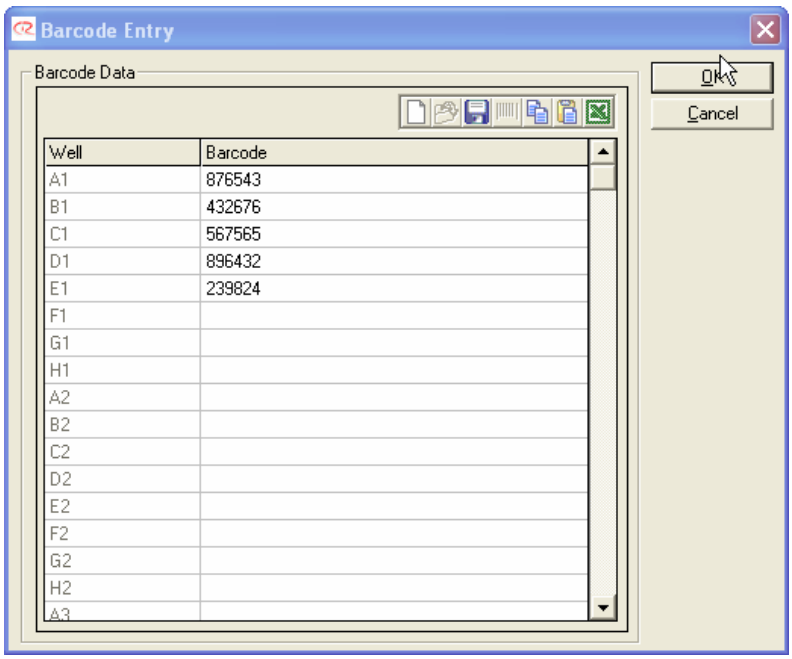
Sample Volume Required and Setting up

Samples can be stored in a variety of plates or tubes. Refer to the section on 'Choosing the Right Plate Type' for further details on selecting an appropriate plate to present samples to the CAS-1820.

The software reports individual sample volumes required in a hover box. The hover box appears when the mouse pointer is positioned over a sample well.

3.9.1 Importing Sample Names Using Barcodes

Sample names can be imported directly using most barcode readers designed to connect to a computer using a Keyboard Wedge. A Keyboard Wedge is an input device inserted between the keyboard plug and the computers keyboard socket. The software accompanying the Keyboard Wedge interprets the barcode read with a barcode reader and converts the barcode information into simulated keyboard strokes. The type of barcode reader used via a Keyboard Wedge must also be able to simulate user TABs or Carriage Returns in addition to reading the barcode. The simulated TABs or Carriage Returns automatically moves the data input point on the data table each time the barcode is read. See the diagram below. This table displays numeric barcode information, alphanumeric barcodes are also available.



As each barcode is scanned, the data is automatically loaded into the Barcode Entry table and the entry point moves to the next point in the barcode column. Once all samples have been scanned,

select 'OK' to update the sample bank table located in the right-hand pane of the software workspace.

3.9.2 Importing Sample Names

Sample names can be imported from any text-based spreadsheet file using the generic file importer.

From software such as Microsoft Excel which does not normally save its data in a text-based format, other formats can typically be exported. For example, from Excel, files can be exported by using the 'Save As…' option and selecting 'CSV (Comma Delimited)' as the file type from the 'Save as Type' selection box.

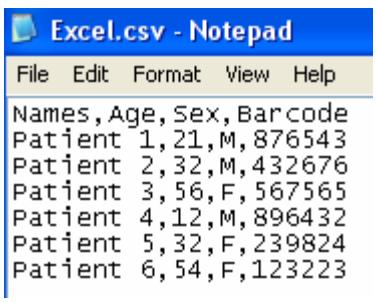
To Import Sample Names:

1. Click on the Sample plate to show the sample right-hand pane. Click on the 'Import...' button. The Import window will appear.

To illustrate the importing of a text-based file, the spreadsheet below was created using Microsoft Excel.

	A	B	C	D	E
1					
2		Names	Age	Sex	Barcode
3		Patient 1	21	M	876543
4		Patient 2	32	M	432676
5		Patient 3	56	F	567565
6		Patient 4	12	M	896432
7		Patient 5	32	F	239824
8		Patient 6	54	F	123223
9					

This spreadsheet was saved as a *.csv file. When opened with Notepad, the csv file looks like this.

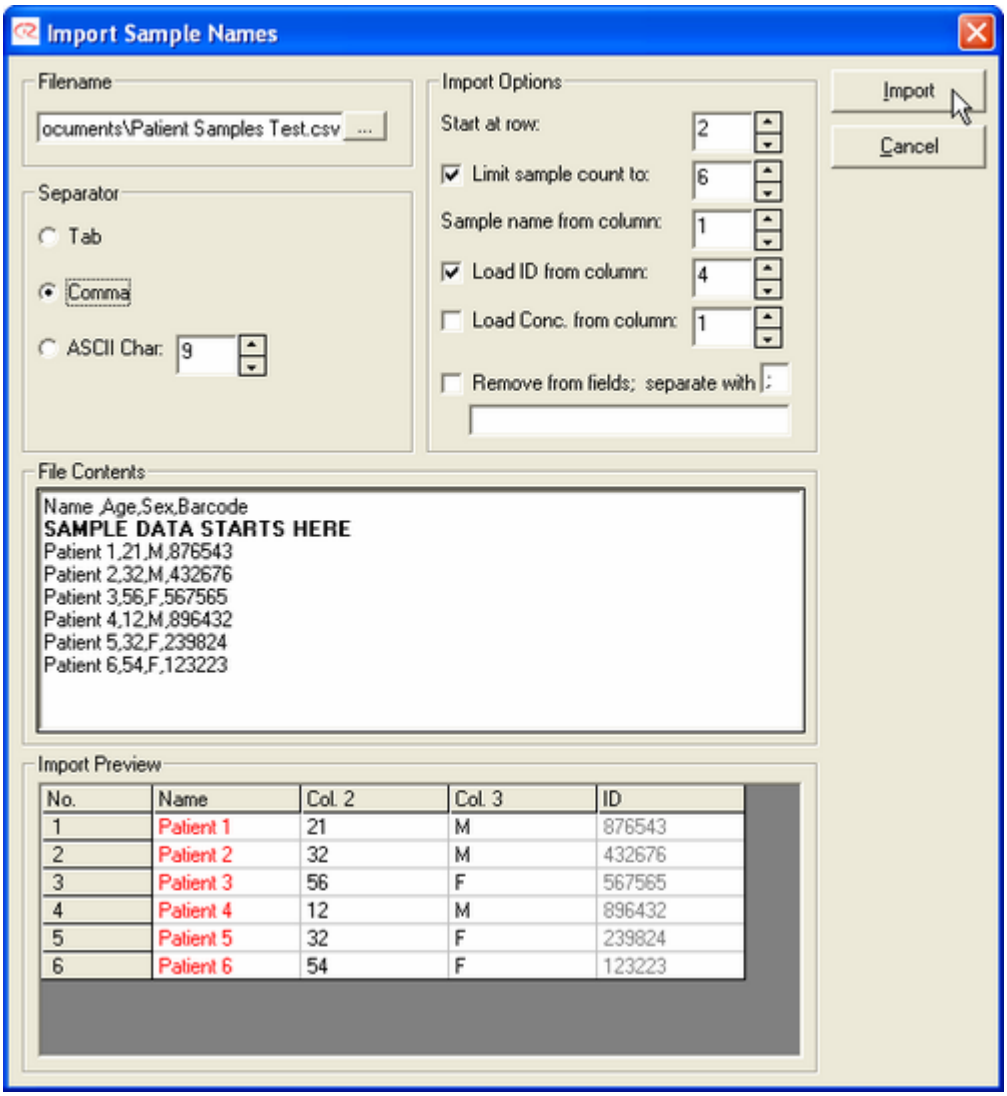


2. In the Import window, in the Filename area click on the ... button. This will allow the user to open a file to import.
3. Ensure that the Separator is selected as the comma. Other separators such as Tab can be used. If needed, the ASCII code for any character can be specified, making the import function universal.
4. In the Import Option area, select the starting row as row number 2. We start importing at row 2, because the first row contains the headings for the columns and no data. In this case there is no need to limit the sample count as only 6 samples are defined. The sample count needs to be limited if a spreadsheet contains more than 96 (or 384) rows of

data. Otherwise the software will try to import more sample names than the spaces available on the plate.

5. Specify from which column the sample names are to be imported. Looking at the *.csv file above, the sample names are in column 1. In the example above, barcodes are listed in column 4. If these barcodes are to be imported, place a tick into the check box to enable the importing of sample IDs. Select column 4 as the import column.
6. The example does not contain information on sample concentrations. If the concentration is to be specified, enable the concentration import by placing a tick in the check box. The column for the sample concentration also needs to be specified.
7. Some text-based spreadsheets contain text or symbols that do not have any meaning. These can include strings of $, " or # symbols. If these are not required as part of the names, they can be removed by typing them into the 'Remove from text' box. Ensure that the check box is also ticked if these kinds of symbols are to be removed.

After all the above options have been set, the 'Import Sample Names' window looks like this:



8. The Import Preview window shows the desired sample names in the 'Name' column, similarly the barcodes are in the 'ID' column. The data is now ready to be imported, click the 'Import' button. The imported data will look as follows in the sample right-hand pane.

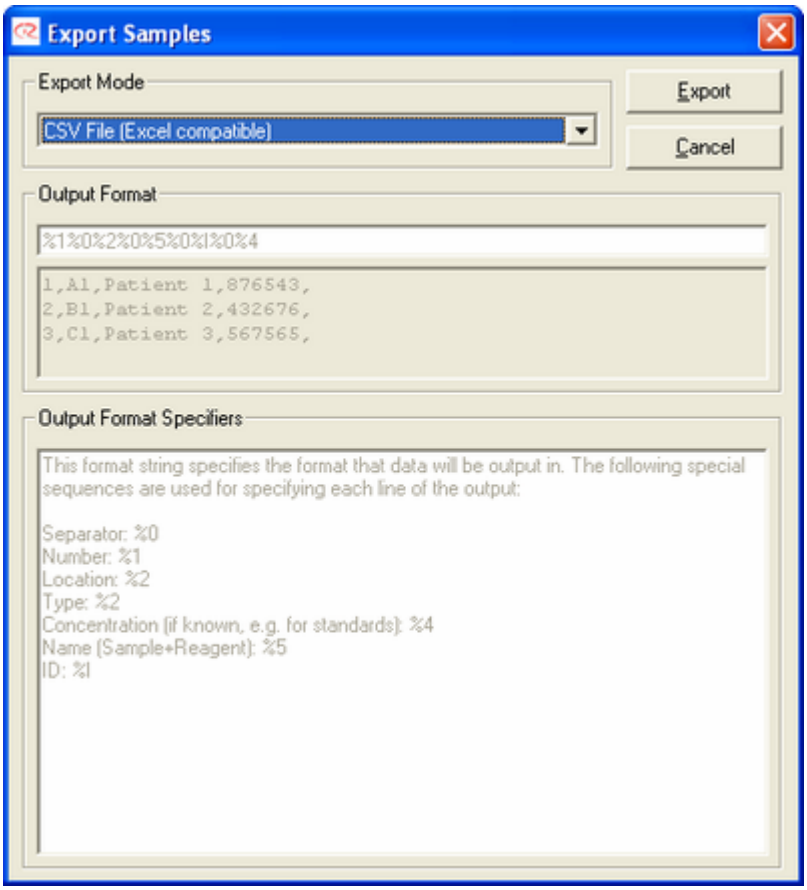
Well	Sample Name	Sample ID	Conc.
A1	Patient 1	876543	0
B1	Patient 2	432676	0
C1	Patient 3	567565	0
D1	Patient 4	896432	0
E1	Patient 5	239824	0
F1	Patient 6	123223	0

9. Please note that the importing of sample data does not automatically make samples part of a sample bank. See the section on Samples for adding wells to sample banks.

Note that any previous sample data in the sample table will be overwritten.

3.9.3 Exporting Sample Names

Any data shown in the sample data table in the right-hand sample pane of the software workspace can be exported in a variety of formats. The export feature allows users to pass sample data directly to the Corbett Rotor-Gene or other data storage systems. Select the 'Export' option in the right-hand sample pane to open the configuration window.



A variety of formats can be chosen under 'Export Mode'. Some of the export modes are configurable so that the exact content of the exported data can be specified. Other modes are 'quick' to use and have the data content pre-determined. The available formats are:

CSV File (Excel compatible) - a *.csv text file with comma separators between columns. The data is fixed and contains the following: number, location, sample name, reagent name, sample type, concentration, and target volume.

Rotorgene v4.4 - a *.sam sample file compatible with the Corbett Rotor-Gene software version 4.4 or earlier. The data is fixed and contains location, sample name and sample type.

Rotorgene v4.6 - a *.smp sample file compatible with the Corbett Rotor-Gene software version 4.6 or later. The data is fixed and contains sample name, sample type and concentration.

Custom, comma separated - a *.csv text file with comma separators between columns. The data can be user defined.

Custom, tab separated - a *.txt text file with tab separators between columns. The data can be user defined.

Export directly into Excel 97 or later - creates an .xls spreadsheet file and if Excel is installed on the PC, automatically launches Excel with the data imported. The data can be user defined.

Available data fields

The data fields available for export and their export string designators are as follows:

- Separator: %0
- Sample Number: %1
- Sample Location: %2
- Sample Type: %3
- Known Concentration: %4
- Sample + Reagent Name: %5
- Sample ID: %I (upper case i)

In the export modes where the data can be freely defined, the user must specify the data content by writing an export string. For example, to generate a *.csv file which contains the Sample Number, the Sample Location and the Sample ID, a valid format string would be:

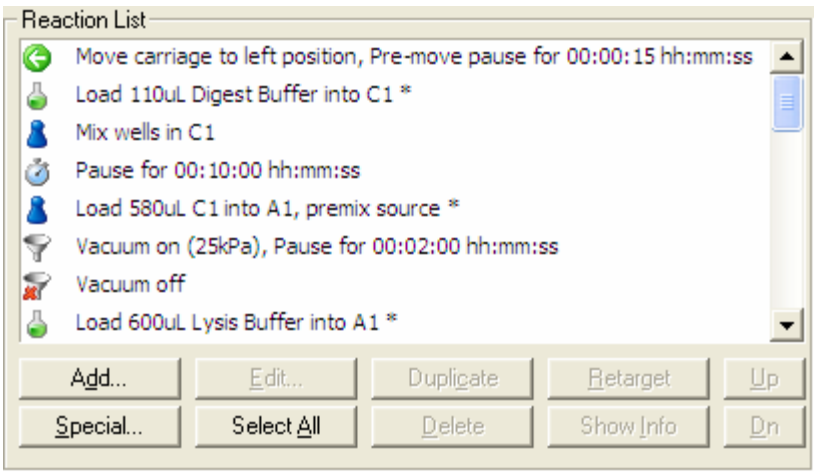
%1 %0 %2 %0 %I

The %0 between the individual data fields indicates that a separator is to be inserted. A separator will result in comma in the case of a *.csv file. In an Excel spreadsheet, a column break would be inserted.

3.10 Reactions

Setting up reactions is fundamental to the operation of the CAS-1820 X-tractor Gene. The DNA Extractor Wizard interface is where most reactions should be compiled. The compiled 'Reaction List' is a series of programmed steps defined as operations. To view these operations after completing the Extraction Wizard, select the capture plate from the software workspace with the mouse pointer, the Reaction List will be displayed in the right-hand pane. The experienced user can compile their own 'Reaction List' or alter a Wizard compiled 'Reaction List' to suit their needs however this is not recommended for the new user because it requires a complete understanding of the run file creation process and knowledge of commands and their actions.

WARNING: Under no circumstance use the DNA Extraction Wizard to open a manually compiled Reaction List or a manually modified Wizard Reaction List. The DNA Extraction Wizard will insert a set of default parameters that may interfere with the correct operation of these files.



Manual editing of the Reaction List

The Reaction List is manipulated by the buttons below the list. These are as follows:

To set up a new simple reaction click the 'Add' button. The Reaction Configuration window will appear.

Existing reactions can be altered by selecting a reaction to be changed (select the item in the reaction list) and select the 'Edit' button. The window appropriate to the reaction will be opened.

Existing reactions can be copied by selecting a reaction to be changed (select the item in the reaction list) and select the 'Copy' button. The user will be prompted to copy the reaction in place or to the end of the list. By choosing the 'in-place' option the reaction copy will appear immediately after the selected reaction. If copied to the end of the list, the reaction copy will be added to the end of the list.

Existing reactions can be deleted by selecting a reaction (select the item in the reaction list) and select the 'Delete' button. Once a reaction is deleted, the operation is irreversible. Multiple reactions can be deleted by selecting multiple items in the list. This can be done while holding down the shift key.

Reactions that involve pipetting operations are bound to a specific target plate. If the target plate is to be changed the reaction can be edited. Alternatively, the reaction can be re-targeted as follows. Select the reaction to be retargeted by selecting it in the reaction list, select the new target plate by left-clicking on it and then click on the 'Retarget' button. The selected reaction will be moved to the new plate.

The '?' button gives a short summary of each reaction type.

The order of reactions may need to be changed. Select the reaction that is to be moved up or down in the reaction list. Then select either the 'Up' or 'Dn' buttons to move the selected reaction up or down. Multiple reactions can be moved up and down by selecting multiple items in the list. This can be done while holding down the shift key.

To set up a new 'Special' reaction select the 'Special' button. The Special Reaction menu will appear.

The 'Select All' button selects all the reactions in the list.

If the total number of reactions configured on one plate exceeds the number of reaction wells a warning will be flagged. This warning must be resolved before the run can be started.

Reactions are added to the reaction plate consecutively. For example, if two reactions are set up in triplicate with a sample bank of 8 samples, the two reactions would each use 24 wells. These 24 wells would be added consecutively to the reaction plate thus using 48 wells.

3.10.1 Special

The special reaction menu lists a number of options that are not available using the standard Reaction Configuration window. These reactions involve pipetting operations (all but the first four items) which have special features that allow for minimum tip usage. However, due to minimum tip usage, in a number of cases cross-contamination may occur. Before using these 'special' reactions, ensure that the function is well understood. It is also recommended that the user perform simulations before using these reactions.

Add pause (30 sec.)

In some reaction set ups a timed pause may be required between pipetting operations. For example, some set ups may require an incubation time before more liquid is added to the plate. By selecting this option, a 30 second pause is inserted into the reaction list.

Add pause (arbitrary)

Similar to the pause option above, but the user is prompted to enter a time which can be any length between one second and one hour.

Add 'reset eject position' (works with samples only)

As the reaction list grows, wells in the reaction plate are used in a consecutive manner. This behaviour cannot be altered in the software. However, a resetting of the ejection location forces the software to start pipetting into a reaction plate from its first well again, typically column A1-H1.

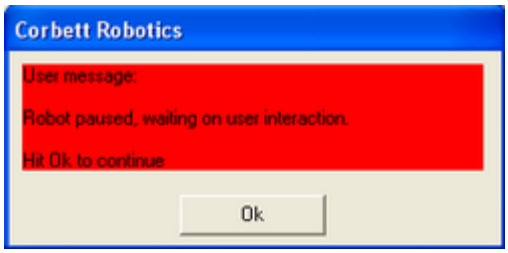
For example, if two reactions are set up in triplicate with a sample bank of 8 samples (1 column), the two reactions would each use 24 wells (3 columns). These 24 wells would be added to the reaction plate thus using 48 wells. In some instances, for example when pooling sample plates, it may be convenient to reset the eject position between reaction set ups. In the example above, the two sets of 24 wells would normally be pipetted consecutively. If a 'reset eject position' was added between

the two reactions in the reaction list, the robot would initially pipette the first set of 24 wells, reset back to well A1, and then pipette the second 24 wells. The result is that the two sets of 24 wells would be pooled on top of one another.

Add 'pause until confirmation'

Similar to the timed pauses above, adding this pause causes the robot to wait. However, these pauses do not have a time limit, these pauses wait for the user to perform a task and then click 'OK' to continue. When setting up this type of pause, the user is prompted to enter a message which is part of the message box that appears during the run.

For example, if a 'pause until confirmation' pause is added, the user is prompted to enter a message. If the user enters 'Robot paused, waiting for user to spin reaction plate', then during the run the following message box will appear.

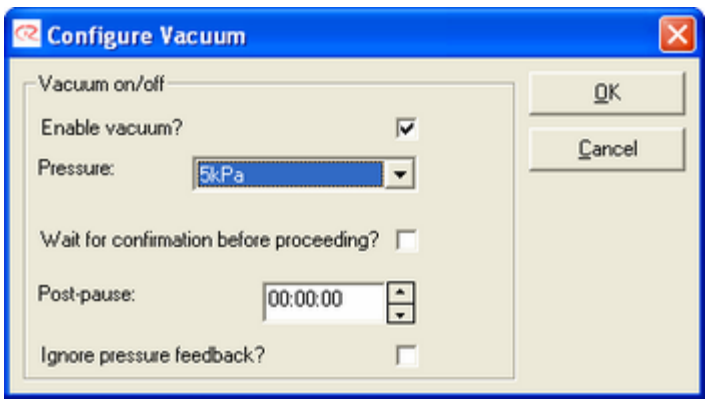


Add move carriage

This function allows the transfer carriage to be moved from the capture position to the elution position and visa versa. Once chosen, the user is prompted for the direction of the move: 'Right' moves the carriage to the elution position from the capture position and 'Left' returns the carriage to the capture position.

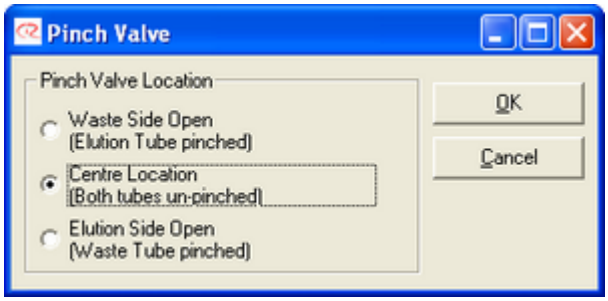
Add Vacuum On & Add Vacuum Off

These functions control the vacuum pump. The parameters for the vacuum pump are entered through the pop-up window. The available options include turning the vacuum on or off. When the vacuum is set to on, the vacuum pressure can then be selected. The user can also set the 'Wait for confirmation before proceeding' option to maintain the vacuum indefinitely. Select the 'Post pause' option to set the length of pause after the vacuum turns off before the next function occurs. The 'Ignore pressure feedback?' option allows the next operation to occur without the vacuum reaching the target vacuum pressure. Note: Only an experienced user should use the latter two functions.



Add Move Pinch Valve

This option allows the user to control the position of the pinch valve in conjunction with the vacuum. Choose 'Pinch Valve Location' and click 'OK'.



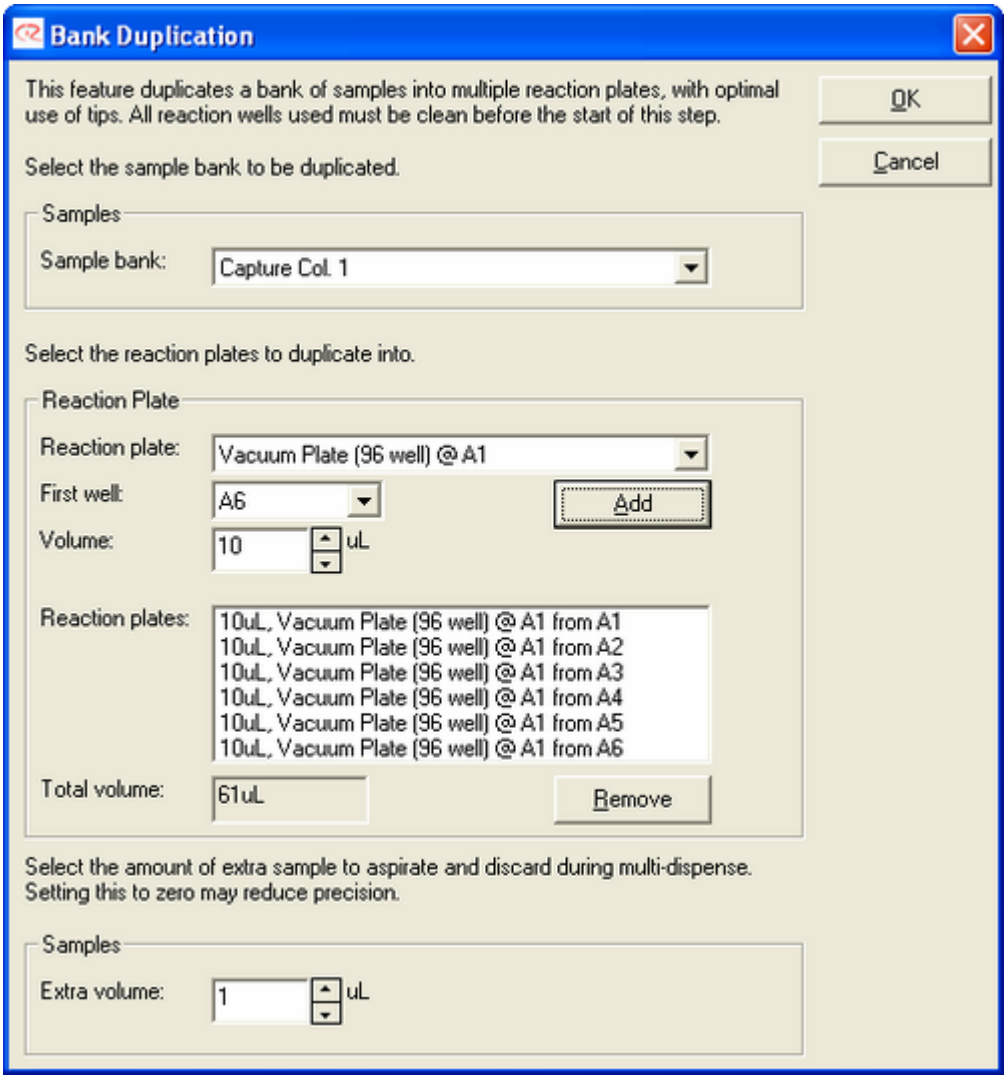
Add sample bank duplication step

This reaction step is used to duplicate sample banks, for example for the purposes of plate copying. Although it is possible to copy sample banks by adding standard reaction steps, this special step conserves tips. If a sample bank of eight samples was duplicated 5 times using the normal technique, 40 tips would be required. Using this method, only eight tips will be used. This method makes use of the multi-eject functionality (see tip re-use options). However, multi-eject does not need to be set up, the bank duplication step uses this feature automatically.

To set up the above example of duplicating one sample bank of eight samples five times, follow these steps:

1. Open the Bank Duplication Window
2. Select the sample bank to be duplicated in the drop-down menu
3. Set the first well to A1 (column A1-H1)
4. Select the volume of the aliquot
5. Click on 'Add'
6. Select the sample bank to be duplicated in the drop-down menu
7. Set the first well to A2 (column A2-H2)
8. Select the volume of the aliquot
9. Click on 'Add'
10. Repeat steps 2 to 5 with first wells of A3, A4, A5, and A6
11. Set the extra volume to the desired amount. The extra volume is the volume that is aspirated over and above the volume required for the duplication. As discussed, an additional extra volume is required to make the multi-eject more accurate. This should be in the range of 1 to 5 µL.
12. Click on 'OK'.

The Bank Duplication Window should look as follows.



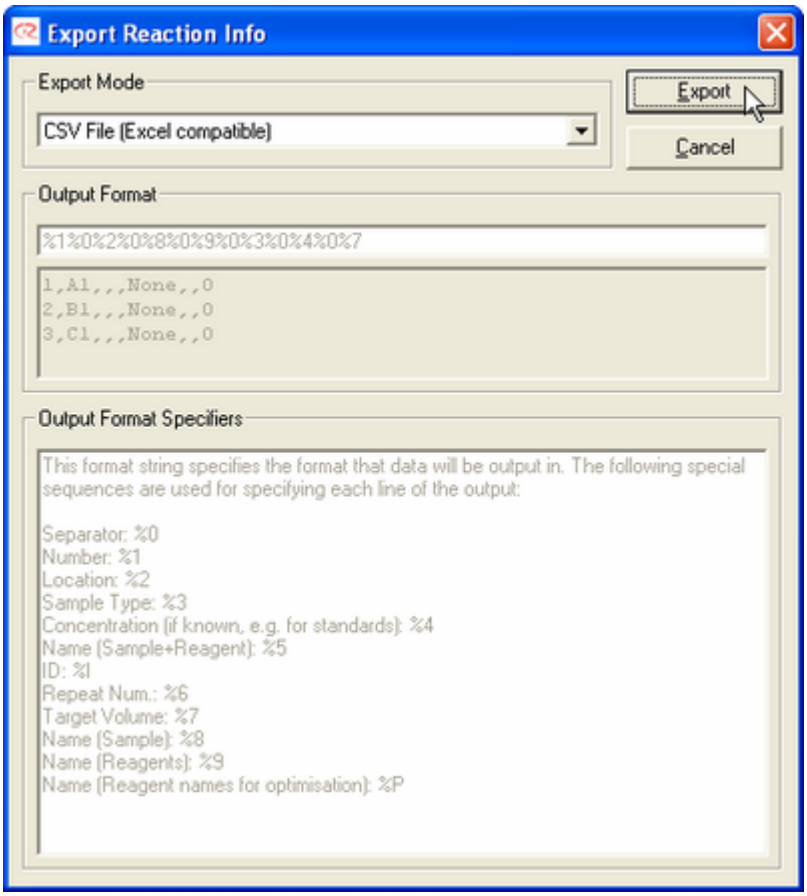
The robot will take 8 tips, aspirate 61 µL/Tip from the first column in the sample bank, and eject 10 µL into wells A1-H1, A2-H2, A3-H3, A4-H4, A5-H5, and A6-H6. The tips will then be discarded. The total tip usage will be 8 tips.

Add Discard Tips

At the completion of a vacuum extraction protocol there may be used tips located in racks that need to be removed from the rack and moved to the ejector chute for disposal. This function controls this action in combination with the software's tip tracking function. Turned on, all used tips will be disposed of automatically.

3.10.2 Exporting Reaction Lists

Any data shown in the reaction list can be exported in a variety of formats. The export feature allows users to pass sample data directly to the Corbett Rotor-Gene or other data storage systems. Upon selecting the 'Export Reaction List' option from the Wizards Menu or the reaction right-hand pane, the Export Reaction Info window is shown.



A variety of formats can be chosen under 'Export Mode'. Some of the export modes are configurable so that the exact content of the exported data can be specified. Other modes are 'quick' to use and have the data content pre-determined. The available formats are:

CSV File (Excel compatible) - a *.csv text file with comma separators between columns. The data is fixed and contains the following: number, location, sample name, reagent name, sample type, concentration, and target volume.

Rotorgene v4.4 - a *.sam sample file compatible with the Corbett Rotor-Gene software version 4.4 or earlier. The data is fixed and contains location, sample name and sample type.

Rotorgene v4.6 - a *.smp sample file compatible with the Corbett Rotor-Gene software version 4.6 or later. The data is fixed and contains sample name, sample type and concentration.

Custom, comma separated - a *.csv text file with comma separators between columns. The data can be user defined.

Custom, tab separated - a *.txt text file with tab separators between columns. The data can be user defined.

Export directly into Excel 97 or later - creates an .xls spreadsheet file and if Excel is installed on the PC, automatically launches Excel with the data imported. The data can be user defined.

Available data fields

The data fields available for export and their export string designators are as follows:

- Separator: %0
- Sample Number: %1
- Sample Location: %2
- Sample Type: %3
- Known Concentration: %4
- Sample + Reagent Name: %5
- Sample ID: %I (upper case i)
- Repeat Number: %6
- Target Volume: %7
- Sample Name: %8
- Reagent Name: %9
- Reagent Names when used in Optimisation Wizard: %P

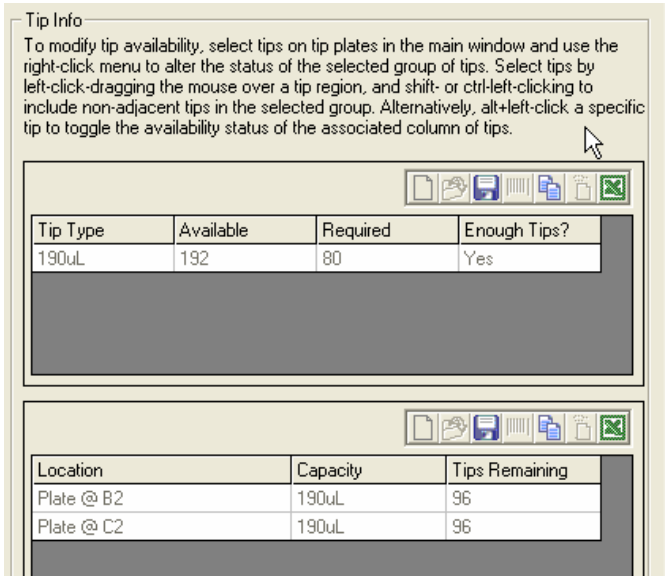
In the export modes where the data can be freely defined, the user must specify the data content by writing an export string. For example, to generate a *.csv file which contains the sample location, the sample name and the expected total volume, a valid format string would be:

%2 %0 %8 %0 %7

The %0 between the individual data fields indicates that a separator is to be inserted. A separator will result in comma in the case of a *.csv file. In an Excel spreadsheet, a column break would be inserted.

3.11 Pipette Tips

The right-hand pane showing tip information appears after selecting any tip plate on the software workspace. In the case of tips, the right-hand pane provides information only. The overall tip status as well as the status of each tip plate is shown. Information on how many tips are available, how many tips are required for the current run and whether or not enough tips are available is presented.



As can be seen in the information presented in the right-hand pane, the software maintains a record of which tips are available. The tip availability is recorded at regular intervals while the software is running and at software shutdown. When re-starting the software, the tip availability should be correct unless tips were physically removed from the robot. Correct tip availability should always be verified before a run is started.

If tip supply is exhausted during a run, please see the section on Exception Handling for further details.

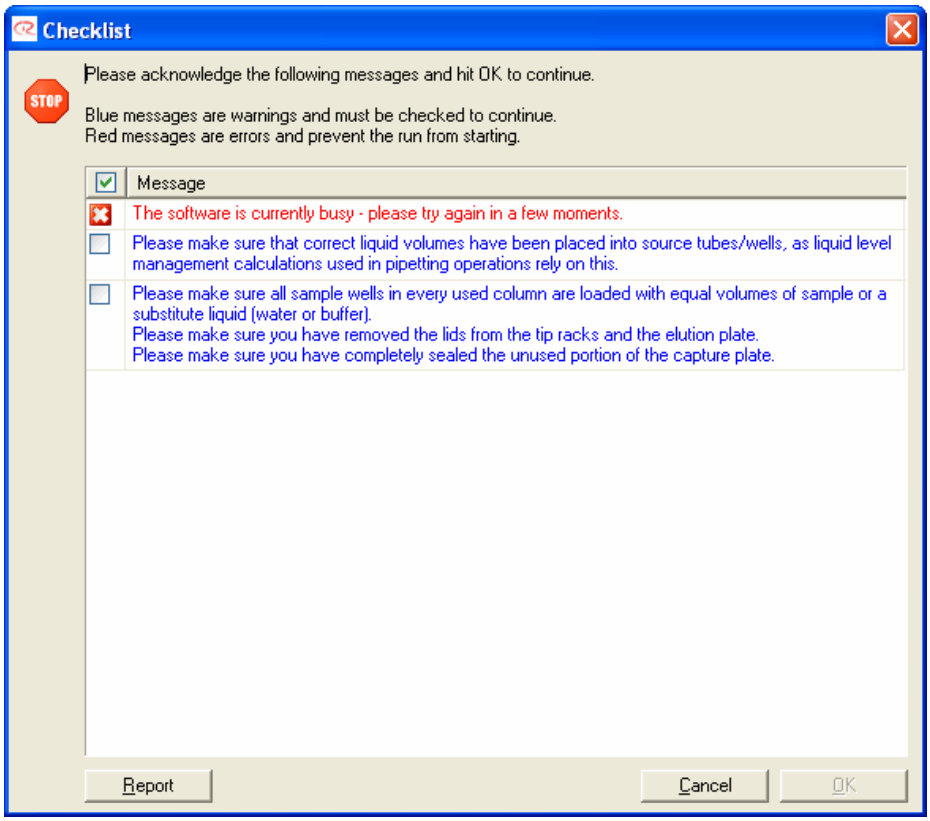
3.12 Starting a Run

Before starting a run, please ensure the following:

1. All tubs and plates are in their correct places and caps have been removed,
2. The correct tips are set as available,
3. Sufficient volumes have been added to reagent and sample wells (all required volumes can be checked by hovering over the wells),
4. Correct plate types have been chosen,
5. All plates are correctly calibrated (position and height).

To start a run, press the  button located on the toolbar.

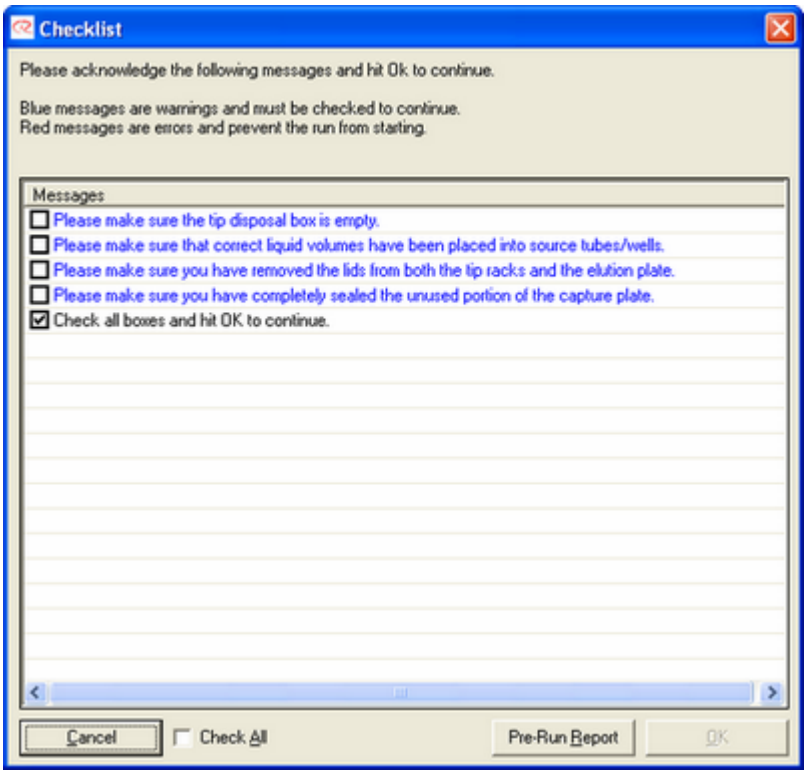
Shortly after clicking the run button, a Checklist Window will appear. The checklist looks similar to this.



The start checklist shows warnings and errors that may exist with the run. Error messages are shown in red and a run cannot start until the errors are resolved. Warning messages are shown in blue and simply require an acknowledgment before the run can start. However, the warnings are presented for a reason and in most cases it is advisable that all warnings be resolved before starting the run.

If the visible warning messages or errors are not enough to determine what the problem may be, more detailed descriptions can be displayed by double-clicking on the messages.

If no warnings or errors exist then the checklist will look like this.



Check to see that the tip disposal box is empty and then click 'Check All'.

If the user wishes to see a pre-run report, click on the 'Pre-Run Report' button.

After selecting 'OK', the run will commence.

Progress of the run will be shown in the right-hand pane.

3.13 Warnings

Warnings and errors are indicated by the  symbol in the toolbar or a red or blue message in the pre-start checklist.

The source of the error or warning can be identified by clicking on the toolbar symbol. Warnings are non-critical and the run can commence with active warning messages. Please note, warning messages and errors are logged in the post-run report and in any support packages.

The following are warnings and do not need to be resolved to start a run but can lead to erroneous results.

1. Insufficient tips - Not Enough Tips available for the run
2. Well Too Full - too much liquid will be ejected into a well
3. Well Too Small - a well cannot contain sufficient liquid to supply the run
4. Eject Volume Too Low - a volume less than 5 µL is ejected into a dry well
5. Will Contaminate - the run contains reaction elements that will contaminate sample wells
6. Volume Too Small - the run requires a volume to be pipetted that is too small

7. Multi Eject - the run is set up with multi-eject functionality which can lead to reduced precision
8. Errors are listed below and must be resolved before the run can start.
9. Not Enough Output Wells - there are not enough output wells in the reaction plate
10. Too Many Standards - the software can accommodate a limited number of dilution series
11. Invalid Sample Config - sample banks are set up incorrectly
12. Operation Impossible - an item in the reaction list or combination thereof cannot be performed
13. Dilution Out Of Range - the sample and target concentration are set to values that are not achievable
14. Bad Plate Config - a plate configuration is not usable
15. Reagent Name - problems with reagent naming in the optimisation wizard have occurred.

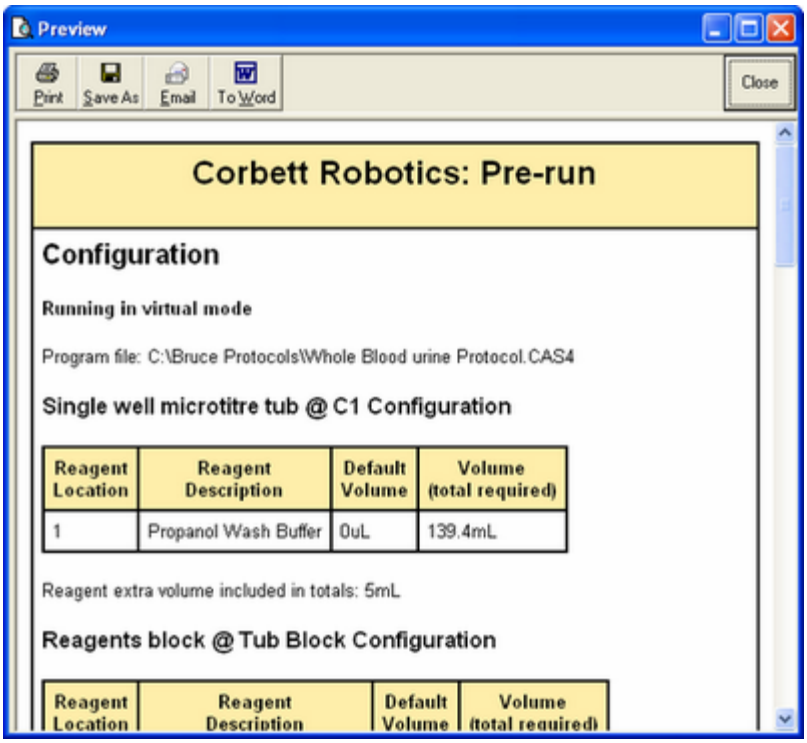
For system error messages and codes see the Appendix.

3.14 Pre-run Report

When selecting the Start button to commence a run the user is presented with the Pre-run Checklist. This checklist allows the user to display the Pre-run Report. This report summarises the run set up in a text-based format. The file format of the report is html and can thus be opened on any computer with a type of Internet Explorer. The Pre-run Report contains information on:

- Whether the run is running in Virtual-Mode or not
- The configuration of reagents, their location and the amount of reagent required during the run
- All program settings including air volumes, pipetting speeds, run settings and other options, and
- The current time and date.

The pre-run report offers options to print the report, save the report (in html format), e-mail the report (only available if the PC has e-mail software set up) and an option to export the report into Microsoft Word. These options are available via the toolbar at the top of the report. The export into Word allows the report to be edited if necessary. Additional information could also be added to the report.



The report can be closed by clicking on the 'X' or the 'Close' button.

The elements of the Pre-run Report are described below.

3.14.1.1 Reagents

The first section of the report details reagents: Plate Location, Reagent Description, Default Volume, and Volume (total required). The 'Default Volume' has an indicated volume of 0 µL because the actual volume of reagent dispensed is controlled by the Extraction Wizard. If a dispensed reagent volume is assigned as part of a manually constructed run file, that volume would be displayed. The Volume required is the total volume of a particular reagent required for the extraction process and is the same as the volume indicated in the pop-up window when hovering over the reagent tub depicted on the software workspace.

Please note that a reagent is only annotated as not in use if it is not used in a run, regardless of whether that reagent's name is blank or its volume set to 0 µL. If reagents are set up in separate blocks (or block segments), two or more tables showing the reagent configuration may be displayed in the report.

Configuration

All configuration settings entered into the vacuum extraction wizard are summarised in this section. At the base of the report the date, time and software version number are recorded. Please see the following example.

Processing

Sample Introduction

Your samples are preloaded into an empty lysis block.

Columns in Use

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12.

Table Setup

Capture Plate - Reaction 800uL flat tube, Whatman.
Incubation Plate - Reaction 2000uL deep well.

Sample Setup

Sample Volume 200uL.
Reagent 1, 100uL of 3X GuHcl Digest Buffer with Proteinase K, Incubate for 00:20:00 hh:mm:ss and mix 10 times for 1 iteration.
Reagent 2, 600uL of 5.25M GuSCN Lysis Buffer, Incubate for 00:10:00 hh:mm:ss and mix 10 times.

Load and Capture Sample

Do not Pre Wet Capture plate.
Load 600uL in 1 vacuum step(s), each for 00:03:00 hh:mm:ss at 25kPa.

Wash Steps

Wash 1, 1 wash using 600uL of 5.25M GuSCN Lysis Buffer.
Wash 2, 2 washes, each using 700uL of Propanol Wash Buffer.
Wash 3, 1 wash using 750uL of Ethanol.

Sample Drying

Dry Sample for 00:05:00 hh:mm:ss at 75kPa.

Product Elution

1 elution using 150uL of Elution Buffer.
Incubate for 00:02:00 hh:mm:ss before eluting for 00:01:00 hh:mm:ss at 31kPa.

This report generated by Corbett Robotics v4.7.96.31 at 21/02/2005 1:06:26 PM

3.15 Aborting a Run

The run can be paused and aborted at any time. To pause a run, select the  button in the toolbar. The robot will finish its current operation (it will never pause with the pipetting head down) and then pause. The user will be presented with an option to select 'OK' to continue or Cancel to abort. Please note that the assignment of the buttons is different to a number of conventions. This is deliberately the case. A run can also be paused by lifting the robot lid. Pausing the run in either of these ways is logged in the post-run report.

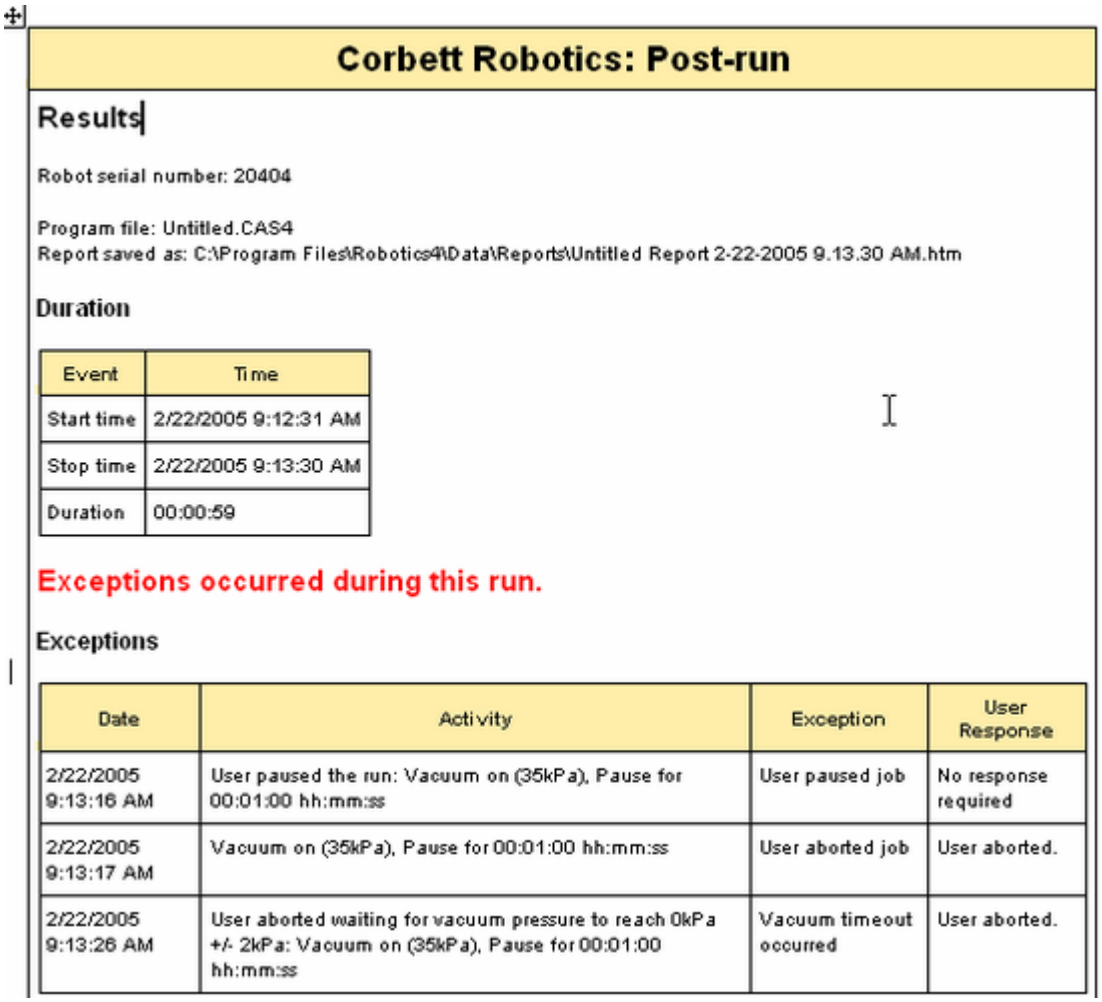
If the user chooses to abort the run, the robot will discard the tip and return to its resting position at the rear right, the lid must be closed for the run to completely abort.

Please note, once a run is aborted, it cannot be restarted at the point where it was aborted.

3.16 Post-run Report

At the completion of a run a Post-Run Report is generated by the software. The report is very similar to the Pre-run Report preserving the same information. However, the report also includes information on any errors or warnings (termed exceptions) that may have occurred during the run. For example, the Post-run report records events such as the opening of the lid during a run, level sensing errors, machine errors and similar such events.

The occurrence of an exception is highlighted in the report by a larger red heading as shown below.



Corbett Robotics: Post-run

Results

Robot serial number: 20404

Program file: Untitled.CAS4
Report saved as: C:\Program Files\Robotics4\Data\Reports\Untitled Report 2-22-2005 9.13.30 AM.htm

Duration

Event	Time
Start time	2/22/2005 9:12:31 AM
Stop time	2/22/2005 9:13:30 AM
Duration	00:00:59

Exceptions occurred during this run.

Exceptions

Date	Activity	Exception	User Response
2/22/2005 9:13:16 AM	User paused the run: Vacuum on (35kPa), Pause for 00:01:00 hh:mm:ss	User paused job	No response required
2/22/2005 9:13:17 AM	Vacuum on (35kPa), Pause for 00:01:00 hh:mm:ss	User aborted job	User aborted.
2/22/2005 9:13:26 AM	User aborted waiting for vacuum pressure to reach 0kPa +/- 2kPa: Vacuum on (35kPa), Pause for 00:01:00 hh:mm:ss	Vacuum timeout occurred	User aborted.

Similar to the Pre-Run report, the Post-run Report may be printed, saved (in html format), e-mailed or exported to Microsoft Word. Please not the Post-run Report is automatically saved in a sub directory called 'Reports' in the current run file save directory (the default directory is c:\Program Files\Robotics4\Data).

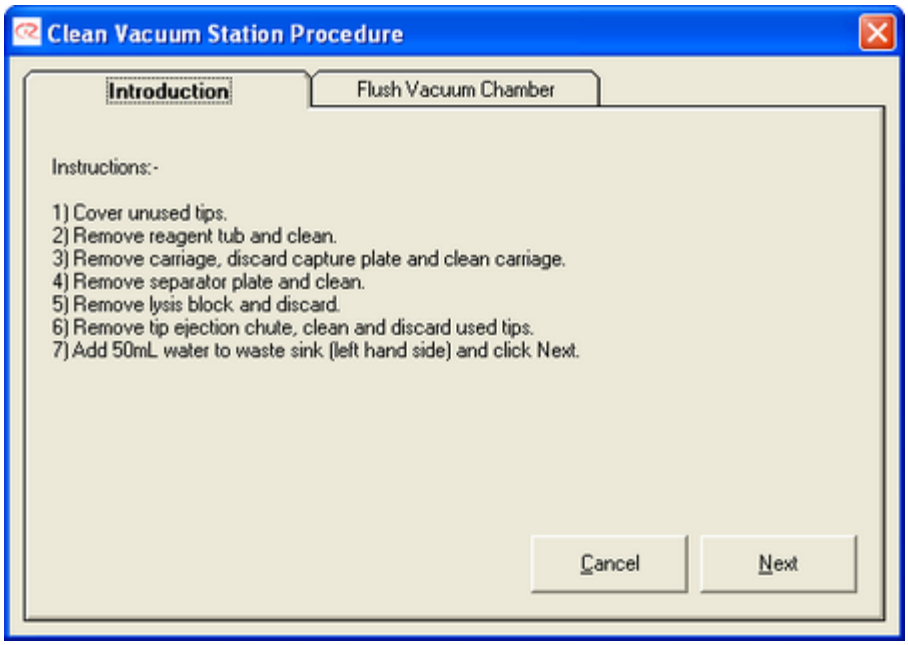
3.17 Exception Handling

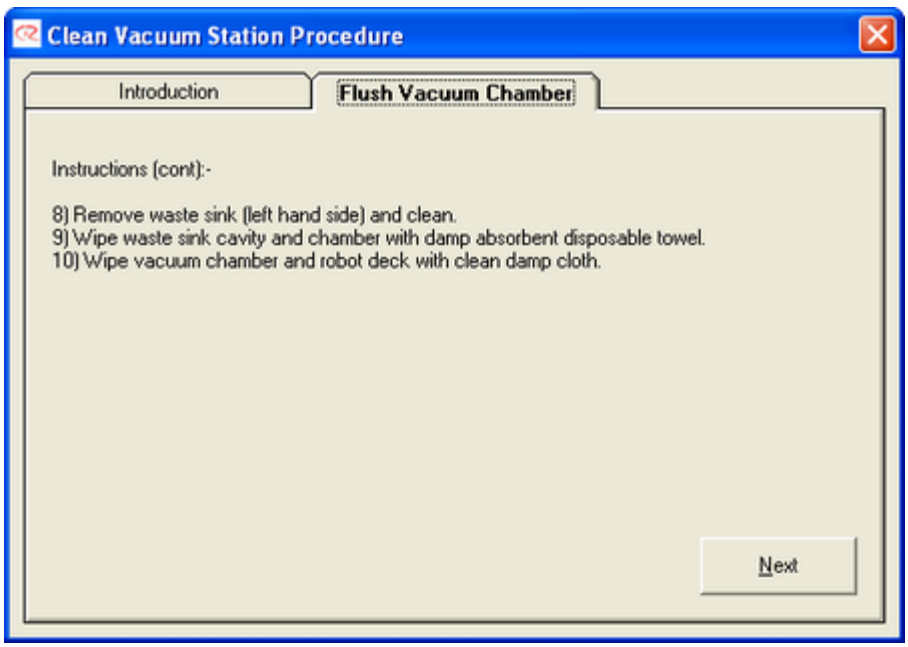
During a run, if an exception (an error or a warning) is encountered, the user will be prompted with a window. As well as the warning windows, an audible warning will sound for the first 30 seconds, then in 30 second intervals. The window contains information on the error that occurred. Selecting 'Abort' will cancel the run. Selecting 'Retry Operation' will cause the robot to dispose of the current tip and move the pipetting head to the rear right of the robot. This gives access to the entire workspace where the error may be resolved. A message box appears prompting the user to click 'OK' to continue the run. If 'Ignore' is selected, the robot will continue its operation as if nothing had happened. Other error messages are covered in the Appendix.

3.18 Shutting Down

The Robotics Software can be shut down by clicking the  in the top right hand corner or by selecting 'Exit' in the File Menu.

When shutting down the Robotics Software you will be prompted to clean the machine. This operation is very straight forward and should always be completed to ensure the CAS-1820 X-tractor Gene is kept in proper working condition. The options are self explanatory within each of two windows.

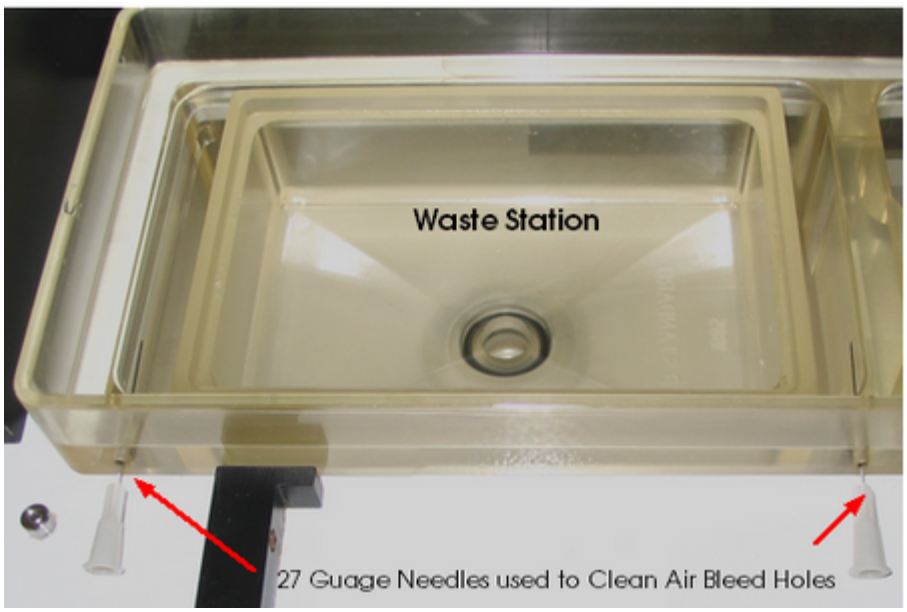




Cleaning the Air Bleed Holes

The CAS-1820 has three Air Bleed Holes position at strategic points on the Vacuum Chamber. These holes serve two distinct purposes: control air flow direction and the vacuum pressure. Each of these holes has a small brass aperture inserted in the hole to specifically control the rate of airflow. All three air bleed holes can be cleaned by passing a 27 or 30 gauge needle through the hole.

The holes in the lysis/waste station side of the vacuum chamber maintain a positive pressure above the separator plate and provide bypass airflow to prevent reagents and sample aerosols from cross-contaminating the samples.



The third Air Bleed Hole is located in the elbow screwed into the base of the elution chamber. This hole provides airflow to stop cross-contamination from the waste chamber to the elution chamber via the adjoining silicon hose. Airflow through this bleed hole maintains a positive flow of air from the elution chamber to the waste bottle.

Moving to a Safety Position

The robot will go through its homing routine and return the pipetting head to a known safe location. The user is prompted when it is safe to shut the robot down. All the robot axes are disengaged and can be moved freely.

4 Vacuum Extraction Protocol Wizard

Most nucleic acid extraction protocols will be constructed using the 'Vacuum Extraction Protocol' Wizard. With experience, the user can further edit these protocols using the edit functions of the right-hand pane exposed by selecting the capture reaction plate.

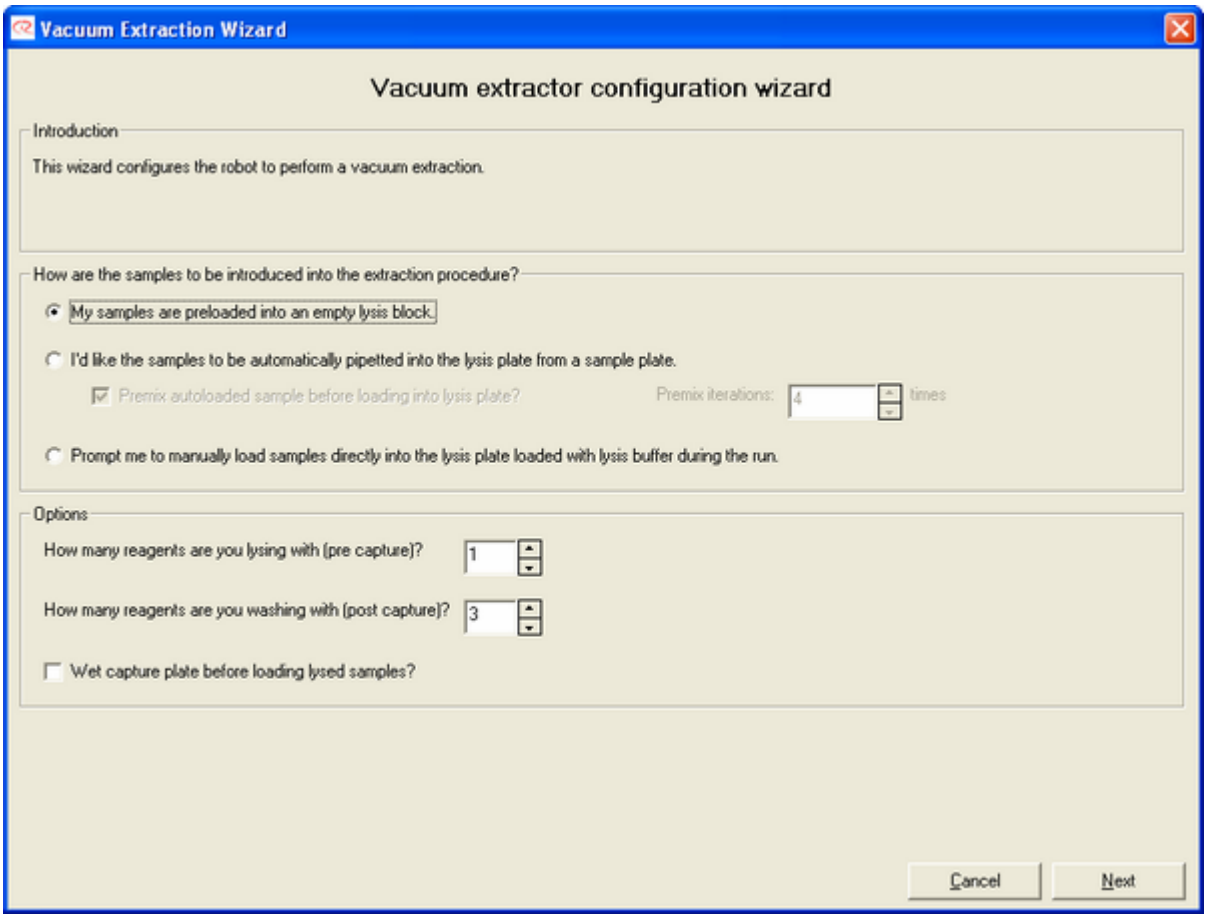
Constructing a Vacuum Extraction Run File (name.cas4) involves making choices via a Wizard. These steps are as follows:

Vacuum Extraction Wizard Configuration

1. Table Setup
2. Configuration [1]
3. Configuration [2]
4. Load Pre-capture Reagent 1
5. Load Pre-capture Reagent 2
6. Wash Steps 1...n
7. Dry Sample
8. Wizard Template Report
9. Wizard Complete Message

4.1 Vacuum Extraction Wizard Configuration

The key elements of this screen are 'How are the samples to be introduced into the extracting procedure?' and 'Options'.



How are the samples to be introduced into the extracting procedure?

Samples may be presented for extraction in three forms:

1. Samples preloaded into a lysis block to which lysis buffer will be added - 'My samples are preloaded into an empty lysis block'.
2. Samples located in a sample block requiring the samples to be transferred to the lysis block - 'I'd like the samples to be automatically pipetted into the lysis plate from a sample plate'. If this option is chosen, the user can also have the samples premixed a selected number of times before the samples are transferred to the lysis plate.
3. The lysis block is first loaded with lysis buffer and then the operation is paused while the user manually loads samples into the lysis block - 'Prompt me to manually load samples directly into the lysis plate loaded with lysis buffer during the run'.

Options

Three further aspects of the extraction protocol involve the use of additional reagents 'How many reagents are you lysing with (pre-capture)', 'How many reagents are you washing with (post capture)', and 'Wet capture plate before loading lysed samples'.

How many reagents are you lysing with (pre-capture)?

- This option provides an opportunity for the user to add additional lysis reagents that may be required for particular sample types. The number of steps chosen here will determine the number of 'Additional Lysis Steps' to be configured during the wizard. For example, if one additional lysis reagent is chosen, the user will be required to configure one additional lysis step within the wizard. It's important to note that these reagents are added to the sample before the lysis buffer. Strong reagents may inhibit enzyme activity if enzymes are added to lysis buffers such as guanidine thiocyanate.

How many reagents are you washing with (post capture)?

- This option allows the user to define the number of reagents to be used during extraction to wash the samples. The number of steps chosen here will determine the number of 'Wash Steps' to be configured during the wizard. For example, if three wash steps are chosen, the user will be required to configure three wash steps within the wizard.

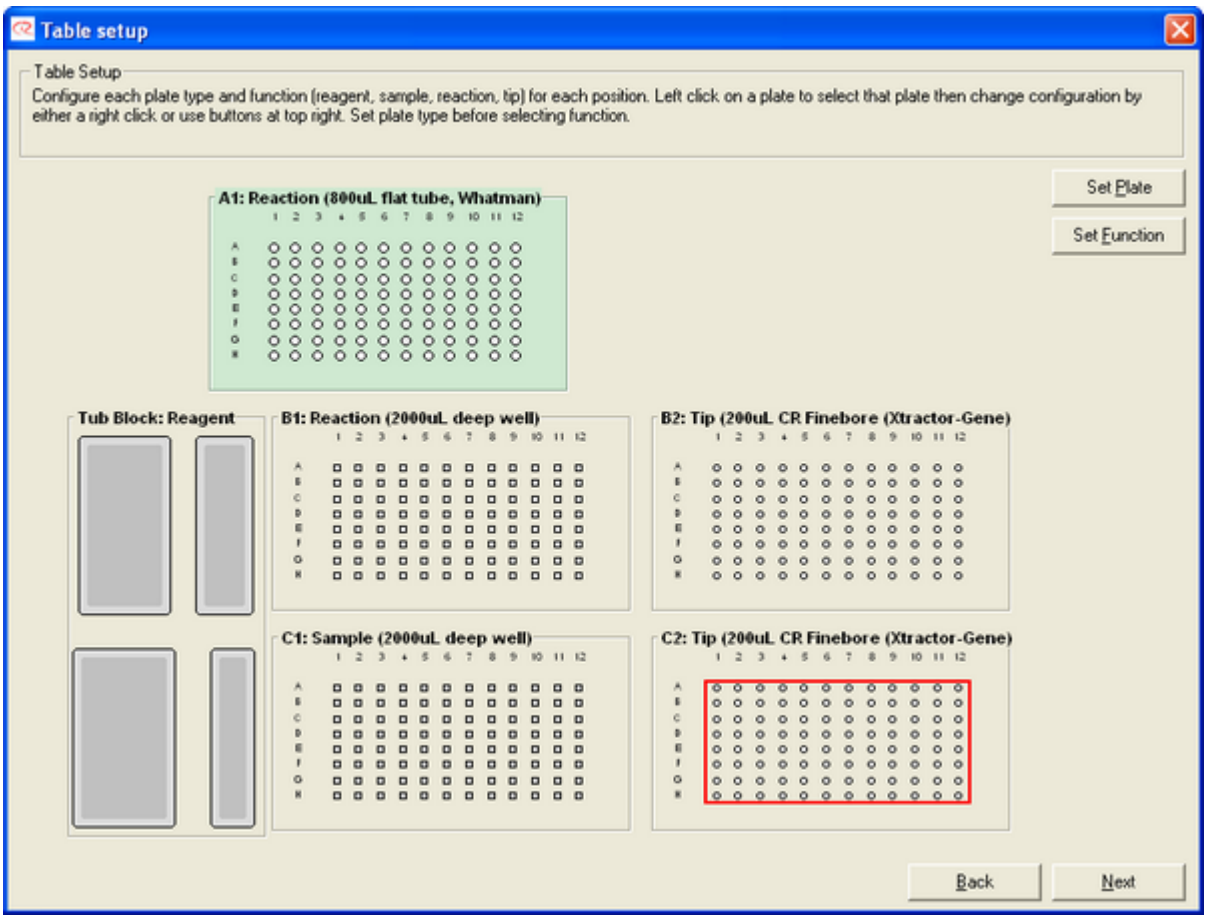
Wet capture plate before loading sample?

- This option allows the user to wet the capture plate before the lysed samples are loaded. This step is not usually required for standard extraction of DNA or RNA.

4.2 Table Setup

Table setup on the CAS-1820 X-tractor Gene means defining in the software what consumables are to be used and where they reside on the workspace. The options screen presented in the vacuum extraction wizard is the equivalent to the right-hand pane seen in the normal software mode. To configure each plate, select the plate with the mouse and select 'Set Plate'. Alternatively, right click on the plate and select 'Change Plate Type' from the drop-down menu. Similarly, set function by using the mouse and left click on the plate and choose 'Set Function' or mouse right click on the plate and select 'Change Plate Function' from the drop-down window.

For a more detailed description of changing plate types and functions see ‘Choosing the Right Plate’.



4.3 Configuration [1]

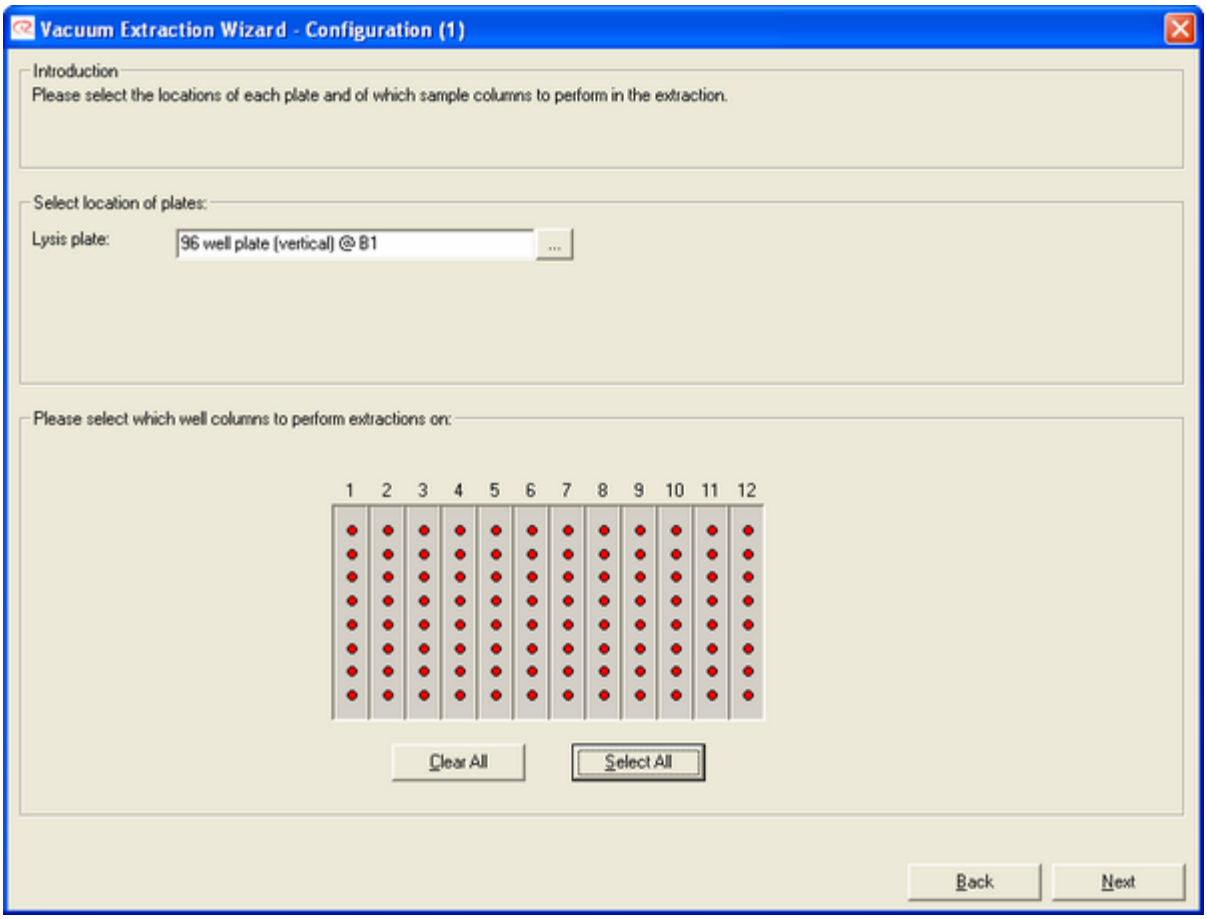
The key elements of this screen are setting the location of the 'Capture Plate' and the 'Lysis Plate' and the number of sample columns to be extracted.

Select location plates:

- The default location of the capture should be left at Vacuum Plate @ A1. The default location (B1) of the lysis plate is the one that is most routinely used. To change the location of the lysis plate, the 'Plate Type' would have been defined under 'Table Setup' with the plate type defined as 'Reaction Plate'.

Please select which columns to perform extractions on:

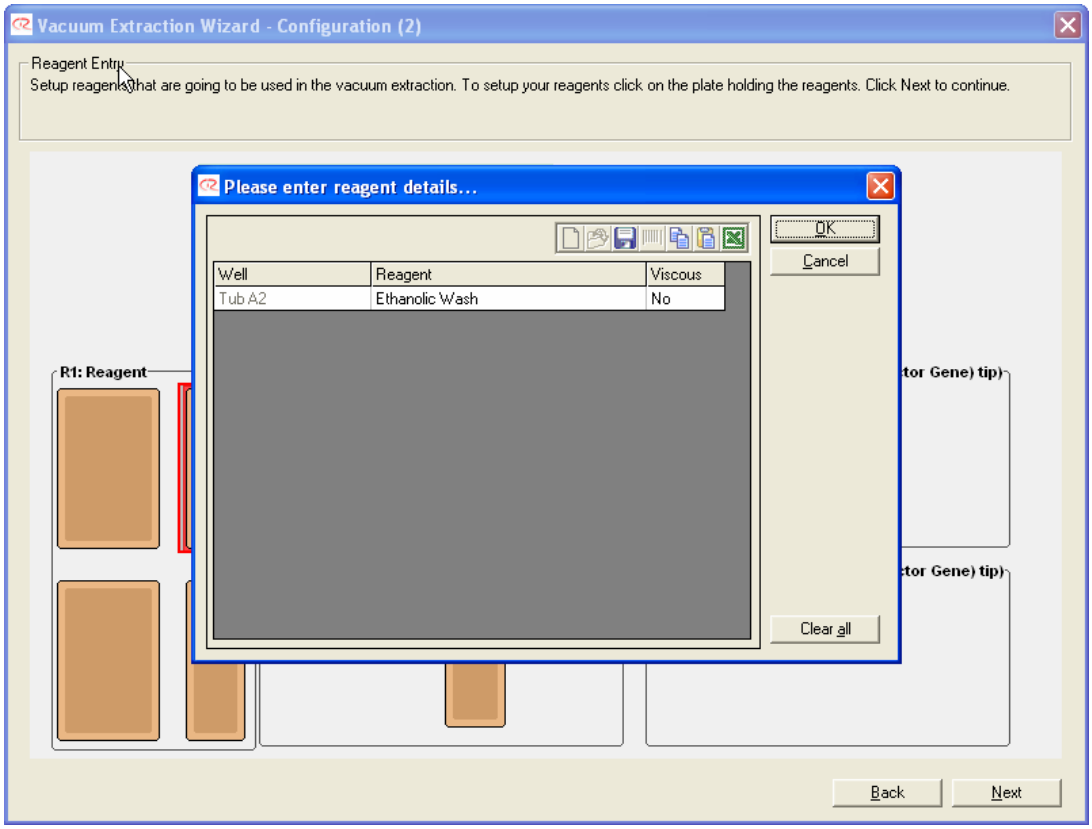
- To select individual columns use the mouse and left click on each of the desired column.
- To select all columns choose 'Select All'. To clear all columns choose 'Clear All'.



4.4 Configuration [2]

To add reagent descriptions, select each reagent tub required (background screen below) and a pop-up window will appear (foreground screen below). Enter a reagent description such as 'Ethanolic Wash' under the 'Reagent' column. If the reagent is viscous, check the box in the column labelled as 'Viscous' in the pop-up window. If you would like the pipette tips to pause briefly over the reagent tub after aspirating reagent, select 'Anti-drip Pause (sec)' and enter the required time to pause. This feature is provided to allow the pipette tips to drain of reagent clinging on the external surface of the pipette tips. This is a very useful feature for viscous liquids.

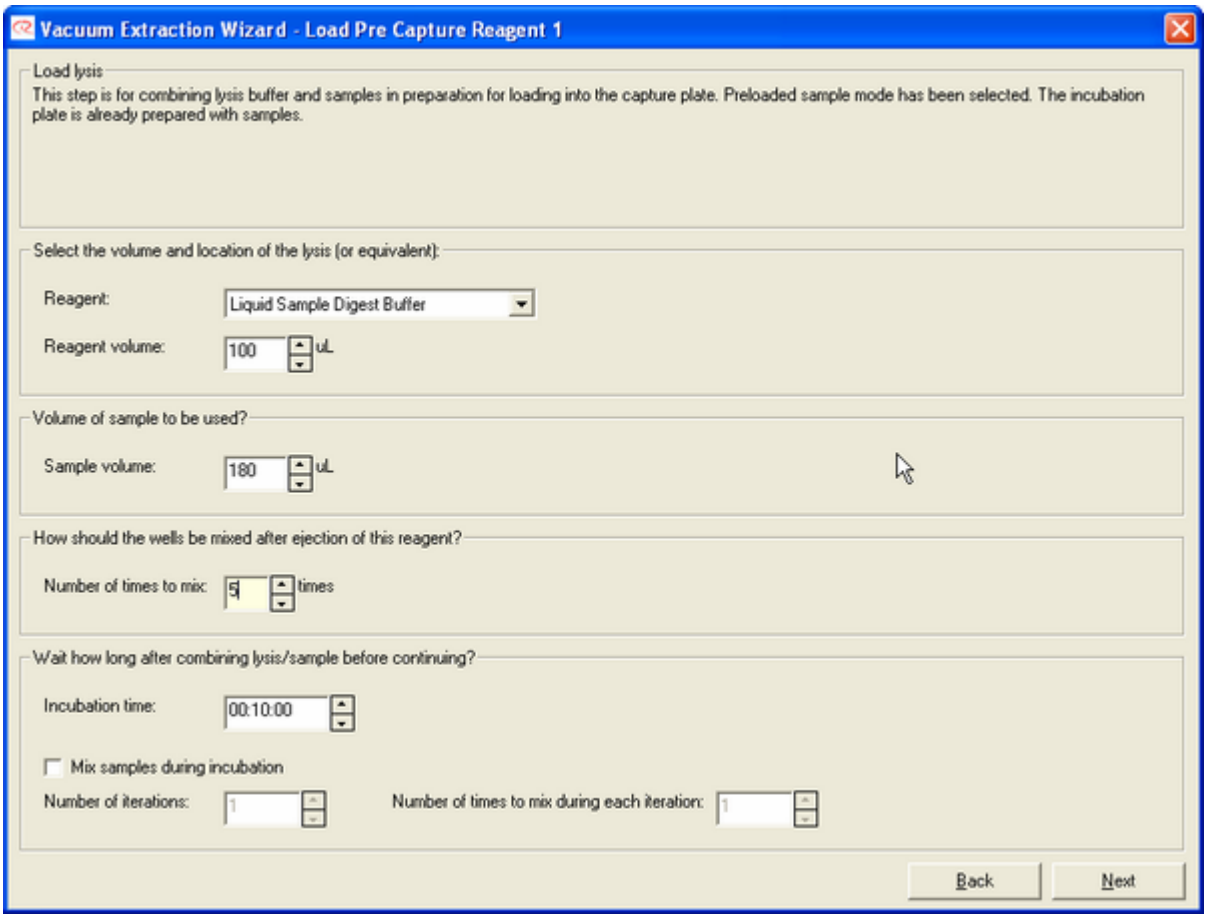
Reagent pop-up window



4.5 Load Pre-capture Reagent 1

This step is for combining lysis buffer and samples in preparation for loading into the capture plate. Depending on which option was selected regarding sample introduction into the extraction procedure, the user will be reminded of this choice in the Load Lysis sub-window of this screen of the wizard. The choice in the 'Vacuum Extraction Wizard Configuration' of 'My samples are preloaded into an empty lysis block' results in the message 'Preloaded samples has been selected. The incubation plate is already prepared with samples' being displayed.

To configure the 'Pre-capture Reagent' window, move through each section, and choose the relevant options.



Select the volume and location of the lysis [or equivalent]

- Choose the appropriate reagent from the drop down window labelled 'Reagent'
- Enter your 'Reagent Volume' required for each sample/well
- Volume of sample to be used?

When considering your sample volume, remember that in addition to the sample, you will need to add two to three volumes of lysis buffer and possibility other lysis reagents. The total volume of sample and lysis buffer must not exceed the working volume for each well of the lysis plate and the capability of the capture plate to process the lysed sample material. For example, using a Whatman 800 µL GF/B filter plate you would use 150 µL of sample and a maximum of 450 µL of lysis buffer.

How should the wells be mixed after ejection of this reagent?

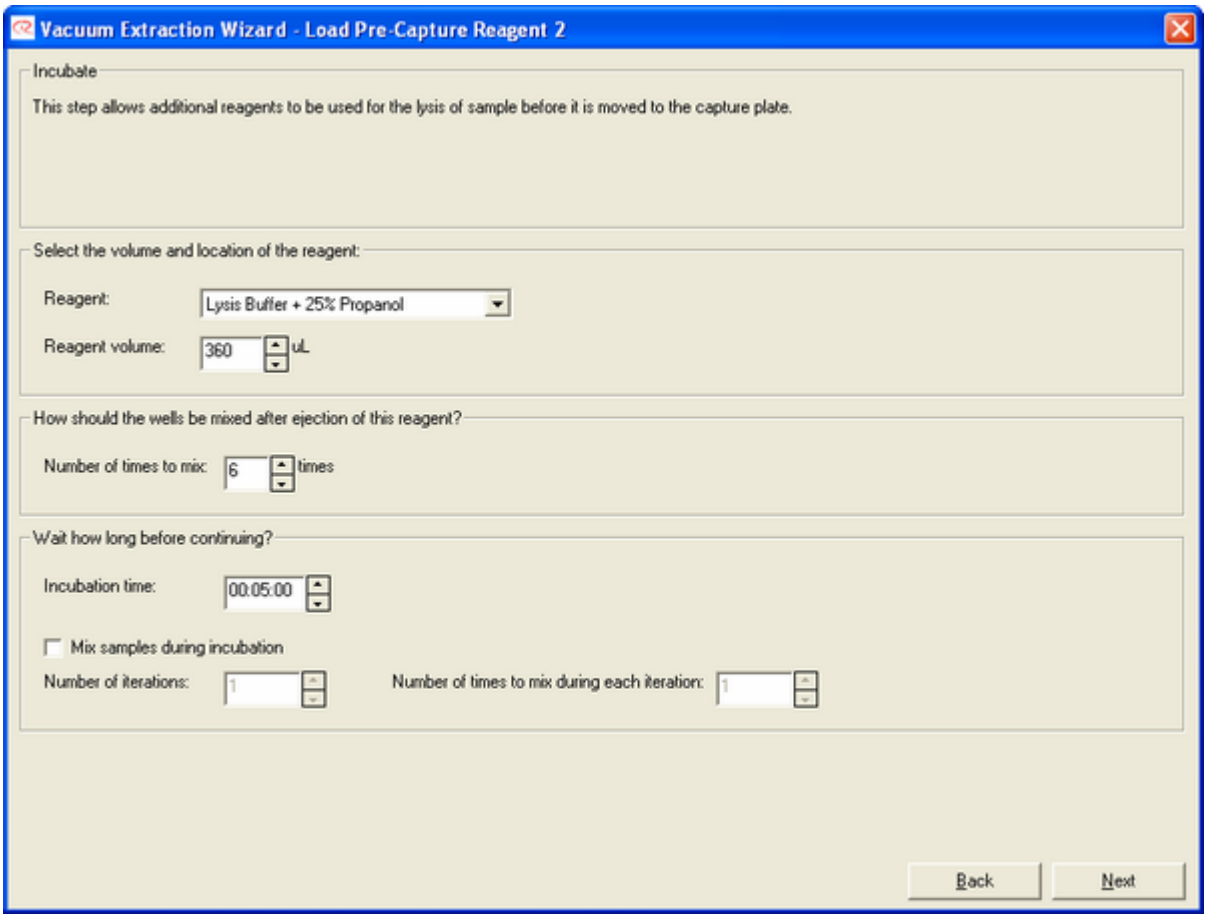
- This option sets the number of times the lysis mixture is to be mixed by pipetting the material up and down.

Wait how long after combining lysis/sample before continuing?

- 'Incubation time' is the time allowed for the lysis buffer to lyse the sample material from which the DNA/RNA will be extracted. If 'The Mix samples during incubation box' is checked, it will force mixing of the samples during the incubation phase. The 'Number of iterations' sets the number of times samples will be mixed during the incubation phase and the 'Number of times to mix during each iteration' is the number of pipetting operations to perform for each mixing routine.

4.6 Load Pre-capture Reagent 2

Because one additional Pre-capture reagent is to be used the user is presented with a second Load Pre-capture Reagent screen in the wizard. This step allows additional reagents to be used for the lysis of sample before it is moved to the capture plate. To configure the 'Pre-capture Reagent 2' window, move through each section and choose the relevant options.



Select the volume and location of the lysis [or equivalent]

- Choose the appropriate reagent from the drop down window labelled 'Reagent'
- Enter your 'Reagent Volume' required for each sample/well

Remember, the total volume of sample and lysis buffer(s) must not exceed the working volume for each well of the lysis plate and the capability of the capture plate to process the lysed sample material.

How should the wells be mixed after ejection of this reagent

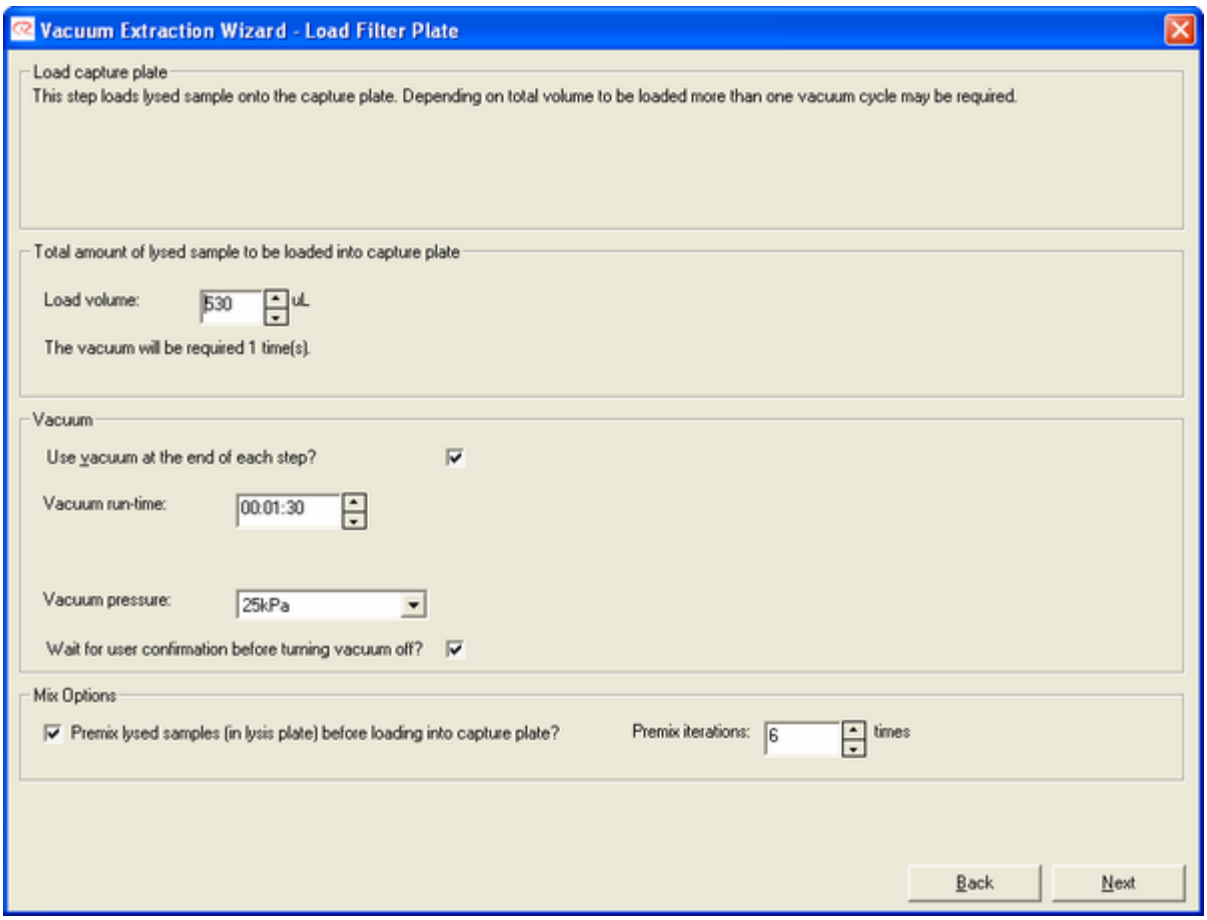
- This option sets the number of times the lysis mixture is to be mixed by pipetting the material up and down.

Wait how long before continuing?

- 'Incubation time' is the time allowed for the lysis buffer to lyse the sample material from which the DNA/RNA will be extracted.
- If the 'Mix samples during incubation box' is checked, it will force mixing of the samples during the incubation phase. The 'Number of iterations' sets the number of times samples will be mixed during the incubation phase and the 'Number of times to mix during each iterations' is the number of pipetting operations to perform for each mixing routine.

4.7 Load Filter Plate

This window configures the amount of lysed sample to be transferred to the capture plate, the vacuum pressure and the duration of the vacuum required to draw the supernatant through the filter membrane.



Total amount of lysed sample to be loaded into the capture plate

- Choose 'Load volume' of the sample required to be filtered. If the volume exceeds the working maximum volume of the capture plate, the volume will be applied over a number of cycles of load volume and vacuum filter until the target volume is achieved. This feature is useful for very dilute samples containing little or no particulate matter or proteins.

Vacuum

- Check the 'Use vacuum at the end of each step?' box if you require the vacuum to draw the sample through the capture plate and enter the desired 'Vacuum run-time' required. Generally, a vacuum will always be used to draw the supernatant through the membrane. The viscosity and the amount of proteins and other materials in the sample will affect the vacuum run-time. The slowest sample will set the vacuum run-time for all samples.
- The user can also set 'Vacuum pressure', however; the user should be cautious and not set the vacuum pressure too high. Excessive vacuum can cause the filter membrane to

collapse, blocking the plate and rendering the samples useless. The default is set at 25 kPa and has been found suitable for most sample types.

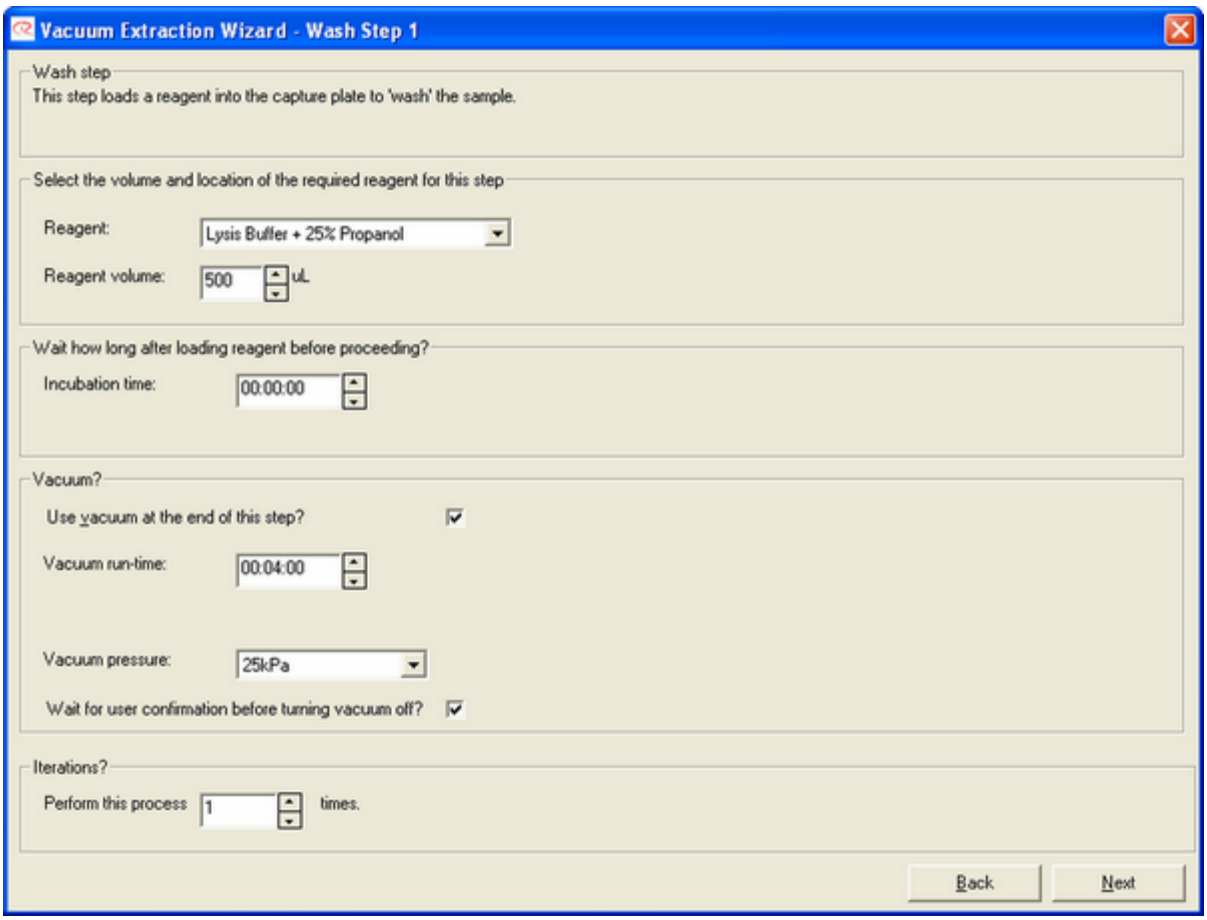
Because the length of vacuum applied is sample-type dependant, the check box 'Wait for user confirmation before turning vacuum off' has been included so that the user can experiment to determine the best vacuum pressure and vacuum time required for each sample type. When this option is checked, the extraction protocol will pause after the vacuum run-time is reached but maintain the desired vacuum until the user clicks the 'OK' button on the pop-up window that appears.

Mix options

- If this option is required check the 'Premix lysed samples [in lysis plate] before loading into capture plate'.
- Enter the number of times to 'Premix' the reagent and the sample. This refers to the number of pipetting operations you require to mix the sample and reagent.

4.8 Wash Step 1 to Wash Step n

To configure the Wash Step window move through each section and choose the relevant options. A wash step is configured for each individual wash buffer to be used, but multiple uses of a particular wash buffer (iterations) are configured within the relevant wash step.



Select the volume and location of the reagent required for this step

- Choose the appropriate reagent from the drop down window labelled 'Reagent'
- Enter the 'Reagent Volume' required for each sample/well

Wait how long after loading reagent before proceeding?

- 'Incubation time' is the time allowed for the wash buffer to absorb proteins and other materials leached from the capture plate filter.

Vacuum?

- Check the 'Use vacuum at the end of each step?' box if you require the vacuum to draw the sample through the capture plate and enter the desired 'Vacuum run-time' required. Generally, a vacuum will always be used to draw the supernatant through the membrane. The viscosity and the amount of proteins and other materials in the sample will affect the vacuum run-time. The slowest sample will set the vacuum run-time for all samples.

The user can also set 'Vacuum pressure', however, should be cautious and not set the vacuum pressure too high. Excessive vacuum can cause the filter membrane to collapse, blocking the plate and rendering the samples useless. The default is set at 20 kPa and is found to be suitable for most sample types.

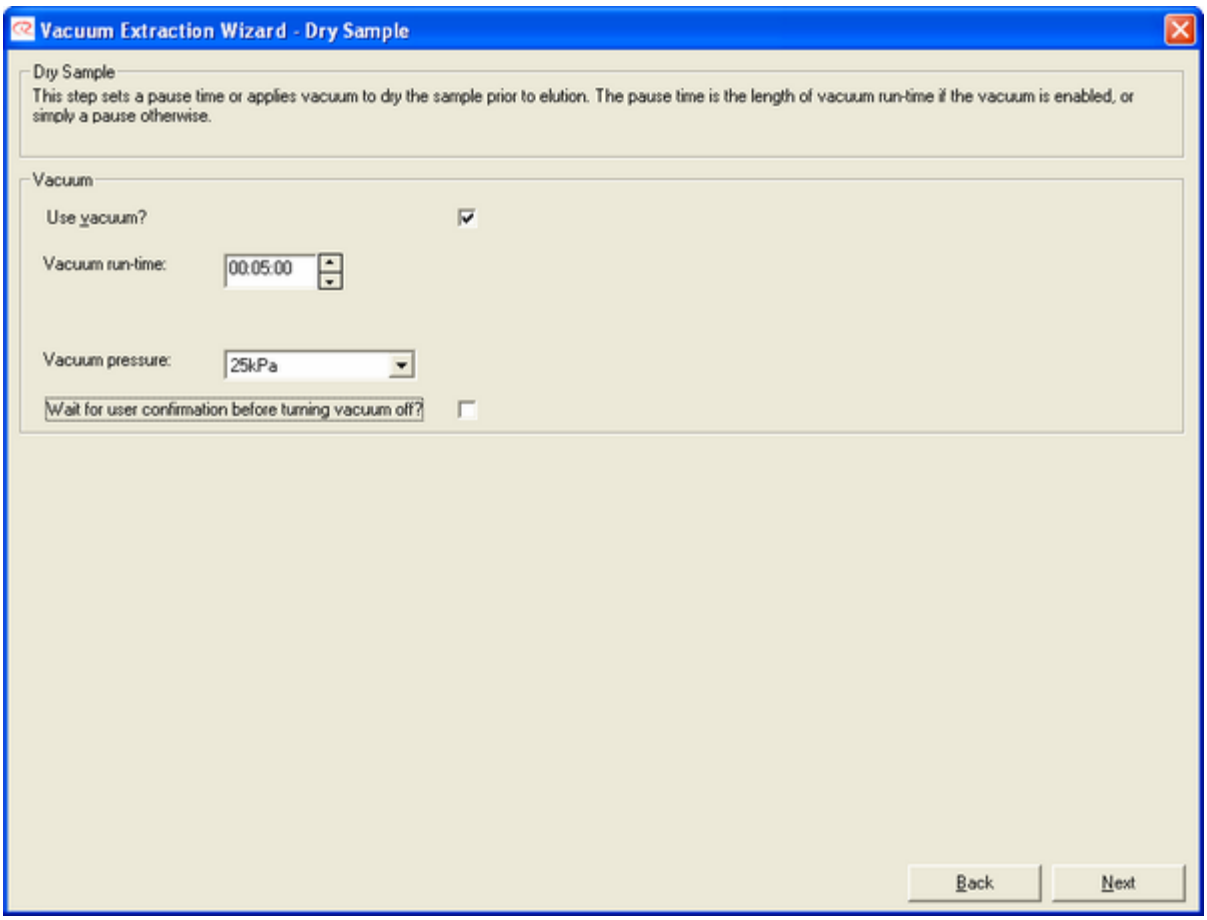
Because the length of vacuum is sample-type dependant, the check box 'Wait for user confirmation before turning vacuum off' has been included so that the user can experiment to determine the best vacuum pressure and vacuum time required for each sample type. When this option is checked, the extraction protocol will pause after the vacuum run-time is reached but maintain the desired vacuum until the user clicks the 'OK' button on the pop-up window that appears.

Iterations?

- Iterations is the number of times to 'Repeat the process' using this particular wash buffer.

4.9 Dry Sample

This step sets a pause time or applies vacuum to dry the sample prior to elution. The pause time is the length of that the vacuum run-time is enabled, or simply a pause otherwise. To configure the 'Dry sample' window, move through each section and choose the relevant options.

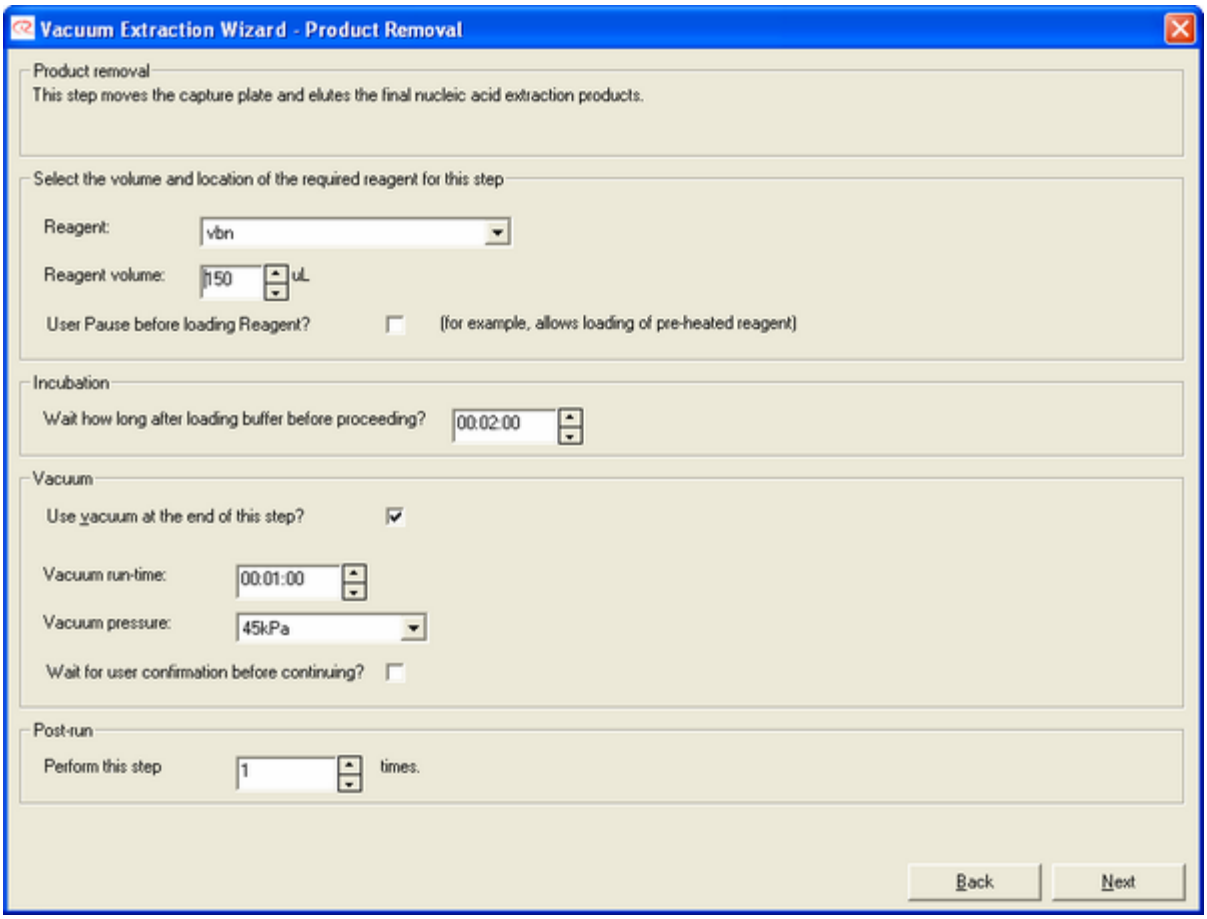


Vacuum

- Check the 'Use vacuum?' box if you require the vacuum to draw the sample and air through the capture plate. Enter the desired 'Vacuum run-time' required to dry the capture plate. Generally, a vacuum will always be used to dry the sample immobilised on the membrane. The viscosity of the sample will affect the vacuum run-time. The slowest sample will set the vacuum run-time for all samples.
- The user can also set 'Vacuum pressure', however, use caution and do not set the vacuum pressure too low. Insufficient vacuum will increase the time required to dry the capture plate. The default is set at 25 kPa and is found to be suitable for most sample types, when the samples have previously been washed with 90% ethanol.
- Because the vacuum run time is sample-type dependant, the check box 'Wait for user confirmation before turning vacuum off' has been included so that the user can experiment to determine the best vacuum pressure and vacuum time required for each sample type. When this option is checked, the extraction protocol will pause after the vacuum run-time is reached but maintain the desired vacuum until the user clicks the 'OK' button on the pop-up window that appears.

4.10 Elute Sample

This step moves the capture plate and elutes the final nucleic acids extraction products. To configure the 'Product removal' window move through each section and choose the relevant options.



Select the volume and location of the reagent required for this step

- Choose the appropriate reagent from the drop down window labelled 'Reagent'.
- Enter your 'Reagent Volume' required for each sample/well.
- 'User pause before loading reagent' gives the user the opportunity to check that the capture plate is indeed dry before the elution buffer is applied to the plate. This pause also provides the user with, for example, the opportunity to heat the elution buffer and to place the heated buffer into the elution tub immediately before its use.

Incubation

- Incubation time is the time allowed for the elution buffer to elute the DNA and/or RNA from the capture plate filter. The incubation time is set by setting the length of time in 'Wait how long after loading reagent before proceeding'.

Vacuum

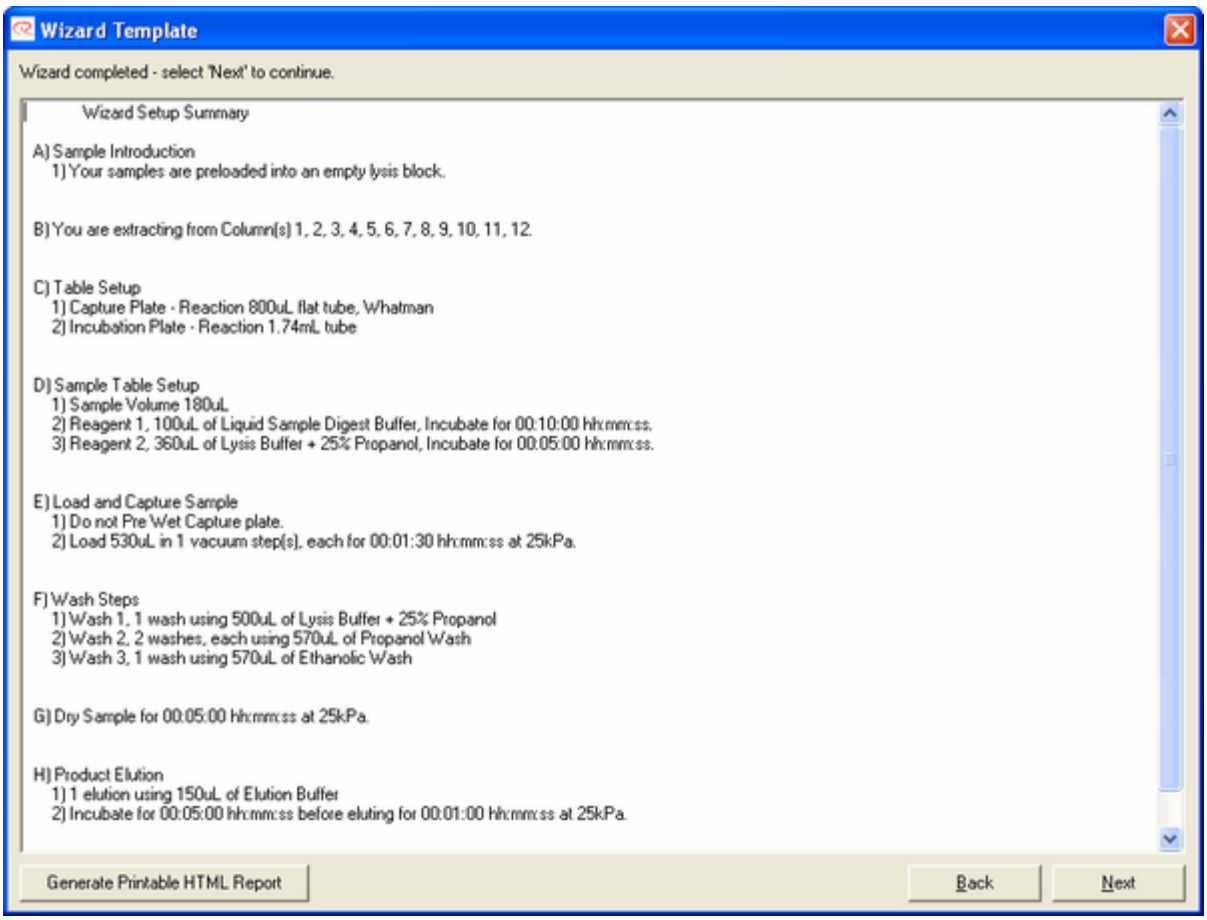
- Check the 'Use vacuum at the end of each step?' box if the vacuum is required to draw the sample through the capture plate and then enter the required 'Vacuum run-time'. Generally, a vacuum will always be used to draw the supernatant through the membrane. The viscosity and the amount of DNA and/or RNA in the sample will affect the vacuum run-time. The slowest sample will set the vacuum run-time for all samples.
- The user can also set 'Vacuum pressure', however, use caution and do not set the vacuum pressure too high. Excessive vacuum can cause the filter membrane to collapse, blocking the plate and rendering the samples useless. The default is set at 30 kPa and is found to be suitable for most sample types. If elution splattering is observed in the tops of the elution tubes the vacuum pressure should be decreased.
- Because the length of vacuum is sample-type dependant, the check box 'Wait for user confirmation before turning vacuum off' has been included so that the user can experiment to determine the best vacuum time for a particular vacuum pressure required for each sample type. When this option is checked, the extraction protocol will pause after the vacuum run-time is reached but maintain the desired vacuum until the user clicks the 'OK' button on the pop-up window that appears.

Post-run

- In some instances the user may wish to recover more of the samples by repeating the elution step. If multiple elution steps are required, enter the number of repeats in the 'Perform this step' box.

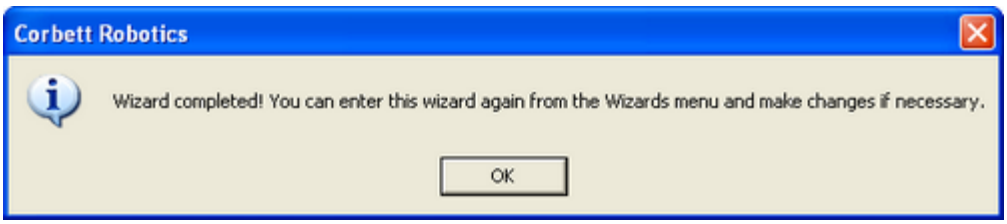
4.11 Wizard Template Report

At the completion of the wizard, the user is presented with a summary describing the run file that is about to be compiled. This report should be checked carefully. Should the user find any errors that require editing of the wizard, the user can use the 'Back' button to cycle backwards through the wizard to correct the problem(s). Otherwise select next to continue. A hard copy of the report can be printed by selecting 'Generate Printable HTML Report'.



4.12 Wizard Complete Message

At completion of the wizard the user will be presented with the following message. When the 'OK' button is pressed, the program will compile the run file that will then be displayed in the right-hand pane of the software interface. The user can then save the run file for later use.



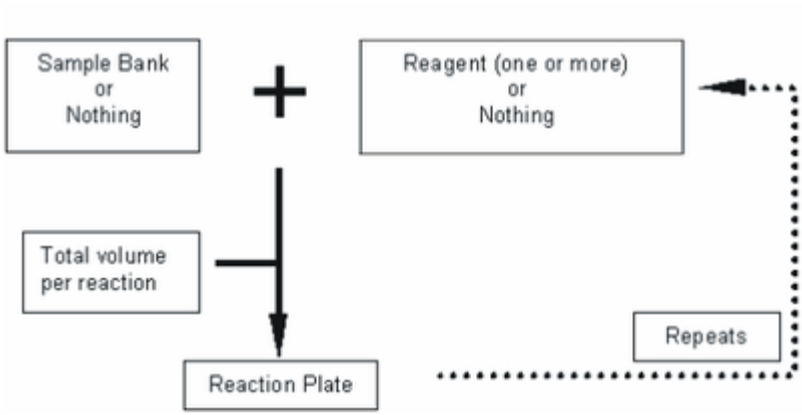
5 Configuring Manual Reaction Run Files

The Reaction Configuration section gives the user access to options to combine liquids. This facility extends the use of CAS-1820 X-tractor Gene beyond its primary purpose of a Wizard driven DNA/RNA extraction system.

To open the Reaction Configuration window select the target reaction plate with the mouse and then select the 'Add...' button on the reaction right-hand pane.

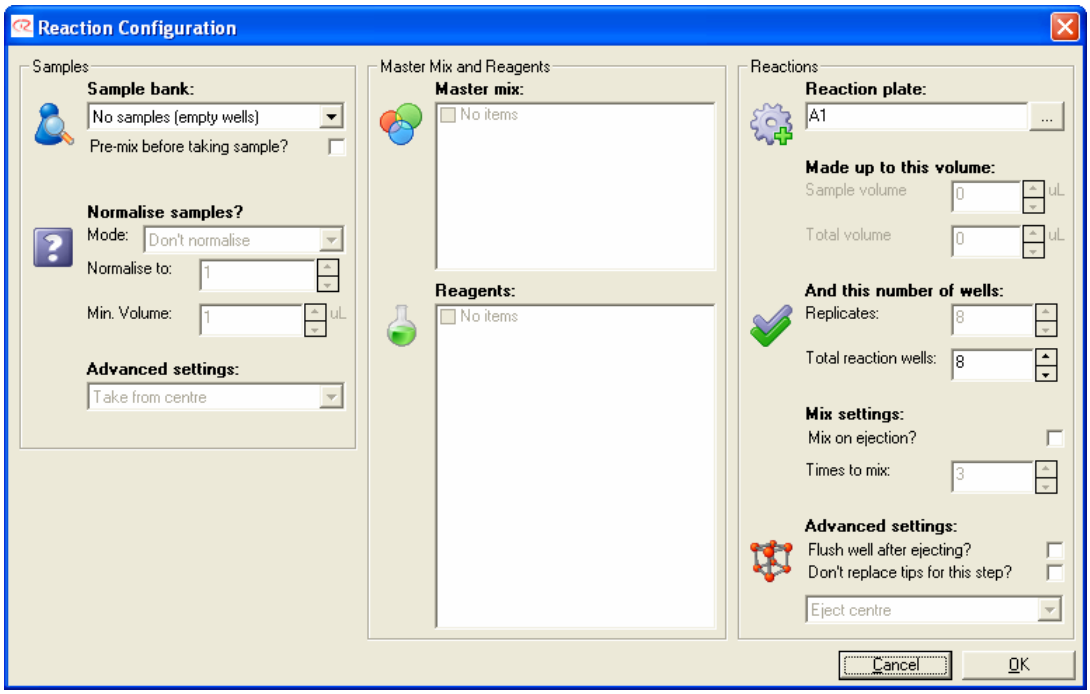
The Reaction Configuration contains a variety of options to combine liquids that have been defined as samples, or reagents. Options exist for pre-mixing or post-mixing, selecting destination plates and others. Unavailable options are greyed out.

The reaction configuration works on the following principle:



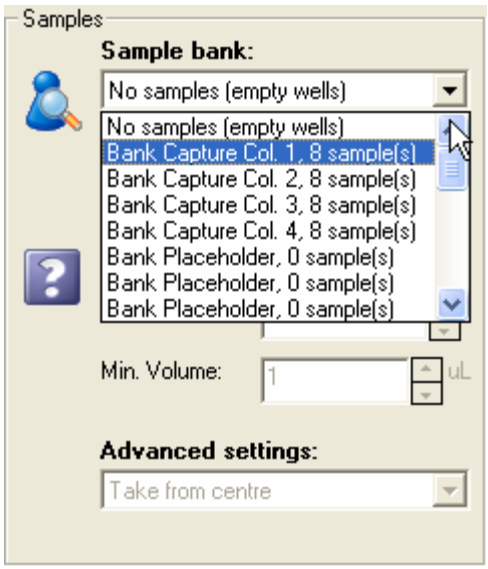
The reaction configuration window is broken down into several sections accordingly. These sections are

- Select ONE of these sets:
- Which will be combined with ALL of these:
- In to THIS reaction plate:
- Made up to this volume:
- Repeating this number of times:
- These sections can be identified in the figure below.



Select ONE of these sets:

- Allows the selection of one available sample bank. No more than one sample bank is allowed per reaction. This would lead to cross-contamination of samples by pooling. By selecting on the drop down menu as shown below, all available sample banks are listed. In this example, 'Blood Samples' represents a plate of 96 samples. If the 'No Samples' option is chosen then no sample bank will be added to the reaction mixture.



- An option is provided to pre-mix samples. If this option is ticked, as an aliquot from the sample tube is taken, the pipetting head repeatedly pipettes up and down before taking the final aliquot. This option can be useful for samples that have a tendency to settle. The pre-mix draws a volume equal to the amount to be aspirated; this volume is fixed and cannot be changed. The pre-mix is repeated five times, this is also fixed.

This will be combined with ALL of these:

- Any reagent that has been configured can be added to the reaction. Place a tick next to the reagent that is to be added to the reaction.

In to THIS reaction plate:

- By clicking on the  button, all available reaction plates will be shown in a window. Choose the reaction plate into which the reagents will be dispensed. If only one reaction plate is available, this plate is selected by default.

Made up to this volume:

- If a sample bank is selected, the volume of the sample bank can be selected. Note that all wells in a sample bank are treated equally. For example, if a sample contains 10 wells and 5 µL is selected as a volume, then 5 µL of each of the 10 wells is used in the reactions.
- The total volume is automatically calculated based on the combination of the sample and reagents.

Repeating this number of times:

- Enter the number of reaction replicates. The maximum number of repeats is defined as 384. The repeats are positioned in such a way that the repeats of one sample well are pipetted into the plate consecutively. For example, if three repeats are selected and the sample bank contains wells A1-A8, then the repeats of well A1-A8 would be pipetted into wells A1-A8, B1-B8 and C1-C8 in a vertical reaction plate.

Other Options

Mix Settings

This option is enabled by placing a tick in the 'Mix on ejection?' check box. The number of mix cycles can also be changed. The volume mixed is the larger of either the well contents or the last volume of liquid added. This option is particularly useful when pipetting a very small volume of sample (less than 3 µL). The mixing 'rinses' the entire sample out of the tip.

Sample Normalisation

Sample normalisation provides functionality to normalise samples of varying concentrations to one final concentration. The sample normalisation option can only be used if the concentrations of the samples are defined and constant across each set of 8 samples. The option is enabled by ticking the 'Use normalisation?' checkbox. If normalisation is selected, the final concentration must be specified. If the range of concentrations is too large, a warning will be flagged. The range of concentrations is directly related to the amount of sample pipetted such that, the lower the concentration, the smaller the volume of the highest concentration that is to be added to the reaction plate.

Pickup and Ejection

The options control the way the reagents or samples are dispensed and following dispensing, whether the tip will be available for reuse or discarded.

6 Appendices

- Appendix A - Helpful Hints
- Appendix B - Maintenance
- Appendix C - Precision vs. Accuracy
- Appendix D - Error Codes
- Appendix E - Capture Plates, Separator Plates, and Transfer Carriages
- Appendix F - Pippetor Servicing
- Appendix G - Decontamination Procedures

6.1 Appendix A - Helpful Hints

The Robotics Software has a number of useful features that are not covered in the body of the manual. In some cases these features are a little obscure but they can make certain setups easier.

Tip availability shortcut

A convenient shortcut has been added to allow individual tips to be toggled between available and unavailable. To do this, simply hold down the 'Alt' button and then click on individual tips. The tip will toggle between available and unavailable.

Cutting and pasting samples into sample banks

It is possible to define which samples belong to a sample bank by cutting and pasting a column of data from a spreadsheet. Refer to the section on Samples or Plate Operations for information on adding samples to sample banks. As described, it is possible to add samples to a sample bank by clicking in the Yes/No column in the sample list. It is possible to copy this Yes/No column from a spreadsheet.

Cutting and pasting sample names

Sample names can not only be imported using the import functionality, but sample names can also be copied and pasted. In a spreadsheet, copy the column that contains the sample name information. In the Robotics Software, right-click on the cell from which the copy is to take place. Select 'Paste to current cell' and the names will be copied.

Copying and Pasting

Copy and paste functionality is available in all tables throughout the software. Simply right-click in desired cell or cells to display copy/cut/paste menu.

Tip re-use and Pipetting

Tip re-use is a useful function that not only saves on tips but also on time. Time is saved because additional tip pickups are not necessary. However, be aware that tip re-use reduces pipetting precision; please see the pipetting precision report in the Appendix.

Volume Calibration and Liquid Retention

Volume calibration has a side effect that more volume than expected is used. This is primarily due to liquid retention in the pipetting tips. Consider a situation where a robot that is not volume calibrated pipettes 19.7 µL when asked to pipette 20 µL. Volume calibration can resolve this short fall. If a volume calibration were to be conducted, the robot would then deliver 20 µL. However, to deliver this volume the robot must take an aliquot of 20.3 µL. The 0.3 µL is a result of liquid retention in the tip. If this volume were delivered over a 96-well plate, the robot would apparently use 30 µL more than it should have. There is no easy solution to this problem other than low retention tips. The user must be aware of this shortfall and account for it when supplying the robot with liquid in reservoirs.

Joystick

The use of a joy pad (a game controller that only has buttons and no stick) can make position calibration significantly easier. A USB joy pad can be connected to the computer that controls the CAS-1820 X-tractor Gene. During calibrations the user can use the joy pad to move the robot arm as this can significantly speed up calibrations, especially position calibrations.

6.2 Appendix B - Maintenance

The CAS-1820 X-tractor Gene requires minimal maintenance. Nonetheless, here are a few helpful hints that will keep your CAS-1820 in good working order.

Pipettor Re-greasing

The pipetting head occasionally needs to be serviced. Tests have shown that after approximately 500,000 pipetting operations, the O-rings in the pipetting head should be replaced and the pistons re-greased. The software has a built-in maintenance counter that will remind the user after 300,000 pipetting operations that the pipetting head needs a service. The service is a straightforward procedure. Your CAS-1820 is delivered with a pipettor service kit. Keep this kit in a safe place until it is needed. The kit contains all parts needed to perform a full service.

Rails

The X, Y, and Z rails are the coated hardened steel rails that support the linear bearings that allow the robot to slide back and forth easily. Do not wipe these rails with a cloth. Any wiping of the rails will only serve to remove the grease. Ensure that a thin film of grease covers the three rails.

The linear bearings incorporate seals to keep the bearing free of dust or grit. The rails and bearings have been tested on the CAS-1820 in excess of 2,000,000 operations without any sign of degradation.

Cleaning

The CAS-1820 needs to be kept clean. All surfaces (with the exception of the rails) can be wiped down with a soft cloth. Diluted bleach can be used on all surfaces. Common alcohols such as isopropanol, ethanol and methanol have been tested and found to be safe. However, the black edges on the outside of the lid should not come in contact with any alcohol or solvent.

The white paint is very hard. It is hard wearing and will resist short wavelength UV exposure. However, due to its hardness, the paint can chip very easily. Take care not to drop any accessories onto the robot workspace as the paint may chip or crack.

Any solvents commonly associated with painting such as mineral turpentine, paint thinners and acetone should never be used near the robot.

Lid

As mentioned above, any alcohol is to be avoided on the painted edges of the lid. The lid is manufactured from highly impact resistant polycarbonate. The polycarbonate is UV absorbent. The polycarbonate also scratches very easily, always use a soft clean cloth to wipe down the lid.

Accessories

The Reagent plates (NOT Tubs) are autoclavable. These can also be placed in a dishwasher if needed.

Service

Should servicing be required, Corbett Robotics Pty. Ltd. or one of its agents will endeavour to service the instrument on site. If it is not possible to service the instrument on site, please contact the Corbett Team.

6.3 Appendix C - Precision vs. Accuracy

Pipetting precision and accuracy are the primary specifications by which to measure any pipetting instrument, either automated or manual. This appendix is written to provide a brief overview of what the terms accuracy and precision refer to.

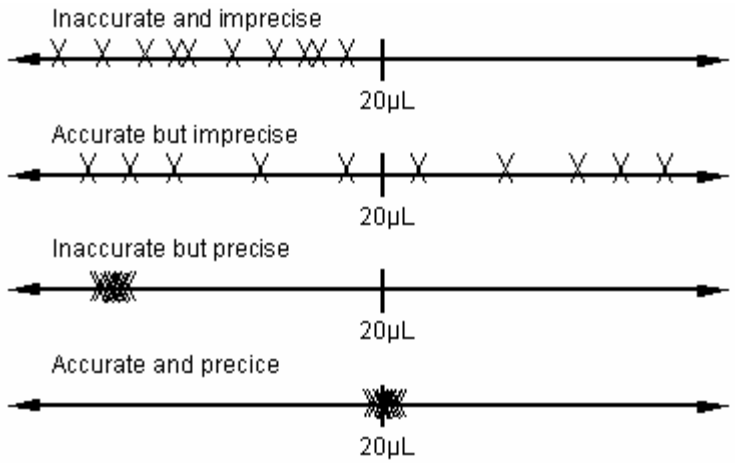
Accuracy

In the case of a pipetting instrument, accuracy can be defined as the closeness of the pipetted volume to the internationally recognised standard of 1 litre (or fraction thereof). Typically accuracy is quoted as an absolute volume variation, i.e. +/- µL at a given volume. Alternatively accuracy can also be expressed as a percentage, i.e. +/- %.

Precision

In the case of a pipetting instrument, precision is a measurement that defines how close the pipetted volume of repeated operations is to one another. Precision is quoted as an absolute volume variation, i.e. +/- µL at a given volume. Alternatively, the convention used for the CAS-1820, precision can be quoted as a statistical definition, coefficient of variation or %C.V.

To illustrate, consider each cross as a pipetting operation or a statistical sample. The line represents a scale of volume that is pipetted. The illustrations shows data of an experiment to pipette 20 µL repeated 10 times.



Coefficient of variation

Coefficient of Variation (C.V.) is defined as

C.V. = (standard deviation / mean) * 100%

What C.V. means (statistically) is that 66% of all samples will fall within plus/minus one standard deviation from the mean (the bell curve principle). Our specification of 1% C.V. means that we guarantee that our standard deviation is less than 1% of the mean, i.e. if you took 50 samples of 20 µL and the mean turns out to be 19.3 µL. We guarantee that 66% of samples fall within 19.107 µL and 19.493 µL. This means that in the set of 50 samples, there will be up to 16 samples that lie outside these limits. It is also possible that a sample might be 18.9 or another sample that might be 20.0 µL. Statistically, it is possible that a sample is only 10.0 µL - although this is very improbable and would indicate an instrument fault.

The 19.3 µL, being 3.5% different from the desired volume of 20 µL, is an accuracy issue. This can be resolved by volume calibrating the instrument.

Accuracy (Volume Calibration)

Different liquids have different surface tension properties and viscosities. These physical properties significantly affect tip retention and allowable maximum pipetting speeds. Depending on which liquid the volume calibration is performed with, different results will be obtained. It is generally recommend that a volume calibration be carried out with pure water (distilled or PCR).

Volume calibration will change slightly upon performing a pipettor service.

Testing Precision

Precision can be tested in a variety of ways. A set of pipetting samples can be weighed and statistically analysed. Although this would be the best way of performing a test, it is very time consuming due to the weighing of the tubes. Alternatively, precision can be measured by pipetting samples of dye (visible of fluorescent) into an optically clear plate and then reading the samples on a plate reader.

Corbett Robotics Pty. Ltd. uses visible dyes and reads these on an absorbance plate reader.

Precision should never be determined by performing an amplification on samples pipetted by the CAS-1820. The amplification process can introduce too many significant variables.

6.4 Appendix D - Error Codes

There are a number of sources of error messages. These include run set up, machine errors or software errors. For run set up errors, refer to the section on warnings.

Software Errors

Error numbers of 55556 are errors generated by the software. The following error strings may be encountered. If any of these are encountered, they can usually be resolved reasonably quickly.

- No tips - there are no tips available, add more
- Robot error - see above
- User aborted job
- User opened lid
- User paused job
- User resumed job
- Software or configuration error - there could be a problem with the installation
- Tip ejection failed - a tip was not ejected from the pipetting head
- Tip pickup failed - a tip was not correctly picked up
- Machine Errors

Machine errors have error numbers starting at 40000. All error codes listed are added to this offset. If these are encountered, contact Corbett Support.

1 FIFO overrun error

2 bad checksum error

3 bad axis number used when an axis number is not supported by command

4 if robot is sent an unknown command

5 if vacuum sensor cannot sense vacuum

6 if an axis is not enabled

9 if the home switch could not be found within limit

10 82C55 I/O pins are unstable

11 an error has occurred in SPI comms

129 an invalid axis was specified

130 the robot did not respond with the first reply

131 the robot did not respond with a second reply

132 the comm port has problems

133 the micro firmware is mismatched

134 an invalid COM port was chosen

135 an attempt was made to move an axis beyond its limit

136 too many replies were specified in sendPacket

137 in a received packet a wrong checksum was encountered

138 the first reply was corrupt

139 the second reply was corrupt

140 the local receive FIFO overflowed

141 an incorrect number of replies was received

142 if specified packet ID could not be found in collection

143 specified accessory port number does not exist

144 specified accessory port unoccupied

145 an unknown accessory was detected

146 a bad temperature reading was taken

147 a bad packet length was received

148 more data was received while processing

6.5 Appendix E - Capture Plates, Separator Plates, Transfer Carriages

The table below summaries which Transfer Carriage is used with what Separator Plate and which Riser Block is to be used in the Elution Chamber. The table also provides the catalogue and part number for each manufacturer.

DNA Capture Plates Adaptor parts and adaptor kits

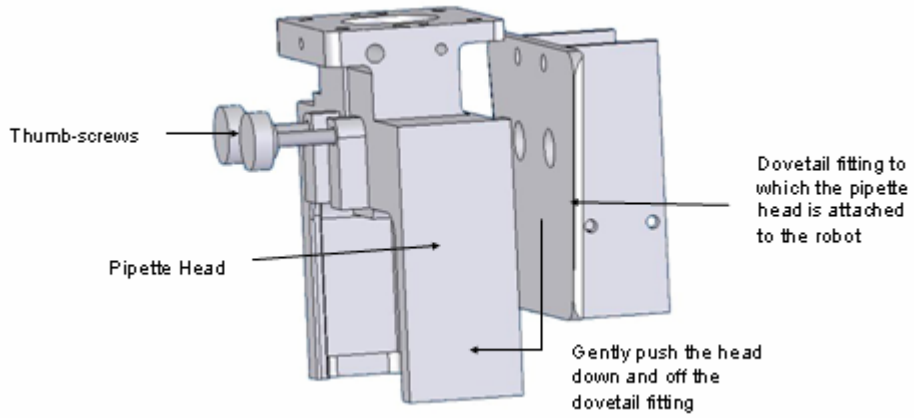
Manufacturer	Plate Description	Cat#	Max Yield	Transfer Carriage to use	Separator Plate to use	Elution Riser Block to use	Kit Name	Capture plate Adaptor Kit PN
Whatman	96 well 800ul GF/B Filter, long drip UniFilter Plate	7700-2803	10 ug	PN:1857 or 2427	PN:1695	PN:1775	NA	2445 (Supplied with X-tractor)
	Corbett 96 well Low skirt capture plate	0913						
Corbett (OEM)	Corbett 96 well High skirt High yield capture plate	1864	40 ug	PN:1675	PN:1697	PN:2443	Adapter Kit High Skirt	2446 (Supplied with X-tractor)
Invitek	96 well High skirt capture plate	Only in Kits	10 ug	PN:2427	PN:2452	PN:2443	Adapter Kit Invitek	2447
Promega	96 well high skirt Binding Plate	A2271 and Kits	40 ug	PN:1675	PN:1697	PN:2443	Adapter Kit High Skirt	2446 (Supplied with X-tractor)
Macherey Nagel	96 well High skirt HTP capture plate	only In Kits	Varied	PN:1675	PN:1697	PN:2443	Adapter Kit High Skirt	2446 (Supplied with X-tractor)
	High skirt 8 Strip system	only In Kits	Varied	PN:1675	PN:1696	PN:2443	Adapter Kit M&N 8 strip	2448

6.6 Appendix F - CAS-1820 Pipettor Servicing

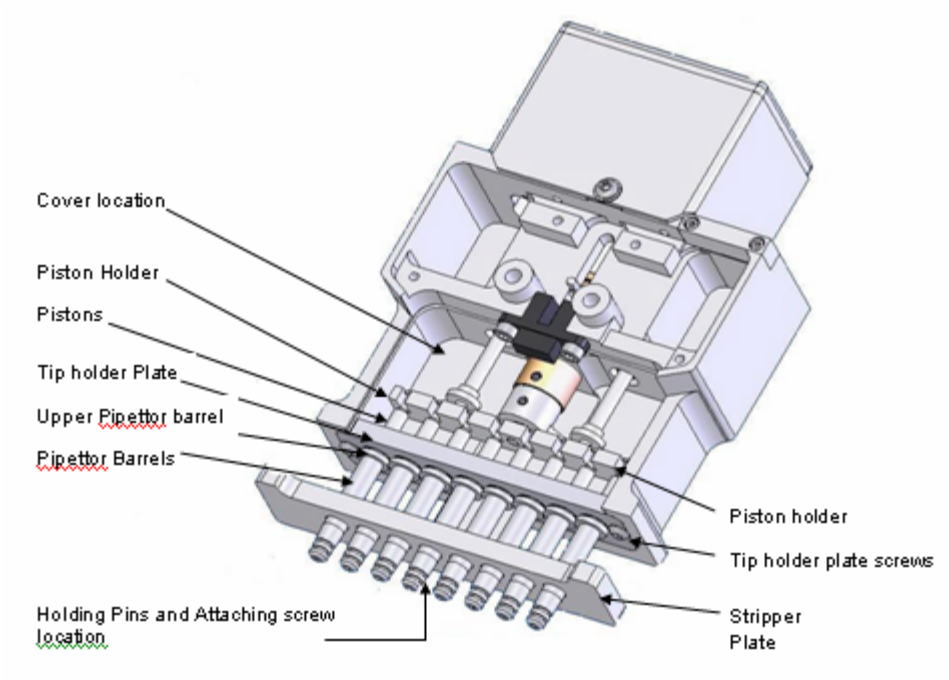
To maintain its precision, the CAS-1820 pipettor head must be serviced. This process is required every 6 to 12 months, or if the robot is heavily used, every 3 months. The following tools are required to complete this service:

- 2.5 mm Allen Key
- 2 mm Allen Key
- 8-Channel Pipettor Barrel Removing Tool (part of service kit – part no. 0827)
- Lint free cloth or tissues (part of service kit – part no. 0827)
- Pipettor Oil (part of service kit – part no. 0827)
- O-rings (part of service kit – part no. 0827)
- Large black o-rings
- Medium clear o-rings
- Small white o-rings.
- Perform the following tasks to service your pipettor.

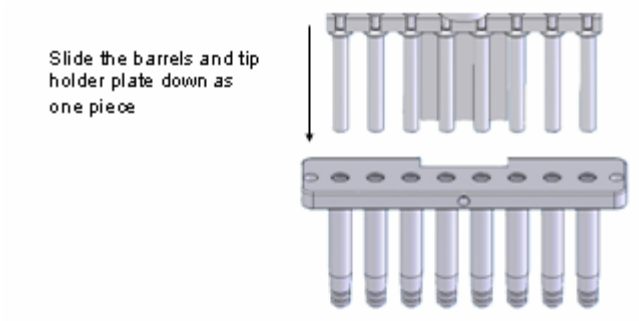
1. Ensure the robot is turned off. Push the pipette head downwards, until the electrical cable plug can be pulled out. Once this is done, remove the thumb-screws and gently push the head down off the dovetail fitting. With the latest model robots, the head will not have a dovetail fitting and can be simply lifted away from the robot after the thumb-screws are removed.



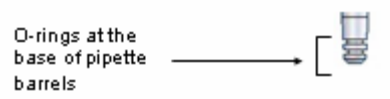
2. Remove the screw attaching the stripper plate to the pipette head, and carefully work the stripper plate free of its two holding pins.



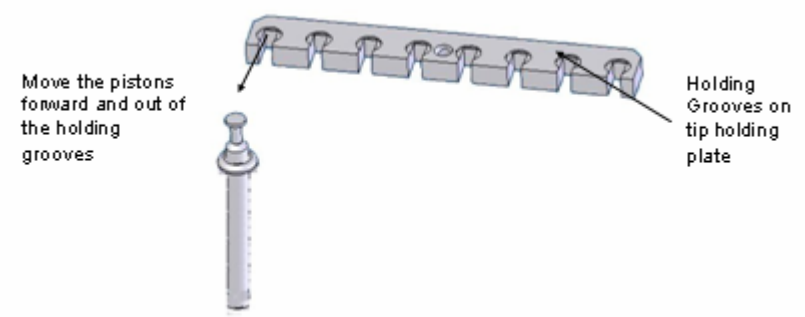
3. Using the 8-Channel pipettor barrel removing tool, loosen all the pipette barrels in sequence from left to right.
4. Remove the screw holding the Perspex cover in place, and slide the cover down and out of position.
5. Using the 2mm Allen key, remove the two screws at either end of the tip holder plate. Slide the barrels and tip holder plate gently down off the pistons as one piece. Once removed, unscrew the barrels from the tip holder plate.



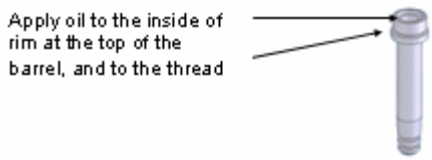
6. Remove the old upper-pipettor barrel o-rings from either the tip holder plate or from inside the top of the pipettor barrels, being sure to have removed all eight.
7. Remove the two o-rings off the base of each of the pipettor barrels, being careful not to scratch or damage the barrels, or o-ring grooves. Fine-tipped tweezers can be used for this, but it is vital to avoid scratching the barrels, therefore, do so with caution.



8. Slide the eight pistons out of the piston holder by moving them forward and out of the holding grooves.

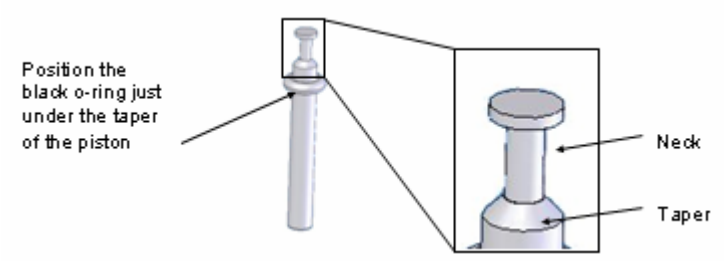


9. Remove the old o-rings from the tops of each of the pistons, being sure to remove all eight
10. Remove any oil and foreign matter from the pipettor barrels, the pistons, tip holder plate, and the stripper plate. Use a lint free cloth moistened with methylated spirits, or similar alcohol.
11. Thoroughly clean the barrels with warm tap water, then ethanol, and allow to completely dry.
12. Inspect all components for any obvious damage. Pay particular attention to the pipettor barrels, as they must be free of any scratches or scoring, which can interfere with their ability to pick up, retain, and eject tips.
13. The pipettor may now be reassembled using pipettor oil. Note that the type of lubricant used has a significant effect on the pipetting precision. Additionally, it should be noted that excessive or insufficient oil can also cause imprecision. To avoid any imprecision, use only the pipettor oil supplied in the kit, and adhere to the following assembly instructions.
14. Apply a small drop of oil to the inside rim, and the thread at the top of each of the barrels.

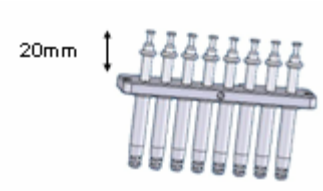


15. Position one medium clear o-ring into each of these rims (one per barrel) and then screw the barrels back into the base of the tip holder plate.

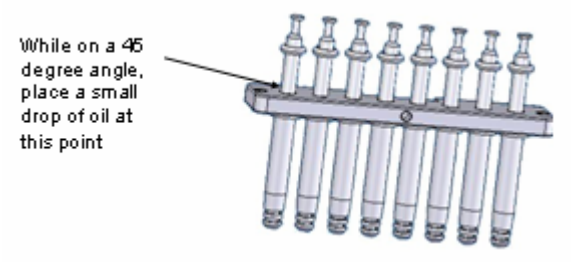
16. Using the new, large black o-rings, slide one o-ring over the lower end of each of the pistons and slide it up into position at the base of the taper.



17. Carefully push the pistons half-way into each of the eight barrels. Allow approximately 20mm of the piston to be exposed above the tip holder plate

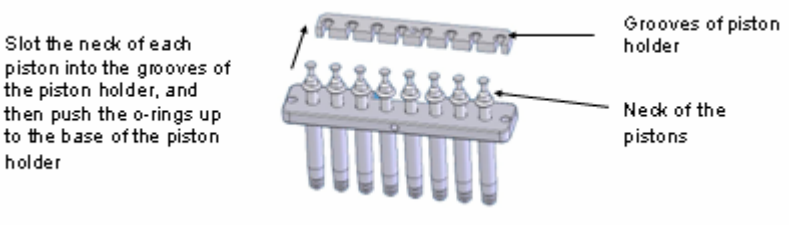


18. Turn the assembly on a 45 degree angle and drop a small drop of oil onto the uppermost part of each of the pistons at the point indicated below.

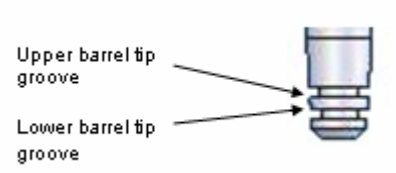


19. Push the piston in and pull it back out to check the amount of oil. A thin film of oil should cover the entire piston with no obvious dry spots or large accumulations. Be careful to avoid getting oil on the large black o-ring.

20. Align the tops of the eight pistons. Holding the assembly by the barrels, and ensuring the Perspex cover screw hole is at the front, slot the taper of each of the pistons into the grooves of the piston holder. Push the black o-rings up to the base of the piston holder, so they lock into the taper of the piston.

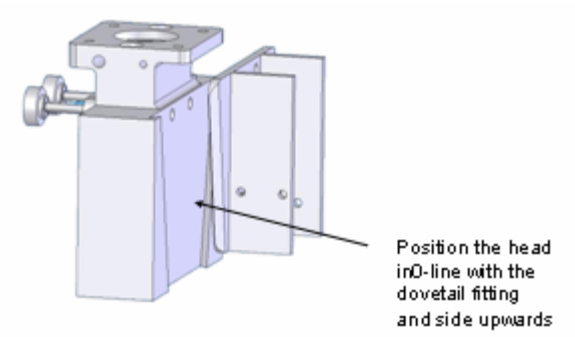


21. Push the tip holder plate up and into position and screw into place on either end.
22. Slide the cover up into position, ensuring it is positioned correctly within its railing. Screw into place.
23. Using a small amount of oil, lubricate each of the new small white o-rings. Carefully place one o-ring over the tip of the pipettor barrel and slide it into the lower groove. Now repeat this procedure with the second o-ring and allowing it to settle in the upper groove. Max sure that the o-rings aren't twisted. Do this with each of the eight pipettor barrels. When all o-rings are in position, use a kimwipe (Kimberly-Clark) to remove any exposed oil from the barrel. Ensure no there is no lint on the o-rings or in the grooves.



24. Ensuring the stripper plate is in the correct orientation (the channel indent should be facing upward), slide over the barrels and locate onto the two holding pins. Secure the plate with the screw, and tighten with a 2.5mm Allen key. Be sure that the two positioning pins are flush with the bottom of the stripper plate.

25. The Pipette head is now ready to be reattached to the robot. Position the pipette head in-line with the dovetail fitting and slide upwards so that the head remains in place with minimal support (As mentioned in step 1 above, the latest models will not have this dovetail fitting, therefore, this aligning step will not apply). Attach the thumb-screws and tighten. Move the head to a position that allows access to the cable socket, and attach the electrical cable plug.



6.7 Appendix G - Decontamination Procedures

Fundamental to most applications of the CAS-1820, is the requirement that the robot remains free of contaminants, especially DNA and RNA. To eliminate cross-contamination and ensure validity of results, the robot must be routinely decontaminated. The following procedure describes this process for the CAS-1820.

Caution:

- Common alcohols, such as isopropanol, ethanol and methanol have been tested and found to be safe. Note however, that the black edges on the outside of the lid should not come into contact with any alcohol or solvent.
-
- Do not use solvents associated with oil based paint, such as mineral turpentine, paint thinners, or acetone. These solvents will damage the painted surface of the robot.
-
- The polycarbonate material of the robot's lid is easily scratched, therefore, never use abrasive materials to clean this. Instead, a clean soft cloth is recommended.
-
- Avoid removing grease from the rails (X and Y axis) that support the linear bearings. These rails support the pipette head and allow it to slide back and forth easily.
-
- Wiping these rails will remove the grease that is vital both to function of the robot and also to preventing corrosion.

Preventative measures:

- Always wear gloves and change them frequently.
- Use filter-barrier pipette tips.
- Use a dedicated set of pipettes to assemble reagent aliquots.

Aliquot reagents to each individual investigator; that way contamination sources can be identified and contained.

Decontamination Procedures

Materials Required:

- Liquid bleach concentrate
- Absolute ethanol
- Sterile, nucleic-acid-free water
- Detergent
- 3 Sterile squirt bottles
- 1 plastic bucket (~ 9 litres) and lid.
- A liquid reagent spray bottle containing household bleach at a final concentration of 1% (10,000 ppm) chlorine: typically a 1:5 or 1:10 dilution of bleach depending on initial sodium hyperchlorite concentration.

- A liquid reagent bottle containing nucleic-acid-free deionised or Milliq water.
- A liquid reagent bottle containing nucleic-acid-free 70% ethanol.
- Soft, clean paper towelling

Steps:

The following decontaminating steps should be used regularly to ensure that the robot is free of DNA/RNA contamination. The free chlorine available in bleach acts to cross-link the DNA in addition to its cleaning and disinfectant properties.

1. Add bleach concentrate to a clean bucket and dilute to 1% free chlorine with sterile nucleic-acid-free water. You require approximately 5 litters of dilute bleach.

2. Remove all plates, reagent blocks, tip racks, and the tip ejector chute from the CAS1820's deck. Wash these with detergent and rinse with clean water. Place the clean components into the bleach solution and soak for 15 to 30 minutes. Rinse the components completely will DNA-free sterile water. Rinse briefly with absolute ethanol and dry with a soft paper towel. Make sure all components are free of detergent and bleach.

3. Spray the workspace of the robot with the dilute bleach and let stand for 15 to 30 minutes to cross-link the DNA. Wipe off the excess bleach of the robot with clean paper towels. Spray the work space with sterile water and dry off. Repeat this process three times. Now, rinse the workspace with ethanol and allow too dry.

4. Place all components back onto the work space and close lid.

5. If your robot is fitted wih the optional UV light, turn the light on and run for 15 minutes.

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