

ClonePix[™] 2

ROBOT MANUAL



Contents

Introduction to ClonePix 25 Technical Specifications
-
External Dimensions and Weight6
Compressed Air Supply6
Operating Environment6
Electrical Supply7
Electrical Connections
Symbols on Equipment8
PC Connections
Filter options10
Robot Enclosure11
Installation
Layout of ClonePix 212
Source and Destination Plate Stackers13
Connecting the Wash Bottles14
Connecting the Ethanol Bottles14
Camera Alignment15
ClonePix 2 Panel15
Health and Safety16
Important Note
Transport and Storage16
Lifting Points16
External Covers16
Electrical Safety
Safety Features17
Drive Safety
Hot Air/Halogen Dryer17
Noise Levels
Service and Maintenance18
Service and Maintenance

System Features	
Pre-Power-Up Checklist	
Power-Up Procedures	
Shutdown procedure	20
General Maintenance	21
Regular Maintenance	
General Precautions	22
Cleaning Procedures	22
Removable Parts	22
Cleaning of Instrument Interior	24
Cleaning of System Fluid (Sterile Water) Supply	24
Incoming Compressed Air Supply	25
Automated Picking Pin Cleaning	25
Sterility Validation Tests	26
Changing the Picking Head	27
Remove Head:	27
Replace Head:	27
Changing or Replacing Picking Pins	27
Replacement Parts and Optional Extras	
Replacement Parts	
Reagents and Supplies	
CloneDetect Detection Reagents	
CloneMatrix	
CloneMedia / XPMedia	
PetriWell Cell Culture Plates	
Other	
Troubleshooting	
Common Problems	
Glossary of Terms	
Index	
Contact Details	
Contact Details	

Table of Figures

Figure 1: ClonePix 2 electrical connections	8
Figure 2: ClonePix 2 PC connections	9
Figure 3: ClonePix 2 layout and dimensions	. 12
Figure 4: Stacker orientation	. 13
Figure 5: Wash bottle connections	. 14
Figure 6: Front Panel	. 15

Introduction to ClonePix 2

Manufactured by Molecular Devices (New Milton) Ltd.

ClonePix[™] 2 is a dedicated solution for imaging, identification, and selective picking of antibody or protein secreting cells. The technology is based on quantitative fluorescence detection. The system is also compatible with direct fluorescence (e.g. GFP expression).

- → Automated delivery and lid removal of 1 well and 6 well micro-plate format plates to on board imaging station and dispersal of picked colonies to 96 well plates.
- \rightarrow Fluorescent imaging using a choice of five filter sets.
- \rightarrow Colonies selected on the basis of size, shape and proximity to neighbors.
- \rightarrow Colonies ranked based on fluorescent intensity of the colony or the secreted product.
- → Automated selective picking using high precision picking pins with proprietary cleaning routine between each pick.

Intuitive Windows[®]-based software allows you to easily set up multiple picking processes and analyze the results. For detailed information about the software please see the ClonePix 2 Software Application Manual.

Note: ClonePix 2 is strictly for research use only and is not intended or recommended for the diagnosis of disease in humans or animals.

If the robot is used in a manner not specified in this manual the protection provided by the equipment may be impaired.

Technical Specifications

External Dimensions and Weight

Size (excluding ancillary equipment): (height)

1010mm (width) x 900mm (depth) x 1495mm

Weight:

Robot - 350 kg (maximum) Compressor - 60 kg

Compressed Air Supply

Minimum Pressure:6 barMinimum volume:80L/min

Operating Environment

Indoor use only	
Temperature:	10°C to 40°C
Humidity:	20 to 80% non-condensing
Altitude:	Up to 2000M
Mains supply:	+/- 10% Rated Voltage
Transient overvoltage:	Installation Category (Overvoltage category) II
Rated pollution:	Pollution degree 2

Electrical Supply

European:			
Voltage:	210 - 250V AC 45-66 Hz single phase		
Power:	Robot - 1250W		
Connections:	IEC Input - Robot Main	s / Halogen Heater	
	IEC Output - PC		
	IEC Output - Monitor		
Fuses:	Input - F3 – T10A	- Robot / Halogen Heater	
	Output - F2 - T5A	- Computer and Monitor	
Compressor:	230V AC 50Hz 3.4A		

USA/Japan:

Voltage: Power:

Halogen Heater

Fuses:

Compressor:

100 – 125V AC 45-66 Hz single phase Robot -1250W Connections: IEC Input - Robot Mains / IEC Output - PC IEC Output - Monitor Input - F3 - T10A - Robot / Halogen Heater Output - F2 - T5A - Computer and Monitor 120V AC 60Hz 7.5A

Electrical Connections

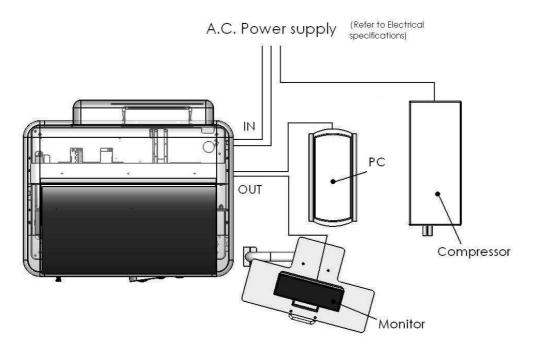


Figure 1: ClonePix 2 electrical connections

Symbols on Equipment

Symbol	Meaning
	Beware moving parts. Refer to Robot Manual for operating instructions.
2011	The year of manufacture is given on the serial number label which is located on the right side of the unit.
CE	European Economic Area (EEA) This mark on the product indicates compliance with the following EEC Directives: 2006/42/EEC, relating to The Supply of Machinery (Safety) Regulations 1992 2004/108/EEC with amendments 92/31/EEC, relating to Electromagnetic Compatibility 2006/95/EEC, the Low Voltage Directive

PC Connections

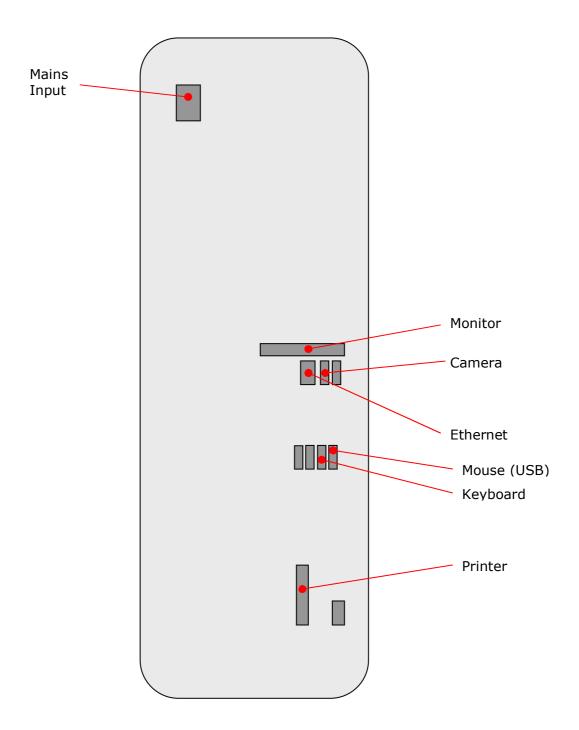


Figure 2: ClonePix 2 PC connections

Filter options

Filter set		Excitation		Emission	
Cat. No.	Suitable for:	Wavelength (nm)	Filter Position	Wavelength (nm)	Filter Position
X4990	CFP AF430	440 (Royal Blue)	4	505 (Cyan)	4
X4991 (standard)	CloneDetect FITC* Fluorescein GFP AF488	470 (Blue)	3	535 (Green)	3
X4992	YFP	500 (Cyan)	2	550 (Yellow)	2
X4993	CloneDetect 549* R-phycoerythrin Rhodamine AF546	530 (Green)	1	590 (Red)	1
X4994	CloneDetect 649* Cy5 FMAT Blue AF647	622 (Red)	6	700 (Far Red)	6

Notes:

- \rightarrow *Refer to <u>Reagents & Supplies</u> section for details on how to order CloneDetect reagents.
- \rightarrow White light imaging uses excitation filter position 7 and emission filter position 5.

Robot Enclosure

ClonePix 2 is designed to provide a controlled environment for sample protection conducive to accurate and reliable mammalian cell picking. Protection within the robot top cover is provided by utilizing a rear mounted HEPA filtered air supply and a front mounted exhaust fan. This creates a horizontal laminar flow of sterile air across the picking area of the bed of the machine whilst the front door is closed.

The main features are:

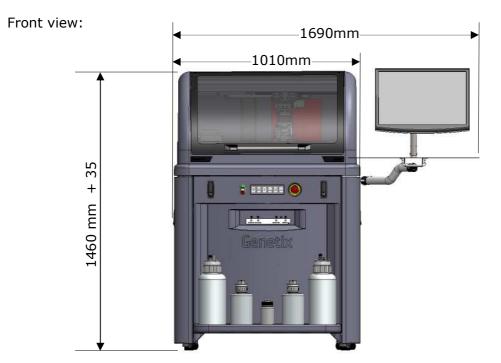
- → Class 100 type enclosure
- \rightarrow Offers product protection from particulates and microbes.
- → HEPA filtration (99.999% at 0.3 microns exclusion).
- \rightarrow Laminar airflow across the bed.
- \rightarrow Air inflow greater than air exhaust maintains positive air pressure.
- \rightarrow Always on when ClonePix 2 is on.
- \rightarrow Integrated UV germicidal lamp with timer.

Note: The ClonePix 2 robot enclosure does not offer operator protection.

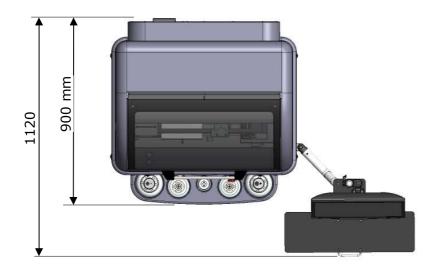
Installation

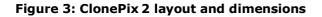
Note: Installation is to be undertaken only by Molecular Devices approved personnel.





Top view:





Source and Destination Plate Stackers

ClonePix 2 uses a stacker system to hold and process the source and destination plates.

The source stacker is located at the rear of the machine and the destination stacker towards the front. The stacker cassettes can accommodate up to 10 microplates. Plates must be loaded into the feed cassette with lids on and <u>well A1 facing towards the imaging station</u>. The plates are automatically fed onto the bed individually and the lid is removed as the plate is pushed through the lid lift mechanism. The lid is replaced as the plate returns through to the cassette.

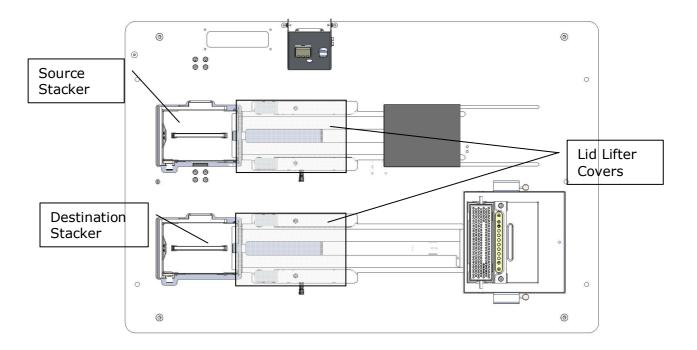


Figure 4: Stacker orientation

To insert the feed cassettes, push firmly into place until the cassette sits in the stacker.

Both the feed and return cassettes have a locking mechanism to secure them while in use. To insert the cassette, pull the lock outwards and lower the cassette into the stacker. Release the lock and check that the lock returns fully into place.



Warning – ENSURE THAT THE LID LIFTER COVERS ARE FITTED DURING NORMAL OPERATION

Connecting the Wash Bottles

The tubing carrying system fluid (sterile water) to the Wash Station and back to the waste bottles will be connected by a Molecular Devices approved engineer on commissioning of **ClonePix 2**. If it is necessary to disconnect these make sure that they are marked so that the tubes can be re-connected correctly. The outlets on the machine are in the same order as the bottles on the wash station.

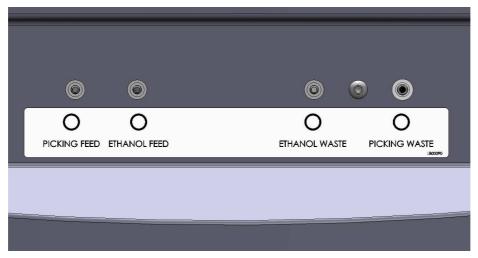


Figure 5: Wash bottle connections

Connecting the Ethanol Bottles

The tubing carrying ethanol to the Ethanol Wash Bath and back to the ethanol waste bottle will be connected by a Molecular Devices approved engineer on commissioning of **ClonePix 2**. If it is necessary to disconnect these make sure that they are marked so that the tubes can be re-connected correctly.

Camera Alignment

To guarantee the most accurate picking, it is crucial that the camera is calibrated and aligned correctly.

The aim of camera alignment is to achieve pin-to-spot accuracy, relating the image pixel coordinates with the robot x, y co-ordinates.

Calibration and alignment are performed before ClonePix 2 is dispatched and will be checked by a Molecular Devices approved engineer when the robot is installed.

If the actuator is subject to a knock or a crash, picking accuracy may be affected. Therefore it may be necessary to re-calibrate the camera. **This should be carried out by a Molecular Devices approved engineer.**

ClonePix 2 Panel



Figure 6: Front Panel

The lights on the panel indicate which elements of the robot are operating. The panel houses the **Start / Stop** button and the **Emergency Stop** button.

Health and Safety

Important Note

Before using the robot, it is very important that you have read this manual and have understood all the safety instructions.



Prior to using the robot, please make sure that you have been through the robot Pre-Power-Up checklist. This checklist ensures that all moving parts on the Robot are correctly situated in their datum positions.

Then follow the procedures as set out in the Power Up section.

Transport and Storage

The robot must be stored and transported in temperatures within the range -25° C to $+55^{\circ}$ C.

The machine is fitted with caster wheels for ease of movement.

Lifting Points

The machine should not be moved after installation. If relocation is necessary, standard lifting gear is adequate but should be undertaken only in the presence of a Molecular Devices approved engineer.

The robot should be moved into position using appropriate handling equipment such as forklift trucks or dolly trucks. The machine should be properly balanced on the forks prior to lifting.

Important note: Do not use any part of the exterior bodywork to lift the instrument as this may cause irreparable damage.

External Covers



Warning – If any of the external covers on ClonePix 2 are removed the power supply is not automatically interrupted. If it is necessary to remove any of the external covers it is essential that the power is switched off first. Do not attempt to use the robot until the covers are replaced.

Electrical Safety



The robot must be connected to a properly earthed power outlet to protect users from the risk of electric shock. The main chassis of the machine is earthed together with all associated electrical components.

Do not remove any of the fixed covers, as there are no user serviceable parts inside. All internal work should be referred to Molecular Devices approved service personnel.

In the event of a liquid spillage into the base cavity of the robot, the power supply must be disconnected at the mains before any attempt is made to clean up the spillage.

There should not be any spillage from the Wash Bath in normal use with the Wash Station correctly set up. However, spill trays have been fitted where appropriate.

Safety Features

Door:

The machine will not run with the front door open. If the door is open an electromagnetic switch prevents the machine from running. This switch should never be tampered with, as it serves two purposes:

- \rightarrow It prevents the motors from running and therefore the potential of any physical damage.
- \rightarrow It disables the UV light therefore preventing the risk of damage from UV radiation.

The door is made from acrylic, and so prevents UV light from passing through during operation.

UV Light:

ClonePix 2 is fitted with a UV germicidal lamp with timer. This is a 30W linear discharge lamp with a sharply defined output at 253.7nm making the lamp an efficient source of germicidal radiation.

Emergency STOP Button:

The location of the Emergency Stop button is shown in Figure 7 (Page 15). Striking the Emergency Stop button will **immediately** stop the machine and cut power from the drives. The button must be pulled out before the machine can be restarted.

Note: If the PC is powered from the robot, power to the PC will also be cut and current data will be lost.

Drive Safety



Be aware that the motors use high-powered magnets.

The linear drive units and encoders are delicate so great care should be taken with them. The Pre-Power-Up checklist should be followed before every routine in order to prevent serious damage to the robot or any of its constituent parts.

Power is removed from the drives when the doors are open.

Hot Air/Halogen Dryer

ClonePix 2 is fitted with an ultra-high temperature halogen dryer. The casing may become hot during the drying cycle.

Noise Levels

During normal operation the level of airborne noise emitted by the robot will not exceed 70db measured at a distance of 1 meter.

Service and Maintenance

It is strongly recommend that maintenance be carried out regularly by a Molecular Devices approved service engineer.

Maintenance contracts can be obtained from Molecular Devices (New Milton) Ltd. Please see contact details below.

The user is responsible for <u>General Maintenance</u> (page 21).

Initial Operation

System Features

ClonePix 2 is constructed within a welded steel framework. The outer measurements are given in the Technical Specifications section on page 6. Be aware that these dimensions do not allow for opening robot doors.

The lifting door on the front of the machine, protected by an electro-mechanical interlock, contains the working volume of the machine.

The bed of the machine contains the source and destination stackers, and the wash system, which is used for cleaning the Picking Pins between cycles in order to eliminate pin blockage and cross-contamination.

The stacker system can hold up to 10 standard microplates microplates.

The Z ball-screw drive carries a unique actuator system that accommodates the head. This system allows for easy exchange and set-up of the head.

Pre-Power-Up Checklist

- → Check that the Emergency Stop button (see Figure 7 on page 15) is released (pulled out). ClonePix 2 will not start if the button is pushed in.
- \rightarrow Check that no items have been left on the bed of the instrument.
- → Check that all motor tracks are free of obstruction.
- \rightarrow Check that there are no obstructions to head movement.
- Check that the main robot axes, XY, are roughly in the centre of their respective travel positions. The axes need to be away from their respective end-stops on 'start up' in order that they may reference themselves correctly to their 'home' positions.
- \rightarrow Check that the lifting door on the front of the robot is closed.
- \rightarrow Ensure that there are no plates or cassettes in the stackers.

Power-Up Procedures

- \rightarrow Turn on the power supply to the compressor.
- → Push the START button on the front panel. The red 'Power On' indicator (on the front panel of the instrument) illuminates. If the system fails to 'power on', it is likely that the lifting door is open or the emergency stop button is pushed in.
- \rightarrow Check that the air supply indicator (on front panel) is on.
- → Once the computer has booted up, click on the ClonePix 2 icon. The first time ClonePix 2 is used, the three axes will sequentially go through their 'Initialize drives' routine. This enables the drives to find their respective datum positions. ClonePix 2 must be allowed to complete this process without interference to ensure that there is no damage to the instrument or its ancillary equipment.

Shutdown procedure

- → Exit from the ClonePix 2 application by selecting Exit from the File menu on the main setup screen.
- → Close down Windows Click the Start menu at the bottom of the screen then click Shut Down. Wait for computer to switch off completely.
- \rightarrow Switch the ClonePix 2 by pushing the STOP button on the front panel.
- \rightarrow Turn the power off at the mains.

General Maintenance

Regular Maintenance

Daily

- \rightarrow Check that the robot interior is clean and free from any obstruction.
- → Ensure sterile water (feed) bottle is connected to the system fluid supply line. Empty the waste bottle every time that a new sterile water bottle is connected.
- → If the system has been sanitized, purge the system fluid supply line on first use to remove air bubbles that will have developed overnight. If there is doubt about sterility, run the 'Prepare for Pick Run' routine which includes a purge of the system fluid supply line with 70% ethanol or Sterilizing Agent (K8080). Sterilizing Agent (K8080) cannot be used in North America. This should be done no more than once a week.
- → During the purge, check that there are no air bubbles in the supply line coming from the sterile water bottle (indicates a fault in the connection), and that there are no leaks around the picking head (indicates a blocked pin). If there is a leak, or if a tube detaches from the head, replace blocked pin with a new one or follow the pin cleaning procedure below.
- → Fill ethanol wash bath with 70% Ethanol. For systems fitted with a replenishing ethanol wash bath, check that there are no kinks in the supply/waste tubing and then run through with 70% ethanol. Check that the bath fills with ethanol and that the waste ethanol flows freely into the waste bottle. On first use, it may be necessary to run through several times.

Weekly

- → In any week that ClonePix 2 is not used, it is important to purge the system fluid supply line with sterile water, and run 70% ethanol through the replenishing ethanol wash bath (if fitted). Failure to keep the pumps regularly primed may lead to system malfunction.
- \rightarrow Autoclave the picking pins.
- \rightarrow Check operation of interlock switch and emergency stop.
- → Check the compressed air filter for signs of moisture. If necessary, push the drain upwards to force moisture out.

Monthly

- \rightarrow Sanitize picking head and silicon supply pipes (picking tubing).
- → Sanitize source plate illumination cover.

Annually

→ Preventative Maintenance Service – only to be carried out by Molecular Devices approved personnel.

As Required

→ If the robot is used in particularly dusty conditions, the HEPA filter or pre-filter should be checked and may need to be replaced more frequently than recommended (by Molecular Devices approved personnel).

General Precautions

- → All wastes, e.g. ethanol, must be disposed of according to local regulation. Ethanol is flammable and should be handled accordingly.
- \rightarrow Do not use in explosive environments.
- → For safety reasons the front door interlock should never be interfered with or overridden. If the door is opened at any stage this will trigger the actuator head or germicidal lamp to stop until the door is closed.

Cleaning Procedures

Removable Parts

The design of ClonePix 2 permits all components coming into close proximity with biological material to be removed and cleaned:

Picking Head

The picking head can be regularly sprayed with 70% ethanol *in situ* and allowed to dry in the HEPA laminar air flow.

For more thorough cleaning use the following procedure:

- 1. Remove picking head from ClonePix 2 (see instructions below), and remove pins and picking tubing.
- 2. Submerge head into a beaker of 70% Ethanol.
- 3. Depress highest piston to allow interior to fill.
- 4. Leave 10 minutes.
- 5. In a sterile environment, lift head out of ethanol.
- 6. Depress highest piston to allow interior to empty.
- 7. Leave to air dry.
- 8. Replace picking head.

An alternative is to treat with Sterilizing Agent (K8080) as follows: (**Note:** This approach cannot be used in North America)

- 9. Remove picking head from ClonePix 2, and remove pins and picking tubing.
- **10.** Make up Sterilizing Agent (K8080) in 1 liter warm water.
- **11.** Submerge head into sterilizing solution.
- **12.** Depress highest piston to allow interior to fill.
- 13. Leave 10 minutes.

Do not leave head in Sterilizing Agent for longer than 10 minutes because acetic acid generated as a by-product may cause discoloration or deterioration of function

- 14. In a sterile environment, lift head out of sterilizing agent.
- **15**. Depress highest piston to allow interior to empty.
- 16. Submerge head into a beaker of sterile water.
- **17.** Depress highest piston to allow interior to fill.
- **18.** Lift head out of water.
- **19**. Depress highest piston to allow interior to empty.
- 20. Repeat steps 8-11 multiple times to remove Sterilizing Agent.
- 21. Hold head over an empty beaker and spray liberally with 100% ethanol including the interior.
- 22. Depress highest piston and ensure that the interior is well-drained.
- 23. Leave to air dry.
- 24. Replace picking head.

Important note: Autoclaving the head is not recommended as this can cause deterioration of function. Molecular Devices recommends that each project/group should have at least one picking head.

Picking Pins, O-rings and Pin Removal Key

The Picking Pins, O-rings and Pin Removal Key should be regularly cleaned and autoclaved using the following procedure:

- 1. Remove pins from picking head (see instructions below).
- 2. Make a 2% solution (50mls) of aQu Clean Pin Cleaning Solution (K2505) in a small plastic beaker.
- 3. Place all the items in the solution and sonicate for 10 minutes.
- 4. Rinse thoroughly in de-ionized water.
- 5. Place items in de-ionized water and sonicate for 10 minutes.
- 6. Autoclave pins, O-rings and pin removal key.

Recommended standard autoclave conditions are 121°C at 15 psi (103 kPa) for 20 minutes. Molecular Devices recommends that each project/group should have at least two sets of Picking Pins.

Picking Tubing

Picking Tubing (X4970) is the 8 silicon tubes that connect between the picking head and the actuator. These tubes can be cleaned along with the picking head, or can be autoclaved using standard conditions: 121°C at 15 psi (103 kPa) for 20 minutes.

Ethanol Wash Bath & Nylon Bristle Insert

The stainless steel Ethanol Wash Bath & Nylon Bristle Insert is filled with 70% ethanol in normal use.

If contamination is suspected the Wash Bath and Bristle Insert may occasionally be autoclaved using standard conditions: 121°C at 15 psi (103 kPa) for 20 minutes. Slight distortion of the nylon block following autoclaving is normal, and this can be forced back to straightness. However, a severely warped block must not be used as this may cause pin / robot damage.

Source Plate Illumination Cover

The Source Plate Illumination Cover can be removed and sprayed/wiped with ethanol or autoclaved as required using standard conditions: 121°C at 15 psi (103 kPa) for 20 minutes.

Wash and Waste Bottles

The Wash (Feed) Bottle must be sterile before use:

- 1. Fill the Wash Bottle with demonized water.
- 2. Replace cap but leave loose. Failure to do this may damage the bottle.
- 3. Seal the air inlet pipe with an autoclavable 0.2µm disposable filter.
- Autoclave using standard conditions: 121°C at 15 psi (103 kPa) for an appropriate time. The time required to ensure sterility will be dependent on the autoclave system used.

The Waste Bottle and Waste Vacuum Bottle can be sanitized if required. Bottle caps must be loose to prevent damage to the bottles.

Molecular Devices recommends that each project/group should have at least three Wash Bottles and one Waste Bottle. One Wash Bottle should be kept for Cleaning of System Fluid Supply with 70% ethanol or Sterilizing Agent (see instructions below).

Silicon Supply Tubes to Wash and Waste Bottles

Silicon supply tubes can be removed and autoclaved as required using standard conditions: 121°C at 15 psi (103 kPa) for 20 minutes.

Cleaning of Instrument Interior

For efficient decontamination of pathogenic micro-organisms, all non-removable parts within the ClonePix 2 top cover should be sprayed or wiped with Sterilizing Agent (K8080) or 70% Ethanol. Sterilizing Agent (K8080) cannot be used in North America.

Abrasive cleaners should not be used, as they will damage the surface of the bed.

ClonePix 2 can be left in a laboratory during formaldehyde vapor fumigation at an appropriate concentration.

Note: Excessive formaldehyde treatment will damage sensitive electrical and optical components.

Cleaning of System Fluid (Sterile Water) Supply

The cell collection process relies on the presence of non-expandable system fluid (sterile water) between the pumps and the picking pins. Only at the tip of the picking pins is a small amount of air (default 2μ I) drawn in from the sterile interior immediately prior to sample collection to separate the sample from the system fluid. The system fluid is fed in from a detachable wash bottle and the waste fluid is voided directly to the waste bottle. The system fluid is also used to purge out the picking pins between each cycle of picking (see Automated Picking Pin Cleaning below).

The Wash and Waste bottles are easily and securely changed using the quick-release connectors with check valves that close when the connection is broken. It is recommended to

liberally spray 70% ethanol onto the connectors before re-connection.

To ensure a sterile supply of system fluid, it is essential to regularly sanitize the system fluid lines using 70% ethanol or Sterilizing Agent (K8080) (Sterilizing Agent (K8080) cannot be used in North America.). Please follow instructions by running 'Prepare for Pick Run' in the software. The required frequency of sanitization is dependent on usage, but should ideally be conducted weekly (but no more than once a week), and after any period of non-usage.

Note: Never clean the ClonePix 2 system fluid lines with bleach.

Incoming Compressed Air Supply

Compressed air is required for the gentle picking action of the picking pins, and for controlling the movement of the illumination cover. The supplied oil-free compressed air unit draws air from the local environment and filters it to 0.02 microns using a 3-stage filtration process.

Automated Picking Pin Cleaning

The reusable Picking Pins are subject to repeated pin cleaning during a picking run. Specifically, they are cleaned prior to the first pick, between each cycle of picking, and at the end of the run. There are 4 components to the cleaning process:

- \rightarrow Unidirectional purge of sterile water to clean the internal bores of the picking pins.
- → Brushing of the picking pins in the Ethanol Wash Bath for effective mechanical cleaning of the pin exterior.
- \rightarrow Halogen dryer to heat the picking pins and remove residual ethanol.
- \rightarrow Air blower blows sterile air over the picking pins to cool them ready for the next pick.
- → The length of each of the above steps can be user defined according to the application but the halogen dryer should not be run for less than 10 seconds.

Note: The automated cleaning is not designed to replace the sterilization of picking pins by autoclaving.

Sterility Validation Tests

Described below are two typical sterility tests conducted on ClonePix 2.

1. Chamber sterility test

A ClonePix 2 was prepared using the cleaning procedures described above. A Trypticase Soy Agar* plate (BD Biosciences #221185) was placed open on the robot bed during a prolonged mammalian cell picking run. After 20 minutes the plate was covered, removed, and replaced with another open plate. This was done with a total of 6 plates to cover a 2 hour period. The plates were incubated at 37°C for 2 weeks to encourage any potential contaminant growth. After this period there was no contamination on any of the agar plates.

*A general purpose medium which supports the growth of fastidious and nonfastidious microorganisms.

2. Mammalian cell picking sterility test

A ClonePix 2 was prepared using the cleaning procedures described above. CHO-S cells were plated in chemically defined medium without antibiotics and rendered semi-solid using CloneMatrix (K8500). The plates were incubated at 37°C for 10 days to grow the cells into clonal colonies. Selected colonies (n=175) were picked using ClonePix 2 into 96 well plates prefilled with 200µl liquid medium without antibiotics. The picked cells were allowed to grow for 12 days, then replenished with fresh liquid medium without antibiotics, and then expanded to exhaustion (4 weeks). After 4 weeks there was no visible contamination in any of the wells.

Changing the Picking Head

Remove Head:

- → Click the Remove Head icon under Utility processes. This will move the head into the appropriate position.
- → Remove all of the picking tubing from the left-hand side of the head. A microplate lid may be placed under the picking head to collect any drops of water.
- → Undo the thumbscrew on the right-hand side, which secures the head to the actuator assembly, taking care not to lose the washer.
- \rightarrow Slide the head out of the actuator, being careful not to damage the pins.

Replace Head:

- → Click the Replace Head icon under Utility processes. This will move the head into the appropriate position.
- \rightarrow Slide the head into the actuator.
- \rightarrow Tighten the thumbscrew on the right-hand side to secure the head.
- → Fit the picking tubing to the tube connectors starting at the back and working forward, making sure that each tube is fitted to the correct connector.
- \rightarrow Follow the instructions to purge and align the pins.

Important note: When handling the picking head take care that the pins are not knocked. Damage to pins may produce unreliable results and can cause serious damage to ClonePix 2. If any pins are suspected to have incurred damage please replace them immediately.

Changing or Replacing Picking Pins

Occasionally the pins may need to be removed from the picking head for cleaning or to change to a different pin type.

Remove the pins from the head by unscrewing each one anti clockwise with the Pin Removal Key (X4948).

Prior to re-fitting, ensure that each pin has an O-ring (X1036) on the thread. Screw the pins back into the head clockwise using the Pin Removal Key just until resistance is felt. Avoid over-tightening.

Replacement Parts and Optional Extras

Please refer to the Molecular Devices website for the latest replacement parts and optional extras <u>www.moleculardevices/genetix.com</u>

Replacement Parts

Code	Description
X4941	Additional picking head populated with eight X4961 (F1 Pins)
X4942	Additional picking head populated with eight X4962 (F2 Pins)
X4949	Additional picking head without pins
X4961	F1 – ClonePix picking pin, 0.40mm diameter (Single pin)
X4962	F2 – ClonePix picking pin, 0.70mm diameter (Single pin)
X1036	O Rings for picking pins (Pack of 8)
X4970	Replacement picking tubing (Pack of 8)
X4948	Picking Pin Removal Key
X3445D	Cassette for stacker system
X4975F	5L Feed (wash) bottle & fixings
X4975W	5L Waste bottle & fixings
X4976	Nylon bristle ethanol bath insert

Reagents and Supplies

Please refer to the website for the latest reagents and supplies <u>www.moleculardevices/genetix.com</u>

CloneDetect Detection Reagents

Cat No.	Description	Pack Size
K8295	Recombination CloneDetect anti-human IgG detection agent. FITC labeled.	10000U / 1ml
K8200	CloneDetect anti-human detection agent. FITC label. Sterile, azide free.	10000U / 1ml
K8201	CloneDetect anti-human detection agent. FITC label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8202	CloneDetect anti-human detection agent, FITC label. Sterile, azide free, BSA free.	10000U / 1ml
K8210	CloneDetect anti-human detection agent. 549 label. Sterile, azide free.	10000U / 1ml
K8211	CloneDetect anti-human detection agent. 549 label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8212	CloneDetect anti-human detection agent. 649 label. Sterile, azide free.	10000U / 1ml
K8213	CloneDetect anti-human detection agent. 649 label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8215	CloneDetect anti-human CIF (Complex Initiation Factor)	10000U / 1ml
K8205	CloneDetect human IgG (Fc-Chain) specific. FITC label. Sterile, azide free.	10000U / 1ml
K8206	CloneDetect human IgG (Fc-Chain) specific. FITC label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8252	CloneDetect anti-human detection agent. Kappa chain specific. FITC label. Sterile, azide-free.	10000U / 1ml
K8250	CloneDetect anti-human detection agent. Kappa chain specific. 549 label. Sterile, azide-free.	10000U / 1ml
K8251	CloneDetect anti-human detection agent. Kappa chain specific. 649 label. Sterile, azide-free.	10000U / 1ml
K8255	CloneDetect anti-human detection agent. IgM specific. FITC label. Sterile, azide-free.	10000U / 1ml
K8253	CloneDetect anti-human detection agent. IgM specific. 549 label. Sterile, azide-free.	10000U / 1ml
K8254	CloneDetect anti-human detection agent. IgM specific. 649 label. Sterile, azide-free.	10000U / 1ml
K8220	CloneDetect anti-mouse detection agent. FITC label. Sterile, azide free.	10000U / 1ml
K8221	CloneDetect anti-mouse detection agent. FITC label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8230	CloneDetect anti-mouse detection agent. 549 label. Sterile, azide free.	10000U / 1ml
K8231	CloneDetect anti-mouse detection agent. 549 label. Sterile, azide free. In Atomizer applicator	10000U / 5ml

K8232	CloneDetect anti-mouse detection agent. 649 label. Sterile, azide free.	10000U / 1ml
K8233	CloneDetect anti-mouse detection agent. 649 label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8225	CloneDetect mouse IgG (Fc-Chain) specific. FITC label. Sterile, azide free.	10000U / 1ml
K8226	CloneDetect mouse IgG (Fc-Chain) specific. FITC label. Sterile, azide free. In Atomizer applicator	10000U / 5ml
K8235	CloneDetect anti-mouse CIF (Complex Initiation Factor)	10000U / 1ml
K8240	CloneDetect anti-rat detection agent. FITC label	10000U / 1ml
K8300	LiveDetect Green (494nm Ex/ 517nm Em). Sterile. In Atomizer applicator	50ul (for 5ml)

CloneMatrix

Cat No.	Description	Pack Size
K8510	CloneMatrix	1 x 40ml (100ml final media volume)
K8500	CloneMatrix	6 x 40ml (6 x 100ml final media volume)
К8530	CloneMatrix: optimized for CHO cells	1 x 40ml (100ml final media volume)
К8520	CloneMatrix: optimized for CHO cells	6 x 40ml (6 x 100ml final media volume)

CloneMedia / XPMedia

Cat No.	Description	Pack Size
K8610	CloneMedia (semi-solid media for hybridomas/myelomas)	1 x 90ml
K8600	CloneMedia (semi-solid media for hybridomas/myelomas)	6 x 90ml
K8685	CloneMedia-HEK (semi-solid media for serum-free HEK 293 cells)	1 x 90ml
K8680	CloneMedia-HEK (semi-solid media for serum-free HEK 293 cells)	6 x 90ml
K8710	CloneMedia-CHO (semi-solid media for CHO-s, CHOK1 and DG44 cells)	1 x 90ml
K8700	CloneMedia-CHO (semi-solid media for CHO-s, CHOK1 and DG44 cells)	6 x 90ml
K8740	CloneMedia-CHO-G (glutamine free semi-solid media for CHO-s, CHOK1 and DG44 cells)	1 x 90ml
K8730	CloneMedia-CHO-G (glutamine free semi-solid media for CHO-s, CHOK1 and DG44 cells)	6 x 90ml
K8725	CloneMedia-CHOK1SV (semi-solid media for CHOK1SV cells; glutamine-free)	1 x 90ml
K8720	CloneMedia-CHOK1SV (semi-solid media for CHOK1SV cells; glutamine-free)	6 x 90ml
K8712	CloneMedia-CHO DHFR (semi-solid media for DG44, DUXB11 and CHO-S cells)	1 x 90ml
K8742	CloneMedia-CHO DHFR (glutamine-free semi-solid media for DG44, DUXB11 and CHO-S cells)	1 x 90ml

K8775	CloneMedia-PER.C6 (semi-solid media for PER.C6 cells)	1 x 90ml
K8650	XP Media (liquid media for cell line expansion of hybridoma/myeloma cells)	500ml
K8750	XP Media-CHO (liquid media for cell line expansion of CHO-S cells)	500ml
K8760	XP Media-CHO-G (Glutamine-free liquid media for cell line expansion of CHO-S cells)	500ml

PetriWell Cell Culture Plates

Cat No.	Description	Pack Size	
W1000	PetriWell-1 Plate. Black with clear base. Polystyrene. TC treated	1/40	
W1005	PetriWell-1 Plate. Black with clear base. Polystyrene. Non TC treated 1/40		
W1007	PetriWell-1 Plate. Black with clear base. Polystyrene. Collagen coated	1/40	
W1009	PetriWell-1 Plate. Black with clear base. Polystyrene. Poly-D-Lysine coated	1/40	
W1050	PetriWell-1 Plate. Black with clear base. EquiGlass. TC treated	1/40	
W1055	PetriWell-1 Plate. Black with clear base. EquiGlass. Non TC treated	1/40	
W1055BC	PetriWell-1 Plate. Black with clear base. EquiGlass. Non TC treated. Bar-coded.	1/40	
W1057	PetriWell-1 Plate. Black with clear base. EquiGlass. Collagen coated	1/40	
W1059	PetriWell-1 Plate. Black with clear base. EquiGlass. Poly-D-Lysine coated	1/40	
W1100	PetriWell-6 Plate. Black with clear base. Polystyrene. TC treated	1/40	
W1105	PetriWell-6 Plate. Black with clear base. Polystyrene. Non TC treated	1/40	
W1107	PetriWell-6 Plate. Black with clear base. Polystyrene. Collagen coated	1/40	
W1109	PetriWell-6 Plate. Black with clear base. Polystyrene. Poly-D-Lysine coated	1/40	
W1150	PetriWell-6 Plate. Black with clear base. EquiGlass. TC treated	1/40	
W1155	PetriWell-6 Plate. Black with clear base. EquiGlass. Non TC treated	1/40	
W1155BC	PetriWell-6 Plate. Black with clear base. EquiGlass. Non TC treated. Bar-coded.	1/40	
W1157	PetriWell-6 Plate. Black with clear base. EquiGlass. Collagen coated	1/40	
W1159	PetriWell-6 Plate. Black with clear base. EquiGlass. Poly-D-Lysine coated	1/40	
W1500	PetriWell-96 Plate. Black with clear base. Polystyrene. TC treated	1/40	
W1505	PetriWell-96 Plate. Black with clear base. Polystyrene. Non TC treated	1/40	
W1507	PetriWell-96 Plate. Black with clear base. Polystyrene. Collagen coated	1/40	
W1509	PetriWell-96 Plate. Black with clear base. Polystyrene. Poly-D-Lysine coated	1/40	
W1510	PetriWell-96 Plate. Clear. Polystyrene. TC treated	1/40	
W1515	PetriWell-96 Plate. Clear. Polystyrene. Non TC treated	1/40	
W1515BC	PetriWell-96 Plate. Clear. Polystyrene. Non TC treated. Bar-coded.	1/40	
W1517	PetriWell-96 Plate. Clear. Polystyrene. Collagen coated	1/40	
W1519	PetriWell-96 Plate. Clear. Polystyrene. Poly-D-Lysine coated	1/40	
W1550	PetriWell-96 Plate. Black with clear base. EquiGlass. TC treated	1/40	
W1555	PetriWell-96 Plate. Black with clear base. EquiGlass. Non TC treated	1/40	

W1557	PetriWell-96 Plate. Black with clear base. EquiGlass. Collagen coated	1/40
W1559	PetriWell-96 Plate. Black with clear base. EquiGlass. Poly-D-Lysine coated	1/40

Other bar-coded plates are available. Please enquire.

Other

Cat No.	Description	Pack Size
K2505	aQu Clean pin cleaning solution	1L
K8080	Sterilizing Agent (1L per Sachet)	10
K8085	Sterilizing Agent (1L per Sachet)	50
K8150	CaliBeads: Fluorescent beads for ClonePix. 200µm diameter. Pan-wavelength.	100ml
K8010	Adherent Cell Picking Reagent – Type A	1 x 500ml
K8020	Adherent Cell Picking Reagent – Type B	1 x 500ml
K8030	Adherent Cell Picking Reagent – Type C	1 x 500ml
K8040	Adherent Cell Picking Reagent – Type D	1 x 500ml
K8005	Adherent Cell Picking Reagent Test Kit Types A – D	4 x 250ml
K8100	ProbeClean: Fluorescently labeled Antibody Clean-up Columns	50

Troubleshooting

Common Problems

Problem	Possible Solutions	
Machine will not turn on	 Check that main power switch is turned on and the emergency stop button is released (pulled out) 	
Computer will not start	- Check that PC power is turned on	
One or more of the axes won't move	 Check that the main power is turned on and the START button has been pressed 	
	 Manually move the actuator to the middle of the bed 	
	 Disconnect and reconnect control cable between PC and right side of ClonePix 2 (a small screwdriver may be necessary). Re-boot ClonePix 2 	
Interior light or UV germicidal lamp will not turn on	- Check that the bulbs are not burned out	
Picking alignment incorrect	 Ensure that pins are correctly aligned Check for any bent pins and replace if necessary 	
Silicon tubing becomes detached from pin during purge	- Pin may be blocked. Replace with new pin	
Water is leaking from picking head or actuator	- Pin may be blocked. Replace with new pin	
Poor picking results	- Air in system. Purge the supply line	
	- Check Z height of pin	
Not depositing	- Pin blocked – run Purge option or replace pin	
	- Dispense volume too low	
No liquid flow to Wash Station	- System fluid supply bottle is empty	
	- Check air supply and liquid pipes for kinks	
	 Check outputs in configuration are correct (consult Customer Support) 	
Wash Station overflowing	- Check no kinks in tubing to waste bottle	
	- Waste bottle full and/or vacuum pump broken	
Pins are catching on holes of the wash station	- Datum point not set accurately.	
	- Pins are damaged	
	- Pin fitted with 2 O-rings	
Water is leaking from top of pins	- Check each pin is fitted with one o-ring	
Pins not firing correctly	- Check that air compressor is working	
	 Pistons may have accumulated residue. Run Pin Fire Test for up to 1 hour. Do not undertake a Pick Run if pins not firing correctly 	
Illumination cover not opening closing correctly	- Check that air compressor is working	
No ethanol flow to Replenishing Ethanol Wash Bath	- Check no kinks in ethanol supply tubing	
	 Peristaltic pump tubing has become blocked (usually after period of inactivity) 	
Replenishing Ethanol Wash Bath overflowing	- Check no kinks in ethanol waste tubing	
	 Peristaltic pump tubing has become blocked (usually after period of inactivity) 	

Glossary of Terms

BARCODE

A unique label for source and destination microplates. Recommended barcoded plate types for colonies suspended in semi-solid medium are PetriWell-1 plates (Catalogue number W1055BC) and PetriWell-6 plates (Catalogue number W1155BC)

COLONY

A clonal group of cells, typically of mammalian origin, that have grown either adherently or suspended in semi-solid medium.

DATUM POINT

A series of X, Y, Z co-ordinates that define a set position on the Robot bed.

DESTINATION PLATE

96-well microplate(s) prefilled with liquid medium to collect picked colonies.

DISPERSAL

Dispersal Option separates out the cells of a picked colony by aspirating and dispensing in the destination plate.

EPI-ILLUMINATION

The white light option normally used for configuring the robot and for visualising colonies during picking, using LEDs located under the source plate being imaged.

EQUIGLASS

A high quality polymer used as the base material for PetriWell plates. It has excellent properties for optimal fluorescent imaging.

HALOGEN DRYER

Proprietary ultra-high temperature dryer used as part of the Pin Sanitise system.

IMAGE ACQUISITION

The capturing of images using pre-defined Acquisition Options.

IMAGE ARCHIVE

Default location for Pick Run and Imaging Run images and data. Accessible via Review Results.

LED INTENSITY

ClonePix2 uses only LEDs for consistent and reliable imaging. The intensity of the LEDs can be adjusted by the user to control image exposure under the Acquisition tab.

LIMS

Laboratory Information Management System.

LIVEDETECT

A reagent for visualising only live cells. Can be used as a fluorescent alternative to imaging colonies by white light. Catalogue number K8300.

.GFP FILE

Format used to save a Process.

.GFT FILE

Format used to save a Process Template.

INTERIOR LIGHT

Illuminates the interior of the instrument. Can be activated or deactivated at any time using the Interior Light icon in the bottom right corner of the screen. Interior Light is not used for imaging and will automatically switch off during imaging

PETRIWELL-1 PLATE

Single well plate optimized for visualizing plated colonies. The non-treated type (Catalogue number W1055) are recommended for colonies suspended in semi-solid medium. The TC-treated type (Catalogue number W1050) are recommended for colonies grown as adherent monolayers.

PETRIWELL-6 PLATE

Six well plate optimized for visualizing plated colonies. The non-treated type (Catalogue number W1155) are recommended for colonies suspended in semi-solid medium. The TC-treated type (Catalogue number W1150) are recommended for colonies grown as adherent monolayers.

PICKING PINS

Reusable stainless steel tools used to collect colonies. F1 pins have an internal tip diameter of $400\mu m$ and are for picking suspended colonies. F2 pins have an internal tip diameter of $700\mu m$ and are for picking adherent colonies (monolayers).

PRIMARY PIN

The picking pin nearest the front of the robot. All other pins are aligned relative to this one.

PRIME CONFIGURATION

The Acquisition Option that will be used for colony detection. Normally this is based on white light imaging.

PRIME PROBE

The type of illumination that will be used for colony detection. Normally, this will be white light by transillumination (trans). The Prime Probe is set up by creating a Prime Configuration.

PROCESS

A standard program for ClonePix 2 to carry out a task such as a series of similar experiments, or a maintenance task. A Process is saved as a .gfp file.

PROXIMITY INDICATORS

Red lines between colonies on the image that show the nearest neighbour for each colony. Note: This feature is not the same as the colony exclusion feature in Groups.

Remote Data Viewer

An optional software program (order code: SL4950-A01) for accessing ClonePix 2 results remotely. Please contact Customer Support for further information.

SEMI-SOLID MEDIUM

A viscous cell culture medium such as CloneMedia used to immobilize cells in suspension to permit growth of clonal colonies.

SOURCE PLATE

One- or 6-well microplate(s) containing colonies for picking.

STERILIZING AGENT

Recommended for sterilizing ClonePix 2 and its components and accessories. Catalogue number K8080. (Sterilization Agent (K8080) cannot be used in North America.)

TEMPLATE

A program from which Processes can be generated. A Template is saved as a .gft file.

TRANSILLUMINATION

The white light option normally used to detect colonies using LEDs positioned on the robot head.

WASH BOTTLE

4 liter bottle of sterile water used to purge the fluid system (1 liter in some older systems).

WASTE BOTTLE

4 liter bottle used to collect purged fluid (1 liter in some older systems).

WHITE LIGHT

Full spectrum LED illumination, of which there are two options: Transillumination (Trans) is normally used to detect colonies. Epi-illumination is normally used for configuring the robot and for visualising colonies during picking.

X DRIVE

Robot axis running from right to left.

XML

Extensible Markup Language. A simple text format derived from SGML Originally designed to meet the challenges of large-scale electronic publishing, XML is also used in the exchange of a wide variety of data on the Web.

Y DRIVE

Robot axis running from back to front.

Z DRIVE

Robot axis running vertically from high to low on the Robot bed.

Index

A

Accuracy	
Actuator	, 19
Air supply	. 19
Airborne noise	. 18

В

Ball-screw	19
Bath	16
Bed	19

С

Calibration	15
Calibration and alignment	
Cleaning	19
Compressed Air Supply	6
Compressed Air Supply:	
Connecting the Wash Bottles	
Cross-contamination	19

D

Dimensions	6,	8,	9,	12,	19
Door				17,	19
Drives				17,	19
Dryer					17

Е

Electrical safety	16
Electrical supply	
Emergency15, 17,	19

G

Germicidal	radiation	 	 17	

Η

Head19

Ι

Initial Operation	19
Installation11, 12,	16
Isolator switch	20

L

Lifting	9
Liquid spillage 16	5

Μ

Maintenance	18
Maximum	6
Mechanical interlock	19
Microplates	19

0

```
Operating environment ......6
```

Ρ

Pin(s)15,	19
Power outlet	16
Power-Up Procedures	19
Pre-Power-Up Checklist	
Power-Up Procedures	19

R

Remove	16
Reset button	15
Run	17

S

Safety Setup		
Setup screen		
Shutdown		20
Shutdown procedure		20
Source plate		13
Spillage		16
Stacker 13,	, 16,	19
System Features		19

Т

Technical Specifications	6
Travel	19

U

UV light	7
----------	---

V

Voltage	 	 	 	/
Volume	 	 	 6,	19

W

Wash bath1	9
------------	---

Wash station14, 16

Waste bottles 14	4
------------------	---

Contact Details

Molecular Devices

Queensway, New Milton Hampshire BH25 5NN, UK

Tel: +44 (0) 1425 624 600 Fax: +44 (0) 1425 624 700 Web: www.moleculardevices.com/genetix

For all technical queries please contact your nearest Customer Support group. Visit www.moleculardevices.com/genetix for latest contact details.

Trademarks

ClonePix[™], CloneSelect[™], CellReporter[™], *Half*BD®, '*N*Rich®, SlidePath[™], Data Arena[™] and Image Arena[™] are trademarks of Molecular Devices (New Milton) Ltd.

Copyright © 2012 by Molecular Devices (New Milton) Ltd

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

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

Revised August 2012