

User Manual for Setting Up and Loading Script Files on the iR-Processor

Automated Construction of TCR/BCR Immunorepertoire Library on the iR-Processor for Next-Gen Sequencing with the Illumina MiSeq



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The iR-Processor™ and iC-System™ components are covered by one or more US and worldwide patents pending. Arm-PCR is covered under US Patent 7,999,092 B2.

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Version Updates

Update Classification	Update Description	Version
Minor	Updated styling	V20200810



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Introduction

This document describes the process for setting up the iR-Processor™ for the first time and updating the iR-Processor™ with script files provided by iRepertoire Inc. in the event that new script files are available. The script files (or .hst files) will be provided as either a zipped file via e-mail or download through a web server. The purpose of the update will be provided with the accompanying files.

The goal of the iR-Processor[™] and iR-Cassette[™] product line is to provide the end-user a solution to amplify the immune repertoire in an automated and closed format. Furthermore, the amplified product, or library, is purified from primer-dimers in the cassette, removing the need for gel extraction of the primary amplification product. This goal is achieved by using the iR-processor[™] and the closed cassette. First, the user adds their template RNA with the RT-enzyme. Two PCR reactions are completed by the instrument and residual primer-dimers are removed by magnetic selection of the primary product. After amplification and primer-dimer removal, the cassette is removed from the iR-Processor[™], and the amplified library is eluted from the magnetic beads in the cassette by the user. The cassette is placed on an iRep magnetic stand (provided with the kit), and the library is pipetted into a clean PCR tube. The amplified immune library is directly ready for quantification, pooling, QC, and sequencing using the Illumina MiSeq next generation sequencing platform.



Safety

Safety Conventions Used in This Document

Please read this manual and become familiar with the information before installing or using the equipment. Do not perform procedures on the iR-Processor™ that are not specifically outlined in this manual, unless directed to do so by iRepertoire technical support.

Informational Notes and Warnings that Appear in this Manual

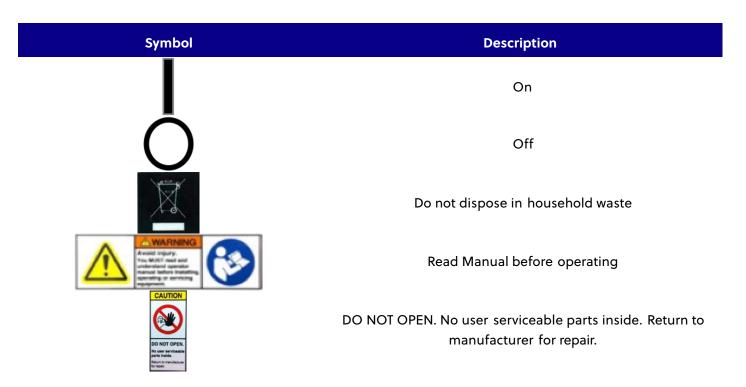
Note: A note provides general helpful information. No safety or performance issues are involved.

(!) Caution: This message is used in cases where the hazard is minor or only potential hazard is present. Failure to comply with the caution may result in potentially hazardous conditions.

(a) Warning: This message is used in cases where danger to the operator or to the performance of the instrument is present. Failure to comply with the warning may result in death or serious injury.

Symbols and Safety Labels on Instrument

These symbols describe warnings, cautions, and general information used in the operation of this instrument. Some of these symbols are further defined under "Safety Precautions."





Specific Safety Hazards

Read the following safety information before setting up or using the iR-Processor™ workstation. A user should be present during operation. This system contains electrical and mechanical components that if handled improperly, are potentially harmful. In addition, biological hazards may be present during system operation. Therefore, we recommend that all system users become familiar with the specific safety advisories below, in addition to adhering to standard laboratory safety practices. The protection provided by the equipment may be impaired or the warranty voided if the system is used in a manner not specified by the instructions or by iRepertoire™ Inc.

The iRepertoire ™ system is designed to be safe to operate when installed in accordance with these installation instructions and operated according to the iR-Processor™ User Manual. Installation hazards include:

- Electrical: (!) Caution: AC Power line voltages are used to power the iRepertoire™ System. Take all precautions
 when handling AC power line voltages and their interfaces.
- **Electrical:** When operated according to this manual, all heater elements are enclosed and do not pose a danger to the operator unless safety interlocks are defeated. Do not operate the system if the unit shows signs of external or internal damage.
- This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This
 device may not cause harmful interference, and (2) this device must accept any interference received, including
 interference that may cause undesired operation.
- Mechanical: When operated according to this manual, all moving parts and other mechanical hazards are
 enclosed and do not pose a danger to the operator unless safety interlocks are defeated. Do not operate the
 system if unit shows signs of external or internal damage.
- (a) Warning: No operator serviceable parts inside; refer servicing to a service trained personnel.
- (a) Warning: For continued fire protection, use specified ~line fuse.



Unpacking and Installation

Site Requirements

iRepertoire™ equipment must be located out of direct sunlight, away from heat sources and electrical interference. The site of installation should be free of excessive drafts, moisture, or dust and should not be subject to large temperature fluctuations.

See the packing materials for a summary of the unpacking steps.

Use a level workbench that is large enough and strong enough to accommodate the computer, iR-Processor™, Notebook and Kvaser Leaflight. Ensure the workbench is dry, clean, not subject to excessive vibration, and has additional space for accessories.

The iRepertoire™ system must be placed within approximately 1.5m of a properly grounded AC power outlet. The power line to the instrument should be voltage regulated and surge protected. The iR-Processor™ has an autoswitching power supply capable of operation at all standard line voltages (115 VAC – 220 VAC, 50-60 Hz, 460 W).

(□) Warning: To ensure that the iR-Processor™ is disconnected from AC power, the unit must be disconnected from the AC mains (power cord). Turning the power switch to the off position does not guarantee that the unit is electrically isolated from AC input power.

To ensure proper ventilation and to have easy access to the iR-Processor™ power switch, maintain a minimum clearance of 10cm at the rear of the iR-Processor™. The iR-Processor™ has additional ventilation at the unit bottom. Openings for ventilation must not be covered or constricted in any way.

Site Requirements

Note: The unit is heavy and it is highly recommended that two people perform this procedure.

Note: iRepertoire™ recommends that all packaging material be saved for possible reshipping of the unit.

Recommended additional equipment

Uninterruptible Power Supply (UPS): iRepertoire™ highly recommends using an uninterruptible power supply (UPS) to protect the system from power outages. Choose one that can provide 1050 Watts for at least 45 minutes. The UPS should be UL listed, CSA and CE marked or otherwise marked appropriately for your region when used internationally.

Surge Protector: If you do not use a UPS, use a surge protector. Choose a protector that meets your needs. Factors to consider include electrical environment, endurance, suppressed voltage rating, and method of protection. It should have six outlets, rated at least 1500 Watts, and be UL listed, CSA certified, and CE marked for non-domestic use or otherwise marked appropriately for your region when used internationally.





1) Place unit near final destination, with taped side up



2) Use scissors to cut banding



3) Remove packing tape. Do not slice with knife.



4) Open top of box



5) Locate all accessory boxes if applicable



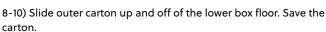




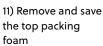




6-7) Remove and save any accessory boxes if applicable









12) Remove the packing tape without damaging the plastic protective



13) Slide the plastic bag down, exposing the unit.



14) iRepertoire™ recommends placing a piece of cardboard down on the work surface to help slide and position the unit once placed.



15) Tip unit back so as to get a front hand hold under the unit, then tip forward to get a rear hand hold



16) Using your legs, lift the unit straight up and out of the lower foam and box



17) Set the unit on the cardboard slide located on the work surface



18) Slide and position the unit to its final operating position and then remove the cardboard slide.



19) Save all packaging materials in case the unit needs to be returned to iRepertoire™



Familiarizing Yourself with the iR-System™

The iR-System™ consists of the iR-Processor™, Netbook and Kvaser Leaflight; it also includes all required interconnect and AC power cables as further described in this manual. Before installing the system, familiarize yourself with the following basic parts of the iR-Processor™:

Figure 1. iR-Processor™: front view and major components

- 1. iR-Cassette™ Slot 1
- 2. iR-Cassette™ Slot 1 abort push button
- 3. iR-Cassette™ Slot 2
- 4. iR-Cassette™ Slot 2 abort push button
- 5. iR-Cassette™ Slot 3 abort push button
- 6. iR-Cassette™ Slot 3

- 7. iR-Cassette™ Slot 4 abort push button
- 8. iR-Cassette™ Slot 4
- 9. iR-Processor™ front bezel
- 10. Magnetic door latch
- 11. Sliding dust cover door

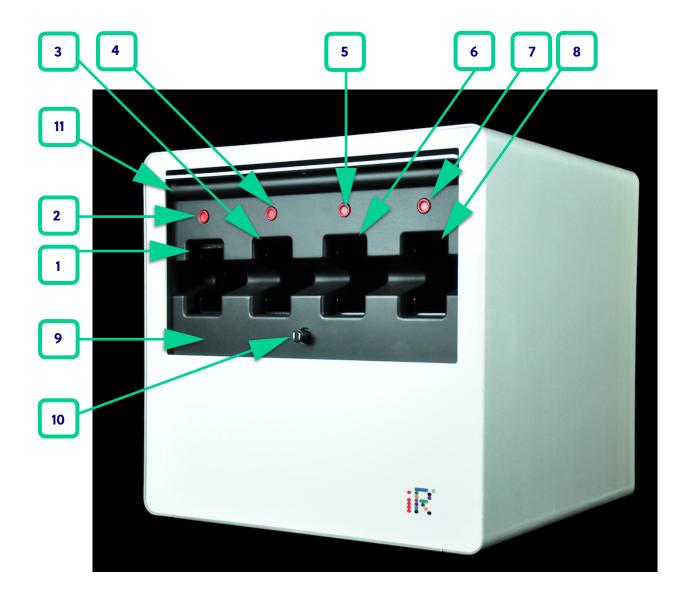
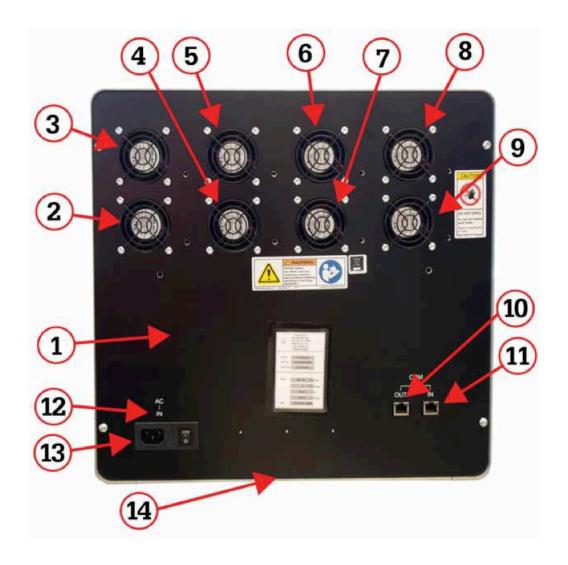




Figure 2. iR-Processor™: rear view and major components

- 1. iR-Processor™ back panel
- 2. iR-Cassette™ slot 4 exhaust fan 2
- 3. iR-Cassette™ slot 4 exhaust fan 1
- 4. iR-Cassette™ slot 3 exhaust fan 2
- 5. iR-Cassette™ slot 3 exhaust fan 1
- 6. iR-Cassette™ slot 2 exhaust fan 1
- 7. iR-Cassette™ slot 2 exhaust fan 2

- 8. iR-Cassette™ slot 1 exhaust fan 1
- 9. iR-Cassette™ slot 1 exhaust fan 2
- 10. Communications interface output connector
- 11. Communications interface input connector
- 12. AC power input connector label
- 13. AC power input connector
- 14. Bottom cooling air intake vents





Dust Cover Operation

iR-Processor™ includes a sliding dust cover door (identified as item 12 in the front view image) which can be retracted into the iR-Processor™ front bezel (Figure 1, item 9).

The processor has a magnetic door latch (Figure 1, item 10) that holds the sliding dust cover door closed when not in use and protects the iR-Processor™ from dust and debris.

To operate the door: Located on the door is a red circle. This circle identifies the location of the magnetic latch hidden behind the door. To open the door, press the red circle until the latch releases. Remove pressure and, while holding the door at the edges, rotate the door up and slide it back into the iR-Processor™ door storage slot. Once the door is slid back into the slot, you can release the door, and it will stay in place being held by the metal disk and front bezel. The metal disk is located on the back side of the door just behind the red circle.

To close the door, lift the front edge of the door, which is located in the door storage slot so the metal disk clears the front bezel. Pull the door straight out until reaching its internal stops. Rotate the door down so it comes into contact with the magnetic latch. Press in on the red circle until you feel the latch engage. The door is now closed and will help protect the iR-Processor™ from dust accumulation. During iR-Processor™ operation, the door can remain open or closed

Cleaning and Decontamination Instructions: The iR-Processor™ is designed in such a way that decontamination by the user will not be necessary. If a contamination event occurs please stop all activity on the affected unit immediately, seal the affected unit in a biohazard bag and contact iRepertoire technical support for further instructions.

To clean the exterior of the iR-Processor™, use a mild detergent mixed with water and wipe the exterior with a soft cloth. Avoid the use of bleach or ethanol, as these substances may cause harm or destruction of the iR-Processor™ components. Before cleaning any unit of the iR-System™, ensure that the unit is powered off and that all cords have been removed.

Do not clean the back panel, as damage could occur.

Do not clean the iR-Cassette™ slots on the iR-Processor™.

Ensure that the unit is completely dry before plugging in any cords and powering the unit back on.

Maintenance Procedures: The iR-System™ is designed in such a way that maintenance by the end user will not be necessary. If the iR-System™ does not operate as expected, contact iRepertoire technical support Department.



Disposal and Recycling Information: The symbol below indicates that the product must be disposed of properly according to local laws and regulations. When the product reaches the end of its useful life, contact local authorities to learn about recycling options. These are likely not home methods of disposal.



Setting Up the iR-Processor™

- Unbox the iR-Processor™ and place it upright on a sturdy bench top as indicated in the "Unpacking and Installing" section.
- 2. Plug the 115 V AC cable provided with the iR-Processor™ into the back of the iR-Processor™ and into an appropriate outlet (see Figure 1).
- 3. Turn the iR-ProcessorTM on by switching "I / O" power switch to the "on" position "I".
- 4. Once powered on, the blades will flash orange and then turn to green once they are ready to accept cassettes.
- 5. The iR-Processor™ is random access. This means that the blades run independently of one another and can be started at different times.
- 6. The iR-Processor™ comes pre-loaded with scripts and is ready to accept cassettes. The remaining instructions provided in this manual are only necessary in the event that the processor needs to be updated.
- 7. Do not run any cassettes or use the USB port for other purposes while updating the instrument.

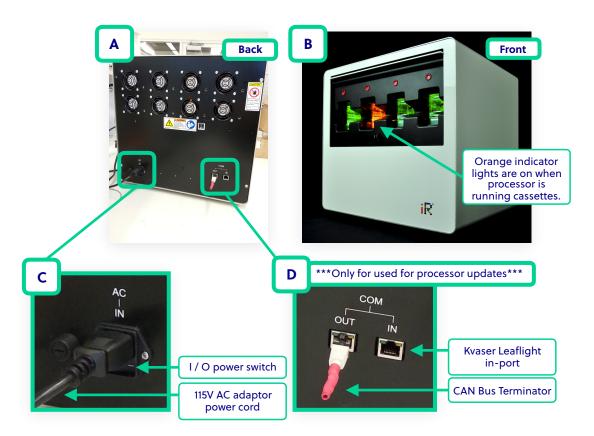


Figure 1: (1A) The back of the iR-Processor ™. (1B) The front of the iR-processor ™. Note: the orange indicator lights for operation. (1C) The 115V AC adaptor power cord and the I / O power switch. (1D) The Kvaser Leaflight in-port and CAN Bus Terminator port. Please see Figures 3 and 4 on page 14 for a detailed view of the Kvaser Leaflight cable.



Preparing the Netbook and .hst files

Method

- These instructions should only be followed in the event that iRepertoire has sent notification of an available update.
- 2. Unbox the netbook.
- 3. Plug the power module into 115V AC outlet.
- 4. Plug the power cord into the netbook.
- 5. Press the power button on the computer so that the computer boots up. The computer is running the Windows 10 Operating System. Allow any updates to install, although the computer does not need to be connected to internet to update scripts.
- 6. Log in with to the username: iRepertoire, and with the password: iRepertoire (if prompted, otherwise no username or password may be required).
- 7. Pre-installed on the computer is the File Transfer Utility software which will be used to load the script files onto the iR-ProcessorTM.
- 8. Once you login, you will see two main folders on the desktop (see Figure 2 below). The folder on the left is the "File Transfer Utility" application, on the right is the "iRepertoire Config Files" folder.
- 9. You were sent via e-mail, or you downloaded from our website a zipped file containing 1 .hst files. You may make a subfolder within the iRepertoire_Config_Files folder and place the .hst files here.
- 10. The single .hst file applies to all four blades and needs only to be loaded once onto the iR-Processor™ via the File Transfer Utility app (see "Using the File Transfer Utility Software to Load Scripts").







Figure 2: After logging into the netbook, two important items are on the desktop in the two black boxes. The folder on the right is the "iRep_Config_Files" folder. This is where you should place downloaded .hst files (after making a subdirectory for them). The application in the left black box is the pre-configured File Transfer Utility application required for updating the iR-ProcessorTM.



Connecting the Netbook to the iR-Processor™

- 1. Plug the Kvaser cable (Figure 3A and 3B) into the laptop using the USB cord on the cable.
- 2. The other end of the cable (an RJ45 connector) will plug into the 'COM IN' port (Figure 4) on the back of the processor.
- 3. Plug the red-tailed CAN Bus Terminator into the 'COM OUT' port. NOTE: No data transmission will happen if the CAN Bus Terminator is not plugged in!
- 4. Continue to the section "Using the File Transfer Utility Software to Load Scripts."

Figure 3: (3A) The red arrow indicates the USB connector to be plugged into the netbook. (3B) The Kvaser Leaflight.

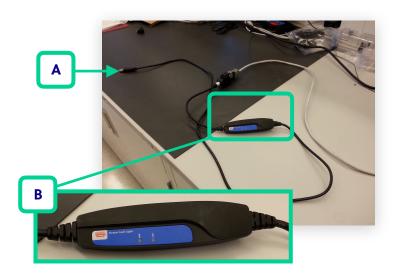
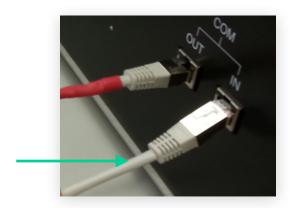


Figure 4: The red arrow indicates the Kvaser Leaflight-RJ45 connecter being plugged into the 'COM IN' port on the back of the processor. NOTE: the red-tailed CAN Bus Terminator should also be plugged into the 'COM OUT' port.





Using the File Transfer Utility Software to Load Scripts

- 1. Open the File Transfer Utility software by double clicking on the File Transfer Utility icon on the desktop (in the box on the left side—see Figure 2).
- 2. Click the "Script File Download" button.
- 3. In the new window, click the "Desktop" tab on the left side.
- 4. From the list that populates, choose the "iRep_Config_Files" folder.
- 5. Browse to the newly generated folder which contains the hst file provided by iRepertoire to be placed on the iR-Processor.
- Double click the new hst file. The upload of the file to the iR-Processor will begin and is represented by the status bar in the File Utility Transfer application. Once the file has finished loading a small window will pop-up to notify the user.
- 7. Repeat this process for any additional hst files provided by iRepertoire.
- 8. Once complete, the Kvaser and netbook can be disconnected from the iR-Processor and stored safely.
- 9. Restart iR-Processor™ by toggling the power on and off.
- 10. The iR-Processor™ loaded with the new script is ready for testing with a blank cassette labelled with the appropriate id indicated by iRepertoire.



How to Check the Newly Loaded Processor Script

- With the .hst file download, you should be notified as to which well the pipette in the cassette should puncture first.
- 2. Insert a test cassette with the correct CID label association.
- 3. The pipette should first move away from the user in order to initialize the cassette.
- 4. Once initialized, the cassette will move to the position designated by the script being run.
- 5. After it reaches this destination, you can turn on and off the processor to eject the cassette.
- 6. Assess the cassette using the numbering in Figure 5 to see if the pipette tip went to the correct well.

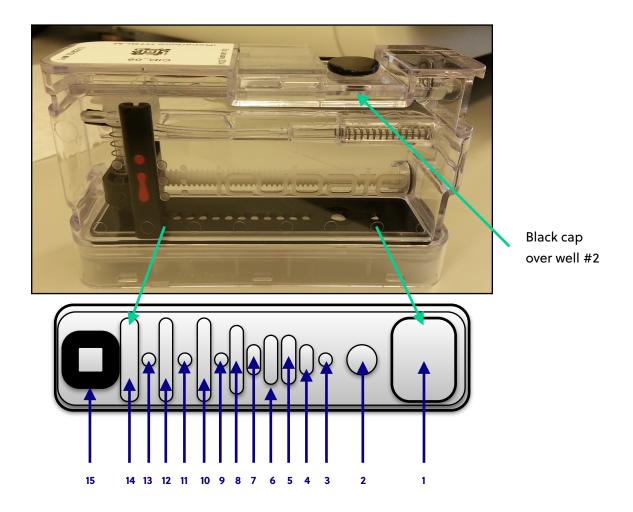


Figure 5: Well #2 is the PCR well where reagents are added in the cassette. For reference, the black cap is blocking well #2 and can be used for orientation. After inserting the cassette, the pipette will move towards well #1 (away from the user) and then to a well designated by the script.



Standalone iR-Processor Firmware Update

Purpose: To be used when notified of update in current firmware by iRepertoire, Inc.

Methods:

Note: This update procedure will require 2 persons. First, follow the instructions in **Section III. Connecting the**Netbook to the iR-Processor™. The Kvaser and netbook will be needed for this type of update.

- 1. Turn Off iR-Processor power
 - (1) Flip the iR-Processor AC power switch (located on the rear panel) to the OFF position.
- 2. Put the iR-Processor in firmware update mode
 - (1) On the iR-Processor front panel, press the Red eject/abort buttons above each slot simultaneously while switching ON the AC power to the iR-Processor. Two people will be required for this task in order to update firmware on all four blades at once.
 - (2) Note: If all four red buttons are not simultaneously pressed while the power is re-cycled on the processor the firmware will fail to update.
 - (3) When the iR-Processor is in firmware update mode, the slot LED will be a non-flashing Red color.
- 3. Open the file Transfer Utility
 - (1) Boot the laptop to the desktop login screen
 - (2) Click on the File Transfer Utility icon to start the application.
- 4. Select the *.hex file to download and start the firmware update process.
 - (1) Click on the Firmware Download button. This will open the directory path menu.
 - (2) Search the file directory path to locate the *.hex file to be downloaded. User's are requested to create and place this file in the "Firmware" folder. Double-click the selected firmware file and this will initiate the file transfer to the iR-Processor.
- 5. Monitor and verify file download
 - (1) As the file download proceeds, the slot lights of the iR-Processor will change color every few seconds (Red, Amber, Green, Black).
 - (2) The progress bar in the file Transfer Utility window should gradually advance from left to right.
 - (3) The entire firmware update process typically takes 3-4 minutes.
 - (4) Once the file transfer is complete, a "Done" pop-up message will appear on the menu screen and shortly thereafter the iR-Processor blades will reset to their initial operating state. The slot lights will turn steady Green.

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- 6. Once the update process is complete, the File Transfer Utility can be closed.
- 7. The Kvaser and netbook can be disconnected from the iR-Processor and stored safely.
- 8. Re-cycle the power of the iR-Processor. The update process is now complete. Continue to "Running a Diagnostic Cassette."



Running a Diagnostic Cassette

Purpose: To validate and diagnose proper working conditions for iR-Processor, scripts, and firmware.

Methods:

Note: This update procedure will require 2 persons. First, follow the instructions in **Section III. Connecting the Netbook to the iR-Processor**TM. The Kvaser and netbook will be needed for this type of update.

- 1. Insert diagnostic cassette labeled CID 15 into the processor.
- 2. The pipette should first move away from the user in order to initialize the cassette.
- 3. Once initialized, the cassette will move to well position number 5 and pause for 5 seconds.
- 4. Assess the cassette using the numbering in Figure 5, page 17 of manual, to see if the pipette tip went to the correct well.
- 5. After the 5 second pause, the diagnostic loop script will begin and continue to run for 13.5 minutes.
- 6. Diagnostic Loop will complete with pipette tip at well #8.
- 7. Assess the cassette using the numbering in Figure 5, page 17 of manual, to see if the pipette tip went to the correct well.
- 8. Diagnostic/Validation Step is now complete and processor is ready for use.



Appendix A: Additional Terms and Conditions

Standard Terms and Conditions for Use of Product

By opening the packaging containing this product ("Product") or by using such Product in any manner, you are consenting and agreeing to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return the Product for a full refund prior to using them in any manner.

Acceptance

ALL SALES ARE SUBJECT TO AND EXPRESSLY CONDITIONED UPON THE TERMS AND CONDITIONS CONTAINED HEREIN, AND UPON RECIPIENT'S ASSENT THERETO. NO VARIATION OF THESE TERMS AND CONDITIONS SHALL BE BINDING UPON IREPERTOIRE ("PROVIDER") UNLESS AGREED TO IN WRITING AND SIGNED BY AN AUTHORIZED REPRESENTATIVE OF PROVIDER. For purposes of this agreement, "RECIPIENT" shall be the party to whom Provider sells or provides Product. Recipient, by accepting the Product shall be deemed to have assented to the terms and conditions set forth herein, notwithstanding any terms contained in any prior or later communications from Recipient and whether or not Provider shall specifically or expressly object to any such terms.

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Appendix B: Hardware Specifications

iR-System™ Hardware Specifications:

iR-Processor™

Processor weight approx. 102 lbs.

* * Contains processing slots

AC input requirements: 100-240 VAC, fused @ 5 amps, 50-60Hz Single Phase

Physical dimensions: 17 inches W x 17 inches D x 17 inches H

Heater operating range: 35°C to 95°C nominal

Kvaser Leaf Light™

The Kvaser Leaf Light (product number 73-30130-00685-0) is in conformity with the essential requirements of the following regulations and directives:

- DIRECTIVE 2012/19/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 July 2012 (WEEE)
- REGULATION (EC) No. 1907/2006 (REACH), Annex XIV (the 'Candidate list') and Annex XVII ('Restriction of Substances')

The Kvaser Leaflight (product number 73-30130-00685-0) also complies with DIRECTIVE 2011/65/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 8 June 2011 (RoHS) and is in conformity with EN 50 581 (2012: Assessment with respect to restriction of hazardous substances). The product listed above also complies with DIRECTIVE 2004/108/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 15 December 2004 (EMC-directive) and is in conformity with EN 55 022 (2010: Class B, radiated. IT equipment), commercial emission and EN 55 024 (2010: IT equipment, commercial immunity).

iRepertoire™ Operating System Environment

Indoor use only

Operating temperature: 15°C to 30°C (59°F to 86°F)

Humidity: 20%-80%, noncondensing

Altitude: Operation up to 2400 m (7874 ft.) above mean sea level

Shipping and storage: The allowable shipping and storage temperature and humidity ranges are 0°C to + 50°C and

20-80% noncondensing, respectively.



Appendix B: Hardware Specifications

iRepertoire Inc.

800 Hudson Way, Suite 2304, Huntsville, AL 35806. (256)-327-0948 iR-System™: User Manual Rev. August 2016

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