Helaxy NGS Fluidic Prep Device
User Manual

Apr 2024

For Research Use Only

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY, AND WILL VOID ANY WARRANTY APPLICABLE TO THE PRODUCT(S).

HELAXY DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE).

Table of Contents

1 General Information

1.1 Operation

Intended Product Use

The Helaxy NGS Fluidic Prep Device allows for sample preparation of various sample types such as nasopharyngeal swabs, plasma, FFPE and stool samples with the Helaxy Fluidic Prep Card for DNA/RNA purification and NGS library preparation. The device is intended for Research Use Only (RUO) and not for in-vitro diagnostics applications.

Information on Product Liability

The protection provided by the device may be impaired and the liability for the function of the device may be passed to the operator if:

- The device is not used in accordance with the operating manual and conditions.
- The device is used outside the range of application described in the operating manual.
- The user made unauthorized service or modifications to the device.
- The user used a detachable power cord of inadequate rating which is not supplied by the manufacturer.

1.2 Safety Guidelines

1.2.1 Symbols on Instrument

Electrical Symbols

Symbol	Description	Symbol	Description
	Indicates the **On** position of the main power switch.		Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates the **Off** position of the main power switch.		Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.

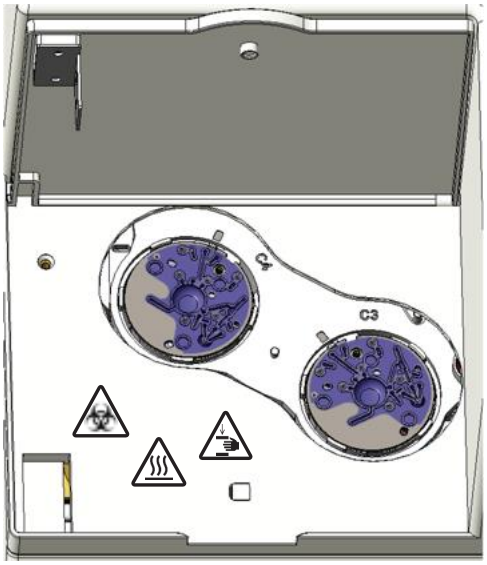
Safety Symbols

The following table describes the safety symbols that may be displayed on Helaxy instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of biohazard waste and product and to proceed with appropriate caution.
	Indicates the protective conductor terminal on the device

Locations of Warnings

The Helaxy NGS Fluidic Prep Device contains warnings at the locations shown below:



1.2.2 General Instrument Safety

PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Helaxy may result in personal injury or damage to the instrument.

SAFETY PRECAUTION.

STRICTLY NO NAKED FLAME allowed in the **VICINITY** of the device.

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs).

PHYSICAL INJURY HAZARD. Use this instrument as specified by Helaxy. Using this instrument in a manner not specified by Helaxy may result in personal injury or damage to the instrument.

Cleaning or Decontaminating the Instrument

Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Servicing the Instrument

Only authorized service personnel from Helaxy shall be allowed to perform service and repair of this instrument:

- Received required service training and certification by Helaxy.
- Read and understood all applicable service procedures and safety requirement.

- Ensure that the device is kept upright during lifting and installation.
- Unplug all electrical cables before lifting device from the base as support.

1.2.3 Chemical Safety

Chemical Hazard Warning

CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

Obtaining MSDSs

The MSDS for any chemical supplied by Helaxy can be obtained online at https://helaxy.com

Chemical Safety Guidelines

To minimize the hazards of chemicals:

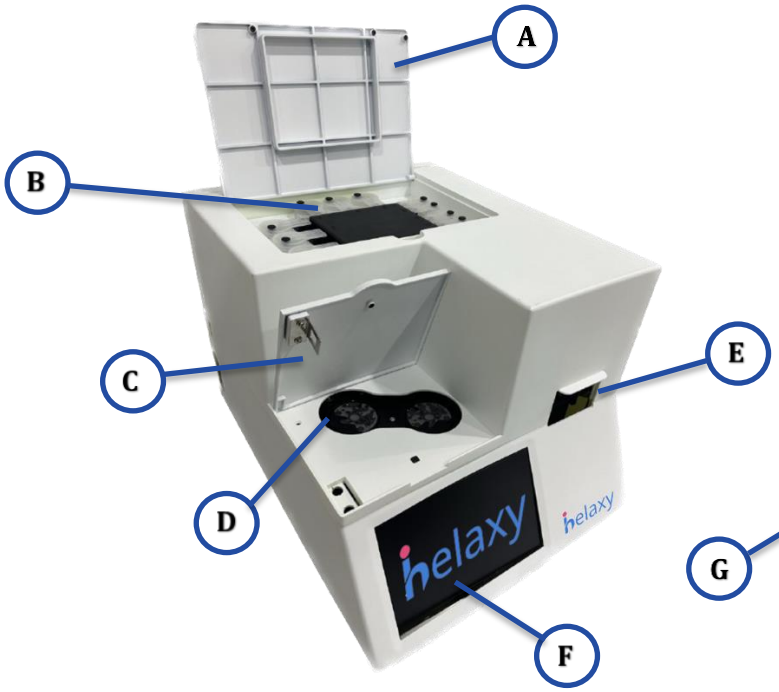
- Read and understand the MSDSs provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

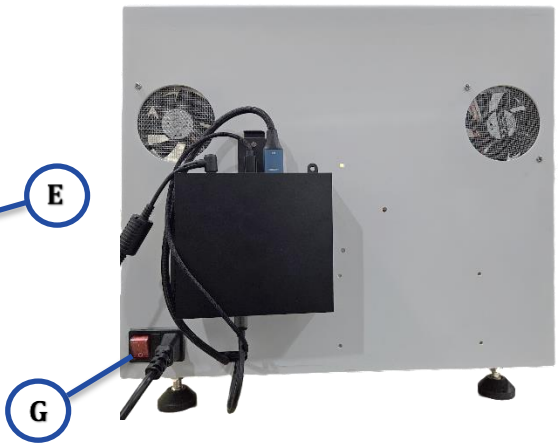
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations

2 Introduction

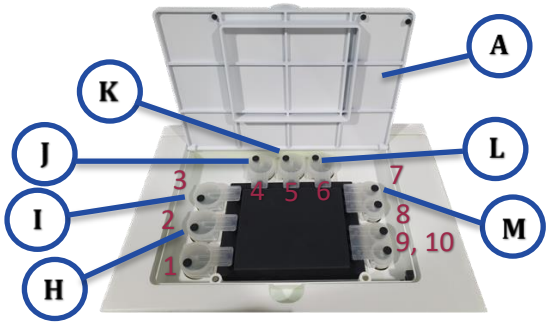
Helaxy NGS Fluidic Prep Device combines proven of next generation sequencing (NGS) technology with an automated revolutionary workflow that lets you go from DNA to analyzed data in as few as thirteen hours with ten minutes of hands-on time.

2.1 Helaxy NGS Fluidic Prep Device

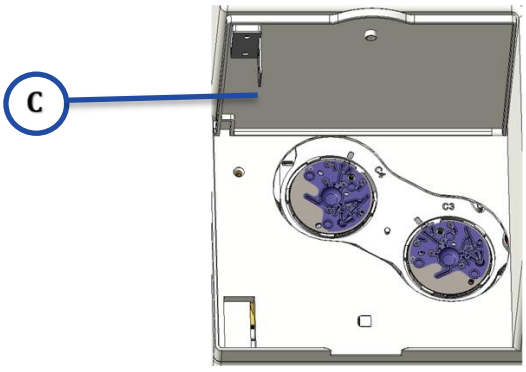




Reagent Warehouse



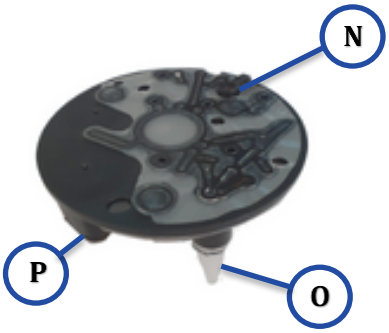
D Fluidic Card Container



- **A** Top Cover
- **B** Reagent Warehouse
- **C** Card Cover
- **D** Fluidic Card Container
- **E** Waste Tray
- **F** Touchscreen User
- **G** Power Switch
- **H** Buffer 2
- **I** Buffer 3
- **J** Buffer 4
- **K** 80% Ethanol
- **L** Nuclease-free Water (NFW)
- **M** Buffer 7

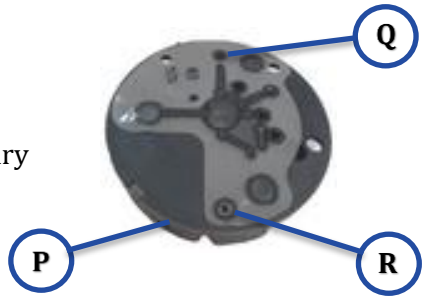
2.2 NGS Library Prep Card

- **N** Sample Port
- **O** PCR Tube
- **P** Waste Chamber



2.3 Size Selection & Normalization Card

- **Q** Input: NGS Library
- **R** Output: Clean NGS Library



2.4 Technical Specifications

Power Supply	
Voltage	100 to 240V ± 10%
Fuses	30A DC
Current Consumption	<30A
Frequency	50Hz to 60Hz ± 5%
Power Consumption	750W
Overvoltage Category	105% ~ 125% rated output power
Protection Class	Constant current limiting, recovers automatically after fault condition is removed
Degree of Pollution	2
Ambient Conditions	
General operation	15 ºC to 25 ºC indoors 55% to 75% relative humidity Up to 2000m above sea level
Storage Conditions	15 ºC to 35 ºC 55% to 75% relative humidity
Weight/Dimensions	
Device (W x D x H) mm	450 x 550 x 450
Weight	18kg
Interfaces	
USB	USB C x 2
HDMI	HDMA x 1

3 Installation, Service and Maintenance

3.1 Installation

Installation of Helaxy Fluidic Prep Device must always be carried out by service personnel of Helaxy Pte. Ltd. Or Helaxy service and distribution partner.

3.2 Delivery Package

The below accessories are included in the standard scope of delivery and required for installation and operation of the device.

Description	Quantity
1. Helaxy Fluidic Prep Device with Mini PC-mounted	1
2. NFW Containers	5 Medium & 1 Small
3. Waste Tray	1
4. Waste Container	1
5. Device Power Cables (compatible to the country where device is installed, UK:US Adaptor is provided)	1
6. Mini PC Power Supply Cord	1

3.3 Deinstallation and Disposal

Deinstallation of Helaxy Fluidic Prep Device must be always be carried out by service personnel of Helaxy Pte. Ltd. Or Helaxy service and distribution partner.

In the event the product is to be disposed of, the relevant legal regulations are to be observed. The disposal of electrical devices is regulated based on EU Directive 2002/96/EC pertaining to waste electrical and electronic equipment. The device may not be disposed of in municipal or domestic waste.

Contact the manufacturer for appropriate return and disposal of device.

Manufacturer Information	Helaxy Pte. Ltd. 16 Ayer Rajah Crescent #03-02 Singapore 139965

3.4 Cleaning and Decontamination

It is recommended that the Helaxy NGS Fluidic Prep Device to be washed if it has been idle for more than 7 days.

Follow the on-screen instructions for routine fluidic cleaning procedure.

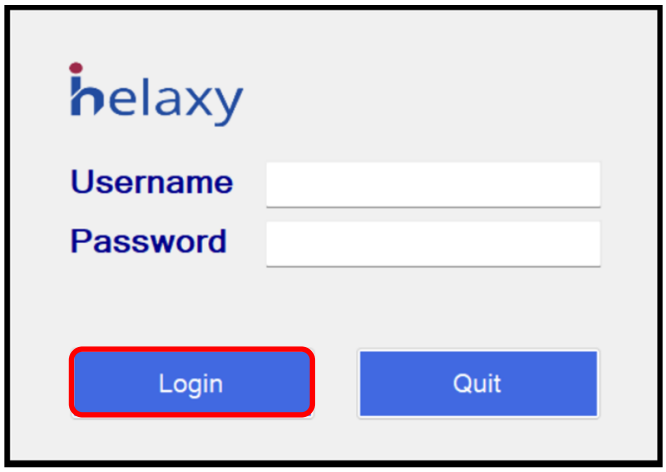
For decontamination, it is recommended that the Helaxy NGS Fluidic Prep Device be wiped down with 70% ethanol or 1% hypochlorite solution. During maintenance, the shell of the device is to be removed and the rotary table and adapters are exposed.

4 Operation of the Instrument

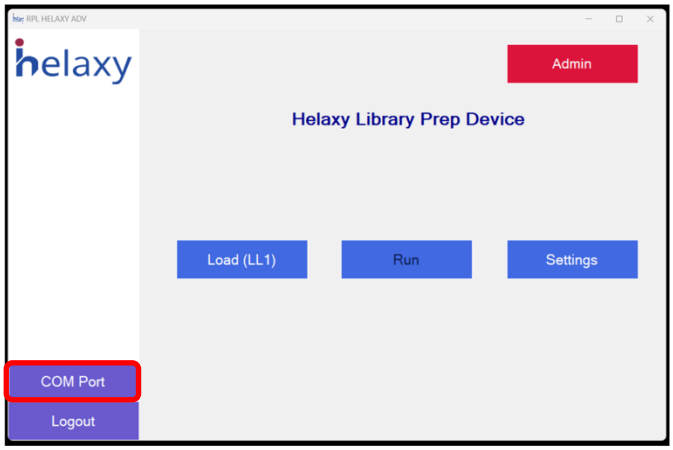
4.1 Pre-run Preparation

4.1.1 Logging into the software

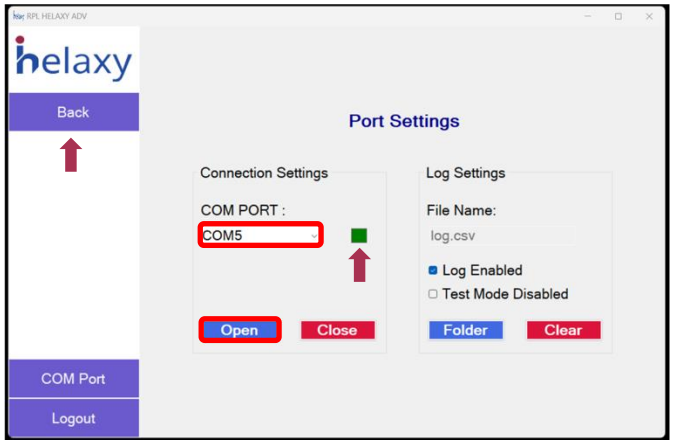
1. Enter Username and Password provide by Helaxy and click **Login**.



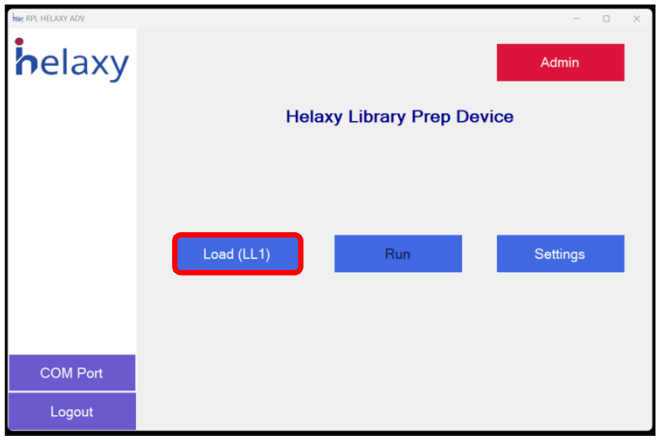
2. Click **COM Port**.



3. Select **COM5** and Click **Open**. Square White Box should change to Green once connected. Click **Back** to return to the Main Menu.

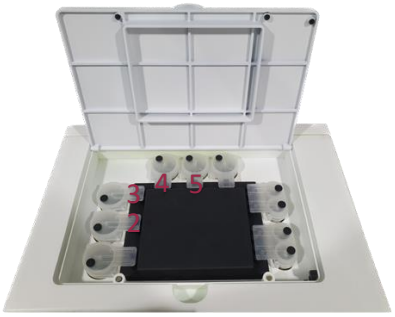


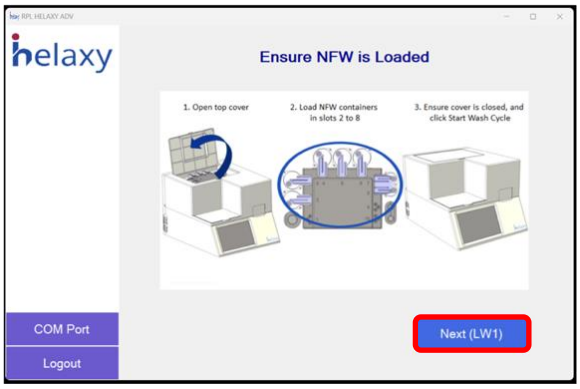
4. Click **Load** to begin the Pre-run Wash.



4.1.2 Pre-run Wash

1. Container loading instructions:
    a. Open the Top Cover.
    b. Take out the washing containers from the spot number 2, 3, 4, 5.
    c. Before first use, fill up the washing containers with 30mL of NFW.
    d. Check the level of NFW in the washing containers is above the minimum level on the reagent cartridge holder. If below the minimum line, add 30mL of NFW.
    e. Load the filled washing containers into the Reagent Warehouse on the spot number 2, 3, 4, 5.





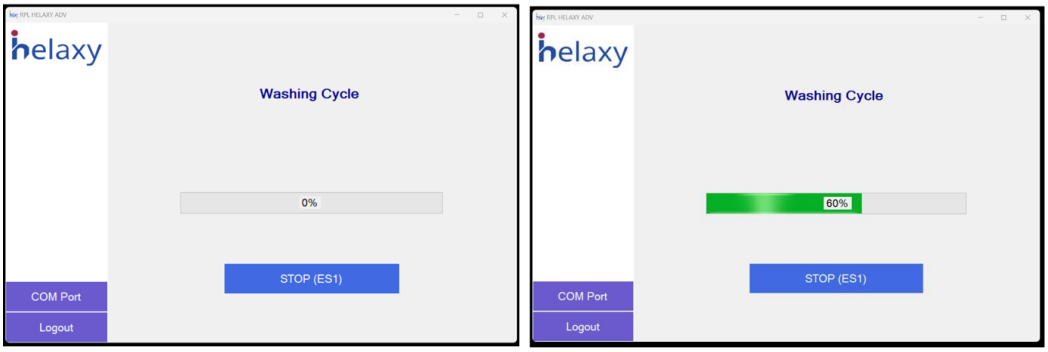
    f. Ensure that the Top Cover is closed.
    g. Click **Next** to begin the Washing Process.

NOTE: Ensure that the volume of NFW in the washing containers is above the minimum line on the reagent cartridge holder.

2. The washing cycle still start upon clicking Next. The status bar indicates the progress of the washing cycle which takes approximately 15 minutes to complete.

   Clicking ‘Stop’ button would abort the washing process.

   Once you stop the cycle, you will return to the main menu.



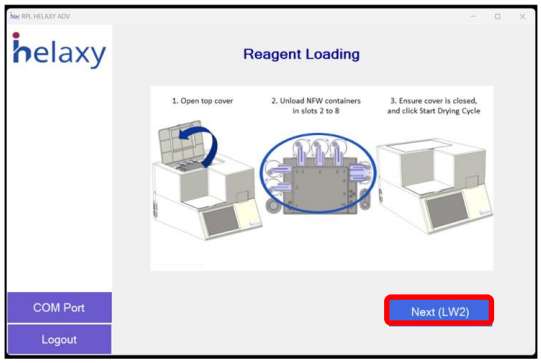
3. Once the Washing Cycle is finished,

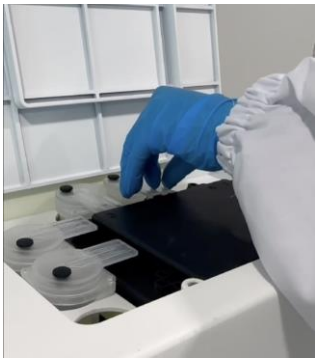
   click **Load Reagent** to proceed to the Drying Cycle.

4. Remove all Washing Containers from the reagent docking station at position 2, 3, 4 and 5.

   To store the washing containers after all the Wash Cycle, the user may use the cardboard insert that is provided.

   Click **Next** to proceed to the Drying Cycle.

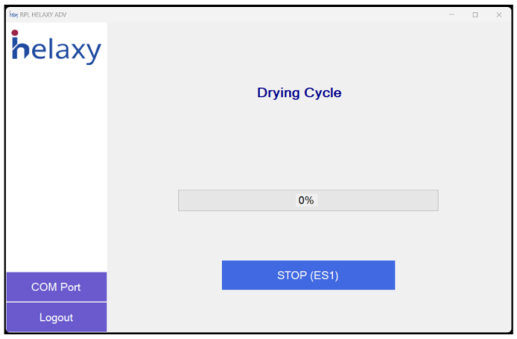


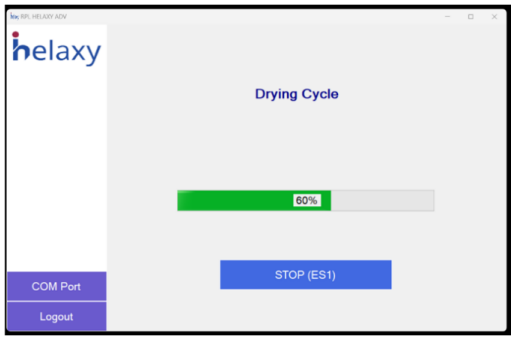


5. The Drying Cycle will start upon clicking Next. The status bar indicates the progress of the drying cycle which takes approximately 15 minutes to complete.

   Clicking 'Stop' button would abort the washing process.

   Once you stop the cycle, you will return to the main menu.





6. Once the Drying Cycle is finished,

   click **Load Reagent** to start the Run Set-up process.

4.2 Setting up a run

4.2.1 Preparation of Device Working Solutions before first use

1. Buffer 2

    a) To reagent container: Add 24mL of absolute ethanol (molecular biology grade).

Remove the cap of the cartridge

Add the absolute ethanol

    b) Cap tightly and swirl to mix.

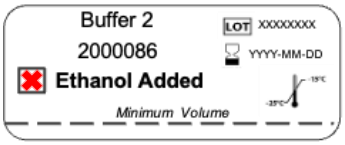
Reached the max. level

Final volume of the solution

    c) Ensure container is upright at all times.

    d) Store at room temperature.

    e) Cross on • of "Ethanol Added" and indicate date of addition of Ethanol.



f) The volume of reconstituted Buffer B2 is sufficient for 10 Fluidic Preps or up to 3 months.

g) Onboard stability experiment is ongoing. Currently, Buffer 2 has passed the 1-month mark.

2. Buffer 3

a) To reagent container: Add 8.8mL of absolute ethanol (molecular biology grade).

Remove the cap of the cartridge

Add the absolute ethanol

b) Cap tightly and swirl to mix.

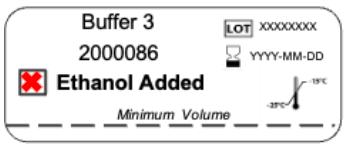
Reached the max. level

Final volume of the solution

c) Ensure container is upright at all times.

d) Store at room temperature.

e) Cross on • of "Ethanol Added" and indicate date of addition of Ethanol.



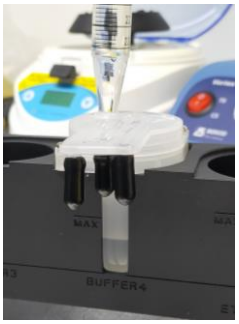
f) The volume of reconstituted Buffer B3 is sufficient for 10 Fluidic Preps or up to 3 months.

g) Onboard stability experiment is ongoing. Currently, Buffer 3 has passed the 1-month mark.

3. Buffer 4

a) To reagent container: Add 8.8mL of absolute ethanol (molecular biology grade).

Remove the cap of the cartridge



Add the absolute ethanol

b) Cap tightly and swirl to mix.

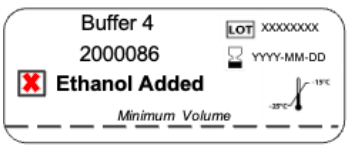
Reached the max. level

Final volume of the solution

c) Ensure container is upright at all times.

d) Store at room temperature.

e) Cross on • of "Ethanol Added" and indicate date of addition of Ethanol.

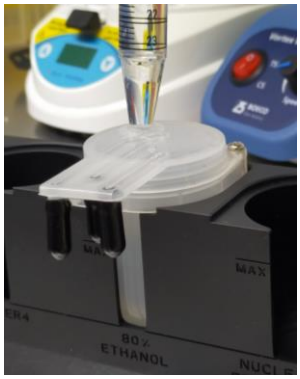


f) The volume of reconstituted Buffer B4 is sufficient for 10 Fluidic Preps or up to 3 months.

g) Onboard stability experiment is ongoing. Currently, Buffer 4 has passed the 1-month mark.

4. 80% Ethanol

a) To empty container: Add 9mL of NFW + 36mL of absolute ethanol (molecular biology grade).



Add NFW and absolute ethanol

b) Cap tightly and swirl to mix.

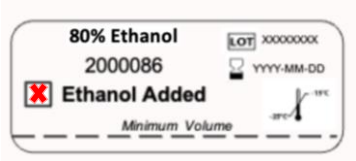
Reached the max. level

Final volume of the solution

c) Ensure container is upright at all times.

d) Store at room temperature.

e) Cross on • of "Ethanol Added" and indicate date of addition of Ethanol.



f) Onboard stability experiment is ongoing. Currently, 80% ethanol has passed the 1-month mark.

5. Nuclease-free Water (NFW)

a) To empty container: Add 50mL of NFW.

Add NFW

Final volume reached the max. level

b) Cap tightly and ensure container is upright at all times.

c) Store at room temperature.

6. Washing Containers (Buffer 2, Buffer 3, Buffer 4, 80% Ethanol)

a) To empty container: Add 30mL of NFW.

b) Cap tightly and ensure container is upright at all times.

c) Store at room temperature.

4.2.2 Reagent Loading

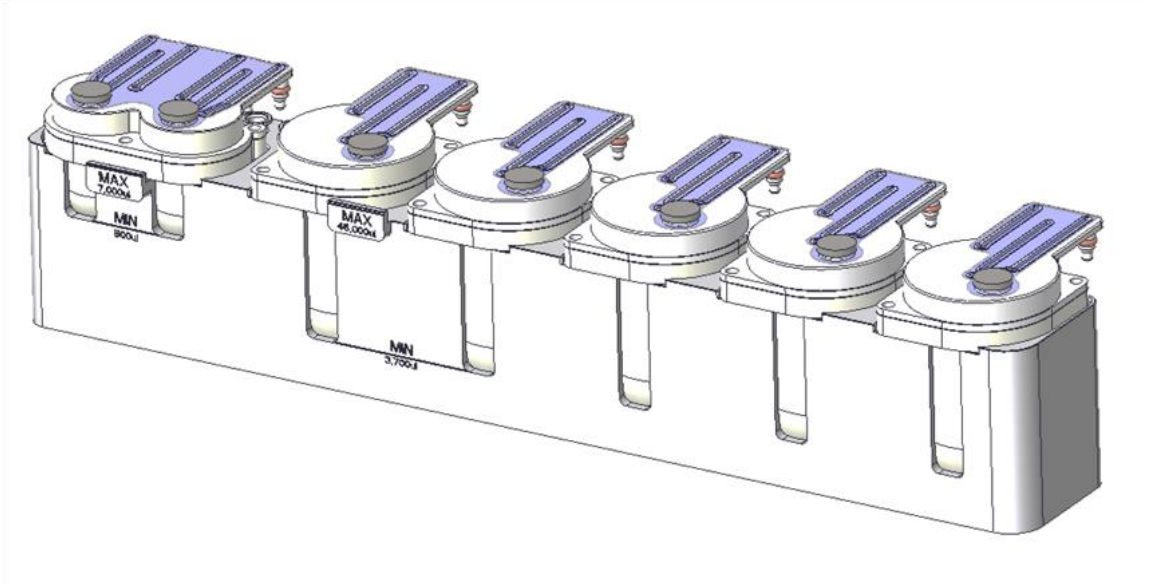
Container loading instructions:

a) Open the Top Cover.

b) Note that the buffer should not be below the minimum level:

- Small Container: 2mL
- Big Container: 8.5mL

Minimum and maximum level will be shown on the label and is indicated as lines in the containers. Minimum and maximum level will be standardized and user will be provided a holder to place these cartridges. The holder is also used to dispense the 80% Ethanol and NFW.

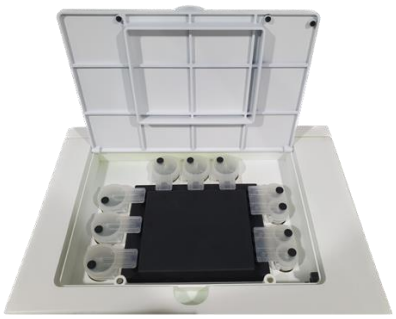


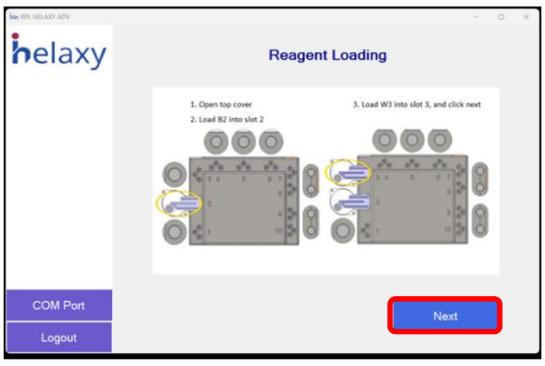
NOTE: Ensure that the buffers within the reagent cartridges are between the minimum and maximum mark.

Buffer	Position	Remarks
Buffer 2	2	Ensure 24mL of ethanol is added before first use.
Buffer 3	3	Ensure 8.8mL of ethanol is added before first use.
Buffer 4	4	Ensure 8.8mL of ethanol is added before first use.
80% Ethanol	5	Ensure 45ml of freshly prepared 80% ethanol is added before first use
NFW	6	Ensure 50mL of NFW is added before first use
Buffer 7	7	Ensure that reagent volume is above minimum level.

Load the Reagent Cartridges onto the reagent docking station according to the Positions defined in the Table above. Refer to the illustration below for further clarification.

c) Load the buffers according to their positions.
d) Load B2 into slot 2 and W3 into slot 3.
e) Click **Next** to proceed loading the next set of buffers.



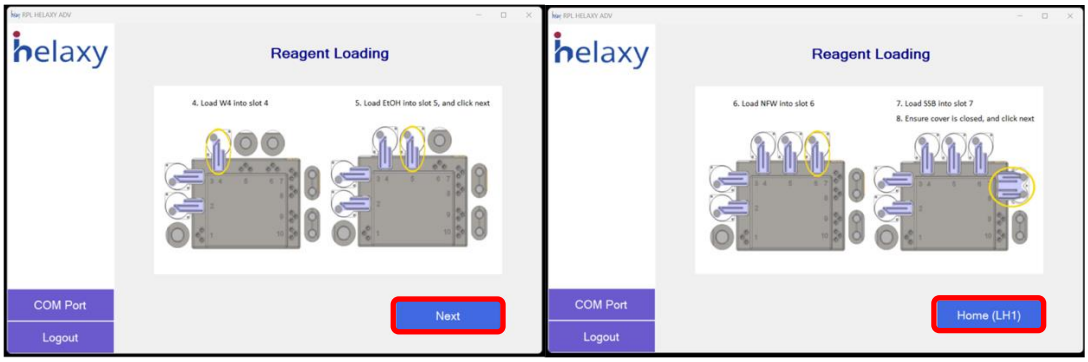


f) Load W4 into slot 4 and 80% Ethanol into slot 5.

g) Click **Next** to proceed loading the next set of buffers.

h) Load NFW into slot 6 and Buffer 7 into slot 7.

i) Uploading all reagent cartridges in their respective positions, click **"Home"** to return to main menu.



4.2.3 Library Prep Card Preparation

1. Remove the cards from the vacuum sealed bag.

2. Separate one 0.1mL PCR tube from the 8-strip from the tube pouch.

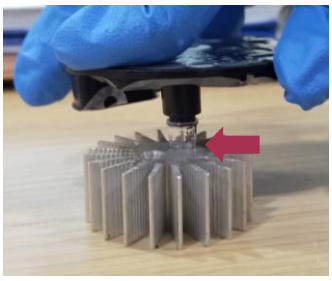
   Add mineral oil or respective master mixes (provide in the respective assay kit) according to respective **workflow** shown below:

Helaxy's DNA Assay Workflow	Helaxy RNA Assay Workflow	User-Customized Assay Workflow
Add 50µL of mineral oil into the 0.1mL PCR tube ⬇ Add two PCR master mixes & primer pellets from the pellet pouch	Add 50µL of mineral oil into the 0.1mL PCR tube ⬇ Add two RT-PCR master mixes & primer pellets from the pellet pouch	Add appropriate master mixes & primers into the 0.1mL PCR tube ⬇ Add 50µL of mineral oil into the 0.1mL PCR tube

3. Carefully transfer the pellet into the 0.1mL PCR tube without touching the pellet.

Two pellets and mineral oil inside the PCR tube

4. Use the tube adapter to fit the 0.1mL PCR tube onto the NGS Lib Prep Card at the position shown below.

Fit the tube to the adapter

5. Mix the cooled lysate with a 1000µL micropipette, by pipetting up and down.
6. To each Helaxy Library Prep card, slowly pipette 600µL of sample lysate into the sample port.


Lid of the sample port

7. Close the lid of the sample port. Ensure lid is tight.

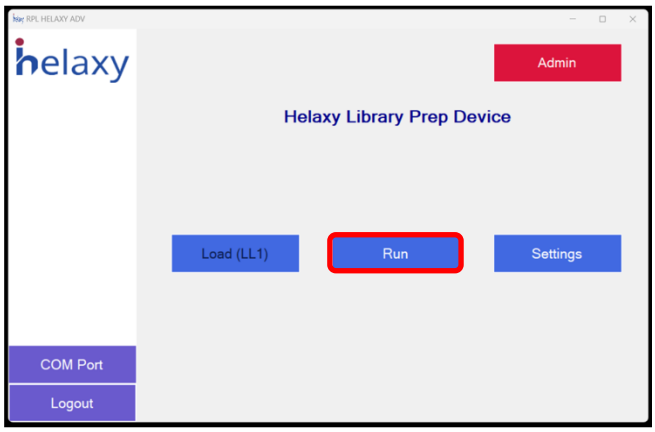
4.2.4 Size Selection & Normalization Card Preparation

1. Remove one card from the vacuum sealed bag.
2. Bring NGS Clean Up Beads to room temperature for at least 30 minutes.
3. Vortex and mix NGS Clean Up Beads before use.
4. Add 30µL of NGS Clean Up beads and 70uL of NFW into a 1.5mL Centrifuge Tube.
5. Vortex and mix the 1.5mL Centrifuge Tube to create a homogeneous bead suspension.
6. Pipette 100µL of bead suspension from the 1.5mL Centrifuge Tube into the port of the size selection card as shown below.
7. Push 40µL of air using a P100 pipette slowly so that all remaining reagent in the channel enters the chamber in the card. Ensure that beads suspension do not enter other channels in the card.

Pipette bead suspension into port. Push air solely to ensure that all beads have entered the chamber.

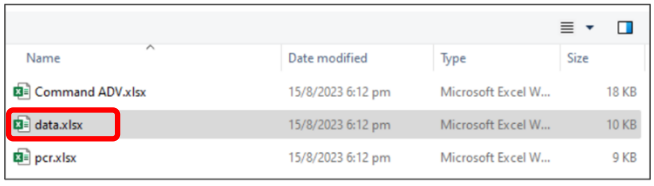
4.2.5 Starting a run on GUI software

1. Click **Run** to begin the process.

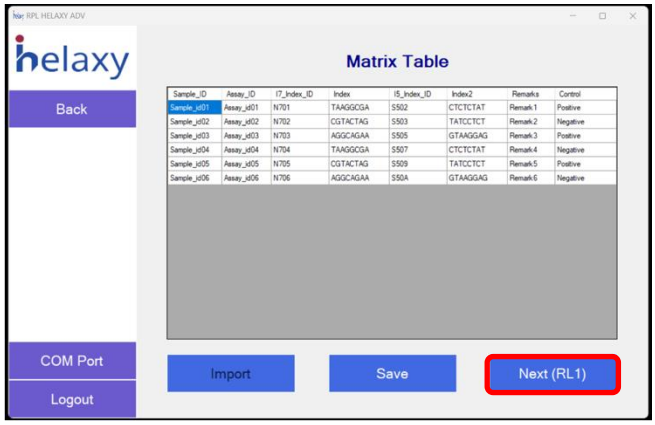


2. Click **Import** to import the Sample List.

3. Select Sample ID file (Excel). Data will be populated into Matric Table.
   Please refer to the section 5.1 for the instruction on how to set up and edit sample ID file.



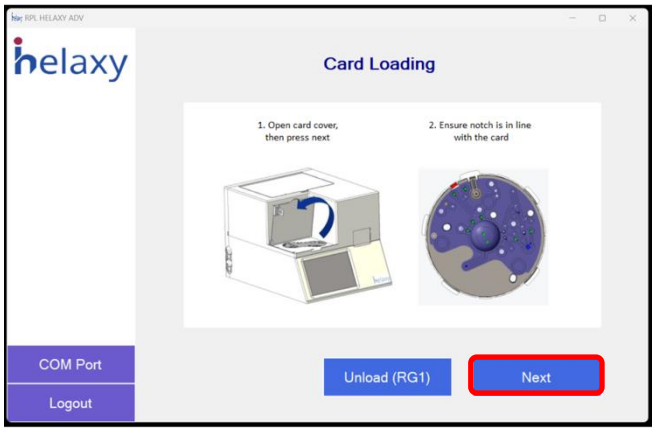
4. Click Save if necessary. Click **Next** to proceed to the Card Loading process.



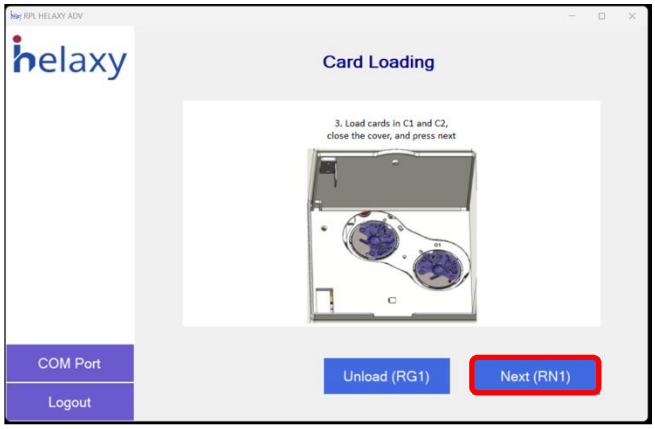
5. Library Prep Card Loading instructions:

   Open the Card Cover and click **Next**.

   In case the user put the cards in the wrong position, the user can click Unload to restart the Card Loading Process.



6. Load Library Prep card with sample in Position **C1** and **C2**. Close the card cover and click **Next**.

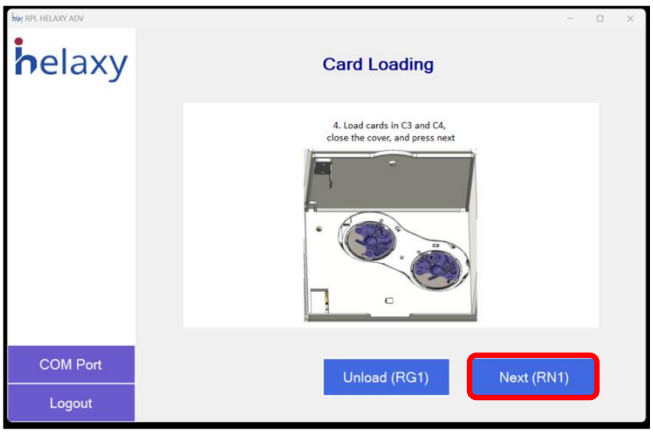


NOTE: Ensure that the notch on the Library Prep Card is alighed with the catch on the card adapter during Card Loading.

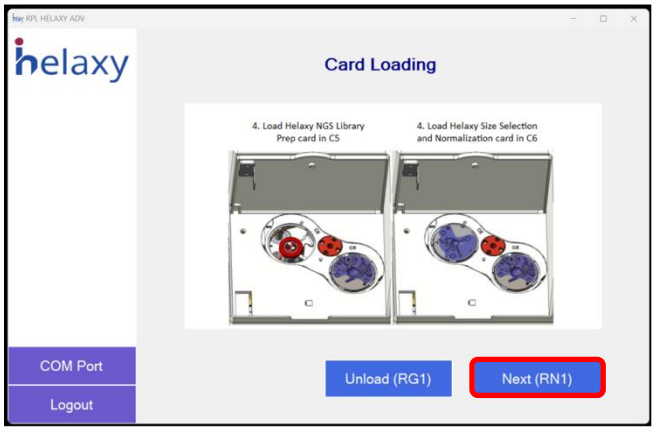
Notch

Catch

7. Load Library Prep card with sample in Position **C3** and **C4**. Close the card cover and click **Next**.



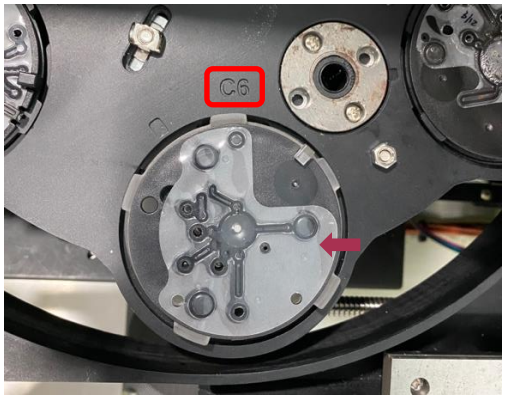
8. Load Library Prep card with sample in Position **C5**. Load Fluidic NGS Clean Up card with Size Selection Beads in Position **C6**. Click **Next**.



Notch

Catch

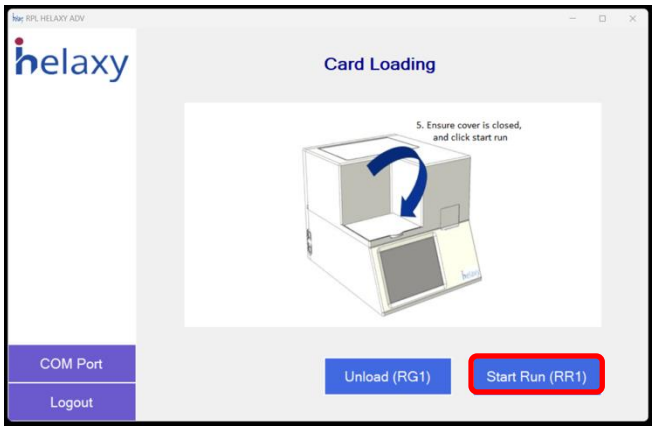
NOTE: Ensure that the notch on the Library Prep Card is alighed with the catch on the card adapter during Card Loading.



NOTE: Ensure that Fluidic NGS Clean Up card with Size Selection Beads is loaded in Position 6.

9. Ensure that the card cover is closed. Click Unload if necessary.

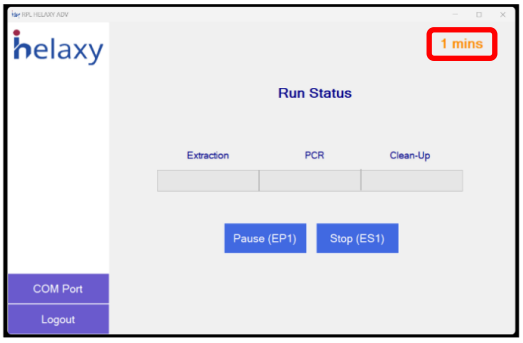
   Click **Start Run**.

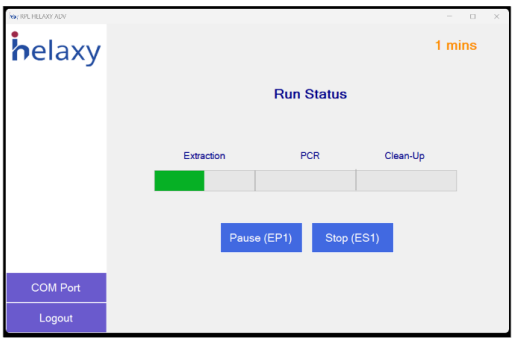


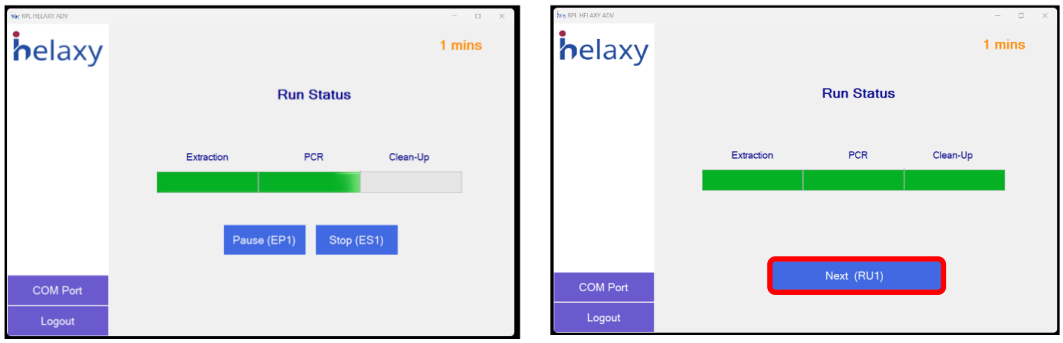
10. Run status bar will progress. The time indicator will be shown on the top right of the screen. Once the Run is finished, Click **Next**.

    Clicking 'Pause' button would halt the run process and the user can continue afterwards.

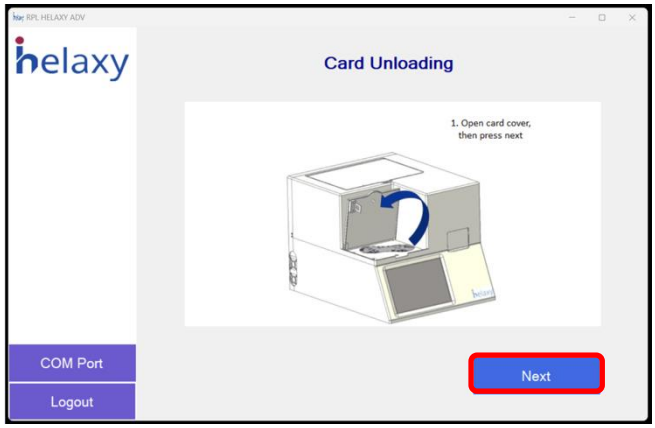
    Clicking 'Stop' button would abort the run process and the user will return to the main menu.



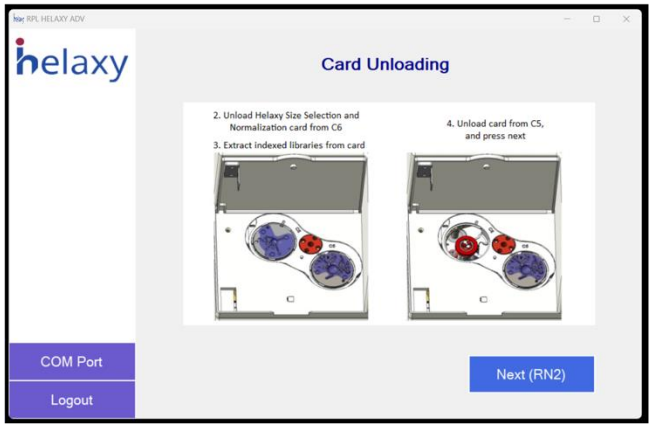




11. Open the card cover. Click **Next** to begin the Card Unloading Process.



12. Unload the Size Selection Card form C6 and proceed to retrieve the cleaned library.

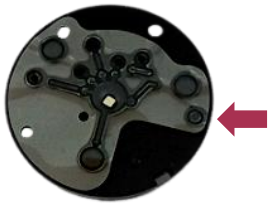


4.3 Post-run Preparation

4.3.1 Retrieving Cleaning Library

1. Retrieve the cleaned library from the Elution chamber of the Helaxy Size Selection and Normalization card using a P100 pipette.

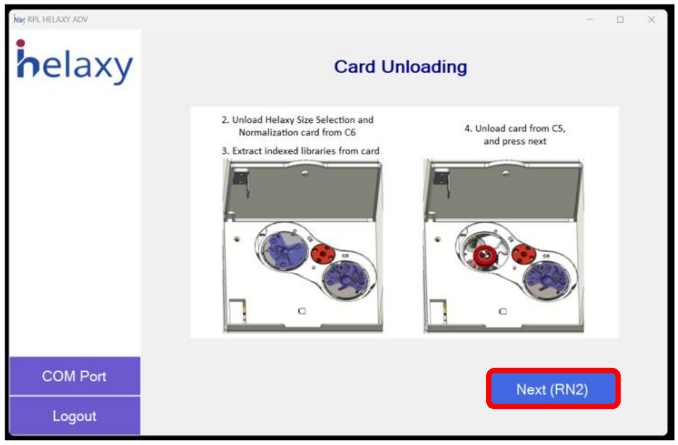
Retrieve the NGS pooled library here



Top view of the card

2. Transfer the cleaned library to a fresh 0.2mL microcentrifuge tube.

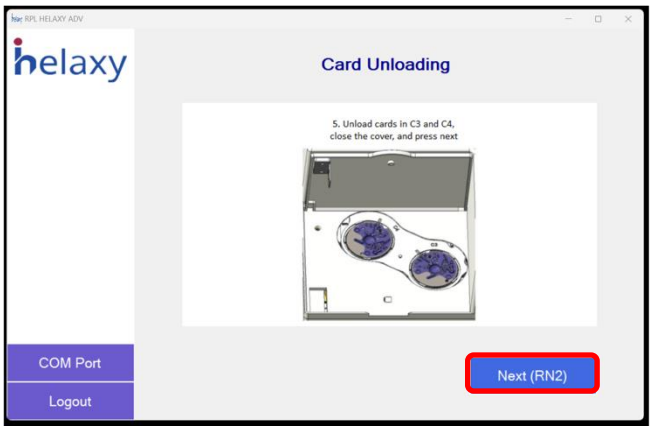
3. Unload Library Prep Card from C5.
    - Extracted DNA/RNA may be retrieved from the Elution storage chamber at the bottom side of the card.
    - Transfer the extracted DNA/RNA to a 0.2mL PCR tube for storage.
    - Close the card cover and click **Next**.



Elution Storage Chamber

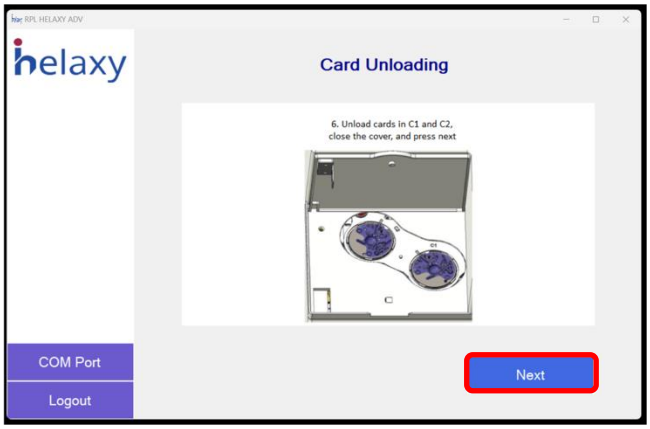
NOTE: Dispose used Library Prep cards in a Biohazard bin.

4. Unload the Library Prep Cards in C3 and C4.
    - Extracted DNA/RNA may be retrieved from the Elution storage chamber at the bottom side of the card.
    - Transfer the extracted DNA/RNA to a 0.2mL PCR tube for storage.
    - Close the card cover and click **Next**.



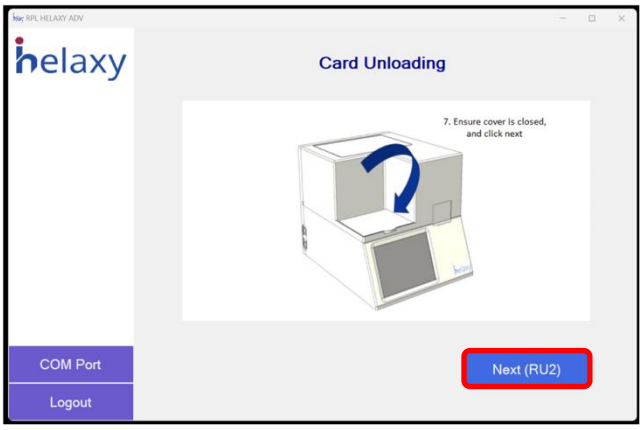
NOTE: Dispose used Library Prep cards in a Biohazard bin.

5. Unload the Library Prep Cards in C1 and C2.
    - Extracted DNA/RNA may be retrieved from the Elution storage chamber at the bottom side of the card.
    - Transfer the extracted DNA/RNA to a 0.2mL PCR tube for storage.
    - Close the card cover and click **Next**.

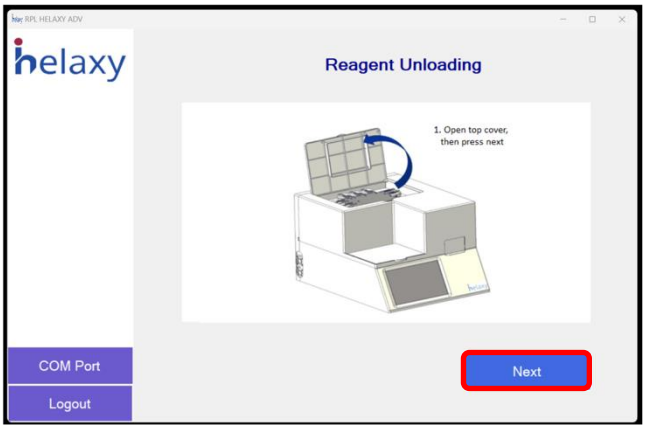


NOTE: Dispose used Library Prep cards in a Biohazard bin.

6. Ensure the card cover is closed and Click **Next**.

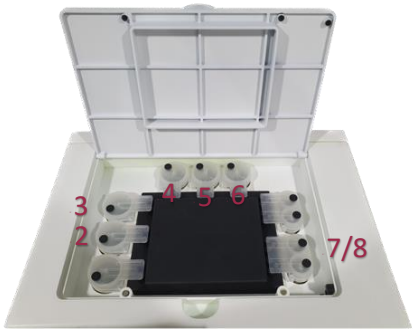


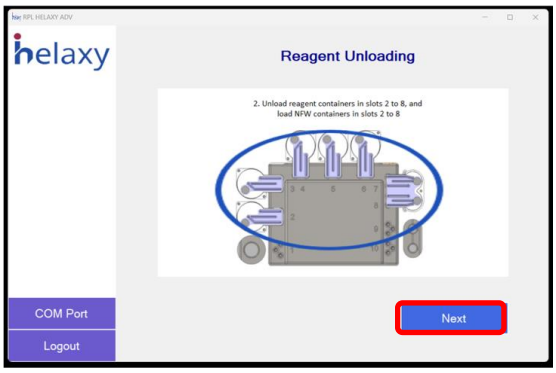
7. Open container cover. Click **Next** to begin the Reagent Unloading Process.



4.3.2 Post-run Wash

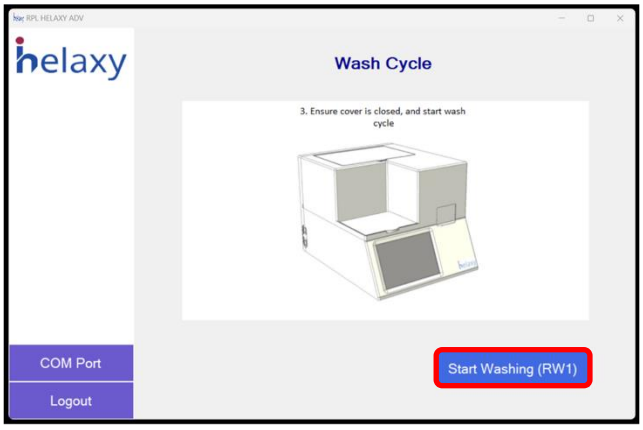
1. Post-run Wash Instructions:
    a) Unload the reagent cartridges from slot 2 to 8.
    b) Check the level of NFW in the washing containers is above the minimum level on the reagent cartridge holder. If below the minimum line, add 30mL of NFW.
    c) Load the NFW containers in slot 2 to 8.
    d) Click **Next**.





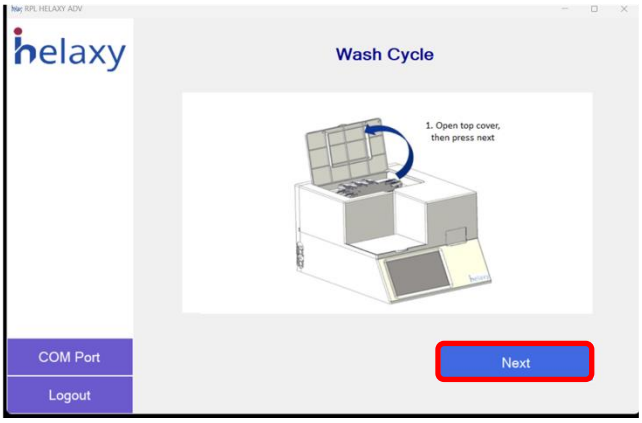
NOTE: Ensure that the volume of NFW in the washing containers is above the minimum line on the reagent cartridge holder.

2. Close the container cover. Click **Start Washing**.

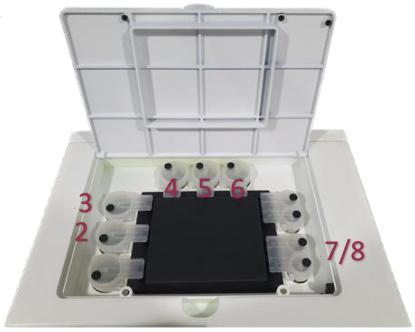


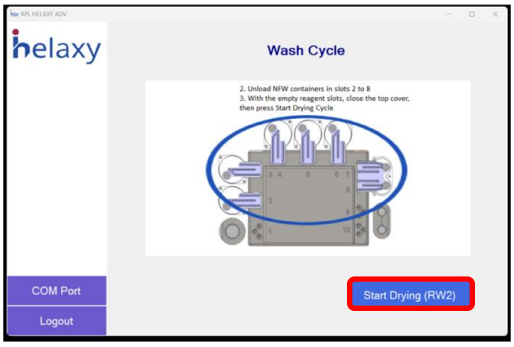
3. The Washing Cycle will start upon clicking Next. The status bar indicates the progress of the washing cycle which takes approximately 15 minutes to complete. Clicking ‘Stop’ button would abort the washing process. Once you stop the cycle, you will return to the main menu.

4. Once the Wash Cycle is finished, open the container cover. Click **Next**.

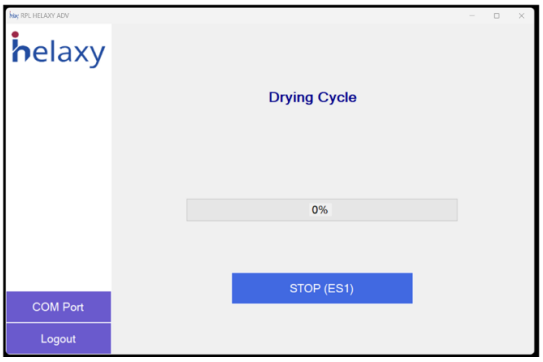


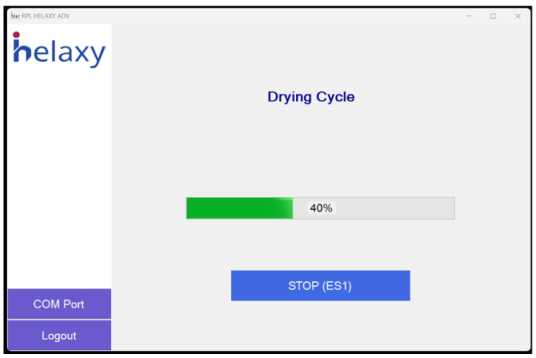
a) Unload the NFW containers from slot 2 to 8.
b) Close the Top Cover.
c) Click **Start Drying**.





5. The Drying Cycle will start upon clicking Next. The status bar indicates the progress of the washing cycle which takes approximately 15 minutes to complete. Clicking 'Stop' button would abort the drying process. Once you stop the cycle, you will return to the main menu.

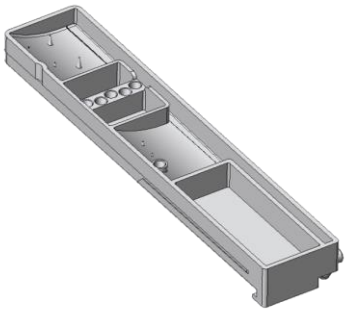




6. Waste Removal Instruction:
    a) Open the waste tray cover.
    b) Pull out the waste tray.
    c) Pull out the waste tube from the waste container.

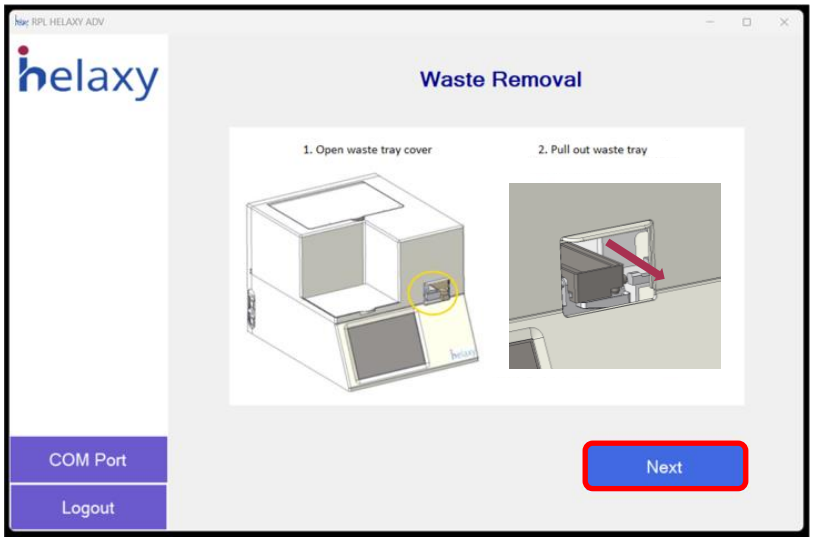
Waste Tube

Waste Tray Cover

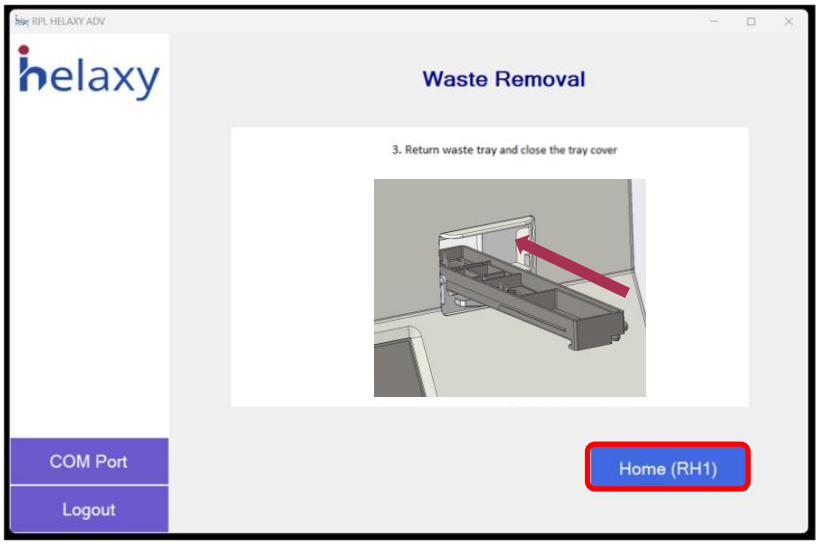


Waste Tray

    d) Wash both waste tray and container.
    e) Click **Next**.



7. Waste Removal Instruction:
    a) Ensure the waste tray is dry before inserting back into the device. Make sure to return the waste tray in the correct orientation.
    b) Close the waste tray cover.
    c) Plug in the waste tube back into the waste container.
    d) Click **Home** to return to the main menu.



5 Appendix

5.1 Sample ID Input

Instruction on how to set up a Sample ID file:

1. Enter Sample ID and Assay for samples to be processed.

Sample_ID	Assay_ID	I7_Index_ID	index	I5_Index_ID	index2	Remarks	Control
Sample_id01	Assay_id01	N701	TAAGGCGA	S502	CTCTCTAT	Remark1	Positive
Sample_id02	Assay_id02	N702	CGTACTAG	S503	TATCCTCT	Remark2	Negative
Sample_id03	Assay_id03	N703	AGGCAGAA	S505	GTAAGGAG	Remark3	Positive
Sample_id04	Assay_id04	N704	TAAGGCGA	S507	CTCTCTAT	Remark4	Negative
Sample_id05	Assay_id05	N705	CGTACTAG	S509	TATCCTCT	Remark5	Positive
Sample_id06	Assay_id06	N706	AGGCAGAA	S50A	GTAAGGAG	Remark6	Negative

2. Enter the I7 and I5 Index ID and Index sequence of the fluidic card in which the sample is processed with.

Table of Index Sequences

Kit A

Item Number	Index Combination
2000059	N701 x S502
2000138	N701 x S503
2000139	N701 x S505
2000140	N701 x S506
2000141	N701 x S507
2000142	N701 x S508
2000143	N701 x S510
2000060	N702 x S502
2000146	N702 x S503
2000147	N702 x S505
2000148	N702 x S506
2000149	N702 x S507
2000150	N702 x S508
2000153	N703 x S502
2000154	N703 x S503
2000155	N703 x S505
2000156	N703 x S506
2000157	N703 x S507
2000158	N703 x S508
2000161	N704 x S502
2000162	N704 x S503
2000163	N704 x S505
2000165	N704 x S506
2000166	N704 x S507
2000167	N704 x S508

Kit B

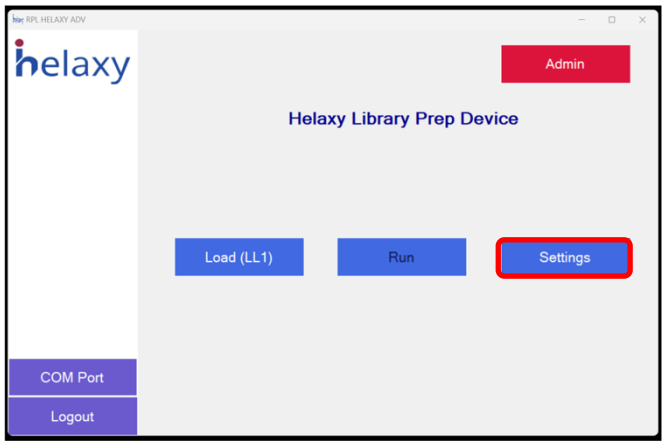
Item Number	Index Combination
2000145	N705 x S502
2000174	N705 x S503
2000176	N705 x S505
2000178	N705 x S506
2000180	N705 x S507
2000182	N705 x S508
2000184	N705 x S510
2000188	N706 x S502
2000190	N706 x S503
2000192	N706 x S505
2000194	N706 x S506
2000196	N706 x S507
2000198	N706 x S508
2000202	N707 x S502
2000204	N707 x S503
2000206	N707 x S505
2000208	N707 x S506
2000210	N707 x S507
2000212	N707 x S508
2000216	N710 x S502
2000218	N710 x S503
2000220	N710 x S505
2000222	N710 x S506
2000224	N710 x S507
2000226	N710 x S508

i5 Sequences	
S502	CTCTCTAT
S503	TATCCTCT
S505	GTAAGGAG
S506	ACTGCATA
S507	AAGGAGTA
S508	CTAAGCCT
S510	CGTCTAAT

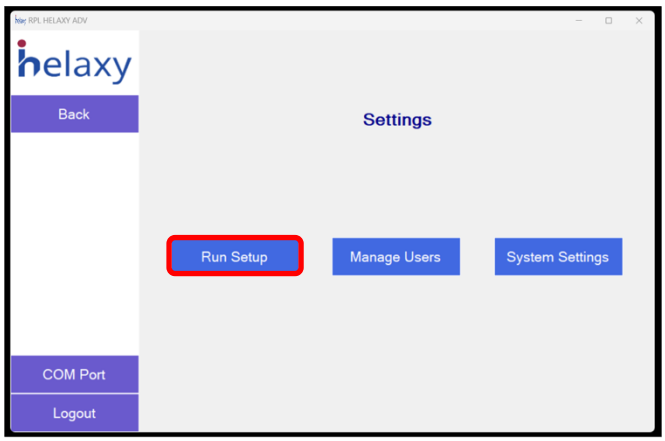
i7 Sequences	
N701	TAAGGCGA
N702	CGTACTAG
N703	AGGCAGAA
N704	TCCTGAGC
N705	GGACTCCT
N706	TAGGCATG
N707	CTCTCTAC
N710	CGAGGCTG

5.2 PCR Profile Input

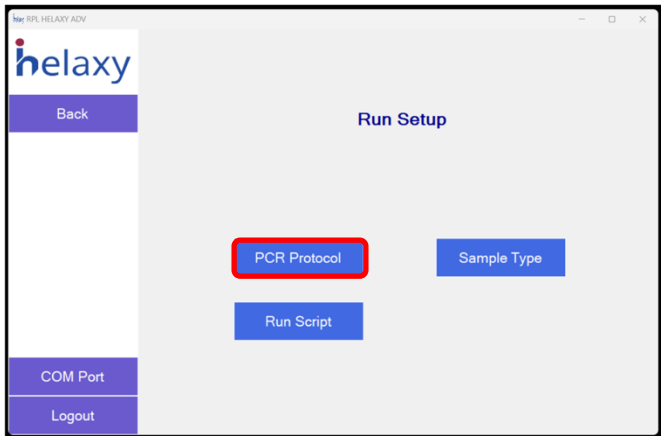
1. Click **Setting**.



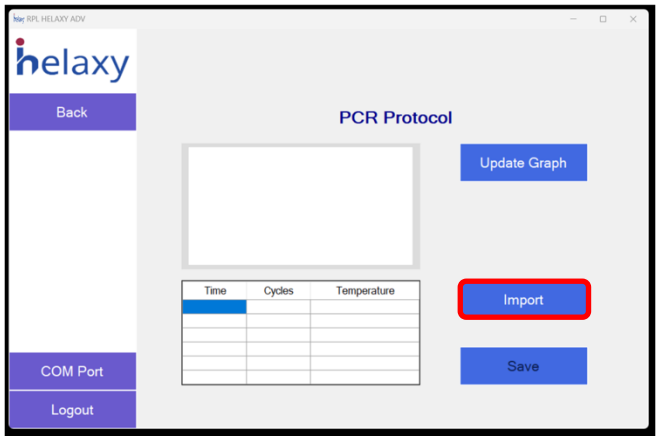
2. Click **Run Setup**.



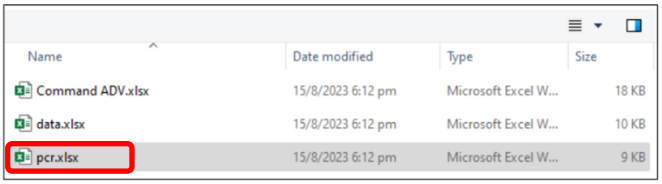
3. Click **PCR Protocol**.



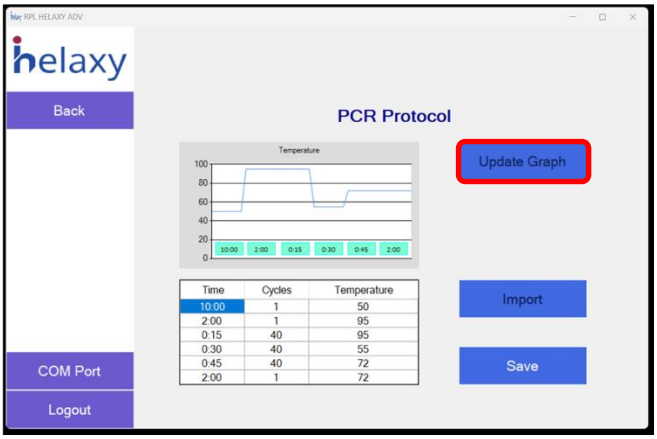
4. Click **Import**.



5. Select PCR Profile file (Excel).
    - User will be provided with a blank file in order to enter PCR Profiles.
    - User can upload into the specified directory.
    - Additionally, user can edit every cell in the excel file.



6. Click **Update Graph**. Click Save if necessary. Click Back 3 times to reach home screen.



5.3 Sample Preparation

5.3.1 Preparation of sample materials

1. Swab Samples:
    - Incubate the swabs in PBS, sodium chloride or universal transport media for at least 5 min.
    - Vortex the mixture with the swab and aliquot the sample mixture into small volumes.
2. Plasma Samples:
    - A sample volume of 525µL of plasma is recommended.
    - When processing less than 525µL of sample, adjust with PBS buffer to a final volume of 525µL.
3. Stool Samples:
    - Mix equal volume of feces with equal volume of PBS buffer.
    - Vortex vigorously for 2 min.
    - Allow the particles to settle and centrifuge at low speed (500 x g) for 1 min twice.
    - Proceed with the clarified supernatant. For difficult to lyse bacteria, mechanical disruption or treatment using suitable beads may be required.
4. FFPE Sample:
    - Samples should be thin sections (3 to 20µm thickness) of human or animal origin obtained by tissue resection or biopsy.
    - Protocol described is suitable for up to 5mg tissue. The amount of paraffin is limited to 15mg when using the standard protocol with Paraffin Dissolver (~7 sections of 10µm x 250mm^2).

5.3.2 Preparation of Lysis Working Solutions before first use

1. Proteinase K (lyophilized):
    - 1 vial (75mg): Add 3.35mL of Proteinase Buffer.
    - Invert to mix to dissolve.
    - Aliquot dissolved Proteinase K into small volumes.
    - Store at -20°C.
    - Reconstituted Proteinase K at -20°C is stable for at least 6 months.
2. Carrier RNA (lyophilized):
    - 1 vial (400µg): Add 500µL of Carrier RNA Buffer.
    - Invert to mix to dissolve.
    - Aliquot dissolved Carrier RNA into small volumes.
    - Store at -20°C for at least 6 months.
    - Reconstituted Carrier RNA at -20°C is stable for at least 6 months.

5.3.3 Sample Lysis A – For all samples types except FFPE

Materials: 1.5mL microcentrifuge tube, 1000µL micropipette tip, 100µL micropipette tip, 10µL micropipette tip

1. Preheat incubator/thermal bath to 56°C.
2. Add the following into each 1.5mL microcentrifuge tube.
    - 30µL of reconstituted Proteinase K.
    - 5µL of reconstituted Carrier RNA.
    - Either 40µL of Lysis Buffer 1 or 90µL of Lysis Buffer 2.

      (If sample type is swabs, use Lysis Buffer 1; otherwise, use Lysis Buffer 2)
3. Add 525µL of sample into the microcentrifuge tube.
4. Add 10µL of Magnetic Beads (Extraction).
5. Ensure lid of tube is tightly capped, invert to mix.
6. Briefly spin down sample in tube.
7. Incubate the sample mix at 56°C for 15min.
8. Ensure lid of tube is tightly capped, invert to mix.
9. Briefly spin down sample in tube and cool to room temperature.

5.3.4 Sample Lysis B – For FFPE samples

Materials: 1.5ml & 2mL microcentrifuge tube, 1000µL micropipette tip, 100µL micropipette tip, 10µL micropipette tip

1. Preheat shaking incubator/shaking thermal bath to 60°C.
2. Put the sample into a 2mL microcentrifuge tube.
3. Deparaffinize sample:
    - Add 400µL of Paraffin Dissolver to the sample.
    - Incubate for 3 min at 60°C to melt the paraffin.
    - Vortex or shake the sample immediately (at 60°C) at a vigorous speed to dissolve the paraffin. Cool down sample briefly to room temperature.
    - Ensure paraffin melts completely during the heat incubation and mix well after melting to completely dissolve the paraffin.

      (If solid particles are observed when cooling before the lysis step, repeat sub-point 2 to 4)
4. Change temperature of shaking incubator/shaking thermal bath to 56°C.
5. Lysis step:
    - Add the following into the lower aqueous phase in the 2mL microcentrifuge tube:
        a) 200µL Lysis buffer 3.
        b) 30µL of reconstituted Proteinase K.
    - Centrifuge at 11,000 x g for 1min.
    - Incubate at 56°C for 1 to 3 hours or overnight with shaking.
6. De-crosslink sample:
    - Set temperature of shaking incubator/thermal bath to 90°C.
    - Centrifuge the tube for 1 min at 11,000 x g.
    - Add 100µL of Buffer D-Link. Mix by pipetting up and down and vortex for 5 sec.
    - Centrifuge the tube for 1 min at 11,000 x g to obtain phase formation.
    - Incubate at 90°C for 30 min.
    - Mix by vortexing for 5 sec. Cool samples to room temperature.
7. Centrifuge the tube for 1 min at 11,000 x g.

8. Remove the blue hydrophobic phase carefully by using a 1000μL micropipette and dispose into an empty 1.5mL microcentrifuge tube.
9. Centrifuge the tube containing the remaining aqueous phase at 11,000 x g for 1min.
10. Using a 1000μL micropipette, insert the tip into the transparent aqueous layer and carefully retrieve the aqueous phase into a fresh 1.5mL microcentrifuge tube. *(Do NOT retrieve any blue hydrophobic phase)*
11. Centrifuge the tube containing the retrieved aqueous phase at 11,000 x g for 1min.

6 Index

A

B

C

D

E

F

H

I

L

M

N

O

P

R

S

T

U

V

W