Samplix

Xdrop® Sort manual

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Learn more about Samplix technology at samplix.com.
For technical support and other queries, contact us at support@samplix.com.

Xdrop and related Samplix technologies are for research use only, not for use in diagnostic procedures.

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Chapter 1: Xdrop Sort

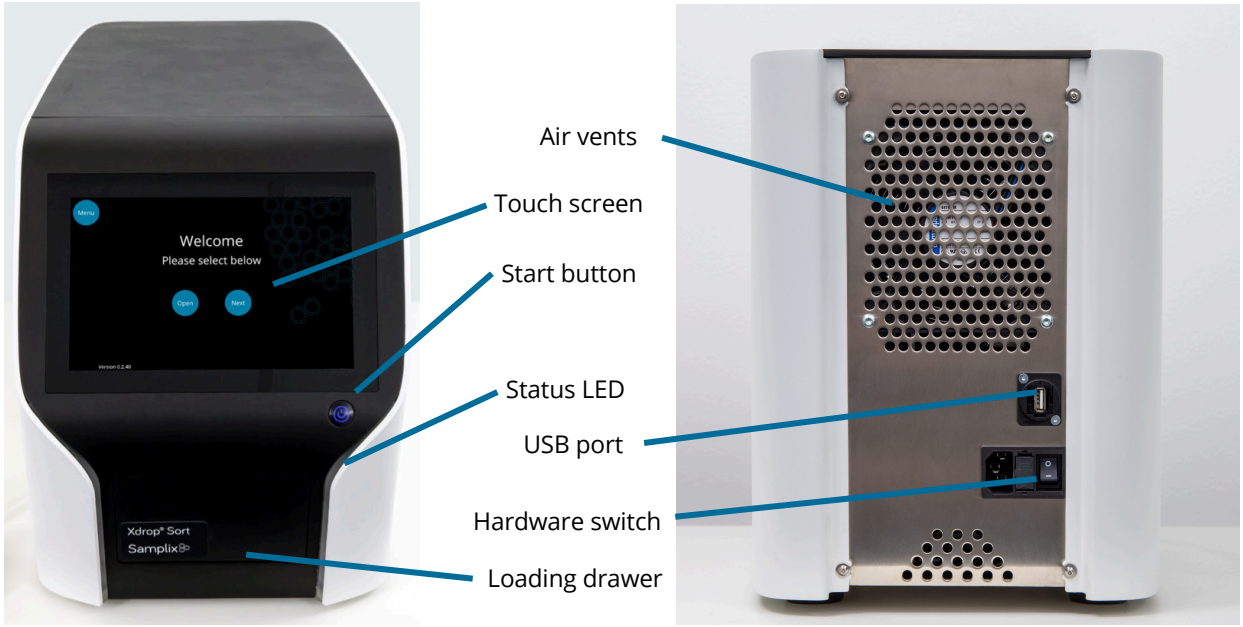
Thank you for selecting Xdrop Sort. This instrument supports the highest-resolution analyses of cells and genomes. It uses proprietary, microfluidics-based technology to encapsulate biological material, such as small living cells, single molecules (including long DNA molecules), or organelles, in highly stable, picoliter-sized, double- or single-emulsion droplets. It can also sort material in double-emulsion droplets based on fluorescence signals.

At the front of the instrument, you will see:

- The start button to activate the instrument, including the touch screen
- The touchscreen to control the instrument
- The status LED, which is purple during initialization and shutdown, green during standby mode, blue during operation, and yellow–green during loading drawer movement
- The loading drawer for the cartridges

At the back of the instrument, you will see:

- The USB port for firmware updates, which are available for download at samplix.com/software
- The air vents, which require a minimum of 30 cm clearance from the wall or other objects
- The hardware switch for full shutdown of the instrument



Xdrop Sort is compatible with:

- Xdrop DE20 Cartridges for generating double-emulsion droplets to encapsulate biological material
- Xdrop DE20 Sort Cartridges for sorting the double-emulsion droplets
- Xdrop SE85 Cartridges for generating single-emulsion droplets

Note: Xdrop SE85 Cartridges must be used with the accompanying holder. All cartridges must be sealed with the corresponding gasket before they are placed into the loading drawer. See the chapters on each droplet workflow for more information on the cartridges.

Products and accessories included in shipping box

Name	Item no.	Quantity	Notes
Xdrop® Sort	IN30100-SF001-EU	1 instrument	To encapsulate and/or sort biological material within droplets
Xdrop® Sort manual		1 booklet	Easy to read manual to help the user get started on various Xdrop Sort workflows
Mains power cord, IEC-320-C13	-	1 power cable	Country-specific power cable to connect the Xdrop Sort instrument to the wall socket.
USB Flash Drive		1 USB key	Contains the latest version of the instrument software.
Xdrop® Sort Lane Opener	ACXSCHOP100	2 lane openers	Used to punch inlet holes in the foil on the Xdrop Sort Cartridges

Specifications

Width 30.5 cm	12 inches	**Height** 36.4 cm	14.3 inches
Length 65.4 cm	25.7 inches	**Weight** 23.5 kg	51.8 lbs
Line frequency	50–60 Hz	**Overvoltage category**	II
Max current	650 mA @ 230 VAC and 1.3A @ 115 VAC		
Voltage requirements	110–240 V	**Degree of ingress protection (IEC 60529)**: IP20	

Support

For technical support, contact us at support@samplix.com.

Warranty

The instrument (Xdrop Sort) and its associated accessories are covered by a standard Samplix ApS warranty. Contact us at support@samplix.com for more details of the warranty.

Safety

We strongly recommend that you follow the safety specifications in this manual.

Xdrop Sort has been tested and found to comply with *Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use* (IEC 61010-1), EMC directive 2014/30/EU, FCC Part 15B (Class A) and CISPR 11 (Class A, Group 1) for radiated and conducted emissions.

These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.

This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications.

FCC Caution: Any changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate this equipment.

Environment and power requirements

The operating conditions for Xdrop Sort are:

Temperature 20–25°C **Relative humidity (RH)** 0–75%.

Altitude max. 2000 m

Xdrop Sort requires a stable power supply and can be powered using mains voltage of 110–240 VAC, 50–60 Hz, mains supply voltage fluctuations +/-10%.

Xdrop Sort must be installed on a flat surface where access to the main power outlet is not restricted. The instrument may not be operated on the floor. The instrument is not to be used against the manufacturer's instructions. Failure to comply with these requirements can result in potential hazards to the instrument and the user.

Instrument safety warnings

The following warning labels refer directly to the safe use of the Xdrop Sort instrument.

Icon	Meaning
	Warning about the risk of harm to body or equipment. Operating Xdrop Sort before reading this manual can constitute a personal injury hazard. Only qualified laboratory personnel should operate this instrument.
	Warning about the risk of harm to body or equipment from electrical shock. Do not attempt to repair or remove the outer case of this instrument, power supply, or other accessories. If you open this instrument, you put yourself at risk for electrical shock and void your warranty. All repairs must be done by an authorized repair service.
	Warning that the installation, adjustment, or removal of laser module, laser cabling and related optical components not specified in this document may result in hazardous radiation exposure. Do not remove or otherwise modify the excitation laser or detection module. Do not remove casing elements or open the front hatch (flap) during run. Do not insert a hand or any tool by opening the front hatch. Caution: use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure This instrument is laser class 1. This instrument contains a laser module classified laser class 3B

Intended use and intended users

Xdrop Sort and consumables are intended for research use purposes only and shall not be used for any other purposes. Xdrop Sort is intended for use by trained laboratory personnel in a clean laboratory environment for biological sample preparation (e.g., cell preparation, DNA preparation) using droplet microfluidic technology.

Transportation and storage

Always transport the instrument in the original Samplix box. Before starting up the instrument, let it stand at room temperature for at least 2 hours.

Maintenance, service, and cleaning

All service and maintenance must be carried out by trained personnel at Samplix or Samplix suppliers. When shipping the instrument to Samplix for maintenance, please ensure that the outer surfaces are cleaned using a cloth and 70% ethanol.

Disposal

The instrument can be disposed of as normal electronic equipment. Before discarding the instrument, please ensure all outer surfaces are cleaned using a cloth and 70% ethanol. Users may hand in the instrument as part of the public disposal system for returning and collecting waste electronic equipment. If no such system exists locally, the instrument can be shipped back to Samplix (Birkerød, Denmark) where it will be disposed of accordingly.

Xdrop Sort installation

1. Place the transport box on a flat surface.
2. Flip out the four lock twisters and turn them counterclockwise to unlock the lid of the transportation box.
3. Remove the lid to gain access to the instrument.
4. Place a hand on each side of the instrument and lift it out of the box.

   Tip: If required, gently lift the back of the instrument 10–15 cm and place it against the foam padding at the back of the box. This should allow you to get a good grip, placing both hands under the instrument.
5. Lift the instrument out of the box and place it on a flat horizontal surface.
6. Leave the instrument unused for at least 2 hours.

   Note: Leaving the instrument at ambient temperatures allows the instrument to equilibrate and reduces the risk of instrument failure.
7. Attach the power cord to the back of the instrument.

   Note: Only use the included power cable.
8. Plug the power cable into an appropriate power outlet.

   Note: Only connect the instrument to a protective earthed wall socket.
9. Turn the hardware switch at the back of the instrument to the ”|” position.
10. Press the start button at the front to power up the instrument.

    Note: The power button needs to be fully pressed for the instrument to start up.

Required items for Xdrop Sort workflows

For double-emulsion droplet production

Name	Item no.	Notes
Xdrop® Sort	IN30100-SF001-EU	
Xdrop® DE20 Cartridge	CADE20A100	
Xdrop® DE Gasket	GADEA100	Included with Cartridge
Storage film	FI00100	
DE20 PCR kit	REFKITDE20PCR100	Required for DNA/PCR applications
Contents:		
Droplet oil (DE) 8 lanes ● (RT)	REOILDEB0850	-
DE PCR buffer (2x) 8 lanes ● (–20°C)	REBUFDE1500	Not required if using a custom buffer
DE PCR mix (2x) 8 lanes ● (-20°C)	REMIXDE0195	
DE Stabilizing Solution for DNA 8 lanes ● (4°C)	REDIVSTABSOL0900	Required if a using custom buffer
DE Stabilizing Solution for cells 8 lanes ● (4°C)	REDIVSTABSOL1500	Required if using a custom buffer

For single-emulsion droplet production

Name	Item no.	Notes
Xdrop® Sort	IN30100-SF001-EU	
Xdrop® SE85 Cartridge	CASE85A100	
Xdrop® SE85 Holder	HOSE85A100	
Xdrop® SE Gasket	GASEA100	Included with Cartridge
Storage film	FI00100	
SE MDA kit	REFKITSEMDA100	Required for DNA/PCR applications
Contents:		
Droplet oil (SE) 8 lanes ● (RT)	REOILSE0640	
SE MDA mix (5x) 8 lanes ● (-20°C)	REMIXSE0035	
SE MDA enzyme 8 lanes ○ (-20°C)	REENZSE0011	

For double-emulsion droplet sorting

Name	Item no.	Notes
Xdrop® Sort	IN30100-SF001-EU	
Xdrop® DE20 Sort Cartridge	CADE20S100	
Xdrop® DE20 Sort Gasket	GADES100	Included with cartridges
Foil for sorting	FI00200	Included with cartridges
Xdrop® Sort DE20 sorting essentials kit	REFKITDE20SRT100	
Contents:		
Xdrop® Blank Oil Droplets 8 lanes ○ (4°C)	REOILBLDRPA0120	
DE sorting buffer (2x) 8 lanes ● (4°C)	REBUFDE4000S	
Xdrop® Sort DE20 DNA sorting kit	REFKITDNAADD100	For use in DNA workflows
Contents:		
DE staining buffer 4 lanes ●	REBUFDE4800S	
Droplet sorting wash buffer 8 lanes ●	REBUFDE3840S	
Xdrop® Sort Lane Opener	ACXSCHOP100	

For downstream analysis

Name	Item no.	Notes
Small volume droplet break kit	REFKITBRESMVL100	Breaks both DE and SE droplets
Contents:		
Droplet break color 8 lanes ●	REBREDESE0024	
Droplet break solution 8 lanes ●	REBREDESE0195	

In addition to listed Samplix products, the user may need the following.

Equipment

LAF (laminar air flow) cabinet

Microcentrifuge

Pipette set from P2 to P1000

Consumables

Nuclease-free PCR tubes and microcentrifuge tubes

Filtered pipette tips

Wide-bore pipette tips for P200 pipette, outer diameter of 1–1.9 mm

Eppendorf DNA LoBind 1.5 ml tubes (cat. no. 0030108051)

Reagents

Nuclease-free water

Chapter 2: Double-emulsion droplet production with the Xdrop DE20 Cartridge

Double-emulsion droplets are generated on the Xdrop Sort instrument using microfluidics-based technology. The sample (e.g., living cells or DNA fragments), assay chemistry (optional), and medium (e.g., growth medium or buffer) are enclosed in an oil shell that is surrounded with medium. Thus, the encapsulation process creates an isolated compartment for the sample and assay. Crucially for single-cell work, the compartment includes the cell's immediate environment, meaning live cells can be encapsulated and kept alive (Fig. 2.1).

Yeast and bacterial cells have been successfully encapsulated in double-emulsion droplets produced with the Xdrop DE20 Cartridge These DE20 droplets are highly stable and robust during incubation.

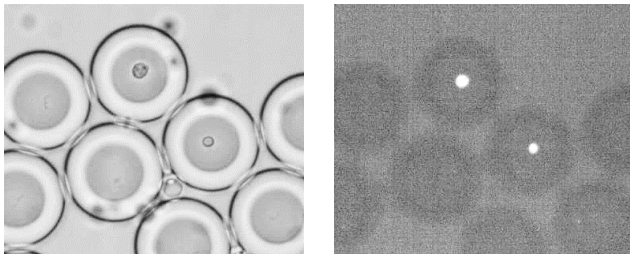


Fig. 2.1. Pictures of DE20 droplets encapsulating yeast cells that are expressing green fluorescent protein. Left: brightfield microscopy, right: fluorescence microscopy. Inner droplet diameter: 15 µm, outer droplet diameter: 20 µm, volume: 1.6 pl.

Considerations for DE20 droplet-based assay design

Size of DE20 droplets

- The outer diameter is 20 µm, the inner diameter is 15 µm, and the volume is 1.6 pl.
- Encapsulated cells should be small, preferably less than 5 µm in diameter. Yeast cells and bacterial cells have been successfully encapsulated in DE20 droplets, but larger cells can block the filters of the cartridge and prevent droplet production.
- When producing DE20 droplets in Xdrop DE20 Cartridges, particles introduced into the cartridge may block the microfluidic channels. If this is suspected, filter buffers or media before use.

About DE20 droplets

- DE20 droplets are highly stable. They can withstand temperatures up to 95°C and pH up to 10. They can also be vortexed, frozen in glycerol, and stored for more than 6 months.
- The oil shell surrounding the DE20 droplets is flexible and semi-permeable. It allows water and small molecules to pass via osmosis, but large molecules, such as DNA or protein, stay within the droplet. This means that if the concentration of solutes differs in the inner and outer phases, the osmotic gradient causes the droplets to swell or shrink within a few minutes. The change in size can be observed when looking in a microscope using a counting chamber (Fig 2.2). DE20 droplet swelling, or shrinkage can affect the concentrations of all molecules inside the droplets, which may impact the assay. Avoid osmotic gradients by adjusting the osmolarity in the outer phase.
- Fluorescence can be detected within cells encapsulated in DE20 droplets and/or in the surrounding inner phase of the droplet.

Buffers and media for DE20 production

- **Do not use detergents such as PEG, Tween, Triton-X, or SDS in the buffers or media**. They prevent droplet production. Check your enzyme stock solutions as they may contain detergents.
- With some buffers and media, the outer phase must be stabilized during droplet production. This can be done by adding DE Stabilizing Solution for DNA • (Cat No. REDIVSTABSOL0900) or DE Stabilizing Solution for cells • (Cat No. REDIVSTABSOL1500) to a custom buffer or growth medium.
- DE Stabilizing Solution is not needed during the incubation of droplets, so the outer phase used for production can be replaced by a custom outer phase after droplet production.
- **When testing DE20 droplet production with a non-standard buffer, medium or other aqueous solution, the first production run should be no more than 10 minutes**. If DE20 droplet production is not feasible with the non-standard solution, foam will form and may enter the instrument. After 10 minutes, press Stop on the screen and check that droplets have been produced, preferably by looking at a sample in a counting chamber or hemocytometer with a cover glass. Examine it using a brightfield microscope (Fig. 2.2). At least 25% of the DE20 droplets should have an appearance like the droplets in Fig. 2.1. Some oil-in-water droplets without an inner aqueous phase are also expected and will not affect droplet sorting. If no DE20 droplets are observed, try to isolate which of the solution components is preventing production.

How to check DE20 droplet production

Note: Use a counting chamber or hemocytometer with a cover glass to check DE20 droplet production. A standard microscope slide with cover glass compresses and flattens the flexible droplets, making them appear larger than their actual size (Fig. 2.2).

1. Place a cover glass on top of the counting chamber.
2. Resuspend the DE20 droplets well. Note that they rapidly sediment.
3. Pipet **5–10 µl** of resuspended DE20 droplets in buffer/media onto the counting chamber.
4. Use a low magnification (e.g., 4x) to identify the DE20 droplets. Switch to a higher magnification (e.g., 20x) to properly visualize DE20 droplets and oil droplets (Fig. 2.2).

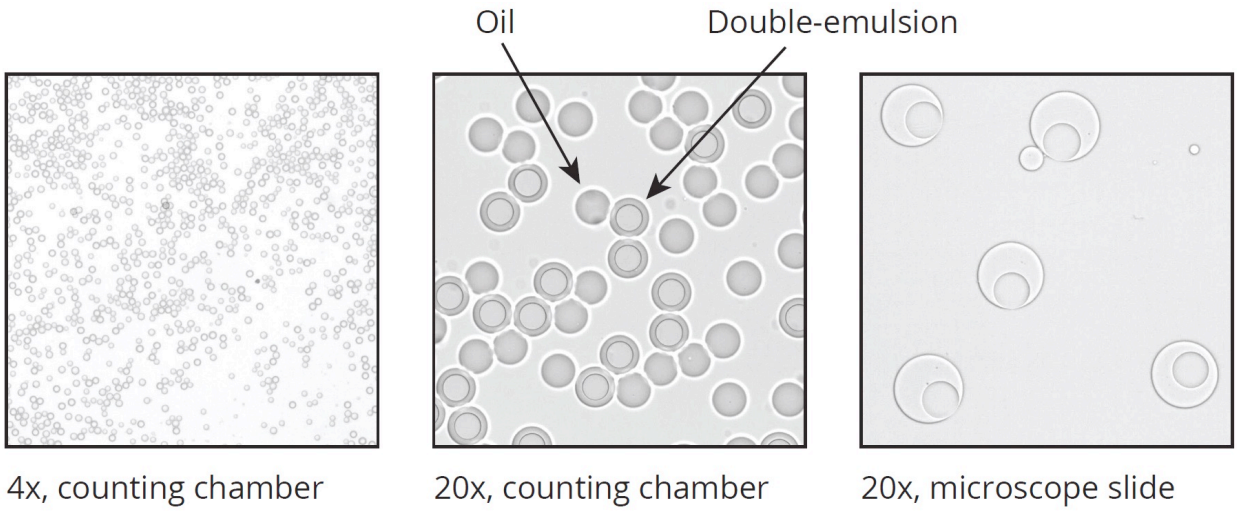


Fig. 2.2. Check the DE20 droplet production using a counting chamber or hemocytometer. Left: 4x magnification of DE20 droplets in a counting chamber. Middle: 20x magnification of the same sample. Both DE20 droplets and oil droplets can be seen. Right: 20x magnification of a sample on a microscope slide. The DE20 droplets appear larger and deformed.

Optimization of DE20 droplet production on Xdrop DE20 cartridges

The chosen inner and outer phase for DE20 droplet production depends on the material to be encapsulated, e.g., DNA fragments or living cells.

For DNA, we recommend 1x DE PCR mix as the inner phase and 1x DE PCR buffer as the outer phase.

For encapsulation of living cells, optimize the cell density in the sample mix by referring to the Cell distribution calculator under **Digital Tools** on our website and perform a droplet production test. The Cell distribution calculator enables the user to calculate the likelihood of how many cells will be present in each of the droplets at a given input number of cells.

For yeast cells, the OD600 nm should not be higher than 10, corresponding to around 50,000 yeast cells per µl or 2 million cells in 40 µl. Using higher concentrations will most likely lead to clogging of the microfluidic chip.

When testing a buffer, medium, or other aqueous solution, the first production run should be no more than 10 minutes. If production is not feasible with the solution, foam will form and may enter the instrument.

To test, produce DE20 droplets as described from the next page forward.

After 10 minutes, press Stop on the screen and check that droplets have been produced as described in the previous section. At least 25% of the DE20 droplets should look like the droplets in Fig. 2.1. Oil droplets without an inner aqueous phase are also expected. They add stability during production and sorting.

If no DE20 droplets are produced, try to isolate which of the solution components is preventing droplet production. Addition of DE Stabilizing Solution • to the outer phase can aid DE20 droplet production.

Preparing and loading the Xdrop DE20 Cartridge

1. Prepare your sample mix (cells, DNA, or other biological material; assay chemistry (if needed); inner phase; and any necessary stabilizing reagent), bearing in mind that the sample mix:
   - Cannot contain elements larger than 6 µm
   - Must have a similar osmolarity to the outer phase
   - Cannot interfere with fluorescence at 488 nm
2. Dilute the outer phase to 1x with molecular-grade water and mix with DE Stabilizing Solution ●. If you are using DE PCR buffer, you do not need to add Stabilizing Solution.
3. Keeping the cartridge in its closed, original packaging, place it at room temperature for 20 min to equilibrate.
4. Unpack the cartridge and place it on a clean, flat surface in a LAF (laminar air flow) cabinet or a similar clean, dust-free environment. The layout of the Xdrop DE 20 Cartridge is shown in Fig. 2.3. A cross-section of the cartridge is shown in Fig. 2.4.

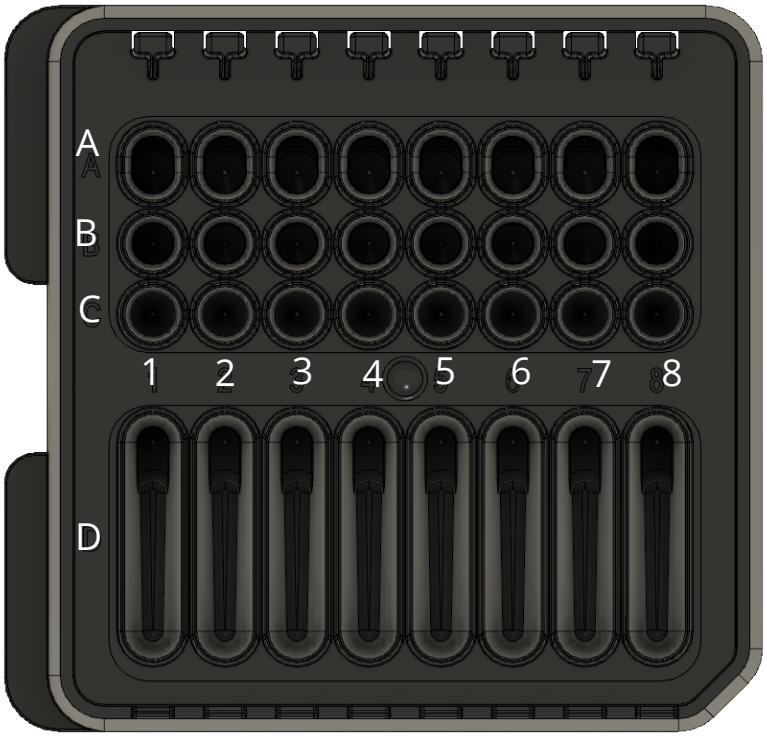


Fig. 2.3. Top view of Xdrop DE20 Cartridge with the wells (A–D) and lanes (1–8) marked.

5. Handle the cartridge as follows:
    - Always use gloves when handling the cartridge.
    - Hold the cartridge by its sides.
    - Do not touch any of the input wells (#A–C) or droplet collection wells (#D).
    - Do not touch the microfluidic chip on the back of the cartridge.
    - Save the cartridge bag for later storage of the cartridge.
6. Do not use the same lane more than once as this will disrupt droplet production. To avoid using the same lane more than once, mark the cartridge bag or the cartridge directly with a permanent marker once a lane has been used.
7. Allow all reagents to reach room temperature before loading them.
8. Load the cartridge with reagents in a LAF cabinet or a similar clean, dust-free environment.
9. When loading the Xdrop DE20 Cartridge, avoid introducing air bubbles by pipetting onto the side wall of the wells.

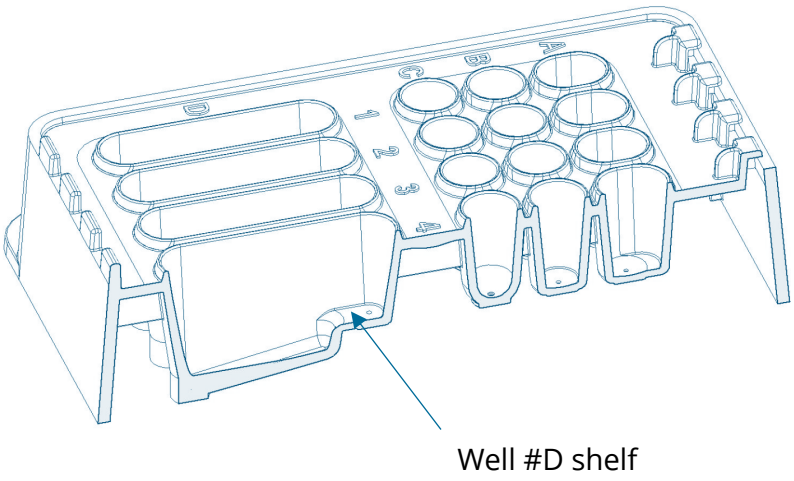


Fig. 2.4. Cross section of the Xdrop DE20 Cartridge. Note the location of the shelf in well #D.

Note: It is important to load the Xdrop DE20 Cartridge in the order described here and to avoid air bubbles by pipetting carefully on the sides of the wells.

10. Load **300 µl** of the outer phase (e.g., medium with Stabilizing Solution DE PCR buffer) into well **#A**.
11. Load **40 µl** of the outer phase (e.g., medium with Stabilizing Solution or DE PCR buffer) onto the shelf of the collection well (**#D**; Fig. 2.4).
12. Load **40 µl** of the sample mix into well **#C**.
13. Load **100 µl** of Droplet oil (DE) ● into well **#B**.

Table 2.1. Summary of loading order

Well	Content
#A	300 µl outer phase
#D	40 µl outer phase onto the shelf
#C	40 µl sample mix
#B	100 µl Droplet oil (DE) ●

14. Place the white rubber gasket onto the cartridge. Orient the gasket to the cartridge using the angled corner (beside lane 8, well #D on the cartridge, Fig. 2.3). Insert the pins through the pinholes and then pull the gasket gently over the T-hooks (Fig. 2.5).
15. Immediately after loading, proceed to production.

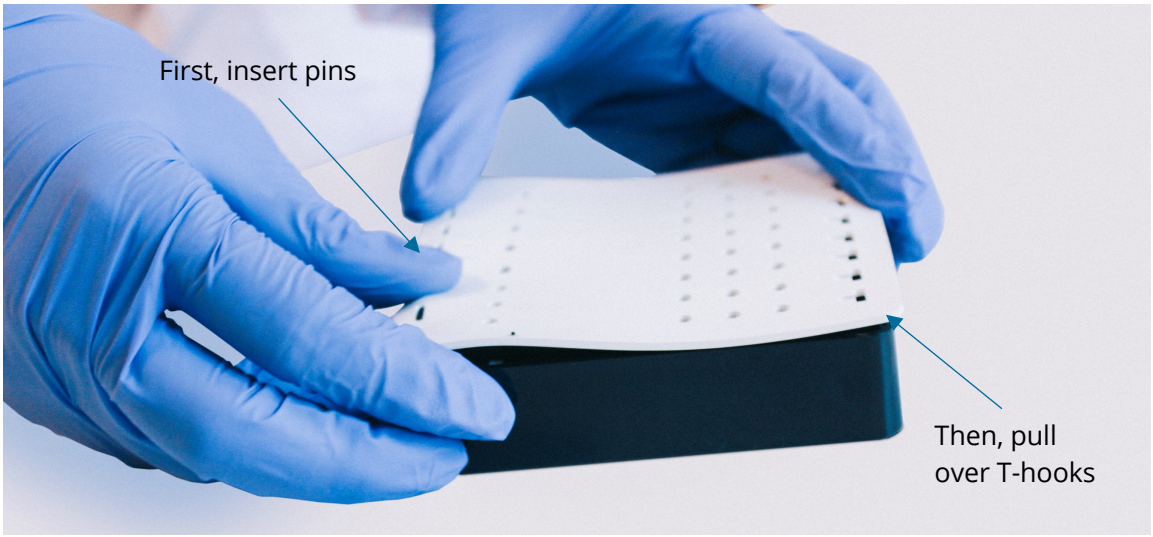


Fig. 2.5. Cover the cartridge with the white rubber gasket with the angled corner on the gasket to angled corner on the cartridge, then insert the pins and pull gently over the T-hooks.

Running DE20 droplet production

Before turning on the instrument, please make sure that the main power switch is in the “I” position. The main switch is located at the back of the instrument.

1. Push the Start button at the front. The Welcome screen will appear (Fig. 2.6).
2. Push Open on the instrument touchscreen to open the drawer (Fig. 2.6).

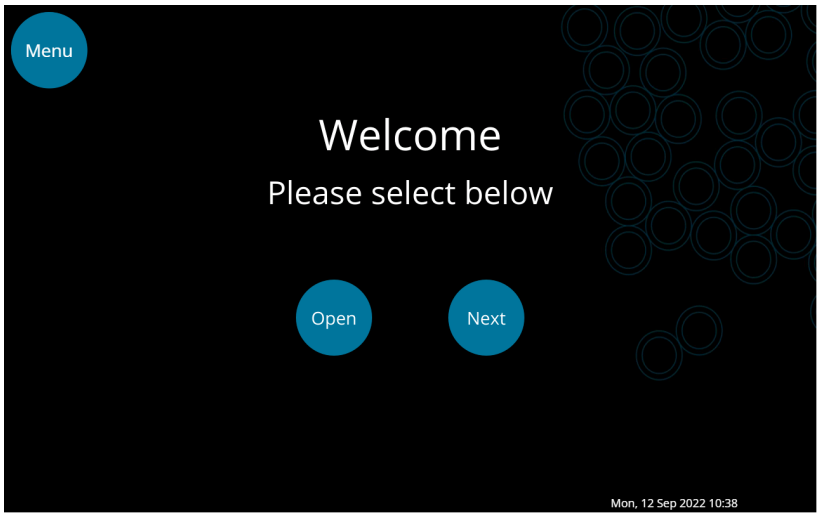


Fig. 2.6. The Xdrop Sort instrument Welcome screen. Press Open to open the drawer.

3. The screen will now show Please insert/remove cartridge and Close. Ensure that the cartridge is correctly positioned into the drawer (Fig. 2.7) as it may otherwise cause damage to the instrument. To position the cartridge correctly, ensure that the angled corner on the cartridge is aligned with the angled corner in the drawer. Press the cartridge carefully but firmly into place. Once the cartridge is correctly inserted, press Close to close the drawer.
4. Once the drawer is fully closed, press Next on the touchscreen.

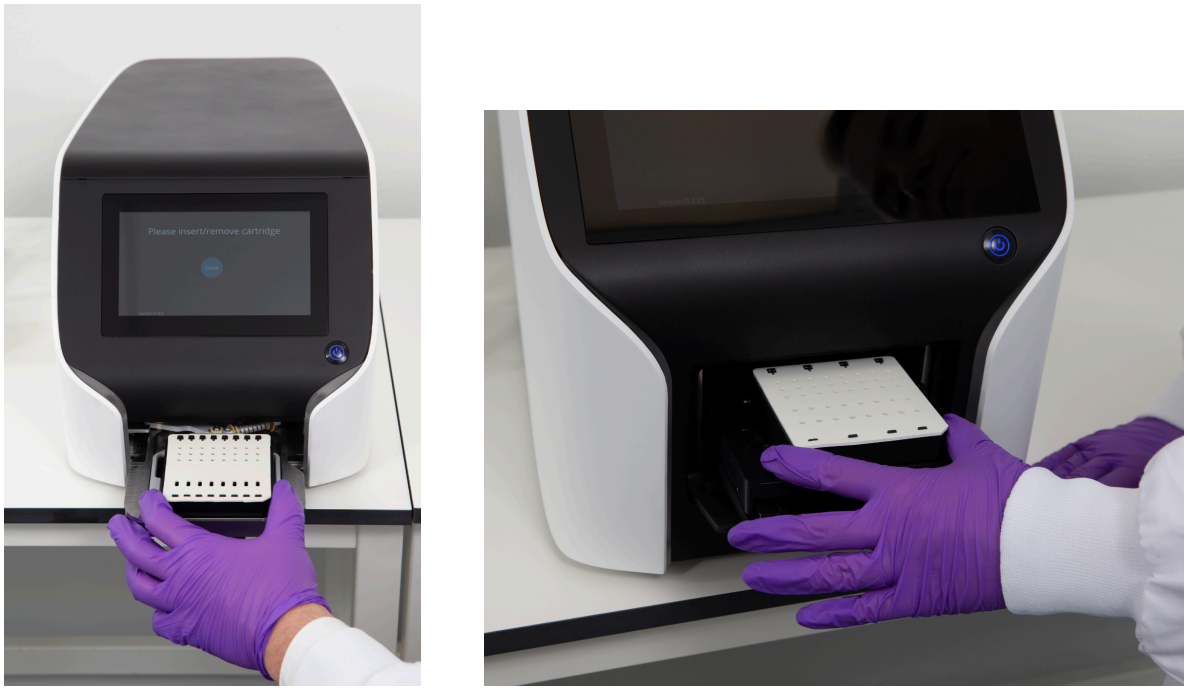


Fig. 2.7. Two views of Xdrop Sort with a correctly inserted Xdrop DE20 Cartridge. Position the cartridge correctly by aligning the angled corner on the cartridge with the angled corner in the drawer. to avoid damaging the instrument or the cartridge.

5. Xdrop Sort can operate with either Xdrop DE20, DE20 Sort, or Xdrop SE85 Cartridges. Choose the DE20 droplet option by selecting DE on the touchscreen (Fig. 2.8).

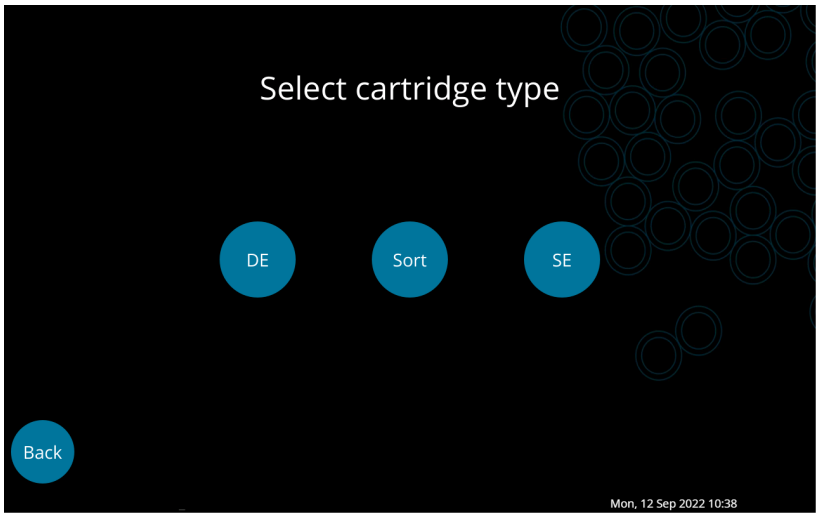


Fig. 2.8. The Xdrop Sort Select cartridge type screen. Select DE for DE20 droplet runs.

6. The lanes to be processed are selected by pressing the numbers for the corresponding lanes 1–8 on the screen (Fig. 2.10).

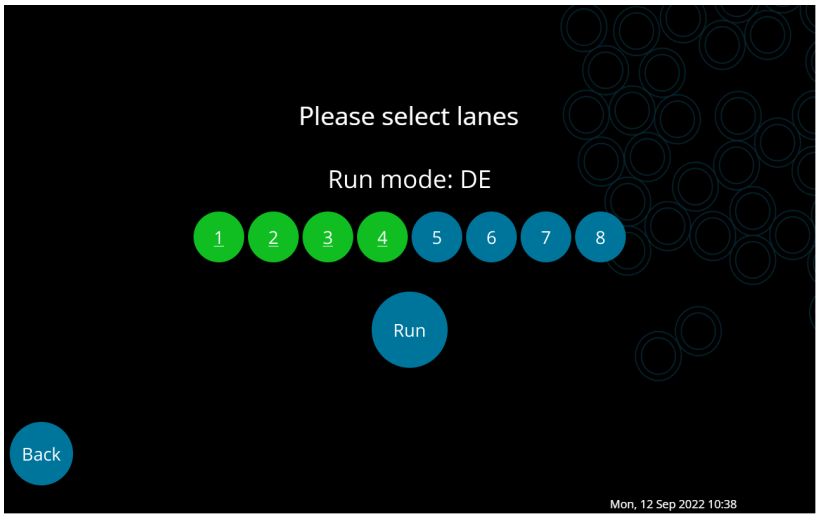


Fig. 2.10. Selecting the lanes to be used. Green indicates the lanes that have been selected (corresponding to lanes in the cartridge) and blue indicates the lanes not yet selected.

7. Press Run.

Once optimal pressures have been reached, the message Making your droplets and the remaining run time is displayed on the screen (Fig. 2.11). Double-emulsion droplet production on Xdrop Sort takes approximately 40 minutes.

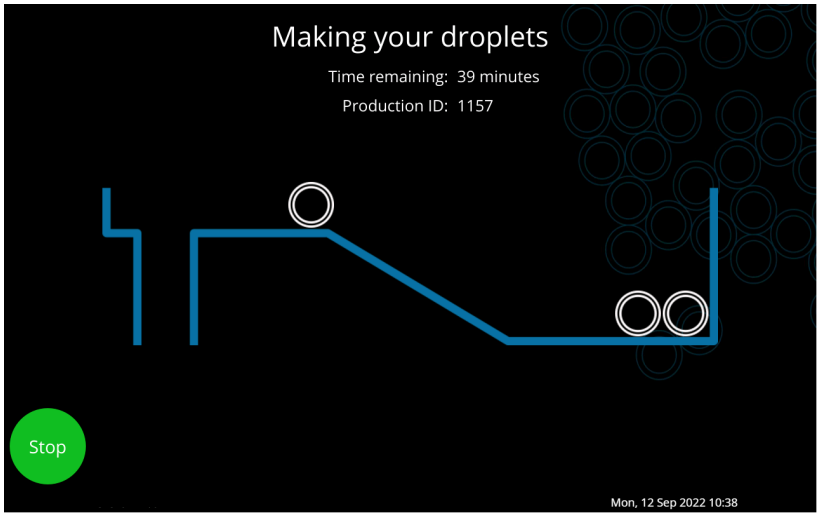


Fig. 2.11. This touchscreen image is displayed during droplet production.

8. When droplet production has been completed, the screen will change to Your droplets are ready.
9. Press Finish to return to the Welcome screen.
10. Press Open to open the cartridge drawer.
11. Carefully remove the cartridge from the instrument and place it in an LAF cabinet.
12. Press Close to close the drawer.
13. Power down the instrument after a completed droplet production to avoid damage to the instrument. Push the Start button at the front to initiate the automatic shutdown procedure and power down the instrument.

Collecting droplets generated with Xdrop DE20 Cartridge

1. Look at the collection well (#D) to confirm that double-emulsion droplets have been produced. Double-emulsion droplets sink to the bottom of the collection well to form a white layer with a clear buffer phase on top. This can also be observed in the collection tubes (Fig. 2.12) where clear separation into droplets, outer phase and foam is seen.

2. Collect droplets from the collection well (#D) into a 0.5 ml or 1.5 ml LoBind tube (Fig. 2.12).

3. Use **200 µl** of the outer phase buffer to wash residual droplets from the shelf inside the collection well (#D). You can use the excess outer phase from well #A for this purpose.

The droplets and buffer collected after droplet production should have a total volume of around 300–400 µl.

Note: If you are proceeding with droplet PCR in a thermal cycler, mix gently and dispense the droplets from the 0.5 ml or 1.5 ml tube into four PCR-tube aliquots (of approximately **80–90 µl** each).

Double-emulsion droplets sediment rapidly during handling. To ensure equal distribution into the aliquots, be sure to mix gently by pipetting up and down between each pipetting step.

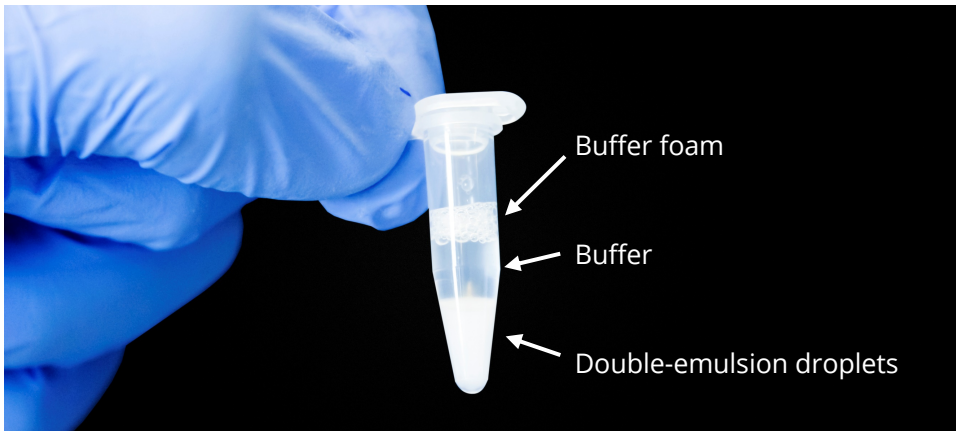


Fig. 2.12. Collection of double-emulsion droplets from the collection well (#D) into a 0.5 ml tube. The double-emulsion droplets are relatively heavy and will sediment at the bottom of the tube.

Xdrop DE20 Cartridge storage after use

Note: Each lane is single-use and will not function properly if re-used. Gaskets are also single use. Reusing a production lane or gasket increases the risk of sample cross-contamination. Unused lanes can be stored for later if stored correctly (see below). If you plan to use the cartridge more than once, we recommend ordering additional gaskets (sold separately, cat. no. GADEA100).

1. Remove the excess liquid from wells #A and #B before storing the cartridge.
2. Seal the cartridge with Storage film (cat. no. FI00100) covering all wells, and store protected from light and dust in the freezer (–20ºC) in a sealed bag.
3. The cartridge can be stored **for up to 4 weeks** in the freezer (–20°C) and used up to a total of three times. We do not recommend using the Xdrop DE20 Cartridge more than three times, as further freeze–thaw cycles could damage the cartridge.

Chapter 3: DE20 droplet sorting

DE20 droplets generated using Xdrop Sort and the Xdrop DE20 Cartridge can be sorted and collected using the Xdrop DE20 Sort Cartridge. In this step, DE20 droplets that contain the biological material of interest (e.g., DNA fragments or microbial cells with the desired fluorescence) are identified and separated from those without the material of interest (negative droplets or other material) based on the signal emitted upon excitation by the 488 nm laser.

Preparing the Xdrop DE20 Sort Cartridge

Xdrop Sort can run up to 8 lanes in parallel, but it is also possible to select fewer lanes. When selecting lanes, note that the lanes are paired (Fig. 3.1) with the threshold level for sorting applying to both lanes in a pair. When running all 8 lanes, choose samples with similar positive droplet fluorescence levels for the paired lanes. If your samples have issues such as low fluorescence or high-level background fluorescence, we advise using only odd or even lanes, and then running the remaining samples in a subsequent run on the opposite lanes. Example: First run: lanes 1, 3, 5, and 7, Next run: lanes 2, 4, 6, and 8. The cartridge can be used twice.

1. Unpack the Xdrop DE20 Sort Cartridge and place it on a clean flat surface in a LAF cabinet or a similar clean, dust-free environment. The layout of the DE20 Sort Cartridge is depicted in Fig. 3.1.

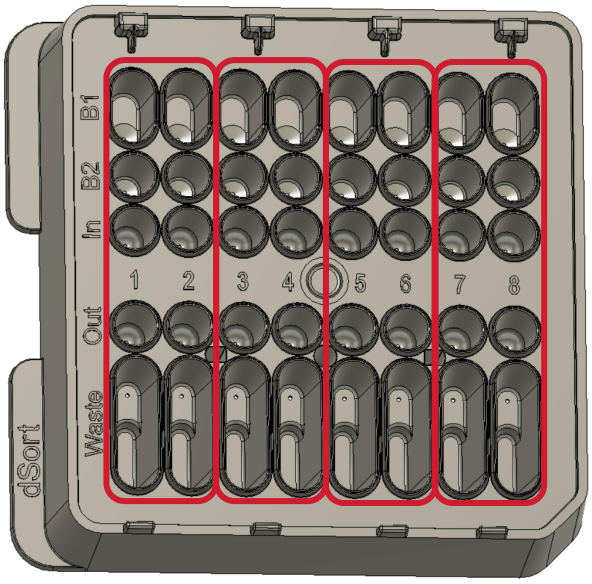


Fig. 3.1. Top view of the Xdrop DE20 Sort Cartridge with the wells (B1, B2, In, Out and Waste) and lanes (1–8) marked. Lane pairs are circled in red.

2. Handle the cartridge as follows:
    - Use gloves when handling the cartridge.
    - Hold the cartridge by its sides.
    - Do not touch any of the input (B1, B2, In) or droplet collection (Out, Waste) wells.
    - Do not touch the microfluidic chip on the back of the cartridge.

3. Seal the entire cartridge with Foil for sorting (cat. No. FI00200), make sure it adheres closely to all wells (Fig. 3.2).

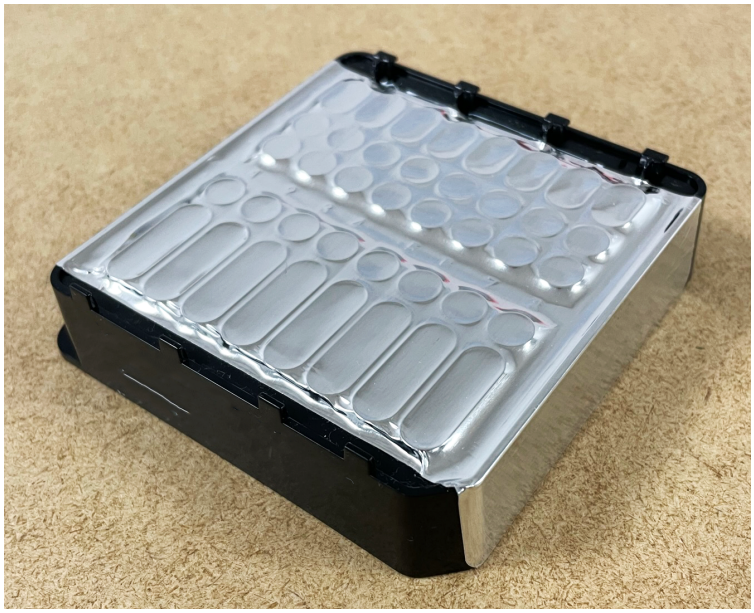


Fig. 3.2. Xdrop DE20 Sort Cartridge sealed with foil for sorting. Make sure to carefully cover all wells.

4. Use the Xdrop Sort Lane Opener to press openings in the sealing foil to the lanes you intend to load (Fig. 3.3). If all lanes are not used in the first run, remaining lanes can be used in a second run after another layer of foil for sorting has been applied.

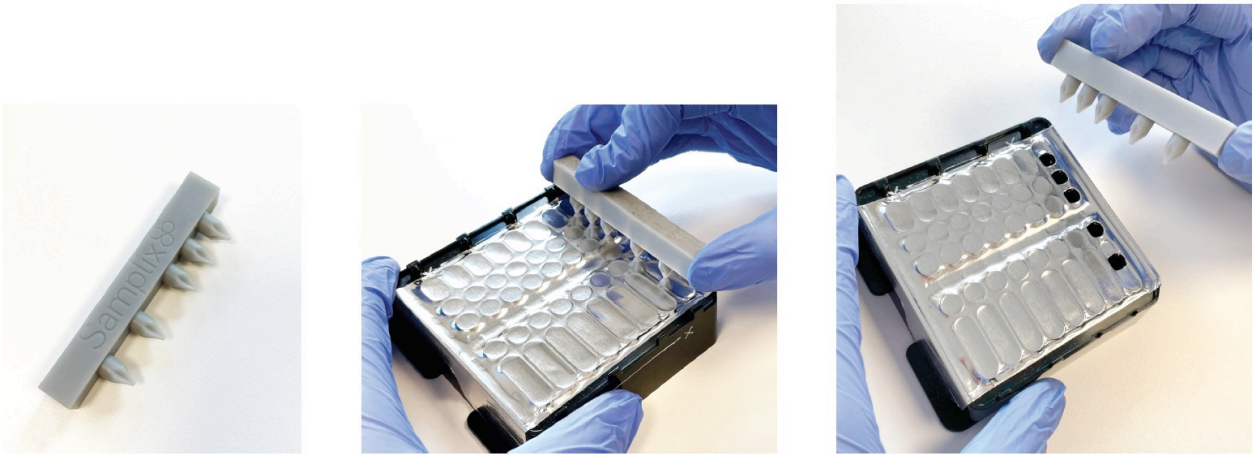


Fig. 3.3. Use the Xdrop Sort Lane Opener as shown to open the lanes to be used. Left: The Xdrop Sort Lane Opener. Middle: Open the lanes to be used by inserting the lane opener and piercing the foil. Right: The appearance of the open wells.

Staining of DNA inside DE20 droplets

If a sample contains purified DNA, the DNA in the DE20 droplets must be stained before sorting. Below, we describe the procedure for staining DNA inside DE20 droplets with DE staining buffer ●. For some workflows, staining may not be necessary e.g., if the assay includes a labeled probe. Please discuss this with one of the Samplix Field Application Scientists.

1. Add 1 ml of DE staining buffer ● to a 1.5 ml tube.
2. Remove the outer phase (DE PCR buffer or custom buffer with Stabilizing Solution) from the tubes with DE20 droplets.
3. Pipette the DE20 droplets into the 1.5 ml tube with the DE staining buffer. Mix gently by pipetting the contents up and down.
4. Incubate the tubes with DE staining buffer and DE20 droplets for 5 min at room temperature while keeping them in the dark.
5. Reserve 300 µl of the stained droplets for loading into the **#In** well of the Xdrop Sort Cartridge

Loading the Xdrop DE20 Sort Cartridge

Note: It is important to load the Xdrop DE20 Sort Cartridge in the order described here and to avoid air bubbles by pipetting carefully on the sides of the wells. All reagents should be at room temperature when loaded.

1. Load the cartridge with reagents in a LAF cabinet or a similar clean, dust-free environment. Allow the reaction mix to reach room temperature before loading the Xdrop DE20 Sort Cartridge. After loading, cover the cartridge with an Xdrop DE Sort Gasket and put the cartridge in the drawer of your Xdrop Sort.
2. Load **5 µl** Blank droplets ○ in well **#Out**

- Ensure that the loaded liquid covers the opening of the microfluidic channel at the bottom of the #Out well. Check this by letting light shine beneath the cartridge. The light observed from above should be diffused.

3. Load **600 µl** 1x DE sorting buffer ● in well **#B1**.
4. Load **300 µl** 1x DE sorting buffer ● into well **#B2**.
5. Load DE20 droplets and bring up the volume to a total of 300 µl by adding DE sorting buffer ● into well **#In**. The total DE20 droplet volume should not exceed 200 µl. E.g. 200 µl DE20 Droplet + 100 µl DE sorting buffer ●.

Note: Ensure that your droplet mix allows positive droplets to emit fluorescent light in the wavelength range 515–1,000 nm once excited by the 488 nm laser. Examples of dyes that fit the Xdrop Sort system are DE staining buffer, FITC, FAM, and GFP.

Note: The **#In** sample well must contain a volume of 300 µl in total. If your DE20 droplet volume is < 200 µl, bring up the total volume to 300 µl by adding DE sorting buffer ●**.**

Note: The concentration of positive droplets must not exceed 10,000 per 100 µl of droplet emulsion (the white phase). If the concentration of positive droplets is higher, dilute with Blank droplets ○ to reach a total volume of 200 µl, add **100 µl** DE sorting buffer ● and load into the **#In** well.

Table 3.1. Summary of loading order

Well	Content
#Out	5 µl Blank droplets into the well
#B1	600 µl 1x DE sorting buffer into well
#B2	300 µl 1x DE sorting buffer into well
#In	300 µl DE20 droplets and DE sorting buffer into the well

6. Remove visible air bubbles from well **#B1**, **#B2**, **#In** and **#Out**.

7. Cover the cartridge with the Xdrop DE20 Sort Gasket (Fig. 3.4). Orient it using the angled corner on the gasket, which should line up with the angled corner on the cartridge. Insert the pins first, then pull the gasket gently over the T-hooks.

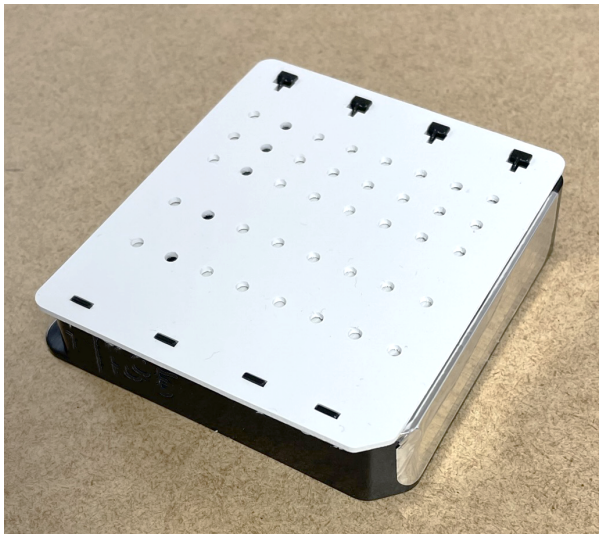


Fig. 3.4. The Xdrop DE20 Sort Cartridge covered with foil and the Xdrop DE20 Sort Gasket.

Starting double-emulsion droplet sorting

Before turning the instrument on, check that the main power switch is in the "I" position. The main switch is located at the back of the instrument. Start the instrument by pushing the start button at the front. The Welcome screen will appear.

1. Push Open on the instrument touchscreen to open the drawer (Fig. 3.5).

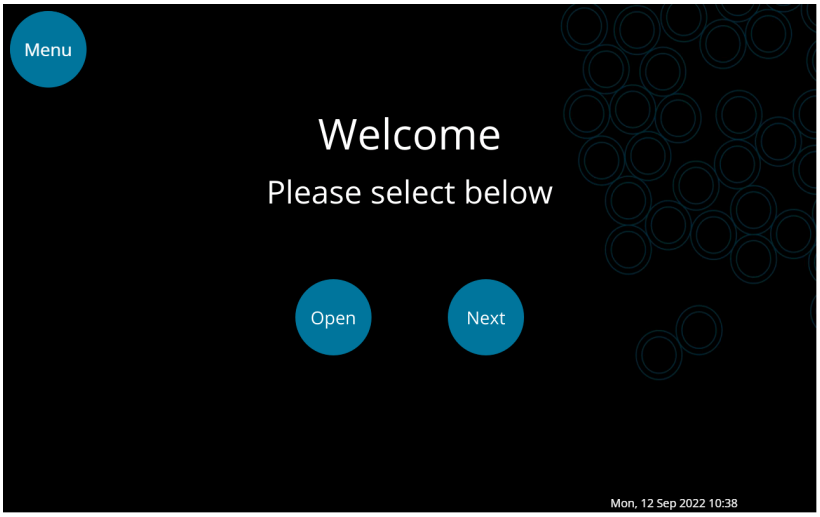


Fig. 3.5. The Welcome screen. Press Open to open the drawer.

2. The screen will now display Please insert/remove cartridge and Close. Make sure that the cartridge is correctly positioned in the drawer (Fig. 3.6) as it may otherwise cause damage to the instrument. To position the cartridge correctly, ensure that the angled corner on the cartridge is aligned with the angled corner in the drawer. Press the cartridge carefully but firmly into place. Once the cartridge is correctly inserted, press Close to close the drawer.

Fig. 3.6. Xdrop Sort with a correctly inserted Xdrop DE20 Sort Cartridge. Position the cartridge correctly to avoid damaging the instrument or cartridge.

3. Make sure droplets sediment for at least 5 minutes in the Xdrop Sort Cartridge before starting the run.
4. After waiting 5 minutes and verifying that the drawer is fully closed, press Next on the touchscreen.
5. Xdrop Sort can operate with Xdrop DE20, DE20 Sort or SE85 Cartridges. Choose the Xdrop DE20 Sort Cartridge option by selecting Sort on the touchscreen (Fig. 3.7).

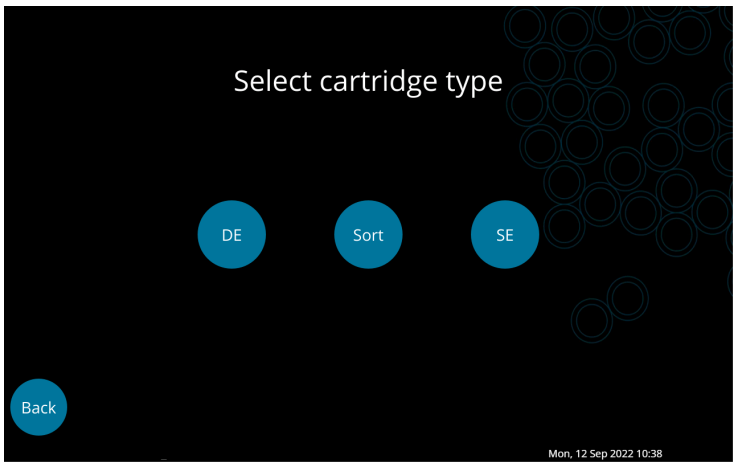


Fig. 3.7. The Select cartridge type screen. Select Sort for double-emulsion droplet sorting.

6. The lanes to be processed are selected by pressing the numbers for the corresponding instrument lanes 1–8 on the screen. When selected, the buttons turn green (Fig. 3.8).

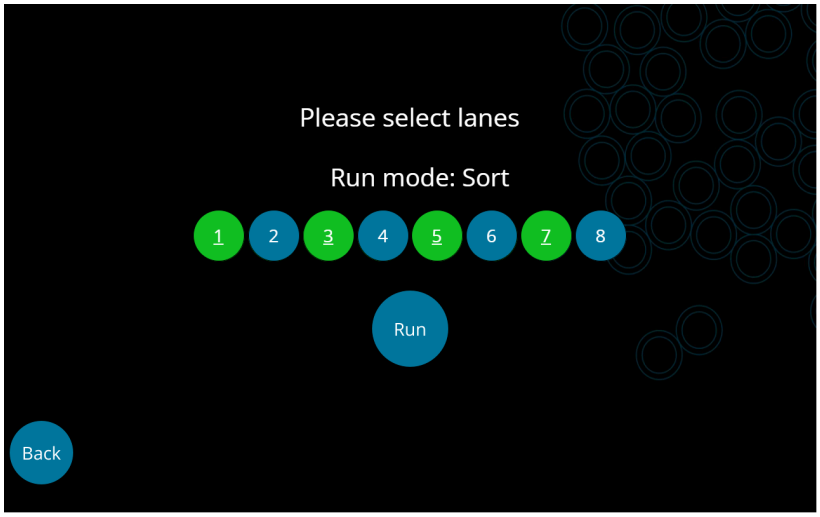


Fig. 3.8. Selecting the lanes to be used. Green indicates selected lanes in the cartridge, and dark blue indicates the remaining lanes not selected.

7. Press Run.

After alignment (~30 sec), sample analysis starts on all selected lanes, but sorting is not yet engaged. The droplet view displays the real-time analysis of the sample (Fig. 3.9).

Note: Do not press Start sorting until thresholds have been set. See Overview of droplet view screen for an in-depth explanation of the various functions.

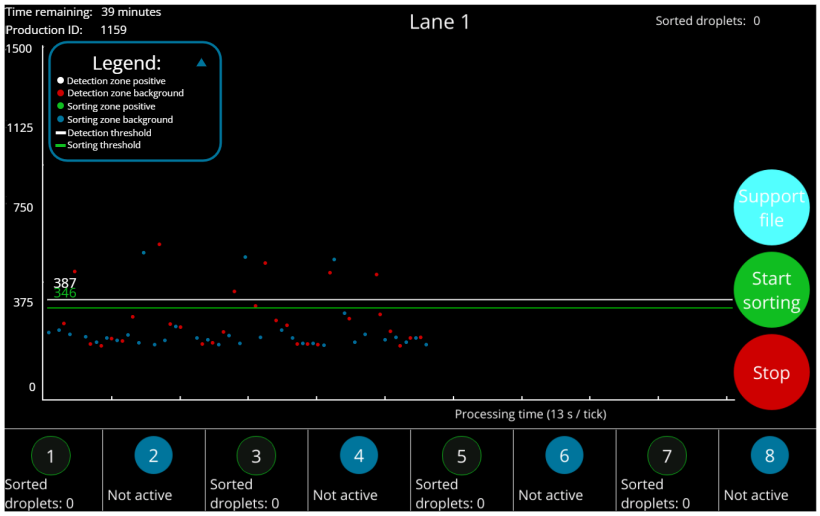


Fig. 3.9. This touchscreen image displays during droplet analysis before sorting is engaged. Note the number of sorted droplets in the upper right corner. Red dots: Detection zone background, blue dots: Sorting zone background.

8. Initially, droplets are shown in the background droplet colors (red and blue). One dot indicates the highest value detected within one second. Thresholds for detection and sorting are set by sliding the white and green lines, respectively, using the touch screen. Move the threshold lines between the lower and upper fluorescent populations. Set the thresholds for each lane pair (see Fig. 3.1 for view of lane pairs) for **all** selected lane pairs, then press Start sorting, which will activate the sorting across all lanes. The Xdrop Sort sorts the droplets from each selected lane and the count of sorted droplets can be followed separately for each lane.
9. When sorting is engaged, pairs of white dots show successful detection (i.e., a DE20 droplet with fluorescence above the detection threshold level has passed the detection laser), while pairs of green dots show successful sorting (i.e., a DE20 droplet with fluorescence above the sorting threshold level has passed the sorting confirmation later) (Fig 3.10). Thus, each successfully sorted droplet appears four times on the screen. Once sorting has started, adjust the threshold as necessary as described in below in Adjustment of sorting threshold.

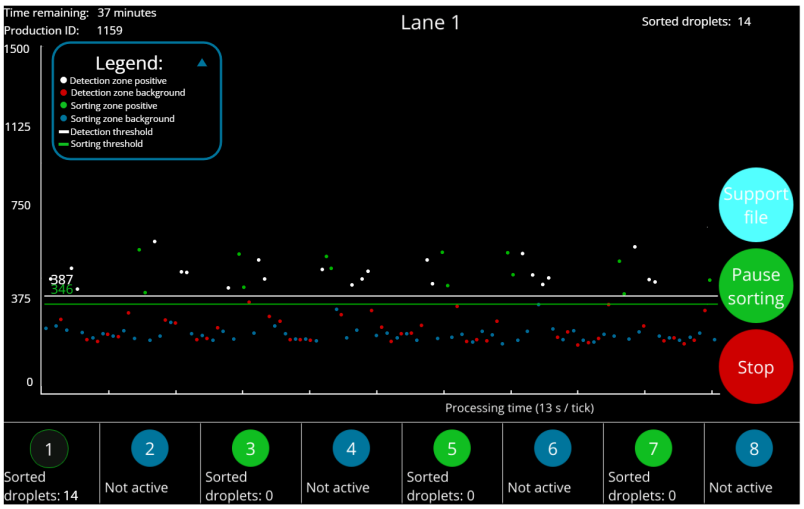


Fig. 3.10. This touchscreen image displays during droplet sorting. Note the number of sorted droplets in the upper right corner. Red dots: Detection zone background, blue dots: Sorting zone background, white dots: Detection zone positive, green dots: Sorting zone positive. Thus, each successfully sorted droplet appears four times on the screen

When sorting has been enabled, the remaining run time and the number of sorted droplets per lane are displayed on the screen together with the real-time droplet detections from the last selected lane. Optimal sorting time is dependent on the frequency of positive droplets in your sample. As a standard, total run time will be set to 40 minutes.

Note: It is very important that the instrument does not run for long with a cartridge in which one or more lanes are out of buffer or droplet sample as this may harm the instrument. In samples with a very low frequency of positive droplets, carefully observe the screen during the run, and manually stop the run using the red Stop button if 10 minutes pass without a sorted droplet. Fig. 3.11 shows an example of the display when a sample has run out.

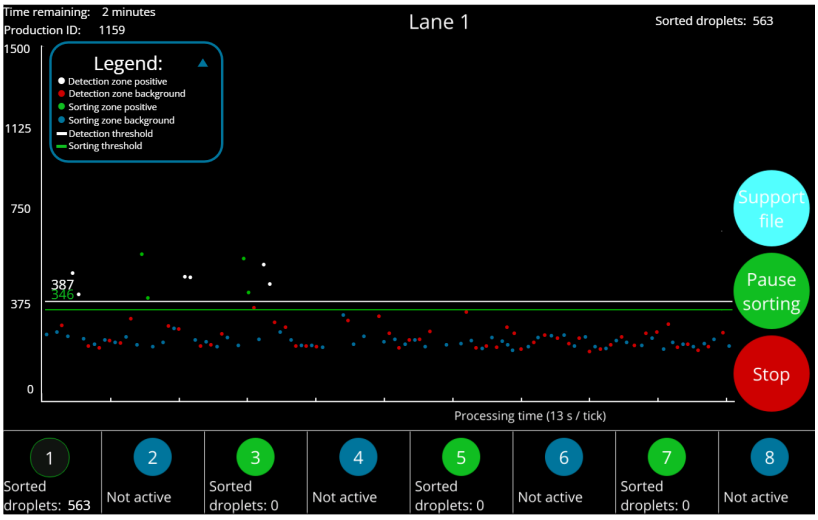


Fig. 3.11. Touchscreen display when the cartridge runs out of sample.

Overview of the droplet view screen

The droplet view (Fig. 3.12) displays the real-time analysis of the samples. Each **sorted** droplet is detected four times (two white signals for its detection, two green signals for its sorting) to ensure high purity and recovery of positive droplets. The first two detections establish the presence of a positive droplet. The third detection activates the sorting and the fourth confirms that sorting has taken place, i.e., that the positive droplet is on its way to the positive sorting well.

Note: While sorting is inactive, only background droplet colors will be displayed.

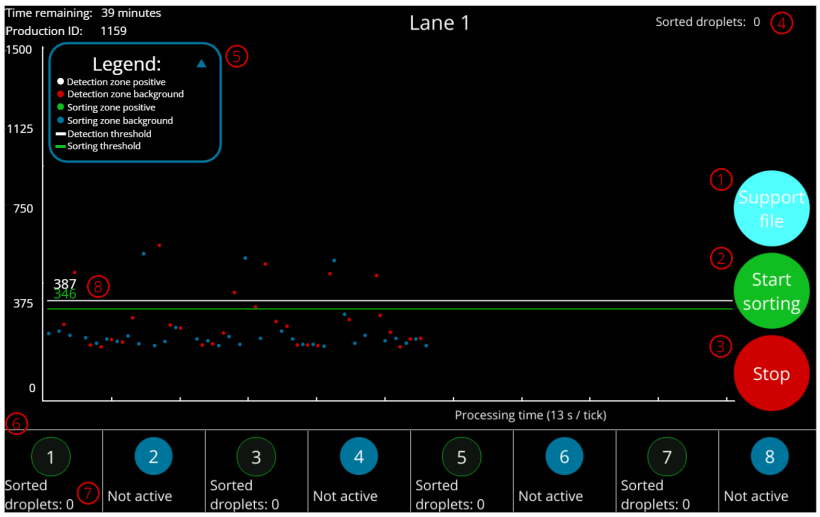


Fig. 3.12. Droplet view screen. When the run has been started without sorting, the Xdrop Sort droplet view screen will show this overview. When sorting has been started, the red and blue dots above the white and green threshold lines will respectively change to white and green, provided they match predefined sorting settings.

1. Support file button. Generate support file. See below for a full description of the process.
2. Start sorting/Pause sorting button. Activate or inactivate the sorting for all lanes.
3. Stop. End the run.
4. Sorted droplets. Counter of sorted droplets for current lane.
5. Legend. You can maximize or minimize the legend by pressing the triangle on the touch screen.
6. Lane view. The bottom of the screen displays the active (selected) and not active lanes. View another lane by pressing the relevant lane button.
7. A sorted droplets counter is also displayed for each active lane.
8. Threshold lines. Set and adjust thresholds to sort. See Adjustment of the sorting threshold below for further explanation.

Adjustment of the sorting threshold

During the run, the user can adjust the fluorescence threshold for each lane pair (see Fig. 3.1 for an overview of lane pairs). Droplets with a fluorescence level higher than the detection and sorting thresholds are sorted into the well **#Out** and will appear as green dots on the droplet view screen. Droplets with a fluorescence level below the respective thresholds are directed to the waste well **#Waste**.

To adjust a threshold, select the lane pair where the threshold needs adjustment. A graph of droplets and their relative fluorescent levels is shown for the selected lane (see Fig. 3.10). Set the threshold by sliding the threshold line to the desired level.

Collecting the sorted droplets

1. When droplet sorting has completed, the screen will change to Run ended.
2. Press View results to generate a results table that can be exported (Fig. 3.13). Insert a USB drive in the USB port in the back and press Export results to export the results table and data for analysis in Droplet viewer (Chapter 4).

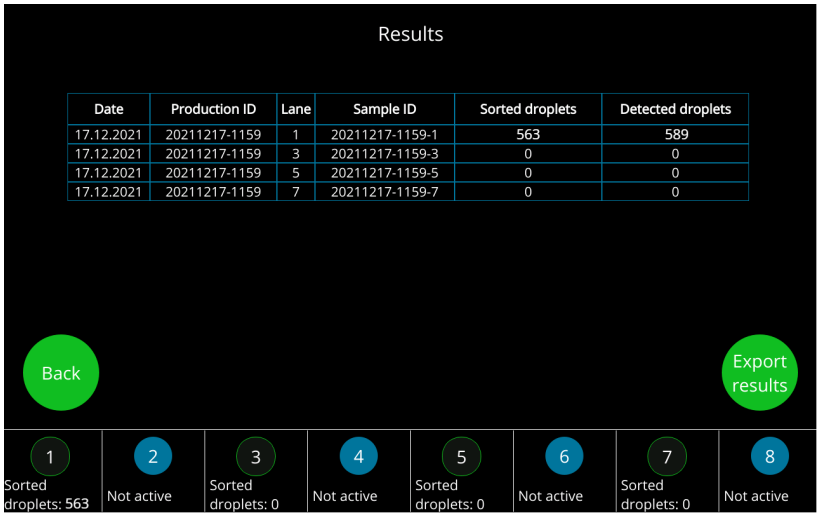


Fig. 3.13. Results table showing the date of sorting, production ID, the lanes applied, sample ID, the number of sorted droplets and detected droplets.

3. Press Back to return to the Welcome screen.
4. Press Open to open the cartridge drawer.
5. Carefully remove the cartridge from the instrument.
6. Press Close to close the drawer

7. Power down the instrument after a completed droplet sorting to avoid damage to the instrument. Push the start button at the front of the instrument to initiate the automatic shutdown procedure and power down the instrument.
8. Carefully and slowly pipette the sorted droplets from the bottom of the #**Out** well into a 1.5 ml tube immediately after removing the cartridge from the instrument. Do not pipet close to the channel connected to the **#Out** well as you may pull out negative droplets from the chip and reduce the sorting purity.
9. Wash the well #**Out** with the remaining Sort buffer from well #**B1** to ensure that you collect all the droplets as some may remain after a single collection. Add the remaining droplets to the collection tube and spin down.
10. Remove the excess liquid from well **#B2** and discard the non-sorted droplets from well **#Waste** before storing the cartridge.

The cartridge can be stored at room temperature for a second use if not all the lanes were used. Seal the entire cartridge with a second foil for sorting to cover the used wells. Before a second run, use the Xdrop Sort Lane Opener to make new holes for the wells of the relevant lanes. Lanes cannot be used a second time and a maximum of two layers of foil can be used.

Generation of support files

It is possible to generate support files during sorting. These support files can help Samplix support engineers find the root cause if no sorting of DE20 droplets is visible in the droplet view screen or if there is a high background signal presented as red or blue dots in the droplet view screen. The support files can be acquired as described in the following section.

1. Once the thresholds have been set on all the lanes press Start sorting (Fig. 3.14).

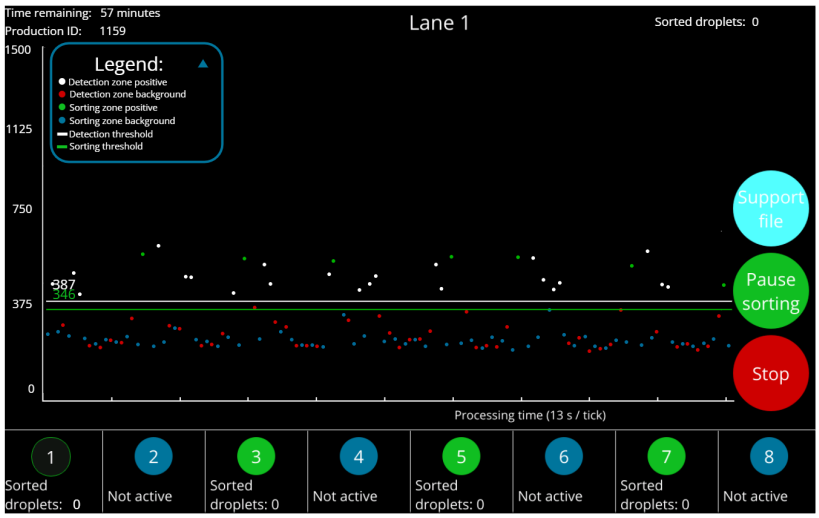


Fig. 3.14. This droplet view screen displays during droplet analysis when sorting is applied.

2. Press Support file (light blue button).
3. A warning message will appear stating that during support file generation sorted droplets are not counted. Press Yes to continue (Fig. 3.15).

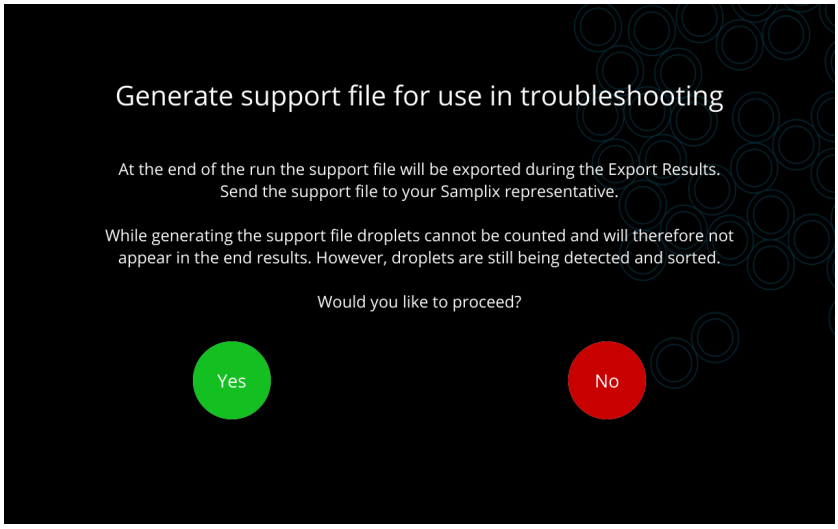


Fig. 3.15. The screen stating that during support file generation droplets cannot be counted and will not appear in the end results.

4. The support files will only be generated for one lane pair at a time. Once the support file has been generated, press Ok to return to droplet view.
5. Proceed to generate support files for the first lane pair. Generate at least 2 support files for each sorting lane pair.
6. Press Stop.
7. The instrument will change to the Run ended view. Note the run ID.
8. Select View Results.
9. Press Export results. The screen will show a list of files that will be exported.
10. Press Copy files. Insert the USB stick provided by Samplix to transfer the files.
11. Once the screen shows Transfer done, press Back to return to the result screen.
12. Remove the USB drive.
13. On the USB drive, the transferred data will be in the folder with the run ID. Send all the files in this folder to your Samplix representative.
14. Proceed to generate support files for the remaining lanes. By repeating steps 6 to 13 for each lane pair, generating at least 2 support files for each sorting lane pair.

Optional: Breaking sorted double-emulsion droplets

For some workflows, e.g., targeted enrichment of DNA, the contents of sorted DE20 droplets must be released by breaking the droplets. This is done using the Small volume droplet break kit (REFKITBRESMVL100), containing: Droplet break solution ● and Droplet break color ● as described below. This is not essential for all workflows.

Note: Before starting, vortex the Droplet break color ● tube upside down and spin it. This is required to ensure that the reagent is fully homogenized and works correctly.

15. Remove the DE sorting buffer without disturbing the pellet.
16. The pellet must be washed twice to remove all DE sorting buffer. To do this, add **200 µl** of fresh Droplet sorting wash buffer ● and spin down the droplets.
17. Remove the Droplet sorting wash buffer and repeat step 2.
18. Remove most of the Droplet sorting wash buffer ● without disturbing the pellet leaving 10-20 µl wash buffer with droplets and add **20 µl** Droplet break solution ● to each tube.
19. Add **1 µl** of Droplet break color ●. This will color the water phase. If coloring is too weak, add an extra **1 µl** of Droplet break color.

    Note: The water phase may be a color ranging from yellow to purple as the Droplet break color functions as a pH indicator.
20. Flick the tube gently and spin it briefly (15–30 seconds). Do not vortex it (see Fig. 3.16).
21. Remove the clear Droplet break solution phase from the bottom of the tube and discard.
22. Repeat steps 6 and 7 to remove all leftover Droplet break solution.
23. Keep the colored water phase, which will contain the contents of the droplets.

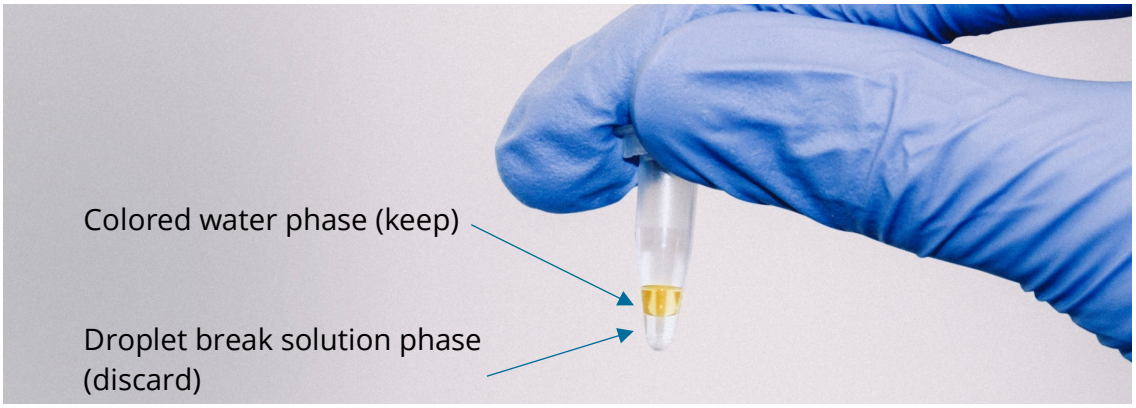


Fig. 3.16. Break the sorted double-emulsion droplets using the Small volume droplet break kit (REFKITBRESMVL100), containing: Droplet break solution ● and Droplet break color ●. Discard the clear Droplet break solution phase at the bottom of the tube. Keep the top, colored water phase.

Chapter 4: Analyzing sorting data with Droplet viewer software

After a sorting run, data can be exported by inserting a USB stick at the back of the instrument and pressing the Export results button (see Fig. 3.13). The CSV files exported from View results include the Summary file and a CSV file for each used lane and can be viewed using the Droplet Viewer, which is one of the Digital Tools on the Samplix website. Interactive plots are automatically generated, and you can choose to view the different zones (detection and sorting) to get an overview of your entire run. The app also allows you to easily download the generated plots.

Log-in

Log-in using your samplix.com user credentials.

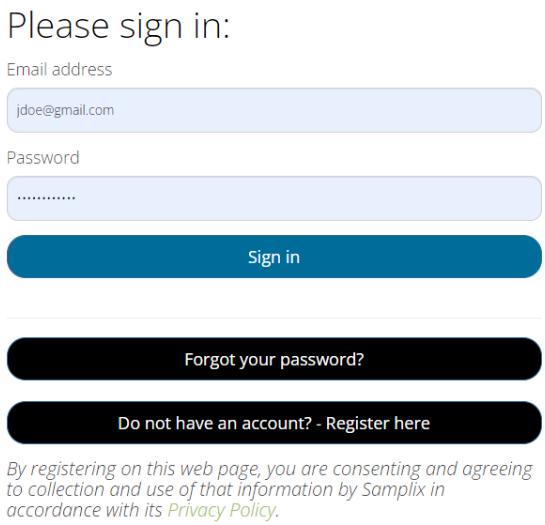


Fig. 4.1. Xdrop Sort Droplet Viewer log-in screen.

Upload files

1. Click Browse under Choose folder to select the whole run folder or Browse under Choose CSV file to select CSV file(s) from your Xdrop Sort run (Fig. 4.2).

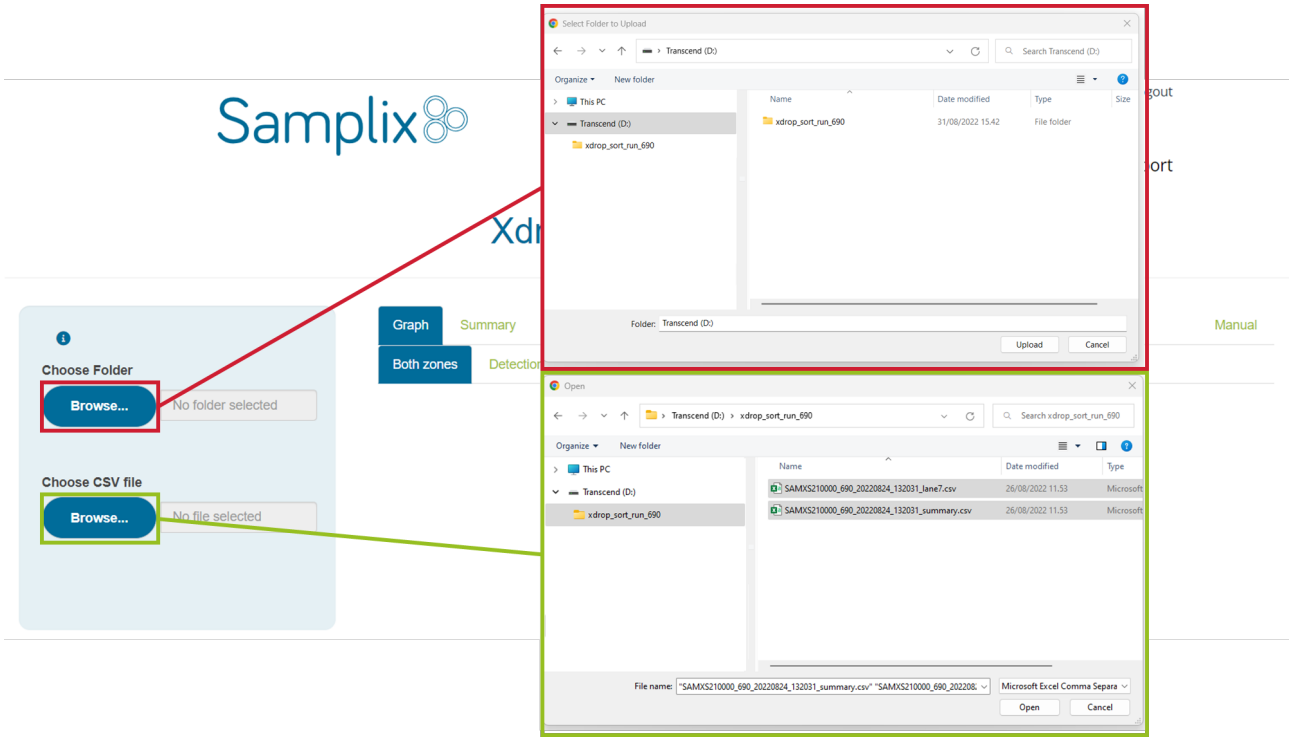


Fig. 4.2. Use the browse button to upload your Xdrop Sort run.

2. If uploading a whole folder, a pop-up will appear asking for confirmation. Click Upload to approve the upload of the files (Fig. 4.3).

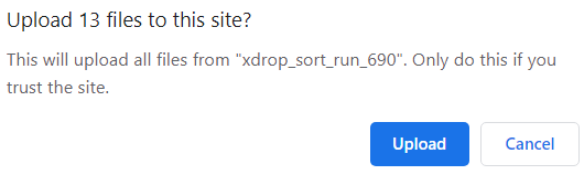


Fig. 4.3. Click Upload to confirm upload of your Xdrop Sort run.

3. Once the upload is complete the runs will appear in the *navigation bar*. To upload multiple files/folders click the Browse button again, and these new runs will appear in the *navigation bar* (Fig. 4.4).

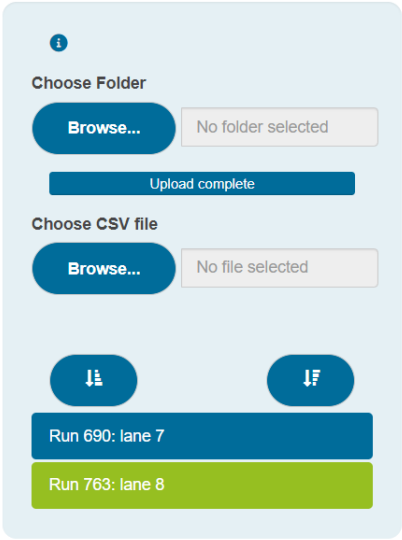


Fig. 4.4. All uploaded runs will appear in the navigation bar.

4. The uploaded runs can be sorted in ascending and descending order using the sorting buttons (Fig. 4.5).

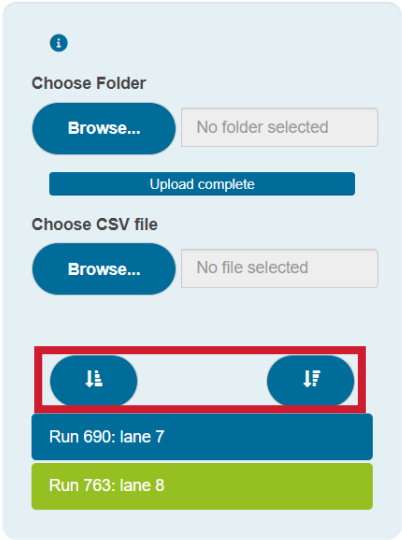


Fig. 4.5. Use the sorting buttons to sort the runs in ascending and descending order.

View graphs

The interactive plots are automatically generated. Use the tabs to navigate between the different graphs: Both zones, Detection zones and Sorting zones. See the section Interactivity below for use of the interactive buttons.

Both zones

The Both zones tab displays the detection and the sorting zone together in one graph, which provides an overview of the entire Xdrop Sort run. Detection zone positive (●) indicates a droplet above the Detection threshold. Detection zone background (●) indicates noise or a droplet under the Detection threshold (—). Sorting zone positive (●) indicates a droplet above the Sorting threshold. Sorting zone background (●) indicates noise or a droplet under the Sorting threshold (—). The Sorting zone sorted (●) indicates that this droplet counted as a sorted droplet in the droplet counter. The Sorted droplets box provides the total number of sorted droplets in this lane, if the summary file has been provided (Fig. 4.6).

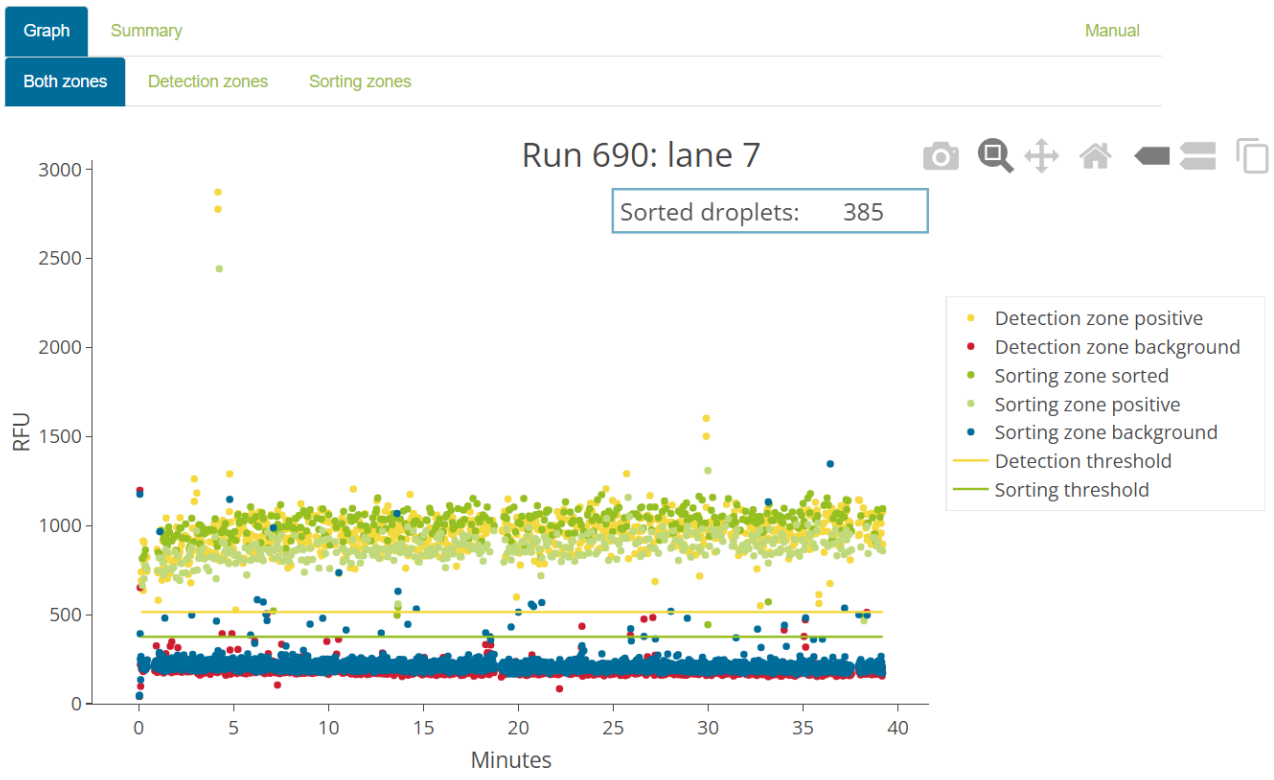


Fig. 4.6. Plot displaying both the detection and sorting zones.

Detection zones

The Detection zones tab displays the detection and the sorting zone together in one graph, which provides an overview of the entire Xdrop Sort run. The Detection zone positive (●) indicates a droplet above the Detection threshold. The Detection zone background (●) indicates noise or a droplet under the Detection threshold (—). The Sorted droplets box provides the total number of sorted droplets in this lane, if the summary file has been provided (Fig. 4.7).

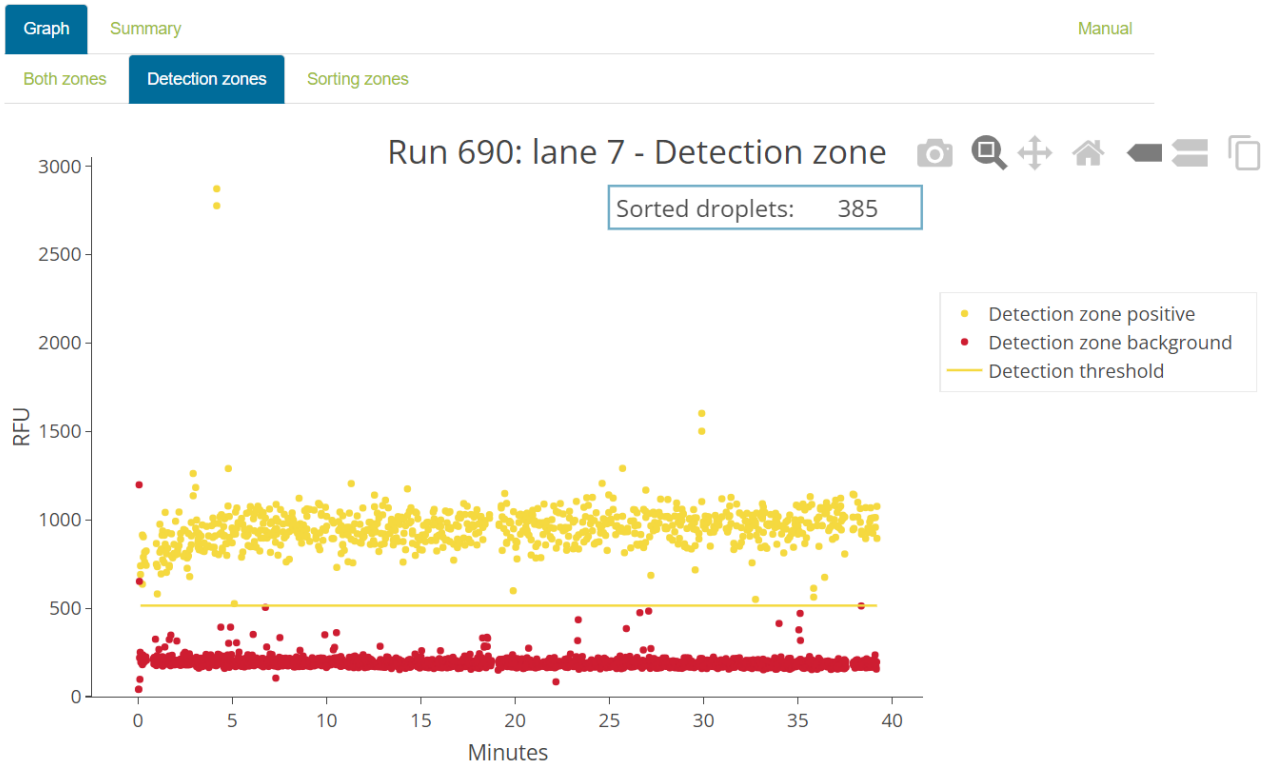


Fig. 4.7. Plot displaying the Detection zones.

Sorting zones

The Sorting zones tab displays the detection and the sorting zone together in one graph, which provides a nice overview of the entire Xdrop Sort run. Sorting zone positive (●) indicates a droplet above the Sorting threshold. Sorting zone background (●) indicates noise or a droplet under the Sorting threshold (—). The Sorting zone sorted (●) indicates that this droplet counted as a sorted droplet in the droplet counter. The Sorted droplets box provides the total number of sorted droplets in this lane, if the summary file has been provided (Fig. 4.8).

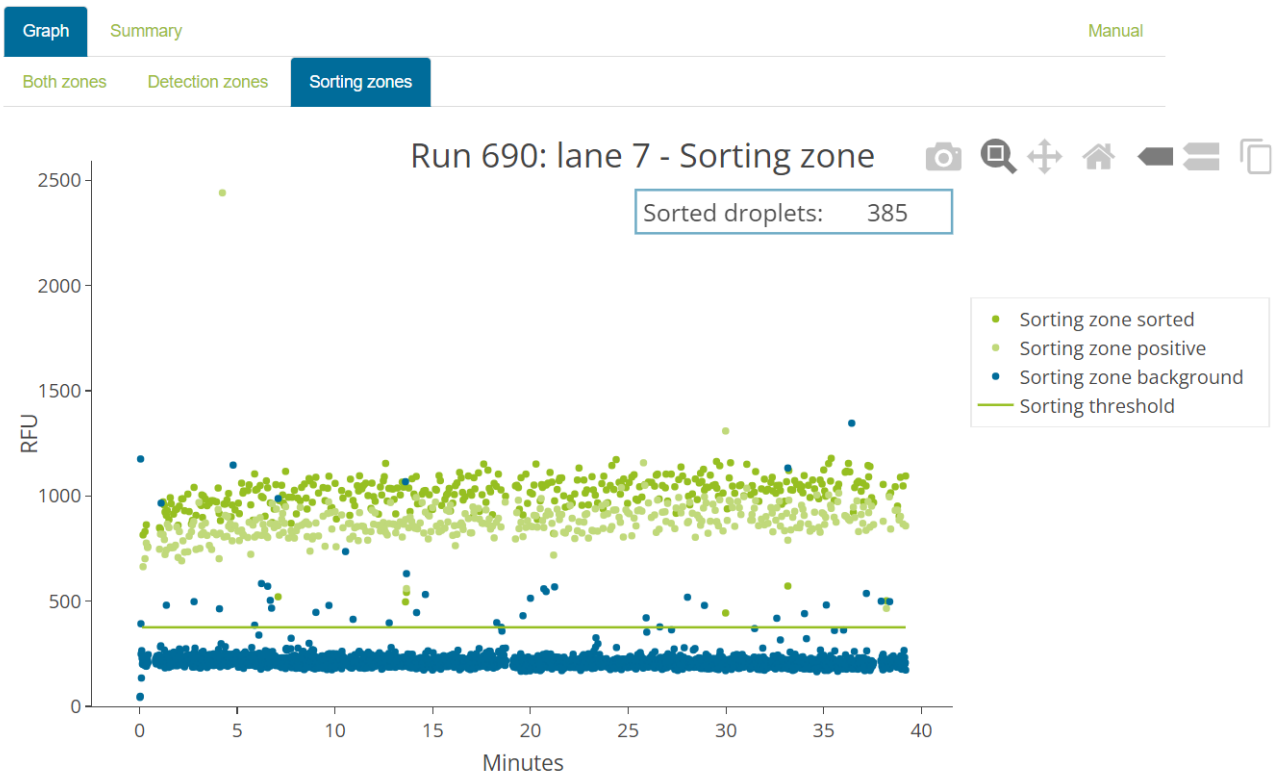


Fig. 4.8. Plot displaying the Sorting zones.

Interactivity

All generated plots are interactive. The following table described the functions of the mode bar in the upper right corner of the screen (Fig. 4.9). Here is a key to the mode bar.

Download plot	Zoom	Pan	Auto-scale	Show closest data on hover	Compare data on hover	Copy plot to clipboard

Fig. 4.9. Use the mouse to select an area of the plot to zoom in on. Use the scroll button on your mouse to zoom in and out.

View summary table

The summary table is automatically generated when a summary csv file is provided together with a lane csv file (Fig. 4.7).

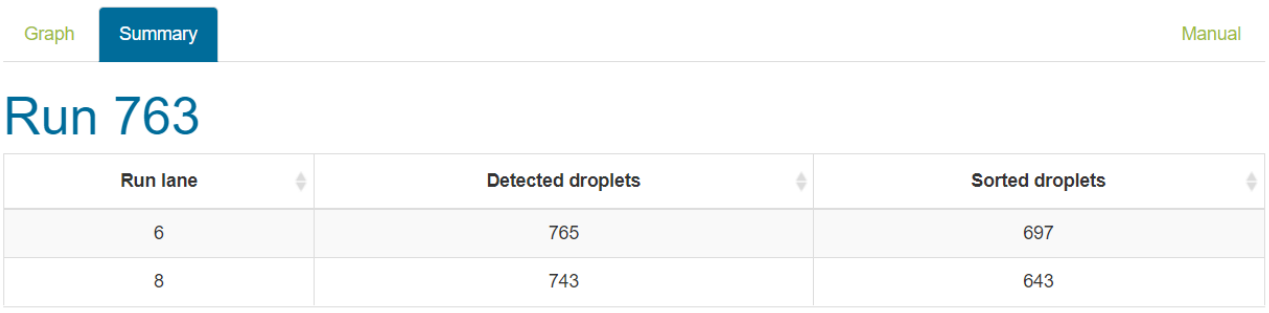


Fig. 4.10. Summary table of run.

Tool tips

Toggle the tool tips on to enable tips when hovering over buttons and tab (Fig. 4.11).

Tool tips can be toggled on/off.

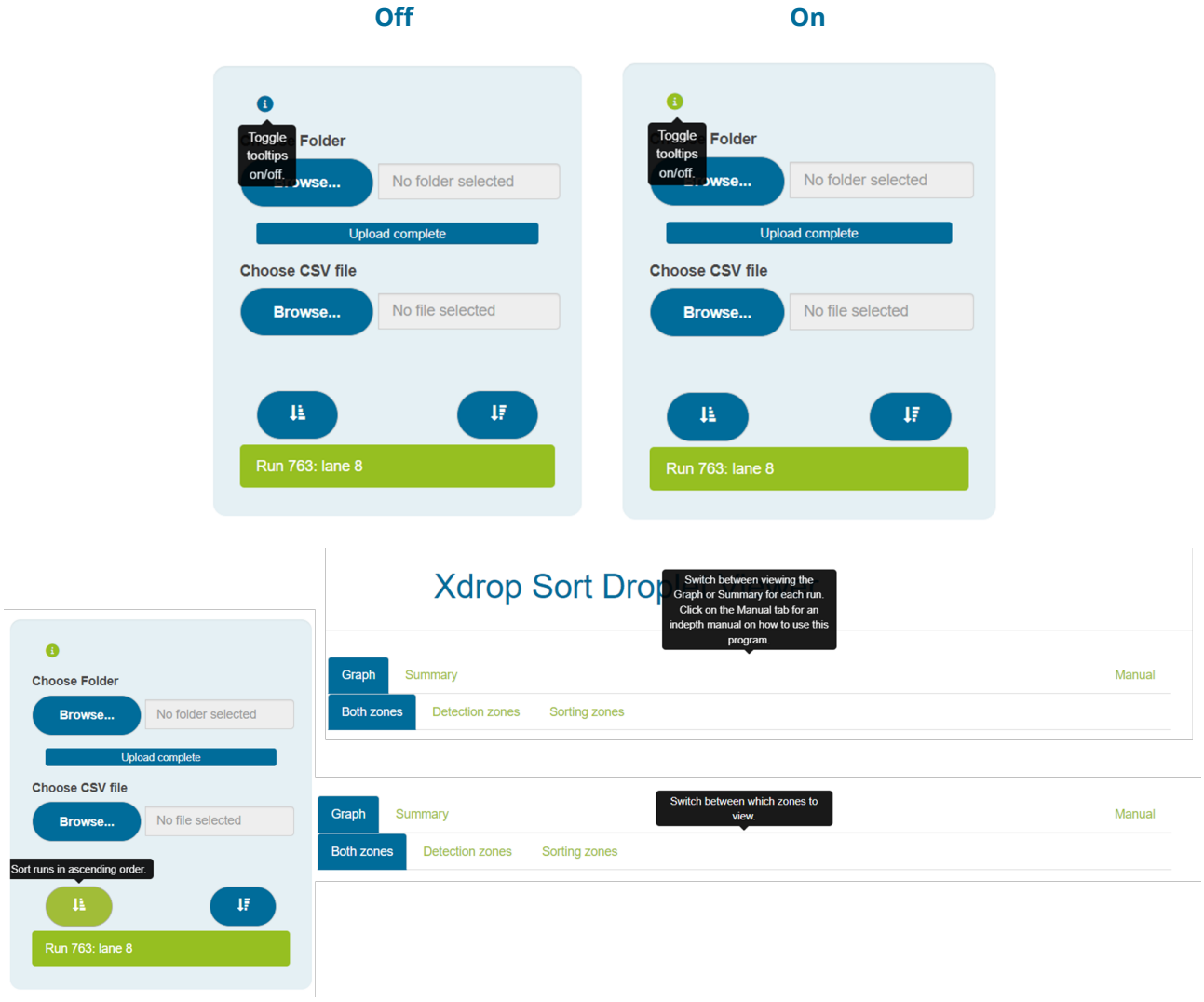


Fig. 4.11. Toggle on tool tips to enable tips when hovering over buttons and tabs.

Chapter 5: Single-emulsion droplet production with Xdrop SE85 Cartridge

Single-emulsion droplets are produced using the Xdrop SE85 Cartridge inserted in the Xdrop SE85 Holder (Fig. 5.1). The Xdrop SE85 Holder is reusable for multiple runs. It is provided together with your Xdrop or Xdrop Sort, but if needed, additional Xdrop SE85 Holders (HOSE85A100) can be purchased separately.

As with the other cartridges, the Xdrop SE85 Cartridge must be loaded in a clean LAF (laminar air flow) cabinet and then sealed with a gasket for droplet production.

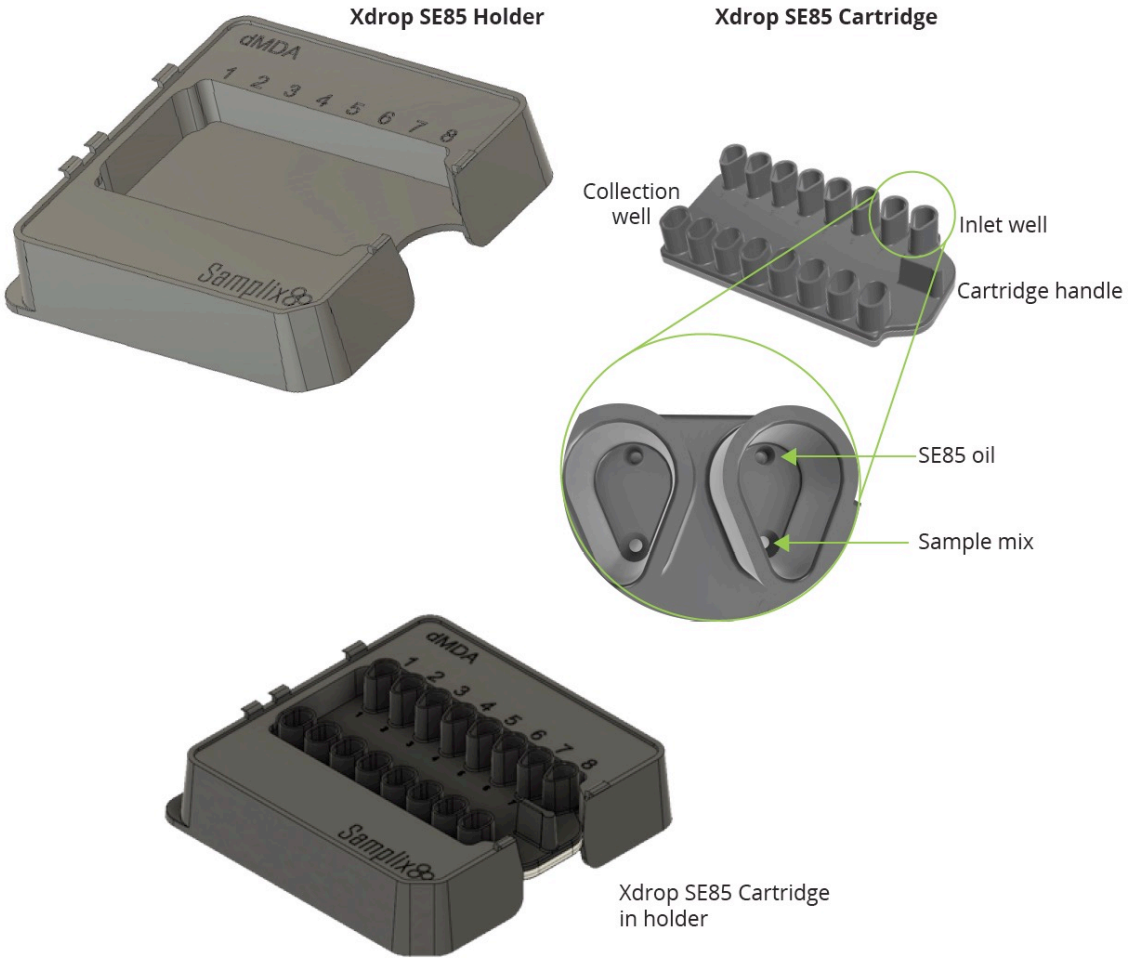


Fig. 5.1. Left: Xdrop SE85 Holder. Right: Top view of Xdrop SE85 Cartridge with inlet well and collection wells marked. Insert: Close-up view of the holes in an inlet well. Bottom: The assembled Xdrop SE85 Cartridge and Holder.

Preparing and loading the Xdrop SE85 Cartridge

1. Wearing gloves, unpack the Xdrop SE85 Cartridge and Holder from their original packaging.
2. Handle the cartridge as follows:
   - Always use gloves when handling the cartridge.
   - Hold the cartridge by its sides or by its handle (see Fig. 5.1).
   - Do not touch any of the inlet wells or droplet collection wells.
   - Avoid DNA contamination throughout loading and handling.
   - If only partially used, cover the cartridge with a protective storage film in a clean, sealed plastic bag.
3. Be careful not to use the same sample lane more than once as this will disrupt droplet production and contaminate your sample. Mark the storage film above used lanes with a permanent marker to avoid repeat usages.
4. Place the Xdrop SE85 Cartridge in the groove of the Xdrop SE85 Holder as shown in Fig. 5.1. The inlet wells go on the side with the numbers and the collection wells on the side with the Samplix logo.
5. Using an appropriate manual pipette and a wide-bore P200 pipette tip with an outer diameter of 1–1.9 mm, collect **20 µl** of your sample mix (e.g., DNA with MDA mix). To avoid using the same lane more than once, mark the storage plastic bag with a permanent marker once a lane has been used.

   Note: Wide-bore P200 pipette tips with an outer diameter of 1–1.9 mm are mandatory for loading samples into the Xdrop SE85 Cartridge to ensure the required fit with the sample mix inlet hole.
6. Place the pipette tip vertically in the inlet hole at the bottom of the inlet well (the narrow end of the teardrop), making a tight connection (Fig. 5.2):

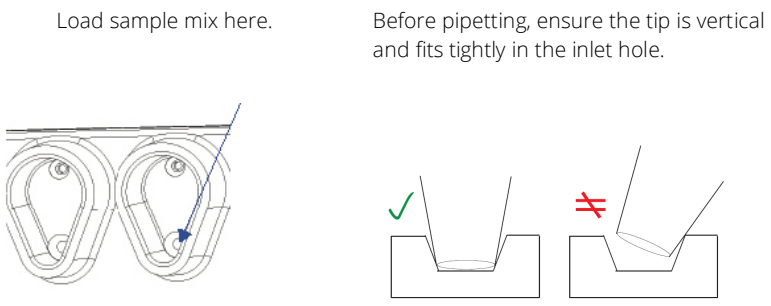


Fig. 5.2. Loading considerations for the Xdrop SE85 Cartridge

7. Slowly inject the sample until you reach the first stop position on the pipette plunger. **Without adding any more pressure to the plunger**, hold it for 15 seconds and ensure that you do not lose the tight connection of the pipette tip and the cartridge (Fig. 5.3).
8. Remove the pipette **while still holding the plunger button in the first position**. The entire sample should have entered the channel in the chip and there should be no liquid visible in the well.
9. Repeat steps 5 to 8 for the next lane to use, if applicable.
10. Add **75 µl** Droplet oil (SE) ● to the side of the inlet well, allowing it to flow gently into the reservoir in the loaded lane(s). Do not inject the oil directly into the upper channel hole.
11. Place the gasket on top of the cartridge and fix it in place using the T-hooks (Fig. 5.4).

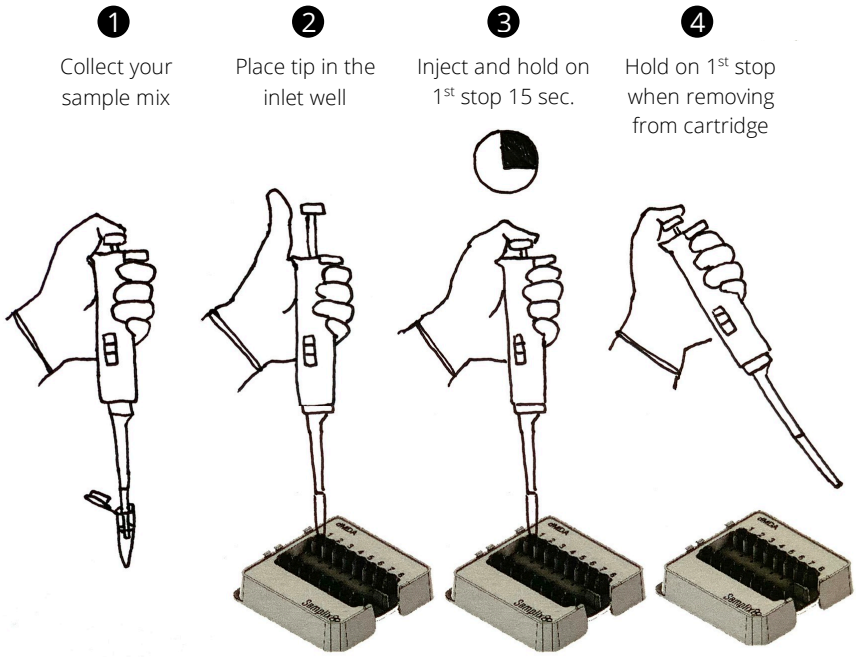


Fig. 5.3. Visualization of steps 5 through 8 for loading of the Xdrop SE85 cartridge.

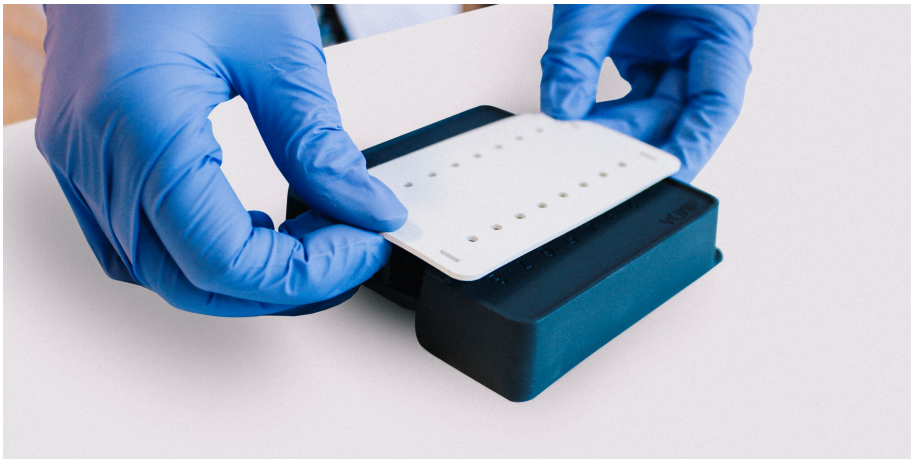


Fig. 5.4. Attach the white rubber Xdrop SE 85 Gasket to T-hooks of the Xdrop SE85 Cartridge. Note that a gasket should only be used once to avoid contamination.

Running single-emulsion droplet production with Xdrop Sort

Before turning the instrument on, check that the main power switch is in the "I" position. The main switch is located at the back of the instrument. Start the instrument by pushing the start button at the front. The Welcome screen will appear.

1. Press Open on the Xdrop Sort instrument touchscreen to open the drawer (Fig. 5.5).

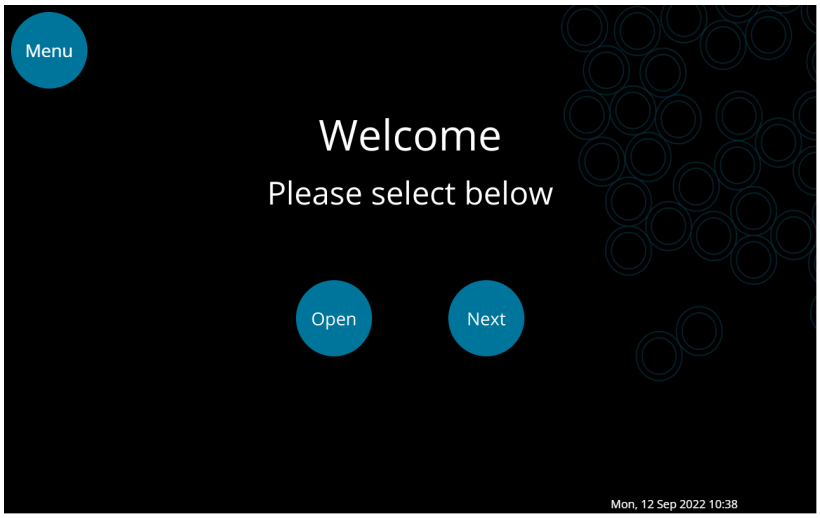


Fig. 5.5. The Welcome screen. Press Open to open the drawer.

2. The screen will now display Please insert/remove cartridge and Close. Make sure that the cartridge is correctly positioned in the drawer (Fig. 5.6) as it may otherwise cause damage to the

instrument. To position the cartridge correctly, ensure that the angled corner on the cartridge is aligned with the angled corner in the drawer. Press the cartridge carefully but firmly into place. Once the cartridge is correctly inserted, press Close to close the drawer.

Fig. 5.6. Photo of Xdrop Sort with a correctly inserted Xdrop SE85 Cartridge.

3. Once the drawer is fully closed, press Next on the touchscreen.

4. Xdrop Sort can operate with Xdrop DE20, DE20 Sort, or Xdrop SE85 Cartridges. Access the Xdrop SE85 Cartridge protocol by pressing SE on the touchscreen (Fig. 5.7).

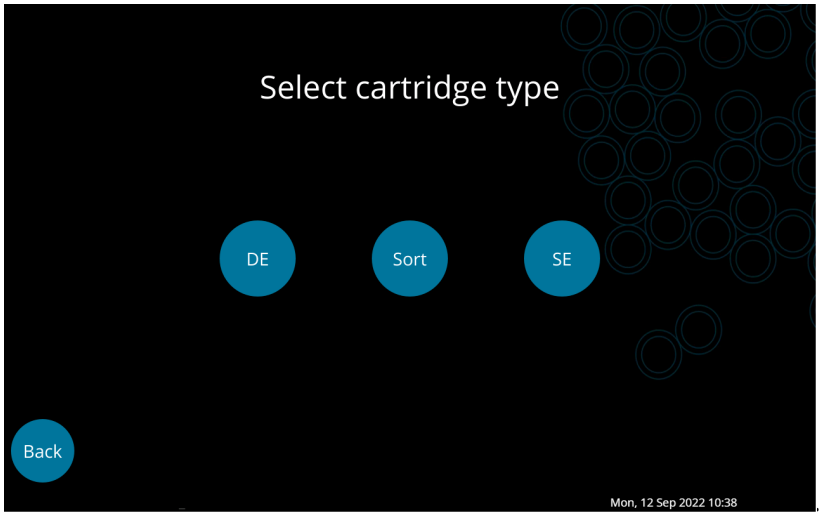


Fig. 5.7. The Select cartridge type screen. Select SE for single-emulsion droplet production.

5. The lanes to be processed are selected by pressing the numbers for the corresponding instrument lanes 1–8 on the screen. When selected, the button turns green (Fig. 5.8).

Fig. 5.8. Select the lanes to be used. Green indicates selected lanes corresponding to lanes in the cartridge and blue indicates the lanes not yet selected.

6. Press Run.

Once optimal pressures have been reached, the message Making your droplets appears and the remaining run time is displayed on the screen (Fig. 5.9). Single-emulsion droplet production on Xdrop Sort takes approximately 45 seconds.

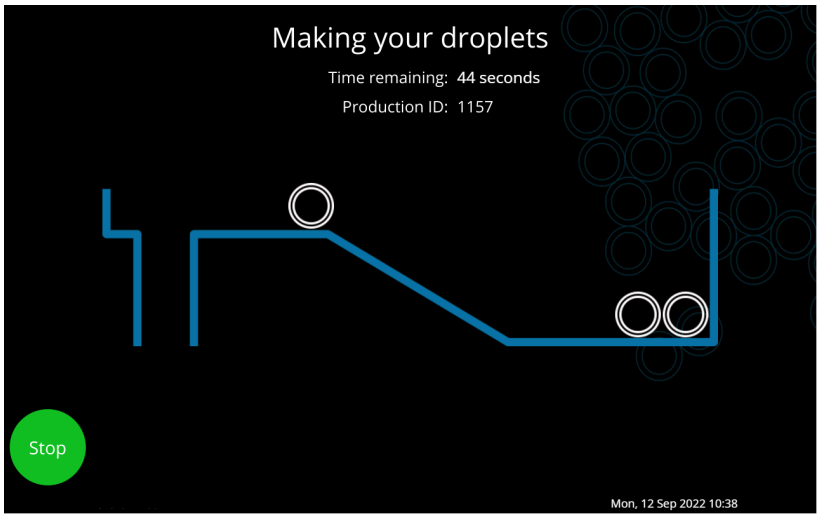


Fig. 5.9. This image appears during droplet production.

7. When droplet production has finished, the screen will change to Your droplets are ready.
8. Press Open to open the cartridge drawer.
9. Carefully remove the cartridge from the instrument and place it in an LAF cabinet.
10. Press Close to close the drawer.
11. Press Finish to return to the Welcome screen.
12. Power down the instrument after a completed droplet production to avoid damage to the instrument. Push the start button at the front to initiate the automatic shutdown procedure and power down the instrument.

Collecting droplets generated with an Xdrop SE85 Cartridge

1. Collect all the single-emulsion droplets from the collection well with a P200 pipette and transfer them into a nuclease- and DNA-free PCR tube. Collect them by slowly pipetting from the sides towards the center of the well. The total volume of single-emulsion droplets and oil in each collection well should be 70–100 µl (Fig. 5.10).

2. Inspect the volume of collected droplets before removing the oil. The droplet layer is on top of the oil phase and should be approximately 2–3 mm thick.
3. Remove all but 1–2 mm of oil from the bottom of the collection tube (Fig. 5.10).

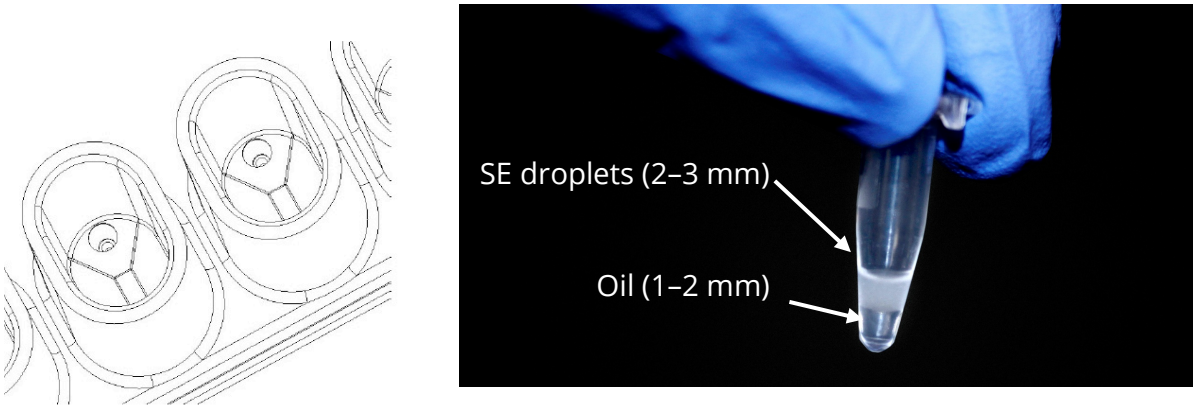


Fig. 5.10. Collection of single-emulsion droplets from the collection well. Left: Drawing of a collection well. Note that the sides slant slightly towards the inlet hole of the well. Collect the droplets by pipetting slowly from the sides to the center of the well. Right: Single-emulsion droplets in a PCR tube after collection. The droplets form a white layer on top with the excess oil at the bottom.

Xdrop SE85 Cartridge storage after use

Note: The production lanes and gaskets are single-use and will not function properly if reused. Note also that reuse of cartridges or gaskets increases the risk of sample cross-contamination.

If some lanes are still unused after a run, place the Samplix Storage film (Cat. No. FI00100) over the cartridge without removing it from its holder (Fig. 5.11). Store the Xdrop SE85 Cartridge and Holder in a Ziplock bag for up to **4 weeks** at room temperature. The Storage film should be placed so that all wells (used and unused) are sealed. Note that the Xdrop SE85 cartridge has a shelf-life of **4 weeks** after the packaging has been opened, provided that this period does not exceed the expiry date indicated on the product.

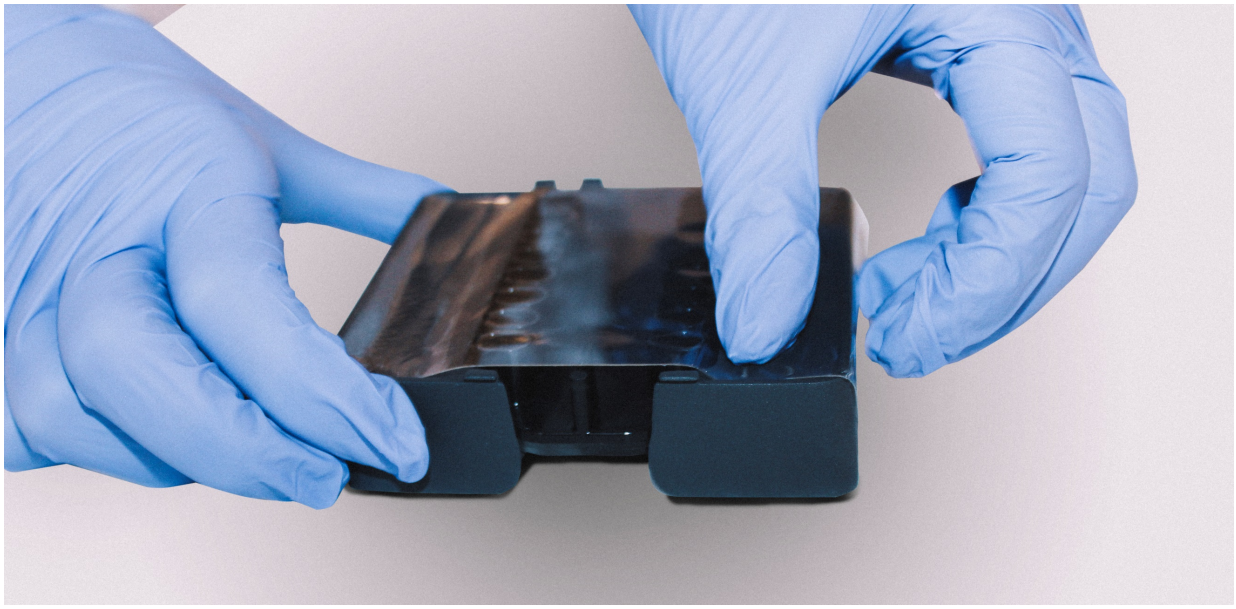


Fig. 5.11. Place a Samplix storage film on the partially used Xdrop SE85 Cartridge to seal all the wells, thus avoiding cross-contamination from used wells to unused wells.

Optional: Breaking single-emulsion droplets

For some workflows, the contents of single-emulsion droplets must be released by breaking the droplets. This is done with Droplet break solution ● and Droplet break color ● as described below. This is not essential for all workflows.

Note: Before starting, vortex the Droplet break color ● tube upside down and then spin it. This is required to ensure that the reagent is fully homogenized and works correctly.

1. Add **20 µl** of Droplet break solution ● to each tube of single-emulsion droplets.
2. Add **1 µl** of Droplet break color ●. This will color the water phase. If coloring is too weak, add an extra **1 µl** of Droplet break color.

   Note: The water phase may be a color ranging from yellow to purple as the Droplet break color functions as a pH indicator.
3. Flick the tube gently and spin it briefly (15–30 seconds). Do not vortex it.
4. Remove the clear Droplet break solution phase from the bottom of the tube and discard.
5. Repeat steps 3 and 4 to remove all leftover Droplet break solution.
6. Keep the colored water phase, which will contain the contents of the droplets (Fig. 5.12).

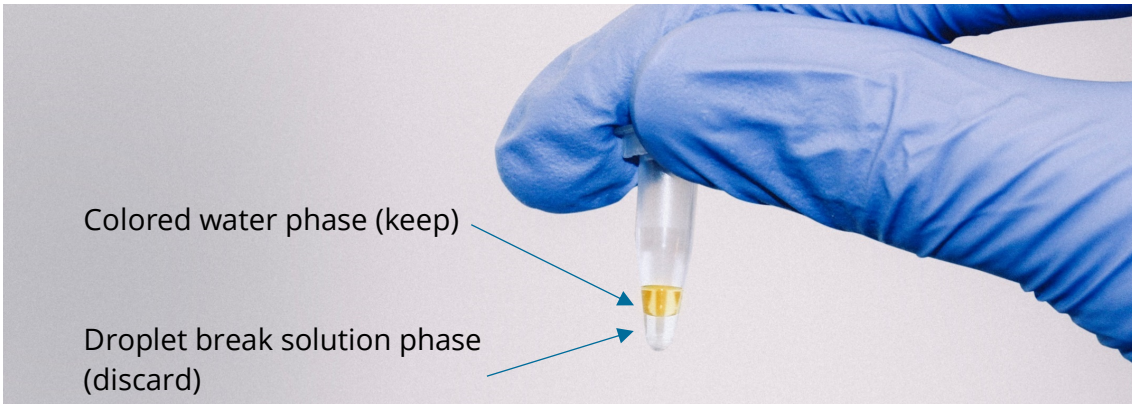


Fig. 5.12. Break single-emulsion droplets with Droplet break solution ● and Droplet break color ●. Discard the clear Droplet break solution phase at the bottom of the tube. Keep the top, colored water phase.