Samplix

# Xdrop Manual for High Throughput Screening

Experimental manual v. 1.2 released August 2021

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# Chapter 1: Xdrop at a Glance

## Workflow overview

Congratulations on your new Samplix® Xdrop® instrument, which we expect will facilitate groundbreaking research.
The Xdrop introduces a new approach for high throughput analysis of cells and molecules. Our innovative technology enables convenient screening of millions of parallel reactions in droplets with the option of recovering selected cells or molecules using a cell sorter.
Using the Xdrop instrument, cartridges, and reagents, samples are partitioned into millions of picolitre-sized droplets. The Xdrop workflow includes six steps:

1. Mixing sample with assay reagents to prepare for the droplet assay.
2. Sample encapsulation in double emulsion droplets (Chapters 2 and 3)
3. Run the detection assay in droplets e.g. by incubation
4. Screening and sorting using a cell sorter (Chapter 4)
5. Releasing target cells or molecules by adding break solution (Chapter 4.5)
6. Recover the cells or molecules for downstream application by culturing (cells), amplification (DNA), or other downstream process (Chapter 5 and 6)

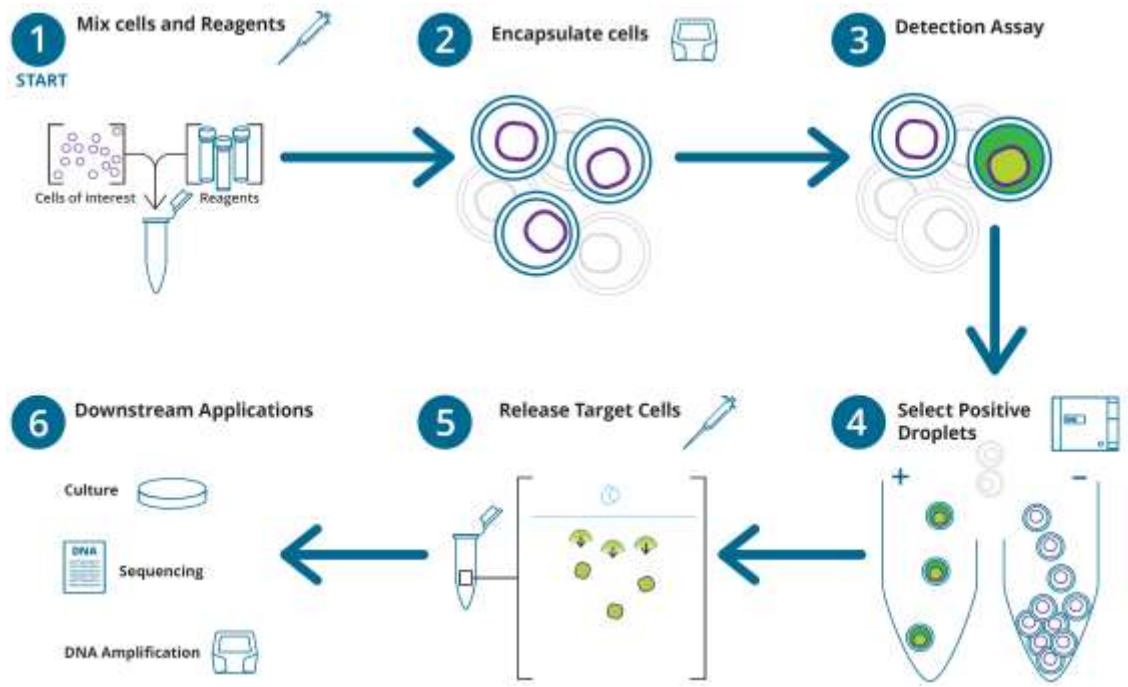



Fig. 1.1. The Xdrop Workflow at a glance. Start with a mixture of molecules or cells and end with a sample enriched for targets with selected properties.

## Xdrop instrument overview

The Xdrop droplet generator instrument is compatible with 2 sets of cartridges; (1) one producing double emulsion droplets (called dPCR cartridges) and the other generating single emulsion droplets (called dMDA cartridges). When using dMDA cartridges, always use the accompanying holder. The Xdrop droplet generator is used for generating both dPCR and dMDA droplets and consists of the following parts (see figure below):

- A start button on the front of the instrument.
- A touchscreen to control the droplet generator with gloved or un-gloved hands.
- A status LED that is purple when initializing and shutting down, green when in standby, blue when operating, and yellow-green when opening and closing the drawer.
- The loading drawer that holds the dPCR or dMDA cartridge.
- A USB port on the back of the instrument to connect a USB flash drive for updating instrument firmware.
- Air vents on the back of the instrument for ventilation.
- A hardware switch on the back of the instrument.

## Specifications

Width: 25 cm / 9.8 inches
Height: 25 cm / 9.8 inches
Length: 48 cm / 18.9 inches
Weight 17 kg / 37.5 lbs.
Voltage requirements: 110 V – 240 V (Line frequency; 50–60 Hz, Max current: 2.3 A)

## Support

For technical support, contact the support team at support@samplix.com

## Warranty

The Xdrop instrument and associated accessories are covered by a standard Samplix ApS warranty. Contact your local Samplix ApS office for the details of the warranty.

## Safety

We strongly recommend that you follow the safety specifications listed in this section and throughout this manual.

Xdrop is produced to comply with Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use (UL 61010-1) and complies with EU (CISPR 11, class A, group 1, 150 kHz – 30 MHz) EMC.

This equipment has also been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. 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. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

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

Xdrop operating conditions are at temperatures 20–25°C, with a relative humidity (RH) 0–75%. Xdrop requires a stable power supply and can be powered using mains voltage of 100–240 VAC, 50–60 Hz. Main power fuses (F3A, 250 V, 5x20 mm) are located behind a small black plastic cover between main switch and the power main plug at the back of the instrument. The Xdrop instrument must be installed on a flat surface where access to the main power outlet is not restricted. The instrument is not to be used against the manufacturer's instructions. Failing to comply with the 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 instrument.

| Icon | Meaning |
|---|---|
|  | Warning about the risk of harm to body or equipment. Operating the Xdrop 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 these instruments, you put yourself at risk for electrical shock and void your warranty. All repairs must be done by an authorized repair service. |
|  | Warning about the risk of harm to hands and fingers. Always keep hands and fingers away from the instrument when the drawer is in motion. |

## Intended use and intended users

The Xdrop instrument is intended for use by trained laboratory personnel in a clean laboratory environment for DNA or cell sample 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 and cleaning

If the instrument is shipped back to Samplix for maintenance, please make sure that the outer surfaces are cleaned using a cloth and 70 % ethanol.

## Xdrop installation and set-up quick guide

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. Slide a hand into the box on each side of the instrument and lift it out of the box.

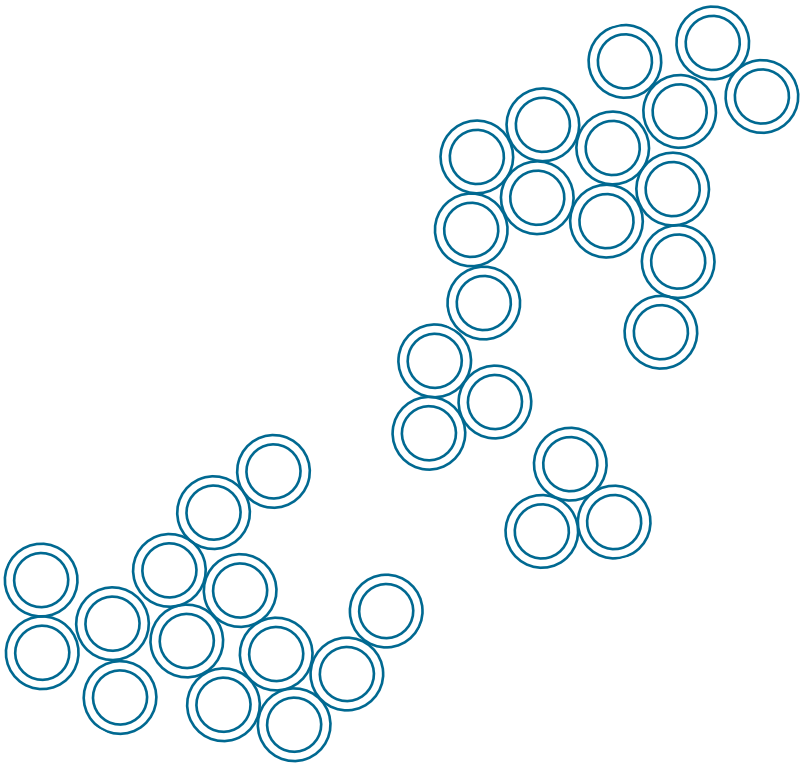
> *Tip: If required, gently lift the back of the instrument 10–15 cm and place it against the foam/padding at the back. This should allow you to get a good grip, placing both hands under the instrument.*

5. Lift out the instrument 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.
9. Turn the main switch at the back of the instrument to the "|" position.
10. Press the Power 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

### dPCR (for double emulsion droplets)

| Name | Cat. No. | |
|---|---|---|
| Xdrop instrument | IN00100 | |
| dPCR cartridge | CA10100 | Shipped at room temperature (RT). Store at –20°C upon receipt. |
| dPCR gasket | GA10100 | |
| Storage film | FI00100 | |
| dPCR oil | 8300100-0850 | (store at 4°C)<br>dPCR oil ● |

### Complementary Samplix items

| Name | Cat. No. | | |
|---|---|---|---|
| Cell sorter control kit | CO10100 | Part 1<br>(store at –20°C)<br>Droplet dye ●<br>dPCR buffer (2x) ● | Part 2<br>(store at 4°C)<br>Control droplets ○ |
| Stabilizing solution | RE10400 | (store at 4°C)<br>Stabilizing solution (4x) ○ | |
| dPCR buffer<br>(outer buffer) | 8100100-1450 | (store at –20°C)<br>dPCR buffer (2x) ● | |
| dPCR mix | 8000100-0500 | (store at –20°C)<br>dPCR mix (2x) ● | |

## dMDA (for single emulsion droplets)

| Name | Cat. No. | | |
|---|---|---|---|
| Xdrop instrument | IN00100 | | |
| dMDA cartridge | CA20100 | | |
| dMDA holder | HO10100 | | |
| dMDA gasket | GA20100 | | |
| Storage film | FI00100 | | |
| dMDA kit | RE20300 | Part 1<br>(store at –20°C)<br>dMDA mix (5x) ●<br>dMDA enzyme ○ | Part 2<br>(store at RT and 4°C)<br>dMDA oil ● (RT)<br>Break solution ● (RT)<br>Break colour ● (4°C) |

## Equipment and reagents for Xdrop droplet production and sorting supplied by the user

In addition to the required and suggested Samplix products, the following laboratory items are needed.

### Equipment

Cell sorter instrument
LAF (Laminar Air Flow) hood
Microcentrifuge
Vortex mixer
Pipette set from P2 to P1000
Brightfield microscope

### Consumables

Nuclease-free microcentrifuge tubes
Eppendorf DNA LoBind tubes 1.5 ml tubes (Catalog No. 0030108051)
Filtered pipette tips
Wide bore pipette tips for P200 pipette, outer diameter of tip: 1 mm – 1.9 mm (only for dMDA cartridges)

### Reagents

Nuclease-free water (only for dMDA)

# Chapter 2: Double Emulsion Droplets: Assays and Considerations

## Considerations for assay design

The Xdrop double emulsion droplets produced using the dPCR cartridge are 20 µm outer diameter and 16 µm inner diameter. It is possible to encapsulate small cells within the droplets, but the following considerations should be made when designing the assay:

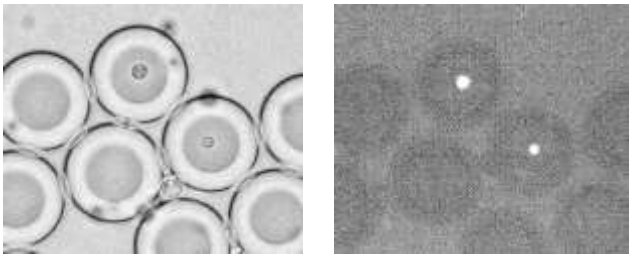


Fig. 2.1. Pictures of double emulsion droplets with yeast cells expressing GFP inside. Inner droplet diameter is around 16 µm, outer diameter is around 20 µm. Left: brightfield microscopy, Right: Fluorescence microscopy.

- Cells should be small, preferably less than 5 µm in diameter. Yeast cells and bacterial cells have successfully been added to the droplets, but larger cells will most likely block the filters of the cartridge and prevent droplet production.
- Particles may block the channels. If this is suspected, buffers can be filtrated before use.
- The droplets are very stable but temperatures above 94 degrees and pH above 10 is known to destabilize them.
- Do not use detergents such as PEG, Tween, Triton-X or SDS in the buffers. This will prevent droplet production. Check your enzyme stock solutions, as they may contain detergents.
- The outer buffer must be stabilized during droplet production and sorting. This can be done by adding the Samplix Stabilizing solution O to a custom buffer (Chapter 2) or by using the Samplix dPCR buffer as outer buffer (recommended for initial trials).
- The Stabilizing solution is not needed during incubation of droplets and can be replaced by a custom outer buffer after droplet production. The stabilizing solution increases viscosity of the outer buffer but does not affect droplet stability. The stabilizing solution is needed both during droplet production and during droplet sorting.

- Fluorescence can be detected within cells and in the surrounding inner buffer. In some cases, it is also possible to detect the number of cells within a droplet from the forward or side scatter on the cell sorters.
- When looking at droplets in a microscope, use a counting chamber. When using a standard microscope slide with cover glass, the flexible droplets will be compressed and will be flattened and appear larger than their actual tridimensional size.
- If testing a non-standard buffer for the first time, run a **maximum of 10 min droplet production on the Xdrop instrument!** If droplet production is not feasible with the new buffer, foam will be created and may enter the instrument. After 10 minutes, simply press "STOP" on the screen, and check that droplets are produced. This can preferably be checked by looking in a brightfield microscope. There should be at least 25% droplets with an appearance like the droplets in figure 2.1. Oil-in-water droplets without an inner aqueous phase are also expected and will not affect droplet sorting. If no double emulsion droplets are observed, try to isolate which of the buffer components is preventing droplet production.
- The oil shell surrounding the droplets is flexible and semi-permeable. It will allow water and small molecules to pass by osmosis, but large molecules such as DNA or protein, will stay within the droplet. This means that, if concentrations inside and outside the droplets is different, an osmotic gradient is created, which will cause 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. Droplet swelling or shrinking can affect the concentrations of all molecules inside the droplets, which may impact the assay, and should be avoided. Osmotic gradients can be avoided by adjusting the concentration of the outer buffer.
- 0.9% NaCl can be used as Inner buffer in combination with Samplix dPCR buffer ● as Outer buffer.

## Examples of double emulsion droplet production tests

Test 1: Droplet production in standard buffers (dPCR mix and dPCR buffer)

| Sample | Inner buffer (40 µL) | Outer buffer (300 µL) |
|---|---|---|
| 0 cells (negative control) | 1X dPCR mix ● | 1X dPCR buffer ● |
| 0.5 – 2 mln cells | 1X dPCR mix ● | 1X dPCR buffer ● |
| 2 - 4 mln cells | 1X dPCR mix ● | 1X dPCR buffer ● |

0.9% NaCl can be used instead of 1X dPCR mix as the Inner buffer.

Run droplet production for 10 minutes and observe in a counting chamber in a microscope that double emulsion droplets have been formed. Measure the inner and outer diameter of the droplets, which should be 14-18 and 18-25 µm, respectively. It is expected that there will be both double and single emulsion

droplets. The latter are present to stabilize the droplet production. Select the number of cells to be used for subsequent tests.

### Test 2: Testing custom buffers

| Sample | Inner buffer (40 µL) | Outer buffer (300 µL) |
|---|---|---|
| Selected number of cells | 1X Custom buffer | 1X dPCR buffer ● |
| Selected number of cells | 1X Custom buffer | 1X Custom buffer<br>1X Stabilizing solution ○ |

Run droplet production for 10 minutes and inspect droplets in a counting chamber using a microscope. Measure the diameter of the droplets immediately after droplet production and after 15 minutes at room temperature. During incubation, water will diffuse in or out of the droplets if the osmotic pressure is different in the inner and outer aqueous solution. This will cause the droplets to shrink or swell and the diameter will increase or decrease.

If Test 2 shows swelling or shrinkage of droplets during the incubation described above, adjust outer buffer composition to increase/decrease osmolarity, e.g. by changing the concentration of the outer buffer to 0.75X, 1.25X or 1.5X while keeping the stabilizing solution at 1X

# Chapter 3: Double Emulsion Droplet Production

## Prepare the cartridge

1. Dilute the 2x dPCR buffer ● to 1x with molecular-grade water or prepare custom outer buffer. Note: Dilute an entire vial of dPCR buffer. Precipitates potentially present in the 2x buffer will dissolve when diluted to 1x. Make sure to mix well by vortexing for 10 seconds or inverting the tube >20 times.
2. Keep the cartridge in the packaging and place it at room temperature for 20 minutes to equilibrate.
3. Unpack the cartridge and place it on a clean flat surface in a LAF (Laminar Air Flow) hood or a similar clean, dust-free environment. The layout of the cartridge is depicted in Fig. 3.1.
4. 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 or droplet exit wells.
    - Do not touch the microfluidic chip on the back of the cartridge.
    - Save the cartridge storage bag for later storage of the cartridge.

5. Be careful not to use the same lane more than once as this will disrupt droplet production. To avoid using the same lane more than once mark the storage plastic bag or the cartridge directly with a permanent marker once a lane has been used.
6. Load the cartridge with reagents in a LAF hood or a similar clean, dust-free environment. Allow the reaction mix to reach room temperature before loading on the dPCR cartridge and then insert the cartridge immediately thereafter into the Xdrop instrument.

Note: it is important to load the reagents onto the cartridge in the order described in this manual.


Fig. 3.1. Top view of dPCR cartridge with wells (A–D) and lanes (1–8) marked.

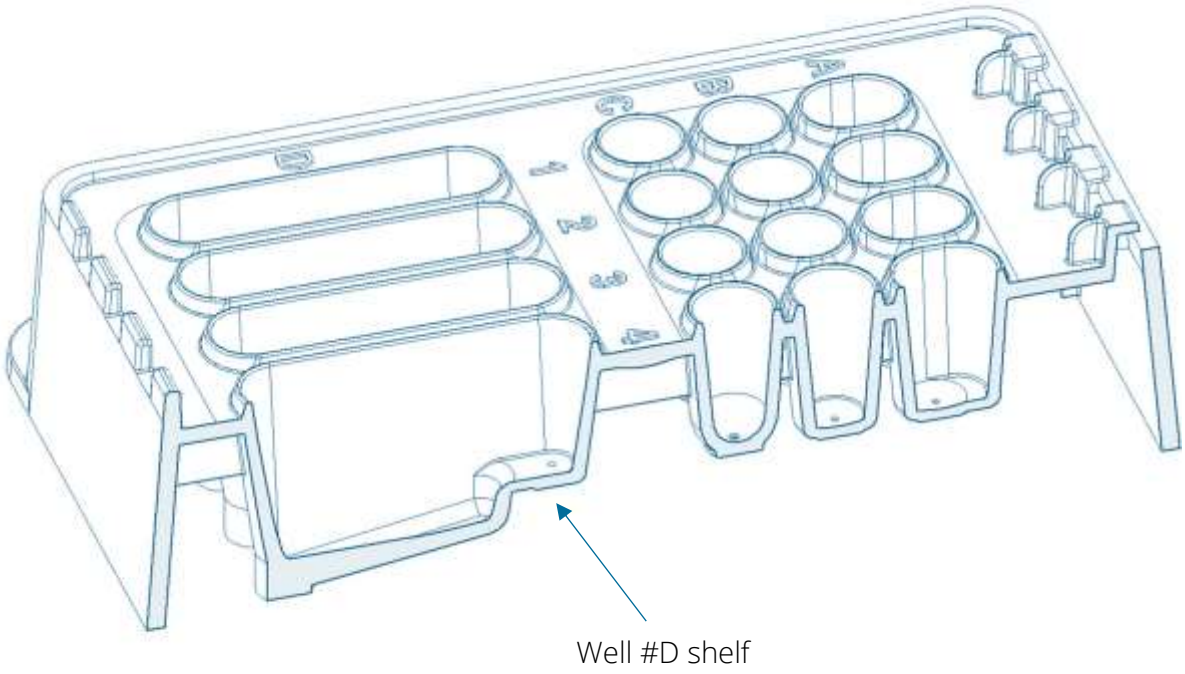



Fig. 3.2. Cross section of the dPCR cartridge. Notice the exact location of the shelf in well #D, where 40 µl of 1x dPCR buffer ● or custom outer buffer is added.

When loading the dPCR cartridge, avoid introducing air bubbles by pipetting on the side wall of the wells.

7. Load **300 µl** 1x dPCR buffer ● or custom inner buffer in the first well (**#A**). It is important to load the cartridge in the order described here and avoid air bubbles by pipetting carefully on the side of the well.
8. Load **40 µl** 1x dPCR buffer ● or custom outer buffer onto the shelf of the collection well **#D** (Fig. 3.2).
9. Load **40 µl** reaction mix into well **#C**.
10. Load **100 µl** dPCR oil ● into well **#B**.

| | | |
|---|---|---|
| **Notice that the order of loading is:** | **300 µl** 1x dPCR buffer ● into the well | **#A** |
| | **40 µl** 1x dPCR buffer ● onto the shelf | **#D** |
| | **40 µl** 1x reaction mix into well | **#C** |
| | **100 µl** dPCR oil ● into well | **#B** |

11. Add a white rubber gasket to the top of the cartridge. Orient the gasket to the cartridge using the angled corner. Attach the gasket to the pins first and then to the T-hooks (Fig. 3.3).

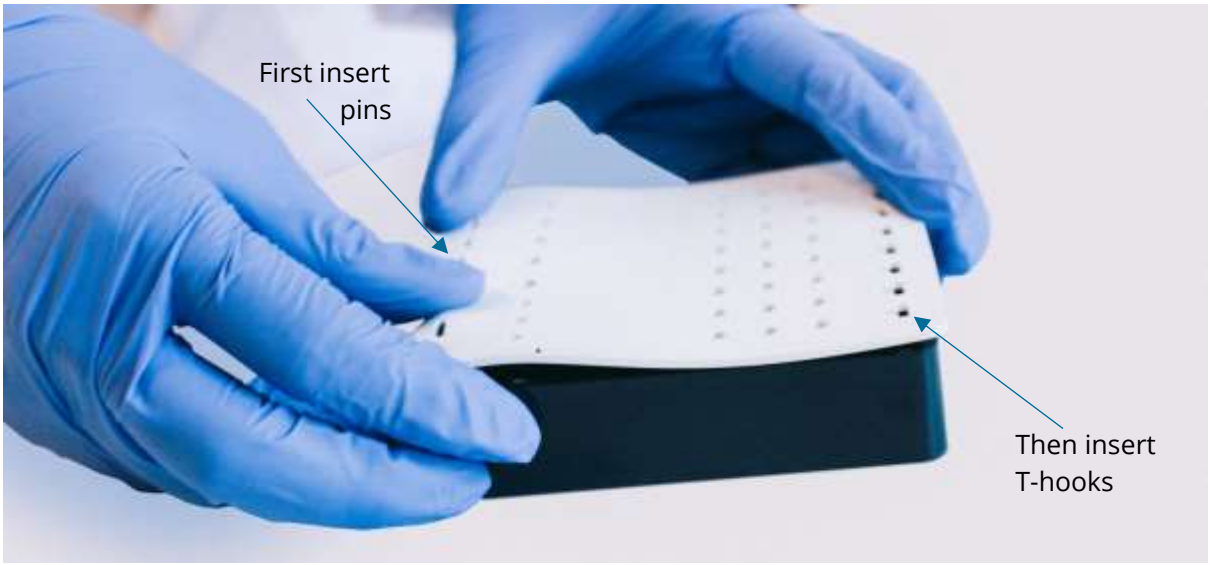



Fig. 3.3. Cover the cartridge with the white rubber gasket while orienting it correctly (angled corner on the gasket to angled corner on cartridge), then attach first the pins and pull gently to attach T-hooks.

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. Start the instrument by pushing the Start/Stop button at the front. The instrument will start initializing and will shift to the "Welcome screen".

12. Push "Open" on the instrument touchscreen to eject the drawer (Fig. 3.4).

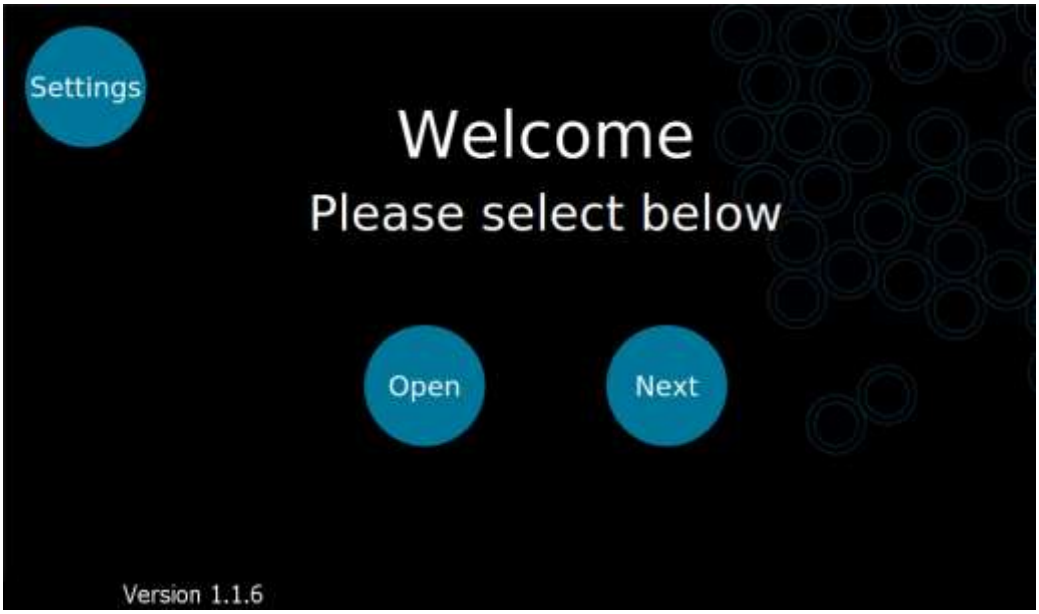



Fig. 3.4. The Xdrop instrument "Welcome" screen. Press "open" to eject the drawer.

13. Upon pressing the “Open” or “Next” button, the screen displays “Please insert/remove cartridge” and “Close”. Make sure that the cartridge is correctly positioned into the drawer (Fig. 3.5) as it may otherwise cause damage to the instrument. Once the cartridge is correctly inserted, press “Close” to retract the drawer into the instrument.


Fig. 3.5. The Xdrop instrument with a correctly inserted dPCR cartridge. Be very careful to position the cartridge correctly to avoid damaging the instrument or cartridge.

14. Once the drawer is fully closed, press “Next” on the touchscreen.
15. The Xdrop instrument can operate with either dPCR or dMDA cartridges. Use the dPCR cartridge option by selecting “dPCR” on the touchscreen (Fig. 3.6).

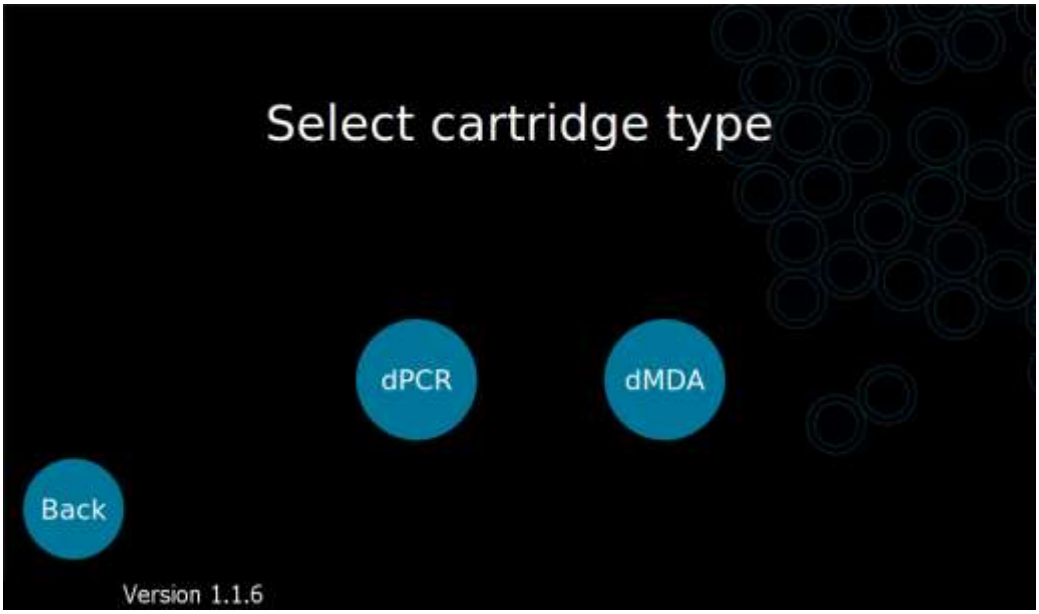



Fig. 3.6. The Xdrop instrument "Select cartridge type" screen. Select dPCR cartridge.

16. The lanes to be processed are selected by pressing the corresponding numbers 1–8 on the screen. When selected, the buttons turn green (green = selected and blue = not selected; Fig. 3.7).

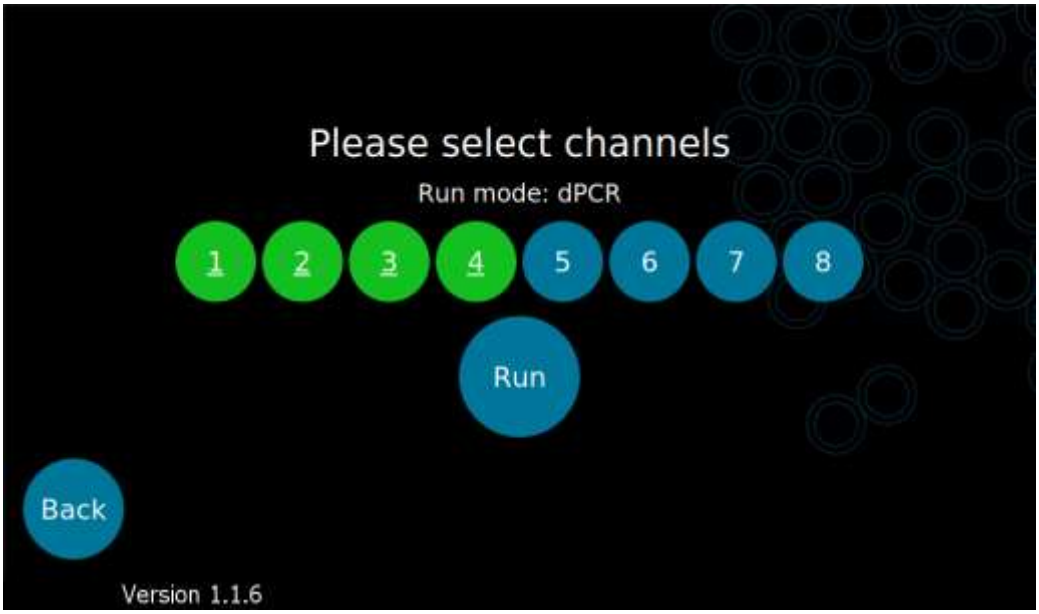



Fig. 3.7. Selecting the lanes to be used. Selected channels will be indicated by green buttons (here: 1 to 4). Blue buttons indicate channels not yet selected.

17. Press "Run".

The instrument will now ramp up the pressures. This process will take around 15 seconds.

Once optimal pressures have been reached, the message “Making your droplets” and the remaining run time are displayed on the screen (Fig. 3.8). The Xdrop instrument will produce double emulsion droplets in approximately 40 minutes. Note: If testing new buffers, please stop the run after 10 minutes (press “Stop” on the screen) and check the droplets in a microscope to confirm correct double emulsion droplets formation.

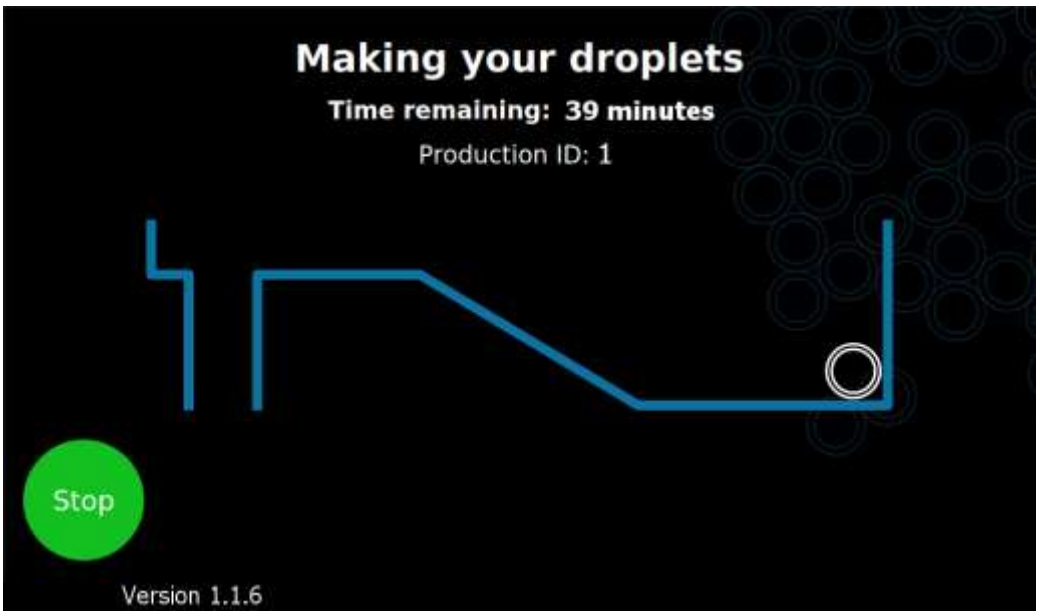



Fig. 3.8. Touchscreen image while producing droplets.

18. When droplet production has been completed, the screen will change to “Your droplets are ready”.
19. Press “Open” to eject the cartridge.
20. Remove the cartridge from the instrument and place it in a LAF hood.
21. Press “Close” to make the instrument retract the drawer back into its closed position.
22. Press “Finish” to return to the Welcome screen.
23. Shut down the instrument after a completed droplet production to avoid damage to the instrument. If the instrument is not to be used for several hours, it should preferably be turned off. To turn the instrument off, push the ON/OFF button at the front to initiate the automatic shutdown procedure and power off the instrument.

## Collect generated droplets

1. After droplet production, confirm that double emulsion droplets have been produced. Double emulsion droplets will sink to the bottom of the collection well and form a white layer with a clear buffer phase on top.
2. Collect droplets from the collection well (#D) into a 0.5 ml or 1.5 ml tube. Use tips that minimize the binding of droplets to the side of the tip.
3. 200 µl dPCR buffer (surplus in well #A) can be used to "wash" residual droplets from the shelf inside the collection well #D.

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

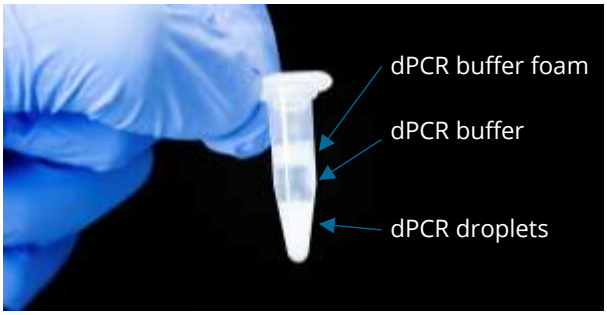



Fig. 3.9. Collection of dPCR droplets from the collection well (#D) into a 0.5 ml or 1.5 ml tube.

4. If any lanes are unused, the cartridge can be stored up to 4 weeks in the freezer (–20°C) and used a second time. We do not recommend using the dPCR cartridge more than two times, as further freeze/thaw cycles could damage the cartridge. Discard the single-use gasket and remove excess liquid in wells #A and #B before storing the cartridge.
5. Seal the cartridge with Storage film (Cat. No. FI00100) covering all wells, and store protected from light and dust in the freezer (–20°C) in a sealed bag.

Notice that the dPCR cartridge has a shelf-life of 4 weeks (at –20°C) after the original packaging has been opened, provided that this period does not exceed the expiry date indicated on the original packaging of the product.

Note: Each production lane and gasket are single-use and will not function properly if re-used. Furthermore, attempting to reuse the gasket and cartridge lanes will increase the risk of cross-contamination of samples.

## Reaction in droplets

The droplets can now be incubated, washed, or otherwise treated as needed to generate a fluorescent signal.

# Chapter 4: Detection and Sorting of Droplets

Double emulsion droplets generated in the Xdrop instrument with the dPCR cartridge can be sorted and collected in a standard cell sorter, thereby capturing the content of interest. In this step, the positive dPCR droplets containing the target of interest are identified and separated from the negative droplets using the fluorescent signal provided by the assay.

## Requirements for flow cytometry cell sorter

- Cell sorter fitted with the correct wavelength laser exciting the fluorophores of interest.
- Optical configuration detecting fluorophores of interest.
- 100 µm nozzle tip/sorting chip minimum.
- Sample probe should be positioned just above and near the bottom of the sample tube.

## Notes to operator

- Double emulsion droplets produced on the dPCR cartridge are stable and relatively heavy. Start with a high sample pressure to get the dPCR droplets into the channels. Be aware that it can take up to 5–10 min before the dPCR droplets reach the point of interrogation and appear on the plot.
- Due to the density of the dPCR droplets, they will quickly sediment at the bottom of the sample tube and will not remain in suspension during sorting. Make sure that the sample probe is positioned near the bottom of the sample tube.
- Use either SSC or FSC as threshold channel to exclude contaminating events (e.g. particles, pure oil droplets).
- Double emulsion droplets produced on the dPCR cartridge have an outer diameter of about 20 µm, therefore the correct events are high in Forward Scatter (FSC) and Side Scatter (SSC) (see Fig. 4.1).
  Note: The "height" setting more clearly displays the population of correct dPCR droplets.
- Doublet discrimination can be performed but is not required. Doublet discrimination will slightly decrease the number of double emulsion droplets analyzed while not contributing greatly to sorting quality.
- Positive fluorescent droplets are likely to be very rare (possibly less than 0.02% of total double emulsion droplets, depending on the assay). Therefore, a positive population can be difficult to

identify. Make sure the live plot of fluorescence shows at least 100,000 events. A histogram plot is not recommended.

- A negative control sample is not required since an internal negative control (double emulsion droplets not containing target) will be present in all samples.

## Preparation of droplets for flow cytometry

1. Make sure the 2x dPCR buffer ● is diluted with molecular-grade water to 1x. Mix well by vortexing for 10 seconds or inverting the tube at least 20 times.
2. Add 15 µl molecular-grade water into the bottom of a 1.5 ml DNA LoBind collection tube and place the collection tube in the appropriate holder in the cell sorter instrument. Alternatively, if the cell sorter is equipped with a plate sorter module, droplets can be sorted directly into a 96- or 384-well plate.
3. Confirm that the sort settings for your cell sorter are correct. Check that the side stream is centered on the collection tube or on the center of the wells for 96 or 384-plates.
4. Load the sample tube containing the double emulsion droplets in 1000 µL 1X dPCR buffer onto the flow cytometer and start analysing (see Fig. 4.1).

## Flow cytometry analysis and sorting of dPCR droplets

5. Identify the dPCR droplet population on a plot of FSC (height) versus SSC (height). dPCR droplets have higher side scatter (SSC) and fluorescence than oil droplets. Note that it could take a few minutes before the heavy dPCR droplets are analyzed and visible in the plot.
6. Use side scatter (SSC) or forward scatter (FSC) as threshold channel and exclude events that are not dPCR droplets (Fig. 4.1).
7. Gating the identified dPCR droplets in a new plot, identify a positive and a negative population of droplets. Plot fluorescence versus side scatter (SSC).
8. Set the gates, taking care to draw a strict gate around the population to be sorted. Note: be aware of potential drift in fluorescence over time and be prepared to move the sorting gate during the sorting if required.
9. Start sorting the positive population into the collection tube containing 15 µl molecular-grade water or a plate as preferred. Remember to acquire data during the sort for your records.

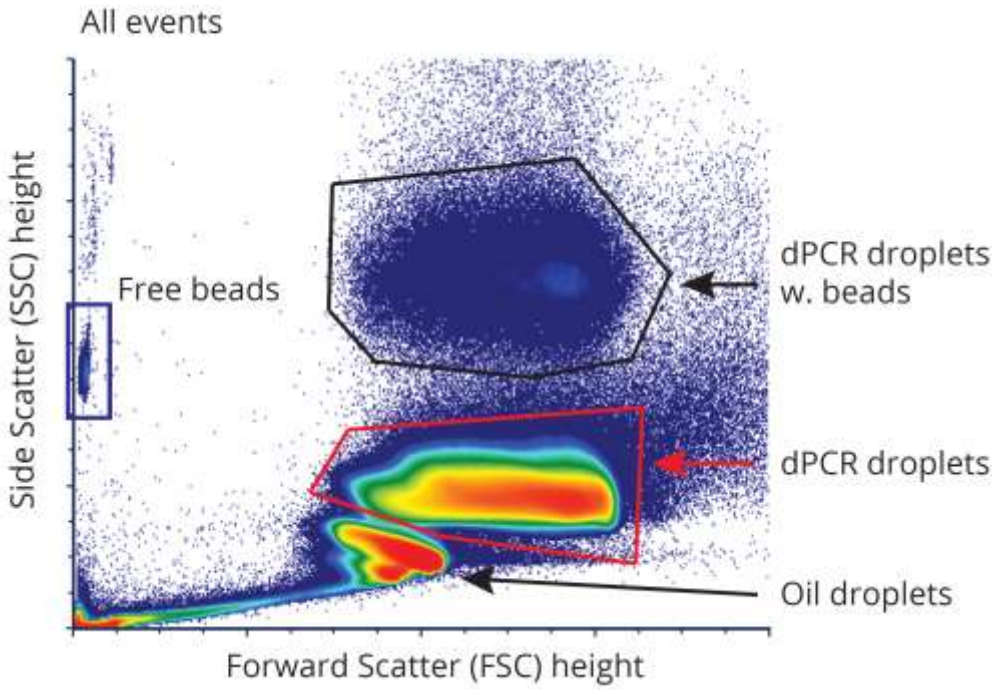



Fig. 4.1. Identification of dPCR droplets. dPCR droplets are higher in SSC than pure oil droplets and dPCR droplets containing beads are higher in SSC than empty dPCR droplets. Free beads are beads of approximately 5 µm not encapsulated in dPCR droplets. Note that the "height" setting more clearly displays the populations of interest.

# Chapter 4.5: Collection of Droplet Content (BETA)

## Breaking the droplets

1. Add an appropriate amount of recovery buffer, depending on the volume required in the subsequent custom assay (e.g. 20 µL if dMDA is performed, or a higher volume, e.g. 200 µL if needed) to the sorted droplets. This buffer should be aqueous and compatible with your downstream processes and could for instance be culture medium or a buffer.
2. Add 20 µl Break solution ● to each tube of sorted droplets with recovery buffer.
3. Optional: Add 1 µl of Break colour ●. This will colour the water phase. If colouring is too weak, add 1 µl extra of Break colour. Note: The water phase may be a colour ranging from yellow to purple as the Break colour functions as a pH indicator.
4. Flick tube gently, do not vortex.
5. Spin tube briefly (15–30 seconds).
6. Remove the clear Break solution phase from the bottom of the tube and discard. Note: Be careful to remove all the Break solution as it may inhibit downstream enzymatic reactions.
7. Repeat steps 3–5 to remove all leftover Break solution.
8. Keep the coloured water phase, which will contain the contents from the positive droplets (Fig. 5.1).

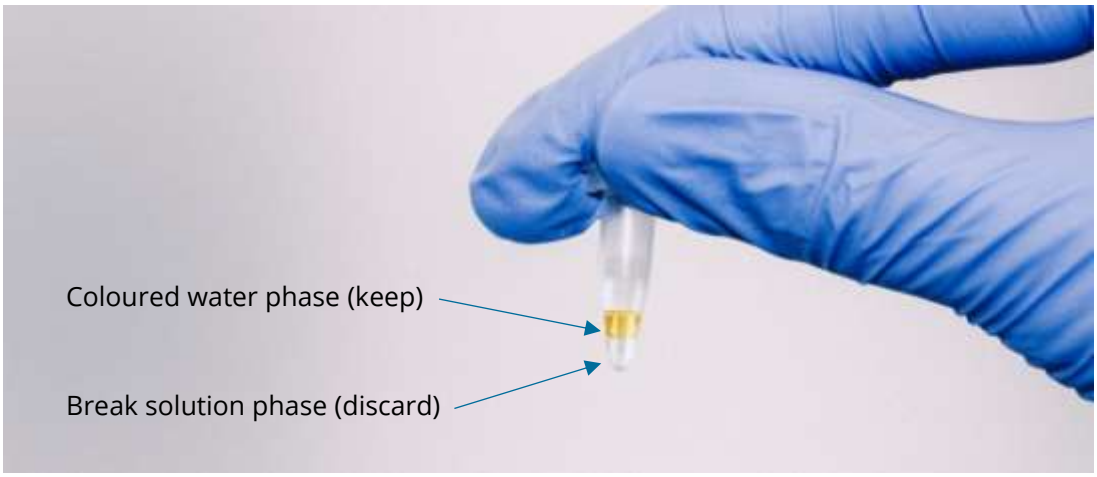



Fig. 5.1. Break sorted droplets with Break solution ● and Break colour ●. Discard the clear Break solution phase at the bottom of the tube. Keep the top, coloured water phase, as it will contain your selected cells/molecules.

# Chapter 5: Multiple Displacement Amplification in droplets (dMDA)

## Overview

Depending on the Xdrop application, it might be relevant to perform DNA amplification after sorting of the selected target. For that purpose, Samplix provides a dedicated cartridge (dMDA cartridge) and reagents to perform multiple displacement amplification (dMDA) in droplets, which provides much better uniformity than bulk MDA.

## Set up dMDA reaction

Note: Do not use any other reagents than Samplix dMDA kit (Cat. No. RE20300) for Xdrop dMDA droplet production as this may compromise droplet production, droplet stability, and downstream enrichment. Thaw and keep all reagents at 4°C or in a cooling block while setting up the reactions, except the oil that should be kept at RT.

The MDA reaction is very susceptible to contamination. Make sure to avoid DNA contaminations of any kind.

Prepare the amplification mix following the instructions below in a LAF hood or similar clean, dust-free environment.

1. Prepare the amplification mix (see table below). Mix gently, do not vortex.

| Amplification mix | 1X |
|---|---|
| water (molecular grade) | 5 µl |
| dMDA mix (5x) ● | 4 µl |
| dMDA enzyme ○ | 1 µl |
| **Total mix** | **10 µl** |
| Template DNA solution | 10 µl |

2. Aliquot the mix in a cooling block. Important! Keep cold at 4°C until use.
3. Add **10 µl** template (DNA from sorted double emulsion droplets after break or genomic DNA 0.1 pg/µl).
4. We recommend including the following control reactions:
   - **10 µl** molecular-grade water (negative control)
   - **10 µl** sheath fluid from flow cytometer (contamination control)
   - **10 µl** genomic DNA (0.1 pg/µl, positive control, provided by the user)

5. Mix gently and keep cold at 4°C until loading on the dMDA cartridge.

Note: When taking aliquots of your samples, always pipette from the center of the coloured phase as an additional precaution to avoid carrying over remaining Break solution, which can hamper the DNA amplification.

## Load the dMDA cartridge

The dMDA reaction takes place inside droplets formed in the dMDA cartridge inserted in the holder (Fig. 5.2). The cartridge must be sealed with a gasket on top during droplet production. Load samples in a clean LAF hood.

Note: The dMDA holder is re-used for all following runs.

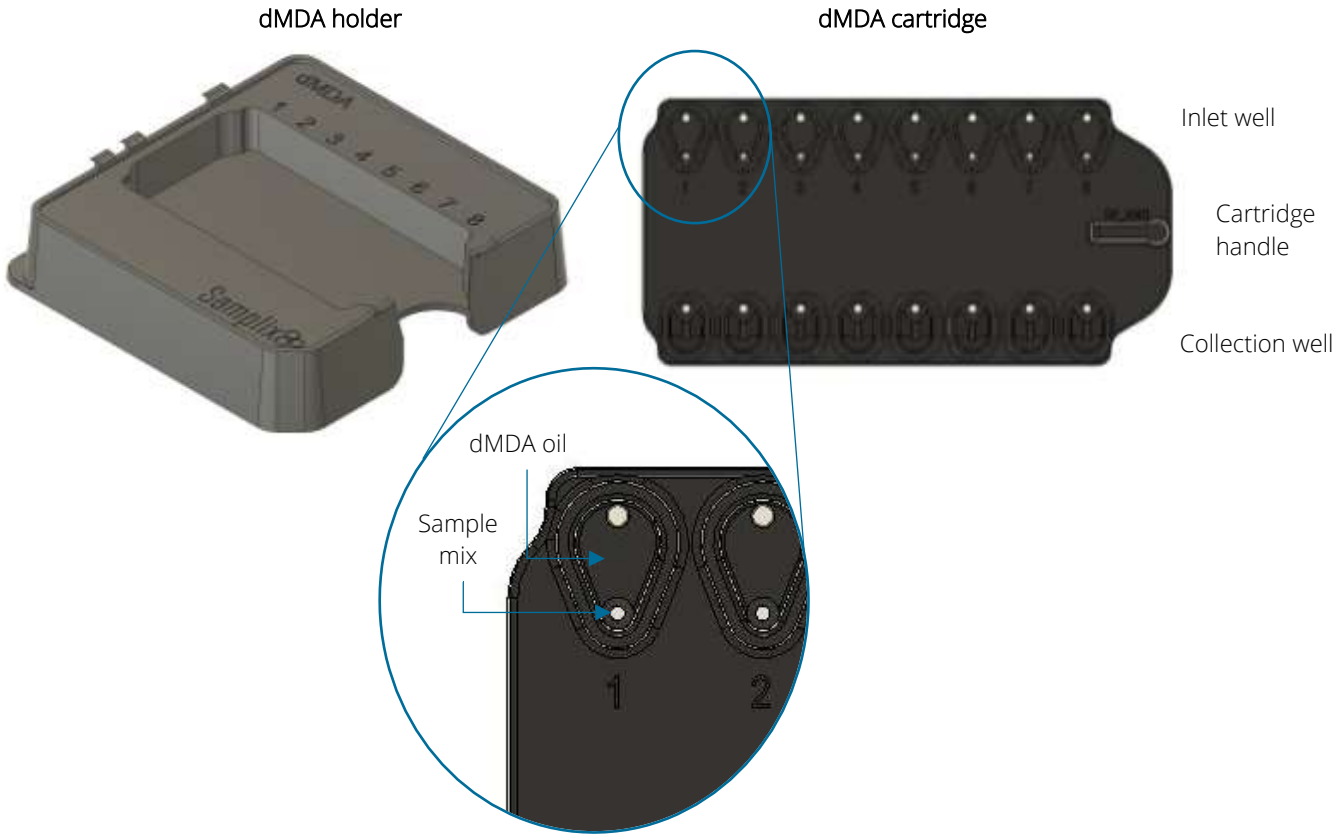



Fig. 5.2. Left: dMDA holder. Right: Top view of dMDA cartridge with inlet well on top and collection well below. Bottom: zoomed view of the holes of the inlet well.

Set up the cartridge:

1. Wearing gloves, unpack the dMDA holder and cartridge from their original packaging.
2. Handle the cartridge as follows:

    - Always wear gloves when handling the cartridge.
    - Hold the cartridge by its sides or by the handle.
    - Do not touch any of the inlet wells or droplet collection wells.
    - Be cautious to avoid DNA contamination at all times.
    - Store the cartridge (if partially used) covered with 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 lead to contamination of your sample. To avoid using the same lane more than once, mark the storage plastic bag with a permanent marker once a lane has been used.


Fig. 5.3. The assembled dMDA cartridge with holder (dark grey) and dMDA cartridge (black) inserted.

Note: It is strictly mandatory that the sample is injected into the dMDA cartridge with wide bore pipette tips P200 with an outer diameter of 1 mm -1.9 mm.

4. Place the dMDA cartridge in the groove of the dMDA holder as shown in Fig 5.3. Inlet wells are placed closest to the numbers on the holder while the collection wells are placed closest to the side with the Samplix logo.

5. Place the wide bore pipette tip (with 20 µl sample mix) in the inlet hole at the bottom of the inlet well making a tight connection (Fig. 5.4 and Fig. 5.5).

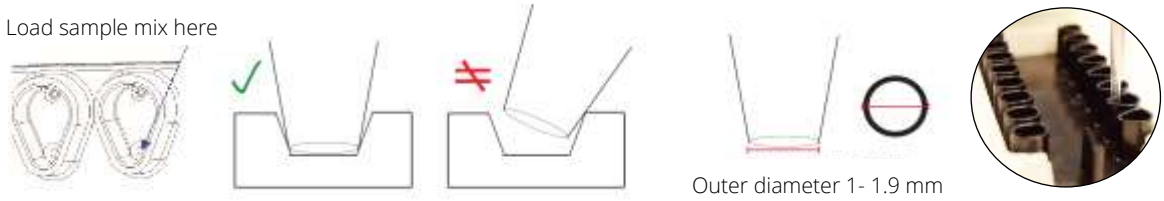



Fig. 5.4. Injection of the sample into the inlet well. From left to right: Cartridge inlet where the sample mix is loaded: the wide bore pipette tip should enter the hole in the pointy end of the tear-shaped inlet well. Correct positioning of the wide bore pipette tip. Incorrect positioning of the wide bore pipette tip. Outer diameter of the wide bore pipette tip (1–1.9 mm). Picture of dMDA cartridge loading of the sample mix.

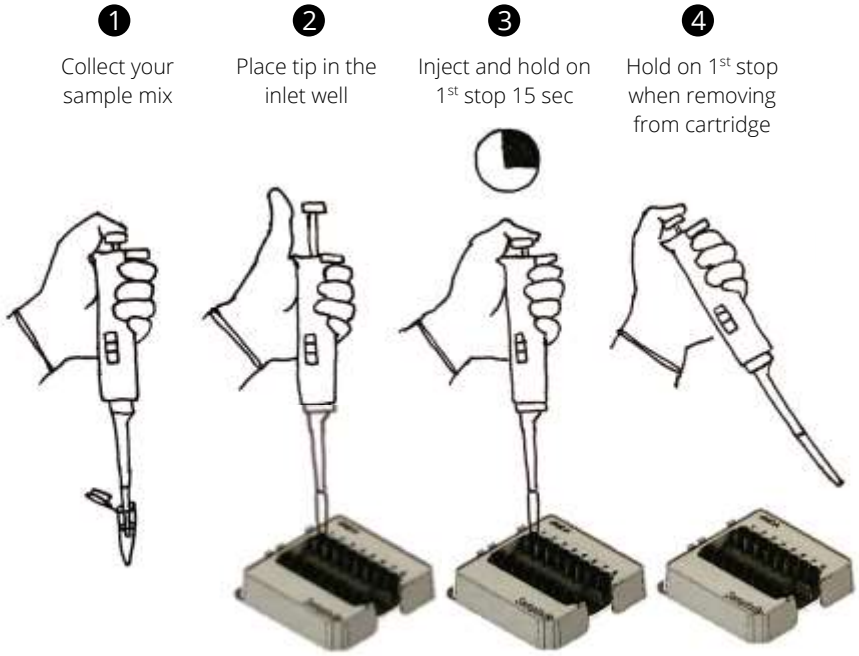



Fig. 5.5. Loading of the dMDA cartridge. 1) Collect your sample mix. 2) Place the wide bore pipette tip in the hole at the pointed end of the tear-shaped inlet well. 3) Load the sample by slowly pressing the plunger to the first stop and keep it pressed for 15 seconds; this allows the mix to enter the microfluidic system. 4) Keep the plunger down at the first stop when removing the tip from the cartridge.

6. Slowly inject the sample and hold the pipette plunger in the first stop position for 15 seconds while still applying moderate pressure on the pipette tip to ensure a tight connection between the pipette and cartridge (see Fig. 5.4 and Fig. 5.5).
7. Remove the pipette while still holding the plunger button in the first position. The entire sample should have entered the channel in the chip, there should be no liquid visible in the well (see Fig. 5.5).
8. Repeat steps 5 to 7 for the next lane to use, if applicable.
9. Add 75 µl dMDA oil ● 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 (see Fig. 5.2).
10. Place the gasket on top of the cartridge and fix using the T-hooks (Fig. 5.6).

**Fig. 5.6.** Attach the white rubber gasket to T-hooks of the dMDA cartridge.

## Generate dMDA droplets on the Xdrop instrument

Before powering up the instrument, please make sure that the main power switch is in the "|" position. The main switch is located at the back of the instrument. Power up the instrument by pushing the Start/Stop button at the front. The instrument will initialize and will shift to the "Welcome screen".

1. Press "Open" on the Xdrop instrument touchscreen to eject the drawer (Fig. 5.7).

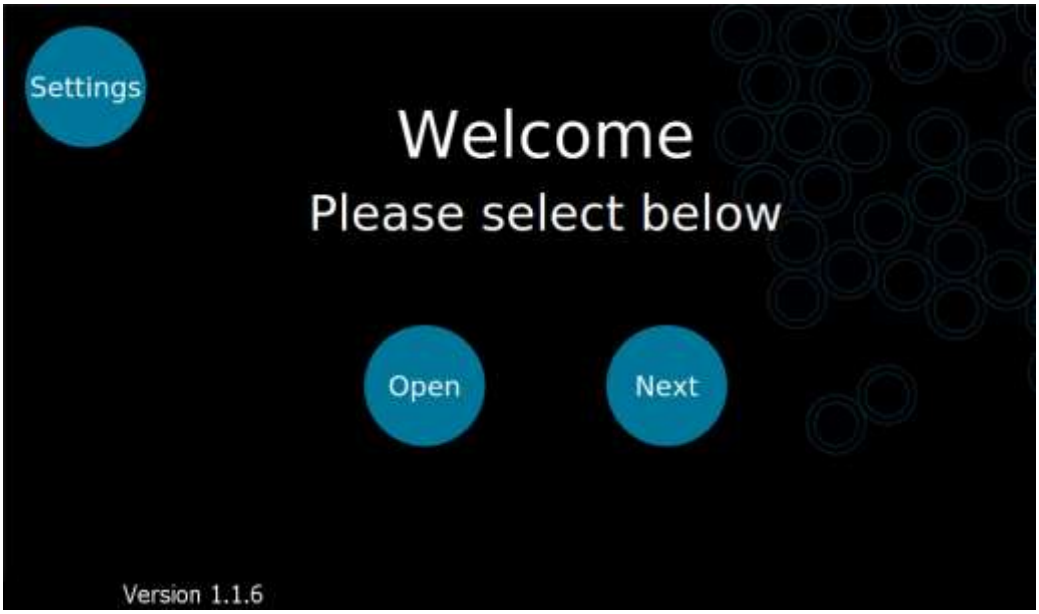



Fig. 5.7. The Xdrop instrument “welcome screen”. Press “open” to eject the drawer.

2. Upon pressing the “Open” or “Next” button, the screen displays “Please insert/remove cartridge” and “Close”.

3. Place the loaded cartridge with the holder into the Xdrop instrument drawer. Make sure that the cartridge is correctly positioned into the drawer by aligning the rounded corner on the holder to the rounded corner of the instrument drawer (Fig. 5.8). Incorrect insertion of the cartridge may cause damage to the instrument. Once the cartridge is correctly inserted, press “close” to retract the drawer into the instrument.

Fig. 5.8. Photo of Xdrop instrument with a correctly inserted dMDA cartridge.

4. Once the drawer is fully closed, press “next” on the touchscreen.
5. The droplet generator instrument can operate with either dPCR or dMDA cartridges. Use the dMDA cartridge with the “dMDA” option on the touchscreen (Fig. 5.9).

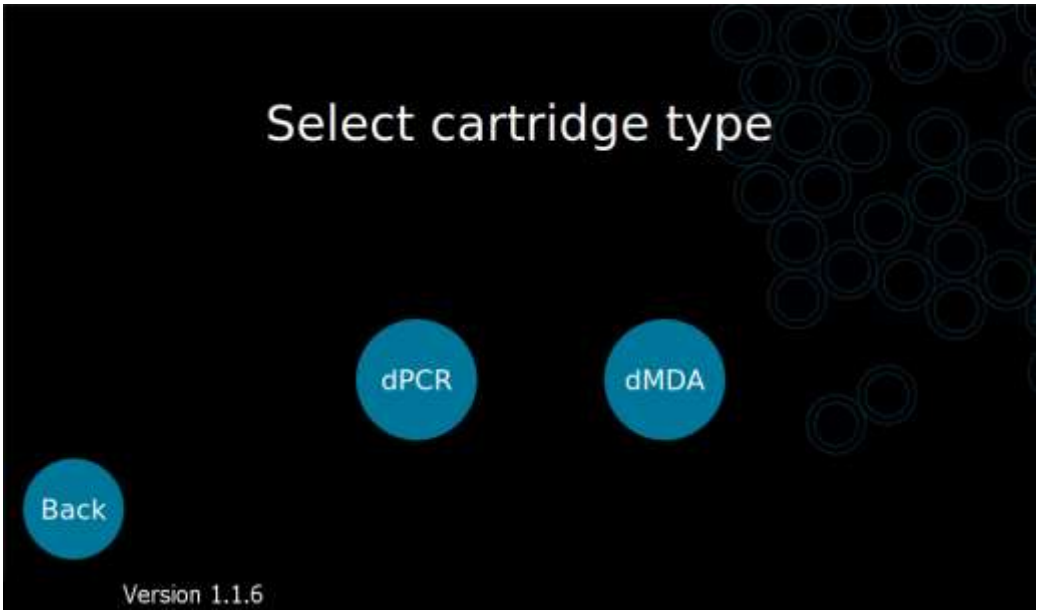


Fig. 5.9. The Xdrop instrument "Select cartridge type" screen. Select dMDA cartridge.

6. The channels to be processed are selected by pressing the corresponding numbers 1–8 on the screen. When selected, the button turns green (green = selected & blue = not selected; Fig. 5.10). Deselect the channels not used.

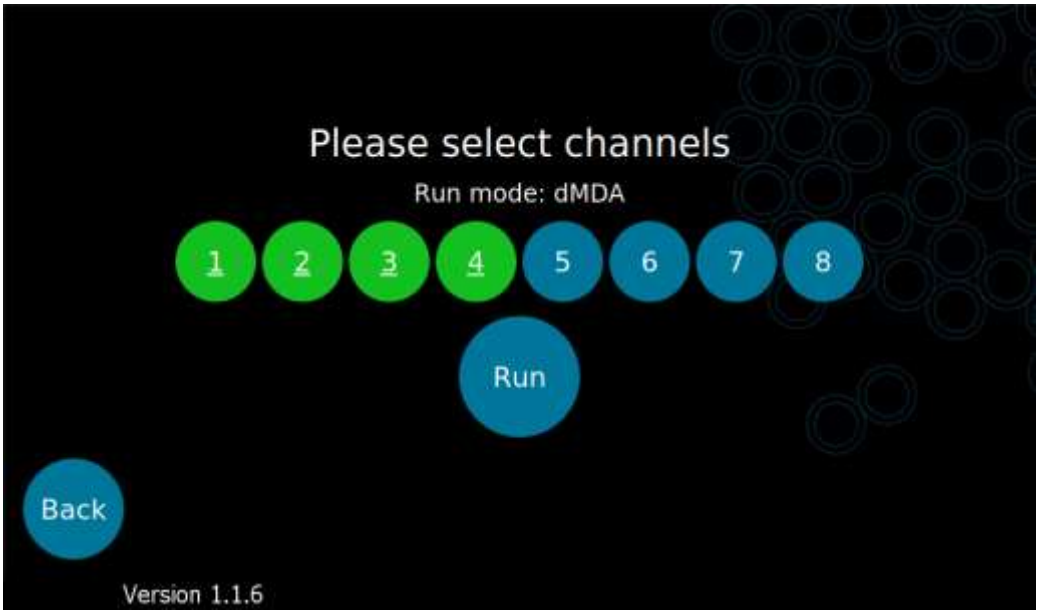


Fig. 5.10. Selecting the channels to be used. Selected channels will be indicated by green buttons (in this figure, channels 1 to 4). Blue buttons indicate channels not yet selected.

7. Press “run”.

The message “Making your droplets” and the remaining run time is displayed on the screen (Fig.5.11). The dMDA protocol will produce droplets in approximately 45 seconds.

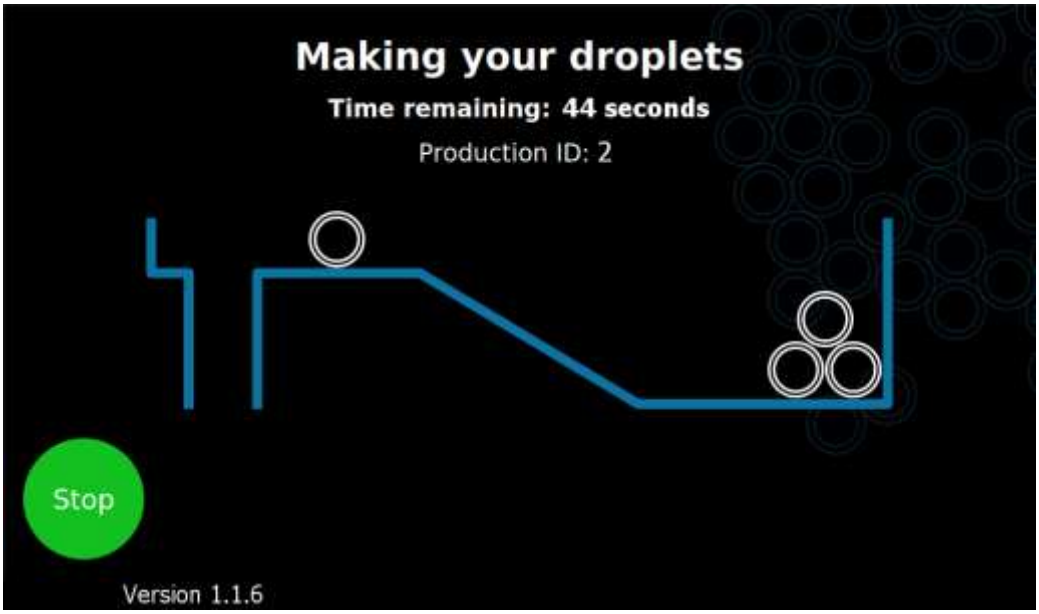



Fig. 5.11. Touchscreen image while producing droplets.

8. Once droplet production has been completed, the screen will change to “Your droplets are ready”.
9. Press “Open” to eject the cartridge.
10. Remove the cartridge from the instrument and place it in a LAF hood.
11. Press “Close” to make the instrument retract the drawer back into its closed position.
12. Press “Finish” to return to the Welcome screen.
13. Shut down the instrument after a completed droplet production to avoid damage to the instrument. If the instrument is not to be used for several hours, it should preferably be turned off. To turn it off, push the ON/OFF button at the front to initiate the automatic shutdown procedure and power off the instrument.
14. Collect all the dMDA droplets from the collection well with a P200 pipette and transfer them into a nuclease- and DNA-free PCR tube. Collect dMDA droplets by slowly pipetting from the sides towards the center/channel of the well. The total volume of dMDA droplets and oil in the collection well is 70–100 µl (Fig. 5.12).

Note: Each production lane and gasket are single-use and will not function properly if an attempt to re-use already processed lanes is made. Furthermore, attempting to reuse the cartridge and gasket will increase the risk of cross-contamination of samples.

15. Inspect the volume of collected droplets before removing the oil (step 16). You can expect approximately 2–3 mm layer of droplets on top of the oil phase.
16. Remove the excess dMDA oil from the bottom of the collection PCR tube. Only 1–2 mm of dMDA oil should be left in the bottom of the tube (see Fig. 5.12).

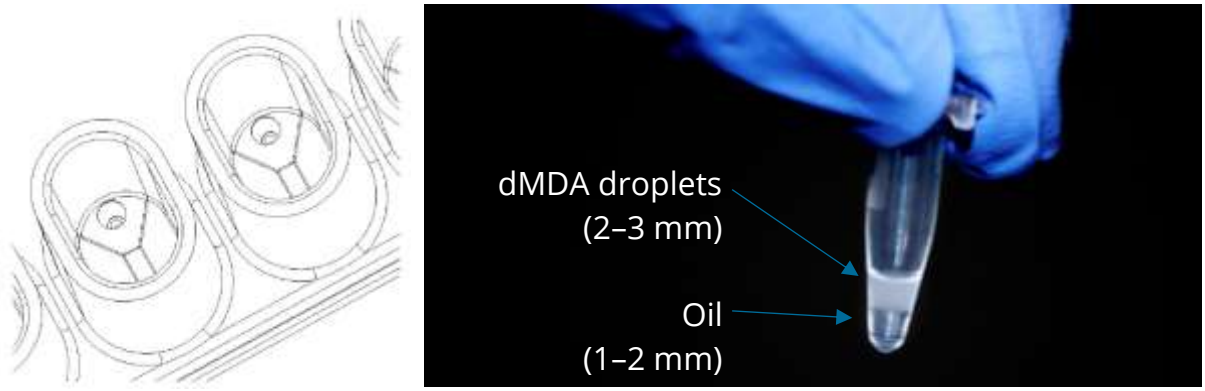



Fig. 5.12. Collection of dMDA droplets from the collection well. Left: Drawing of a collection well. Notice that the sides are slightly slanting towards the inlet hole of the well. Collect the dMDA droplets by pipetting gently. Right: dMDA droplets in a PCR tube after collection. dMDA droplets will form a white layer on the top with the excess oil at the bottom.

17. Incubate dMDA droplets in a thermal cycler at 30°C for 16 hours followed by 10 minutes at 65°C. Run the following program on a thermal cycler:

| Temperature | Duration |
|---|---|
| 30°C | 16 hours |
| 65°C | 10 min |
| 4°C | ∞ |

18. If some lanes are still unused when all samples have been run, place the Samplix Storage film (Cat. No. FI00100) over the cartridge while still in the holder (Fig. 5.13) and store the dMDA cartridge and holder in a Ziplock bag 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 dMDA 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.

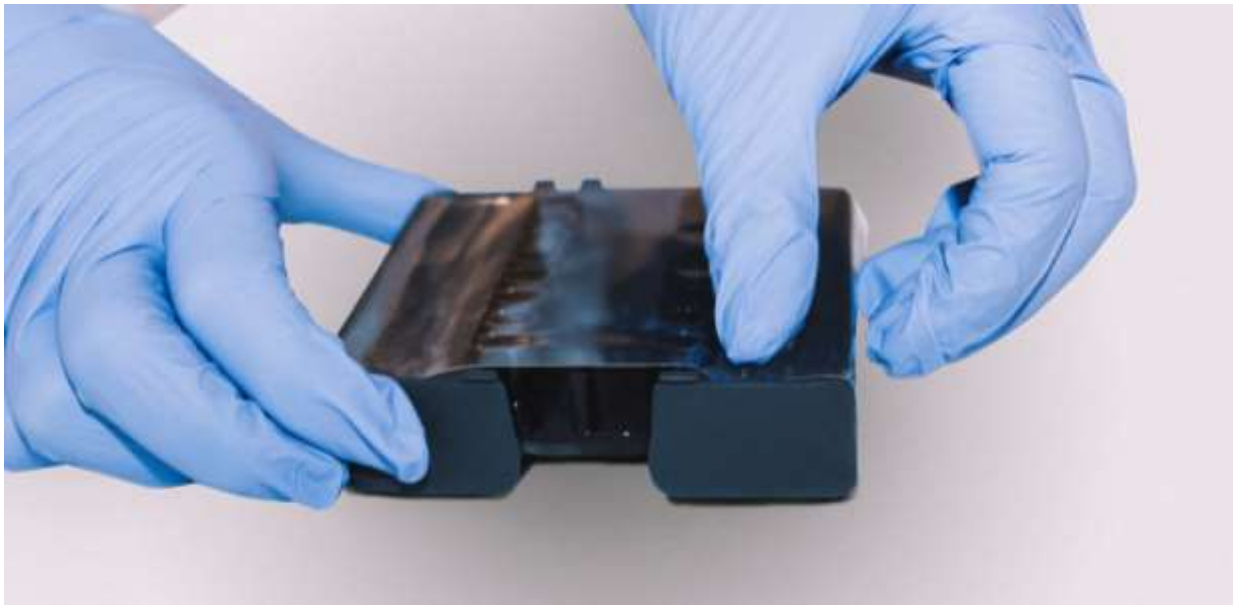


Fig. 5.13. Place a Samplix storage film on the dMDA cartridge to seal the wells and avoid cross-contamination.

# Chapter 6: Breaking droplets after dMDA

## Breaking the droplets

After dMDA incubation, break the dMDA droplets with Break solution ● and Break colour ● (Fig. 6.1).

1. Add 20 µl Break solution ● to each tube.
2. Add 1 µl of Break colour ●. This will colour the water phase. If colouring is too weak, add 1 µl extra of Break colour. Note: The water phase may be a colour ranging from yellow to purple as the Break colour is functioning as a pH indicator as well.
3. Flick tube gently, do not vortex.
4. Spin tube briefly (15–30 seconds).
5. Remove the clear Break solution phase from the bottom of the tube and discard.
6. Repeat steps 3–5 to remove all leftover Break solution. It is important to remove all the Break solution as residual Break solution may inhibit downstream enzymatic reactions.
7. Keep the coloured water phase, which will contain the amplified DNA (Fig. 6.1).

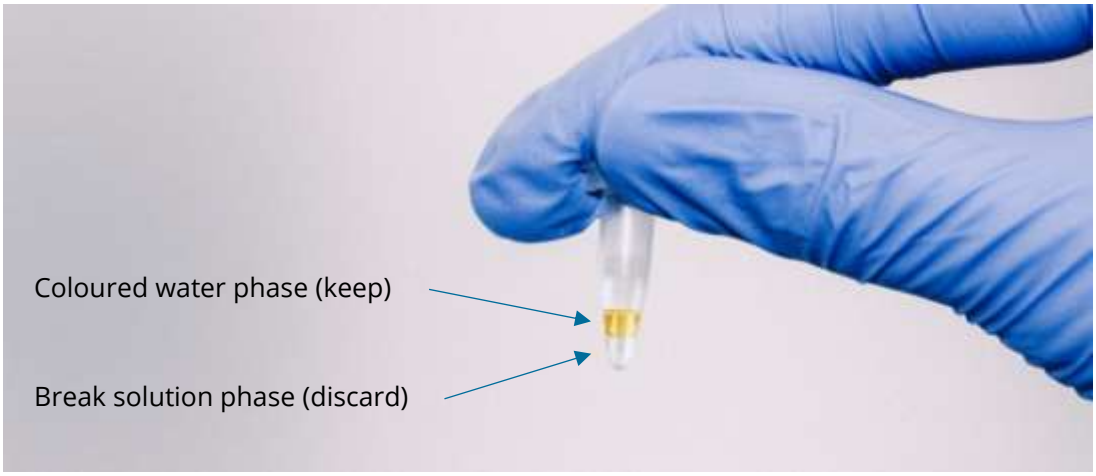



Fig. 6.1. Break post incubation dMDA droplets with Break solution ● and Break colour ●. Discard the clear break solution phase at the bottom of the tube. Keep the top, coloured water phase as it will contain your DNA molecules.

Good pause point: Store released DNA at 4°C for one week and at –20°C longer.

Measure the total amount of enriched and amplified DNA using a reliable and sensitive method such as with Qubit™, Bioanalyzer™, TapeStation™, FEMTO Pulse™ or similar. If possible, evaluate the size of sorted and amplified DNA fragments. If relevant, proceed with the downstream analysis of choice (e.g. library preparation and sequencing).