Single cells or nuclei amplification with Xdrop

This protocol describes the lysis and DNA denaturation of single cells or isolated nuclei and the dMDA amplification (multiple displacement amplification in droplets) in single emulsion droplets using the dMDA microfluidic cartridge.

The procedure includes the following steps:

- Lysis of the single cells / nuclei and DNA denaturation
- Mixing with dMDA reagents
- dMDA droplets generation
- dMDA incubation
- Isolation of amplified DNA

We also include recommendations for:

- DNA quality control after dMDA
- Next Generation Sequencing

The general Xdrop Instrument description, detailed handling and loading of the dMDA cartridge as well as the enzyme mix recipes are described in the Xdrop manual.

NOTE: when working with single cells or nuclei, only one copy of each allele is present and all possible measures to avoid losing any material should be taken. Therefore, it is important to minimise handling of lysed material before the amplification has occurred. We recommended minimising excess mixing of volumes and using low bind plastic tubes and pipette tips.

Lysis of single cells / nuclei and DNA denaturation

Many different techniques can be used to isolate single cells or nuclei. The most common method for single cell isolation is cell sorting using Fluorescent Assisted Cell Sorter (FACS) technology. We recommend adding a cell or nuclei into a small volume of lysis buffer in a microtiter plate, 0.2 mL low bind PCR vial or similar.

The following protocol is made for single cells sorted into a in a low bind 0.2 mL PCR vials.

Add 2.8* µL Lysis buffer (200 mM KOH, 5 mM EDTA (pH 8) and 40 mM 1.4 DTT) in a low bind 0.2 mL PCR vial and spin down briefly.

Sort one cell/nucleus into each PCR vial and incubate for 5 minutes at room temperature to allow the cell lysis and DNA denaturation . Then add 1.4 µL neutralization buffer (400 mM HCl and 600 mM Tris HCl (pH 7.5)) and incubate for additional 5 min at room temperature, then place the vials on ice.

* Different technologies have different requirements for volumes and the lysis buffer can be diluted with up to 2 uL of PCR grade water just before use. Dilutions may however mean that extended incubation times are needed to properly lyse and denature the cell and its components (particularly its DNA). We do not

recommend to use more lysis buffer since its high salt concentrations may interfere with the downstream droplet production and stability.

Mixing with dMDA reagents

Add 15.8 µL dMDA of mastermix (Samplix dMDA kit item# RE20300) to each well (Table 1). Mix the reagents carefully with the lysed cell / nucleus and spin down in a centrifuge. Then, using a wide bore pipette tip, inject the 20 µL dMDA amplification mixture into the Inlet well and hold the pipette plunger for 15 seconds before releasing (holding the plunger). Then add 75 µL dMDA oil (Samplix dMDA kit item # RE20300) into the side of the Inlet well.

dMDA master mix	1x	
dMDA mix (5x)	4 µl	15.8µL dMDA mastermix
dMDA enzyme	1 µl	
H_2O	10.8 µl	
Total volume (mastermix only)	**15.8 µl**	
Lysed/neutralized nuclei or cell	4.2 µl	
Total volume (including lysed sample)	**20 µl**	

Table 1: dMDA mastermix.

dMDA droplets generation

Close the dMDA cartridge with a gasket, place it into the dMDA holder and insert into the Xdrop instrument. Start the dMDA program, which will produce single emulsion droplets within ~ 45 seconds. Transfer the droplets from the collection well of the dMDA cartridge into low bind 0.2 ml PCR vials. Make sure to harvest as much droplets as possible by taking extra oil from the bottom of the 0.2 mL well PCR vial and wash the collection well. After all droplets are collected, allow then to settle at the top of the oil and the oil excess is removed from the collection PCR vials leaving only 2-3 mm of dMDA oil at the bottom of each vial.

dMDA incubation

Move the dMDA droplets in the 0.2 mL collection PCR vials to a thermal block and incubate the MDA droplets at 30°C for 16 hours and then heat inactivated at 65°C for 10 minutes. If using the thermal block with a hot lid make to set the lid temperature to 75° C during the incubation.

Isolation of amplified DNA

Break the droplets by adding 20 µL of Break solution and 1 µL of Break colour (Samplix dMDA kit item # RE20300) (Samplix ApS, Herlev, Denmark) and mix moderately by flicking (not vortex). Spin down briefly and remove the oil phase from the bottom but keep the aqueous phase (pink/reddish). Repeat spinning to fully remove the oil from the aqueous phase if needed.

DNA quality control

The DNA material from Xdrop droplet MDA may be quantified using Qubit ™ Fluorometer (ThermoFisher Inc., Waltham, MA, USA) and the DNA integrity investigated using Fragment Analyzer or a Tapestation (Agilent Inc., Santa Clara, CA, USA) according to the manufacturer's instructions. Note that dMDA material can be branched and may behave differently from genomic DNA of the same size.

Next Generation Sequencing

It is recommended to make DNA libraries using TruSeq PCR free DNA library kit (Cat. No. 20015962, Illumina Inc., San Diego, CA, USA), or similar methodologies limiting PCR cycling of the library, after standard mechanical fragmentation (for example Covaris, Woburn, MA, USA) or enzymatic fragmentation (for example NEBNext® Ultra™ II FS DNA Module from New England Biolabs, cat. No. E7810S).

Appendix

Extra reagents needed (must be prepared in your own lab):

Lysis buffer: 200 mM KOH + 5 mM EDTA + 40 mM DTT

- Store in aliquots at -80°C for no longer than three months.

Neutralization buffer: 400 mM HCl + 600 mM Tris-HCl, pH 7.5

- Store in aliquots at -80°C for no longer than three months.