Xdrop® droplet compatibility with common growth media

Rikke Stick Hansen and Cristina Gamba

Samplix ApS, Birkerød, Denmark

Summary

- Xdrop double-emulsion droplets are compatible with a broad range of media for growing bacteria, fungi, and mammalian cells

Introduction

Droplet microfluidics has revolutionized the study of single cells. Using picolitre droplets to isolate cells facilitates the extraction of a wide range of insights, including genomic, transcriptomic, proteomic, and metabolomic data.

The encapsulation process creates an isolated compartment for a single cell's immediate environment. The high throughput supports the analysis of thousands to millions of single cells, making it suitable for heterogeneous population analysis and rare event discovery. For such applications, the droplets must be compatible with common growth media.

Xdrop technology only requires low input volumes, making large screening efforts fast and affordable. Furthermore, the double-emulsion droplets (Figure 1) are compatible with droplet- and cell-sorting technology.

Here, we report on the compatibility of Xdrop double-emulsion droplets with various media for growing bacteria, fungi, or mammalian cells as well as some solutions commonly used in cell biology.

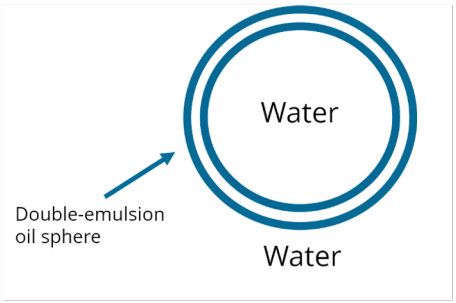

Figure 1. Double-emulsion droplet structure. They are composed of an oil sphere filled and surrounded by aqueous solutions (referred to as water-in-oil-in water).

Methods

All media and solutions listed in Table 1 were tested in using an Xdrop, Xdrop DE20 Cartridges and Gaskets, and DE oil. The inner buffers for the double-emulsion droplets were prepared with or without supplements and sterile filtered. The outer buffer was either the standard Samplix dPCR buffer (1x) or the inner buffer mixed with Samplix Stabilizing Solution (1x).

The procedure is Xdrop high-throughput screening, as described in the Xdrop Manual.

Results

In 80% of cases, the media tested were compatible with the formation of double-emulsion droplets, both when coupled with the standard outer buffer (dPCR buffer) and when the inner and outer media were the same (the outer in combination with Samplix Stabilizing Solution). Of media and solutions tested, 68% of them where always successful (100% success rate, Table 1), 18% were partially successful (ranging from 33% success rate to 87%) and 14% were not successful. Droplet images for some of the media tested are shown in Figure 2.

Conclusion

The high success rate demonstrates that the Xdrop double-emulsion droplet system has the potential to encapsulate bacterial, yeast or mammalian cells with a wide variety of growth media. Cell growth potential is further supported by the possibility to replace the outer buffer with growth media, including supplements, and the partial permeability of the oil layers of the double-emulsion shell, potentially allowing for nutrient exchange.

Learn more about Xdrop at samplix.com/applications and samplix.com/technology.

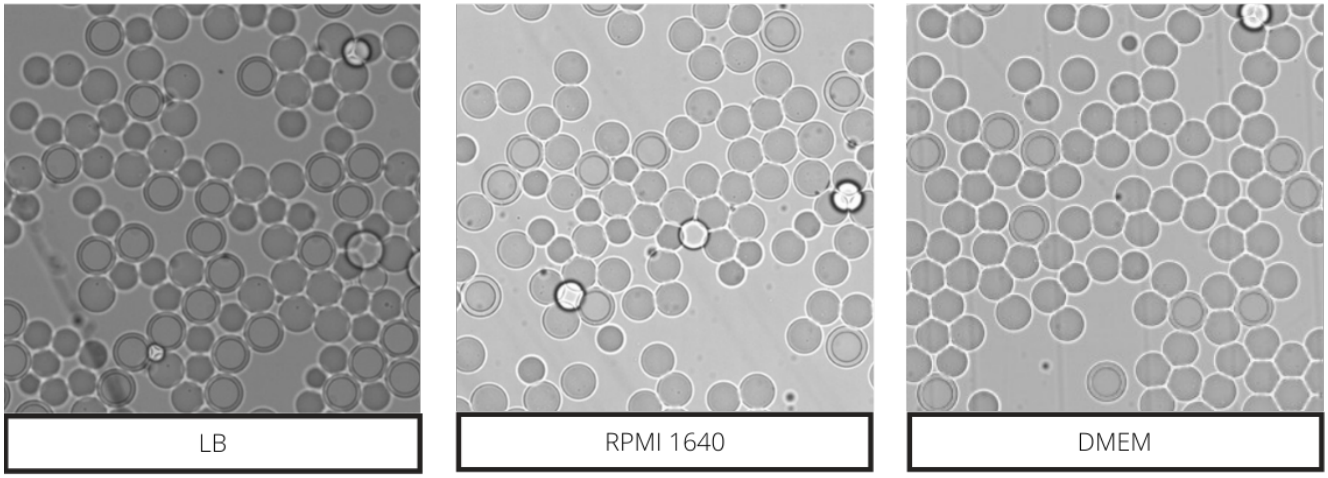

Figure 2. Microscope images (magnification 10x) of the droplets produced with three of the tested media. In all three cases, the outer buffer was the same as the growth medium plus Samplix Stabilising Solution. Standard DE oil was used for droplet formation.

Table 1. Compatibility list for Xdrop double-emulsion droplets and growth media. The success ratio was calculated as the number of successful droplet productions (successes) over the total number of droplet productions (tests). Supp = supplements: FBS (fetal bovine serum; 10%), HEPES (10–25 mM), Na pyruvate (1 mM), non-essential amino acids (1:100). Pen-strep = Penicillin–streptomycin (100 U/ml). SS = Samplix Stabilizing Solution

Application	Inner medium or solution	Outer medium or solution	Success ratio (%)
Bacterial cells	LB: Lysogeny broth, mainly for bacterial growth	dPCR buffer	100
		Inner buffer+ SS	87.5
	M2: Basic culture media, used to maintain microorganisms	dPCR buffer	0
		Inner buffer+ SS	100
	PDB: Potato dextrose broth, primarily used to grow fungi and bacteria	dPCR buffer	50
	M-17: Primarily used to isolate lactic *Streptococci*	dPCR buffer	0
		Inner buffer+ SS	100
	MRS: De Man, Rogosa and Sharp, primarily used to grow and isolate *Lactobacillus* species	dPCR buffer	0
		Inner buffer+ SS	100
Mammalian cells	RPMI 1640: Roswell Park Memorial Institute 1640 solution, primarily used to grow lymphoblastoid cells in suspension	dPCR buffer	100
		Inner buffer+ SS	100
	RPMI 1640 + Supp: As above, including supplements	dPCR buffer	100
		Inner buffer+ SS	83
	RPMI 1640 + Supp + Pen-Strep: As above, including penicillin–streptomycin	dPCR buffer	100
		Inner buffer+ SS	50
	DMEM: Dulbecco's modified Eagle's medium, used to grow a variety of mammalian cells	dPCR buffer	100
		Inner buffer+ SS	100
	DMEM + Supp: As above, including supplements	dPCR buffer	100
		Inner buffer+ SS	100
	Eagle's MEM: Eagle's minimal essential medium, primarily used to grow attached cell lines	dPCR buffer	100
		Inner buffer+ SS	100
	MEM Eagle + Supp: As above, including supplements	Inner buffer+ SS	100
Yeast	YDP: Yeast extract, peptone, and dextrose broth, for propagating yeast	Inner buffer+ SS	100
Other solutions	Unfiltered skimmed milk	dPCR buffer	0
		Inner buffer+ SS	100
	PBS: Phosphate-buffered saline	dPCR buffer	33.33
	10 mM Tris hydrochloride (Tris-HCl)	dPCR buffer	100
	0.9% NaCl	dPCR buffer	100