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BIO ONE BIOPRINTING PROTOCOL

CELLINK XPLORE

This is a suggested procedure, please adjust it according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for dispensing droplets and bioprinting multi-layered grids with CELLINK XPLORE using the BIO ONE bioprinter. Droplets and grids printing reveals the CELLINK XPLORE training ink printability of simple and complex structures for training and visualization of different compartments within a construct. This document covers procedures for printing without cells on Petri dishes or in 96-well plates in unsterile condition, including suggested bioprinting parameters as well as post-print ionic crosslinking.

Materials

- CELLINK XPLORE*
- 3 mL BD Plastipak™ Syringes with Luer-Lok™ Tip (Ref#309658)
- Conical bioprinting nozzles, 22-27G recommended*
- BIO ONE 3D bioprinter*
- Well plate or Petri dish*

*The product can be purchased in the CELLINK shop at www.cellink.com/shop.

Protocol

The protocol has been optimized for use with the BIO ONE bioprinter. This protocol can be performed with printhead and printbed at room temperature, where room temperature is between 20-25°C.

1. Preparation for printing

MATERIAL

CELLINK XPLORE

3 mL BD syringes with Luer lock connections

Female/female Luer lock adaptor

Conical bioprinting nozzles, 22-27G recommended.

DESCRIPTION

- Let CELLINK XPLORE reach room temperature.
- Transfer CELLINK XPLORE to the 3 mL syringe using the Luer lock adapter and cap the syringe with a printing nozzle of choice, 22-27G recommended.
- Place the syringe into the printhead. Rotate the syringe plunger holder arm over the plunger and twist the syringe by the tabs counterclockwise to ensure it is locked in place.

Note:

- It is not recommended to blend cells with CELLINK XPLORE as it is intended for use in demonstration purposes and for visualization of different compartments within a construct.

2. Calibration and nozzle priming

MATERIAL

BIO ONE

Well plate or Petri dish

DESCRIPTION

- Place a Petri dish or well plate on the printbed and perform either manual- or automatic calibration. Perform calibration each time a new syringe is placed in the printhead. If needed, perform Autobed levelling.
- Right before each print, prime the nozzle by extruding a couple of drops of material. Make sure the nozzle tip is clean before starting the print.

Notes:

- Before starting the print, test the flow of the bioink using the Test extrude button with the recommended starting parameters in Table 1 or even lower extrusion rate to avoid losing material during the nozzle priming procedure.
- If the system has been idle for an extended period, the bioink in the nozzle can dry causing it to clog. If this occurs purge the nozzle by extruding 10 to 50 µL of the bioink or until the dried part is extruded. If the clog cannot be removed, replace with a new nozzle. Always ensure the nozzle is fully primed with bioink prior to printing.

3. Printing

MATERIAL

BIO ONE

Well plate or Petri dish

DESCRIPTION

- Dispense droplets or print three-layered grids with parameters according to Table 1 or Table 2 respectively, in a well plate or Petri dish.
- See Figure 1 for reference droplets and Figure 2 for three-layered grid structures.
- If the printed structures are not as desired, adjust the extrusion rate up/down by 0.1 µL to extrude more/less material or refer to the BIO ONE Protocol Parameter Guidelines.

Notes:

- The values are only a reference of starting parameters. The actual values needed to print will vary depending on the preparation procedures well as the print surface.

Table 1. Recommended settings in DNA Studio Core used for dispensing 5 µL droplets of CELLINK XPLORE through a 22G nozzle in a 96-well plate using the Droplet Print function on the BIO ONE bioprinter.

Parameters	
Well plate	96-well plate
Printbed temperature	-
Printhead temperature	-
Extrusion rate	60 µL/s
Extrusion volume	20 µL
Retract volume	15 µL
Z-offset	0.3 mm
Extra preflow volume	0 µL
Retract rate	60 µL/s
Postflow stop time	0.5 s

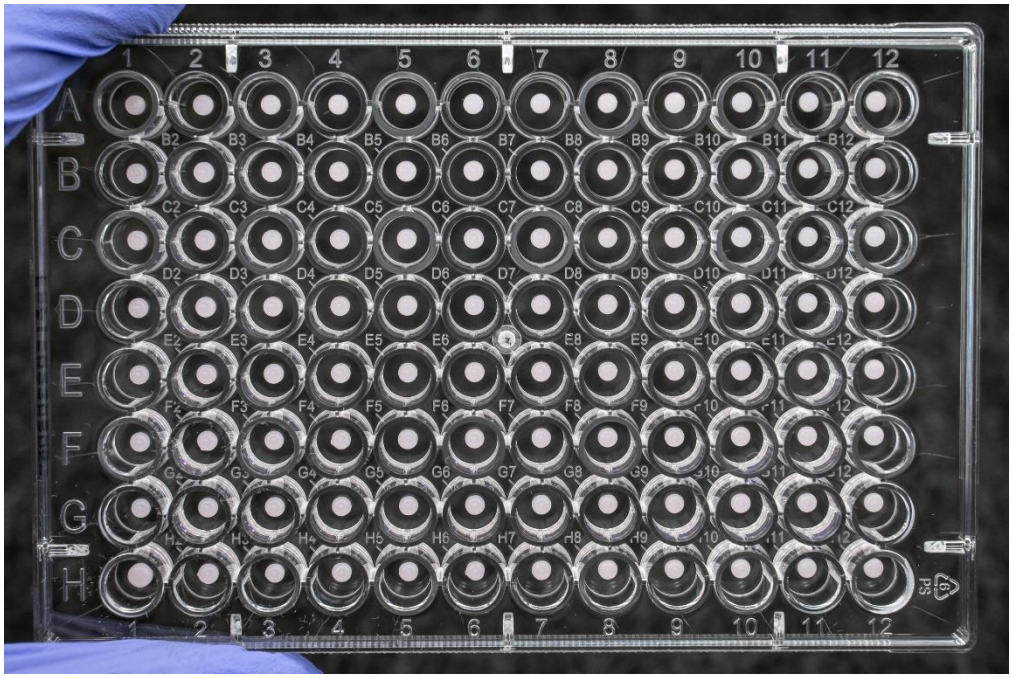

Figure 1. 5 µL droplets of CELLINK XPLORE dispensed in a 96-well plate, using parameters in Table 1.

Table 2. Recommended settings in DNA Studio Core used for printing three-layered grids (20 x 20 mm) of CELLINK XPLORE.

Parameters	
Surface	Petri dish
Printbed temperature	-
Nozzle	0.2 mm (27G)
Speed	10 mm/s
Printhead temperature	-
Preflow volume	35 µL
Extrusion rate	2.5 µL/s
Retract volume	35 µL
Z-offset	0.2 mm
Extra preflow volume	10 µL
Infill extrusion multiplier	100%
Retract rate	60 µL/s
Extra retract	0 µL
Postflow stop time	0.3 s
Z-lift	3.0 mm

Figure 2. Three-layered grid structure, 20 x 20 mm, acquired after printing with the parameters in Table 2 with CELLINK XPLORE. The picture was taken prior to ionic crosslinking.

4. Crosslinking

MATERIAL

Crosslinking Agent

Hanks' Balanced Salt solution containing calcium

DESCRIPTION

- CELLINK XPLORE is crosslinked using the $CaCl_2$-containing Crosslinking Agent. Submerge the constructs in the Crosslinking Agent for 30 seconds to 5 minutes depending on construct size, infill density and desired construct stiffness.
- Replace the Crosslinking Agent with the Hanks' Balanced Salt solution containing calcium.