C1 MiniMetagenomics Qiagen Repli-g sc Protocol

Reagents and materials to UV treat (for 30min)
DLB2 (made from DLB+IM DTT)
H2O (high quality, PCR water)
PBS
1% NaCl
Blocking Solution for C1
Cell wash buffer (DNA seq)
Tris-Cl Ph 8.0 (10mM)
Repli-g stop solution
Preload Reagent for C1
1.5 mL Eppendorf tubes (about 6)
C1 chip

Other Reagents Not UV Treated
Harvest reagent for C1
10% tween
Lysozyme Stock
Repli-g Single Cell Polymerase
Repli-g Single Cell Reaction Buffer

Cell Sample Preparation:

Centrifuged quickly for the dirt to settle in the tube (typically using 2 mL tube)
Transfer clear sample into a clean tube
Spin down the sample at 5000g for 7 min
Resuspend in 1% UV treated NaCl
Dilute until ~2 cells per nL (2*10^6 cells / mL).

Prime the Chip:

10 uL of C1 blocking reagent in cell input and output ports
200 uL harvest reagent in top and bottom rubber sealed chambers
20 ul in 4 wells (2+2) on the left and right side of the chip
20 uL harvest reagent in 4*9 wells
Strip film from bottom of chip
Load chip
Run Mini-Metagenomics Prime Script

Load Cells:

Remove C1 blocking reagent
Put 10-15 uL of the properly diluted cell mixture only in the left cell load port
20 uL C1 pre-load reagent in well #2
Load chip
Run Mini-Metagenomics Random Capture Script

Making Master Mixes:

Make 10% tween in clean water

Make DLB2 solution
DLB 33 uL (44 ul)
DTT 1M 3 uL (4 ul)
UV treat for 30 min

Make Lysozyme Stock (Once):
Lysozyme Stock at 36,000 U/uL 2.2 uL
UV treated Tris-Cl (10mM) 197.9 uL
Final Lysozyme Concentration 400 U/uL

A	**Make Lysozyme Enzyme Master Mix** 400 U/uL Lysozyme Stock 10 uL UV treated Tris-Cl (10mM) 25 uL 10% Tween 5 uL Final Lysozyme Concentration 100 U/uL
B	**Make Lysis solution** DLB2 solution 19 uL 10% Tween 1 uL Total : 20 uL
C	**Make Stop Mix** Repli-g stop solution 19 uL 10% Tween 1 uL Total: 20 uL
D	**Make Salt Addition Solution with Master Mix** DLB2 2.25 uL Repli-g stop solution 2.25 uL Repli-g Single Cell Reaction Buffer 13 uL UV treated water 16.25 uL Total: 33.75 uL
E	**Make MDA Master Mix** Repli-g Single Cell Reaction Buffer 30.75 uL Repli-g single cell polymerase 3 uL Total: 33.75 uL

Running MDA:

Add 7 uL lysozyme enzyme mix (A) to well #3
Add 7 uL lysis mix (B) to well #4
Add 9 uL Stop mix (C) to well #6
Add 24 uL (12 ul+12 ul) Make Salt Addition Solution with Master Mix (D) to well #7
Add 24 uL (12 ul+12 ul) Make MDA Master Mix (E) into well #8
Top up preload reagent to 20 uL in well #2
Add 180 uL harvest reagent to the 4 troughs
Load chip and run the Mini-Metagenomics Qiagen Lysis MDA script