

## 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

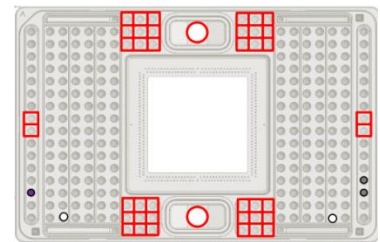
Other Reagents Not UV Treated
Harvest reagent for C1
10% tween
Lysozyme Stock
Repli-g Single Cell Polymerase
Repli-g Single Cell Reaction Buffer

### 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 ( $2 \times 10^6$  cells / mL).

### Prim the Chip:

10 uL of C1 blocking reagent in cell input and output ports (white circles)  
200 uL harvest reagent in top and bottom rubber sealed chambers (red circles)  
20 uL harvest reagent in  $4 \times 9 + 4$  wells (labeled with red squares)  
20 uL C1 pre-load reagent in well #2  
20 uL cell wash buffer in well #1 and #5  
Strip film from bottom of chip  
Load chip  
Run Standard 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  
Top up pre-load reagent in well #2 (~5ul)  
Load chip  
Run Load Cell Script  
Use the custom script (named Random Dispersion) for longer loading time

### 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	<b>Make Lysozyme Enzyme Master Mix</b> 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	<b>Make Lysis solution</b> DLB2 solution 19 uL 10% Tween 1 uL Total : 20 uL
C	<b>Make Stop Mix</b> Repli-g stop solution 19 uL 10% Tween 1 uL Total: 20 uL
D	<b>Make Salt Addition Solution with Master Mix</b> 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	<b>Make MDA Master Mix</b> Repli-g Single Cell Reaction Buffer 30.75 uL Repli-g single cell polymerase 3 uL Total: 33.75 uL

### Running MDA:

Add 10 uL lysozyme enzyme mix (A) to well #3

Add 10 uL lysis mix (B) to well #4

Add 10 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 24 uL in well #2

Add 200 uL harvest reagent to the 4 troughs

Load and Run Custom MDA Script