

## Supplementary file 1: Library prep protocol

### MODIFIED TRUSEQ NANO LIBRARY PREP PROTOCOL

Step	Reagent volume (uL)	Vendor; catalogue number
<b>1 End Repair</b>		
Input cfDNA (1.5ng/uL)	20	
End Repair Mix 2	12	Illumina; 15036418, kit FC-121-4003
<b>Total Volume (uL)</b>	<b>32</b>	

Thermocycler conditions: End repair

30°C, 30 min

70°C, 10 min

10°C, 5 min, then hold

### 2 dA-Tailing

End-repaired cfDNA	32	
A-Tailing Mix	32	Illumina; 1503641, kit FC-121-4003
<b>Total Volume (uL)</b>	<b>64</b>	

Thermocycler conditions: dA-Tailing

37°C, 30 min

70°C, 5 min

10°C, 5 min and then hold

### 3 Ligation

dA-Tailed DNA	64	
Ligation Mix 2	6	Illumina; 15036183, kit FC-121-4003
Adapter	5	Illumina; kit FC-121-4003

### STANDARD TRUSEQ NANO LIBRARY PREP PROTOCOL

Input DNA	60
End Repair Mix 2	40
<b>Total Volume (uL)</b>	<b>100</b>

30°C, 30 min

4°C hold

2 rounds of DNA purification and size selection

End-repaired DNA	17.5
A-Tailing Mix	12.5
<b>Total Volume (uL)</b>	<b>30</b>

Thermocycler conditions: dA-Tailing

37°C, 30 min

70°C, 5 min

10°C hold

dA-Tailed DNA	30
Ligation Mix 2	2.5
Resuspension Buffer (RSB)	2.5

**Total Volume (uL) 75**

**4 Stop ligation**

Thermocycler conditions: Stop ligation

20°C, 30min

Stop Ligation buffer 5 Illumina; 15012546, kit FC-121-4003

**Total Volume (uL) 80**

**5 Sample purification**

AMPure XP bead 64 Beckman Coulter; cat# A63880  
Elute in 23 uL RSB. Transfer 20 uL Illumina; kit FC-121-4003

**6 PCR amplification of ligation product**

5X KAPA HiFi Fidelity buffer 10  
10 mM dNTP Mix 1.5 Kapa BioSystems; cat# KK2621  
HiFi HotStart DNA Polymerase 1  
PPC 5 Illumina; kit FC-121-4003  
H2O 12.5  
adapter-ligated library 20  
**Total Volume (uL) 50**

Thermocycler conditions: PCR amplification of ligation product

98°C 45 sec  
98°C 15 sec  
60°C 30 sec  
72°C 30 sec  
72°C 1 min  
} 9 cycles (>10 ng cfDNA input), OR 12 cycles (<10 ng cfDNA input)

Adapter 3

**Total Volume (uL) 38**

Thermocycler conditions: Stop ligation

30°C, 10 min

Stop Ligation Buffer 5

**Total Volume (uL) 43**

2 rounds of DNA purification with AMPure XP beads

Ligated Product 25

PPC primer 5

Enhanced PCR Mix 20

**Total Volume (uL) 50**

95°C 3 min  
98°C 20 sec  
60°C 15 sec  
72°C 30 sec  
72°C 5 min  
} 9 cycles

4°C Hold

**7 Sample purification**

AMPure XP bead 50 Beckman Coulter; cat# A63880  
Elute in 25 uL RSB. Transfer 23 uL to Targeted Panel Enrichment

4°C Hold

AMPure XP bead 90  
Elute in 31.5 uL RSB. Transfer 30 uL to Targeted Panel Enrichment