SCI-seq: Sequencing thousands of single-cell genomes with combinatorial indexing

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Abstract

Single-cell genome sequencing has proven valuable for the detection of somatic variation, particularly in the context of tumor evolution. Current technologies suffer from high library construction costs which restrict the number of cells that can be assessed and thus impose limitations on the ability to measure heterogeneity within a tissue. Here, we present Single cell Combinatorial Indexed Sequencing (SCI-seq) as a means of simultaneously generating thousands of low-pass single cell libraries for somatic copy number variant detection.

Introduction

Here we present the protocol for Single cell Combinatorial Indexing and Sequencing (SCI-seq) as a means of producing thousands of low-pass single cell genome sequencing libraries for the purpose of copy number variant and aneuploidy detection. The protocol requires nucleosome depletion in order to provide uniform sequence reads throughout the genome. We provide two alternative methods: Lithium Assisted Nucleosome Depletion (LAND) and crosslinking with SDS (xSDS). LAND typically produces greater read counts per cell at the cost of decreased coverage uniformity, whereas xSDS produced more uniform coverage at the cost of reduced read counts.

Reagents

- Phosphate Buffer Saline (PBS, Thermo Fisher, Cat. 10010023)
- 0.25% Trypsin (Thermo Fisher, Cat. 15050057)
- Tris (Fisher, Cat. T1503)
- HCl(Fisher, Cat. A144)
- NaCl (Fisher, Cat. M-11624)
- MgCl2 (Sigma, Cat. M8226)
- Igepal CA-630 (Sigma, I8896)
- Protease Inhibitors (Roche, Cat. 11873580001)
- Lithium 3,5-diiodosalicylic acid (Sigma, Cat. D3635) - LAND Only
- Formaldehyde (Sigma, Cat. F8775) – xSDS Only
- Glycine (Sigma, Cat. G8898) – xSDS Only
- HEPES (Fisher, Cat. BP310) – xSDS Only
- NEBuffer 2.1 (NEB, Cat. B7202) – xSDS Only
- SDS (Sigma, Cat. L3771) – xSDS Only
- Triton-X100 (Sigma, Cat. 9002-93-1) – xSDS Only
- DAPI (Thermo Fisher, Cat. D1306)
- TD buffer and NPM from Nextera kit (Illumina, Cat. FC-121-1031)
- 96 Indexed Transposomes (either assembled using published methods or obtained from Illumina, oligos shown in Table 1)
- Indexed i5 and i7 PCR primers (Table 2)
- SYBR Green (FMC BioProducts, Cat. 50513)
- Qiaquick PCR purification kit (Qiagen, Cat. 28104)
- dsDNA High Sensitivity qubit (Thermo Fisher, Cat. Q32851)
- High Sensitivity Bioanalyzer kit (Agilent, Cat. 5067-4626)
- NextSeq sequencing kit (High or Mid 150-cycle)
- Sequencing primers (Table 3)

Equipment

- Dounce Homogenizer
- 35µM Cell Strainer (BD Biosciences, Cat. 352235)
- Sony SH800 cell sorter (Sony Biotechnology, Cat. SH800) or other FACS instrument capable of DAPI-based single nuclei sorting
- CFX Connect RT Thermal Cycler (Bio-Rad, Cat. 1855200) or other real time thermocycler
- Qubit 2.0 Flourometer (Thermo Fisher, Cat. Q32866)
- 2100 Bioanalyzer (Agilent, Cat. G2939A)
- NextSeq 500 (Illumina, Cat. SY-415-1001)

Procedure

1) Preparation of Nucleosome Depleted Nuclei (i: LAND or ii: xSDS)

i) LAND Nuclei Preparation & Nucleosome Depletion

Suspension Cell Culture	Adherent Cell Culture	Tissue Sample
a) Triturate gently to break up cell clumps. b) Pellet cells by spinning at 500xg for 5 minutes. c) Wash with 500 µL ice cold PBS.	a) Aspirate media and wash cells with 10 mL of PBS at 37°. b) Add enough 0.25% Trypsin at 37° to cover monolayer. c) Incubate at 37° for 5 minutes or until 90% of cells are no longer adhering to surface. d) Add 37° media at 1:1 ratio to quench Trypsin. e) Pellet cells by spinning at 500xg for 5 minutes. f) Wash with 500 µL ice cold PBS.	a) Place tissue sample in 2 mL dounce homoginzer on ice. b) Add 2 mL of NIB buffer (10mM TrisHCl pH7.4, 10MM NaCl, 3mM MgCl2, 0.1% igepal, 1x protease inhibitors)to sample and allow to incubate on ice for 5 minutes. c) Dounce 5 times with loose pestle followed by 15 strokes with tight pestle. d) Put sample through 35µM cell strainer. (it may be necessary to use more than one strainer)

a) Pellet cells or nuclei by spinning at 500xg for 5 minutes.
b) Resuspend in 200 µL 12.5 mM LIS in NIB buffer (2.5 µL 1M LIS + 197.5 µL NIB buffer).
c) Incubate on ice for 5 minutes.
d) Add 800 µL NIB buffer and 5 µL DAPI(5 mg/mL).
e) Gently pass through 35µM cell strainer.

ii) xSDS Nuclei Preparation & Nucleosome Depletion

Suspension Cell Culture	Adherent Cell Culture	Tissue Sample
a) Triturate gently to break up cell clumps. b) To 10 mL of cells in media add 406 µL of 37% formaldehyde and incubate at room temp for 10 minutes with gentle shaking. c) Add 800 µL of 2.5 M Glycine and incubate on ice for 5 minutes. d) Centrifuge at 550xg for 8 minutes at 4°. e) Wash with 10 mL of ice cold PBS. f) Resuspend cells in 5 mL of ice cold NIB (10mM TrisHCl pH7.4, 10MM NaCl, 3mM MgCl2, 0.1% igepal, 1x protease inhibitors). g) Incubate on ice for 20 minutes with gentle mixing.	a) Aspirate media and wash cells with 10 mL of PBS at 37°. b) Add enough 0.25% Trypsin at 37° to cover monolayer. c) Incubate at 37° for 5 minutes or until 90% of cells are no longer adhering to surface. d) Add 37° media at 1:1 ratio to quench Trypsin. e) Bring volume to 10ml with media. f) Resuspend in 10 mL media, add 406 µL of 37% formaldehyde, and incubate at room temp for 10 minutes with gentle shaking. g) Add 800 µL of 2.5 M Glycine and incubate on ice for 5 minutes. h) Centrifuge at 550xg for 8 minutes at 4°. i) Wash with 10 mL of ice cold PBS. j) Resuspend cells in 5 mL of ice cold NIB. k) Incubate on ice for 20 minutes with gentle mixing.	a) Place tissue sample in 2 mL dounce homoginzer on ice. b) Add 2 mL of HEPES NIB (20mM HEPES, 10MM NaCl, 3mM MgCl2, 0.1% igepal, 1x protease inhibitors) buffer to sample and allow to incubate on ice for 5 minutes. c) Dounce 5 times with loose pestle followed by 15 strokes with tight pestle. d) Put sample through 35µM cell strainer. (it may be necessary to use more than one strainer) e) Bring volume up to 10ml with HEPES-NIB f) To the 10 ml, add 406 µL of 37% formaldehyde. g) Add 800 µL of 2.5 M Glycine and incubate on ice 5 minutes.

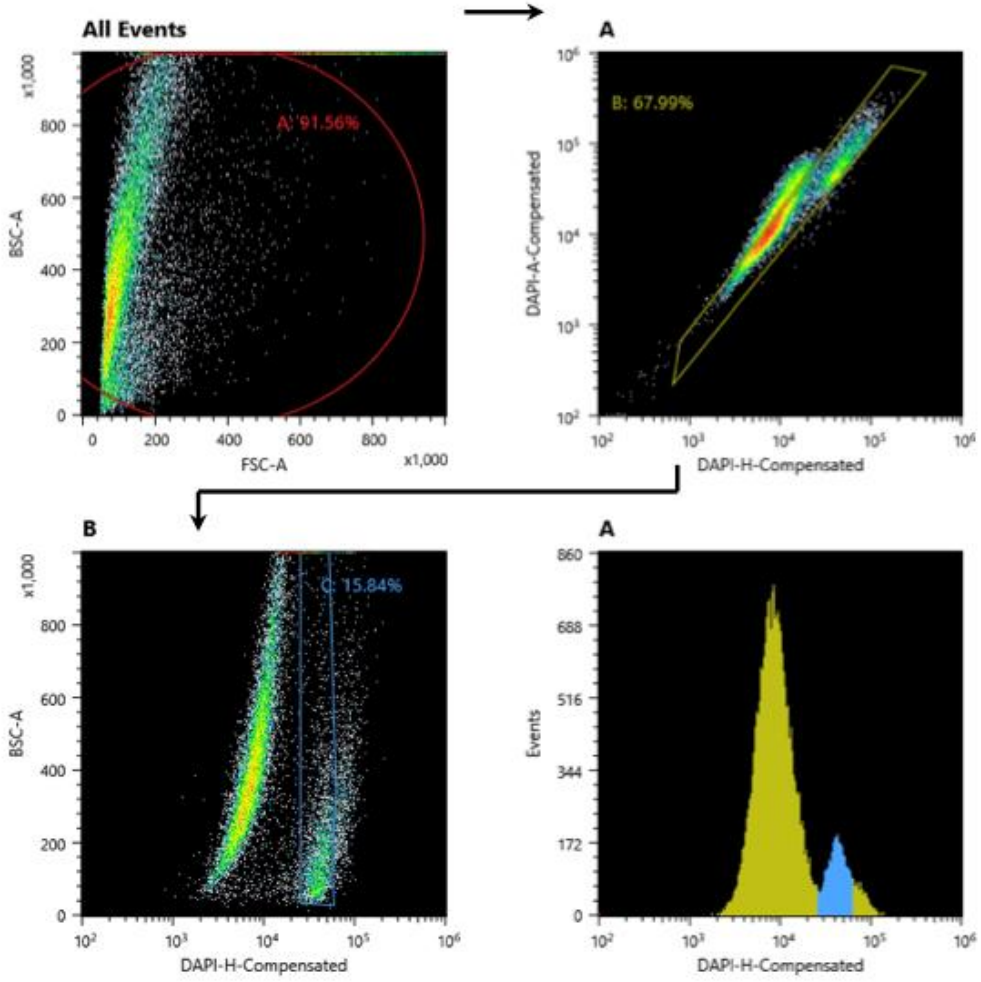
f) Pellet cells or nuclei by spinning at 500xg for 5 minutes and wash with 900 µL of 1x NEBuffer 2.1
g) Spin 500 x g for 5 minutes.
h) Resuspend in 800 µL 1x NEBuffer 2.1 with 12 µL of 20% SDS and incubate at 42°C with vigorous shaking for 30 minutes.

i) Add 200 µL of 10% Triton-X and incubate at 42°C with vigorous shaking for 30 minutes.
j) Add 5 µL of 5mg/mL DAPI and pass through a t 35 µm cell strainer.

2) Sorting and Tagmentation

a) Prep tagmentation plate with 10 µL 1x TD buffer (for 1 plate: 500 µL NIB buffer + 500 µL TD buffer)
b) Sort 2000 single nuclei into each well of the tagmentation plate.
NOTE: At this step the number of nuclei per well can be varied slightly as long as the number of nuclei per well is consistent for the whole plate.

Gating scheme as follows:



c) Spin down plate.
d) Add 1 µL 2.5 µM of uniquely indexed transposome to each well.
e) Seal plate and incubate at 55° for 15 minutes with gentle shaking.
f) Let plate return to room temperature then place on ice.
g) Pool all wells, add 5 µL DAPI(5 mg/mL), and pass through 35µM cell strainer.

3) Second Sort and PCR Indexing

a) Prepare a master mix for each well with:
   I. 0.25 µL 20mg/mL BSA
   II. 0.5 µL 1% SDS
   III. 7.75 µL H_2O
b) Add 8.5 µL of master mix and 2.5 µL of each (i5 and i7) 10 µM primer to each well of a 96 well plate.
c) Sort 15-22 single nuclei into each well using the most stringent sort settings.
d) Spin down plate.
e) LAND ONLY: Incubate for 5 min at 55° to denature transposase.
f) xSDS ONLY: Incubate at 68° for 45 minutes to denature transposase and reverse crosslinks.
g) Add 12 µL (7.5 µL NPM + 4 µL H2O + 0.5 µL 100x sybr green) to each well of strip tube (for 1 plate: 750 µL NPM + 400 µL H2O + 50 µL sybr)
h) Perform the following PCR cycles:

Cycles	Temp	Time
1	72°C	5:00
1	98°C	0:30
99*	98°C	0:10
	63°C	0:30
	72°C	1:00
	-- Plate Read --	
	72°C	0:10

i) *Run until majority of wells are exponentially amplifying.

4) Library Clean Up and Quantification

a) Pool 5 µL from each well of the PCR plate
b) Purify using Qiaquick PCR Purification column and elute in 30 µL of EB.
c) Run 2 µL of cleaned up pooled library on dsDNA HS qubit.
d) Use qubit reading to dilute library to ~4 ng/µL and run 1 µL on a High Sensitivity Bioanalyzer chip.
e) Use bioanalyzer concentration results for the range of 200 bp - 1 kb to dilute the pool to 1 nM for sequencing.

5) Sequencing

a) Set up NextSeq run as per manufacturers instructions for a 1 nM sample except for the following changes.
b) Library pool should be loaded at a concentration of 0.8 pM and a total volume of 1.5 mL.
c) Custom R1, R2, I1, and I2 primers should be loaded into the appropriate wells at a concentration of 0.6 µM.
d) Nextseq should be operating in standalone mode. Choose the SCIseq custom chemistry recipe (Amini et. al. 2014). Select dual index. Enter appropriate number of read cycles (50 recommended). And 18 cycles for each index. Select the custom checkbox for all reads and indices.

Cartridge position	Reagent	Concentration	Total Volume (dilute in HT1)	Stock oligo (100 uM)	HT1
7	Custom Read 1	0.6 uM	1.5 mL	9 µL	1491 µL
8	Custom Read 2	0.6 uM	1.5 mL	9 µL	1491 µL
9	Custom Index 1 & 2	Each 0.6 uM	3 mL	18 µL each	2964 µL
10	Library	0.8 pM (<800 bp)	1.5 mL		

Oligo Sequence Tables

Table 1: Tagmentation Oligos

Name	Sequence (5'->3')
Mosaic End Sequence	/5Phos/CTGTCTCTTATACACATCT
CPT_TS_i5_1	TCGTCGGCAGCGTCTCCACGCTATAGCCTGCGATCGAGGACGGCAGATGTGTATAAGAGACAG
CPT_TS_i5_2	TCGTCGGCAGCGTCTCCACGCATAGAGGCGCGATCGAGGACGGCAGATGTGTATAAGAGACAG
CPT_TS_i5_3	TCGTCGGCAGCGTCTCCACGCCCTATCCTGCGATCGAGGACGGCAGATGTGTATAAGAGACAG
CPT_TS_i5_4	TCGTCGGCAGCGTCTCCACGCGGCTCTGAGCGATCGAGGACGGCAGATGTGTATAAGAGACAG
CPT_TS_i5_5	TCGTCGGCAGCGTCTCCACGCAGGCGAAGGCGATCGAGGACGGCAGATGTGTATAAGAGACAG
CPT_TS_i5_6	TCGTCGGCAGCGTCTCCACGCTAATCTTAGCGATCGAGGACGGCAGATGTGTATAAGAGACAG
CPT_TS_i5_7	TCGTCGGCAGCGTCTCCACGCCAGGACGTGCGATCGAGGACGGCAGATGTGTATAAGAGACAG
CPT_TS_i5_8	TCGTCGGCAGCGTCTCCACGCGTACTGACGCGATCGAGGACGGCAGATGTGTATAAGAGACAG
CPT_TS_i7_1	GTCTCGTGGGCTCGGCTGTCCCTGTCCCGAGTAATCACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_2	GTCTCGTGGGCTCGGCTGTCCCTGTCCTCTCCGGACACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_3	GTCTCGTGGGCTCGGCTGTCCCTGTCCAATGAGCGCACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_4	GTCTCGTGGGCTCGGCTGTCCCTGTCCGGAATCTCCACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_5	GTCTCGTGGGCTCGGCTGTCCCTGTCCTTCTGAATCACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_6	GTCTCGTGGGCTCGGCTGTCCCTGTCCACGAATTCCACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_7	GTCTCGTGGGCTCGGCTGTCCCTGTCCAGCTTCAGCACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_8	GTCTCGTGGGCTCGGCTGTCCCTGTCCGCGCATTACACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_9	GTCTCGTGGGCTCGGCTGTCCCTGTCCCATAGCCGCACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_10	GTCTCGTGGGCTCGGCTGTCCCTGTCCTTCGCGGACACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_11	GTCTCGTGGGCTCGGCTGTCCCTGTCCGCGCGAGACACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
CPT_TS_i7_12	GTCTCGTGGGCTCGGCTGTCCCTGTCCCTATCGCTCACCGTCTCCGCCTCAGATGTGTATAAGAGACAG

Table 2: PCR Primers

Name	Sequence (5'->3')
i7-T119-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATaatgccgcttGTCTCGTGGGCTCGG
i7-T120-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATtatagacgcaGTCTCGTGGGCTCGG
i7-T121-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATtcaatcgcatGTCTCGTGGGCTCGG
i7-T122-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATttcttaataaGTCTCGTGGGCTCGG
i7-T123-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATgtcctagaggGTCTCGTGGGCTCGG
i7-T124-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATatattgatacGTCTCGTGGGCTCGG
i7-T125-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATccgctgccagGTCTCGTGGGCTCGG
i7-T126-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATcctagtacgtGTCTCGTGGGCTCGG
i7-T127-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATcaattaccgtGTCTCGTGGGCTCGG
i7-T128-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATggccgtagtcGTCTCGTGGGCTCGG
i7-T129-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATcgattacggcGTCTCGTGGGCTCGG

i7-T130-NEX2cpt-A	CAAGCAGAAGACGGCATACGAGATtaatgaacgaGTCTCGTGGGCTCGG
i7-T131-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATccgttccttaGTCTCGTGGGCTCGG
i7-T132-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATggtaccatatGTCTCGTGGGCTCGG
i7-T133-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATccgattcgcaGTCTCGTGGGCTCGG
i7-T134-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATatggctctgcGTCTCGTGGGCTCGG
i7-T135-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATgtataatacgGTCTCGTGGGCTCGG
i7-T136-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATatcagcaagtGTCTCGTGGGCTCGG
i7-T137-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATggcgaactcgGTCTCGTGGGCTCGG
i7-T138-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATttaattgaatGTCTCGTGGGCTCGG
i7-T139-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATttaggaccggGTCTCGTGGGCTCGG
i7-T140-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATaagtaagagcGTCTCGTGGGCTCGG
i7-T141-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATccttggtccaGTCTCGTGGGCTCGG
i7-T142-NEX2cpt-B	CAAGCAGAAGACGGCATACGAGATcatcagaatgGTCTCGTGGGCTCGG
i7-T143-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATttatagcagaGTCTCGTGGGCTCGG
i7-T144-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATttacttggaaGTCTCGTGGGCTCGG
i7-T145-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATgctcagccggGTCTCGTGGGCTCGG
i7-T146-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATacgtccgcagGTCTCGTGGGCTCGG
i7-T147-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATttgactgacgGTCTCGTGGGCTCGG
i7-T148-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATttgcgaggcaGTCTCGTGGGCTCGG
i7-T149-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATttccaaccgcGTCTCGTGGGCTCGG
i7-T150-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATtaaccttcggGTCTCGTGGGCTCGG
i7-T151-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATtcaagccgatGTCTCGTGGGCTCGG
i7-T152-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATcttgcaacctGTCTCGTGGGCTCGG
i7-T153-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATccatcgcgaaGTCTCGTGGGCTCGG
i7-T154-NEX2cpt-C	CAAGCAGAAGACGGCATACGAGATtagacttcttGTCTCGTGGGCTCGG
i7-T231-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATtgcgcgatgcGTCTCGTGGGCTCGG
i7-T232-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATattgagattgGTCTCGTGGGCTCGG
i7-T233-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATttgatatattGTCTCGTGGGCTCGG
i7-T234-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATcggtaggaatGTCTCGTGGGCTCGG
i7-T235-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATaccagcgcagGTCTCGTGGGCTCGG
i7-T236-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATcgaatgagctGTCTCGTGGGCTCGG
i7-T237-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATagttcgagtaGTCTCGTGGGCTCGG
i7-T238-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATttggacgctgGTCTCGTGGGCTCGG
i7-T239-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATatagactaggGTCTCGTGGGCTCGG
i7-T240-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATtatagtaagcGTCTCGTGGGCTCGG
i7-T241-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATcggtcgttaaGTCTCGTGGGCTCGG
i7-T242-NEX2cpt-D	CAAGCAGAAGACGGCATACGAGATatggcggatcGTCTCGTGGGCTCGG
i7-T243-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATctctgatcagGTCTCGTGGGCTCGG
i7-T244-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATggccagtccgGTCTCGTGGGCTCGG
i7-T245-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATcggaagatatGTCTCGTGGGCTCGG
i7-T246-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATtggctgatgaGTCTCGTGGGCTCGG
i7-T247-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATgaaggttgccGTCTCGTGGGCTCGG
i7-T248-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATgttgaaggatGTCTCGTGGGCTCGG
i7-T249-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATccattcgtaaGTCTCGTGGGCTCGG
i7-T250-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATtgcgccagaaGTCTCGTGGGCTCGG
i7-T251-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATcgaataattcGTCTCGTGGGCTCGG

i7-T252-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATgcgacgccttGTCTCGTGGGCTCGG
i7-T253-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATatcaacgattGTCTCGTGGGCTCGG
i7-T254-NEX2cpt-E	CAAGCAGAAGACGGCATACGAGATgttctgaattGTCTCGTGGGCTCGG
i7-T255-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATgctaacctcaGTCTCGTGGGCTCGG
i7-T256-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATcaagcaactgGTCTCGTGGGCTCGG
i7-T257-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATggagcggccgGTCTCGTGGGCTCGG
i7-T258-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATcgcgtacgacGTCTCGTGGGCTCGG
i7-T259-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATcgatggcgccGTCTCGTGGGCTCGG
i7-T260-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATtggtattcatGTCTCGTGGGCTCGG
i7-T261-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATgataaggcaaGTCTCGTGGGCTCGG
i7-T262-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATgccggtcgagGTCTCGTGGGCTCGG
i7-T263-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATtgcgccatctGTCTCGTGGGCTCGG
i7-T264-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATaagtcttccgGTCTCGTGGGCTCGG
i7-T265-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATagactcaagcGTCTCGTGGGCTCGG
i7-T266-NEX2cpt-F	CAAGCAGAAGACGGCATACGAGATgcaggcgacgGTCTCGTGGGCTCGG
i5-T155-NEX1cpt-A	AATGATACGGCGACCACCGAGATCTACACgtccttaagaTCGTCGGCAGCGTC
i5-T156-NEX1cpt-A	AATGATACGGCGACCACCGAGATCTACACagtaacggtcTCGTCGGCAGCGTC
i5-T157-NEX1cpt-A	AATGATACGGCGACCACCGAGATCTACACgttcgtcagaTCGTCGGCAGCGTC
i5-T158-NEX1cpt-A	AATGATACGGCGACCACCGAGATCTACACcgcctaatgcTCGTCGGCAGCGTC
i5-T159-NEX1cpt-A	AATGATACGGCGACCACCGAGATCTACACaccggaattaTCGTCGGCAGCGTC
i5-T160-NEX1cpt-A	AATGATACGGCGACCACCGAGATCTACACtaggccatagTCGTCGGCAGCGTC
i5-T161-NEX1cpt-A	AATGATACGGCGACCACCGAGATCTACACtaactcttagTCGTCGGCAGCGTC
i5-T162-NEX1cpt-A	AATGATACGGCGACCACCGAGATCTACACtatgagttaaTCGTCGGCAGCGTC
i5-T163-NEX1cpt-B	AATGATACGGCGACCACCGAGATCTACACtatcatgatcTCGTCGGCAGCGTC
i5-T164-NEX1cpt-B	AATGATACGGCGACCACCGAGATCTACACgagcatatggTCGTCGGCAGCGTC
i5-T165-NEX1cpt-B	AATGATACGGCGACCACCGAGATCTACACtaacgatccaTCGTCGGCAGCGTC
i5-T166-NEX1cpt-B	AATGATACGGCGACCACCGAGATCTACACcggcgtaactTCGTCGGCAGCGTC
i5-T167-NEX1cpt-B	AATGATACGGCGACCACCGAGATCTACACcgtcgcagccTCGTCGGCAGCGTC
i5-T168-NEX1cpt-B	AATGATACGGCGACCACCGAGATCTACACgtagctccatTCGTCGGCAGCGTC
i5-T169-NEX1cpt-B	AATGATACGGCGACCACCGAGATCTACACttgccttggcTCGTCGGCAGCGTC
i5-T170-NEX1cpt-B	AATGATACGGCGACCACCGAGATCTACACtgctaattctTCGTCGGCAGCGTC
i5-T171-NEX1cpt-C	AATGATACGGCGACCACCGAGATCTACACgtcctacttgTCGTCGGCAGCGTC
i5-T172-NEX1cpt-C	AATGATACGGCGACCACCGAGATCTACACggtaggttagTCGTCGGCAGCGTC
i5-T173-NEX1cpt-C	AATGATACGGCGACCACCGAGATCTACACgagcatcattTCGTCGGCAGCGTC
i5-T174-NEX1cpt-C	AATGATACGGCGACCACCGAGATCTACACccgctccggcTCGTCGGCAGCGTC
i5-T175-NEX1cpt-C	AATGATACGGCGACCACCGAGATCTACACttcttccggtTCGTCGGCAGCGTC
i5-T176-NEX1cpt-C	AATGATACGGCGACCACCGAGATCTACACaggagagaacTCGTCGGCAGCGTC
i5-T177-NEX1cpt-C	AATGATACGGCGACCACCGAGATCTACACtaactcaattTCGTCGGCAGCGTC
i5-T178-NEX1cpt-C	AATGATACGGCGACCACCGAGATCTACACactataggttTCGTCGGCAGCGTC
i5-T207-NEX1cpt-D	AATGATACGGCGACCACCGAGATCTACACtaacgaattgTCGTCGGCAGCGTC
i5-T208-NEX1cpt-D	AATGATACGGCGACCACCGAGATCTACACtgagaaccaaTCGTCGGCAGCGTC
i5-T209-NEX1cpt-D	AATGATACGGCGACCACCGAGATCTACACttattctgagTCGTCGGCAGCGTC
i5-T210-NEX1cpt-D	AATGATACGGCGACCACCGAGATCTACACttattatggtTCGTCGGCAGCGTC
i5-T211-NEX1cpt-D	AATGATACGGCGACCACCGAGATCTACACatatgagccaTCGTCGGCAGCGTC
i5-T212-NEX1cpt-D	AATGATACGGCGACCACCGAGATCTACACcaaccagtacTCGTCGGCAGCGTC
i5-T213-NEX1cpt-D	AATGATACGGCGACCACCGAGATCTACACcatccgactaTCGTCGGCAGCGTC

i5-T214-NEX1cpt-D	AATGATACGGCGACCACCGAGATCTACACatcatggctgTCGTCGGCAGCGTC
i5-T215-NEX1cpt-E	AATGATACGGCGACCACCGAGATCTACACccgcaagttcTCGTCGGCAGCGTC
i5-T216-NEX1cpt-E	AATGATACGGCGACCACCGAGATCTACACcttctcattgTCGTCGGCAGCGTC
i5-T217-NEX1cpt-E	AATGATACGGCGACCACCGAGATCTACACcaggaggagaTCGTCGGCAGCGTC
i5-T218-NEX1cpt-E	AATGATACGGCGACCACCGAGATCTACACgatatcggcgTCGTCGGCAGCGTC
i5-T219-NEX1cpt-E	AATGATACGGCGACCACCGAGATCTACACccagtcctctTCGTCGGCAGCGTC
i5-T220-NEX1cpt-E	AATGATACGGCGACCACCGAGATCTACACcatagttcggTCGTCGGCAGCGTC
i5-T221-NEX1cpt-E	AATGATACGGCGACCACCGAGATCTACACcgtaatgcagTCGTCGGCAGCGTC
i5-T222-NEX1cpt-E	AATGATACGGCGACCACCGAGATCTACACccgttcggatTCGTCGGCAGCGTC
i5-T223-NEX1cpt-F	AATGATACGGCGACCACCGAGATCTACACccataagtccTCGTCGGCAGCGTC
i5-T224-NEX1cpt-F	AATGATACGGCGACCACCGAGATCTACACggcaatgagaTCGTCGGCAGCGTC
i5-T225-NEX1cpt-F	AATGATACGGCGACCACCGAGATCTACACcggttatgccTCGTCGGCAGCGTC
i5-T226-NEX1cpt-F	AATGATACGGCGACCACCGAGATCTACACtggccggcctTCGTCGGCAGCGTC
i5-T227-NEX1cpt-F	AATGATACGGCGACCACCGAGATCTACACagctgcaataTCGTCGGCAGCGTC
i5-T228-NEX1cpt-F	AATGATACGGCGACCACCGAGATCTACACtggccatgcaTCGTCGGCAGCGTC
i5-T229-NEX1cpt-F	AATGATACGGCGACCACCGAGATCTACACtgacgctccgTCGTCGGCAGCGTC
i5-T230-NEX1cpt-F	AATGATACGGCGACCACCGAGATCTACACaactgctgccTCGTCGGCAGCGTC

Table 3: Sequencing Primers

Name	Sequence (5'->3')
Read 1 sequencing primer	GCGATCGAGGACGGCAGATGTGTATAAGAGACAG
Read 2 sequencing primer	CACCGTCTCCGCCTCAGATGTGTATAAGAGACAG
Index 1 sequencing primer	CTGTCTCTTATACACATCTGAGGCGGAGACGGTG
Index 2 sequencing primer	CTGTCTCTTATACACATCTGCCGTCCTCGATCGC