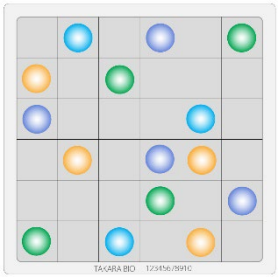

Archival nanowell sequencing

I. Introduction

Archival nanowell sequencing (Arc-well) describes high-throughput single-cell DNA-seq library preparation from nuclei isolated from fresh-frozen and FFPE tissues. The protocol was developed by scientists in Dr. Nicholas Navin's lab at the UT MD Anderson Cancer Center, and was originally published in Wang, Kaile et al. Archival single-cell genomics reveals persistent subclones during DCIS progression. *Cell* **186,** 3968–3982.e15 (2023).
Access the paper »

NOTE: This user-generated protocol is provided for general information only and is not directly supported, endorsed, or validated by Takara Bio. Takara Bio gives no warranties and makes no claims about the provided protocol. For questions, including dispense program setup and pre-experiment consultation, please contact our Field Support team at field_support@takarabio.com.

The Arc-well assay on the ICELL8® cx Single-Cell System has six dispense steps as listed below:

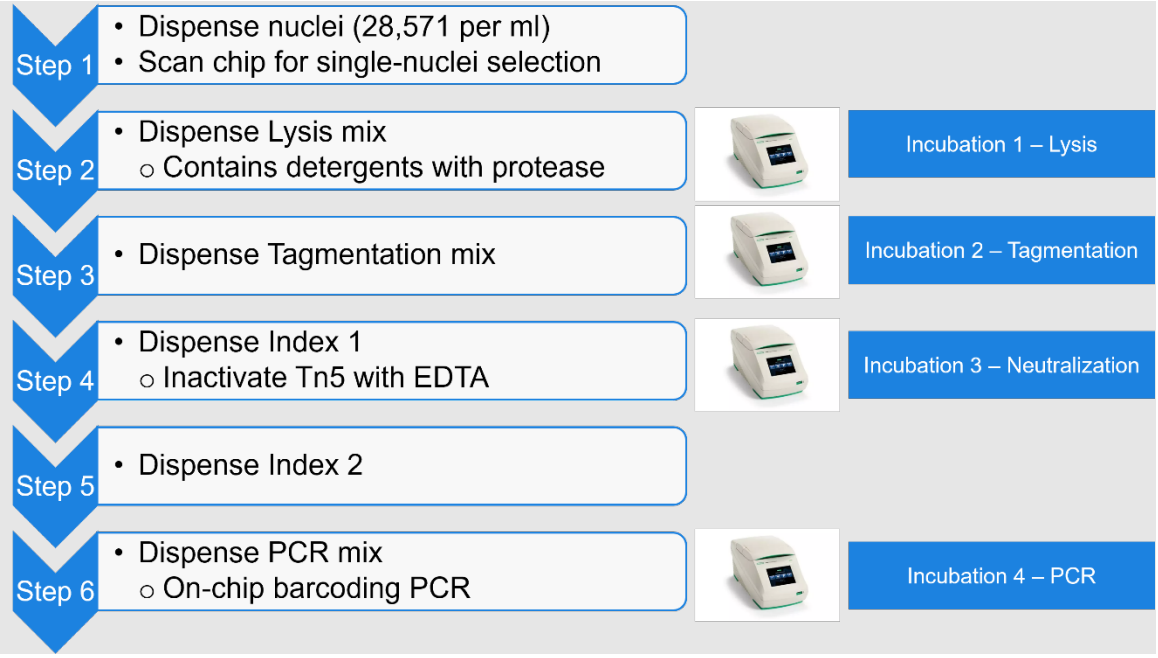

Figure 1. Schematic overview of Arc-well workflow on the ICELL8 cx system.

Using ICELL8 cx CELLSTUDIO® Software, set up an Arc-well application with the dispense steps shown in Figure 2. For additional information, please see the ICELL8 cx CELLSTUDIO Application Creation Quick Start Guide. The software will generate a 384-well source plate layout file, which you can use as a guide when aliquoting nuclei suspension or reagent master mix into the corresponding wells.

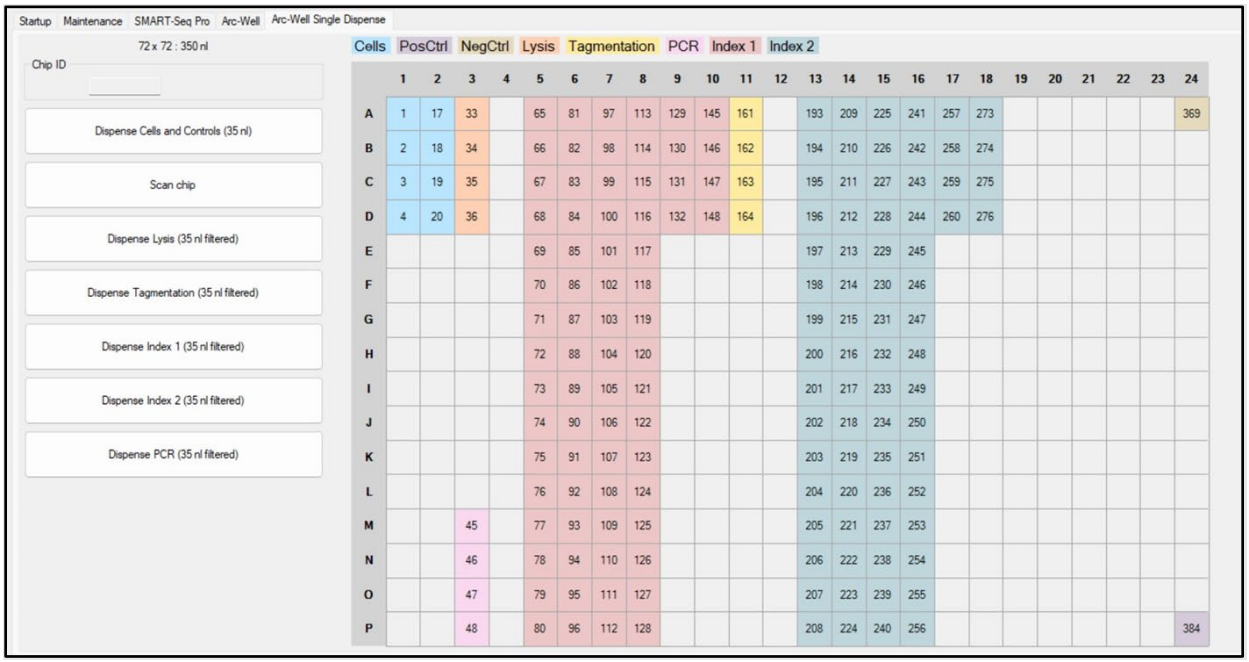

Figure 2. Arc-well dispense protocol and plate setup in the ICELL8 cx CELLSTUDIO software. Individual dispense and chip-scanning steps are shown on the left. Color-coded wells on the 384-well source plate indicate the correct position for aliquoting (right).

II. Additional Materials Required

Nuclei isolation

Reagents and equipment	Source	Catalog number
Formalin solution, neutral buffered, 10%	Millipore Sigma	HT5012-1CS
Finesse ME+	Thermo Scientific	A77500016
gentleMACS Dissociator with Heaters	Miltenyi Biotec	130-096-427
FFPE Tissue Dissociation Kit	Miltenyi Biotec	130-118-052
1.5 ml LoBind tubes	Fisher Scientific	22431021
Dimethyl sulfoxide (DMSO)	Millipore Sigma	D2650

NST-DAPI buffer for nuclei suspension

Reagents and equipment	Source	Catalog number
NaCl	Invitrogen	AM9760G
$MgCl_2$	Invitrogen	AM9530G
Tris base	Fisher Scientific	BP154-1
$CaCl_2$	Sigma-Aldrich	21115-1ML
BSA	Sigma-Aldrich	A2058-100G
Nonidet P-40	US Biological	N3500
DAPI	Invitrogen	D1306

Nuclei dispense

Reagents and equipment	Source	Catalog number
ICELL8 350v Chip	Takara Bio	640019
ICELL8 Blank Chip Reagent Kit (contains Second Diluent)	Takara Bio	640196
ICELL8 Loading Kit – B	Takara Bio	640206
ICELL8 Collection Kit – L	Takara Bio	640212
MSND 384-Well Source Plate and Seals (430-000025)	Takara Bio	640018
DPBS	Millipore Sigma	D8537

Lysis mix

Reagents and equipment	Source	Catalog number
Tris-HCl (pH 7.4)	Millipore Sigma	T2194
Tween-20	Andwin Scientific	NC9022994
Triton X-100	Fisher Scientific	ICN19485483
QIAGEN Protease (30 AU)	Qiagen	19157

Tagmentation mix

Reagents and equipment	Source	Catalog number
Illumina® Tagment DNA Enzyme and Buffer Large Kit	Illumina	20034198

Index 1, Index 2, and PCR mix

Reagents and equipment	Source	Catalog number
KAPA HotStart PCR Kit, with dNTPs	Roche	KK2502
0.5 M EDTA	Thermo Scientific	R1021
Nuclease-free water	Takara Bio	0912
$MgCl_2$	Invitrogen	AM9530G

Please see the Appendix for additional Index information.

III. Protocol

A. Nuclei isolation from FFPE tissues

1. Prepare NST-DAPI buffer using the recipe below. Filter-sterilize the NST-DAPI buffer and store at 4ºC in the dark.

Component	Volume
NST solution • 146 mM NaCl • 10 mM Tris base (pH 7.8) • 1 mM $CaCl_2$ • 21 mM $MgCl_2$ • 0.05% (w/v) BSA • 0.5% (v/v) NP-40	800 ml
DAPI solution • 106 mM $MgCl_2$ • 10 mg DAPI	200 ml
Total volume	1,000 ml

2. Section FFPE blocks to generate 1–3 50 mM scrolls using a microtome.
3. Generate single-cell suspensions from the scrolls using the gentleMACS Dissociator with Heaters and the FFPE tissue dissociation kit according to the manufacturer's recommendations with the following changes:
   a. After the last wash with ice-cold Buffer W, transfer single-cell suspensions to 1.5 ml LoBind tubes and spin at 500*g* for 5 min at 4ºC.
   b. Resuspend the cell pellet in an appropriate volume of NST-DAPI buffer (200 µl–5 ml) to generate nuclei suspensions.
      NOTE: Nuclei suspensions can be stored long term in 10% DMSO.
   c. Flow-sort nuclei suspensions with the BD FACSMelody, where DAPI intensity is used to gate the desired diploid/aneuploid populations.

B. Arc-well scDNA-seq library prep on the ICELL8 cx Single-Cell System

1. Dispense nuclei and scan chip

1. Wash nuclei suspensions with 0.5X DPBS. Follow the table below and combine Second Diluent with the nuclei suspension in 0.5X DPBS for a final concentration of 28,571 nuclei per ml.

Component	Diluted stained nuclei suspension	Volume per source well (for each sample)*
Second Diluent (100X)	10 µl	1 µl
Nuclei suspension	Dilute to 28,571 nuclei per ml	Dilute to 28,571 nuclei per ml
0.5X DPBS	Up to 1,000 µl	Up to 100 µl
Total volume	1,000 µl	100 µl

*You can load up to eight different samples per chip run.

2. Using a 200 µl pipette tip, carefully load 80 µl of nuclei suspension into wells A1, A2, B1, B2, C1, C2, D1, and D2 of a 384-well source plate (blue; Figure 2). If applicable, prepare positive and negative control samples, and refer to Figure 2 to aliquot to corresponding source wells.
3. Load the 350v chip in the chip nest, place the 384-well source plate in the ICELL8 cx Single-Cell System with the A1 corner positioned at the top-right corner of the plate nest.
4. Click [Dispense cells and Controls (35 nl)].
5. Make sure the chip and source plate are properly inserted, remove the seals, and click [Done] to confirm.
6. When the dispense is completed, remove the chip for blotting, and seal the chip with RC Film using a film-sealing roller.
7. Centrifuge the chip at 1,000*g* for 5 min at 4ºC.
8. Load the chip, remove the RC Film, and click [Scan chip].
9. Follow steps in the ICELL8 cx Single-Cell System User Manual (Section X.E, Page 62) to acquire imaging data (make sure to scan the chip under the DAPI channel), perform automatic data analysis, and save the result in the ICELL8 cx CellSelect® software.

2. Dispense Lysis mix

1. Prepare Lysis mix as below:

Component	Volume
Lysis buffer • 30 mM Tris-HCl, pH 8.0 • 5% Tween • 0.5% Triton X-100	180 µl
Protease (1.36 AU/ml)	20 µl
Total volume	200 µl

4. Aliquot 50 µl of freshly prepared Lysis mix into wells A3, B3, C3, and D3 (orange; Figure 2) of a 384-well source plate and seal the plate.
5. Load chip and source plate in the correct orientation. Remove imaging film from the chip and sealing film from the source plate.
6. Click [Dispense Lysis (35 nl filtered)].
7. When the dispense is completed, remove the chip for blotting, and seal the chip with RC Film using a film-sealing roller.
8. Centrifuge the chip at 1,000*g* for 5 min at 4ºC.
9. Put the chip onto the ICELL8 cx thermal cycler and run the Lysis program below:

59.7ºC	5 sec
54.5ºC	30 min
79ºC	11 sec
75.3ºC	15 min
4ºC	hold

3. Dispense Tagmentation mix

1. Prepare Tagmentation mix as below:

Component	Volume
2x TD buffer	144 µl
TDE1, Illumina	16 µl
Total volume	160 µl

2. Aliquot 40 µl of freshly prepared Tagmentation mix into wells A11, B11, C11, and D11 (yellow; Figure 2) of a 384-well source plate and seal the plate.
3. Load chip and source plate in the correct orientation. Remove RC Film from the chip and sealing film from the source plate.
4. Click [Dispense Tagmentation (35 nl filtered)].
5. When the dispense is completed, remove the chip for blotting, and seal the chip with RC Film using a film-sealing roller.
6. Centrifuge the chip at 3,220*g* for 5 min at 4°C.
7. Put the chip onto the ICELL8 cx thermal cycler and run the Tagmentation program below:

59.7°C	5 sec
54.5°C	• 8 min (for FFPE sample) • 12 min (for fresh sample)

4. Dispense Index 1 (with Neutralization mix)

1. Prepare Neutralization master mix as below:

Component	Volume in master mix (for 72 Index wells)
5x Kapa Hifi Fidelity buffer	850 µl
25 mM dNTP	136 µl
0.5 M EDTA	85 µl
Nuclease-free water	544 µl
Total volume	1,615 µl

2. Aliquot 19 µl of the Neutralization master mix into each well for wells A5–P8, A9–D9, and A10–D10 (coral; Figure 2) on the source plate. Spike in 1 µl of Index 1 and seal:

Component	Per well in wells A5–P8, A9–D9, and A10–D10
Neutralization master mix	19 µl
100 µM Index 1	1 µl
Total volume per Index well	20 µl

3. Centrifuge the source plate and the chip (with RC Film on) at 3,220*g* (minimum 2,600*g*) for 3 min at 4ºC.
4. Load chip and source plate in the correct orientation. Remove RC Film from the chip and sealing film from the source plate.
5. Click [Dispense Index 1 (35 nl filtered)].
6. When the dispense is completed, remove the chip for blotting, and seal the chip with RC Film using a film-sealing roller.
7. Centrifuge the chip at 3,220*g* for 5 min at 4ºC.
8. Put the chip onto the ICELL8 cx thermal cycler and run the Neutralization program below:

54.9ºC	5 sec
49.4ºC	30 min
4ºC	hold

5. Dispense Index 2

1. Prepare master mix as below:

Component	**Volume in master mix (for 72 Index wells)**
5x Kapa Hifi Fidelity buffer	850 µl
1 M $MgCl_2$	29.58 µl
Nuclease-free water	735.42 µl
Total volume	1,615 µl

2. Aliquot 19 µl of the above master mix into each well for wells A13–P16, A17–D17, and A18–D18 (cyan; Figure 2) on the source plate. Spike in 1 µl of Index 2 and seal:

Component	Per well in wells A13–P16, A17–D17, and A18–D18
Master mix	19 µl
100 µM Index 2	1 µl
Total volume per Index well	20 µl

3. Centrifuge the source plate and the chip (with RC Film on) at 3,220*g* (minimum 2,600*g*) for 3 min at 4ºC.
4. Load chip and source plate in the correct orientation. Remove RC Film from the chip and sealing film from the source plate.
5. Click [Dispense Index 2 (35 nl filtered)].
6. When the dispense is completed, remove the chip for blotting, and seal the chip with RC Film using a film-sealing roller.
7. Centrifuge the chip at 3,220*g* for 5 min at 4ºC.

6. Dispense PCR mix

1. Prepare PCR mix as below:

Component	Volume
5x Kapa Hifi Fidelity buffer	40 µl
KAPA HiFi HotStart DNA Polymerase (1 U/µl)	40 µl
Nuclease-free water	120 µl
Total volume	200 µl

2. Aliquot 50 µl of freshly prepared PCR mix into wells M3, N3, O3, and P3 (pink; Figure 2) of a 384-well source plate and seal the plate.
3. Load chip and source plate in the correct orientation. Remove RC film from the chip and sealing film from the source plate.
4. Click [Dispense PCR (35 nl filtered)].
5. When the dispense is completed, remove the chip for blotting, and seal the chip with RC Film using a film-sealing roller.
6. Centrifuge the chip at 3,220*g* for 5 min at 4ºC.
7. Put the chip onto the ICELL8 cx thermal cycler and run the PCR program below:

72.1ºC	8 min
99.6ºC	30 sec
X cycles*:	
99.6ºC	20 sec
57.5ºC	5 sec
62.7ºC	30 sec
72.1ºC	1 min
72.1ºC	2 min
4ºC	hold

*10-12 cycles for frozen samples; 14-16 cycles for FFPE samples

8. Centrifuge the chip at 3,220*g* for 5 min at 4ºC.

7. Extract, purify, and QC the library

1. Open ICELL8 Collection Kit – L and label the Collection Tube with the engraved chip number.
2. Assemble the collection module by attaching the Collection Tube to the Collection Fixture.
3. Carefully peel the film from the chip.
4. With the nanowells facing down, place the chip into the assembled collection module.
5. Seal the chip and the top of the collection module with a Collection Film.
6. Using a balance or blank chip, assemble another collection module.
7. Centrifuge both collection modules at 3,220g (minimum 2,600g) for 10 min at 4°C.

8. Perform 1.8X bead purification with AmPure XP beads on 50 µl of the library.
9. QC the library with Agilent BioAnalyzer 2100. Use the bioanalyzer results to determine library quality and average size for qPCR.
10. Quantitate the library using the Library Quantification Kit (Cat. No. 638324). Refer to the Library Quantification Kit User Manual for instructions. Use the average size as determined by the bioanalyzer to calculate the molar library concentration.
11. Use the qPCR results from the Library Quantification Kit to determine the library quantity for sequencing.
12. The library can be sequenced on Illumina NextSeq® 2000 or NovaSeq™ 6000 with 8 bp dual Indexing sequencing at a target of 1 million reads per cell.

IV. Appendix

Index 1		
Well position	Name	Sequence
A5	ArcS501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTC
B5	ArcS502	AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTC
C5	ArcS503	AATGATACGGCGACCACCGAGATCTACACAGAGTAGATCGTCGGCAGCGTC
D5	ArcS504	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGTCGTCGGCAGCGTC
E5	ArcS505	AATGATACGGCGACCACCGAGATCTACACACTGCATATCGTCGGCAGCGTC
F5	ArcS506	AATGATACGGCGACCACCGAGATCTACACAAGGAGTATCGTCGGCAGCGTC
G5	ArcS507	AATGATACGGCGACCACCGAGATCTACACCTAAGCCTTCGTCGGCAGCGTC
H5	ArcS508	AATGATACGGCGACCACCGAGATCTACACCGTCTAATTCGTCGGCAGCGTC
I5	ArcS509	AATGATACGGCGACCACCGAGATCTACACTCTCTCCGTCGTCGGCAGCGTC
J5	ArcS510	AATGATACGGCGACCACCGAGATCTACACTCGACTAGTCGTCGGCAGCGTC
K5	ArcS511	AATGATACGGCGACCACCGAGATCTACACTTCTAGCTTCGTCGGCAGCGTC
L5	ArcS512	AATGATACGGCGACCACCGAGATCTACACCCTAGAGTTCGTCGGCAGCGTC
M5	ArcS513	AATGATACGGCGACCACCGAGATCTACACGCGTAAGATCGTCGGCAGCGTC
N5	ArcS514	AATGATACGGCGACCACCGAGATCTACACCTATTAAGTCGTCGGCAGCGTC
O5	ArcS515	AATGATACGGCGACCACCGAGATCTACACAAGGCTATTCGTCGGCAGCGTC
P5	ArcS516	AATGATACGGCGACCACCGAGATCTACACGAGCCTTATCGTCGGCAGCGTC
A6	ArcS517	AATGATACGGCGACCACCGAGATCTACACTTATGCGATCGTCGGCAGCGTC
B6	ArcS518	AATGATACGGCGACCACCGAGATCTACACTAAGCGTTTCGTCGGCAGCGTC
C6	ArcS519	AATGATACGGCGACCACCGAGATCTACACTCCGTCTTTCGTCGGCAGCGTC
D6	ArcS520	AATGATACGGCGACCACCGAGATCTACACTTCTGTGTTCGTCGGCAGCGTC
E6	ArcS521	AATGATACGGCGACCACCGAGATCTACACTCTGCTGTTCGTCGGCAGCGTC

F6	ArcS522	AATGATACGGCGACCACCGAGATCTACACTTGGAGGTTCGTCGGCAGCGTC
G6	ArcS523	AATGATACGGCGACCACCGAGATCTACACTGATACGTTCGTCGGCAGCGTC
H6	ArcS524	AATGATACGGCGACCACCGAGATCTACACTGCATAGTTCGTCGGCAGCGTC
I6	ArcS525	AATGATACGGCGACCACCGAGATCTACACTGCGATCTTCGTCGGCAGCGTC
J6	ArcS526	AATGATACGGCGACCACCGAGATCTACACTTCCTGCTTCGTCGGCAGCGTC
K6	ArcS527	AATGATACGGCGACCACCGAGATCTACACTAGTGACTTCGTCGGCAGCGTC
L6	ArcS528	AATGATACGGCGACCACCGAGATCTACACTACAGGATTCGTCGGCAGCGTC
M6	ArcS529	AATGATACGGCGACCACCGAGATCTACACTGTGGTTGTCGTCGGCAGCGTC
N6	ArcS530	AATGATACGGCGACCACCGAGATCTACACTACTAGTCTCGTCGGCAGCGTC
O6	ArcS531	AATGATACGGCGACCACCGAGATCTACACTCGAAGTGTCGTCGGCAGCGTC
P6	ArcS532	AATGATACGGCGACCACCGAGATCTACACTAACGCTGTCGTCGGCAGCGTC
A7	ArcS533	AATGATACGGCGACCACCGAGATCTACACTTGGTATGTCGTCGGCAGCGTC
B7	ArcS534	AATGATACGGCGACCACCGAGATCTACACTGAACTGGTCGTCGGCAGCGTC
C7	ArcS535	AATGATACGGCGACCACCGAGATCTACACTACTTCGGTCGTCGGCAGCGTC
D7	ArcS536	AATGATACGGCGACCACCGAGATCTACACTCTCACGGTCGTCGGCAGCGTC
E7	ArcS537	AATGATACGGCGACCACCGAGATCTACACGAGACGTGTCGTCGGCAGCGTC
F7	ArcS538	AATGATACGGCGACCACCGAGATCTACACTTGCCTAATCGTCGGCAGCGTC
G7	ArcS539	AATGATACGGCGACCACCGAGATCTACACCTTTAACATCGTCGGCAGCGTC
H7	ArcS540	AATGATACGGCGACCACCGAGATCTACACCGTAGACCTCGTCGGCAGCGTC
I7	ArcS541	AATGATACGGCGACCACCGAGATCTACACTATTTGCGTCGTCGGCAGCGTC
J7	ArcS542	AATGATACGGCGACCACCGAGATCTACACATCCAGGATCGTCGGCAGCGTC
K7	ArcS543	AATGATACGGCGACCACCGAGATCTACACTCTGGCGATCGTCGGCAGCGTC
L7	ArcS544	AATGATACGGCGACCACCGAGATCTACACAATCTACATCGTCGGCAGCGTC
M7	ArcS545	AATGATACGGCGACCACCGAGATCTACACCGATAGGGTCGTCGGCAGCGTC
N7	ArcS546	AATGATACGGCGACCACCGAGATCTACACGGTGAAGGTCGTCGGCAGCGTC
O7	ArcS547	AATGATACGGCGACCACCGAGATCTACACATCGAATGTCGTCGGCAGCGTC
P7	ArcS548	AATGATACGGCGACCACCGAGATCTACACTCAAGAGCTCGTCGGCAGCGTC
A8	ArcS549	AATGATACGGCGACCACCGAGATCTACACGCCCACGTTCGTCGGCAGCGTC
B8	ArcS550	AATGATACGGCGACCACCGAGATCTACACCCCTTGGATCGTCGGCAGCGTC
C8	ArcS551	AATGATACGGCGACCACCGAGATCTACACATTACCGTTCGTCGGCAGCGTC
D8	ArcS552	AATGATACGGCGACCACCGAGATCTACACAGTCCGAGTCGTCGGCAGCGTC
E8	ArcS553	AATGATACGGCGACCACCGAGATCTACACACTTGTTGTCGTCGGCAGCGTC

F8	ArcS554	AATGATACGGCGACCACCGAGATCTACACGTAATACATCGTCGGCAGCGTC
G8	ArcS555	AATGATACGGCGACCACCGAGATCTACACGGCGTCTATCGTCGGCAGCGTC
H8	ArcS556	AATGATACGGCGACCACCGAGATCTACACGCGCTGCTTCGTCGGCAGCGTC
I8	ArcS557	AATGATACGGCGACCACCGAGATCTACACGTGCCATTTCGTCGGCAGCGTC
J8	ArcS558	AATGATACGGCGACCACCGAGATCTACACAACACCTATCGTCGGCAGCGTC
K8	ArcS559	AATGATACGGCGACCACCGAGATCTACACCTCCGAACTCGTCGGCAGCGTC
L8	ArcS560	AATGATACGGCGACCACCGAGATCTACACCAACGGCATCGTCGGCAGCGTC
M8	ArcS561	AATGATACGGCGACCACCGAGATCTACACCAATGTAGTCGTCGGCAGCGTC
N8	ArcS562	AATGATACGGCGACCACCGAGATCTACACGGCTACCCTCGTCGGCAGCGTC
O8	ArcS563	AATGATACGGCGACCACCGAGATCTACACAAAGTCCGTCGTCGGCAGCGTC
P8	ArcS564	AATGATACGGCGACCACCGAGATCTACACTTCCGCGGTCGTCGGCAGCGTC
A9	ArcS565	AATGATACGGCGACCACCGAGATCTACACAGGCACTTTCGTCGGCAGCGTC
B9	ArcS566	AATGATACGGCGACCACCGAGATCTACACCTTCAGTGTCGTCGGCAGCGTC
C9	ArcS567	AATGATACGGCGACCACCGAGATCTACACGCCGGTAGTCGTCGGCAGCGTC
D9	ArcS568	AATGATACGGCGACCACCGAGATCTACACTTCAATCCTCGTCGGCAGCGTC
A10	ArcS569	AATGATACGGCGACCACCGAGATCTACACCCACACACTCGTCGGCAGCGTC
B10	ArcS570	AATGATACGGCGACCACCGAGATCTACACATATTATCTCGTCGGCAGCGTC
C10	ArcS571	AATGATACGGCGACCACCGAGATCTACACCCGAAGCATCGTCGGCAGCGTC
D10	ArcS572	AATGATACGGCGACCACCGAGATCTACACGTATCGGTTCGTCGGCAGCGTC

Index 2		
Well position	Name	Sequence
A13	ArcN701	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGG
B13	ArcN702	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGG
C13	ArcN703	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGG
D13	ArcN704	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGG
E13	ArcN705	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGG
F13	ArcN706	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGG
G13	ArcN707	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGG
H13	ArcN708	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGG
I13	ArcN709	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGG
J13	ArcN710	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGG

K13	ArcN711	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGG
L13	ArcN712	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGG
M13	ArcN713	CAAGCAGAAGACGGCATACGAGATTCATGAGCGTCTCGTGGGCTCGG
N13	ArcN714	CAAGCAGAAGACGGCATACGAGATCCTGAGATGTCTCGTGGGCTCGG
O13	ArcN715	CAAGCAGAAGACGGCATACGAGATTAGCGAGTGTCTCGTGGGCTCGG
P13	ArcN716	CAAGCAGAAGACGGCATACGAGATGTAGCTCCGTCTCGTGGGCTCGG
A14	ArcN717	CAAGCAGAAGACGGCATACGAGATTACTACGCGTCTCGTGGGCTCGG
B14	ArcN718	CAAGCAGAAGACGGCATACGAGATAGGCTCCGGTCTCGTGGGCTCGG
C14	ArcN719	CAAGCAGAAGACGGCATACGAGATGCAGCGTAGTCTCGTGGGCTCGG
D14	ArcN720	CAAGCAGAAGACGGCATACGAGATCTGCGCATGTCTCGTGGGCTCGG
E14	ArcN721	CAAGCAGAAGACGGCATACGAGATGAGCGCTAGTCTCGTGGGCTCGG
F14	ArcN722	CAAGCAGAAGACGGCATACGAGATACTGATCGGTCTCGTGGGCTCGG
G14	ArcN723	CAAGCAGAAGACGGCATACGAGATTAGCTGCAGTCTCGTGGGCTCGG
H14	ArcN724	CAAGCAGAAGACGGCATACGAGATGACGTCGAGTCTCGTGGGCTCGG
I14	ArcN725	CAAGCAGAAGACGGCATACGAGATATCACGTTGTCTCGTGGGCTCGG
J14	ArcN726	CAAGCAGAAGACGGCATACGAGATCGATGTTTGTCTCGTGGGCTCGG
K14	ArcN727	CAAGCAGAAGACGGCATACGAGATTTAGGCATGTCTCGTGGGCTCGG
L14	ArcN728	CAAGCAGAAGACGGCATACGAGATTGACCACTGTCTCGTGGGCTCGG
M14	ArcN729	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGG
N14	ArcN730	CAAGCAGAAGACGGCATACGAGATGCCAATGTGTCTCGTGGGCTCGG
O14	ArcN731	CAAGCAGAAGACGGCATACGAGATCAGATCTGGTCTCGTGGGCTCGG
P14	ArcN732	CAAGCAGAAGACGGCATACGAGATACTTGATGGTCTCGTGGGCTCGG
A15	ArcN733	CAAGCAGAAGACGGCATACGAGATGATCAGCGGTCTCGTGGGCTCGG
B15	ArcN734	CAAGCAGAAGACGGCATACGAGATGGCTACAGGTCTCGTGGGCTCGG
C15	ArcN735	CAAGCAGAAGACGGCATACGAGATTGGTTGTTGTCTCGTGGGCTCGG
D15	ArcN736	CAAGCAGAAGACGGCATACGAGATTCTCGGTTGTCTCGTGGGCTCGG
E15	ArcN737	CAAGCAGAAGACGGCATACGAGATTAATAAGAGTCTCGTGGGCTCGG
F15	ArcN738	CAAGCAGAAGACGGCATACGAGATACTAAGTCGTCTCGTGGGCTCGG
G15	ArcN739	CAAGCAGAAGACGGCATACGAGATGCTGGTCTGTCTCGTGGGCTCGG
H15	ArcN740	CAAGCAGAAGACGGCATACGAGATCTGTATTTGTCTCGTGGGCTCGG
I15	ArcN741	CAAGCAGAAGACGGCATACGAGATTTTCATAAGTCTCGTGGGCTCGG
J15	ArcN742	CAAGCAGAAGACGGCATACGAGATGACCCAAGGTCTCGTGGGCTCGG

K15	ArcN743	CAAGCAGAAGACGGCATACGAGATTTATTTGGGTCTCGTGGGCTCGG
L15	ArcN744	CAAGCAGAAGACGGCATACGAGATTTTAACGCGTCTCGTGGGCTCGG
M15	ArcN745	CAAGCAGAAGACGGCATACGAGATCTTACTCCGTCTCGTGGGCTCGG
N15	ArcN746	CAAGCAGAAGACGGCATACGAGATGGGAACCGGTCTCGTGGGCTCGG
O15	ArcN747	CAAGCAGAAGACGGCATACGAGATGCATTAAAGTCTCGTGGGCTCGG
P15	ArcN748	CAAGCAGAAGACGGCATACGAGATGACCGTTTGTCTCGTGGGCTCGG
A16	ArcN749	CAAGCAGAAGACGGCATACGAGATTTTGGATCGTCTCGTGGGCTCGG
B16	ArcN750	CAAGCAGAAGACGGCATACGAGATATCATCATGTCTCGTGGGCTCGG
C16	ArcN751	CAAGCAGAAGACGGCATACGAGATCGTGTTGGGTCTCGTGGGCTCGG
D16	ArcN752	CAAGCAGAAGACGGCATACGAGATTGTTGTTAGTCTCGTGGGCTCGG
E16	ArcN753	CAAGCAGAAGACGGCATACGAGATGGTTTACCGTCTCGTGGGCTCGG
F16	ArcN754	CAAGCAGAAGACGGCATACGAGATGGTCGATGGTCTCGTGGGCTCGG
G16	ArcN755	CAAGCAGAAGACGGCATACGAGATGTTCCCATGTCTCGTGGGCTCGG
H16	ArcN756	CAAGCAGAAGACGGCATACGAGATATTGGCCGGTCTCGTGGGCTCGG
I16	ArcN757	CAAGCAGAAGACGGCATACGAGATTCATTCCCGTCTCGTGGGCTCGG
J16	ArcN758	CAAGCAGAAGACGGCATACGAGATCCGAATACGTCTCGTGGGCTCGG
K16	ArcN759	CAAGCAGAAGACGGCATACGAGATATAGCTGAGTCTCGTGGGCTCGG
L16	ArcN760	CAAGCAGAAGACGGCATACGAGATAGATAAATGTCTCGTGGGCTCGG
M16	ArcN761	CAAGCAGAAGACGGCATACGAGATATCTCGGGGTCTCGTGGGCTCGG
N16	ArcN762	CAAGCAGAAGACGGCATACGAGATAGACATTAGTCTCGTGGGCTCGG
O16	ArcN763	CAAGCAGAAGACGGCATACGAGATGAATTGGCGTCTCGTGGGCTCGG
P16	ArcN764	CAAGCAGAAGACGGCATACGAGATGCACGGCGGTCTCGTGGGCTCGG
A17	ArcN765	CAAGCAGAAGACGGCATACGAGATTCGGTCAGGTCTCGTGGGCTCGG
B17	ArcN766	CAAGCAGAAGACGGCATACGAGATTCGAAATGGTCTCGTGGGCTCGG
C17	ArcN767	CAAGCAGAAGACGGCATACGAGATTGGCAAGCGTCTCGTGGGCTCGG
D17	ArcN768	CAAGCAGAAGACGGCATACGAGATTGGTAGAAGTCTCGTGGGCTCGG
A18	ArcN769	CAAGCAGAAGACGGCATACGAGATCGTCACGTGTCTCGTGGGCTCGG
B18	ArcN770	CAAGCAGAAGACGGCATACGAGATCGCGGACAGTCTCGTGGGCTCGG
C18	ArcN771	CAAGCAGAAGACGGCATACGAGATAAGTTTAAGTCTCGTGGGCTCGG
D18	ArcN772	CAAGCAGAAGACGGCATACGAGATGTTGTGGTGTCTCGTGGGCTCGG

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