Cell, Volume *186*

Supplemental information

Archival single-cell genomics reveals persistent subclones during DCIS progression

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Table S1. Detailed experimental steps and chemistry of Arc-well and ACT

Steps		Reagents	Arc-well		ACT
single cell preparation	Microtome Sectioning		Yes		No
	deparaffinization		Yes		No
	nuclei isolation		Yes		Yes
	FACS sorting		1 time sorting per sample		1 sorting per plate (Multiple times sorting per sample)
	single cell dispense		yes		No
	morphology checking (imaging based single cell selection)		yes		No

			one-time cell dispensing protocol	two-times cell dispensing protocol	
single cell dispensing	reaction volume		35 nl	50 nl	< 5 nl
	reaction reagents	PBS	0.5X	0.5X	1X

single cell lysis	reaction volume		35 nl (70nl)	35 nl (85nl)	200 nl (200 nl)
	reaction reagents	Tris-HCl(ph 8.0)	13.5 mM	11.12 mM	27 mM
		Tween 20	2.25%	1.85%	4.50%
		TritonX-100	0.25%	0.21%	0.45%
		protease	0.068 AU/mL	0.056 AU/mL	0.136 AU/mL
	reaction conditions		59.7 °C for 5 s 54.5 °C for 30 min 79 °C for 11 s 75.3 °C for 15 min		55 °C for 10 min 75 °C for 15 min

tagmentation	reaction volume		35 nl (105 nl)	35 nl (120 nl)	600 nl (800 nl)
	reaction reagents	TDE1 (Illumina #20034211)	0.033X	0.029X	
		ATM (Illumina # FC-131-1096)			0.25X
		TD buffer	0.6X	0.53X	1X
	reaction conditions		59.7 °C for 5 s 54.5 °C for 8 min		55 °C for 5 min

neutralization + indexing	reaction volume		35 nl (140 nl)	35 nl (155 nl)	200 nl (1000 nl)	neutralization (ACT only)
	neutralization + Index Arc-S5XX (Arc-well only)	KAPA Fidelity Buffer with Mg2+	0.625X	0.565X		
		dNTP	0.5 mM	0.45 mM		
		EDTA	6.25 mM	5.64 mM		
		Arc-S5XX (forward PCR index)	1.25 uM	1.13 uM		
		NT buffer (Illumina # FC-131-1096)			0.2X	
	reaction conditions		54.9°C 5s 49.4°C 30min,		25 °C for 5 min	
	reaction volume		35 nl (175 nl)	35 nl (190 nl)	150 nl (1150 nl)	Index S5XX and N7XX (ACT only)
	Index Arc-N7XX (Arc-well only)	KAPA Fidelity Buffer with Mg2+	1X	0.92X		
		MgCl2	3.48 mM	3.21 mM		
		Arc-N7XX (reverse PCR index)	1 uM	0.92 uM		
		ACT-S5XX(forward PCR index)			6.52 uM	
		ACT-N7XX(reverse PCR index)			6.52 uM	

PCR	reaction volume		35 nl (210 nl)	35 nl (225 nl)	5350 nl (5500 nl)
	reaction reagents	KAPA Fidelity Buffer with Mg2+	1X	0.93X	
		KAPA HotStart DNA Polymerase	0.033X	0.031X	
		KAPA HIFI Hot start master mix			1X
	reaction conditions		72.1 °C for 8 min 99.6 °C for 30 s 10-16X cycles for (99.6 °C for 20s 57.5 °C for 5s 62.7 °C for 30s 72.1 °C for 1min) Then 72°C for 2 min		72 °C for 3 min 98 °C for 30 s 18 cycles for (98 °C for 10s 63 °C for 30s 72 °C for 30s) Then 72°C for 5 min

Library collection	reaction volume		1 step centrifuge with chip face down		manually pipette each well to transfer into 1 tube
Total volume			210 nl	225 nl	6500 nl (6.5 ul)

NOTE: The concentration in each step is the final concentration of one reagent, such as: in neutralization step, 0.5mM dNTP = 2 mM (dNTP con in 35 nl) * 35nl / 35nl*4

Table S1. Detailed experimental steps and chemistry of Arc-well and ACT, related to Figure 1.

This table provides a detailed description of the experimental steps and chemistry used to perform Arc-well using the one round and two rounds of nuclei dispensing protocol and the ACT protocol.

Table S2. Clinical information for DCIS and recurrence cancer patients																									
	Patient	Sample_ID	Tissue_ID	Timepoint	ER status	PR status	HER2 status	Grade	Side of Recurrence	Histology	Surgery	Dominant growth pattern (DCIS)	Size (mm)	pT	pN	pM	Adjuvant Treatment of primary DCIS	Age of Diagnosis	Sex	Time to recurr. (Years)	Sample Year	Age of the block (Years)	Margins	DNA integrity number (DIN)	Percentage of DCIS present in IBC
single time point samples	P1	P1R	DUKE249RE (DUKE27)	Recurrence	Pos	Neg	Unk	High	Ipsilateral	DCIS	Lumpectomy	Solid/Cribriform	12	Tis	0	0		51	female	1.1	2017	4		Unk	Only DCIS
	P2	P2P	NKI17P	Primary	Neg	Neg	Pos	High	Ipsilateral	DCIS	Lumpectomy	Solid	Unk	-	-	-	None	66	female	1.9	1999	22	Clear	2.4	Only DCIS
10 paired samples	P3	P3P	DUKE248P (DUKE24)	Primary	Pos	Pos	Neg	Intermediate		DCIS	Lumpectomy	Solid	22	Tis	0	0	None	69	female	1.6	2015	6	Clear	Unk	Only DCIS
		P3R	DUKE248RE (DUKE25)	Recurrence	Pos	Pos	Unk	Intermediate	Ipsilateral	DCIS		Solid Cribriform	25	Tis	0	0					2017	4		Unk	Only DCIS
	P4	P4P	DUKE254P (DUKE30)	Primary	Pos	Pos	Neg	High		DCIS	Lumpectomy	Cribriform	12	Tis	0	0	Unk	50	female	10.9	2000	21	Clear	Unk	Only DCIS
		P4R	DUKE254RE (DUKE31)	Recurrence	Pos	Pos	Neg	High	Ipsilateral	DCIS		Cribriform	10	T1b	0	0					2011	10		Unk	>95% DCIS
	P5	P5P	NKI23P	Primary	Pos	Pos	Neg	Intermediate		DCIS	Lumpectomy	Solid	Unk	-	-	-	None	74	female	1.9	1995	26	Clear	2.4	Only DCIS
		P5R	NKI23RE	Recurrence	Pos	Pos	Neg	High	Ipsilateral	IDC with DCIS (NST)		-	17	T1c	0	Unk					1997	24		2.1	<2%
	P6	P6P	NKI26P	Primary	Neg	Neg	Pos	Intermediate		DCIS	Lumpectomy	Clinging/FEA	Unk	-	-	-	None	34	female	6.2	1997	24	Clear	5.4	Only DCIS
		P6R	NKI26RE	Recurrence	Pos	Pos	Pos	High	Ipsilateral	Invasive (NST)		-	13	T1c	0	0					2004	17		4.7	0
	P7	P7P	NKI28P	Primary	Neg	Neg	Pos	High		DCIS	Lumpectomy	Solid	Unk	-	-	-	None	61	female	15.7	1990	31	NA	2.5	Only DCIS
		P7R	NKI28RE	Recurrence	Neg	Neg	Pos	High	Ipsilateral	IDC with DCIS (NST)		-	15	T1c	1A	0					2006	15		2.5	<10%
	P8	P8P	NKI19P	Primary	Pos	Pos	Neg	Intermediate		DCIS	Lumpectomy	Cribriform	Unk	-	-	-	None	50	female	10.8	1994	27	NA	1.9	Only DCIS
		P8R	NKI19RE	Recurrence	Pos	Pos	Neg	Intermediate	Ipsilateral	Invasive (NST)		-	20	T1c	0	0					2005	16		2.3	0
	P9	P9P	NKI22P	Primary	Neg	Neg	Pos	High		DCIS	Lumpectomy	Solid	18	-	-	-	None	69	female	6.0	1994	27	NA	3.4	Only DCIS
		P9R	NKI22RE	Recurrence	Neg	Neg	Pos	High	Ipsilateral	Invasive micropapillary carcinoma		-	20	T1c	1B	Unk					2000	21		2.5	0
	P10	P10P	NKI15P	Primary	Pos	Pos	Neg	Intermediate		DCIS	Lumpectomy	Solid	Unk	-	-	-	None	46	female	5.1	1991	30	Clear	2.6	Only DCIS
		P10R	NKI15RE	Recurrence	Pos	Pos	Neg	Intermediate	Ipsilateral	IDC with DCIS (NST)		-	12	Unk	1B	0					1996	25		Unk	60%
	P11	P11P	NKI31P	Primary	Pos	Neg	Neg	High		DCIS	Lumpectomy	Solid	Unk	-	-	-	None	52	female	1.9	2001	20	Clear	5.4	Only DCIS
		P11R	NKI31RE	Recurrence	Pos	Pos	Neg	High	Ipsilateral	IDC with DCIS (NST)		-	50	T2	2A	0					2003	18		3.5	<5%
	P12	P12P	NKI12P	Primary	Pos	Pos	Neg	Intermediate		DCIS	Lumpectomy	Solid	25	-	-	-	None	68	female	4.2	2004	17	Clear	3.6	Only DCIS
		P12R	NKI12RE	Recurrence	Pos	Pos	Neg	Intermediate	Ipsilateral	IDC with DCIS (NST)		-	14	T1c	0	0					2008	13		Unk	<5%

Table S2. Clinical information for DCIS and recurrence cancer patients, related to Figure 1.

This table provides clinical information of the 12 breast cancer patients that were analyzed by Arc-well. ER and PR positive status is defined by Immunohistochemistry (IHC) with greater than 10%, while HER2 positive status is defined by IHC with scores of 3+ or 2+ with a positive fluorescence in situ hybridization (FISH)/ (chromogenic in-situ hybridization) CISH cytology test. Histopathological analysis of the tissue was used to classify the lesions as ductal carcinoma in situ (DCIS), invasive ductal carcinoma (IDC) or synchronous IDC with DCIS. All patients had their primary DCIS removed by surgical lumpectomy with no other treatment. The percentage of DCIS present in the recurrence samples that were synchronous DCIS/IDC were assessed by histopathological analysis of the H&E slides. The surgical margins were also assessed by histopathology and classified as 'clear' when no premalignant DCIS cells were identified.

The abbreviations are as follows: Positive (Pos), Negative (Neg), Unknown (Unk), Not Available (NA).

Table S3. Arc-well QC metrics for samples used to evaluate technical performance												
Samples	Sample_ID	Tissue_ID	Cells passed sequencing QC[1]	Cells passed processing QC[2]	Percent cells passed QC (%)[3]	Diploid cells	Aneuploid cells	Frac. Of Aneuploid (%) - pass QC	Total reads per cell (mean)	Reads retained per cell (mean)	Percent PCR Dups per cell (mean) (%)	Median Bin Counts per cell (mean)
Aneuploid cell line	MDA231	MDA-MB-231_p38	1,205	969	80%	0	969	100%	1,045,101	871,511	16.43%	129
	fresh_MDA231	MDA231_p37_non_fixed	1,454	1,138	78%	0	1,138	100%	757,184	683,234	9.47%	51
	formalin_MDA231	MDA231_p37_fixed	1,328	1,048	79%	0	1,048	100%	860,492	757,331	11.81%	57
Diploid cell line	fresh_315A	315A_non_fixed	2,164	1,932	89%	1,932	0	0%	536,284	448,813	16.06%	34
	formalin_315A	315A_formalin	1,660	1,272	77%	1,272	0	0%	663,351	510,868	22.01%	38
Species mixture	A20/GM12878	A20_GM12878	1,607	NA	NA	NA	NA	NA	NA	NA	NA	NA
IDC	IDC-Frozen	BCIS28-Frozen	1,478	1,118	76%	0	1,118	100%	803,834	681,559	15.02%	50
	IDC-FFPE	BCIS28-FFPE	1,583	797	50%	15	782	98%	708,805	617,606	12.68%	45
Lung cancer	Lung-P1	Lung30-p1	1,202	961	80%	21	940	98%	1,578,980	1,235,015	21.21%	88
	Lung-P2	Lung31-p2	1,206	864	72%	62	802	93%	1,557,470	1,260,960	18.56%	86
prostate cancer	Prostate-P1	prostate-p1-ax4bl	554	342	62%	24	318	93%	3,648,715	2,282,741	36.55%	169
	Prostate-P2	prostate-p2-pcf169	905	764	84%	167	597	78%	1,801,460	1,375,097	21.75%	101

Table S3. Arc-well QC metrics for samples used to evaluate technical performance, related to Figure 1.

QC and sequencing metrics for the aneuploid breast cancer cell line (MDA-MB-231), the diploid lymphoblast cell line (315A), and the frozen and freshly prepared FFPE IDC samples, as well as 2 human lung adenocarcinoma samples and 2 human castration-sensitive prostate cancer samples. Abbreviations include Not Available (NA) for calculations that are related to the species mixture experiments. The following columns are defined as:

[1] '*Cells passed sequencing QC'* – this column includes cells that passed sequencing QC by the following 3 metrics: (1) mapping quality (Q>=1), (2) read counts > 100k when average read number per cell is around 1M, (3) cells with <=10% bins with no mapped reads. For species mixture experiment, this number represent cells that passed the first two QC metrics (1,2) but not the third metric (3).

[2] *'Cells passed processing QC'* – this column includes cells that passed processing QC metrics by the following 3 criteria: (1) passing CBS correlation filtering (>=0.8, as described in the methods section: Filtering of low-quality copy number profiles), (2) passing HDBSCAN filtering and (3) at least have 6 cells in each subclone from each timepoint

[3] *'Percent cells passed QC'*, was calculated by diving: (Cells passed processing QC / Cells passed sequencing QC) * 100

Table S4. Arc-well data quality control and sequencing metrics for the 22 breast cancer FFPE samples													
	Patient	Sample_ID	Tissue_ID	Cells passed sequencing QC[1]	Cell passed processing QC[2]	Percent Cell passed QC (%)[3]	Diploid cells	Aneuploid cells	Percent Aneuploid (%) - pass QC	Total reads per aneuploid cell (mean)	Reads retained per aneuploid cell (mean)	Percent PCR Dups per aneuploid cell (mean) (%)	Median Bin Counts per aneuploid cell (mean)
single time point samples	P1	P1R	DUKE249RE/DUKE27	1,082	888	82.07%	4	884	99.55%	1,644,322	1,260,150	22.75%	91
	P2	P2P	NKI17P	1,057	762	72.09%	0	762	100.00%	1,331,874	606,274	53.27%	40
10 paired samples	P3	P3P	DUKE248P/DUKE24	975	497	50.97%	0	497	100.00%	2,216,662	854,717	57.75%	67
		P3R	DUKE248RE/DUKE25	953	242	25.39%	5	237	97.93%	2,666,682	1,365,951	48.42%	98
	P4	P4P	DUKE254P/DUKE30	878	712	81.09%	1	711	99.86%	1,595,370	390,018	74.36%	25
		P4R	DUKE254RE/DUKE31	1,416	637	44.99%	400	237	37.21%	1,363,481	430,941	60.59%	28
	P5	P5P	NKI23P	1,164	893	76.72%	21	872	97.65%	1,275,353	723,687	42.34%	101
		P5R	NKI23RE	1,208	1,004	83.11%	4	1,000	99.60%	1,271,747	581,608	52.90%	73
	P6	P6P	NKI26P	1,919	498	25.95%	49	449	90.16%	1,905,937	1,120,058	37.23%	79
		P6R	NKI26RE	1,134	943	83.16%	15	928	98.41%	1,264,355	1,060,215	15.64%	74
	P7	P7P	NKI28P	719	531	73.85%	2	529	99.62%	1,480,202	496,312	64.79%	32
		P7R	NKI28RE	1,090	733	67.25%	17	716	97.68%	871,038	505,772	40.34%	33
	P8	P8P	NKI19P	1,114	713	64.00%	89	624	87.52%	746,817	310,941	54.24%	22
		P8R	NKI19RE	1,105	922	83.44%	41	881	95.55%	868,527	373,932	55.53%	25
	P9	P9P	NKI22P	1,137	511	44.94%	236	275	53.82%	1,677,450	962,500	41.07%	64
		P9R	NKI22RE	1,134	667	58.82%	112	555	83.21%	1,285,348	700,438	41.14%	44
	P10	P10P	NKI15P	1,083	322	29.68%	38	284	88.20%	1,543,503	910,189	40.33%	65
		P10R	NKI15RE	1,053	463	44.05%	238	225	48.60%	1,727,981	897,855	47.31%	61
	P11	P11P	NKI31P	1,051	505	48.05%	2	503	99.60%	1,827,116	863,747	51.62%	56
		P11R	NKI31RE	556	444	79.86%	0	444	100.00%	1,990,501	196,934	89.54%	12
	P12	P12P	NKI12P	1,058	710	67.11%	184	526	74.08%	1,329,074	1,001,594	23.83%	68
		P12R	NKI12RE	1,098	587	53.46%	258	329	56.05%	1,103,280	735,718	32.21%	50

Table S4. Arc-well data quality control and sequencing metrics for the 22 breast cancer FFPE samples, related to Figures 2 and 3. Quality control metrics for the 12 breast cancer patients in this study. The following columns are defined as described below:

[1] '*Cells passed sequencing QC'* – this column includes cells that passed sequencing QC by the following 3 metrics: (1) mapping quality (Q>=1), (2) read counts > 100k when average read number per cell is around 1M, (3) cells with <=10% bins with no mapped reads. For species mixture experiment, this number represent cells that passed the first two QC metrics (1,2) but not the third metric (3).

[2] *'Cells passed processing QC'* – this column includes cells that passed processing QC metrics by the following 3 criteria: (1) passing CBS correlation filtering (>=0.8, as described in the methods section: Filtering of low-quality copy number profiles), (2) passing HDBSCAN filtering and (3) at least have 6 cells in each subclone from each timepoint

[3] *'Percent cells passed QC'*, was calculated by diving: (Cells passed processing QC / Cells passed sequencing QC) * 100

Table S5. Arc-well reagent distribution settings in the source plate of the Takara iCell8cx System																							
Step 1		Sample, PC and NC																					
Step 2		Lysis buffer																					
Step 3		Tagmentation reagents																					
Step 4		Index S5 with EDTA;																					
Step 5		Index N7 MgCl2;																					
Step 6		PCR mix																					

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	Sample (80ul/well)		Lysis buffer (50ul/ well)		ArcS501	ArcS517	ArcS533	ArcS549	ArcS565	ArcS569	PCR mix (50ul/well)		ArcN701	ArcN717	ArcN733	ArcN749	ArcN765	ArcN769						NC
2					ArcS502	ArcS518	ArcS534	ArcS550	ArcS566	ArcS570			ArcN702	ArcN718	ArcN734	ArcN750	ArcN766	ArcN770						25ul
3					ArcS503	ArcS519	ArcS535	ArcS551	ArcS567	ArcS571			ArcN703	ArcN719	ArcN735	ArcN751	ArcN767	ArcN771						
4					ArcS504	ArcS520	ArcS536	ArcS552	ArcS568	ArcS572			ArcN704	ArcN720	ArcN736	ArcN752	ArcN768	ArcN772						
5					ArcS505	ArcS521	ArcS537	ArcS553	20ul/well				ArcN705	ArcN721	ArcN737	ArcN753	20ul/well							
6					ArcS506	ArcS522	ArcS538	ArcS554					ArcN706	ArcN722	ArcN738	ArcN754								
7					ArcS507	ArcS523	ArcS539	ArcS555					ArcN707	ArcN723	ArcN739	ArcN755								
8					ArcS508	ArcS524	ArcS540	ArcS556					ArcN708	ArcN724	ArcN740	ArcN756								
9					ArcS509	ArcS525	ArcS541	ArcS557					ArcN709	ArcN725	ArcN741	ArcN757								
10					ArcS510	ArcS526	ArcS542	ArcS558					ArcN710	ArcN726	ArcN742	ArcN758								
11					ArcS511	ArcS527	ArcS543	ArcS559					ArcN711	ArcN727	ArcN743	ArcN759								
12					ArcS512	ArcS528	ArcS544	ArcS560					ArcN712	ArcN728	ArcN744	ArcN760								
13			Tagmentation mix (40ul/ well)		ArcS513	ArcS529	ArcS545	ArcS561					ArcN713	ArcN729	ArcN745	ArcN761								
14					ArcS514	ArcS530	ArcS546	ArcS562					ArcN714	ArcN730	ArcN746	ArcN762								
15					ArcS515	ArcS531	ArcS547	ArcS563					ArcN715	ArcN731	ArcN747	ArcN763								25ul
16					ArcS516	ArcS532	ArcS548	ArcS564					ArcN716	ArcN732	ArcN748	ArcN764								PC

Table S5. Arc-well reagent distribution settings in the source plate of the Takara iCell8cx System, related to STAR Methods.

Reagents source plate settings for the Takara iCell8cx nanowell system. NC: negative control, PC: positive control.

Table S6. DNA Sequences of PCR index primers

Forward PCR primer sets (S5XX)		
Well Position	**Name**	**Sequence**
A5	ArcS501	AATGATACGGCGACCACCGAGATCTACAC**TAGATCGC**TCGTCGGCAGCGTC
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	AATGATACGGCGACCACCGAGATCTACAC**GTATCGGT**TCGTCGGCAGCGTC

Reverse PCR primer sets (N7XX)

Well Position	Name	Sequence
A13	ArcN701	CAAGCAGAAGACGGCATACGAGAT**TCGCCTTA**GTCTCGTGGGCTCGG
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	CAAGCAGAAGACGGCATACGAGAT**GTTGTGGT**GTCTCGTGGGCTCGG

Table S6. DNA Sequences of PCR index primers, related to STAR Methods.

The nanowell position (in the source plate), names and sequences of the forward and reverse PCR index primers are provided in this table. The nucleotides in bold indicates the DNA barcode index for exemplary primers.