nature portfolio

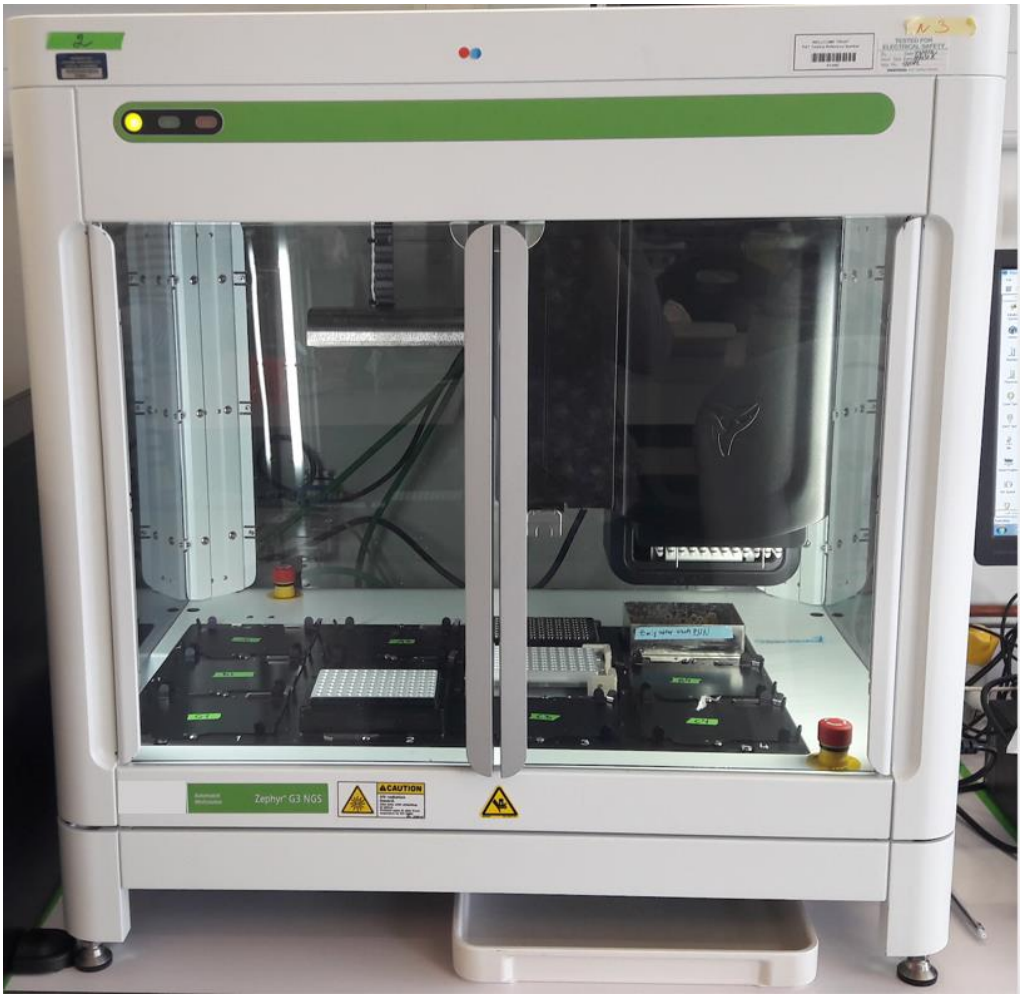
https://doi.org/10.1038/s41596-021-00523-3

Supplementary information

High-throughput full-length single-cell RNA-seq automation

In the format provided by the authors and unedited

Supplementary Figures

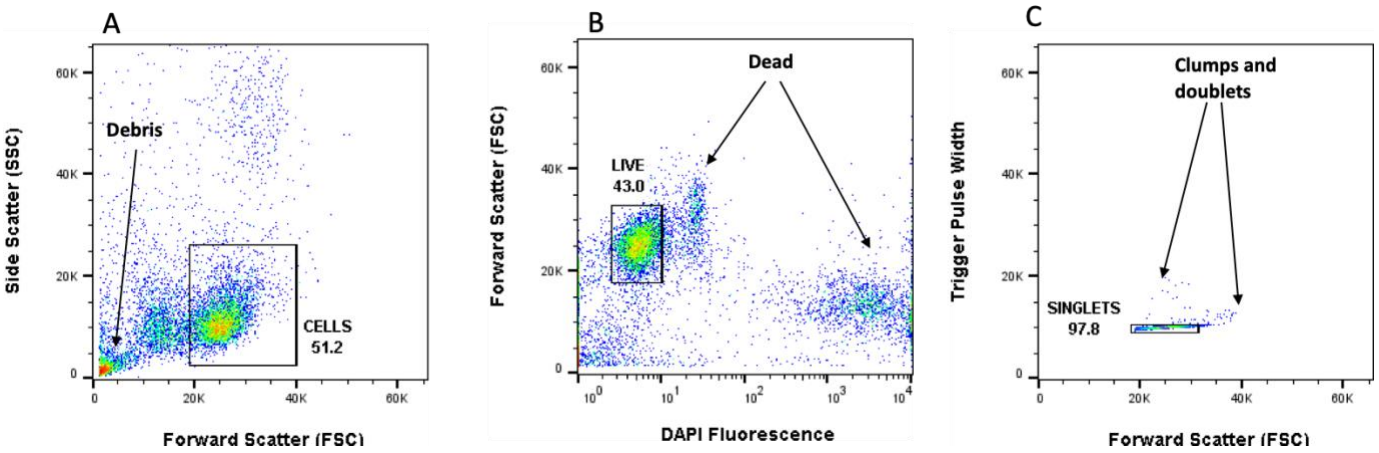


Supplementary Figure 1. Zephyr G3 NGS Workstation

This photo shows an example of the benchtop NGS workstation from PerkinElmer, which can be used as an automation platform in Procedure 1.

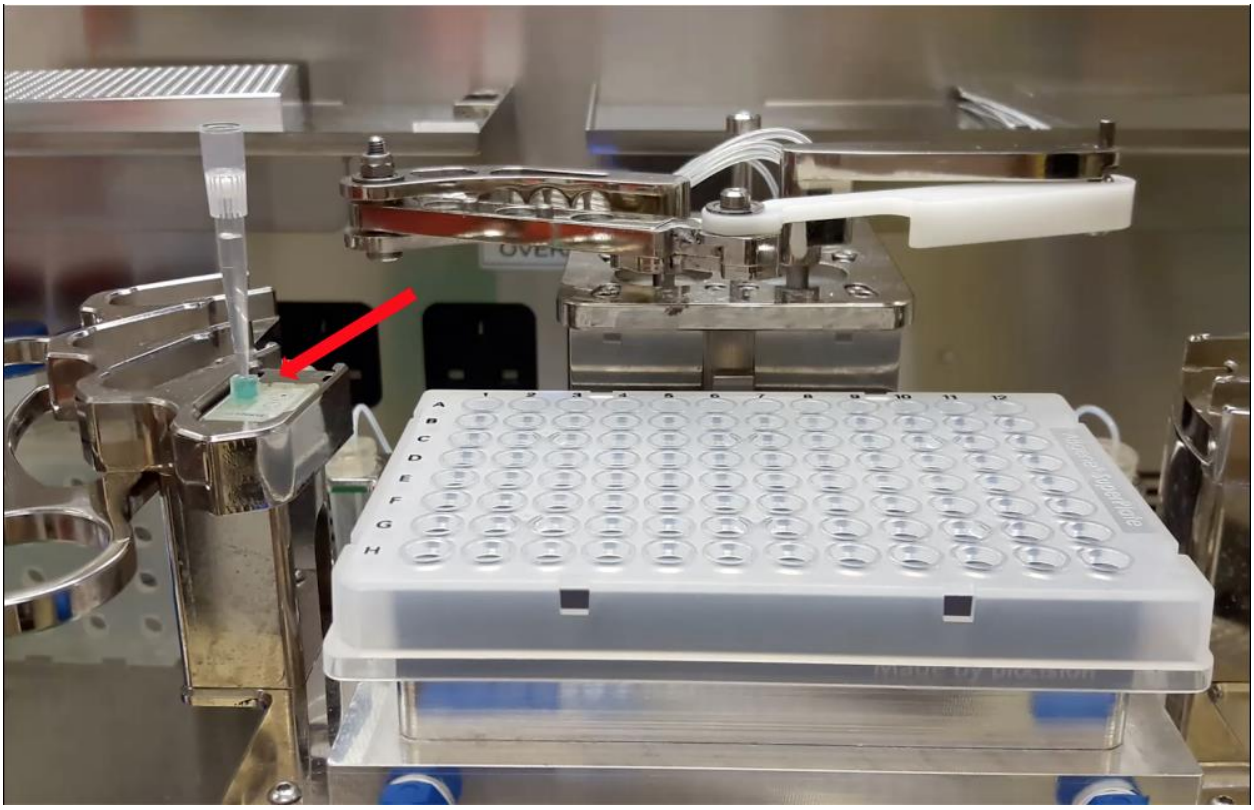
Supplementary Figure 2. Formulatrix Mantis microfluidic liquid handler

This photo shows an example of the benchtop liquid dispenser from Formulatrix, which can be used for reagent dispensing in Procedure 1.



Supplementary Figure 3. Density plots displayed of mouse splenocytes stained with DAPI.

The stained cell preparation was analysed on a BDInflux™ cell sorter (A) Region gate CELLS, created on FSC versus SSC to gate out the cellular debris from the main cell population. (B) Region gate LIVE, created on DAPI fluorescence versus FSC to exclude dead cells which have a compromised plasma membrane and stained with a higher DAPI fluorescence intensity than live cells. (C) Region gate SINGLETS, created on FSC versus Pulse Width to exclude clumps and doublets. Live single cells were isolated into 96 well plate based on this gating strategy.

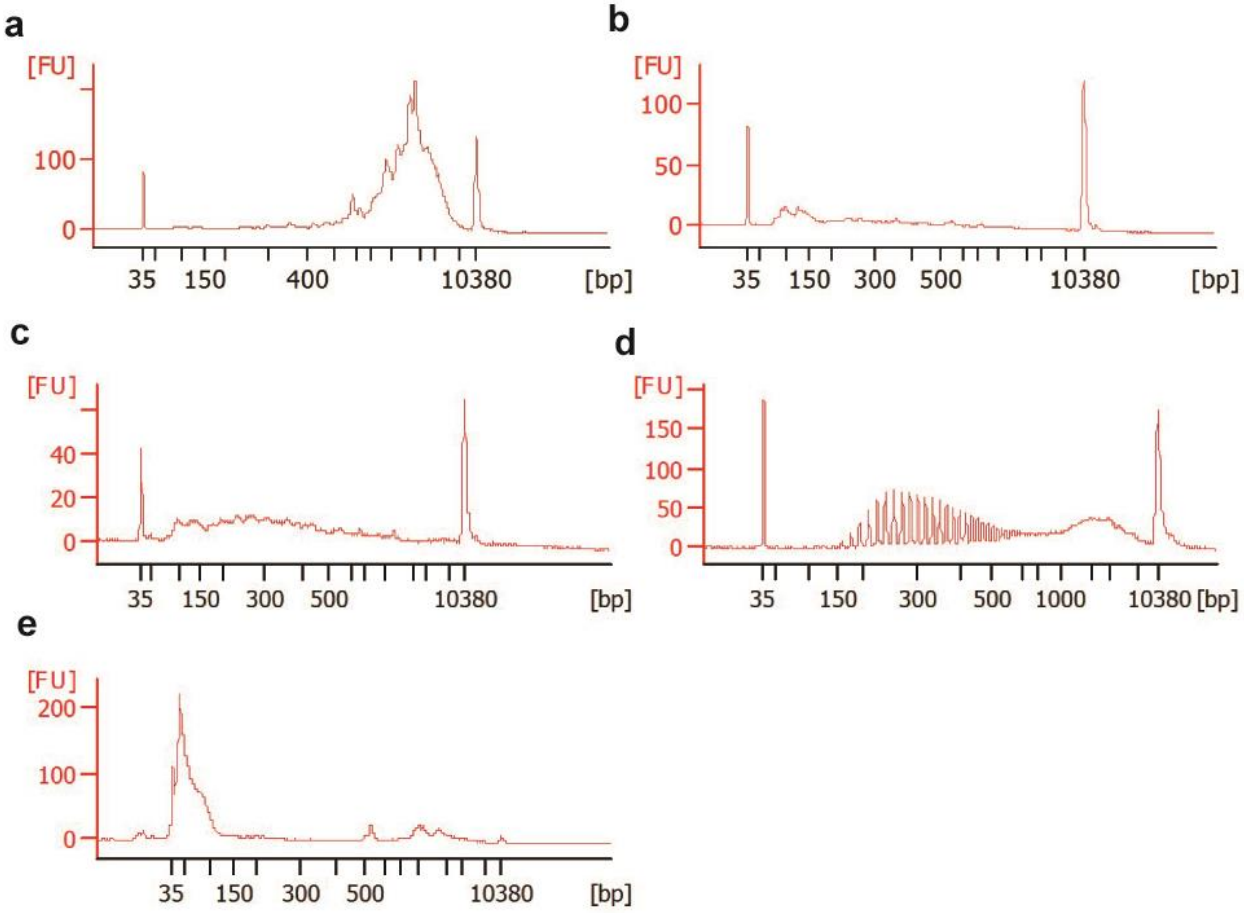


Supplementary Figure 4. Reagent dispensing on the Formulatrix Mantis microfluidic liquid handler.

The reagents are dispensed from a 0.2 ml or 1 ml tip inserted into the High Volume (HV) Silicone-based Mantis chip.

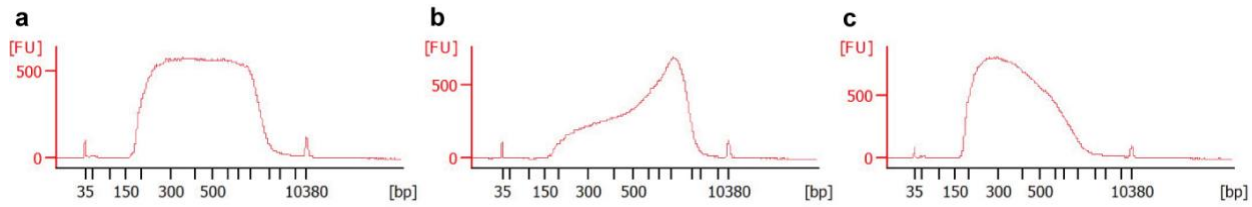
Supplementary Figure 5. Nextera Index preparation.

This image shows the schematic layout of the individual vials with indexes for creating the Indexe set mix (A-B-C-D).



Supplementary Figure 6. Bioanalyser profile electropherogram plots for profiles for a Single-Cell cDNA amplification step.

(a) A successful single-cell cDNA amplification profile, with fragments in the range of 0.2-8kb. FU: fluorescence units. (b) Failed cDNA amplification, probably due to the missalignment during FACS sorting and absence of a cell in the well. (c) Failed cDNA amplification, possibly due to degraded samples in the well. (d) A representative example of a sample with a 'hedgehog' profile, most probably due to TSO concatemers. (e) A representative example of cDNA from a partially degraded or a specific cell subtype or development stage. It is critical to pay attention to the Signal intensity (FU). Low cDNA peaks might be due to initial low RNA present in the cell or the cell's developmental stage.



Supplementary Figure 7. Bioanalyser profile electropherogram plots for representative profiles of Nextera sequencing libraries.

(a) A pool of good quality Nextera libraries has a distribution of fragments between 150bp and 1kb. It is also possible to obtain Nextera libraries with other shapes. A wide-spread range of fragments might be due to under-fragmentation (long fragments) (b) and over-fragmentation (short fragments) (c) of cDNA. FU, fluorescence units.

Supplementary Tables

Supplementary Table 1. Timelines and throughput*

	Number of plates	**Processing time**
Procedure 1	1 Plate	3 days
	<=4 Plates	3-4 days
	<=16 Plates**	5 days
Procedure 2	1 Plate	3 days
	<=4 Plates	3 days
	<=16 Plates**	3 days

* - two people processing the plates.

** - does not include sequencing

Supplementary Table 2. Cost Efficiency for Single-Cell RNA-Seq kits per cell based on online UK prices

Method	Capacity per week	Kit size	cDNA cost per cell	Library cost per cell	Total library cost per cell
SmartSeq (in house automated with SMT)[a]	12 of 96w plates	96	£2.5/£2.8*	£5.5	£8/8.3*
SmartSeq (in house automated with Maxima)[a]	10 of 96w plates	96	£1.6/£1.9*	£5.5	£7.1/7.4*
NEBNext® Single Cell/Low Input RNA Library Prep Kit (in house automated)[b]	40 of 96w or 384w plates	96 or 384	£15	Cost included in the kit	£15
NEBNext® Single Cell/Low Input RNA Library Prep Kit	96w	96	£37.5	Cost included in the kit	£37.5
Takara (SMART-Seq® Single Cell Kit)	Manual	96	£58.7	Provided by user	£58.7
Takara(SMART-Seq® Single Cell PLUS Kit)	Manual	96	£72.7	Cost included in the kit	£72.7
QIAseq FX Single Cell RNA Library Kit	Manual	96	£40	Cost included in the kit	£40

* scRNA cleanup inclusive

[a]: the cost for the automated system in procedure 1 is estimated ~ £100,000;

[b]: the cost for the automated system in procedure 2 is estimated ~ £150,000.

Supplementary Table 3. Lysis buffer benchmarking for various cell types.

Lysis buffer	Cell type
Triton	T cells B cells Epidermal cells Thymocytes Natural killer cells Monocytes Mesenteric Lymph Node cells Human Hematopoietic Stem Cells Fibroblasts
RLT	Macrophages Phagocytes Mast cells Neutrophils Eosinophils Stromal cells Trophoblasts Epithelial cells Myeloid cells LSK cells*

* LSK cells are cells sorted by FACS according to Lin-Sca1+Kit- in the expression.

This table shows the cell type distribution according to the lysis buffer that has been used to lyse the cells with subsequent successful cDNA synthesis prior to Nextera library construction.

Supplementary Methods

Data processing:

The raw sequencing data and quality scores for the Illumina HiSeq4000 were assessed based on Wellcome Sanger Institute sequencing quality control guidelines. The read quality was assessed using FastQC[47], and overrepresented sequences and adapters (>0.5% of total reads) were filtered using Cutadapt[38].

Reads Mapping:

To measure the sensitivity and accuracy of sequencing according to the previously published method, we quantified the abundances of reference sequences for each demultiplexed scRNA-seq run using Salmon version 1.1.0 [39], with library type parameter '-l A' and the optional flag '--posBias --gcBias --writeUnmappedNames --numBiasSamples 100000 --numAuxModelSamples 100000 --numPreAuxModelSamples 100000'. The Salmon transcriptome mapping reference was built by adding External RNA Controls Consortium (ERCC)[48] sequences to cDNA sequences from Ensembl 90 cDNA annotation of GRCm38[49]. To understand the effects of sequencing depth on the sequencing sensitivity and accuracy, the raw reads from each single-cell were randomly downsampled to 10^6, 10^5 and 10^4 reads. As a high-speed pseudo-aligner, Salmon facilitates the reads mapping repeated at different downsamplings.

To quantify the reads mapped to different genomic regions, we use STAR aligner[40] to map the reads to the reference genome, which was mm10 from UCSC[50]. The reads were mapped with the following parameters: '--outSAMtype BAM SortedByCoordinate --outSAMstrandField intronMotif --outFilterType BySJout --outFilterMultimapNmax 10 --outFilterScoreMinOverLread 0 --outFilterMatchNminOverLread 0 --outFilterMatchNmin 0 --outFilterMismatchNmax 2 --quantMode TranscriptomeSAM'. The aligned 'bam' files were sorted, indexed and transformed to 'sam' files with SAMtools[41].

Feature count:

The gene expression profile was quantified according to the sorted 'sam' files using HTseq[42], with the parameters 'htseq-count -r name --nonunique all --stranded=no -i gene_id'. Further, we used qualimap2[44] to quantify the percentages of reads mapped to the exonic, intronic, intergenic regions. We used featureCounts[43]to quantify the percentage of reads mapped to the ribosomal RNA. The gene body coverages were calculated from STAR aligned results using RSeQC (geneBody_coverage.py) after sorting and indexing the bam files with samtools. From the RSeQC website (https://sourceforge.net/projects/rseqc/files/BED/Mouse_Mus_musculus/) we downloaded the reference housekeeping genes to calculate the gene body coverages. The gene body coverage density of each cell is normalised to 1 for comparison.

All the commands used in the reads mapping and feature count steps are provided at GitHub (https://github.com/chichaumiau/SCQUA).

Performance metrics (Accuracy and Sensitivity):

The input concentration of ERCC is known *a priori*, and is measured by multiplying the molecules per microlitre from the manufacturer with the volume used and the final dilution in lysis mix of the scRNA-seq protocol.

If a spike-in RNA has a Transcripts per Million mapped reads (TPM) greater than 1, it is considered as detected[13]. The sensitivity, which is also known as detection limit, is calculated using a logistic regression model and as previously published[13]. The spike-in detecting probability at a given input level is modelled as a logistic function:

$$p(\,detected) = \frac{1}{1 + e^{-\left(a \times \log\left(M_i\right) + b\right)}} + \varepsilon$$

Where M_i is the input level for spike-in RNA i, and ε is the variances. a and b are two parameters to be fit for the model. The LogisticRegression class in the scikit-learn Python package, which uses a liblinear solver, was used to fit the model. The detection limit (number of detected molecules) was chosen as the molecular abundance where the logistic regression model passes 50% detection probability, which can be defined using the a and b parameters:

$$detection\ limit = -\frac{b}{a}$$

The scRNA-seq accuracy is estimated using Pearson product correlation between input spike-in concentration and measured TPM expression. The accuracy is only calculated when > 7 spike-ins types are detected.

To understand the relationship between sequencing depth and performance metrics (sensitivity and accuracy) for individual protocols, we use all the downsampled data to fit a linear model with a quadratic read depth term to capture diminishing returns on investment[13]. The effect of sequencing depth is considered global with a performance parameter for each protocol. The linear models were fitted using OLS regression function in Python package statsmodels.

Supplementary Protocol

Supplementary Protocol. Procedure 2 high-throughput automated scRNA-seq 384 well workflow

Perform steps 1-17 in a designated RNAse free and pre-cDNA amplification area, keeping reagents chilled at all times. We prepare lysis buffer plates and perform cDNA generation on the Formulatrix Mantis and Agilent Bravo NGS platform respectively, but these steps can be performed on alternative liquid handling platforms.

Preparation of lysis buffer plates and FACS Timing 1-4 hours

1. Prepare the cell lysis buffer, as described in the table below, which will provide sufficient volume for one 384-well plate. Mix thoroughly by pipetting up and down and keep chilled on ice. Dispense 2 µl of lysis buffer into each well of a 384-well plate. If required add a diluted stock (1/500,000) of ERCCs into the lysis buffer.

Reagent	**Volume (µl)**
NEBNext Cell Lysis Buffer (10x)	96
Murine RNase Inhibitor	48
Nuclease-free water	816
Total	**960**

CRITICAL STEP We recommend to seal dispensed plates and centrifuge them immediately (1,000g for 1 min at 4^{o}C) to spin down the lysis buffer to the bottom of the well to prevent a sample degradation.
PAUSE POINT Lysis buffer plates can be stored at -80 ^{o}C prior to cell sorting for < 6 months.

CRITICAL STEP When FACS sorting, take care of plate calibration/priming prior to single-cell deposition. If many plates are deposited at the same time, repeat the calibration/priming at least every 8 plates. We recommend defrosting lysis buffer plates on ice prior to cell sorting, centrifuge (1,000g for 1 min at 4^{o}C) and keep chilled on ice.

2. FACS sort single cells into the plate. Immediately centrifuge (1,000g for 1 min at 4 ^{o}C) plates containing cells and keep them chilled on ice or at -80 ^{o}C until further processing.

PAUSE POINT Samples can be stored at -80 °C for < 6months. We have successfully processed samples after 1 year in storage. The quality of the data depends on the cell type and duration of storage.

Primer annealing for first-strand synthesis Timing 20 minutes

3. Prepare the primer annealing mix, as described in the table below, which will provide sufficient volume for one 384-well plate. Mix thoroughly by pipetting up and down, and keep chilled on ice.

Reagent	**Volume (µl)**
NEBNext Single Cell RT Primer Mix	200
Nuclease-free water	800
Total	**1000**

4. Place a fresh 96-well plate on a chilled position of the robot deck and add 10 µl of annealing mix to each well.
5. Thaw and centrifuge (1,000g for 1 minute at 4 °C) the 384-well working plate containing the lysed cells. Place on a chilled position of the robot deck.
6. The robot will perform 4 x 96 well stamps from the reagent plate, and combine 1.6 µl of primer annealing mix with 2 µl of lysed cells and mix by pipetting.
7. Seal and transfer the plate to a thermocycler with the heated lid set to 100 °C and run the following program:

Temperature	Time
70 °C	5 minutes
4 °C	Hold

Reverse Transcription (RT) and Template Switching Timing 2 hours

8. Prepare the RT Mix, as described in the table below, which will provide sufficient volume for one 384-well plate. Mix thoroughly by pipetting up and down, and keep chilled on ice.

Reagent	Volume (µl)
NEBNext Single Cell RT Buffer	1000
NEBNext Template Switching Oligo	200
NEBNext Single-Cell RT Enzyme	600
Nuclease-free water	400
Total	**2200**

9. Place a fresh 96-well plate on a chilled position of the robot deck and add 22 µl of RT mix to each well.
10. Collect the lysed cell plate from the thermocycler and place it on a chilled position of the robot deck.
11. The robot will perform 4 x 96 well stamps from the reagent plate, and add 4.4 µl of the RT mastermix to each well and mix by pipetting.
12. Seal and transfer the plate to a thermocycler with the heated lid set to 100 ^{o}C and run the following program:

Temperature	Time
42 ^{o}C	90 minutes
70 ^{o}C	10 minutes
4 ^{o}C	Hold

cDNA PCR Amplification Timing 1 hour 15 minutes → 1 hour 45 minutes

13. Prepare the cDNA amplification mix, as described in the table below, which will provide sufficient volume for one 384-well plate. Mix thoroughly by pipetting up and down, and keep chilled on ice.

Reagent	Volume (µl)
NEBNext Single Cell cDNA PCR Master Mix	7500
NEBNext Single Cell cDNA PCR Primer Mix	400
Nuclease-free water	3100
Total	**11,000**

14. Place a fresh 96-well plate on a chilled position of the robot deck and add 100 µl of cDNA amplification mix to each well.
15. Collect the RT plate from the thermocycler and place onto a chilled position of the robot deck.
16. The robot will perform 4 x 96 well stamps from the reagent plate, and add 22 µl of cDNA amplification mastermix to each well and mix by pipetting.
17. Seal the plate, transfer to a dedicated post-PCR cDNA amplification laboratory, place in a thermocycler with the heated lid set to 100 ^{o}C, and run the following program:

Temperature	Time	Cycles
98 ^{o}C	45 seconds	1
98 ^{o}C	10 seconds	16-25 cycles depending on the cell type
62 ^{o}C	15 seconds	
72 ^{o}C	3 minutes	
72 ^{o}C	5 minutes	1
4 ^{o}C	Hold	1

PAUSE POINT Amplified cDNA Samples can be stored at -20 ^{o}C for several months before purification.

Purification of the amplified cDNA Timing 45 minutes

Perform steps 18-33 in a dedicated post-cDNA amplification room. We perform cDNA purification on the Agilent Bravo NGS or Hamilton STAR platform, but these steps can be performed on alternative liquid handling platforms.

18. Collect the amplified cDNA plate from the thermocycler, and centrifuge at 1,000g for 1 minute.
19. Place the amplified cDNA plate on the robot deck at room temperature.
20. Allow Agencourt AMPure XP beads to equilibrate to room temperature for 30 minutes before use. Ensure the solution is homogeneous prior to use, mixing gently by inversion.
21. Add 18 µl of Agencourt AMPure XP beads to each well of the 384-well plate containing the PCR-amplified cDNA molecules (0.6:1 ratio), mix well by pipetting.
22. Incubate the mixture at room temperature for 5 minutes.
23. Move the plate to a magnet, and allow the beads to settle for 2 minutes.
24. Carefully remove and discard the supernatant. Do not disturb the beads.
25. Keeping the 384-well plate on the magnet, wash the beads with 180 µl of freshly prepared 80% (vol/vol) ethanol for 30 seconds, and then remove and discard the ethanol wash. Do not disturb the beads.
26. Repeat Step 25 once.
27. Allow beads to dry for 5 minutes.
28. Remove the plate from the magnet, add 25 µl of nuclease-free water to the beads, and mix by pipetting.
29. Incubate the mixture at room temperature for 2 minutes.
30. Return the 384-well plate to the magnet, and allow beads to settle for 5 minutes.
31. Transfer the supernatant, which contains the purified cDNA to a new 384-well plate at room temperature. Do not disturb the beads.

Quality Control of Amplified cDNA TIMING 30 minutes

32. We measure the concentration of amplified cDNA for each sample using a fluorescence-based assay (Accuclear, Biotium) according to the manufacturer's instructions. To streamline our laboratory workflow, we avoid normalising each sample to a fixed concentration for library preparation. Instead, we calculate an average concentration and transfer a fixed volume such that 5-25 ng of each successfully amplified cDNA sample enters library preparation. In addition to quantification of cDNA, we assess cDNA quality by subjecting a small number of samples to analysis with a Bioanalyzer high sensitivity DNA kit (Agilent Technologies) an example can be seen in figure S6(a).
33. Taking an average across the plate. Transfer ~ 10 ng of cDNA into a fresh 384-well plate for sequencing library preparation.

PAUSE POINT The amplified cDNA can be stored at -20 °C for several months prior to library preparation.

Illumina Sequencing Library Preparation TIMING 3 hours

Continue the protocol in the same post cDNA amplification laboratory until DNA amplification (step 70), then move to the post library amplification laboratory. We perform library preparation on the Agilent Bravo NGS platform, but these steps can be performed on alternative liquid handlers.

34. Centrifuge the 384-well plate containing ~ 10 ng of purified cDNA at 1000g for 1 minute, and place onto a chilled position of the robot deck.
35. Allow Agencourt AMPure XP beads to equilibrate to room temperature for 30 minutes before use. Ensure the solution is homogeneous prior to use, mixing gently by inversion.

36. Add a 0.9 X volume of Agencourt AMPure XP beads to each well of the 384-well plate containing the PCR-amplified cDNA molecules, mix well by pipetting.
37. Incubate the mixture at room temperature for 5 minutes.
38. Move the plate to a magnet, and allow the beads to settle for 2 minutes.
39. Carefully remove and discard the supernatant. Do not disturb the beads.
40. Keeping the 384-well plate on the magnet, wash the beads with 180 µl of freshly prepared 80% (vol/vol) ethanol for 30 seconds, and then remove and discard the ethanol wash. Do not disturb the beads.
41. Repeat Step 40 once.
42. Allow beads to dry for 5 minutes.
43. Remove the plate from the magnet, add 13 µl of TE pH 8.0 to the beads, and mix by pipetting.
44. Incubate the mixture at room temperature for 2 minutes.
45. Return the 384-well plate to the magnet, and allow beads to settle for 5 minutes.
46. Transfer 12.4 µl of the supernatant from step 45 to a new 384-well plate without disturbing the beads.
47. Prepare Fragmentation/End prep Mix, as described in the table below, which will provide sufficient volume for one 384-well plate. Mix thoroughly by pipetting up and down, and keep chilled on ice.

Reagent	**Volume (µl)**
NEBNext Ultra II FS Reaction Buffer	1344
NEBNext Ultra II FS Reaction Enzyme	384
Total	**1728**

48. Place the plate containing the purified cDNA on a chilled position of the robot deck.
49. The robot will add 3.6 µl of fragmentation/end prep mastermix to each well and mix by pipetting.
50. Seal and transfer the plate to a thermocycler with the heated lid set to 100 ^{o}C and run the following program:

Temperature	**Time**
72 ^{o}C	15 minutes
65 ^{o}C	30 minutes

4 °C	Hold

51. Prepare the ligation mix as described in the table below, which will provide sufficient volume for one 384-well plate. Mix thoroughly by pipetting up and down, and keep chilled on ice.

Reagent	**Volume (µl)**
NEBNext Ultra II Ligation Master Mix	5760
NEBNExt Ultra II Ligation Enhancer	192
Duplexed Adapter (100 µM)	432
Nuclease-free water	48
Total	**6432**

52. Place a fresh 384-well plate onto a chilled position of the robot deck and add 13.4 µl of ligation mix from step 51 to each well.
53. Collect the fragmentation/end prep plate (step 50) from the thermocycler and place onto a chilled position of the robot deck.
54. Transfer the entire contents of the fragmentation/end prep plate into the 384-well plate containing the ligation mix (step 52). Mix thoroughly by pipetting up and down.
55. Incubate for 15 minutes at 20 °C on the robot deck.
56. Allow Agencourt AMPure XP beads to equilibrate to room temperature for 30 minutes before use. Ensure the solution is homogeneous prior to use, mixing gently by inversion.
57. Add 20 µl of Agencourt AMPure XP beads to each well of the 384-well plate containing adapter ligated material (0.7:1 ratio), mix well by pipetting.
58. Incubate the mixture at room temperature for 5 minutes.
59. Move the plate to a magnet, and allow the beads to settle for 2 minutes.
60. Carefully remove and discard the supernatant. Do not disturb the beads.
61. Keeping the 384-well plate on the magnet, wash the beads with 180 µl of freshly prepared 80% (vol/vol) ethanol for 30 seconds, and then remove and discard the ethanol wash. Do not disturb the beads.
62. Repeat Step 61 once.
63. Allow beads to dry for 5 minutes.

64. Remove the plate from the magnet, add 10 µl of nuclease-free water to the beads, and mix by pipetting.
65. Incubate the mixture at room temperature for 2 minutes.
66. Return the 384-well plate to the magnet, and allow beads to settle for 5 minutes.
67. Place a new 384-well plate containing 5 µl of unique dual indexed iPCR-Tag primers (40 µM) onto a chilled position of the robot deck or on ice. This plate will become the PCR reaction plate.
68. Transfer 15 µl of KAPA HiFi HotStart ReadyMix (2x) to each well containing indexed primers.
69. Keeping the plate on the magnet transfer 10 µl of the adapter-ligated library from Step 66 to the plate containing the KAPA mix and iPCR-Tags (Step 68). Mix thoroughly by pipetting up and down.
70. Seal the plate, transfer to a dedicated post-PCR amplification laboratory, place in a thermocycler with the heated lid set to 100 ^{o}C, and run the following program:

Temperature	**Time**	**Cycles**
98 ^{o}C	45 seconds	1
98 ^{o}C	10 seconds	8
62 ^{o}C	15 seconds	
72 ^{o}C	3 minutes	
72 ^{o}C	5 minutes	1
4 ^{o}C	Hold	1

PAUSE POINT Amplified libraries can be stored at -20 °C for several months prior to library purification.

Pooling and purification of amplified libraries Timing 45 minutes

We use a Hamilton Microlab STAR to perform pooling and purification (step 71-83), but these steps can be performed on alternative liquid handling platforms or manually.

71. Create an equal volume pool of the 384 amplified libraries by combining 3 µl of each amplified library.

72. Allow Agencourt AMPure XP beads to equilibrate to room temperature for 30 minutes before use. Ensure the solution is homogeneous prior to use, mixing gently by inversion.
73. In a fresh deepwell 96-well plate combine 200 µl of the pooled libraries with 190 µl of Agencourt AMPure beads (1:0.95 ratio), mix well by pipetting.
74. Incubate the mixture at room temperature for 5 minutes.
75. Move the plate to a magnet, and allow the beads to settle for 2 minutes.
76. Carefully remove and discard the supernatant. Do not disturb the beads.
77. Keeping the 96-well plate on the magnet, wash the beads with 180 µl of freshly prepared 80% (vol/vol) ethanol for 30 seconds, and then remove and discard the ethanol wash. Do not disturb the beads.
78. Repeat Step 77 once.
79. Allow beads to dry for 5 minutes.
80. Remove the plate from the magnet, add 200 µl of nuclease-free water to the beads, and mix by pipetting.
81. Incubate the mixture at room temperature for 2 minutes.
82. Return the 96-well plate to the magnet, and allow beads to settle for 5 minutes.
83. Keeping the 96-well plate on the magnet transfer 200 µl of adapter amplified library from Step 86 to a new 1.5 ml Eppendorf tube.

Quality control of sequencing libraries and normalisation for sequencing TIMING 45 minutes

84. Run 1 µl of the eluted sample from step 83 in triplicate on an Agilent Bioanalyser using a DNA 1000 assay kit.
85. Note the molarity of each reading and take an average of the 3 triplicates discarding any outliers.
86. In a fresh 1.5 ml tube, combine the amplified library pool with nuclease-free water to produce a final concentration of 2.8 nM. 5 µl of this pool will enter the workflow for HiSeq 4000 sequencing.

Library sequencing TIMING 2 Days

87. Libraries are submitted for sequencing on one lane of an Illumina HiSeq 4000 instrument (paired-end, 75-bp reads) according to the manufacturer's protocol. We typically aim for an average depth of 1 million reads per cell. Alternative sequencing options to achieve comparable sequencing depth are; the NextSeq 550 with high output flow cell or NovaSeq 6000 SP flow cell on one lane of an XP workflow.

Transcriptome data analysis—reads mapping and statistics TIMING approximate 6-18 hours

Refer to steps 63-68 of in-house automated scRNA-seq workflow.

Sequencing benchmarking—sensitivity and accuracy TIMING approximate 1 hr

Refer to steps 69-73 of in-house automated scRNA-seq workflow.