



# cDNA synthesis using the mosquito HV genomics with the SMART-Seq® Single Cell Kit

## I. Introduction

The [SMART-Seq Single Cell Kit](#) (SSsc; Cat. # 634472), is designed to generate high-quality, full-length cDNA directly from single cells. The cDNA synthesis protocols presented in the [SMART-Seq Single Cell Kit User Manual](#) (Sections V.A–C) can be automated using the mosquito HV genomics (SPT Labtech) with a series of customized scripts. For a copy of these scripts, please contact the vendor directly at: [mosquitosupport@sptlabtech.com](mailto:mosquitosupport@sptlabtech.com).

The following user guide describes how to perform cDNA synthesis with these scripts using a 384-well plate and 1/8 of the reaction volumes specified in the user manual. Please read the user manual, which provides essential details needed to perform the cDNA synthesis protocols, before referring to this user guide, which contains abbreviated versions of the protocols.

The mosquito HV genomics tips provide a maximum capacity of 5 µl and a minimum dispense capability of 500 nl and either 9-mm or 4.5-mm spacing; the 4.5-mm spacing is ideal for use with a 384-well plate. As such, the protocols we describe below assume this configuration: 4.5-mm-pitch tips with a 384-well plate.

For any technical support regarding the Mosquito HV Genomics instrumentation, please contact SPT Labtech directly at: [mosquitosupport@sptlabtech.com](mailto:mosquitosupport@sptlabtech.com).

For any technical support regarding the SMART-Seq Single Cell Kit, please visit: [takarabio.com/support](http://takarabio.com/support).



**Figure 1. Additional plateware required.** From left to right: Eppendorf 384-well PCR plate; SPT Labtech LVSD plate; SPT Labtech magnetic bead separation block.

## II. Additional Materials Required

- 384-well PCR plates (Eppendorf twin.tec PCR Plate 384, Cat. # 951020702)
- 384-Well LVSD plate (SPT Labtech P/N 4150-05829)
- 384-well magnetic bead separation block (SPT Labtech P/N 3268-02008)
- NucleoMag NGS Clean-up and Size Select (Cat. # 744970.5, 744970.50, or 744970.500)
- Adhesive sealing tape for PCR plates (e.g., Thermo Scientific Adhesive PCR Plate Seals, Cat. No. AB0558)
- Minicentrifuge for PCR plates
- Single-channel pipette: 10  $\mu$ l, 20  $\mu$ l, and 200  $\mu$ l
- Eight-channel or 12-channel pipette (recommended): 20  $\mu$ l and 200  $\mu$ l
- Filter pipette tips: 2  $\mu$ l, 20  $\mu$ l, and 200  $\mu$ l

### III. First-Strand cDNA Synthesis

These protocols describe how to use the mosquito HV genomics to transfer small volumes of samples, reagent master mixes, ethanol, and elution buffers from a 384-well source plate to a 384-well reaction plate using a series of customized scripts.

#### A. CDS Primer Annealing Protocol

This protocol corresponds to Section V.A, Steps 1–6 of the User Manual. It describes how to anneal the RNA in single-cell samples to the primer needed to perform cDNA synthesis. It uses the mosquito HV genomics with the “Step 1. SSsc\_CDSpriming\_384” script.

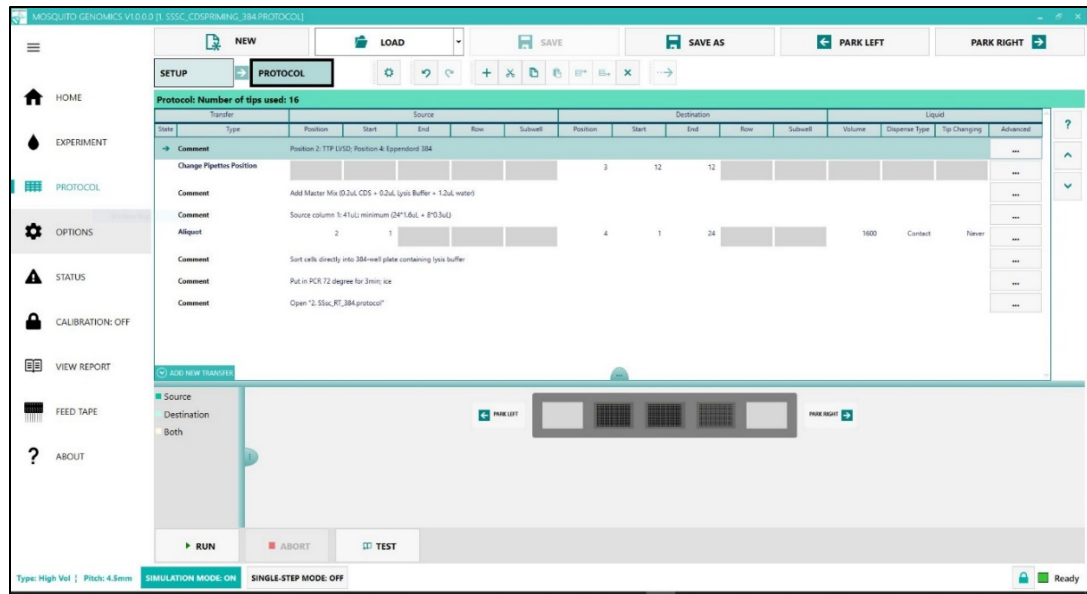


Figure 2. “Step 1. SSsc\_CDSpriming\_384” script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples (refer to Figure 9 in Appendix for larger view).

1. Prepare a master mix of CDS Sorting Solution (CSS) by combining the following reagents in the order shown in a 1.5-ml nuclease-free tube at room temperature. Make sure to prepare 10X Reaction Buffer just before preparing the master mix.

**NOTE:** As a general guide, additional master mix has been added for each aspiration by the mosquito HV genomics.

Component	1 rxn (µl)	96 rxn (µl)† (6 columns)	192 rxn (µl)† (12 columns)	384 rxn (µl)† (24 columns)
10X Reaction Buffer	0.2	21	42	84
CDS Primer	0.2	21	42	84
Nuclease-free water	1.2	126	252	504
Total volume	1.6	168	336	672
Volume per source plate well		10.5	21	42

†additional volume for mosquito aspirations included

2. Aliquot the required volume per well of CSS, as shown in “Volume per source plate well” from the table above, into Column 1 of the LVSD source plate.
3. In the mosquito HV genomics software, open “Step 1. SSsc\_CDSpriming\_384” script. On the deck of the mosquito HV genomics, place your LVSD source plate in Position 2 and your 384-well PCR reaction plate in Position 4. Run to aliquot 1.6 µl of CSS per well into Columns 1 through 6, 12, or 24 of the reaction plate for 96, 192, or 384 samples, respectively.

**NOTE:** If using a different number of samples than shown above, be aware that reagent volumes may not scale linearly. For best results, please consult your SPT Labtech Field Application Scientist prior to starting experiment.

4. Use flow cytometry to dispense single-cell samples sorted directly into the 1.6 µl of CSS per well of the reaction plate.

**NOTE:** In this protocol, we are assuming that FACS sorting of the cells will not change the volume of liquid in the plate wells. If your sorter dispenses a non-negligible amount of sheath fluid, adjust the volume of the CSS mix by reducing the amount of nuclease-free water to maintain a total volume of 1.6 µl per well.

5. Transfer the reaction plate to a preheated thermal cycler and run the following program:
  - 72° C for 3 min
  - 4° C forever
6. Spin down the reaction plate at 2,000 rpm for 30–60 sec after the program is done.

## B. Reverse Transcription Protocol

This protocol corresponds to Section V.A, Steps 7–12 of the User Manual. It describes how to perform the reverse transcription reaction on the samples from Section II.A, Step 8 of this user guide using the mosquito HV genomics with the “Step 2. SSsc\_RT\_384” script.

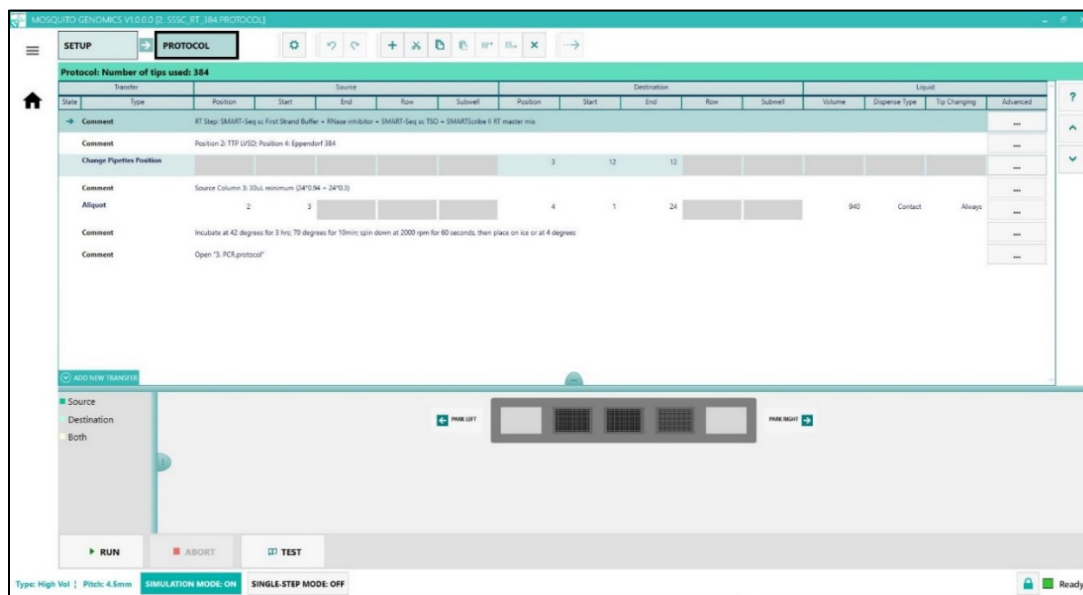


Figure 3. “Step 2. SSsc\_RT\_384” script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples (refer to Figure 10 in Appendix for larger view).

1. While the reaction is in the thermal cycler, make the RT Master Mix in a 1.5-ml nuclease-free tube at room temperature by combining the following reagents in the order shown.

**NOTE:** Place the RT Master Mix on ice after adding the first three components, then add SMARTScribe™ II Reverse Transcriptase to the master mix after Step 2 and proceed to Step 3.

Component	1 rxn (µl)	96 rxn (µl)†	192 rxn (µl)†	384 rxn (µl)†
		(6 columns)	(12 columns)	(24 columns)
SMART-Seq sc First Strand Buffer	0.5	63	126	252
RNase Inhibitor (40 U/µl)	0.0625	8	16	32
SMART-Seq sc TSO	0.125	16	32	64
SMARTScribe II Reverse Transcriptase	0.25	32	64	128
<b>Total volume</b>	<b>0.94</b>	<b>119</b>	<b>238</b>	<b>476</b>
Volume per source plate well		7.5	15	30

†additional volume for mosquito aspirations included

2. As soon as the thermal cycler reaches 4°C, place the reaction plate on ice for 2 min, then spin down the plate at 2,000 rpm for 30–60 sec.
3. Aliquot the required volume per well of the RT Master Mix, as shown in “Volume per source plate well” from the table above, into Column 3 of the LVSD source plate.
4. In the mosquito HV genomics software, open “Step 2. SSsc\_RT\_384” script and adjust to reflect the actual number of columns desired. On the deck of the mosquito HV genomics, place your LVSD source plate in Position 2 and your 384-well PCR reaction plate in Position 4 and run. The script will aliquot 0.94 µl of RT Master Mix into each well.

**NOTE:** If plate warping has occurred as a result of the thermocycler, place your reaction plate in the PCI Plate Clamp supplied with the mosquito HV genomics instrument.

5. Remove the reaction plate from the mosquito HV genomics instrument and seal with adhesive tape.
6. Vortex the reaction plate gently and thoroughly, then spin down the plate at 2,000 rpm for 30-60 sec.
7. Insert the reaction plate into the thermal cycler and run the following program:
  - 42° C for 180 min
  - 70° C for 10 min
  - 4° C forever
8. Spin down the reaction plate at 2,000 rpm for 30–60 sec after the RT reaction is done.

**SAFE STOPPING POINT:** The plate can be stored at 4°C overnight.

### C. PCR Amplification Protocol

This protocol corresponds to Section V.B, Steps 1–4 of the User Manual. It describes how to perform cDNA amplification by LD-PCR on the cDNA samples from Section II.B, Step 10 of this user guide using the mosquito HV genomics with the “Step 3. SSsc\_PCRamplification\_384” script.

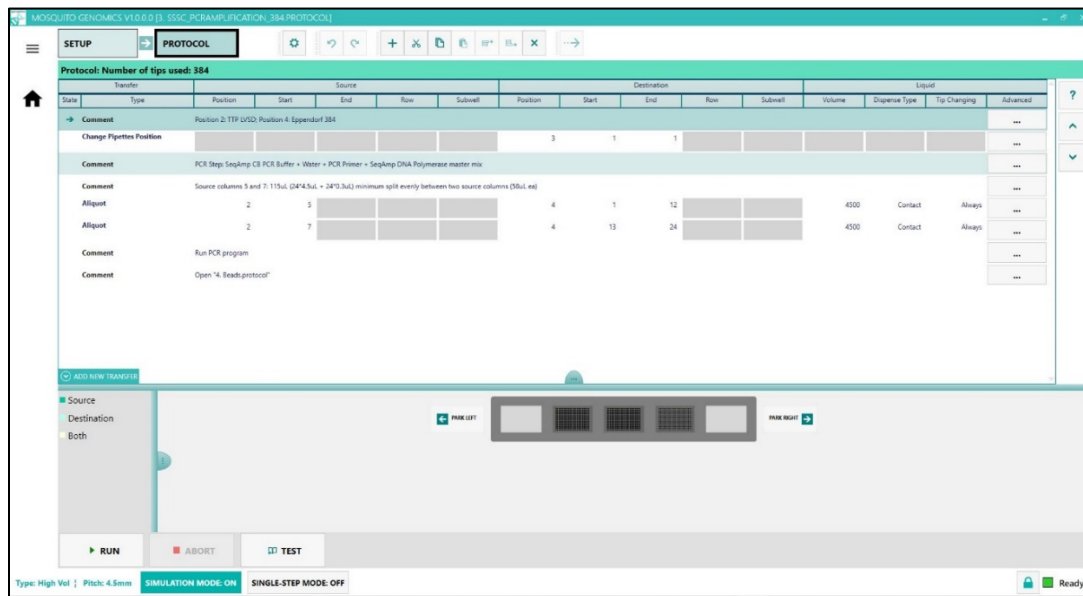


Figure 4. “Step 3. SSsc\_PCRamplification” script from the mosquito HV genomics software GUI. The setup shown here is for 384 samples (refer to Figure 11 in Appendix for larger view).

1. Make the PCR Master Mix by combining the following reagents in the order shown in a 1.5-ml nuclease-free tube on ice. Be sure to add SeqAmp™ DNA Polymerase last and just before use. Mix gently by pipetting up and down.

Component	1 rxn (µl)	96 rxn (µl)† (6 columns)	192 rxn (µl)† (12 columns)	384 rxn (µl)† (24 columns)
SeqAmp CB PCR Buffer (2X)	3.5	358	716	1,433
Nuclease-free water	0.73	75	150	300
PCR Primer	0.13	13.5	27	54
SeqAmp DNA Polymerase	0.14	14.5	29	58
Total volume	4.5	461	922	1,845
Volume per source plate well		29	58	58*

†additional volume for mosquito aspirations included; \*Two source columns required.

2. Aliquot the required volume per well of the PCR Master Mix, as shown in "Volume per source plate well" from the table above, into Column 5 of the source plate (if there are 384 samples, aliquot into both Columns 5 and 7).
3. Spin down the reaction plate at 2,000 rpm for 30–60 sec after the RT is finished.

4. Place the source plate in Position 2 and the reaction plate (in the booster, if warped by the thermocycler) in Position 4 on the mosquito HV genomics.
5. Load the "Step 3. SSsc\_PCRamplification\_384" script, adjusting the number of columns desired accordingly, and run. The script will aliquot 4.5 µl to each well.
6. Remove the reaction plate from the mosquito HV genomics and seal with adhesive tape.
7. Vortex the reaction plate gently and thoroughly, then spin down the plate at 2,000 rpm for 30–60 sec.
8. Place the reaction plate in the thermal cycler and run the following program:

```

95°C           1 min
X cycles*:
    98°C    10 sec
    65°C    30 sec
    68°C    3 min
72°C           10 min
4°C            hold
    
```

\*Use the following table to as a guideline to help determine the optimal number of PCR cycles for your input:

Cell type	Approximate RNA equivalent (per cell)	Typical # of PCR cycles
K-562 or HEK293 cells	10–15 pg	17
PBMCs	1–5 pg	20
Jurkat cells	5 pg	17
Lymphoblastoid cells	2–15 pg	17–19

9. Spin down the reaction plate at 2,000 rpm for 30–60 sec after the PCR reaction is done.

**SAFE STOPPING POINT:** The plate can be stored at 4°C overnight.



## D. Library Purification Using NucleoMag NGS Clean-up and Size Select

This protocol corresponds to Section V.C, Steps 1–6 of the User Manual. It describes how to purify the PCR-amplified cDNA from Section II.C, Step 12 of this user guide using NucleoMag Clean-up and Size Select beads and the mosquito HV genomics with the “Step 4. SSsc\_Beads\_384” script.

### NOTES:

- Aliquot magnetic beads into 1.5-ml tubes upon receipt in the laboratory.
- Before each use, bring bead aliquots to room temperature for at least 30 minutes and mix well to disperse.
- Prepare fresh 80% ethanol for each experiment.
- You will need 400 µl per sample.
- You will need a magnetic separation device for 0.2-ml tubes, strip tubes, or a 96-well plate.

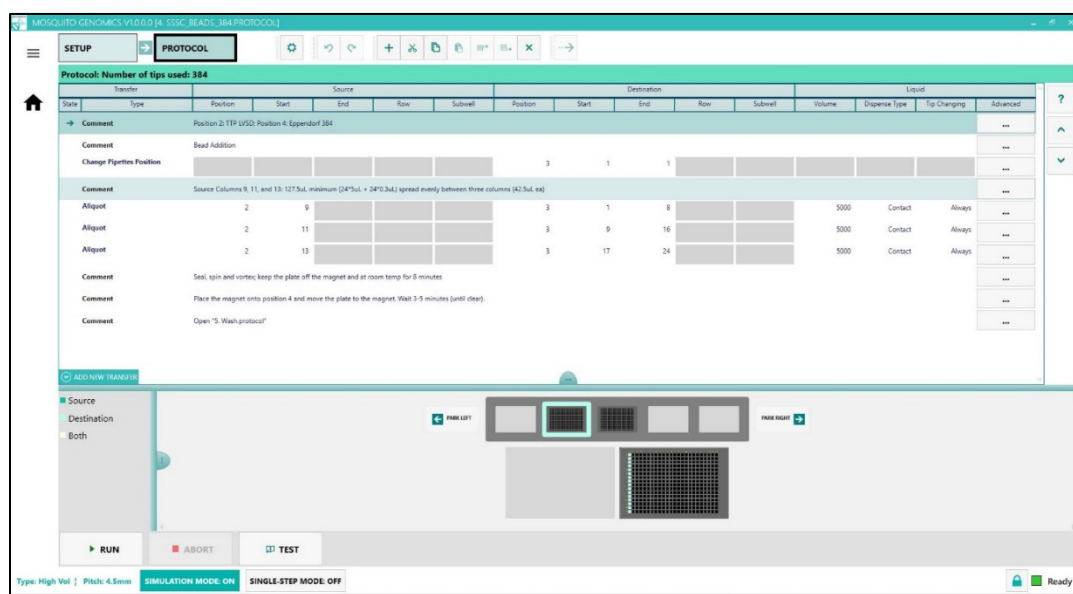


Figure 5. “Step 4. SSsc\_Beads\_384” script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples (refer to Figure 12 in Appendix for larger view).

1. Vortex magnetic beads until evenly mixed.
2. In the LVSD source plate, add 42.5 µl of magnetic beads to each well in Columns 9, 11, and 13.
3. Place the source plate in the mosquito HV genomics in Position 2 and the reaction plate (in the booster, if desired) in Position 3.
4. Load the “Step 4. SSsc\_Beads\_384” script and adjust the desired number of columns accordingly.

**NOTE:** Use one column per 16 samples. If the number of samples is not divisible by 16, it is still necessary to use an entire column. For example, use one column for 1–16 samples, two columns for 17–32 samples, three columns for 33–48 samples, etc.

5. Run the “Step 4. SSsc\_Beads\_384” script.
6. Take the reaction plate with the beads in it; seal the plate with adhesive tape.

7. Briefly spin and thoroughly vortex the reaction.
8. Leave the plate at room temperature for 8 min.
9. After 8 min, put the reaction plate on top of the 384-well magnetic plate (supplied with the mosquito HV genomics).
10. Wait for 3–5 min or until the supernatant is clear; then put the magnetic plate in Position 4.

### E. Ethanol NucleoMag NGS Clean-up and Size Select Wash Protocol

This protocol corresponds to Section V.C, Steps 7–8 of the User Manual. It describes how to wash impurities from the magnetic beads in Section II.D, Step 12 of this user guide using the mosquito HV genomics with the “Step 5. SSc\_Wash\_384” script.

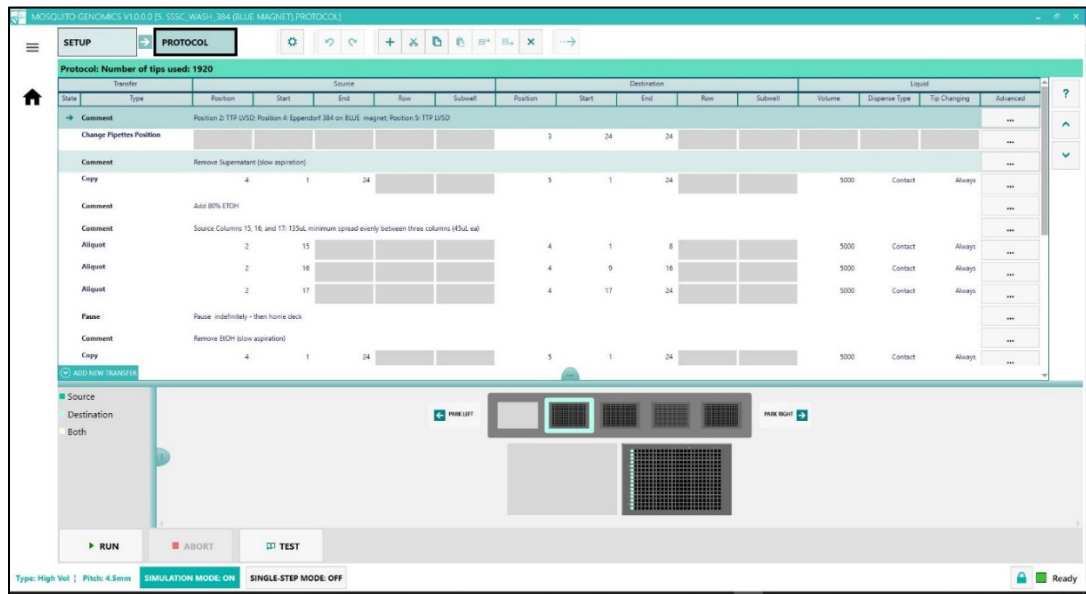


Figure 6. “Step 5. SSc\_Wash\_384” script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples (refer to Figure 13 in Appendix for larger view).

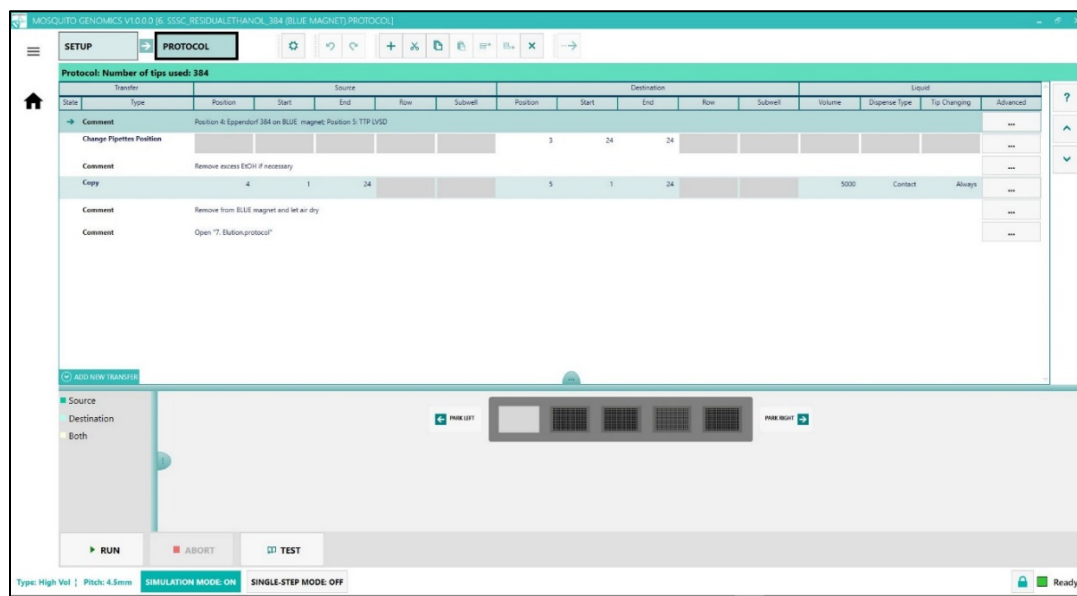
- For the 384 samples, in the LVSD source plate in Position 2, add 45  $\mu$ l of 80% ethanol to each well in Columns 15,16, and 17 for the first wash, and add 45  $\mu$ l of 80% ethanol to each well in Columns 18, 19, and 20 for the second wash. For fewer samples, adjust source columns accordingly.

**NOTES:** Because of the volume of wash required for 384 samples, it is necessary to add ethanol to more than one column of the source plate. The first wash will be performed using the ethanol in Columns 15–17. The second wash will be performed using the ethanol in Columns 18–20. If fewer than 384 samples are processed, fewer columns may be needed.

- For 1–128 samples, use Columns 15 and 18 as the respective source columns for the two ethanol wash steps.
  - For 129–256 samples, use Columns 15–16 and 18–19.
  - For 257–384 samples, use Columns 15–17 and 18–20.
- Add a clear source plate to Position 5 to collect discarded supernatant and wash buffer.
  - Load the “Step 5. SSsc\_Wash\_384” script, adjusting the program to the desired number of columns.
  - Run the “Step 5. SSsc\_Wash\_384” script.

## F. Removing Residual Ethanol Protocol

This protocol corresponds to Section V.C, Steps 9–10 of the User Manual. It describes how to aspirate the remaining liquid from the magnetic beads in Section II.E, Step 4 of this user guide using the mosquito HV genomics with the “Step 6. SSsc\_ResidualEthanol\_384” script.



**Figure 7.** “Step 6. SSsc\_ResidualEthanol\_384” script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples (refer to Figure 14 in Appendix for larger view).

- After the wash protocol is finished, if there is a visible amount ethanol remaining above the pellet, load the “Step 6. SSsc\_ResidualEthanol\_384” script to remove the excess supernatant.

- Run the “Step 6. SSsc\_ResidualEthanol\_384” script.
- Remove the reaction plate from the 384-well magnetic plate and air dry at room temperature until the beads are dry (approximately 3–4 min).

## G. Elution Protocol

This protocol corresponds to Section V.C, Steps 11–15 of the User Manual. It describes how to elute purified PCR-amplified cDNA from the dried magnetic beads in Section II.F, Step 3 of this user guide using the mosquito HV genomics with the “Step 8. SSsc\_Elution\_384” script.

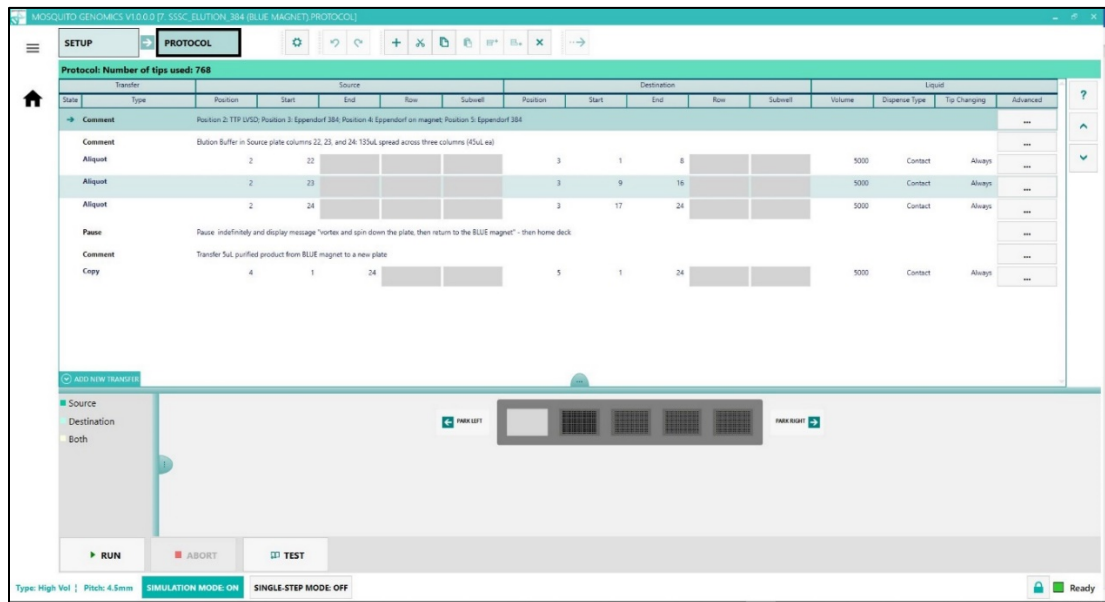


Figure 8. “Step 7. SSsc\_Elution\_384” script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples (refer to Figure 15 in Appendix for larger view).

- For 384 samples, in the LVSD source plate in Position 2, add 45  $\mu$ l of Elution Buffer to each well in Columns 22, 23, and 24.

### NOTE:

- For 1–128 samples, use only Column 22.
- For 129–256 samples, use Columns 22–23.
- For 257–384 samples, use Columns 22–24.

- Place the reaction plate with the dry beads (in the booster if desired) into Position 3.
- Load the “Step 7. SSsc\_Elution\_384” script, adjusting the desired number of columns accordingly.
- Run the “Step 7. SSsc\_Elution\_384” script (the script will pause after the elution buffer has been added to the beads).
- Take the reaction plate from the mosquito HV genomics and seal with adhesive tape, vortex the reaction plate thoroughly, then spin down the plate at 2,000 rpm for 30–60 sec.
- Leave the plate at room temperature for 2–3 min.

7. Put the reaction plate onto the 384-well magnet in Position 4 until the supernatant is clear.
8. Place a new collection plate in Position 5.
9. Resume the “Step 7. SSsc\_Elution\_384” script. The new collection plate will contain 5  $\mu$ l of the purified product.
10. Transfer clear supernatant containing purified cDNA from each well to a nuclease-free, low-adhesion tube. Label each tube with sample information and store at  $-20^{\circ}\text{C}$  until ready for library preparation.

**SAFE STOPPING POINT:** The samples can be stored at  $-20^{\circ}\text{C}$  for several months.

## H. Quantification and Library Preparation

Refer to the [SMART-Seq Single Cell Kit User Manual](#) for quantification and library preparation options.

## IV. Technical Support

For any technical support regarding the Mosquito HV Genomics instrumentation, please contact SPT Labtech directly at: [mosquitosupport@sptlabtech.com](mailto:mosquitosupport@sptlabtech.com)

For any technical support regarding the SMART-Seq Single Cell Kit, please visit: [takarabio.com/support](http://takarabio.com/support)

## Appendix

The images in this section represent running customized scripts in the mosquito HV genomics GUI as they pertain to each specific protocol. These figures are already embedded in the main document but are included again here in higher resolution and so they can be printed out as a reference.

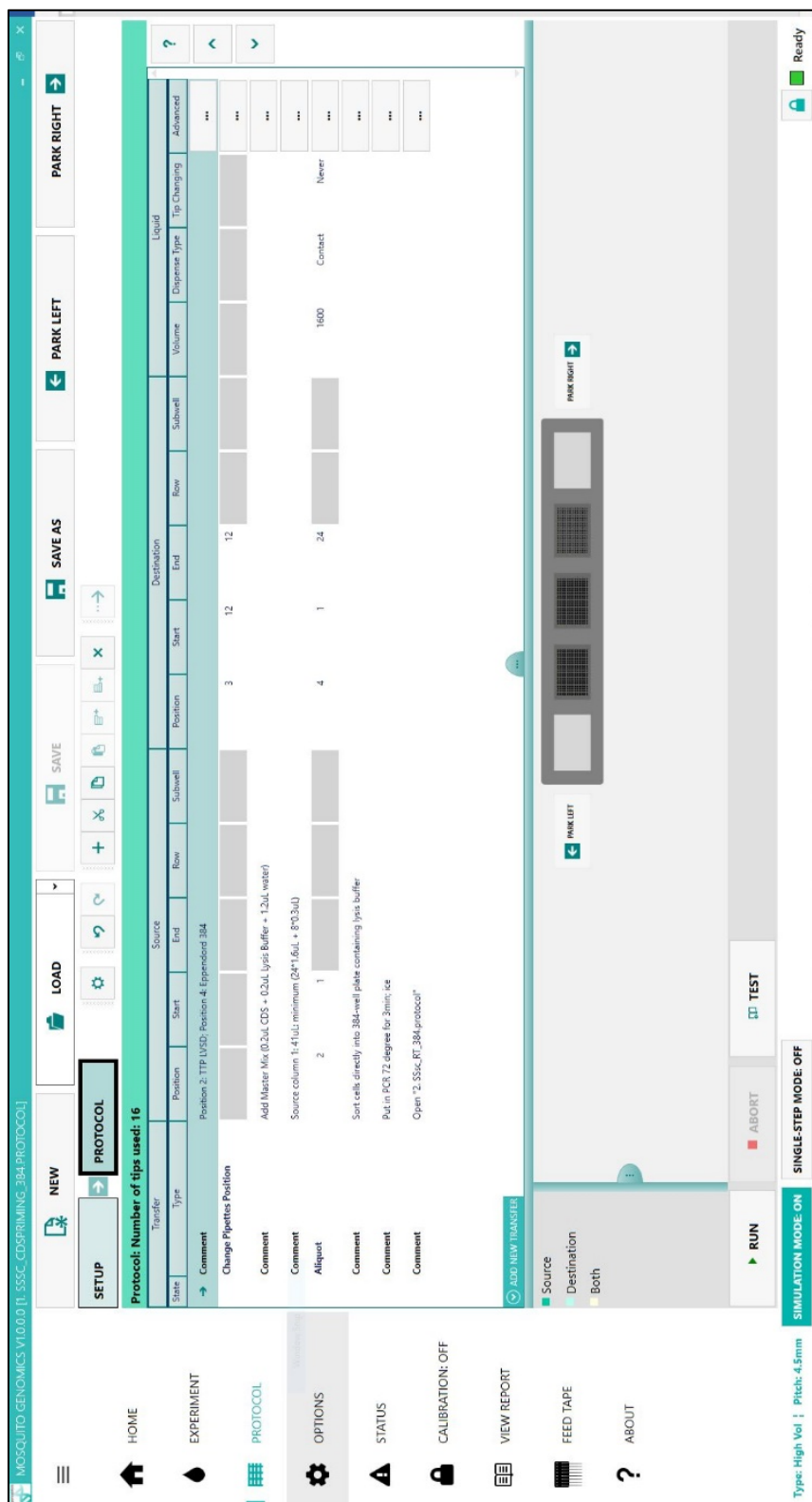


Figure 9. "Step 1. SSsc\_CDspriming\_384" script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples.

MOSQUITO GENOMICS V10.0.0 [2: SSSC\_RT\_384-PROTOCOL]

**Protocol: Number of tips used: 384**

State	Type	Transfer	Position	Start	End	Row	Subwell	Volume	Duplex Type	Tip Changing	Advanced
→	Comment	RT Step: SMART-Seq v4: First Strand Buffer + RNase inhibitor + SMART-Seq v4: TSO + SMART-Seq II RT master mix									...
	Comment	Position 2: TTP; UCD; Position 4: Eppendorf 384									...
	Change Pipette Position		3	12	12						...
	Comment	Source Column 3: 30uL minimum (24°0.04 - 24°0.3)									...
	Aliquot		2	3	3						...
	Comment	Incubate at 42 degrees for 3 hrs; 70 degrees for 10mins; spin down at 2000 rpm for 60 seconds; then place on ice or at 4 degrees									...
	Comment	Open "3_PCS_protocol"						940	Contact	Always	...

ADD NEW TRANSFER

Source Destination Both

PINK LIGHT

PINK LIGHT

▶ RUN

■ ABORT

⏸ TEST

SIMULATION MODE ON

SINGLE-STEP MODE OFF

Type: High Vol | Pitch: 4.5mm

Ready

Figure 10. "Step 2. SSSC\_RT\_384" script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples.

MOSQUITO GENOMICS V10.0.0 [ SSSc\_PCRAMPLIFICATION\_384\_PROTOCOL ]

**SETUP** **PROTOCOL**

Protocol: Number of tips used: 384

Transfer	Type	Position	Start	End	Source	Row	Subwell	Position	Start	End	Destination	Row	Subwell	Volume	Dispense Type	Tip Changing	Advanced	
→	Comment	Position 2: TTP LVSD: Position 4: Eppendorf 384																
	Change Pipette Position							3		1								...
	Comment	PCR Steps: SeqAmp CB PCR Buffer + Water + PCR Primer + SeqAmp DNA Polymerase master mix																
	Comment	Source columns 5 and 7: 115µL (24*4.5µL + 24*0.3µL) minimum split evenly between two source columns (5&6, 6&7)																
	Aliquot		2	5				4		1		12		4000	Contact	Always	...	
	Aliquot		2	7				4		13		24		4000	Contact	Always	...	
	Comment	Run PCR program																
	Comment	Open "4. Beads protocol"																

ADD NEW TRANSFER

Source  
Destination  
Both

▶ RUN  
▶ ABORT  
▶ TEST

Simulation Mode: ON  
Single-Step Mode: OFF

Type: High Vol | Pitch: 4.5mm

Ready

Figure 11. "Step 3. SSSc\_PCRamplification" script from the mosquito HV genomics software GUI. The setup shown here is for 384 samples.



**MOSSQUITO-GENOMICS V1.0.0.9 [4\_S5SC\_BEADS\_384.PROTOCOL]**

**PROTOCOL**  
**Protocol: Number of tips used: 384**

State	Transfer			Source			Destination			Liquid						
	Type	Position	Subwell	Start	End	Row	Subwell	Position	Start	End	Row	Subwell	Volume	Dispense Type	Tip Changing	Advanced
→	Comment	Position 2:TIP LVSD: Position 4: Eppendorf 384														
	Comment	Bead Addition														
	Change Pipettes Position															
	Comment	Source Columns 9, 11, and 13: 127.5ul, minimum (24*5ul + 24*0.3ul) spread evenly between three columns (47.5ul ea)														
	Aliquot	2	9	3	1	8							5000	Contact	Always	...
	Aliquot	2	11	3	9	16							5000	Contact	Always	...
	Aliquot	2	13	3	17	24							5000	Contact	Always	...
	Comment	Seal, spin and vortex; keep the plate off the magnet and at room temp for 8 minutes														
	Comment	Place the magnet onto position 4 and move the plate to the magnet. Wait 3.5 minutes (until clear).														
	Comment	Open '5. Wash.protocol'														

**ADD NEW TRANSFER**

Legend: ■ Source, ■ Destination, ■ Both

Buttons: **RUN**, **ABORT**, **TEST**

Mode: **SIMULATION MODE: ON** | **SINGLE-STEP MODE: OFF**

Footer: Type: High Vol | Pitch: 4.5mm | Ready

Figure 12. "Step 4. S5sc\_Beads\_384" script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples.

**PROTOCOL**  
 Protocol: Number of tips used: 1920

State	Type	Position	Start	End	Row	Subwell	Position	Start	End	Row	Subwell	Volume	Dispersive Type	Tip Changing	Advanced
Comment	Change Pipettes Position	Position 2: TTP LVSD; Position 4: Eppendorf 384 on BLUE magnet; Position 5: TTP LVSD													...
Copy	Remove Supernatant (low aspiration)		4	1	24		5	1	24			5000	Contact	Always	...
Comment	Add 80% EtOH														...
Comment	Source Columns 15, 16, and 17: 135uL minimum spread evenly between three columns (45uL ea)														...
Alliquot			2	15			4	1	8			5000	Contact	Always	...
Alliquot			2	16			4	9	16			5000	Contact	Always	...
Alliquot			2	17			4	17	24			5000	Contact	Always	...
Pause	Pause indefinitely - then home deck.														...
Comment	Remove EtOH (low aspiration)														...
Copy			4	1	24		5	1	24			5000	Contact	Always	...

**ADD NEW TRANSFER**

Source: [Color] Destination: [Color] Both: [Color]

MAKE LEFT MAKE RIGHT

**RUN** **ABORT** **TEST**

Simulation Mode: ON SINGLE-STEP MODE: OFF

Type: High Vel | Pitch: 4.5mm

Ready

Figure 13. "Step 5. SSsc\_Wash\_384" script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples.

MOSQUITO GENOMICS V10.0.0 [6: S5SC\_RESIDUALETHANOL\_384 [BLUE\_MAGNET].PROTOCOL]

Protocol: Number of tips used: 384

State	Type	Source				Destination				Liquid				
		Position	Start	End	Row	Subwell	Position	Start	End	Row	Subwell	Volume	Dispense Type	Tip Changing
→	Comment	Position 4: Eppendorf 384 on BLUE magnet Position 5: TTP USSD												
	Change Pipettes Position						3	24	24					
	Comment	Remove excess ECH if necessary												
	Copy	4	1	24			5	1	24			5000	Contact	Always
	Comment	Remove from BLUE magnet and let air dry												
	Comment	Open '7_Eulow.protocol'												

ADD NEW TRANSFER

Source Destination Both

PARK LEFT PINK LEFT PINK RIGHT PINK RIGHT

RUN ABORT TEST

SIMULATION MODE ON SINGLE-STEP MODE OFF

Type: High Vol | Pitch: 4.5mm

Ready

Figure 14. "Step 6. S5sc\_ResidualEthanol\_384" script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples.

MOSQUITO GENOMICS V10.0.0 [P: SSsc\_ELUTION\_384 (BLUE MAGNET) PROTOCOL]

Protocol: Number of tips used: 768

Transfer	Source					Destination									
	Type	Position	Start	End	Row	Subwell	Position	Start	End	Row	Subwell	Volume	Dispense Type	Tip Changing	Advanced
Comment															...
Comment															...
Aliquot		2	22				3	1	8			5000	Contact	Always	...
Aliquot		2	23				3	9	16			5000	Contact	Always	...
Aliquot		2	24				3	17	24			5000	Contact	Always	...
Pause															...
Comment															...
Copy		4	1	24			5	1	24			5000	Contact	Always	...

Position 2: TIP LVSD; Position 3: Eppendorf 384; Position 4: Eppendorf on magnet; Position 5: Eppendorf 384  
 Elution Buffer in Source plate columns 22, 23, and 24; 15µL spread across three columns (5µL ea)

Pause indefinitely and display message "vortex and spin down the plate, then return to the BLUE magnet" - then home deck  
 Transfer 5µL purified product from BLUE magnet to a new plate

ADD NEW TRANSFER

Source  
 Destination  
 Both

MARK LEFT  
 MARK RIGHT

RUN  
 ABORT  
 TEST

Simulation Mode ON  
 Simulation Mode OFF

Type: High Vol | Pitch: 4.5mm  
 Ready

Figure 15. "Step 7. SSsc\_Elution\_384" script in the mosquito HV genomics software GUI. The setup shown here is for 384 samples.

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