Smart-seq2 V3

This protocol is based on Picelli et al (2013) Nature Methods.

Acronyms or shorthands:

EtOH	ethyl alcohol
M1	
RT	room temperature
RT mix	reverse transcriptase mix
TD buffer	tagment DNA buffer
TE solution	buffer containing Tris-HCl and EDTA
s or sec	second

RNA extraction, first-strand synthesis, and amplification:

1. Take tubes (cells) out of **-80°C**. Keep on ice for **3 min**.
2. Add 4 µL of M1+lysis buffer mix. Spin for **10 s**. Place on ice for **1 min**, vortex for **30 s**, on ice for **1 min**, spin for **10 s**, place on ice for **3 min**.
3. Incubate under Program 1.

   Program 1:

   4 °C – hold
   72 °C – 3 min
   4 °C – hold
4. Place on ice. Add 6 µL of RT mix (Mix 2) to each sample. Vortex for **20 sec**. Spin for **10 sec**. Place immediately on ice.
5. Incubate under Program 2. (**175 min**)

   Program 2:

   4 °C – hold
   42 °C – 90 min
   50 °C – 2 min
   42 °C – 2 min
   70 °C – 15 min
   x10
   4 °C – hold
6. Add 15 µL of Mix 3 to each tube. Vortex for **25 sec** to mix. Spin for **10 s**, place on ice.
7. Incubate under Program 3. (**125 mins**)

   Program 3:

   4 °C – hold
   98 °C – 3 min
   98 °C – 20 sec
   67 °C – 15 sec
   72 °C – 6 min
   72 °C – 5 min
   x19
   4 °C – hold

---------- ***PAUSE POINT*** ----------*Store at -20 C or -80* C ----------

Clean-up:

8. Leave AMPure XP beads at **RT**.
9. Vortex beads to mix well. Then, add 20 µL (1:0.8 ratio) beads to each sample, mix thoroughly by pipetting 10 times.
10. Leave (caps on) at **RT** for **8 min**.
11. Place undisturbed on magnet stand for **5 min**.

12. Carefully remove the liquid with the tubes still on the magnet stand.
13. Add 100 µL of 75% EtOH with tubes still on the magnet. Incubate for **30 s** at **RT**. Remove ethanol.
14. Again-Add 100 µL of 75% EtOH with tubes still on the magnet. Incubate for **30 s** at **RT**. Remove ethanol.
15. Remove as much EtOH as possible this time using a P20 pipette.
    Let the beads dry completely- turn the hood blower OFF. Leave at **RT** on the magnet stand for **10 min**. It's dry enough if you start seeing cracks. *While the beads dry, prepare fresh PCR tubes for step below.*
16. Add 20 µL TE solution. Take the tubes off the magnet, mix 10 times to resuspend fully and leave at **RT** for **3 min**.
17. Place the tubes back on the magnet stand. Leave it for **5 min** or until solution appears clear.
18. Remove exactly 17.6 µL of the supernatant without disturbing the beads and transfer it to fresh PCR tubes.
19. Check size distribution on an Agilent high-sensitivity DNA chip. Expected yield is >0.5 ng/µL.

Tagmentation:

20. Normalize cDNA to a concentration of 0.2 ng/µL. Prepare tagmentation mix on ice. Add 0.625 µl DNA to 1.875 µL Mix 4 per tube. Total reaction volume: 2.50 µL.
21. Perform tagmentation (Program 4)
    Program 4:
    10 min — 55 °C
22. Stop tagmentation reaction by adding 0.625 µL of Neutralize Tagment (NT) buffer.
    Total volume: 3.125 µl.
    Pipette a few times to mix. Incubate for **5 min** at **RT**.

Enrichment:

23. Prepare Mix 5. Add 3.125 µL to each tube. Total volume: 6.25 µL
24. Perform PCR (Program 5). **23 min**.
    Program 5:
    4 °C – hold
    72 °C – 3 min, 95 °C —30 sec
    95 °C – 10 sec
    55 °C – 30 sec
    72 °C – 1 min
    72 °C – 5 min
    x12
    4 °C – hold

Clean-up (pooled):

25. Leave Ampure XP beads at **RT**.
26. Pool 2 µL of each library into a 1.5 mL centrifuge tube.
27. Vortex beads to mix well. Then, add x µL (1:0.9 ratio) beads to the pooled sample, mix thoroughly by pipetting 10 times.
28. Leave (caps on) at **RT** for **8 min**.
29. Place undisturbed on magnet stand for **5 min**.
30. Carefully remove the liquid with the tubes still on the magnet stand.
31. Add 1 mL of 75% EtOH with tubes still on the magnet. Incubate for **30 s** at **RT**. Remove ethanol.
32. Again-Add 100 µL of 80% EtOH with tubes still on the magnet. Incubate for **30 s** at **RT**. Remove ethanol.
33. Remove as much EtOH as possible this time using a P20 pipette.
    Let the beads dry completely- turn the hood blower OFF. Leave at **RT** on the magnet stand for **10 min**. It's dry enough if you start seeing cracks. *While the beads dry, prepare fresh PCR tubes for step below.*

34. Add 50 µL nuclease-free water. Take the tubes off the magnet, mix 10 times to resuspend fully and leave at **RT** for **5 min**.
35. Place the tubes back on the magnet stand. Leave it for **5 min** or until solution appears clear.
36. Remove exactly 48 µL of the supernatant without disturbing the beads and transfer it to fresh polyallomer tubes.

    Expected concentration: 10-30 ng/µL with size distribution of 300-700 bp

Library enrichment and barcode addition:

2. 5 µL TD Buffer
1.25 µL tagment DNA enzyme Mix
1.25 µL DNA

5 µL Total

Incubate at **55°C** for **10 min**

Add 1.25 µL NT Buffer
Keep at RT for **5 min**.

Add 3.75 µL Nextera PCR Mastermix
2.5 µL Barcode mix each
Mix, centrifuge
Amplify

Program 5:

4 °C – hold
72 °C – 3 min, 95 °C —30 sec
95 °C – 10 sec
55 °C – 30 sec
72 °C – 1 min
72 °C – 5 min
x12
4 °C – hold