

I. Introduction

This demonstrated protocol provides instructions for the use of Trekker® Single-Cell Spatial Mapping Kits with the Singulator platform from Precision Cell Systems for nuclei isolation. Follow the appropriate Trekker protocol from the list below, with the modifications described in this protocol-at-glance:

- [Trekker U Single-Cell Spatial Mapping Kit User Manual](#)
- [Trekker Q Single-Cell Spatial Mapping Kit User Manual](#)
- [Trekker 5C Single-Cell Spatial Mapping Kit User Manual](#)

II. Required Materials

This protocol applies to the following Takara Bio products:

- Trekker U 10x10 Bundle (Cat. No. SK017)
- Trekker Q 10x10 Bundle (Cat. No. SK027)
- Trekker 5C 10x10 Bundle (Cat. No. SK014)

Additional Materials Required (Not Provided):

- NIC+ Cartridge (Precision Cell Systems, Cat. No. 100-215-389)
- Singulator platform
 - Singulator 100 (Precision Cell Systems, Cat. No. 100-067-764) or
 - Singulator 200 (Precision Cell Systems, Cat. No. 100-243-621) or
 - Singulator 200+ (Precision Cell Systems, Cat. No. 100-320-689)

III. Protocol Overview

Integrating two protocols

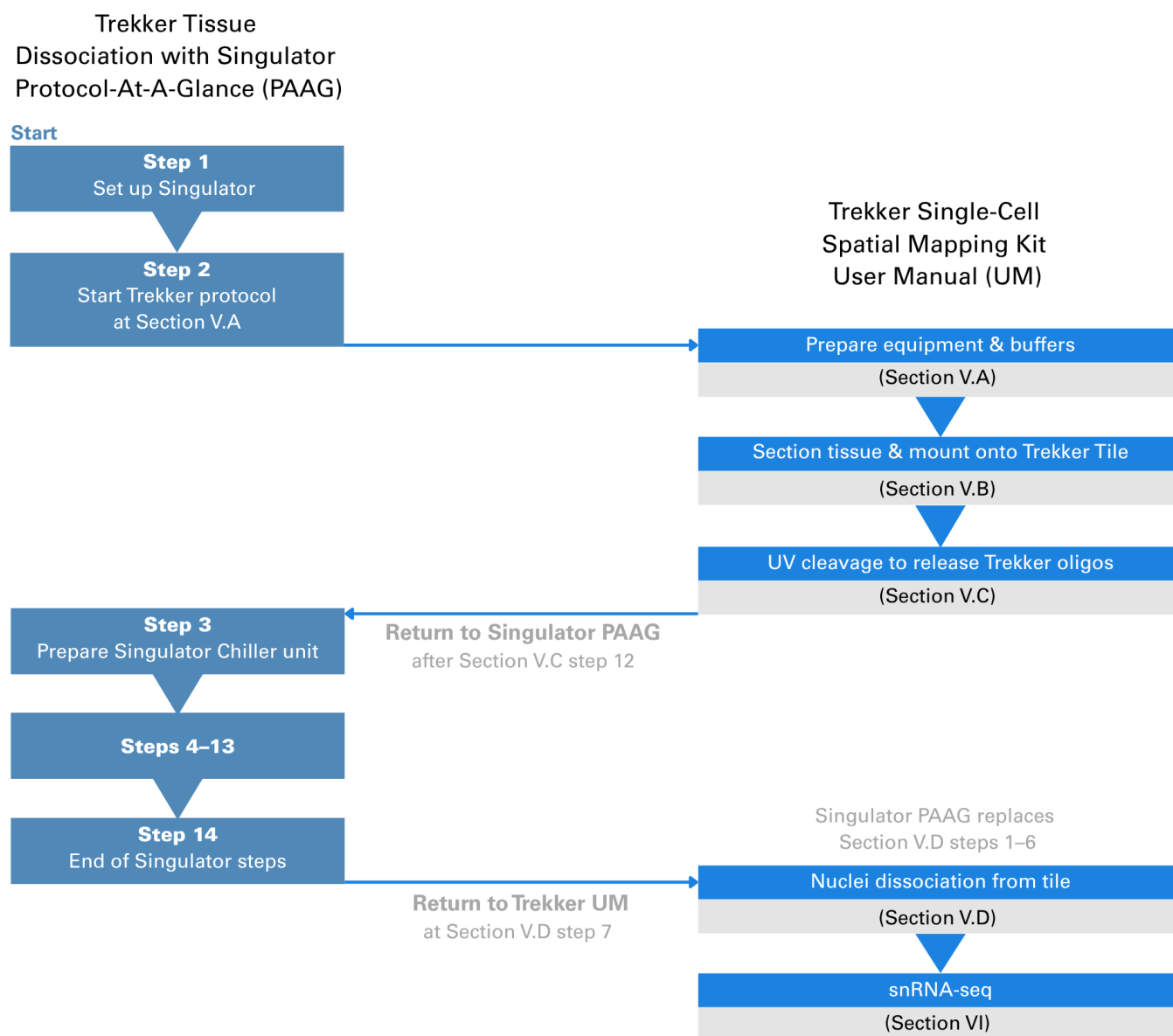


Figure 1. Schematic of how this Singulator Protocol-At-A-Glance integrates with standard Trekker protocols.

IV. Protocol

A. Single-Nucleus RNA-seq on Singulator

1. Singulator setup
 - a. Place the NIC+ cartridge(s) in a –20°C freezer overnight or for at least 20 minutes before use.
 - b. Turn on the Singulator by pressing the power button on the top of the tablet interface.
 - c. Pre-cool the Singulator by sliding the toggle in the upper right of the **User Home Screen** to **Cool** and tap the icon to initiate pre-cooling. The bar will turn orange indicating pre-cooling is in progress, and will turn green and display **On** upon completion.
 - d. Refer to the [Frozen Tissue Nuclei Isolation](#) protocol from Precision Cell Systems for additional details on instrument setup.
2. Follow the standard Trekker protocol through the end of Section V.C. “Protocol: UV Cleavage and Tissue Dissociation from Tile”.
3. Add 3 mL of Buffer B from Section V.A step 9 to the Singulator Chiller unit.
4. Remove the NIC+ cartridge from the –20°C freezer and remove the cap from the cartridge.
5. Use a P1000 pipette to transfer the contents of the well containing dissociated tissue from Section V.C step 12 to the Dissociation Chamber of the NIC+ cartridge and replace the cap.
6. Lift the door of the Singulator open and slide out the Cartridge Tray while lifting the red knob.
7. Place the NIC+ cartridge on the Cartridge Tray and turn the white cartridge lock counterclockwise to lock the cartridge in place.
8. Slide in the Cartridge Tray by pushing the back of the tray until the red knob fully drops into place.



IMPORTANT: Do not use the red knob to push the cartridge tray.

9. Close the door of the Singulator.
10. Select the desired protocol.
 - a. Select **Run a Protocol** from the **User Home Screen**.
 - b. Select the **Nuclei** button to toggle to nuclei protocols.
 - c. Select **FFPE Nuclei Isolation**.
 - d. Select **Next**.
 - e. On the Run Notes Screen, add notes if desired to be saved in the internal log files, then select **Next**.
11. Select **Run**—the nuclei isolation takes approximately 17 minutes.
12. After completion of the run, the instrument will display a Run Complete Screen. Lift the door then lift the red knob up, slide the Cartridge Tray and cartridge out of the instrument. Close the door. Rotate the cartridge lock clockwise and remove the cartridge from the Cartridge Tray.
13. Pierce the foil seal of the Output Chamber with a 1 mL pipette and transfer the sample into two cold 1.5 mL microfuge tubes. Tap the cartridge on the benchtop to get any remaining sample out of the filter unit.
14. Proceed to Section V.D step 7 and follow the standard Trekker protocol for the remaining steps.

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This document has been reviewed and approved by the Quality Department.