

Video Article

Erratum: Isolation of Adult Spinal Cord Nuclei for Massively Parallel Single-nucleus RNA Sequencing

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Abstract

An erratum was issued for: *Isolation of Adult Spinal Cord Nuclei for Massively Parallel Single-nucleus RNA Sequencing*. The Protocol section was updated.

Step 3.1 was updated from:

Place the lumbar spinal cord in a pre-chilled Dounce homogenizer and add 500 mL pre-chilled detergent lysis buffer.

to:

Place the lumbar spinal cord in a pre-chilled Dounce homogenizer and add 500 μ L pre-chilled detergent lysis buffer.

Step 3.6 was updated from:

Pass an additional 1 mL low sucrose buffer over the 40 mm strainer, bringing the final volume to 3 mL of the low sucrose buffer and 500 mL of the lysis buffer.

to:

Pass an additional 1 mL low sucrose buffer over the 40 mm strainer, bringing the final volume to 3 mL of the low sucrose buffer and 500 μ L of the lysis buffer.

Step 5.5 was updated from:

Once the centrifugation is complete, immediately decant the supernatant in a flicking motion.

NOTE: A residual volume (less than 400 mL) of sucrose buffer can be discarded if desired to produce a lower volume and cleaner final sample, but this residual volume does contain nuclei and can be preserved to maximize nuclei yield

to:

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NOTE: A residual volume (less than 400 μ L) of sucrose buffer can be discarded if desired to produce a lower volume and cleaner final sample, but this residual volume does contain nuclei and can be preserved to maximize nuclei yield

Step 5.6 was updated from:

Using 100 mL - 1 mL of resuspension solution, resuspend the nuclei remaining on the wall. Avoid the myelin 'frown' that remains with the detergent-based preparation.

to:

Using 100 μ L - 1 mL of resuspension solution, resuspend the nuclei remaining on the wall. Avoid the myelin 'frown' that remains with the detergent-based preparation.

Steps 6.1.1 - 6.1.4 were updated from:

1. Adjust nuclei to a final concentration of 225 nuclei per mL.
2. Prepare barcoded beads at a concentration of 250 beads per mL.
3. Prepare the lysis buffer with 0.7% sarkosyl.
4. Adjust the flow rates to 35 mL per min for beads, 35 mL per min for nuclei, and 200 mL per min for oil.

to:

1. Adjust nuclei to a final concentration of 225 nuclei per μ L.
2. Prepare barcoded beads at a concentration of 250 beads per μ L.
3. Prepare the lysis buffer with 0.7% sarkosyl.

4. Adjust the flow rates to 35 μL per min for beads, 35 μL per min for nuclei, and 200 μL per min for oil.

Protocol

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Disclosures

No conflicts of interest declared.