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Nuclei counting after Trypan Blue staining

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We use this protocol and it's working

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Abstract

Nuclei counting after Trypan Blue staining

Guidelines

Be wary of nuclei integrity when counting for snRNA-seq. Some minor blebbing of nuclei can still be considered acceptable, but broken up nuclei are considered debris and should not be counted, as they have lost single-cell characteristics.
Trypan Blue also stains debris, so for counting it is important to have a clean nuclei suspension.

Materials

Nuclei suspension
Hemocytometer
Trypan Blue 0.4%
Microscope
Tally counter
0.2 ml tube

1 Prepare nuclei suspension from samples.

2 In order to increase accuracy, prepare duplicates or triplicates per sample.

3 Add 9 µl of 0.4% Trypan Blue to a 0.2 ml tube

4 Add 1 µl of nuclei suspension to the Trypan Blue solution. Do not dispense to the walls of the tube.

5 Mix slowly by pipetting up and down 10 times. If the nuclei preparation is prone to clumping or losing integrity (breakage and release of chromatin), use a 20 µL pipette and further reduce mixing speed.

6 Fill the counting chamber. Observe at 20X

7 Calculate the average concentration obtained across duplicates/triplicates of the sample