Sample Preparation Guideline for 10x Chromium Single Cell 3' Reagent Kits v3

Cell Line Preparation for Single Cell Sequencing

This protocol is intended to provide BGI customers a guideline to prepare frozen tissue for Single Cell Sequencing.

Cell line sample preparation

We recommend the preparation steps to be performed in the sterile condition.
Before start, please prepare the following items and reagents first:

<table>
<thead>
<tr>
<th>Check</th>
<th>Item/reagents</th>
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<tbody>
<tr>
<td></td>
<td>Prechilled sterilized Phosphate buffered saline (PBS) (pH 7.4)</td>
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<td></td>
<td>Sterilized 15mL centrifuge tube</td>
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<td>1mL pipette</td>
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<td>200μL pipette</td>
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<td>Desktop refrigerated centrifuge</td>
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<td>Fetal bovine serum (FBS), dimethyl sulfoxide (DMSO)</td>
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<td></td>
<td>2mL cryotube</td>
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<td>Cell Freezing Container</td>
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1. Digest the cells into single cell solution according to the customer’s experience.
2. Transfer cell suspension to a 15mL centrifuge tube, spin down at 500X g in 4°C for 5 minutes.
3. Check the suspension in the tube, if the suspension appears to be cloudy, then repeat the centrifuge.
4. Remove and discard the supernatant. Do not disturb cell pellets at the bottom of the tube.
5. Wash the cell pellets one more time by sterilized PBS and spin down at 500X g in 4°C for 5 minutes.
6. Remove and discard the supernatant. Do not disturb cell pellets at the bottom of the tube.
7. Prepare enough cryopreservation solution, we recommend 10% to 15% DMSO in FBS (85% - 90%). Usually, the volume ratio of cell to cryopreservation solution is 1:9. Mix cryopreservation solution well and put on ice.
8. Slowly resuspend cell pellets in cryopreservation solution at the cell density of 1x10^6 cells/mL.
9. Transfer 1 - 1.5mL cell suspension to a 2mL cryotube.
10. Put the cryotube in a gradient freezing container with isopropyl alcohol at room temperature, move the container to -80°C and keep freezing overnight.
11. After 24 hours, the cryotubes can be shipped to us with dry ice.
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