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

Tissue Sample Preparation for Single Cell Sequencing

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

Tissue sample preparation

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

check	Item/reagents
	Prechilled sterilized Phosphate buffered saline (PBS) (pH 7.4)
	ice cold ice box
	Sterilized ophthalmic scissors or scalpel
	Sterilized 50 mL centrifuge tube
	Sterilized 6 cm petri dish
	1mL wide-bore pipette tips
	1mL pipette
	200µL pipette
	Desktop refrigerated centrifuge
	Fetal bovine serum (FBS), dimethyl sulfoxide (DMSO)
	2mL cryotube
	Cell Freezing Container

1. Once the fresh tissue has been obtained, it should be put in a 50mL centrifuge tube or a petri dish. Add sterilized ice-cold PBS immediately to cover the tissue mass.
2. Leave the tissue sample with container on ice.
3. Use a sterilized ophthalmic scissors or a clean scalpel to cut samples into small pellets at the diameter of 3 - 4 mm. The tissue needs to be immersed in ice-cold PBS solution and the tissue container needs to be on ice through the whole process of this tissue trimmer step. It is also critical that the ophthalmic scissors or scalpel is razor sharp to minimize the squeezing which could damage the cells.
4. Transfer tissue pellets to a 50mL centrifuge tube, spin down at 500X g in 4°C for 5 minutes.
5. Check the suspension in the tube, if the suspension appears to be cloudy, then centrifuge one more time.
6. Remove and discard the supernatant. Do not disturb tissue 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 tissue pellets in cryopreservation solution by wide-bore pipette tips.
9. Transfer 1 - 1.5mL tissue pellets 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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