Freezing and Thawing Cells for Use with the Illumina Bio-Rad Single-Cell Sequencing Solution

Abstract

Maintaining primary cells and cell lines in continuous culture is time-consuming and increases the occurrence of genetic drift, microbial contamination, and cell senescence. These protocols provide instructions for freezing and thawing cells for use with the Illumina Bio-Rad Single-Cell Sequencing Solution.

Freezing Cells

1. Prepare 1 ml of chilled cryopreservation medium for every 5 x 10^6 cell. Use appropriate sample-specific cell propagation medium + 20% FBS +10% DMSO.

2. Place a CoolCell FTS30 or equivalent cell freezing container in a 4°C refrigerator.

   Note: Proceed to Step 6 if you are working with non-adherent cells.

3. Remove medium from cultured cells and rinse culture vessels with 1X PBS.

4. Detach cells by incubating them for 2–3 min in 2.5 ml of TrypLE Express Enzyme per T-75 Flask.

   Note: It is best to use cells that have been recently thawed, that are within the first five passages, and are in the log phase of growth (70–90% confluency).

5. Add 7.5 ml culture media to inactive TrypLE Express Enzyme.

6. Collect cells in Falcon Tubes and count them using the Bio-Rad TC20™ Cell Counter. Make note of the cell count and viability. Recommended viability is at least 95%. Lower viability will result in background noise.

7. Centrifuge cells at 300 rcf for 3 min and resuspend cell pellets in growth medium by pipetting up and down 10 times.

   Note: Concentrate to the desired number of cells. If the concentration is too high to achieve an accurate cell count, further dilute the cells with growth medium, thoroughly mix the resulting cell suspension, and recount.

8. Repeat the count for a total of four counts to ensure an accurate cell count. Make note of the total count and viability each time and use the average of the four counts as the final count or concentration.

9. Based on total volume (V) and concentration (C), calculate total cell number (N = C x V)

10. Calculate the total volume of cells required to achieve the desired concentration.

11. Centrifuge cells at 300 rcf for 5 min.

12. Resuspend the pellet in an appropriate volume of cryopreservation medium (prepared in Step 1) to achieve the required cell concentration in each cryovial.

13. Dispense 1-ml aliquots of cells into cryovials. While dispensing, make sure that the cells are thoroughly resuspended and that no cells settle to the bottom.

14. Remove the chilled CoolCell FTS30 Cooling Unit from the 4°C refrigerator and place the cryovials in the cooling unit.

15. Place the chilled CoolCell FTS30 Unit in the –80°C freezer for at least 4 hr. Ensure that the bottom and top vents of the cooling unit are not obstructed to allow for adequate air flow.

16. After 4 hr or overnight storage, transfer the cryovials to liquid nitrogen for long-term storage.

Thawing Cells

1. Set a water bath to 37°C and ensure that it has reached the desired temperature before starting this protocol.
2. Prepare 1X PBS with 0.1% BSA (1 mg/ml) for the final resuspension. Keep chilled.
3. Remove a single cryovial of frozen cells from liquid nitrogen.

   Note: Wear cryogloves, safety glasses, a lab coat, and closed-toe shoes and carefully follow all instructions and precautions while handling liquid nitrogen.
4. Place the cryovial in the 37°C water bath and let it thaw for no more than 1–3 min. Remove vial from the water bath when the cells are almost completely thawed and only a tiny ice crystal remains in the tube.

   Note: Do not leave the vial in the water bath for more than 5 min.
5. Mix the cells by gently pipetting 10 times and transfer the entire volume to a 1.5-ml microcentrifuge tube.
6. Centrifuge cells at 200 rcf for 3 min.
7. Carefully remove the supernatant without disturbing the pellet.
8. Add 1 ml of cold 1X PBS with 0.1% BSA (created in Step 2 of this protocol) to the tube and gently pipet 5 times to slowly dislodge and resuspend the pellet.
9. Centrifuge cells at 200 rcf for 3 min.
10. Carefully remove supernatant without disturbing the pellet.
11. Repeat steps 8–10 for a total of two washes.
12. Resuspend pellet in 1 ml of 1X PBS with 0.1% BSA (PBS/BSA). If there are too few cells, resuspend in 500 µl of 1X PBS/BSA.
13. Carefully filter the cells by passing them through a 5-ml, 35-µm Falcon Tube or general cell strainer on ice.
14. Pulse vortex the cells for 1 sec three times.
15. Mix 10 µl of vortexed cells with 10 µl of 0.4% trypan blue and determine the cell count using a TC20 Cell Counter. Make note of the count and viability and ensure that viability is >95%.

    Note: To ensure even sampling of the cell mixture, aspirate 10 µl of cells from the middle of the tube within 5 seconds of vortexing before counting on the TC20 Cell Counter.
16. Repeat steps 14 and 15 for a total of four counts. Take an average of the four viable cell counts and use this number to dilute the cells to the desired cell concentration in 1X PBS/BSA.

    Note: For repeat counts the addition of trypan blue is optional.
17. Determine and record the final cell count and viability. Take two readings and use the average. If the final count is not within ±10% of the target concentration, adjust accordingly. The final viability should be >95%.

The single cell suspension can remain on ice for up to 1 hour before loading on the ddSEQ™ Single-Cell Isolator.

Bovine serum albumin, BSA; Dulbecco's Modified Eagle Medium, DMEM; dimethyl sulfoxide, DMSO; fetal bovine serum, FBS; phosphate buffered saline, PBS; relative centrifugal force, rcf

For Research Use Only. Not for use in diagnostic procedures.

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