**SOP Preparing Frozen Cell Pellets Recommended (Generation 2)**

**Preparing Frozen Cell Pellets Recommended Buffer**

1. Prepare Stabilizer Buffer by combining 980 μl of Bionano Cell Buffer + 20 μl Bionano DNA Stabilizer for each of the pellets you plan to prepare. These reagents can be directly purchased from Bionano. Please, contact Manasi Pimpley and Amy Dieterle from Bionano Genomics for a quote.

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**PREPARING FROZEN CELL PELLETS PROCEDURE**

1. Count Cells in Stock Cell Culture
   1. Resuspend stock cell culture to create a uniform cell suspension for counting.
   2. Count number of viable cells with a cell counting device.

**Note:** Cells should be in log phase with high percent cell viability (≥ 70%) as this maximizes quality and size of isolated gDNA. Record number/percent of viable cells.

* 1. Calculate the volume of original stock cell culture required for up to twelve cell pellets, each containing 1.5E+06 viable cells. If the viable cell density is < 1.25E+06 viable cells/ml, proceed to Step 2. If viable cell density is ≥ 1.25E+06 viable cells/ml, proceed to Step 3.

1. Concentrate Cells (if cell concentration is low)
   1. Transfer appropriate volume of stock cell culture into a 15 ml conical tube.
   2. Centrifuge 15 ml conical tube at room temperature at 500 x g for five minutes in a swinging bucket rotor to pellet cells.
   3. Remove supernatant and resuspend cells with a smaller volume of growth media to obtain a live cell concentration of at least 1.25E+06 live cells/ml.
   4. Count number of viable cells with a cell counting device.
   5. Calculate concentrated stock cell culture volume to yield 1.5E+06 viable cells per pellet.
2. Aliquot Cells
   1. Pipette mix stock cell culture suspension to ensure homogenous cell suspension.
   2. Aliquot the target cell volume of stock cell culture suspension into each pre-labeled, pre-chilled 1.5 ml Protein LoBind tube. Place on ice.
3. Pellet Cells
   1. Centrifuge cells at 4°C at 500 x g for five minutes in a fixed angle rotor microcentrifuge.
   2. Remove entire supernatant without disturbing the pellet. Discard supernatant in the 50 ml conical tube containing bleach. Place sample on ice.
4. Wash Cells with Cold Stabilizing Buffer
   1. Add 1 ml of cold Stabilizing Buffer to each pellet.
   2. Resuspend pellet by pipetting up and down three times with a P1000 pipette set to 1,000 μl.
   3. Centrifuge the cells at 4°C at 2,200 x g for two minutes in a fixed angle rotor microcentrifuge.
   4. After centrifugation, place samples on ice.
   5. Aspirate entire supernatant and discard into the 50 ml conical tube containing bleach. Use a P200 pipette to remove residual liquid from cell pellet.
   6. Keep samples on ice until all supernatants have been removed.

6. Freeze Cell Pellets on Ice

1. Place cell pellets on dry ice and incubate for five minutes to snap-freeze.
2. Transfer snap-frozen cell pellets to pre-labeled, pre-chilled (-80°C) cryobox.

**Note:** Frozen cell pellets can be used for gDNA isolation the next day.