Freezing 96-well master plates

Materials:

2X Freezing media = 80% FBS + 20% tissue-culture grade DMSO

Methods:

1. Change media 2-4 hours prior to freezing. Cells must be 80% confluent and healthy.
2. Prepare 2X freezing media and pre-chill on ice.
3. When cells are ready to be frozen, aspirate off media and wash with 150ul pre-warmed PBS. Add PBS gently to avoid disrupting the cells.
4. After aspirating PBS, add 30ul of pre-warmed trypsin. Be sure to cover the entire plate by carefully swirling it.
5. Incubate in a 37C incubator for 5 minutes and check under the microscope. If you see big clumps of cells, incubate additional 2-5 minutes. Do not over-trypsinize.
6. While cells are incubating, place an ice bucket inside the hood to chill the plates. Starting this step, the plates need to be on the ice bucket.
7. Once majority of the cells are uniformly dispersed into small clumps or single cells, inactivate trypsin by adding 70ul of medium. Gently pipette up and down using P200 Rainin multichannel pipette 10-15 times without making bubbles.
8. Add 100ul of freezing media, bringing up the total volume to 200ul per well. The final concentration of DMSO must be 10%. Gently pipette up and down to mix well, as DMSO will sink to the bottom.
9. Once the plates are ready to be frozen, securely tape the plates by wrapping it around the entire plate once. At -80C many commercial tapes lose their stickiness. Clearly label the date and name.
10. Carefully place the plates into the Styrofoam box and close it. Frost will form around the plate if the box is missing the top, increasing the chance of contamination.
11. Place the box inside the -80C freezer to store.