

**Freezing ES cells**

## 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 label cryovials with date/name/number of cells. Pre-chill on ice.
3. When cells are ready to be frozen, aspirate off old media and wash with 10ml pre-warmed PBS. Add PBS gently to the side of the plate to avoid disrupting the cells.
4. After aspirating off PBS, add 2ml 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, add 5ml of warmed medium into 15ml Falcon tube.
7. Once majority of the cells are uniformly dispersed into small clumps or single cells, inactivate trypsin by adding 5ml of medium. Gently pipette up and down using 10ml pipette 10-15 times without making bubbles.
8. Transfer the cells into the 15ml Falcon tube and take a small amount to count the number of cells.
9. Spin down the cells by centrifuging for 5 minutes at 1000rpm, and meanwhile count the cells using hemocytometer. Aspirate off the supernatant and resuspend the pellet in pre-chilled medium.
10. Add equal volume of freezing media slowly while swirling. The final concentration of DMSO must be 10%.
11. Aliquot 0.5-1ml into each cryovial. 2-4 million cells/vial can be revived to a 10cm plate.