FREEZING CELLS

Materials:

Fetal Bovine
Sera
DMSO (Sigma D-5879)
1.8ml cryovials (Nunc/Nalge 368 632)
1xPBS
media/10%FBS
1xtrypsin

Freezing Solution

90% FBS
10% DMSO

Procedure:

1) Grow cells until ~80% confluency.

80% confluency	amount of freezing solution	number of cryovials
25 cc flask	1 ml	2
100mm plate	1 ml	2
150mm plate	2ml	4

2) Wash cells with 1xPBS, trypsinize (if necessary), then add 10ml fresh media/10%FBS and transfer to a 15ml conical tube.
3) Spin cells 2000rpm 2 min.
4) Label cryovial with:
    - cell line name
    - date
    - your initials
5) Discard media and resuspend cell pellet in freezing solution (mix well).
6) Aliquot 0.5ml into the labeled cryovials.
7) Cells need to freeze slowly. Place vials in the top chamber rack of the blue liquid nitrogen tank **overnight** to freeze. Vials will be above the level of the liquid nitrogen and will freeze gradually.
8) Put the frozen vials into a storage box in one of the tanks.
9) Fill in the information in the logbook and database record sheet.