Inactivated primary MEFs are used to promote growth and prevent differentiation of embryonic stem cells. MEFs can be inactivated either by chemical treatment or irradiation. When access to an irradiator is limited, treatment with mitomycin C is recommended.

Care must be taken when handling mitomycin C as it is genotoxic. Wear gloves, sleeve, and a lab coat when working with the substance. Solutions containing this chemical should be disposed of according to the MSDS.

1. Reconstitute mitomycin C (Calbiochem #475820) to make 1 mg/mL mitomycin C solution. Mix thoroughly.
2. Add the necessary amount of 1 mg/mL mitomycin C solution to flasks containing 80–90% confluent fibroblasts and MEF culture medium to achieve a 10 ug/ml final concentration (i.e., 400 µl into 40 mL).
3. Incubate the flasks for 2.5–3 hrs at 37°C in humidified incubator with 5% CO2.
4. Carefully remove culture medium containing mitomycin C and store in a designated waste bottle for proper disposal.
5. Wash each flask with 10 mL PBS. Remove the wash and add it to the mitomycin C waste bottle.
6. Wash again with 10 mL PBS.
7. Add appropriate amount of 0.25% trypsin-EDTA to the flask and incubate for 1 min. in a humidified, 37°C incubator with 5% carbon dioxide.
8. While tapping the flask, observe the cells under an inverted microscope until cells detach (1.5–2 min.).
9. Add equal amount of culture medium to inactivate the trypsin and rinse surface of the flask to detach all cells.
   Gently pipetting up and down will break cell clumps.
10. Transfer the cell suspension into a centrifuge bottle or tube. Wash the flask with medium.
11. Centrifuge at 270 x g for 5 min. Discard the supernatant.
12. Resuspend the pellet in the culture medium.
13. Perform cell count and dilute the cell suspension to twice the final concentration. The final concentration can be up to 12 x 10^6 cells/vial.
14. Add an equal volume of cold 2X freezing medium to the cell suspension.
15. Aliquot 1 mL of the suspension into each cryovial.
16. Place the vials in an appropriate insulated freezing container and place in a -80°C freezer overnight. The next day transfer the vials into liquid nitrogen.

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2X Freezing Medium
20% DMSO  (Mediatech 25-950-CQC)
80% FBS    (AMSBIO GSM-6001)

MEF Culture Medium
DMEM          (Mediatech 15-013-CV)
15% FBS       (AMSBIO GSM-6001)