PROCEDURE FOR MICROMASS CELL CULTURE
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Using nearly confluent C3H10T1/2 cells from 4x T-75 flasks.

Cell Passage

1. Remove the medium.
2. Add 3ml trypsin to T-75 flask.
3. Place in incubator for 1 minute. (Time may vary for other cell lines 1-5 min.)
4. Shake the flask and add 9ml complete BME medium to T-75 flask.
5. Mix cell resuspension well by pipetting.
6. Transfer total volume from all T-75 flasks into a 50ml conical flask (for example 4 flasks x 12ml = 48ml).
7. Pass desired volume of mixture into flaks for maintenance of cell line (for example 5ml into 2 T-75 flasks) and add complete BME medium to these flasks (about 15ml each).

Micromass Cell Culture Plating

8. Spin down remaining volume (~38ml) in the 50ml conical flask at ½ speed for 5 minutes in the centrifuge.
9. Remove supernatant being careful not to disturb cells.
10. Resuspend pellet of cells with desired volume of complete BME medium to have adequate volume for spotting (for example 8ml).
11. Vortex resuspended cells briefly (about 5 seconds) to ensure mixing.
12. Spot 75µl of resuspended mix, using a pipette, onto center of wells for a 24-well plate (Sufficient volume for ~106 wells).
13. Place plates in incubator (37ºC, 5%CO_2) for 4 hours to allow cells to attach.
14. Add complete BME medium SLOWLY using transferring pipette to cover the bottoms surface of well and spotted cells (~0.5ml for wells of 24-well plate).