Video Article

From MEFs to Matrigel I: Passaging hESCs in the Presence of MEFs

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Abstract

This video demonstrates how to grow human embryonic stem cells (hESCs) on mouse embryonic fibroblast (MEF) feeder cells.

Video Link

The video component of this article can be found at https://www.jove.com/video/722/

Protocol

Splitting human embryonic stem cells (hESCs) plated on mouse embryonic fibroblasts (MEFs)

Usually a confluent hESC plate can be split 1:6 to 1:10, depending on the particular hESC line. The split plate will become confluent again 5-7 days after splitting.

1. Two days before splitting, gelatinize plates using a 0.1% gelatin solution. For a 6-well plate, add 2ml into each well and incubate plate in a 37°C, 5% CO_2 tissue culture incubator overnight.
2. Plate MEFs on gelatinized 6-well plates the day before you plan on splitting the hESCs. (NOTE: MEFs can be plated as many as 3 days earlier if needed. After ~ 3 days, MEFs become flatter and more spread-out and cannot sustain hESCs as well as fresher MEFs can.) Take a vial of g-irradiated or mitomycin C treated CF1 MEFs, containing 5-6 x 10^6 cells, from liquid nitrogen and thaw for 2 min in a 37°C water bath. Wash cells once with warm MEF Culture Media in a 50ml falcon tube and resuspend in the final volume of warm MEF Culture Media. At 5-6 x 10^6 cells, this is enough to plate two or three 6-well plates.
3. While the MEFs are being washed, take out the gelatinized plates, remove all solution from the wells and add 2.5 ml of MEF Culture Media per well.
4. Add 0.5 ml of MEF suspension per well to achieve 3ml as a final volume in each well. Make sure MEFs are evenly dispersed and place plates back in the incubator overnight to settle.
5. On the day of hESC splitting, prepare fresh or use < 2 week-old sterile collagenase IV solution at 1mg/ml. Remove all the media from the hESC wells you want to split, wash once with 2ml per well of warm 1×PBS, pH 7.4, and add 1ml of collagenase IV solution. Incubate at 37°C for 5-10 min. Take the 6-well hESC plate out of the incubator and add 1ml of ES media (without bFGF) to each well. Using the solution in each well, with a 1ml pipette suck up the media and blow the stem cells off of the plate. For each well this will take about 5 to 10 repetitions. Then, transfer the suspended hESCs into a 50ml falcon tube. Do this for all the wells being split and combine in one 50ml falcon tube. Pellet the hESCs at room temperature at 200g for 5 min and wash one time with ES media lacking bFGF.
6. While the hESCs are being washed, take out the MEF plates, remove all media from the wells and wash once with sterile, warm 1×PBS, pH 7.4 then add 2.5 ml of ES media (now supplemented with 10 ng/ml bFGF) per well.
7. Resuspend the pelleted hESCs in an appropriate volume of ES media, supplemented with 10ng/ml bFGF. (NOTE: When the hESCs are resuspended, they should be of roughly uniform colony size and shape. The resuspension volume depends on the splitting ratio.) Carefully pipette the hESC suspension up and down a few times to make the colonies smaller and more uniform, but not so much that single cells or very small colonies are generated. Add 0.5 ml of hESC suspension per well to the MEF plates to achieve 3 ml as a final volume in each well. Visually check to make sure that the hESCs are distributed evenly before placing the plates back in the incubator overnight to settle. After plating, it will usually take a few days for colonies to take on their characteristic shape and border appearance.

Discussion

This video demonstrates how to grow human embryonic stem cells (hESCs) on mouse embryonic fibroblast (MEF) feeder cells. In the last step before plating, when the hESCs are resuspended, they should be of roughly uniform colony size and shape. Carefully pipette the hESC suspension up and down a few times to make the colonies smaller and more uniform, but not so much that single cells or very small colonies

are generated.Immunofluorescence staining and microscopy or flow cytometry for hESC pluripotency markers, such as Oct-4 and SSEA-4, are needed to confirm maintenance of hESCs in an undifferentiated state during culture.

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