Video Article

Passaging HuES Human Embryonic Stem Cell-lines with Trypsin.

Erin Trish[1], John Dimos[1], Kevin Eggan[1]

[1]Department of Molecular and Cell Biology, Harvard

Correspondence to: Erin Trish at etrish@mcb.harvard.edu, John Dimos at jdimos@fas.harvard.edu, Kevin Eggan at eggan@mcb.harvard.edu

URL: https://www.jove.com/video/49
DOI: doi:10.3791/49

Keywords: Cellular Biology, Issue 1, embryonic stem cells, ES, tissue culture

Date Published: 10/12/2006

Citation: Trish, E., Dimos, J., Eggan, K. Passaging HuES Human Embryonic Stem Cell-lines with Trypsin.. *J. Vis. Exp.* (1), e49, doi:10.3791/49 (2006).

Abstract

In this video we demonstrate how our lab routinely passages HuES human embryonic stem cell lines with trypsin. Human embryonic stem cells are artifacts of cell culture, and tend to acquire karyotypic abnormalities with high population doublings. Proper passaging is essential for maintaining a healthy, undifferentiated, karyotypically normal HuES human embryonic stem cell culture. First, an expanding culture is washed in PBS to remove residual media and cell debris, then cells are overlaid with a minimal volume of warm 0.05% Trypsin-EDTA. Trypsin is left on the cells for up to five minutes, then cells are gently dislodged with a 2mL serological pipette. The cell suspension is collected and mixed with a large volume of HuES media, then cells are collected by gentle centrifugation. The inactivated trypsin media mixture is removed, and cells resuspended in pre-warmed HuES media. An appropriate split ratio is calculated (generally 1:10 to 1:20), and cells re-plated onto a 1-2 day old plate containing a monolayer of irradiated mouse embryonic fibroblast feeder cells. The newly seeded HuES culture plate is left undisturbed for 48 hrs, then media is changed every day thereafter. It is important not to trpsinize down to a single cell suspension, as this increases the risk of introducing karyotypic abnormalities.

Video Link

The video component of this article can be found at https://www.jove.com/video/49/

Protocol

General

We recommend thawing no more than one sample at a given time to ensure easy handling. The handler should take care not to leave cultures at room temperature and low CO2 for long periods of time. All centrifugation of live cells is done at 500-600 x g for 5 min at room temperature.

MEFs (Mouse Embryonic Feeder Cells)

1. Pre-warm MEF media to 37°C. Remove MEF vial from -80°C and immediately submerge the bottom half of tube in a 37°C water bath. It should take about 30-45 seconds before the cells are 80% thawed (small frozen portion left).
2. Quickly bring the tube to the laminar flow hood, spray down with 70% alcohol, and transfer cells to pre-warmed media.
3. Aspirate the gelatin solution from plates prepared earlier.
4. Aliquot 2 ml of MEF solution in a drop wise manner into each of the six wells. Be sure to distribute the MEFs evenly about the well. Date the MEF plate, and place in a 37°C incubator overnight to allow MEFs to attach to plate.
5. After 6 hours MEFs will be attached to the plate. It is best to use MEF plates 24-48 hours after plating. DO NOT use a MEF plate that is over 4 days old.
6. Pre-warm HuES media and 0.05% Trypsin/EDTA to 37°C Under the hood, prelabel one sterile 50 ml conical tube.
7. Carefully aspirate the HuES media from the culture to be split. Gently wash the wells with 2mL of 1X phosphate buffered solution (PBS) per well. Aspirate the PBS. Add 0.75 ml 0.05% Trypsin/EDTA to the well.
8. Replace lid, and observe the cells under 4x magnification. The MEFs surrounding the HuES colonies should begin to retract. When the MEFs are sufficiently rounded and the borders of the HuES colonies are rough, return the plate to the hood. This process should take about 5 mins, absolutely NO longer than 10 mins or your cells will not re-grow.
9. Gently pipette up and down, washing the bottom of the well, until the MEF monolayer has completely detached (monolayer is sticky and may remain in one piece).
10. Transfer the cell suspension to an 50 ml conical tube containing additional HuES media. Spin the tube of cells 500xg for 5 mins, Aspirate off the media, be careful not to disturb the cell pellet.
11. To plate onto a new plate of MEFs, add the appropriately determined volume of cells to additional HuES media, and pipette the solution 5-7 times with an automated pipette.
12. Remove the MEF media from a fresh plate of MEFs.
13. Aliquot the HuES solution drop wise to each well, making sure to distribute the drops evenly about the well. Without shaking the plate, carefully return the cells to a 37°C incubator overnight to let HuES colonies seed.

14. HuES cells coming out of a split should behave similarly to those coming out of a thaw.

Discussion

In this video we demonstrated how we routinely passage HuES human embryonic stem cell lines in our laboratory. Efficient and regular splitting and passaging is essential for maintaining a healthy human embryonic stem cell line. Trypsin mediated passage is a significant advantage of HuES cell lines, however, it is important not to over trypsinize cultures or to have a large proportion of single cells during re-platting.

References

1. Cowan, C.A. et al. Derivation of Embryonic Stem Cells from Human Blastocysts (2004), NEJM 1997; 336(23):1650-1656.

