



PRINCIPE FELIPE
CENTRO DE INVESTIGACION

BANCO NACIONAL DE
LÍNEAS CELULARES
NODO COMUNIDAD VALENCIANA

“MECHANIC PASSAGE OF HUMAN EMBRYONIC STEM CELLS (hESC)”

PROCEDURE

1. Evaluate the plate; size of the colonies, % differentiation and number of colonies. Establish the ratio of splitting.
2. Select irradiated feeders and change the foreskin medium to hESC medium (3 ml). Add bFGF 20ng/ml (final volume of 4ml).
3. Stretch sterile Pasteur glass pipettes under flame to sharp ones to cut the colonies and to handle ones to remove them.
4. Change the media in the plates to be expanded.
5. Cut the colonies with the sharp pipettes making from 3 to 4 parallel and perpendicular cuts.
6. With the handle pipette get up the fragments and left them floating in the medium.
7. Pick up the medium with the fragments of the colonies and distribute it in the new wells.
8. Move the plate inside the incubator to distribute the fragments homogeneously in the well.
9. Evaluate the plate; size of the colonies, % differentiation and number of colonies every 48 hours.