BCRJ Code:	0275
Cell Line:	HFF-1
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Skin, Foreskin
Cell Type:	Fibroblast
Morphology:	Fibroblast
Disease:	Normal
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	newborn /
Applications:	Can be used to produce feeder cells
Biosafety:	1
Addtional Info:	it is not recommended to use them past passage no. 50 (P50). It is recommended that the feeder cells be plated 24 hours before use at 5X104 cells/cm2 in order to obtain a supportive monolayer for stem cell growth.
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose and 15% of fetal bovine serum.

Subculturing:	Cells should be split when they reach confluency. Volumes used in this protocol are for 150 or 225 cm2; proportionally reduce or increase amount of dissociation medium for culture flasks of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum, which contain trypsin inhibitor. Add 5 mL of Trypsin-EDTA (0.25% (w/v) Trypsin-0.53 mM EDTA solution to flask and incubate for 1 minute, gently tapping the flask observe cells under an inverted microscope until cells detach (usually within 1 to 2 minutes). Add 6.0 to 8.0 mL of complete growth medium and rinse surface of the flask to detach all cells. Gently pipetting up and down will break cell clumps. Transfer all cells into a centrifuge bottle or tube and centrifuge at 270 xg for 5 minutes. Remove and discard the supernatant. Add 10 mL complete growth medium to cell pellet and with 10 mL pipette resuspend the cells gently (create a single-cell suspension). Add more complete growth medium to cell suspension as needed to plate cells at approximately 5x106/T225 flask. Place flasks in incubator 37°C with a 5% CO2 in air atmosphere. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.
Subculturing Medium Renewal:	Twice a week or as pH decreases
Subculturing Subcultivation Ratio:	A split ratio of 1:5 to 1:7 is recommended.
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:	SAFETY PRECAUTION: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris. 1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions. 3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes. 4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio). 5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).
References:	Amit M, et al. Human feeder layers for human embryonic stem cells. Biol. Reprod. 68: 2150-2156, 2003; Hovatta O, et al. A culture system using human foreskin fibroblasts as feeder cells allows production of human embryonic stem cells. Hum. Reprod. 18: 1404-1408, 2003; Andrews P, et al. Human embryonic fibroblast feeder cells. International Patent Application WO 03/078611 A1.
Depositors:	Cristina Pacheco Soares
Cellosaurus:	CVCL_3285