BCRJ Code:	0309
Cell Line:	OP9
Species:	Mus musculus
Vulgar Name:	Mouse
Tissue:	Bone Marrow/Stroma
Cell Type:	Embryonic Stem Cells, Macrophage
Morphology:	Fibroblast-Like
Growth Properties:	Adherent
Age/Ethinicity:	newborn /
Derivation:	The OP9 cell line was established from newborn op/op mouse calvaria.
Applications:	OP9 cells can be used to coculture mouse embryonic stem cells (ES cells) to induce the differentiation of embryonic stem (ES) cells into blood cells of erythroid, myeloid, and B cell lineages. Cocultivation with OP9 does not require exogenous growth factors or complex embryoid structures. This system will facilitate the study of molecular mechanisms involved in development and differentiation of hematopoietic cells.
Biosafety:	1
Addtional Info:	The cells do not produce functional macrophage colony-stimulating factor (M-CSF) due to an osteopetrotic mutation in the gene encoding M-CSF. The presence of M-CSF had inhibitory effects on the differentiation of embryonic stem (ES) cells to blood cells other than macrophages
Culture Medium:	Alpha Minimum Essential Medium without ribonucleosides and deoxyribonucleosides and with fetal bovine serum to a final concentration of 20% .

Subculturing:	NOTE: Maintain cultures at a cell concentration between 4 X 10e3 and 1 X 10e4 cells/cm2. Volumes are given for a 75 cm2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Note: Cell density is important. If the subculture ratio is too low, the culture will not reach confluence. However, do not overgrow. Very large cells tend to appear after overgrowth and these cells are a warning sign that the OP9 cells will not support the maintenance of hematopoietic cells. Subculture just before confluence. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum which contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Transfer cell suspension to a centrifuge tube and spin at approximately 125 X g for 5 to 10 minutes. Discard supernatant. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. Population Doubling Time: 26 hrs 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:	Every 2 to 3 days
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:	Nakano T, et al. Generation of lymphohematopoietic cells from embryonic stem cells in culture. Science 265: 1098-1101, 1994.
Depositors:	ANA FLAVIA POPI - ECB
Cellosaurus:	CVCL_4398