U-CH1 and U-CH2 (Chordoma)

Prepared by: Duke/UNC/UTA/EBI ENCODE group

Source of cells: Chordoma Research Foundation (http://www.chordomafoundation.org/research/). For questions email David Alcorta (da51@Duke.edu) or Josh Sommer (Joshua.Sommer@Duke.edu). This cell line was created by Silke Bruederlein (silke.bruederlein@uniklinik-ulm.de) at the University of Ulm, Germany

Lineage of cells: The human chordoma cell line U-CH1 was established from a recurrence of a sacrococcygeal chordoma (Scheil et al., 2001). Chordoma tumors are attributed to neoplastic transformation of notochordal remnants.

Karyotype: der(1)t(1;22), del(4), +del(5), +del(6), +7, del(9), del(10), +der(20)t(10;20), +21

Sex: 46 year old male

Media for growth:

Passaging Cells

1) Coat 75 cm^3 flasks with 3ml 0.1% gelatin (Sigma, G1393) solution, and allow to dry for 30+ minutes. Aspirate remaining solution and allow to dry for 30+ minutes in tissue culture hood at room temperature. Alternatively precoated flasks can be used.
2) Remove cell-containing flasks from incubator, aspirate media, and wash with 10 ml PBS
3) Add 1.5 mL of preheated 1x trypsin/EDTA (Invitrogen/GIBCO 25200-056)
4) Seal flask caps and swirl gently
5) Return to CO2 incubator for 8-10 minutes Remove from incubator and shake vigorously to dislodge cells from flask wall
6) Quickly add 50mL media
7) Pipette out 25mL into each freshly-coated flask (split 1:2)

Notes:

Cells grow relatively slowly – doubling time is approximately 5-7 days. Change media twice per week and split cells every 7 days. U-CH1 is comprised mainly of physaliferrous (bubbly) cells containing various numbers and sizes of vacuoles, however not all cells are physaliferrous. U-CH2 has a lower proportion of physaliferrous cells than U-CH1. Unlike some other cell lines, the presence of vacuoles is a sign of optimal growth, rather than stress. Once cells reach >80% confluence the morphology begins to change: cells loose vacuoles and become smaller. This differentiation is not completely reversible even after splitting to lower confluence. U-CH1 cells tend to be quite large and as a result grow at low density compared to other cell lines. A T-75 flask at 80% confluence will contain between 0.6-0.8 x 10^6 cells. Cells are mostly adherent in a gelatin-coated flask. Without coating they are only slightly adherent and can be grown well in suspension, forming floating clusters.

Identification:

U-CH1 and U-CH2 both express brachyury, CD24 and cytokeratin. These cell lines can be positively identified by genotyping 8 STR markers from the CODIS panel. The STR marker signatures are as follows:

Cell line	CSF1PO	D13S317	D16S539	D5S818	D7S820	THO1	TPOX	vWA
U-CH1	10,11	11,13	12,13	11,12	9,12	7	8,11	17
U-CH2	11,12	11	12	10,11	8,12	6,9.3	8	17

Media

IMDM (Invitrogen 12440)/RPMI 1640(Sigma R8758) four to one ratio - (4:1)
10% FBS (Invitrogen/GIBCO 16000-044)
[optional]100 u/mL penicillin-streptomycin (Invitrogen/GIBCO 15140-122). (Note: treating with antibiotics can mask a mycoplasma contamination so the use of antibiotics is not recommended, however using pen/strep does not affect cell growth or viability.)

Media preparation: 400 ml IMDM, 100ml RPMI, 50ml FBS, 5ml pen-strep

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Reference for cell source:

Stefanie Scheil, Silke Bruderlein, Thomas Liehr, Heike Starke, Jochen Herms, Michael Schulte, and Peter Moller. (2001) Genome-wide Analysis of Sixteen Chordomas by Comparative Genomic Hybridization and Cytogenetics of the First Human Chordoma Cell
Line, U-CH1. *GENES, CHROMOSOMES & CANCER 32:203–211*