Chordoma Foundation

Cell Line Validation

U-CHCF359

Cell morphology, growth and Brachyury expression analysis

February 20, 2020

CHORDOMA FOUNDATION

Validation Report

Cell line: ***U-CHCF359***

Growth conditions

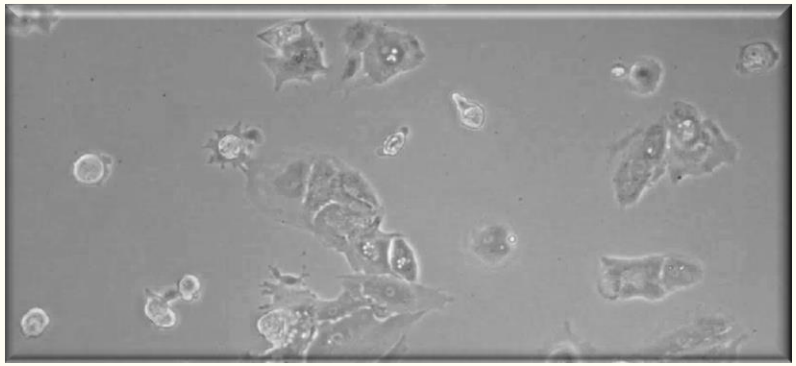
Media: 4:1 IMDM : RPMI1640 +10% FCS, L-glutamine, Pen/Strep

- Change medium every 3-4 days
- Do not passage cells before reaching 80-100% confluency!
- Max. split ratio 1:2
- Cell expansion: 2x 25cm² flasks (80-100% confluent) ➔ 1x 75cm² flask
  2x 75cm² flasks (80-100% confluent) ➔ 1x 175 or 225cm² flask

Percentage viable cells after thawing: >95%

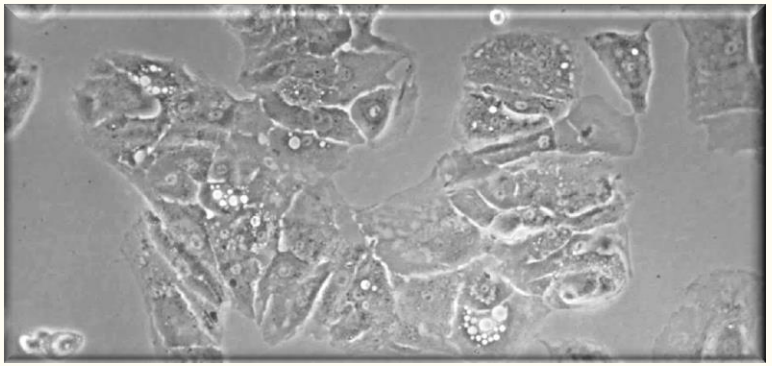
Morphology[1]**:**

partly physaliferous



Cells 24 hours post thawing
(~90% adherent)

physaliferous

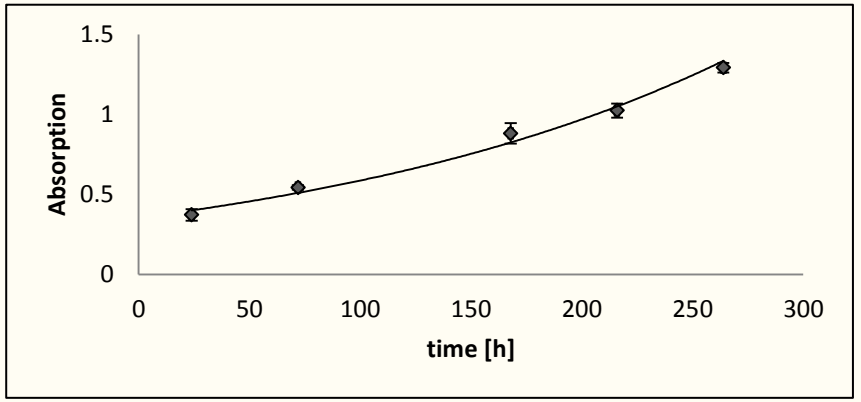


Cells 7 days post thawing

Population doubling time[2]:

5-6 days

(at recommended density of 5000-7500 cells per cm²)

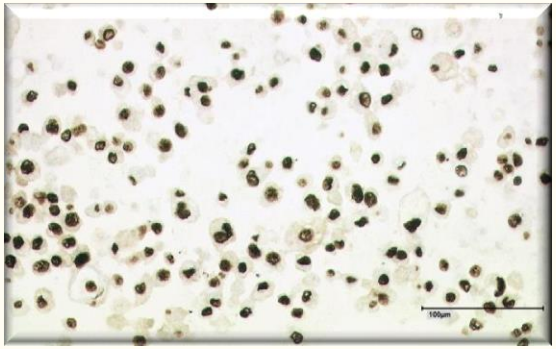


STR profile:

	AMEL	D13S317	D7S820	D16S539	Penta E	THO1	D18S51	D3S1358	D8S1179	TPOX	CSF1PO	Penta D
U-CHCF359	X	9	10 11	11	12	7 9.3	16	15 18	12 15	8 12	11	9 10

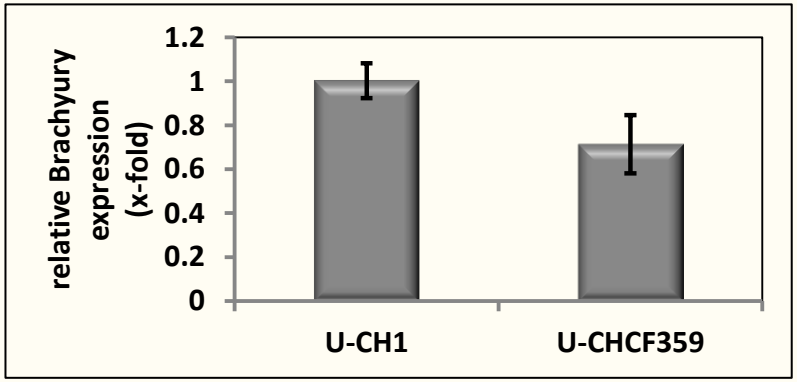
Brachyury expression:

Immunocytology[3]



~100% of the cells show positivity for Brachyury

mRNA levels (qPCR detection[4])



0.7-fold expressed compared to U-CH1

<u>Validation result</u>: The cell line meets the criteria for being a chordoma cell line.

1. Cell morphology was monitored and documented using an invert phase contrast microscope. Typical physaliferous morphology of the cells may vary between different cell lines.

2. Population doubling was estimated by seeding the cells in recommended densities in 96 well plates. Measurement of viable cells was performed using MTS assays at several time points (n=6 per time point).

3. Nuclear positivity for Brachyury was tested using standard immunocytochemistry sections of FFPE cell bocks were used. Stainings were performed using a rabbit monoclonal Brachyury antibody, anti rabbit antibodies and either red or pink or brown dyes.

4. Relative *T* gene expression was calculated using the ΔΔCT method (fold change in relation to *T* expression of U-CH1).