

# **Chordoma Foundation**

## **Cell Line Validation**

### **U-CH18**

Cell morphology, growth and Brachyury  
expression analysis

February 20, 2020

# Validation Report

## Cell line: U-CH18

### Growth conditions

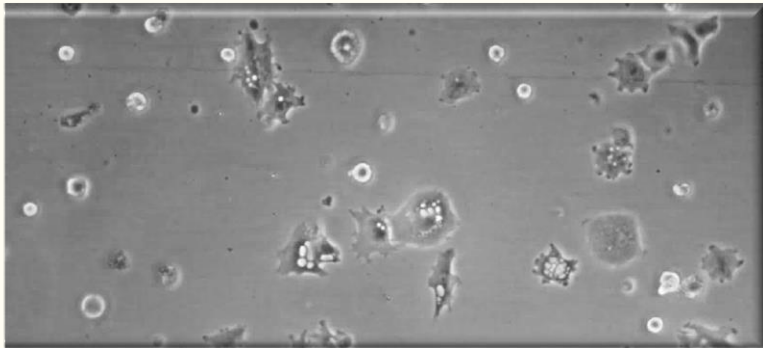
Media: 4:1 IMDM : RPMI1640 +10% FCS, L-glutamine, Pen/Strep

- Change medium every 5-7 days
- Do not passage cells before reaching 90-100% confluency!
- Max. split ratio 1:2 (growing speed is cell density dependent)
- Cell expansion: 2x 25cm<sup>2</sup> flasks (95% confluent) → 1x 75cm<sup>2</sup> flask  
2x 75cm<sup>2</sup> flasks (95% confluent) → 1x 175 or 225cm<sup>2</sup> flask

Percentage viable cells after thawing: >90%

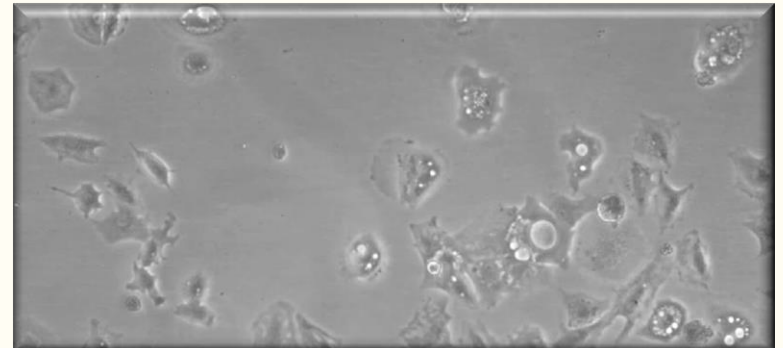
### **Morphology<sup>1</sup>:**

mainly physaliferous



Cells 24 hours post thawing  
(60-70% adherent)

mainly physaliferous

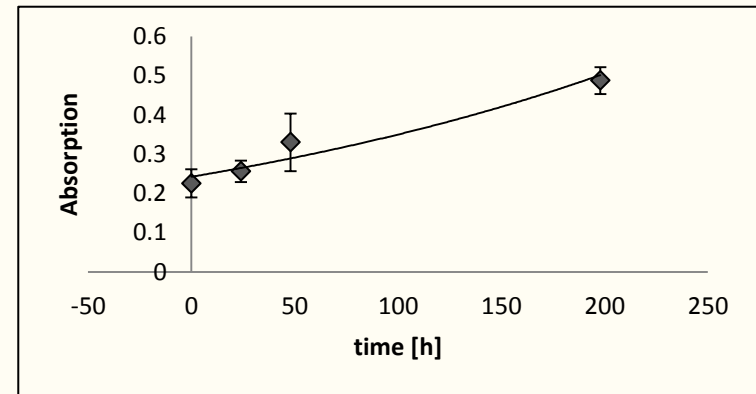


Cells 7 days post thawing

## Population doubling time<sup>2</sup>:

approx. 7-8 days

(at recommended density of 5000-7500 cells per cm<sup>2</sup>)

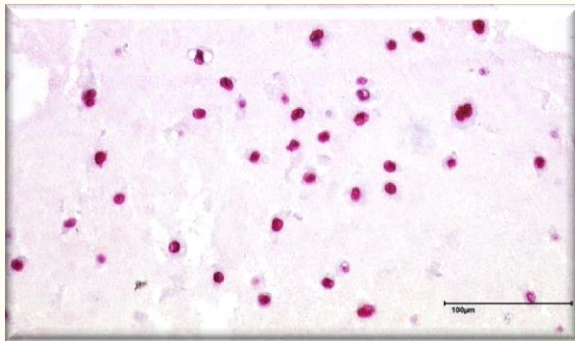


## STR profile:

	AMEL	D13S317	D7S820	D16S539	Penta E	THO1	D18S51	D3S1358	D8S1179	TPOX	CSF1PO	Penta D
U-CH18	X Y	12 13	9 10	10 12	7 13	9.3	17	16	13 14	8 11	11 12	9 13

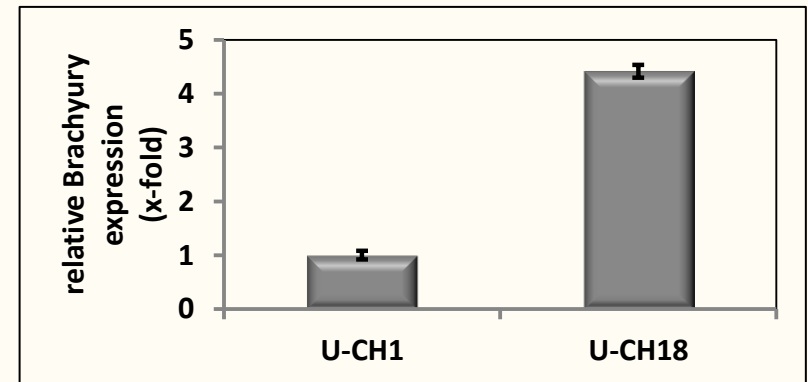
## Brachyury expression:

### Immunocytology<sup>3</sup>



~100% of the cells show positivity for Brachyury

### mRNA levels (qPCR detection<sup>4</sup>)



4.4-fold expressed compared to U-CH1

**Validation result:** 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 blocks 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  $\Delta\Delta CT$  method (fold change in relation to *T* expression of U-CH1).