

# Chordoma Foundation Cell Line Validation

## U-CH12

Cell Line Phenotype and Expression  
Analysis Report

March 3, 2016

# Cell Line Receiving

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Frozen Vials	October 7, 2015	N/A	3	p (0), frozen February 26, 2015	$6.48 \times 10^5$	59.2%

## Growth Conditions

Media:

4:1 IMDM/RPMI + 10% FBS

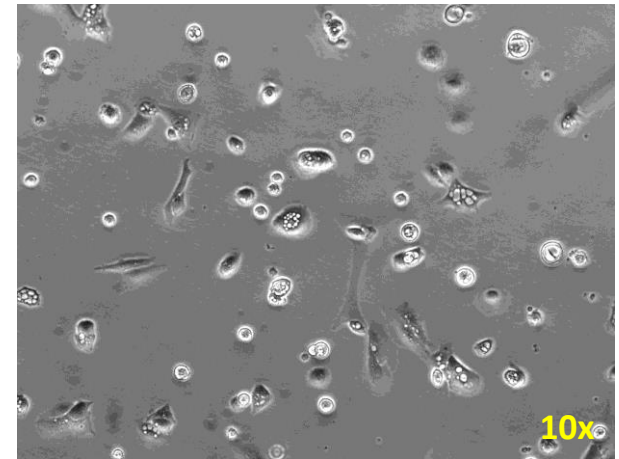
+ l-Glutamine + Pen/Strep +ITS-G

→ Passage when ~80-90% confluent (no more than 1:2)

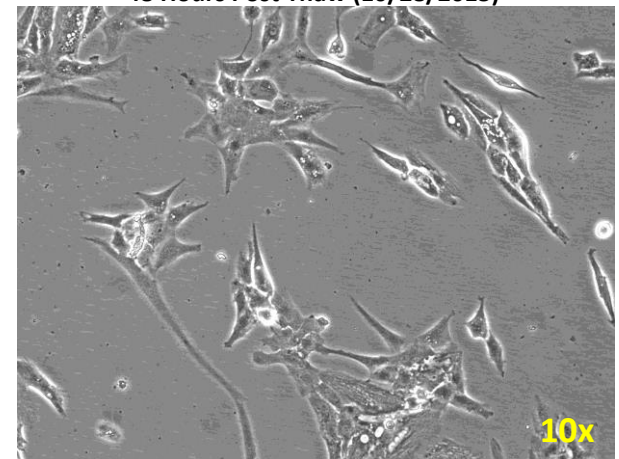
→ Change media every 3-4 days

## Phase Contrast Image Review

Cells were settled but still mostly rounded up post thaw. They flattened out over time and have maintained a flat morphology with subsequent passaging.



48 Hours Post Thaw (10/28/2015)

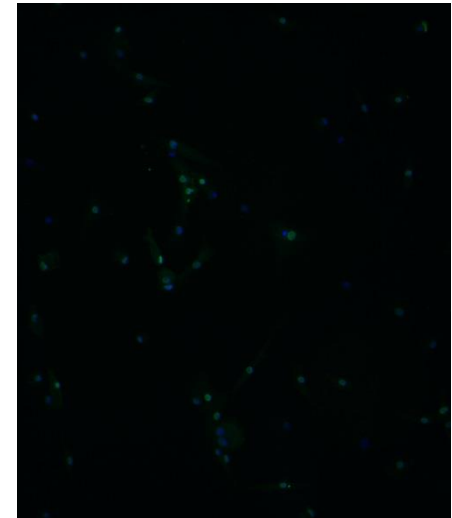
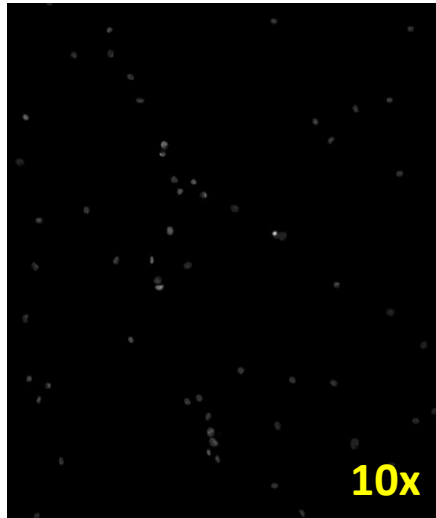


Pre Immunofluorescent Validation (11/11/2015)

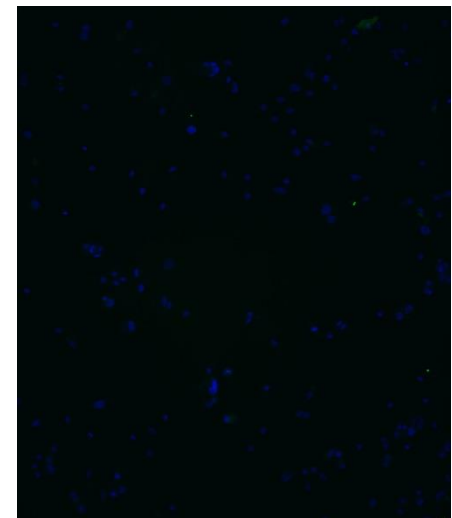
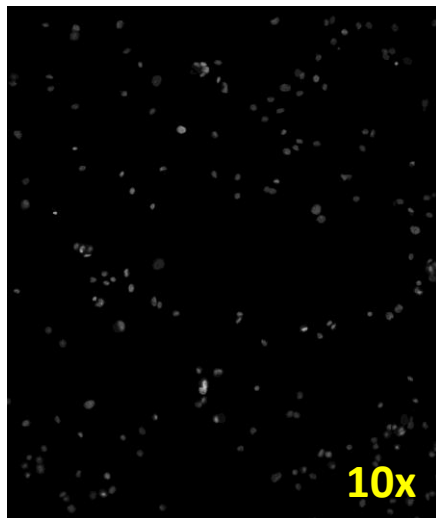
# Cell Line Immunofluorescence Validation

U-CH12 p.(1) Anti-Brachyury versus Secondary-only Negative Control

U-CH12



U-CH12  
2°  
Only  
Control



Hoechst Nuclear Stain

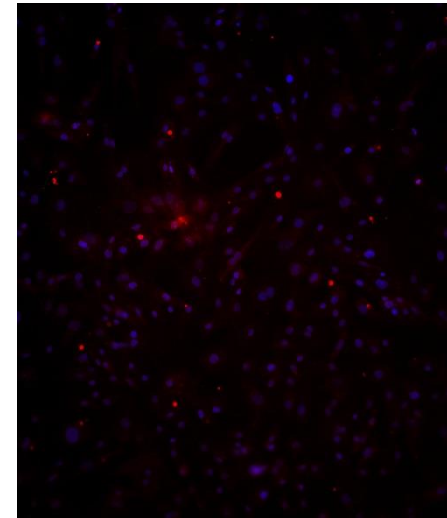
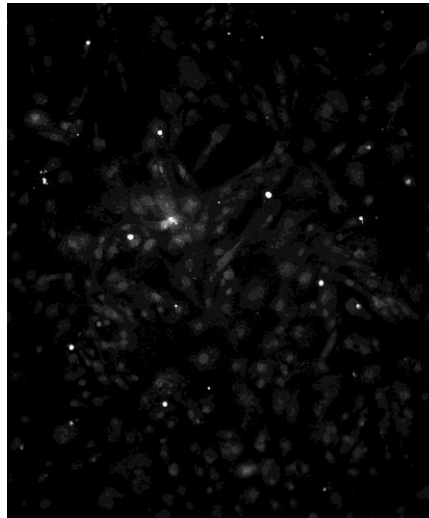
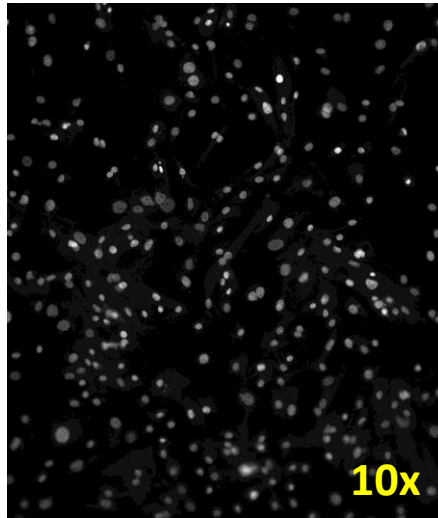
Brachyury

Color Composite

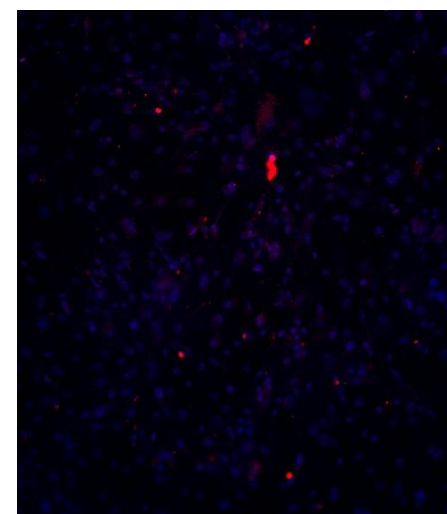
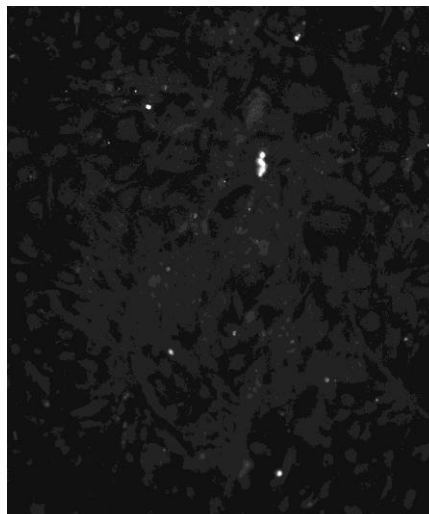
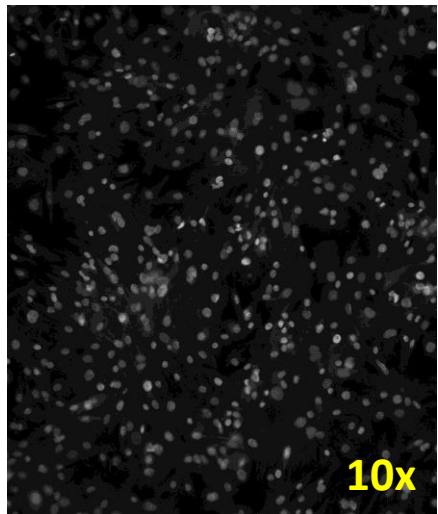
# Cell Line Immunofluorescence Validation

U-CH12 p.(1) Anti-CD24 versus Secondary-only Negative Control

U-CH12



U-CH12  
2°  
Only  
Control



Hoechst Nuclear Stain

CD24

Color Composite

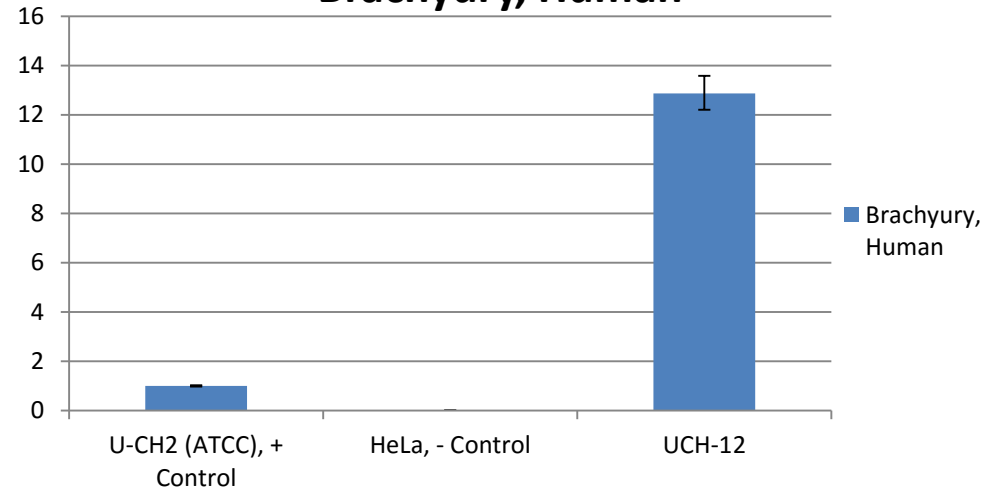
# Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in U-CH12 cell line

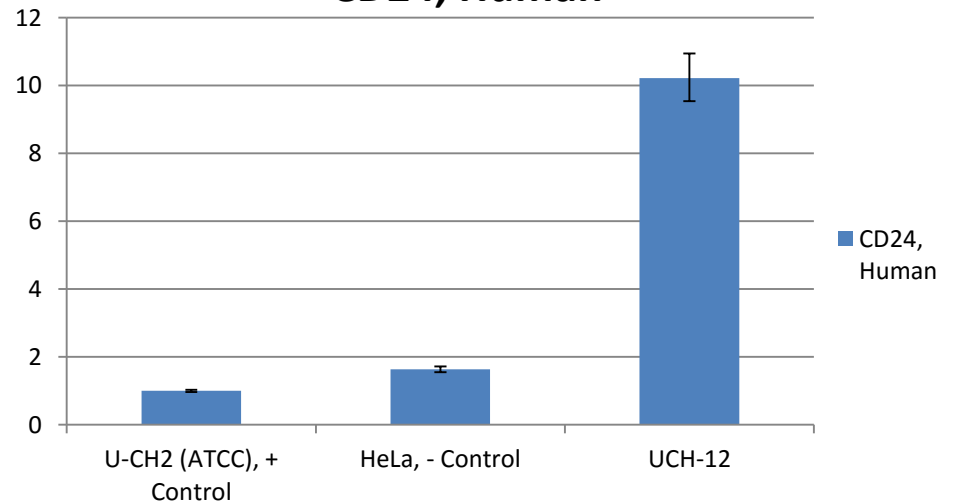
Sample Name	Brachyury, Human	Neg Error	Pos Error
U-CH2 (ATCC), + Control	1	0.0254	0.0261
HeLa, - Control	0.0122	0.0008	0.0009
UCH-12	12.8709	0.6714	0.7083

Sample	CD24, Human	Neg. Error	Pos. Error
U-CH2 (ATCC), + Control	1	0.032	0.033
HeLa, - Control	1.632	0.086	0.091
UCH-12	10.214	0.681	0.729

### Brachyury, Human



### CD24, Human



Tables and associated graphs depict relative quantification of N (top table and graph) and Z (bottom table and graph) gene expression/RNA in TEST cell samples. Gene expression across all assessed lines is set relative to the positive control sample, which is set at 1. The X-axis represents cell lines assessed and the Y-axis represents gene expression relative to positive control.

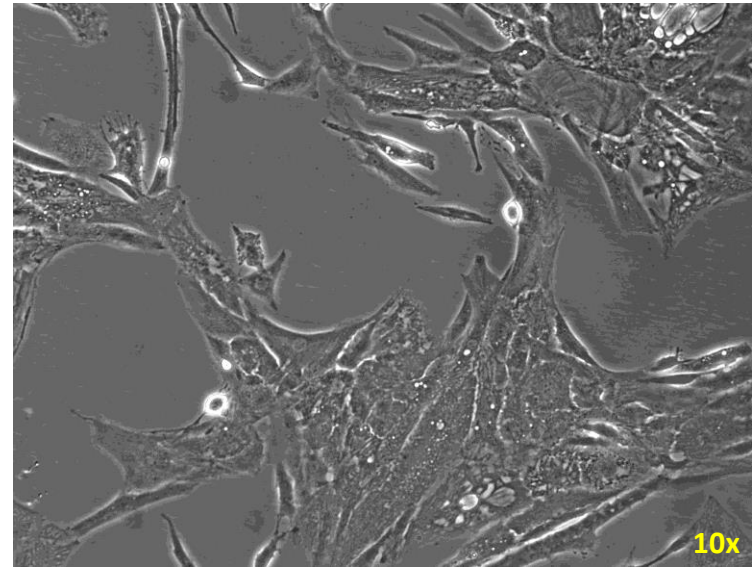
# Cell Line Validation Results

Results summary report of U-CH12

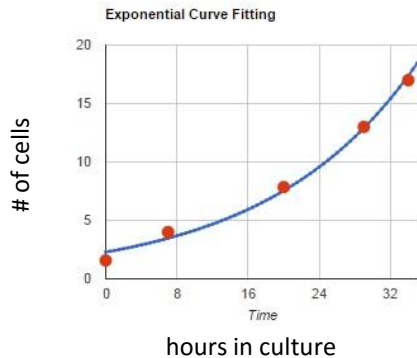
TEST	SPECIFICATION	RESULTS
Cell Growth	Immortalized	Pass
STR Analysis	Human, unique	Pass
IF Validation	Signal in nucleus	Pass
PCR Validation	Expressing Brachyury and CD24	Pass

Cell lot generated

Stock Lot#	1021-079
Cells per vial	500k
Lot Viability	98%
Passages	p.8



**Cell Line Growth:** Cell doubling time= 11-13 days



Cell growth rates were calculated from an actively growing culture for four passages. Growth rates will likely be slower when calculated from a fresh thaw.

**U-CH12 Thaw of lot Vala 1021-079**