

Chordoma Foundation Cell Line Validation

U-CH6

Cell Line Phenotype and Expression
Analysis Report

February 20, 2018

Cell Line Receiving

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Frozen Vials	January 12, 2017	Frozen	3	P(0)	200k	90%

Growth Conditions

Media:

4:1 IMDM/RPMI + 10% FBS

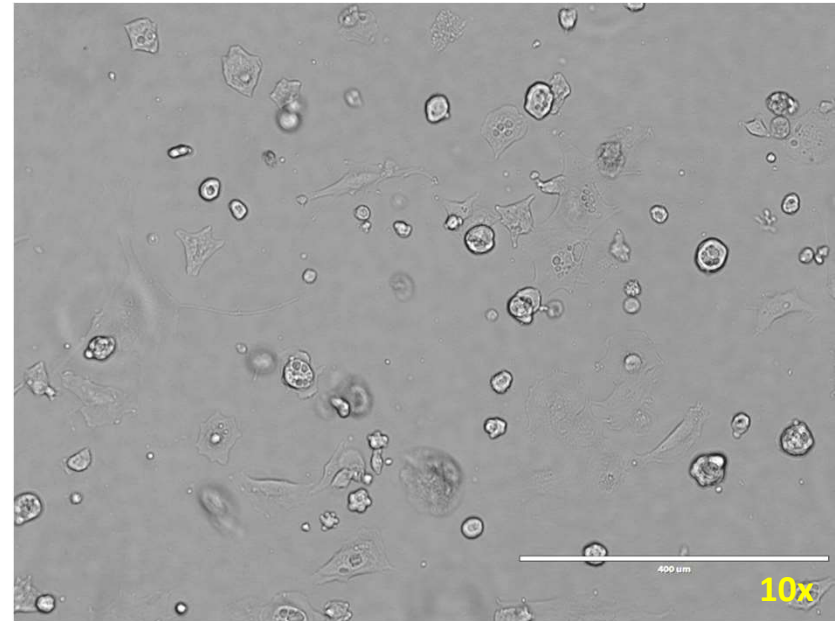
+ l-Glutamine + Pen/Strep +ITS-G

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

→ Change media every 3-4 days

Phase Contrast Image Review

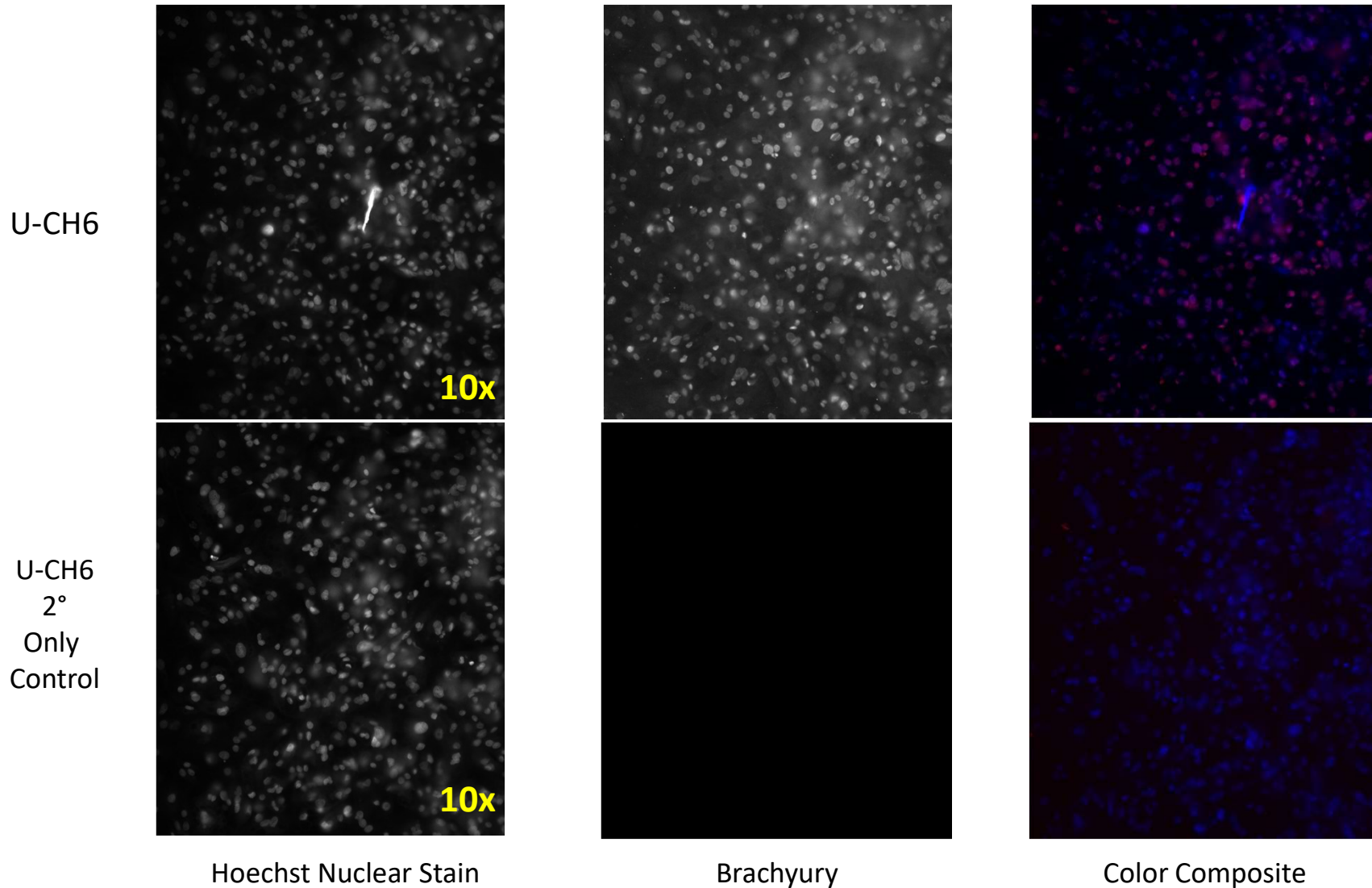
Cells were settled but still mostly rounded up post thaw. They flattened out over time and have maintained a flat morphology with subsequent passaging.



U-CH6 24 hours post thaw, in T25 flask.

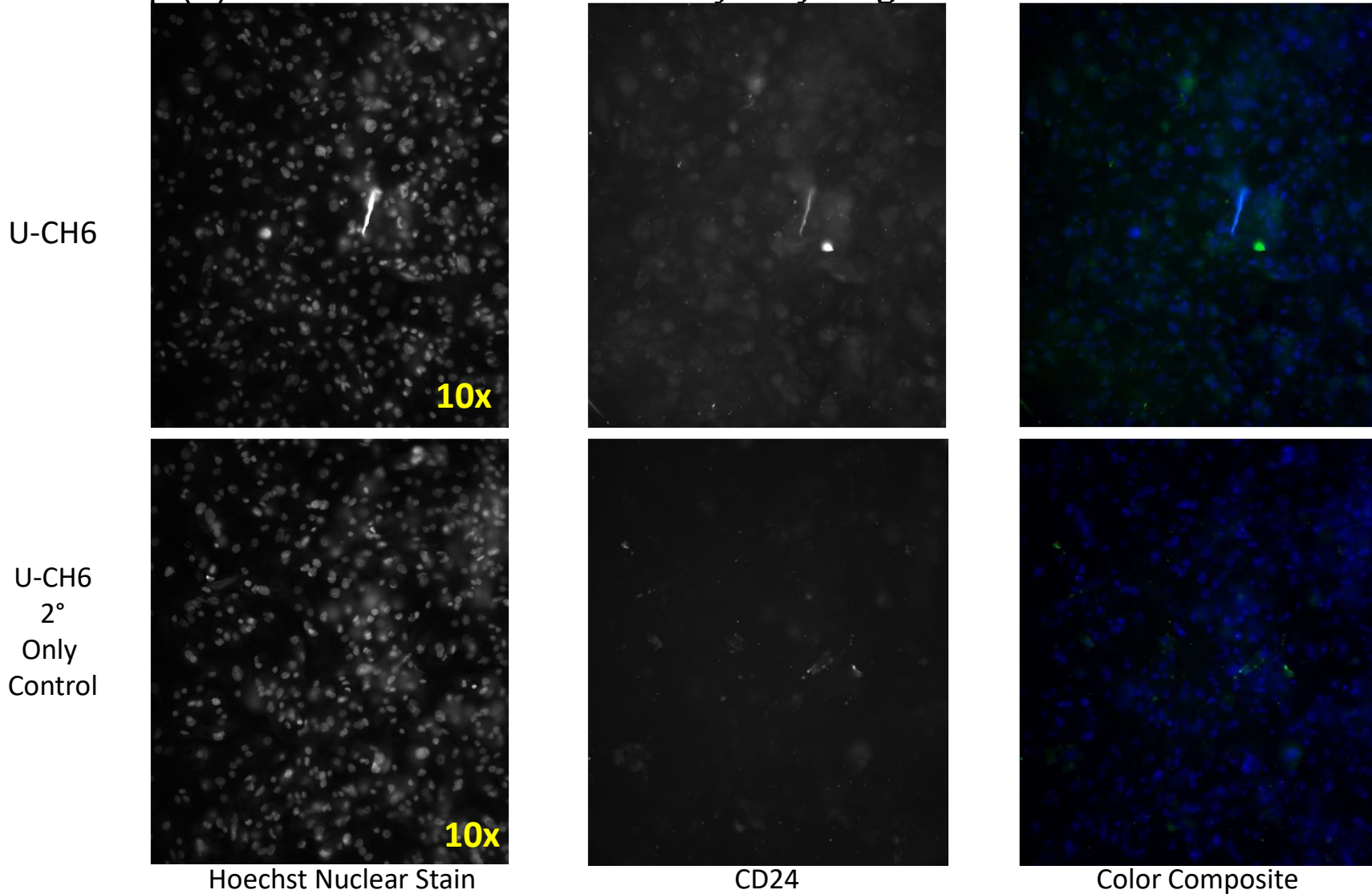
Cell Line Immunofluorescence Validation

U-CH6 p.(1) Anti-Brachyury versus Secondary-only Negative Control



Cell Line Immunofluorescence Validation

U-CH6 p.(1) Anti-CD24 versus Secondary-only Negative Control

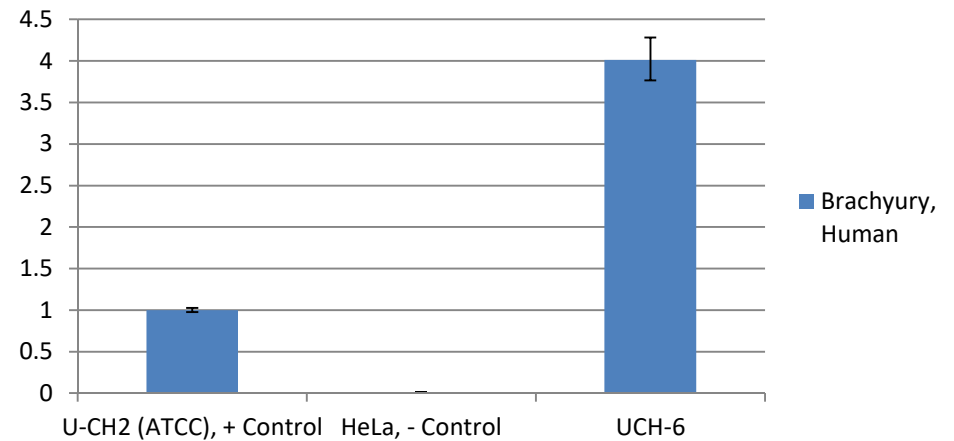


Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in U-CH6 cell line

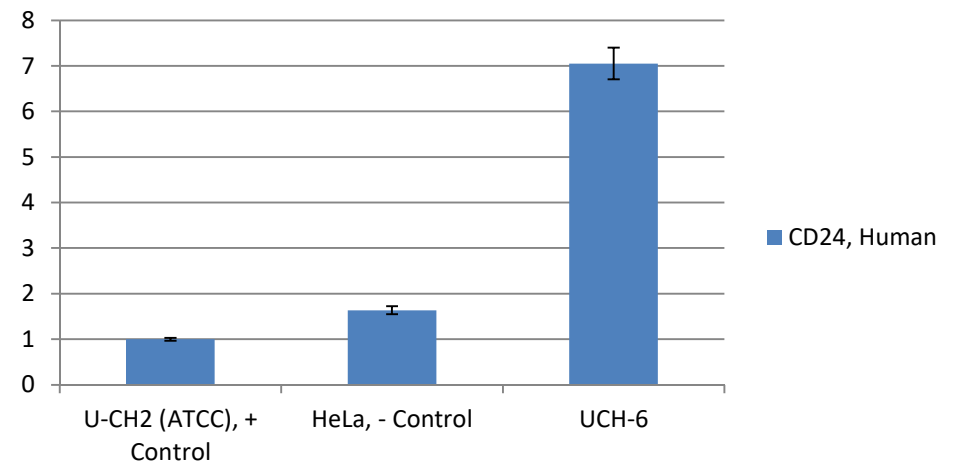
Sample Name	Brachyury, Human	Neg Error	Pos Error
U-CH2 (ATCC), + Control	1	0.0254	0.0261
HeLa, - Control	0.0122	0.0008	0.0009
UCH-6	4.0140	0.2499	0.2665

Brachyury, Human



CD24, Human

Sample	CD24, Human	Neg. Error	Pos. Error
U-CH2 (ATCC), + Control	1	0.0315	0.0326
HeLa, - Control	1.6318	0.0861	0.0908
UCH-6	7.0463	0.3400	0.3572



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-CH6

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

Cell lot generated

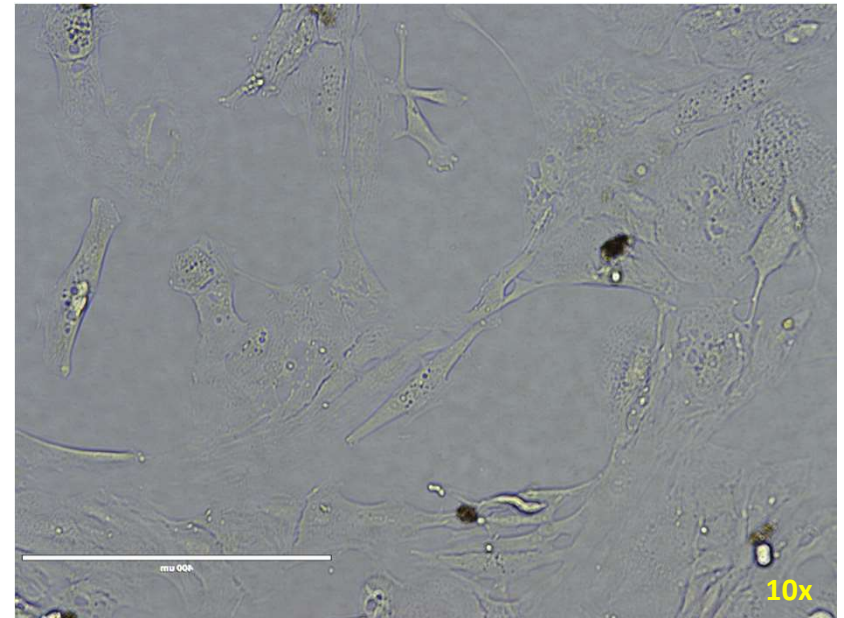
Stock Lot#	1027-082
Cells per vial	375k
Lot Viability	97%
Passages	8

Cell Line Growth: Cell doubling time=

of cells

hours in culture

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-CH6, p (8) pre lot freeze down 1027-082.