

# Chordoma Foundation Cell Line Validation

## U-CH11R

Cell Line Phenotype and Expression  
Analysis Report

June 19, 2017

# Cell Line Receiving

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Frozen Vials	January 12, 2017	N/A	3	p (0)	$7.98 \times 10^5$	68%

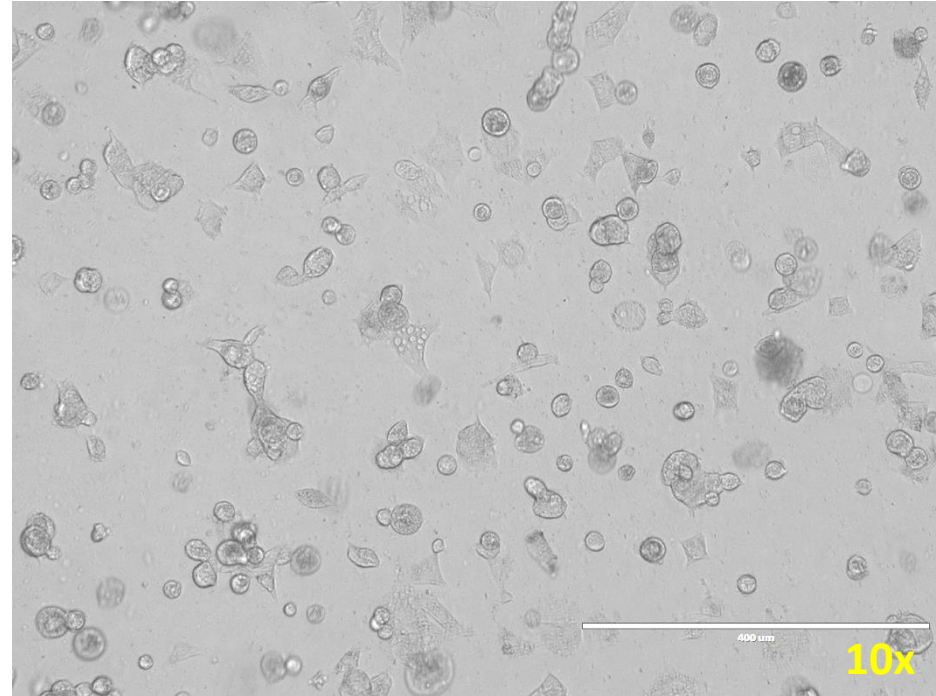
## Growth Conditions

Media:

4:1 IMDM/RPMI + 10% FBS + l-Glutamine +  
Pen/Strep

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

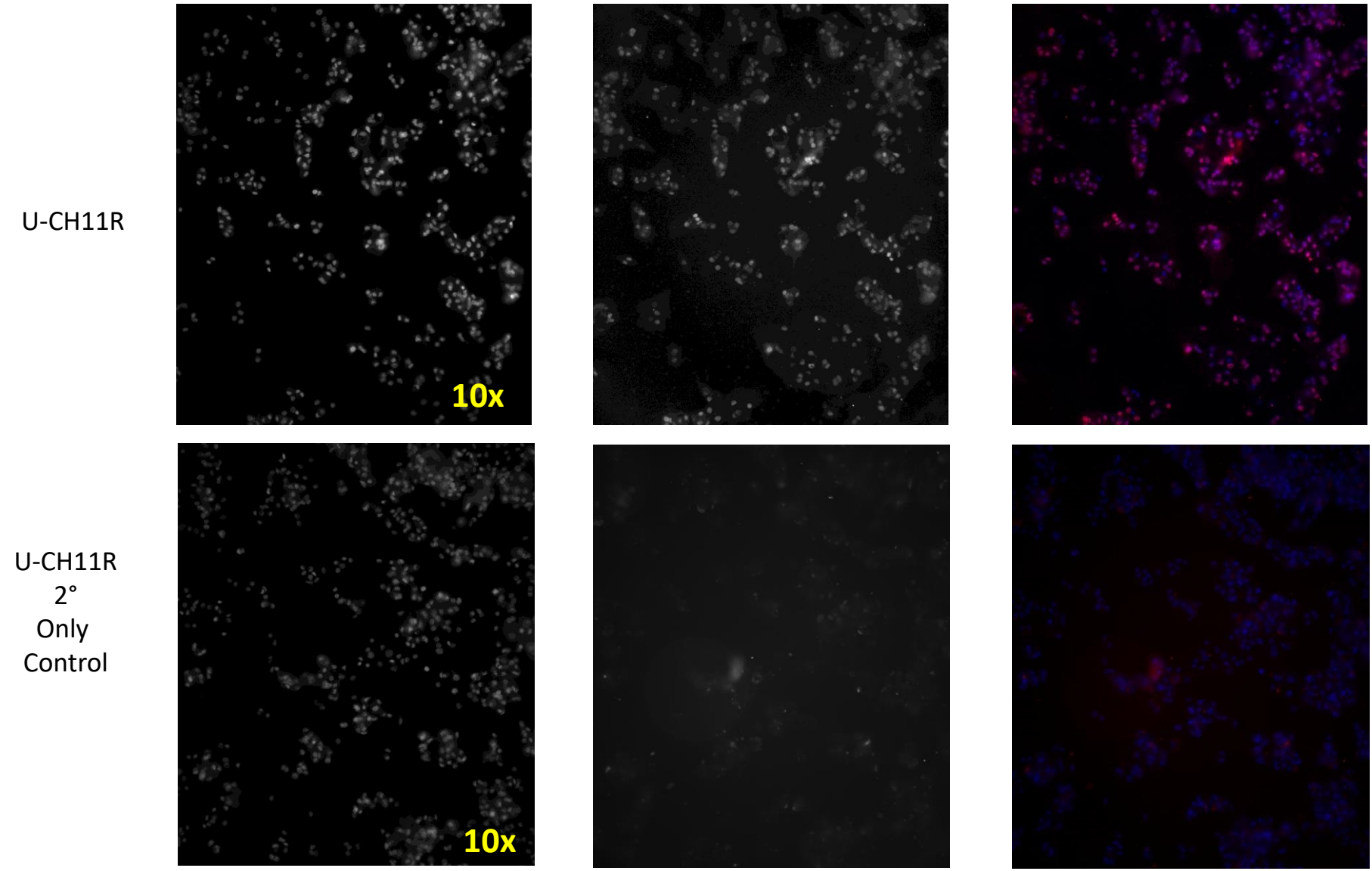
→ Change media every 5-7 days



U-CH11R 24hrs post thaw.

# Cell Line Immunofluorescence Validation

U-CH11R Anti-Brachyury versus Secondary-only Negative Control



U-CH11R

10x

U-CH11R  
2°  
Only  
Control

10x

Hoechst Nuclear Stain

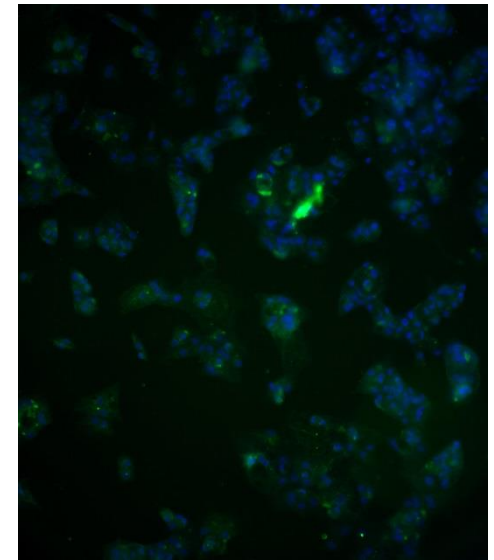
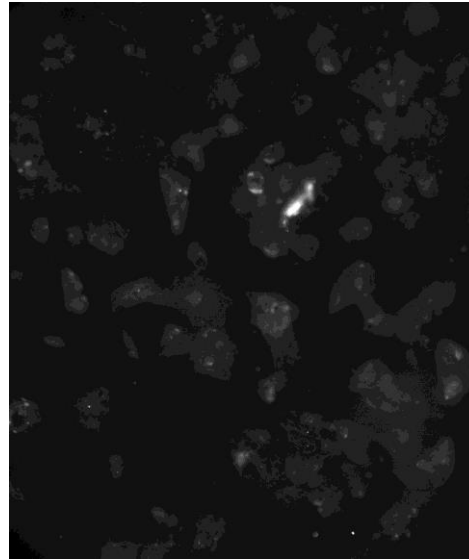
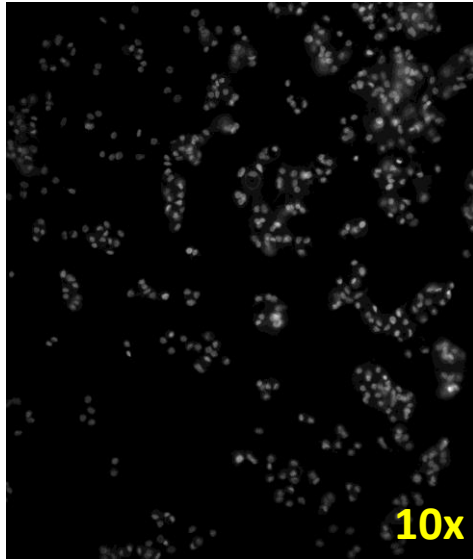
Brachyury

Color Composite

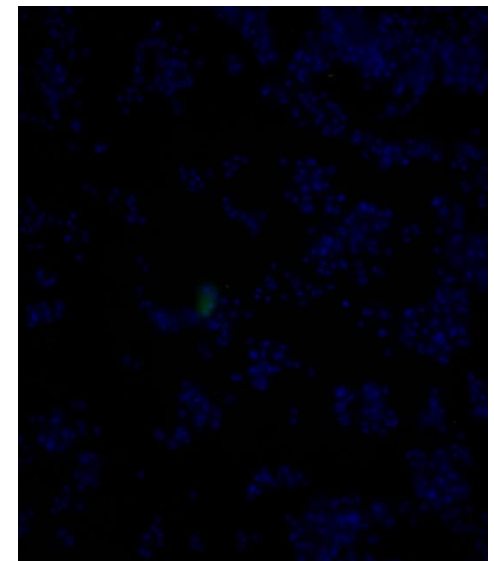
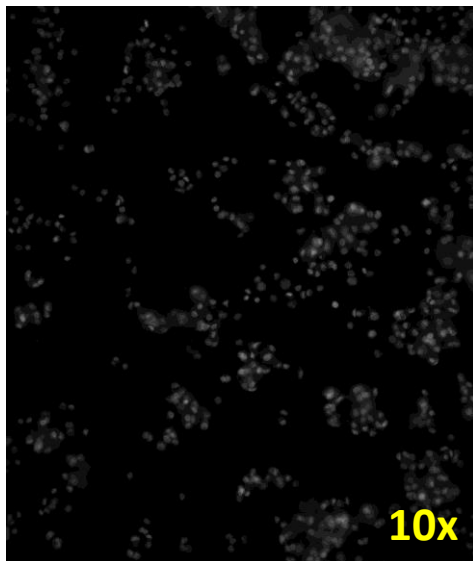
# Cell Line Immunofluorescence Validation

U-CH11R Anti-CD24 versus Secondary-only Negative Control

U-CH11R



U-CH11R  
2°  
Only  
Control



Hoechst Nuclear Stain

CD24

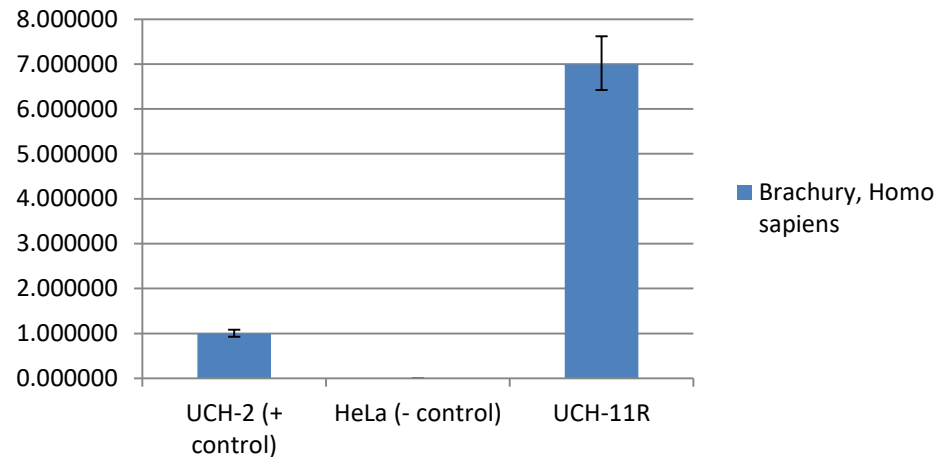
Color Composite

# Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in U-CH11R cell line

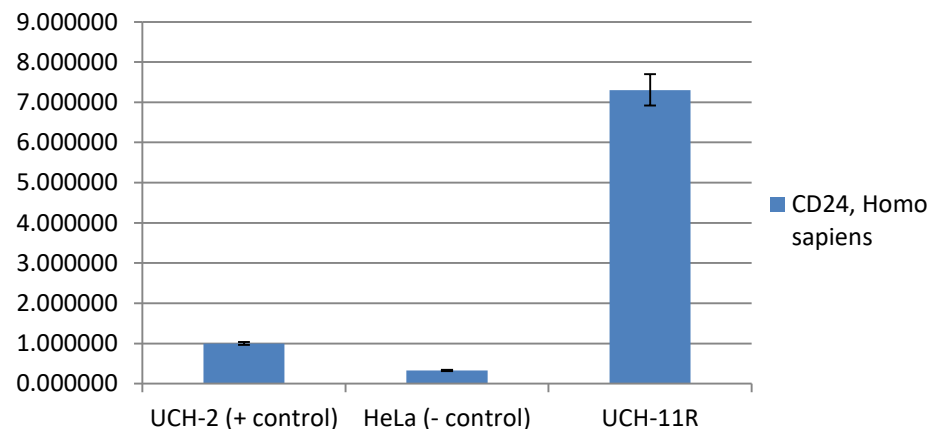
Sample	<u>Brachyury, Homo sapiens</u>	<u>Neg. Error</u>	<u>Pos. Error</u>
UCH-2 (+ control)	1.000000	0.076062	0.082324
HeLa (- control)	0.000820	0.000052	0.000055
UCH-11R	6.992499	0.573949	0.625272

## Brachyury, Homo sapiens



Sample	<u>CD24, Homo sapiens</u>	<u>Neg. Error</u>	<u>Pos. Error</u>
UCH-2 (+ control)	1.000000	0.032074	0.033137
HeLa (- control)	0.326105	0.017748	0.018769
UCH-11R	7.299600	0.382430	0.403574

## CD24, Homo sapiens



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

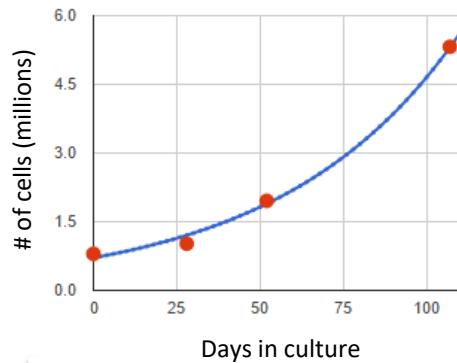
Results summary report of U-CH11R

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

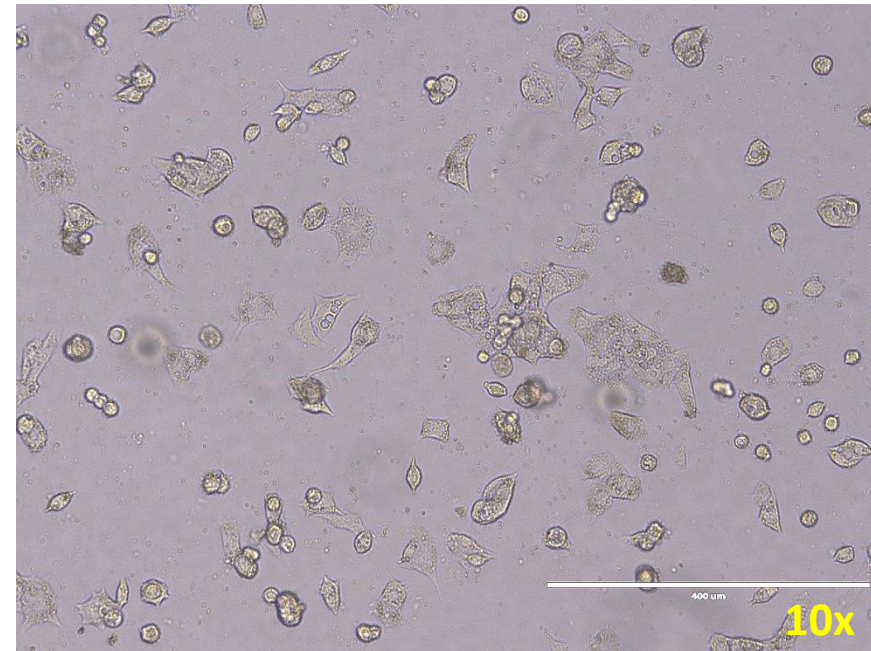
Cell lot generated

Stock Lot#	1027-032
Cells per vial	500k
Lot Viability	94%
Passages	5

**Cell Line Growth:** Cell doubling time= 35 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-CH11R, lot#1027-032S