## Chordoma Foundation Cell Line

# U-CH2

### Cell Line Phenotype and Expression Analysis Report June 22, 2015



### **Cell Line Receiving**

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Flasks (T25)	November 26, 2013	good	2	p. 26	N/A	N/A

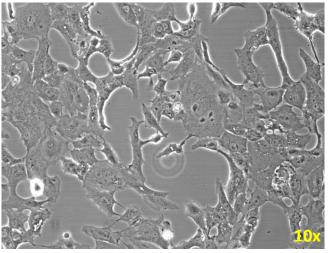
#### **Growth Conditions**

Media:

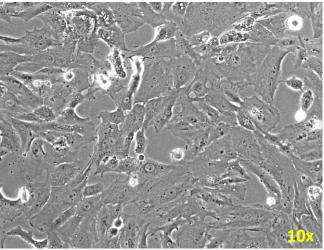
- 4:1 IMDM/RPMI 1640 + 10% HI FBS
- + Pen/Strep
- $\rightarrow$ Use flasks coated with 0.1% gelatin
- $\rightarrow$  Passage when ~80-90% confluent (1:3, 1:5)
- $\rightarrow$ Change media every 3 days

#### **Phase Contrast Image Review**

Cells arrived live, in 2 T25 flasks. They looked healthy. They are visibly clear of contamination and grow well.



U-CH2 arrival day (11/26/2013)

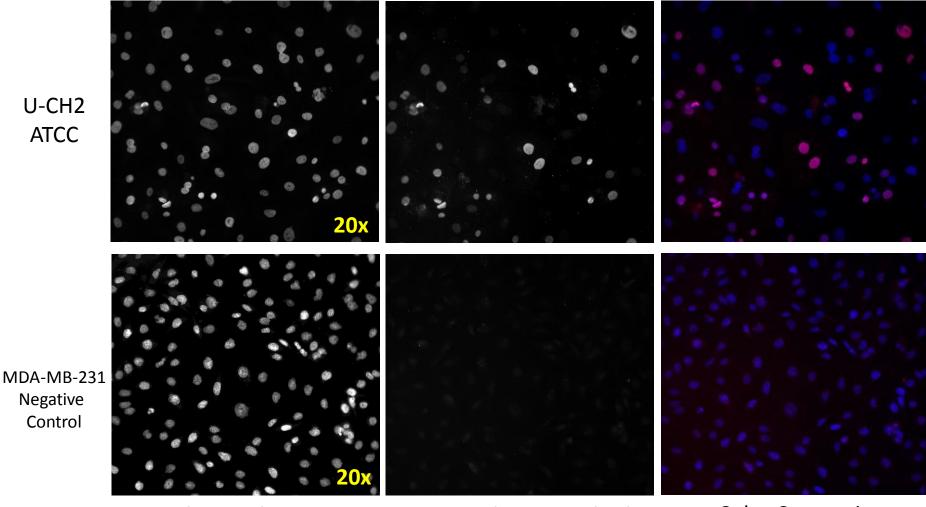


24 Hours After Receiving (11/27/2013)



### **Cell Line Immunofluorescence Validation**

U-CH2 (Supplied by Duke University) p.37 versus Non-Chordoma Negative Control



Hoechst Nuclear Stain

Anti-Brachyury Antibody

**Color Composite** 

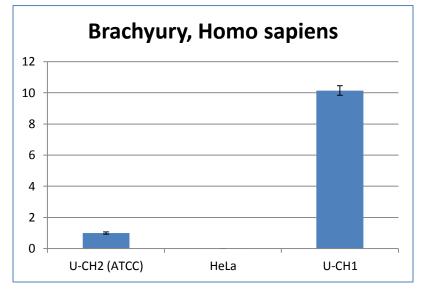


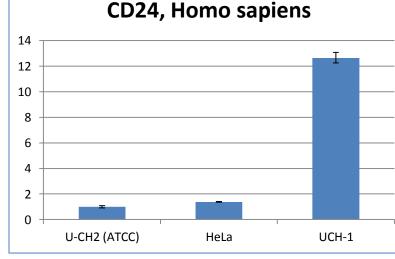
### Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in U-CH2\* cell line

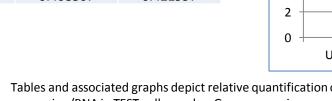
\*PCR data was normalized to U-CH2 (the positive control)

<u>Sample</u>	<u>BRACHYURY,</u> <u>Homo sapiens</u>	<u>Neg. Error</u>	<u>Pos. Error</u>
U-CH2 (ATCC)	1	0.071225	0.076687
HeLa	0.009183	0.00052	0.00055
U-CH1	10.143163	0.301634	0.310879





Sample	<u>CD24,</u> Homo sapiens	<u>Neg. Error</u>	Pos. Error
U-CH2 (ATCC)	1	0.078939	0.085705
HeLa	1.386653	0.03058	0.03127
UCH-1	12.643509	0.408367	0.421997



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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**

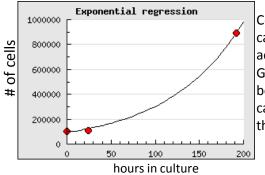
#### Results summary report of U-CH2

TEST	SPECIFICATION	RESULTS
Cell Growth	Immortilized	Doubling time = 3 days
STR Analysis	Human, unique	Pass
IF Validation	Signal in nucleus	Pass
PCR Validation	Expressing Brachyury and CD24	Pass

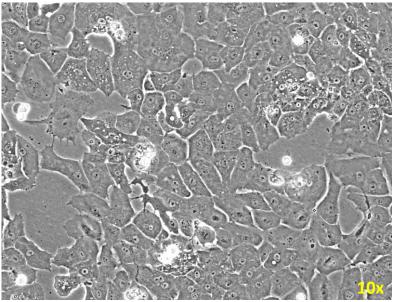
#### Cell lot generated

Stock Lot#	EB1013-050
Cells per vial	2.5x10 <sup>6</sup>
Lot Viability	97.7%
Passages	p.34

#### Cell Line Growth: Cell doubling time= 3 days



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



U-CH2 Vala cell lot EB1013-050 viability thaw

