

Chordoma Foundation Cell Line Validation

CH-22

Cell Line Phenotype and Expression
Analysis Report

June 12, 2015

Cell Line Receiving

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
1 vial	January 06, 2015	NA	1	p. (0) -no passage given	1.9×10^6	91.5%

Growth Conditions

Media:

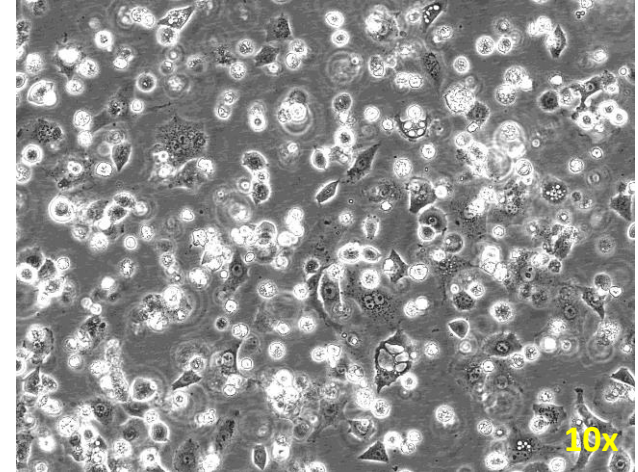
RPMI + 10% FBS + Pen/Strep

→ Passage when ~80-90% confluent (1:2, 1:3)

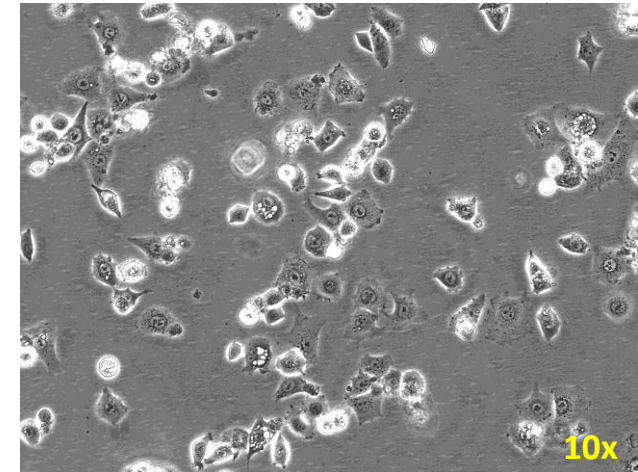
→ Change media every 2-3 days

Phase Contrast Image Review

One vial of cells arrived. Were thawed on 1/6/2015. Thaw produced considerable 24 hour cell death but culture was visibly clear of contamination and recovered well after media change.



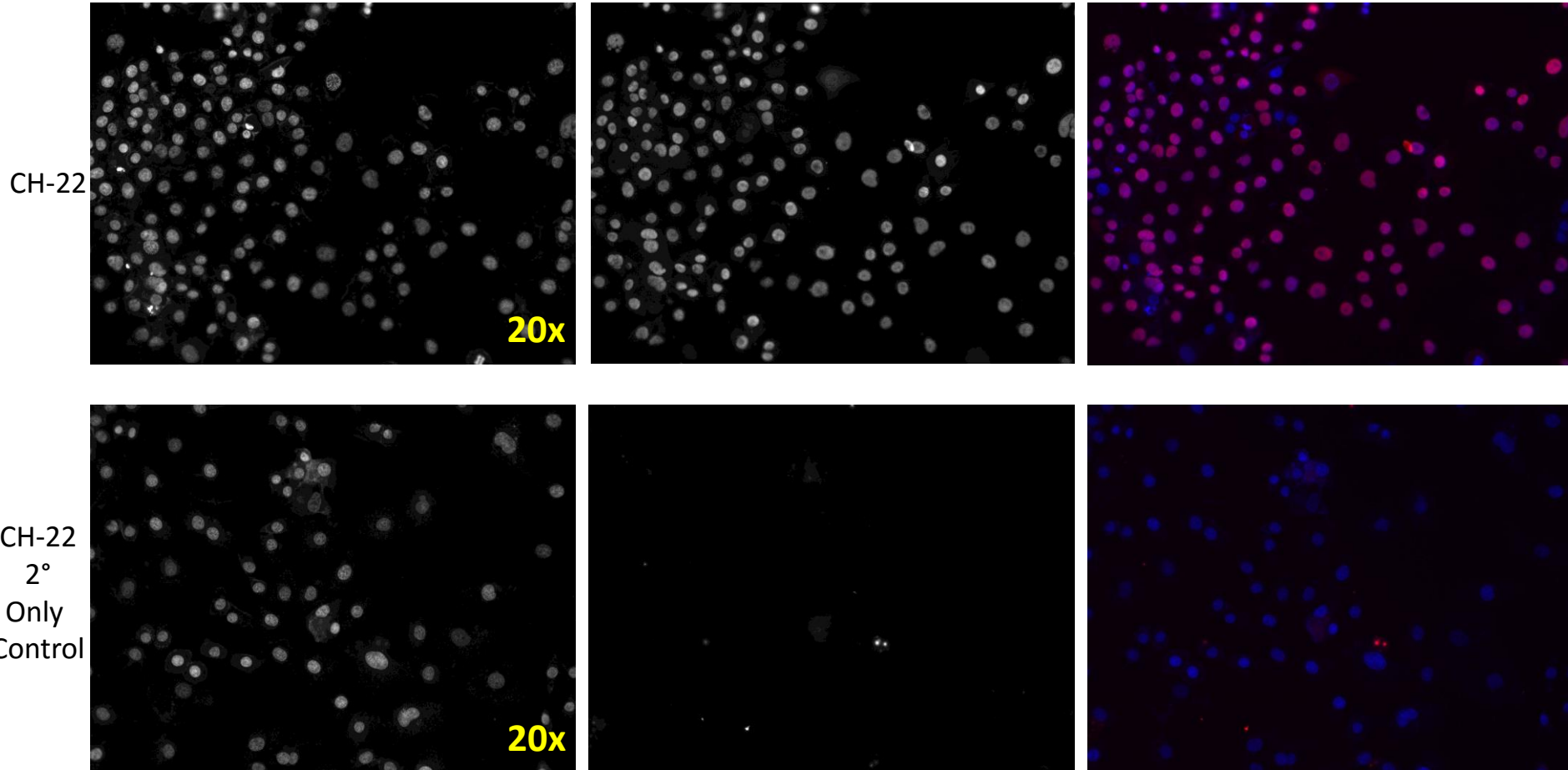
24 Hours Post Thaw, Pre-media change (10/08/2013)



24 Hours Post Thaw, Post-media change (10/08/2013)

Cell Line Immunofluorescence Validation

CH-22 p.(4) Anti-Brachyury versus Secondary-only Negative Control



Hoechst Nuclear Stain

Anti-Brachyury Channel

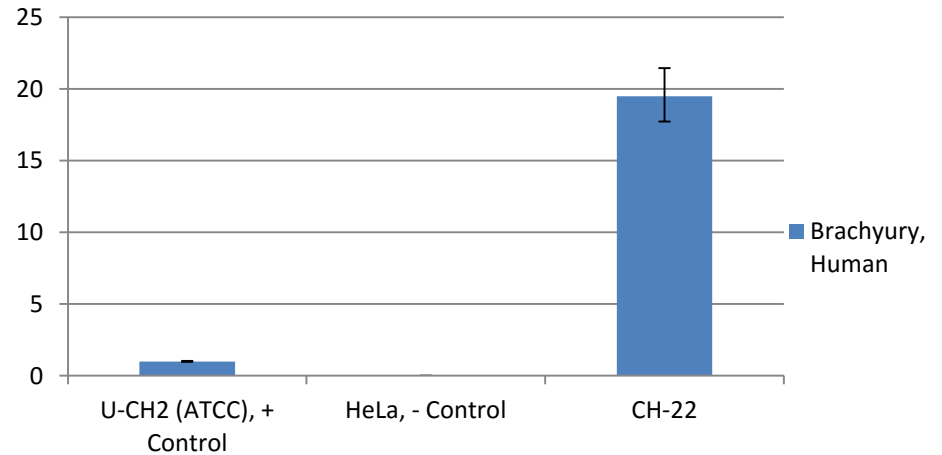
Color Composite

Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in CH-22 cell line

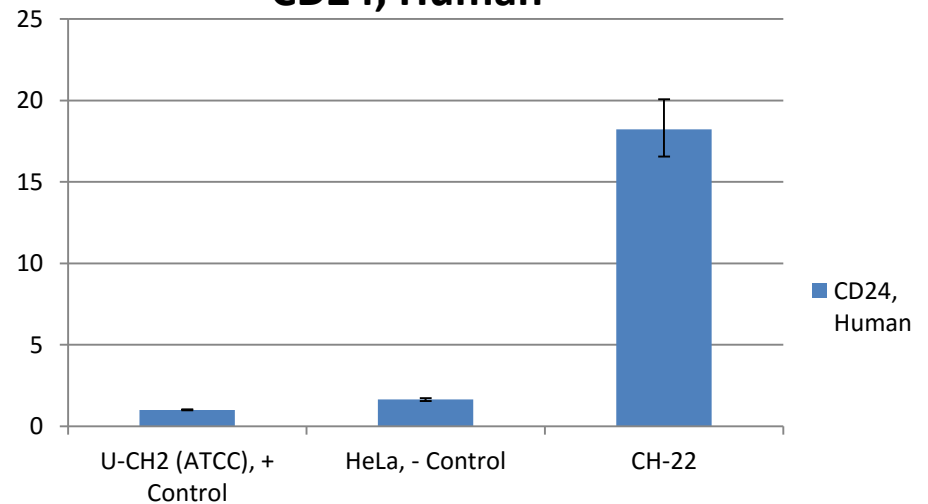
Sample Name	Brachyury, Human	Neg Error	Pos Error
U-CH2 (ATCC), + Control	1	0.025434	0.026098
HeLa, - Control	0.012224	0.000844	0.000906
CH-22	19.49005	1.77822	1.956749

Brachyury, Human



Sample	CD24, Human	Neg. Error	Pos. Error
U-CH2 (ATCC), + Control	1	0.03154	0.032568
HeLa, - Control	1.631788	0.086057	0.090848
CH-22	18.23151	1.669121	1.837332

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.

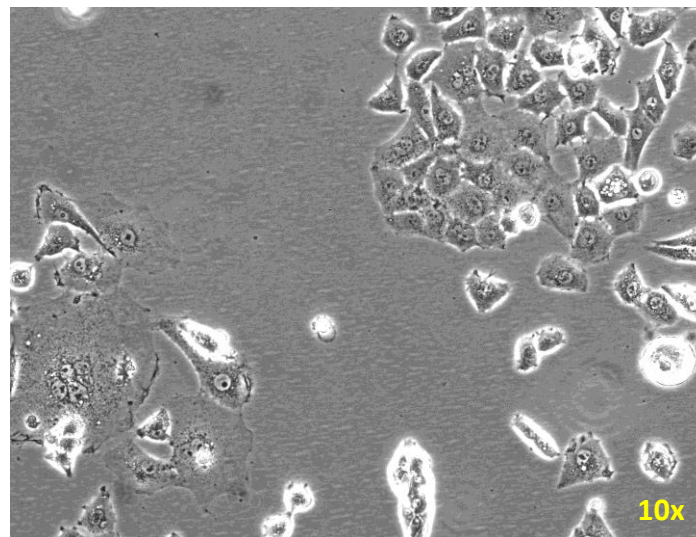
Cell Line Validation Results

Results summary report of CH-22

TEST	SPECIFICATION	RESULTS
Cell Growth	Immortalized	Doubling time = 2.3 days
STR Analysis	Human, unique	Pass
IF Validation	Signal in nucleus	Pass
PCR Validation	Expressing Brachyury and CD24	Pass

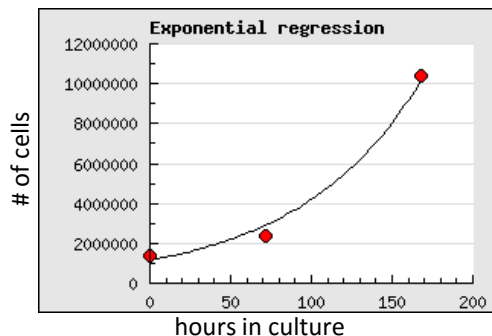
Cell lot generated

Stock Lot#	EB1018-031
Cells per vial	5.0×10^5
Lot Viability	97%
Passages	p. (4)



CH-22 Vala cell lot EB1018-031

Cell Line Growth: Cell doubling time= 2.3 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.