Chordoma Foundation Cell Line Validation

MUG-Chor1

Cell Line Phenotype and Expression Analysis Report 2/20/2018



Cell Line Receiving

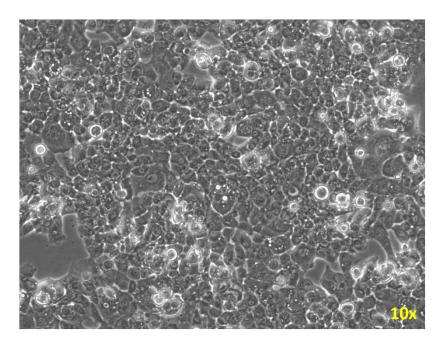
Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Frozen	November 21, 2013	good	2	p. (0)	4.4 x 10 ⁶	99%

Growth Conditions

Media: IMDM/RPMI 4:1 + 10% FBS + ITS+ 2mM glutamine + 1X Pen-Strep

Phase Contrast Image Review

Cells grow well after thaw and are visibly free of contamination.

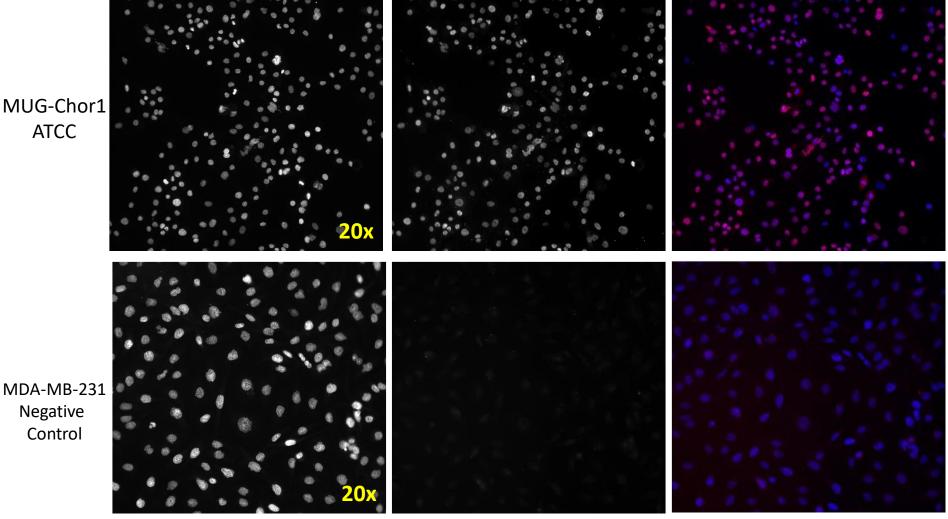


MUG-Chor1 48 hours post thaw, before first split.



Cell Line Validation Service

Immunofluorescent Validation of Chordoma Lines. Images **MUG-Chor1** versus Non-Chordoma Negative Control



Hoechst Nuclear Stain

Anti-Brachyury Antibody

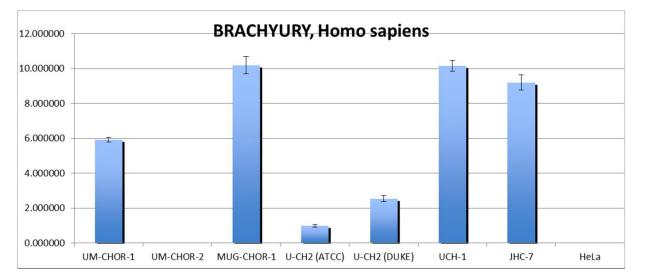
Color Composite

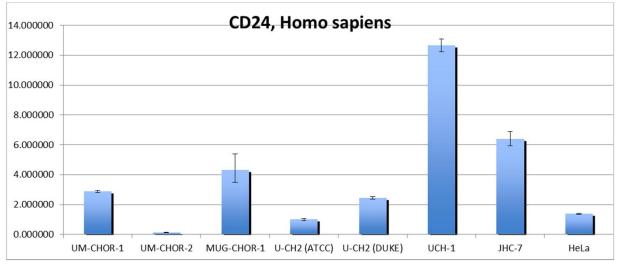
*Passages since thawed and cultured at Vala Sciences, Inc.

Cell Line Validation Service

Relative quantification of Brachyury and CD24 in MUG-Chor1 Chordoma cell line

<u>Sample</u>	<u>BRACHYURY,</u> Homo sapiens	<u>Neg. Error</u>	<u>Pos. Error</u>
UM-CHOR-1	5.912447	0.135544	0.138725
UM-CHOR-2	0.000023	0.000017	0.000069
MUG-CHOR-			
1	10.174857	0.483211	0.507303
U-CH2			
(ATCC)	1.000000	0.071225	0.076687
U-CH2			
(DUKE)	2.538421	0.173369	0.186077
UCH-1	10.143163	0.301634	0.310879
JHC-7	9.195996	0.43329	0.45471
HeLa	0.009183	0.00052	0.00055





Sample	<u>CD24, Homo</u> sapiens	Neg. Error	Pos. Error
UM-CHOR-1	2.884621	0.072260	0.074117
UM-CHOR-2	0.137998	0.004368	0.004510
MUG-CHOR-			
1	4.324368	0.849385	1.056999
U-CH2			
(ATCC)	1.000000	0.078939	0.085705
U-CH2			
(DUKE)	2.441440	0.080156	0.082877
UCH-1	12.643509	0.408367	0.421997
JHC-7	6.398626	0.44710	0.48069
HeLa	1.386653	0.03058	0.03127

Tables and associated graphs depict relative quantification of brachyury (top table and graph) and CD24 (bottom table and graph) gene expression/RNA in chordoma cell samples. Gene expression across all assessed lines is set relative to the positive control sample, U-CH2 (ATCC), 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

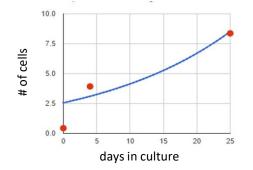
Results summary report of MUG-Chor1

TEST	SPECIFICATION	RESULTS
Cell Growth	Immortilized	Doubling time = 15 days
STR Analysis	Human, unique	Pass
IF Validation	Signal in nucleus	Pass
PCR Validation	Expressing Brachyury and CD24	Pass

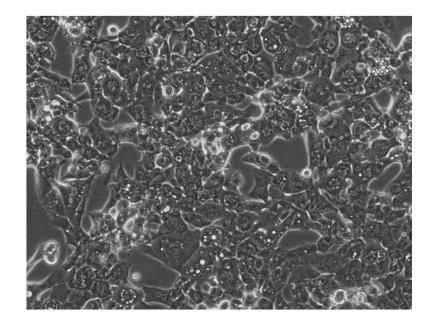
Cell lot generated

Stock Lot#	1013-028
Cells per vial	5.0x10 ⁵
Lot Viability	99%
Passages	p. (5)

Cell Line Growth: Cell doubling time= 12-14 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.



MUG-Chor1 p(5) lot# EB1013-028

