

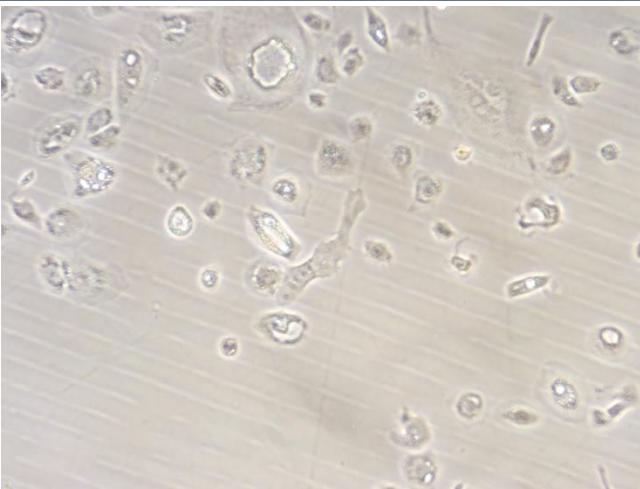
# Cell Line Validation

## UM-Chor7; UM-Chor7-CAF; UM-Chor7-L

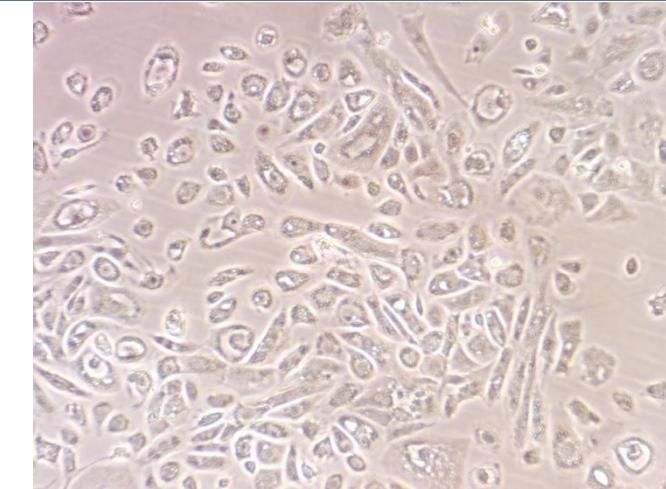
Cell morphology, growth and Brachyury  
expression analysis

January 5, 2026

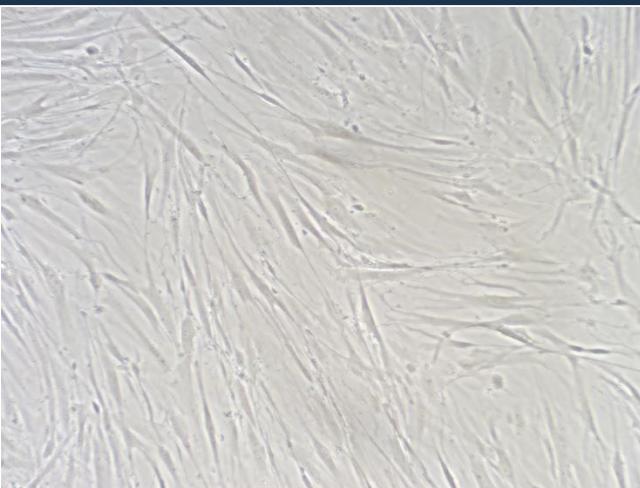
# CELL LINE MORPHOLOGY



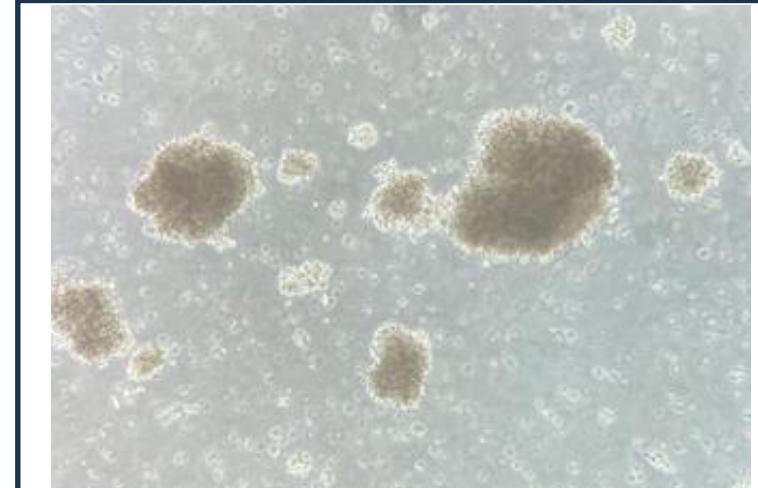
UM-Chor7 low density



UM-Chor7 high density



UM-Chor7-CAF



UM-Chor-L

# CELL GROWTH CONDITIONS

## UM-Chor7 GROWTH CONDITIONS

**Media:** 4:1 IMDM/RPMI 1640 + 10% HI FBS + Pen/Strep + Ampho B

**Coating:** Polystyrene flasks

**Passaging:** At >60% confluence

**Media Change Frequency:** Every 7 Days

## UM-Chor7-CAF GROWTH CONDITIONS

**Media:** DMEM + 10% HI FBS + Pen/Strep

**Coating:** Polystyrene flasks

**Passaging:** At >60% confluence

**Media Change Frequency:** Every 3 Days

## UM-Chor7-L GROWTH CONDITIONS

**Media:** Immunocult-XF T Cell Medium + Pen/Strep + CD3/CD28/CD2 T Cell Activator + IL-2

**Coating:** Suspension growth

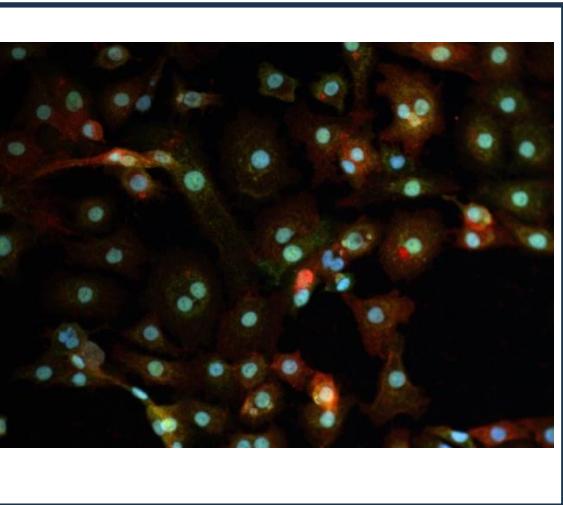
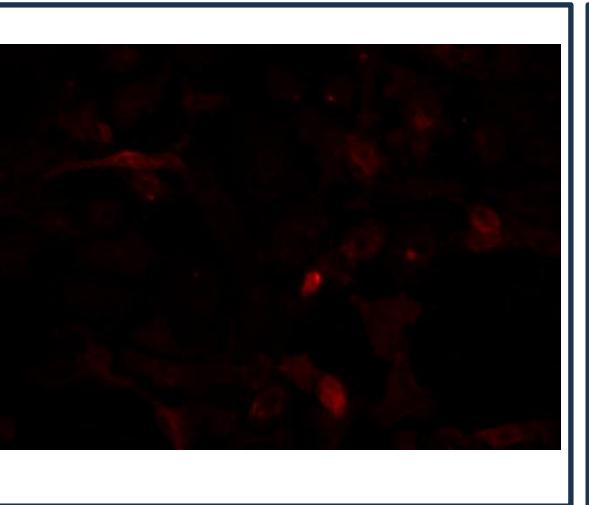
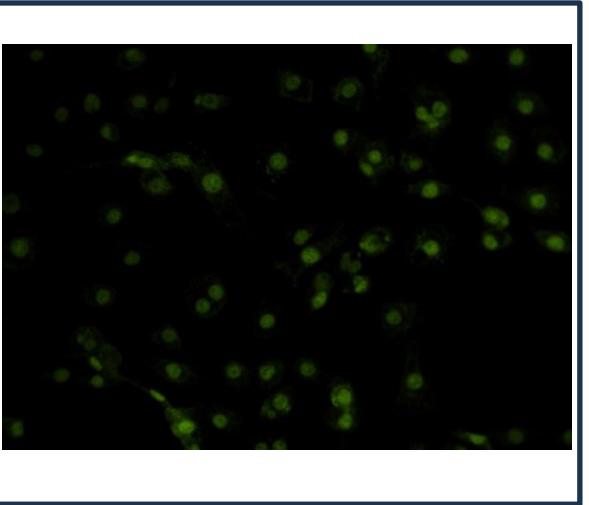
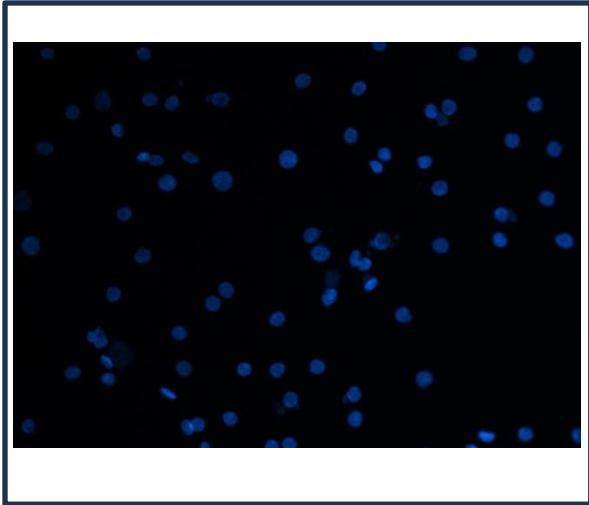
**Passaging:** After 7 Days of cellular division

**Media Change Frequency:** Every 3 Days

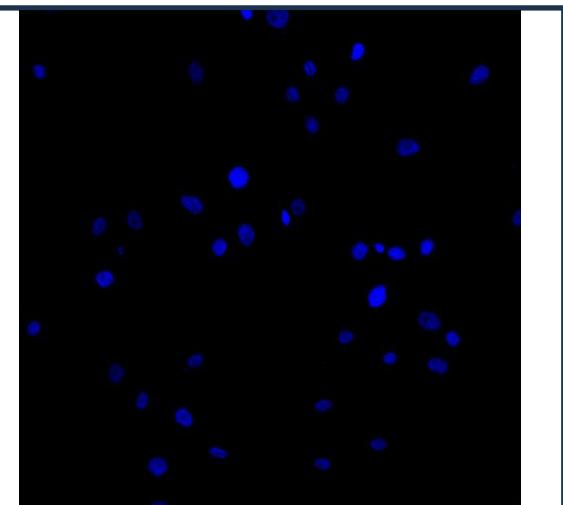
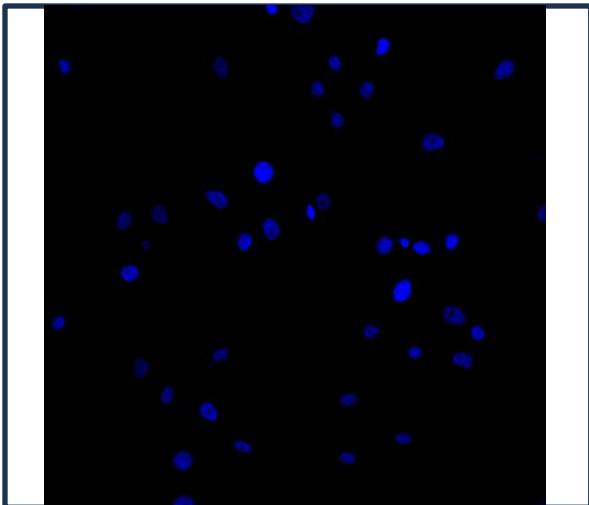
# CELL LINE IMMUNOFLUORESCENCE VALIDATION

UM-Chor7 (supplied by University of Michigan) p12 versus Non-Chordoma Negative Control UM-Chor7-CAF

UM-Chor7



UM-Chor7-CAF



DAPI NUCLEAR STAIN

ANTI-BRACHYURY ANTIBODY

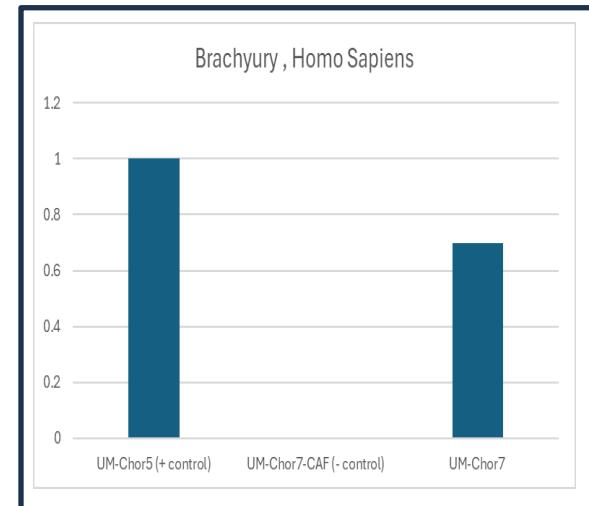
ANTI-CD24 ANTIBODY

COLOR COMPOSITE <sub>4</sub>

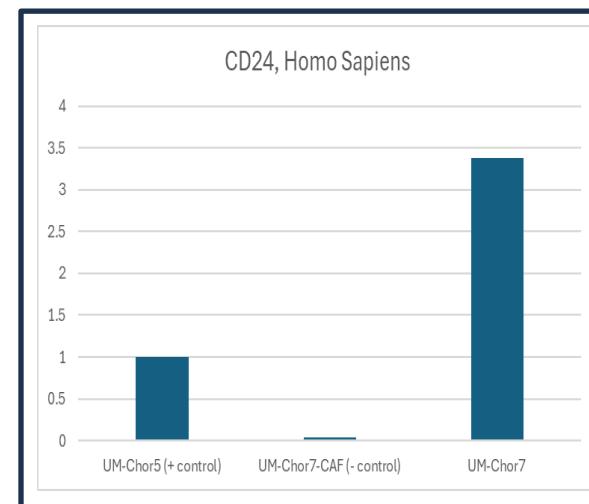
# CELL LINE PCR VALIDATION

Relative quantification of Brachyury and CD24 gene in UM-Chor7 cell line

Brachyury , Homo Sapiens	
UM-Chor5 (+ control)	1
UM-Chor7-CAF (- control)	0
UM-Chor7	0.6972



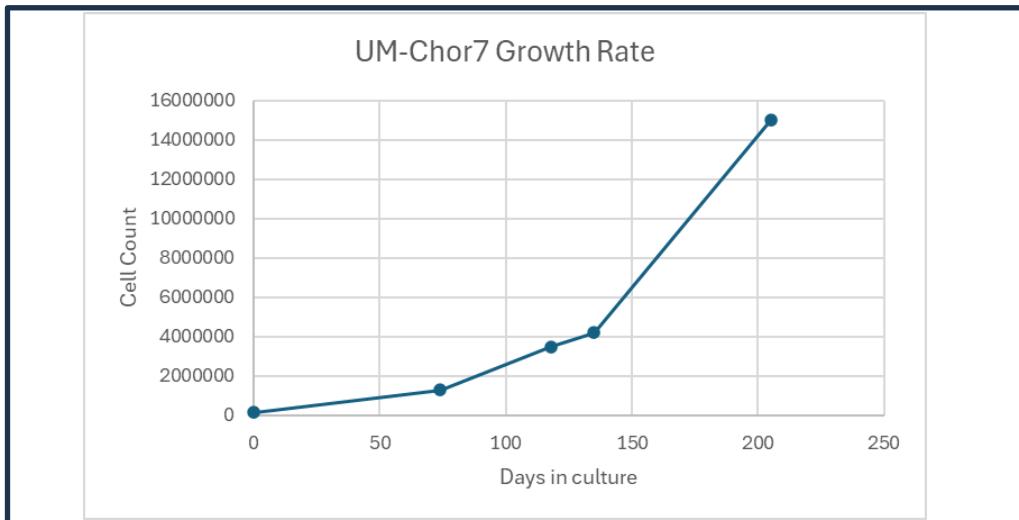
CD24, Homo Sapiens	
UM-Chor5 (+ control)	1
UM-Chor7-CAF (- control)	0.0431
UM-Chor7	3.3814



Description: Gene expression is set relative to the positive control UM-Chor5, which is 1.

# STR ANALYSIS

- POPULATION DOUBLING TIME: 30.86 Days



Cell growth rates were calculated from an actively growing culture for four passages.

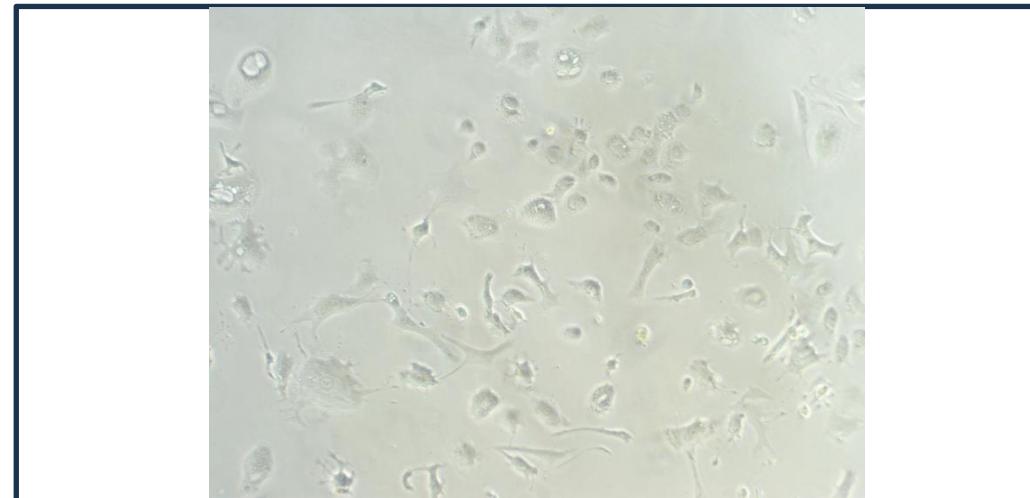
- STR PROFILE:

Sample	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	Penta_D	Penta_E	TH01	TPOX	vWA
UM-Chor7	X	12	13, 14	13	14	30	15	12	10, 11	10, 13	22, 25	12	14, 17	6, 9.3	8, 9	18
UM-Chor7-CAF	X	12	13, 14	13	12, 14	28, 30	15, 16	12	10, 11	10, 13	22, 25	11, 12	14, 17	6, 9.3	8, 9	17, 18
UM-Chor7-L	X	12	13, 14	13	12, 14	28, 30	15, 16	12	10, 11	10, 13	22, 25	11, 12	14, 17	6, 9.3	8, 9	17, 18

# CELL LINE VALIDATION RESULTS

TEST	SPECIFICATION	RESULTS
CELL GROWTH	IMMORTILIZED	DOUBLE TIME = 30.86 Days
STR ANALYSIS	HUMAN, UNIQUE	PASS
IF VALIDATION	SIGNAL IN NUCLEUS	PASS
PCR VALIDATION	EXPRESSING BRACHYURY AND CD24	PASS

**VALIDATION RESULT:** The cell line meets the criteria for being a chordoma cell line.



CELL LINE VIABILITY THAW