Introduction

The JHC7 cell line was developed by Sagar Shah and Alfredo Quiñones-Hinojosa at Johns Hopkins University from a primary sacral chordoma presenting in a 56 year old female. It is one of the faster growing and easier to maintain chordoma cell lines.

Media formulation

Materials

- DMEM/F12 (Gibco, Cat# 11320033)
- FBS, Certified, US (Gibco, Cat# 16000044) or equivalent quality FBS
- Nalgene Rapid-Flow Vacuum Filter (Thermo Scientific, Cat# 5660010)

DMEM/F12 + 10% FBS media preparation protocol

- 1. Prepare media in the bio-safety cabinet using aseptic technique. This media is made without antibiotics so extra precautions need to be taken.
- 2. Douse items in 70% Ethanol and place in bio-safety cabinet. Wait at least 10 minutes so ethanol has a chance to disinfect. Check for liquid at base of cap, if present, aspirate.
- 3. Create 500mL of media using volumes to the right by pipetting the media components into the upper compartment of a 500mL Nalgene vacuum filter, filter the components, and then store media at 4°C.

Component	Volume (mL)
DMEM/F12	450
FBS	50
Total	500

Culturing characteristics

This cell line is thawed using CF Labs general thawing protocol, <u>found here</u>. This line can be grown on standard tissue culture treated, non-collagen coated vessel surfaces as this line's viability and growth are not dependent on collagen. Using the above media formulation and grown in the absence of collagen, JHC7 cells exhibit a doubling time of approximately three days. The cell line exhibits a heterogeneous physaliferous morphology. It is important to note, the doubling time of JHC-7 slows approximately two months after thawing. This line is generally only grown for two months and then tossed. **(A)** JHC-7 representative image. When approaching 80-90% confluence, the cells will form tightly packed clusters. Cells in the center of the cluster begin to bleb and appear to undergo cell death. When culturing, it is important to monitor the health of the cells at the center of the cluster. Cells dying in the middle of the cluster may skew experimental data. **(B)** In a 96-well plate, plating 2,000 cells/well (6,250 cells/cm²) will yield 15% confluence 36 hours after plating, labeled as day 0 where 36 hours is the time allowed for cells to adhere and spread out on the plate. The cells reach 90% confluence after seven days. This information can be extrapolated to other vessel types.

