

Introduction

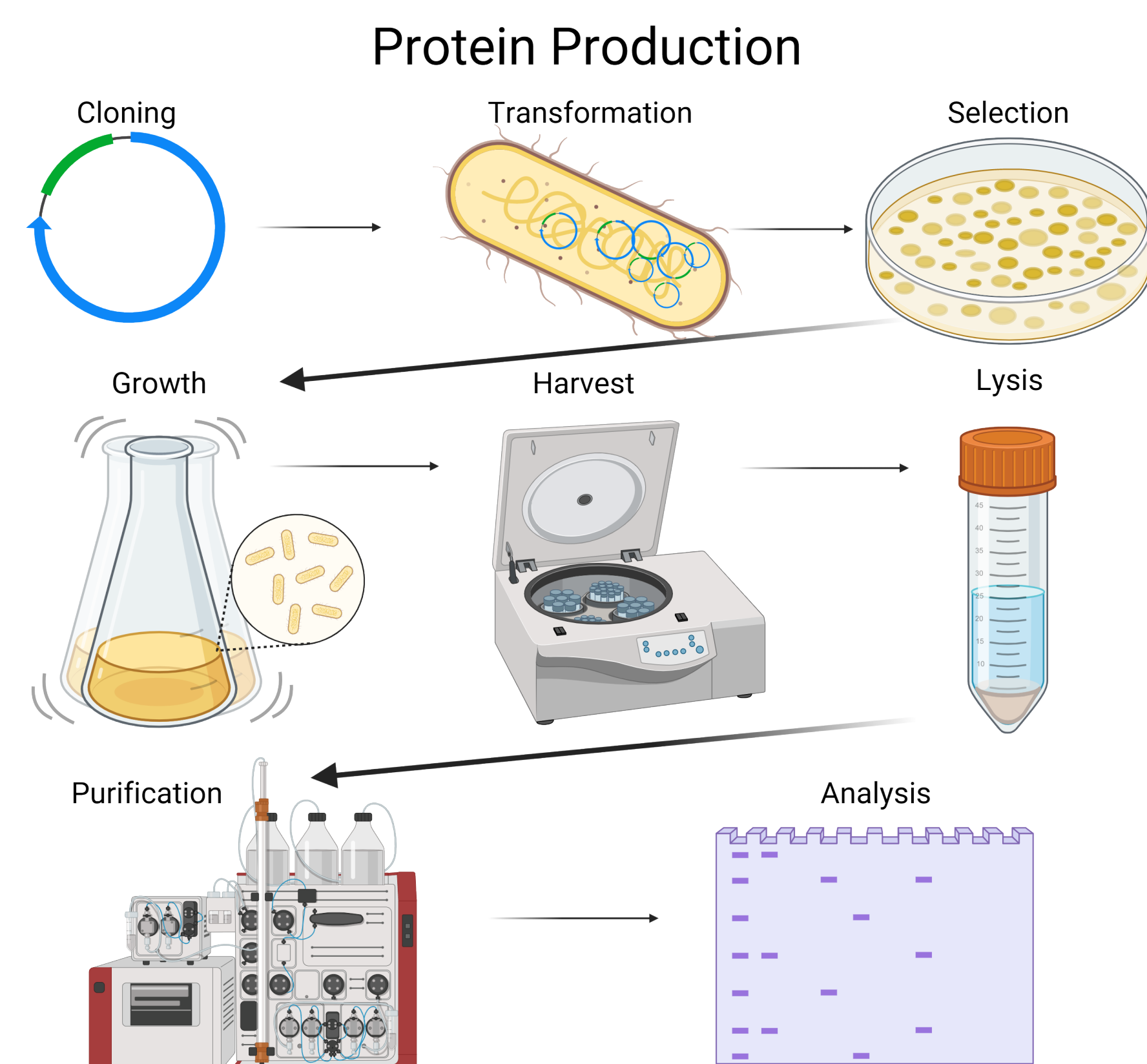
Human induced pluripotent stem cells (iPS cells) utilize media as a nutrient source. Serum-containing media have historically been used and contain hormones, growth factors, and other components critical for cell growth.

Cons of serum-containing media:

- batch to batch variation of serum
- undefined components
- components that can inhibit cell growth

Defined Bioscience is dedicated to making media that are fully defined and serum-free as a way to address these problems. This project focuses on manufacturing and testing FGF2-G3, a hyperstable growth factor component of HiDef-B8 critical for cell proliferation and maintaining stem cell pluripotency.

Methods



Results

FGF2-G3 confirmation of purification via SDS page gel analysis

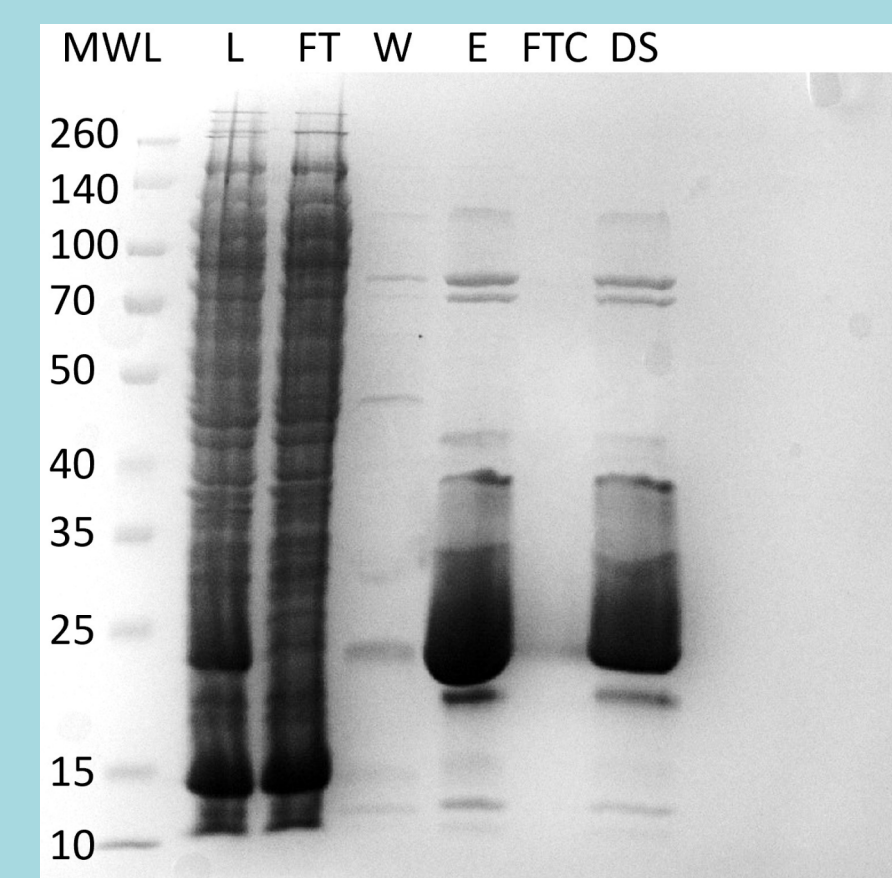


Figure 1. SDS-PAGE analysis of FGF2-G3 purification via Ni immobilized metal affinity chromatography. Lanes correspond to: SpectraBR molecular weight ladder (MWL; numeric labels in kDa), column load (L), column flowthrough (FT), column wash (W), column elution (E), flowthrough from sample concentration (FTC), and desalted fraction (DS).

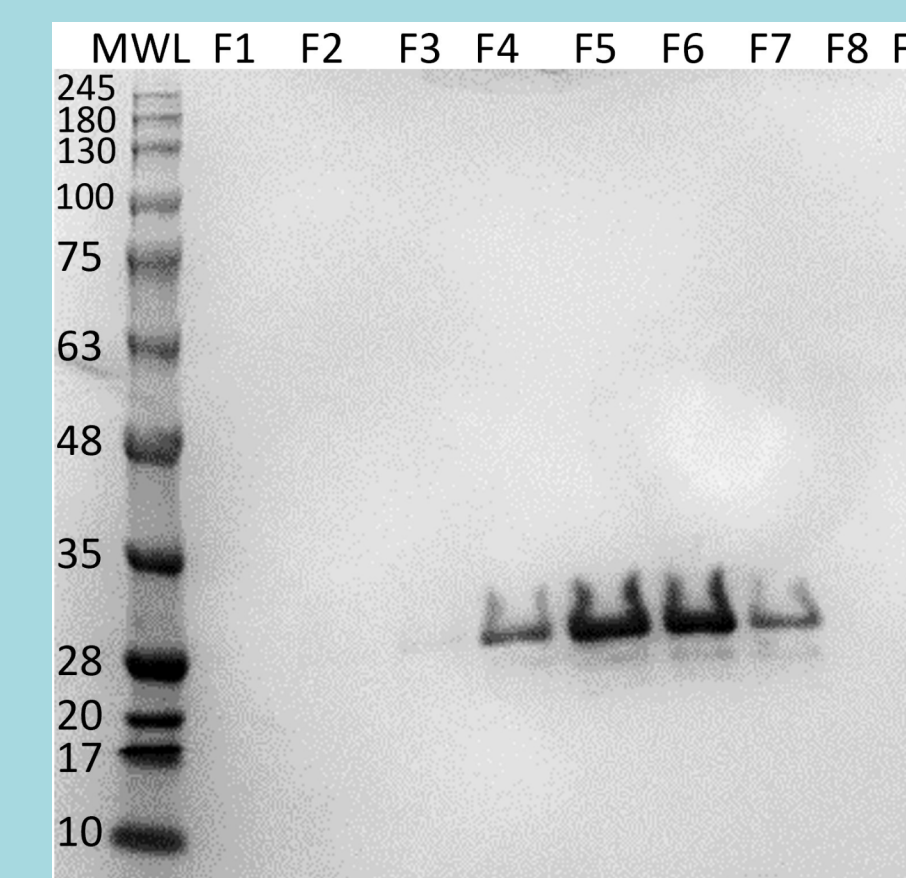


Figure 2. SDS-PAGE analysis of FGF2-G3 characterization via size-exclusion chromatography. Percentage purity (~98%) confirmed by MS/MS analysis. Mass corresponds to the monomeric form of FGF2-G3 with associated N-terminal tags (20.9 kDa). Lanes correspond to: RBG BroadRange molecular weight ladder (MWL; numeric labels in kDa), and SEC fractions 1-9 (F1-F9).

FGF2-G3 confirmation of pluripotency and single cell passaging

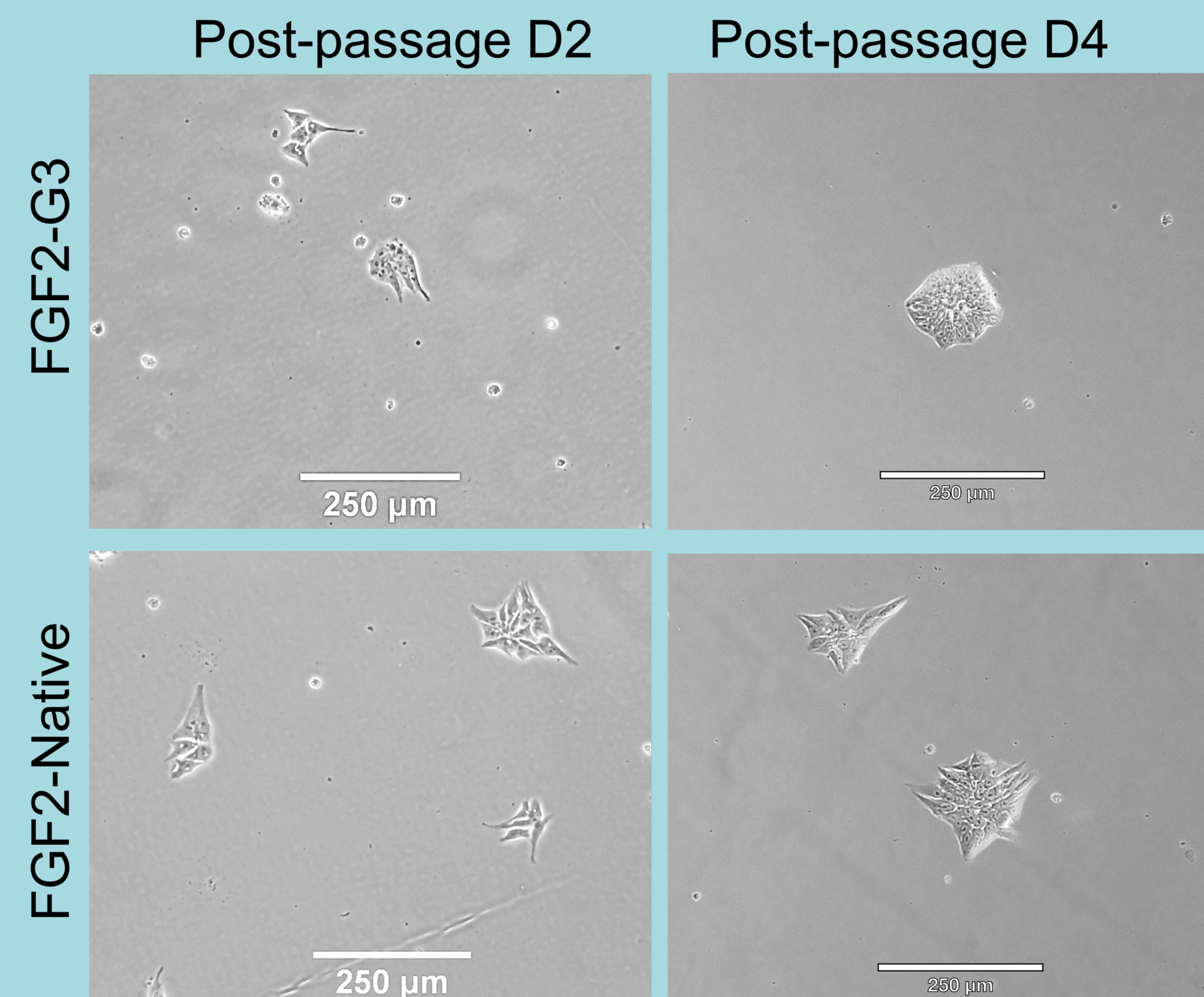
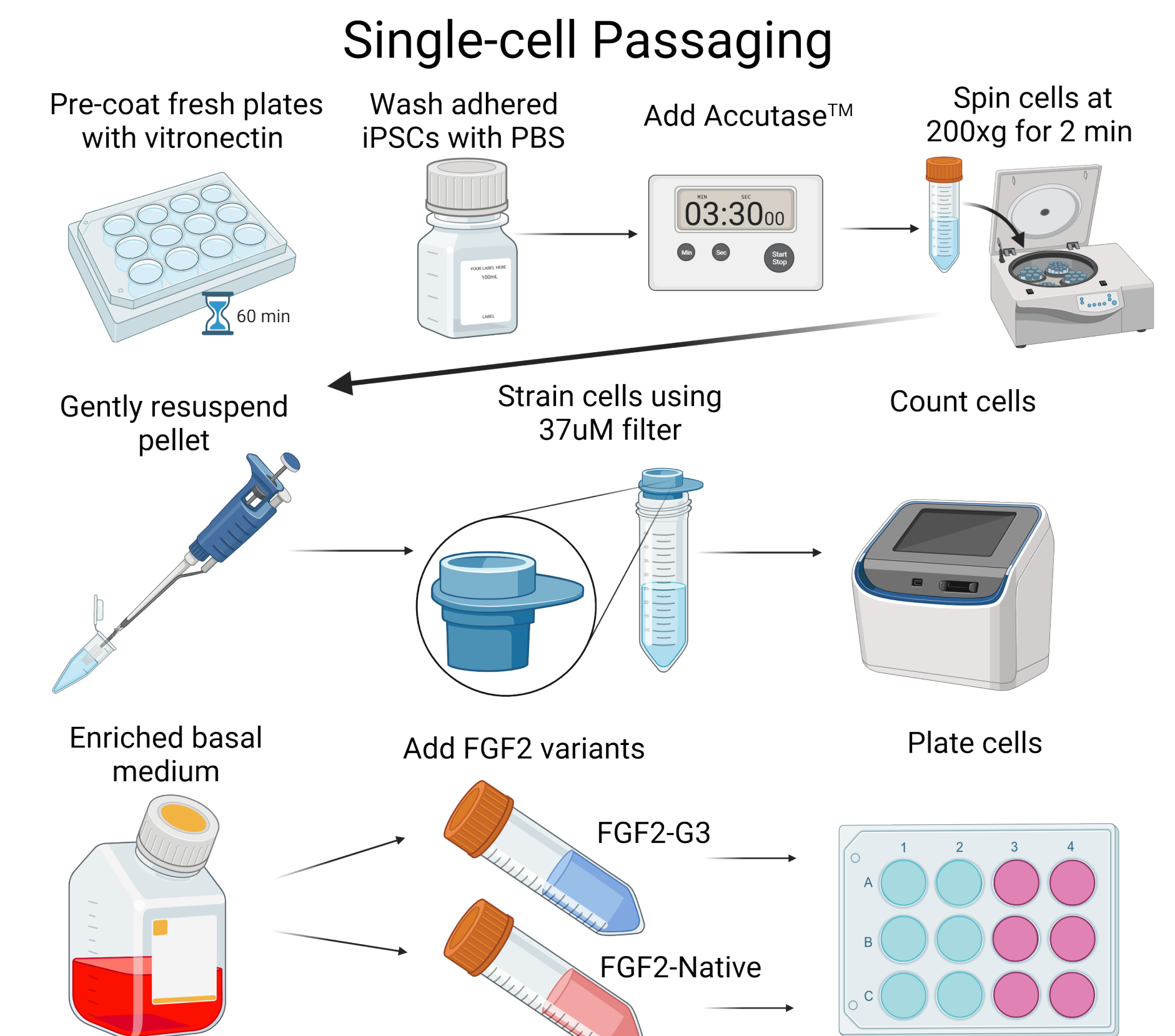


Figure 3. Single-cell suspension passaging comparing hyperstable FGF2-G3 to FGF2-Native. Top left - FGF2-G3 post passage day 2. Top right - FGF2-G3 post passage day 4. Bottom left - FGF2-Native post passage day 2. Bottom right - FGF2-Native post passage day 4.

Methods



Future Directions

This research demonstrated FGF2-G3 as a key component of HiDef-B8 for maintenance of human iPS cell phenotype and pluripotency.

Defined Bioscience continues to explore hyperstable and species-specific FGF2 variants using FGF2-G3 as a performance benchmark running more analyses including flow cytometry and metabolic assays.

Defined Bioscience is continuing to assess new growth factor leads for use in stem cell culture.

Acknowledgements