High Efficiency Cell Line Development of a Density-Dependent Hepatic Cell Line

Cell Microsystems, Inc., Durham, NC USA

Background

- Hepatic cell lines like Huh7, HepG2, and Hep3B are important tools for studying liver cancer, drug toxicity, and hepatic physiology.
- Traditional single cell cloning methods, such as single-cell dispensing and limiting dilution, leave a single cell alone in a well lacking growth factors secreted by neighboring cells. This leads to low outgrowth efficiency and cellular differentiation.
- An academic laboratory focused on hypoxia-related cell signaling pathways found that stressors associated with single cell cloning of Hep3B cells resulted in a permanent loss of hypoxia-induced erythropoietin expression.
- CellRaft® Technology increases cell line development efficiency by allowing single cells to share media while maintaining spatial separation and monoclonality.
- This poster presents an integrated workflow for cell line development of hundreds of Hep3B cells that maintain hypoxia-induced erythropoietin expression on just one CellRaft® Array. Expansion of single cells to large colonies with confirmed monoclonality required less than 2 weeks and less than 2 hours of hands-on time.

CellRaft® Workflow

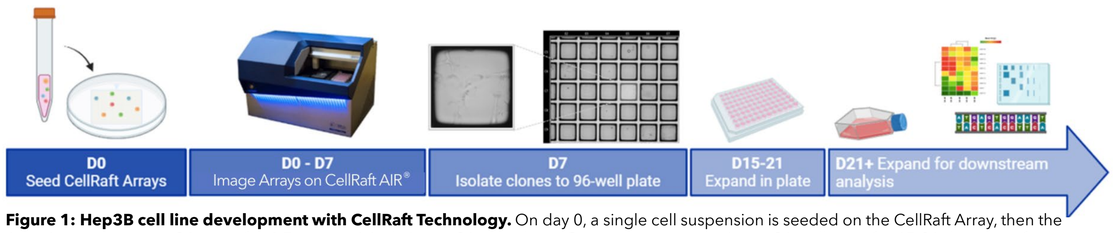

Figure 1: Hep3B cell line development with CellRaft Technology. On day 0, a single cell suspension is seeded on the CellRaft Array, then the Array is scanned every 24 hours for 4-7 days until isolation to 96-well plates. Clones are expanded in the 96-well plate for 15-21 days, then further expanded for downstream analysis. Figure created with BioRender.com.

Visual confirmation of monoclonality

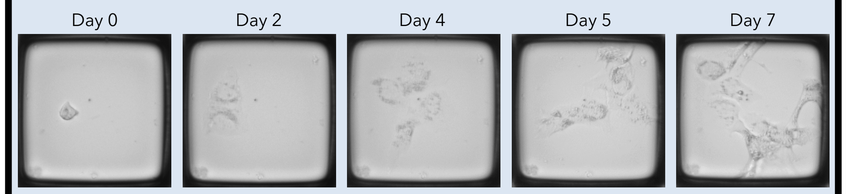

Figure 2: Representative time-course images of a single Hep3B cell proliferating on a 200 µm CellRaft. CellRaft Arrays were imaged on the CellRaft AIR System 4 hours post-seeding to identify single cells and then every subsequent 24 hours to monitor clonal growth until the isolation of clonal colonies on day 7.

Software-guided clonal selection identified monoclonal colonies within the CellRaft Array

Figure 3: Representative example of identification of monoclonal colonies using CellRaft Cytometry™. Two population sets, single cells on day 0 and confluent colonies on day 7, were overlayed to identify CellRafts containing monoclonal colonies.

Hundreds of single cells and monoclonal colonies were identified on each CellRaft Array

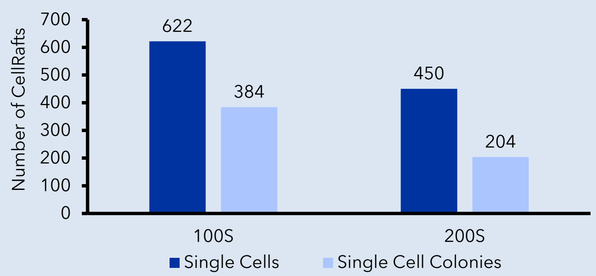

Figure 4: Number of single cells on the day of seeding and single cell-derived colonies at day 4 (100S) or day 7 (200S) post-seeding on CS-100S and CS-200S CellRaft Arrays. Hundreds of monoclonal colonies were available for isolation and further expansion from each Array.

Hep3B clones expanded from isolated CellRafts

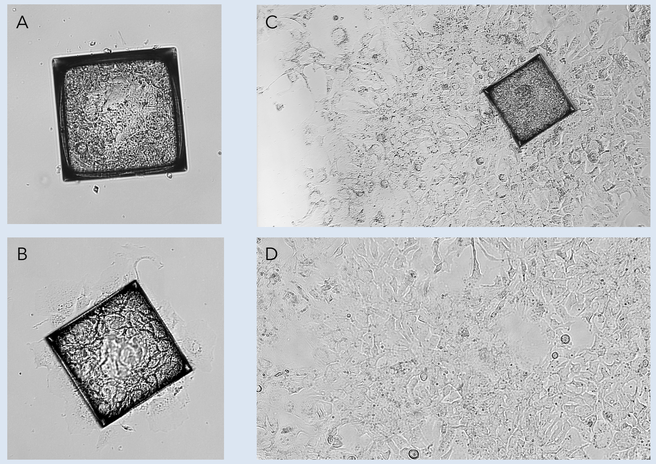

Figure 5: Hep3B clones post-isolation from 200S Array in conditioned or unconditioned culture media. (A) Hep3B clone in unconditioned media 9 days post-isolation failed to expand off Raft (20X magnification). (B) Hep3B clone expanding off Raft in conditioned media at 8 days post-isolation (20X magnification, (C) 15 days post-isolation (10X magnification), and (D) expanded to a T-75 culture flask (10X magnification).

Conditioned media improved monoclonal colony outgrowth after isolation to plates

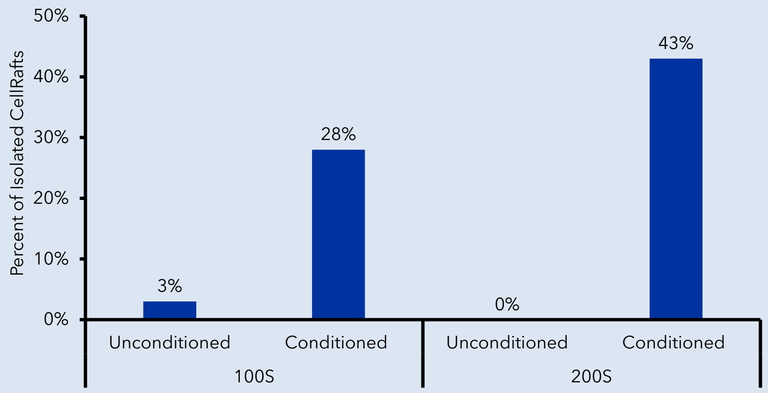

Figure 6: Outgrowth of Hep3B monoclonal colonies isolated from CellRaft Arrays into conditioned or unconditioned culture media. Conditioned media resulted in significantly higher outgrowth efficiency from both Array types. Optimal outgrowth was obtained from the 200S CellRaft Array isolated into conditioned media.

Monoclonal Hep3B populations exhibited a more homogeneous morphology than parental

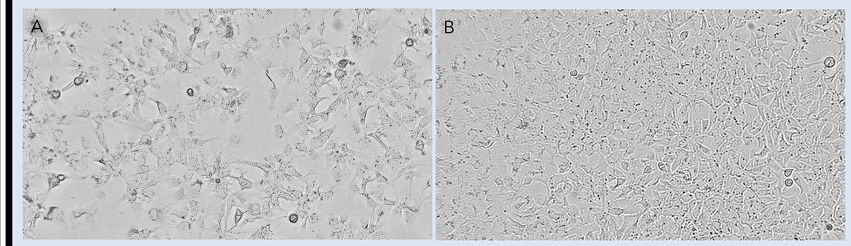

Figure 7: Monoclonal Hep3B populations exhibited a more homogeneous morphology than the parental polyclonal population. (A) Hep3B parental polyclonal population and (B) a monoclonal population expanded from a CellRaft (10X magnification).

Hep3B clones from CellRaft Arrays retained hypoxia-induced erythropoietin expression

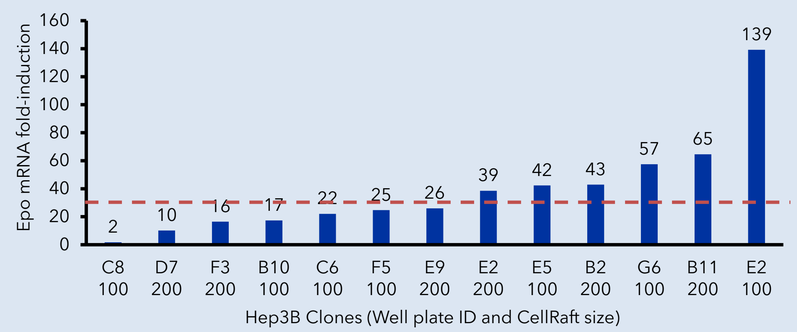

Figure 8: Hep3B clones exhibit high levels of Epo mRNA. Erythropoietin (Epo) mRNA fold-induction from hypoxic Hep3B clones (1% oxygen, 8 hours). Expected induction is 30-50-fold based on previous work with polyclonal Hep3B cells (red dashed line).

Summary

- One CellRaft Array generated hundreds of colonies, nearly half of which retained the expected hypoxia-induced erythropoietin expression, a phenotype previously lost due to the stressors of traditional single cell cloning workflows like limiting dilution.
- Over 100 cell lines have been validated on the CellRaft Technology platform, demonstrating significantly increased efficiency compared to limiting dilution and without the use of conditioned media. However, Hep3B colonies, rather than single cells, still failed to expand off-Raft when isolated into fresh media, emphasizing their need for growth factors from neighboring cells in shared media.

References

1. Biorender. Retrieved March 28, 2024, from https://biorender.com/.