FGF2, part two: evaluation of homologs for use in human and animal cell culture for the life sciences and cultivated meat

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Abstract

Fibroblast growth factor 2 (FGF2, or basic fibroblast growth factor) has been instrumental in the maintenance and differentiation of stem cells, particularly in serum-free medium formulations. While recent improvements in FGF2 stability and performance have centered on the human isoform[1] for use in conspecific cell culture, earlier work showed differential performance of cross-species homologs. Considering the rapidly expanding interest in food-grade cultivation of agriculturally relevant animal tissue stem cells, these data suggest that cross-species usage of high-performing homologs could provide benefit for both the life science and cultivated meat industries. Following previous work exploring nearly a dozen species homologs of FGF2 via chromatography, we evaluated several candidates for proliferation of human- and animal-derived cell lines. These results highlight the value of growth factor homolog screening when optimizing cell culture conditions.

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References

[1]Dvorak P, Bednar D, Vanacek P, Balek L, Eiselleova L, Stepankova V, Sebestova E, Kunova Bosakova M, Konecna Z, Mazurenko S, Kunka A, Vanova T, Zoufalova K, Chaloupkova R, Brezovsky J, Krejci P, Prokop Z, Dvorak P, Damborsky J. Computer-assisted engineering of hyperstable fibroblast growth factor 2. Biotechnol Bioeng. 2018 Apr;115(4):850-862. doi: 10.1002/bit.26531. Epub 2018 Jan 24. PMID: 29278409.

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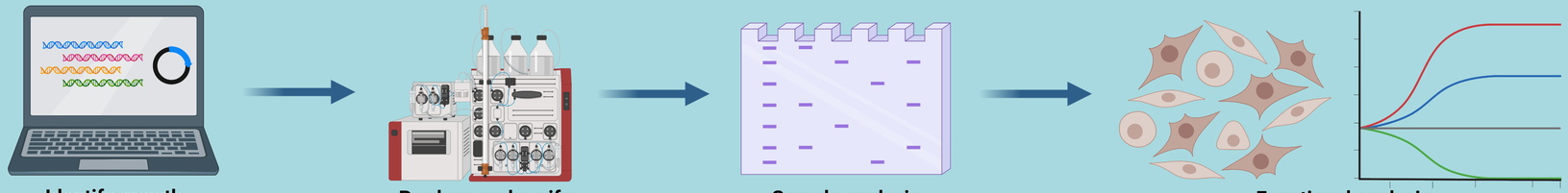
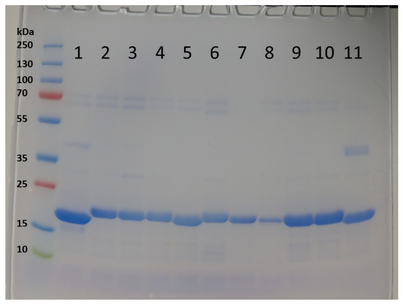
Workflow for FGF2 variant production and functional validation

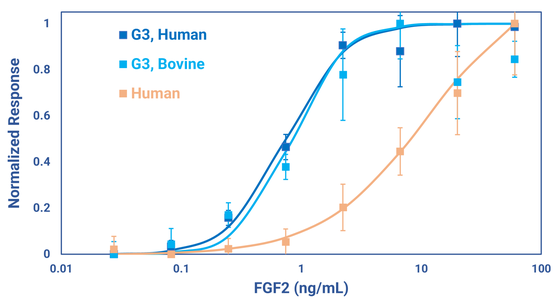
Defined Bioscience leverages expertise in protein engineering and stem cell technologies to create defined and robust solutions for stem cell culture, including purified growth factors, stem cell medium formulations, and protocols for downstream applications. With feedback from academic and industry professionals, we identified species and growth factors key to the life science and cultivated meat fields, and use our in-house pipeline to initiate production and validation trials.



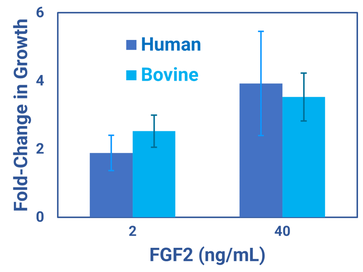
Human and Bovine FGF2-G3



Purified FGF2 variants: cow (1), atlantic salmon (2), sheepshead minnow (3), king salmon (4), red salmon (5), chicken (6), keta salmon (7), rainbow trout (8), tuna (9), coho salmon (10), and zebrafish (11).

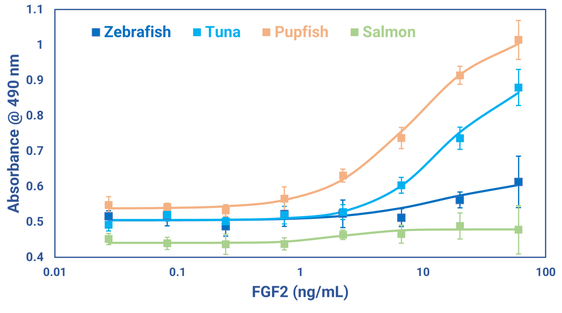


FGF2-G3 outperformed FGF2-native in an outgrowth assay of murine 3T3 cells, which were starved and exposed to FGF2 before analysis via MTS assay (absorbance at 490 nm). FGF2-G3 performed similarly when the same mutations were applied to the native bovine FGF2 sequence. Error bars reflect standard deviation (n=6).



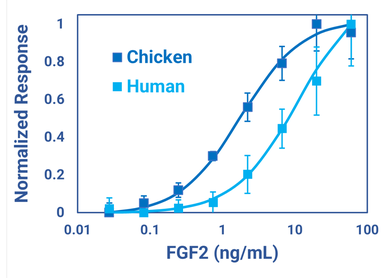
Additionally, both G3 homologs performed similarly in a growth assay of bovine satellite cells (BSCs) in HiDef-B8 supplemented with albumin (n=4), (performed by the Kaplan lab).

Piscine FGF2 homologs



An initial screen of FGF2 homologs from zebrafish, yellowfin tuna, pupfish (sheepshead minnow), and atlantic salmon demonstrated variable performance in initial 3T3 outgrowth assays, evaluated via MTS (n=6).

Chicken FGF2



The native sequence of chicken FGF2 showed a slightly improved response in the 3T3 outgrowth assay compared with human native FGF2 (n=6), though remaining less effective than FGF2-G3.

Future Directions

Defined Bioscience aims to further explore the potential benefits of FGF2 homologs in the culture of cross-species cells.

Validation in cell lines relevant to cultivated meat will help to generate new FGF2 variants with improved efficacy.

Continued research across species will enable convergence on homologs with broad effectiveness, increasing cell culture accessibility.