



Pioneering the future of high-value finfish:

Cultivated seafood product development



My background

- B.S/M.S in Cell & Molecular Biology and Ph.D. in Neuroscience
- President of the Association for Women in Science (AWIS)
- From microbes to neurodevelopment and schizophrenia, my research led me to cell line development and ultimately to BlueNalu.
- Key accomplishments at BlueNalu:
 - Developing finfish cell lines
 - Developing lipid loading in finfish





**BlueNalu is a next
generation seafood
company delivering
renewable protein, without
taking fish from the ocean.**



hIGH-VALUE finfish Prototypes

Yellowtail amberjack – fall 2019



Mahi mahi – summer 2021

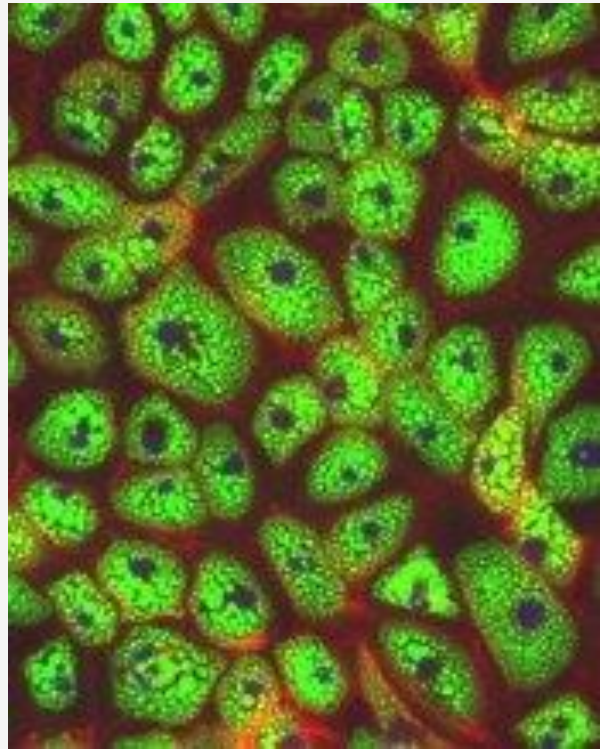


Bluefin tuna – summer 2022





Scalable process from cell to sushi





Cell line development



Flexible cell line platform technology

BlueNalu developed a platform technology to routinely generate cell lines from a wide range of fish species

Species Selection Strategy

- Vulnerable in the wild
- Difficult to farm-raise
- High in contaminants
- Primarily imported
- Drives premium price point

Cell types of interest include muscle, fat, & connective tissue

CLD Guideposts

- ✓ Non-GMO cells
- ✓ Scalable cell-line
- ✓ Cells in suspension
- ✓ Animal-Component Free Media

CELL LINE DEVELOPMENT OVERVIEW

- **Stable line generation (2D)**

- Primary cells are stabilized in adherent culture to achieve reproducibility and longevity

- **Differentiation and lipid loading**

- Identify lines with high differentiation to muscle or fat
- Lipid loading enables decreased cost of production

- **Suspension cell line generation**

- Adaptation from 2D to suspension enables easier scale-up of cell cultures
- Faster growth rate improves commercial production volumes

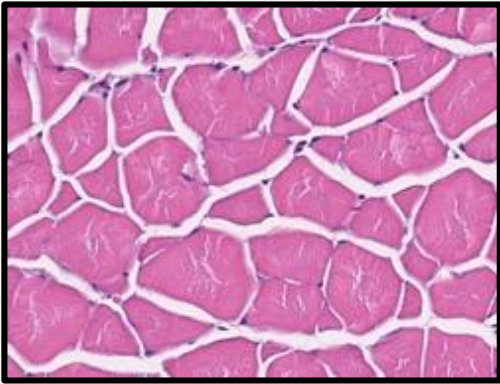
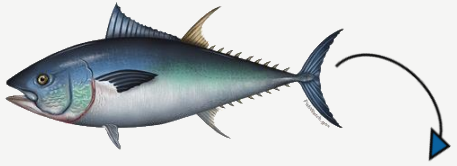
- **Cryopreservation optimization**

- Optimized cryopreservation process allows for long-term storage, batch to batch uniformity, and global distribution of cell banks

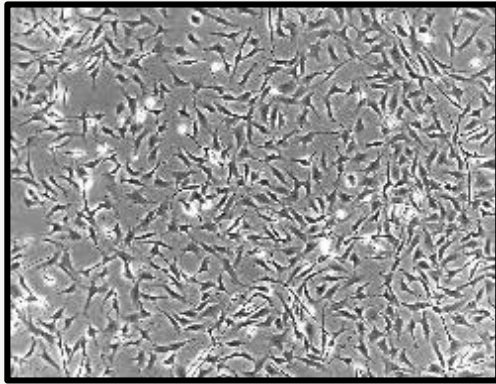
- **GMP cell banking**

- Master cell banks are starting ingredient in manufacturing process and must be validated for regulatory approval

DEVELOPING 2D STABLE CELL LINES



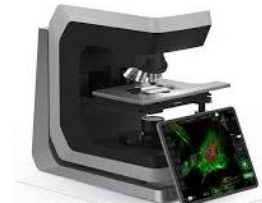
Muscle samples are procured from species of interest



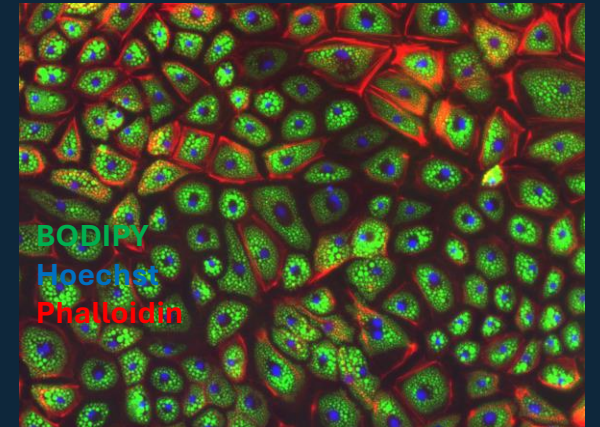
Muscle cells are isolated and cultured *in vitro*, grown in media with serum. Many growth factors, media components and media were tested to develop our in-house media formulation



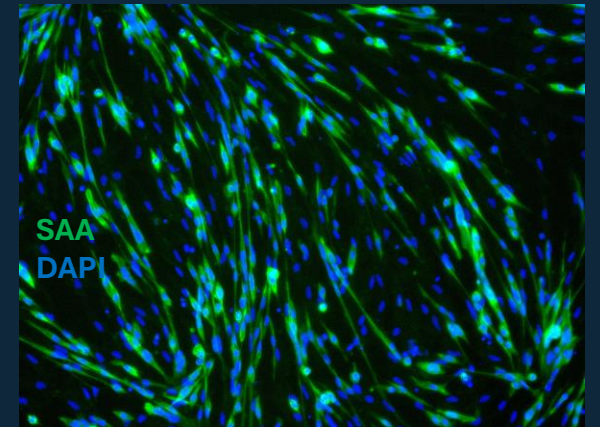
Cells are grown in static incubator in 2D



Cells imaged on microscope



Pre-adipocytes readily load with lipids such as Omega-3 fatty acids

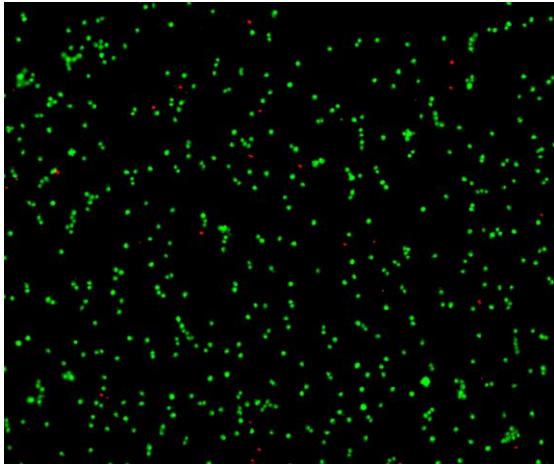


Cells form elongated and multinucleated myotubes

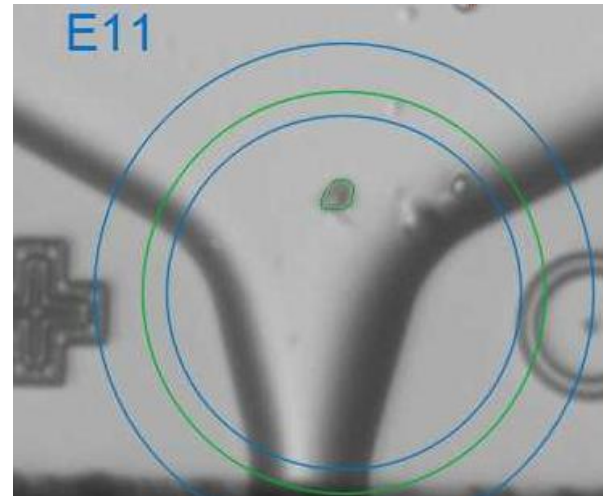
Myotubes express sarcomeric alpha-actinin with visible striations

Adaptation to Suspension & Generating Monoclonal Cell Lines

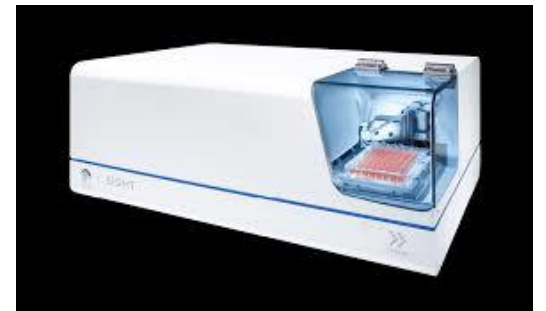
- Cells must survive, thrive, and be optimized in suspension culture
- Optimization includes transitioning media to food grade (FG), antibiotic free and animal-component free media (ACF)
- Single cell cloning and line selection were performed to ensure genetic uniformity



Example image of bluefin tuna cells in suspension culture. Live cell staining (green) shows the high percentage of viable cells.



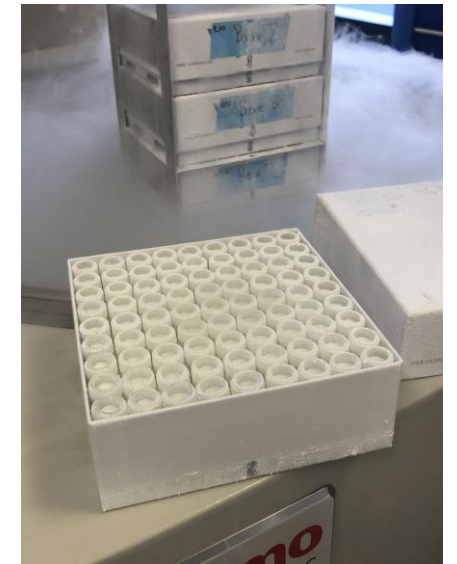
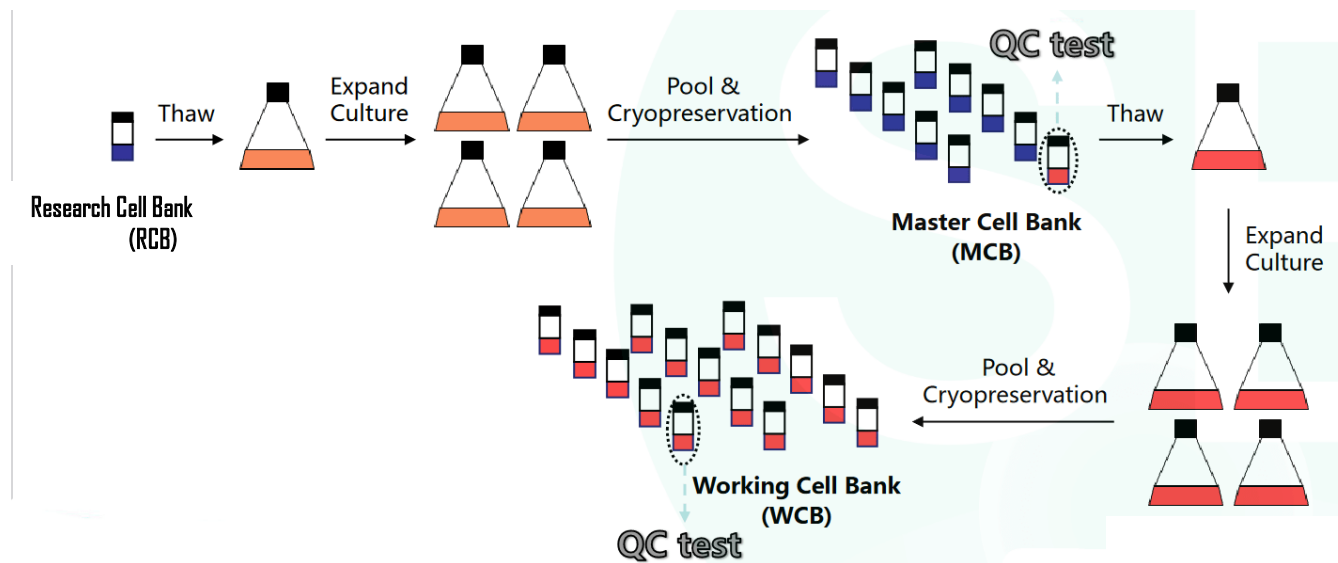
Single cell confirmation for uniform cell line development via C. Sight & CSI



Single cell cloning via C- Sight

Cell Banking of Suspension Cell Lines

- FG ACF adapted clonal line cryopreserved (proprietary) and GMP banks generated
- GMP Master and Working cells banks are required for manufacturing
- Cryopreserved cell banks are the starting point for manufacturing
 - Back-up cell banks are stored at a geographically distinct GMP storage location
- Upon generation of banks, cell line source information and cell bank testing are required for regulatory safety review



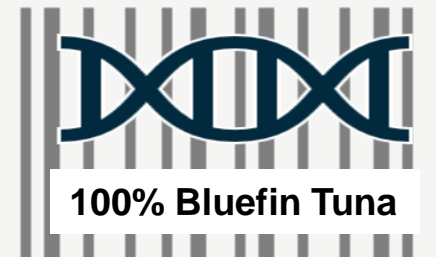


Cell bank validation & Food safety



Cell bank validation

- Pathway developed in collaboration with FDA and risk-based testing panel established
- Identity
 - Consider source material
 - Test for cross-contamination
- Stability
 - Growth performance metrics
 - Protein expression
 - Metabolism
- Sterility
 - Bacteria / Fungi
 - Viruses
 - Mycoplasma



CONTROLLED MANUFACTURING YIELDS PRODUCT SAFETY BENEFITS

- BlueNalu has a GMP pilot production facility, capable of producing our cell-cultivated product
- Through risk assessment of species, raw materials & handling, manufacturing controls mitigate otherwise common seafood risks
- Testing of BlueNalu product shows non-detectable microbial counts and mercury which is not typical of conventional product

Comparison of Cell-cultivated to Conventional Bluefin Tuna

Tests	BlueNalu Bluefin tuna Cells (n=3) & toro block (n = 2)	Bluefin tuna Market (Range; n=5)
APC	<10/g	180 – 12,000/g
Coliform	<10/g	<10 – 170/g
Yeast	<10/g	<10 – 180/g
Mercury*	<0.005 ppm	0.566 – 1.79ppm

BlueNalu's Commitment to Food Safety and Quality

Food Safety is a number one priority as we prepare for commercial production, and we have developed systems to comply with applicable US Food Safety Regulations.

- System follows 21 CFR 123 – Fish and Fishery Products
- Quality Management System and our first-of-its-kind, cell-cultured seafood HACCP Program.
- Applies best practices from industry guidance like the National Fisheries Institute and international standards, such as the International HACCP Alliance.



- BlueNalu has a commitment to quality assurance and food safety on a global level.
- We plan to obtain third-party food safety certification via a Global Food Safety Initiative (GFSI) recognized scheme (e.g., SQF, BRC, FSSC 22000, or equivalent).





SCALING FOR MANUFACTURE

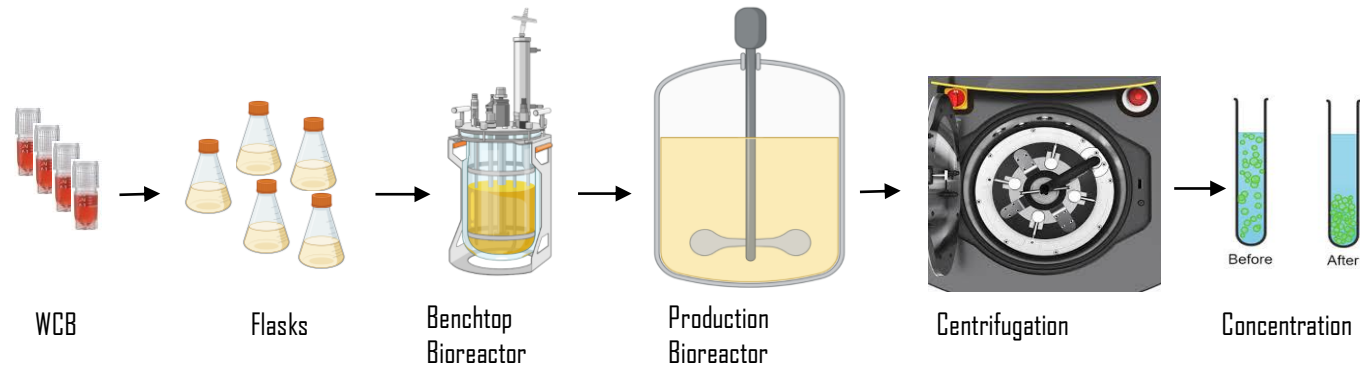


Scaling for manufacturing

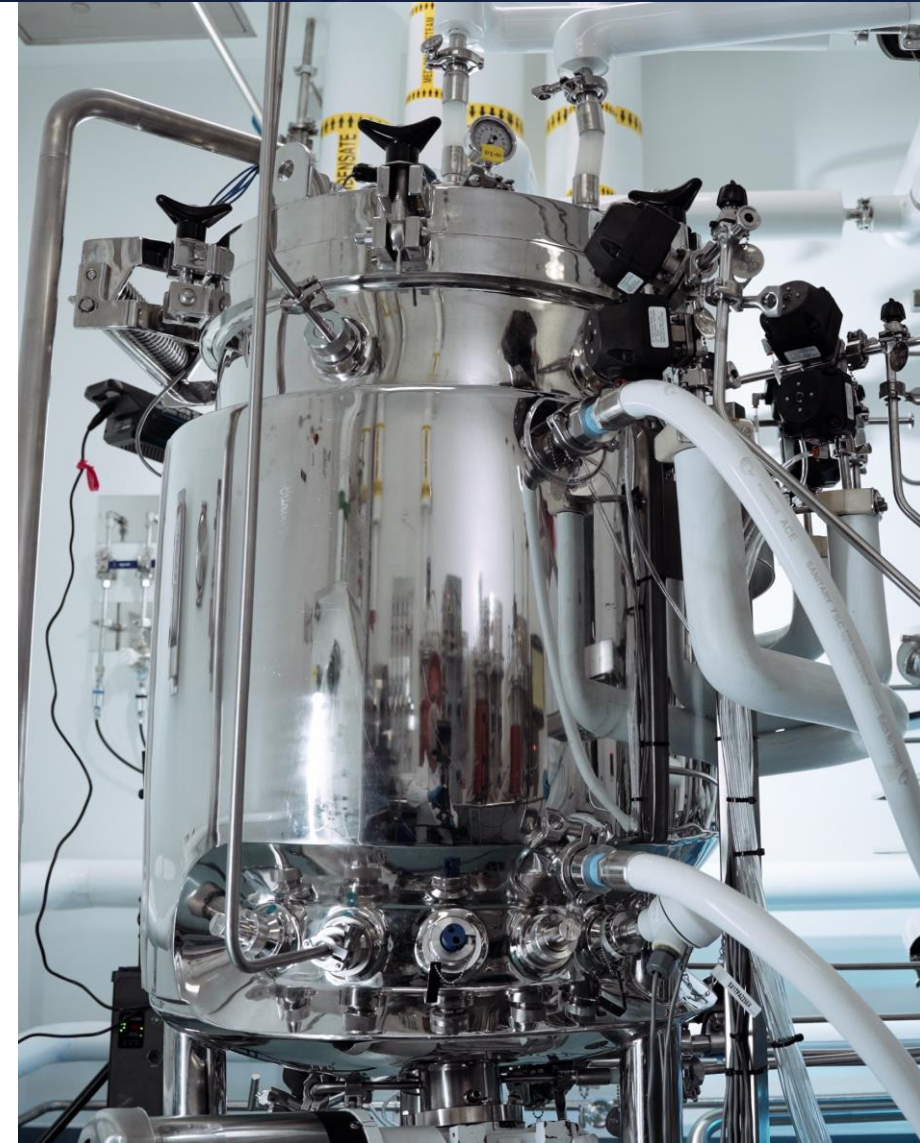
- Cell lines must be capable of performing during development and manufacturing scales
- Non-GMO process eases regulatory pathway and provides easier consumer acceptance
- Cells must perform and meet expectations for various metrics
 - Longevity
 - Growth rate
 - Differentiation rate
 - Stability
 - Scalability



Cell-Cultured Seafood Production



BlueNalu Bluefin tuna toro Saku Block



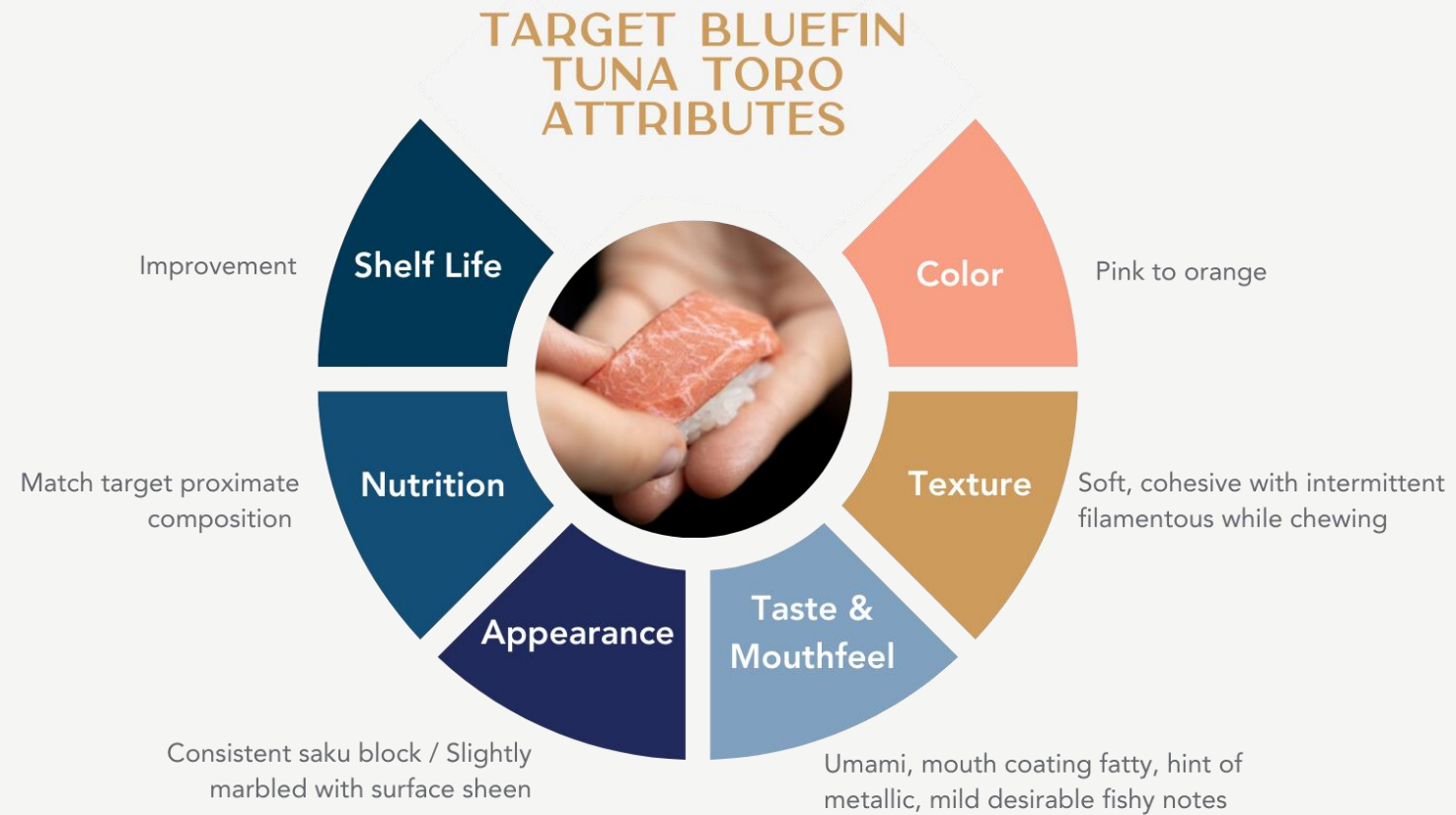


Product

BLUEFIN TUNA PRODUCT CONSIDERATIONS & OPTIMIZATION



- Partner with chefs to test culinary performance & sensory qualities of prototype
- Chefs provide quantitative ratings across applications
- Iterative sessions track progress and optimize prototypes





First commercial product: bluefin tuna toro

Bluefin tuna is the most prized fish in the world, but intense global demand has severely depleted supply. Toro, the most valuable cut, is a sought-after delicacy – also known as the ‘wagyu beef of the sea’



- ✓ Free from mercury
- ✓ No parasites or antibiotics
- ✓ Consistent quality
- ✓ 100% yield
- ✓ Year-round availability
- ✓ Non-GMO cells
- ✓ Better for the ocean

BLUENALU'S FOCUS ON HIGH QUALITY, SAFE, DELICIOUS SEAFOOD

Starting with Bluefin Tuna Toro





QUESTIONS?

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Thank you

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