

UNLOCKING BETTER ZEBRAFISH ANTIBODIES

The Secret Art of Antigen Design





TODAY'S ROADMAP

01

Current Zebrafish Antibody Dilemma

Great potential, lacking
antibodies

02

BOSTER Revolutionary Solutions

Game-changing innovation

03

Techniques For Antigen Design

See how antigen magic works

04

Q&As

Join The Antibody Revolution!

Current Zebrafish Antibody Dilemma

ONE OF THE MOST
INFLUENTIAL MODEL
ORGANISMS

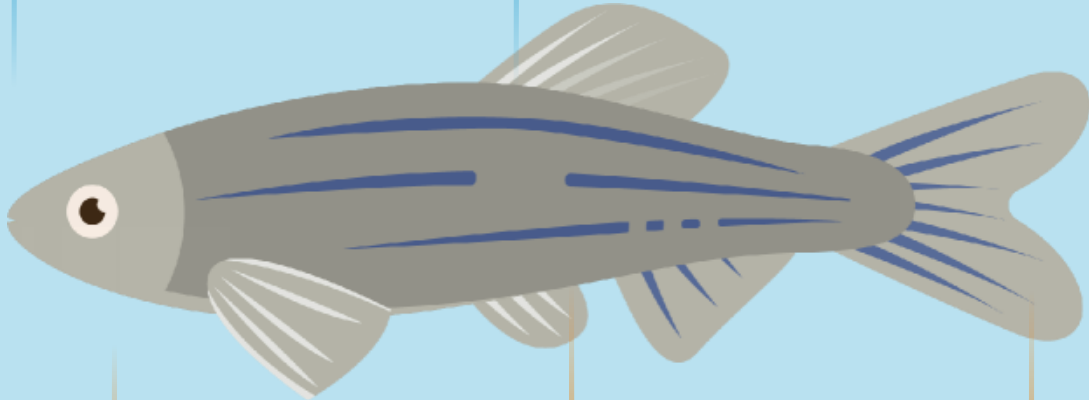
Transparency

01

Relevance to
Human Diseases

02

Unique Qualities:



Other Traits:

Ease of Genetic
Manipulation

01

High
Fecundity

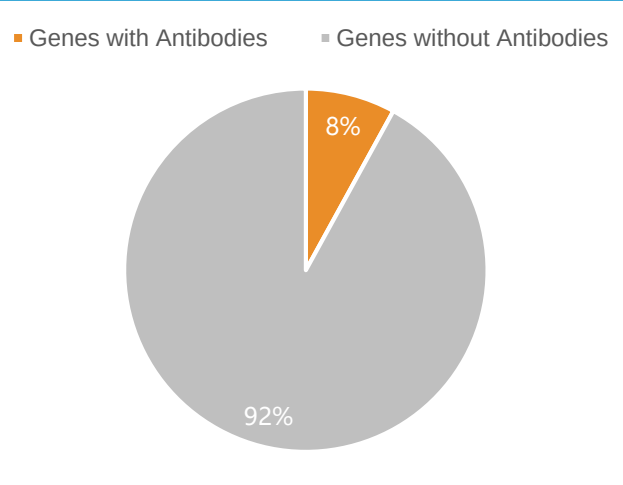
02

Rapid
Development

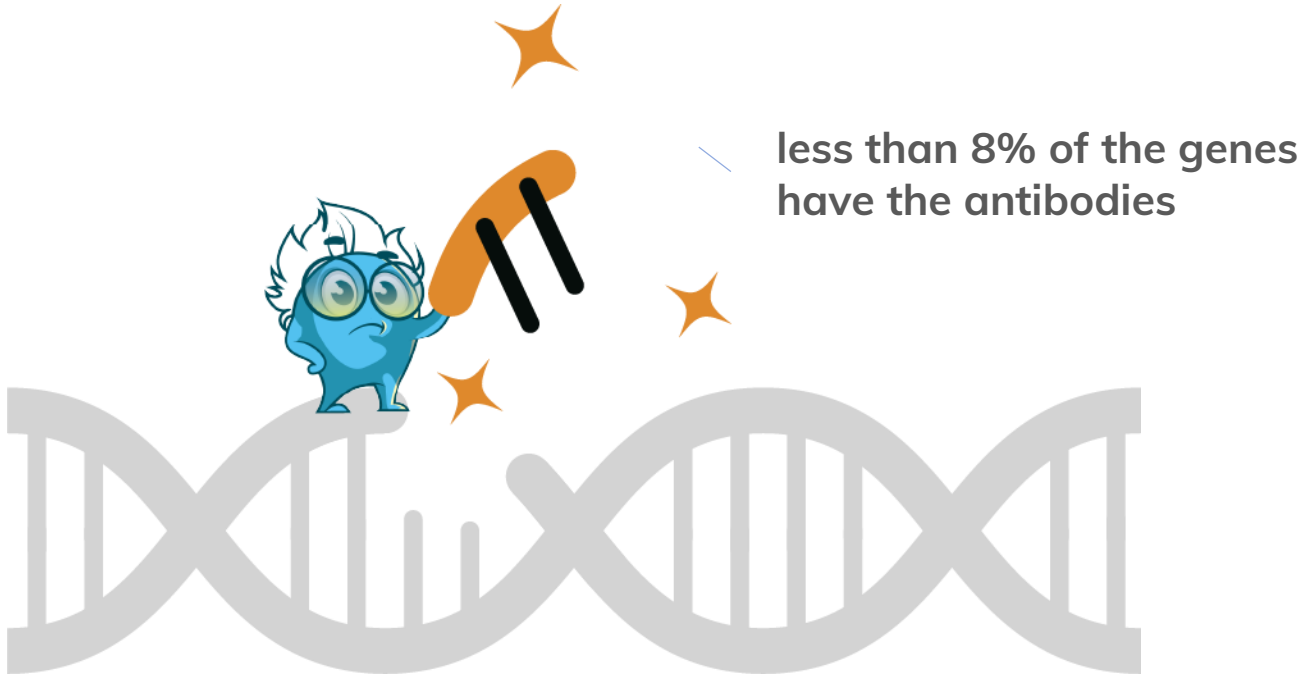
03

Current Zebrafish Antibody Dilemma

The Antibody Challenge



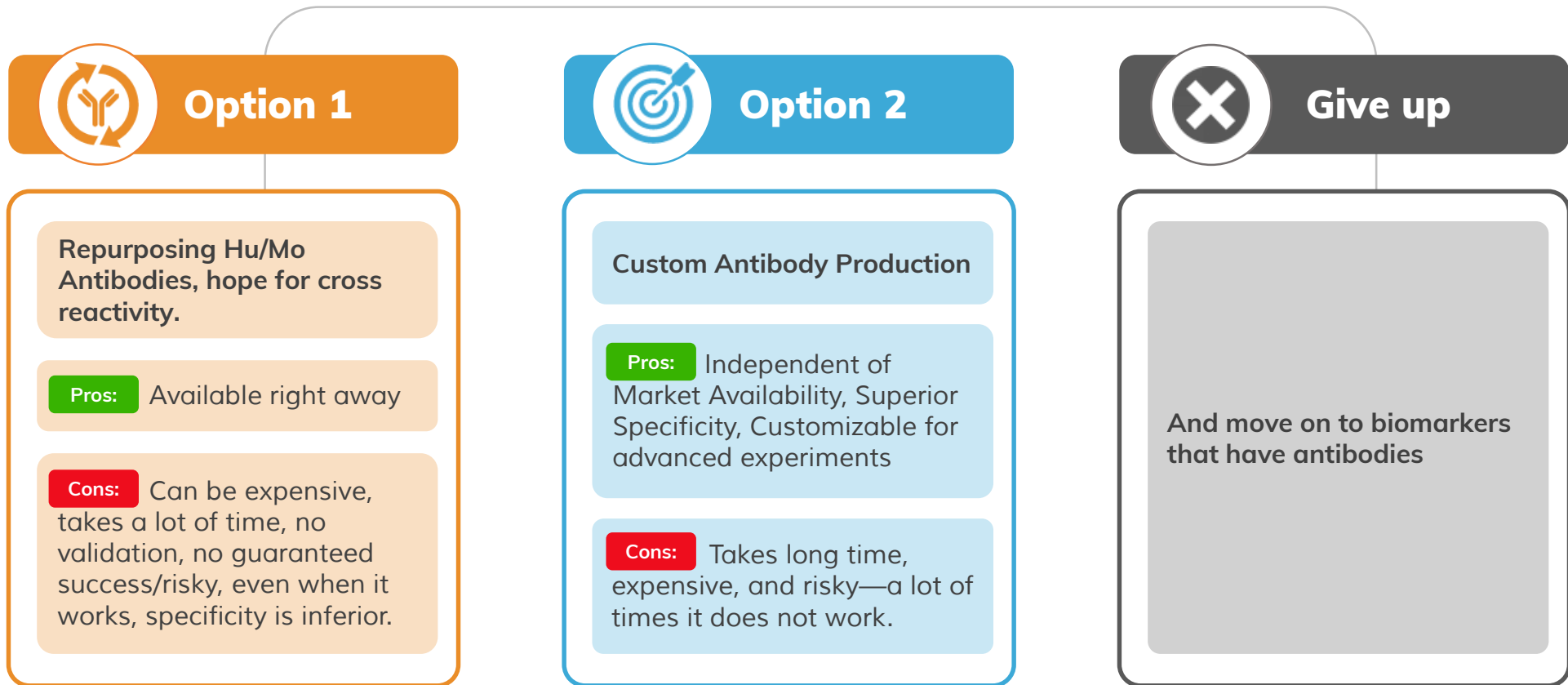
The Antibody Challenge in Zebrafish Research



Limited Availability

92%+ protein-encoding genes in Zebrafish have no antibodies on zfin.org

Options When There are No Antibodies



With the Boster Zebrafish Solution:

- ✓ Get results in 2-3 days
- ✓ We do the validation experiments for you
- ✓ Validate for free, only buy after knowing it works.
- ✓ 9000+ participating antibodies on bosterbio.com

Boster's Solution For Repurposing Antibodies

Solution 1

Free validation using Zebrafish:

Boster Bio is the only antibody company that offers a free antibody validation program for zebrafish users. Have your antibodies custom tested on relevant samples (most tissues and 5000+ cell lines available).

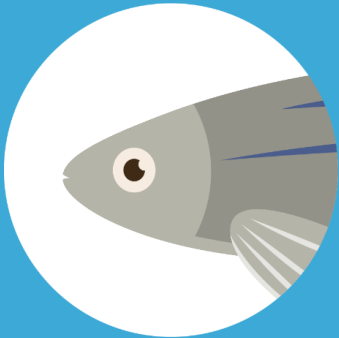
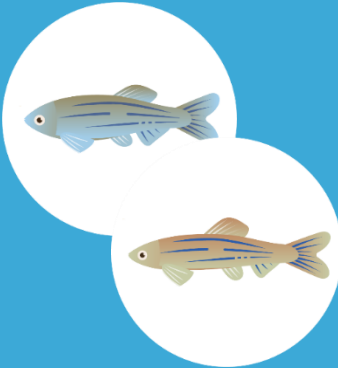
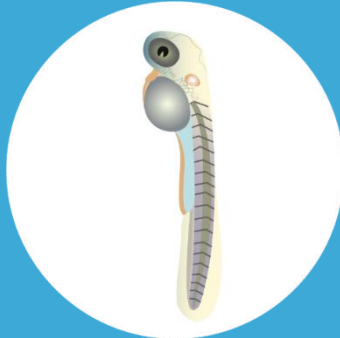
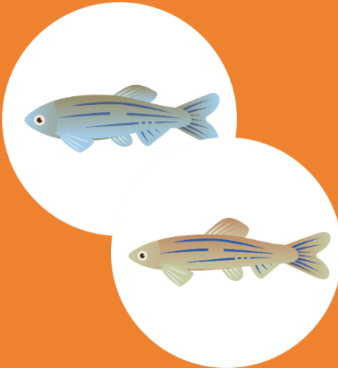
Available assays:

WB, IHC/IF, IP, FC. Validation is done based on YOUR custom experiment requirements on demand. Takes 2-3 days to get results back.



Old Challenges:

- ✗ Takes time to receive antibody and validate
- ✗ Takes days to do the experiments
- ✗ Cost money upfront to buy the antibodies
- ✗ Have to search all over to find viable antibodies

WB, IP	 Head lysate	 Adult Whole-body lysate	 48 HPF Embryo lysate
IHC, IF		 Adult whole-body paraffin sections	 48 HPF Embryo paraffin sections

With the Boster Zebrafish Solution:

- ✓ \$600 per antibody
- ✓ High success rate (we will dive into this in later slides)

Boster's Solution For Custom Antibodies

Boster Bio offers a \$600 custom antibody service for making antibodies for zebrafish targets.

It is full service, covering all steps from antigen synthesis to antibody purification.



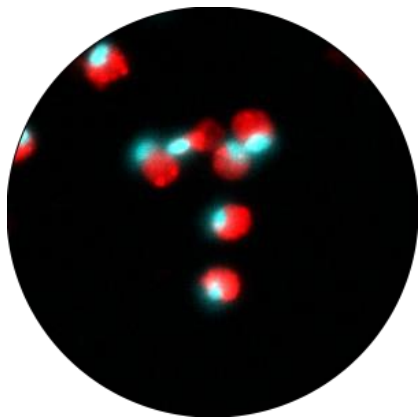
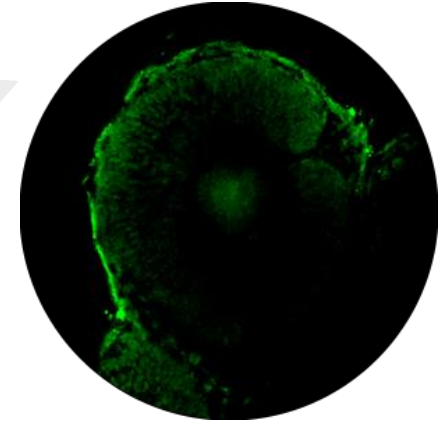
Solution 2

Old Challenges:

- ✗ Expensive, between \$3000 to \$5000
- ✗ Low success rate

Testimonies

This antibody shows some promising results in whole mount applications using 24hpf embryos. Applications with cryosections may be more precise.

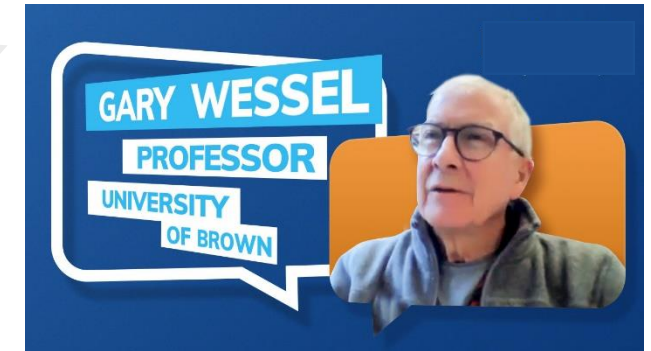


We ordered this antibody as a custom antibody from Boster Bio to stain for zebrafish eosinophils in FFPE tissue sections and fixed cells. It works wonderfully: very bright, with no relevant background or off-target staining, and highly reproducible.



Testimonies

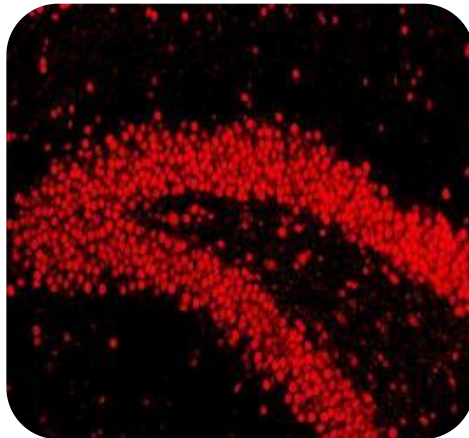
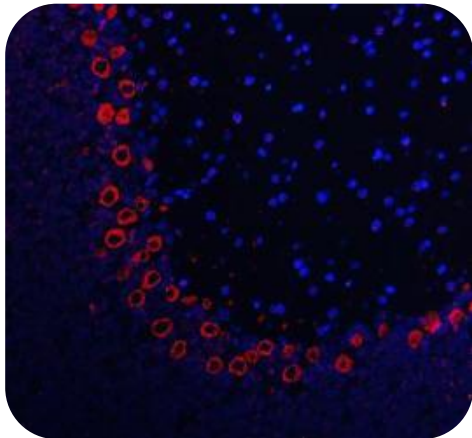
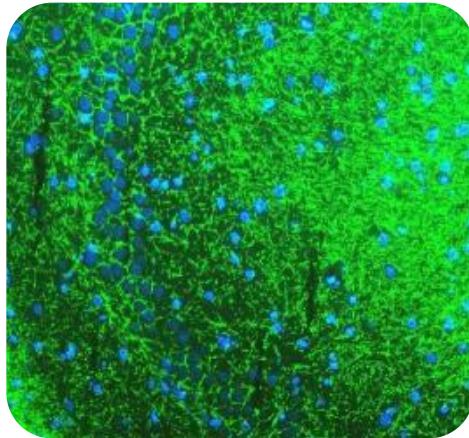
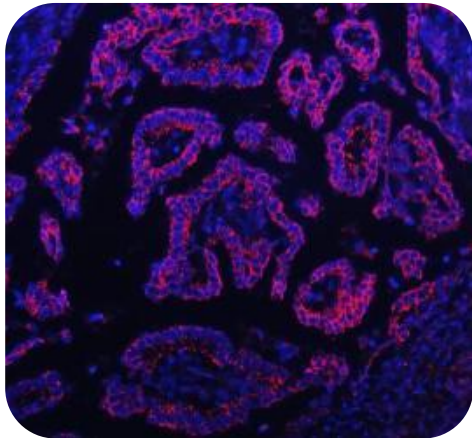
I can imagine the ability for people now to rely on you all for quality products and not guesswork products. So keep doing what you are doing!



When we saw this deal, my first impression was: Is this for real? Because it sounds almost too good to be true



Fluorescent Dye-Conjugated Primary Antibodies



Bosterbio provides a wide range of fluorophore-conjugated primary antibodies specifically designed for **immunofluorescence** applications. Our popular monoclonal and polyclonal antibodies are now directly conjugated with fluorescent dyes such as **Cy3**, **FITC**, **APC**, **PE**, **Dylight 488**, **Dylight 550**, **Dylight 594**, and **iFluor 647**. These antibodies are ideal for immunofluorescence, requiring no secondary.

01

Commonly known best practices

- Avoid homologous sequences
- Pick epitopes according to application needs (surface accessibility, functional epitopes, etc.)

02

Unique insights from Boster Bio

- Use recombinant protein instead of peptides
- Pick the right hydrophobicity mix, hydrophilic flanking if necessary
- Predict and maximize Immunogenicity (based on local conformational contrasts)

Avoid Homologous Sequences

neurotrophin-3 isoform 2 preproprotein [Homo sapiens]

Sequence ID: [NP_002518.1](#) Length: 257 Number of Matches: 1

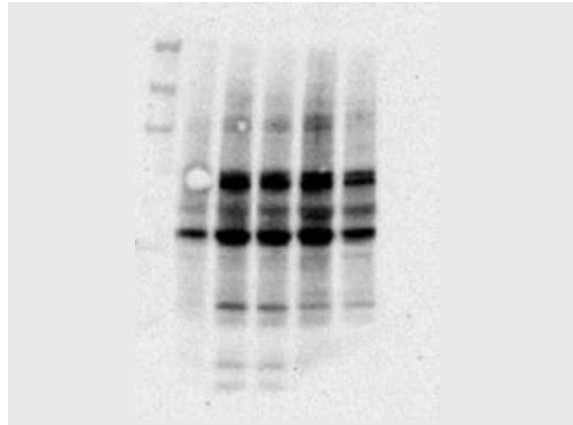
[See 8 more title\(s\)](#) [See all Identical Proteins\(IPG\)](#)

Range 1: 60 to 256 [GenPept](#) [Graphics](#) [Next Match](#) [Prev](#)

Score	Expect	Method	Identities	Positives	Gaps
174 bits(440)	2e-52	Compositional matrix adjust.	95/212(45%)	128/212(60%)	19/212(8%)
Query 40	HGTLESVNGPKAGSRGLTSLADTFEHVIE---	ELLDEDQKVRPNEENNKDADLYTSRVML	96		
	TL P+ RG + F+ VI ELL + ++	+ RV+L			
Sbjct 60	QSTLPKAEAPREPERGGPA-KSAFQPVIAMDTELLRQRRYN-----	SPRVLL	106		
Query 97	SSQVPLEPPLLFLLEEYKNYLDAANMSMRVRRHSD-PARRGELSVCDSEWVTAADKKT	155			
	S PLEPP L+L+E+Y AN + R +R+++ + RGE SVCDS S WVT DK +				
Sbjct 107	SDSTPLEPPPLYLMEDYVGSPVVANRTSRRKRYAEHKSHRGEYSVCDSESLWVT--DKSS	164			
Query 156	AVDMSGGTVTVLEKVPVSKGQLKQYFYETKCNPMGYTKEGCRGIDKRHWNSQCRTTQSYV	215			
	A+D+ G VTVL ++ +KQYFYET+C K GCRGID +HWNSQC+T+Q+YV				
Sbjct 165	AIDIRGHQVTVLGEIKTGNSPVKQYFYETRCKEARPVKNGCRGIDDKHWNSQCKTSQTYV	224			
Query 216	RALTMDSKKRIGWRFIRIDTSCVCTLTIKRGR	247			
	RALT ++ K +GWR+IRIDTSCVC L+ K GR				
Sbjct 225	RALTSENNKLVGWRWIRIDTSCVCALSRKIGR	256			

Commonly known best practices

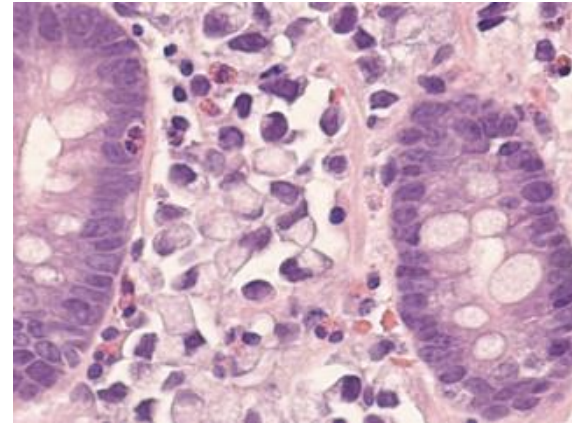
Pick Epitopes According To Application needs



Aplication: Western Blot

Antigen retrieval: Very Aggressive

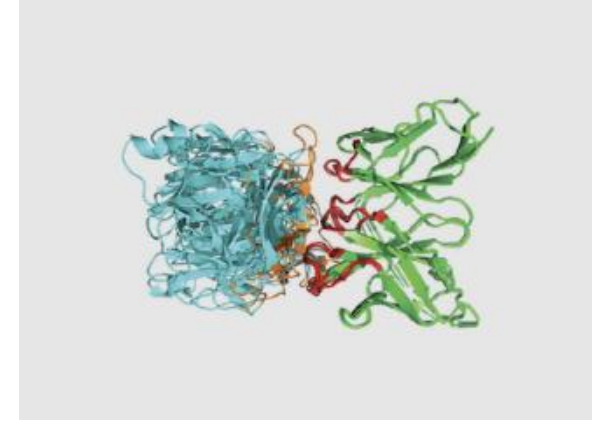
Epitope selection priority: Immunogenicity



Immunohistochemistry

Strong

Immunogenicity + Surface
Accessibility



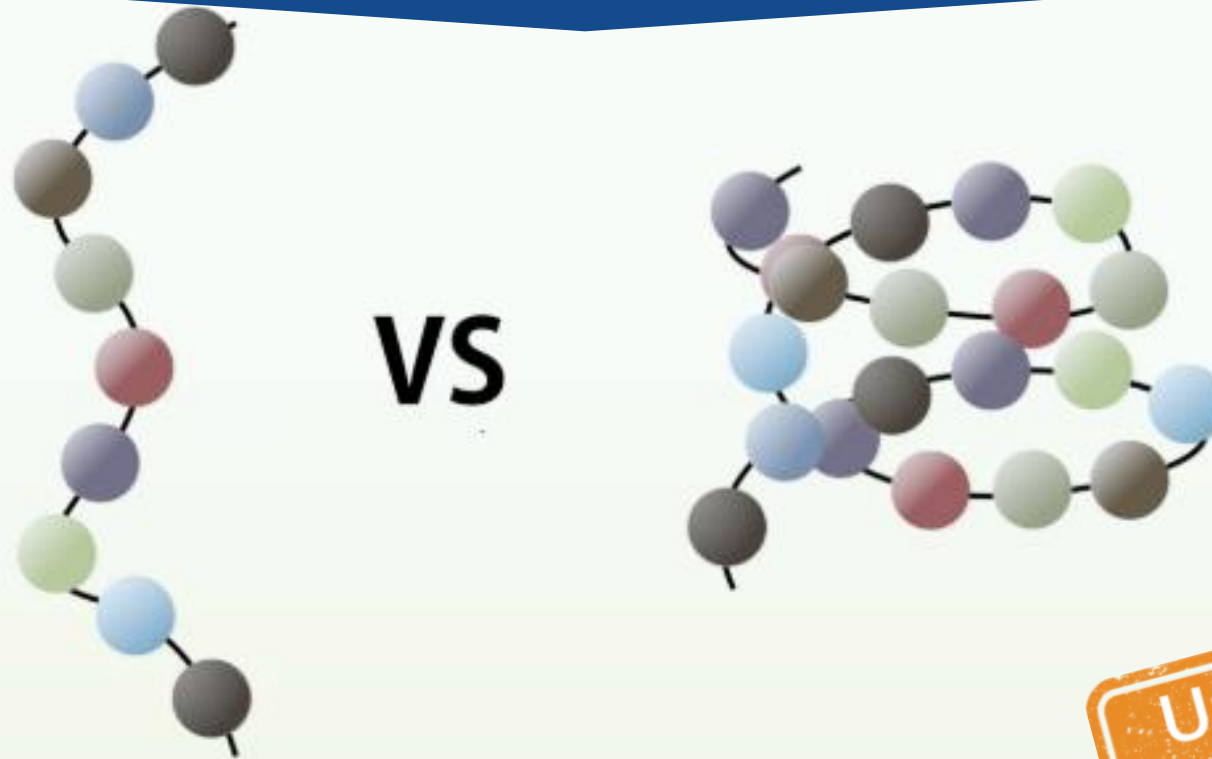
Functional Assay

None

Target Functional Domain

**Commonly known
best practices**

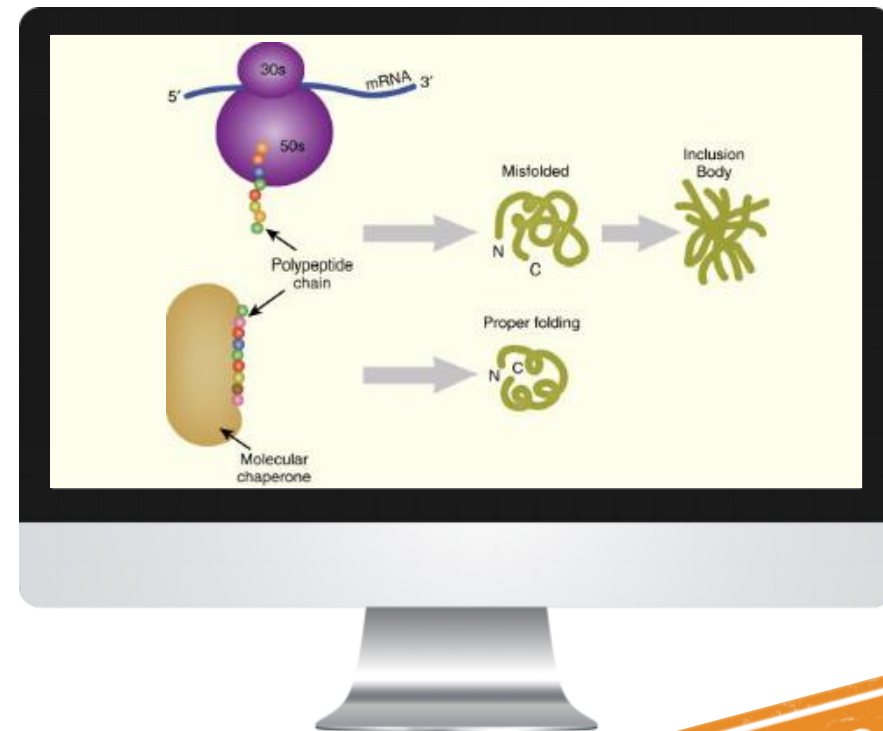
Use recombinant protein
instead of peptides



Unique insights
from Boster Bio

Hydrophobicity Mix & Hydrophilic Flanking

Highly hydrophobic sequences lead to protein clustering and formation of inclusion bodies. Hydrophobic interactions are also ($>10x$) stronger than hydrogen bonds and can lead to non-specific interactions. Avoiding highly hydrophobic sequences, and when you have to use them, flank these hydrophobic sequences with hydrophilic ones to mitigate the above issues.



Unique insights
from Boster Bio

Predict and maximize Immunogenicity



The interaction between Complementarity Determining Region (CDR) and epitopes is like grabbing onto rocks. The more **local contrast** between neighboring amino acids, the easier it is for immune system to come up with a CDR with strong affinity towards that epitope.

Unique insights
from Boster Bio

Opportunities and Q&As.



Be the first lab to have antibodies for critical biomarkers and publish Groundbreaking Papers



Many low-hanging opportunities just opened Many biomarkers' research were hindered by lack of antibodies, which is no longer the case.



With that, we will take questions.

InVivo Biosystems: *C. elegans* and zebrafish preclinical models

Amy Robbins, Ph.D. – Scientist I at IVB specializing in custom zebrafish models of rare disease

10

Patents

30

Number of Employees

30%

PhDs on Staff

4000+

Genetic Models Created

247

Customer Publications

18

Average Years of
Experience in Genetics

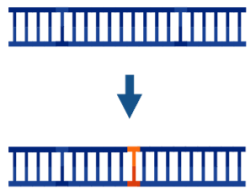
IVB is passionate about partnership. We help biotechnology, pharmaceutical, and academic clients in:

- RapidGen™ Preclinical Platform: Custom Genetic Modeling
- Drug Discovery
- Toxicity Testing
- Compound Efficacy Testing

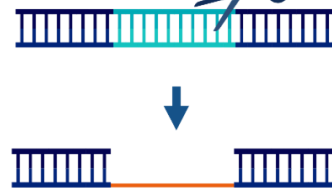


Industry leaders and innovators in CRISPR genome editing of *C. elegans* and Zebrafish

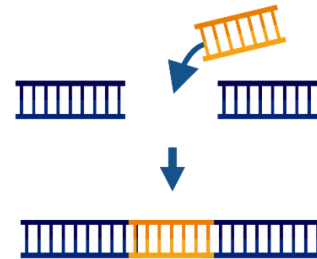
Precise Point
Mutation



Knockout (indel,
deletion, F0
crisprants)

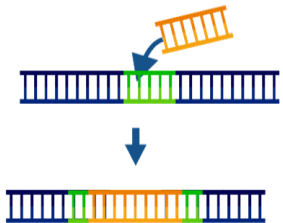


Knock-in

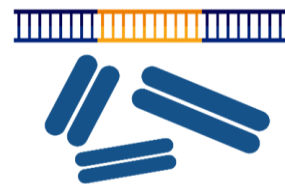


We select or create
the right model for
your research.

MosSCI



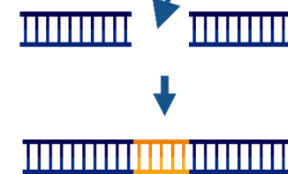
Extrachromosomal
Array



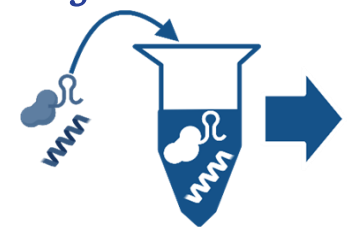
Inject Express



Tol2
Insertion



Custom
Injection Mixes



Antibody and Immunostaining Applications

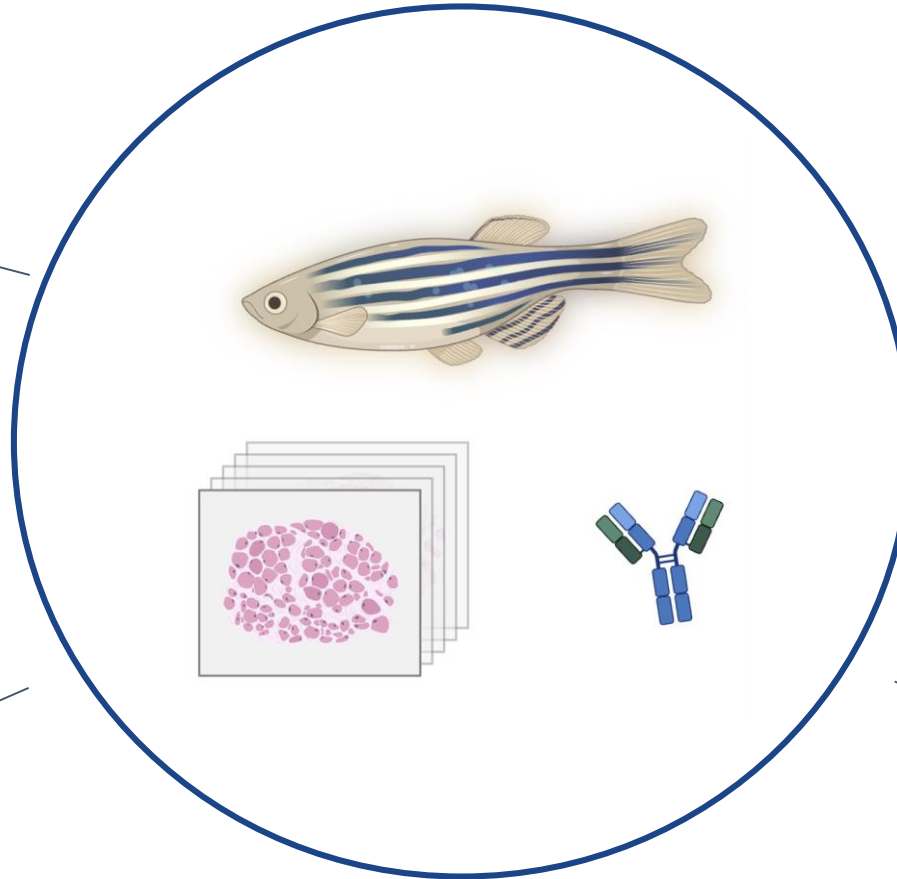
Validation of
Genetic Models

Disease Phenotyping

Compound Efficacy

Toxicity Testing

Enhanced Molecular Understanding of Zebrafish Models



InVivo Biosystems

sales@invivobiosystems.com

Custom Antibody Utility

- Sourcing reliable antibodies in zebrafish work is a well-known problem
- Example: No antibody tested in zebrafish available for a protein of interest, manually compiled a list of 46 antibodies with any immunogen information available, used protein homology tools to identify best candidates and tested 4 antibodies before (luckily) finding a usable antibody
 - Lost time, labor, money, reagents, disease samples
 - Also explored ISH options in parallel because no guarantee we would find a working antibody
 - This story is not unique
- With Boster Bio Custom Antibody offerings, could have designed and tested antibodies first!

Additional Advantages of Boster Bio Antibodies

- **Fluorophore-conjugated primary antibodies**
 - Allows for multiplexing in antibodies derived from the same host animal (e.g. rabbit)
- **Tissue-specific validation available**
 - Save labor and optimization efforts
- **Affordable custom antibodies!**
 - \$600 is the typical price of off-the-shelf antibodies; custom orders are often > \$3k

THANK YOU FOR WATCHING!

