Customer Story: Looking at Gene Paralogs across both zebrafish and *C. elegans* Model Organisms

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InVivo Biosystems

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> - Dr Jeffrey Amack

Overview

Dr David Pruyne and Dr Jeff Amack are Associate Professors of Cell and Developmental Biology at Upstate Medical University. Dr Prunye’s lab primarily utilizes a *C. elegans* model system to study muscle cell growth, but recently teamed up with Dr Amack to investigate whether vertebrates show similar effects.

Challenge

Pruyne and Amack wanted to disrupt the genes fhod3a and fhod3b in zebrafish, as they are associated with familial hypertrophic cardiomyopathy - a condition where heart muscle grows abnormally thick and makes it hard for the heart to pump.

But, when working with these genes it is necessary to target very specific point mutations within the exons because these genes are subject to alternative splicing which, in turn, can obscure the isoform specific effect.

“With your guy’s expertise we’re more confident that we’re going to be able to insert these very specific point mutations and begin to tease out isoform specific differences.”- Dr Pruyne

Solution

Pruyne and Amack leveraged InVivo Biosystems’ Custom Validated Injection Mix offering to design and create a validated injection mix for the generation of a novel zebrafish line carrying a targeted in frame deletion to disrupt fhod3a and fhod3b.

Benefits

Utilizing these custom mixes, Pruyne and Amack’s labs have been able to isolate deletions that are in frame as well as a collection of other types of deletions. They are now in the process of comparing the zebrafish’s phenotypes and defects. This work also enables Pruyne and Amack to look at the paralogs of these birth defects-associated genes in a vertebrate zebrafish model and Pruyne’s primary nematode *C. elegans* model. Comparing the effects of these proteins across model organisms can help inform the design of future studies - attracting funding and better understanding the translatability between invertebrate and vertebrate muscle development.

Background:
Dr David Pruyne is an Associate Professor of Cell and Developmental Biology at Upstate Medical University. His lab is focused on understanding how muscle cells organize their actin cytoskeleton. Dr Pruyne primarily utilizes a *C. elegans* animal model, but worked with a zebrafish animal model for this project in collaboration with Dr Jeff Amack who has a neighboring lab at Upstate Medical University.

Dr Jeff Amack is also an Associate Professor of Cell and Developmental Biology, but has slightly different research interests than Pruyne. Amack's lab is focused on investigating how organs take shape during embryonic development, specifically looking at left-right asymmetry, biophysical interactions with organ formation, and cilia development. While Amack always thought he would start a mammalian research lab, he began working with zebrafish during his post-doc and ultimately decided to have zebrafish be his model of choice. He explained that their use as a model organism has only continued to grow in power and popularity

Amack has been working with zebrafish since his postdoc, and although he thought he would use a mammalian model system when he started his own lab, he explained that when it came time, he chose to continue working with zebrafish as, their use as a model organism has only "continued to grow in power and popularity."

Why zebrafish?
Jeff specifically is interested in utilizing zebrafish as a model for early developmental events, which zebrafish are extremely well-suited to model due to their external development and translucent embryos.

"zebrafish offer the ability to study and address questions that are more difficult to do in other vertebrate animals." - Dr Amack

While David typically utilizes *C. elegans* in his research, he explained that zebrafish offer advantages for certain projects; for instance, he found that funding agencies tended to be more interested in studies on vertebrate muscle cells than invertebrate muscle cells as the translatability between vertebrate and invertebrate muscle cells is still unknown. David further noted that zebrafish are a particularly useful model for investigating skeletal muscle as, unlike mice, zebrafish's skeletal muscle can actually begin to develop before the heart develops. And so, even if the heart does not develop, which is one of the phenotypes David is studying, it is possible to see how the skeletal muscle itself is affected.

"Having it [zebrafish knockouts] outsourced by your scientists that do this for a living was a really easy decision." - Dr Amack

Why Invivo Biosystems?

He explained that in academic labs like his, there is a lot of variability introduced through the inevitable rotation of grad students - some students get results that are 95% effective and others won't have any success at all. Working with InVivo Biosystems enabled Jeff to, "take care of some of that variability and get tools that we know were going to work and can be really confident about."

Pruyne also noted the advantage that InVivo Biosystems brings to project design, as an expert in both zebrafish and *C.elegans* models: "its valuable having somebody who does work on the different model systems because then they can very easily go to the databases and find what it is that you're talking about." He explained that it is vital to find good targets and good designs for them.

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The Data

The knockout mixes that InVivo Biosystems created for Pruyne and Amack empowered them with a validated way to look at the effect of gene mutations in the fhod3 gene associated with abnormal heart muscle growth.

First, InVivo Biosystems worked with Pruyne and Amack to design injection mixes which produced deletions that targeted the critical domain of the proteins encoded by fhod3a and fhod3b. Then, we at InVivo Biosystems evaluated the sgRNA cutting efficiency to validate the mies, utilizing our in-house screening tools to mitigate failure rates. Finally, InVivo Biosystems sent the reagents to Pruyne and Amack, enabling them to create these KO zebrafish lines, and then analysis the phenotypes in zebrafish and compare them with gene

[Working with InVivo Biosystems] you're not only paying for the reagents, but you're also paying for the support (...) I think this technology moves very fast, so I definitely appreciate your technical insight and expertise." - Dr Amack

InVivo Biosystems' team of genome editing experts were able to get Pruyne and Amack the injection mix tool they needed in a timely manner, accelerating their study's timeline. Furthermore, being validated mixes, Pruyne and Amack were able to confidently plan the rest of their study as they didn't have to worry about the variability that comes from attempting the intensive process of reagent sourcing, designing, and validating a CRISPR injection mix/SgRNA validation in your own lab.

About InVivo Biosystems

Founded in Eugene, Oregon in 2011, InVivo Biosystems is working to accelerate deep in-vivo insights into human biology and enable researchers to develop and deliver solutions that improve human health. An expert in CRISPR genome editing, InVivo Biosystems provides a unique capability for creating custom genome edited zebrafish and *C. elegans* that enable therapeutic research on genetic models of aging, developmental, and neurodegenerative disease, uncovering potential cures. The company's *in vivo* analytical testing platforms and technologies provide faster, cost-effective investigations that focus on proof-of-principle experiments for rapid go/no go decision making so that biopharma and nutraceutical companies around the world can better understand aging and aging related diseases and explore potential treatments.

All our projects include on-call project status updates, as well as regularly scheduled communication. We also provide on-call consulting and interpretation with our Ph.D. level, subject-matter experts.

What we do:

- Deliver scientific data on test results in less than 5 months.
- Produce the best outcome measures for anti-aging products.
- Provide information about mechanisms of action (MoA).
- Support your Marketing and IP claims with real science.

Contact us to start a conversation about how our services can support your innovation.


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