Screening for HSP:Gal4 Homozygous Transgenic Fish

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ABSTRACT

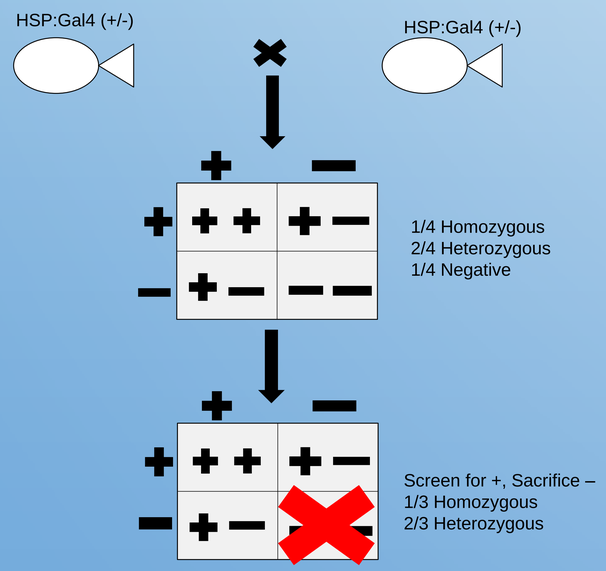
About 100 trillion microorganisms inhabit the mammalian body – these microorganisms outnumber us about 100 to 1. Most of these microorganisms reside in the small intestine. Our mucosal immune system provides protection against pathogenic microbes on mucosal surfaces. Intestinal epithelial cells provide a barrier between the commensal bacteria in the intestine and the underlying tissue. The Traver lab is interested in investigating the role that microbial signals have in shaping the intestinal immune system.

The Gal4-UAS system is used to study the function of genes. Gal4 is a yeast protein and works as a transcription factor. Gal4 binds UAS (Upstream Activation Sequence), and this binding initiates transcription of downstream genes. This system is used in zebrafish to analyze the function of genes. The Traver lab had tanks of HSP:Gal4 transgenic fish, which express a heat shock promoter upstream of Gal4. There are also tanks of UAS:GFP transgenic fish that express UAS upstream of GFP. The HSP:Gal4 fish were a mix of heterozygous and homozygous fish, and ththe UAS:GFP fish were all homozygous. My project was to determine which of the HSP:Gal4 fish were heterozygous or homozygous.

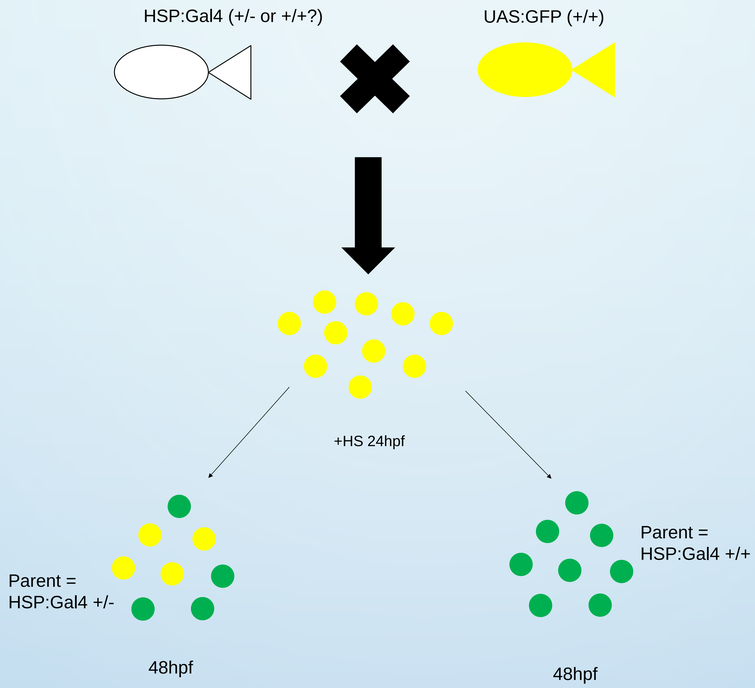
We mated HSP:Gal4 and UAS:GFP fish. Upon heat shocking the embryos, transcription factors will bind to the heat shock promoter to initiate the transcription of Gal4. As a protein, Gal4 will bind to the UAS DNA sequence and initiate the transcription of GFP. If the embryos are all fluorescent (GFP+), the parent HSP:Gal4 fish is homozygous, where as if only half of the embryos are fluorescent, the parent HSP:Gal4 is heterozygous.

Identifying homozygous HSP:Gal4 fish is important, as they will be used to validate a number of UAS lines in the lab. These UAS lines will be used to overexpress dominant negative proteins that will inhibit signaling pathways downstream of innate immune receptors. These studies will help elucidate the roles that microbes have in shaping the intestinal immune system.

Screening for HSP:Gal4+ Transgenic Fish by PCR

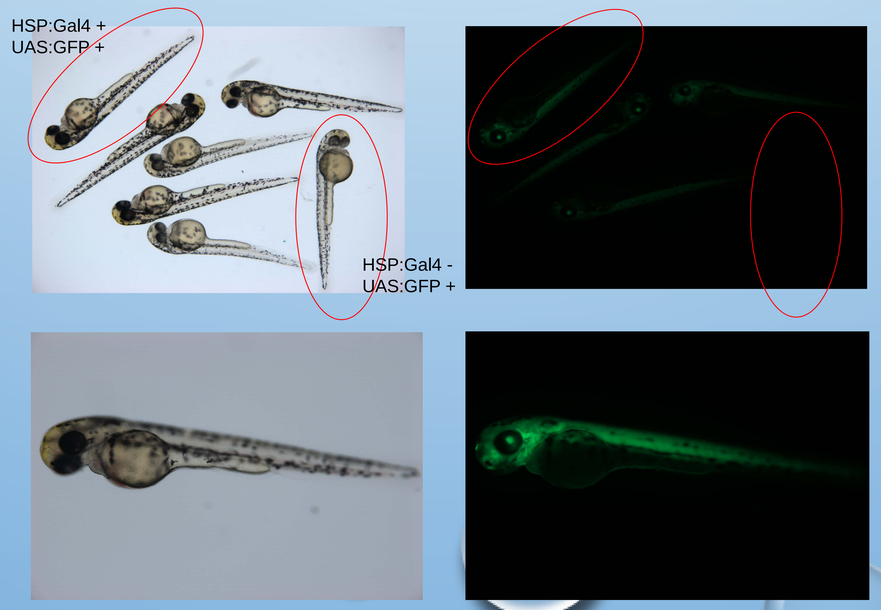


Screening for HSP:Gal4 Homozygous and Heterozygous fish



Screening Fish for GFP Fluorescence

HSP:Gal4 (+/-) x UAS:GFP (+/+) 48 hpf



GFP Fluorescence

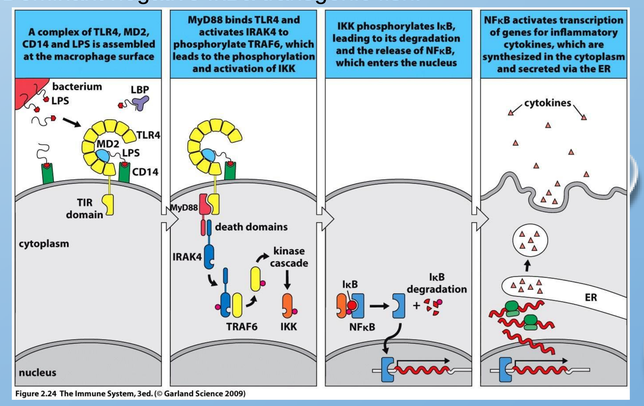
Parent fish	48 hpf larvae Positive vs. Negative (GFP)	Is the parent heterozygous or homozygous?
#1	33 Positive 11 Negative	Heterozygous
#2	1 Positive 2 Negative	Heterozygous
#3	4 Positive 7 Negative	Heterozygous
#4	50 Positive 0 Negative	Homozygous
#5	15 Positive 35 Negative	Heterozygous
#6	23 Positive 27 Negative	Heterozygous
		1/6 Homozygous **5/6 Heterozygous**

CONCLUSIONS

Out of the 6 fish we screened, we hypothesized that 2 fish would be homozygous. We found 1 homozygous fish. This data is consistent with our hypothesis. We may have had inconclusive results with fish #2 because there were only three embryos in the clutch.

FUTURE DIRECTIONS

The homozygous HSP:Gal4 fish identified will be used to validate the UAS:Dominant negative IkBa transgenic fish.



HSP:Gal4(+/+) x UAS:Dominant negative IkBa (+/-)
HS 24 hpf embryos → 5 hours later, inject embyos with LPS → 2 hour later, take samples and assess cytokine production

Hypothesis: Reduced cytokine production in UAS:DN-IkB+ fish compared to UAS:DN-IkB- fish.