

Differences in habenula kisspeptin expression and its effects on stress coping styles in zebrafish, *Danio rerio*

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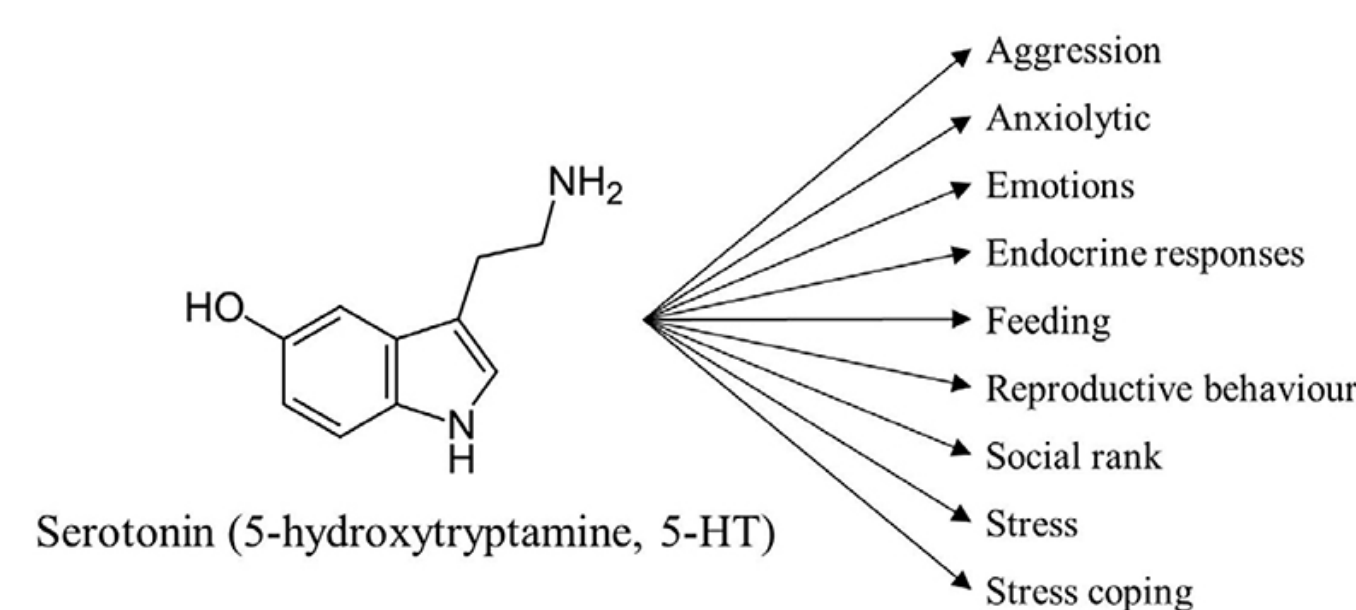
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Background

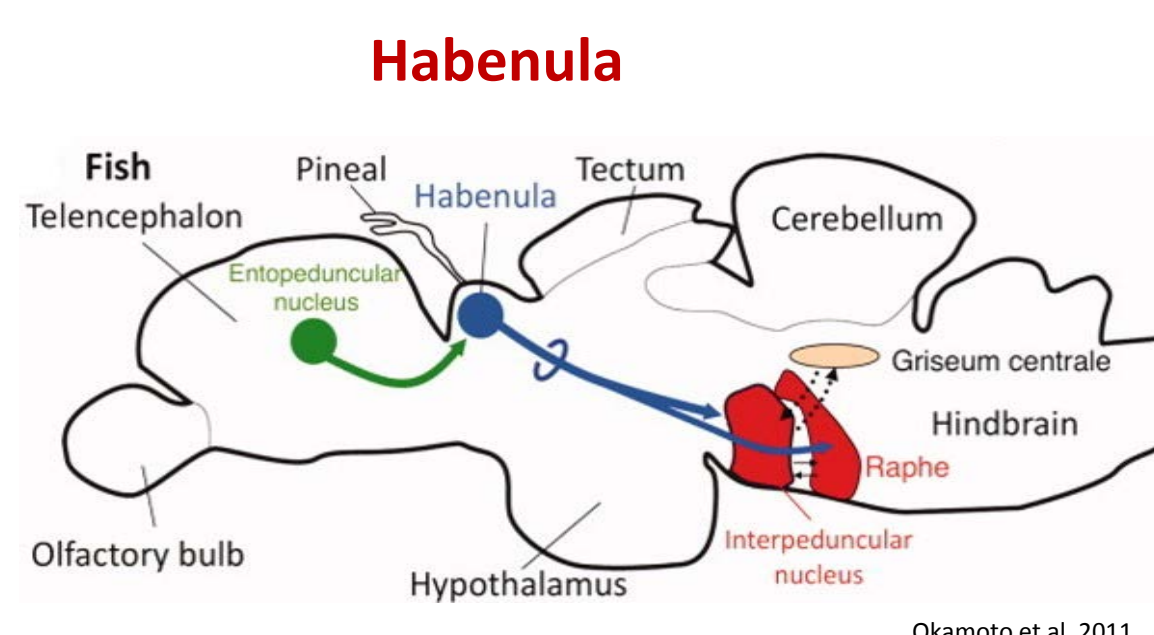
Stress Coping Styles

	Proactive	Reactive
BEHAVIORAL CHARACTERISTICS		
Aggression	High	Low
Conditioned immobility	Low	High
Routine formation	High	Low
PHYSIOLOGICAL CHARACTERISTICS		
HPA axis reactivity	Low	High
Parasympathetic reactivity	Low	High
Sympathetic reactivity	High	Low

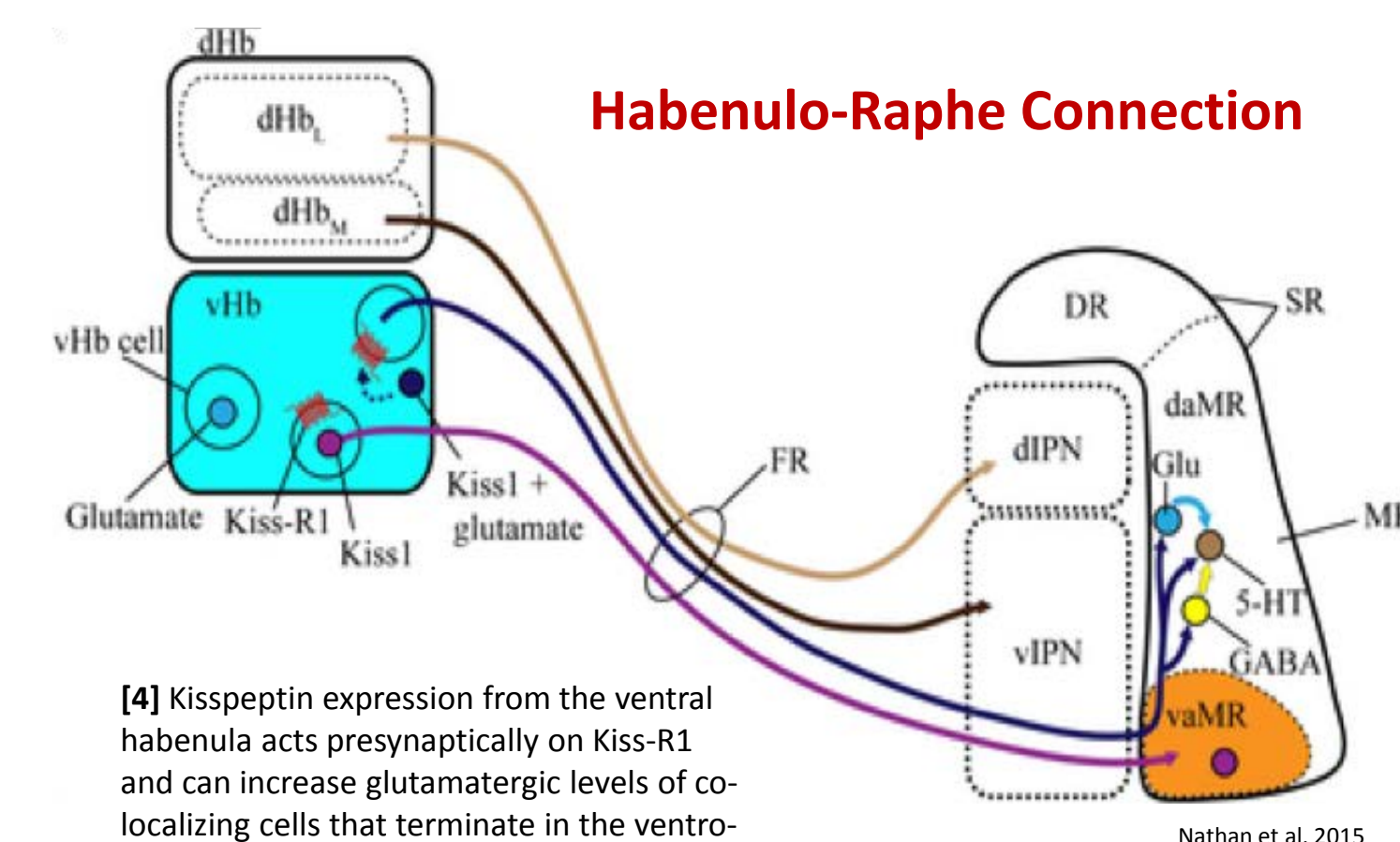
[1] Teleosts, such as zebrafish, display two distinct stress coping styles that are also seen in many other vertebrates. These coping styles are defined by several behavioral and physiological differences.



[2] 5-HT plays a distinct role in many aspects of an animals existence. In particular, proactive and reactive individuals have differences in 5-HT expression



[3] The habenula has afferent and efferent connections from major forebrain networks (ex. limbic system, basal ganglia) and the monoaminergic hindbrain (ex. raphe, ventral tegmental area)



[4] Kisspeptin expression from the ventral habenula acts presynaptically on Kiss-R1 and can increase glutamatergic levels of co-localizing cells that terminate in the ventro-anterior median raphe.

Objective
Our objective is to investigate whether there is a difference in kisspeptin sensitivity between proactive and reactive stress coping styles

Hypothesis
Proactive zebrafish will be more sensitive to kisspeptin and this plays a role in the increased 5-HT expression

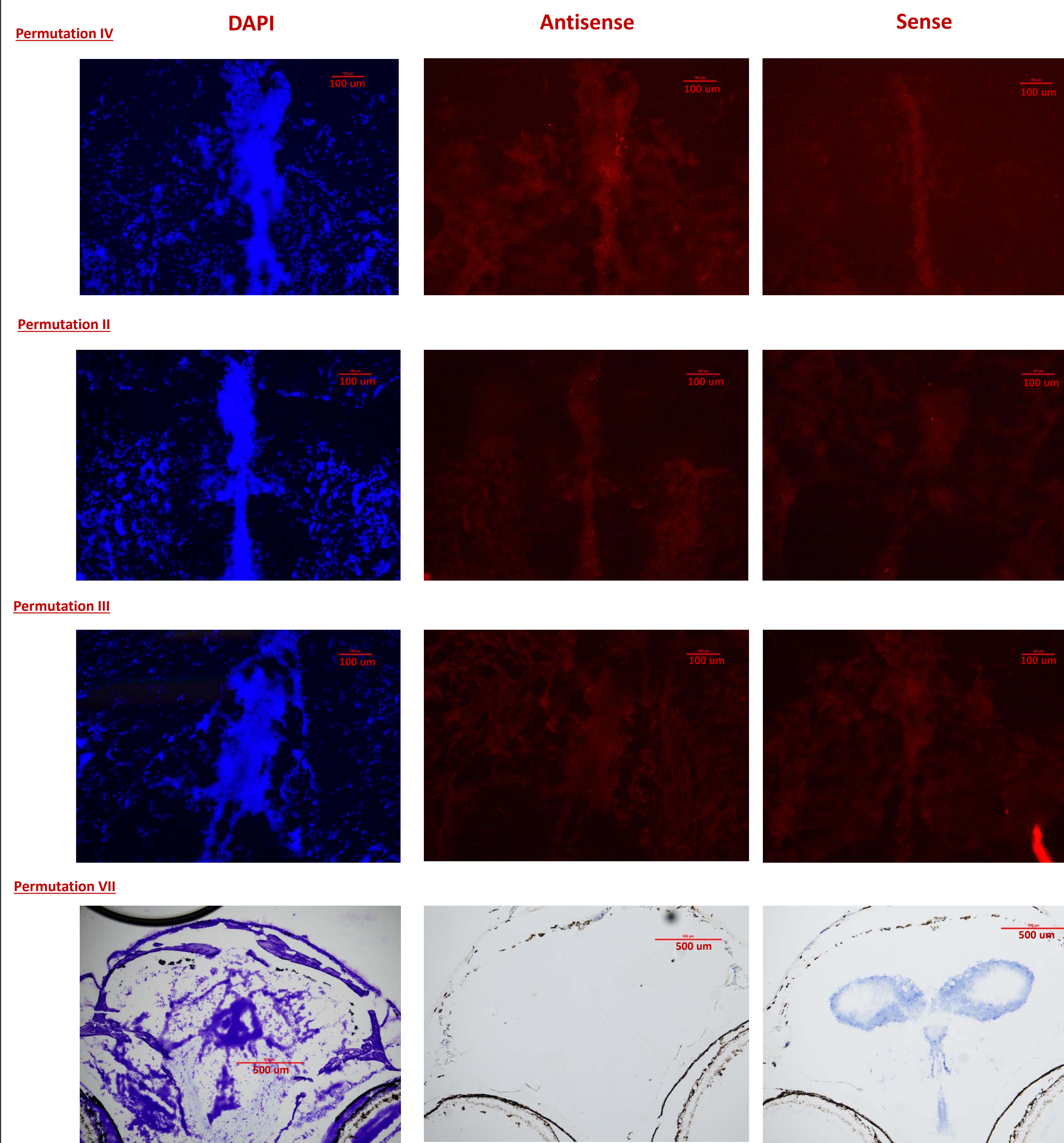
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Results

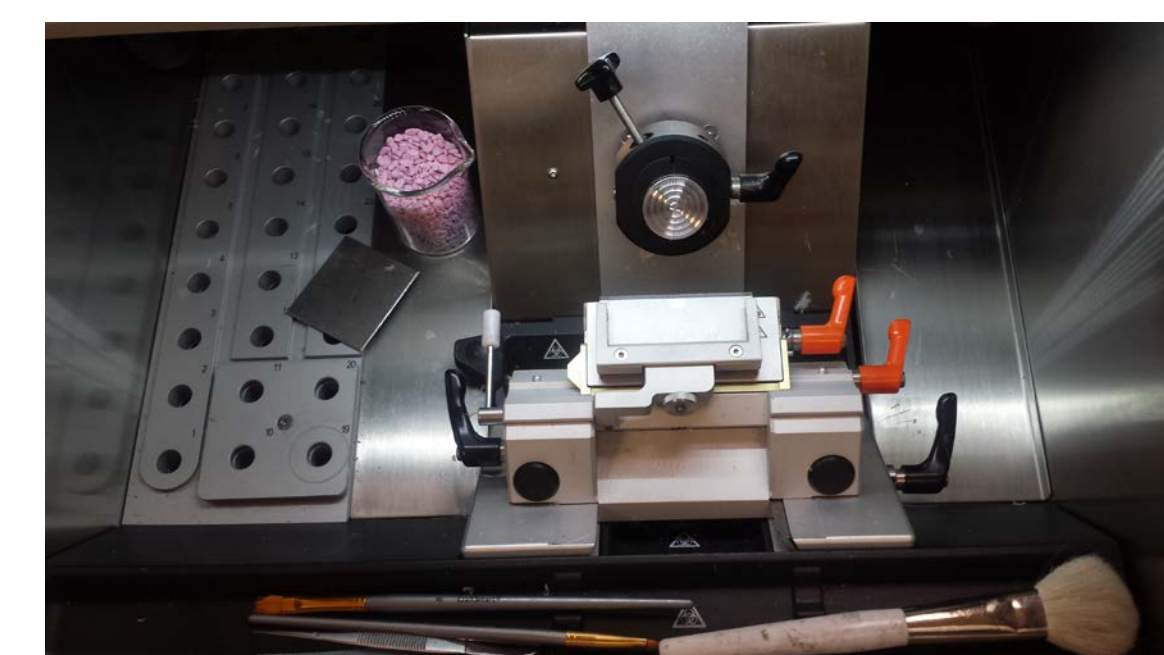
	Hybridization Temp		Blocking Buffer			Antibody		Detection	
Permutation	65°C	67°C	1X	0.5X	0.25X	HRP	Poly-HRP	Fluorescent	Colormetric
I	X					X		X	
II	X		X			X		X	
III	X			X		X		X	
IV	X				X	X		X	
V		X			X	X		X	
VI	X						X	X	
VII	X					X			X

All permutations were attempted under low, medium, and high wash stringencies



Materials and Methods

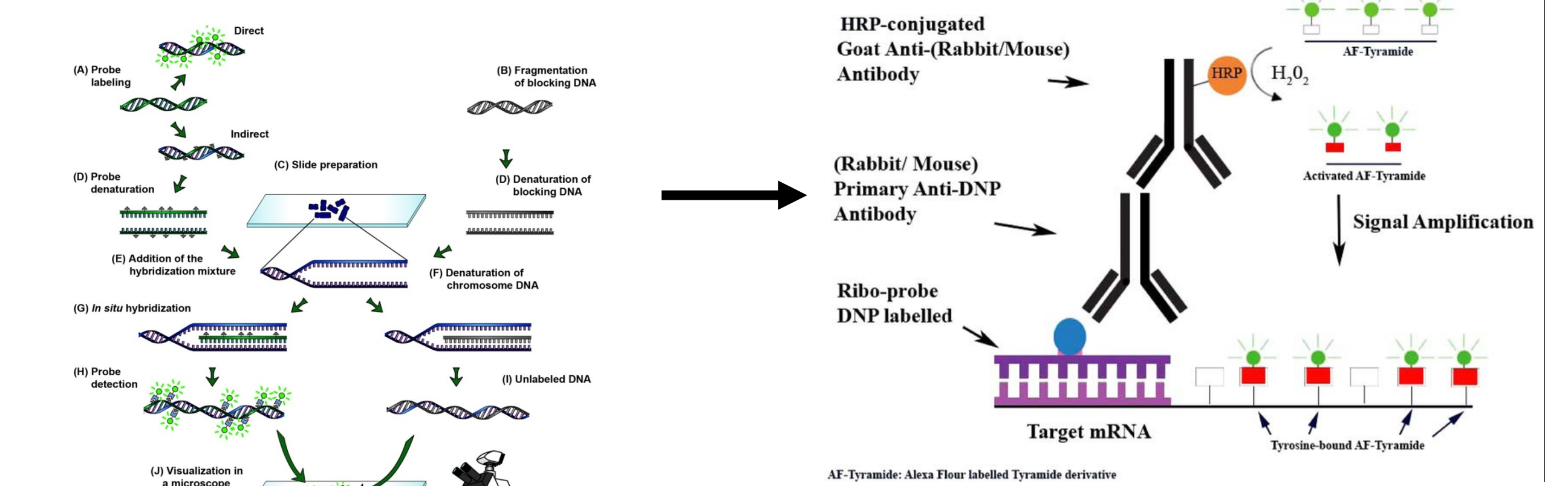
Cryosection



- 12 proactive and 12 reactive [N=24] zebrafish were sacrificed
- Half male/half female
- 16µm sections



In situ Hybridization



- Created complementary probes 595bp long for *kiss1ra* mRNA
- Synthesized sequence with digoxigenin
- Fluorescence *in situ* determined via tyramide signal amplification

Conclusion and Discussion

- Despite trying to optimize the in situ hybridization reaction parameters by running ten different permutations, we have yet to identify a parameter that demonstrates our probe is specifically binding to *kiss1ra* mRNA
- Further investigation will be required to determine the specific issue
- Given that we see signal on both antisense and sense it is possible that the issue lies in the probes specificity for binding to only *kiss1ra*
- Alternate primers for *kiss1ra* mRNA will be reviewed

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