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Developing Techniques to Observe The Effects of Bisphenol AF on Guppy Embryo Development

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Introduction

BPAF is a chemical compound that has been used to produce plastic since BPA has been more strictly regulated. The purpose of this research is to develop the techniques for examining the effects of BPAF in guppies *(Poecilia reticulata)*. The techniques developed include isolating DNA and RNA and determining the sex of guppies in their embryonic phase.

Questions/Hypotheses

1. Will high doses of BPAF will cause a decrease in apoptosis in the ovaries and result in poor embryo development
2. Will there be a higher level of female offspring in guppies that have been exposed to BPAF than in guppies that have not been exposed

*In order to address 1 & 2, we needed to develop good techniques for studying guppy gene

Method

DNA extraction

Tissue was exposed to 50mM NaOH, then heated using a hot water bath for 10 minutes. We then added 1 MTris (PH8), and centrifuged the tissue for 2 minutes.

Sex determination

PCR was used to detect a sequence in the DNA that identifies if the guppy is male or female. PCR products were observed in agarose gels and quantified using imageJ. Additionally, we developed a run schedule for PCR using modified techniques from Dreyer et. al.

RNA extraction

Initially, we attempted to extract RNA using and Rneasy kit from Qiagen. The results indicated a low amount of RNA. Guppy eggs have more yolk than zebra fish eggs, and therefore need a different method for RNA extraction. We modified a procedure using TRIzol and chloroform to extract RNA.

Why Guppies?

1. Guppies are viviparous fish; the embryos are retained in the female ovary and she gives birth to fully formed larvae
2. Guppies have short gestational periods ranging from 20-30 days (Whitney & Hahnell)
3. The maternal environment influences the way that the embryos develop (Yang et al.)

Figure 1. The first PCR that we ran used zebra fish sex determining primers against a 1 Kb ladder. The wells contained guppy tissue from tail muscle (M), tail fin (T), side fin (F), and embryo (E). We assumed that the band at the bottom were primer dimers, especially considering the band in the NTC.

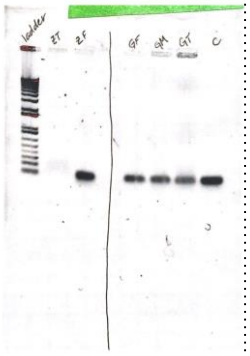

Figure 2. To observe if the zebra fish sex primers worked with zebra fish, tissue we compared tissue samples from guppies (G) and zebra fish (Z). The samples included tail fin (T), side fin (F), tail muscle (M) and the control. Using a 1kb ladder for comparison, we saw a band in the control which suggested primer dimers.

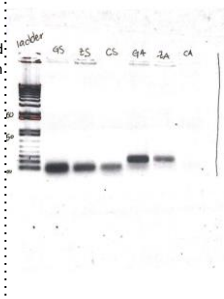

Figure 3. In order to determine that the PCR system we were using was working, we tested actin primers (A) with guppy (G) and zebra fish (Z) tissue as well as the sex determining primers (S) against a 1kb ladder. All tissue was from the tail muscle. There was no band in the control, and the bands were higher in the gel which indicated that our procedure was working, and not just producing primer dimers.

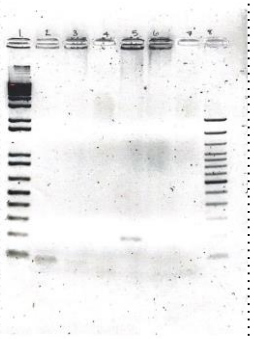

Figure 4. It was determined that the sequences we tried to observe were smaller than anticipated, we ran the gel for longer. Figure 4 shows a band in lane 2 and 5 from the zebra fish actin and zebra fish sex primers. Against the 1 kb ladder on the left, and 100 bp ladder on the right. Additionally, there is a faint band in lane 3 where the guppy tissue was, indicating that the sex primers worked for guppies as well.

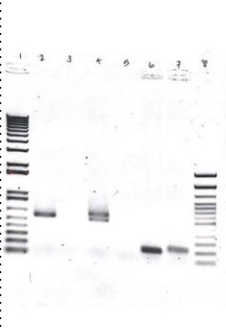

Figure 5. After determining that the procedure worked for the gels, we tested the method again, using Beta Catenin primers against a 1kb ladder (left) and a 100 bp ladder (right) The lines in wells 2 and 3 are from Beta Catenin, and 6 and 7 are from the sex primers.

Conclusion

This research is important because it provides us with the tools we need to study the effects of BPAF on embryonic development in guppies. Understanding how BPAF effects are passed from mother to offspring is an important area of study. Specifically, if guppies are exposed to certain chemicals, and experience negative side effects, will their offspring have those same side effects? Little is known about if the chemicals are passed from mother to guppy during the gestational period, and this research could give further insight. In addition, this research could relate to other animals' interactions with BPAF, and how chemicals affect the gestational period with an affinity for estrogen receptors.

References

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