



October 14, 2003

Mr. Alan Blake
Chief Executive Officer
Yorktown Technologies

Re: Temperature Sensitivity of Fluorescent Transgenic Zebrafish

Dear Mr. Blake:

I am writing to inform you of the results of our temperature sensitivity study of fluorescent transgenic zebrafish expressing DsRed and EGFP. As you know, zebrafish are freshwater tropical fish, and are only found in warm waters. To date, there have been no reports of zebrafish (*Danio rerio*) establishing a permanent reproducing population in the United States. As transgenic zebrafish are brought to the ornamental fish markets, it is important to ensure that the temperature sensitivity of the transgenic fish is not altered in a manner that would allow them to survive and establish populations in the waters of the United States.

With this in mind, we compared the temperature sensitivities of wild-type zebrafish to transgenic zebrafish expressing DsRed and EGFP. This experiment was conducted over two days to allow the fish to acclimate slowly to lower temperatures and determine the temperatures at which their behavior changed and death was observed. The data we gathered from this experiment is summarized in Exhibit A, and provided in its raw form in Exhibit B.

In analyzing the data, we found all of the zebrafish demonstrated significant distress at temperatures below 67°F (19°C). Below these temperatures, there were significant differences between the average temperatures at which death occurred in wild-type zebrafish compared to the DsRed zebrafish ($p < 0.01$). The EGFP zebrafish similarly demonstrated lower average temperature fitness, although the difference was not statistically significant in this study. In sum, the average point at which the DsRed zebrafish died was 48.6°F (9.2°C), while the EGFP zebrafish died at an average of 43.6°F (6.4°C), and the wild-type zebrafish died at an average of 41.5°F (5.3°C).

While the temperatures at which behavior changes and death occurs may vary over longer experimental periods of time, based on the data presented in this study, increased temperature sensitivity of the DsRed zebrafish is expected in all cases, and such increased sensitivity, while not statistically significant in this study, appears likely in EGFP zebrafish. Therefore, it is expected that DsRed zebrafish, and possibly EGFP zebrafish, would have an overall reduced fitness in the wild compared to wild-type zebrafish, particularly in non-tropical environments.

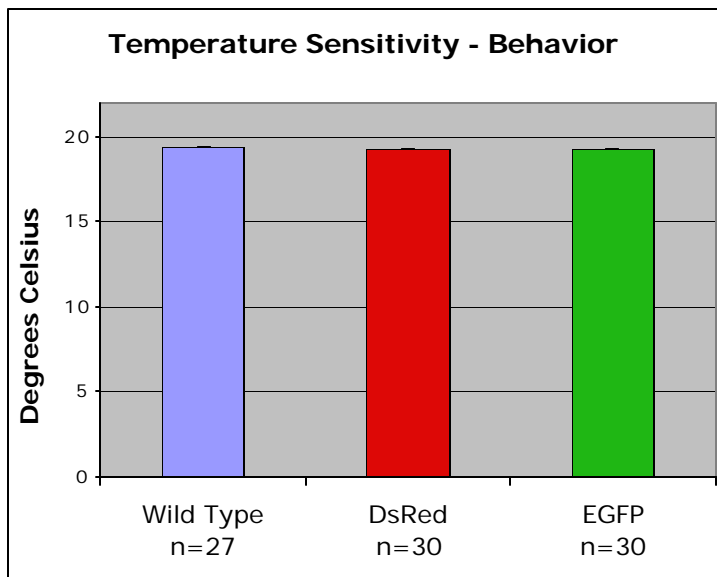
Sincerely,

A handwritten signature in black ink that reads "Jeffrey J. Essner". The signature is written in a cursive style with a large, prominent 'J'.

Jeffrey J. Essner, Ph.D.
Director of Operations
Discovery Genomics, Inc.

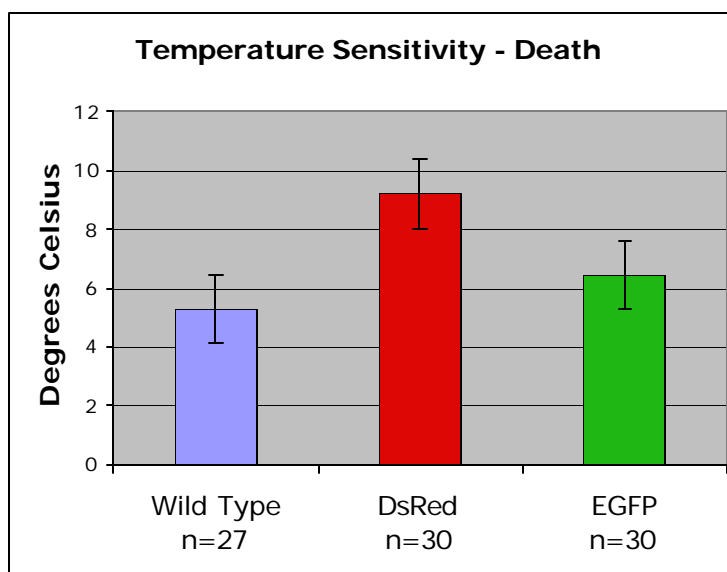
Exhibit A – Summary of Temperature Sensitivity Data

A. No difference in temperature sensitivity with respect to behavior (active swimming) was observed among transgenic and wild-type zebrafish.



Legend: The first behavior change was that previously active zebrafish moved to the bottom of the tank and did not swim unless startled.

B. DsRed zebrafish were more sensitive to cold temperatures with respect to death than wild-type zebrafish ($p < 0.01$). EGFP zebrafish also showed greater sensitivity, but the difference was not statistically significant in this study.



Legend: Death was assessed as lack of a beating heart and the inability to be resuscitated at a higher temperature.

Exhibit B - Raw Temperature Sensitivity Data

Fish	Temperature - Stress			Temperature - Death		
	Wild Type (°C/°F)	DsRed (°C/°F)	EGFP (°C/°F)	Wild Type (°C/°F)	DsRed (°C/°F)	EGFP (°C/°F)
1	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	11.5 / 52.7	8.5 / 47.3
2	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	11.5 / 52.7	8 / 46.4
3	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	11.5 / 52.7	6.5 / 43.7
4	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	11.5 / 52.7	6.5 / 43.7
5	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	11.5 / 52.7	6.5 / 43.7
6	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	11.5 / 52.7	5 / 41
7	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	10 / 50	5 / 41
8	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	9.5 / 49.1	5 / 41
9	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	9 / 48.2	5 / 41
10	21 / 69.8	21 / 69.8	21 / 69.8	5 / 41	8 / 46.4	5 / 41
11	18 / 64.4	18 / 64.4	18 / 64.4	8 / 46.4	11 / 51.8	8.5 / 47.3
12	18 / 64.4	18 / 64.4	18 / 64.4	8 / 46.4	11 / 51.8	8.5 / 47.3
13	18 / 64.4	18 / 64.4	18 / 64.4	8 / 46.4	11 / 51.8	8 / 46.4
14	18 / 64.4	18 / 64.4	18 / 64.4	7.5 / 45.5	11 / 51.8	8 / 46.4
15	18 / 64.4	18 / 64.4	18 / 64.4	6.8 / 44.2	10 / 50	8 / 46.4
16	18 / 64.4	18 / 64.4	18 / 64.4	5.5 / 41.9	9.5 / 49.1	8 / 46.4
17	18 / 64.4	18 / 64.4	18 / 64.4	5.5 / 41.9	9 / 48.2	8 / 46.4
18	18 / 64.4	18 / 64.4	18 / 64.4	5.5 / 41.9	9 / 48.2	6.8 / 44.2
19	18 / 64.4	18 / 64.4	18 / 64.4	5.5 / 41.9	9 / 48.2	6.8 / 44.2
20	18 / 64.4	18 / 64.4	18 / 64.4	5.5 / 41.9	9 / 48.2	6.8 / 44.2
21	19 / 66.2	19 / 66.2	19 / 66.2	5 / 41	10 / 50	8.5 / 47.3
22	19 / 66.2	19 / 66.2	19 / 66.2	4 / 39.2	7.5 / 45.5	6.5 / 43.7
23	19 / 66.2	19 / 66.2	19 / 66.2	4 / 39.2	7.5 / 45.5	6.5 / 43.7
24	19 / 66.2	19 / 66.2	19 / 66.2	3.5 / 38.3	7.5 / 45.5	6 / 42.8
25	19 / 66.2	19 / 66.2	19 / 66.2	3.5 / 38.3	7.5 / 45.5	6 / 42.8
26	19 / 66.2	19 / 66.2	19 / 66.2	3.5 / 38.3	6.5 / 43.7	5 / 41
27	19 / 66.2	19 / 66.2	19 / 66.2	3.5 / 38.3	6.5 / 43.7	4 / 39.2
28		19 / 66.2	19 / 66.2		6.5 / 43.7	4 / 39.2
29		19 / 66.2	19 / 66.2		6 / 42.8	4 / 39.2
30		19 / 66.2	19 / 66.2		5.8 / 42.4	4 / 39.2
Average	19.4 / 66.9	19.3 / 66.8	19.3 / 66.8	5.3 / 41.5	9.2 / 48.6	6.4 / 43.6
St. Dev.	1.3 / 2.4	1.3 / 2.3	1.3 / 2.3	1.3 / 2.4	1.9 / 3.4	1.5 / 2.8

Legend: Numbers are the temperatures in which either behavior changed or death occurred either for wild-type, DsRed, or EGFP zebrafish. The first behavior change was that previously active zebrafish moved to the bottom of the tank and did not swim unless startled.