

18 August 2003

Mr. Alan Blake  
Yorktown Technologies



Discovery Genomics, Inc.  
[www.discoverygenomics.net](http://www.discoverygenomics.net)

Dear Mr. Blake

You have asked me to comment on safety issues concerning genetic engineering of tropical fish. Specifically, you propose to market existing lines of zebrafish that have new coloration patterns based on the introduction of fluorescent protein genes that will be expressed in a variety of tissues. I have been involved with evaluation of safety issues regarding transgenic fish for the past 15 years and have been engaged in making such fish for about 18 years. I have written several reviews and testified in Washington on the consequences of transgenic fish. I am an associate editor of the journals *Marine Biotechnology* and *Transgenic Research*. I summarize the issues below:

The issues are:

- 1) Will transgenic tropical fish survive should they escape home aquaria?
- 2) If the fish were to survive, would they pose any risk to the environment?
- 3) If the fish did not survive, might the transgene escape and have unexpected consequences?

The answers to all three questions is NO. Here is why:

- 1) Tropical fish are unable to withstand the environments of most communities. Chlorine (chloramines) and temperature levels would be lethal to the fish. Any tropical fish, most of which are relatively brightly colored, that managed to survive for a period of time soon would become snack food for naturally adapted aquatic species. With respect to zebrafish in particular, the validity of this conclusion is obvious when one realizes that millions have been introduced into the United States and yet there have been no confirmed reports of populations that have established themselves in the wild.
- 2) Were a fish to survive, it would be essentially alone. As such, it would not be able to breed. Were multiple fish to escape and survive, they and their eggs/offspring would be food for natural populations. Tropical fish are not equal in size to natural fish, which would prey on the very conspicuous tropical fish. The odds of an established transgenic population are too low to be realistically estimated by scientific methods. But, what if they did – would there be a risk to the environment? No. Fluorescent protein genes would essentially be a refinement to vivid coloration patterns that already exist in tropical fish. Moreover, after many years of detailed research and direct observation, these fish have consistently demonstrated that they have no adaptive ability with regard to reproduction or viability when compared to wild type tropical fish. The bottom line is that if the fish escaped and survived against all odds,

there is no scientific reason to believe that the transgenic tropical fish would have an environmental impact that differed from their wild-type parents.

- 3) All animals naturally consume genes. For instance, the average human consumes more than 100,000,000,000,000 genes per day in the food he/she normally eats. Essentially none make their way into the chromosomes of a cell. If they did, one consequence would be cancer or some other genetic disease. Transgenes are no different. Moreover, even if a gene were to be incorporated into the chromosome of a cell in the gut, it would not be passed on to the offspring or otherwise become established because this can only happen when germ-line (sperm or egg) cells take up foreign DNA. Germ-line cells are elaborately protected against invasion of all kinds to protect the integrity of the natural genes in the animal. Again, the chances of such an event happening are too low to be scientifically measured or even estimated based on evidence available.

In sum, there is no basis for assuming that transgenic tropical fish pose a threat in any way to the environment. This evaluation is supported by the United States Department of Agriculture (USDA). Beginning in the late 1980s there were concerns of escape of transgenic food fish such as salmonids (including trout) that contained extra growth hormone genes. As a consequence, the USDA and the American Fisheries Society proposed guidelines. In the past 10 years of development of guidelines, tropical fish were considered to be essentially risk-free and thus are not subject to the guidelines. At the University of Minnesota, where issues of transgenic fish escape were raised, the aquaculture facility that was to house transgenic fish had elaborate safeguards to prevent large fish or their eggs from escaping. No provisions were deemed necessary for tropical fish models, including zebrafish, of which thousands of transgenics have been made.

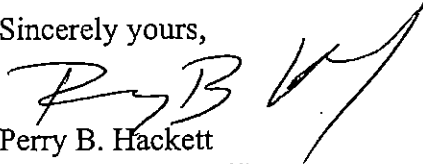
In your letter to me requesting my opinion, you mentioned the efficacy of further safeguards. These safeguards include methods to 1) make the transgenic fish sterile (effectiveness estimated to be about 1 fish in 1000 will escape the procedure), 2) produce populations of single sex (about the same effectiveness rate) and 3) introduce modifications that would render the fish vulnerable to some selective agent (or its absence). All of these methods have been suggestion for large, commercial food fish. There is a reason for this. Large fish natural to the outside environment can both survive and breed with natural populations, thereby upsetting natural populations. In distinct contrast, the tropical fish cannot. The modifications are unnecessary – the odds of a problem arising from a fluorescent transgenic tropical fish are so low as to be incalculable. It is meaningless to call for lowering a number that is, for all practical purpose, zero.

You raised the question of ethics, which I take to mean the ethics of introducing a gene into an animal. Genetic engineering in its broadest form is thousands of years old – beginning before written history when humans selected for particular mutants or variants, such as dogs that were bred into a large number of species. Today, this is accomplished by selective introduction of genes into chromosomes to speed up the process and obtain specific traits without other random mutations. Although concerns have been voiced by a

very few rather vocal individuals and groups, transgenic crops and animals are here to stay. Currently, a very large percentage of corn, soybeans, and other crops grown in the United States have been genetically engineered to produce products that have fewer pesticide contaminants than non-transgenic crops. Transgenic organisms are becoming more prevalent every year throughout the world. Consumers have a choice to purchase or not purchase transgenic products, including tropical fish, and there is no reasonable basis for such fish to elicit any negative comments.

In summary, there are many issues concerning growth-enhanced, transgenic, commercial fish such as Atlantic salmon. There are no scientifically established environmental consequences with respect to fluorescent transgenic tropical fish.

Sincerely yours,

A handwritten signature in black ink, appearing to read 'Perry B. Hackett', with a large, sweeping flourish extending to the right.

Perry B. Hackett  
Chief Sciences Officer  
and  
Professor (on leave of absence)  
Department of Genetics, Cell Biology and Department  
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