

## Identification and Analysis of Weak Linear Banding Patterns of Fish Chromosomes with a Computer-Based Densitometric Method

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Identification of individual chromosomes by banding patterns is an essential step for physical genetic mapping of fish and other species where chromosomes are numerous and similar in size. Many techniques, such as Giemsa banding, reverse banding and restriction enzyme banding can generate well-defined patterns on mammalian chromosomes. However, these linear banding techniques are not useful with fish species, presumably due to structural homogeneity of fish chromosomes (4). Efforts have been made to produce banding patterns on fish chromosomes with techniques such as replication banding, in which DNA is labeled chemically during replication (10). However, the resultant patterns are difficult to analyze with the naked eye because distinct borders do not exist between dark and light bands, thus making these observations subjective and labor-intensive. Computer-assisted methods have been utilized to automate karyotyping and to examine subtle differences in chromosome size (2,8). Use of densitometry-based techniques has been reported in the field of molecular biology for quantitation of radiographic assays, electrophoretic bands (3,6) and DNA hybridization signals on chromosomes (7). Little information exists on application of densitometry to karyotyping or numerical analysis of chromosomal bands, especially for problematic groups such as fish. We report in this study, a computer-based densitometric method for analysis of weak banding patterns, thus providing a fast and objective solution to this problem.

Pro-metaphase chromosomes were prepared from cultured leukocytes of channel catfish (*Ictalurus punctatus*) with standard fluorouracil-bromodeoxyuridine replication banding procedures and stained by fluorochrome plus Giemsa (5,10). Images of the chromosomes were captured directly with a 24-bit video capture board (Imaging Technology, Bedford, MA, USA) at 1000× magnification from a Microphot™-SA Light Microscope (Nikon, Melville, NY, USA) equipped with a high-resolution RGB color video camera (Model A206A; Microimage Video Systems, Boyertown, PA, USA). The Op-

timas® computer software package (Bioscan, Edmonds, WA, USA), a Windows®-based application, was used for processing of captured images.

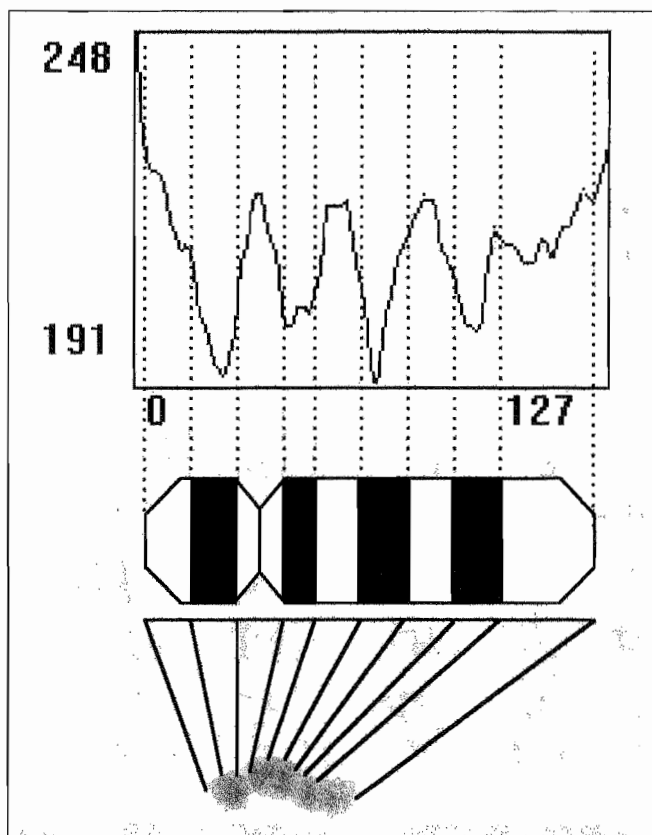
The linear measurement tool was used to draw a line along the long axis of individual chromosomes. By using this line as a transect, length and luminance of chromosomes were measured simultaneously. In this program, the line transect can be analyzed as 1–4096 sample units (segments), and a luminance value can be acquired from each. The number of units used varied with chromosome size and was standardized in preliminary trials. In channel catfish, chromosomes were divided into 32 sample units per 1% of relative length, which was calculated using the following formula:

$$\text{Relative length (\%)} = (\text{length of homologous pair} / \text{total length of chromosome complement}) \times 100$$

The smallest chromosome of channel catfish (relative length, 2.0%) was divided into 64 sample units, and the largest chromosome (relative length, 5.1%) was divided into 163 units (10).

Luminance patterns were derived from the average grayscale values of each sample unit along a chromosome. The linear luminance pattern of each chromosome was displayed as a line chart, in which the Y-axis represented grayscale values ranging from black (a value of 0) to white (a value of 255), and the X-axis corresponded to the length of the chromosome (Figure 1). An idiogram was created for each chromosome with Microsoft® PowerPoint™ (Version 4.0; Microsoft, Redmond, WA, USA), indicating size (based on length), centromeric position and the banding pattern based on the luminance chart (Figure 1).

This rapid and semi-automated procedure provided an objective method for analysis of weak or ambiguous chromosome bands of fish. With this technique, we have produced a standard banded karyotype of channel catfish (9) and American oyster, *Crassostrea virginica* (unpublished). Establishment of such analysis systems need not be expensive to most laboratories (1). Software such as NIH Image (written by W. Rasband at the NIH and available from the Internet by anonymous file transfer protocol [FTP] from [zippy.nimh.nih.gov](http://zippy.nimh.nih.gov)), which has functions similar to Optimas for analysis of grayscale images, can be downloaded at no charge from the Internet. Assuming that a microscope and a computer are



**Figure 1. Analysis of chromosome banding patterns by densitometry.** The pattern (bottom), produced by replication banding and stained by Hoechst 33258 and Giemsa (10), was expressed as a luminance plot (top), in which the X-axis indicates chromosome segments and the Y-axis indicates relative grayscale value. The idiogram (middle) was created for the chromosome based on the luminance plot.

available, a video camera with a digitizing board can be obtained at a cost of about \$3000 or a digital camera can be obtained at a cost of about \$1200. Alternatively, photographs can be digitized with a standard flatbed scanner and analyzed by the image analysis software (scanners cost about \$1000).

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