**Data Analysis Problem**

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to accompany

*The Cell: A Molecular Approach,* Eighth Edition

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**5.2 Spectral Karyotyping**

This Data Analysis Problem does not appear in the textbook.

**Source:** Trask, B. 2002. Human cytogenetics: 46 chromosomes, 46 years and counting. *Nature Reviews Genetics* 3: 769–778.

**Corresponding chapter(s) in the textbook:** Chapter 5

**Review the following terms before working on the problem:** fluorescence *in situ* hybridization, metaphase cell, interphase cell, autosomes, sex chromosomes, DNA library, restriction endonucleases, cloning vector, fluorescence microscope, homologous chromosomes

**Experiment**

The figure shows a micrograph of fluorescence *in situ* hybridization (FISH). A spread from human lymphocytes in metaphase was analyzed. (The figure shows a cell in interphase, as well). Hybridization probes were prepared as follows. The 24 different chromosomes from a human cell sample (22 autosomes, X, and Y) were separated from each other based on size. A subgenomic DNA library was created from each chromosome by digesting the DNA of the chromosome with a restriction endonuclease and ligating the DNA fragments into appropriate vector molecules. The restriction fragments from each library were labeled *in vitro* with either a single fluorescent dye or a unique combination of dyes so that each of the 24 different sets of fragment probes could identify a specific chromosome by its unique color. Metaphase chromosomes were hybridized with a mixture of all 24 different probes and visualized under a fluorescence microscope.

**Figure**



*An interphase cell (upper left corner) and*

*a set of metaphase chromosomes.*

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**Questions**

1. How would you prepare a lymphocyte sample enriched with cells in metaphase?

2. How would you separate the DNA molecules of the 24 different chromosomes from each other?

3. How can homologous chromosomes be identified in the figure? See how many pairs you can find.

4. What does the fluorescent image of the interphase nucleus tell you?

5. How could this technique be applied in clinical genetics?