

technical limitations of the antibody staining technique.

3. Methodology Tip—FISH Technique

In situ hybridization was invented by Gall and Pardue (54). This technique can determine the location of a given gene on a chromosome, to identify the particular chromosome, and to measure the number of copies of that gene on the chromosome.

FISH means *fluorescence in situ hybridization*. This term appears on the Kaplan–Meier plot from the Vogel study (55), as described earlier in this chapter. FISH involves contacting a fluorescent-tagged nucleic acid with a permeabilized cell, and allowing the fluorescent nucleic acid to diffuse to the gene of interest and to hybridize with the gene of interest, thereby producing a fluorescent signal that is associated with the gene in a stable manner.

The signal is proportional to the number of copies of the gene in the cell. In an article discussing the reliability of the FISH technique for measuring *HER2* expression in breast cancer tumors, Gunn et al. (56) compared the FISH technique, which measures the number of *HER2* genes, with immunological techniques, which measures the amount of *HER2* protein residing on the membrane of the cell.

This concerns gene amplification. Gene amplification can occur in mammals (57), bacteria (58), protozoans (59), and archaeobacteria (60). Gall (61) has been credited with the discovery of gene amplification (62). Schimke and co-workers (63,64) conducted most of the early, detailed research on the mechanisms of gene amplification, in the context of studies explaining how cancer cells in patients treated with methotrexate became resistant to that drug.

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