2. Fluorescent or silver in situ hybridization (FISH or SISH).

In FISH, fluorescently tagged DNA or RNA probes are used to identify genomic sequences of interest. FISH may be used to identify sequences of interest in tissue sections, an advantage that permits correlation of probe hybridization with tissue morphology. When coupled to conventional cytogenetics, FISH provides high resolution for identification of specific abnormalities, e.g., gene amplification, deletions, and translocations.

FISH requires denaturation, hybridization with a probe, and washing. First, a probe specific for the target of interest is applied to the slide, along with a nuclear counterstain and reagents or heat that enhance denaturation of target DNA and reduce background. The slides are sealed and incubated in a humid environment under conditions that denature the DNA, allowing hybridization to occur between the probe and its cDNA sequence. The unbound probe is then removed by washing, and patterns of fluorescence are interpreted by fluorescence microscopy.

Principles of hybridization

* DNA is double stranded.
* Bonds between complementary bases hold stands together (Cytosine ⟷ Guanine; Adenine ⟷ Thymine).
* Heat/alkalinize DNA – separation of strands ('denaturation') occurs.
* Cool separated strands – *complementary* double strands re-form.
* Labelled complementary single-strand DNA can identify a DNA sequence (e.g. a gene) in intact cells or disrupted cell preparations.