Fluorescence *in situ* hybridization (FISH)

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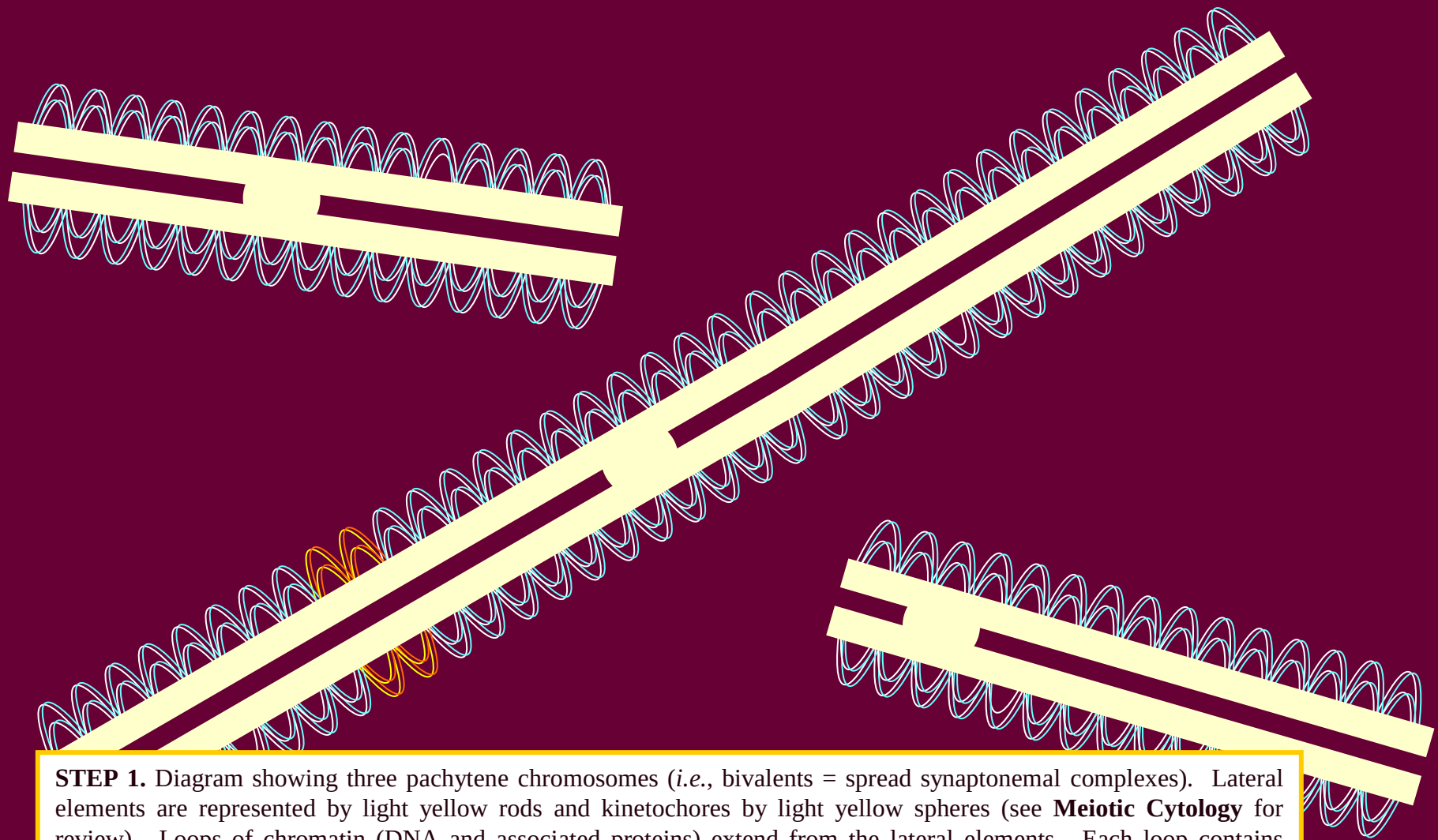
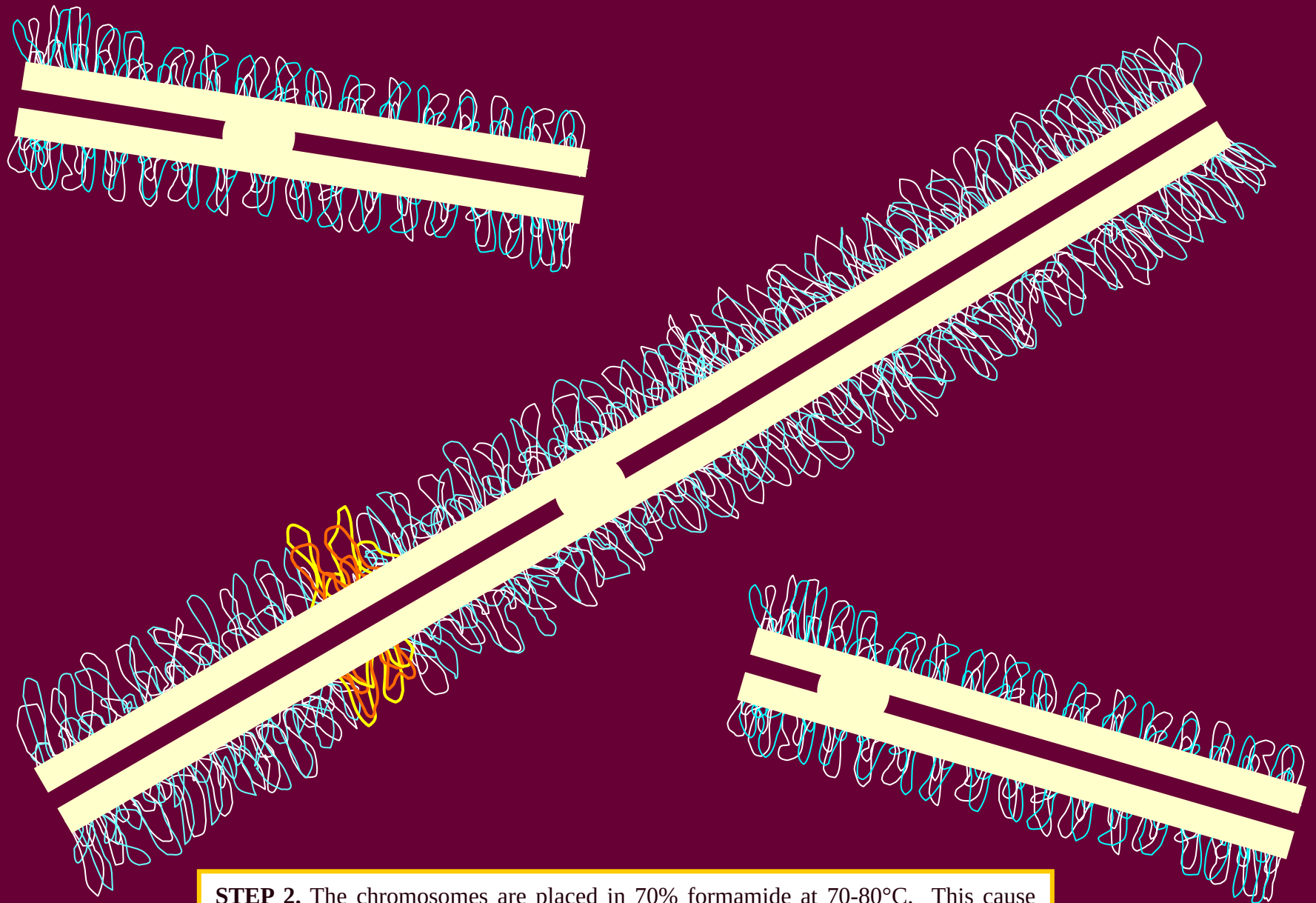
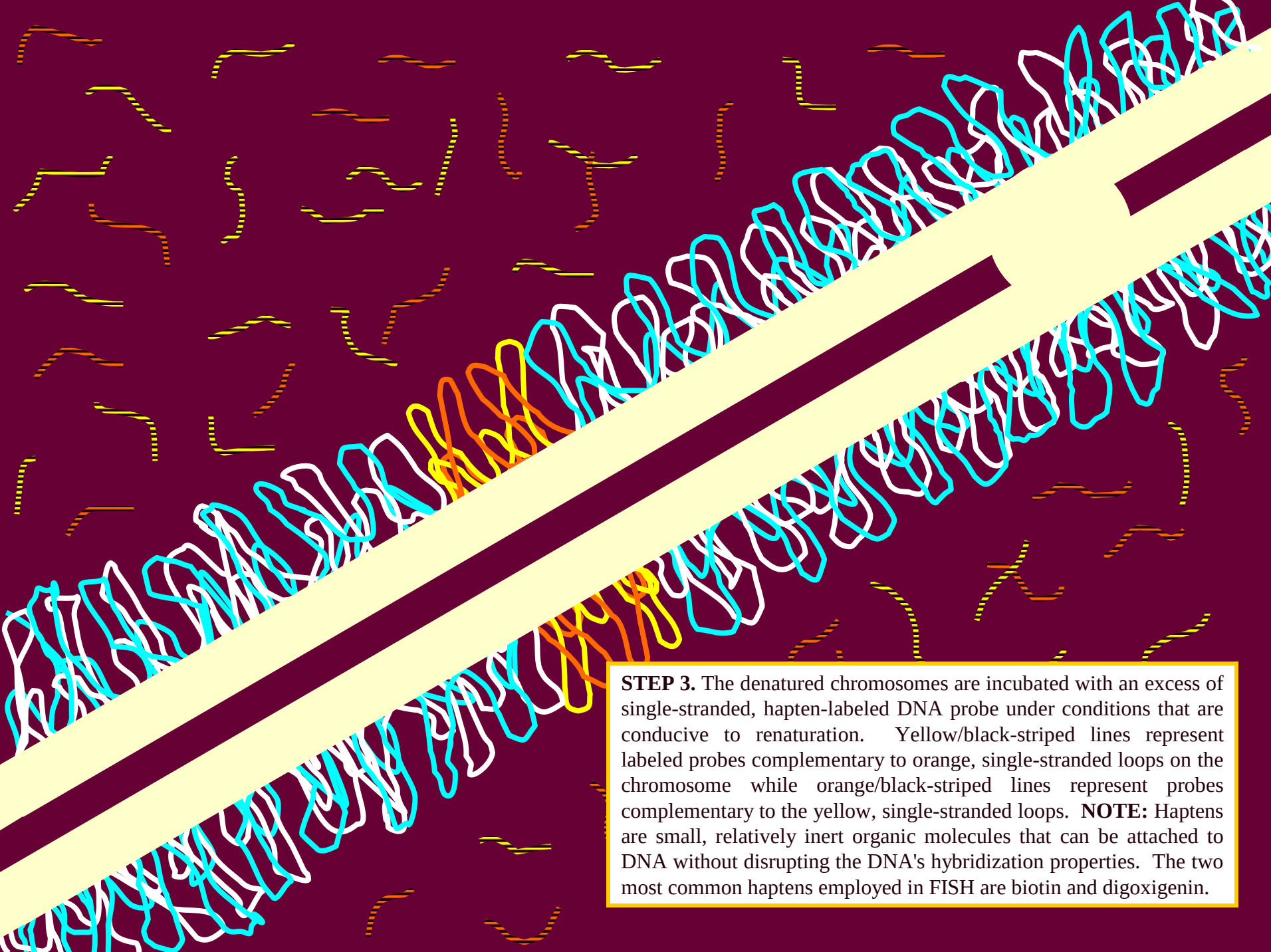


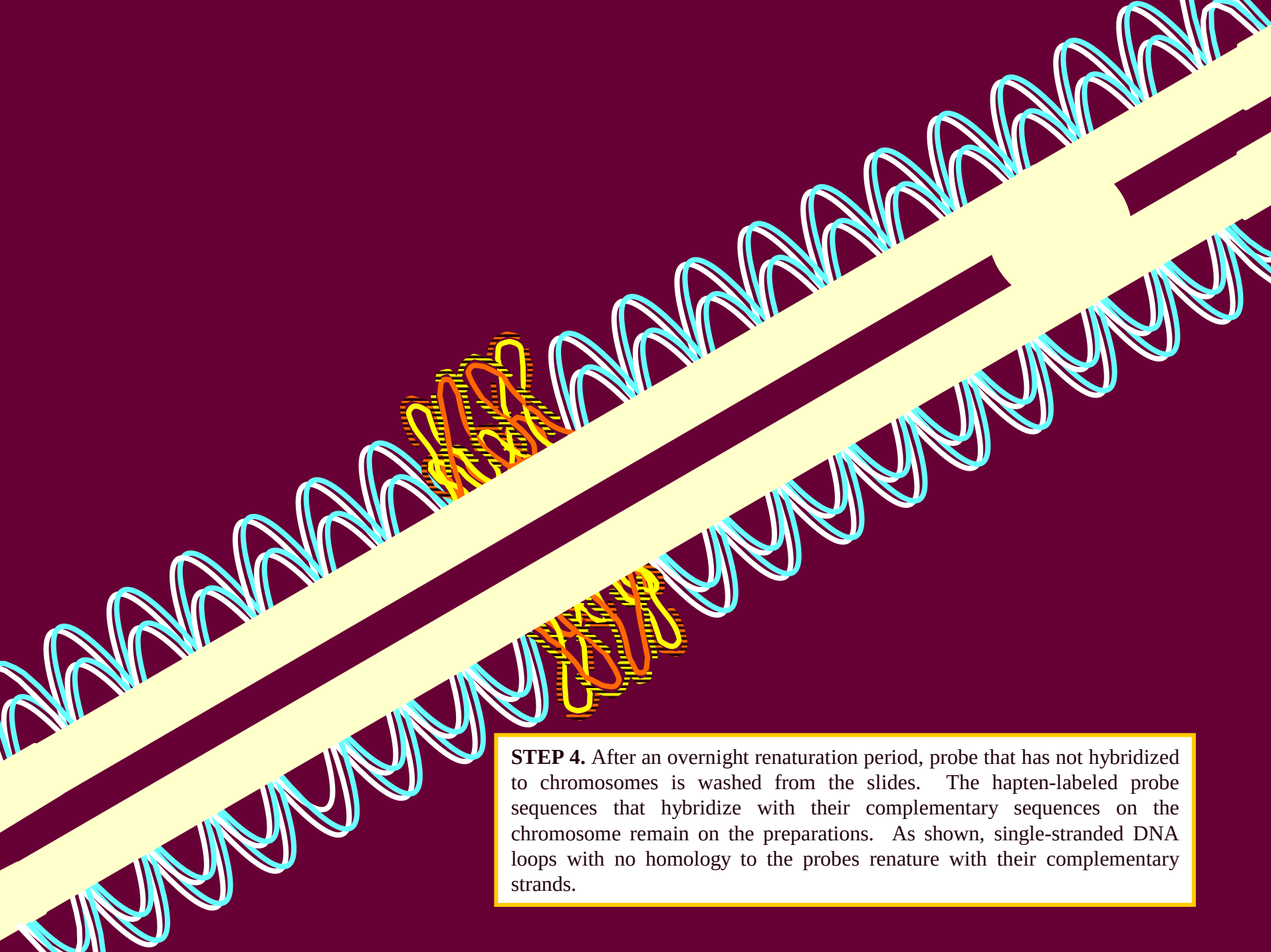
STEP 1. Diagram showing three pachytene chromosomes (*i.e.*, bivalents = spread synaptonemal complexes). Lateral elements are represented by light yellow rods and kinetochores by light yellow spheres (see **Meiotic Cytology** for review). Loops of chromatin (DNA and associated proteins) extend from the lateral elements. Each loop contains double-stranded DNA (complementary DNA strands are represented by the colors white and light blue). As each homologue is composed of two sister chromatids, two loops extend from each locus along a lateral element. The chromosomal sequence of interest (*i.e.*, the sequence with homology to the probe being used) is represented by the yellow/orange loops on the longest chromosome in the diagram (yellow represents one strand of the target DNA molecule while orange represents its complementary strand).



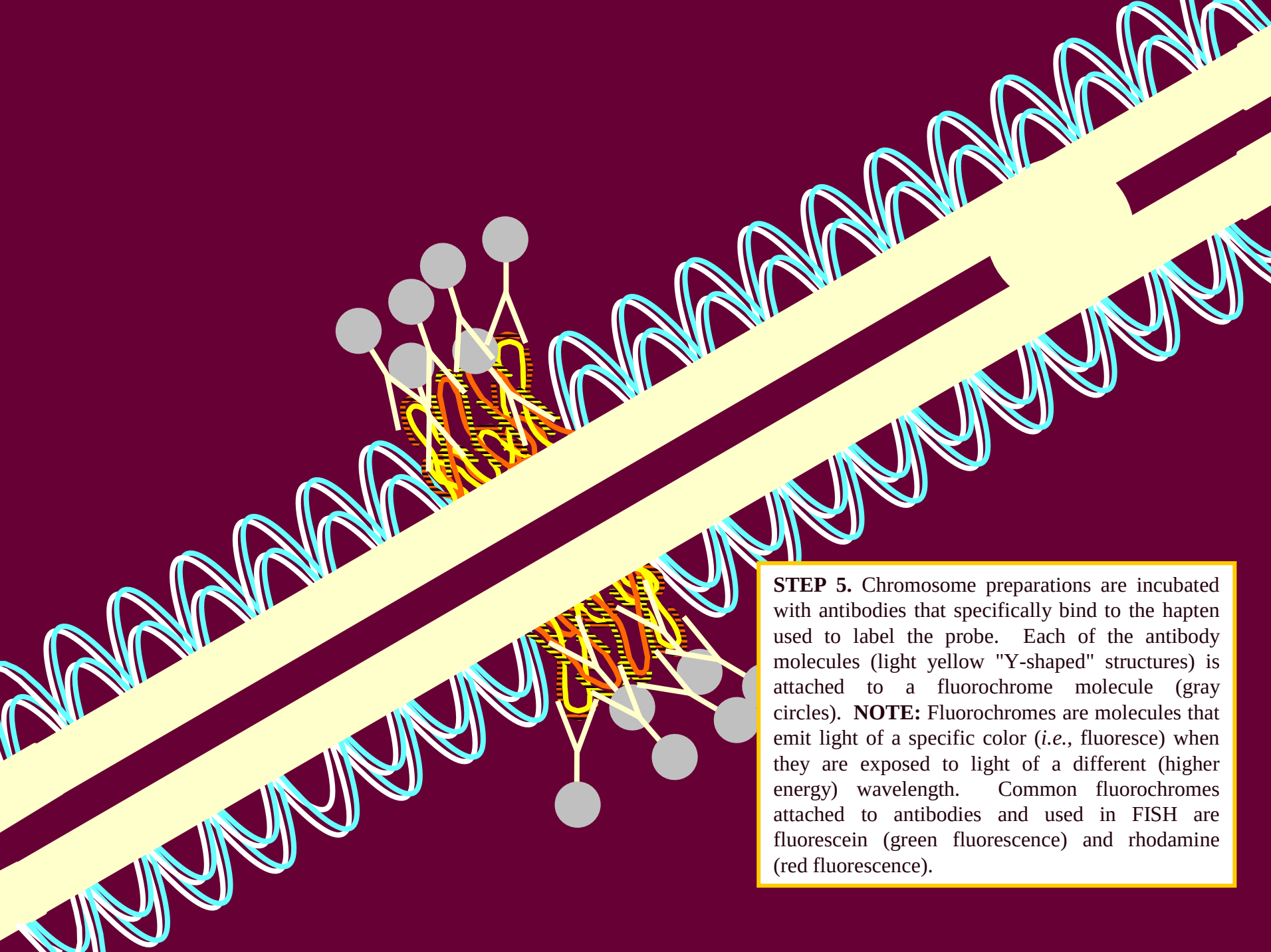
STEP 2. The chromosomes are placed in 70% formamide at 70-80°C. This cause DNA duplexes to come apart resulting in single-stranded molecules (*i.e.*, the DNA is denatured).



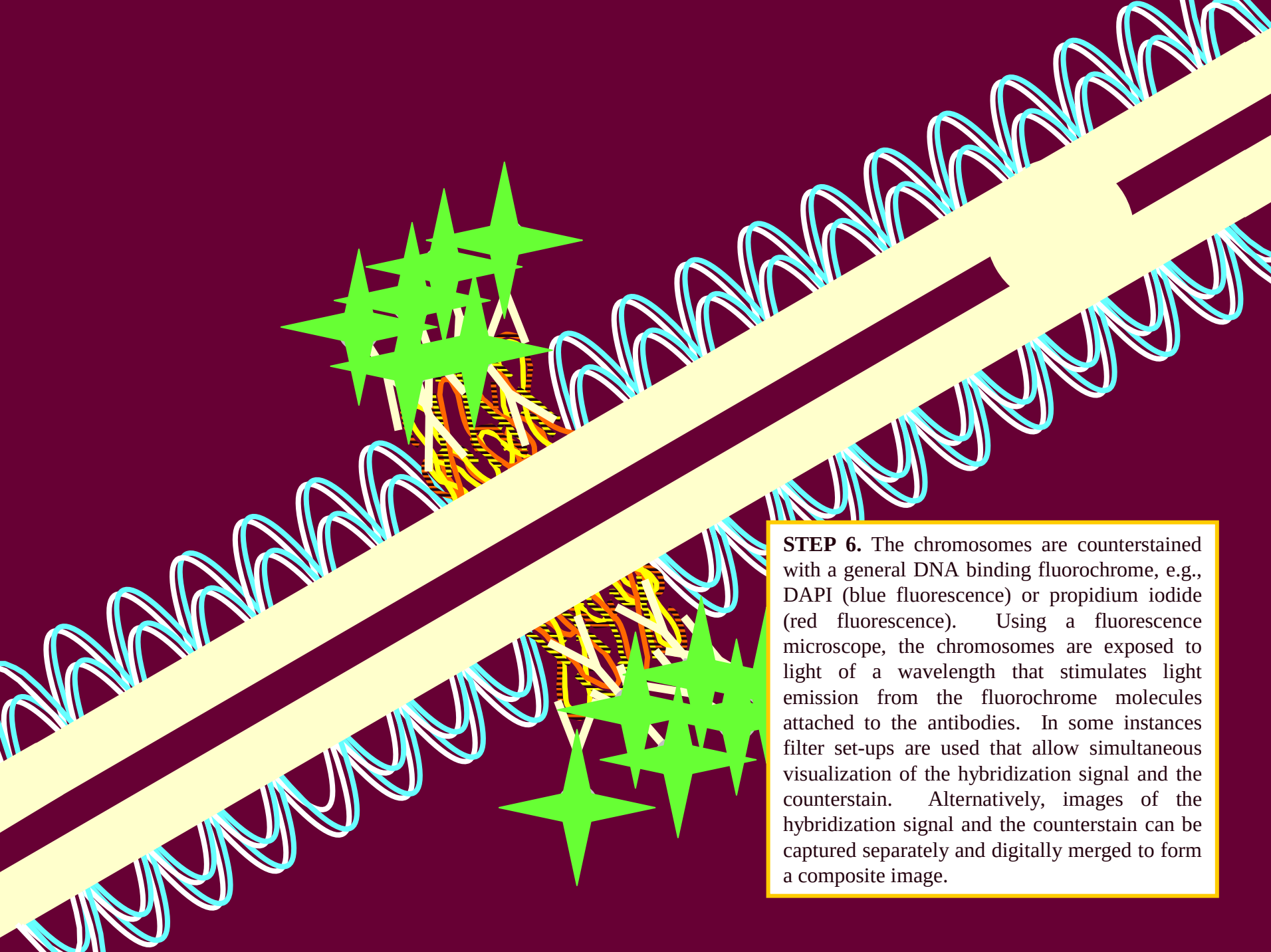
STEP 3. The denatured chromosomes are incubated with an excess of single-stranded, hapten-labeled DNA probe under conditions that are conducive to renaturation. Yellow/black-striped lines represent labeled probes complementary to orange, single-stranded loops on the chromosome while orange/black-striped lines represent probes complementary to the yellow, single-stranded loops. **NOTE:** Haptens are small, relatively inert organic molecules that can be attached to DNA without disrupting the DNA's hybridization properties. The two most common haptens employed in FISH are biotin and digoxigenin.



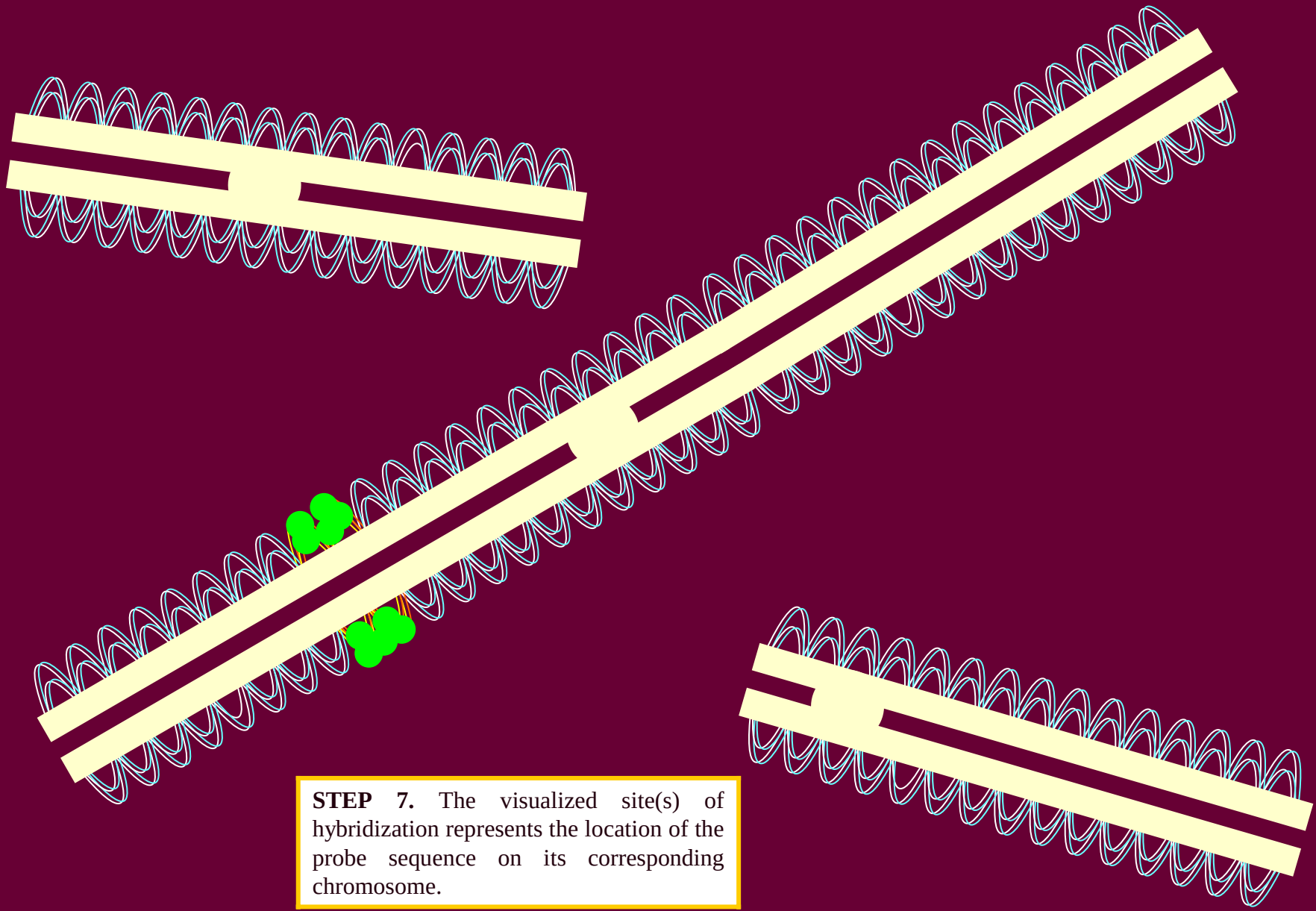
STEP 4. After an overnight renaturation period, probe that has not hybridized to chromosomes is washed from the slides. The hapten-labeled probe sequences that hybridize with their complementary sequences on the chromosome remain on the preparations. As shown, single-stranded DNA loops with no homology to the probes renature with their complementary strands.



STEP 5. Chromosome preparations are incubated with antibodies that specifically bind to the hapten used to label the probe. Each of the antibody molecules (light yellow "Y-shaped" structures) is attached to a fluorochrome molecule (gray circles). **NOTE:** Fluorochromes are molecules that emit light of a specific color (*i.e.*, fluoresce) when they are exposed to light of a different (higher energy) wavelength. Common fluorochromes attached to antibodies and used in FISH are fluorescein (green fluorescence) and rhodamine (red fluorescence).



STEP 6. The chromosomes are counterstained with a general DNA binding fluorochrome, e.g., DAPI (blue fluorescence) or propidium iodide (red fluorescence). Using a fluorescence microscope, the chromosomes are exposed to light of a wavelength that stimulates light emission from the fluorochrome molecules attached to the antibodies. In some instances filter set-ups are used that allow simultaneous visualization of the hybridization signal and the counterstain. Alternatively, images of the hybridization signal and the counterstain can be captured separately and digitally merged to form a composite image.



STEP 7. The visualized site(s) of hybridization represents the location of the probe sequence on its corresponding chromosome.