9.4: Diagnosing Human Chromosome Abnormalities

Bright Field Microscopy

How can we confirm that a person has a specific chromosomal abnormality? The first method was simply to obtain a sample of their cells, stain the chromosomes with **Giemsa** dye, and examine the results with a light microscope (Figure \(\PageIndex{1}\)). Each chromosome can be recognized by its length, the location of its centromere, and the characteristic pattern of purple bands produced by the Giemsa. For example, if mitotic cells from a person consistently contained forty seven chromosomes in total with three chromosome 21s this would be indicative of Down syndrome. Bright field microscopy does have its limitations though - it only works with mitotic chromosomes and many chromosome rearrangements are either too subtle or too complex for even a skilled cytogeneticist to discern.

Figure \(\PageIndex{1}\): Human chromosomes. One way to obtain chromosomes is to take a blood sample, culture the cells for three days in the presence of a T-cell growth factor, arrest the cells in metaphase with a microtubule inhibitor, and then drop the cells onto a slide. The cells burst and the chromosomes stick to the slide. The chromosomes can then be stained or probed. Because the cells are in metaphase it is possible to see 46 replicated chromosomes here. There
Fluorescence In Situ Hybridization

The solution to these problems was fluorescence in situ hybridization (FISH). The technique is similar to a Southern blot in that a single stranded DNA probe is allowed to hybridize to denatured target DNA (see Section 8.6). However, instead of the probe being radioactive it is fluorescent and instead of the target DNA being restriction fragments on a nylon membrane it is denatured chromosomes on a glass slide. Because there are several fluorescent colours available it is common to use more than one probe at the same time. Typically the chromosomes are also labeled with a fluorescent stain called DAPI which gives them a uniform blue colour. If the chromosomes have come from a mitotic cell it is possible to see all forty six of them spread out in a small area. Alternatively, if the chromosomes are within the nucleus of an interphase cell they appear together within a large blue circle.

Using FISH to Diagnose Down Syndrome

Most pregnancies result in healthy children. However in some cases there is an elevated chance that the fetus has trisomy-21. Older women are at a higher risk because the non-disjunction events that lead to trisomy become more frequent with age. The second consideration is what the fetus looks like during an ultrasound examination. Fetuses with trisomy-21 and some other chromosome abnormalities have a swelling in the back of the neck called a nuchal translucency. If either or both factors is present the woman may choose amniocentesis. In this test some amniotic fluid is withdrawn so that the fetal cells within it can be examined. Figure 2 shows a positive result for trisomy-21. Based upon this image the fetus has two X chromosomes and three chromosome 21s and therefore has a karyotype of 47,XX,+21.

Using FISH to Diagnose Cri-du-Chat Syndrome

A physician may suspect that a patient has a specific genetic condition based upon the patient's physical appearance, mental abilities, health problems, and other factors. FISH can be used to confirm the diagnosis. For example, Figure
shows a positive result for cri-du-chat syndrome. The probes are binding to two long arms of chromosome 5 but only one short arm. One of the chromosome 5s must therefore be missing part of its short arm.

![Diagram showing positive result for cri-du-chat syndrome](image)

Figure 3: A positive result for cri-du-chat syndrome. This diagram is based upon actual results. Cells from a patient's blood were prepared to show an interphase nucleus (a) and mitotic chromosomes (b). The DNA has been coloured blue with DAPI. The green fluorescent probe is binding to the tip of the short arm of chromosome 5 (shown here as open circles). This is the region absent in cri-du-chat. The red fluorescent probe is binding to the middle of the long arm of the same chromosome (filled circles). This probe is used as a control. Source: Figure 1 in Fang J.-S. et al. 2008 Cytogenetic and molecular characterization of three-generation family with chromosome 5p terminal deletion. Clinical Genetics 73:585-590 PubMed ID: 18400035.

**Newer Techniques**

FISH is an elegant technique that produces dramatic images of our chromosomes. Unfortunately, FISH is also expensive, time consuming, and requires a high degree of skill. For these reasons, FISH is slowly being replaced with PCR and DNA chip based methods. Versions of these techniques have been developed that can accurately quantify a person's DNA. For example a sample of DNA from a person with Down syndrome will contain 150% more DNA from chromosome 21 than the other chromosomes. Likewise DNA from a person with cri-du-chat syndrome will contain 50% less DNA from the end of chromosome 5. These techniques are very useful if the suspected abnormality is a deletion, a duplication, or a change in chromosome number. They are less useful for diagnosing chromosome inversions and translocations because these rearrangements often involve no net loss or gain of genes.

In the future all of these techniques will likely be replaced with DNA sequencing. Each new generation of genome sequencing machines can sequence more DNA in less time. Eventually it will be cheaper just to sequence a patient's entire genome than to use FISH or PCR to test for specific chromosome defects.