Abstract

The method of fluorescence in situ hybridisation (FISH) is based on the fundamental property of DNA strands to anneal to one another in a complementary fashion. However, there are different classes of DNA probes available for FISH investigations, but not all of them are suitable for all targets. Locus-specific probes are derived from single-copy DNA and produce a specific signal. In contrast, painting probes are heterogeneous DNA libraries, composed of different DNA classes, namely single copy sequences as well as repetitive sequences. Furthermore, DNA probes constituted of repetitive sequences only target specific structures of the chromosomes, i.e. the centromeres and the telomeres. All these probes have in common that they are directly or indirectly labelled and after hybridisation are visualised under an epifluorescence microscope. Comparative genomic hybridisation (CGH), a method to determine genetic imbalances with a resolution between 2 and 10 Mb, uses normal metaphase spreads as the target and the fluorescently labelled test DNA is hybridised together with differently labelled normal reference DNA. The resolution of CGH has been improved tremendously by the introduction of array-CGH, where the target chromosomes are replaced by DNA from, for example, bacterial artificial chromosomes with a spacing of 1 Mb and beyond. FISH has developed into a valuable tool for genetic investigations in clinical as well as tumour cytogenetics for detection of microdeletions, amplifications, specific fusion genes and others.

18.1 Introduction

Fluorescence in situ hybridisation (FISH) was introduced in 1986 (Pinkel et al. 1986). In general, FISH is applied to slides prepared from standard cytogenetic cell suspensions, but it can be carried out on tissue sections, blood or bone marrow smears as well. The method of FISH is based on the fundamental property of DNA strands to anneal to one another in a complementary fashion (complementary base pairing). Owing to this basic feature of single-stranded DNA, a specific DNA probe will hybridise to its complementary target DNA
sequence, no matter whether it is present in a metaphase chromosome, an interphase nucleus or as naked filamentary DNA (fibre-FISH).

There are different classes of DNA probes available for FISH investigations, but not all of them are suitable for all targets. For example, a whole chromosome painting probe is constituted of a library of different DNA sequences from the same chromosome, but specific for a variety of different loci on the respective chromosome. Such a probe is informative on metaphase chromosomes, but will contribute only little to no information on a DNA fibre.

FISH is carried out for different purposes. By using locus-specific probes, the presence or absence of a specific DNA sequence can be determined, or specific DNA probes can be assigned to particular chromosomal sites. In clinical cytogenetics, FISH investigations provide clarification of aberrations that were observed using standard cytogenetic techniques (GTG-banding). Furthermore, FISH is able to detect cryptic or submicroscopic chromosomal aberrations (e.g. microdeletions).

18.2 The Different Classes of DNA Probes

The human DNA is composed of different DNA classes (Table 18.1), which are of variable abundance. The basic nature of these DNA classes is of importance for the technical aspect of FISH experiments. A single copy sequence is present only once in the human haploid genome and, thus, will produce a specific signal.

In contrast, painting probes are heterogeneous DNA libraries and include all DNA classes. In order to avoid cross-hybridisation with repetitive DNA

<table>
<thead>
<tr>
<th>Class</th>
<th>Copy number</th>
<th>Distribution</th>
<th>Percentage of the human genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single copy sequence</td>
<td>1</td>
<td>Unique copies throughout the whole genome</td>
<td>50</td>
</tr>
<tr>
<td>Low copy number repeat</td>
<td>2–20</td>
<td>Mainly throughout the euchromatin</td>
<td>10</td>
</tr>
<tr>
<td>Moderately repeated DNA</td>
<td>50–5,000</td>
<td>Scattered throughout the entire genome</td>
<td>25</td>
</tr>
<tr>
<td>Highly repeated DNA</td>
<td>10,000–500,000</td>
<td>Clustered in heterochromatin, like satellite DNA but also interspersed throughout the genome</td>
<td>15</td>
</tr>
</tbody>
</table>