DNA and RNA Hybridization

Principles of nucleic acid hybridization:

Nucleic acid hybridization involves mixing single strands of two sources of nucleic acids, a probe which typically consists of a homogeneous population of *identified molecules* (e.g. cloned DNA or chemically synthesized oligonucleotides) and a **target** which typically consists of a *complex, heterogeneous population* of nucleic acid molecules. If either the probe or the target is initially double-stranded, the individual strands must be separated, generally by heating or by alkaline treatment. After mixing single strands of probe with single strands of target, strands with complementary base sequences can be allowed to reassociate. Complementary probe strands can reanneal to form *homoduplexes*, as can complementary target DNA strands.

Southern blot hybridization

In this procedure, the target DNA is digested with one or more restriction endonucleases, size-fractionated by agarose gel electrophoresis, denatured and transferred to a nitrocellulose or nylon membrane for hybridization.

Following electrophoresis, the test DNA fragments are denatured in strong alkali. As agarose gels are fragile, and the DNA in them can diffuse within the gel, it is usual to transfer the denatured DNA fragments by blotting on to a durable nitrocellulose or nylon membrane, to which single-stranded DNA binds readily. The individual DNA fragments become immobilized on the membrane at positions which are a faithful record of the size separation achieved by agarose gel electrophoresis. Subsequently, the immobilized single-stranded target DNA sequences are allowed to associate with labeled single-stranded probe DNA. The probe will bind only to related DNA sequences in the target DNA, and their position on the membrane can be related back to the original gel in order to estimate their size.

Northern blot hybridization

Northern blot hybridization is a variant of Southern blotting in which the target nucleic acid is RNA instead of DNA. A principal use of this method is to obtain information on the expression patterns of specific genes. Once a gene has been cloned, it can be used as a probe and hybridized against a Northern blot containing, in different lanes, samples of RNA isolated from a variety of different tissues. The data obtained can provide information on the range of cell types in which the gene is expressed, and the relative abundance of transcripts.

Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridisation (FISH) allows the visualisation of prokaryotic cells in their natural environment. In short, cells are fixed (i.e., they are not viable anymore and the status quo of their DNA and RNA is preserved), permeabilised to facilitate access of the probe to the target site and then hybridised with nucleic acid probes. The probes are either directly labelled with a fluorochrome or the dye is introduced in a secondary detection step. The samples can then be analysed by epifluorescence or laser scanning microscopy or flow cytometry. The classic FISH technique relied solely on (usually 16S) rRNA as probe target. The rRNA immediately suggests itself as the ideal target because it is present in all living cells in relatively high copy numbers. Furthermore, since it is traditionally used as phylogeneticmarker a lot of sequence data is available for probe design.

Since its origins some 20 years ago this technique has become an invaluable tool for environmental microbiologists and has spawned numerous variations. The reasons for this popularity are obvious:
(1) FISH allows the detection of cells regardless of their culturability.
With as little as 0.3% of bacteria in soil and <0.1% in marine water being culturable FISH offers a glimpse at the full bacterial biodiversity.
(2) The possibility to detect cells in situ allows an insight into the structure of microbial communities and may help to unveil their ecological function.

Apart from permeability issues, the main reason for weak signals with the classic rRNA targeted FISH is the low ribosome content found in slowly growing or metabolically inactive cells in environmental samples. Problems of this nature have led to a number of different approaches for signal amplification being developed in recent years. This improved sensitivity of the method ultimately negated the dogma that FISH requires high copy numbers of the target molecule and paved the way for new applications, allowing microbiologists to move away from 16S rRNA and instead target other nucleic acids present in lower copy numbers, such as mRNA, plasmids or even single copy genes. This trend is further aided by the increasing amount of available sequence data gathered from multiple genome projects.