

Understanding
chromosome
disorders

Unique



Fluorescence *in situ* hybridisation (FISH)

Fluorescence *in situ* hybridization (FISH) Chromosomes

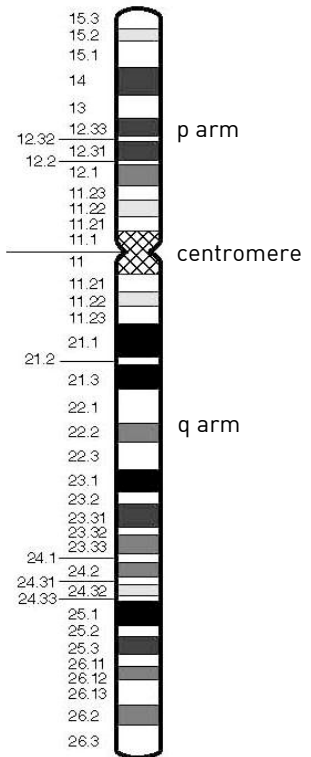
Chromosomes are structures that contain the genetic information (DNA) that tells the body how to develop and function. The cells in the body have 46 chromosomes which are arranged in pairs. Each pair contains a chromosome from each parent and they are arranged approximately according to size and numbered from 1 to 22. The last pair contains the sex chromosomes. Girls have two Xs (XX) and boys have an X and a Y (XY). Cytogenetic (chromosome) testing looks for changes in the number and structure of the chromosomes and for losses (deletions) and gains (duplications) of genetic material. People with these types of changes in their cells may have an increased risk of birth defects, developmental delay, behavioural problems and intellectual disability.

Looking at chromosomes (chromosome analysis)

Chromosomes can be seen if they are stained and magnified under the microscope. Chromosomes have a short (p) arm and a long (q) arm held together at a structure called the centromere. Each chromosome has a distinct pattern of light and dark bands which are numbered outwards from the centromere. The chromosome diagram on the right shows the banding pattern of chromosome 10. Clinical scientists use microscope analysis to look for changes in the shape of the chromosomes and their banding patterns. If the change is large enough, rearrangements and imbalances (deletions and duplications) involving chromosomes or parts of the chromosomes can be seen. Clinical scientists who do the analysis are very skilled at detecting very small and often very subtle changes. However, chromosome rearrangements and the loss or gain of material can often be extremely small and frequently cannot be observed with a routine chromosome test even by the most skilled of scientists. FISH testing allows some of these changes to be detected.

What is FISH?

FISH is a method that can be used to detect small deletions and duplications that are not visible using microscope analysis. It can also be used to detect how many chromosomes of a certain type are present in each cell and to confirm rearrangements that are suspected after microscope analysis. FISH looks specifically at the one specific area of a chromosome only. It uses a very small chemical that glows brightly when it detects the specific region on a chromosome. A scientist uses a special microscope to look at the chromosome and see how many bright spots are present. When a person has a deletion, only one bright spot can be seen instead of two (one on each chromosome). When a person has a duplication, three bright spots can be seen instead of just two.



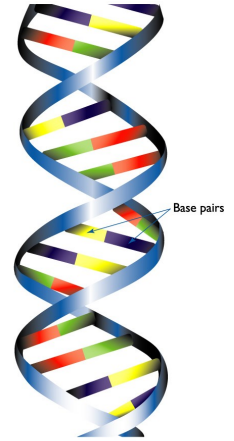
Chromosome 10

How does FISH work?

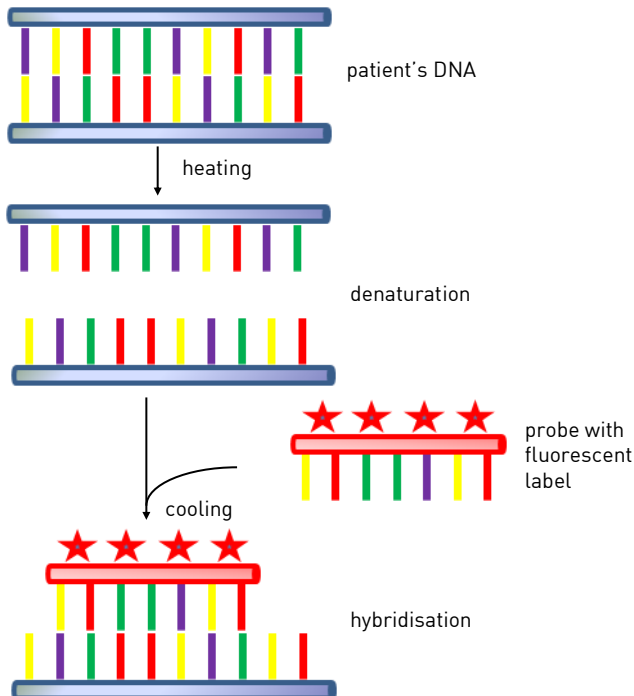
The DNA in our cells contains two strand-like molecules coiled together into a structure known as a double helix (see right). Each strand has a sequence containing a mixture of four bases (A, T, G and C). The bases in each strand are able to bind to each other and hold the DNA together, but can only do so if they are properly matched, or complementary, to those in the other strand (an A can only bind to a T and a C can only bind to a G). When two complementary sequences find each other they will bind together, or hybridise. FISH works by exploiting the ability of one DNA strand to hybridise specifically to another DNA strand.

FISH uses small DNA strands called probes that have a fluorescent label attached to them. The probes are complementary to specific parts of a chromosome. When DNA is heated, the patient's two DNA strands break apart, or denature, and the probes are able to hybridise to their complementary sequence in the patient's DNA (see diagram below).

If a small deletion is present in the region complementary to the probe, the probe will not hybridise. If a duplication is present, more of the probe is able to hybridise.



Two strands of DNA are held together in the shape of a double helix by the bonds between base pairs.



An overview of the FISH test



FISH testing for a deletion. Two probes are usually used; The first probe (green) is a control probe used to identify both copies of the chromosome under test. It hybridises to a sequence that is not part of the deletion, so a signal is observed on each chromosome. The second probe (red) hybridises to the sequence that may be deleted. A deletion is usually found in only one of the chromosomes in a pair, therefore the probe can bind to the intact chromosome, but is unable to bind to the deleted chromosome and only one signal is seen.

What types of probes are used?

Many FISH probes bind to specific gene-containing parts of the DNA and are used to detect small imbalances. These are known as locus-specific probes. Other probes bind to the centromere or the telomeres (the ends of the chromosome arms) and are used to count the number of certain chromosomes and to detect rearrangements. Paint probes are also available which bind to the whole chromosome.

What samples are needed for FISH testing?

FISH is most commonly performed on blood samples from adults and children. FISH can also be used as a prenatal test for aneuploidy (extra copies of whole chromosomes) using amniotic fluid from an amniocentesis or placental samples from chorionic villus sampling (CVS). Less commonly it is used as a prenatal test for deletions, also using amniotic fluid or CVS samples.

Why has FISH been offered for your child?

FISH is often performed alongside standard microscope analysis if your child has characteristics that are strongly suggestive of a particular deletion syndrome or another syndrome for which a FISH test is available. Your geneticist may request microscope analysis and FISH testing together or may request FISH testing if microscope analysis gives a normal result.

How will we be given the results?

The results are most likely to be given to you by your geneticist who will talk you through your child's results and you will almost certainly receive a follow up letter.

FISH results are often included with the results of microscope analysis in a combined report. The first line of a report shows the results as a karyotype. If your child's chromosomes are not deleted, the karyotype may look something like this:

46,XX,ish 22q11.2(TUPLE1x2)(ARSAx2)

46	The total number of chromosomes present
XX	The sex chromosomes present (XX for girls, XY for boys)
ish	The analysis was by fluorescent in situ hybridisation (FISH)
22	The FISH test was performed on chromosome 22
q11.2	The chromosome has two breakpoints, both in band 22q11.2 and material between these two breakpoints is missing
(TUPLE1x2)	The DNA segment (probe) called TUPLE is present in two copies (as expected)
(ARSAx2)	The DNA segment (probe) called ARSA2 is present in two copies (as expected)

If a deletion is present, the karyotype is written slightly differently, as shown below

46,XX,ish del(22)(q11.2q11.2)(TUPLE1-)

46	The total number of chromosomes present
XX	The sex chromosomes present (XX for girls, XY for boys)
ish	The analysis was by fluorescent in situ hybridisation (FISH)
del	A deletion, or material is missing
(22)	The deletion is from chromosome 22
(q11.2q11.2)	The chromosome has two breakpoints, both in band 22q11.2 and material between these two breakpoints is missing
(TUPLE1-)	One copy of the DNA segment (marker) called TUPLE is missing

How long do the results take?

With blood testing, results are usually available in 4 weeks and can be available in under two weeks for priority cases such as newborn babies. Results may be available much sooner if a blood sample has been provided previously for microscope analysis, as the same sample can be used for the FISH test.

What are the advantages of FISH?

- FISH is able to detect many small deletions, duplications and rearrangements that are not visible with standard microscope analysis
- A diagnosis from FISH may avoid your child having to undergo many other tests

What are the benefits of FISH?

- It may help you and your doctor watch for common health problems associated with your child's chromosome imbalance
- It may help to predict what to expect as your child gets older
- It may show which specific genes are included in your child's deletion or duplication. If the gene(s) has been associated with a particular feature or health problem it may help to guide management or treatment for your child
- It can help you to obtain specialist services for your child
- You can choose to join a support group to meet other parents facing similar challenges
- Parents and other family members can be tested to see if they are carriers of changes in their DNA that put them at risk of having more children with a chromosome change

What are the limitations of FISH?

- FISH testing does not usually screen all chromosomes for changes. Most FISH probes are specific for one particular deletion or duplication within one band of one chromosome so it will only find what it is looking for
- FISH probes are only available for the most well characterised deletion and duplication syndromes. Your child may have a small imbalance that cannot be detected with the standard FISH tests currently available
- FISH has limited ability to precisely define which genes and breakpoints are involved in an imbalance
- It can be difficult for clinical scientists to see a duplication using FISH, as the attachment of extra probes is not always easy to see

What other tests are available if FISH does not reveal any rearrangements?

If microscope analysis and FISH testing do not reveal a rearrangement, your child may be referred for microarray-based comparative genomic hybridisation (array CGH). This test examines all chromosomes for losses and gains of DNA and can detect smaller imbalances than those detected with FISH or microscope analysis.

Notes

Inform Network Support



Rare Chromosome Disorder Support Group

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Join Unique for family links, information and support.

Unique is a charity without government funding, existing entirely on donations and grants. If you can please make a donation via our website at www.rarechromo.org

Please help us to help you!



This leaflet is not a substitute for personal medical advice. Families should consult a medically qualified clinician in all matters relating to genetic diagnosis, management and health. The information is believed to be the best available at the time of publication. It was compiled by Unique and reviewed by Dr Shehla Mohammed and Dr Caroline Ogilvie, Guy's Hospital, London. UK and Professor Maj Hultén, Professor of Reproductive Genetics, University of Warwick, UK. 2011, 2013 Version 2.1 (SW)

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