

How to Conquer a Chromosome Abnormality— What is a FISH analysis?



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What is a FISH analysis?

Special points of interest:

- * Fluorescence *in situ* hybridization
- * Using FISH probes
- * Using pooled FISH probes
- * Detecting small copy number changes

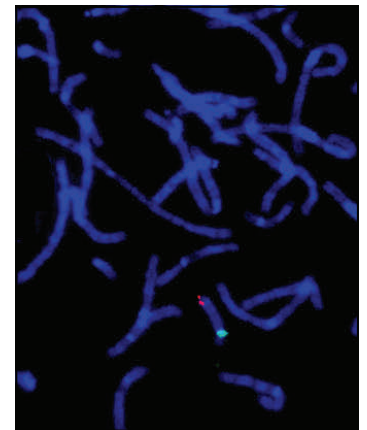
Many of you have heard of FISH analysis as a confirmatory test for a karyotype result. Here we will explain what this test can tell you and what it cannot.

FISH is an acronym for fluorescence in situ hybridization. Hybridization is just a fancy word for “attach.” This means that a fluorescently-labeled probe is used to attach to a certain spot on the chromosome on the microscope slide.

In our laboratory, we have over 400 different FISH probes that are specific for every part of chromosome 18. This way we can look for the presence or absence of any part of the chromosome.

In this particular example of a FISH, we used a probe for the chromosome 18 centromere shown in green and for the end of chromosome 18q arm, the 18q telomere, shown in red. This tells us that this particular chromosome 18 does not have a deletion from the end of the long arm.

Because this particular technique does not show the bands, it does not tell us much else about chromosome 18, or any other chromosome for that matter. For example, there could be a deletion of the end of the p arm and you would not necessarily know that from this particular test because you are only looking for the presence or absence of the FISH probes.



Inside this issue:

Whole chromosome paints	2
Karyotype and FISH comparison	2
Summary	2

FISH probe tests

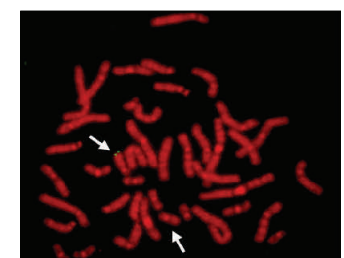
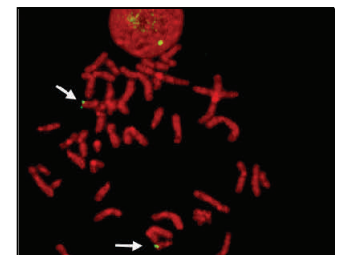
Here is another example. This experiment also uses a probe for the end of the 18q, the long arm—the 18q telomere.

In the top picture, the white arrows point to the two copies of chromosome 18. Each of these chromosomes has the green FISH probe attached to it at the telomere; therefore, these chromosomes do not have a deletion that involves the end of 18q. We cannot conclude that these are normal chromosomes—just that there are two 18q telomeres. There could be a small deletion that the probe does not attach to.

In the bottom picture the arrows point to each copy of chromosome 18. Here you

can see that only one copy of the chromosome has the green probe attached to it. Therefore, one of these chromosomes has an 18q deletion at the end but we have no way of knowing how big the deletion is.

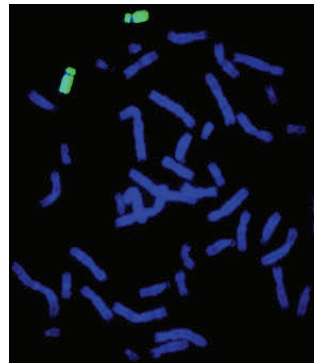
On the positive side, chromosomes that appear normal in the standard karyotype can actually have small deletions or duplications that can be detected using the FISH technique. But you have to know just which probe to use in order to detect the deletion or duplication. FISH is useful when you already suspect that a particular region has a copy number change.



Whole Chromosome Paints

Another similar strategy is to use whole chromosome paints. These are pooled FISH probes that cover an entire chromosome and are labeled with a single color. In this experiment you can see that two chromosome 18s have the fluorescent probe attached. This means that we can determine that there is no chromosome 18 that has been translocated to another chromosome. We can also see that each of these two copies of chromosome 18 is entirely made up of chromosome 18 material. There is no material from another chromosome

translocated onto them. This technique is helpful if you suspect that there is a translocation between two different chromosomes.



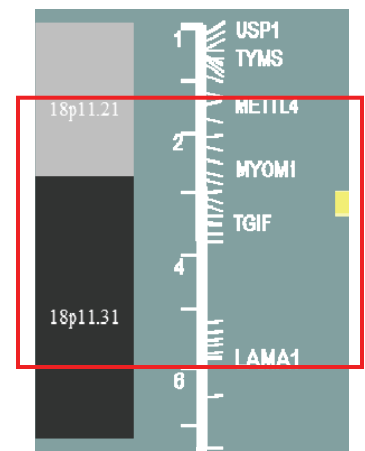
“FISH can be used to detect small copy number changes but the probe is still bigger than many genes.”

Comparing Standard Karyotype and FISH techniques

Even though FISH probes are small and allow the detection of small deletions, they are still relatively large.

In the diagram to the right, the shaded boxes represent the chromosome 18 bands at the tip of the p arm. To the right is the base pair scale going from 1 at the top to over 7 million base pairs at the bottom. To the right of that are the location of the genes, shown by the white lines. Some of the gene name abbreviations are also shown. The red box shows the smallest detectable change using the standard cytogenetic karyotype. The little

yellow bar shows the size of a FISH probe relative to the base pair scale. On our football field, it would be five inches long. You can see that the probe is very small compared to the smallest detectable cytogenetic change. This means that it can be used to detect small changes. On the other hand, the probe is still bigger than many genes. It could, in fact, cover several genes. It still is not specific enough if the goal is to know exactly which genes might exist in one or even three copies instead of the normal two copies.



Summary

To summarize what we have learned about FISH:

- * It is not a good screening tool for assessing all chromosomes and discovering what might be different from normal. You need to know what region of the genome you will be assessing when you do the experiment. It is, however, a good tool for confirming a diagnosis.
- * It has the potential for missing a really small deletion because, while the probe is small, it is still bigger than many genes.
- * Most clinical cytogenetic laboratories perform FISH, however, they usually only use a limited number of probes with proven clinical significance.



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Our Motto

To provide individuals and families affected by chromosome 18 abnormalities with comprehensive medical and educational information with a focus on treatment options.

We are on the web!

<http://pediatrics.uthscsa.edu/centers/chromosome18/>

Information provided by The Chromosome 18 Clinical Research Center to:



<http://www.chromosome18.org/>

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