



R.C.P.U. NEWSLETTER

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R.C. Philips Research and Education Unit
A statewide commitment to the problems of mental retardation

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Review of Genetic Testing Techniques

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Review of Genetic Testing

As we learn more about the human genome and the science of genetics our ability to perform genetic testing is increasing at a rapid pace. Genetic testing is often the best way to confirm a diagnosis in a patient with symptoms and features suggestive of a genetic disease. Today, there are hundreds of different genetic tests for disorders ranging from those that are relatively common, such as cystic fibrosis, to very rare disorders. Not all disorders will be tested for in the same way - there are many different types of tests (or techniques for genetic testing) which can be performed for various genetic diseases. A genetic test is defined as "the analysis of human DNA, RNA, chromosomes, proteins, and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes" (Hotlzman, 1999). Below, some of the most common techniques used for genetic testing are reviewed. Genetic testing can be done directly (testing a particular gene or chromosomal locus) as in cytogenetics and methylation analysis, or indirectly. Indirect testing is typically done through linkage analysis.

A. Direct Testing

1. Chromosome analysis

Cytogenetic tests are used to diagnose chromosomal disorders, in which chromosomes or chromosomal segments are duplicated, deleted, or moved (translocated) to different chromosomes. These tests make it possible to identify the chromosomal basis of many different conditions which are caused by the presence of extra chromosomes, missing and incomplete chromosomes or rearranged chromosomes. The simplest and most common cytogenetic test is a chromosome analysis.

Chromosomes are made up of genes which consist of different amounts of DNA. These genes serve as the instruction manual for our biological development. Thus, missing or extra genes can cause significant problems within our bodies systems and with our development. Every individual has 46 chromosomes which are inherited in pairs. One copy of each pair is inherited from the mother and one from the father; thus half of every person's genetic material comes from each parent. A chromosome analysis provides a picture or karyotype (Figure 1) that enables identification of missing or extra chromosomes, large pieces of missing or extra DNA, or rearrangements of chromosomal material (translocations). This type of testing can diagnose conditions like Down syndrome.

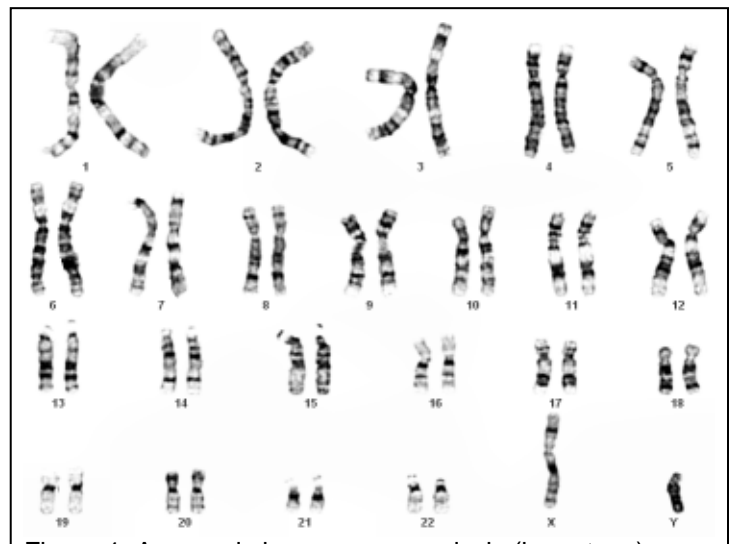


Figure 1: A normal chromosome analysis (karyotype). Notice the banding patterns of the chromosomes. Each band contains, on average, over 100 genes. The chromosomes are grouped in pairs and labeled # 1 through #22. The last two chromosomes are an X and a Y (male).

FISH analysis

One cytogenetic technique, fluorescence *in situ* hybridization (FISH), identifies specific chromosomal regions through the use of fluorescent DNA probes. In this technique a DNA probe is labeled with fluorescent molecules so that it can be seen with a microscope. The DNA probe is added to the cell where it hybridizes (attaches) to its complementary target DNA. Once hybridized, the probe will fluoresce allowing identification of the precise location of the target DNA. If the probe does not fluoresce, it means that the target DNA is deleted (Figure 2).

FISH analysis can pinpoint small chromosomal duplications and deletions missed by conventional chromosome analysis (Burke, 2002). One common region of the genome tested with by FISH is the 22q11.2 region, known to be deleted in Velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS). FISH analysis is a versatile testing method because, depending upon the design of the probe DNA, it can detect many different types of genetic changes. Thus when ordering a "FISH test" one must specify which region of which chromosome the test is being performed, e.g. 22q11.2, 15q11.2, 17p11.2 etc.

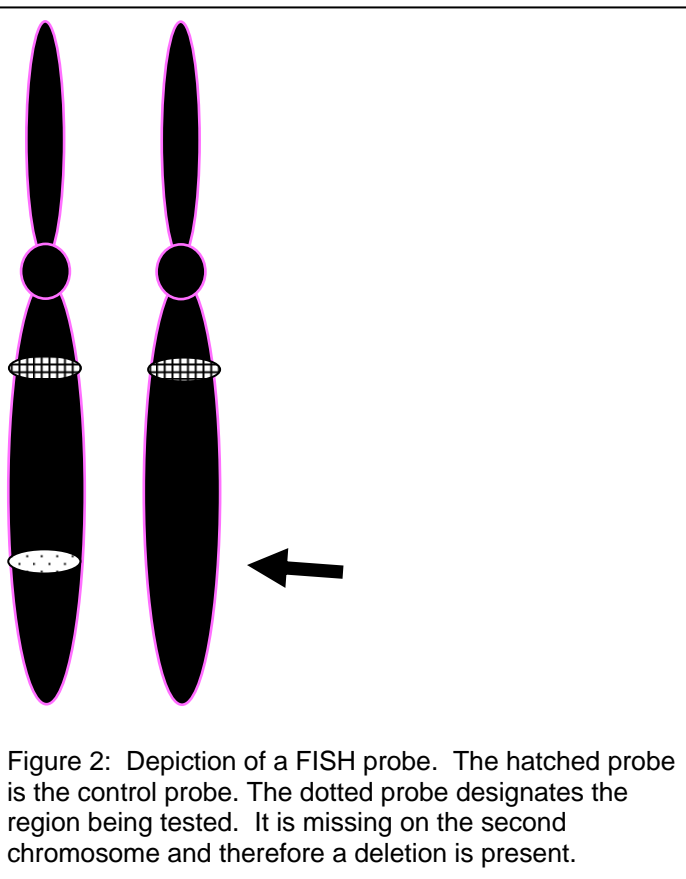


Figure 2: Depiction of a FISH probe. The hatched probe is the control probe. The dotted probe designates the region being tested. It is missing on the second chromosome and therefore a deletion is present.

Subtelomere analysis

Subtelomere analysis is another cytogenetic technique which utilizes FISH analysis. In subtelomere analysis, many FISH probes are used at the same time. These FISH probes are specific for the ends of the chromosomes (telomere regions) (Figure 3). These regions are difficult to assess on a conventional chromosome analysis and are gene-rich areas of the genome. It is estimated that in 2-8% of individuals with

mental retardation and minor anomalies, duplications or deletion in the telomeric region can be identified (Kok et al, 2005).

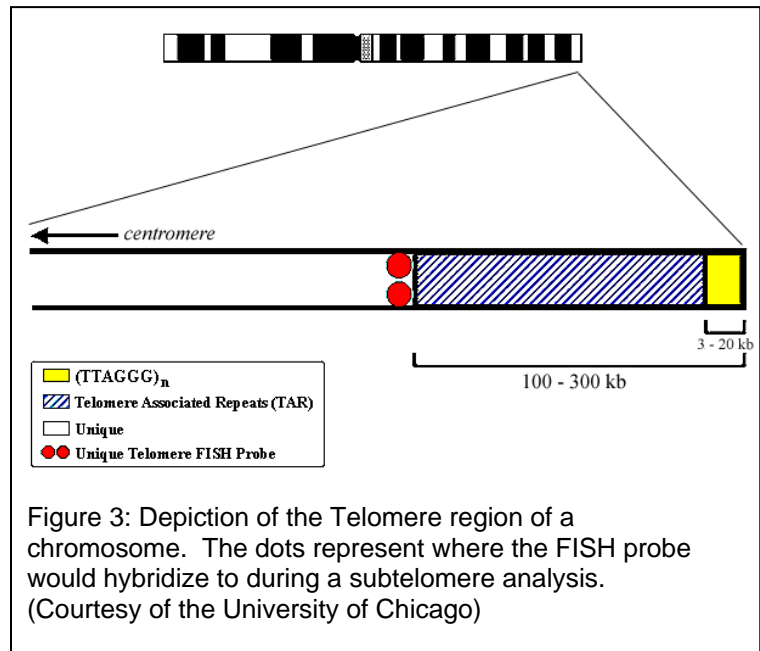


Figure 3: Depiction of the Telomere region of a chromosome. The dots represent where the FISH probe would hybridize to during a subtelomere analysis. (Courtesy of the University of Chicago)

Comparative Genomic Hybridization

Comparative Genomic Hybridization (CGH) is a new genetic testing method which also uses the FISH technique, but on a larger scale. CGH was developed to measure alterations in dosage of DNA throughout the entire genome in a single experiment (Kallioniemi et al, 1992). CGH uses comparative hybridization of differentially labeled genomic DNA from an abnormal cell population ("test" genome) to a normal cell population ("reference" genome). By measuring the ratios of the hybridization intensities, copy number variations in the test genome relative to the reference genome are able to be detected.

In Table 1 the drawbacks and benefits of the different type of cytogenetic techniques are compared and contrasted.

Table 1: Comparison of Cytogenetic Testing Techniques

	Chromosome analysis	FISH analysis	CGH Analysis
Labor Intensity	+++	+	+
Speed of Test	-	++	++
Resolution of Testing	+	++	++++
Need for prior Knowledge	-	+	-
Ability to detect Balanced Rearrangements	++	+	-

2. Molecular Testing:

Methylation

Methylation is an analysis that detects the parent-of-origin for a specific region in the genome using the fact that some regions of the genome have a different and specific pattern of methylation when they are maternally inherited from when they are paternally inherited. It detects the methylation imprint, thus methylation analysis is sometimes used interchangeably with the term "imprinting". Methylation analysis is able to detect if only one pattern is present in a child's genome, i.e. only a maternal pattern or only a paternal pattern. When only one pattern is present, it is referred to as uniparental disomy (UPD). Methylation analysis will be abnormal for both deletions and UPD. In these cases, FISH analysis for deletions will be necessary to determine if methylation analysis is abnormal because of a deletion or because of UPD. Methylation studies are most often done for Prader Willi and Angelman syndromes.

Sequence analysis

This type of analysis is used when multiple different mutations can cause a genetic disorder. In this type of testing, the sequence of amino acids in a particular gene is obtained to determine if there is a deleterious alteration. Conditions such as Neurofibromatosis, Noonan syndrome and a host of others are evaluated by this methodology.

B. Indirect Testing

Linkage analysis

Linkage analysis is an alternative approach typically used when direct testing is not available or is unsuccessful. Linkage analysis is not as accurate as direct testing and does not provide information for all families. Linkage analysis is only possible if two or more family members are affected and available for testing. This type of testing identifies patterns of DNA markers located near the disease gene that can be associated with the disease gene in a particular family. This type of testing is sometimes used with Duchenne muscular dystrophy when the causative gene mutation or deletion can not be identified.

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About the RCPU

The Raymond C. Philips Research and Education Unit began in 1978 when the legislature established section 393.20, F.S., of what is now known as the "prevention" legislation. It is named after Raymond C. Philips, who was the Superintendent of Gainesville's Tacachale (formerly Sunland) Center for 38 years, and was an acknowledged state and national leader in services for mentally retarded persons. The Unit is located on the Tacachale campus and is funded through a contract with the Department of Children and Families and the Department of Health.

The purpose of the R.C.P.U. is to treat, prevent, and/or ameliorate mental retardation through medical evaluations, education and research. The unit provides direct evaluations and counseling to families and promotes service, education, and prevention projects.

Some of the conditions currently under study at the RCPU involve Angelman, Velo-Cardio-Facial, Prader-Willi, Fragile X, Williams and Smith-Lemli-Opitz syndromes.

The R.C. Philips Unit is a resource for all Floridians interested in the diagnosis, treatment and prevention of mental retardation. Staff members are available for consultation and for educational programs for health professionals and for the community at large.

Acknowledgments:

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