FISH-ing for Genes:

Modeling Fluorescence *in situ* Hybridization

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Teaching methods of genetic analysis such as fluorescence *in situ* hybridization (FISH) can be an important part of instructional units in biology, microbiology, and biotechnology. Experience, however, indicates that these topics are difficult for many students. While several authors offer excellent reviews of the technique (for example, Beatty, 2002; Lewis, 2002; Teixeira, 2002), it was our idea to create an activity that effectively simulates FISH using a paper model and fluorescent dyes. The following hands-on procedure is designed for use as either a laboratory or classroom exercise. Instructors may use the templates provided or create their own. Research has shown that adapting manipulatives to fit topics being presented enhances instruction, student satisfaction, and understanding.

The purpose of this activity is to promote both content mastery and critical thinking through self-discovery. In the process, students will:

- improve their scientific reasoning and communication skills
- generate and test ideas about FISH
- explain how the assay is used for gene mapping and identifying chromosomal abnormalities
- draw conclusions and communicate their reasoning.

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This activity is considered non-hazardous, but regular safety procedures should be followed when using these materials.

Cytogenetics & Karyotyping

Before beginning this activity, students should be familiar with DNA structure and function. Traditionally, chromosome analysis has been done using images of chromosomes arranged by size, centromere location, and shape into a karyotype or a diagrammatic representation termed an ideogram. Each chromosome is numbered from the largest to the smallest. The sex chromosomes are shown last. When arranged in this fashion, some chromosomes appear identical. Fortunately, they differ in another important characteristic. When stained with special dyes, such as Geimsa or quinacrine, light and dark bands appear that help distinguish one chromosome from another. It is not exactly certain what the function of the bands might be. However, the light bands are thought to contain most active genes.

This banding pattern is useful when describing a chromosomal location. For example, 17q21 describes the location of the BRAC1 gene recently associated with breast cancer. The first number, 17, refers to the chromosome on which the gene is located. The q refers to the long arm of the chromosome. If a gene is located on the short arm, the letter in the designation would be p. The arms are separated by a centromere. Chromosomes are always arranged with the p arm towards the top of the karyotype. The last number, 21, refers to the band in which the gene is located. Bands are numbered from the centromere out. This standard

cytogenetic nomenclature is essential when discussing the genes and genetic locations used for this activity.

FISH-Based Techniques

Traditional karyotyping is labor intensive. An effective alternative that has become a mainstay in modern cytogenetic laboratories is FISH. The technique is useful for identification of genes, chromosomes, and genetic abnormalities. It relies on DNA probes specific for a gene, chromosomal segment, or complete chromosome. The probes usually carry a fluorescent label or antigen recognized by a fluorescent antibody. When these probes bind, or hybridize, to their complementary DNA sequences, they mark the spot with a bright color that can be visualized using a fluorescence microscope or other special instrumentation. Some probes are used to mark entire chromosomes. By using different combinations of fluorescent dyes, cytotechnologists are even able to mark each human chromosome with a distinct color, creating a very colorful karyotype. While some of the details of probe action may be more complex than required for your biology course, students will benefit from exploring the process at a basic level. Figure 1 shows how the fluorescent labeled probe hybridizes to the target sequence.

Figure 1. Hybridization of fluorescent labeled probe to the target DNA sequence.

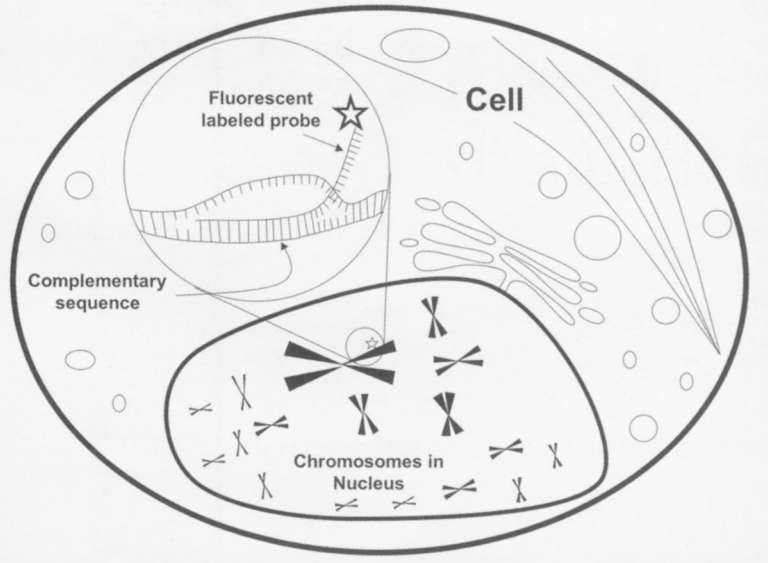
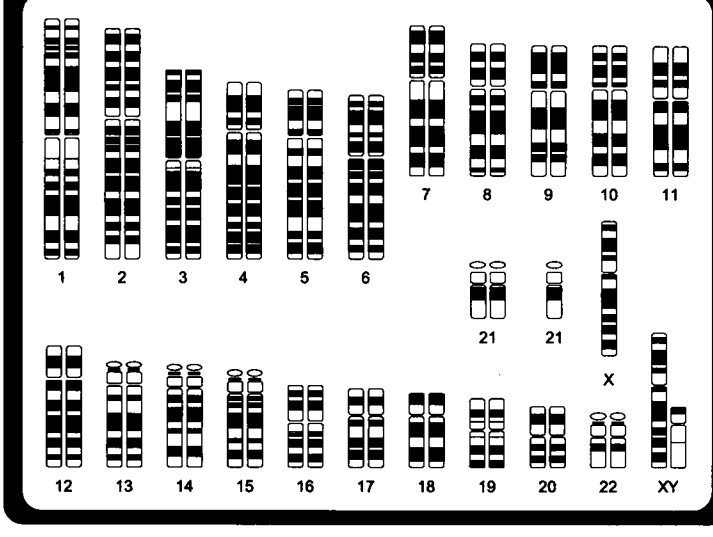


Figure 2. Chromosome set.



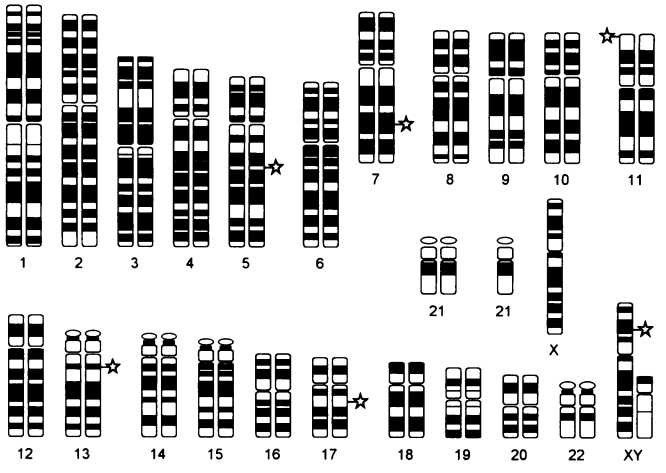
Information gained from FISH analysis can be used to detect a wide variety of genetic changes. This includes deletions, duplications, inversions, translocations, and changes in chromosome number. Conditions such as aneuploidy, the addition or loss of a single chromosome, or polyploidy, the addition of entire sets of chromosomes, can be easily detected. For example, FISH analysis is extremely useful in diagnosing trisomy, a condition in which an extra chromosome is present, such as occurs in Trisomy 21, or Down syndrome. Since many of the genetic mutations associated with cancer initiation are sub-microscopic, high resolution techniques have been developed capable of detecting amplifications or rearrangements of single genes. At present, the technique is not able to detect smaller point mutations involving relatively few nucleotides. For more information on various FISH-based cytogenetic techniques see Teixeira (2002).

In preparation for this activity, you will want to cut out one set of chromosomes from the copied page for each group of students to work with (Figure 2). Each set should be marked with a fluorescent pen at one of the genetic sites shown in the Karyotype Key (Figure 3). You may use the genetic conditions given or create your own. An extra X chromosome has been included so that it is easier to create a female karyotype or model an anueploid condition such as Klinefelter syndrome (47,XXY). You may also wish to demonstrate Turner syndrome (45,X), a monosomic condition in which there is only one copy of the X chromosome. We place the completed chromosome cut-outs in a large resealable sandwich bag labeled with a simulated patient number.

Learning To FISH

Students can work individually or in small groups (see Table 1). To prepare for the activity, the instructor should photocopy and prepare one set of chromosomes (Figure 2) for each group of students. This diagram of a human

Figure 3. Karyotype key.



LOCATION	GENE DESCRIPTION
Chromosome 5q21-22	**APC.** A mutant form of this gene causes a condition termed Familial adenomatous polyposis (FAP) in which individuals develop hundreds or thousands of polyps in their colon. These may progress into colorectal cancer.
Chromosome 7q31.2	**CFTR.** Mutations in this gene are associated with cystic fibrosis, a condition that affects secretions of the respiratory, digestive, and reproductive systems. Thick secretions are produced that interfere with organ function.
Chromosome 11p15.5	**HBB.** This gene codes for one portion of the hemoglobin molecule that functions in red blood cells to carry oxygen. Mutation in this gene causes the sickle shape observed in the red blood cells of sickle cell anemia.
Chromosome 13q12.3	**BRCA2.** Multiple mutations in this region have been identified. These have been associated with an increased risk for breast, ovarian, prostate, and pancreatic cancers. BRCA2 is a tumor suppressor gene that is inherited in an autosomal dominant manner.
Chromosome 17q21	**BRCA1.** Mutations in this gene have been linked to an inherited susceptibility to breast cancer. The gene was discovered in families with a history of early-onset of the disease.
Chromosome 21	**Trisomy 21.** An extra copy of Chromosome 21 is associated with the mental and physical features of Down syndrome. Partial trisomy of Chromosome 21 is also associated with the condition.
Chromosome Xp21	**DMD.** The Duchenne muscular dystrophy gene. The product of this gene is a protein termed dystrophin. Certain abnormalities in this gene lead to progressive muscle degeneration.
Chromosome 47, XXY	**Klinefelter syndrome.** An aneuploid condition typified by males who are sterile and exhibit hypogonadism and gynecomastia.
Chromosome 45,X	**Turner syndrome.** A monosomic condition in which only one copy of the X chromosome is inherited. Affected individuals are females who are shorter than average and fail to develop secondary sex characteristics at puberty.

karyotype represents a complete human genome. Using the key in Figure 3, place a fluorescent mark at one of the genetic locations described. Both copies of the chromosome pair should be marked. Note that for an anueploid condition, such as Trisomy 21, or Down syndrome, the entire chromosome should be labeled. Cut out the chromosomes, removing the numbers as you do so. The chromosomes are arranged in pairs that should be separated for a more realistic exercise.

Each group should first roughly organize its chromosomes by size from largest to smallest. They can then use the Karyotype Key and the banding patterns on each chromosome to match up chromosome pairs and determine the appropriate number for each pair. The instructor can include two X chromosomes or an X and Y when setting up the activity to create male and female genotypes. Using an ultraviolet light source or "black" light, the fluorescent mark on one of the chromosome pairs can be visualized. This is analogous to the procedure followed by scientists. Note: Appropriate safety procedures must be followed when using ultraviolet lights. For example, do not look directly into the blacklight or expose skin surfaces to it. Follow all of the manufacturer's recommendations for safety. Students readily see that the fluorescent dye makes a gene or chromosome visible when illuminated with the proper light source.

Next, students identify their fluorescent gene or chromosome by comparing size, shape, and banding patterns with the labeled chromosome key. We then have students look up information regarding the specific gene or chromosomal abnormality they have identified and report on the conditions associated with it. Case studies may also be used to further the simulation.

Teaching Tips

Monitor understanding by asking students to describe each step in the procedure. Provide feedback to

Table 1. Student Guide.

Name _____ Date _____

Purpose:

1) To improve your scientific reasoning and communication skills
2) To generate and test ideas about fluorescence *in situ* hybridization - FISH
3) To explain how the FISH reaction is used to identify gene and chromosomal abnormalities
4) To draw conclusions and communicate your reasoning

Materials needed:

1 copy of the Karyotype Key (labeled diagram of human karyotype)
1 set of chromosome cut-outs per group
Blacklight

Procedure:

1. Select a partner to work with. Begin by obtaining a copy of the Karyotype Key and one set of chromosome cut-outs.
2. Observe your Karyotype Key carefully. It represents a labeled diagram of the human karyotype. You will use it to identify the specific gene or chromosomal abnormality of your patient.
3. Next, arrange and carefully observe your chromosome cut-outs. They represent the karyotype of your patient. Record the patient number and any observations about this karyotype.
4. When you are ready, your teacher will help you visualize your karyotype under a blacklight or ultraviolet light source. **Caution:** do not look directly into the blacklight or expose skin surfaces to it. Follow all of the manufacturer's recommendations for safety.
5. Next, identify the fluorescent gene or chromosome by comparing size, shape, and banding patterns with the labeled Karyotype Key.
6. Work with your lab partner. Analyze the data you have obtained so far and answer the following questions:
   a. What specific gene or chromosomal abnormality was labeled with the fluorescent probe?
   b. Describe a specific disease or condition associated with the gene or chromosomal abnormality you have identified.
7. Record your ideas in your laboratory notebook. Include your reasoning. Be prepared to discuss your answers with the class.

Table 2. Application Questions.

1. What is fluorescence *in situ* hybridization (FISH)?
2. How does FISH work?
3. Define the following:
   a. DNA
   b. probe
   c. fluorescence
   d. chromosome
   e. gene
4. Describe specific differences between the real chromosomes and fluorescent dyes used in the FISH procedure and the materials you used.
5. Explain how FISH is used for gene mapping and identifying chromosomal abnormalities.

ensure there is comprehension before going on to the next step. You may wish to use simple inventory control, like labeling kits, to aid with materials management. Make sure to announce a clean-up policy at the start of the activity and stick with it. You may also select a member of each group to act as the materials manager. Emphasize the importance of the material manager role.

Classroom Discussion

At this point in the exercise, define the terms generated in the lesson, such as *DNA, probe, fluorescence, chromosome, gene*, and any others that may have been used by yourself or the students. Encourage students to use these terms in subsequent investigations. For a more detailed explanation of the concepts and terms listed here, check one of the following referenced texts.

Evaluation & Extension

To assess understanding, use open-ended questions that allow students to apply the concept of the FISH procedure (see Table 2). Students should be reminded that this activity is a simulation. The models used are two dimensional and the shape of actual DNA is three dimensional.

Ideally, evaluation of inquiry activities should emphasize both content and process skills. For example, while

the class is engaged in the activity, we observe each student's performance. We use portfolio-type research notebooks to collect products of individual student and group work such as lab worksheets, drawings, journals of observations, self-evaluations, and answers to assigned questions. We also observe students as they make presentations to the class, interact with peers, and use computers. We have them conduct research on the Internet to find Web sites that cover the gene they have identified and report their findings to the class. Always ask what other questions their results have brought to mind.

Conclusion

Research indicates that the effectiveness of instruction is enhanced when it incorporates materials that actively engage students in the generation of scientific explanations. To this end, the present exercise allows students to model the fluorescence *in situ* hybridization (FISH) technique using readily-available resources. Students' comments indicate this hands-on experience to be beneficial. As one student responded when asked on a survey to comment about this laboratory exercise, "The opportunity to work hands-on with the FISH activity provided me with the visualization I needed to fully understand."

Internet Resources

http://www.accessexcellence.org/AB/GG/fish.html

http://gslc.genetics.utah.edu/units/disorders/karyotype/

http://www.biology.arizona.edu/human_bio/activities/karyotyping/karyotyping.html

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