The 2 parental strains used in the cross have the phenotypes and genotypes shown in Table 1. The allele of a particular gene (wild-type or mutant) that causes the phenotype is displayed to the right of the phenotype. Parental strain 1 is yellow (because of a mutation in the *yA* gene) and requires PABA (p-aminobenzoic acid)—but not biotin—in the growth medium. The requirement for PABA in the growth medium is because of a mutation in the *pabaA* gene that results in an inability to synthesize PABA. Parental strain 2 is green and requires biotin—but not PABA—in the growth medium. The requirement for biotin is because of a mutation in the *biA* gene.

If the *yA* and *pabaA* genes are closely linked on a chromosome, the following result would be expected when progeny from the cross are analyzed: most of the yellow progeny should require PABA (should not grow on medium lacking PABA), and most of the green progeny should not require PABA (should grow on medium lacking PABA). That is, most of the progeny that have the mutant *yA* allele should, like the yellow parent, also have the mutant *pabaA* allele. Most of the progeny that have the wild-type *yA* allele should, like the green parent, also have the wild-type *pabaA* allele. Only when genetic recombination (crossing-over) occurs between the *yA* and *pabaA* genes will progeny be produced that are yellow—and do not require PABA—or that are green—and do require PABA. Figure 3 shows this to be the case.

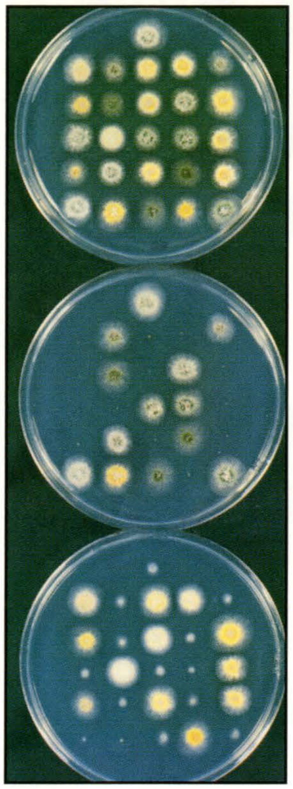

Figure 3 Progeny from the cross provided in the kit are tested for growth on 3 different types of media and have been replicated onto the same postition of all 3 plates.

Figure 3 shows 26 progeny from the cross inoculated onto 3 different media: (1) medium containing PABA and biotin (top plate), (2) medium containing biotin but not PABA (middle plate), and (3) medium containing PABA but not biotin (bottom plate). The same progeny have been replicated onto the same position of all 3 plates. By comparing the top and middle plates, students can see that all but one of the progeny able to grow without PABA (middle plate) are green. (The second colony on the bottom row is yellow.) Similarly, all but one of the progeny unable to grow without PABA (middle plate) are yellow. (The first colony on the third row of 5 colonies does not grow without PABA and is green.) The color of the progeny that does not grow without PABA can be determined by looking at the same position on the top plate, as the top plate contains both PABA and biotin, allowing all progeny to grow.

Therefore, most of the progeny have the same combination of wild-type and mutant alleles for *yA* and *pabaA* as one of the 2 parents. Genetic recombination (crossing-over) must occur between the *yA* and *pabaA* genes to produce yellow progeny that can grow in the absence of PABA and green progeny that cannot grow in the absence of PABA (recombinant progeny). It can also be seen that *biA* is linked to *yA*: All of the progeny able to grow in the absence of biotin (bottom plate) are yellow, and all but one of the progeny unable to grow without biotin are green. (Compare the top and bottom plates.) The second colony on the bottom row, which does not grow in the absence of biotin, is yellow. Crossing-over between *yA* and *biA* has occurred to produce that progeny.

Have each group of 3 students (in a class of 30) produce one complete set of test plates as shown in Figure 3. Students quickly see in a qualitative way the result of close linkage between 2 genes. There is a predominance of the parental types because crossing-over between 2 closely linked genes is rare.

In addition, the experiment generates quantitative data. Pool the data generated by each group of 3 students into a set of data for the entire class. Using the class data, students can determine the linkage distances for each gene pair in the cross: *yA-biA*, *yA-pabaA*, and *biA-pabaA*. The linkage distance for any gene pair is calculated as the percentage recombinant progeny in the cross. They can then construct a linkage map like the one below to show that these 3 genes are all closely linked.

biA ---- 7 ---- yA ---- 15 ---- pabaA

Because the sexual life cycle of *Aspergillus* is rapid, occurring in 14 days, this entire experiment can be done in less than 30 days. Because the 2 strains of *Aspergillus* used in the cross are haploid, only a single cross is needed to demonstrate linkage. All haploid progeny from a cross demonstrate phenotypically the combination of genetic markers received from meiosis. This experiment is an ideal way for students to get hands-on experience to help them understand linkage and recombination, topics that many find difficult.

Table 1: Phenotypes and Genotypes of *A. nidulans* Strains Used in Cross Experiment

Parental Strain 1:	
Phenotype	**Genotype**
Produces yellow conidia (spores)	Mutant allele of *yA* gene
Requires PABA in growth	Mutant allele of *pabaA* gene medium
Doesn't require biotin in *biA* gene	Wild-type allele of growth medium
Parental Strain 2:	
Phenotype	**Genotype**
Produces green conidia (spores)	Wild-type allele of *yA* gene
Doesn't require PABA in medium	Wild-type allele of *pabaA* growth gene
Requires biotin in growth medium	Mutant allele of *biA* gene

Further Resources

Denison, S. H. 2000. *Aspergillus Genetics Kit Manual.* Carolina Biological Supply Company, Burlington, NC.

Klug, W. S., and M. R. Cummings. 1999. *Essentials of Genetics,* 3rd edition. Prentice Hall, New York.

Web Sites

www.fgsc.net/teaching/labfungi.htm
www.fgsc.net/teaching/highschool.htm
www.gla.ac.uk/Acad/IBLS/molgen/aspergillus