TIPS, TRICKS & TECHNIQUES

Karyotype Analysis Activity: A Constructivist Learning Design

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Abstract

This classroom activity is based on a constructivist learning design and engages students in physically constructing a karyotype of three mock patients. Students then diagnose the chromosomal aneuploidy based on the karyotype, list the symptoms associated with the disorder, and discuss the implications of the diagnosis. This activity is targeted at undergraduates in a nonmajors genetics course, but the goals align with AP Biology Big Idea 3 and Next Generation Science Standards HS-LS3. The activity illustrates the relationship between genotype and phenotype, reinforces the chromosome theory of inheritance, and includes mapping of meiotic nondisjunction events.

Key Words: *Karyotype; chromosomes; aneuploidy; constructivist learning design; chromosome theory of inheritance; genotype; phenotype; meiosis; meiotic nondisjunction; AP Biology; Next Generation Science Standards.*

Karyotype analysis is the visualization of an individual's chromosomes for the purpose of determining chromosome size and number. This karyotype analysis activity was designed for undergraduates in a nonmajors genetics course but is appropriate for high school students because it reinforces some basic themes of genetics, including the chromosome theory of inheritance, the relationship between genotype and phenotype, and meiosis and meiotic nondisjunction as sources of new genetic information.

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The activity is modeled after a constructivist learning design (CLD) outlined by Gagnon and Collay (2001) and consists of six elements: Situation, Groupings, Bridge, Questions, Exhibit, and Reflections. Students work in groups to complete the karyotype of three mock patients, diagnose their disorder, and list symptoms associated with the aneuploidy identified. Students are also asked what meiotic nondisjunction events could have led to aneuploidy in these patients. The activity concludes with questions that guide students in synthesizing this information and discussing the ethics of prenatal diagnosis.

Purpose of Karyotype Analysis

As the first element of the CLD, Situation is an overview of the entire activity and establishes its purpose (Gagnon & Collay, 2001, pp. 17–34). The purpose of karyotype analysis is to look at the gross anatomical structures of the chromosomes of an individual. This can allow us to visualize the number and size of each chromosome found within each cell. This test can be performed using almost any tissue from an adult or amniotic fluid from a developing fetus (http://www.nlm.nih.gov/medlineplus/ency/article/003935.htm). Although this technology is a century old, it is still accepted by the American College of Obstetricians and Gynecologists (ACOG) as a definitive diagnostic test for identifying aneuploidy in a developing fetus and, unlike newer technologies, can be used to identify triploidy or balanced translocation events, where genetic information has moved from one location to another without any loss of information (ACOG Committee Opinion nos. 545 and 581).

Activity Preparation

This portion of the activity is rooted in the CLD element Groupings, in which students work together in small groups, help each other, and generate shared meaning (Gagnon & Collay, 2001, pp. 35–50). Students are divided into groups of three or four students and supplied with scissors and tape. They should generate one set of karyotypes per group. This activity requires 60–90 minutes to complete, depending on the amount of pre-activity and Reflection discussion that occurs.

The CLD element Bridge involves reviewing what your students should know and linking what they know with what they are going to

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learn (Gagnon & Collay, 2001, pp. 51–63). Students should review the structure of chromosomes and the steps of meiosis before the activity. The Bridge can consist of a pre-activity to confirm that they have the basic information they need to complete the activity and identify any misconceptions.

For example, you could ask students to draw a cell with two pairs of homologous chromosomes. Students should label each chromosome either A, a, B, or b, where A/a and B/b represent homologous pairs of chromsomes. The instructor should confirm that students have not drawn sister chromatids at this point, which students typically draw as an X.

Next, ask students to draw the same cell after it has completed S phase and the DNA has been replicated. This is an opportunity to confirm that all students recall that this stage consists of 4 chromosomes, each with sister chromatids that are attached at the centromere region, as well as an opportunity to reinforce the genetic difference between chromatids, homologous chromosomes, and nonhomologous chromosomes.

Students can then be asked to draw this cell at the end of meiosis I. At this point, the instructor can confirm that all students have drawn two cells, each containing only two chromosomes, one of each homologous pair, arranged as sister chromatids still attached at the centromeres. Students should then be asked to draw the cell at the end of meiosis II, and the instructor can confirm that each student has drawn four cells, each containing two chromosomes, one of each homologous pair.

Students can label each cell as "n" or "2n" according to the number of chromosomes in the original cell, and the instructor can confirm that the cells drawn at the end of meiosis I and II are all labeled "n". Lastly, the instructor can have the students label each cell with the number of chromosomes you find in a human germ cell at each time point indicated. The instructor can confirm that the cells drawn before the start of meiosis are labeled "46" and that the cells drawn after the end of meiosis I are labeled "23".

○ Karyotype Activity Worksheet

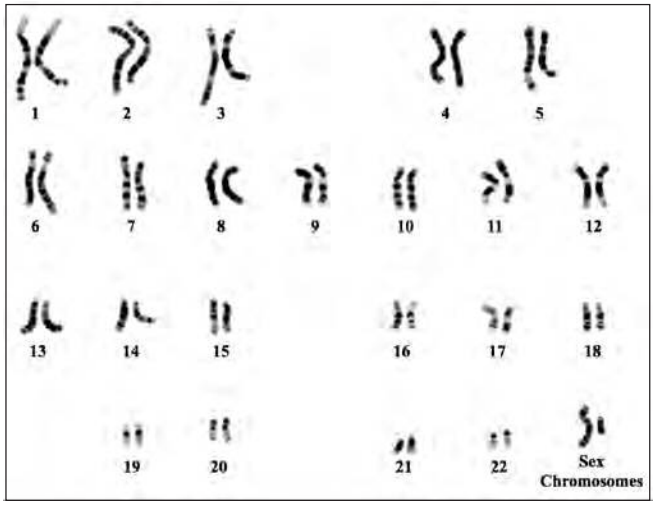
The karyotype activity consists of two sets of chromosomes, "Mom's" chromosomal contribution and "Dad's" chromosomal contribution. The activity worksheet includes an example of a normal karyotype from a human male (Figure 1) and Mom's chromosomes for three patients (Figures 2–4), to which each group will add Dad's chromosomes (Figure 7). Dad's chromosomes need to be cut out and taped onto Mom's set, completing the karyotype. Each group needs to arrange and count the number of each type of chromosome to diagnose the patient.

The questions on the first page of the worksheet are designed to make sure that students understand that chromosomes are arranged by size in a karyotype (Figure 1, question 1) and that males have XY whereas females have XX for sex chromosomes (Figure 1, question 2). The questions in Figures 2–4 ask the students to diagnose the condition. They can look up symptoms using their textooks or at http://ghr.nlm.nih.gov/chromosomes. The correct diagnoses are as follows:

- Patient 1 has Down syndrome, or Trisomy 21; an extensive list of symptoms can be found at http://ghr.nlm.nih.gov/condition/down-syndrome (Figure 2).

Karyotype Activity Worksheet

Background: Karyotyping is routinely used to diagnose disorders caused by abnormal chromosomes. A **karyotype** is the arrangement and classification of the complete set of chromosomes in an individual. Cells are suspended while in mitosis, when the chromosomes have condensed and are easily visualized using a light microscope. A dye is introduced to the cell, which stains certain regions of the chromosomes, producing a banding pattern (also called "G-bands"). An image is taken of the chromosomes, and they are digitally rearranged so that members of each pair are next to each other and chromosomes are arranged according to size. The analysis involves comparing chromosome number, length, placement of the centromere, and the banding pattern. Below is an example of a karyotype taken from a normal individual.



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1. How are the chromosomes arranged?
2. Is this karyotype of a male or female individual?

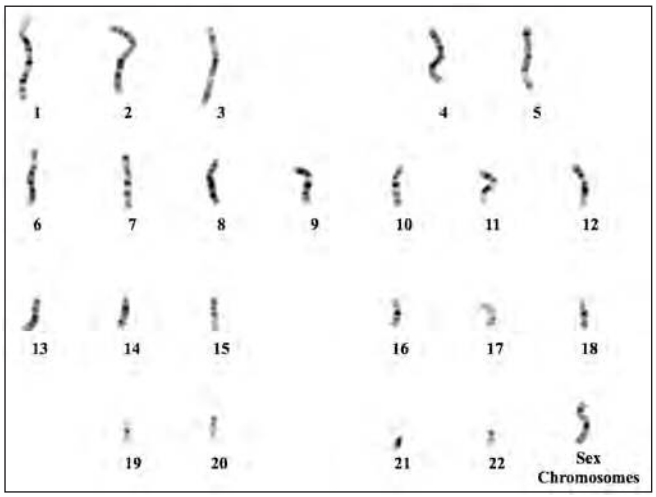
Figure 1. Activity page no. 1: Introduction to karyotypes.

- Patient 2 has Jacob's syndrome, or 47,XYY. This patient would be slightly taller than average and may have a mild autism-spectrum disorder. A description of the disorder and list of symptoms can be found at http://ghr.nlm.nih.gov/condition/47xyy-syndrome (Figure 3).
- Patient 3 has Turner syndrome, or monosomy X; an extensive list of symptoms can be found at http://ghr.nlm.nih.gov/condition/turner-syndrome (Figure 4).

Questions 9–11 (Figure 5) require students to draw the nondisjunction events that occurred during spermatogenesis in the "Dad" for all three patients. They are told to draw only the chromosome affected (cause of the aneuploidy) and to draw the germ cell premeiosis, at the end of meiosis I, and at the end of meiosis II. This group of questions simplifies the outcomes of the nondisjunction events by limiting the error to having come only from the father and tracking the nondisjunction of only one pair of homologous chromosomes (or sex chromosomes) per event for each patient. They should draw that the nondisjunction event occurred in meiosis II for patient 2 (question 10), but nondisjunction could have occurred in either meiosis I or II for patients 1 and 3 (questions 9 and 11). The instructor should confirm that groups have drawn haploid gametes at the end of meiosis II. As a follow-up to questions 9–11, you can ask each group to think of a way a geneticist might be able to distinguish between whether the nondisjunction event occurred in

You have three patients who are in need of karyotyping. Cut out the chromosomes for each patient (on the last two pages) and match them to their pair(s). On the basis of their karyotype, diagnose the disorder they have. You may need to refer to your book or Internet resources.

Patient 1: Patient 1 is the nearly full-term fetus of a 40-year-old female. Chromosomes were obtained from fetal epithelial cells acquired through amniocentesis.



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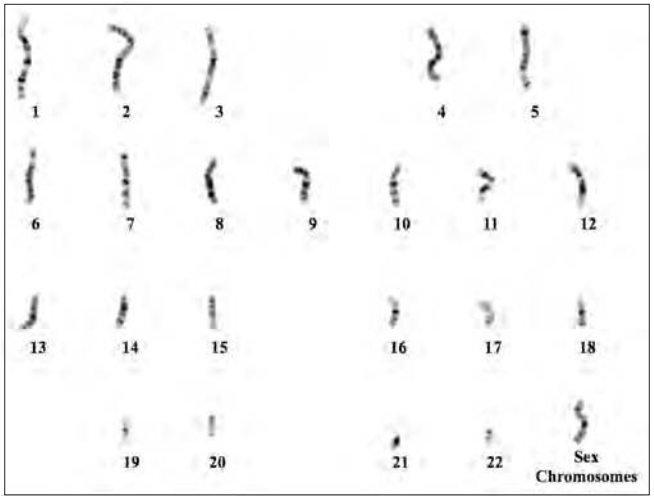
3. What disorder does patient 1 have?
4. What symptoms might this patient have?

Figure 2. Activity page no. 2: "Mom's" chromosomes for patient 1.

Patient 2: Patient 2 is a healthy, 28-year-old male who fathered a daughter with Down syndrome.



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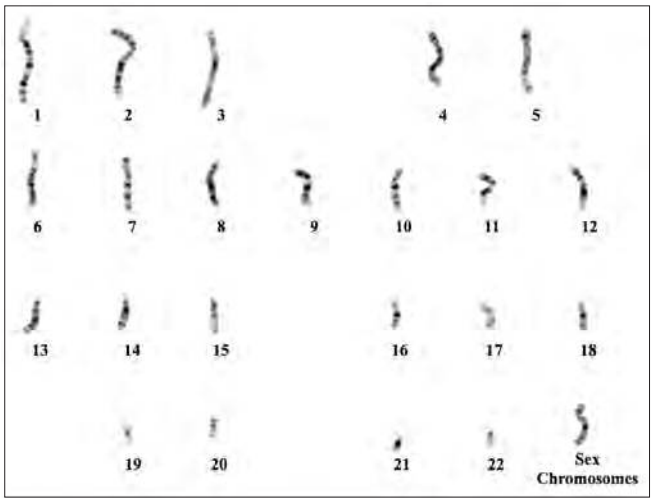
5. What disorder does patient 2 have?
6. What symptoms does the man have?

Figure 3. Activity page no. 3: "Mom's" chromosomes for patient 2.

meiosis I or meiosis II. After groups have completed questions 1–11, the instructor should give groups the opportunity to exhibit what they did. Groups can either take turns answering the questions or volunteer their answers.

Patient 3: Patient 3 died shortly after birth. Chromosomes were obtained from a tissue sample.



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7. What disorder did patient 3 have?
8. If the child had lived, what symptoms might she have had?

Figure 4. Activity page no. 4: "Mom's" chromosomes for patient 3.

If the chromosomes in this packet were from "Mom" and the chromosomes you cut out and pasted were from "Dad," draw the nondisjunction event that would have had to occur in spermatogenesis to produce each karyotype. **Draw one germ cell pre-meiosis, at the end of meiosis I and at the end of meiosis II. Include only the chromosome affected.**

9. Patient 1:

10. Patient 2:

11. Patient 3:

Figure 5. Activity page no. 5: Mapping the nondisjunction events.

○ Additional Discussions & Activities

The questions in Figure 6 are designed to make students reflect on the activity with regard to chromosomes being units of genetic information and that we can visualize chromosomes using karyotype analysis. Question 12 deals with the problem of "gene dosage" in individuals with aneuploidy. Having one extra copy or lacking two copies of some of the genes on that chromosome leads to the symptoms associated with that aneuploidy. Students can explore the genes located on chromosomes 21, X, and Y at http://www.ncbi.nlm.nih.gov/genome/51. Question 13 should help students make the connection between genotype and phenotype, as the symptoms (phenotype) seen in the aneuploidy patients correlate directly with the genes located on that chromosome (genotype). The variability in symptoms depends on the number of genes located on

12. **Given that every human has two pairs of autosomes, why would the addition or subtraction of one chromosome cause a disorder?**

13. **Why is there variability in severity of symptoms caused by abnormal chromosome number between the different chromosomes?**

14. The banding pattern results from a dye that stains A and T nucleotides. Given that one of the pairs of chromosomes is from the mother and the other from the father and each contributes their own genes, **why is the banding pattern so similar between the two pairs of chromosomes?**

15. New, noninvasive techniques allow for prenatal diagnosis of aneuploidy in the developing fetus as early as the first trimester. **What are the benefits and consequences of knowing? Would you want to know?**

Figure 6. Activity page no. 6: Reflection questions.

Cut out the chromosomes below for each patient (# indicated). Match them to their pair to complete the karyotype.

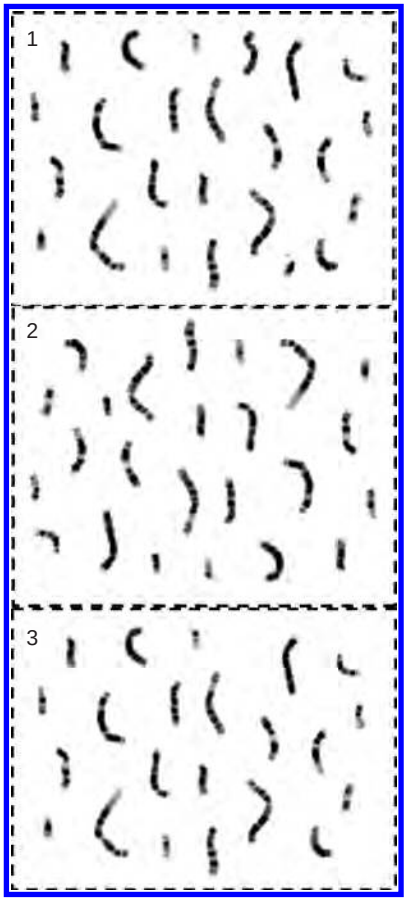

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Figure 7. "Dad's" chromosomes for patients 1, 2, and 3.

the chromosomes and the function of the gene products. Students can explore the various genes located on the other chromosomes. The instructor can use this question as an opportunity to discuss the implications of translocation, deletion, and duplication events, and how individuals in which such events have occurred may look phenotypically similar to individuals with aneuploidy.

Questions 14 and 15 are designed to have students think about the limitation of karyotyping as a diagnostic tool and allow the instructor the opportunity to have students explore new technologies. Question 14 specifically addresses a limitation of karyotyping, in that it looks at highly condensed chromatin and the short segments of DNA that are highly polymorphic or variable between most humans and are too small to be seen by this technology. Karyotype analysis is not useful for detecting small translocation, deletion, or duplication events.

To identify copy-number variants and/or single-nucleotide polymorphisms, fluorescent in situ hybridization (FISH) or chromosomal microarray analysis can be performed. FISH analysis involves identifying specific sequences on chromosomes using a smaller piece of fluorescently labeled DNA that base-pairs with the DNA within the chromosomes. Unlike karyotype analysis, FISH analysis allows for the specific localization of known DNA sequences and can be used to detect translocation, deletion, and duplication events a few nucleotides long. However, FISH requires creating a DNA probe using a known sequence and, therefore, cannot detect all translocation, deletion, or duplication events, since they have not all been identified yet.

Chromosomal microarray analysis involves fragmenting fetal DNA into short fragments, fluorescently labeling these fragments, letting the fragments base-pair with known sequences arranged in an array on a chip, and comparing the intensity of fluorescent signal with a known control to identify differences in DNA sequence. Depending on the type of microarray used, the differences detected may be as small as a single nucleotide change or as large as the gain or loss of a whole chromosome. Since the whole genome is used for the comparison, many translocation, deletion, and duplication events can be identified, even those not previously identified. However, microarray analysis requires cutting the DNA up into small fragments and having a computer put the pieces back together and, therefore, this technique is not useful for identifying balanced inversions or translocation events, in which DNA moves to a new location but is not duplicated or deleted, since the original location of each fragment is lost.

The ACOG 2013 Committee Opinion offers a good comparison between microarray and karyotype analysis (ACOG Committee Opinion no. 581). Karyotype, chromosomal microarray, and FISH analysis require the collection of fetal DNA by amniocentesis (removal of fluid from the uterus) or chorionic villus sampling (removal of tissue from the placenta) and carry the risk of causing bleeding, infection, or miscarriage. The A.D.A.M. online medical encyclopedia has synopses of both procedures (amniocentesis: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0004369/; chorionic villus sampling: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0003884/). Noninvasive prenatal testing involves isolating cell-free fetal DNA from the plasma of the mother's blood. This fetal DNA can be sequenced using new technology called "massively parallel genomic sequencing" to detect DNA abnormalities, including aneuploidy, although this technology is not currently considered as precise as technologies that utilize amniocentesis or chorionic villus sampling

(ACOG Committee Opinion no. 545). The small number of individuals whose genomes have been sequenced currently limits the capacity to interpret the results, but as more genomes are sequenced and added to the collective database, the diagnostic capacity of this technology will improve. The ACOG 2012 committee Opinion offers a good review of the use of cell-free fetal DNA to test for aneuploidy (ACOG Committee Opinion no. 545).

Question 15 should spark a dynamic group discussion and serves as a good ending point since there is no right answer to this question. Rather than having the group give a collective answer, it might be beneficial to have each student reflect on whether they would want to know and why. Questions 12–15 are part of the last element of the CLD, Reflection, which allows students to make a personal reflection on what they have learned as a result of this activity (Gagnon & Collay, 2001, pp. 97–111).

Alignment with AP Biology & NGSS Curricula

This activity addresses 2012 AP Biology Big Idea 3 and, in particular, Enduring Understanding 3.A and 3.C (College Board, 2011). Construction and interpretation of the karyotype reinforce Essential Knowledge 3.A.2, 3.A.3, 3.A.4, 3.C.1, and 3.C.2. The activity addresses Learning Objectives 3.9 and 3.12 by giving a specific visual representation of chromosomes after meiosis and fertilization in the form of karyotypes. Since the three mock patients are the result of meiotic nondisjunction events, which will not follow Mendelian inheritance patterns, this activity can also address Learning Objectives 3.16 and 3.17. Learning Objective 3.13 is addressed by question 15 (Figure 6). The questions in Figure 5 regarding the specific meiotic nondisjunction events that lead to the aneuploidy in these patients address Learning Objectives 3.11 and 3.15, since students draw when the nondisjunction event could have happened on the basis of the "evidence," which is the patient's karyotype.

This activity also addresses the *Next Generation Science Standards* HS-LS3-1 by connecting chromosomes with meiosis and fertilization and giving specific examples of the genotype–phenotype relationship (NGSS Lead States, 2013). The activity extends HS-LS3-2 to include meiotic nondisjunction as another source of genetic variation. To address Crosscutting Concepts, students are asked to interpret data and make connections between cause and effect. They are given a specific example of how technology has advanced science and are asked to think about how this technology can influence society.

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Links

A.D.A.M. Medical Encyclopedia
http://www.ncbi.nlm.nih.gov/pubmedhealth/

Medline Plus
http://www.nlm.nih.gov/medlineplus/

National Center for Biotechnology Information
http://www.ncbi.nlm.nih.gov/

Genetics Home Reference
http://ghr.nlm.nih.gov/

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