

E - Bio Worksheet

HUMAN GENETIC DISORDERS

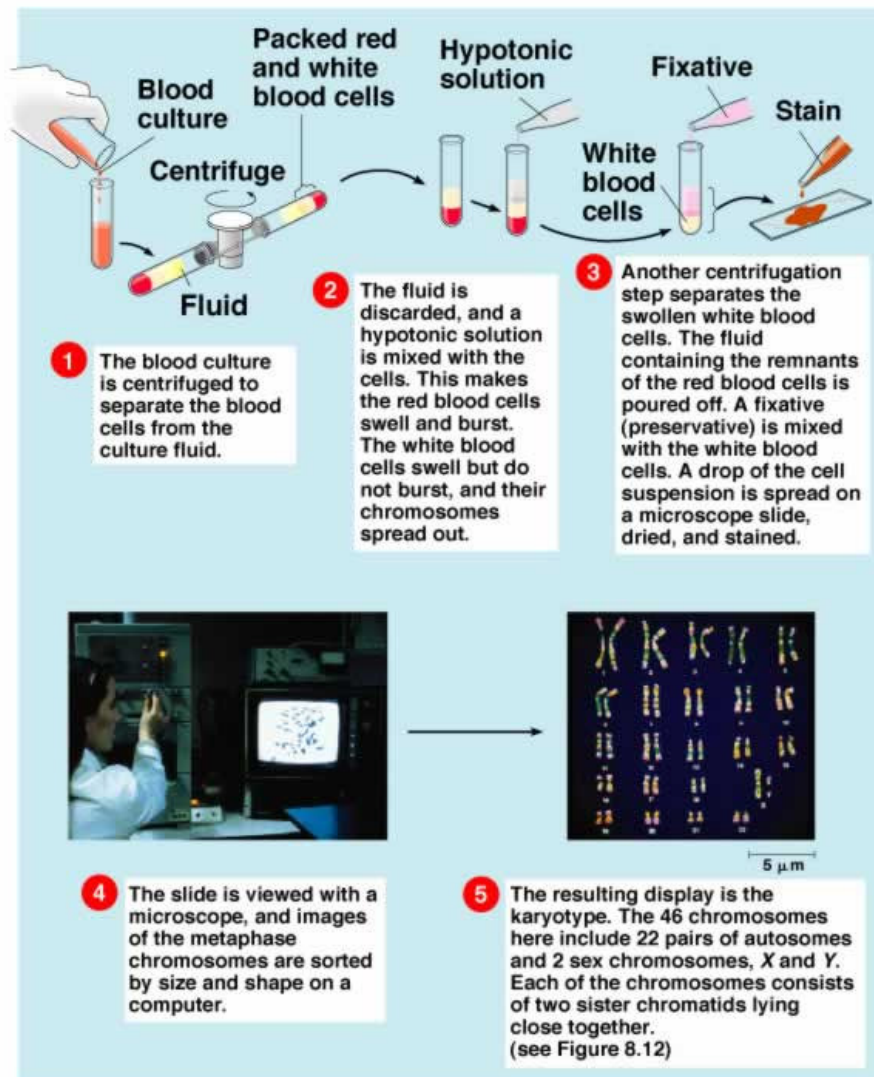
Background Reading - Pages 556 - 564 in Nelson Biology

Purpose - To explain and prepare a human karyotype to identify specific genetic disorders

Introduction

Each species has a characteristic number of chromosomes; for example, corn cells have 20 chromosomes, mouse cells have 40 chromosomes, and human cells have 46 chromosomes. In order to view the chromosomes so that they may be counted, a cell will be allowed to reproduce and colchicine is added to stop the cell division during metaphase. The resulting cells are placed in a hypotonic solution that causes the cell membranes to rupture. The chromosomes are stained and photographed. The chromosomes may then be cut out of the photograph and arranged by homologous pairs. The resulting display is called a **karyotype**. See **Figure I.**

Figure I: Karyotyping Procedure



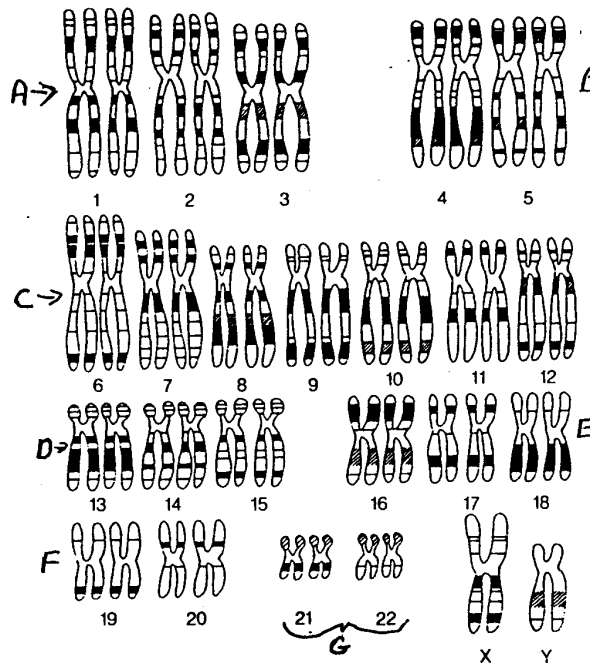
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Part I - The Normal Human Karyotype

The normal human karyotype is composed of **SEVEN** groups of chromosomes (**A - G**) plus the sex chromosomes (**X and Y**). The chromosomes are grouped according to **size, position of the centromere and the characteristic banding pattern**. The first seven groups are called the **autosomes** while the larger **X** and smaller **Y** chromosomes are called the **sex chromosomes**.

1. Observe the normal human karyotype chart found in **FIGURE II**.

Figure II - Human Karyotype Chart



- Q1** - What is the total number of chromosomes found in this cell?
Q2 - How many autosomal chromosome pairs are visible in the above karyotype?
Q3 - What are the **THREE** chromosome characteristics used to organize the karyotype?
Q4 - Which pair is not given a number? Why?
Q5 - What is the sex of the above individual?
Q6 - In many karyotype charts, the **X** chromosome is placed in the **C** group and the **Y** chromosome at the end of row four. Suggest a reason why this is done.
Q7 - Could two individuals have the same karyotype? Explain.
Q8 - At what point in the cell life cycle is the cell best for photographing? Why?

Table I - Genetic Conditions

| Chromosomal Makeup | Clinical Condition |
|------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 46 XX | Female (normal). |
| 46 XY | Male (normal). |
| 13 trisomy (three number 13 chromosomes) | D ₁ syndrome. Multiple congenital anomalies; fatal by 1 year in most cases. |
| 18 trisomy | E syndrome. Multiple congenital anomalies; fatal by age 3 months in most cases. |
| G trisomy | Down syndrome. Mental retardation and distinctive facial characteristics (e.g., skin fold at inner corners of eyes). |
| XXY | Klinefelter syndrome. Male appearance; testes underdeveloped, breasts enlarged. Usually sterile; often mentally retarded. |
| XO | Turner syndrome. Female, anatomically and physiologically; rudimentary ovaries, no menstruation or ovulation. (YO male condition does not seem to be a viable one. None have been found.) |
| XXX, XXXX, XXXXX, XXXY, XXXXY | Multiple sex chromosomes. Some degree of mental retardation, some degree of anatomical or physiological abnormality, or both. Individuals may be fertile; may produce normal offspring. |
| D-G translocation | Small fragments are broken off one 15 (D) and one 21 (G) chromosome, and the damaged chromosomes fuse together. Individuals in which this occurs will be normal themselves, but will produce some gametes containing both the D-G translocation and chromosome 21 (G). If such a gamete is involved in zygote formation, the embryo will be affected with G trisomy (Down syndrome). |
| G-G translocation | Similar to D-G translocation. (Chromosome 22 instead of 15 is involved.) |
| Partial deletion short arm B | Cri-du-chat syndrome. Rounded, moonlike faces; feeble, plaintive catlike cries; mental and physical retardation. |
| Partial deletion short arm 18 | Found in cases of mental retardation and congenital malformations. |
| Partial deletion long arm G | Philadelphia chromosome. Found in patients with chronic myeloid leukemia. Involves one 22 (G) chromosome. |
| Partial deletion long arm Y | Found in some cases of infertility. |

From E. P. Volpe, and DeGruy, C. V. 1983. *Biology and Human Concerns Laboratory Manual*, 3d ed., Dubuque, IA: Wm. C. Brown Publishers.

Part II - Identifying Genetic Disorders

Karyotypes can be used to identify a number of **chromosomal mutations**. Translocation defects, inversion mutations, addition and deletion mutations are all chromosome structure mutations. nondisjunction and polyploidy are chromosome number mutations.

Nondisjunction is the failure of chromosomes to separate during anaphase of cell division. This results in monosomy and trisomy conditions.

Q9 - What is the difference between monosomy and trisomy?

Q10 - How could nondisjunction result in an individual with Down syndrome?

1. Obtain a copy of a karyotype photograph from your instructor. It represents highly enlarged representative chromosomes. Carefully cut out each chromosome and then match the pairs. Using **Figure II** as your comparison model, prepare a **NEAT** karyotype chart by taping the chromosomes to the karyotype template.

Q11 - What is the sex of the individual?

Q12 - What is the total chromosome count? Is this normal?

Q13 - Using **TABLE I** identify the condition present in this individual.

Internet Activity (Questions 14 – 19)

The following site has an interactive karyotype activity. Go to the site and read the introduction and then click on patient histories.

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

Start with Patient A and complete the karyotype. **Answer the questions at the end of the karyotype.** There are 3 different situations each asking the same questions for a total of **6 questions.**

What notation would you use to characterize Patient A's karyotype?

What diagnosis would you give patient A?

Do the same for Patients B and C.

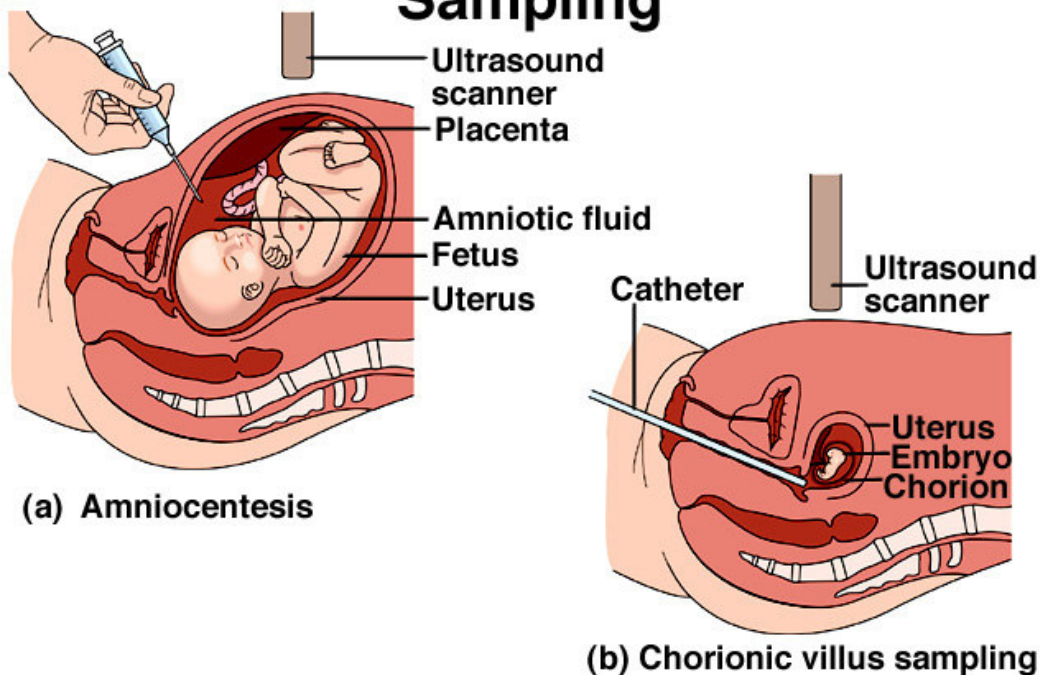
FETAL ANALYSIS

Both CVS and amniocentesis and early amniocentesis are acceptable methods for obtaining fetal tissue. Make a T-chart with both of these topics as headings. Under each side of the chart answer the following.

- How early in pregnancy can it be done?
- What tissue is sampled?
- What instrument is used to obtain the sample?
- What are the dangers?

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Amniocentesis & Chorionic Villus Sampling



Lab Reading:

Prenatal Diagnosis Options

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There are currently several options available to pregnant women for the prenatal diagnosis of chromosomal abnormalities and for numerous single gene disorders. These procedures are amniocentesis, early amniocentesis and chorionic villus sampling. The availability of these techniques may be limited in your region, and testing options will change over time.

Chorionic villus sampling

Chorionic villus sampling (CVS) is the procedure currently available the earliest in pregnancy. It is usually performed between 10 and 12 menstrual weeks gestation. Consult with your regional genetics center regarding dating parameters and recommendations for prior dating ultrasounds or cervical cultures. This procedure is performed either transvaginally using a catheter, or transabdominally using a needle. The device is inserted into the developing placenta under ultrasound guidance and the villi are aspirated into the attached syringe. The tissue is brought to the laboratory where the villi are dissected for either chromosomal analysis or processed further for DNA or biochemical analysis. From the mother's perspective, the transvaginal CVS feels very similar to a routine PAP smear, except for the full bladder necessary for better ultrasound visualization. The aspiration is usually painless, however, the positioning (full bladder, speculum, and abdominal pressure from the ultrasound probe) is often uncomfortable.

Transabdominal CVS feels similar to amniocentesis in that there may be discomfort and/or uterine cramping at the site of the needle insertion. Common complications following CVS include mild cramping and vaginal spotting. Most women feel well enough after CVS to resume normal activities. However, as with the other procedures, they are advised to avoid strenuous activities for 24-48 hours and to seek medical care in the event of more serious complications.

Potential risks involved with CVS include pregnancy complications which could result in miscarriage, an inability to provide conclusive results, and a possible increased risk for limb defects in the fetus. Inconclusive results (e.g., chromosomal mosaicism) may necessitate a second procedure. Precise risks may be site specific, and may vary among genetics centers. The risk for procedure-related pregnancy loss is slightly greater for CVS than amniocentesis. However, the exact risks are more difficult to ascertain due to the higher background rate of pregnancy loss during the first trimester. Several studies have reported a higher incidence of terminal transverse limb reduction defects (i.e., missing fingers and toes or portions thereof) following CVS, while other large-scale collaborative studies have disputed this association. Although the magnitude of the risk is controversial, it is most likely <1% and probably <1/1000. This risk may be higher when the procedure is performed before the 10th week, and may also be related to the specific device used for sampling and the size of the sample.

Limitations include the inability to perform a high resolution ultrasound at the early gestational age when CVS is performed, and the inability to screen for neural tube defects. Some cytogenetics laboratories report that chromosomes from CVS are usually too short to identify micro-deletions or subtle chromosomal abnormalities. Certain metabolic disorders are not expressed in villus cells, preventing prenatal diagnosis by CVS.

Benefits of CVS include the early gestational age at which the results are received, providing early reassurance or the ability to make decisions about terminating the pregnancy at a time when the decision can be more private; that DNA analysis for single gene disorders may be much faster and easier with CVS since the cells may not need to be cultured prior to analysis; and that specific

metabolic disorders that are not expressed in amniocytes, preventing prenatal diagnosis by amniocentesis, can be diagnosed using CVS.

Amniocentesis

Amniocentesis is usually performed at 15-16 weeks gestation by inserting a 22 gauge spinal needle into the amniotic sac. This procedure is usually done under ultrasound guidance so that there is continuous visualization of the fetus and needle. In terms of discomfort, the procedure is generally comparable to a blood draw; however, some women report uterine cramping, especially if the uterus contracts when the needle is in place. Generally, women feel relieved following amniocentesis and resume their normal activities. They are advised to avoid strenuous activity for 24-48 hours and to contact their care providers in the event of complications.

Still considered the “gold standard” for prenatal diagnosis in many ways, benefits of amniocentesis include a lower risk for significant pregnancy complications (<1/200), including miscarriage. Amniocentesis also has the lowest risk for cell culture failure and fetal damage. Amniotic fluid can be studied for neural tube and abdominal wall defects, infections, biochemical disorders, as well as chromosomal abnormalities.

Limitations of amniocentesis include the relatively late gestational age at which the procedure is performed and results received. Results may not be available until 18 weeks gestation when quickening often occurs. At this time, the pregnancy is more obvious, making decision-making more difficult and less private. The concept of the procedure (i.e., a needle in the abdomen) can be very distressing to some women with needle anxiety. It may also take longer for the results of DNA testing if cultured cells are needed for analysis.

Early Amniocentesis

Early amniocentesis (EA) generally refers to any amniocentesis performed prior to 15 weeks gestation. However, the gestational ages when it is performed may vary from center to center. In some centers this procedure is performed from 10-14 weeks gestation; while other centers may only perform EA at 14 weeks gestation. Each center may also have specific criteria for offering the procedure, such as evidence of chorioamniotic fusion, a posterior placenta, or absence of maternal obesity. For these reasons, women who are scheduled for an early amniocentesis are more likely to be rescheduled for a later date than women who are scheduled for routine amniocentesis or CVS. The procedure is identical to routine amniocentesis except that a smaller amount of fluid is extracted.

Risks involved with early amniocentesis are still not clearly defined. Published rates of pregnancy loss following EA range from <1% to 5.3%. Some authors report losses comparable to routine amniocentesis, others report losses comparable to CVS, while others suggest a higher post-procedural loss rate than CVS or routine amniocentesis. There may also be a higher rate of amniotic fluid leakage following EA. Some studies have also suggested a higher than expected incidence of orthopedic and respiratory problems among children exposed to EA; and some earlier studies reported a higher incidence of cell culture failure, but this does not appear to be as prevalent as first reported. Since a smaller quantity of fluid is extracted, it generally takes longer to receive test results because fewer cells are present to initiate the cell culture.

Limitations include the absence of norms for amniotic fluid alpha-fetoprotein medians at early gestational ages, so that neural tube defects may not be identifiable. A few enzyme-based metabolic disorders may also lack values for comparison at early gestational ages. Benefits of EA are similar to the benefits of CVS. The early gestational age at which the results are received enables early reassurance, or early decision-making. The quality of the chromosome study is comparable to routine amniocentesis, making it less likely that a small chromosome deletion would be missed.

Your Karyotype Worksheet

| | | | | | | |
|----|----|----|----|----|----|----|
| 1 | 2 | 3 | 4 | 5 | | |
| 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 13 | 14 | 15 | 16 | 17 | 18 | |
| 19 | 20 | 21 | 22 | X | Y | |