

## Teaching Guide

### Module 0: Introduction to Genetics

Slide 1: Title.

Slide 2: Learning objectives.

Slide 3: History of Genetics. Fun fact: Mendel did not use the words “gene” or “genetics.” “Genetics” was named in 1906 by English biologist William Bateson. The chromosomal theory explained that genes had specific locations on chromosomes and that their locations were related to other genes.

Slide 4: The idea of X-ray crystallography was first provided by Maurice Wilkins. Rosalind Franklin was able to obtain images of DNA. These images allowed Watson and Crick to advertise the double-helix model of DNA. In 1962, Watson, Crick, and Wilkins received the Nobel Prize. Franklin, who died from cancer, was not honored and the reasons for this are still under speculation.

Slide 5: Additional history provided.

Slide 6: This slide is shown in several of our other modules. The central dogma is the framework to understand how genetic information flows. The Central Dogma is comprised of DNA replication, transcription (DNA → RNA), and translation (RNA → Protein).

Slide 7: Human somatic cells are diploid (46 chromosomes or 23 pairs of chromosomes). Note: human gametes are haploid (23 chromosomes). To address the location of a gene, use the following pronunciation:

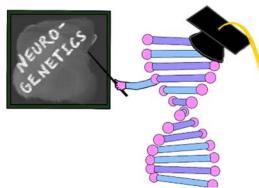
- 46, if diploid
- Chromosome 15, long arm (q), region 1, band 3, sub-band 3
  - 15q13.3 - “Fifteen Q One Three Point Three” [NOT “Fifteen q thirteen”]
- A gene is the building unit of heredity that determines a trait
- 20K human genes
- An allele is a copy of a gene that differs in the sequence of bases from another copy

Slide 8: Text on slide.

Slide 9: Additional definitions:

- Exon: the part of a gene that ends up in an mRNA molecule
- Intron: the part of a gene that does not end up in the mRNA molecule
- Intergenic region: the non-coding area in between genes

(Not necessary to include in the discussion - however if there is a question about the figure- TE stands for transposable elements. These are DNA sequences with the ability to move with genomes)



Slide 10: Transcription creates pre-mRNA molecules that will be processed into mature mRNA. Mature mRNA includes a 5' cap, 3' poly-A tail, and RNA splicing. RNA splicing removes introns. Alternative splicing allows different mature mRNAs to be created, resulting in different proteins.

Slide 11: Degenerate means redundant. Multiple combinations of codons may lead to the same amino acid. H. Gobind Khorana won the Nobel Prize in 1968 for describing the cell's synthesis of proteins.

- Single nucleotide substitutions: where one nucleotide base is changed.
  - Synonymous (Silent) variant: the base change in the DNA codes for the same amino acid (**remember the genetic code is degenerate/redundant!**)
  - Missense variant: the base change in the DNA codes for a different amino acid
  - Nonsense variant: the base change in the DNA codes for a STOP codon
- INDELS: where a base or multiple bases are inserted or deleted.
  - The example given in the PowerPoint is a deletion.
  - Insertions and deletions change the reading frame, resulting in premature truncation.

Slide 12: Text on slide.

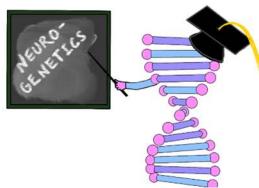
Slide 13-14. Slides presented in most of the modules. Some additional definitions below:

- Homozygous - Same allele at the same locus on both chromosomes.
- Heterozygous - Two different alleles at the same locus on each chromosome.
- Compound heterozygous - Two different mutant alleles, with one on each chromosome.
- Hemizygous - having or characterized by one or more genes (as in a genetic deficiency or in an X chromosome paired with a Y chromosome) that have no allelic counterparts.
  - Ex. male who has an abnormal allele for a gene located on the X chromosome.

Slide 15. Additional definitions.

- Examples:
  - Pleiotropy: multiple phenotypes are expressed → Marfan syndrome: mutation of the FBN1 gene can cause problems in multiple organ systems
  - Genetic heterogeneity: There are many genes which can result in epilepsy.
  - Penetrance: Polydactyly → a dominant disorder but not everyone who has the dominant allele will have extra digits
    - Complete penetrance: gene or trait is expressed in everyone who has the genotype.
    - Incomplete penetrance: gene or trait is expressed in only part of the population.
  - Variable expressivity: Polydactyly → an extra digit may be full-sized or less than full-sized; With relationship to epilepsy- variants in some epilepsy genes can result in a range of phenotypes-like SCN1A variants have a range from febrile seizures to severe myoclonic infancy of epilepsy.

Slide 16. This is a traditional metaphase G-banded karyotype. Metaphase allows for the detection of deletions and duplications of 5-10 megabases. High-resolution banding in prometaphase uses chromosome staining (used for 2-3 megabase range).



- Method of karyotyping: To prepare a short-term culture that is suitable for cytogenetic analysis of these cells, a sample of peripheral blood is obtained. The white blood cells are collected, placed in tissue culture medium, and stimulated to divide. After a few days, the dividing cells are arrested in **metaphase** with chemicals that inhibit the mitotic spindle. Cells are treated with a hypotonic solution to release the chromosomes, which are then fixed, spread on slides, and stained by one of several techniques, depending on the diagnostic procedure being performed. They are then ready for analysis.
- G-banded = stained with Giesma
  - This is the most common staining method. With this method, each chromosome has a unique pattern of light and dark bands used in chromosome identification.

Slide 17. The short arm of the chromosome is known as the p arm (p = petit). The long arm is the q arm. "q" was chosen because it follows "p" in the alphabet. The light and dark bands are numbered moving outwards from the centromere.

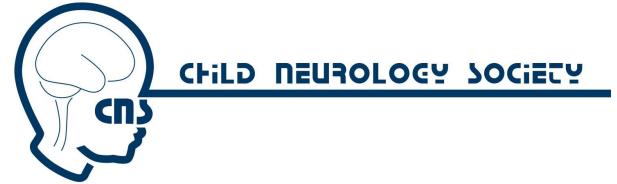
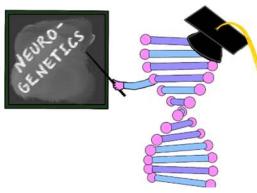
Slide 18. This example shows a ring chromosome 20. Structural changes such, as ring chromosome or translocations, are visible with the karyotype technique.

Slide 19. "Know what you're 'fishing' for." FISH is used when looking for a specific region on a chromosome. Example: if a newborn has a congenital heart defect and has seizures secondary to hypocalcemia, FISH analysis for 22q11.2 region can be done to assess for this common deletion. In this Figure, A shows a normal signal. B shows that the red signal corresponding to the 11.2 region of chromosome 22 is missing one of the chromosomes, diagnostic of a deletion in this region.

Slide 20. Chromosomal microarray is a genetic test that can detect missing (deletions) or extra pieces (duplications) of chromosomes. These deletions and duplications are called copy number variants (CNVs). This test has a much greater resolution than conventional karyotyping and can detect deletions/duplications down to a 50-100 KB level (Smallest resolution of karyotype is > 5 MB- much larger!)

Slide 21. First practiced by Fred Sanger, a British biochemist, in 1977, this method is also known as chain termination sequencing. He used this method to sequence small fragments of DNA. The bases in the fragments are overlapped to assemble larger sequences. This is still known as the gold standard of DNA sequencing.

- Steps:
  1. Targeted DNA sample (what is supposed to be sequenced) is put into a tube with primer, DNA polymerase, deoxynucleotides, and dye-labeled dideoxynucleotides. This mixture is heated (annealed) to denature the target DNA strand.
  2. DNA polymerase starts to synthesize the new DNA strand until one of the dye-labeled dideoxynucleotides is added to the chain. The reaction cannot continue beyond addition of the dideoxynucleotides and a fragment is created-the end of which is dye-labeled. As this reaction occurs thousands of times - fragments of every length are created.
  3. The fragments are sent through gel electrophoresis.



4. The end of the electrophoresis detects the dye-labeled nucleotides and this is the target sequence.

Slide 22. Next Generation Sequencing (NGS) is a slightly modified, digital, and vastly scaled-up implementation of Sanger sequencing. In both methodologies, a polymerase copies template molecules by incorporating nucleotides from a pool, that is, either partially (Sanger) or entirely (NGS) composed of dyed and unextendible bases. Extension, arrangement, and detection are shared steps in both protocols but occur in different order, with NGS alone having a restoration step that converts bases to the undyed and extendable form.

Slide 23. Text on slide.

Slide 24. Text on slide.

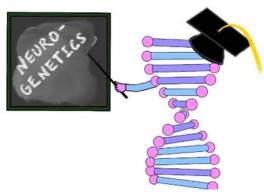
Slide 25. Understanding a genetic report.

- 1) arr[hg19]2q33.1(198,356,789-203,491,035)X1:
  - a) A chromosomal microarray report → indicates a deletion on the long arm of chromosome 2 at position 33.1
  - b) hg 19 is the human genome version used
  - c) x1 means only one copy of the DNA is present in the sample
  - d) 198,356,789-203,491,035 are the base pairs missing
- 2) c.346G>C (p.G116R)
  - a) A missense variant → resulting in the amino acid in the sequence changing from glycine to arginine at position 116 in the protein.
- 3) c.1945dupT (p.S649FfsX40)
  - a) Insertion → The thymine at position 1945 is duplicated, resulting in a substitution of phenylalanine from serine. This results in a frameshift from this point onwards in the protein sequence, and premature truncating 40 amino acids down.
- 4) c.847C>T (p.R283X)
  - a) A nonsense variant → amino acid arginine is changed to a stop codon (resulting in a premature truncation of protein).
- 5) c.487+1G>T
  - a) An intronic variant at the first position in the intron. Given its location, it will likely result in altered splicing.

Slide 26. Segregation is the separation of two alleles during the formation of gametes. Segregation is important because it ensures each gamete receives only one allele.

Slide 27. There are 20 amino acids and they are split into categories based on their differing physiochemical properties: nonpolar, polar, and charged (acidic or basic). Changes in amino acids that have similar physiochemical properties tend to be more tolerated than changes to a radically different amino acid.

- Similar change: Glycine to Alanine
- Radical change: Glycine to Serine



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Slide 28. Conservative = amino acid replacement with similar biochemical properties. Non-conservative = differing biochemical properties

Slide 29. ACMG provides guidance on variant classification. This is ADVANCED READING. It is helpful to know it exists.

Slide 30. Suggested reading.

Slide 31. Acknowledgements.