



GEN PHILIC

Department of Biotechnology

Fergusson College (Autonomous), Pune.

Issue 3

September 2020

Methods in Genetics

What? Why? & How?



Dr. Sonali Joshi

Head of The Department of Biotechnology

Dr. Gayatri Gurjar
Editor in Chief

The Science of tree building

Molecular Phylogenetics

Human beings have always been involved in systematically organizing, cataloguing and classifying the biodiversity on the planet. Historically, the classification was entirely based on the morphological similarities and the differences between the species. The species that share a common ancestor look similar to each other morphologically, since such species acquire at least a part of their phenotype through heredity.

Today, we know that all organisms on the planet are somehow related to each other. However, some species look more similar to each other in comparison to several other species. For example, *Homo sapiens* (Human beings) looks similar to *Pan troglodytes* (Chimpanzees), than to *Drosophila melanogaster* (fruitfly). The similarities and the differences between these species in terms of morphology are obvious. However, if you are asked the question- which species of big cats are more closely related to each other, or how are various species of lizards related to each other, or when did the most recent common ancestor of all the grasses live; the answer may not be that obvious. Further, many unrelated taxa or species show the presence of similar characters, which have evolved independently in those lineages, and not through common ancestry, but through an evolutionary event referred to as morphological convergence. For example, the birds and the insects do not share a very recent common ancestor, birds are more closely related to the vertebrate order reptiles, whereas the insects are invertebrates. However, the possibility of clustering birds and insects together cannot be ruled out, since the two show the presence of common character – the wings, which have evolved independently in both the birds and the insects.

When morphology fails to give clear answers one needs to turn towards the factors that shape the morphology – the molecules. A phylogenetic tree built using molecular information is called as molecular phylogenetic reconstruction. Molecular phylogenetics certainly has clear advantages over establishing evolutionary relationships based on morphological data. Firstly, the number of morphological characters is limited, you could probably examine only up to a hundred morphological characters, depending upon your patience and determination. Again, every character could have multiple character states. The shape of the leaf, for example, is a character, but it could have multiple character states, such as acicular, acuminate, aristate, bipinnate, cordate and so on, which makes

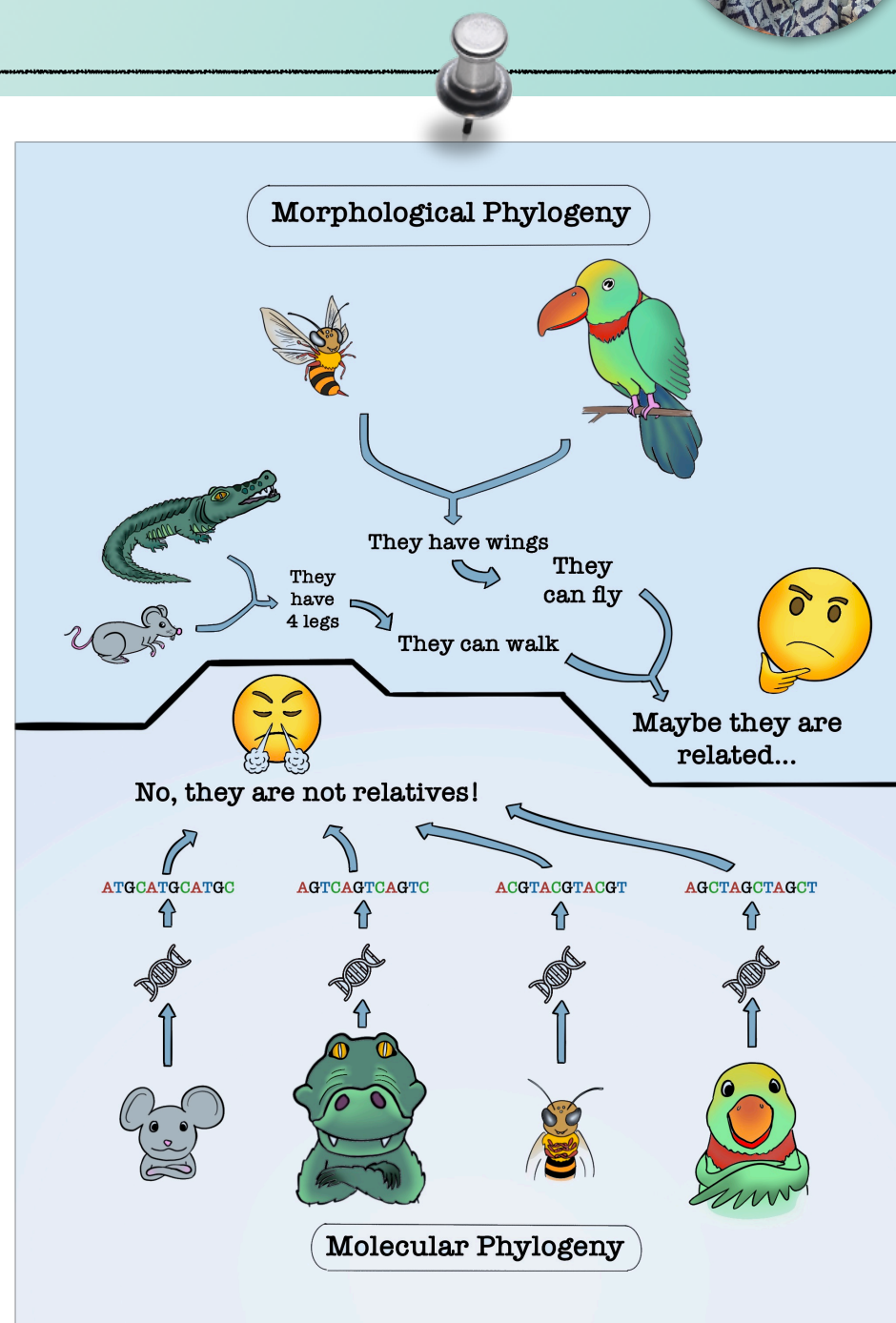


Illustration 1 : Morphological and molecular phylogeny

Illustrator : Hrishikesh Hardikar
S. Y. BSc. Biotechnology

Mudra Deshpande

S. Y. BSc Biotechnology
(Editor)Divya Bhardwaj
S. Y. BSc Biotechnology
(Editor)

morphological assessment even more time consuming. Then again, there could exist species of trees with leaves that can neither be called obtuse, nor elliptical. Molecular phylogenetics, on the other hand, has only one character, the nucleotides of the DNA, and only four character states – A, G, C, T. Also, the character states in molecular phylogenetics are discrete, the states of the character could be either A, G, C or T, and there isn't an intermediate state between A and G or C and T; unlike morphological data, which could be continuous and could have several intermediate character states. Furthermore, sequencing only a small region of the DNA (a gene fragment), can yield at least two hundred base pair data, so the problem of sample size is also ruled out. Another important fact that needs consideration for morphological classification is the need for trained taxonomists, a profession that is now almost extinct. The number of molecular biologists, on the other hand, is certainly increasing, and molecular biologists find molecular phylogenetics easier to learn over classical taxonomy. Lastly, molecular phylogenetics also helps to deal with morphological convergence, and on a phylogenetic tree, birds cluster with reptiles, and insects cluster with invertebrates appropriately.

But then what is a phylogenetic tree? As the name suggests, a phylogenetic tree is a dendritic arrangement of the sampled species on the tip of the branches, as leaves, with the most closely related leaves connected to each other by a single, relatively smaller branch, whereas, the more distantly related species connected by longer branches. The branches are called internodes, the point where an internode bifurcates is where the ancestral species is placed, the point of bifurcation is called as the node. The node also indicates the speciation event, or the time when the two daughter species split from each other.

Molecular phylogenetics has multiple applications, including those in systematics, where phylogenetics is employed to identify species (DNA barcodes) and also unearth the hidden or the cryptic diversity. The science also finds its importance in biogeography, to find out where the ancestral species would occur on the earth, and what events (dispersal, vicariance) led the ancestral species giving birth to the daughter species. Molecular phylogenetics is also used in understanding how parts of the body have evolved, or lost, in a species. An interesting example is “limblessness” in vertebrates wherein molecular phylogenetics has been utilized to find out the specific genes that are responsible for the development of limbs. Finally, one can also find out the age of the species using molecular phylogenetics, with the application called dating trees, which considers the rate at which the gene evolves and the number of substitutions that have taken place. This is one of the reasons why molecular phylogeneticists are well-known for their “tree-dating” abilities!



By Gaurang Gowande.
Assistant Professor
Department of Biotechnology

Looking for diseases in the Gene Map

Linkage Analysis

Genetic linkage analysis is one of the DNA indirect diagnosis methods. It is a study aimed at establishing linkage between genes and is considered as a powerful tool to detect the chromosomal location of genes that cause diseases. Linkage analysis basically refers to a group of statistical methods to map a gene to the region of the chromosome in which it is located. It is based on the observation that genes whose loci is close to each other on a chromosome are inherited together during the meiosis phase of sexual reproduction. This is the phenomenon which is referred to as genetic linkage. In other words, if two genes on a chromosome are nearer, they will have a lower chance of recombination and thus, they are likely to get inherited together. To identify these loci on the chromosome and their inheritance patterns, scientists make use of genetic markers. The term genetic marker is used to denote a segment of DNA with an identifiable physical location on a chromosome whose inheritance can be followed.

The first challenge faced by any researcher is to identify the chromosome on which the gene is located. Without knowing either the gene or it's product, it is necessary to use a statistical procedure called Linkage Analysis to achieve this goal. Linkage analysis begins with a host of genetic loci called markers or marker loci. A marker locus is a polymorphic locus with a known location on a chromosome. It is important to note that this method does not try to locate the precise position of the disease gene. Rather, it tries to identify which marker loci are close to the disease gene. In this way, a rough area of a particular chromosome will be targeted for further analysis.

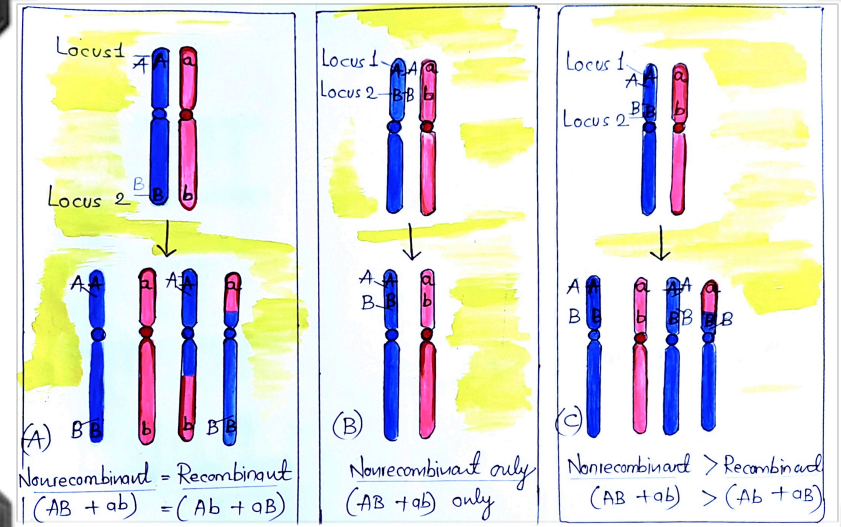


Illustration 2 : Possibilities of chromosomal recombination in various loci arrangements



Illustrator : Aqsareha Mujawar
S. Y. BSc Biotechnology

Linkage analysis is either parametric or non-parametric. Parametric linkage analysis also called Model-based Linkage Analysis or LOD score linkage analysis is the traditional approach which is used when the relationship between phenotypic and genotypic similarity are known. In other words, this technique assumes that the genetic models underlying both the trait in question and the associated marker loci are known. The statistical estimate of whether two genes are likely to lie near each other on a chromosome and are likely to be inherited together is called the LOD score [logarithm (base 10) of odds]. Positive LOD scores show that linkage is present and negative LOD scores show that presence of linkage is less likely. By convention, a LOD score greater than 3.0 is considered evidence for linkage and a LOD score less than -2.0 is considered evidence to exclude linkage. LOD score analysis is a simple way to assess complex family pedigrees which can help determine linkage between Mendelian traits. Non-parametric or model-free linkage analysis does not require parameters for mode of inheritance for the

trait being linked to marker loci.

Linkage analysis has been instrumental in the identification of genes that cause or increase the risk of many diseases and birth defects. For example, the identification of the genes that cause Mendelian disorders such as cystic fibrosis (CFTR) and Huntington’s disease (HTT). Linkage analysis is also used to study aggregation of complex diseases that leads to identification of high-risk alleles in certain genes. Should these alleles be present in an individual, there’s a high likelihood of them being afflicted with said complex disease, for example, BRCA1 and BRCA2 genes and breast cancer. This study also identifies genes with rare risk alleles that cause moderate increase in the risk for complex diseases such as NOD2 gene and inflammatory bowel disease. It is also used to study quantitative traits e.g. activity of an enzyme Dopamine β-hydroxylase that catalyses conversion of dopamine to norepinephrine and was localised to chromosome 9q34 region and specific variants that were responsible for the variation of trait activity.

Genetic linkage analysis still remains the cornerstone of disease gene localisation and identification of Mendelian disorders and will still hold its importance even after the development of newer techniques. Novel genetic markers, automated genotyping and new mathematical models permit application of linkage analysis to complex Non-Mendelian disorders too. This technique is here to stay and will be revamped more and more as time goes by.



By Sifa Lalani
S. Y. BSc. Biotechnology



And Pragyananda Choudhury
S. Y. BSc. Biotechnology

Back to the Basics with Friendly Ghost Grandpa Mendel!

You know the legendary Gregor Mendel. You might not know the legends in the race to find secret of life! Come, discover the story with Mendel himself, and see where the journey takes you! (Note : Read from left to right)

Continued from Issue 2..

WATSON AND CRICK’S JOKE OF A MODEL INCURRED THE WRATH OF THE KING’S TEAM BOSS, SIR. J. T. RANDALLWHO CALLED UP THEIR BOSS, SIR LAWRENCE BRAGG TO COMPLAIN ABOUT THEIR BEHAVIOUR

How dare Watson and Crick make use of our unpublished data? Have they forgotten all gentlemanly behaviour?

I apologise on their behalf!

LAWRENCE BRAGG

Bragg to Watson and Crick - I’m furious with the two of you. I’m kicking you off this case. Your model building equipment should be sent off to King’s.

IT WAS A BAD DAY FOR OUR ENGLISH BOYS BUT CRICK HAD SOME GOOD NEWS – ERWIN CHARCAFF, A TRUE CHEMIST, EXPLAINED HIS RULE OF A=T AND G=C TO THE “IMMATURE” WATSON AND CRICK DESPITE HIS SHEER DISLIKE OF THEM OVER DINNER.

The DNA molecule is made up of four nitrogen bases – Adenine (A), Guanine (G), Cytosine (C) & Thymine (T). In a particular species, amount of A equals to amount of T & the amount of G equals amount of C, always.

Oh, tell us more about your discovery!

How exactly did you find this out?

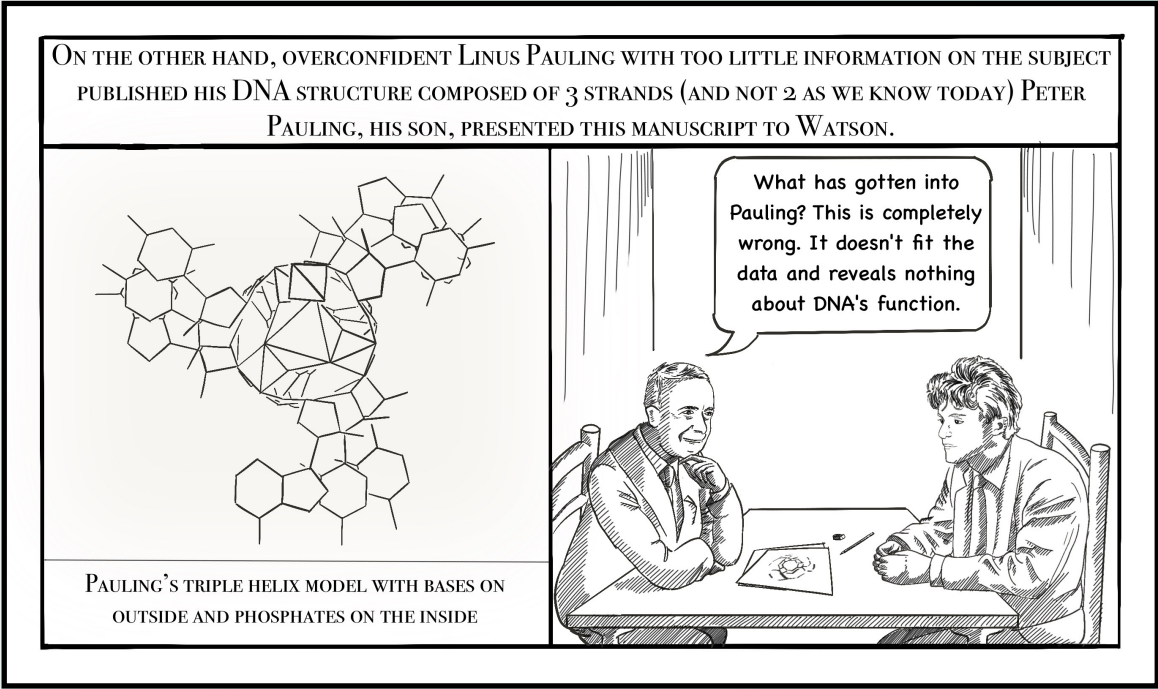
MEANWHILE THE OTHER TWO RACERS HAD MADE RAPID LEAPS. AT KING’S COLLEGE, ROSALIND MADE HER BEST DISCOVERY YET. THE HELICAL STRUCTURE OF DNA AS REPRESENTED BY AN X-RAY CRYSTALLOGRAPHIC IMAGE SHE CLICKED WITH GOSLING - PHOTO 51

PHOTO-51

But Professor how did Rosalind manage to take such a detailed and revolutionary image that too in 1952?

Good you noted that, kid! Rosalind definitely possessed exceptional skills. Because of the work of scientists like them, today we have advanced techniques like Pedigree Analysis, Karyotyping, Phylogenetics and so on. Want to know more about them? Read this issue further! (Self- Promo 101)

Back to Basics



Want to know what will Watson's next move be? Find out in our next issue!



Author : Aarjvi Jain
S. Y. BSc. Biotechnology



Illustrator : Hrishikesh Hardikar
S. Y. BSc. Biotechnology

Journey through the Chromosome

Karyotyping and Cytogenetics

Ever wondered how our chromosomes look? A way to visualise them is through karyotyping, a cytogenetic procedure practised to obtain images of your karyotype, the collection of chromosomes you harbour by analysing and studying them. This technique is used to identify and evaluate the size, shape, and number of chromosomes in a sample of somatic cells. It is also used to diagnose genetic disorders like Down Syndrome, Edwards's Syndrome, Klinefelter syndrome and Turner Syndrome. Karyotyping is carried out in dividing the cells that are arrested during metaphase, the time of greatest chromosome condensation. This is performed by disruption of the spindle fibres using drugs such as colchicine that prevent microtubule polymerisation by binding to the tubulin component.

Another known way to visualise the chromosomes is cytogenetics, a branch of genetics that is concerned with how the chromosomes relate to cell behaviour, particularly to their behaviour during mitosis and meiosis.

This DNA is condensed during cell division and forms chromosomes. Cytogenetics studies the number and morphology of these chromosomes. It also includes the study of factors that cause chromosomal changes.

Other many techniques used include analysis of G-banded chromosomes and other cytogenetic banding techniques as well as molecular cytogenetic techniques such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH). What is the source of such cells?

Usually it is subject's blood sample, bone marrow, amniotic fluid or cells of chorionic villi that are collected and cultured in vitro. These cells are then restricted at metaphase or prometaphase followed by treatment using hypotonic solution causing the nuclei to swell and the cells to lyse. Finally, fixative and subsequent staining is done to visualise the chromosomes under the microscope.

In order to make the analysis even more efficient, various staining procedures are employed which give rise to characteristic banding patterns on the chromosome enabling structural features to be highlighted.

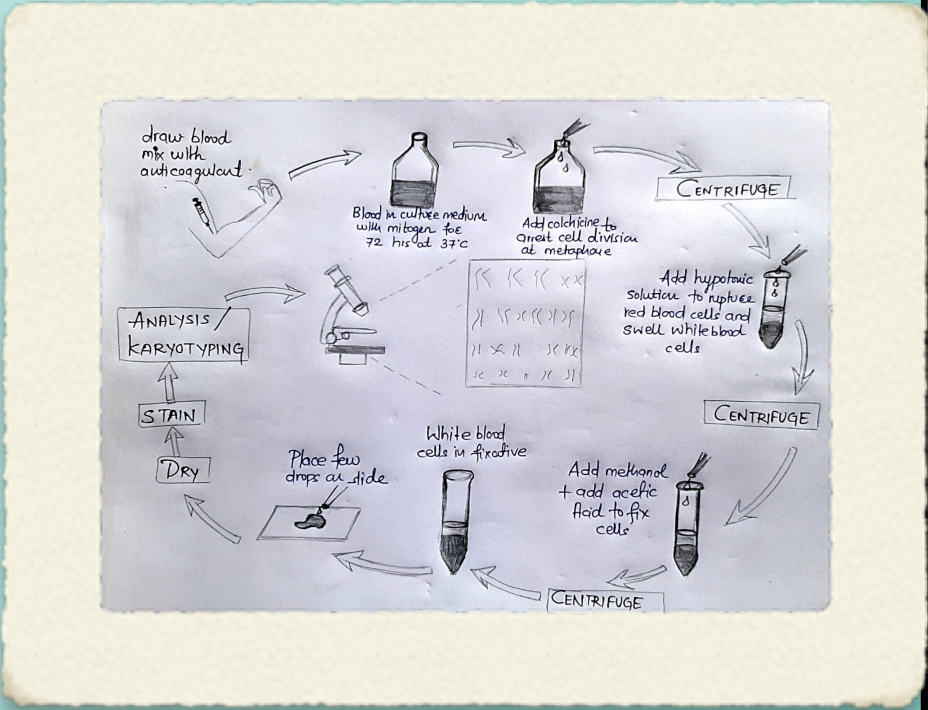


Illustration 3 : Production of Metaphase chromosomes for cytogenetic analysis



Illustrator : Aqsareha Mujawar
S. Y. BSc. Biotechnology

These banding patterns are:- 1. G banding 2. R banding 3. C banding 4. Q banding 5. NOR Banding

Here, chromosome is initially treated with trypsin that degrades proteins. Thus, relaxing the chromatin structure followed by the application of Geimsa stain which offers higher resolution of individual bands and can be analysed with bright-field microscopy.

This type of banding method selectively darkens the heterochromatin region of chromosome that is rich in A-T base pairs and is relatively poor with respect to gene quantity when compared to euchromatin, a region rich in G-C base pairs which is lightly stained. Since this method is highly reproducible, it is widely used.

This gave better resolution than Q-banding, allowed permanent preparations, and did not necessarily need the use of fluorescence microscopy.

R Banding

Like the G Banding, this method also employs Giemsa stain but only after the chromosome is treated by heat. This causes preferential loosening of the helix at the A-T rich regions, resulting in their lighter staining and creating darker bands at G-C rich regions. Hence, this method is often referred to as the 'reverse of G banding'. R banding or Reverse banding technique was first described by Dutrillaux and Lejeune. If we try to observe the chromosomes after this method, we will be able to see that the regions on the chromosomes that are stained lightly appear to be heterochromatic and all the dark regions appear to be euchromatic. This method is mainly used to determine chromosomal deletions.

C Banding

It's a specialized Giemsa technique that primarily stains the non-coding regions of

chromosomes at the centromeres. It is rarely used for diagnostic purposes. However, this technique has proved useful for chromosome characterization and identification because the C-banding patterns are heritable and remain discernible throughout the mitotic and meiotic cell cycles as they stain the heterochromatic regions. C-bands are localised in the heterochromatic regions of the chromosomes. Many chromosomes have regions that differ among individuals but have no pathological importance. These polymorphic regions can be visualised optimally by using C-band method. C-banding is also useful to show chromosomes with multiple centromeres and to distinguish between donor and recipient cells in bone marrow transplantation.

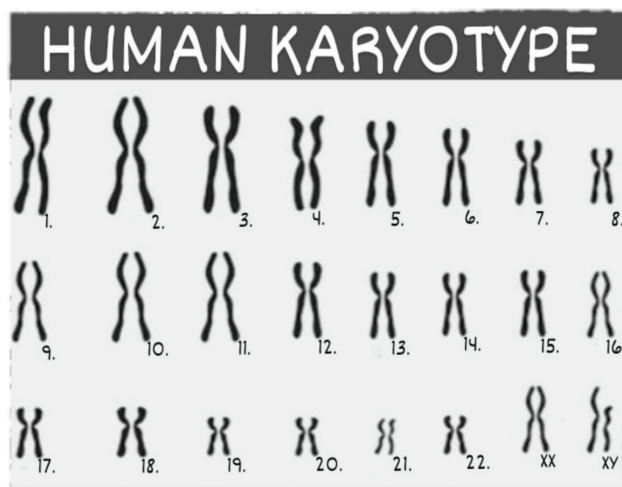


Illustration 4 : Human Karyotype



Illustrator : Divyashi Motwani.
S. Y. BSc Biotechnology

Q banding

This involves staining of chromosomes with Quinacrine which goes onto alkylate DNA and fluoresce on exposure to UV light. A disadvantage of the technique is that the fluorescent intensity fades rapidly and thus, observations and photographs must be made within a few

minutes of staining. This technique, however, was less favourable for routine studies as the fluorescent staining quickly decreased the fluorescence intensity. Hence, several other banding techniques were developed, for example, G, R, C and NOR banding, each having its own specific properties and applications.

NOR Banding

Nucleolar organizer region or NOR is a region in the chromosome around which the nucleolus forms. It contains genes for ribosomal RNA. Treating chromosomes with silver nitrate solution selectively stain the NORs. Since the NORs are located on the satellite stalks of the acrocentric chromosomes, silver staining will yield dark regions in these areas.

It is important to note that all the chromosomes must have their centromeres aligned along a horizontal axis with the P arm above and the Q arm below the axis, respectively.

After performing any of the mentioned procedures for karyotyping, these stained chromosomes need to be organized into a karyogram – with the chromosomes numbered and arranged in decreasing order of their sizes. When schematically represented, a karyogram is called an ideogram.

Such an arrangement helps to identify numeral abnormalities, translocations and chromosomal aberrations.

Cytogenetic Analysis in AML

Cytogenetic analysis plays a critical role in the diagnosis, classification, prognosis, and management of acute myeloid leukemia (AML). It has become an essential technique that helps doctors identify leukemia and provide immediate guidance. Cytogenetic testing is usually performed on the bone marrow of the patients who are suffering from AML. It not only helps in characterizing the aggressiveness of leukemia but also assists in determining treatment response and overall prognosis. AML risk factors are determined by identifying the underlying cytogenetic abnormalities, including chromosomal duplications, deletions, or substitutions and also under- or over-expression of some proteins. Abnormalities in chromosomes cause several genetic disorders leading to developmental delay, congenital malformations, mental retardation, and infertility.

Cytogenetic analysis is very crucial in the diagnosis of oncologic and hematologic disorders. It helps in the diagnosis and classification of disease as well as in planning treatment regimens and monitoring the status of disease. And although a time consuming and a laborious process, Karyotyping provides a single snapshot of the cell's chromosome making it very convenient for cancer diagnosis. It provides clear images of the intricate banding patterns on chromosome aiding in pre-natal diagnostics too!



Article by : Nivedita Sivakumar
S. Y. BSc. Biotechnology



And Akanksha Mahajan
S. Y. BSc Biotechnology

Let the DNA Glow!

Fluorescent *in situ* hybridization

In any normal procedure, treatment is preceded by diagnosis, and diagnosis is inherently dependant on the cause. When we track the cause of a genetic disease, disorder or abnormality, things can get a little tricky, as we conduct the search on a molecular level.

However, imagine a scenario wherein, the cause glows for you, saving you the effort to try and pinpoint what lies in the dark. This can be realised by Fluorescence *in situ* hybridization (FISH).

FISH is a laboratory technique for detecting and locating a specific DNA sequence on a chromosome or in specific cells. The technique relies on exposing chromosomes to a small DNA sequence called a probe that has a fluorescent molecule attached to it and it must be large enough to hybridize. The probe sequence binds to its corresponding complementary sequence on the chromosome which then can be viewed under UV light.

DNA fragments that are to be made into a probe are not directly isolated from the source. They are usually first cloned in artificial chromosomes while keeping to strict size guidelines. A method called Nick translation is then employed to incorporate labelled nucleotides into this sequence. The labelling is done using fluorophores such as fluorescein, biotin, cyanines etc.

A variation of this cytogenic lab technique which is so widely used today was first utilized in 1969 where the scientists named Pardue and Gall then tagged rRNA probes with with an isotope of hydrogen to visualise RNA coding in genes in cytological preparations of *Xenopus laevis*.

It was then further developed by biomedical researchers in 1980s to detect and localize the presence or absence of specific DNA and RNA sequences, as well as entire chromosomes.

Not only does the process of Fluorescence *in situ* hybridization (FISH), give good results but is also easy to conduct in a lab. It is carried out in the following simple steps:

1. A fluorescent dye is attached to a purified piece of DNA. This piece of DNA functions as a probe.
2. This probe is then incubated with the full set of chromosomes from the originating genome, which have been attached to a glass microscope slide.
3. The probe with fluorescently labeled DNA finds its matching segment on one of the chromosomes, where it binds to it in a complementary fashion.
4. By looking at the chromosomes under a microscope, a researcher can find the region where the probe is bound because of the fluorescent dye attached to it.
5. This information thus reveals the location of that piece of DNA in the starting genome.

The technique is used in a wide array of applications. Some really phenomenal research that you must have read about in recent times would be impossible without FISH!

FISH technique has played a very vital role in mapping genes and polymorphic loci onto metaphase chromosomes for constructing a physical map of the human genome. FISH technology enabled us to detect and diagnose genetic disorders as well as chromosomal abnormalities in the human genome. The prenatal screening is used to detect abnormalities and aneuploidies due to increase and decrease of sex chromosomes (X and Y) as well as some autosomal chromosomes such as chromosome 13, 18 and 21. Chromosomal abnormalities including translocations, deletions, duplications, and gene amplifications have been identified using this method.

FISH assay used for the *Plasmodium* genus uses specific probes that detect all five species of *Plasmodium* that cause the disease in humans. The FISH assay is preferable to Giemsa staining method as it has greater sensitivity. FISH technology is preferred by scientists due to the immense advantages it provides. Some of which are listed below:

- It has been proved to have high sensitivity and specificity when it comes to recognizing the target DNA and RNA sequences.
- Due to the freedom imparted by this technique where the size of the target is concerned, for example, an entire chromosome, it holds an advantage as compared to its counterparts.
- Owing to the principle it's based on, i.e. fluorescence and complementary binding, FISH is not restricted for use only in metaphase in contrast to traditional karyotyping. It can also be applied during the interphase while having high resolution.
- FISH allows the use of differently coloured (fluorescent) probes and hence is also to study chromosomal translocations and structural rearrangements. Conventional karyotyping does not have this application.
- FISH also has a high signal-to-noise ratio, which translates to the fact that it mostly gives the desired results without adding background false data which can lead to mistaken observations and wrong conclusions.

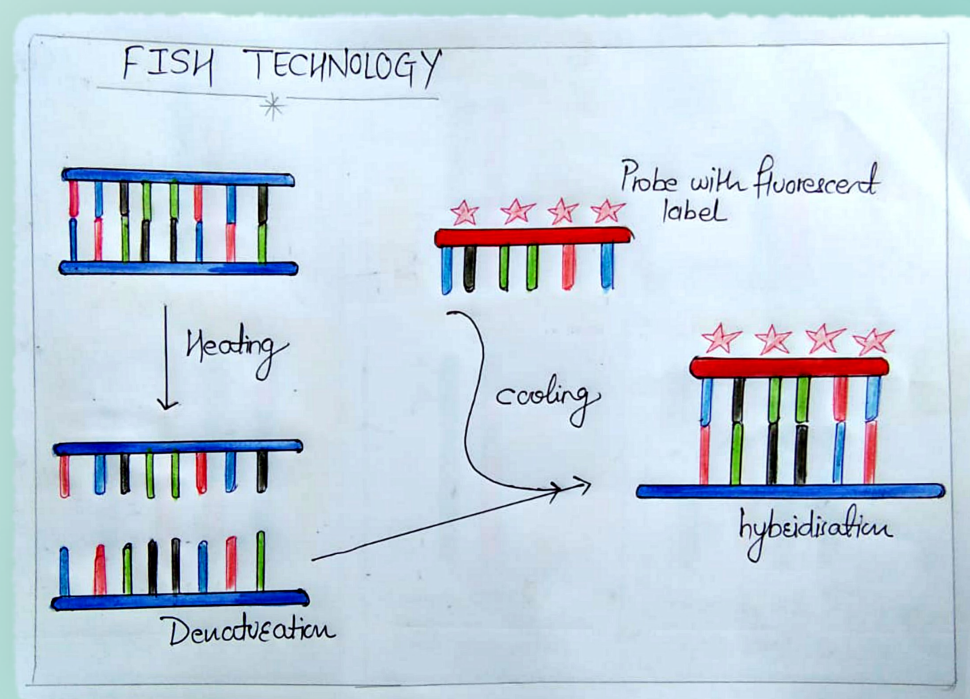


Illustration 5 : FISH Technology



Illustrator : Aqsareha Mujawar
S. Y. BSc. Biotechnology

- The basic idea behind FISH has been modified such that it has many types. One such type being Q-FISH, which can be used to study aging and progression of diseases such as cancer.
- A FISH assay can also be used to study at the level of a single cell by cataloguing its fluorescent intensity/activity.
- One of the advantages that this technique enjoys above all else is its ability to be performed and provide results for both, fresh and aged samples.

That is not to say that FISH is perfect and comes without limitations. Analysis of thin sections can prove to be a cumbersome and thankless task as most of the nuclei are lost during sectioning. This can lead to false-positive results when observing chromosomal deletions and studying aneuploidy.

Another disadvantage being that FISH requires a high number of DNA and RNA copies and has a tough time locating targets when deprived of those. Nevertheless, great strides are being made in this direction to improve the sensitivity of this technique by amplifying the amount of target sequences.

Even while carrying its limitations, FISH has made a good name for itself in the arena of biomedical research and has been greatly favoured by scientists far and wide.



Article by: Anam Namazi
S. Y. BSc. Biotechnology

Secrets of the Family Tree

Pedigree Analysis

Has it ever happened that you wondered whether you might have a disease which has frequently bared its fangs in your ancestry? Or maybe why so many of your family members have a widow's peak? The answer to these questions lies in pedigree analysis. Inheritance of traits in plant and animal species can be studied using controlled crosses. But what about humans, where controlled crosses are not possible? In such a case, researchers analyse pedigree or family trees to understand how human traits and diseases are inherited. A pedigree is a diagram displaying a family's history of a trait (usually a disease) across generations. Common procedure involves the collection of phenotypic data from several generations and construction of a pedigree, following which careful analysis allows one to determine :

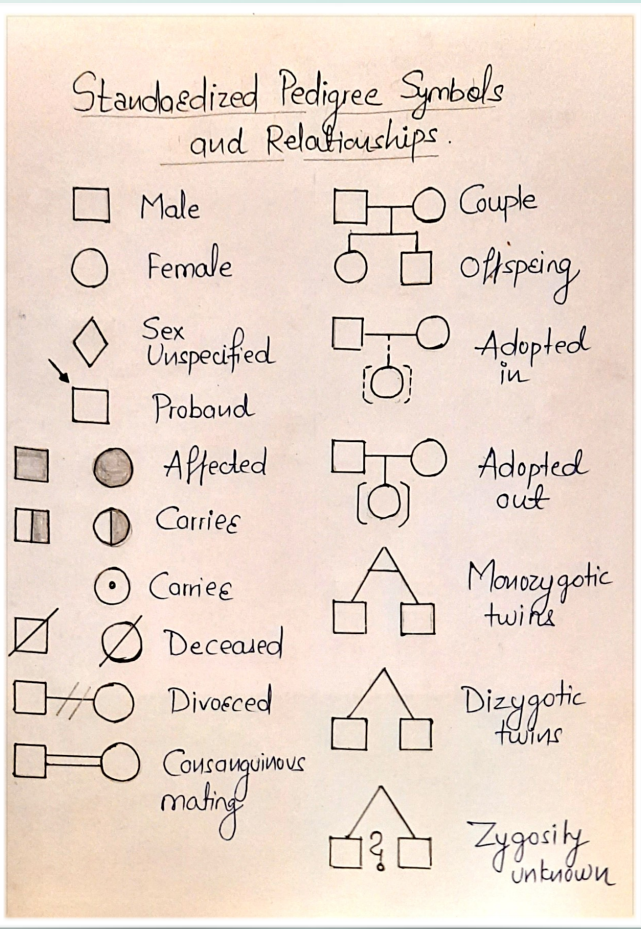


Illustration 6 : Standardised symbols for Pedigree analysis

- Dominance - whether the disease alleles are dominant or recessive
- Linkage - whether the disease alleles are X-linked (present on the X chromosome) or autosomal (alleles present on either of the remaining 22 chromosomes).
- Given below is the clarification for some of the characteristics whose occurrence though common, can be a bit confusing at times.

X-linked or Autosomal?

Clearing Misconceptions about Pedigree

- 1) The presence of many affected individuals in a family does not mean that the trait is dominant.
- 2) You may not always be able to determine the genotype of an individual based on a Pedigree.

Do you know?

- 1) In a polymorphism, the contrasting morphs or phenotypes are often determined by alleles of a single autosomal gene.
- 2) Autosomal recessive disorders appear more frequently in children of consanguineous marriages.
- 3) Although it is uncommon, a trait may be recessive but still show up in all generations of a Pedigree.
- 4) Careful pedigree analysis can identify relatives who may be at risk of inheriting the disease.

Task for you

- 1) Which type of pedigree chart will be applicable for cystic fibrosis?
- 2) What is Rett syndrome and why is it categorised as an X-linked dominant disorder?

- When most of the males in the family tree are affected, the disorder would be categorised as X-linked.
- When equal amounts of both males and females are affected, the disorder would be autosomal.

(note that the corresponding mutated disease-causing gene when located on the X chromosome results in an X-linked trait; while on the other hand, autosomal trait is a result of a mutation on either of the chromosomes from 1 to 22)

Dominant or Recessive?

- To categorise any disorder as dominant, either of the parents must be affected
- On the other hand, for a disorder to be recessive, neither parent is necessarily affected because of the possibility of them being heterozygous for the trait.

Analysis of a pedigree chart can be a cumbersome process. Keeping a few common characteristic points in mind for the easy recognition of these pedigrees reduces the amount of time that needs to be invested.

Autosomal Dominant

A characteristic feature of an autosomal dominant disorder is that in each generation males and females are equally at risk of being affected. An autosomal dominant disorder:

- Generally appears with equal frequency in both the sexes. Moreover, the trait can be transmitted from both sexes to the offsprings.
- The traits usually do not skip generations
- Unless a new mutation originates, affected offsprings usually have affected parents; this clearly indicates, unaffected parents won't transmit the trait to the offsprings.

So, approximately 50% of the offsprings get affected if one parent is unaffected and the other is affected (heterozygous).

Autosomal Recessive

The appearance of the phenotype in the progeny (both male and female) of unaffected individuals usually determines an autosomal recessive disorder. Autosomal Recessive disorder:

- Usually appears with equal frequency in both sexes.
- Due to the recessive behaviour, traits skip generations.
- Usually affected offsprings are born to unaffected parents and therefore, approximately 1/4th of the progeny gets affected as a result of both parents being heterozygous for the trait.

X-Linked Dominant

An X-linked dominant disorder is determined by; passing on of the trait from either of the affected parents resulting in an unequal distribution of the trait among both sexes in the progeny. In an X-linked disorder:

- Often more females are seen to be affected as compared to males suggesting that frequency of appearances of the trait in both the sexes is unequal.
- Traits are not generally seen to skip generations.
- Sons who are affected must have an affected mother; but in case of affected daughters either the mother or the father can be affected.
- Affected fathers pass the trait to all their daughters, whereas heterozygous mothers who are affected pass the trait on to 50-50% of both their sons and daughters.

X-Linked Recessive

In X-linked Recessive disorders, the catch is that females are rarely or almost never affected, instead they are carriers for the disease and so carrier mothers (have the allele/gene responsible for the disease but do not show any symptoms of the disease) mostly pass the trait to their sons. In an X-linked recessive disorder:

- Often more males are seen to be affected as compared to females in the family tree.
- Usually there is a 50% possibility of unaffected (carrier) mothers giving birth to affected sons, thus we can say, the trait skips generations.
- The trait is never passed on from father to son.
- Daughters of affected fathers are usually carriers.

Was it a bit confusing? A few examples might help clear the understanding of pedigree analysis.

Haemophilia A

An example of an X-linked recessive condition is the blood clotting disorder known as haemophilia A. This condition arises due to a mutation in the gene coding for the production of blood clotting factor VIII. The symptoms observed in this condition are excessive and uncontrolled bleeding after cuts or wounds. The disease illustrates the fact that the amount of product made can vary and the factor VIII activity determines the severity of the disease. Due to the fact that fathers pass on the Y chromosome to sons, an affected father cannot transmit the disease to a son. However, on the other

hand, the daughter of an affected father receives the defective X chromosome and thus, can be either a carrier or an affected individual (if the mother is also a carrier or afflicted with the condition).

Thus, the disease often tends to show a pattern of skipping a generation. In females, owing to X-inactivation i.e. the phenomenon of one X chromosome being typically inactivated, female carriers usually acquire adequate factor VIII activity and are hence asymptomatic.

Huntington’s disease

Huntington’s disease is an autosomal dominant genetic disorder that affects the central nervous system in humans. Nerve cells degenerate in certain parts of the brain, giving rise to symptoms like dementia, memory loss, severely decreased mental capacity, muscle rigidity and loss of muscle coordination. On the account of this being a autosomal dominant disease, it affects both the sexes in the progeny equally and very rarely does it skip a generation. The chances of the offspring of a heterozygous individual and a normal individual being discommoded by this disease is 50%.

Examples of Autosomal recessive disorder: Cystic fibrosis, Sickle cell anaemia.

Examples of X-linked dominant disorder: Rett syndrome, Vitamin D resistant rickets.

Pedigree method is an excellent analysis technique which can determine the mode of inheritance of a disease or a trait, as well as predict the probability of it’s appearance in the offsprings. Therefore, pedigree analysis is thought to be a crucial tool in research and genetic counselling.

Nonetheless, some drawbacks still linger. Only a large family unit can be examined through pedigree analysis. Moreover, there is always some possibility of errors in the factual information required for Pedigree analysis, especially when relying on data from family members. In Real pedigrees, further complications arise due to incomplete penetrance i.e. the condition wherein an individual carrying the defective gene does not show outward phenotypic characteristics of the the disease and variable expressivity of disease alleles.

In this era of high throughput sequencing and analytic methods, methods like pedigree analysis might have been left behind in the race. Though, it is needless to mention, the utmost importance of pedigree analysis in genetics.

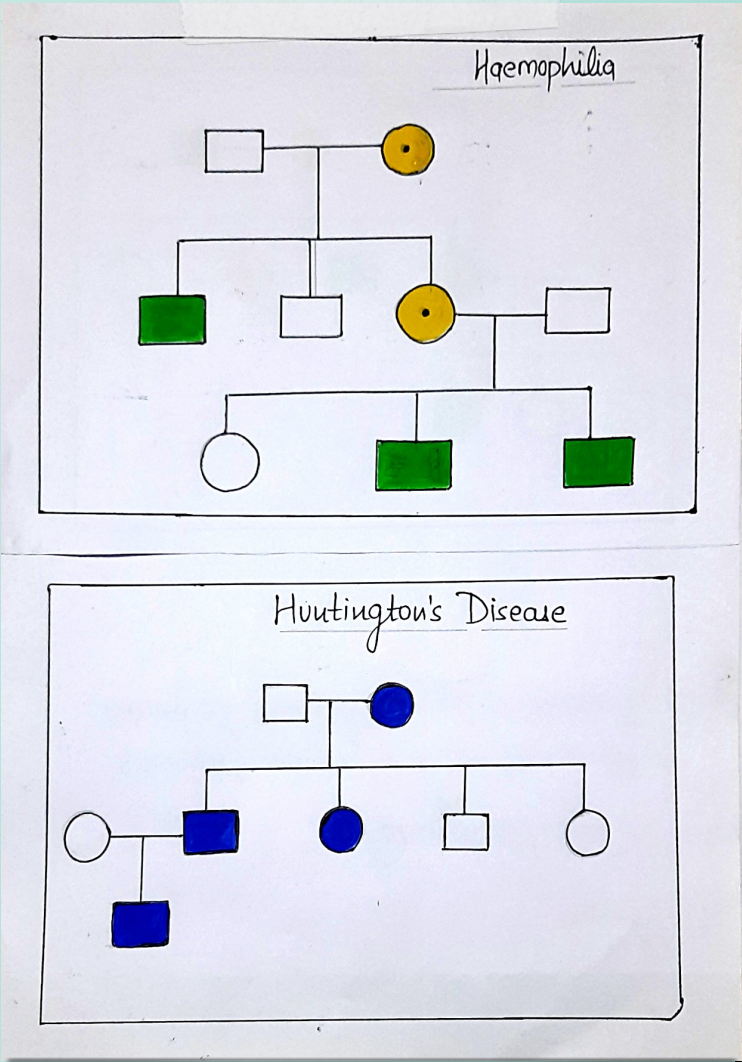
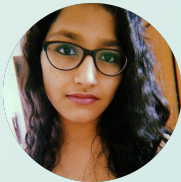


Illustration 7: Haemophilia and Huntington’s disease



Article by: Sanskriti Deshmukh
S. Y. BSc. Biotechnology



And Aqsareha Mujawar
S. Y. BSc. Biotechnology

Illustrations by: Aqsareha Mujawar

It’s Gaming Time

The Merry Game-te Corner

1. Code Decode

Fill in the blanks and unjumble the highlighted letters to decode the word of this issue.

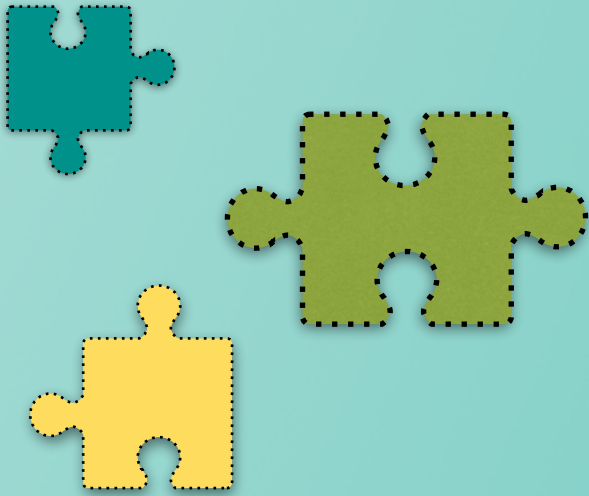
- i. Motion of dispersed particles under the influence of uniform electric field : _____
- ii. Chromosomal landmarks used to trace or identify specific region of a gene: _____
- iii. A small circular DNA strand in the cytoplasm used in the manipulation of genes: _____
- iv. Procedure for measuring biochemical or immunological activity of a sample: _____
- v. First mammal cloned from an adult somatic cell: _____

Word of the Issue: _____

2. Heredity Hunt

Find the words listed in the word search below:
Find Us:

- 1. ARRAY
- 2. IMMUNOFLUORESCENCE
- 3. HEREDITYTECHNIQUE
- 4. PCR CLONE
- 5. PROBE VECTOR
- 6. CYTOGENETICS
- 7. CRISPR



K J P S E N O T Y P E G H L O R F N I T
S E W C N F D E R B E R U P Z T U F E E
U N R I L A I G I D T G C N E T I C S E
O O T T L R B A E B O R P J H R H A O Y
G L R E B R M I R A I P E O O N D U W T
Y C L N N A I C E S O I N T I R I N G I
P J A E D Y L N P R C P C Q T E Q V F D
O E W G E V Y R J N E E U T S Q U A R E
M K K O L R O T C E V E Y E P O Y B N R
O Q E T G E N P N V D S P Q F L O R D E
H U N Y O A L V C E O B E D M E N W M H
E C E C N E C S E R O U L F O N U M M I

3. Gags By Gregor

Q. Why did the scientist try to genetically sequence his chicken sandwich?

A. He wanted to make it CRISPR.

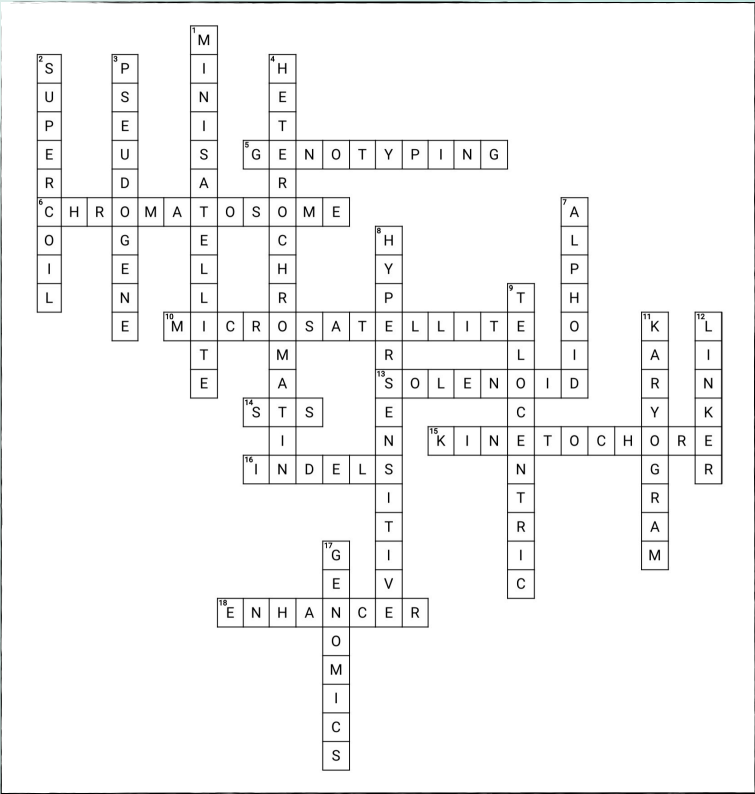


Created by : Divya Bharadwaj
S. Y. BSc. Biotechnology

Having tough time solving it? Stay tuned for next issue!

Key to the crossword of Genophilic, 2nd Issue

We know, we know you solved it. But still, here’s the key!



How did you find it? Any feedback? Suggestions?

We would like to know what you thought of this issue of Genophilic, any suggestions are welcome.

Our next issue will be on the topic ‘Genetics and Diseases’, and we would appreciate any contributions on said topic. Contact us on : editor.genophilic@gmail.com

References:

Linkage Analysis:

<https://jamanetwork.com/journals/jamaneurology/fullarticle/775035>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3267991/>

Karyotyping and Cytogenetics

<https://geneticeducation.co.in/karyotyping-definition-steps-procedure-and->

<https://www.webmd.com/baby/what-is-a-karyotype-test#1>

<https://www.nature.com/scitable/topicpage/karyotyping-for-chromosomal->

<http://www.pathology.washington.edu/galleries/Cytogallery/main.php?>

https://www.mun.ca/biology/scarr/Human_Karyotype.html

<https://www.nature.com/scitable/topicpage/cytogenetic-methods-and-disease-flow->

<https://onlinelibrary.wiley.com/doi/pdf/10.1038/npg.els.0001444>

https://www.researchgate.net/publication/221920029_Cytogenetic_Techniques_in_Di

Fluorescent in-situ Hybridization:

<https://anatomypubs.onlinelibrary.wiley.com/doi/full/10.1002/ar.22944>

https://geneticeducation.co.in/a-brief-introduction-to-fish-fluorescence-in-situ-hybridization/#Different_types_of_FISH

<https://www.frontiersin.org/articles/10.3389/fcell.2016.00089/full>

<https://www.genome.gov/genetics-glossary/Fluorescence-In-Situ-Hybridization>

<https://link.springer.com/article/10.1007/s00776-007-1215-1>

Pedigree Analysis:

<https://www.ncbi.nlm.nih.gov/books/NBK21257/>

<https://www.cs.cmu.edu/~genetics/units/instructions/instructions-PBA.pdf>

http://www.bogari.net/Bogari/Medical_Genetics_files/3-1%20Patterens%20of%20Inheritance.pdf

<http://ar.utmb.edu/webpath/genhtml/genet054.htm>

<https://vanhornhuntingtonsdisease.weebly.com/>

[https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3638020/#:~:text=The%20ofundamental%20disadvantage%20of%20pedigree,meiotic%20process%20\(Boehnke%201994\)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3638020/#:~:text=The%20ofundamental%20disadvantage%20of%20pedigree,meiotic%20process%20(Boehnke%201994))

<https://www.ndsu.edu/pubweb/~mcclean/plsc431/mendel/mendel9.htm>

<https://www.jove.com/science-education/10775/pedigree-analysis>

[https://bio.libretexts.org/Bookshelves/Genetics/Book%3A_Online_Open_Genetics_\(Nickle_and_Barrette-Ng\)/05%3A_Pedigrees_and_Populations/5.1%3A_Pedigree_Analysis](https://bio.libretexts.org/Bookshelves/Genetics/Book%3A_Online_Open_Genetics_(Nickle_and_Barrette-Ng)/05%3A_Pedigrees_and_Populations/5.1%3A_Pedigree_Analysis)

<https://medicine.uiowa.edu/humangenetics/resources/how-draw-pedigree/instructions-how-draw-pedigree>

<https://www.khanacademy.org/science/high-school-biology/hs-classical-genetics/hs-pedigrees/a/hs-pedigrees-review>