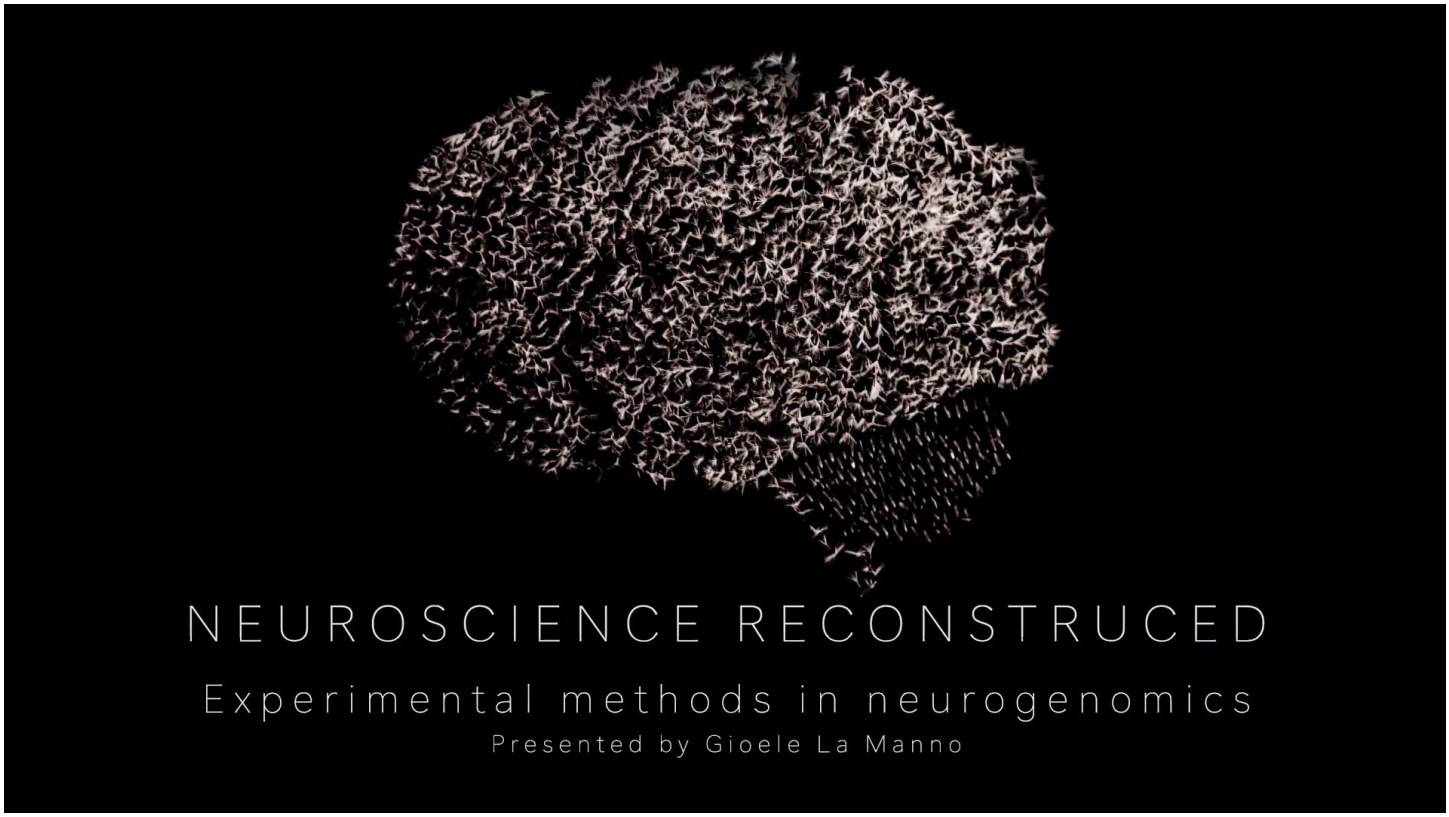

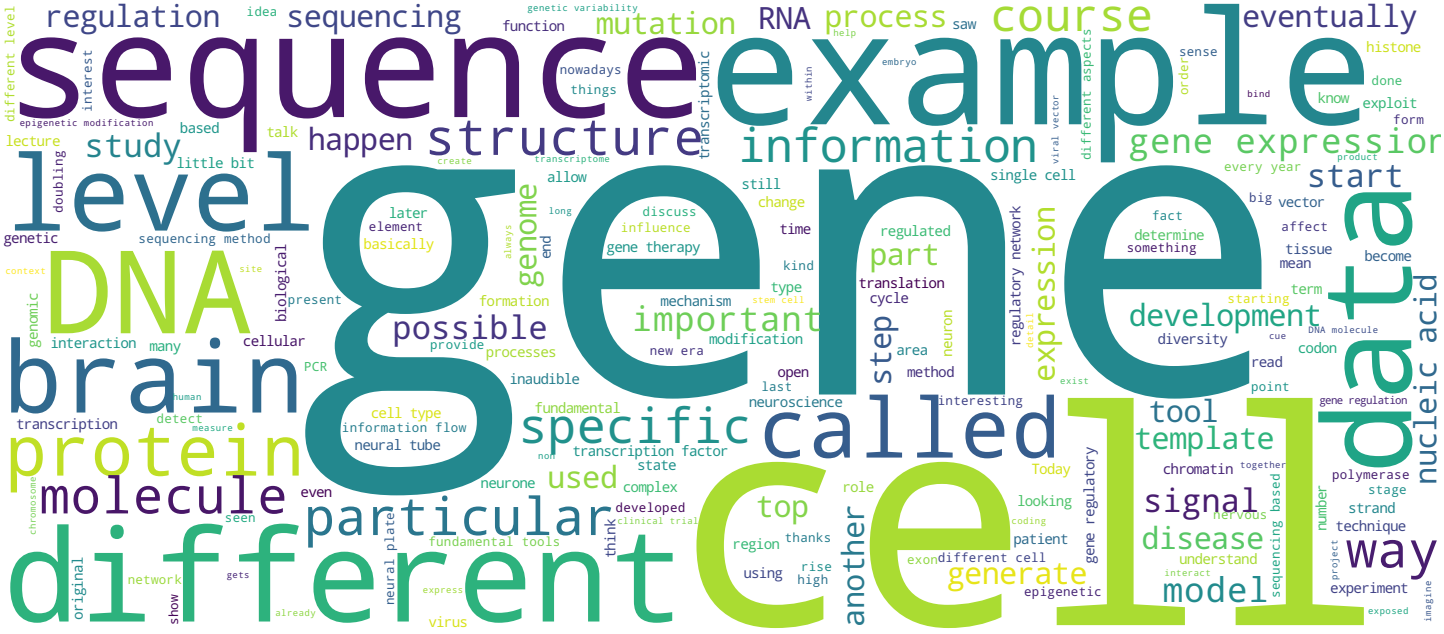

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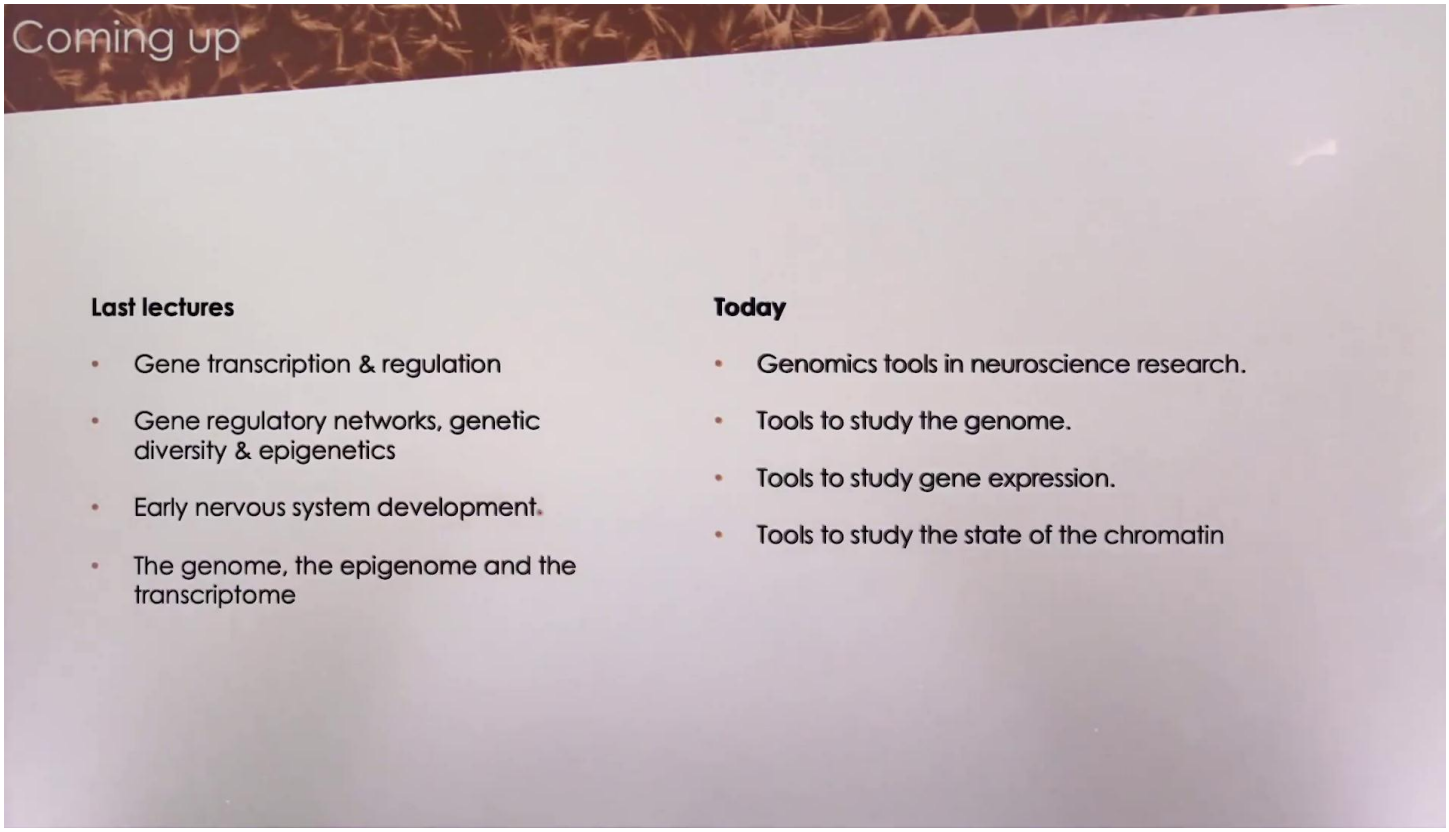
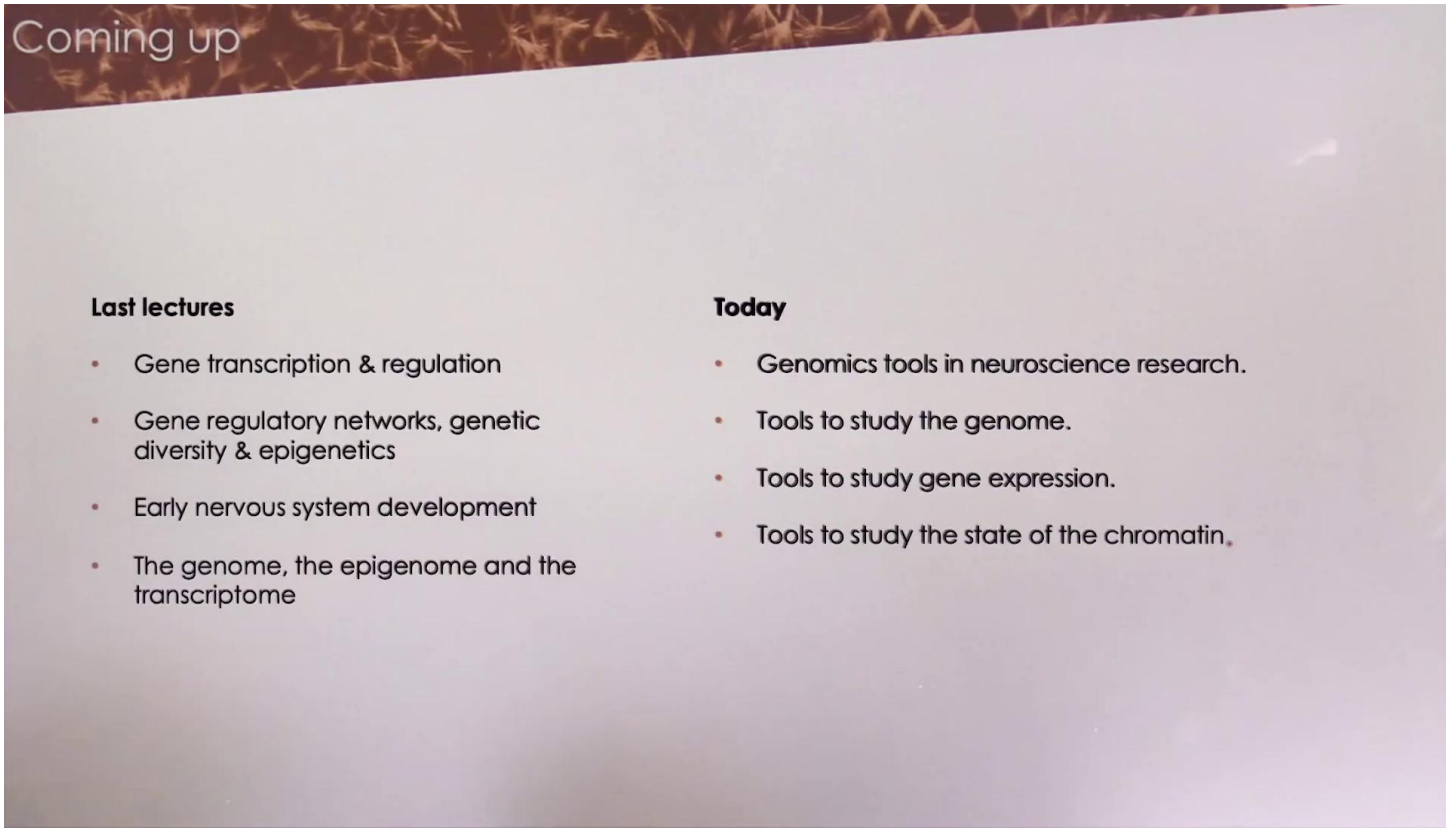


Hello everyone, I am Gioele La Manno and welcome to another lecture on neuroscience reconstructed. Today we're going to talk about experimental methods that are fundamental tools to make possible research in genetics of brain development and a molecular aspect of neuroscience. Before starting, let me just give you a quick summary on what we've been covering over the last few lecture. We have been discussing transcription and gene regulation. We have seen how different genes can interact with each other, activating each other. For example, transcription factor can generate networks or cascade that generate the diversity that we see in different cell types. For example, at the level of the tissue this can be regulated and modulated by epigenetic aspects and we saw this all together making it possible to generate from one single stem cell the dragos a full nervous system through the discussion of the first and key early steps of neurodevelopment. Also been discussing the genome, the epigenome and the transcriptome or information flow between them. Today, we are going to focus some methods. Those are fundamental tools that researchers need to actually probe the biological system.

Notes

Summary

0m 10s

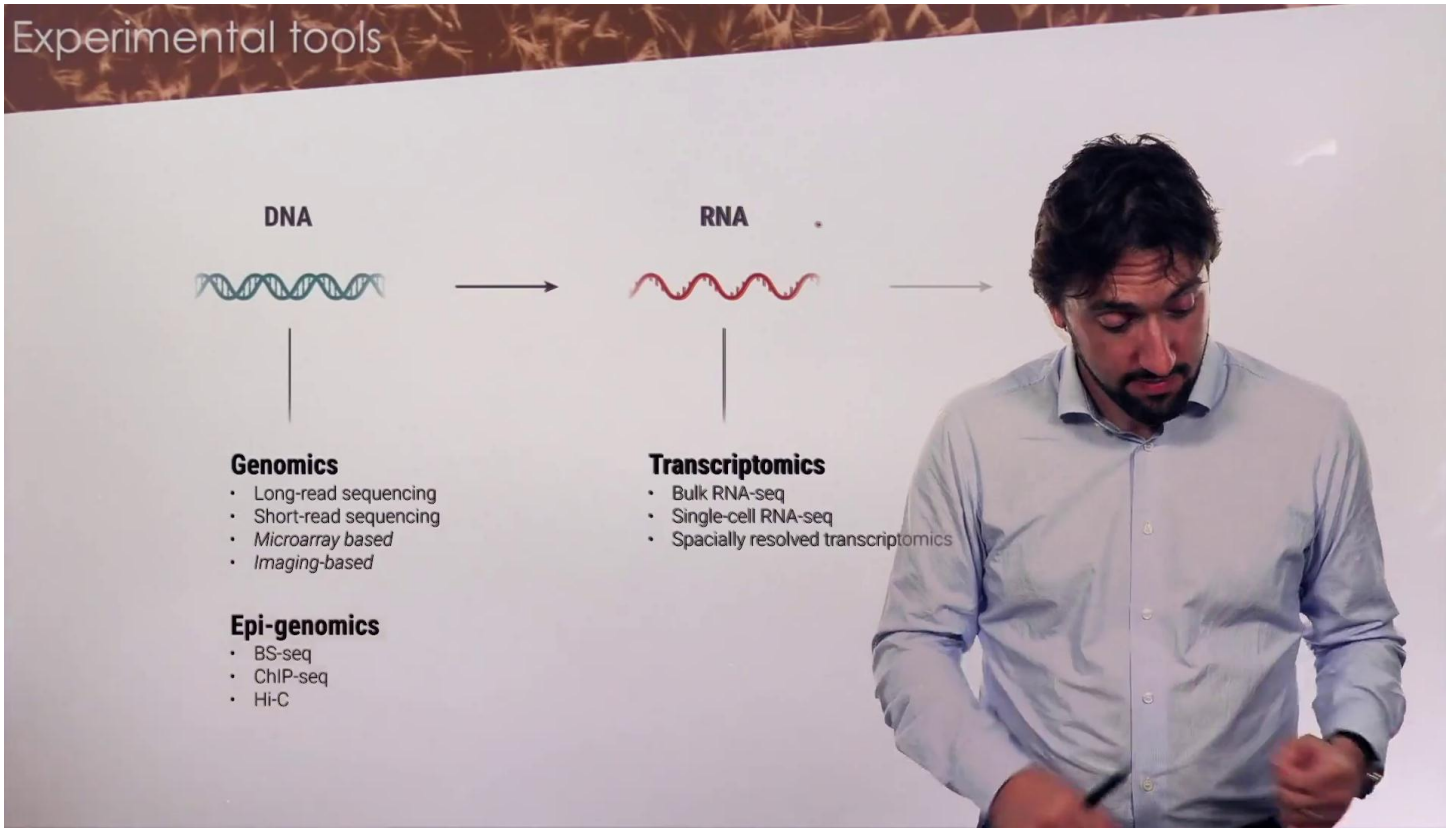


Do neurobiology looking at molecules and nucleic acid in particular. And we're going to look at the tools to study the genome. The tools to study... The related tools that are used to study gene expression, and finally, also the state of the chromatin. So information now that is not at the level of the sequence but is still at the level of the nucleic material inside nuclei.

Notes

Summary

1m 42s



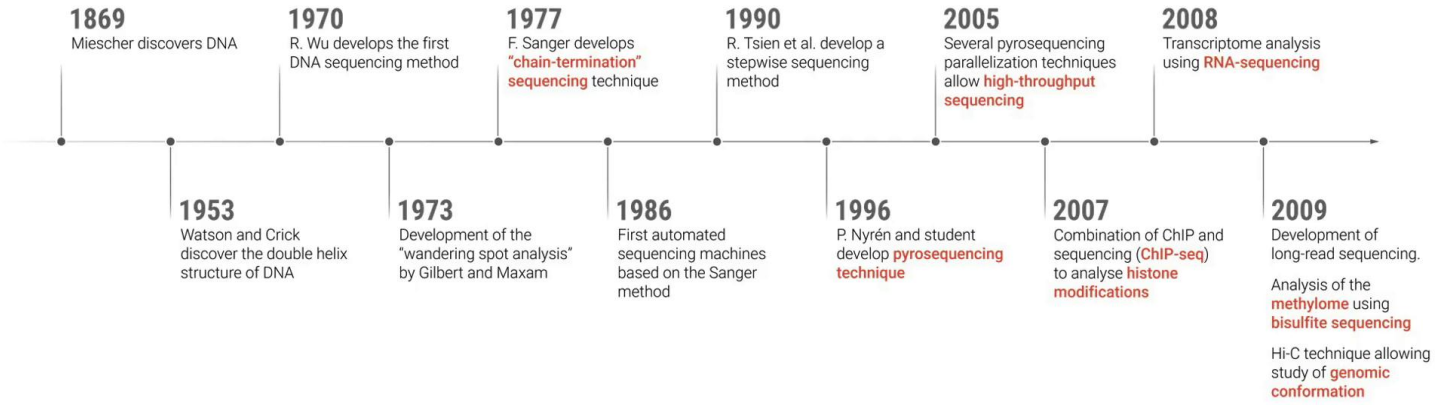
Just to project this on top of the central dogma molecular biology the information flow from DNA to RNA to protein. We know that each step there could be modulation of information. It could be a regulation of expression. There could be regulation of translation. And among these three classes of molecules, two are subtly special because they are nucleic acids. And as we're going to see, thanks to some key technologies that exploit the fact that nucleic acid cannot act as a template on which reverse complement strand can be synthesised. It's possible to study nucleic acid in a way that is much, much simpler than for protein that indeed do not have this property. Therefore, today we're going to discuss only DNA and RNA. So genomics and transcriptomics. The genomics will founded on the study specifically of the sequence, transcriptomics on the study on the levels of expression of specific genes so how much each gene is transcribed there are polymerases and how these affect the functions and processes that are regulated in a level in the cells to also eventually the translation to protein. Then we're also looking at epi-genomics, so the aspect that is still related to DNA but not specifically related to the sequence of the DNA but to further modification that can change the structure and accessibility of the chromatin to different process of regulation or silence.

Notes

Summary

2m 13s

A long way to NGS



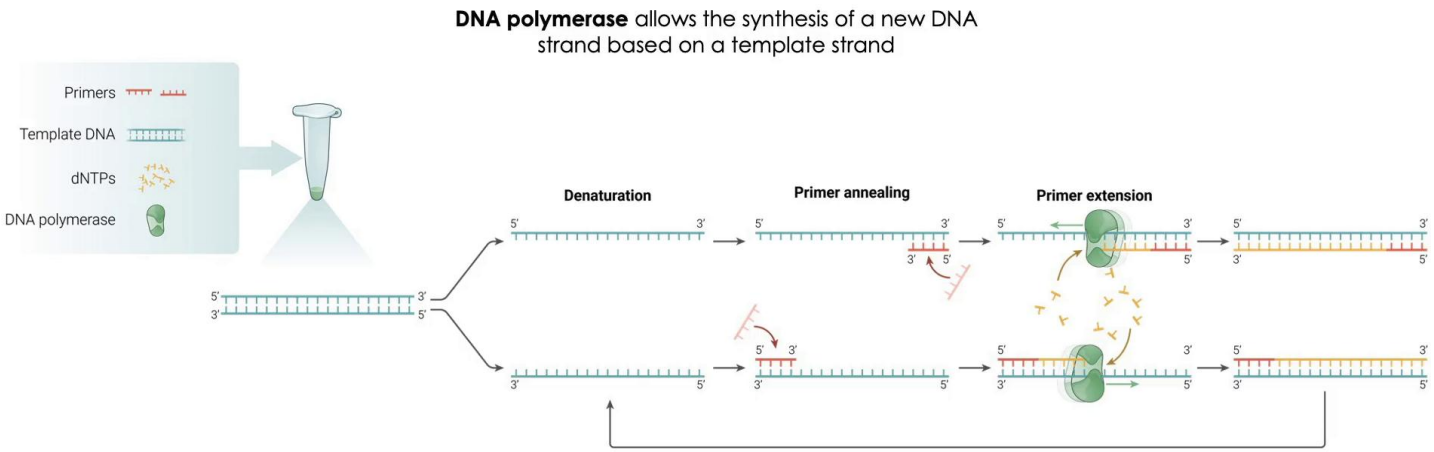
I want to show you before starting getting into the methods a little bit of a timeline of what has been the development of sequencing based methods over the years. We are moving from the leader discovery of DNA very fast through almost a century where eventually the structure of DNA was resolved and this really exposed a very interesting fact the complementarity of the two strengths and on which many of the techniques you're going to see are going to be based. The first sequencing method was developed in the '70s, eventually fundamental method in 1977, Sanger sequencing based on chain termination. The attempt to, of course, make this more scalable through automation and eventually, key step the invention of pyrosequencing. That opens the new era that will be instrumental to open the new era by triple sequencing that really can be dated to the 2000s where we see after this [inaudible 00:05:01] devolution over every year, new technologies and nowadays of dozens of technologies related to sequencing, estimation of different aspects... Measurement of different aspects of gene expression, genome sequence, modification of chromosome at different level, single-cell special level are developed every year.

Notes

Summary

3m 48s

DNA polymerase and PCR



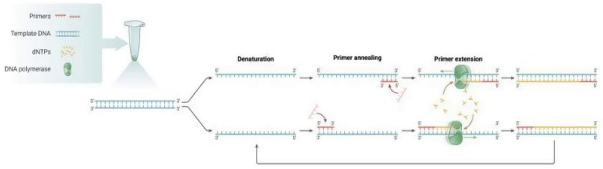
So now we entered this next generation sequencing era where actually sequencing becomes a tool that can sometimes even indirectly tells us about things that are not in the sequence, are really exploiting nowadays. Really sequencing is exploited to study also other axes of cellular and genetic variability that are not maybe strictly related to the sequence of molecules persa. So I would like now to introduce the polymerase chain reaction or PCR. This is a fundamental technology that enables all the sequencing methods and without this basically nothing what we're going to discuss in the lecture will be possible. It's a technique that is based on this fundamental insight that since the DNA is double-stranded, you can always use one of the strands as template and they exist in nature enzymes called polymerases that we can exploit to synthesise a strand on top of the template. You can see here from this scheme you can use increased temperature to denaturate two strands of your DNA molecule. You can design primers. In other words, small stretches of DNA oligonucleotides, the hybridised from a stramity of the sequence of interest. And then polymerases will extend the primer synthesizing, adding new nucleotides to the new DNA strand.

Notes

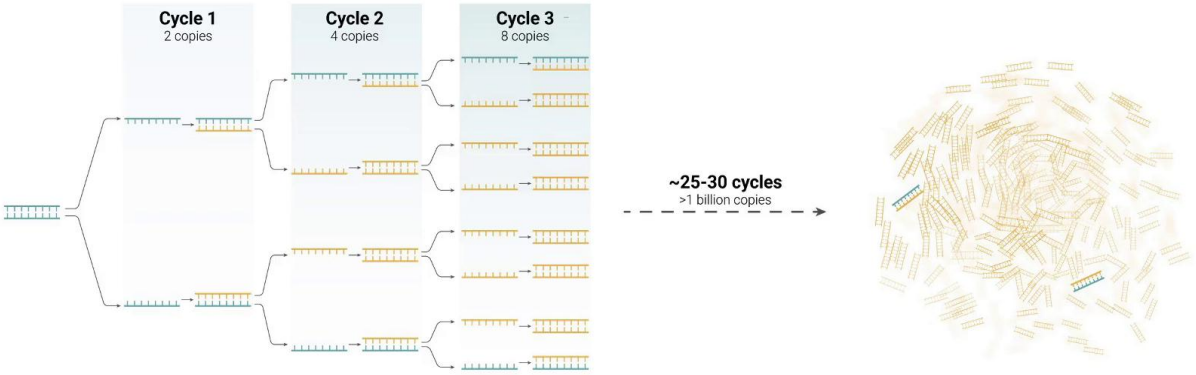
Summary

5m 27s

DNA polymerase and PCR



Polymerase Chain Reaction (PCR) is used to amplify DNA, quantify the amount of a specific DNA sequence, or in sequencing.



And eventually what you get at the end is two molecules identical to the original one. Then what is called first or one cycle of PCR. The idea is that repeating these over each cycle there is a doubling of number of molecules in your test tube and eventually over for example 25-30 cycle of this doubling you get up to 1 billion copies from one single molecule. You really get an amplification of material but the conservation of the same sequence all the molecule being amplified starting from original one will have the same sequence. This is fundamentals, you can have high concentration lot of molecules. A lot of material that is easiest to detect and to measure. But all could conserve the same sequence of the molecules you started with. That's the fundamental idea behind PCR.

Notes

Summary

7m 03s