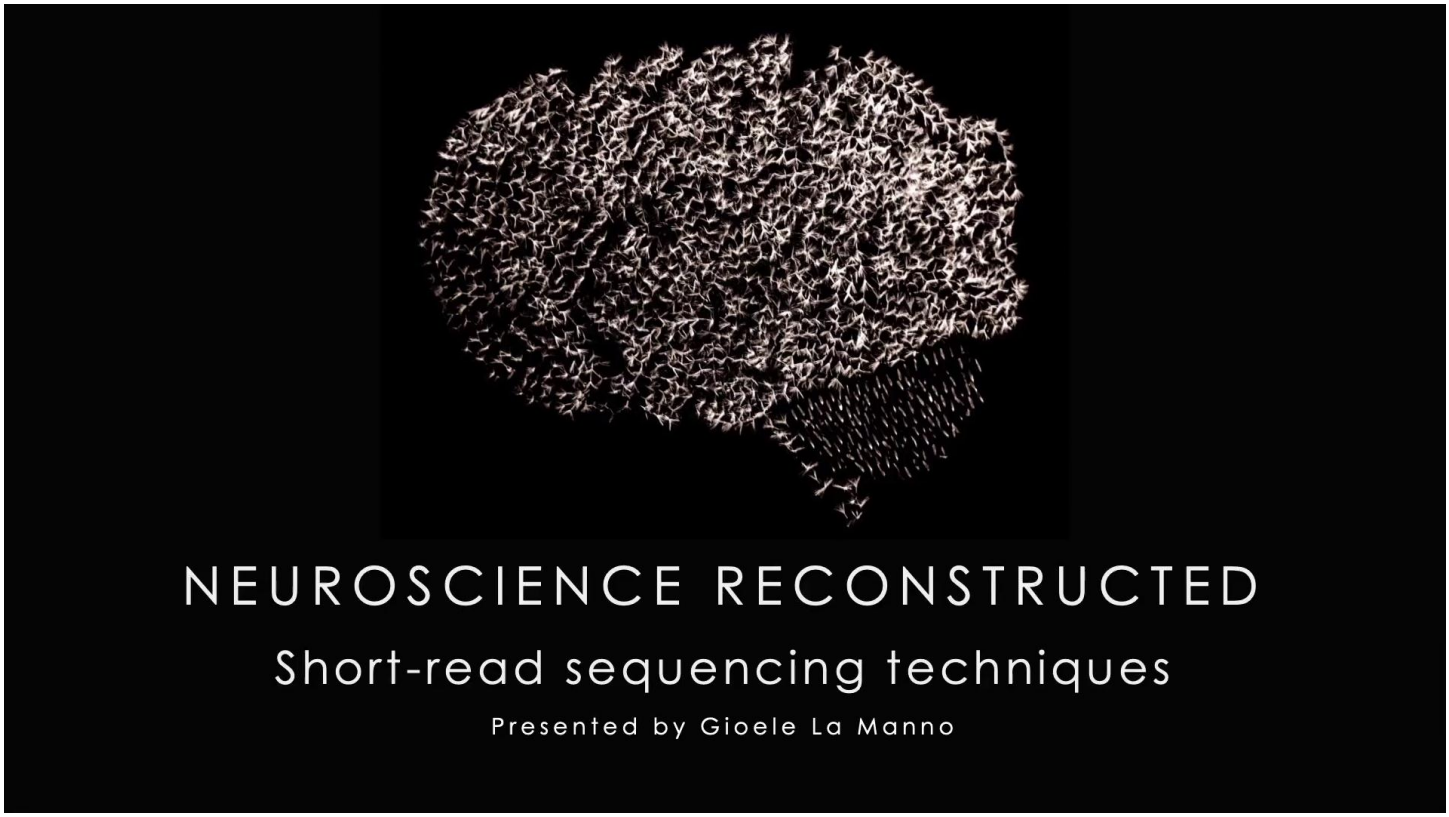

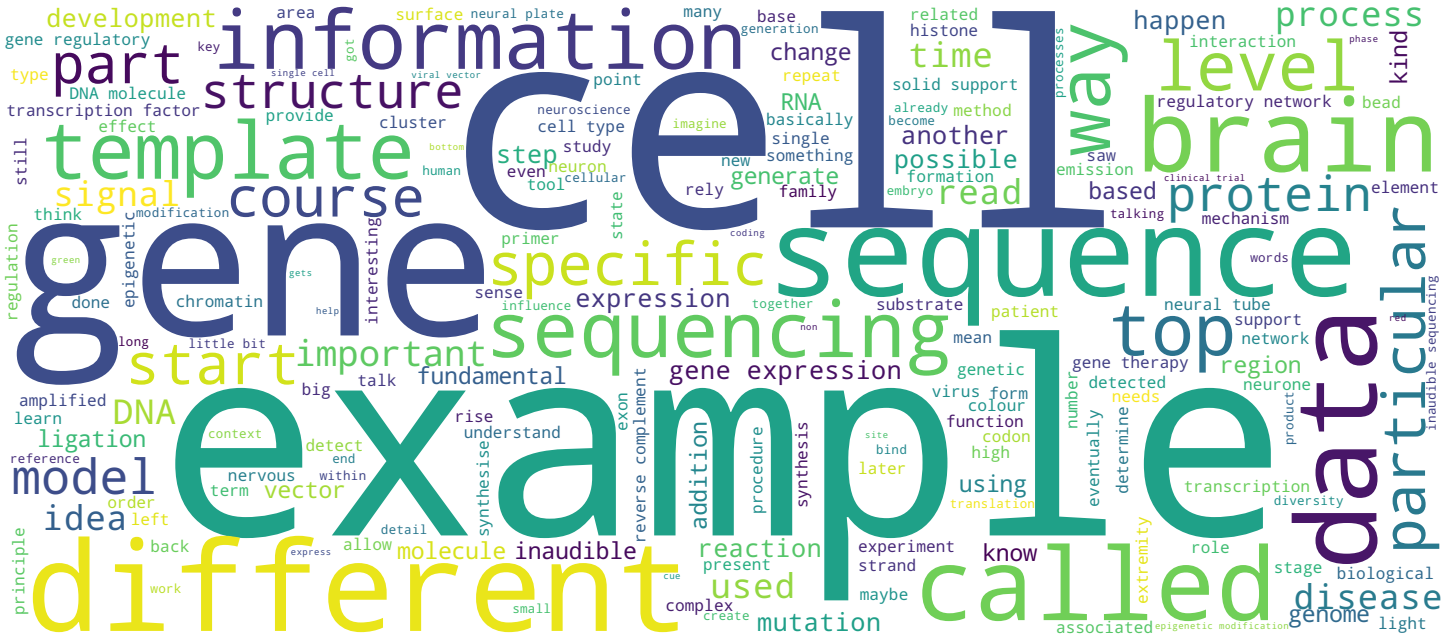

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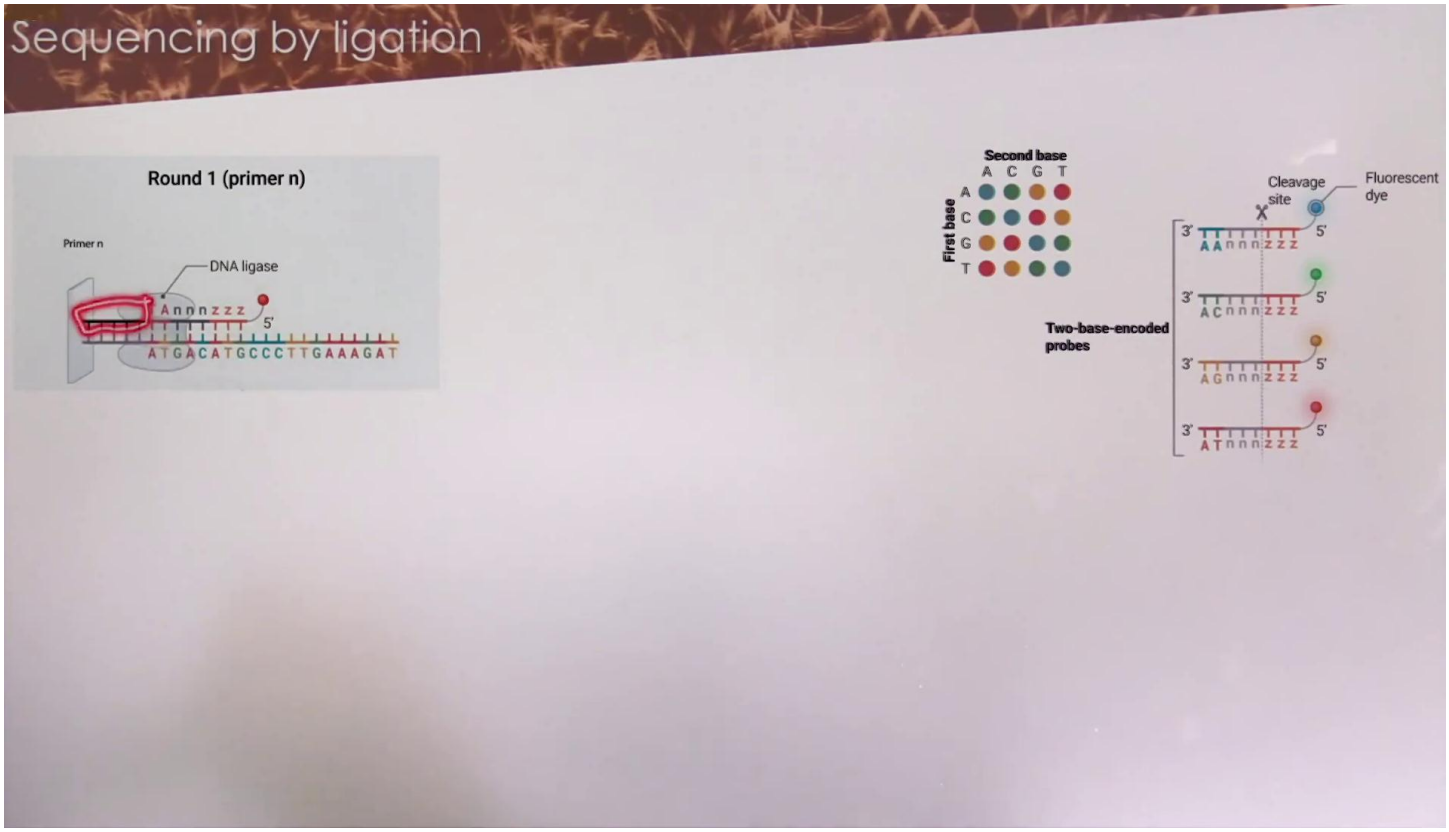
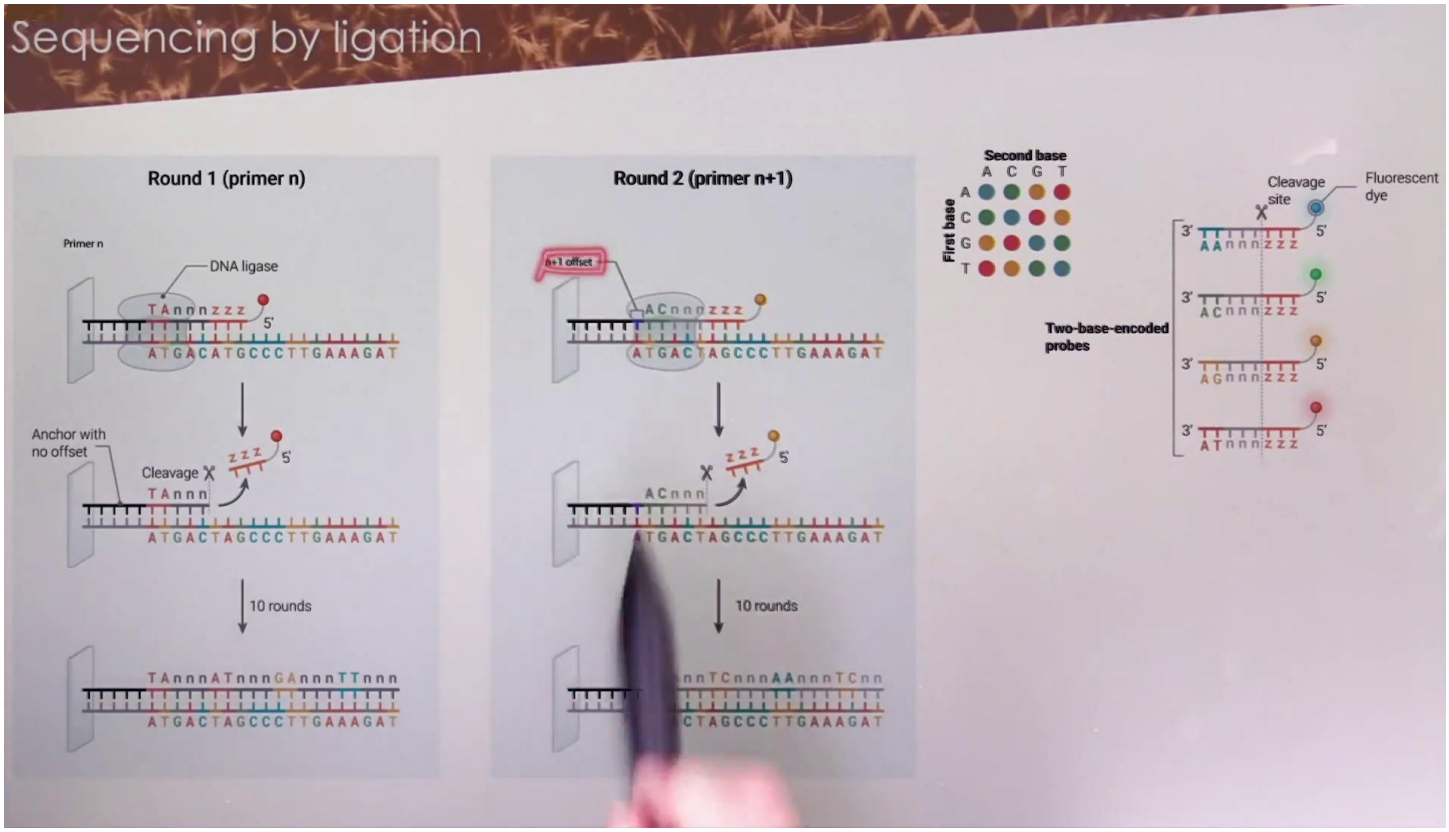


One of the first fundamental matters about [inaudible 00:00:10] sequencing, aiming for [inaudible 00:00:12], is sequencing by allegation. Again, here the starting material is beads. It's quite a solid support on top of which when have been amplified, a cluster of a particular template. Been amplified by PCR specifically, on top of a specific support. On top of this support that is then fixed... On top of this support, we have the template. And the template is going to be sequenced here by a sequence of ligation cleavage cycles that will give partial information. We're going to see on the sequence and then they are then repeated, shifted. This is going to make sense in a couple of minutes. Coupling the reaction of ligation, in other words the condensation diffusion of two DNA molecules thanks to an enzyme, DNA ligase, that when oligonucleotides are perfectly nicely hybridised, allows the ligations of two DNA stretches. This step, the ligase here is providing sensitivity. Specificity, that really makes possible then to rely on the sequencing. The way it works is the following here. I'm showing the template on the bottom, and your primer on the left.

Notes

Summary

0m 05s

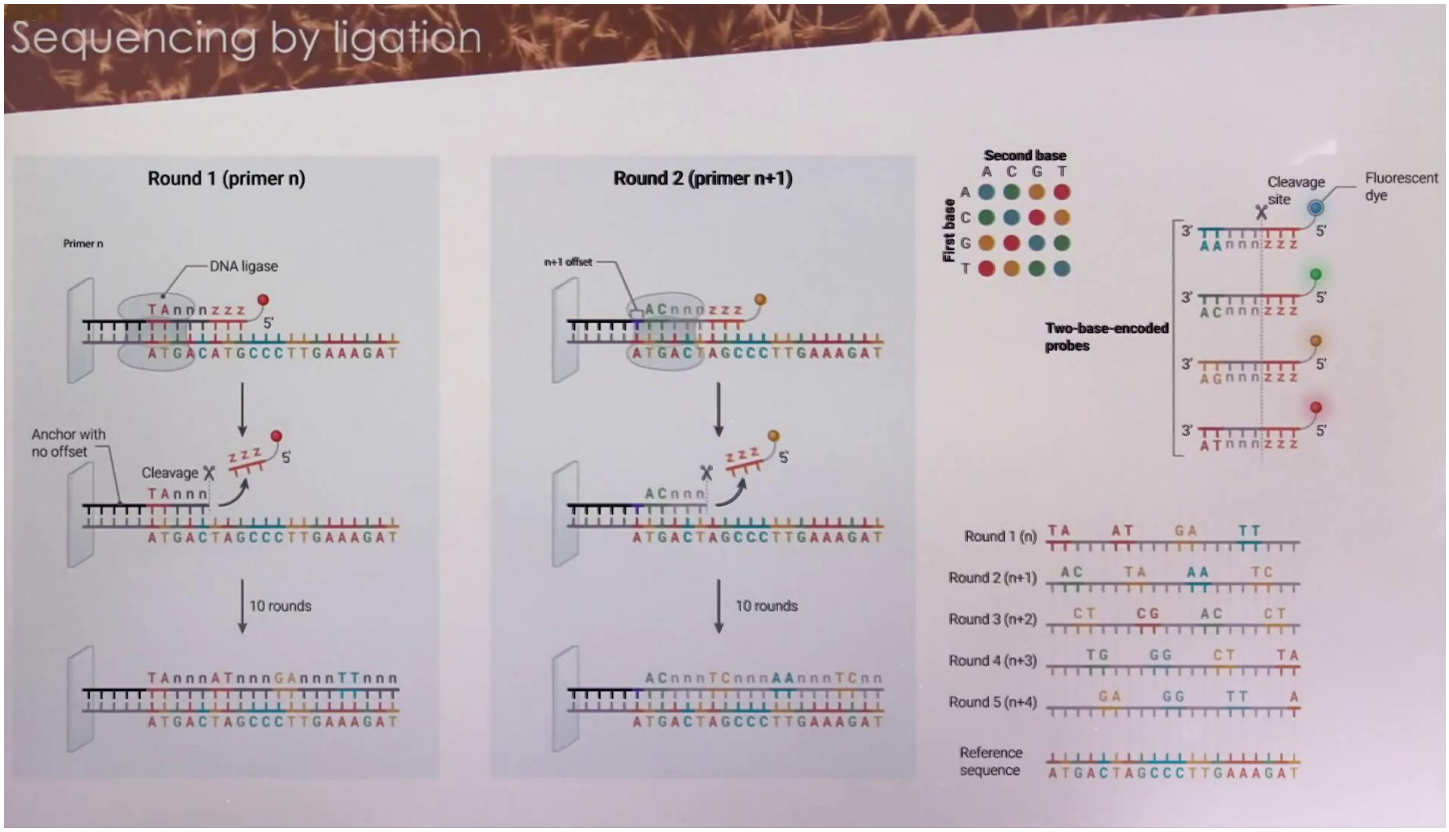


This part over here is a eight basis oligonucleotide, that hybridises, becomes complementary to this sequence. and which is associated with fluorophore that can be detected by the emission spectrum with a particular colour that is being specifically associated to the two bases of the five prime extremity of this oligo. If this particular oligonucleotide [inaudible 00:02:28], we have information that can be read out here in this reference lookup table. We have read so we know it needs to be one of these possible pairs, for example. Oligonucleotides could be, for example, TA GC, CG or AT. After we got this information we can cleave out the part of these nucleoids we don't need any more. After this cleavage, we can go in and repeat the ligation over and over each time and get information on the identity of one of these doublets, one of these pairs of nucleotides. We can actually not fully disambiguate which one of these possible reds or these possible yellows we have been sequencing. That's why we repeat the same procedure on the same template. We now strip out the previous template and we can rerun this, but now shifted with an offset to one base.

Notes

Summary

1m 51s

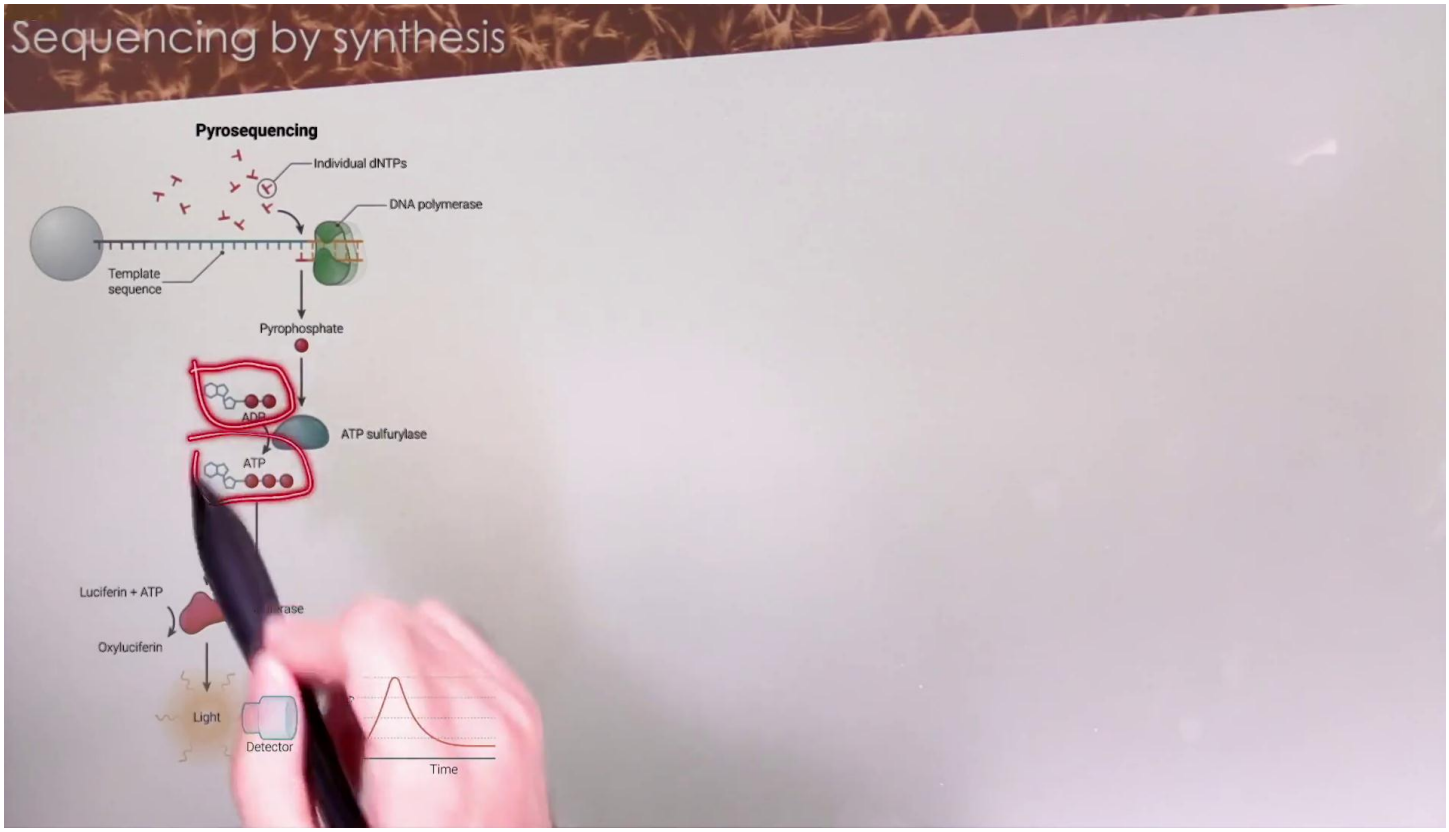


We can do this simply providing a primer that is one base longer and we will repeat the same procedure. Now we get information shifted by one. Now, combining the information of our example, this was red and this was green. We can, of course, now finally learn for example one of the two, that we are almost sure, and eventually with the next offset we're going to learn by consensus of these colours what is the sequence template that we want to sequence. For this case it, for example, requires five different rounds with different shifts. By collecting this information on these colours it's possible to then get information on the sequence. This is much more scalable because it does not require electrophoresis to read out. This can be done on a solid support and it does not rely on termination, terminators, or polymerases but just ligation and specific hybridisation.

Notes

Summary

3m 44s

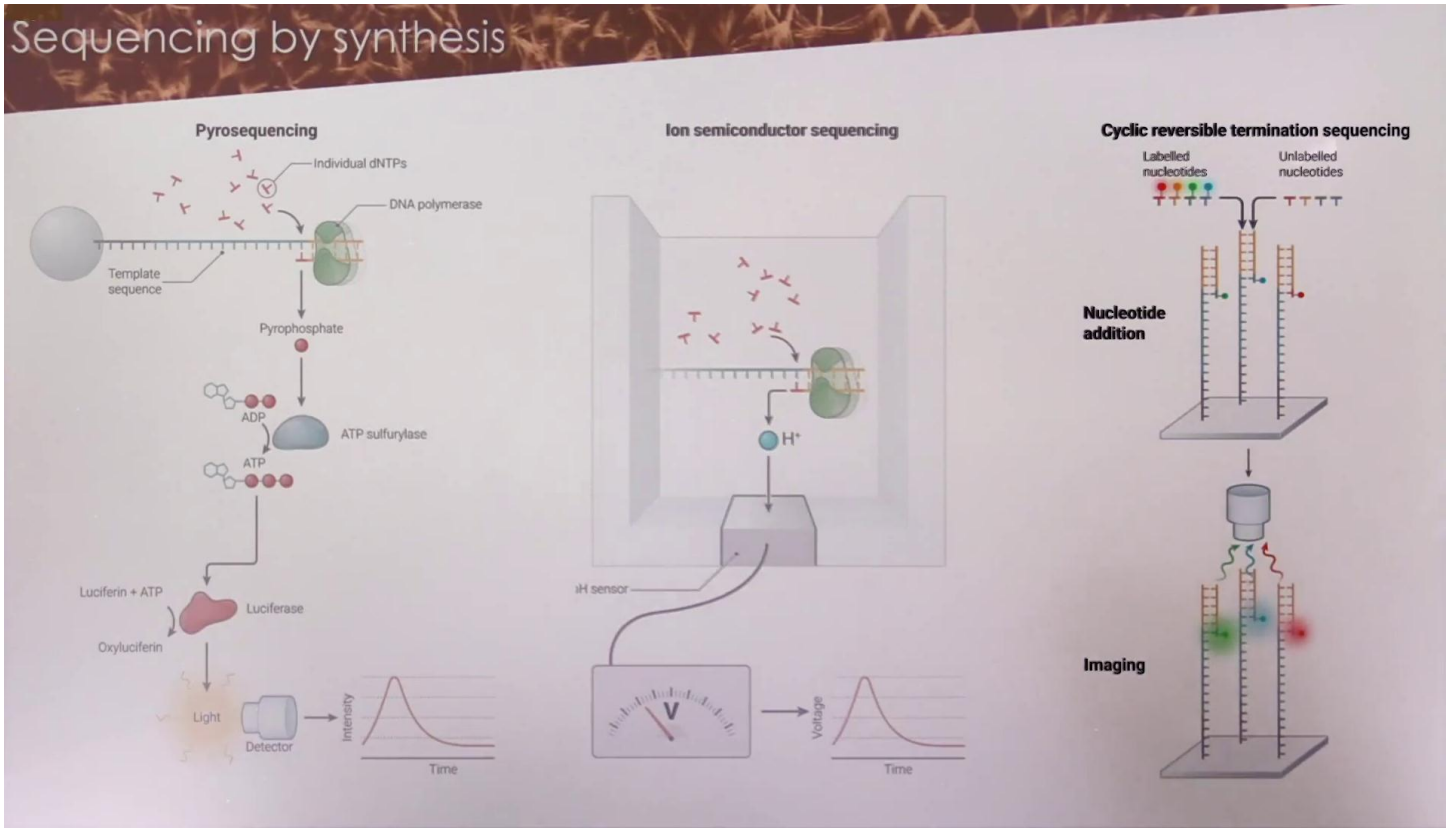


Now we'll be talking about sequencing by synthesis. This is actually a family of technologies that are based on the idea that you can synthesise a molecule without actually explicitly terminating in the same way we solved for [inaudible 00:05:04] sequencing. In a more multiplex and scalable way, you can achieve information on different sequences at the same time. The idea, for example with pyrosequencing, is that you can have a template that is first amplified on top of a solid support, a bead is coating it completely. On that support, we can then start an elongation procedure of reaction starting from DNA primer again here. This is possible to design because we have previously ligated an adapter sequence into an extremity of our template sequence. From this primer, we can start to synthesise the reverse complement thanks to a DNA polymerase. The addition of a new nucleotide, that is associated with adding to the reaction a particular dNTP. Let's say that we now add to the bead G, guanosine. If guanosine is the reverse complement or if there was C in our template, we will add guanosine. Phosphate is going to be released. Phosphate can be used as substrate for ATP sulfurylase to transform ADP in ATP.

Notes

Summary

4m 51s

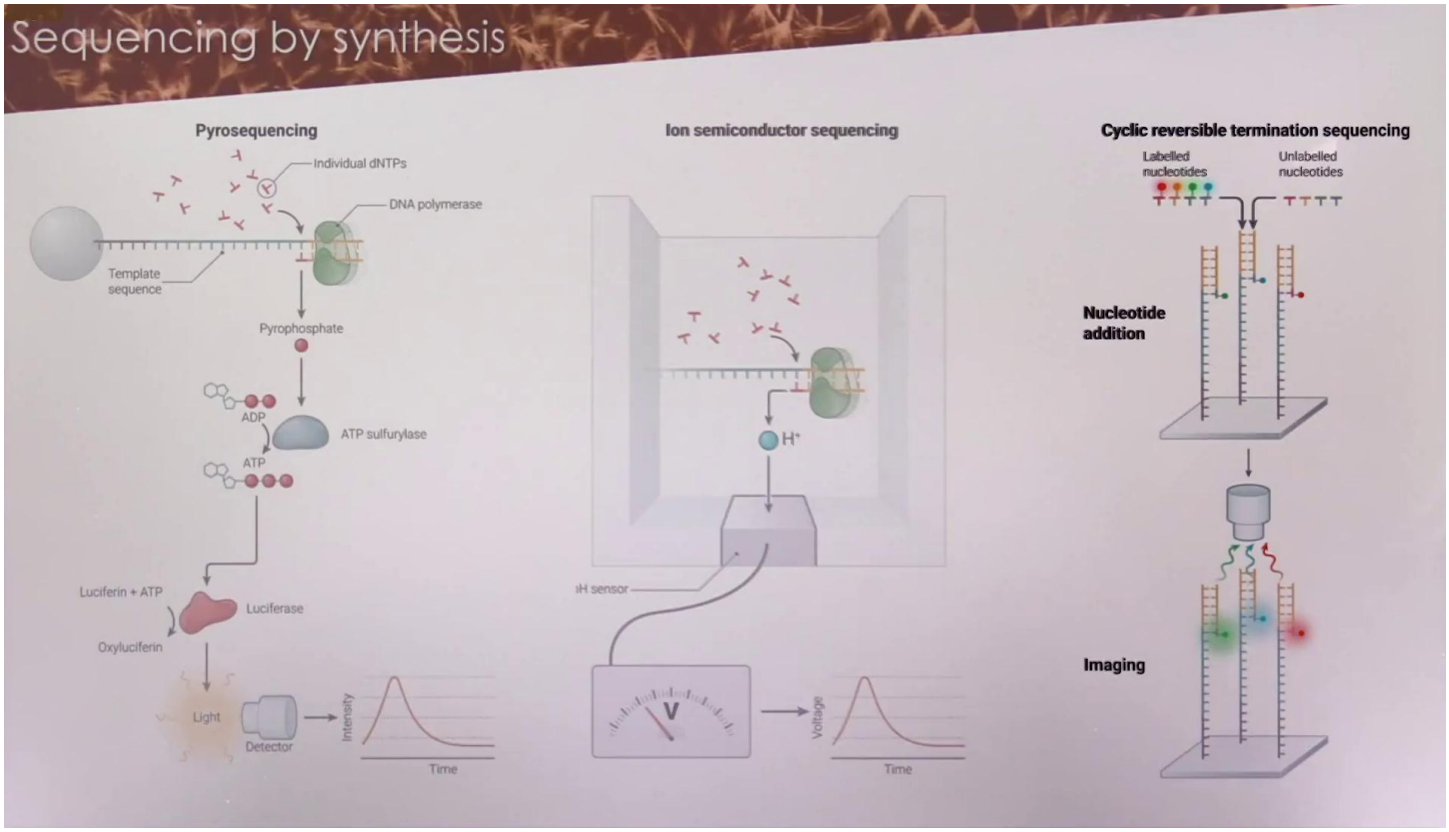


ATP can be then used as a substrate to catalyse the reaction of luciferin into oxyluciferin with emission of light. The light can be detected by detector. But now we have the associated adding into the reaction mix the right base. For example, G, with an emission of light, without, explicitly, a termination step. The same kind of association between synthesis, and the detection band can be achieved, for example, by what is called ion semiconductor sequencing. That uses the same principle, now miniaturising wells coupled with a pH sensor, basically, a semiconductor chip that detects the change of voltage associated with the release of a proton. That is one of the effect of the addition of one of the byproducts of the reaction of the addition of a nucleotide to the strand, to reverse complement strand of our template. Finally, and maybe more importantly, because this is a base technology on which Illumina Sequencer, one of the most popular technologies, nowadays is based on. One can do what is called a cyclic reversible termination sequencing. Again here we go back to the idea of termination but now we will be completely free from the idea of having to run electrophoresis.

Notes

Summary

6m 22s



The idea is that one can form on top of a surface. The surface is a set of clusters where those are spots that are densely packed with the same kind of template that originates from one molecule in the original input for sequencing. We can start synthesis from one extremity of the molecules that are the present of this specific cluster and then that we can do it within termination. Then the old cluster is going to light up quite strongly because a lot of molecules strongly enough that can be detected by a microscope. This allows basically to directly read the sequence onto a 2D surface, on top of which the different DNA molecules have been currently bound.

Notes

Summary

8m 01s