What is Next Generation Sequencing (NGS)?

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Hello, my name is Nirali M. Patel. I am currently an assistant professor of pathology and laboratory medicine at the UNC School of Medicine. Today I'll be talking about next generation sequencing and giving a brief overview of what the technology is.

Where did we get started with sequencing? There are currently many methods of sequencing nucleic acids, both DNA and RNA. Previous methods prior to next generation sequencing included things such as Sanger sequencing. The limits of some of these methods included things like the need for a high tumor content. So up in the upper right-hand side, you see a pink background of what we see as cells under the microscope in a cancer specimen. Circled in black is the actual tumor cells of a patient's lung cancer. As you can see, they're only a very tiny proportion

of all the cells on the slide. Therefore, an old method such as Sanger sequencing would not be able to pick up the DNA from that very small amount of cells in a larger background of the tumor.

In addition, we were only able to look at one specific segment of DNA at a time. For example, in lung cancer we currently need to look at anywhere from 10 to 15 genes to get a comprehensive view of what therapies may be best for the patient. With old methodologies, we could only look at these one at a time, leading to very long turnaround times for test results and a high cost for testing.

Finally, old methods of sequencing were only able to look at the entire group of DNA pieces for a certain segment. This is like me walking into a room and saying, "Hey, half the people in here are guys and half the people are girls." With things like next generation sequencing I can now say, "Yes, half the people in this room are males and they are Bob, Joe, and Steve. The other half are females and they are Tasha, Karen, and Jessica."

How does next generation sequencing do this? Well, its alternate name, also known as massively parallel sequencing, gives us a view. Basically, there's many different, very scientific ways we can do this, but all of these technologies let you see each molecule entirely on its own. You can see in the bottom left-hand corner that each of these molecules has a slightly different sequence. With traditional Sanger sequencing, we would only be able to tell that half of this first letter was T's and half of this first letter was G's. Whereas with next generation sequencing, I can tell exactly what each of these molecules is saying.

Finally, how do we use this vast amount of data to really impact patient care? Well, the ability to individually identify these molecules gives the potential ability to identify multiple types of changes. These are things such as mutations, which is when a small number of nucleotides, or the letters of the DNA, is changed.

A good example of this is regular words. Changing the word “cat” to the word “bat”; it’s a one-letter change, but it still makes a difference in how you see that. Cats and bats are two very different things. You can also detect changes called translocations. So these are when large pieces of DNA (the equivalent of words) change places and combine in new ways. So the sentences here, “Amanda chases rabbits” and “George eats tomatoes” combine to make “Amanda chases tomatoes” and “George eats rabbits.” The effect this has on proteins can be very varied. “George eats rabbits” makes sense. “Amanda chases tomatoes” doesn’t make sense. In the same way, these translocations in cancers can either make proteins that help the cell grow or make proteins that are useless to the cell.

Finally, you can detect copy number changes. This is going from the word “ah!” with one “a” to “AAAAAAAAAAh!” Very, very different outcomes. And so, this is the way we can use next generation sequencing technology to well-characterize tumors.