

Wellcome Genome Campus | OC4_1-21_Sample_quality_control

Hi, I am Paola Niola, and I am an Application Specialist and Teaching Fellow at UCL Genomics. Here, I'm going to talk about the Qubit. You will learn how the Qubit works.

The Qubit is a quantitation method useful to quantify DNA, RNA, or proteins. Quantitation is one of the crucial step of a genomics workflow. And the Qubit is considered to be more accurate compared to the Nanodrop or the Tapestation because it is a quantification method based on fluorescence.

The dye, which is combined with the Qubit buffer, binds specifically the DNA or RNA. When this happens, the dye shows a fluorescence and intensity of the signal is proportional to the concentration of our sample. So higher the signal is, higher the concentration of our sample is.

For this quantification, we need to prepare a working solution using a buffer and the dye, which is DNA- or RNA-specific. Then we combine the working solution together with our samples in two standards, which are used for the machine calibration. When we insert the sample, the Qubit emits light that travels through the solution and is able to excite the dye specifically bound to DNA or RNA.

The amount of fluorescence detected by the machine is then converted in nanograms per microliter providing, a concentration value. So the Qubit is pretty simple to use. It's a good method for quantification. It's more sensitive and accurate compared to other methods, but doesn't give any information about purity and integrity.

So as I said in the previous presentations, this is the reason why it's always better to combine more methods for an accurate and complete quality control of our samples before starting any genomics experiments.

Here, I'm going to talk about the Tapestation. You will learn how the Tapestation works and how to interpret quality control data.

What is that Tapestation? The Tapestation is a modern version of the traditional electrophoresis agarose gel. In other words, it is an automated electrophoresis system. Electrophoresis is a process which separates charged biomolecules in a fluid using a field electrically charged. Based on this process, the tapestation is able to provide information about the size, the concentration, and the integrity of both RNA and DNA.

But how does it work? One of the Tapestation components is the screentape. The screentape is a device where the electrophoresis happens. It has 16 channels. Each of them has a buffer chamber where the DNA or RNA is loaded by the machine, the gel, which is inside the channel, and the electrodes where the electric charge is applied.

As you may know, both DNA and RNA have a negative charge. And when an electric field is applied to the gel, they migrate towards the positive charge and separate based on their size and weight. Based on this, the detection software can calculate and provide information about the size, the concentration, and integrity of our sample.

But now, let's focus on the RNA quality control. For this analysis, we need the RNA ladder, the RNA buffer, our sample, and finally, the RNA screentape. The RNA ladder is a set of known RNA band size that goes from 25 nucleotide up to 6,000 nucleotides. Basically, our RNA sample is compared to the ladder. The ladder is then a reference that is used to assess the size and mass of our sample.

Now, let's see how the tapestation quantifies the RNA sample. This is a Tapestation trace, and it's a typical trace of a good and intact RNA. The y-axis indicates the fluorescence, while the x-axis indicates the length in nucleotide.

When we prepare our sample for the tapestation analysis, we need to combine our sample-- we need to combine a few microliters of our sample together with a fluorescence buffer. Inside the buffer, there is a small RNA of 25 nucleotides. This small RNA is the small peak that you can see in the trace, and it's called lower marker, and it's used as an internal control to align the sample to the ladder.

The two big peaks are the 18 and 28S ribosomal fractions of the total RNA. The ribosomal RNA represents 80% of the total RNA. For this reason, it's used to assess both quantity and quality of the RNA.

Now, having all this information, the Tapestation is able to calculate the concentration. The concentration is calculated based on the area under the peak-- 18 and 28S-- compared to the area under the ladder.

Why the integrity is assessed using the integrity of ribosomal RNA? But why? Because when the RNA is degraded or is starting to degrade, the first portion of RNA to degrade is the 28 subunit, which is usually the longest of the RNAs. The tapestation software assess the integrity, calculating a ratio between 28 and 18S.

And it gives a number. This number is called the RNA integrity number. It's basically a scale that goes from 1 to 10 and tell us how intact the RNA is. Higher the number, better the quality of RNA is.

Now, let's see some examples of RNA Tapestation traces. The right side, we have an example of a trace of an intact RNA. On the left, we have the gel image of different RNA samples. The first column is the ladder, and you can see all the different band size.

The green band at the bottom is the lower marker of 25 nucleotides. The first band is the five subunit of the ribosomal RNA. And around the 2,000 nucleotides, we have the 18S band. And around 6,000 nucleotides, the 28S.

As you can notice from the gel, sample D has a RIN of 5. This means that this sample is starting to be degraded. Therefore, the 28S band is almost gone.

For each sample, the tapestation gives an RNA integrity number. And we can say that sample with a RIN higher or equal to 7 is considered good for any downstream application, like sequencing.

This is, instead, a typical trace of a degraded RNA. From the trace on the right, we can't see any ribosomal peaks because the sample is completely degraded. It's completely fragmented in shorter size.

And in the gel image on the left, we can't see any bands. But we just see a smear of RNA. Therefore, for these samples, the RNA integrity number is around 1.

Now, let's focus on the DNA tapestation analysis. For the analysis, we need the DNA ladder, buffer, our sample, and finally the DNA screentape. Also, here, we have the DNA ladder. The DNA ladder is a set of different and known DNA band size used to assess the size and mass of our DNA sample.

The smallest peak in this trace is the lower marker used as an internal control to align the sample to the ladder. This is a physical trace of an intact, genomic DNA. The y-axis indicates the fluorescence intensity. The x-axis, the length in base pairs.

While the first small peak is the lower marker, and in this case, this concentration is calculated based on the height of the DNA peak, while the integrity is based on how wide the peak is. When the peak is narrow, means that the DNA is intact. Why? When the peak is wider and spread along the base pair axis means that the DNA is chopped in smaller fragments, so degraded.

Also, in this case, that tapestation gives a number to indicate the integrity of the samples. And it's called DNA integrity number.

To summarise, the tapestation is the best method to set the integrity providing the RNA or DNA integrity number which is a scale of how intact the sample is. It provides information about the size, the quantity, and the quality of our sample. But it doesn't give any information about the purity. So we can say that for a better quality control on our sample, we need to combine more methods. Thanks for listening.