Homework #9

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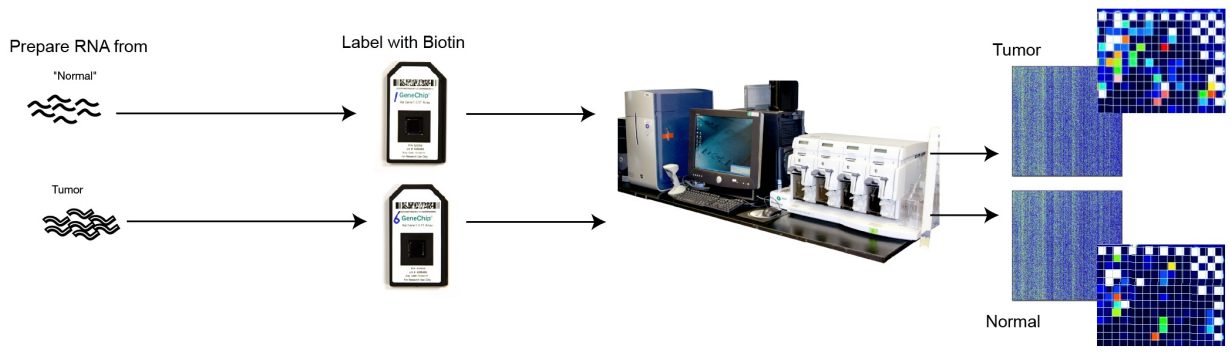
Context

Microarray technology is a technique, first used in 1983, to recognize differences in genes for a particular individual. For example if DNA is analyzed then using this technique it can be determined if an individual has a mutation on the DNA sequence analyzed. This technology is used on single-stranded DNA, ie RNA, and essentially consists of comparing two samples of healthy cells and diseased cells. By comparatively analyzing the two samples, one can identify genes that differ from the RNA of diseased cells (RNA from DNA). This is very important because it can determine the genes that play a key role in a disease.

In the case of a disease caused by a mutation, genes can also be overexpressed or underexpressed, and this can be determined by comparing the analyzed gene with a healthy one. Today this technique is used in clinical diagnosis for a number of diseases: such as cancers. Or to determine which drugs are better for one person than for another, because a mutation involves abnormal behavior of organisms regarding the chemistry of drugs (if that gene is involved). [1, 2, 3]

Technique



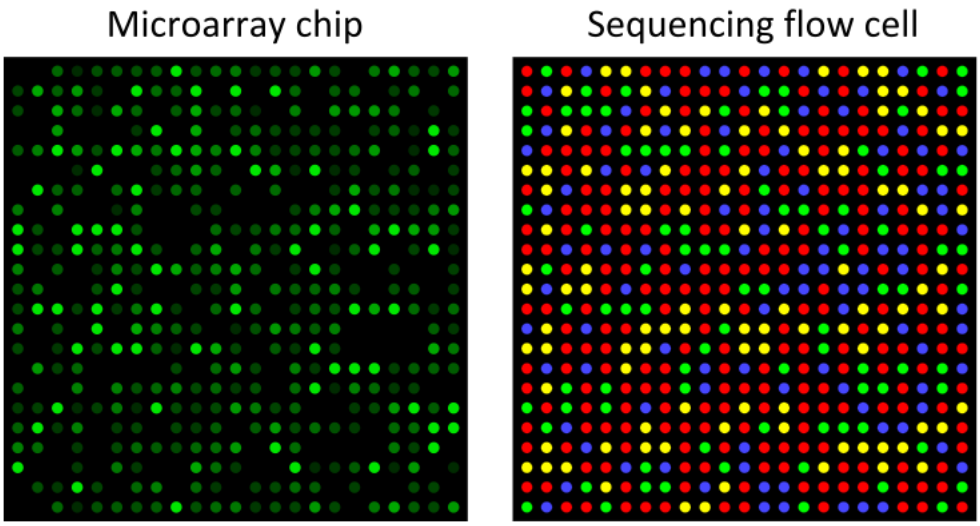
The test begins by taking two DNA or RNA samples: one suspected of being sick - from the patient, and a healthy one, called a control sample (which does not contain a mutation in the gene of interest). If DNA is received, then no problem, it separates into single-stranded DNA, RNA with equivalent information by heating at 90-98 degrees Celsius (the temperature at which the hydrogen chains yield) then cooling. The RNA is then purified with a Chromatin immunoprecipitation protein (eg Trizol) which isolates much of the RNA. As a result of this process, the RNA can be damaged. To study the quality of genetic material, electrophoresis can be done to determine the quality and quantity of RNA. If there is more than 1 microgram of quality RNA then the experiment can continue.

The next step involves cutting the long RNA chain into several pieces of shorter length RNA that are easier to manage (RNA can be multiplied using PCR, this step is optional). The RNA is then labeled as follows: the one from the patient who has just been cut is added to a green fluorescent solution and the one from the control sample (the reference one) is added to a red fluorescent solution (so is the standard option, but there are many ways to do the labeling). The samples are added to a solution that increases the rate of hybridization (functions as an enzyme / catalyst) such as dextran sulfate.

The last step involves inserting these pieces of RNA into specific places in the gene chip, and they are allowed to hybridize (usually 12 hours), then the microarray is washed with citric acid or sodium dodecyl sulfate and left to dry. The microarray is read with laser technology that excites the dyes. After reading of course there is also a step of normalizing the data: the simplest method to do this is to subtract the average and divide by the standard deviation.

If the individual does not have a mutation in the gene of interest then both the green RNA and the red RNA will bind to the samples on the gene chip (normal for a gene that has not moved). But if the individual made a mutation in the gene of interest then the green RNA will bind to the RNA on the moved chip and the red sample (red RNA) will remain unbound. [1, 2, 3, 4]

Manufacturing



A microarray chip looked a lot like a computer chip. And over time, with the development of technology both computer chips have benefited from an increase in the number of features and these chips have increased in number of features (from 10 to 5 million slots) that have the possibility of incorporating as much information as possible from the genome of a species (especially human). They contain, as we specified above, both normal genes (in known places) and mutated genes that have been discovered (in known places). Initially, these tests were also used for sequencing, but now sequencing is done with other techniques, but it remains a cheaper option than sequencing. [2, 5]

References

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