## False negatives: How to tackle them?

In the past, one of the ways to unravel new drug targets was by randomly mutate genomes and then select for different phenotypes. The process was time consuming, expensive and a shot in the dark when it comes to acquire valuable results. In order to overcome this issue, a more focused technique was developed: CRISPR screens. The theory behind this technique is using molecular scissors to introduce mutations in DNA [1].

To facilitate the detection of the mutations, an element of interest is conjugated with a barcode. As in the products of the grocery store, in this technique a barcode is a small specific sequence used to be easily detected during the sequencing. In this way it is possible to find the positive results, i.e. cells with the required mutations. In the specific case of CRISPR screening the element of interest is a small RNA molecule (single-guide RNA - sgRNA) that will recognize the gene of interest.

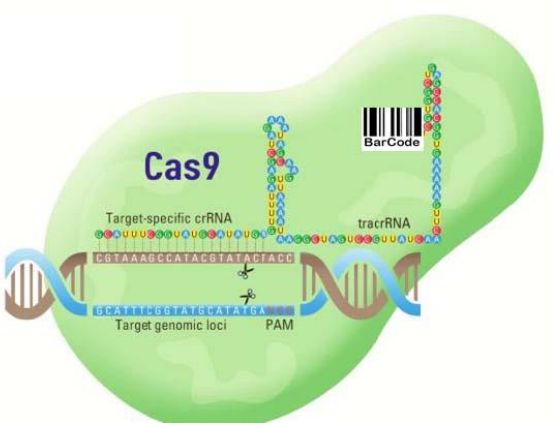


*Figure 1 - Barcode associated CRISPR/Cas9 system. (Adapted from Thermofisher website) [2]*

In this specific case, one of the problems is the occurrence of false negative results, which are caused by the uncoupling of the sgRNA from its respective barcode. The uncoupling can occur in two different phases: during the production of the delivery system and in the mutation-detection phase.

The delivery system used to introduce the CRISPR/Cas9 machinery into mammalian cells is through a lentiviral vector by using its capability to infect cells as a way to insert the molecular scissors into them [1]. The production and infection of the vector leads to the uncoupling problem. Moreover, after the delivery process it is necessary to detect the cells that were successfully infected. The sgRNA-associated barcode detection is performed by sequencing, but first the template has to be amplified by PCR, which can also lead to uncoupling.

To optimize this detection system, the authors suggested that the distances between the sgRNA and its barcode in the vector should be reduced as well as the number of PCR cycles and the amount of templates need to be minimized. By implementing these modifications, the system is more accurate and less prone to the occurrence of uncoupling events, therefore reducing the number of false negatives.

CRISPR screenings have several applications, such as, detecting cancer drug resistance and pathogen virulence, identifying essential genes in cancer lines, understanding gene networks in immune cells, among others [3]. The type of optimization studies presented in this article are crucial for the development of improved screening systems leading to a breakthrough in healthcare.

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