New approaches for viral vector genome integrity evaluation

David Dobnik, Nejc Košir, Rebecca Vollmeier Kovačić, Nejc Jakoš, Mojca Janc, Katarina Bačnik, Polona Kogovšek
National Institute of Biology, Ljubljana, Slovenia

In the production of viral vectors for gene therapy, the emphasis is on pure, safe, and effective products. The absence of impurities and the presence of a complete vector genome play a crucial role. Currently the focus is still on analysing the ratio of full and empty viral vector capsids, although this does not provide information on the actual capsid content. This brings us to one of the emerging attributes of viral vectors, namely the integrity of the viral genome. The recombinant viral genome contains all the information required for the therapeutic effect of the manufactured construct (e.g., promoter, enhancer, gene of interest, polyA tail). If the viral genome is not complete, no therapeutic effect or a much lower therapeutic effect can be expected; moreover, such fragments may generate neoantigens in cells leading to unexpected immune responses. The integrity of the viral vector genome can be assessed by several methods, each of which has its advantages and disadvantages. We have addressed the problem of AAV vector genome integrity by developing an advanced dPCR multiplex approach. We have shown that different conditions in upstream processes can lead to up to fourfold differences in the amount of full-length vector genome. We are also developing other new approaches that do not involve PCR amplification. Existing methods such as dPCR multiplex and long read sequencing will be presented and discussed in parallel with the approaches under development. The goal of all methods is to provide an accurate quantitative result on genome integrity. Their application in sample testing could better guide process development with the goal of obtaining as many full-length vector genomes as possible.