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RECOMBINANT LENTIVIRAL VECTOR PURIFICATION USING ANION EXCHANGE CHROMATOGRAPHY

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Introduction. Over the past two decades, recombinant lentiviral vectors (rLVs) have been widely studied. Scientists have been developing rLVs for genetic engineering. However, challenges related to the manufacturing and purification of this vectors remain unresolved. Therefore, my scientific paper focuses on optimizing the process of purifying recombinant lentiviral vectors.

A recombinant lentiviral vector is based on the genome of the human immunodeficiency virus type 1 (HIV-1) (Family: Retroviridae) and is used in gene delivery. It can transduce dividing and nondividing cells, target tissues and integrate the gene of interest (GOI), thereby providing long-term stable gene expression[1].

rLV is an enveloped viral vector that is produced using mammalian cell lines, such as HEK293 cells.[3] Production of lentiviral vectors is based on transient cotransfection by three plasmids into cell culture. After transfection, there is a cultivation stage when viral particles are being produced. When cultivation is finished, cell culture supernatant containing rLV is sent to purification stage.

The standard viral envelope for pseudotyping remains the glycoprotein of the vesicular stomatitis virus (VSV-G), which recognizes the low-density lipoprotein receptor (LDL-R) for cellular attachment and entry[2].

The main objectives of the purification stage are:

1) Increase titers of infectious rLV particles;
2) Increase yield of infectious rLV particles;
3) Reduce the amount of impurities.

Purification of recombinant lentiviral vectors is a complex multi-stage process that includes various methods: ultracentrifugation, ultrafiltration, tangential flow filtration, precipitation methods, chromatographic methods.

Anion exchange chromatography (AEX) is used for intermediate purification of recombinant lentiviral vectors . This method is carried out in sorption-desorption mode. Lentiviral vector particles carry a negative surface charge in neutral pH and bind on a positively charged matrix. Positively charged groups are most often represented by quaternary amines (QA) or diethylaminoethyl (DEAE) ligands[4]. Bonded particles can be eluted with the use of high salt concentrations from 0,65 M to 1,5 M NaCl[5,6].

Optimization of chromatographic purification of recombinant lentiviral vectors based on the new monolithic anion exchange sorbent is relevant, since this type of chromatography a rapid, reproducible and scalable method.

Main part. Loading buffer is used for applying the sample to the chromatographic column and it is one of the the crucial components of the purification process. The experiments were performed to determine the optimal concentration of NaCl in the loading buffer. Four buffers with different concentrations of NaCl were selected for the study: №1 - buffer A + 0 mM NaCl; №2 - buffer A + 43.33 mM NaCl; №3 - buffer A + 86.7 mM NaCl; №4 - buffer A + 130 mM NaCl. Thus, four sequential chromatographic purification processes were carried out using four buffers with different concentrations of NaCl for loading samples. Fractions from four series of chromatography runs were analyzed to determine the physical and functional titer, HCP, and residual plasmid DNA.

As a result of the experiments, it was determined that:

1) An increase the concentration of NaCl does not significantly affect the sorption of viral particles.

2) An increase the concentration of NaCl contributes to the effective removal of producer cell proteins at the stages of chromatographic purification and, consequently, to a reduced content of HCP in the eluate.
3) An increase the concentration of NaCl does not contribute to the removal residual plasmid DNA. Nucleic acids bind to a positively charged matrix and are eluted together with viral vectors. As a result, high concentrations of residual plasmid DNA are observed in the eluate.
4) An increase the concentration of NaCl contributes to a slight increase in the lentiviral vectors content in the flow-through fraction.
5) The obtained rLV preparations have approximately the same transducing activity, so the increase the concentration of NaCl does not significantly affect the transducing activity of the vector.

Therefore, buffers with a high concentration of NaCl are the optimal buffers for loading the sample into the chromatographic column.

Conclusion. The obtained results indicate that the optimal concentration of NaCl is represented in the number four buffer with the maximum concentration of NaCl. Under these conditions, contaminants such as host cell proteins are removed effectively while maintaining the yield of recombinant lentiviral vectors.

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