DEVELOPMENT OF IMPROVED PRODUCTION AND PURIFICATION PROTOCOLS FOR PRECLINICAL GRADE LENTIVIRAL VECTORS

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Major issues impacting the successful use of lentiviral vectors for preclinical studies in animals include low titers and the lack of standardized methods for titration. Other problems that impact the use of such vectors are poor purity and difficulties related to their large-scale production. We have implemented a number of improvements allowing more efficient lentivirus vector manufacturing. One improvement is related to the efficiency of production of 2\textsuperscript{nd} and 3\textsuperscript{rd} generation lentiviral vectors by transient transfection and the effects of culture additives during virus production. Another improvement concerns the relationships between p24 concentrations, transduction units, integration units, and virus RNA particle titers. A third improvement is related to contaminating host cell proteins. We utilized ultracentrifugation techniques involving density gradients made with Iodixanol to aid in understanding the levels of virus proteins and host cell proteins in lentivirus vector preparations. Finally we developed a scaleable protocol that both can result in viral concentration with little volume limitation, and improved purification from serum proteins and cellular contaminants. We have used Mustang Q anion exchange cartridges (PALL) to concentrate and purify lentiviral vectors from crude cell culture supernatants. Mustang Q chromatography units are capable of replacing large chromatography columns due to the rate of mass transfer to the functional quarternary (Q) ammonium group. Thus, virus binding is not limited by slow diffusion into and out of the column pores because the flow is directly through convective pores. Consequently, the flow rate of Mustang Q chromatography units is very high.