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Salt tolerant endonucleases for the removal of host cell DNA in Downstream Processing of enveloped viruses

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SALT TOLERANT ENDONUCLEASES FOR THE REMOVAL OF HOST CELL DNA IN DOWNSTREAM PROCESSING OF ENVELOPED VIRUSES

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Host cell DNA is a critical impurity in downstream processing of enveloped viruses. For vaccine applications, host cell DNA content should be below 10 ng per dose in the final product. Enveloped viruses exhibit an overall negative net charge on their surface and therefore have binding properties similar to DNA. Consequently, separation of virus from DNA can be cumbersome. In addition to DNA in its naked form, host cell DNA is present in virus preparations in form of chromatin. Chromatin (Figure 1) consists of complex and large structures which include DNA and highly positively charged histones. Therefore, different types of interactions of chromatin with chromatographic material and membranes can be observed, electrostatic interaction through negative charges of DNA and positive charges histones and hydrophobic interaction through hydrophobic patches of histones. Moreover, chromatin is often similar in size to viruses, further complicating their separation. We evaluated the performance of four different endonucleases, two salt tolerant endonucleases and two sensitive to salt, in the downstream processing of recombinant Measles virus. Endonuclease treatment was performed after clarification and followed by a purification step using flowthrough chromatography with Capto™ Core 700 resin. Nanoparticle tracking analysis (NTA) was used to determine size and particle concentration and TCID50 to determine the infectivity of the viruses. DNA and histones presence (in process and purified samples) were determined using PicoGreen™ assay and Western blot analysis using detecting anti-histone antibodies. The salt tolerant endonucleases are more efficient in the removal of chromatin and consequently in the removal of host cell DNA. A 97 % reduction of DNA could be observed.

Application of a salt tolerant endonuclease may reduce costs as they are more efficient and the process gets more robust, because operating at a wide temperature range is possible and endonuclease treatment can be also conducted a low temperature.

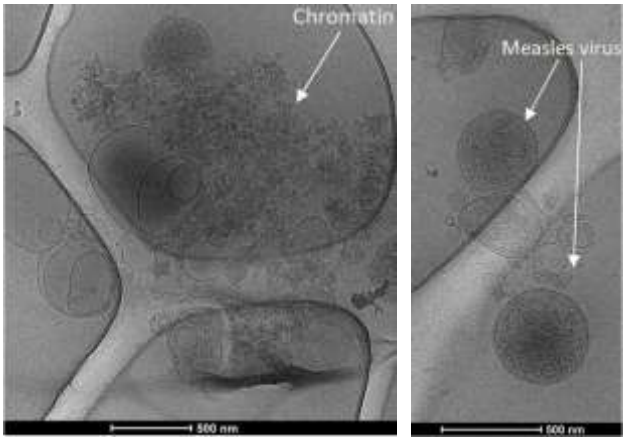

Figure 1: cryo-EM picture of chromatin found in recombinantly produced Measles virus supernatant. On the right, Measles viruses with SARS-CoV-2 spikes are shown for comparison.