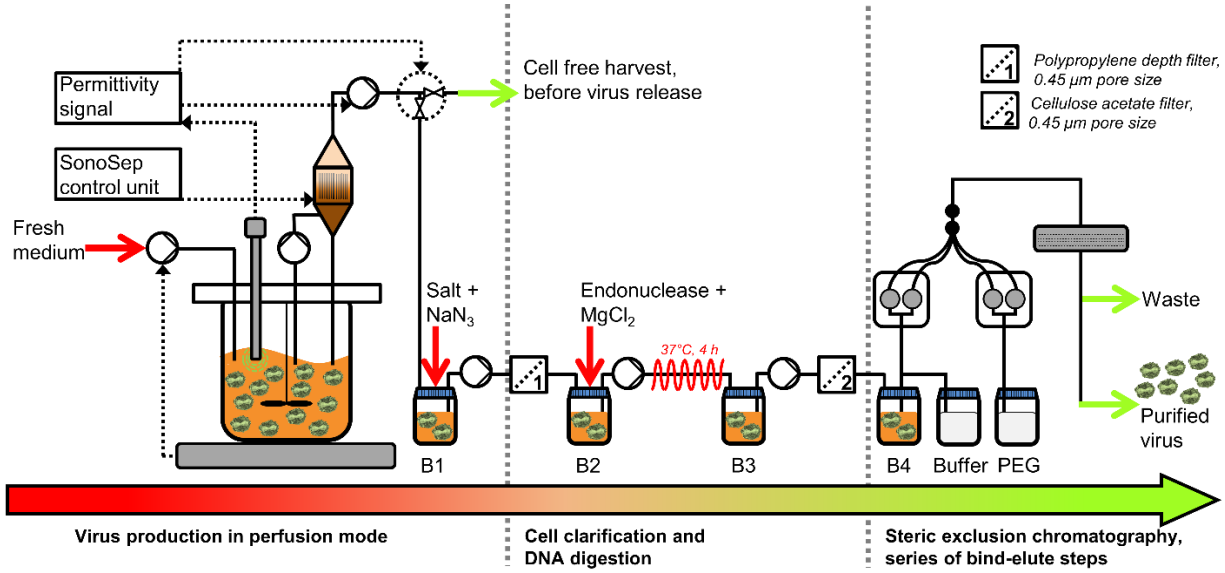

Figure 1: Scheme of an integrated process for cell culture-based virus production in perfusion mode. The integrated MVA production is separated in three main steps, separated by grey vertical dotted lines: 1) Virus production in perfusion mode using an acoustic filter, 2) cell clarification and DNA digestion, and 3) steric exclusion chromatography (SXC) as a series of bind-elute steps. MVA is produced using AGE1.CR.pIX cells grown in suspension in a stirred tank bioreactor. To achieve high cell concentrations, the cells are retained in the bioreactor while cell free medium is continuously harvested through the acoustic chamber controlled by the SonoSep control unit (acoustic filter as perfusion system). To allow a constant bioreactor working volume and weight, fresh medium is added into the bioreactor through a peristaltic pump controlled by a scale. During the cell growth phase, the harvest flow rate is controlled based on the estimation of the viable cell concentration using a capacitance sensor. After infection, a decrease in the permittivity signal indicates virus particle release, and initiates cell clarification and subsequent chromatography steps. The harvest containing MVA (which was first cell clarified through the acoustic settler) is collected into bottle B1. Salt and sodium azide (NaN3) are added to bottle B1 as well. The virus harvest is then continuously filtered through a polypropylene depth filter with 0.45 µm pore size (Filter 1). For continuous endonuclease digestion (addition of endonuclease and magnesium chloride (MgCl2) in bottle B2), the harvest is incubated into a plug-flow reactor (indicated with the coiled red tube) at 37°C with a residence time of 4 h. The endonuclease-digested product is continuously collected into bottle B3. After another filtration step using cellulose acetate depth filter with 0.45 µm pore size (Filter 2), the harvest is collected into bottle B4 at 4°C. An ÄKTA Pure 25 system is used to purify the virus harvest using membrane-based SXC operated in a semi-continuous bind-elute mode; the composition of buffer solutions (including buffer solution with PEG) used in purification are described in section 2.3.3. Finally, purified MVA is collected into 50 mL tubes (not illustrated). The color of the horizontal arrow going from red to green illustrates the stepwise purification of the MVA and the removal of contaminating host cell DNA.