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Lab-Scale Harvest and Clarification of Lentiviral Vectors Using Vacuum Filtration Units

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Abstract

In this study, Sartolab® RF50 and Sartoclear Dynamics® Lab V50 (Sartorius), as well as other manufacturers' filters, named products A, B, and C, were evaluated for clarification of lentiviral vectors produced by transient transfection of HEK293T/17 SF suspension cells. Filtration performance was evaluated regarding the volume filtered, filtration time, filter capacity, flux, turbidity reduction and removal of contaminants, as well as lentiviral vector titer recovery. With products A, B, and C early filter clogging occurred, while with Sartoclear Dynamics® Lab V50 and Sartolab® RF50 complete filtration of the sample volume was possible. Thus, in terms of filter capacity, filtration time, and flux, Sartoclear Dynamics® Lab V50 and Sartolab® RF50 performed best. Moreover, these filter units are most suitable for safe handling given their filter unit design. The highest turbidity reduction was achieved with Sartoclear Dynamics® Lab V50, while Sartolab® RF50 provided the highest recoveries of LV particles and infectious titers.

Introduction

The importance of viral vectors for gene and gene-modified cell therapy has increased in recent years. Because viruses are intracellular parasites, they have evolved as efficient vehicles for DNA or RNA delivery to target cells [1]. The number of clinical trials involving gene and gene-modified cell therapy is increasing steadily, with lentiviral vectors (LV) used in 10.1 % of all gene therapy trials in 2019 and near a half of CAR-T trials [2]. Hence, the development of efficient LV purification methods is required to meet the increasing demand of LV-mediated gene therapy products and CAR-T [3]. Lentiviral vectors are typically produced by transient transfection of HEK293T cells with multiple vector plasmids [5]. The LV is released into the supernatant and requires a process to separate the cells from the LV-containing cell culture liquid [4]. The aim of the clarification process is to eliminate major contaminants and to reduce turbidity of the solution, while maintaining vector activity critical in CAR-T workflows.

To simplify CAR-T research workflows, Sartorius T-Cell Screening Solution offers a semi-automated multiplexed way for the discovery of novel targets and development of efficient CAR constructs (Figure 1). Sartorius T-Cell Screening Solution enables production, optimization, and purification of the viral vector. While laboratory-scale lentivirus harvest often uses centrifugation and clarification with subsequent microfiltration [5], single-step membrane filtration with the use of filter aids like diatomaceous earth (DE) may be more suitable for the CAR-T workflow due to the rapid filtration, safe handling, and preservation of viral activity they enable.

The Sartolab® RF50 with a 0.45 µm polyethersulfone (PES) membrane is ideal for clarification of aqueous solutions. It provides fast flow rates and ensures low protein binding and extractables. The Sartoclear Dynamics® Lab V50 kit contains Sartolab® RF50 filter units and diatomaceous earth (DE). The filter aid consists of fused skeletal remains of diatoms with a highly porous structure [6]. After the upstream process the culture broth is mixed with the filter aid first, then applied on a filtration membrane. DE forms a nearly incompressible porous cake, and its high porosity allows liquid flow around the particles, preventing filter clogging [7]. The use of filter aids enables harvest and clarification of mammalian cell culture in a single step, eliminating the centrifugation step and reducing processing time.

Clarification trials with Sartolab® RF50 units and the Sartoclear Dynamics® Lab V50 kit were compared to other commercially available filters on the market (products A, B, and C). Except for the Sartoclear Dynamics® Lab V50 kit, harvest and clarification were performed by a centrifugation step and subsequent filtration of the supernatant. Filtration performance was evaluated regarding the volume filtered, filtration time, filter capacity, flux, turbidity reduction, removal of contaminants, as well as recovery of lentiviral vector particles and infectious titers.

Materials and Methods

The lentiviral vector was expressed by transient transfection with four plasmids using HEK293T/17SF cells cultivated in suspension by means of an Ambr® 250 bioreactor system (Sartorius). The plasmids, transfection method and cultivation parameters used are described by Labisch et al. [12].

The Sartolab® RF50 filtration units (Sartorius, 180F01--------2) consist of a funnel, a 50 mL conical tube and a tube connector for vacuum connection. The filter is made of a 0.45 µm PES membrane. Centrifugation of 50 mL cell culture broth was performed at 800 x g for 5 min.

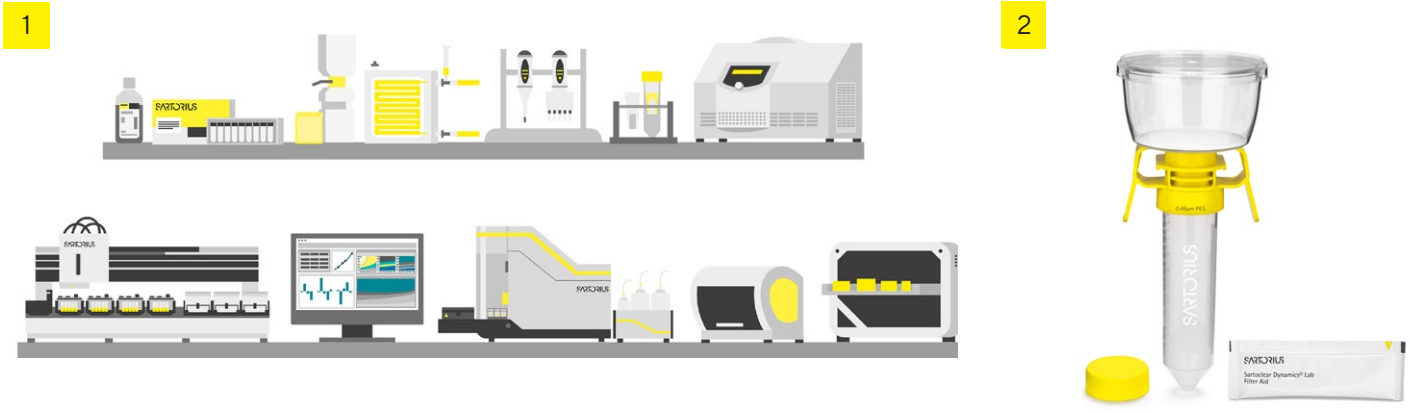

Figure 1: Sartorius T-Cell Screening Solution to enable discovery of novel targets and development of efficient CAR constructs (1). Sartoclear Dynamics Lab® V50 to rapidly clarify lentivirus, while maintaining vector activity (2).

Afterwards, the supernatant was clarified using a Sartolab® RF50 filtration unit or a different manufacturer's filter with the same pore size, named product A, B or C. The Sartoclear Dynamics® Lab V50 kit contains Sartolab® RF50 with a 0.45 µm PES membrane and DE. The kit is supplied with a choice of standard quantities of 1 g of DE (Sartorius, SDLV-0050-01F0-2) or 2 g of DE (Sartorius, SDLV-0050-02F0-2). A DE concentration of 5 g/L was used in the trials. Therefore, the respective amount of DE was added to 50 mL of the cell culture broth and mixed to obtain a homogeneous suspension first, then immediately passed through each of the filters. Vacuum filtration with Sartorius filters was performed with the Sartolab® MultiStation (Sartorius, SDLC01), which is a stand specially designed to hold one to six Sartolab® RF50 vacuum filtration units, allowing simultaneous filtration of up to six samples. Each filtration trial was performed in triplicate.

To determine the clarification performance, the following parameters were determined according to the suppliers' respective instructions: turbidity (Orion™ AQUAfast AQ3010 turbidity meter, Thermo Fisher Scientific); total dsDNA concentration (Quant-iT™ PicoGreen™ Assay, Thermo Fisher Scientific); and total protein concentration (Pierce™ Coomassie Bradford protein assay kit, Thermo Fisher Scientific). Measurements were performed before and after the respective filtration step. Furthermore, the LV particle titer was determined by performing p24-ELISA (QuickTiter™ Lentivirus Titer Kit, Cell Biolabs) according to the suppliers' instructions. The infective lentiviral particle titer was measured by transduction of adherent HEK293T cells with virus samples and measurement of transgene expression (green fluorescent protein) 32 h post-infection by a real-time imaging approach with the IncuCyte® S3 Live-Cell Analysis System (Sartorius).

Results and Discussion

Filter Performance und Handling

Harvest and clarification were performed using Sartolab® RF50 0.45 µm PES, the Sartoclear Dynamics® Lab V50 kit with 5 g/L DE or a different manufacturer's vacuum filter, named product A, B or C. Upon harvesting the cell culture, the cell density measured was 1.52 x 10^6 cells/mL and the turbidity was 109 NTU.

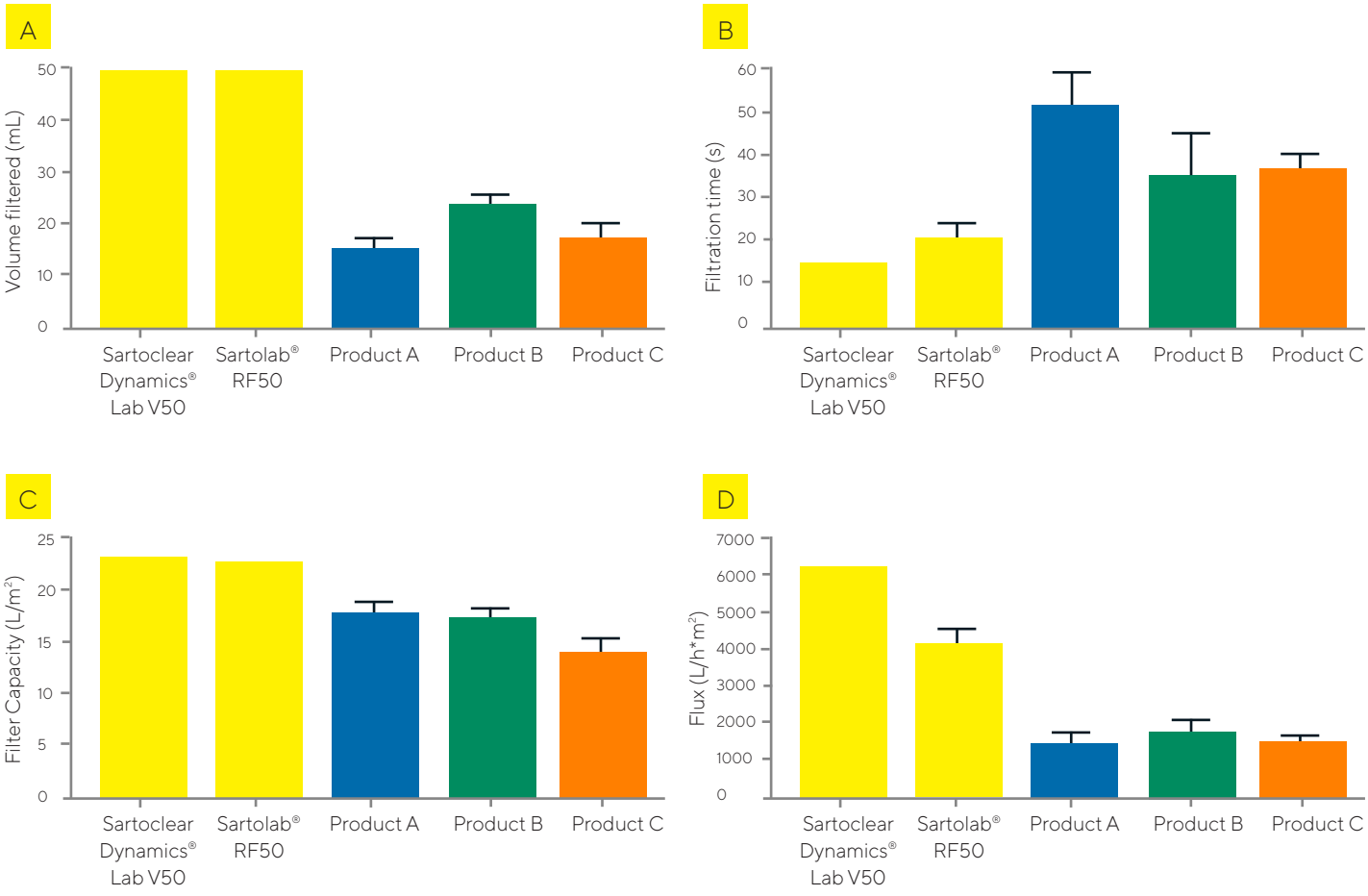

Figure 2: Filtration performance of Sartoclear Dynamics® Lab V50, Sartolab® RF50 and products A, B, and C. A: Volume filtered until filter clogging occurred (max. volume 50 mL); B: Filtration time (until filter clogging); C: Filter capacity given in L/m²; D: Flux given in L/m²h. Shown here are the mean values of trials performed in triplicate, as well as the respective standard deviations.

Clarification with products A, B, and C resulted in early filter clogging: after 14 mL with product A, 24 mL with product B, and after 20 mL with product C (Figure 2A). In contrast, the entire 50 mL of LV solution was filtered by Sartoclear Dynamics® Lab V50 and Sartolab® RF50. The use of products A, B, and C would require higher usage of lab consumables to filter the same quantity of LV material. The filtration time was measured until the filter became clogged or until the entire volume was completely filtered. The fastest filtration run of 14 s was obtained using Sartoclear Dynamics® Lab V50, followed by that of 20 s using Sartolab® RF50 (Figure 2B). Filtration with products A, B, and C took between 38 s and 45 s until filter clogging occurred. The filter capacity was calculated based on these results. Products A, B, and C had a filter capacity of 19.5, 18.8, and 14.7 L/m², respectively (Figure 2C). The 23.8 L/m² that was achieved with Sartoclear Dynamics® Lab V50 and Sartolab® RF50 does not reflect the maximum filter capacity as these filters did not become clogged during the experiment. Therefore, the maximum filter capacity (throughput) was determined independently in a further experiment at 63.5 L/m² when an LV batch with 3.7×10^6 cells/mL and a turbidity of 398 NTU at the time of harvest were used [8]. Therefore, the loading capacity of Sartoclear Dynamics® Lab V50 was at least three-fold higher compared with all of the competitor products. The highest flux of 6,120 L/h·m² was calculated for the Sartoclear Dynamics® Lab V50, followed by 4,180 L/h·m² for Sartolab® RF50, and between 1,390 L/h·m² and 1,820 L/h·m² for products A, B, and C (Figure 2D).

The design and handling of the filtration units was evaluated in Table 1 with a focus on their suitability for clarification of lentiviral vectors classified as biosafety level 2.

The use of Sartoclear Dynamics® Lab V50 reduced the handling time as it eliminates the need for a centrifugation step and therefore facilitated fast handling. The handling of products A and B was evaluated as very unsafe due to the high risk of spillage, which is a great risk for the operator since lentiviral vectors are classified as biosafety level 2. By contrast, Sartolab® RF50 features an excellent filter device design that enables safe handling by minimizing the risk of spillage of the virus material.

Filter	Design	Design Score	Handling	Handling Score
Sartoclear Dynamics® Lab V50 0.45 µm PES	Material looks well processed; large filter area; multi parallel usage possible together with MultiStation	***	Large funnel →Safe handling DE eliminates centrifugation step → Reduces handling time	***
Sartolab® RF50 0.45 µm PES	Material looks well processed; large filter area; multi parallel usage possible together with MultiStation	***	Large funnel → Safe handling; centrifugation step required	**
Product A	Small filter area; no parallel filtration of multiple units possible	*	Virus solution must be inverted → Risk of spillage if the 50 mL tubes are not screwed on correctly. After filter clogging occurs, the partially filled 50 mL tube must be unscrewed → Risk of spillage → Unsafe handling for S2 material; centrifugation step required	*
Product B	Small funnel; no parallel filtration of multiple units possible	*	Small funnel →Spillage of the virus solution possible when filtering 50 mL → Unsafe handling for S2 material; centrifugation step required	*
Product C	No parallel filtration of multiple units possible	**	Sufficiently large funnel → Safe handling; centrifugation step required	**

Table 1: Design and handling of vacuum filtration units tested for safe handling with lentiviral material. *** gives the highest score.

Removal of Impurities and Lentiviral Vector Titer Recovery

The turbidity was measured before and after clarification to calculate the reduction in turbidity.

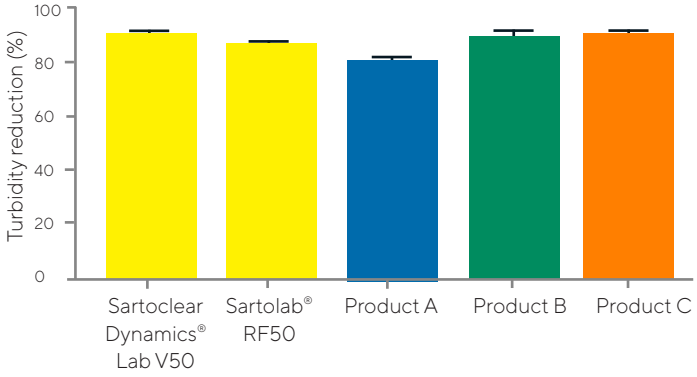

Figure 3: Turbidity reduction after clarification of the LV supernatant with Sartoclear Dynamics® Lab V50, Sartolab® RF50 and products A, B, and C. Shown are mean values of triplicates with standard deviations.

The greatest turbidity reduction of about 90 % was achieved using Sartoclear Dynamics® Lab V50 as well as products B and C (Figure 3). The lowest turbidity reduction, 80 %, was observed for product A.

Another major aim besides product recovery is to remove contaminants. The efficiency of impurity removal was determined by measurement of the total protein content and the total dsDNA amount before and after clarification.

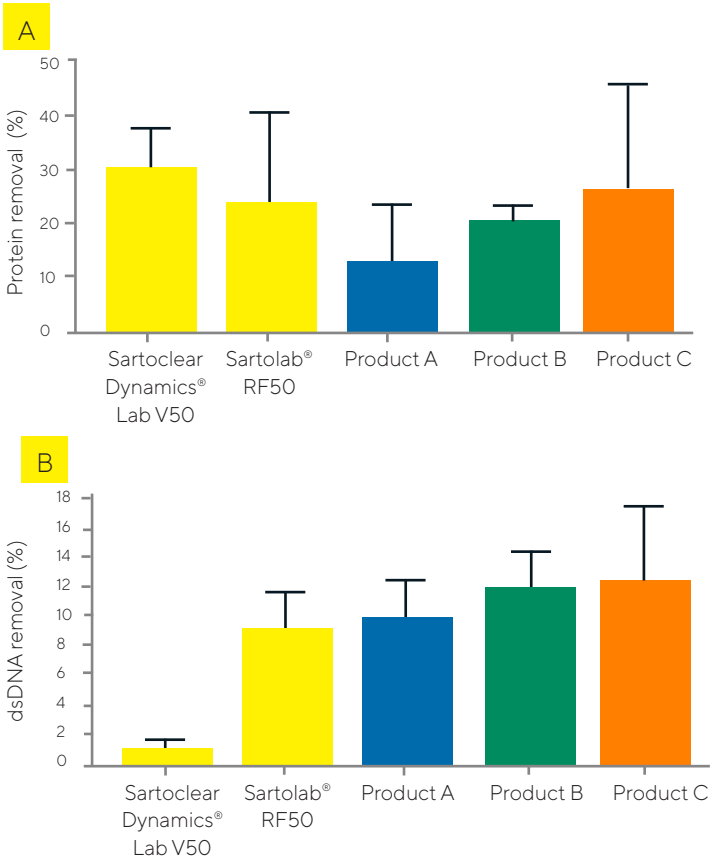

Figure 4: Protein (A) and dsDNA (B) removal after clarification of the LV supernatant using Sartoclear Dynamics® Lab V50, Sartolab® RF50, and products A, B, and C. Shown here are the mean values of the trials performed in triplicate, along with their respective standard deviations.

The protein concentration of the centrifuged non-filtered material was 74 µg/mL, and the dsDNA concentration was 130 ng/mL. Removal of protein was best using Sartoclear Dynamics® Lab V50, Sartolab® RF50 and product C, in a range of 25 - 30 % (Figure 4A). The highest removal of DNA was achieved with products B and C, by about 12 % (Figure 4B). The lowest DNA removal was observed for Sartoclear Dynamics® Lab. A reason could be that a centrifugation step removes DNA more effectively than the filtration step itself, since with Sartoclear Dynamics® Lab V50, no centrifugation was performed.

The most important objective, however, is to recover the product of interest, the lentiviral vector. The LV titer can be measured by considering all LV particles or by measuring the concentration of infectious particles.

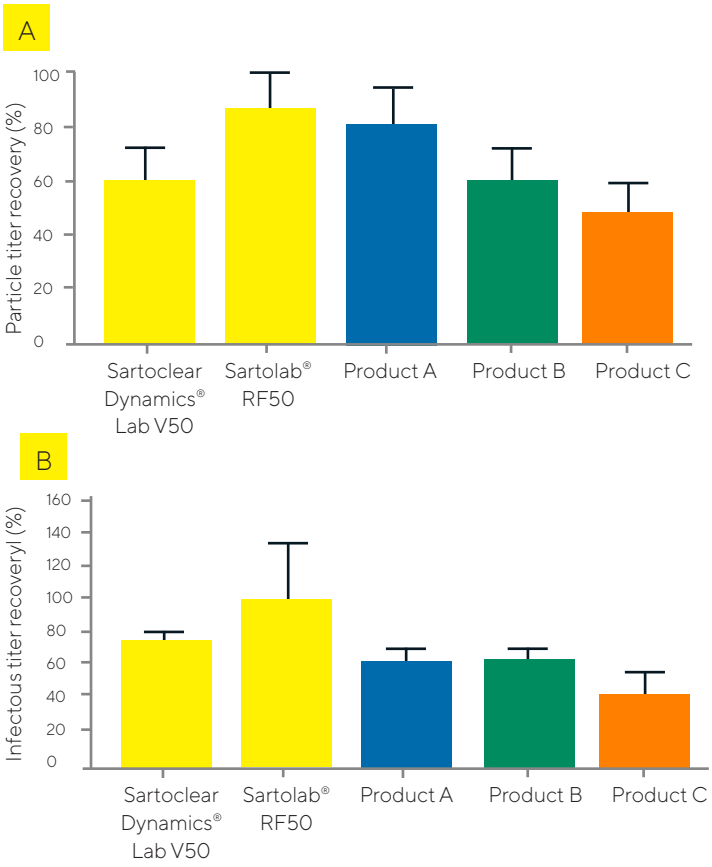

Figure 5: Particle titer recovery (A) and infective LV titer recovery (B) after clarification with Sartoclear Dynamics® Lab V50, Sartolab® RF50, and products A, B, and C. Shown here are the mean values of the trials performed in triplicate, along with their respective standard deviations

Sartolab® RF50 achieved the best results in lentiviral particle recovery at 87 % (Figure 5). Similar particle recoveries were obtained with product A. Particle recovery after clarification with Sartoclear Dynamics® Lab V50 was 62 %. The infective titer was fully recovered using Sartolab® RF50. With Sartoclear Dynamics® Lab V50, 75 % of the infectious virus particles were recovered. The infectious titer recovery with product A, B, or C was between 39 % and 63 %.

Conclusion

Since filter clogging occurred while clarification was performed with products A, B, and C, the filter units of choice are Sartolab® RF50 and Sartoclear Dynamics® Lab V50 as they both have a greater filter capacity, a higher flux and faster filtration performance. The loading capacity of Sartoclear Dynamics® Lab V50 is at least three-fold higher compared with all of the competitor products. Moreover, on the basis of their filter unit design, Sartoclear Dynamics® Lab V50 and Sartolab® RF50 are best suited for safe handling of lentiviral vectors, which are classified as biosafety level 2. Turbidity reduction was best with the Sartoclear Dynamics® Lab V50 (90 %), while the use of the Sartolab® RF50 resulted in an LV particle titer recovery of 87 % and an infectious titer recovery of 100 %. These findings suggest Sartoclear Dynamics® Lab V50 and Sartolab® RF50 as filtration units of choice for LV clarification in gene and gene-modified cell therapy applications.

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Abbreviations

(ds)DNA	(Double stranded) desoxyribonucleic acid
DE	Diatomaceous earth
HEK	Human embryonal kidney cells
LV	Lentiviral vector
PES	Polyethersulfone
RNA	Ribonucleic acid
TU	Transducing units
VP	Viral particles

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