CELL & GENE THERAPY INSIGHTS

LATEST DEVELOPMENTS IN VIRAL & NON-VIRAL VECTOR MANUFACTURING

SPOTLIGHT

EXPERT INSIGHT

Challenges and progress in lentiviral vector bioprocessing

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Cell and gene therapies provide potential treatment alternatives to conventional medicine, opening a new era of targeted medicine for patients with cancers and rare diseases. Chimeric antigen receptor (CAR) T-cell therapies, some of which have been granted FDA approval, and TCR T-cell therapy, amongst which specific peptide enhanced affinity receptor (SPEAR) T cells are in Phase 1–2 trials, are two immunotherapy options that are shaping the future of medicine. In both CAR and TCR T-cell therapies, a patient's own T cells are engineered *ex vivo* to express the therapeutic gene, often using lentiviral vectors. As a consequence, both approaches face similar process challenges in getting treatment to patients. Herein, we examine challenges and progress in lentiviral vector bioprocessing.

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INTRODUCTION

Retroviruses, including the lentivirus subfamily, have two single-stranded RNA (ssRNA) genomes of 7-11 kilobases packaged in lipid-enveloped viral particles of ~80–120 nm in diameter [1–3]. Complex retroviruses, such as lentiviruses, can transduce quiescent cells and exhibit reduced insertional mutagenesis. These properties have led to lentiviruses becoming increasingly popular tools in the gene therapy field [4,5]. Lentiviral vectors (LVs) have the ability to stably integrate into the target cell genome, allowing persistent expression of the gene of interest, and they can also transduce post-mitotic cells and accommodate larger transgenes than γ-retrovirus vectors [4,5].

The ability of LVs to transduce quiescent and post-mitotic cells makes them very useful for gene therapy applications [6]. In addition, self-inactivating LVs appear to have reduced genotoxicity and an

improved safety profile compared to other vector systems [5]. LVs also have a safety advantage due to the lack of innate and cellular responses against vector-associated proteins [7]. Historic safety concerns about establishment of replication competent lentivirus (RCL) that required a lengthy and expensive product release assay have been substantially mitigated by current LV designs and substantial patient experience. The US Food and Drug Administration (FDA) issued a Guidance in July 2018 concerning RCL that includes an approach for elimination of RCL product release testing [8].

There is growing interest in the use of LVs, particularly for cancer immunotherapy and the treatment of monogenic diseases. Manufacturing of LVs can be challenging, primarily due to cytotoxic effects of LV components resulting in lower cell culture titers and LV instability leading to low purification yields. In addition, currently used processes are typically not easily scalable, as they rely on adherently cultured cells. LV manufacturing is associated with high cost of goods (COGs), and clinical trials have highlighted the elevated cost of LV production as one of the main challenges in gene therapy [9–11]. LV manufacturing mostly relies on the transient transfection of the packaging plasmids and therapeutic gene plasmid into a host cell to be able to package LV particles. Transient transfection offers significantly reduced development times and increased flexibility for LV manufacturing compared to the generation of stable packaging and producer cell lines, but it increases batch-to-batch variability and cost of manufacturing [12]. Moreover, the majority of reported protocols rely on transient transfection of adherent cell lines [13], whilst a more desirable approach is transient transfection of suspension-adapted cell lines, due to the ease of scale-up and the advantages associated with culture in controllable bioreactors [12,14].

UPSTREAM CHALLENGES: ADHERENT APPROACH

Adherent human embryonic kidney 293T (HEK293T) cells have been widely used as a mammalian system to rapidly supply LV to Phase 1 clinical trials. LV particles have been generated by the co-transfection of packaging and transgene plasmids in HEK293T [15]. The common method of separating constructs to three or four plasmids is used to reduce the possibility of recombination to replication-competent virus [16].

To date, LV material for clinical application has largely been generated by adherent methods [9]. Bioreactor designs compatible with adherent cell growth typically require scale-out methods, in which a unit operation is replicated to increase volume, rather than scale-up strategies for increased production, in which a unit operation is simply increased in size to augment production volumes. This approach leads to labor intensive processes that are unsuitable for large-scale production [17]. Most developers are using adherent processes, as these are the processes that have been established in Good Manufacturing Practice (GMP) facilities. Progressing a clinical candidate that uses LV produced by adherent means is not impossible, but it creates a manufacturing capacity burden and drives non-optimal COGs. This approach

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is expensive and laborious; however, it does not have the complexity of a biologics process in bioreactors. Therefore, the adherent approach can be developed somewhat more simply and rapidly and can serve the needs of small, Phase 1 clinical trials adequately. Although adherent Cell Factory™ processes can be set up easily, they are inherently less robust and less controlled than processes in bioreactors. This approach carries a higher risk for deviations and batch-to-batch variability. Adherent processes may require a high number of aseptic manipulations during manufacturing, increasing the opportunity for contamination and operator error. Moreover, clean room space requirements for such processes is likely to be larger than bioreactor processes, which contributes to an increased cost per batch.

Packed or fluidized bed bioreactors and fixed bed bioreactors have been described as systems for LV production [18]. Hollow fiber bioreactors have also been investigated for LV production but are associated with diffusion gradients among other disadvantages. The use of a hollow fiber bioreactor for LV production was recently reported with LV titer comparable to that from Cell Factory™ systems [19].

A limitation of packed bed, fixed bed and hollow fiber bioreactors is their limited scalability [18]. An exception is the iCELLis fixed bed bioreactor, which can be used for LV manufacturing in single-use mode with a maximum culture area of 500 m^2 [20]. These types of bioreactors allow anchorage-dependent cells to adhere to a fixed bed core that is continuously fed with fresh media using a perfusion approach, in this way replacing media nutrients to maintain cell viability during LV production. Fixed-bed bioreactors, although interesting for Phase 2/3 clinical trials, may still present challenges in regards to how the inoculum is generated and the use of serum-containing medium. Thus, careful considerations should be made for the inoculum strategy, perhaps inoculating the fixed-bed reactor at low density to minimize the Cell Factories™ needed for the inoculum. Moreover, serum remains an issue, not only for its limited supply but also for its batch-to-batch variability and the risk of using animal sourced components due to adventitious agents, including transmissible spongiform encephalopathies (TSE).

UPSTREAM CHALLENGES: SUSPENSION APPROACH

Unlike adherent processes, suspension processes have the ability to scale up in a single unit operation, making it easier to produce larger amounts of LV and reducing COGs and labor costs in a clean room. Moreover, concerns regarding TSE due to the use of bovine products have been removed by the use of serum-free chemically defined media. Suspension bioreactors grow suspension-adapted cells using stirred impellers to promote air mass transfer, allowing greater numbers of cells to grow in a 3D environment compared to adherent formats, where cells grow in a monolayer or on beads and become rapidly confluent. Size of reactors can scale up to thousands of liters, making this option viable for commercial applications.

Other suspension methods, such as the rocking motion bioreactor, have also been trialed for LV production. The rocking motion

bioreactor has a more gentle hydrodynamic environment and bubble-free aeration, which are beneficial for production of shear-sensitive LVs [21,22]. However, scalability of these types of reactors are limited to few hundred liters of LV harvest.

Running bioreactors has its own set of challenges. Bioreactors are complex and require skilled operators. There have only been a few reports of LV production from suspension-growing cells, and as such, there are still unanswered questions on the performance of these processes for LV manufacturing [12,14,21–25]. Although maximum viral titers were in the order of 10^7 TU mL^{-1} or above, demonstrating scalability in stirred tank reactors (STRs) and the validity of using suspension mammalian processes for LV manufacturing, there is still little experience using suspension-derived GMP-manufactured LV in clinical trials as opposed to the conventional adherent format [26–28].

Another area that requires careful consideration when scaling up and characterizing suspension bioreactor processes is the engineering fundamentals. Considerations will include whether to scale the bioreactor impeller agitation by power per unit volume (P/V) or tip speed and identifying bubble size, KLa coefficient of gas mass transfer and a suitable and scalable gassing strategy that can replicate conditions across bioreactor scales. LV stability is dependent upon a number of physicochemical conditions. Therefore, critical physicochemical parameters need to be established in the bioreactor system, such as temperature, pH, dissolved oxygen and media and supplements to be added during growth and production phases in the bioreactor. Both engineering fundamentals and physicochemical parameters play an intricate and in some cases interactive role in building a scalable and robust process. Thus, application of design of experiment modalities can aid in identifying Critical Process Parameters (CPPs) and how they affect LV's Critical Quality Attributes (CQAs). CQAs for a LV, when used as a starting material for *ex vivo* cell therapy application, typically refer to the biological infectivity of the LV, its potency in transducing T cells and the particle to infectivity ratio that highlight the quality of the LV preparation used in the T-cell process.

DOWNSTREAM CHALLENGES

The most significant challenge for upstream processing is the low product titers, whereas the challenge for downstream processing is maintaining biological infectivity and maximizing the recovery of the physical particles. There are a number of reports that indicated how LV bioprocessing is influenced by LV characteristics [17]. For example, components present in the cell culture media reduced functional titer [29], host cell-derived impurities inhibited cell transduction [3], or temperature affected LV infectivity [30]. The mechanisms of LV inactivation are poorly characterized, and, as a result, technical solutions are largely unknown and require further investigation and may need to be empirically understood during process development activities.

Other considerations include the level of impurities generated by a suspension system in bioreactors, their effect on clarification

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and purification of LV, and their impact on overall yield. There are uncharacterized impurities, such as chromatin impurities, that need attention [31]. There are host cell, DNA and other cell-membrane impurities that could interfere with LV purification.

Technology solutions are primarily aimed at production and purification of recombinant proteins; as a result, total downstream recoveries are typically low and variable in the setting of LV bioprocessing [11,32]. Another contributing factor to downstream recoveries being lower than in conventional protein purification is the absence of targeted affinity purification methods aimed to increase LV purification recoveries. Chromatography-based methods have been explored as a large-scale purification option. There is growing interest in the use of monolithic resins and membrane adsorbers for virus bioprocessing [33]. Monolithic adsorbents are highly porous, allowing mass transfer to occur predominantly by convection, which results in retention of dynamic binding capacity and resolution at high flow rates [33–35]. Most viruses carry a net negative charge at neutral pH [36], which makes purification of LVs by anion exchange chromatography a suitable option. Both strong and weak anion exchange ligands have been used with similar recoveries and purification factors [37]. Heparin affinity chromatography has also been employed for LV purification, achieving high yields and low impurity profiles [14,38].

Another downstream challenge is streamlining the concentration of LV without loss of functionality. Loss in functional virus has been ascribed to hydrodynamic shear forces, long processing times and co-concentration of virus with inhibitors of transduction. Concentration of the feed-stream at an early stage during downstream processing is beneficial, as it reduces the feed volume for subsequent steps [3]. Moreover, ultrafiltration (UF) allows for the use of gentle processing conditions in contrast to centrifugation and precipitation, and it does not require a change of phase [3]. UF is scalable with a potentially high purification factor, and it can be used for diafiltration for buffer exchange or formulation [39].

SUPPLY CHAIN CHALLENGES & A FULLY INTEGRATED CMC STRATEGY

The complexity of LV GMP manufacturing starts from plasmid manufacturing and ends in the release of a GMP LV lot. This complexity can be reduced by employing the right expertise and support functions, especially in relation to supply chain and stock control. It requires a fully integrated CMC team with functions such as Quality, Manufacturing and Regulatory to execute a CMC strategy that drives a therapeutic candidate from discovery to market.

Plasmid manufacturing is likely to be outsourced to contract manufacturing organizations (CMOs), and the management of those can be costly and time consuming. Plasmid manufacture can take up to three months to fully release for use in LV production. Plasmid manufacturing slots need to be agreed upfront and need to be linked with both internal and external plasmid forecasting for both developmental

and GMP work. This in itself can be very challenging, as flexibility must be maintained as direction of work can change in medium-sized organizations and therapeutic candidates' priority can change dependent on clinical results. Formulating a risk-based approach to bioprocessing allows medium-sized biotech companies to be savvy in their approach through clinical stages, informing how to best position the workforce and their expertise.

CMOs can offer supply of compliant LV for clinical trials. However, facilities that provide the correct grade of clean rooms to manufacture cell and gene therapy products are scarce, and when available, they come with high costs. Building HVAC clean rooms with all the auxiliary QC, warehouse and microbiology infrastructures is a large capital expenditure for small to medium-sized biotech companies. For LV GMP manufacturing, in-house capabilities allow a more streamlined CMC approach to 'marry' security of LV supply to the needs of the clinical trials without relying on external organizations.

A CMO removes the risk of capital investment early in the development lifecycle, when novel therapeutic products are being developed. Additionally, if only a limited number of lots per year are required, a CMO option may be more cost effective than running an in-house manufacturing facility not at full capacity. The advantage of having an in-house manufacturing capability is that it allows more flexibility and control of manufacturing slots solely dedicated to the product pipeline without having to rely and align with a third party's manufacturing slots. When LV production is performed internally, the process of controlling capacity to meet the needs of the company are more straightforward, technology transfer activities are more streamlined and protection of the process know-how is easier. Moreover, having in-house capabilities will likely be more cost effective if the facility is used at full capacity.

There are initiatives that offer a 'hybrid' approach in which it is possible to lease out a clean room module. This model reduces capital risk upfront, controlling both the capacity and technology transfer internally and retaining know-how. However, fixed costs are still a commitment that needs consideration.

Another important mechanism for success is the choice of technology. Therefore, the equipment purchased must match the efforts performed at small scale as well as what will be required for GMP scale manufacturing. Before starting the process of installation qualification, operational qualification and performance qualification, if acquiring a new piece of equipment, design specifications will be required. The logistics for bioreactor purchase, delivery, commissioning and regular maintenance in GMP clean rooms can be cumbersome, with long lead-times. Another aspect to consider is the long lead times for consumables, especially for single use bioreactors. All of these steps must be considered, as they can impact clinical timelines.

CONCLUDING REMARKS

LV bioprocessing is likely to move towards less transient and more stable suspension approaches of manufacture [40–43], reducing COGs and batch-to-batch variation and

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improving the DSP challenge by producing more consistent feed streams. Continuous harvest of LVs should be achievable in both fixed bed and suspension bioreactors, as already shown by employment of bioreactors operated in perfusion mode [26,44,45]. Moreover, VSV-g specific affinity chromatography for LV purification are gaining traction and should become available in the future [46,47]. This will not only enhance downstream recoveries but also have an impact on COGs, lowering the cost per dose and making cell therapy products more accessible to patients. The use of low shear methods, chilled product steps and the product stabilizers will inevitably increase LV yields. Continuous processing, both upstream and downstream, will not only reduce processing times but also intensify LV productivity in future. In-house LV manufacturing is likely to take precedence over CMO LV manufacturing as cell and gene therapy companies move towards Phase 2/3 clinical trials allowing flexibility and security of LV supply for clinical trials.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

The authors are full time employees of Adaptimmune, who have stock options. They have no other relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock options or ownership, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

REFERENCES

1. Vogt VM, Simon MN. Mass determination of Rous sarcoma virus virions by scanning transmission electron microscopy. *J. Virol.* 1999; 73: 7050–5.
2. Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. *Nat. Med.* 2001; 7: 33–40.
3. Segura MM, Kamen A, Garnier A. Downstream processing of oncoretroviral and lentiviral gene therapy vectors. *Biotechnol. Adv.* 2006; 24: 321–37.
4. Lewis P, Hensel M, Emerman M. Human immunodeficiency virus infection of cells arrested in the cell cycle. *EMBO J.* 1992; 11: 3053–8.
5. Mátrai J, Chuah MKL, VandenDriessche T. Recent advances in lentiviral vector development and applications. *Mol. Ther.* 2010; 18, 477–490.
6. Naldini L. *Ex vivo* gene transfer and correction for cell-based therapies. *Nat. Rev. Gen.* 2011; 12: 301–15.
7. Abordo-Adesida E, Follenzi A, Barcia C *et al.* Stability of lentiviral vector-mediated transgene expression in the brain in the presence of systemic antivector immune responses. *Hum. Gene Ther.* 2005; 16: 741–51.
8. Guidance for Industry: Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up, July 2018.
9. Ansorge S, Henry O, Kamen A. Recent progress in lentiviral vector mass production. *Biochem. Eng. J.* 2010; 48: 362–77.
10. Waehler R, Russell SJ, Curiel DT. Engineering targeted viral vectors for gene therapy. *Nat. Rev. Genetics* 2007; 8: 573–87.
11. Merten O-W, Charrier S, Laroudie N *et al.* Large scale manufacture and characterisation of a lentiviral vector

produced for clinical *ex vivo* gene therapy application. *Hum. Gene Ther.* 2010; 22: 343–56.

12. Ansorge S, Lanthier S, Transfiguracion J, Durocher Y, Henry O, Kamen A. Development of a scalable process for high-yield lentiviral vector production by transient transfection of HEK293 suspension cultures. *J. Gene Med.* 2009; 11: 868–76.

13. Kuroda H, Kutner RH, Bazan NG, Reiser J. Simplified lentivirus vector production in protein-free media using polyethylenimine-mediated transfection. *J. Virol. Meth.* 2009; 157: 113–21.

14. Segura MM, Garnier A, Durocher Y, Coelho H, Kamen A. Production of lentiviral vectors by large-scale transient transfection of suspension cultures and affinity chromatography purification. *Biotechnol. Bioeng.* 2007; 98: 789–799.

15. Naldini L, Blömer U, Gallay P *et al. In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 1996; 272: 263–7.

16. Binder GK, Dropulic B. Lentivirus Vectors. In: *Concepts in genetic medicine*. Dropulic B, Carter B (eds) 2008; Wiley, NJ, USA, 19–37.

17. Andreadis ST, Roth CM, Le Doux JM, Morgan JR, Yarmush ML. Large-scale processing of recombinant retroviruses for gene therapy. *Biotechnol. Prog.* 1999; 15: 1–11.

18. Warnock JN, Merten O-W, Al-Rubeai M. Cell culture processes for the production of viral vectors for gene therapy purposes. *Cytotechnology* 2006; 50: 141–62.

19. Sheu J, Beltzer J, Fury B *et al.* Large-scale production of lentiviral vector in a closed system hollow fiber bioreactor. *Mol. Ther. Meth. Clin. Dev.* 2015; 2: 15020.

20. Wang X, Olszewska M, Qu J *et al.* Large-scale clinical-grade retroviral vector production in a fixed-bed bioreactor. *J. Immunother.* 2015; 38: 127–35.

21. Broussau S, Transfiguracion J, Jabbour N *et al.* Inducible packaging cells for large-scale production of lentiviral vectors in serum-free suspension culture. *Mol. Ther.* 2008; 16: 500–7.

22. Guy HM, McCloskey L, Lye GJ, Mitrophanous KA, Mukhopadhyay TK. Characterization of lentiviral vector production using microwell suspension cultures of HEK293T-derived producer cells. *Hum. Gene Ther. Meth.* 2013; 24: 125–39.

23. Lesch HP, Laitinen A, Peixoto C *et al.* Production and purification of lentiviral vectors generated in 293T suspension cells with baculoviral vectors. *Gene Ther.* 2011; 18: 531–8.

24. Segura MM, Garnier A, Durocher Y, Ansorge S, Kamen A. New protocol for lentiviral vector mass production. *Meth. Mol. Biol.* 2010; 614: 39–52.

25. Witting SR, Li L-H, Jasti A *et al.* Efficient large volume lentiviral vector production using flow electroporation. *Hum. Gene Ther.* 2012; 23: 243–9.

26. Manceur AP, Kim H, Misic V *et al.* Scalable Lentiviral Vector Production Using Stable HEK293SF Producer Cell Lines. *Hum. Gene Ther. Meth.* 2017; 28(6): 330–9.

27. Hebben, M, Pre-industrial Manufacturing of Lentiviral Vectors by Transient Transfection in Single Use Systems. Presented at: the Spring Meeting of ISBiotech 9–11 March 2015; Washington/DC, USA.

28. Merten, O-W, Hebben, M, Bovolenta C, Production of lentiviral vectors. *Mol. Ther. Meth. Clin. Dev.* 2016; 16017: 1–14.

29. Slingsby JH, Baban D, Sutton J *et al.* Analysis of 4070A envelope levels in retroviral preparations and effect on target cell transduction efficiency. *Hum. Gene Ther.* 2000; 11: 1439–51.

30. Carmo M, Dias JD, Panet A *et al.* Thermosensitivity of the reverse transcription process as an inactivation mechanism of lentiviral vectors. *Hum. Gene Ther.* 2009; 20: 1168–76.

31. Gagnon P. The antagonistic effects of chromatin on biomolecule purification. Monolith Summer School, 15–20 June 2018; Portoroz, Slovenia.

32. Slepushkin V, Chang N, Cohen R *et al.* Large-scale purification of a lentiviral vector by size exclusion chromatography or Mustang Q ion exchange capsule. *Bioprocessing J.* 2003; 2: 89–95.

33. Jungbauer A, Hahn R. Polymethacrylate monoliths for preparative and industrial separation of biomolecular assemblies. *J. Chromatography* 2008; 1184: 62–79.

34. Barut M, Podgornik A, Brne P, Štrancar A. Convective Interaction Media short monolithic columns: enabling chromatographic supports for the separation and purification of large biomolecules. *J. Sep. Sci.* 2005; 28: 1876–92.

35. Podgornik A, Barut M, Štrancar A. Construction of large-volume monolithic columns. *Anal. Chem.* 2000; 72: 5693–9.

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36. Michen B, Graule T. Isoelectric points of viruses. *J. Appl. Microbiol.* 2010; 109: 388–97.

37. Bandeira V, Peixoto C, Rodrigues AF *et al.* Downstream processing of lentiviral vectors: releasing bottlenecks. *Hum. Gene Ther. Methods* 2012; 23: 255–63.

38. Segura MM, Kamen A, Trudel P, Garnier A. A novel purification strategy for retrovirus gene therapy vectors using heparin affinity chromatography. *Biotechnol. Bioeng.* 2005; 90: 391–404.

39. Cooper AR, Patel S, Senadheera S, Plath K, Kohn DB, Hollis RP. Highly efficient large-scale lentiviral vector concentration by tandem tangential flow filtration. *J. Virol. Meth.* 2011; 177: 1–9.

40. Stewart HJ, Fong-Wong L, Strickland I *et al.* A stable producer cell line for the manufacture of a lentiviral vector for gene therapy of Parkinson's disease. *Hum. Gene Ther.* 2011; 22: 357–69.

41. Stornaiuolo A, Piovani BM, Bossi S *et al.* RD2-MolPack-Chim, a packaging cell line for stable production of lentiviral vectors for anti-HIV gene therapy. *Hum. Gene Ther. Meth.* 2013; 24: 228–40.

42. Sanber KS, Knight SB, Stephen SL *et al.* Construction of stable packaging cell lines for clinical lentiviral vector production. *Sci. Rep.* 2015; 5: 1–10.

43. Wielgosz MM, Kim Y-S, Carney GG *et al.* Generation of a lentiviral vector producer cell clone for human Wiskott-Aldrich syndrome gene therapy. *Mol. Ther. Meth. Clin. Develop.* 2015; 2: 14063.

44. Shirgaonkar IZ, Lanthier S, Kamen A. Acoustic cell filter: a proven cell retention technology for perfusion of animal cell cultures. *Biotechnol. Adv.* 2004; 22: 433–444.

45. Ansorge S, Lanthier S, Transfiguracion J, Durocher Y, Henry O, Kamen A. Development of a scalable process for high-yield lentiviral vector production by transient transfection of HEK293 suspension cultures. *J. Gene Med.* 2009; 11: 868–76.

46. Terova, O *et al.*, Enabling custom solutions for downstream processing. Thermo Fisher Scientific, 35 Wiggins Ave, Bedford, MA, 01730, USA, Thermo Fisher Scientific, J.H. Oortweg 21, 2333 CH, Leiden, Netherlands.

47. Pabst TM *et al.* Camelid VHH affinity ligands enable separation of closely related biopharmaceuticals. *Biotechnol. J.* 2016; 11: 1–10.

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