

Development of an affinity chromatography process for lentiviral vectors

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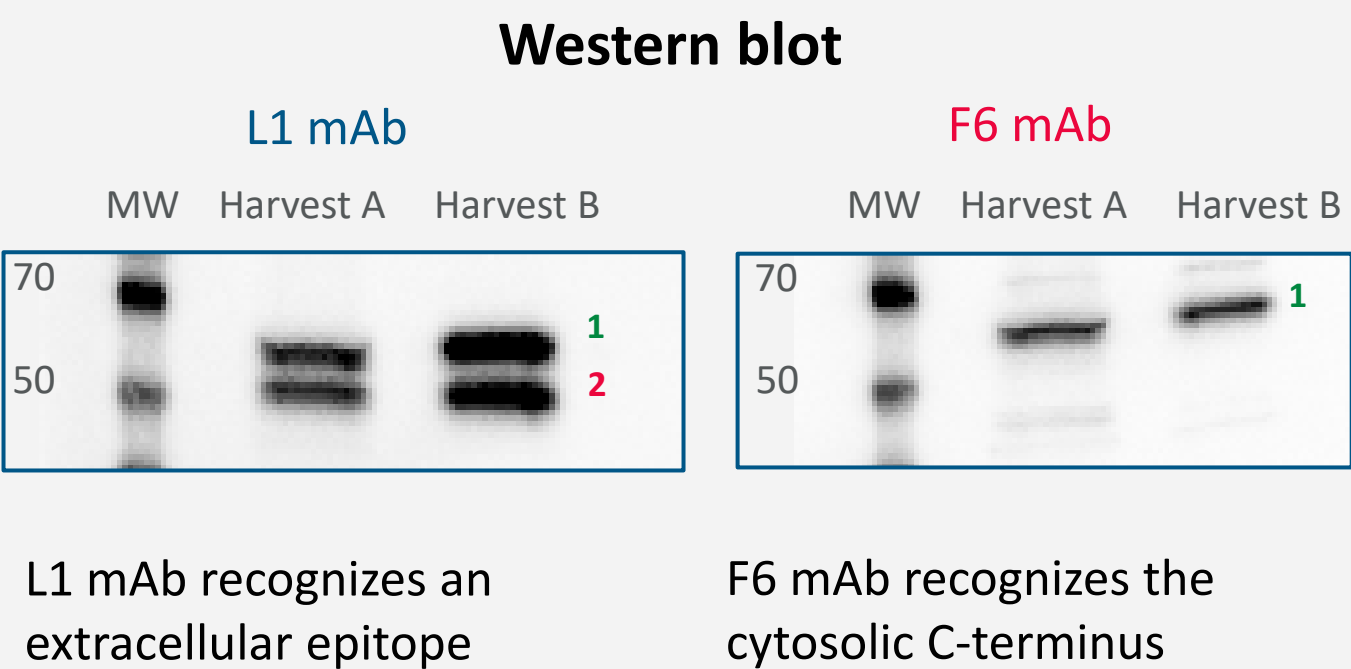
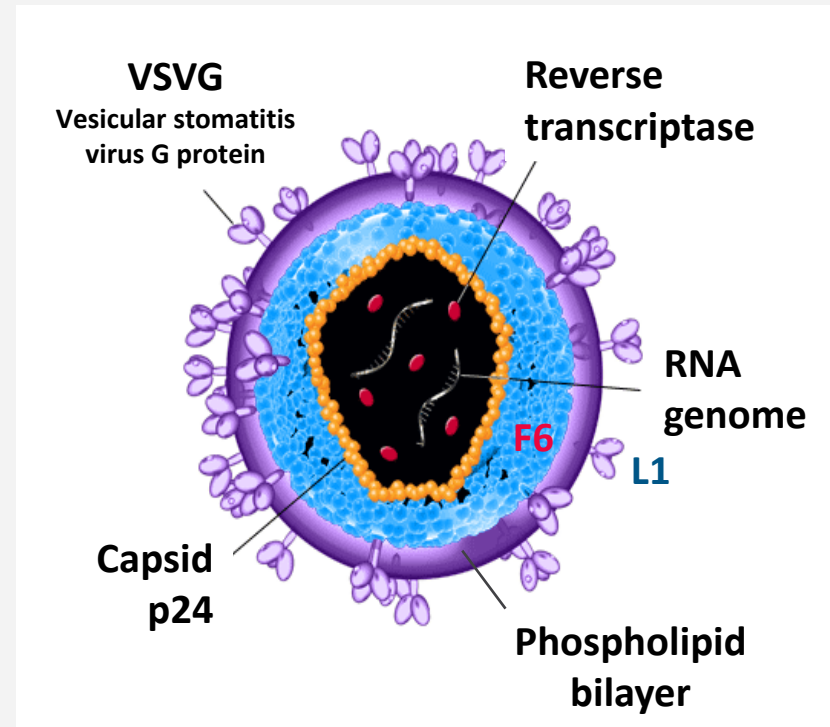


A REPLIGEN COMPANY

Lentiviral vector affinity purification

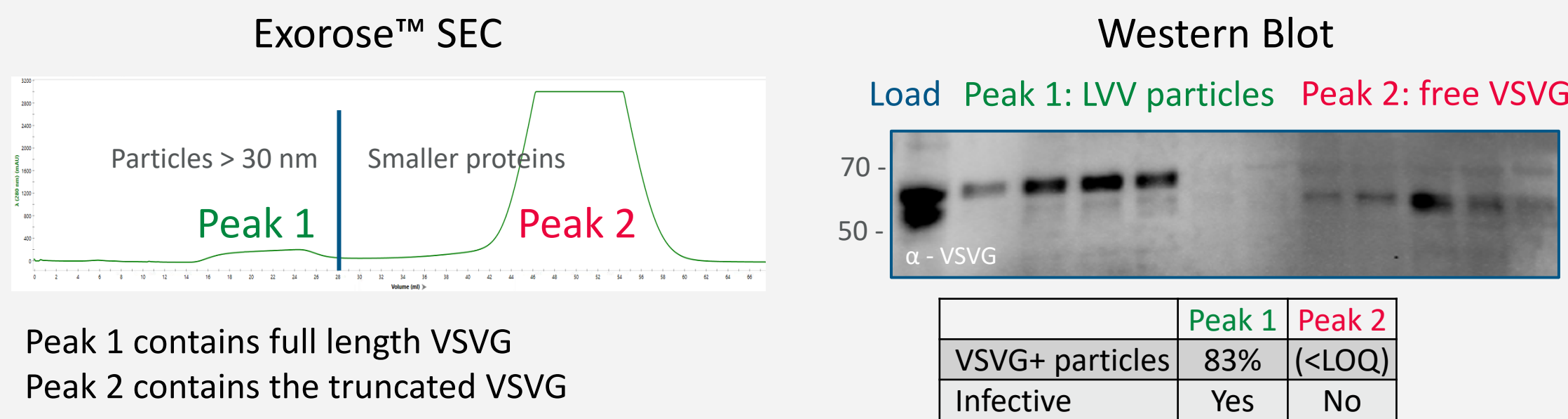
- Free VSVG produced in bioreactor
- 2 –step affinity process required
- High yields
- Great purity
- NaOH stable

2 VSVG species are produced in the bioreactor



F6 only recognizes the upper band, hence only the upper band contains the cytoplasmic tail.
The lower band is a cleaved VSVG, presumably prior to the transmembrane region and near the particle surface.

SEC shows the truncated VSVG is soluble protein and not associated with particles



2–step process to purify LVV

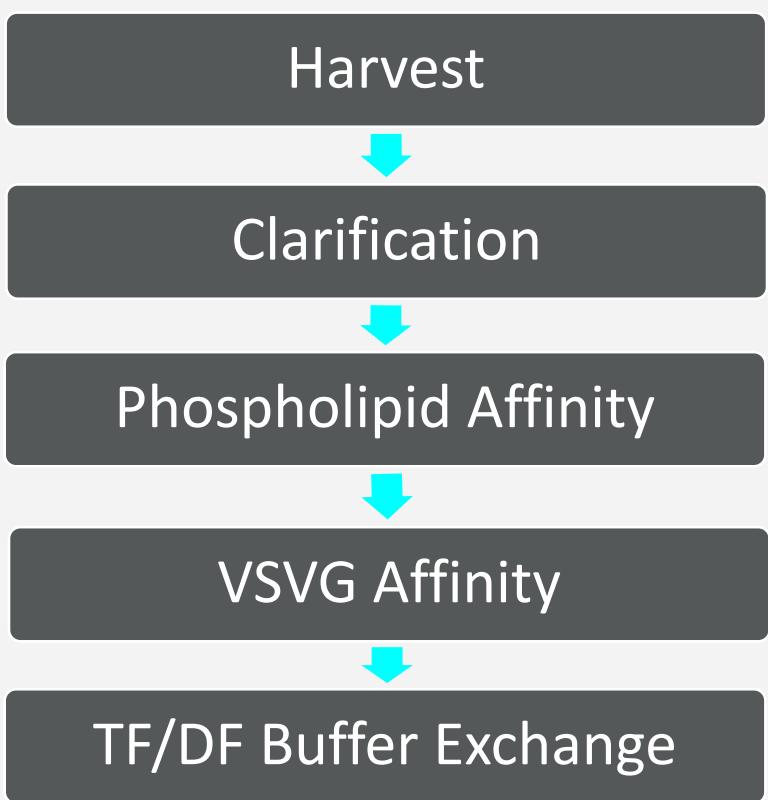
Free VSVG negates our VSVG affinity ligands as a 1-step process
But we have an alternative:

We have developed a resin that recognizes the phospholipid membrane for

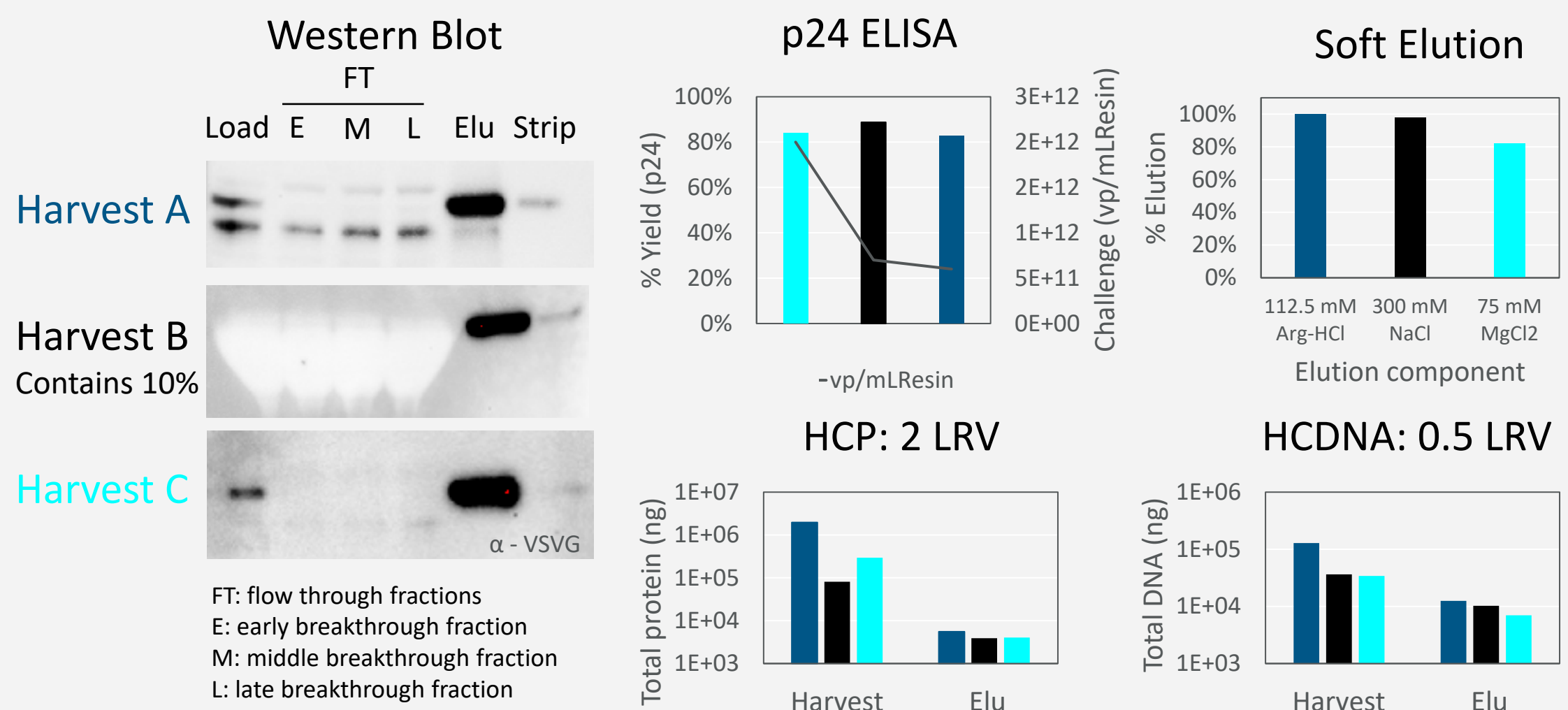
- Viruses
- Exosomes
- Extracellular vesicles
- Virus-like particles

We envisage a 2-step process

- AVIPure® PL
- Purify Lentivirus particles from free VSVG
- AVIPure® Lenti Polish
- Purify Lentivirus from other particles/EVs

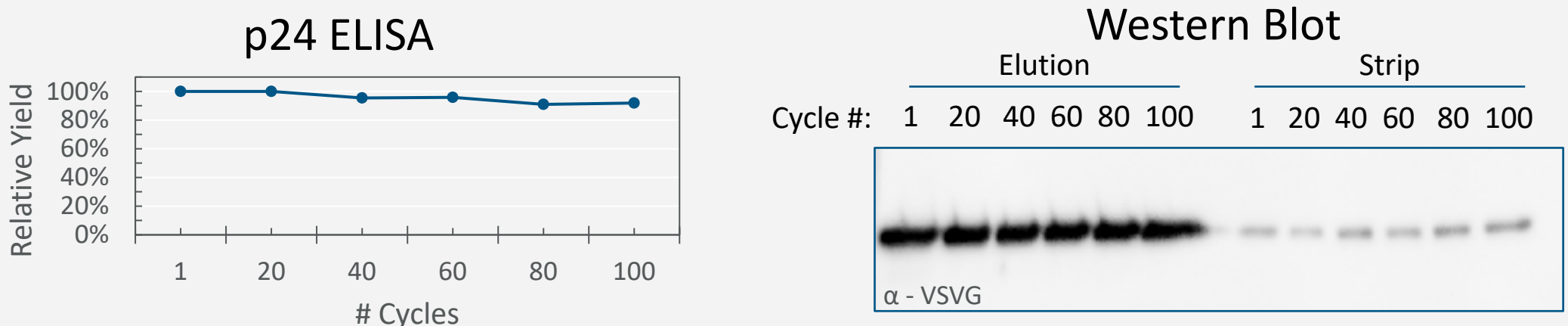


AVIPure® PL: high capacity, step yield, and purity

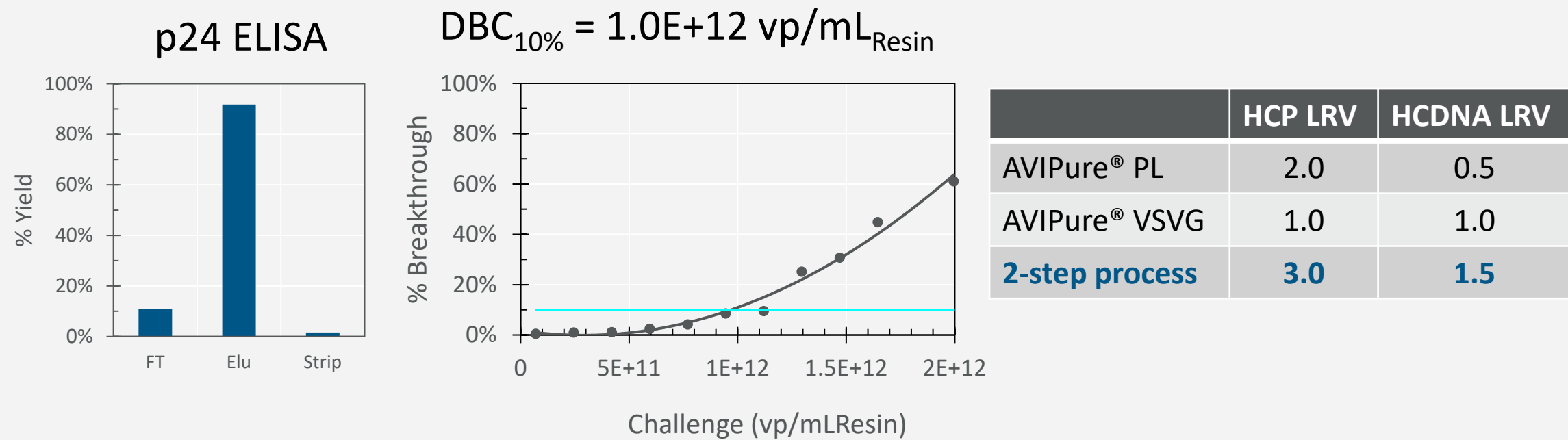


>100 Re-use cycles with 0.5 N NaOH CIP

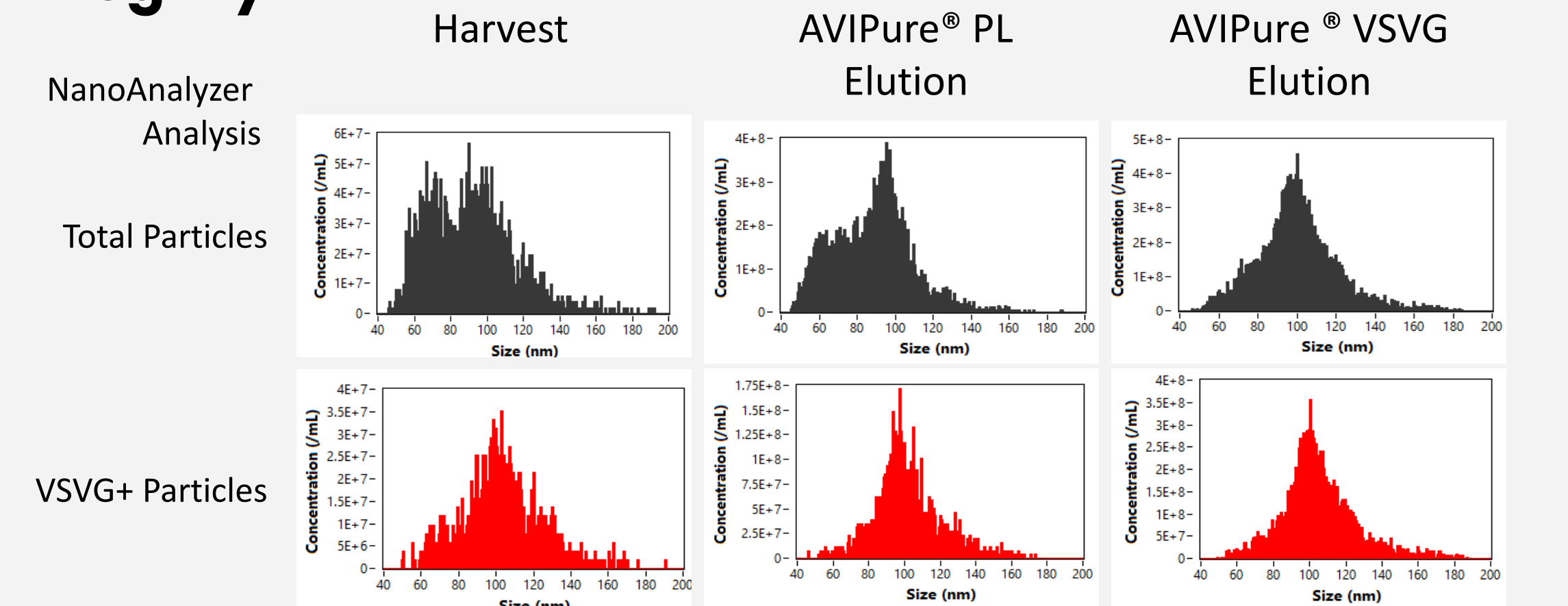
Simulated 0.5 N NaOH cycling with harvest material over AVIPure PL resin



AVIPure® Lenti Polish selectively purifies Lentivirus particles from AVIPure® PL eluate



AVIPure® PL and AVIPure® Lenti Polish maintain LVV integrity



Summary

AVIPure® PL and AVIPure® Lenti Polish operate @ 15 second residence time

Total batch time for 50 L bioreactor < 2 hours using 250 mL devices

>80% step yields

3 LRV HCP and 1.5 LRV HCDNA

Maintains LVV integrity

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