Introduction to USP – Seed Train Development

Your Objectives:

At the end of the lesson, you should be able to sequence the steps in seed train development

A **seed train** serves to generate an adequate number of cells for the inoculation of a production bioreactor. From volumes used for either cell thawing or cell line maintenance, the cell number will have to be increased, rendering it both time and cost intensive.

A typical cell culture process begins with the thawing of a cryopreserved cell-bank vial, followed by successive expansions into larger culture vessels, such as shake flasks, spinners, Wave bags, and stirred bioreactors.

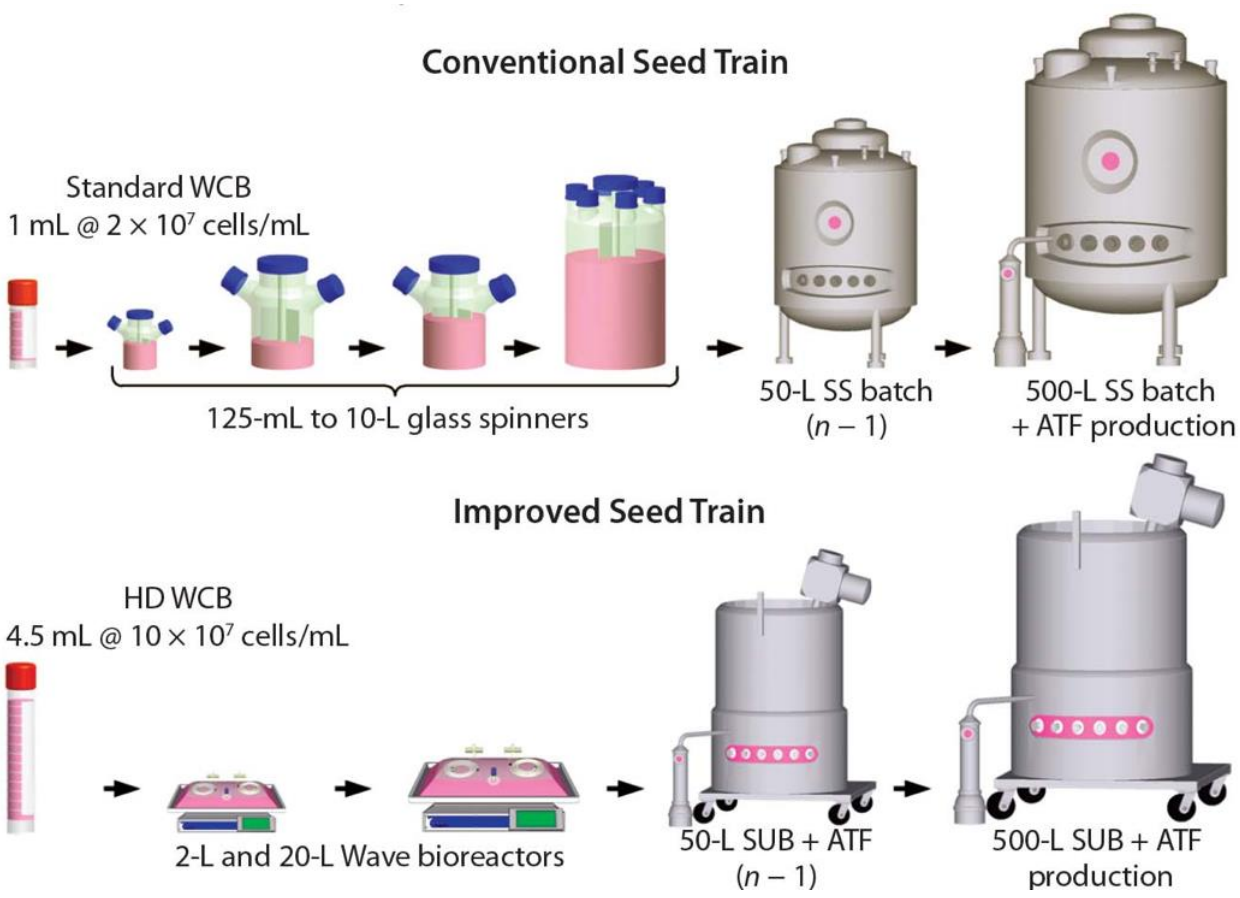
This approach presents several challenges: Shake flasks or spinners used in the initial stages require manual manipulations inside a laminar flow hood, making them vulnerable to contamination and operator error. In addition, since a conventional cryopreserved cell-bank vial typically contains low cell numbers, the seed-train process is time consuming. It takes longer and becomes even more complex with much larger production bioreactors because additional large-volume culture vessels are required to support scale up to a production bioreactor. Finally, production bioreactors being usually inoculated at a density of <0.5 × 106 viable cells/mL also means a necessary growth phase of 5 to 10 days, which is basically unfavourable since, during that period, cell concentrations is rather low (as is the initial product yield).

Previous studies have shown that high-density (HD) cell banking can be effective at reducing the number of seed-train steps required whilst improving operational success in seed-train processes, namely using HD cell banks containing 450 million viable cells/vial to directly inoculate a 20-L Wave bag. One particular study revealed that the use of HD cell-banks would eliminate several intermediate shake-flask expansion steps thereby reducing process times by up to nine (9) days.

Another study combined HD cell banks with a single-seed bioreactor capable of operating at several working volumes, thus reducing seed-train expansion duration by as much as 60 to 70 percent.

Single-use technology actually offers numerous advantages over stainless-steel systems, including enhanced facility flexibility, time savings in set-up, cleaning, and sterilization, not to mention a reduction in capital investment. Disposable Wave bioreactors are, therefore, routinely used in seed-train expansion processes because they also decrease contamination risk (typically requiring no manipulations in laminar-flow hoods). Because such bioreactors are disposable, no extra cumbersome and costly resources are needed for assembly, cleaning or sterilization.

Perfusion cell-culture processes is in greater demand in this industry as perfusion bioreactors have shown success in manufacturing viable commercial therapeutics. Perfusion processes can reach high cell densities in relatively small bioreactor volumes and will remove unstable product from cultures more quickly than the more conventional batch and fed-batch processes. Because of the aforementioned advantages (and others), perfusion has become the preferred production method over seed expansion.



The illustration compares a novel seed-train process using HD cell banking, disposable Wave bioreactors, and Refine Technology's Alternating Tangential Flow (ATF) perfusion cell-culture technologies over a conventional one. An example of this process for a 500-L production perfusion bioreactor with a conventional seed-train process.

Using the HD cell bank approach involves eliminating two intermediate spinner steps, by allowing for direct inoculation into a 2-L Wave bioreactor. Replacing spinners (125 mL to 10 L) with Wave bioreactors (2 L and 20 L) means significantly reducing the number of required manipulations in a **laminar flow cabinet** (**tissue culture hood**)*, improving operational success by allowing operation under a "closed" system. Additionally, using ATF perfusion technology at the n – 1 stage (50-L bioreactor with ATF perfusion device) enables cell densities to be ≥50 × 106 viable cells/mL, allowing for an inoculation density of 5 × 106 viable cells/mL in the 500-L production bioreactor rather than 0.5 × 106 viable cells/mL in the conventional cell culture process. Higher seeding density helps reduce the 500-L production bioreactor growth phase by approximately five days, saving manufacturing plant time for a 500-L bioreactor operating for 50 days.

*A **laminar flow cabinet**, or **tissue culture hood**, is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials.

The Bioreactor Train

Biogen bioreactors are organized into trains, each being larger than the next. A bioreactor trains consists of different volumes:

- 60 litre
- 100 litre
- 235 litre
- 750 litre
- 950 litre
- 2000 litre
- 3750 litre
- 15000 litre
- 18500 litre

Each bioreactor feeds into the next. The train is a continuation of the scale up process that begins with the inoculum preparation stage and ends usually with the harvesting process in the largest bioreactor.