An intensified perfusion one-step seed process with high-density cell banks

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Abstract and introduction
This work describes how the use of perfusion in the seed culture expansion process, in combination with the use of high-density cell banks, drastically can reduce processing time, simplify operations, and maximize equipment utilization (Fig. 1). A high-density cell bank was created using a method that does not require centrifugation. One vial from this cell bank was used to inoculate a bioreactor culture that, when operated in perfusion mode, enabled a one-step seed culture process that could be used to seed a 2000 L production bioreactor.

Results
The described method allowed for the generation of ~ 400 vials of 4.5 mL cryopreserved high-density cell bank at 50 × 10^6 cells/mL (> 95% viability) from a 2 L culture. Cells were successfully revived from cryopreservation at a viability of > 90%, which is similar to the performance of the original conventional cell bank. One vial of the high-density cell bank was used to inoculate a 20 L Cellbag perfusion culture chamber at a starting volume of 1 L. The culture volume was further expanded to 20 L, after which perfusion was started. A final density of > 50 × 10^6 viable cells/mL (> 95% viability), with cells in full exponential growth phase, was achieved. Results are displayed in Figures 3 to 8.

Materials and methods
High-density cell banks were created by growing Chinese hamster ovary (CHO) DG44 cells (licensed from Celltech GmbH) in a 2 L ActiCHROM™ P medium. Culturing was performed in perfusion mode in a disposable 10 L Cellbag™ bioreactor with a floating internal filter using the ReadyToProcess™ WAV25 System (Fig. 2). For cryopreservation, chilled, fresh medium with DMSO was added (1:1 ratio to the bioreactor, whereupon the culture was concentrated back to 50 × 10^6 cells/mL, before harvested, dispensed, and frozen in 4.5 mL cryovials. Cells from one vial of the cryopreserved cell bank were recovered and directly transferred to a new 20 L Cellbag bioreactor at 1 L. The volume was stepwise expanded to a final working volume of 10 L, whereupon the culture was continued in perfusion mode.

Target criteria:
- Cell bank generation (2 L): > 50 × 10^6 viable cells/mL (> 95% viability)
- Viability at revival from cryopreservation: > 90% viability
- Seed culture (10 L): > 50 × 10^6 viable cells/mL (> 95% viability)

Conclusion
The described culture process allowed:
- A simple method for generation of high-density cell banks in a closed system, without the need for a separate centrifugation step.
- Direct inoculation of a disposable bioreactor culture from one vial of the cryopreserved high-density cell bank, without prior cell expansion in shake flasks.
- Efficient one-step seed culture process, generating high cell densities at a culture volume that obviates the need for intermediate seed bioreactors.

Perfusion culturing in combination with the high density cell bank enabled expansion of cell numbers sufficient for seeding of a 2000 L bioreactor in 10 days from revival of the cell bank.