HIGH CELL DENSITY CRYOPRESERVATION STRATEGY

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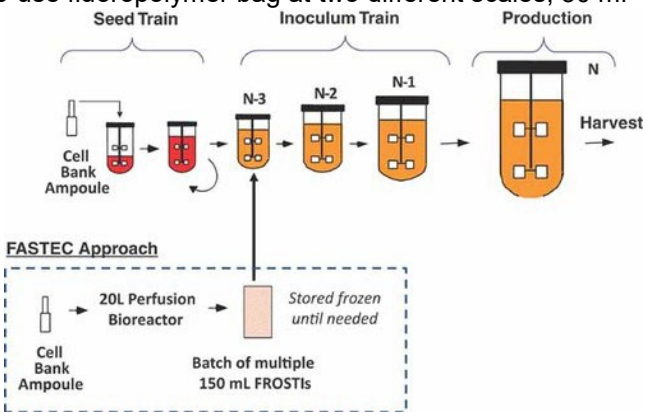
- *Cryopreservation*
- *Seed train reduction*
- *Single Use fluoropolymer bag*
- *High density cell banking*

The scientific strategy of this study is to amplify cells, freeze them in their single-use storage bags and in vials (which is the reference), keep them for a shorter or longer time in liquid nitrogen, then defrost directly in culture and compare the yields obtain in single-use storage bags versus in vials. This study follows 2 steps described below.

PART I aims at evaluating cell freezing in Aramus single-use fluoropolymer bag at two different scales, 50 ml and 500 ml respectively. Two approaches will be used for the freezing step:

- ultra-fast freezing rate
- slow freezing rate (-0.5 to -3 °C/min)

The first method is simple: bags will be immersed into liquid nitrogen until complete freezing, leading to amorphous solid structure. The second method requires a precise control of freezing rate during incubation in -80°C freezer, and until reaching at least -40°C. To achieve this goal a customized “Cryobox” is necessary to mimic passive devices used for freezing in vials. The prototype Cryobox was based on polyethylene foam of known thermal capacity. A thickness of 7 mm was calculated to be enough to modulate a cooling rate comparable to the reference in the commercial device Mr. Frosty.



Conventional seed train process flow versus FASTEC approach. From Seth et al. (2012)

PART II focus is made on freezing of a suspension cell in 50 ml bag by two methods:

- slow freezing rate (-0.5 to -3 °C/min) using Cryobox
- ultra-fast freezing rate by immersion in liquid nitrogen (LiN).

Chinese Hamster Ovary (CHO) cells were selected for this study as they are widely used in the industry. Cells were amplified until the desired number of cells were obtained, then concentrated in freezing media and transferred to Aramus bag for freezing. The bags were then kept in LiN tank for several days before thawing of cells and evaluation of cell survival and cell growth rate. Protocol efficiency was evaluated by comparing with classical vials incubated in Mr Frosty box as control. Cells frozen by immersion in LiN did not survive the operation. In contrast, the Cryobox method showed excellent results compared to the reference vials, with survival and growth rate slightly superior to the control method. We also discuss results obtained at a higher scale (500 ml).