Cell bank

- Mutational changes, aging and evolution continually occur in all actively growing cell cultures.
- The longer cultures are maintained the more these changes accumulate.

- As a result, cell cultures often lose important genotypic and/or phenotypic characteristics, such as biochemical features and alterations in functional properties.

- Frozen stocks of early passage cells can provide the assurance of a homogeneous culture supply as well as provide an important retained backup supply for replenishing occasional losses due to contamination or accidents.

- Fortunately, cryogenically preserved cultures do not undergo any detectable changes once they are properly frozen and stored below -130°C.

- Therefore, the biological effects of *in vitro* cellular aging and evolution may be minimized by periodic regeneration from your frozen stock cultures every two to three months depending on your needs and situation.

- This approach allows ongoing long-term culture experiments to be successfully completed without these unwanted variables.

- In addition, the process of establishing a certified working cell stock enhances the value of the cell line by ensuring suitability for validation studies of the cell line in the event of industrial applications.
- Ideally, each frozen cell stock would contain enough vials to provide fresh cultures for as long as needed.

- A more flexible and practical approach for creating cell stocks is to adapt cell banking methods used in the biopharmaceutical industry for cell lines used in manufacturing of biological products including recombinant proteins, monoclonal antibodies and vacci and so on.

- In the first stage, a smaller amount of cells is grown, harvested, pooled and frozen to create a **master cell stock** containing only ten to twenty vials.
- Then from this master stock, a single vial is thawed and cultured until there are enough cells to produce the initial **working cell stock**, also containing only ten to twenty vials.

- At this point, the working stock must be thoroughly tested to ensure their quality.
- One vial is recovered from the working stock and carefully tested to verify the following:

- 1)Viability
- 2)Absence of mycoplasma (bacteria that lack a cell wall around their cell membrane. Without a cell wall, they are unaffected by many common antibiotics such as penicillin or other antibiotics that target cell wall synthesis) or other microbial contaminants
- 3) Cell line identity
- 4) Important cell line characteristics.
- Future needs for these cells are then met by drawing vials only from the working stock.

- Once the first working stock has been used up, a second working stock is produced by thawing another vial from the original master seed stock and repeating the process.
- A vial from this new working stock should be tested in the same manner as the original working stock.

- Additional working stocks can be produced from the remaining master seed stock vials as required.
- Once the working stock has completed the validation process, it is important to record as much information as is available about the culture's characteristics, requirements and results.

- The master cell bank will have the capacity to generate nine additional working stocks of frozen cells over its lifetime.
- This will only require growing $2x10^7$cells to create the master cell stock and an equal number of cells to produce the first working stock.

- **Prepare the cultures:** Before freezing, the cells should be actively growing to ensure maximum health and a good recovery.
- Ideally, the culture medium should be changed the previous day. It is best if the cultures are maintained in antibiotic-free medium for at least two weeks prior to cryopreservation.

- This practice of antibiotic-free cultivation is recommended to help reveal any cryptic (hidden) or low level microbial contamination within the cell culture system.

- The avoidance of antibiotics will enhance the reliability of quality control testing.
- Consequently, the use of antibiotics should be avoided when establishing cell banks, wherever possible

- A cell bank is a facility that stores cells of specific genetic lines for the purpose of future use in a product or medicinal needs.

- Before putting the donated cell lines into storage, they are first proliferated and multiplied into a large number of identical cells before being stored in a number of cryovials.

- These cryovials are then placed into a tray, which is labelled with the genetic line data and then they are all frozen in "the liquid or vapor phase of liquid nitrogen typically between -196 and -70 degrees Celsius.

- This temperature serves to stop all cell growth within the cryovials and preserves the cell lines.