



Cell Culture in Process Development

Genentech, Inc.

Cell Line development

- Produce transformed cells
insert genetic material using a plasmid
- Select for expression +/-
Test for production of product or surrogate marker
- Select for expression productivity
Quantitate production

Example: pGLO plasmid used to express GFP in *E. coli*



Cell Line Selection

- Clone cells to ascertain single cell production
- Test again for product production
- Select for highest productivity

Test for quality after selection of clone

- Restriction map to verify presence of DNA sequence
- Sequence the isolated gene to verify sequence
- Verify copy number
- Test for stability of transformant
- Test for contaminants = viruses , mycoplasma, fungi, bacteria

Creation of Master cell bank

- Production of the Master Cell Bank (MCB) is one of the first steps in therapeutic drug development
- A very large lot of cells produced under the highest standards possible
- Supports clinical development of the product
- Ultimately, the commercial supply phase, a period that can last decades.

Working Cell Bank

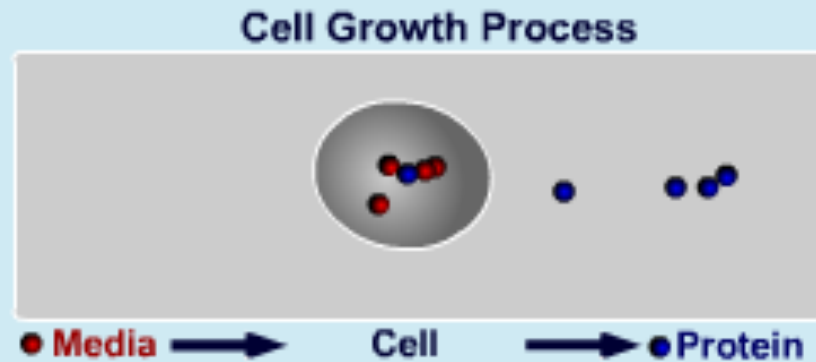
- a Working Cell Bank (WCB) is usually required for the later stages of development and manufacture.
- The use of large WCB's can yield capacity and cost benefits in routine production of products far outweighing the cost of manufacture.

Scale up Process

■ Master Cell Bank	(0.5 ml)
■ Working Cell Bank	(0.5ml)
Lab inoculum	5-10 ml
Fermentor seed train	100 ml
Fermentor seed train	2 liters
Fermentor seed train	20 liters
Production run fermentor	200 liters

Cell Culture in Media

Cell Culture Fluid (CCF) utilizes media for growth. Over a period of time cells grow and duplicate producing protein used to manufacture product.



Media



Millions of the genetically engineered, protein producing E. coli or CHO (Chinese Hamster Ovary) cells are added to a nutrient-rich growth medium

Source : Technology Review (www.technologyreview.com/BioTech) Photo Essay "Biotech Drug Factory"

Seed Train

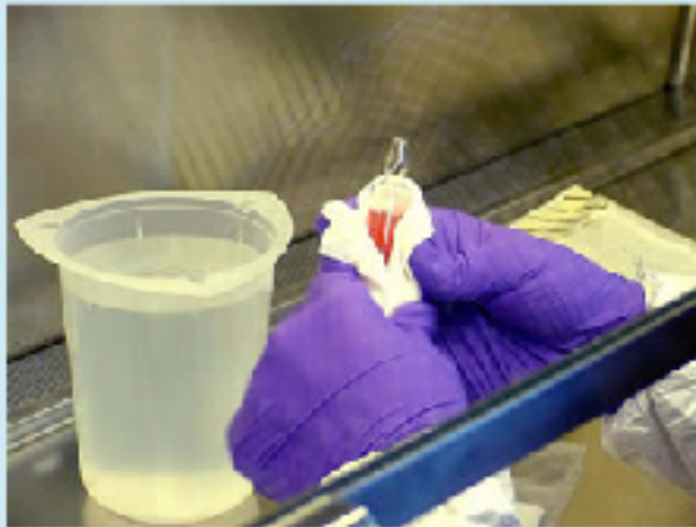


Seed Train is maintained under chemical selective pressure.

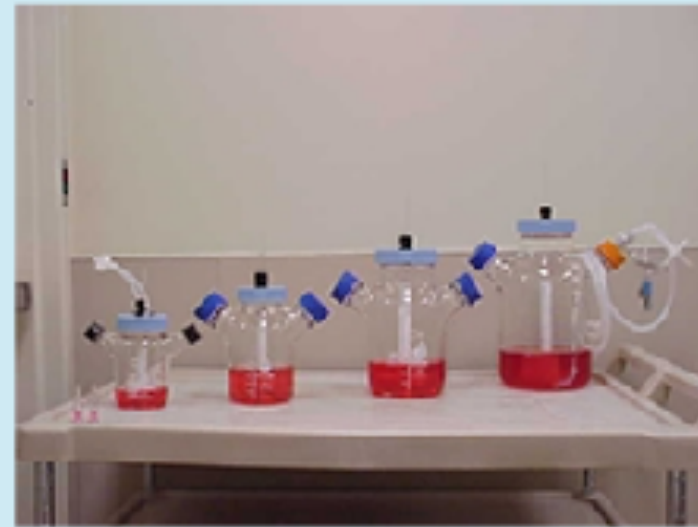
Source : Technology Review (www.technologyreview.com/BioTech) Photo Essay "Biotech Drug Factory"

Thaw of Ampule & Spinner Sizes

The cell culture process begins with a frozen ampule, which is thawed into a small, primary spinner. A spinner is a container that holds Cell Culture Fluid (CCF). The resulting culture is passaged (maintenance process of the spinner culture) into larger stock spinners. These stock spinners are eventually used to inoculate a 20L Fermentor.



Ampule



Spinners

Seed Train Scale Up

Two 1ml ampule's are thawed using a Laminar Flow Hood, which provides a clean working environment for aseptic operations, into a 250ml spinner. As the cells grow they are transferred to a larger container in the following order: 500ml spinner, 1L spinner and 3L spinner. From the 3L spinner flask, the cells are inoculated in a 20L fermentor.

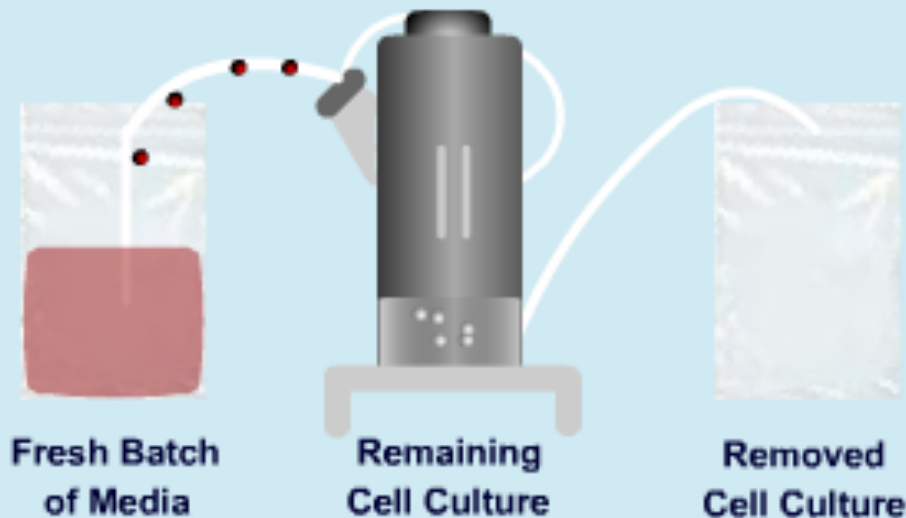
Thaw of 1ml ampule to spinners



Solera of 20L Batch Refeed

The 20L Fermentor also uses a process called the batch refeed or solera. During this process, a pre-calculated volume of cell culture is removed from the tank after a specified duration of growth (usually 3 or 4 days). A fresh batch of media is added to the remaining cells and the culture is again allowed to grow.

Solera Process



Inoc Trains



Inoc trains are built into process to maximize cell density.



Genentech's manufacturing process, automated with constant human monitoring, operate seven days a week, 24 hours a day.

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