HTGAA Bootcamp

Culturing cells

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Goals

- Cell culturing is the process of growing cells **outside** of their natural environment.
- This technique allows to study cells in a controlled setting, providing valuable insights into cellular processes, disease mechanisms and life in general

Goals

- **Identification**: Culturing allows scientists to identify specific microorganisms present in a sample, which is crucial for diagnosing infections and understanding microbial communities

- **Pathogen Study**: Pure cultures are essential for studying the virulence, antibiotic susceptibility, and genome sequences facilitating development of treatments.

Types of Cell Cultures

- **Batch** – one time culture is done, medial is consumed, cells are harvested and culture is discarded

  Continuous – the media and debris are periodically refreshed and removed
- **Solid growth media**- Petri style cultures using Agar as base
- **Liquid cultures/Broth** without using Agar

The Art of Cell Culture: Key Techniques

Aseptic Technique:
Maintaining a sterile environment to prevent contamination

- **Cell Passaging**: also known as subculturing, is the process of transferring cells from one culture to a fresh growth medium to create new cultures

Sterile/Aseptic technique

- Usually the media is autoclaved (heated to 115 Celsius) before antibiotic is added. That usually kills all unwanted organisms
- Usually the environment is sterilized with alcohol, chlorine, UV etc. Sometimes a hood with fine air filter is used

- The lab operators wear nitrile gloves
- A flame from a Benson burner placed near the media creates upward air currents. That reduces the chance of dust particles carrying organisms falling in the media

Mammalian cell culture

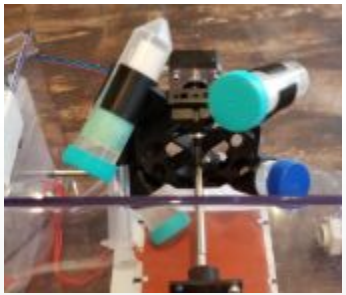
- **Fetal bovine serum** (FBS) is the most widely use serum-supplement for the in vitro cell culture of eukaryotic cells. This is due to it having a very low level of antibodies and containing more growth factors, allowing for versatility in many cell culture applications. Fetal bovine serum is derived from the blood drawn from a bovine fetus via a closed system of collection at the slaughterhouse

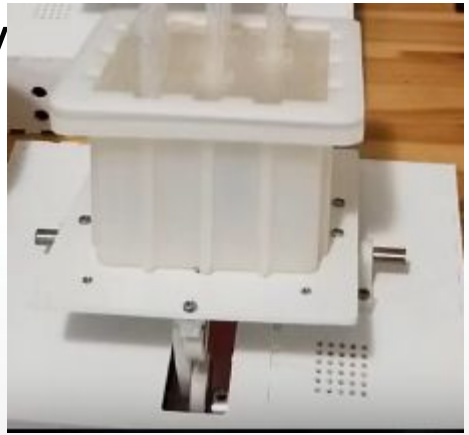
Closed flasks are sometimes used to avoid contamination of the cells

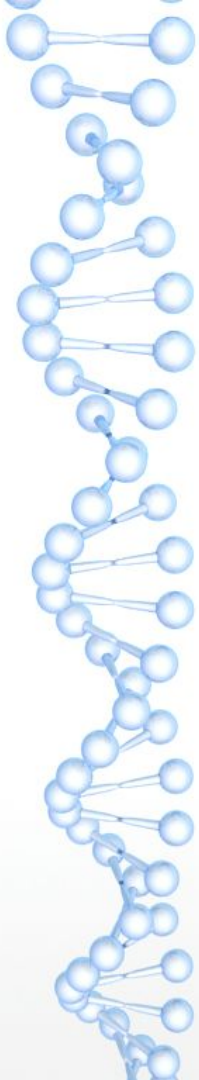
For some cells CO_2 needs to be controlled

Liquid culture

- When a large quantity of cells is needed we usually use a liquid culture
- Many organisms requires some oxygen to survive so they need to be exposed to air from time to time. That is done with an orbital shaker or a bioreactor or a tube rotator. The tube rotator can be used for smaller batches because the oxygen depleted after sev







Most research starts with culturing bacteria

- **Rite of passage in molecular biology**

Why?

- **It's fast** – doubles about every 20 minutes
- It's **cheap** and that applies to organisms, media and labware
- It's more **resistant** to contamination. One cell is a full organism not a piece of one
- It's better **understood**, easier to **control**
- Devices for culturing bacteria are **easy**. Just control the temp in an incubator

●Petri Dishes!

Most commonly, cultures are done in Petri dishes

Most common bacterial media is LB-Agar: 10 g peptone, 5 g yeast extract, 5 g sodium chloride, 12 g agar, and 1 L water. Usually we need minimum 0.37 grams LB-Agar and 10 mL water per Petri dish

- Agar is a jelly like base made of chopped sea weed, not the food! It's great as a transparent support

Other Additives

Usually the cells we grow have a gene that makes the cells resistant a particular antibiotic

- For that reason we can also add an antibiotic to our media usually Ampicillin, Kanamycin etc. That kills all the cells that are not resistant to the specific antibiotic and protects our cultured cells