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Basic cell culture techniques

Basic cell culture techniques are foundational skills in biology and biotechnology labs. Here's a concise overview to get you started:

🔬 1. Lab Setup & Sterility

- **Biosafety cabinet (laminar flow hood):** Use a Class II cabinet to maintain a sterile environment.
- **Sterilization:** Always sterilize hands (gloves), surfaces, and equipment with 70% ethanol before and after use.
- **Aseptic technique:** Avoid introducing contamination—work near the flame (if using a Bunsen burner) or within the sterile airflow.

🧪 2. Media Preparation

- **Culture media:** Contains essential nutrients (e.g., DMEM, RPMI, MEM) and often supplemented with:
  - 10% FBS (fetal bovine serum)
  - Antibiotics (e.g., penicillin-streptomycin)
  - Glutamine
- **pH and temperature:** Media is typically pH ~7.2–7.4 and incubated at 37°C with 5% CO_2.

🧫 3. Cell Thawing

- Warm frozen vial quickly in 37°C water bath (~1-2 min).
- Transfer to a culture flask with pre-warmed complete medium.
- Centrifuge if necessary to remove DMSO (cryoprotectant).

🧍 4. Cell Seeding

- **Adherent cells:** Seed directly into flasks, dishes, or well plates. Cells will attach to the surface.
- **Suspension cells:** Stay in the medium and can be gently agitated.

🔄 5. Subculturing (Passaging)

- Done when cells are ~70–90% confluent.
- For **adherent cells**:
  - Remove media
  - Wash with PBS
  - Add trypsin (or other detachment enzyme), wait ~2-5 min
  - Neutralize with media, centrifuge, and re-plate at appropriate dilution
- Keep track of **passage number** to avoid genetic drift.

👀 6. Monitoring Growth

- Check under microscope daily.
- Look for confluency, morphology changes, or signs of contamination (e.g., cloudiness, unusual pH/color, motile bacteria).

❄ 7. Cryopreservation

- Use freezing medium (usually 10% DMSO + FBS + media).
- Freeze slowly to -80°C (using a controlled-rate freezer or isopropanol freezing container), then transfer to liquid nitrogen for long-term storage.

8. Contamination Prevention

- Common contaminants: Bacteria, fungi, and mycoplasma.
- Always use proper aseptic technique.
- Regularly test for contamination if doing long-term culture.