

Techniques in Molecular Genetics

2009 edition

H.E. Schellhorn

Day 3

- Cell Fractionation
- PAGE gels
- Spectroscopy and Enzyme Assays
- Stain and Destain gels
- Inoculate Cultures

Techniques in Molecular Genetics

Cell Fractionation and
Processing

H.E. Schellhorn

Quiz Format

- 10 multiple Choice (10 marks)
- 5 short answer (15 marks)
- Total 25 marks (worth 20% of final grade)

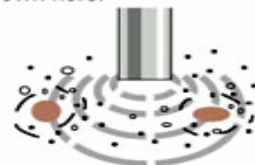
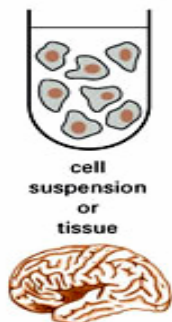
Cell Fractionation

BREAKING CELLS AND TISSUES

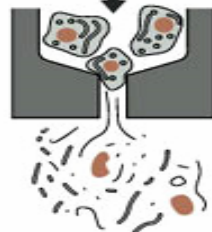
The first step in the purification of most proteins is to disrupt tissues and cells in a controlled fashion.

Using gentle mechanical procedures, called homogenization, the plasma membranes of cells can be ruptured so that the cell contents are released. Four commonly used procedures are shown here.

The resulting thick soup (called a homogenate or an extract) contains large and small molecules from the cytosol, such as enzymes, ribosomes, and metabolites, as well as all the membrane-bounded organelles.



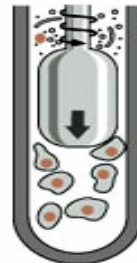
- ① break cells with high frequency sound



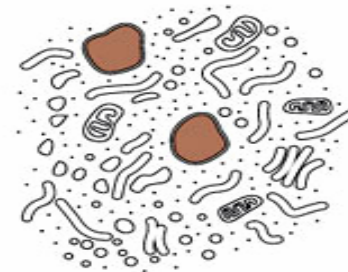
- ③ force cells through a small hole using high pressure



- ② use a mild detergent to make holes in the plasma membrane



- ④ shear cells between a close-fitting rotating plunger and the thick walls of a glass vessel



When carefully applied, homogenization leaves most of the membrane-bounded organelles intact.

Cell Fractionation

While it fairly easy to break mammalian cells, other types of cells, including bacterial cells, spores, plant cells, can be very difficult.

Three main methods for lysing bacterial cells

1. Enzyme detergent (e.g. lysozyme, EDTA, SDS)
2. Sonication
3. Explosive Decompression (French Pressure Cell)

Cell Fractionation

Lysozyme/SDS well suited for DNA isolation but will denature most proteins. Can be used for proteins when this is not an issue (e.g. Westerns)

Sonication and French pressure cells are generally used in protein purification.

Sonication - fairly gentle, can process many samples.
Has low capacity (low protein yeild)

French Pressure Cells - very disruptive, can only process one sample at time, high yield of protein (can be very concentrated)

Sonication



How Does a Sonicator Work?

The ultrasonic electronic generator transforms AC line power to a 20KHz signal that drives a piezoelectric convertor/transducer. This electrical signal is converted by the transducer to a mechanical vibration due to the characteristics of the internal piezoelectric crystals.

The vibration is amplified and transmitted down the length of the horn/probe where the tip longitudinally expands and contracts. The distance the tip travels is dependent on the amplitude selected by the user through the amplitude control knob.

In liquid, the rapid vibration of the tip causes *cavitation*, the formation and violent collapse of microscopic bubbles. The collapse of thousands of cavitation bubbles, releases tremendous energy in the cavitation field. Objects and surfaces within the cavitation field are “processed”.

From Misonix Web Site

French Pressure Cell

Cells are placed in a cell “Bomb” and pressurized using a hydraulic press. When fully pressurized, a small orifice is slowly opened and the sample is extruded from the device. Rapid depressurization causes outgassing and cell lysis.

French Pressure Cell



Mechanical Press

