Techniques in Molecular Genetics

Cell Fractionation and Processing

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Day 3

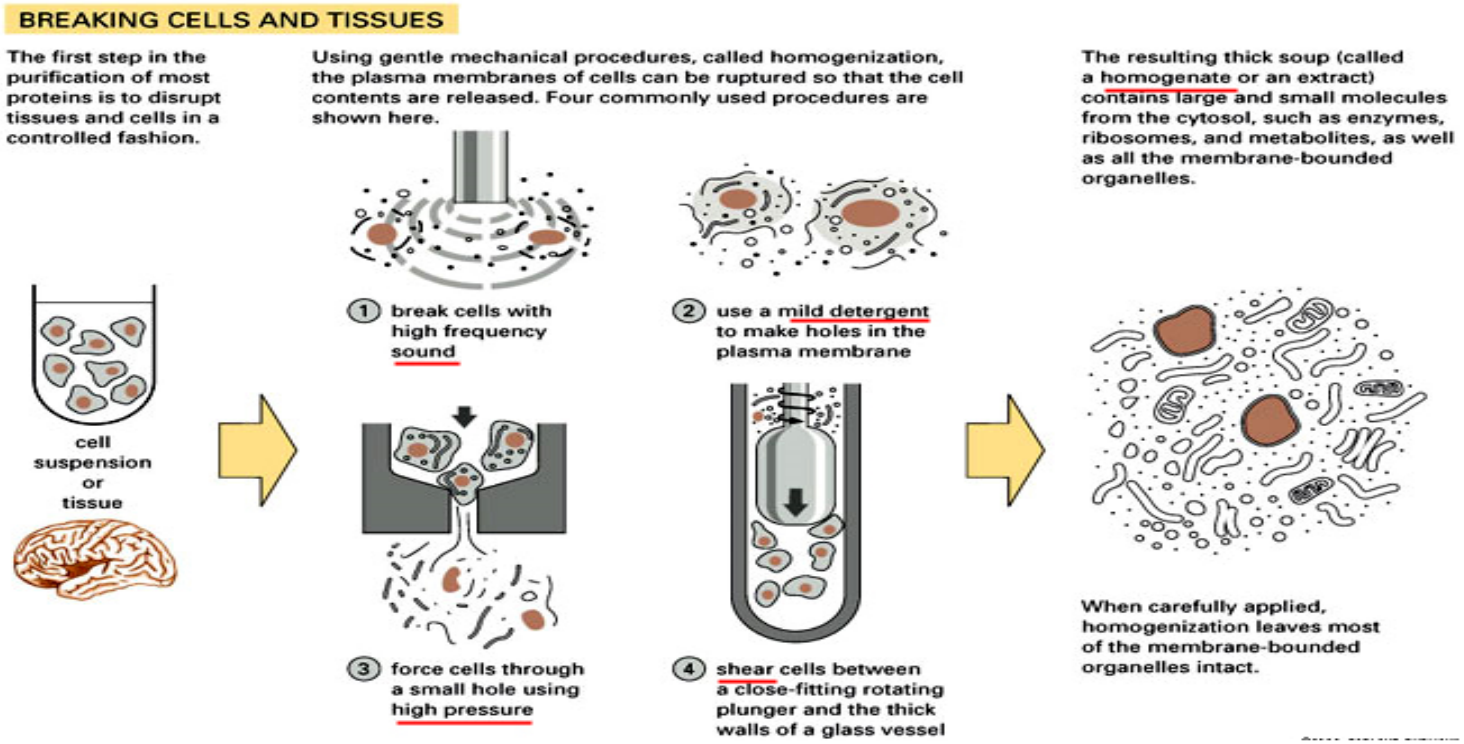
- Cell Fractionation
- PAGE gels
- Spectroscopy and Enzyme Assays
- Stain and Destain gels
- Inoculate Cultures

Quiz Format

- Friday May 10.
- 10 multiple Choice (10 marks)
- 5 short answer (15 marks)

- Total 25 marks (worth 20% of final grade)

Cell Fractionation



http://www.bio.miami.edu/~cmallery/255/255tech/ecbxp5x4x1.jpg

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)

http://www.bio.miami.edu/~cmallery/255/255tech/ecbxp5x4x1.jpg

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 yield)

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

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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

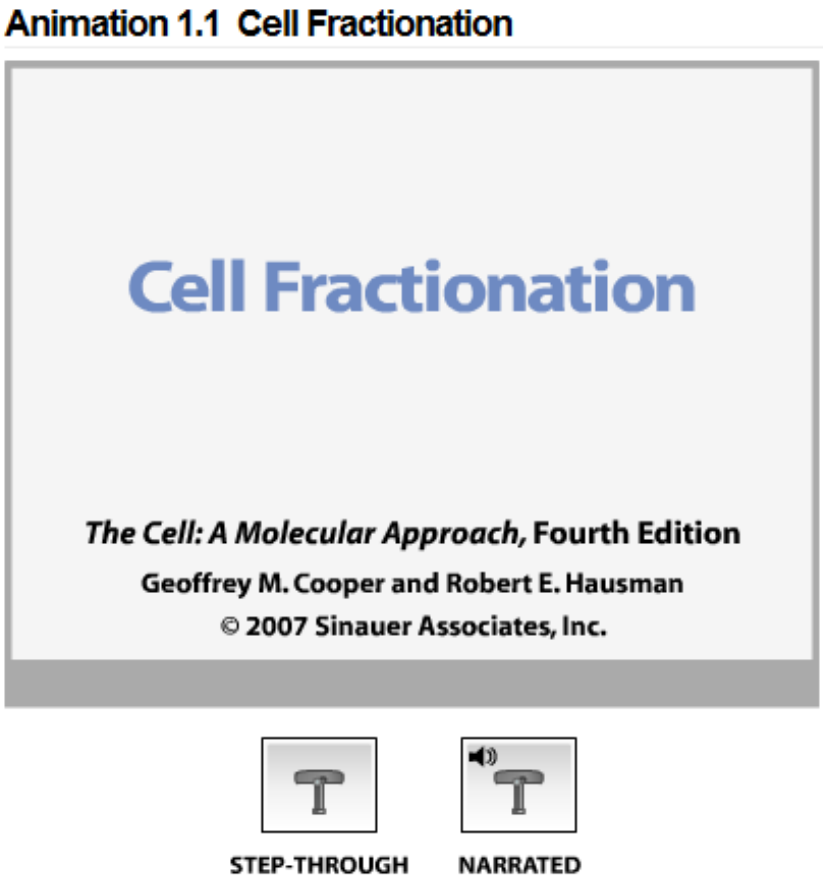
French Pressure Cell

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

Sonication and cellular fractionation



See video at
http://www.sinauer.com/cooper/4e/animations0101.html