Guide to Cell Modification & Re-Engineering Solutions

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No matter what your cell biology application Harvard Apparatus has the solution

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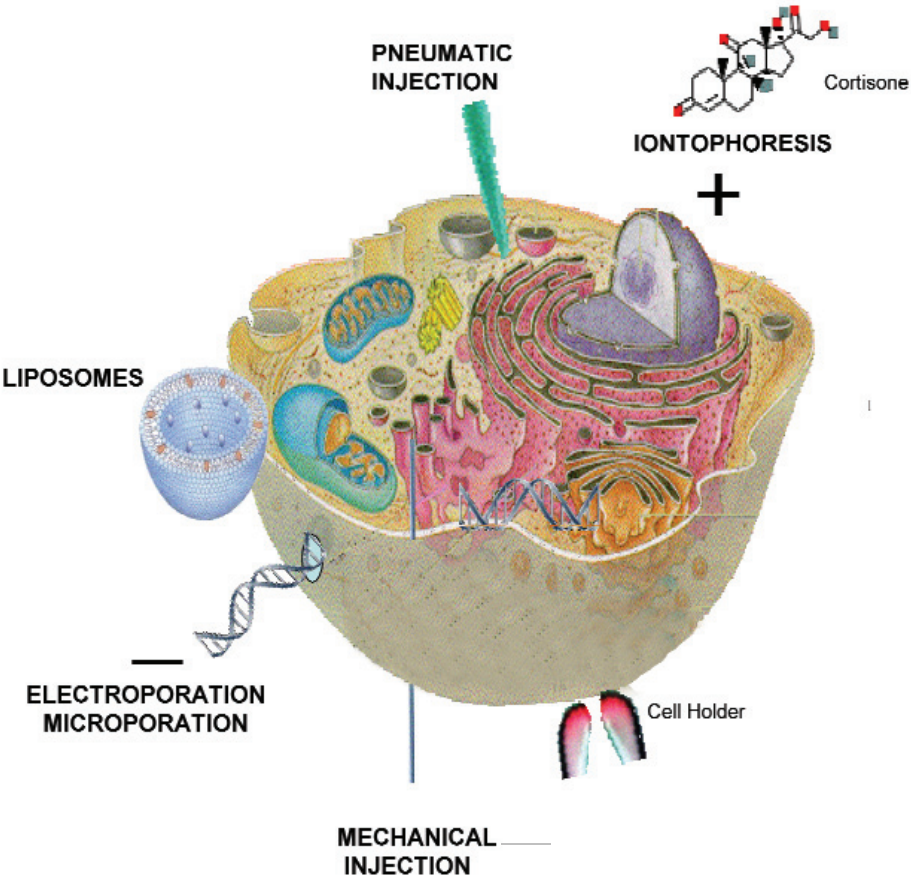


Introduction

The Harvard Apparatus Advantage…Serving cell biologists with exceptional products and support.

Introducing materials into cells such as, bacteria, DNA, drugs and ions, for the purpose of:
- Monitoring the cells behavioral to the materials
- Modifying and re-engineering the cells structure and function requires many different techniques, to get the cells to incorporate the materials into the cell to get desired effects

When you choose a techniques you must ask the following questions to select the optimal technique for your applications:

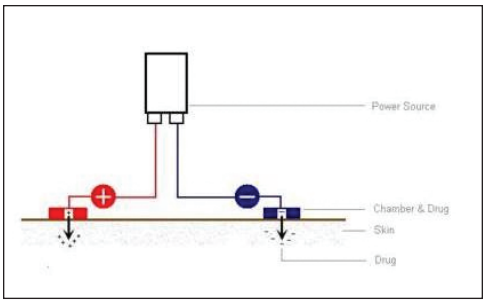
1. How many cells do I need for my experiment?
2. In what period of time do I need this number of cells?
3. Are my cells sensitive to certain materials in a negative way?
4. Of the cells modified do I need a high or low yield rate?
5. Are my cells temperature sensitive?
6. Are you going to keep them observed for longer than 4 hours?
7. Are my cells free floating or fixed?
8. How big is the substance going into the cell?
9. Do you only want some cells in a mixture handled?
10. Do I work with organs, tissue or cells clusters or individual cells?

Select Proper Technique

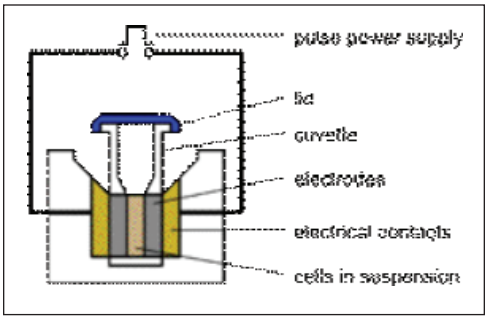
Harvard Apparatus has many ways for you to handle the introduction of samples into the Cells that best meets your experimental goals. The following chart will assist you in your selection:

	Electroporation	Iontophoresis	Liposomes	Pneumatic Injectors	Mechanical
Example	**BTX**	**BH-2**	**Liposomat**	**PLI-100**	**Pico plus**
Technical driving principal	High Voltage Opens Pores	Charge thru Existing Pores	Fat Ball thru Cell Wall	Push in w/Air	Push in w/Liquid
Average Price	$ 8000	$ 10000	$ 3000	$ 4000	$ 2000
One Cell at a Time	Not Typical	Not Typical	Not Typical	Yes	Yes
Transfect Hundreds of Cells Simultaneously	Yes	Yes	Yes	No	No
Transfect Thousands of Cells Simultaneously	Yes	No	Yes	No	No
Cell Container	Cuvette or In Vivo	In Vivo	Culture or In Vivo	Culture	Culture
Typical yields	30-70%	30-70%	50-90%	>90%	>90%
Gentle to Cells	Harsh to Low	Medium to Low	Low	Low	Low
Labor Intensive	Yes	Yes	Yes	No	No
Require Lots of Technique	Littlle	Little	Some	Yes	Yes
Need to Hold Cells Rigidly	No	No	No	Recommended	Recommended
Needs Cell Rigidly Held	Not Need	Not Need	Not Need	Yes	Yes
Equipment:					
Microscope	No	No	No	Yes	Yes
Capillary Glass Puller	No	No	No	Yes	Yes
Microforge	No	No	No	Yes	Yes
Applications:					
Large Particles and Molecules	Yes	No	No	Yes	Yes
Small Particles and Molecules	Yes	Yes	Yes	Yes	Yes
Bacteria	Yes	No	No	Yes	Yes
DNA	Yes	No	No	Yes	Yes
Virus	Yes	No	Yes	Yes	Yes
RNA	Yes	No	No	Yes	Yes
Drugs	Yes	Yes	Yes	Yes	Yes
Proteins	Yes	No	No	Yes	Yes
Plasmids	Yes	Yes	Yes	Yes	Yes

Technique Definitions

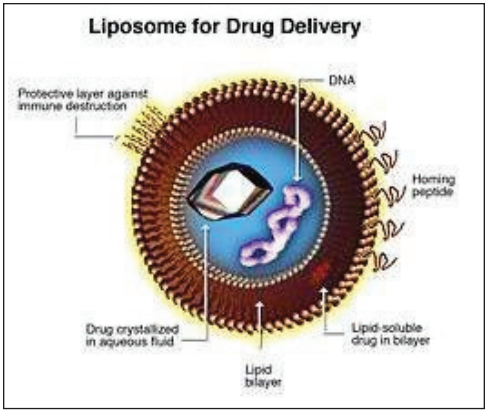


Iontophoresis is a non-invasive method of propelling high concentrations of a charged substance, normally medication or bioactive-agents, transdermally by repulsive electromotive force using a small electrical charge applied to an iontophoretic chamber containing a similarly charged active agent and its vehicle. To clarify, one or two chambers are filled with a solution containing an active ingredient and its solvent, termed the vehicle. The positively charged chamber, termed the anode will repel a positively charged chemical, whilst the negatively charged chamber, termed the cathode, will repel a negatively charged chemical into the skin.

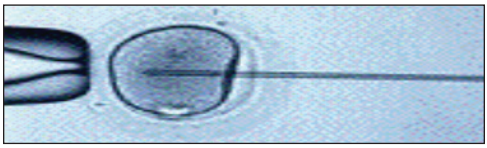


Electroporation, or electropermeabilization, is a significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field. It is usually used in molecular biology as a way of introducing some substance into a cell, such as loading it with a molecular probe, a drug that can change the cell's function, or a piece of coding DNA. Pores are formed when the voltage across a plasma membrane exceeds its dielectric strength. If the strength of the applied electrical field and/or duration of exposure to it are properly chosen, the pores formed by the electrical pulse reseal after a short period of time, during which extracellular compounds have a chance to enter into the cell. However, excessive exposure of live cells to electrical fields can cause apoptosis and/or necrosis -the processes that result in cell death.

Technique Definitions (cont.)



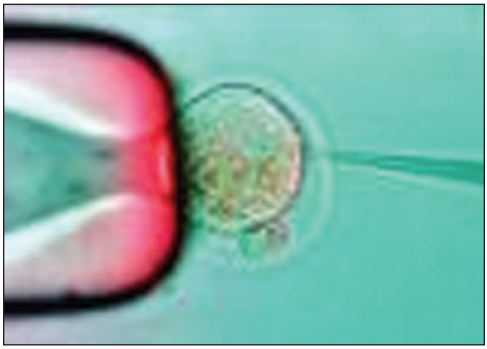
A **liposome** is a spherical vesicle with a membrane composed of a phospholipid and cholesterol bilayer (see on the right). Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidylethanolamine), or of pure surfactant components like DOPE (dioleolylphosphatidylethanolamine). Liposomes. Liposomes are used for drug delivery due to their unique properties. A liposome encapsulates a region on aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes can not readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane)they can be (indiscriminately) delivered past.



An **injector** is a pump-like device, which charges or discharges containers under pressure with suitable arrangements. Motive force is gained at the inlet from a suitable gas or liquid that is under pressure. Pneumatic injectors can deliver 10–15L femtolitre of materials and specialized mechanical pumps are capable of 10–12L picolitre delivery volumes.

Pneumatic Injectors

PLI-100 and PLI-90 Pico-Injector Series



- **5 pressures: inject, balance, clear, fill and hold**
- **Popular applications:**
  - Injection of mouse, frog, zebrafish and other oocytes
  - Extracellular brain injections
  - Injection of DNA, mRNA, microbeads, eurotransmitters, kinases and other proteins
- **Most Published Injector**

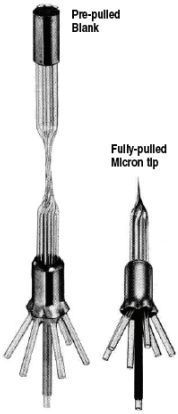
The PLI-100 Pico-Injector reliably delivers a wide range of volumes through micropipettes by applying a regulated pressure for a digitally set period of time. Compressed gas allows the user to deliver desired volumes from femtoliters to microliters while simultaneously holding a cell. Whether you need to do large injections into capillaries or very small injections into mammalian nuclei, the PLI-100 is well suited for your experiment.

The **PLI-100** has become a favorite of prestigious national microinjection workshops like Cold Spring Harbor Laboratories and other researchers worldwide. Other companies have tried to design similar systems, but the PLI-100 remains unparalleled in terms of ease of use, durability, precision, and cost. Pico- Injector, like the reliable PLI-100 Pico-Injector, controls the precise and reproducible regulation of injection pressure and time.
Because the **PLI-90** features only the injection, balance, and clearing positive pressures, it is a lower cost alternative to the PLI-100. This simplicity makes it even easier to use than the PLI-100.

Iontophoresis

Neuro Phore BH-2

- **Modular design; buy what you need now and add additional modules later as your protocol evolves**
- **Accommodates 7 barrel micropipettes supports the most complex and demanding injection and recording protocols**
- **Extremely low noise, ±105 volts compliance, allows rapid iontophoretic injection with even the smallest micropipette tips**
- **Automatic current neutralization for minimal electrical artifacts**
- **Digitally controlled eject and pause timing utilizes easy to read and set digital panel switches on each iontophoretic or pressure module/channel**



The **Neuro Phore BH-2 System** was developed under the guidance of active researchers with extensive experience in Iontophoresis techniques. These researchers needed a system to provide precise stimulation and quantitative control for ejection of drugs in their pharmacological studies of drug evoked responses such as neuro-synaptic discharges, contraction, and changes in chemical concentration. What emerged was a reliable, accurate, easy to use, Neuro Phore BH-2 system that is capable of accommodating high impedance multi-barreled micro-electrode pipettes.

Mechanical Injector

- **Infuse/ withdraw capability**
- **Dual syringes for broad flow rate range**
- **Easy-to-read display, the injection rate can be changed from 0.0550 pl/sec to 0.0073 ml/sec**
- **Remote to micromanipulator injections are easy non-obstructed viewing and no heavy weight to hinder positioning**
- **This pump has exceptional milliliter, microliter, nanoliter and picoliter smooth and accurate flow, easily set through the keyboard**
- **The injection can be controlled by initiating the manual start button, RS232 through your computer or with the included foot pedal**
- **Operating parameters are set with the membrane keypad and VFD display**
- **From the keypad the user can set pump to:**
  -Infusion mode
  -Withdrawal mode (reversing switch on back panel)
- **All micro tubing and connectors are available**
- **Volume mode -enter a target volume, pump will stop when value achieved**
- **Adjustable infusion limit switch and adjustable withdraw mechanical stop**

The **Pump 11 Pico Plus** is the lowest flow rate pump that Harvard Apparatus manufactures. It can accurately delivery volumes down to 3.3 picoliters/minute. It is ideal for applications such as cellular injections or for providing the micro flow required for FIA or capillary LC. It has both infusion and withdrawal capabilities as well as RS-232 communications.

Applications:

•Cellular injection i.e. oocytes
•Micro flow for FIA or capillary LC
•Micro reaction delivery
•HPLC post-column reaction pump

Electroporation

- **Specialty electrodes**
- **Cuvettes**
- **High throughput 96-well plates**

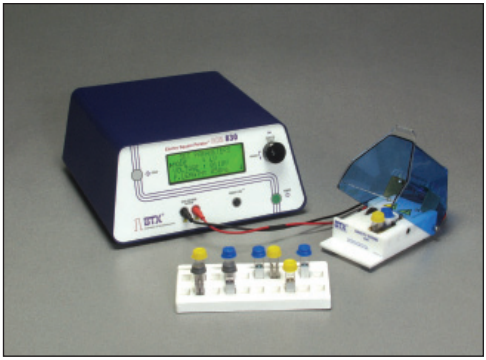
Electroporation is a techniques used to perform research on embryonic stem cells (HESC), adult stem cells, somatic cell nuclear transfer, therapeutic cloning and cancer research.

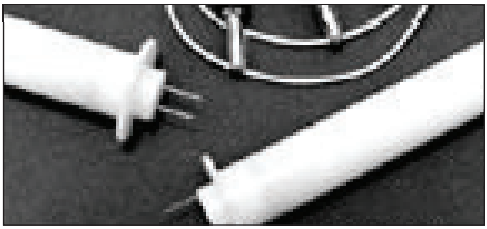
Applications:

- Transfection of Mammalian Cells
- In Vitro, In Vivo, Ex Vivo and In Ovo

Experiments

- Nuclear Transfer
- Plant Tissue and Protoplast Transfection
- Transformation of Bacteria and Yeast



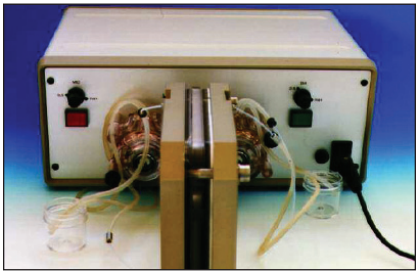


Liposomes

- **Liposomes are used for studying protein-liposome interactions with compounds such as serum lipoproteins, lectins, toxins and clotting components.**
- **Entrapment of anti-tumor agents, anti-microbial drugs, anti-inflammatory and immunomodulatory agents and CNS-active drugs. The therapeutic applications are presently include cancer therapy, arthritis, metal chelation therapy, enzyme replacement therapy, hemophilia (factor VIII), myocardial infarction, and as radiopharmaceutical markers.**
- **Liposomes are used for studying in vitro and in vivo liposome-cell interactions.**
- **They are used for reconstitution experiments especially for ion transport systems.**
- **In molecular biology, liposomes are used for the mediated delivery of macromolecules into eukaryotic cells.**
- **The use of liposomes in gene-based technology and in broader biotechnology and pharmaceutical applications is increasing day by day.**

Today liposomes are an important part of biological, pharmaceutical, and medical research. Because liposomes are the most effective carriers for the introduction of many different types of agents into cells, the applications of liposome-based samples and products are extremely wide.

Liposomat



- **The Liposomat is ideal for the preparation of liposomes of volumes from 3 ml to 50 ml or higher.**
- **The system has two serpentine channels superimposed on each other and separated by a membrane.**
- **Each channel has a volume of 3 ml and a length of 3 meters. The mixed lipid/detergent micelles run through one of the channels while the buffer flows through the other channel.**
- **Due to controlled dialysis and the high surface area in the system, liposomes can be formed within 30 minutes.**

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Harvard Apparatus Information

GETTING TO KNOW HARVARD APPARATUS!

Since 1901, **Harvard Apparatus** has been serving the global bioresearch community by developing solutions for the thousands of experimental challenges in animal, organ, tissue, cellular, and molecular bioresearch. Harvard Apparatus offers "Total Solutions"which are utilized in the area of studies of: Physiology, Electrophysiology, Biology, Neurology, Cardiology, Toxicology, Pharmacology , Molecular Biology. Harvard Apparatus is made up of seven groups developing specialized products:

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Harvard Apparatus Sample Preparation -	CPK molecule models to SPE columns for de-salting and equilibrium dialysis in 1 or 96 well plates for T3/T4 measurements or protein-lig and binding studies

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CELLS

Harvard Apparatus/ Warner Instruments	Observing, modifying, recording and maintaining cells under a microscope or ion transport studies through a cell membrane i.e. Ussing Chambers
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