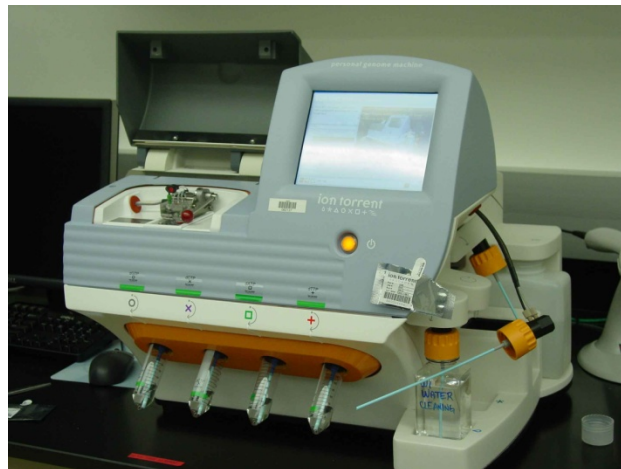


BINF 6350/8350
Fall 2011
Genomic Biotechnology Lab

<http://webpages.uncc.edu/~jweller2>



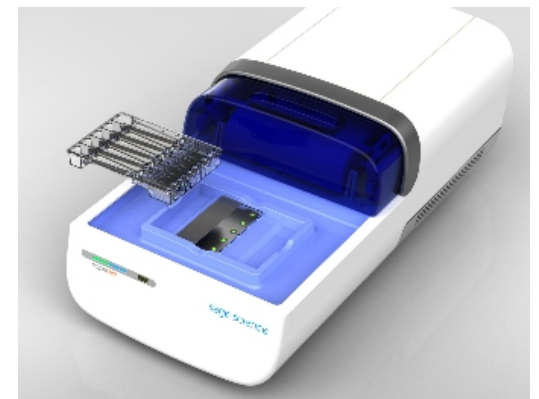
Contents

- Course Conduct, overview
- Genomics versus Bioinformatics
- On-line Industry 'zines
- Methods



Goals

- Learn some basic techniques
 - (Micro)Pipetting
 - Making solutions and dilutions, order of operations
 - Using spectrophotometers and running gels
- Disrupt tissue and purify nucleic acids
 - Experimental design
 - Yields versus representation
- Process nucleic acids to a usable form
 - Microarrays
 - Sequences
 - qPCR



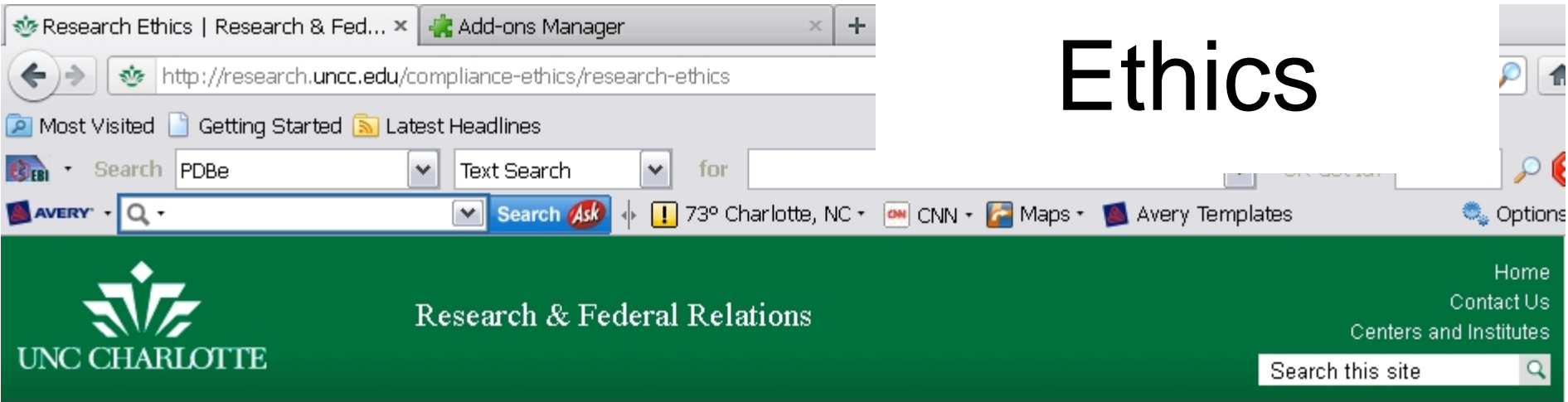
Student Responsibilities

- At the end: read a protocol describing how a sequencing library was created, or set of microarrays, be able to describe what each step does and if any are missing, carry them out yourself and know what QC steps should be done, and explain how the choices affect the way an analysis should be carried out, if any biases were likely caused by the methodology.

Grades

- Come to class! Prepared! (50%)
 - Coordinate with your partner when extra-class attention is needed
- Keep a lab notebook – in pen. (10%)
 - I will check it every two weeks and provide criticism.
- Write up experiments – 4 summaries with full Introduction, Materials and Methods, Results, Discussion, Figures + Tables, References. (20%)
- Project – pick one aspect of library preparation or Ion Torrent that needs improvement. (20%)
 - Describe in complete detail your idea for improving the outcome, explain how you would test it - where the samples come from, exact protocols to use etc.

Ethics



Research Ethics | Research & Fed... x Add-ons Manager x +

http://research.uncc.edu/compliance-ethics/research-ethics

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UNC CHARLOTTE Research & Federal Relations Home Contact Us Centers and Institutes Search this site

– The Responsible Conduct of Research is an essential part of a research scientists job.

- What does it mean in the context of genomics
- At UNCC: <http://research.uncc.edu/compliance-ethics/research-ethics>

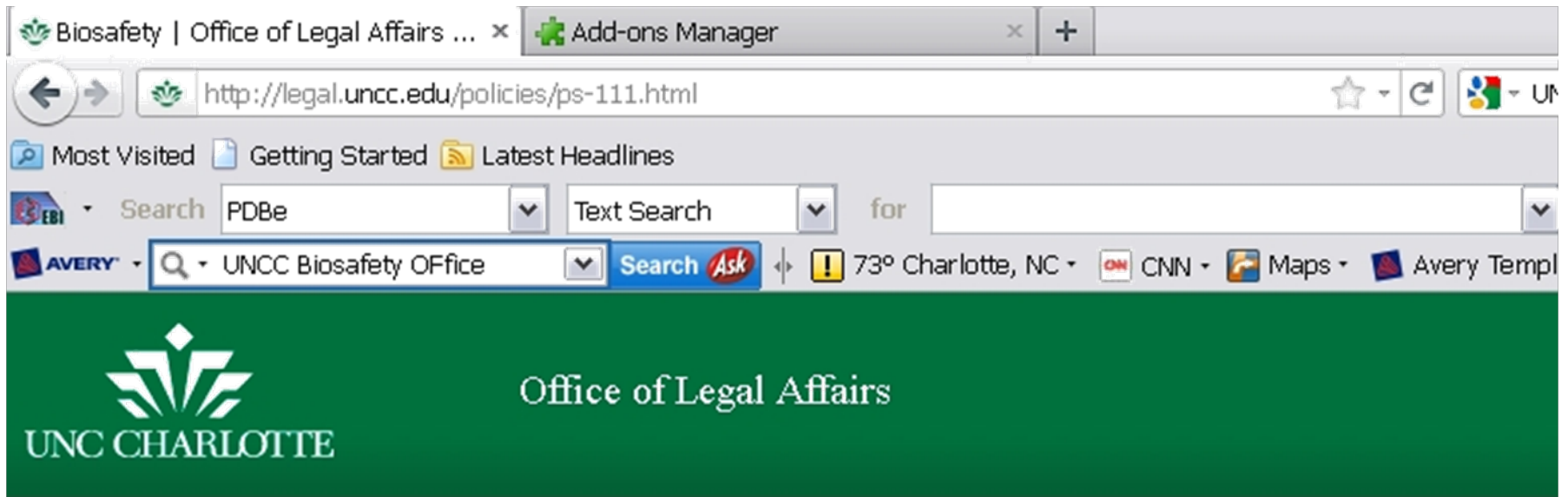
– Academic Integrity policies for the University must be followed.

<http://library.uncc.edu/display/?dept=instruction&format=open&page=920>

in Scientific Research - Harvard Medical School
Search Video Series

ity- US Dept. of Health and Human Services

led from lectures given by Professor Henry Bauer in the Chemistry Department at Virginia Tech



Office of Legal Affairs

Biosafety

Legal Topics

Policies, Regulations, &
Procedures

Legal Services

Meet the Staff

Website Use Policies

- The Molecular Biology Survival Manual – draft form, mostly describes safe use and disposal of materials, how to keep records.
 - A working draft is posted, I will update it.
- UNCC BioSafety site

Material Safety Data Sheets

HC-12a/HC-22a

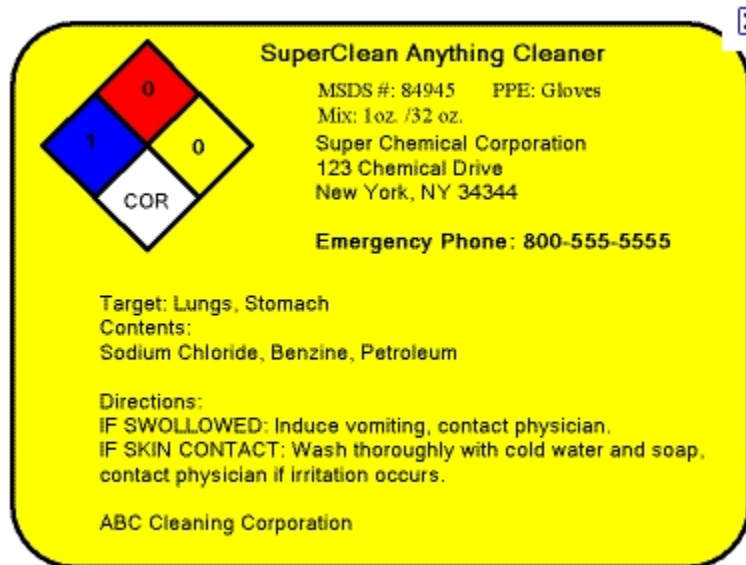
MATERIAL SAFETY DATA SHEET

(Complies with OSHA Communication Standard 29 CFR 1910.1200 Department of Labor)

IDENTITY:		Compressed Gas - Flammable NOS		24-Hour Emergency Telephone Number	
HC-12a		Liquefied Petroleum		Telephone Number	
HC-22a		UN 1954 Class 2		(208) 755-3087	
Section I:					
Manufacturer's Name		Emergency Telephone Number		(208) 687-7000	
Address		Telephone Number for Information		(208) 687-7000	
OZ Technology, Inc.		Date Prepared		April 11, 2002	
10278 N. Church Rd.		Signature of Preparer (Optional)		Not Applicable	
Rathdrum, ID 83858, U.S.A.					
Section II: Hazardous Ingredients / Identity Information					
Hazardous Components (Specific Chemical Identity; Common Name(s))		OSHA PEL	ACGIH	Other Limits Recommended	%(Optional)
Trade Secret - HC-12a/HC-22a					
Compressed Hydrocarbon Mixture		TWA/PEL	OSHA	1800 Mg	100%
		Asphyxiant			
Section III: Physical / Chemical Characteristics					
Boiling Point		Specific Gravity (H ₂ O = 1)			
HC-12a: -29.0° F / HC-22a: -40° F		0.552			
Vapor Pressure (PSIG)		Melting Point			
HC-12a: 72 @ 70° F. / HC-22a: 110 @ 70° F		Not Applicable			
Vapor Density (Air = 1)		Evaporation Rate (Butyl Acetate = 1)			
1.770		Not Available			
Solubility in Water		Ignition Temperature (Method used: Heated Metal Surface)			
Soluble		1490° F.			
Appearance and Odor		Auto-ignition Temperature			
Colorless gas with natural gas odor		1627° F.			
Section IV: Fire and Explosion Hazard Data					
Flash Point (Method Used)		Flammable Limits		LEL	MEL
Not Determined		% Upper 8.5; % Lower 1.9		N/A	N/A
Extinguishing Media					
Use a water spray to cool fire-exposed containers, structures, and to protect personnel.					
Special Fire Fighting Procedures					
Shut off source of flow. Do not extinguish fire if gas source cannot be shut off. Use water spray to disperse gas or vapor and to protect personnel attempting to stop a leak.					
Unusual Fire and Explosion Hazards					
Heavy concentrations of vapor may form flammable mixtures with air. Heavy concentrations of vapor or gas may spread to distant ignition sources and flash back. Vapor or gas may accumulate in low or confined areas. Dangerous when exposed to flame or high temperature sparks. Containers may rupture when heated above their rated pressure values.					

Section V: Reactivity Data			
Stability	Unstable	Conditions to Avoid	
	Stable	X	Heat, Strong oxidizers, Peroxides, Plastics, and Chlorine dioxide
Incompatibility (Materials to Avoid)			
Strong oxidizers, Peroxides, Plastics, and Chlorine dioxide			
Hazardous Decomposition or By-products			
When burned in a deficiency of oxygen, CO can form			
Hazardous Polymerization	May occur	Conditions to Avoid	
	Will not occur	X	Strong oxidizers, Peroxides, Plastics, and Chlorine dioxide
Section VI: Health Hazard Data			
Route(s) of Entry	Inhalation ?	Skin ?	Ingestion ?
	Yes	Yes	Not Applicable
Health Hazards (Acute and Chronic)			
Central nervous system depressant. Asphyxiant Heavy exposure may cause anemia and irregular heart rhythm, respiratory arrest, and death.			
Carcinogenicity	NIP ?	ARC Monographs ?	OSHA Regulation ?
		Presently not on any list	
Signs and Symptoms of Exposure			
Difficulty in breathing, dizziness, euphoria, and irritation of nose and throat. Contact with liquefied material may cause frostbite.			
Medical Conditions Generally Aggravated by Exposure			
Hydrocarbons may sensitize the heart to epinephrine and other circulating catecholamines.			
Emergency and First Aid Procedures			
Do not give epinephrine. Immerse frostbite in cool-warm water. Inhalation: remove from place of exposure. Insure breathing. Give oxygen or CPR if needed.			
Section VII: Precautions for Safe Handling and Use			
Steps to Be Taken in Case Material is Released or Spilled			
No flares or open flames in hazard area. Do not touch or walk through spilled materials. Use water spray to reduce vapors. Isolate and ventilate area until gas has dispersed. No special procedures are required for clean up. Avoid methods resulting in water pollution.			
Waste Disposal Method			
This material is not specifically listed as hazardous waste, but can be classified as hazardous waste when contaminated or if seen as ignitable under (40 CFR261).			
Precautions to Be Taken in Handling and Storing			
Store in tightly closed containers in cool, dry, isolated, well ventilated area away from heat and sources of ignition.			
Other Precautions			
Empty containers may contain flammable or combustible residue vapors. Do not cut, grind, drill, weld, or reuse containers without adequate precautions.			
Section VIII: Control Measures			
Respiratory Protection (Specify Type)			
NIOSH Approved			
Ventilation	Local Exhaust	Yes	Special None
	Mechanical (General)	None	Other None
Protective Gloves		Eye Protection	
Use if in contact with liquid material		Use proper eye protection	
Other Protective Clothing or Equipment			
Long sleeves and long pants			
Work / Hygienic Practices			
Avoid open flames or ignition sources in excess of 1490° F			

Quick MSDS Labels



SuperClean Anything Cleaner

MSDS #: 84945 PPE: Gloves
Mix: 1oz /32 oz.
Super Chemical Corporation
123 Chemical Drive
New York, NY 34344

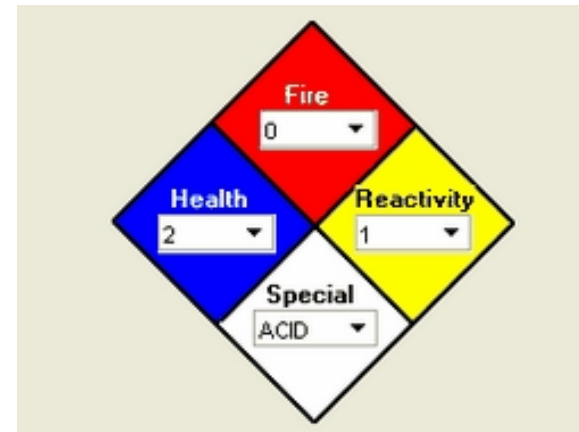
Emergency Phone: 800-555-5555

Target: Lungs, Stomach
Contents:
Sodium Chloride, Benzine, Petroleum

Directions:
IF SWALLOWED: Induce vomiting, contact physician.
IF SKIN CONTACT: Wash thoroughly with cold water and soap,
contact physician if irritation occurs.

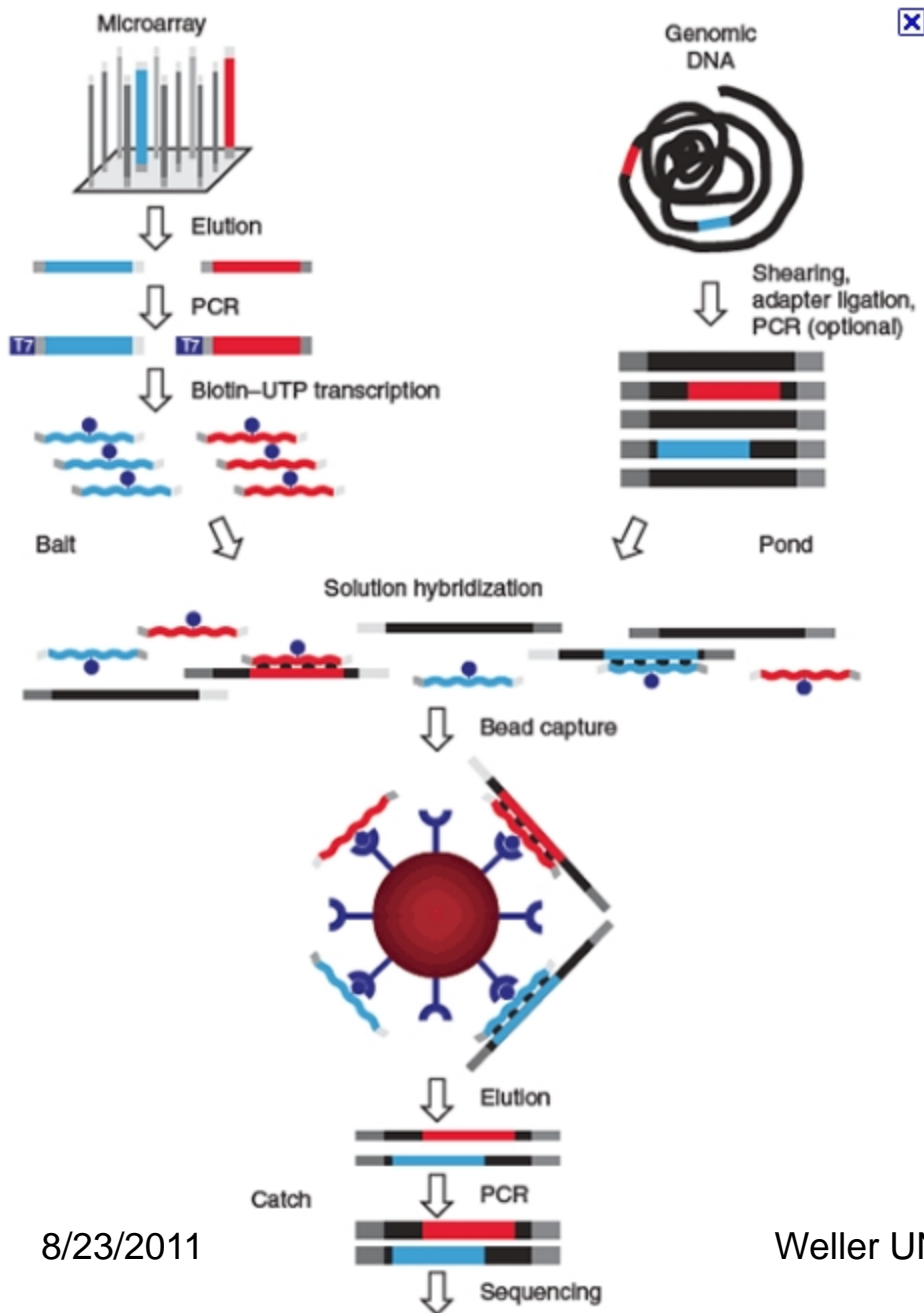
ABC Cleaning Corporation

The label features a diamond-shaped hazard pictogram with four colored sections: red (top) with '0', blue (left) with '1', yellow (right) with '0', and white (bottom) with 'COR'.



An interactive hazard diamond with four colored sections and dropdown menus:

- Red (top): **Fire**, dropdown menu showing '0'.
- Blue (left): **Health**, dropdown menu showing '2'.
- Yellow (right): **Reactivity**, dropdown menu showing '1'.
- White (bottom): **Special**, dropdown menu showing 'ACID'.



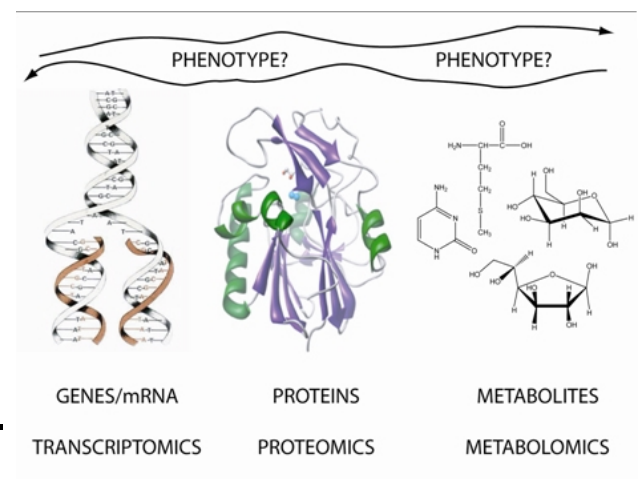
✕

Semester Schedule

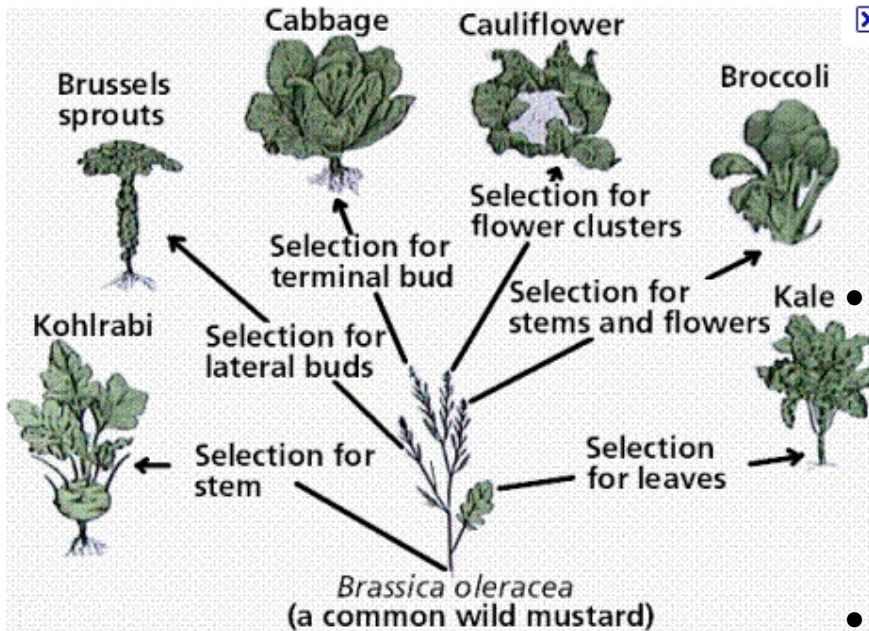
- First 3 weeks: pipetting, dilutions, spectrophotometry, gels, experimental design
- Next 3 weeks: grind up plants, purify DNA and RNA, use Bioruptor on gDNA, do RT-PCR on RNA, design and use PCR primers for specific genes
- Next 4 weeks: purify the amplicons, polish the ends, quantify and do emulsion PCR to make a 'library', perform and Ion Torrent run
- Next 4 weeks: Analyze data, perform Sanger sequencing and analyze that, perform microarray hybridization and analyze that.

Overview: Biotechnology and 'Omics

- Biotechnology: processes in which living organisms convert material to goods and services.
 - Understand the processes (R & D)
 - Scale-up (technology transfer)
 - Monitoring and QC
 - Packaging, marketing, shipping, and sales
- 'Omics - a way of studying biological systems that catalogs the complete 'parts list'
 - genomics (DNA)
 - transcriptomics (mRNA)
 - proteomics (proteins)
 - metabolomics (small molecules)
 - glycomics (carbohydrates)
 - eco-nomics (oh, wait, that's taken)



Origins



- Biotechnology: the 20th century name for the selection applied by humans to the evolution of organisms

- Material + organism → desired transformation.

Useful organisms were identified by accident.

- Offspring were culled.

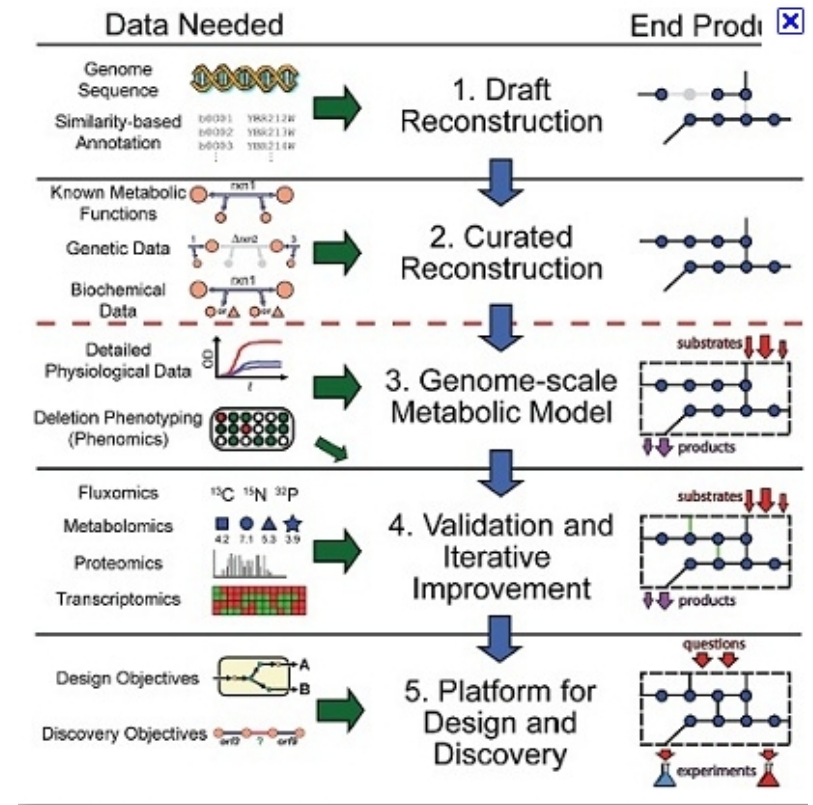
- Record keeping was needed to track recessive traits

- Microorganisms are usually haploid

- Preserving/passaging cultures - eye of newt?

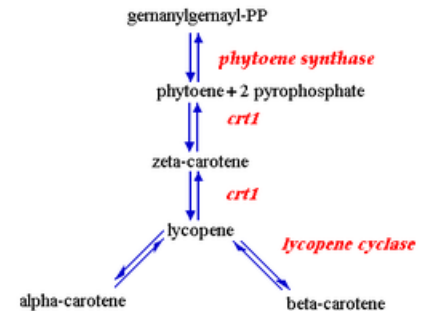
Technology Raiding Disciplines

- Biological Sciences
 - Genetics
 - Molecular biology
 - Biochemistry
 - Microbiology, organismal biology
- Engineering
 - Systems analysis
 - Instrumentation
- Medicine
 - Clinical medicine
 - Immunology
 - Diagnostics
- Computer science
 - Information technology,
- Mathematics
 - Modeling, statistics
- Business- related fields (marketing, etc).



Examples of Goals

- Plants: make naturally resistant to pathogens; fix nitrogen; **include important nutrients (Vitamin A)**
 - The Good: ‘**golden rice**’
 - The Bad: Frankenfood
- Engineer microorganisms to produce
 - Active isomers
 - Enzymes degrading hazardous compounds
- Compared to bulk chemical reactions, aim for more efficient and selective processes.



http://en.wikipedia.org/wiki/Golden_rice

Bioinformatics and Genomics in Technology

- Take a systems approach (fewer unintended consequences)
 - Identify component molecules
 - Model complex processes and interactions
 - Document data handling and analysis for regulatory agencies
 - Knowledge Discovery: pattern-finding, new analysis and visualization tools

Issues of current interest

- Biomedical (majority of members)
regulation
 - Stem Cell research
 - Reproductive medicine
- Biodefense, forensics, food and water monitoring
- Climate change, alternate energy sources
 - Cellulosic biofuels
- Bioremediation

BIO: Biotechnology Industry Organization

Bio membership
Join, renew, or learn about BIO member benefits

Monday, January 18, 2010

Biotech in the Home

Biotechnology is all around us, especially in our homes. From new household cleaners and detergents made with biotech enzymes, to our contact solution and the paper we use every day at home and in the office.

BioBytes: Biotech in the home

★★★★★

Find the latest information about Biosimilars here

Bio International Convention
The Global Event for Biotechnology
May 3-6, 2010
McCormick Place
Chicago, IL

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BIO News

January 8, 2010

Leaders in Science, Advocacy, Media to Judge 2010 Biotech Humanitarian Award

Today, the Biotechnology Industry Organization (BIO) announced a distinguished panel of judges who will evaluate nominations for [The Biotech Humanitarian Award](#) and select the 2010 Honoree.
[Read the news release](#)
January 8, 2010

Provisions in the Senate Health Care Bill Help Patients, Promote Innovation, Encourage Job Growth

BIO Applauds Provisions in Senate Health Reform Bill. Provisions provide early Christmas present to patients while promoting continued innovation and job growth.
[Read the news release](#)
Thursday, December 24

News & Insights from [Biotech Now](#)

[Nominate Your Local Biotech Here](#)
[BioNJ to Kick Off 2010 with 17th Annual Meeting to Support New Jersey's Biotech Innovation](#)
[BioBytes: Biotechnology and Endangered Animals Download Podcast](#)
[Touring CA Biotech Companies with Governor Howard Dean, MD](#)
[This Week in Biotechnology](#)

Upcoming Events

[7th Annual BIO Asia Partnering Conference](#)
January 25-26, 2010
Tokyo, Japan

[12th Annual BIO CEO & Investor Conference](#)
February 8-9, 2010
New York, NY

- Note that this is an industry site.
- It has an Ethics section, as well as new products, patents and emerging market information, meetings and job ads.
- <http://www.bio.org/>

On-line “Trade Rag” for Genomics

The screenshot shows the homepage of the Genome Technology website. The header includes the 'genomeweb' logo and 'Genome Technology' text, with a 'Log in or Register' link. A navigation menu contains 'Home', 'News', 'Magazine', 'Blogs', and 'Careers'. Below this is a search bar with 'Google Custom Search' and a 'GO' button. A secondary menu lists 'Arrays', 'Dx/PGx', 'Informatics', 'PCR', 'Proteomics', 'RNAi', and 'Sequencing'. The date 'Monday, January 18, 2010' is displayed in the top right.

The main content area is divided into several sections:

- Cover Story:** 'Reaching for Potential' with a sub-headline 'Kudos to this year's young investigators.' and a thumbnail image of a magazine cover titled 'Tomorrow's Pls'.
- Feature Story:** 'Making Sense of the Neandertal Genome' by Ed Green.
- Gene Fusions and Transcriptome Sequencing** by Christopher Maher.
- Getting to the Nanoscale** by Vincent Tabard-Cossa.
- Sensing Small Stuff for Medicine** by Ryan Bailey.
- Developing Algorithms for Cancer Genes** by Gurinder Abwal.
- Exploring Autoimmune Diseases with GWAS** by Jeffrey Barrett.

On the right side, there are several promotional and news blocks:

- Most Viewed:** A list of five 'GenomeWeb Daily News' items, including AstraZeneca/Dako team on Companion Dx, NIH issues request for SBIR/STTR applications, court approval of Decode assets sale, JP Morgan Healthcare Conference, and Life Technologies early-access program.
- Choose Biosearch for Kit Manufacturing & OEM Services:** An advertisement for Biosearch Technologies.
- Trust your research to IDT Quality oligos. Every time. 100%:** An advertisement for IDT DNA with the website 'WWW.IDTDNA.COM'.
- Hamamatsu:** An advertisement for Hamamatsu equipment with the headline 'Gear-up to win the throughput race' and a photo of a piece of equipment. Below the photo are links for 'Young Investigator Profile', 'Blog', 'Papers of Note', and 'People on the Move'. A note states 'Accelrys has appointed Michael Piraino to be senior'.
- Applied Biosystems:** An advertisement for Applied Biosystems with the headline 'A Portfolio of Real-Time Possibilities. Just for Me.' and a photo of a man.

Search site GO



[\(view larger image\)](#)

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Thermo SCIENTIFIC Accurate and consistent results are easier to obtain
Solaris Assays are now available to the mouse genome

Solaris qPCR Gene Expression Assays

Breaking News *updated throughout the day*

Algeta Taps the Institute for Energy Technology for Commercial Manufacture of Alpharadin

Scientists Report Successful Use of Ex Vivo-Expanded Cord Blood as Transplants in Leukemia Patients

Researchers Identify Gene Variants Influencing Insulin/Glucose Regulation

Studies Find Link between Alzheimer Disease, Down Syndrome, and Atherosclerosis

Quintiles and Invivodata Join Forces to Offer ePRO Solutions

[VIEW BY SUBJECT](#) | [MORE NEWS](#) | [RSS](#)

Industry Alerts

Owens & Minor to Release 4th Quarter & Full Year 2009 Financial Results on Monday, February 8, 2010

Carl Zeiss Meditec Continues Successful Growth Strategy with New Chief Executive Officer

Diabetes epidemic in First Nations adults, especially women in prime reproductive years

Four Leading Pharmaceutical Companies Select Cegedim Dendrite's Customer Master Data Management Solution

British Columbia Securities Commission: Updated InvestRight Guide Makes It Easy for Investors to Work With Their Investment Advisors

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Special Report

Stem Cell Utility Limited by Lack of Ethnic Diversity

In December 2009, NIH director, Francis S. Collins, M.D., Ph.D., approved 40 human embryonic stem cell (hESC) lines for NIH-funded research under the NIH Guidelines for Human Stem Cell Research adopted last July. While investigators breathed a sigh of relief, scientists from Cedars Research Institute



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WEBINARS

GEN Webinars: Use of Human Cells For Manufacturing Human Therapeutics

Date: Wednesday, January 27, 2010

This webinar will provide a brief history of the development of various cell lines and their current... [Learn more.](#)

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Omics

The complete sequencing of the human genome has ushered in a new era of systems biology referred to as omics. This has transformed cell biology in academia and industry from a cottage industry in which one gene or protein is studied at a time to a world in which whole organelles and pathways are studied simultaneously. The term omics refers to the comprehensive analysis of biological systems. A variety of omics subdisciplines have begun to emerge, each with their own set of instruments, techniques, reagents and software. The omics technology that has driven these new areas of research consists of DNA and protein microarrays, mass spectrometry and a number of other instruments that enable high-throughput analyses. Likewise, the field of bioinformatics has grown in parallel and with the help of the internet, rapid data analysis and information exchange is now possible. Omics will not only have an impact on our understanding of biological processes, but the prospect of more accurately diagnosing and treating disease will soon become a reality. However, new technology is developing constantly and quickly, so it is important that researchers keep up to date with the latest protocols, commercial products and other sources of information. OmicsWorld was developed as a portal to link investigators to the wide variety of resources that are currently available in specific omics fields. We hope that this site will serve as a valuable tool in this endeavor.

MicroarrayWorld

RESOURCES FOR
MICRO ARRAYS
AND EXPRESSION
PROFILING

ProteomicWorld

RESOURCES FOR
PROTEOMICS AND
PROTEIN EXPRESSION

GenomicWorld

RESOURCES FOR
GENOMICS AND
GENETICS

ApoptosisWorld

RESOURCES FOR
STUDYING
CELL DEATH

RNA Interference

Database of
siRNA
sequences

Real Time Primers

Validated Primer Sets
for Quantitative PCR

Gen-omics

Genomics may be described as the comprehensive analysis of DNA structure and function. Understanding biological diversity at the whole genome level will yield insight into the origins of individual traits and disease susceptibility. Though organisms such as humans are quite similar at the genetic level, differences exist at a frequency of about 1 in every 1000 nucleotide bases. This translates into approximately 3 million base differences between each individual. Such changes are referred to as single nucleotide polymorphisms (SNPs) and a significant effort is now underway in the research community to map the individual SNPs in humans and other organisms. SNPs may be found within gene coding regions or in non-coding regions. Their effects may be subtle yielding slight changes in protein function or profound, leading to the development of disease. A polymorphism is distinct from a mutation. The latter is considered rare, affecting less than one percent of the species, whereas a polymorphism is relatively common and its prevalence is no different to what is considered normal. Over the last decade, there has been an unprecedented surge of data directed at sequencing and categorizing all of genes in the human genome as well other organisms. There has also been a concomitant acceleration in the technology dedicated to genomics research including instrumentation, reagents, software and databases.

Bioinformatics

<http://www.bioinformaticsweb.org/>

Bioinformaticsweb.org

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Breaking Story

Statistical Aspects of Datamining-Video Course-Watch Online

Comments (0)

Posted on 27 August 2009

Data Mining is used to discover patterns and relationships in data, with an emphasis on large observational data bases. Despite the obvious connections between data mining and statistical data analysis, most of the methodologies used in Data Mining have so far originated in elds other than Statistics.

 [Video, bioinformatics](#)

Paperless Ph.D Workflow-Free Video Tutorial

07 July 2009

This work flow includes RSS feeds from PubMed, reading abstracts in NetNewsWire, and finally downloading and archiving the article PDF

 [Tutorials & Tips](#)

Comments (0)

Introduction to Machine Learning-Free Video Lecture

07 July 2009

Provides a broad introduction to machine learning and statistical pattern recognition. Topics include supervised learning, unsupervised learning, learning theory, reinforcement learning and adaptive control. Recent applications of machine

BACKGROUND

The Cell Cycle-Video tutorial

Posted on 19 February 2009

The Cell Cycle-Video tutorial The cell cycle, or cell-division cycle, is the series of events that take place in a cell leading to its replication. In prokaryotes, the cell cycle occurs via a process termed binary fission. In eukaryotes, the cell cycle can be divided in two brief periods: interphase —during which the cell grows, accumulating nutrients [...]

BIOLOGY BASICS

The Cell Cycle-Video tutorial

Posted on 19 February 2009

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CAREER

Bioinformatics-India-List of Institutes offering Weller UNCC



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biotechnology.jhu.edu

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8/23/2011

In the Lab (12:30-4:45)

- Safety walk-around (labcoats, safety glasses, fire extinguisher, eyewash, shower)
- Equipment recognition, safety
 - Centrifuges, UV light, chemicals, sharps
- Partners and bench space
- The micropipette and balance
- Lab notebook
- Activities

Use and Maintenance of Micro-pipettes



Introduction

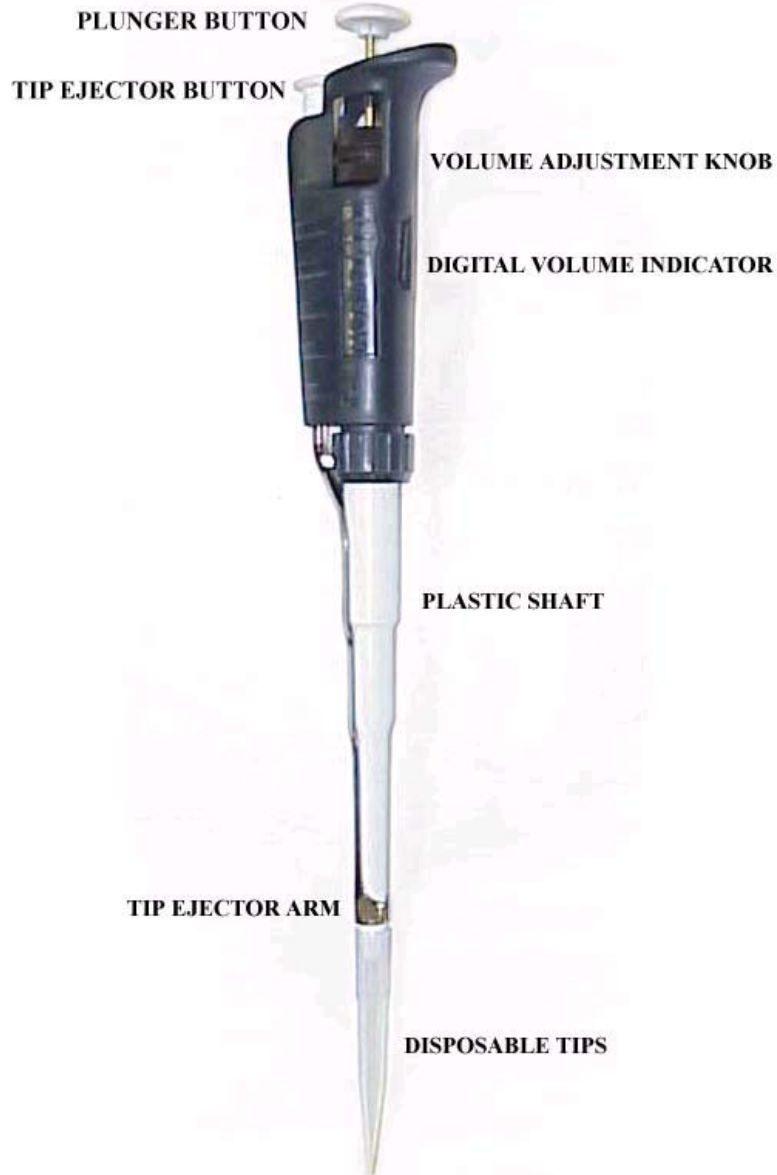
- Automatic pipettes are used to accurately transfer small liquid volumes
- Glass pipettes are not highly accurate for volumes less than 1 milliliter (1 ml), but the automatic pipettes are both accurate and precise
- These are continuously adjustable digital pipettes
- Each pipette can be set to transfer any volume within its own volume

The Automatic Pipette

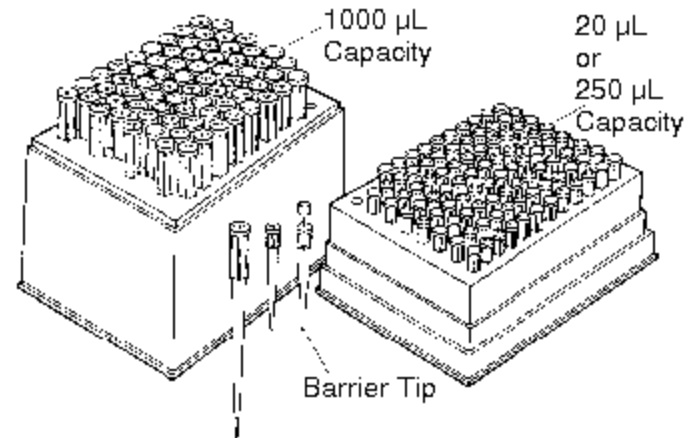
Make sure you know how to

- Select the proper automatic pipette to transfer a specified volume of sample
- Set a specified volume on the pipette volume indicator using the volume adjustment knob
- Read a digital volume setting in both micro liter (μl) and milliliter (ml) units
- Demonstrate the correct technique to accurately transfer a sample of a stock solution to another vessel
- Correctly answer questions based on the material for the Automatic Pipette

Parts of the Automatic Pipettor



Parts of the Pipette



Operating the Micropipette

Step 1: Set the Volume



Operating the Micropipette

Read the Volume



(a): P-20 Model
6.86 μl = 0.00686
or 6.86 x 10⁻³ ml



(b): P-200 Model
132.4 μl = 0.1324
or 1.324 x 10⁻¹ ml



(c): P-1000 Model
262 μl = 0.262
or 2.62 x 10⁻¹ ml

Operating the Micropipette

Step 2: Attach the Disposable Tip



Operating the Micropipette

Step 3: Depress the Plunger to the First Stop



Step 4: Immerse Tip in Sample



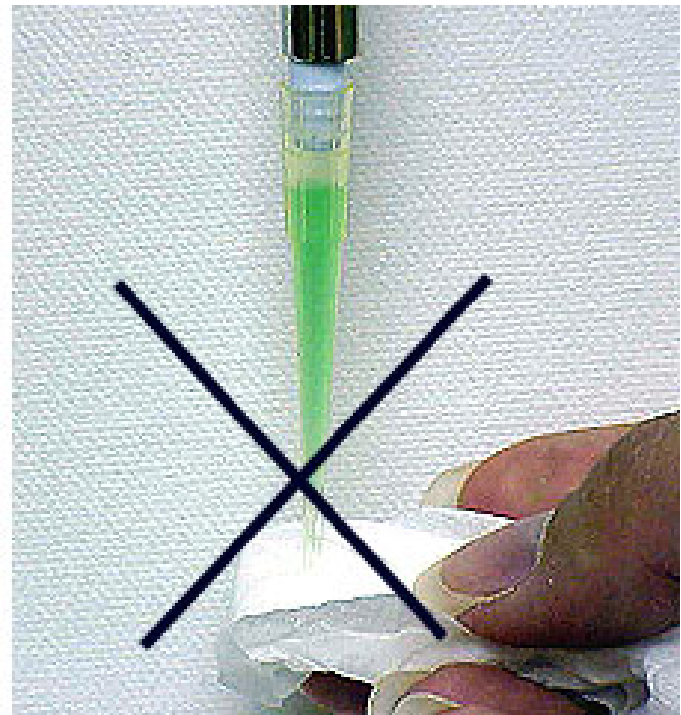
To aspirate the sample into the tip, allow the pushbutton to return slowly and smoothly to the fully extended UP POSITION.

NEVER LET THE PLUNGER SNAP UP! This draws the exact calibrated volume into the tip if the tip remains below the liquid surface during withdrawal.

Wait a few seconds to ensure that the full volume of sample is drawn into the plastic tip. **WAIT LONGER FOR LARGER VOLUMES. WAIT LONGER FOR MORE VISCOUS ("SYRUP-LIKE") SUBSTANCES.**

Operating the Micropipette

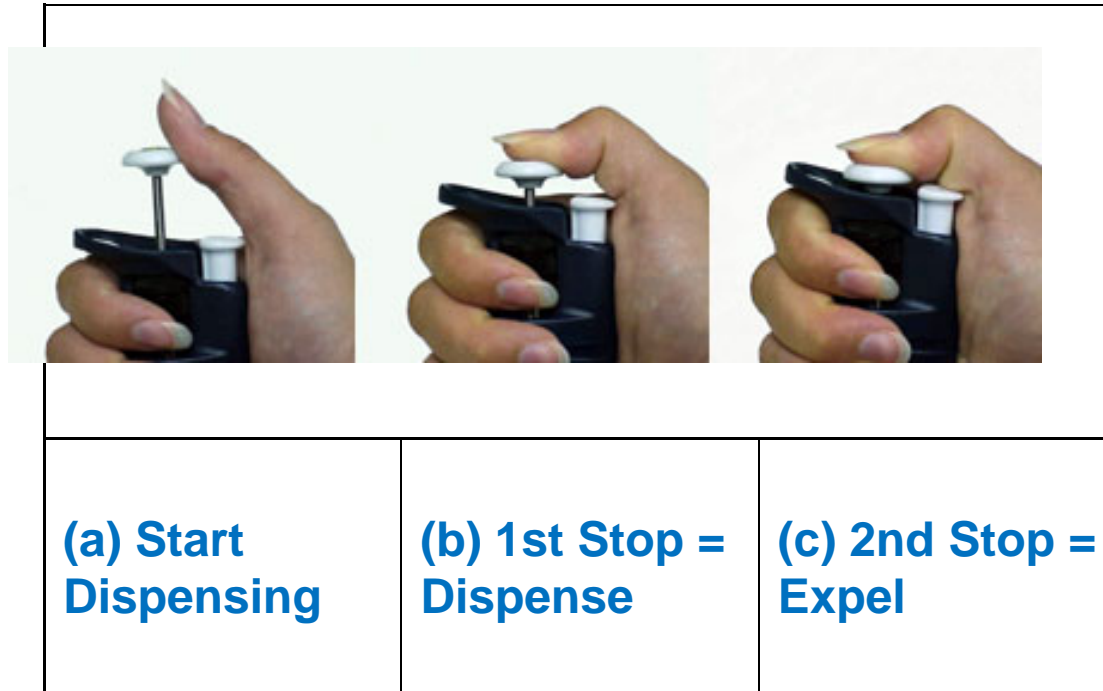
Remove the tip from the sample liquid. No liquid should remain on the **OUTSIDE** of the tip. Wipe away any droplets on the outside of the tip with a lint-free tissue, such as **KIMWIPES**, but only wipe droplets from the side of the tip. **NEVER TOUCH THE TIP OPENING** or you may absorb part of your sample.



Operating the Micropipette

To dispense the sample from the pipette:

- a) Touch the tip end to the side wall of the receiving vessel and
- b) Depress the plunger to the **FIRST STOP**.
- c) Pause for at least one second-- 1-2 seconds for P-1000, 2-3 seconds for P-5000, or longer for viscous liquids.
- d) Press the plunger to the **SECOND STOP** (the second point, of greater resistance, at the bottom of the stroke) to expel any residual liquid in the tip (like "blowing out" a glass pipette).



Operating the Micropipette

Step 9: Withdraw the Pipette

With the plunger fully depressed, withdraw the pipet from the receiving vessel carefully, sliding the tip along the wall of the vessel. Holding the tip against the side of vessel is especially important when transferring small volumes of liquid.



Step 10: Release the Plunger

Gently allow the plunger to return to the UP position. **DO NOT** allow it to **SPRING BACK!**



Equipment and Supplies

- A set of micropipettes and tips
- Several capped sample vials
- Sample solution to practice volume transfer



Step-wise Operation of the Automatic Pipette

- 1) Set the volume
- (2) Attach disposable tip
- (3) Depress the plunger to the first stop
- (4) Immerse tip in sample
- (5) Draw up the sample
- (6) Pause
- (7) Withdraw the tip
- (8) Dispense the sample
- (9) Withdraw the pipette
- (10) Release plunger
- (11) Discard the tip



Accuracy and Precision

- Accuracy means the closeness with which the dispensed volume approximates the volume set on the pipette
- Accuracy is specified as mean error, the average deviation of replicate measurements from the expected set volume
- Precision is the "scatter" or reproducibility of individual measurements of the same volume
- Precision can also be expressed as standard deviation

Accuracy and Precision (Continued)

- Relative accuracies are generally about 1% or less
- Precision is less than 0.5 % except when transferring the smallest recommended volume for a given pipette model
- Using the pipettes to transfer volumes which are below the recommended range will introduce larger errors

Pipetting Guidelines and Precautions

For optimal reproducibility, use the following pipetting procedures:

- (1) Consistent **SPEED** and **SMOOTHNESS** when you press and release the **PLUNGER**
- (2) Consistent pressure on the **PLUNGER** at the **FIRST STOP**
- (3) Consistent and sufficient **IMMERSION DEPTH**
- (4) Nearly **VERTICAL POSITIONING** of pipette
- (5) **AVOID ALL AIR BUBBLES:** Since the plastic pipette shaft can be damaged if liquids are drawn beyond the tip into the shaft
- (6) **NEVER** lay the pipette on its **SIDE** nor **INVERT** the pipette if liquid is in the tip

Practice with Pipettes

- Practice using the pipette
- Practice setting a few volumes
- Practice reading the digits of set volumes
- Practice drawing up and dispensing samples
- Get the "feel" of the 1st and 2nd stops
- Practice "blowing out" the pipette



Checking the volume

- If you know the density of the liquid you are pipetting, and you have a calibrated mass balance, you can pipette a specified volume into a container and weight it
 - Weigh the container first (or tare it)



Keeping a lab notebook

- Capture the workflow, both intended and real.
- Name and Date on the notebook, waterproof ink for all notations.
- Leave room at the beginning for a table of contents
- Experiments are recorded
 - » The plan with detailed notes (what to prepare ahead)
 - » What happened – measurements, source of reagents, observations, loss of attention, etc.
 - » Explanations as the process occurs
- Make time at the end of the day to summarize results and observations, recommendations for the next lab
- Do not skip pages

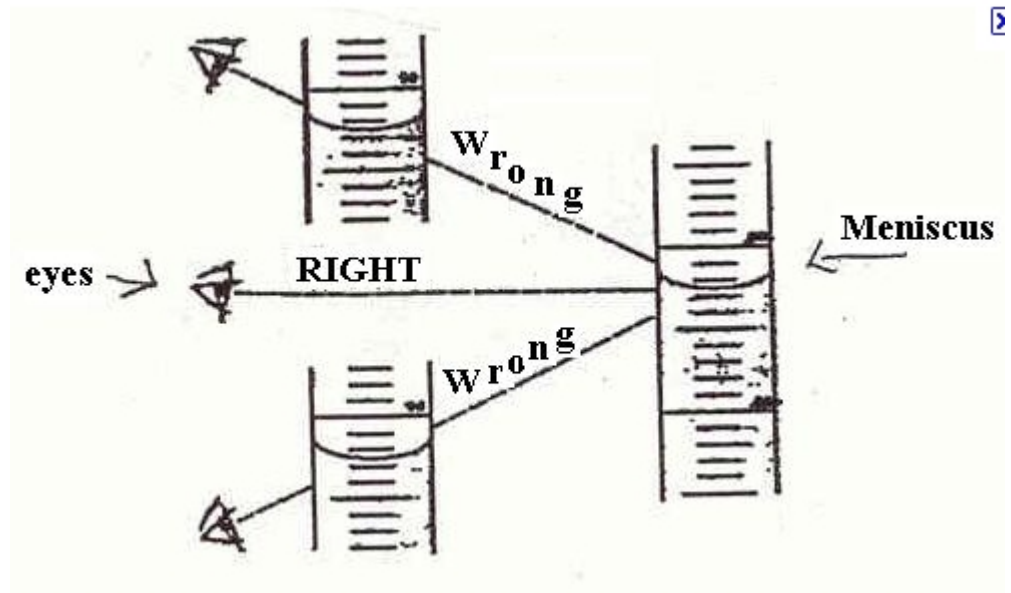
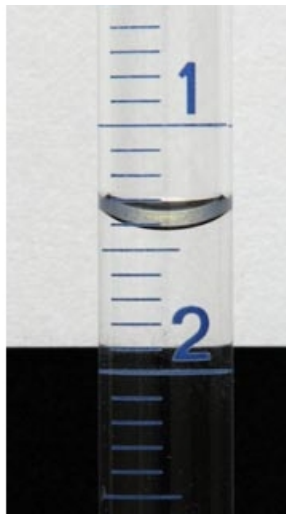
Solutions and Dilutions

- Solutions are of two types
 - Molar solutions
 - Percents: 1% is 1 part per 100, for example (mass-volume versus volume-volume)
- Reagents are combinations of stock solutions:
TE buffer is 10mM Tris-HCL, 1mM EDTA
 - Stocks are 1M Tris-HCl, 100mM EDTA
- How much should you make at one time?
Should you aliquot it? Where should you store it?
- **Label EVERYTHING!!**

Handling solutions

- You are using very small volumes and biological materials
 - How do you check?
- Temperature matters
- Shearing matters
 - Mix by pipetting
 - Mix by vortexing
- Loss of solution to surface tension matters
 - Usual to quick-spin in a microfuge (balance!!!)
 - How do you check?

Pipette use pointers



8/23/2011

Solution Delivery

