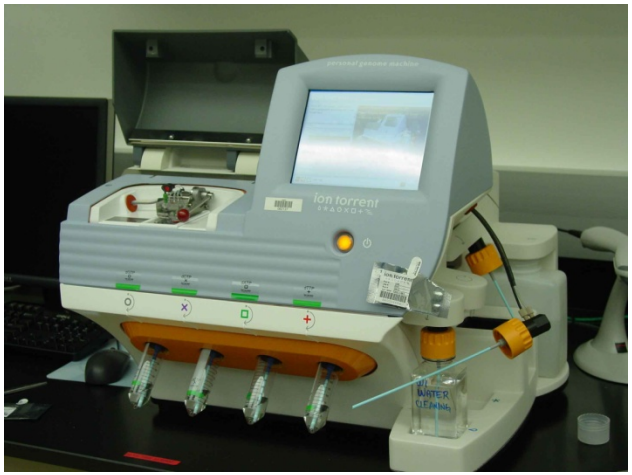
BINF 6350/8350
Fall 2011
Genomic Biotechnology Lab

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

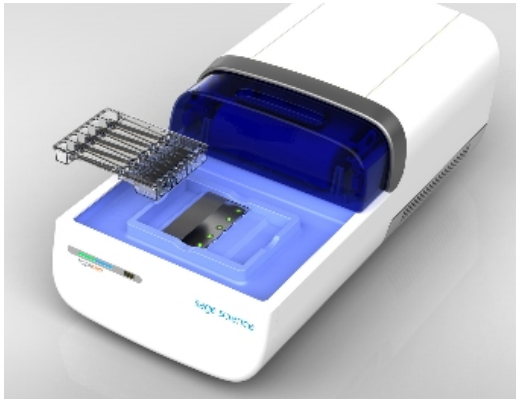


Contents

- 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.

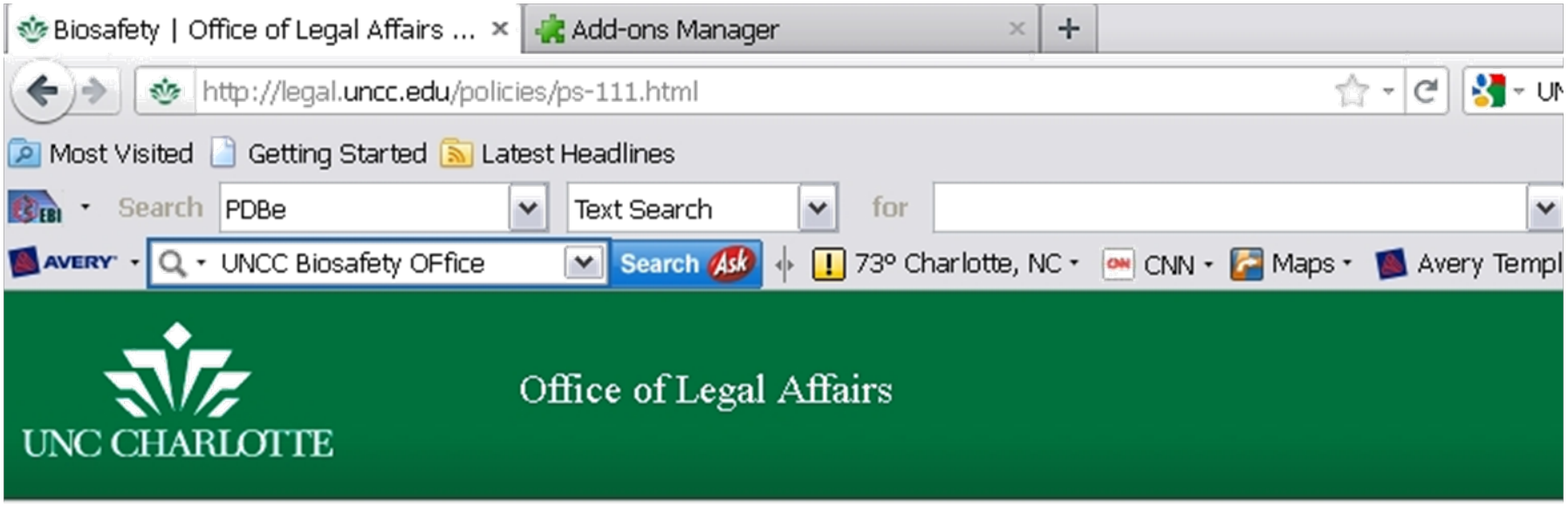
Grades

- 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

- 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



- 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:
HC-12a
HC-22a

Compressed Gas - Flammable NOS
Liquefied Petroleum
UN 1954 Class 2

24-Hour Emergency Telephone Number
(208) 755-3087

Section I:

Manufacturer's Name
OZ Technology, Inc.

Address
10278 N. Church Rd.
Rathdrum, ID 83858, U.S.A.

Emergency Telephone Number
(208) 687-7000

Telephone Number for Information
(208) 687-7000

Date Prepared
April 11, 2002

Signature of Preparer (Optional)
Not Applicable

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
HC-12a: -29.0° F / HC-22a: -40° F

Specific Gravity (H_2O = 1)
0.552

Vapor Pressure (PSIG)
HC-12a: 72 @ 70° F. / HC-22a: 110 @70° F

Melting Point
Not Applicable

Vapor Density (Air = 1)
1.770

Evaporation Rate (Butyl Acetate = 1)
Not Available

Solubility in Water
Soluble

Ignition Temperature (Method used: Heated Metal Surface)
1490° F.

Appearance and Odor
Colorless gas with natural gas odor

Auto-Ignition Temperature
1627° F.

Section IV: Fire and Explosion Hazard Data

Flash Point (Method Used)
Not Determined

Flammable Limits
% Upper 8.5; % Lower 1.9

LEL N/A

UEL 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			Conditions to Avoid
	Unstable		
	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			Conditions to Avoid
	May occur		
	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 anomia 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

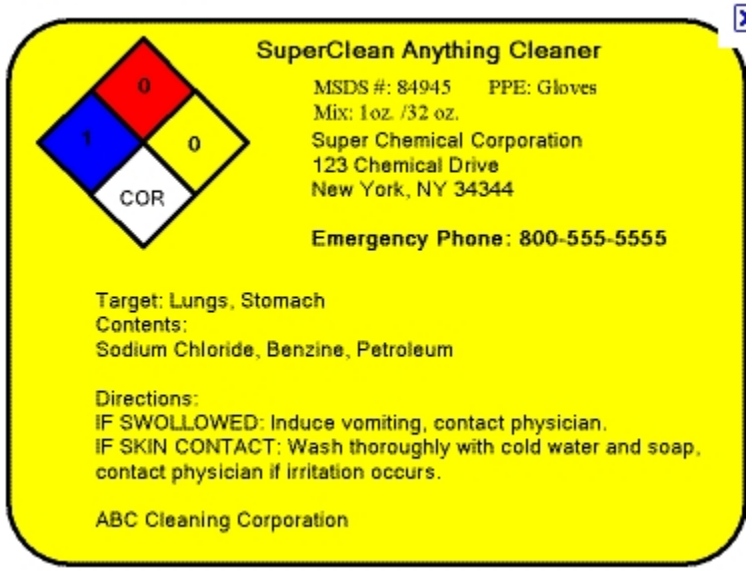
Protective Gloves
Use if in contact with liquid material

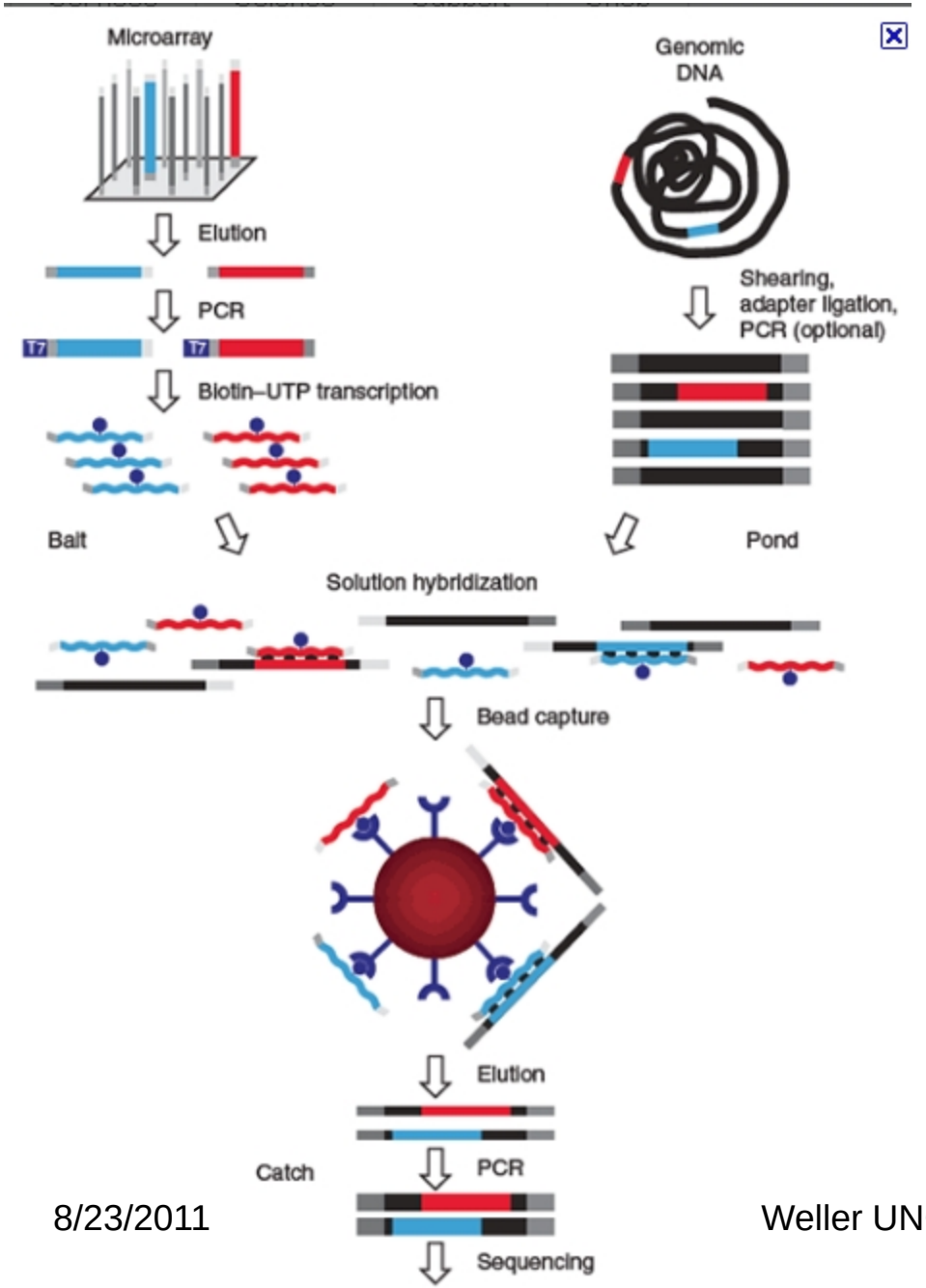
Eye Protection
Use proper eye protection

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



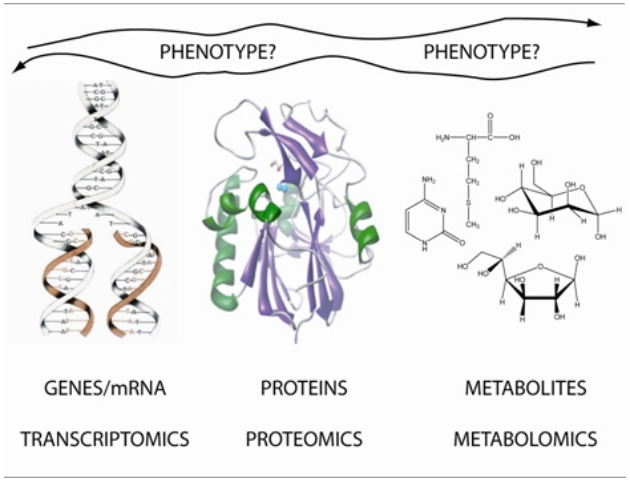


Semester Schedule

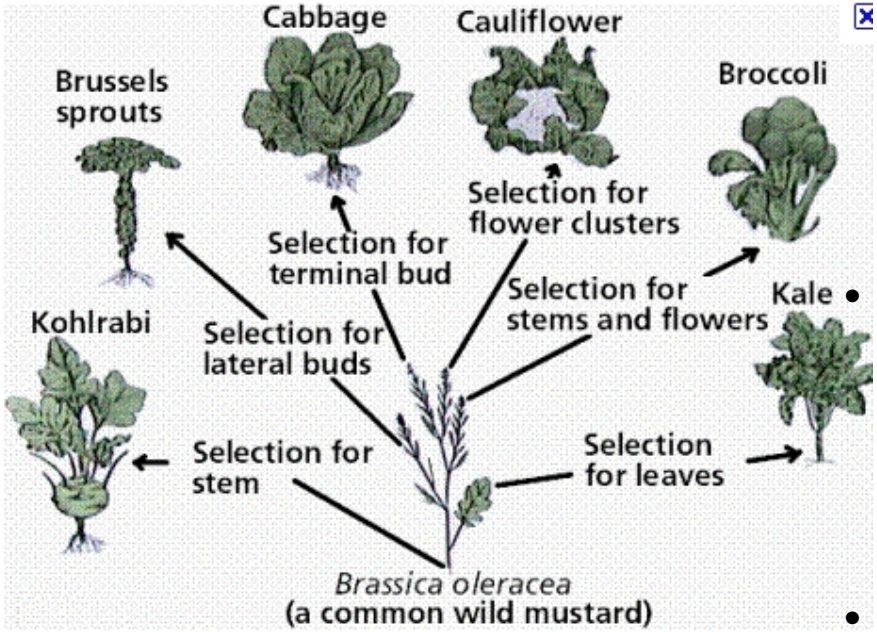
- First 3 weeks: pipetting, dilutions, spectrophotometry, gels, experimental design
- Next 3 weeks: grind up plants, purify DNA and RNA, use Bioruptor on gDNA, do RT-PCR on RNA, design and use PCR primers for specific genes
- Next 4 weeks: purify the amplicons, polish the ends, quantify and do emulsion 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.

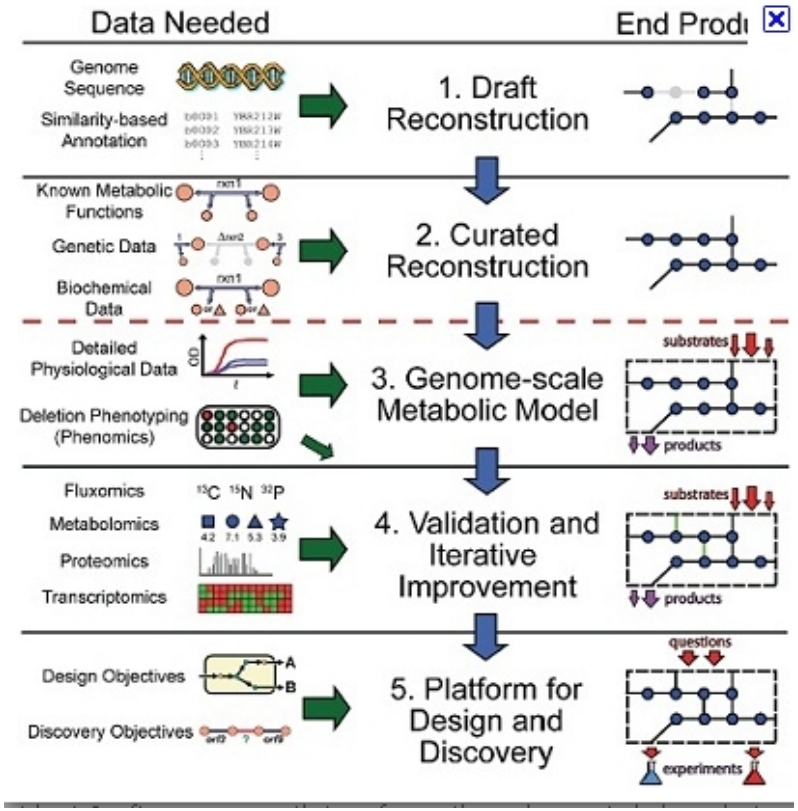
- Offspring were culled.
  - Record keeping was needed to track recessive traits

Microorganisms are usually haploid

- Preserving/passaging cultures - eye of newt?

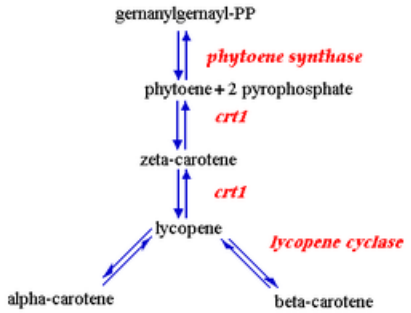
Technology Raiding Disciplines

- 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

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

- Compared to bulk chemical reactions, aim for more efficient and selective processes.

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

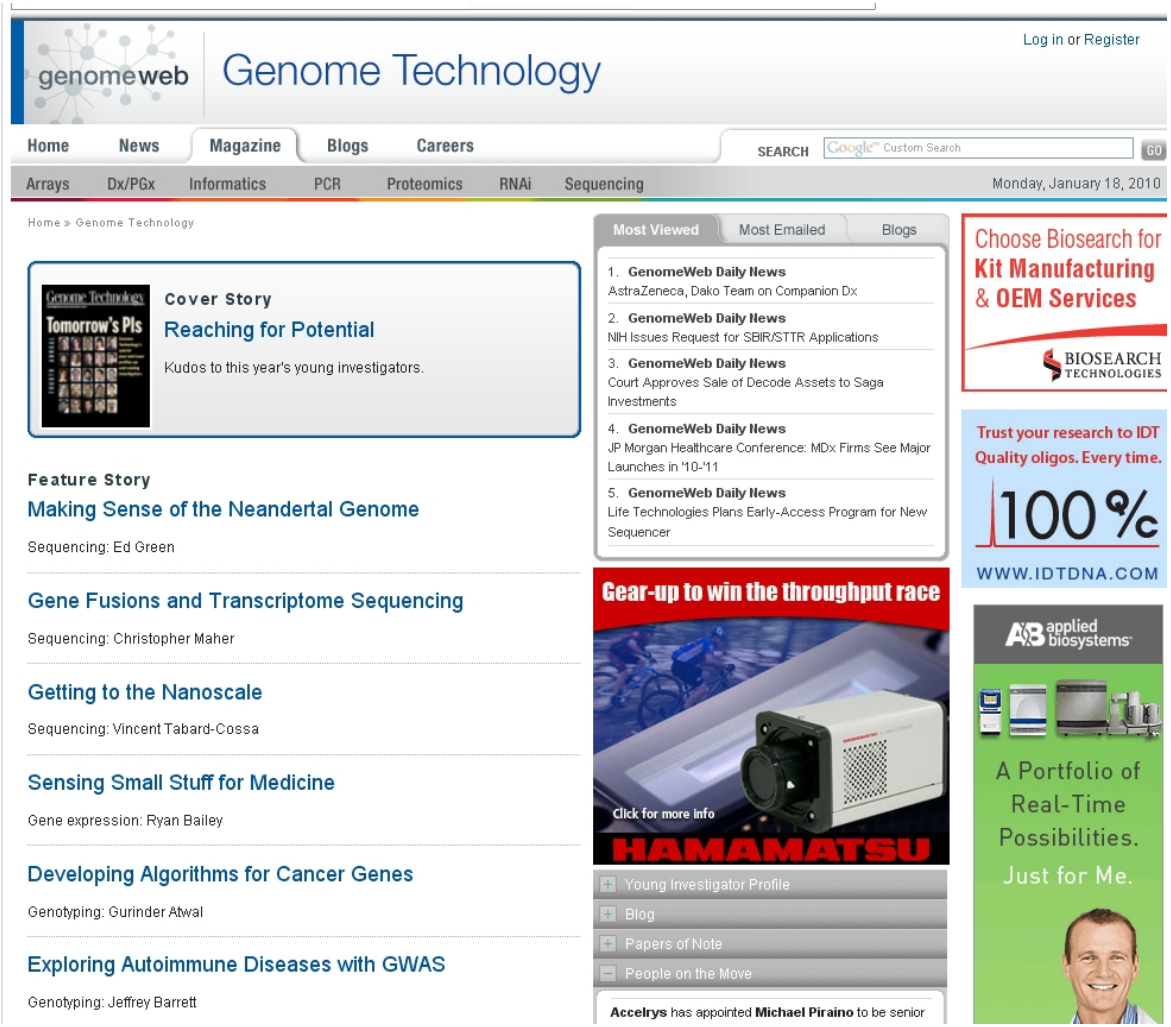
Issues of current interest

- 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

- 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



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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

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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,

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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.

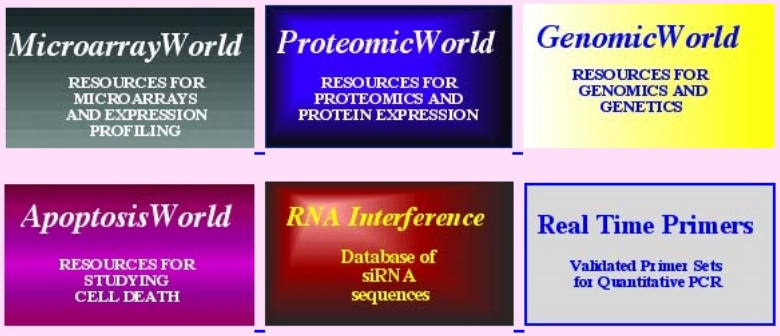
view all webinars

OmicsWorld

RESOURCES FOR GENOMICS, TRANSCRIPTOMICS AND PROTEOMICS

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.



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/

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

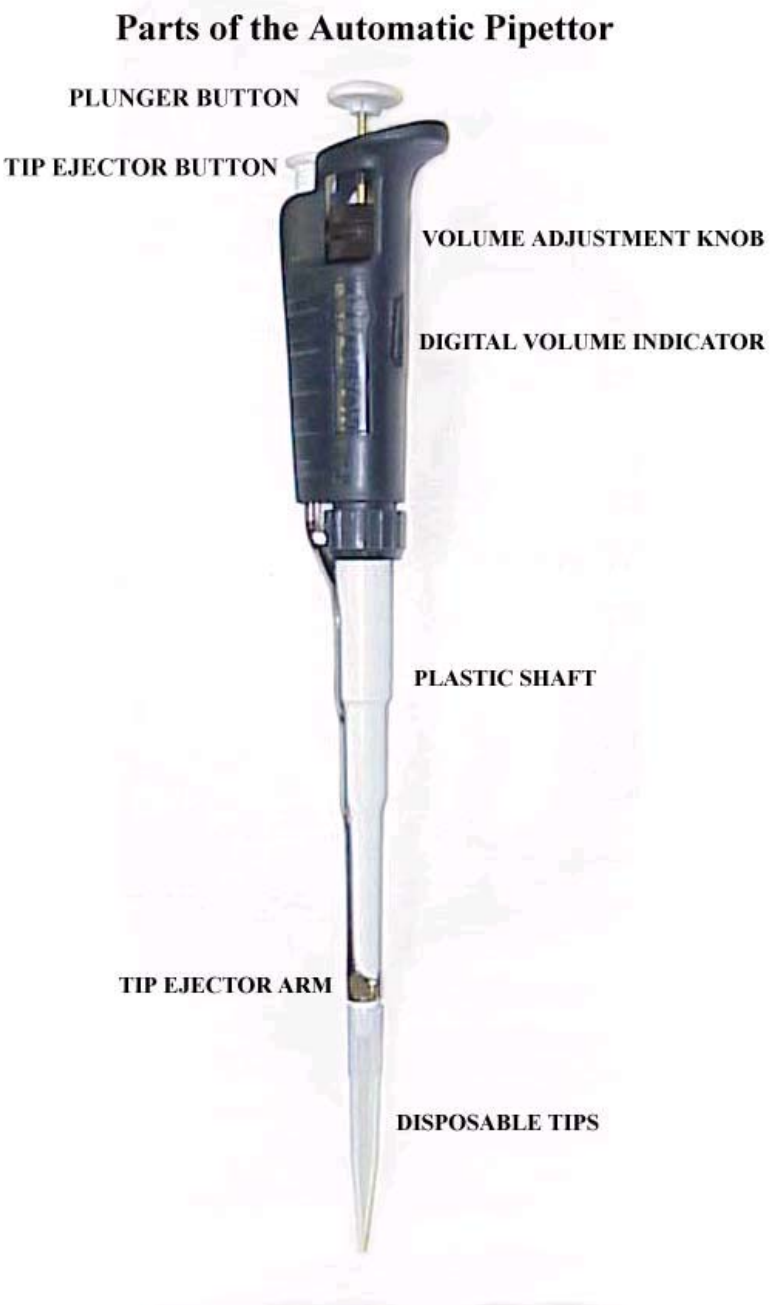
- Automatic pipettes are used to accurately transfer small liquid volumes
- Glass pipettes are not highly accurate for volumes less than 1 milliliter (1 ml), but the automatic pipettes are both accurate and precise
- These are continuously adjustable digital pipettes
- Each pipette can be set to transfer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

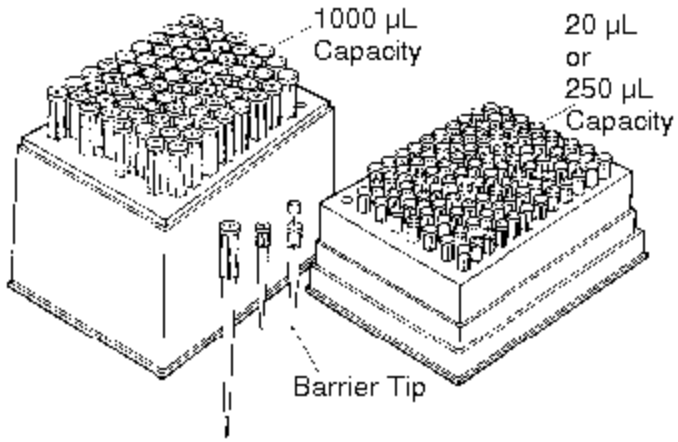
The Automatic Pipette

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 Pipette





Operating the Micropipette

Step 1: Set the Volume

Operating the Micropipette

Read the Volume

		
(a): P-20 Model **6.86 μ l = 0.00686** **or 6.86 x 10^{-3} ml**	**(b): P-200 Model** **132.4 μ l = 0.1324** **or 1.324 x 10^{-1} ml**	**(c): P-1000 Model** **262 μ l= 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

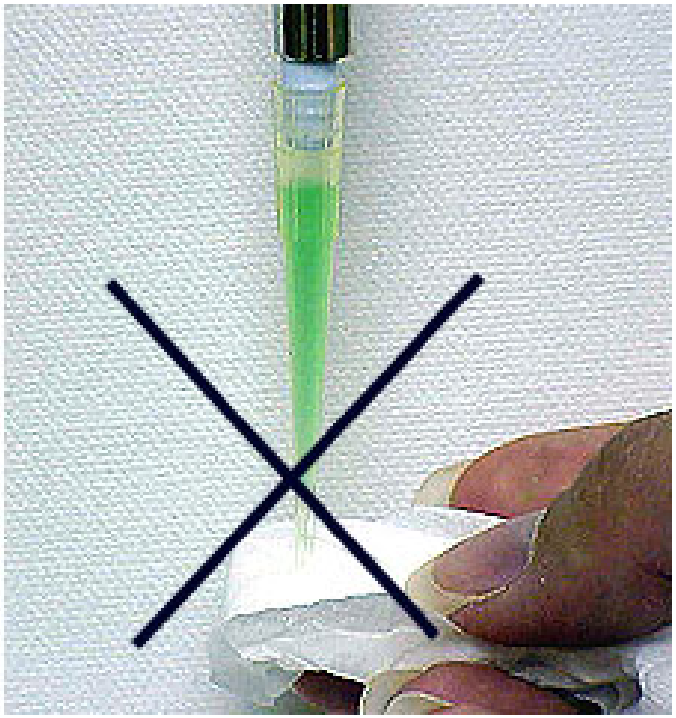
To aspirate the sample into the tip, allow the pushbutton to return slowly and smoothly to the fully extended UP POSITION.

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).

(a) Start Dispensing	(b) 1st Stop = Dispense	(c) 2nd Stop = Expel

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

Pippetting 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

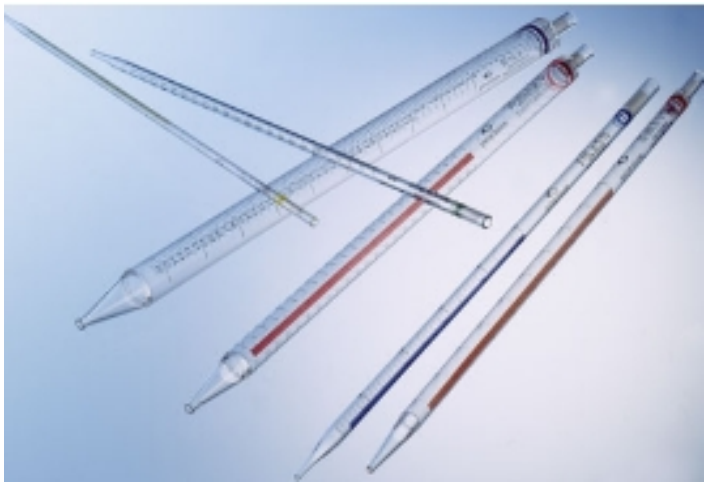
Solutions and Dilutions

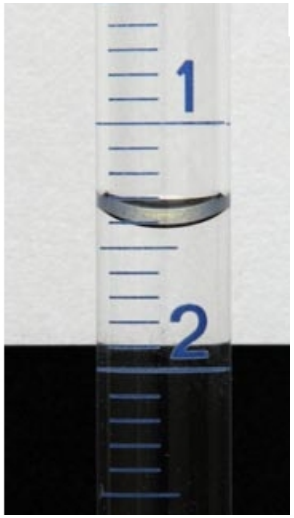
- Solutions are of two types
  - Molar solutions
  - Percents: 1% is 1 part per 100, for example (mass-volume versus volume-volume)
- Reagents are combinations of stock solutions: TE buffer is 10mM Tris-HCL, 1mM EDTA
  - Stocks are 1M Tris-HCl, 100mM EDTA
- How much should you make at one time? Should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

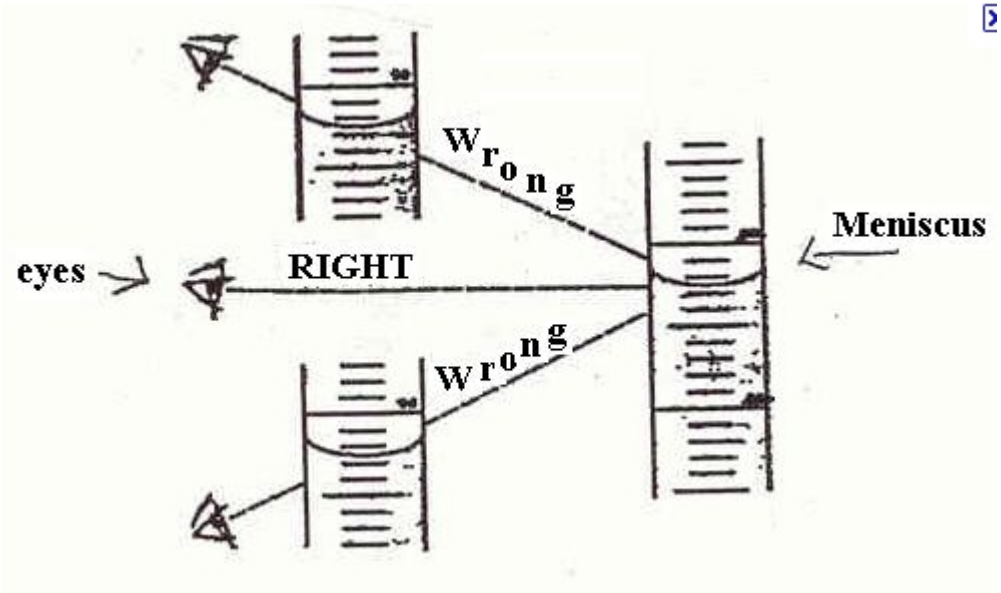
Handling solutions

- 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







Solution Delivery