

University of Utah  
DNA Sequencing & Genomics Core Facility



# DNA Sequencing Core Workflow & Customer Interaction

Helaman Escobar

# University of Utah DNA Sequencing & Genomics Core Facility



HSC Core Research Facilities – University of Utah  
http://www.cores.utah.edu/

## HSC CORE RESEARCH FACILITIES

Home    Work Authorization Form    Useful Links    Downloads    Contact    Cores Newsletter

- Biostatistics
- Cell Imaging/ Fluorescence Microscopy
- DNA Sequencing
- DNA/Peptide
- Electron Microscopy
- Flow Cytometry
- Genomics
- Mass Spectrometry and Proteomics
- Microarray
- Microarray/Bioinformatics
- NMR
- Protein Interaction
- Small Animal Imaging
- Transgenic/Gene-Targeting Mouse Facility
- C. elegans 'Worm' Facility
- CZAR (zebrafish)

**Amaya nucleofector**  
- nucleofector login request

### Core Facility News/Announcements

from Jerry Kaplan, Associate Dean for Research, School of Medicine

1. We have recently obtained two new pieces of equipment that may be used by faculty.

a. The Huntsman General Clinical Research Center (GCRC) in the School of Medicine recently acquired an AUTOPURE automated DNA extraction system. This system is capable of processing up to 96 samples per day and can be programmed to prepare DNA from whole blood, tissue culture cells and buccal swabs.

The GCRC will be accepting applications from the entire University of Utah campus to provide DNA preparation service. Applications in the form of an abbreviated protocol will be available from the GCRC and will be screened for approval by the GCRC Advisory Committee. This service will be free of charge to investigators with approved DNA preparation only protocols. If sample numbers for a single protocol are high, the principal investigator will be asked to cover the cost of reagents.

DNA samples will be verified by gel electrophoresis, A260/280 ratios will be provided for each sample and returned at a standard concentration of 200 ug/ml. Details and protocol forms will be posted soon on the GCRC website <http://crc-gw.med.utah.edu/index.cfm>.

b. The Health Science Center has purchased four mouse metabolic cages, each one houses one mouse during a typical experiment. These cages allow one to measure oxygen consumption, CO2 evolution, heat generation, locomotor activity, food intake, water intake and related parameters on normally behaving mice in real time. This equipment will be housed in the Department of Biochemistry. For additional information please contact Jared Rutter, Ph.D., Office: 581-3340, Lab: 581-8718 [Rutter@biochem.utah.edu](mailto:Rutter@biochem.utah.edu)

### Research Resources

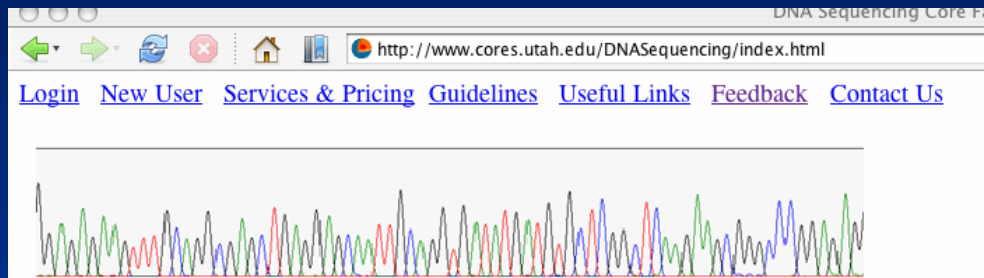
Grant information, training grants that exist on campus, location of supply centers/freezer programs, etc.

A photograph showing a wide view of a mountain range under a blue sky with some clouds, likely in Utah.

- Biostatistics
- Cell Imaging
- DNA Sequencing & Genomics
- Electron Microscopy
- Flow Cytometry
- Proteomics
- Microarray
- Bioinformatics
- NMR
- Protein Interaction
- Small Animal Imaging
- Transgenic Mouse
- C. elegans
- Zebrafish
- Metabolomics

www.cores.utah.edu

# University of Utah DNA Sequencing & Genomics Core Facility



[Login](#) [New User](#) [Services & Pricing](#) [Guidelines](#) [Useful Links](#) [Feedback](#) [Contact Us](#)

### DNA Sequencing Core Facility

The DNA Sequencing Core Facility at the University of Utah Health Science Center uses state of the art fluorescent DNA sequencing methods to produce high quality DNA sequencing at competitive [price](#) to the University community and off-campus users. The facility is equipped with one ABI3730 96-capillary sequencer and one ABI3100 16-capillary sequencer. The average turnaround time is two working days.

#### Lastest News

01/12/06	The Core is now hosting a Freezer Program for Applied Biosystems Lab Consumables. Get it cheaper, get it faster and pay no shipping (if we don't have it in stock). Download the order form and item list <a href="#">here</a> (pdf file) or call us for more info.
04/22/05	Sequencher™ 4.5 for Mac and PC is now available.
01/13/05	You can now send us feedback on any issue concerning our services through this web site. Click on the <a href="#">feedback</a> link above.

How can I open and edit my sequence files? Click on the link below:  
[Sequencher™ 4.5 for MacOS 9.2 or 10.2 and higher: includes OS X users](#)



Mark Hoff



Mary Lue Nelson



Chris Walls



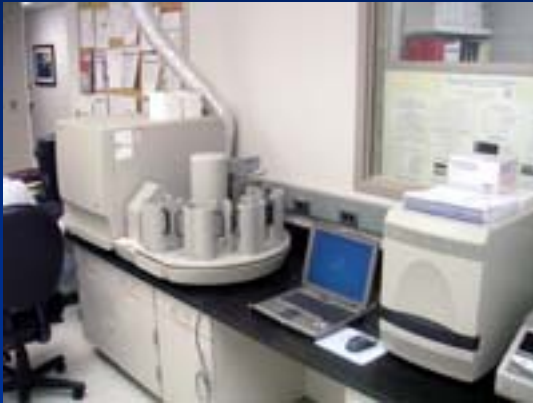
Mike Klein



University of Utah  
DNA Sequencing & Genomics Core Facility



# Equipment



ABI 7900 HT



ABI 3130xl



ABI 3730xl



Robotics



Biomek FX



Other

## Lab Profile – DNA Sequencing

- Not subsidized
- 210 PIs and 550 users (techs + students)
- 5 pick-up locations on campus
- 100K sequencing samples (cycle seq)
- 30K samples for re-sequencing projects
- 40% of work requests - 4 samples or less

## Basic Workflow

1. Re-array all samples into 96-well plates
2. Transfer to 384-wells before cycling
3. EtOH Precipitation
4. Electrophoresis in formamide
5. Review data using LIMS + SeqA or SeqScape

University of Utah

DNA Sequencing & Genomics Core Facility



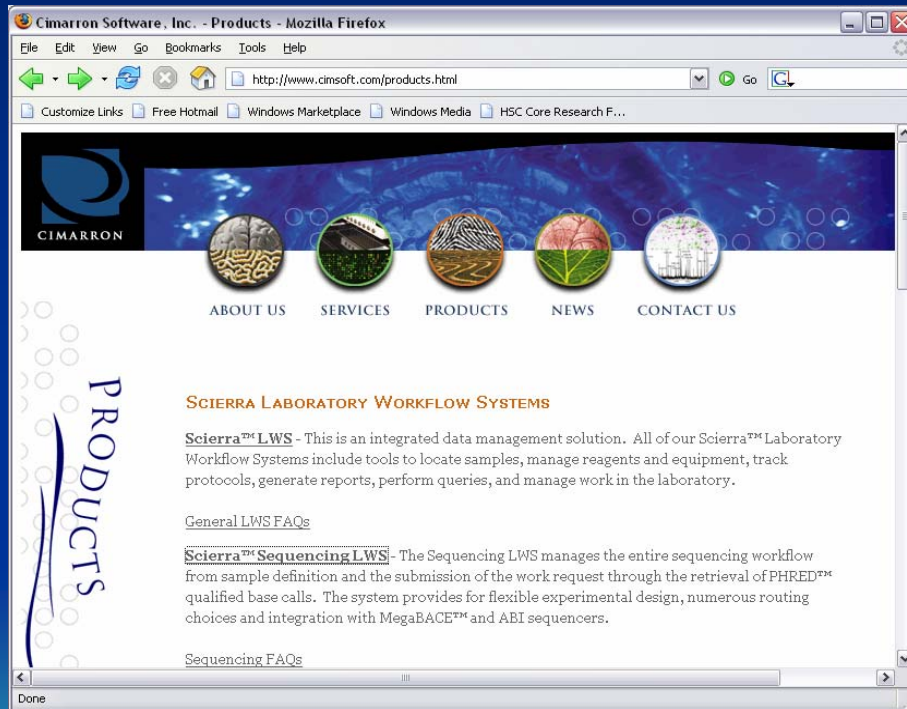
## Workflow Highlights

- LIMS
- Use robotics - Velocity11 Vprep Station
- We pre-mix and aliquot BD 3.1
  - 1/32 BD dilution
- Same plate from reaction to instrument
- 5 ul rxns:
  - 0.5 pmoles of primer
  - 5 -10 ug PCR or 100-150 ug of plasmid
  - Very long array life – 700 to 1100 runs
- 2% dropout rate



University of Utah  
DNA Sequencing & Genomics Core Facility

# Sierra from Cimarron



- Flexibility
- Oracle Database
- Other Core modules
- Tracking every process step
- Barcode enabled
- Flag problems
- Reassign samples
- Built-in inventory
- Records protocols and processes



## Do I need a LIMS?

- Increase your productivity, minimized human error, free time and please your customers
- More than 15 – 20K samples
- Out-of-the-box – decide what features are must
- If building your own:
  - Make sure you have a Requirements Specification Document
  - Hire professionals

# The Core Lab Business Predicament

- Quality
  - Seq Data quality
  - Customer service/support
- Turn over time
- Price

What should be my focus?

# Business Approach

- Assembly line
- Adhere to a Schedule
  - Clear time deadlines
- Consistent Processing
  - Detailed protocols
  - Streamline your format
  - Delegate specific tasks to technicians

## Pros:

Predictability  
Minimize human error  
Less stress  
Consistent results  
Time saving

## Cons:

Takes a certain type of staff  
Goes against academic culture  
Extra consumables cost

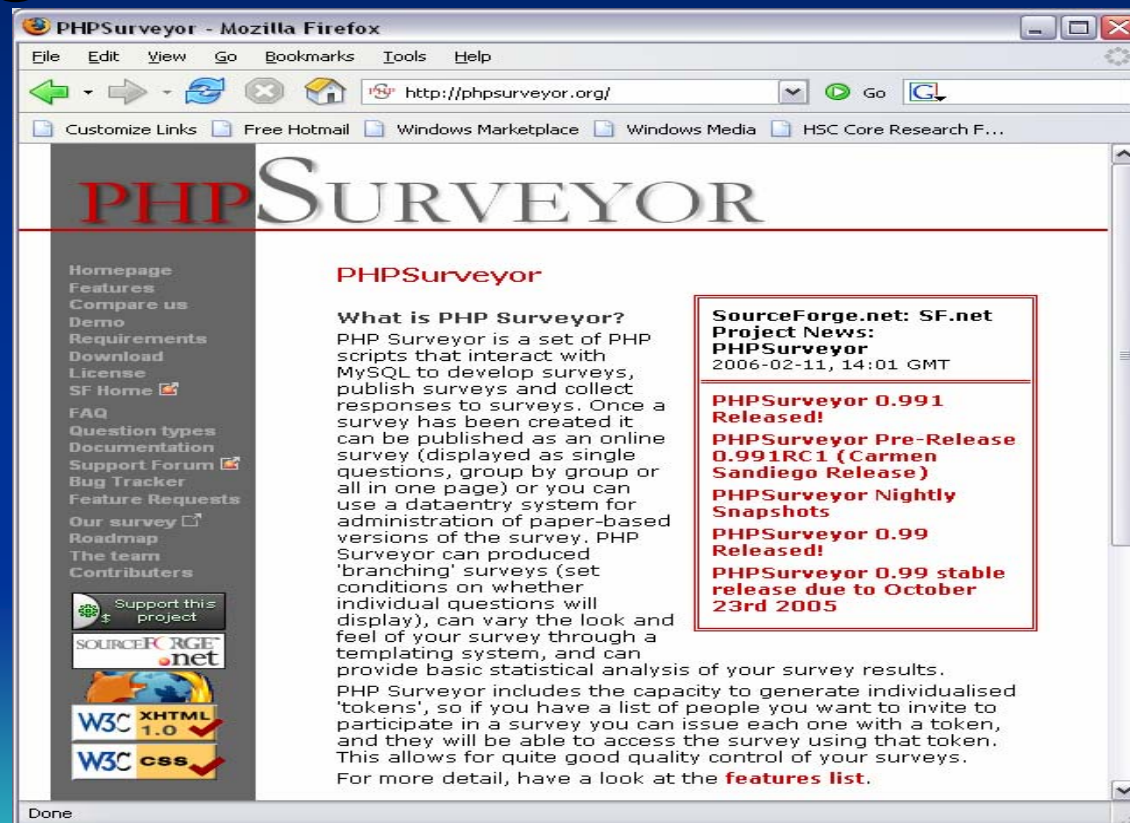
## Turnover and Quality

- We check most sequences individually
- Redo dropouts automatically
- Contact users having systematic problems
- 25 hrs turnover time
- Perform surveys
- Hold Lab Open houses and sponsor seminars
- Common lab area

# How to get to your Customers

- Redundancy
- Redundancy
- Redundancy

# Surveys



<http://phpsurveyor.org>

## Survey Caveats

- Make them short!!
- Ask meaningful questions
- Do not do them too often!
- Act on them quickly!!
- Share the data
- Personally contact those who raised issues and gave feedback

# Case Study: Post-sequencing Analyzes

- 26 questions – most of them multiple choice
- 4 min to complete
- Opened for 2 weeks
- Major questions:
  - Sequence software
  - Computer platform
  - Expectations from the Core



# Business Approach

- Assembly line
- Adhere to a Schedule
  - Clear time deadlines
- Consistent Processing
  - Detailed protocols
  - Streamline your format
  - Delegate specific tasks to technicians

## Pros:

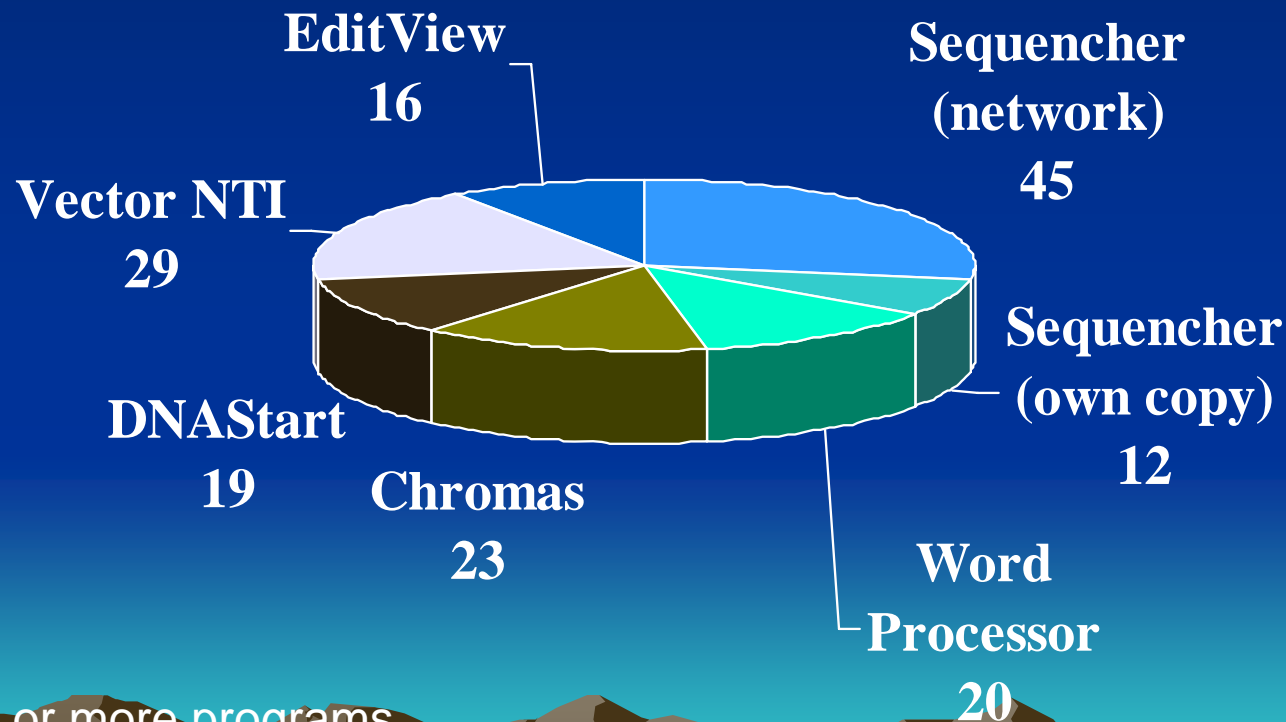
Predictability  
Minimize human error  
Less stress  
Consistent results  
Time saving

## Cons:

Takes a certain type of staff  
Goes against academic culture  
Extra consumables cost

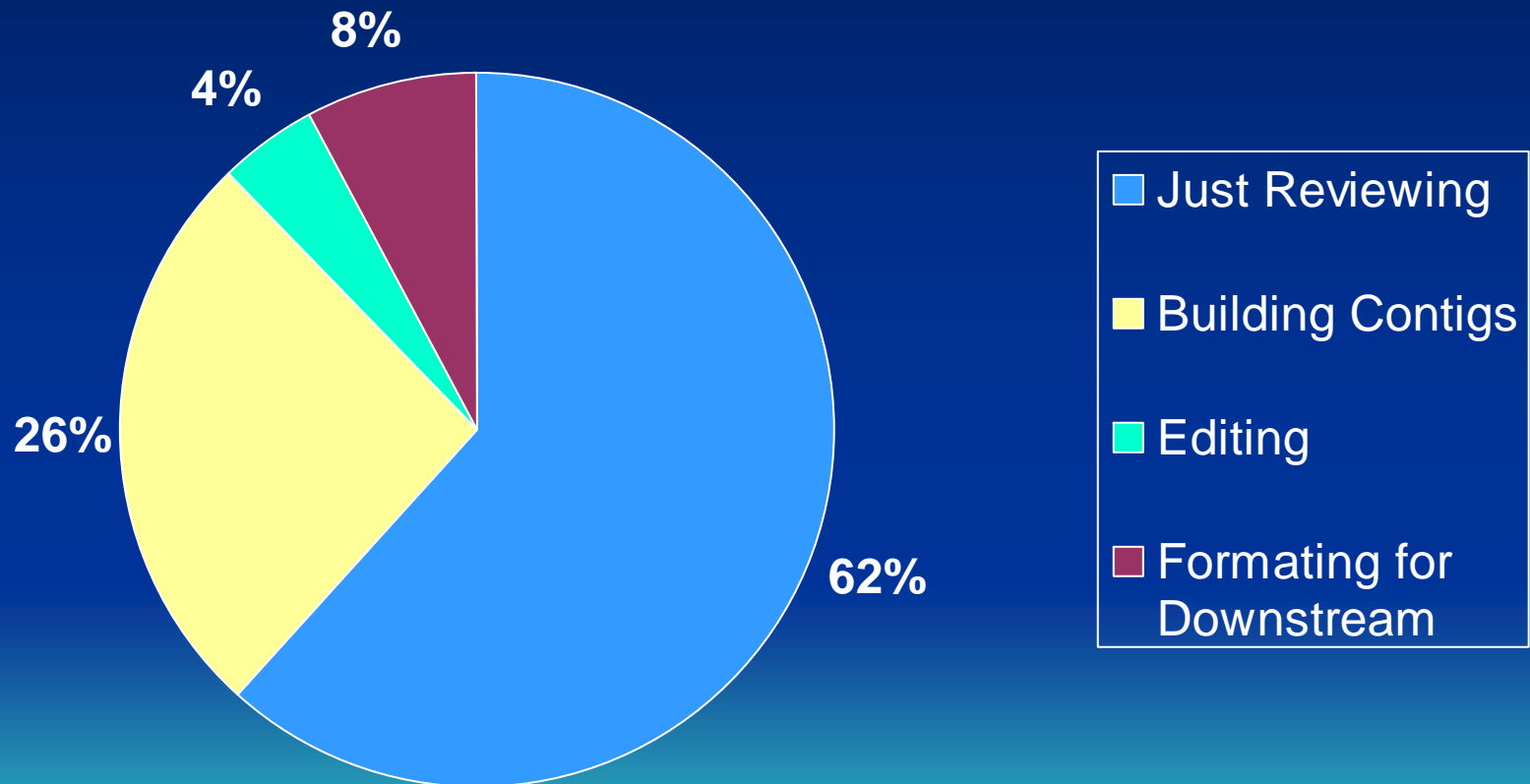
## Most Common Sequence Editors

N=124



53 labs use 2 or more programs  
Including 10 labs using 4 programs

## Post Sequencing Activity



# Feedback

- Software training session for Sequencher
- A Sequencing Troubleshooting Guide
- Forum of a Blog where users can exchange information
- Training on other Sequence Editors
- Phred files posted.

## List of Common Editors

- Sequencher
- Chromas
- MacVector
- DNA Stars
- DNAMAN
- DNA Strider
- Vector NTI
- BioEdit
- 4Peaks
- Ape
- Multalin (web)
- ClustalW
- GCG
- Staden Pkg
- NCBI

## Survey Conclusions

- I don't need more licenses for Sequencher
- I don't need to upgrade Sequencher
- I need to provide training for downstream software
- Software they use might be influencing their Core perceived quality

# Review

- Run your Core like a small business
- Decide the focus for your business
- Be consistent with your workflow and sample processing
- Develop good communication with your customers
- Enjoy It