Science Skills Boot Camp

- 9:00-10:15 NIH and Research Culture/Resources (Phil Ryan (OITE) and NIDA/NIA staff)
- 10:15-10:30 Coffee Break
- 10:30-11:30 Reading a Scientific Paper (Vani Paryadath (NIDA))
- 11:30 -12:30 Group Lunch (students must provide their own lunch)
- 12:30-2:00 Communicating Science
  - Keeping a Lab Notebook (Phil Ryan (OITE))
  - Giving Group Meeting(Roza Selimyan (NIA))
  - Writing an Abstract (Tom Keck (NIDA))
- 2:00-2:15 Break
- 2:15-2:35 What makes a good research question? (Darryl Murray (OITE))
- 2:40-3:00 Animal Models (Roza Selimyan (NIA))
- 3:05-3:30 Biostatistics/Epidemiology (Nai-Lin Cheng (NIA))

Navigating the NIH:

Getting the Most from Your NIH Summer Internship

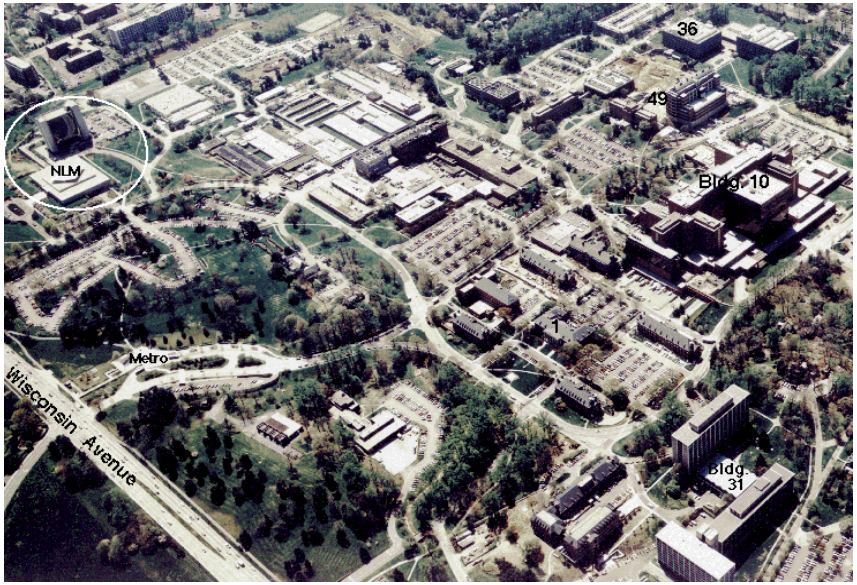
Philip Ryan, PhD
Director of Student Services
NIH Graduate Partnerships Program
Office of Intramural Training and Education

Office of Intramural TRAINING & EDUCATION
NATIONAL INSTITUTES OF HEALTH

Four Sections

- Understanding the NIH
- Settling into your research group and meeting your science goals
- Using NIH resources to meet your career and professional goals
- Understanding the research culture

What is the NIH?



The Nation's Biomedical Research Institution

- 27 Institutes &Centers (ICs)
- Biomedical, behavioral, and social science research at all levels - basic, translational and clinical
- Campuses in MD, NC, MT, AZ, MI and MA
- Two main divisions: **intramural** and **extramural**
- Learn more at www.nih.gov

Bench-To-Bedside Research At NIH

- 240 beds
- 7,000 inpatient admissions/yr
- 72,600 outpatient visits/yr
- 900 active clinical protocols

For information on clinical trials, please visit: http://clinicaltrials.gov/
We seek diversity in our clinical trials!

Researchers at NIH

- 1100 Faculty (PIs)
- 1500 Staff Scientists & Clinicians
- 3800 Postdoctoral Fellows
- 480 Clinical Fellows
- 485 Graduate Students
- 100 Medical Students
- 600 Postbacs
- YOU! ~1000 Summer interns

People You May Meet in Your IC

- Your Summer Coordinator, Training Director and Training Office staff
  - Be sure to attend your IC orientation
- The SD and Deputy SDs
- Your Branch or Lab Chief
- Other PIs in your Lab/Branch
- Your AO

First -- Define your goals

Focus on Development In Three Areas:

- Science
- Career
- Personal

To get the most out of your summer:

Make a plan

Research goals

Professional goals

Personal goals

Share it with your mentor!

Important Research Relationships

- Supervisor
  - Someone who directs the work of another
  - Is responsible for ensuring that someone does their job

- Mentor
  - Someone who passes on skills, knowledge, and wisdom to another person
  - Works to help develop someone's career by providing honest feedback, encouragement and guidance

There is No Typical Research Experience

- Differences in lab and research groups
  - Group dynamics and atmosphere
  - Level of day-to-day engagement of the PI
  - Level of independence, at experimental level and beyond
  - Types of rules and how they are imposed
  - Type and frequency of feedback
- Work hours, work environments and expectations vary; be sure you understand expectations in your group

Expectations Your Supervisors and Mentors Have of You

- Engagement in planning your research
- Honest communication regarding research progress
- Careful thought and regard for all elements of scientific ethics
- Energy and focus on your lab notebook
- Active participation in group activities and attention to fostering collegial relationships in your research group
- Good work ethic; balance internship and outside responsibilities effectively

Expectations You Should Have of Your Supervisors and Mentors

- Intellectual support and guidance
- Assistance in developing a project within the framework of on-going work in the group
- Availability when needed; guidance in planning the next step
- Honest but supportive feedback
- Support for professional development activities outside of lab
- Honest feedback regarding letters of recommendation

Mentoring Relationships Are Key To Your Success

- Successful scientists have many mentors -- in all areas
- Mentoring is a two-way relationship
- There is help to resolve issues:
  - Your IC Summer Coordinator and/or Training Director
  - Branch Chiefs and others in your research group
  - OITE staff and career counselors
  - Office of the Ombudsman, Center for Cooperative Resolution
  - NIH Employee Assistance Program (EAP)

To Achieve Your Science Goals

- Appreciate that first impressions are key
- Meet with your supervisors - EARLY & OFTEN
- Read papers in your field; work to be sure you understand how to read a paper
- Focus on the “big picture” AND the details of your work
- Use “down-time” in lab wisely
- Actively participate in research group meetings
- Attend seminars, in and outside of your field
- Present your work at Summer Poster Day

To Achieve Your Career Goals

- Use the online Summer Handbook and IC materials to find information on useful career development activities
- Work with your mentor to create a summer plan
- Attend the Graduate & Professional School Fair if appropriate
- Use informational interviews to explore careers
- Make use of online resources from OITE
  - Follow us on twitter @NIH_OITE
- Participate in Summer Poster Day
- E-mail me if you have questions: ryanp@od.nih.gov

Take Care of Your Whole Self

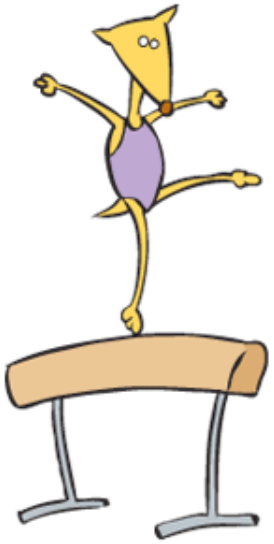
- Make certain you always feel your best
  - Exercise
  - Eat well
  - Stay connected to family/friends
- Get to know interns in your research group and IC
- Explore your local area

A tale of 2 PIs

- Hands-on
- Found in the lab/office or close-by
- Talks with students and fellows all the time
- Called by his/her first name

- Hands-off
- Locked in his/her office or away at meetings
- Meets with trainees for formal meetings scheduled in advance
- Called Dr._____

Interacting with your mentor



- Be respectful and courteous
- Adapt to his/her mentoring style
- Accept criticism with grace
- Ask when you have questions
- Find balance between being independent and asking for help
- Address problems before they escalate

Be a good lab citizen

- Keep common space clean
- Don’t disturb other people’s work
- Be extremely careful with common reagents and equipment
  - Don’t contaminate!
  - Replace common reagents if they are running low
  - **Ask for help** if you don’t know how to use equipment
  - Tell somebody if equipment is broken

“Research is formalized curiosity. It is poking and prying with a purpose.”

Zora Neale Hurston

Scientific research . . .

- Is a team effort

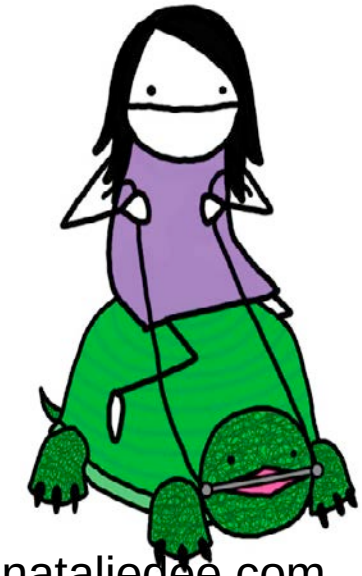
www.barcelonafootballblog.com

Scientific research . . .

- Is a team effort
- Involves asking small, manageable questions

Scientific research . . .

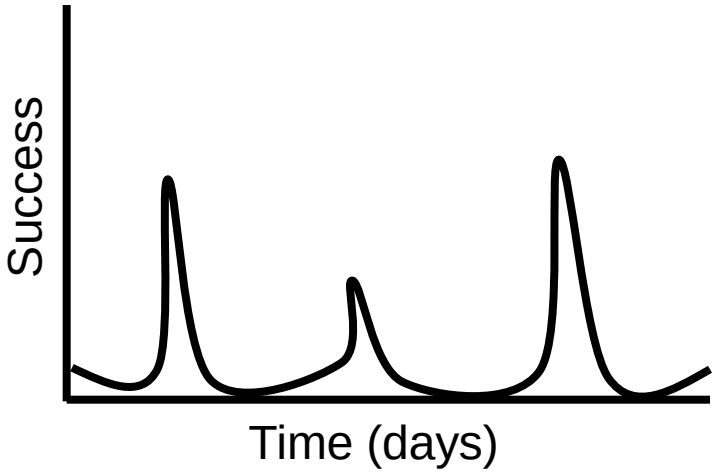
- Is a team effort
- Involves asking small, manageable questions
- Often moves slowly



nataliedee.com

Scientific research . . .

- Is a team effort
- Involves asking small, manageable questions
- Often moves slowly
- Contains periods of frustration between successes



Scientific research . . .

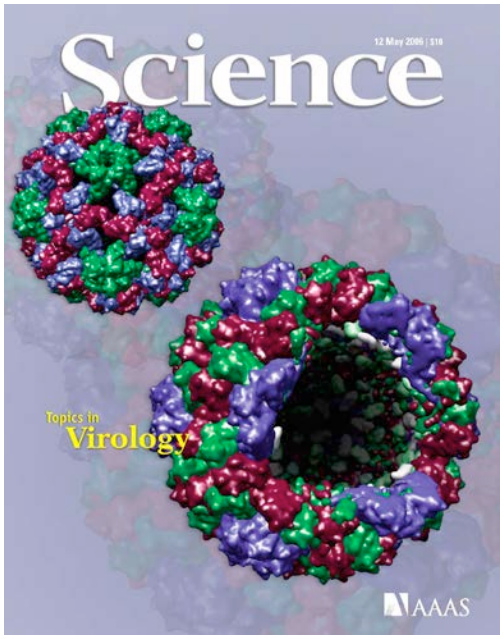
- Is a team effort
- Involves asking small, manageable questions
- Often moves slowly
- Contains periods of frustration between successes
- Is not a 9-5 job

Scientific research . . .

- Is a team effort
- Involves asking small, manageable questions
- Often moves slowly
- Contains periods of frustration between successes
- Is not a 9-5 job
- Must always be documented

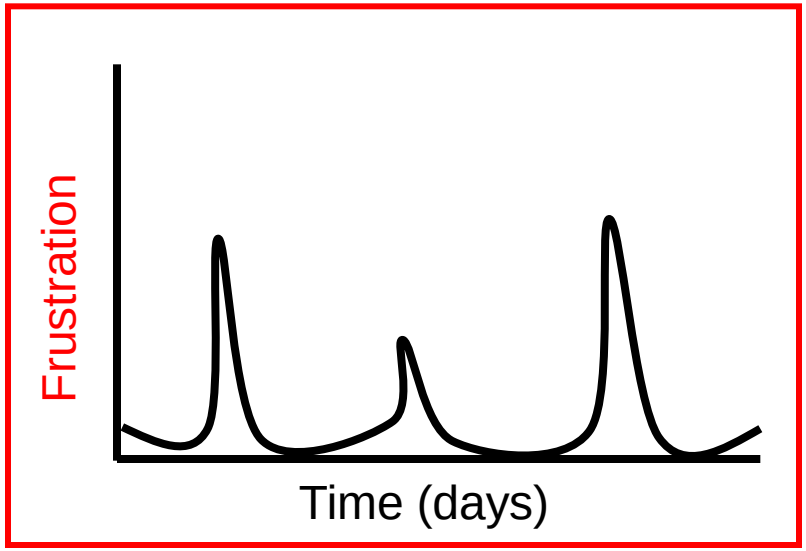
For data to be publishable, your work must be:

- Meticulous
- Reproducible
- Well-documented



Science can be unpredictable

- Don’t get frustrated!
- Ask for help
- Be creative
- Be flexible with your research plan



What if you don’t have any work to do?

- Read papers
- Think about your project
- Plan experiments
- Learn about other people’s projects
- Volunteer to help others
- Start your poster

This is your summer job . . . But it's also a training experience!

- Attend seminars and workshops
- Take advantage of NIH resources
- Talk to other researchers
- Network
- Have fun

What if something really bad happens?

You are not alone!

- Your principle investigator
- Your IC training director
- Dr. Sharon Milgram, OITE Director
  milgrams@mail.nih.gov
- Dr. Pat Sokolove, Director Postbaccalaureate and Summer Intern Programming
  sokolovp@mail.nih.gov

How to read a scientific article

Vani Pariyadath, Ph.D.

06/27/2012

Why are you reading the paper?

- To replicate a study
- To understand a technique/field better
- For a journal club
- To include as a reference for a manuscript
- The title of the paper sounded vaguely interesting

The reason will determine how you go about reading the paper.

Abstract

- A good abstract should contain
  - The rationale for the study
  - Methodology (broadly)
  - Results
  - Principal conclusions
- This is generally the best place to start!
  - Try reading through 1-2 times before reading the main text, and once again after you finish the whole paper.

Pattern of Intake and Drug Craving Predict the Development of Cocaine Addiction-like Behavior in Rats

David Belin, Eric Balado, Pier Vincenzo Piazza, and Véronique Deroche-Gamonet

Background: Clinical observations suggest that cocaine addiction often emerges with new patterns of use. Whether these changes are a cause of addiction or its consequence is unknown. We investigated whether the development of an addiction-like behavior in the rat is associated with the pattern of cocaine intake and with cocaine craving, a major feature of cocaine addiction.

Methods: To determine whether changes in the pattern of cocaine use and enhanced craving precede or parallel the onset of addiction, we used a rat addiction model that incorporates core features of human addiction. For this purpose, the pattern of inter-infusion intervals (a measure of pattern of cocaine intake), sensitivity to cocaine-induced reinstatement (a measure of cocaine craving), and addiction-like behaviour were assessed over several months of intravenous cocaine self-administration.

Results: We found that, even at early stages of cocaine self-administration, both the pattern of cocaine intake and the intensity of drug-induced reinstatement predict the severity of cocaine use, measured after 75 days of self-administration.

Conclusions: Our results identify key predictors of cocaine addiction—intensified pattern of drug use and high drug-induced craving—that may help in the identification of subjects at risk for subsequent development of severe cocaine addiction.

nature neuroscience

BRIEF COMMUNICATIONS

A unique adolescent response to reward prediction errors

Jessica R Cohen[1,4], Robert F Asarnow[2], Fred W Sabb[2,3], Robert M Bilder[1–3], Susan Y Bookheimer[1–3], Barbara J Knowlton[1,3] & Russell A Poldrack[1–4]

Previous work has shown that human adolescents may be hypersensitive to rewards, but it is not known which aspect of reward processing is responsible for this. We separated decision value and prediction error signals and found that neural prediction error signals in the striatum peaked in adolescence, whereas neural decision value signals varied depending on how value was modeled. This suggests that heightened dopaminergic prediction error responsivity contributes to adolescent reward seeking.

Introduction

- Provides the context
- <u>Quickly summarizes relevant findings in the field, and identifies a gap.</u>
- States the hypotheses and what methods will be used to test them
- Sometimes even gives away the punch-line!

Adolescence is a unique period in psychological development that is characterized by increased risky choices and actions as compared with children and adults. This may reflect the relatively early functional development of limbic affective and reward systems in comparison with prefrontal cortex[1], causing adolescents to make poor decisions and risky choices more often than both children (who are not yet fully sensitive to rewards) and adults (who are sensitive to rewards, but have the ability to exert control over reward-driven urges).

According to behavioral decision theories, choices are driven by the value assigned to each potential choice (decision value)[2]. The decision value is computed by a system in the medial prefrontal cortex that serves as a common pathway for value representation[3,4]. However, to behave adaptively in a changing or noisy world, these values must be updated on the basis of experience. Reward prediction error signals reflect the difference between the expected value of an action and the actual outcome of the action[5] and are coded by phasic activity in the mesolimbic dopamine system[6]. In functional magnetic resonance imaging, they are usually observed in the ventral striatum, reflecting dopaminergic output[7]. The nature of prediction error signals in children or adolescents is unknown. Adolescents may have a hypersensitive striatal response to reward[8,9], although this finding is somewhat inconsistent[10,11]. Using a probabilistic learning procedure[12], we examined whether adolescence is associated with unique changes in either decision value or prediction error signals (**Fig. 1**

Methods

- Typically the hardest portion of the article
- If you're new to the field, and are hoping to gain depth, read every line.
- Look up references to previously published material on the method if necessary.
- If you're not planning on using the method, don't dwell on the details. Move on.
- As you gain experience, you'll probably skim through this section.

Methods and Materials

Subjects, Surgery, and Apparatus

Male Sprague-Dawley rats ($n = 32$) weighing 280–300 g at the beginning of the experiments were used. The rats' housing and surgical conditions and the self-administration setup are described in Supplement 1.

Procedures

The details of the experimental procedures are provided in Supplement 1 and are based on our previous work (6).

Cocaine Self-Administration Training. The daily self-administration session was composed of three drug components (60 min each, fixed ratio five schedule of reinforcement) separated by 15-min drug-free periods. Cocaine HCl (.8 mg/kg/infusion) availability was signaled by a blue cue light. Drug unavailability was signaled by the complete illumination of the self-administration box.

Progressive-Ratio Reinforcement Schedule. During this schedule, cocaine availability was signaled by the blue cue light. The number of responses per infusion was increased after each infusion (see progression in Supplement 1). The session ceased after either 5 hours or when a period of 1 hour had elapsed since the previously earned infusion.

Resistance to Punishment Procedure. During these sessions, rats were placed for 40 min in the self-administration chamber. The blue cue light signaling drug availability was on. The schedule was the following: the first response led to the illumination of the green cue light signaling the presence of the shock. After three additional responses, rats received an electric shock (.8 mA, 2 sec), and after the fifth response, rats received both an electric shock (.8 mA, 2 sec) and a cocaine infusion (.8 mg/kg), associated with the corresponding conditioned stimulus (white cue light). Then the green cue light was turned off. The schedule could reinitiate at the end of the timeout period (i.e., 40 sec after the infusion). If within 1 minute rats did not complete response requirements leading to shock and shock plus infusion, respectively, the green cue light turned off and the sequence was reinitiated (i.e., the following response turned on the green cue light).

Cocaine-Induced Reinstatement. Cocaine-induced reinstatement was performed over two sessions as previously described (6,10). On session 1, following a 90-min period of extinction, the intravenous infusions of four unit volumes (20, 40, 80, and 160 μL in ascending order) of vehicle (saline) were triggered by the computer at one infusion every 30 min. On session 2, the same schedule was applied except that the vehicle solution was replaced by a cocaine solution (.2 mg/kg/20μL), leading to cocaine priming doses of .2, .4, .8, and 1.6 mg/kg.

Pattern of Cocaine Self-Administration. General pattern of cocaine self-administration was determined according to III. A

If you're lucky, the gist and important details will be contained in a figure

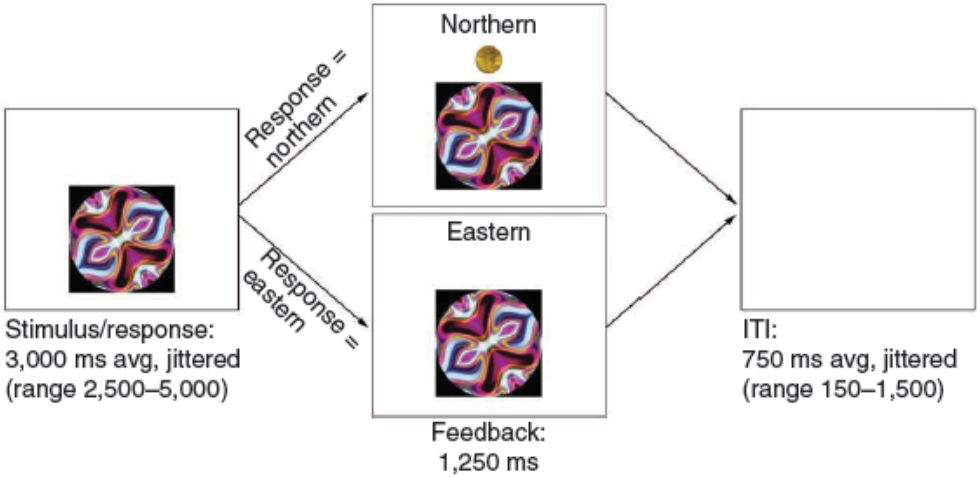


Figure 1 Experimental design. 45 healthy participants (18 children aged 8–12, 16 adolescents aged 14–19, and 11 adults aged 25–30) performed a probabilistic learning task during fMRI acquisition. Written informed consent was obtained. Participants classified abstract stimuli into one of two categories (Northern and Eastern) and were given feedback displaying the correct response at the end of each trial. If their response matched the outcome, feedback included a monetary reward. We paid participants according to the reward they received to ensure motivation. There were two stimulus types, predictable (associated 83% of the time with one of the two categories) and random (associated 50% of the time with each category). There were also two rewards, large ($0.25) and small ($0.05).

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Adolescence is a unique period in psychological development and the risky, reward-seeking behavior that occurs during this period can result in substantial morbidity and mortality, including accidental death and the onset of drug addiction. Understanding the neural basis of adolescent decision-making is a critical challenge. Our results suggest that enhanced prediction error signals contribute to adolescent reward-seeking, which provides a target for future studies of this important period in development.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

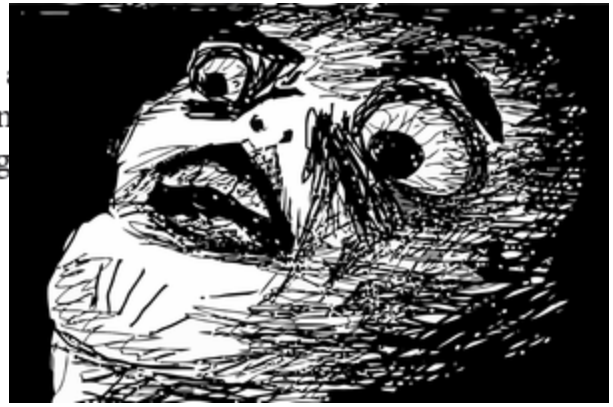
This research was supported by the National Institute of Mental Health (5R24 MH072697), the National Institute of Drug Abuse (5F31 DA024534), the James S. McDonnell Foundation and the Della Martin Foundation.

AUTHOR CONTRIBUTIONS

J.R.C. helped design the experiments, conducted data
wrote the manuscript. R.F.A., R.M.B. and S.Y.B. design
contributed to data acquisition. B.J.K. and R.A.P. desig
helped write the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.



Results

- If you understood the hypotheses and the methods, this part should be a breeze.
- Sometimes it helps to peruse the figures before reading the text.
- In a time-crunch, look for the money-figure.

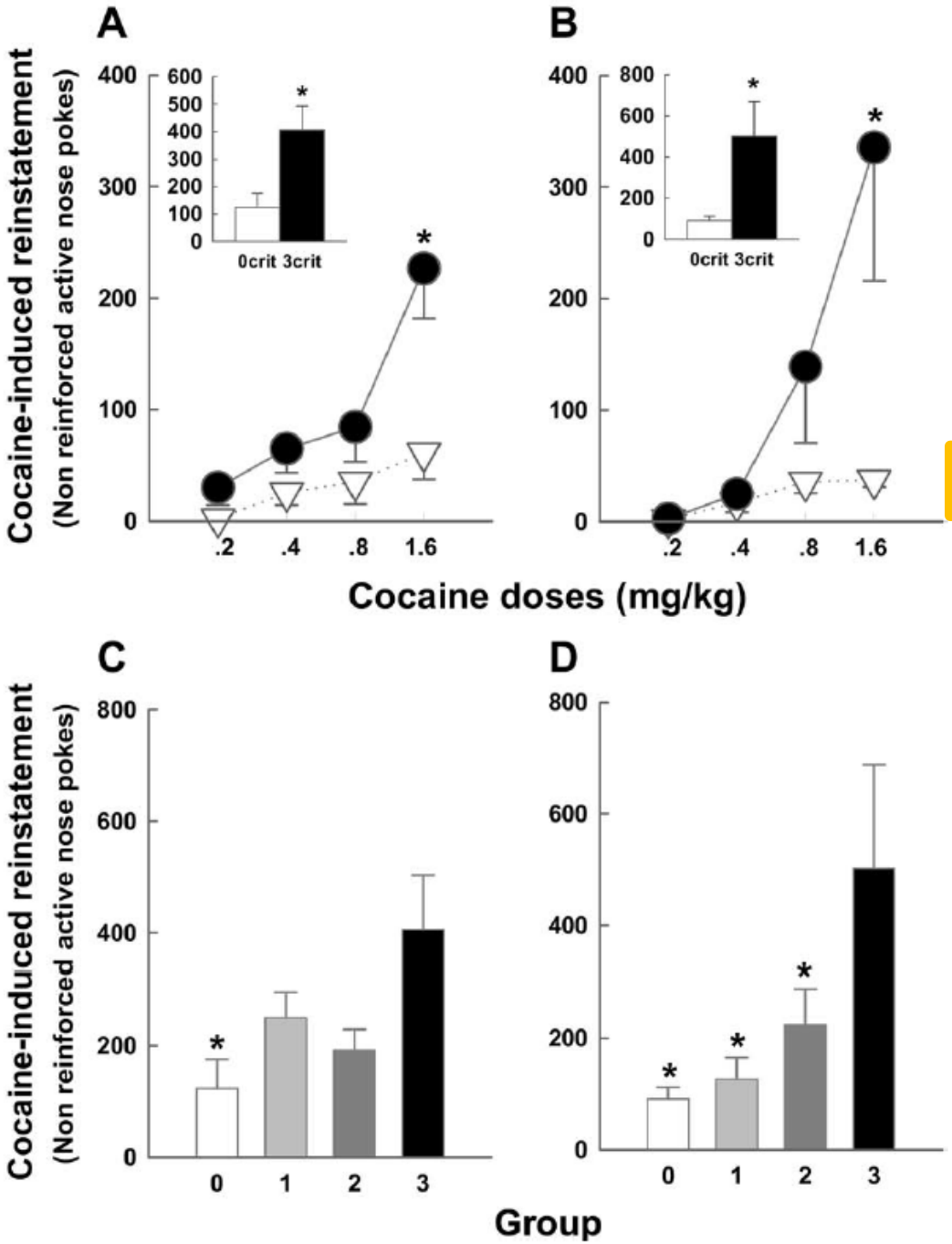


Figure 3. Cocaine addiction is specifically associated with and preceded by higher cocaine-induced reinstatement. Compared with 0crit rats (white triangles), 3crit rats (black circles) showed an upward shift in the dose-response curve for cocaine-induced reinstatement after both 30 (**A**) and 80 (**B**) days of cocaine self-administration. Insets and (**C**) and (**D**) represent the sum of the responses cumulated over the first 15 min that followed each of the 4 noncontingent infusions of cocaine. High cocaine-induced reinstatement was a feature specific to 3crit rats after both 30 (**C**) and 80 days of self-administration (**D**). * $p < .05$ versus 3crit.

Discussion

- Summarizes key findings
- Places data in context with other studies
- Discusses potential issues with the current study (and why they're not important :P)
- Suggests future directions for the field based on the study

- The least important section in a paper, and the least likely to withstand the test of time

Other types of publications

- Atypical styles – Letters to Nature, Brevia (*Science*), etc.
- Review articles, book chapters
  - Great place to turn to for overviews of a topic
- Methods papers

Reading is an active task

- Ask yourself these questions as you read the article:
  - What are the expected results?
  - What are the limitations to the methodology?
  - Is this the appropriate method to test the hypothesis?
  - Are the findings convincing?
  - Is there an alternative explanation?
  - How does this method or finding relate to what I'm doing?

Reading is an active task

- At the end of the introduction, can you summarize what the hypotheses are?
- Try to predict what the outcome will be.
- At the end of the results section, can you summarize what the principal findings were?
- Come up with your own explanations for any unexpected findings before you reach the Discussion.

When you finish…

- When you're done reading the paper, summarize the methods and findings and file away with the paper. Future you will thank you for this!
- If you're old school, put this on a post-it.
- If you prefer electronic versions, use the Papers app or Mendeley (free!).

Don’t get bogged down by the details

- Get the big picture first
- Key words to look for in the paper:
  - “we hypothesize that…” or “ we predicted that…”
  - “Surprisingly”
- Note down the bits you didn’t understand and move on. Later, ask someone what these mean. Google and Wikipedia are your friends.
- Don’t be too hard on yourself – sometimes a paper is hard to follow because it was written poorly. ☺

Journal Clubs, or how to now present what you've learned to someone else

- Don't just read the paper out loud!
- Explain the bigger picture first – the context behind the study (you know where to look for this) in 3-4 sentences.
- Then go through each section either in order (Intro → Methods → Results → Disc), or as a story. One approach is to walk them through the figures.
- Summarize the key information in each section. Again, <u>don't get caught up in the details</u>.

Bioinformatics

Some tools you might need to use

Arjun Prasad
Research Fellow, Genome Technology Branch
National Human Genome Research Institute
arjun.prasad@nih.gov

What is bioinformatics?

What is bioinformatics?

Using computers to study biology

A few resources

- Entrez
- PubMed
- GenBank
- OMIM
- BLAST
- UCSC Genome Browser
  - In-silico PCR

How to find out more

- Google
- Bitesize Bio (http://bitesizebio.com)
  - short articles on specific laboratory technique
- OpenHelix (http://www.openhelix.com/)
  - Video tutorials on genomics / bioinformatics techniques (Subscription service, but some free)

National Center for Biotechnology Information (NCBI)

- Central repository for biological information and databases for the US (and the world)
- Part of the National Library of Medicine
- Gigantic databases…
- Lots of databases…
- Entrez as entry point

http://www.ncbi.nlm.nih.gov/

Entrez cross-database searc

www.ncbi.nlm.nih.gov/sites/gquery

PubMed | UCSC | tiki | Journals | Admin | binfo | CiteULike | CiteULike2 | CiteULike Lib | Calendar | Weather | Readability | Read Now | ~ Notforest ~ | Other Bookmarks

NCBI

Entrez, The Life Sciences Search Engine

HOME | SEARCH | SITE MAP | PubMed | All Databases | Human Genome | GenBank | Map Viewer | BLAST

Search across databases [ ] GO Clear Help

Welcome to the Entrez cross-database search page

- **PubMed:** biomedical literature citations and abstracts
- **PubMed Central:** free, full text journal articles
- **Site Search:** NCBI web and FTP sites
- **Books:** online books
- **OMIM:** online Mendelian Inheritance in Man

- **Nucleotide:** Core subset of nucleotide sequence records
- **EST:** Expressed Sequence Tag records
- **GSS:** Genome Survey Sequence records
- **Protein:** sequence database
- **Genome:** whole genome sequences
- **Structure:** three-dimensional macromolecular structures
- **Taxonomy:** organisms in GenBank
- **SNP:** short genetic variations
- **dbVar:** Genomic structural variation
- **Gene:** gene-centered information
- **SRA:** Sequence Read Archive
- **BioSystems:** Pathways and systems of interacting molecules
- **HomoloGene:** eukaryotic homology groups
- **Probe:** sequence-specific reagents
- **BioProject:** aggregated biological research project data
- **dbGaP:** genotype and phenotype
- **UniGene:** gene-oriented clusters of transcript sequences
- **CDD:** conserved protein domain database
- **Clone:** integrated data for clone resources
- **UniSTS:** markers and mapping data
- **PopSet:** population study data sets
- **GEO Profiles:** expression and molecular abundance profiles
- **GEO DataSets:** experimental sets of GEO data
- **Epigenomics:** Epigenetic maps and data sets
- **PubChem BioAssay:** bioactivity screens of chemical substances
- **PubChem Compound:** unique small molecule chemical structures
- **PubChem Substance:** deposited chemical substance records
- **Protein Clusters:** a collection of related protein sequences
- **OMIA:** online Mendelian Inheritance in Animals
- **BioSample:** biological material descriptions

- **NLM Catalog:** catalog of books, journals, and audiovisuals in the NLM collections
- **MeSH:** detailed information about NLM's controlled vocabulary

| Counts in XML | Entrez Utilities | Disclaimer | Privacy statement | Accessibility |

NCBI

Entrez, The Life Sciences Search Engine

HOME | SEARCH | SITE MAP | PubMed | All Databases | Human Genome | GenBank | Map Viewer | BLAST

Search across databases GARS GO Clear Help

- Result counts displayed in gray indicate one or more terms not found

290	**PubMed:** biomedical literature citations and abstracts	66	**Books:** online books
391	**PubMed Central:** free, full text journal articles	11	**OMIM:** online Mendelian Inheritance in Man
none	**Site Search:** NCBI web and FTP sites		

42776	**Nucleotide:** Core subset of nucleotide sequence records	none	**dbGaP:** genotype and phenotype
63	**EST:** Expressed Sequence Tag records	18	**UniGene:** gene-oriented clusters of transcript sequences
12	**GSS:** Genome Survey Sequence records	1	**CDD:** conserved protein domain database
9849	**Protein:** sequence database	1673	**Clone:** integrated data for clone resources
17	**Genome:** whole genome sequences	16	**UniSTS:** markers and mapping data
36	**Structure:** three-dimensional macromolecular structures	34	**PopSet:** population study data sets
1	**Taxonomy:** organisms in GenBank	6162	**GEO Profiles:** expression and molecular abundance profiles
2427	**SNP:** short genetic variations	1	**GEO DataSets:** experimental sets of GEO data
95	**dbVar:** Genomic structural variation	none	**Epigenomics:** Epigenetic maps and data sets
2099	**Gene:** gene-centered information	103	**PubChem BioAssay:** bioactivity screens of chemical substances
9	**SRA:** Sequence Read Archive	none	**PubChem Compound:** unique small molecule chemical structures
637	**BioSystems:** Pathways and systems of interacting molecules	28	**PubChem Substance:** deposited chemical substance records
2	**HomoloGene:** eukaryotic homology groups	36	**Protein Clusters:** a collection of related protein sequences
231	**Probe:** sequence-specific reagents	none	**OMIA:** online Mendelian Inheritance in Animals
2	**BioProject:** aggregated biological research project data	2	**BioSample:** biological material descriptions

NCBI

Entrez, The Life Sciences Search Engine

HOME | SEARCH | SITE MAP | PubMed | All Databases | Human Genome | GenBank | Map Viewer | BLAST

Search across databases GARS GO Clear Help

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17	**Genome:** whole genome sequences	16	**UniSTS:** markers and mapping data
36	**Structure:** three-dimensional macromolecular structures	34	**PopSet:** population study data sets
1	**Taxonomy:** organisms in GenBank	6162	**GEO Profiles:** expression and molecular abundance profiles
2427	**SNP:** short genetic variations	1	**GEO DataSets:** experimental sets of GEO data
95	**dbVar:** Genomic structural variation	none	**Epigenomics:** Epigenetic maps and data sets
2099	**Gene:** gene-centered information	103	**PubChem BioAssay:** bioactivity screens of chemical substances
9	**SRA:** Sequence Read Archive	none	**PubChem Compound:** unique small molecule chemical structures
637	**BioSystems:** Pathways and systems of interacting molecules	28	**PubChem Substance:** deposited chemical substance records
2	**HomoloGene:** eukaryotic homology groups	36	**Protein Clusters:** a collection of related protein sequences
231	**Probe:** sequence-specific reagents	none	**OMIA:** online Mendelian Inheritance in Animals
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Neurogenetics Branch, National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), 35 Convent Drive, Bethesda, MD 20892-3705, USA.

Abstract

Charcot-Marie-Tooth disease type 2D, a hereditary axonal neuropathy, is caused by mutations in glycyl-tRNA synthetase (GARS). The mutations are distributed throughout the protein in multiple functional domains. In biochemical and cell culture experiments, some mutant forms of GARS have been indistinguishable from wild-type protein, suggesting that these in vitro tests might not adequately assess the aberrant activity responsible for axonal degeneration. Recently, mouse and fly models have offered new insights into the disease mechanism. There are still gaps in our understanding of how mutations in a ubiquitously expressed component of the translation machinery result in axonal neuropathy. Here, we review recent reports, weigh the evidence for and against possible mechanisms and suggest areas of focus for future work.

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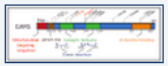

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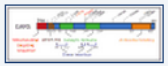

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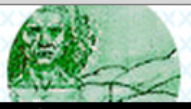

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CHARCOT-MARIE-TOOTH DISEASE, AXONAL, TYPE 2D; CMT2D

Alternative titles; symbols

CHARCOT-MARIE-TOOTH DISEASE, NEURONAL, TYPE 2D
CHARCOT-MARIE-TOOTH NEUROPATHY, TYPE 2D

Phenotype Gene Relationships

Location	Phenotype	Phenotype MIM number	Gene/Locus	Gene/Locus MIM number
7p14.3	Charcot-Marie-Tooth disease, type 2D	601472	GARS	600287

Phenotypic Series

Clinical Synopsis

TEXT

A number sign (#) is used with this entry because Charcot-Marie-Tooth disease type 2D can be caused by mutation in the gene encoding glycyl tRNA synthetase (GARS; 600287). Distal spinal muscular atrophy type V (DSMAV; 600794) is an allelic disorder with a similar phenotype.

For a phenotypic description and a discussion of genetic heterogeneity of axonal CMT type 2, see CMT2A1 (118210).

Clinical Features

Ionasescu et al. (1996) reported results of clinical, electrophysiologic, and genetic linkage studies on a large pedigree with autosomal dominant Charcot-Marie-Tooth axonal neuropathy type 2, which they designated CMT2D. The pedigree consisted of 38 members, 14 of which were affected. Onset of the disease was between 16 and 30 years of age with weakness of the hands. Affected members had severe weakness and atrophy of the hands and mild to moderate weakness of the feet. Deep tendon reflexes were absent in the upper extremities and decreased in the lower extremities. There was distal hypesthesia for touch, proprioception, and vibration

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Manage Filters

Top Organisms [Tree]

- Homo sapiens *(4)*
- Mus musculus *(1)*

Find related data

Database:

Select

Find items

Search details

CMT2d[All Fields]

Display Settings: Full Report Send to:

GARS glycyl-tRNA synthetase [*Homo sapiens*]

Gene ID: 2617, updated on 13-May-2012

Summary

Official Symbol	GARS provided by HGNC
Official Full Name	glycyl-tRNA synthetase provided by HGNC
Primary source	HGNC:4162
See related	Ensembl:ENSG00000106105; HPRD:02617; MIM:600287; Vega:OTTHUMG00000152769
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Homo sapiens
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as	HMN5; CMT2D; DSMAV; GlyRS; SMAD1
Summary	This gene encodes glycyl-tRNA synthetase, one of the aminoacyl-tRNA synthetases that charge tRNAs with their cognate amino acids. The encoded enzyme is an (alpha)2 dimer which belongs to the class II family of tRNA synthetases. It has been shown to be a target of autoantibodies in the human autoimmune diseases, polymyositis or dermatomyositis. [provided by RefSeq, Jul 2008]

Table of contents
Summary
Genomic context
Genomic regions, trans products
Bibliography
Phenotypes
Interactions
General gene info
General protein info
Reference sequences
Related sequences
Additional links

Links
Order cDNA clone
BioProjects
BioSystems
Books
CCDS

BLAST

- Basic Local Alignment Search Tool
- Search for sequences
- Homology search (evolutionary hypothesis of relatedness)
- What sequence / sequences in the database are closely related to the sequence I'm searching with?

BLAST example

- You get sequences for your plasmid, and they're not what you expect.
- http://blast.ncbi.nlm.nih.gov

TATTCTCATGACAGCTCGCACTGTTTATGATGATGCTGCTAGACGTGTTTGGACACTGATGAATG
TCATTACACTTGTTTACAAAGTCTACTATGGTAATGCTTTAGATCAAGCTATTTCCATGTGGGCCTT
AGTTATTTCTGTAACCTCTAACTATTCTGGTGTCGTTACGACTATCATGTTTTTAGCTAGAGCTATA
GTGTTTGTGTGTGTTGAGTATTACCCATTGTTATTTATTACTGGCAACACCTTACAGTGTATCATGC
TTGTTTATTGTTTCTTAGGCTATTGTTGCTGCTGCTACTTTGGCCTTTTCTGTTTACTCAACCGTTA
CTTCAGGCTTACTCTTGGTGTTTATGACTACTTGGTCTCTACACAAGAATTTAGGTATATGAACTC
CCAGGGGCTTTTGCCTCCTAA

BLAST: Basic Local Alignmen

blast.ncbi.nlm.nih.gov/Blast.cgi

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NCBI/ BLAST Home

BLAST finds regions of similarity between biological sequences. more...

New Aligning Multiple Protein Sequences? Try the COBALT Multiple Alignment Tool. Go

BLAST Assembled RefSeq Genomes

Choose a species genome to search, or list all genomic BLAST databases.

- Human
- Mouse
- Rat
- *Arabidopsis thaliana*
- *Oryza sativa*
- *Bos taurus*
- *Danio rerio*
- *Drosophila melanogaster*
- *Gallus gallus*
- *Pan troglodytes*
- *Microbes*
- *Apis mellifera*

Basic BLAST

Choose a BLAST program to run.

nucleotide blast	Search a **nucleotide** database using a **nucleotide** query *Algorithms:* blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query *Algorithms:* blastp, psi-blast, phi-blast, delta-blast
blastx	Search **protein** database using a **translated nucleotide** query

News

DELTA-BLAST

DELTA-BLAST performs more sensitive protein-protein searches.
Mon, 23 Apr 2012 12:00:00 EST

More BLAST news...

Tip of the Day

Use Genomic BLAST to see the genomic context

If you are interested in the evolution of a particular gene or gene family it is often intetesting to examine the intro-exon structure even across species

Nucleotide BLAST: Search nu

blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS...

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NCBI/ BLAST/ blastn suite

Standard Nucleotide BLAST

blastn | blastp | blastx | tblastn | tblastx

BLASTN programs search nucleotide databases using a nucleotide query. more...

Reset page
Bookmark

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) Clear

Query subrange

From

To

Or, upload file Choose File No file chosen

Job Title

Enter a descriptive title for your BLAST search

☐ Align two or more sequences

Choose Search Set

Database ○ Human genomic + transcript ○ Mouse genomic + transcript ⊙ Others (nr etc.):

Nucleotide collection (nr/nt)

Organism
Optional

Enter organism name or id--completions will be suggested ☐ Exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Exclude
Optional

☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Nucleotide BLAST: Search nu

blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS...

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blastn | blastp | blastx | tblastn | tblastx

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Reset page
Bookmark

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) Clear

TATTCTCATGACAGCTCGCACTGTTTATGATGATGCTGCTAGACGTGTTTGGACACTGA
TGAATGTCATTACACTTGTTTACAAAGTCTACTATGGTAATGCTTTAGATCAAGCTATT
TCCATGTGGGCCTTAGTTATTTCTGTAACCTCTAACTATTCTGGTGTCGTTACGACTAT
CATGTTTTTAGCTAGAGCTATAGTGTTTGTGTGTGTTGAGTATTACCCATTGTTATTTA
TTACTGGCAACACCTTACAGTGTATCATGCTTGTTTATTGTTTCTTAGGCTATTGTTGC

Query subrange

From

To

Or, upload file Choose File No file chosen

Job Title

Enter a descriptive title for your BLAST search

☐ Align two or more sequences

Choose Search Set

Database ○ Human genomic + transcript ○ Mouse genomic + transcript ⊙ Others (nr etc.):

Nucleotide collection (nr/nt)

Organism
Optional

Enter organism name or id--completions will be suggested ☐ Exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Exclude
Optional

☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Nucleotide BLAST: Search nu

blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS...

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Enter a descriptive title for your BLAST search

☐ **Align two or more sequences**

Choose Search Set

Database

○ Human genomic + transcript ○ Mouse genomic + transcript ⊙ Others (nr etc.):

♦ Nucleotide collection (nr/nt)

Organism
Optional

Enter organism name or id--completions will be suggestec ☐ Exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Exclude
Optional

☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Entrez Query
Optional

Enter an Entrez query to limit search

Program Selection

Optimize for

⊙ Highly similar sequences (megablast)

○ More dissimilar sequences (discontiguous megablast)

○ Somewhat similar sequences (blastn)

Choose a BLAST algorithm

BLAST

Search **database Nucleotide collection (nr/nt)** using **Megablast (Optimize for highly similar sequences**

☐ **Show results in a new window**

⊕ **Algorithm parameters**

Note: Parameter values that differ from the default are highlighted in yell

marked with ♦ sign

NCBI Blast:Nucleotide Sequer

blast.ncbi.nlm.nih.gov/Blast.cgi

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BLAST®

Basic Local Alignment Search Tool

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[Sign In] [Register]

NCBI/ BLAST/ blastn suite/ Formatting Results - WGRZE2NA01S

Edit and Resubmit | Save Search Strategies | Formatting options | Download

Nucleotide Sequence (420 letters)

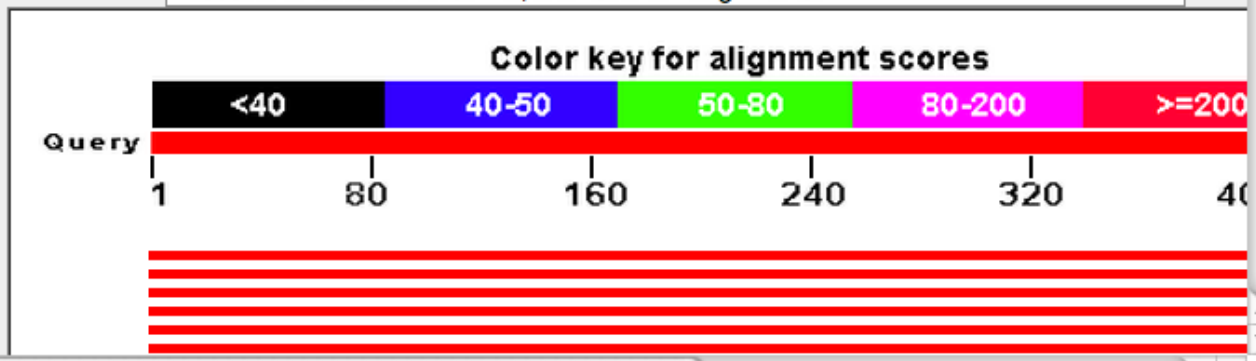
Query ID	lcl|12969	**Database Name**	nr
Description	None	**Description**	All GenBank+EME GSS,environment
Molecule type	nucleic acid	**Program**	BLASTN 2.2.26+
Query Length	420		

Other reports: Search Summary [Taxonomy reports] [Distance tree of results]

Graphic Summary

Distribution of 100 Blast Hits on the Query Sequence

Mouse over to see the defline, click to show alignments



NCBI Blast:Nucleotide Sequer
blast.ncbi.nlm.nih.gov/Blast.cgi
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Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer PubChem BioAssay

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverag
JQ316196.1	SARS coronavirus HKU-39849 isolate UOB, complete genom	776	776	100%
JF292922.1	SARS coronavirus ExoN1 isolate c5P1, complete genome	776	776	100%
JF292915.1	SARS coronavirus MA15 isolate d4ym5, complete genome	776	776	100%
JF292909.1	SARS coronavirus MA15 isolate d2ym4, complete genome >g	776	776	100%
JF292906.1	SARS coronavirus MA15 ExoN1 isolate d3om5, complete gen	776	776	100%
JF292903.1	SARS coronavirus MA15 ExoN1 isolate d4ym5, complete gen	776	776	100%
HQ890541.1	SARS coronavirus MA15 isolate d2ym1, complete genome >g	776	776	100%
HQ890538.1	SARS coronavirus MA15 ExoN1 isolate d2om5, complete gen	776	776	100%
HQ890535.1	SARS coronavirus MA15 ExoN1 isolate d2om2, complete gen	776	776	100%
HQ890532.1	SARS coronavirus MA15 ExoN1 isolate d4ym2, complete gen	776	776	100%
HQ890531.1	SARS coronavirus MA15 ExoN1 isolate d4ym1, complete gen	776	776	100%
HQ890529.1	SARS coronavirus MA15 ExoN1 isolate d2ym4, complete gen	776	776	100%
HQ890526.1	SARS coronavirus MA15 ExoN1 isolate d2ym1, complete gen	776	776	100%
GU553365.1	SARS coronavirus HKU-39849 isolate TCVSP-HARROD-00003	776	776	100%
GU553364.1	SARS coronavirus HKU-39849 isolate TCVSP-HARROD-00002	776	776	100%
GU553363.1	SARS coronavirus HKU-39849 isolate TCVSP-HARROD-00001	776	776	100%
FJ882960.1	SARS coronavirus ExoN1 isolate P3pp34, complete genome	776	776	100%

NCBI Blast:Nucleotide Sequer

blast.ncbi.nlm.nih.gov/Blast.cgi

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JF292903.1	SARS coronavirus MA15 ExoN1 isolate d4ym5, complete gen	776	776	100%
HQ890541.1	SARS coronavirus MA15 isolate d2ym1, complete genome >g	776	776	100%
HQ890538.1	SARS coronavirus MA15 ExoN1 isolate d2om5, complete gen	776	776	100%
HQ890535.1	SARS coronavirus MA15 ExoN1 isolate d2om2, complete gen	776	776	100%
HQ890532.1	SARS coronavirus MA15 ExoN1 isolate d4ym2, complete gen	776	776	100%
HQ890531.1	SARS coronavirus MA15 ExoN1 isolate d4ym1, complete gen	776	776	100%
HQ890529.1	SARS coronavirus MA15 ExoN1 isolate d2ym4, complete gen	776	776	100%
HQ890526.1	SARS coronavirus MA15 ExoN1 isolate d2ym1, complete gen	776	776	100%
GU553365.1	SARS coronavirus HKU-39849 isolate TCVSP-HARROD-00003	776	776	100%
GU553364.1	SARS coronavirus HKU-39849 isolate TCVSP-HARROD-00002	776	776	100%
GU553363.1	SARS coronavirus HKU-39849 isolate TCVSP-HARROD-00001	776	776	100%
FJ882960.1	SARS coronavirus ExoN1 isolate P3pp34, complete genome	776	776	100%

BLAST

- Looks for homology
- What sequences in the database are evolutionary similar to this one?
- Can search DNA, RNA, Proteins
- Lots of tutorials and explanations on the web

UCSC Genome browser

- Get a genomic context view of human and some other reference genomes
  - Mouse, fly, cat, dog, panda, turkey, orangutan, naked mole rat, etc.
- Annotation tracks
- What's going on at a particular locus
- http://genome.ucsc.edu

UCSC Genome Bioinformatics

About the UCSC Genome Bioinformatics Site

Welcome to the UCSC Genome Browser website. This site contains the reference sequence and working draft assemblies for a large collection of genomes. It also provides portals to the ENCODE and Neandertal projects.

We encourage you to explore these sequences with our tools. The Genome Browser zooms and scrolls over chromosomes, showing the work of annotators worldwide. The Gene Sorter shows expression, homology and other information on groups of genes that can be related in many ways. Blat quickly maps your sequence to the genome. The Table Browser provides convenient access to the underlying database. VisiGene lets you browse through a large collection of *in situ* mouse and frog images to examine expression patterns. Genome Graphs allows you to upload and display genome-wide data sets.

The UCSC Genome Browser is developed and maintained by the Genome Bioinformatics Group, a cross-departmental team within the Center for Biomolecular Science and Engineering (CBSE) at the University of California Santa Cruz (UCSC). If you have feedback or questions concerning the tools or data on this website, feel free to contact us on our public mailing list.

News

News Archives ▶

To receive announcements of new genome assembly releases, new software features, updates and training seminars by email, subscribe to the genome-announce mailing list.

15 May 2012 - New Fugu (fr3) Assembly Now Available in the Genome Browser

We are pleased to announce the release of a Genome Browser for the October 2011 fugu, *Takifugu rubripes* (JGI v5.0, UCSC version fr3). Whole genome shotgun assembly was provided by the International Fugu Genome Sequencing Consortium, led by JGI and the Singapore Institute of Molecular and Cell Biology (IMCB).

Bulk downloads of the sequence and annotation data are available via the Genome Browser FTP server or the Downloads page. These data have specific conditions for use. The fugu (fr3) browser annotation tracks were generated by UCSC and collaborators worldwide. See the Credits page for a detailed list of the organizations and individuals who contributed to the success of this release.

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Human (Homo sapiens) Genc

genome.ucsc.edu/cgi-bin/hgGateway?hgsid=276435845&clade=mammal&org=Human&db=0

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Human (*Homo sapiens*) Genome Browser Gateway

The UCSC Genome Browser was created by the Genome Bioinformatics Group of UC Santa Cruz.
Software Copyright (c) The Regents of the University of California. All rights reserved.

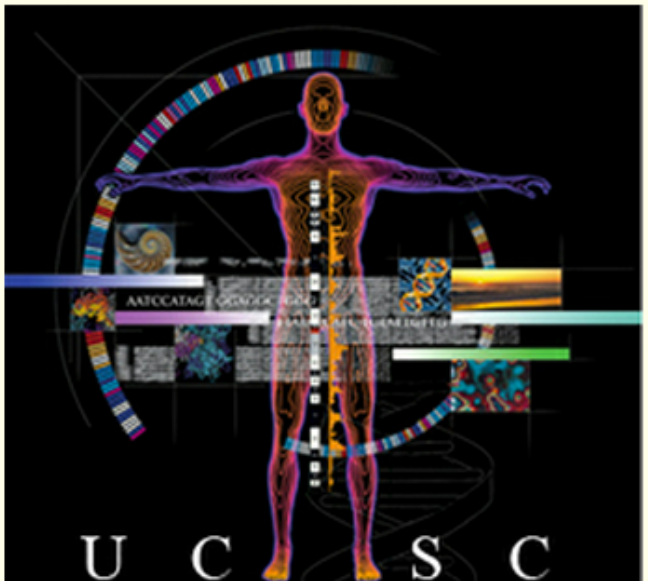
clade	genome	assembly	position or search term	gene	
Mammal	Human	Feb. 2009 (GRCh37/hg19)	GARS		submit

Click here to reset the browser user interface settings to their defaults.

track search | add custom tracks | track hubs | configure tracks and display | clear position

About the Human Feb. 2009 (GRCh37/hg19) assembly (sequences)

The February 2009 human reference sequence (GRCh37) was produced by the Genome Reference Consortium. For more information about this assembly, see GRCh37 in the NCBI Assembly database.



Sample position queries

A genome position can be specified by the accession number of a sequenced genomic clone, an mRNA or EST or STS marker, a chromosomal coordinate range, or keywords from the GenBank description of an mRNA. The following list shows examples of valid position queries for the human genome. See the User's Guide for more information.

Human GARS - UCSC Genom
genome.ucsc.edu/cgi-bin/hgTracks?hgHubConnect.destUrl=..%2Fcgi-bin%2FhgTracks&clade=mammal&org=Human&db=hg1...
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UCSC Genes

GARS (uc003tbm.3) at chr7:30634181-30673648 - Homo sapiens glycyl-tRNA synthetase (GARS), mRNA.
GART (uc010gmd.3) at chr21:34876238-34915195 - Homo sapiens phosphoribosylglycinamide formyltransfera
GART (uc002yrz.3) at chr21:34876238-34915195 - Homo sapiens phosphoribosylglycinamide formyltransfera
GART (uc002yry.3) at chr21:34876238-34915198 - Homo sapiens phosphoribosylglycinamide formyltransfera
GART (uc002yrx.3) at chr21:34876238-34914464 - Homo sapiens phosphoribosylglycinamide formyltransfera

RefSeq Genes

GARS at chr7:30634181-30673648 - (NM_002047) glycine--tRNA ligase precursor

Non-Human RefSeq Genes

gars at chr7:30638412-30672065 - (NM_001093178) glycyl-tRNA synthetase
gars at chr7:30634682-30673476 - (NM_001007491) glycyl-tRNA synthetase
GARS at chr7:30638412-30673476 - (NM_001031510) glycine--tRNA ligase
GARS at chr7:30634449-30673645 - (NM_001133870) glycine--tRNA ligase precursor
GARS at chr7:30634523-30673656 - (NM_001097566) glycine--tRNA ligase
Gars at chr7:30634680-30673659 - (NM_180678) glycine--tRNA ligase

Basic Gene Annotation Set from ENCODE/GENCODE

GARS at chr7:30634297-30673649

Comprehensive Gene Annotation Set from ENCODE/GENCODE

GARS at chr7:30634297-30673649

Human chr7:30,634,181-30

genome.ucsc.edu/cgi-bin/hgTracks

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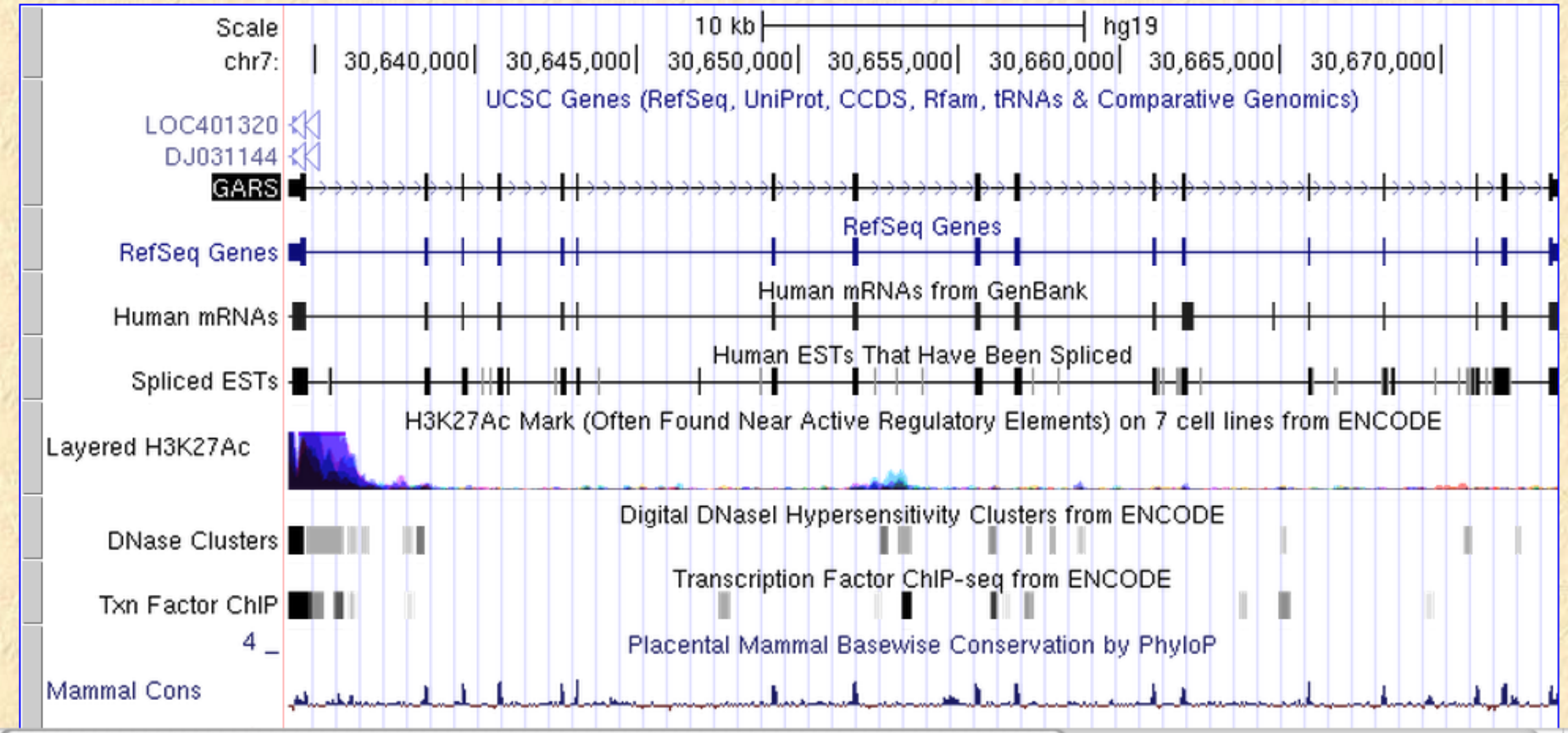
Home | Genomes | Blat | Tables | Gene Sorter | PCR | DNA | Convert | PS/PDF

UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

position/search chr7:30,634,181-30,673,648 gene jump clear size 39,468 bp. configure



Human chr7:30,634,181-30

genome.ucsc.edu/cgi-bin/hgTracks

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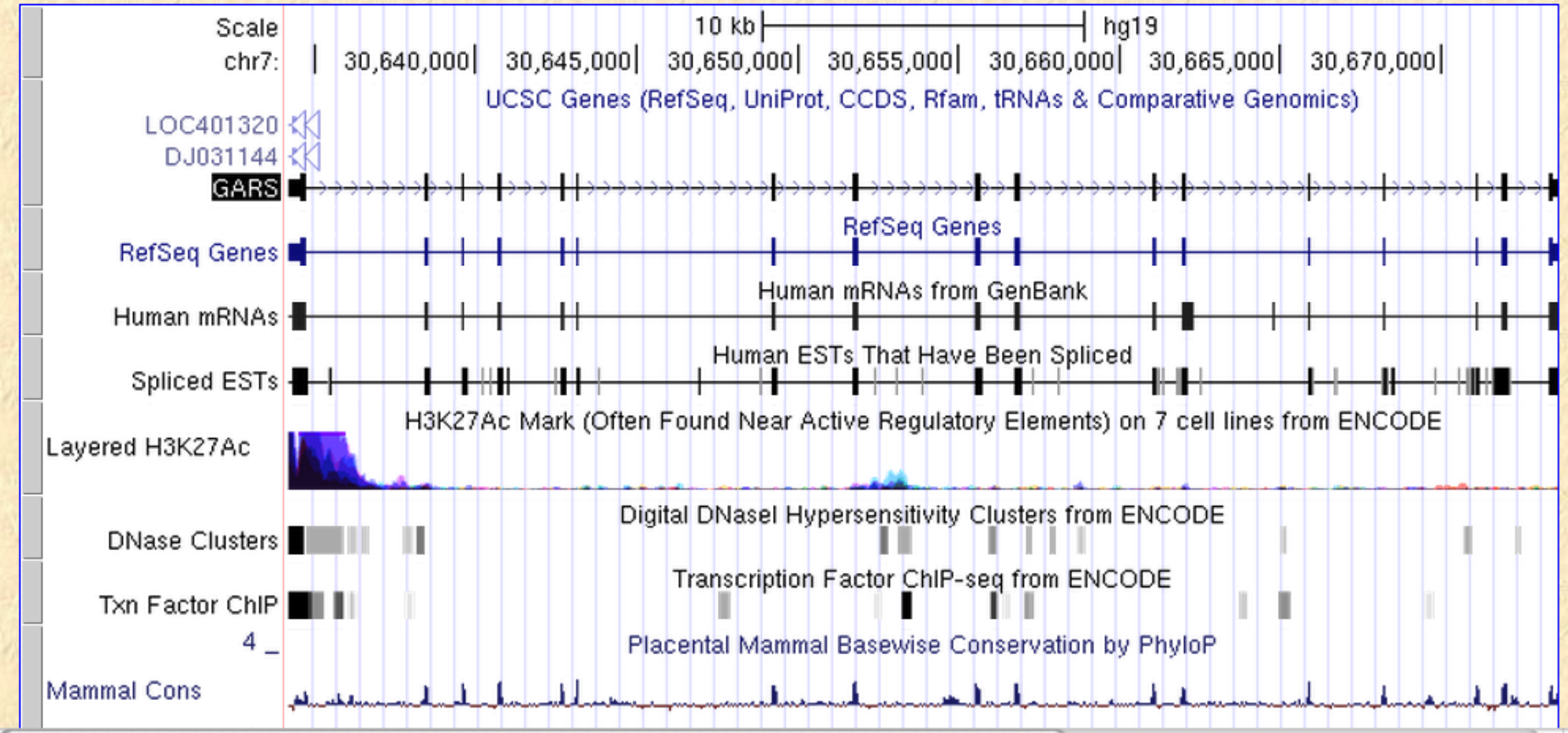
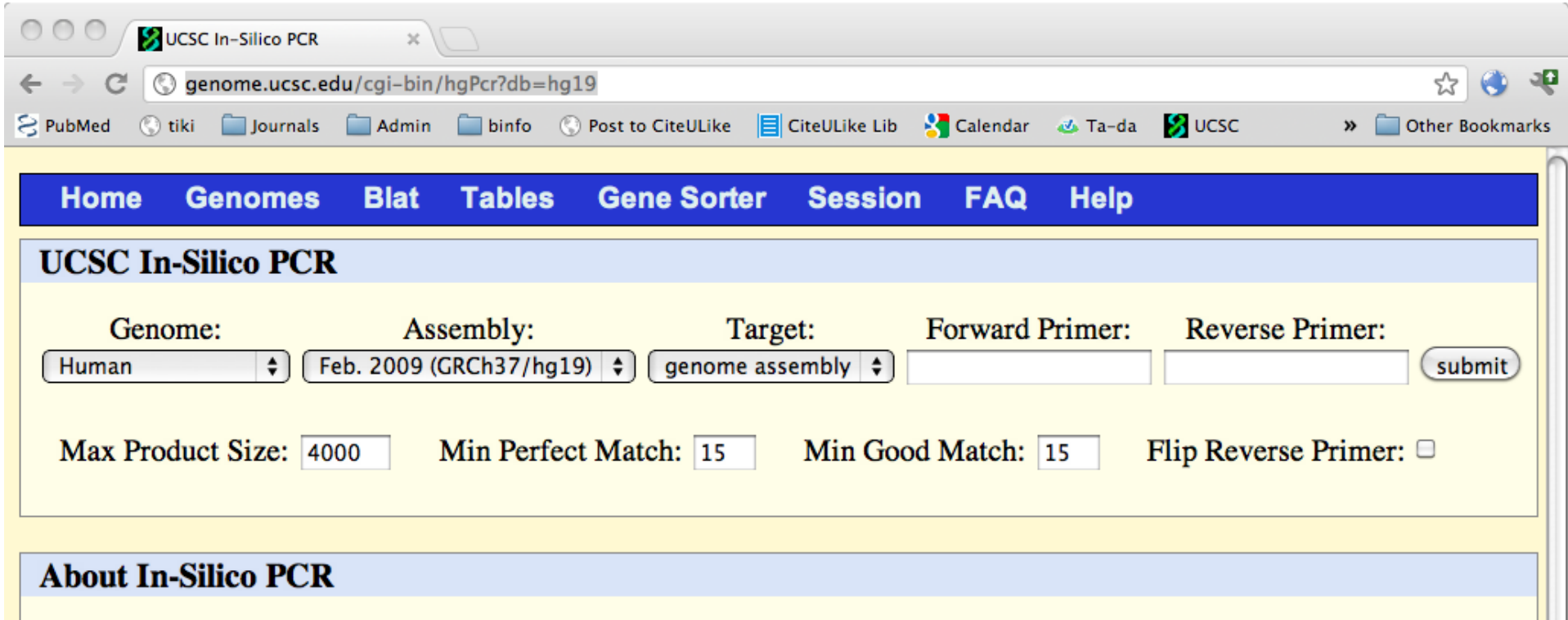
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

position/search chr7:30,634,181-30,673,648 gene jump clear size 39,468 bp. configure



UCSC In-silico PCR

- Quickly check where your primers map in a genome.
- http://genome.ucsc.edu/cgi-bin/hgPcr?db=hg19



Any Questions?

arjun.prasad@nih.gov

Keeping a Lab Notebook

Basic Principles and Best Practices

Philip Ryan, PhD
Director of Student Services
NIH Graduate Partnerships Program
Office of Intramural Training and Education

Office of Intramural TRAINING & EDUCATION
NATIONAL INSTITUTES OF HEALTH

Overview

- Introductory material
  - What is a lab notebook?
  - What are the different types of lab notebooks?
- Lab notebook do’s and don'ts
  - Structure and organization
  - Lab notebook ethics
- Examples of good notebooks

A Lab Notebook Is…

- Complete record of procedures, reagents, data, and thoughts to pass on to other researchers
- Explanation of why experiments were initiated, how they were performed, and the results
- Legal document to prove patents and defend your data against accusations of fraud
- Scientific legacy in the lab

A Lab Notebook Is Not…

- A journal
- A record of communications
- A place to compile lab protocols/manuals
- Yours to take home

Different Types of Lab Notebooks

- Bound/Stitched Notebook
- Loose Leaf/Three Ring Binder Notebook
- Electronic Notebook

Advantages/Disadvantages

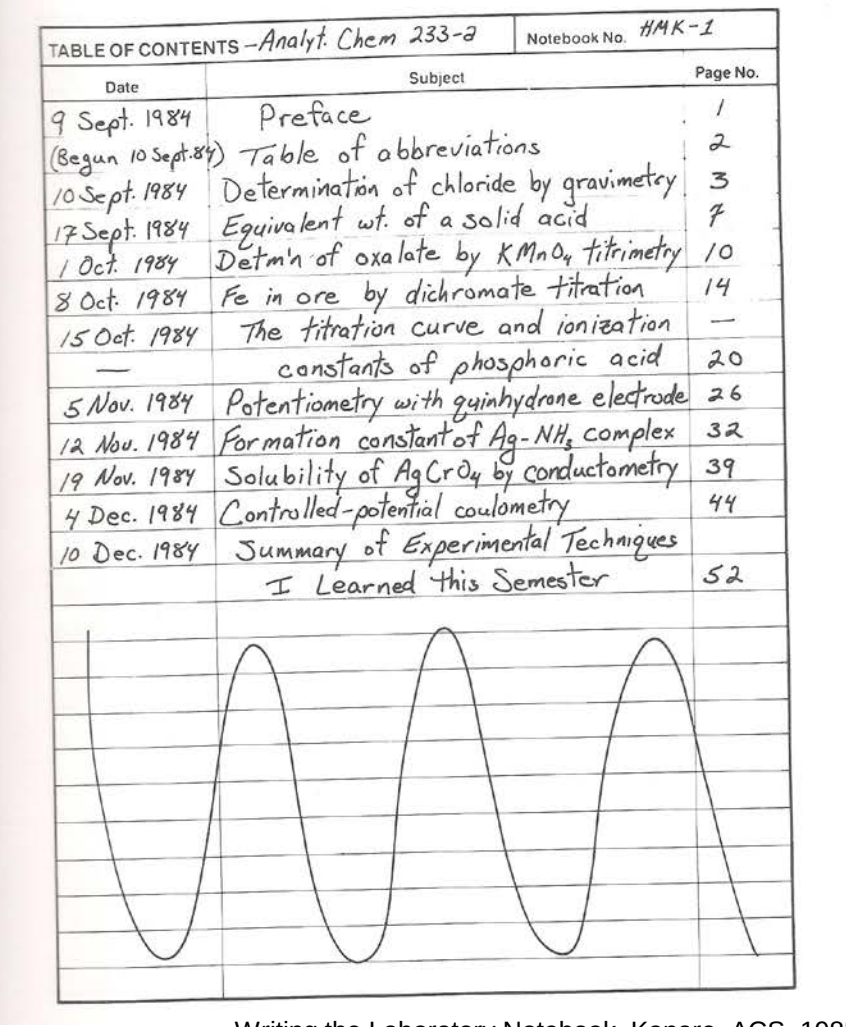
Type of Notebook	Advantages	Disadvantages
Bound/Stitched	No lost pages, legally stronger	Difficult to copy, not logically organized, requires references to data stored elsewhere
Loose Leaf/Binder	Organized by experiment, data stored together	Sheets fall out, difficult to authenticate
Computer/Electronic	Easy to search, easy to read, digital data easy to store	Requires electronic security, corrupted files, software compatibility issues

What Goes in the Lab Notebook

- Notebook name
- Inside cover or cover page
  - Your name and year
  - General project name
  - Lab mailing address
- Table of Contents
- Body of notebook
  - Experimental entries

Table of Contents

- Table of Contents
  - Page number
  - Date
  - Subject/Experiment



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Writing the Laboratory Notebook, Kanare, ACS, 1985

Experimental Entries

- Date
- Title
- Hypothesis or Goal: Brief statement of purpose
- Background
- How: Protocols, calculations, reagents, equipment
- Observations:
  - All that happens (planned or unplanned)
  - Raw experimental data
  - Taped in information or reference to data location
- Data analysis:
  - Processing of raw data, graphs, interpretations
- Ideas for future experiments

The Details of “How”

- Reagents: source, product number, lot number, expiration date, how and where stored
- Solutions and how they were made
- Cells used: type, source, passage number, growth medium
- Instruments: type, name, location, serial number
- Number and volume of washes
- Centrifuge speeds and duration of spins
- Heating rates and levels of agitation
- Time between and during steps
- Gel percentages
- Type of water used

Ethics

- All data go in to the notebook
  - Even "bad" data points or “outliers”
  - Failed experiments or contradictory experiments
- No pages come out of the notebook
  - Do not take any pages out or remove any data
  - Do not skip pages in your notebook
  - Cross out any unused parts of a page
- Correct mistakes, do not remove them
  - Cross out mistakes with a single line
  - Paste in corrections without covering anything
  - Sign and date all corrections
- Honesty is the best policy

Example

Co-IP of IKEPP & Myotpn from fractionated cells

1/19/05

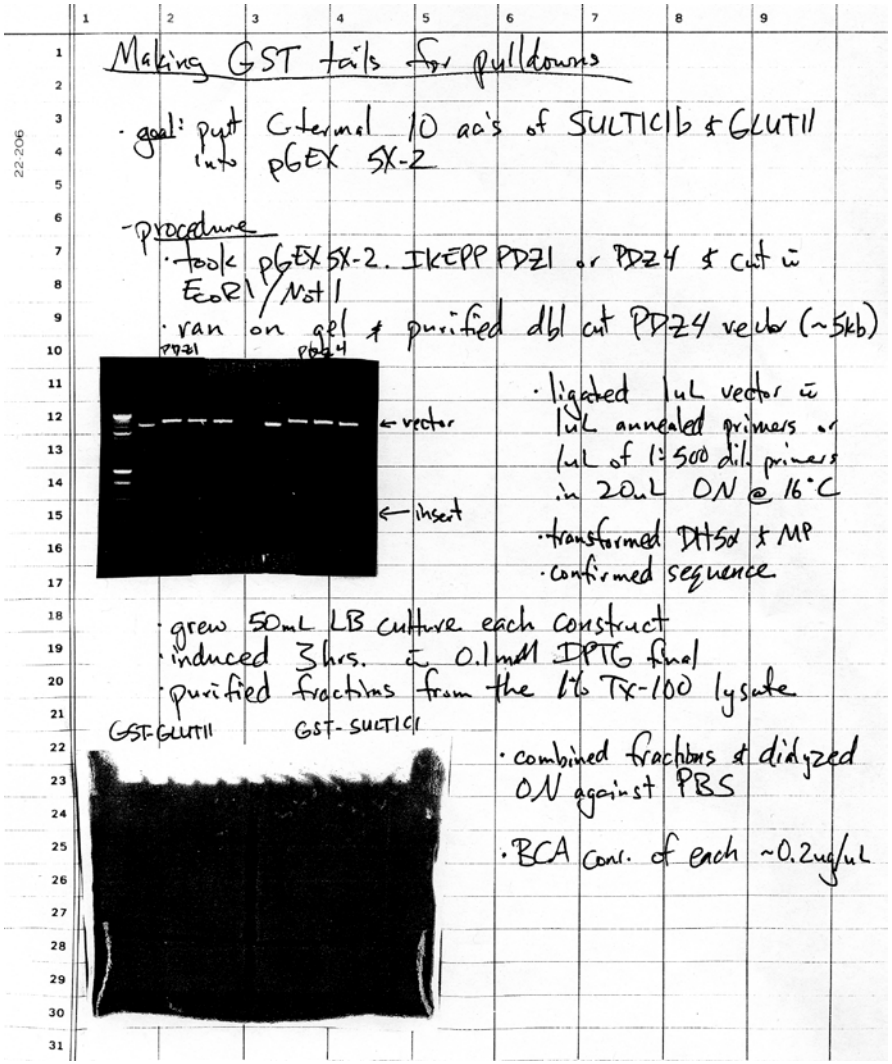
goal: to see if diff. to co-IP from cytosol & mem.

procedure
- took 100mm dishes of transfected Cos cells & made 400uL equal vol. of mems & cytosol
- precleared each on 30uL of Protein A for one hour
- added lysate to Roche HA-beads (25uL) for 3 hours 1:30-4:30
- washed 6X w/ lysis bfr (0.3% Tx-100)
- eluted from beads by heating 2X CFTR disaggregation bfr w/out BME 3 min.; inputs in normal SB
- ran gel & blotted for IKEPP

result
- see potential co-IP band in cytosol
- also see IK signal at top of gel maybe b/c no BME in sample bfr
- in inputs, IK is mostly cytosol but w/ myotpn 50:50 cyt:mem

next step
- strip & reprobe w/ 2° alone to see if high bands just IgG
- will repeat w/ correct buffer

Example



Making GST tails for pulldowns

· goal: put C-terminal 10 aa's of SULTIC1b & GLUT11 into pGEX 5X-2

-procedure

· took pGEX 5X-2, IKEPP PDZ1 or PDZ4 & cut w/ EcoR1/Not1

· ran on gel & purified dbl cut PDZ4 vector (~5kb)

PDZ1 PDZ4

← vector

← insert

· ligated 1uL vector w/ 1uL annealed primers or 1uL of 1:500 dil. primers in 20uL ON @ 16°C

· transformed DH5α & MP

· confirmed sequence

· grew 50mL LB culture each construct

· induced 3hrs. w/ 0.1mM IPTG final

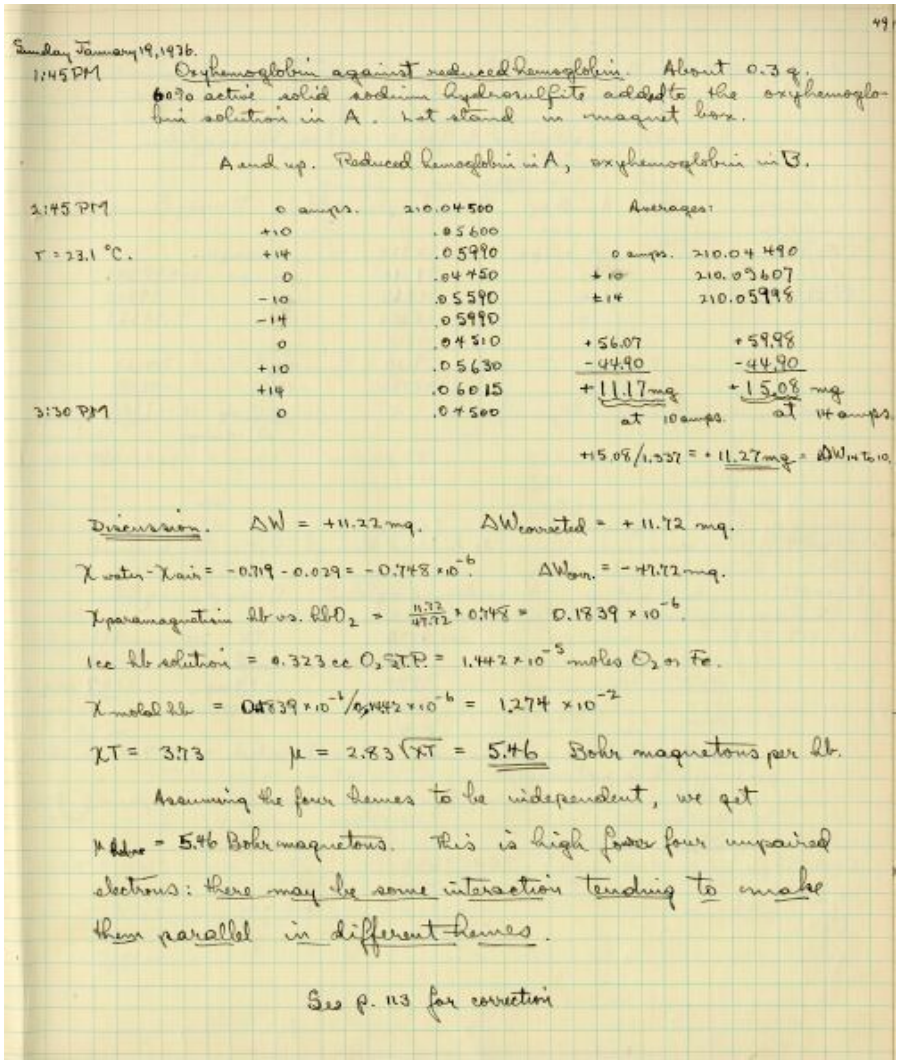
· purified fractions from the 1% Tx-100 lysate

GST-GLUT11 GST-SULTIC1

· combined fractions & dialyzed ON against PBS

· BCA conc. of each ~0.2ug/uL

Example



49

Sunday January 19, 1936.

1:45 PM Oxyhemoglobin against reduced hemoglobin. About 0.3 g. 60% active solid sodium hydrosulfite added to the oxyhemoglobin solution in A. Let stand in magnet box.

A end up. Reduced hemoglobin in A, oxyhemoglobin in B.

2:45 PM	0 amps.	210.04500	Averages:	
	+10	.05600		
T = 23.1 °C.	+14	.05990	0 amps.	210.04490
	0	.04450	+10	210.05607
	−10	.05590	+14	210.05998
	−14	.05990		
	0	.04510	+56.07	+59.98
	+10	.05630	−44.90	−44.90
	+14	.06015	+11.17 mg	+15.08 mg
3:30 PM	0	.04500	at 10 amps.	at 14 amps.

+15.08/1.337 = +11.27 mg = $\Delta W_{14 \to 10}$

Discussion. ΔW = +11.72 mg. $\Delta W_{corrected}$ = +11.72 mg.

$\chi_{water} - \chi_{air} = -0.719 - 0.029 = -0.748 \times 10^{-6}$. $\Delta W_{corr.}$ = −47.72 mg.

$\chi_{paramagnetic}$ Hb vs. HbO$_2$ = $\frac{11.72}{47.72} \times 0.748 = 0.1839 \times 10^{-6}$.

1 cc Hb solution = 0.323 cc O$_2$ S.T.P. = 1.442×10^{-5} moles O$_2$ or Fe.

$\chi_{molal\ Hb} = 0.1839 \times 10^{-6} / 0.1442 \times 10^{-6} = 1.274 \times 10^{-2}$

χT = 3.73 $\mu = 2.83\sqrt{\chi T}$ = 5.46 Bohr magnetons per Hb.

Assuming the four hemes to be independent, we get

$\mu_{Hb\ one}$ = 5.46 Bohr magnetons. This is high for four unpaired electrons: there may be some interaction tending to make them parallel in different hemes.

See p. 113 for correction

Taken from the online *Linus Pauling Research Notebooks*, http://osulibrary.orst.edu/specialcollections/rnb/index.html

References

- Guidelines for Scientific Record Keeping in the Intramural Research Program at the NIH
  - http://sourcebook.od.nih.gov/ethic-conduct/RECORDKEEPING.pdf
- Writing the Laboratory Notebook, Howard Kanares, ACS 1985
- The Oregon State Library Special Collections
  - http://osulibrary.orst.edu/specialcollections/rnb/index.html

Acknowledgements

- Charu Chaudhry, PhD
  - NICHD
- Garland Crawford, PhD
  - NIAMS
- Nicholas Fritzkee, PhD
  - NIDDK

SUMMER INTERN SCIENCE SKILLS BOOT CAMP

Giving An Effective Presentation

Roza Selimyan, Ph.D.

06.27.2012

Presenting at a group meeting should help:

1) the **audience** understand what you are doing

Presenting at a group meeting should help:

1) the **audience** understand what you are doing

2) **<u>you</u> with your research**

The 3P rule:

PLAN

PREPARE*

PRESENT

WITH CARE

Possible presentation scheme:

1. **start with welcoming courtesies, introduce yourself**
2. **state the purpose of your talk**
3. **give a route map** (also tell them how long will your presentation take)
4.* **give the rules** (e.g., questions in the end or can be interrupted)

Communicate what you want the audience to know

1) Tell them what you are going to tell them

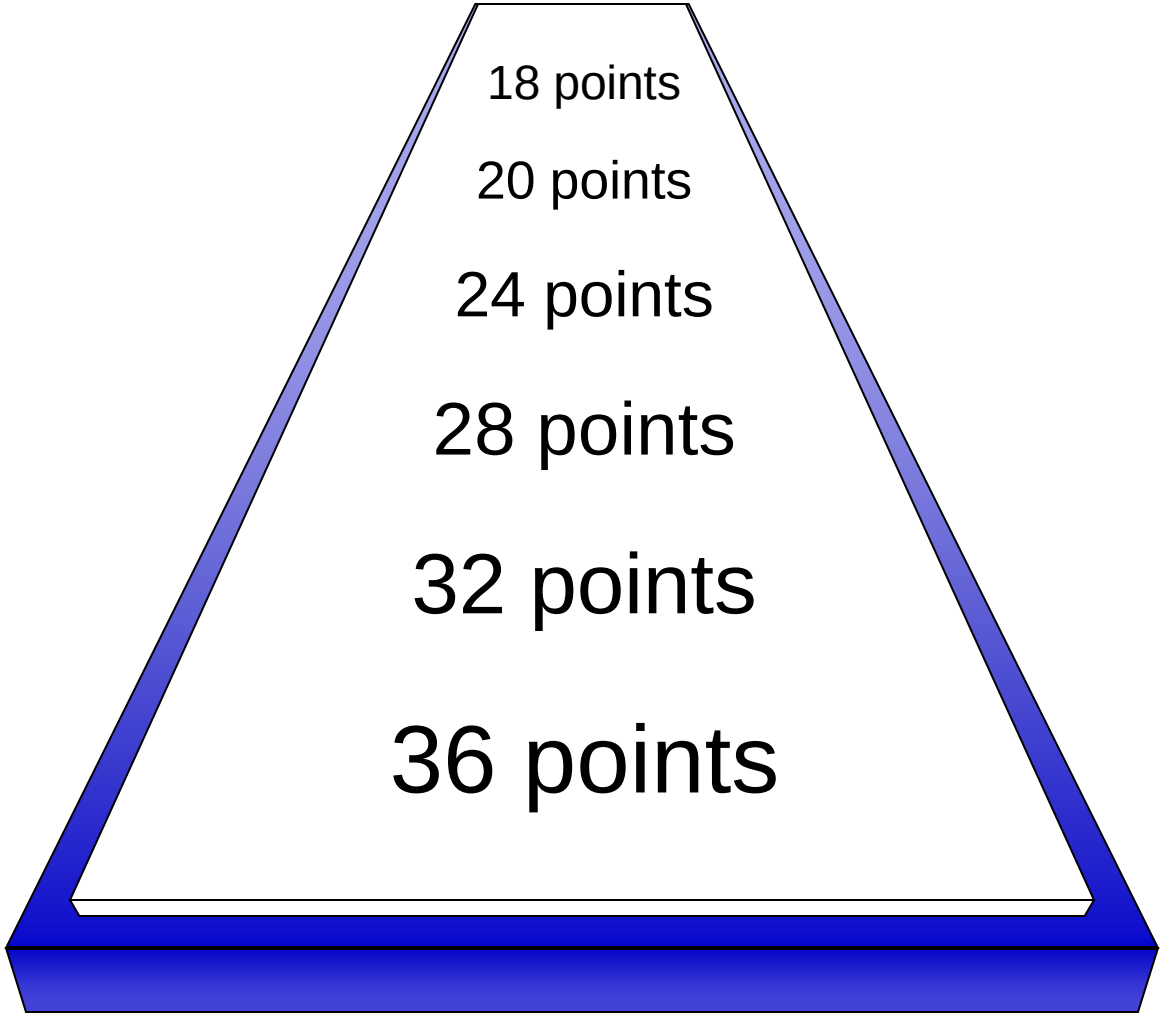
2) Tell them

3) Tell them what you told them

Preparing the slides

- Keep the layout and style as consistent as possible
- Every slide should have a heading
- If it’s possible, make a statement

Type size should be 18 points or larger



* References can be smaller (≥14 points)

Make your slides readable

use proper font size

Make your slides readable

use proper font size

Make your slides readable

Use contrasting colors

Make your slides readable

Use contrasting colors

Make your slides readable

Lots of people can't read this

Make your slides readable

Lots of people can't read this –

and even if they could,

it makes your eyes hurt.

Other color combinations can be equally bad!

Other color combinations can be equally bad!

1. Transcription control of gene regulation is controlled by:

1. **Promoters**
   - **Occur upstream of the transcription start site.**
   - **Some determine where transcription begins (e.g., TATA), whereas others determine if transcription begins.**
   - **Promoters are activated by specialized transcription factor (TF) proteins (specific TFs bind specific promoters).**
   - **One or many promoters (each with specific TF proteins) may occur for any given gene.**
   - **Promoters may be positively or negatively regulated.**

2. **Enhancers**
   - **Occur upstream or downstream of the transcription start site.**
   - **Regulatory proteins bind specific enhancer sequences; binding is determined by the DNA sequence.**
   - **Loops may form in DNA bound to TFs and make contact with upstream enhancer elements.**
   - **Interactions of regulatory proteins determine if transcription is activated or repressed (positively or negatively regulated).**

Transcriptional control of gene regulation

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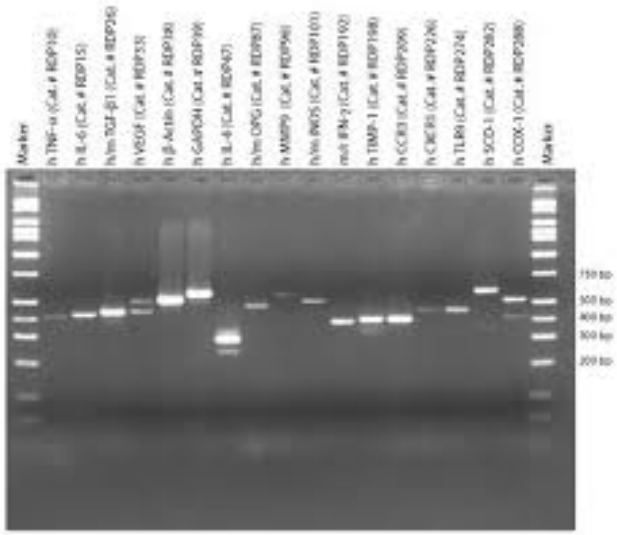
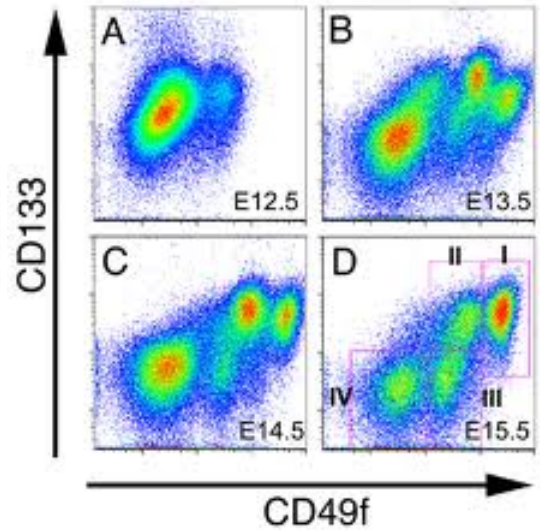
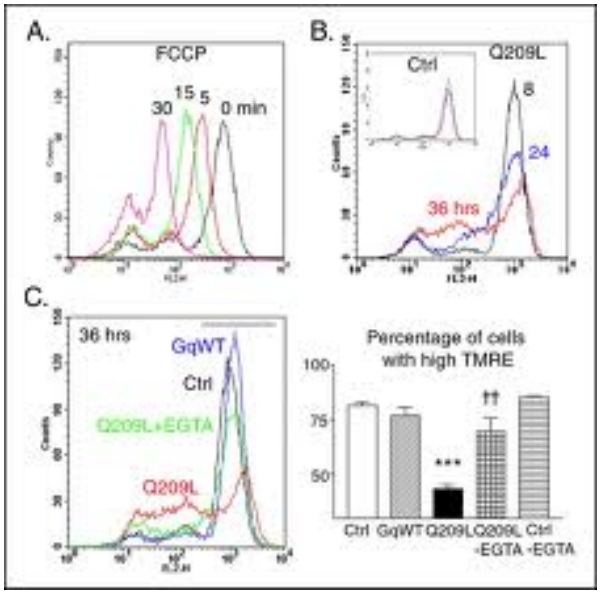
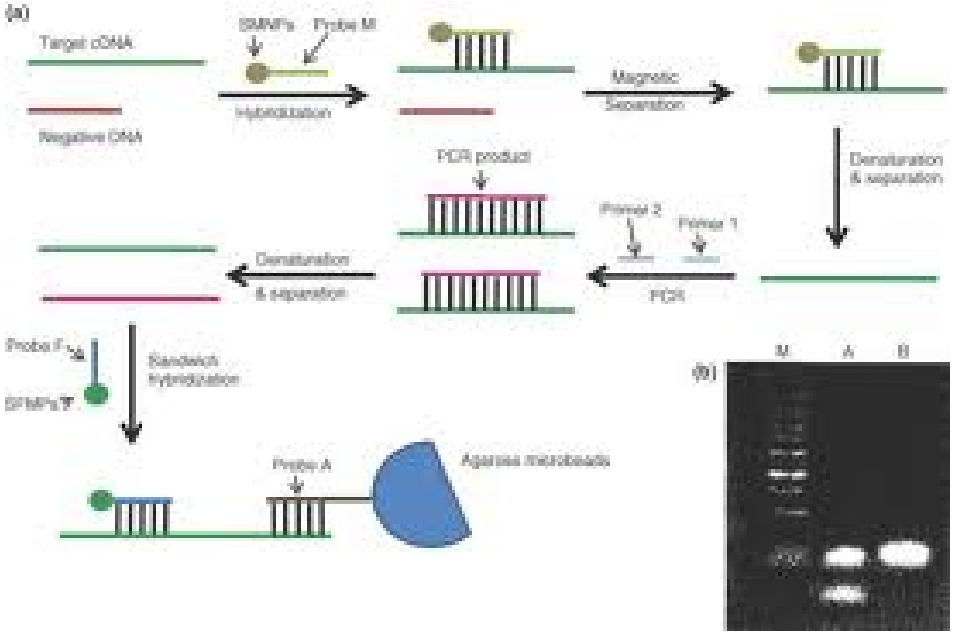
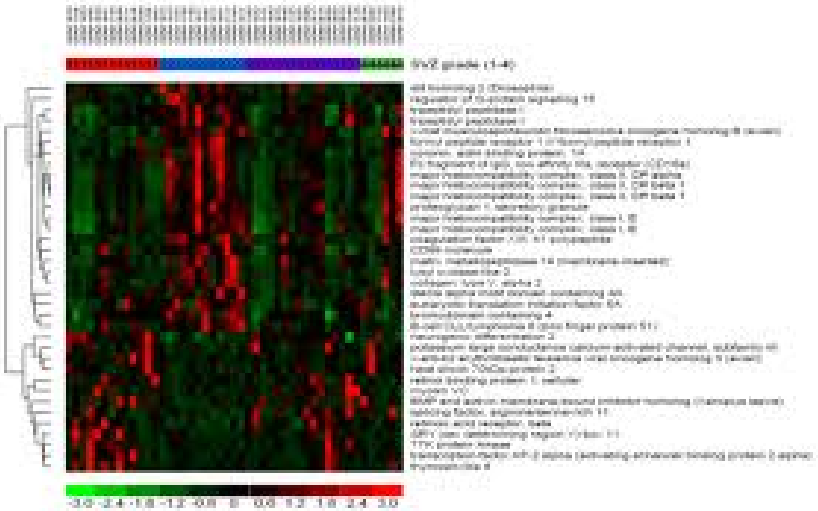
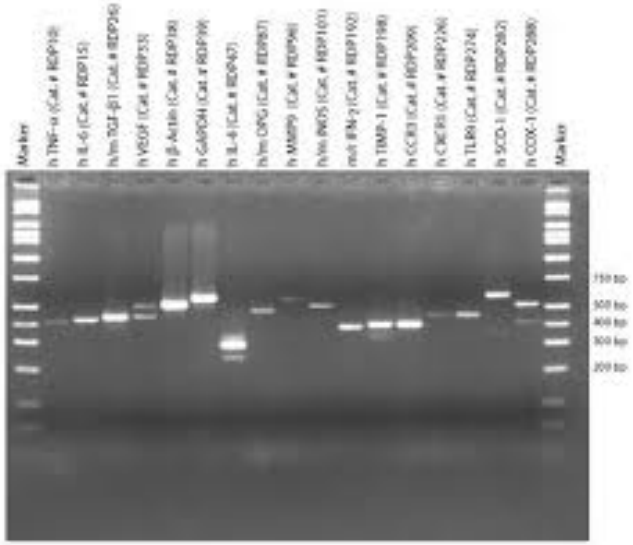

Marker
h TNF-α (Cat. # RDP10)
h IL-6 (Cat. # RDP15)
h/m TGF-β1 (Cat. # RDP35)
h/rat VEGF (Cat. # RDP33)
h β-Actin (Cat. # RDP100)
h GAPDH (Cat. # RDP99)
h IL-8 (Cat. # RDP47)
h/m DPG (Cat. # RDP87)
h MMP9 (Cat. # RDP96)
h/m iNOS (Cat. # RDP101)
mus IFN-γ (Cat. # RDP102)
h TIMP-1 (Cat. # RDP198)
h CCR3 (Cat. # RDP209)
h CXCR5 (Cat. # RDP226)
h TLR9 (Cat. # RDP274)
h SCD-1 (Cat. # RDP282)
h COX-1 (Cat. # RDP288)
Marker
750 bp
500 bp
400 bp
300 bp
200 bp


CD133
CD49f
A
E12.5
B
E13.5
C
E14.5
D
E15.5
I
II
III
IV


A.
FCCP
30
15
5
0 min
B.
Ctrl
Q209L
8
24
36 hrs
C.
36 hrs
GqWT
Ctrl
Q209L+EGTA
Q209L
Percentage of cells with high TMRE
100
75
50
***
††
Ctrl
GqWT
Q209L
Q209L -EGTA
Ctrl -EGTA


(a)
Target cDNA
SMNPs
Probe M
Hybridization
Negative DNA
Magnetic Separation
Denaturation & separation
PCR product
Primer 2
Primer 1
PCR
Denaturation & separation
Probe F
SFMPs
Sandwich hybridization
Probe A
Agarose microbeads
(b)
M
A
B




Marker
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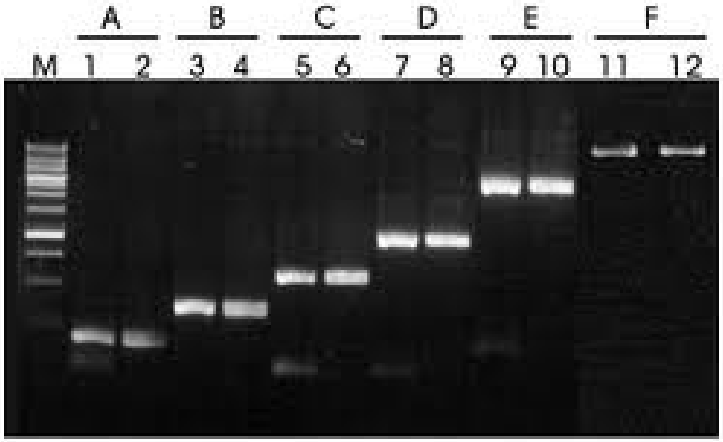
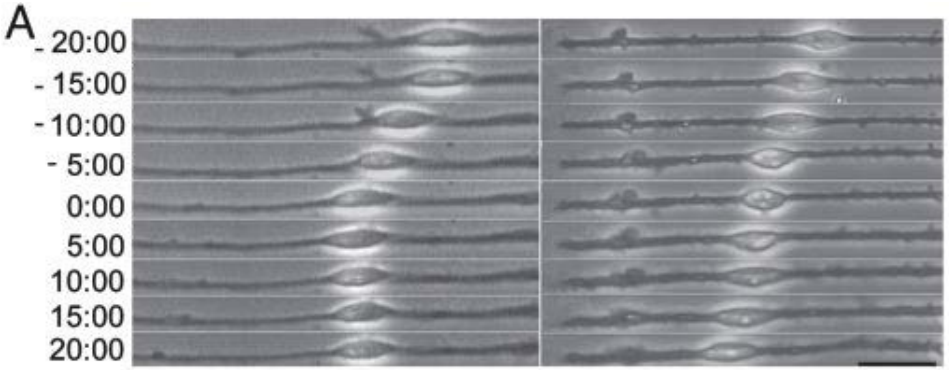
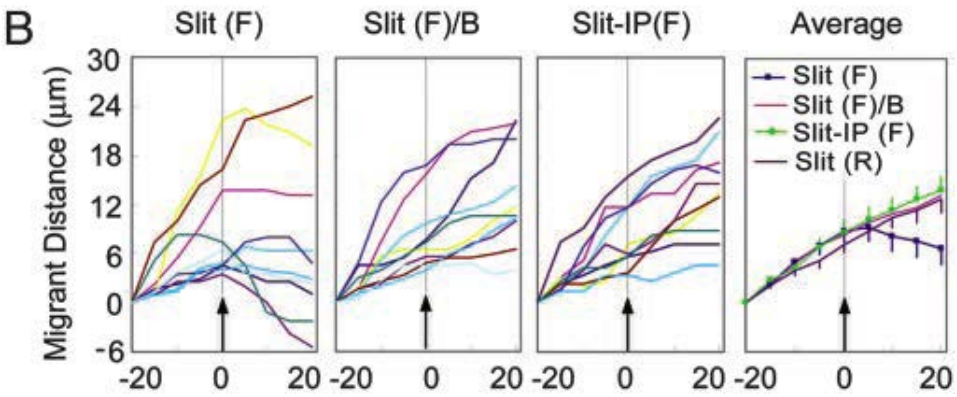
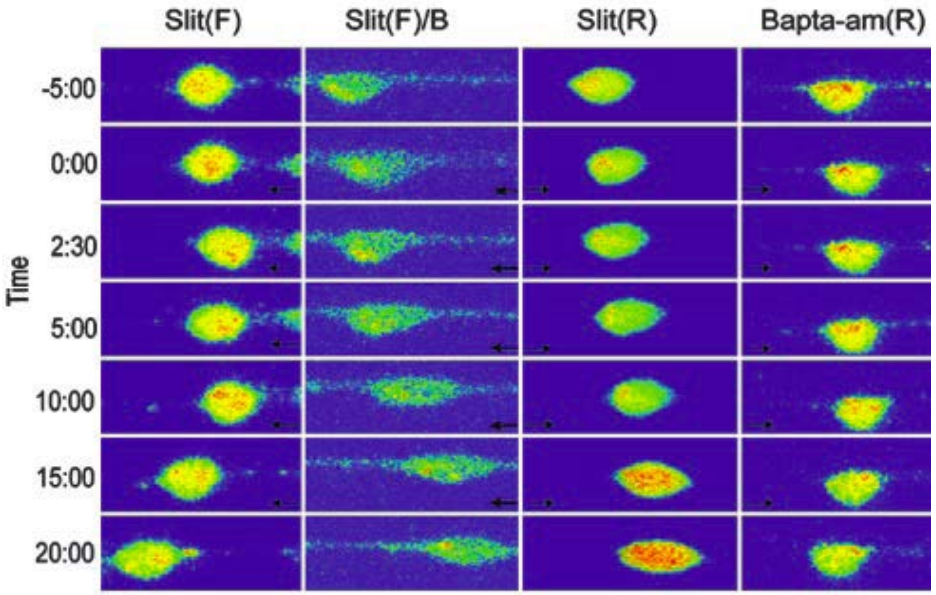
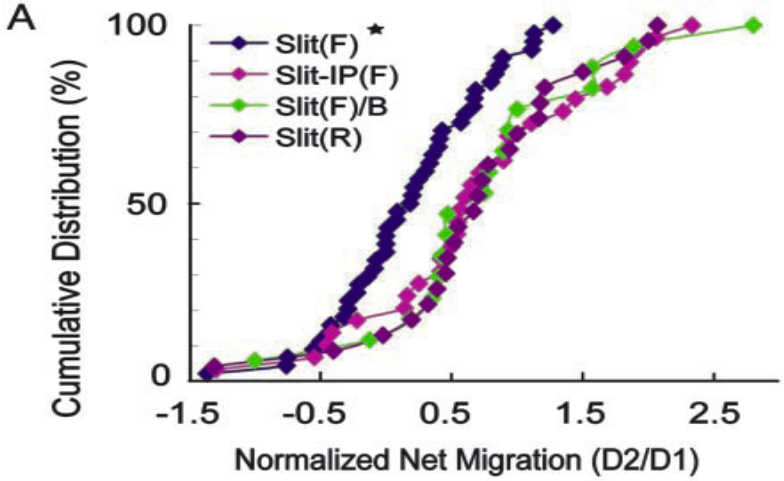
h IL-6 (Cat. # RDP15)
h/m TGF-β1 (Cat. # RDP20)
h VEGF (Cat. # RDP23)
h β-Actin (Cat. # RDP30)
h GAPDH (Cat. # RDP39)
h IL-8 (Cat. # RDP47)
h/m OPG (Cat. # RDP87)
h MMP9 (Cat. # RDP96)
h/m iNOS (Cat. # RDP101)
m/r IFN-γ (Cat. # RDP102)
h TIMP-1 (Cat. # RDP198)
h CCR3 (Cat. # RDP209)
h CXCR5 (Cat. # RDP236)
h TLR9 (Cat. # RDP274)
h SOD-1 (Cat. # RDP282)
h COX-1 (Cat. # RDP288)
Marker
750 bp
600 bp
400 bp
300 bp
200 bp

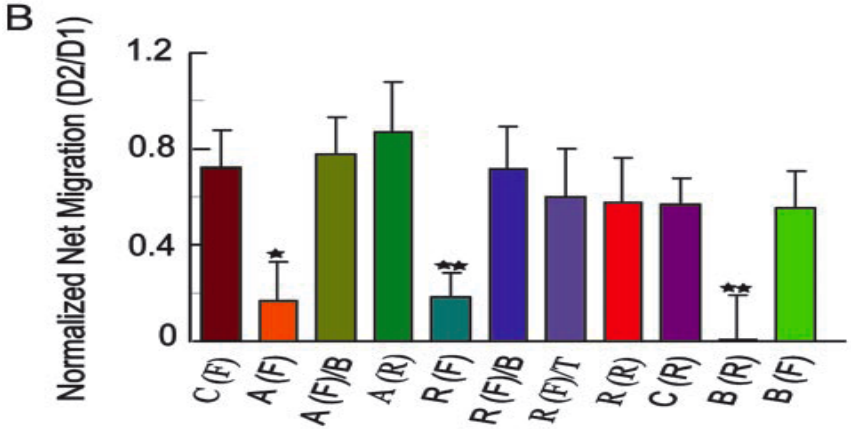

A
B
C
D
E
F
M 1 2 3 4 5 6 7 8 9 10 11 12

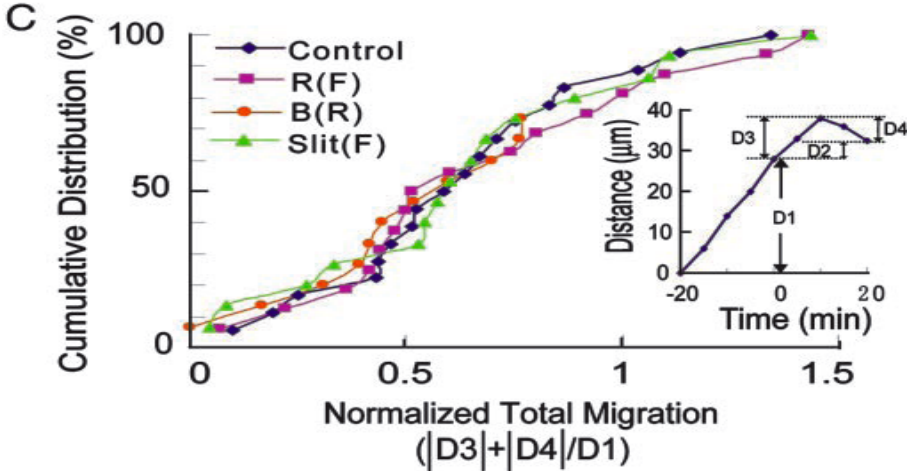

A
- 20:00
- 15:00
- 10:00
- 5:00
0:00
5:00
10:00
15:00
20:00

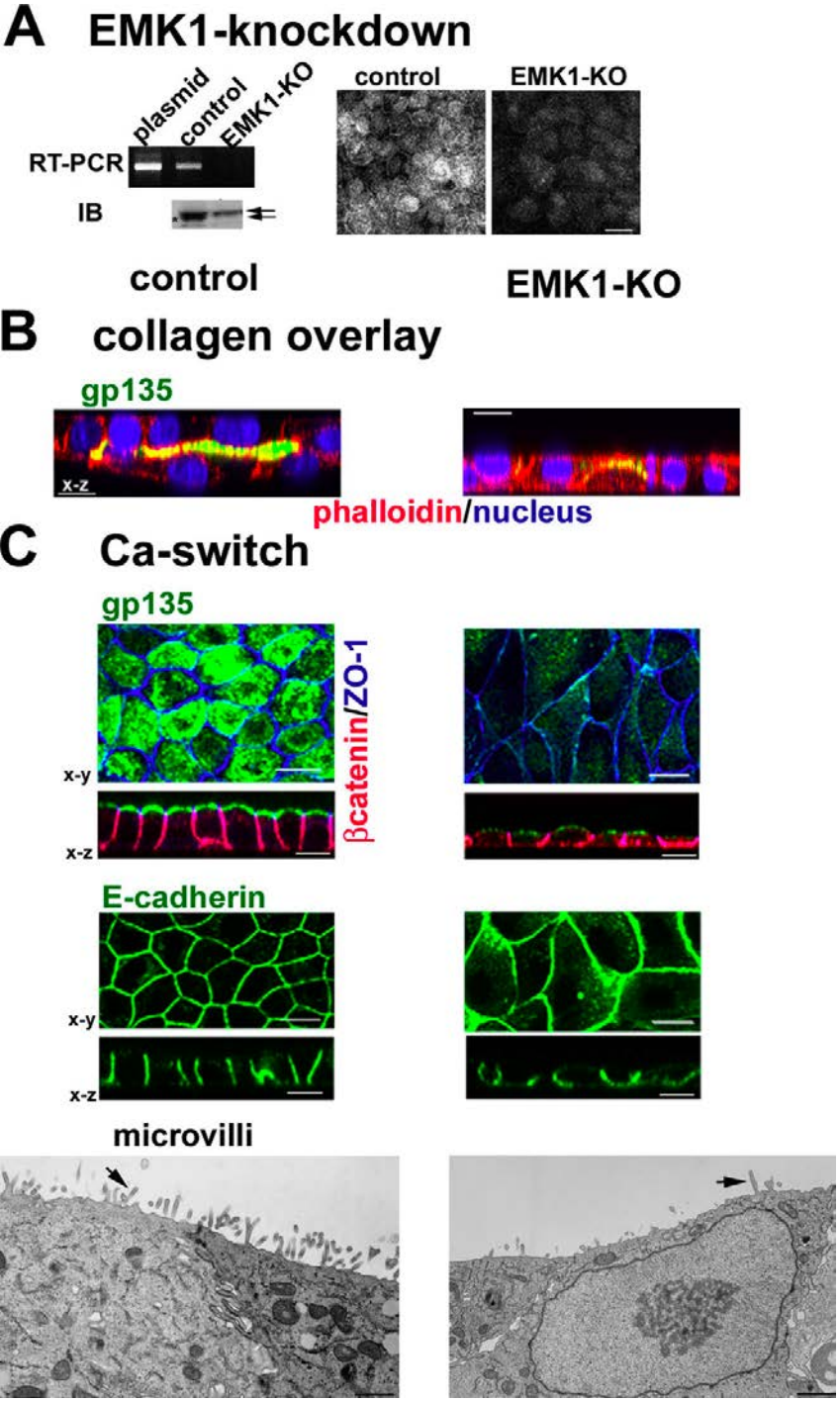

B
Slit (F)
Slit (F)/B
Slit-IP(F)
Average
Migrant Distance (µm)
30
24
18
12
6
0
-6
-20
0
20
Slit (F)
Slit (F)/B
Slit-IP (F)
Slit (R)


Slit(F)
Slit(F)/B
Slit(R)
Bapta-am(R)
Time
-5:00
0:00
2:30
5:00
10:00
15:00
20:00


A
Cumulative Distribution (%)
100
50
0
Slit(F) *
Slit-IP(F)
Slit(F)/B
Slit(R)
-1.5
-0.5
0.5
1.5
2.5
Normalized Net Migration (D2/D1)


B
Normalized Net Migration (D2/D1)
1.2
0.8
0.4
0
*
**
**
C (F)
A (F)
A (F)/B
A (R)
R (F)
R (F)/B
R (F)/T
R (R)
C (R)
B (R)
B (F)


C
Cumulative Distribution (%)
100
50
0
Control
R(F)
B(R)
Slit(F)
0
0.5
1
1.5
Normalized Total Migration
(|D3|+|D4|/D1)
Distance (µm)
40
30
20
10
0
D3
D4
D2
D1
-20
0
20
Time (min)



Emk1 knockdown inhibits lumen formation in MDCK cells:

-RT-PCR: EMK1 is effectively knocked down in MDCK cells 24 hours after transfection with P-SUPER (control) or P-SUPER-siEMK1 plasmid; knockdown confirmed on the right with antibodies to EMK1.

- Collagen overlay assay: cells cultured 24 h on collagen I before being overlaid with additional collagen on the apical surface, analyzed 24 h later. Note the lack of lumen in EMK1-KO cultures.

- Ca switch: control or EMK1-KO cells were plated in low Ca medium 24 h upon transfection with pSUPER or pSUPER-KO. After 12 h, cultures were switched to normal medium for 24 h. Transmission EM of cells sectioned perpendicular to the substratum shows lack of microvilli in EMK1-KO cells.

Preparation of text

- Avoid too much detail
- Avoid jargon
- Use plain English
- Do not overload the slide with Information

Preparation of text

The reason for limiting text to only couple of lines per text block is that when the text block goes on and on forever, people in the audience are going to have to make a huge effort to read the text, which will preclude them from paying attention to what you are saying. Every time you lose their focus, your presentation suffers!

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Here are some of the things many listeners want from a talk:

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Content

Conveys new information
Poses an interesting question
Conveys how people in other fields think
Describes important ideas
Novel discovery

Clarity and Organization

Understandable
Avoids jargon
Uses clear and simple visual aids
Well organized
Doesn’t run over time

Style and Delivery

Keeps me awake
Varies voice
Conveys enthusiasm
Doesn’t stay in one place
Friendly and approachable

Expertise

Credible
Inspires trust and confidence
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Know your topic!

1) Understand **everything** you present.

2) Think of what questions you could be getting.

   Often that will make you understand something new and important about your work.

3) Practice, practice, practice…

Thank you!

Writing a fantastic scientific abstract

Thomas M. Keck, Ph.D.

2012 Summer Student Boot Camp

What is an abstract?

- A brief outline of a research project containing the most salient information
- Uses:
  - Beginning of journal articles
  - Beginning of grant applications
  - Scientific meeting presentations
- Goals:
  - Concise, accurate presentation of scientific goals and findings
  - Comprehensible to target audience
  - …which may include those outside your field

How is an abstract structured?

- Most abstracts are subject to a word or character limit
  - 150-250 words ≈ 1000-2000 characters
- Main components:
  - Informative title
  - Authors & affiliations
  - Introduction & project aims
  - Research methodology
  - Results
  - Conclusions/discussion

How is an abstract structured?

- No citations unless absolutely critical
- Avoid abbreviations
  - Define nonstandard abbreviations that *are* used
- Be specific to project you're discussing, not the larger research program
- Avoid non-essential details
- Concise, honest, accurate
- Spelling & grammar matter!

Informative title

TECHNOLOGY TRANSFER PROGRAM: DEVELOPMENT OF AUTOMATED PRIZE-BASED CONTINGENCY MANAGEMENT SOFTWARE FOR DISTRIBUTION TO COMMUNITY TREATMENT CLINICS

Authors & affiliations

Michelle L Jobes[1], D H Epstein[1], W A Elgie III[2], J L Lin[1], M Mezghanni[2], K L Preston[1], M Vahabzadeh[1]; [1]NIDA IRP Treatment Section, Baltimore, MD, [2]Johns Hopkins Bayview Medical Center, Baltimore, MD

Introduction & project aims

Aims: Hundreds of studies have shown contingency management (CM) to be one of the most effective known behavioral treatments for substance dependence. The main barriers to its implementation in community clinics are its cost and its technical complexity. The latter problem is addressed by Motivational Incentives Implementation Software (MIIS), which we developed as part of collaborative technology-transfer project between NIDA and SAMHSA. MIIS, which is available to clinics at no cost, is a user-friendly stand-alone program designed for settings where there may be only one computer, and where that computer may be outmoded. MIIS tracks and helps reinforce behaviors such as drug abstinence and counseling attendance. The reinforcement schedule, target behaviors, and prizes can be tailored to the specific needs of the clinic and its patients.

Research methodology

Methods: As part of the development process, we conducted a field test in 25 outpatient volunteers who were also enrolled in other treatment studies in our clinic. Our counseling staff used working versions of MIIS to reinforce participants' attendance at up to 12 counseling sessions for up to 14 weeks. Participants earned opportunities to draw for prizes for each on-time attendance to their regularly scheduled weekly counseling session.

Results

Results: The MIIS software was well received by both staff and participants and improved participant counseling attendance. We used feedback on usability, functionality, and simplicity from staff and participants to modify the software iteratively until a final version was created.

Conclusions/ discussion

Conclusions: We have evaluated MIIS for usability and robustness under conditions simulating those of a community treatment program with minimal technology support. Availability of MIIS may improve technology transfer of a proven effective treatment to the community.

Financial Support: Supported by the Intramural Research Program (IRP) of the National Institute on Drug Abuse (NIDA), National Institutes of Health.

Financial support

CPDD 74th Annual Meeting • Palm Springs, CA

Informative title

Authors & affiliations

Introduction & project aims

Research methodology

Results

Conclusions/ discussion

Identifying novel mGluR5 negative allosteric modulators as tools for in vivo investigation in addiction

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The excitatory neurotransmitter glutamate regulates neuronal firing via ionotropic and eight metabotropic glutamate receptor (mGluR) subtypes. mGluR5 is a G protein-coupled receptor with high expression in mesocorticolimbic regions of the brain. Primarily localized post-synaptically, mGluR5 modulates neuronal firing through Gq signaling coupled to phospholipase C, increasing cytosolic calcium upon receptor activation. Attenuation of mGluR5 signaling has shown promising results in preclinical models for conditions as diverse as anxiety, fragile X syndrome, gastroesophageal reflux disease and drug abuse. The orthosteric glutamate binding site, located within a large bi-lobed N-terminal domain, has high sequence homology across the mGluRs. Targeting an allosteric site located within the transmembrane region allows for much greater subtype selectivity and highly selective agents can be designed that can either negatively or positively modulate glutamate's actions at mGluR5. The prototypic mGluR5 negative allosteric modulators (NAMs), MPEP (K_i = 16 nM) and MTEP (K_i = 42 nM), have served as critical research tools, but notable limitations on receptor selectivity and metabolic stability have limited clinical development. Herein, a series of aryl-substituted alkynyl analogues were synthesized to evaluate structure-activity relationships and identify further modifications to the MPEP pharmacophore that optimize binding affinity at mGluR5. Radioligand displacement of [^{3}H]MPEP binding in rat brain membranes showed that several of these novel alkynyl compounds had nanomolar affinities for mGluR5, with up to a 23-fold improvement in receptor affinity over MPEP. Modification at the 6-position of the pyridyl ring of MPEP along with various 3'-CN, 5'-substitutions were generally well tolerated, however when the 5'-CN was reduced to $CONH_2$, inactive compounds resulted. All of the active analogues (K_i < 150 nM) had cLogP values in the 2-5 range. Selected analogues were evaluated in mouse models of anxiety-like behaviors, including open-field locomotor activity, light-dark box and marble burying. The novel high-affinity NAM MFZ 10-7 (K_i = 0.67 nM) produced *in vivo* behavioral effects consistent with anxiolysis more potently than MPEP or MTEP. Future evaluation in behavioral models of drug abuse, including cocaine self-administration and reinstatement, are planned. Collectively, these studies will enable us to identify novel mGluR5 NAMs as potential therapeutic tools for *in vivo* investigation.

NIDA Intramural Research Program

NEUROSCIENCE 2011

Financial support

General blueprint

- What's the problem and why should we care?
- How can we address this problem?
- What strategy was used in this study? What are the predictions of this strategy?
- What were the results of the designed experiments?
- How do you interpret these results?
- What are the strengths and weaknesses of your chosen approach?
- What's the next step?
- What should others take away from your research findings?

Exercise!

Place the following clauses in the proper order…

A. **5** The p60$^-$ strain displayed no defect in viability, testing normally in standard *in vitro* pathogenicity assays. However, when p60$^-$ was tested *in vivo* in mice, a 1000-fold reduction in virulence was observed.

B. **1** Intracellular pathogens, such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*, cause very high mortality rates in the United States. Deciphering the mechanisms through which intracellular pathogens cause disease is of great interest.

C. **7** Future studies will focus on the precise role of p60 in *Listeria* pathogenesis.

D. **3** p60 is an antigen secreted by *Listeria* that has been implicated in regulated bacterial cell wall breakdown. The objective of this study was to determine whether p60 is essential for *Listeria* viability and whether p60 is a virulence factor in *Listeria*?

E. **8** This work increases our understanding of such diseases as tuberculosis, various food poisonings, and meningitis.

F. **4** To examine these questions, a *Listeria* strain lacking p60 (p60$^-$) was developed.

G. **6** These findings support the hypothesis that p60 is a key factor in the disease-causing ability of *Listeria*, but is not essential for viability.

H. **2** Previous studies have demonstrated that *Listeria* causes illness by secreting molecules, called virulence factors, to the outside of the bacterial cell in order to affect the host organism.

Answer!

Intracellular pathogens, such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*, cause very high mortality rates in the United States. Deciphering the mechanisms through which intracellular pathogens cause disease is of great interest. Previous studies have demonstrated that *Listeria* causes illness by secreting molecules, called virulence factors, to the outside of the bacterial cell in order to affect the host organism. p60 is an antigen secreted by *Listeria* that has been implicated in regulated bacterial cell wall breakdown. The objective of this study was to determine whether p60 is essential for *Listeria* viability and whether p60 is a virulence factor in *Listeria*? To examine these questions, a *Listeria* strain lacking p60 ($p60^-$) was developed. The $p60^-$ strain displayed no defect in viability, testing normally in standard *in vitro* pathogenicity assays. However, when $p60^-$ was tested *in vivo* in mice, a 1000-fold reduction in virulence was observed. These findings support the hypothesis that p60 is a key factor in the disease-causing ability of *Listeria*, but is not essential for viability. Future studies will focus on the precise role of p60 in *Listeria* pathogenesis. This work increases our understanding of such diseases as tuberculoses, various food poisonings, and meningitis.

(195 words, 1306 characters)

FAQ

- I don't have any results…what the heck do I write about?
  - If the experiments didn't work, explain the obstacles and proposed solutions
  - If the experiments are pending, describe the results that would support or falsify your hypothesis
- How do I know if I'm doing this right?
  - Share it! If your peers—especially those not working on the same project—can't understand what you're describing, you probably need to clarify it
  - Your boss/advisor/mentor has probably written dozens or hundreds of these things—I bet they have some suggestions…
- Any tips to make this less painful?
  - Start early: multiple rounds of revisions usually helps *a lot*, so give yourself ample time to complete it

Good luck!

Any questions?

What Makes a Good Scientific Question?

Philip Ryan, PhD
Director of Student Services
NIH Graduate Partnerships Program
Office of Intramural Training and Education

Office of Intramural TRAINING & EDUCATION
NATIONAL INSTITUTES OF HEALTH

Biology

- What is the question?
  - What is Biology?
  - What's the answer?
    - The Study of Life
- What are the next questions?
  - What is life?
  - How do we study it?
- Life is defined by a set of criteria
  - Once we know that criteria, we can ask the question…
  - "Is X alive?

What Makes a Good Question?

- Has not already been answered!
  - Background information is paramount
  - Literature searches, database searches
  - Reading scientific papers

- Can be answered
  - With the resources you have
    - Time, technology, reagents, expertise, etc.
  - Conclusively
    - While some evidence may suggest that prehistoric toads liked softer lily pads to more coarse ones….one may never know for sure.

Relevance

- So what?
  - How does it relate to the big picture?
  - Who will be impacted?

- Builds on what is already known
  - That is why we publish…so others can add to it
    - Background information is important
  - If there is previous work, it is likely important to someone

Hypothesis Driven

- There is reason to believe that the answer is X?
  - Builds on what is already known
    - Background information: notice a pattern yet?
  - Logically fits with what is understood
- Proteins Y and Z interact. Protein Z has been found in the nucleus.
    - Hypothesis: Protein Y can be found in the nucleus.
    - Question: Is Protein Y in the nucleus under the same conditions as when Protein Z is in the nucleus?

Different Approaches

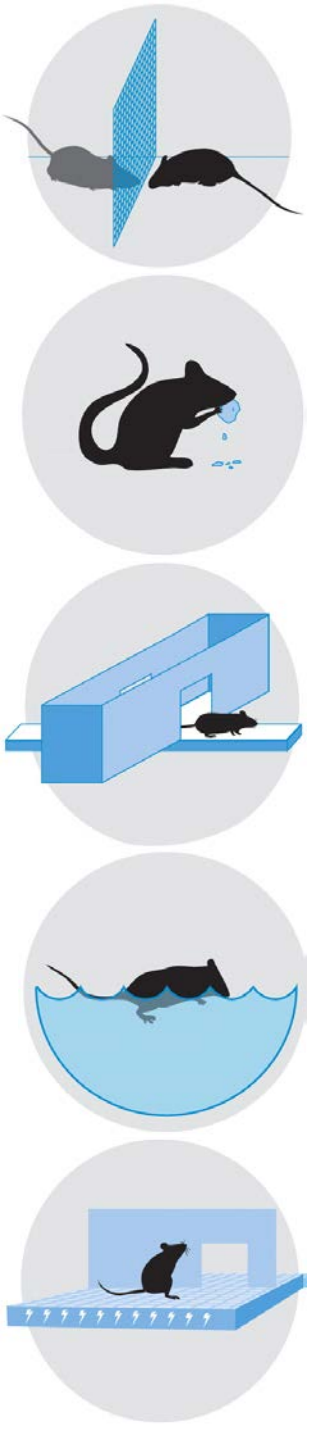
- More than one way to approach answering the question.
  - Sometimes your first approach does not give you an answer either way
  - Answering a question only one way, often is misleading
- Different approaches let you adjust the question as you go along
  - A “no” to the original question can be redirected to a related question to answer

Further Questions

- Good scientific questions lead to more questions when they are answered
- If “no” means the end of the project…then its not a good question
  - Note: That does not mean it should not be asked and answered! Just not something to base a project on

Answers lead to Questions

- Proteins Y and Z interact. Protein Z has been found in the nucleus and affects transcription of gene B.
  - Hypothesis: Protein Y can be found in the nucleus.
  - Question: Is Protein Y in the nucleus under the same conditions as when Protein Z is in the nucleus?
    - If yes, hypothesis two: Protein Y can affect transcription of gene B
    - Question: Does Protein Y affect transcription of gene B?
      - If yes…how?
      - If no, hypothesis three: Protein Y can affect the transcription of other genes
      - Question: Does Protein Y affect the transcription profile in cell X?



SUMMER INTERN SCIENCE SKILLS BOOT CAMP

Animal Models in Biomedical Research

Roza Selimyan, Ph.D.

06.27.2012

Animal Models in Biomedical Research

Animal models are used to:

- help researchers understand the fundamental ways, in which diseases affect living tissue;
- develop and test treatments for illness or injury;
- train future scientists and physicians.

Opposition to Animal Research

Animal welfare is not the same as animal rights.

-Animal welfare is fully supported by the scientific community, and says we should treat animals with compassion & provide for their humane treatment.

Opposition to Animal Research

Animal welfare is not the same as animal rights.

-Animal welfare is fully supported by the scientific community, and says we should treat animals with compassion & provide for their humane treatment.

Some philosophers argue that animals have the same rights as humans and should not be used even to preserve human life or cure human disease.

Groups that believe in this philosophy work actively to end the use of animals in research & education.

Examples of animal rights groups views on animal research

People for Ethical Treatment of Animals (PETA) advocates abolishing all animal research.

"Even if animal research resulted in a cure for AIDS, we'd be against it."

"I wish everyone would get up and go into the labs and get the animals out and burn them down."

--Ingrid Newkirk, PETA Director

What have we learned from animal research?

Animal research has played a major role in nearly all medical advances for both humans and other animals.

These include, but are not limited to:

Angiograms; X-Rays; CAT scans; cardiac pacemaker; hypertension medications; blood pressure measurement; insulin for diabetes; numerous vaccines; cardiac catheters; kidney dialysis; cataract surgery; MRI; burn treatments; heart valve replacements; artificial hips…

FASEB

Alternatives to Animal Models

Cell Culture

- Preliminary screen to check the toxicity of substances
- Can answer fundamental questions about biology

- Cannot provide information about potential impacts on entire living organism

Computer Models

- Simulate specific molecular and chemical structures and their interactions

---

- Limited by programming and knowledge of how the physiological system works

The Limitations of Animal Models

No model is perfect. The term ‘‘model’’ implies deviation from reality, usually by simplifying and reducing variables.

Species differences: Even among rodent species, correlations of only 70%are typically found.

Pharmaco- and toxicokinetics of substances are different in humans; humans are not 70 kg rats

Many health effects are multifactorial. However, the disease models are mostly based on **only one and often-artificial cause**.

And more…

Animal Models in Biomedical Research

Animals serve as good models to help us understand how living tissues function and the biology underlying disease.

The interaction of cells, tissues and organs within the body is very complex, and can often only be studied in the whole animal.

Only by understanding how disease or injuries affect living organisms can we develop treatments or cures.

Animal Models in Biomedical Research

"Virtually every major medical advance for both humans and animals has been achieved through biomedical research by using animal models to study and find a cure for a disease and through animal testing to prove the safety and efficacy of a new treatment."

Dr. Everett Koop
former U.S. Surgeon General

Animal Models in Biomedical Research

"The use of animals in research, teaching, and testing is acceptable ONLY if it promises to contribute to understanding of fundamental biological principles, or to the development of knowledge that can reasonably be expected to benefit humans or animals."

Canadian Council for Animal Care

Animal Models in Biomedical Research

There are a number of federal and local laws, regulations and institutions, as well as nonprofit organizations, that ensure animals used in research & education are being treated humanely.

These include:

- Animal Welfare Act
- Public Health Service
- IACUCs
- AAALAC

FASEB

Regulation of Animal Research

Federal Animal Welfare Act set standards regarding the housing, feeding, cleanliness, and medical care of research animals

Institutional Review Boards are present at each institution; researcher must prove the need to use animals, select the most appropriate species, and devise a plan for using as few animals as possible

The 3R Principle:

- ✓ **R**eplace animal models wherever possible
- ✓ **R**educe numbers of animals used
- ✓ **R**efine animal use procedures

Animal Models in Biomedical Research

A. Invertebrate models

B. Vertebrate models

- Pharmaceutical research including the development of biologics
- Toxicology testing
- Development and testing of new medical devices
- Surgical research
  a) development of new surgical techniques e.g. techniques of gastrectomy, open heart surgery, coronary artery surgery, microsurgery, endoscopy etc.
  b) the development of new therapies e.g. organ and tissue transplantations, cardiopulmonary resuscitation etc.
- Pathophysiological research

and many others…

In vivo Models in Biomedical Research

Similarity in biochemical and developmental features

Physiology/behavior: rabbit, dog, monkey, ape, bird, rat, mouse

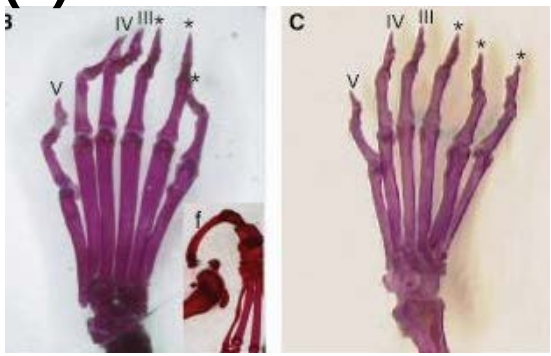
Development: mouse, frog, chick, fish, sea urchin, fruit fly, nematode

Genetics: mouse, rat, zebrafish, fruit fly, nematode, yeast, bacteria

Polydactyly in:

(a) man

(b) mouse



(c) chick

Characteristics of a good animal model

The animal model should (adapted from Isselhard, Kushe 1986):

- ✓ closely reproduce the disease or condition under study;
- ✓ be easily available to many researchers, that is, not a rare or exclusive animal. This allows validation and stimulates further investigations;
- ✓ be large enough for multiple biological sampling, e.g., tissue, blood etc. (In the case of a vertebrate model);
- ✓ fit into available animal facilities of the average institution;
- ✓ be easily handled by most investigators;
- ✓ be available in multiple sub-species;
- ✓ survive long enough for results to be meaningful;
- ✓ be sufficiently robust for the purpose of the study.

<u>However</u>…

Characteristics of a good animal model

<u>However…</u>

…Transgenic animal models, spontaneous animal models and highly specialized animal models such as non-human primates do not fit these traditional guidelines. Such ***special animal models*** are, however, increasingly used in biomedical research.

What have we learned from animal research?

Animal research has played a major role in nearly all medical advances for both humans and other animals.

These include, but are not limited to:

Angiograms; X-Rays; CAT scans; cardiac pacemaker; hypertension medications; blood pressure measurement; insulin for diabetes; numerous vaccines; cardiac catheters; kidney dialysis; cataract surgery; MRI; burn treatments; heart valve replacements; artificial hips…

Let's look at some specific examples of how animals have helped in medical advances…

FASEB

What have we learned from animal research?

Infant Mortality

- Studies in sheep and lambs led to use of steroids in treatment of respiratory distress syndrome (formerly hyaline membrane disease), a major cause of death in premature infants.
- Advances in understanding and treatment of sudden infant death syndrome (SIDS) came from studies in rats, mice, dogs, and sheep.

FASEB

What have we learned from animal research?

AIDS

- Numerous animal models in studies to understand the disease and how it attacks the immune system.
- Current anti-AIDS treatment developed in animal models have greatly extended life expectancy and quality of life for AIDS victims.
- AIDS vaccines being developed in monkeys.

FASEB

What have we learned from animal research?

Stroke

- Stroke kills over 150,000 people in the U.S. each year and causes major disability that can include paralysis, inability to speak, loss of vision and loss of cognitive function.
- Tissue plasminogen activator (TPA), a new treatment for acute stroke, and one that can reverse disability due to stroke was first studied in rats with experimental stroke.
- Other potential treatments, even cures, for stroke are now being studied in experimental animals.

FASEB

Animal Models in Biomedical Research

Nematode -	model of Parkinson's disease model of spinal muscle atrophy
Drosophila -	complete classical genetic map model for cancer therapy model as human glioma
Zebrafish -	forward genetic screens model for polycistic kidney disease model for melanoma

Models used to study genetics and human diseases

Model Organism	Common Name	Research Applications
Saccharomyces cerevisiae	Yeast	Cellular processes (e.g., mitosis) and diseases (e.g., cancer)
Pisum sativum	Pea plant	Patterns of inheritance (by G. Mendel)
Drosophila melanogaster	Fruit fly	Variety of studies from early gene mapping to large scale mutant screens
Caenorhabditis elegans	Roundworm (nematode)	Development of simple nervous systems & the aging process
Danio rerio	Zebra fish	Mapping & identifying genes involved in organ development
Mus musculus	House mouse	Genetic principles and human disease
Rattus norvegicus	Brown rat	Genetic principles and human disease

Animal Models in Biomedical Research

Thank you!

2012 NIH Boot Camp
-Biostatistics/Epidemiology

Nai-Lin Cheng

Postdoctoral fellow
Lymphocyte Differentiation Section
Laboratory of Molecular Biology & Immunology
National Institute on Aging, NIH

Statistics

- Making sense of information
- Drawing inferences about a body of data when only a part of the data is collected and measured.

  - **Systematically recorded information with context (5 W and 1 H approach)**
  - **Analyzing data with proper procedures and techniques**
  - **Sample number (how many is enough?)**

Bio-statistics

The application of the mathematical tools used in statistics to the fields of biological sciences, including epidemiology, medical sciences, health sciences, educational research and environmental sciences.

Observational

- Analyzing the effect of treatment
- Determining behavior patterns

Experimental

- Characterizing the molecular-molecular interactions

.....

To find the order underlying the apparent chaos

Experimental design

Validity & Reliability

Unreliable & Unvalid

Unreliable, But Valid

Reliable, Not Valid

Both Reliable & Valid

http://en.wikipedia.org/wiki/Validity_(statistics)

Null hypothesis (H_0)

- The assumption of whatever is proposed as a cause has no effect on the variable being measured

"While one can not "prove" a null hypothesis one can test how close it is to being true with a power test"

- Error
  - Type I error
  - Type II error
- Significance (P-value)

Mathmatical presentation

- Measure of central tendency
  - Median
  - Arithmetic mean (average)

- Measure of dispersion
  - Range
  - Variance
  - Standard deviation
  - Standard error

7

7 7

3

7 7

8

7 11

Mean=7
SD=0

Mean=7
SD=0.63

Mean=7
SD=2.92

b overexpression and elevated NF-κB occurred independently of these genetic alterations. Future investigations should define whether rarer mutations in NF-κB regulators are present in these cases, or whether the activation of NF-κB derives from microenvironmental factors that are epigenetically sustained by miR-125a/b–mediated suppression of *TNFAIP3*.

In this work, we identified and characterized miR-125a and miR-125b as regulators of TNFAIP3 expression and function, and consequently NF-κB activity. The fine-tuning of TNFAIP3 levels mediated by miR-125 expression had a striking impact on K-63 ubiquitination of TRAF2 and RIP1, IκBα degradation, p65 nuclear accumulation, and transcription of NF-κB target genes. These events defined an antiapoptotic profile compatible with constitutive NF-κB activation and enhanced the fitness of B-lymphoma cells. Together, our data provide a coherent mechanism for a miR-125 role in immune cell activation and oncogenesis, and point to a unique model for constitutive activation of the NF-κB pathway in DLBCL.

Materials and Methods

Cell Lines and Primary Tumors. Human DLBCL cell lines were cultured as we previously described (33) and are detailed in *SI Materials and Methods*. The relevant features of the primary DLBCLs analyzed have been described (13) and are summarized in the Table S1.

Reporter Assays. The two TNFAIP3 miR-125a/b binding sites (WT or mutant) were cloned in the 3′-UTR of the luciferase gene and these constructs, used described (33) and are detailed in *SI Materials and Methods*.

Expression of NF-κB Target Genes and NF-κB Activity Assay. Expression of *CD83*, *BIRC3*, *NFKB2*, *TNF*, *IRF1*, and *TNIP1* was determined by quantitative real-time RT-PCR, in DLBCL cell lines stably expressing miR-125a, miR-125b, or empty MSCV vector, as described in *SI Materials and Methods*. DNA-binding activity of p65 was examined in protein extracts of DLBCL cell lines, as well as primary DLBCL biopsies, according to the manufacturer's instructions (TransAM p65 kit; Active Motif).

Immunoprecipitations. TRAF2 and RIP1 were immunoprecipitated from DLBCL cell lines genetically modified to express miR-125a/b and pulldowns immunoblotted to detect K63 ubiquitination. Immunoprecipitation of AGO2-containing RISC in a relevant DLBCL model was performed as described (25). Both assays are detailed in *SI Materials and Methods*.

Cell Growth, Soft Agar Colony-Forming Assays, and Apoptosis Rate. Cell growth, soft agar colony-forming assays, and apoptosis rate, defined in multiple DLBCL cell lines genetically modified to express miR-125a and miR-125b, are described in detail in *SI Materials and Methods*. All assays were performed in triplicate and repeated three times.

Statistics. The Mann–Whitney test was used to determine the significance of the difference in TNFAIP3 expression in primary DLBCL. For all other assays, the statistical analyses were performed with two-tailed Student's *t* test. $P < 0.05$ was considered significant. Data analyses were performed in the Prism software (version 5.0; GraphPad) and Excel software (Microsoft).

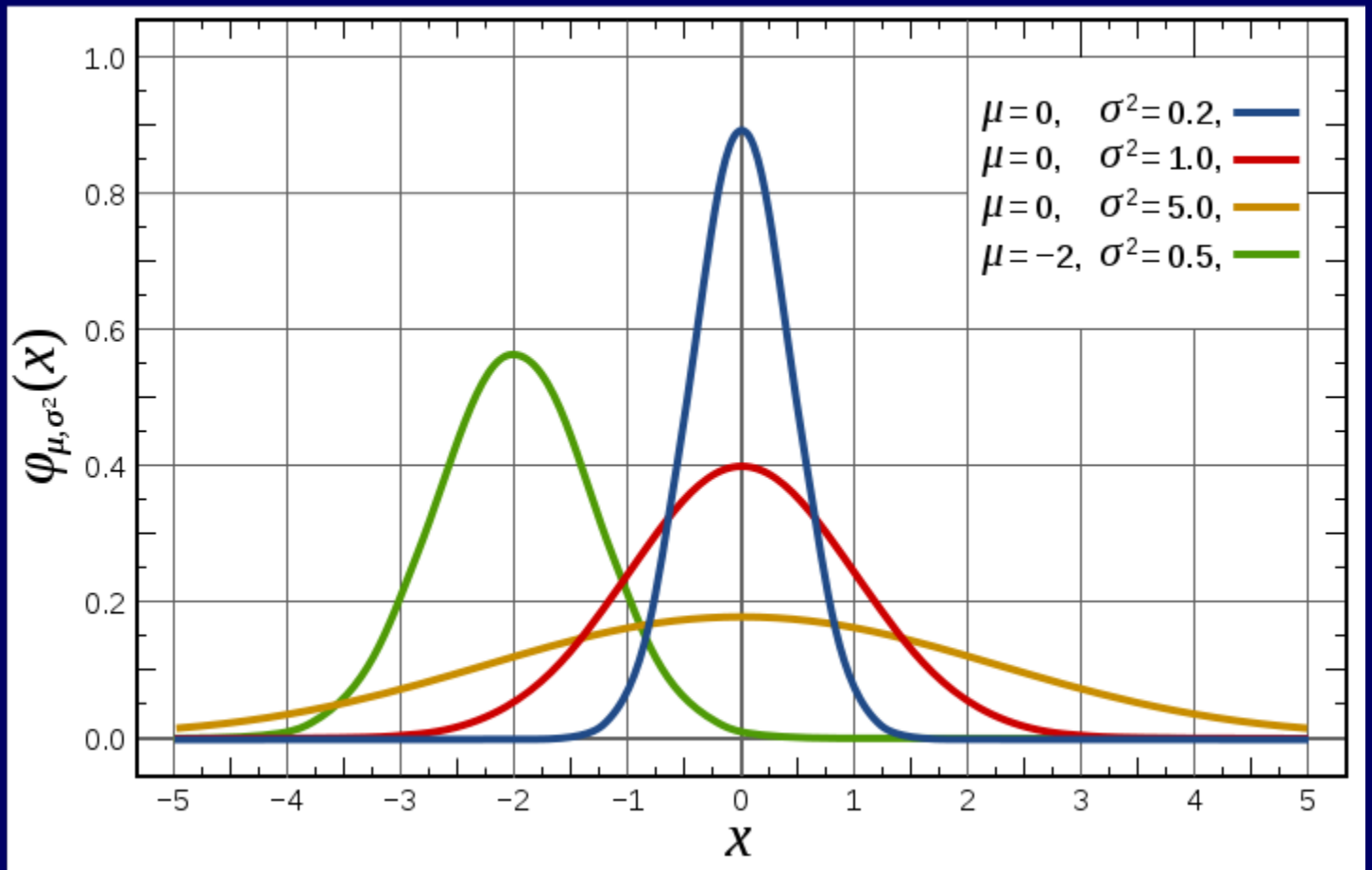
Click on Sign to place signature

Examples

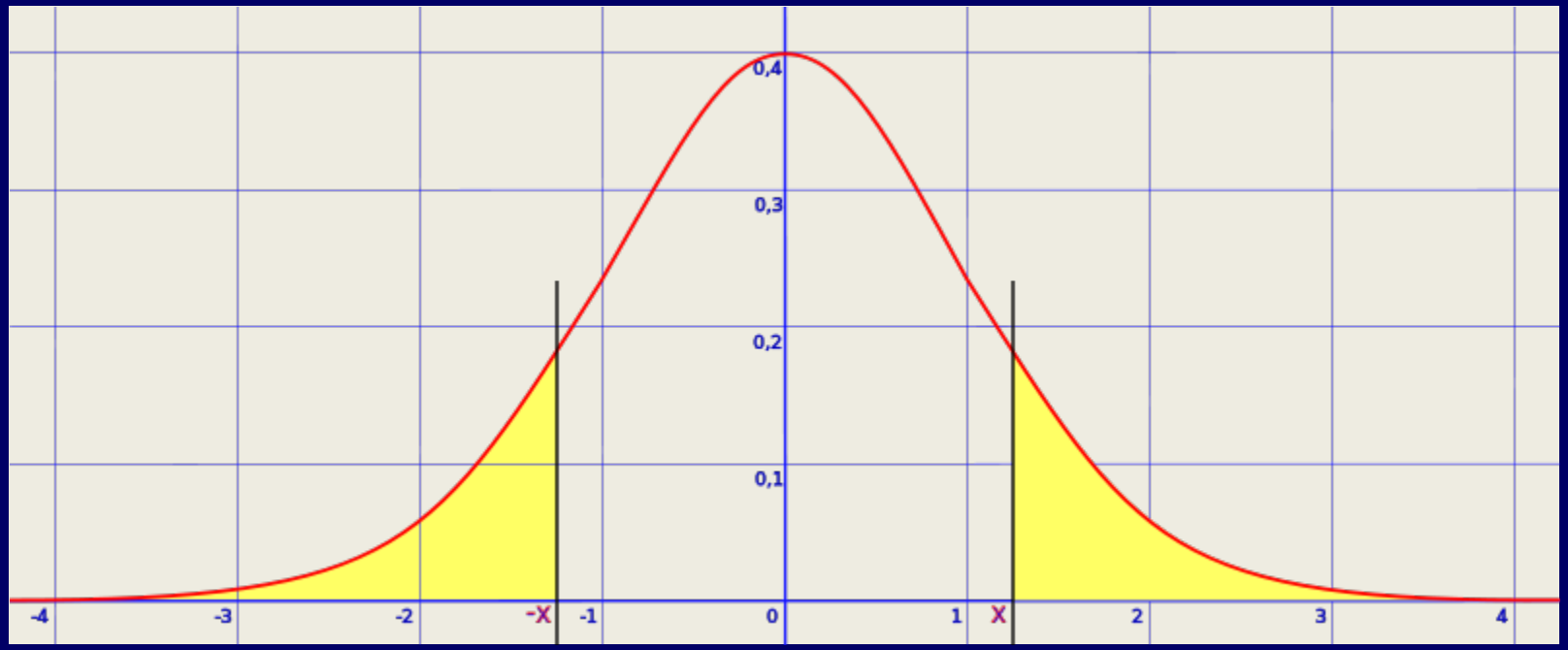
- Student's *t*-test
- Analysis of variance (ANOVA)

Student's *t*-test

- When the test statistic would follow a normal distribution



- One-tailed test
- Two-tailed test



Analysis of variance (ANOVA)

- Whether the means of several groups are all equal
- Generalizing t-test to more than two groups

To do Statistics right

- Think about scenario (null hypothesis) you are testing
- Analyze through proper calculation and making meaningful display
- Tell what we can learned from the data

Thank you!

Have fun!!!