Science Skills Boot Camp

Workshop 2: Asking Scientific Questions

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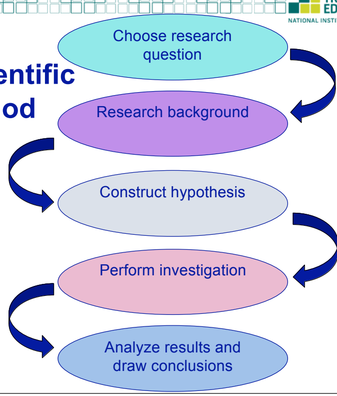
www.training.nih.gov

“Science is a way of thinking much more than it is a body of knowledge.”

Carl Sagan

The scientific method

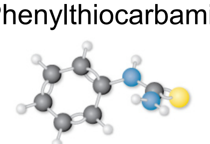


Where does this fit into the scientific method:

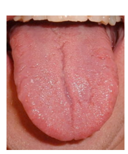
Prove that your hypothesis is true

Today’s investigation:

Phenylthiocarbamide (PTC)



The human gene TAS2R38 encodes taste receptor that detects PTC



http://learn.genetics.utah.edu/content/begin/traits/ptc/

Class data collection 1:

Taste the PTC paper and pick one description:

- ✧This tastes like paper
- ✧This tastes a little bitter
- ✧This tastes horribly bitter

Please dispose of used PTC paper into the ziploc.

Step 1:
Choosing a research question

What makes a good research question?

- Testable
- Not too broad, but not too narrow
- Fits appropriately into the existing information
- Realistic in terms of resources
- Contributes to the field no matter what you find

Some possible questions:

- How does the sense of taste work?
- Do factors other than genetics affect the ability to taste PTC?
- How did PTC sensitivity evolve?
- Are supertasters cuter than non-tasters?
- Are there gender differences in PTC sensitivity?
- Do PTC tasters have a specific DNA polymorphism?

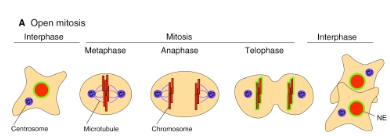
Step 2:
Researching background

Finding background information

- Primary literature
- Review articles
- Textbooks
- Your colleagues
- Internet resources

Review articles are a great place to start!

- Provide an overview of the field
- Often written by experts in the field
- Summarize many primary papers
- Often contain useful diagrams



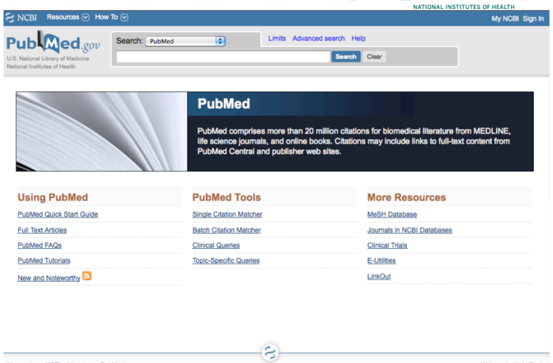
Webster et al. Journal of Cell Science. 2009. 122: 1477-1486

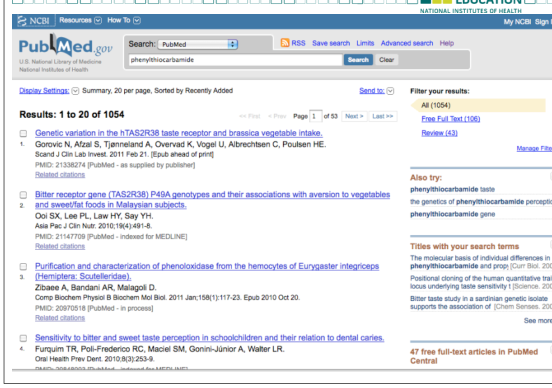
Primary literature

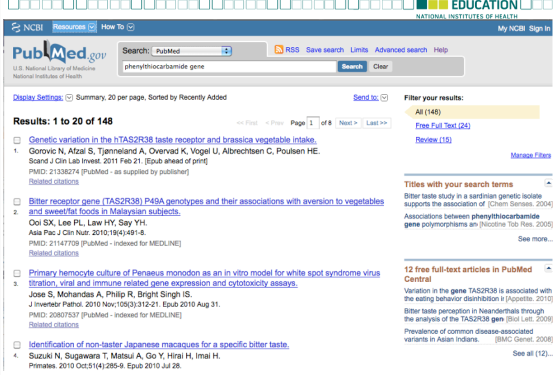
- This is where the actual data is!
- Describes the experiments in detail
  - Introduction
  - Methods
  - Results
    - Figures
    - Tables
  - Discussion
  - References

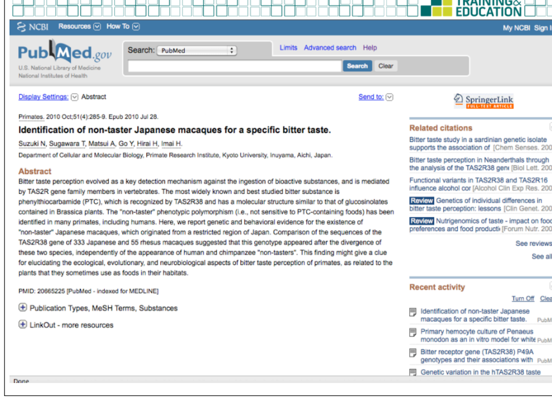
All about PubMed

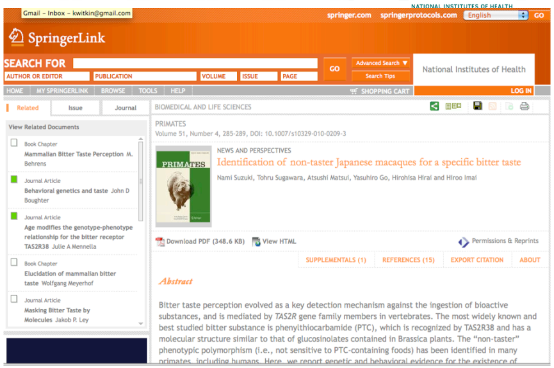
- Free database of biomedical and life sciences literature
- Maintained by the National Library of Medicine
- Contains over 20 million citations
- http://www.ncbi.nlm.nih.gov/pubmed/





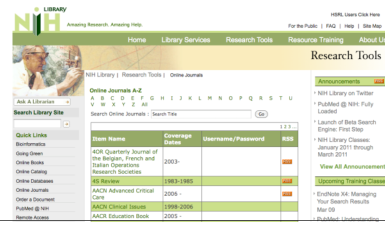






If there's no link from PubMed, the NIH library may still have it

- Go to NIH library webpage: http://nihlibrary.nih.gov/Pages/default.aspx
- Under Research Tools, click on Online journals



If there's no Online access. . .

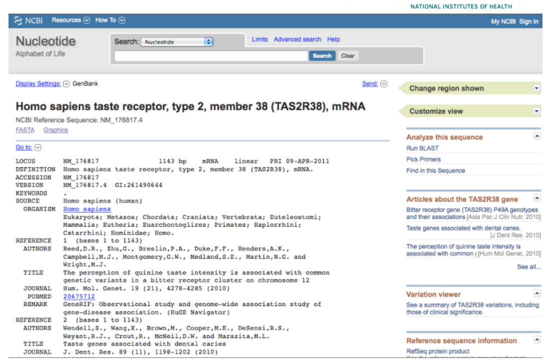
- Go to the library (Building 10)
- Loansome doc
  - On PubMed abstract page, click "Send to" then click "order"
  - Need to sign up for an account (On NIH library Web site, left sidebar, click on "Sign up for pubmed doc del")
- Order through the NIH library
  - On NIH library Web site, click on "services" then "order a document"

Where else can you find scientific papers?

- EMBASE: Biomedical and pharmacological database
- SCOPUS: Broad coverage of scientific, technical, medical, and social science literature, including arts and humanities
- Web of Science: Coverage of ~12,000 journals and conference proceedings. Not limited to biomedical sciences.

Also on the Internet: Bioinformatics tools

- GenBank
  - Free annotated collection of DNA sequences, run by NCBI (NIH)
  - http://www.ncbi.nlm.nih.gov/genbank/
  - Search by gene name to get DNA or protein sequence
  - "Blast" a DNA or protein sequence to find matches in the database



More bioinformatics tools

- Sequence alignment programs
- Structure prediction programs
- Gene expression and regulation databases
- Organism-specific databases
- Pathway analysis
- Promoter/SNP prediction

And many others!

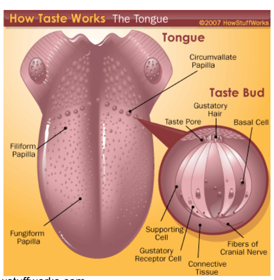
Step 3: Constructing a hypothesis

Hypothesis:
Tentative statement predicting the outcome of an experiment

If . . ., **then** . . .

What we know about taste:

Taste is controlled by receptors in the tongue

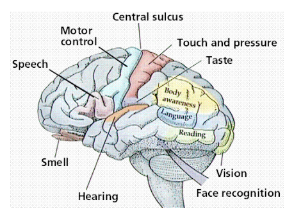


http://health.howstuffworks.com

What we know about taste:

Taste perception involves additional factors

- Smell
- Temperature
- Visual cues
- Texture



What we know about taste:

Studies show that smelling **mint** while eating makes it difficult to identify food by taste.

Hypothesis:
If you smell mint while tasting PTC paper, **then** you will be less sensitive to the bitter taste.

Step 4: Perform investigation

Planning

Doing

Planning experiments

For each experiment:

- Define your objective
- Plan your general strategy
- Decide on experimental details

Planning experiments

For each experiment:

- Define your objective
  - To determine whether strong smells decrease sensitivity to PTC paper
- Plan your general strategy
  - Participants will taste PTC paper and report on the taste. They will then smell mint while re-tasting PTC paper and report on the taste.
- Decide on experimental details
  - How many participants? What kind of mint? How will they rate PTC taste? What will be the controls?

What are some features of a good experiment?

- Has a clear purpose
- Answers one question definitively
- Has appropriate **controls**
- Has limited **variables**
- Has a large enough sample size
- Uses available reagents and equipment
- Can be repeated by you and others

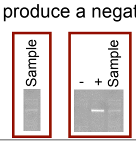
Common controls

- **Positive controls**

  Show that everything is working well, and that your conditions are able to achieve a positive result, even if your samples turn out all negative

- **Negative controls**

  Show the base-line background in your experiment, using known samples that should produce a negative result



Our experiment today:

Each subject will taste PTC paper and report whether there is no taste, weak bitter taste, or strong bitter taste

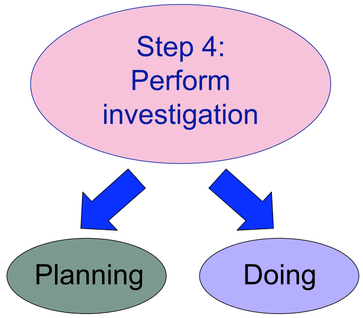
Experimental Group

Half of the subjects will re-taste PTC paper while smelling mint and will report whether there is no taste, weak bitter taste or strong bitter taste

Control Group

Half of the subjects will re-taste PTC paper while smelling parsley and will report whether there is no taste, weak bitter taste or strong bitter taste

- What are the controls in this experiment?
- What are the variables?
- Are there other controls that we're missing?



Tips for good experiments

- Be prepared
- Be organized
- Be meticulous
  - Work deliberately and carefully
  - Follow protocol closely
  - Note any deviations from protocol
- Minimize bias
- Document everything

Learning a new technique

- Find a protocol
- Read it carefully
- Consult with your mentor or other experts
- Make or acquire reagents ahead of time
- Learn how to use required equipment
- Do a “dry run”
- Allow plenty of time for the first run

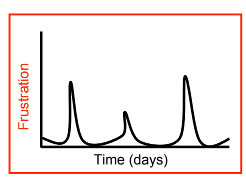
Making or acquiring reagents

- If ordering reagents, do it as early as possible
- Research how each reagent should be used and stored
- If making up solutions
  - Make sure you know what solvent to use
  - Brush up on molarity and serial dilutions, if necessary
  - Check and re-check all calculations

Common mistakes

- Doing huge experiments with too many samples
- Not thinking carefully about your controls before you start
- Waiting until the last minute before tracking down reagents
- Forgetting to grow up the cells you need ahead of time

What if your experiment doesn’t work??



When your experiment doesn't work: Troubleshooting

- Identify possible sources of error
  - Reread protocol
  - Check calculations
  - Consider whether reagents or equipment might be suspect
  - Think about repeating experiment as is
- Consult with mentor
- Consider whether your hypothesis might be flawed
- Don't get frustrated!

Class data collection- Part 2

1. Taste the PTC paper while smelling the mint or parsley and pick one description:
   - ✧This tastes like paper
   - ✧This tastes a little bitter
   - ✧This tastes horribly bitter

2. Dispose of all waste into the ziploc

3. Class data will be collected and recorded on the board

Step 5: Analyzing Results and Drawing Conclusions

- How many people changed their responses after smelling the mint?
- How many people changed their responses in the control group?
- Can we draw any conclusions?
- Does our data suggest future experiments?
- How do we want to present this data?

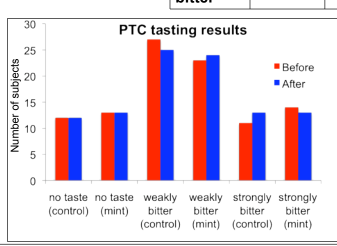
Raw data: Recorded in your notebook

Subject	Mint or parsley	Before	After
1	M	No	No
2	M	Strong	Strong
3	M	Strong	Weak
4	M	Weak	Weak
5	P	No	No
6	P	Weak	Strong
7	P	Weak	Weak

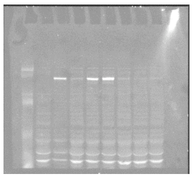
Other types of raw data:
Gels
Blots
Photographs
Observations

Processed data:

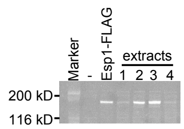
	Parsley		Mint	
	Before	After	Before	After
No taste	12	12	13	13
Weakly bitter	27	25	23	24
Strongly bitter	11	13	14	13



Another example:



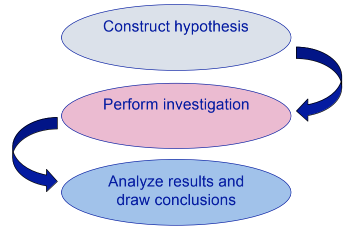
Raw data



Processed data

Is the data consistent with your hypothesis?

If not, then start again at step 3!



Data vs interpretation

Data: 48/50 study participants reported no change in PTC taste sensitivity after smelling mint, compared to 49/50 in the control group.

Interpretations:

•Smell does not affect taste

•Smelling mint does not affect taste

•The smell of mint does not affect PTC taste sensitivity

Question:

How could we design an experiment to better test the broader conclusion that smell does not affect taste?

Does your data mean what you think it means?

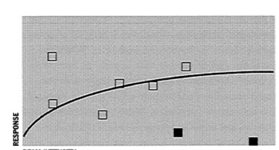
- Is it statistically significant?
- Are you doing the right statistical analysis?
- Do you have a large enough sample size or enough repetitions?
- Are there alternative explanations?
- Are there confounding factors?

An example of a difficult ethical question

Predicted data:
A curve similar to this one

Actual data:
All of these data points

Possible source of error:
Power supply fluctuations



The ethical question:
Can you discard the two filled squares as outliers?

Adapted from "On Being a Scientist: Responsible Conduct in Research," 2nd ed., National Academy Press, 1995.

Pitfalls in data analysis

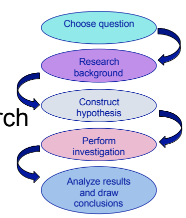
- Only considering specific data points
- Over-interpretation of data
- Ignoring confounding factors
- Using too small of a sample size

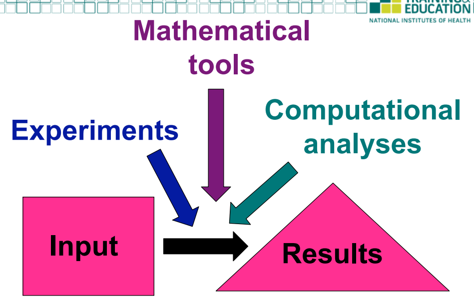
Scientific misconduct

- **Falsifying data**
  Can happen accidentally when you "process" data
- **Fabricating data**
  This is always wrong!
- **Plagiarism**
  Includes using other's IDEAS as well as WORDS

Same basic scientific method no matter what kind of research you do

- Basic research
- Clinical research
- Translational research
- Social and behavioral research
- Epidemiology
- Computational research
- Mathematical modeling





Does the data support my hypothesis??

Special considerations for epidemiologists

- Are you surveying an appropriate population?
- Do you have enough study participants?
- Are you using the appropriate analytical tools?
- Have you considered potential alternative explanations?
  - Confounding factors
  - Bias
  - Chance
  - Reverse causality

Special considerations for clinical research

- Bioethics
- Professionalism
- Confidentiality
- Possibility of health risks for investigator
- Critical to have appropriate study design and meticulous technique
  - Institutional Review Board (IRB)
  - Data Safety Monitoring Board
  - Double-blind studies

More on **bioethics**: The 7 major ethical principles that guide clinical research

Each study must have:

- Social and clinical value
- Scientific validity
- Fair subject selection
- Favorable risk-benefit ratio
- Independent review
- Informed consent
- Respect for potential and enrolled subjects

Special considerations when using animals in research

- Oversight by the NIH Office of Laboratory Animal Welfare
- Must have an Animal Care and Use Protocol
- Ensuring humane and responsible use
  - Carefully designed experiments
  - Minimizing the number of animals used
  - Avoiding/minimizing pain and stress
  - Appropriate housing conditions
  - Appropriate sedation or anesthesia
  - Veterinary care, when necessary

Ethical considerations for all kinds of scientists

- Honesty
- Objectivity
- Integrity
- Carefulness
- Respect for intellectual property
- Responsible publication
- Respect for colleagues

Last but not least . . .

Communicate your results