Introducing bioinformatics

NextGen sequencing and analysis tools

Dr. Mark Peterson

2014-Jan-20

An overview

An overview

1. Biology I
2. Chemistry & Physics

An overview

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2. Chemistry & Physics
3. Computers

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6. Facebook and Twitter

An overview

1. Biology I
2. Chemistry & Physics
3. Computers
4. Stats
5. Biology II
6. Facebook and Twitter
7. Conclusions

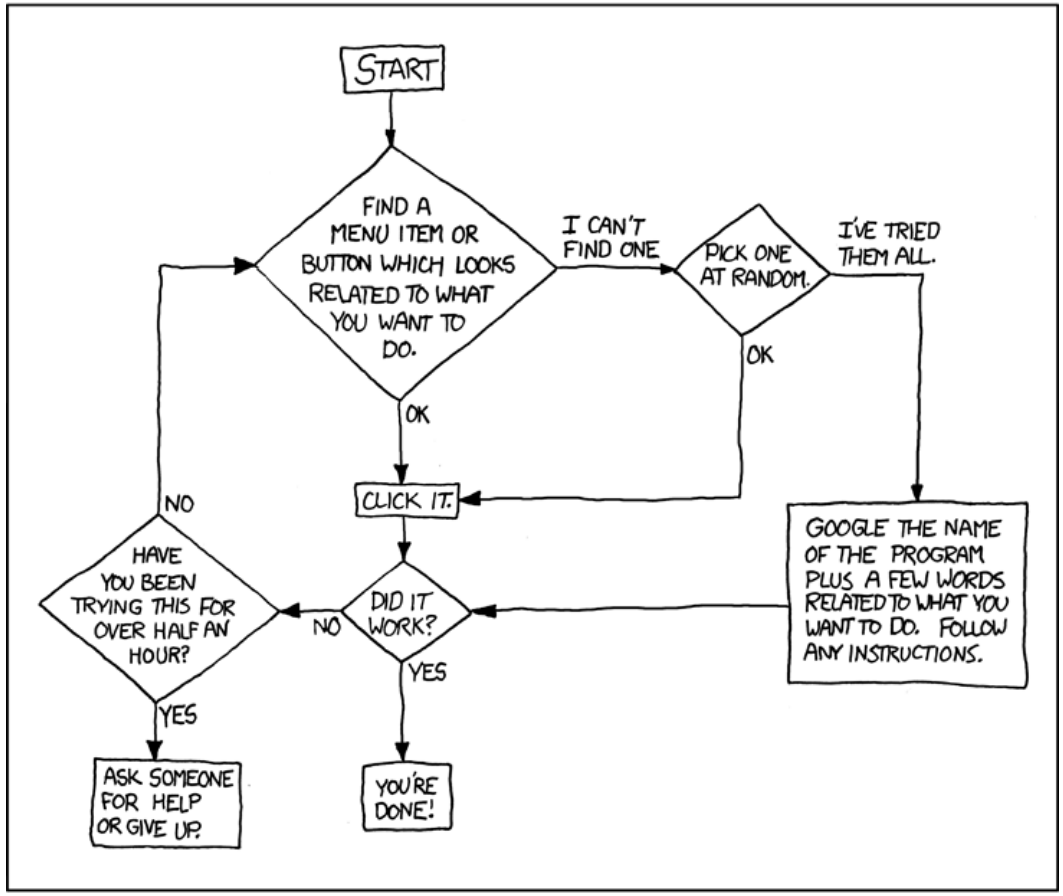
Most of genomics work

Computers

Computers

DEAR VARIOUS PARENTS, GRANDPARENTS, CO-WORKERS, AND OTHER "NOT COMPUTER PEOPLE."

WE DON'T MAGICALLY KNOW HOW TO DO EVERYTHING IN EVERY PROGRAM. WHEN WE HELP YOU, WE'RE USUALLY JUST DOING THIS:



PLEASE PRINT THIS FLOWCHART OUT AND TAPE IT NEAR YOUR SCREEN. CONGRATULATIONS; YOU'RE NOW THE LOCAL COMPUTER EXPERT!

xkcd.com/627

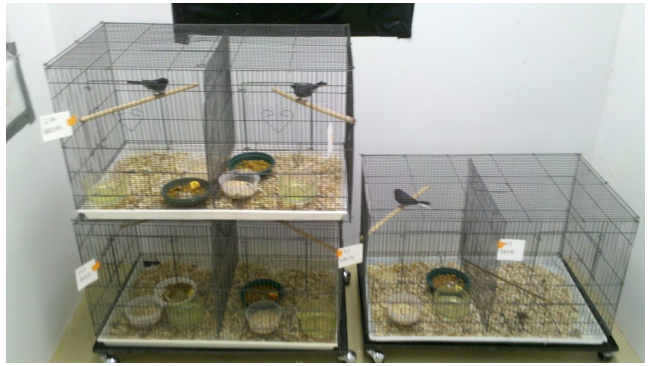
Experimental Design

Experimental Design

- Study question
  - How are males and females different?

Experimental Design

- Study question
  - How are males and females different?
- Sample size
  - How many can I get?



Sample Collection

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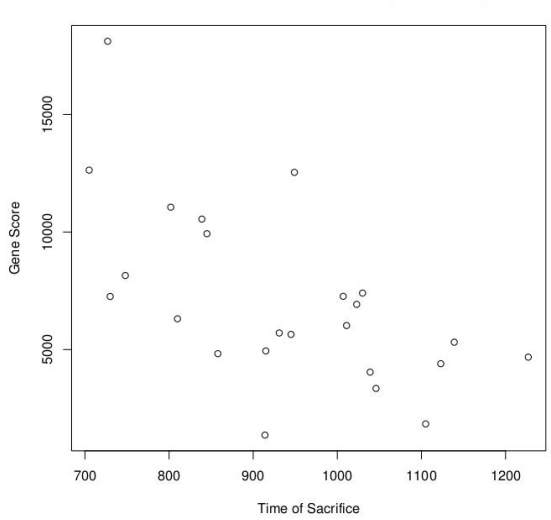
- Covariates
  - Will time of death affect expression?



Sample Collection

- Covariates
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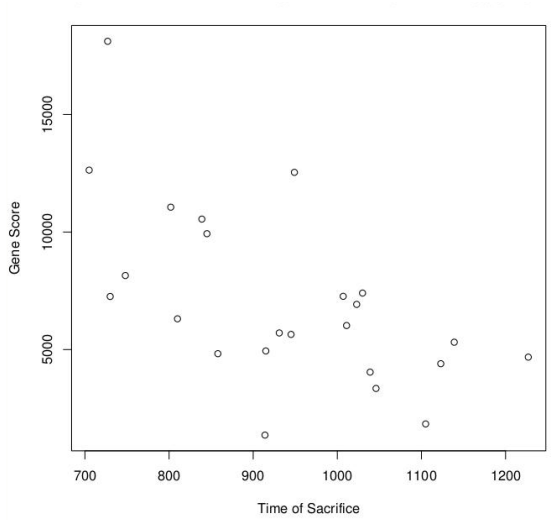
- RNA degrades rapidly
  - Deep freeze immediately



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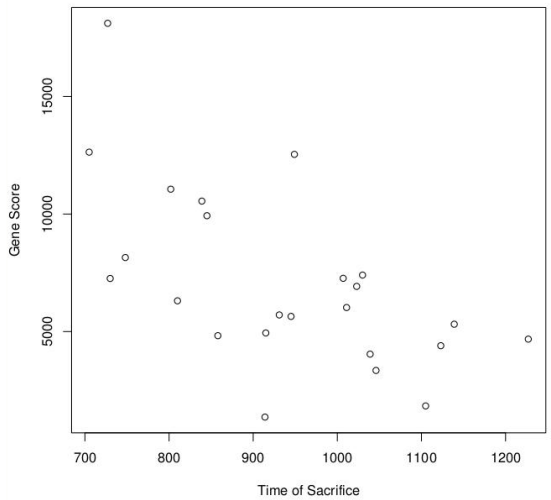
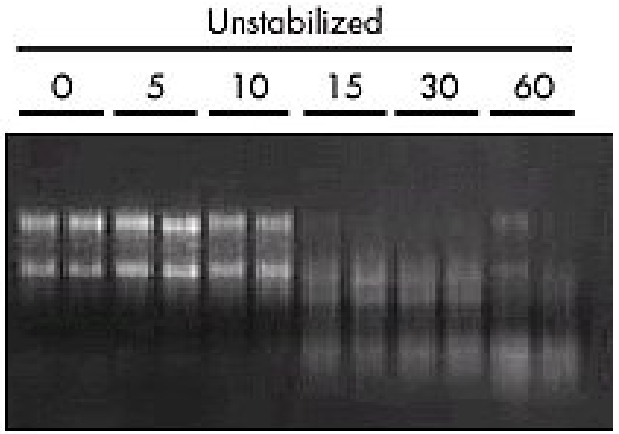
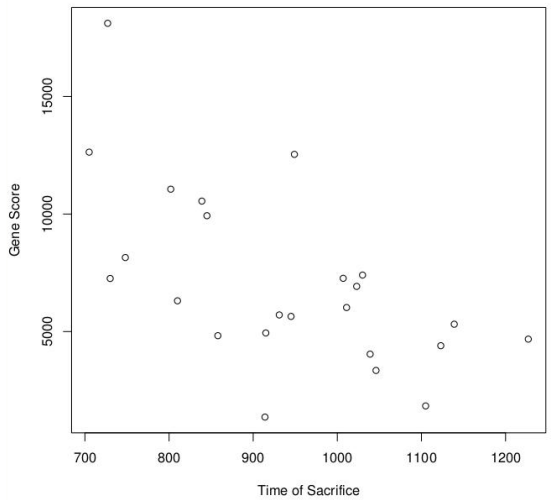
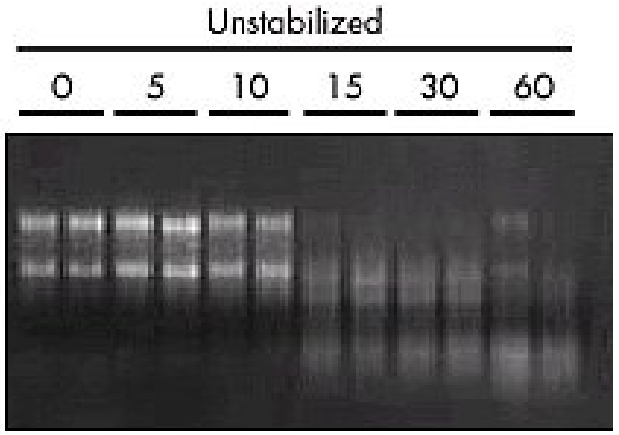




Sample Collection

- Covariates
  - Will time of death affect expression?
- RNA degrades rapidly
  - Deep freeze immediately
- Get enough tissues
  - Balance time and questions

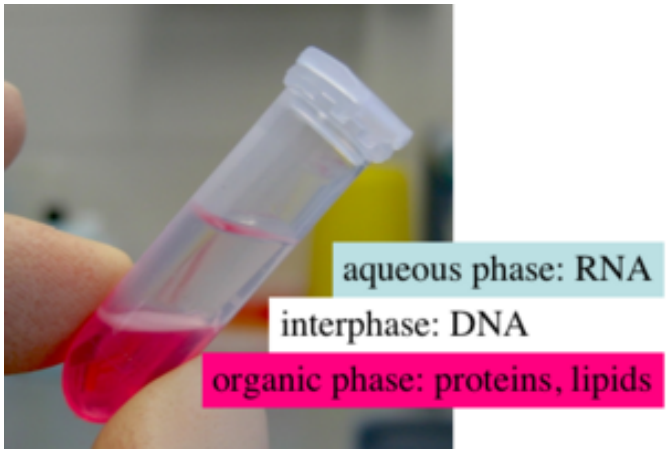




Sample prep

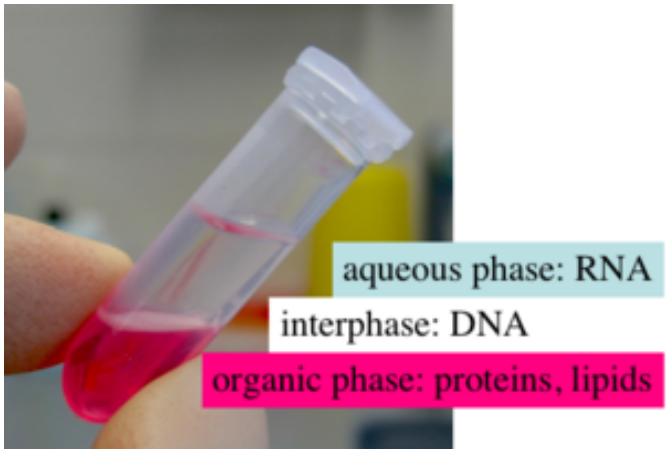
Sample prep

- Extract RNA
  - Phenol-chloroform, multi-step



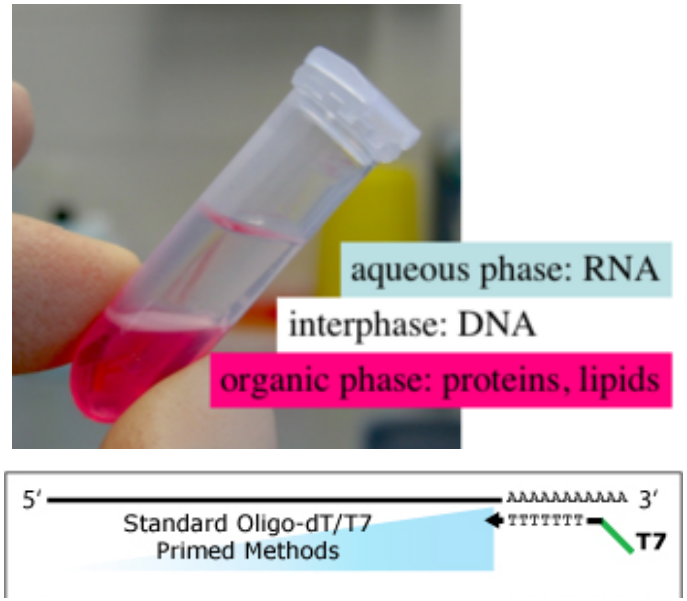
Sample prep

- Extract RNA
  - Phenol-chloroform, multi-step
- Deplete rRNA



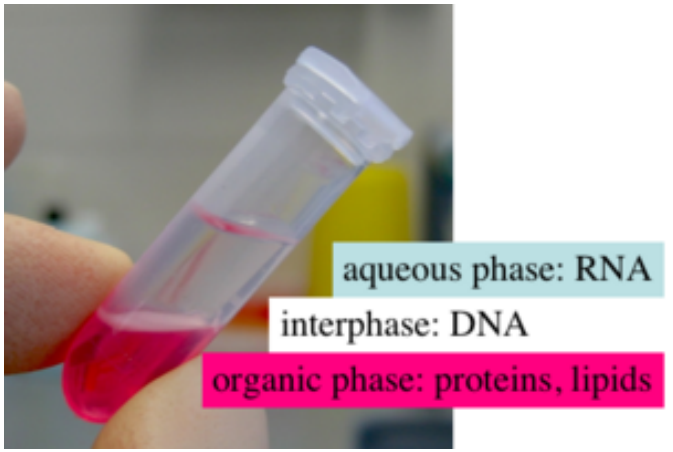
Sample prep

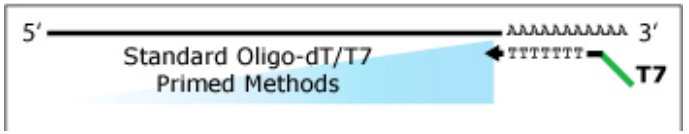
- Extract RNA
  - Phenol-chloroform, multi-step
- Deplete rRNA
  - Select poly-A



Sample prep

- Extract RNA
  - Phenol-chloroform, multi-step
- Deplete rRNA
  - Select poly-A
  - Use magnets to target rRNA

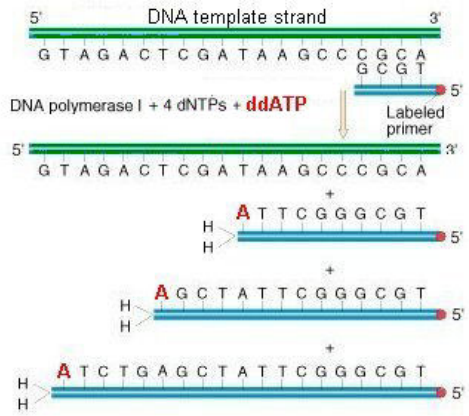




Sanger Sequencing

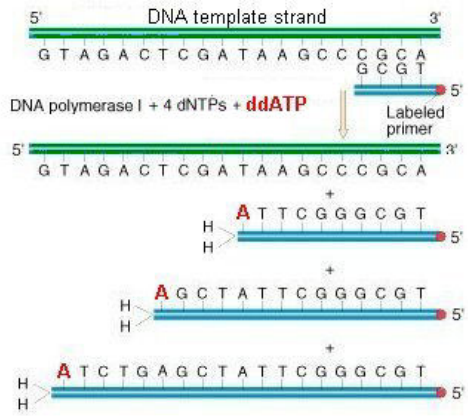
Sanger Sequencing

- Chain terminal sequencing
  - Compare length of fragments



Sanger Sequencing

- Chain terminal sequencing
  - Compare length of fragments
- Measure size of each fragment



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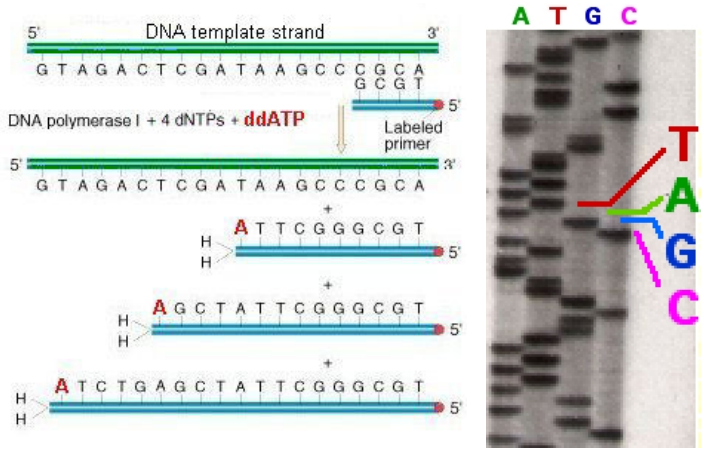
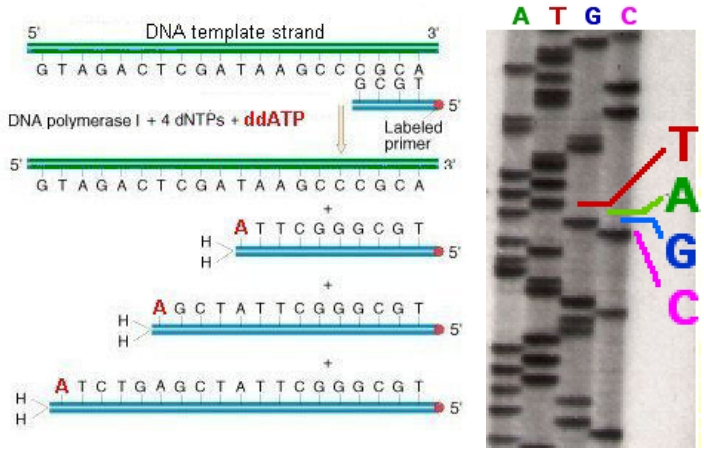
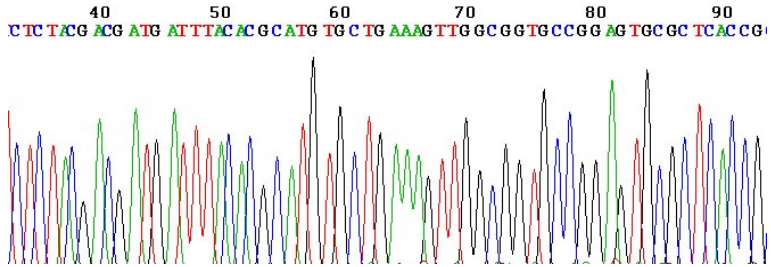
  - Gel, one reaction per base



Sanger Sequencing

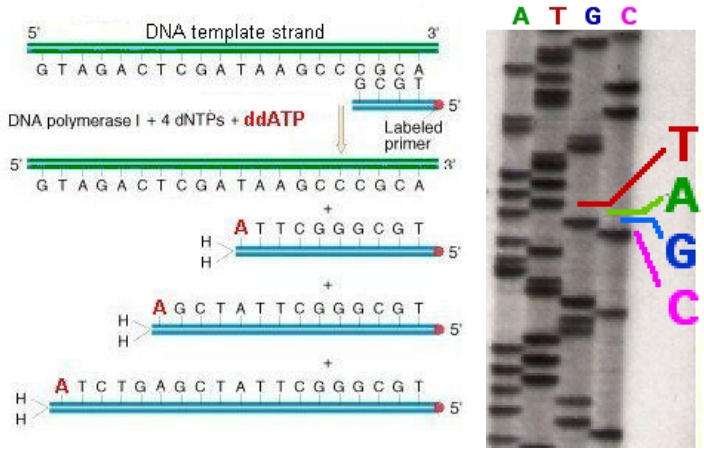
- Chain terminal sequencing
  - Compare length of fragments
- Measure size of each fragment
  - Gel, one reaction per base
  - Capilary, single reaction with color

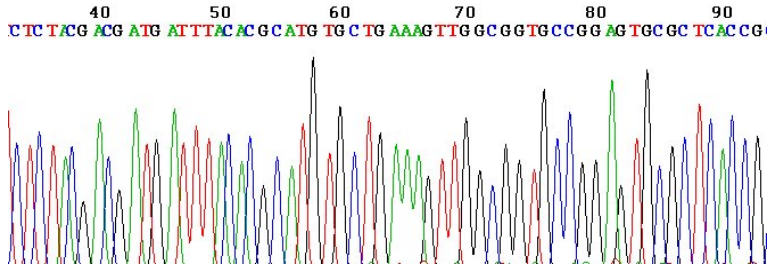




Sanger Sequencing

- Chain terminal sequencing
  - Compare length of fragments
- Measure size of each fragment
  - Gel, one reaction per base
  - Capilary, single reaction with color
- Slow, expensive, requires specifc primers





Illumina sequencing

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- Parallel sequencing
  - Many simultaneous reactions

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- Parallel sequencing
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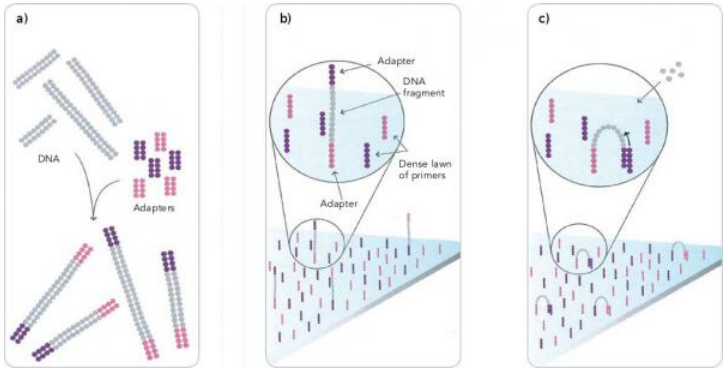
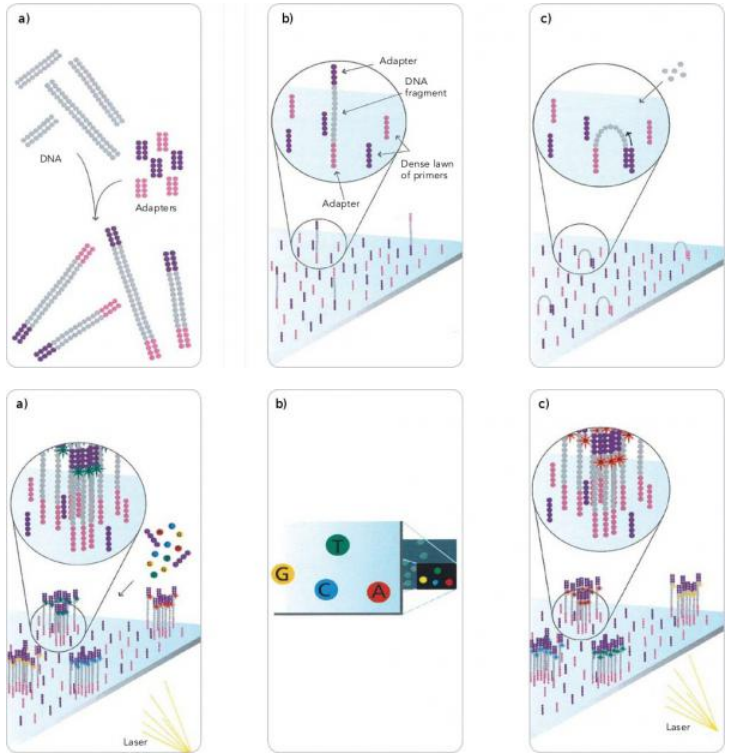
- Bind to plate
  - PCR replicate clusters



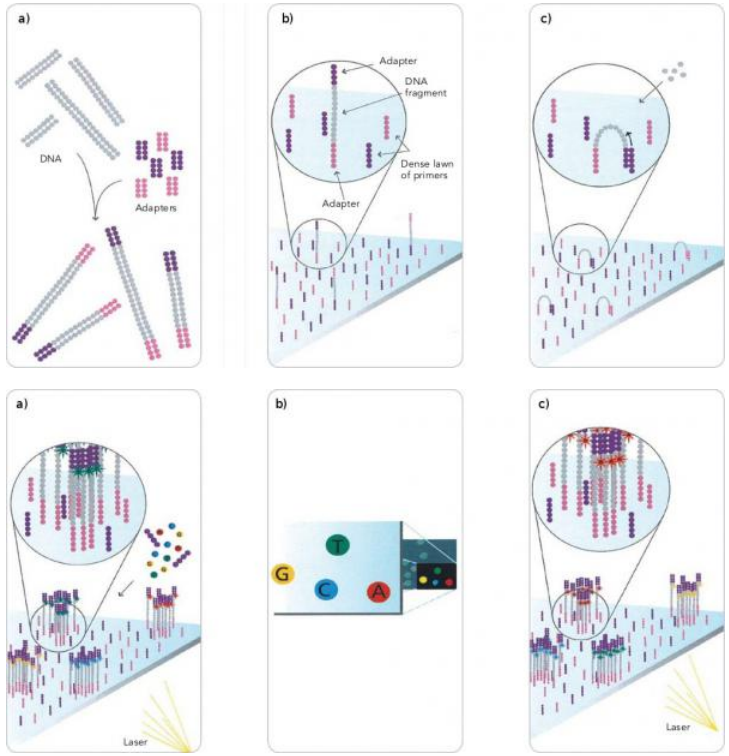
Illumina sequencing

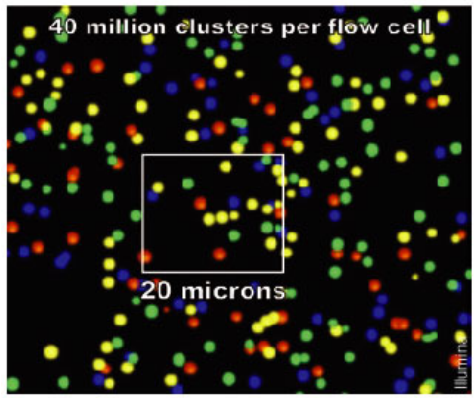
- Parallel sequencing
  - Many simultaneous reactions
- Bind to plate
  - PCR replicate clusters
- Like Sanger, use terminators
  - However, these are reversible



Illumina sequencing

- Parallel sequencing
  - Many simultaneous reactions
- Bind to plate
  - PCR replicate clusters
- Like Sanger, use terminators
  - However, these are reversible
- Read individual spots





454 sequencing

454 sequencing

- A different approach

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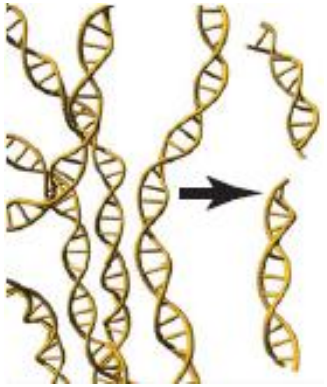
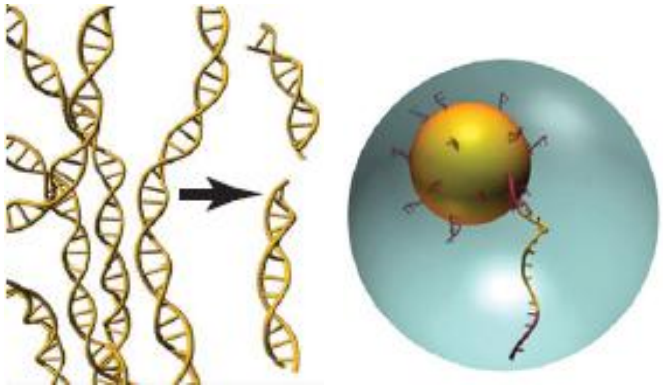
- A different approach
- Random cleaving
  - Add tags



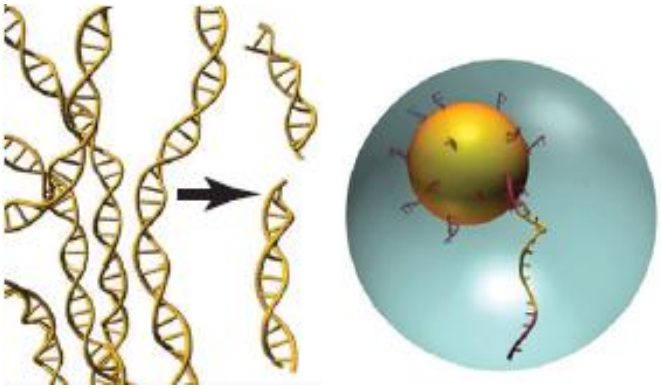
454 sequencing

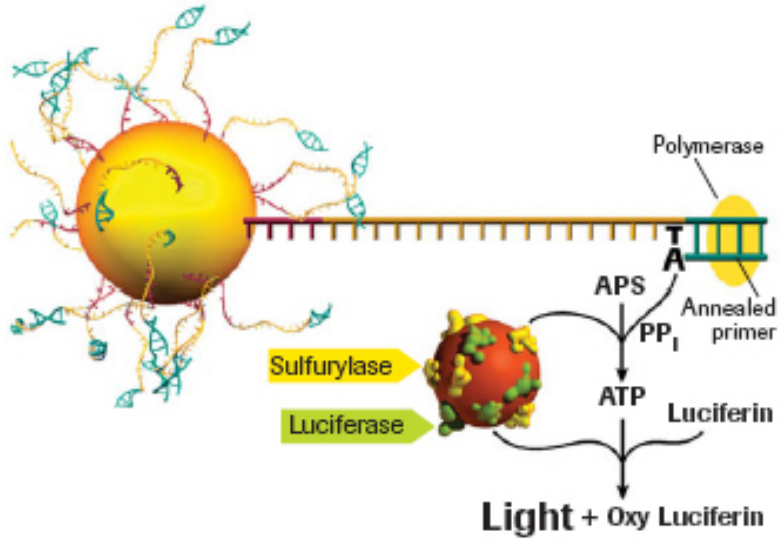
- A different approach
- Random cleaving
  - Add tags
- Emulsion PCR
  - Clonally amplify for reading



454 sequencing

- A different approach
- Random cleaving
  - Add tags
- Emulsion PCR
  - Clonally amplify for reading
- Pyrosequencing
  - Read flashes





PacBio sequencing

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- Single Molecule Real Time (SMRT)

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  - High (~10%) error
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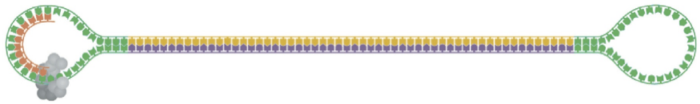
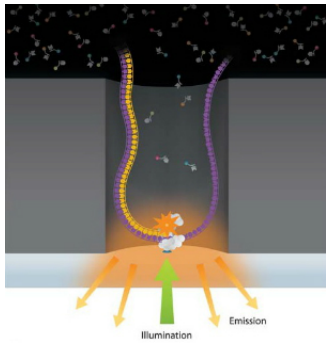
- Gets *incredibly* long reads, starting 2013-Oct-03
  - Mean length 8,500 bases
  - N50 of 10k bases
  - Max reads of 30k bases





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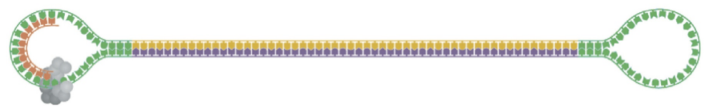
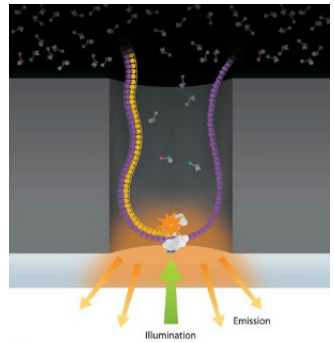
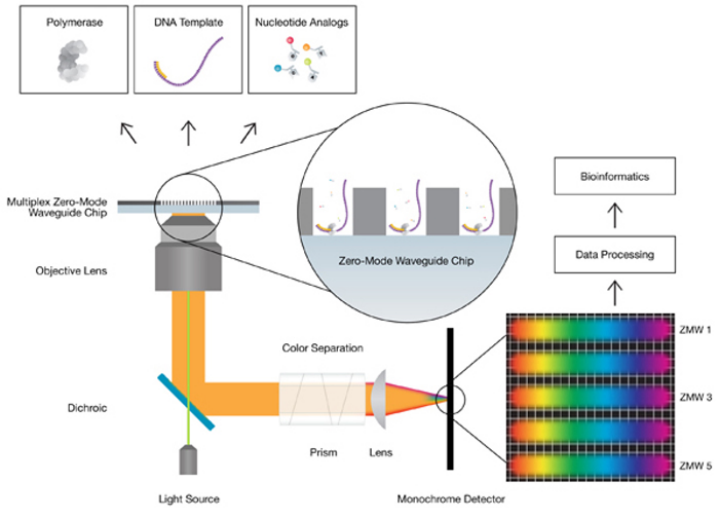
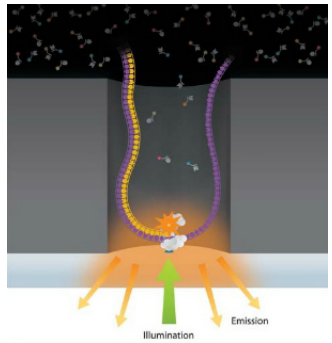


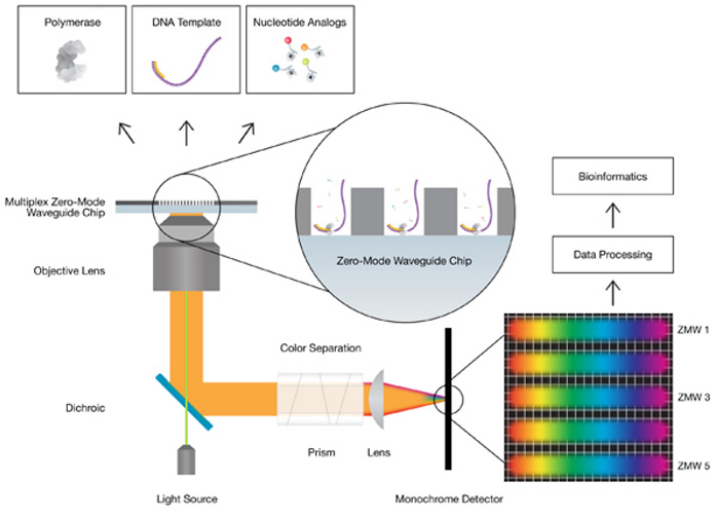




PacBio sequencing

- Single Molecule Real Time (SMRT)
- Circularizes, reads single molecule
  - High (~10%) error
  - Uses consensus from multiple reads
- Gets *incredibly* long reads, starting 2013-Oct-03
  - Mean length 8,500 bases
  - N50 of 10k bases
  - Max reads of 30k bases
- Fewer (50k) reads





Nanopore Sequencing

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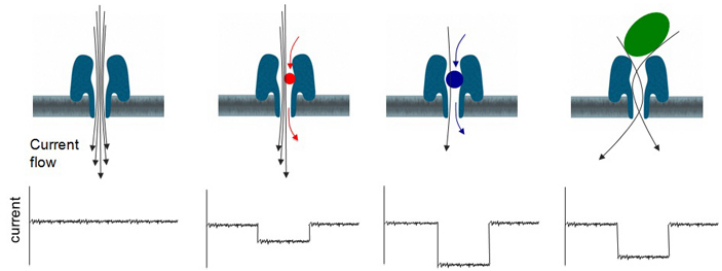
- Create a *tiny* pore with electrical potential

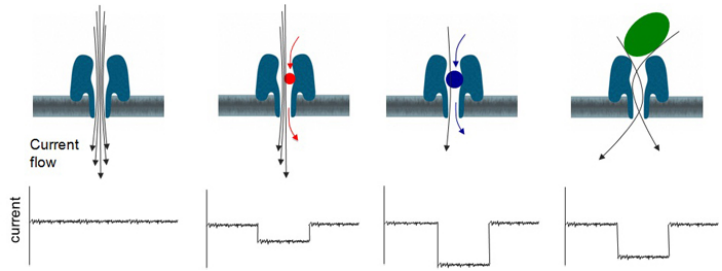
Nanopore Sequencing

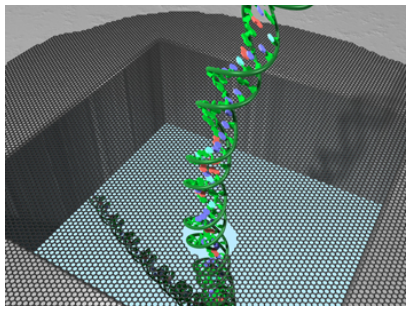
- Create a *tiny* pore with electrical potential
- Current changes as it is blocked



Nanopore Sequencing

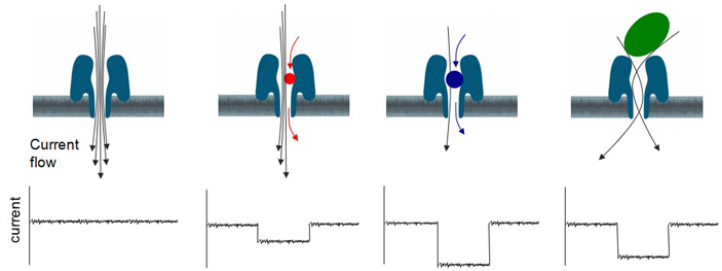
- Create a *tiny* pore with electrical potential
- Current changes as it is blocked
- Pass DNA through

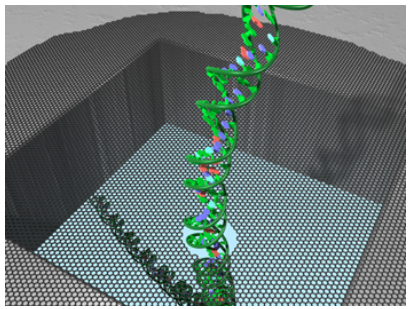


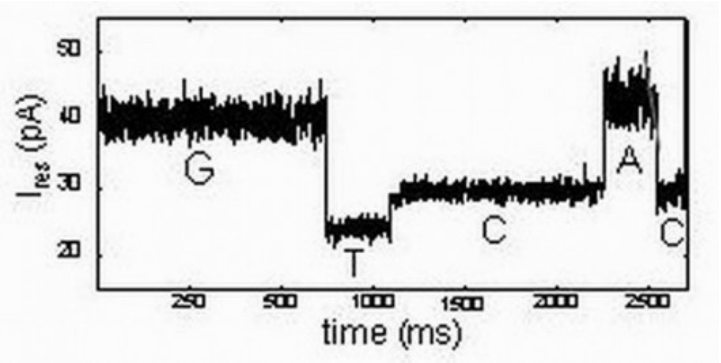


Nanopore Sequencing

- Create a *tiny* pore with electrical potential
- Current changes as it is blocked
- Pass DNA through
- Read current changes

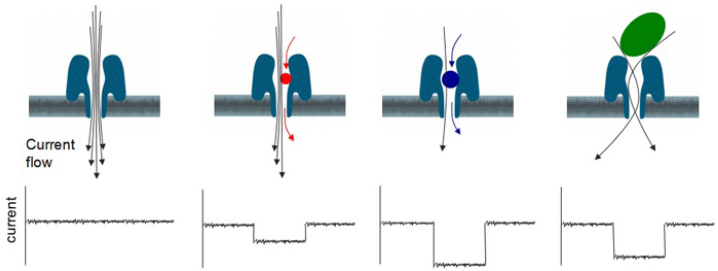


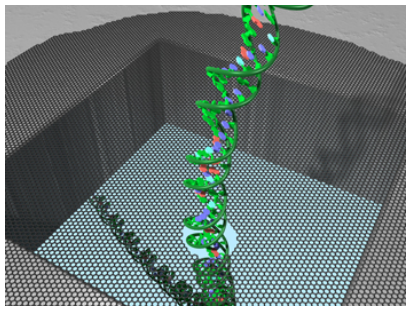


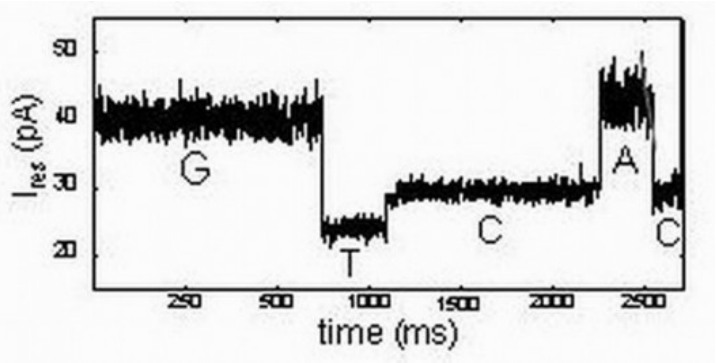


Nanopore Sequencing

- Create a *tiny* pore with electrical potential
- Current changes as it is blocked
- Pass DNA through
- Read current changes
- Arbitrary length reads, but many challenges:
  - DNA moves too fast
  - How are homopolymers addressed?
  - Making good pores







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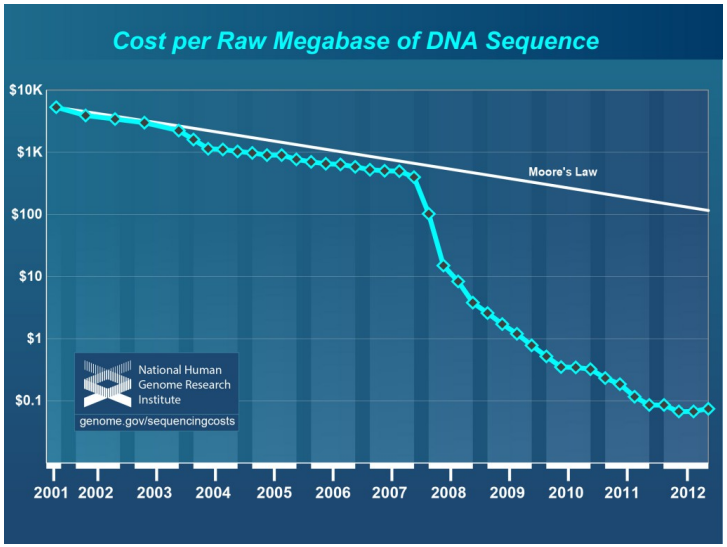

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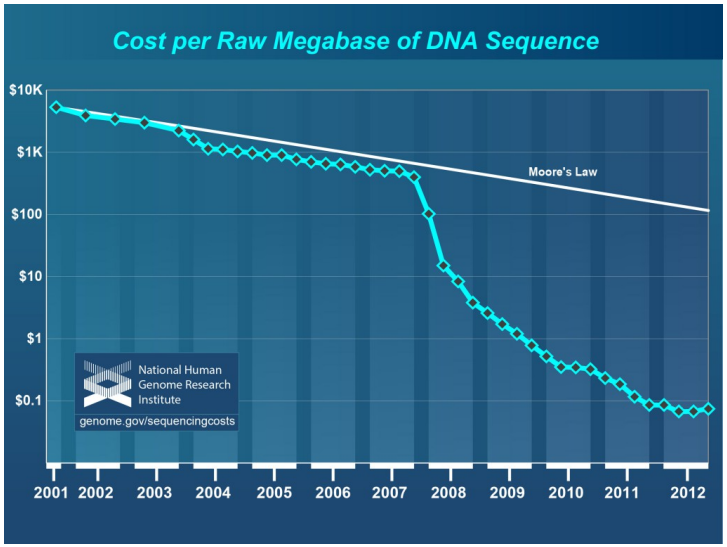

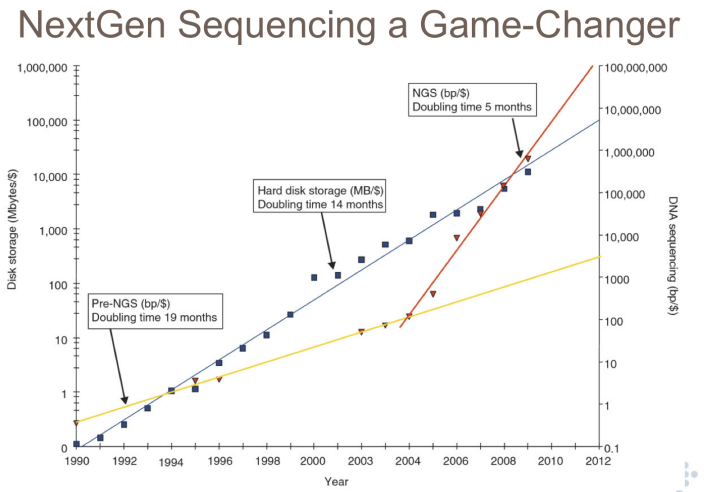

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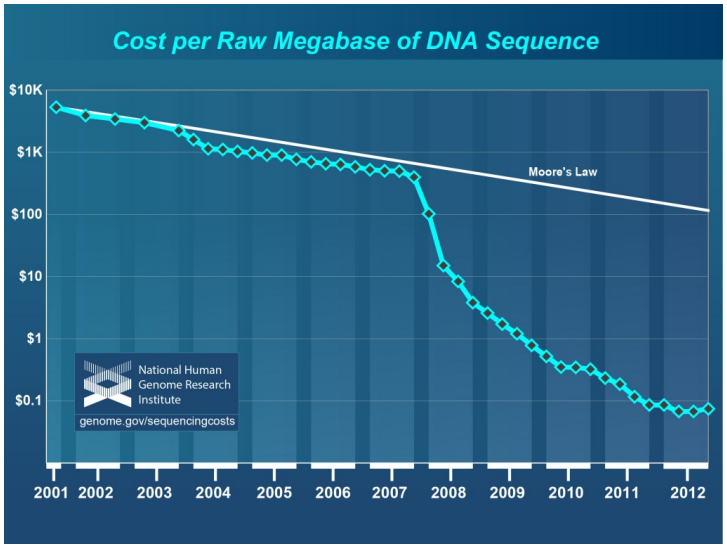
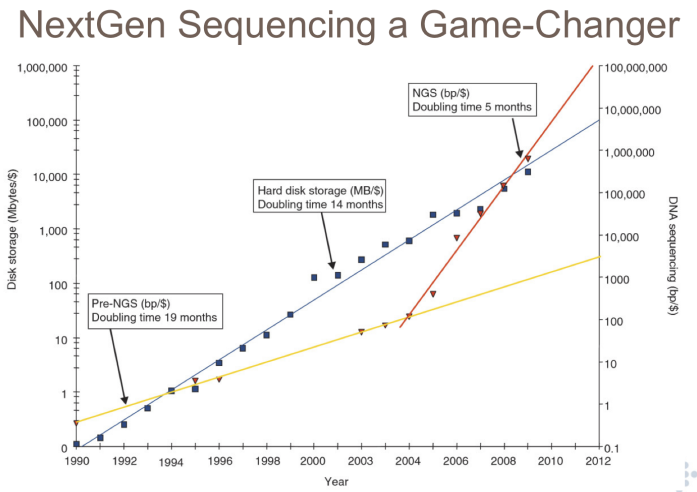
- Most Data files are shared
  - GEO; SRA; GenBank; EMBL; etc.





Splitting and trimming

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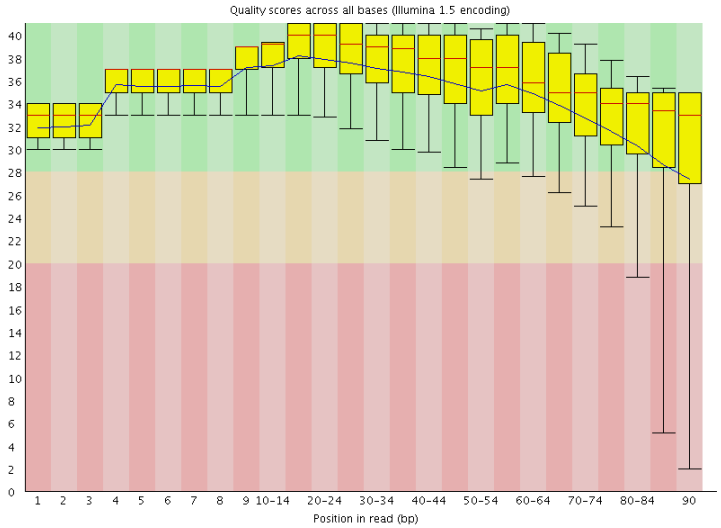

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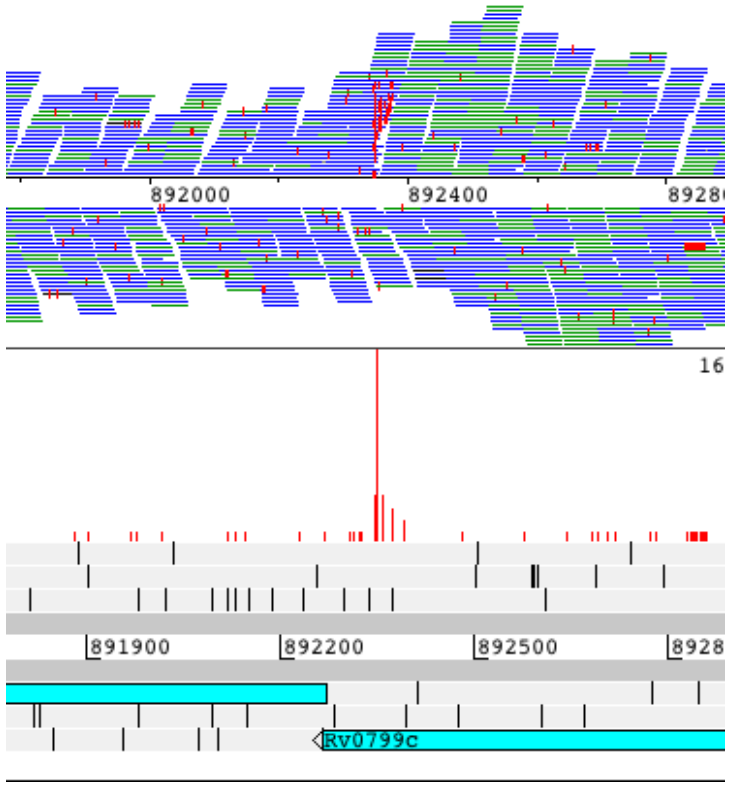

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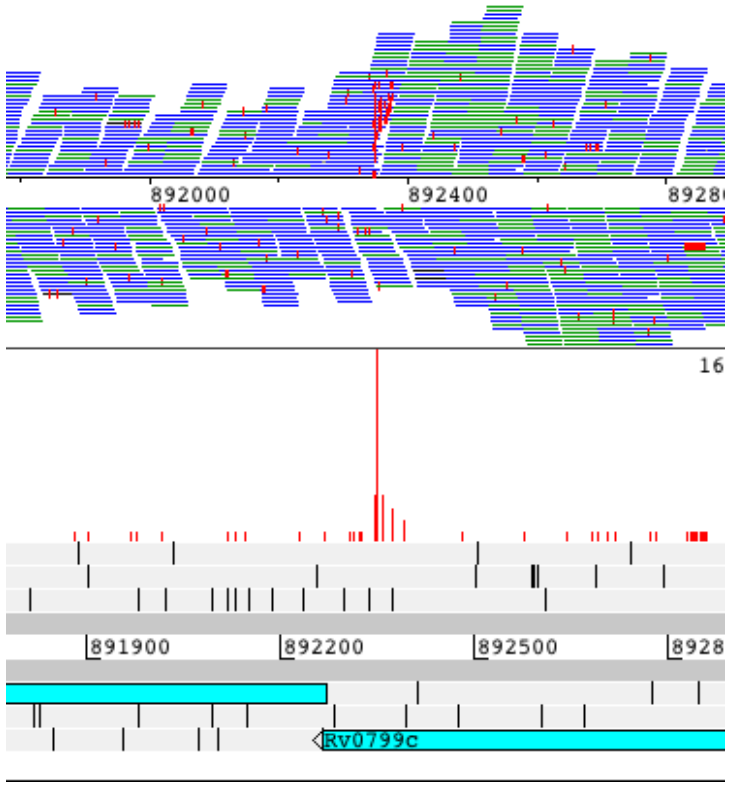

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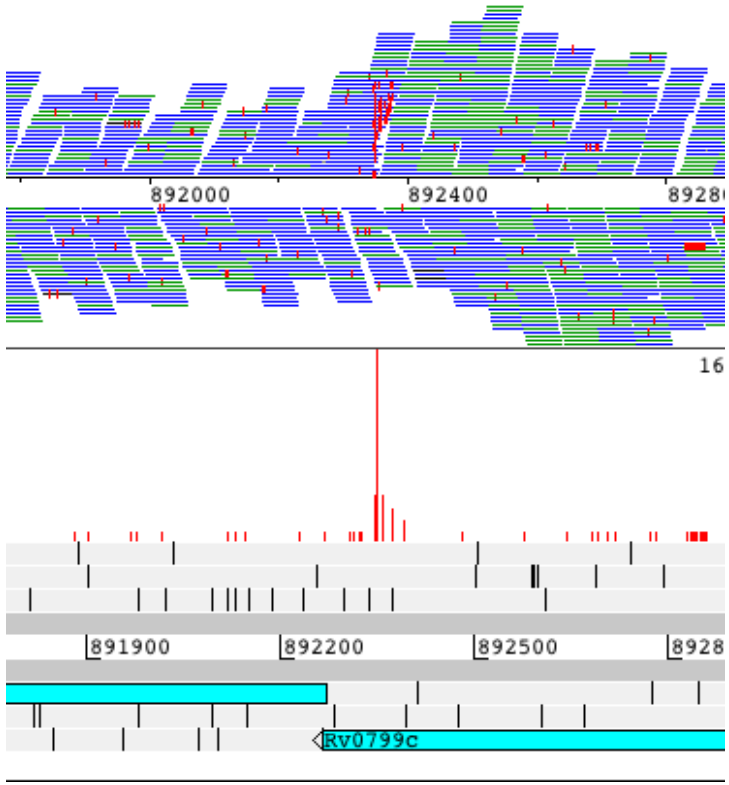

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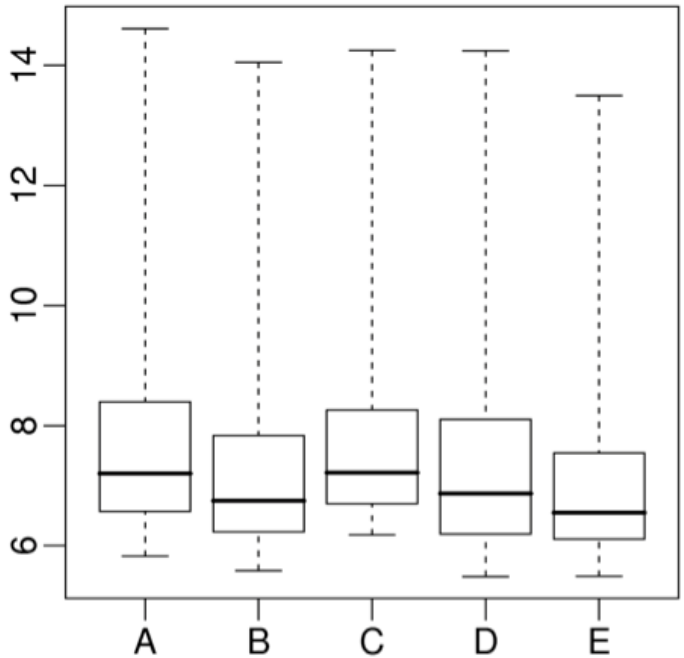

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- Fragments per kilobase per million fragments - FPKM
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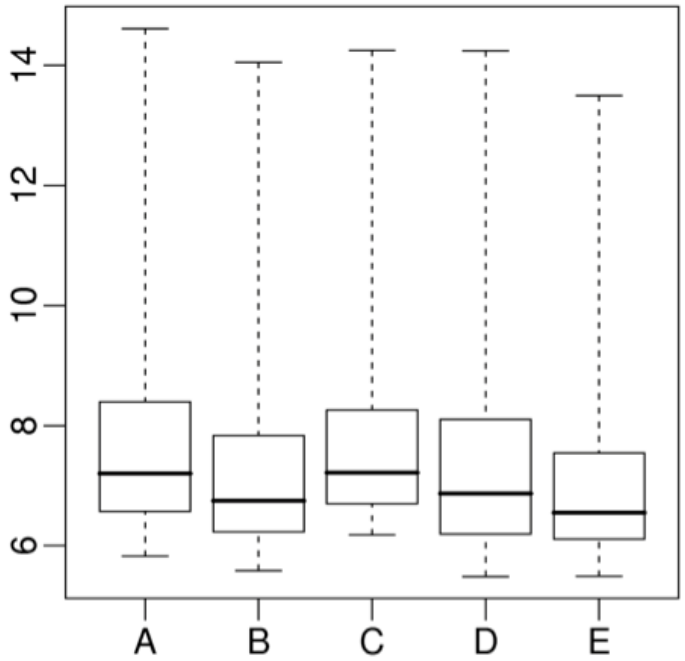

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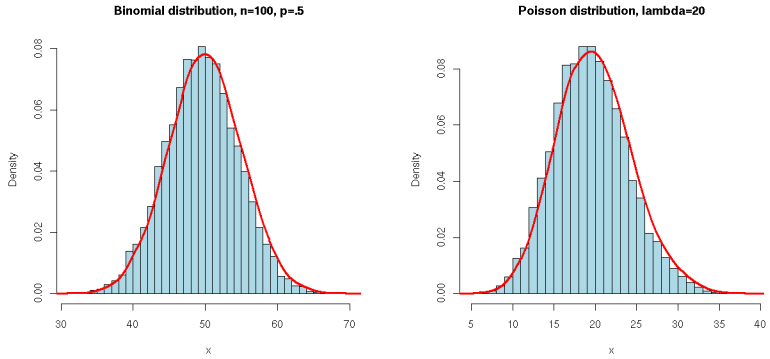

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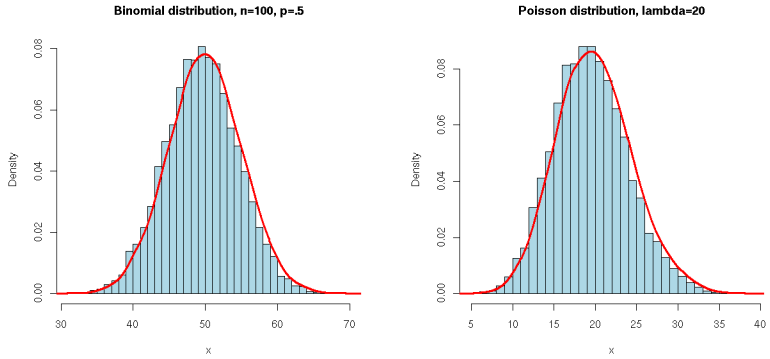

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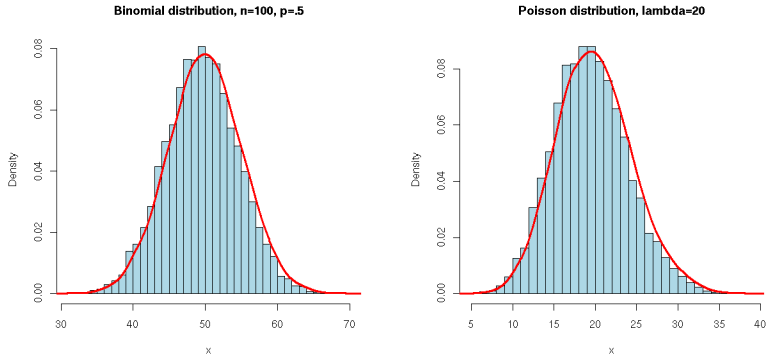

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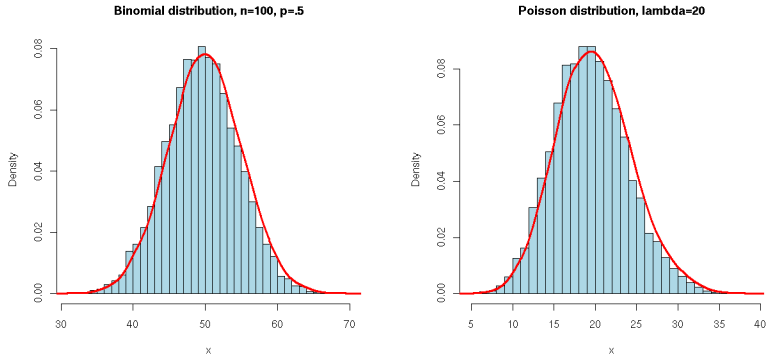

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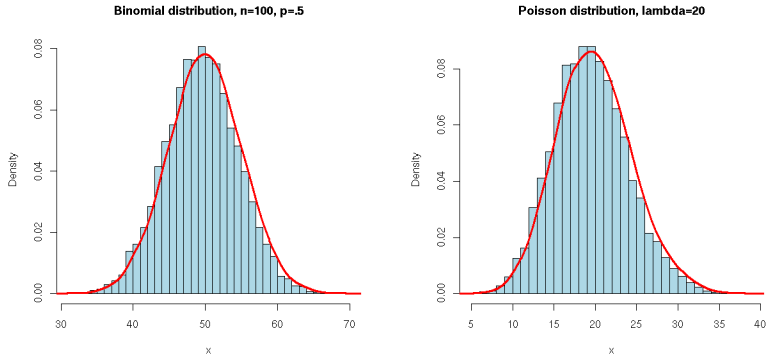

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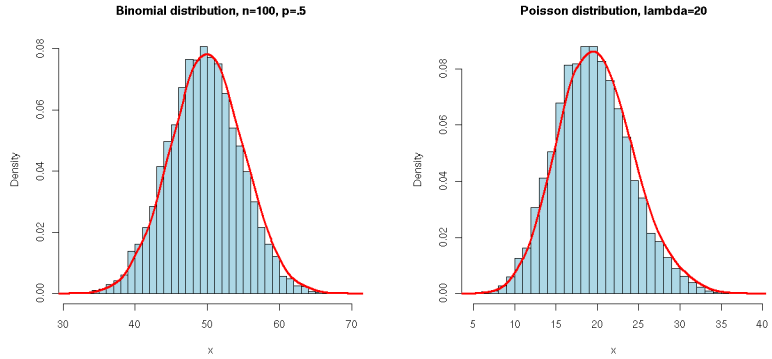

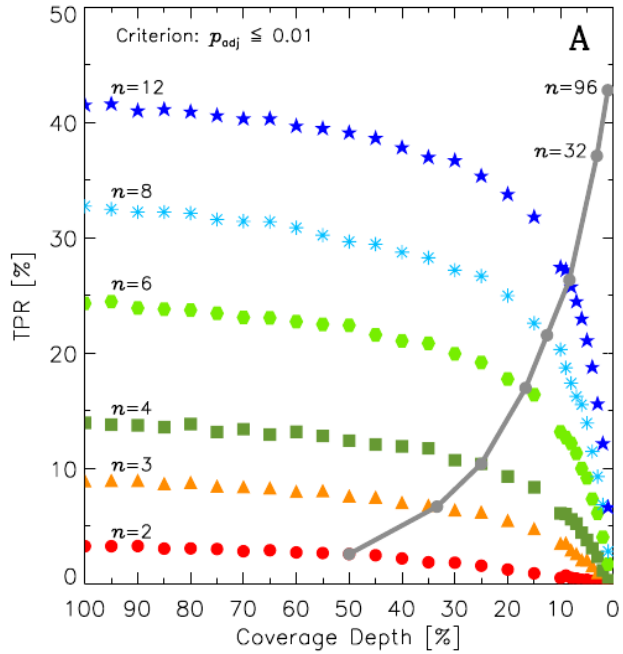

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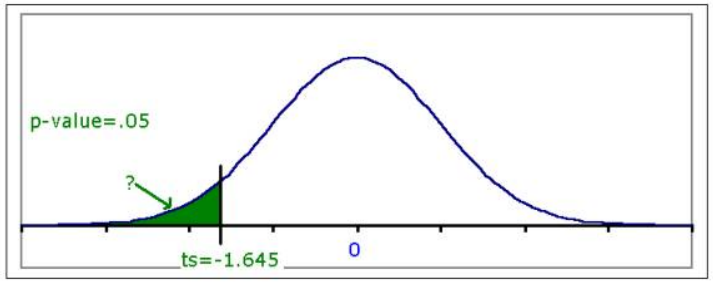

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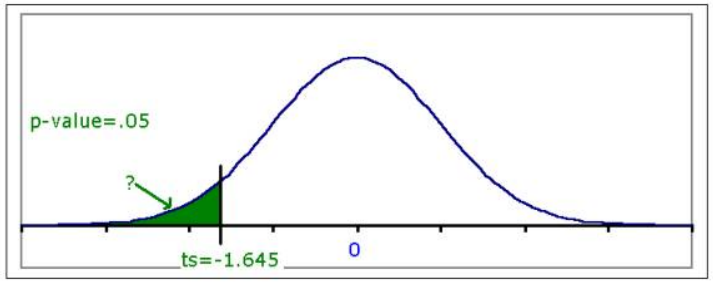

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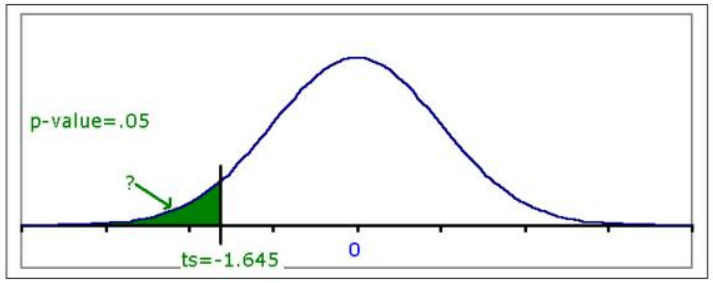

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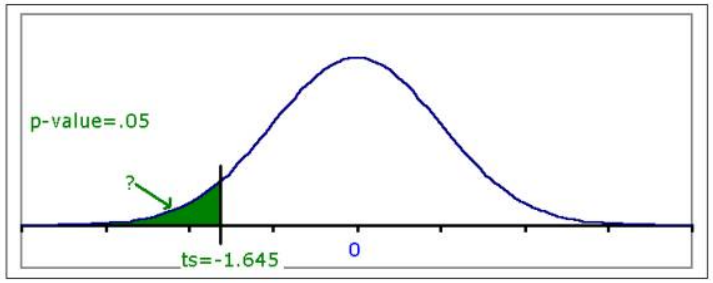

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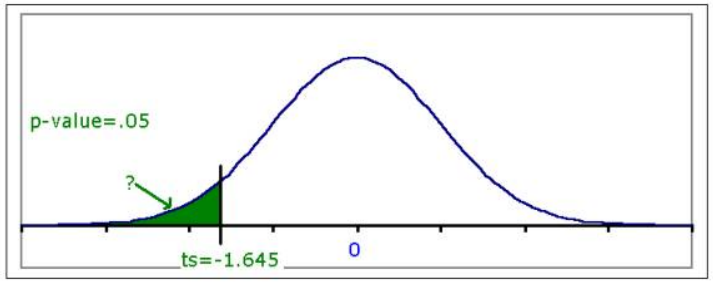

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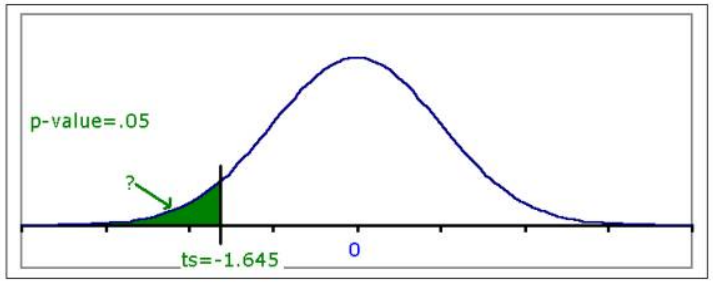

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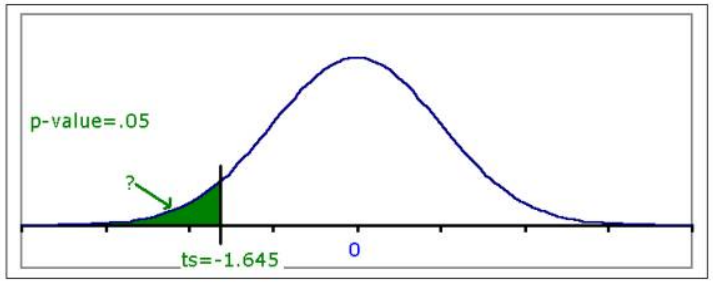

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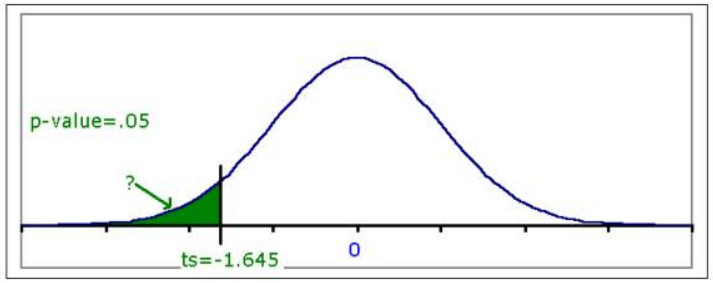

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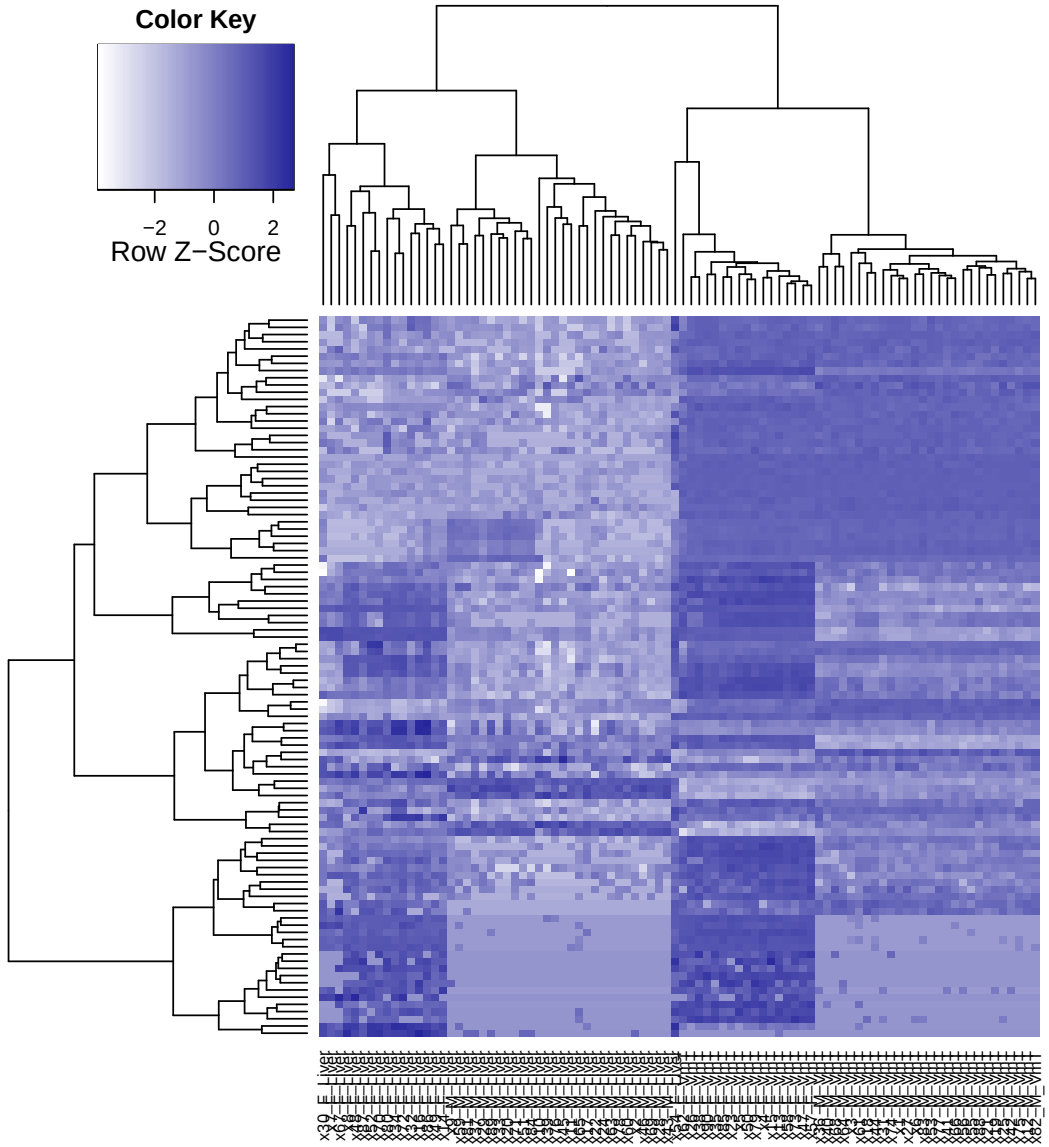

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Microarray Expt

RNAseq Experiment

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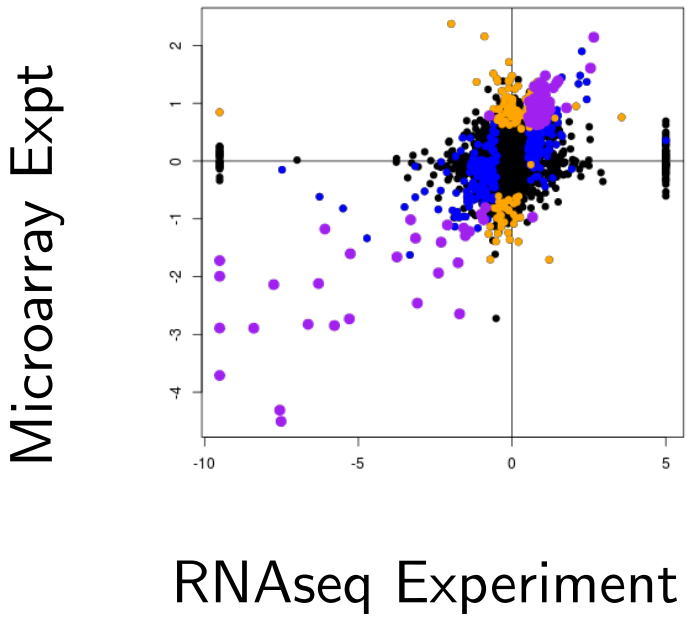

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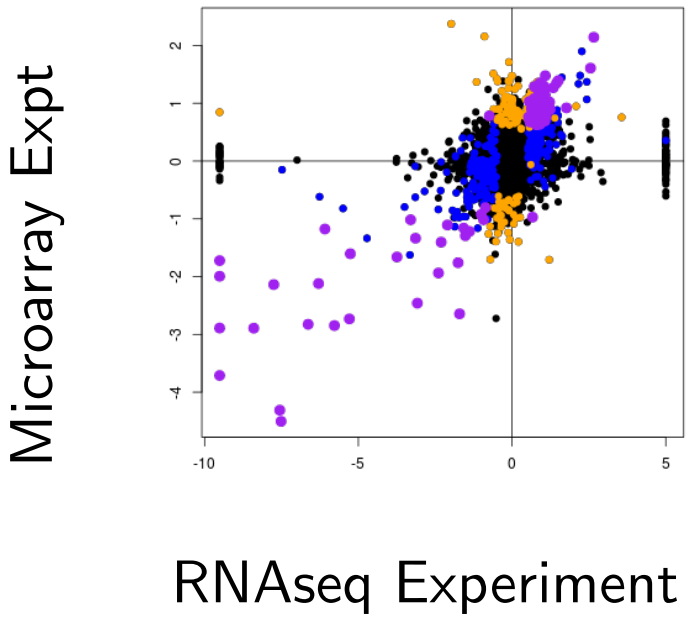

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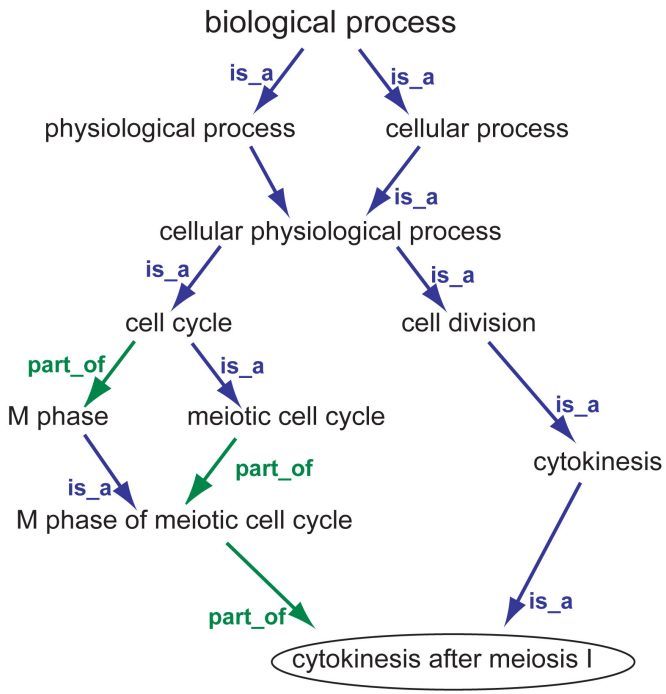

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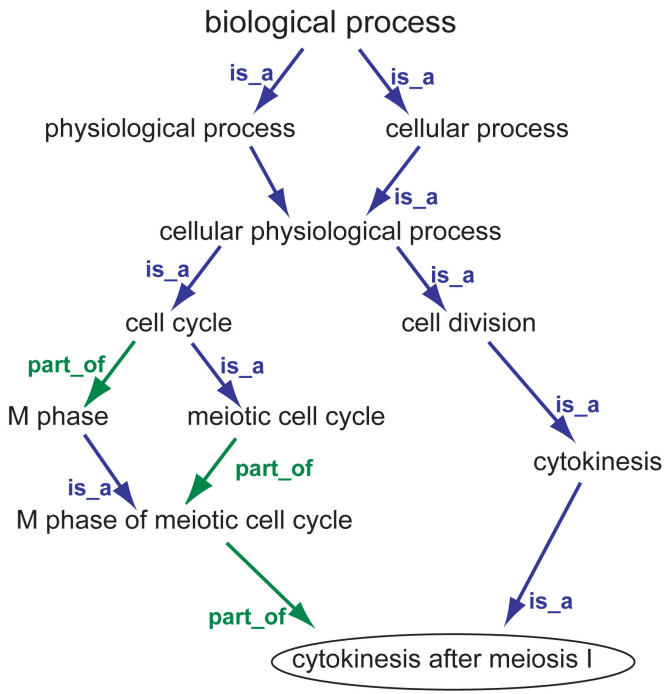

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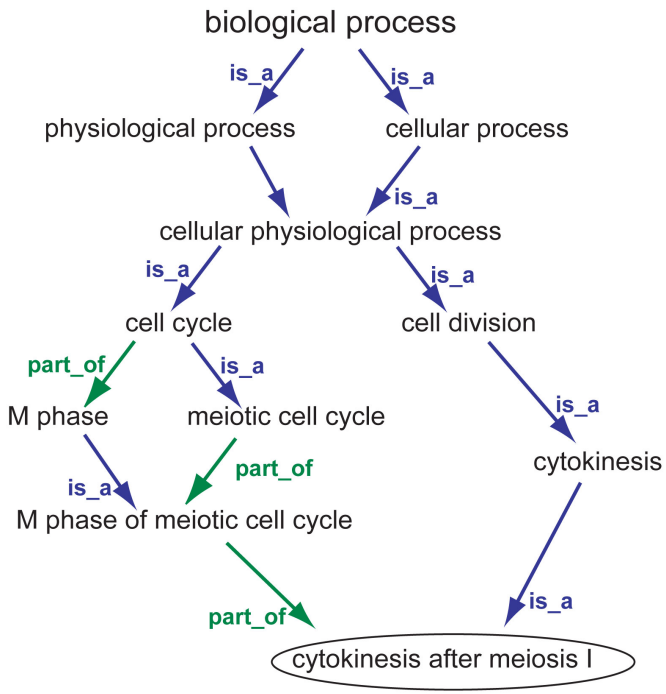

	is Sig DE	not Sig DE
Has GO term	A	C
Not this GO	B	D

GO analysis

- Look for over-represented functions
- Need heirarchical, conistent annotation
  - Gene Ontology
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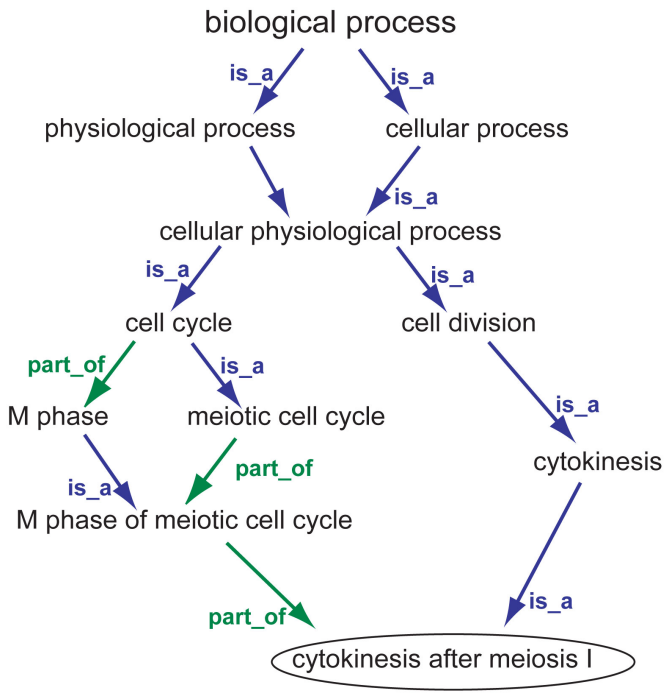

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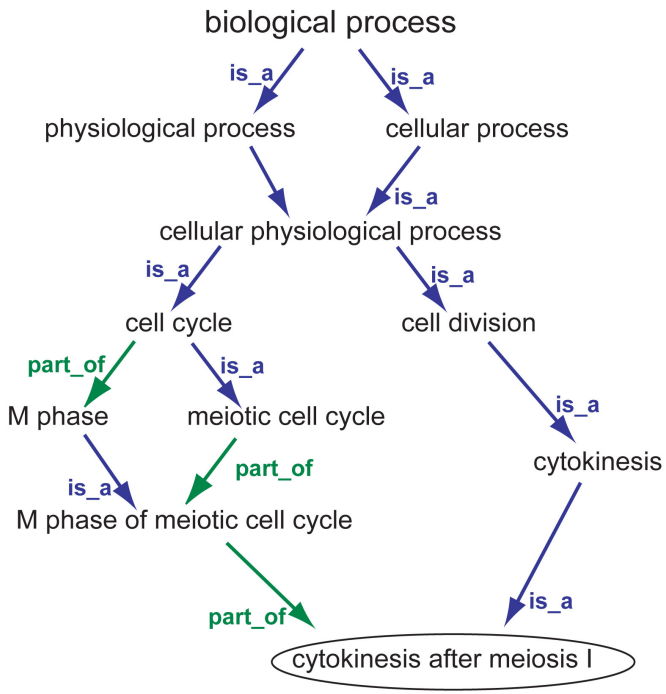

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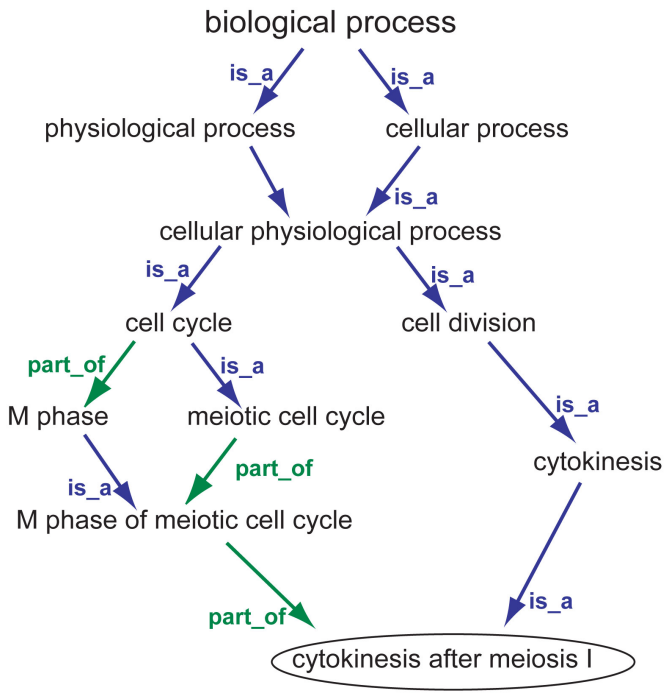
- Several alternatives
  - Chi-square
  - Use heirarchy



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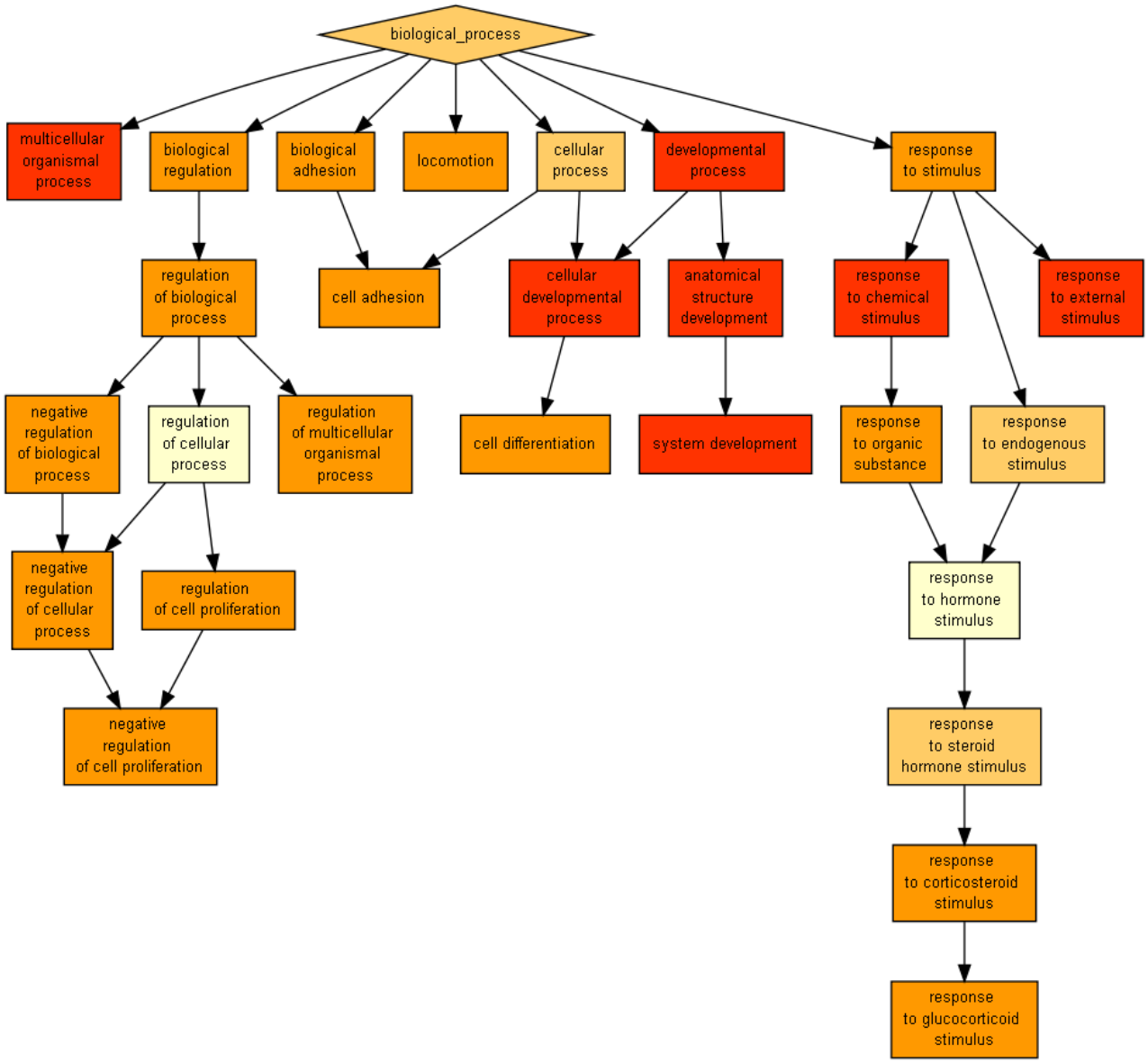

A bigger example

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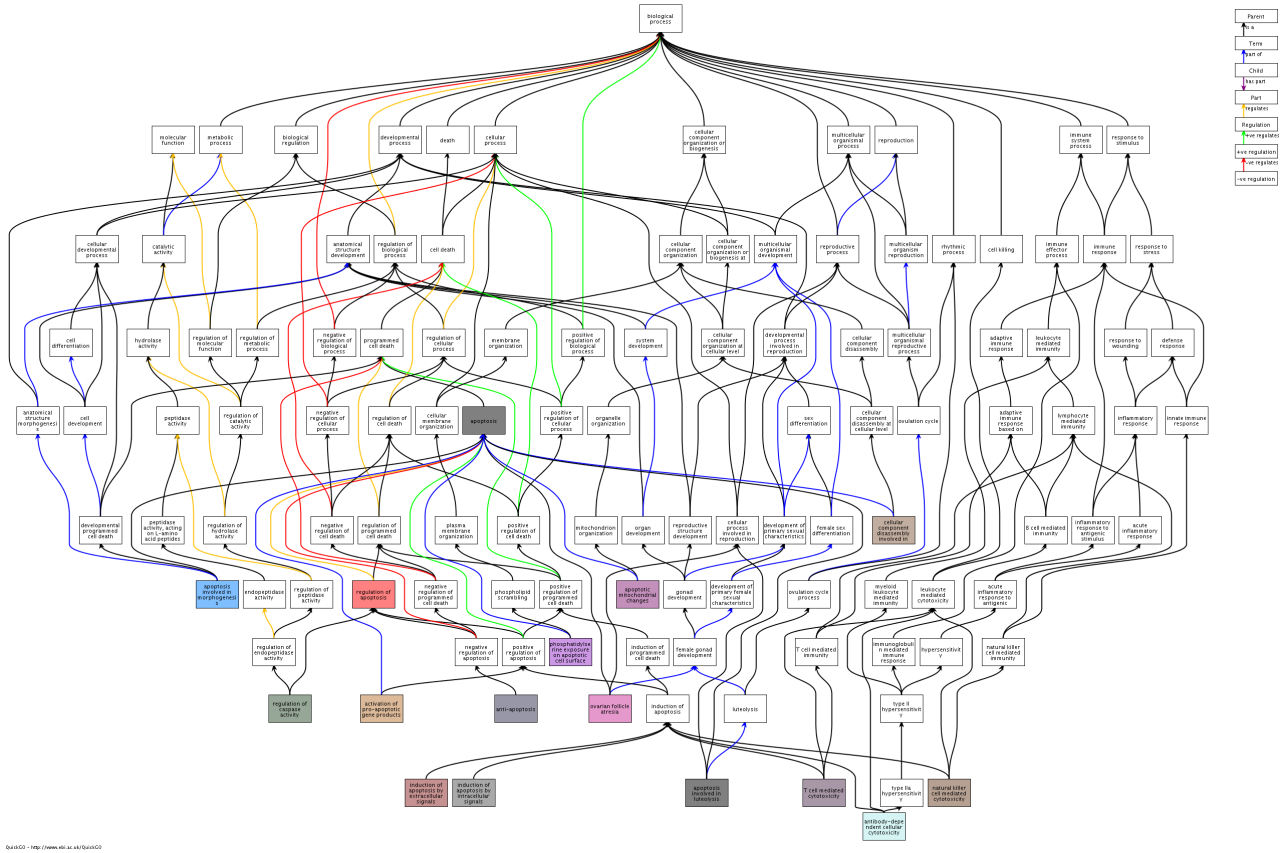

Facebook and Twitter analysis

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- Facebook networks are similar to gene networks

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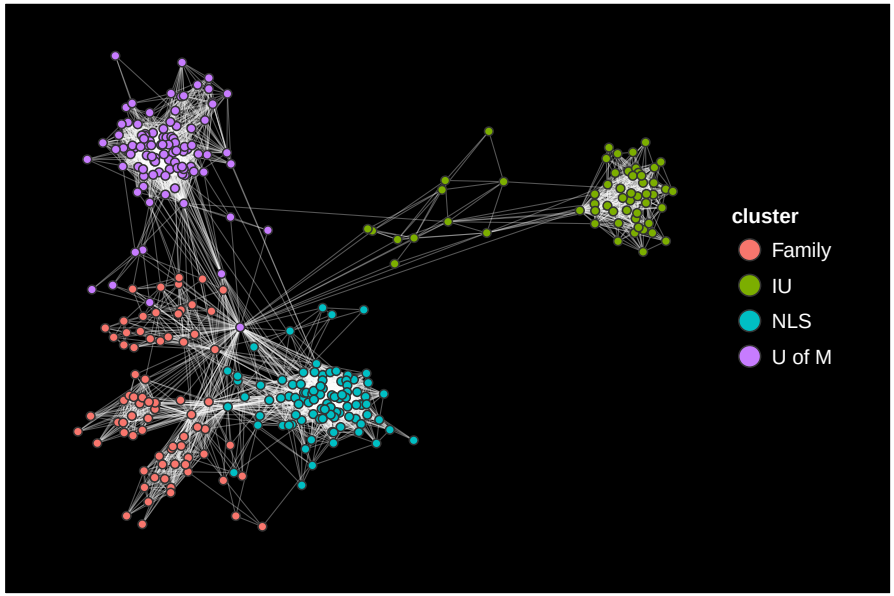

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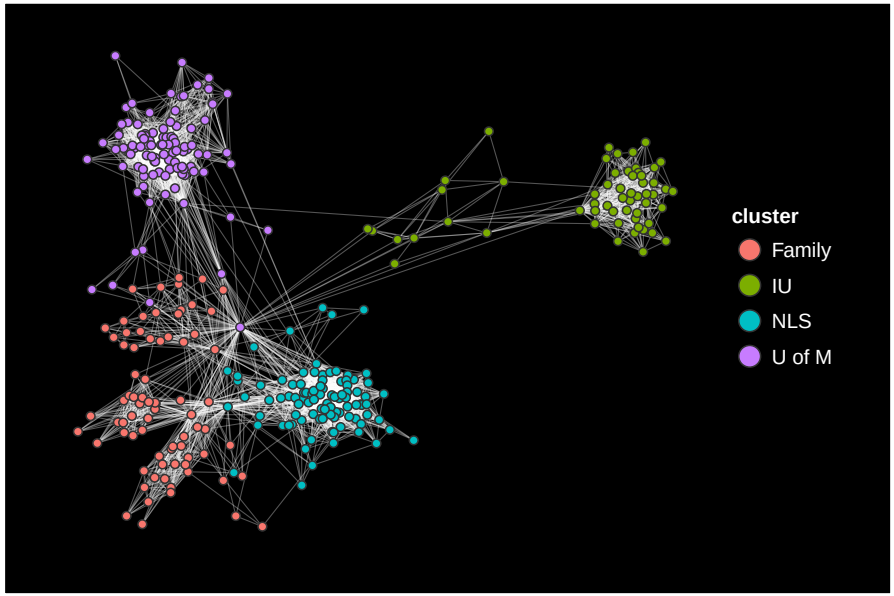
- Both Facebook and Twitter offer ability to access data



Facebook and Twitter analysis

- Facebook networks are similar to gene networks
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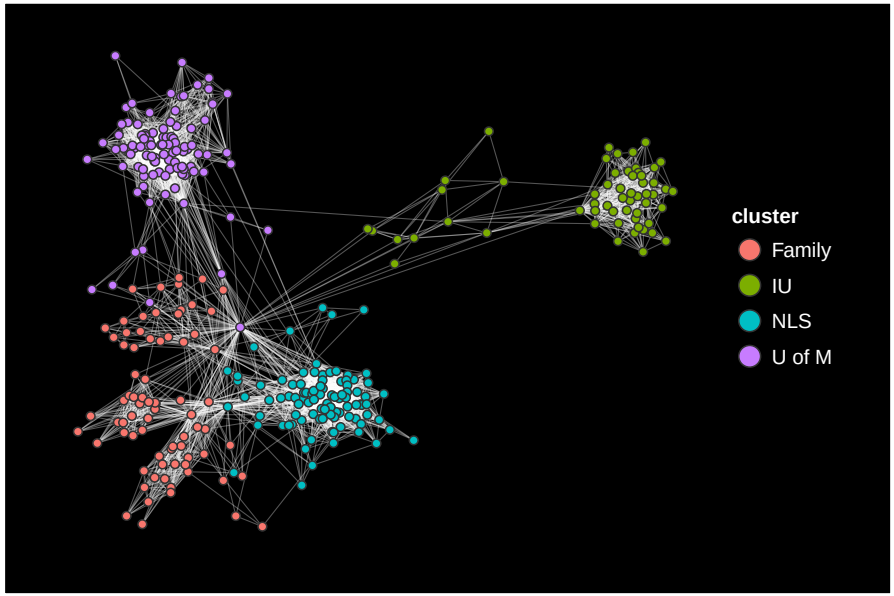
  - My friends network
- Both Facebook and Twitter offer ability to access data
  - Planning text analysis



Conclusions

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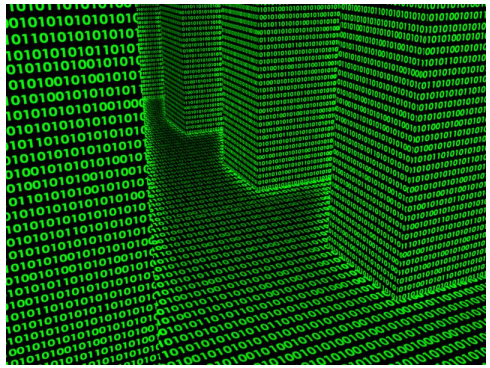

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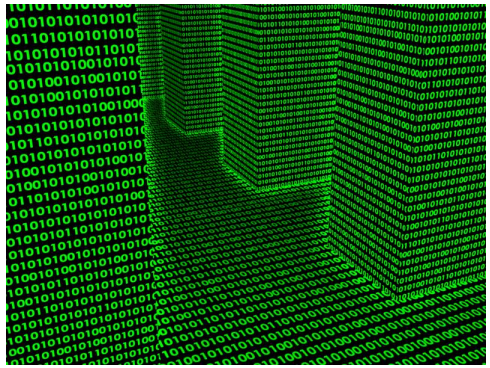

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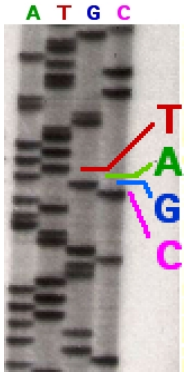

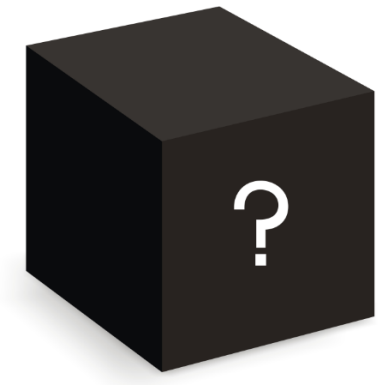

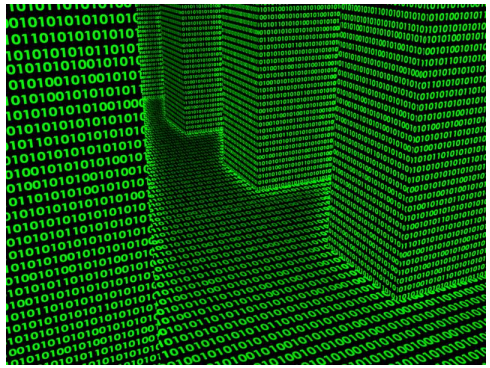

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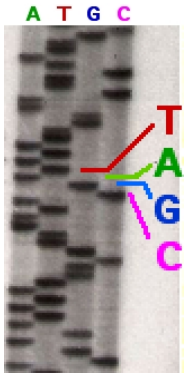

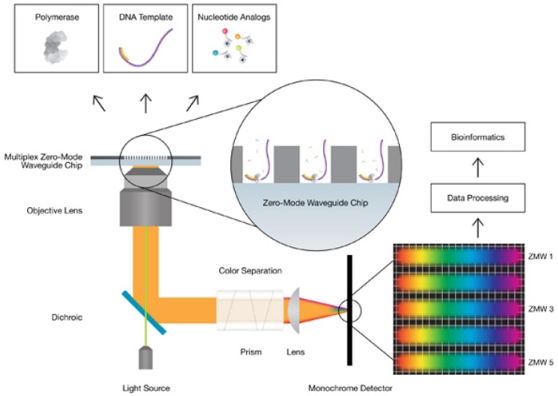

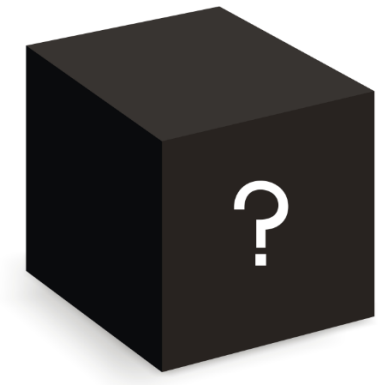

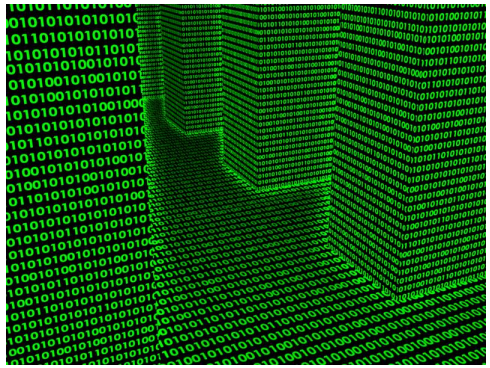

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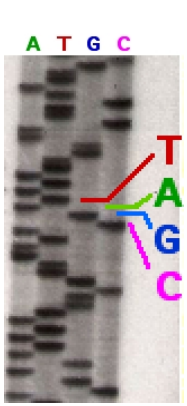

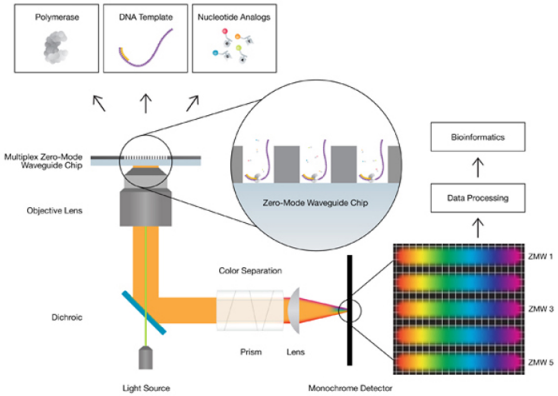

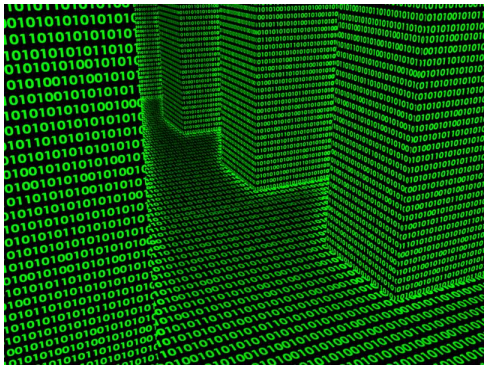

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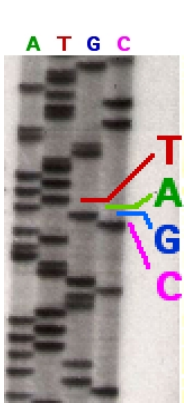

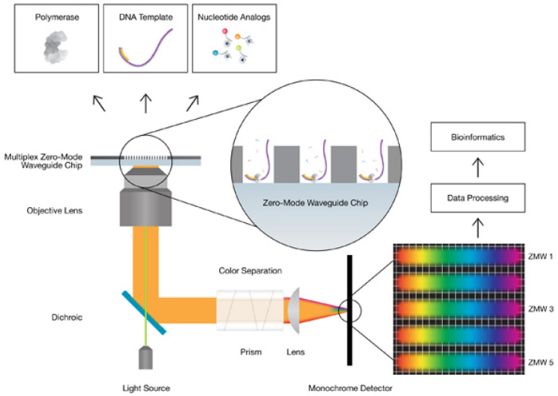

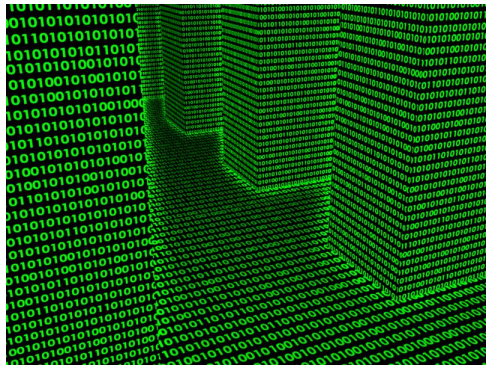

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