

Questions

Use the next 15+ minutes to answer and send me your responses: guertin@uchc.edu

1. Have you ever made high throughput sequencing (HTS) libraries?
2. Does your thesis project involve HTS experiments or analysis? —if so, please describe
3. Have you previously analyzed HTS data?
 - If so, did you use the command line or web-based tools?
4. How would you rate your abilities in the terminal/command line?
 - Rate 1 to 5: 1 = *The Terminal...the 2004 movie starring “America’s dad” Tom Hanks? (wow, my professor is hip to the contemporary cinematic features!); 5 = my stack overflow name is shellHacker1976*
5. How would you rate your abilities in R?
 - Rate 1 to 5: 1 = *I haven’t had a lecture on “R” since kindergarten; 5 = statistics, parsing, figures...all the things.*
6. Are you familiar with any programming languages? —if so, please list them
7. What type of computer and operating system will you be using for this course?

Bookmark this page:
<https://github.com/guertinlab/meds5420>

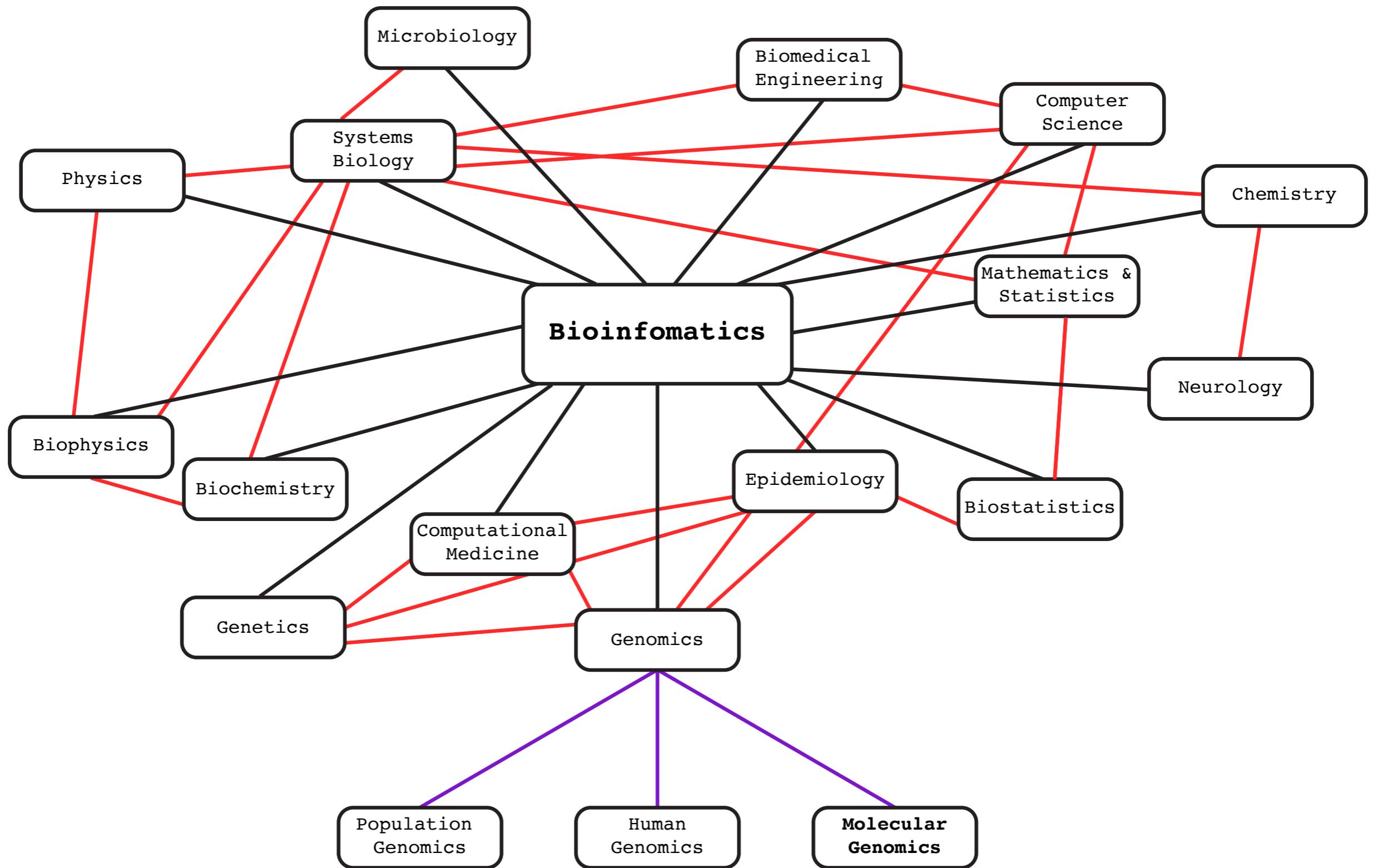
MEDS 5420: Molecular Genomics Practicum



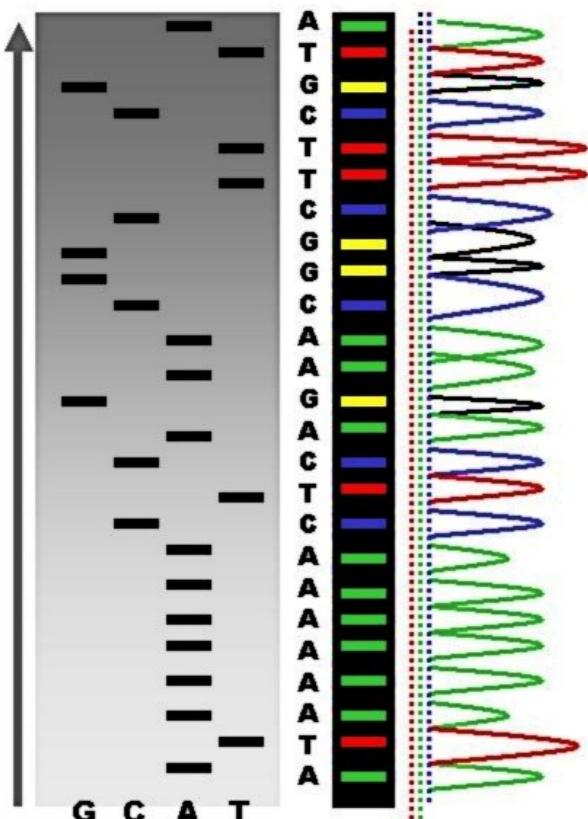
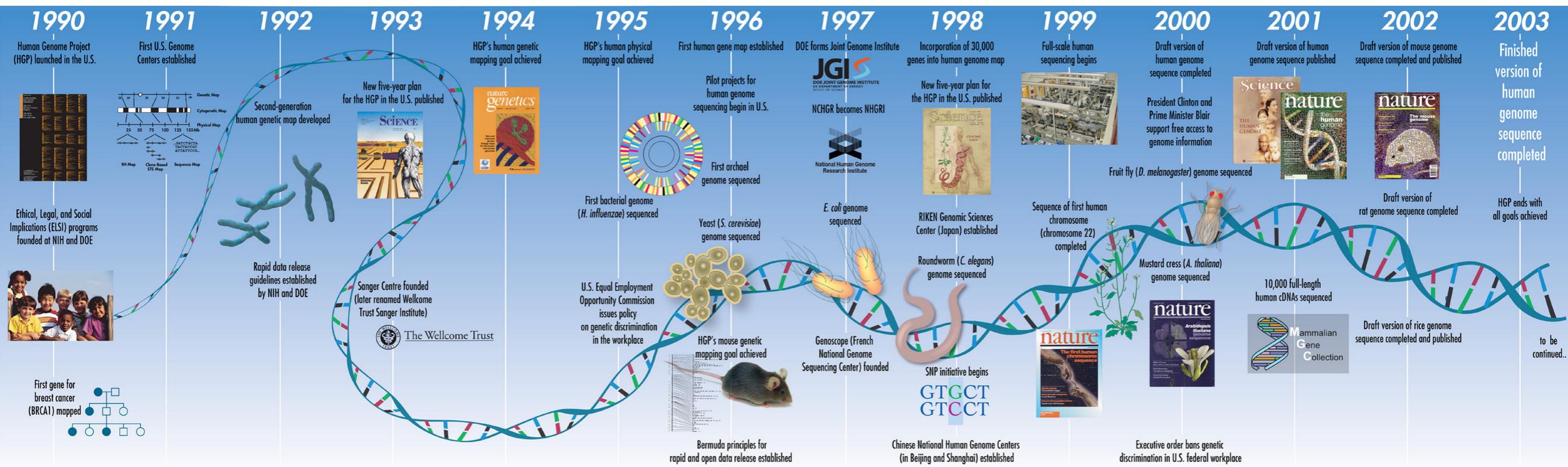
Spring 2024
Mike Guertin
Featuring Pedro Miura

This course is adapted from UConn Professor Leighton Core's course MCB 5430

Why *molecular genomics* and not **bioinformatics**?



Human Genome Project



Sanger sequencing

Cost: 2.7 billion

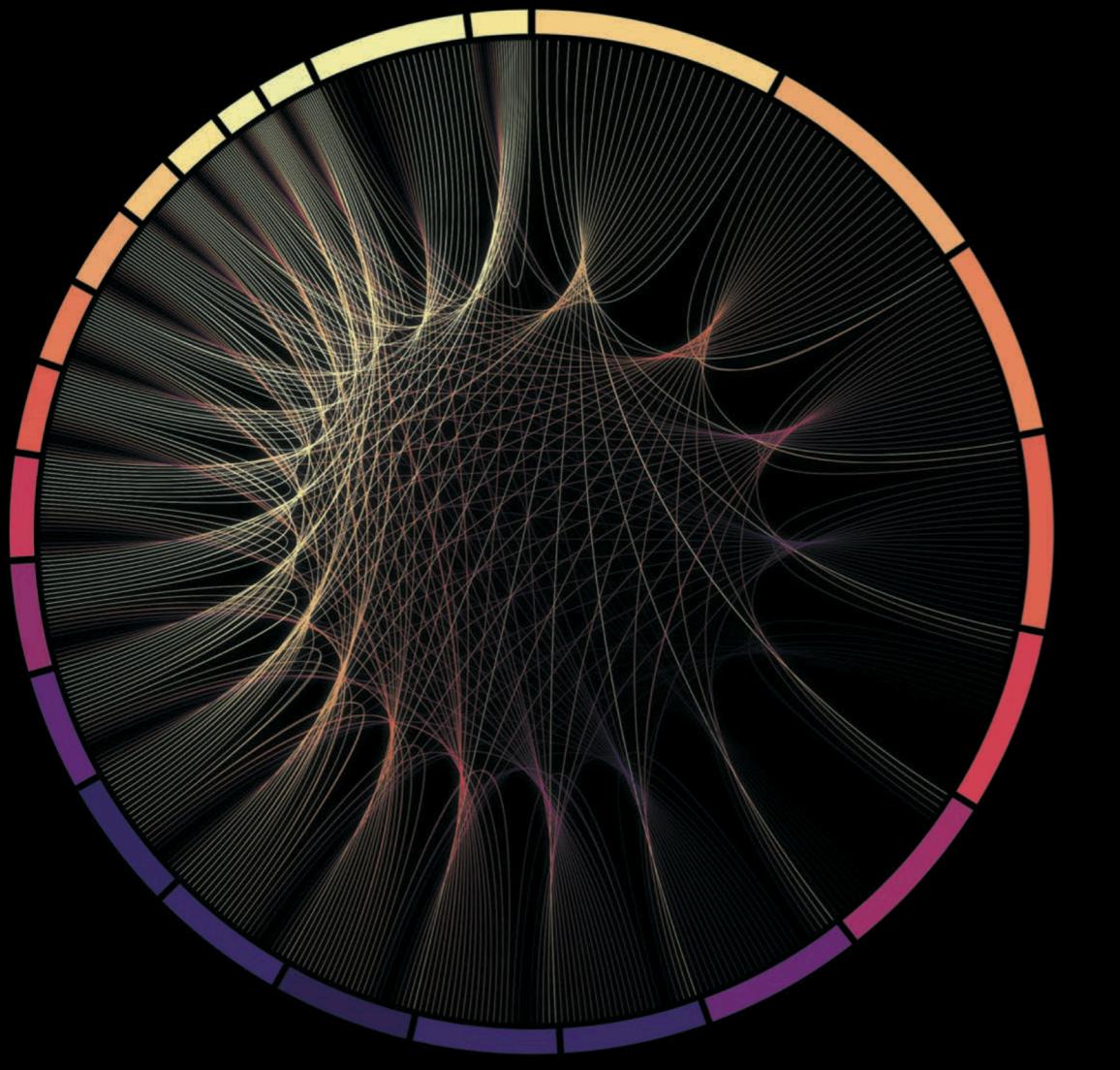
Contemporary milestones in genomics

www.nature.com/collections/genomic-sequencing-milestones

February 2021

nature milestones

Genomic sequencing



Produced by:

Nature, Nature Genetics and
Nature Reviews Genetics

With support from:

illumina®

<https://www.nature.com/immersive/d42859-020-00099-0/pdf/d42859-020-00099-0.pdf>

Genomics of human variation

Epigenomics

Population Genomics

Functional Genomics

Genomics?!

Microbiome Genomics

Metagenomics

Medical Genomics

Structural Genomics

Molecular Genomics: coupling classic molecular biology techniques to HTS for nucleic acid quantification

How do we begin to understand the genome?

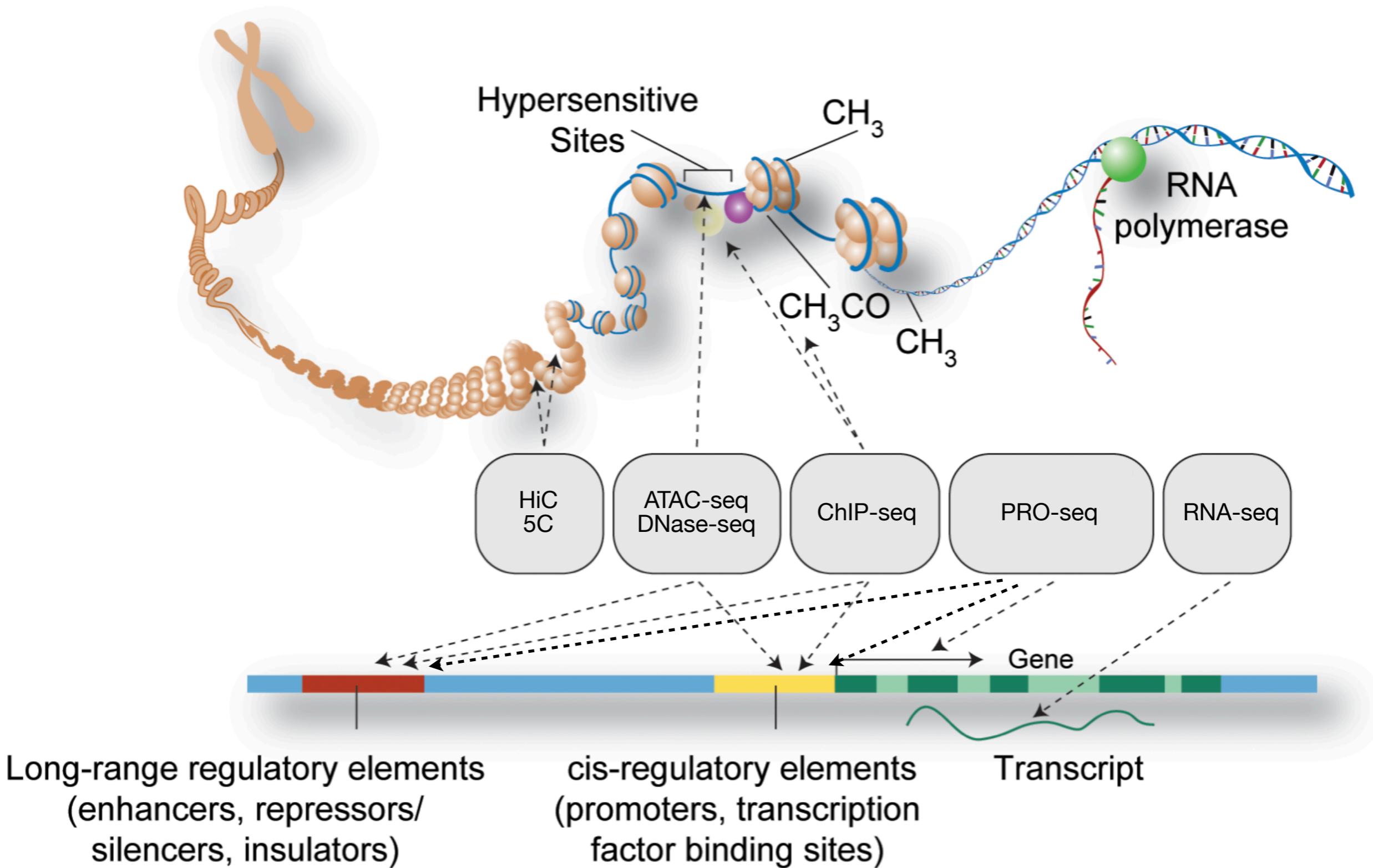


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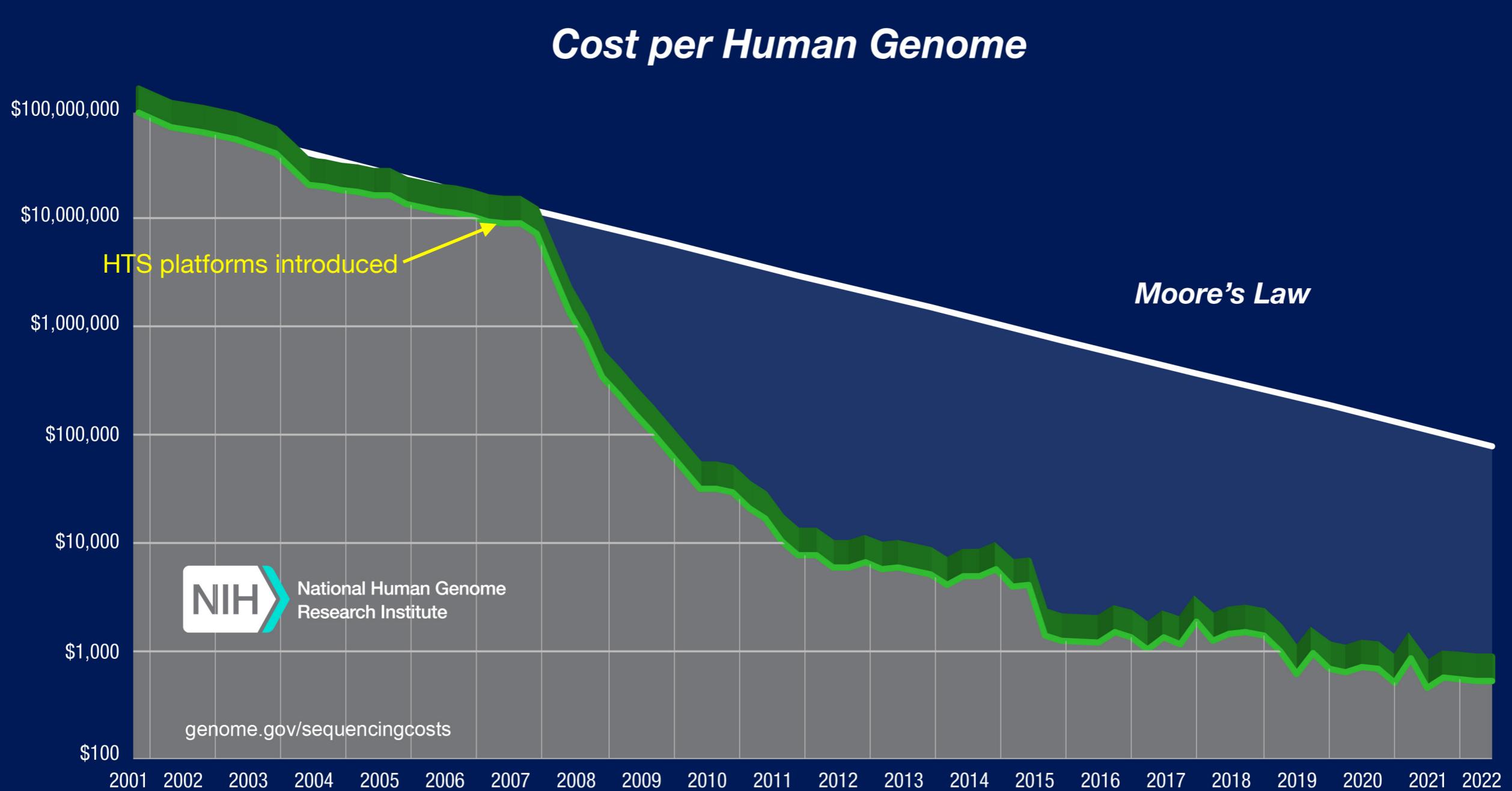
Questions that can begin to be addressed with Molecular Genomics

- How much of the genome is functional?
- Where are the functional elements?
- How are elements organized 3 dimensionally?
- What constitutes the molecular makeup of regulatory regions?
- How do regulatory regions change throughout development, upon environmental perturbation, or in the presence of mutations?

Molecular genomics assays



High throughput sequencing costs drove the genomics revolution



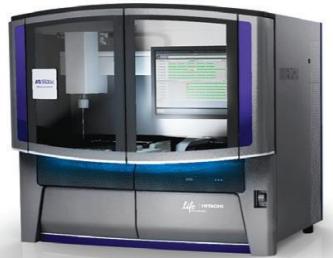
High throughput sequencing technologies



Roche 454



Ion Torrent



ABI Solid

Long Read (> 1kb)



Oxford Nanopore



Pacific Biosciences

Illumina



GALIx



HiSeq 2500



iSeq 100



MiniSeq



NextSeq 550



NextSeq 1000 & 2000



NovaSeq 6000

High throughput sequencing technology

Platform	Instrument	Reads/unit	Read Length (bp)	Read Type	Error Type
Illumina	NovaSeq 6000 S4	10,000,000,000	300	SR & PE	substitution
Illumina	NextSeq 550 High-Output	1,200,000,000	300	SR & PE	substitution
Illumina	HiSeq High-Output v4	250,000,000	250	SR & PE	substitution
Illumina	GALx	42,075,000	300	SR & PE	substitution
Illumina	MiSeq v3	25,000,000	600	SR & PE	substitution
Illumina	MiniSeq High-Output	25,000,000	300	SR & PE	substitution
Ion	Proton I	60,000,000	200	SR	indel
Ion	PGM 314	400,000	400	SR	indel
PacBio	PacBio Sequel	370,000	20,000	NA	indel
PacBio	PacBio RS II (P6)	55,000	15,000	NA	indel
Roche 454	GS FLX+ / FLX	700,000	700	NA	indel
SOLID	5500xl W	266,666,667	100	SR & PE	A/T Bias
SOLID	5500xl	81,500,000	100	SR & PE	A/T Bias
Oxford Nanopore	PromethION 48	depends on size (300 Gb total)	length of molecule up to 4,000,000	NA	sub/indel

Genomics at UConn

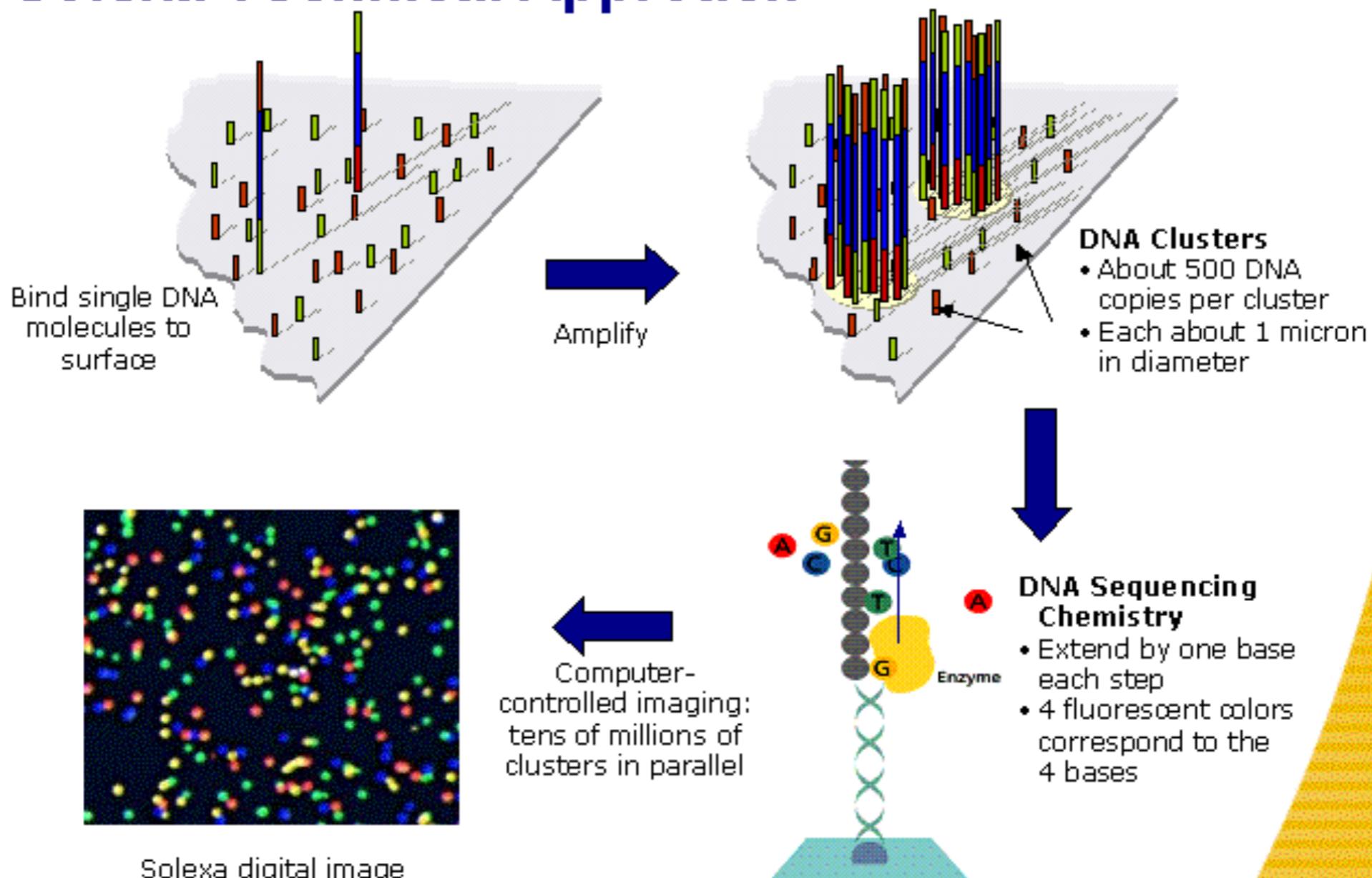


NovaSeq 6000 can sequence the equivalent of 48 human genomes per run at 30x coverage!

Illumina (formerly Solexa) Sequencing Technology: Clonal PCR colonies and Reversible Terminators



Solexa Technical Approach

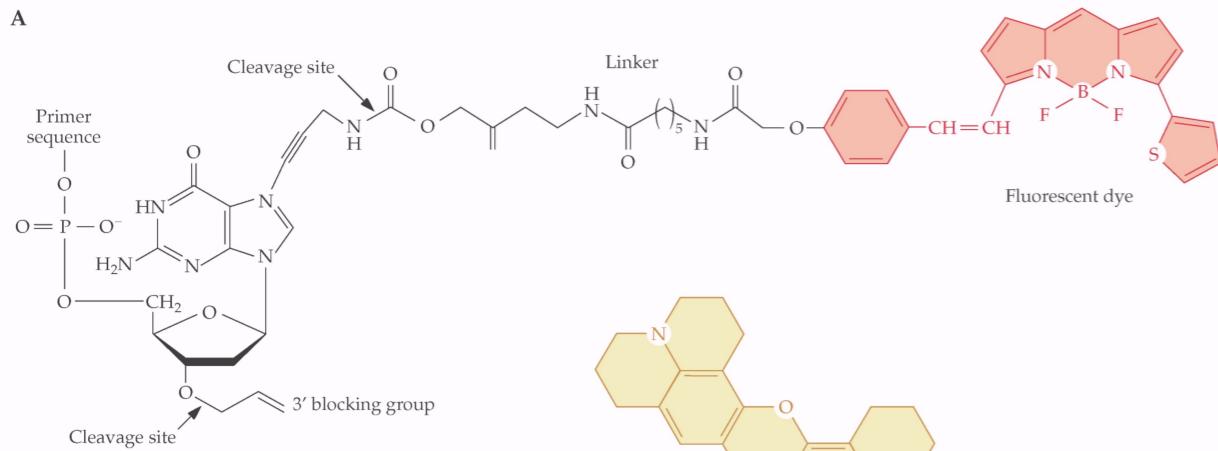


November 2006

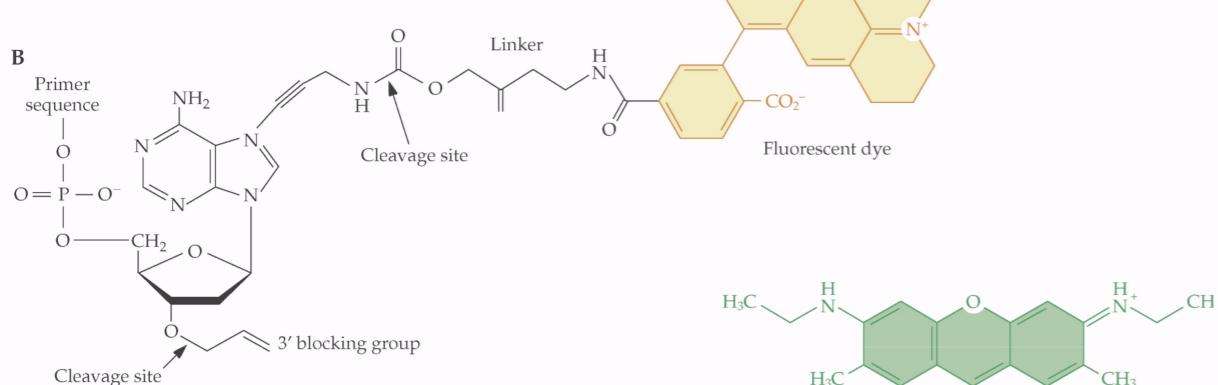
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Illumina Sequencing Technology: Dye and Reversible Terminators

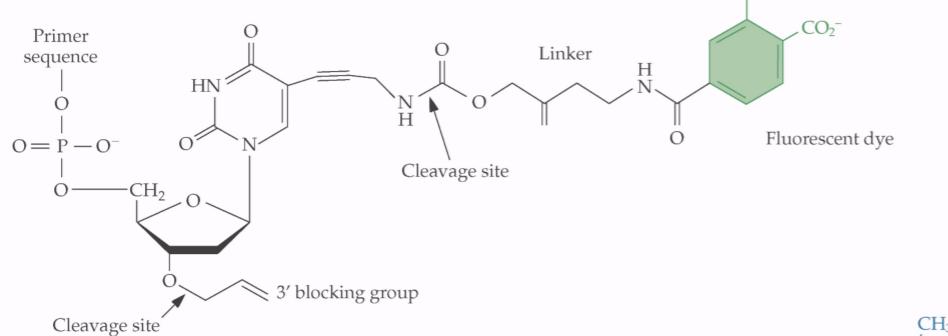
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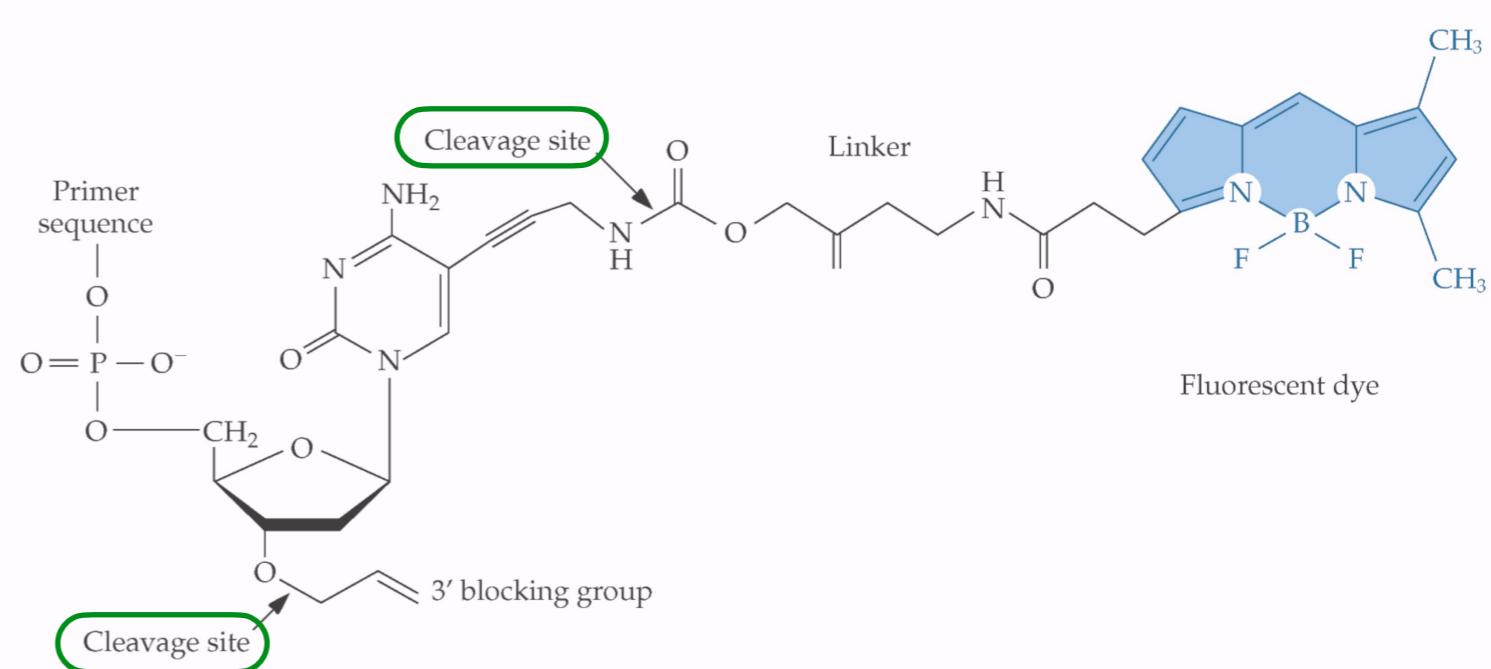
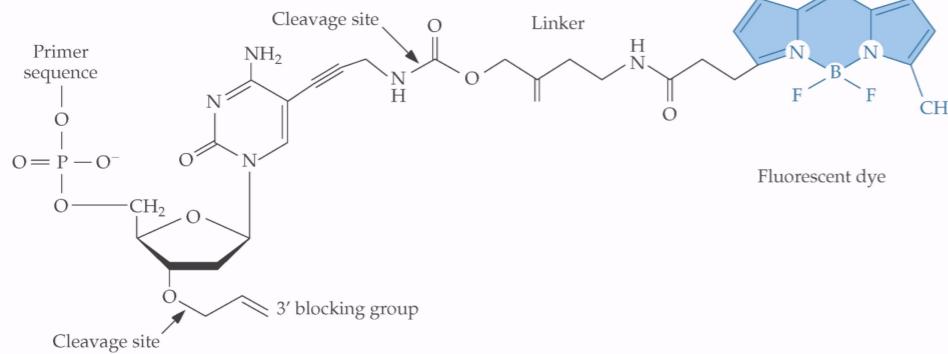
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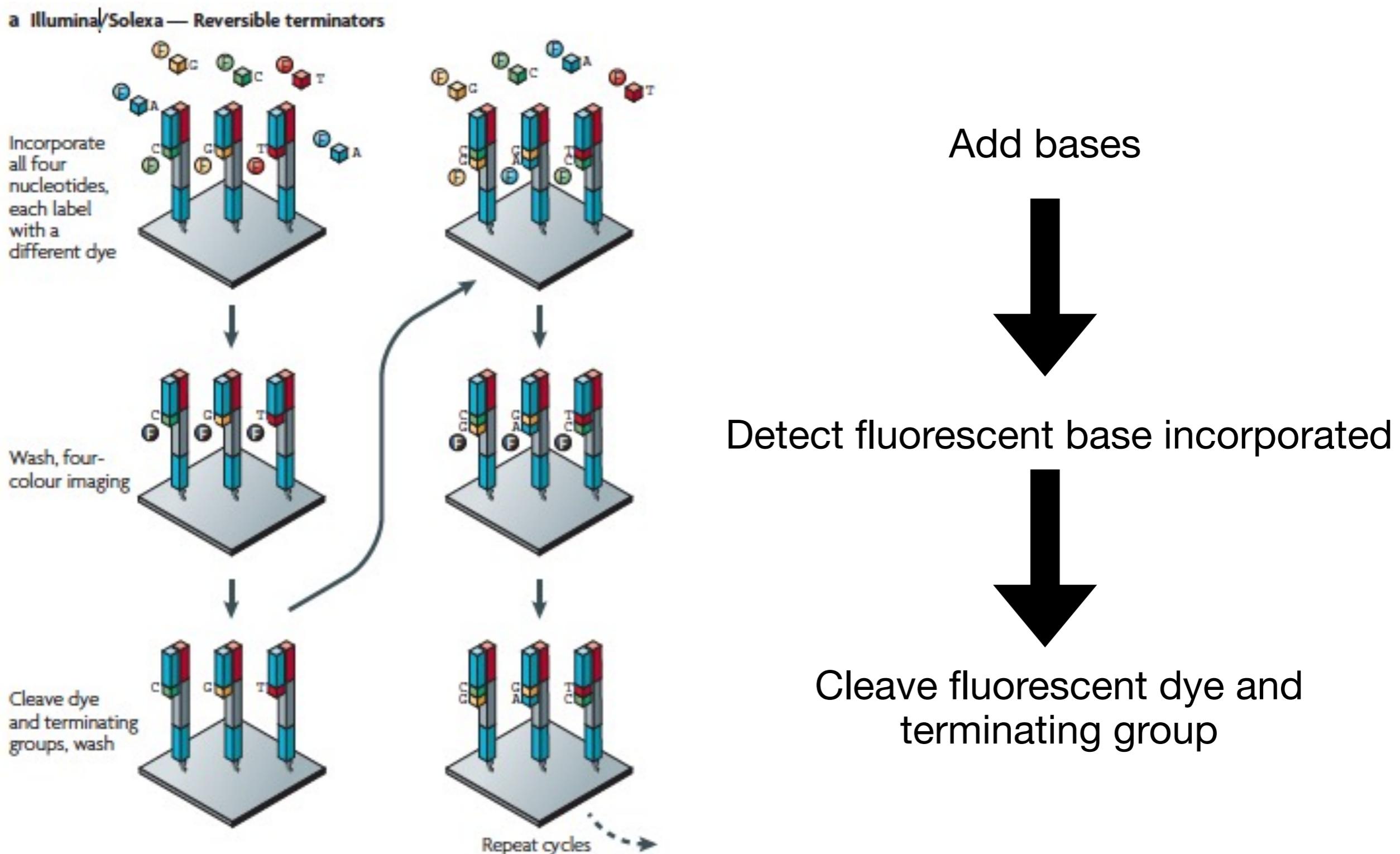
C



D



Illumina Sequencing Technology: Dye and Reversible Terminators



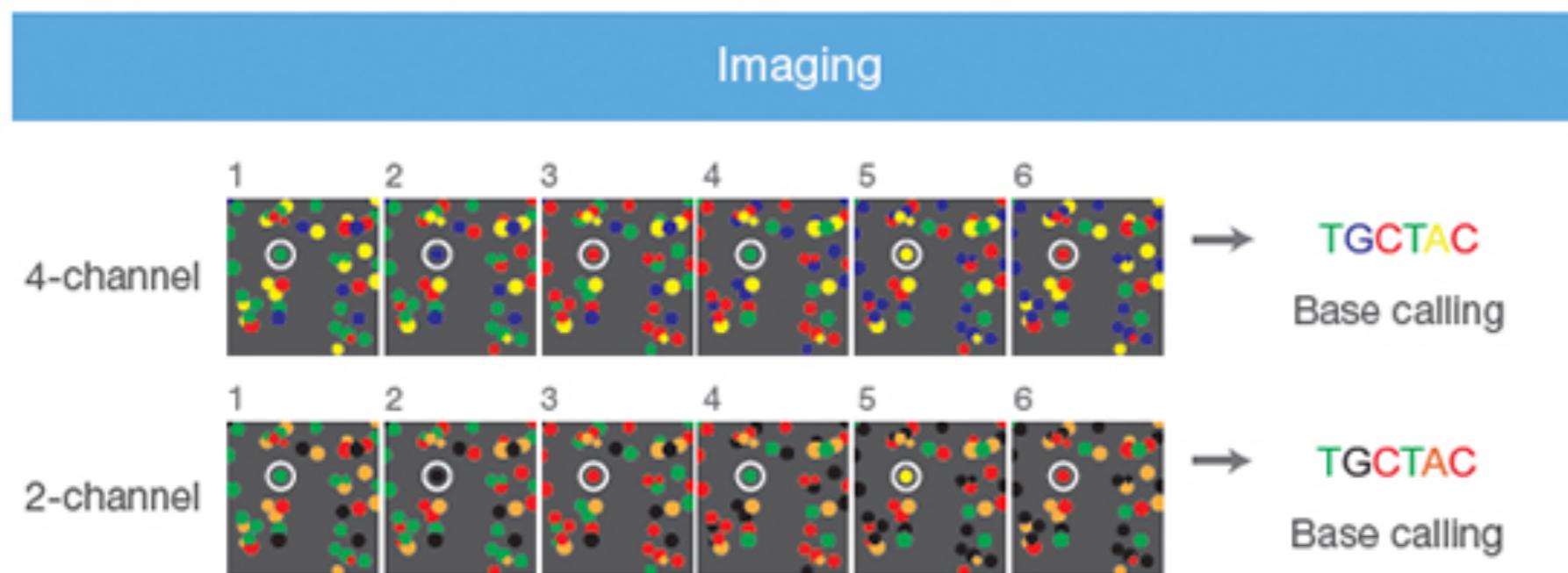
Illumina sequencing by synthesis (SBS) updates: 2 color imaging



Benefits:

Fewer images (2 vs 4):
= less data acquisition and processing time
= faster sequencing.

User experience unaffected



Current acquisition method for all Illumina devices

Illumina SBS updates: patterned flow cell

Distinct, Ordered Nanowell Design

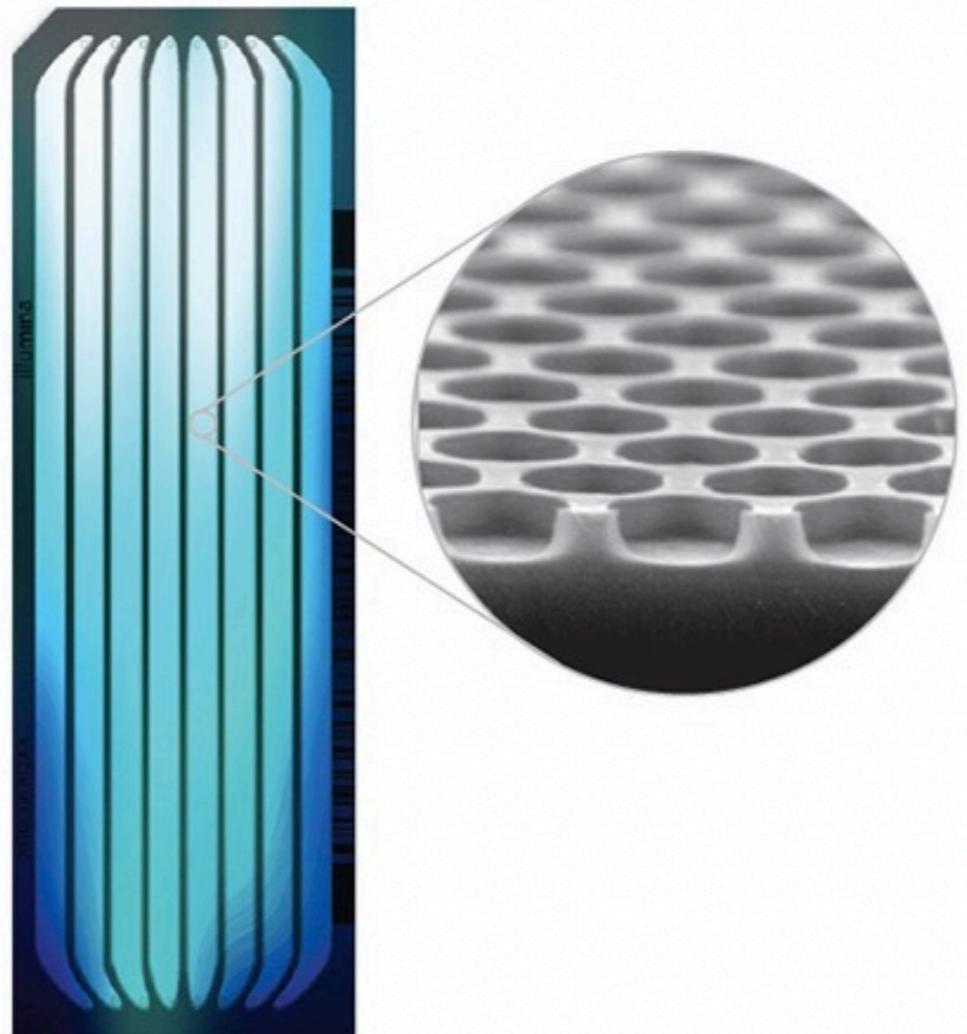


Figure 1. Advanced Patterned Flow Cell Design Enables Maximum Throughput.

Patterned flow cells contain billions of nanowells at fixed locations, providing even cluster spacing and uniform density.

Benefits:

- Location of clusters known
- Less cluster overlap
- Exclusion Amplification (ExAmp) allows multiple clusters from a single molecule

Pitfalls:

- Nanowells favor clustering small adapter/adapter products
- ExAmp creates PCR duplicates—good for genome coverage; bad for quantification of molecular genomics experiments

Currently used on NextSeq2000, NovaSeq

(page 7 of the patent provides an explanation of the technology)

<https://patentimages.storage.googleapis.com/f5/8f/f7/a0c052678df60e/WO2013188582A1.pdf>

Videos of HTS technologies

Roche 454: <https://www.youtube.com/watch?v=rsJoG-AuINE>

Ion Torrent: <https://www.youtube.com/watch?v=zBPKj0mMcDg>

Pac bio: <https://www.youtube.com/watch?v=v8p4ph2MAvI>

Illumina: <https://www.youtube.com/watch?v=HMyCqWhwB8E>

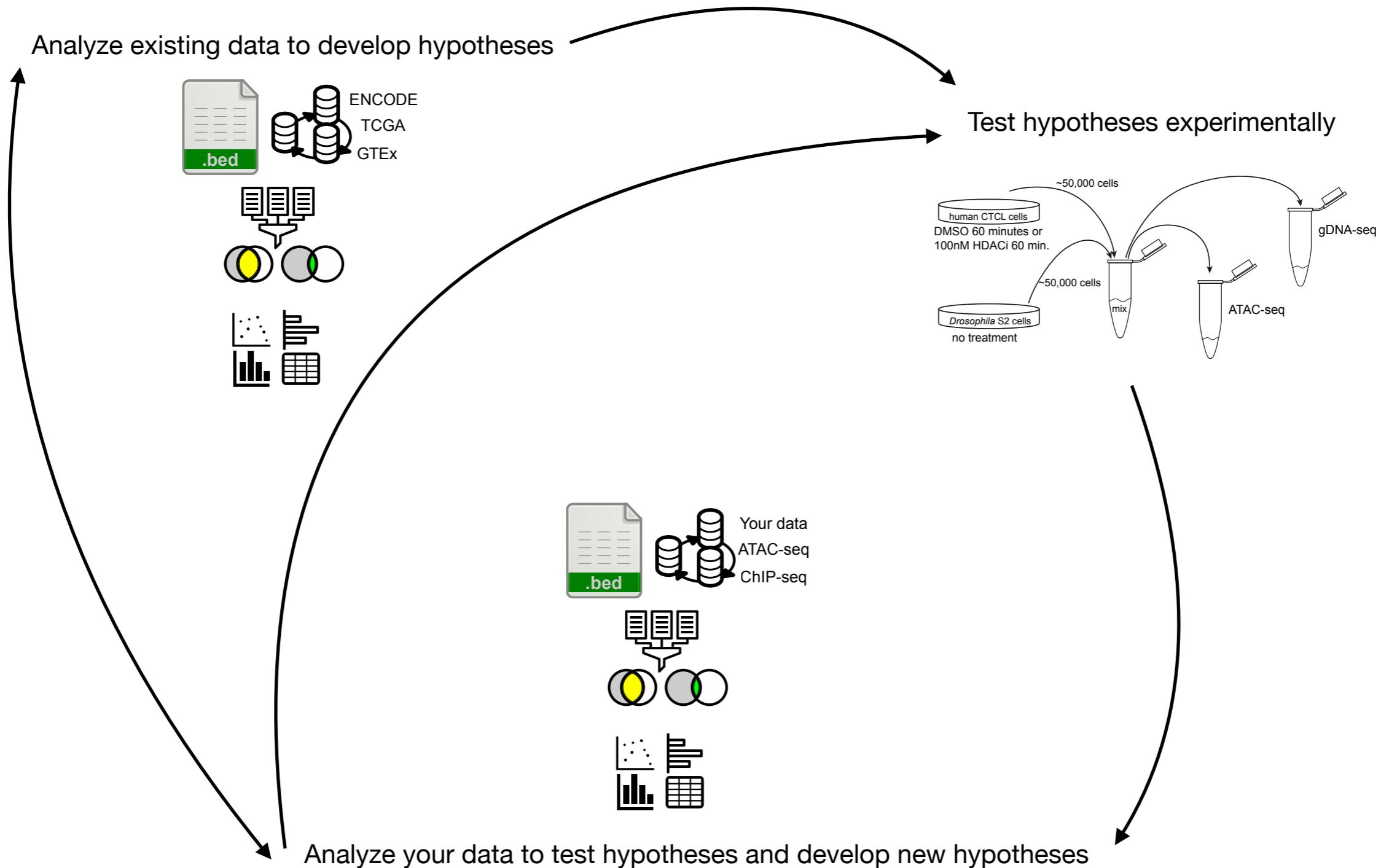
ABI solid: <https://www.youtube.com/watch?v=nIvyF8bFDwM>

Nanopore: <https://www.youtube.com/watch?v=3UHw22hBpAk>

Challenges that arise when working with big datasets

- Computational resources
 - Data storage
 - Processing power
 - RAM
 - CPUs
- Computational competency
 - Adept in a command line environment
 - Knowledge about available utilities
 - Programming languages
 - Pipeline development

A need for versatile scientists

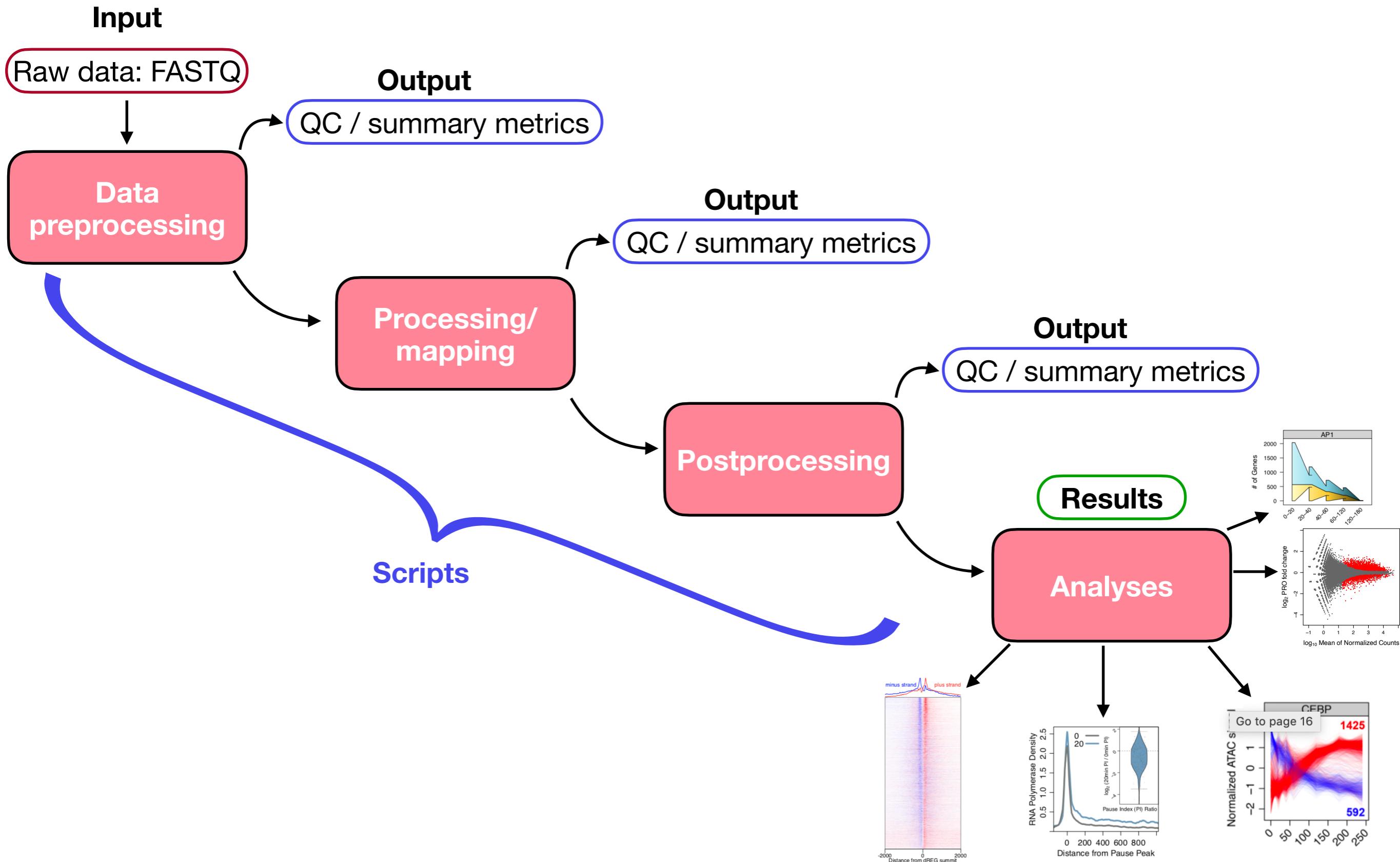


Scientists need to be able to move between the bench and bioinformatics

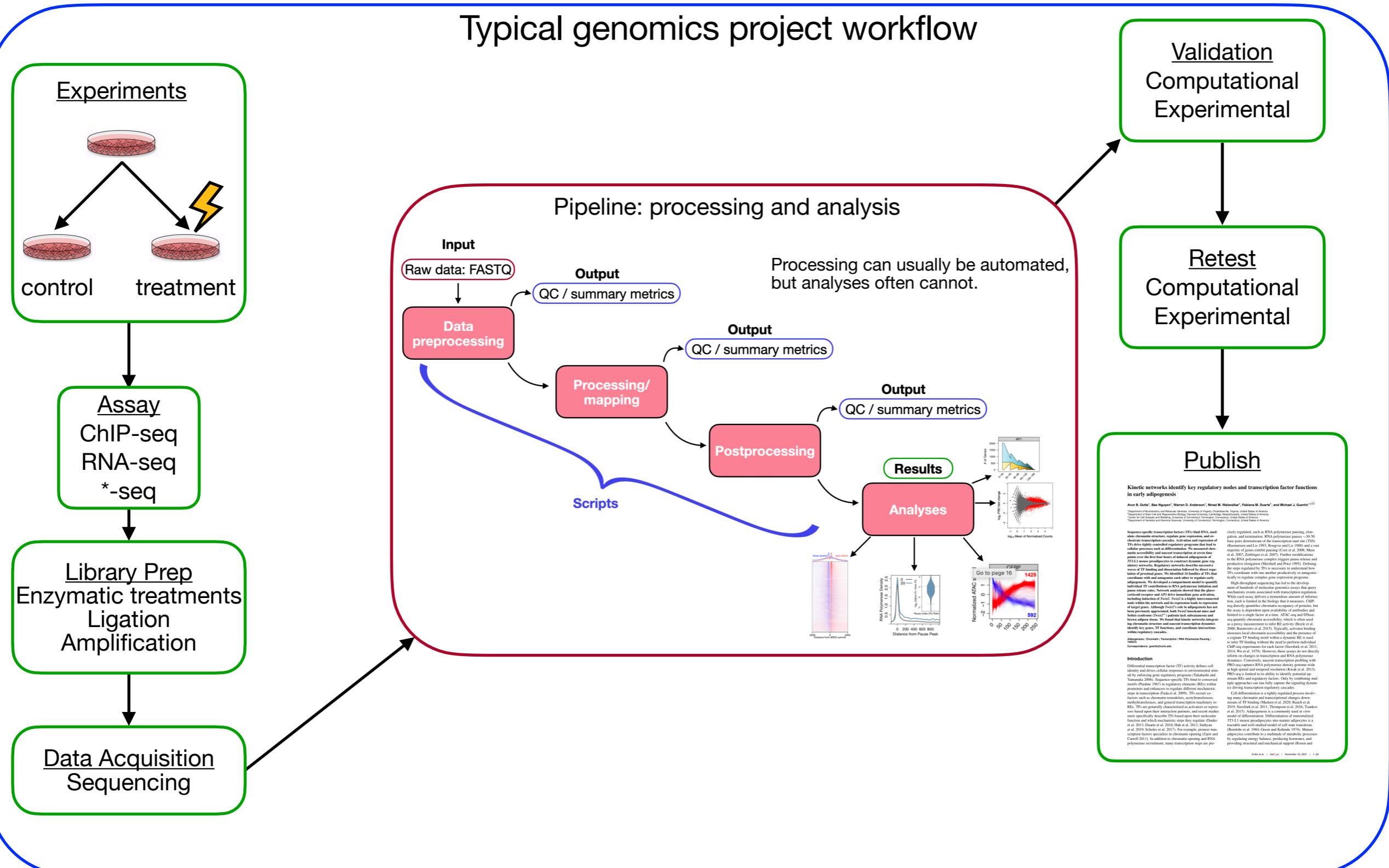
Terminology

- **Script**
 - Executable document or program listing computer interpretable commands to be executed in sequence.
- **Pipeline**
 - Often a series of independent scripts
 - Output from one script becomes input for next until desired result is achieved
 - Once defined requires limited user effort
 - Most processes that are routine enough to be automated in a pipeline are limited in the biological insights they can provide. Exploratory analyses are not usually pipelined.
- **Workflow**
 - A series of steps to be followed in sequence with varying levels of effort
 - May involve one or more pipelines
 - Can encompass entire project starting from experiments at the bench and ending with detailed analyses

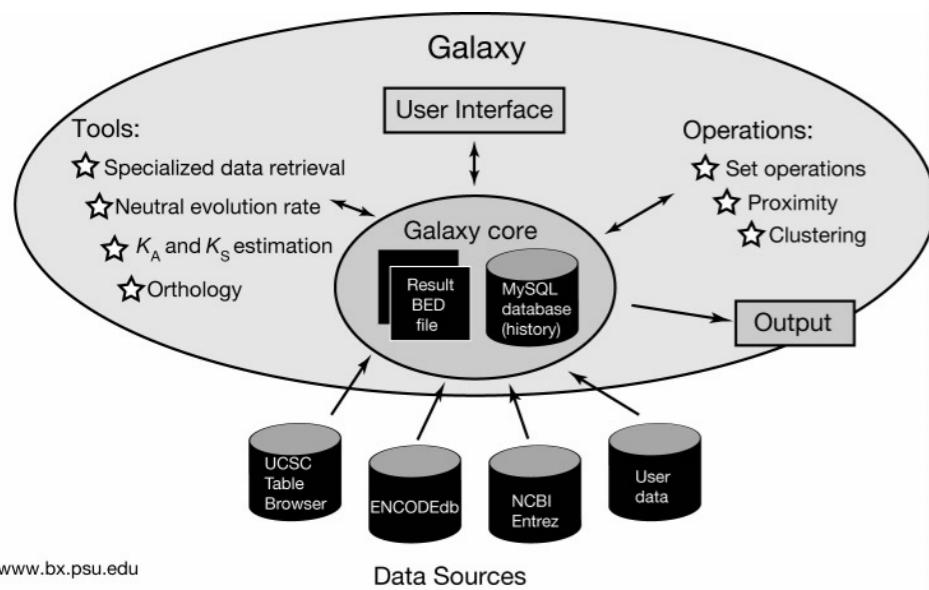
General analysis pipeline for genomics



Workflow can encompass projects and analysis pipelines

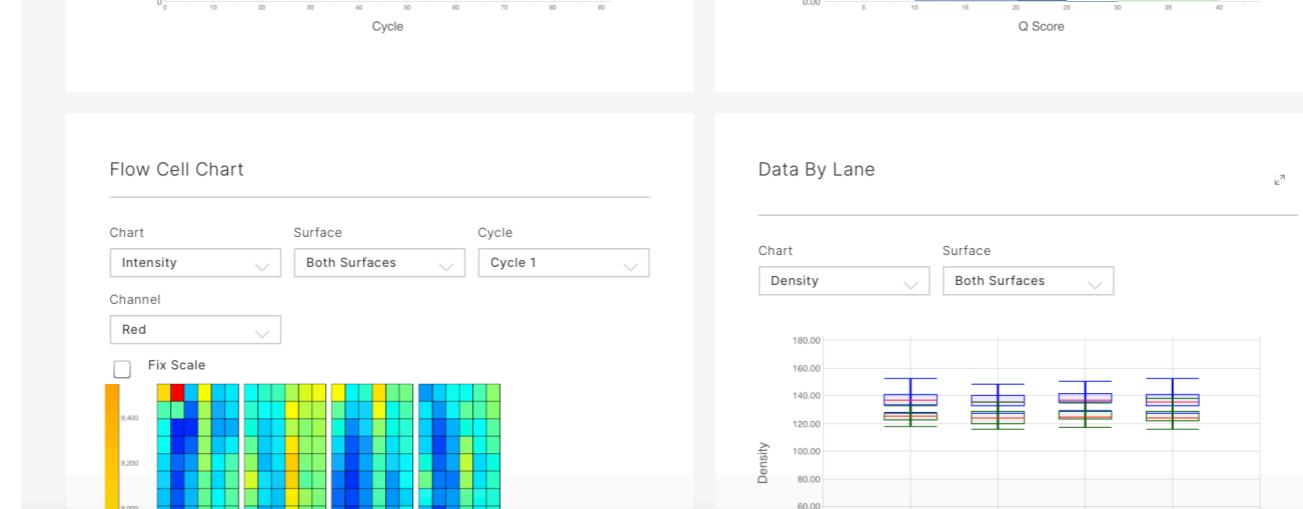
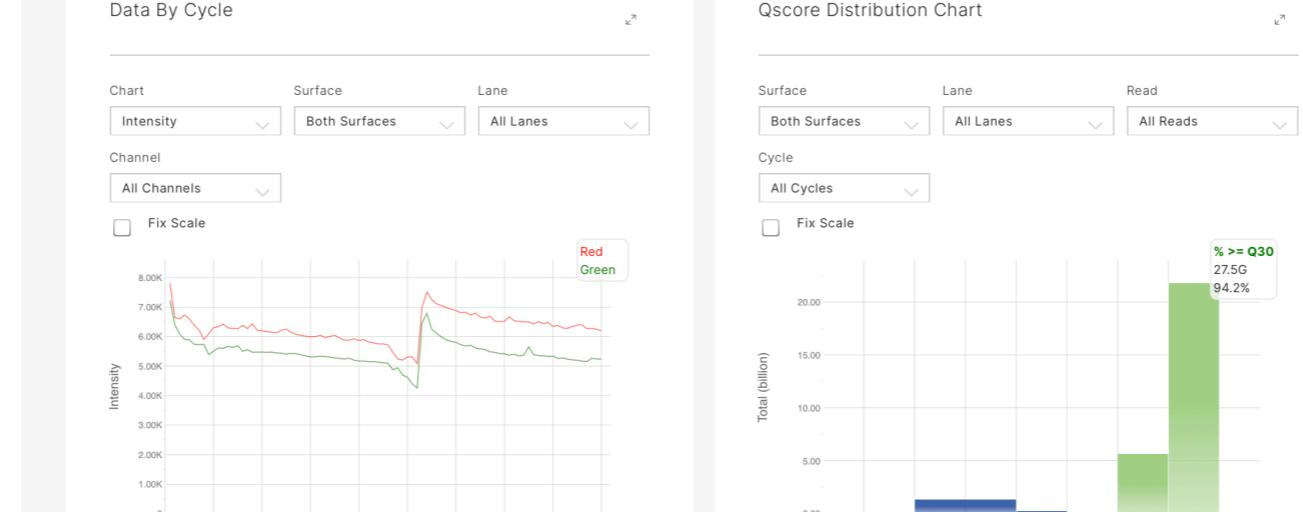
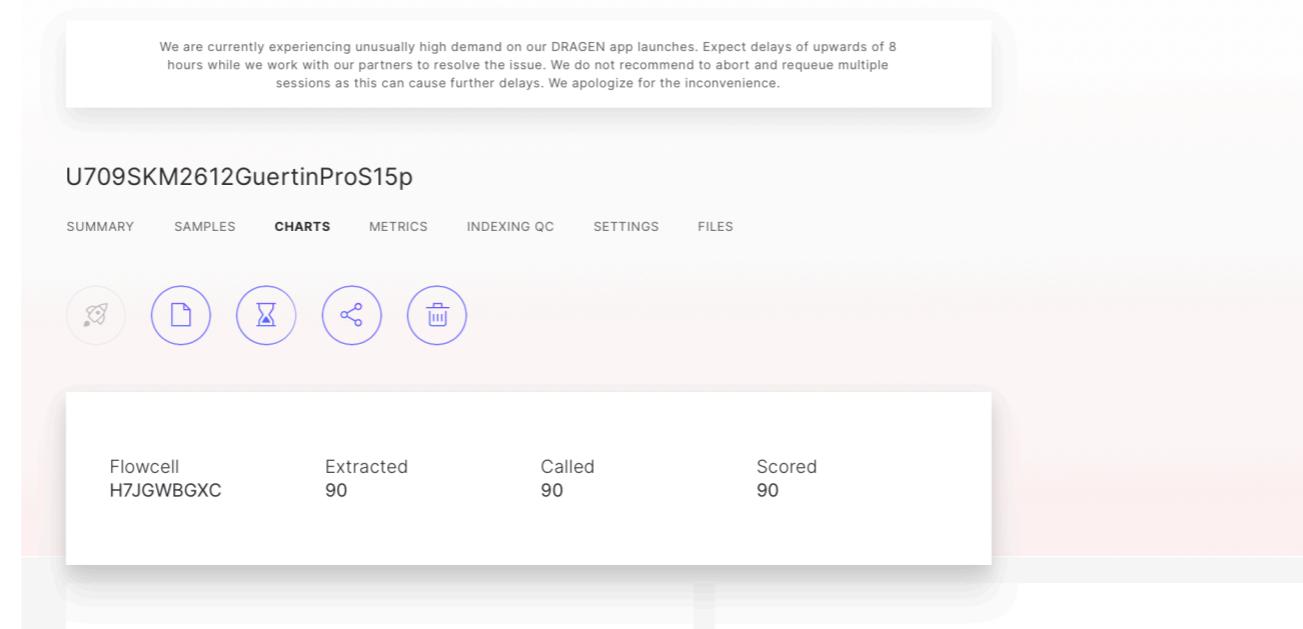


Web-based solutions for building pipelines



The screenshot shows the Galaxy Workflow Canvas interface. On the left, a sidebar lists various tools under 'Tools'. A workflow is being constructed on the canvas, starting with an 'Input dataset' step, followed by a 'Map with BWA' step, and finally a 'SAM Filter' step. The 'SAM Filter' step is currently selected, with its details shown in the 'Details' panel on the right. The 'Details' panel shows the configuration for the 'SAM Filter' tool, including the 'File to filter' input ('input1 (sam)'), the 'Optional field to filter on' dropdown set to 'Edit Distance', and the 'Value to require for flag' input set to '1'. There is also an 'Edit Step Attributes' section with an annotation field.

Web-based solutions for building pipelines



Web-based solutions for building pipelines

The screenshot shows the official website for GenomeSpace, a platform for bioinformatics tool integration. The header features the GenomeSpace logo (a cloud with DNA helixes) and navigation links: What is GenomeSpace?, Tools, Recipes, Documentation, Developers, Support, and About.

GENOMESPACE

Frictionless connection of bioinformatics tools

STATUS 11.18.19 06:02PM

With the discontinuation of NHGRI funding for GenomeSpace we have shut down the servers.

GenomeSpace Recipes can be found at <http://recipes.genomespace.org/> however data transfer through GenomeSpace will not be available.

More details can be found at <http://www.genomespace.org/news/>

Citing GenomeSpace

To cite your use of GenomeSpace, please reference Qu K, Garamszegi S, Wu F, et al. [Nature Methods](#). 2016 Jan 18. doi: 10.1038/nmeth.3732.

F1000 Research Check out our [F1000 GenomeSpace Channel](#) for published, community-contributed [recipes](#).

WHAT'S NEW

News Highlights **GenomeSpace Blog**

The GenomeSpace project is ending

The GenomeSpace project servers are shutting down on November 15, 2019 due to expiration of its NHGRI funding. We would like to thank all GenomeSpace users for their support and for all the important science they have done on the platform over the last nine years. [More >>](#)

[See All News Highlights](#)

Calendar of Upcoming Events

Tweets by @genomespace

GenomeSpace Team @genomespace
The GenomeSpace project ends *tomorrow* November 15, 2019 due to expiration of its NHGRI funding. Please save any data from your GenomeSpace account by transferring it to your own storage before that date. More details at [genomespace.org/news/the-genom...](#)

Thank you!

Nov 14, 2019

GenomeSpace Team @genomespace
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Sep 23, 2019

MEDS 5420 is a GUI-free zone



Why go GUI-free?

- Less use of system resources
- Generally better for large data
- Remote access to servers
- Easier creation of pipelines and automation
- Flexibility with diverse software
- Customization of parameters and pipelines

MEDS 5420: what will you gain?

- Learn how to access and navigate your computer via the command line for simple and moderately complex tasks.
- Learn programming strategies useful for processing, parsing, and analysis of data.
- Basic script construction and execution.
- Ability to string together commands (and / or scripts) and bioinformatics tools into processing pipelines and analysis scripts.
- Visualize data – figure making in R.
- **Google strategies and key words**
- **How to articulate questions and prompts for GPT3**
- Confidence to analyze genomic data and tackle more complex analyses

Course goals: Programming languages

Command line

January 18	Overview of Molecular Genomics and High Throughput Sequencing Technology
22	Introduction to the Command Line: directories, head, wc, etc.
25	Introduction to the Command Line: pipes, compression, and grep
29	Introduction to the Command Line: find, cut, variables, scripting, and permissions (Homework 1 assigned)
February 1	Introduction to the Command Line: awk
5	Introduction to the Command Line: Logical operators, loops, and Xanadu
8	Introduction to the Command Line: Batch scripts, interactive sessions, and software installs
12	Illumina data format, QC, and preprocessing (HW1 due)
15	Illumina preprocessing: fastx tools
19	Aligning Illumina data (Homework 2 assigned)
22	Transcription Factors and ChIP-seq lecture
26	Post-mapping processing with samtools and bedtools
29	The UCSC genome browser (Dr. Guertin may pre-record or lecture remotely)
March 4	ChIP-seq Analysis: ChIP-seq peak calling
7	ChIP-seq Analysis: Analyze ChIP-seq peaks with bedtools and awk (HW 2 due) (Midterm assigned)
18	ChIP-seq Analysis: de novo motif analysis (pre-recorded) Dr. Guertin is in Utah
21	ChIP-seq Analysis: Motif queries to genomes and databases
25	Introduction to R
28	RNA-seq lecture

R

April 1	R functions and plotting (Midterm due) (Homework 3 assigned)
4	RNA-seq Analysis: RNA-seq mapping
8	RNA-seq Analysis: visualization
11	RNA-seq Analysis: transcript assembly (recommended CCAM seminar at 4pm in Grossman Auditorium at 400 Farmington: Dr. Robin Dowell)
15	RNA-seq Analysis: Differential Expression (HW3 due) (Final Assigned)
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22	ATAC-seq Analysis
25	ATAC-seq Analysis

Course goals: Molecular Genomics assays and analysis

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ATAC-seq

ChIP-seq

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ATAC-seq

ChIP-seq

*ATAC-seq analysis is not tested in this course.
2023 ATAC Lectures were not well-attended.
2023 feedback did not address the usefulness of the ATAC lectures.

We can omit the ATAC and spend more time with other material or keep this as “optional” for the class.

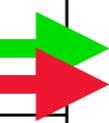
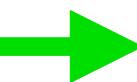
Course goals: Creating processing and analysis pipelines

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**Analysis and interpretation
processing and QC**

Course goals: important dates

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15	RNA-seq Analysis: Differential Expression (HW3 due) (Final Assigned)		
18	RNA-seq Analysis: Gene Set Enrichment analysis		
22	ATAC-seq Analysis		
25	ATAC-seq Analysis		

Dates are subject to change based on our progress. Midterm and final due dates will be officially determined when assigned.

Up to the midterm

- Command line usage
- Basic shell scripting
- Server access, usage, etiquette—Xanadu
- QC and preprocessing of Illumina data (ChIP-seq)
- Mapping (alignment to a genome)
- Additional QC and converting files
- Genome browsers
- ChIP-seq analyses:
 - Peak calling
 - Quantification of reads in genomic intervals / windows
 - Sequence motif discovery
 - Transcription factor database queries

midterm to final

- R language syntax, data types, and resources
- Plotting data (base R and lattice)
- RNA-seq
 - Experimental design
 - Preprocessing, mapping
 - Paired-end vs. single-end processing and visualization in browsers
 - Differential gene expression analysis (DESeq2)
 - Gene set enrichment analysis

Syllabus: contact and references

MEDS 5420: Molecular Genomic Practicum

Mon, Thurs. 1:00-3:00pm

400 Farmington Ave.
Room: R 1401

Instructor: Michael Guertin; guertin@uchc.edu

Office hours: by appointment

Text references:

Practical Computing for Biologists. Steven H. D. Haddock & Casey Dunn (2018).

Getting started with R: an Introduction for Biologists. Andrew P. Beckerman & Owen L. Petchey (2012)

R in Action: Data Analysis and Graphics with R. Robert I. Kabacoff (2011).

R Graphics 3rd Edition. Paul Murrell (2018)—<https://www.stat.auckland.ac.nz/~paul/RG3e/>

Although not necessary for this class, these books can be helpful. Ask your PI to purchase these books.

Syllabus: assignments and grading

Homework: Homework assignments will be announced in class. All assignments will be posted on GitHub and announced in class. Homework will be submitted via email to guertin@uchc.edu. **Assignments should be named with the NetID and assignment number (e.g. xyx15002_HW1).** Assignments are due by 5pm on the scheduled due date. Late assignments will lose 5% of total points per day, including weekends.

Course Components	Weight
In class exercises	20%
Homework	30%
Midterm project	25%
Final project	25%

Grading Scale for MED 5420:

Grade	Letter Grade	GPA
180-200	A	4.0
165-179	A-	3.7
130-164	B+	3.3
120-129	B	3.0
110-119	B-	2.7
105-109	C+	2.3
100-104	C	2.0
95-99	C-	1.7
92-94	D+	1.3
90-91	D	1.0
88-89	D-	0.7
<88	F	0.0

Server access at UConn Health

We have access to a special queue on the Xanadu server for this course. I will distribute usernames and passwords during the second week of classes. I recommend using this user account even if you have your own already. This will avoid confusion with directory tree structure and problems with access when the server gets busy. **You will need to transfer your data to your own account before the end of the semester.** To request a personal account fill out the form here: <https://bioinformatics.uconn.edu/contact-us/>

Useful links from UConn Computational Biology Core

Understanding the UConn Xanadu cluster:

<https://bioinformatics.uconn.edu/resources-and-events/tutorials-2/xanadu/>

Unix basics:

<http://bioinformatics.uconn.edu/unix-basics>

Other CBC tutorials:

<http://bioinformatics.uconn.edu/resources-and-events/tutorials/>

First task: identify / install shell terminal

1. If you're laptop is >3 years old check with me about what type and OS.
2. Mac users will use built in Unix shell called 'Terminal' located in: Applications > Utilities > Terminal.app
3. *PC user resources (posted in syllabus):

Ubuntu (Linux) is available at Microsoft Store, instruction here:

<https://tutorials.ubuntu.com/tutorial/tutorial-ubuntu-on-windows#0>

Shell terminal is also now available for Windows 10:

<https://www.laptopmag.com/articles/use-bash-shell-windows-10>

PuTTY, a SSH tool for connecting to server:

<https://www.putty.org/>

<https://mediatemple.net/community/products/dv/204404604/using-ssh-in-putty->

WinSCP, a tool for file transfer between server and user's local machine:

<https://winscp.net/eng/download.php>

Another option for PC users (<Windows 10):

Partition your hard drive and install linux on your computer:

Linux download: <http://www.ubuntu.com/download/desktop>

Instructions for partitioning:

<https://help.ubuntu.com/community/HowtoPartition>

*I have never owned a PC and haven't used a PC in 15+ years. However, I am confident that we will figure it all out! Any PC experts with command line or remote ssh experience please help out

Let's get started!