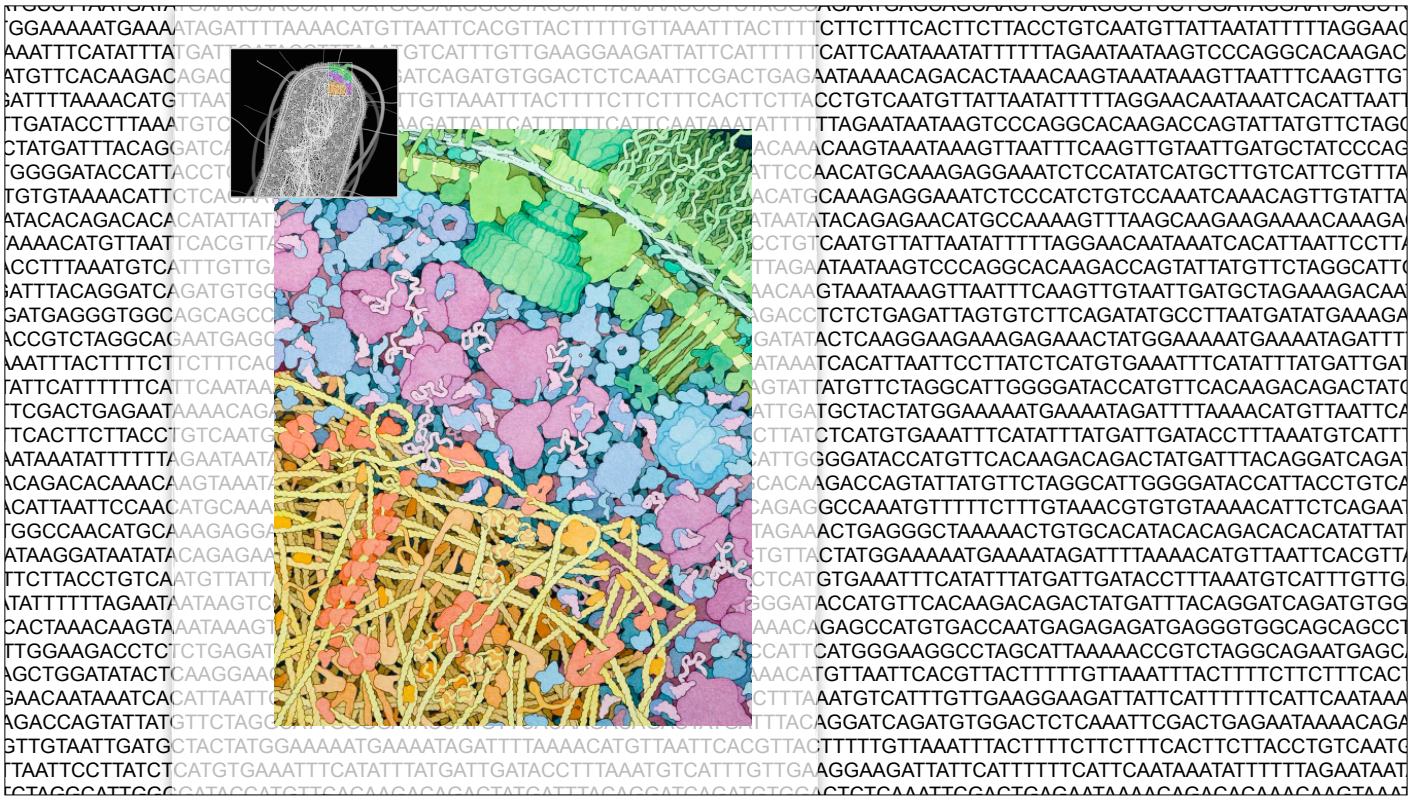
Genomics I

Biomedical Data Science: Mining and Modeling
CB&B 752 • MB&B 452
Matt Simon
Jan 23, 2023



What is genomics?

1. The **global** study of how biological **information** is encoded in genome sequence

   Genes
   Regulatory sequences
   Genetic variation

2. How this information is **read out** to produce distinct **biological outcomes**

   Gene expression and regulation
   Cellular identity, differentiation and development
   Phenotypic variation among individuals and species

In practice, many experiments that involve **deep sequencing** are considered genomics.

Overview

Genomics I: (today's lecture): Focus on sequencing technology and genomes.

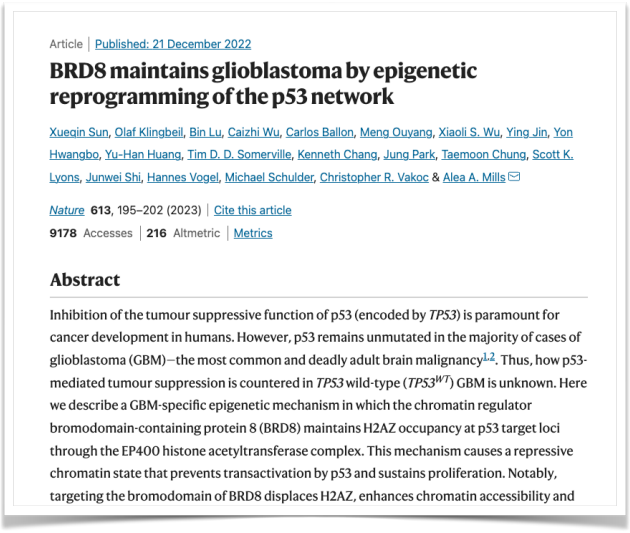
Genomics II: (Wednesday's lecture): Focus on applications of sequencing technology.

Overview

- Sequencing data: from wet lab to fastq.
- Applications to studying genomes and much much more.

✳ Sophisticated use of data from genomics requires an integrated understanding of the biological experiment, sample preparation and down stream computational analyses of the data.

Importance of genomics data: these data are central to most biomedical and biological



Article | Published: 21 December 2022

BRD8 maintains glioblastoma by epigenetic reprogramming of the p53 network

Xueqin Sun, Olaf Klingbeil, Bin Lu, Caizhi Wu, Carlos Ballon, Meng Ouyang, Xiaoli S. Wu, Ying Jin, Yon Hwangbo, Yu-Han Huang, Tim D. D. Somerville, Kenneth Chang, Jung Park, Taemoon Chung, Scott K. Lyons, Junwei Shi, Hannes Vogel, Michael Schulder, Christopher R. Vakoc & Alea A. Mills

Nature **613**, 195–202 (2023) | Cite this article

9178 Accesses | **216** Altmetric | Metrics

Abstract

Inhibition of the tumour suppressive function of p53 (encoded by *TP53*) is paramount for cancer development in humans. However, p53 remains unmutated in the majority of cases of glioblastoma (GBM)—the most common and deadly adult brain malignancy[1,2]. Thus, how p53-mediated tumour suppression is countered in *TP53* wild-type ($TP53^{WT}$) GBM is unknown. Here we describe a GBM-specific epigenetic mechanism in which the chromatin regulator bromodomain-containing protein 8 (BRD8) maintains H2AZ occupancy at p53 target loci through the EP400 histone acetyltransferase complex. This mechanism causes a repressive chromatin state that prevents transactivation by p53 and sustains proliferation. Notably, targeting the bromodomain of BRD8 displaces H2AZ, enhances chromatin accessibility and

Methods: ChIP-seq, ATAC-seq, RNA-seq

Data availability

The ChIP-seq and RNA-seq data generated in this study is available at the GEO database (https://www.ncbi.nlm.nih.gov/geo/) with accession number GSE158551. The RNA-seq data of GBM cells isolated from patient specimens are from datasets GSE84465 and GSE121720 in the GEO database.

https://www.nature.com/articles/s41586-022-05551-x

Raw data can be found in genomics databases

Experiment attributes:
GEO Accession: GSM4802676

Links:
NCBI link: NCBI Entrez (gds)

Runs: 1 run, 43.5M spots, 3.3G bases, 1.1Gb

Run	# of Spots	# of Bases	Size	Published
SRR15101033	43,549,074	3.3G	1.1Gb	2022-11-10

- Most journals require authors to submit their data to a database (e.g.,GEO) prior to publication.
- These databases entries contain raw data and processed data.
- These data can be use to examine the authors' claims, but also to test new hypotheses.

What is the output from an Illumina sequencing experiment?

One read (fastq format)

```
@HWI-ST1239:178:H0KPNADXX:2:1101:3120:1979 1:N:0:TGACCA
NCTGTAGGCTGCGTAGCCTCCCTGCAGGGTAAGTGGGAGGAGAGAGAGCAGAGGGACTTAGTGGGGCTCCCCAGGG
+
#1=DDFFFHHHHHIJIJJJIJJJJJIJJJ?FHIDGIJ=GIHGIIIHGIJIHEHIHHGFFFFEEEDDDDDDDDDDDD
```

1. Read identifier
2. **Sequence**
3. Quality score identifier "+"
4. Quality score

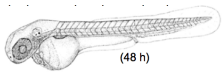
Central questions

Where do these data come from?

How does the way we collect it influence what we know?

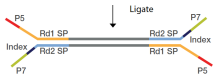
Workflow

1. Isolation of sample.



e.g., Isolate DNA and shear.

2. Library preparation



e.g., Add known sequences to the ends.

3. Sequencing

e.g., Illumina Novaseq

4. Analysis

e.g., Map to genome and interpret.

Metrics for evaluating sequencing technology

Throughput:
- Number of high quality bases per unit time
- Number of independent samples run in parallel
- Difficulty of sample preparation

Yield
- Number of useful reads per sample
- Read length

Cost
- Per run cost
- Per base cost
- Equipment
- Reagents
- Labor
- Analysis

Quality
- Accuracy per base

What is sequencing?

One-at-a-time methods

a. Maxam-Gilbert Sequencing
b. Sangar Sequencing

Short read deep sequencing

a. Illumina Sequencing
b. Ion Torrent

Long read deep sequencing

a. **Nanopore** based
b. **Pacific Bioscience** Sequencing

Sequencing technology	Platform	Data type	Read length (kb)		Read accuracy (%)	Throughput per flow cell (Gb)		Estimated cost per Gb (US$)	Maximum throughput per year (Gb)[a]
			N50	Maximum		Mean	Maximum		
Pacific Biosciences (PacBio)	RS II[b]	CLR	5–15	>60	87–92	0.75–1.5	2	333–933[c]	4,380
	Sequel	CLR	25–50	>100		5–10	20	98–195[d]	17,520
	Sequel II	CLR	30–60	>200		50–100	160	13–26[e]	93,440
		HiFi	10–20	>20	>99	15–30	35	43–86[e]	10,220
Oxford Nanopore Technologies (ONT)	MinION/GridION	Long	10–60	>1,000	87–98	2-20	30	50–500[f]	21,900 (MinION) 109,500 (GridION)
		Ultra-long	100–200	>1,500		0.5–2	2.5	500–2,000[f]	913 (MinION) 4,563 (GridION)
	PromethION	Long	10–60	>1,000		50–100	180	21–42[f]	3,153,600
Illumina	NextSeq 550	Single-end	0.075–0.15	0.15	>99.9	16–30	>30	50–63[g]	>47,782
		Paired-end	0.075–0.15 (×2)	0.15 (×2)		32–120	>120	40–60[g]	>70,080
	NovaSeq 6000	Single-end	0.05–0.25	0.25		65–3,000	>3,000	10–35[h]	>1,194,545
		Paired-end	0.05–0.25 (×2)	0.25 (×2)					

The technology will change, but your need to critically understand the input and output will not.

Logsdon (**2020**) *Nat Rev Genetics*

The steps of sequencing experiments

1. Sample preparation

   a. Isolation
   b. Library construction

2. **Sequencing**

   a. Flow cell loading
   b. Cluster generation
   c. Sequencing
   d. Processing image files
   e. De-multiplexing samples

3. Data analysis

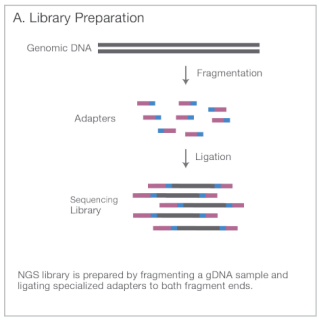
   a. Read filtering
   b. Alignment to a genome
   c. Diverse analyses

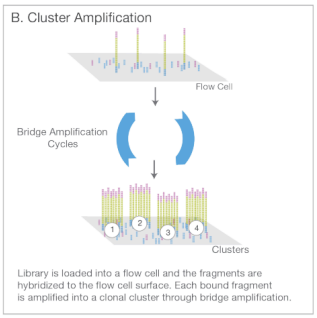
Yale Center for Genome Analysis (YCGA) ≡ MENU

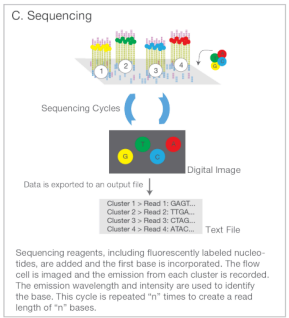
HiC Library Prep	$516	$677
cfDNA extraction	$56	$78
Whole genome methylation(NEB enzymatic)	$70	$96
RNA Library prep (poly A selection)	$77	$105
RNA library prep (smRNA)	$164	$218
RNA Library prep (ribosomal depletion)	$164	$219
RNA Library prep (low input)	$178	$236
RNA Library prep (FFPE)	$250	$331
Analysis	$508	$666
Consultation per hour	$527	$690
MiSeq 500 Cycle	$1,708	$2,227
NovaSeq S1 2x100	$3,412	$4,442
NovaSeq S1 2x150	$4,025	$5,238
NovaSeq SP 2x150	$2,526	$3,290
NovaSeq S4 2x150	$4,568	$5,944

Retrieved Jan 23, 2023:
https://medicine.yale.edu/keck/ycga/services/illuminaprices/

Where do these reads come from?

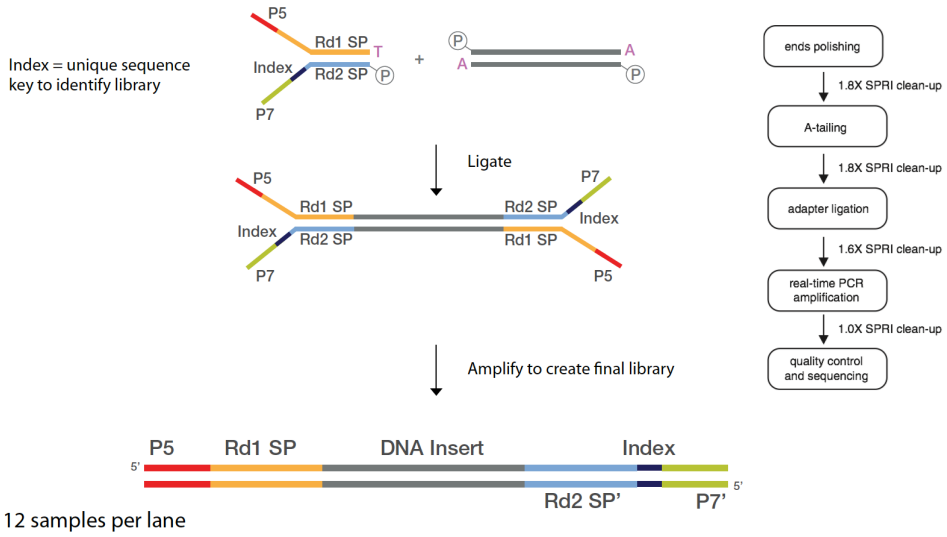






https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

Optional: Library preparation using ligation



Potential sources of bias:

1. Selective PCR amplification (issue of duplicates).
2. Size selection.
3. Enzyme specificities.

Challenging but possible to analyze pg quantities of DNA. (In humans, ~6 pg DNA/cell).

Optional: Library preparation using tagmentation

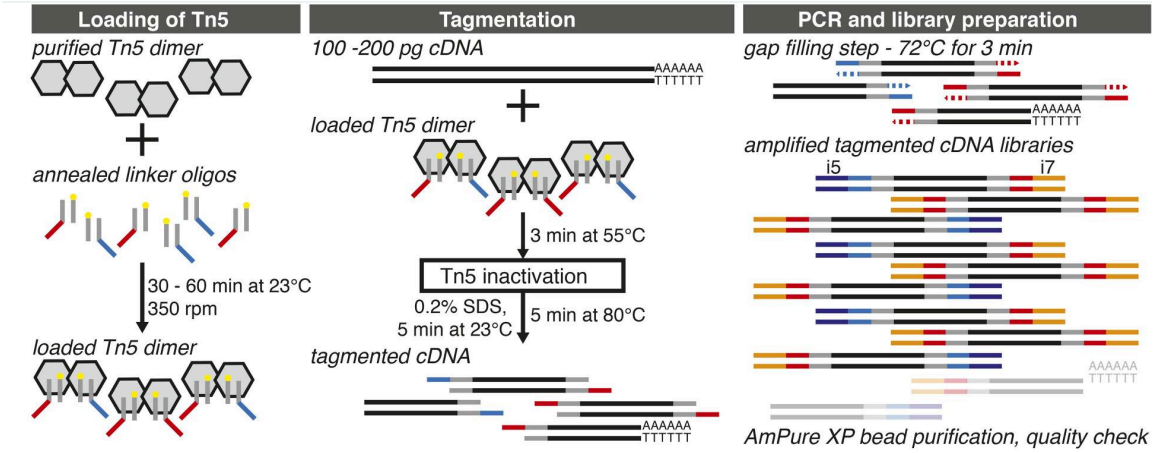
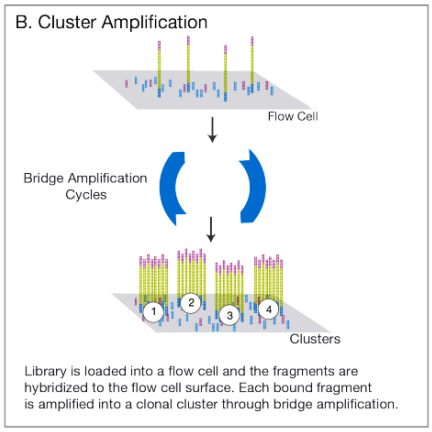
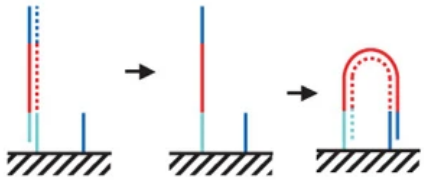


Figure from: Hennig BP, Velten L, Racke I, Tu CS, Thoms M, Rybin V, Besir H, Remans K, Steinmetz LM. G3 (Bethesda). 2018 Jan 4;8(1):79-89. PMID: 29118030.

Cluster amplification.



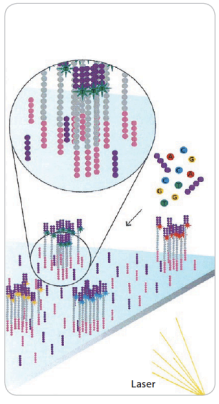


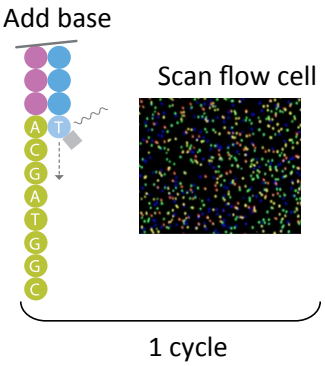
- Separate each individual molecule (randomly).
- Give each molecule an address (spatial location).
- Pack as many on as possible but avoid overlaps.

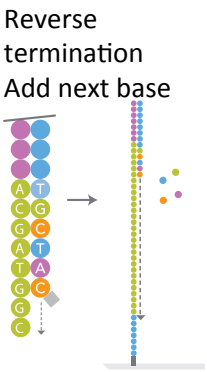
https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

Sequencing by synthesis

Sequencing by synthesis with reversible dye terminators







https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

How long are the reads?

```
TATTGCAATATGTTAACAATCTAACAAGGAAAAAATACCCCACACAAAACAAAACACAACCCTTAGAACTGTGCTG
```


75 nt

While there are other technologies that can give longer read lengths, Illumina reads are generally 50 nt - 250 nt

What limits the insert size and read length?

One read (fastq format)

```
@HWI-D00306:498:HBB89ADXX:1:1101:1180:1882 1:N:0:CGATGT
NCATCACTTTCTGCACCAGCCATGACGTCAATCTTCGTCCGAACCCCAAACTCGAGATCGGAAGAGCACACGTCTG
+
#11BBDDDFFDFBFFFIIIIIIIIIIIIIIIFEGIIIIIFIGAGIIFIII=FEEEEEFFFDDD=@9A@BBBBB=?BB<
```

- For each single end read: Incomplete incorporation of bases.
- For the size of the insert (especially for paired end analysis): Ability to get consistent clusters.

What is the output from an Illumina sequencing experiment?

Paired read (fastq format)

```
@HWI-ST1239:178:H0KPNADXX:2:1101:3120:1979 1:N:0:TGACCA
NCTGTAGGCTGCGTAGCCTCCCTGCAGGGTAAGTGGGAGGAGAGAGAGCAGAGGGACTTAGTGGGGCTCCCCAGGG
+
#1=DDFFFHHHHHIJIJJJIJJJJJIJJJ?FHIDGIJ=GIHGIIIHGIJIHEHIHHGFFFFEEEDDDDDDDDDDDD

@HWI-ST1239:178:H0KPNADXX:2:1101:3120:1979 2:N:0:TGACCA
NNACCTAGCCATCTGCAGTCCTCGGTCCTGTGTTAGACCAGAACTAGGTGCCCAGGCCAGGTACCACCTAATCCTT
+
##4<@@@@@@@@@@?@@@?@@??????@@??@?????????????????>???????????@>???@@@?@@??????
```

1. Read identifier
    a. Instrument
    b. Flow cell
    c. Read ID
    d. Coordinates
    e. Which read from a paired end sample
    f. Which index for multiplexed read
2. Sequence
3. Quality score identifier “+”
4. Quality score

What is the output from an Illumina sequencing experiment?

Many reads…

```
@HWI-D00306:498:HBB89ADXX:1:1101:1180:1882 1:N:0:CGATGT
NCATCACTTTCTGCACCAGCCATGACGTCAATCTTCGTCCGAACCCCAAACTCGAGATCGGAAGAGCACACGTCTG
+
#11BBDDDFFDFBFFFIIIIIIIIIIIIIIIFEGIIIIIFIGAGIIFIII=FEEEEEFFFDDD=@9A@BBBBB=?BB<

@HWI-D00306:498:HBB89ADXX:1:1101:1167:1902 1:N:0:CGATGT
TATTGCAATATGTTAACAATCTAACAAGGAAAAAATACCCCACACAAAACAAAACACAACCCTTAGAACTGTGCTG
+
B@@FFDFFHFHHHJJIJIGIIJJJJJIJJHFIJJJJJIJJJEHHJJIJJJJIIJJJJJJGHHHHFBDFFFE>CEEC
@HWI-D00306:498:HBB89ADXX:1:1101:1190:1928 1:N:0:CGATGT
ACCAAGCCACAATAAGTTAGTGTTTCCATAGTACATGCTGAGTTATTTGATCCCGTATCTATACACTGCTACTGTC
+
@<@DDDDD8CDDDGE?2<AFFBCCEEHEIEGHIIEGEIDD@CDGFFFEFIDGCFCDABFG>FBFGFGIEIFFFDDD
@HWI-D00306:498:HBB89ADXX:1:1101:1157:1931 1:N:0:CGATGT
CTGAGATTCTTTGCCATAGTCCTTAACCACTACGCAACTGCAACCAACCACCTTCCGTGGTTTGCCCTCTCGATCG
+
CCCFFFFFHHHHHHIJJIIJJJIIGHHIJGGJIGIJJJJJJJIJIIIJJJIIJJJIIJGJJHCHFBDFFFDDECB
```

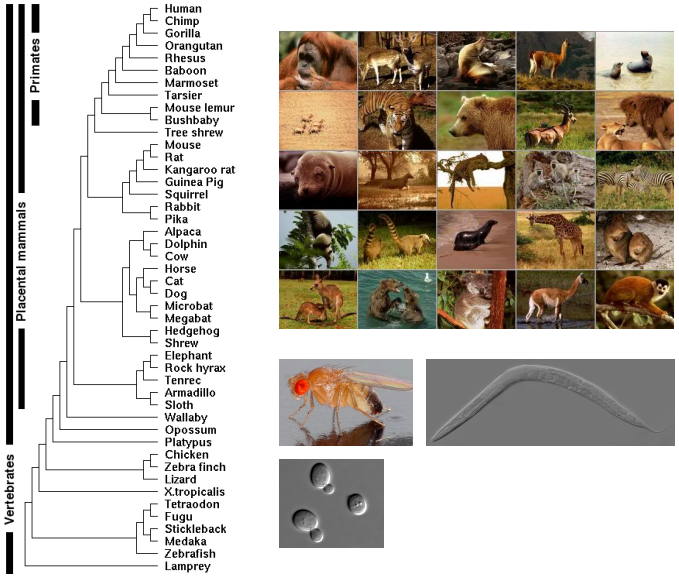
Generally ~ 2,000,000,000 reads/sequencing lane

Note: This is for an Illumina NovaSeq with current chemistry, but this number changes

What do I do with my sequencing reads?

Source: Slate via Noonan

Many reference genomes are available

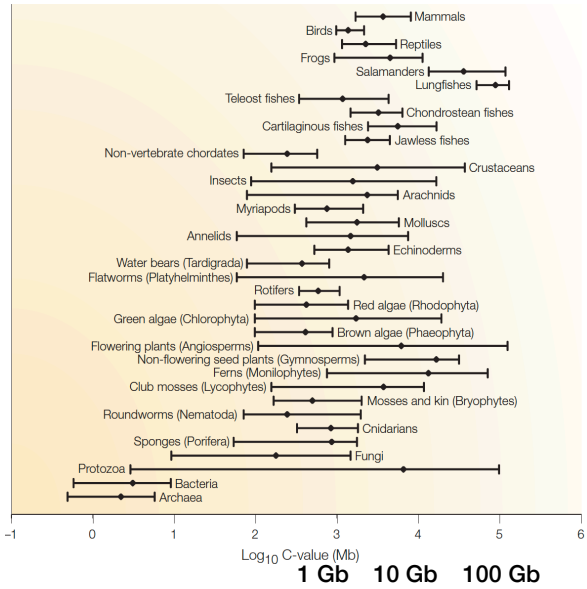


There is a wide range of genome sizes.

kb = 1000 bp
Mb = 1x10^6 bp
Gb = 1x10^9 bp
Tb = 1x10^{12} bp

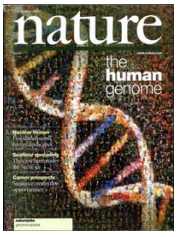
Human haploid genome ~ 3 Gb

75 nt x 3x10^8 reads/lane is about the right scale, but the amount of **coverage** necessary depends on application.



Sequencing of the human genome

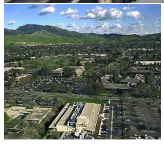
Victory declared **2003**



- Industrialization of Sanger sequencing, library construction, sample preparation, analysis, etc.
- $3 billion total cost
- 1 Gb/month at largest centers (2005)

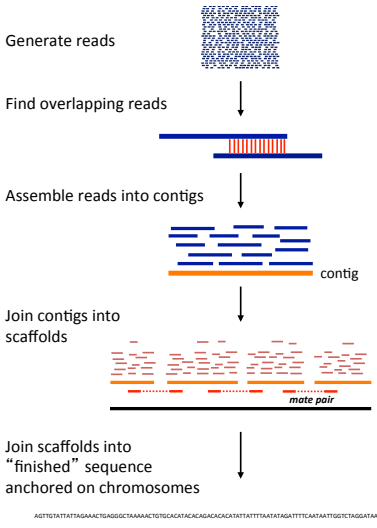
National Human Genome Research Institute

wellcome trust sanger institute



Novaseq 2 billion reads 2x150 bp. $5000 -> <$100/genome.

How to assemble a genome



Assembly quality criteria:

Accuracy: number of errors
(Human << 1/100,000 bp)

Contiguity: number of gaps
(Human: est. 357)

Coverage:
Average number of reads representing a particular position in the assembly

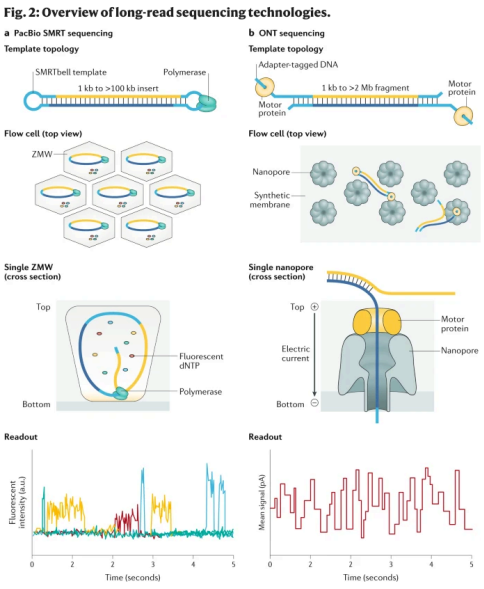
Human, Mouse, Rat: > 20x
Chimpanzee: ~6x
Squirrel: ~2x

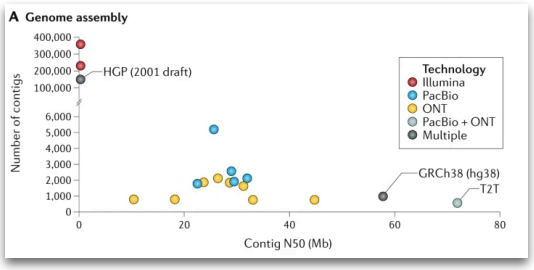
Scaffold_0: 12,865,123 – 12,965-110

AGTTGTATTATTAGAAACTGAGGGCTAAAAACTGTGCACATACACAGACACACATATTATTTTAATATAGATTTTCAATAATTGGTCTAGGATAAGGATAATATACAG

Chr5: 133,876,119 – 134,876,119

The importance of long read sequencing





Logsdon (**2020**) *Nat Rev Genetics*

The importance of long read sequencing

SPECIAL SECTION COMPLETING THE HUMAN GENOME

RESEARCH ARTICLE

HUMAN GENOMICS

The complete sequence of a human genome

Sergey Nurk[1], Sergey Koren[2], Arang Rhie[3], Mikko Rautiainen[4], Andrey V. Bzikadze[5], Alla Mikheenko[6], Mitchell R. Vollger[7], Nicolas Altemose[8], Lev Uralsky[9,10], Ariel Gershman[11], Sergey Aganezov[12], Savannah J. Hoyt[13], Mark Diekhans[14], Glennis A. Logsdon[4], Michael Alonge[15], Stylianos E. Antonarakis[16], Matthew Borchers[17], Gerard G. Bouffard[18], Shelise Y. Brooks[18], Gina V. Caldas[18], Nae-Chyun Chen[19], Haoyu Cheng[20,21], Chen-Shan Chin[22], William Chow[23], Leonardo G. de Lima[24], Philip C. Dishuck[4], Richard Durbin[25,26], Tatiana Dvorkina[6], Ian T. Fiddes[27], Giulio Formenti[27,28], Robert S. Fulton[29], Arkarachai Fungtammasan[30], Erik Garrison[31,32], Patrick G. S. Grady[33], Tina A. Graves-Lindsay[29], Ira M. Hall[34], Nancy F. Hansen[35], Gabrielle A. Hartley[36], Marina Haukness[37], Kerstin Howe[38], Michael W. Hunkapiller[39], Chirag Jain[3,40], Miten Jain[41], Erich D. Jarvis[27,28], Peter Kerpedjiev[42], Melanie Kirsche[5], Mikhail Kolmogorov[43], Jonas Korlach[39], Milinn Kremitzki[29], Heng Li[44,27], Valerie V. Maduro[45], Tobias Marschall[46], Ann M. McCartney[6], Jennifer McDaniel[47], Danny E. Miller[4,16], James C. Mullikin[48,49], Eugene W. Myers[42], Nathan D. Olson[50], Benedict Paten[51], Paul Peluso[39], Pavel A. Pevzner[52], David Porubsky[53], Tamara Potapova[54], Evgeny I. Rogaev[55,56,57], Jeffrey A. Rosenfeld[58], Steven L. Salzberg[59,60], Valerie A. Schneider[61], Fritz J. Sedlazeck[62], Kishwar Shafin[63], Colin J. Shew[64], Alaina Shumate[65], Ying Sims[38], Arian F. A. Smit[66], Daniela C. Soto[67], Ivan Sović[68,69], Jessica M. Storer[66], Aaron Streets[3,47], Beth A. Sullivan[45], Françoise Thibaud-Nissen[50], James Torrance[38], Justin Wagner[20], Brian P. Walenz[2], Aaron Wenger[39], Jonathan M. D. Wood[27], Chunlin Xiao[20], Stephanie M. Yan[70], Alice C. Young[29], Samantha Zarate[4], Urvashi Surti[30], Rojin C. McCoy[40], Megan Y. Dennis[64], Ivan A. Alexandrov[71,72], Jennifer L. Gerton[45,61], Rachel J. O'Neill[30], Winston Timp[63], Justin M. Zook[20], Michael C. Schatz[3,41], Evan E. Eichler[4,16], Karen H. Miga[35]*, Adam M. Phillippy[2]*

Since its initial release in 2000, the human reference genome has covered only the euchromatic fraction of the genome, leaving important heterochromatic regions unfinished. Addressing the remaining 8% of the genome, the Telomere-to-Telomere (T2T) Consortium presents a complete 3.055 billion–base pair sequence of a human genome, T2T-CHM13, that includes gapless assemblies for all chromosomes except Y, corrects errors in the prior references, and introduces nearly 200 million base pairs of sequence containing 1956 gene predictions, 99 of which are predicted to be protein coding. The completed regions include all centromeric satellite arrays, recent segmental duplications, and the short arms of all five acrocentric chromosomes, unlocking these complex regions of the genome to variational and functional studies.

The current human reference genome was released by the Genome Reference Consortium (GRC) in 2013 and most recently patched in 2019 (GRCh38.p13) (*1*). This reference traces its origin to the publicly funded Human Genome Project (*2*) and has been continually improved over the past two decades. Unlike the competing Celera effort (*3*) and most modern sequencing projects based on "shotgun" sequence assembly (*4*), the GRC assembly was constructed from sequenced bacterial artificial chromosomes (BACs) that were ordered and oriented along the human genome by means of radiation hybrid, genetic linkage, and fingerprint maps. However, limitations of BAC cloning led to an underrepresentation of repetitive sequences, and the opportunistic assembly of BACs derived from multiple individuals resulted in a mosaic of haplotypes. As a result, several GRC assembly gaps are unsolvable because of incompatible structural polymorphisms on their flanks, and many other repetitive and polymorphic regions were left unfinished or incorrectly assembled (*5*).

The GRCh38 reference assembly contains 151 mega–base pairs (Mbp) of unknown sequence distributed through the present genome, including pericentromeric and subtelomeric regions, recent segmental duplications, ampliconic gene arrays, and ribosomal DNA (rDNA) arrays, all of which are necessary for fundamental cellular processes (Fig. 1A). Some of the largest reference gaps include human satellite (HSat) repeat arrays and the short arms of all five acrocentric chromosomes, which are represented in GRCh38 as multimegabase stretches of unknown bases (Fig. 1, B and C). In addition to these apparent gaps, other regions of GRCh38 are artificial or are otherwise incorrect. For example, the centromeric alpha satellite arrays are represented as computationally generated models of alpha satellite monomers to serve as decoys for resequencing analyses (*6*), and sequence assigned to the short arm of chromosome 21 appears falsely duplicated and poorly assembled (*7*). When compared with other human genomes, GRCh38 also shows a genome-wide deletion bias that is indicative of incomplete assembly (*8*). Despite finishing efforts from both the Human Genome Project (*9*) and GRCt2 that improved the quality of the reference, there was limited

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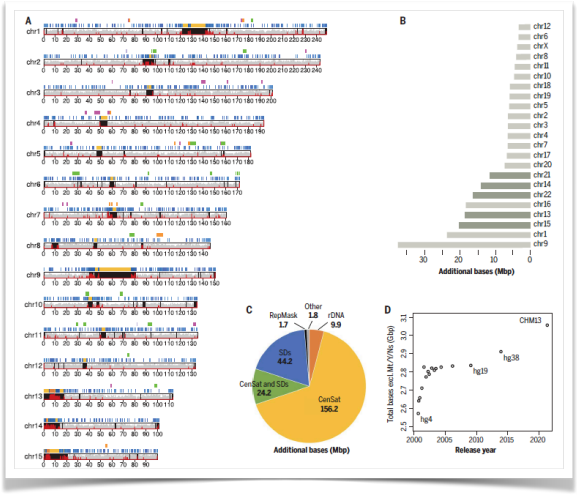

https://www.science.org/doi/10.1126/science.abj6987

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What types of annotation do we have/want?

~3 billion bp

Genes:
- Coding, noncoding, miRNA, etc.
- Isoforms
- Expression

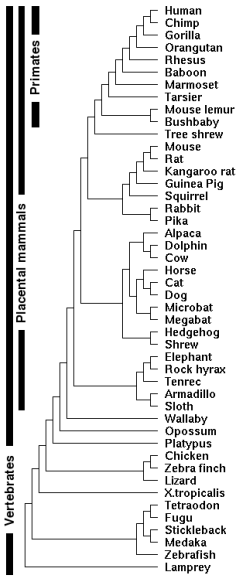
Genetic variation:
- SNPs and CNVs

Sequence conservation

Regulatory sequences:
- Promoters
- Enhancers
- Insulators

Epigenetics:
- DNA methylation
- Chromatin

Degrees of genomic annotation vary widely



ENCODE and modENCODE

Human, Mouse (Fly, Worm, Yeast):
- Chromosome assemblies
- Dense gene and regulatory maps, variation, etc.

Other models (Dog, Chicken, Zebrafish):
- Chromosome assemblies
- Partial gene maps; variation; little regulatory data

Low coverage vertebrate genomes:
- Scaffold assemblies
- Few annotated genes
- Used for comparative purposes

Where do you look for existing annotations?

UCSC Genome Browser (genome.ucsc.edu):
Visualization, data recovery, simple analysis
(also http://genome-preview.ucsc.edu/)

ENSEMBL (ensembl.org):
Visualization, data recovery, simple analysis

Integrative Genomics Viewer
(broadinstitute.orgsoftware/igv/):
Local genome viewer (visualize local and remote data)

Galaxy (main.g2.bx.psu.edu):
Complex data analysis and workflows

Example of a genome browser track (UCSC)

Chr5: 133,876,119 – 134,876,119

Our specific example:

```
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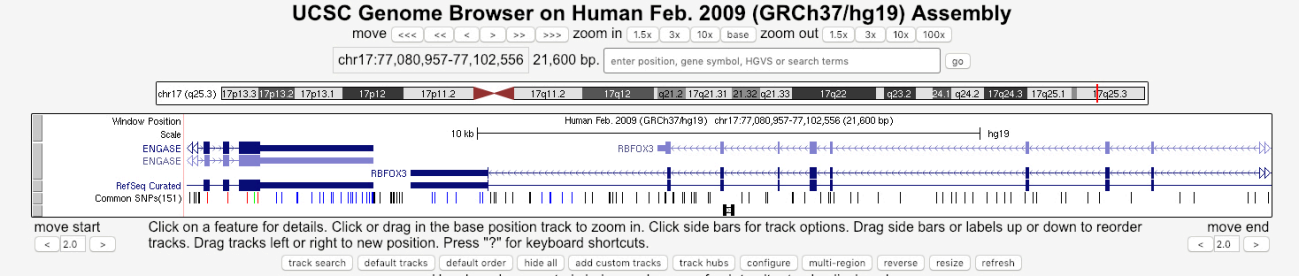
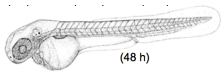


Workflow

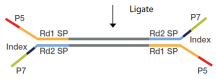
1. Isolation of sample.

e.g., Isolate DNA and shear.



2. Library preparation

e.g., Add known sequences to the ends.



3. Sequencing

e.g., Illumina Novaseq

4. Analysis

e.g., Map to genome and interpret.

Using sequencing to annotate the genome

1. Where are the cis-acting regulatory elements in DNA?
   A. DNase I hyper-sensitivity mapping (DNase-Seq).
   B. FAIRE to map regulatory elements.
2. Where do transcription factors bind?
   C. ChIP-seq of transcription factors (or in high res, ChIP-exo)
   D. Nucleosome mapping (MNase-Seq).
3. Where are different histone modifications found?
   E. ChIP-Seq of histone modifications.
   F. ChIP-Seq of chromatin writers, readers and erasers.
4. Where is RNA polymerase transcribing?
   G. ChIP-Seq of polymerase.
   H. GRO-Seq, NET-Seq and TT-Seq to measure RNA in the polymerase active site..
5. How is the genome organized in 3D?
   I. 4C/5C/Hi-C to measure chromatin conformation.

Applications of sequencing technology next class.

Conclusions

- Sequencing technology is central to our understanding of biology.
- The decrease in cost and increase in throughput make sequencing data increasingly ubiquitous.

✳ Sophisticated use of data from genomics requires an integrated understanding of the biological experiment, sample preparation and down stream computational analyses of the data.