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Genome Informatics

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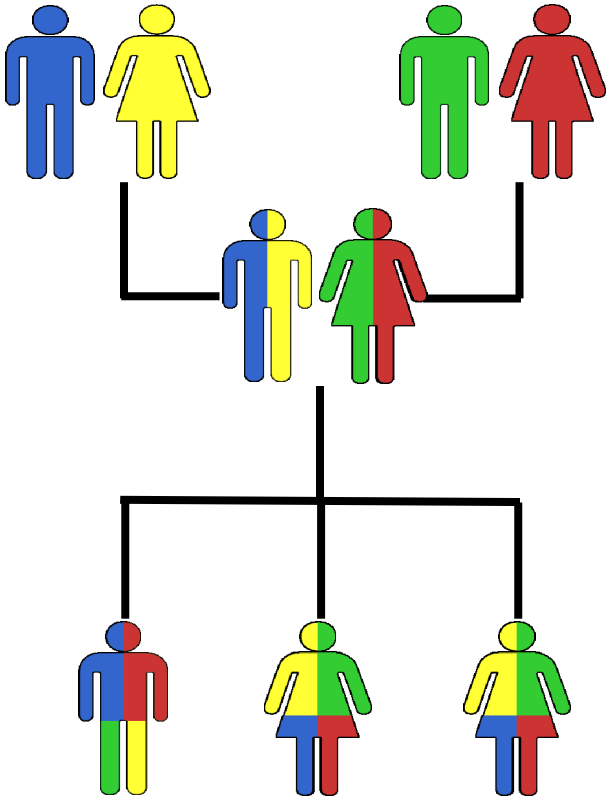
Genetics and Genomics

- Genetics is primarily the study of individual genes, mutations within those genes, and their inheritance patterns in order to understand specific traits.

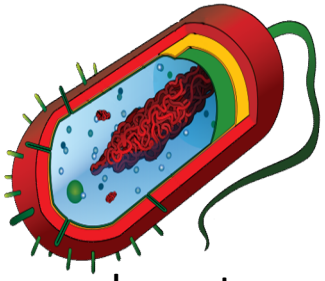
- Genomics expands upon classical genetics and considers aspects of the entire genome, typically using computer aided approaches.

What is a Genome?

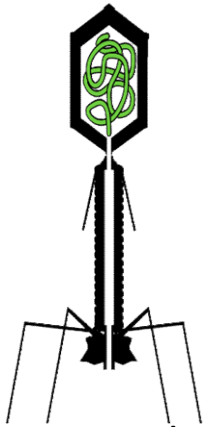
The total genetic material of an organism by which individual traits are encoded, controlled, and ultimately passed on to future generations



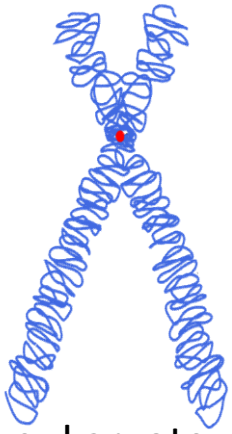
Genomes come in many shapes



prokaryote



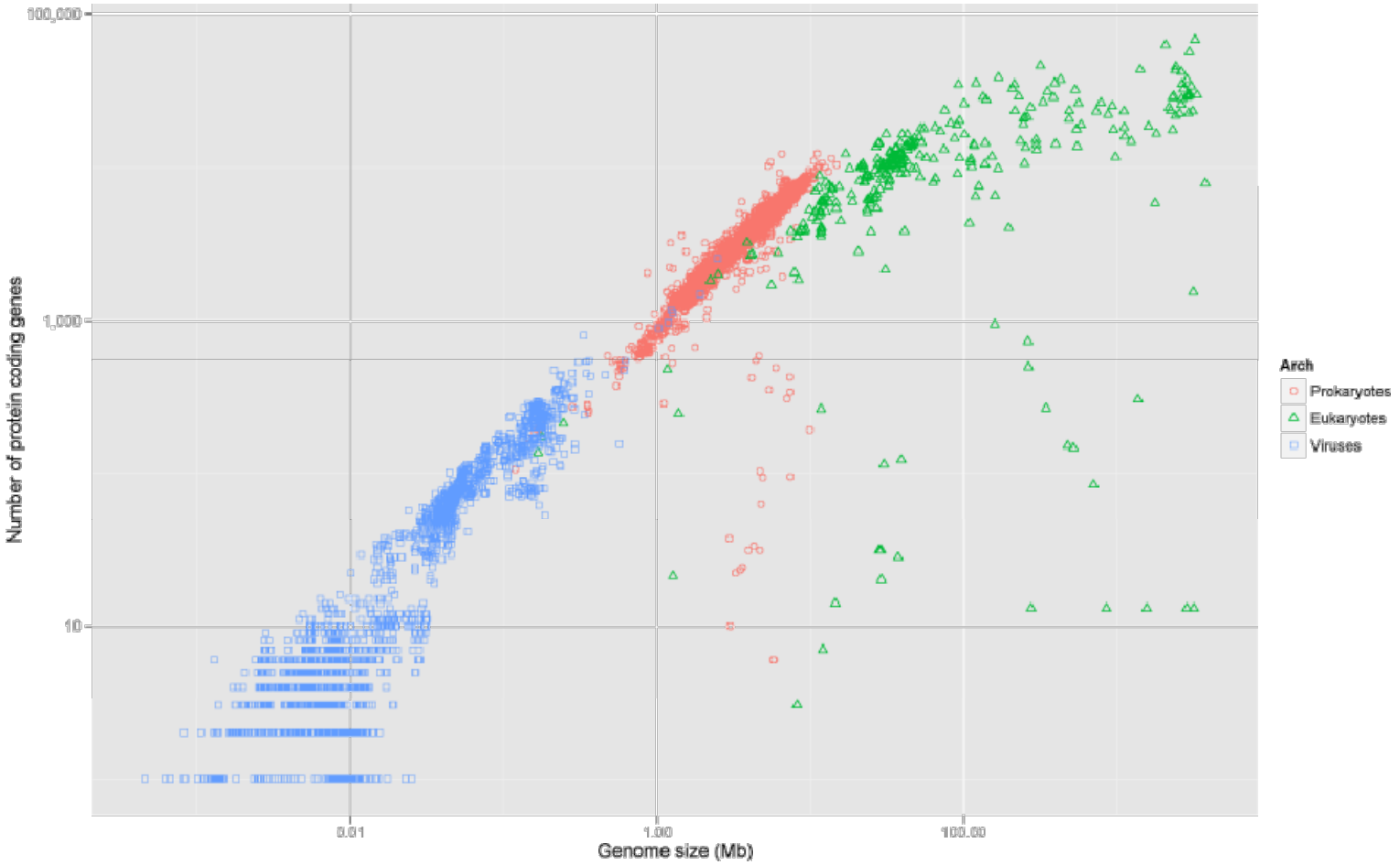
bacteriophage



eukaryote

- Primarily DNA, but can be RNA in the case of some viruses
- Some genomes are circular, others linear
- Can be organized into discrete units (chromosomes) or freestanding molecules (plasmids)

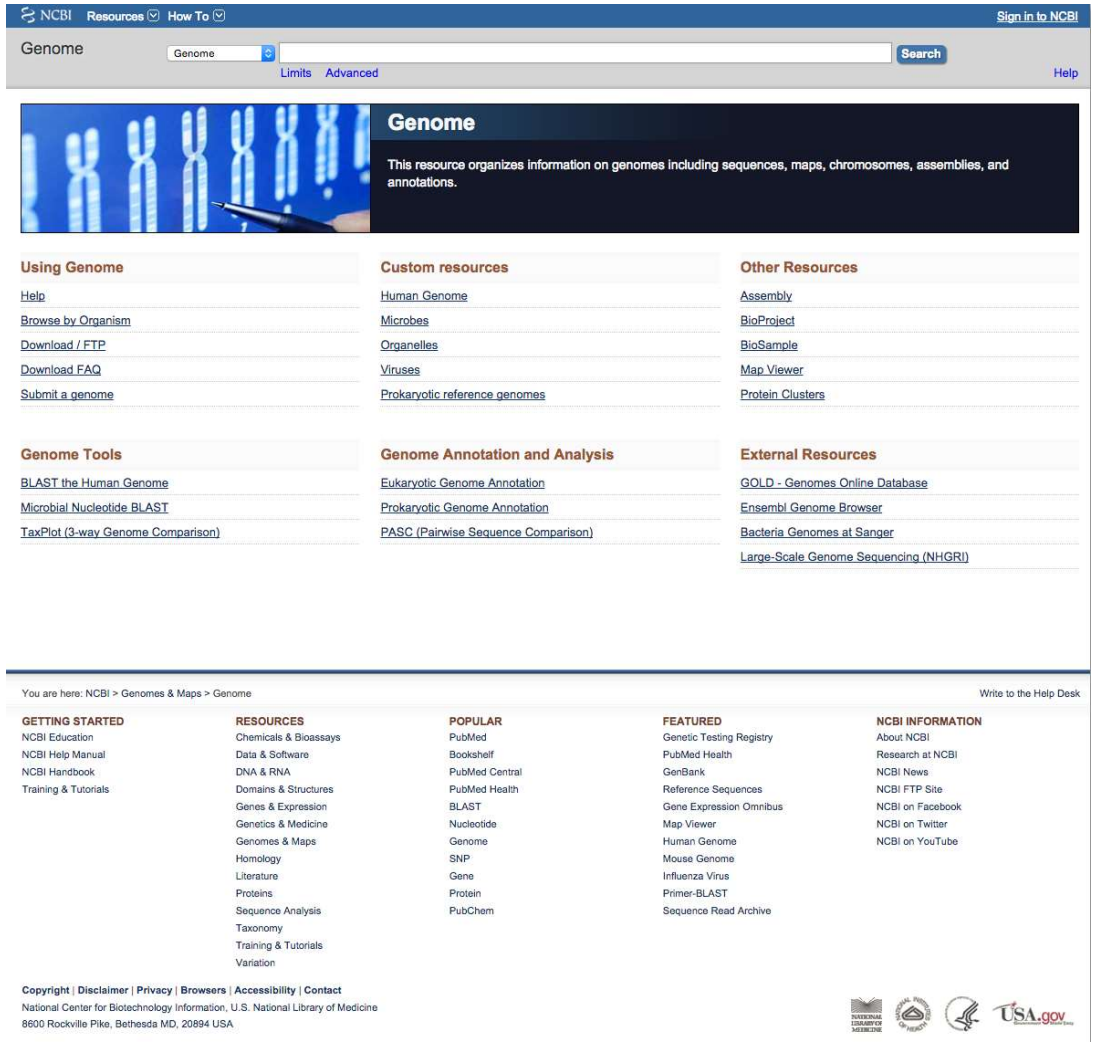
Genomes come in many sizes



Genome Databases

NCBI Genome:

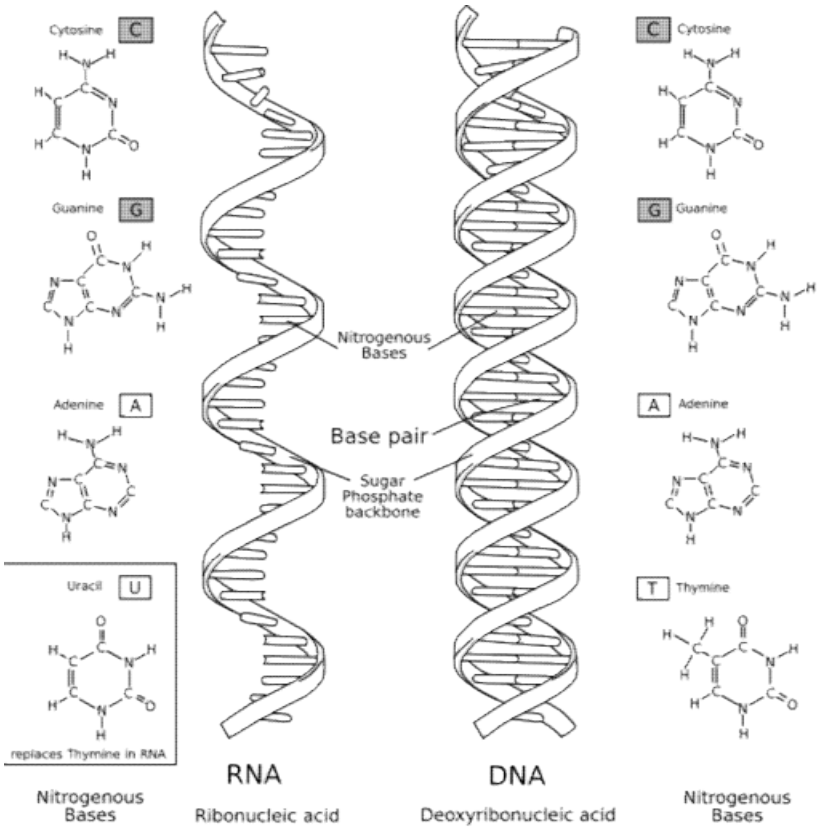
http://www.ncbi.nlm.nih.gov/genome



Characteristics of Genomes

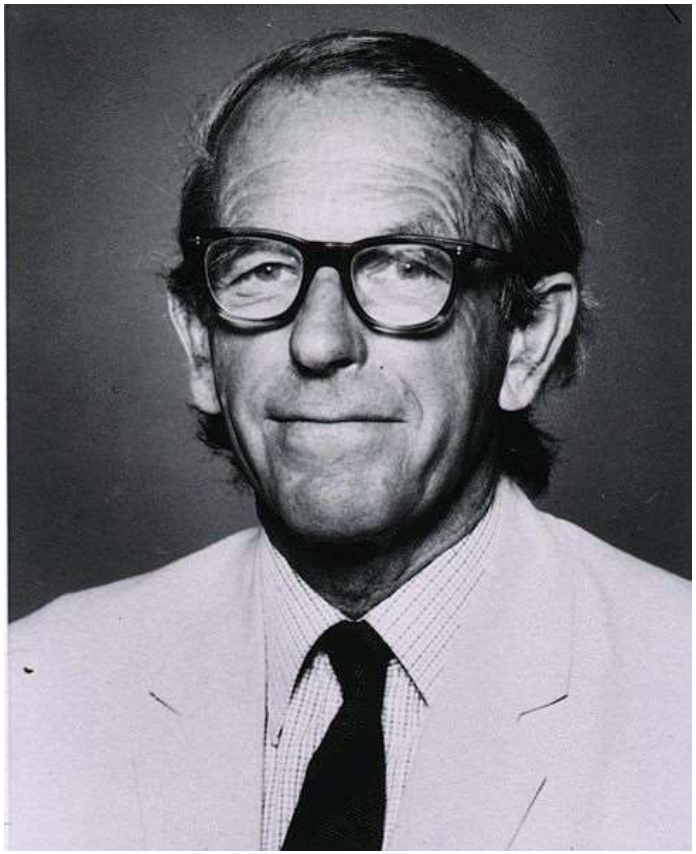
- All genomes are made up of nucleic acids
  - DNA and RNA: Adenine (A), Cytosine (C), Guanine (G)
  - DNA Only: Thymine (T)
  - RNA Only: Uracil (U)
- Typically (but not always), DNA genomes are double stranded (double helix) while RNA genomes are single stranded
- Genomes are described as long sequences of nucleic acids, for example:

  GGACTTCAGGCAACTGCAACTACCTTAGGA



Darryl Leja, Courtesy: National Human Genome Research Institute.

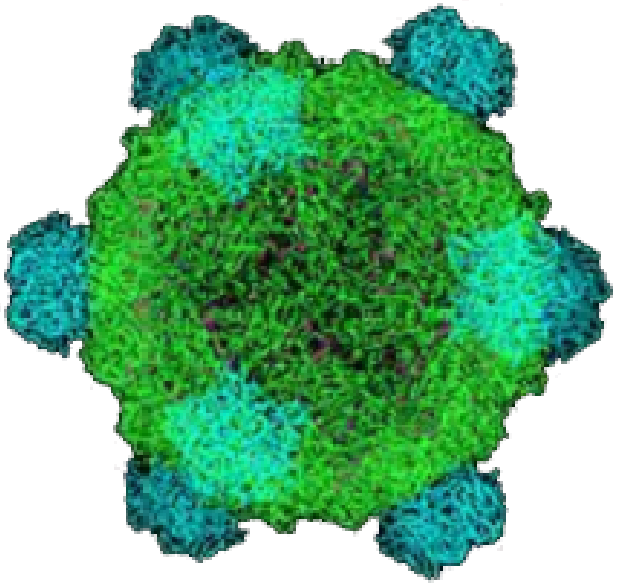
Early Genome Sequencing



- Chain-termination "Sanger" sequencing was developed in 1977 by Frederick Sanger, colloquially referred to as the "Father of Genomics"
- Sequence reads were typically 750-1000 base pairs in length with an error rate of ~1 / 10000 bases

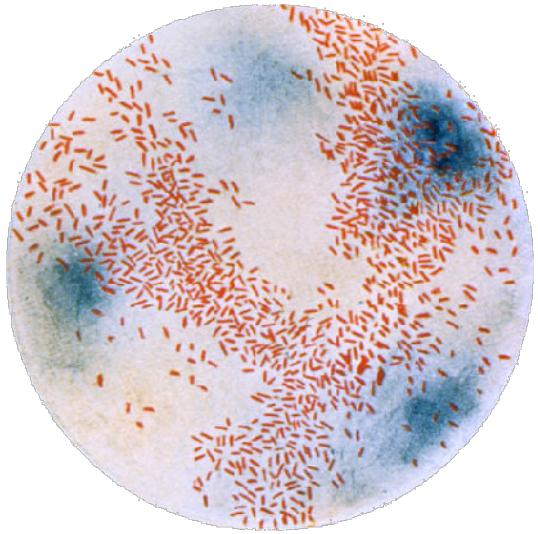
http://en.wikipedia.org/wiki/Frederick_Sanger

The First Sequenced Genomes



Bacteriophage ϕ-X174

- Completed in 1977
- 5,386 base pairs, ssDNA
- 11 genes



Haemophilus influenzae

- Completed in 1995
- 1,830,140 base pairs, dsDNA
- 1740 genes

http://en.wikipedia.org/wiki/Phi_X_174

http://phil.cdc.gov/

The Human Genome Project

- The Human Genome Project (HGP) was an international, public consortium that began in 1990
  - Initiated by James Watson
  - Primarily led by Francis Collins
  - Eventual Cost: $2.7 Billion
- Celera Genomics was a private corporation that started in 1998
  - Headed by Craig Venter
  - Eventual Cost: $300 Million
- Both initiatives released initial drafts of the human genome in 2001
  - ~3.2 Billion base pairs, dsDNA
  - 22 autosomes, 2 sex chromosomes
  - ~20,000 genes

Jane Ades, Courtesy: National Human Genome Research Institute.

What can we do with a Genome?

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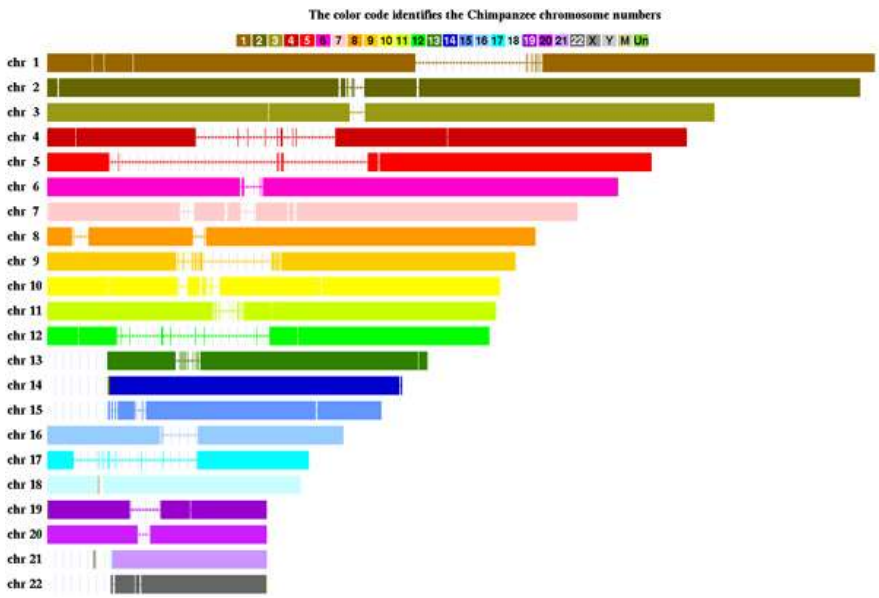
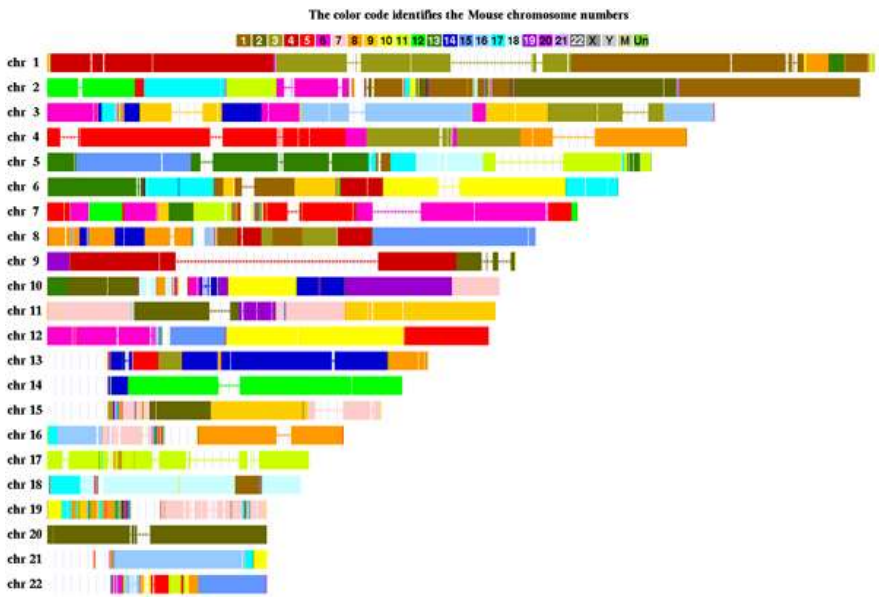
- We can *compare* genomes, both within and between species, to identify regions of variation and of conservation
- We can *model* genomes, to find interesting patterns reflecting functional characteristics
- We can *edit* genomes, to add, remove, or modify genes and other regions for adjusting individual traits

Comparative Genomics

~6-7 million years



~60-70 million years



http://cbse.soe.ucsc.edu/research/comp_genomics/human_chimp_mouse

Conservation Suggests Function

- Functional regions of the genome tend to mutate slower than nonfunctional regions due to selective pressures
- Comparing genomes can therefore indicate segments of high similarity that have remained conserved across species as candidate genes or regulatory regions

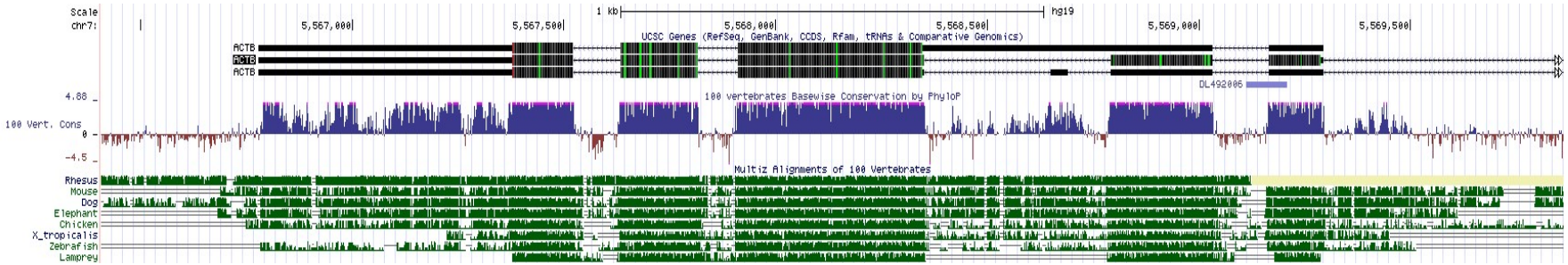


figure generated from: http://genome.ucsc.edu/

Conservation Indicates Loss

- Comparing genomes allows us to also see what we have lost over evolutionary time
- A model example of this is the loss of “penile spines” in the human lineage due to a human-specific deletion of an enhancer for the androgen receptor gene (McLean et al, Nature, 2011)

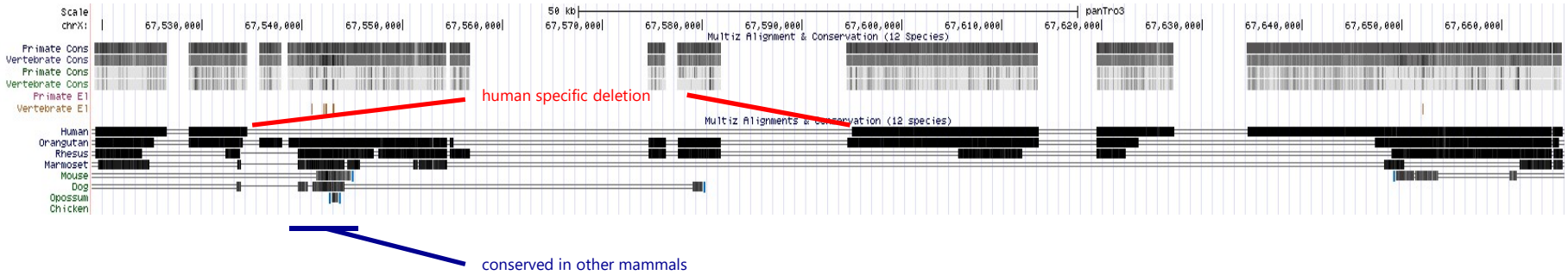


figure generated from: http://genome.ucsc.edu/

Gene Modeling and Prediction

Genomic features such as codon usage patterns can be modeled to identify novel genic regions

Genic Regions:

```
ATGACGGTCTGACTGACCATTCAT…
ATGCGGTATGCCTGGAAGCCAGCC…
ATGCTATGGCAAGCCATTGGAGAG…
ATGCCGTTGTTATATGAGCAGATA…
```

```
P(AAA) = 0.001
P(AAC) = 0.020
P(AAG) = 0.025
P(AAT) = 0.003
.
.
P(TTT) = 0.022
```

Intergenic Regions:

```
TTAGATTAAAGCCAGGACTGAACG…
TAATGAATGGAACCATACAGAACG…
GACTTAGCCCGAATTTATAGATAC…
TAACTGAATCGATTTAGCCAGGAA…
```

```
P(AAA) = 0.020
P(AAC) = 0.001
P(AAG) = 0.003
P(AAT) = 0.040
.
.
P(TTT) = 0.030
```

Gene Prediction Software

GeneMark:

http://exon.gatech.edu/GeneMark/

GeneMark

A family of gene prediction programs developed at
Georgia Institute of Technology , Atlanta, Georgia, USA.

What's New: A new algorithm, **BRAKER1**, an RNA-seq based eukaryotic genome annotation pipeline -- using GeneMark-ET and AUGUSTUS

Supported by NIH

Gene Prediction in Bacteria, Archaea, Metagenomes and Metatranscriptomes

Novel genomic sequences can be analyzed either by the self-training program **GeneMarkS** (sequences longer than 50 kb) or by **GeneMark.hmm with Heuristic models**. For many species pre-trained model parameters are ready and available through the **GeneMark.hmm** page. Metagenomic sequences can be analyzed by **MetaGeneMark** , the program optimized for speed.

Gene Prediction in Eukaryotes

Novel genomes can be analyzed by the program **GeneMark-ES** utilizing unsupervised training. Note that GeneMark-ES has a special mode for analyzing fungal genomes. Recently, we have developed a semi-supervised version of GeneMark-ES, called GeneMark-ET that uses RNA-Seq reads to improve training. For several species pre-trained model parameters are ready and available through the **GeneMark.hmm** page.

Gene Prediction in Transcripts

Sets of assembled eukaryotic transcripts can be analyzed by the modified **GeneMarkS** algorithm (the set should be large enough to permit self-training). A single transcript can be analyzed by a special version of **GeneMark.hmm with Heuristic models**. A new advanced algorithm GeneMarkS-T was developed recently (manuscript sent to publisher); The GeneMarkS-T software (beta version) is available for download .

Gene Prediction in Viruses, Phages and Plasmids

Sequences of viruses, phages or plasmids can be analyzed either by the **GeneMark.hmm with Heuristic models** (if the sequence is shorter than 50 kb) or by the self-training program **GeneMarkS**.

All the software programs mentioned here are available for download and local installation.

The software of GeneMark line is a part of genome annotation pipelines at NCBI, JGI, Broad Institute as well as the following software packages:

- QUAST : quality assessment tool for genome assemblies
  -- using GeneMarkS
- MetAMOS : a modular and open source metagenomic assembly and analysis
  -- using MetaGeneMark
- MAKER2 : a eukaryotic genome annotation pipeline
  -- using GeneMark-ES (along with SNAP and AUGUSTUS)
- BRAKER1 : an RNA-seq based eukaryotic genome annotation pipeline
  -- using GeneMark-ET and AUGUSTUS

For more information see Background and Publications.

Borodovsky Group
Group news

Gene Prediction Programs

- GeneMark
- GeneMark.hmm
- GeneMarkS
- Heuristic models
- MetaGeneMark
- Mirror site at NCBI
- GeneMarkS+
- BRAKER1

Information

- Publications
- Selected Citations
- Background
- FAQ
- Contact

Downloads

- Programs
- Prebuild species models

Other Programs

- UnSplicer
- GeneTack
- Frame-by-Frame
- IPSSP

In silico Biology International Conferences

- 2013
- 2011
- 2009
- 2007
- 2005
- 2003
- 2001
- 1999
- 1997

Bioinformatics Studies at Georgia Tech

- MS Program
- PhD Program
- Center for Bioinformatics and Computational Biology
- Lectures

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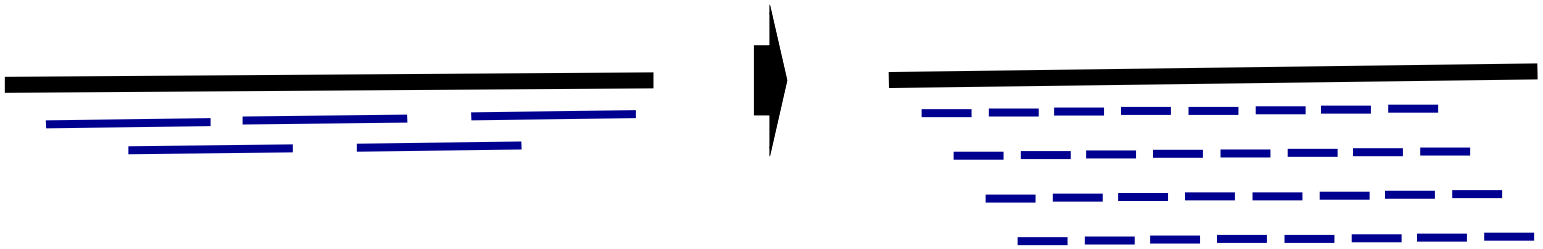
Modern Genome Sequencing

- Next Generation Sequencing (NGS) technologies have resulted in a paradigm shift from long reads at low coverage to short reads at high coverage
- This provides numerous opportunities for new and expanded genomic applications



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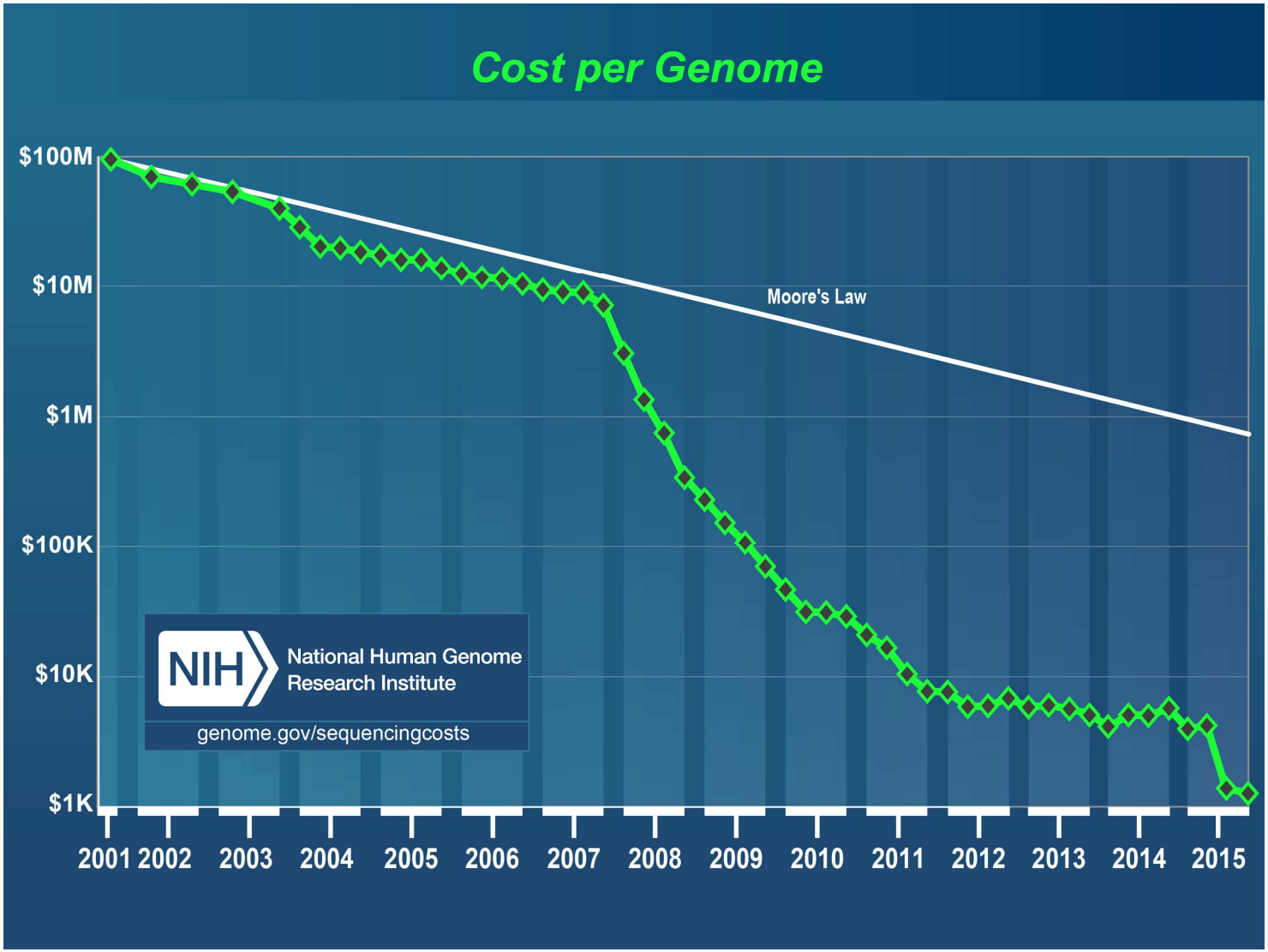

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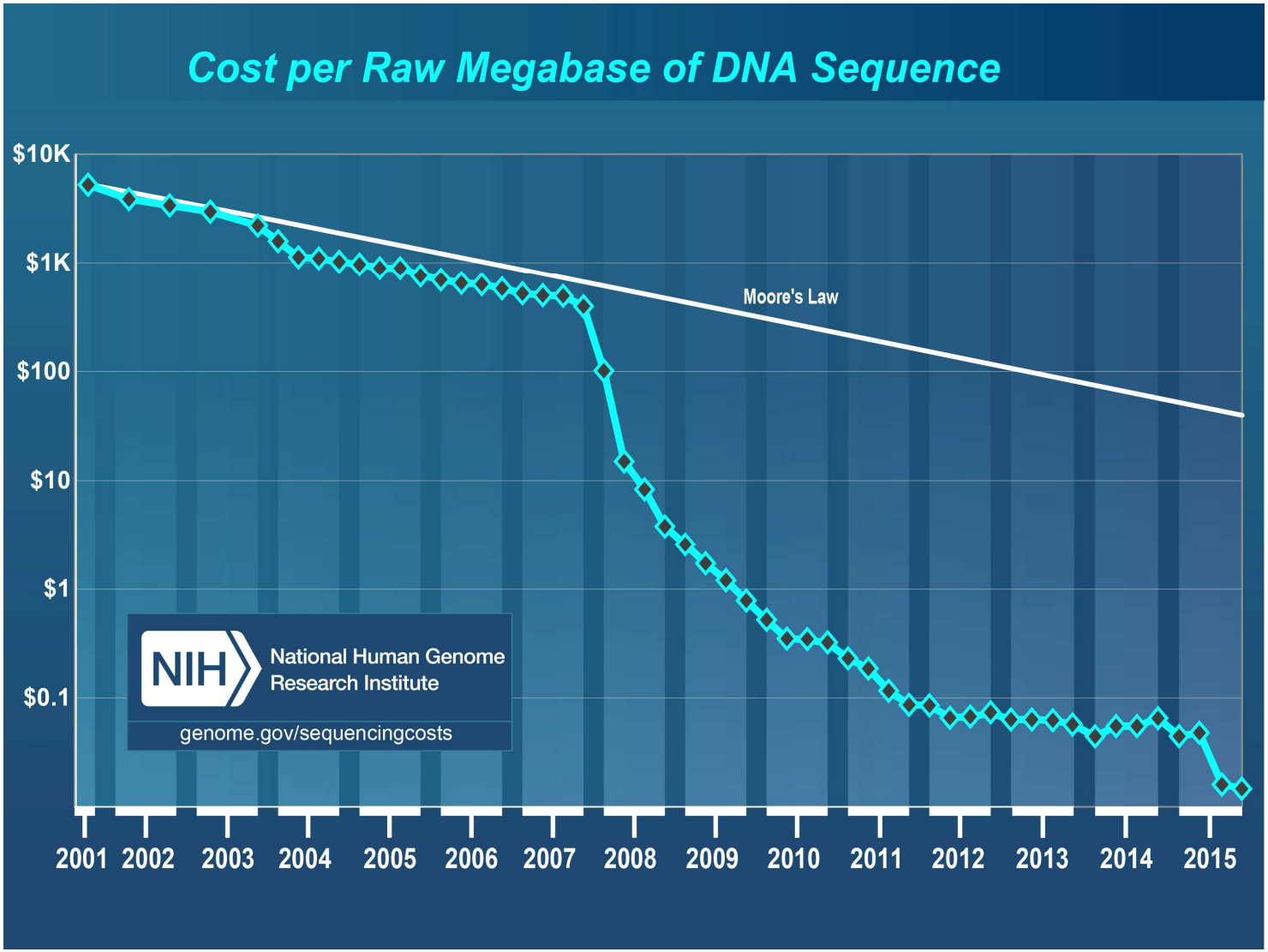
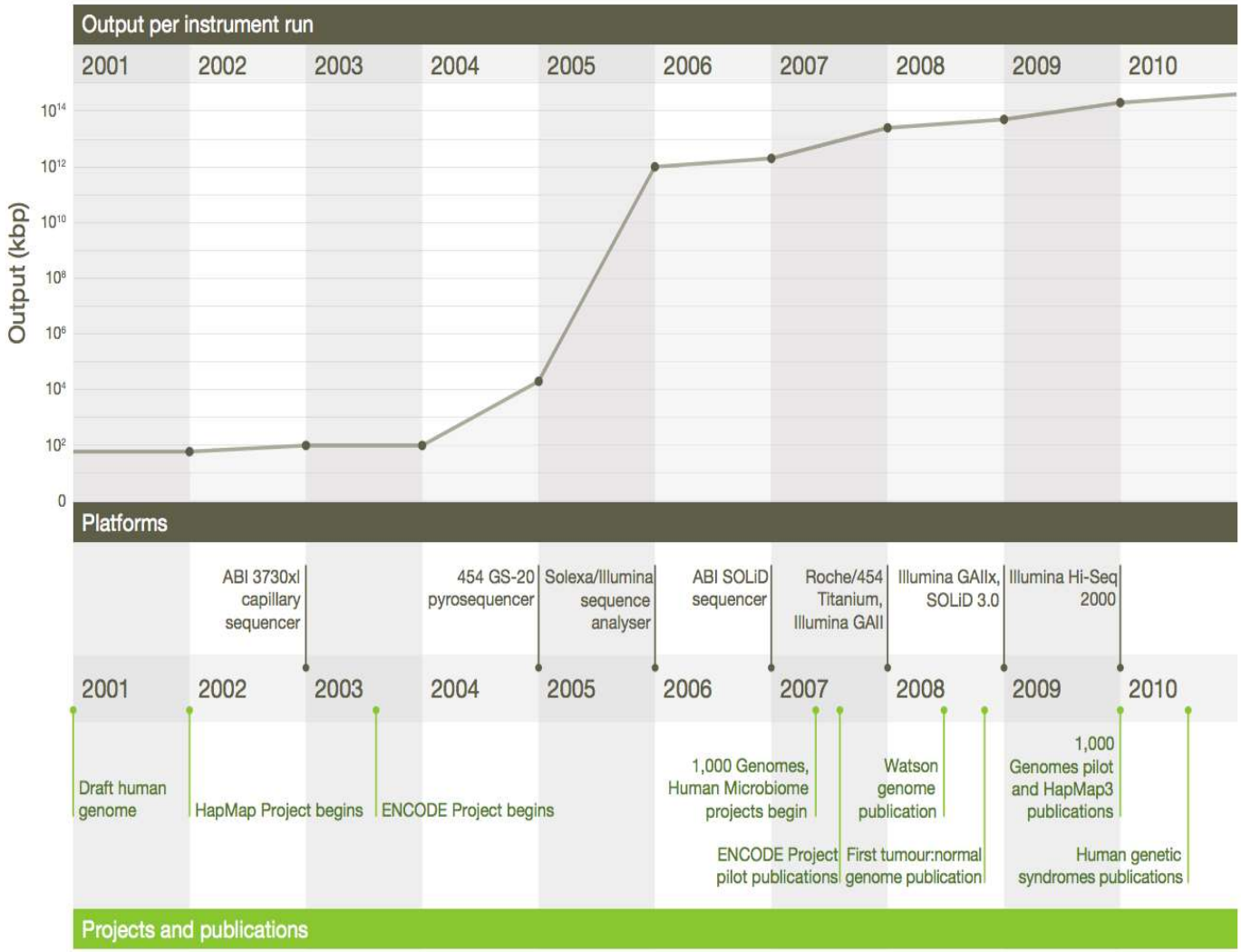


Timeline of Sequencing Capacity



Mardis, ER (2011), Nature, 470, pp. 198-203

DNA Sequencing Concepts

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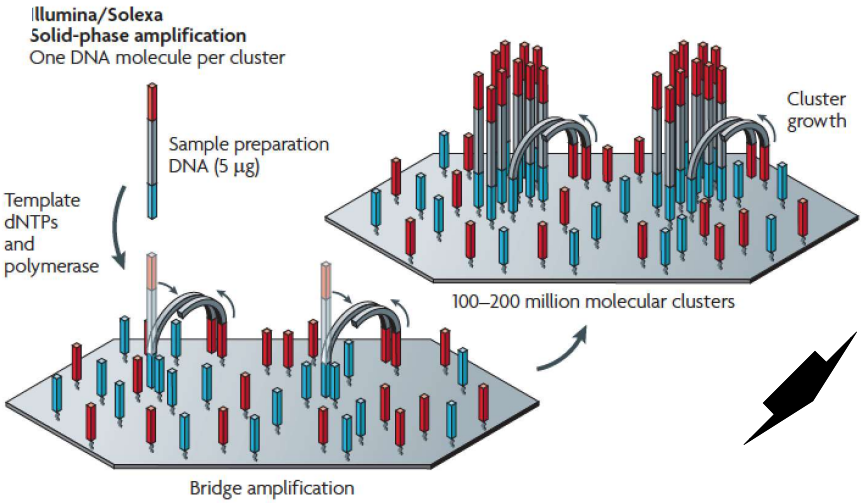
- Sequencing by Synthesis: Uses a polymerase to incorporate and assess nucleotides to a primer sequence
  - 1 nucleotide at a time
- Sequencing by Ligation: Uses a ligase to attach hybridized sequences to a primer sequence
  - 1 or more nucleotides at a time (e.g. dibase)

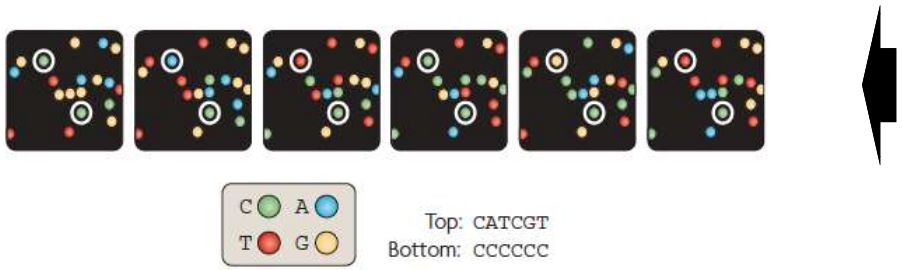
Modern NGS Sequencing Platforms

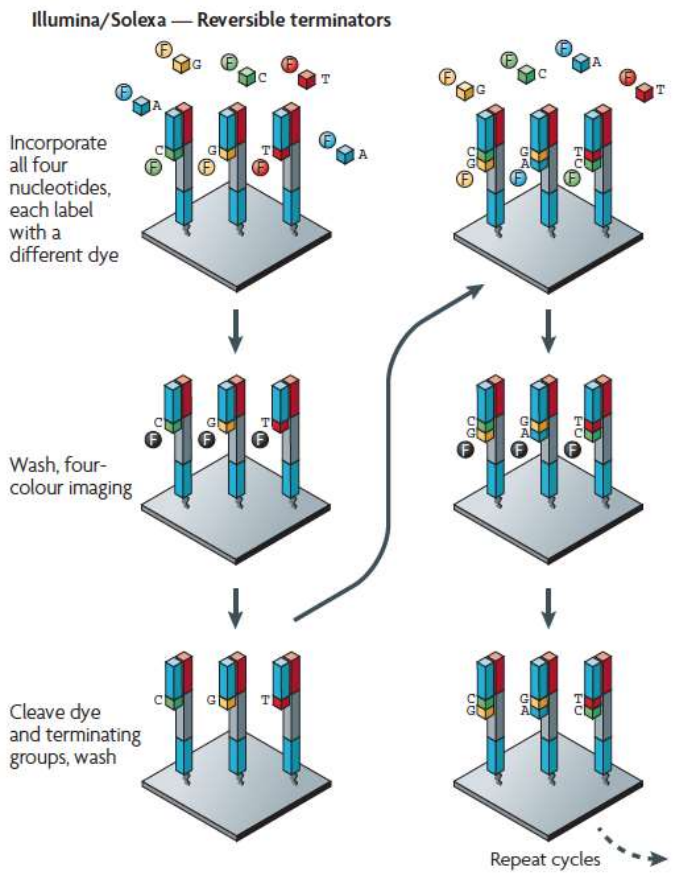
	Roche/454	Life Technologies SOLiD	Illumina Hi-Seq 2000
Library amplification method	emPCR* on bead surface	emPCR* on bead surface	Enzymatic amplification on glass surface
Sequencing method	Polymerase-mediated incorporation of unlabelled nucleotides	Ligase-mediated addition of 2-base encoded fluorescent oligonucleotides	Polymerase- mediated incorporation of end-blocked fluorescent nucleotides
Detection method	Light emitted from secondary reactions initiated by release of PPi	Fluorescent emission from ligated dye-labelled oligonucleotides	Fluorescent emission from incorporated dye-labelled nucleotides
Post incorporation method	NA (unlabelled nucleotides are added in base-specific fashion, followed by detection)	Chemical cleavage removes fluorescent dye and 3′ end of oligonucleotide	Chemical cleavage of fluorescent dye and 3′ blocking group
Error model	Substitution errors rare, insertion/deletion errors at homopolymers	End of read substitution errors	End of read substitution errors
Read length (fragment/paired end)	400 bp/variable length mate pairs	75 bp/50+25 bp	150 bp/100+100 bp

Modified from Mardis, ER (2011), Nature, 470, pp. 198-203

Illumina – Reversible terminators







(other sequencing platforms summarized at end of slide set)

Metzker, ML (2010), *Nat. Rev. Genet*, 11, pp. 31-46

Illumina Sequencing - Video

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https://www.youtube.com/watch?src_vid=womKfikWlxM&v=fCd6B5HRaZ8

NGS Sequencing Terminology

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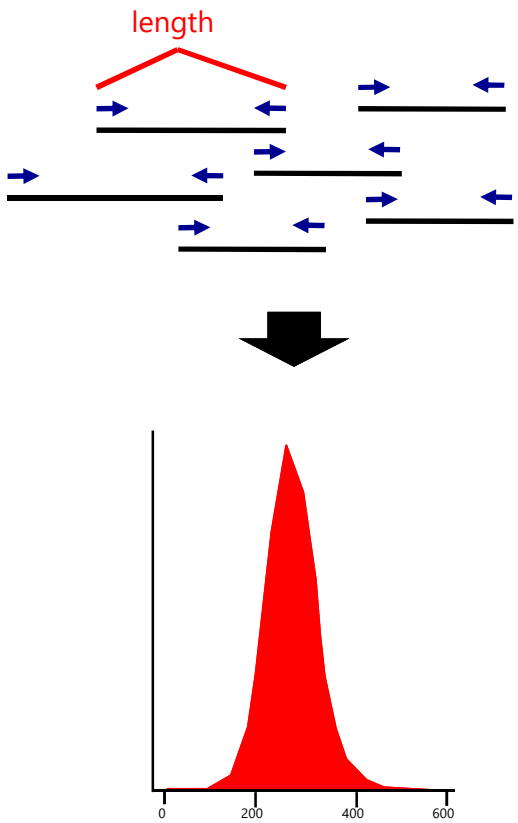
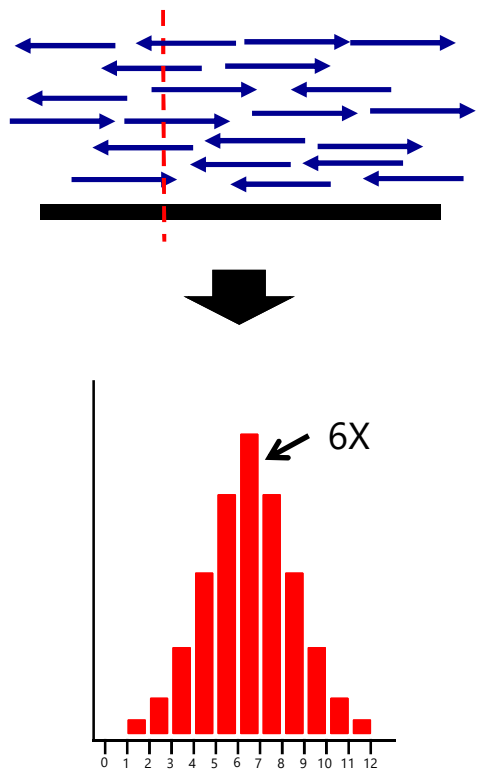
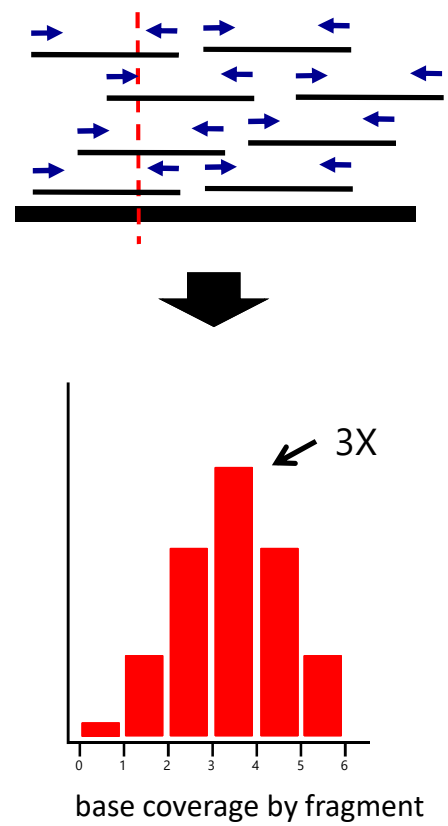
Insert Size



Sequence Coverage



Physical Coverage



Third Generation Sequencing

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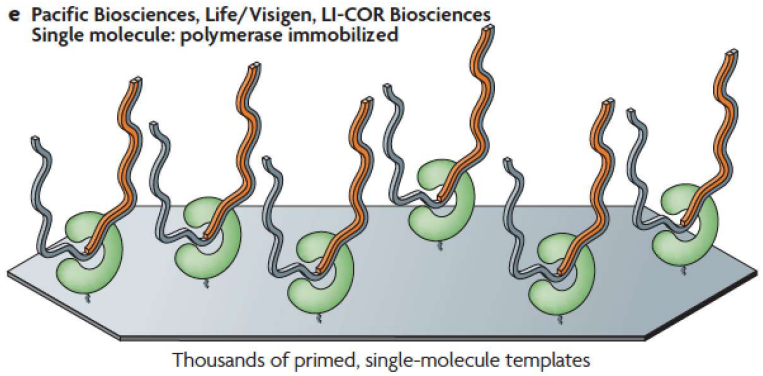
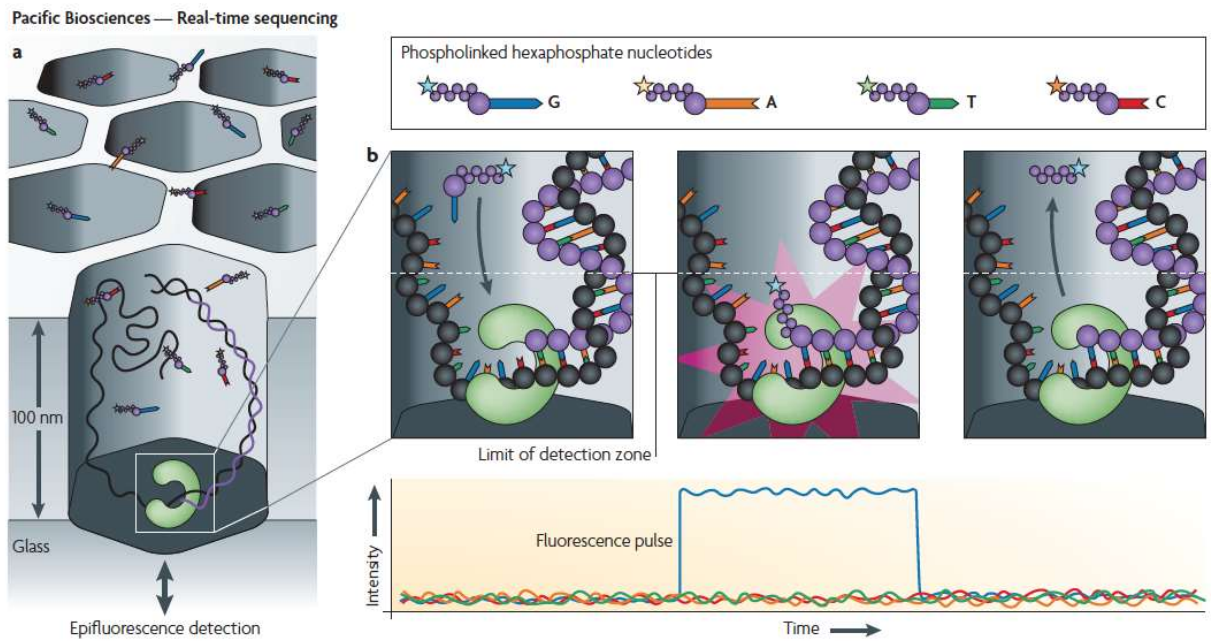
- Currently in transition / development
- Hard to define what "3rd" generation means
- Typical characteristics:
  - Long (1,000bp+) sequence reads
  - Single molecule (no amplification step)
  - Often associated with nanopore technology
    - But not necessarily!

Pacific Biosystems – Real Time Sequencing



zero mode waveguides



Metzker, ML (2010), *Nat. Rev. Genet*, 11, pp. 31-46

Pacific Biosystems – Circular Consensus

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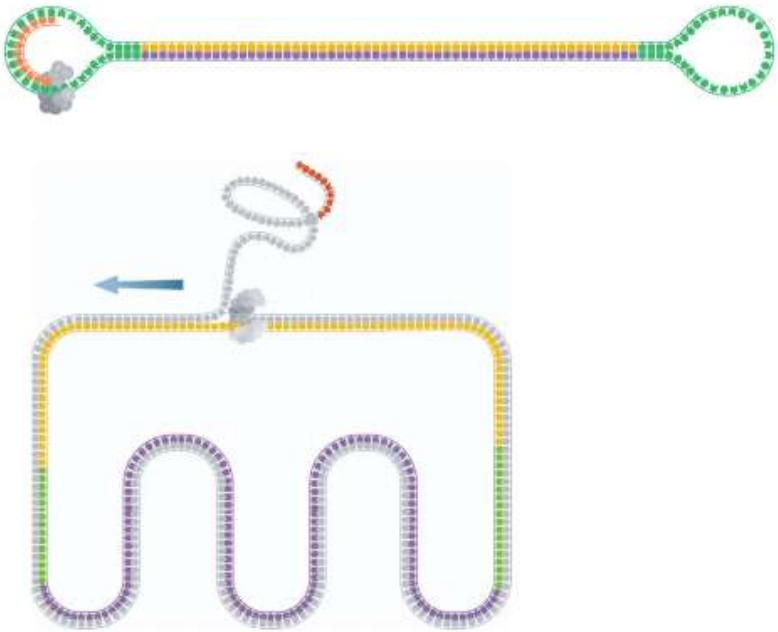
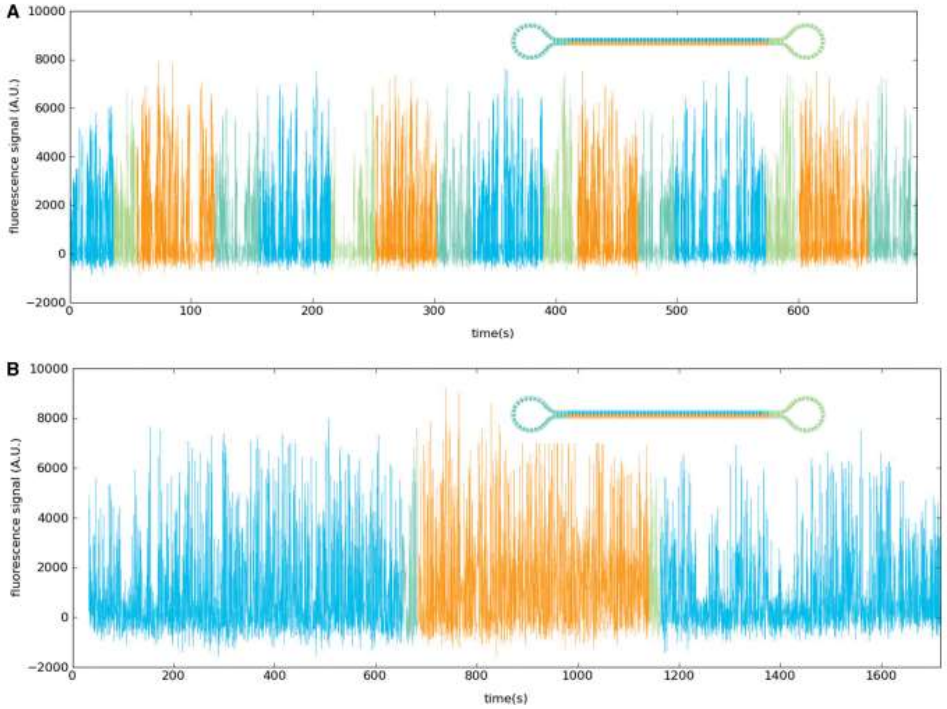
SMRTbell template



Subread Consensus Sequencing



Travers, KJ et al (2010), *Nucl. Acids. Res.*, 38(15) pp. e159

Summary: Generations of DNA Sequencing

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	First generation	Second generation[a]	Third generation[a]
Fundamental technology	Size-separation of specifically end-labeled DNA fragments, produced by SBS or degradation	Wash-and-scan SBS	SBS, by degradation, or direct physical inspection of the DNA molecule
Resolution	Averaged across many copies of the DNA molecule being sequenced	Averaged across many copies of the DNA molecule being sequenced	Single-molecule resolution
Current raw read accuracy	High	High	Moderate
Current read length	Moderate (800–1000 bp)	Short, generally much shorter than Sanger sequencing	Long, 1000 bp and longer in commercial systems
Current throughput	Low	High	Moderate
Current cost	High cost per base Low cost per run	Low cost per base High cost per run	Low-to-moderate cost per base Low cost per run
RNA-sequencing method	cDNA sequencing	cDNA sequencing	Direct RNA sequencing and cDNA sequencing
Time from start of sequencing reaction to result	Hours	Days	Hours
Sample preparation	Moderately complex, PCR amplification not required	Complex, PCR amplification required	Ranges from complex to very simple depending on technology
Data analysis	Routine	Complex because of large data volumes and because short reads complicate assembly and alignment algorithms	Complex because of large data volumes and because technologies yield new types of information and new signal processing challenges
Primary results	Base calls with quality values	Base calls with quality values	Base calls with quality values, potentially other base information such as kinetics

Schadt, EE et al (2010), *Hum. Mol. Biol.*, 19(RI2), pp. R227-R240

SeqAnswers Wiki

A good repository of analysis software can be found at http://seqanswers.com/wiki/Software/list

Log in

Page | Discussion | Read | View source | View history | Go | Search

Software/list

< Software

Below is (one of many possible) dynamic tables of software data, created from pages in the wiki. To add a package to the list, use the following form:

new package name | Add

CSV

JSON

SEQanswers
Forums

wiki navigation
Main page
Recent changes
Random page
Help

Software
Software hub
Browse software
Software list

Toolbox
What links here
Related changes
Special pages
Printable version
Permanent link
Browse properties

Name	Summary	Bio Tags	Meth Tags	Features	Language	Licence	OS
4peaks	Allows viewing sequencing trace files, motif searching trimming, BLAST and exporting sequences.	Sequencing	Sequence analysis			Freeware	Mac OS X
AB Large Indel Tool	Identifies deviations in clone insert size that indicate intra-chromosomal structural variations compared to a reference genome.	InDel discovery Sequencing	Mapping		Perl	GPL	Linux 64
AB Small Indel Tool	The SOLiD™ Small Indel Tool processes the indel evidences found in the pairing step of the SOLiD™ System Analysis Pipeline Tool (Corona Lite).	InDel discovery Sequencing	Mapping Alignment		Perl C++	GPL	Linux 64
ABBA	Assembly Boosted By Amino acid sequence is a comparative gene assembler, which uses amino acid sequences from predicted proteins to help build a better assembly	Genomic Assembly	Assembly Scaffolding			Artistic License	Linux
ABMapper	Maps RNA-Seq reads to target genome considering possible multiple mapping locations and splice junctions	Genomics Transcriptomics	Mapping Alignment		C++ Perl	GPLv3	Linux
ABySS	ABySS is a de novo sequence assembler designed for short reads and large genomes.	De-novo assembly	Assembly De Bruijn graph	MPI OpenMP	C++	Free for academic use	POSIX Linux Mac OS X
Adapter Removal	Removes adaptor fragments from raw short read	General	Adapter Removal	Trimming	Java	Custom Licence	Linux 64

Generic Workflow for NGS

- There are many different ways to analyze sequences generated from NGS, depending on the specific question you are investigating
- For the analysis of genomic sequence data, a typical (if generic) approach is as follows

Quality Control (QC)

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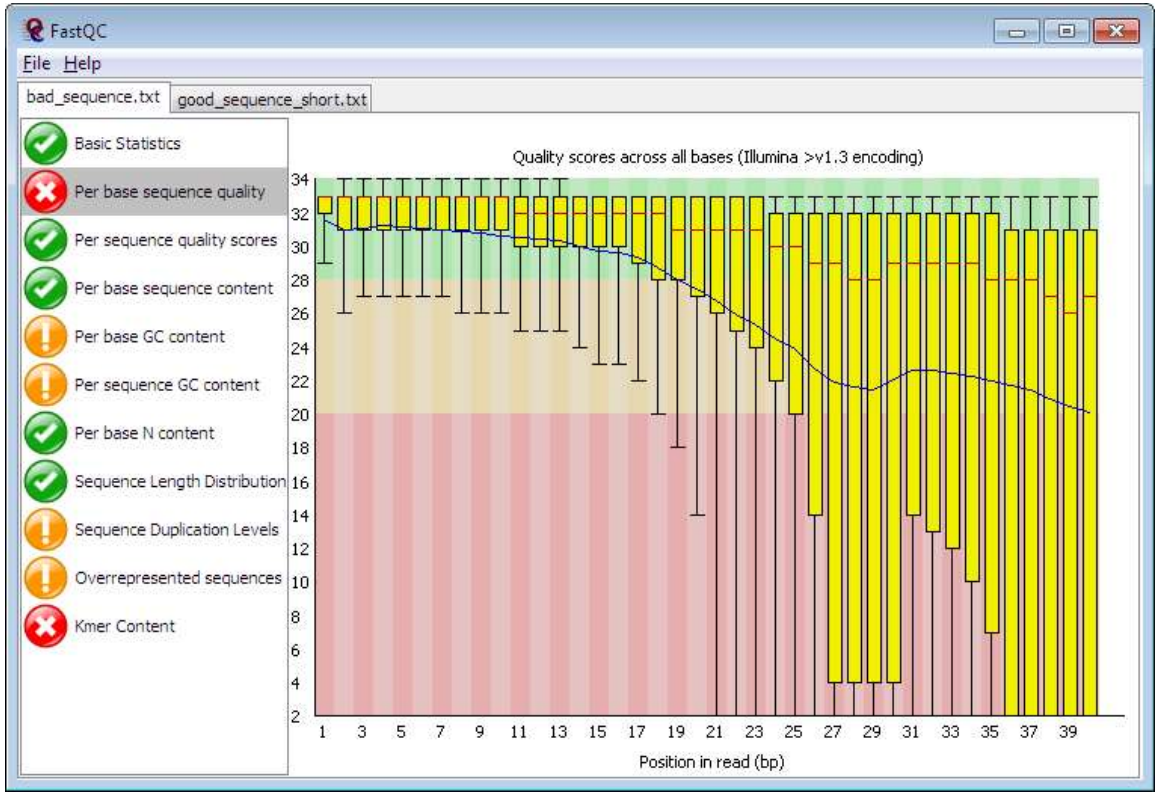
- Quality checks of raw sequence data are *very* important
- Common problems can include:
  - Sample mix-up
  - Sample contamination
  - Machine interruption
  - DNA quality
- It is crucial that investigators examine their sequences upon first receipt before any downstream analysis is conducted

FASTQC

FASTQC is one approach which provides a visual interpretation of the raw sequence reads

- http://www.bioinformatics.babraham.ac.uk/projects/fastqc/



Sequence Alignment

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- Once sequence quality has been assessed, the next step is to align the sequence to a reference genome
- There are *many* distinct tools for doing this; which one you choose is often a reflection of your specific experiment and personal preference

BWA	BarraCUDA	RMAP
Bowtie	CASHx	SSAHA
SOAP2	GSNAP	etc
Novoalign	Mosiak	
mr/mrsFast	Stampy	
Eland	SHRiMP	
Blat	SeqMap	
Bfast	SLIDER	

SAM Format

- Sequence Alignment/Map (SAM) format is the almost-universal sequence alignment format for NGS
  - binary version is BAM
- It consists of a header section (lines start with '@') and an alignment section
- The official specification can be found here:
  - http://samtools.sourceforge.net/SAM1.pdf

Example SAM File

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Header section

```
@HD	VN:1.0	SO:coordinate
@SQ	SN:1	LN:249250621	AS:NCBI37	UR:file:/data/local/ref/GATK/human_g1k_v37.fasta	M5:1b22b98cdeb4a9304cb5d48026a85128
@SQ	SN:2	LN:243199373	AS:NCBI37	UR:file:/data/local/ref/GATK/human_g1k_v37.fasta	M5:a0d9851da00400dec1098a9255ac712e
@SQ	SN:3	LN:198022430	AS:NCBI37	UR:file:/data/local/ref/GATK/human_g1k_v37.fasta	M5:fdfd811849cc2fadebc929bb925902e5
@RG	ID:UM0098:1	PL:ILLUMINA	PU:HWUSI-EAS1707-615LHAAXX-L001	LB:80	DT:2010-05-05T20:00:00-0400	SM:SD37743	CN:UMCORE
@RG	ID:UM0098:2	PL:ILLUMINA	PU:HWUSI-EAS1707-615LHAAXX-L002	LB:80	DT:2010-05-05T20:00:00-0400	SM:SD37743	CN:UMCORE
@PG	ID:bwa	VN:0.5.4
```

Alignment section

```
1:497:R:-272+13M17D24M	113	1	497	37	37M	15	100338662	0
	CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG	0;==-==9;>>>>>=>>>>>>>>>>>=>>>>>>>>>>	XT:A:U	NM:i:0	SM:i:37	AM:i:0	X0:i:1
	X1:i:0	XM:i:0	XO:i:0	XG:i:0	MD:Z:37
19:20389:F:275+18M2D19M	99	1	17644	0	37M	=	17919	314
	TATGACTGCTAATAATACCTACACATGTTAGAACCAT	>>>>>>>>>>>>>>>>>>>><<>>><<>>4::>>:<9	RG:Z:UM0098:1	XT:A:R	NM:i:0	SM:i:0	AM:i:0
	X0:i:4	X1:i:0	XM:i:0	XO:i:0	XG:i:0	MD:Z:37
19:20389:F:275+18M2D19M	147	1	17919	0	18M2D19M	=	17644	-314
	GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT	;44999;499<8<8<<<8<<><<<<><7<;<<<>><<	XT:A:R	NM:i:2	SM:i:0	AM:i:0	X0:i:4
	X1:i:0	XM:i:0	XO:i:1	XG:i:2	MD:Z:18^CA19
9:21597+10M2I25M:R:-209	83	1	21678	0	8M2I27M	=	21469	-244
	CACCACATCACATATACCAAGCCTGGCTGTGTCTTCT	<;9<<5><<<<><<<><><><><><9>><><>>9>>><>	XT:A:R	NM:i:2	SM:i:0	AM:i:0	X0:i:5
	X1:i:0	XM:i:0	XO:i:1	XG:i:2	MD:Z:35
```

http://genome.sph.umich.edu/wiki/SAM

SAM Utilities

- Samtools is a common toolkit for analyzing and manipulating files in SAM/BAM format
  - http://samtools.sourceforge.net/
- Picard is a another set of utilities that can used to manipulate and modify SAM files
  - http://picard.sourceforge.net/
- These can be used for viewing, parsing, sorting, and filtering SAM files as well as adding new information (e.g. Read Groups)

Post-alignment Optimization

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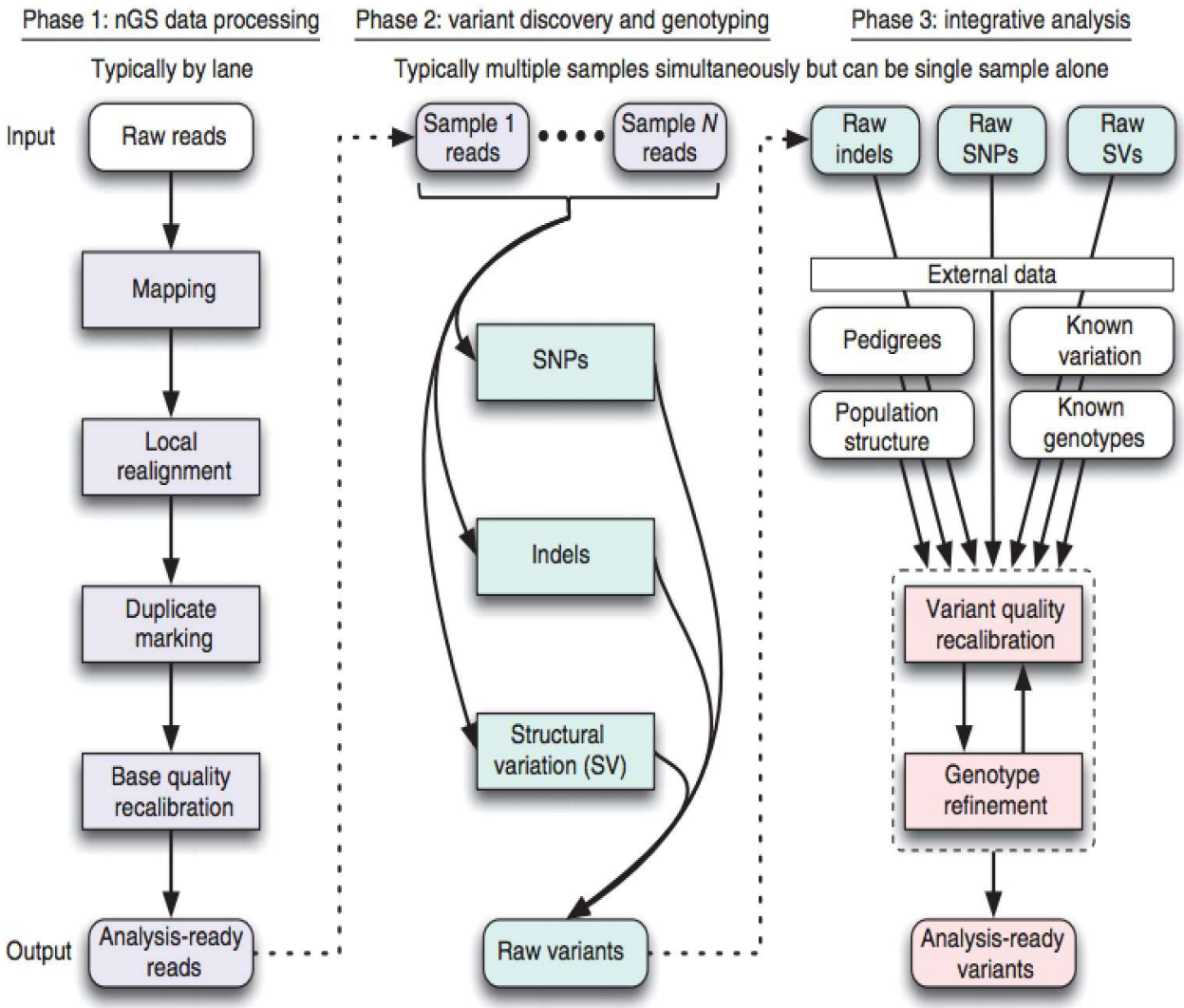
- A lot of research has been conducted to improve and optimize sequence alignments
- However, genomic sequences are very complex and by their very nature can preclude the ability to accurately determine where a sequence read originated
- New tools and approaches have been developed to help address these shortcomings and improve our overall ability to interpret the alignments

Genome Analysis Toolkit (GATK)

University of Michigan Medical School

- Developed in part to aid in the analysis of 1000 Genomes Project data
- Includes many tools for manipulating, filtering, and utilizing next generation sequence data
- http://www.broadinstitute.org/gatk/

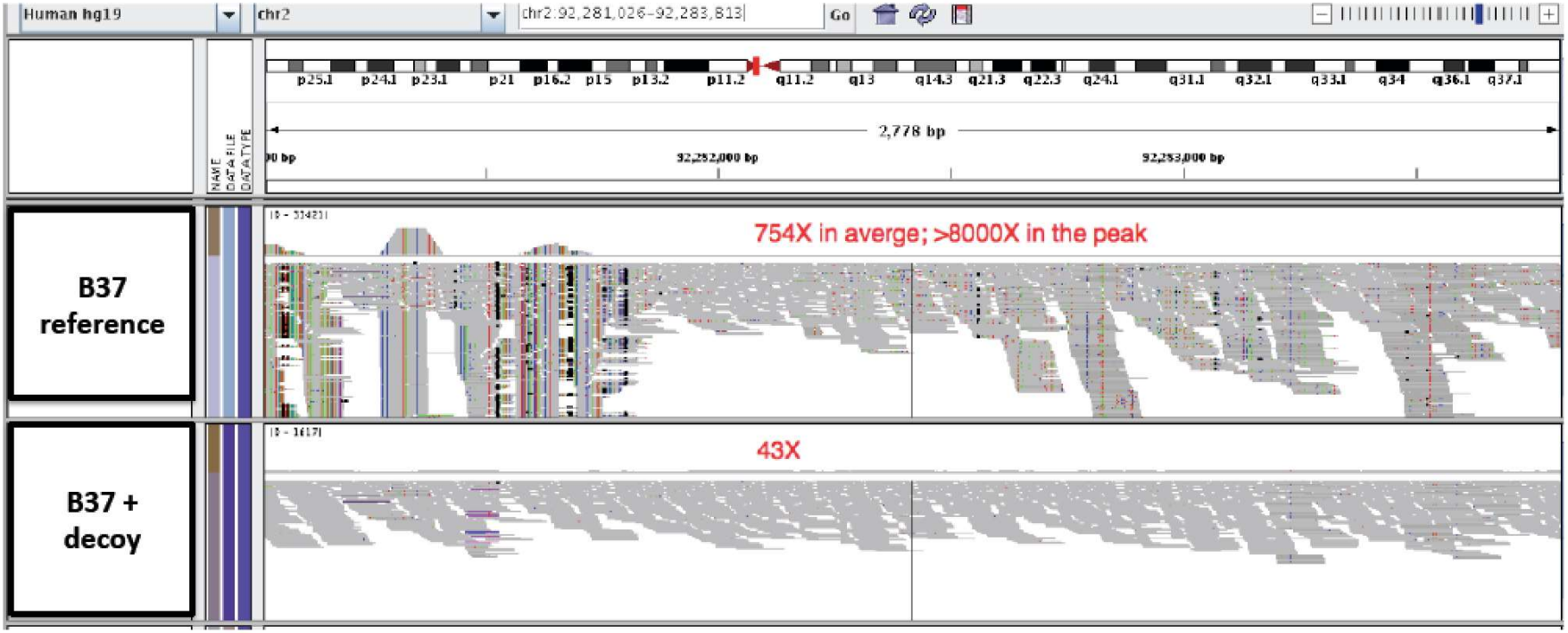
GATK Workflow



DePristo MA et al. (2011) Nat. Genet. 43, pp. 491-498

Decoy Mapping

One approach is to allow reads to map to a “decoy” alignment of extra-chromosomal or unassembled sequences



from Heng Li

Realignment around INDELs

- Insertions and deletions in samples can cause misalignments, resulting in false variant detection
- By identifying regions with known INDELs or reads which may have INDEL characteristics and performing multiple sequence alignments, these alignments can be rescued

HiSeq data, raw BWA alignments

HiSeq data, after MSA

DePristo MA et al. (2011) Nat. Genet. 43, pp. 491-498

Marking/Removing Duplicate Sequences

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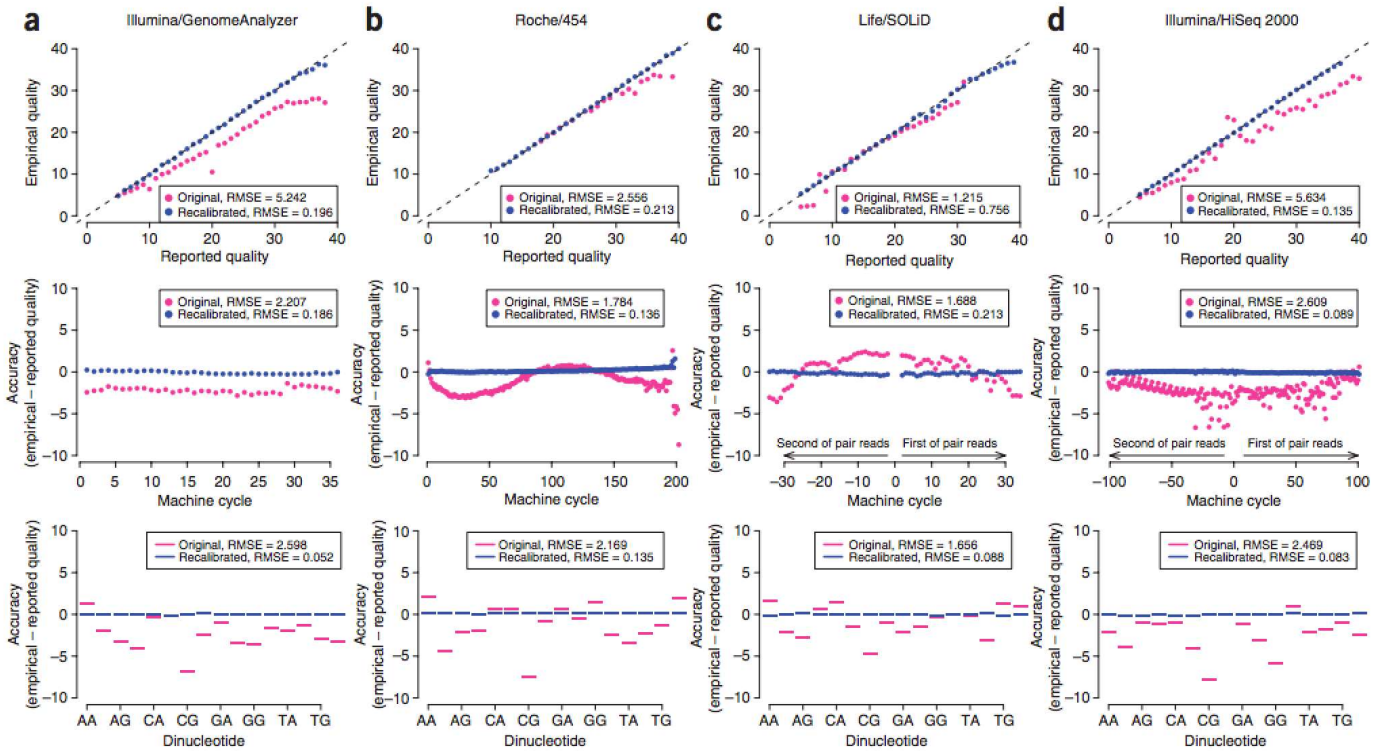
- Sequence biases can arise from PCR amplification effects during the construction of the library
- There can also be optical duplicates which occur when sequences from one cluster are accidentally identified as arising as well from adjacent clusters
- Both Picard (MarkDuplicates) and Samtools (rmdup) have utilities for addressing one or both of these issues

Base Recalibration

- Provides empirically accurate base quality scores for each base in every read
- Also corrects for error covariates like machine cycle and dinucleotide content



DePristo MA et al. (2011) Nat. Genet. 43, pp. 491-498

Population Scale Analysis

We can now begin to assess genetic differences on a very large scale, both as naturally occurring variation in human and non-human populations as well somatically within tumors

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Medical School

"Variety's the very spice of life"

-William Cowper, 1785

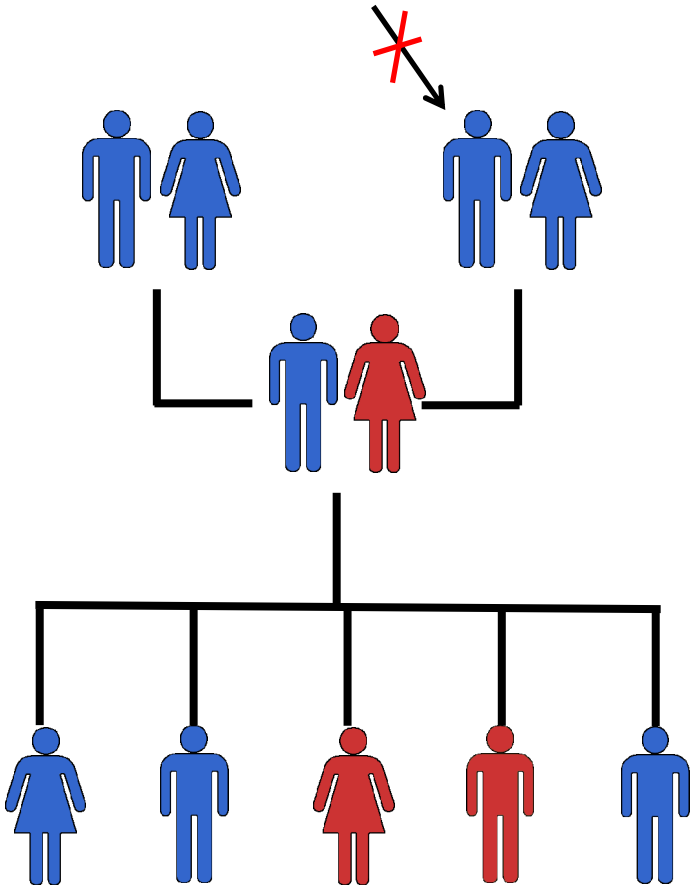
"Variation is the spice of life"

-Kruglyak & Nickerson, 2001

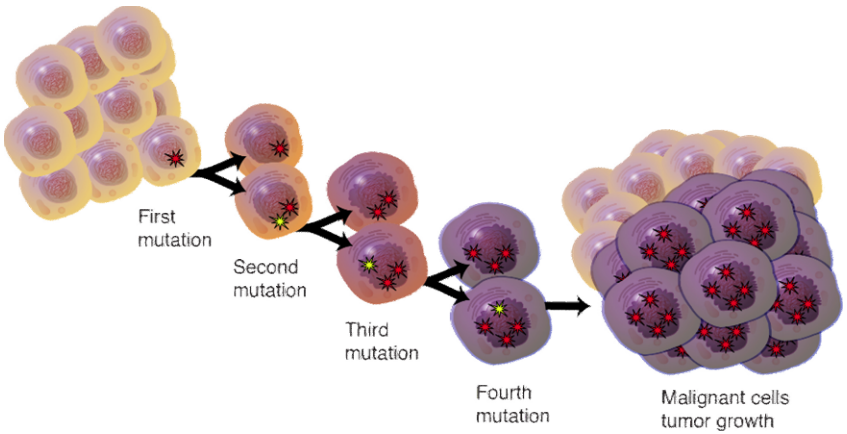
- While the sequencing of the human genome was a great milestone, the DNA from a single person is not representative of the millions of potential differences that can occur between individuals
- These unknown genetic variants could be the cause of many phenotypes such as differing morphology, susceptibility to disease, or be completely benign.

Germline Variation

- Mutations in the germline are passed along to offspring and are present in the DNA over every cell
- In animals, these typically occur in meiosis during gamete differentiation



Somatic Variation



- Mutations in non-germline cells that are not passed along to offspring
- Can occur during mitosis or from the environment itself
- Are an integral part in tumor progression and evolution

Darryl Leja, Courtesy: National Human Genome Research Institute.

Mutation vs Polymorphism

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- A mutation must persist to some extent within a population to be considered polymorphic
  - >1% frequency is often used
- Germline mutations that are not polymorphic are considered rare variants

"From the standpoint of the neutral theory, the rare variant alleles are simple those alleles whose frequencies within a species happen to be in a low-frequency range (0,q), whereas polymorphic alleles are those whose frequencies happen to be in the higher-frequency range (q, 1-q), where I arbitrarily take q = 0.01. Both represent a phase of molecular evolution."

-Motoo Kimura

Kimura M (1983) Mol. Biol. Evol., 1(1), pp. 84-93

Types of Genomic Variation

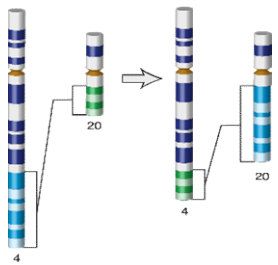
- Single Nucleotide Polymorphisms (SNPs) – mutations of one nucleotide to another

```
AATCTGAGGCAT
AATCTCAGGCAT
```

- Insertion/Deletion Polymorphisms (INDELs) – small mutations removing or adding one or more nucleotides at a particular locus

```
AATCTGAAGGCAT
AATCT--AGGCAT
```

- Structural Variation (SVs) – medium to large sized rearrangements of chromosomal DNA



Darryl Leja, Courtesy: National Human Genome Research Institute.

Variant Subtypes: Repetitive Elements

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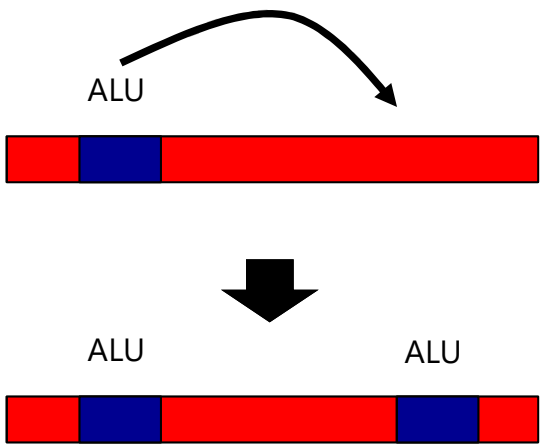
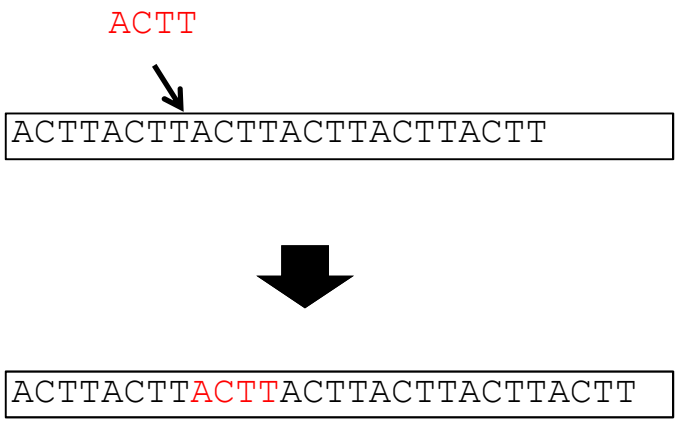
Mobile Elements / Retrotransposons

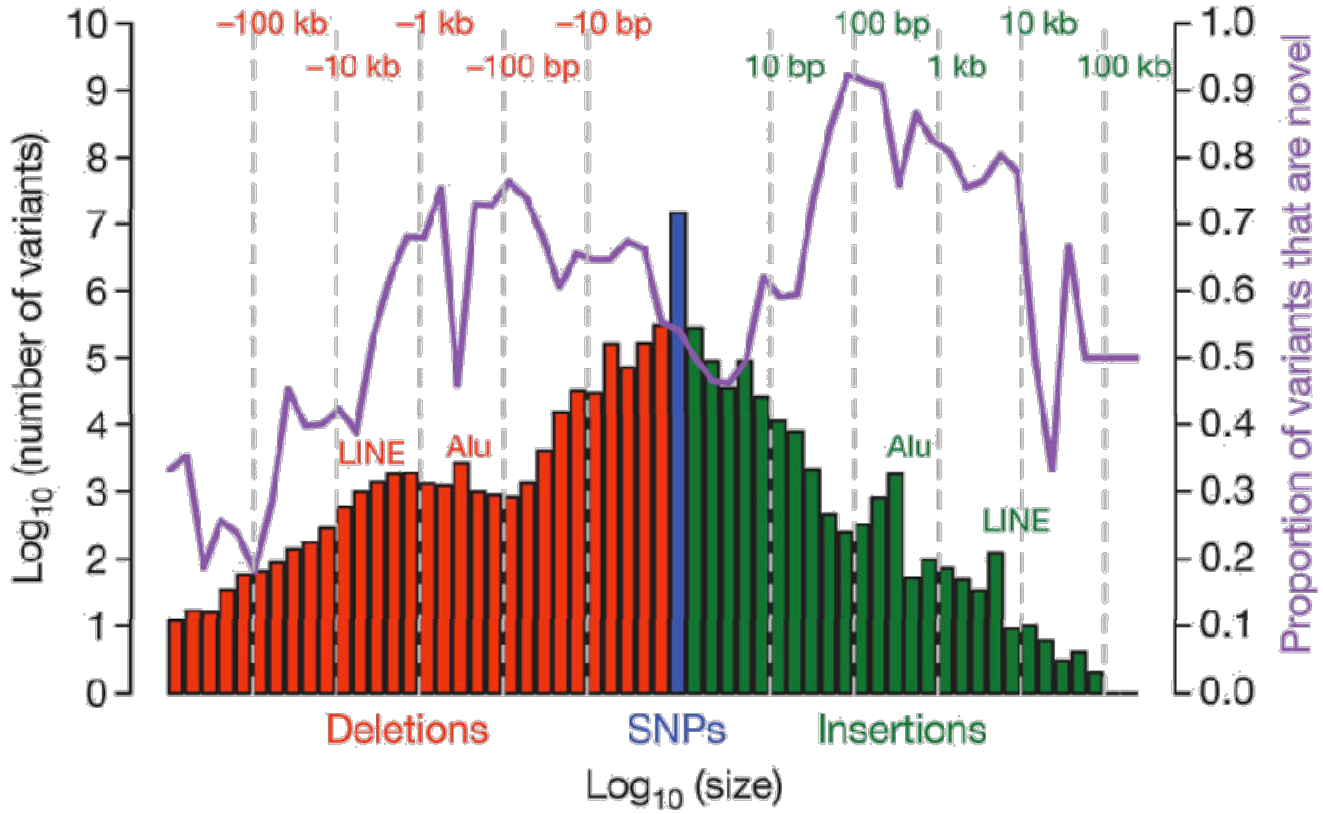


(in humans, primarily ALU, LINE, and SVA)

Repeat Expansions



Variant Length Distribution



1000 Genomes Project, Nature, 2010

Differences Between Individuals

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The average number of genetic differences in the germline between two random humans can be broken down as follows:

- 3,600,000 single nucleotide differences
- 344,000 small insertion and deletions
- 1,000 larger deletion and duplications

Numbers change depending on ancestry!

1000 Genomes Project, Nature, 2012

Discovering Variation: SNPs and INDELs

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Medical School

- Small variants require the use of sequence data to initially be discovered
- Most approaches align sequences to a reference genome to identify differing positions
- The amount of DNA sequenced is proportional to the number of times a region is covered by a sequence read
  - More sequence coverage equates to more support for a candidate variant site

Discovering Variation: SNPs and INDELs

SNP

```
ATCCTGATTCGGTGAACGTTATCGACGATCCGATCGA
ATCCTGATTCGGTGAACGTTATCGACGATCCGATCGA
        CGGTGAACGTTATCGACGATCCGATCGAACTGTCAGC
         GGTGAACGTTATCGACGTTCCGATCGAACTGTCAGCG
           TGAACGTTATCGACGTTCCGATCGAACTGTCATCGGC
           TGAACGTTATCGACGTTCCGATCGAACTGTCACCGGC
           TGAACGTTATCGACGTTCCGATCGAACTGTCAGCGGC
                GTTATCGACGATCCGATCGAACTGTCAGCGGCAAGCT
                 TTATCGACGATCCGATCGAACTGTCAGCGGCAAGCT
```

sequencing error
or genetic variant?

ATCCTGATTCGGTGAACGTTATCGACGATCCGATCGAACTGTCAGCGGCAAGCTGATCGATCGATCGATGCTAGTG

reference genome

```
TTATCGACGATCCGATCGAACTGTCAGCGGCAAGCT
   TCGACGATCCGATCGAACTGTCAGCGGCAAGCTGAT
         ATCCGATCGAACTGTCAGCGGCAAGCTGATCG  CGAT
          TCCGAGCGAACTGTCAGCGGCAAGCTGATCG  CGATC
          TCCGATCGAACTGTCAGCGGCAAGCTGATCGATCGA
           GATCGAACTGTCAGCGGCAAGCTGATCG  CGATCGA
                AACTGTCAGCGGCAAGCTGATCG  CGATCGATGCTA
                   TGTCAGCGGCAAGCTGATCGATCGATCGATGCTAG
                     TCAGCGGCAAGCTGATCGATCGATCGATGCTAGTG
```

sequencing error
or genetic variant?

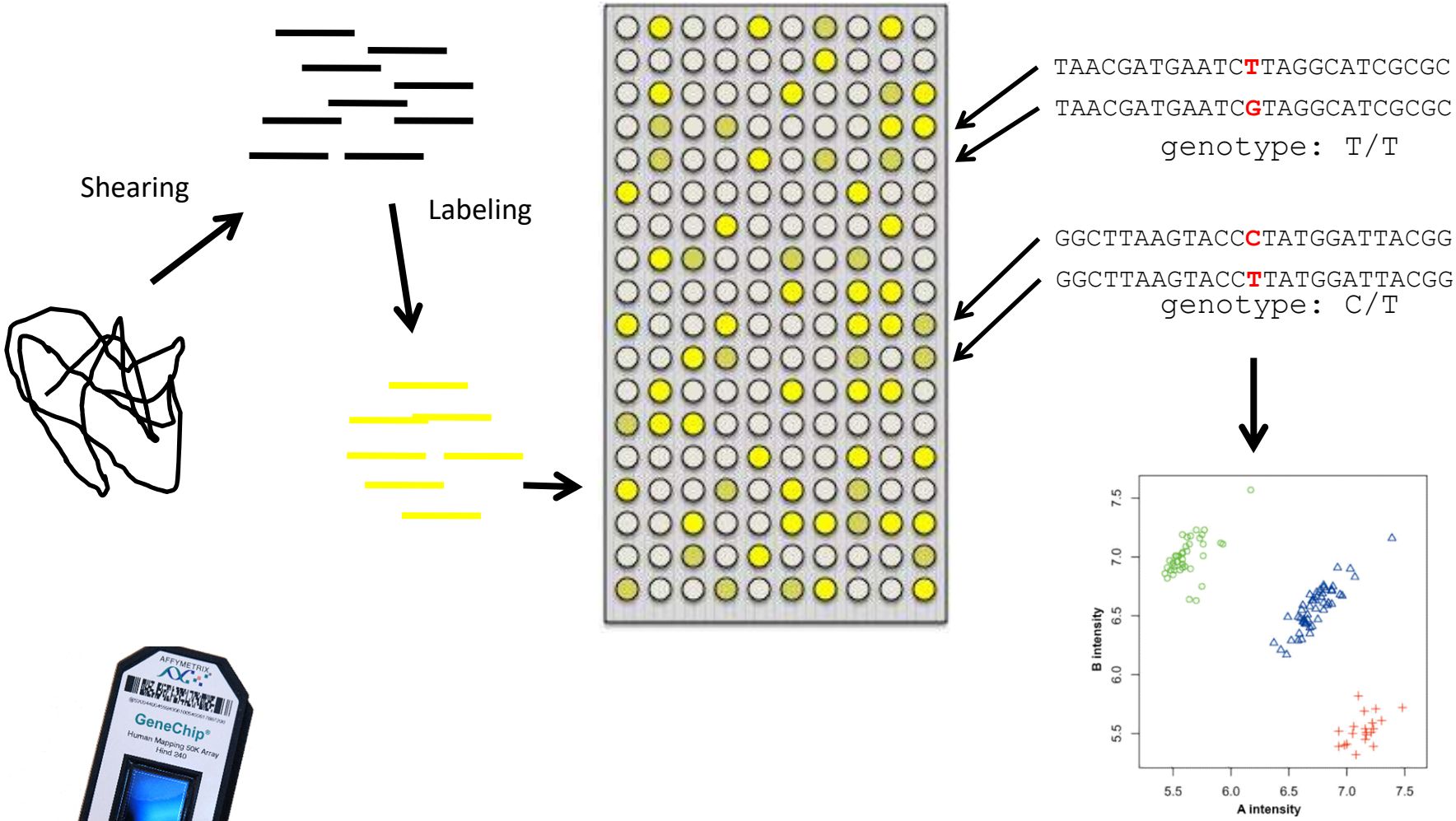
INDEL

Genotyping Small Variants

University of Michigan Medical School

- Once discovered, oligonucleotide probes can be generated with each individual allele of a variant of interest
- A large number can then be assessed simultaneously on microarrays to detect which combination of alleles is present in a sample

SNP Microarrays



Maggie Bartlett, Courtesy: National Human Genome Research Institute.

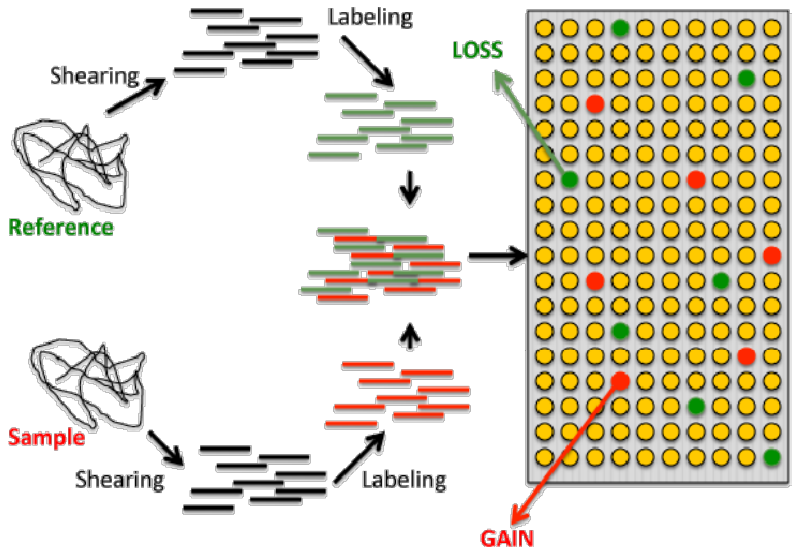
Discovering Variation: SVs

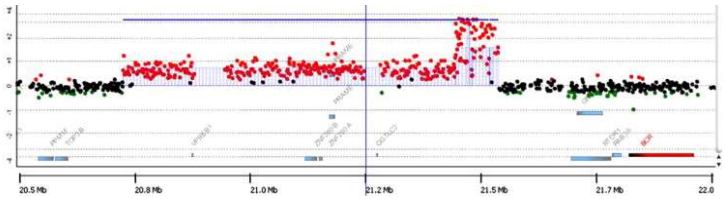
University of Michigan Medical School

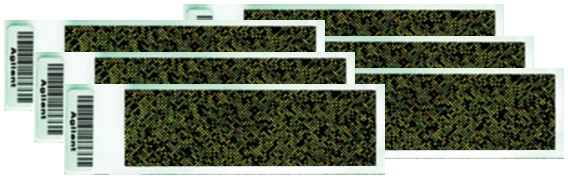
- Structural variants can be discovered by both sequence and microarray approaches
- Microarrays can only detect genomic imbalances, specifically copy number variants (CNVs)
- Sequence based approaches can, in principle, identify all types of structural rearrangements

Microarray-based CNV Discovery

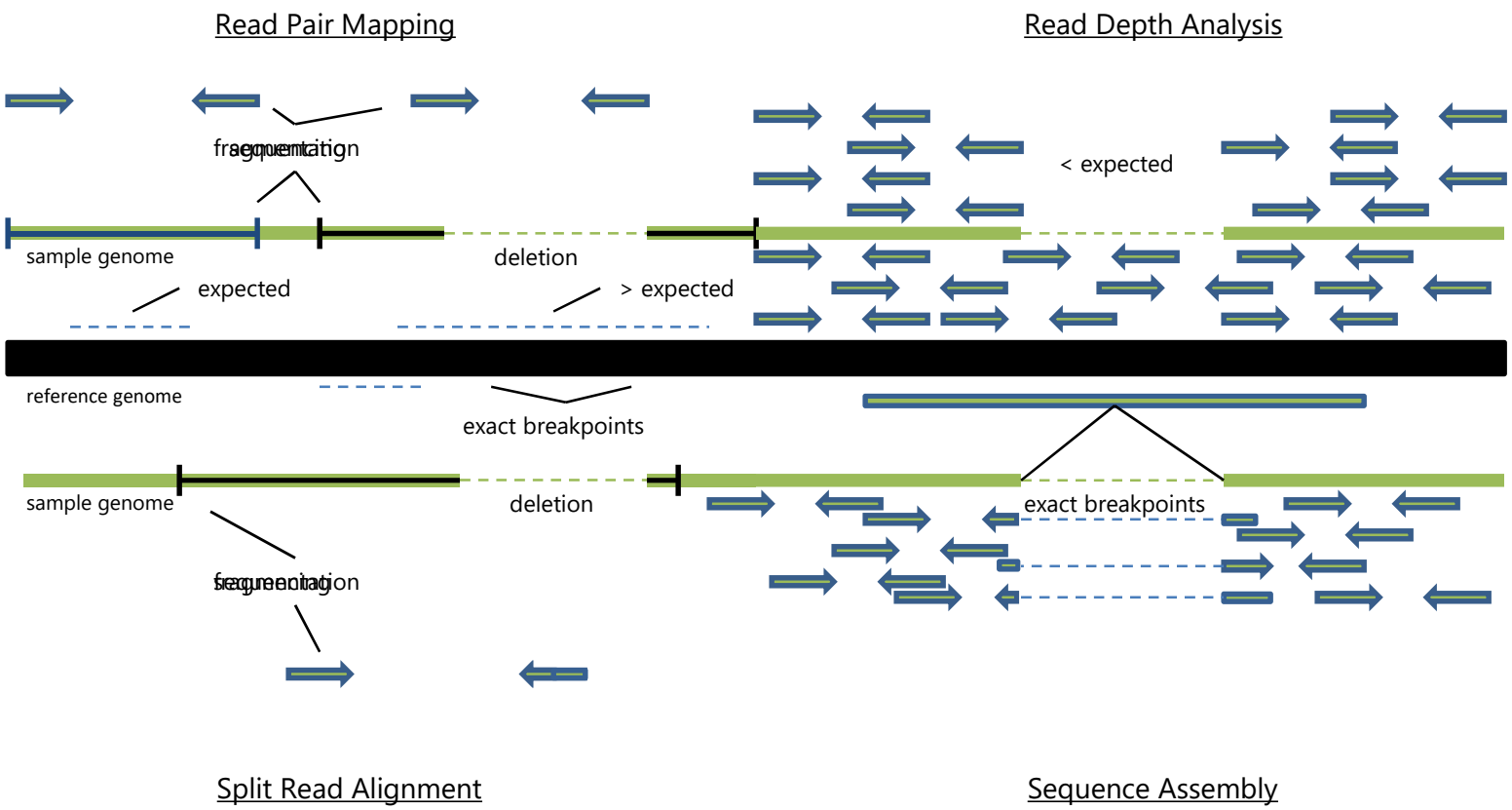
Comparative Genomic Hybridization (CGH)







Sequenced-based SV Discovery



Variant Databases and Formats

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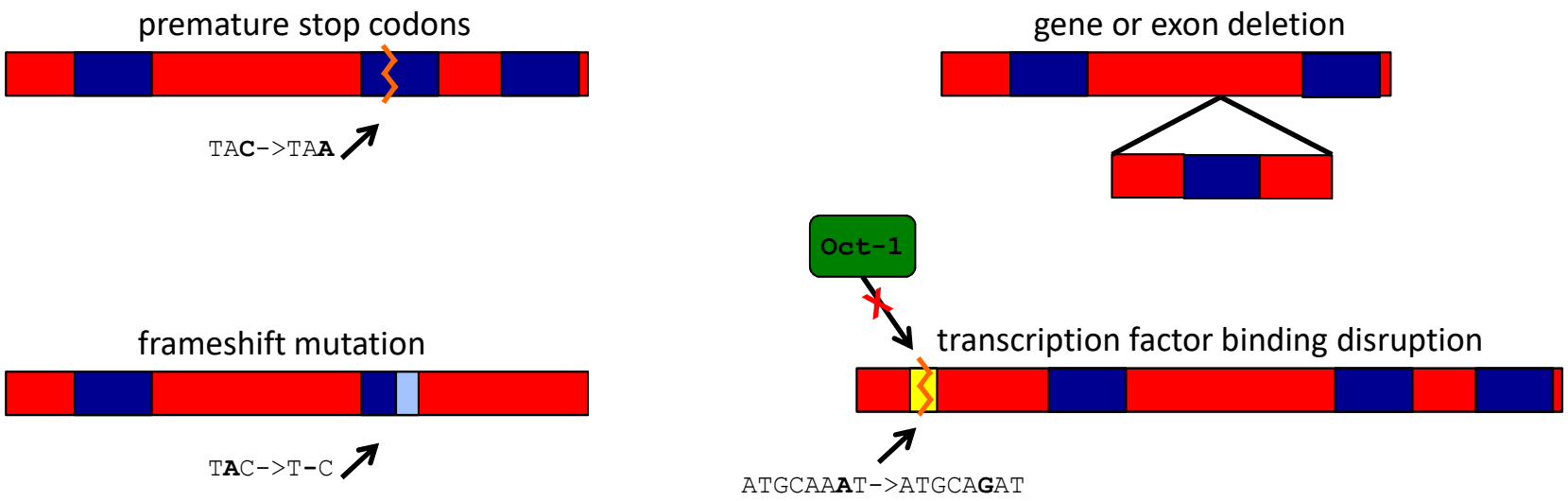
- dbSNP – repository for SNP and small INDELs
  - http://www.ncbi.nlm.nih.gov/SNP/
- VCF – variant call format for reporting variation
  - https://github.com/samtools/hts-specs

VCF Format Example

```
##fileformat=VCFv4.2
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data”>
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth”>
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency”>
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele”>
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129”>
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership”>
##FILTER=<ID=q10,Description="Quality below 10”>
##FILTER=<ID=s50,Description="Less than 50% of samples have data”>
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype”>
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality”>
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth”>
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality”>
#CHROM POS     ID         REF   ALT     QUAL   FILTER   INFO                              FORMAT       NA00001         NA00002         NA00003
20     14370   rs6054257  G     A       29     PASS     NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ  0|0:48:1:51,51  1|0:48:8:51,51  1/1:43:5:.,.
20     17330   .          T     A       3      q10      NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ  0|0:49:3:58,50  0|1:3:5:65,3    0/0:41:3
20     1110696 rs6040355  A     G,T     67     PASS     NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ  1|2:21:6:23,27  2|1:2:0:18,2    2/2:35:4
20     1230237 .          T     .       47     PASS     NS=3;DP=13;AA=T                   GT:GQ:DP:HQ  0|0:54:7:56,60  0|0:48:4:51,51  0/0:61:2
21     1234567 microsat1  GTC   G,GTCT  50     PASS     NS=3;DP=9;AA=G                    GT:GQ:DP     0/1:35:4        0/2:17:2        1/1:40:3
```

Impact of Genetic Variation

There are numerous ways genetic variation can exhibit functional effects



Variant Annotation

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- Variants are *annotated* based on their potential functional impact
- For variants falling inside genes, there are a number of software packages that can be used to quickly determine which may have a functional role (missense/nonsense mutations, splice site disruption, etc)
- A few examples are:
  - ANNOVAR (http://www.openbioinformatics.org/annovar/)
  - VAAST (http://www.yandell-lab.org/software/vaast.html)
  - VEP (http://http://grch37.ensembl.org/Homo_sapiens/Tools/VEP)
  - SeattleSeq (http://snp.gs.washington.edu/SeattleSeqAnnotation134/)
  - snpEff (http://snpeff.sourceforge.net/)

Variant Annotation Classes

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High Impact

- exon_deleted
- frame_shift
- splice_acceptor
- splice_donor
- start_loss
- stop_gain
- stop_loss
- non_synonymous_start
- transcript_codon_change

Medium Impact

- non_syn_coding
- inframe_codon_gain
- inframe_codon_loss
- inframe_codon_change
- codon_change_del
- codon_change_ins
- UTR_5_del
- UTR_3_del
- other_splice_variant
- mature_miRNA
- regulatory_region
- TF_binding_site
- regulatory_region_ablation
- regulatory_region_amplification
- TFBS_ablation
- TFBS_amplification

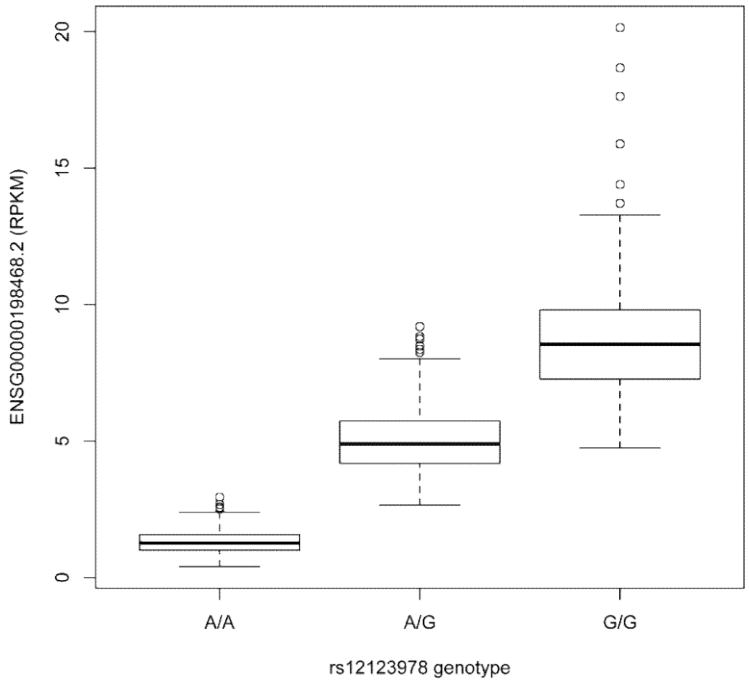
Low Impact

- synonymous_stop
- synonymous_coding
- UTR_5_prime
- UTR_3_prime
- intron
- CDS
- upstream
- downstream
- intergenic
- intragenic
- gene
- transcript
- exon
- start_gain
- synonymous_start
- intron_conserved
- nc_transcript
- NMD_transcript
- transcript_codon_change
- incomplete_terminal_codon
- nc_exon
- transcript_ablation
- transcript_amplification
- feature elongation
- feature truncation

GEMINI, http://gemini.readthedocs.org/

Variation and Gene Expression

- Expression quantitative trait loci (eQTLs) are regions of the genome that are associated with expression levels of genes
- These regions can be nearby (cis) or far away (trans) from the genes that they affect
- Genetic variants in eQTL regions are typically responsible through changes to regulatory elements



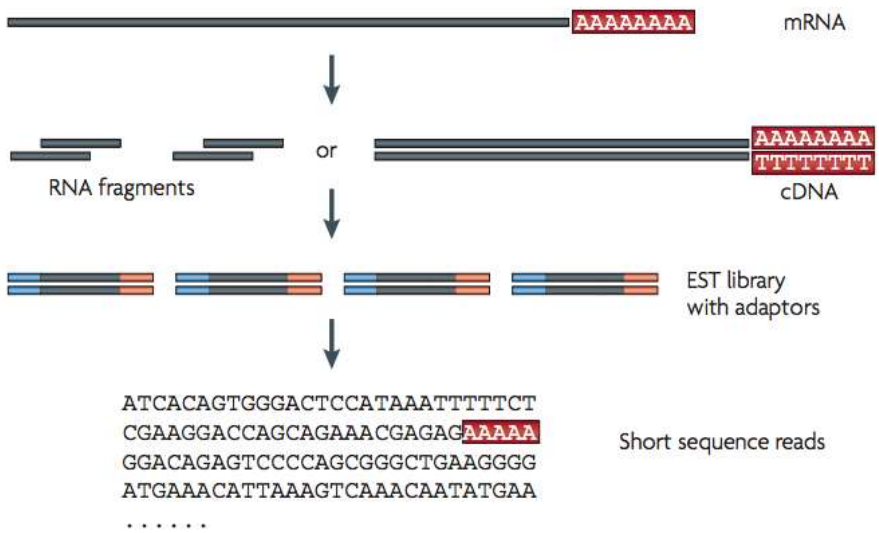
Data generated from http://www.geuvadis.org/

Geuvadis Consortium

http://www.geuvadis.org/web/geuvadis

RNA Sequencing

- Uses same technologies as DNA sequencing
- Primary difference is in library preparation
  - RNA converted to cDNA through reverse transcription



Wang, Z et al (2009), *Nat Rev Genet.*, 10, pp. 57-63

RNA-Seq Overview

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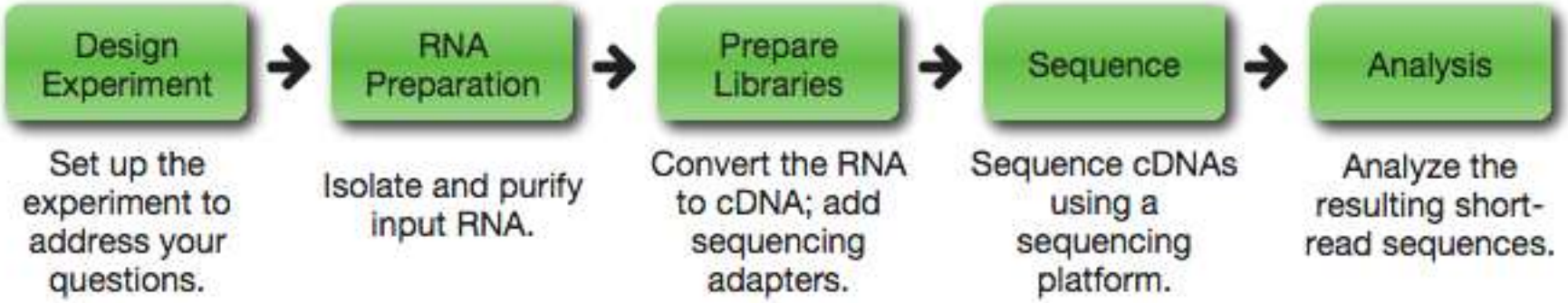
Sample Preparation



Sequencing	Read Alignment	Transcript compilation	Gene identification	Differential expression
RNA-seq reads (2x100bp)	Bowtie/Tophat Alignment (genome)	Cufflinks	Cufflinks (cuffmerge)	Cuffdiff

Analysis Pipeline (Tophat)

http://rnaseq.uoregon.edu/

Types of RNA-Seq Libraries

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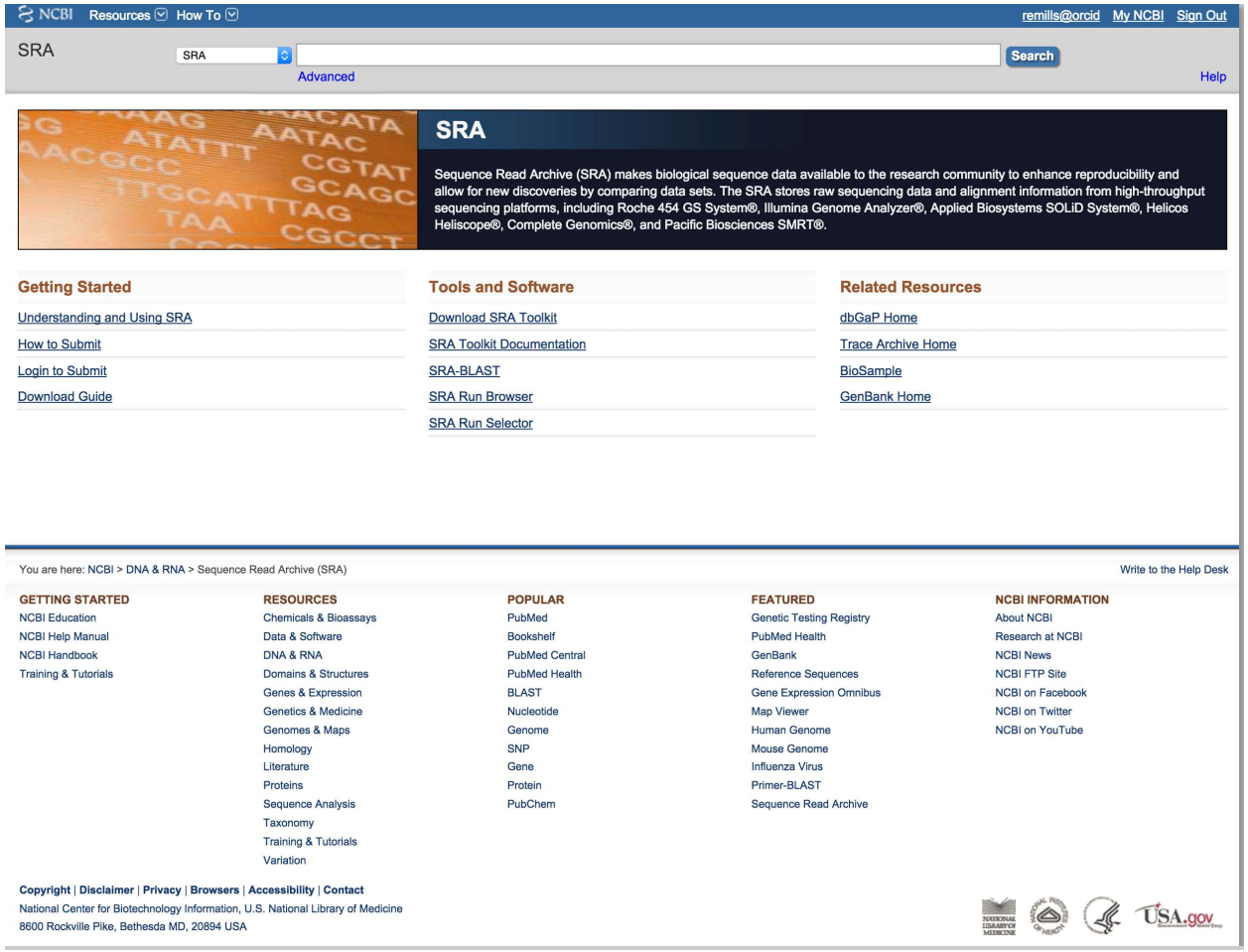
- poly(A) capture – utilizes oligo(dT) to prime off of mature mRNA
  - Won't amplify non-coding mRNA (e.g. lincRNAs, miRNAs, etc)
  - Has enrichment biases between 3' and 5' ends due to RT drop-offs
  - Won't work with fragmented RNA
- random hexamer priming – primes at random positions along the transcript
  - Will work with fragmented or degraded RNA (e.g. FFPE samples)
  - Removes positional biases of poly(A) capture
  - Requires some type of rRNA removal (e.g. Ribo-Zero) to address its overabundance

DNA- and RNA-Seq Databases

NCBI Short Read Archive (SRA):
http://www.ncbi.nlm.nih.gov/sra

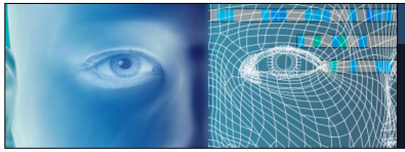


Protected Data - dbGaP

NCBI Database of Genotypes and Phenotypes (dbGaP):
http://www.ncbi.nlm.nih.gov/sra

NCBI Resources How To remills@orcid My NCBI Sign Out

dbGaP dbGaP Search
Limits Advanced Help



dbGaP

The database of Genotypes and Phenotypes (dbGaP) was developed to archive and distribute the results of studies that have investigated the interaction of genotype and phenotype.

Getting Started
- dbGaP Tutorial
- Overview
- FAQ
- How to Submit
- Browse Top Level Studies

Access dbGaP Data
- Collections
- Apply for Controlled Access Data
- Public Data via ftp Download
- Association Results Browser
- Phenotype-Genotype Integrator

Important Links
- Summary Statistics
- dbGaP RSS Feed
- Code of Conduct
- Security Procedures
- Contact Us

Latest Studies

Important notice: NIH has established a collection of dbGaP samples designated as appropriate for general research use (GRU) by submitting institutions, which indicates that there are no further limitations on secondary research use beyond those outlined in the Genomic Data User Code of Conduct. For details, visit the collection's page.

Study	Embargo Release	Details	Participants	Type Of Study	Links	Platform
phs000790.v1.p1 Comparative Analysis of Primary and Metastatic Colorectal Cancer	Version 1: 2015-01-29	V D A S	4	Cohort	Links	HiSeq 2000
phs000848.v1.p1 Autosomal recessive TPP2 mutations cause a new human immunodeficiency	Version 1: 2015-12-16	V D A S	3	Case-Control	Links	Genome Analyzer IIX
phs000842.v1.p1 PediGFR	Version 1: passed embargo	V D A S	1572	Multicenter, Prospective, Observational, Cohort	Links	HumanOmni2.5-Quad
phs000007.v25.p9 Framingham Cohort	Versions 1-22: passed embargo Version 23: 2015-04-25 Version 24: 2015-09-25 Version 25: 2015-12-23	V D A S	15173	Longitudinal	Links	HuGeneFocused50K_Affy Mapping250K_Nsp Mapping250K_Sty Mapping50K_Hind240 Mapping50K_Xba240
phs000825.v1.p1 Whole Genome Sequencing of HUES63 and HUES64	Version 1: passed embargo	V D A S	2	Control Set	Links	HiSeq 2000 HiSeq 2500

List Top Level Studies

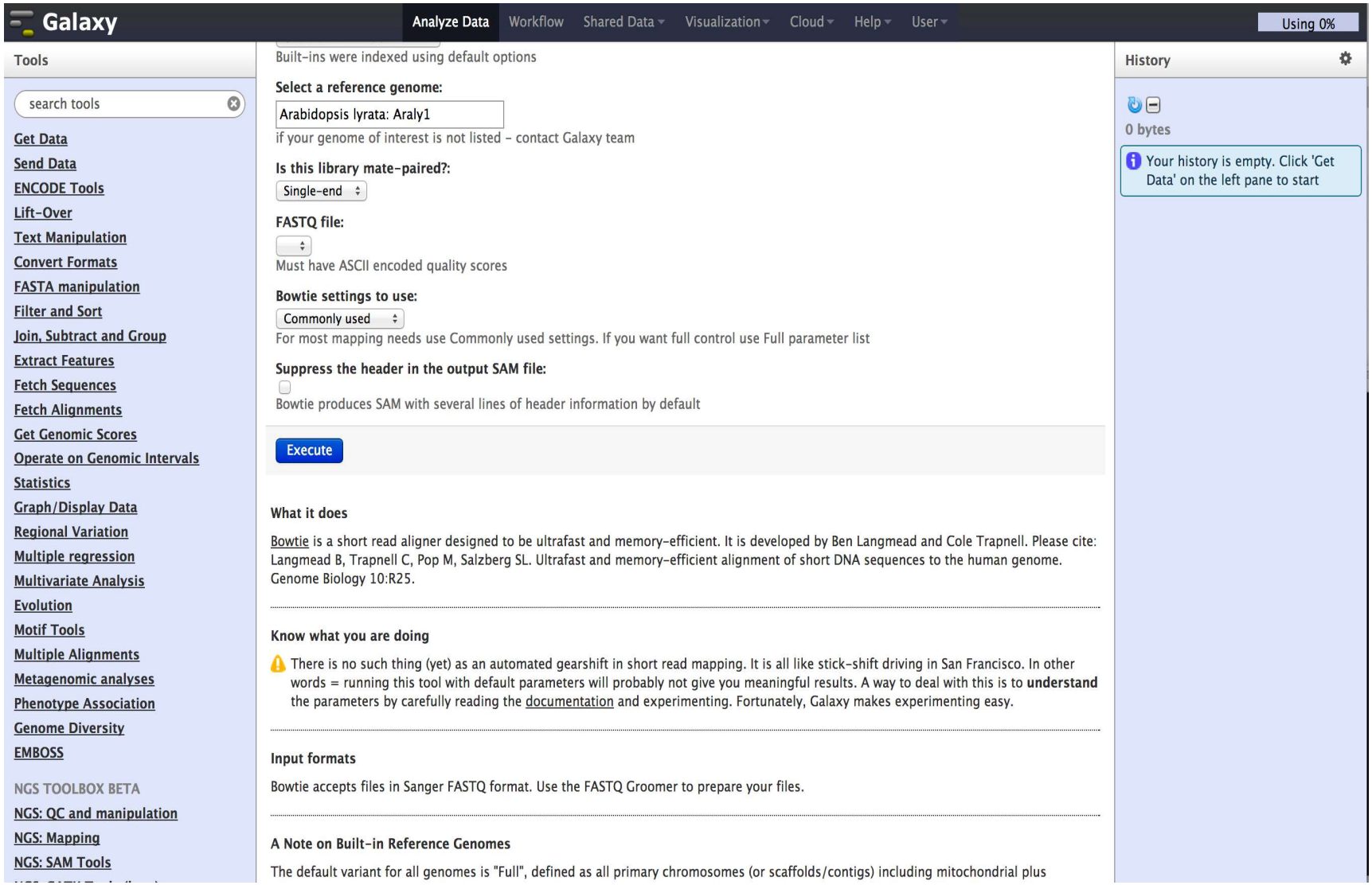
You are here: NCBI > Genetics & Medicine > Database of Genotypes and Phenotypes (dbGaP) Write to the Help Desk

Galaxy

- Galaxy is a useful web-based application for the manipulation of NGS and non-NGS data sets
  - https://main.g2.bx.psu.edu/
- It contains many of the same utilities discussed today, and provides a more standardized approach to analyzing NGS
- However, it requires the uploading of data to their server, which typically precludes its application to protected data sets (e.g. human samples)
- You are also limited to only those tools which have been incorporated into their system

Galaxy Website

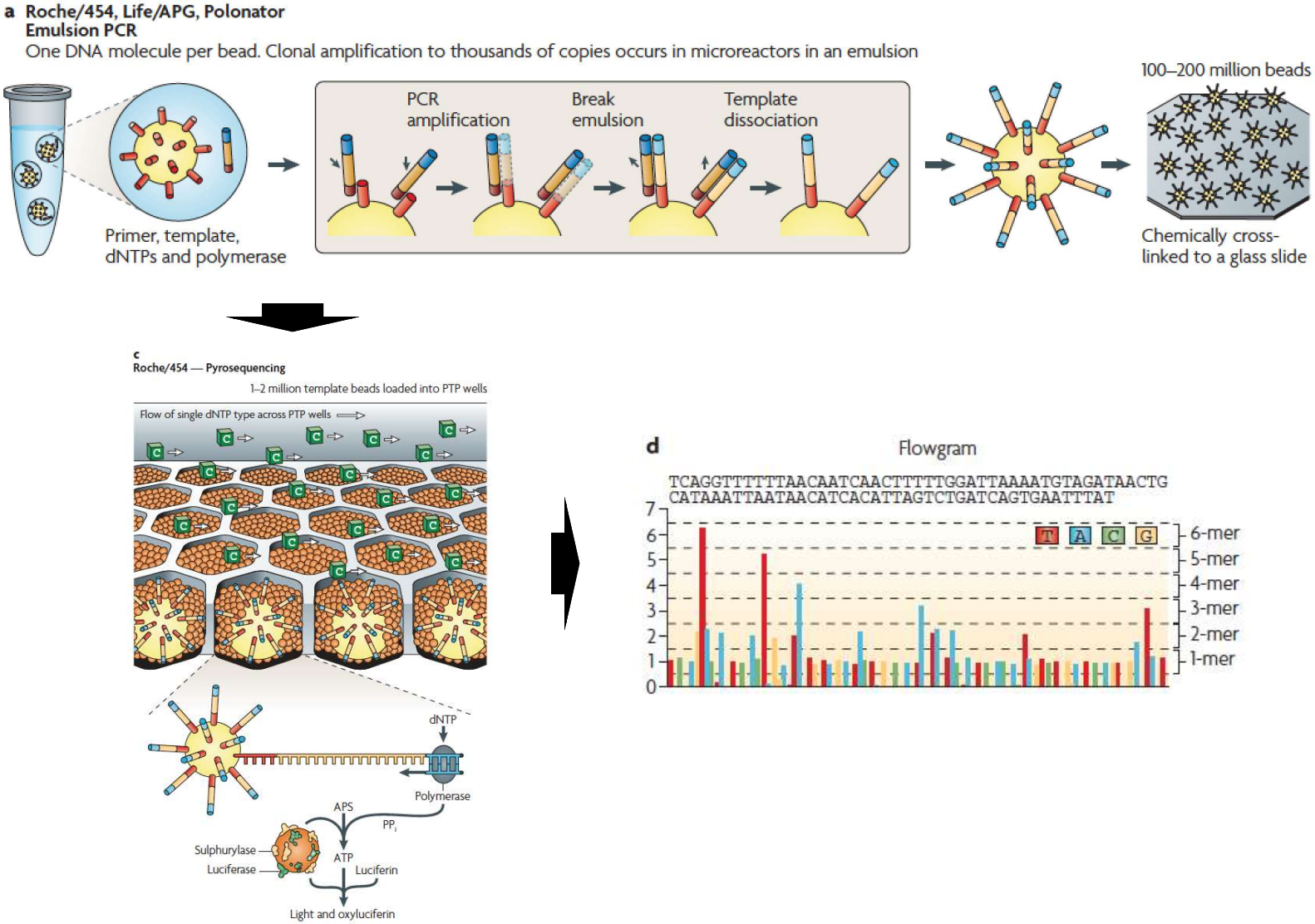
University of Michigan
Medical School



Additional Slides for Reference

University of Michigan
Medical School

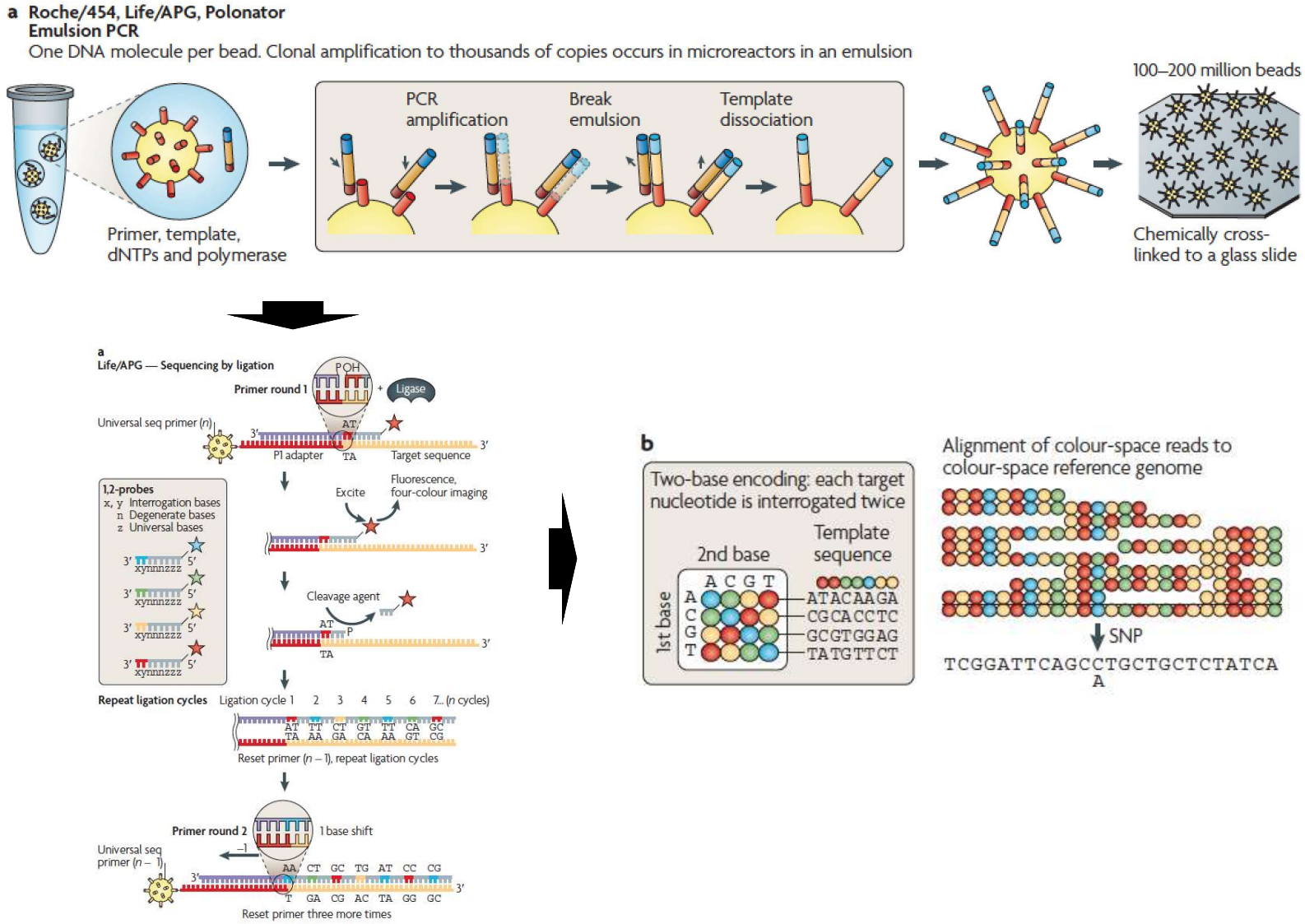
Roche 454 - Pyrosequencing



Metzker, ML (2010), *Nat. Rev. Genet*, 11, pp. 31-46

Life Technologies SOLiD – Sequence by Ligation

University of Michigan Medical School

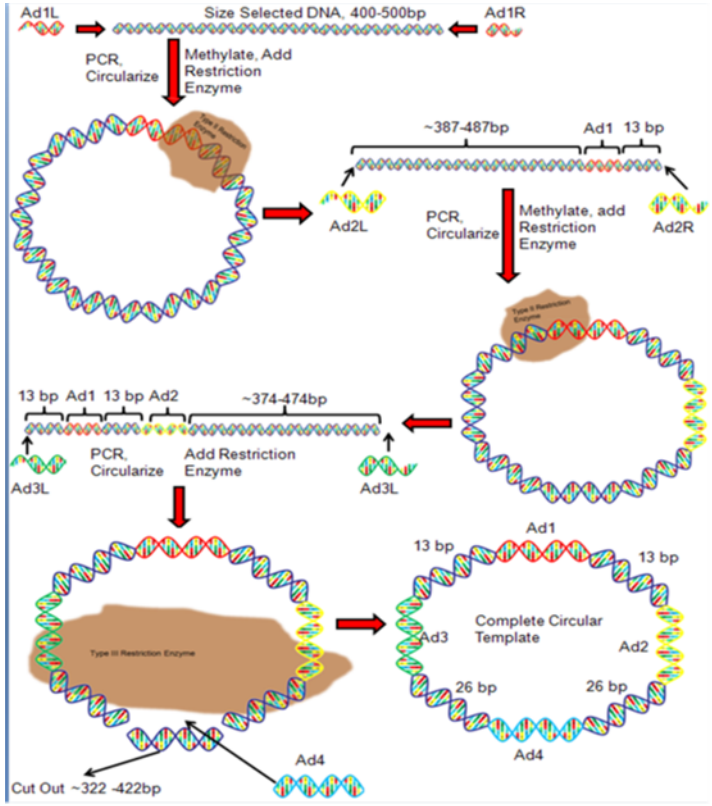


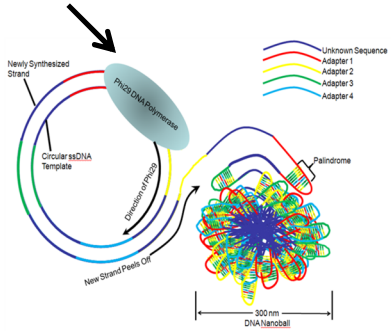
Metzker, ML (2010), *Nat. Rev. Genet*, 11, pp. 31-46

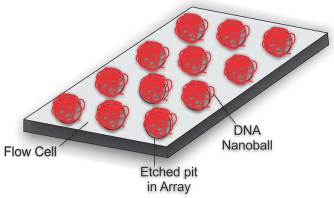
Complete Genomics – Nanoball Sequencing

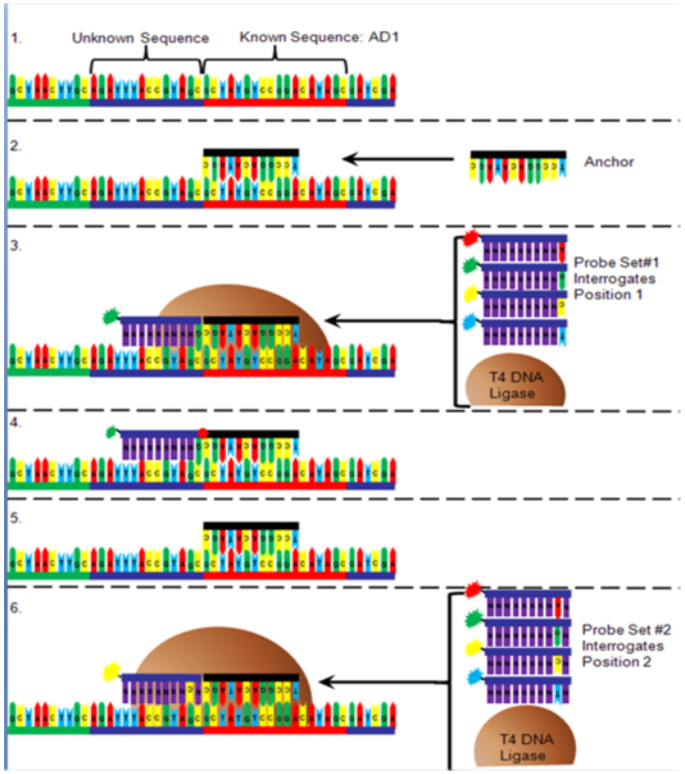
University of Michigan Medical School

Has proofreading ability!









Niedringhaus, TP et al (2011), *Analytical Chem.*, 83, pp. 4327-4341

Wikipedia, "DNA Nanoball Sequencing", September 26, 2012

"Benchtop" Sequencers

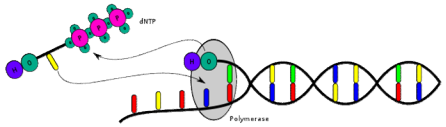
University of Michigan Medical School

- Lower cost, lower throughput alternative for smaller scale projects
- Currently three significant platforms
  - Roche 454 GS Junior
  - Life Technology Ion Torrent
    - Personal Genome Machine (PGM)
    - Proton
  - Illumina MiSeq

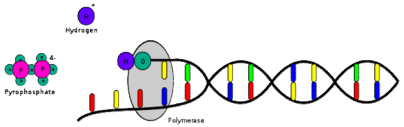
Platform	List price	Approximate cost per run	Minimum throughput (read length)	Run time	Cost/Mb	Mb/h
454 GS Junior	$108,000	$1,100	35 Mb (400 bases)	8 h	$31	4.4
Ion Torrent PGM						
(314 chip)	$80,490[a,b]	$225[c]	10 Mb (100 bases)	3 h	$22.5	3.3
(316 chip)		$425	100 Mb[d] (100 bases)	3 h	$4.25	33.3
(318 chip)		$625	1,000 Mb (100 bases)	3 h	$0.63	333.3
MiSeq	$125,000	$750	1,500 Mb (2 × 150 bases)	27 h	$0.5	55.5

Loman, NJ (2012), *Nat. Biotech.*, 5, pp. 434-439

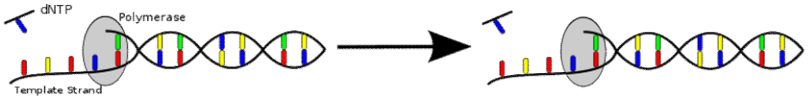
PGM - Ion Semiconductor Sequencing



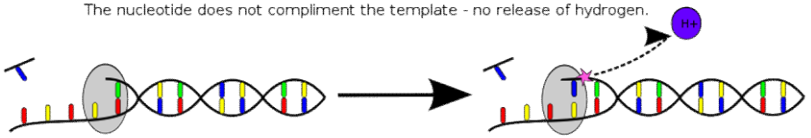
Polymerase integrates a nucleotide.



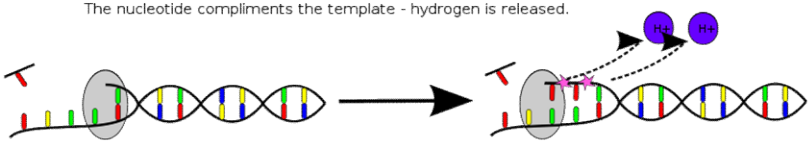
Hydrogen and pyrophosphate are released.



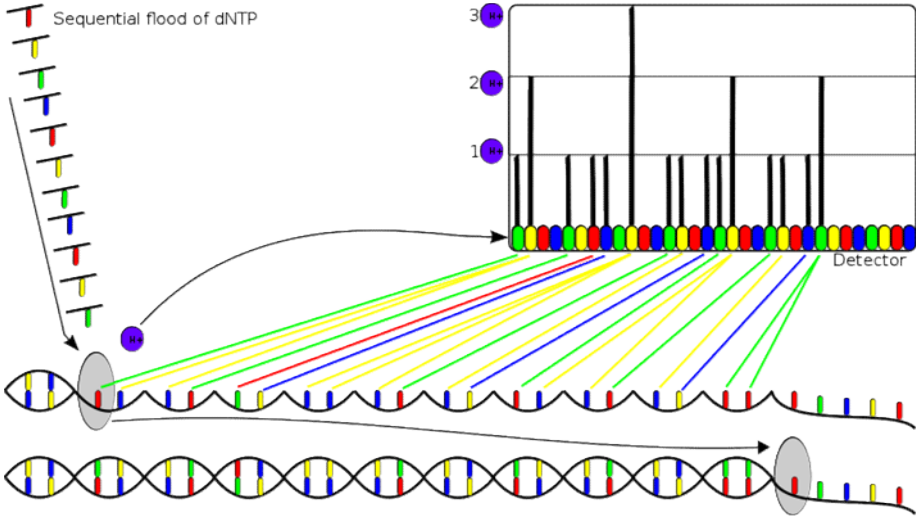
The nucleotide does not compliment the template - no release of hydrogen.



The nucleotide compliments the template - hydrogen is released.



The nucleotide compliments several bases in a row - multiple hydrogen ions are released.



Wikipedia, "Ion Semiconductor Sequencing", September 26, 2012