CS681: Advanced Topics in Computational Biology

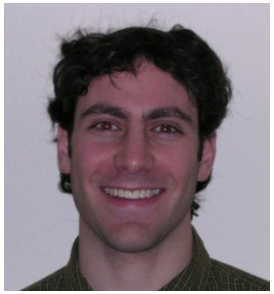
Week 1, Lectures 2-3

Can Alkan
EA509
calkan@cs.bilkent.edu.tr

http://www.cs.bilkent.edu.tr/~calkan/teaching/cs681/

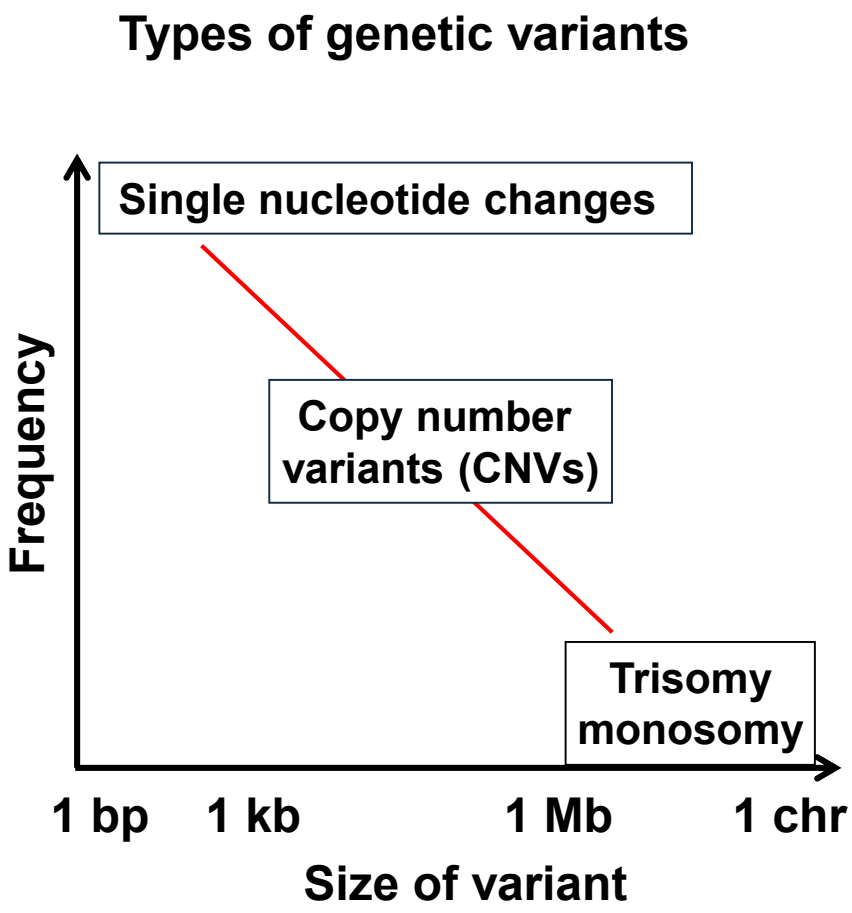
GENOMIC VARIATION:
CHANGES IN DNA SEQUENCE

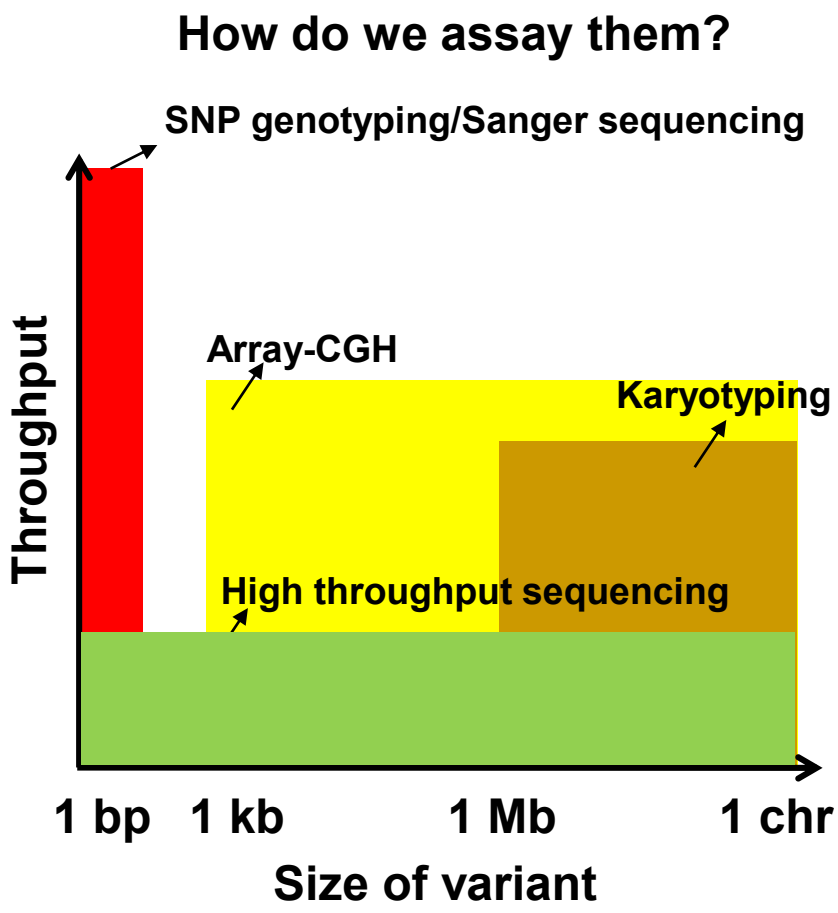
Human genome variation



- Genomic variation
  - Changes in DNA sequence
- Epigenetic variation
  - Methylation, histone modification, etc.

Human genetic variation





Size range of genetic variation

- Single nucleotide (SNPs)
- Few to ~50bp (small indels, microsatellites)
- >50bp to several megabases (**structural variants)**:
  - Deletions
  - Insertions
    - Novel sequence
    - Mobile elements (*Alu*, L1, SVA, etc.)
  - Segmental Duplications
    - Duplications of size ≥ 1 kbp and sequence similarity ≥ 90%
  - Inversions
  - Translocations
- Chromosomal changes

CNVs

Genetic variation

If a mutation occurs in a codon:

- Synonymous mutations: Coded amino acid doesn't change
- Nonsynonymous mutations: Coded amino acid changes

GTT → Valine

GTA → Valine

SYNONYMOUS

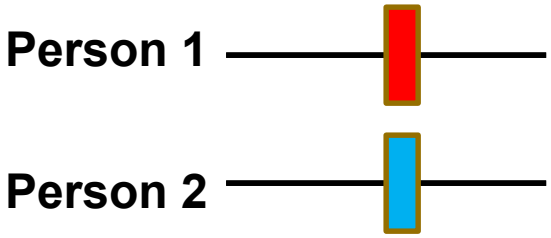
GTT → Valine

GCA → Alanine

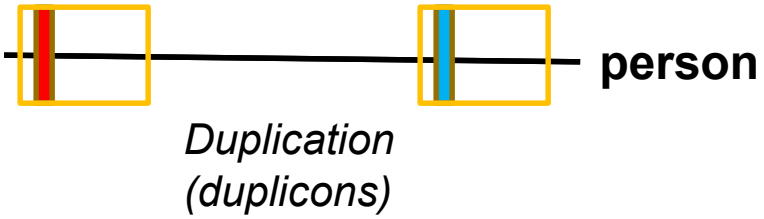
NONSYNONYMOUS

Genetic variation

Where in the genome?



ALLELIC VARIATION



NONALLELIC (PARALOGOUS) VARIATION

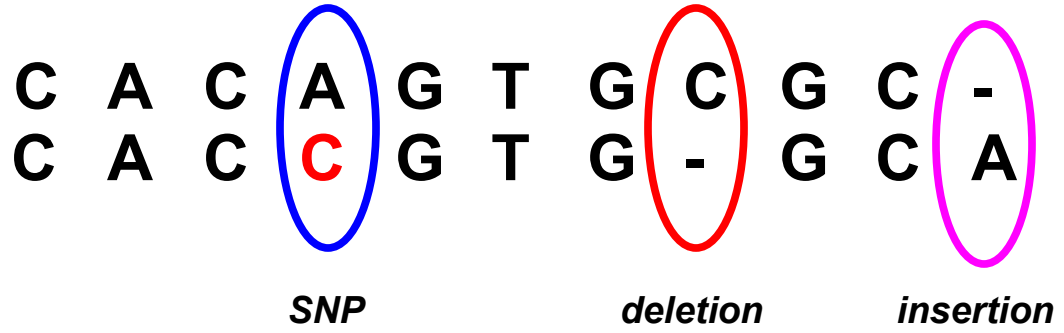
Where in the body?

Germ cells or gametes (sperm egg) -> Transmittable -> Germline Variation

Other (somatic cells) -> Not transmittable -> Somatic Variation

SNPs & indels

SNP: Single nucleotide polymorphism (substitutions)
Short indel: Insertions and deletions of sequence of length 1 to 50 basepairs



- Neutral: no effect
- Positive: increases fitness (resistance to disease)
- Negative: causes disease
- Nonsense mutation: creates early stop codon
- Missense mutation: changes encoded protein
- Frameshift: shifts basepairs that changes codon order

Short tandem repeats

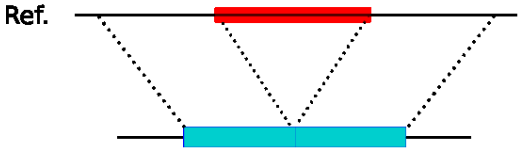
reference: **C A G C A G C A G C A G**

sample: **C A G C A G C A G *C A G* C A G**

- Microsatellites (STR=short tandem repeats) 1-10 bp
  - Used in population genetics, paternity tests and forensics
- Minisatellites (VNTR=variable number of tandem repeats): 10-60 bp
- Other satellites
  - Alpha satellites: centromeric/pericentromeric, 171bp in humans
  - Beta satellites: centromeric (some), 68 bp in humans
  - Satellite I (25-68 bp), II (5bp), III (5 bp)
- Disease relevance:
  - Fragile X Syndrome
  - Huntington's disease

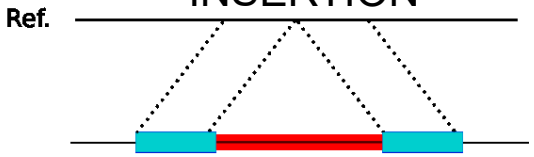
Structural Variation

DELETION

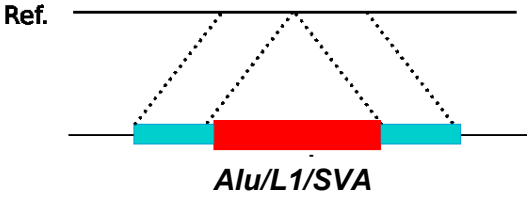


Autism, mental retardation, Crohn's

NOVEL SEQUENCE INSERTION

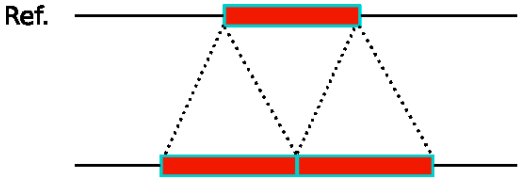


MOBILE ELEMENT INSERTION

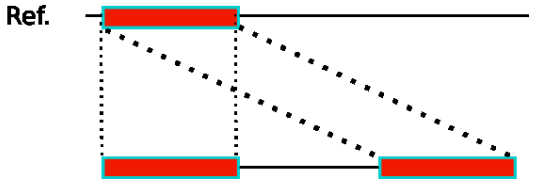


Haemophilia

TANDEM DUPLICATION

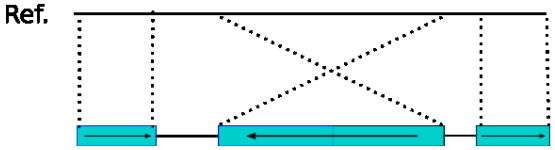


INTERSPERSED DUPLICATION

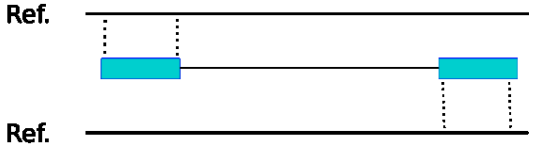


Schizophrenia, psoriasis

INVERSION



TRANSLOCATION



Chronic myelogenous leukemia

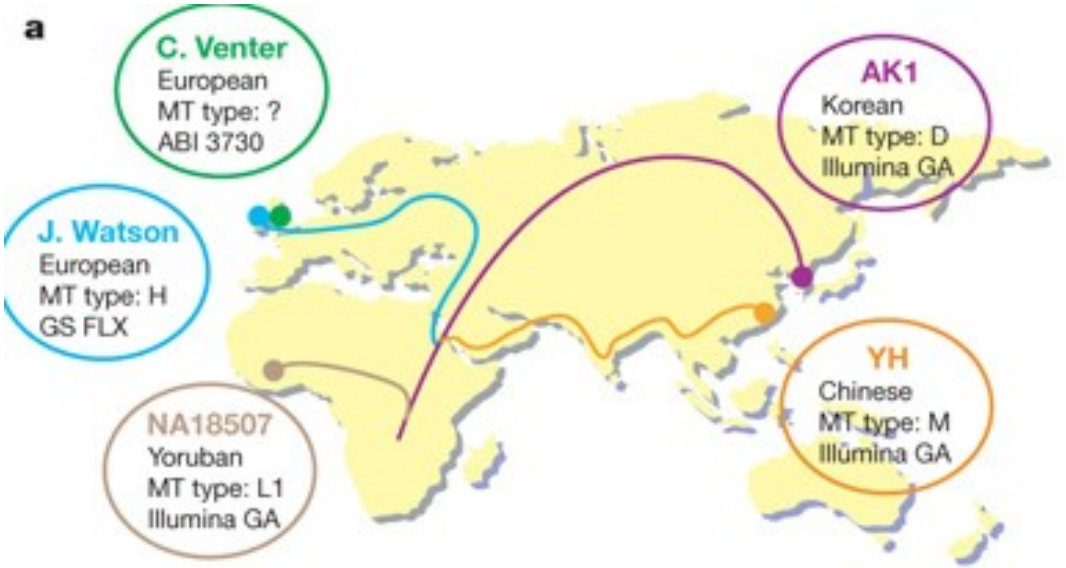
Chromosomal changes

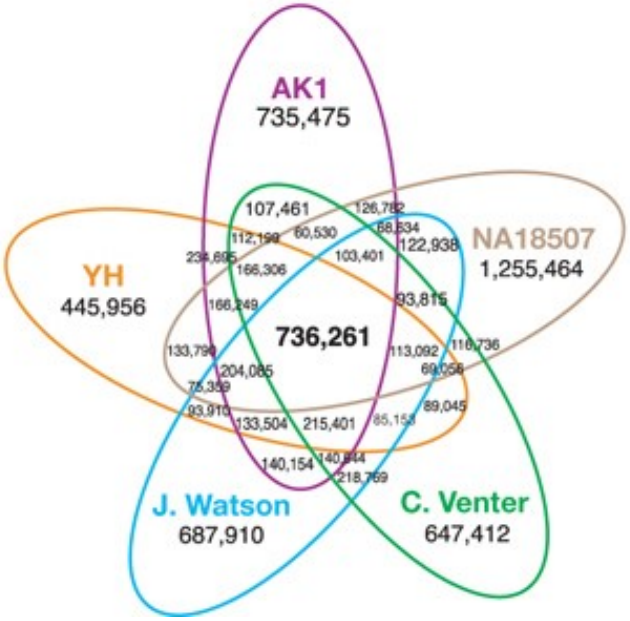
- “Microscope-detectable”
- Disease causing or prevents birth
- Monosomy: 1 copy of a chromosome pair
- Uniparental disomy (UPD): Both copies of *a* pair comes from the same parent
- Trisomy: Extra copy of a chromosome
  - chr21 trisomy = Down syndrome

Genetic variation among humans

Single nucleotide variants in four human genomes		
	(n)	**In dbSNP (%)**
J. Craig Venter's genome	3,213,401	91.0
James D. Watson's genome	3,322,093	81.7
Asian genome	3,074,097	86.4
Yoruban genome	4,139,196	73.6
Structural variants in the Venter genome		
	(n)	**length (bp)**
Block substitutions	53,823	2–206
Indels (heterozygous)	851,575	1–82,711
Inversions	90	7–670,345
Copy number variants	62	8,855–1,925,949

Genetic variation are "shared"

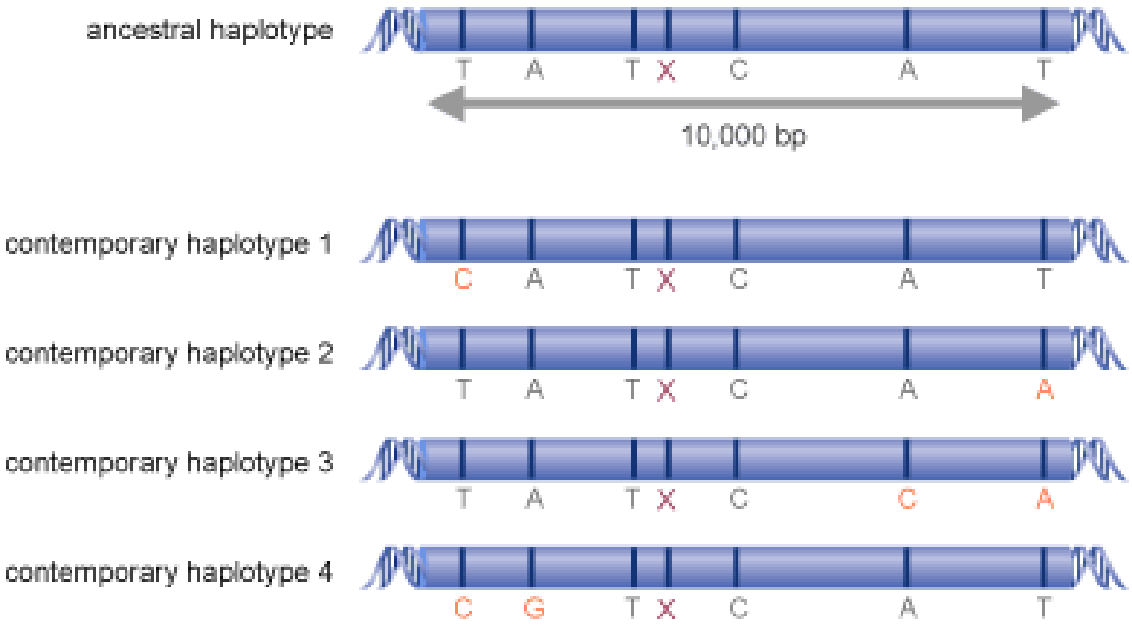




Kim *et al.* Nature, 2009

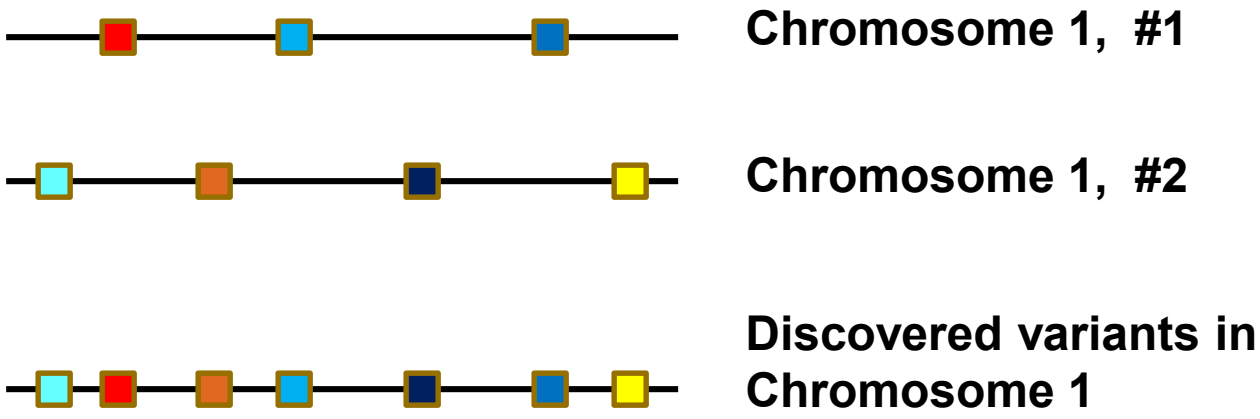
Haplotype

- "Haploid Genotype": *a combination of alleles at multiple loci that are transmitted together on the same chromosome*



Haplotype resolution

- Variation discovery methods do not directly tell which copy of a chromosome a variant is located
- For heterozygous variants, it gets messy:



Haplotype resolution or haplotype phasing: finding which groups of variants "go together"

Discovery vs. genotyping

- Discovery: no *a priori* information on the variant
- Genotyping: test whether or not a "suspected" variant occurs

Variation discovery & genotyping

- Targeted methods:
  - SNP:
    - PCR
    - SNP microarray (genotyping)
  - Indel
    - PCR
    - “Indel microarray” (genotyping)
  - Structural variation
    - Quantitative PCR
    - Array Comparative Genomic Hybridization (array CGH)
    - Fluorescent *in situ* Hybridization (FISH) if variant > 500 kb
  - Chromosomal:
    - Microscope

Variation discovery & genotyping

- Targeted methods are:
  - Cheap(er), but limited:
    - Variants that are not in reference genome cannot be found
    - One experiment yields one type of variant
    - Not always genome-wide
- Alternative:
  - Whole genome resequencing
    - More expensive – getting cheaper
    - (Theoretically) comprehensive
    - Computational challenges

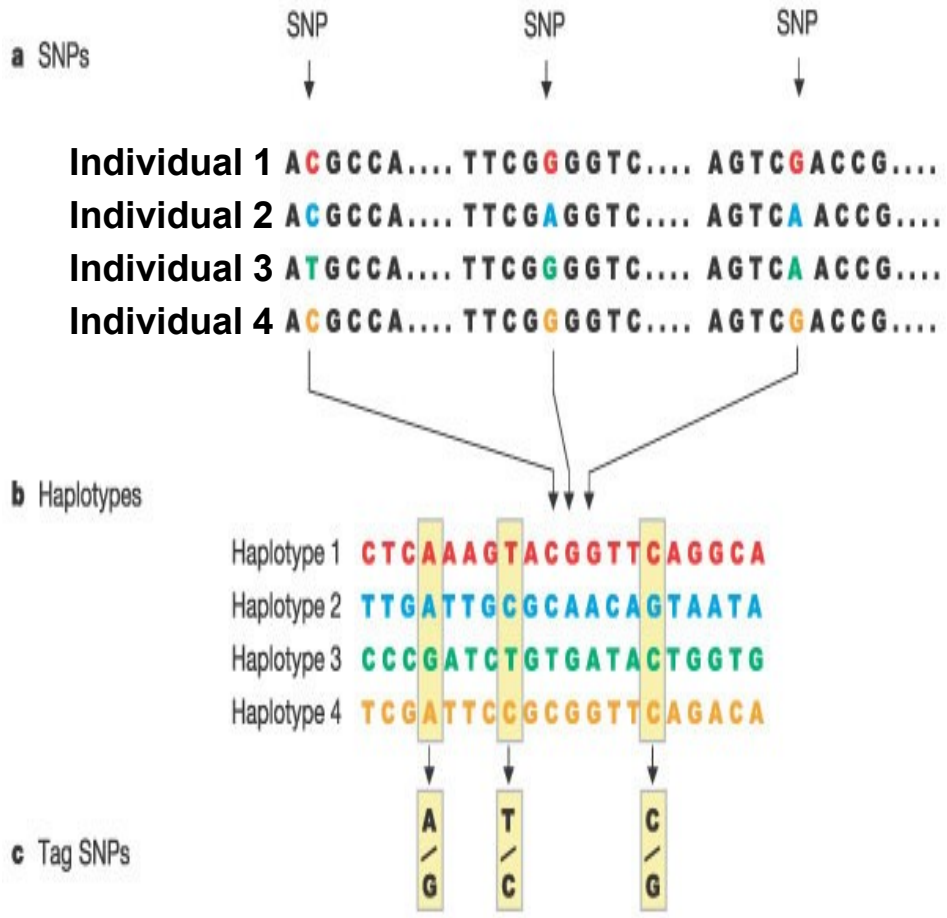
PROJECTS FOR GENOMIC VARIATION DISCOVERY

International HapMap Project

- Determine genotypes & haplotypes of 270 human individuals from 3 diverse populations:
  - Northern Americans (Utah / Mormons)
  - Africans (Yoruba from Nigeria)
  - Asians (Han Chinese and Japanese)
- 90 individuals from each population group, organized into parent-child **trios**.
- Each individual genotyped at ~5 million roughly evenly spaced markers (SNPs and small indels)

http://www.hapmap.org

HapMap Project



Step 1: SNPs are identified in DNA samples from multiple indivduals

Step 2: Adjacent SNPs that are inherited together are compiled into "haplotypes."

Step 3: "Tag" SNPs within haplotypes are identified that uniquely identify those haplotypes

By genotyping just the three tag SNPs shown above, one can identify which of the four haplotypes shown here are present in each individual.

Human Genome Diversity Panel

- More extensive set of genomic variation
- One aim is to build DNA resource libraries for large scale discovery & genotyping projects
- 1.050 human individuals from 52 populations

Initial HapMap and HGDP did not sequence the genomes of any samples.

ARTICLE

doi:10.1038/nature18964

The Simons Genome Diversity Project: 300 genomes from 142 diverse populations

Mallick et al., 2016

Why?

- To understand “normal” human genomic variation
- To understand genetic transmission properties
- To understand *de novo* mutations
- To understand population structure, migration patterns
- To understand human disease
  - Find causal variants
  - Diagnose
  - Guide treatment

Human disease

- Rare variant common disease:
  - Most “complex” diseases, including neuropsychiatric diseases
- Common variant common disease
  - More “common”; diseases that follow Mendelian inheritance
    - If a common disease is caused by a recessive mutation, it can be found at high frequency in a population
      - MAF (minor allele frequency) > 5%

Why sequence whole genomes?

- SNP/indel/arrayCGH platforms are mainly designed for individuals of West European descent
- For a disease common in somewhere else, like India:
  - Variants at high frequency in India may not be represented in the available platforms
  - Genome is a big entity; SNP/indel/arrayCGH can not cover the entire genome:
    - Largest has 2.1 million markers (compare to 3 billion)

High Throughput Sequencing

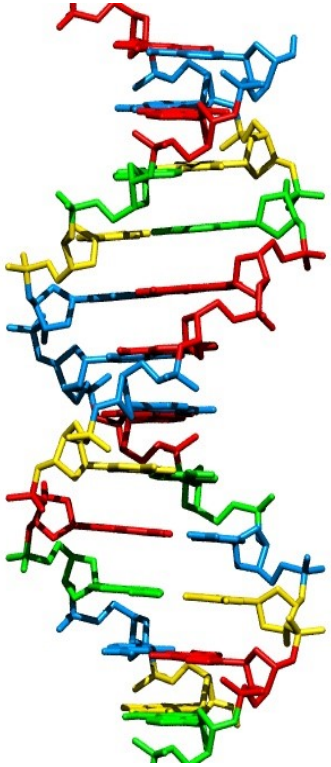
- 2007: “Sanger”-based capillary sequencing; one human genome (WGS): ~ $10 million (Levy et al., 2007)
- 2008: First “next-generation” sequencer 454 Life Sciences; genome of James Watson: ~$2 million (Wheeler et al., 2008)
- 2008: The Illumina platform; genome of an African (Bentley et al, 2008) and an Asian (Wang et al., 2008): ~$200K each
- 2009: The SOLiD platform: ~$200K
- Today with the Illumina platform: ~$1K/ genome

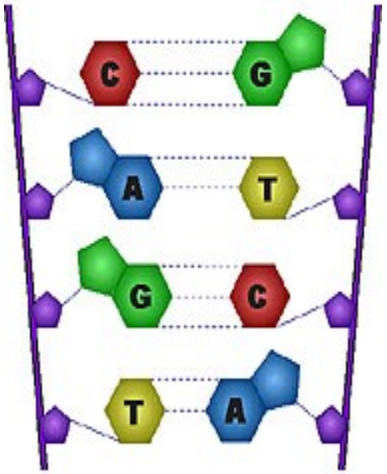
Sequencing-based projects

- The 1000 Genomes Project Consortium (www.1000genomes.org)
  - Large consortium: groups from USA, UK, China, Germany, Canada
  - 2.504 humans from 29 populations
- Independent
  - South African (Schuster et al., 2010), Korean, Japanese, UK (UK100K project), Ireland, Netherlands (GoNL project), France, US All of Us, …
- Ancient DNA: Neandertal (Green et al., 2010); Denisova (Reich et al., 2010)

DNA sequencing

How we obtain the sequence of nucleotides of a species





...ACGTGACTGAGGACCGTG
CGACTGAGACTGACTGGGT
CTAGCTAGACTACGTTTTA
TATATATATACGTCGTCGT
ACTGATGACTAGATTACAG
ACTGATTTAGATACCTGAC
TGATTTTAAAAAAATATT...

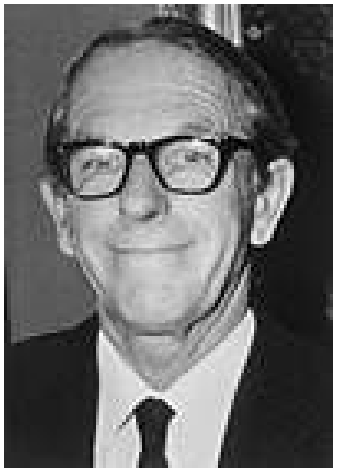
DNA Sequencing

GENERAL CONCEPTS AND CAPILLARY (SANGER) SEQUENCING

DNA Sequencing: History

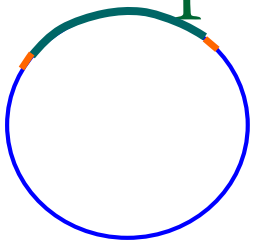
Sanger method (1977): labeled ddNTPs terminate DNA copying at random points.

Gilbert method (1977): chemical method to cleave DNA at specific points (G, G+A, T+C, C).

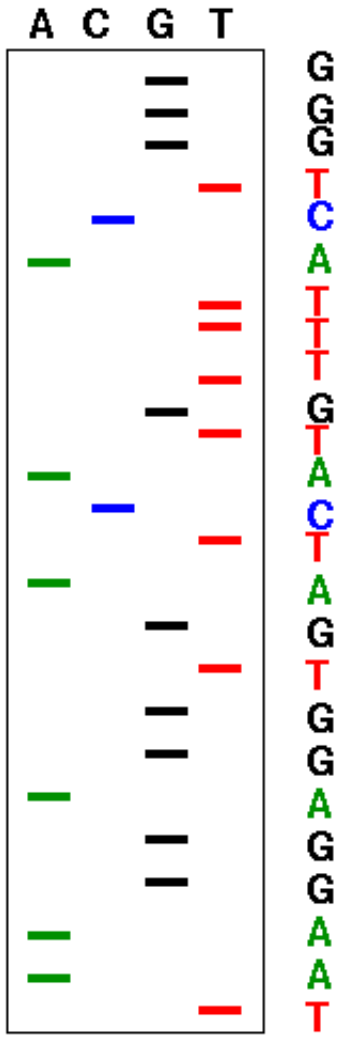


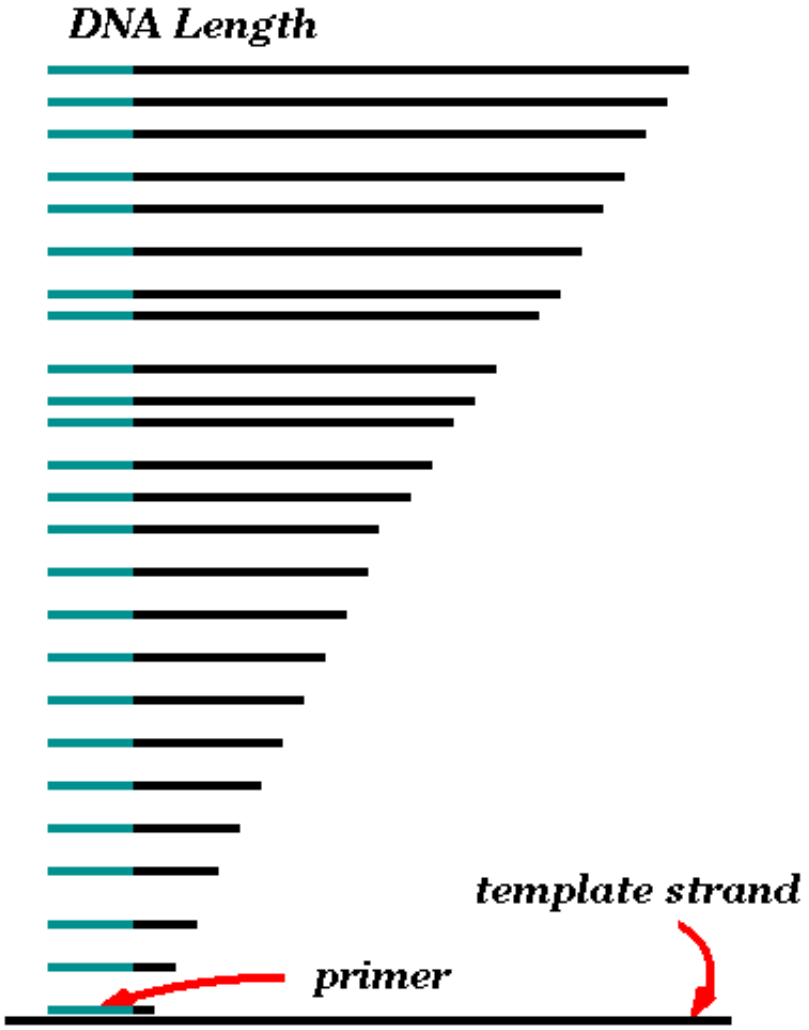
Both methods generate labeled fragments of varying lengths that are further electrophoresed.

DNA sequencing – gel electrophoresis



1. Start at primer (restriction site)
2. Grow DNA chain
3. Include dideoxynucleotide (modified a, c, g, t)
4. Stops reaction at all possible points
5. Separate products with length, using gel electrophoresis

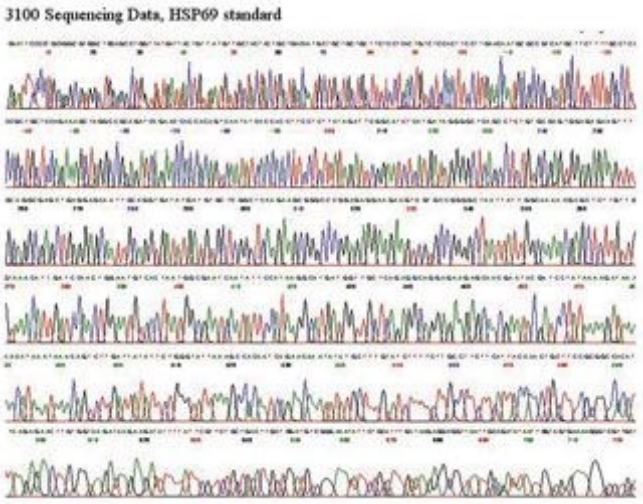




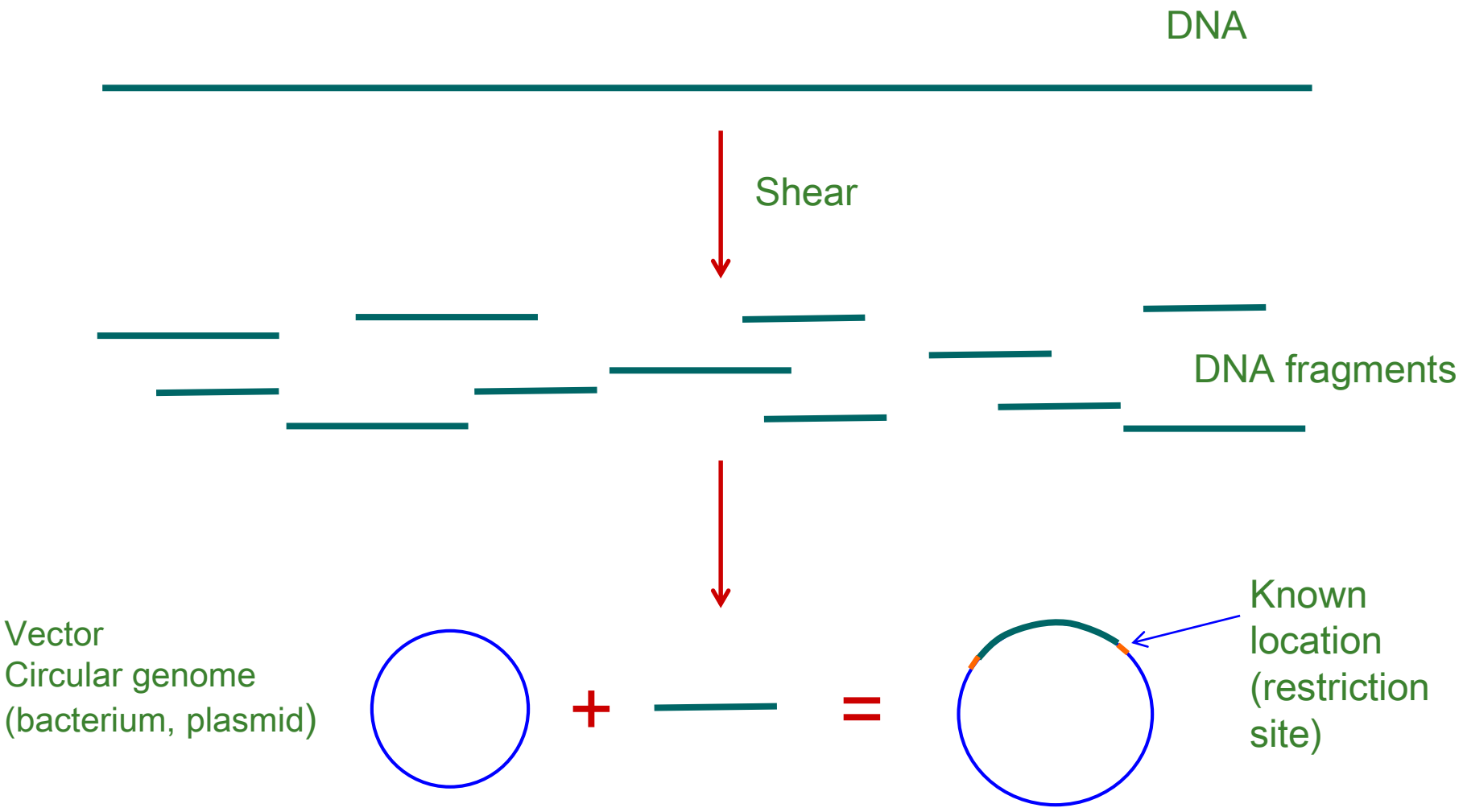
Capillary (Sanger) sequencing

Capillary sequencing (Sanger):

Can only sequence ~1000 letters at a time

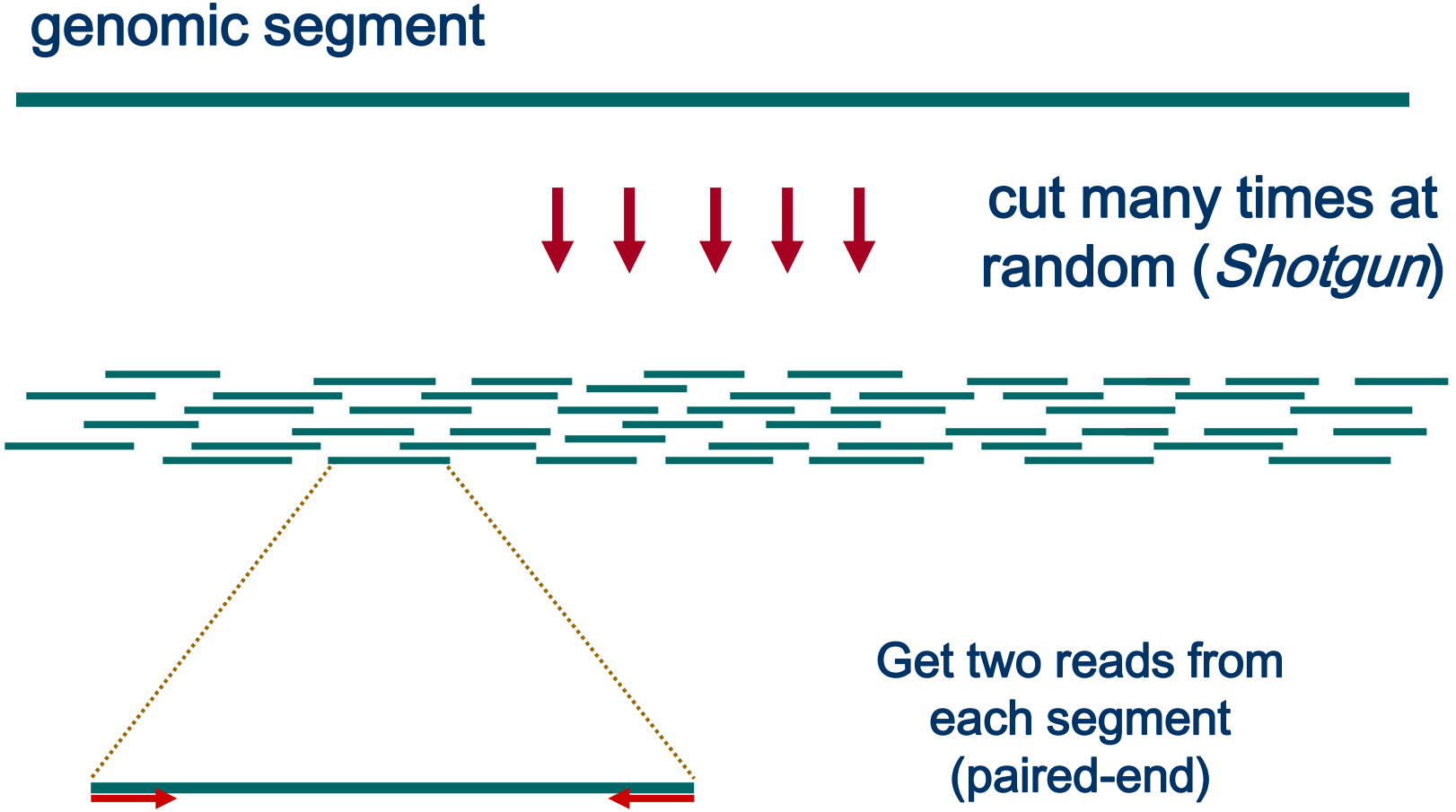


Traditional DNA Sequencing

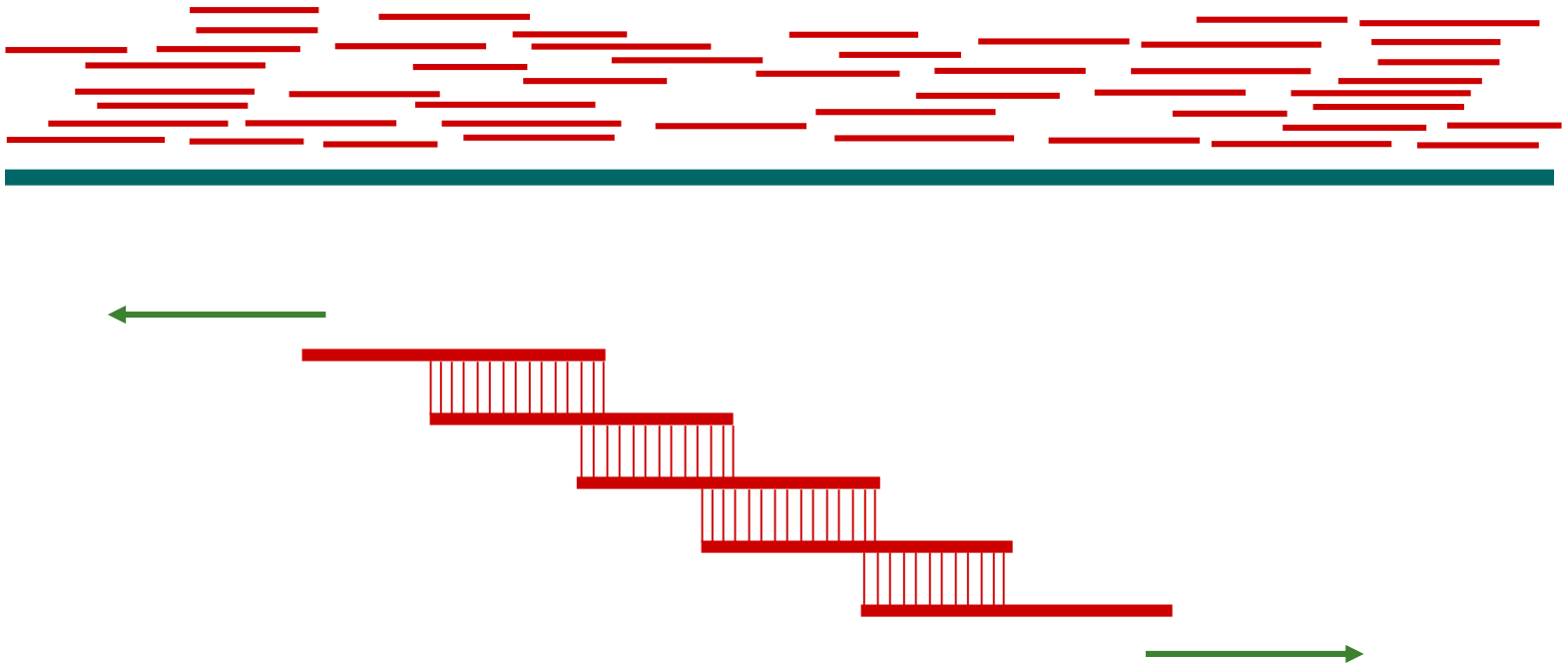


Double-barreled / paired-end sequencing

genomic segment



Reconstructing The Sequence



Need to cover region with >7-fold redundancy (7X) if you use Sanger technology

Overlap reads and extend to reconstruct the original genomic region

Definition of Coverage

Length of genomic segment: **L**
Number of reads: **n**
Length of each read: **l**

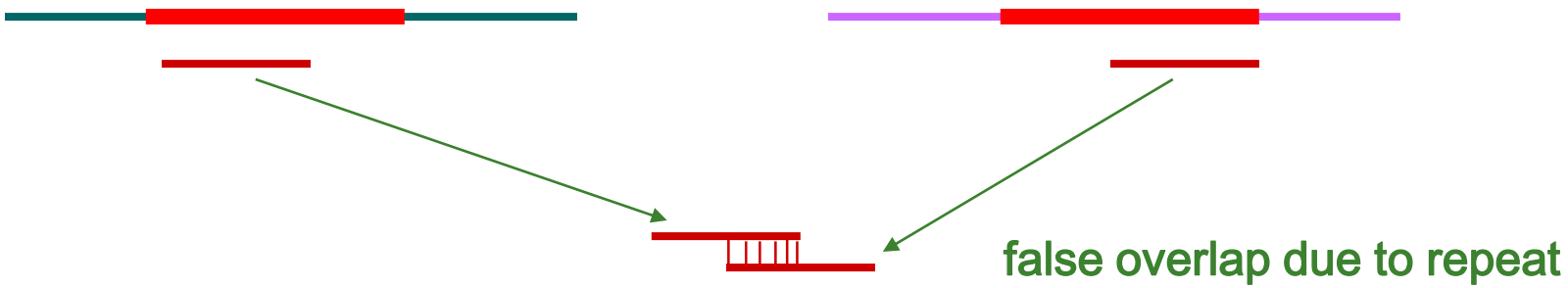
Definition: Coverage **C = n l / L**

How much coverage is enough?

Lander-Waterman model:
Assuming uniform distribution of reads, C=10 results in 1 gapped region /1,000,000 nucleotides

Challenges with Fragment Assembly

- **Sequencing errors**
  ~0.1% of bases are wrong
- **Repeats**



- **Computation: ~ O(N^2) where N = # reads**

Sanger sequencing

- Advantages
  - Longest read lengths possible today (>1000 bp)
  - Highest sequence accuracy (error < 0.1%)
  - Clone libraries can be used in further processing
- Disadvantages
  - The most expensive technology
    - $1500 per Mb
  - Building and storing clone libraries is hard & time consuming

HIGH THROUGHPUT SEQUENCING

Human genome reference

- 1986: Announced (USA+UK)
- 1990: Started
- 1999: Chromosome 22 sequenced
- 2001: First draft
- 2004: Finished

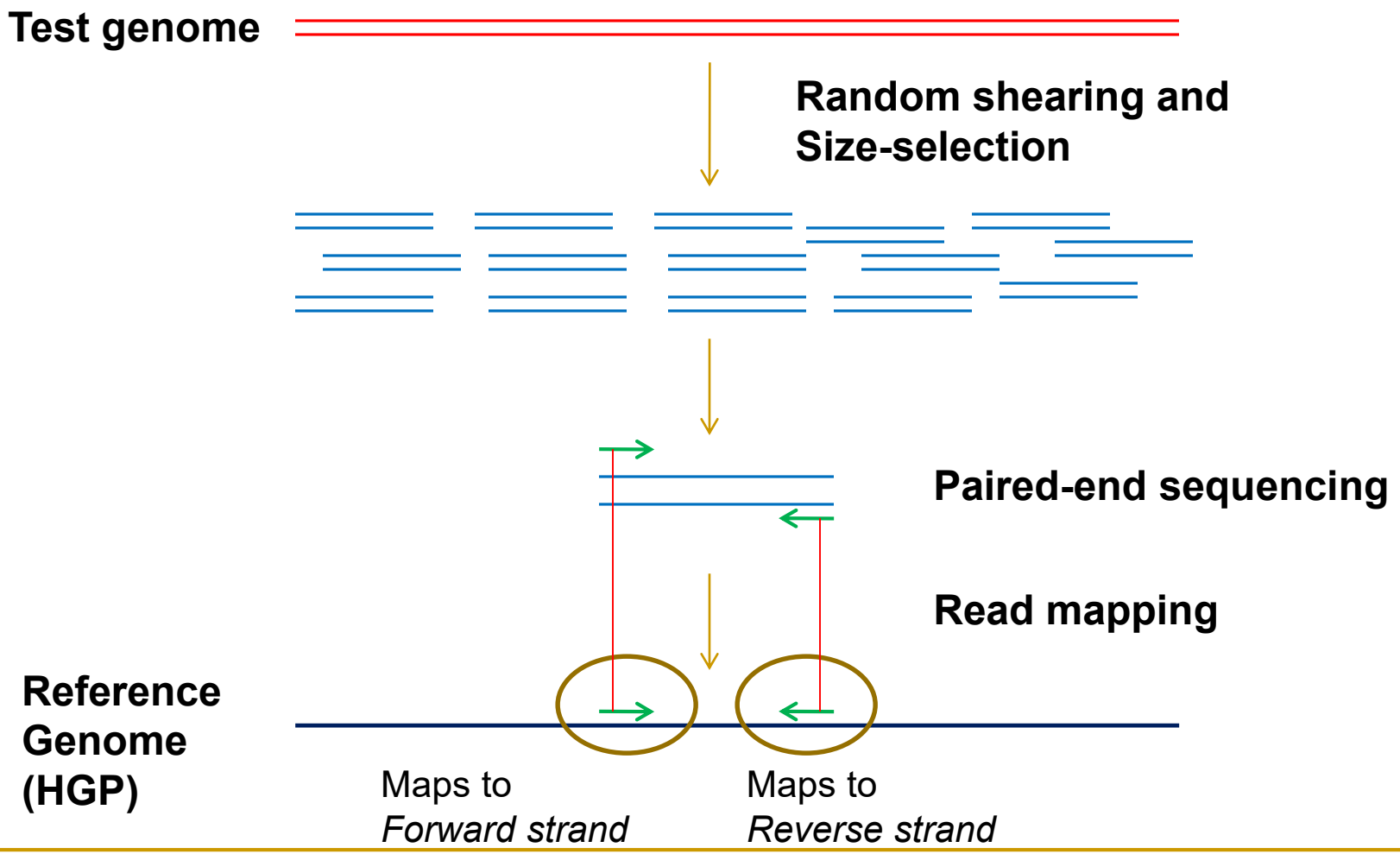
4 human samples, 14 years, 3-10 billion dollars

Current version: hg38

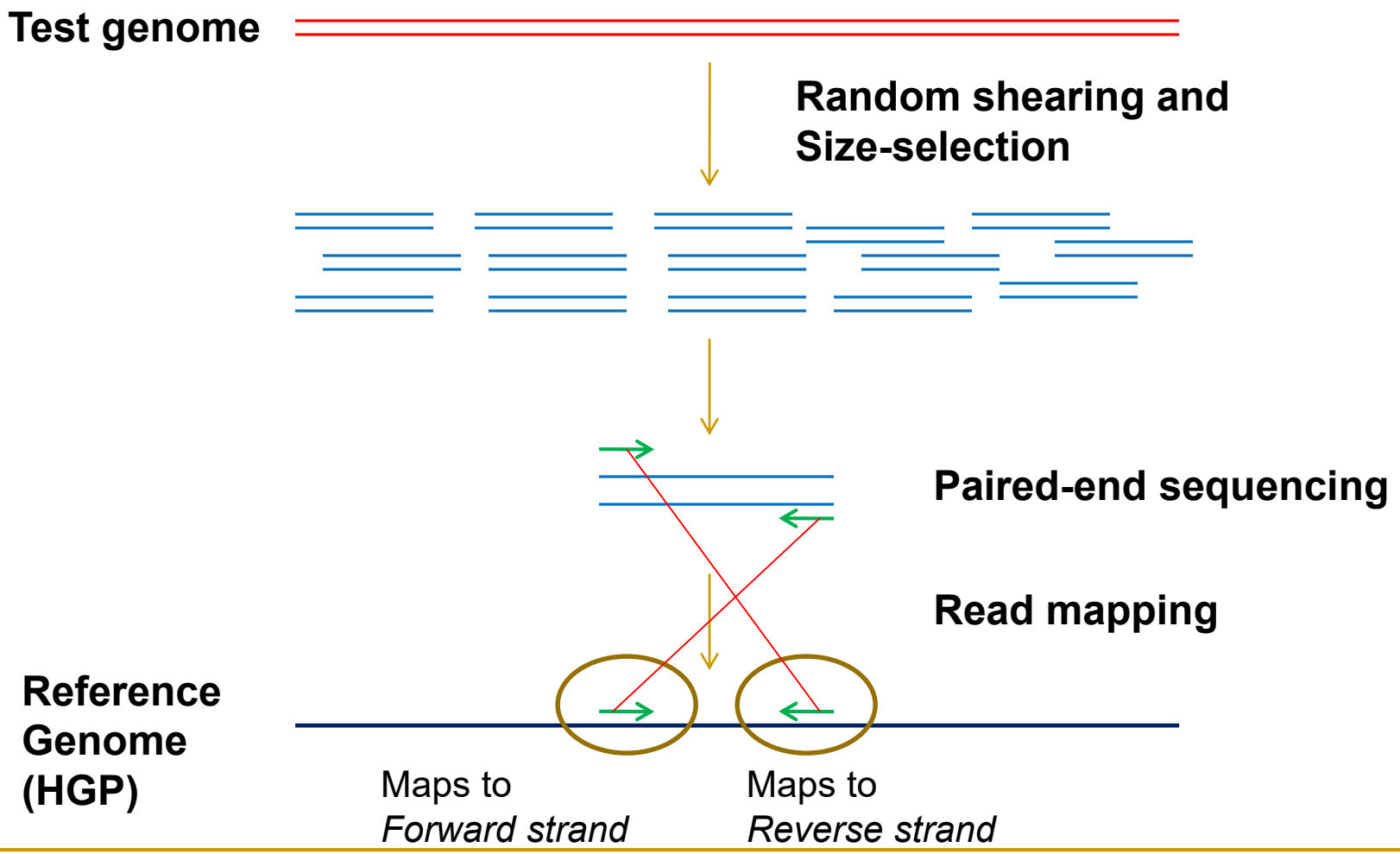
https://www.ncbi.nlm.nih.gov/grc

Chromosomes 1-22, X, Y, MT
Alternative haplotypes
HLA haplotypes

WGS revisited



WGS revisited

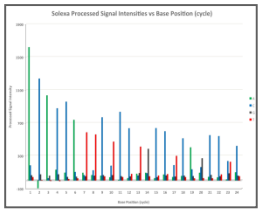


HTS Technologies

- Short read:
  - 454 Life Sciences: the first, acquired by Roche -- dead
    - *Pyrosequencing*
  - Illumina (Solexa): current market leader
    - *GAIIx, HiSeq2000, MiSeq, HiSeq2500, NovaSeq*
    - *Sequencing by synthesis*
  - Applied Biosystems -- dead
    - *SOLiD: "color-space reads"*
- Long Read:
  - Pacific Biosciences Single Molecule Real Time
    - *RSII, Sequel*
  - Oxford Nanopore Technologies:
    - *MinION, Flongle, PromethION, GridION*

Fundamental informatics challenges

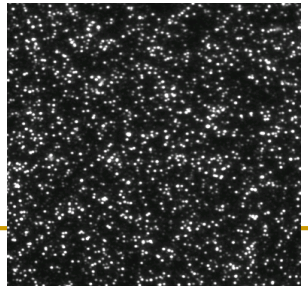
1. Interpreting machine readouts – base calling, base error estimation



```
>B_TITR_1_1_668_35 TIME: Tue Feb 20 02:26:06 2007
ATATCGGATGACACAATATGGGAGGTTGAC
>B_TITR_1_2_843_403 TIME: Tue Feb 20 02:26:06 2007
TGTAGCTTTTCATGACAATTTTATAGGTGT
>B_TITR_1_1_668_35
27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 27 6
>B_TITR_1_2_843_403
26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 24
>B_TITR_1_3_618_922
```

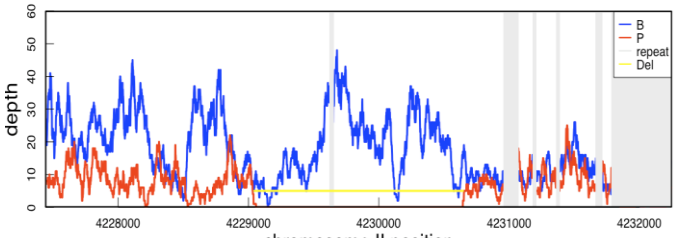
2. Data visualization

3. Data storage & management
Gzip compressed raw data for one human genome > 100 GB (Illumina)

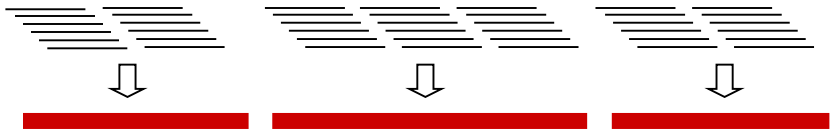


Informatics challenges (cont'd)

4. SNP, indel, and structural variation discovery



5. *De novo* Assembly



What can we use them for?

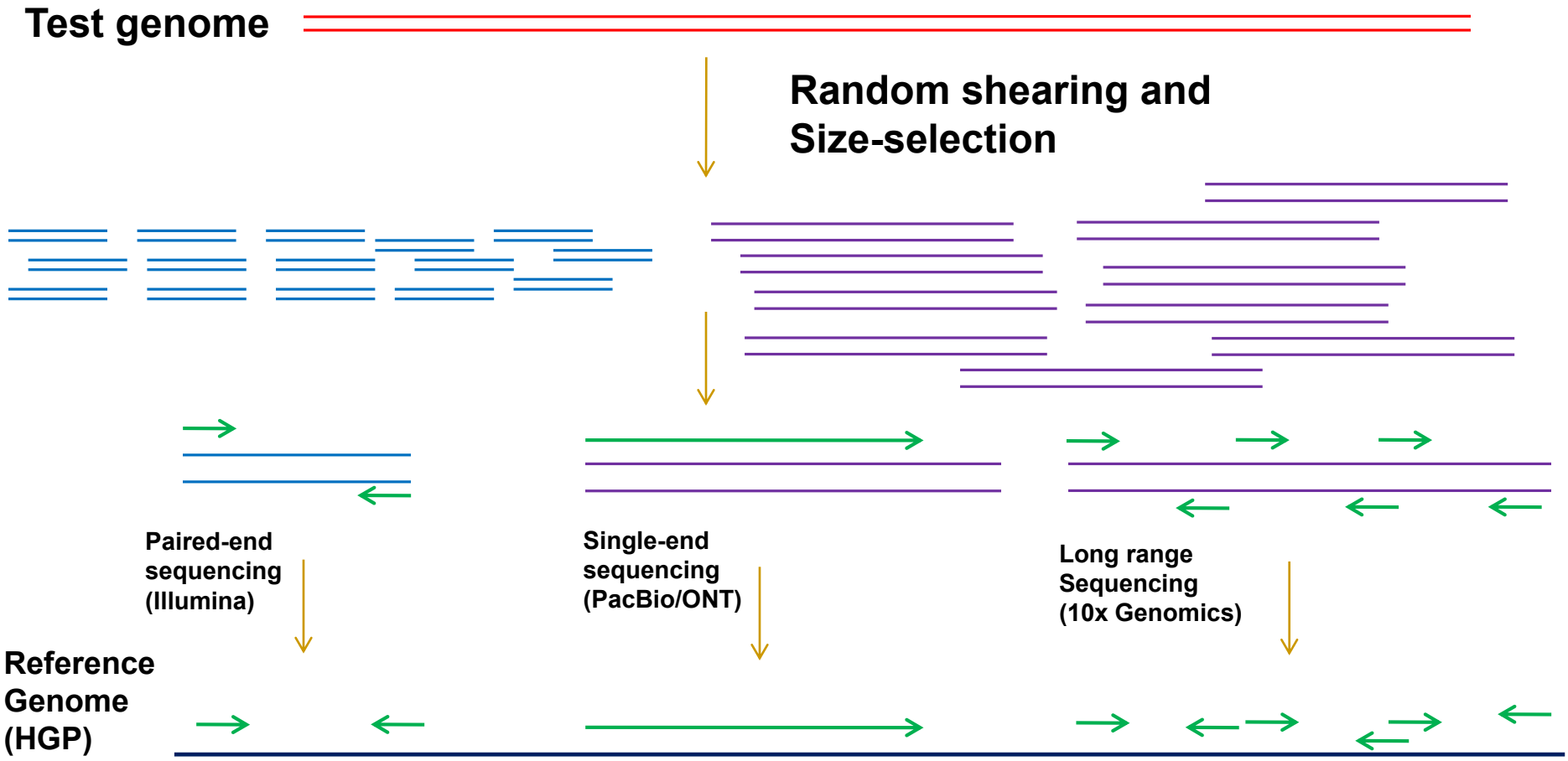
	Sanger	Illumina	PacBio	ONT
De novo assembly	Fragmented	Heavily Fragmented	Fragmented, needs polishing	Less Fragmented, needs polishing
SNP Discovery	Yes	Yes	Yes	Yes
Larger events	Yes	Mid-range	Yes	Yes
Transcript profiling	No	Yes	Somewhat	Somewhat

CURRENT PLATFORMS

Features of HTS data

- Short sequence reads
  - 150 - 300 bp Illumina
- Long, but error prone sequence reads
  - Average ~50 Kb PacBio - 12% error
  - Up to 1 Mb ONT – 20% error
- Huge amount of sequence per run
  - Up to terabases per run (3 Tbp for Illumina/NovaSeq 6000)
- Huge number of reads per run
  - Up to billions
- Higher error (compared with Sanger)
  - Illumina: mostly substitutions
  - PacBio / ONT: mostly indels

Whole Genome Sequencing



Sequencing technologies

Short-Read

Illumina

- 100-200bp
- Paired-end
- Billions of reads
- < 0.1% error

Long Read

PacBio and Oxford Nanopore

- > 10 Kb, up to 1 Mb
- Single-end
- Hundreds of millions of reads
- 12-20% error – indel dominated

Long Range

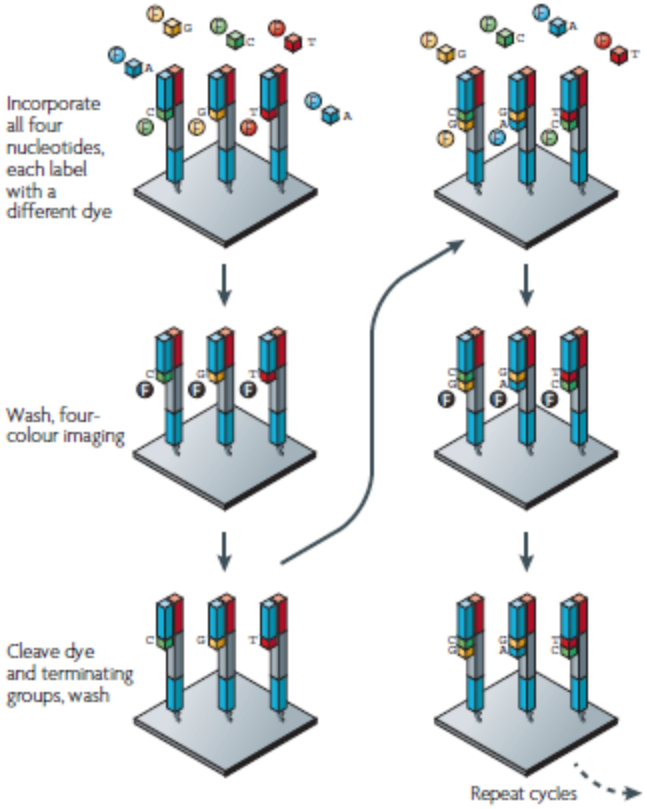
10X + Illumina

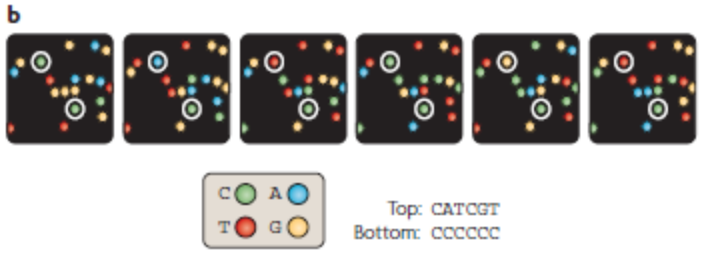
- 100-200bp
- Paired-end
- Billions of reads
- < 0.1% error
- Barcoded: 30-50 Kb molecule range

Illumina

- Current market leader
- Based on *sequencing by synthesis*
- Current read length 150-300bp
- Paired-end easy, longer matepairs harder
- Error ~0.1%
  - Substitution errors dominate
- Throughput: Up to 3 Tbp in one run (2 days)
- Cheapest sequencing technology
  - Cost: ~ $1,000 per human

Illumina





NovaSeq

MiSeq

HiSeq 2000/2500

Illumina – FASTQ output

Read and Quality (1)

@FC81ET1ABXX:3:1101:1215:2154/1
TTTTTCAAATGTTTGTTGCCTATTTTTATATCTTCTTTTGAGAATTGTCTGTTCATGTCNTNNGNNCNCNNTNTCANGGGATTGTTTGTT
+
HHGHHHHHGHHHHDHFHHHHHHHFHHHHHHEHHEHHHHEGGDEF2CGDCDFB0>DA####################################

Read and Quality (2)

@FC81ET1ABXX:3:1101:1215:2154/2
AAGCCANNTNNNNNNNNNNNNACTGGATCCTCATAGCTCACCTTATGCAAAAATCAACTCAAGATGGATGAAGGTCTTAAACCTAATAC
+
HHHBH?##;##############:83<9:;7FDFBFEFE;BEEBE8C>2D8@BBACDFG=E@=CDDHEGGDB;<,:19*23?=@#######

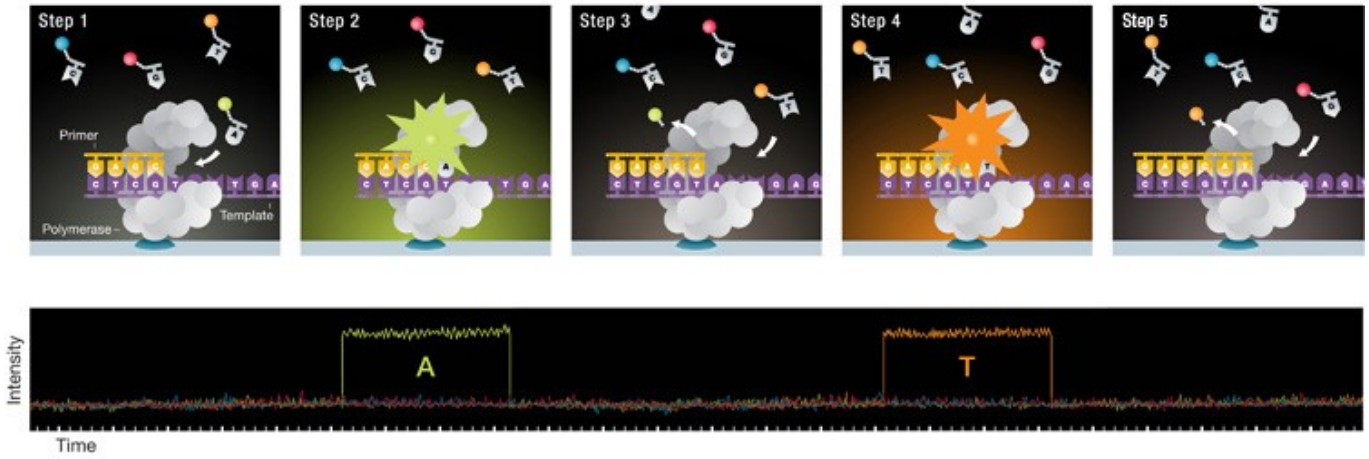
- Read length and quality string length are the same
- All read/1s are the same length in the same run
- All read/2s are the same length in the same run

Illumina

- Read mapping:
  - mrsFAST, BWA-MEM, minimap2, Bowtie2, BFAST, many more
- *De novo* assembly:
  - SPAdes, Velvet, ABySS, SGA, ALLPATHS, ….

Pacific Biosciences

- “Third generation”; single molecule real time sequencing (SMRT)
- No replication with PCR
- Phosphates are labeled. Watches DNA polymerase in real-time while it copies single DNA molecules.
- Premise: long sequence reads in short time (median 1.4 kbp)
- Errors: ~12%; indel dominated
- ~$ 3,000 / human

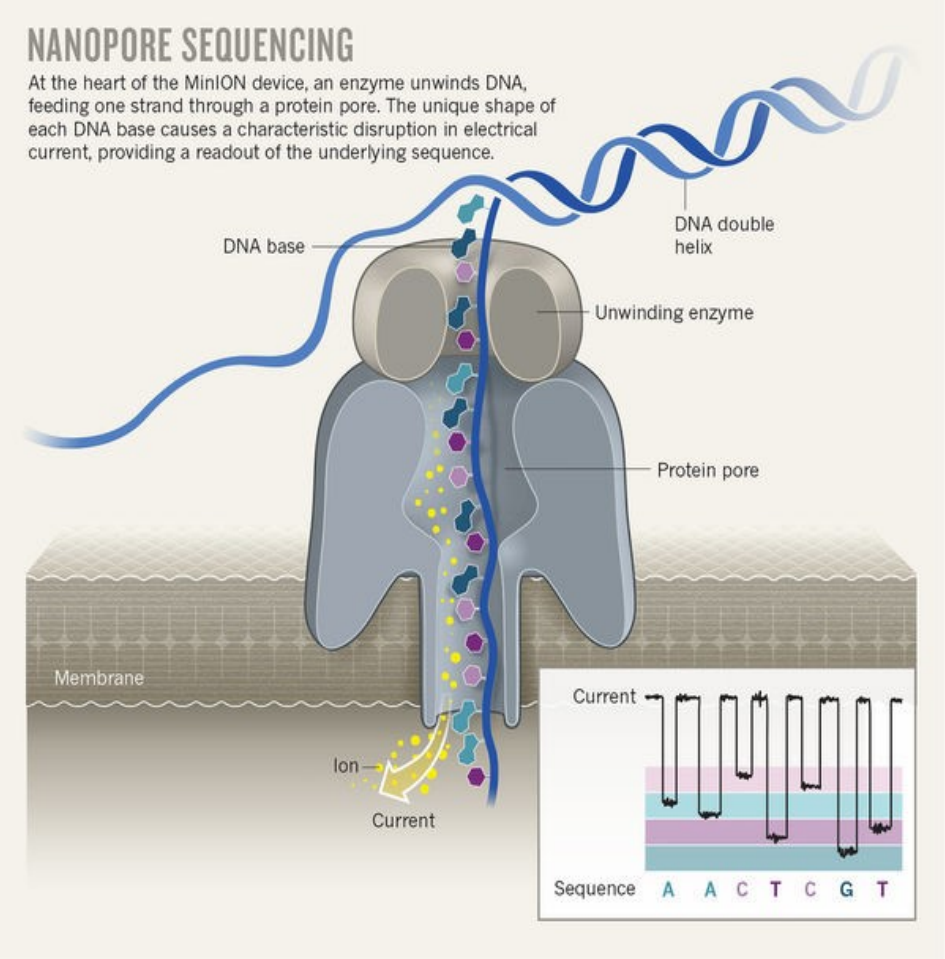


Pacific Biosciences

- For any DNA polymerase you can read a total of ~60 kb (median) sequence
- Two sequencing protocols:
  - CLR: single read
  - CCS: Make a circle, re-read the same molecule 5-6 times
    - Multiple sequence alignment to correct errors
    - Median length = 60000 / 6= 10 Kbp
    - > 99% accuracy

Nanopore sequencing

- Up to 2 Mbp reads
  - 15-20% error, indel dominated
- Real-time analysis supported
- RNN-based basecallers



Nanopore sequencing technology and tools for genome assembly: computational analysis of the current state, bottlenecks and future directions FREE

Damla Senol Cali ✉, Jeremie S Kim, Saugata Ghose, Can Alkan, Onur Mutlu

Briefings in Bioinformatics, bby017, https://doi.org/10.1093/bib/bby017
Published: 02 April 2018 **Article history** ▾

Nanopore sequencing

PacBio & ONT

- Read mapping:
  - Minimap2, MashMap, NGM-LR, …
- *De novo* assembly:
  - Canu, Flye, FALCON

HTS: Computational Challenges

- Data management
  - Files are very large; compression algorithms needed
- Read mapping
  - Finding the location on the reference genome
  - All platforms have different data types and error models
  - Repeats!!!!
- Variation discovery
  - Depends on mapping
  - Again, all platforms has strengths and weaknesses
- *De novo* assembly
  - It's very difficult to assemble short sequences and/or long sequences with high errors