TOPIC 1: Introduction to Next-Gen Sequencing

Bill 525D - Bioinformatics for Evolutionary Biology

Instructors

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Course Objective

1. Introduction: Scope of course, goals and overview of technology [GREG]
2. Programming for biologists [GREG]
3. Fastq files and quality checking/trimming [KAY]
4. Alignment: algorithms and tools [GREG]
5. Assembly: transcriptome and genome assembly [KAY]
6. RNAseq + differential expression analysis [KAY]
7. SNP and variant calling [GREG]
8. Population genomics and plotting in R (Part 1) [GREG]
9. Population genomics and plotting in R (Part 2) [GREG]
10. Phylogenetic inference [GREG]

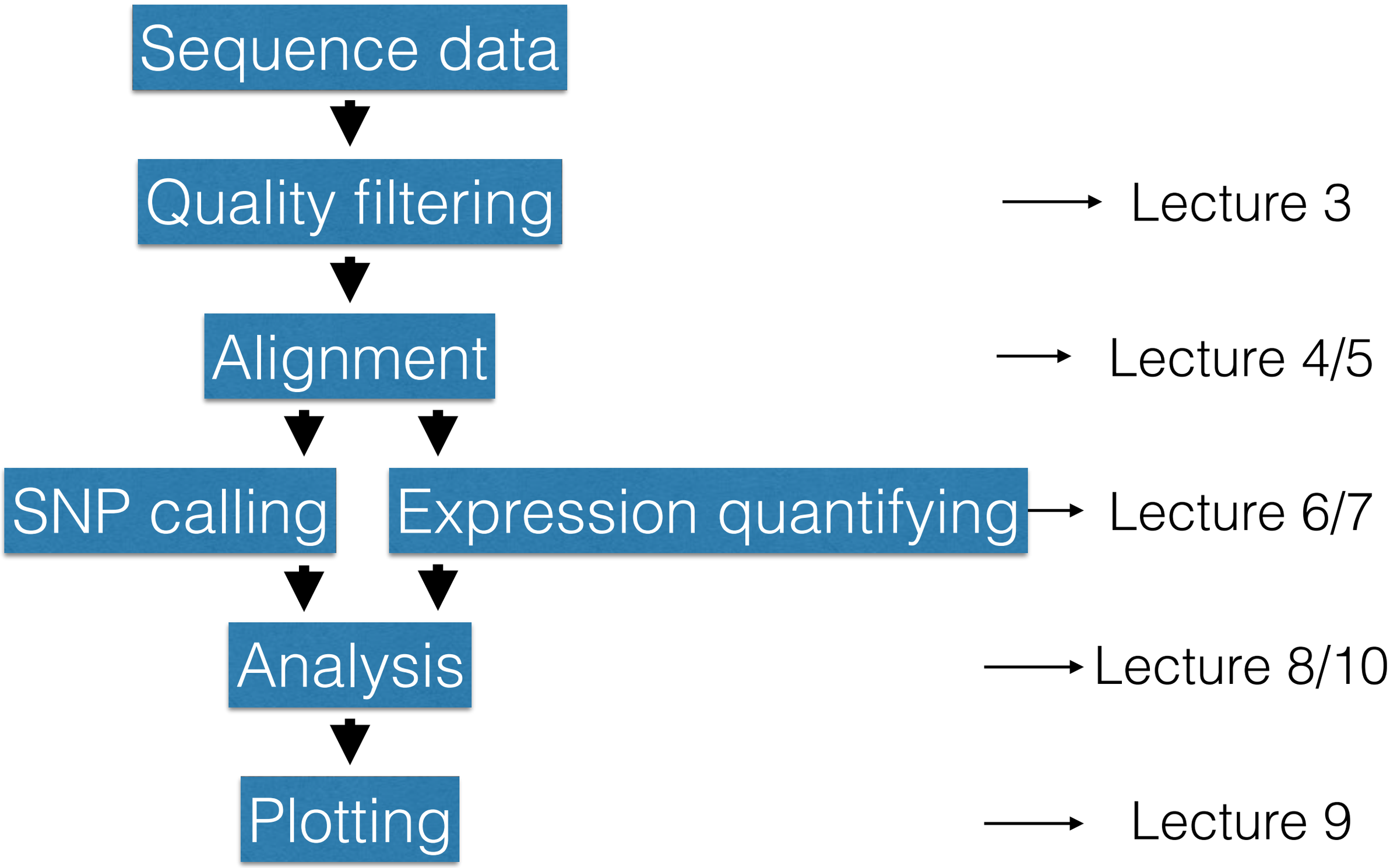
Goals

Raw sequence data

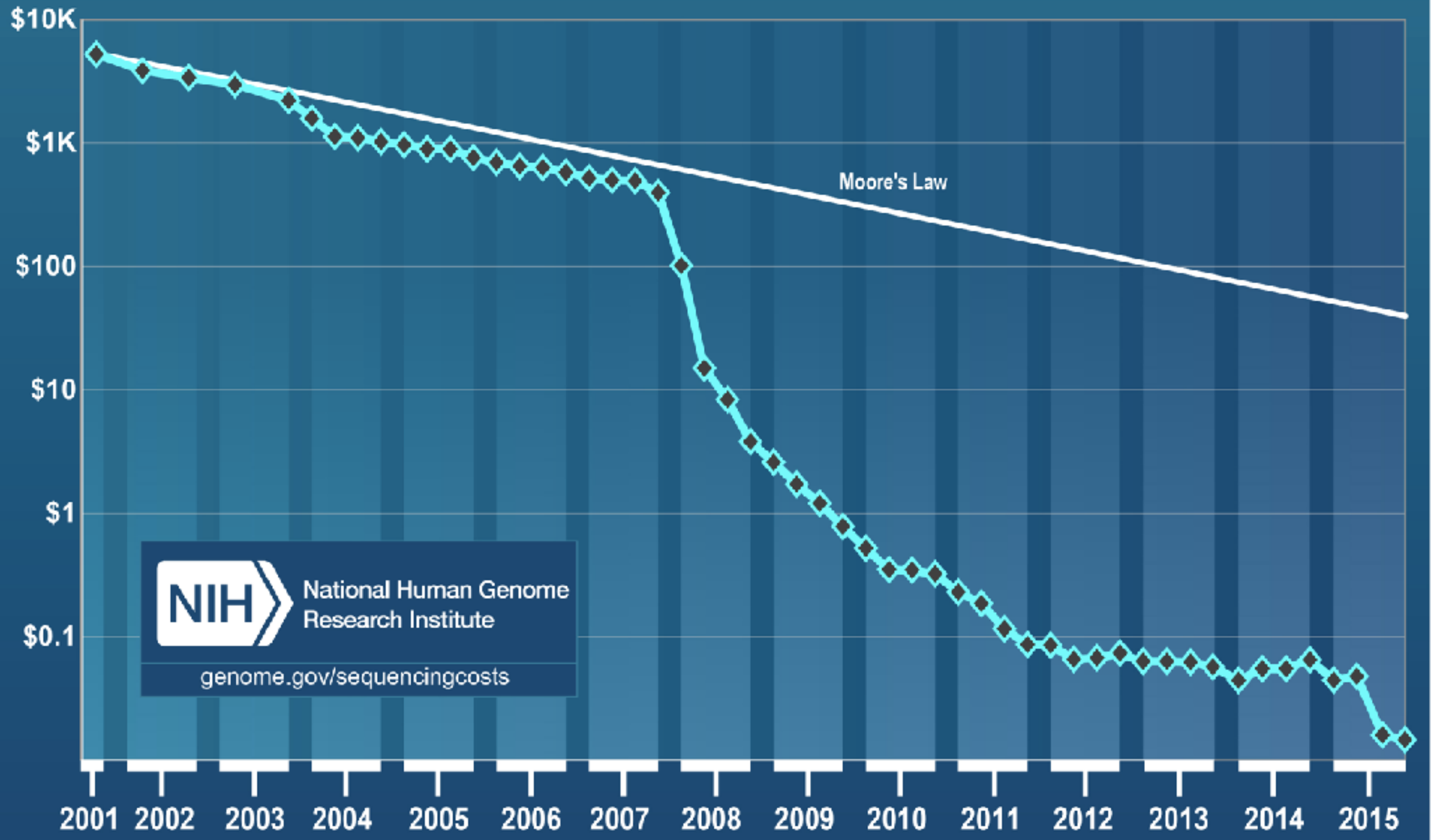
????

Results and Figures

Goals

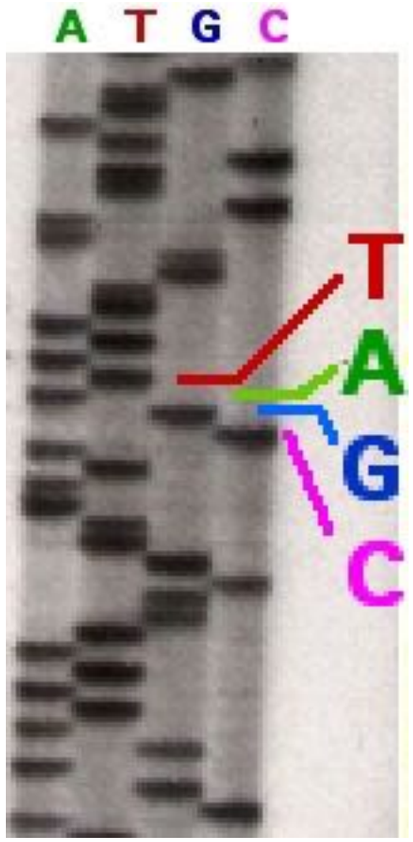

Sequence data
Quality filtering
Lecture 3
Alignment
Lecture 4/5
SNP calling
Expression quantifying
Lecture 6/7
Analysis
Lecture 8/10
Plotting
Lecture 9

Cost per Raw Megabase of DNA Sequence



First Generation Sequencing

- Maxam-Gilbert: Chemical modification and cleavage followed by gel electrophoresis
- Sanger: Selective incorporation of chain-terminating dideoxynucleotides followed by gel electrophoresis
- Became full automated using flourescently labeled dideoxy bases
- Dominant sequencer up until 2007
- Only one fragment sequenced per reaction
- Still used for sequencing individual PCR products



Sanger

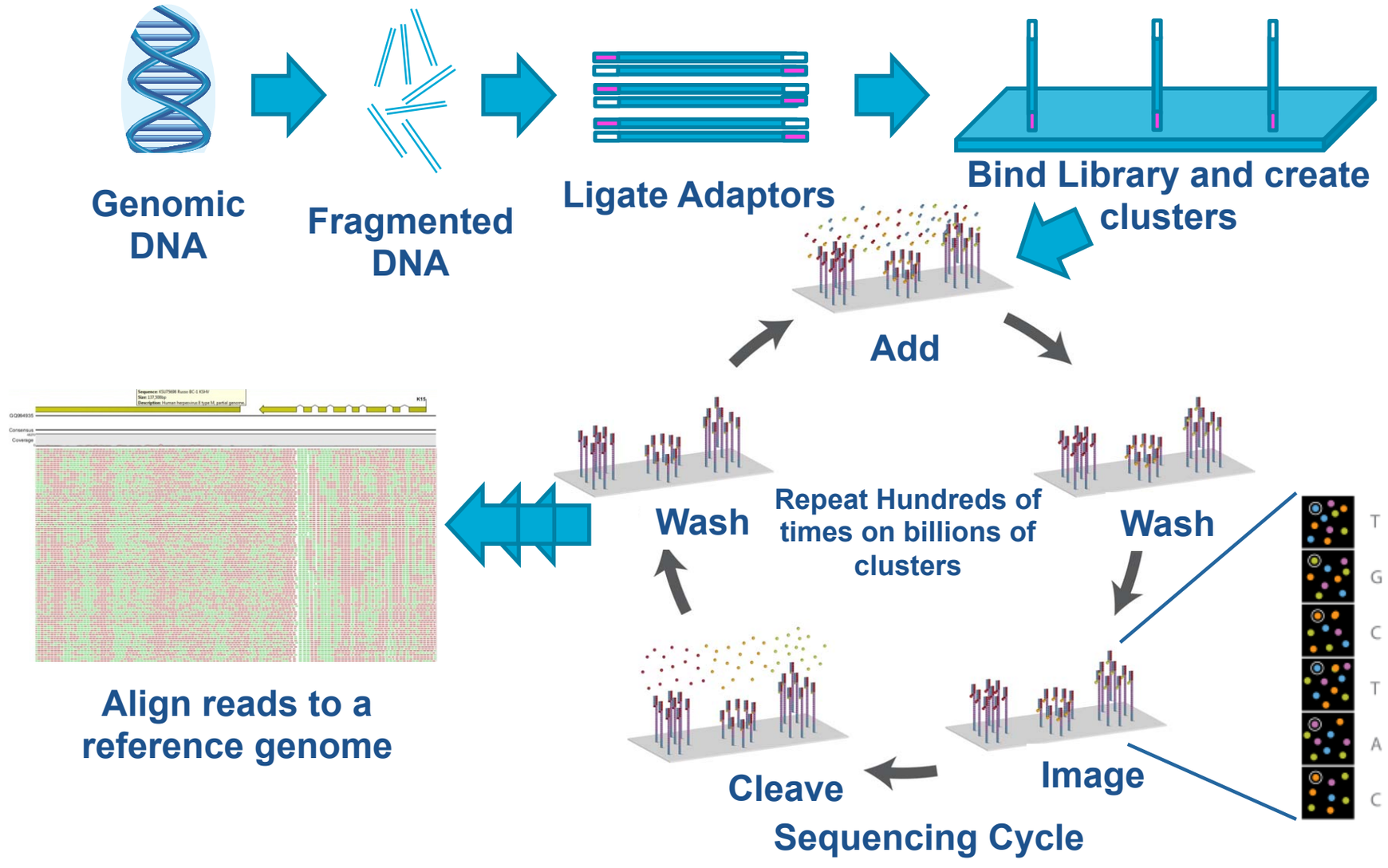
Second generation sequencing

- Sequences many molecules in parallel
- Don’t need to know anything about the sequence to start.
- Main technologies:
  - Illumina
  - Ion torrent
  - 454 (Pyrosequencing)
  - PacBio

Second generation sequencing

Technology	Read Length	Accuracy	Reads/run	Uses
Illumina	50-300bp	99.9%	2-3 billion	Resequencing General depth
MinION	5kb-50kb	80-90%	~3GBase	Microbial genomes Genome assembly
PacBio	10kb-40kb	87%	up to 1Gbase	Genome assembly Structural variants

Illumina sequencing



Challenges of short read technology

- Rely on amplification, which can introduce errors (10^{-6} -10^{-7}).

- Assembling and aligning reads challenging in repetitive regions

- Difficulty with both large and small structural variants.

Long read sequencing

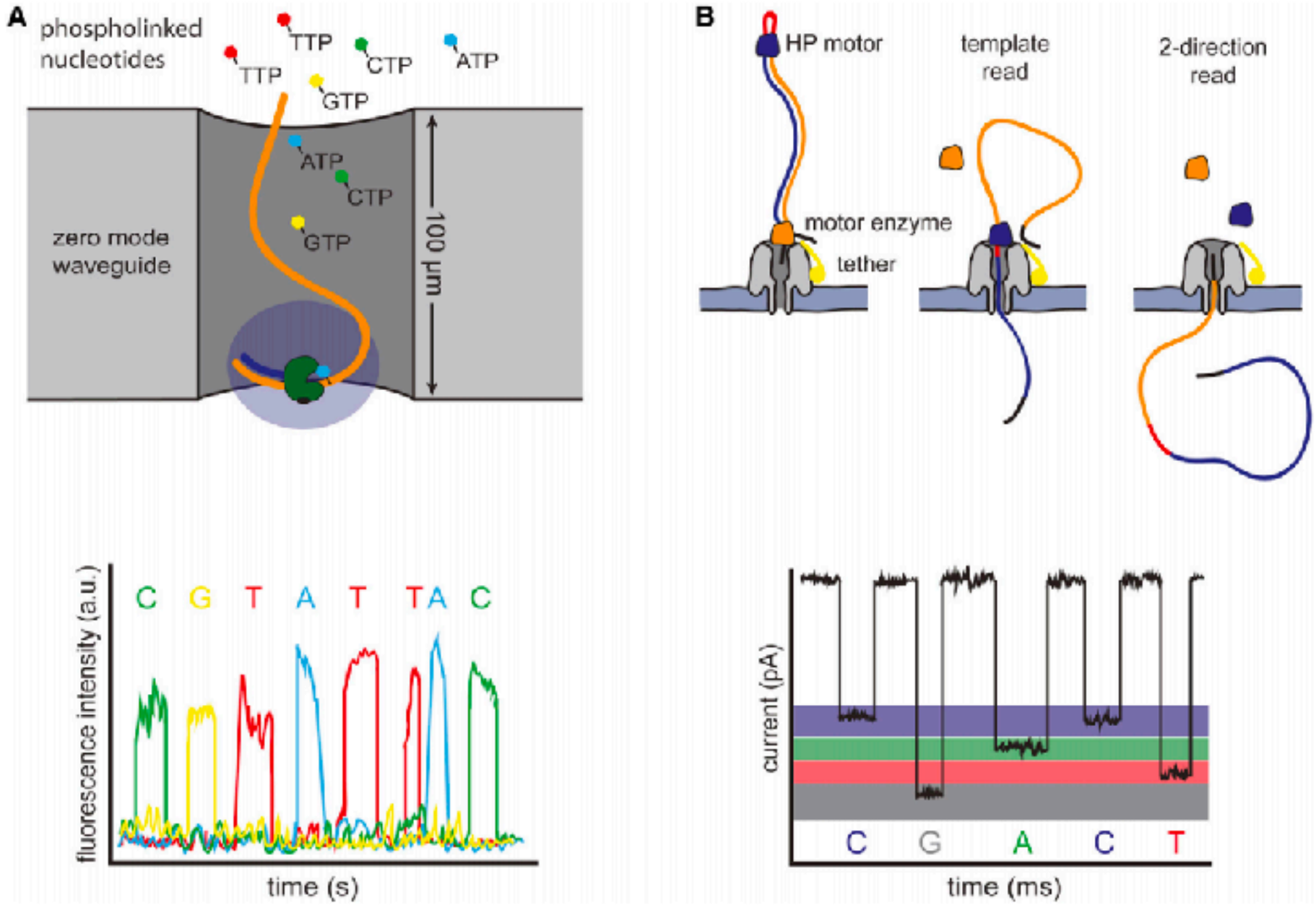


Figure 3. Single Molecule Sequencing Platforms

(A) Pacific Bioscience's SMRT sequencing. A single polymerase is positioned at the bottom of a ZMW. Phosphate-labeled versions of all four nucleotides are present, allowing continuous polymerization of a DNA template. Base incorporation increases the residence time of the nucleotide in the ZMW, resulting in a detectable fluorescent signal that is captured in a video.

(B) Oxford Nanopore's sequencing strategy. DNA templates are ligated with two adapters. The first adaptor is bound with a motor enzyme as well as a tether, whereas the second adaptor is a hairpin oligo that is bound by the HP motor protein. Changes in current that are induced as the nucleotides pass through the pore are used to discriminate bases. The library design allows sequencing of both strands of DNA from a single molecule (two-direction reads).

Challenges of long read technology

- Too expensive to be used for population level sequencing.

- High error rate.

Illumina = more and more data

2018 release

	HiSeq 2500	HiSeq 4000	HiSeq X	NovoSeq S1	NovoSeq S2	NovoSeq S3	NovoSeq S4
Reads per lane (million)	250	350	430	800	1650	1650	2500
Throughput per lane (Gb)	62.5	105	129	240	495	495	750
500 Mb genome coverage	120X	210X	260X	480X	990X	990X	1500X
500 Mb 10X genomes	12	21	26	48	99	99	150

Flavours of sequencing

- Whole Genome Sequencing
- Pool Seq
- RNAseq
- Amplicon Sequencing
- Sequence Capture
- Reduced-Representation Sequencing (RADseq/GBS)
- RADcapture
- GT-seq

Whole Genome Sequencing

- Randomly sheer DNA and sequence all fragments
- May use double-stranded nuclease treatment to reduce repetitive elements

Pros:
-All sites possible
-Simple library prep

Cons:
-Expensive per sample
-Bioinformatic challenges at high sample number

Number of SNPs: 10+ million

Pool Seq

- Whole genome sequencing with pooled DNA of multiple individuals

- Produces a measure of allele frequency but not individual genotypes

Pros:
-All sites possible
-Simple library prep
-Cheaper than individual WGS

Cons:
-Limited analysis options
-No haplotype information

Number of SNPs: 10+ million

RNAseq

- Convert RNA to cDNA, randomly sheer and sequence.

- Only sequences expressed RNA

Pros:
-Many sites and only in genes.
-Also get expression information
-Relatively easy to assemble

Cons:
-Expression differences complicate SNP calling
-Expensive for pop gen level sampling

Number of SNPs: ~1 million

Amplicon Sequencing

- Use PCR to amplify target DNA. Sequence many barcoded samples in one lane.

- Used to characterize microbiome by sequencing 16s rRNA

Pros:
-Get incredible depth at single locus.
-Simple bioinformatics.

Cons:
-Limited to one or few loci.
-Mutations in primer site don't sequence

Number of SNPs: <100

Sequence Capture

- Design probe sequences from genome resources, synthesis attached to beads

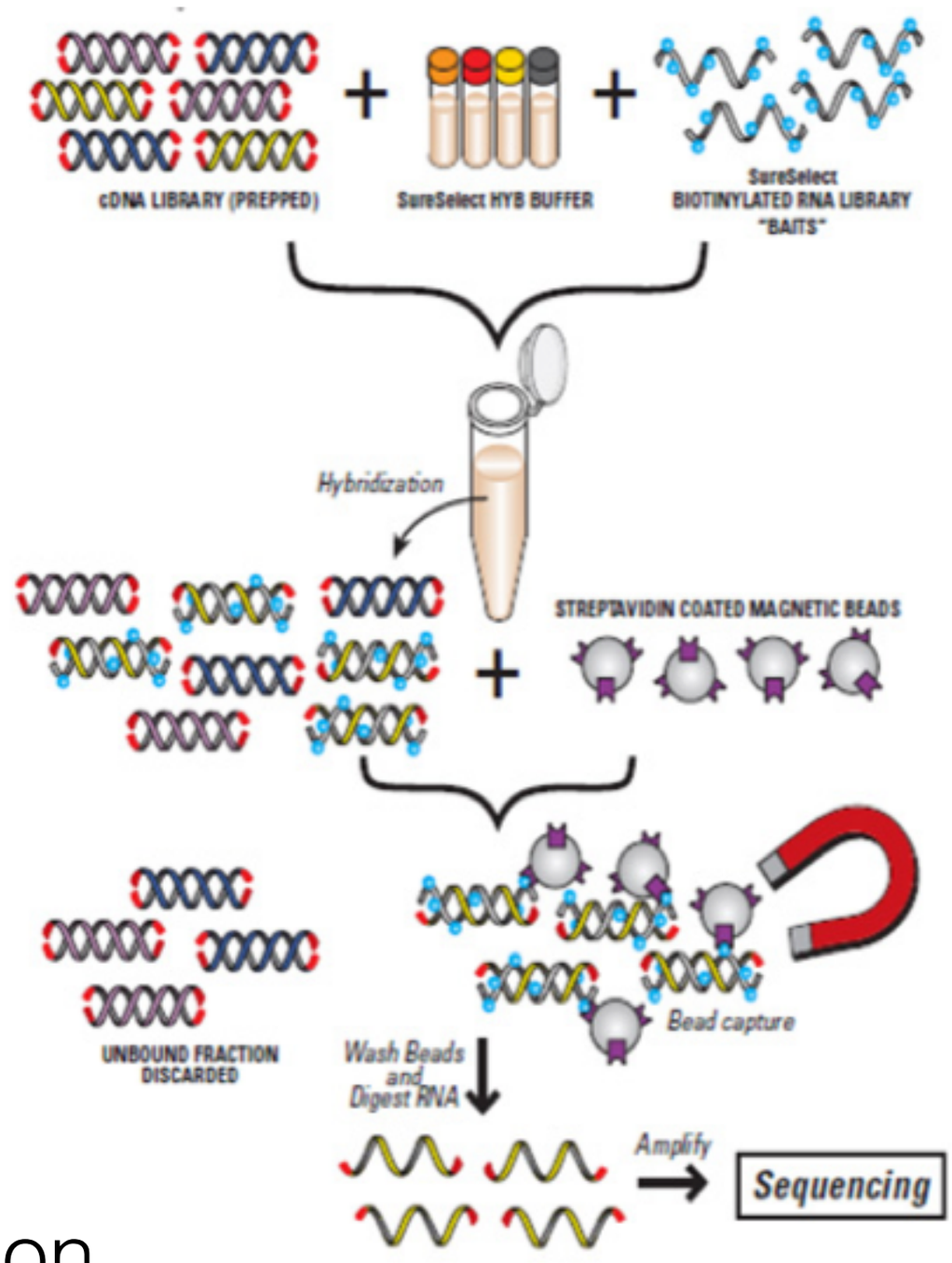
- Make WGS library, hybridize with probe set. Matching sequence will be captured, all others washed away.

- Collect capture sequence, amplify and sequence

Sequence Capture

Pros:
-Relatively cheap per sample.
-Good depth at targeted sites

Cons:
-Requires designing probes.
-Long library prep.

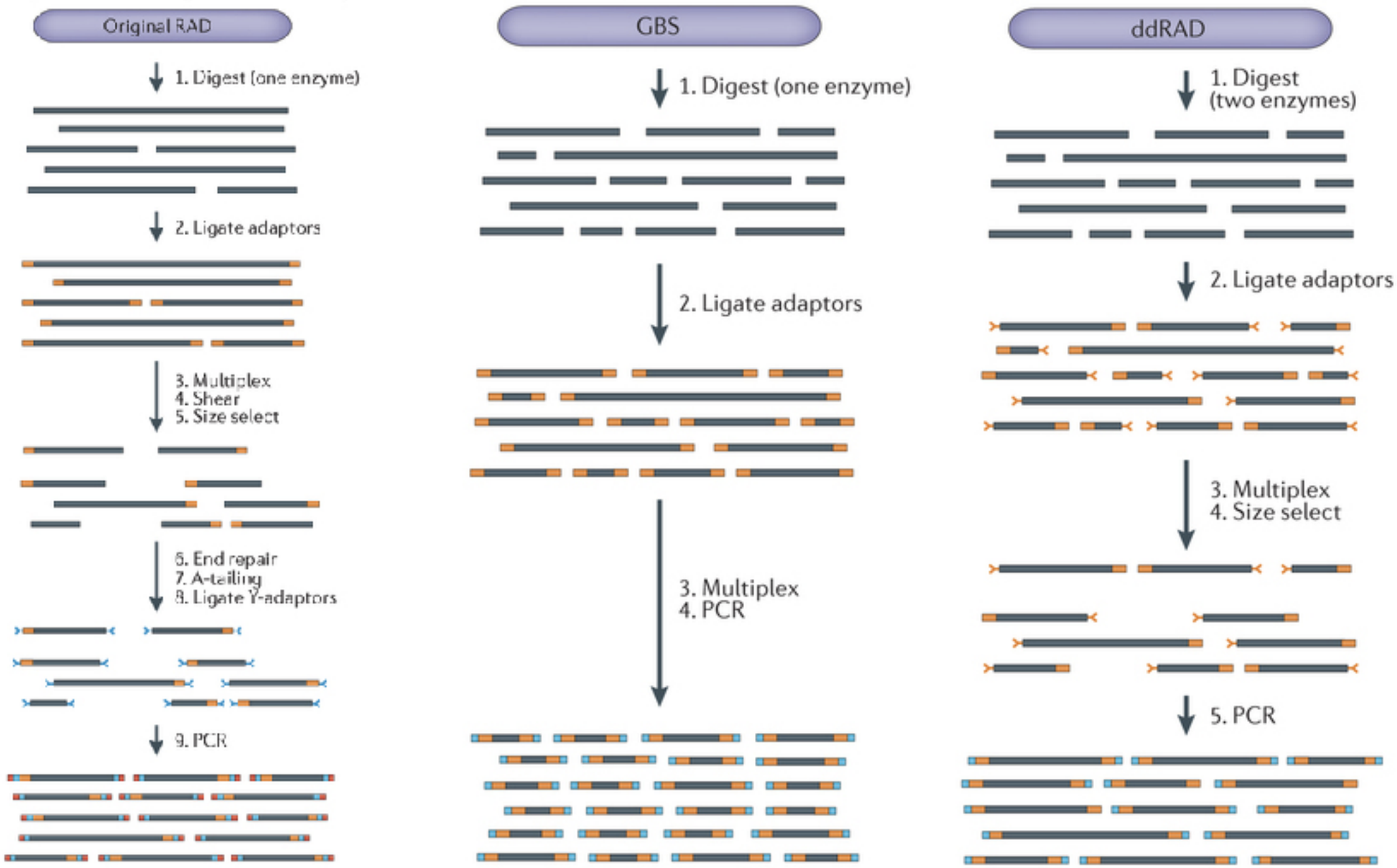


Number of SNPs: 100k - 1 million

Genotyping-by-Sequencing types

- Digest DNA with restriction enzyme. Attach barcode and sequencing tags. Sequence many samples in one library.
- Many different flavours:
    - GBS, RAD, ddRAD

Genotyping-By-Sequencing



Genotyping-By-Sequencing

Pros:
-Quick library prep for hundreds of samples.
-Cheap per sample cost (<$10/sample)

Cons:
-Relatively sparse SNPs compared to other methods
-Can have problems overlapping different library preps

Number of SNPs: 5k - 50k

RADcapture

- Digest DNA with restriction enzyme. Attach barcode and sequencing tags. **Sequence capture before sequencing**. Sequence many samples in one library.

- Different flavours

    - Rapture, RADcap

RADcapture

Pros:
-Quick library prep for hundreds of samples.
-Cheap per sample cost (<$10/sample)
-More overlap of reads = more SNPs
-Can be good for poor quality samples (e.g. herbarium)

Cons:
-Relatively sparse SNPs compared to other methods
-Requires extra step to make capture probes
-Less well established

GT-seq

- Genotyping by Thousands
- Multiplex PCR amplify ~200 known SNPs and then sequence pooled PCR products.
- Very cheap (<$5/sample), and bioinformatically simple.
- Useful for genotyping thousands or tens of thousands of samples.

Illumina = more and more

2018 release

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500 Mb genome coverage	120X	210X	260X	480X	990X	990X	1500X
500 Mb 10X genomes	12	21	26	48	99	99	150
RAD samples per lane (approximate)	100	200	250	500	1000	1000	1500

Recommendations

- GT-seq
  - **Large** scale genetic monitoring (e.g. fisheries)

Recommendations

- RAD/RADcapture
  - Short projects
  - Population structure
  - Phylogenetic
  - Genetic maps / QTL maps
  - Species ID
  - Genome scans

Recommendations

- Whole genome sequencing
  - Fine scale genome analysis
  - Association mapping
  - Small genome organisms

Recommendations

- Sequence capture
  - Large genomes
  - Bigger or longer projects
  - Fine scale genome analysis

Computing options

- Mid sized personal server (~12 cores, 100 GB ram)
  - Works for small/medium scale analyses
  - Too slow for genome assembly
  - Hard to expand capacity
  - Upfront cost ($5-10k)
  - Complete control

Computing options

- Lab supercomputer (~30 cores, 300 GB ram)
  - Works for small to high scale analyses
  - Managing load between users can be troublesome
  - High upfront cost ($50-100K)
  - Need server management!

Computing options

- Westgrid
  - Potentially hundreds of cores
  - Less control and 3 day limit on jobs
  - Free
  - Virtual machines available, but limited.

Computing options

- Zoology computing cluster
  - ~100 cores over several servers
  - Don’t need to submit jobs, but limited installing privileges.
  - Storage space limitations
  - Often clogged by users
  - ~$100 per year

Computing options

- Cloud services (Google, Amazon)
  - Infinitely expandable
  - Can get expensive fast
  - Taking data off cloud servers is expensive