

**CONS_V
503A**

101

Topics in Conservation (A)

Bioinformatics for Evolutionary Biology and Conservation

TOPIC 1:

Introduction to the workshop

Instructors

Dr Tom Booker



Dr Bernadette Johnson



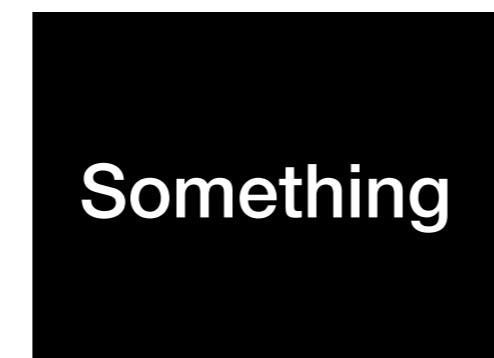
thomas.booker@ubc.ca

An example genomics project

Biological question
(see previous slide)



Genomic analysis

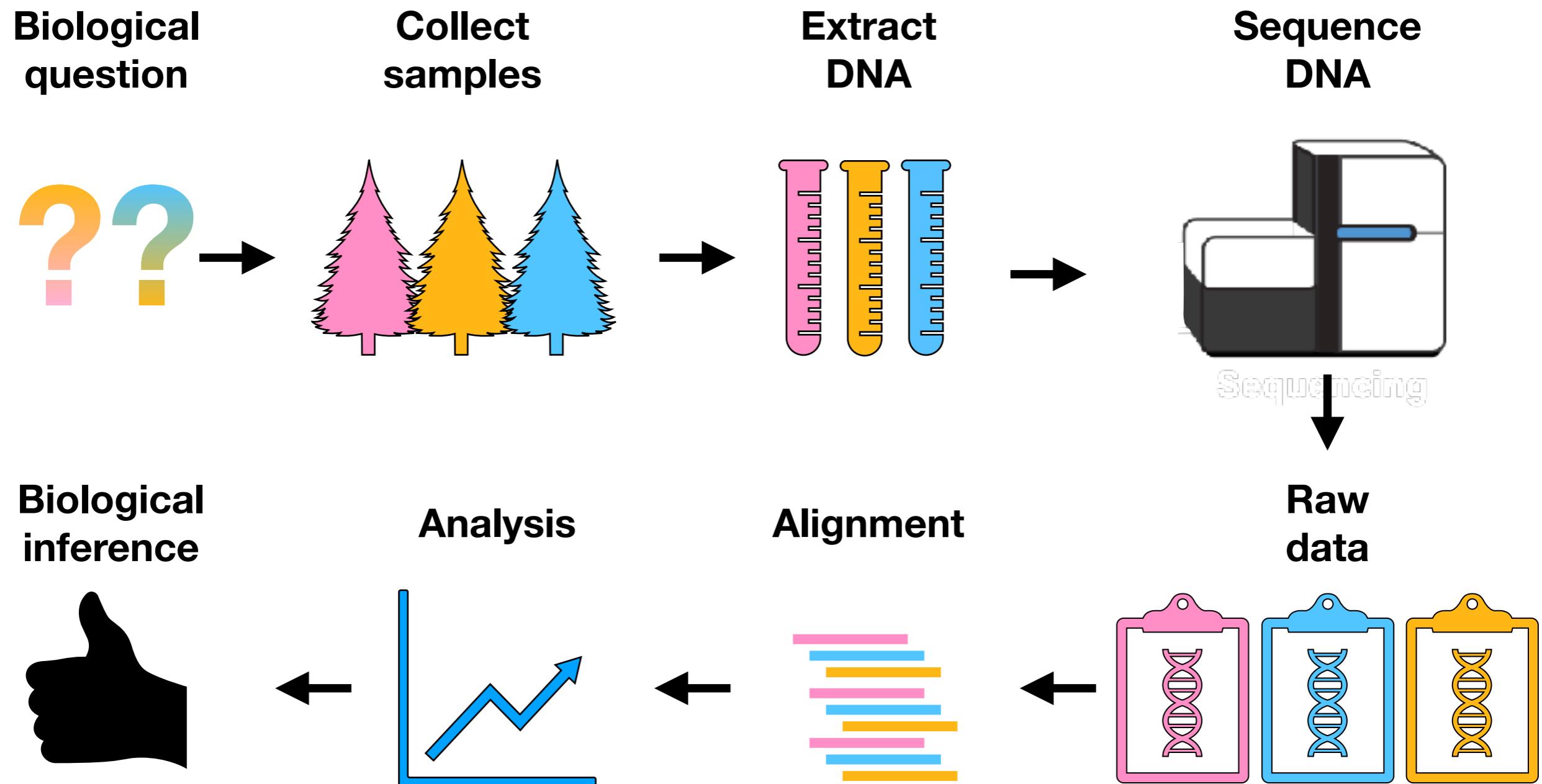


Biological inference/application

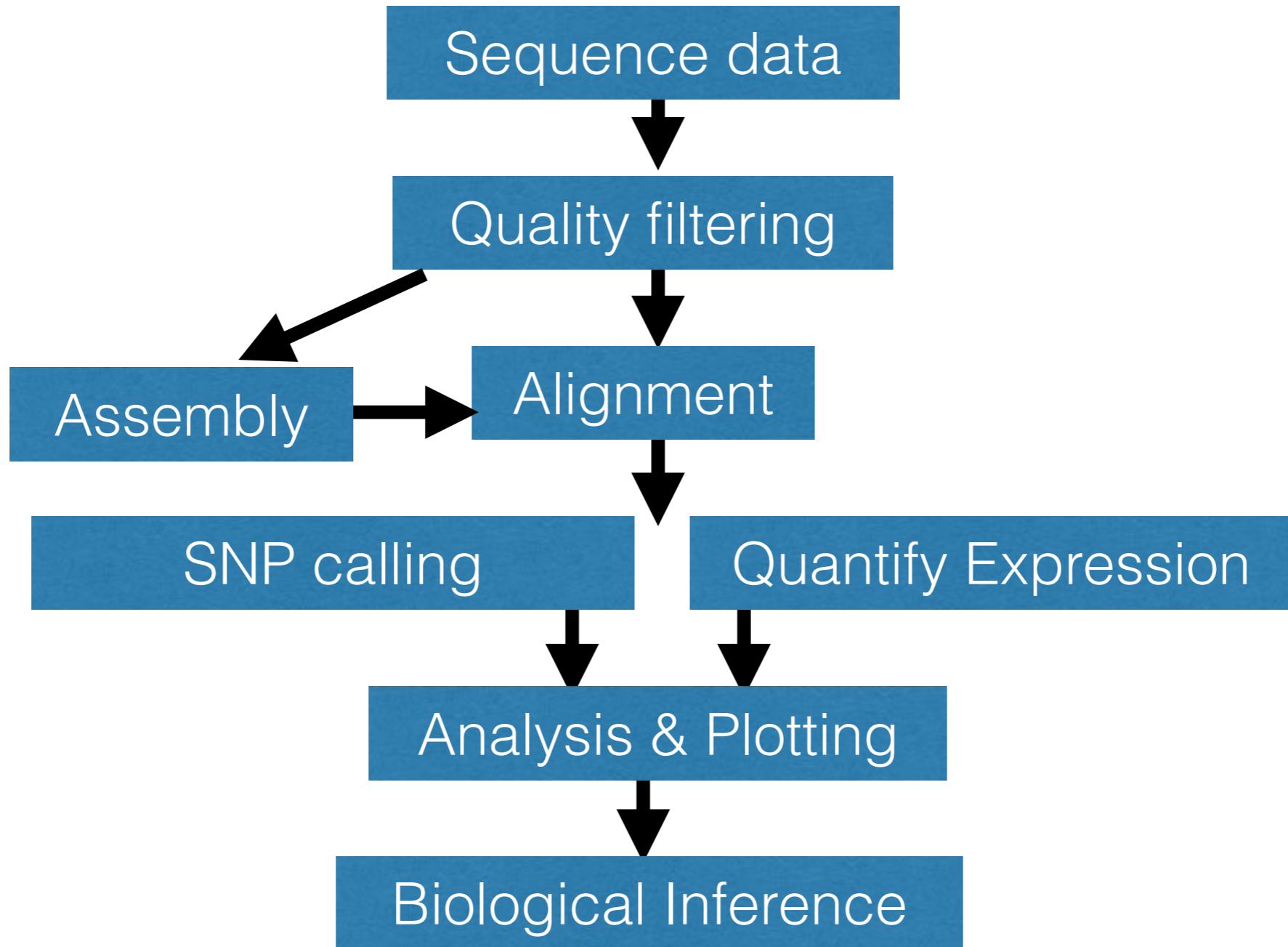


What's in the black box?

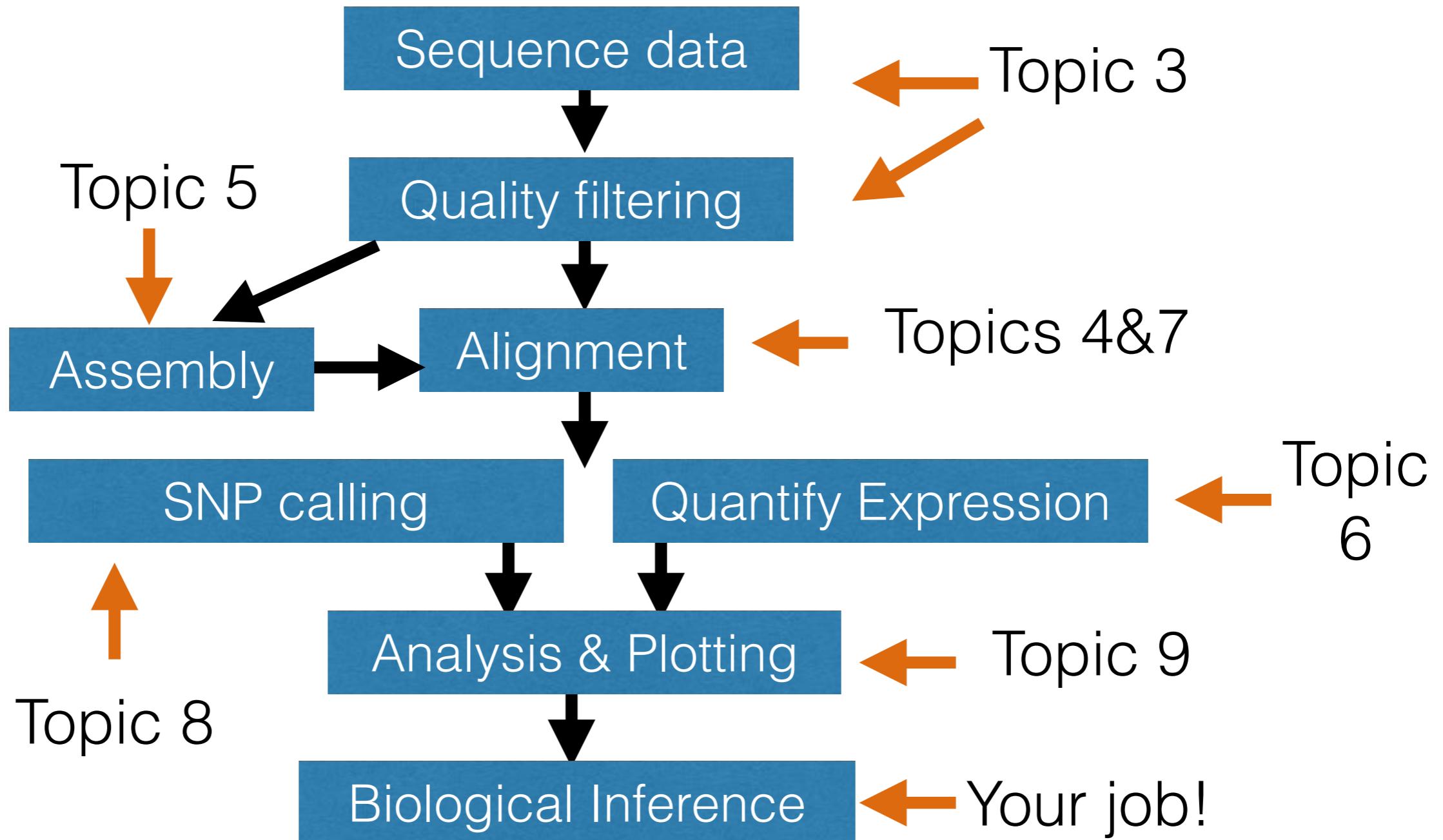
An example genomics project



Rough outline



Rough outline



Overview of the week

[Topic 1 Introduction](#)

[Topic 2 Command Line Basics](#)

[Topic 3 Sequence Data](#)

[Topic 4 Sequence Alignment](#)

[Topic 5 Genome Assembly](#)

[Topic 6 RNAseq Analysis](#)

[Topic 7 Read Mapping](#)

[Topic 8 SNP Calling](#)

[Topic 9 SNP Filtering and Analysis](#)

[Topic 10 Data visualization in R](#)

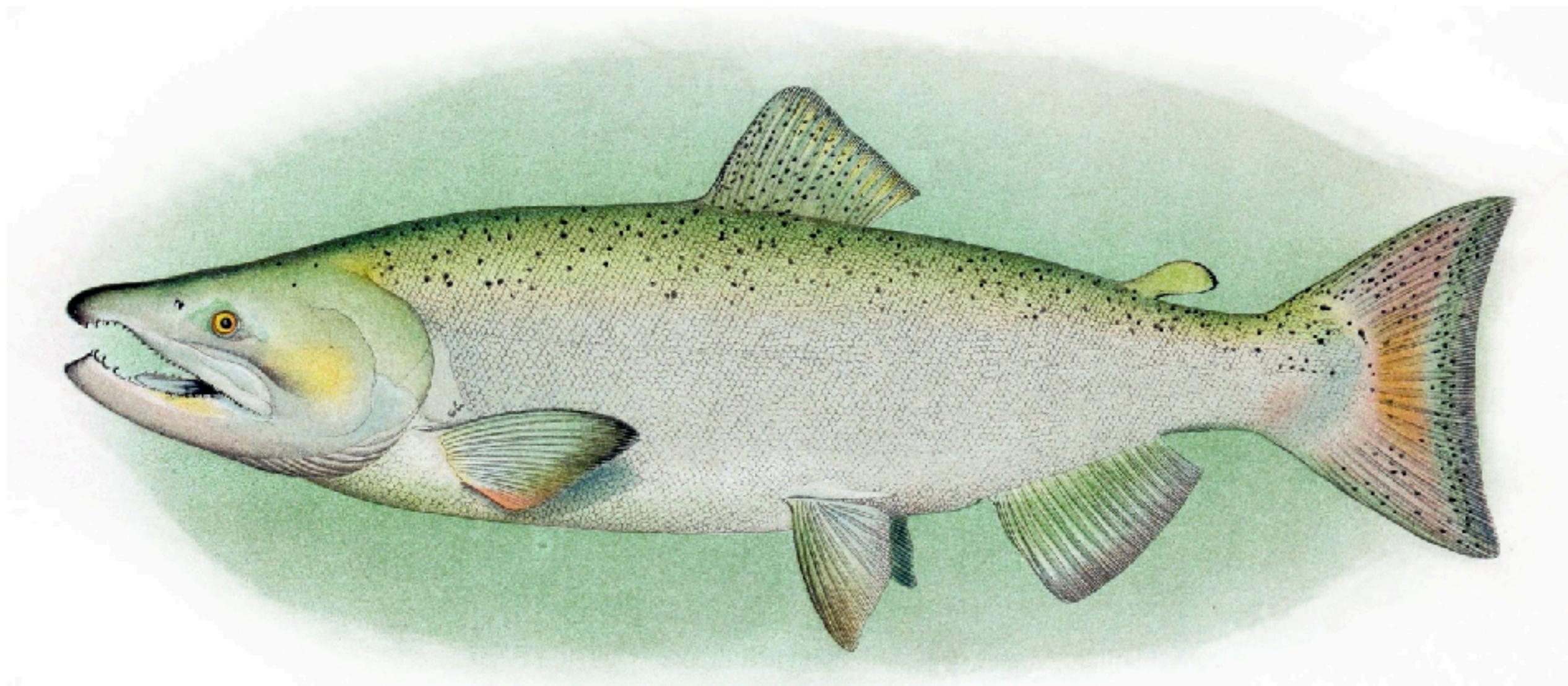
Learning outcomes

The field of bioinformatics is rapidly evolving

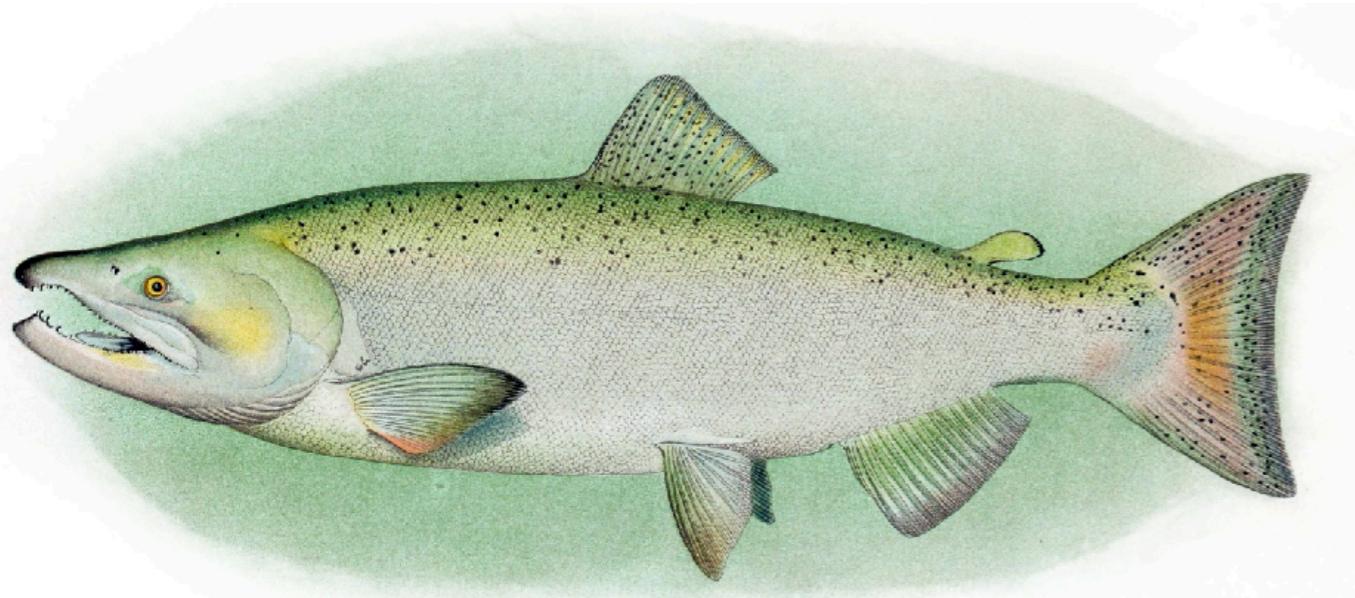
A general grasp of concepts and approaches is very valuable

Common file formats

Chinook



Chinook



Why use simulated data?

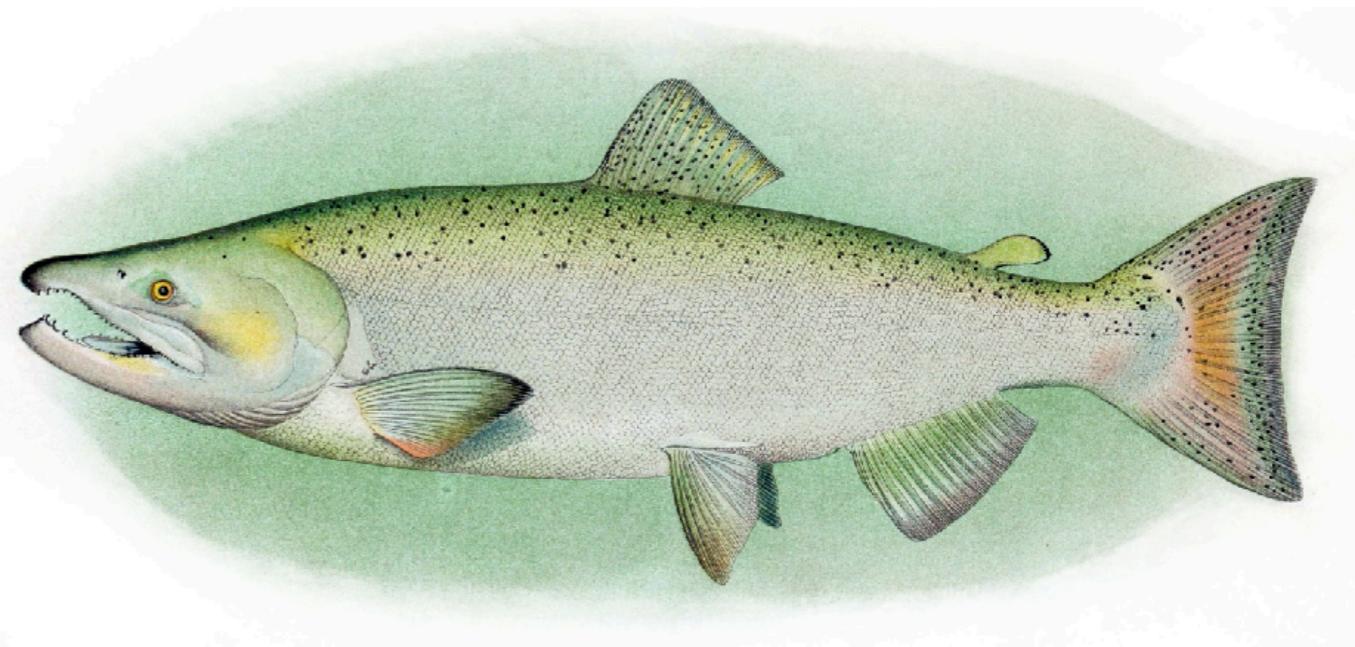
Chinook

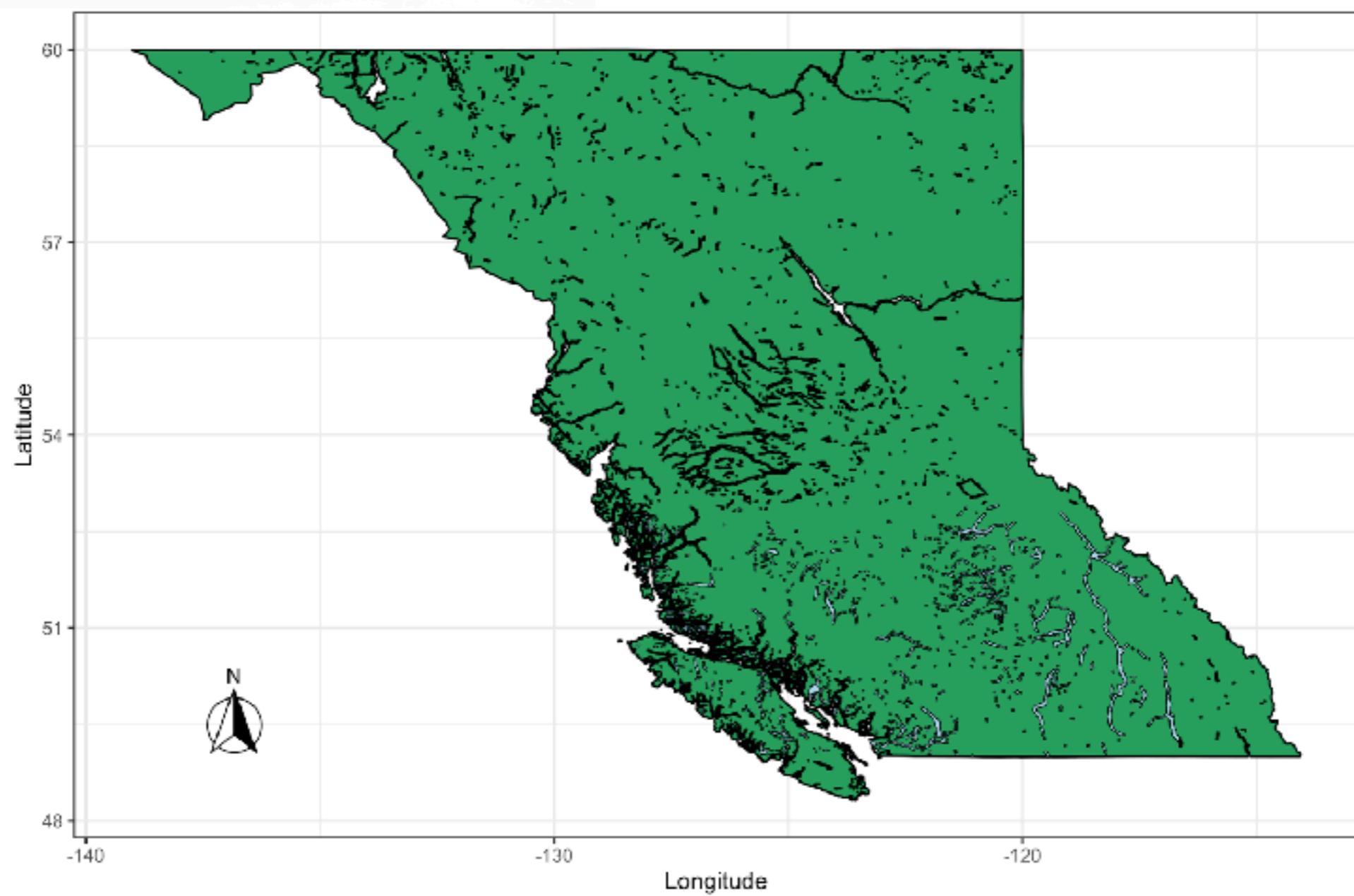
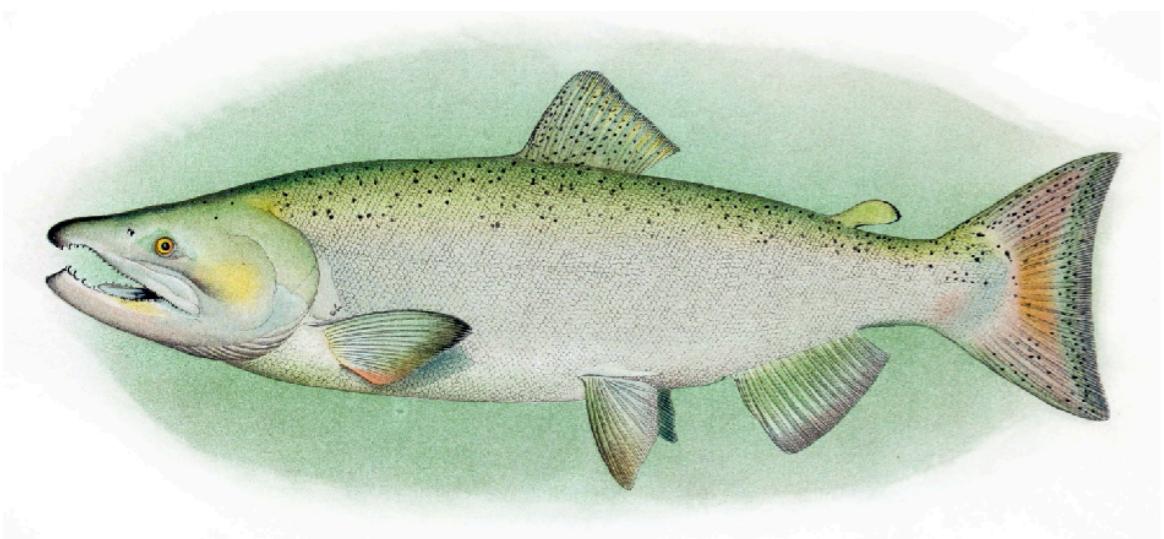
Chinook Salmon

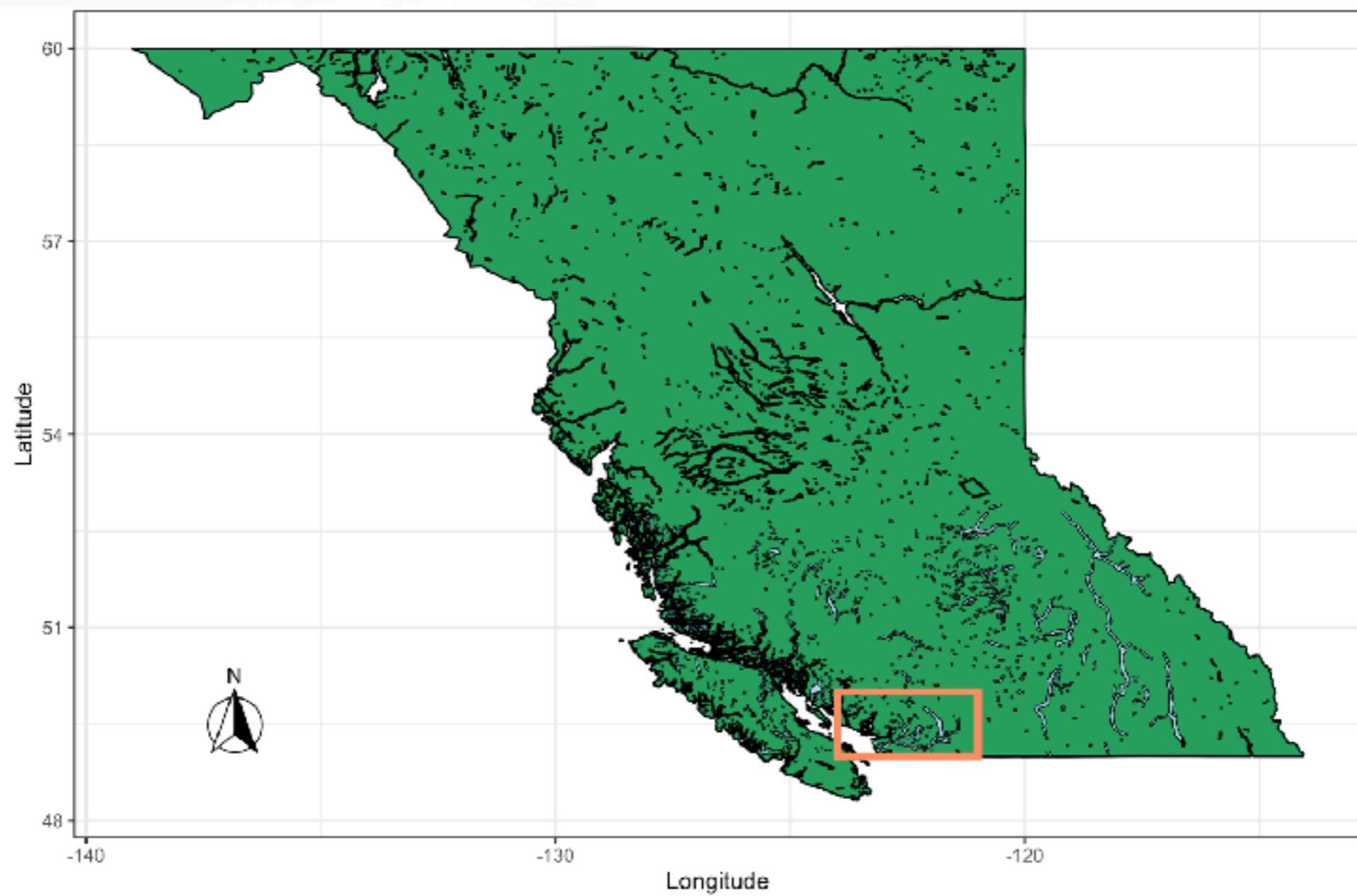
Oncorhynchus tshawytscha

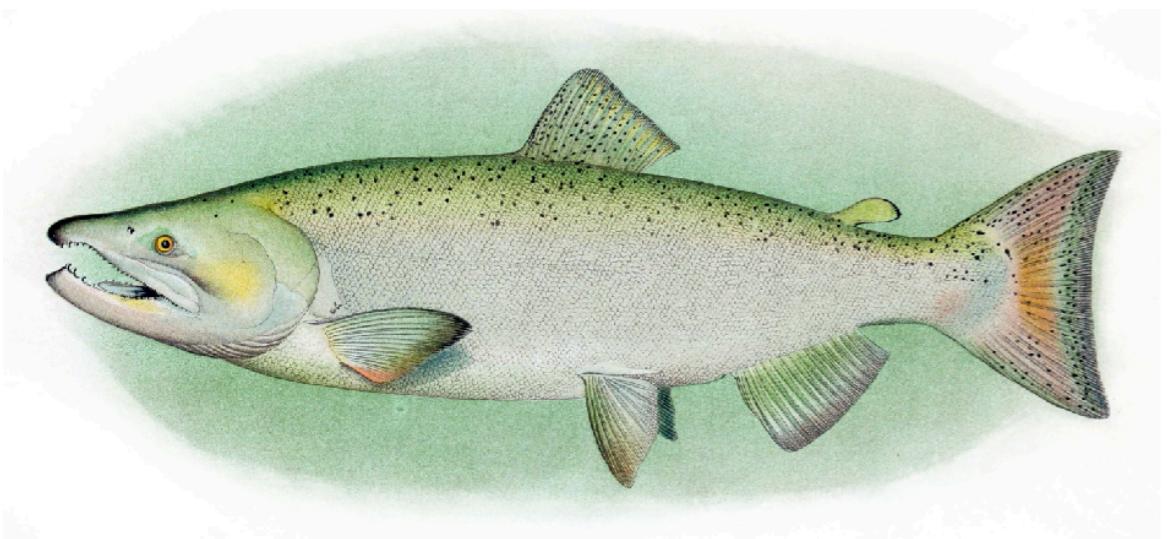
>2.4 Gbp Genome

32 chromosomes

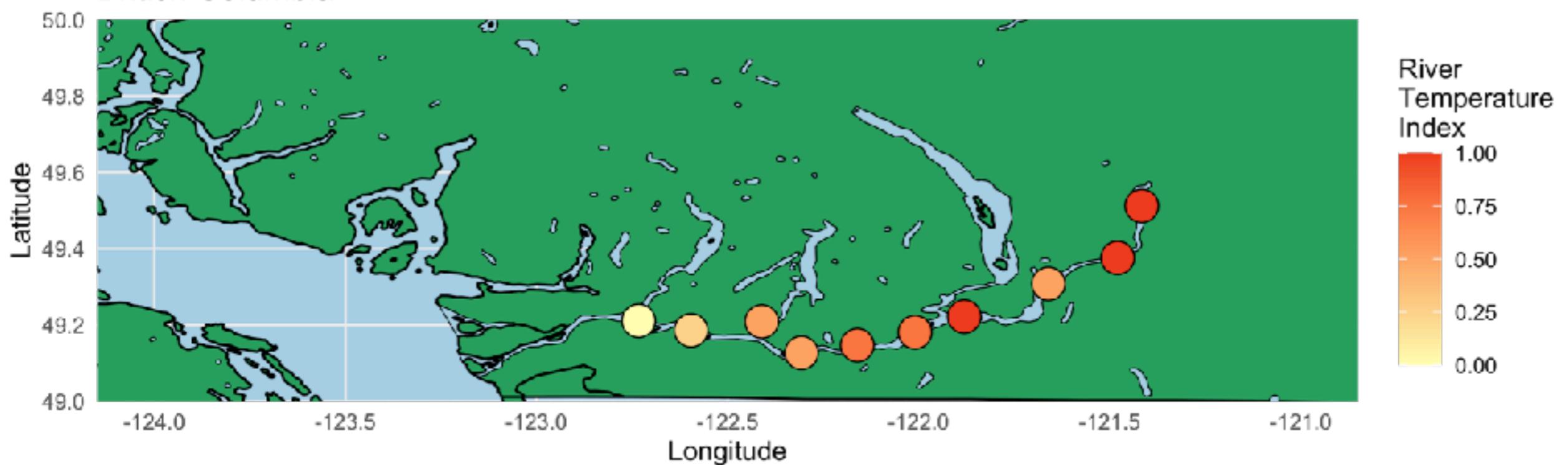


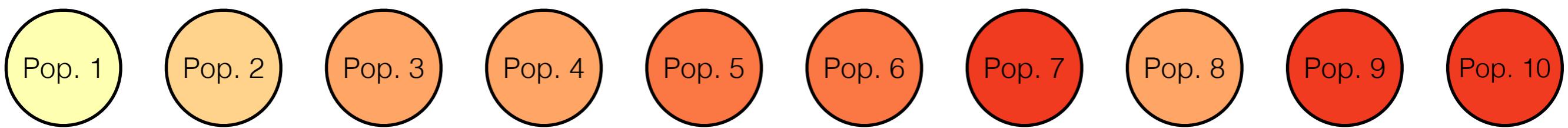
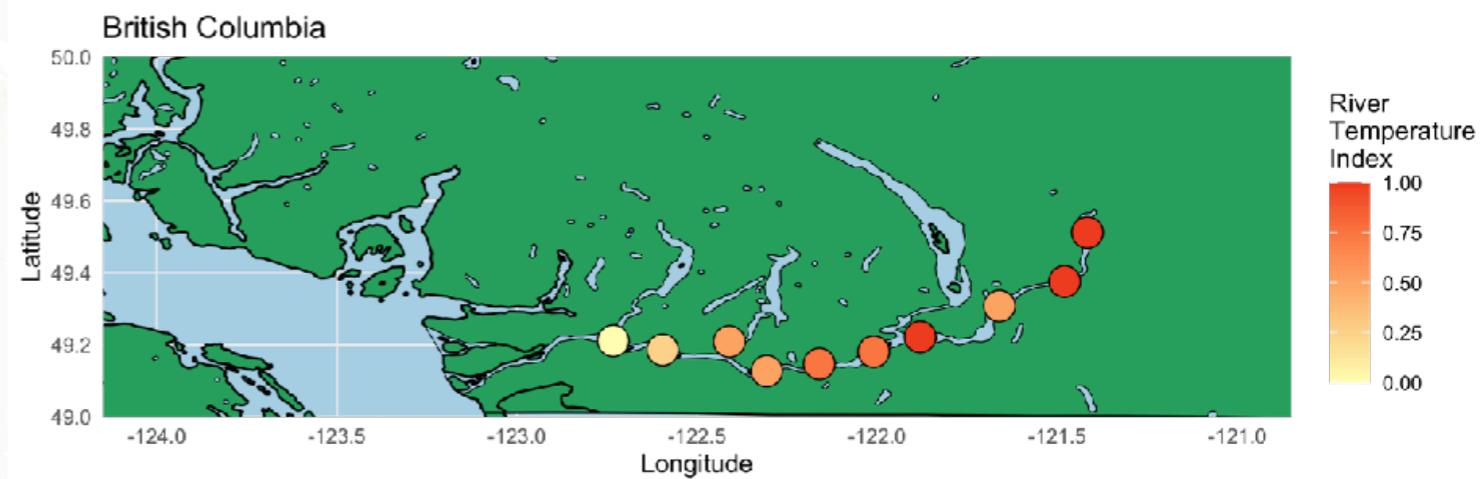
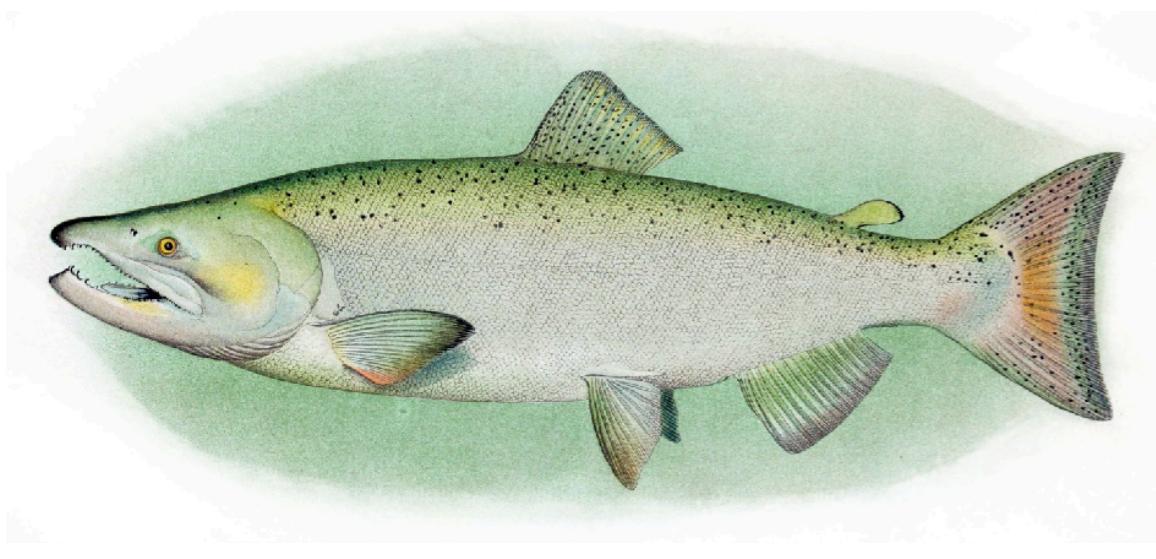


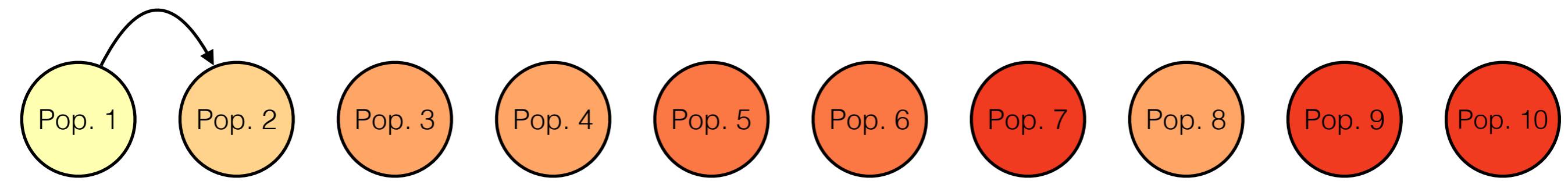
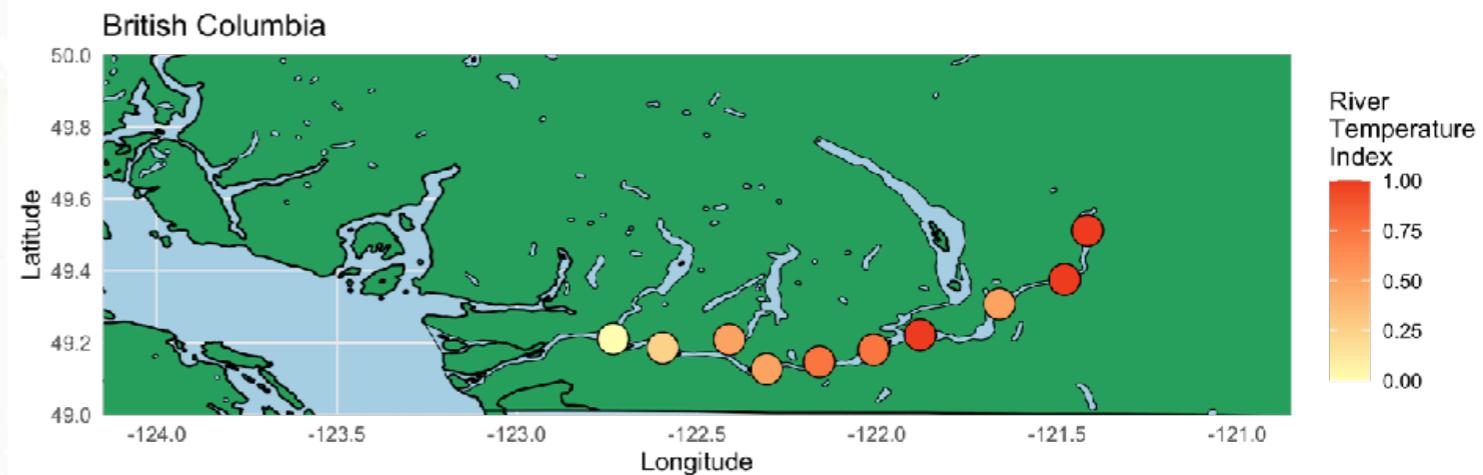
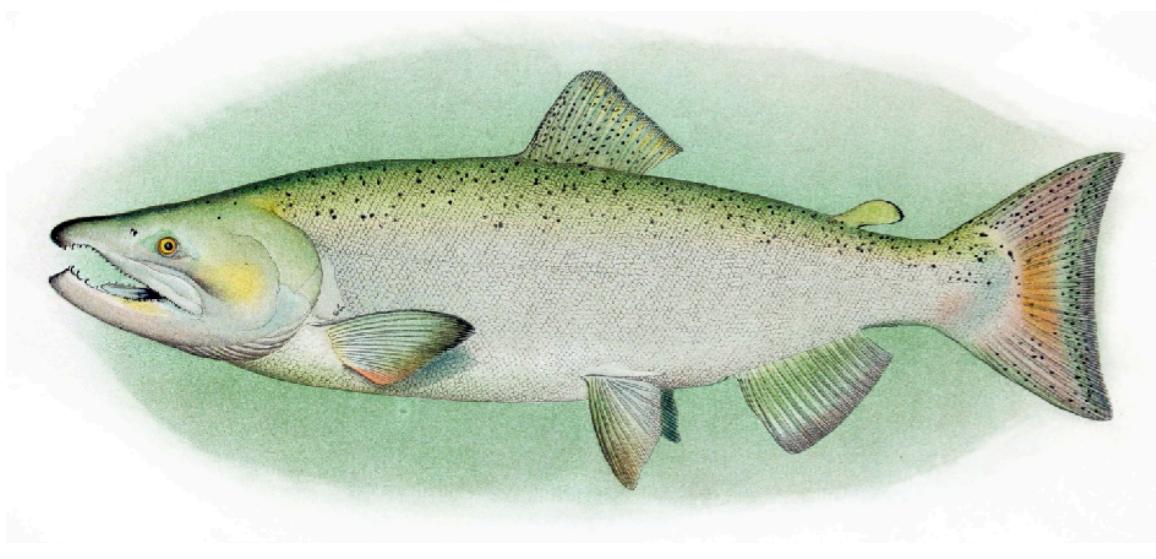


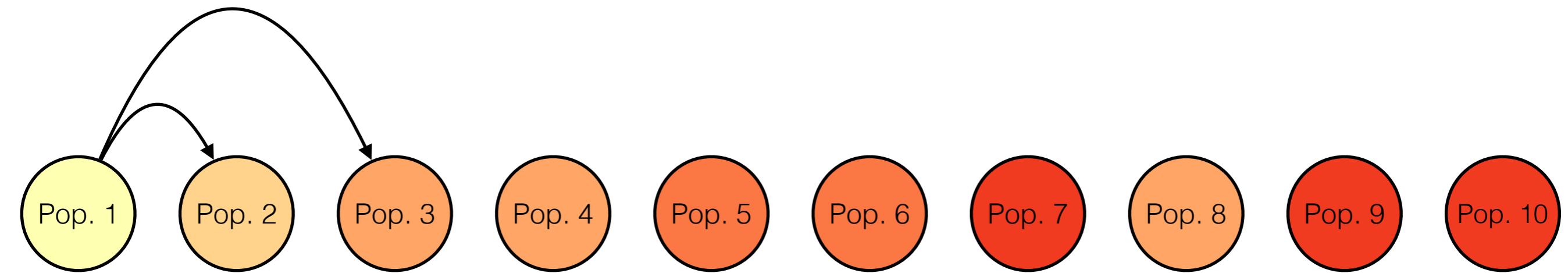
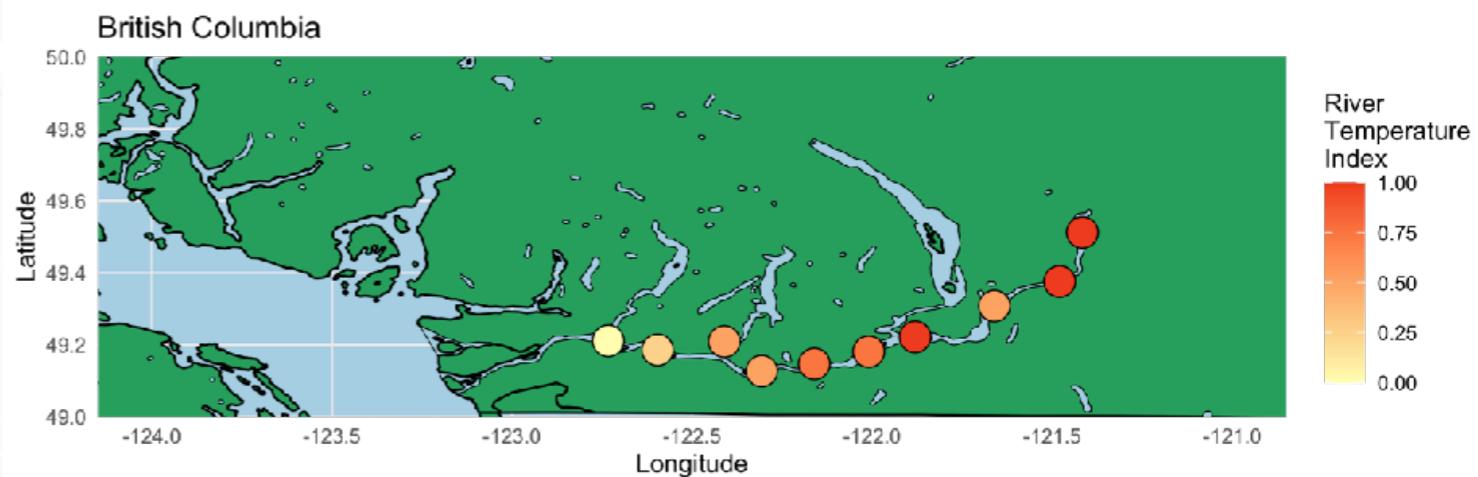
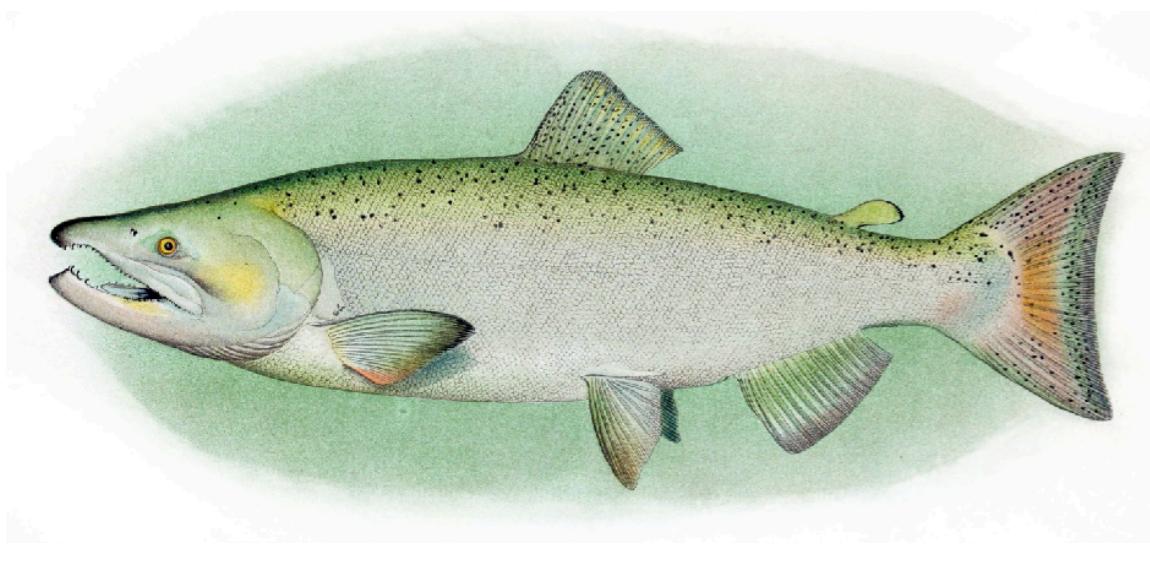


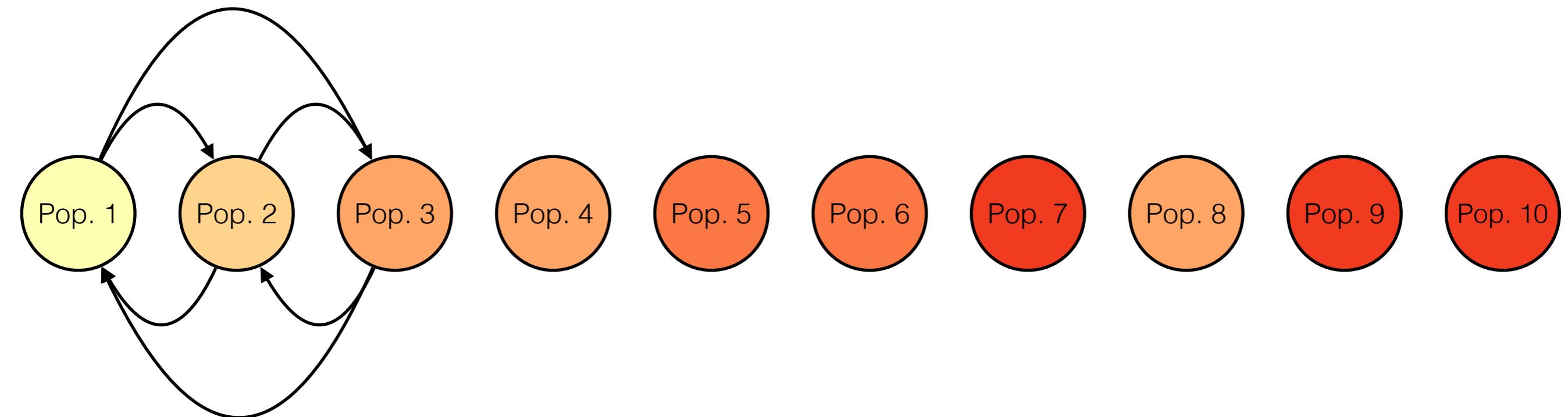
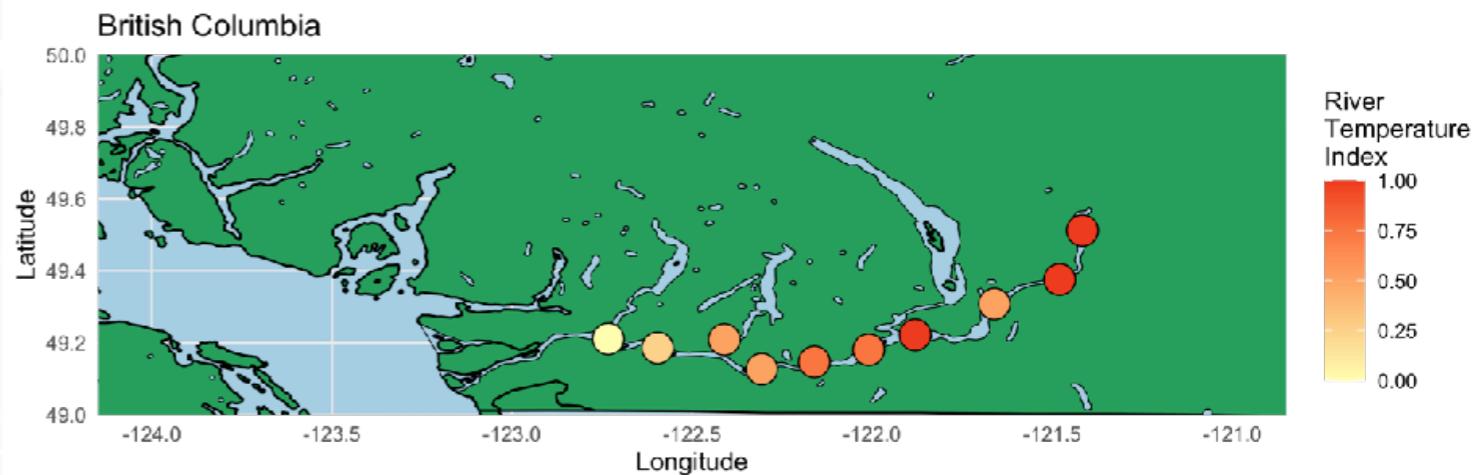
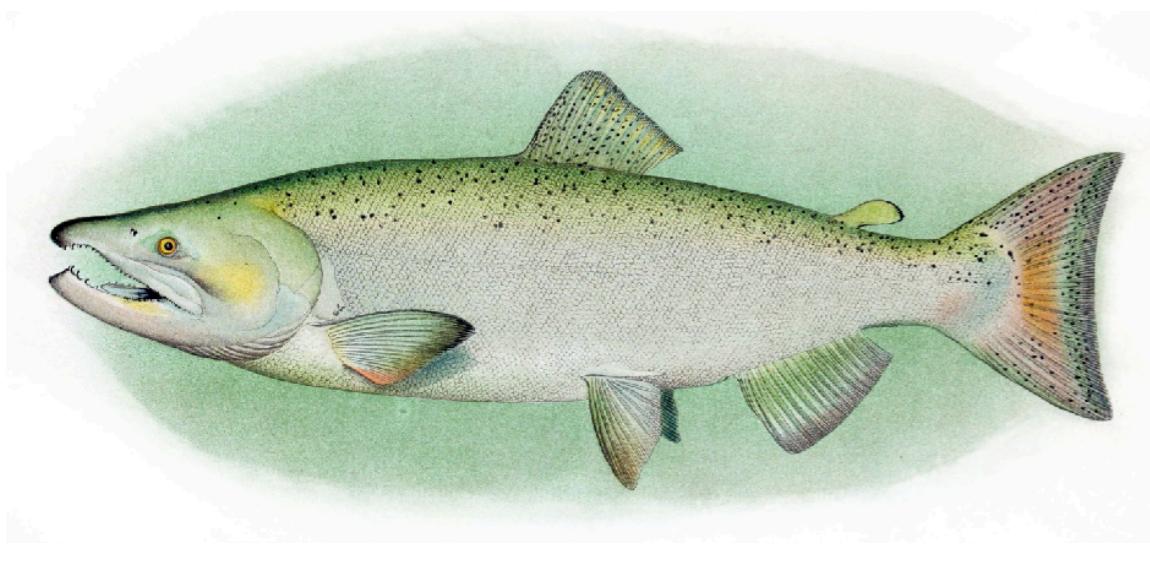
British Columbia

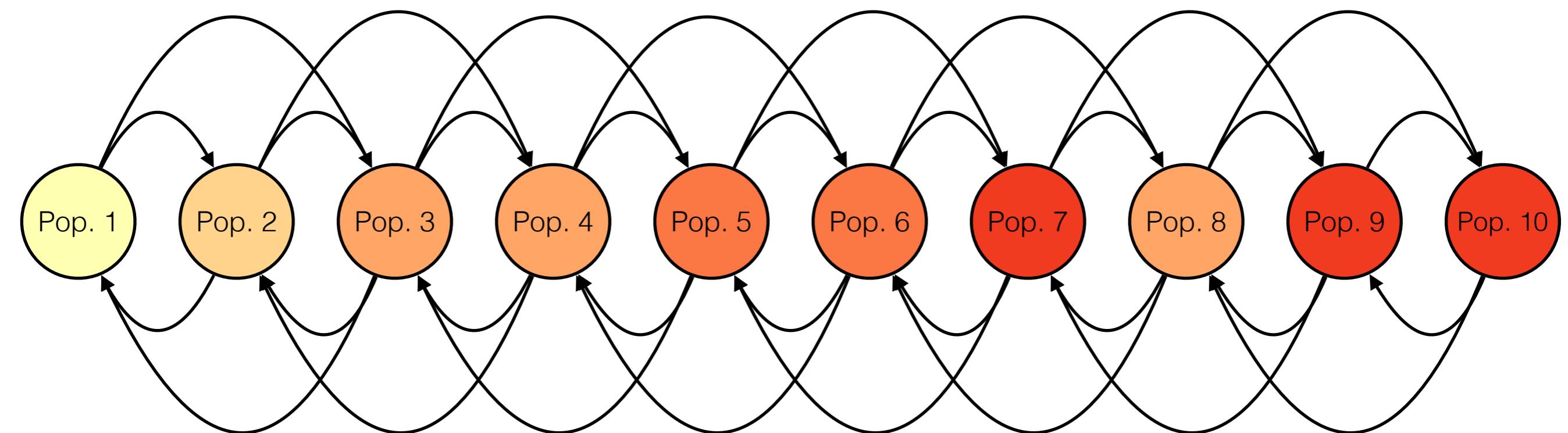
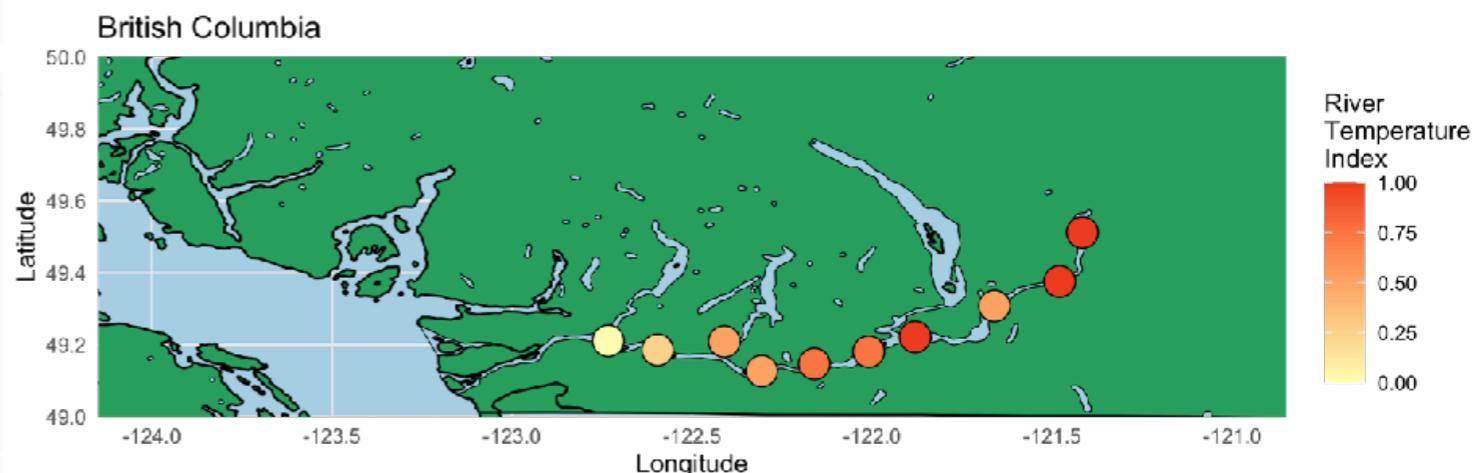
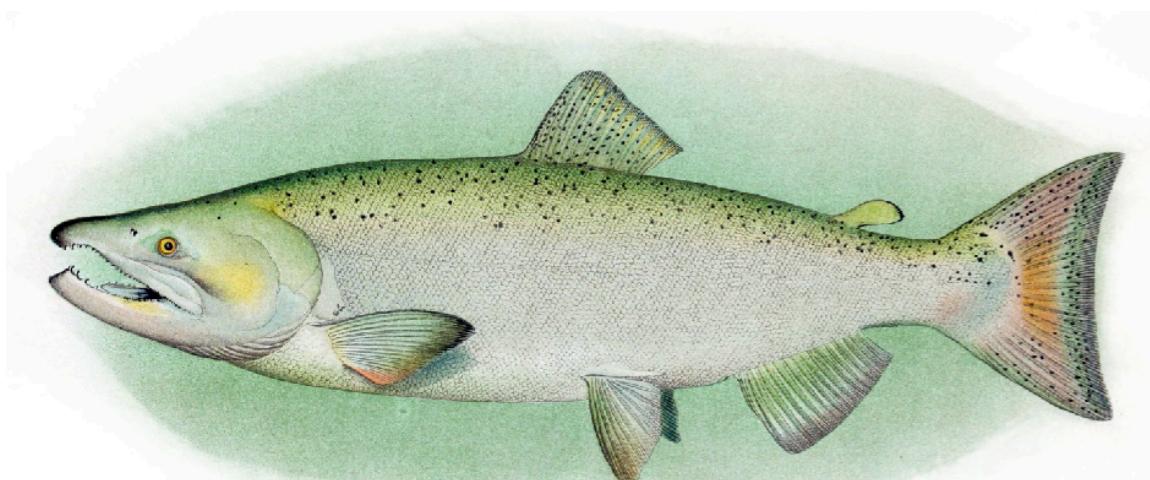


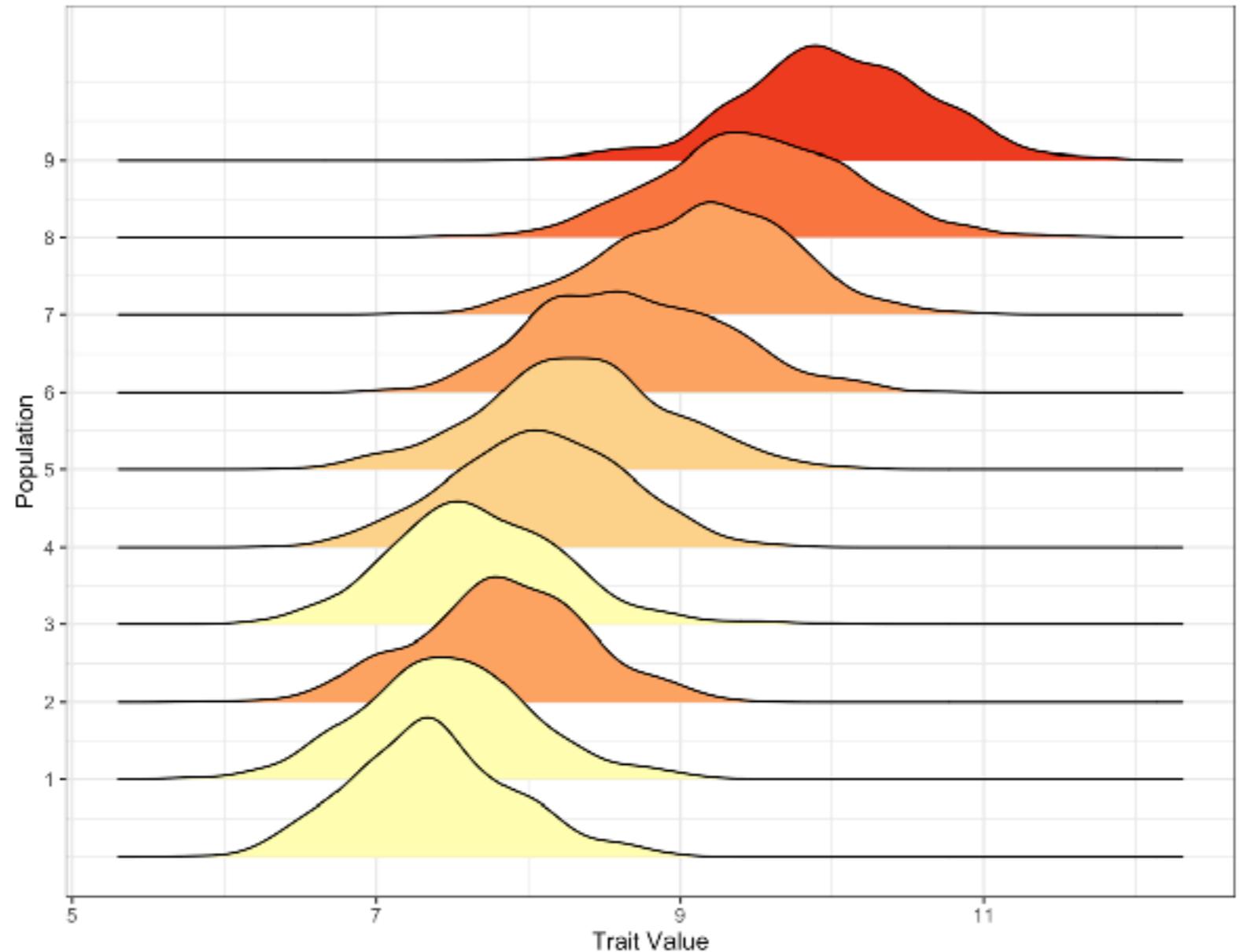
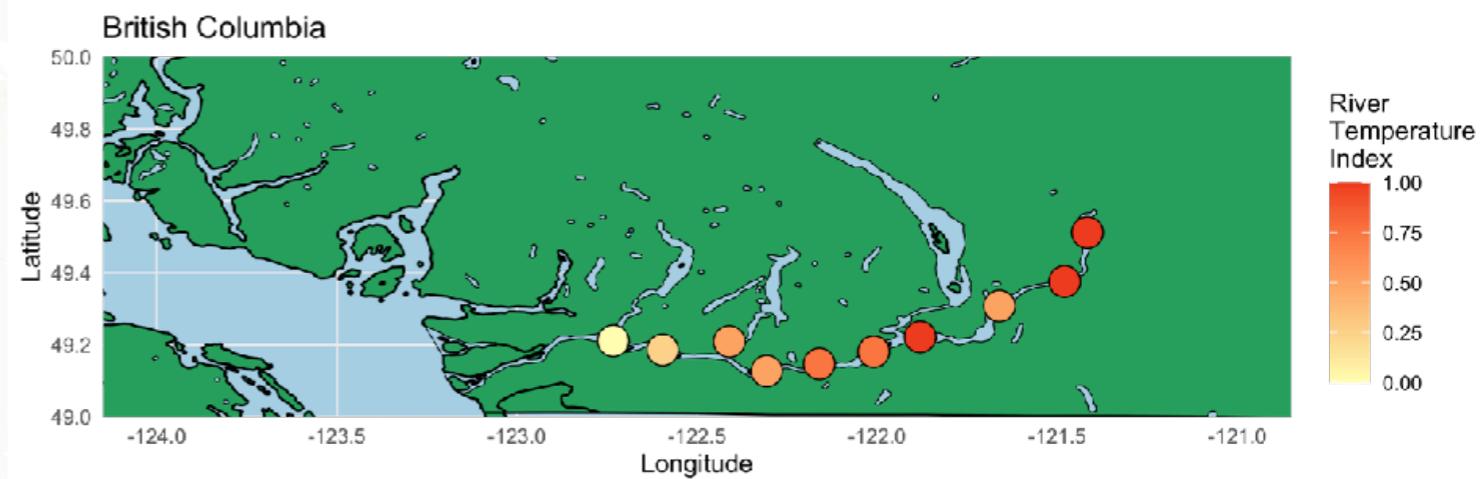
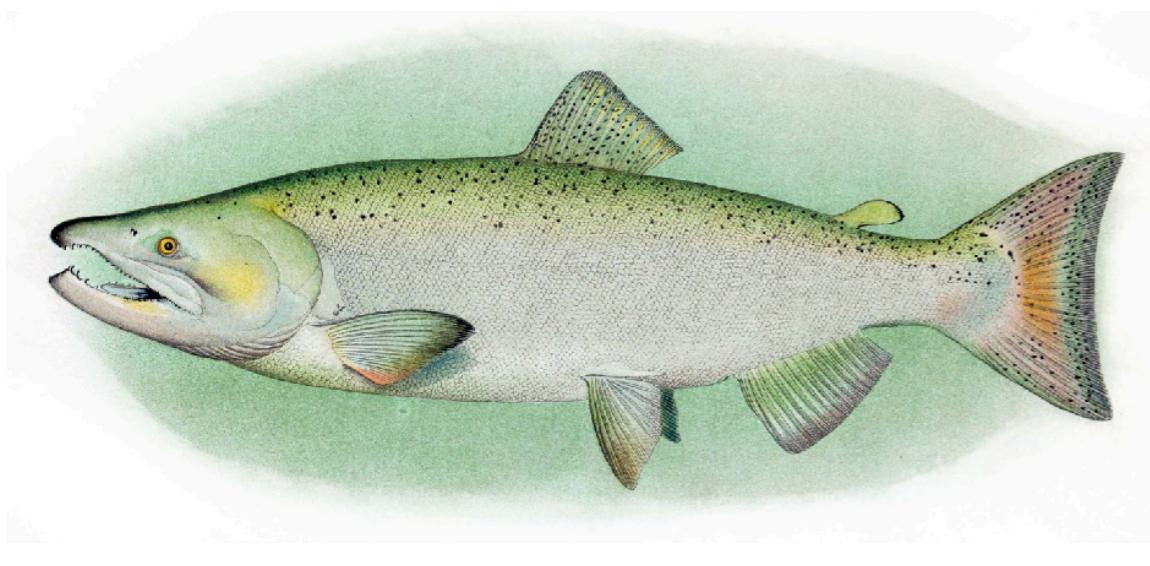


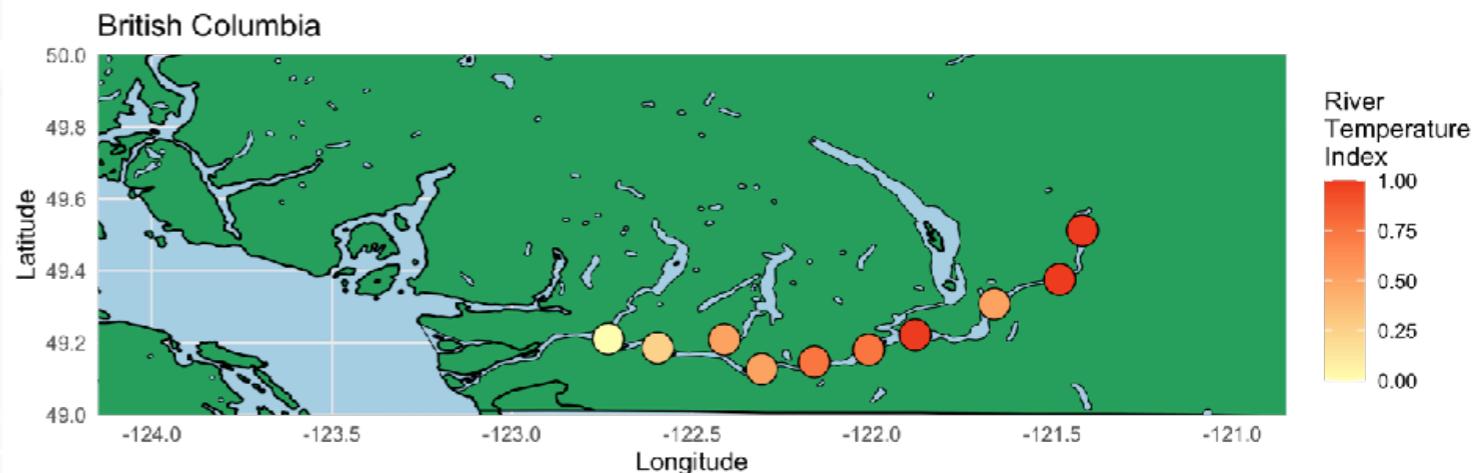
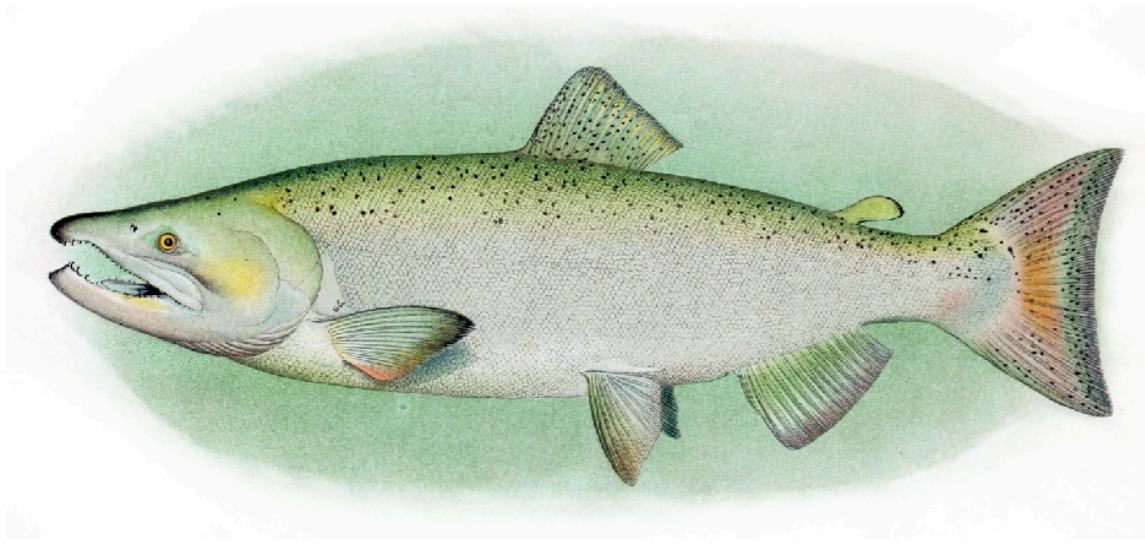






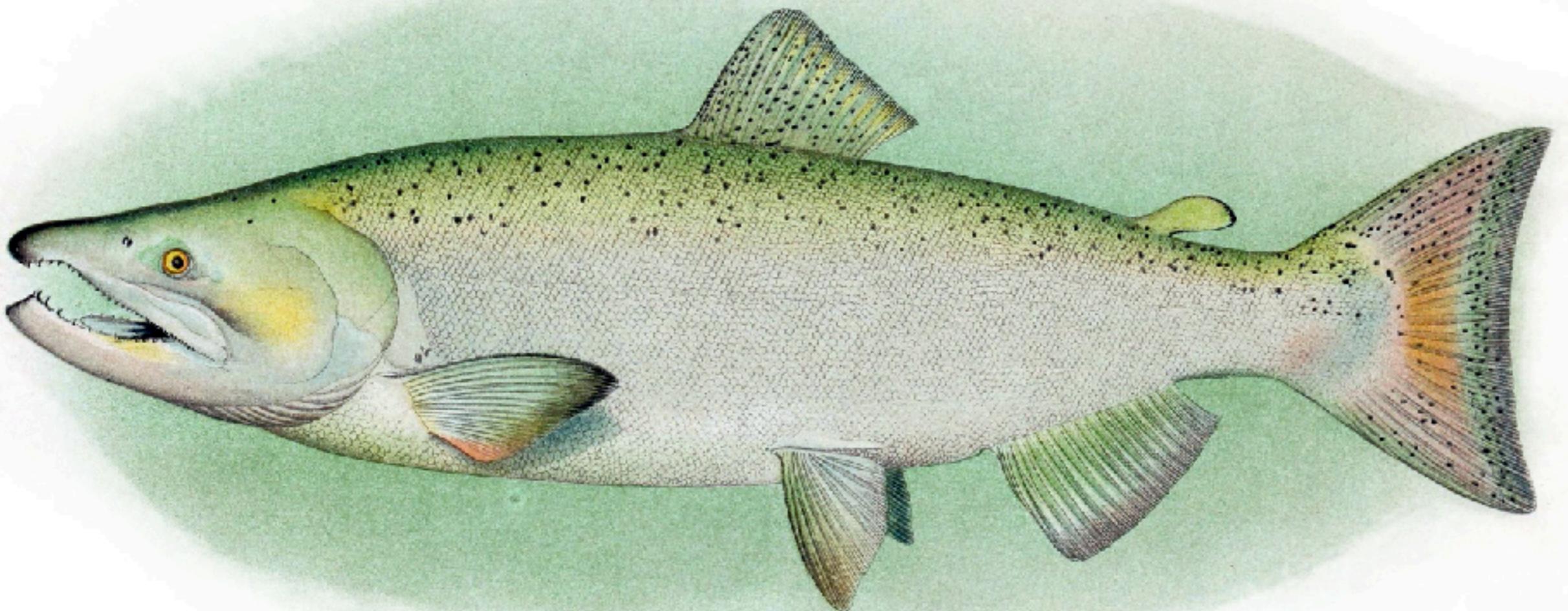






This week we will use the simulated data to:

- Assemble a genome (Wednesday morning)
- Align sequencing reads (Wednesday afternoon and Thursday morning)
- Quantify gene expression (Wednesday afternoon)
- Call variants (Thursday afternoon)
- Examine population structure (Friday morning)
- Analyse the genetic basis of a quantitative trait (Friday morning/afternoon)



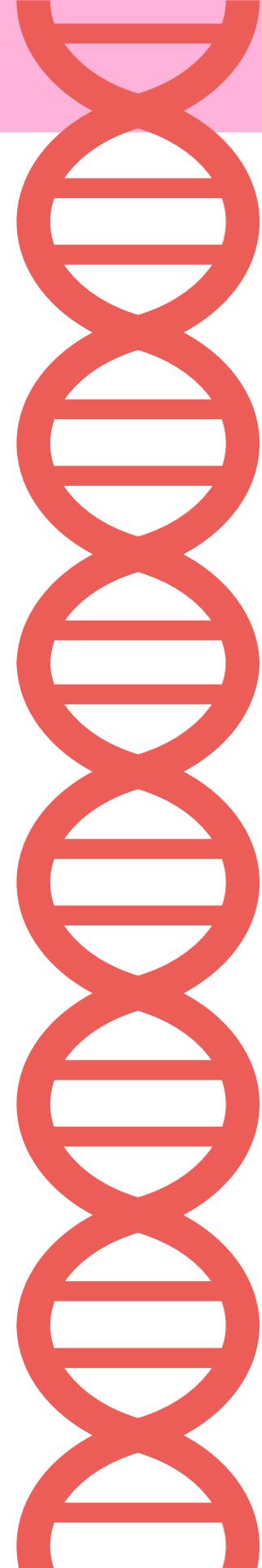
Questions?

Topic 1 Tutorial

In this first tutorial, we will introduce a number of core concepts in bioinformatics.

By the end of this tutorial we will have done the following:

- Played with a reference genome and the FASTA format
- Explored high-throughput sequence alignments (SAM/BAM files)
- Examined genome annotations stored in the General Feature/Transfer Format (GFF/GTF) and BED formats
- Assessed genetic variants called from genome sequencing data

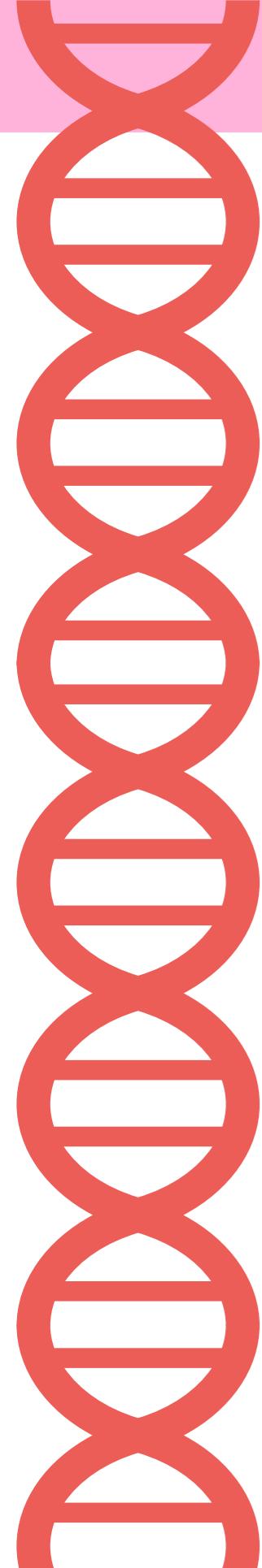


A brief history of DNA sequencing

Genome milestones

- 1977: *Bacteriophage ΦX174*
- 1982: *Bacteriophage lambda*
- 1995: *Haemophilus influenzae*
- 1996: *Saccharomyces cerevisiae*
- 1998: *Caenorhabditis elegans*
- 2000: *Drosophila melanogaster*
- 2000: *Arabidopsis thaliana*
- 2001: *Homo sapiens*
- 2002: *Mus musculus*
- 2004: *Rattus norvegicus*
- 2005: *Pan troglodytes*
- 2005: *Oryza sativa*
- 2007: *Cyanidioschyzon merolae*
- 2009: *Zea mays*
- 2010: Neanderthal
- 2012: Denisovan
- 2013: The HeLa cell line
- 2013: *Danio rerio*
- 2017: *Xenopus laevis*

Excerpted and edited from Box 1 and 2 - Shendure et al 2017 Nature



A brief history of DNA sequencing

Technological milestones

1953: Sequencing of insulin protein

1965: Sequencing of alanine tRNA

1968: Sequencing of cohesive ends of phage lambda DNA

1977: Maxam–Gilbert sequencing

1977: Sanger sequencing

1990: Paired-end sequencing

2000: Massively parallel signature sequencing by ligation

2003: Single-molecule massively parallel sequencing-by-synthesis

2003: Zero-mode waveguides for single-molecule analysis

2003: Sequencing by synthesis of in vitro DNA colonies in gels

2005: Four-colour reversible terminators

2005: Sequencing by ligation of in vitro DNA colonies on beads

2007: Large-scale targeted sequence capture

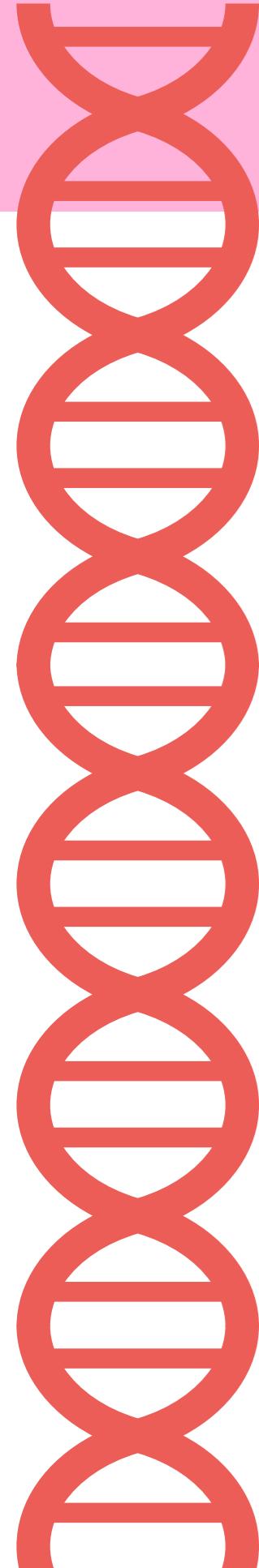
2010: Direct detection of DNA methylation during single-molecule sequencing

2010: Single-base resolution electron tunnelling through a solid state detector

2011: Semiconductor sequencing by proton detection

2012: Reduction to practice of nanopore sequencing

2012: Single-stranded library preparation method for ancient DNA



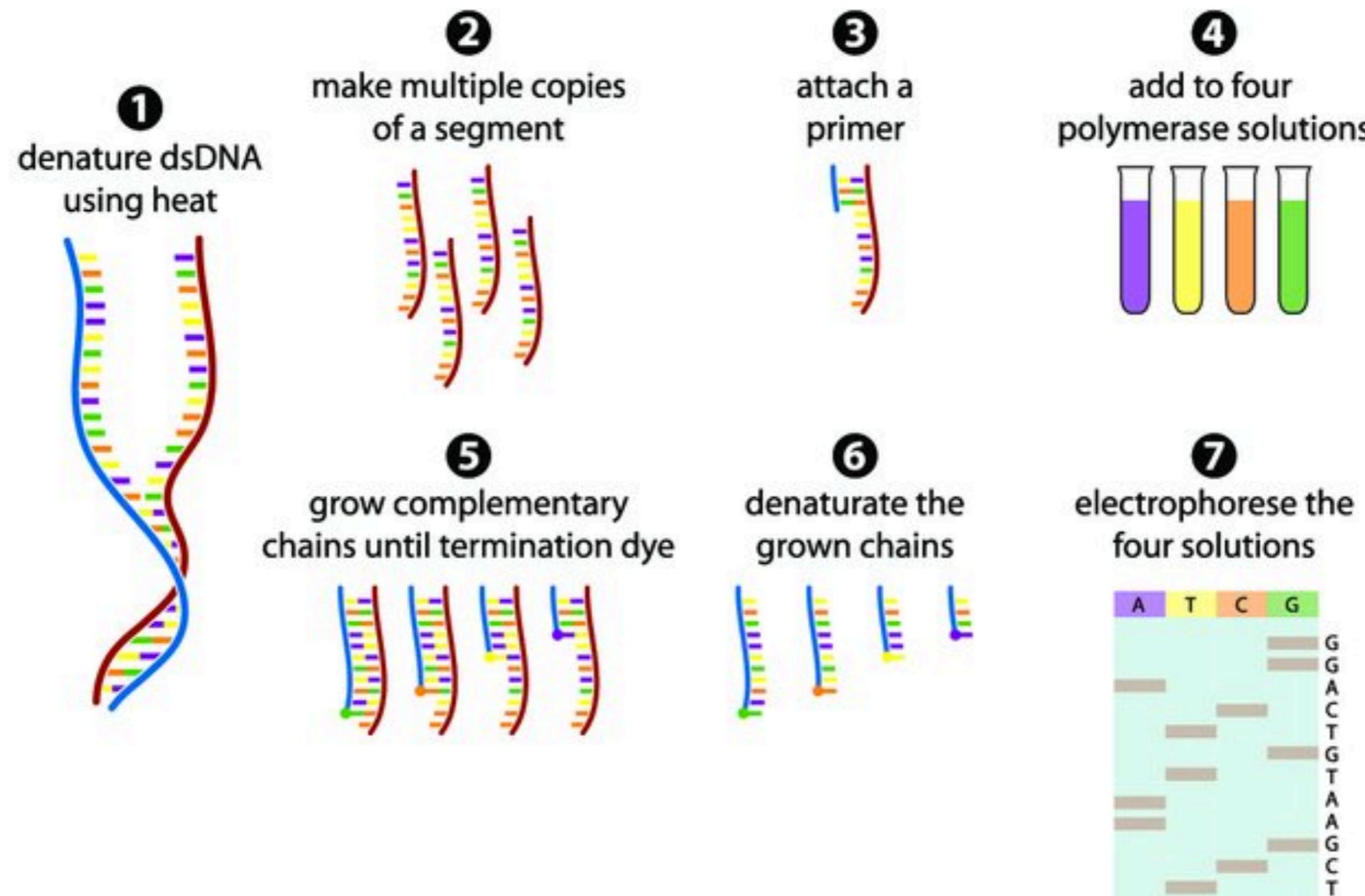
First Generation Sequencing

Maxam-Gilbert: Chemical modification and cleavage followed by gel electrophoresis

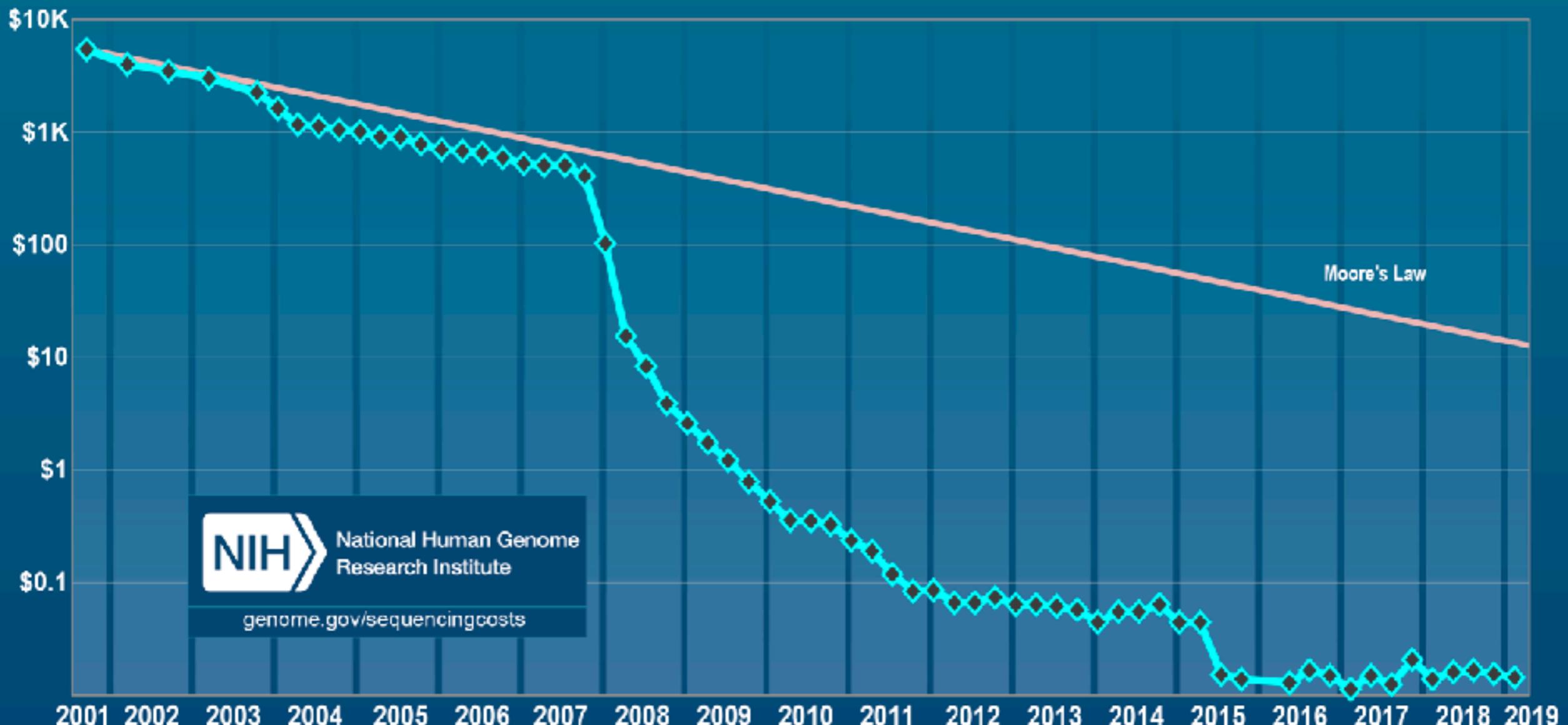
Sanger: Selective incorporation of chain-terminating dideoxynucleotides followed by gel electrophoresis

- Became fully automated using fluorescently labeled dideoxy bases
- Dominant sequencer up until 2007
- Only one fragment sequenced per reaction
- Still used for sequencing individual PCR products

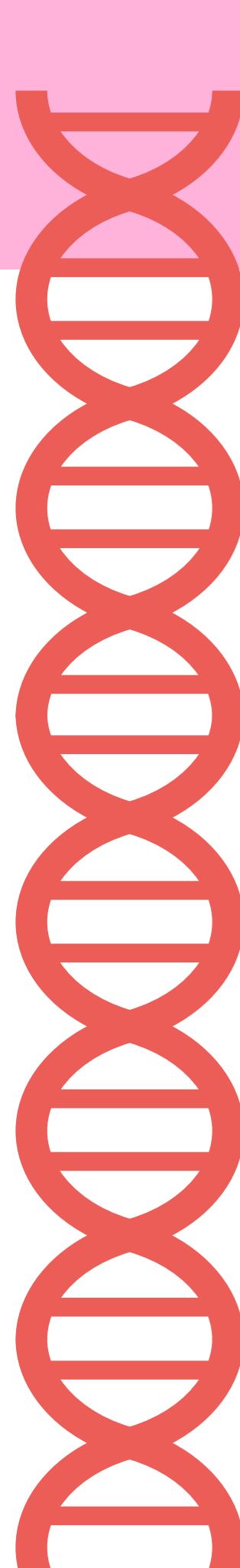
Sanger sequencing



Cost per Raw Megabase of DNA Sequence



*Moore's law stated that the number of transistors on a microchip doubled every two years, while costs halved



Second (Next-gen) and third generation sequencing

Sequences many molecules in parallel

Don't need to know anything about the sequence to start

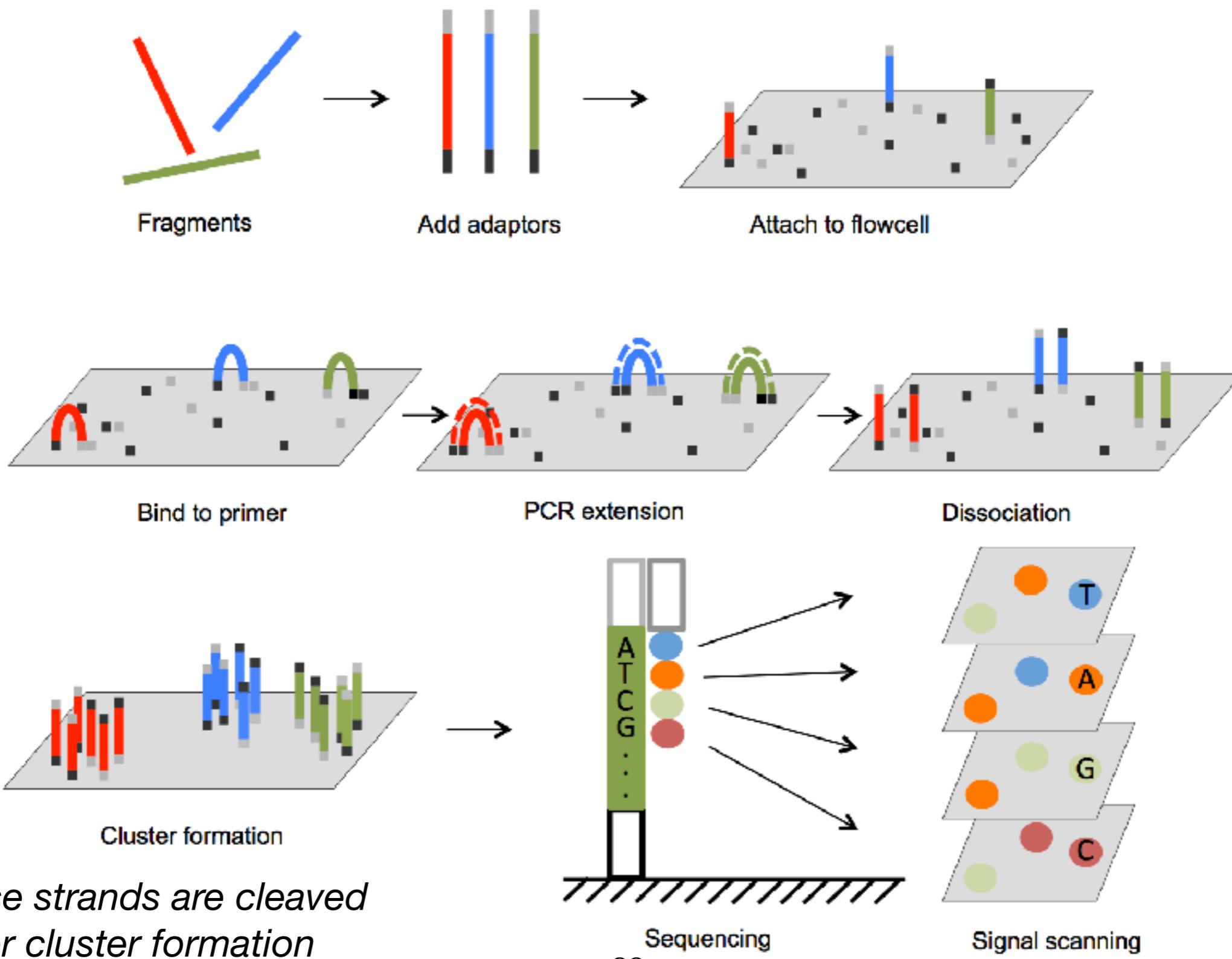
Main technologies:

- Illumina
- Ion torrent
- 454 (Pyrosequencing)
- PacBio

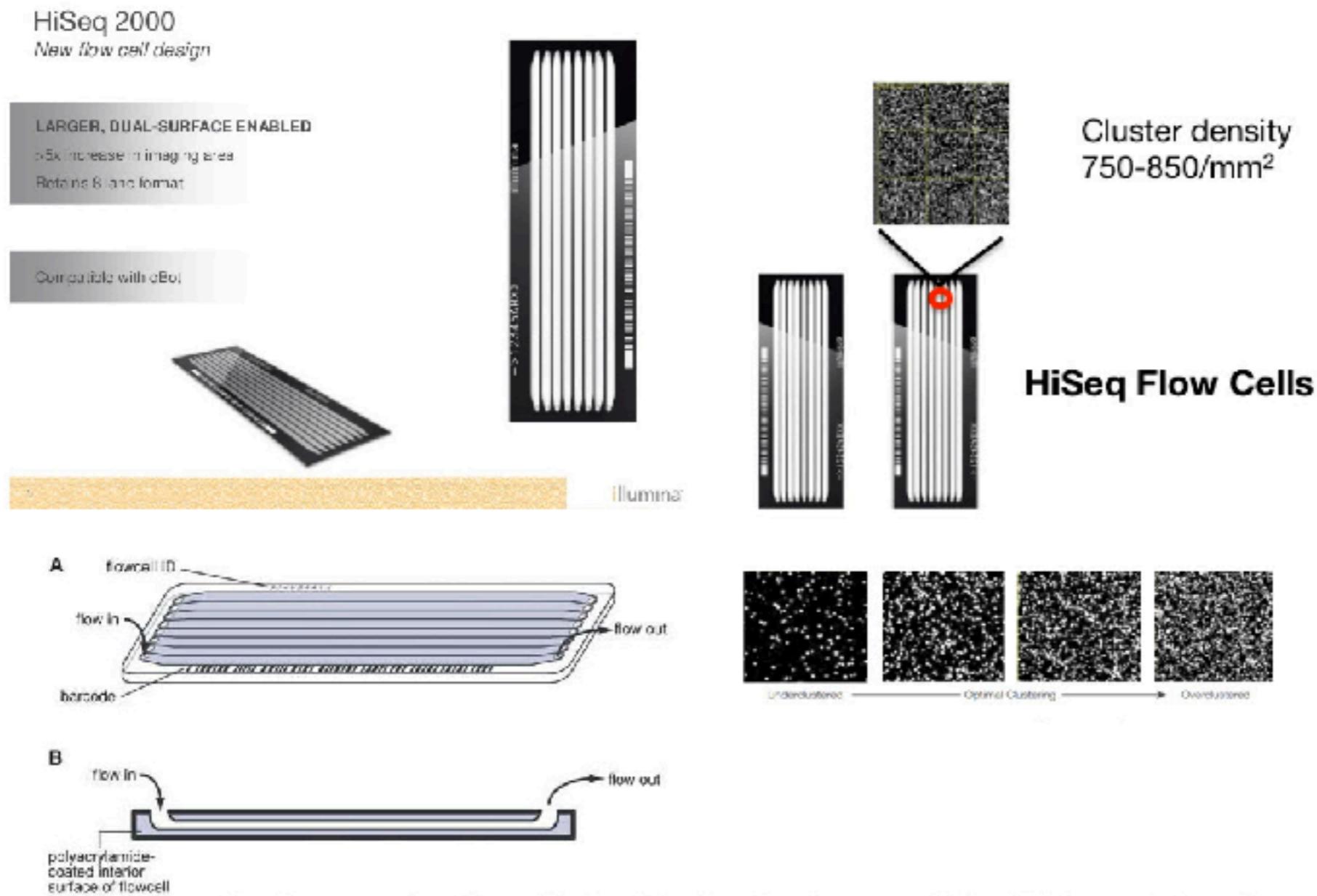
Second generation sequencing

Technology	Read Length	Accuracy	Bases/run	Uses
Illumina	50-600bp	99.9%	500-600 GBase	Resequencing General depth
Oxford Nanopore	5kb-100kb	85-95%	10-30GBase	Microbial genomes Genome assembly
PacBio	10kb-40kb	85-90%	5-10Gbase	Genome assembly Structural variants

Illumina sequencing



Illumina sequencing



From hackteria.org
<https://www.hackteria.org/wiki/File:FlowCell.jpg>

Open up the website to the
Topic 1 tutorial

Part 1: Setting up the tutorial

Install IGV

- Follow the links on the Topic 1 page to install the IGV from the Broad institute if you haven't done so already

Part 1: Setting up the tutorial

Download data for the tutorial

- Follow the links in the tutorial to obtain the data for this practical session
- Make sure you have all the items listed on the Topic 1 page
- ***OPTIONAL:*** Check data integrity

Part 2: Reference genomes

A reference genome is a representation of the average genome for a species/population

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Typically stored as a FASTA file (pronounced like pasta, or fast-a if you're fancy)

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A reference genome is a representation of the average genome for a species/population

Typically stored as a FASTA file (pronounced like pasta, or fast-a if you're fancy)

What a FASTA file looks like:

```
>chr_1
TGGGCAAGGCTGATGAACAGCAGCTGCATAAATTCTCCCCTAATTATATTGTAAATAGCT
GCAGCACACAATAAAGCTTGTAGAGACATCTAGAGAATCACACACTGCATCTGTTCT
GCCGCTCTCCCTCTTGCTCTGTTCTGAGAAGCACTGTTCACTGATTCTGGGTTGTATT
TGTGTTTTCATGCTAACATTGTTATTTGTTGCCTAGAAAGTTCTTGATTGGCCAA
ATTAGTCGATTTAAAGAGTGCACCTCTCTAGTGCATGTAATCTATGTGGACATCTCAAT
AGCTGCTTAATTGTTAGTGGTAATCTCCTCTGAACAGAGAGAAAGGCCTACATGCAGC
CCTCAGAGGAGAGGTGTCAATCTCTCTTGAATTCTCTTGTGTTCCCTTCAGAAGAATC
ATTCTAATCTGGTATTGTACAAGAGGAAATAATGGGACTAAAACCAGGCATGCACCATC
TGATAGATTCACATCCCTAGAAGACTTTGTTGTGTTCAAGTGGAGAGCCTGCTG
```

FASTAs are plain text files

Part 2: Reference genomes

A reference genome is a representation of the average genome for a species/population

Typically stored as a FASTA file (pronounced like pasta, or fast-a if you're fancy)

What a FASTA file looks like:

Sequence
name

```
>chr_1
TGGGCAAGGCTGATGAACAGCAGCTGCATAAATTCTCCCCTAATTATATTGTAAAATAGCT
GCAGCACACAATAAAAGCTTTGTTAGAGACATCTAGAGAATCACACACTGCATCTGTTCT
GCCGCTCTCCCTCTTGCTCTGTTCTGAGAAGCACTGTTCACTGATTCTGGGTTGTATT
TGTGTTTTCATGCTTAACATTGTTATTTGTTGCCTAGAAAGTTCTTGATTGGCCAA
ATTAGTCGATTTAAAGAGTGCACCTCTCTAGTGCATGTAATCTATGTGGACATCTCAAT
AGCTGCTTAATTGTTAGTGGTAATCTCCTCTGAACAGAGAGAAAGGCCTACATGCAGC
CCTCAGAGGAGAGGTGTCAATCTCTCTTGAATTCTCTTGTGTTCCCTTCAGAAGAAC
ATTCTAATCTGGTATTGTACAAGAGGAAATAATGGGACTAAAACCAGGCATGCACCATC
TGATAGATTCACATCCCTAGAAGACTTTGTTGTGTTCAAGTGGAGAGCCTGCTG
```

Nucleotide
sequence

FASTAs are plain text files

Part 2: Reference genomes

Load the reference genome into IGV and explore it a bit

Part 2: Reference genomes

Load the reference genome into IGV and explore it a bit

1. *How many chromosomes do our Salmon have?*

Part 2: Reference genomes

Load the reference genome into IGV and explore it a bit

- 1. How many chromosomes do our Salmon have?*
- 2. What is the length of each chromosome?*

Part 2: Reference genomes

Load the reference genome into IGV and explore it a bit

- 1. How many chromosomes do our Salmon have?*
- 2. What is the length of each chromosome?*
- 3. What is the nucleotide sequence corresponding to chr_1:666-670?*

Flavours of DNA sequencing

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

Different approaches have various pros and cons
(we'll discuss these tomorrow)

This is not an exhaustive list, but based on what people in the BRC do

Part 3: Sequence alignments

Using any of the methods above, most projects will involve some form of sequence alignment

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In this part of the tutorial, we will explore several sequence alignments obtained using different methods

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Flavours of DNA/RNA sequencing

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

Part 3: Sequence alignments

Whole genome resequencing

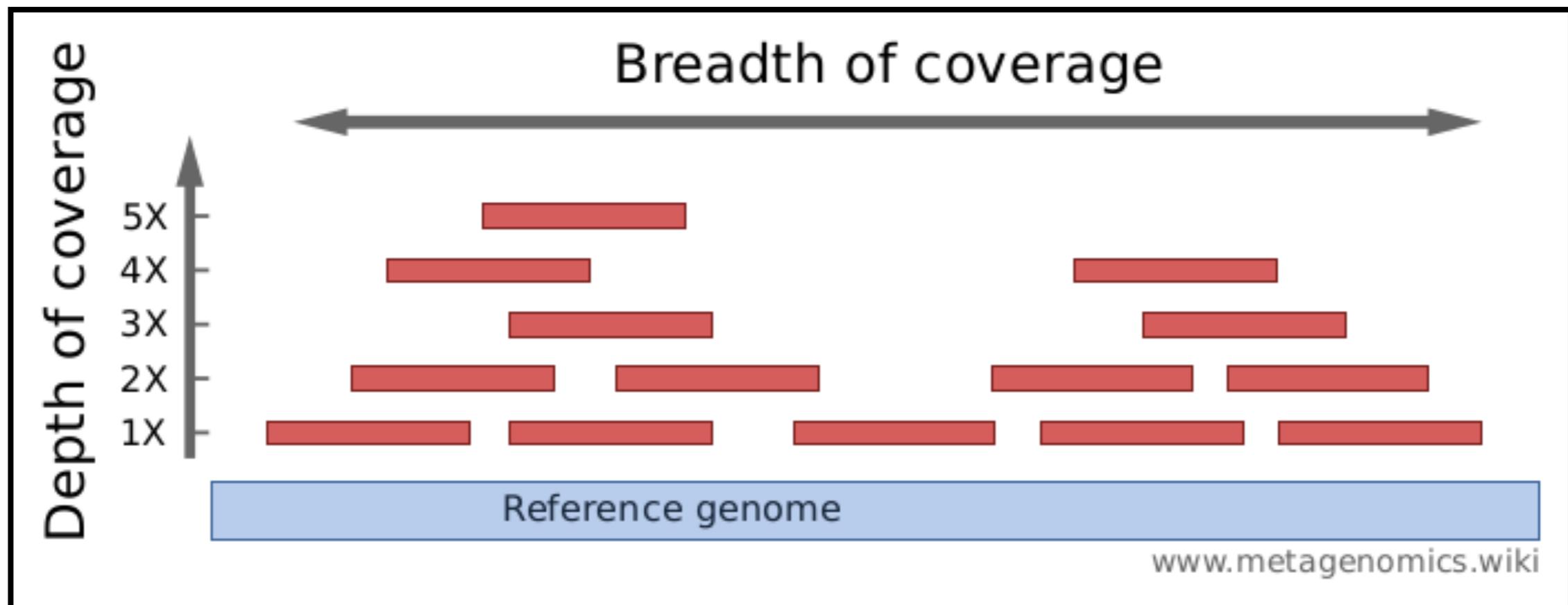
**Follow the instructions under Part 3.1 of tutorial
on the website**

Load the alignment files (BAM files) as indicated on the tutorial page and explore them using IGV

*Start by comparing Salmon.HiSeq.10x.bam to
Salmon.HiSeq.5x.bam*

Part 3: Sequence alignments

Whole genome resequencing



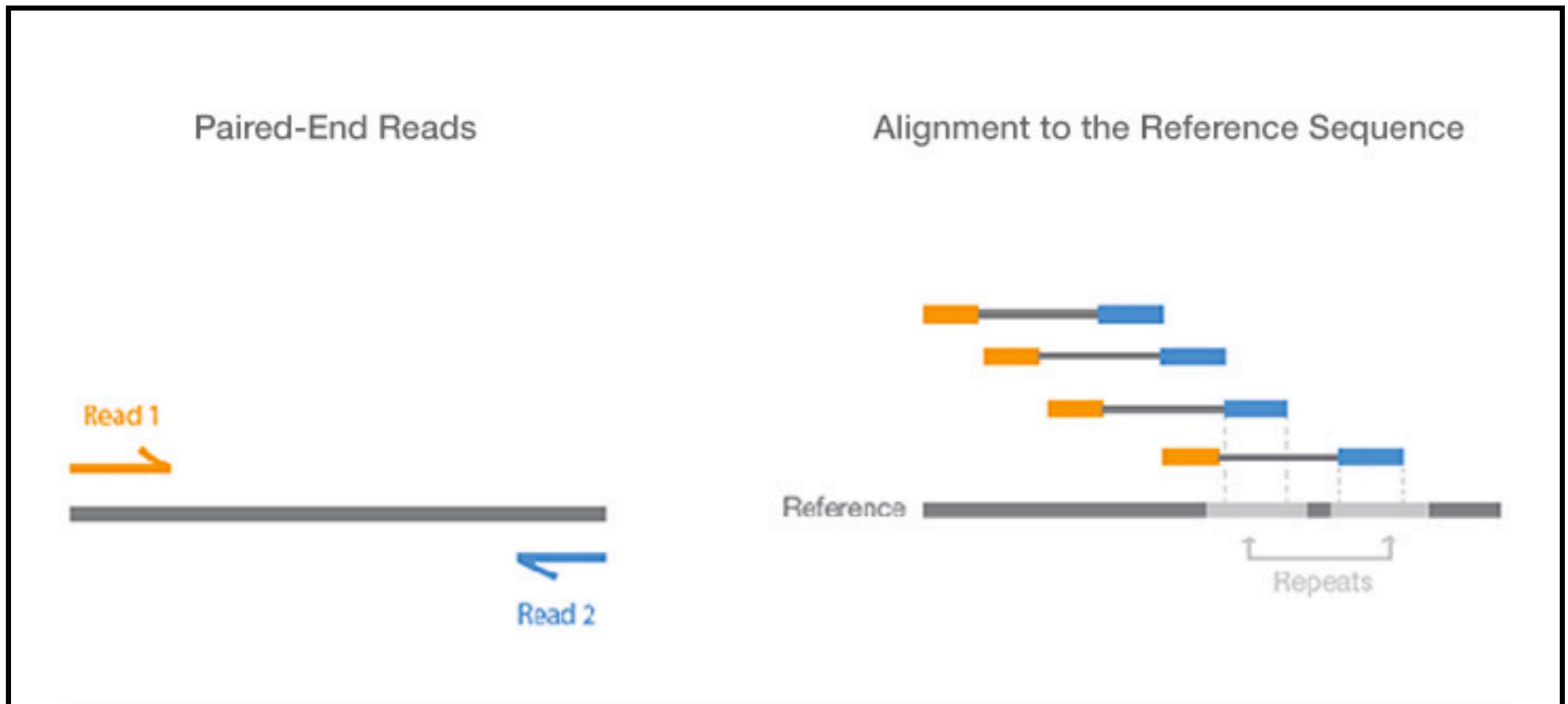
What does increasing sequencing depth give you?

What does increasing breadth give you?

Part 3: Sequence alignments

Whole genome resequencing

Why paired ends?



Picture from Illumina website

Illumina Machines



Name	MiSeq	HiSeq 4000	NovaSeq 6000
Sequencing Capacity	8Gbp	50Gbp	500-600Gbp
Cost (/lane)	~\$1,500	~\$3,000	~\$8,000

We've been looking at HiSeq data

Long read sequencing

Two dominant companies are PacBio and Oxford Nanopore

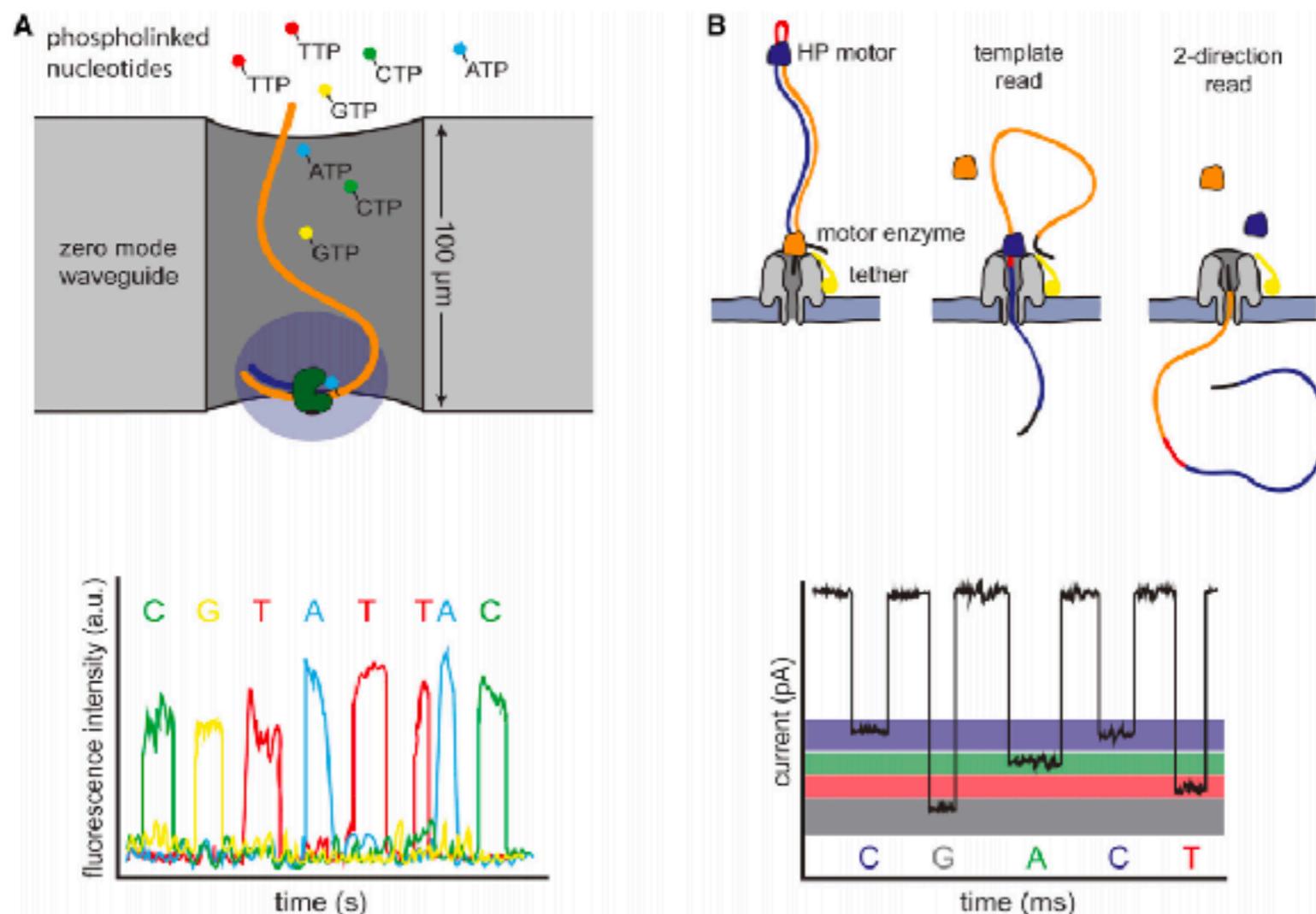


Figure 3. Single Molecule Sequencing Platforms

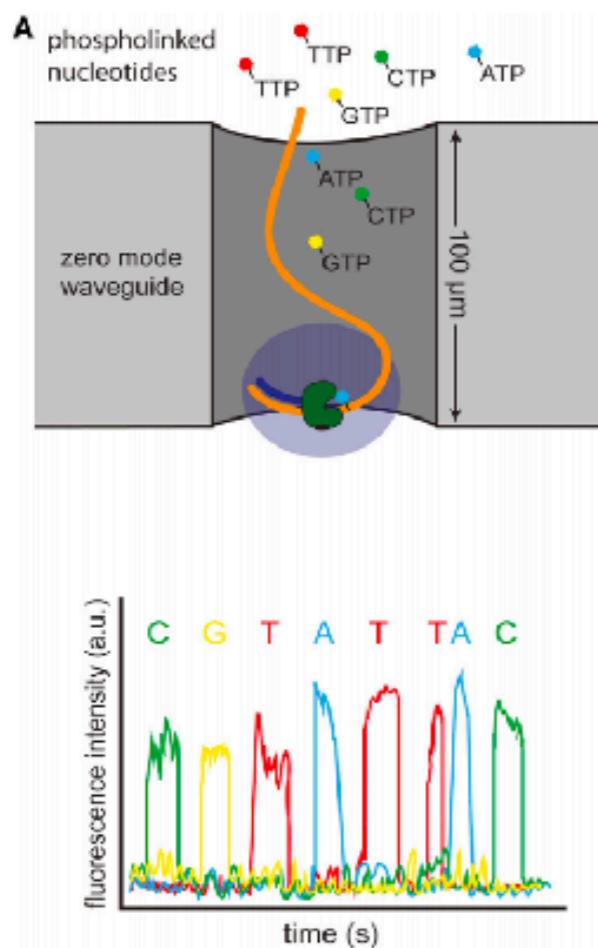
(A) Pacific Bioscience's SMRT sequencing. A single polymerase is positioned at the bottom of a ZMW. Phosphate-labeled versions of all four nucleotides are present, allowing continuous polymerization of a DNA template. Base incorporation increases the residence time of the nucleotide in the ZMW, resulting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

(B) Oxford Nanopore's sequencing strategy. DNA templates are ligated with two adaptors. 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).

Excerpted from Reuter et al 2015 - Molecular Cell

Long read sequencing

PacBio - Pacific Biosciences



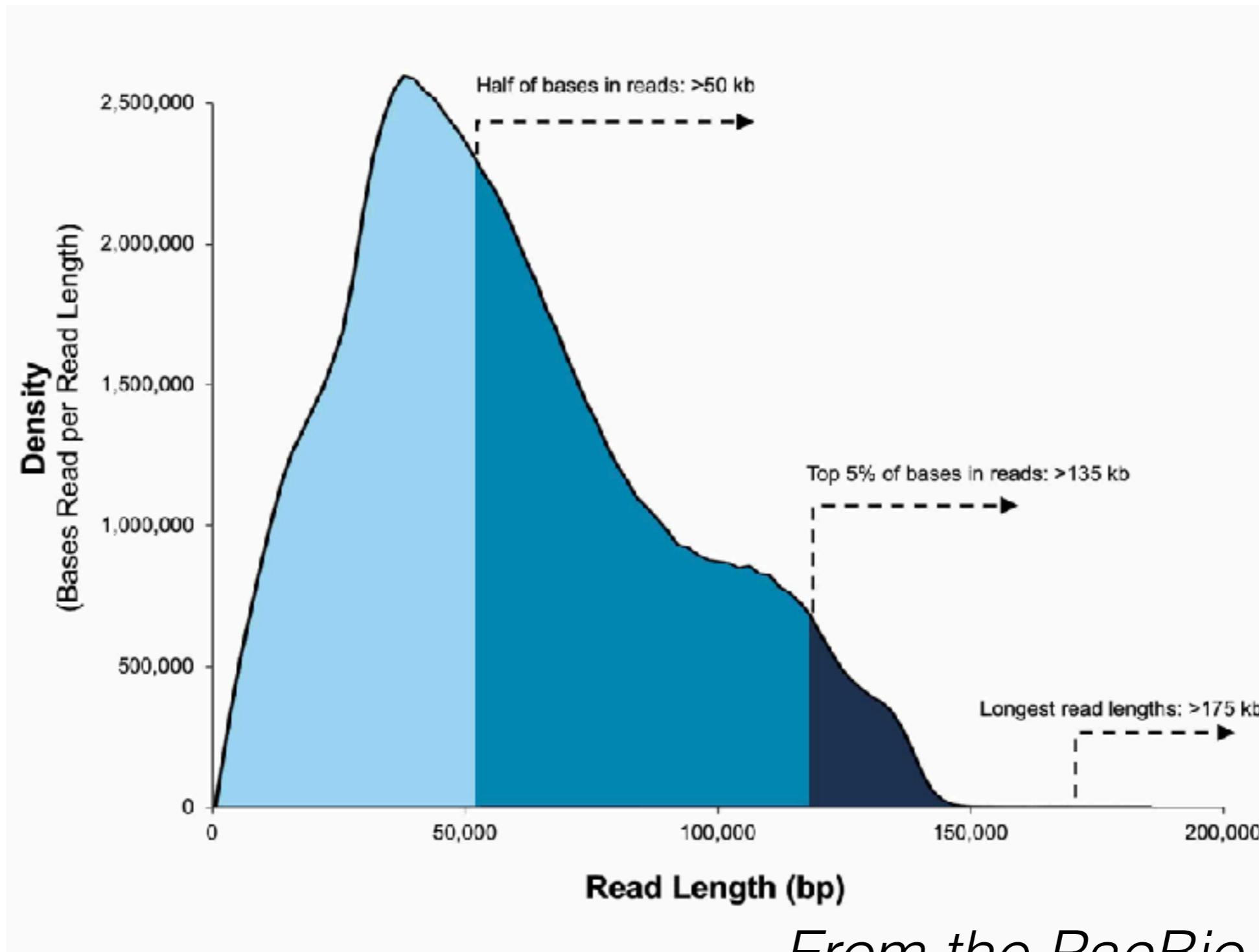
Sequel II

1-10Gb/flowcell

~\$500/flowcell

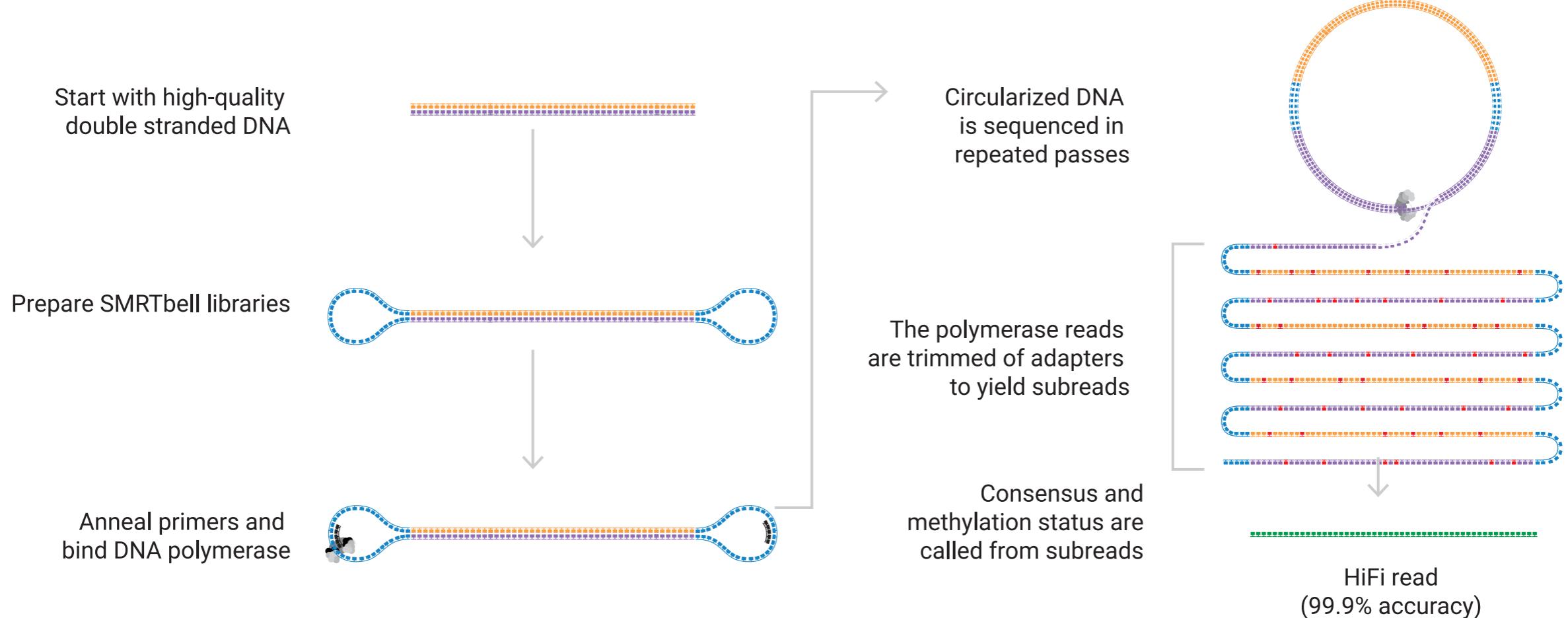
13% error rate

Pacific Biosciences



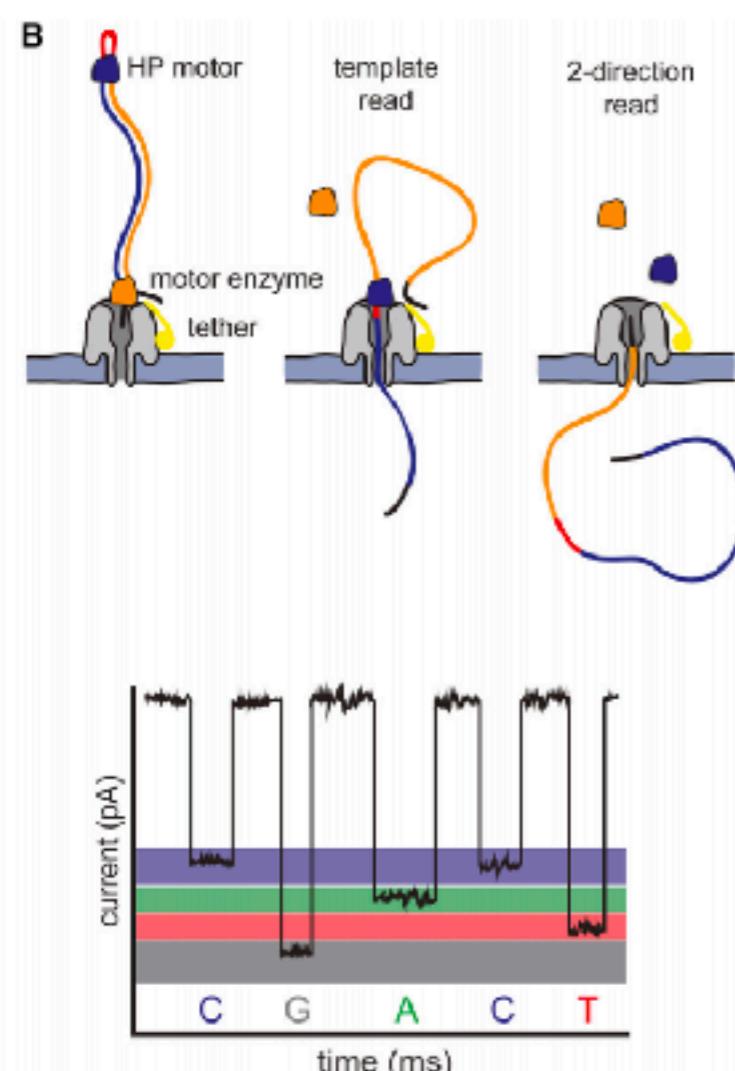
PacBio High Fidelity Sequencing (yet another game changer)

single-molecule, real-time sequencing technology (SMRT)



Long read sequencing

Oxford Nanopore



MinION

15-30Gb/flowcell

~\$1000/flowcell

2-13% error rate



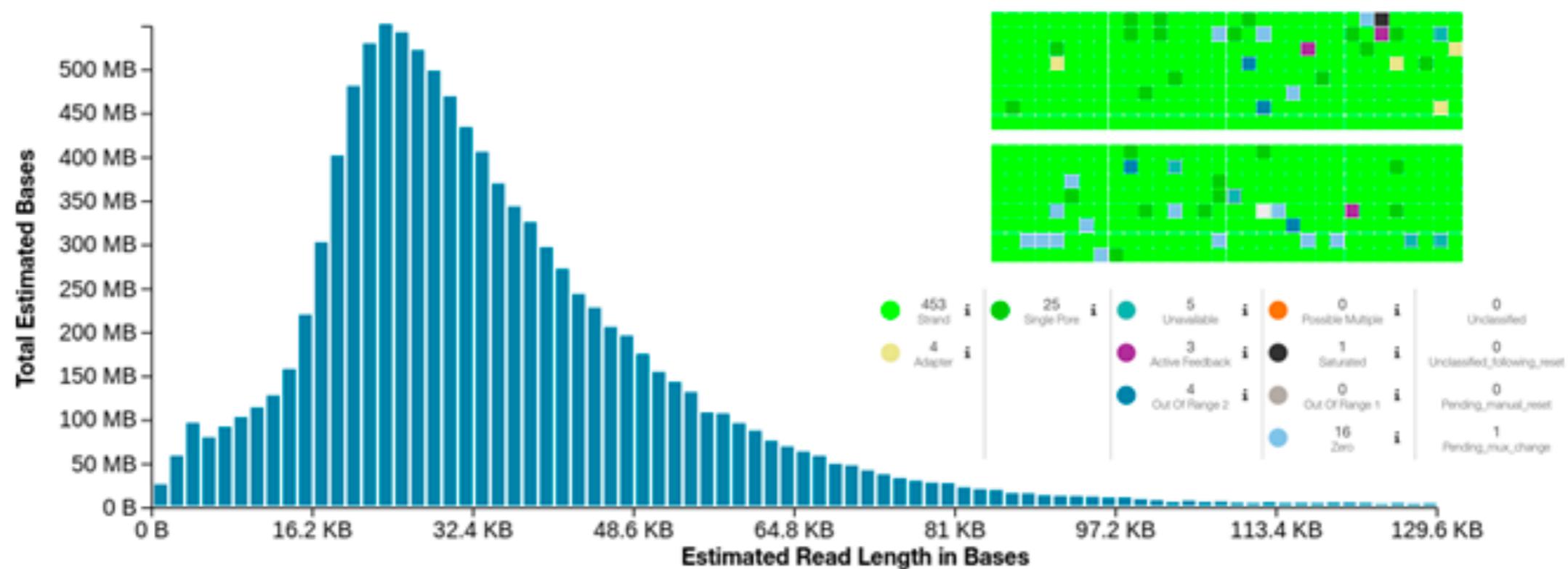
PromethION 24

100-180Gb/flowcell

~\$2000/flowcell

Oxford Nanopore

(C) *Eucalyptus albens*; end ligation library prep (SQK-LSK109). Output: 12.50 Gb.



Part 3: Sequence alignments

Whole genome resequencing

**Follow the instructions under Part 3.2 of tutorial
on the website**

Load up Salmon.16x.PacBio.bam and explore it using IGV

Part 3: Sequence alignments

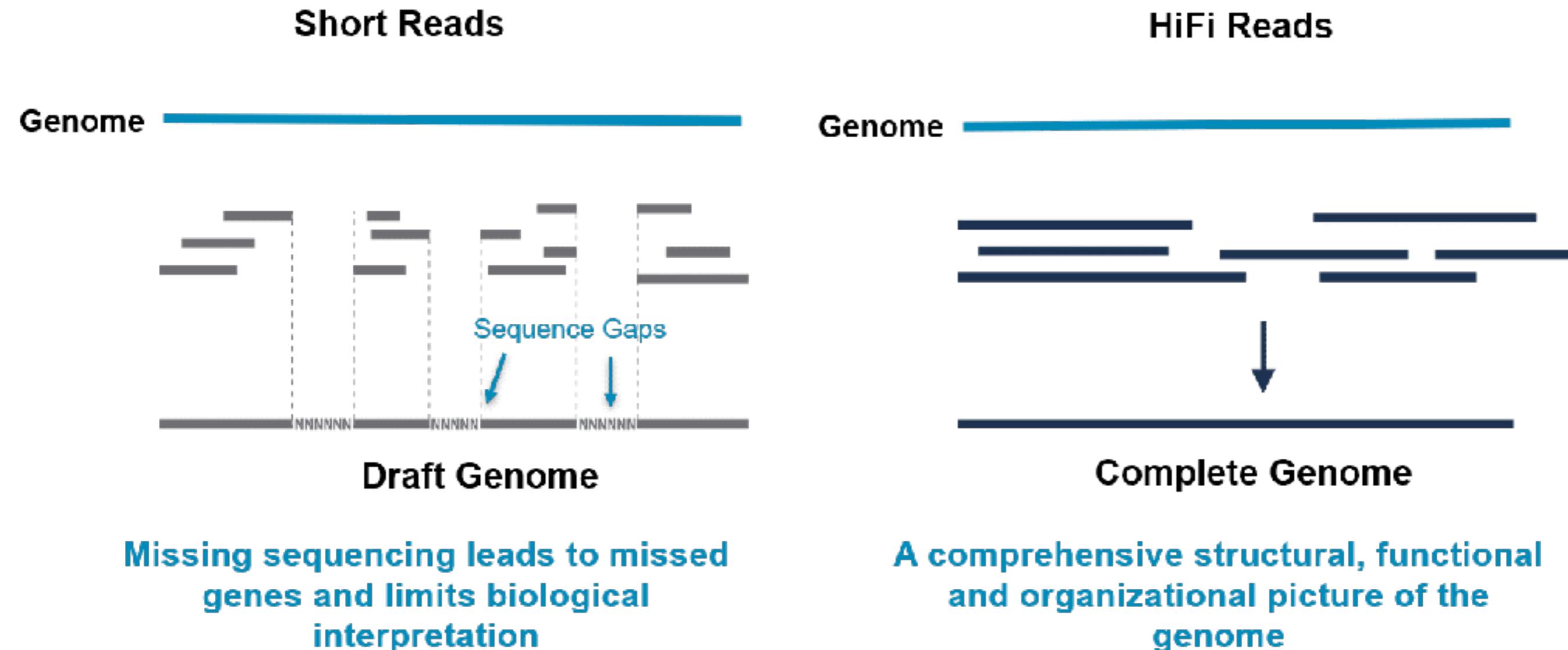
Whole genome resequencing

What do you think the longer reads would be useful for?

What are long reads bad at?

Part 3: Sequence alignments

Whole genome resequencing



Part 3: Sequence alignments

Whole genome resequencing

Short Reads		Long Reads	
Pros	Cons	Pros	Cons
Extremely accurate for complex regions	Rely on amplification, which can introduce errors (at a rate of around 10^{-6} - 10^{-7} /bp).	Great for genome assembly <ul style="list-style-type: none">• 30-60X coverage from ion torrent or PacBio will produce a nice draft genome.	More difficult library prep
Allele frequencies can be scored at many sites across the genome	Assembling and aligning short reads in repetitive regions is very challenging -> impossible	Can characterise alternate splicing of genes.	May be too expensive to be used for population level sequencing.
Very cost-effective	Both large and small structural variants pose difficulties	Structural rearrangement discovery and genotyping.	High error rate. <i>If you have the cash this is not true!</i>

Part 3: Sequence alignments

Reduced representation sequencing

**Follow the instructions under Part 3.3 of tutorial
on the website**

Load up Salmon.ddRAD.bam and explore it using IGV

Part 3: Sequence alignments

Reduced representation sequencing

What do you get with the ddRAD approach?

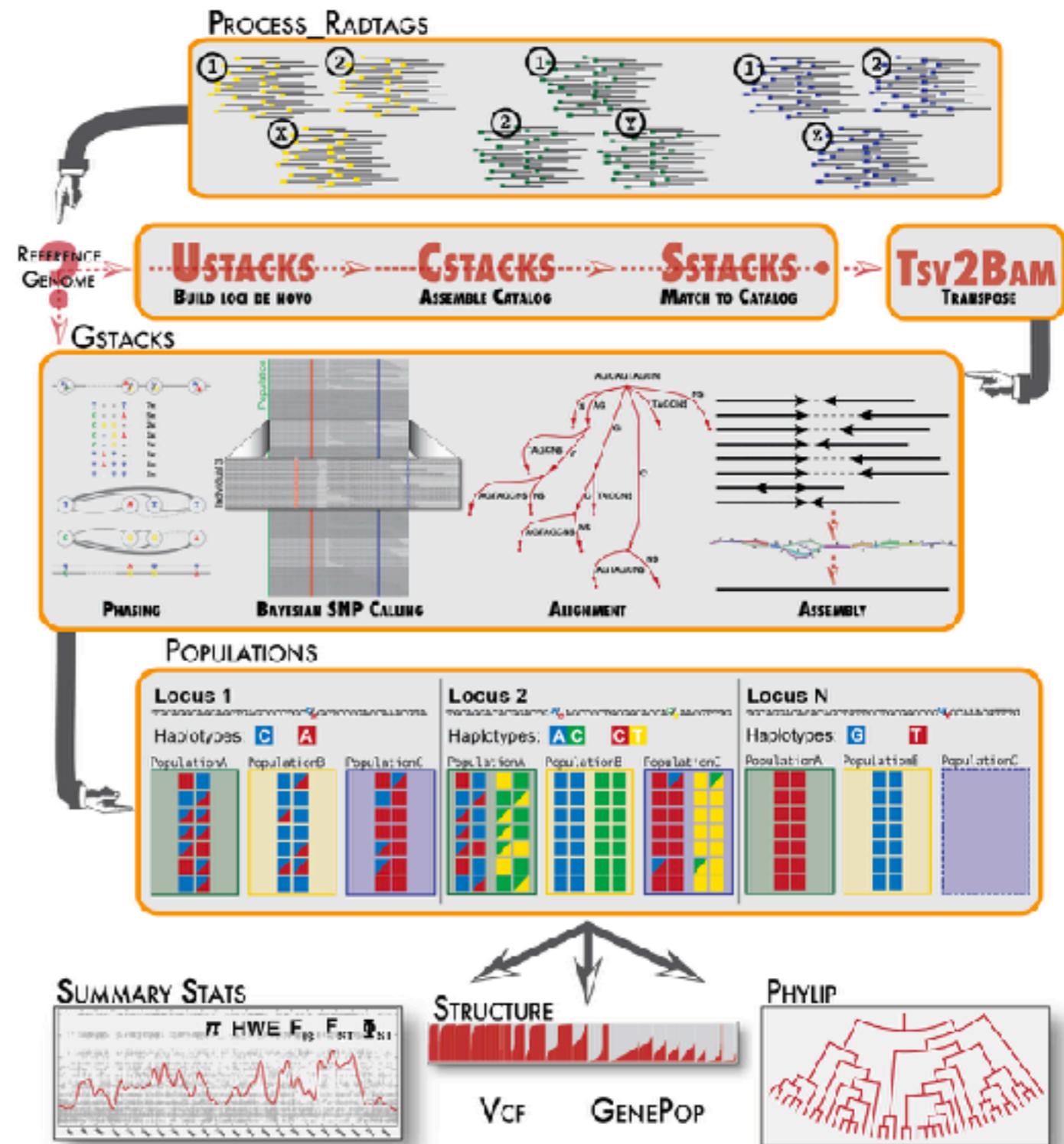
Can you think of some uses for this kind of data?

Part 3: Sequence alignments

Reduced representation sequencing

STACKS is an established pipeline for analysing reduced representation data (>2000 citations)

A real benefit is that you do not need a reference genome at all



Part 3: Sequence alignments

RNA-seq

**Follow the instructions under Part 3.4 of tutorial
on the website**

*Load up coldWaterSalmon.RNA.bam and explore it using
IGV*

Part 3: Sequence alignments

RNA-seq

You'll immediately notice the splice junctions that are inferred by STAR (the alignment software)

Can you think of any difficulties that might arise when trying to align RNA reads to a reference genome?

Part 4: Identifying variants

**Follow the instructions under Part 4 of tutorial
on the website**

*Re-load Salmon.HiSeq.5x.bam and
Salmon.HiSeq.10x.bam as well as
Salmon.HiSeq.20x.vcf.gz*

Part 4: Identifying variants

Scroll around and inspect some variants

What are some features of sequencing that you would think would be useful when identifying variants?

How to choose?

The different technologies and methodologies have different pros and cons

What you use will obviously be informed by budget, but the biological question should also drive your choice

Further reading

PDFs are available on the GitHub page for this topic:

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics*, 17(2), 81.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS one*, 7(5), e37135.

Shendure, J., Balasubramanian, S., Church, G. M., Gilbert, W., Rogers, J., Schloss, J. A., & Waterston, R. H. (2017). DNA sequencing at 40: past, present and future. *Nature*, 550(7676), 345-353.

Production cost per 30x Human genome over 18 years

