Outline for today

“lost in a field of genomics and code CGI style”

My background: largely in RADseq methods

What the structure of today is (focused on WGS and red-rep data)

Key is to look at your data in as many ways as possible!

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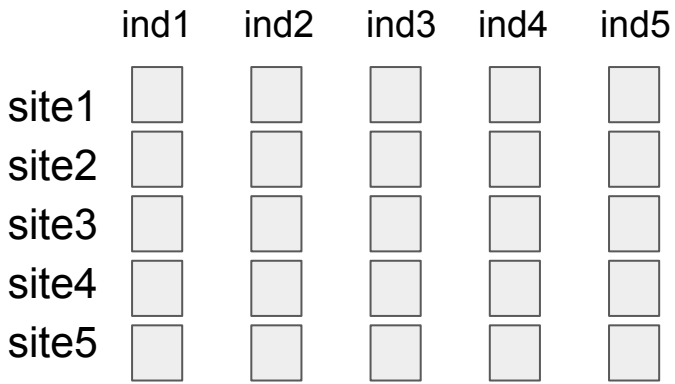

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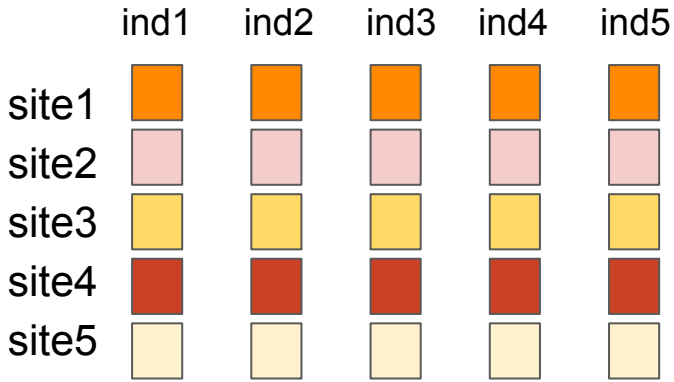

Exercise 1: across sites

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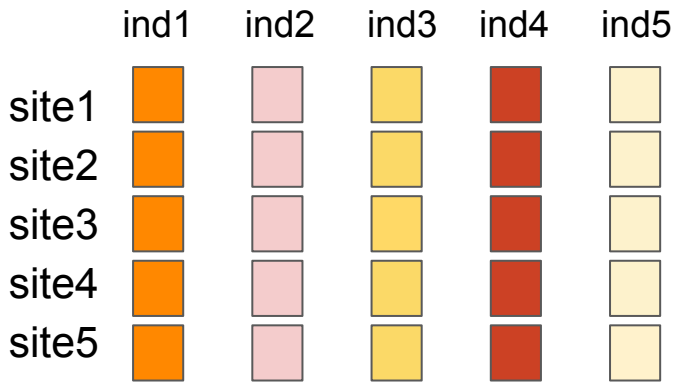

Exercise 1: across sites

Exercise 2: across individuals

An overview of ‘omics approaches

Genomics

- The study of the genome
- We’ll be talking about whole genome sequencing (WGS) and reduced-representation sequencing (sequencing a subset of the genome)

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An overview of ‘omics approaches

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Metagenomics

- The study of nucleotide sequences from all organisms in a sample
- Sometimes called “community genomics”, alluding to the study of specific communities
- Can involve sequencing a specific marker from all organisms of a specific type (e.g., 16S from all bacteria) or sequencing all extracted DNA or RNA

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Transcriptomics
- The study of the transcriptome - the complete set of RNA transcripts that are produced by the genome
- Often the goal is to compare what genes are expressed in specific environments, circumstances, or tissue/cell types

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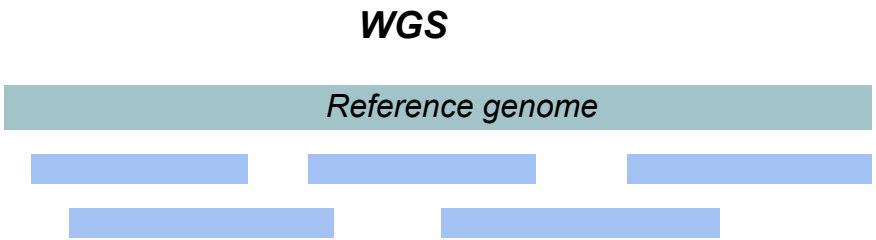
WGS and reduced-representation sequencing



WGS and reduced-representation sequencing

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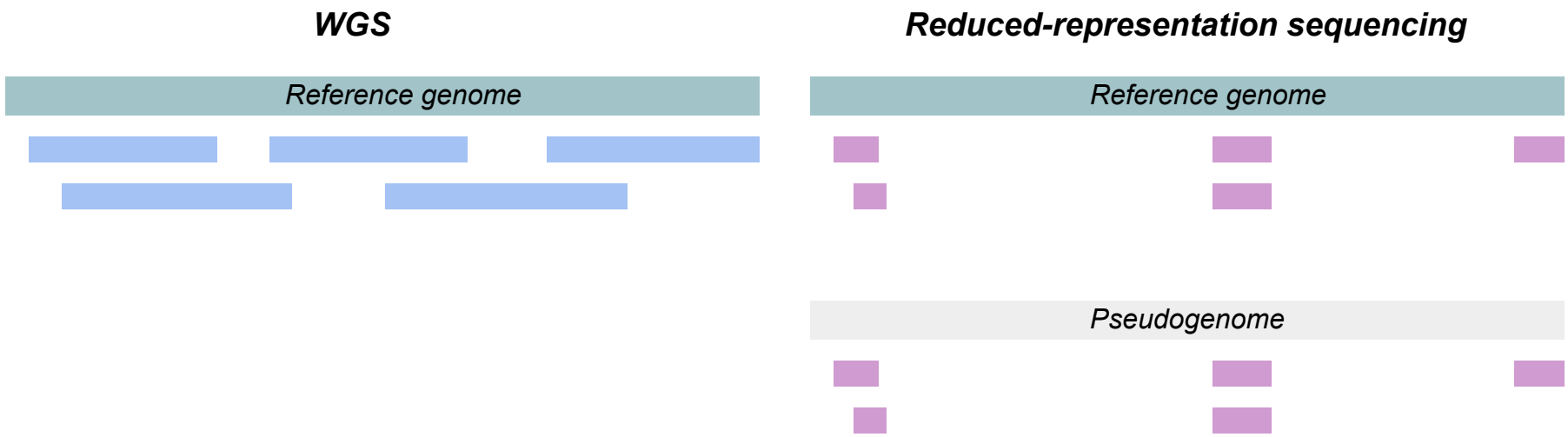

Practical considerations

- Financial & computational resources
- Sample quality (high-quality DNA is critical for sequencing methods that require long, contiguous DNA; low-quality DNA is fragmented)
- Sample selection (outgroups, etc.)
- Number of loci
- Number of reads (depth of coverage)
  - 0.1–3X for probabilistic genotyping (takes into account uncertainty)
  - 10–30X for “hard genotyping”

Adapted (with permission) from R.D. Tarvin

Reference genomes: the (very) basics

Genome

Reads

Contigs

Scaffold (chromosome?)

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***Coverage**: number of reads we have overlapping any given position, or:*
Average sequenced bp / total genome length

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                    CTTCGATGTG
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              GCAGCTCCCTT
           CGGCAGCTCC
      TGGATTCGGC
      TGGATTCGG
```

genome **TGGATTCGGCAGCTCCCTTCGATGTG**

Total sequenced bases = 61

Length = 26

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genome **TGGATTCGGCAGCTCCCTTCGATGTG** Length = 26

Total sequenced bases = 61

Coverage: 61 / 26 = 2.3X

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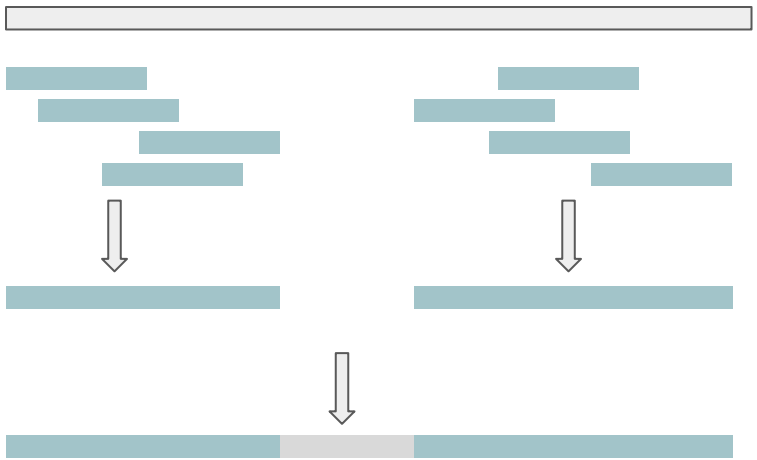

Genome

Reads

Contigs

Scaffold (chromosome?)

Comparing genome assemblies: **N50** and **L50** usually referred to

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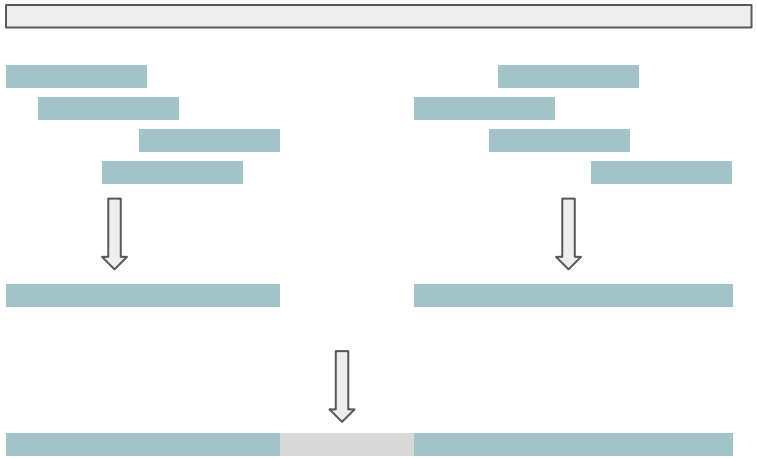

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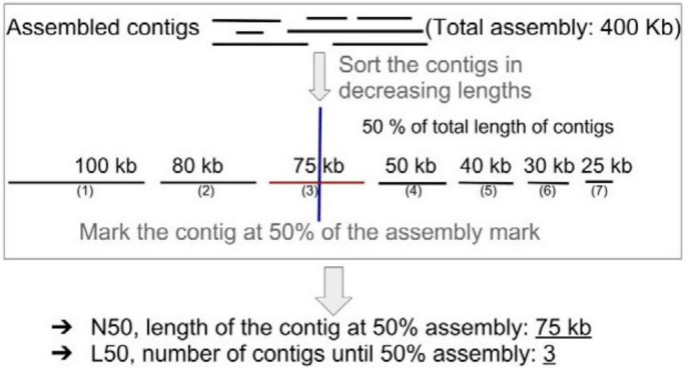

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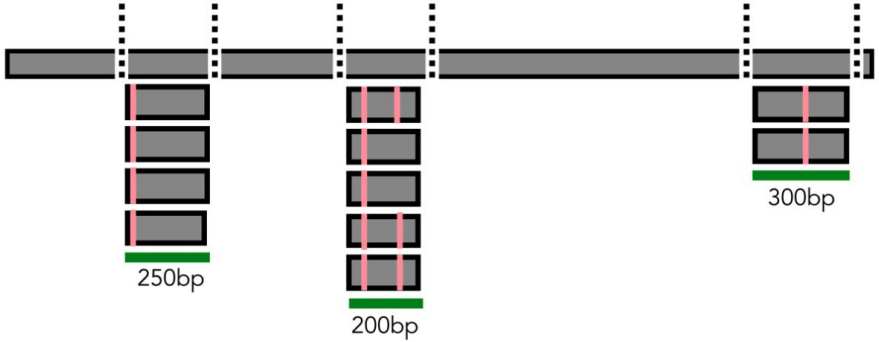
Calculating your sequencing needs



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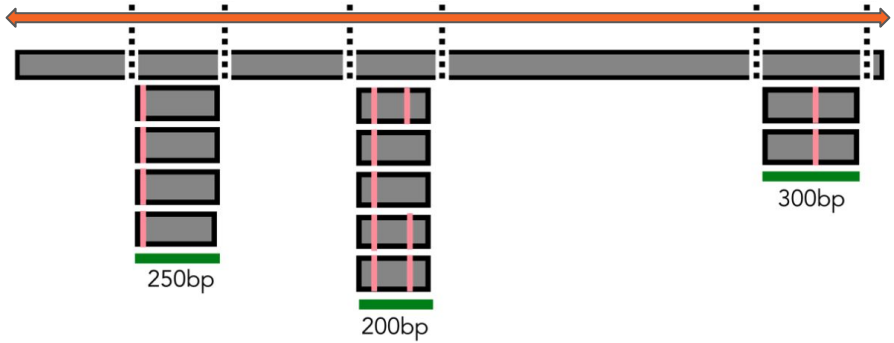
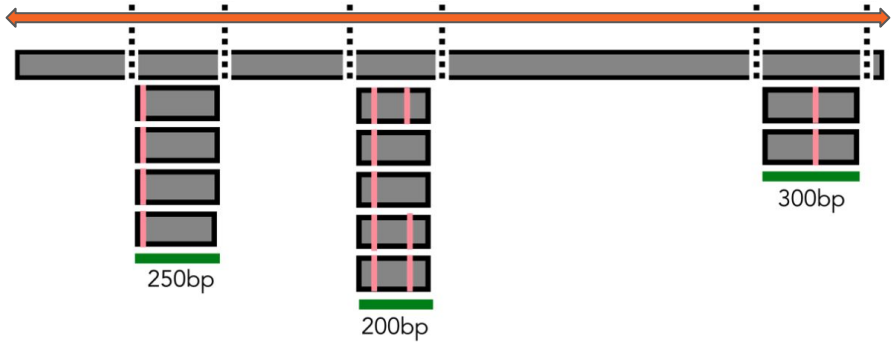


For RADseq libraries:

Genome size (bp)

Calculating your sequencing needs



For RADseq libraries:

Genome size (bp) x **% genome in size range**

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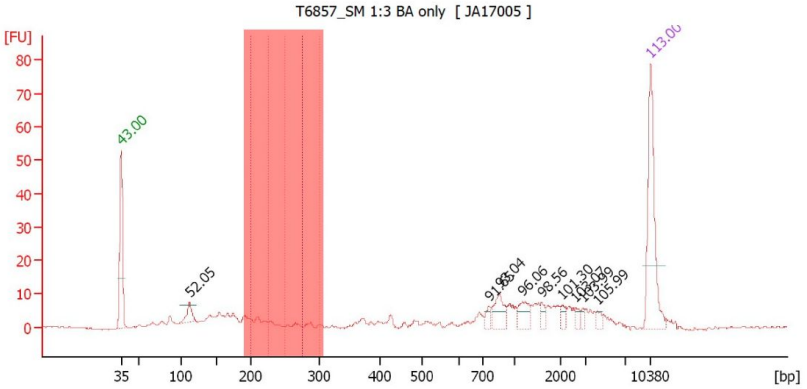

Region table for sample 3 : **T6857_SM 1:3 BA only**

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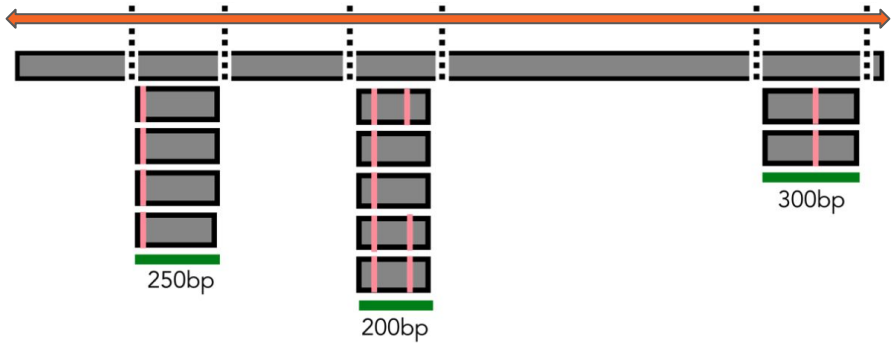

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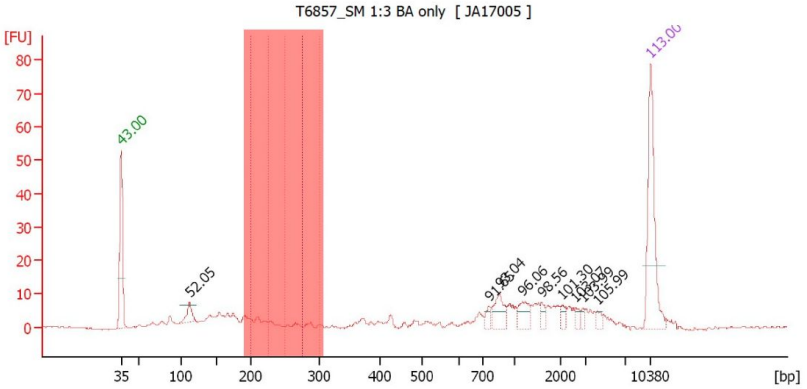

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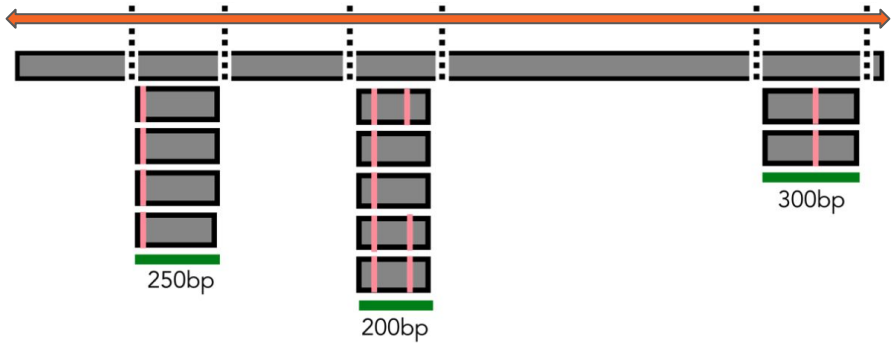

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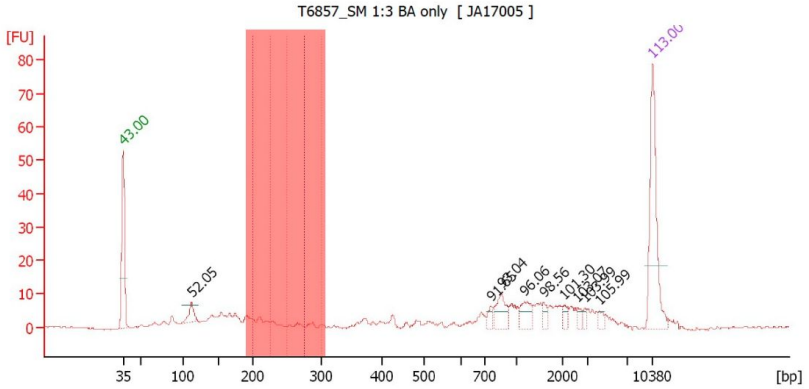

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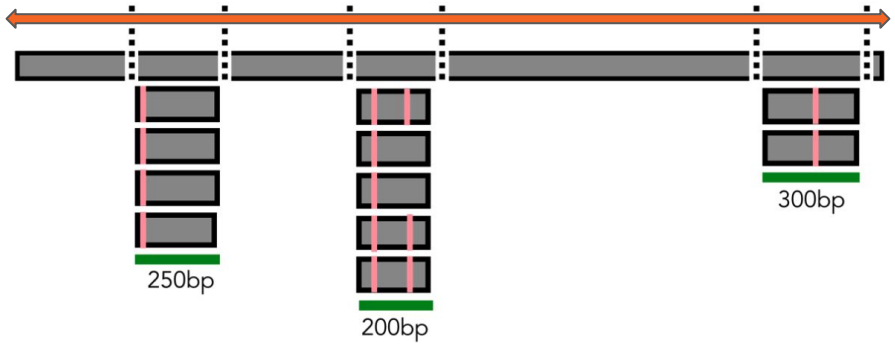

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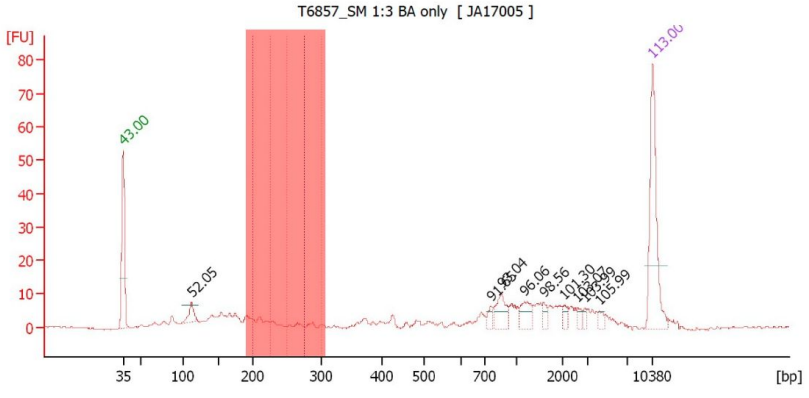

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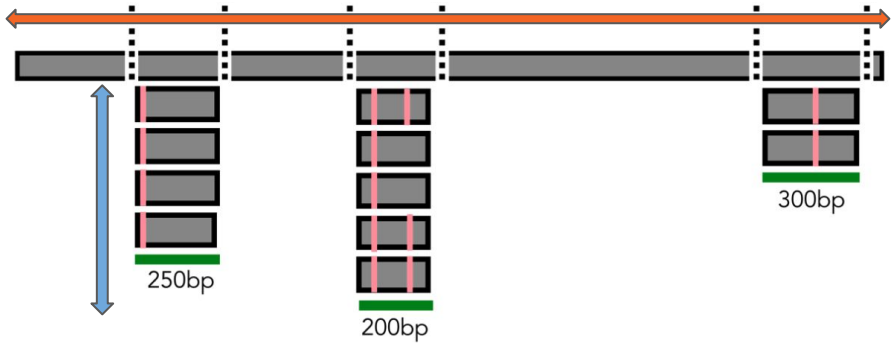

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$$\frac{\text{Genome size (bp)} \times \text{\% genome in size range}}{\text{Average fragment length (bp)}} \times \text{desired coverage}$$

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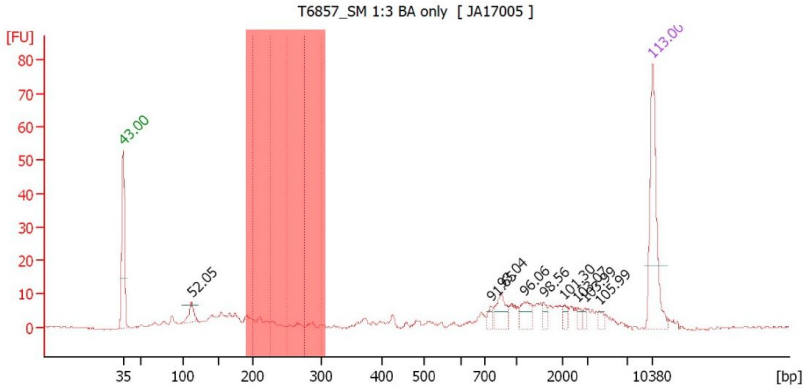

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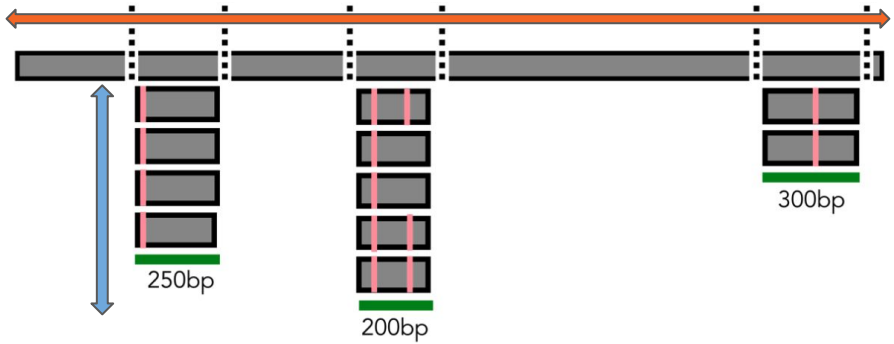

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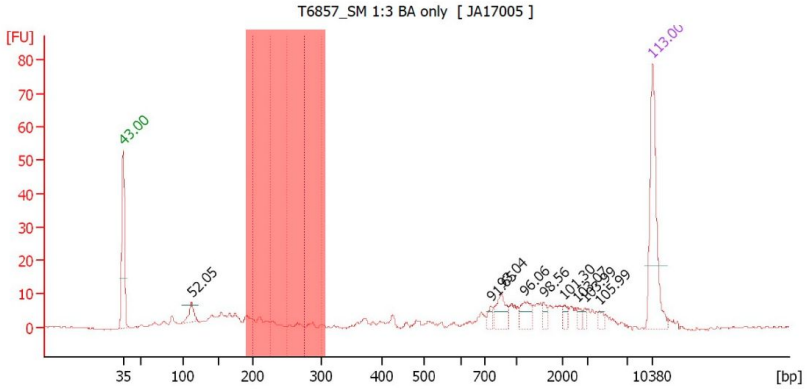

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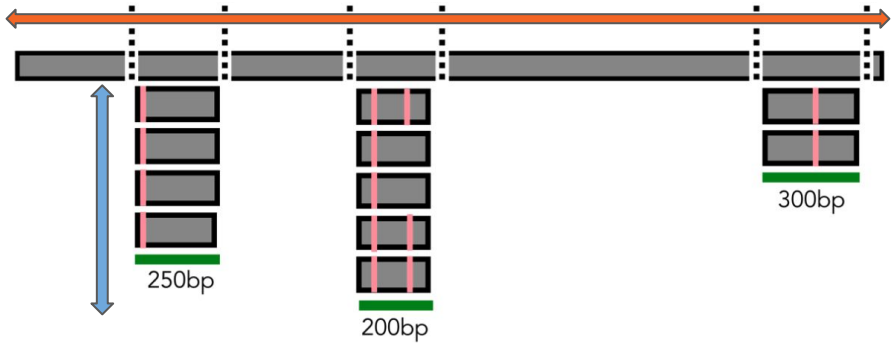

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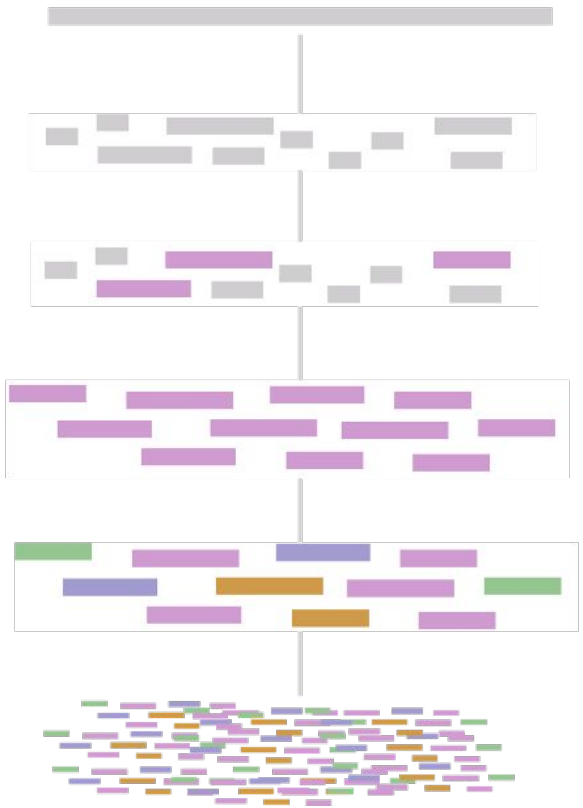
$$\frac{\text{2Gb genome} \times \text{2\%}}{\text{275bp avg fragment length}} \times \text{10X coverage} = 1{,}454{,}545 \text{ reads}$$

WGS and reduced-representation sequencing: **library preparation**



Genomic DNA

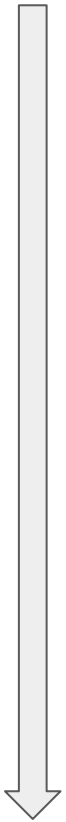
Fragment genome
& ligate adaptors

Size selection

Unique identifiers
based on sample

PCR to add seq primers
and enrich copies

WGS and reduced-representation sequencing: **bioinformatics**



Raw reads

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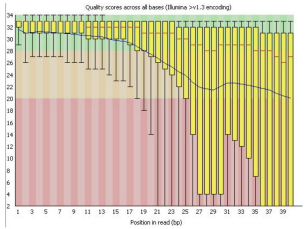

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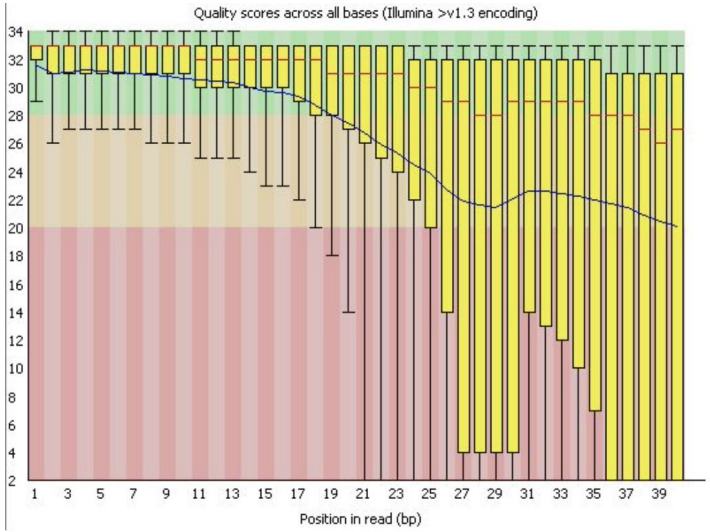
Quality check (fastqc)



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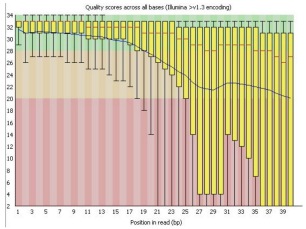

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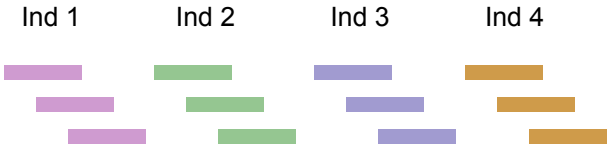

Demultiplexing
& trimming

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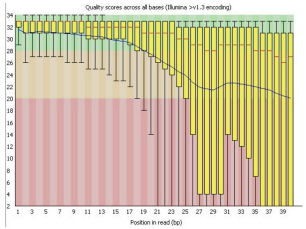

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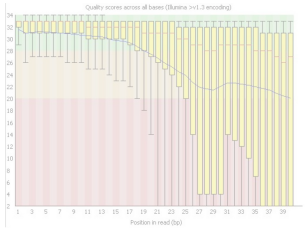

Ind 1 Ind 2 Ind 3 Ind 4

Read depth!

Mapping/alignment
bwa, bowtie2

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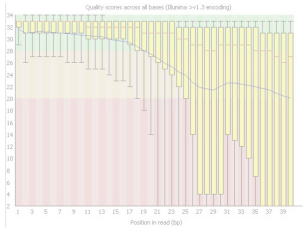

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Variant calling
GATK,
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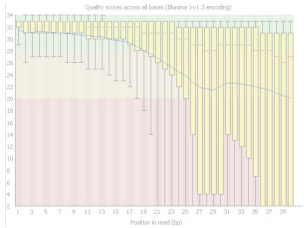

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Further filtering for analyses

What we'll be doing today!

Biases in genomic data

Where do biases arise from? How can we go about identifying them?

De novo assembly errors are going to differ from those produced with a reference genome

Errors from sequencing:
PCR duplicates, genotyping and sequencing errors, read mapping errors

Other types of errors/biases:
Contamination, wrong species (misidentifications), close relatives sequenced, low coverage, differences in library preparation

Think about these biases all the way through your pipeline:

RADseq

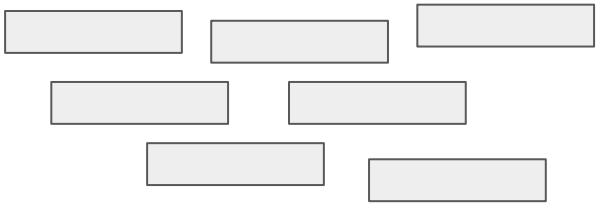
Paralogs are a problem

Think about these biases all the way through your pipeline:

RADseq

Paralogs are a problem

Individual 1: *cluster 1*



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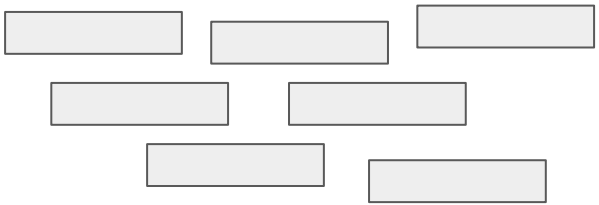

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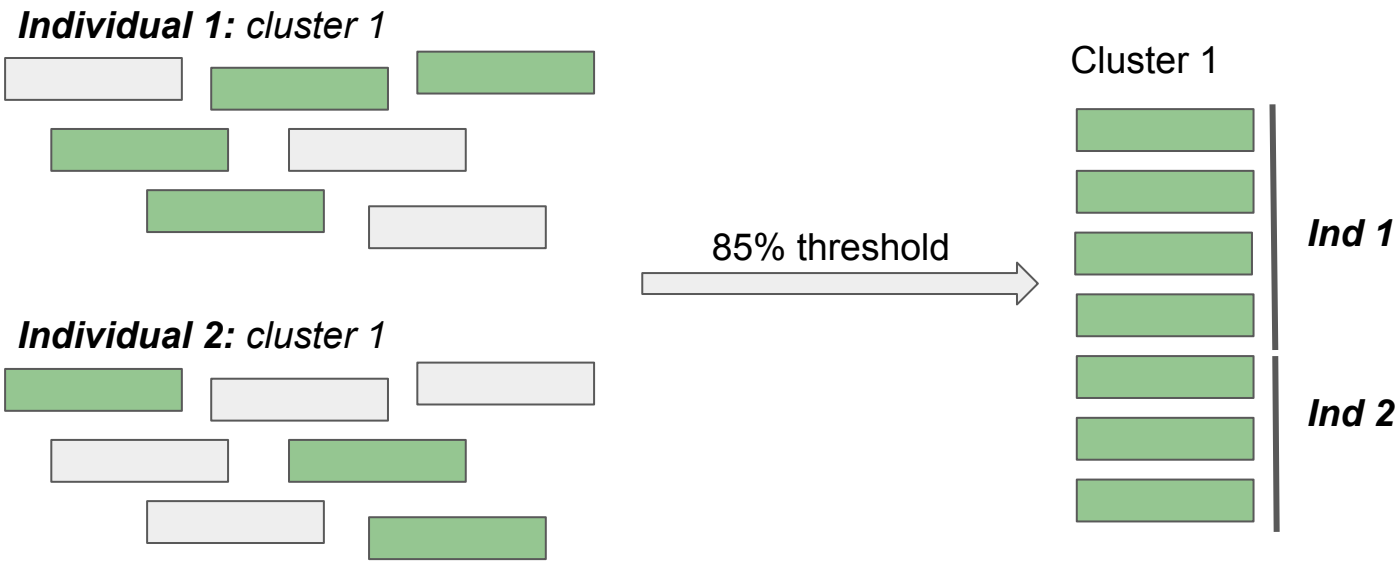

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RADseq

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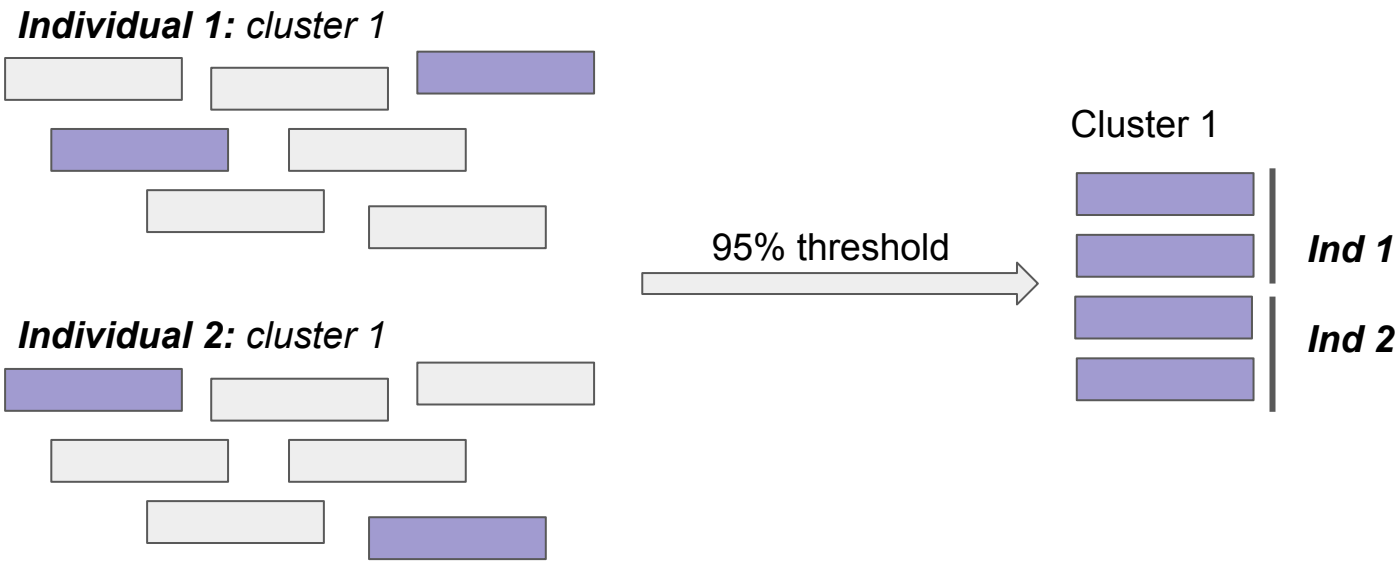


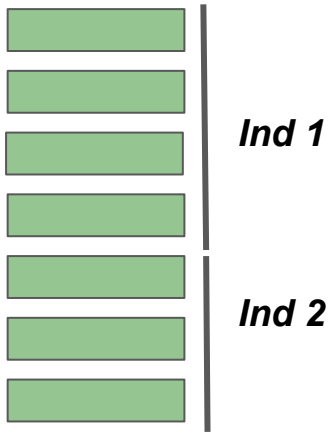
Think about these biases all the way through your pipeline:

RADseq

Paralogs are a problem

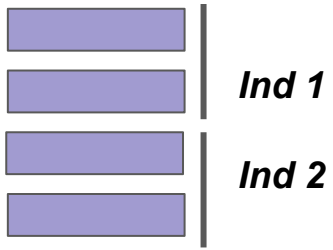
Too relaxed:

Higher likelihood of clustering paralogs



Too stringent:

Higher likelihood of losing homologs



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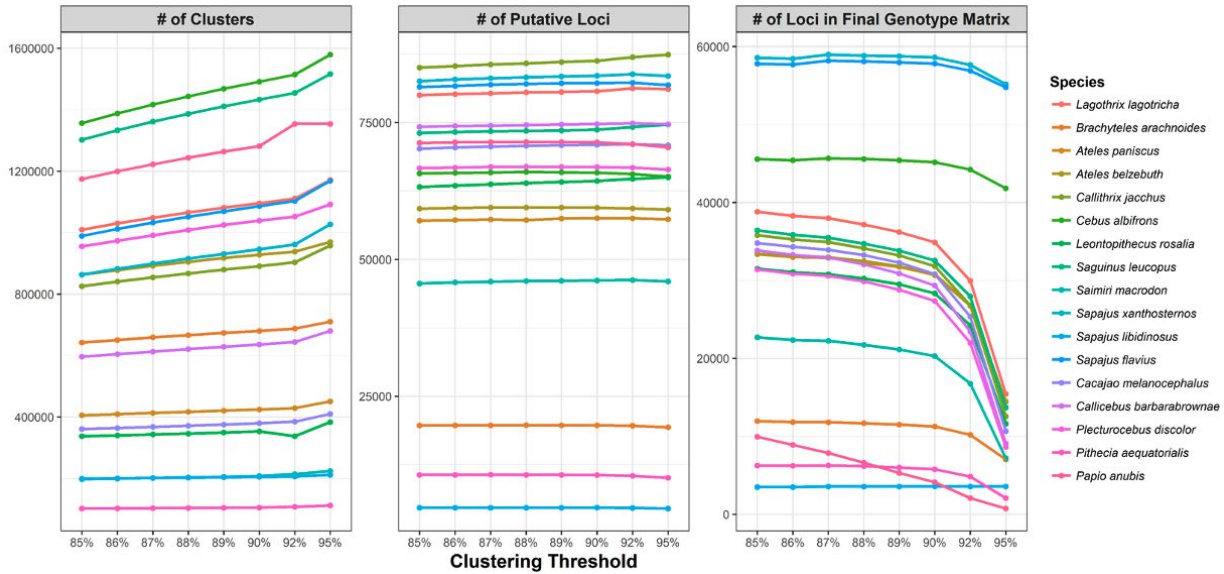


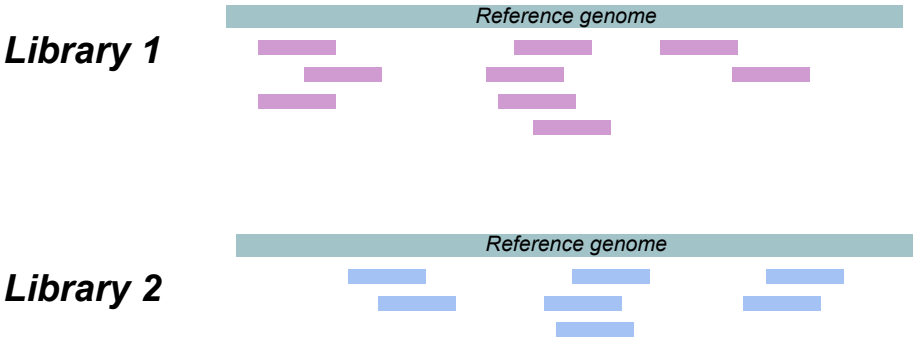
Valencia et al. (2018) PLOS ONE 13(8):e0201254

Think about these biases all the way through your pipeline:

RADseq

Paralogs are a problem

Biases across **different libraries**, making recovery of shared sites (fragments) between individuals difficult



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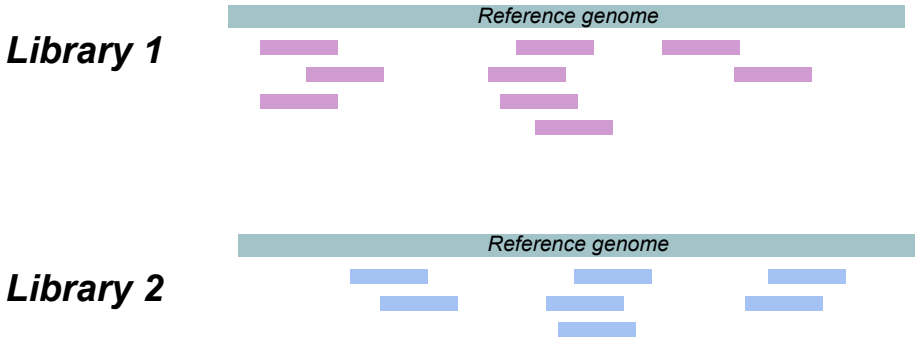

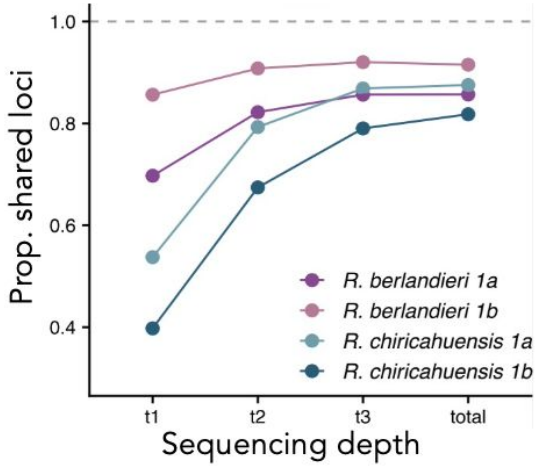

Think about these biases all the way through your pipeline:

RADseq

Paralogs are a problem

Biases across **different libraries**, making recovery of shared sites (fragments) between individuals difficult

Missing data (especially in big genomes!)

- May need to retain sites with lots of MD simply because you can't lose outgroups
- Helpful to think about the source of missing data

Think about these biases all the way through your pipeline:

WGS

PCR duplicates cause some variants to be amplified more than others; **read mapping errors** produce erroneous variant calls

Adapted (with permission) from J. McLaughlin

Think about these biases all the way through your pipeline:

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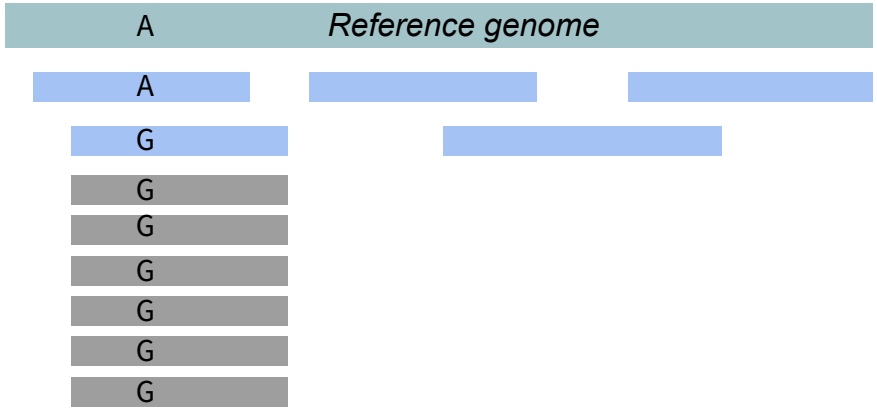

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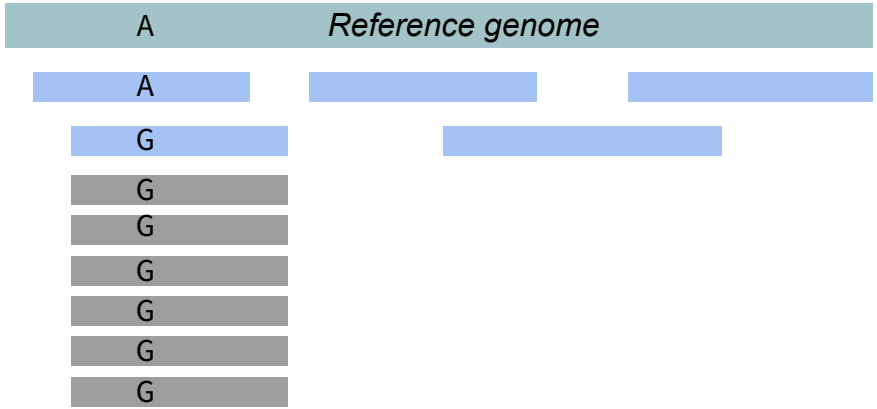

Calculate F-statistics that compare observed vs expected heterozygosity in your data

Adapted (with permission) from J. McLaughlin

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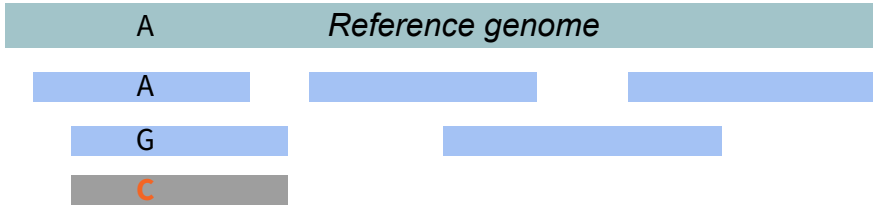

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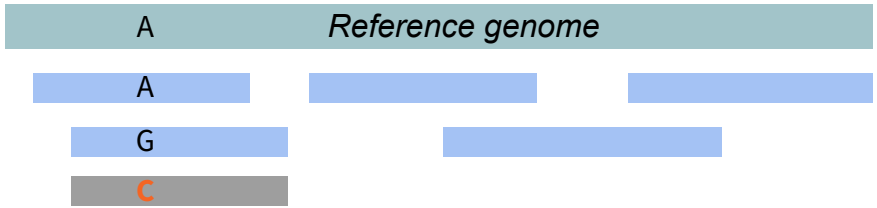


Remove any sites with minor allele frequencies lower than a given threshold

But see Linck & Battery (2019): https://doi.org/10.1111/1755-0998.12995

Think about these biases all the way through your pipeline:

WGS

PCR duplicates cause some variants to be amplified more than others; **sequencing and read mapping errors** produce erroneous variant calls

Low coverage assemblies

Can run a pipeline like ANGSD that generates genotype likelihoods which reduces the number of lost sites

Think about these biases all the way through your pipeline:

WGS

PCR duplicates cause some variants to be amplified more than others; **sequencing and read mapping errors** produce erroneous variant calls

Low coverage assemblies

Linkage among variants to be used for downstream analyses

Think about these biases all the way through your pipeline:

WGS

PCR duplicates cause some variants to be amplified more than others; **sequencing and read mapping errors** produce erroneous variant calls

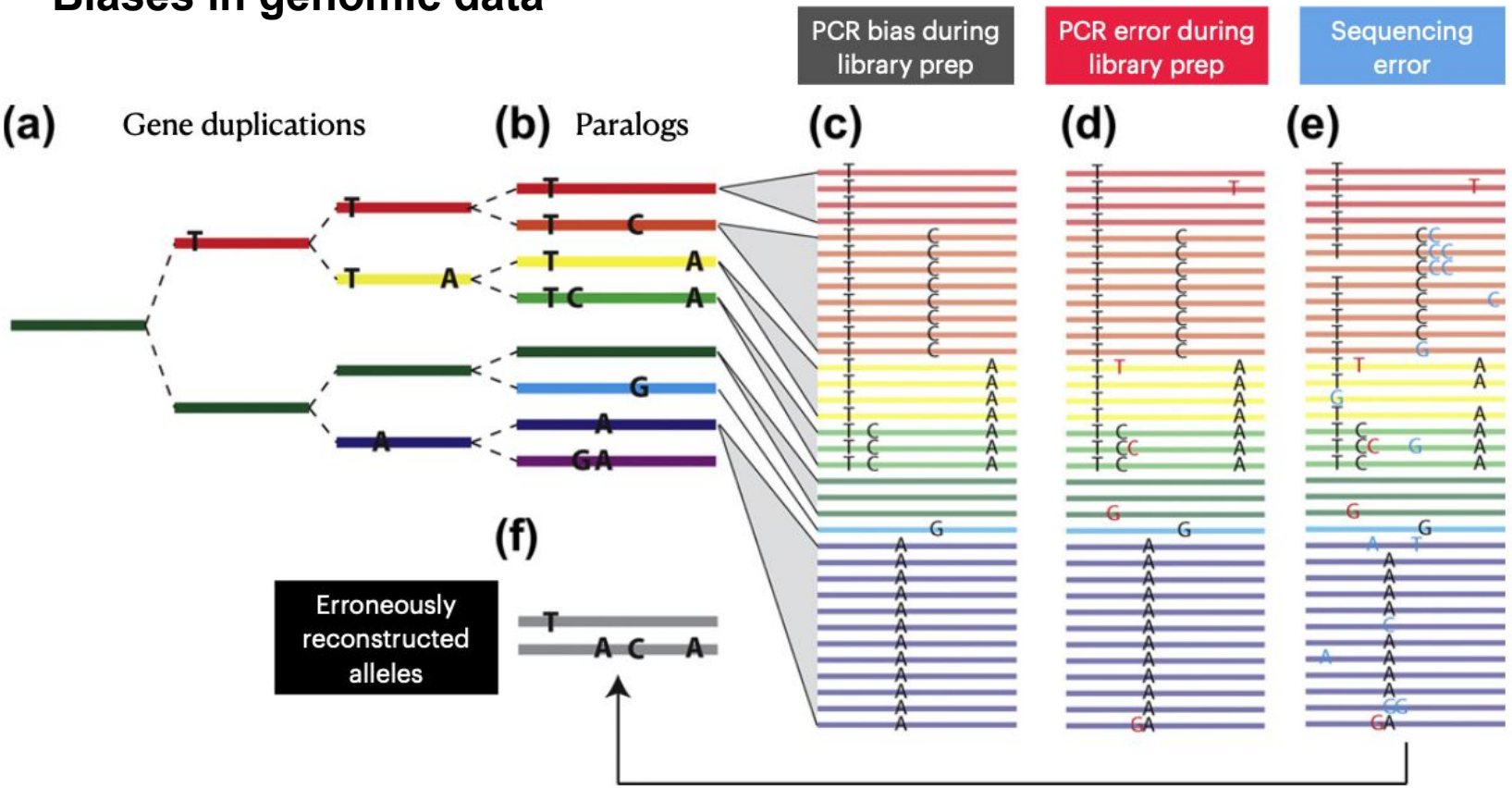
Low coverage assemblies

Linkage among variants to be used for downstream analyses

Should perform linkage disequilibrium (LD)-pruning on any datasets intended for things like population structure

Takes in window size, how much to move window, and correlation

Biases in genomic data



McCormack et al. (2013) https://doi.org/10.1016/j.ympev.2011.12.007

A field guide to common file types

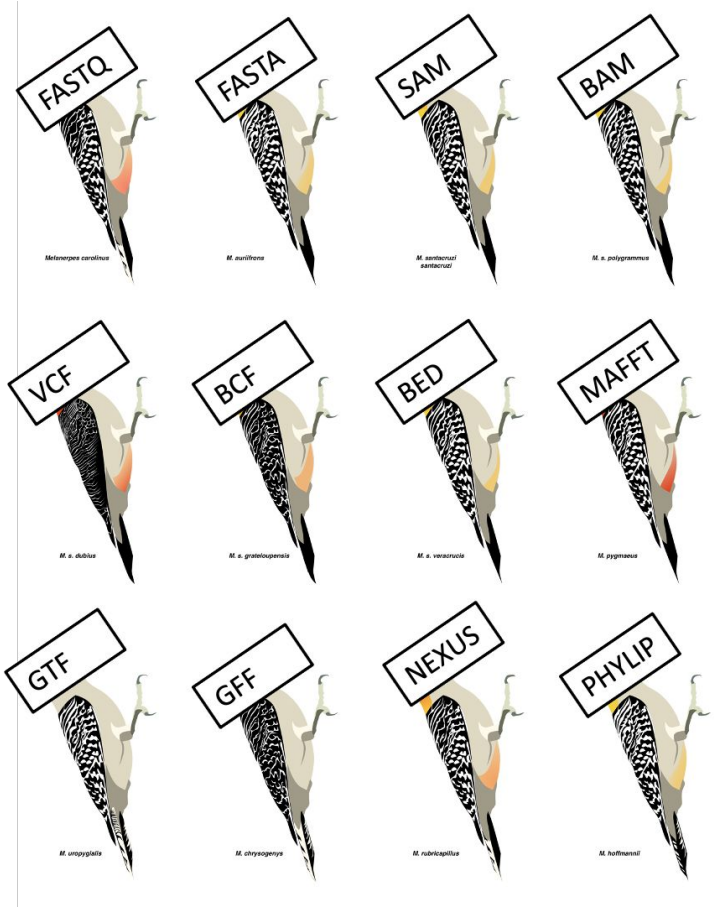


Illustration (with permission): J.F. McLaughlin

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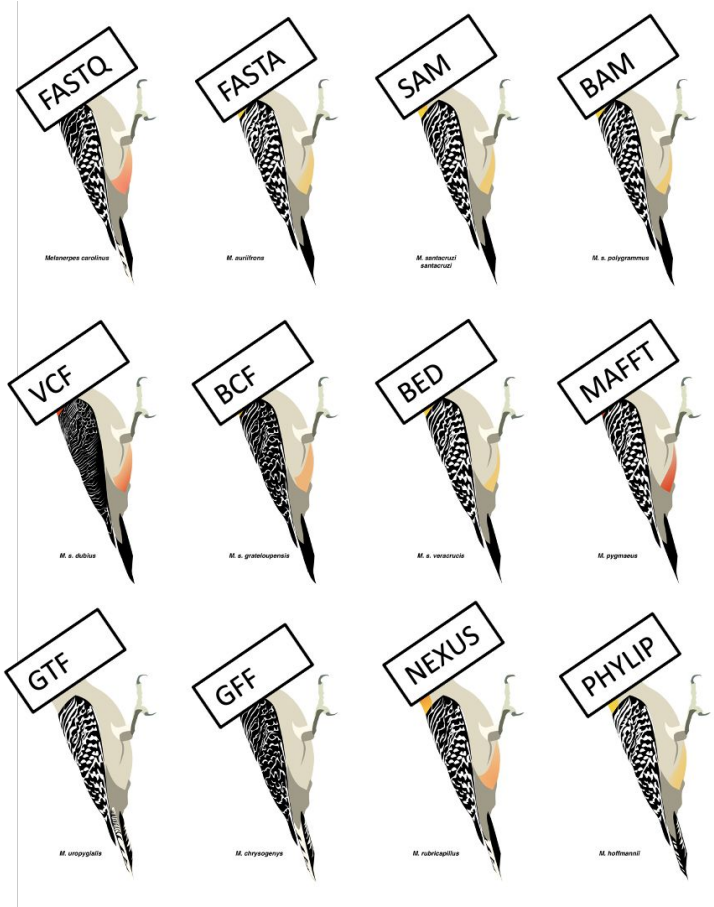
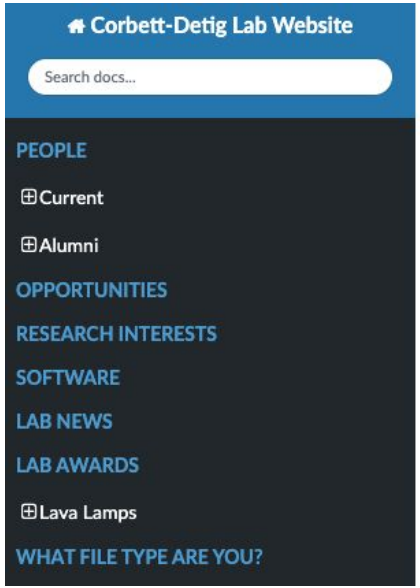
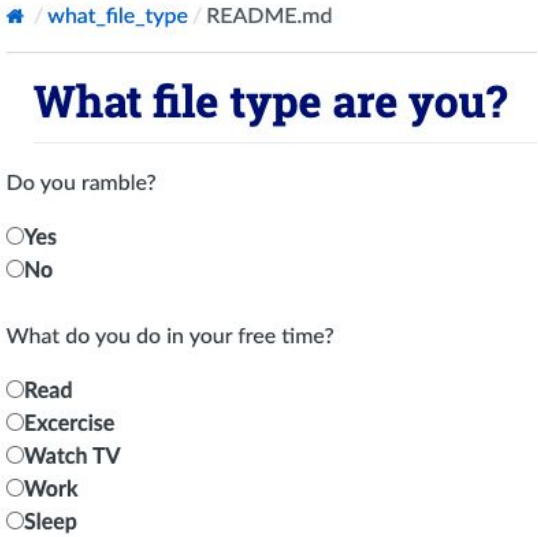


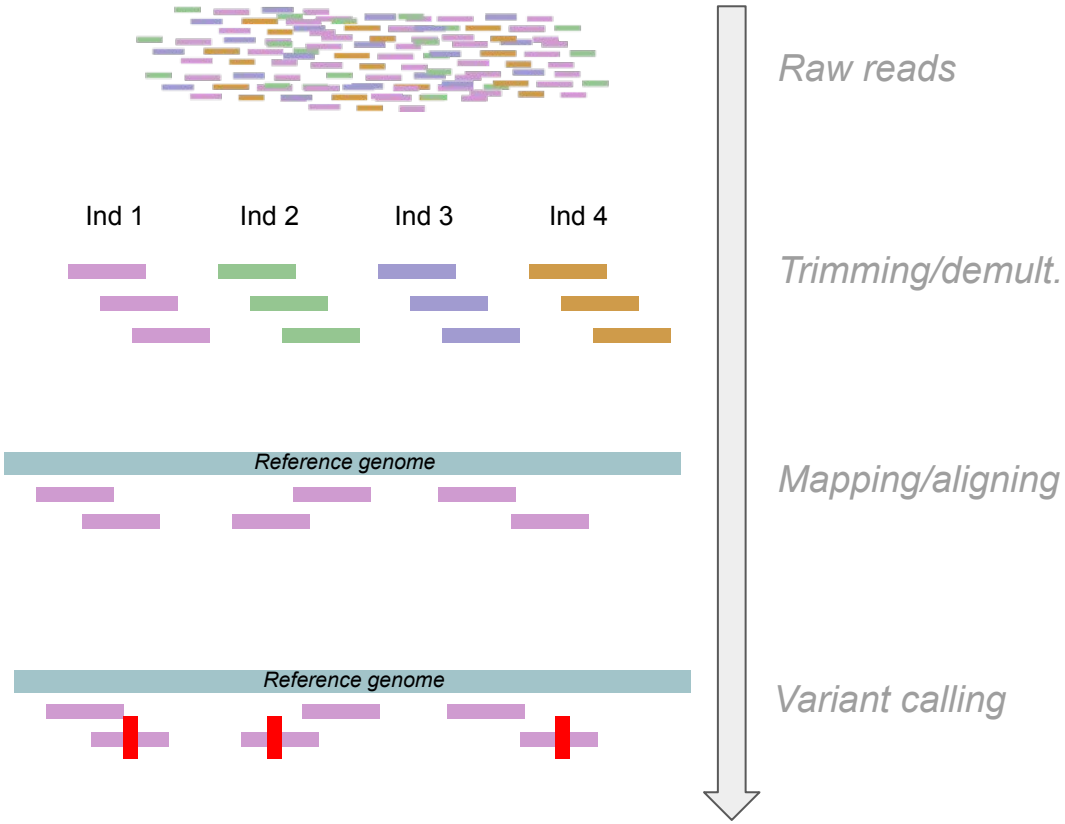
Illustration (with permission): J.F. McLaughlin





https://corbett-lab.github.io/what_file_type/

A field guide to common file types



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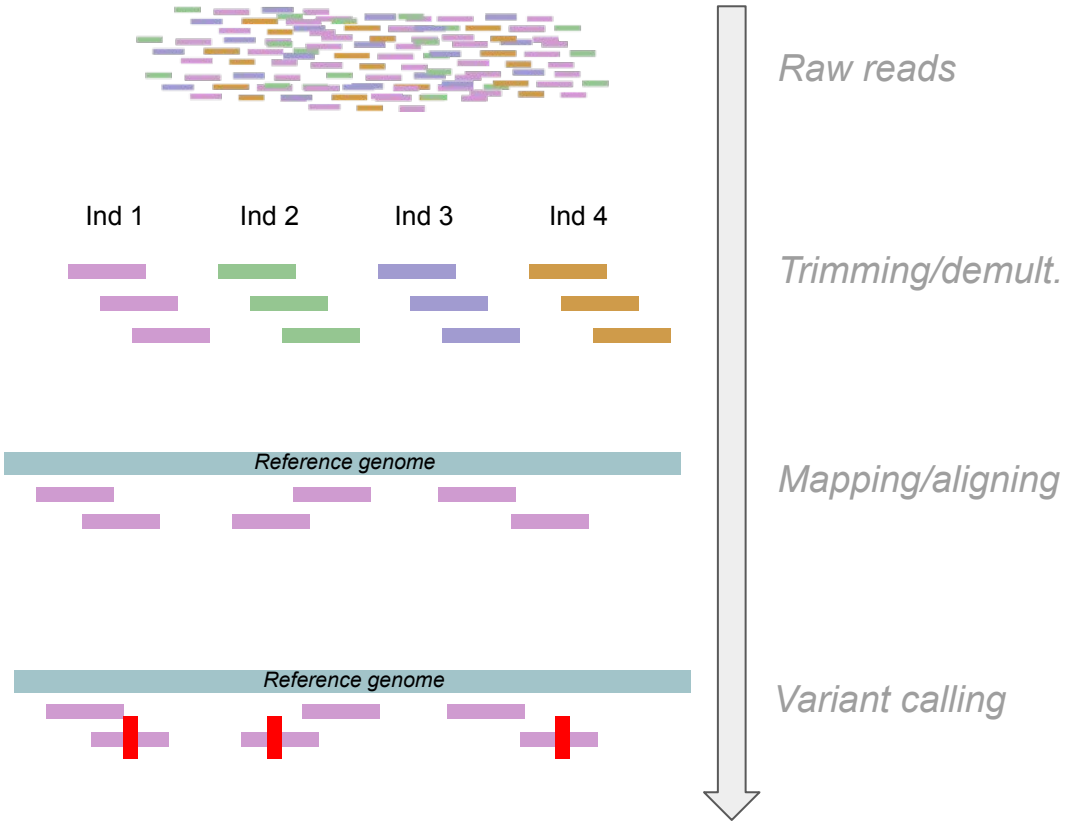

Raw reads and quality scores
(may be paired):
.fastq (fastq.gz) or **.fq**

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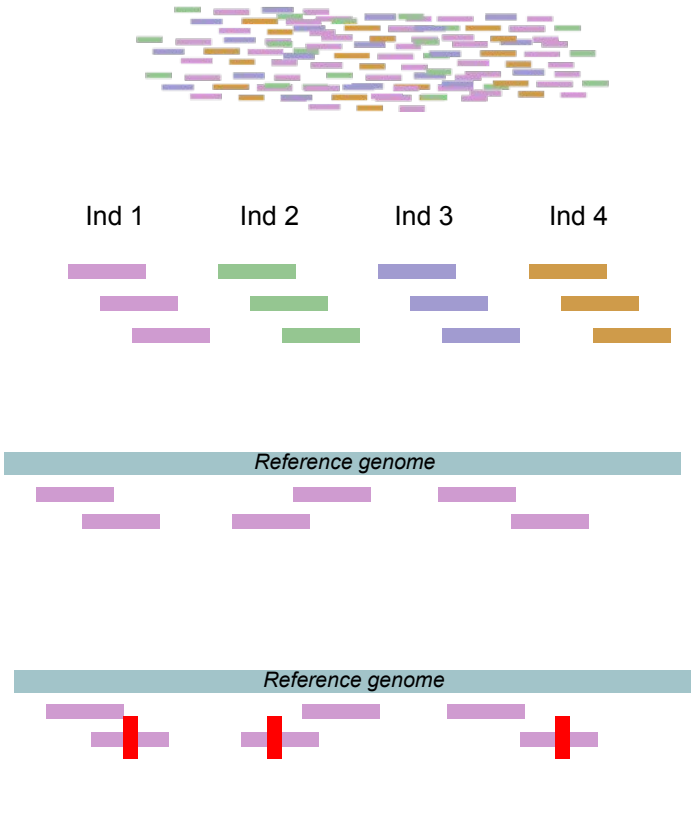

Raw reads

Trimming/demult.

Mapping/aligning

Variant calling

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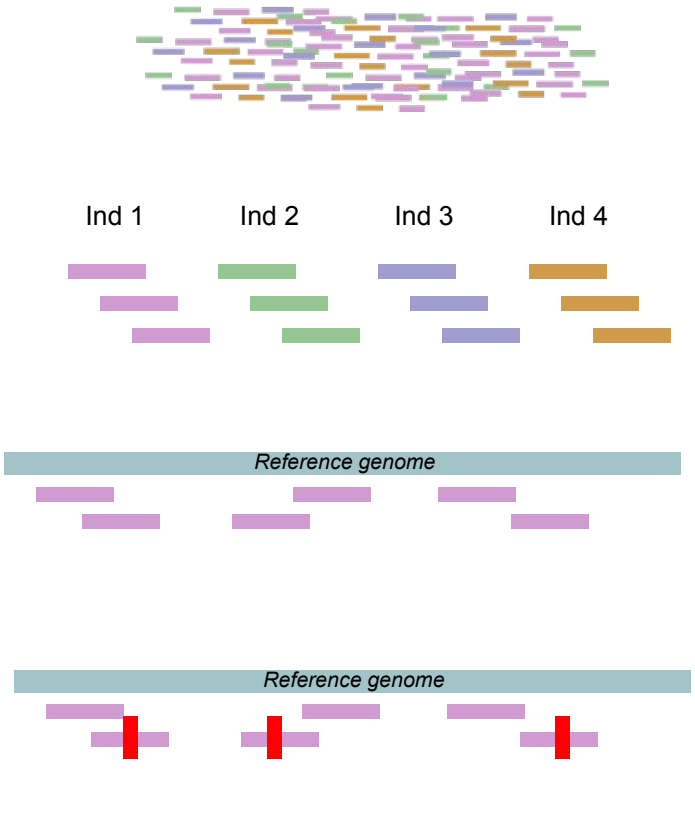

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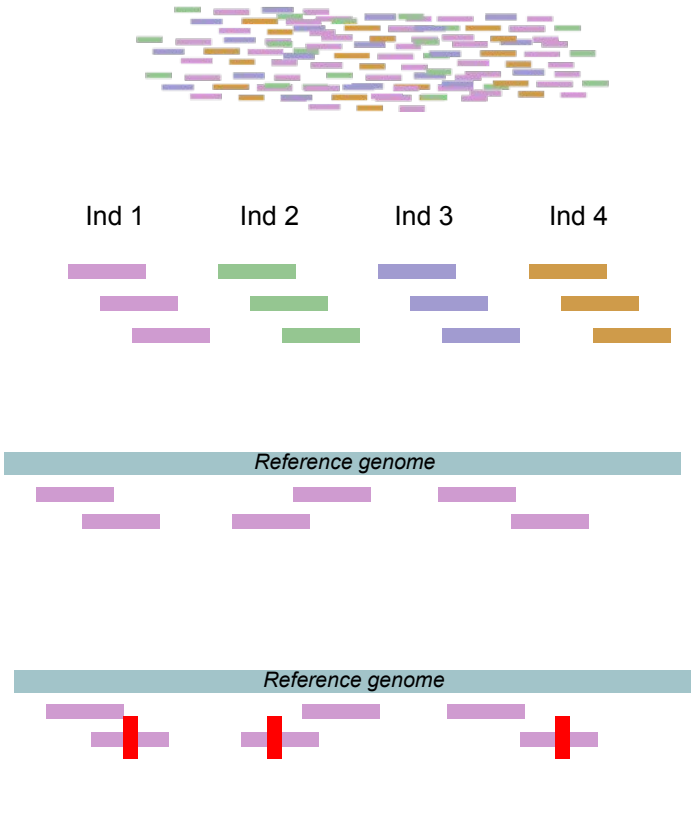

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Trimming/demult.

Mapping/aligning

Alignments and sequences:
.sam, .bam, or **.fasta**

Variant calling

Variant calls:
.vcf (vcf.gz)

These contain both SNPs and indels!

Variant call format (VCF) file

```
##fileformat=VCFv4.2
##fileDate=20220812
##source=PLINKv1.90
##contig=<ID=0,length=2147483645>
##INFO=<ID=PR,Number=0,Type=Flag,Description="Provisional reference allele, may
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
#CHROM  POS ID  REF ALT QUAL  FILTER  INFO  FORMAT  ALT3_ALT3 ANG8_ANG8 BAR360_B
0 10  Locus_10  G C . . PR  GT  0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0
0 15  Locus_15  T C . . PR  GT  0/0 0/0 0/0 0/0 ./. 0/0 0/0 ./. 0/1 0/0 0/0 0/0
0 22  Locus_22  T C . . PR  GT  0/0 0/0 0/0 0/0 0/0 0/0 ./. ./. 0/0 0/0 ./. 0/0
0 28  Locus_28  A C . . PR  GT  0/0 0/0 ./. ./. 0/0 ./. ./. ./. ./. 0/0 ./. 0/0
0 32  Locus_32  C A . . PR  GT  0/0 0/0 0/0 0/0 ./. 0/0 0/0 ./. 0/0 0/0 0/0 0/0
0 35  Locus_35  G A . . PR  GT  ./. 0/0 0/0 0/0 0/0 0/0 0/0 ./. 0/0 0/0 0/0 0/0
0 37  Locus_37  G A . . PR  GT  ./. ./. 0/0 ./. 0/0 ./. ./. 0/0 0/0 0/0 0/0 ./.
0 61  Locus_61  C T . . PR  GT  1/1 ./. 1/1 0/0 0/1 0/0 0/0 0/0 ./. 0/0 0/0 0/0
0 71  Locus_71  G A . . PR  GT  0/0 0/0 0/0 ./. ./. 0/0 0/0 ./. ./. 0/0 0/0 ./.
0 72  Locus_72  A T . . PR  GT  0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 ./. 0/0 0/0
0 82  Locus_82  C T . . PR  GT  0/0 0/1 0/0 0/0 0/0 0/0 0/0 0/0 ./. 0/0 0/0 ./.
0 84  Locus_84  A G . . PR  GT  0/0 0/0 0/0 0/0 ./. 0/0 0/0 0/0 0/0 0/0 0/0 0/0
0 90  Locus_90  C T . . PR  GT  0/0 0/0 ./. 0/0 0/0 0/0 0/0 0/1 ./. 0/0 0/0 0/0
0 91  Locus_91  T A . . PR  GT  0/0 0/0 0/0 0/0 ./. 0/0 0/0 1/1 ./. 0/0 0/0 0/0
0 96  Locus_96  C T . . PR  GT  0/0 ./. 0/0 0/0 0/0 ./. 0/0 0/0 0/0 0/0 0/0 ./.
0 104 Locus_104 T G . . PR  GT  0/0 0/0 0/0 ./. 0/0 ./. 0/0 0/0 ./. 0/0 ./. ./.
0 122 Locus_122 C G . . PR  GT  ./. ./. ./. ./. ./. ./. 0/0 ./. ./. ./. ./. ./.
0 125 Locus_125 T G . . PR  GT  0/0 0/0 ./. ./. ./. 0/0 ./. ./. 0/0 0/0 ./. ./.
0 131 Locus_131 G T . . PR  GT  0/1 ./. ./. ./. 0/0 ./. ./. ./. ./. ./. ./. ./.
0 133 Locus_133 C T . . PR  GT  0/0 0/0 ./. ./. ./. 0/0 ./. ./. 0/0 ./. ./. 0/0
0 138 Locus_138 G A . . PR  GT  0/0 ./. 0/0 ./. ./. ./. ./. ./. ./. ./. ./. ./.
0 154 Locus_154 A G . . PR  GT  0/0 0/0 ./. 0/0 ./. 0/0 ./. ./. ./. ./. ./. ./.
0 162 Locus_162 C T . . PR  GT  0/1 0/1 0/0 ./. ./. 0/0 0/0 ./. 0/0 0/0 1/1 0/0
0 166 Locus_166 C T . . PR  GT  0/0 ./. 0/0 ./. 0/0 ./. ./. 0/1 ./. ./. 0/0 ./.
0 177 Locus_177 G C . . PR  GT  0/0 ./. 0/0 ./. ./. 0/0 0/0 ./. ./. ./. ./. ./.
```

Variant call format (VCF) file

```
##fileformat=VCFv4.2
##FILTER=<ID=PASS,Description="All filters passed">
##FILTER=<ID=FS_SOR_filter,Description="(vc.isSNP() && ((vc.hasAttribute('FS') && F
##FILTER=<ID=MQ_filter,Description="vc.isSNP() && ((vc.hasAttribute('MQ') && MQ < 4
##FILTER=<ID=QUAL_filter,Description="QUAL < 30.0">
##FILTER=<ID=RPRS_filter,Description="(vc.isSNP() && (vc.hasAttribute('ReadPosRankS
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths for the ref
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read depth">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=PGT,Number=1,Type=String,Description="Physical phasing haplotype
##FORMAT=<ID=PID,Number=1,Type=String,Description="Physical phasing ID inform
##FORMAT=<ID=PL,Number=G,Type=Integer,Description="The phred-scaled genotype
##GATKCommandLine=<ID=VariantFiltration,CommandLine="VariantFiltration --outp
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes, f
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency, for each ALT
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in c
##INFO=<ID=BaseQRankSum,Number=1,Type=Float,Description="Z-score from Wilcoxo
##INFO=<ID=ClippingRankSum,Number=1,Type=Float,Description="Z-score From Wilc
##INFO=<ID=DP,Number=1,Type=Integer,Description="Combined depth across sample
##INFO=<ID=ExcessHet,Number=1,Type=Float,Description="Phred-scaled p-value fo
##INFO=<ID=FS,Number=1,Type=Float,Description="Phred-scaled p-value using Fis
##INFO=<ID=InbreedingCoeff,Number=1,Type=Float,Description="Inbreeding coeffi
##INFO=<ID=MLEAC,Number=A,Type=Integer,Description="Maximum likelihood expect
##INFO=<ID=MLEAF,Number=A,Type=Float,Description="Maximum likelihood expectat
##INFO=<ID=MQ,Number=1,Type=Float,Description="RMS mapping quality">
##INFO=<ID=MQRankSum,Number=1,Type=Float,Description="Z-score From Wilcoxon r
##INFO=<ID=QD,Number=1,Type=Float,Description="Variant Confidence/Quality by Depth
##INFO=<ID=ReadPosRankSum,Number=1,Type=Float,Description="Z-score from Wilcoxon ra
##INFO=<ID=SOR,Number=1,Type=Float,Description="Symmetric Odds Ratio of 2x2 contin
##SentieonCommandLine.GVCFtyper=<ID=GVCFtyper,Version="sentieon-genomics-202112.04"
##SentieonCommandLine.Haplotyper=<ID=Haplotyper,Version="sentieon-genomics-202112.0
##contig=<ID=SCAF_1,length=88620470,assembly=unknown>
##contig=<ID=SCAF_2,length=80884353,assembly=unknown>
##contig=<ID=SCAF_3,length=68994874,assembly=unknown>
##contig=<ID=SCAF_4,length=60156485,assembly=unknown>
```

```
##reference=file://ccgp-workflow-results/41-Cyanocitta/data/genome/bCyaSte1.NCBI.p_
##source=VariantFiltration
##bcftools_viewVersion=1.12+htslib-1.12
##bcftools_viewCommand=view -S ccgp-workflow-results/41-Cyanocitta/results/41-Cyanc
#CHROM  POS ID  REF ALT QUAL  FILTER  INFO  FORMAT  MVZCCGP-Cst1_I-A01  MVZCCGP-Cst
SCAF_1  8234  . C T 9034.03 . AC=48;AF=0.16;AN=300;BaseQRankSum=-0.174;ClippingRank
SCAF_1  14861 . C T 665.39  . AC=6;AF=0.02;AN=300;BaseQRankSum=0.431;ClippingRankSu
SCAF_1  21459 . T C 13570 . AC=66;AF=0.221;AN=298;BaseQRankSum=0.21;ClippingRankSum
SCAF_1  36143 . T C 3685.22 . AC=27;AF=0.0906;AN=298;BaseQRankSum=-0;ClippingRankSu
SCAF_1  47228 . T C 3681.84 . AC=17;AF=0.057;AN=298;BaseQRankSum=-0;ClippingRankSum
SCAF_1  48964 . C A 751.02  . AC=6;AF=0.0201;AN=298;BaseQRankSum=-0;ClippingRankSum
SCAF_1  58848 . A T 6379.99 . AC=31;AF=0.103;AN=300;BaseQRankSum=-0;ClippingRankSum
SCAF_1  65432 . T C 2476.73 . AC=19;AF=0.0633;AN=300;BaseQRankSum=0.253;ClippingRan
SCAF_1  76112 . C T 7906.82 . AC=40;AF=0.133;AN=300;BaseQRankSum=0;ClippingRankSum=
SCAF_1  87858 . T C 465.78  . AC=3;AF=0.01;AN=300;BaseQRankSum=0.711;ClippingRankSu
SCAF_1  93071 . G C 774.35  . AC=4;AF=0.0133;AN=300;BaseQRankSum=-0;ClippingRankSum
SCAF_1  102600  . G A 3434.05 . AC=19;AF=0.0638;AN=298;BaseQRankSum=-0;ClippingRank
```

Programs we're working with today

bcftools: helpful for data processing (can also do variant calling)

https://samtools.github.io/bcftools/bcftools.html

Different commands we'll use in exercises: ***query*** and ***view***

```
bcftools COMMAND [OPTIONS] file.vcf
```

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bcftools query -f '%CHROM' file.vcf | head -3
```

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bcftools query -f '%CHROM' file.vcf | head -3
```

-q, --min-af *FLOAT*[*:nref*|*:alt1*|*:minor*|*:major*|*:nonmajor*]

vcftools and Plink

vcftools and **plink**: helpful for summary statistics

Plink works a lot with .ped and .bed files

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Plink works a lot with .ped and .bed files

```
vcftools --vcf file.vcf --out outfile_name --OPTION
```

vcftools and Plink

vcftools and **plink**: helpful for summary statistics

Plink works a lot with .ped and .bed files

```
vcftools --vcf file.vcf --out outfile_name --OPTION

vcftools --vcf file.vcf --out file_name --missing-indv
```

vcftools and Plink

vcftools and **plink**: helpful for summary statistics

Plink works a lot with .ped and .bed files

```
vcftools --vcf file.vcf --out outfile_name --OPTION

vcftools --vcf file.vcf --out file_name --missing-indv

plink --vcf file.vcf --out prefix_name --distance square --make-bed --recode vcf
```

Dataset we're working with today

Lampropeltis triangulum
ddRAD data (~50K SNPs)

EXERCISE 1: gathering basic statistics

Download the vcf file on GitHub here:
https://github.com/eachambers/EvoGeno-Methods-Workshop/blob/main/Workshop1/Data/lampro.vcf

Download the worksheet here:
https://github.com/eachambers/EvoGeno-Methods-Workshop/blob/main/Workshop1/Exercises/EvoGenomics_Ws1_Ex1.txt

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# ================================================================================================
#                                        EXERCISE 1
# ================================================================================================

# bcftools is a great program for processing data (and is also actively maintained), whereas
# vcftools and plink are great for generating summary statistics. In this exercise, we'll
# use all three of these programs in the Terminal.

# ======================== 1. BASIC EXAMINATION OF THE VCF DATA FILE ======================

# Answer the following questions using bash.

#       (1a) Take a look at the lampro.vcf file. How was this vcf generated?
#               *** YOUR ANSWER HERE ***

#       (1b) What info is contained (per site) within the vcf?
#               *** YOUR ANSWER HERE ***
```