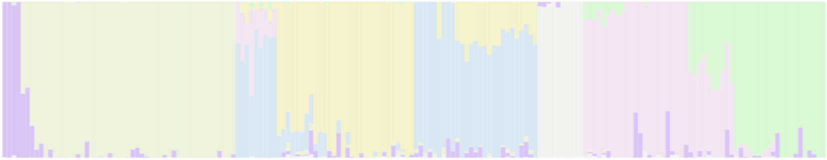
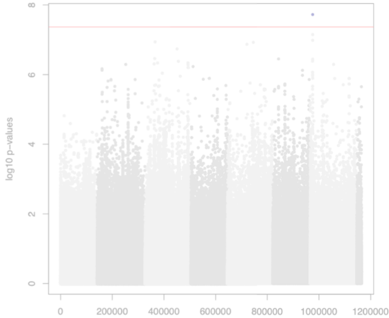
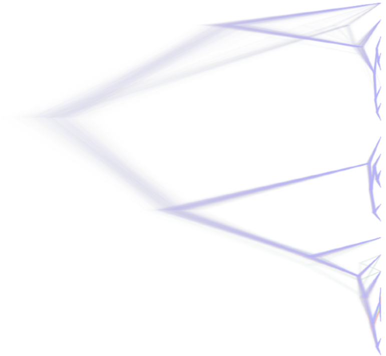
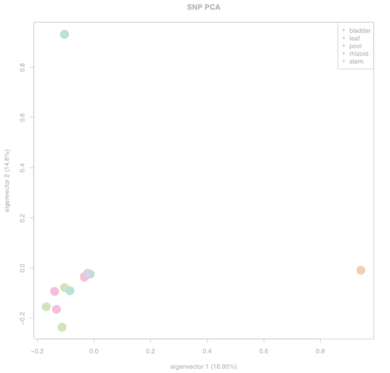






AG2PI: Introduction to SNP Data Analysis



Jacob B. Landis

School of Integrative Plant Science

Cornell University

and

BTI Computational Biology Center

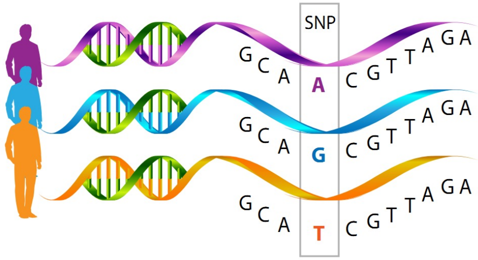
May 20th, 2021

@JLandisBotany

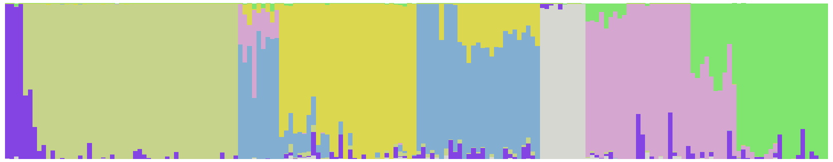
jbl256@cornell.edu

Outline

- What are SNPs and why do we want to call them?

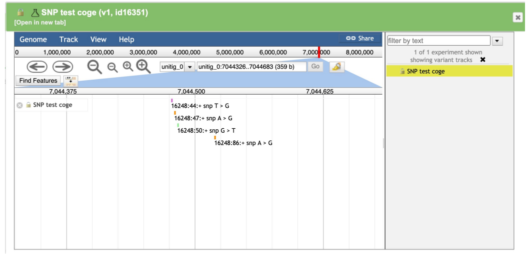


- What data can be used for calling SNPs?



- Approaches to calling SNPs (CyVerse Discovery Environment)

- Setting up for downstream applications (CoGe)


SNP test coge (v1, id16351)
[Open in new tab]

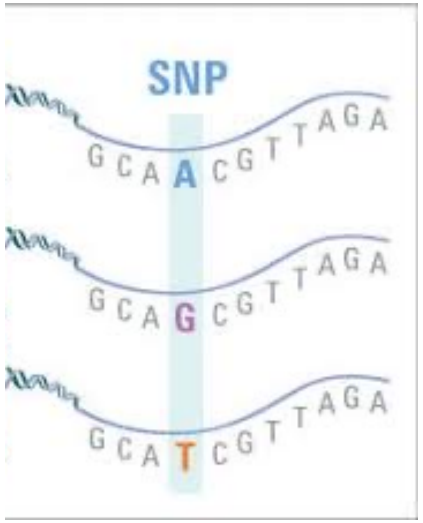
unitig_0:7044326..7044683 (359 b)

1 of 1 experiment shown
showing variant tracks

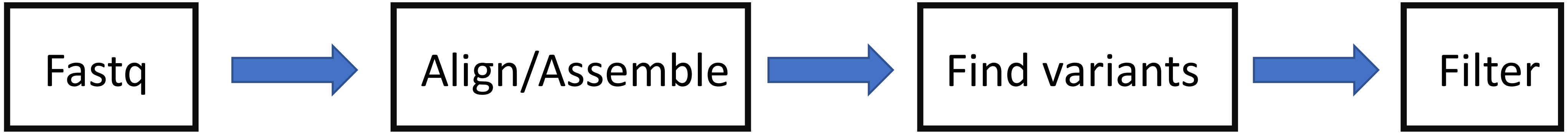
SNP test coge

16248:44:+ snp T > G
16248:47:+ snp A > G
16248:50:+ snp G > T
16248:86:+ snp A > G

Before we get started…What is SNP analysis?



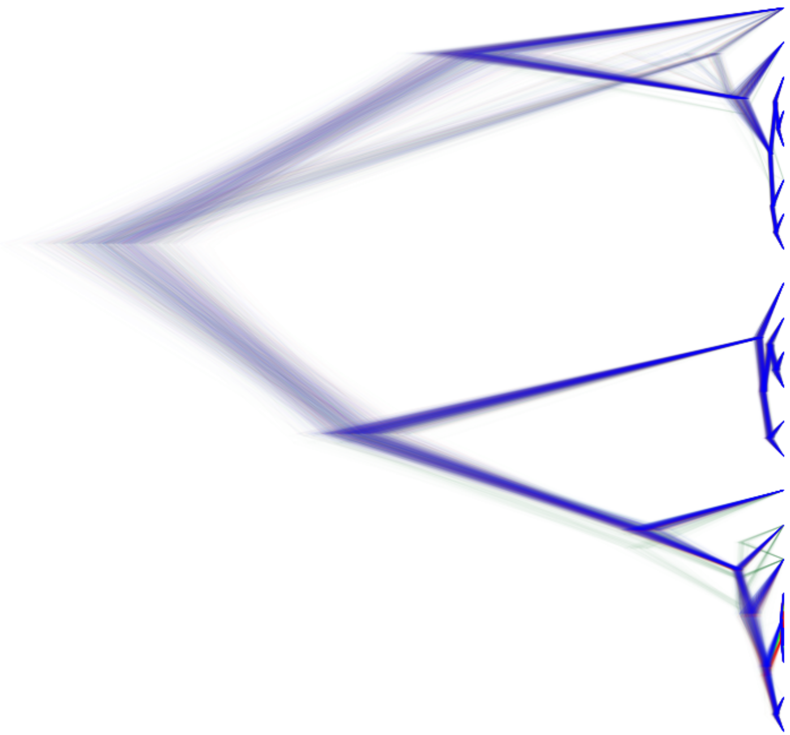
- At its simplest it is Single Nucleotide Polymorphism
- Why is this important?
  - Most common genetic variation
  - Can be linked to phenotype, environment, or heredity
- What is the basic workflow?



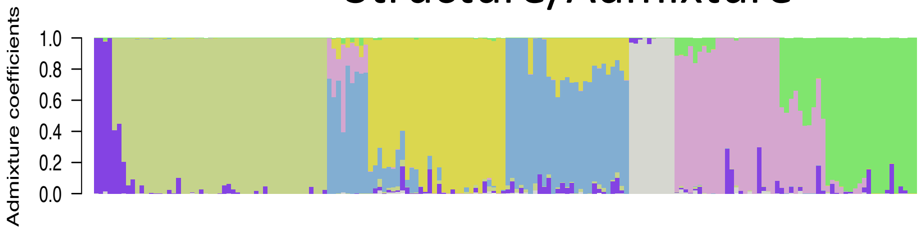
Fastq → Align/Assemble → Find variants → Filter

When I think of SNP analyses

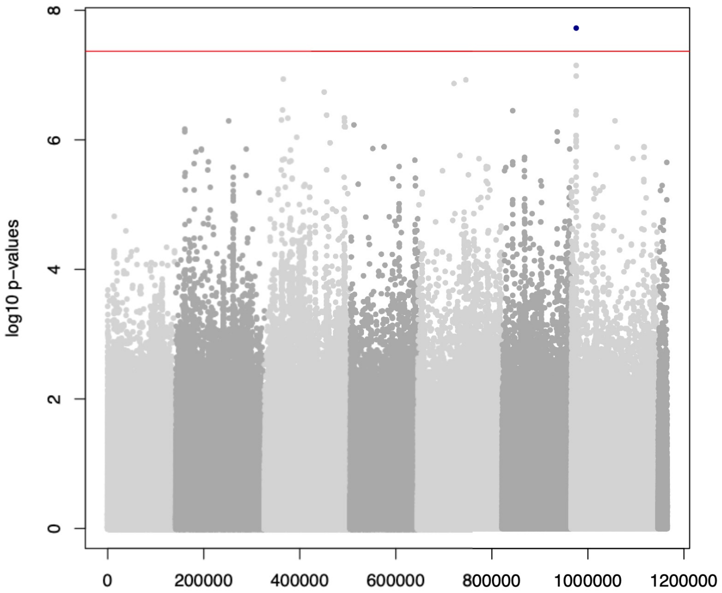
Coalescent tree



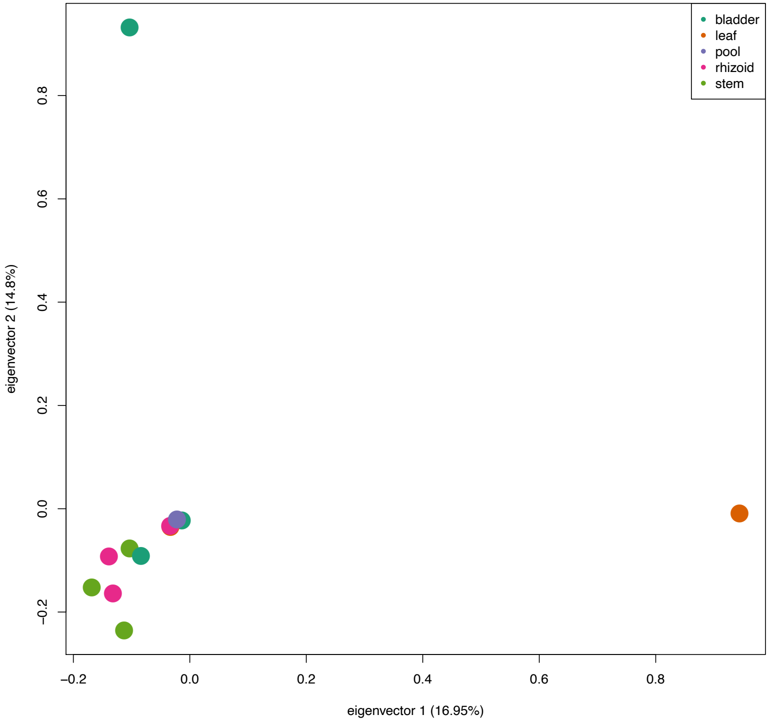
Structure/Admixture



GWAS



PCA



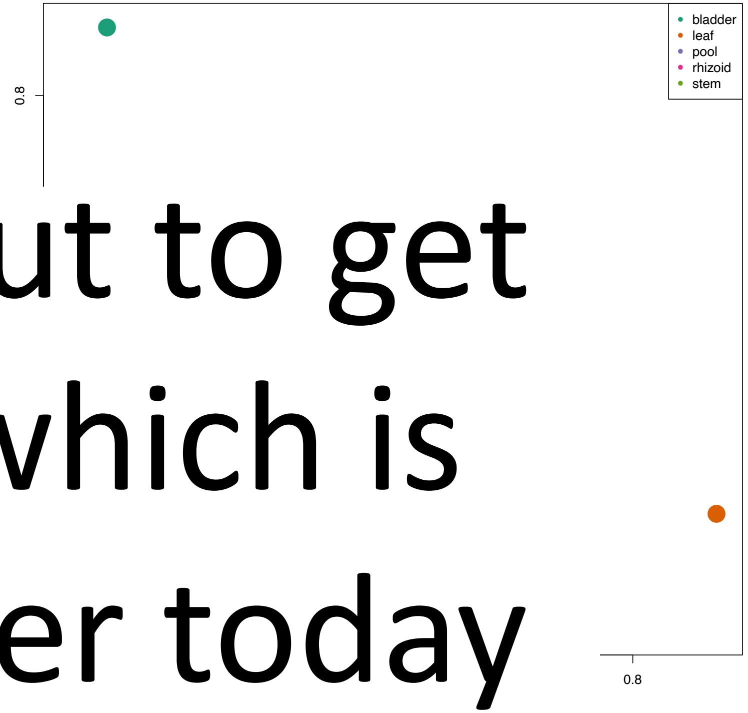
When I think of SNP analyses

Coalescent tree

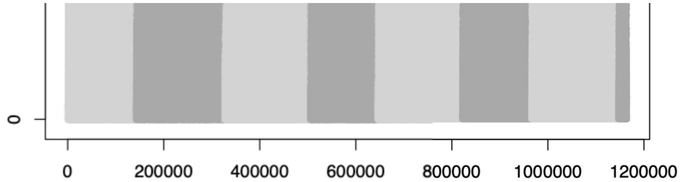
Structure/Admixture

dmixture coefficients
1.0
0.8
0.6
0.4
0.2
0.0

PCA

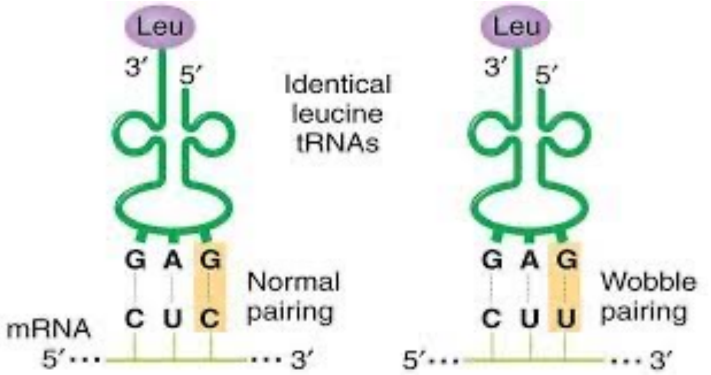


These are the goals but to get there takes work … which is what we are going over today



Evolutionary Relationships

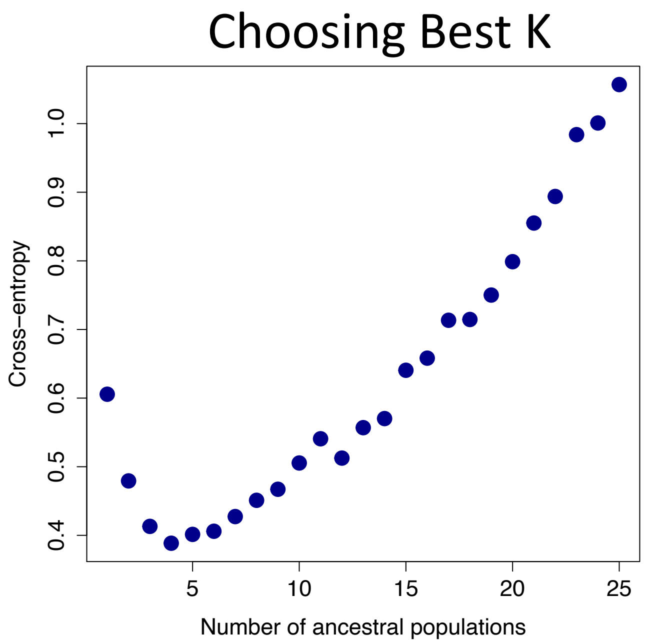
- Which is better to use, SNPs or orthologous genes?
  - I think it depends on the question of interest and the scale interested in
- Very fast changing nucleotides may hide the true signal in deep relationships
- Coverage needed for high confidence differs
  - Orthologous genes 20-50x coverage; SNPs ~6x for homozygous sites and 15x for heterozygous sites
- Inclusion of invariant sites?
  - Necessary for appropriate branch lengths and summary statistics for both

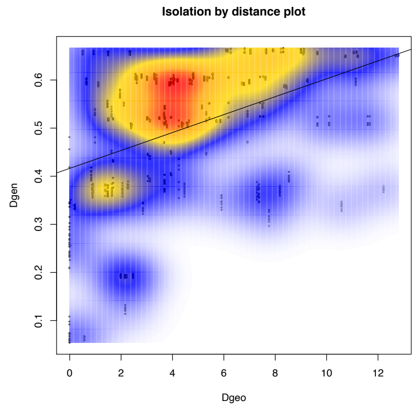


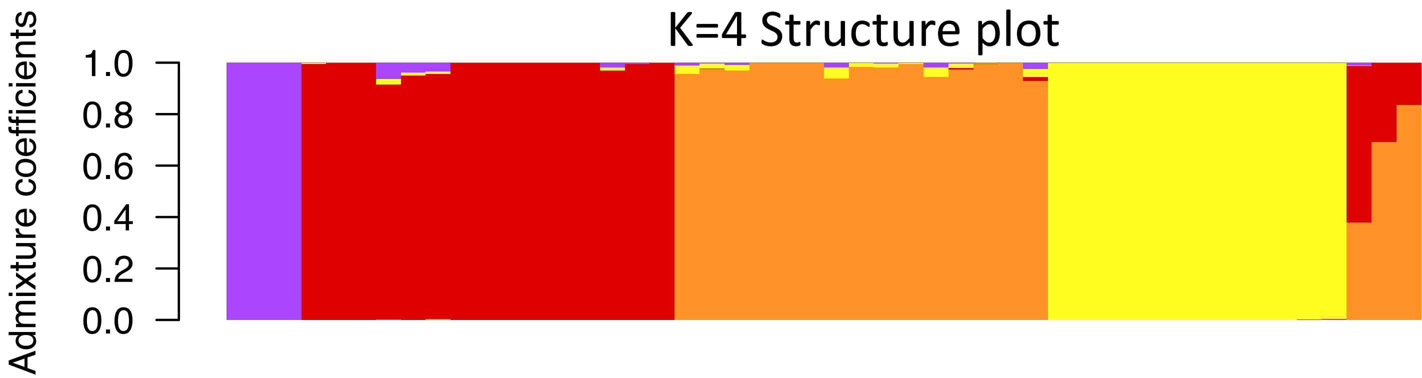
```
Reference CCGTTAGAGTTACAATTCGA
Read 2       TTAGAGTAACAA
Read 3    CCGTTAGAGTTA
Read 4             TTACAATTCGA
Read 5          GAGTAACAA
Read 6       TTAGAGTAACAAT
```

Investigation Gene Flow

- Can estimate the best number of ancestral populations
- Identify individuals that are genetically similar and visualize differences where they occur
- Are individuals that are geographically close genetically similar?

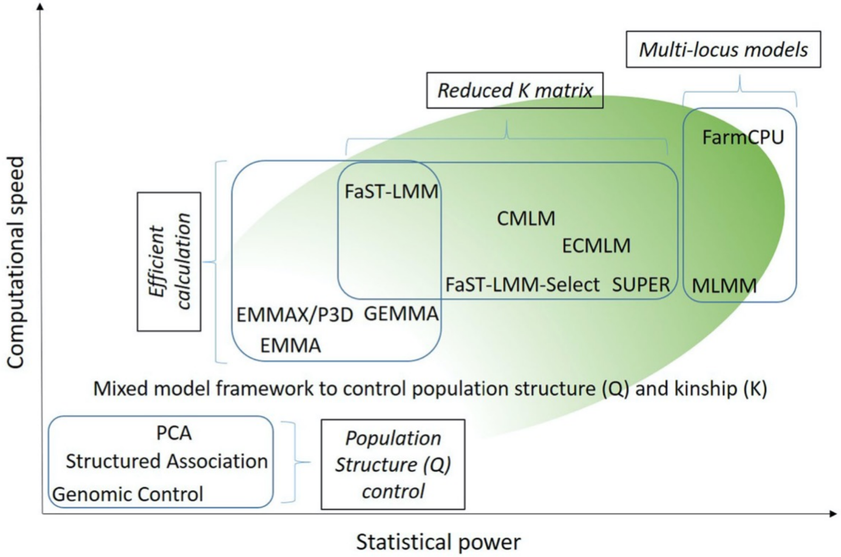

Choosing Best K




K=4 Structure plot

Genome Wide Association

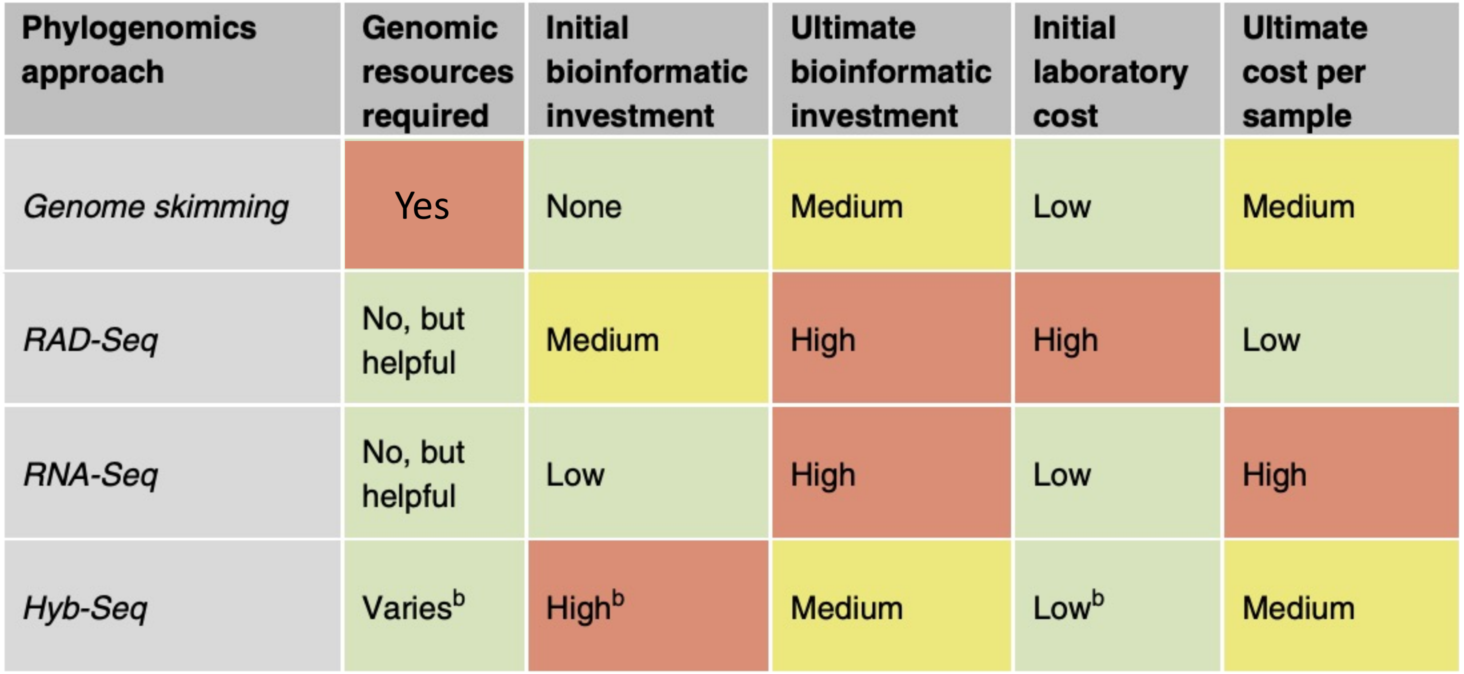
- Scan markers to look for association with SNPs and phenotypes of interest
- Considerations – normalize phenotype data, quantitative continuous data, make sure sample size is large enough, fairly dense sampling of SNPs
- Most methods are designed for reference genome data
  - Low number of contigs/chromosomes
  - *de novo* aspects have issues with LD and lack of coverage across the genome



Cortes et al. 2021; *Plant Genome*

Options for generating SNPs

- Many factors go into deciding the most appropriate option
- Different levels of investments in terms of wet lab and bioinformatic
- Size of genome, number of individuals, how much of the genome do you need to sequence, and ultimate goal for analyses



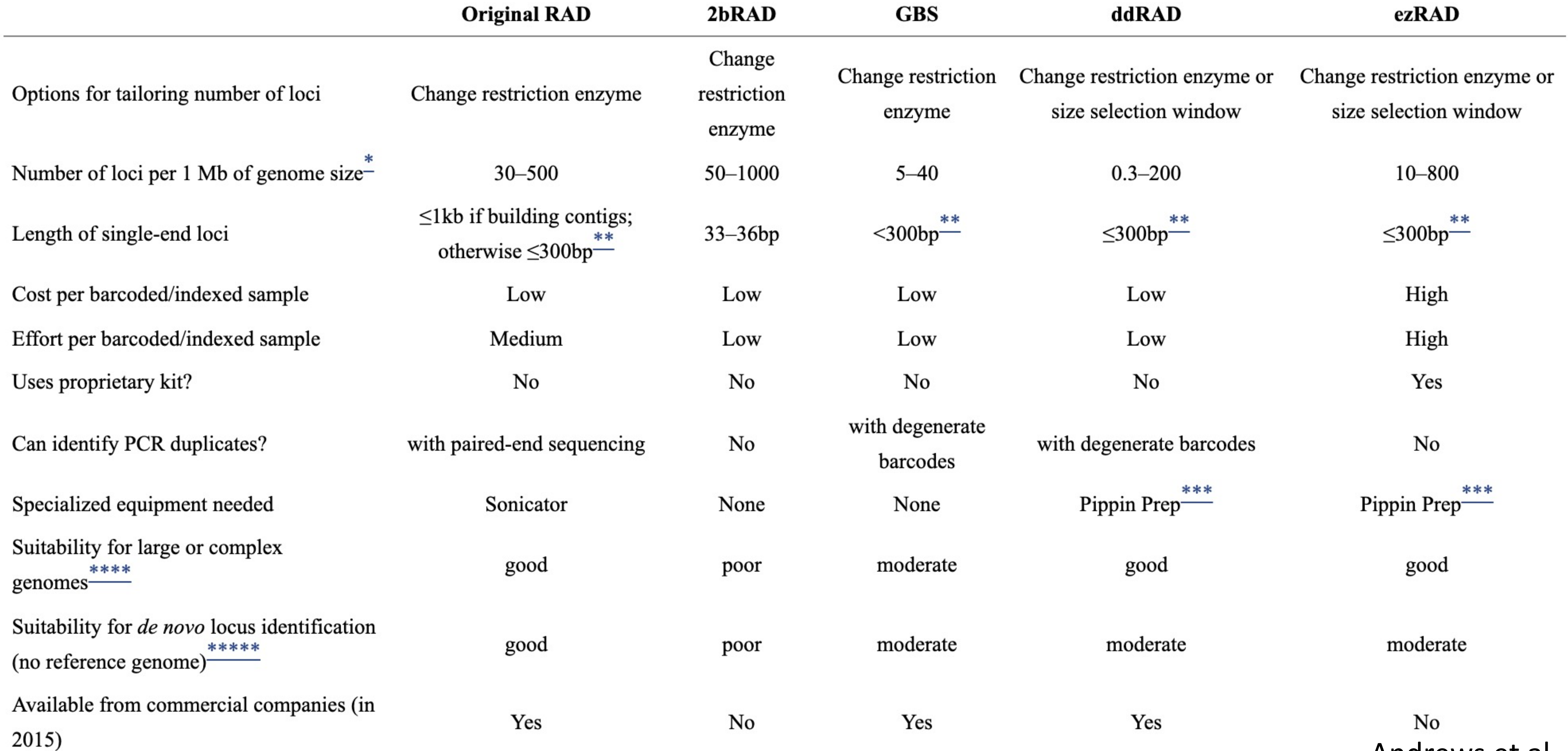
Phylogenomics approach	Genomic resources required	Initial bioinformatic investment	Ultimate bioinformatic investment	Initial laboratory cost	Ultimate cost per sample
Genome skimming	Yes	None	Medium	Low	Medium
RAD-Seq	No, but helpful	Medium	High	High	Low
RNA-Seq	No, but helpful	Low	High	Low	High
Hyb-Seq	Varies[b]	High[b]	Medium	Low[b]	Medium

Modified from Dodsworth et al., 2019

RAD-Seq

- Pros
  - Reduced representation of the genome; higher coverage in sequenced libraries
  - Allows for sequencing more individuals, especially with large genomes
  - Cheaper than other methods, around $15 per sample
  - Do not need a reference genome but this helps
- Cons
  - Do not get the whole genome, so may be missing things
  - Hard to integrate data sets unless they use the same enzymes
  - Biases between species and/or degraded samples if mutations are in the enzyme cut site

RAD-Seq Comparisons

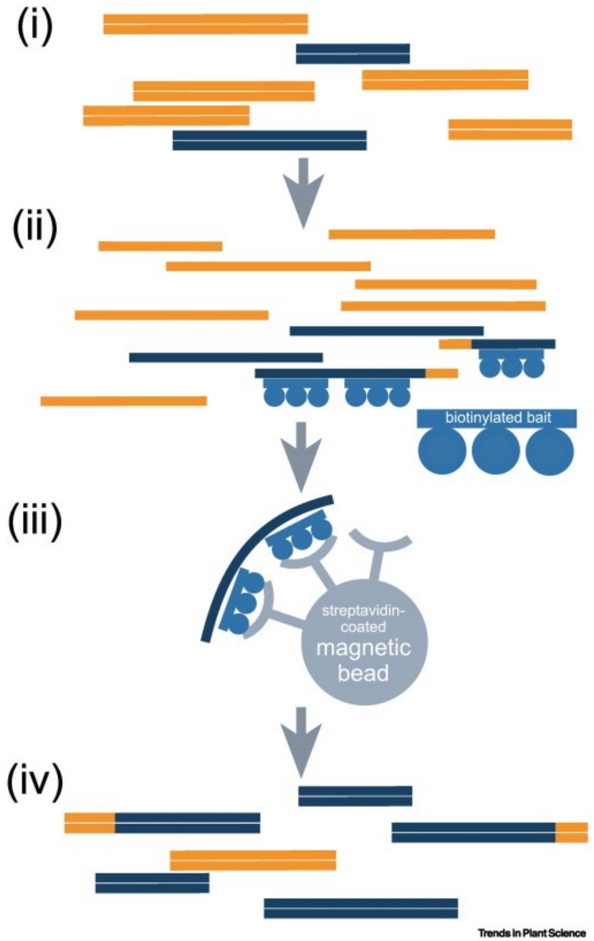


	Original RAD	2bRAD	GBS	ddRAD	ezRAD
Options for tailoring number of loci	Change restriction enzyme	Change restriction enzyme	Change restriction enzyme	Change restriction enzyme or size selection window	Change restriction enzyme or size selection window
Number of loci per 1 Mb of genome size*	30–500	50–1000	5–40	0.3–200	10–800
Length of single-end loci	≤1kb if building contigs; otherwise ≤300bp**	33–36bp	<300bp**	≤300bp**	≤300bp**
Cost per barcoded/indexed sample	Low	Low	Low	Low	High
Effort per barcoded/indexed sample	Medium	Low	Low	Low	High
Uses proprietary kit?	No	No	No	No	Yes
Can identify PCR duplicates?	with paired-end sequencing	No	with degenerate barcodes	with degenerate barcodes	No
Specialized equipment needed	Sonicator	None	None	Pippin Prep***	Pippin Prep***
Suitability for large or complex genomes****	good	poor	moderate	good	good
Suitability for *de novo* locus identification (no reference genome)*****	good	poor	moderate	moderate	moderate
Available from commercial companies (in 2015)	Yes	No	Yes	Yes	No

Andrews et al. 2016

RNA-Seq and Hyb-Seq

- RNA-Seq
  - Only get genes expressed in a particular tissue at a particular time
  - Lots of coverage for sequenced loci
  - Phenotypic differences may not be linked to the sequence of the coding region but in the promoter region; would miss this change
- Hyb-Seq
  - Probe sets can be expensive and need to have reference sequence
  - Can generate probe sets to capture the full exome of a species
  - Do not cover the entire genome, but greater depth at regions sequenced

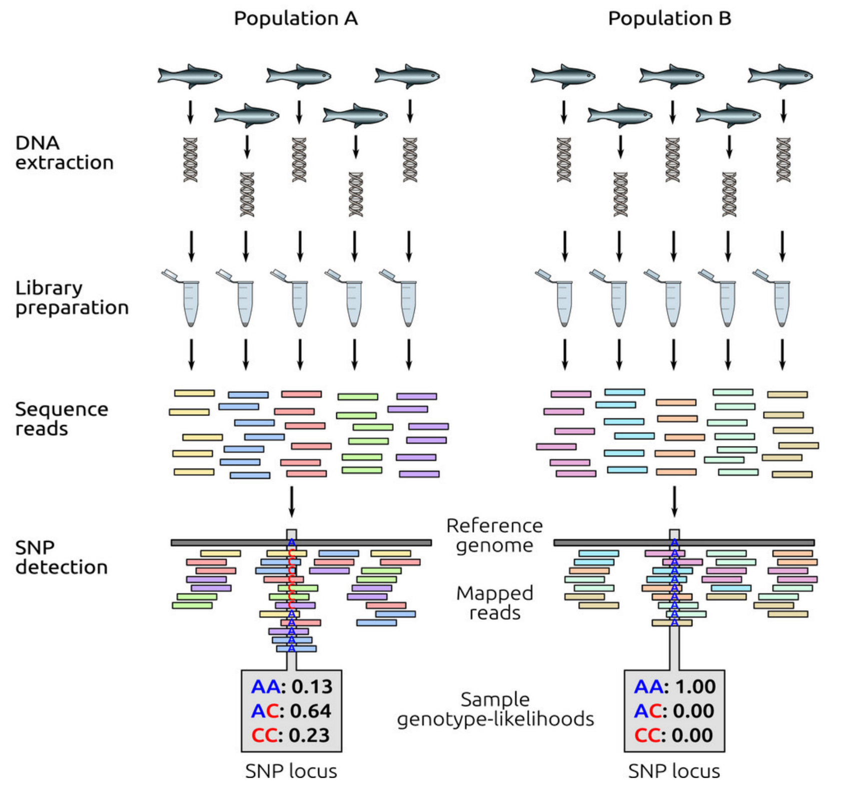


Dodsworth et al. 2019

Genome Resequencing

- Preferred method in most studies but not always possible
- Covers the entire genome
- Silica dried or old tissues works just fine, usually needs to be sheared anyway
- Does not involve any special library prep such as enzymes or probes
- Need a reference genome to align reads
- Not feasible for large-genome species (over 1 GB) even though sequencing costs are always going down

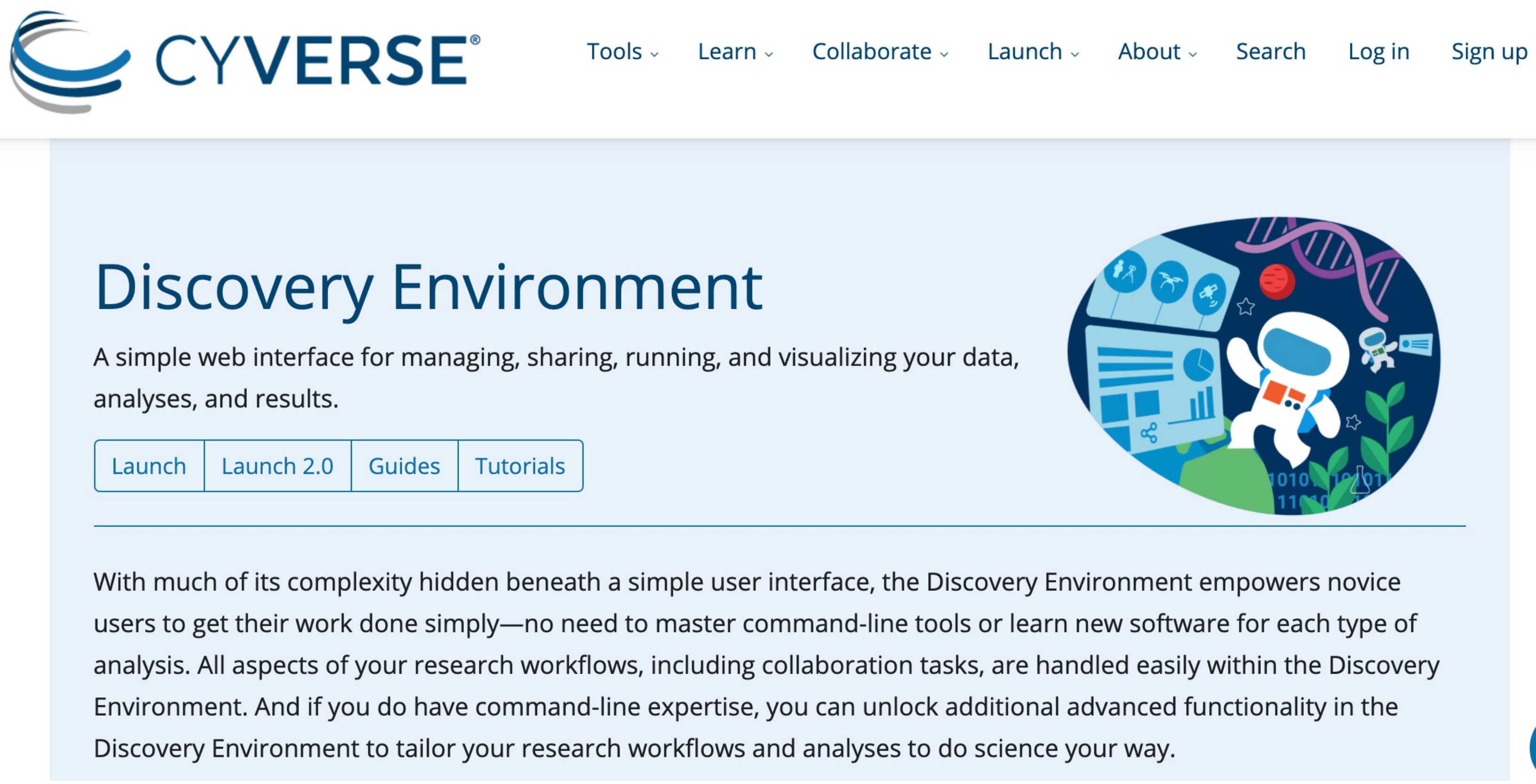
(b) Low-coverage whole-genome resequencing of individuals from a population (lcWGR)



Fuentes-Pardo and Ruzzante 2017

CyVerse Discovery Environment

- Point and click option
- Does not require knowledge of command line
- Works great for small data sets, but will need more resources for large projects
- Often do not have full functionality of all options



Discovery Environment

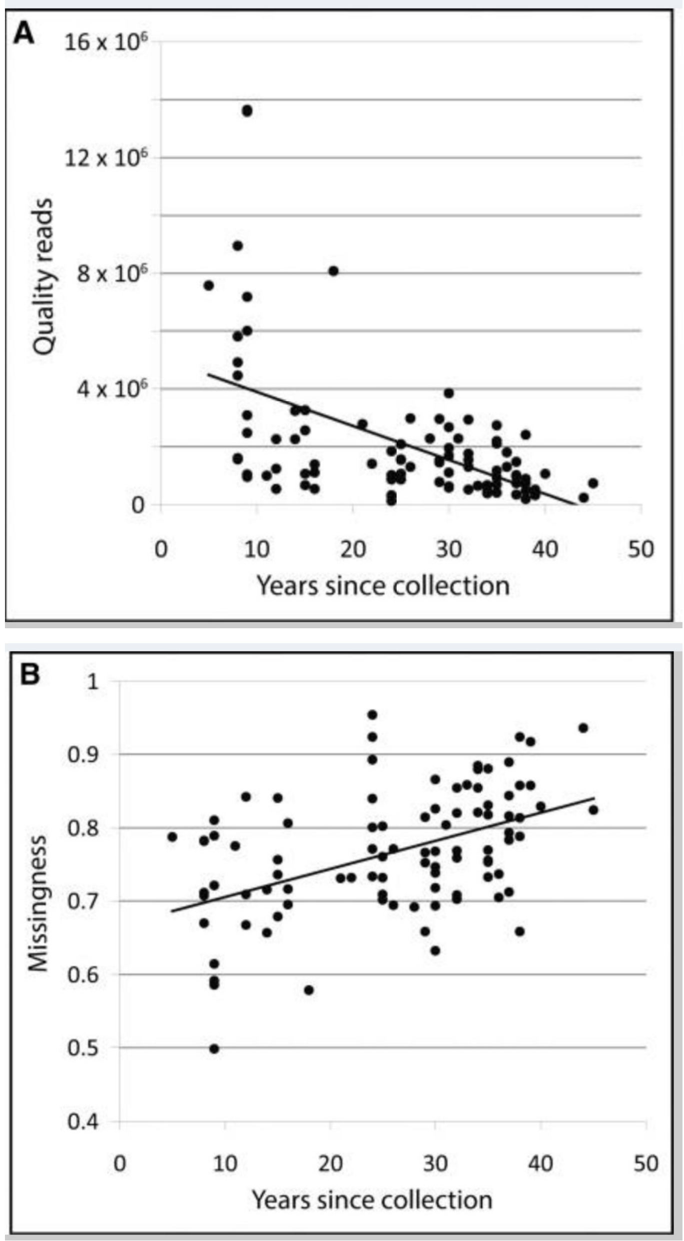
A simple web interface for managing, sharing, running, and visualizing your data, analyses, and results.

With much of its complexity hidden beneath a simple user interface, the Discovery Environment empowers novice users to get their work done simply—no need to master command-line tools or learn new software for each type of analysis. All aspects of your research workflows, including collaboration tasks, are handled easily within the Discovery Environment. And if you do have command-line expertise, you can unlock additional advanced functionality in the Discovery Environment to tailor your research workflows and analyses to do science your way.

de novo RAD-Seq

- Basic CTAB or similar DNA extraction
- Lots of options for enzymes with different frequency of cut sites
- Silica dried material works great
- Herbarium samples or degraded samples can work
- iPyRad or Stacks

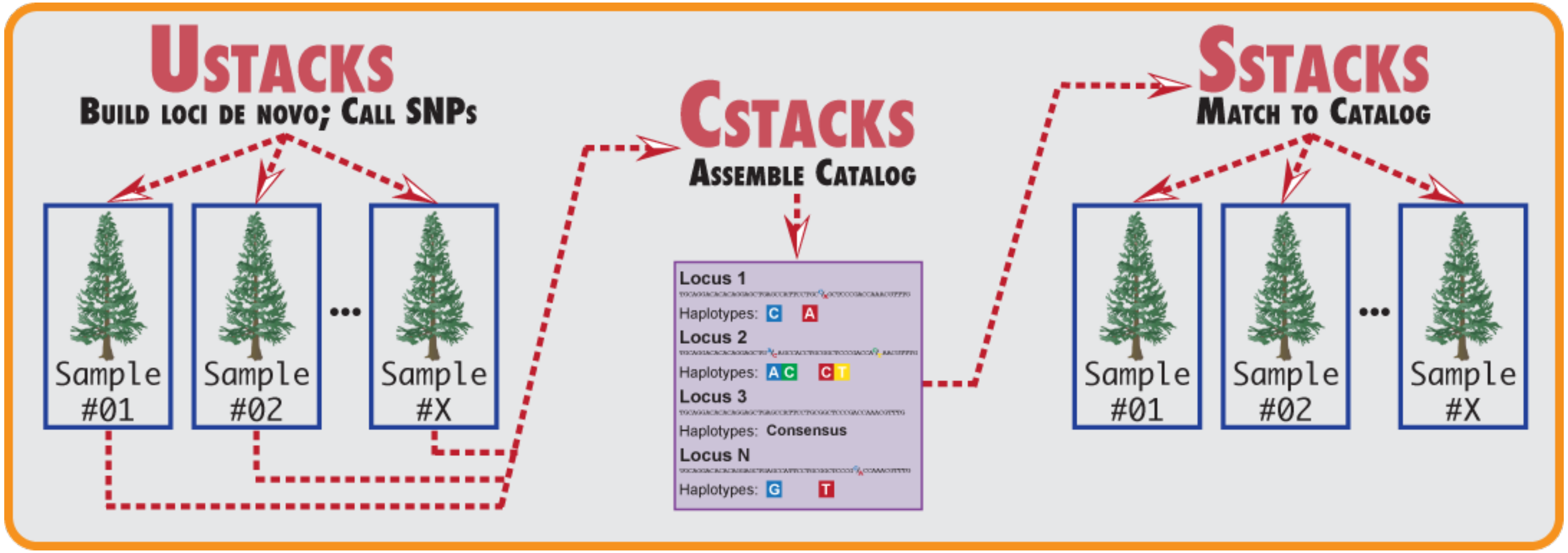
Change over time



Beck and Semple, 2015

de novo RAD-Seq

- Stacks denovo_map.pl script -> specify fastq files and population map



- Assembles loci in each individual and allows specification of number of nucleotide differences to define a locus, then assembles a catalog of all loci, then matches each sample to catalog for SNP calling

Stacks user manual

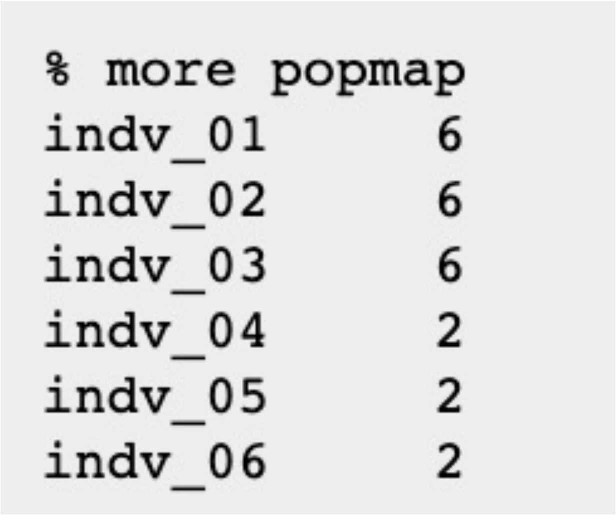
de novo RAD-Seq input

Calling the program input input

~/stacks/2.X/bin/denovo_map.pl --samples fastq_files/ --popmap population_map.txt -o de_novo_wrapper/ -T 8

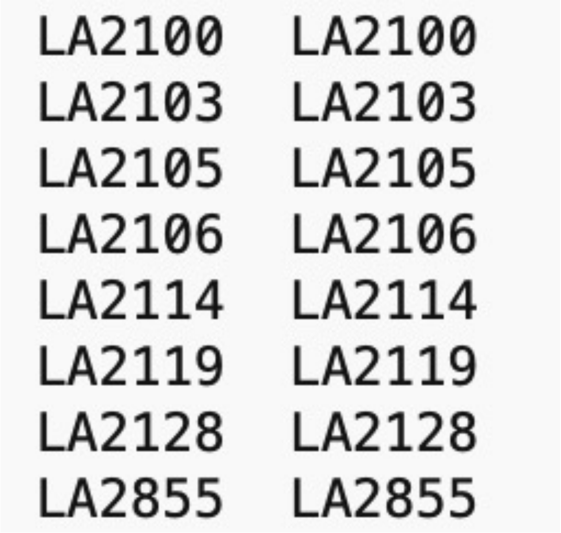
output additional options

Example population map - populations



```
% more popmap
indv_01      6
indv_02      6
indv_03      6
indv_04      2
indv_05      2
indv_06      2
```

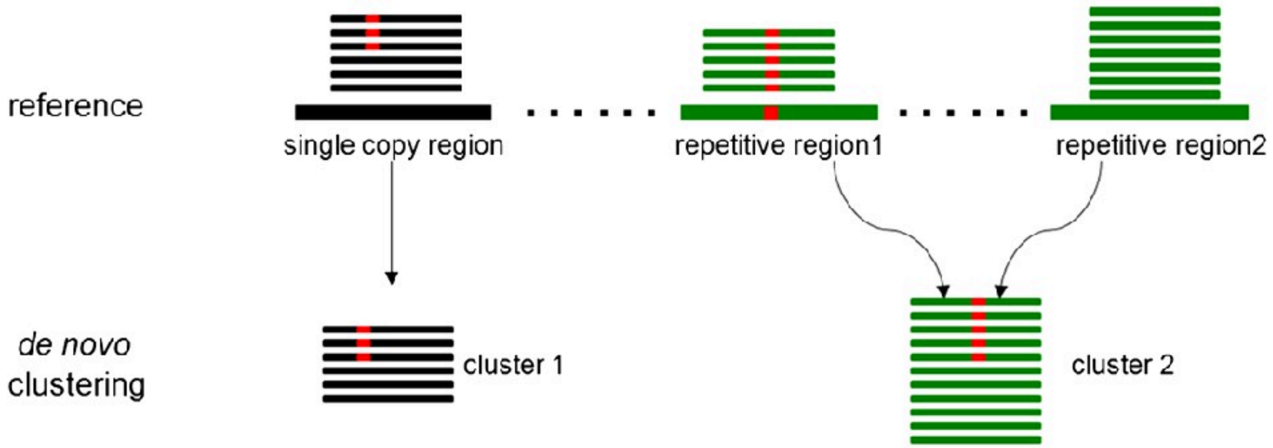
Example population map - individuals



```
LA2100  LA2100
LA2103  LA2103
LA2105  LA2105
LA2106  LA2106
LA2114  LA2114
LA2119  LA2119
LA2128  LA2128
LA2855  LA2855
```

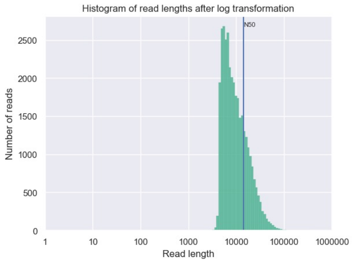
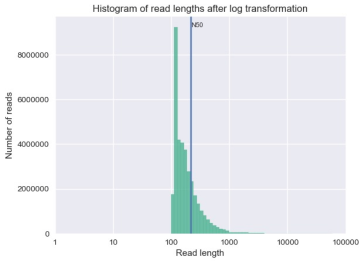
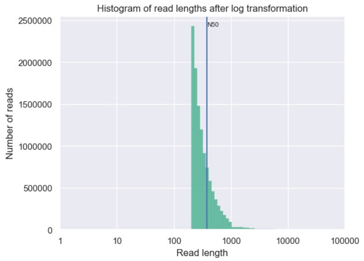
Reference based RAD-Seq

- Wet lab preparation same as for *de novo* approach
- Need to have some form of reference genome to map reads to
- Helps make sure nonhomologous loci are not collapsed

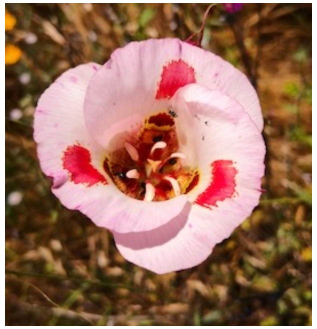


Dou et al. 2011

Does a reference genome help?

	de novo	Nanopore	Illumina	Hybrid
Genome Assembly	🚫	397 MB N50= 14,352 bp 	3 GB N50= 220 bp 	4.3 GB N50= 377 bp 
SNPs				
Raw	5,903	50,723	203,143	258,958
Filtered	2,188	4,976	8,660	15,533

Adriana Hernandez



Calochortus venustus
Estimated genome size of 5.5 GB

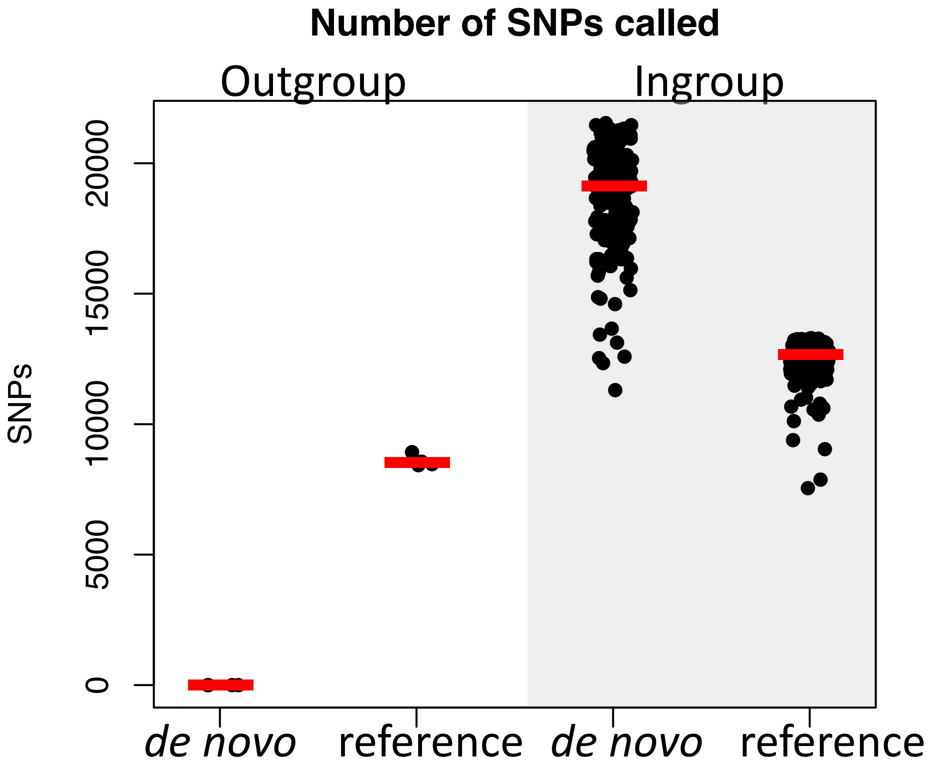
Even a poor draft genome increases the ability to call SNPs

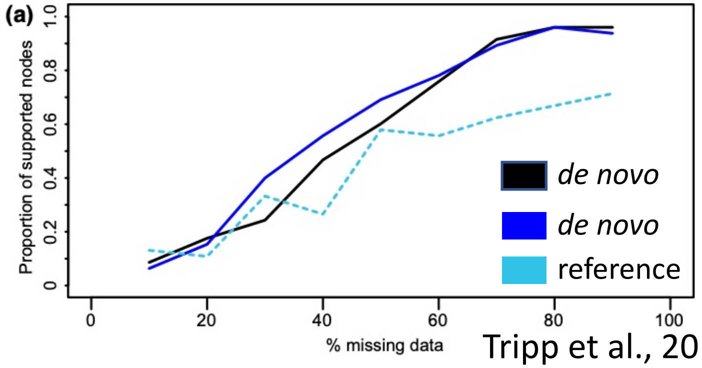
Does a reference genome help?

- For many analyses having an outgroup is helpful if not necessary

- If the outgroups are quite distinct genetically calling SNPs in *de novo* framework may leave them out

- Some concern that using a reference my lead to some bias





Tripp et al., 2017

Lorena Villanueva

Washingtonia filifera

How do I get a reference genome?

- Assemble your own using short- and long-read sequencing data

For a 1GB genome

- 50X Illumina:
  - 50Gb x $26.5/Gb = **$1,325**
- 50X nanopore:
  - 50Gb x $40/Gb = **$2,000**

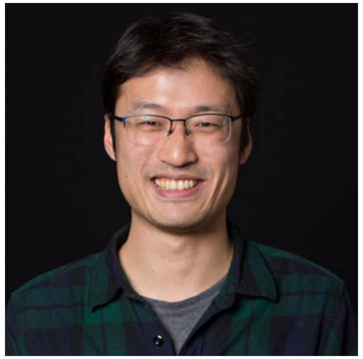
$3,325

- Organized a collaborative workshop covering genome assembly and annotation at Botany 2020

https://github.com/bcbc-group/Botany2020NMGWorkshop

CYVERSE®

Susan Strickler

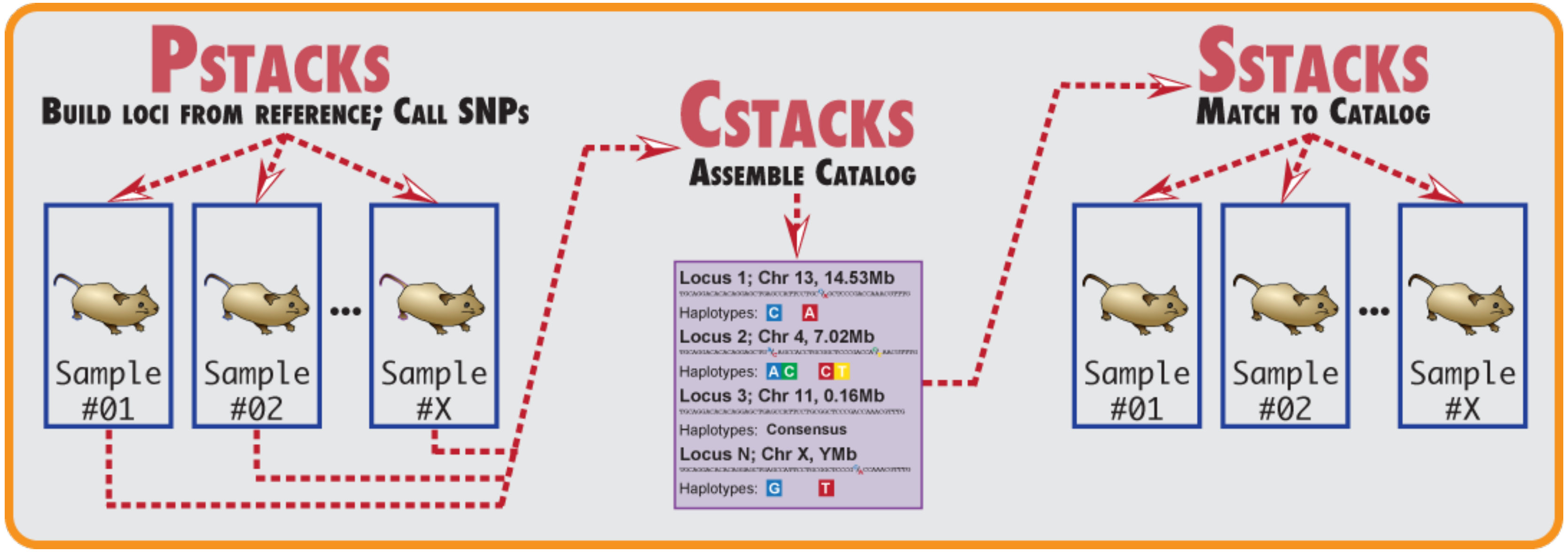


Fay-Wei Li

Andrew Nelson

Reference based RAD-Seq

- Map reads to reference
- refmap.pl -> specify bam files and population map



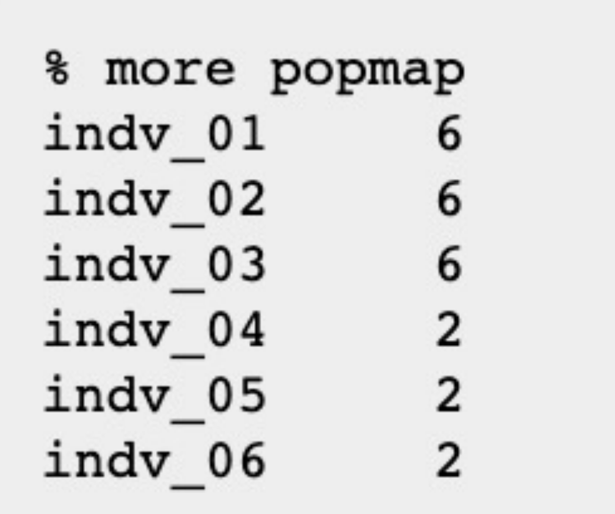
- Take aligned reads and calling SNPs in each locus, then make catalog and match loci based on genomic location not sequence similarity

Stacks user manual

Reference based RAD-Seq code

```
~/stacks/2.X/bin/ref_map.pl --samples sorted_bam_files/ --popmap population_map.txt -o ref_wrapper/ -T 8
```

Example population map - populations



```
% more popmap
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indv_03      6
indv_04      2
indv_05      2
indv_06      2
```
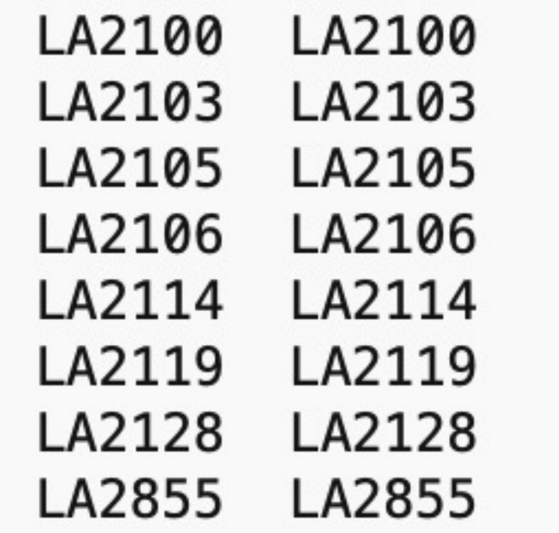

Example population map - individuals



```
LA2100  LA2100
LA2103  LA2103
LA2105  LA2105
LA2106  LA2106
LA2114  LA2114
LA2119  LA2119
LA2128  LA2128
LA2855  LA2855
```

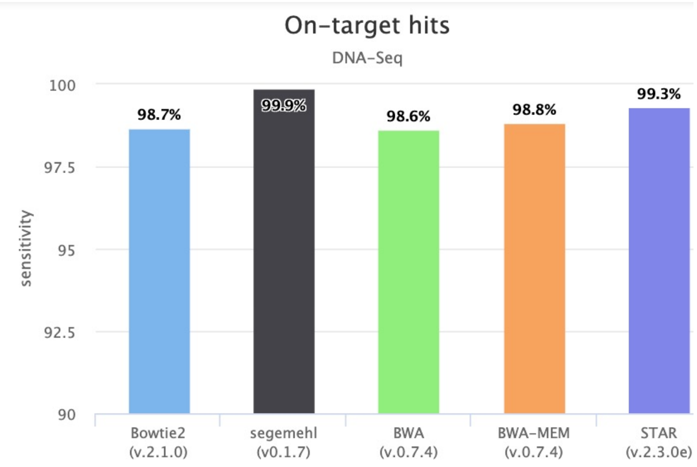
Read mapping is often overlooked

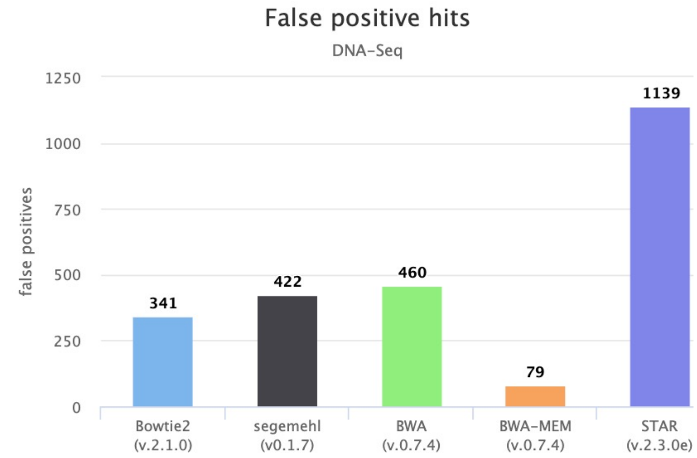
- Many different options for mapping genomic data to a reference include BWA MEM, minimap2, bowtie, etc.

- “the portion of reads that can be mapped is one factor, but not necessarily the most appropriate one”

- BWA MEM often performs the best in comparisons

- To save computation space, convert SAM to BAM





http://merenlab.org/2015/06/23/comparing-different-mapping-software/

Otto et al., 2014

BWA MEM code

- Need to index the fasta file first to specify genetic coordinates

```
bwa index Genome_assembly.fasta
```

- Map reads from each sample to the reference using Read Group(RG) information for easy identification of samples
  - ID: is unique identifier of the samples
  - SM: is the sample name
  - PL: is the sequencing equipment
  - PU: is the run identifier
  - LB: is the library count

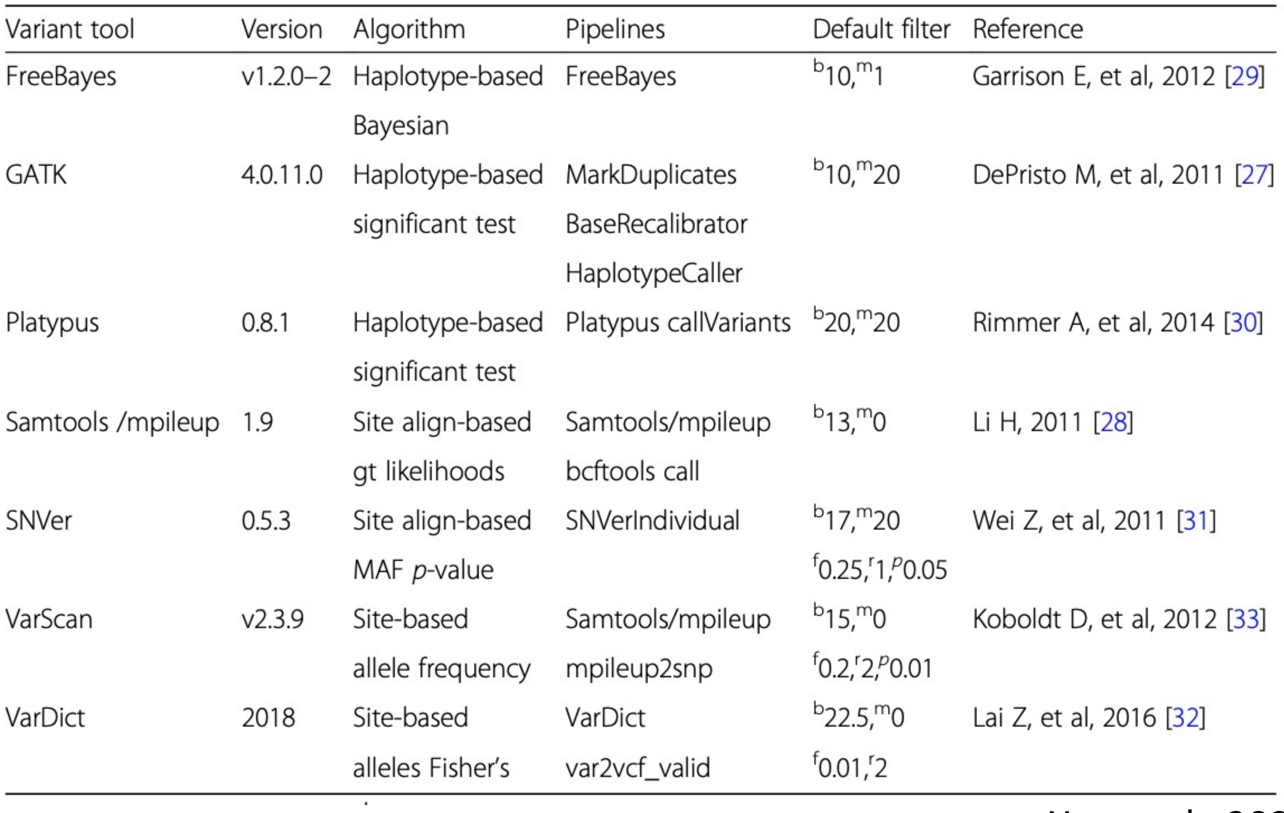
```
bwa mem -t 8 -R "@RG\tID:Sample1_A01\tSM:Sample1\tPL:HiSeq\tPU:HTNMKDSXX\tLB:RNA-Seq" Genome_assembly.fasta Sample1_R1.fastq.gz Sample1_R2.fastq.gz > Sample1.sam
```

Genome Forward Read Reverse Read SAM file as output

Hyb-Seq and Genome resequencing

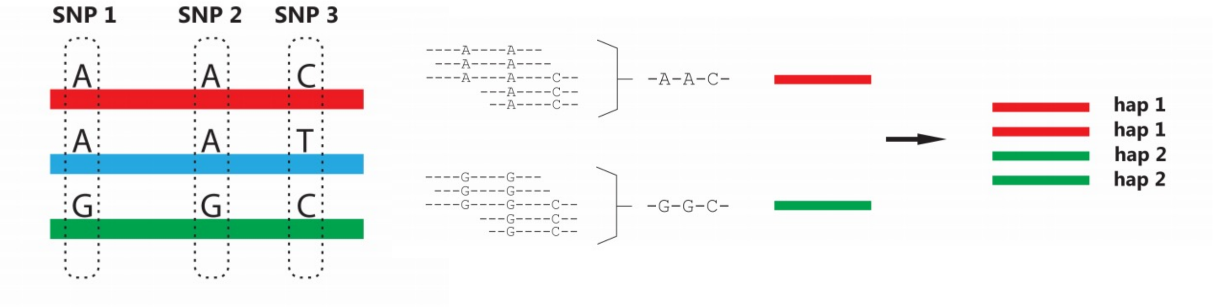
- No shortage in available programs or comparisons between programs
- Differences include maximum-likelihood vs Bayesian
- Haplotype vs site based

Commonly used programs



Variant tool	Version	Algorithm	Pipelines	Default filter	Reference
FreeBayes	v1.2.0–2	Haplotype-based Bayesian	FreeBayes	$^{b}10,^{m}1$	Garrison E, et al, 2012 [29]
GATK	4.0.11.0	Haplotype-based significant test	MarkDuplicates BaseRecalibrator HaplotypeCaller	$^{b}10,^{m}20$	DePristo M, et al, 2011 [27]
Platypus	0.8.1	Haplotype-based significant test	Platypus callVariants	$^{b}20,^{m}20$	Rimmer A, et al, 2014 [30]
Samtools /mpileup	1.9	Site align-based gt likelihoods	Samtools/mpileup bcftools call	$^{b}13,^{m}0$	Li H, 2011 [28]
SNVer	0.5.3	Site align-based MAF *p*-value	SNVerIndividual	$^{b}17,^{m}20$ $^{f}0.25,^{r}1,^{p}0.05$	Wei Z, et al, 2011 [31]
VarScan	v2.3.9	Site-based allele frequency	Samtools/mpileup mpileup2snp	$^{b}15,^{m}0$ $^{f}0.2,^{r}2,^{p}0.01$	Koboldt D, et al, 2012 [33]
VarDict	2018	Site-based alleles Fisher's	VarDict var2vcf_valid	$^{b}22.5,^{m}0$ $^{f}0.01,^{r}2$	Lai Z, et al, 2016 [32]

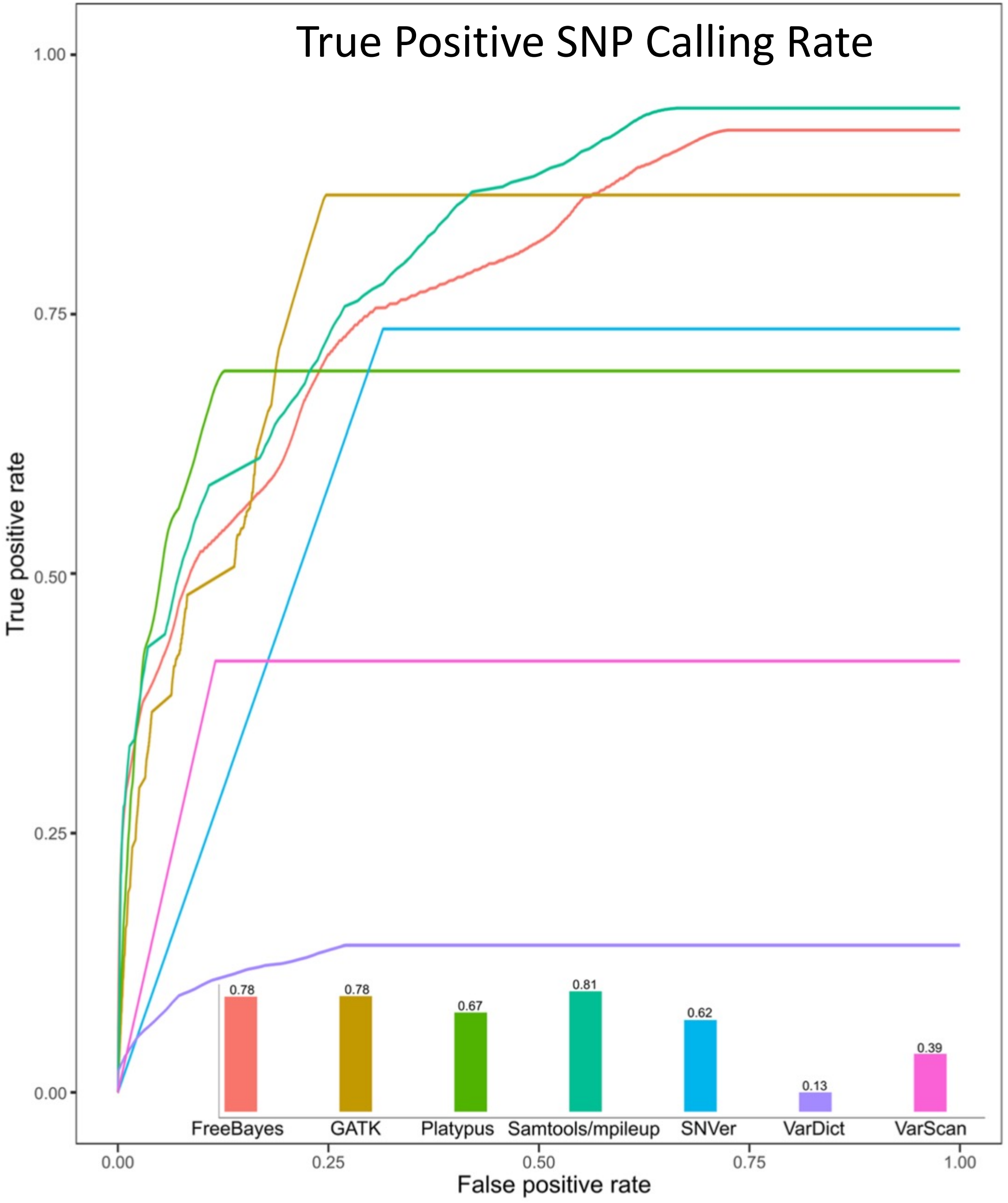
Yao et al., 2020



Bourke et al., 2018

Which SNP caller to use?

- **All SNP callers are NOT created equal**
- FreeBayes, GATK, and Samtools/mpileup had the lowest number of missed calls
- FreeBayes, VarScan and VarDict were most sensitive to unique calls
  - High sensitivity could result in a higher false positive rate
- Testing for true positives Samtools/mpileup called 81%, while GATK called 78.1% and FreeBayes called 77.7%

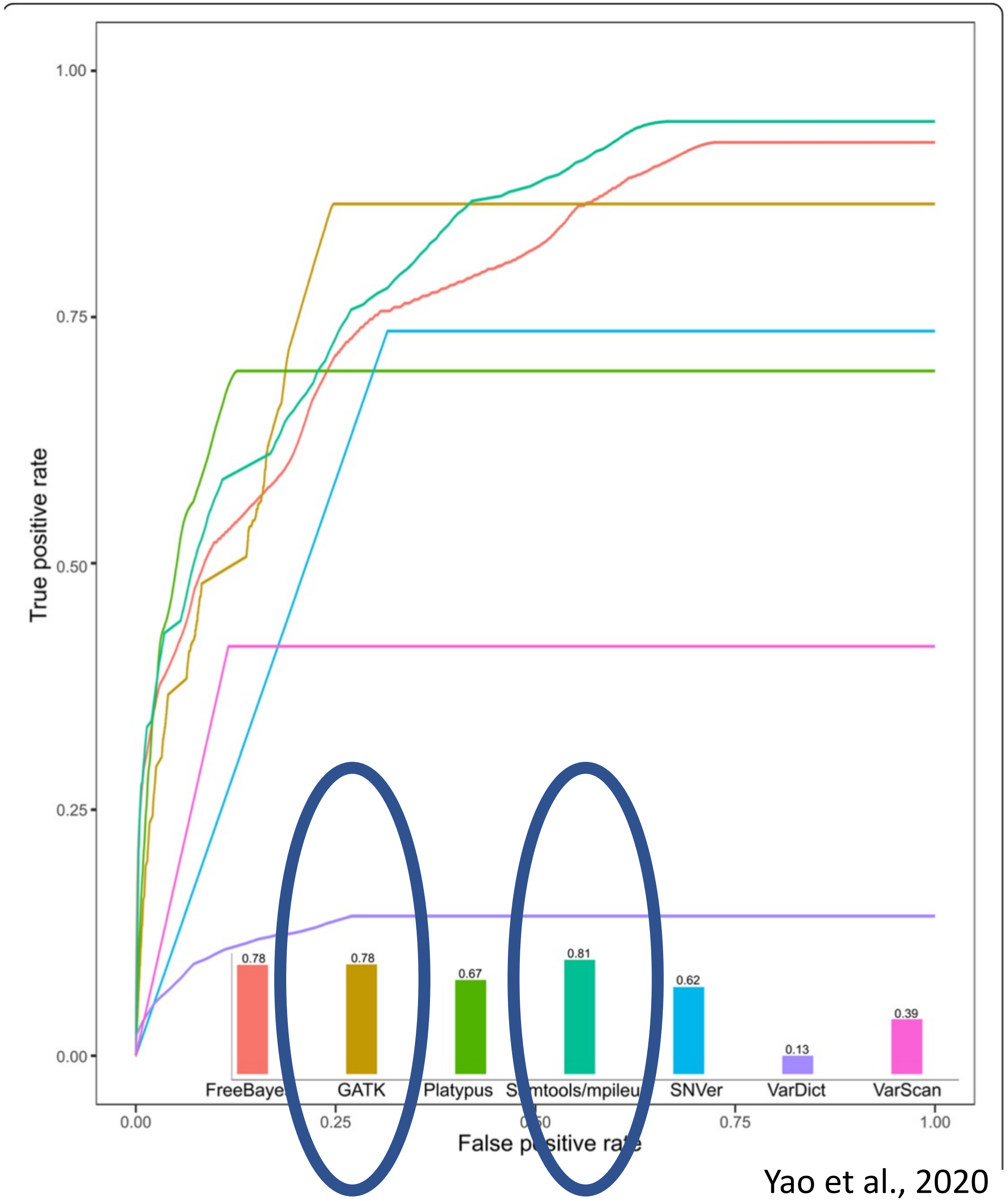


True Positive SNP Calling Rate

Yao et al., 2020

Which SNP caller to use?

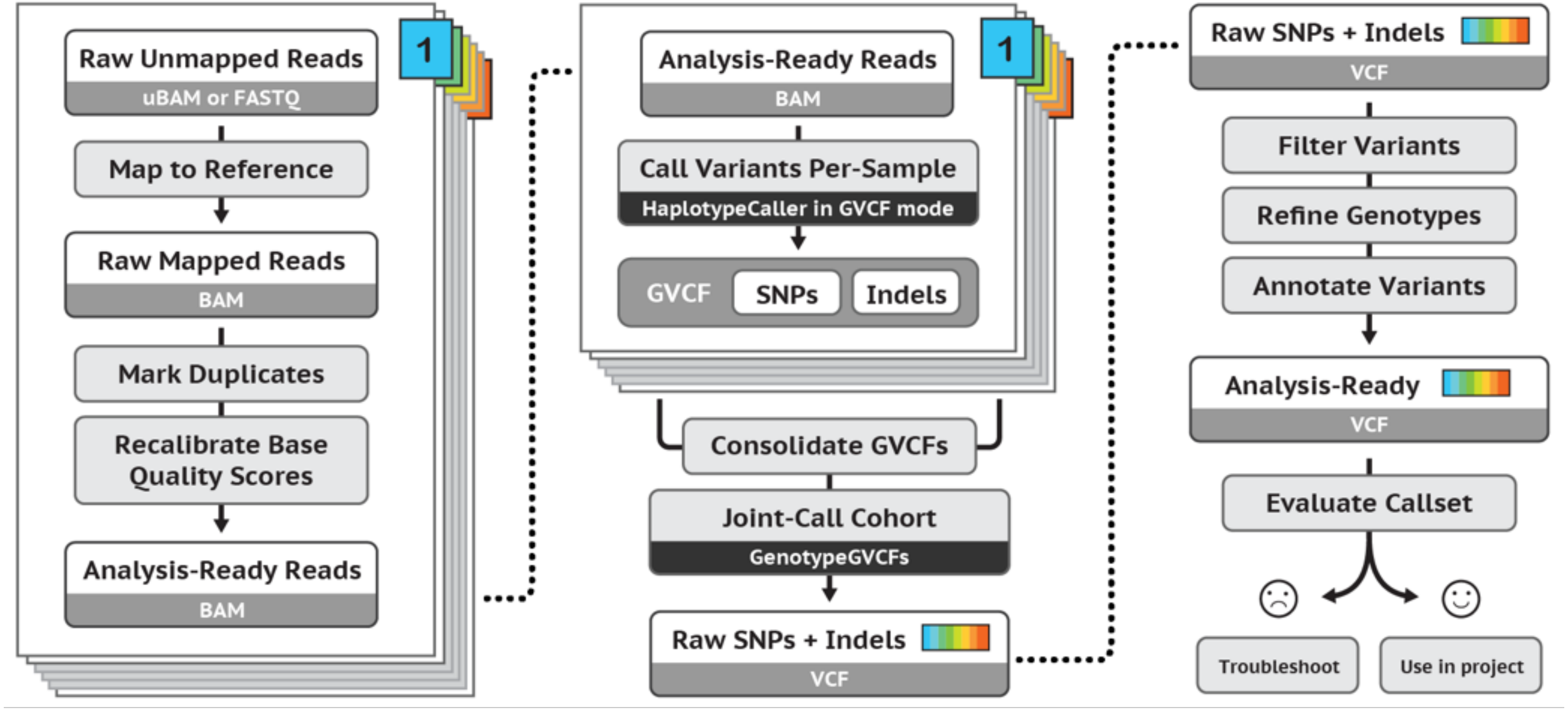
- **All SNP callers are NOT created equal**
- In many comparisons BWA MEM + GATK found to be the best for most genomes
- For complex genomes such as the large, polyploid wheat genome, BWA MEM + Samtools/mpileup is recommended



Yao et al., 2020

GATK

- GATK Best Practices: https://gatk.broadinstitute.org/hc/en-us/sections/360007226651-Best-Practices-Workflows



GATK code

- Prep the reference similar to how we did for BWA MEM

```
gatk CreateSequenceDictionary -R Genome_assembly.fasta -O Genome_assembly.dict
```

```
samtools faidx Genome_assembly.fasta
```

- Each sample that was mapped to the genome will need to be indexed then call SNPs and indels via local re-assembly of haplotypes

```
samtools index Sample1.bam
```

```
gatk HaplotypeCaller -R Genome_assembly.fasta -I Sample1.bam -O
Sample1.g.vcf.gz -ERC GVCF
```

GATK code continued

- We technically have now called SNPs on each sample but only the variants for each sample individually
- We want a file representing all individuals and all variants
- Need to combine the files and the do joint genotyping

```
gatk CombineGVCFs -R Genome_assembly.fasta -V samples.list --output
All_samples_combined.g.vcf.gz
```

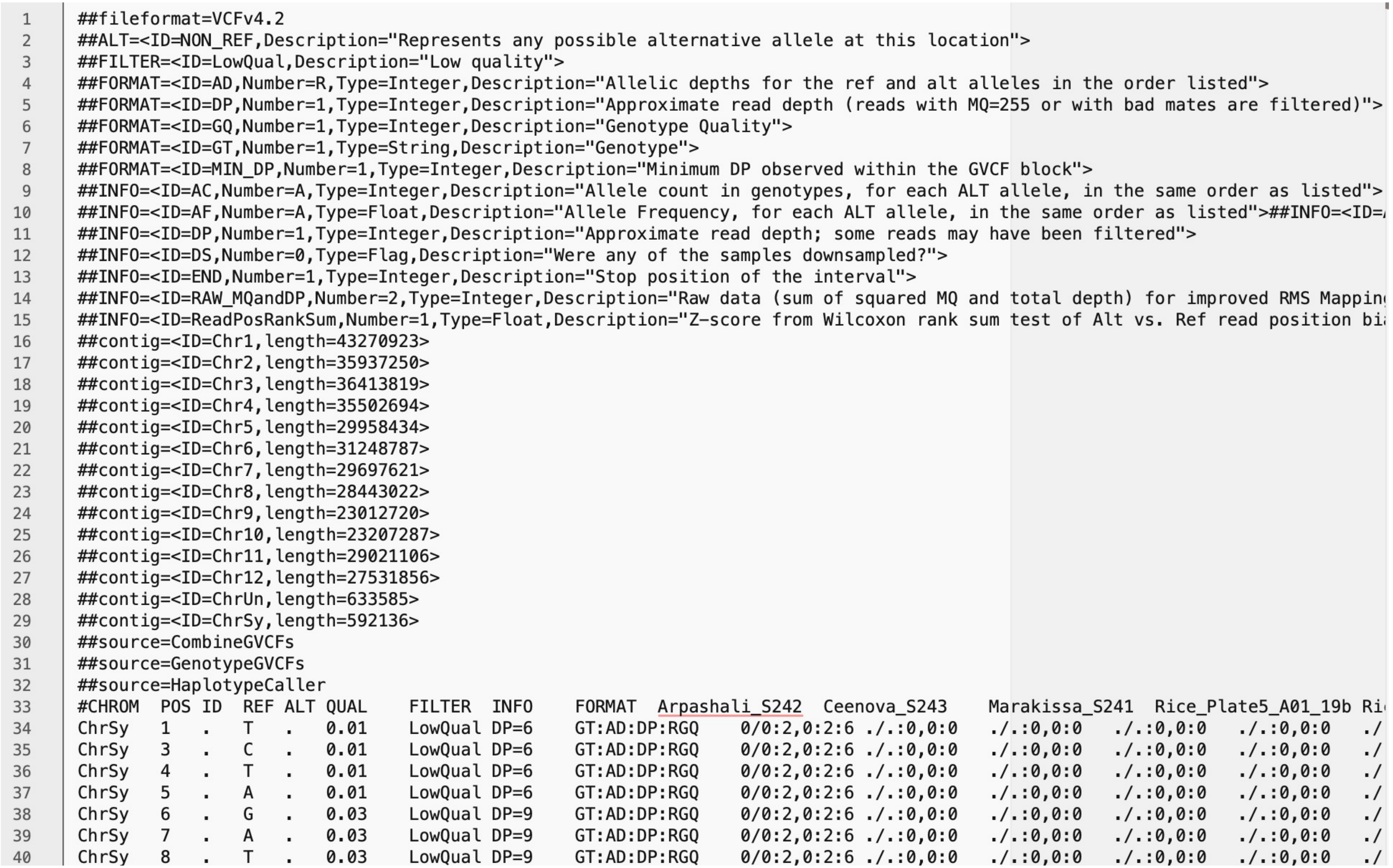
```
gatk GenotypeGVCFs -R Genome_assembly.fasta --variant
All_samples_combined.g.vcf.gz --output All_samples_variants.vcf.gz
```

Resulting file - Variant Call Format

Formatting and info about what is included for each score

Each contig and how big they are

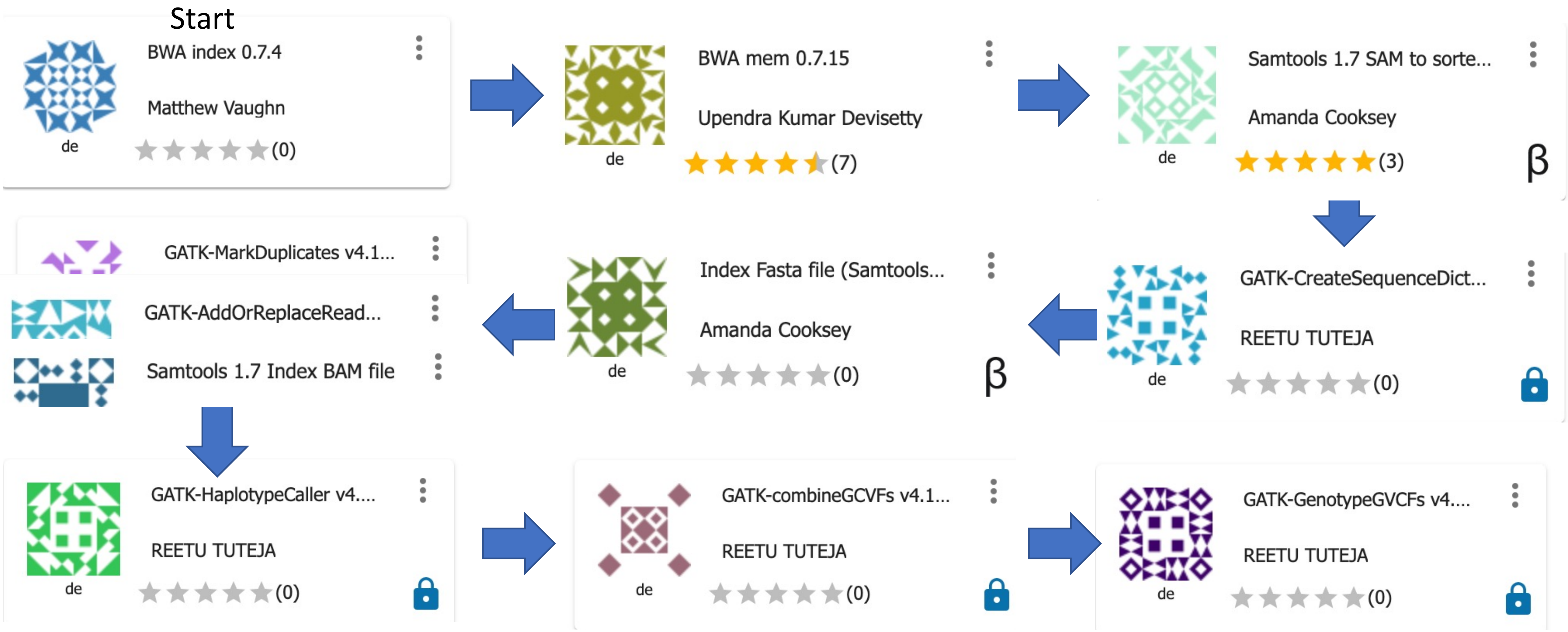
Each line is a variant, each column is a sample



```
##fileformat=VCFv4.2
##ALT=<ID=NON_REF,Description="Represents any possible alternative allele at this location">
##FILTER=<ID=LowQual,Description="Low quality">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths for the ref and alt alleles in the order listed">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth (reads with MQ=255 or with bad mates are filtered)">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=MIN_DP,Number=1,Type=Integer,Description="Minimum DP observed within the GVCF block">
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes, for each ALT allele, in the same order as listed">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency, for each ALT allele, in the same order as listed">##INFO=<ID=
##INFO=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth; some reads may have been filtered">
##INFO=<ID=DS,Number=0,Type=Flag,Description="Were any of the samples downsampled?">
##INFO=<ID=END,Number=1,Type=Integer,Description="Stop position of the interval">
##INFO=<ID=RAW_MQandDP,Number=2,Type=Integer,Description="Raw data (sum of squared MQ and total depth) for improved RMS Mappin
##INFO=<ID=ReadPosRankSum,Number=1,Type=Float,Description="Z-score from Wilcoxon rank sum test of Alt vs. Ref read position bi
##contig=<ID=Chr1,length=43270923>
##contig=<ID=Chr2,length=35937250>
##contig=<ID=Chr3,length=36413819>
##contig=<ID=Chr4,length=35502694>
##contig=<ID=Chr5,length=29958434>
##contig=<ID=Chr6,length=31248787>
##contig=<ID=Chr7,length=29697621>
##contig=<ID=Chr8,length=28443022>
##contig=<ID=Chr9,length=23012720>
##contig=<ID=Chr10,length=23207287>
##contig=<ID=Chr11,length=29021106>
##contig=<ID=Chr12,length=27531856>
##contig=<ID=ChrUn,length=633585>
##contig=<ID=ChrSy,length=592136>
##source=CombineGVCFs
##source=GenotypeGVCFs
##source=HaplotypeCaller
#CHROM  POS ID  REF ALT QUAL    FILTER  INFO    FORMAT  Arpashali_S242  Ceenova_S243    Marakissa_S241  Rice_Plate5_A01_19b Ri
ChrSy   1   .   T   .   0.01    LowQual DP=6    GT:AD:DP:RGQ    0/0:2,0:2:6 ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./
ChrSy   3   .   C   .   0.01    LowQual DP=6    GT:AD:DP:RGQ    0/0:2,0:2:6 ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./
ChrSy   4   .   T   .   0.01    LowQual DP=6    GT:AD:DP:RGQ    0/0:2,0:2:6 ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./
ChrSy   5   .   A   .   0.01    LowQual DP=6    GT:AD:DP:RGQ    0/0:2,0:2:6 ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./
ChrSy   6   .   G   .   0.03    LowQual DP=9    GT:AD:DP:RGQ    0/0:2,0:2:6 ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./
ChrSy   7   .   A   .   0.03    LowQual DP=9    GT:AD:DP:RGQ    0/0:2,0:2:6 ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./
ChrSy   8   .   T   .   0.03    LowQual DP=9    GT:AD:DP:RGQ    0/0:2,0:2:6 ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./.:0,0:0   ./
```

More info: https://samtools.github.io/hts-specs/VCFv4.2.pdf

GATK CyVerse Discovery Environment



Start

BWA index 0.7.4
Matthew Vaughn
de
☆☆☆☆☆(0)

BWA mem 0.7.15
Upendra Kumar Devisetty
de
★★★★★(7)

Samtools 1.7 SAM to sorte...
Amanda Cooksey
de
★★★★★(3)
β

GATK-CreateSequenceDict...
REETU TUTEJA
de
☆☆☆☆☆(0)
🔒

Index Fasta file (Samtools...
Amanda Cooksey
de
☆☆☆☆☆(0)
β

GATK-MarkDuplicates v4.1...

GATK-AddOrReplaceRead...

Samtools 1.7 Index BAM file

GATK-HaplotypeCaller v4....
REETU TUTEJA
de
☆☆☆☆☆(0)
🔒

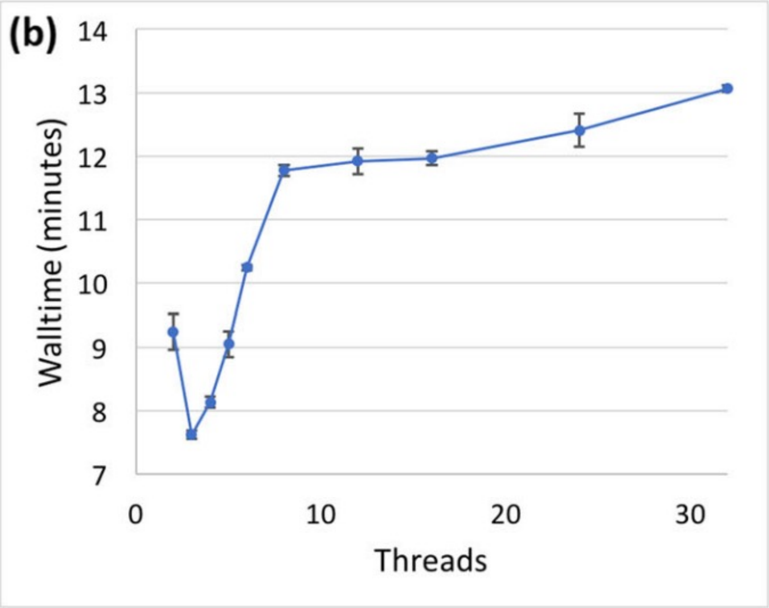
GATK-combineGCVFs v4.1...
REETU TUTEJA
de
☆☆☆☆☆(0)
🔒

GATK-GenotypeGVCFs v4....
REETU TUTEJA
de
☆☆☆☆☆(0)
🔒

- Limited resources: 8 CPU and 16 GB RAM

Possible issues with GATK

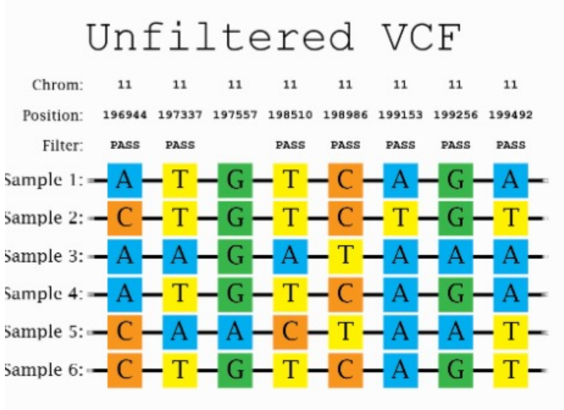
- Can be a difficult program to learn, however there is an extensive and active discussion board and tutorials available
- Scalability – Using more threads/processors doesn't always speed up analyses
- Version issues are real
  - When updates come out, some commands change with little documentation
  - Need to look at the updated tutorials from the Broad Institute



Heldenbrand et al., 2019

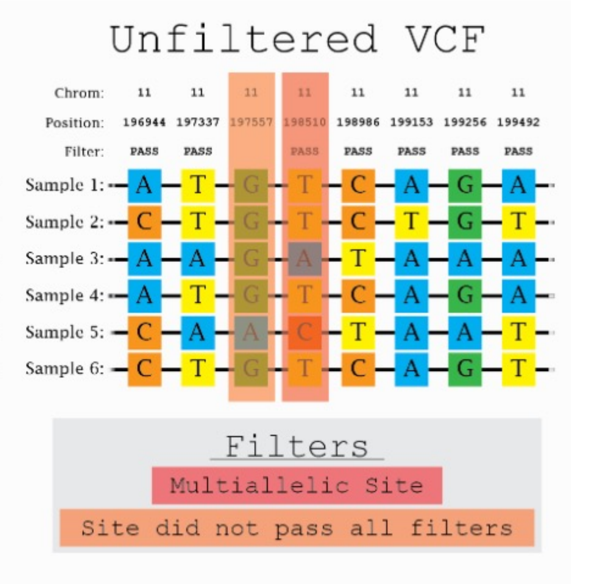
Filtering data

- VCFtools
  - Easy to implement; not very picky on specific formatting
  - Limited to options, but a clear user manual
  - Can be slow on large data sets (hundreds of taxa and millions of SNPs)
  - Cannot handle polyploid data
- BCFtools
  - Harder to implement for basic filtering, but more powerful
  - Much faster with large data sets and can handle polyploid data
  - Actively supported and distributed alongside Samtools
- GATK methods: https://gatk.broadinstitute.org/hc/en-us/articles/360035890471-Hard-filtering-germline-short-variants



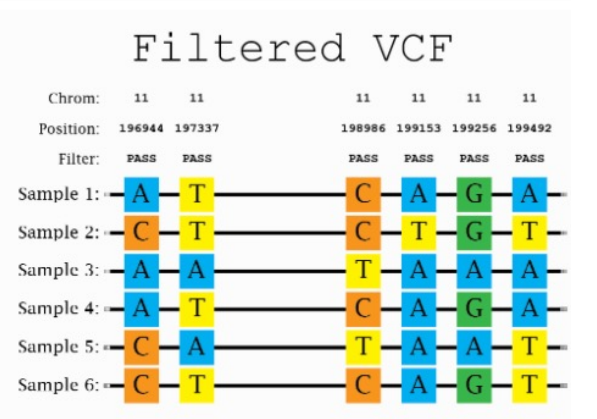
Unfiltered VCF

Chrom:	11	11	11	11	11	11	11	11
Position:	196944	197337	197557	198510	198986	199153	199256	199492
Filter:	PASS	PASS		PASS	PASS	PASS	PASS	PASS
Sample 1:	A	T	G	T	C	A	G	A
Sample 2:	C	T	G	T	C	T	G	T
Sample 3:	A	A	G	A	T	A	A	A
Sample 4:	A	T	G	T	C	A	G	A
Sample 5:	C	A	A	C	T	A	A	T
Sample 6:	C	T	G	T	C	A	G	T



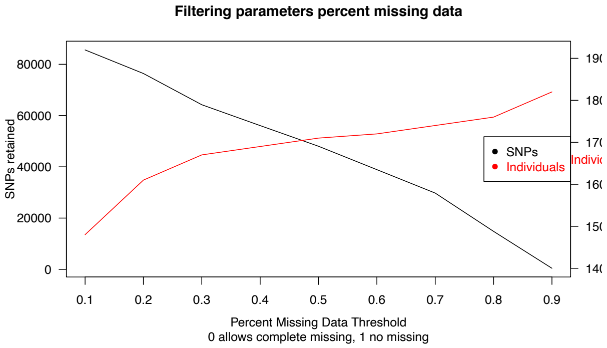
Unfiltered VCF

Chrom:	11	11	11	11	11	11	11	11
Position:	196944	197337	197557	198510	198986	199153	199256	199492
Filter:	PASS	PASS		PASS	PASS	PASS	PASS	PASS
Sample 1:	A	T	G	T	C	A	G	A
Sample 2:	C	T	G	T	C	T	G	T
Sample 3:	A	A	G	A	T	A	A	A
Sample 4:	A	T	G	T	C	A	G	A
Sample 5:	C	A	A	C	T	A	A	T
Sample 6:	C	T	G	T	C	A	G	T



Filtered VCF

Chrom:	11	11	11	11	11	11
Position:	196944	197337	198986	199153	199256	199492
Filter:	PASS	PASS	PASS	PASS	PASS	PASS
Sample 1:	A	T	C	A	G	A
Sample 2:	C	T	C	T	G	T
Sample 3:	A	A	T	A	A	A
Sample 4:	A	T	C	A	G	A
Sample 5:	C	A	T	A	A	T
Sample 6:	C	T	C	A	G	T



VCFtools code

- If you wanted to keep only sites that were biallelic sites, at most 50% missing data, a read depth between 3-30x coverage, and a minor allele frequency of at least 5%

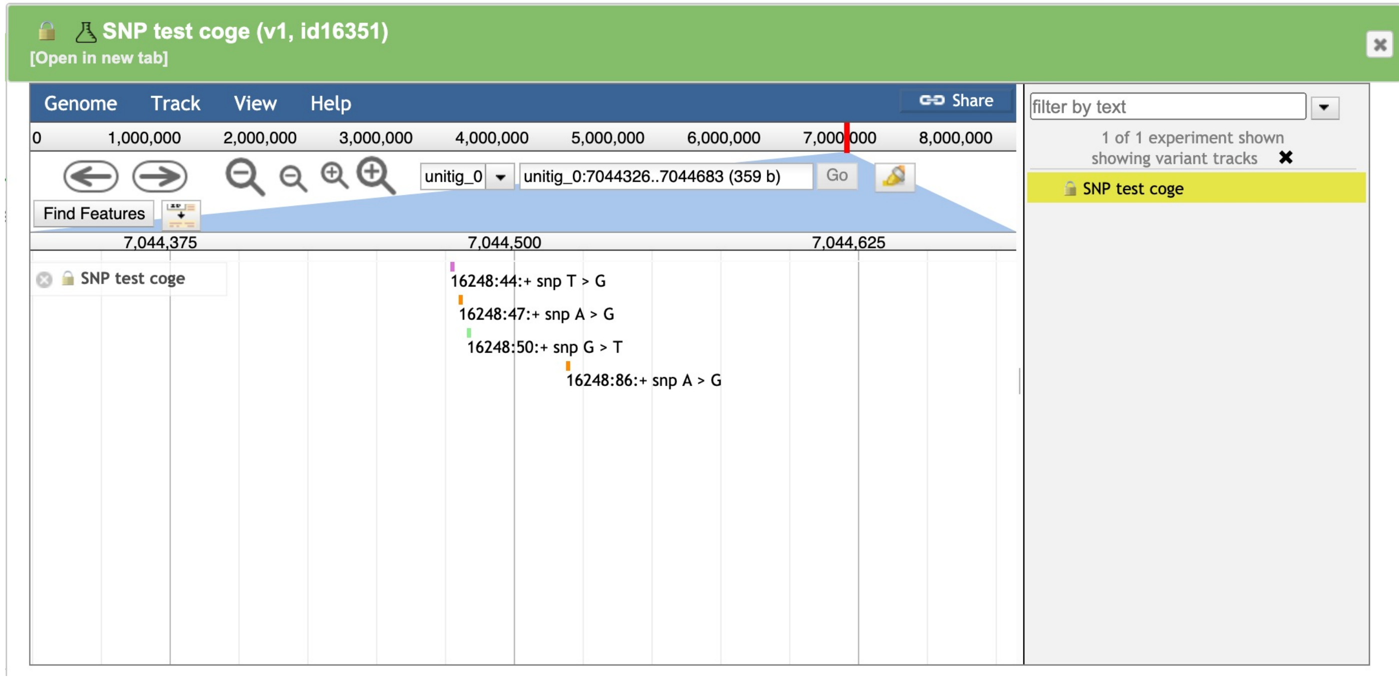
```
vcftools --vcf original.snps.vcf --max-missing 0.5 --min-alleles 2 --max-alleles 2 --min-meanDP 3
--max-meanDP 30 --maf 0.05 --recode --recode-INFO-all --out Filtered_SNPs
```

- Also very easy to in VCFtools to report read depth for each individual and percent of missing data

```
vcftools --vcf Filtered_SNPs.recode.vcf --depth
vcftools --vcf Filtered_SNPs.recode.vcf --missing-indiv
```

CoGe (Comparative Genomics)

- Over 54,000 genomes from 20,515 stored, with most available to the public
- Can upload our resulting VCF file and visualize where the SNPs occur
- Many other options that can be done but that is for a different workshop



SNP test coge (v1, id16351)

[Open in new tab]

unitig_0:7044326..7044683 (359 b)

16248:44:+ snp T > G

16248:47:+ snp A > G

16248:50:+ snp G > T

16248:86:+ snp A > G

1 of 1 experiment shown

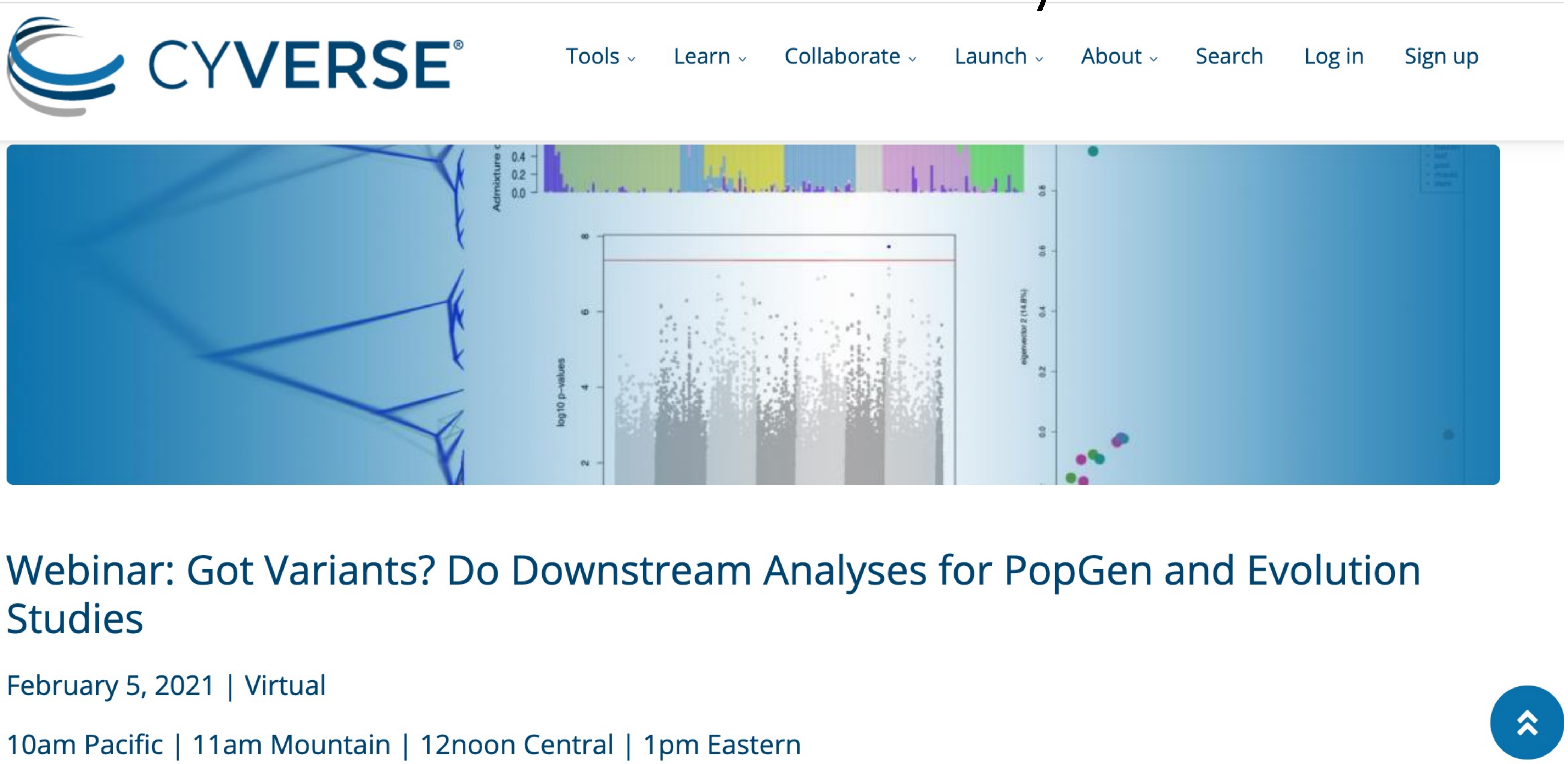
showing variant tracks

GitHub tutorial with *U. gibba*

- SNP calling walkthrough available: https://github.com/jblandis/AG2PI_SNP_Workshop_May2021
- Incorporates publicly available data using a high-quality genome assembly and RNA-Seq data for multiple organ types
    - Bladder, leaf, rhizoid, and stem
- Small data set that can be run on a local machine
- Examples for command line and Discovery Environment
- SNP calling using both Stacks and GATK
- Filtering and PCA using SNP data

Utricularia gibba
Humped bladderwort

More Downstream Analyses



Webinar: Got Variants? Do Downstream Analyses for PopGen and Evolution Studies

February 5, 2021 | Virtual

10am Pacific | 11am Mountain | 12noon Central | 1pm Eastern

https://github.com/bcbc-group/CyVerse_Variant_Analyses

Conclusions

- Every project may demand a modified SNP calling approach
- Things that may influence your methods may be large genomes, polyploidy events, availability and quality of a reference genome
- SNP filtering in some ways is an art; each data set should be explored to see what happens when adjusting parameters
- Hopefully this is a good start on the SNP calling journey but there are many intricacies to each of these programs along the way

TM

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