

# Reads2Map

**Developing best practices for genotyping-by-sequencing  
analysis using linkage maps as benchmarks**

Cristiane Taniguti

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# Motivation: GBS data

Issues while building linkage maps

- ▶ Computational intensive
- ▶ Time consuming
- ▶ Wrong grouping
- ▶ Wrong ordering
- ▶ Inflated linkage maps



- Since 2007 - 52k downloads
- Diploid species
- Bi-parental populations
- Backcross, RILs, F2 and outcrossing
- Biallelic and Multiallelic markers

Maintainer since 2017  
Updates in version 3.0



Augusto Garcia  
Marcelo Mollinari  
Gabriel Margarido

# Motivation

## Users feedbacks



# Motivation: GBS data

Issues while building linkage maps

- ▶ Computational intensive
- ▶ Time consuming
- ▶ Wrong grouping
- ▶ Wrong ordering
- ▶ Inflated linkage maps

Tips and  
Tricks:



RGC11 - Poster



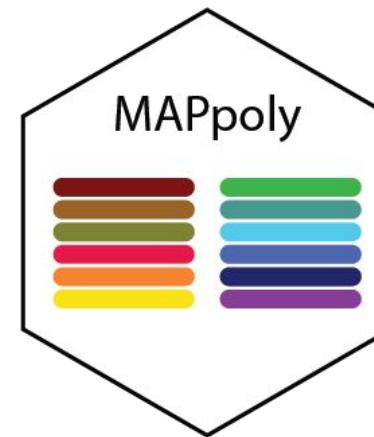
Augusto Garcia



Gabriel Gesteira



Marcelo Mollinari

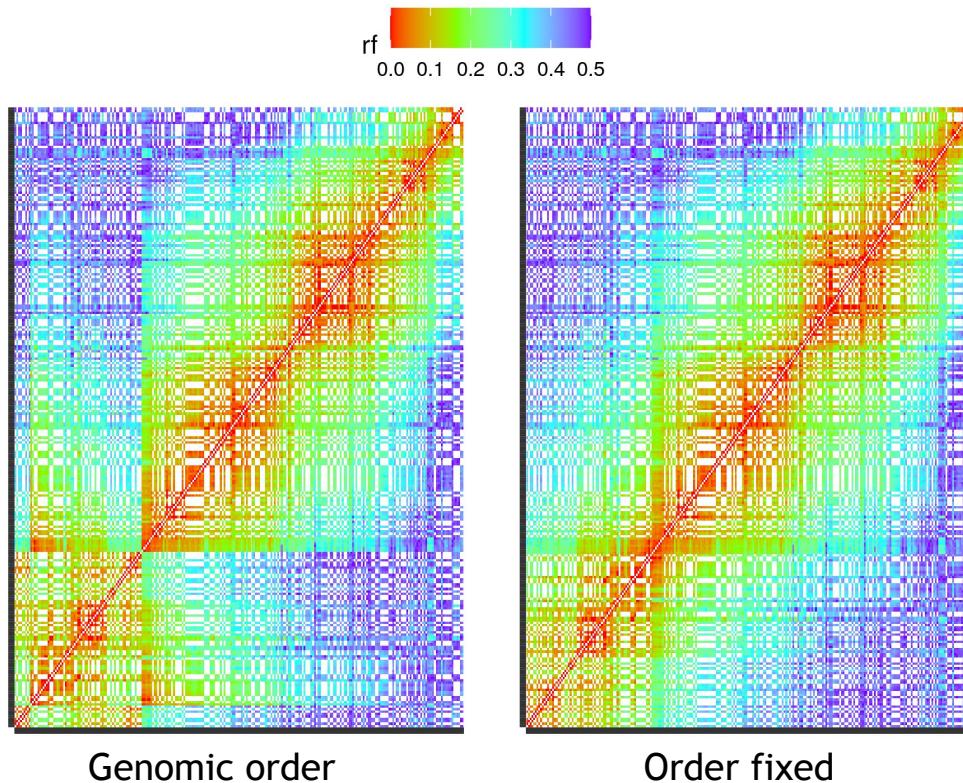


- Since 2018
- Diploid and polyploid species
- Bi-parental populations
- Outcrossing
- All dosages markers
- **Updates by Marcelo and Gabriel**

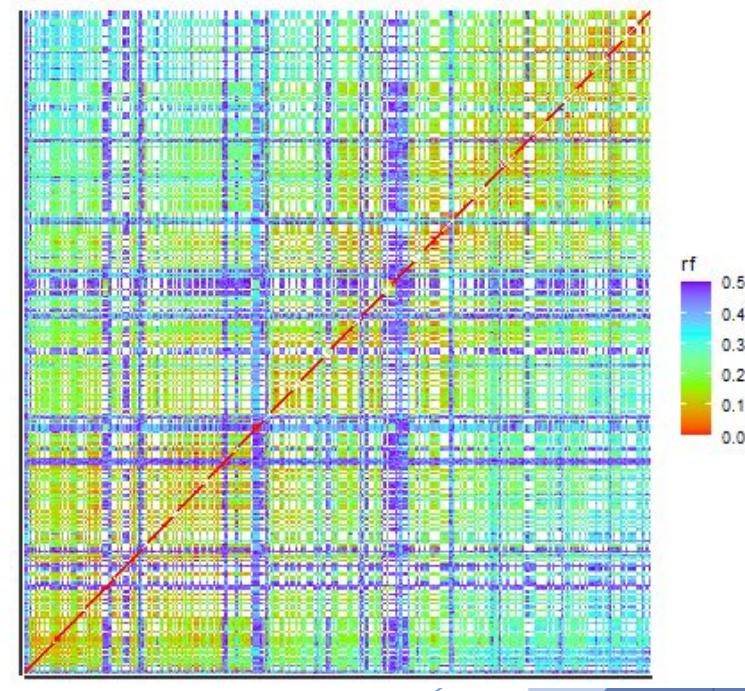
# Recombination fraction matrix as benchmarks

## Inversion

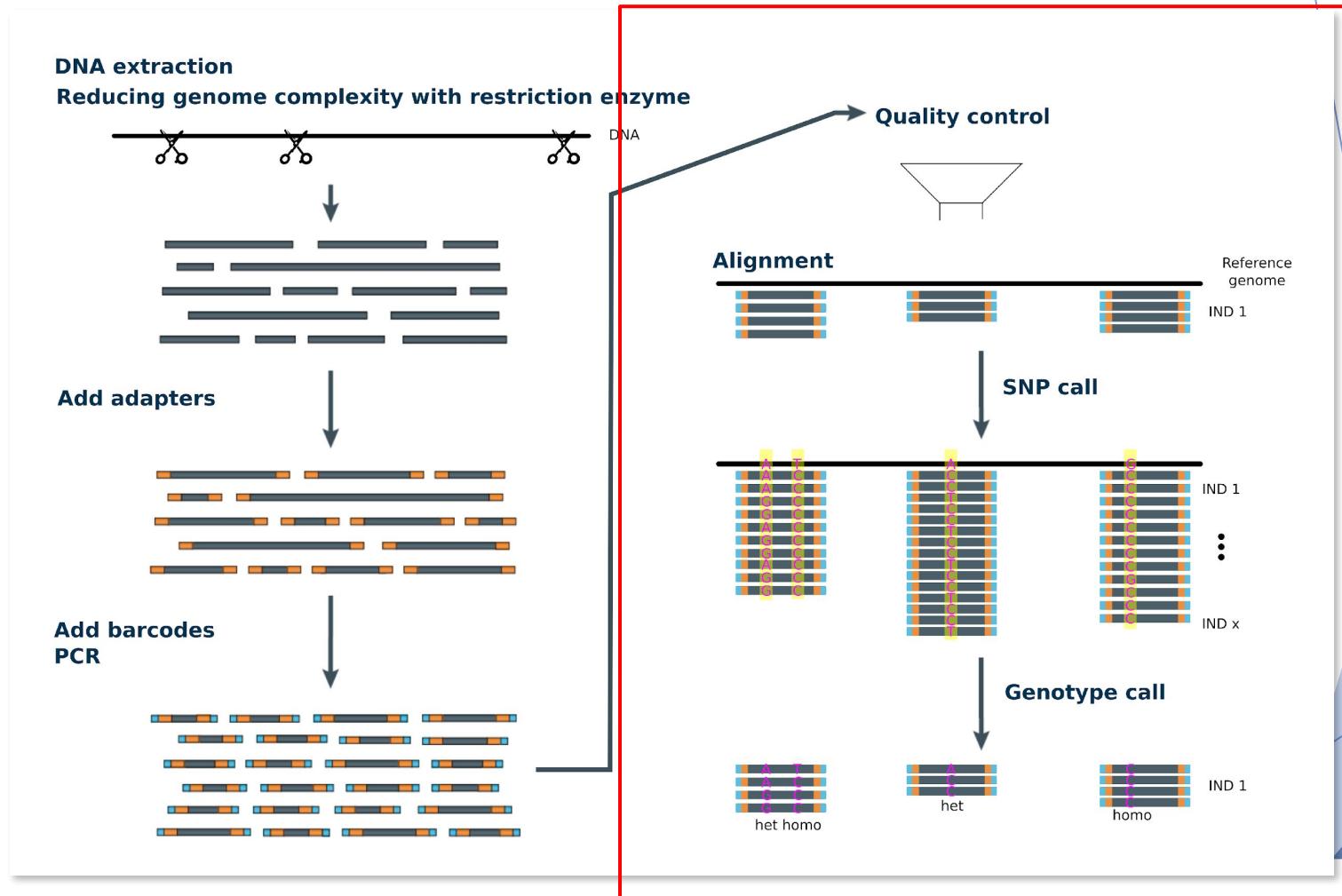
- Aspen chromosome 12



Most likely bad data



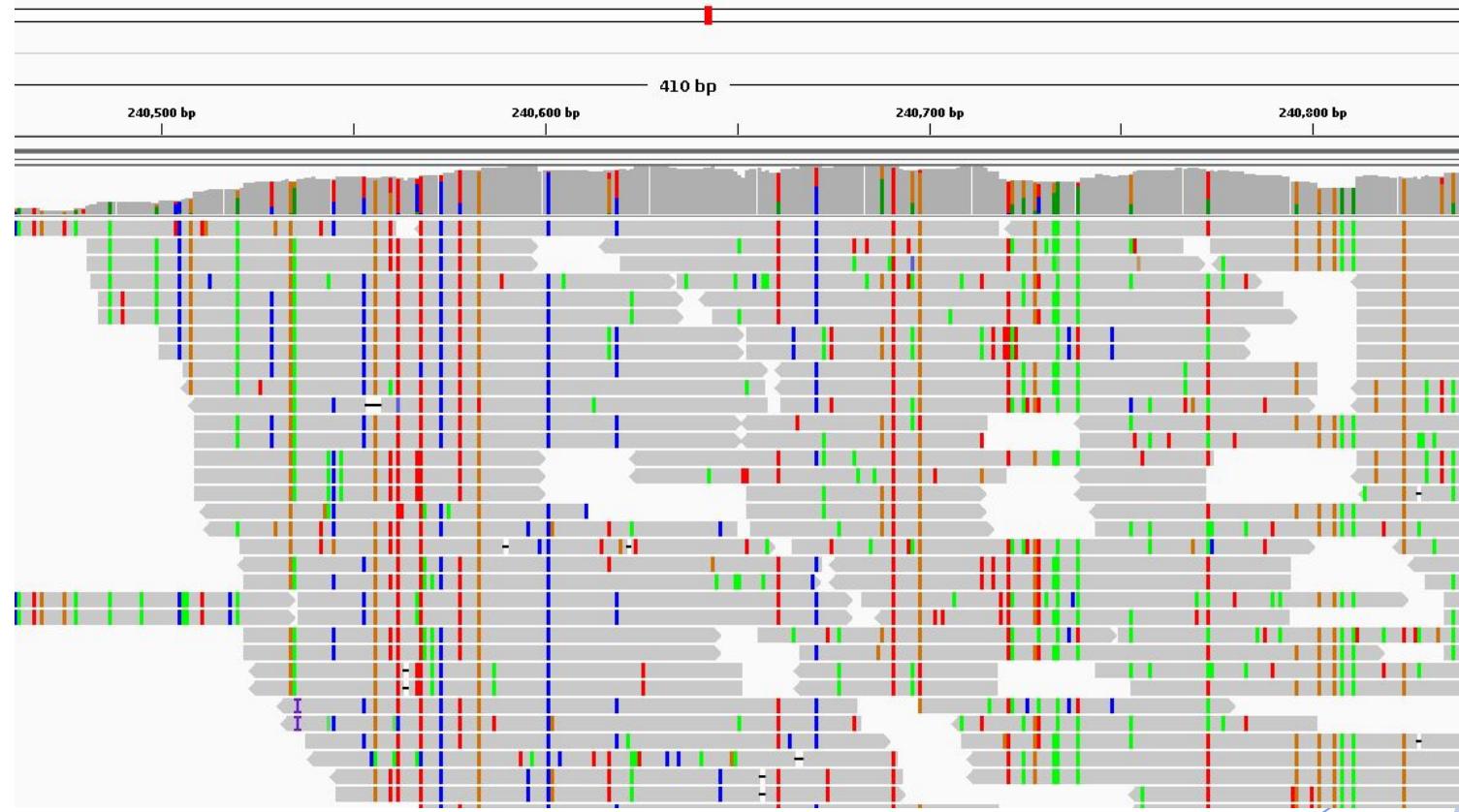
# GBS Overview



# SNP Calling

- Whole Genome Sequencing (WGS)

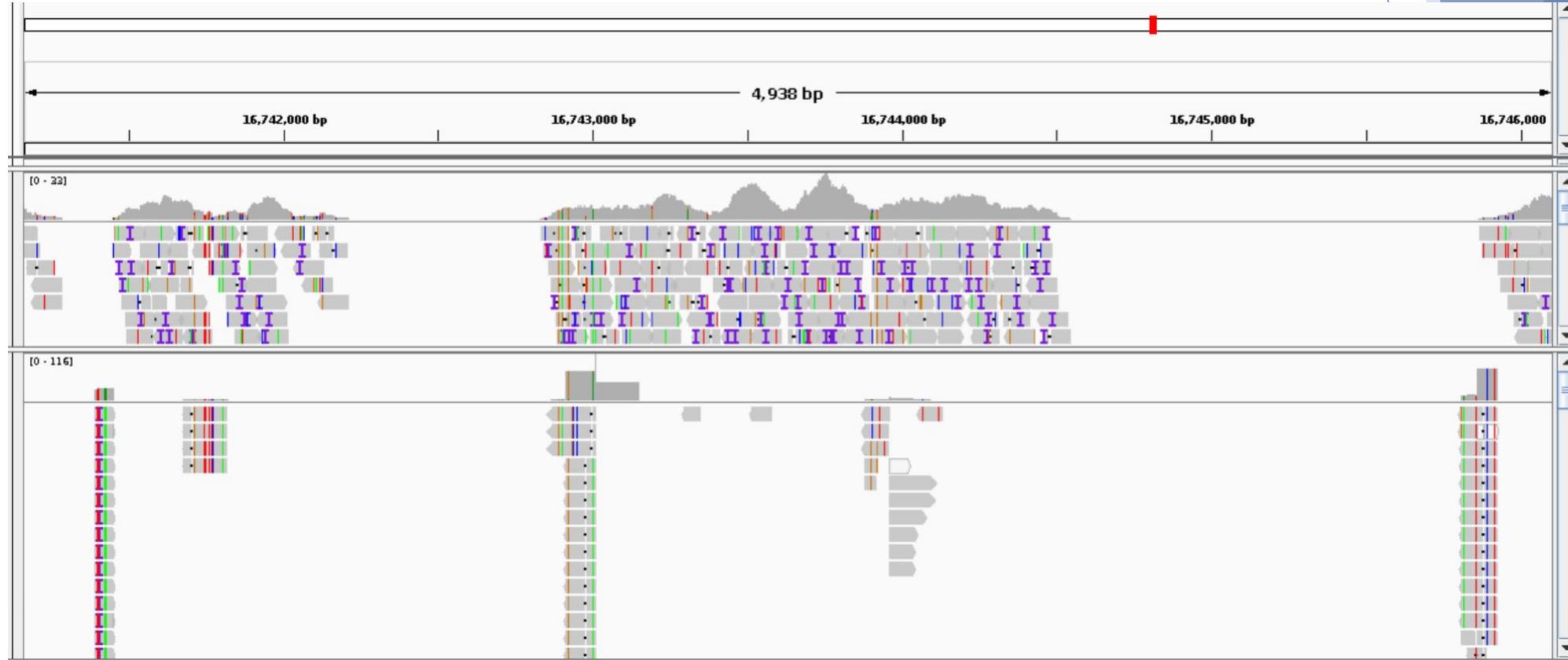
Image: IGV



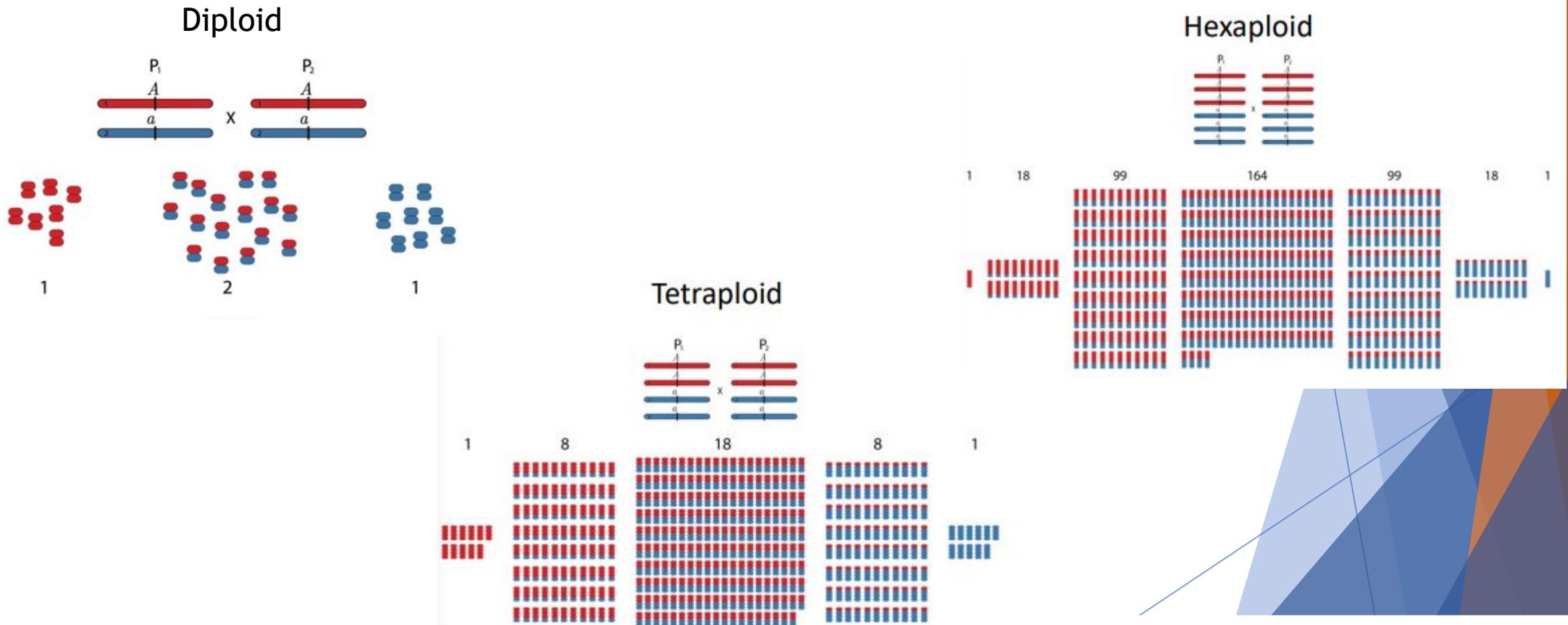
# SNP Calling

- Exome sequencing (top) and Genotyping-by-Sequencing (bottom)

Image: IGV

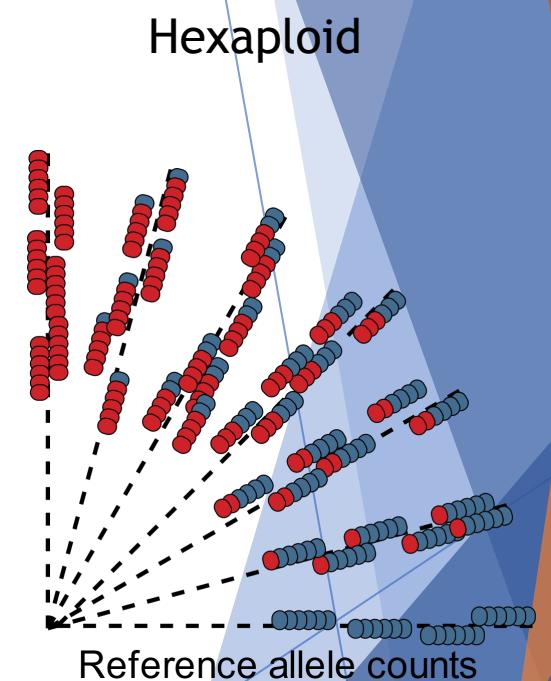
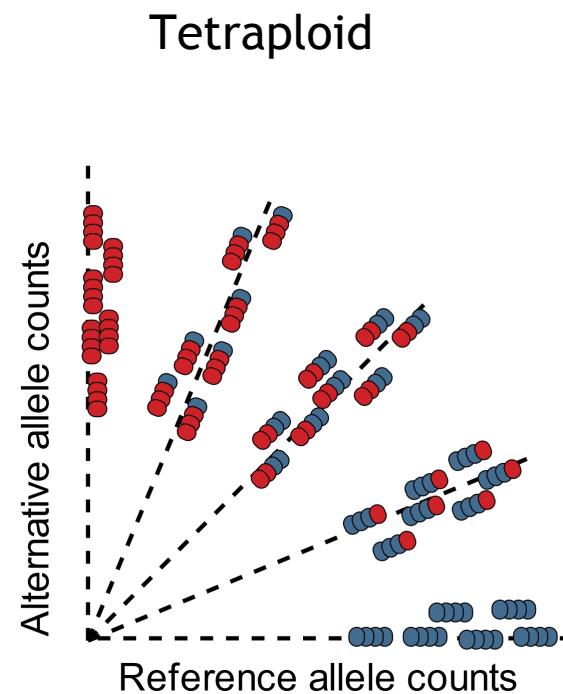
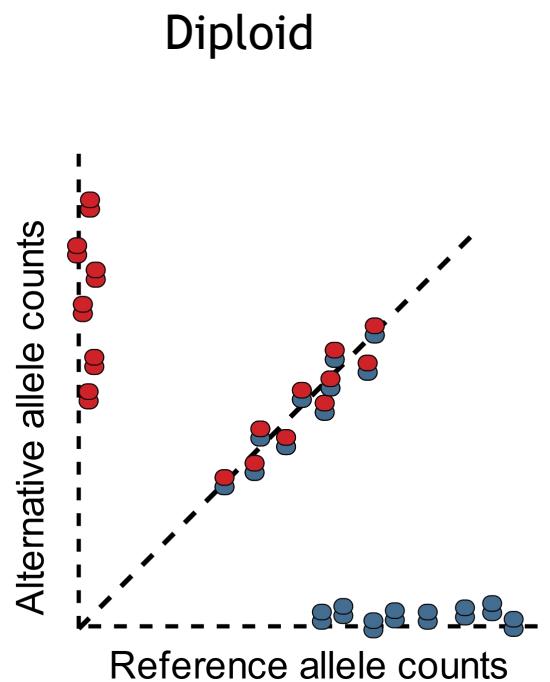


# Dosage calling



# Dosage Calling

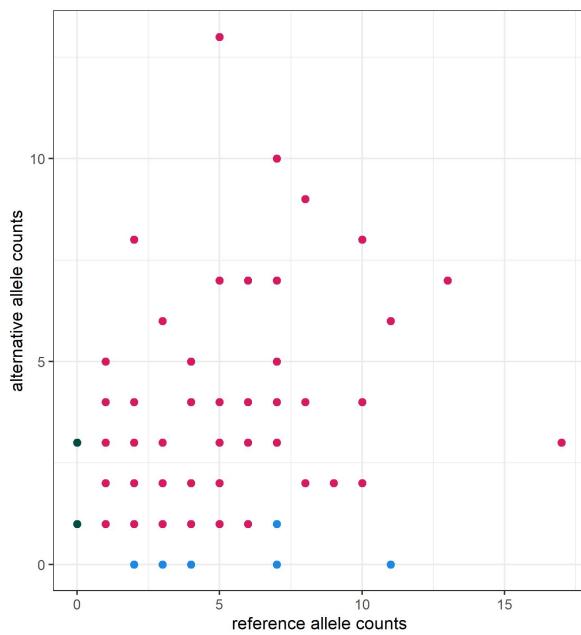
- The theory



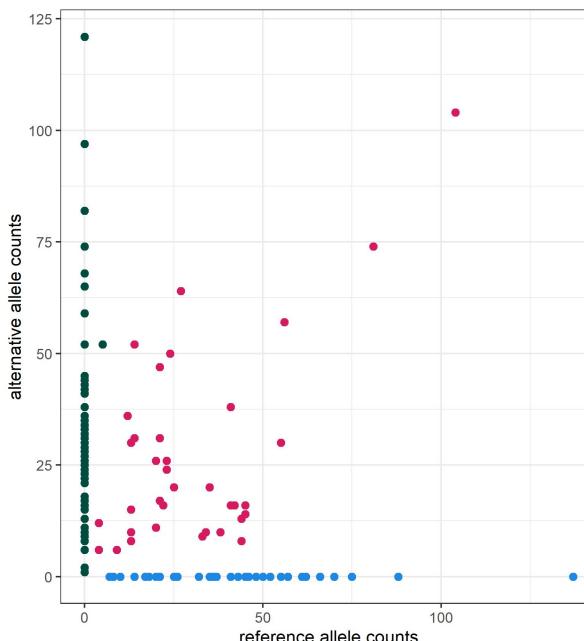
# Dosage Calling

- The reality

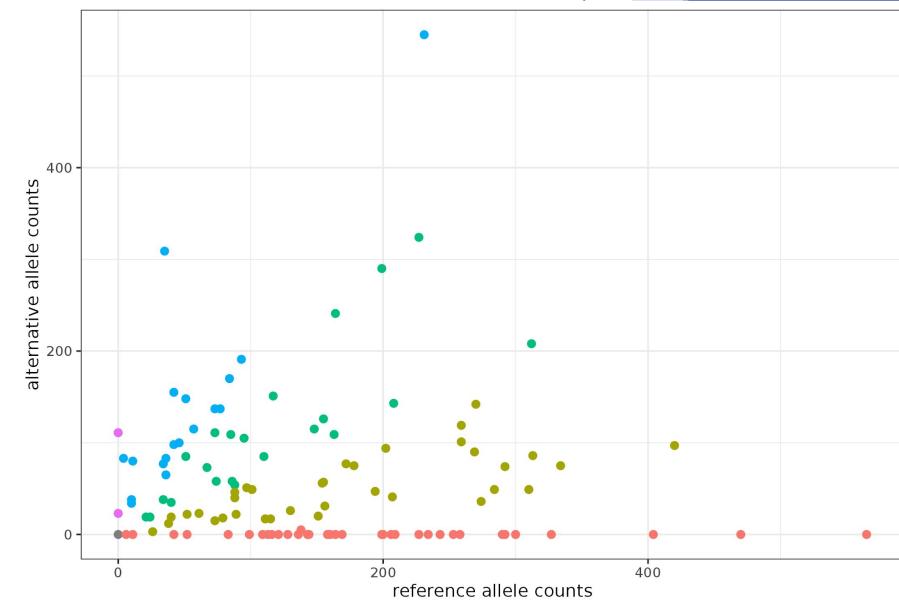
Diploid (mean depth 6)  
N = 200  
 $Aa \times Aa$



Diploid (mean depth 96)  
N = 138  
 $Aa \times Aa$

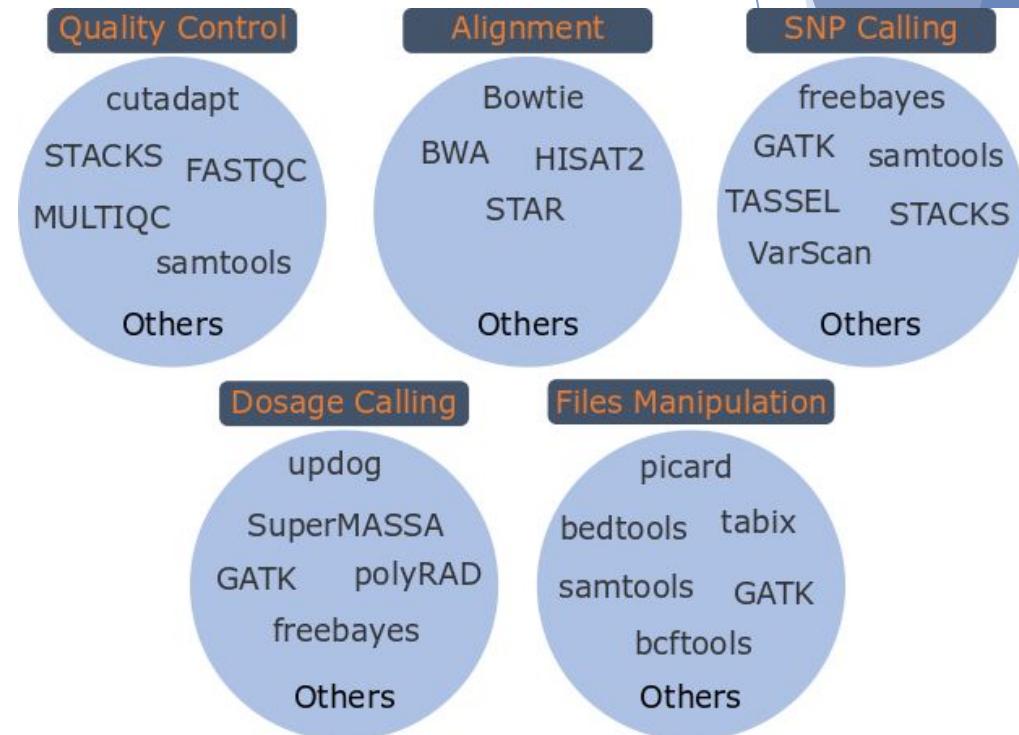


Tetraploid (mean depth 83)  
N = 114  
 $AAaa \times AAaa$



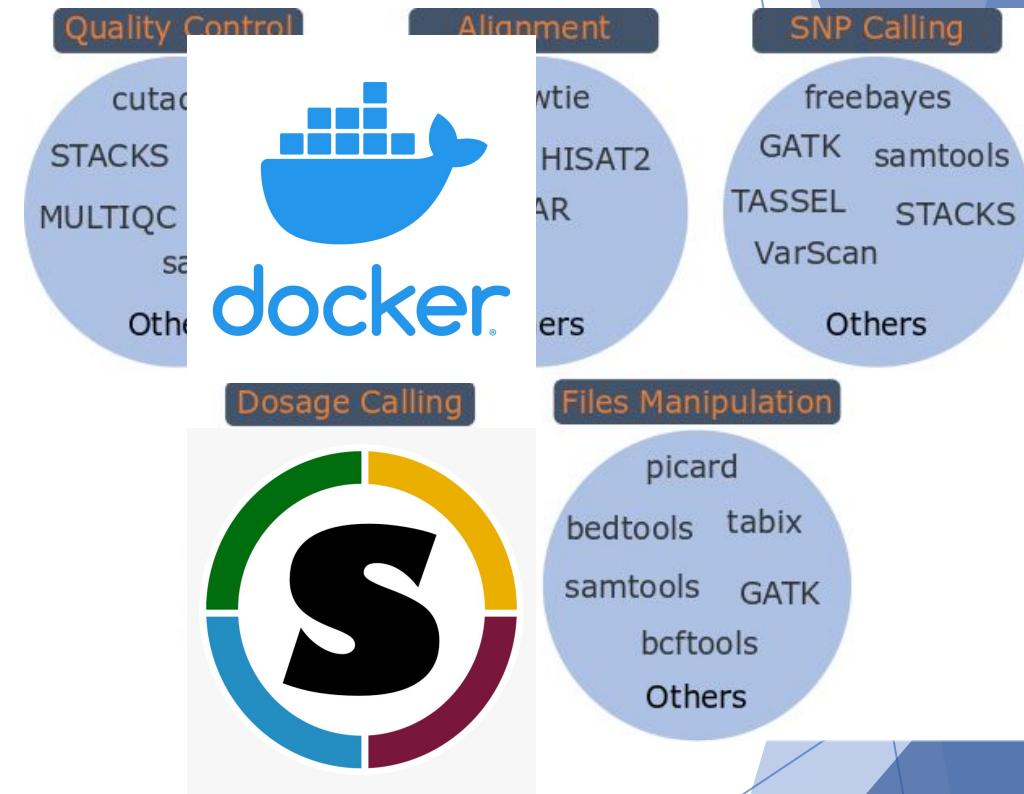
# Sequencing Data - Technical Difficulties

- ▶ Large files
- ▶ Many software
- ▶ Many programming languages
- ▶ Different Operational Systems
- ▶ Updates



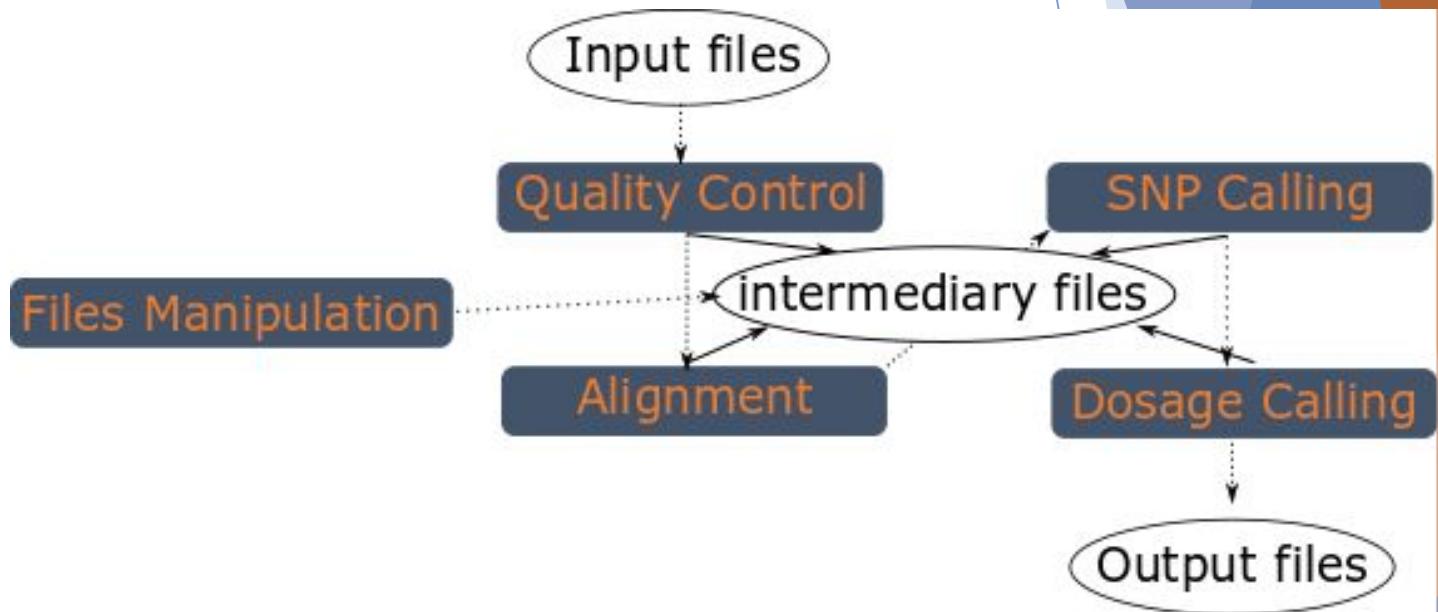
# Sequencing Data - Technical Difficulties

- ▶ Large files
  - ▶ High Performance Computing (HPC)
  - ▶ Management systems (SLURM, SGE)
  - ▶ Cloud (Google, Amazon)
- ▶ Many software
- ▶ Many programming languages
- ▶ Different Operational Systems
- ▶ Updates
  - ▶ Containers
    - ▶ Docker
    - ▶ Singularity (usually available in HPC)
    - ▶ [BioContainers](#)



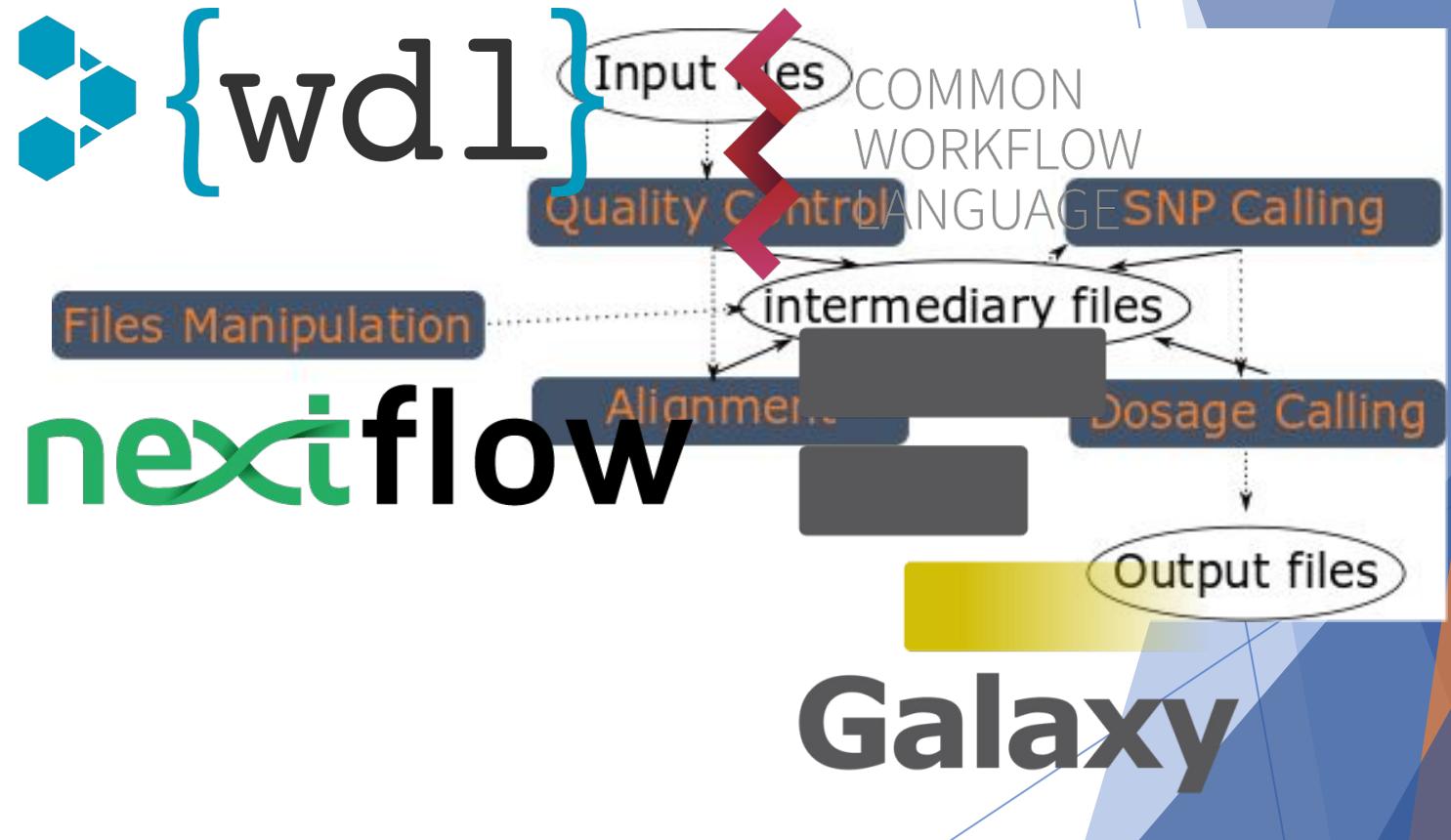
# Sequencing Data - Technical Difficulties

- ▶ Many steps
- ▶ Many file formats



# Sequencing Data - Technical Difficulties

- ▶ Many steps
- ▶ Many file formats
  - ▶ Workflows systems
    - ▶ Galaxy
    - ▶ Nextflow
    - ▶ Snakemake
    - ▶ CWL
    - ▶ WDL
  - ▶ Workflows repositories
    - ▶ [Dockerstore](#)
    - ▶ [WorkflowHub](#)
  - ▶ Run workflows on Cloud
    - ▶ Galaxy
    - ▶ DNAexus
    - ▶ Terra
    - ▶ AnVIL
    - ▶ SevenBridges

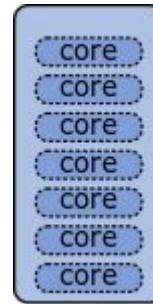


# Sequencing Data - Technical Difficulties

- ▶ Resources optimization
  - ▶ Time
  - ▶ Cores
  - ▶ Nodes
  - ▶ RAM memory

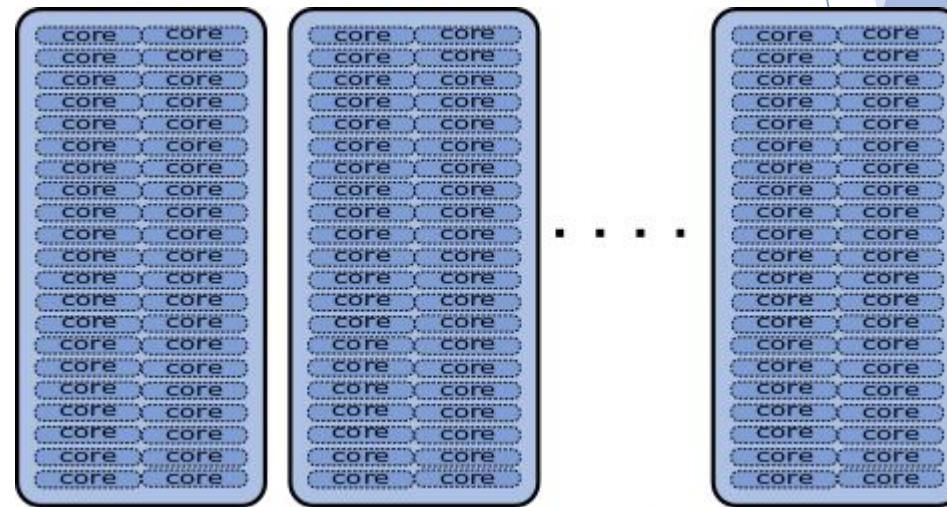
Personal Computer:

4GB RAM; 8 cores; 1 node

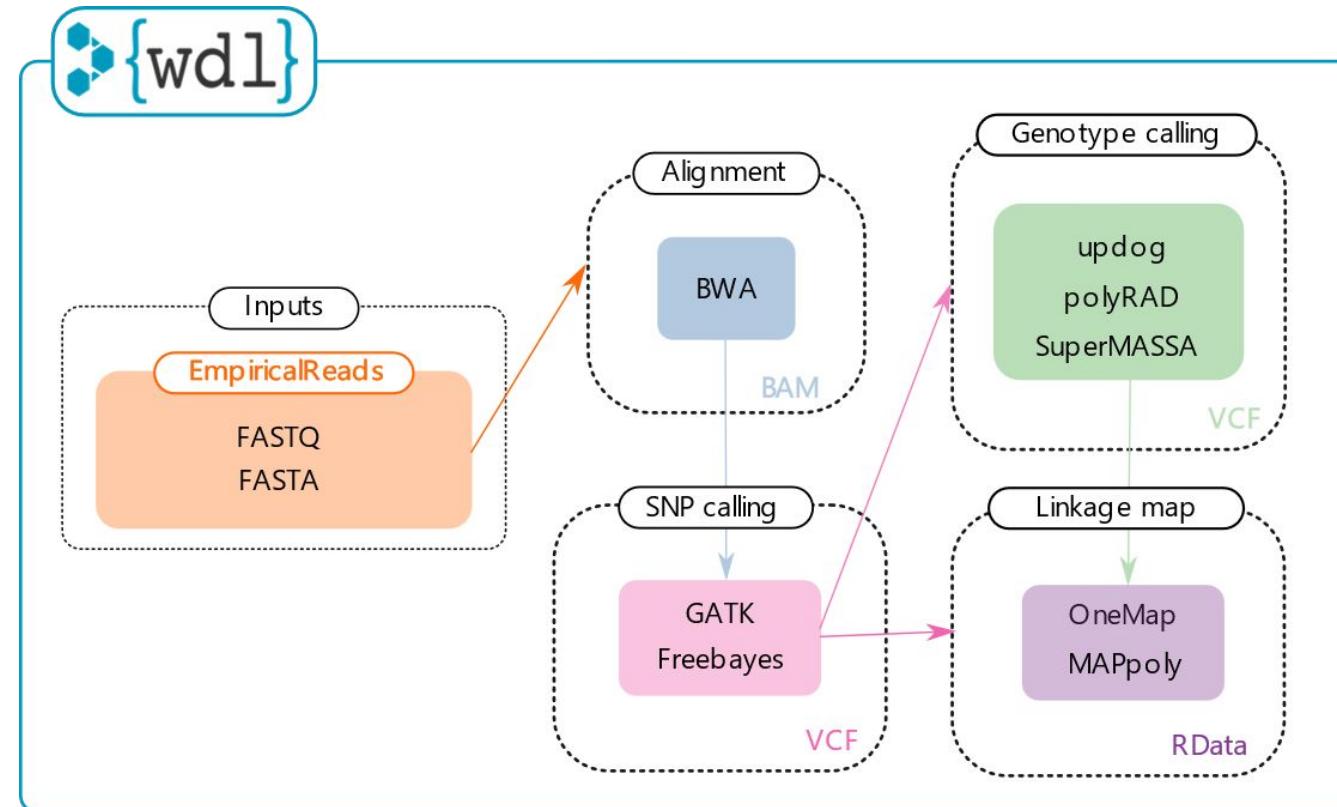


High Performance Computing (Texas A&M):

384GB; 48 cores per node; 900 nodes



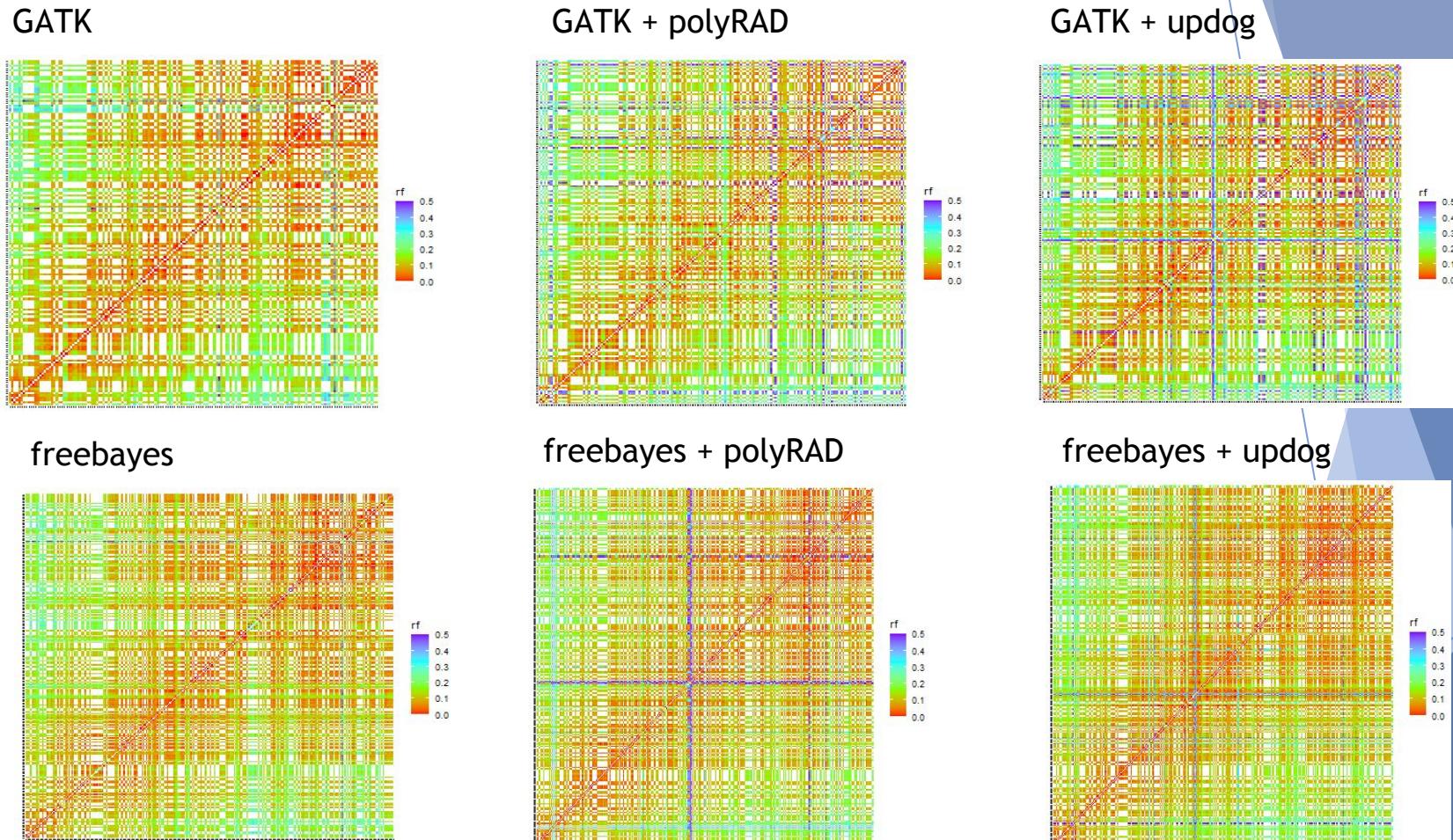
# How we solved it: Reads2Map



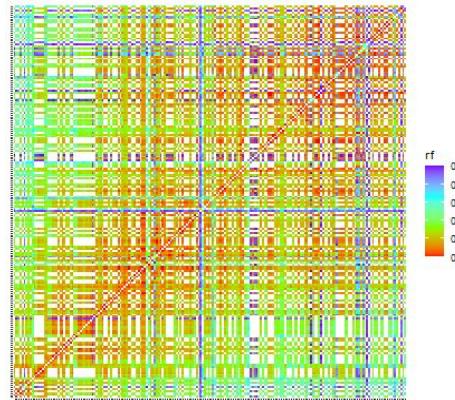
Available in [Github](#), [Dockerstore](#) and [WorkflowHub](#)

# Reads2Map results - Diploid roses

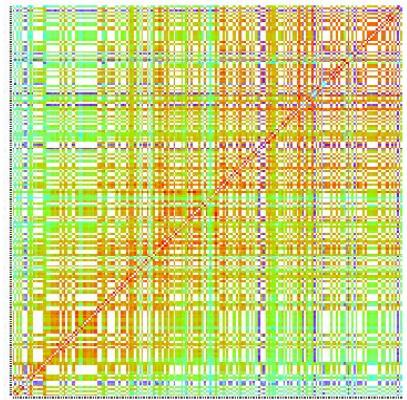
- 37% of chromosome 1
- ~ 38 cM
- Sequencing depth ~ 94X



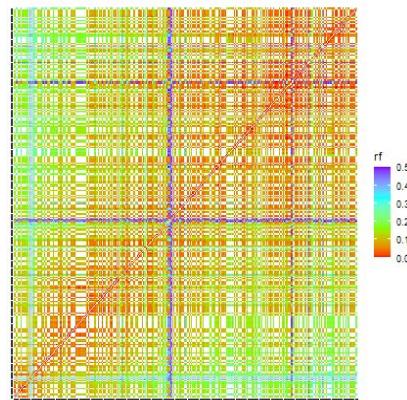
GATK + updog



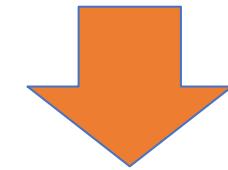
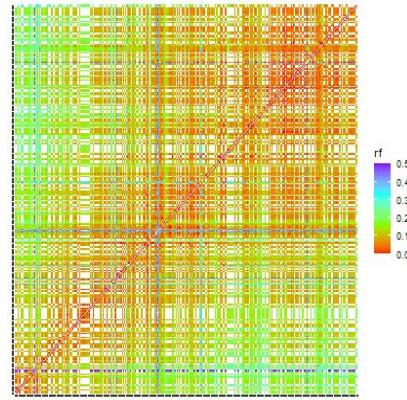
GATK + polyRAD



freebayes + polyRAD



freebayes + updog

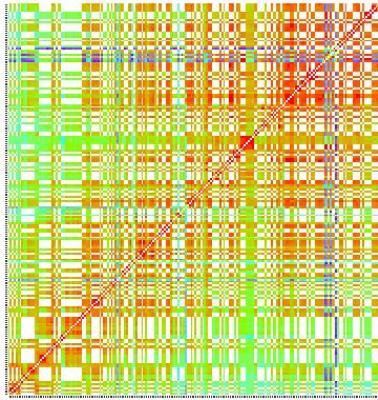


Simulation studies: 10,880 maps  
Empirical studies: 816 maps

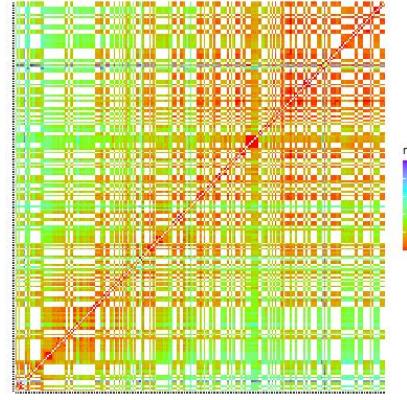
Filters:

- genotype probabilities
- non-informative markers
- replace AD by missing when GT is missing

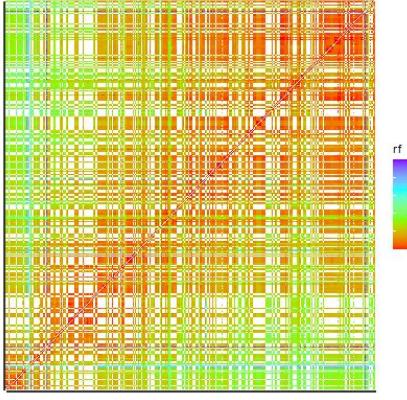
GATK + updog



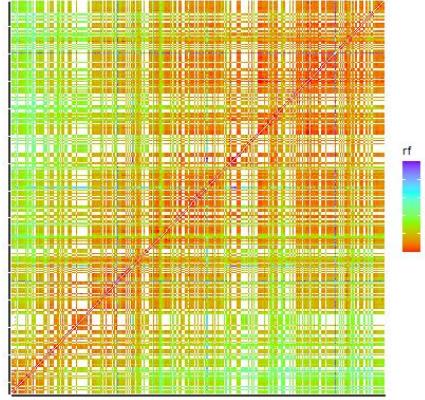
GATK + polyRAD



freebayes + polyRAD

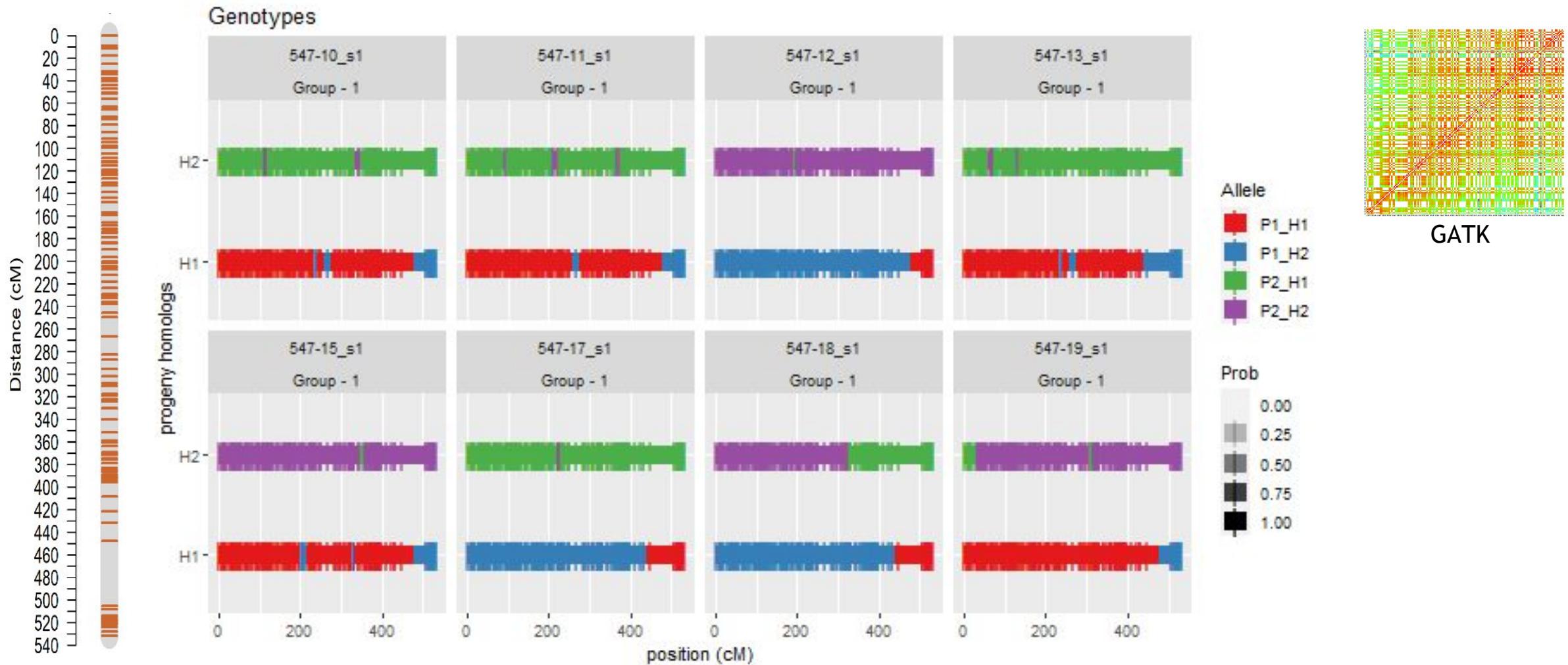


freebayes + updog



# Map size

37% of chromosome 1 ~38cM



# Map size

- Hidden Markov Model Emission Function
  - global error rate
  - genotype probabilities (PL)

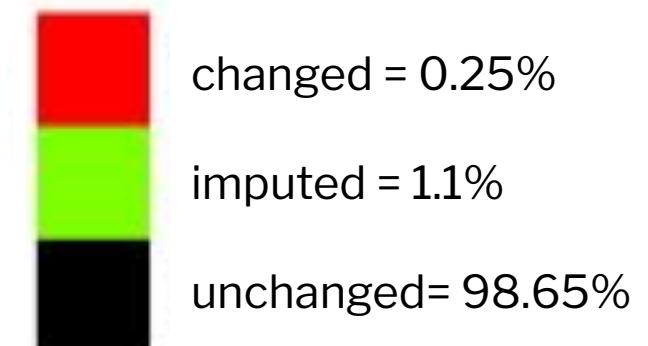
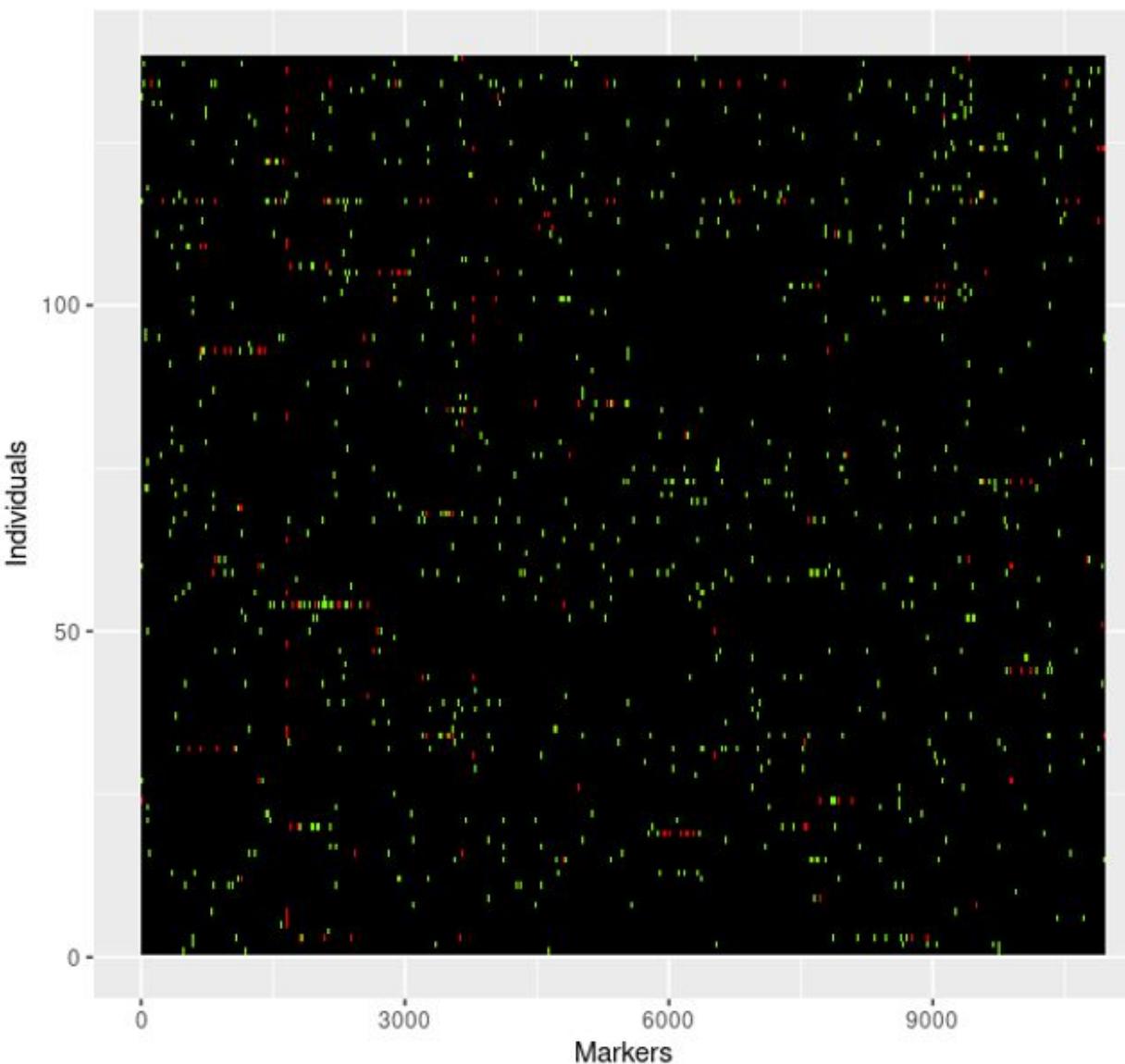
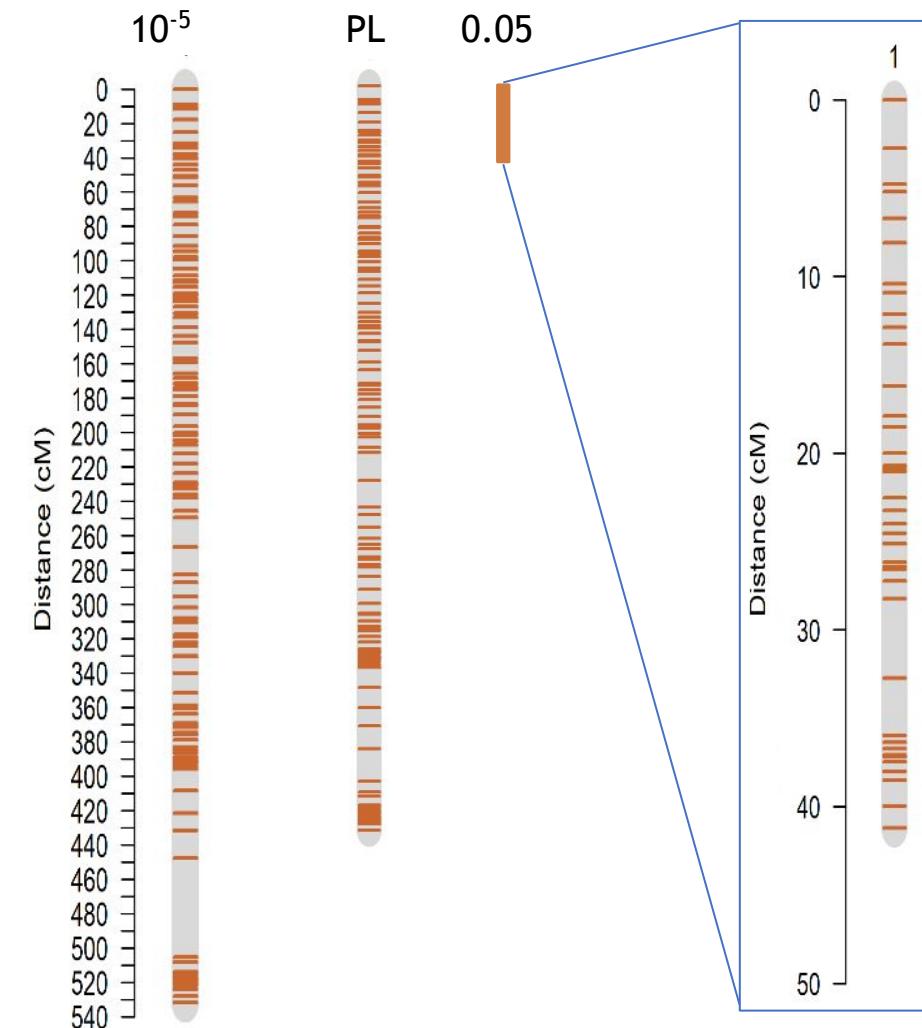
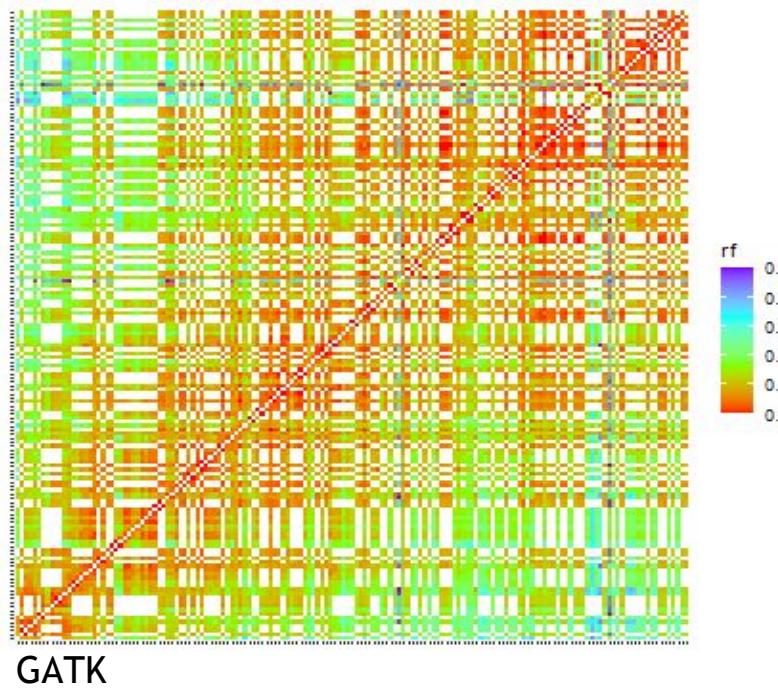


Figure by Jeekin Lau

# Map size

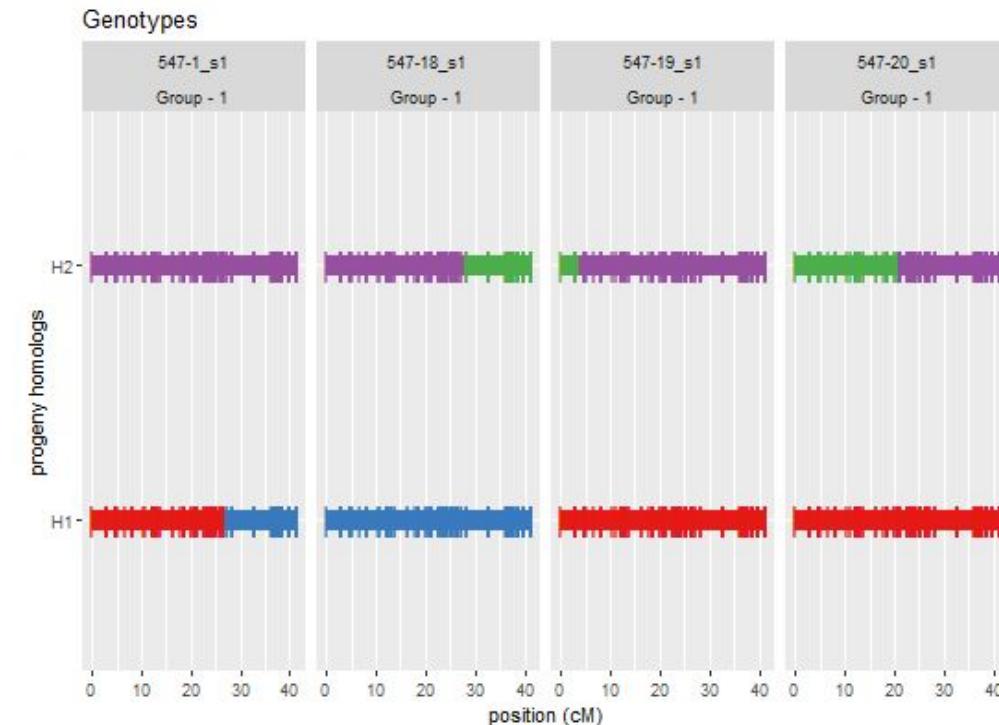
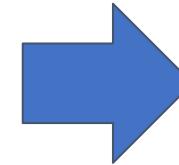
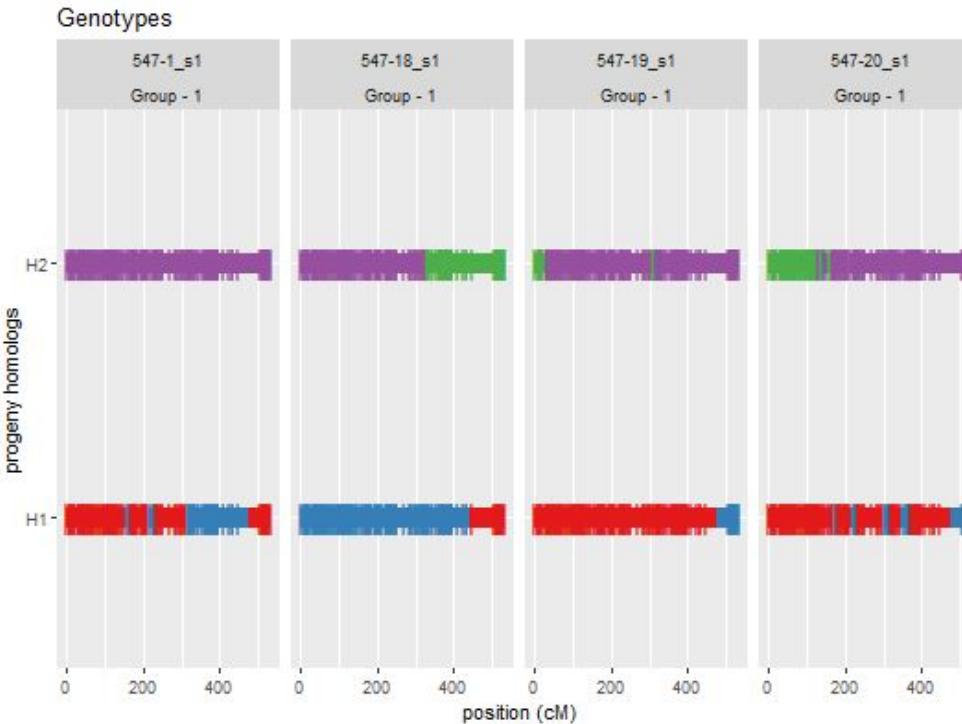
37% of chromosome 1 ~38cM

- Hidden Markov Model Emission Function
  - global error rate
  - genotype probabilities (PL)



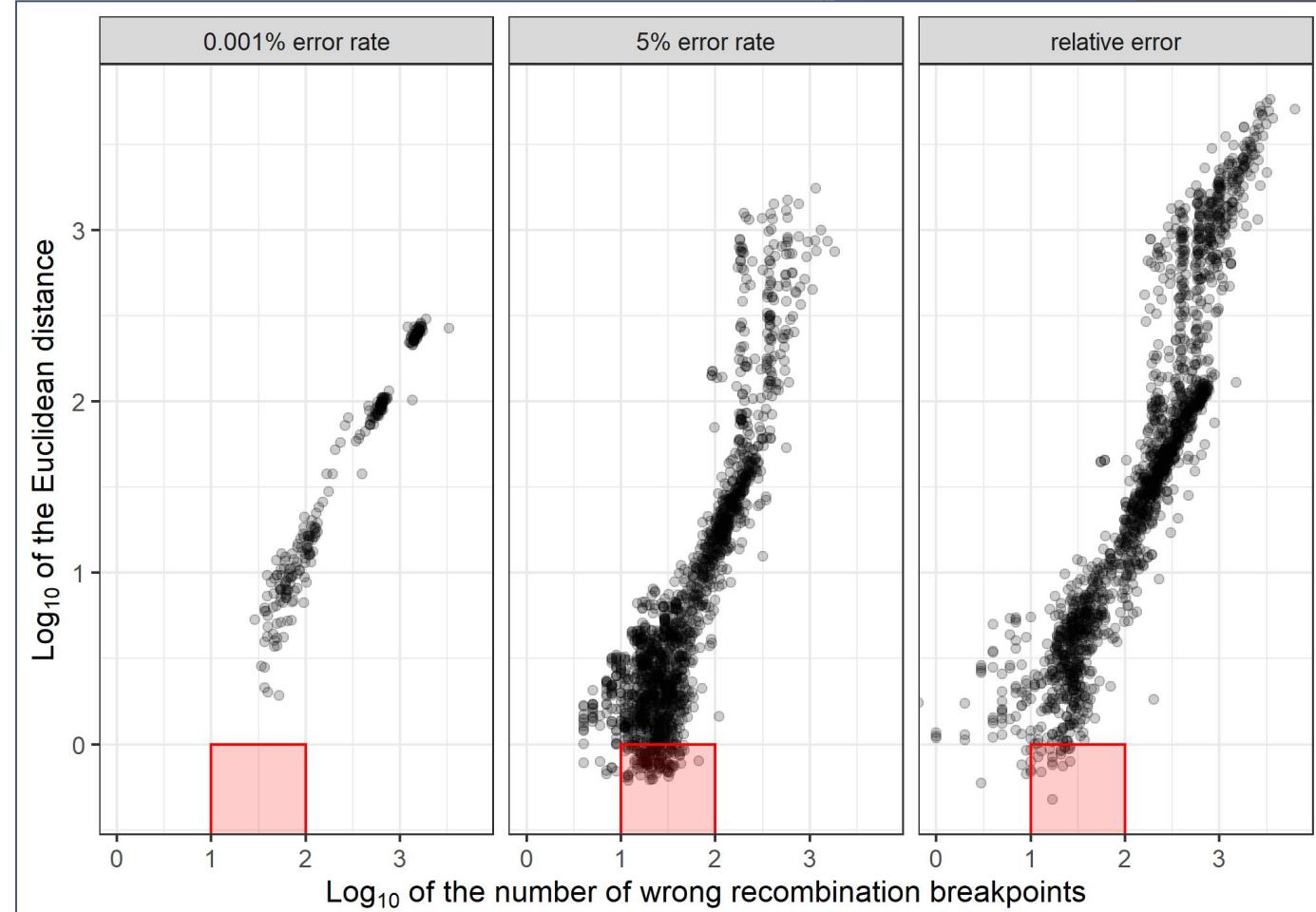
# Map size

37% of chromosome 1 ~38cM



# Simulation study

- Match recombinations breakpoints
  - Large maps - always bad
  - Small maps - not always good
- Other tested scenarios with:
  - Segregation distortion
  - Contaminants samples
  - Multiallelic markers



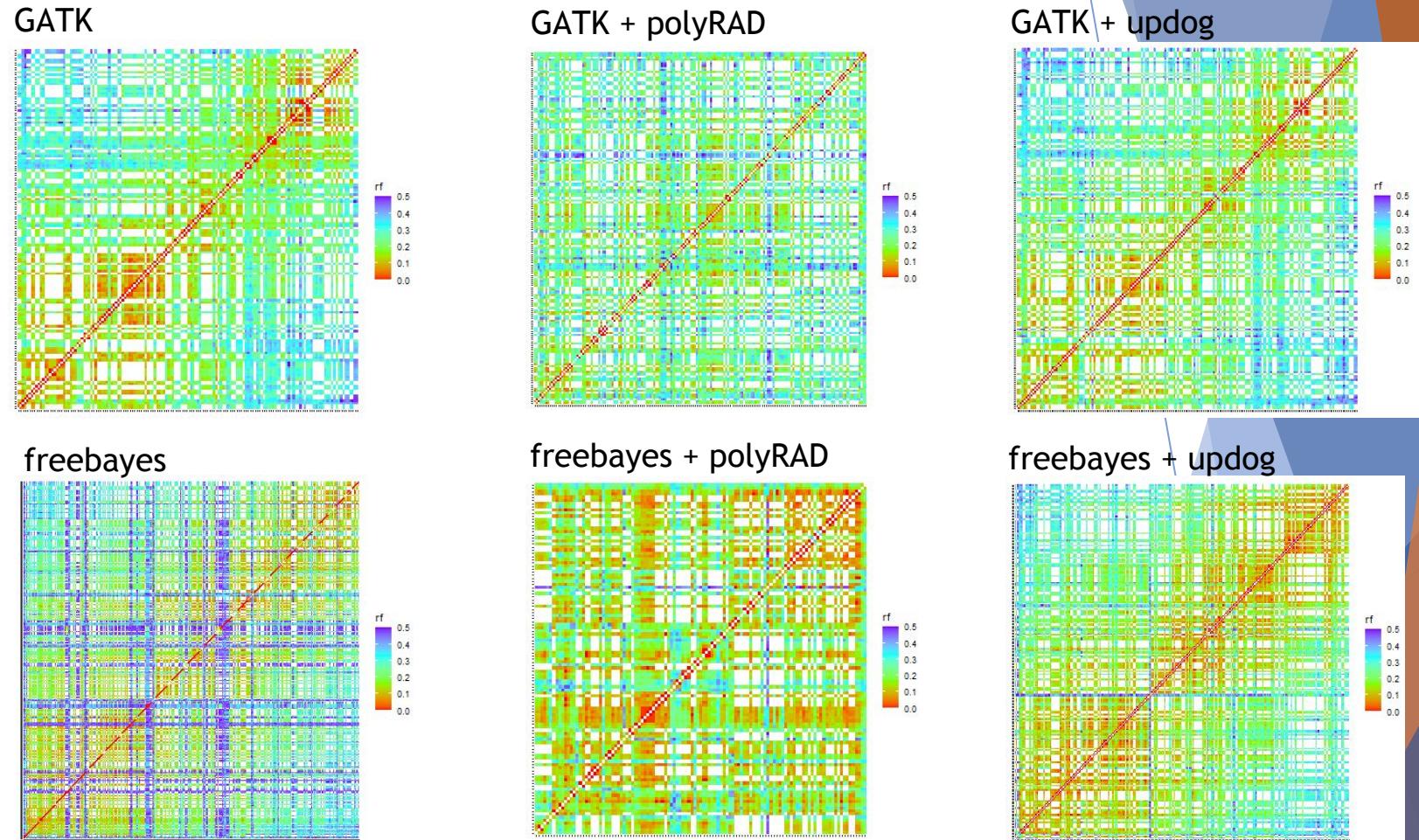
Red square: no inflated size (1 or less Euclidean distance) but have from 10 to 100 wrong recombination breakpoints

# Preprint

The image shows a screenshot of the bioRxiv preprint server interface. At the top left is the CSHL logo (Cold Spring Harbor Laboratory) with its name in blue. To the right is the large red and black bioRxiv logo with the tagline "THE PREPRINT SERVER FOR BIOLOGY" below it. A yellow rectangular box contains a reminder message: "bioRxiv posts many COVID19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive." Below this, on the left, is the "New Results" section. On the right, there is a "Follow this preprint" button with a bell icon. The main title of the preprint is "Developing best practices for genotyping-by-sequencing analysis using linkage maps as benchmarks". Below the title is a list of authors, each preceded by a green circular icon with a white ID symbol: Cristiane Hayumi Taniguti, Lucas Mitsuo Taniguti, Rodrigo Rampazo Amadeu, Jeekin Lau, Gabriel de Siqueira Gesteira, Thiago de Paula Oliveira, Getulio Caixeta Ferreira, Guilherme da Silva Pereira, David Byrne, Marcelo Mollinari, Oscar Riera-Lizarazu, and Antonio Augusto Franco Garcia. At the bottom left is the DOI: <https://doi.org/10.1101/2022.11.24.517847>.

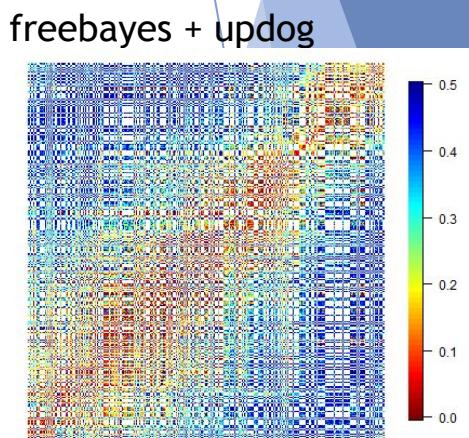
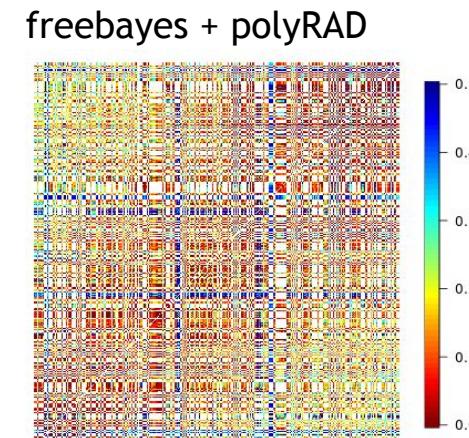
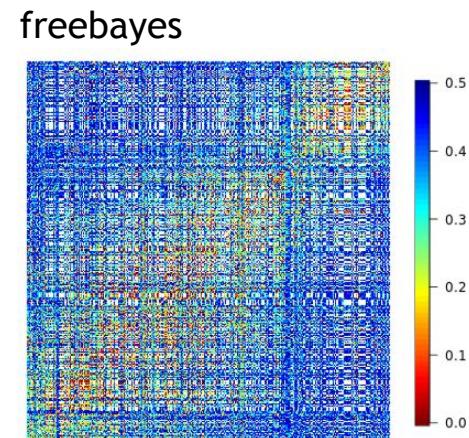
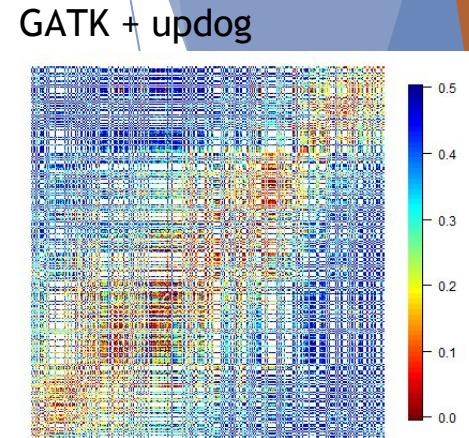
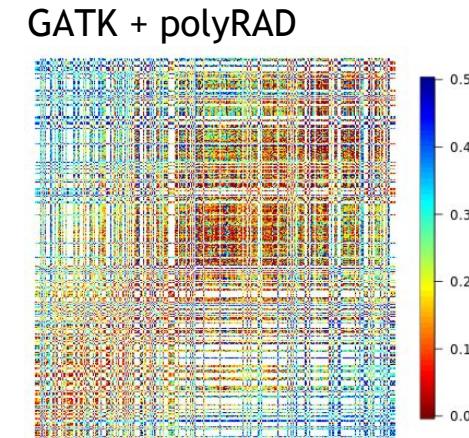
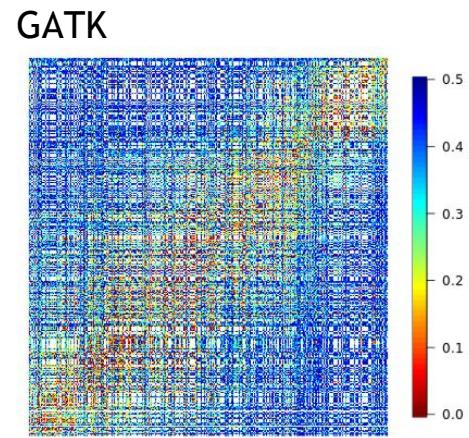
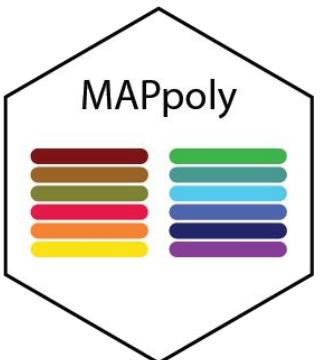
# Diploid Aspen

- 37% of chromosome 10
- Sequencing depth ~ 6X



# Tetraploid rose

- Chromosome 2
- Sequencing depth ~ 50X



# Hands-on Workshop!!

<https://github.com/Cristianetaniguti/Reads2Map>

Tutorial:

[bit.ly/Reads2Map2023](https://bit.ly/Reads2Map2023)

# Reads2Map

Cristianetaniguti / Reads2Map Public

<> Code ⚡ Issues ⚡ Pull requests ⚡ Actions Projects 1 Wiki Security

main ▾

6 branches

13 tags

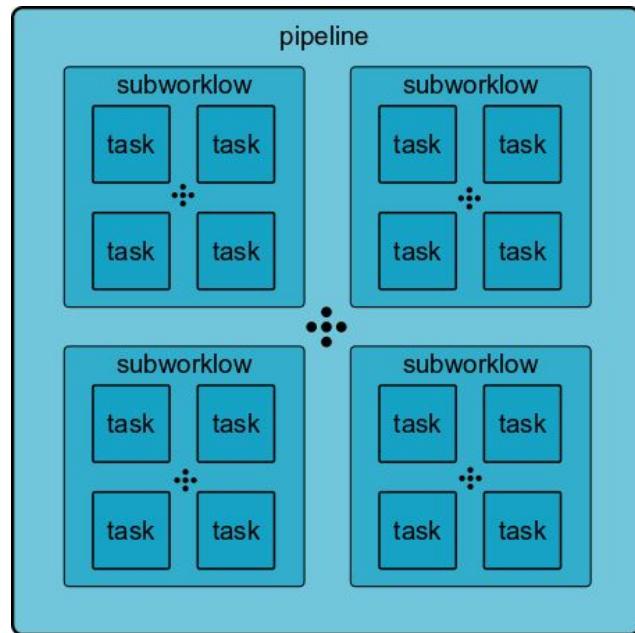
Go to file



Cristianetaniguti Merge pull request #58 from Cristianetaniguti/config\_d...  
...

✓ acca101 on I

📁 .circleci	build: correct env var for prod env
📁 .configurations	fix typo
📁 .dockerfiles	add more tests
📁 .scripts	build: configure to also release to main
③ ➔📁 pipelines	wf specific name
📁 structs	refactor: remove not used struct
② ➔📁 subworkflows	freebayes split ok
① ➔📁 tasks	joint wf
📁 tests	refactor: move test files to tests module
📄 .dockstore.yml	fix path



# Reads2Map

- Cloud environments
  - [terra.bio](#)
- HPC
  - [Cromwell](#)
  - [MiniWDL](#)
  - [dxWDL](#)

inputs.json

```
{  
    "SNPCalling.max_cores": 2,  
    "SNPCalling.ploidy": 4,  
    "SNPCalling.rm_dupli": false,  
    "SNPCalling.replaceAD": false,  
    "SNPCalling.run_gatk": true,  
    "SNPCalling.run_freebayes": true,  
    "SNPCalling.hardfilters": true,  
    "SNPCalling.n_chrom": 1,  
    "SNPCalling.chunk_size": 2,  
    "SNPCalling.samples_info": "tests/data/polyploid/fastq/samples_info.txt",  
    "SNPCalling.gatk_mchap": false,  
    "SNPCalling.references": {  
        "ref_fasta": "tests/data/polyploid/RchinensisV1.0/Chr04_sub.fasta",  
        "ref_dict": "tests/data/polyploid/RchinensisV1.0/Chr04_sub.dict",  
        "ref_ann": "tests/data/polyploid/RchinensisV1.0/Chr04_sub.fasta.ann",  
        "ref_sa": "tests/data/polyploid/RchinensisV1.0/Chr04_sub.fasta.sa",  
        "ref_amb": "tests/data/polyploid/RchinensisV1.0/Chr04_sub.fasta.amb",  
        "ref_pac": "tests/data/polyploid/RchinensisV1.0/Chr04_sub.fasta.pac",  
        "ref_bwt": "tests/data/polyploid/RchinensisV1.0/Chr04_sub.fasta.bwt",  
        "ref_fasta_index": "tests/data/polyploid/RchinensisV1.0/Chr04_sub.fasta.fai"  
    }  
}
```

```
$ java -jar /path/to/cromwell.jar run -i EmpiricalSNPCalling/inputs.json EmpiricalSNPCalling.wdl
```

main ▾

Reads2Map / tasks /

# task



Cristianetaniguti joint wf

..

BWA.wdl

JointReports.wdl

bcftools.wdl

chunk\_lists.wdl

cutadapt.wdl

freebayes.wdl

gatk.wdl

gusmap.wdl

mchap.wdl

pedigree\_simulator.wdl

gusmap.wdl

mchap.wdl

pedigree\_simulator.wdl

pedigree\_simulator\_utils.wdl

pirs.wdl

radinitio.wdl

simuscop.wdl

stacks.wdl

utils.wdl

utilsR.wdl

vcf2diploid.wdl

# task

## example: freebayes.wdl

```
task RunFreebayes {  
    > input { ... }  
  
    > Int disk_size = ceil(size(reference, "GiB") + size(bam, "GiB") + 50)  
    > Int memory_size = ceil(size(bam, "MiB") * 25 * max_cores + 10000)  
  
    > command <<<...  
    >>>  
  
    > runtime { ... }  
  
    > meta { ... }  
  
    > output { ... }  
}
```

```
input {  
    File reference  
    File reference_idx  
    File bam  
    File bai  
    Int max_cores  
    Int ploidy  
}  
  
Int disk_size = ceil(size(reference, "GiB") + size(bam, "GiB") + 50)  
Int memory_size = ceil(size(bam, "MiB") * 25 * max_cores + 10000)
```

### inputs.json

```
{  
    "reference": "tests/data/PtrichocarpaV3.0/Chr10.2M.fa",  
    "reference_idx": "tests/data/PtrichocarpaV3.0/Chr10.2M.fa.fai",  
    "bam": "tests/data/Ptremula_PRJNA395596_subset/merged.bam",  
    "bai": "tests/data/Ptremula_PRJNA395596_subset/merged.bam.bai",  
    "max_cores": 2,  
    "ploidy": 4  
}
```

# task

example: freebayes.wdl

```
task RunFreebayes {  
    > input { ... }  
  
    > Int disk_size = ceil(size(reference, "GiB") + size(bam, "G:  
    > Int memory_size = ceil(size(bam, "MiB") * 25 * max_cores +  
    >  
    > command <<<...  
    >>>  
    >  
    > runtime { ... }  
    >  
    > meta { ... }  
    >  
    > output { ... }  
    >  
}  
  
    command <<<  
  
        ln -s ~{bam} .  
        ln -s ~{bai} .  
  
        freebayes-parallel <(fasta_generate_regions.py ~{reference_idx} 100000) ~{max_cores} \  
        --genotype-qualities --ploidy ~{ploidy} -f ~{reference} *bam > "freebayes.vcf"  
    >>>
```

# task

example: freebayes.wdl

```
task RunFreebayes {  
    > input { ... }  
  
    > Int disk_size = ceil(size(reference, "GiB") + size(bam, "GiB") + 50)  
    > Int memory_size = ceil(size(bam, "MiB") * 25 * max_cores + 10000)  
  
    > command <<<...  
    >>>  
  
    > runtime { ... }  
  
    > meta { ... }  
  
    > output { ... }  
}
```

```
runtime {  
    docker: "cristaniguti/freebayes:0.0.1"  
    cpu: max_cores  
    # Cloud  
    memory: "~{memory_size} MiB"  
    disks: "local-disk " + disk_size + " HDD"  
    # Slurm  
    job_name: "RunFreebayes"  
    mem: "~{memory_size}M"  
    time: "48:00:00"  
}
```

# task

example: freebayes.wdl

```
task RunFreebayes {
>   input { ... }

>   Int disk_size = ceil(size(reference, "GiB") + size(bam, "GiB") +  50)
>   Int memory_size = ceil(size(bam, "MiB") * 25 * max_cores + 10000)

>   command <<<...
>>>
>   runtime { ... }
>   meta {
>     author: "Cristiane Taniguti"
>     email: "ctaniguti@tamu.edu"
>     description: "Split genomic regions and runs [freebayes](https://github.com/freebayes/freebayes) parallelized."
>   }
>   output { ... }
>
}
```

# task

example: freebayes.wdl

```
task RunFreebayes {  
    > input { ... }  
  
    Int disk_size = ceil(size(reference, "GiB") + size(bam, "GiB") + 50)  
    Int memory_size = ceil(size(bam, "MiB") * 25 * max_cores + 10000)  
  
    > command <<<...  
    >>>  
  
    > runtime { ... }  
  
    > meta { ... }  
  
    > output { ... }  
}
```

inputs.json

```
{  
    "reference": "tests/data/PtrichocarpaV3.0/Chr10.2M.fa",  
    "reference_idx": "tests/data/PtrichocarpaV3.0/Chr10.2M.fa.fai",  
    "bam": "tests/data/Ptremula_PRJNA395596_subset/merged.bam",  
    "bai": "tests/data/Ptremula_PRJNA395596_subset/merged.bam.bai",  
    "max_cores": 2,  
    "ploidy": 4  
}
```

```
output {  
    File vcf = "freebayes.vcf"  
}
```

```
miniwdl run --task RunFreebayes -i tests/tasks/freebayes/inputs.json tasks/freebayes.wdl
```



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# subworkflow example

```
workflow FreebayesGenotyping {
>   input { ... }

>   call chunk_lists.CreateChunksBamByChr { ... }

    scatter (chunk in zip(CreateChunksBamByChr.bams_chunks, CreateChunksBamByChr.bais_chunks)) {

>     call freebayes.RunFreebayes { ...
        }
    }

>   call utils.mergeVCFs { ... }

>   call norm_filt.Normalization { ...
    }

    Map[String, Array[File]] map_bams = {"bam": CreateChunksBamByChr.bams_chunks, "bai": CreateChunksBamByChr.bais_chunks}

>   if(replaceAD){ ...
    }

    Array[File] freebayes_vcfs = select_all([Normalization.vcf_norm, ReplaceAD.bam_vcf])
    Array[String] freebayes_software = select_all([Normalization.software, ReplaceAD.software])
    Array[String] freebayes_counts_source = select_all([Normalization.source, ReplaceAD.source])

>   output { ... }
    }
}
```

```
$ java -jar /path/to/cromwell.jar run -i freebayes_genotyping/inputs.json
freebayes_genotyping.wdl
```



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# pipeline example

```
workflow SNPCalling {  
    > input { ...  
    }  
  
    > call fam.CreateAlignmentFromFamilies { ...  
    }  
  
    if(run_gatk){  
        > call gatk.GatkGenotyping { ...  
        }  
    }  
  
    if(run_freebayes){  
        > call freebayes.FreebayesGenotyping { ...  
        }  
    }  
  
    Array[Array[File]] vcfs_sele = select_all([GatkGenotyping.vcfs, FreebayesGenotyping.vcfs])  
    Array[Array[String]] software_sele = select_all([GatkGenotyping.vcfs_software, FreebayesGenotyping.vcfs_software])  
    Array[Array[String]] source_sele = select_all([GatkGenotyping.vcfs_counts_source, FreebayesGenotyping.vcfs_counts_source])  
  
    > output { ...  
    }  
}
```

```
$ java -jar /path/to/cromwell.jar run -i EmpiricalSNPCalling/inputs.json EmpiricalSNPCalling.wdl
```



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# Thank you



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Rodrigo Amadeu  
Getulio Caixeta

Marcelo Mollinari  
Gabriel Gesteira



Lucas Taniguti

Guilherme Pereira

Thiago Oliveira

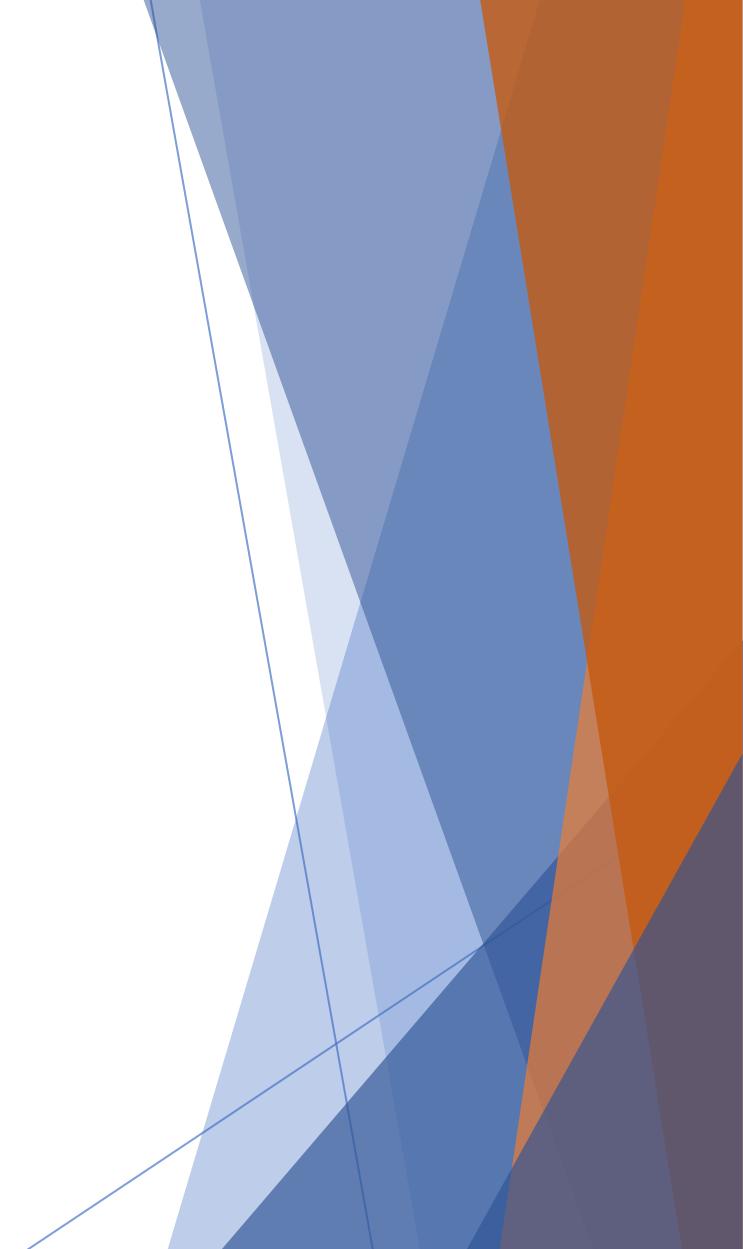


# Thank you!!

- ▶ Susan Thomson
- ▶ Cecilia Deng
- ▶ Ben Warren



**Plant & Food<sup>TM</sup>**  
**Research**  
Rangahau Ahumāra Kai



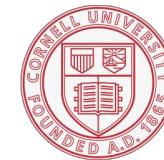
# Project Members



Plant & Food  
Research  
Rangahau Ahumāra Kai



NC STATE  
UNIVERSITY



Cornell University



# Other Collaborators



# Links for Tutorials

- ▶ [polyRAD tutorial](#)
- ▶ [updog tutorial](#)
- ▶ [fitPoly tutorial](#)
- ▶ [\(TASSEL\) Variant and Genotype Calling in Highly Duplicated Genomes \(Lindsay Clark\)](#)
- ▶ [Using Reads2Map workflows for SNP and dosage calling in polyploid sequencing data](#)

More cool tools related to Workflows systems:

- ▶ [cromwell-cli \(by my brother\)](#)
- ▶ [cromwell server](#)
- ▶ [womtools](#)

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