

Reads2Map

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

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NC STATE
UNIVERSITY



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

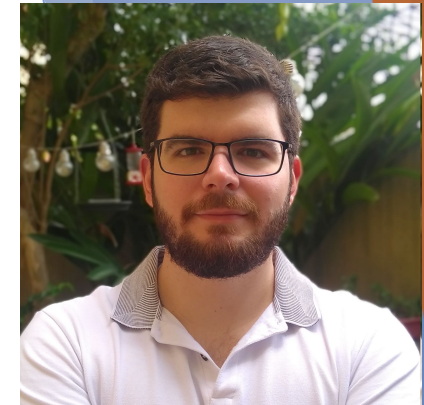
Tips and
Tricks:



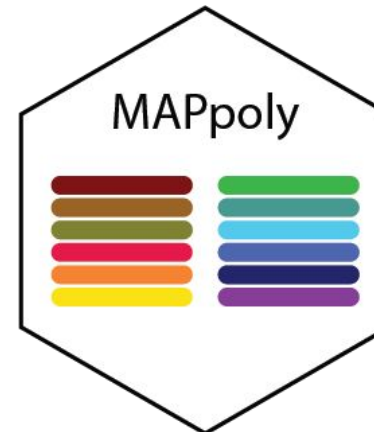
RGC11 - Poster



Augusto Garcia



Gabriel Gesteira



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

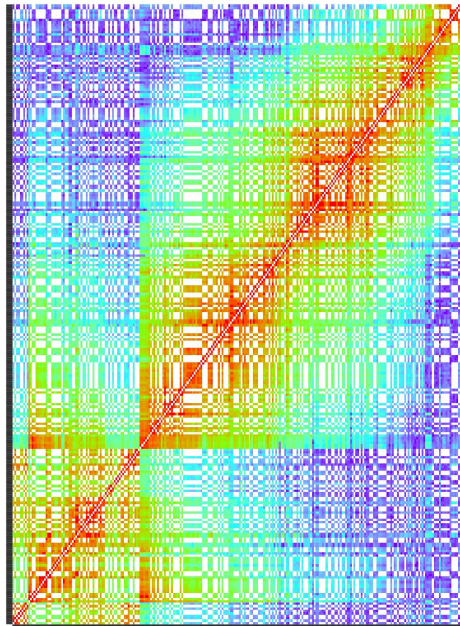
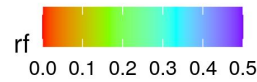


Marcelo Mollinari

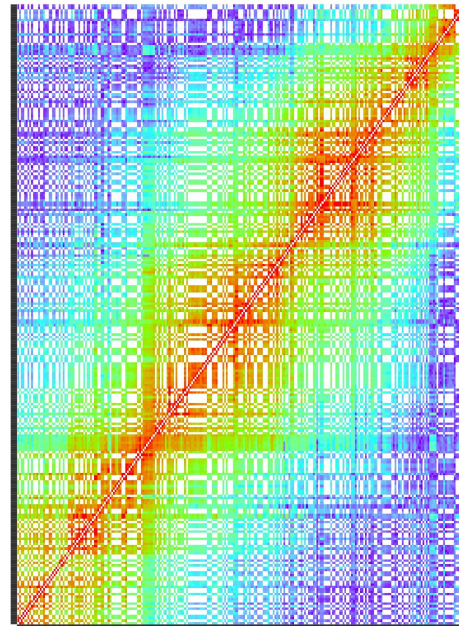
Recombination fraction matrix as benchmarks

Inversion

- Aspen chromosome 12

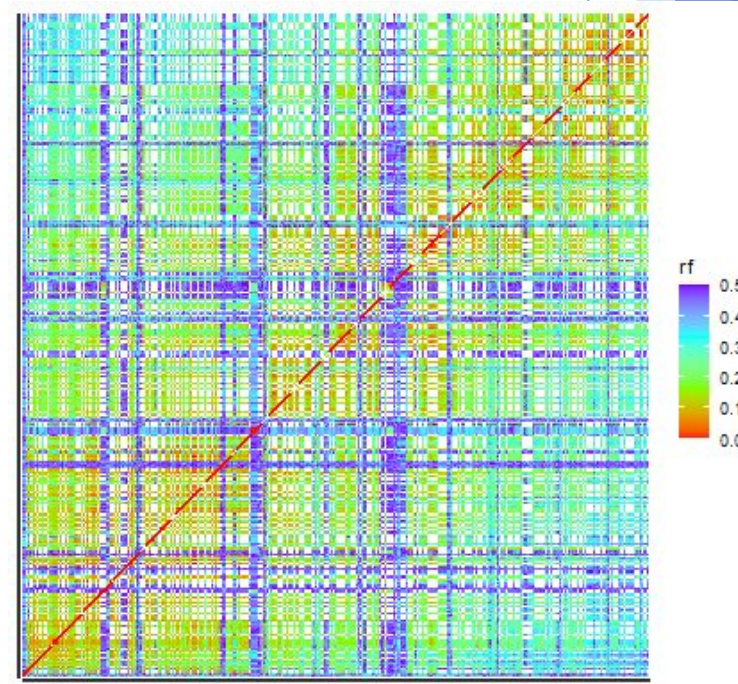


Genomic order

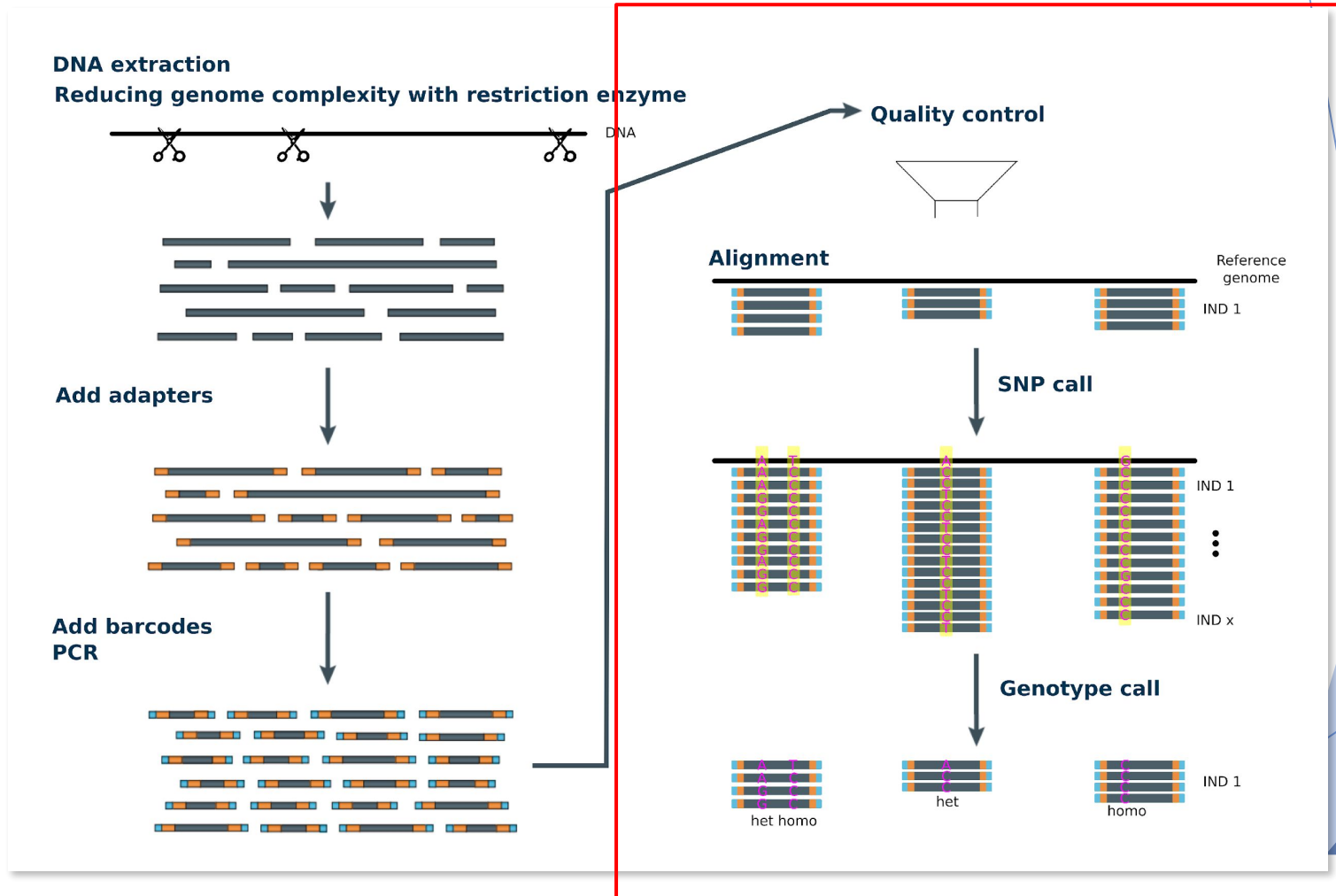


Order fixed

Most likely bad data



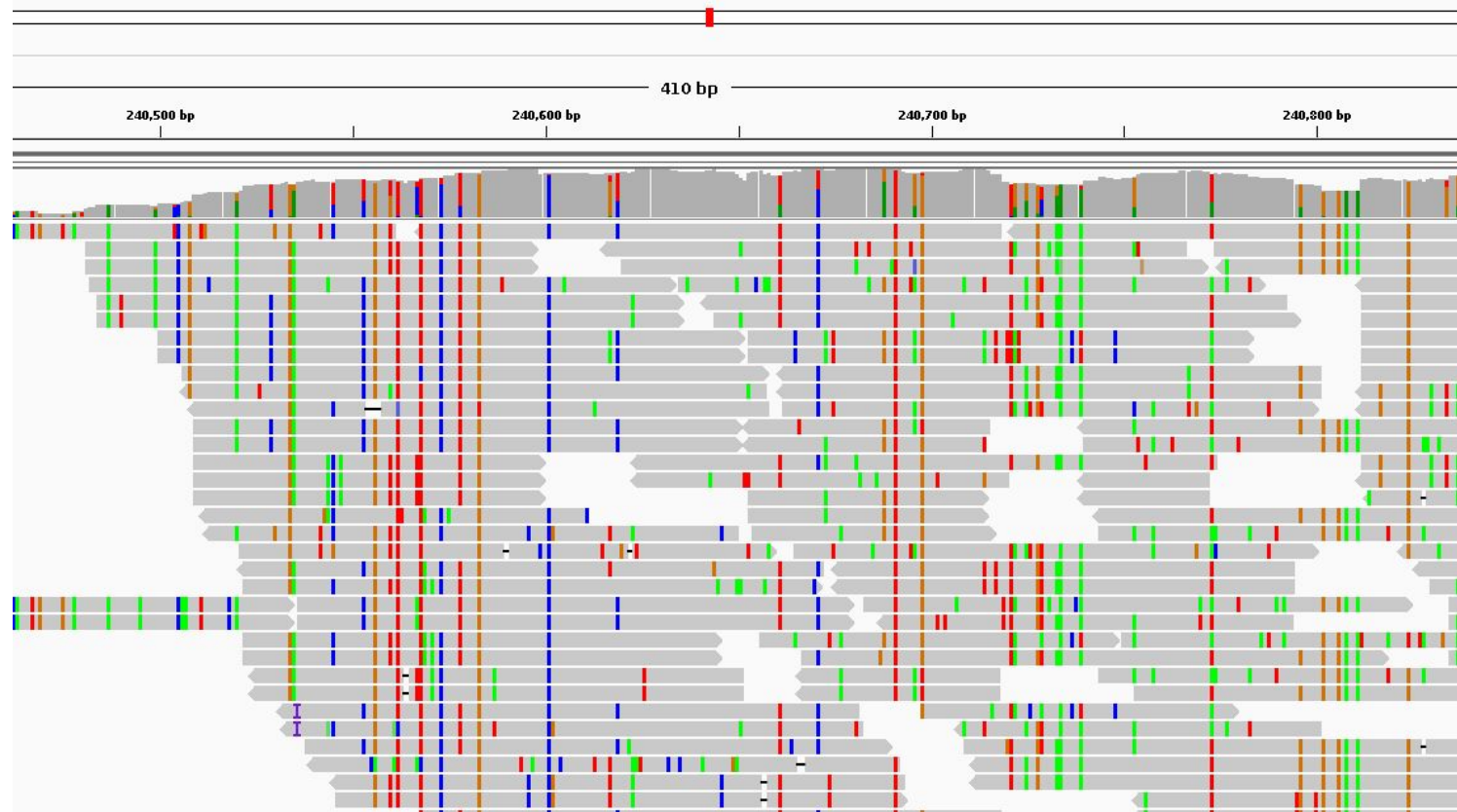
GBS Overview



SNP Calling

- ▶ Whole Genome Sequencing (WGS)

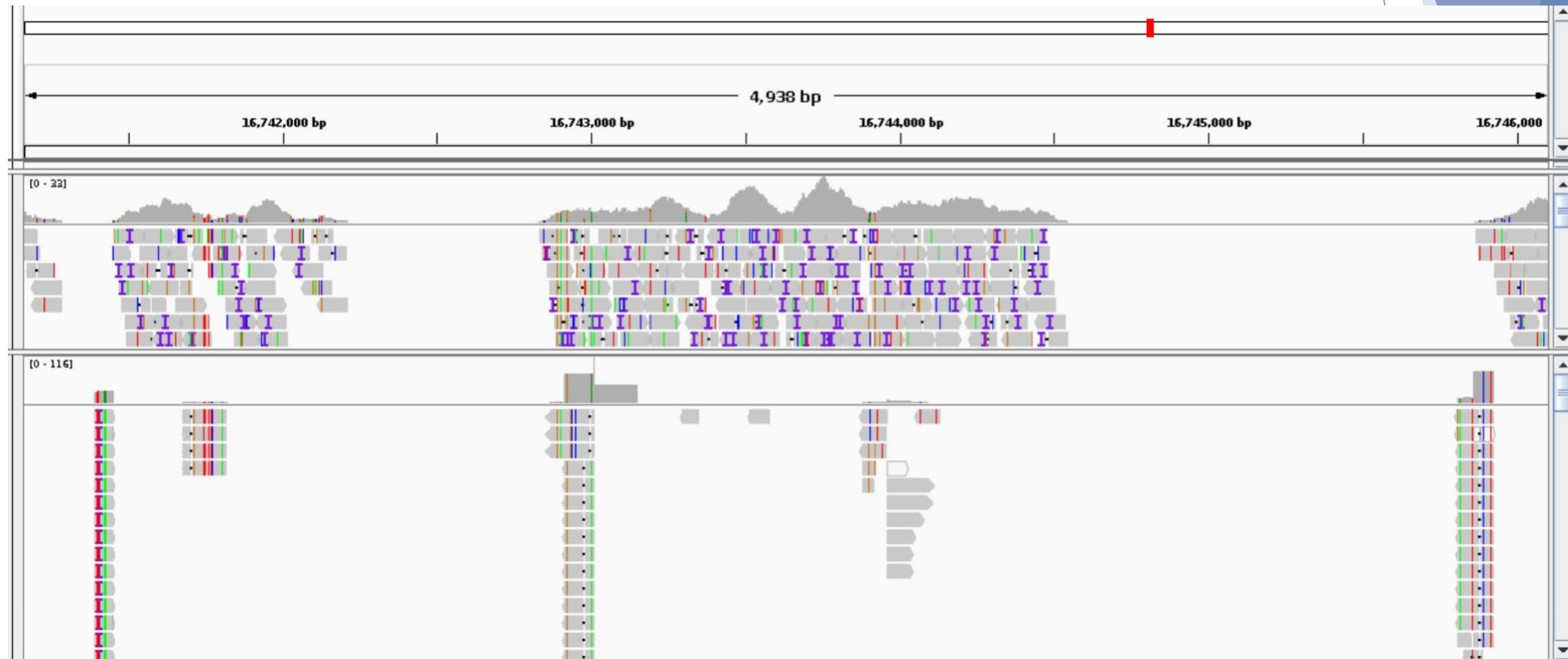
Image: IGV



SNP Calling

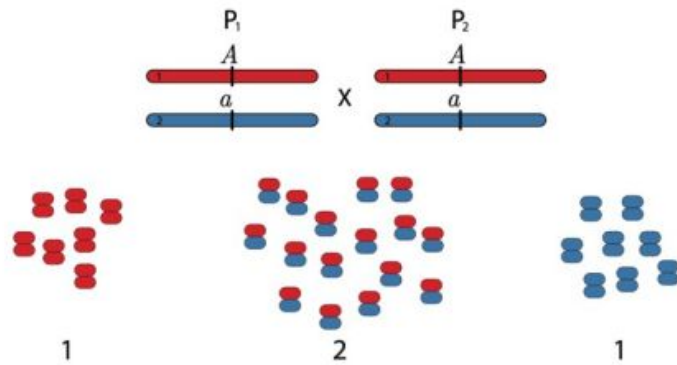
- ▶ Exome sequencing (top) and Genotyping-by-Sequencing (bottom)

Image: IGV

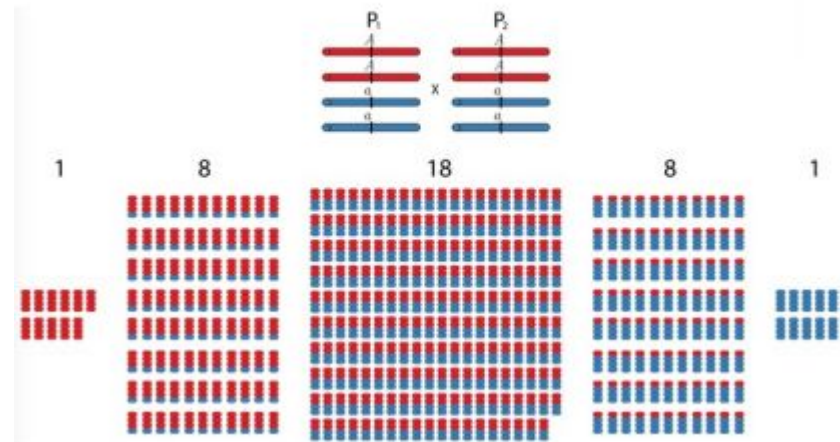


Dosage calling

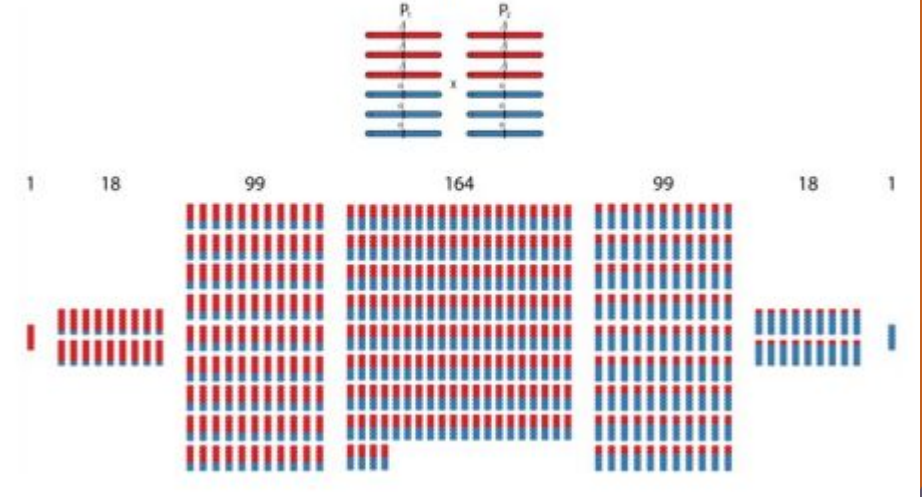
Diploid



Tetraploid



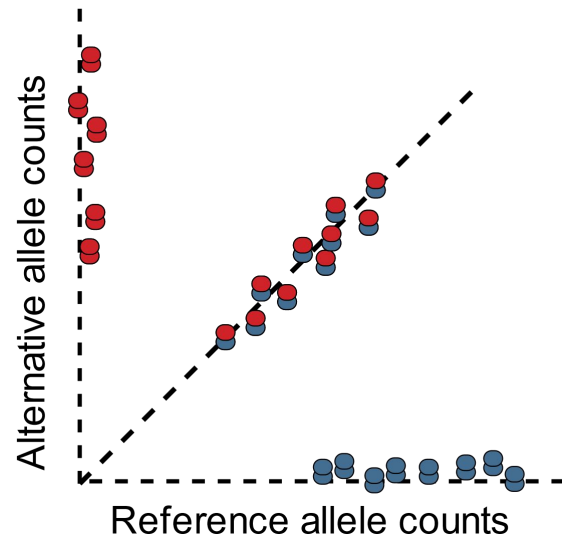
Hexaploid



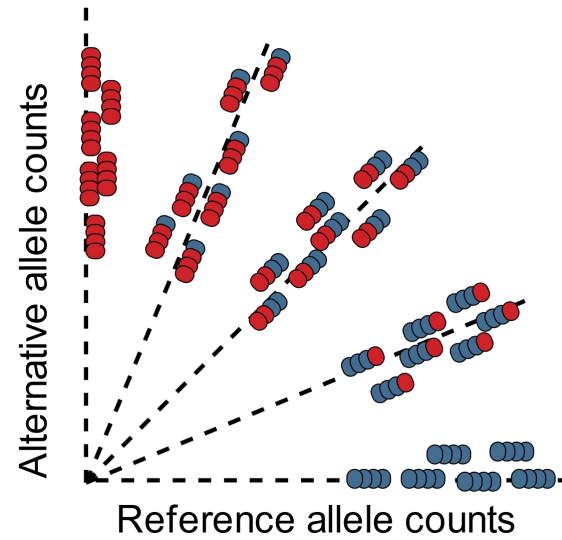
Dosage Calling

- ▶ The theory

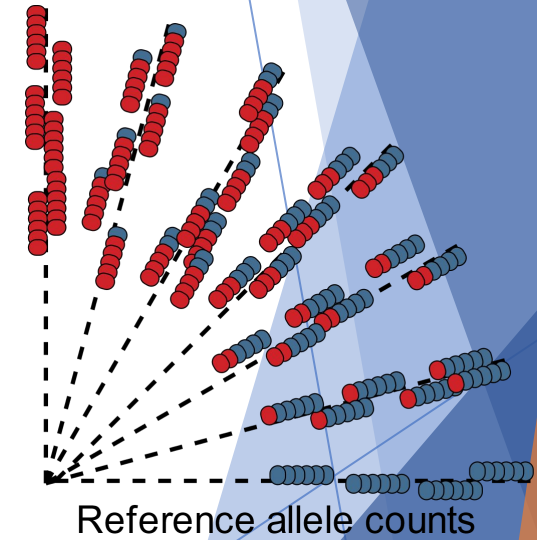
Diploid



Tetraploid



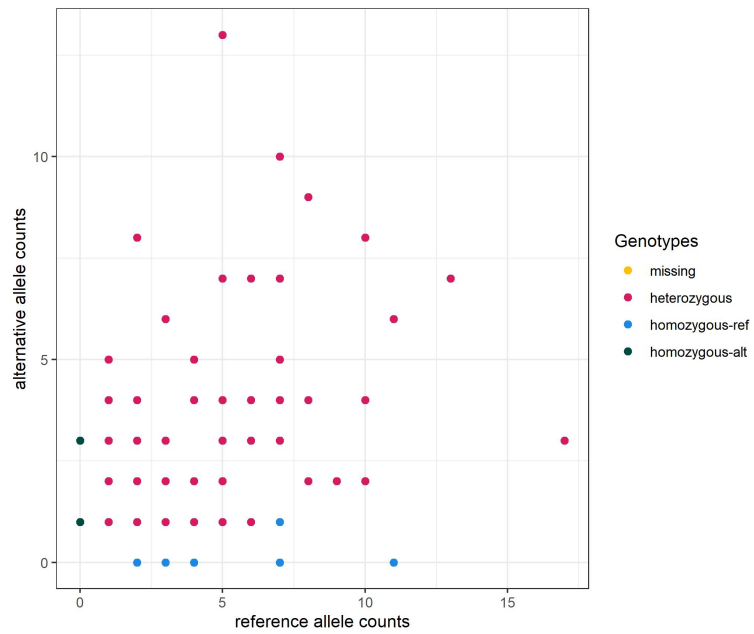
Hexaploid



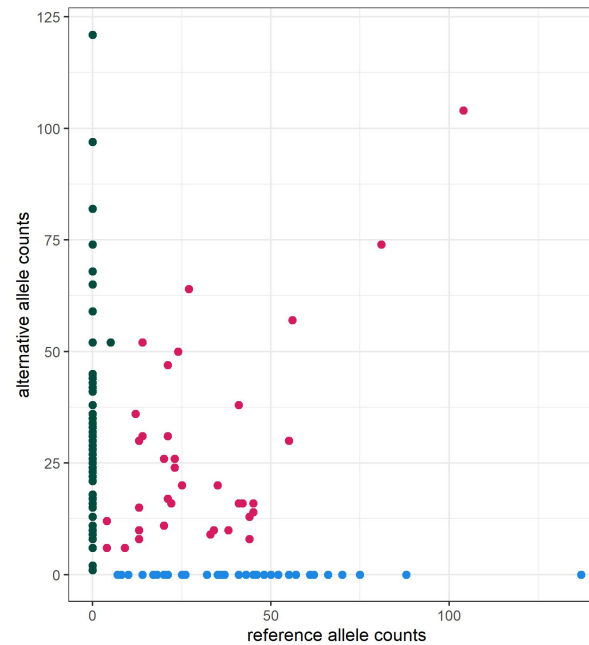
Dosage Calling

► The reality

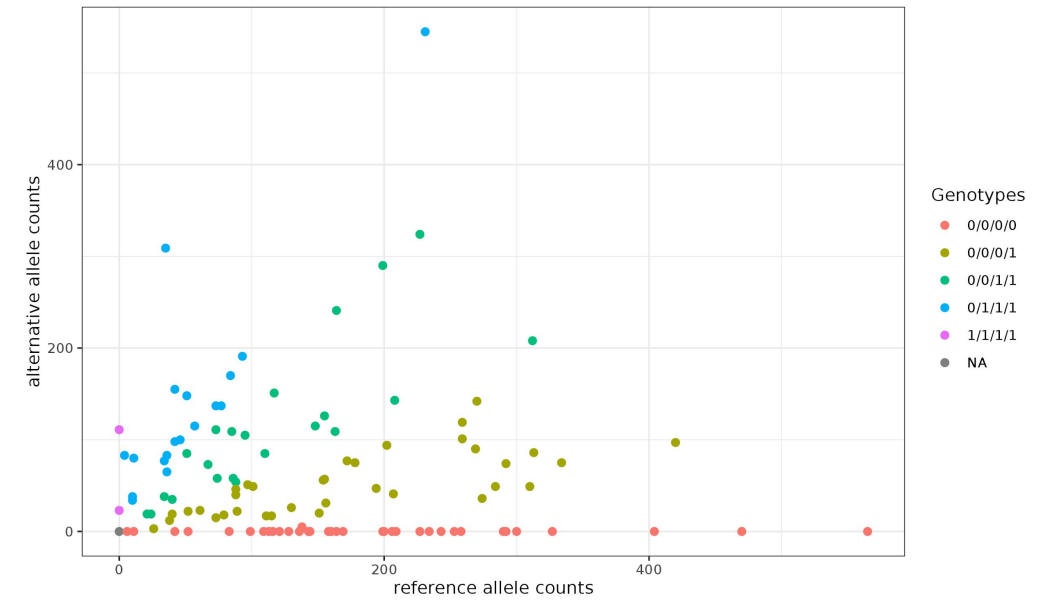
Diploid (mean depth 6)
N = 200
Aa x Aa



Diploid (mean depth 96)
N = 138
Aa x Aa

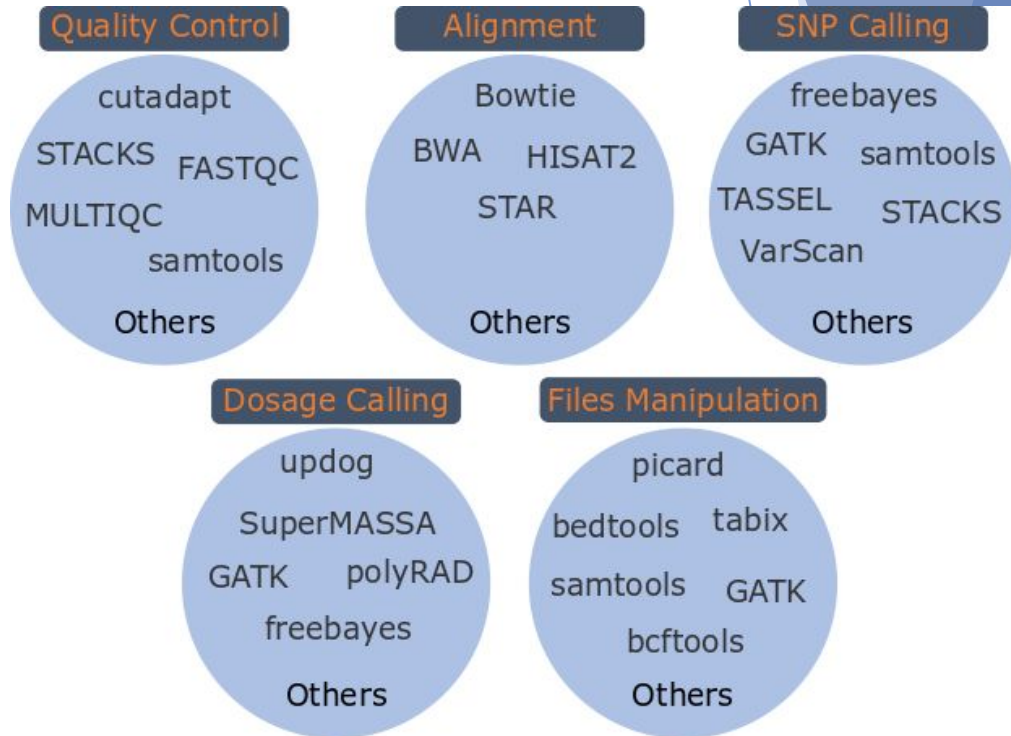


Tetraploid (mean depth 83)
N = 114
AAaa x AAaa



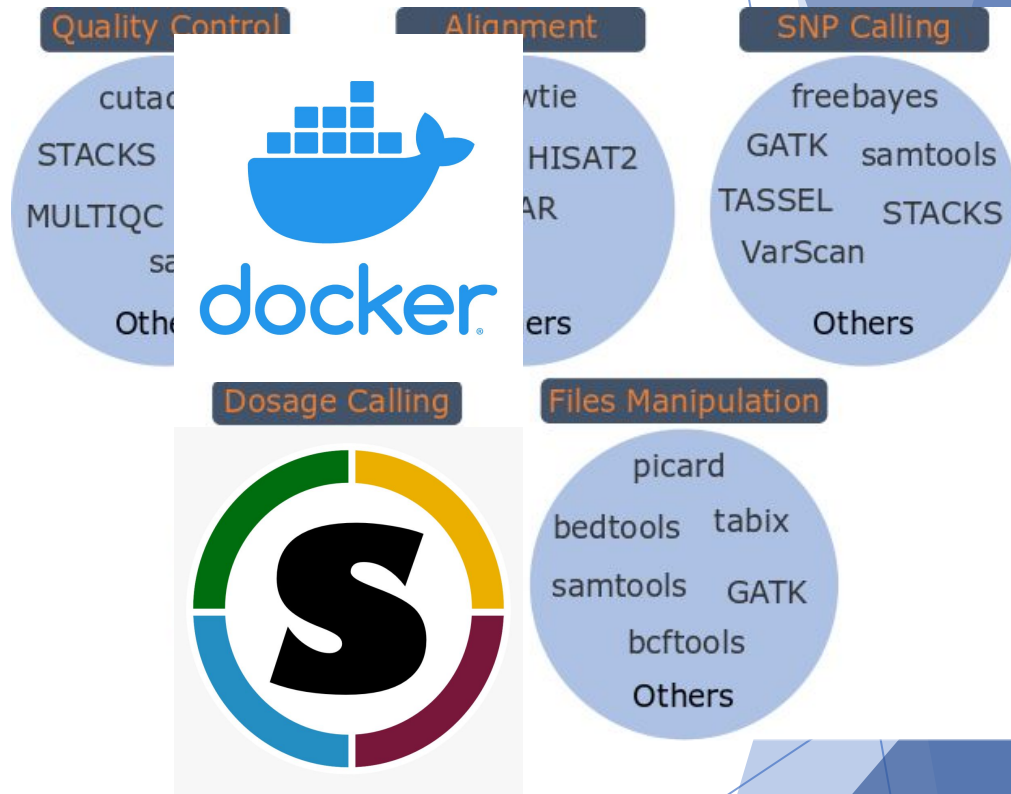
Sequencing Data - Technical Difficulties

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



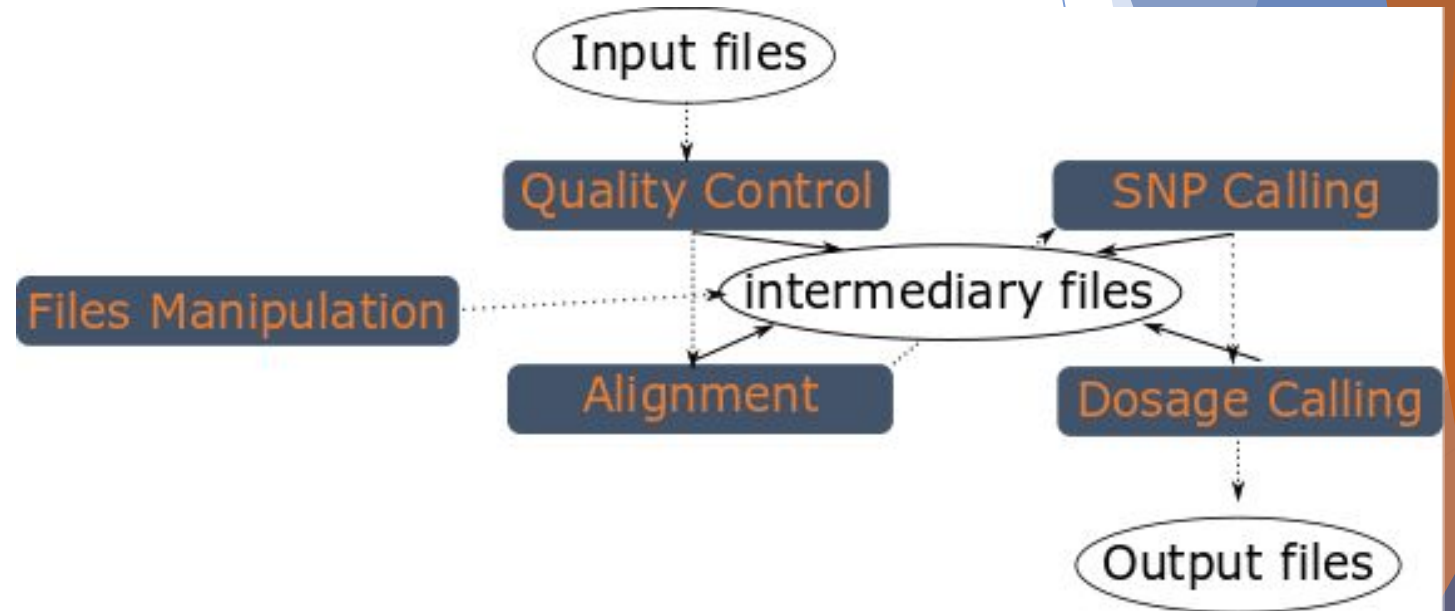
Sequencing Data - Technical Difficulties

- ▶ 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](#)



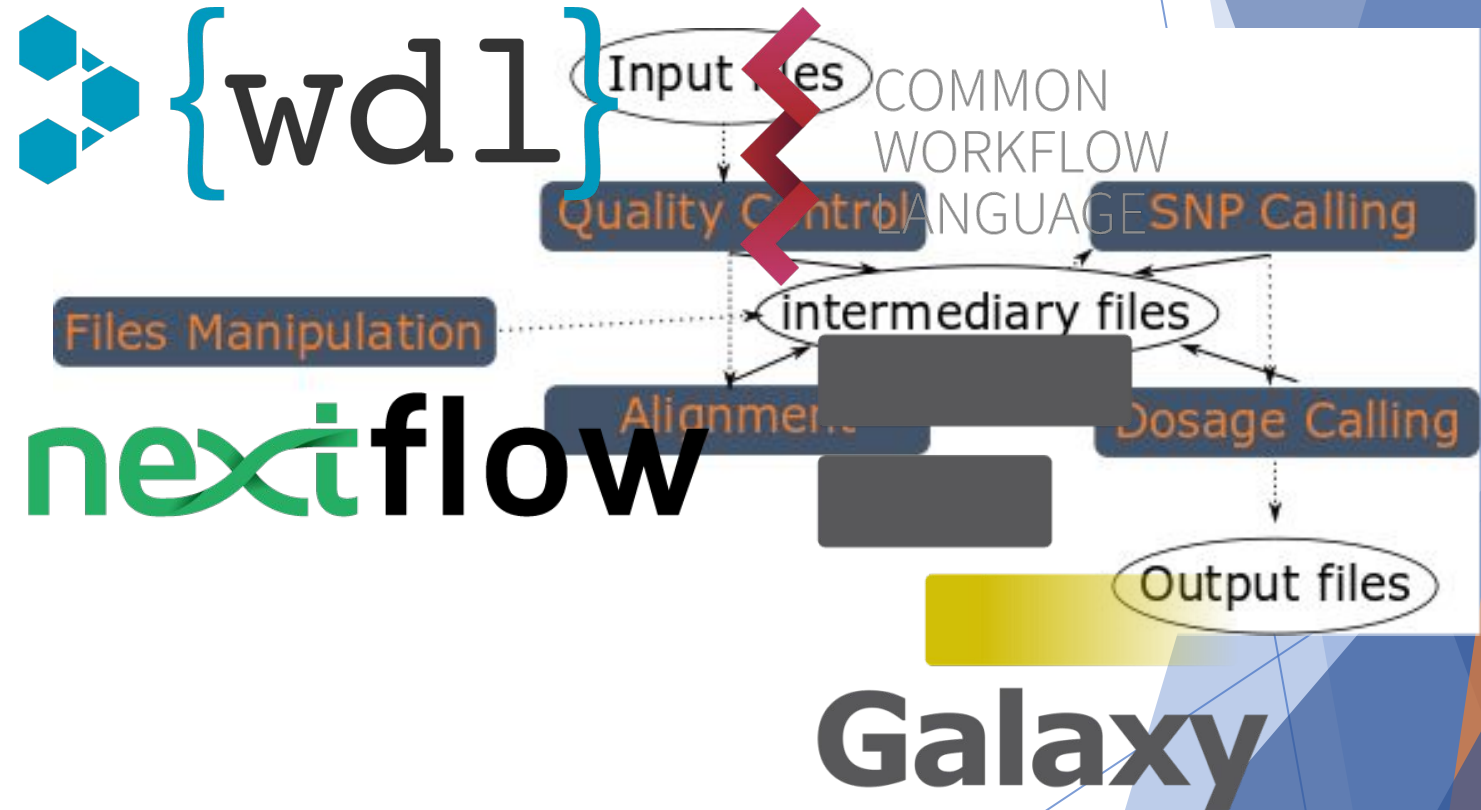
Sequencing Data - Technical Difficulties

- ▶ 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
 - ▶ DNAnexus
 - ▶ Terra
 - ▶ AnVIL
 - ▶ SevenBridges

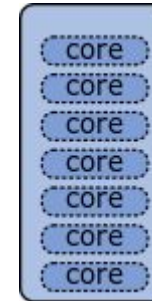


Sequencing Data - Technical Difficulties

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

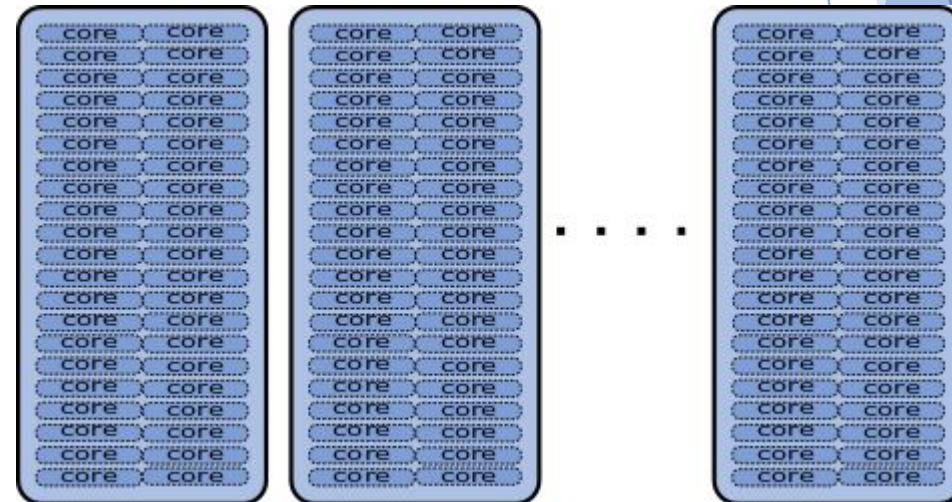
Personal Computer:

4GB RAM; 8 cores; 1 node

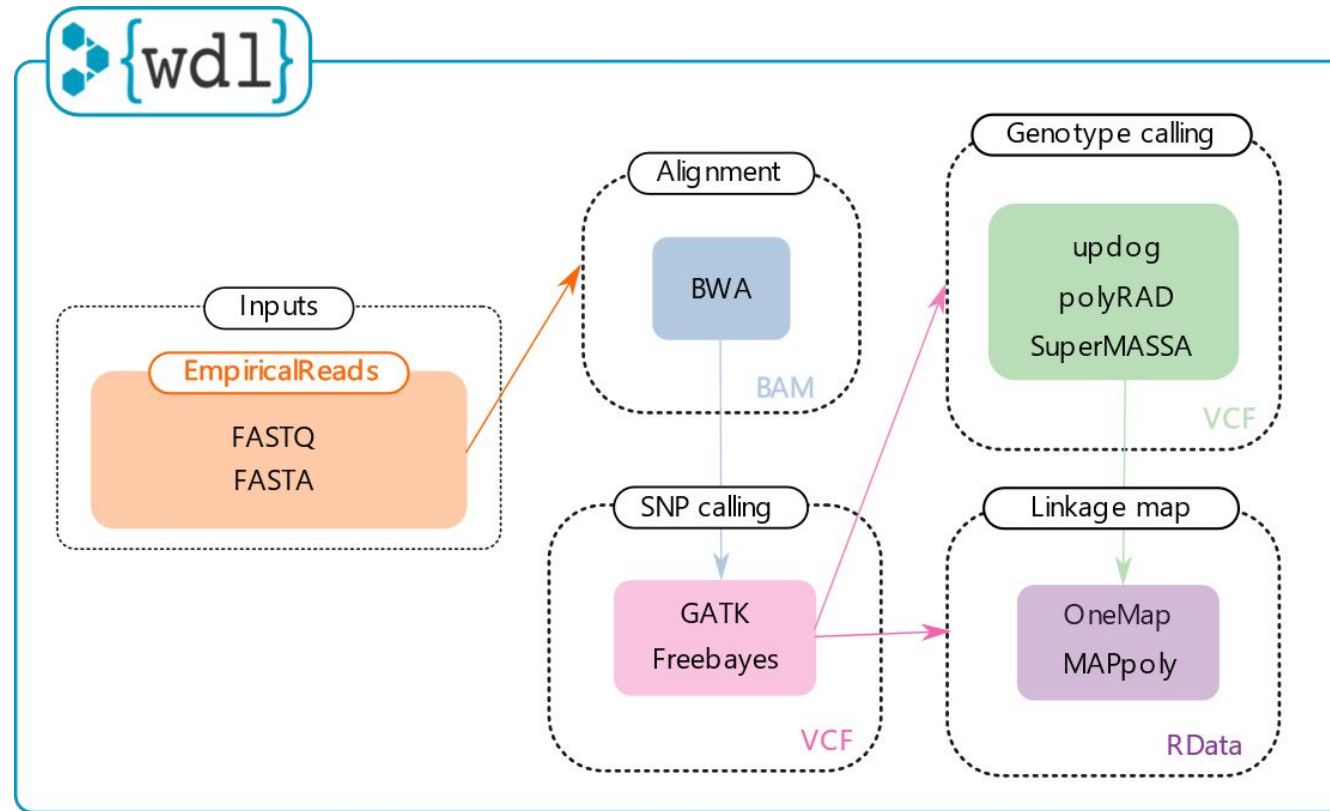


High Performance Computing (Texas A&M):

384GB; 48 cores per node; 900 nodes



How we solved it: Reads2Map



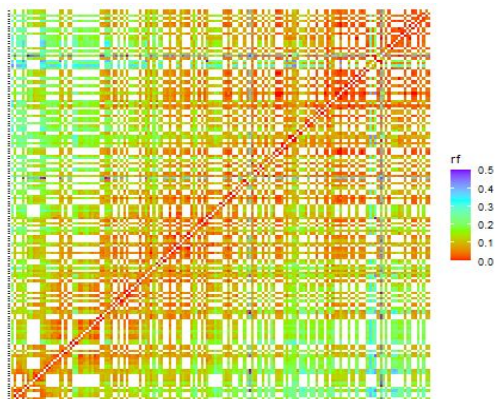
Available in [Github](#), [Dockerstore](#) and [WorkflowHub](#)

Reads2Map results - Diploid roses

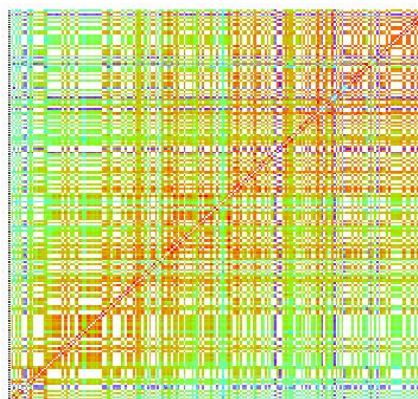
- 37% of chromosome 1
- ~ 38 cM
- Sequencing depth ~ 94X



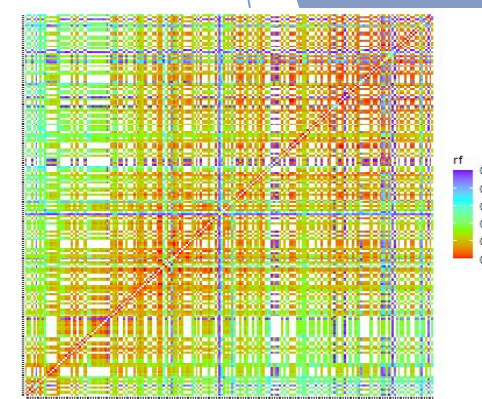
GATK



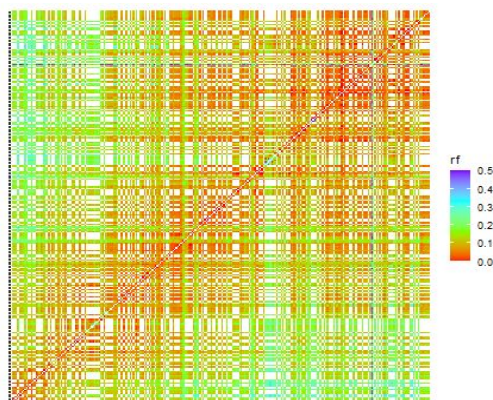
GATK + polyRAD



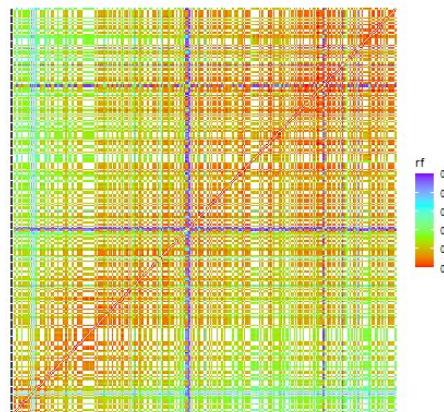
GATK + updog



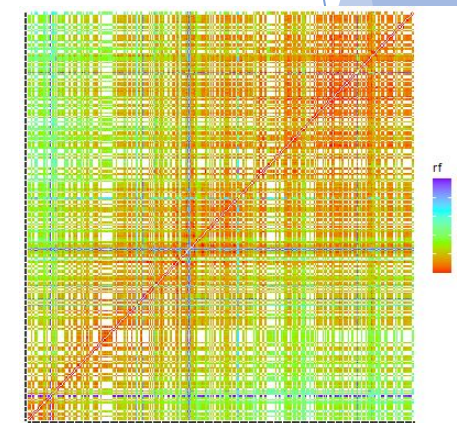
freebayes



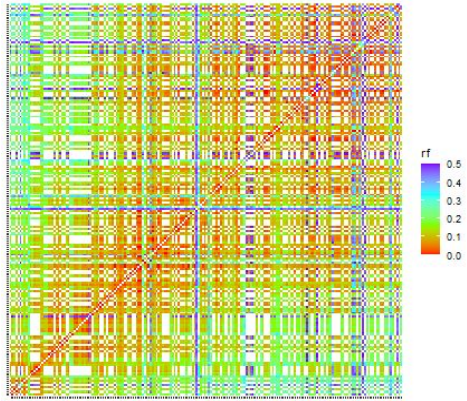
freebayes + polyRAD



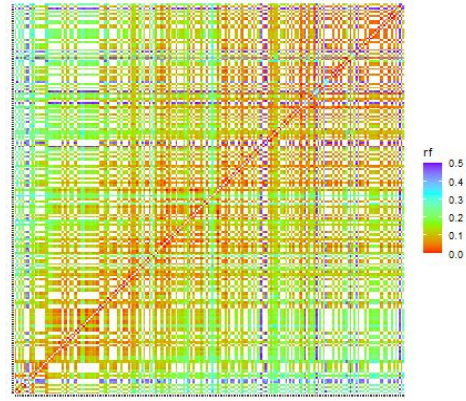
freebayes + updog



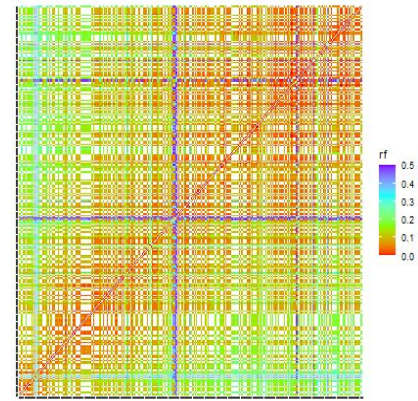
GATK + updog



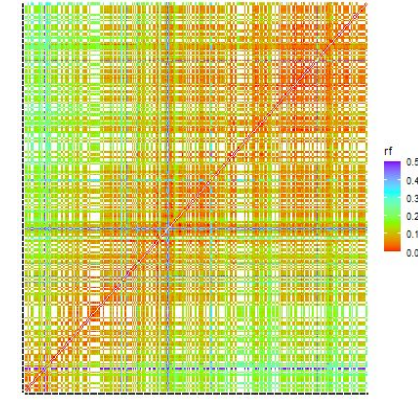
GATK + polyRAD



freebayes + polyRAD



freebayes + updog



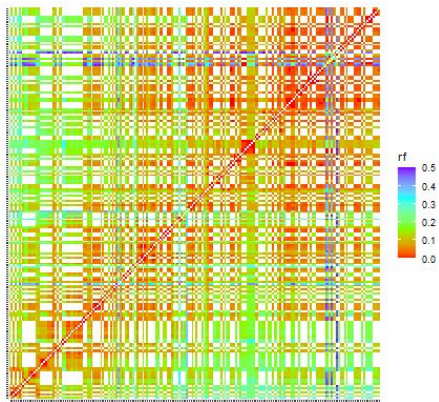
Simulation studies: 10,880 maps
Empirical studies: 816 maps

Filters:

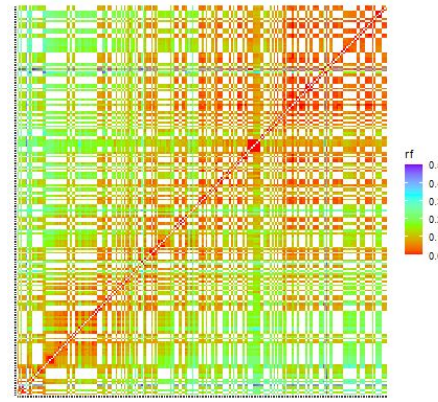
- genotype probabilities
- non-informative markers
- replace AD by missing when GT is missing



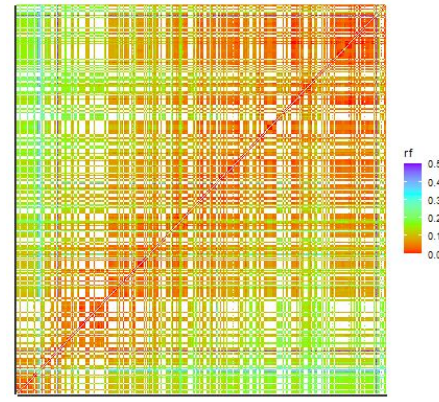
GATK + updog



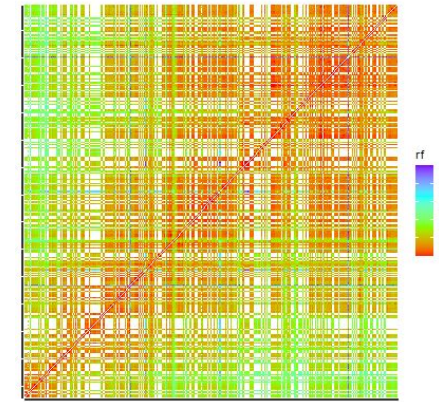
GATK + polyRAD



freebayes + polyRAD

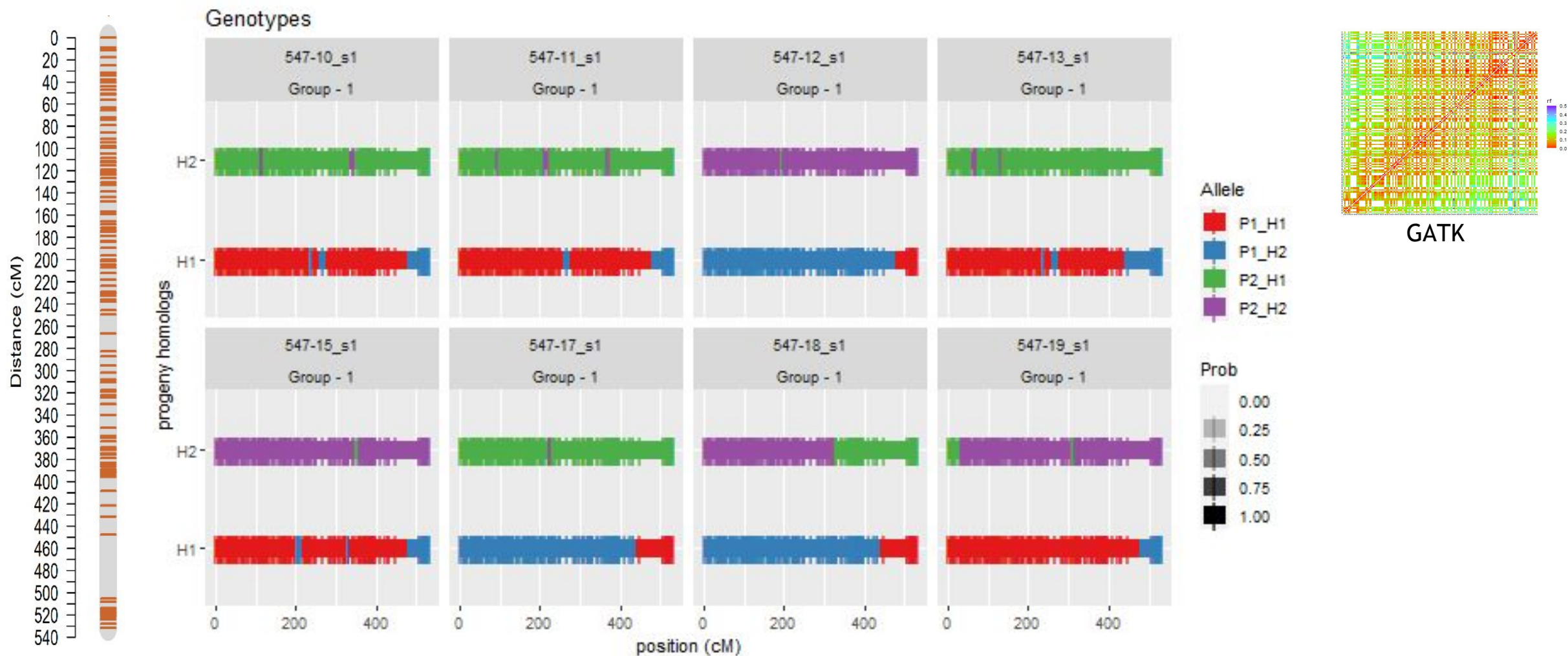


freebayes + updog



Map size

37% of chromosome 1 ~38cM



Map size

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

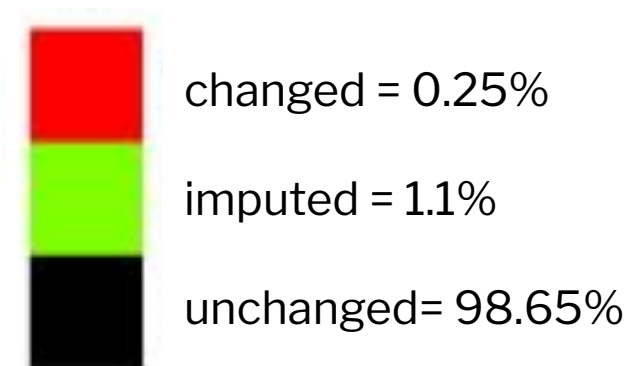
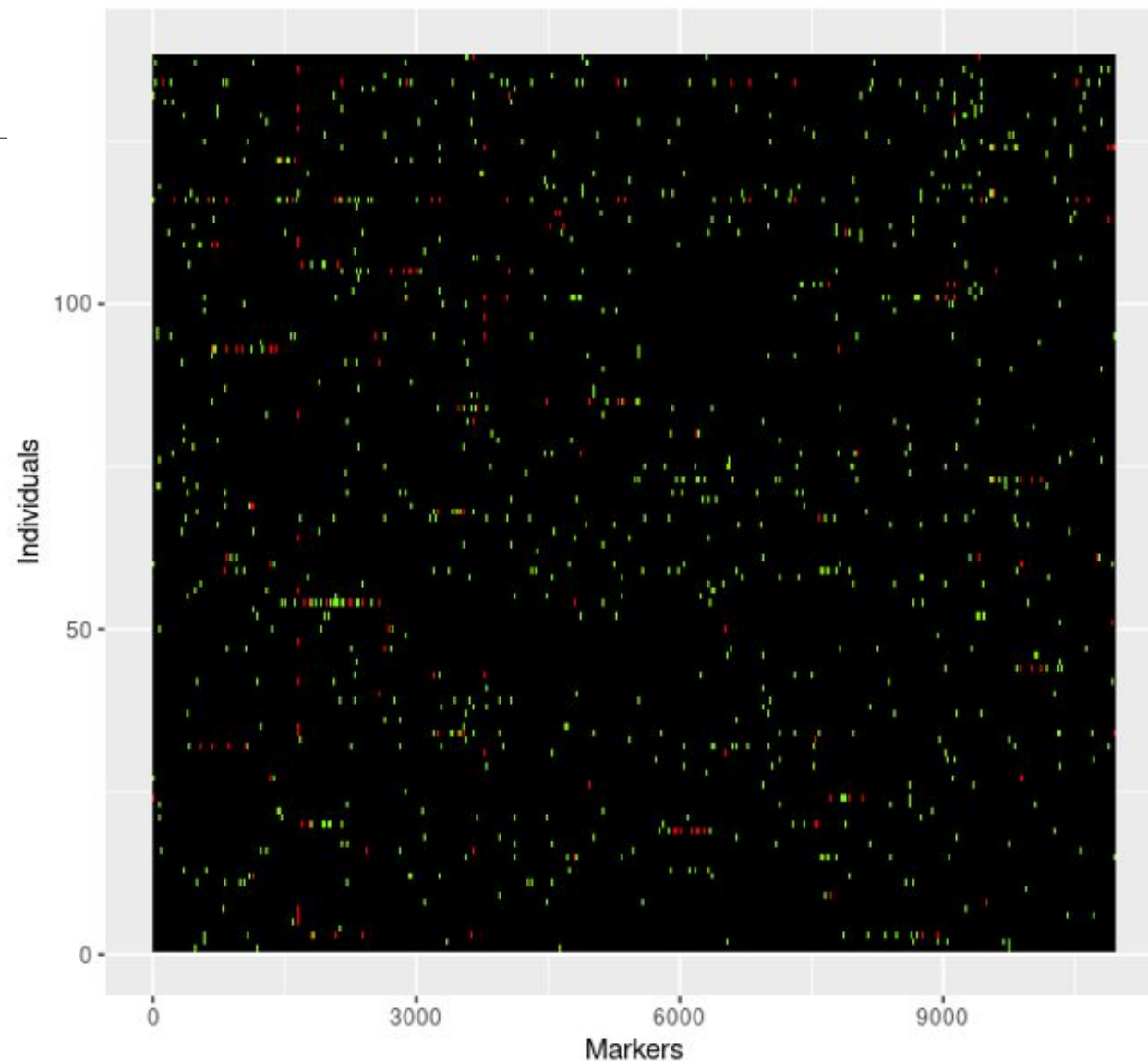


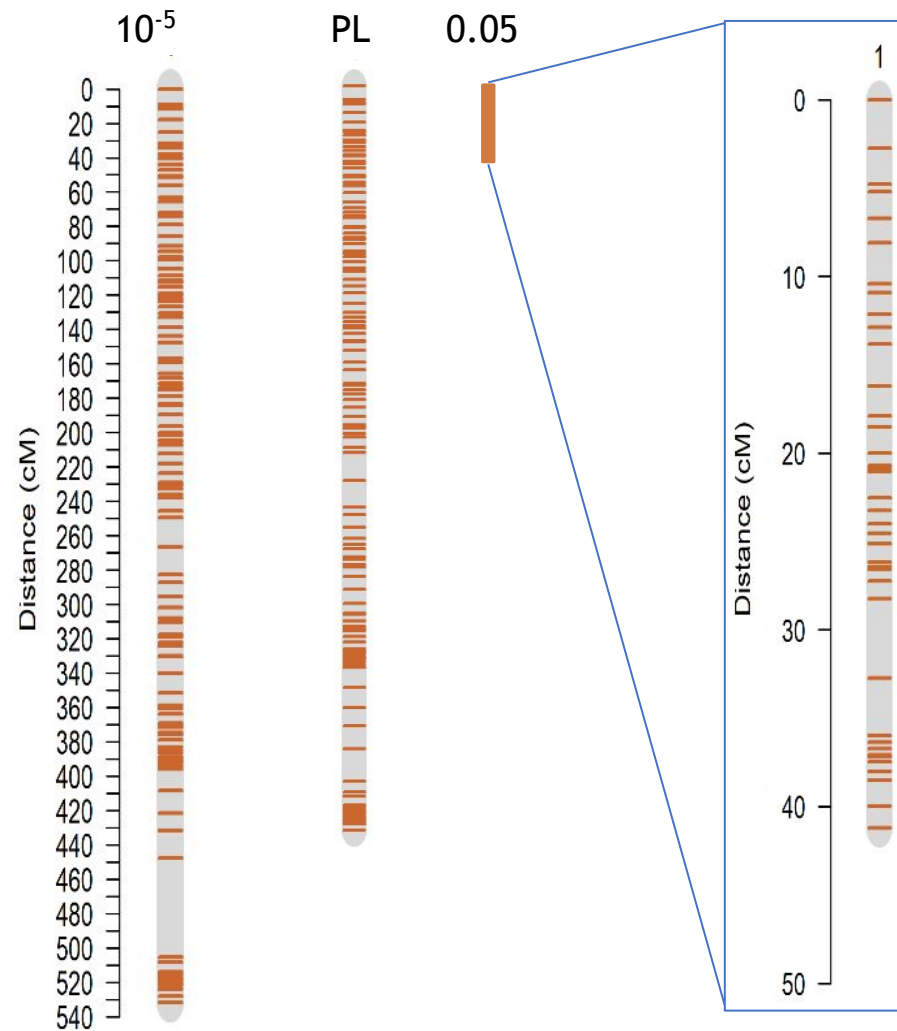
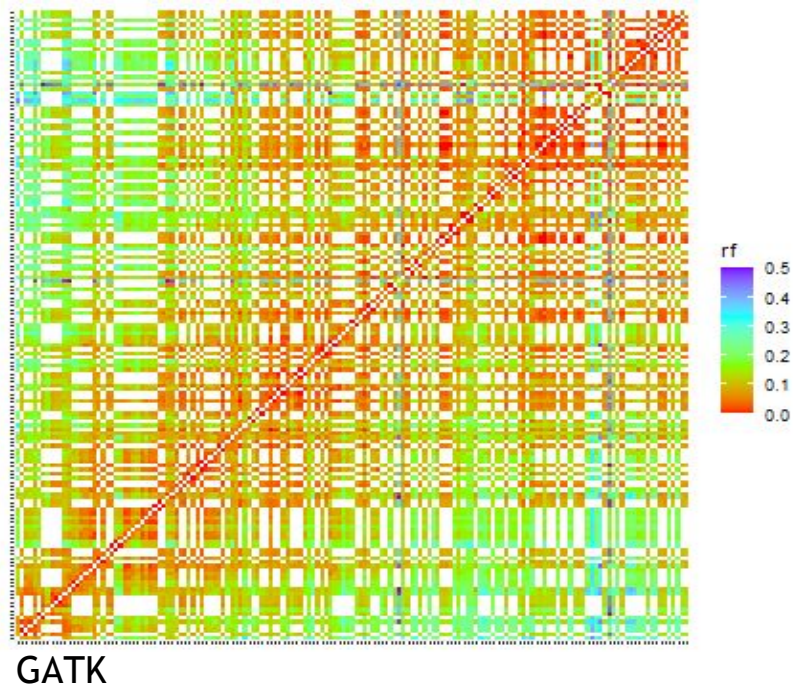
Figure by Jeekin Lau

Map size

37% of chromosome 1 ~38cM

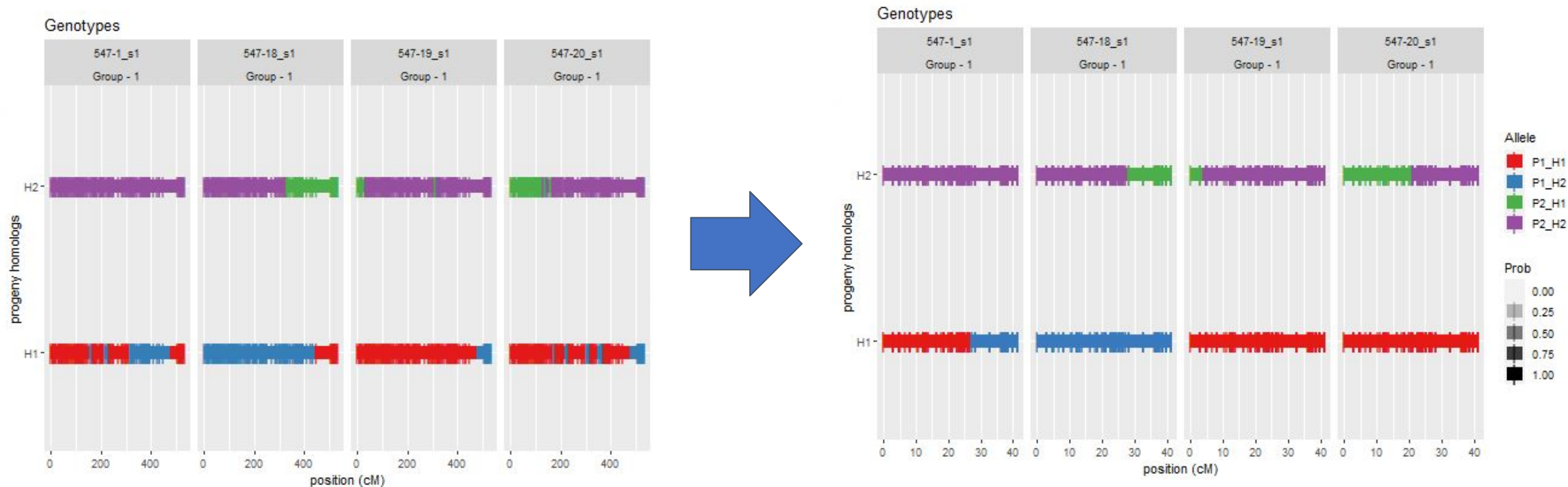
- Hidden Markov Model Emission Function

- global error rate
- genotype probabilities (PL)



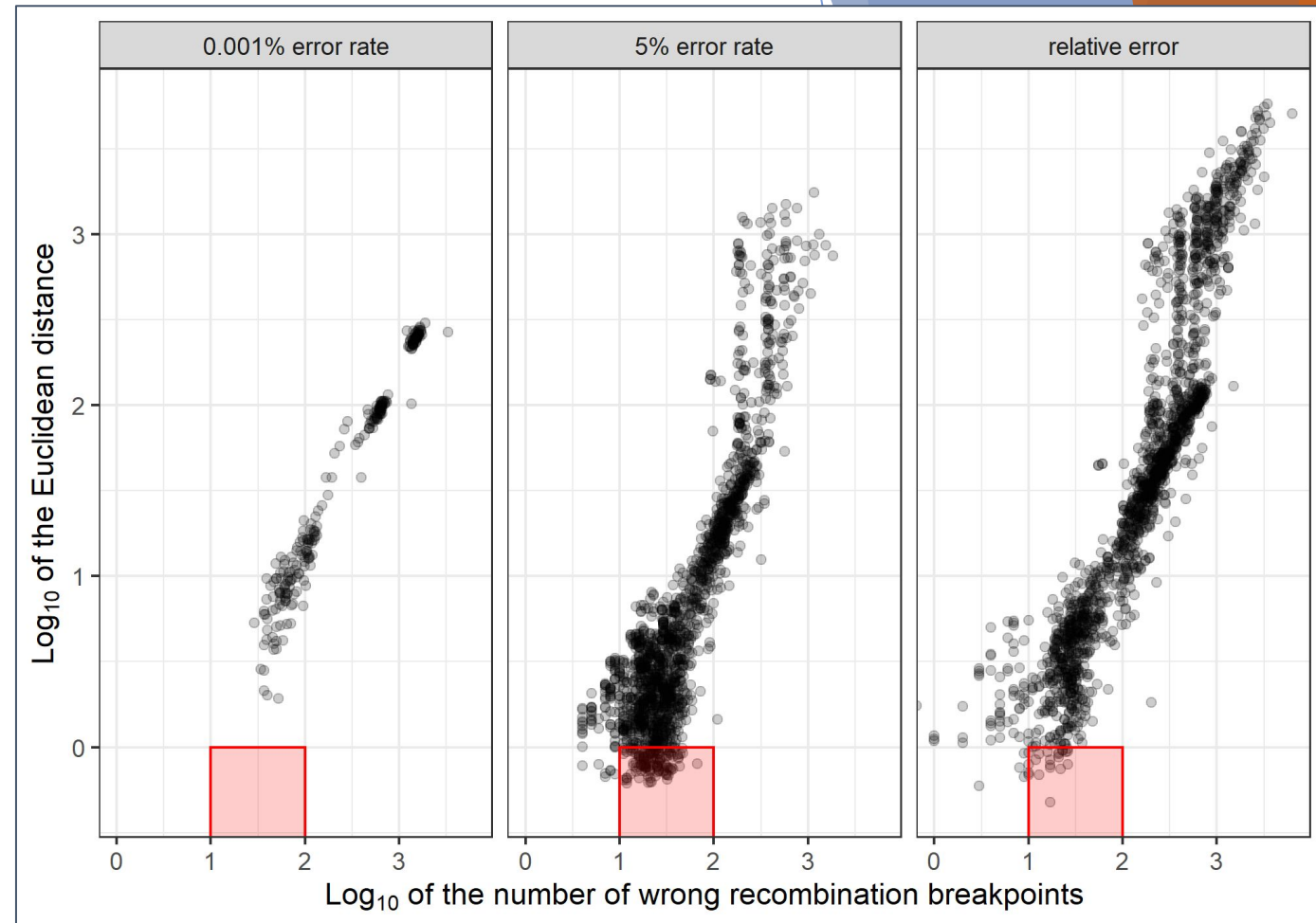
Map size

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



bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

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.

New Results

Follow this preprint

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

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, Antonio Augusto Franco Garcia

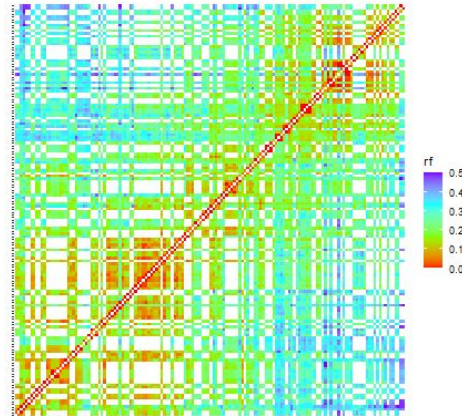
doi: <https://doi.org/10.1101/2022.11.24.517847>

Diploid Aspen

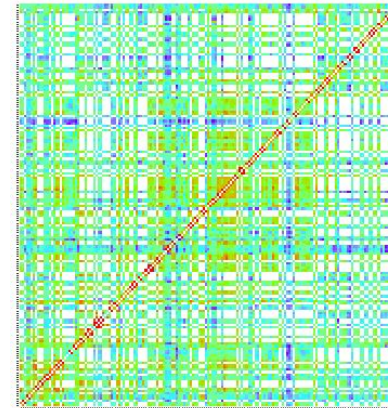
- 37% of chromosome 10
- Sequencing depth ~ 6X



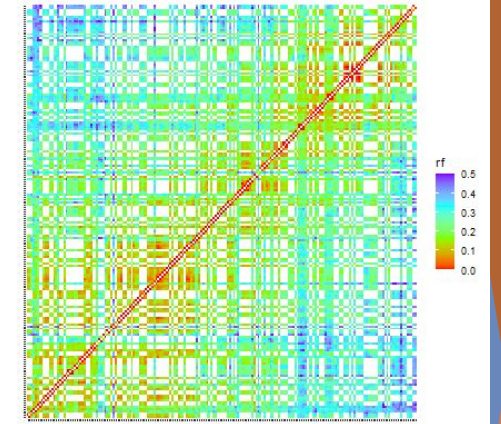
GATK



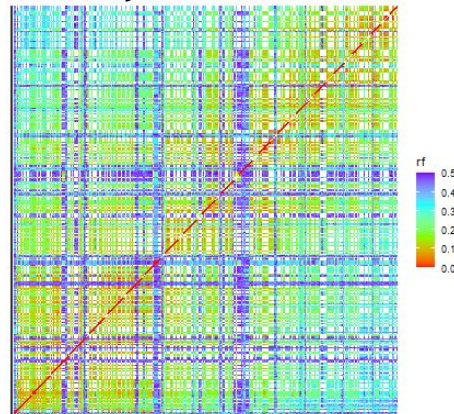
GATK + polyRAD



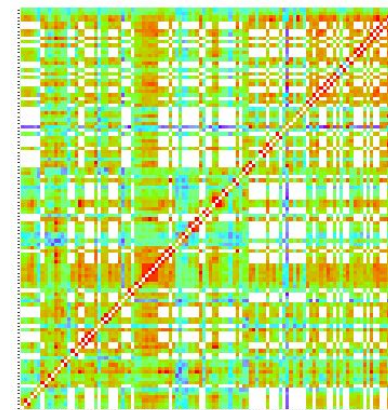
GATK + updog



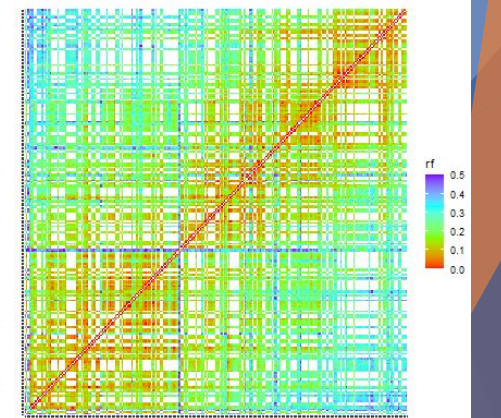
freebayes



freebayes + polyRAD

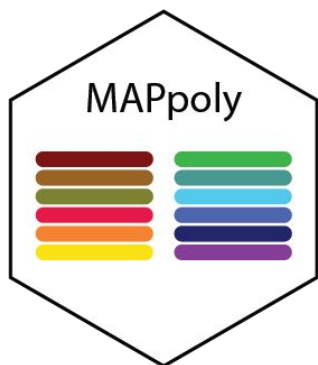


freebayes + updog

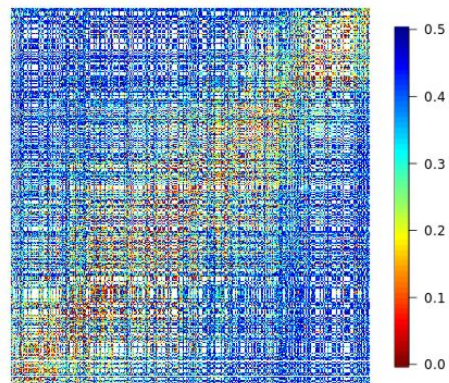


Tetraploid rose

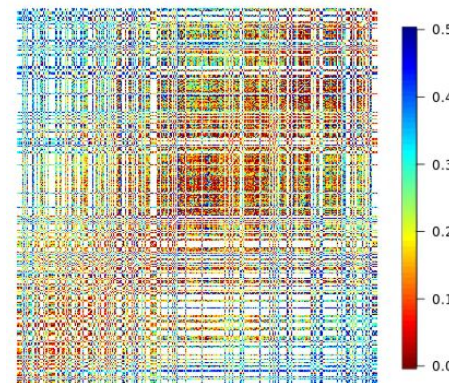
- Chromosome 2
- Sequencing depth ~ 50X



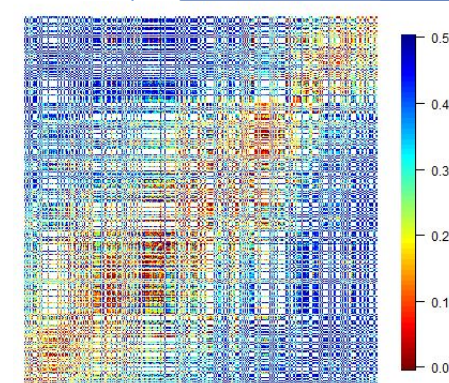
GATK



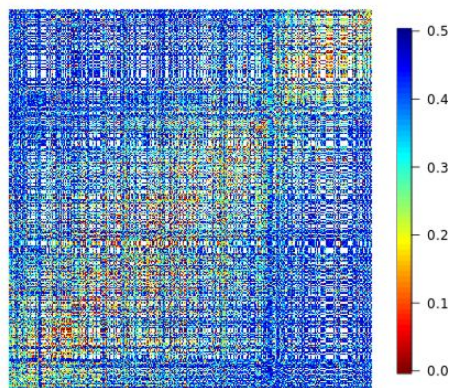
GATK + polyRAD



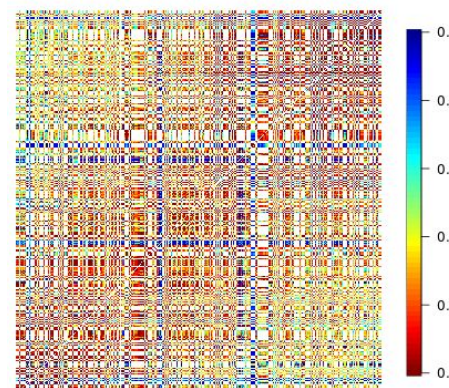
GATK + updog



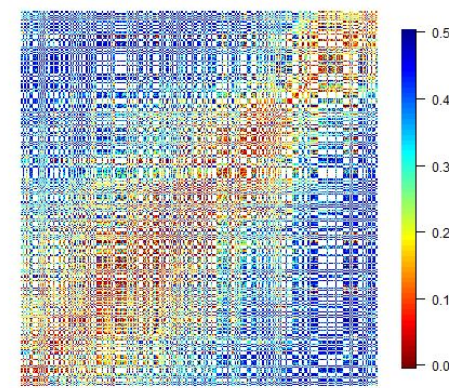
freebayes



freebayes + polyRAD



freebayes + updog



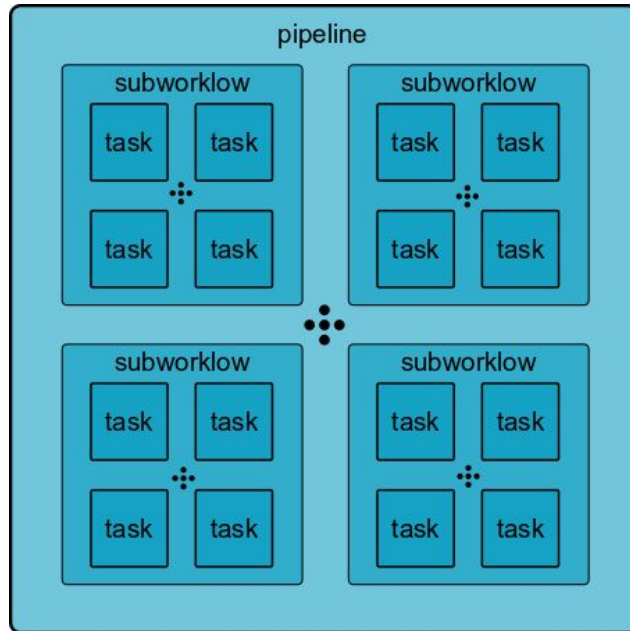
Hands-on Workshop!!

<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_do...

✓ acca101 on 1

.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

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](https://www.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
```












task

main ▾ Reads2Map / tasks /

 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 {...
}

> output {...
}
}

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

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
```

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": CreateChunk

>   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
```

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
```

Thank you



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- ▶ Cecilia Deng
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Plant & Food[™] Research

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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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