

Reads2Map

Practical and reproducible workflows to build polyploid linkage maps from sequencing data

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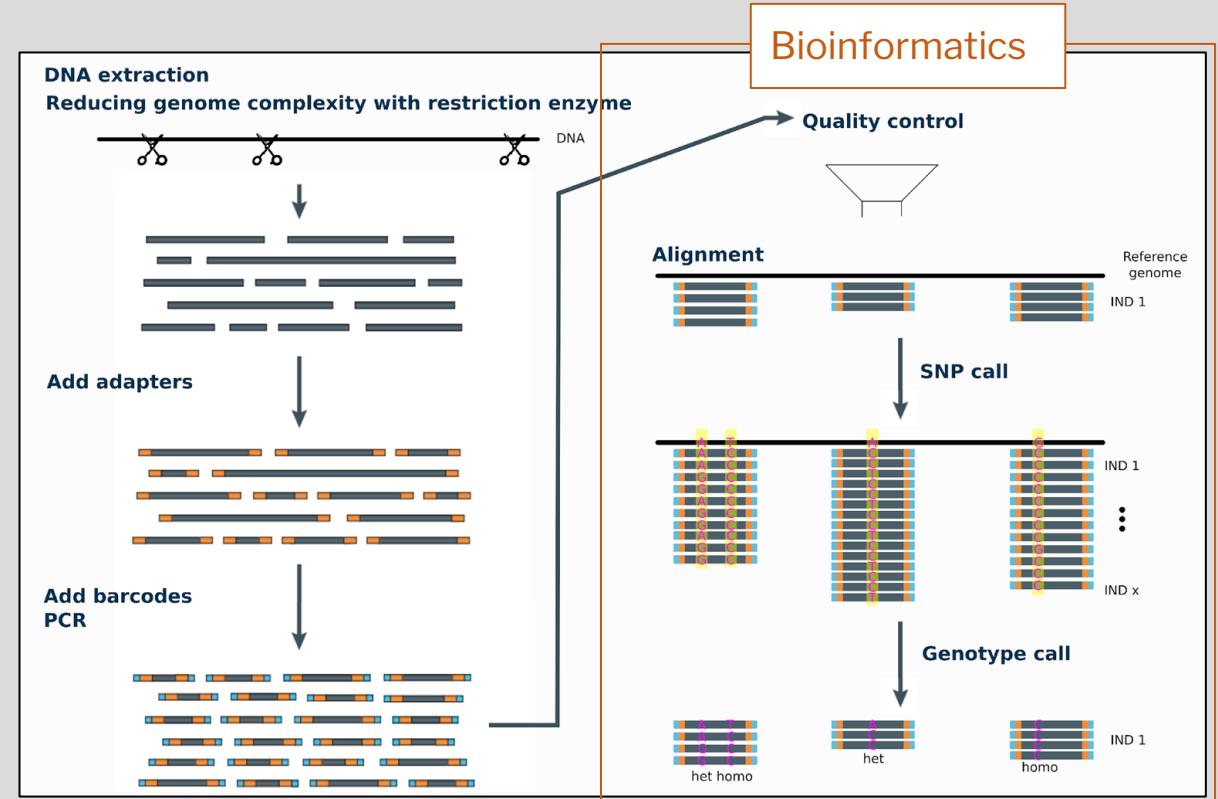
This project is funded by USDA NIFA Specialty Crop Research Initiative
Award # 2020-51181-32156 (09/01/20 - 08/31/24)



Motivation

A genotyping-by-sequencing story

- My first GBS data (2015)
 - Biology background



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Motivation

A genotyping-by-sequencing story

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 - Biology background
 - File formats

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+SRR6249787.8 8 length=143
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FASTQ

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Chr10 99721 Chr10_99721 C A . . NS=123;DP=2236;LU=2;HH=0.613787693283989
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```

VCF



This project is funded by USDA NIFA Specialty Crop Research Initiative
Award # 2020-51181-32156 (09/01/20 - 08/31/24)

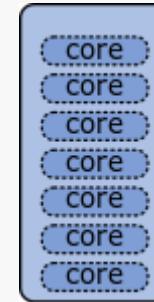


Motivation

A genotyping-by-sequencing story

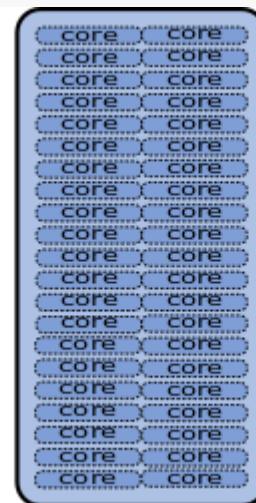
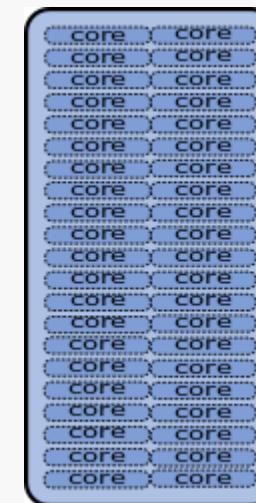
- My first GBS data (2015)
 - Biology background
 - File formats
 - High Performance Computing
 - Linux
 - Surviving without sudo permissions

Personal computer

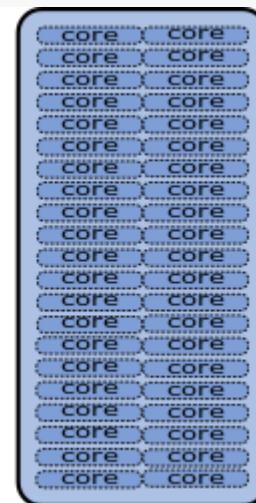


4GB RAM; 8 cores; 1 node

High Performance Computing



...

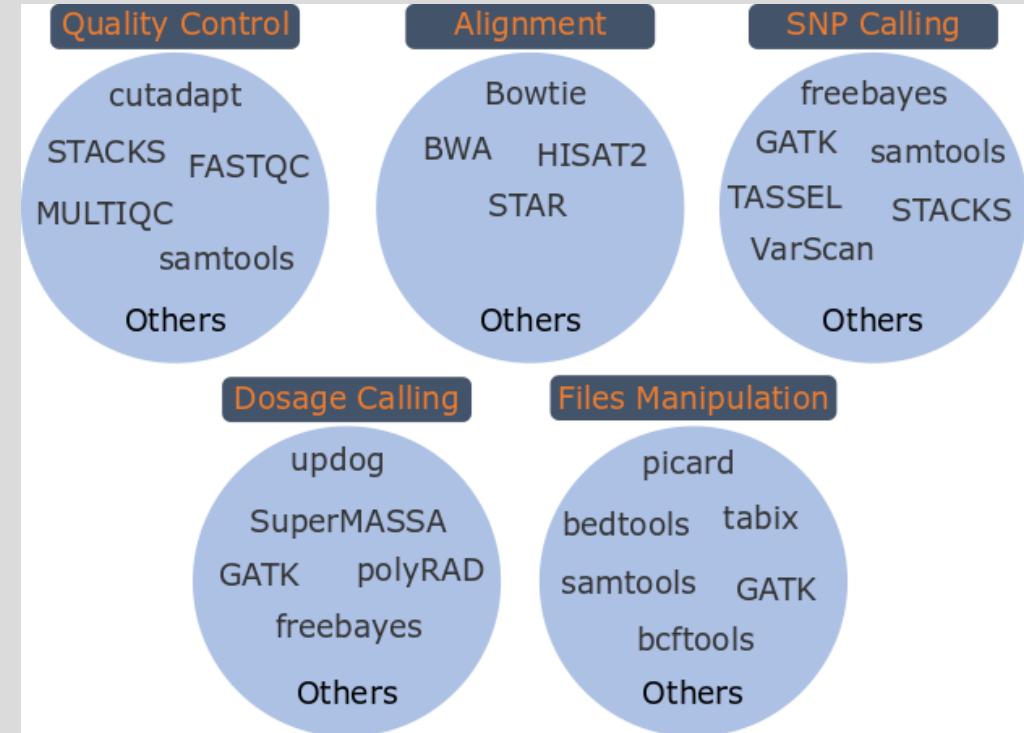


384GB; 48 cores per node; 900 nodes

Motivation

A genotyping-by-sequencing story

- My first GBS data (2015)
 - Biology background
 - File formats
 - High Performance Computing
 - Linux
 - Surviving without sudo permissions
 - Many software and parameters
 - Many programming languages
 - Different Operational Systems
 - Updates



Motivation

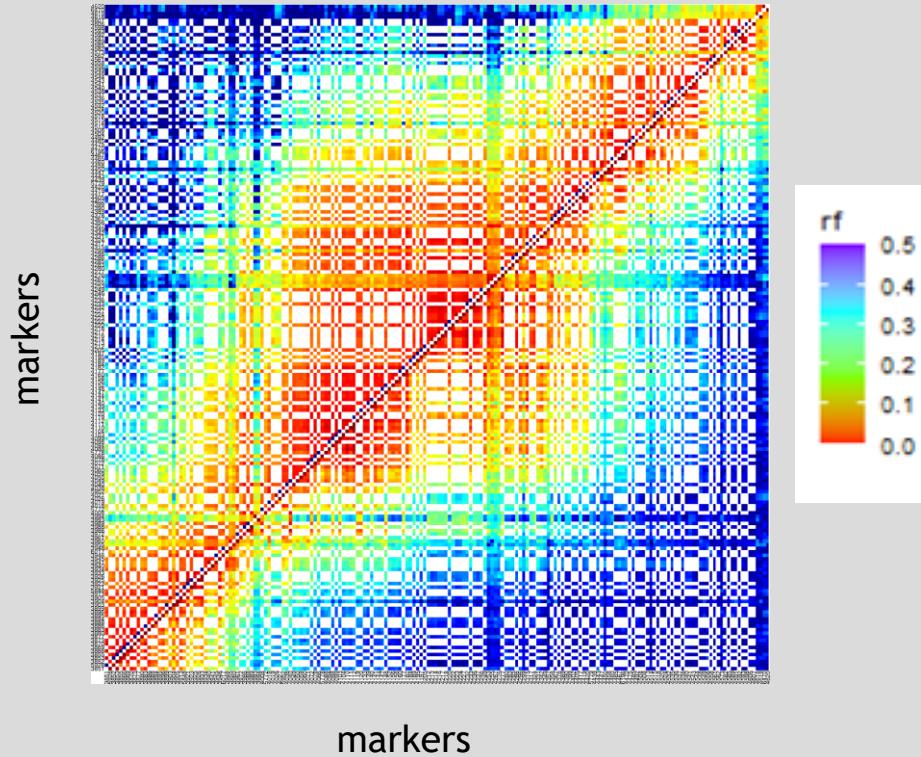
A genotyping-by-sequencing story

- My first diploid outcrossing population linkage map
 - Estimating also phases
 - Hidden Markov Model multipoint approach
 - OneMap
 - R



- Diploid species
- Bi-parental populations
- Backcross, RILs, F2 and outcrossing
- Biallelic and Multiallelic markers

Recombination fraction matrix

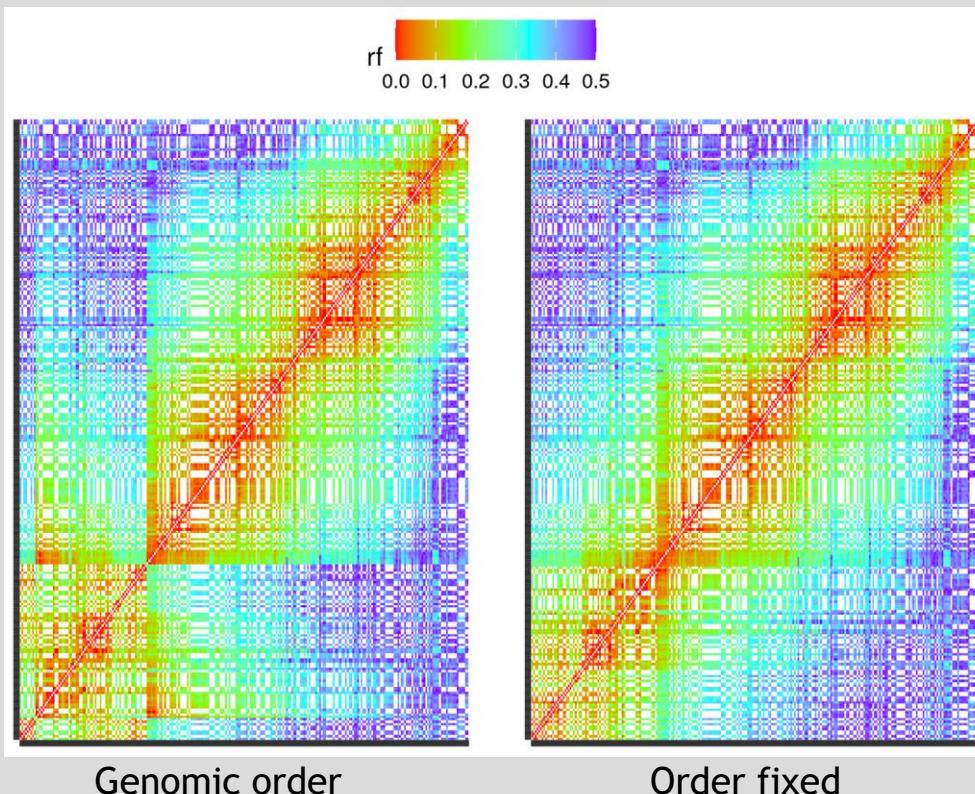


- # recombinations between two markers during meiosis
- Genomic order
 - Example: Eucalyptus chromosome 10

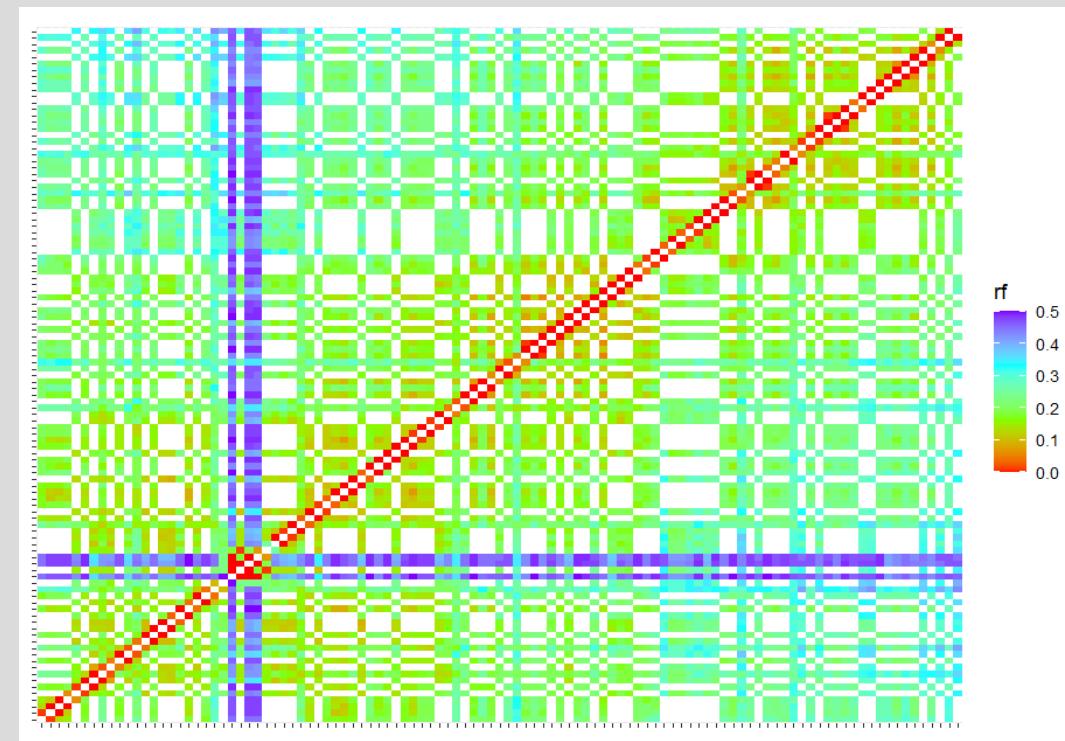
Recombination fraction matrix

Inversion

- Aspen chromosome 12



Most likely bad data



Motivation

Experiences building Linkage Maps

- Using sequencing markers
 - Recombination fraction matrix not always pretty
 - Slow analysis
 - Inflated map size
- OneMap - Since 2007
- OneMap maintainer - Since 2017



- Updates in version 3.0

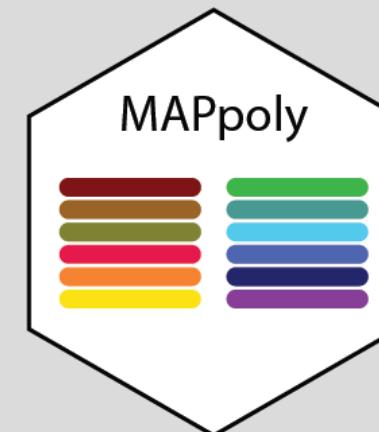
Motivation

Experiences building Linkage Maps

- Using sequencing markers
 - Recombination fraction matrix not always pretty
 - Slow analysis
 - Inflated map size
- OneMap - Since 2007
- OneMap maintainer - Since 2017
- MAPpoly – Since 2018



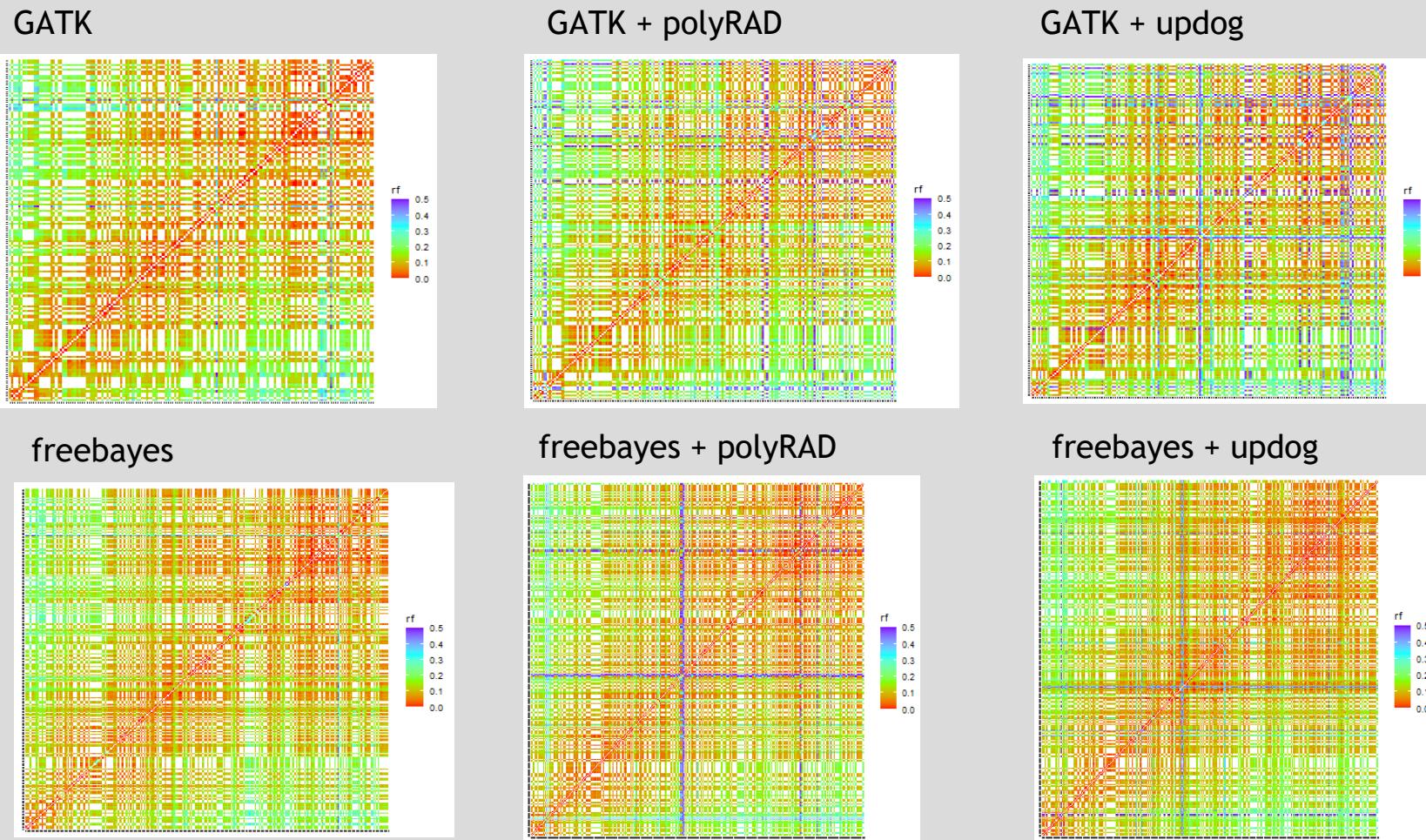
- Updates in version 3.0



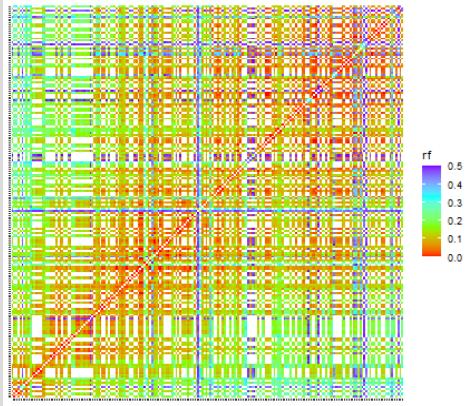
- Diploid and polyploid species
- Bi-parental populations
- Outcrossing
- All dosages markers
- Updates - Marcelo and Gabriel talk

Diploid roses

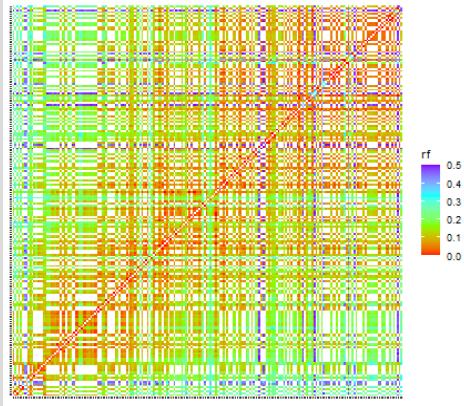
- 37% of chromosome 1
- ~ 38 cM
- Sequencing depth ~ 94X



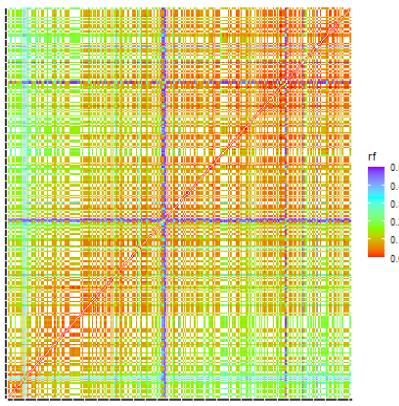
GATK + updog



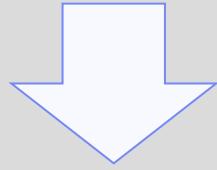
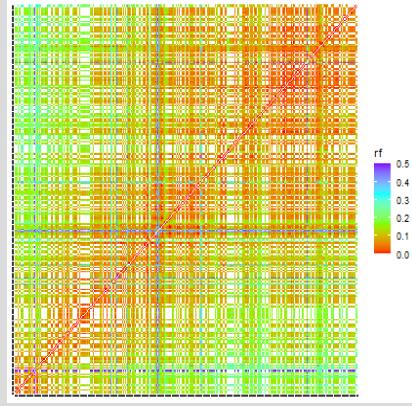
GATK + polyRAD



freebayes + polyRAD



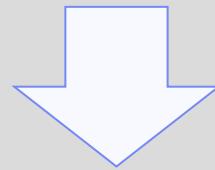
freebayes + updog



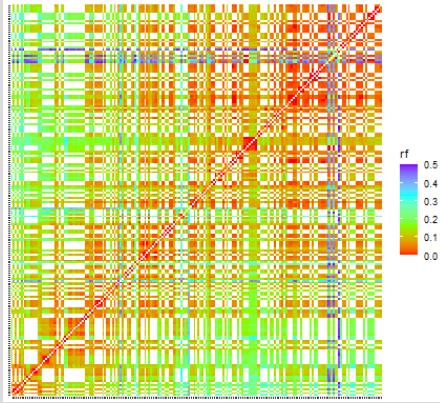
Simulation studies: 10,880 maps
Empirical studies: 816 maps

Filters:

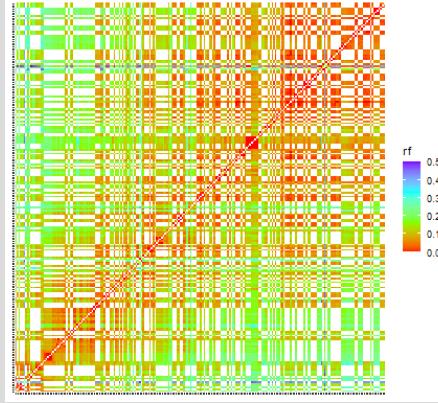
- genotype probabilities
- non-informative markers
- replace AD by missing when GT is missing



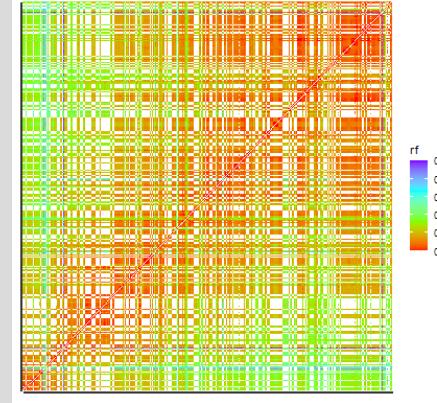
GATK + updog



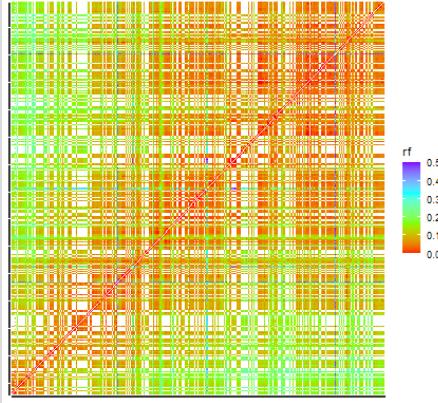
GATK + polyRAD



freebayes + polyRAD

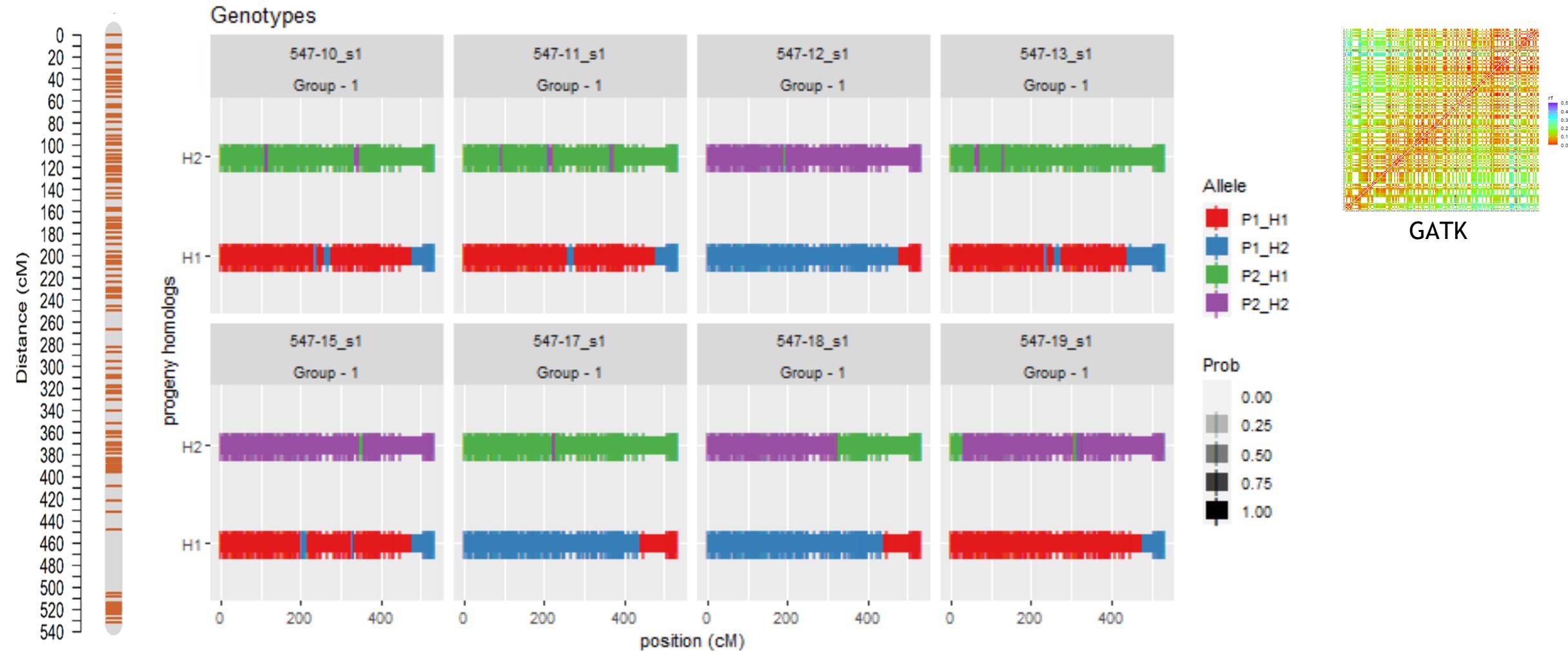


freebayes + updog



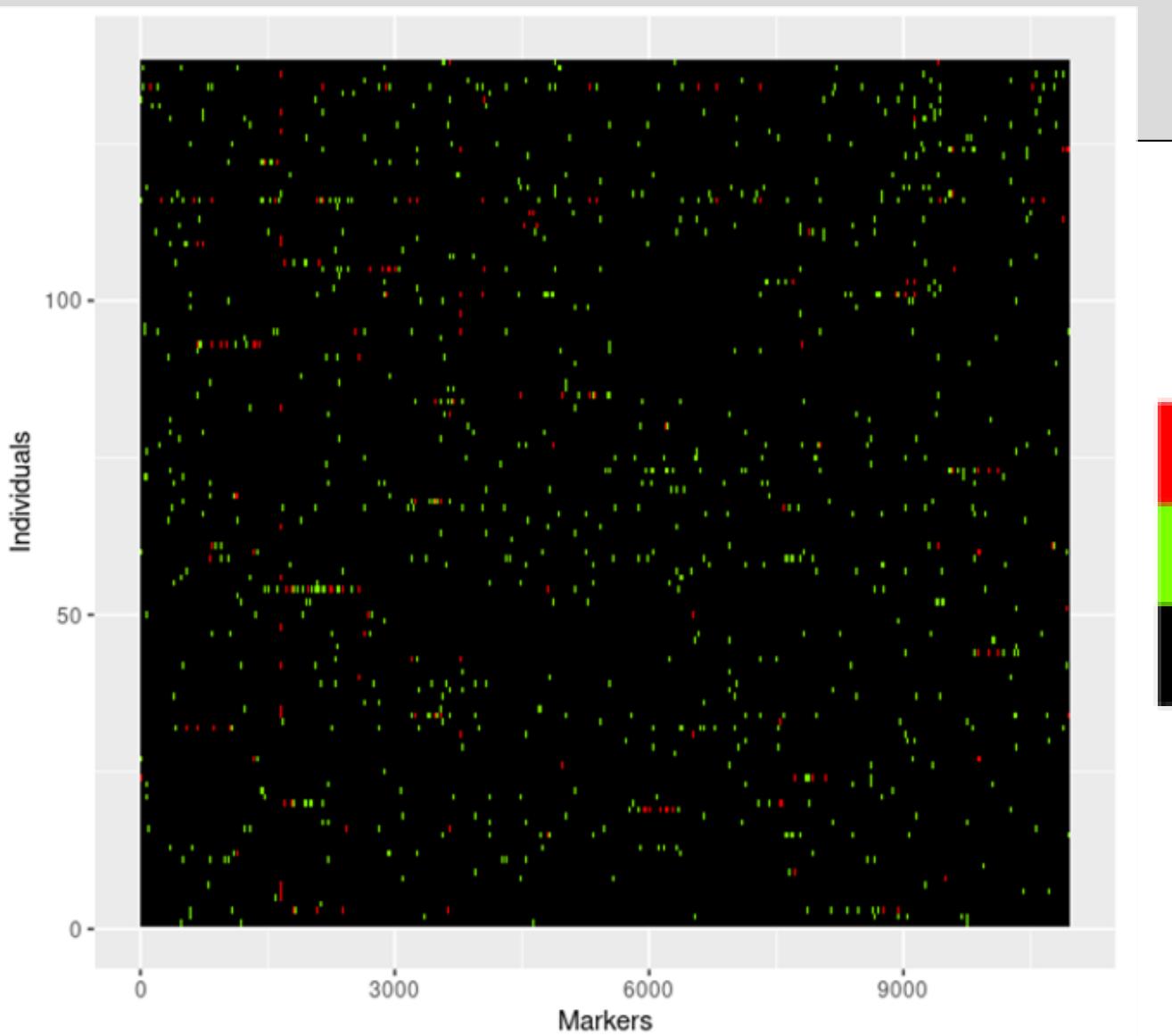
Map size

37% of chromosome 1 ~38cM



Map size

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



changed = 0.25%

imputed = 1.1%

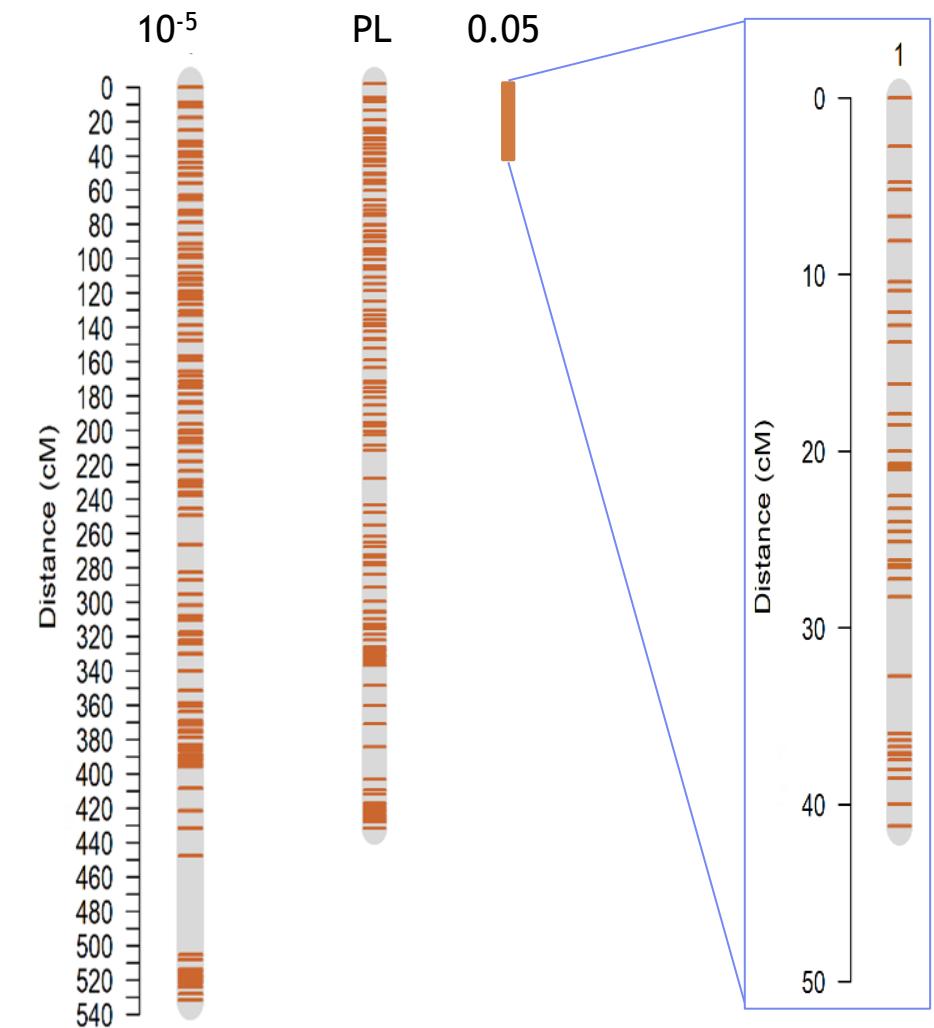
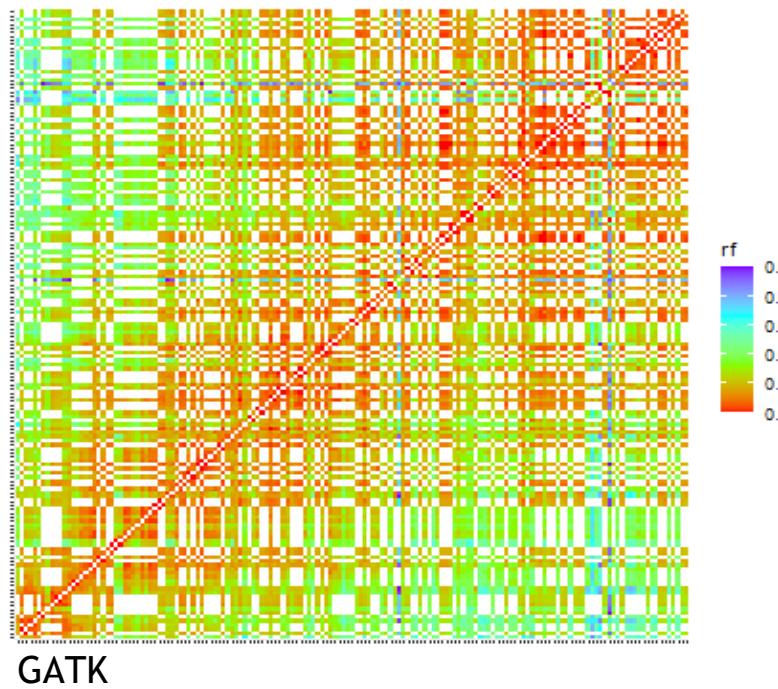
unchanged = 98.65%

Figure by Jeekin Lau

Map size

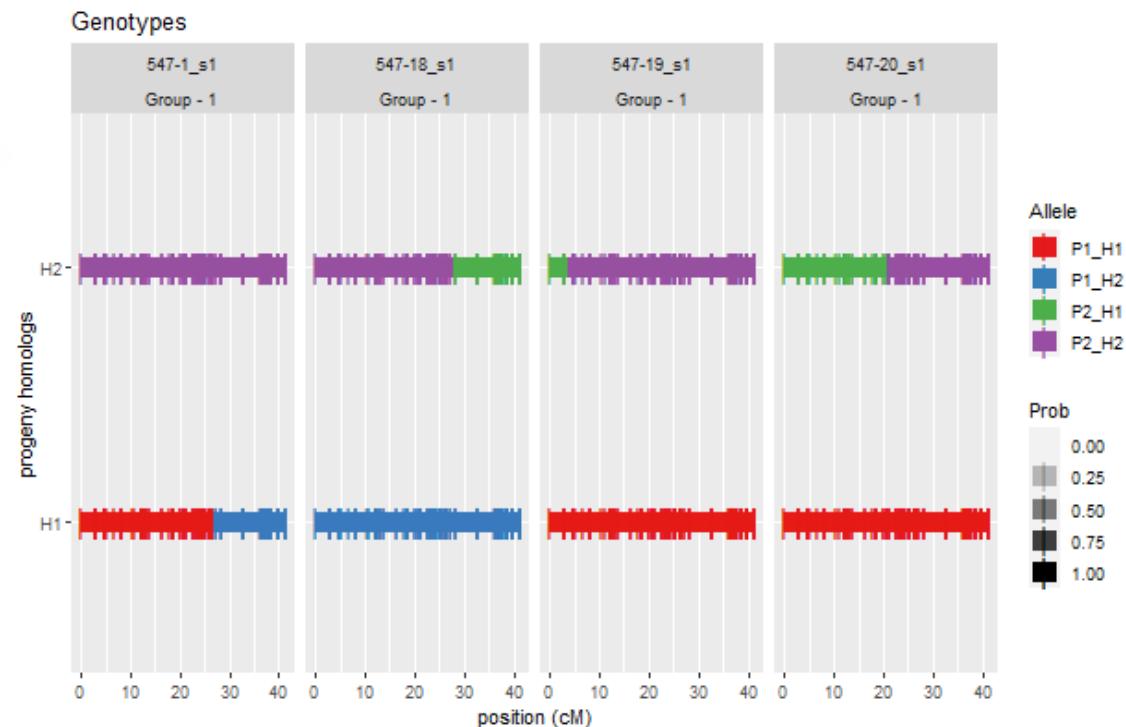
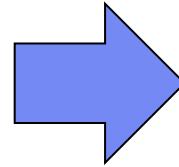
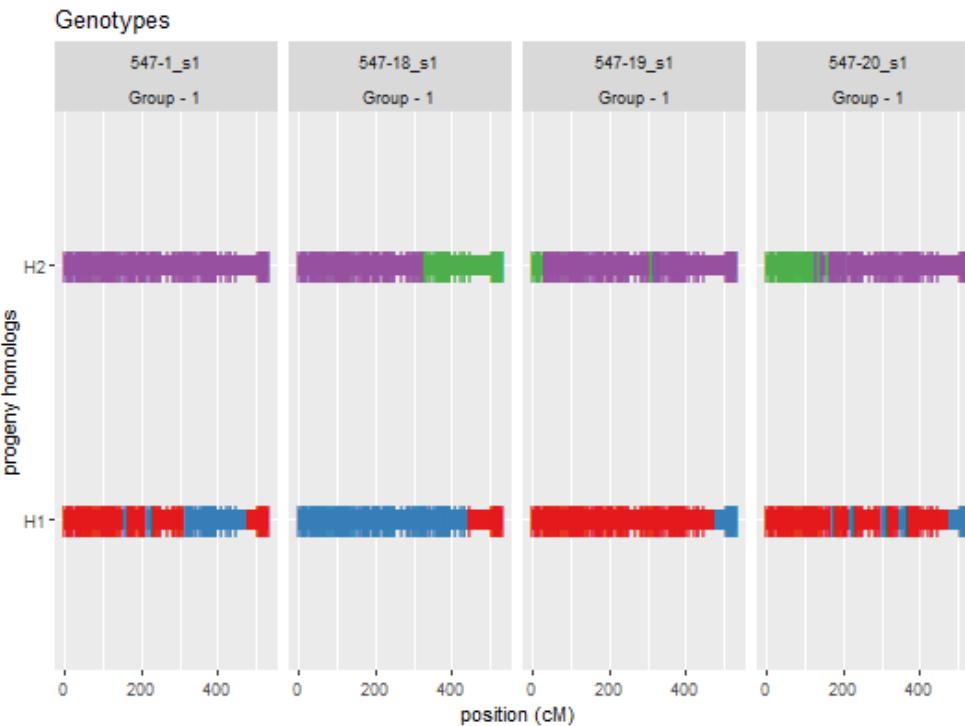
37% of chromosome 1 ~38cM

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



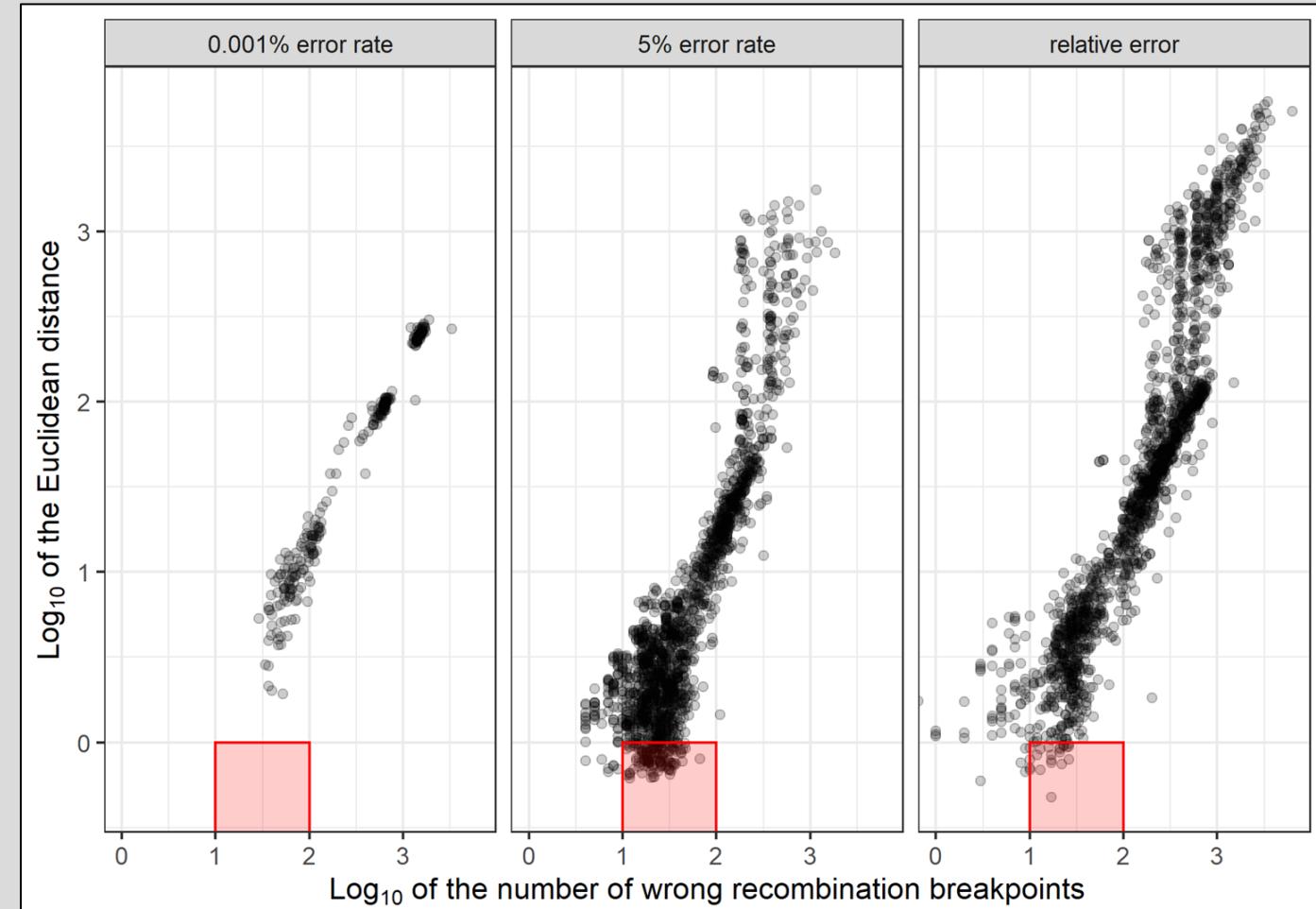
Map size

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



Cold
Spring
Harbor
Laboratory

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

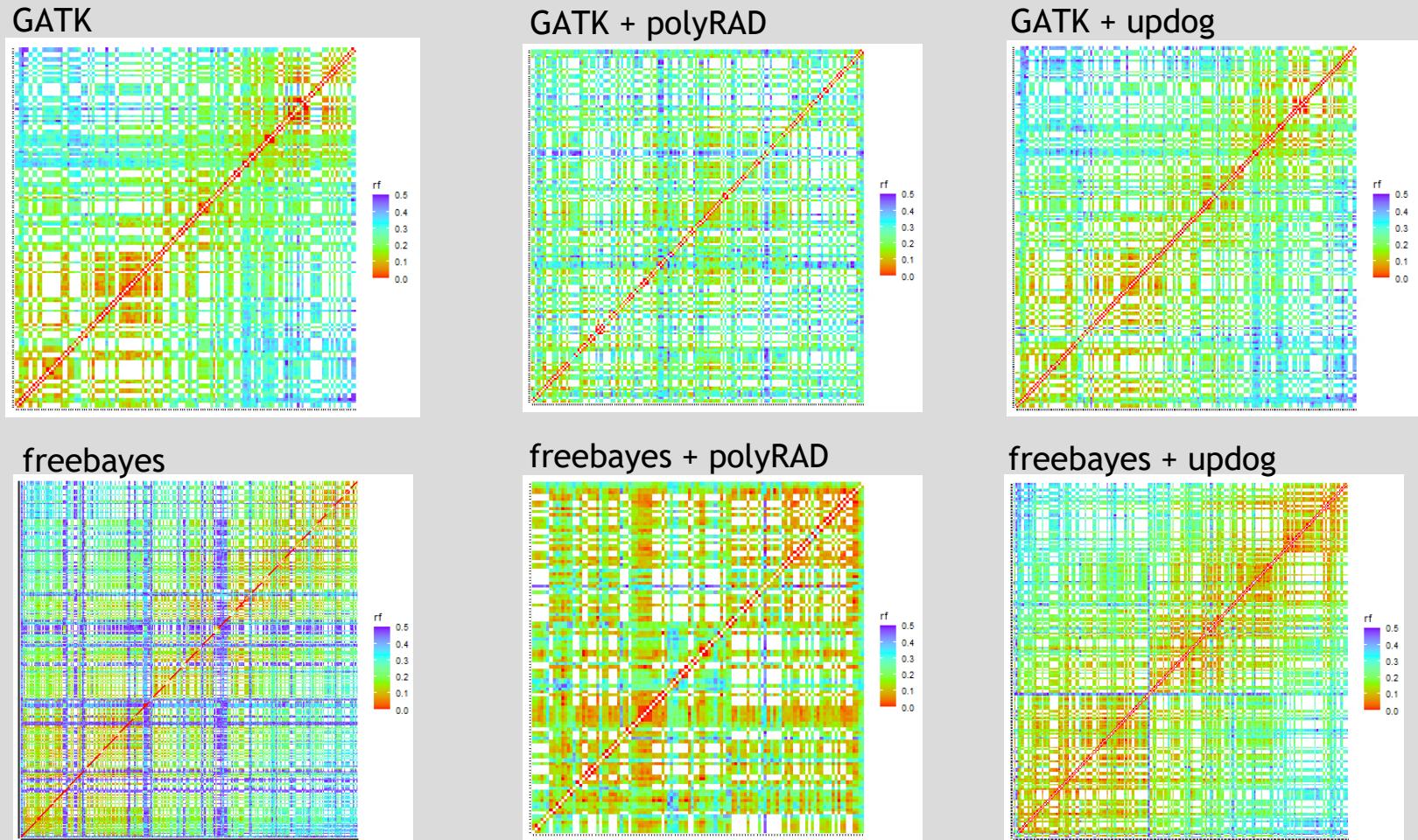
Developing best practices for genotyping-by-sequencing analysis using linkage maps as benchmarks

✉ 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>

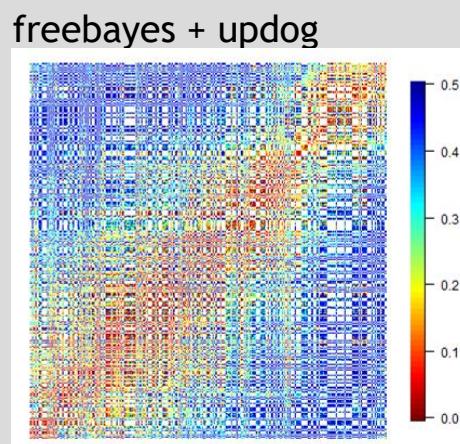
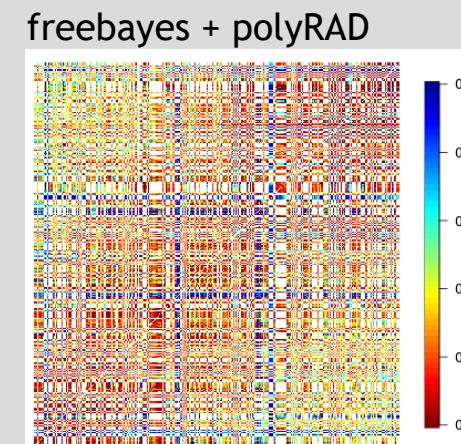
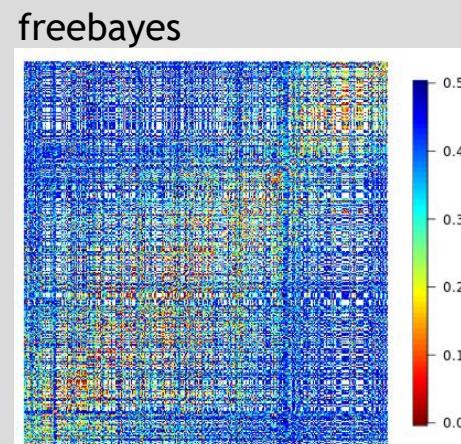
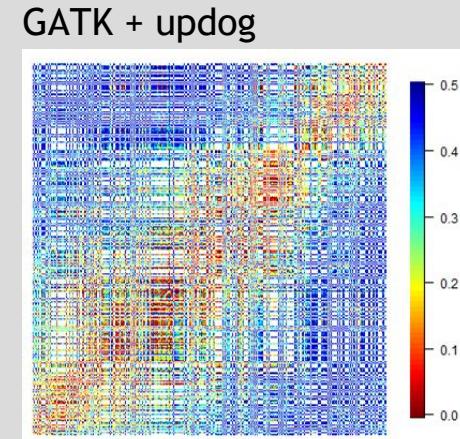
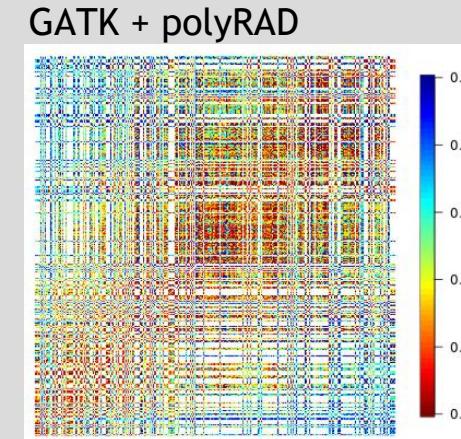
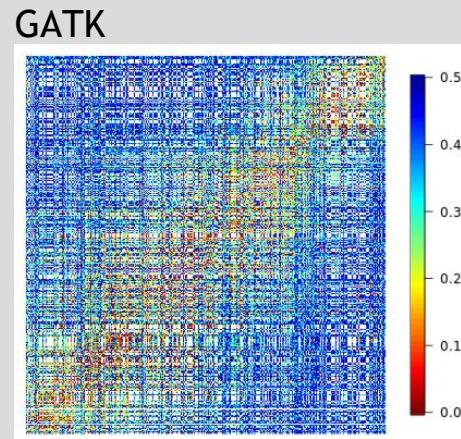
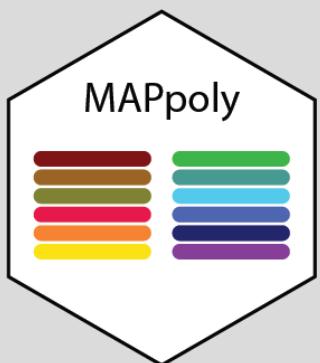
Diploid Aspen

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



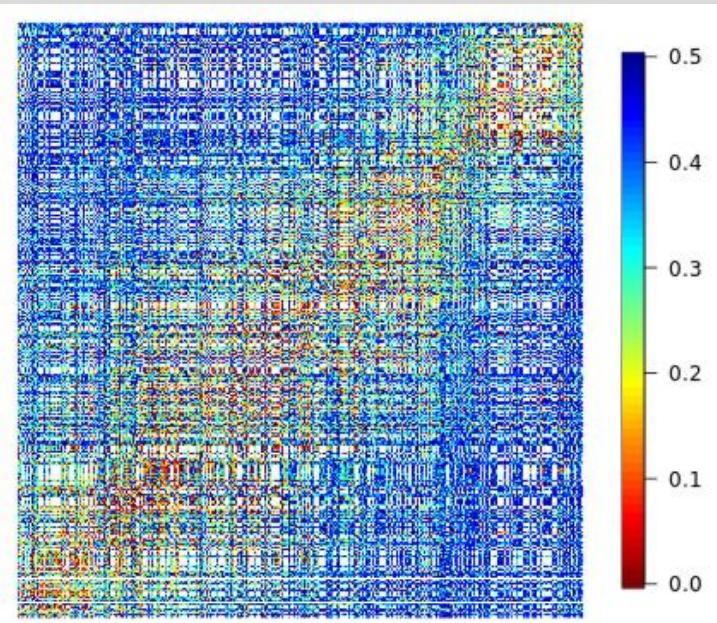
Tetraploid rose

- Chromosome 2
- Sequencing depth ~ 50X



Map size

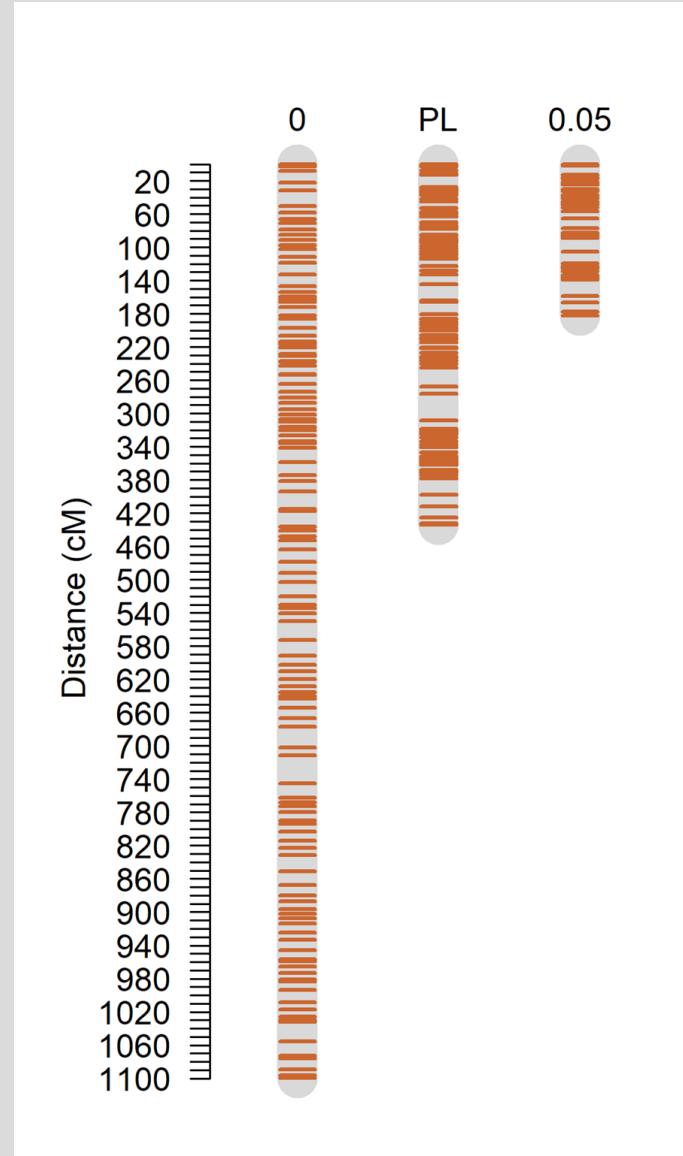
- Chromosome 2
- Sequencing depth ~ 50X



GATK

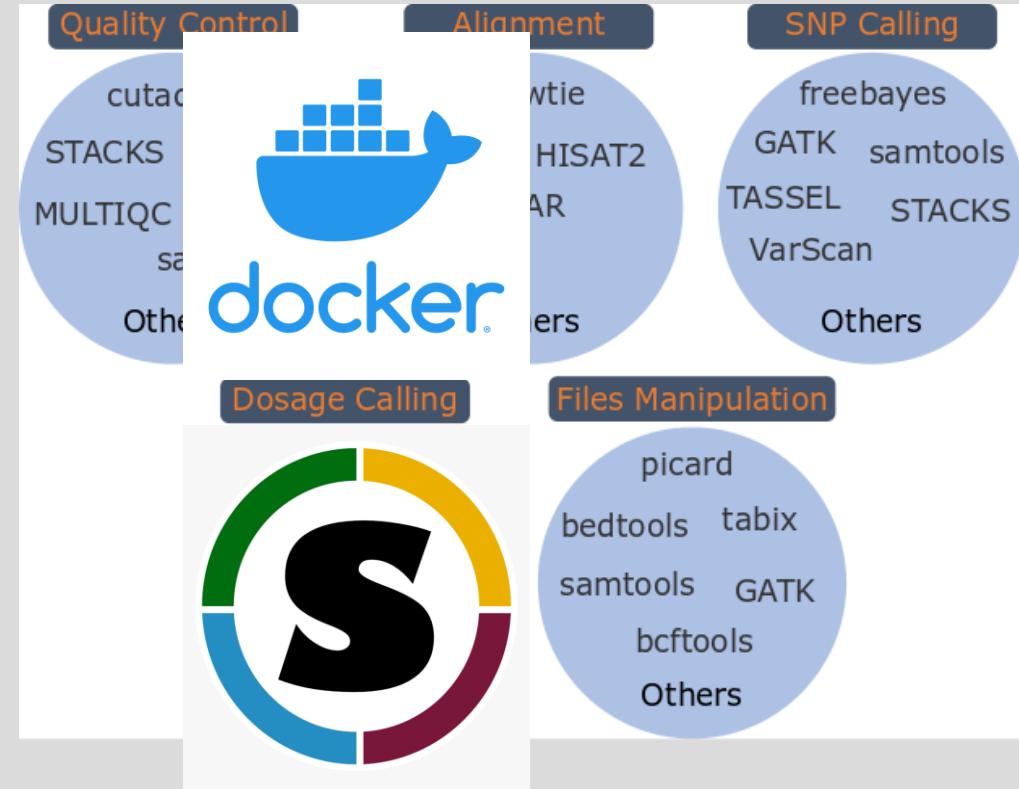


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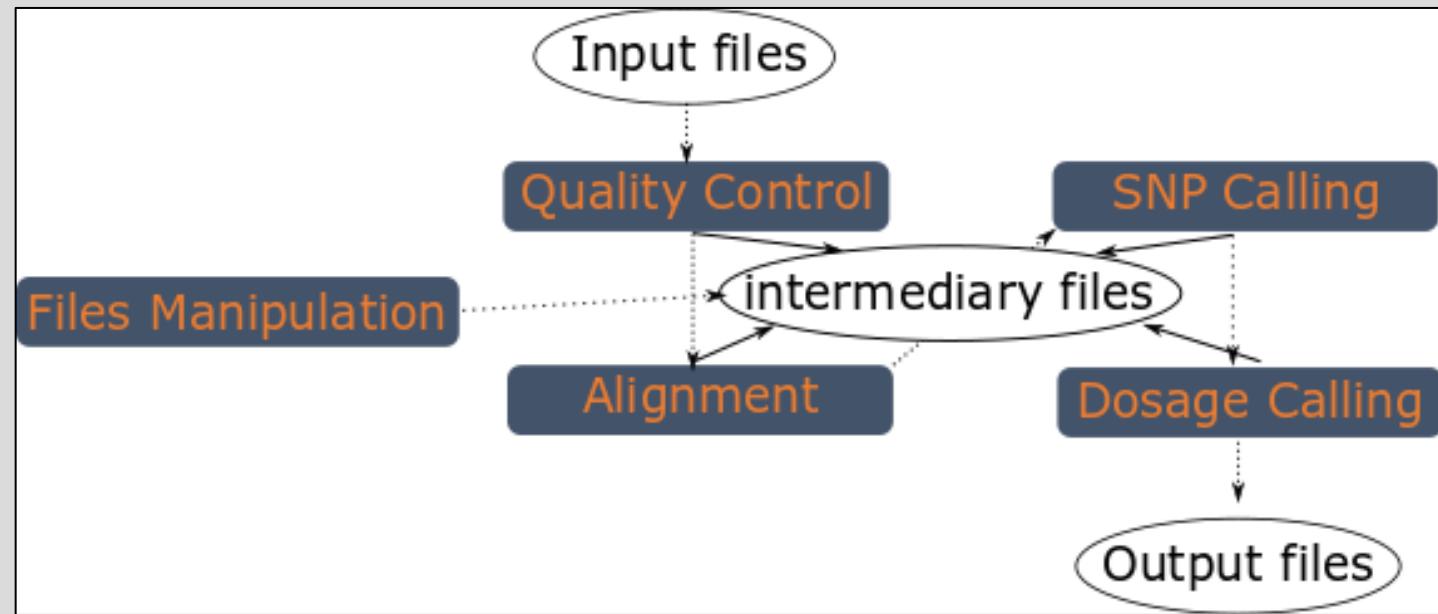
Overcoming Technical Difficulties

- 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



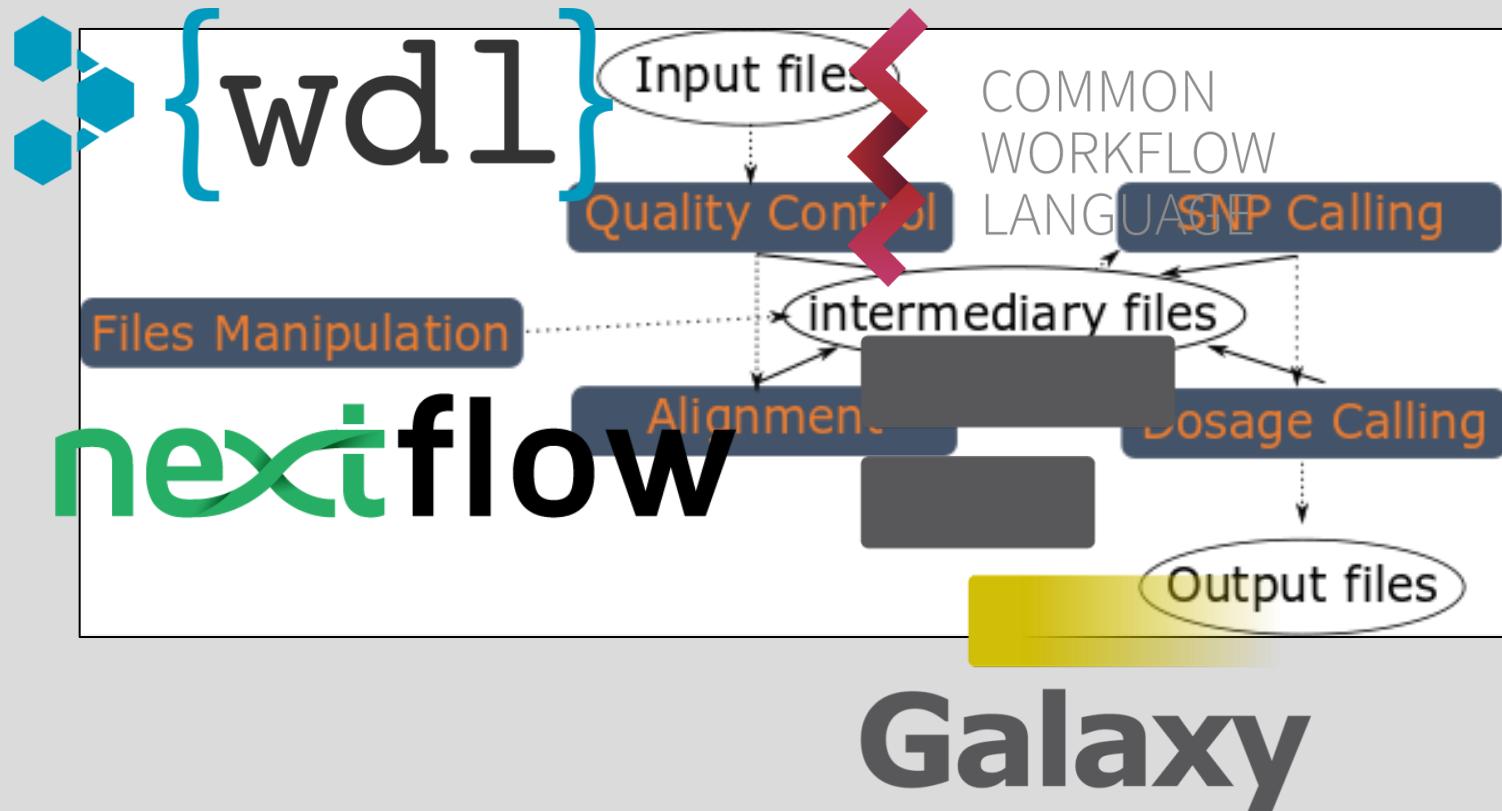
Overcoming Technical Difficulties

- Many steps
- Many file formats



Overcoming 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



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 l

📁 .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 ➔📁 structs	refactor: remove not used struct
① ➔📁 subworkflows	freebayes split ok
📁 tasks	joint wf
📁 tests	refactor: move test files to tests module
📄 .dockstore.yml	fix path



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Award # 2020-51181-32156 (09/01/20 - 08/31/24)



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,  
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    "SNPCalling.gatk_mchap": false,  
    "SNPCalling.references": {  
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        "ref_pac": "tests/data/polyploid/RchinensisV1.0/Chr04_sub.fasta.pac",  
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        "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 { ... }  
        | author: "Cristiane Taniguti"  
        | email: "chtaniguti@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
```

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 { ...  
    }  
}
```

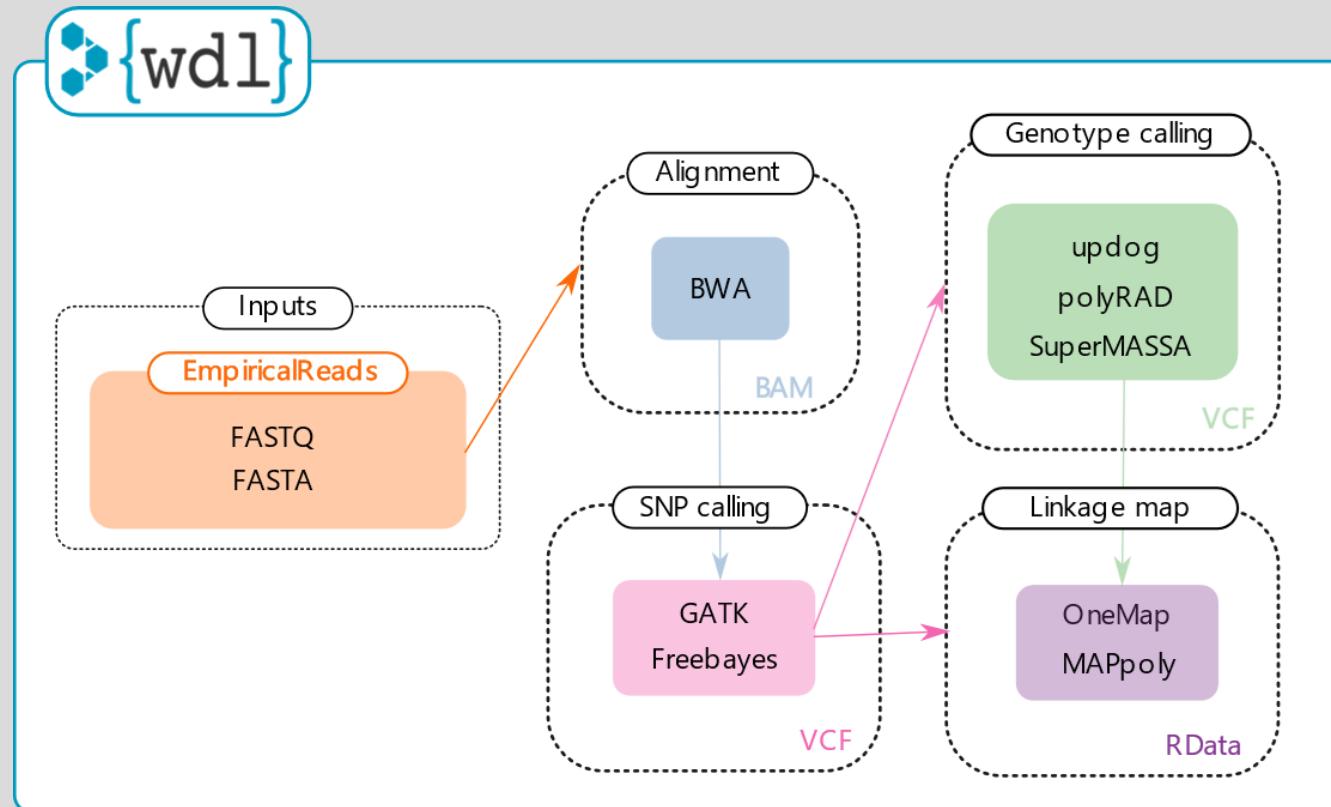
```
$ java -jar /path/to/cromwell.jar run -i EmpiricalSNPCalling/inputs.json EmpiricalSNPCalling.wdl
```



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Reads2Map



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

Summary

- Linkage map quality is very sensitive to upstream choices
- There is no single suggestion for every data set
- Reads2Map uses software's default/tutorials parameters
- Developers, sorry if I used any of the parameters wrongly (let me know)
- Easy to test updates or modification of parameters
- Reads2Map is an organized structure for reproducibility and scalable production

Thank you



Oscar Riera-Lizarazu
David Byrne
Jeekin Lau
Tessa Hochhaus

Augusto Garcia
Rodrigo Amadeu
Getulio Caixeta

Marcelo Mollinari
Gabriel Gesteira



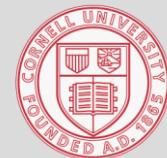
Lucas Taniguti

Guilherme Pereira

Thiago Oliveira



Project Members



Cornell University



NC STATE
UNIVERSITY



UNIVERSITY OF
ARKANSAS



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Other Collaborators



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