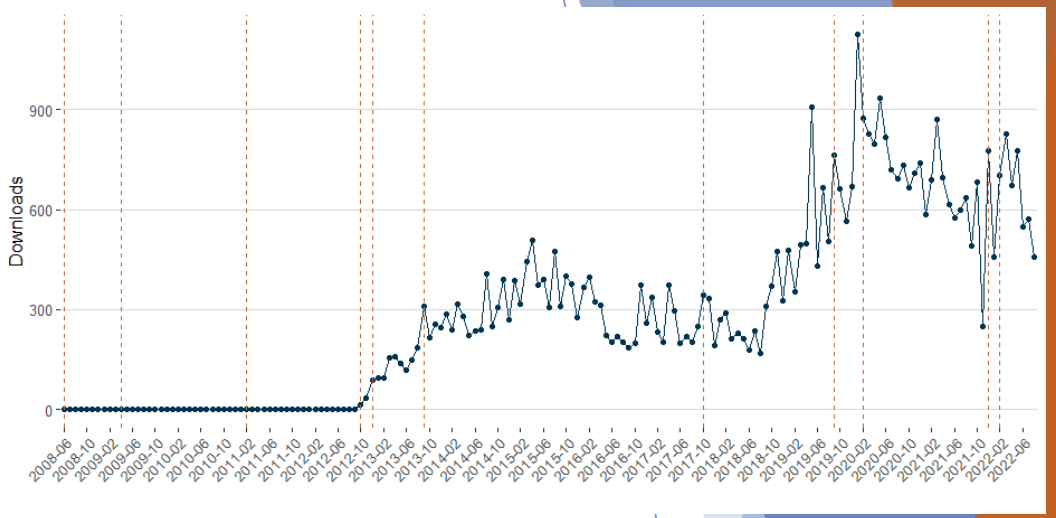
Building highly saturated genetic maps with OneMap 3.0

New approaches using workflows

Cris Taniguti

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

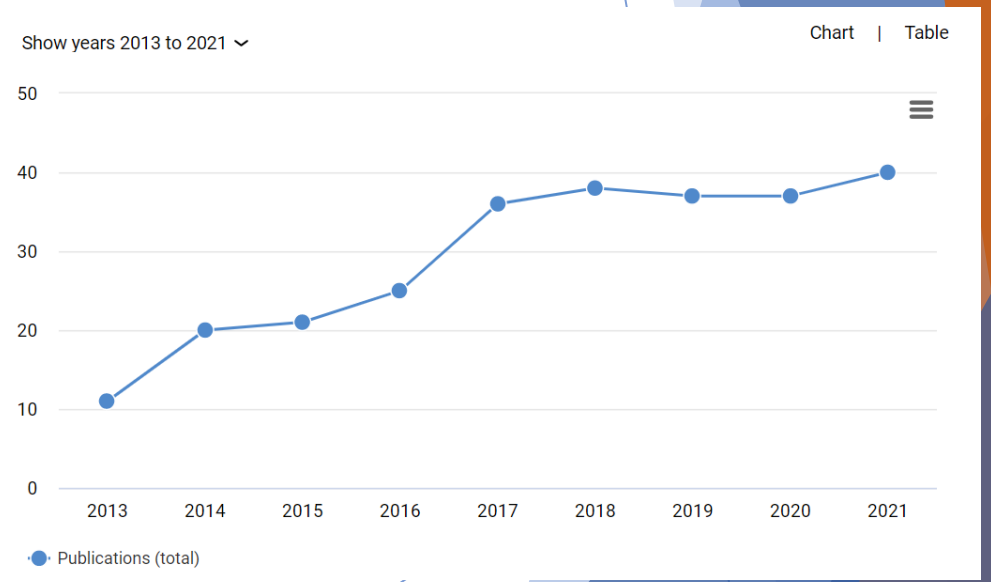
OneMap package



Margarido et al., 2007

OneMap: software for genetic mapping in outcrossing species

- R package
- Diploids
- Inbred and outcrossing populations
- Integrated genetic maps
- Tutorials
- Diagnostic graphics
- Updates by Statistical Genetics Lab members
- CRAN
- GitHub: Cristianetaniguti/onemap



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OneMap users

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OneMap updates

- **R language**: Didactic tutorials, courses and utilities functions
- **Maintenance**: Package “house keeping” and dependencies upgrades
- **VCF file conversion**: new features to deal with all VCF format versions
- **Group markers**: new features to consider physical position and UPGMA method
- **Markers ordering**: MDS method
- **Processing time and memory**: RAM optimization and process parallelization
- **Graphical visualization**: sequencing depth, genotypes, segregation, haplotypes and recombination breakpoints counts

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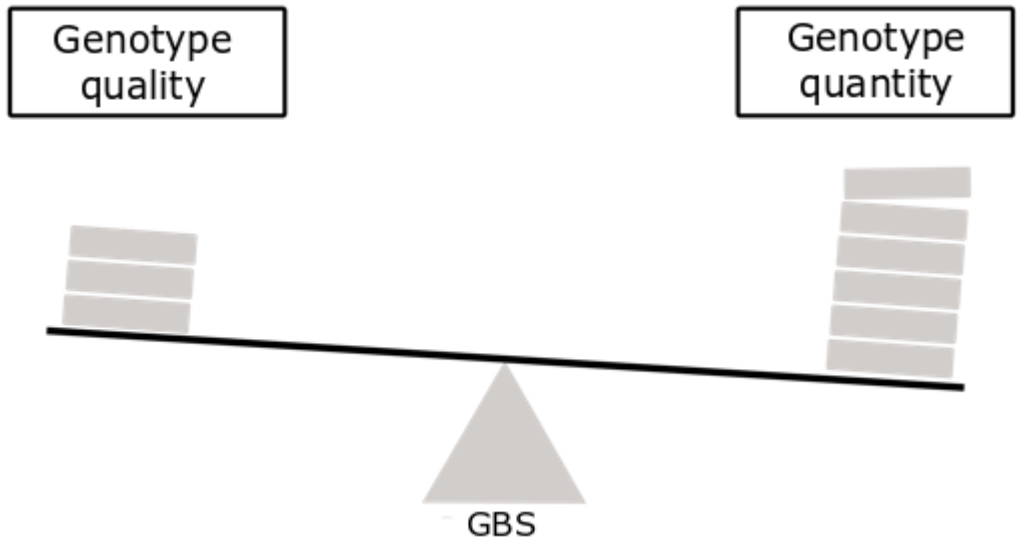
Motivation

- Sequencing based markers
- Characteristics:
  - Thousands of markers
  - Different types of libraries
  - Lower cost
  - Genotyping errors
  - Biallelic markers
- Consequences:
  - Requires bioinformatic skills
  - Requires computational resources
  - Wrong number of recombination events
  - Difficulties in ordering markers



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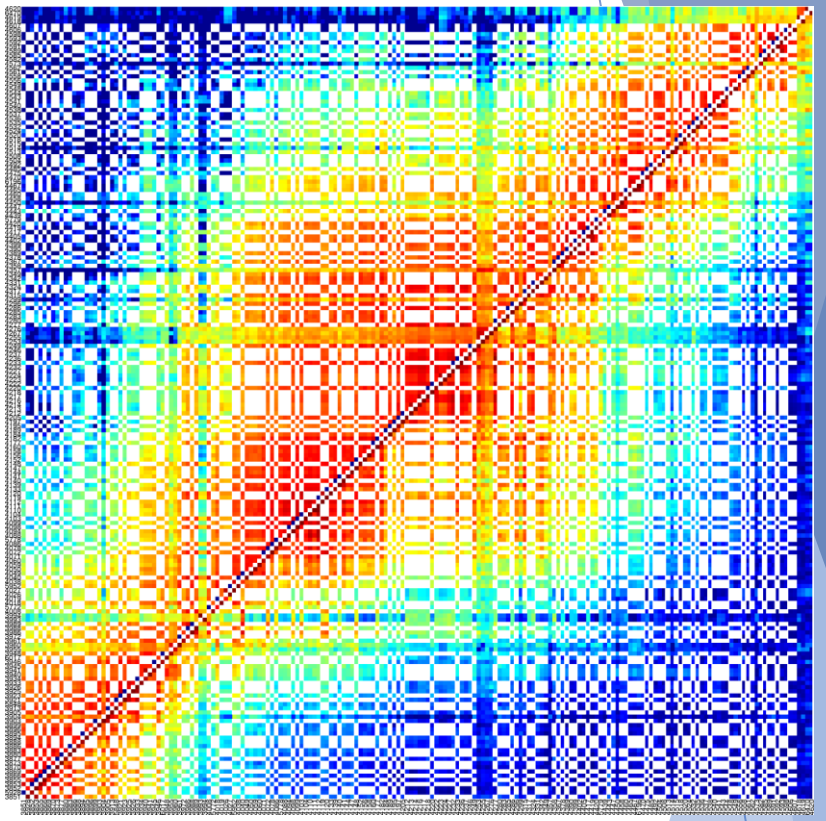
OneMap updates - HMM

- Genotyping errors
- Hidden Markov Model (HMM)
- create_probs(global_error = 0.05)
- create_probs(genotype_prob =
  prob_data.frame)
- create_probs(genotype_error =
  prob_matrix)



833 cM

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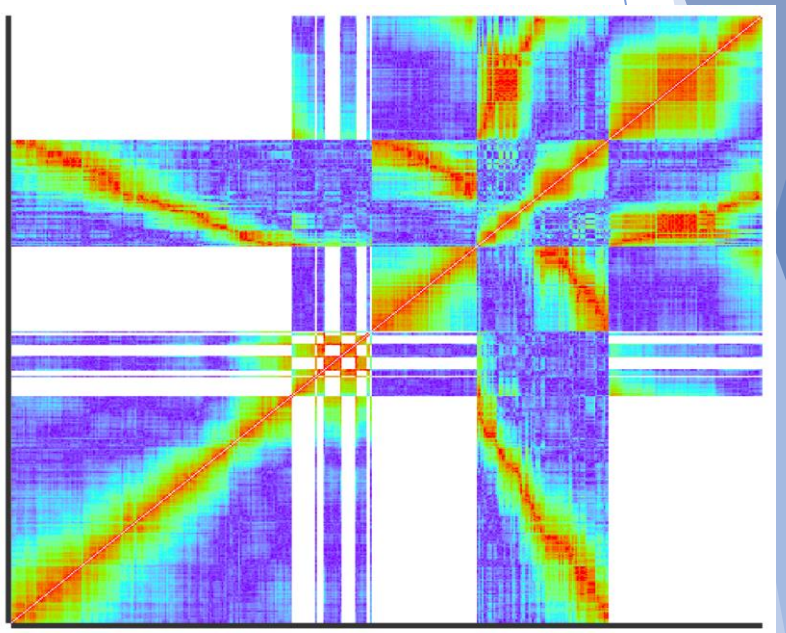
OneMap updates - Multiallelics

- Difficulties in ordering
- Biallelics marker types

  ab x ab
  aa x ab
  ab x aa



- Haplotype-based markers
- onemap_read_vcfR(only_biallelics = FALSE)

  ab x cd

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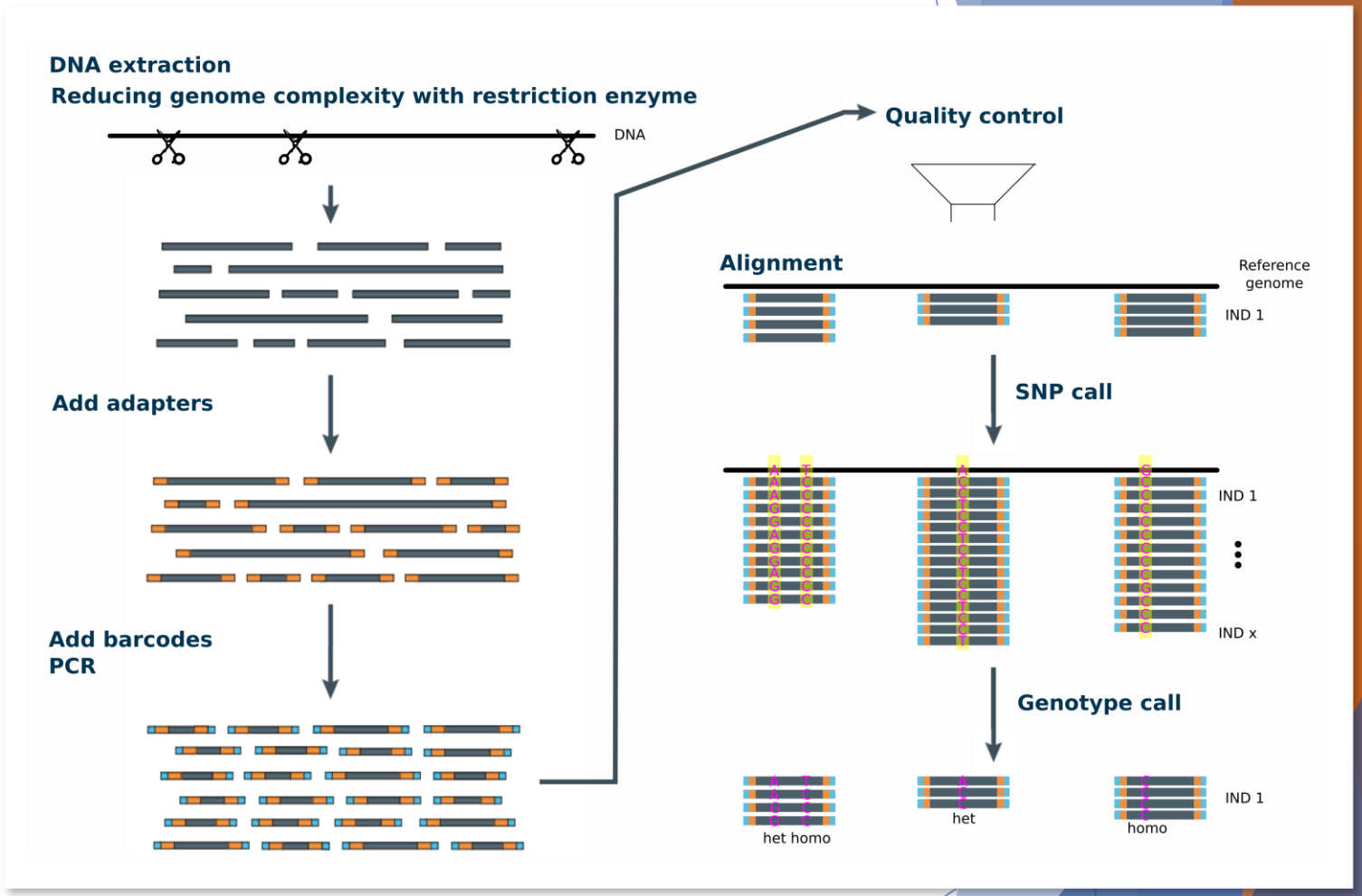
Motivation

- Genotyping-by-Sequencing (GBS) markers
- Genetic map quality is related to upstream processes



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Motivation - Polyploid species

- Organisms that have multiple copies of the complete set of chromosomes

MAPpoly

Mollinari and Garcia, 2019
Linkage Analysis and Haplotype Phasing in Experimental Autopolyploid Populations with High Ploidy Level Using Hidden Markov Models

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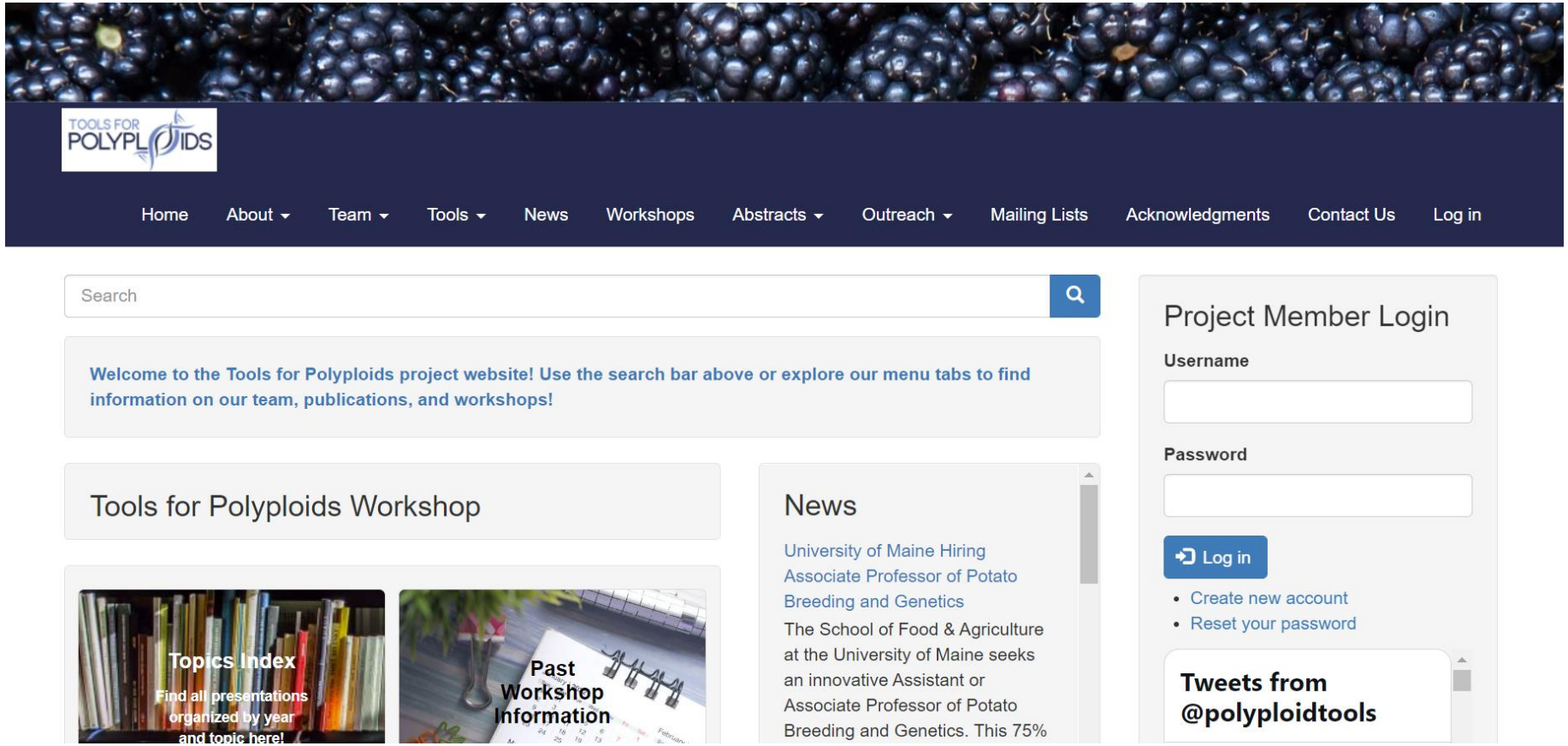
polyploids.org

- Tools for Polyploids Workshop 2023 (January 12-13)



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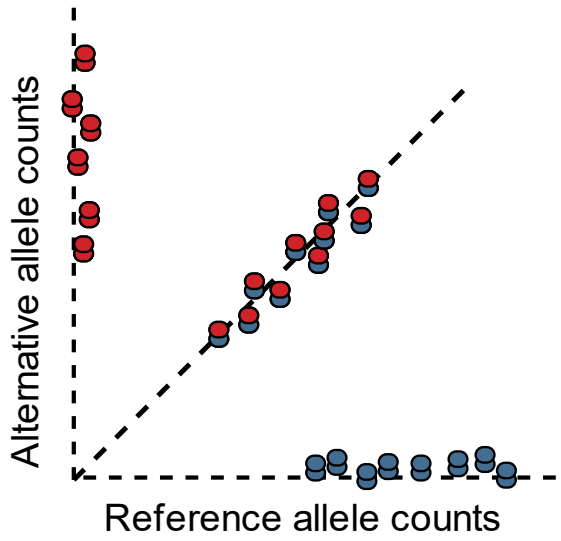
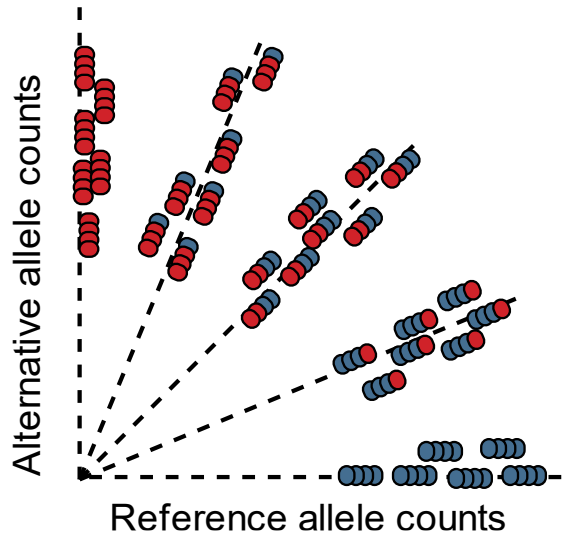
Dosage calling

- The theory

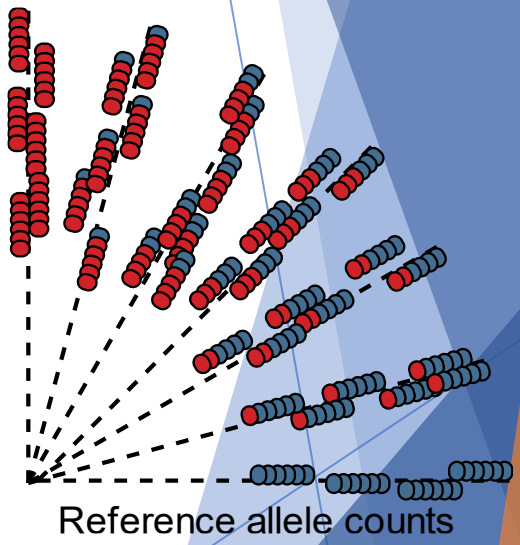
Diploid



Tetraploid



Hexaploid



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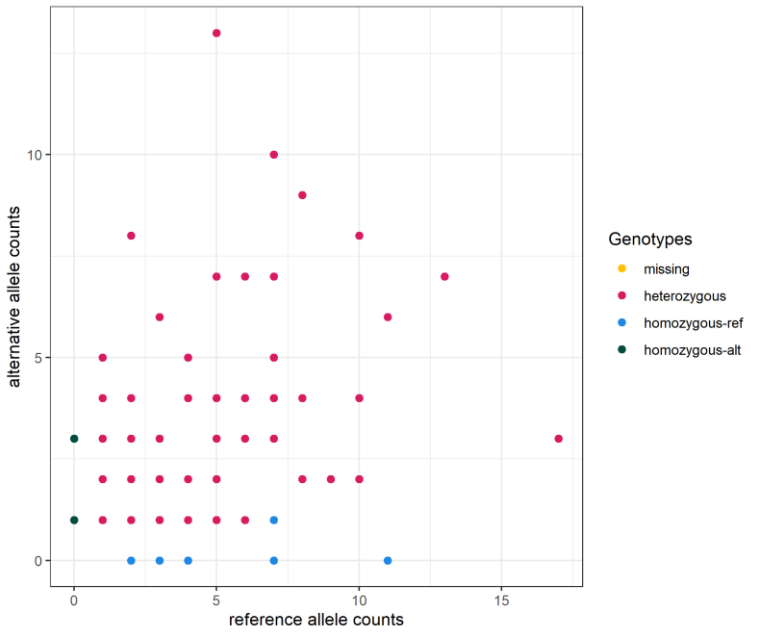
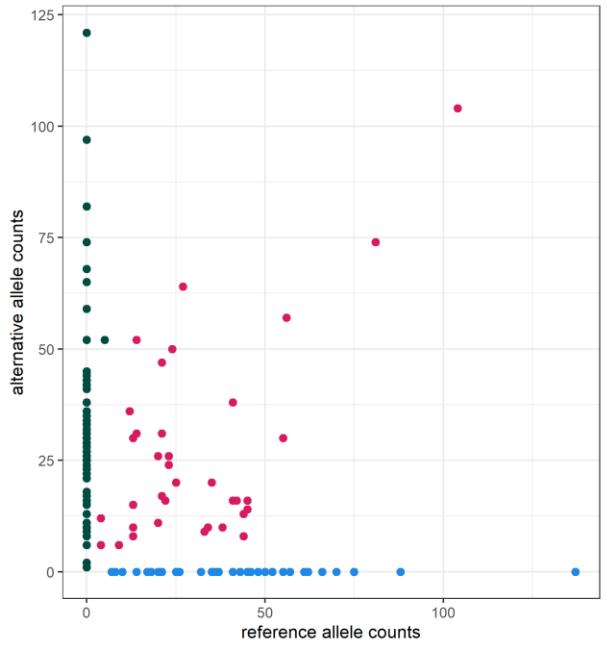
Source of errors

- The reality

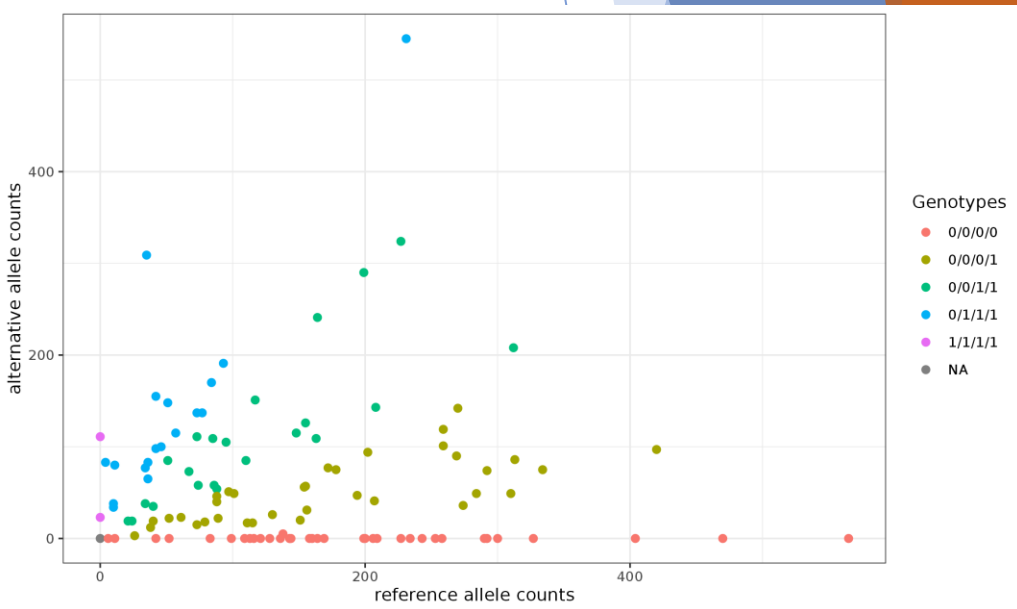
Diploid (mean depth 6)
N = 200
Aa x Aa



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



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



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Dosage calling

- Freebayes (Garrison and Marth, 2012)
  - Alignment quality
  - Haplotype based multiallelic markers
  - Base call quality around indels
  - Depth
- GATK (McKenna et al., 2010)
  - Alignment quality
  - Base call quality of SNPs and indels
  - Depth
  - Hard filtering

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Dosage calling

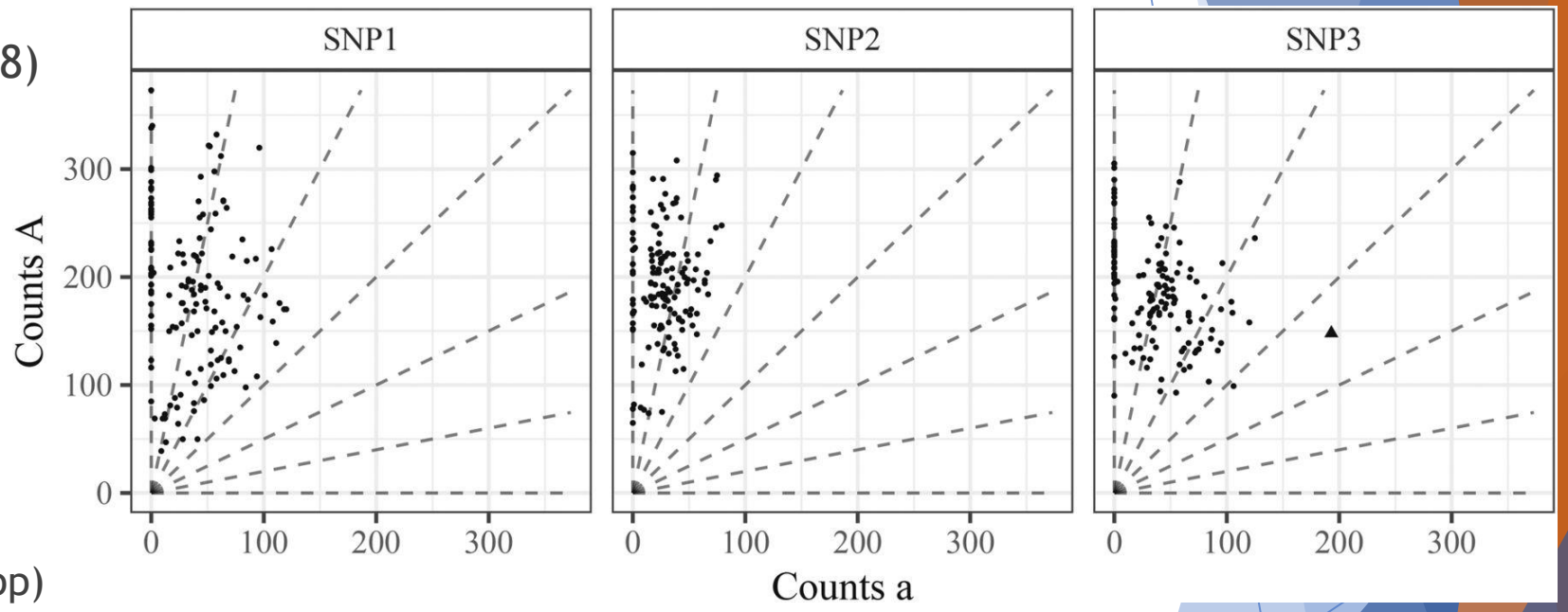
- updog (Gerard et al., 2018)
  - Any ploidy
  - Allelic bias
  - Overdispersion
  - Sequencing errors
  - Outliers
  - Population structure

    (F1, S1, HW, F1pp, S1pp)

Overdispersion

Allelic bias

Outlying observations



Gerard et al., 2018

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Dosage calling

- SuperMASSA (Serang et al., 2012)
  - Any ploidy and variable ploidy
  - Overdispersion
  - Population structure (F1 and HW)
- polyRAD (Clark et al., 2019)
  - Any ploidy
  - Sequencing errors
  - Population structure (F1, S1 and HW)

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Which is the best pipeline?

- Challenges:
  - Many software, many dependencies
  - Different input and output formats
  - Computational resources
  - Explore and visualize results
  - Feedback for developers
  - Adapt to software updates
  - Reproducibility
  - Quality criteria
- Useful tools:
  - Containers (Docker and singularity)
  - Workflow Description Language (WDL)
  - GitHub
  - HPC and Google Cloud
  - Shiny
  - Linkage map

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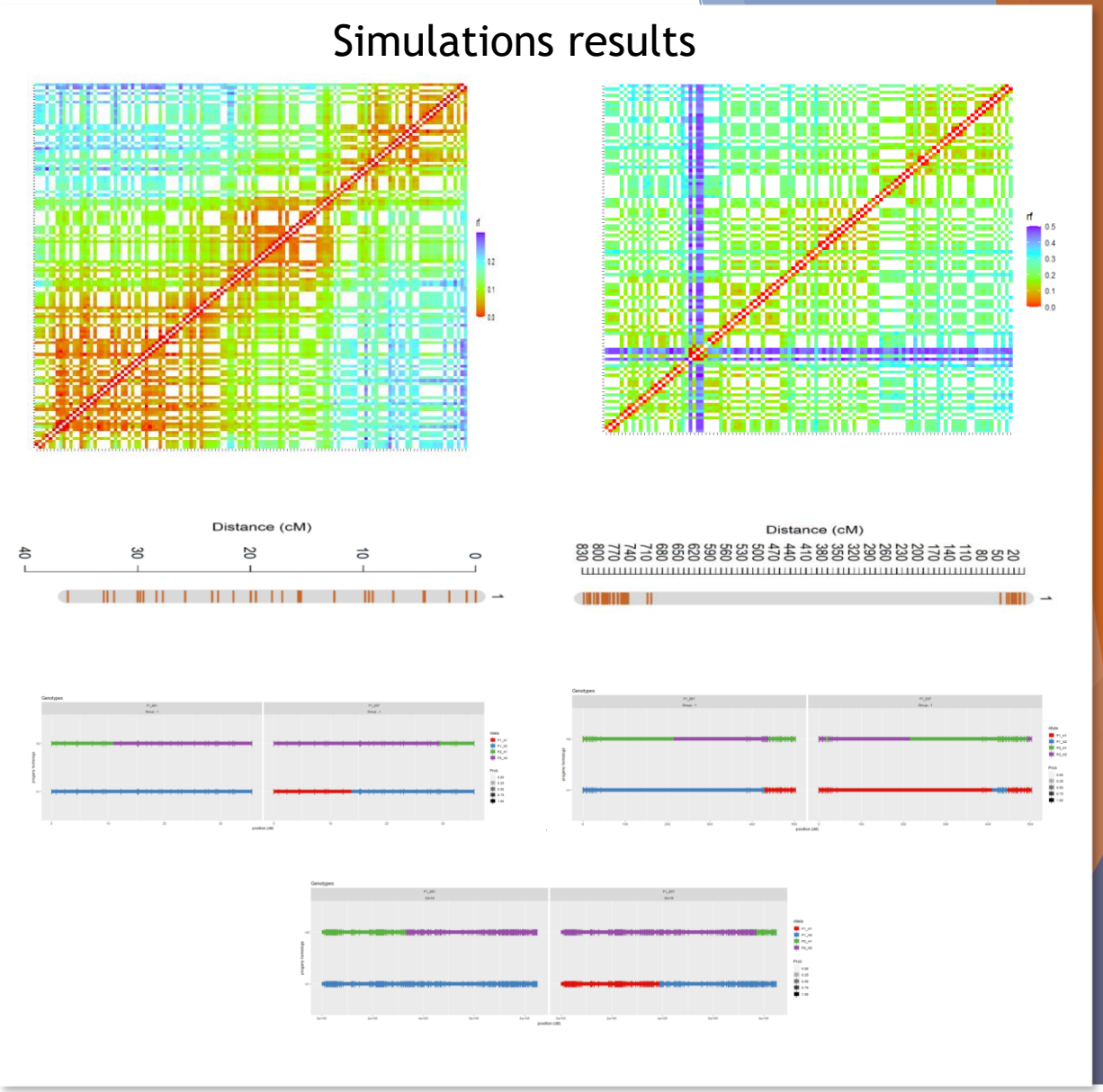
Maps as benchmarks



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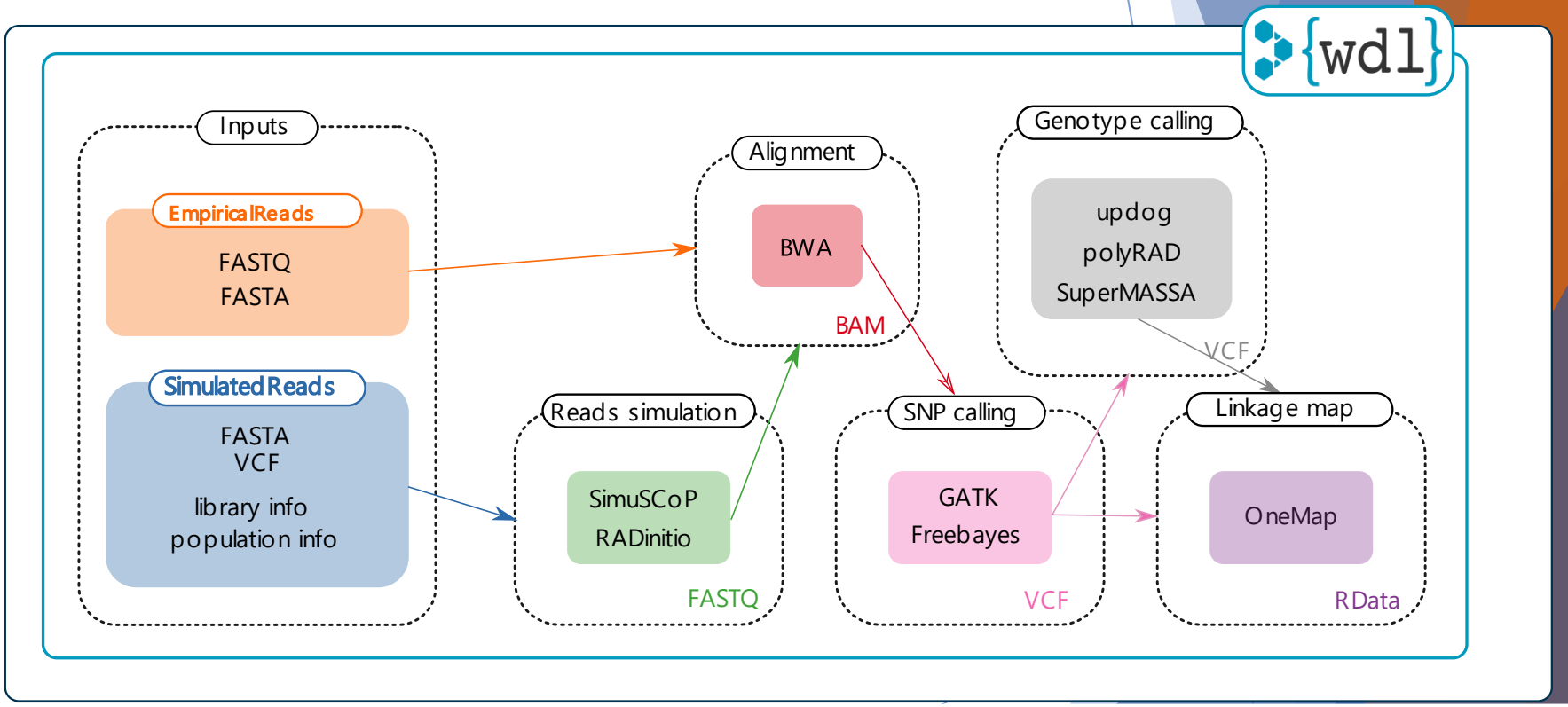
Implementation

- Join several bioinformatics and statistical analyses
- Workflow Description Language (WDL - “widdle”) – Broad Institute (human genome research)
- Best practices
- EmpiricalReads2Map
- SimulatedReads2Map
- Diploid species



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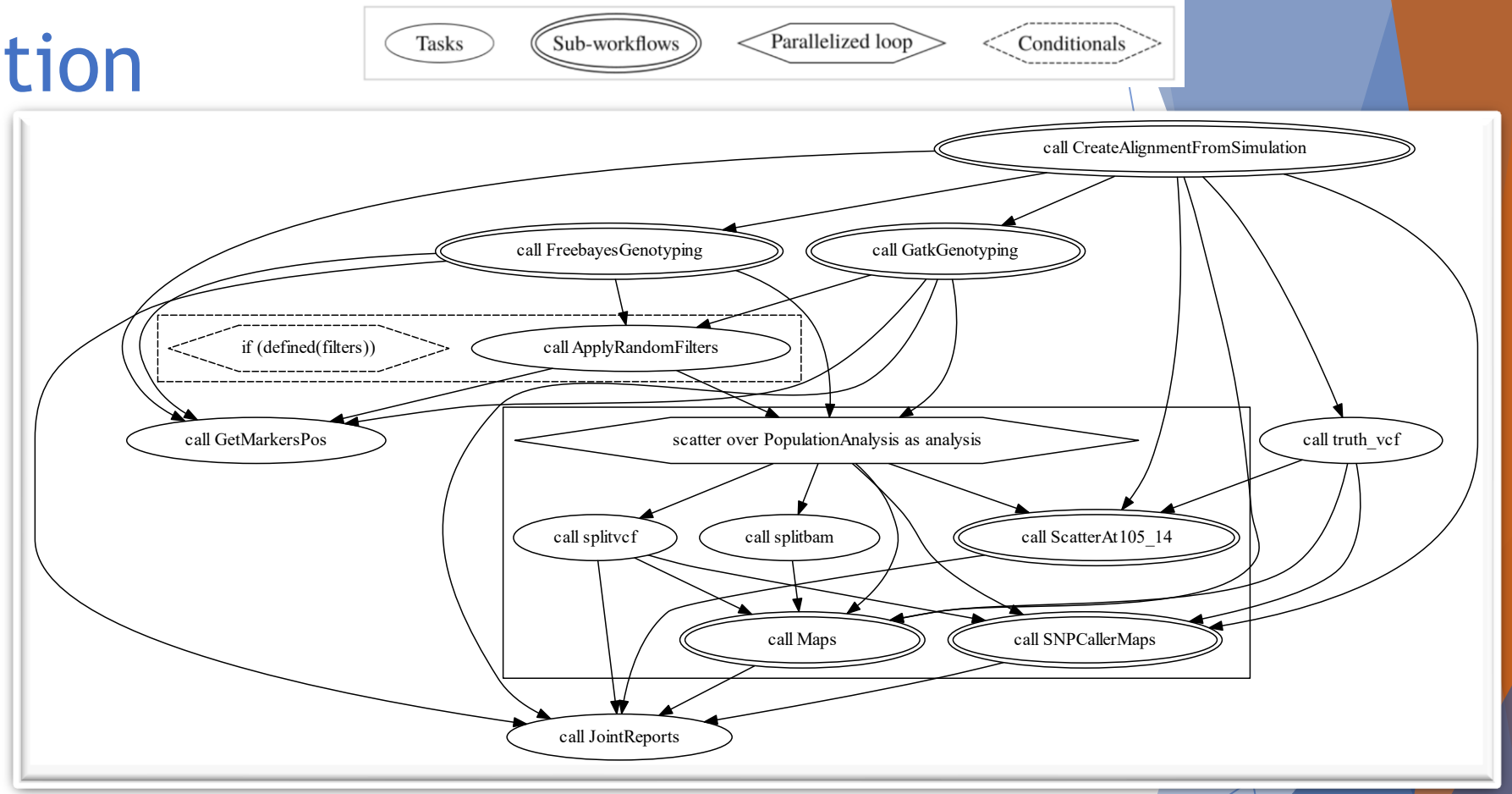
Implementation

- Workflows
  - Sub-workflows
    - Tasks




```
$ java -jar /path/to/womtool.jar graph tasks/SimulatedSingleFamily.wdl > SimulatedSingleFamily.dot
$ dot –Tsvg SimulatedSingleFamily.dot –o SimulatedSingleFamily.svg
```

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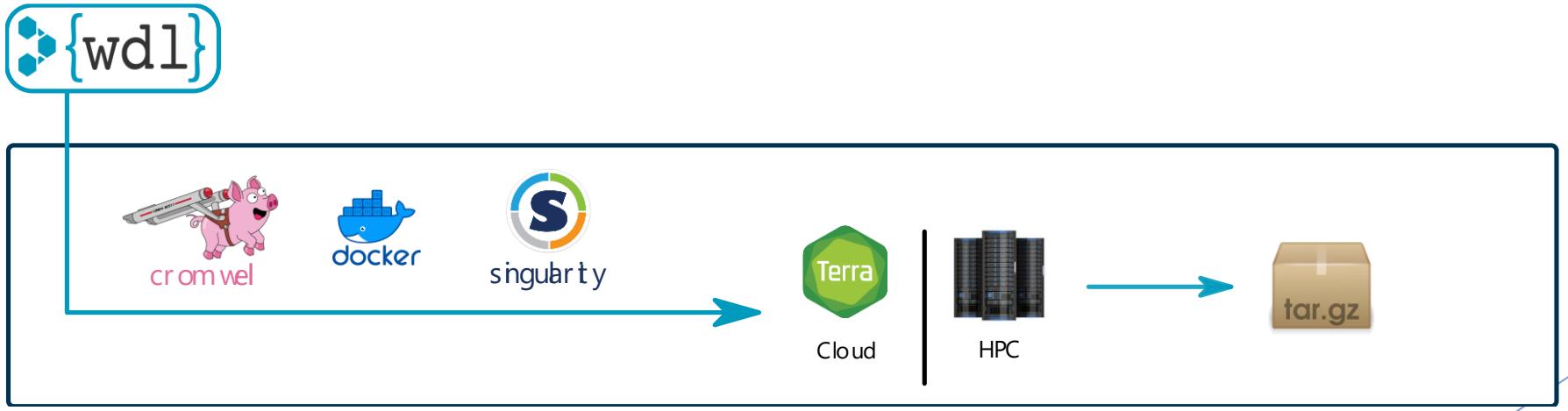
Implementation

- Containers
- High Performance Computing (HPC) or Cloud environments (terra.bio)

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



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More about WDL and usage in Cloud

Streaming live on YouTube
https://youtu.be/3AMJ-LlWRtE

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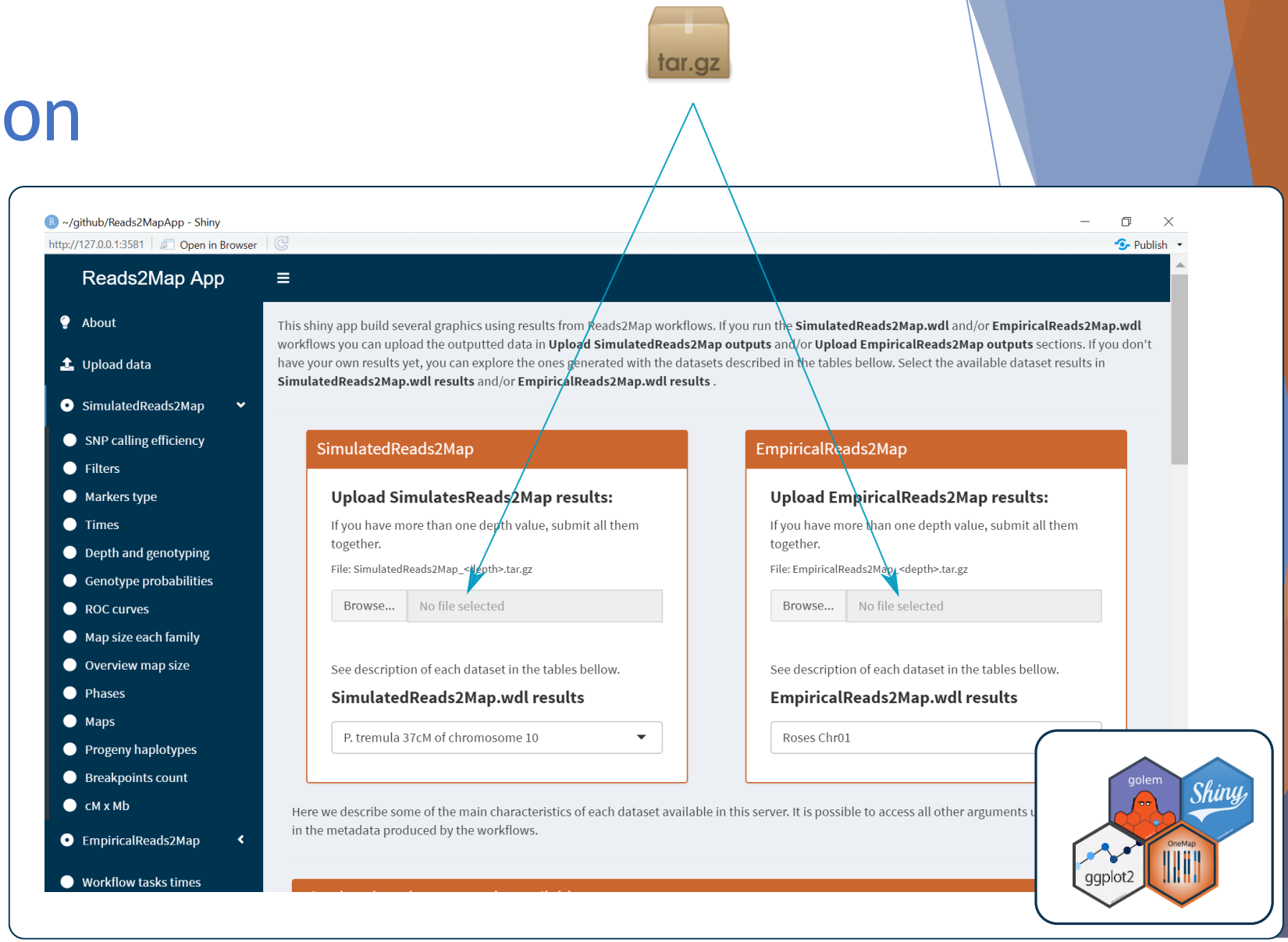
Implementation

- Visualization and exploration



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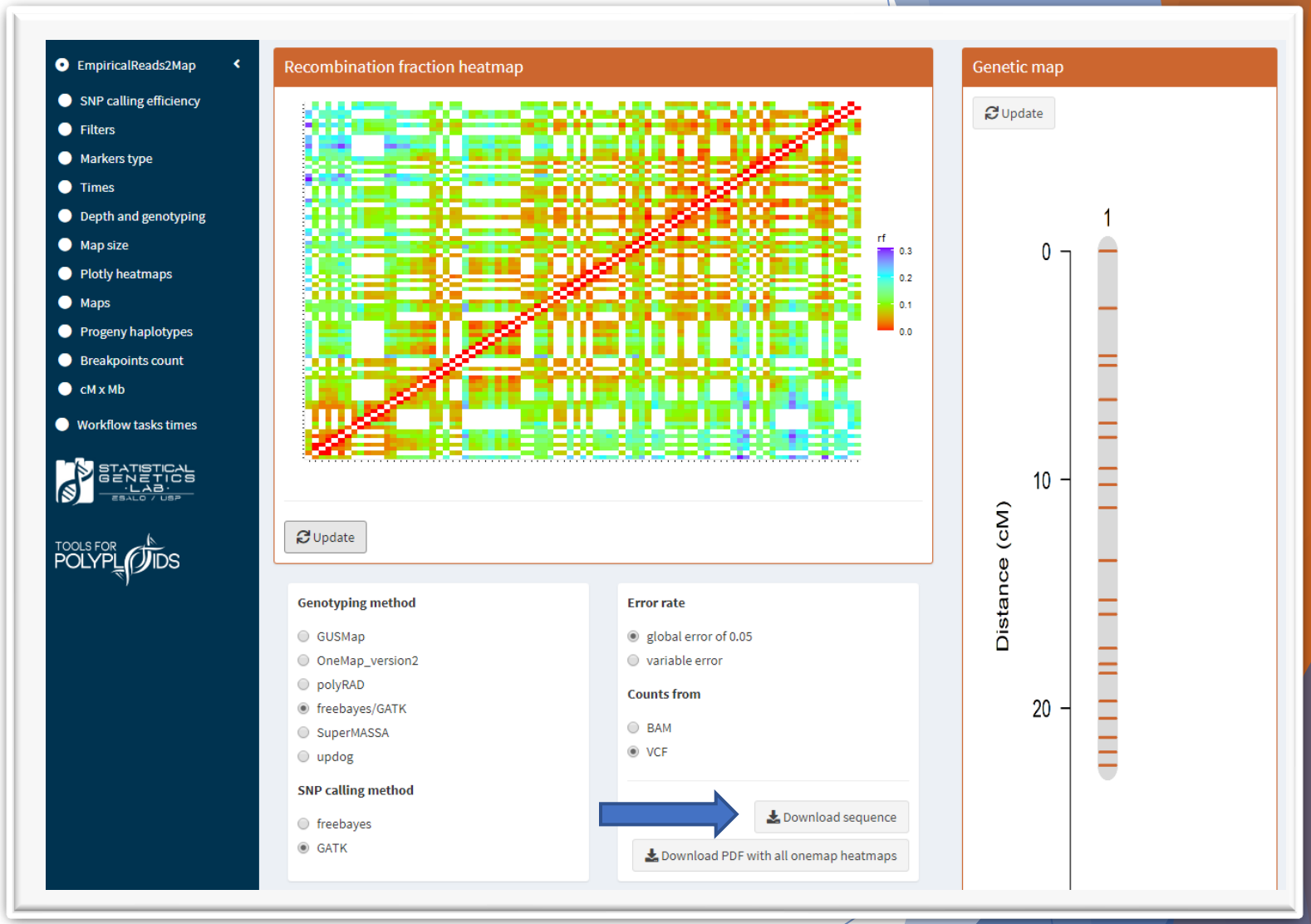
Example results -Diploids

- Outputted maps:
  - Empirical: 34
  - Simulated: 68

- Test only a subset of one group and repeat the pipeline to others



Results for different scenarios

- Playing with parameters:
  - 8160 simulated maps
  - 816 empirical maps

- Effect of filters
- Effect of multiallelic markers
- Effect of contaminants
- Effect of segregation distortion
- Comparison with GUSMap (Bilton et al, 2018)
- Select best pipeline

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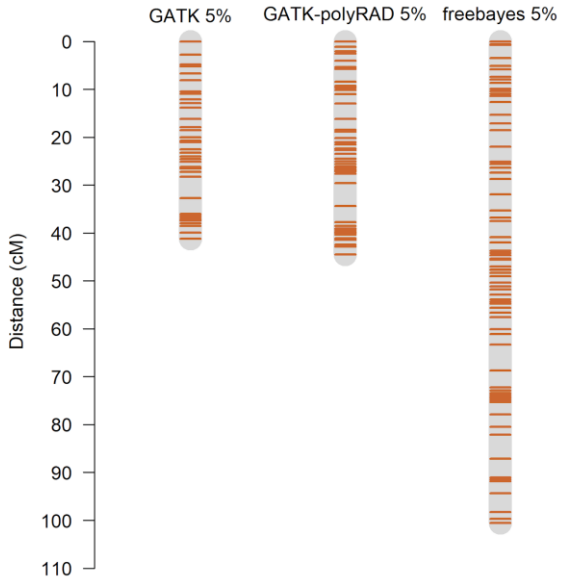
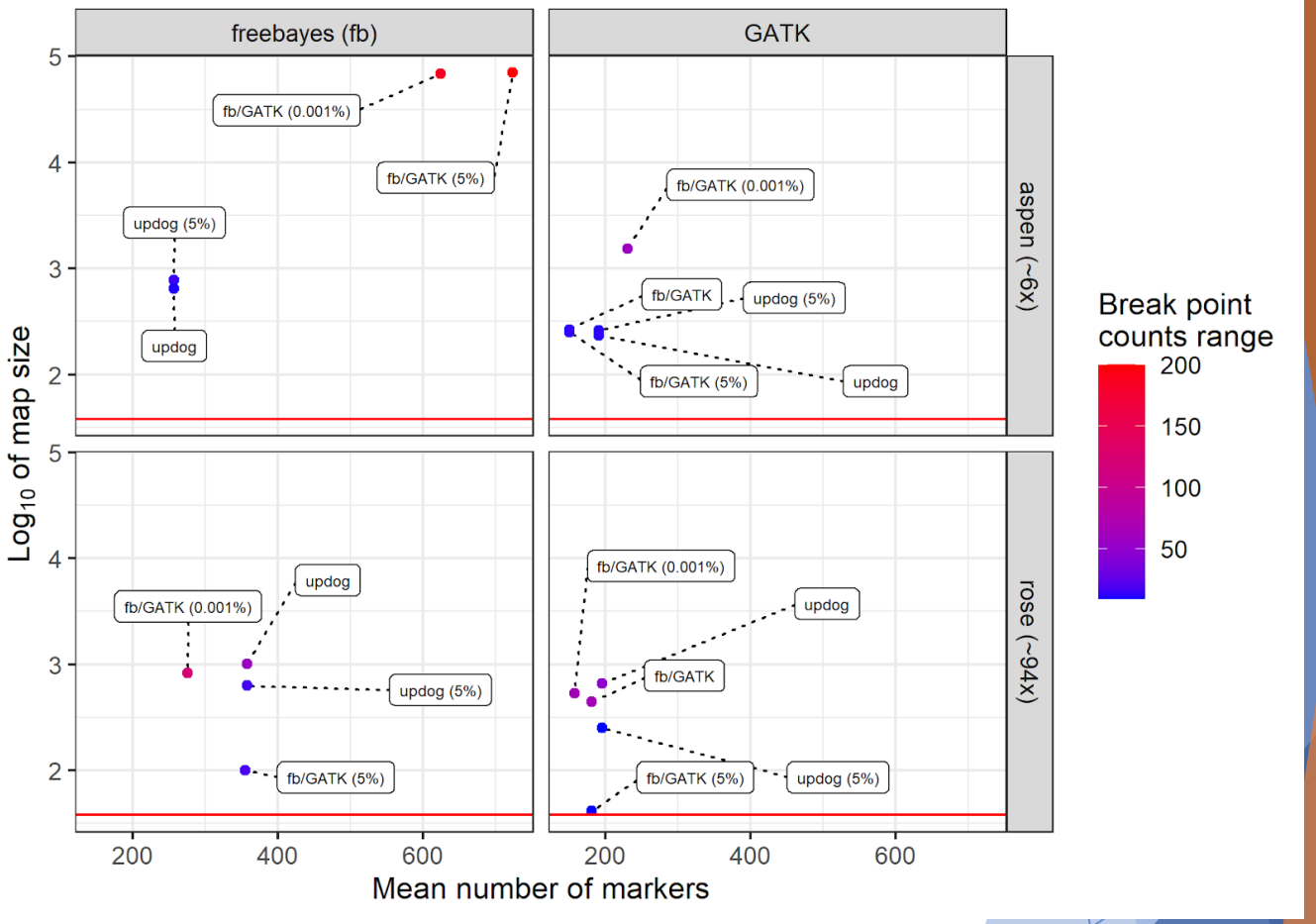
Results for different scenarios

- Selecting best





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Thanks!

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- Authors:

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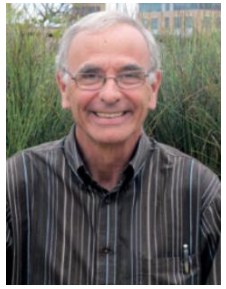

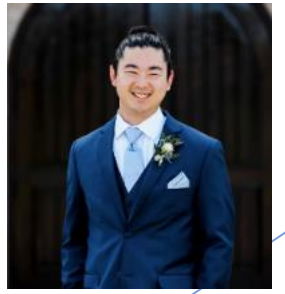


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Other funding agencies

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Other Collaborators

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VacCAP

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CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

Driscoll's

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