

Whole Genome Sequencing of Polyclonal *P. vivax* Relapses in Cambodia

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Disclaimer

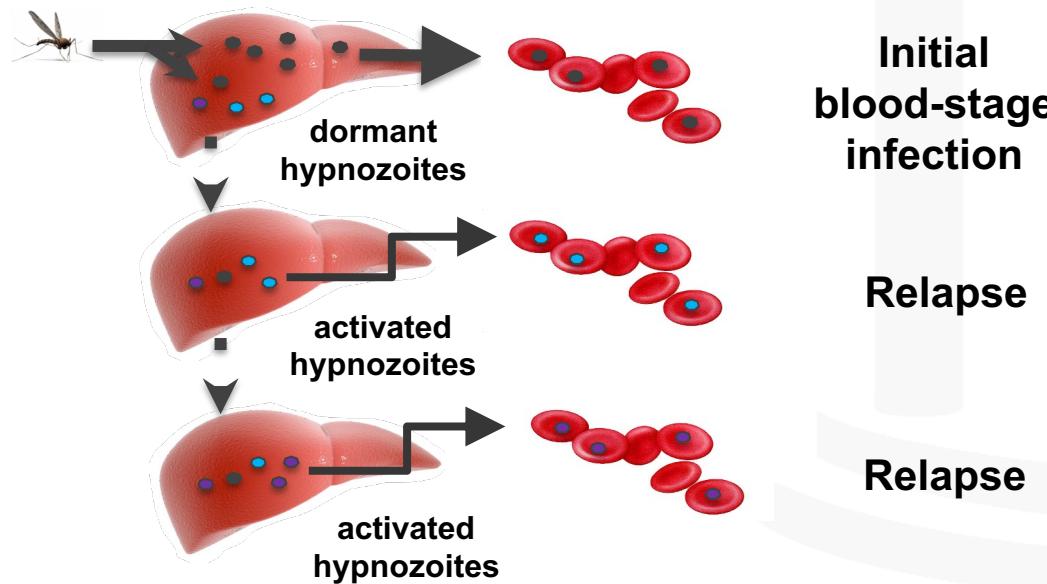
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Vivax Relapse Genotyping from endemic cohorts

Clonal activation of heterologous hypnozoites

Imwong/White. J Infect Dis 2007.

Chen/Cheng. J Infect Dis 2007.



Initial
blood-stage
infection

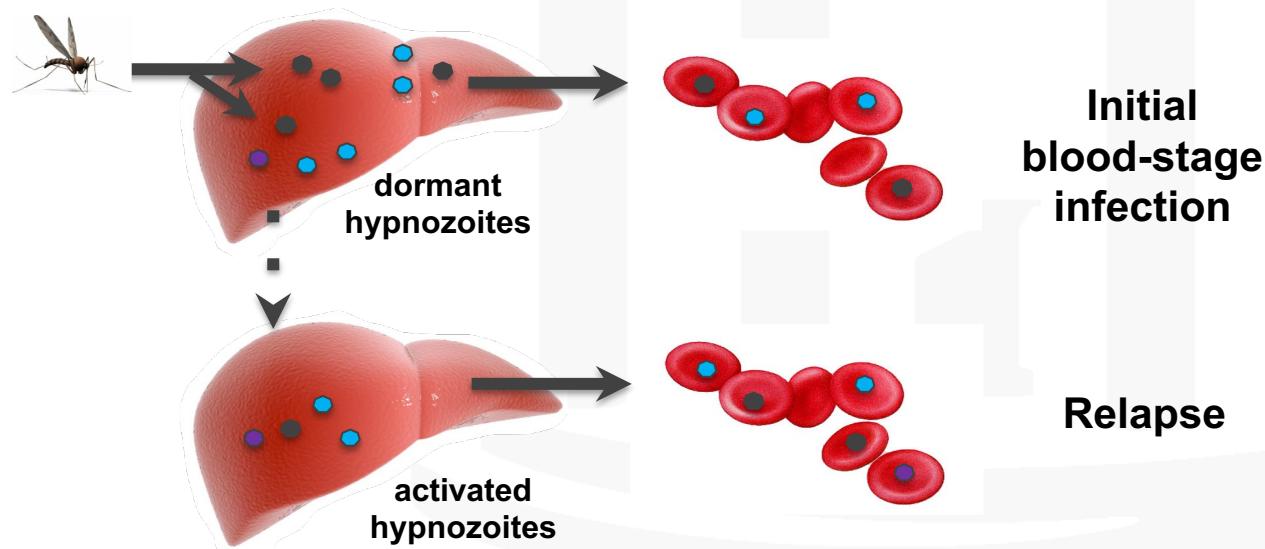
Relapse

Relapse

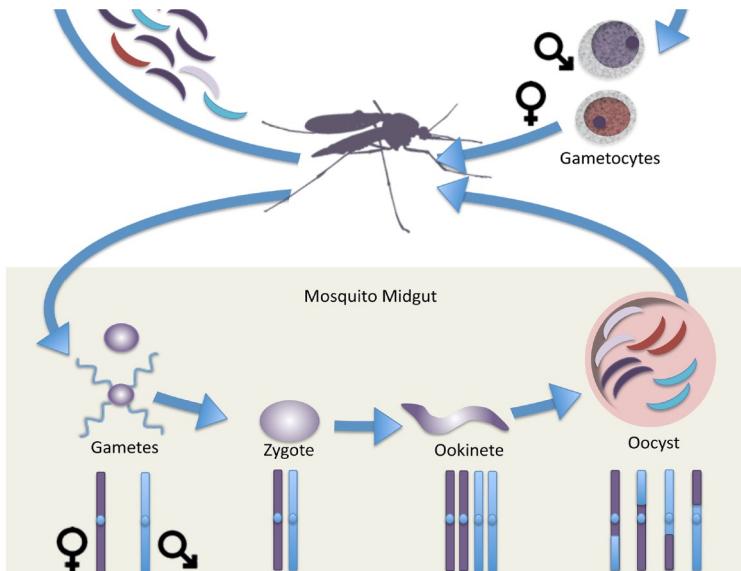
Vivax Relapse Genotyping from endemic cohorts

Multiclonal activation of hypnozoites

De Araujo/de Brito. PLoS One 2012.
Lin/Juliano. J Infect Dis 2015.

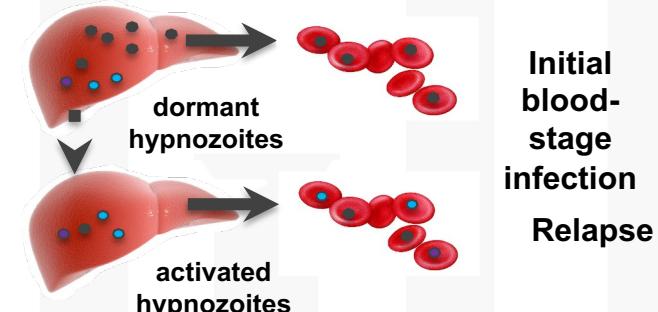


Vivax Relapse Genotyping from endemic cohorts



Bright/Winzeler, PLoS NTD 2014

Multiclonal activation of hypnozoites
De Araujo/deBrito. PLoS One 2012.
Lin/Juliano. J Infect Dis 2015.



Initial
blood-
stage
infection
Relapse

Background Summary

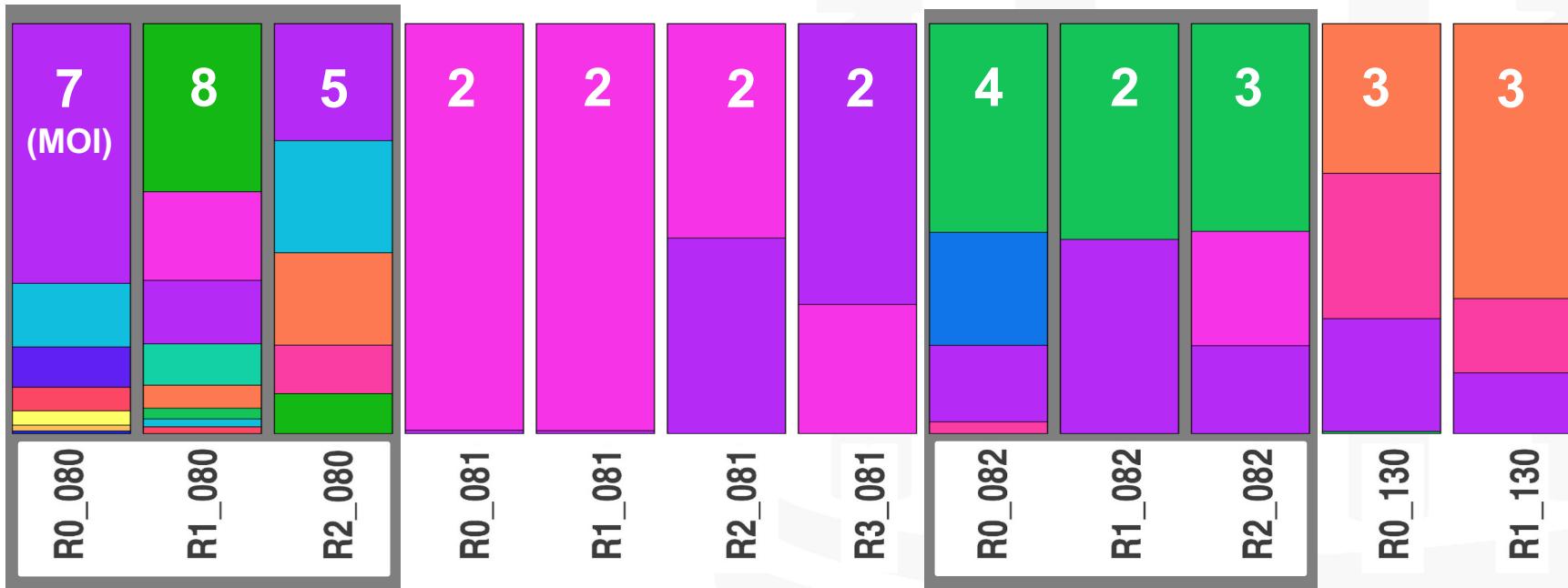
- *P. vivax* relapses from endemic settings may be **are frequently** multiclonal
- *P. vivax* successive relapse infections are expected to be more related due to recent recombination (than 2 independent infections)

Malaria cohort study in northern Cambodia

- Sept 2010 - Mar 2011
- Primaquine radical cure given at end of study
- Approximately one-third of subjects (22/69) developed recurrent *P. vivax* infection
- **We focused on 4 subjects that suffered from multiple recurrences**



Haplotypes from *msp1* Amplicon by SeekDeep



Aims

1. Determine the Multiplicity of Infection (complexity of infection)

- » Does WGS estimate match amplicon estimates from SeekDeep?

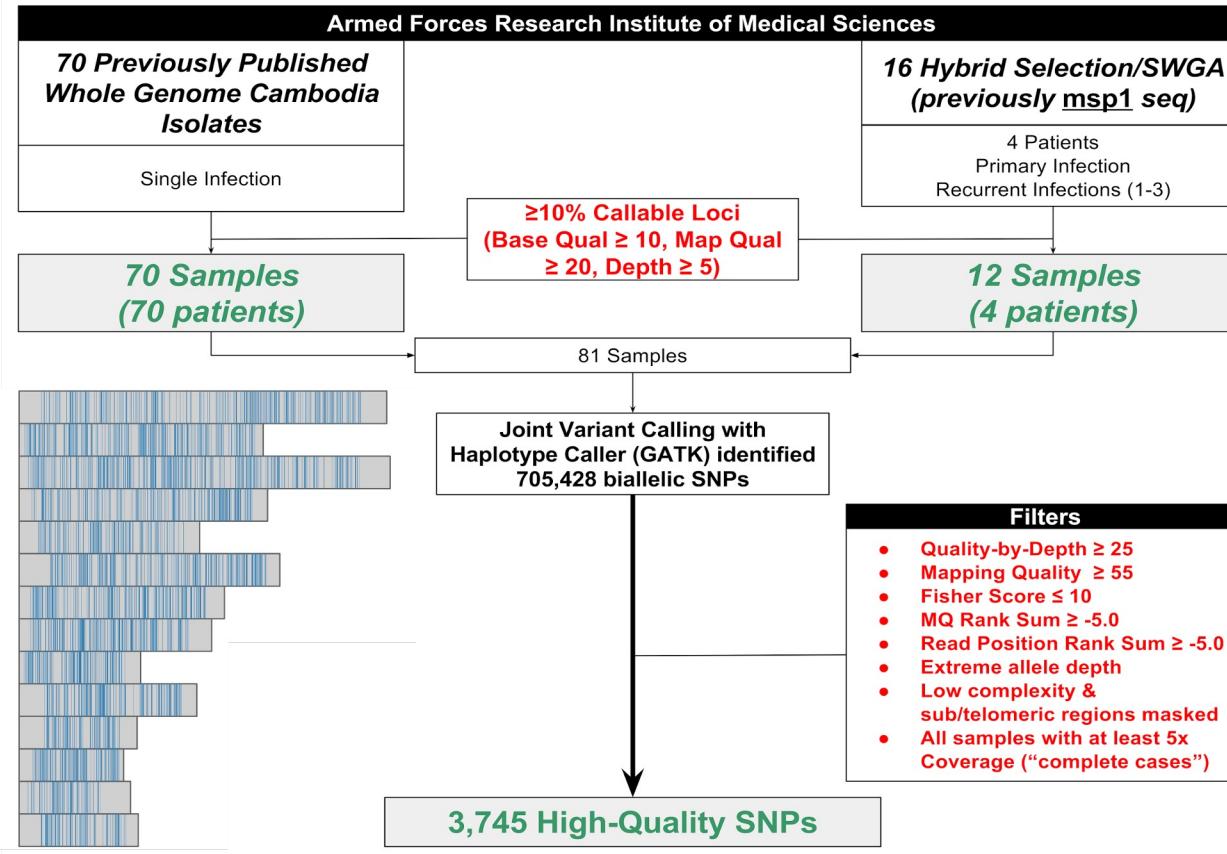
2. Identity genetic relatedness (or “structure”) among successive *P. vivax* episodes

- » Are consecutive relapses more related to each other than to the general *P. vivax* Cambodian population?

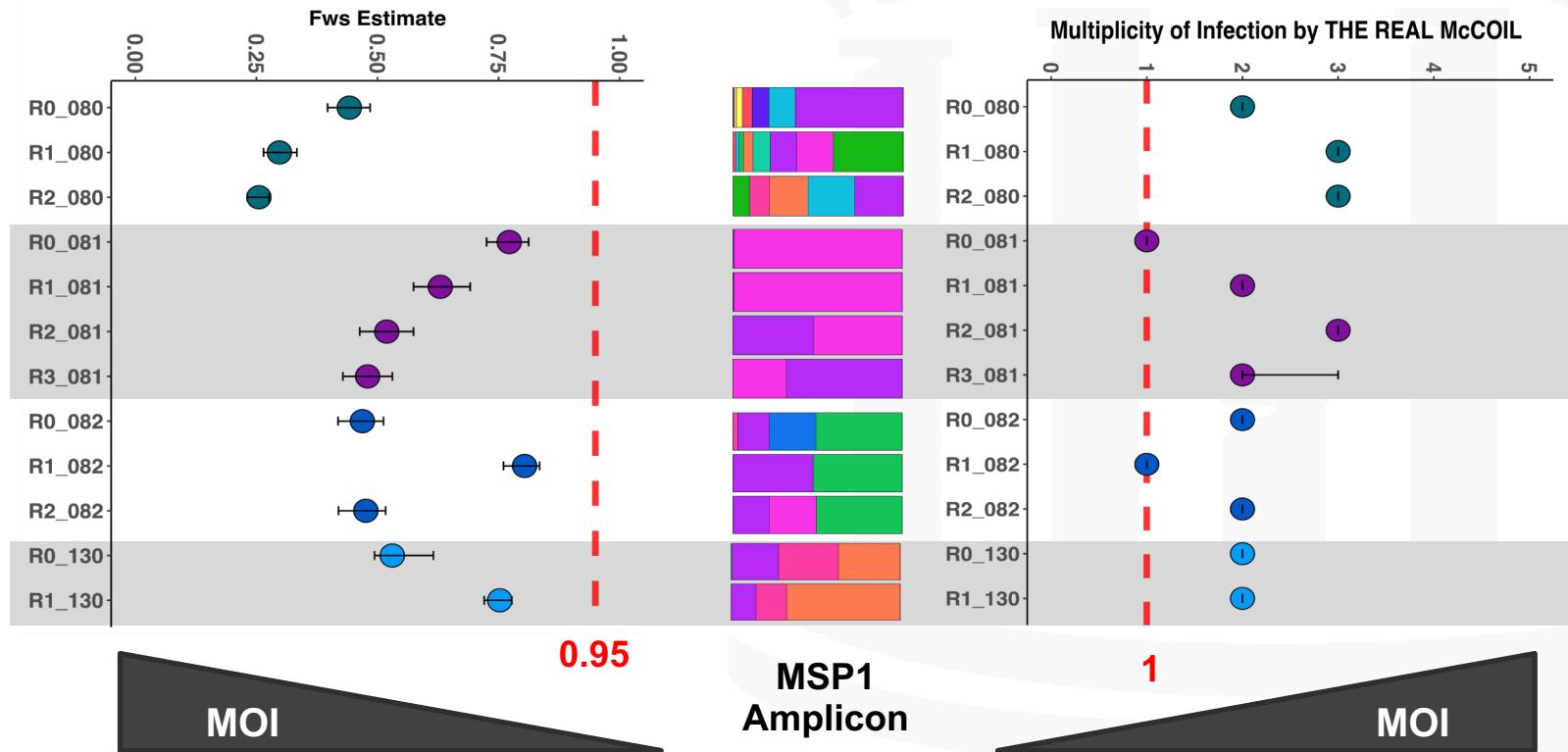
SNP & Sample Selection



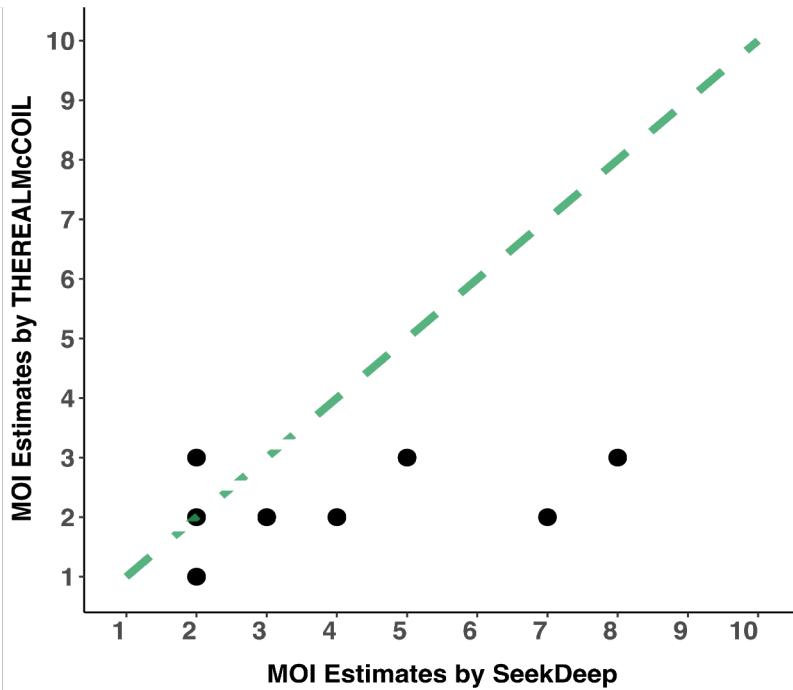
Parobek/Juliano
PNAS 2016



High Multiplicity of Infection (MOI) reflected by Whole Genome Analysis



SeekDeep (*Amplicon*) vs THEREALMcCOIL (*WGS*) Correlation/Summary



- **Multiplicity of Infection by WGS underestimated amplicon estimates**
 - » Depth of coverage
 - 5-fold coverage too low for high MIOI
 - » Too stringent SNP filtering...
 - 0 Passed SNPs in *msp1*
 - » Allelic dropout
- **Goal of analysis**
 - » Depth (amplicon) versus Breadth (WGS)

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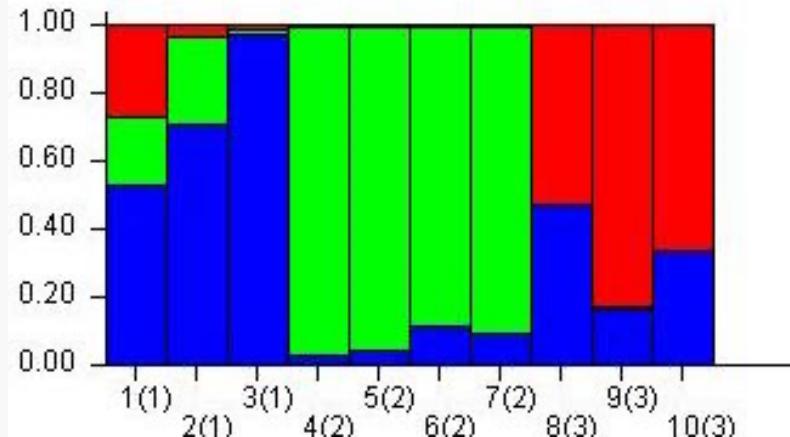
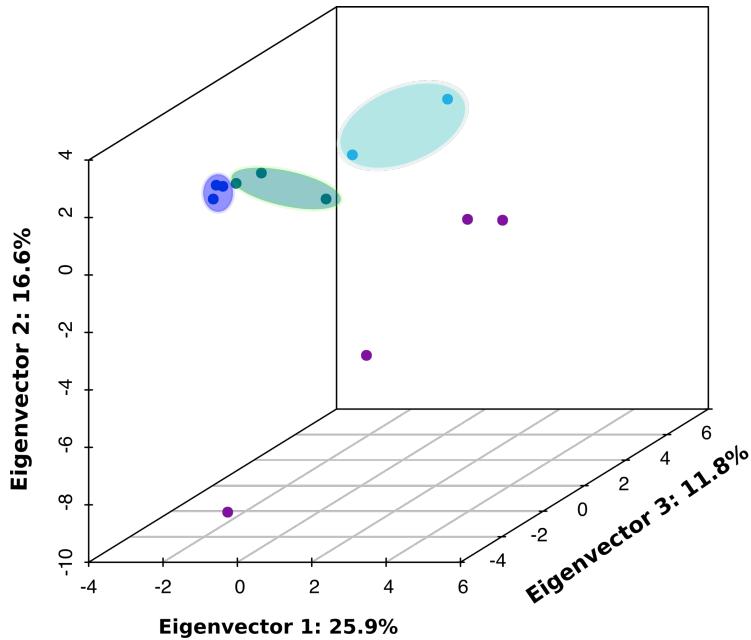
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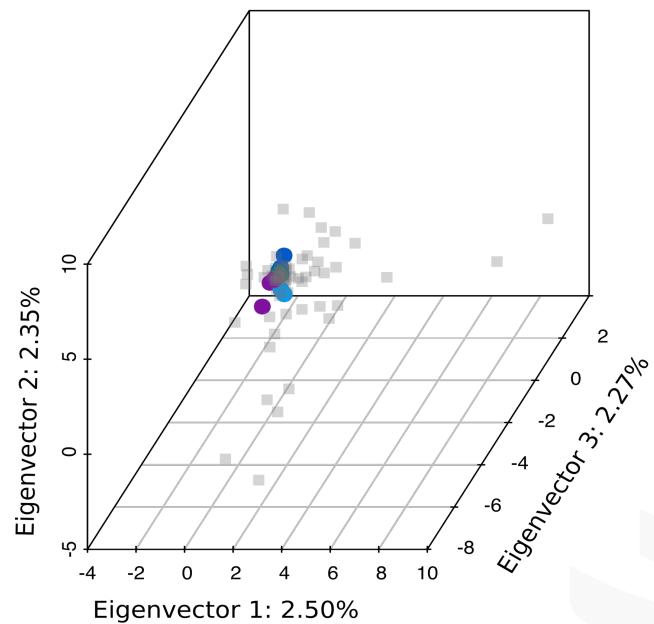
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PCA & STRUCTURE (*relapse samples*)

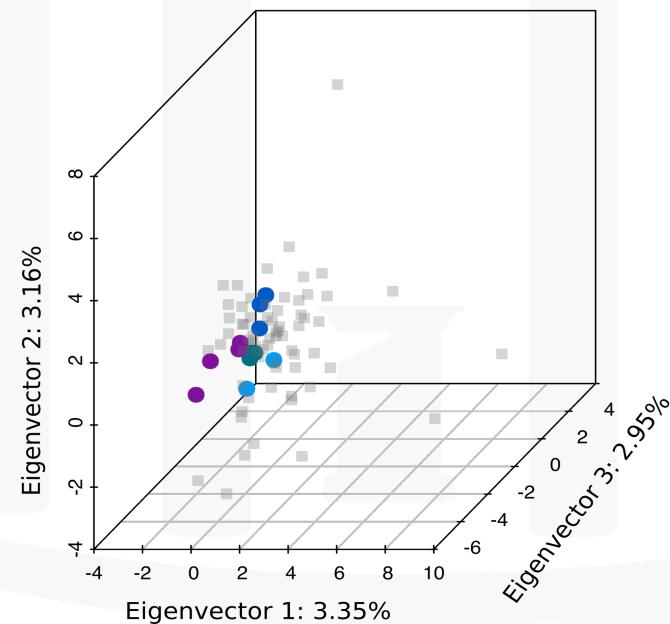


Structure Based on PCA (*all samples*)

PCA: Masked Heterozygote Calls (Diploid)

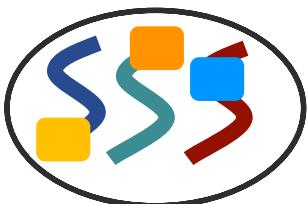


PCA: Dominant Allele (Haploid)

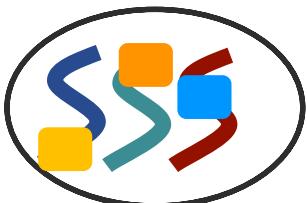


polyIBD Goal

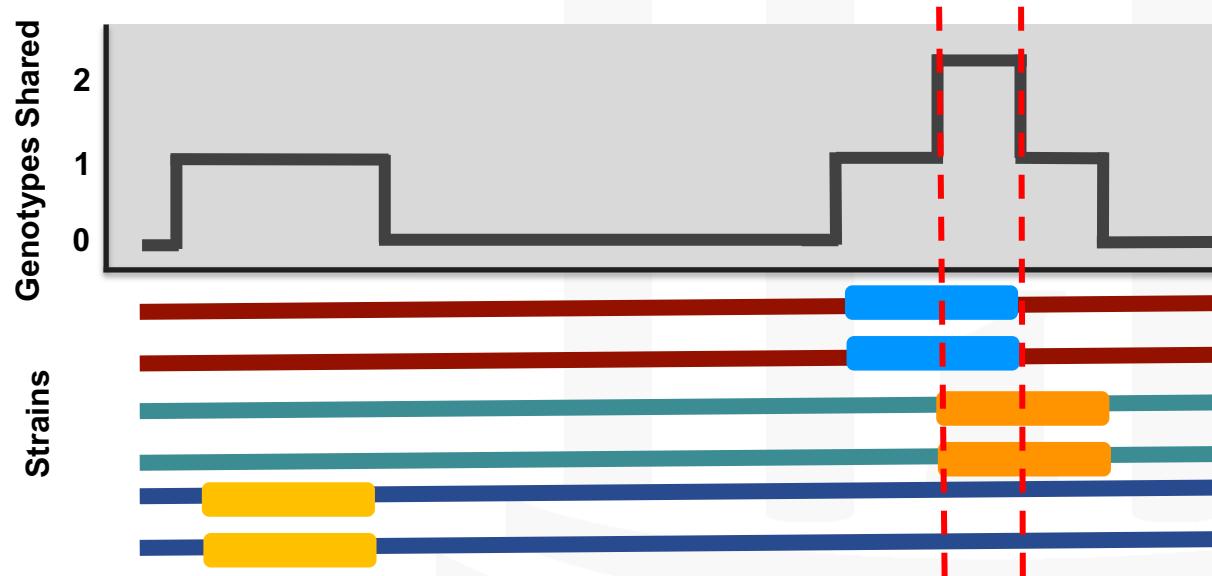
MCMC Multi-state Hidden Markov Model for detecting IBD blocks among unconvoluted genotypic data



Sample 1

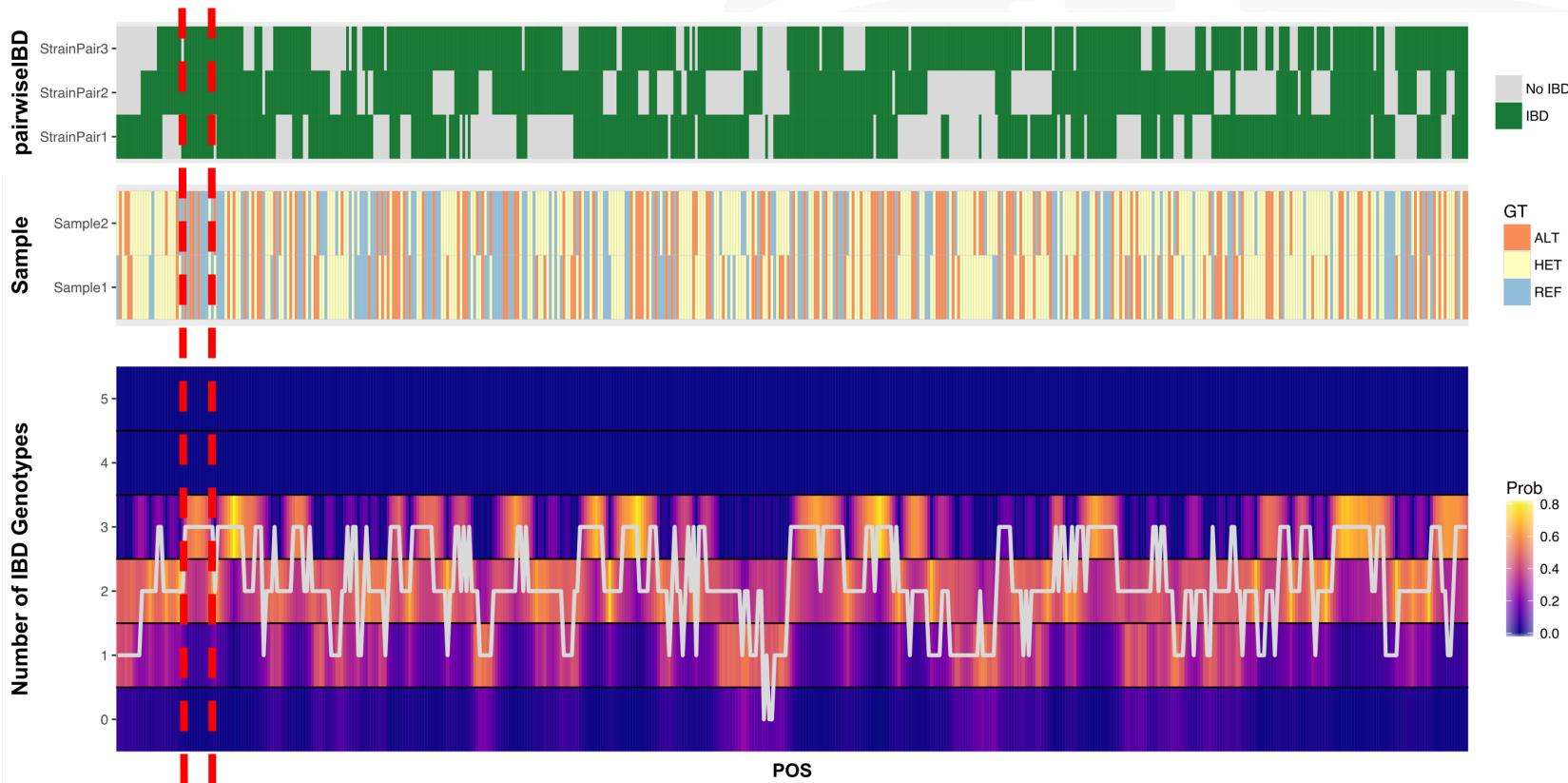


Sample 2



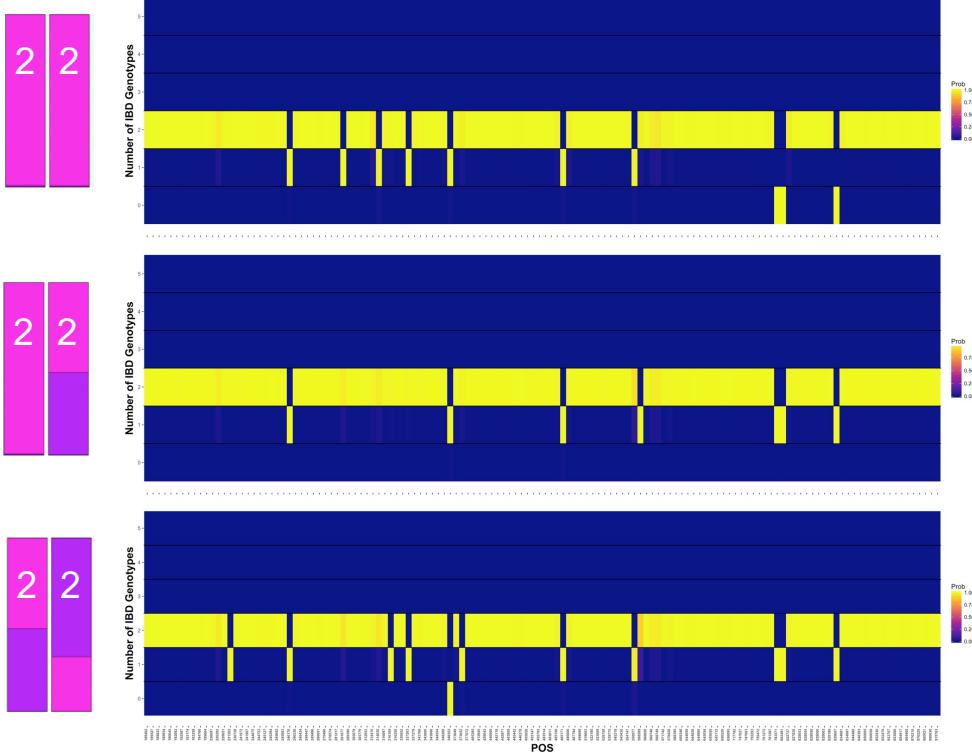
isoRelate (Henden L/Bahlo M, BioArxiv, 2016)
hmmIBD (Schaffner S/Neafsey D, BioArxiv, 2017)

polyIBD Simulation



polyIBD Preliminary

Pt 81



- **polyIBD still under development**
 - » Optimization of parameter estimation
 - » Speed
 - » Simulations to quantify model power and determine any model bias
- **Subsetted/Preliminary Analysis**
 - » Signal of IBD among successive infections within patient

Genetic Relatedness Summary

- Traditional approaches that do not leverage heterozygote variant calls may underestimate genetic relatedness
- Future work aims to use polyIBD to infer genetic relatedness and identify meiotic siblings among successive infections

Aims

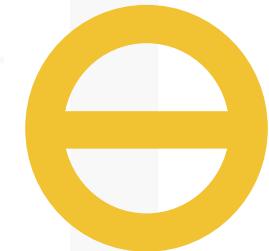
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Acknowledgements

IDEEL@UNC
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UNC MD-PhD Program

Supplementary Information

Allelic Dropout with WGS versus Amplicon

