Estimation of Viral Population Structure from Amplicon-Based Reads

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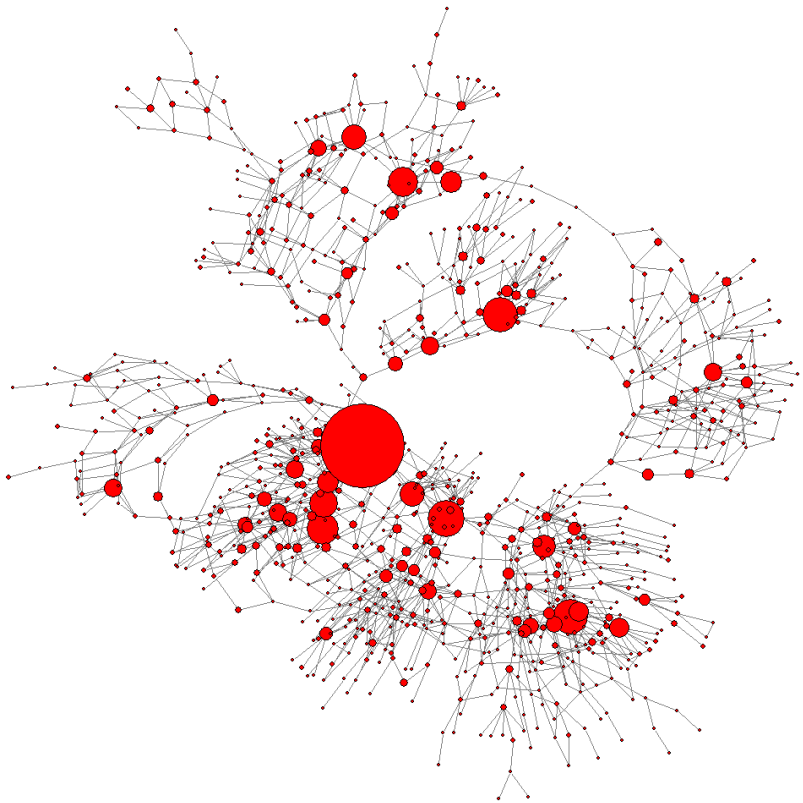
Overview

Viral Quasispecies

- RNA virus replication relies on RNA polymerase
- High mutation rate ($\approx 10^{-4}$)
- Recombination events occur
- HIV, HCV, Influenza



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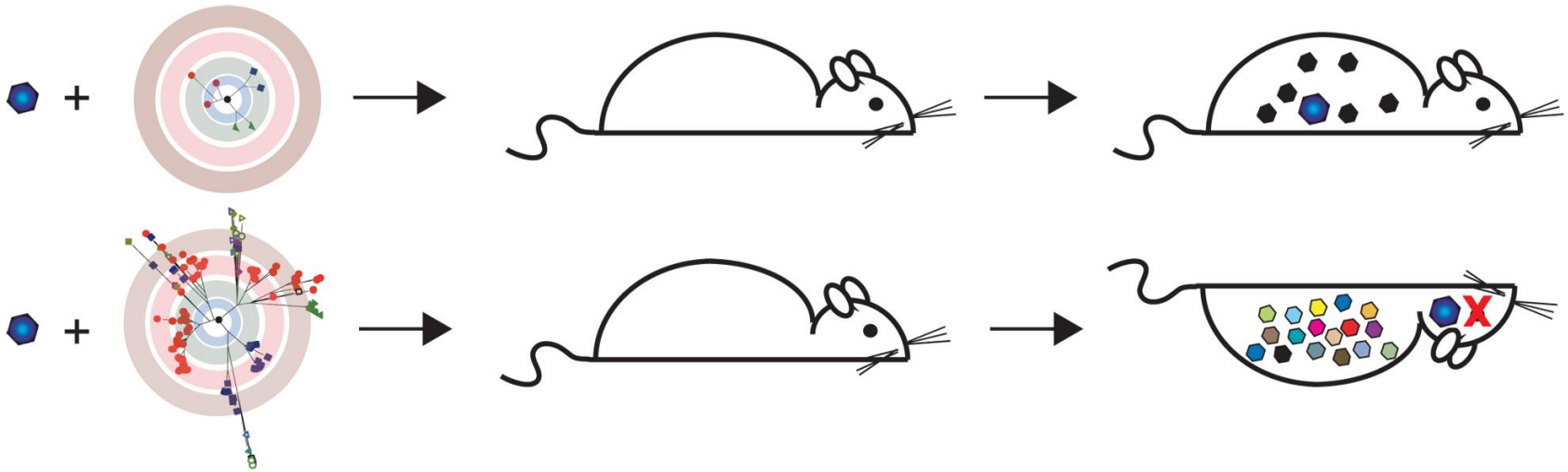
Viral Quasispecies

Populations may differ in

- Virulence
- Escape immune response
- Resistance to antiviral therapies



Lauring AS, Andino R. PLoS Pathog. 2010

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Hepatitis C

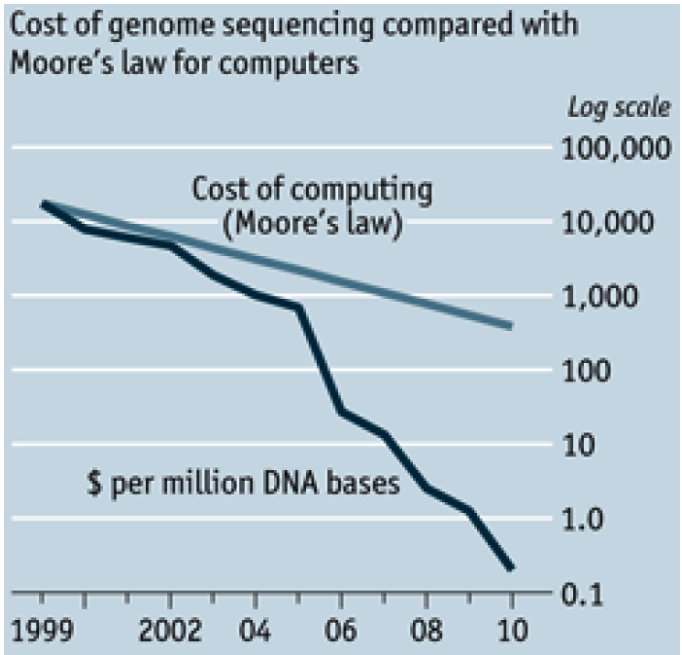
HCV infects 2.2% of the world's population

- No vaccine
- Current interferon and ribavirin therapy effective in 50%-60% of patients
- Therapy is expensive and uncomfortable

Skums et. al., CAME 2011

- Prediction method for interferon outcome
- Highly dependent on accuracy of viral population structure

High Throughput Sequencing



http://www.economist.com/node/16349358

Illumina HiSeq 2000
Up to 6 billion PE reads/run

35 — 100bp read length

Ion Proton Sequencer
Up to 10 billion reads/run

20 — 200bp read length

Roche/454 FLX Titanium
1 million reads/run

400 — 600bp read length

SOLiD 4
1.4-2.4 billion PE reads/run

35 — 50bp read length

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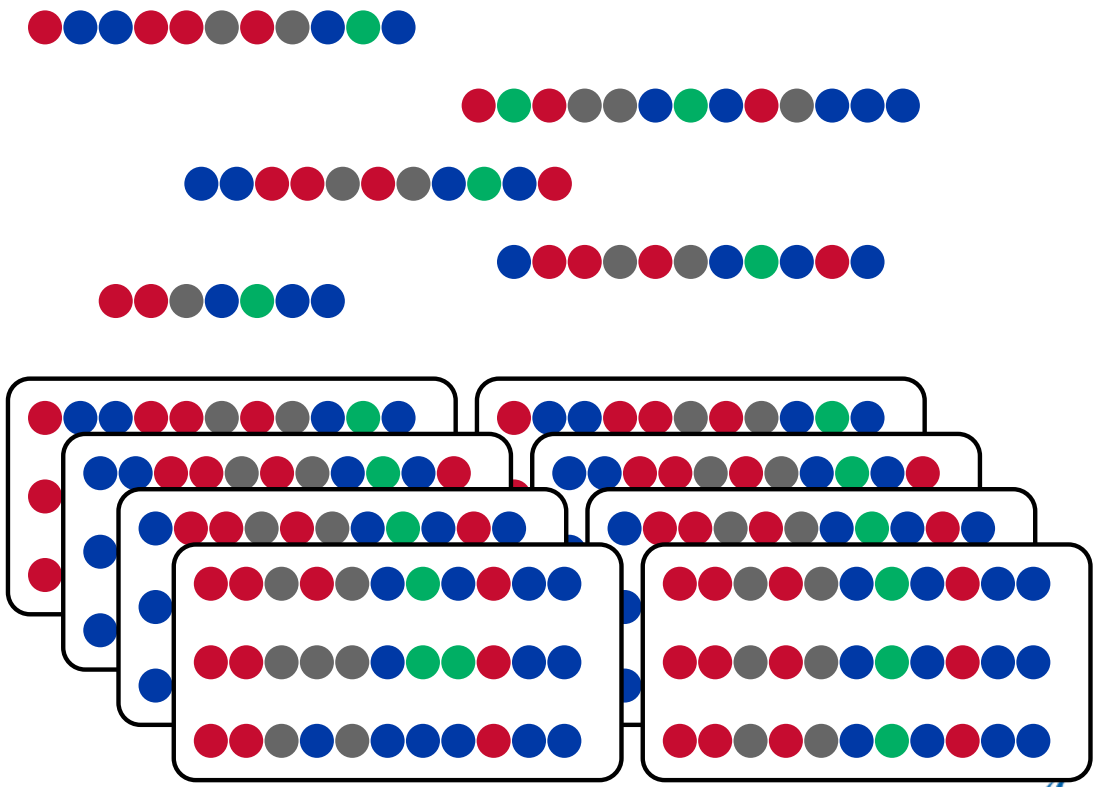
Shotgun and Amplicon Reads

- Shotgun reads have start positions distributed uniformly
- Amplicon reads have start/end positions determined by allele-specific primers



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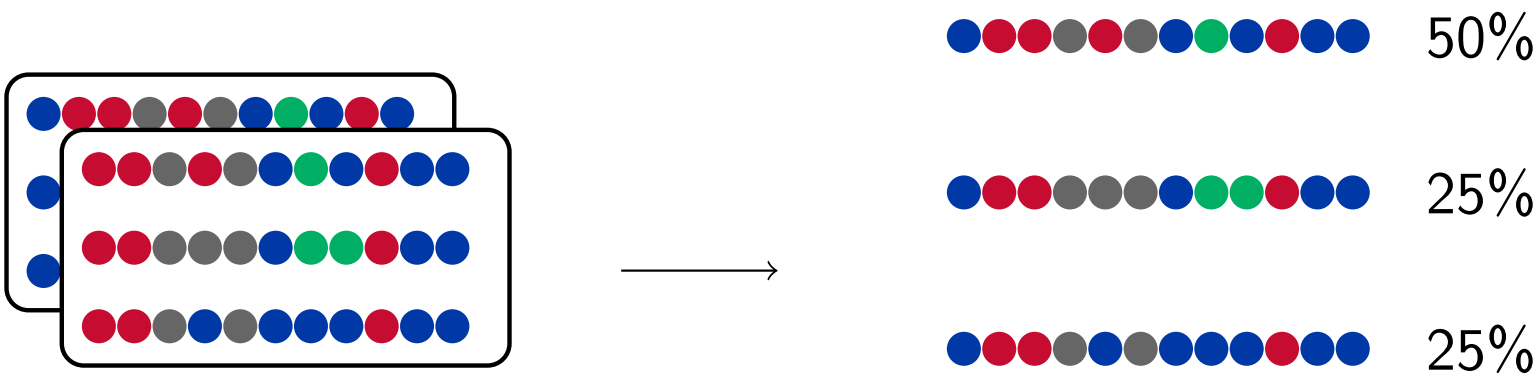
Viral Quasispecies Reconstruction Problem

Problem

Given a collection of amplicon reads generated from a viral sample, assemble the quasispecies, i.e., the set of sequences and respective frequencies of the sample population.



Viral Quasispecies Reconstruction

Local Reconstruction

- Focus on primer-flanked region
- KEC, QuasiRecomb, *k*GEM

Global Reconstruction

- Focus on larger genomic regions
- Typically use read-graph approach to "stitch" locally reconstructed regions together
- ShoRAH, ViSpA, QuRe

VirA is a tool for global quasispecies reconstruction

Global Viral Reconstruction Challenges

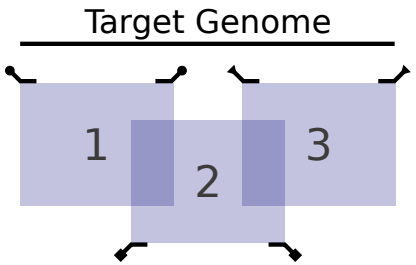
Conserved Regions

Relatively few mutations in long regions obfuscate true population

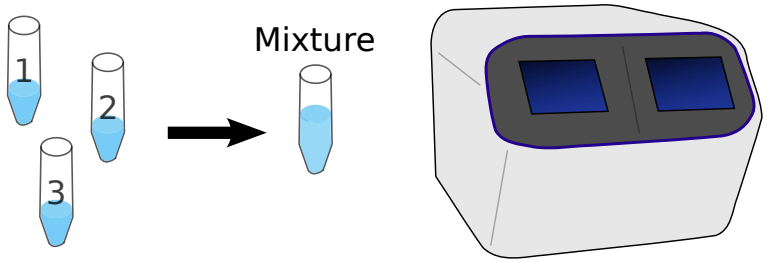
Sequencing Errors

- Homopolymer errors
- Base call errors
- Insertion errors
- Deletion errors

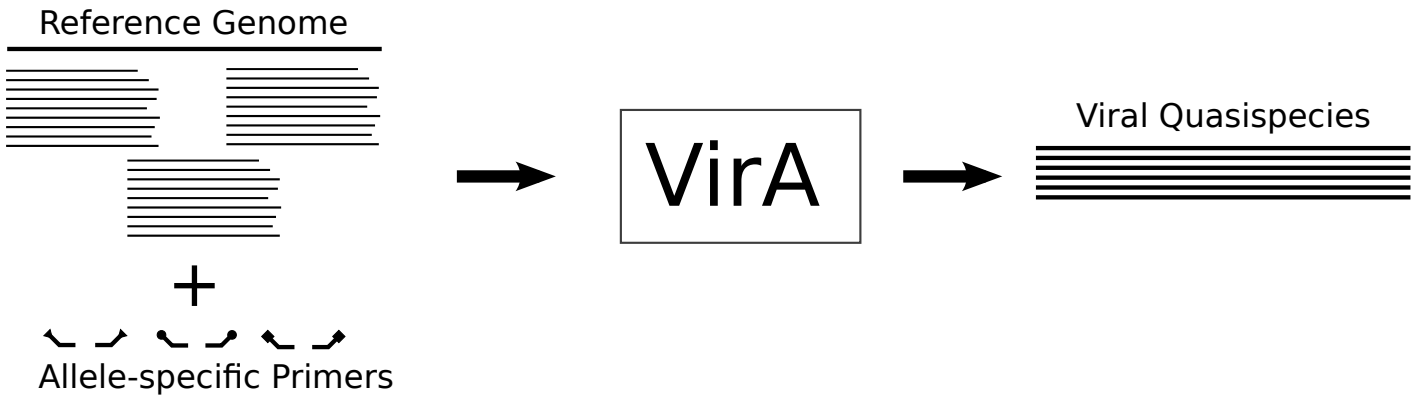
Library Preparations & Workflow



a)



b)



c)

VirA Workflow

1. Align reads to reference
2. Align allele-specific (target) primers to reference
3. Infer amplicon intervals from primers
4. Locally reconstruct in each amplicon
5. Globally reconstruct over read-overlap graph

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Amplicon Inference

Infer amplicons from flanked regions

- Each pair forms interval
- Impose ordering over intervals
- Read belongs in interval if covers significant sub-interval & overlaps with neighboring intervals

Local Reconstruction/Error Correction

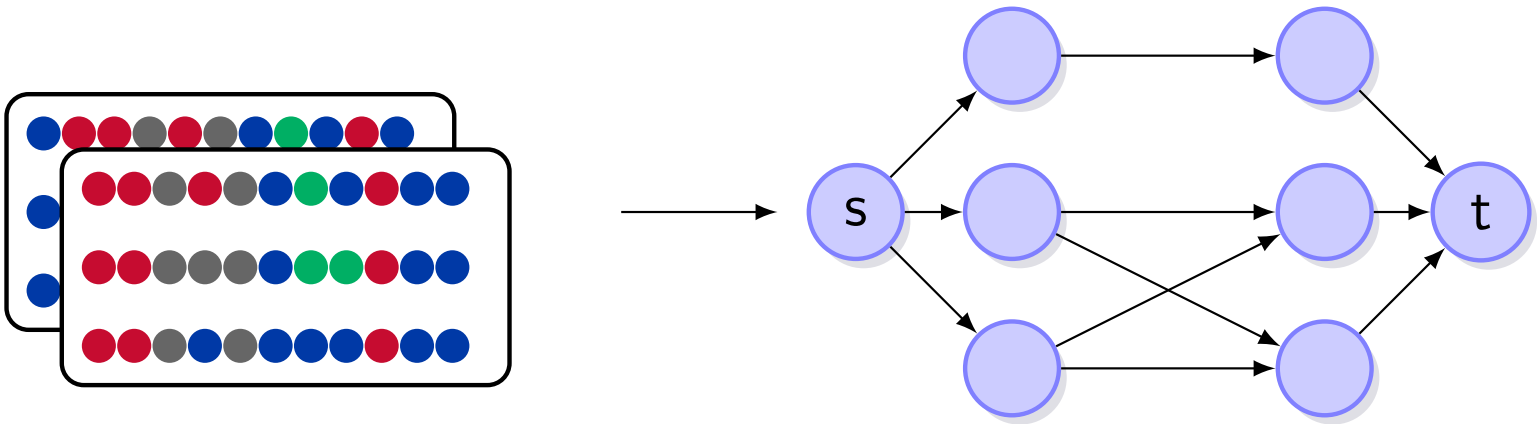
Correct errors with kGEM

- Cluster reads by hamming distance
- Produce local consensuses
- Estimate consensus frequencies
- Estimate allele frequencies
- Repeat until convergence

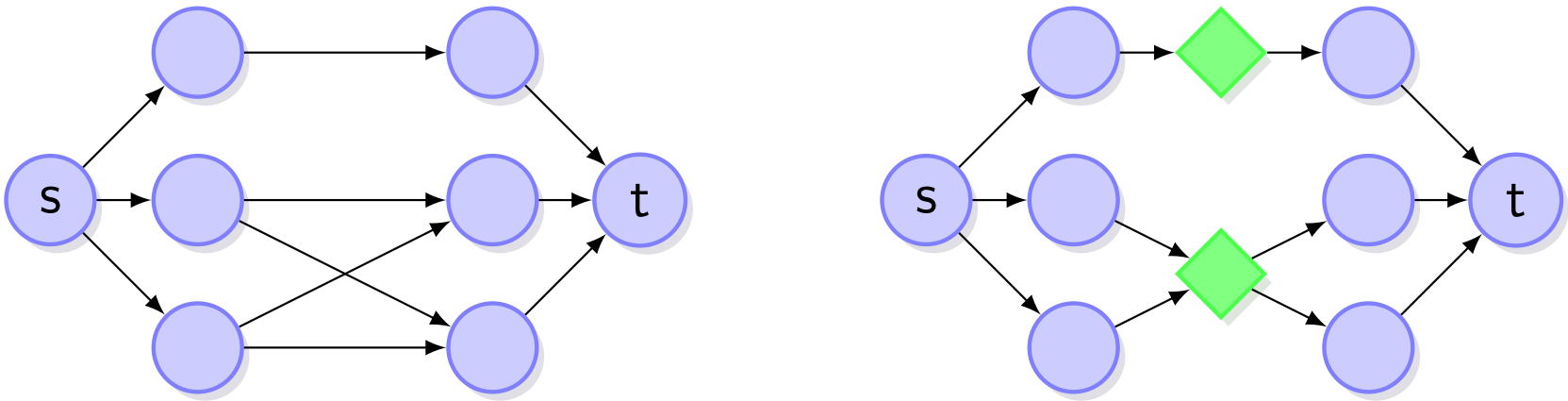
Read Graph

K amplicons represented by K-staged read graph

- Vertices $\Leftrightarrow$ distinct reads
- Edges $\Leftrightarrow$ reads with consistent overlap
- Vertices have count function $c(v)$

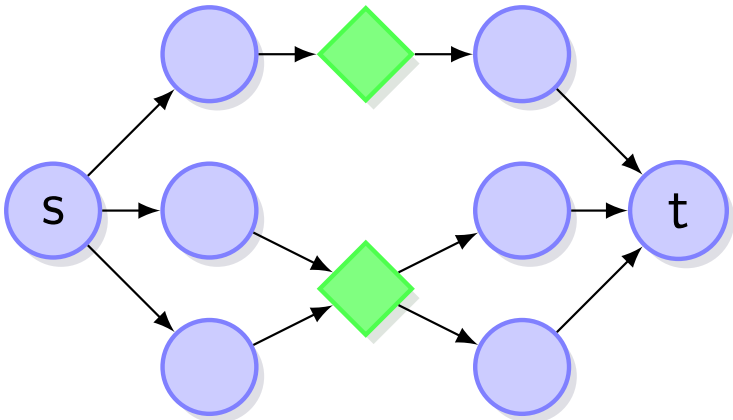


Graph Transformation



Maximum-Bandwidth Paths

- Find simple path containing most possible flow
- Repeat until graph is saturated
- Modified Dijkstra's algorithm
- Mancuso et al, In Silico 2012



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Multi-commodity Flow Formulation

- Finding maximum-bandwidth paths
- Multi-commodity flow k = upper bound on variants
- Minimizes total flow while covering all reads
- ILP on CPLEX
- Skums et al, BMC Bioinformatics 2013

Experimental Setup

- 1734bp HCV E1E2 region
- 43 sequences $\rightarrow$ 10 datasets of 10 variants
- Abundance followed powerlaw ($\alpha = 2$) and uniform distributions
- 7-12 amplicons to cover region
- Reads generated with Grinder version 0.5
- Compared with QuRe [Prosperi et al]

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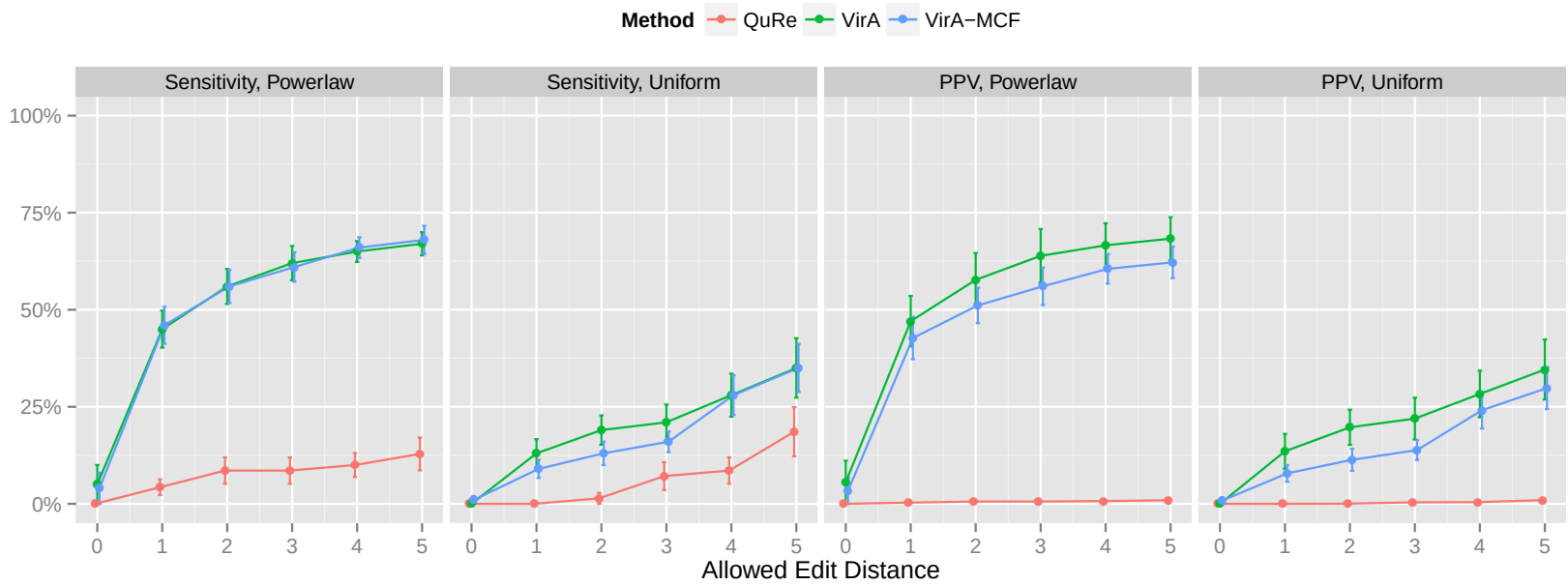
Results


Method
QuRe
VirA
VirA−MCF
Sensitivity, Powerlaw
Sensitivity, Uniform
PPV, Powerlaw
PPV, Uniform
100%
75%
50%
25%
0%
0
1
2
3
4
5
Allowed Edit Distance

Conclusions and Future Work

- Global quasispecies reconstruction is difficult
- VirA
- `http://alan.cs.gsu.edu/vira`

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Thanks

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Funding

Q and A

Thank you!

Questions?