Is Sharing Caring?

Elucidating the Effects of the Presence of CRISPR-Cas Systems on Rates of Horizontal Gene Transfer Using Network Analysis

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MolBiol 4C12 Thesis

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McMaster University

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CRISPR-Cas systems

What Are They?

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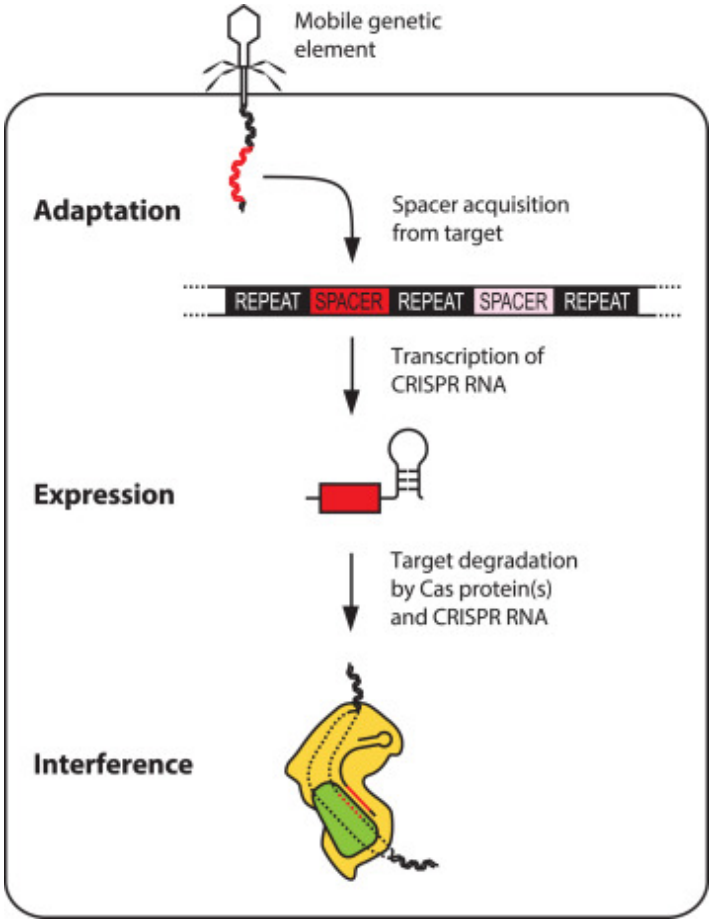
- Adaptive Bacterial Immune System



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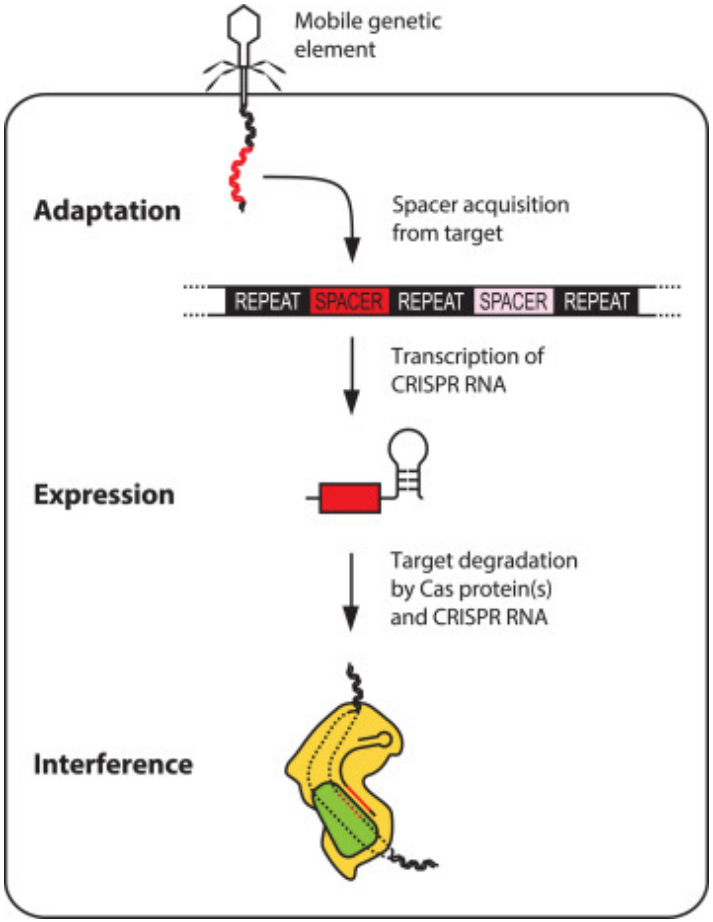

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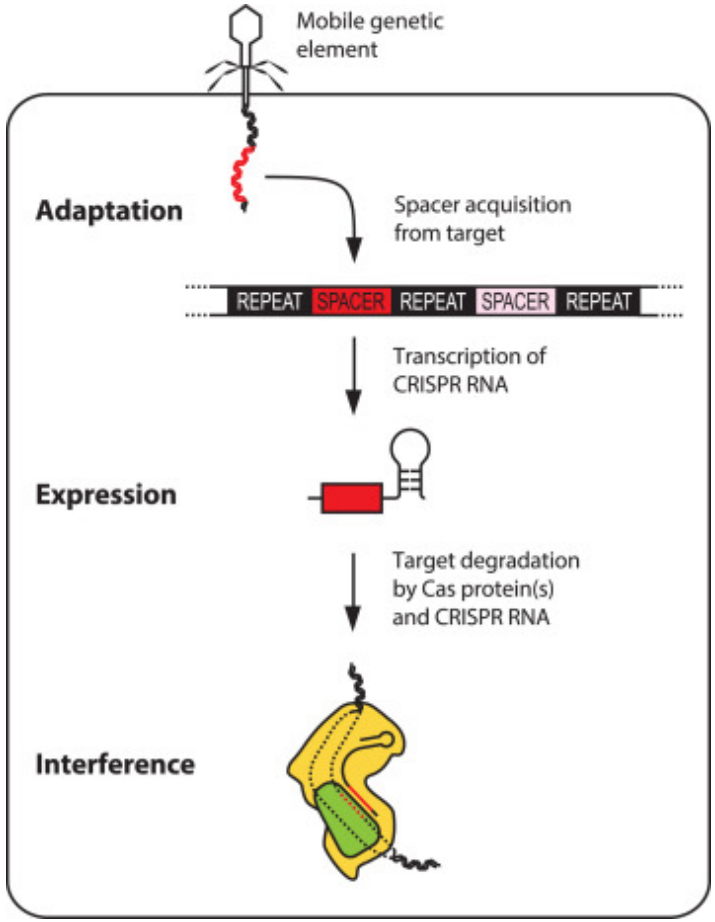
- Protects against foreign DNA
- Requires Cas proteins and CRISPR loci



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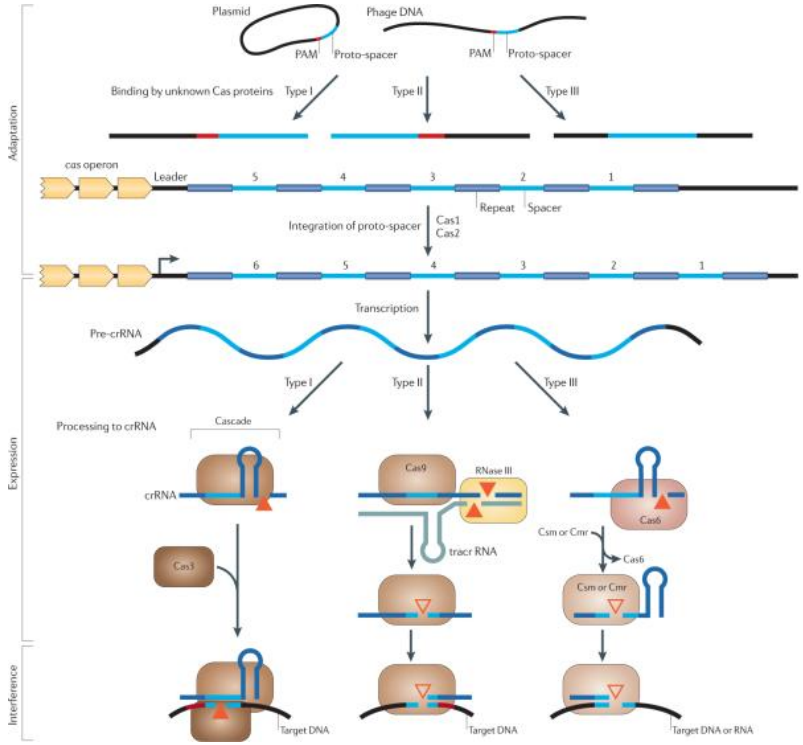

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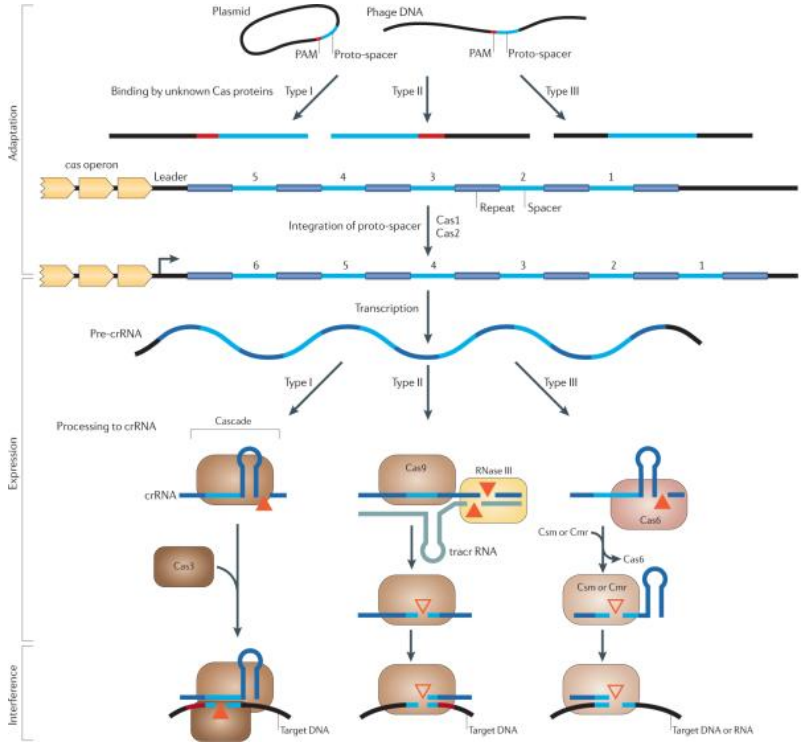

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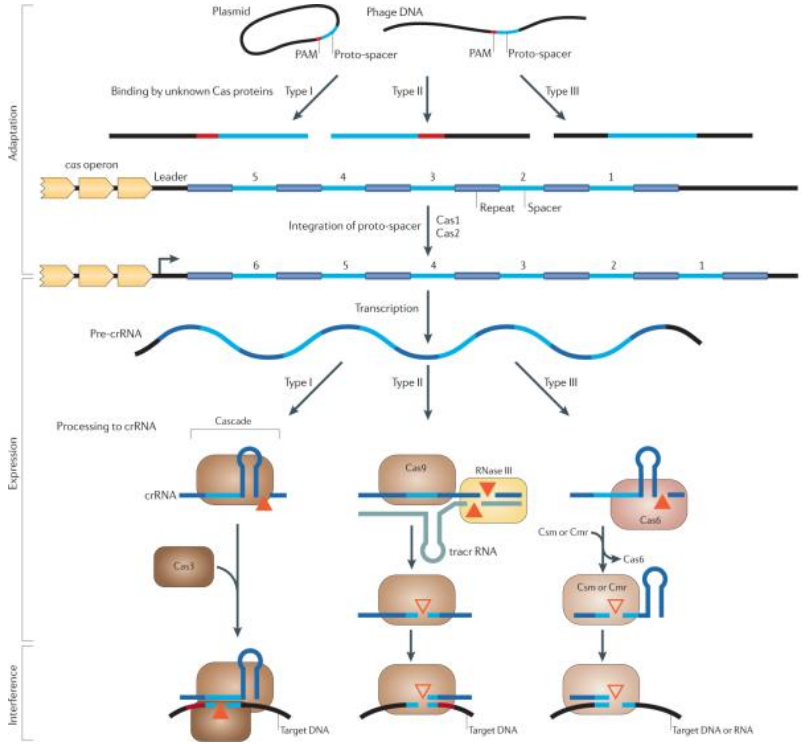

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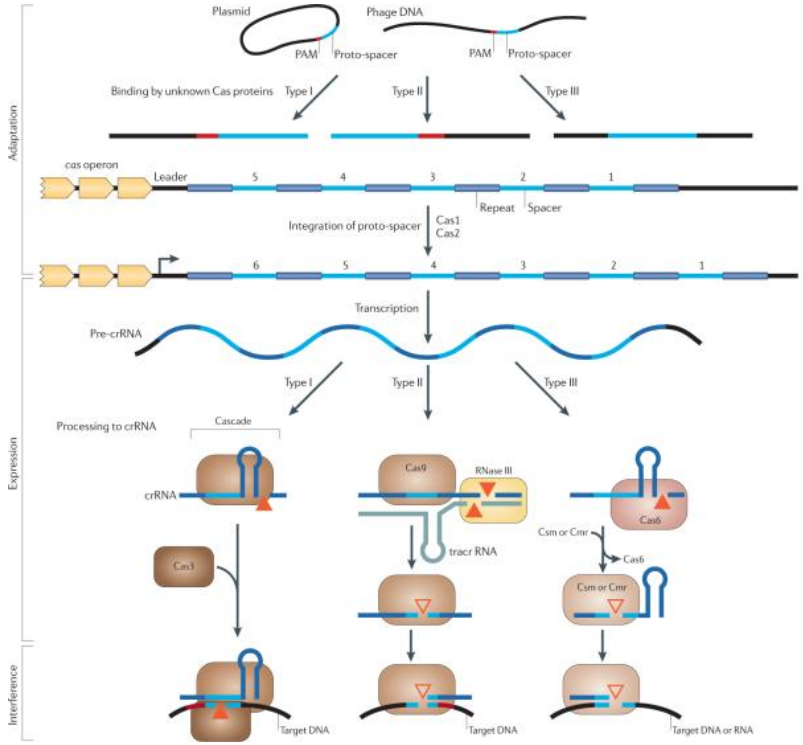
- $11\% - 28\%$ are false or orphaned CRISPR loci[4]



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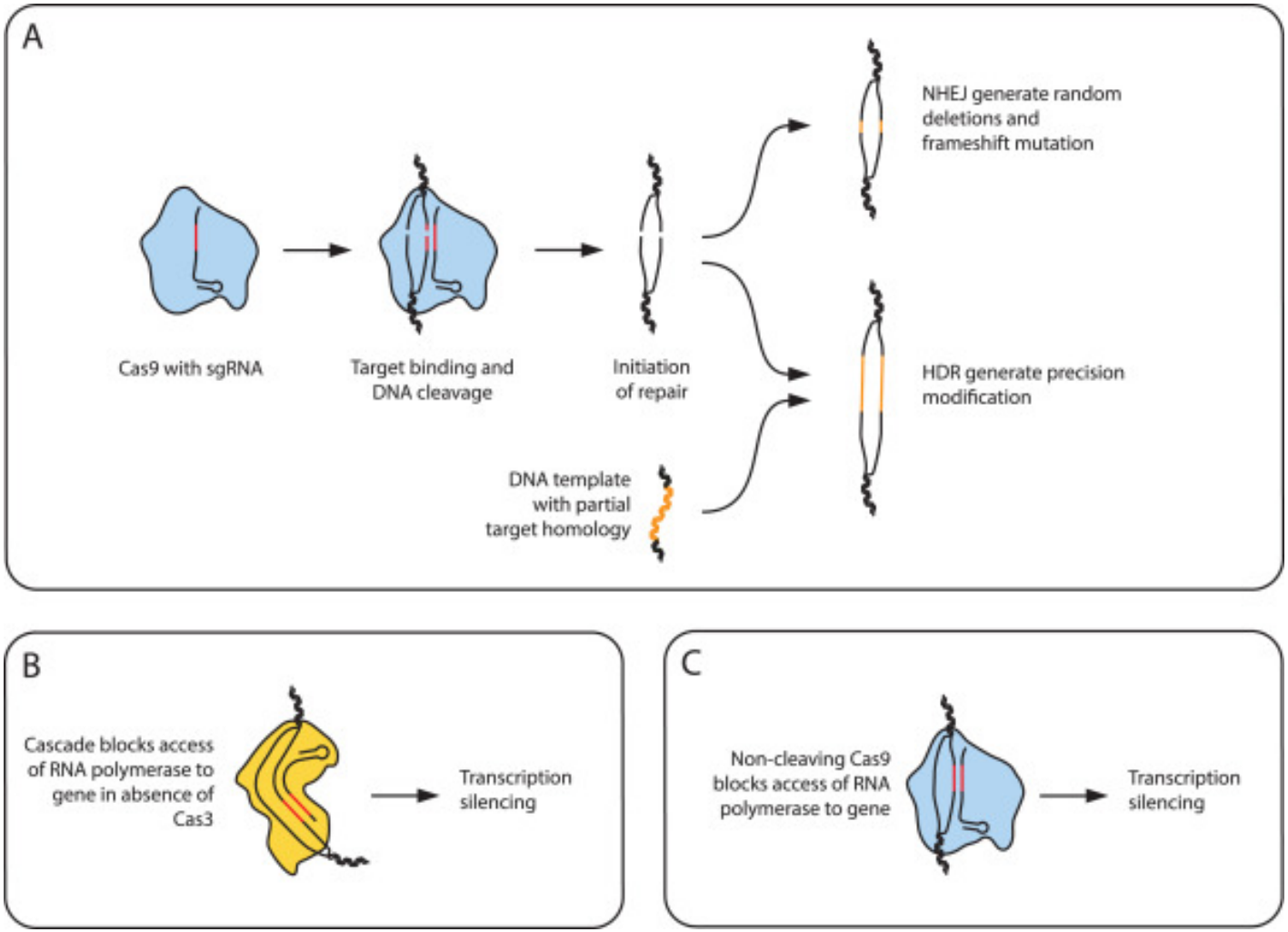
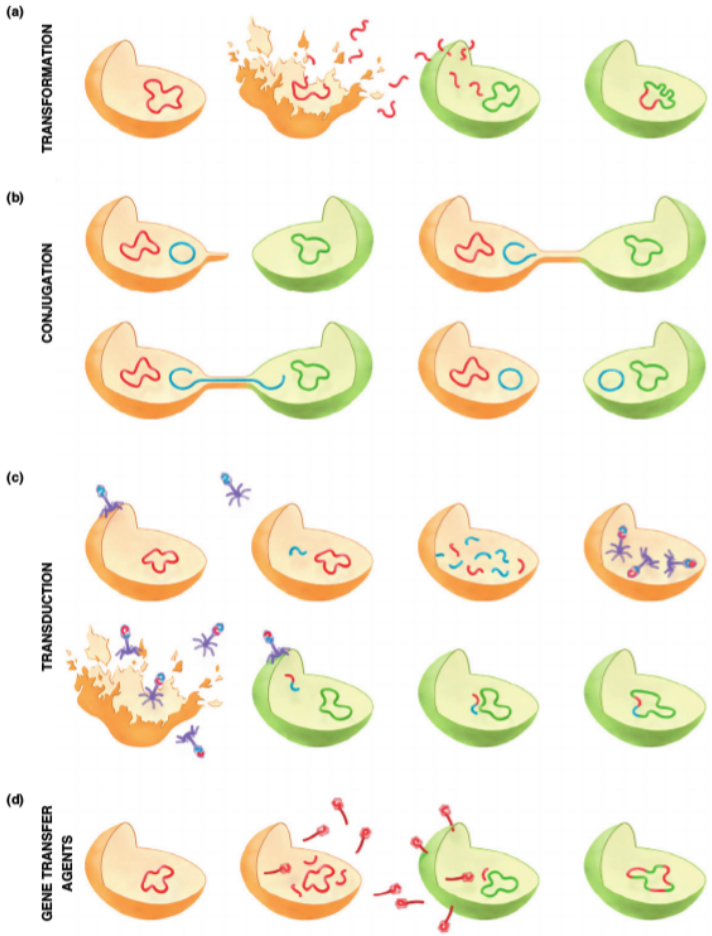


Horizontal Gene Transfer

Mechanisms



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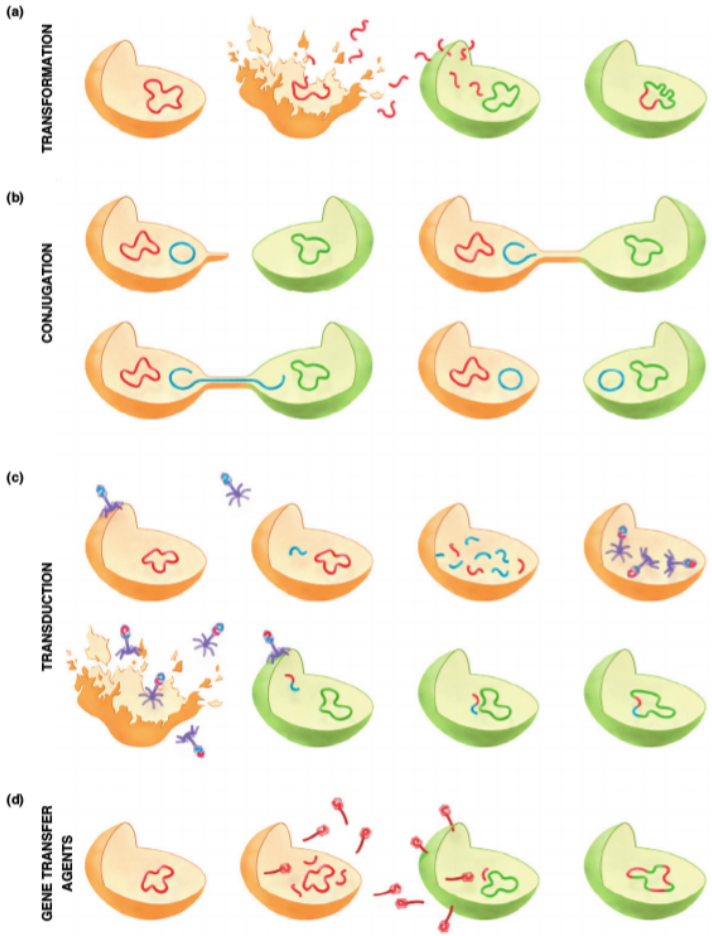

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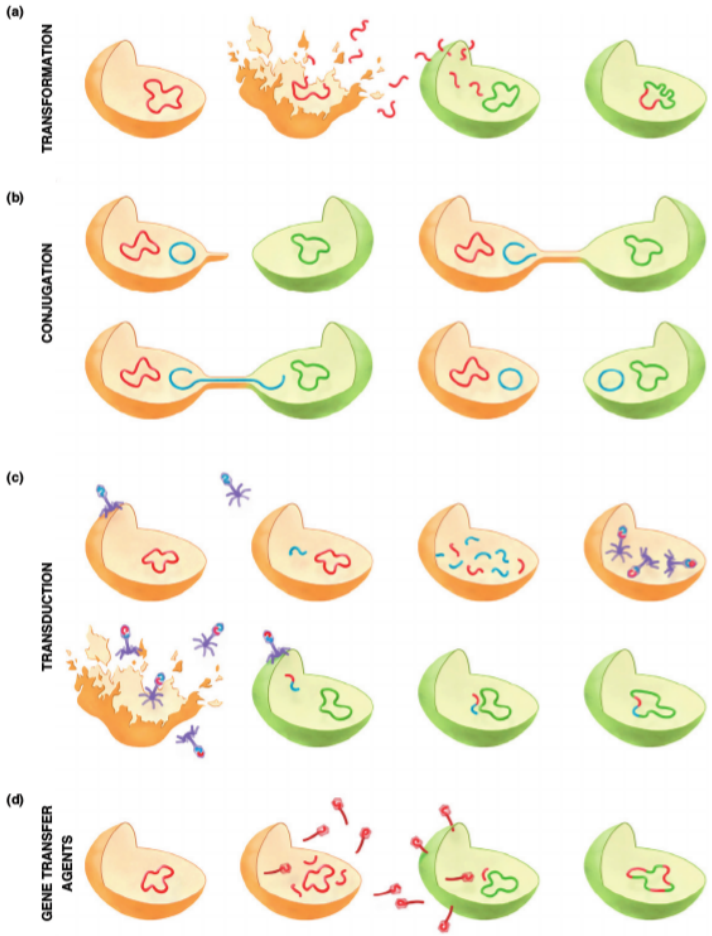

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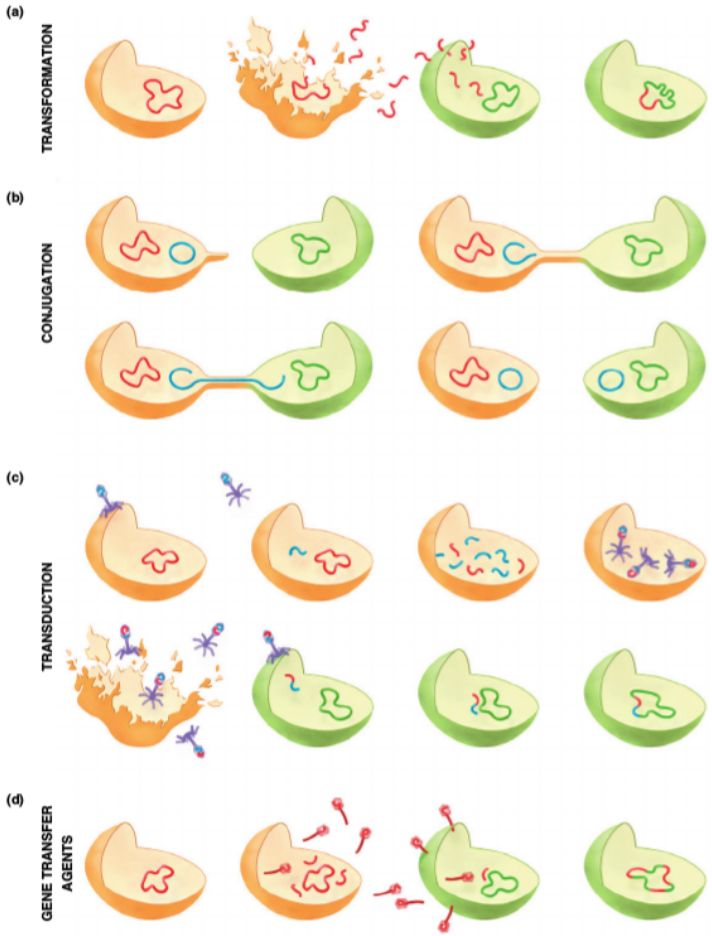

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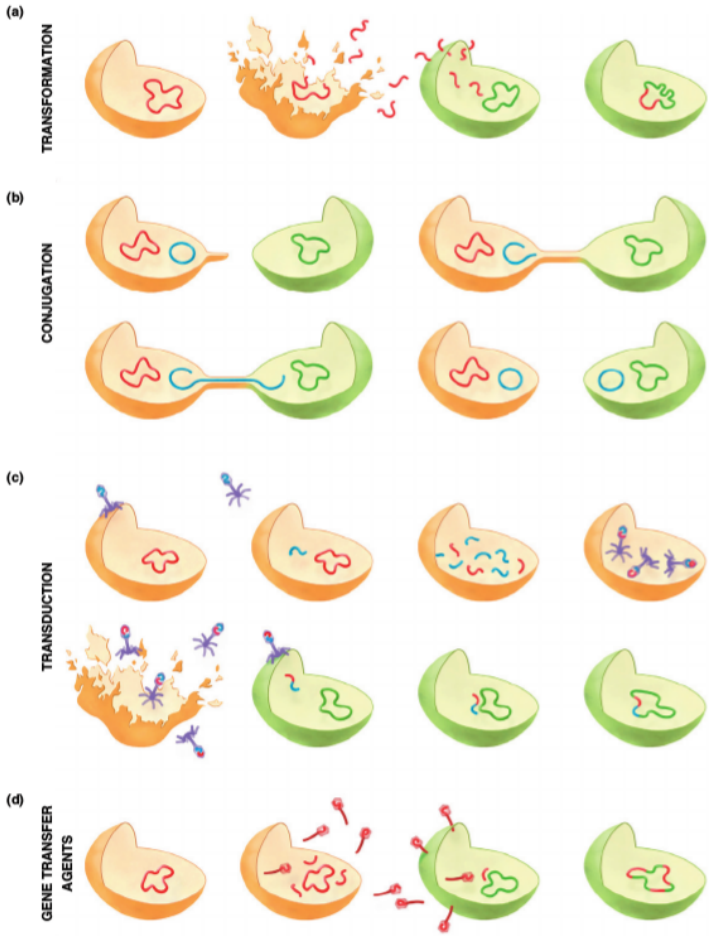

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- Conjugation: Transfer of DNA through cell-cell connections[6]
- Transformation: Incorportaion of free-floating DNA into the genome[6]
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- **CRISPR-Cas directly affects Transduction and Transformation**[6]

Pan-Genomes

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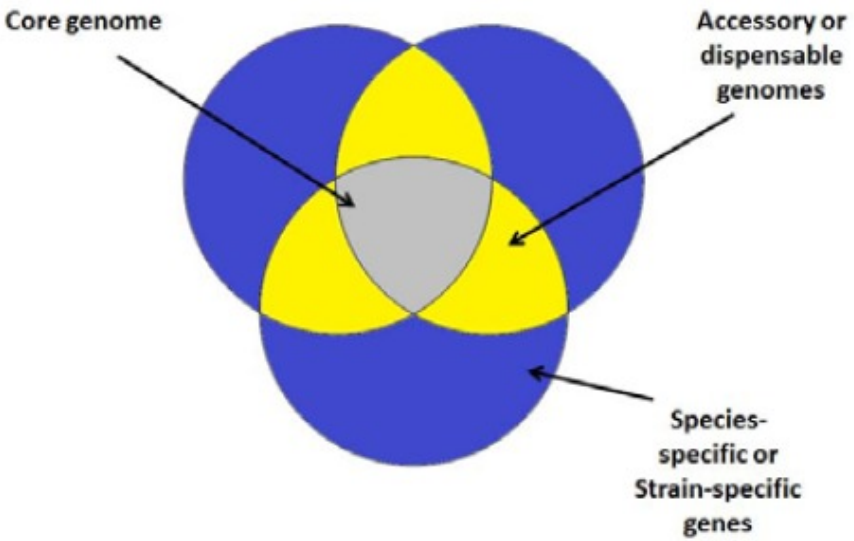

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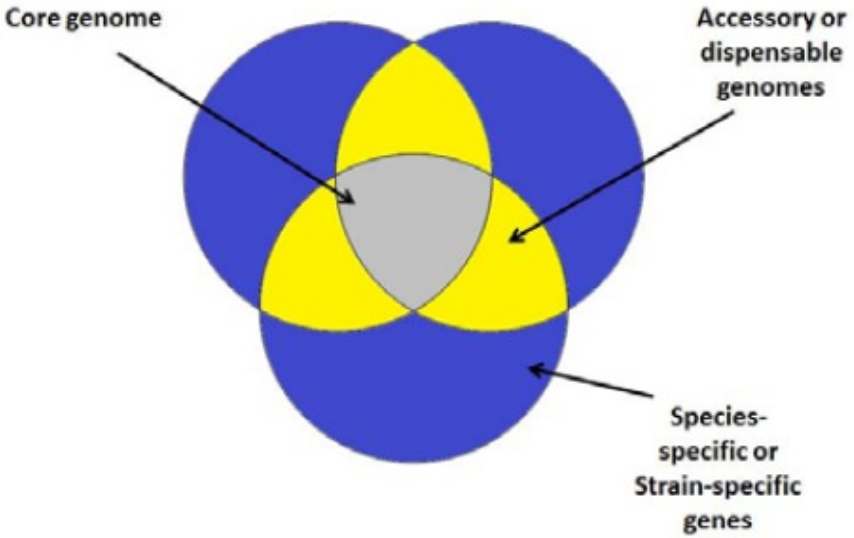

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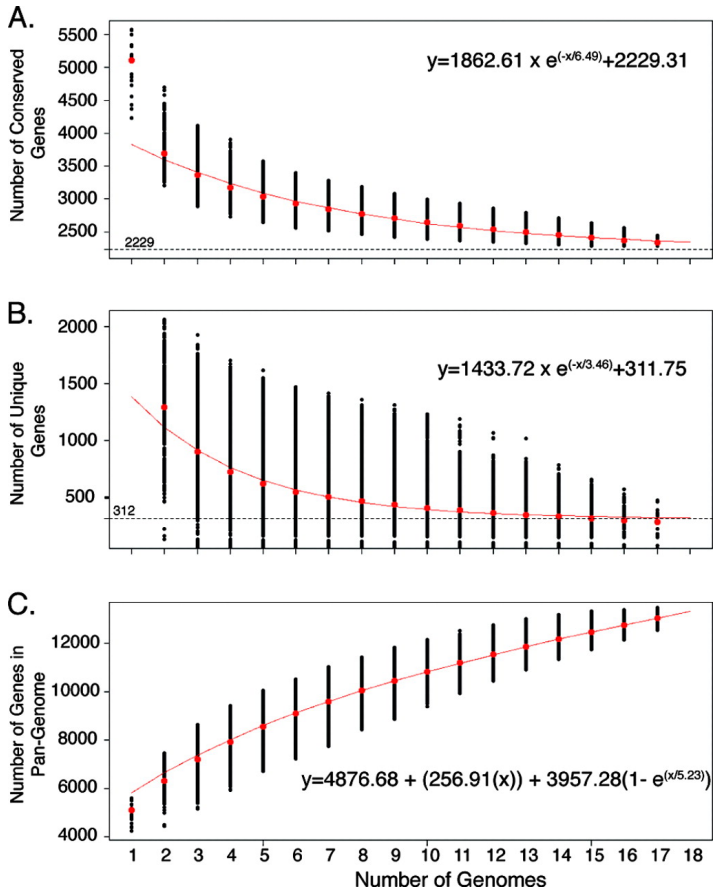

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Rate Influencing Factors

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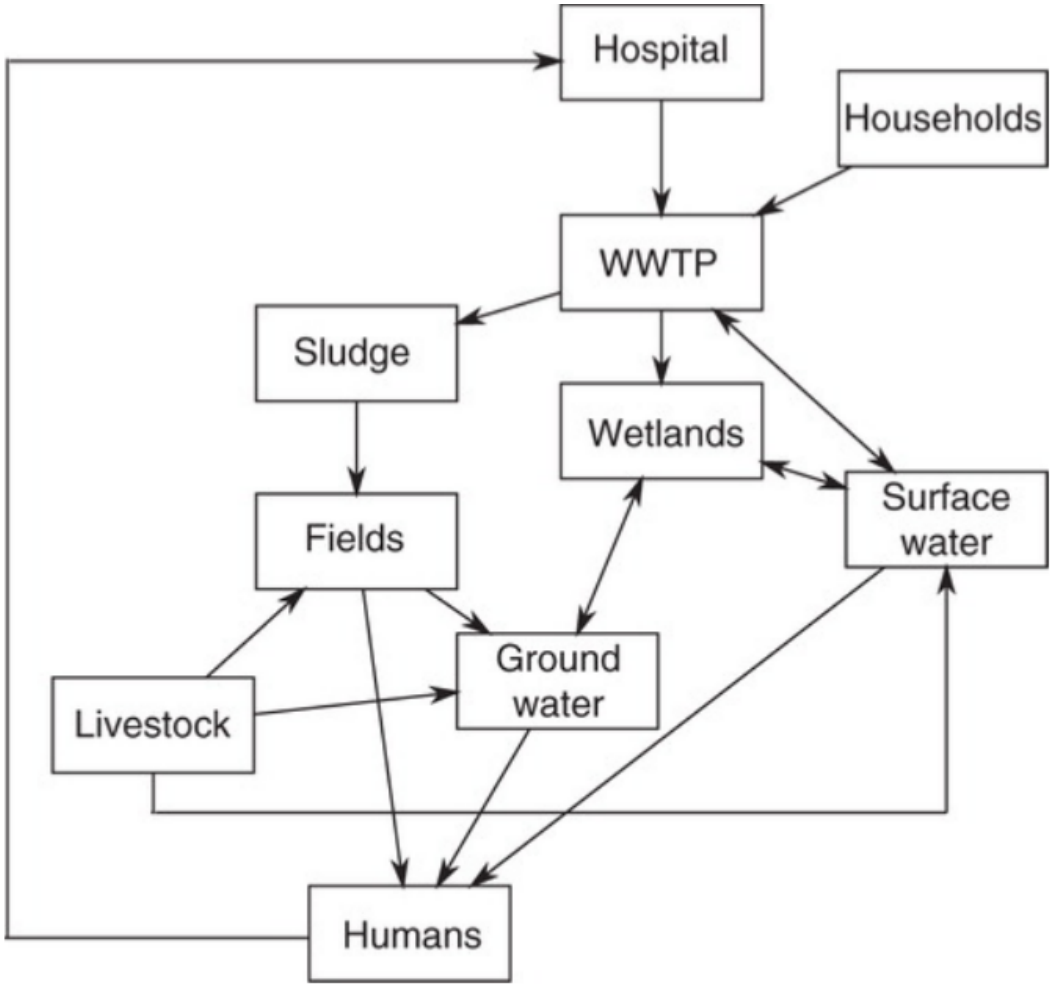

Phylogenomic Networks

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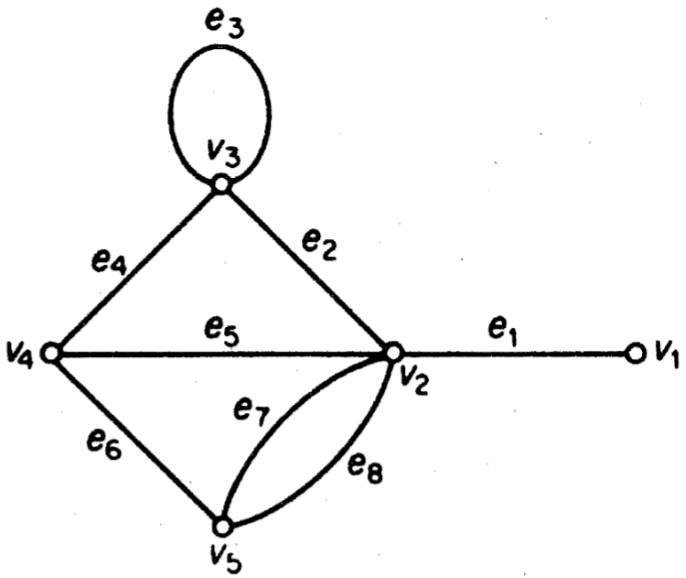

- Useful mathematical abstraction of real world system

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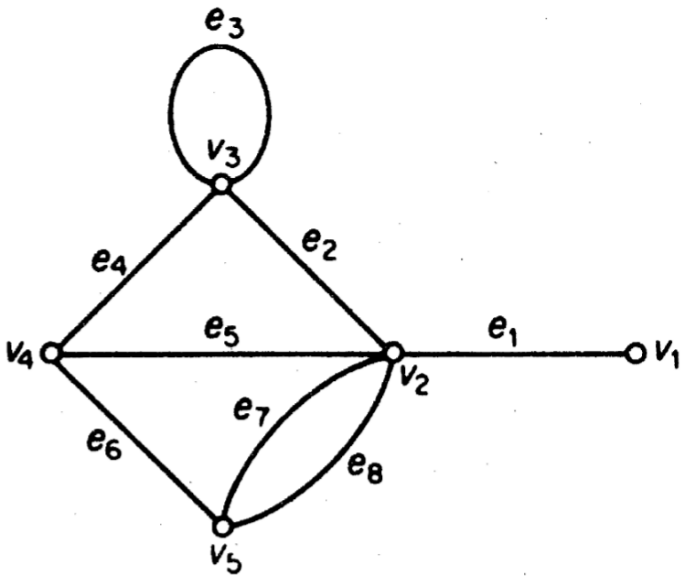

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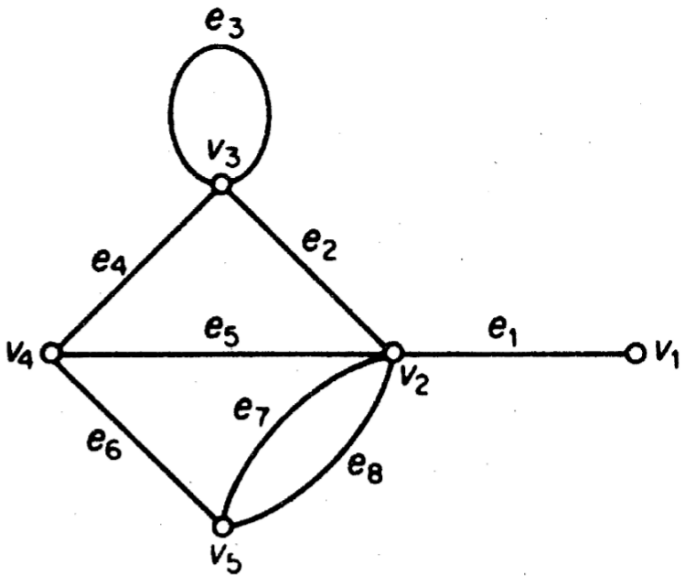

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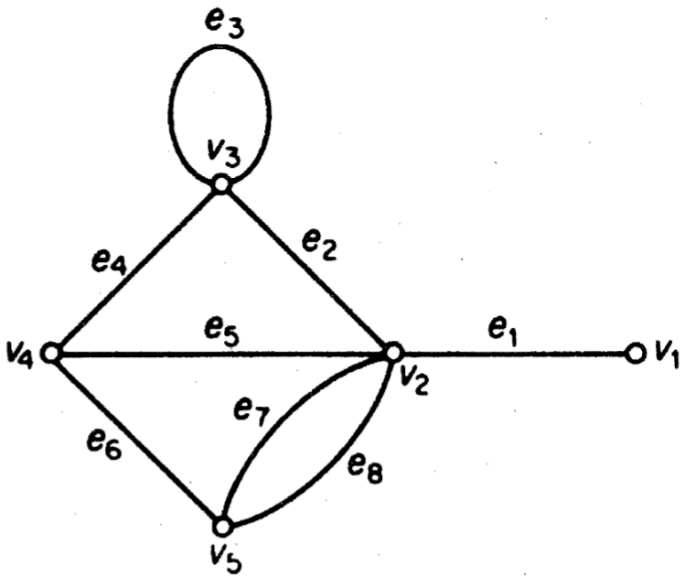

- Useful mathematical abstraction of real world system
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Prokaryotic "Net of Life"

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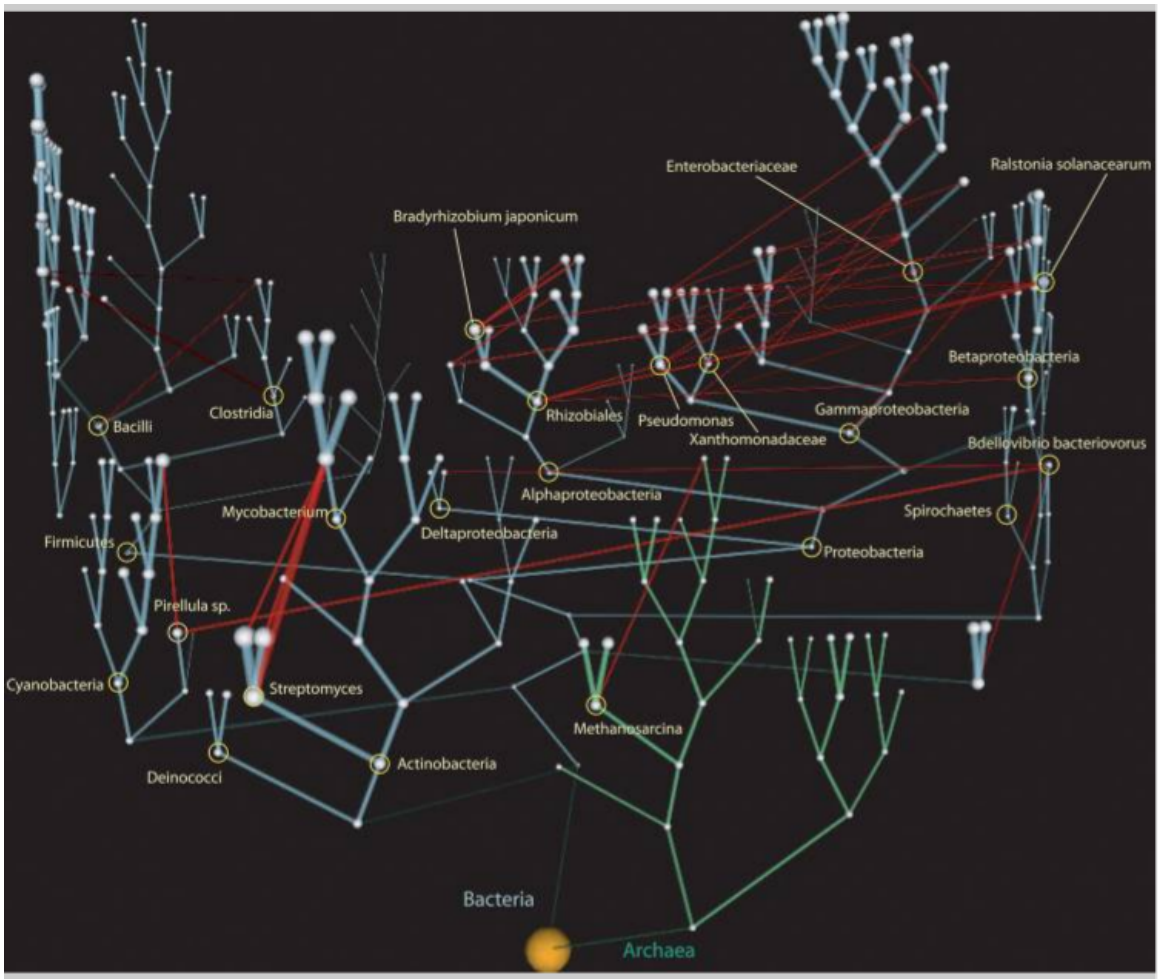
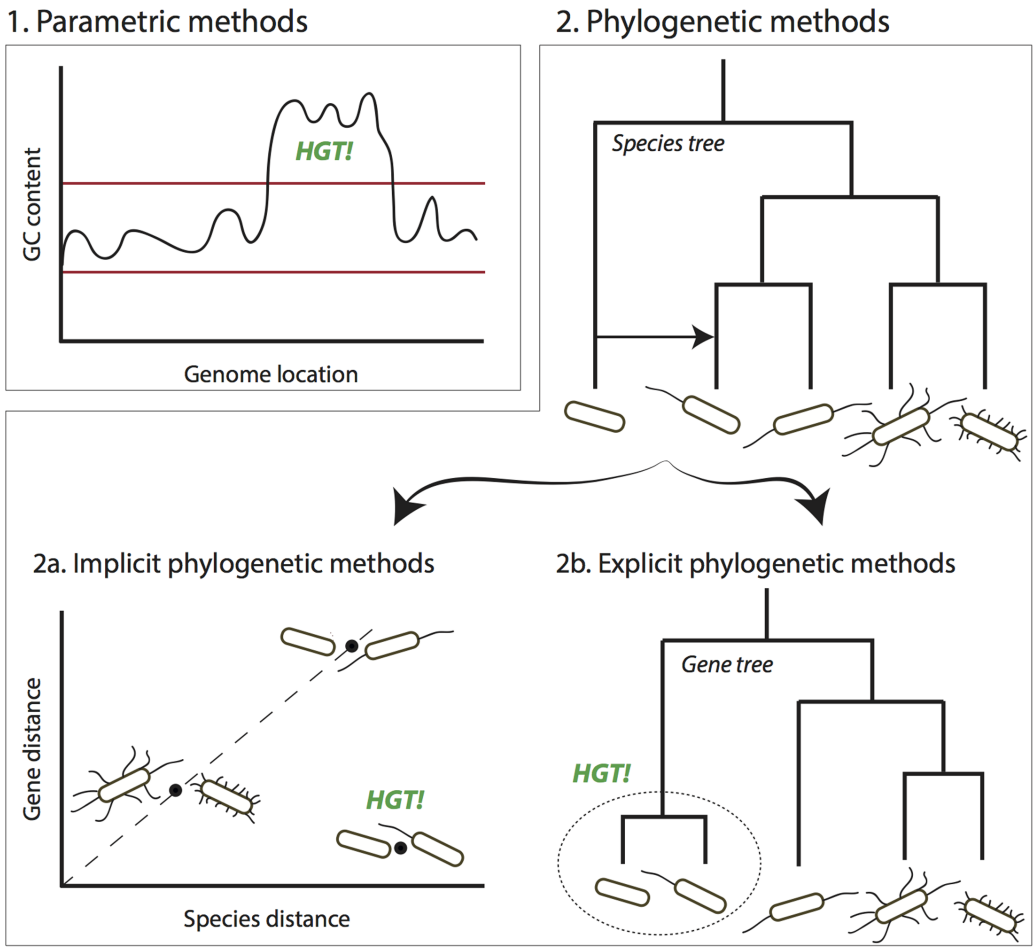


Construction



Do CRRISPR Systems Affect Horizontal Gene Transfer?

Yes

CRISPR Cost Complexity

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- Cost tradeoff factors:

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  - Prophage abundance[15]

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- Selective CRISPR inactivation[1]
- CRISPR can enhance transduction-mediated HGT[15]

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  - Can see inhibitory effects of CRIPSR on HGT over short evolutionary time scales
  - Higher gene indel rates for CRISPR containg genera than non-CRISPR containing outgroups

My Project

Hypothesis

Null Hypothesis

Bacterial strains or genera with known CRISPR systems will show no significant differences in network statistics compared to those strains or genera without known CRISPR systems.

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Alternative Hypothesis

Bacterial strains or genera with known CRISPR systems will show a significant difference in at least 1 network statistic compared to those strains or genera without known CRISPR systems.

Objectives

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Within Network Comparisons

For genera with CRISPR conatining strains, compare the node statistics of CRIPSR-containing strain to non-CRISPR-containing strains.

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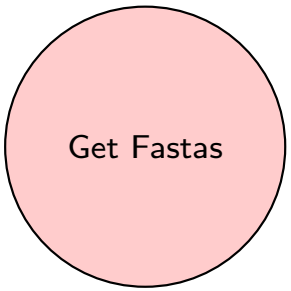
Gene Indel Rates vs. Network Statistics

Compare gene InDel rates to node/network statistics for CRISPR-containing and non-CRISPR-containing strains/genera.

Workflow



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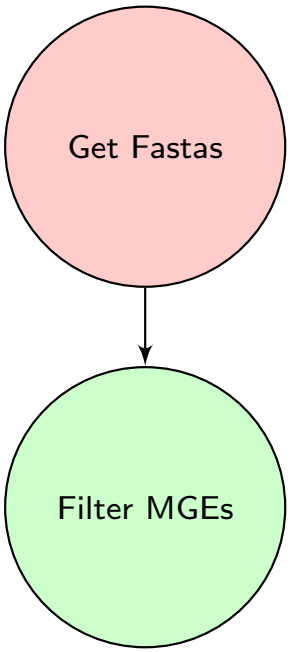

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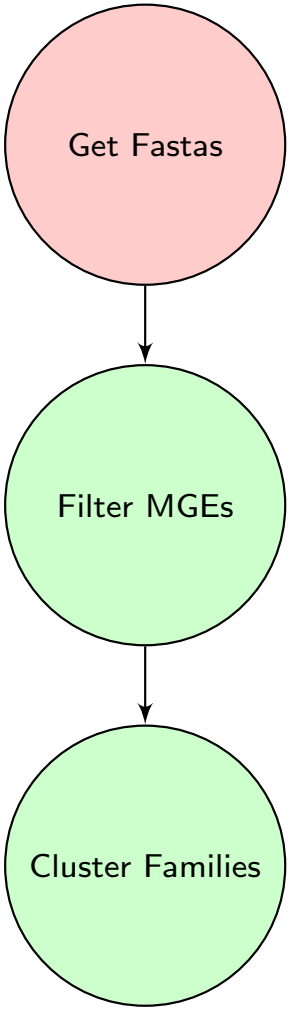

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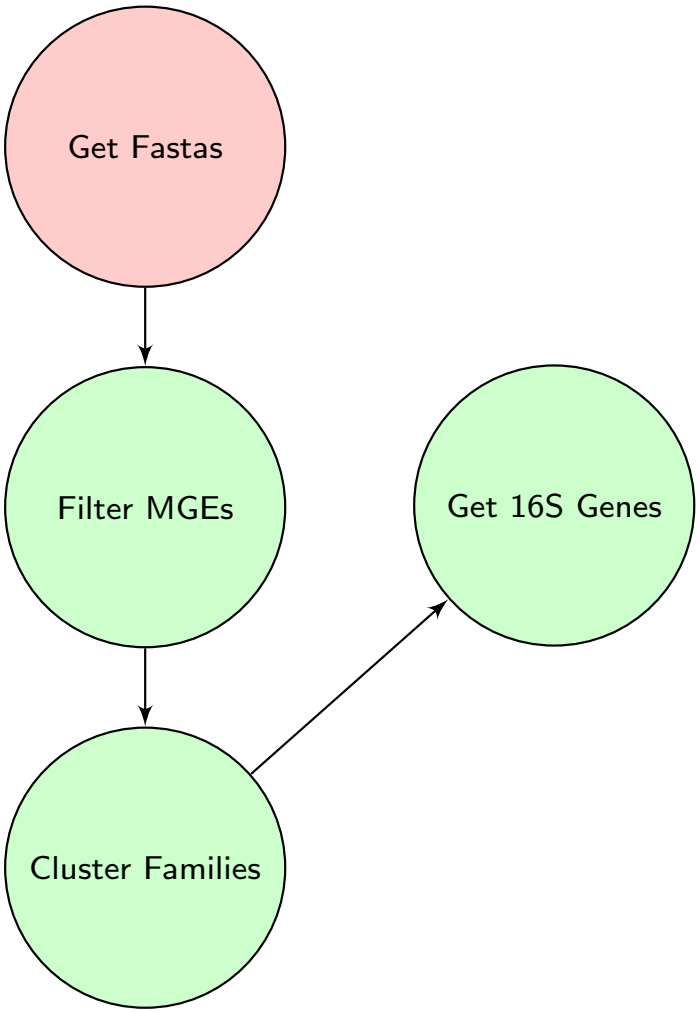

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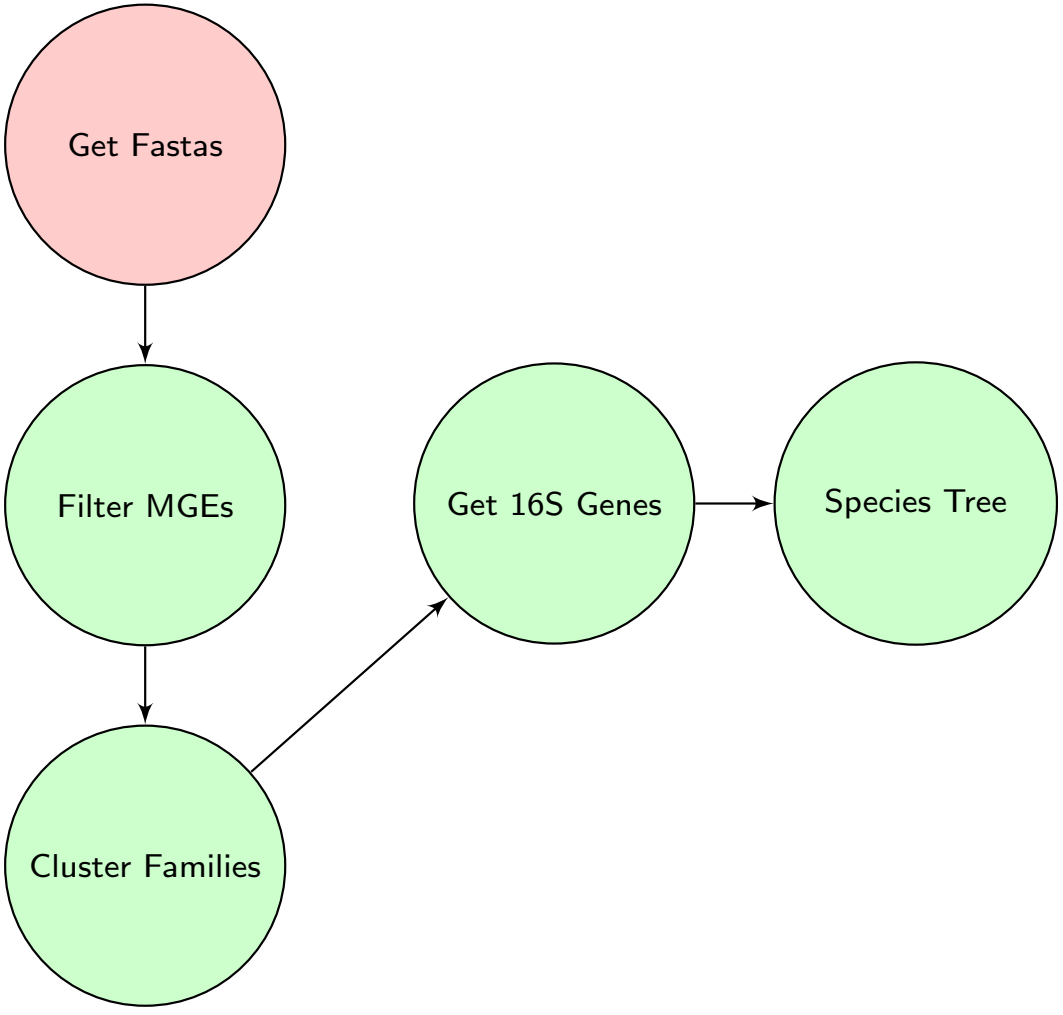
Get Fastas
Filter MGEs
Cluster Families
Get 16S Genes
Species Tree

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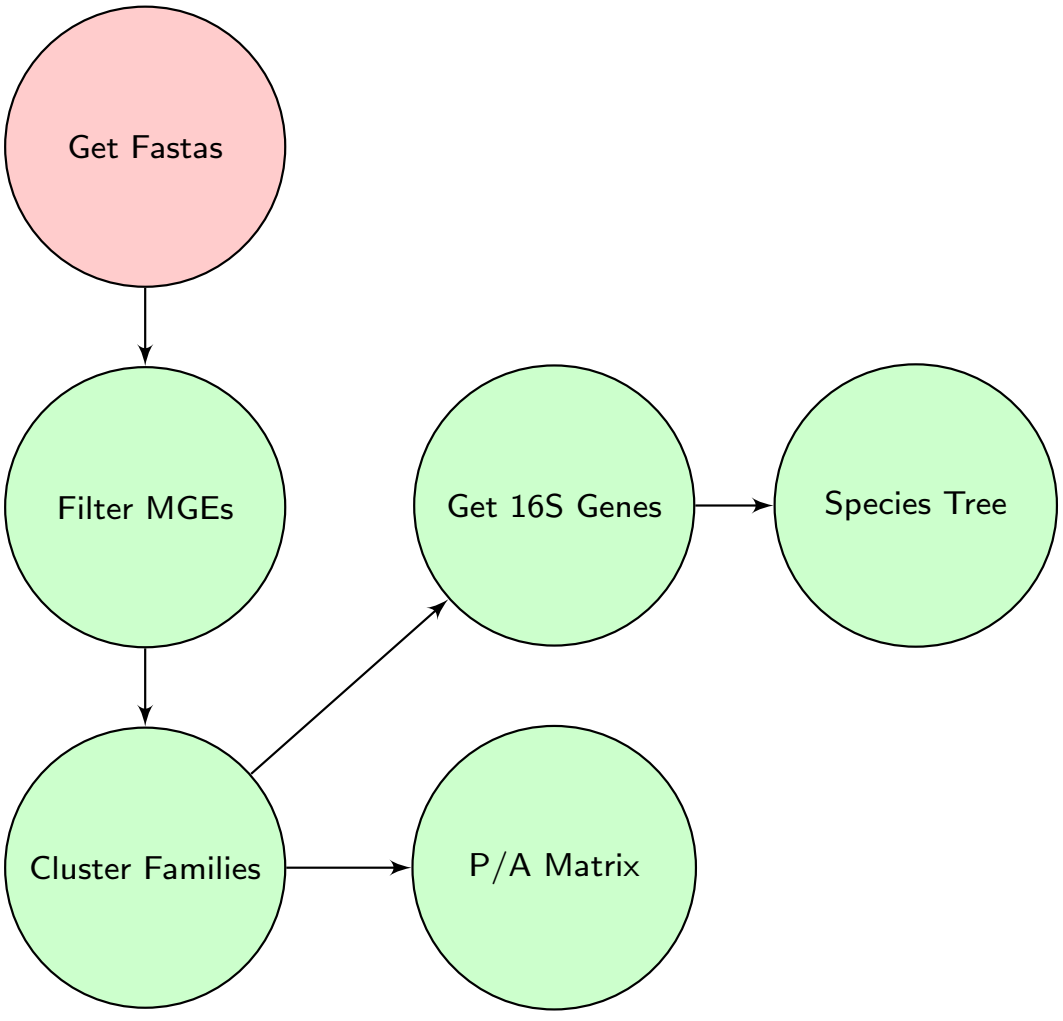
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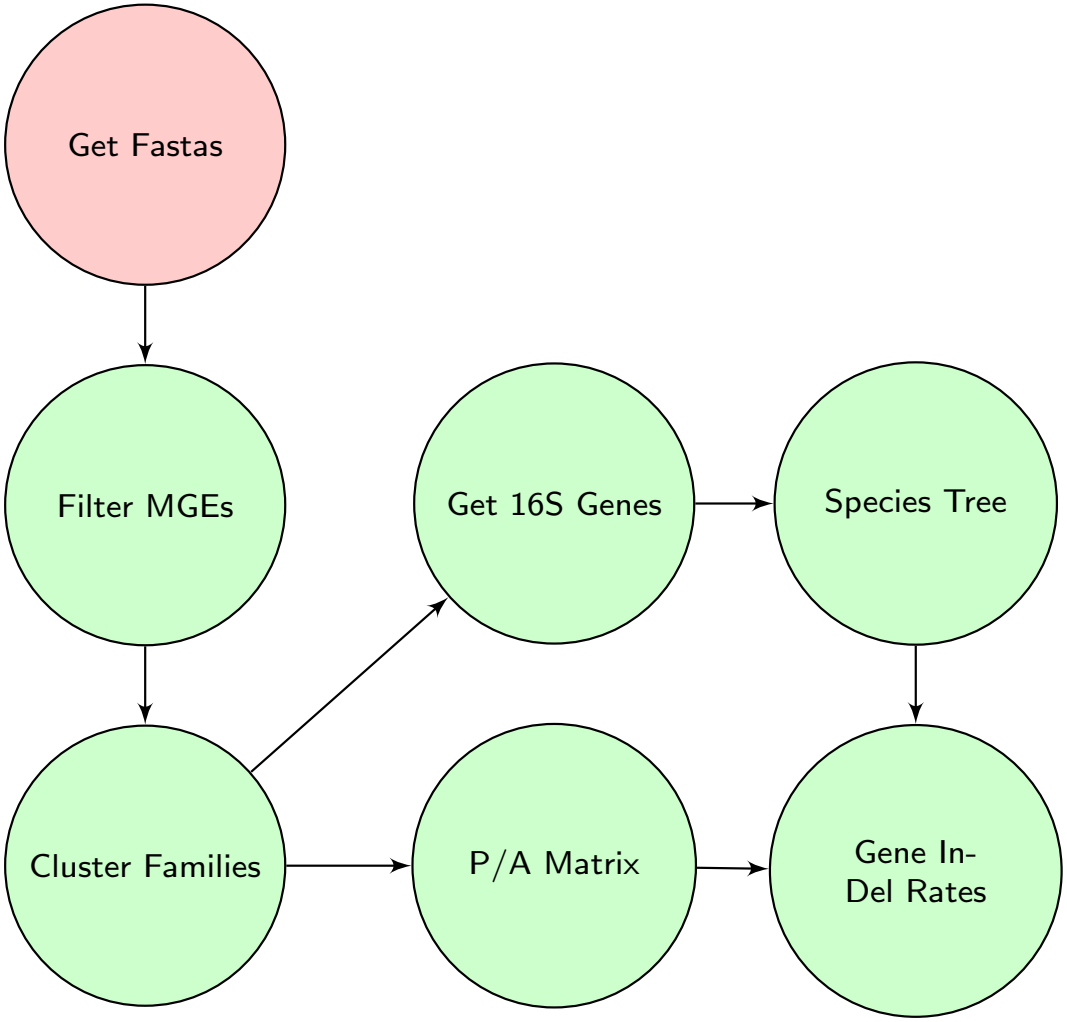
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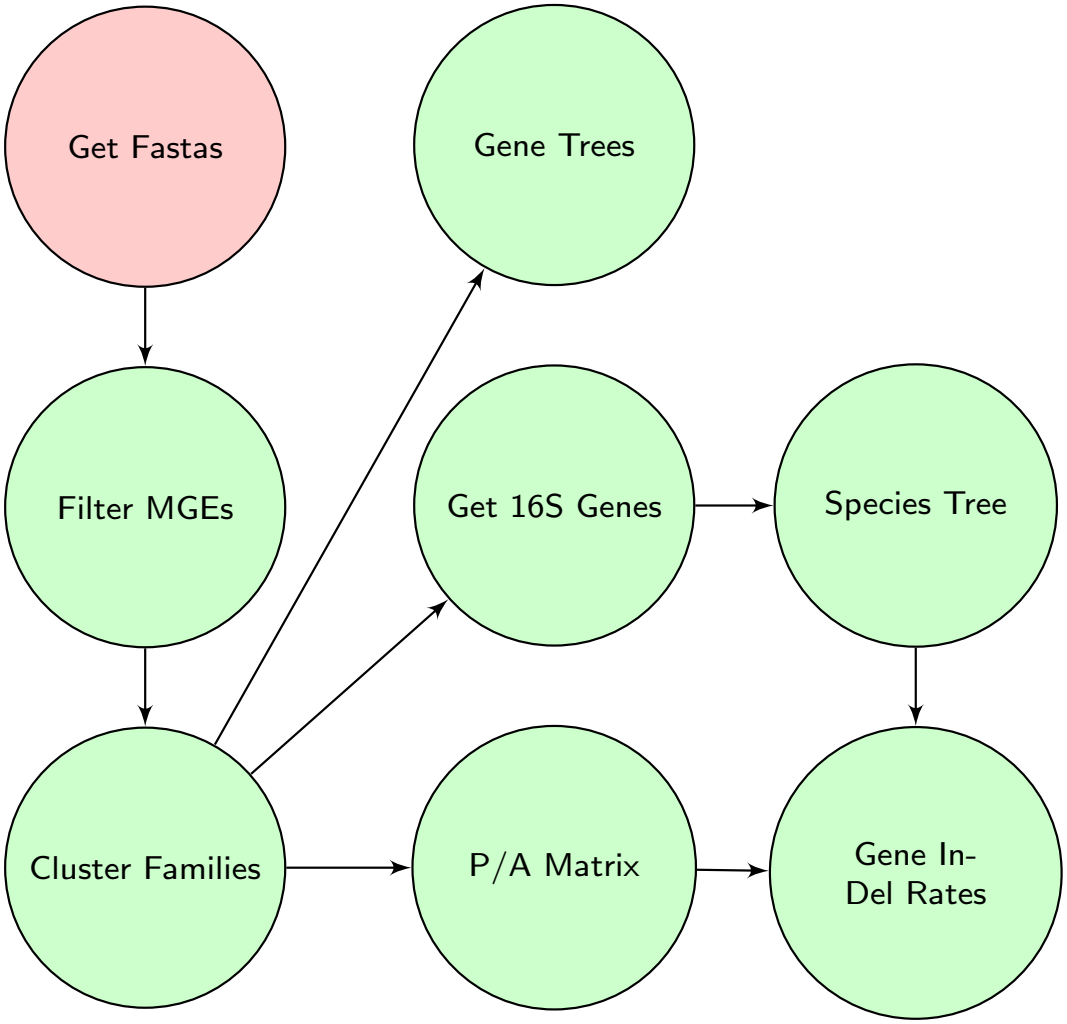
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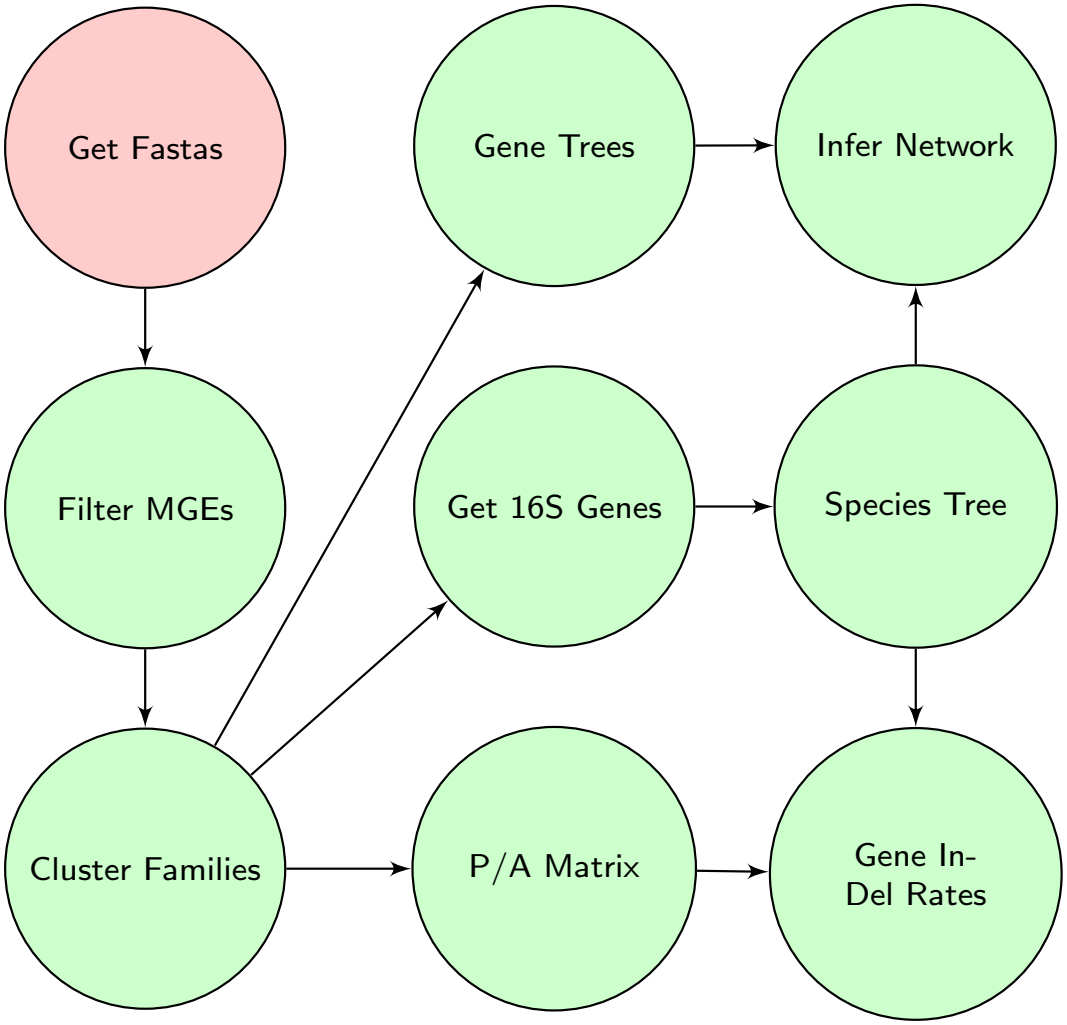
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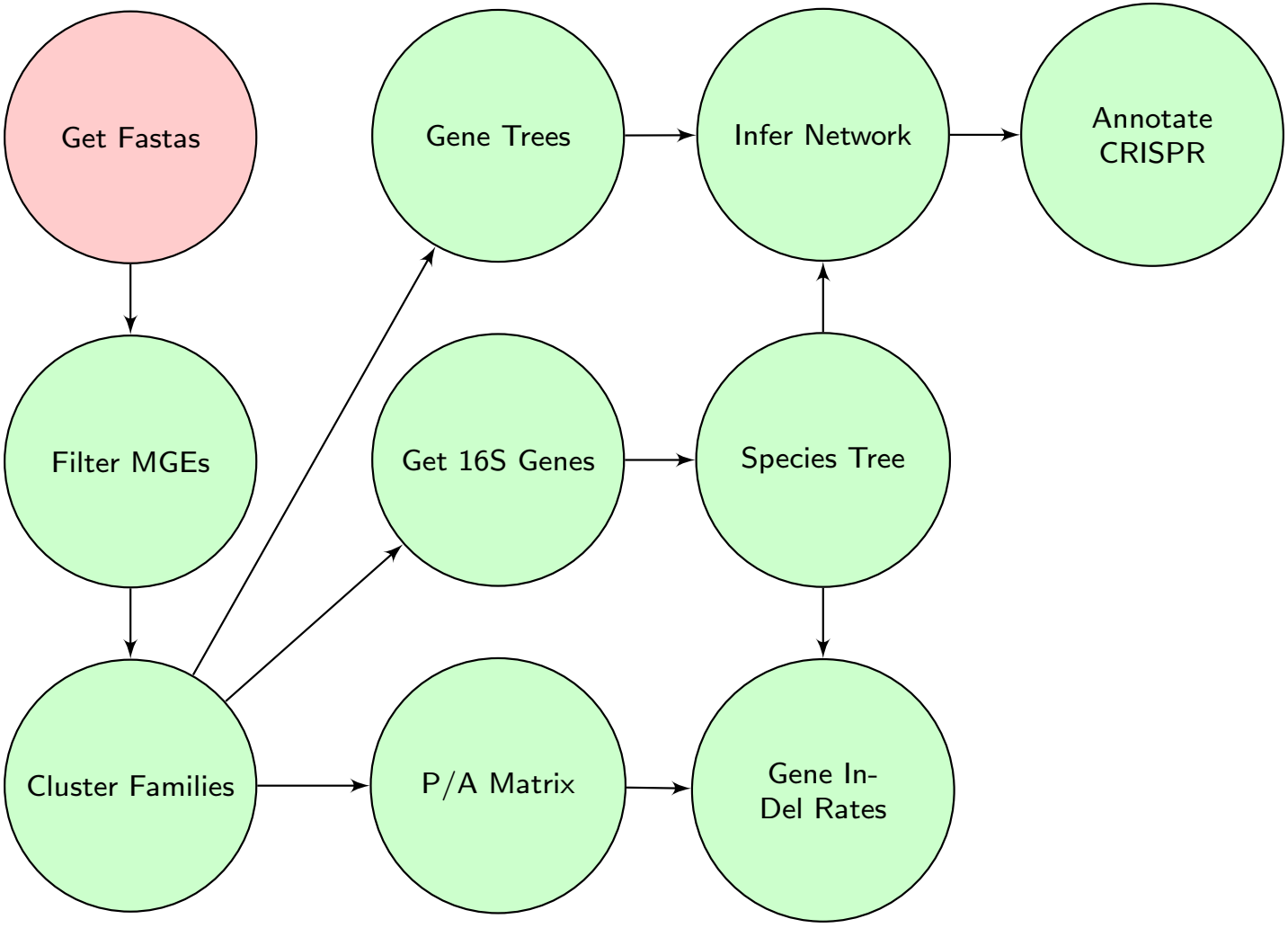
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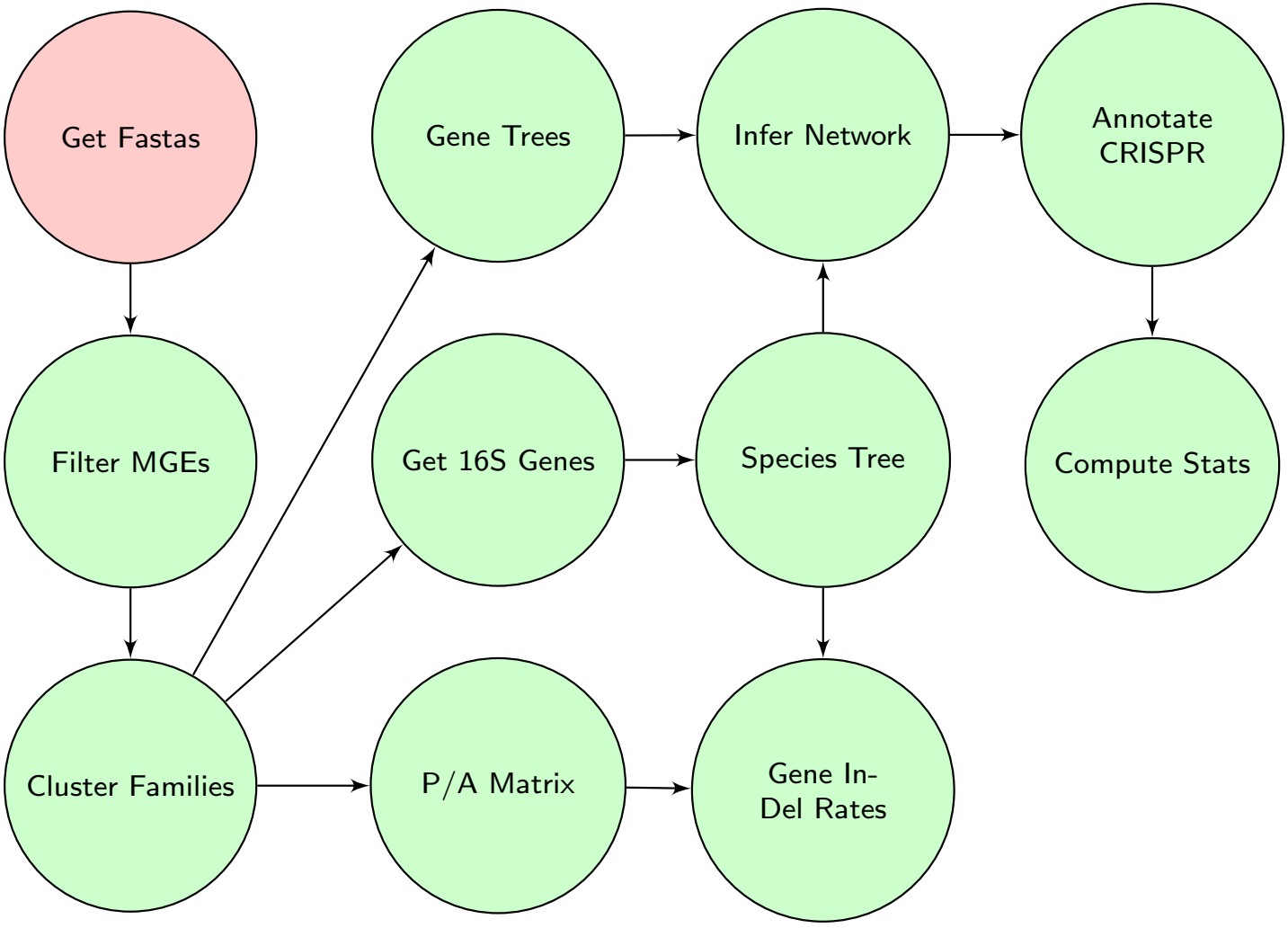
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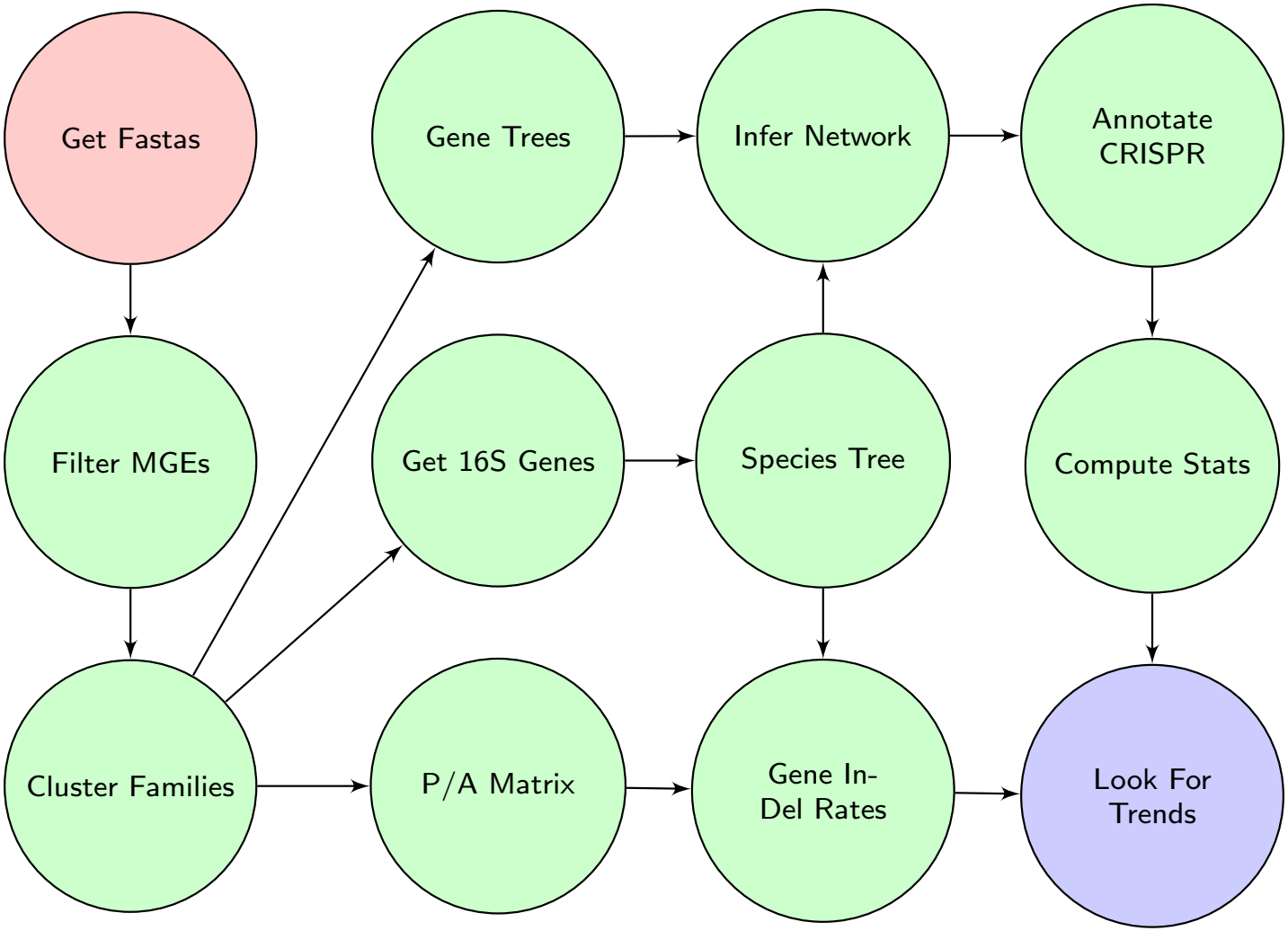
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Look For Trends

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Network Statistics

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- **Average Edge Weight**: $\frac{1}{N_c}\sum_i w_i$, The average edge weight for all nodes with CRISPR or without CRISPR
- **Node Clustering Coefficient**: $\frac{1}{k_u(k_u-1)}\sum_{vw}^{T(u)}\left(\hat{w}_{uw}\hat{w}_{vw}\hat{w}_{uv}\right)^{\frac{1}{3}}$ where $T(u)$ is the set of traingles containing u[18]

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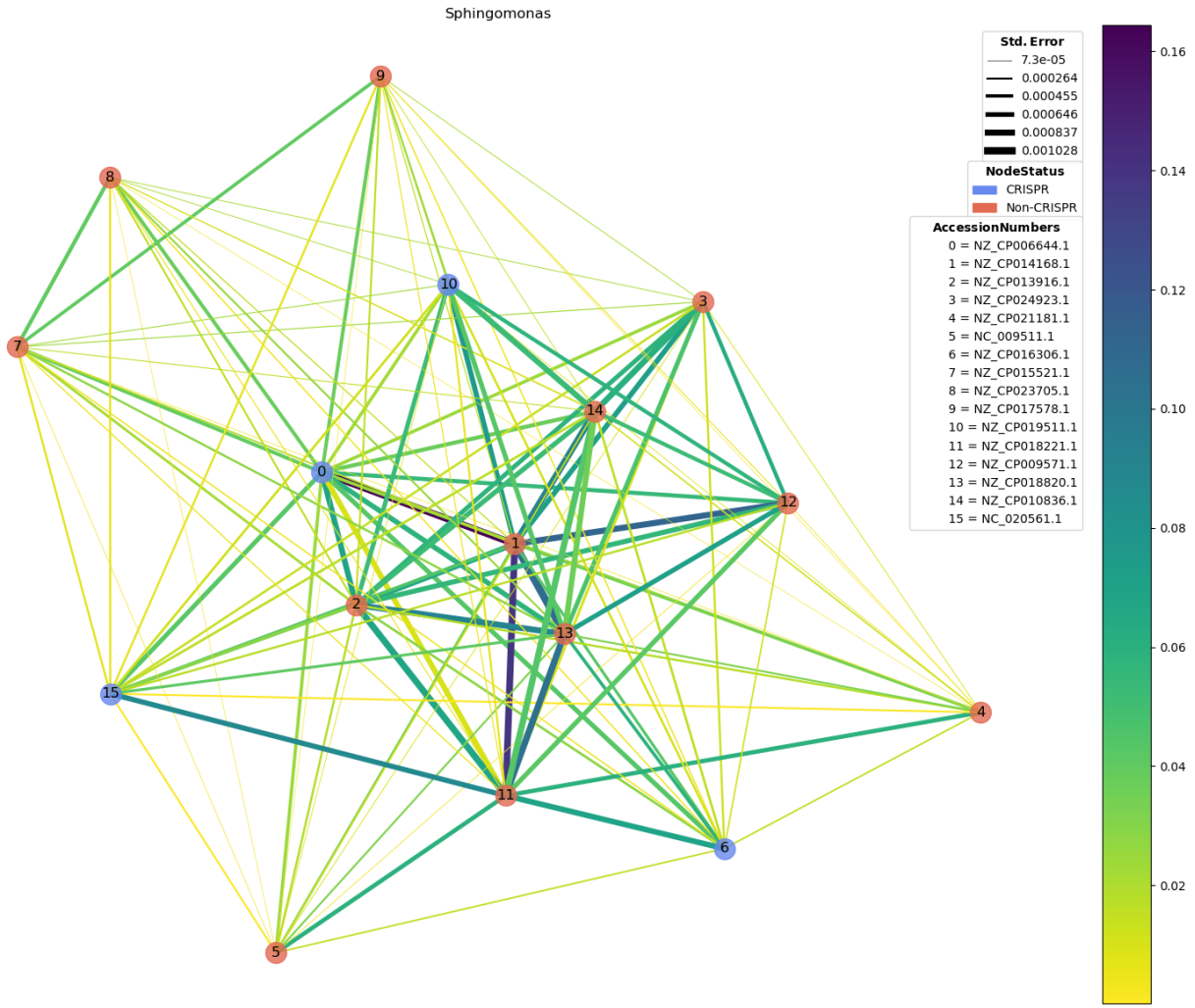
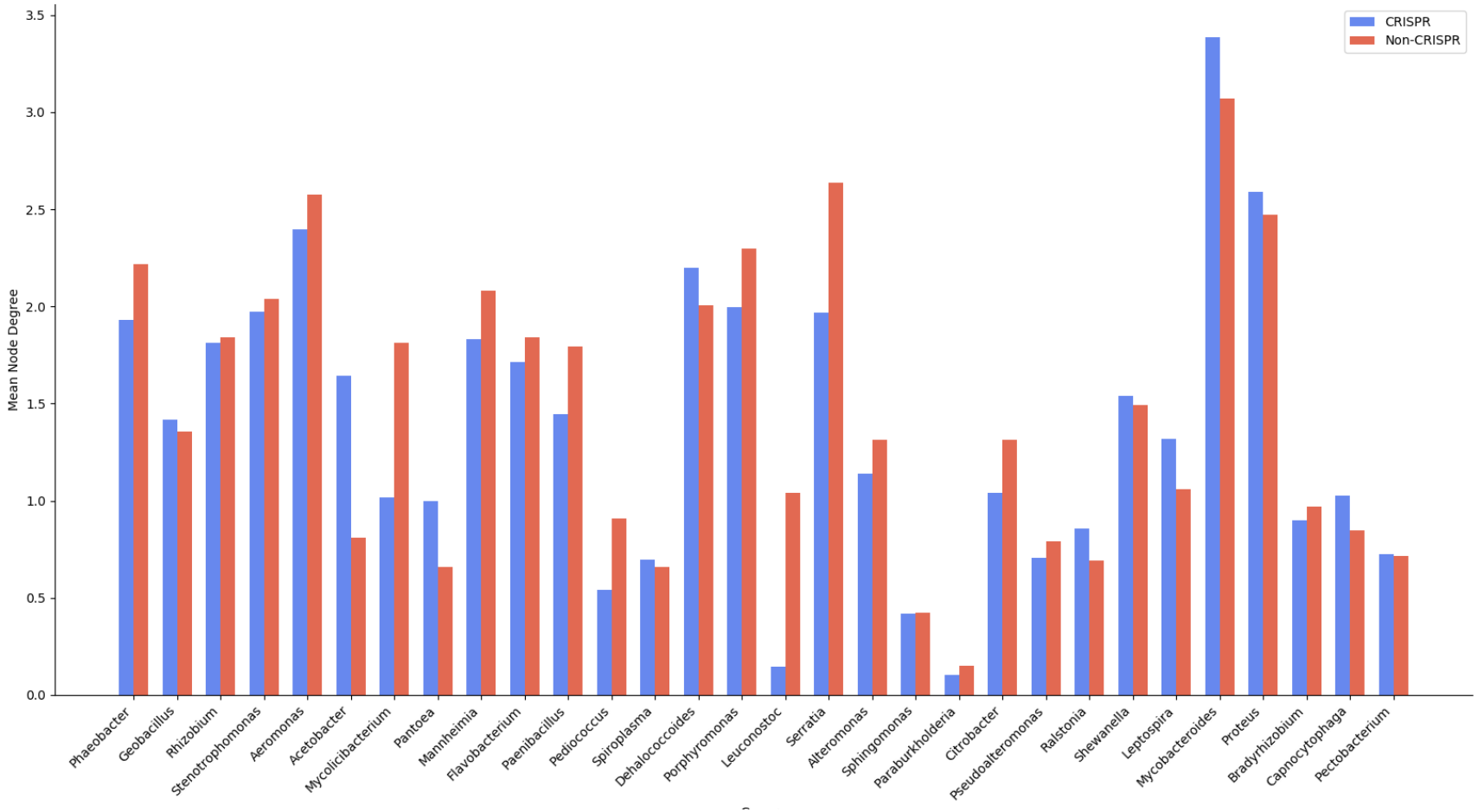
- **Network Modularity**: $Q = \frac{1}{2m}\sum_{uv}^{W}[W_{uv} - \frac{k_u k_v}{2m}]\delta(u,v)$ where m is the total weight of alledges, k_u is the degree of u and $\delta(u,v)$ is 1 if u and v both have or do not have CRISPR systems and 0 otherwise. $Q \in [-1,1]$[20]

Results

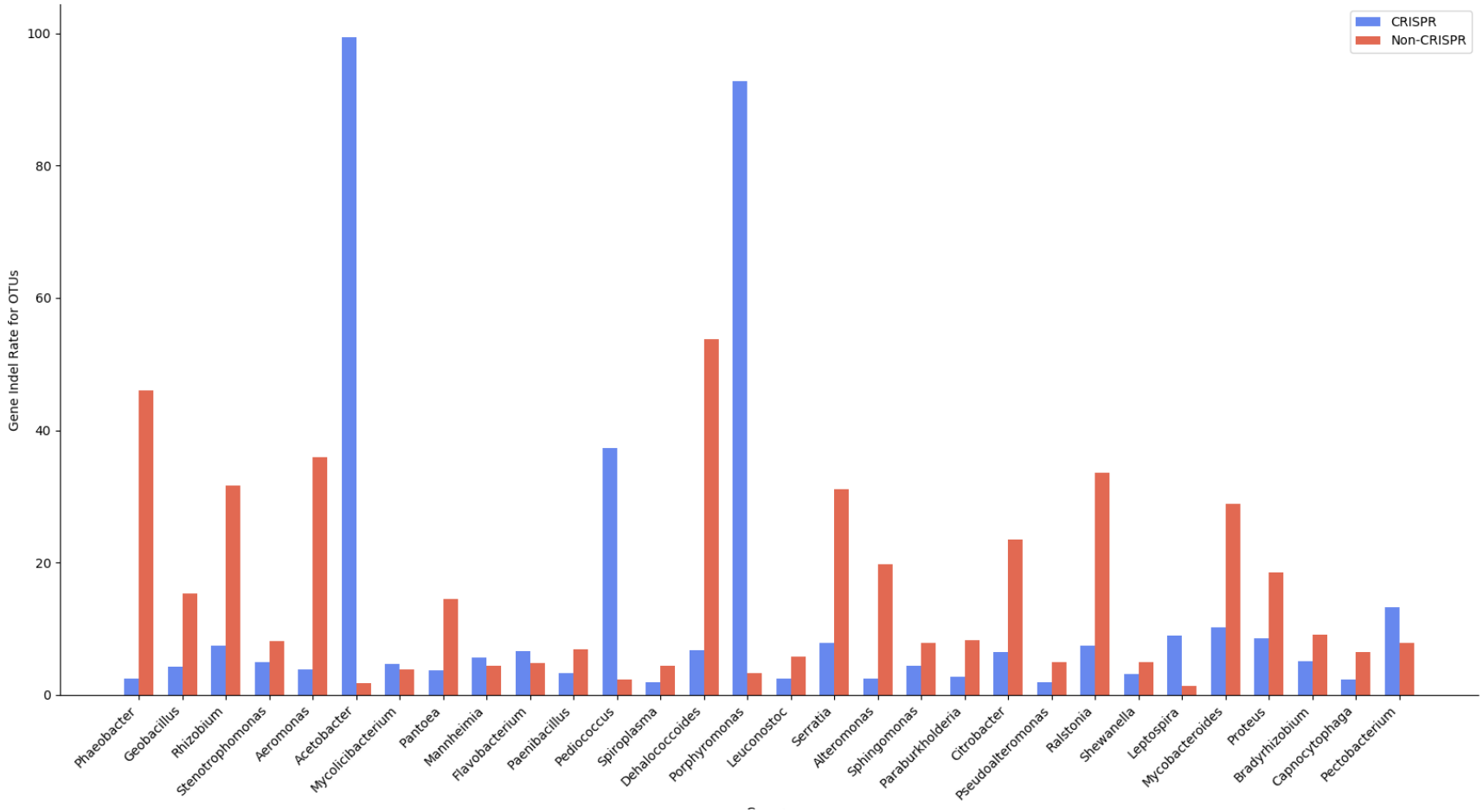
Example "Consensus" Network



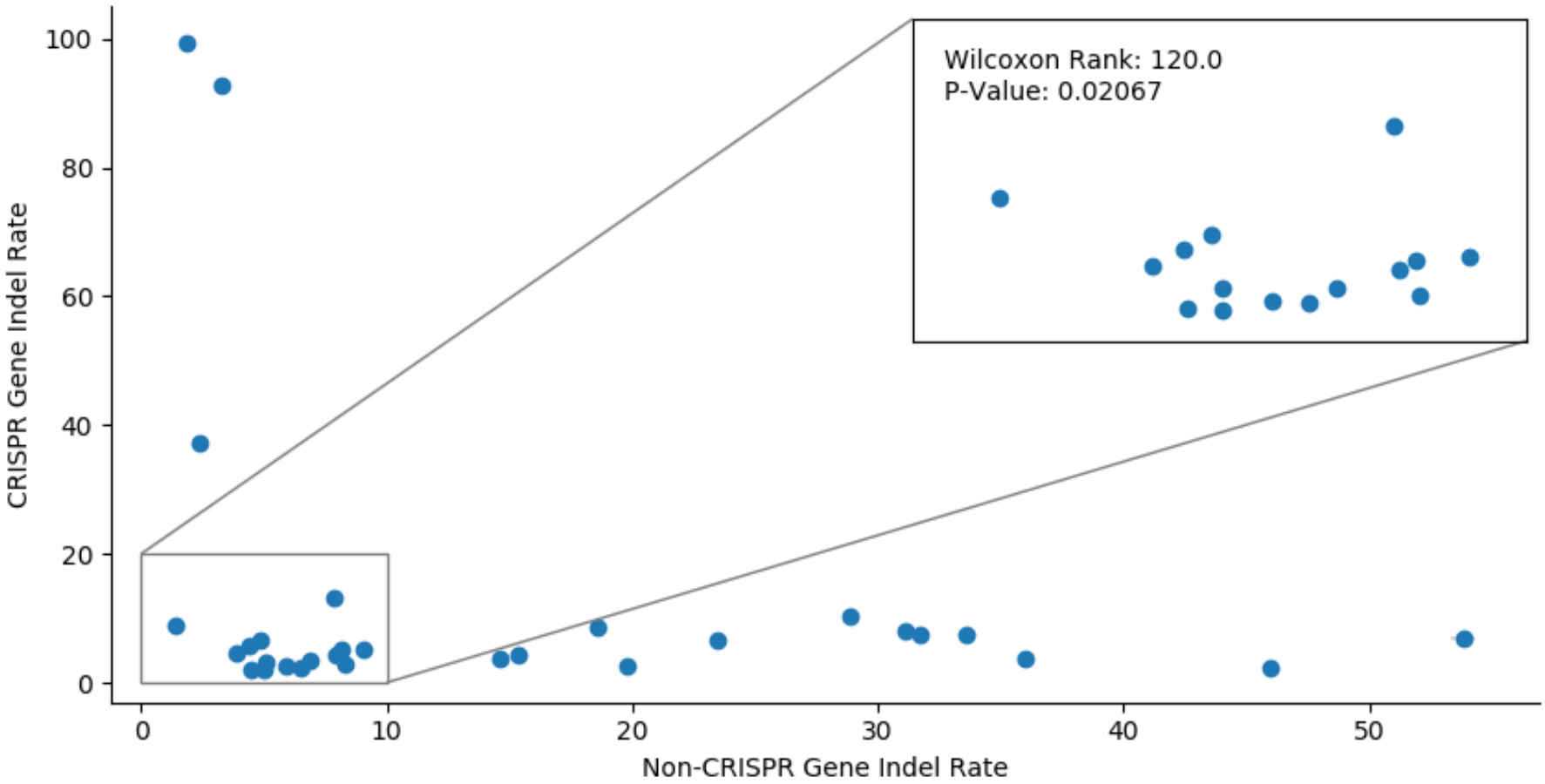
Mean Node Degree



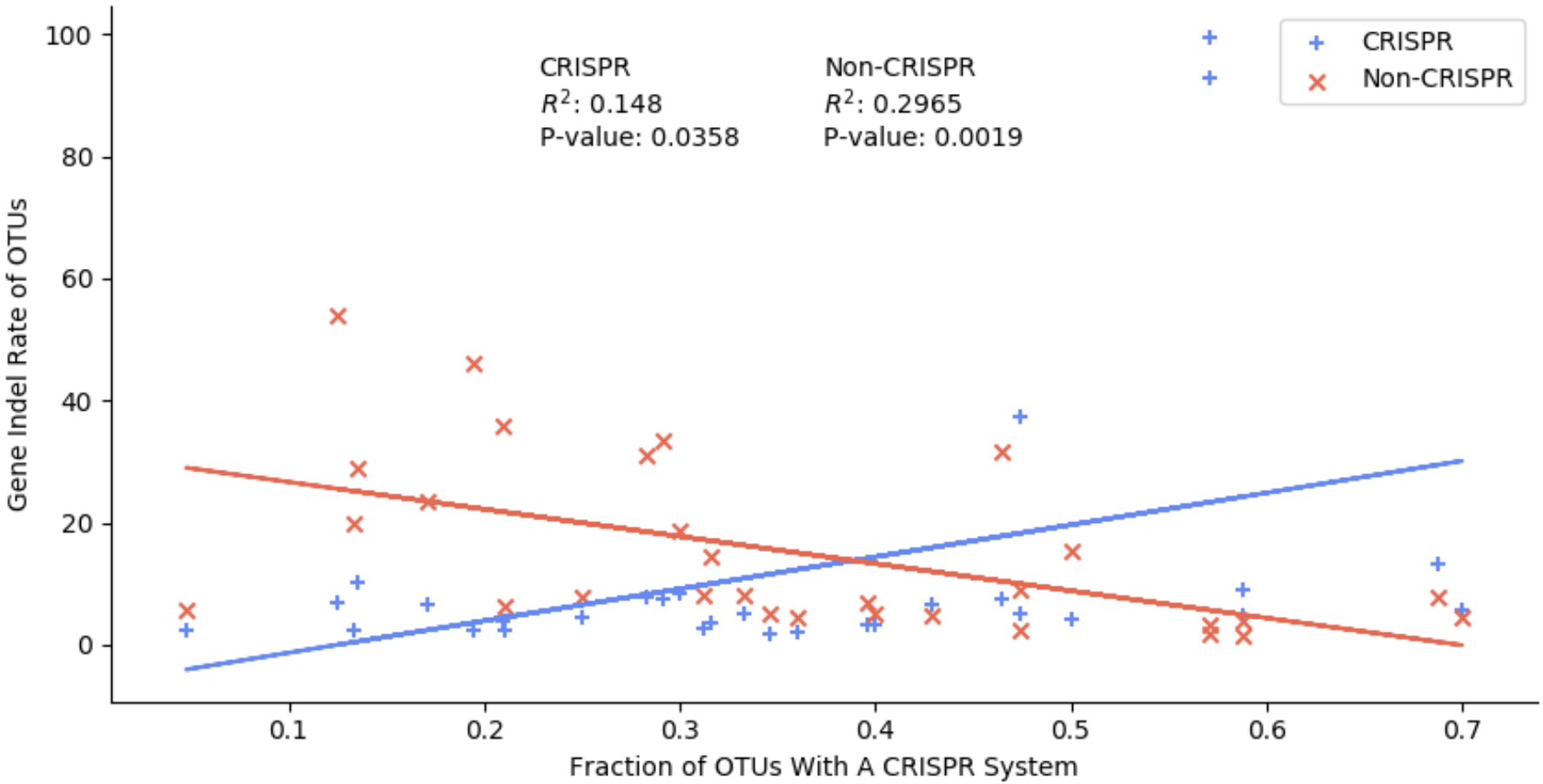
Gene Indel Rates



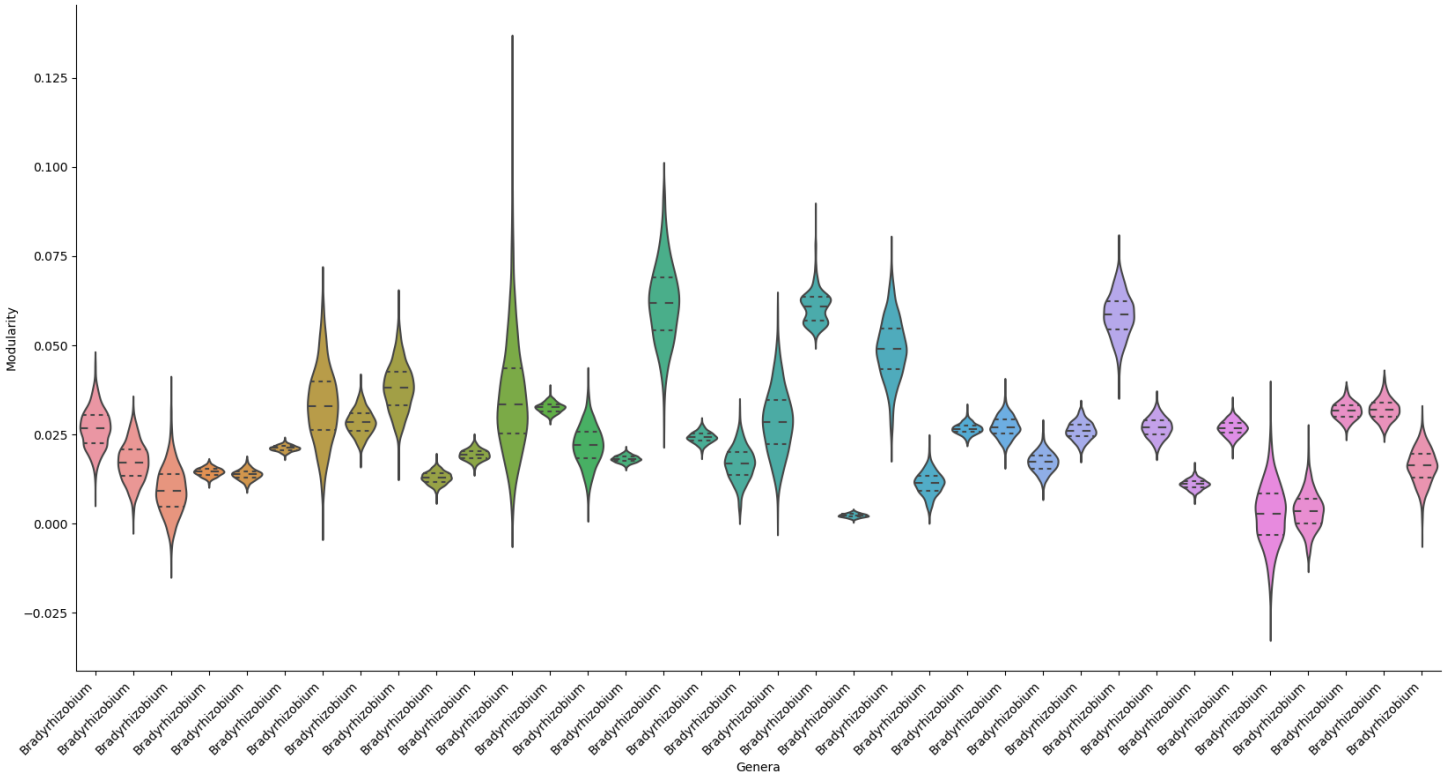
Gene Indel Rates



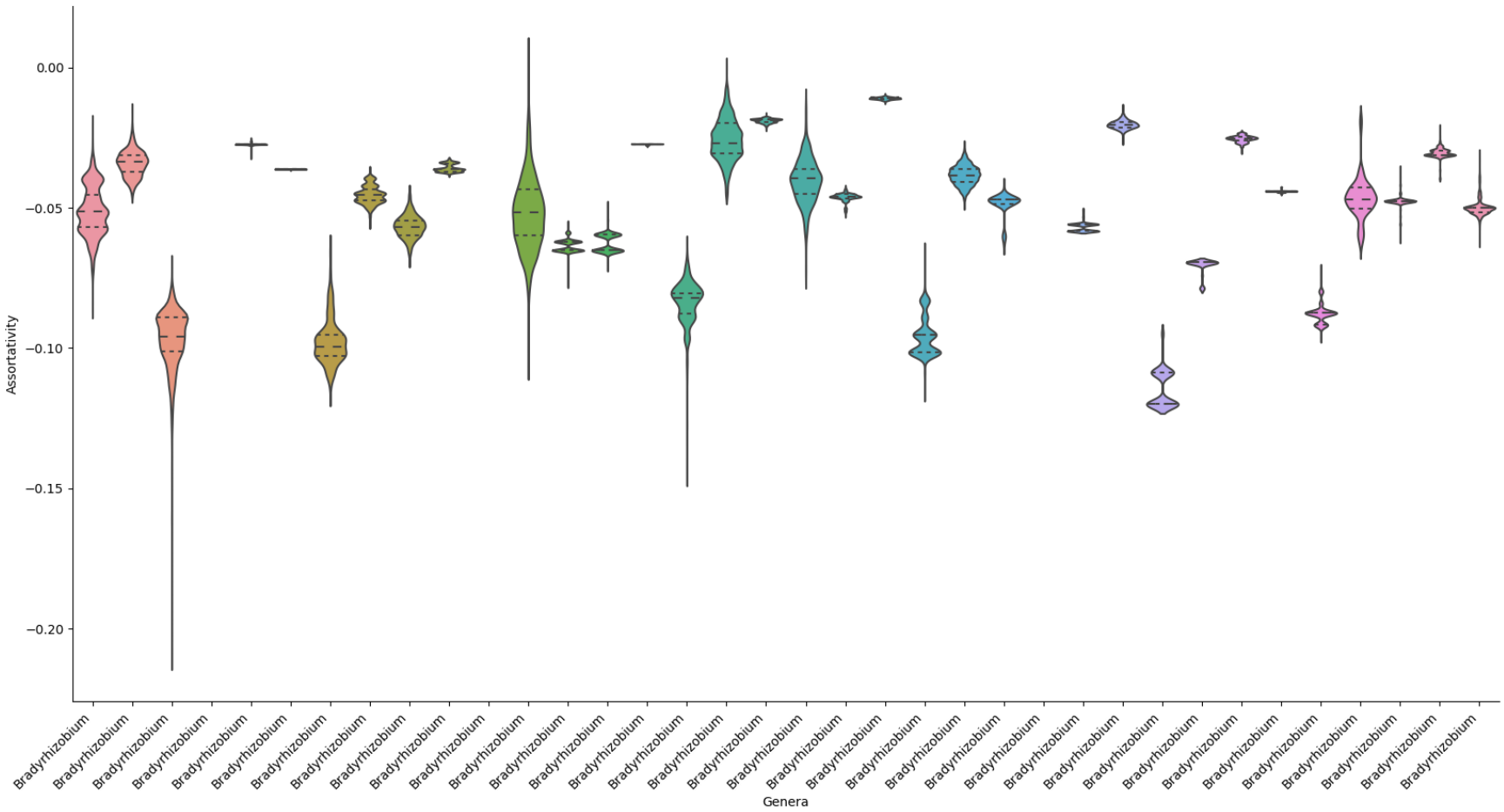
Gene Indel Rate Difference Vs. Fraction CRISPR Species



Modularity Distributions



Assortativity Distributions



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- **Multifurcation Error**: Some species trees contained multifurcations, which were resolved randomly to generate a bifurcating tree. Estimating this error by examining variance over different resolutions is possible.

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- **Considering bacterial ecology and environments**: Consider geographically close OTUs or differences between networks due to environmental factors

Conclusion

Thanks

Thank you to

- Dr. G. Brian Golding
- Dr. Ben Evans
- The Golding lab
  - Caitlin Simopoulos
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  - Lucy Zhang
  - Brianne Laverty
  - Nicole Zhang
- Everyone here for listening

All code used for this project is available at https://github.com/DJSiddharthVader/thesis_SidReed

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