

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



Golding Lab,
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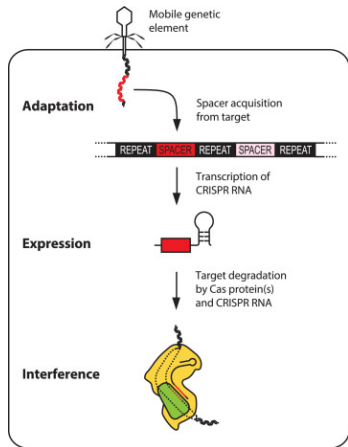
1. Background
2. My Project
3. Results
4. Conclusion

CRISPR-Cas systems

What Are They?

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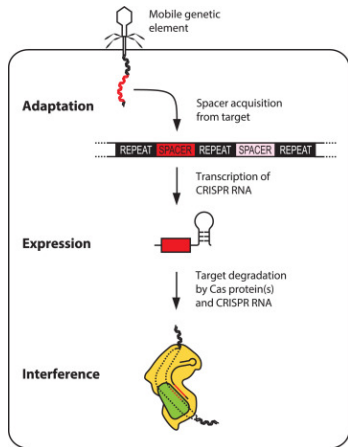
- Adaptive Bacterial Immune System



(Rath et al., 2015)

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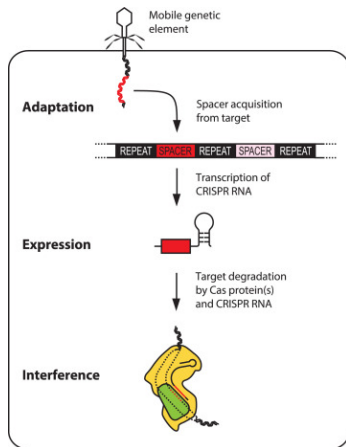
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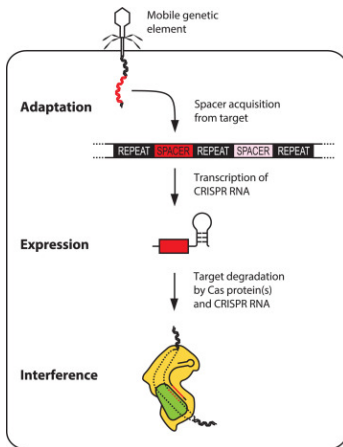
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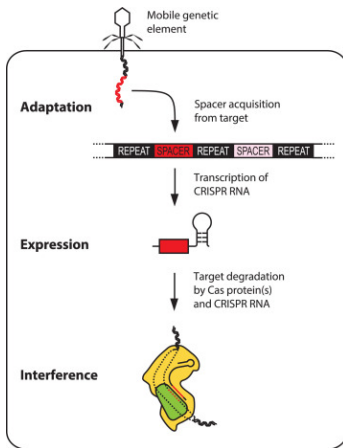
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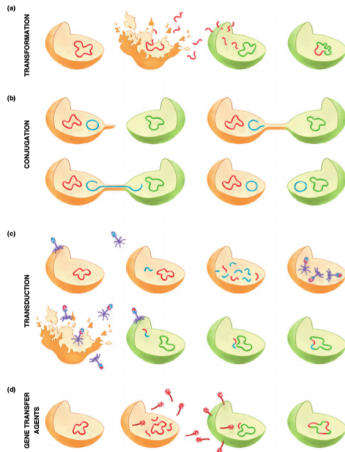
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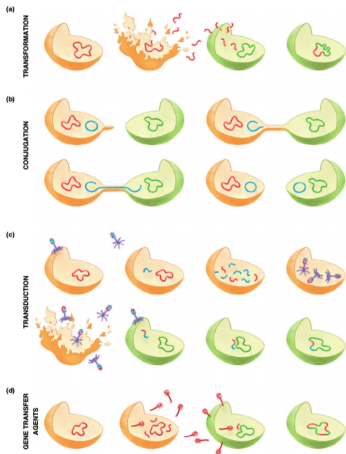
Horizontal Gene Transfer

Horizontal Gene Transfer Mechanisms



(Popa and Dagan, 2011)

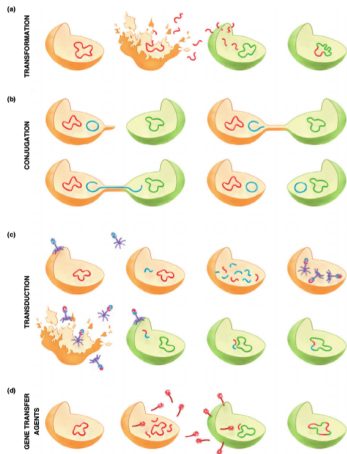
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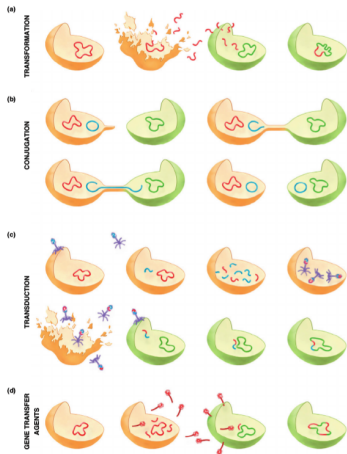
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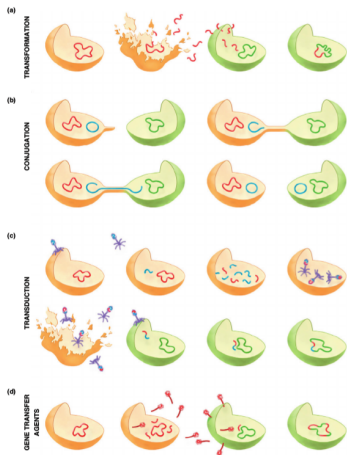
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- **CRISPR-Cas directly affects HGT** (Popa and Dagan, 2011)

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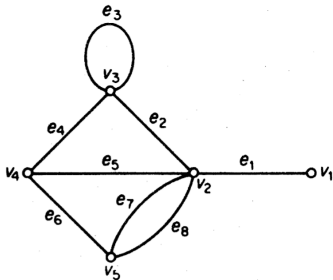
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Phylogenomic Networks

What is A Network?

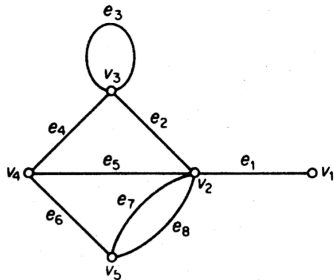
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- Useful mathematical abstraction of real world system

(Bondy and Murty, 2002)

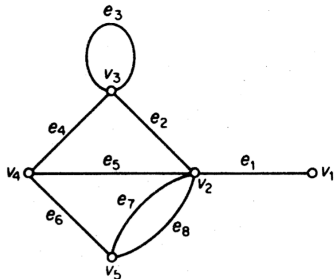
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- Edges can have weights

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Do CRISPR Systems Affect Horizontal Gene Transfer?

Yes

CRISPR Cost Complexity and Curbing It

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 - CRISPR can enhance transduction-mediated HGT (Watson, Staals, and Fineran, 2018)

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

My Project

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Within Network Comparisons

For genera with CRISPR containing OTUs, compare the node statistics of CRISPR containing OTUs to non-CRISPR containing OTUs.

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Gene Indel Rates vs. Network Statistics

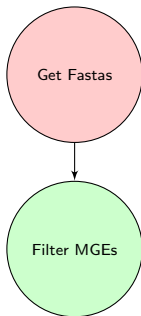
Compare gene Indel rates to node/network statistics for CRISPR containing and non-CRISPR containing OTUs

Workflow (Per Genus)

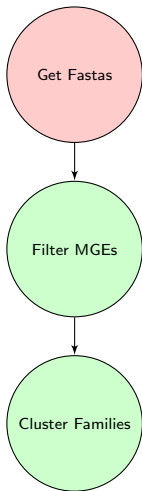


Get Fastas

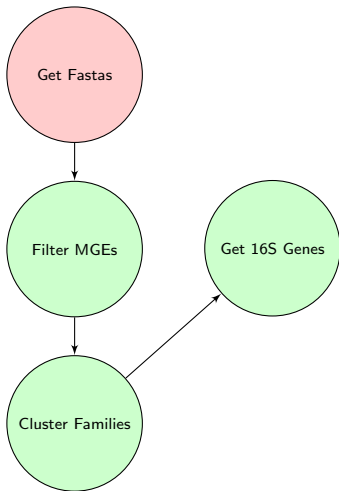
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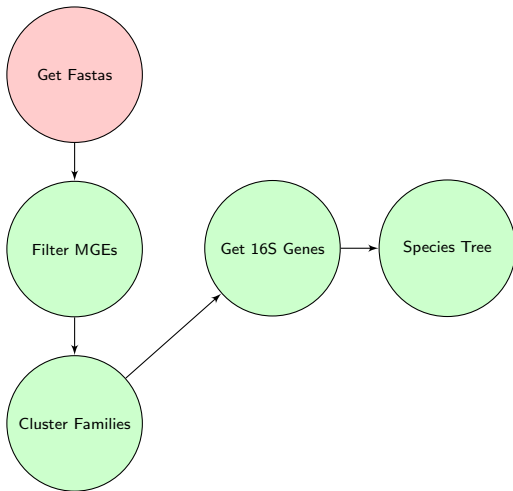
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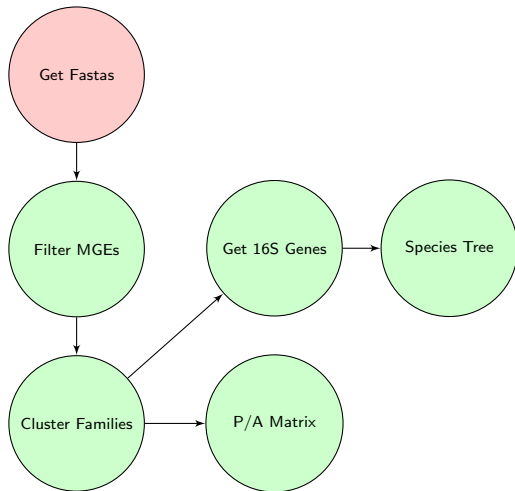
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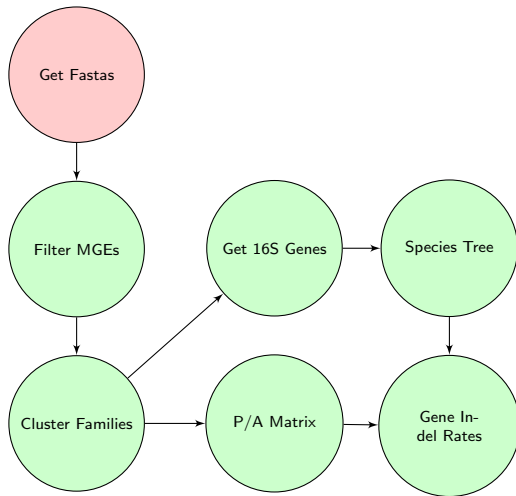
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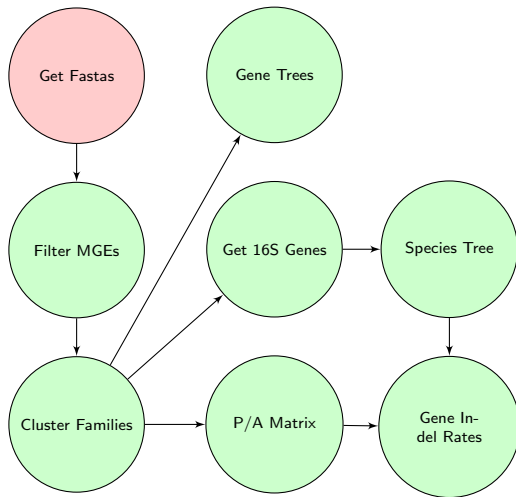
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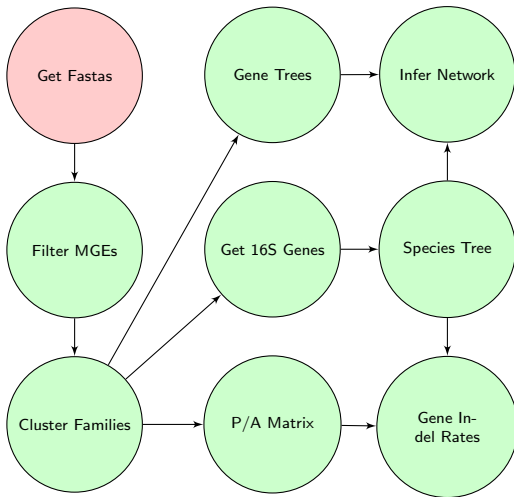
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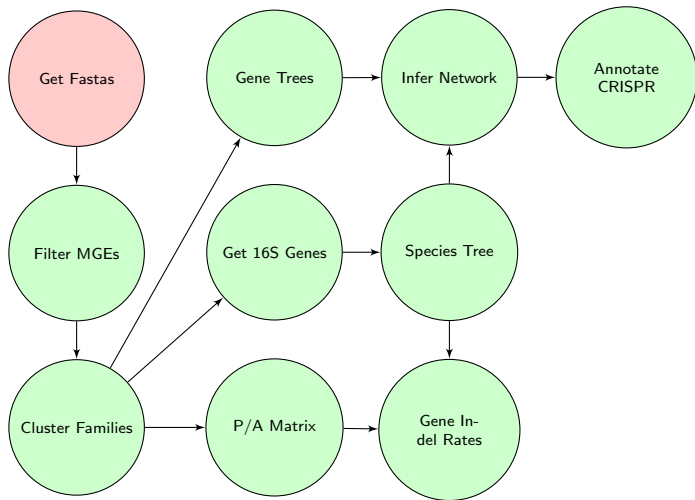
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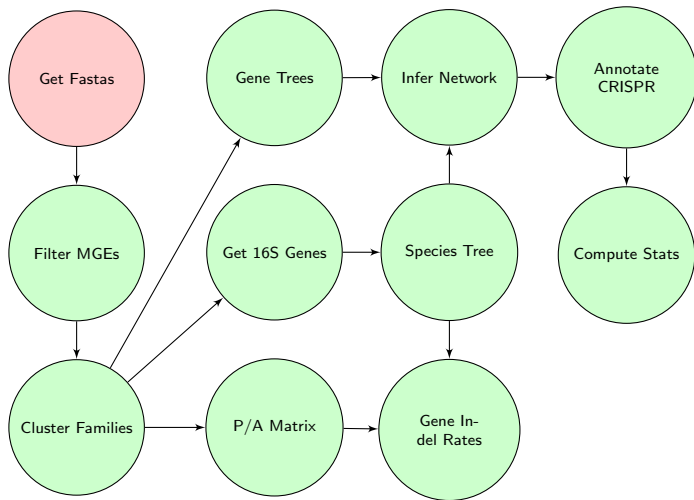
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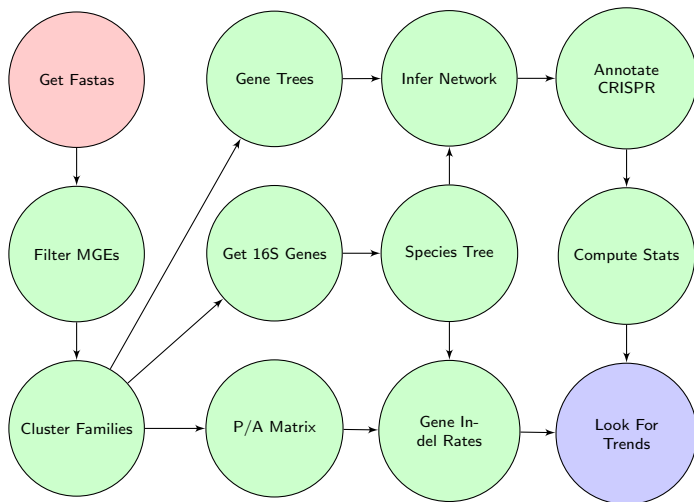
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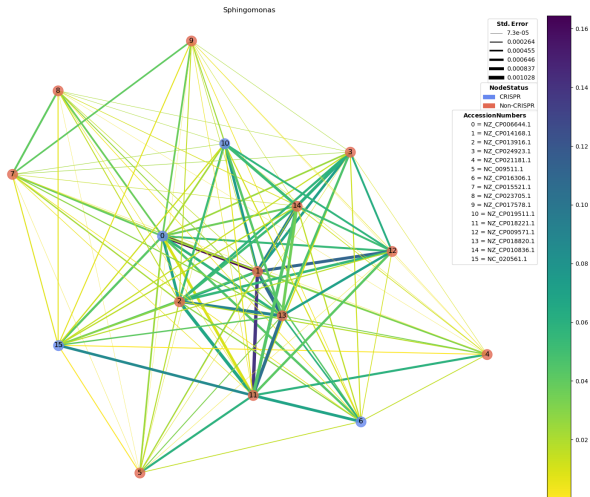


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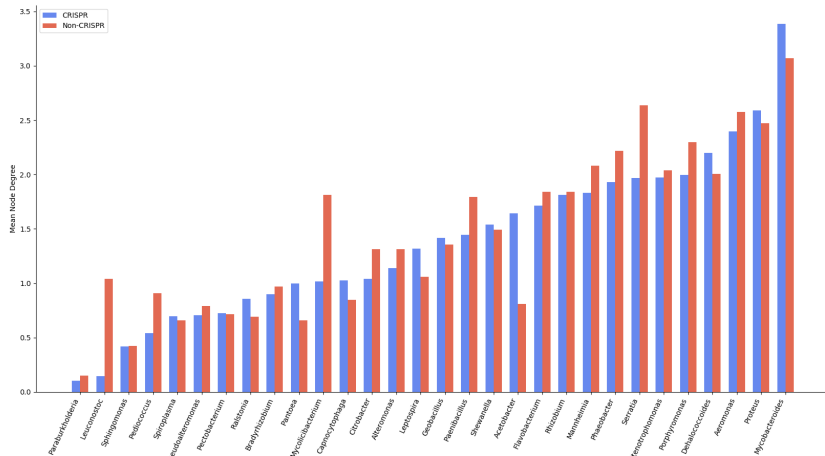


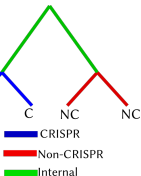
Results

Example "Consensus" Network

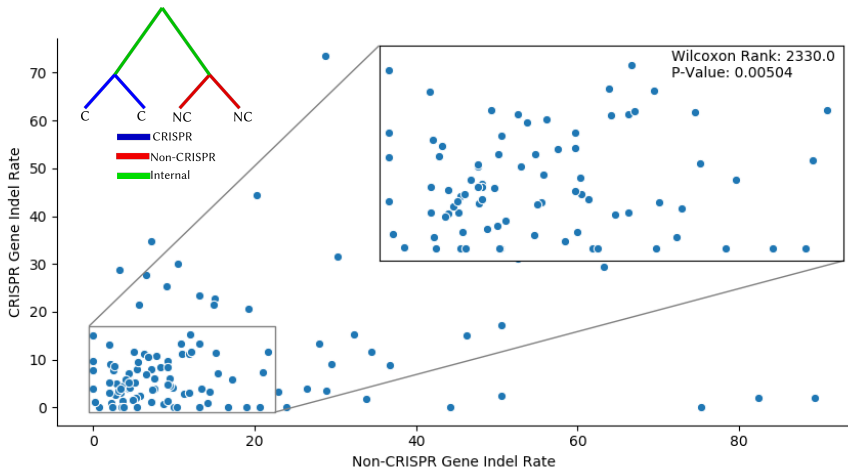


Mean Node Degree

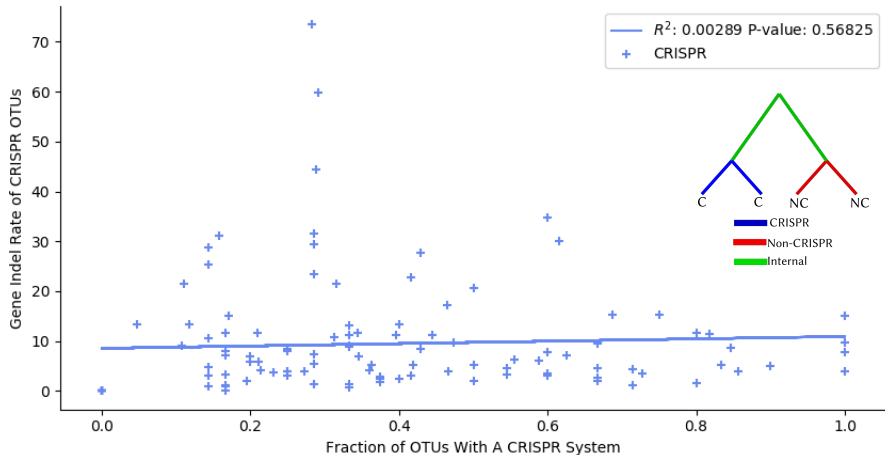




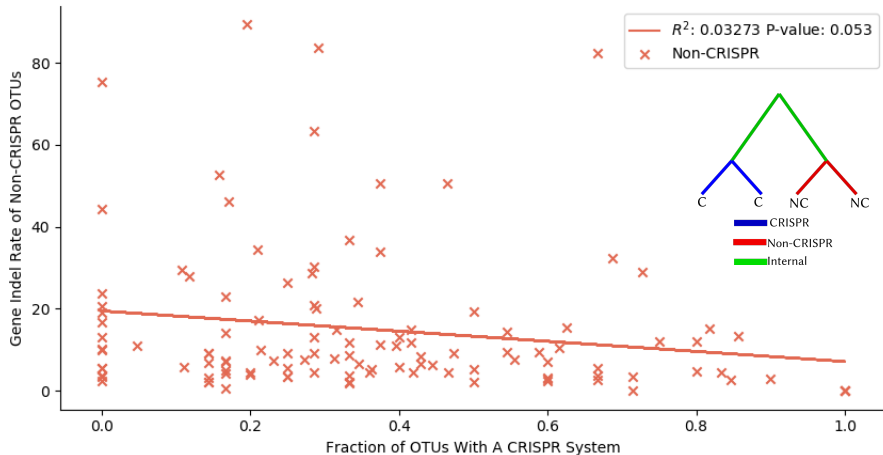
Gene Indel Rates



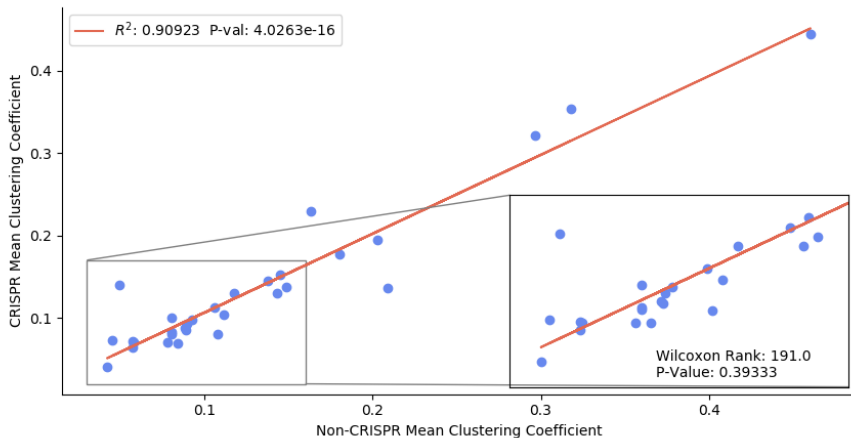
Gene Indel Rate Vs. Fraction of CRISPR OTUs



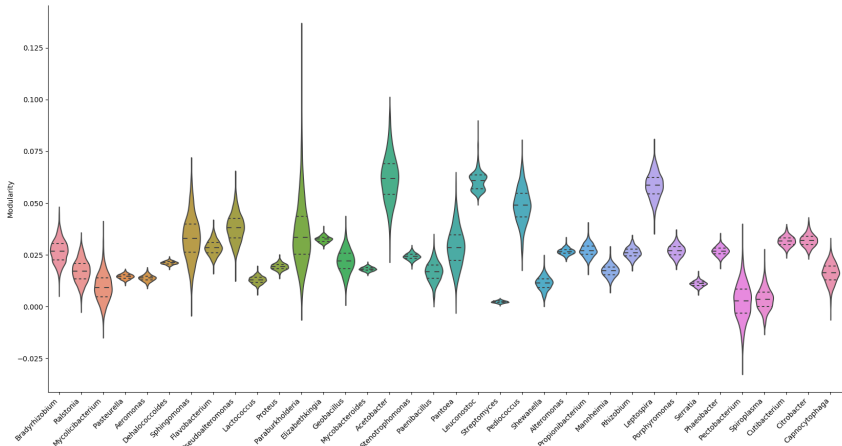
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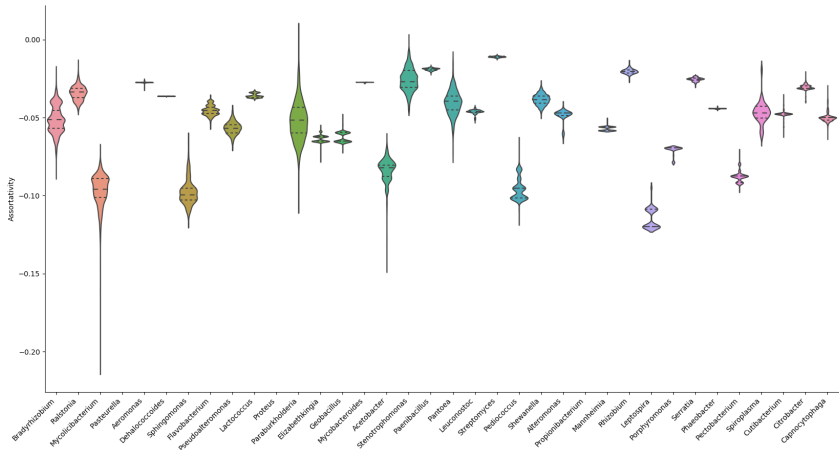
Mean Node Weighted Clustering Coefficient



Modularity Distributions



Assortativity Distributions



Conclusion

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- CRISPR-Cas systems broadly associated with lower HGT rates, with prominent exceptions
- Population level effects of CRISPR-Cas systems may decrease HGT rates
- Interplay of CRISPR-Cas systems and HGT is complex and warrants further study

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- **Intergenic comparisons:** Combine any set of fasta files from OTUs for analyzing transfer dynamics
- **Considering bacterial ecology and environments:** Consider geographically close OTUs or differences between networks due to environmental factors

Is Sharing Caring?

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Yes, for researchers

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Yes, for researchers
Jury's still out for bacteria

Thanks

Thank you to

- Dr. G. Brian Golding
- Dr. Ben Evans
- The Golding lab
 - Caitlin Simopoulos
 - Daniella Lato
 - Zachery Dickson
 - Sam Long
 - Geoge Long
 - 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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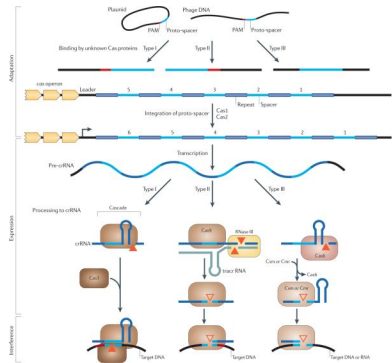
Newman, M. E. (2002). “Assortative mixing in networks”. In: *Phys. Rev. Lett.* 89.20, p. 208701.



– (2004). “Analysis of weighted networks”. In: *Phys Rev E Stat Nonlin Soft Matter Phys* 70.5 Pt 2, p. 056131.

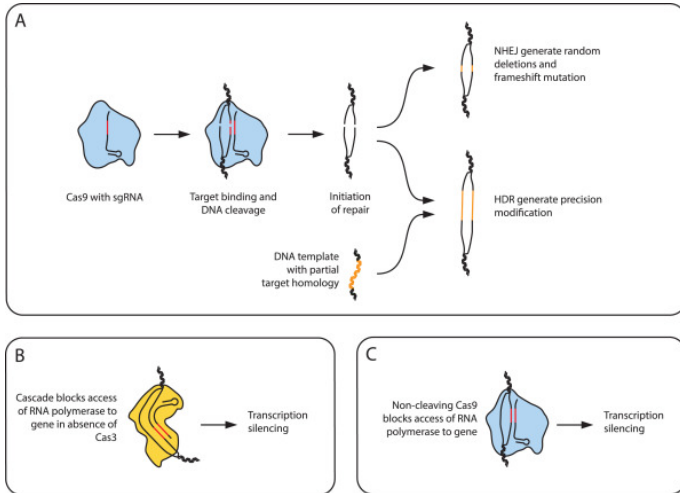
Diversity & Ubiquity

- 45% of bacteria have CRISPR loci ($n = 6782$) (Grissa, I. and Drevet, C. and Couvin, D., 2017)
- 3 Main Types, multiple sub types (Bondy-Denomy and Davidson, 2014)
- CRISPR arrays represent unique life history of an organism
- 11% – 28% are false or orphaned CRISPR loci (Zhang and Ye, 2017)



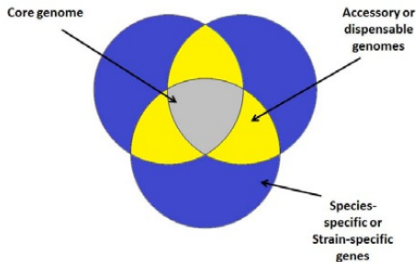
(Makarova et al., 2011)

CRISPR Biotech Application

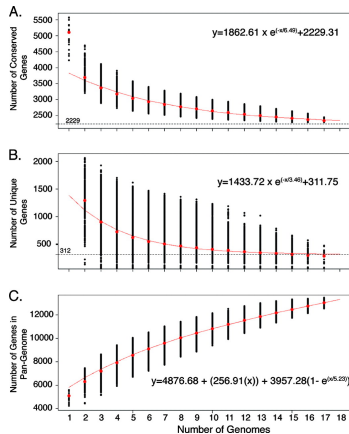


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Pan-Genomes

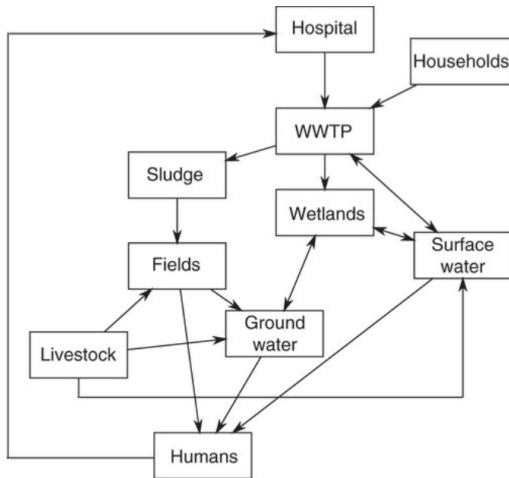


(Guimaraes et al., 2015)



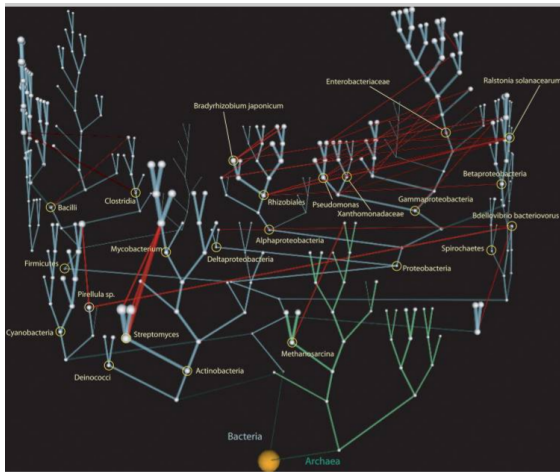
(Rasko et al., 2008)

HGT Applications



(Berglund, 2015)

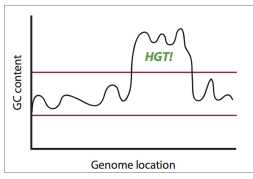
Prokaryotic “Net of Life”



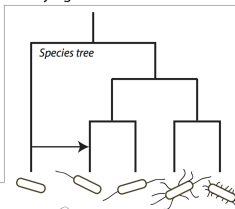
(Kunin et al., 2005)

Phylogenomic Network Construction

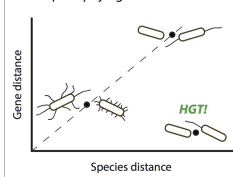
1. Parametric methods



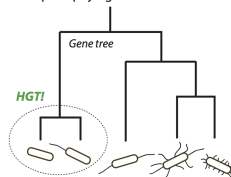
2. Phylogenetic methods



2a. Implicit phylogenetic methods

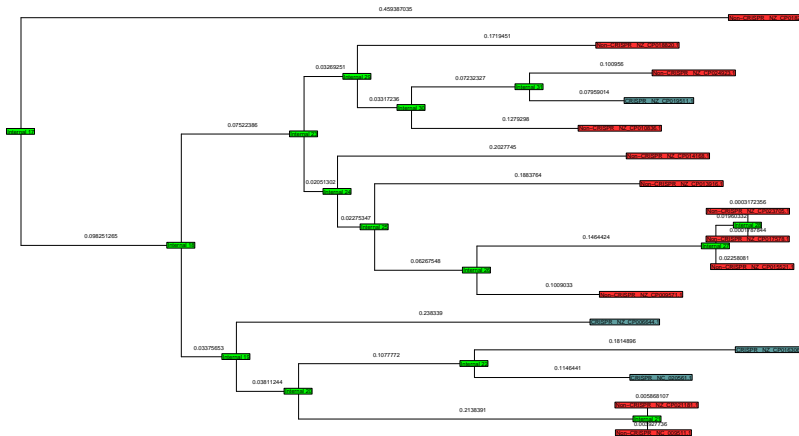


2b. Explicit phylogenetic methods



(Ravenhall et al., 2015)

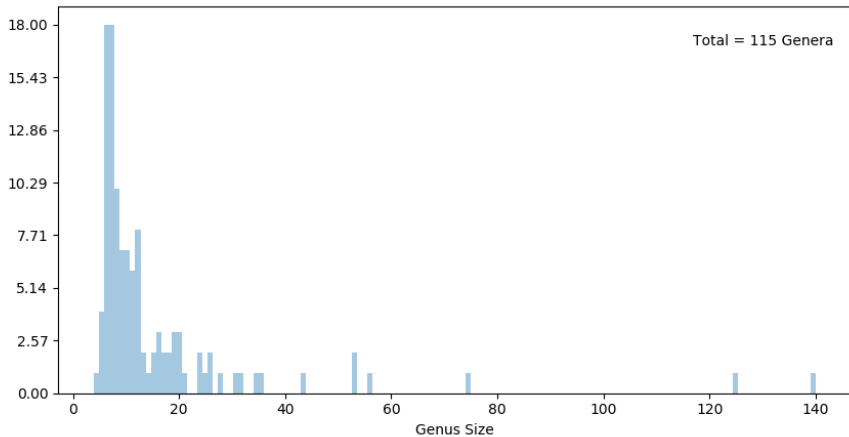
Sphingomonas Species Tree



Network Statistics

- **Average Node Degree:** $\frac{1}{|N_u|} \sum_{uv} w_{uv}$ where N_u is the set of nodes incident to u
- **Node Clustering Coefficient:** $\frac{1}{k_u(k_u-1)} \sum_{vw}^{T(u)} (\hat{w}_{uw} \hat{w}_{vw} \hat{w}_{uv})^{\frac{1}{3}}$ where $T(u)$ is the set of triangles containing u (Onnela et al., 2005)
- **Node Assortativity:** $A = \frac{Tr(M) - ||M^2||}{1 - ||M^2||}$ Where M is the mixing matrix of a given attribute and $||M||$ is the sum of all elements of M . $A \in [-1, 1]$. (Newman, 2002)
- **Network Modularity:** $Q = \frac{1}{2m} \sum_{uv} [W_{uv} - \frac{k_u k_v}{2m}] \delta(u, v)$ where m is the total weight of all edges, 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. $Q \in [-1, 1]$ (Newman, 2004)

Genus Size Distribution



Branch Partition Example

