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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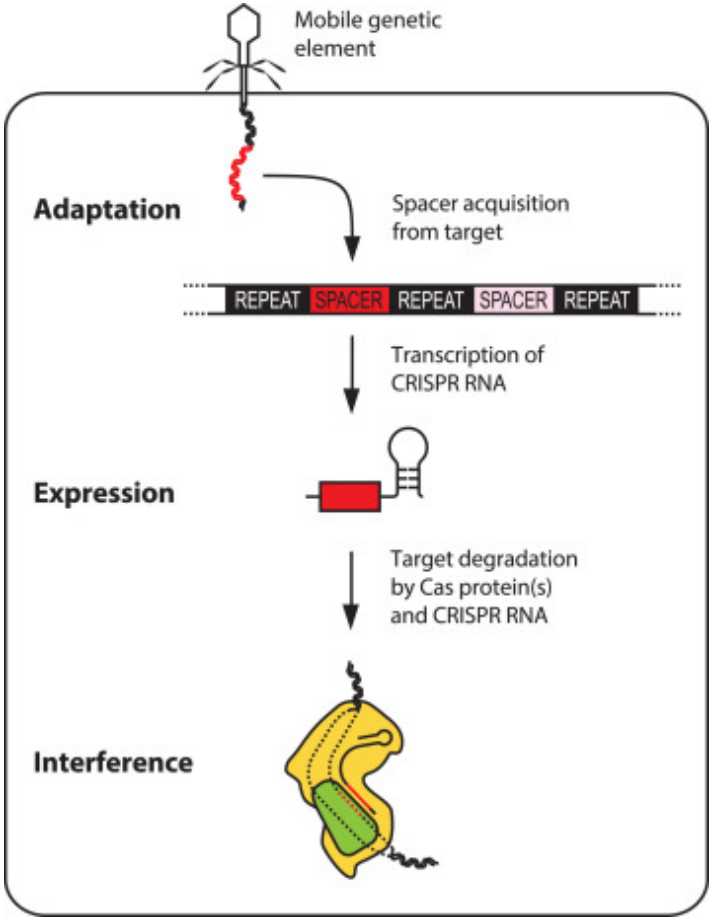
- Adaptive Bacterial Immune System



(Rath et al., 2015)

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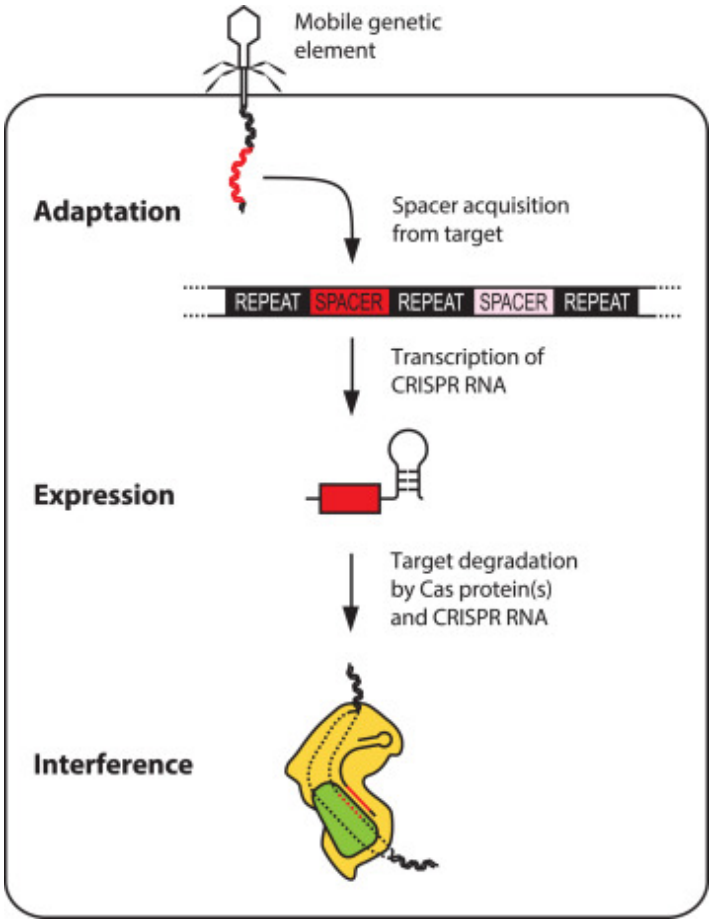

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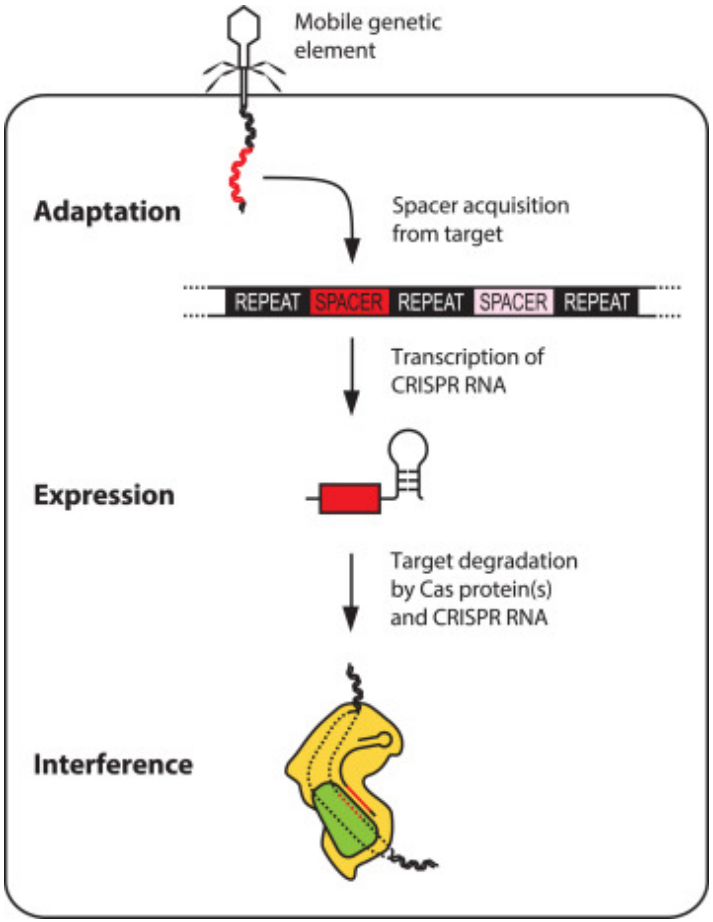

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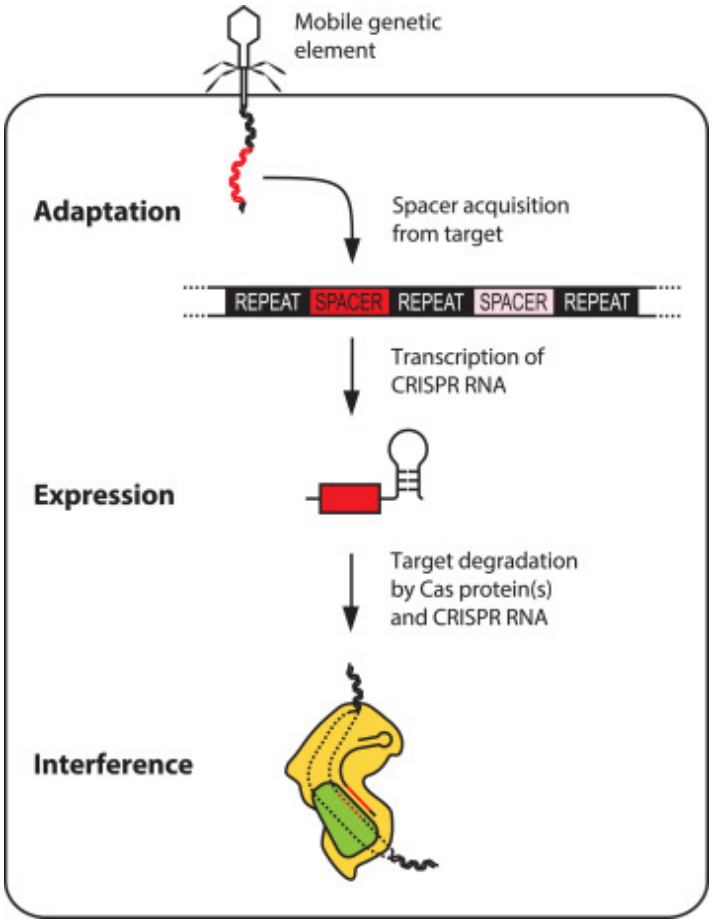

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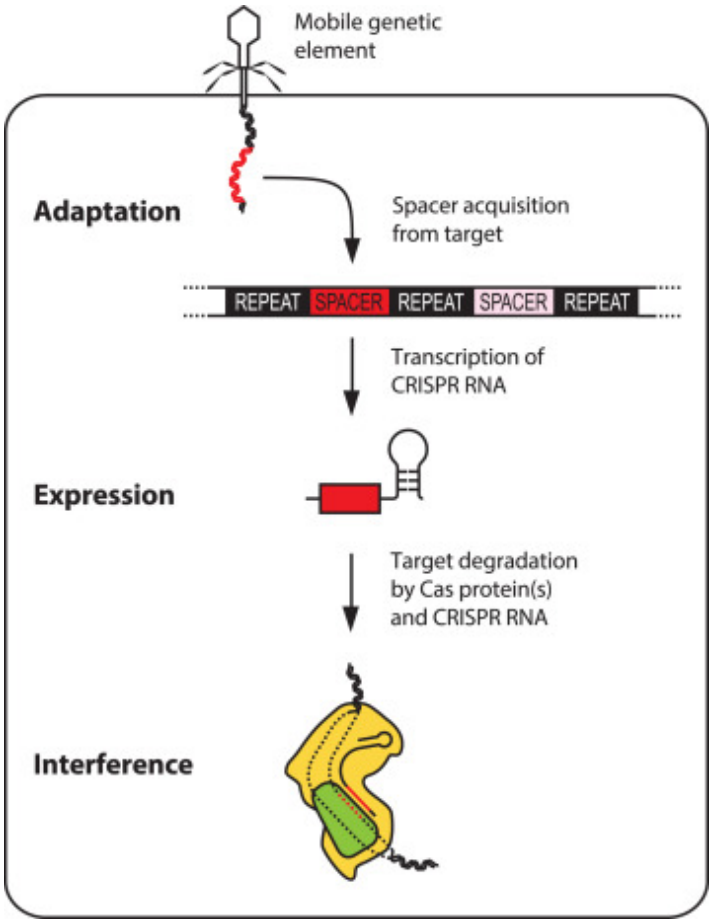
- 45% of bacteria have CRISPR loci ($n = 6782$) (Grissa, I. and Drevet, C. and Couvin, D., 2017)



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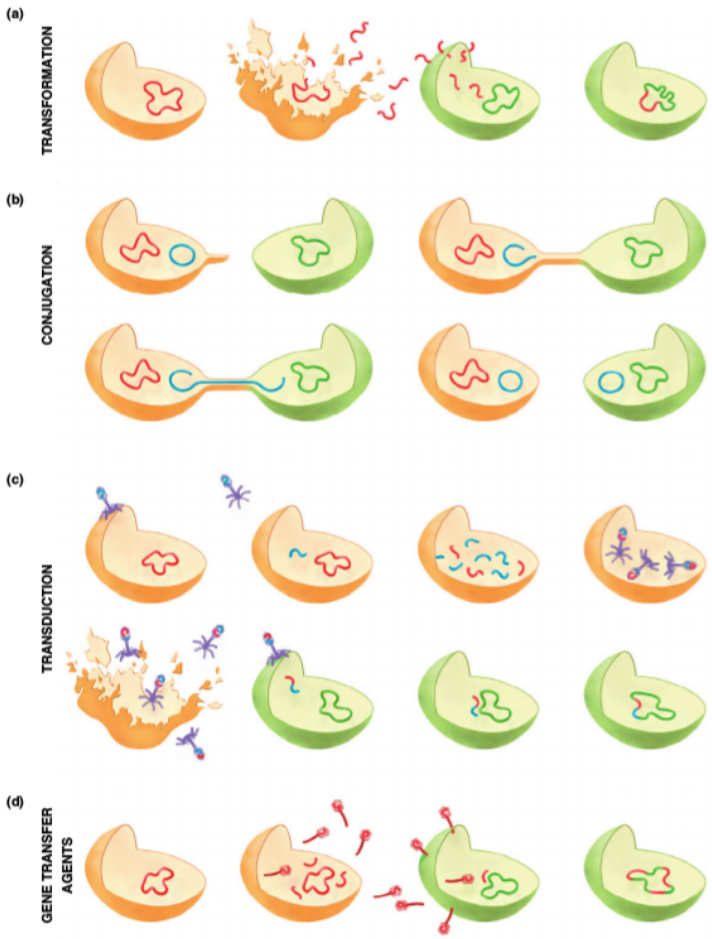
Horizontal Gene Transfer

Horizontal Gene Transfer Mechanisms



(Popa and Dagan, 2011)

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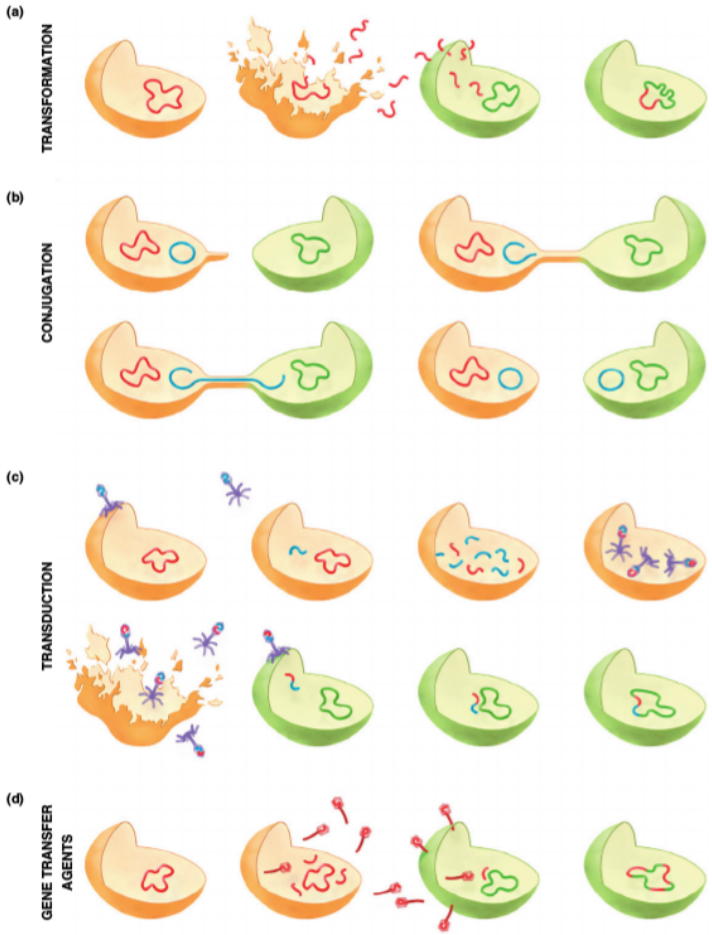

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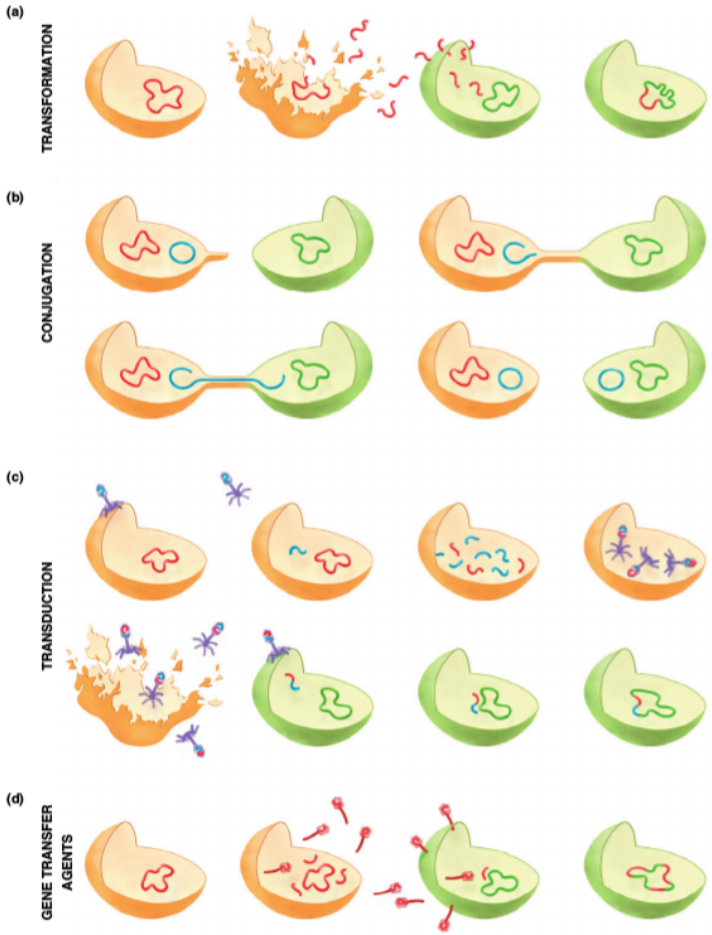

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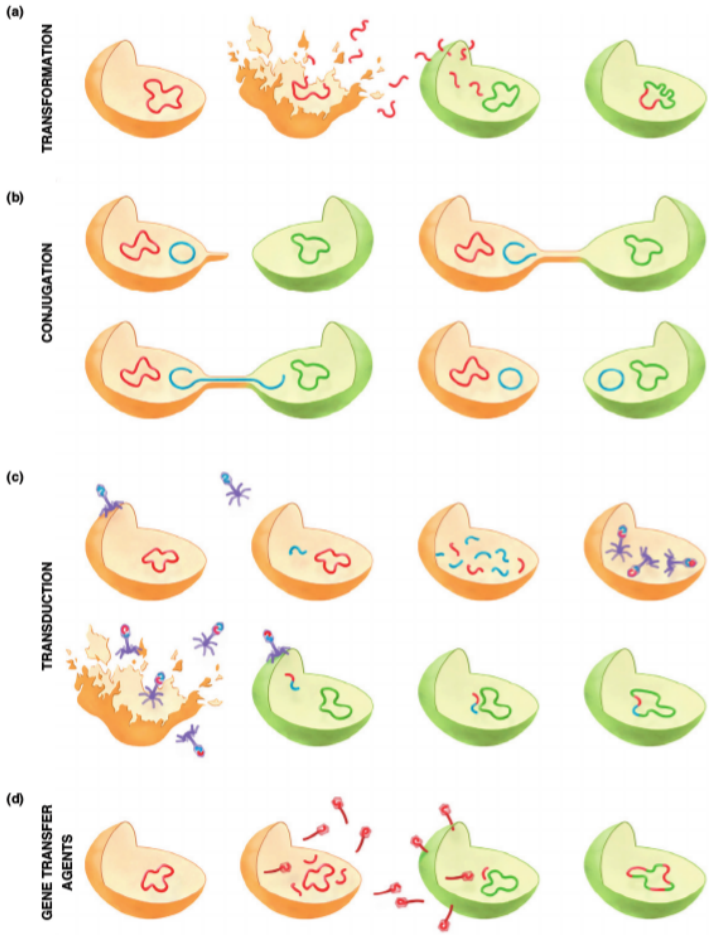

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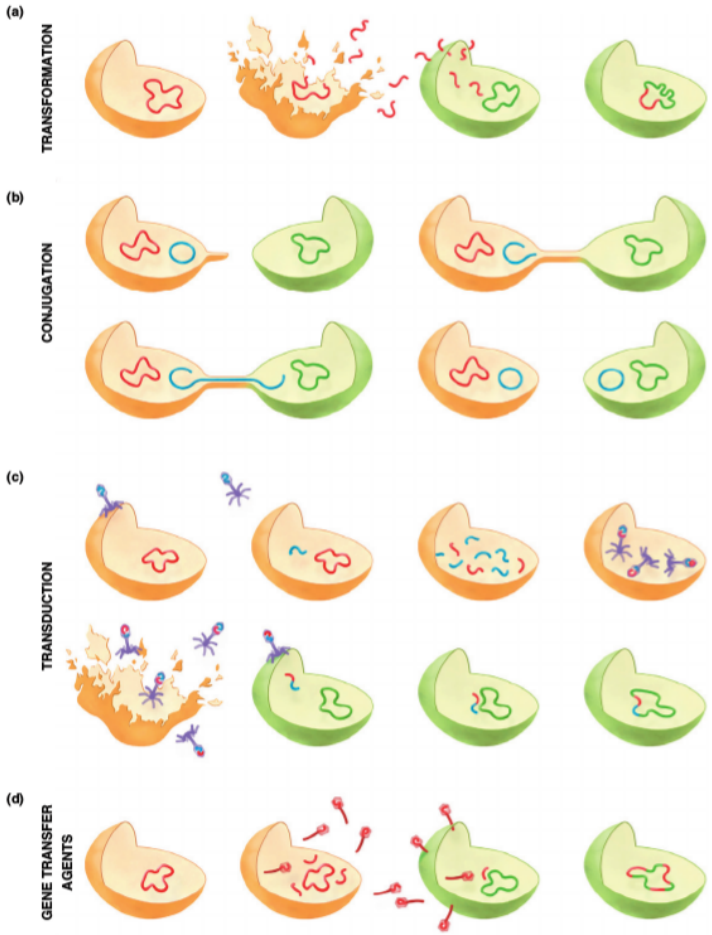

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- **CRISPR-Cas directly affects HGT** (Popa and Dagan, 2011)

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- Amount of exogenous DNA/cell density/phage density

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Phylogenomic Networks

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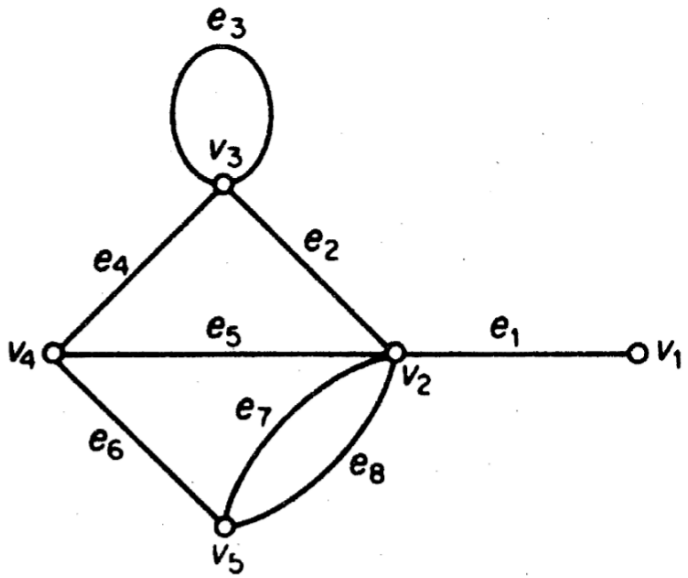

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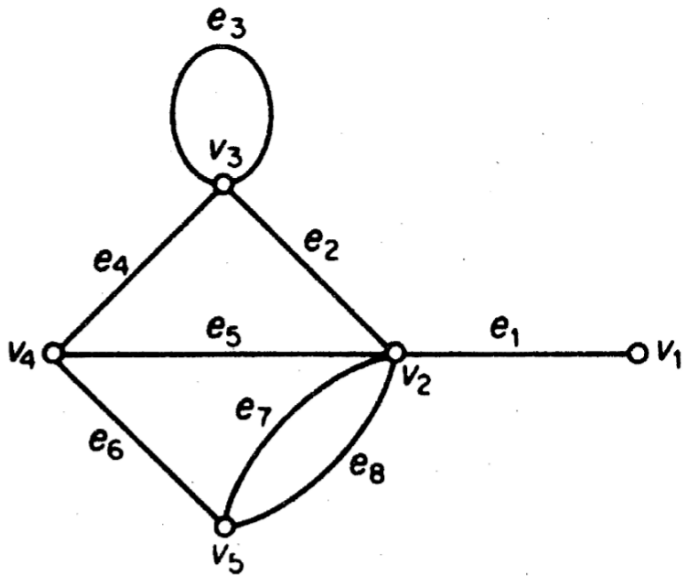

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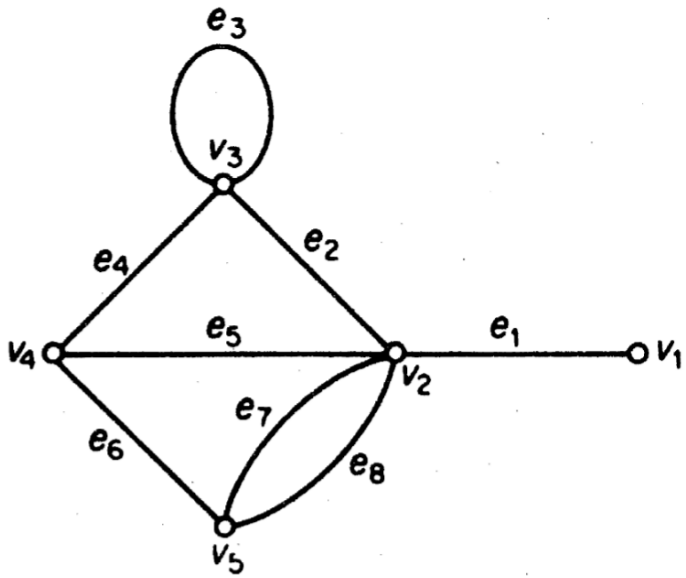

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- Useful mathematical abstraction of real world system
- Nodes can have attributes
- Edges can have weights

Do CRISPR Systems Affect Horizontal Gene Transfer?

Yes

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

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  - Can see inhibitory effects of CRIPSR 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 CRIPSR 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

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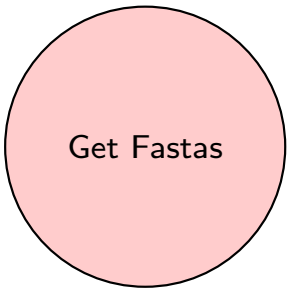

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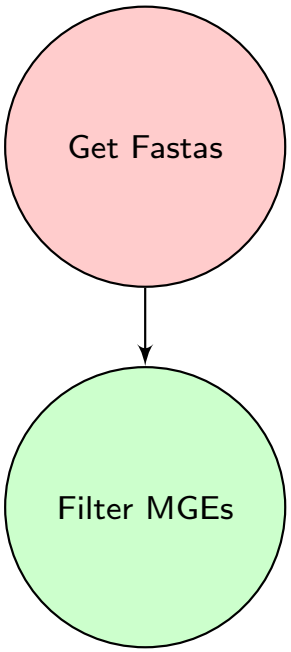

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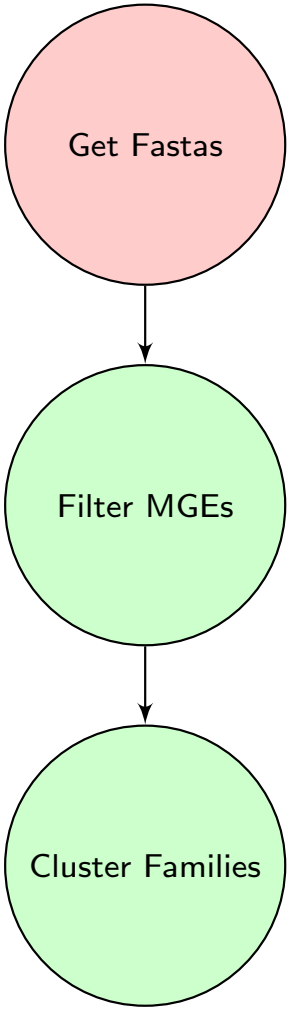

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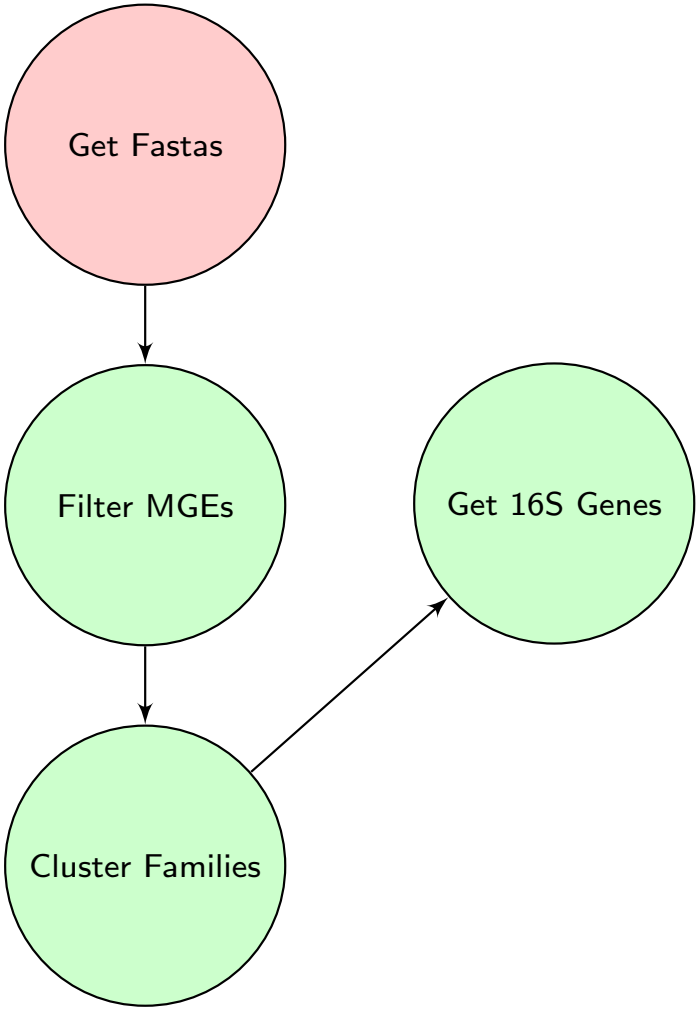

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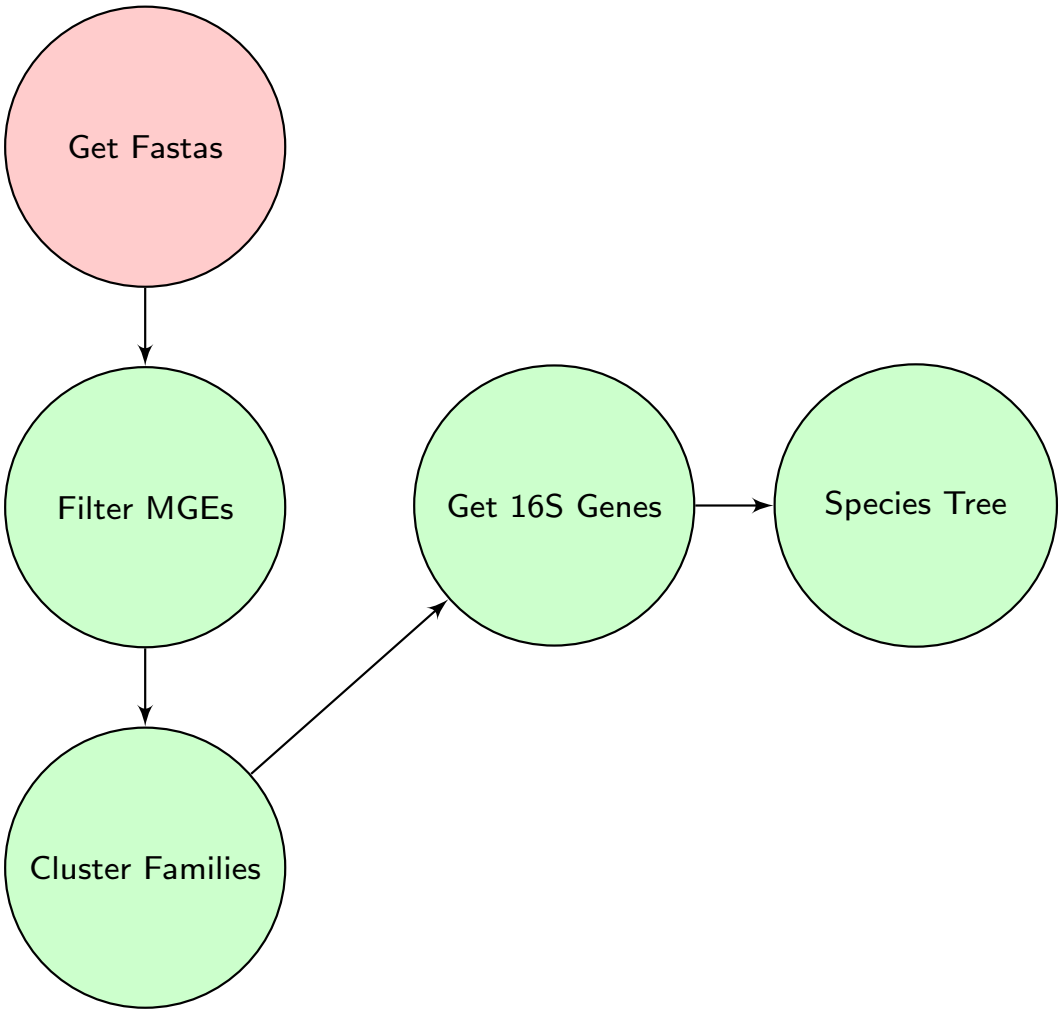

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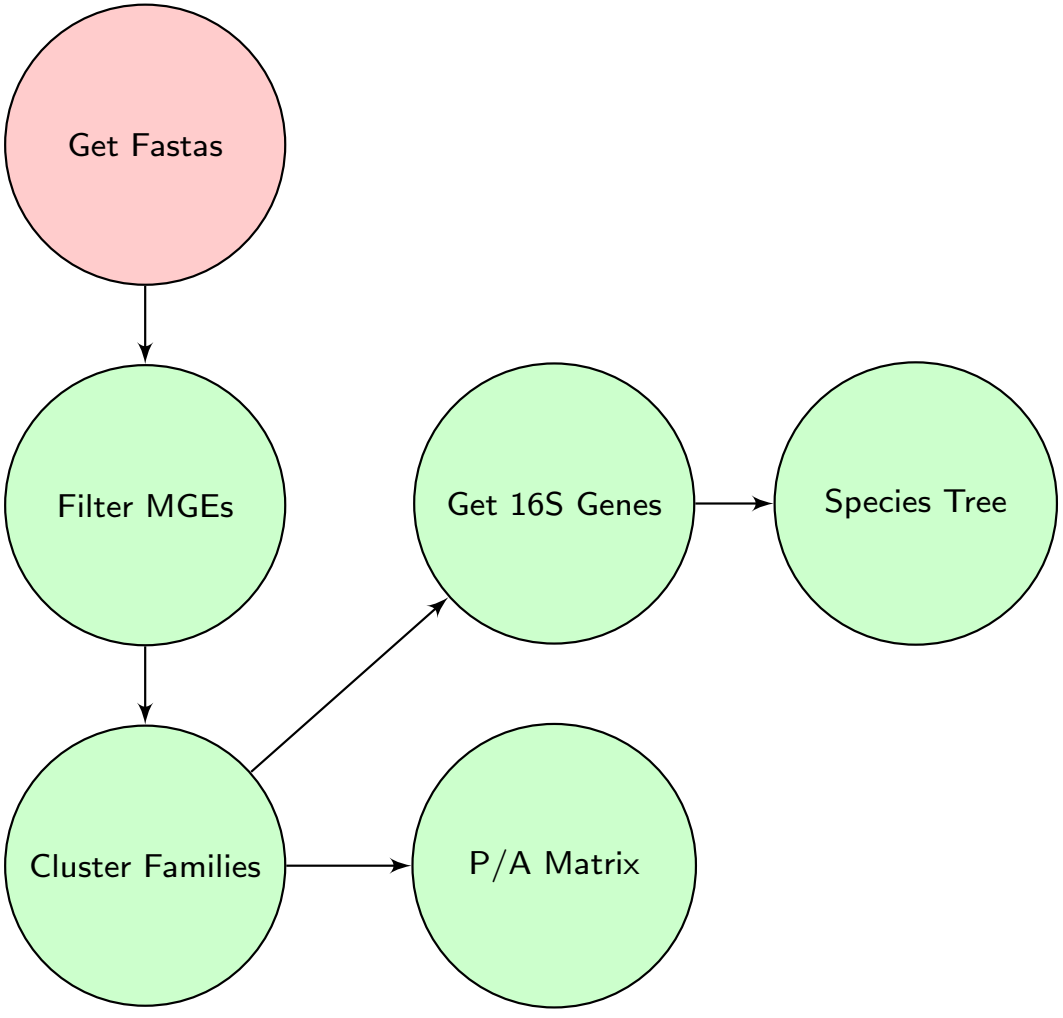

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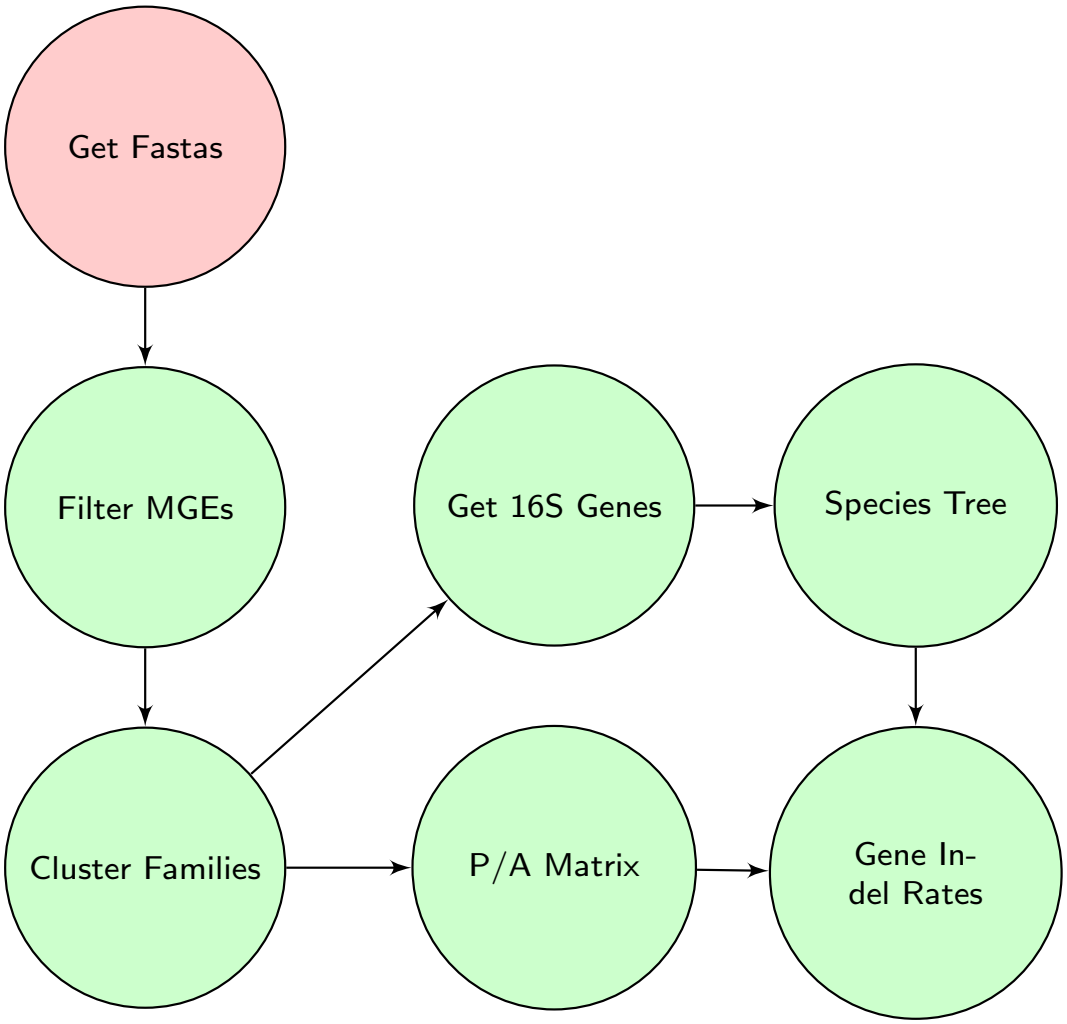

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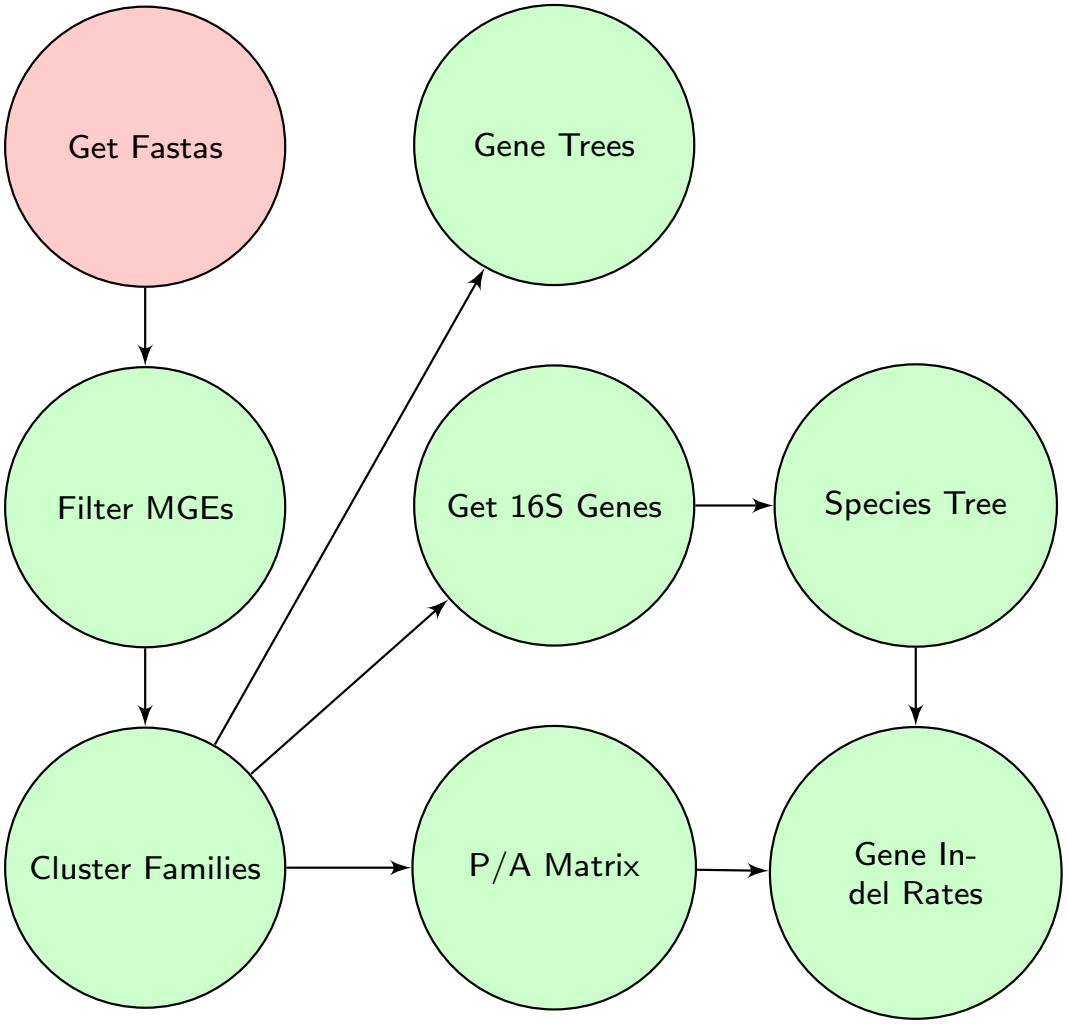

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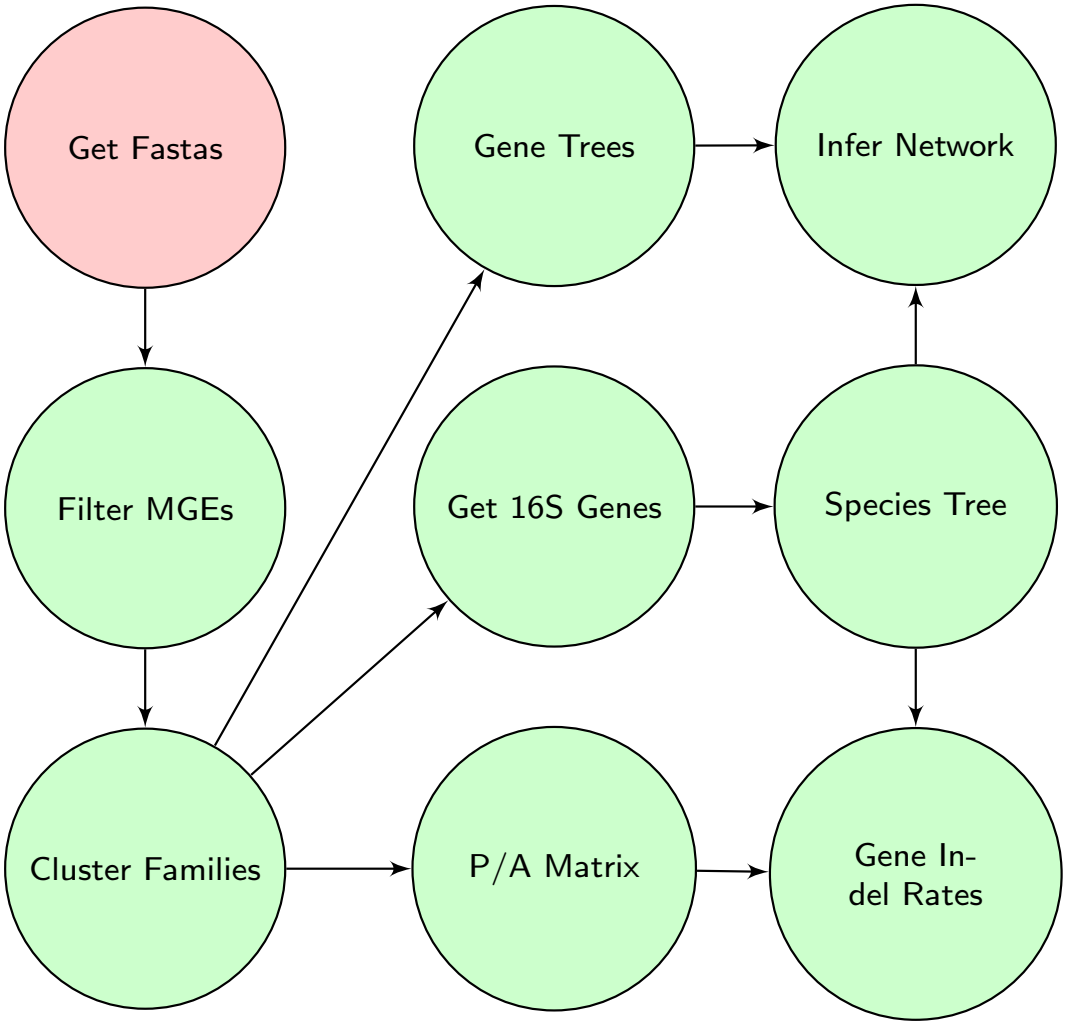

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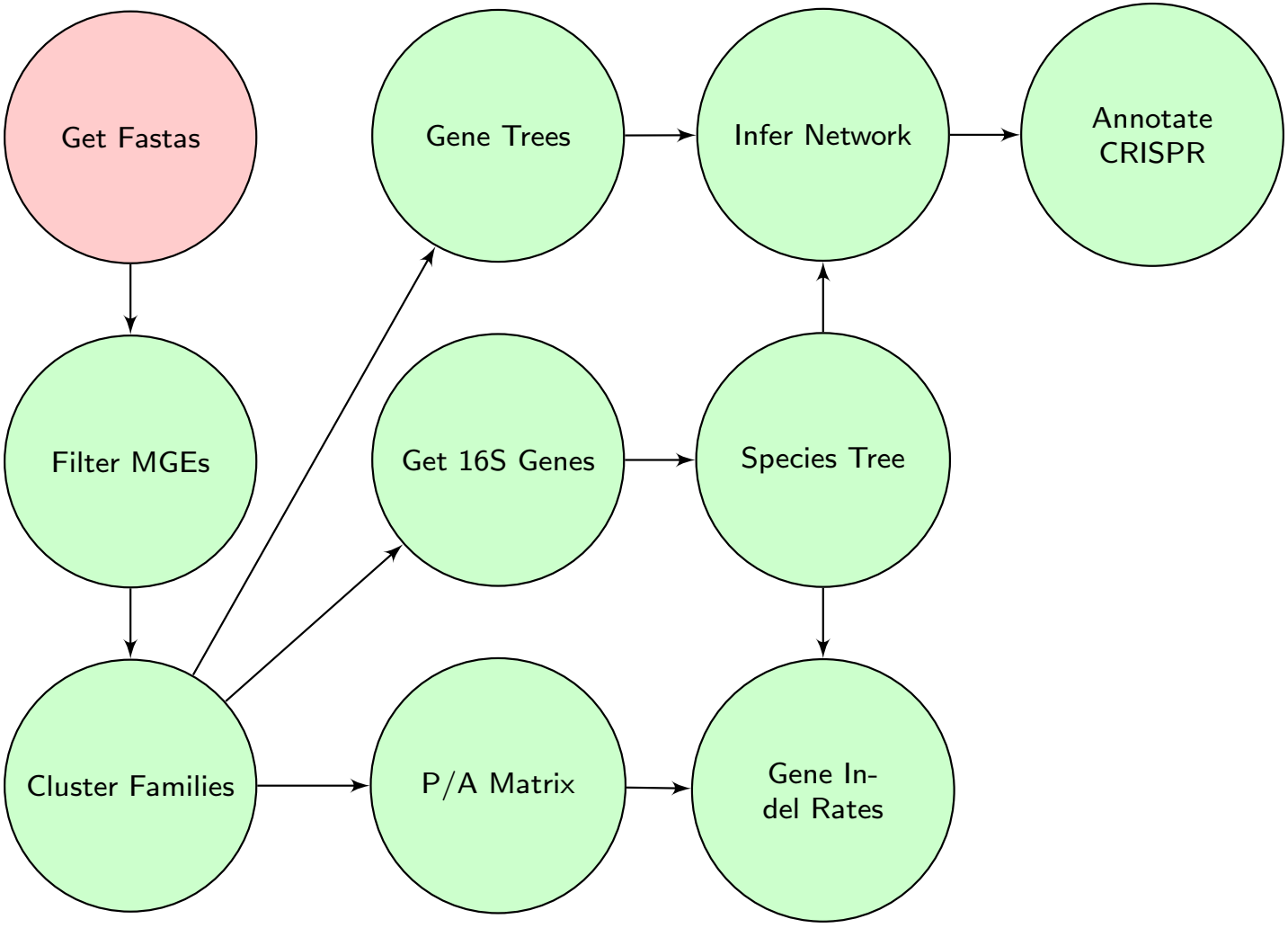

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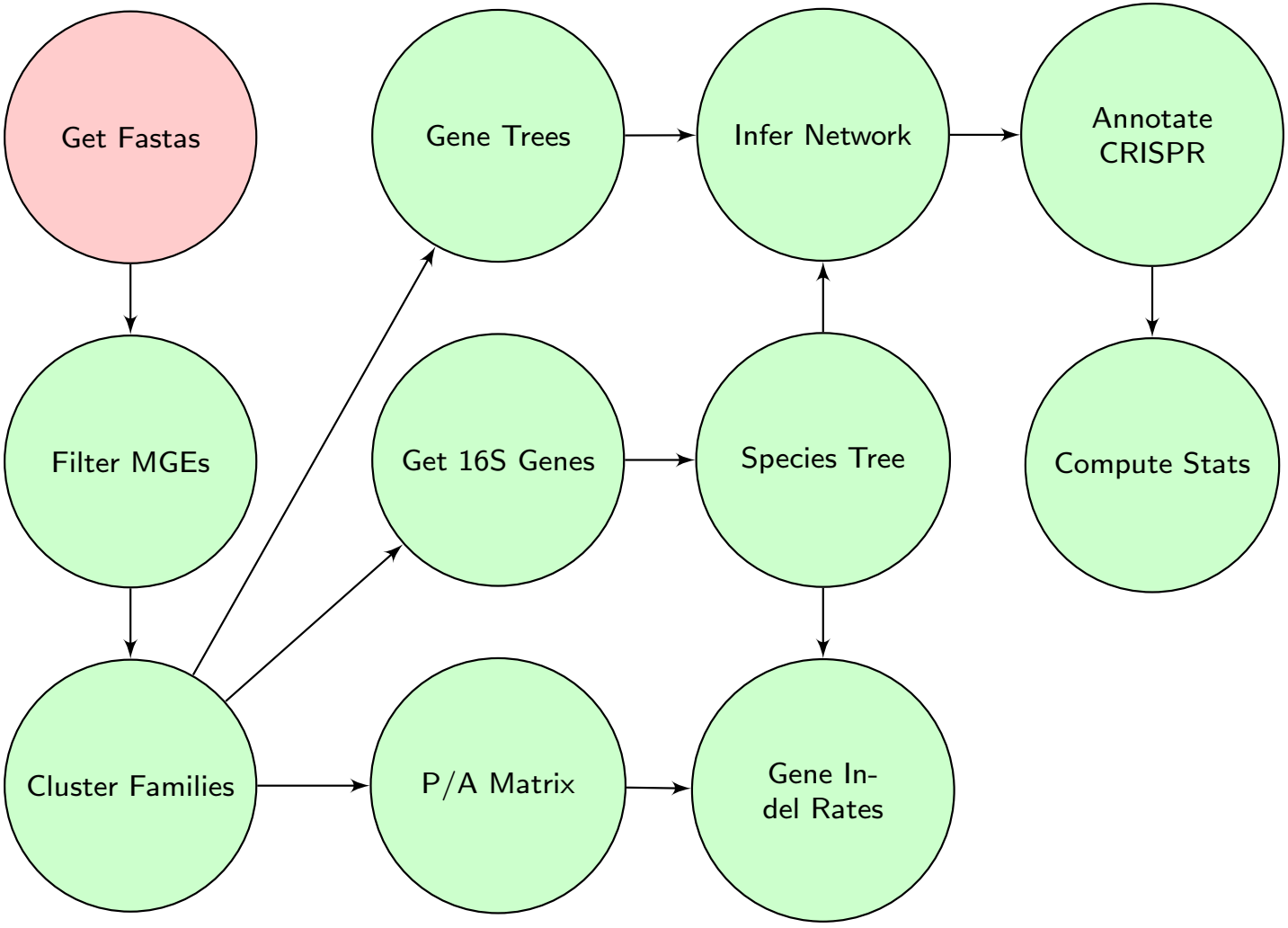

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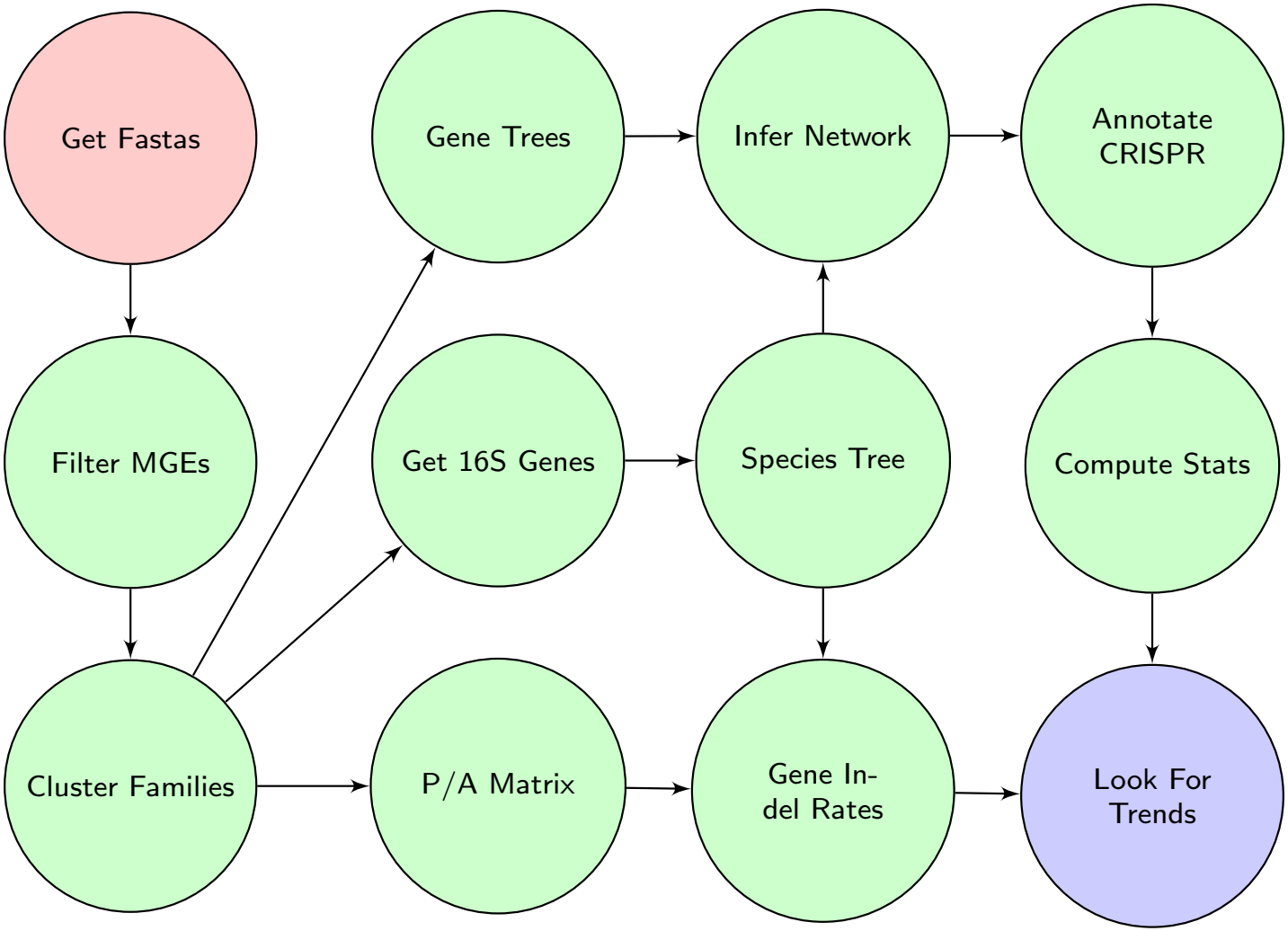
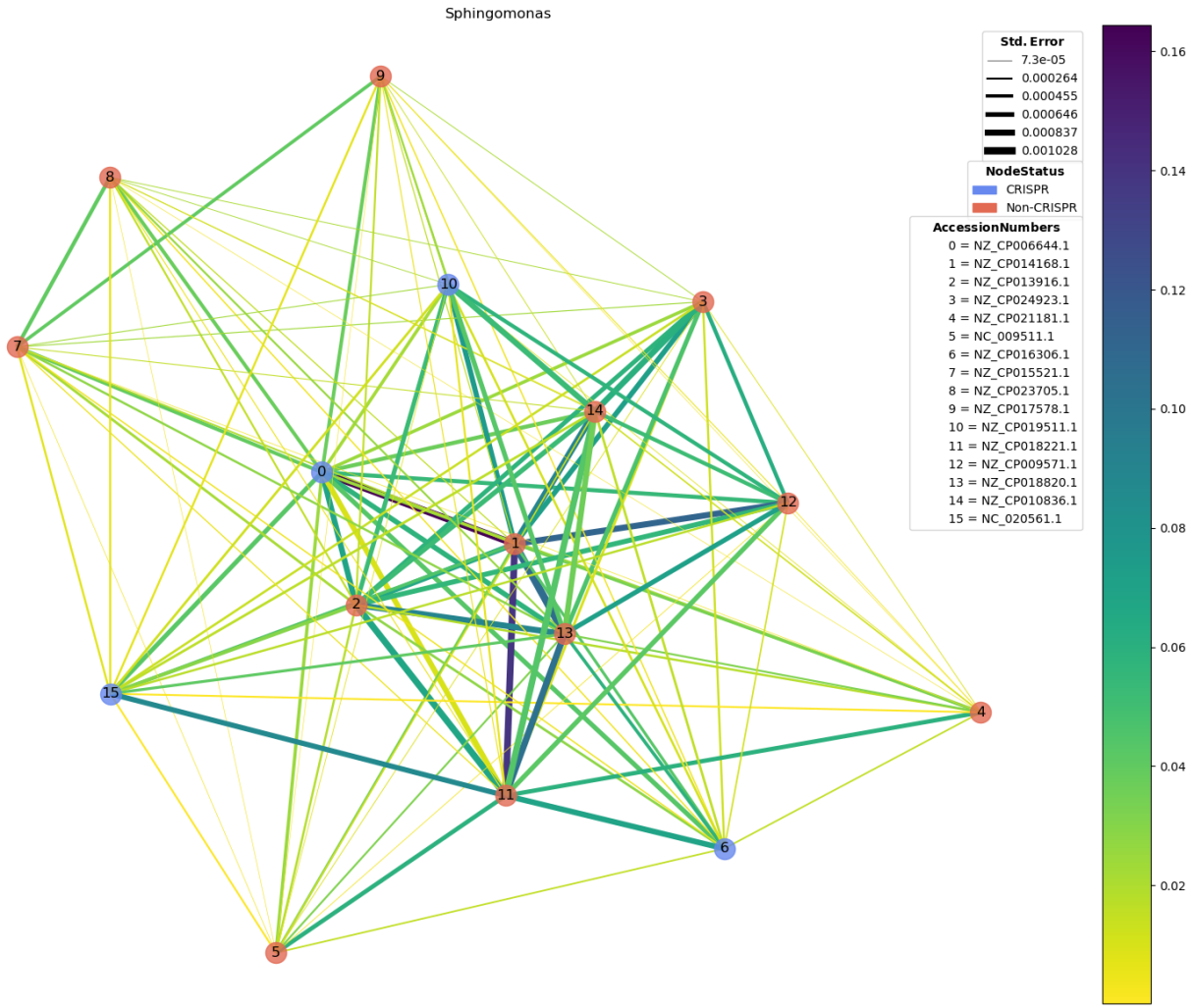
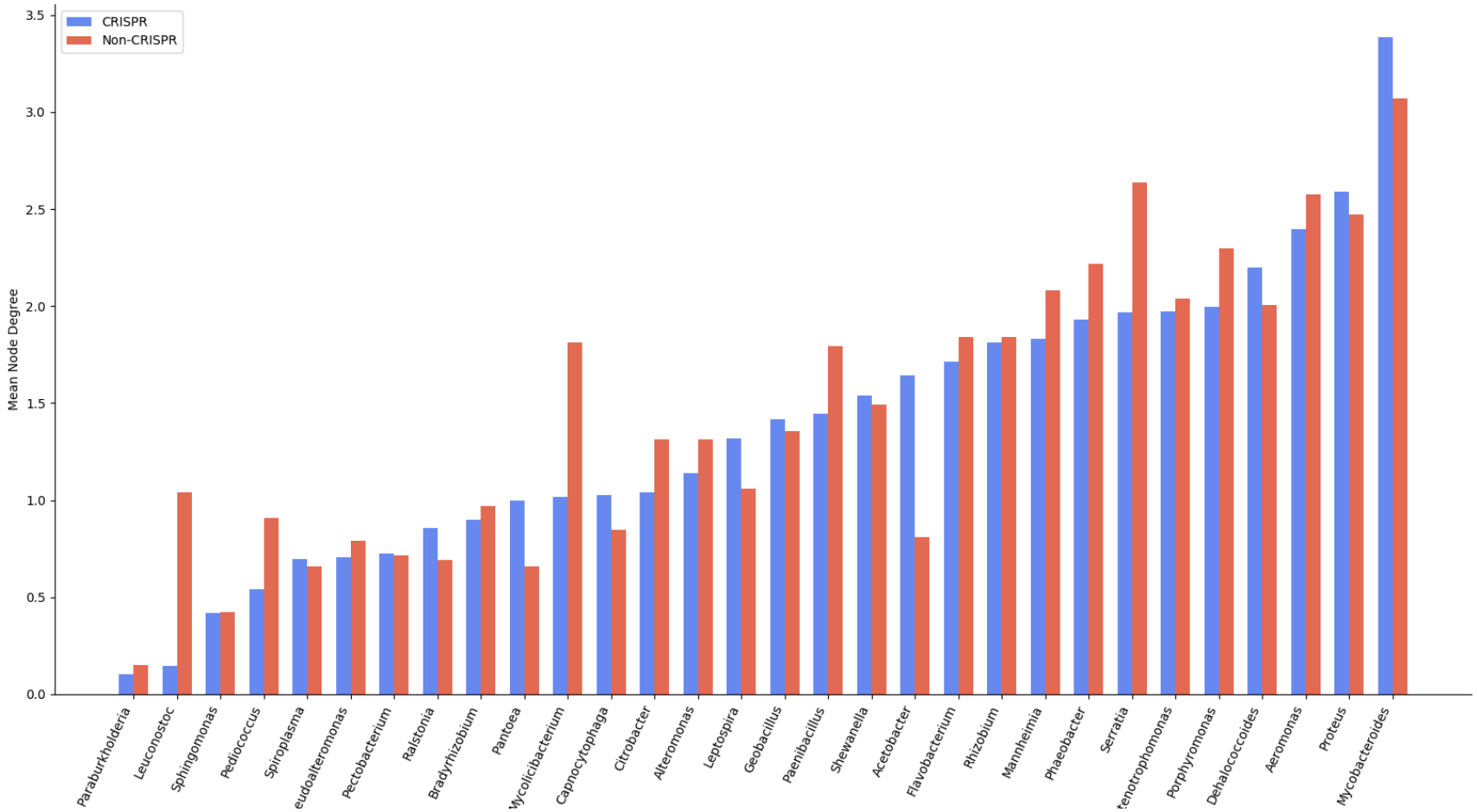


Results

Example "Consensus" Network



Mean Node Degree



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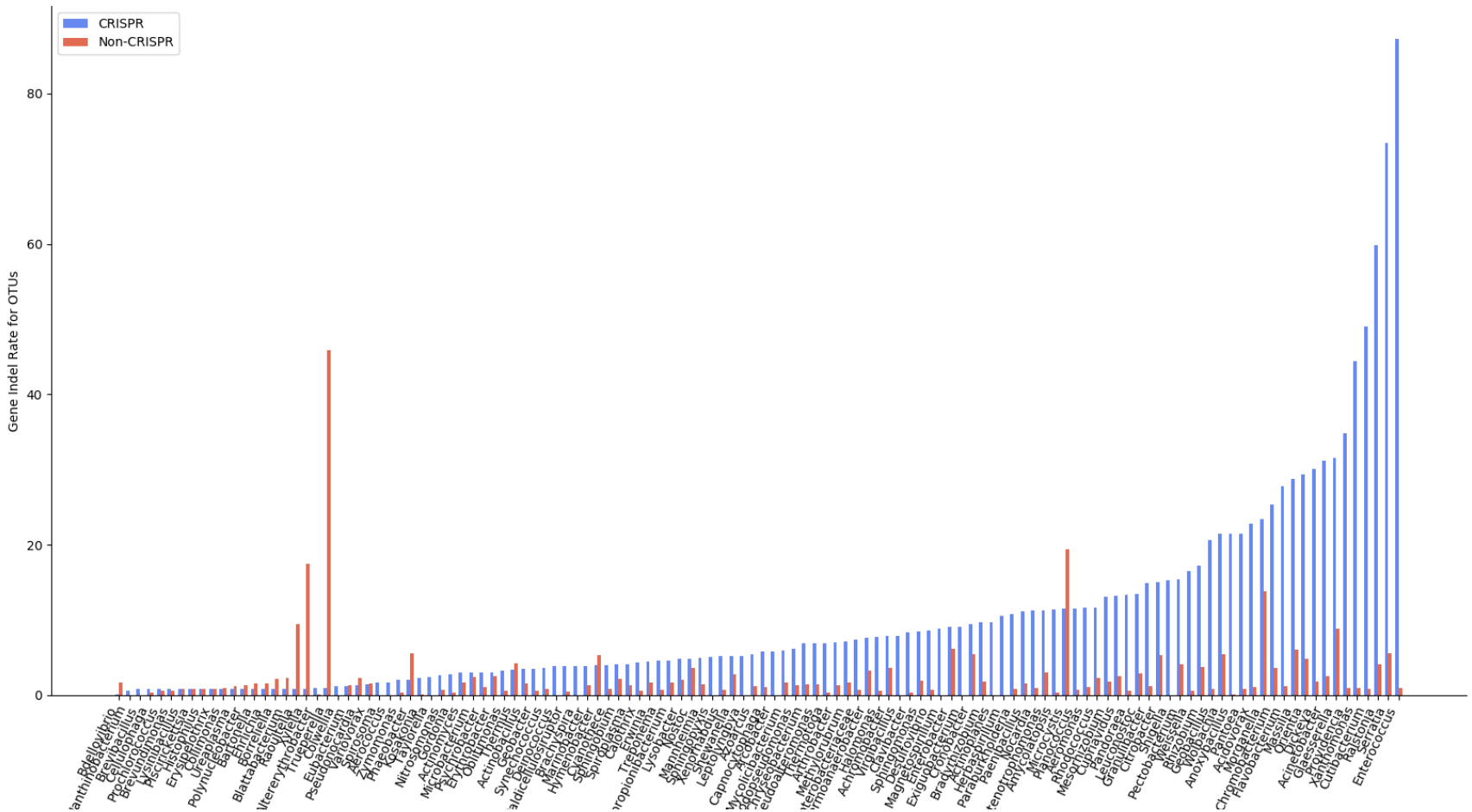

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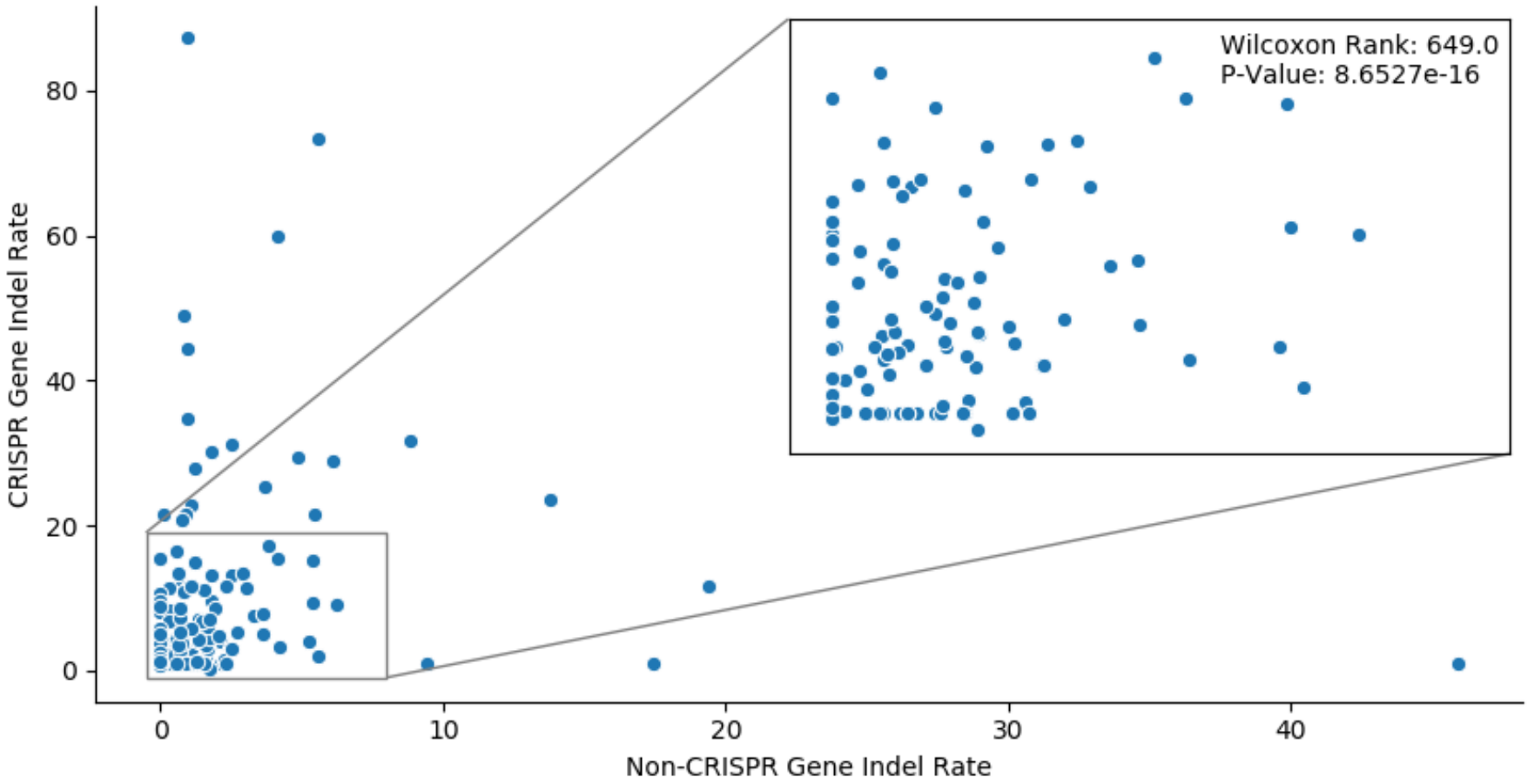
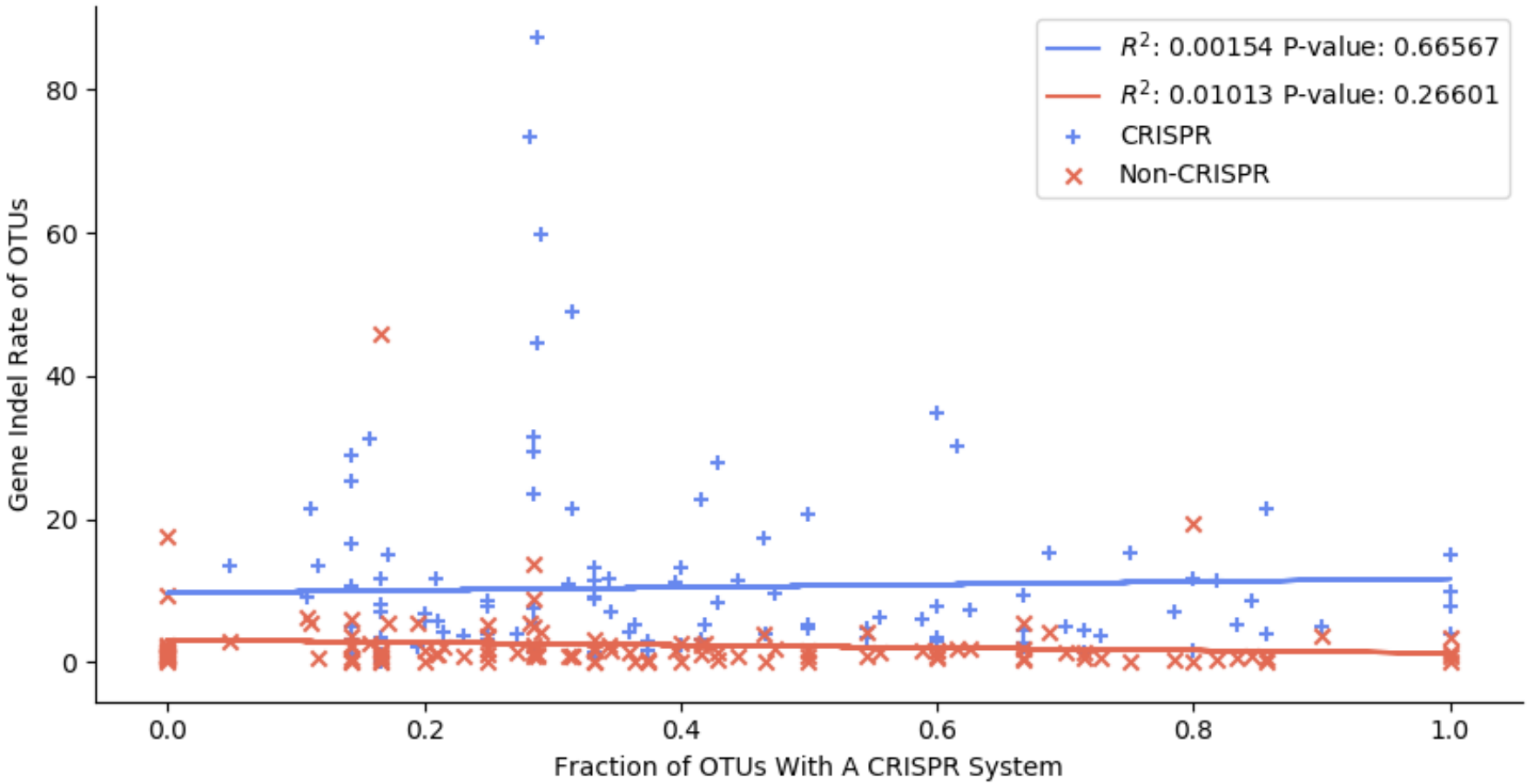
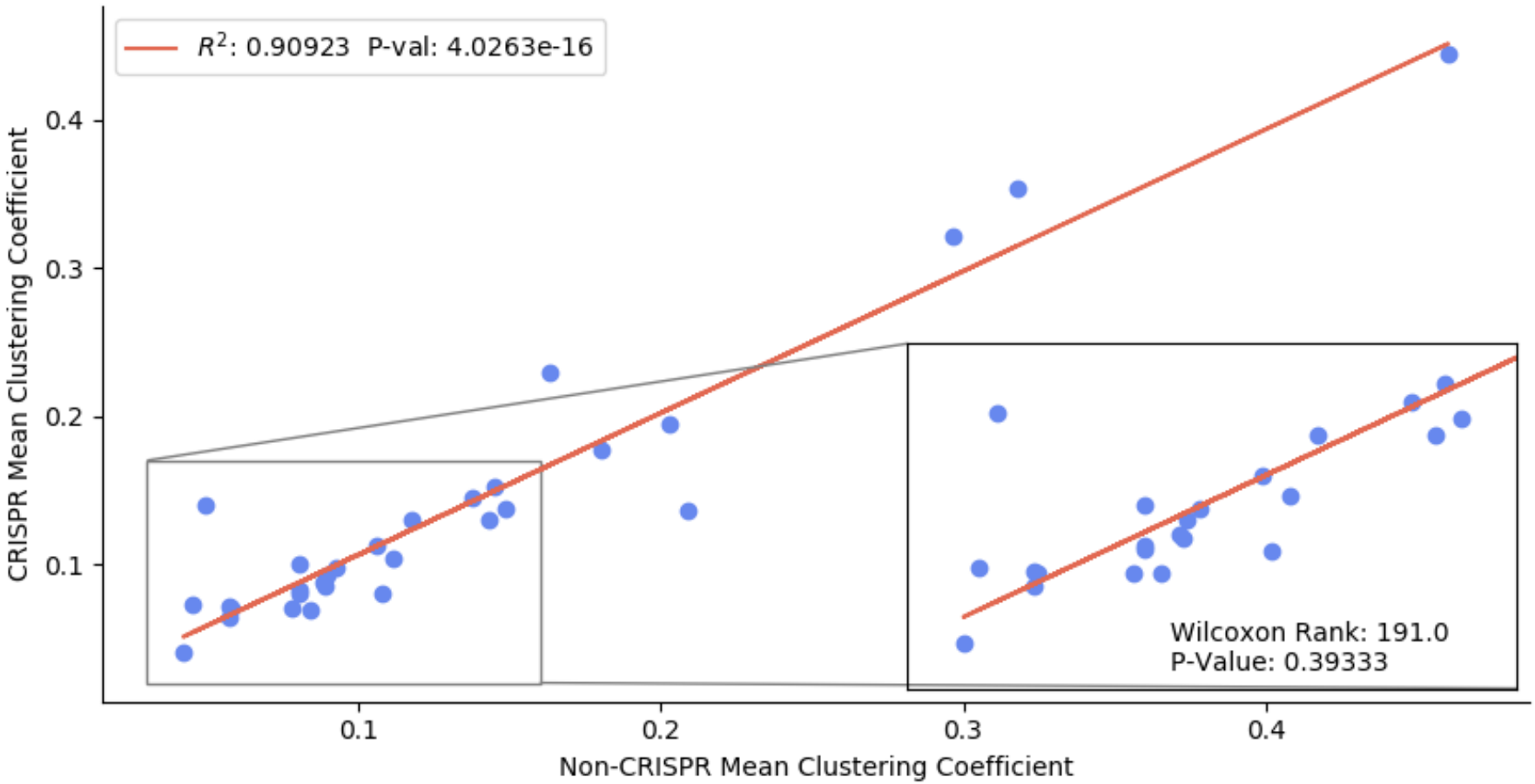


Gene Indel Rate Vs. Fraction of CRISPR OTUs



Mean Node Weighted Clustering Coefficient



Conclusion

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

Is Sharing Caring?

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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

References (1)

- Grissa, I. and Drevet, C. and Couvin, D. (2017). *CRISPRdb*. http://crispr.i2bc.paris-saclay.fr/. Online; accessed 22 October 2018.
- Rath, Devashish et al. (2015). "The CRISPR-Cas immune system: Biology, mechanisms and applications". In: *Biochimie* 117. Special Issue: Regulatory RNAs, pp. 119–128. ISSN: 0300-9084.
- Popa, Ovidiu and Tal Dagan (2011). "Trends and barriers to lateral gene transfer in prokaryotes". In: *Current Opinion in Microbiology* 14.5. Antimicrobials/Genomics, pp. 615–623. ISSN: 1369-5274.
- Bondy, J. A. and U. S. R. Murty (2002). *Graph theory with applications*. Wiley.
- Stern, Adi et al. (2010). "Self-targeting by CRISPR: gene regulation or autoimmunity?" In: *Trends in Genetics* 26.8, pp. 335–340. ISSN: 0168-9525.

References (2)

- Dzidic, Senka and Vladimir Bedeković (2003). "Horizontal gene transfer-emerging multidrug resistance in hospital bacteria". In: *Acta pharmacologica Sinica* 24.6, pp. 519–526.
- Bondy-Denomy, J. and A. R. Davidson (2014). "To Acquire Or Resist:The Complex Biological Effects Of CRISPR-Cas systems". In: *Trends Microbio.* 22.4, pp. 218–25.
- Watson, Bridget N. J., Raymond H. J. Staals, and Peter C. Fineran (2018). "CRISPR-Cas-Mediated Phage Resistance Enhances Horizontal Gene Transfer by Transduction". In: *mBio* 9.1. Ed. by Joseph Bondy-Denomy and Michael S. Gilmore.
- Godde, James S. and Amanda Bickerton (June 2006). "The Repetitive DNA Elements Called CRISPRs and Their Associated Genes: Evidence of Horizontal Transfer Among Prokaryotes". In: *Journal of Molecular Evolution* 62.6, pp. 718–729. ISSN: 1432-1432.

References (3)

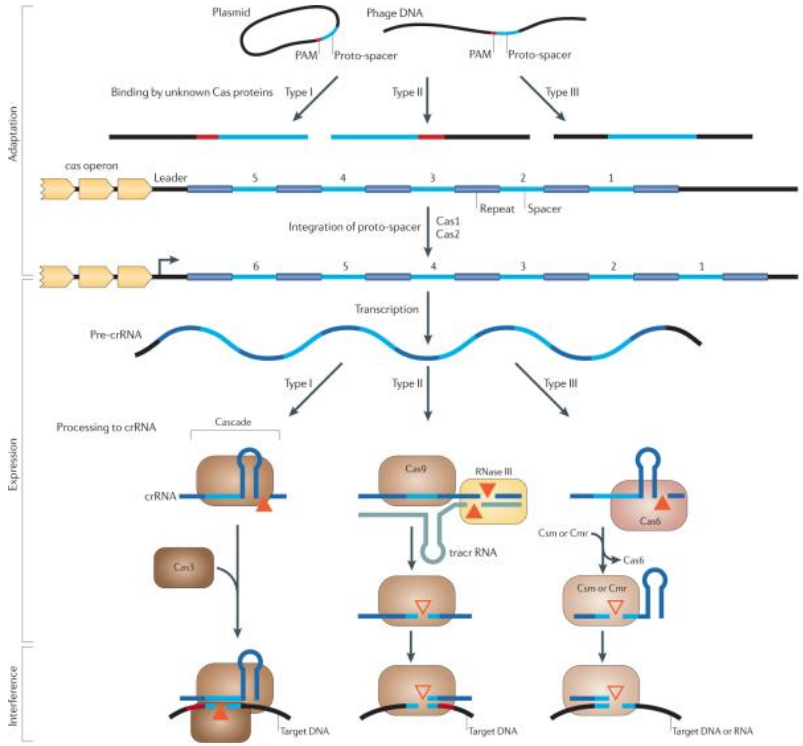
- Zhang, Quan and Yuzhen Ye (Feb. 2017). "Not all predicted CRISPR–Cas systems are equal: isolated cas genes and classes of CRISPR like elements". In: *BMC Bioinformatics* 18.1, p. 92. ISSN: 1471-2105.
- Makarova, K. S. et al. (2011). "Evolution and classification of the CRISPR-Cas systems". In: *Nat. Rev. Microbiol.* 9.6, pp. 467–477.
- Guimaraes, L. C. et al. (2015). "Inside the Pan-genome - Methods and Software Overview". In: *Curr. Genomics* 16.4, pp. 245–252.
- Rasko, David A. et al. (2008). "The Pangenome Structure of Escherichia coli: Comparative Genomic Analysis of E. coli Commensal and Pathogenic Isolates". In: *Journal of Bacteriology* 190.20, pp. 6881–6893. ISSN: 0021-9193.

References (4)

- Berglund, Björn (2015). "Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics". In: *Infection Ecology & Epidemiology* 5.1, p. 28564.
- Kunin, V. et al. (2005). "The net of life: reconstructing the microbial phylogenetic network". In: *Genome Res.* 15.7, pp. 954–959.
- Ravenhall, Matt et al. (May 2015). "Inferring Horizontal Gene Transfer". In: *PLoS Computational Biology* 11.5, pp. 1–16.
- Onnela, J. P. et al. (2005). "Intensity and coherence of motifs in weighted complex networks". In: *Phys Rev E Stat Nonlin Soft Matter Phys* 71.6 Pt 2, p. 065103.
- 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.

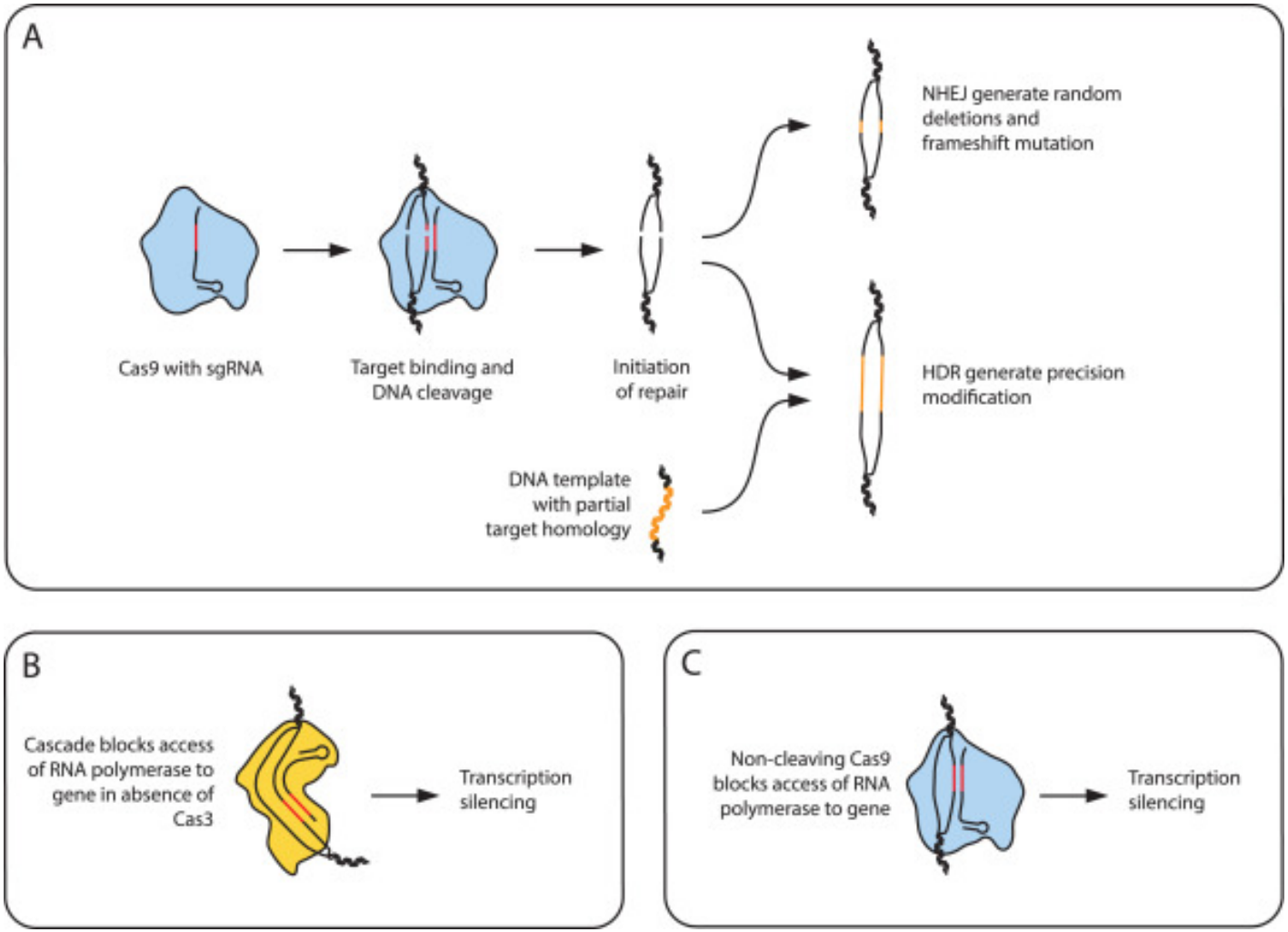
Diversity & Ubiquity

- 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)



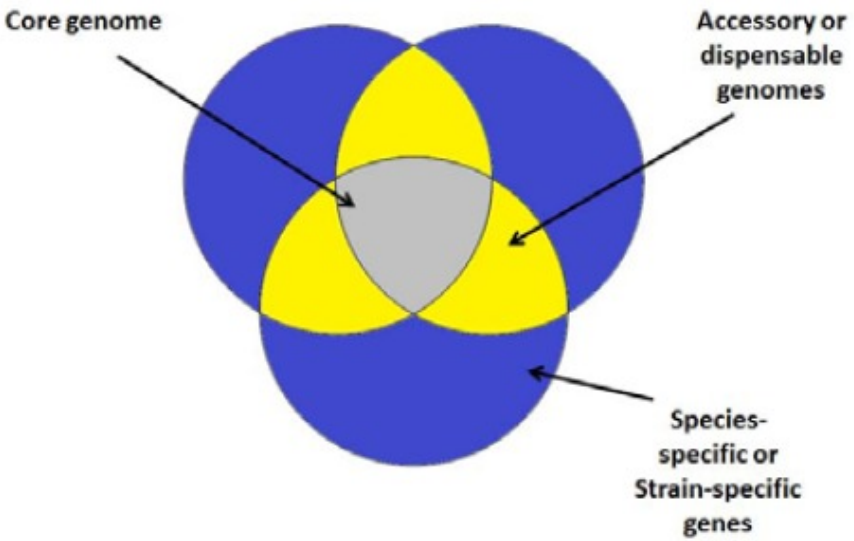
(Makarova et al., 2011)

CRISPR Biotech Application

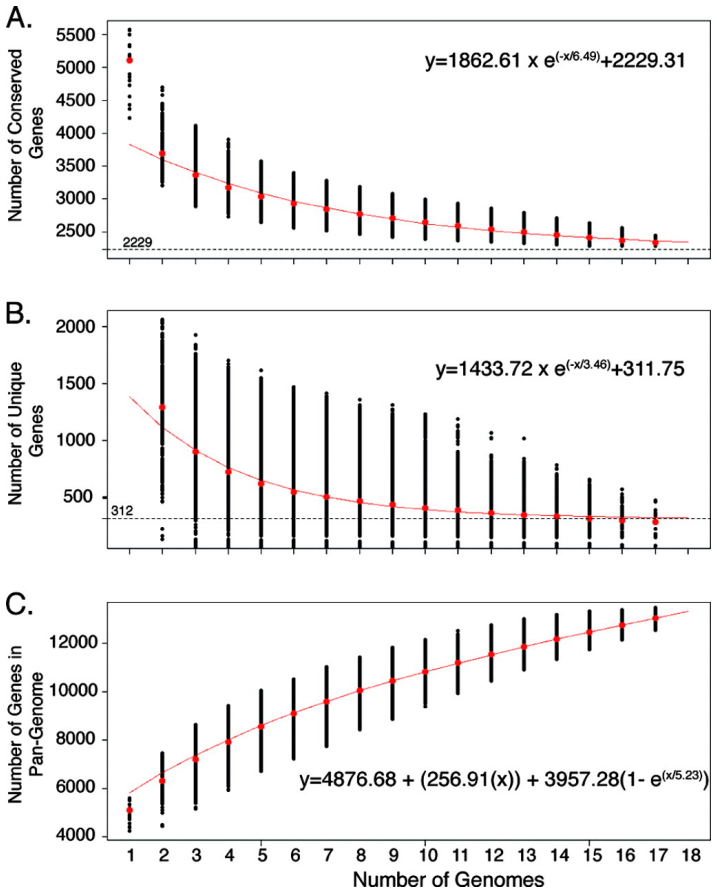


(Rath et al., 2015)

Pan-Genomes

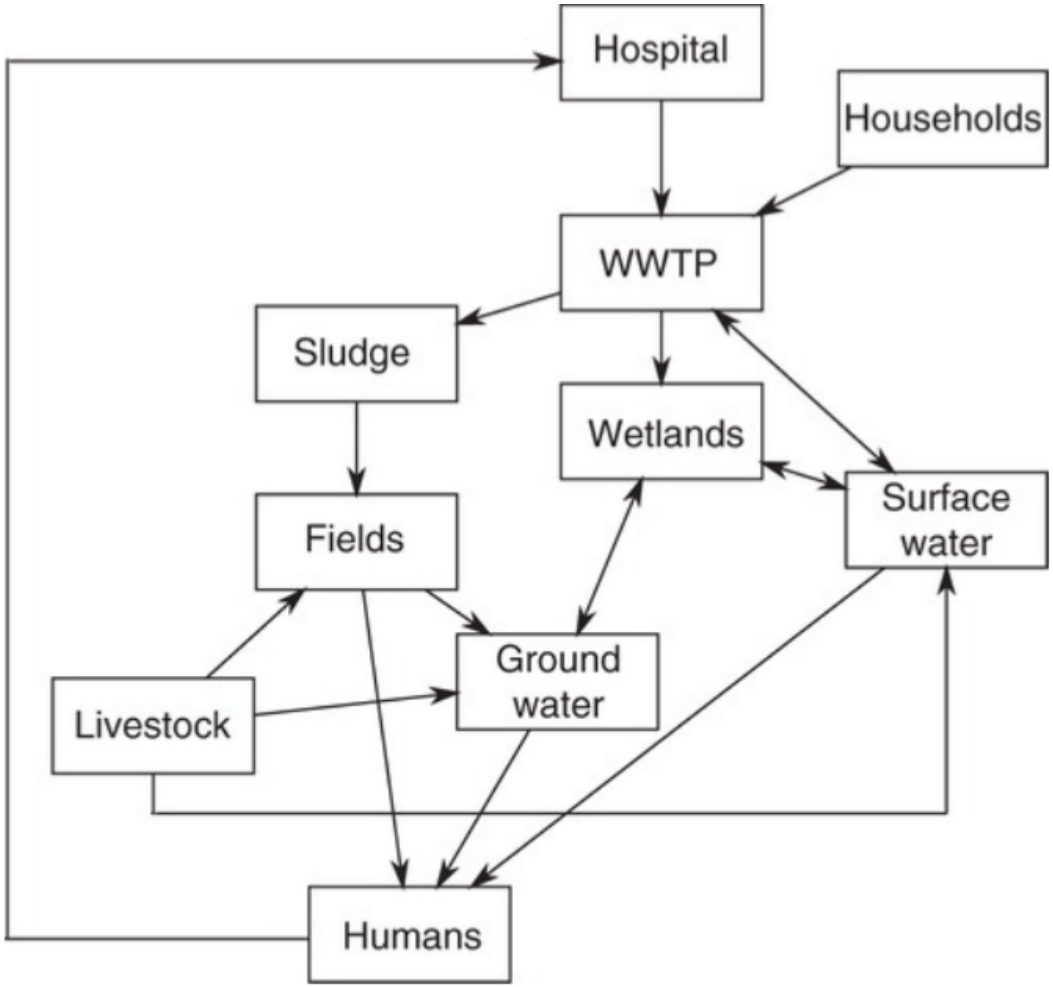


(Guimaraes et al., 2015)



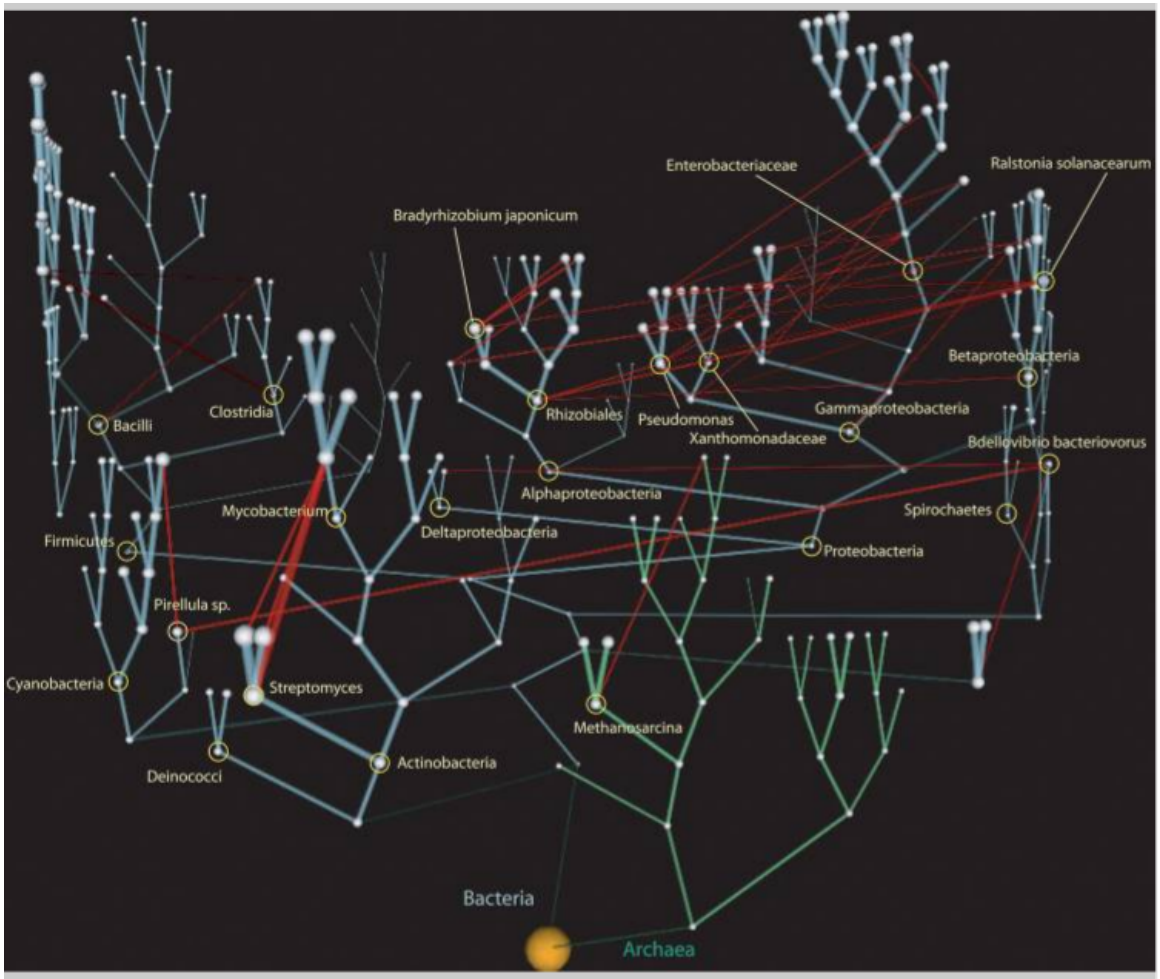
(Rasko et al., 2008)

HGT Applications



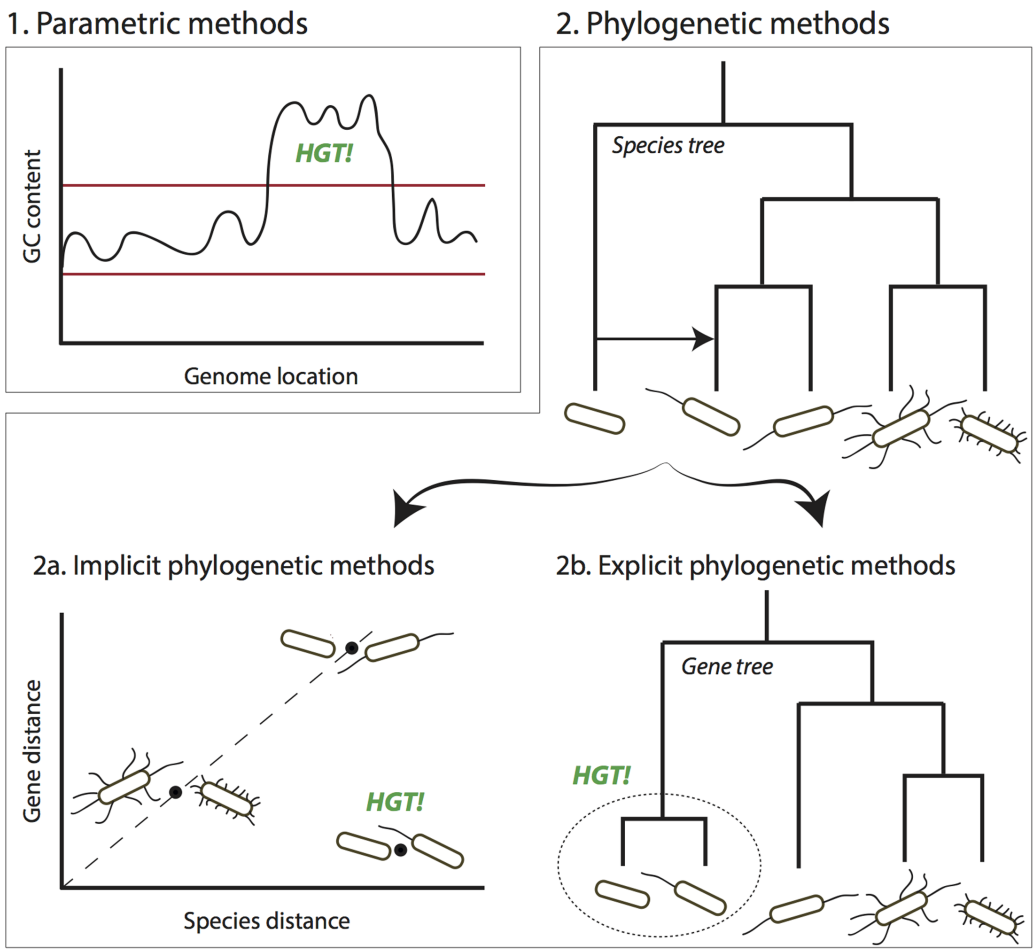
(Berglund, 2015)

Prokaryotic "Net of Life"



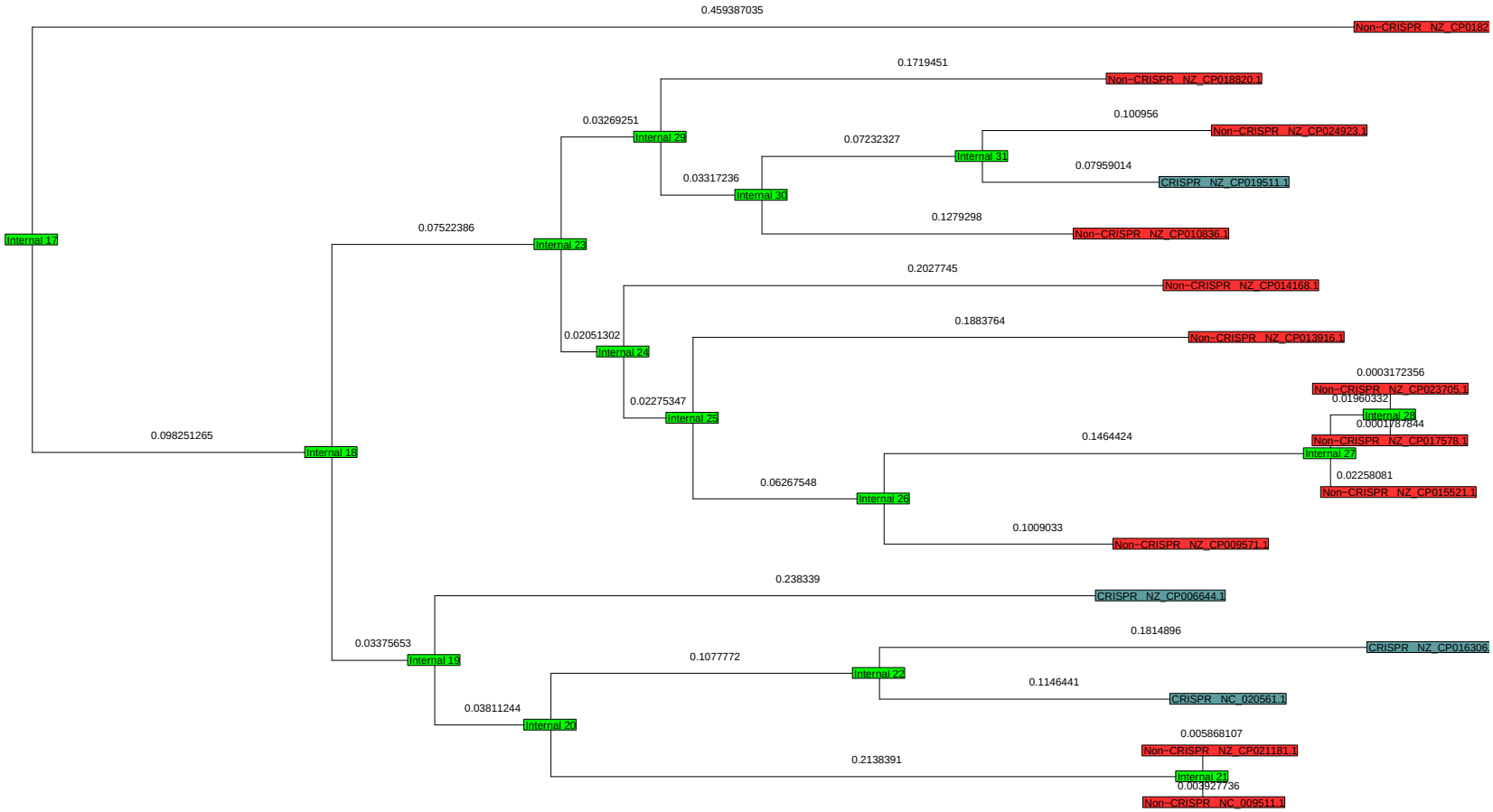
(Kunin et al., 2005)

Phylogenomic Network Construction



(Ravenhall et al., 2015)

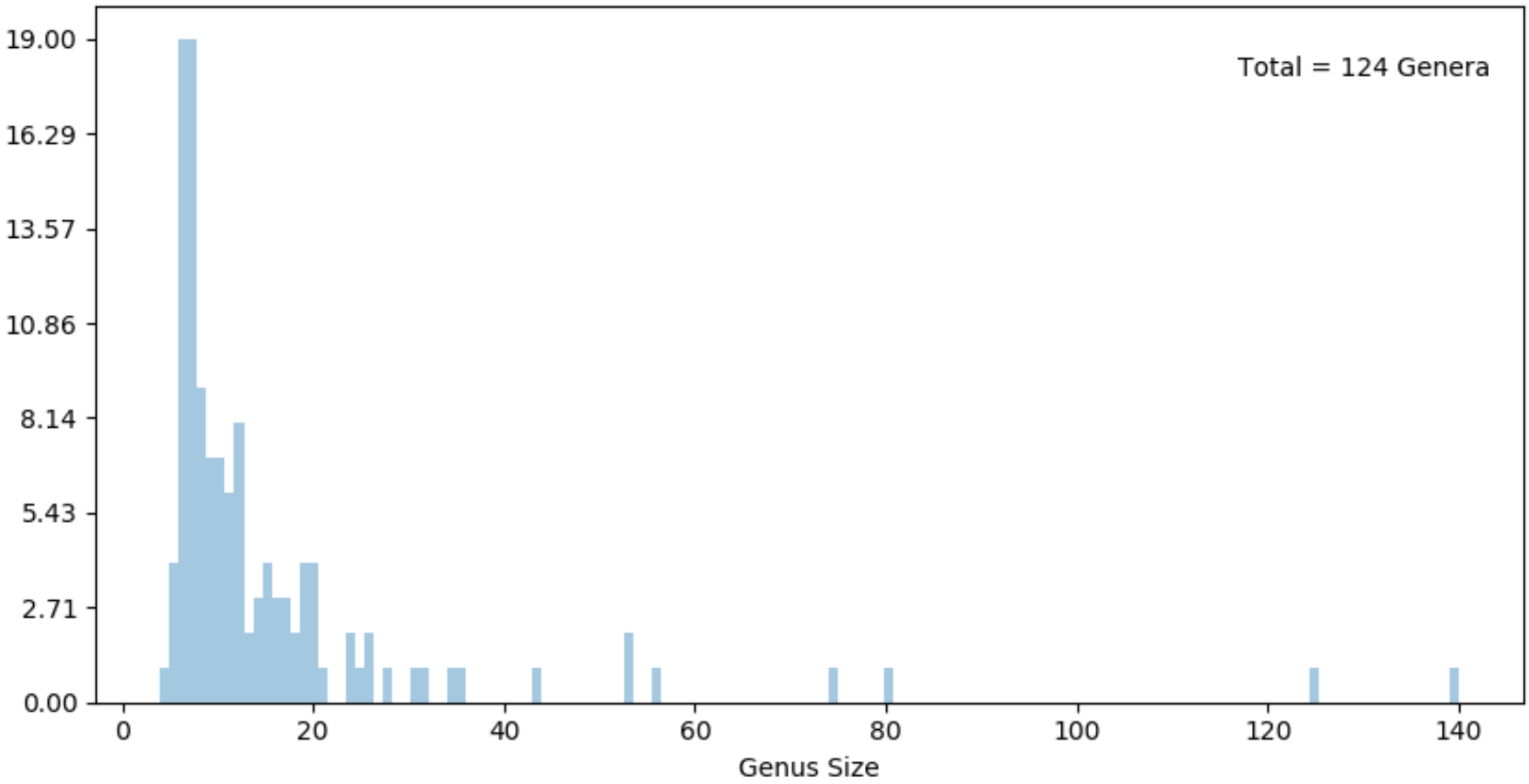
Sphingomonas Species Tree



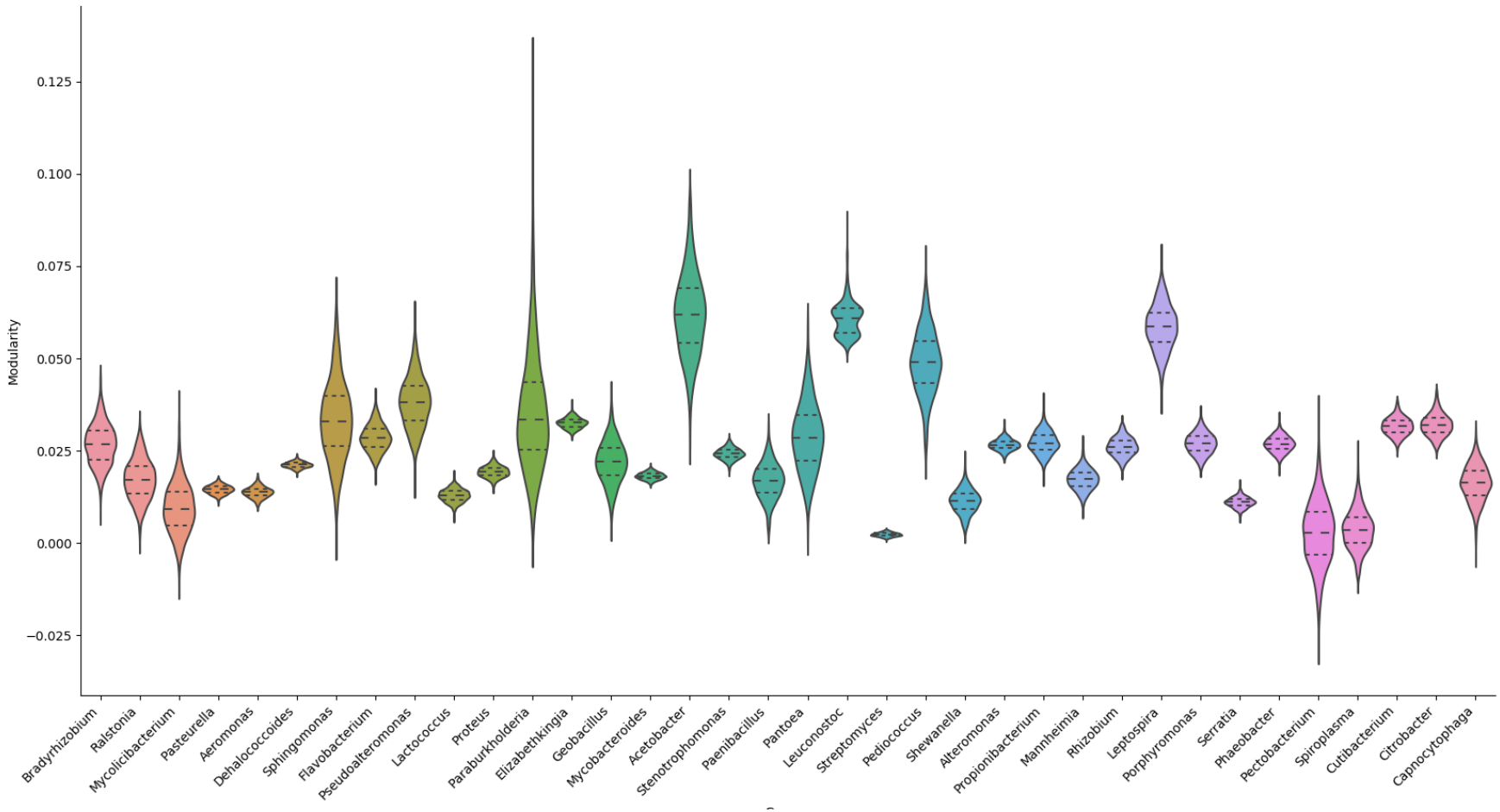
Network Statistics

- **Average Node Degree**: $\frac{1}{|N_u|}\sum_{uv}^{N_u} w_{uv}$ 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**: $Q = \frac{1}{2m}\sum_{uv}^{W}[W_{uv} - \frac{k_u k_v}{2m}]\delta(u, v)$ 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. $Q \in [-1, 1]$ (Newman, 2004)

Genus Size Distribution



Modularity Distributions



Assortativity Distributions