

# Is Sharing Caring?

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

Siddharth Reed  
MolBiol 4C12 Thesis



Golding Lab,  
Biology Department,  
McMaster University

April 7, 2019

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1. Background
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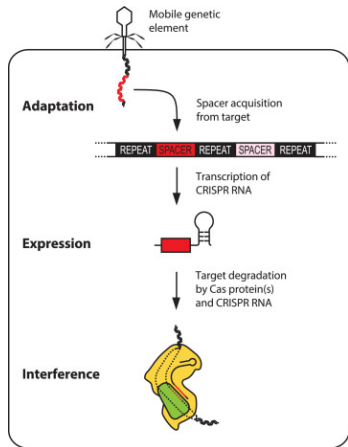
# CRISPR-Cas systems

# What Are They?

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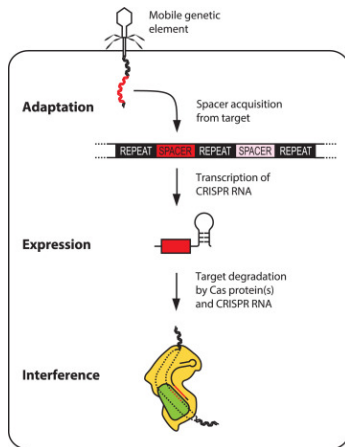
- Adaptive Bacterial Immune System



(Rath et al., 2015)

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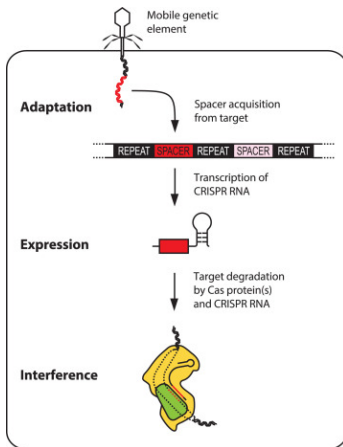
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- Failed “infection” → spacer acquisition → targeted degradation for next “infection”



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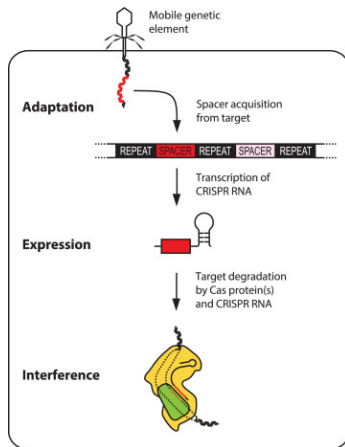
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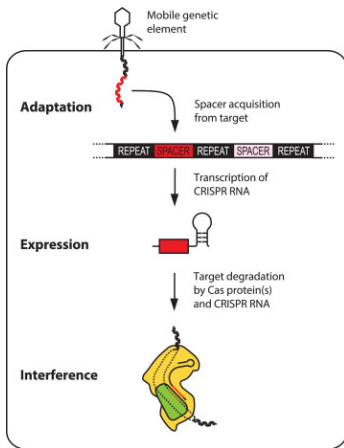


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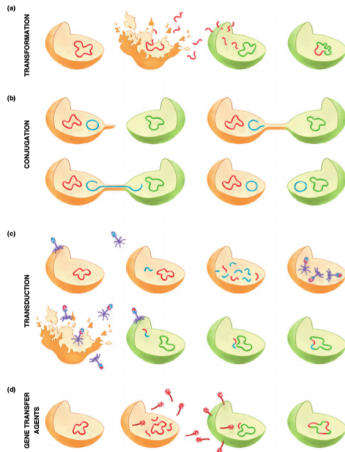
- Adaptive Bacterial Immune System
- Failed “infection” → spacer acquisition → targeted degradation for next “infection”
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- 45% of bacteria have CRISPR loci ( $n = 6782$ ) (Grissa, I. and Drevet, C. and Couvin, D., 2017)



(Rath et al., 2015)

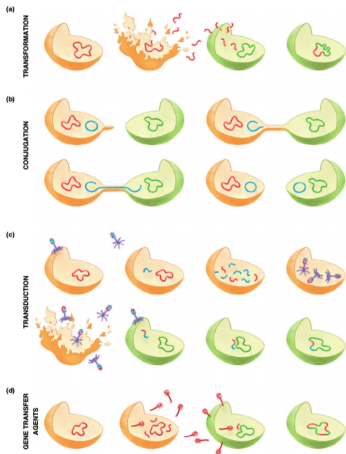
# Horizontal Gene Transfer

# Horizontal Gene Transfer Mechanisms



(Popa and Dagan, 2011)

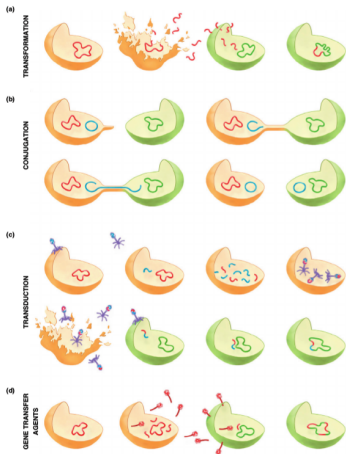
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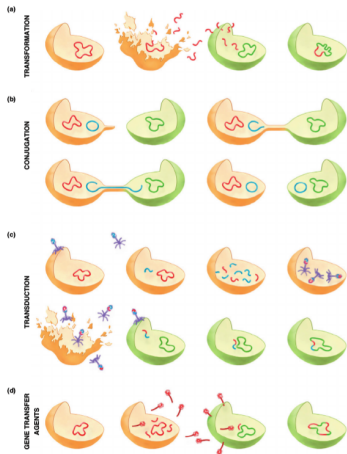
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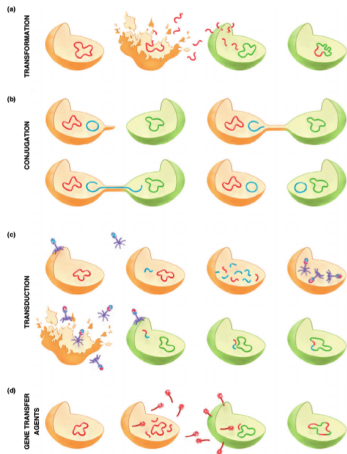
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- **CRISPR-Cas directly affects HGT** (Popa and Dagan, 2011)

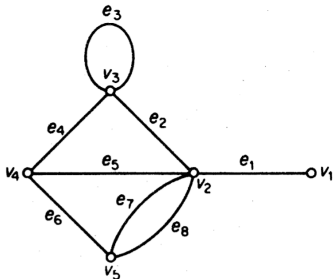
# Phylogenomic Networks



# What is A Network?

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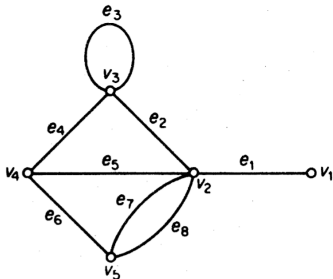
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- Useful mathematical abstraction of real world system

(Bondy and Murty, 2002)

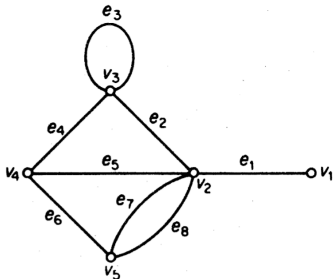
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# Do CRISPR Systems Affect Horizontal Gene Transfer?

Yes

# CRISPR Cost Complexity and Curbing It

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  - Anti-CRISPR systems (Bondy-Denomy and Davidson, 2014)
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  - Selective CRISPR inactivation (Rath et al., 2015)
  - CRISPRs themselves can be transferred  $\implies$  population level immunity (Godde and Bickerton, 2006)

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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

# Objectives

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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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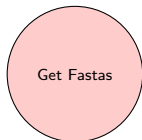
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## Gene Indel Rates vs. Network Statistics

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

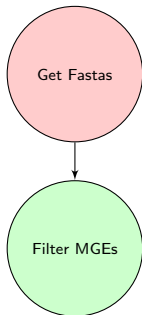
# Workflow (Per Genus)

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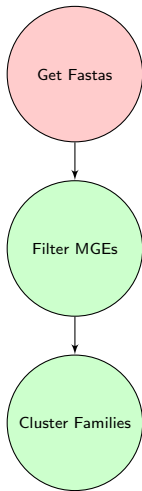
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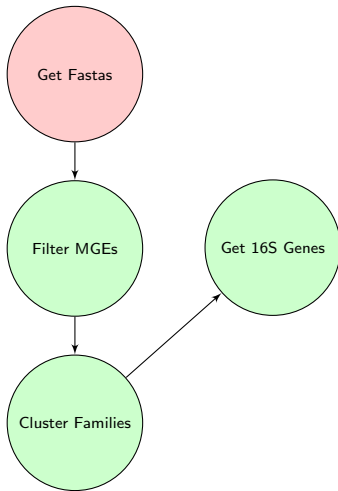


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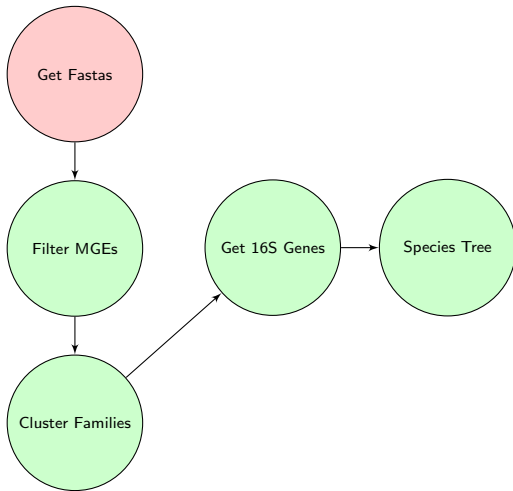
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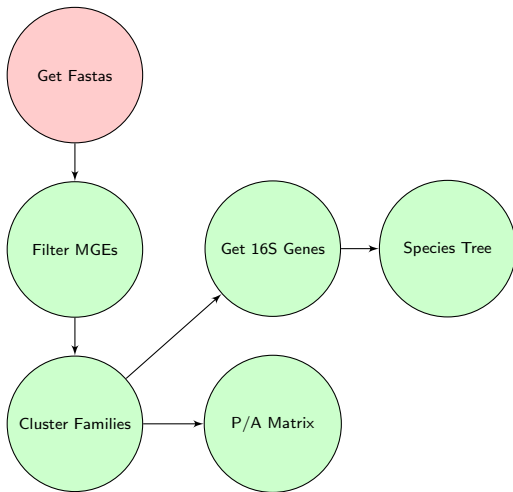
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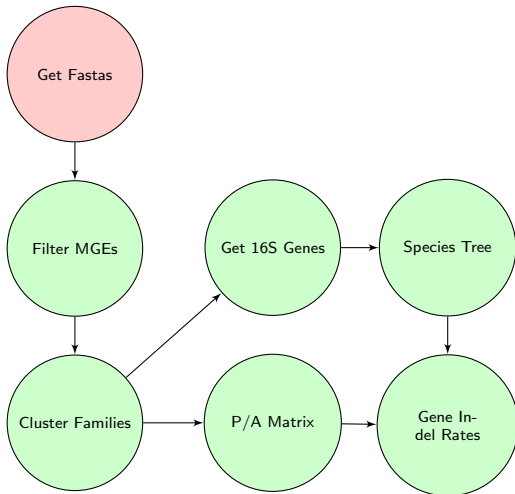
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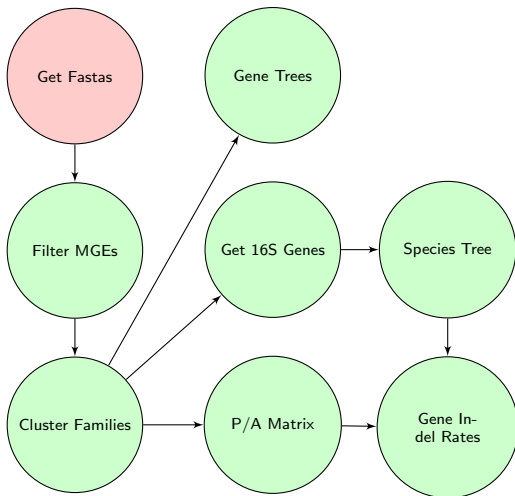


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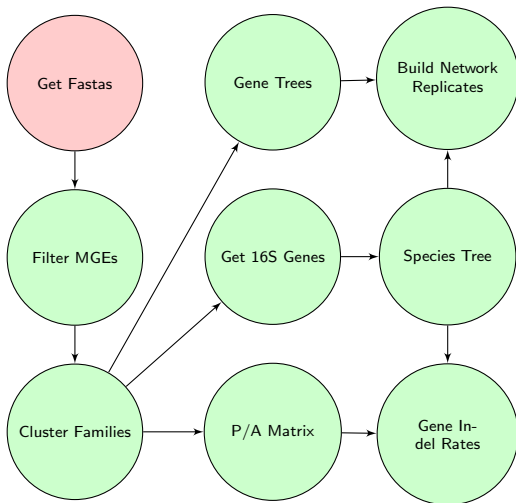




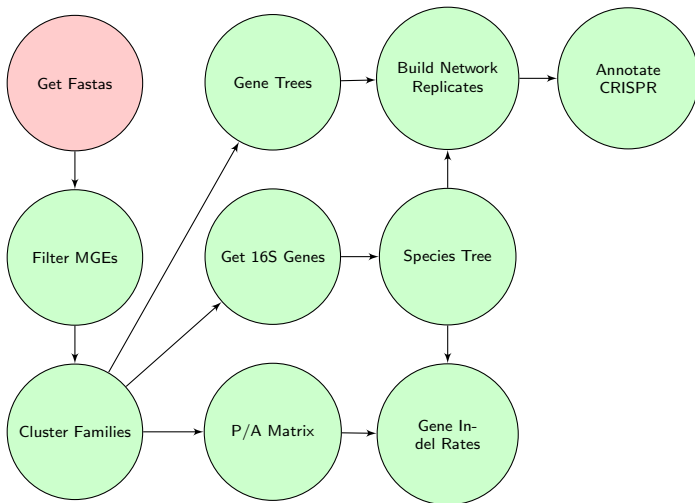
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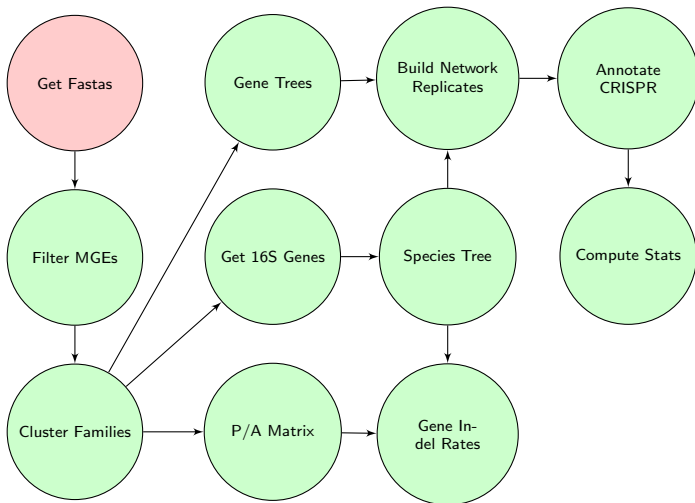
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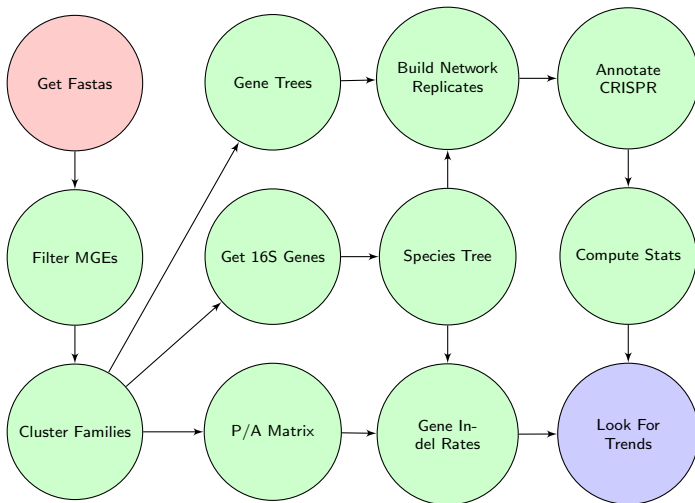
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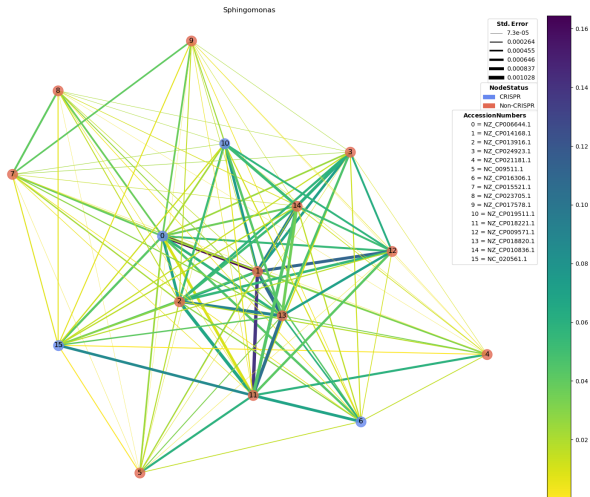


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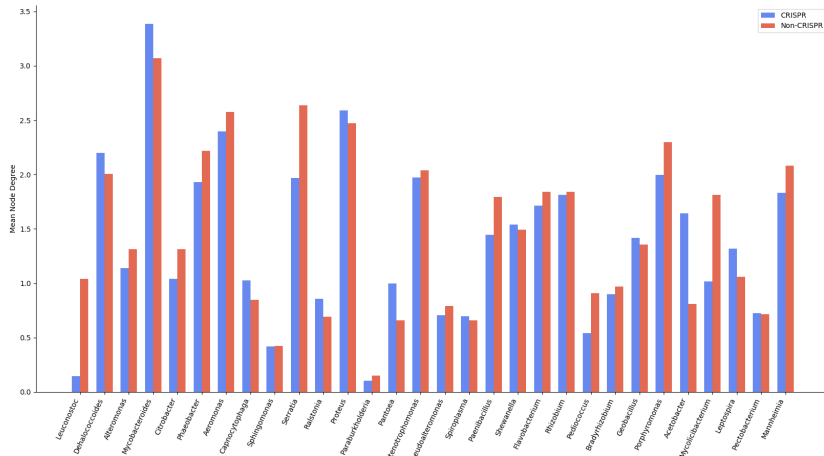


# Results

# Example "Consensus" Network

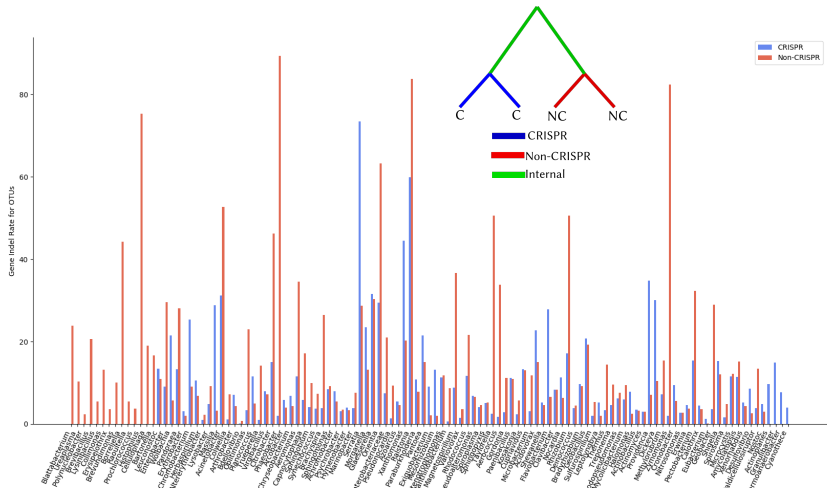


# Mean Node Degree

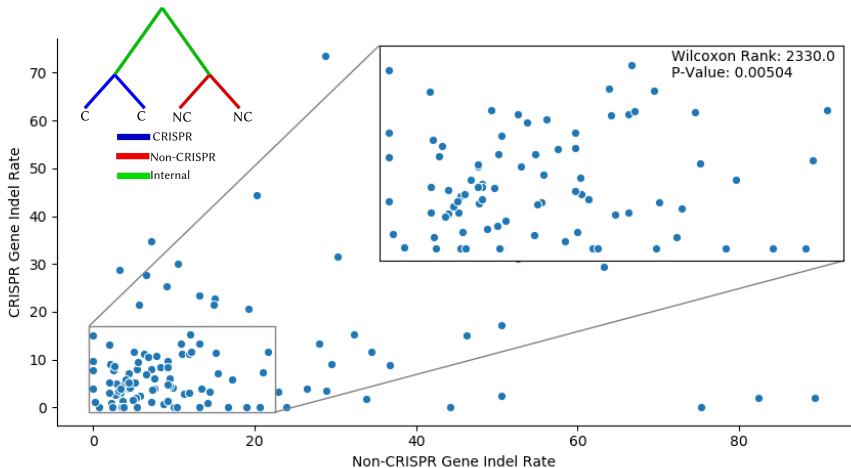




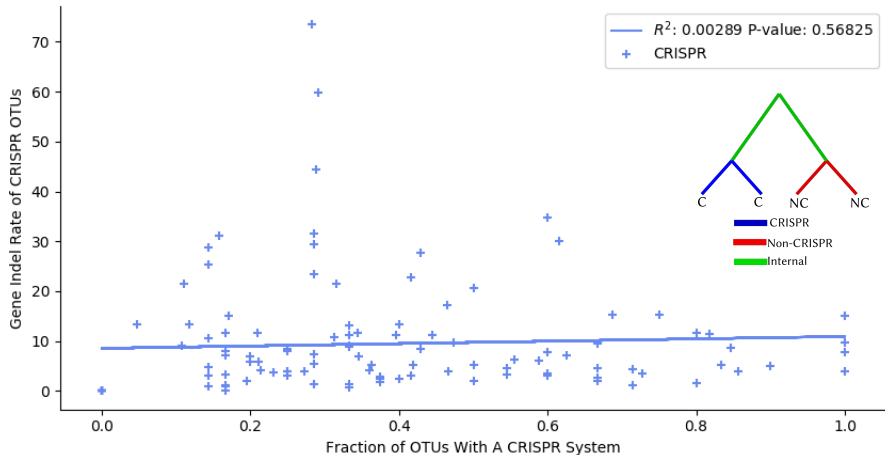
## Gene Indel Rates



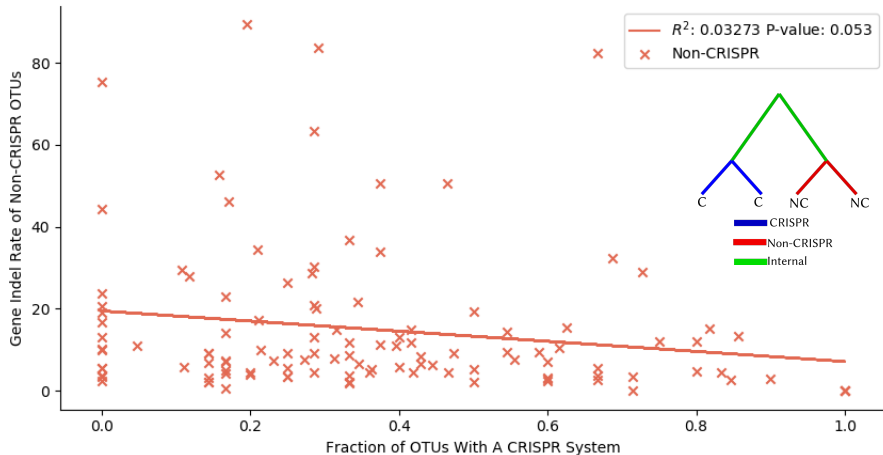
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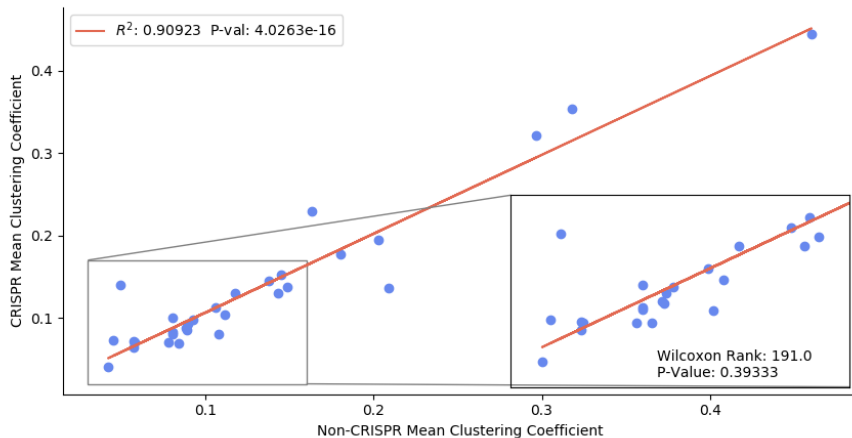
# Gene Indel Rate Vs. Fraction of CRISPR OTUs



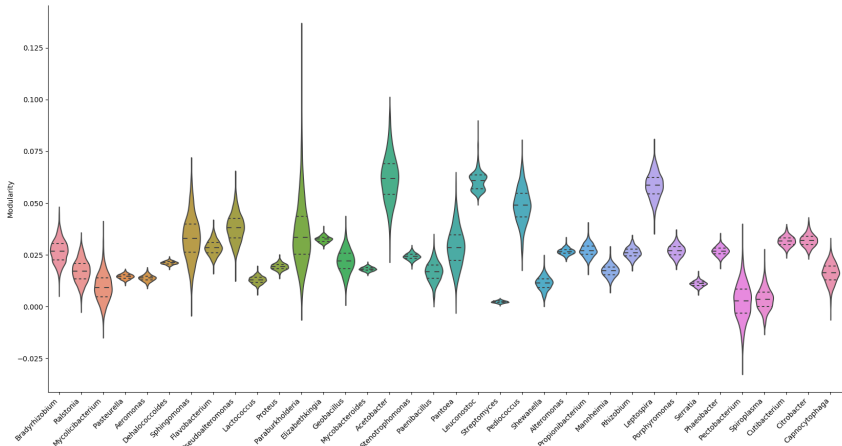
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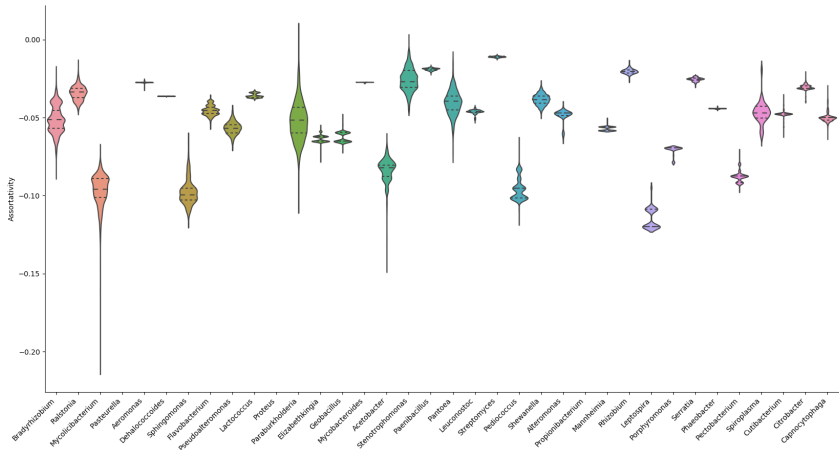
# Mean Node Weighted Clustering Coefficient



# Modularity Distributions



# Assortativity Distributions



# Conclusion



# Findings

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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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- **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

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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](https://github.com/DJSiddharthVader/thesis_SidReed)



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Makarova, K. S. et al. (2011). “Evolution and classification of the CRISPR–Cas systems”. In: *Nat. Rev. Microbiol.* 9.6, pp. 467–477.

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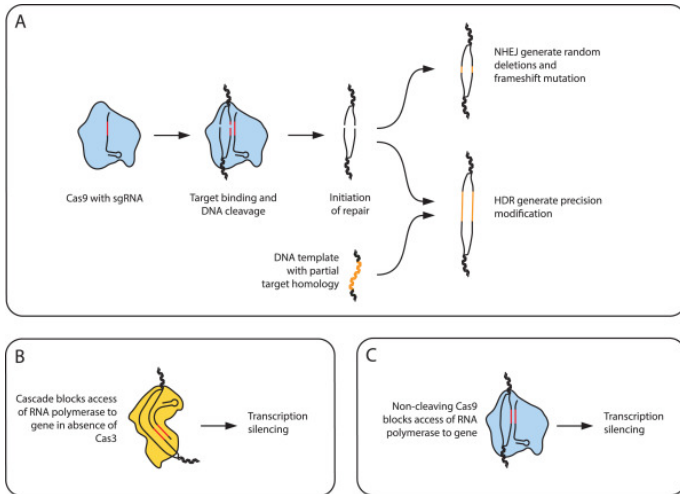
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- Indel are inferred to be the result of HGT

# CRISPR Biotech Application



(Rath et al., 2015)

# Rate Influencing Factors

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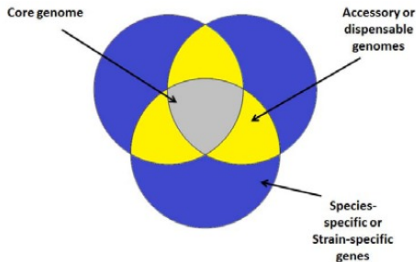
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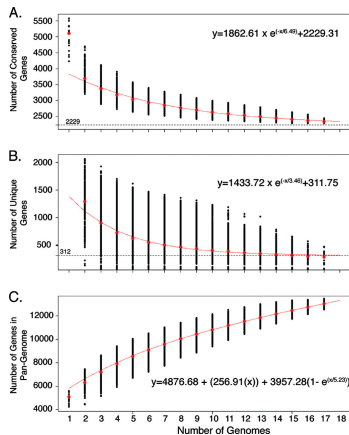
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- Amount of exogenous DNA/cell density/phage density
- Selective pressures
- Metabolic costs
- Sequence compatibility

# Pan-Genomes



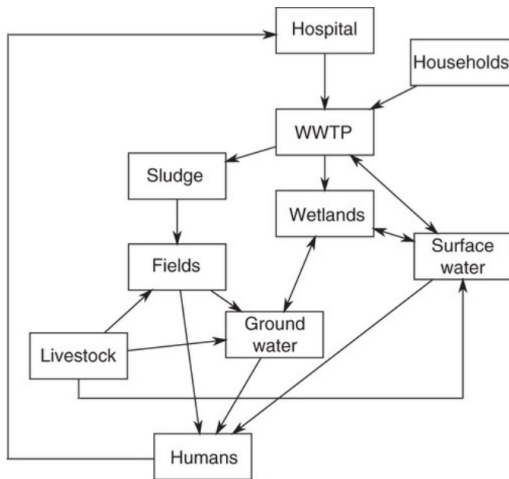
(Guimaraes et al., 2015)



(Rasko et al., 2008)

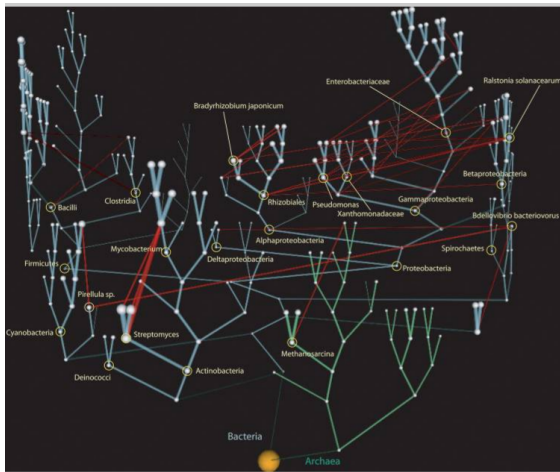


# HGT Applications



(Berglund, 2015)

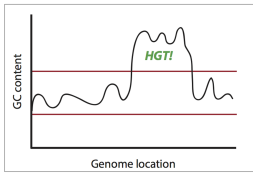
# Prokaryotic “Net of Life”



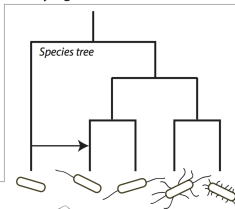
(Kunin et al., 2005)

# Phylogenomic Network Construction

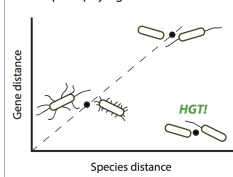
## 1. Parametric methods



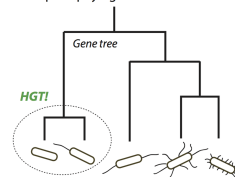
## 2. Phylogenetic methods



### 2a. Implicit phylogenetic methods



### 2b. Explicit phylogenetic methods



(Ravenhall et al., 2015)

# CRISPR Cost Complexity and Curbing It (Expanded)

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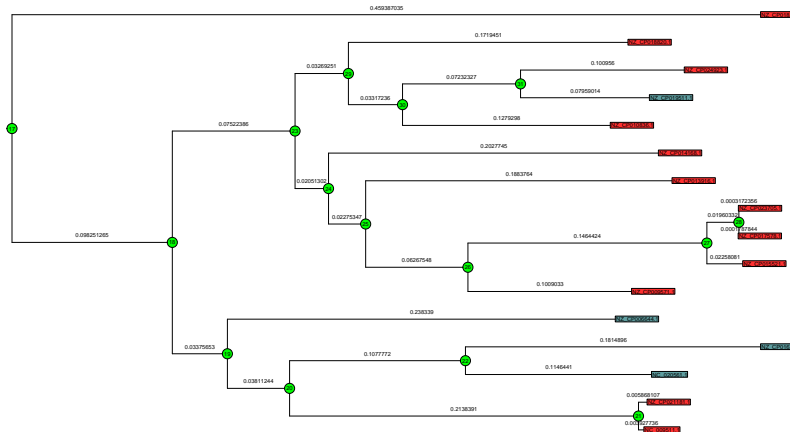
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  - CRISPR can enhance transduction-mediated HGT (Watson, Staals, and Fineran, 2018)

# Sphingomonas Species Tree

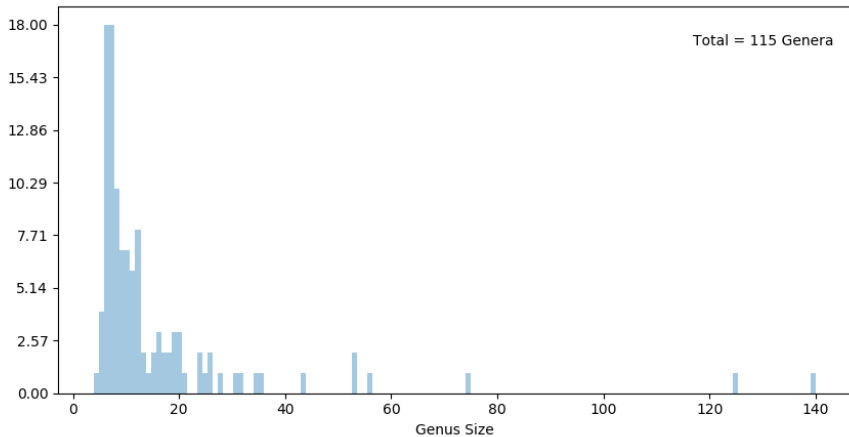


## Network Statistics

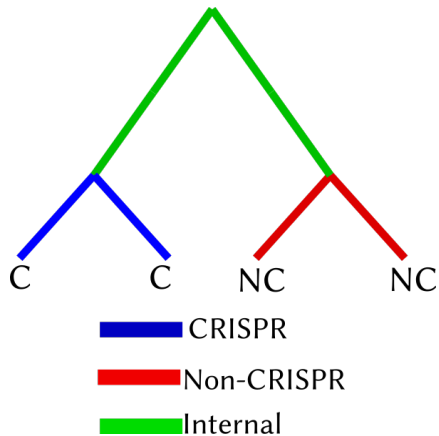
- **Average Node Degree:**  $\frac{1}{|N_u|} \sum_{uv} w_{uv}$  where  $N_u$  is the set of nodes incident to  $u$
- **Node Clustering Coefficient:**  $\frac{1}{k_u(k_u-1)} \sum_{vw}^{T(u)} (\hat{w}_{uw} \hat{w}_{vw} \hat{w}_{uv})^{\frac{1}{3}}$  where  $T(u)$  is the set of triangles containing  $u$  (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)



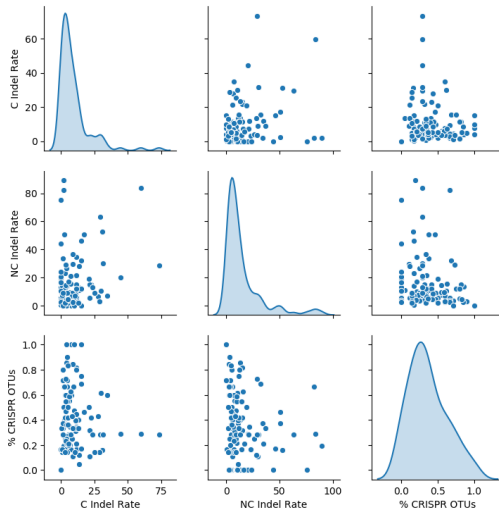
# Genus Size Distribution



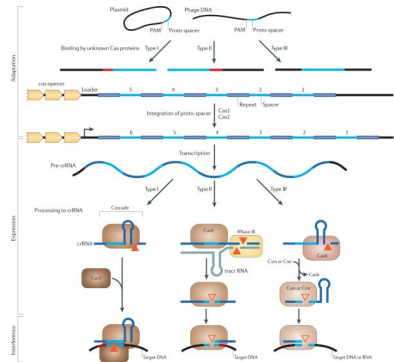
## Branch Partition Example



# Indel Rate Pair Plot



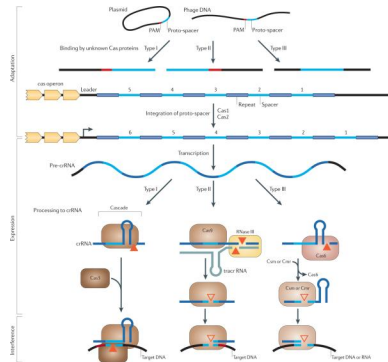
# Diversity & Ubiquity



(Makarova et al., 2011)

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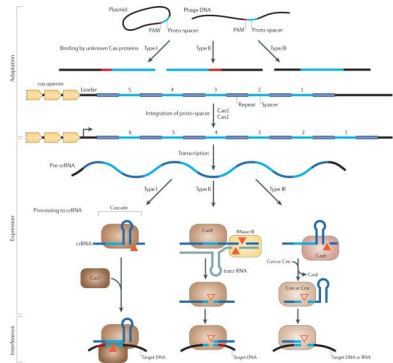
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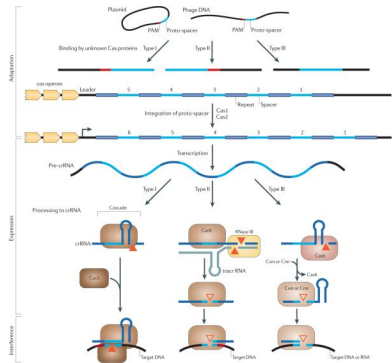
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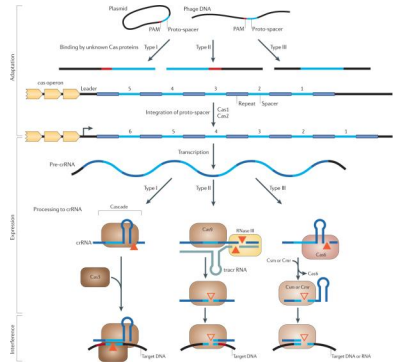
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- 11% – 28% are false or orphaned CRISPR loci (Zhang and Ye, 2017)



(Makarova et al., 2011)