Bayesian analysis

Identifying essential genes by mutagenesis

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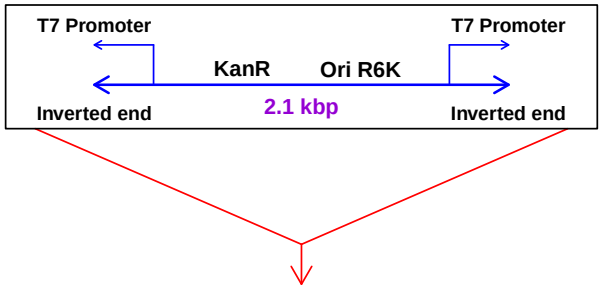
Mycobacterium tuberculosis

- The organism that causes tuberculosis.
  - Cost for treatment: ∼$15,000
  - Other bacterial pneumonias: ∼$35
- 4.4 Mbp circular genome, completely sequenced
- 4250 known or inferred genes

Goal: identify the essential genes

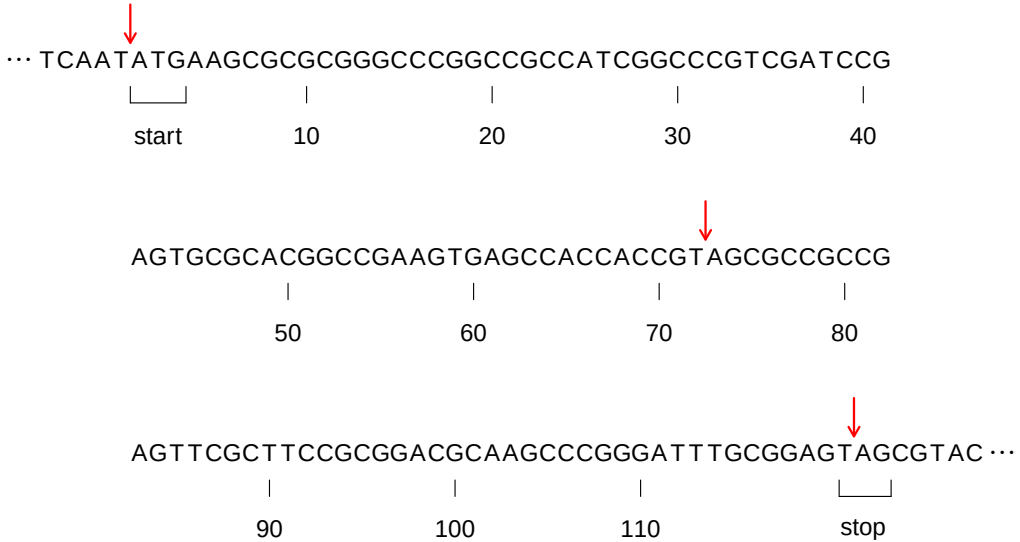
Method: random transposon mutagenesis

Himar1 transposon



Note: $\geq$ 30 stop codons in each reading frame

Sequence of the gene MT598

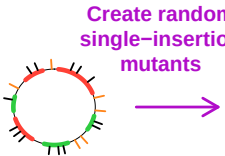


Random transposon mutagenesis

Red = essential

Green = non−essential

Random transposon mutagenesis



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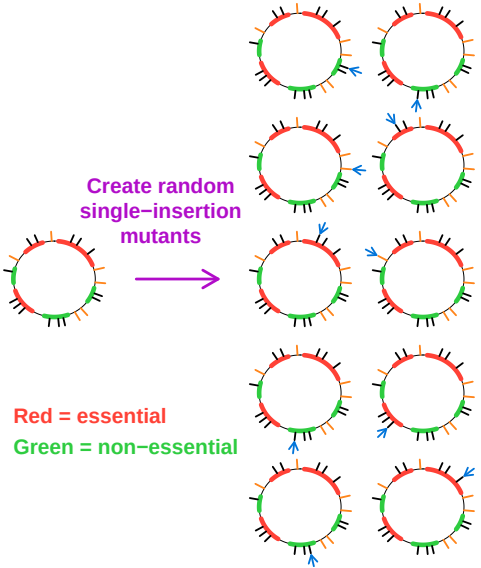
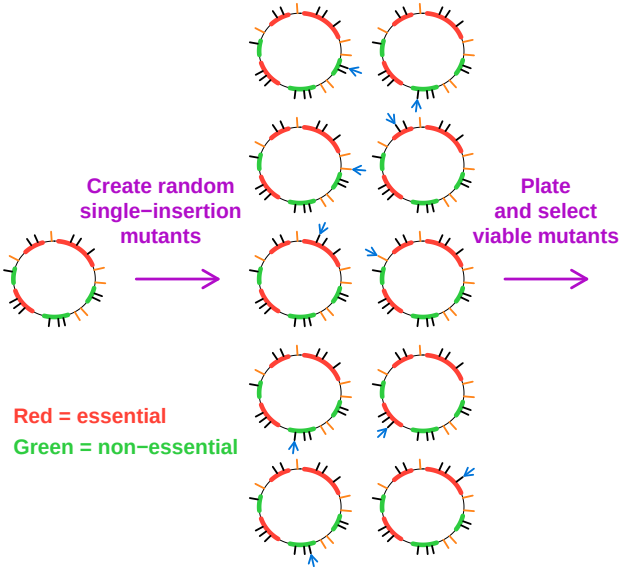
Random transposon mutagenesis



Random transposon mutagenesis



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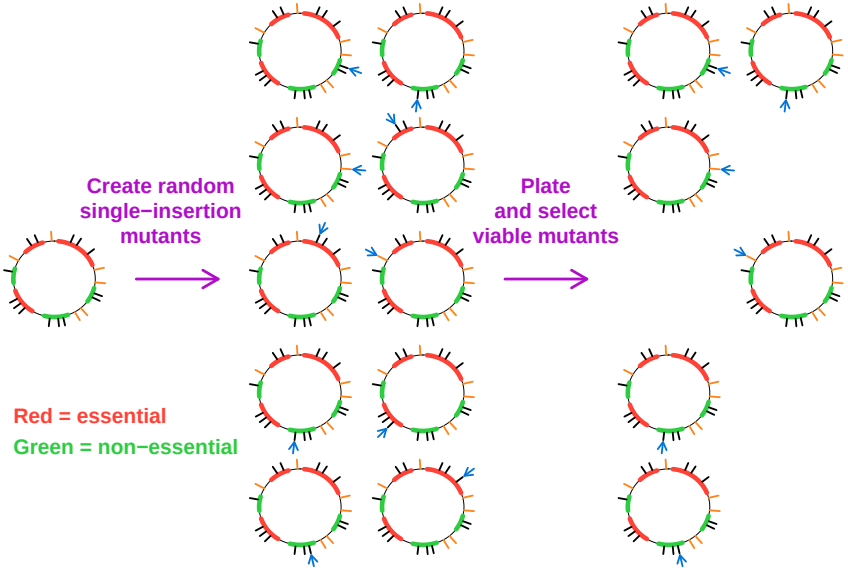
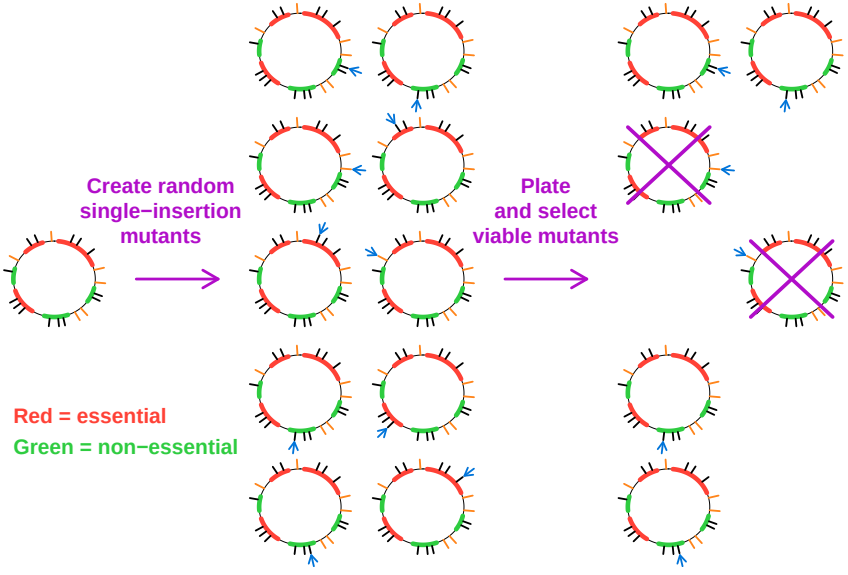


Random transposon mutagenesis



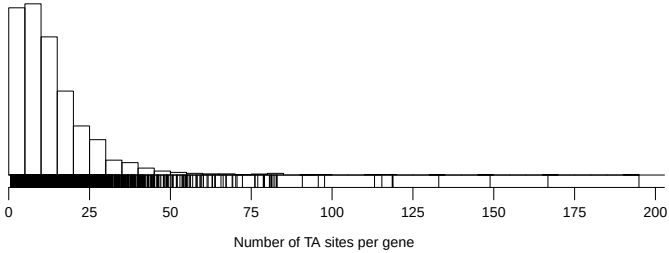
Random transposon mutagenesis

- Location of transposon insertion determined by sequencing across junctions
- Viable insertion within a gene $\Longrightarrow$ gene is non-essential
- Essential genes: we will never see a viable insertion
- Complication: Insertions in the very distal portion of an essential gene may not be sufficiently disruptive.
  Thus, we omit from consideration insertions sites within the last 20% and last 100 bp of a gene.

The data

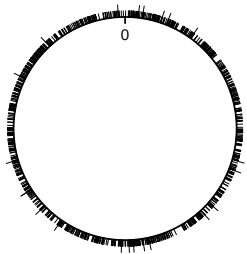
- Number, locations of genes
- Number of insertion sites in each gene
- n viable mutants with exactly one transposon insertion
- Location of the transposon insertion in each mutant

TA sites in M. tuberculosis



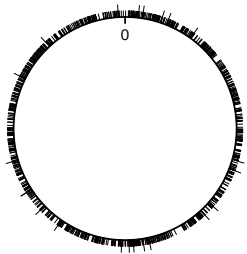
- 74,403 sites
- 65,649 sites within a gene
- 57,934 sites within proximal portion of a gene
- 4204/4250 genes with at least one TA site

1425 insertion mutants



- 1425 insertion mutants
- 1025 within proximal portion of a gene
- 21 double-hits
- 770 unique genes hit

1425 insertion mutants



- 1425 insertion mutants
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Questions: Proportion of essential genes in M. tuberculosis?

Which genes are likely essential?

Model

Transposon inserts completely at random

- Each TA site equally likely
- Genes are either completely essential or completely non-essential

Model

N genes x_i = no. TA sites in gene i

n mutants y_i = no. mutants with insertion in gene i.

$$\theta_i = \begin{cases} 1 \\ 0 \end{cases} \text{ if gene } i \text{ is } \begin{matrix} \text{non-essential} \\ \text{essential} \end{matrix}$$

Model: $\boldsymbol{y} \sim \text{multinomial}(n, \boldsymbol{p})$ where $p_i = x_i\theta_i / \sum_j x_j\theta_j$

Goal: Estimate $\theta_+ = \sum_i \theta_i$ or $1 - \theta_+/N$

The likelihood

$$L(\boldsymbol{\theta} \mid \boldsymbol{y}) = \binom{n}{y} \prod_i (x_i\theta_i)^{y_i} / \sum_j (x_j\theta_j)^n$$

$$\propto \begin{cases} (\sum_i x_i\theta_i)^{-n} & \text{if } \theta_i = 1 \text{ whenever } y_i > 0 \\ 0 & \text{otherwise} \end{cases}$$

Notes:

- Depends only on which $y_i > 0$ and not on the specific values
- The MLE is $\hat{\theta}_i = 1\{y_i > 0\}$

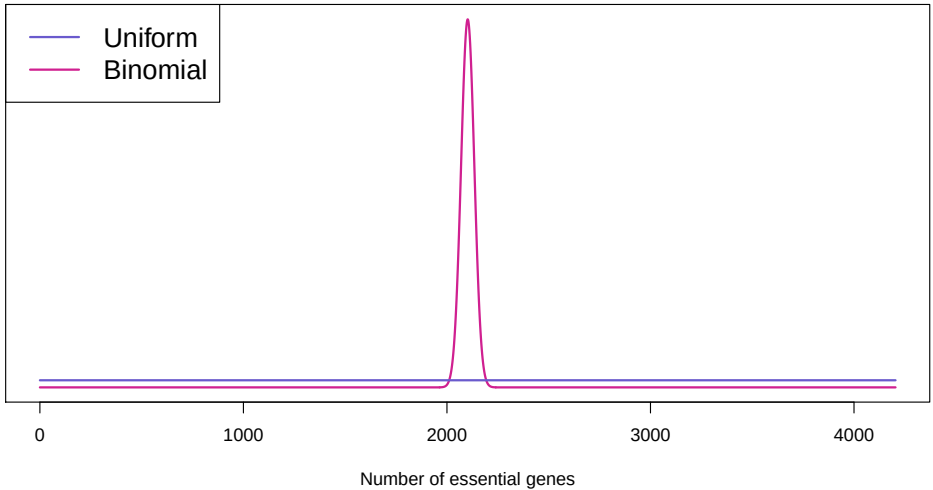
The prior

$\theta_+ \sim$ uniform on { 0, 1, ..., N }

$\boldsymbol{\theta} \mid \theta_+ \sim$ uniform over all sequences of 0's and 1's with θ_+ 1's

Notes:

- We are assuming that Pr$(\theta_i = 1) = 1/2$
- This is quite different from taking θ_i iid Bernoulli(1/2)
- We are assuming that θ_i is independent of x_i and the length of the gene
- We could make use of information about the essential status of particular genes (e.g. known viable knock-outs)


Uniform
Binomial
0
1000
2000
3000
4000
Number of essential genes

A Gibbs sampler

Goal: Estimate $\Pr(\boldsymbol{\theta} \mid \boldsymbol{y})$

Gibbs sampler:

- Begin with some initial assignment $\boldsymbol{\theta}^{(0)}$
- For iteration s, consider each gene one at a time
  - Let $\boldsymbol{\theta}_{-i}^{(s)} = (\theta_1^{(s+1)}, ..., \theta_{i-1}^{(s+1)}, \theta_{i+1}^{(s)}, ..., \theta_N^{(s)})$
  - Calculate $\Pr(\theta_i = 1 \mid \boldsymbol{\theta}_{-i}^{(s)}, \boldsymbol{y})$
  - Assign $\theta_i^{(s)} = 1$ at random with that probability
- Repeat many times

This is an example of Markov chain Monte Carlo (MCMC).

MCMC in action

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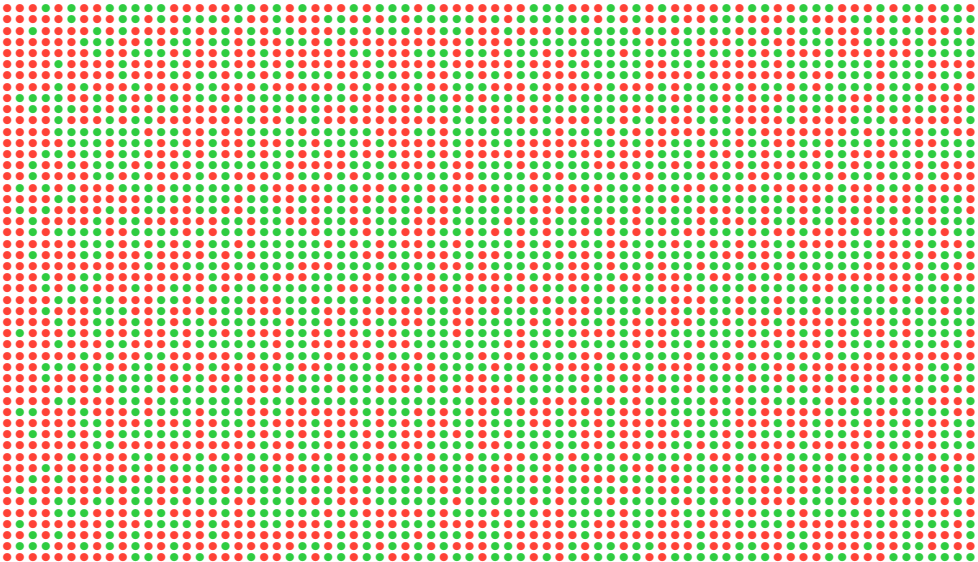
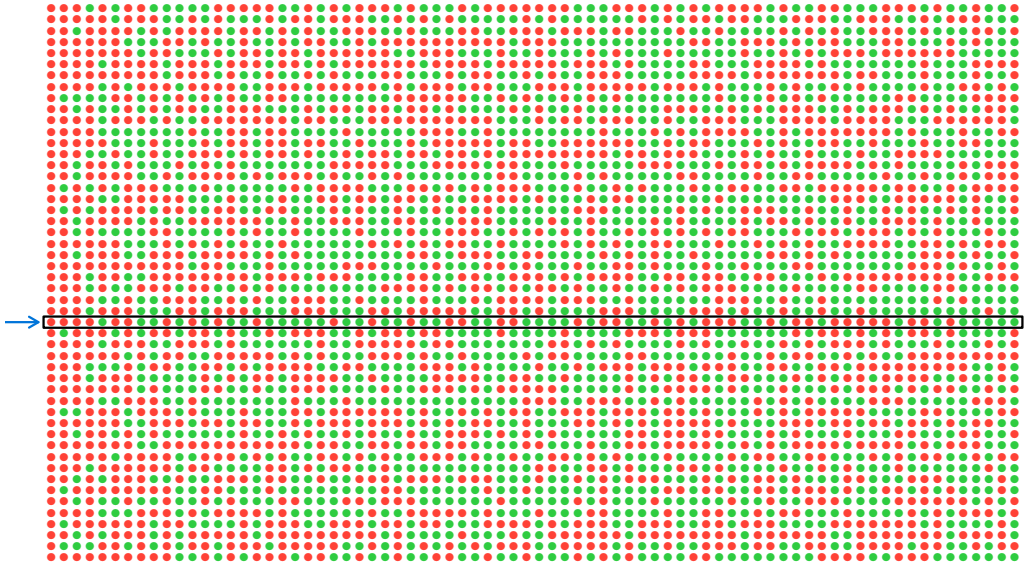


MCMC in action



The conditional probabilities

If $y_i > 0$, then $\Pr(\theta_i = 1 \mid \boldsymbol{y}, \boldsymbol{\theta}_{\text{-i}}^{(\text{s})}) = 1$

If $y_i = 0$,

$$\begin{aligned} \text{Let A} &= \textstyle\sum_{\text{j<i}} \theta_j^{(\text{s+1})} + \sum_{\text{j>i}} \theta_j^{(\text{s})} \\ \text{B} &= \textstyle\sum_{\text{j<i}} x_j\, \theta_j^{(\text{s+1})} + \sum_{\text{j>i}} x_j\, \theta_j^{(\text{s})} \end{aligned}$$

$$\begin{aligned} \text{Then } \Pr(\boldsymbol{\theta}_{\text{-i}}^{(\text{s})}, \theta_i = \text{k}) &= \tbinom{n}{\text{A+k}}/n \\ \Pr(\boldsymbol{y} \mid \boldsymbol{\theta}_{\text{-i}}^{(\text{s})}, \theta_i = \text{k}) &= (\text{B} + \text{k}\, x_i)^{\text{-n}} \end{aligned}$$

$$\begin{aligned} \text{And so } \Pr(\theta_i = 1 \mid \boldsymbol{y}, \boldsymbol{\theta}_{\text{-i}}^{(\text{s})}) &= \ldots \\ &= \frac{(1 + x_i/\text{B})^{\text{-n}}}{(1 + x_i/\text{B})^{\text{-n}} + (n - \text{A})/(\text{A} + 1)} \end{aligned}$$

Estimators

The Gibbs sampler produces $\boldsymbol{\theta}^{(0)}, \boldsymbol{\theta}^{(1)}, \ldots, \boldsymbol{\theta}^{(S)}$

We discard the first 200 or so samples ("burn-in").

Estimated number of non-essential genes: $\mathsf{E}(\theta_+ \mid \mathbf{y})$

$$\theta_+^{(s)} = \sum_i \theta_i^{(s)} \qquad \longrightarrow \qquad \hat{\theta}_+ = \frac{1}{S-200} \sum_{s=201}^{S} \theta_+^{(s)}$$

Probability that gene i is non-essential: $\mathsf{E}(\theta_i \mid \mathbf{y}) = \mathsf{Pr}(\theta_i = 1 \mid \mathbf{y})$

$$\hat{\theta}_i = \frac{1}{S-200} \sum_{s=201}^{S} \theta_i^{(s)}$$

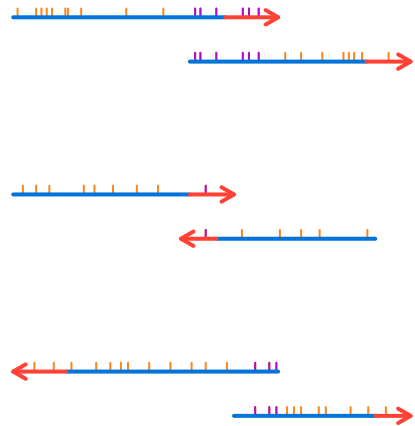
or Rao-Blackwellize:

$$\hat{\theta}_i^{\star} = \frac{1}{S-200} \sum_{s=201}^{S} \mathsf{Pr}(\theta_i = 1 \mid \mathbf{y}, \boldsymbol{\theta}_{-i}^{(s)})$$

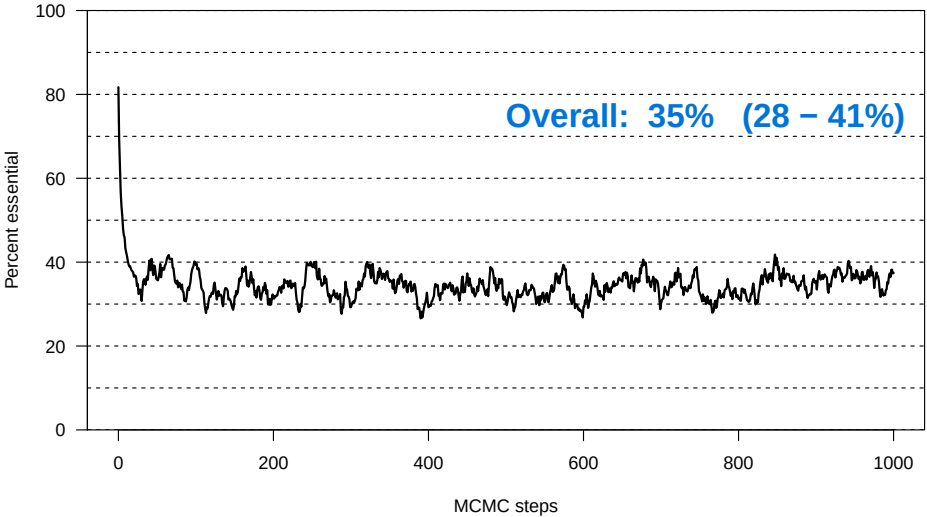
A further complication

Many genes overlap

- Of 4250 genes, 1005 pairs overlap (mostly by exactly 4 bp).
- The overlapping regions contain 547 insertion sites.
- Omit TA sites in overlapping regions, unless in the proximal portion of *both* genes.
- The algebra gets a bit more complicated.



Percent essential genes in M. tb.



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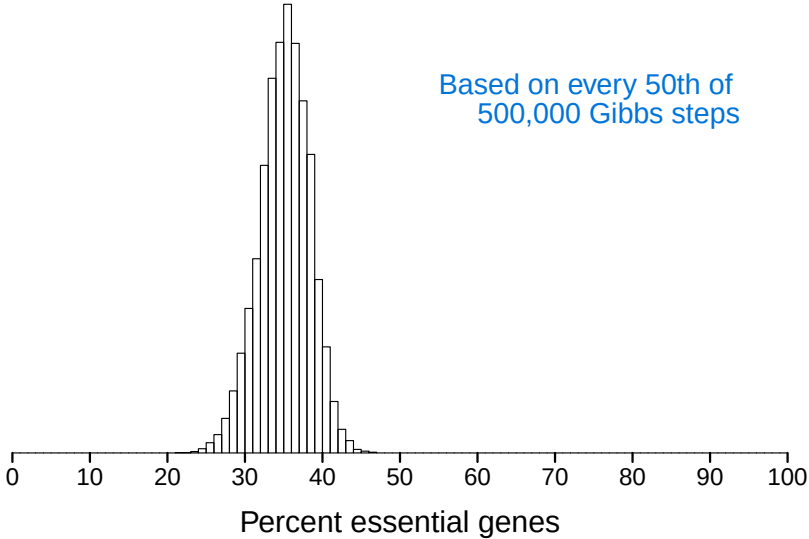
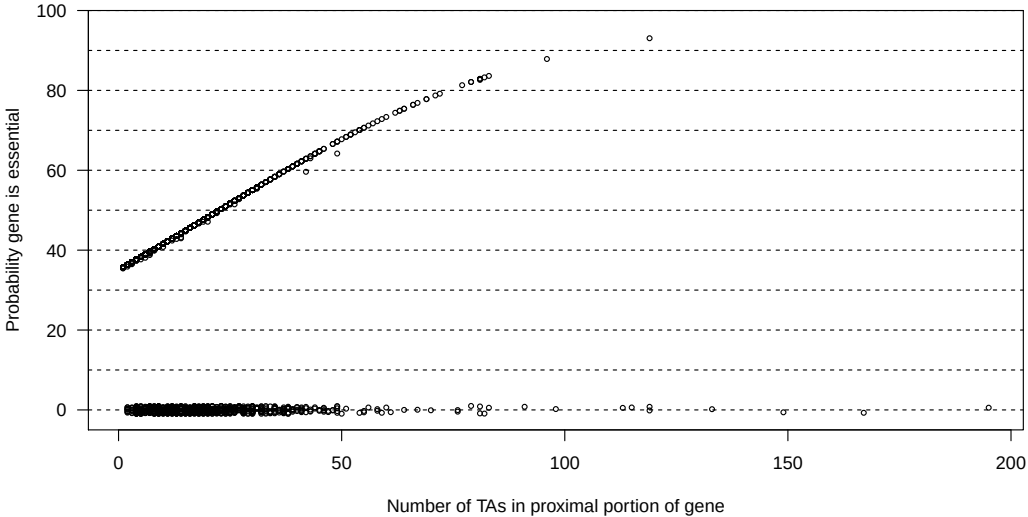


Probability each gene is essential



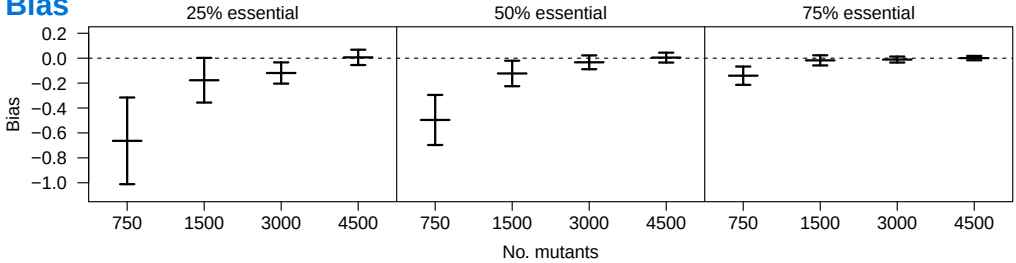
Yet another complication

Operon: A group of adjacent genes that are transcribed together as a single unit.

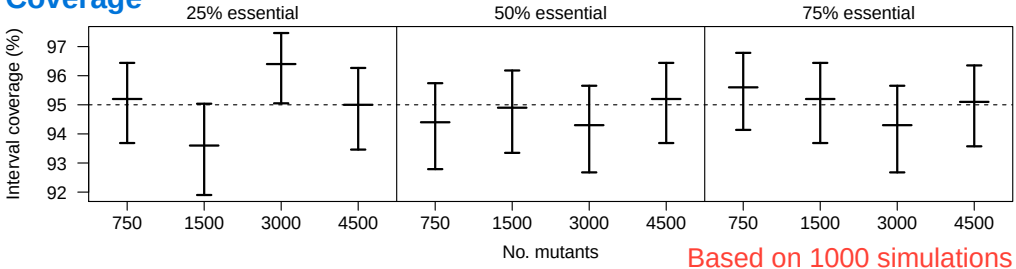
- Insertion at a TA site could disrupt all downstream genes
- If a gene is essential, insertion in any upstream gene would be non-viable
- Re-define the meaning of “essential gene”.
- If operons were known, one could get an improved estimate of the proportion of essential genes.
- If one ignores the presence of operons, estimates should still be unbiased.

Frequentist properties

Bias



Coverage



Based on 1000 simulations

Summary

- Bayesian method, using MCMC, to estimate the proportion of essential genes in a genome with data from random transposon mutagenesis.
- Crucial assumptions:
  - Randomness of transposon insertion.
  - Essentiality is an all-or-none quality.
  - No relationship between essentiality and no. insertion sites.
  - The 80% rule.
- For *M. tuberculosis*, with data on 1400 mutants:
  - 28 – 41% of genes are essential
  - 20 genes which have $\geq$ 64 TA sites and for which no mutant has been observed, have $>$ 75% chance of being essential.

References

- Lamichhane et al. (2003) Proc Natl Acad Sci USA 100:7213-7218 doi:10.1073/pnas.1231432100
- Blades and Broman (2002) Tech Report MS02-20 bit.ly/ms0220
- R/negenes package cran.r-project.org/package=negenes