Identifying essential genes in *M. tuberculosis* by random transposon mutagenesis

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Joint work with Natalie Blades, Gyanu Lamichhane, and William Bishai

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

Aim

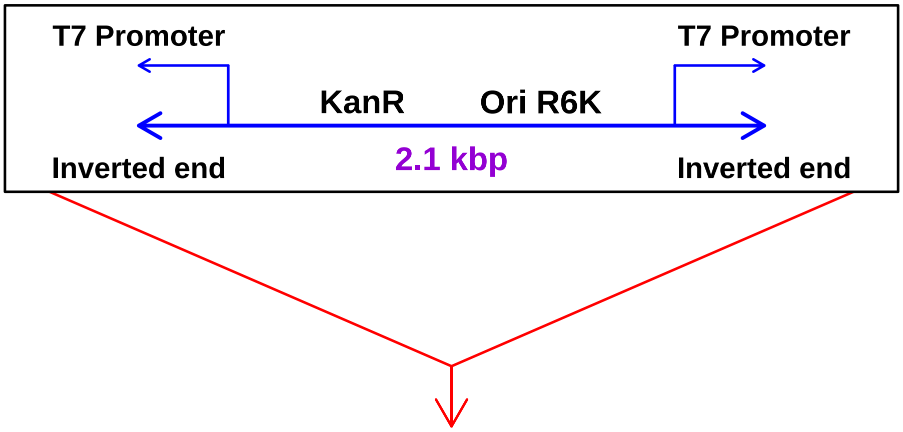
Identify the essential genes

(knock-out ⟹ non-viable mutant)

Method

Random transposon mutagenesis

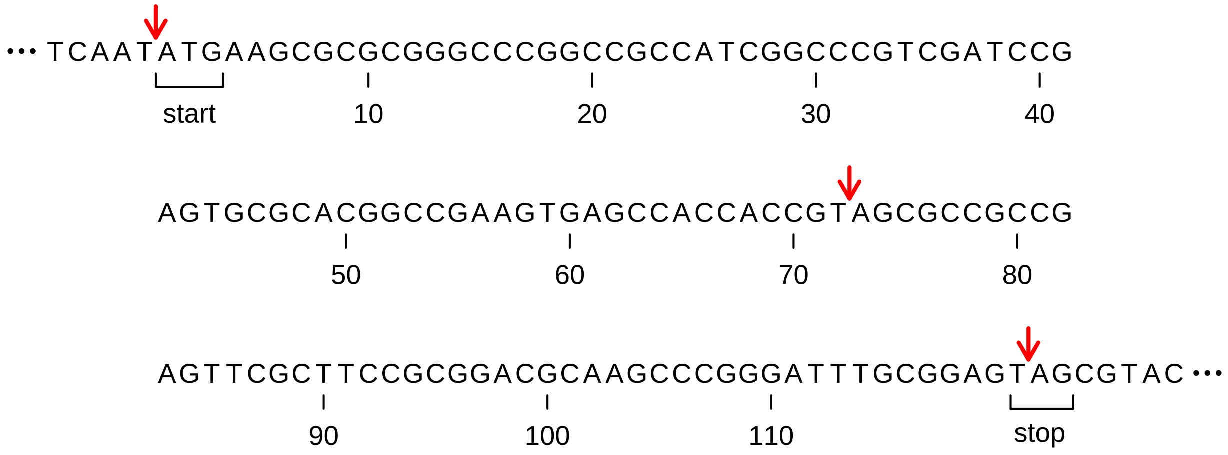
Himar1, a mariner-derived transposon



```
5′-TCGAAGCCTGCGACTAACGTTTAAAGTTTG-3′
3′-AGCTTCGGACGCTGATTGCAAATTTCAAAC-5′
```

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

Sequence of the gene MT598

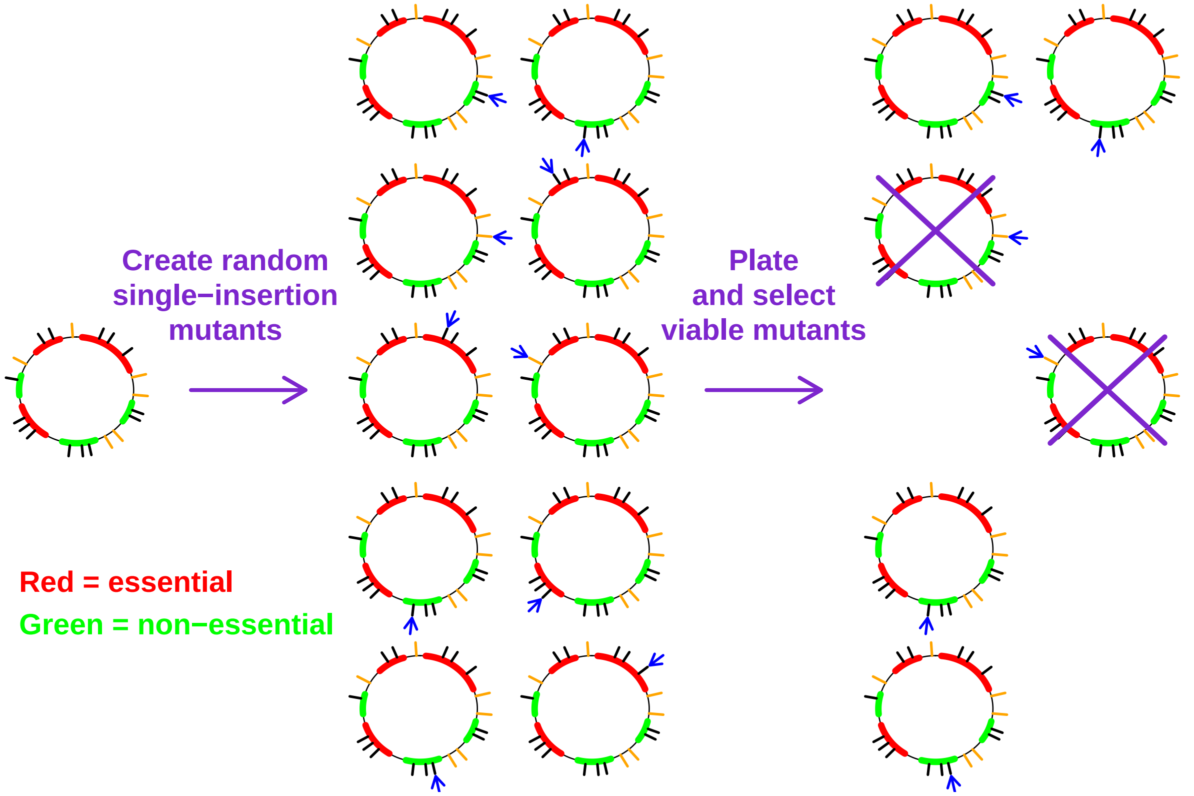


```
••• TCAATATGAAGCGCGCGGGCCCGGCCGCCATCGGCCCGTCGATCCG
start 10 20 30 40

AGTGCGCACGGCCGAAGTGAGCCACCACCGTAGCGCCGCCG
50 60 70 80

AGTTCGCTTCCGCGGACGCAAGCCCGGGATTTGCGGAGTAGCGTAC •••
90 100 110 stop
```

Random transposon mutagenesis



Create random single−insertion mutants

Plate and select viable mutants

Red = essential

Green = non−essential

Random transposon mutagenesis

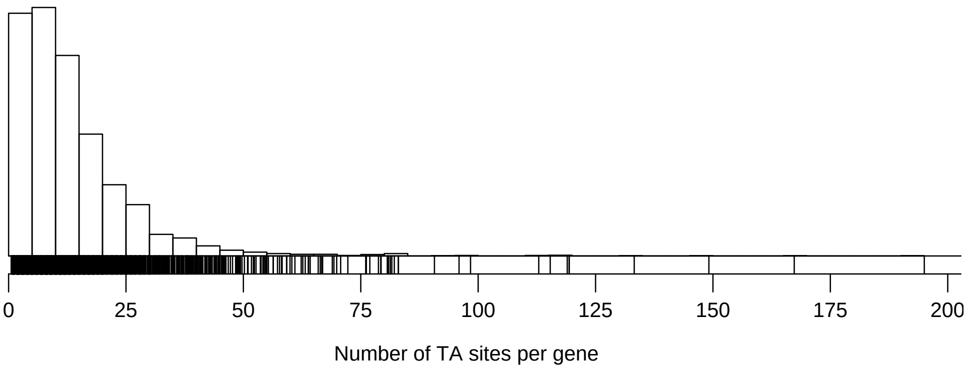
- Location of transposon insertion determined by sequencing across junctions
- Viable insertion within a gene $\Longrightarrow$ gene is non-essential
- Essential genes: we will never see a viable insertion
- Complication: Insertions in the very distal portion of an essential gene may not be sufficently disruptive.

  Thus, we omit from consideration insertion sites within the last 20% and last 100 bp of a gene.

The data

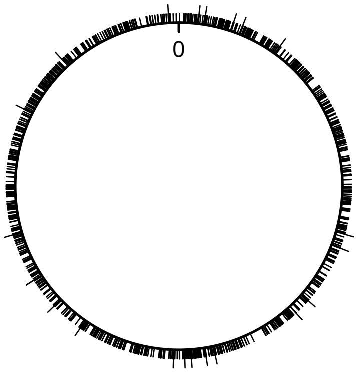
- Number, locations of genes.
- Number of insertion sites in each gene.
- n viable mutants with exactly one transposon insertion.
- Location of the transposon insertion in each mutant.

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

Questions:

- Proportion of essential genes in M. tb.?
- Which genes are likely essential?

Statistical method

Model: Transposon inserts completely at random

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

Prior:

- Number of ess'l genes $\sim$ Uniform$\{0, 1, \dots, 4204\}$
- Given no. ess'l genes, each possible subset is equally likely

Bayes by Markov chain Monte Carlo (MCMC):

Approximate calculation of

- Pr(gene i is essential $|$ data)
- Distribution of no. essential genes given the data

Data and model

N genes $\qquad$ x_i = no. TA sites in gene i

n mutants $\qquad$ y_i = no. mutants with insertion in gene i

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

Model: $\mathbf{y} \sim \text{multinomial}(n, \mathbf{p})$ 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

$$\mathrm{L}(\boldsymbol{\theta} \mid \mathbf{y}) = \binom{\mathrm{n}}{\mathbf{y}} \prod_{\mathrm{i}} (\mathrm{x_i}\, \theta_{\mathrm{i}})^{\mathrm{y_i}} \,/\, (\sum_{\mathrm{j}} \mathrm{x_j}\, \theta_{\mathrm{j}})^{\mathrm{n}}$$

$$\propto \begin{cases} (\sum_{\mathrm{i}} \mathrm{x_i}\, \theta_{\mathrm{i}})^{-\mathrm{n}} & \text{if } \theta_{\mathrm{i}} = 1 \text{ whenever } \mathrm{y_i} > 0 \\ 0 & \text{otherwise} \end{cases}$$

Notes:

- Depends only on which $\mathrm{y_i} > 0$, and not directly on the particular values of $\mathrm{y_i}$.
- MLE: $\hat{\theta}_{\mathrm{i}} = 1\{\mathrm{y_i} > 0\}$

The prior

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

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

Notes:

- We are assuming that Pr(θ_{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 $\mathrm{x_i}$ and the length of the gene.
- We could make use of information about the essential or non-essential status of particular genes (e.g., known viable knock-outs).

A Gibbs sampler

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

Gibbs sampler:

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

The conditional probabilities

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

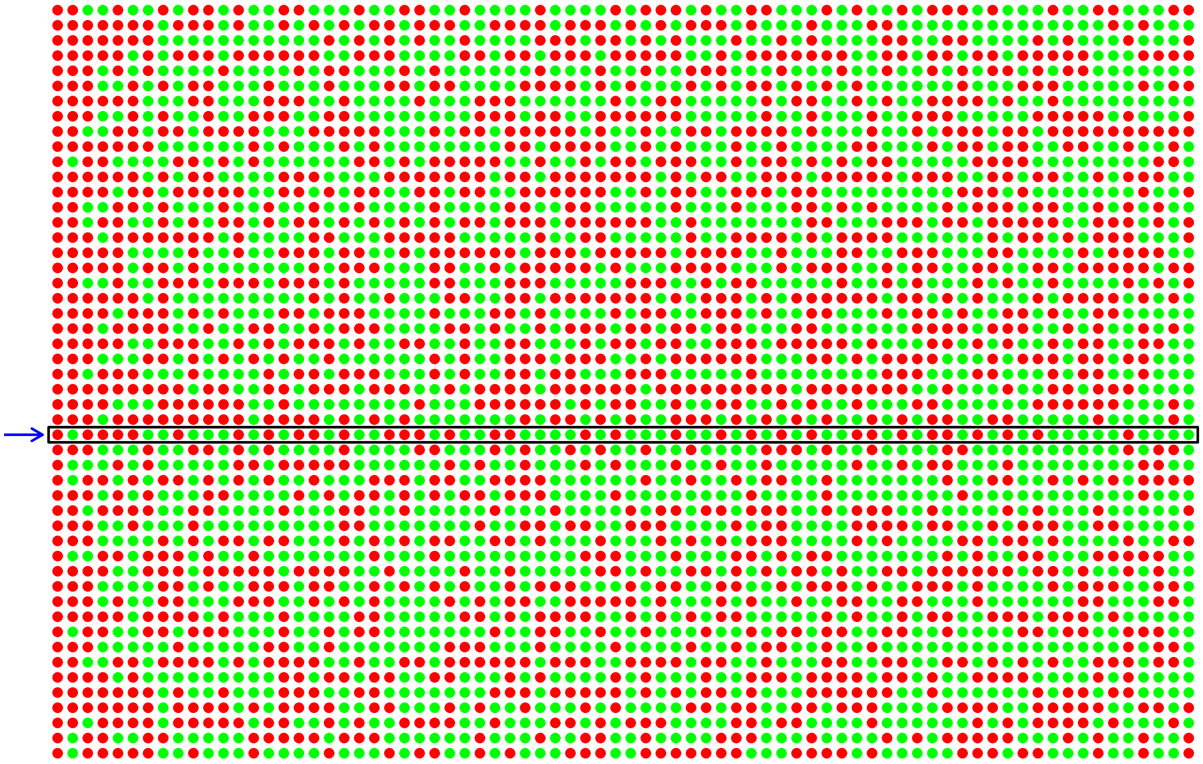
If y_i = 0,

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

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

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

MCMC in action



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: $\mathrm{E}(\theta_+ \mid \mathbf{y})$

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

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

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

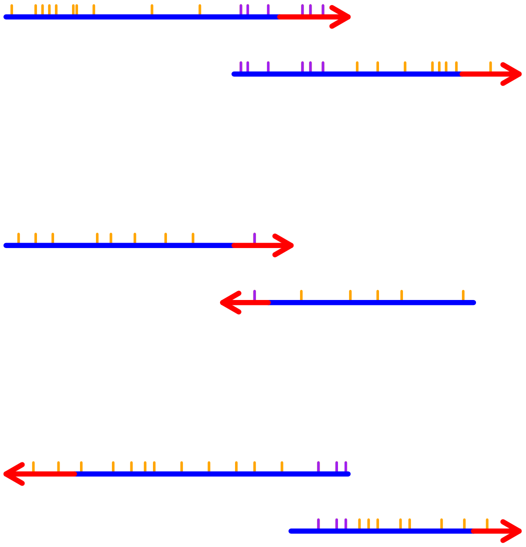
or Rao-Blackwellize:

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

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.

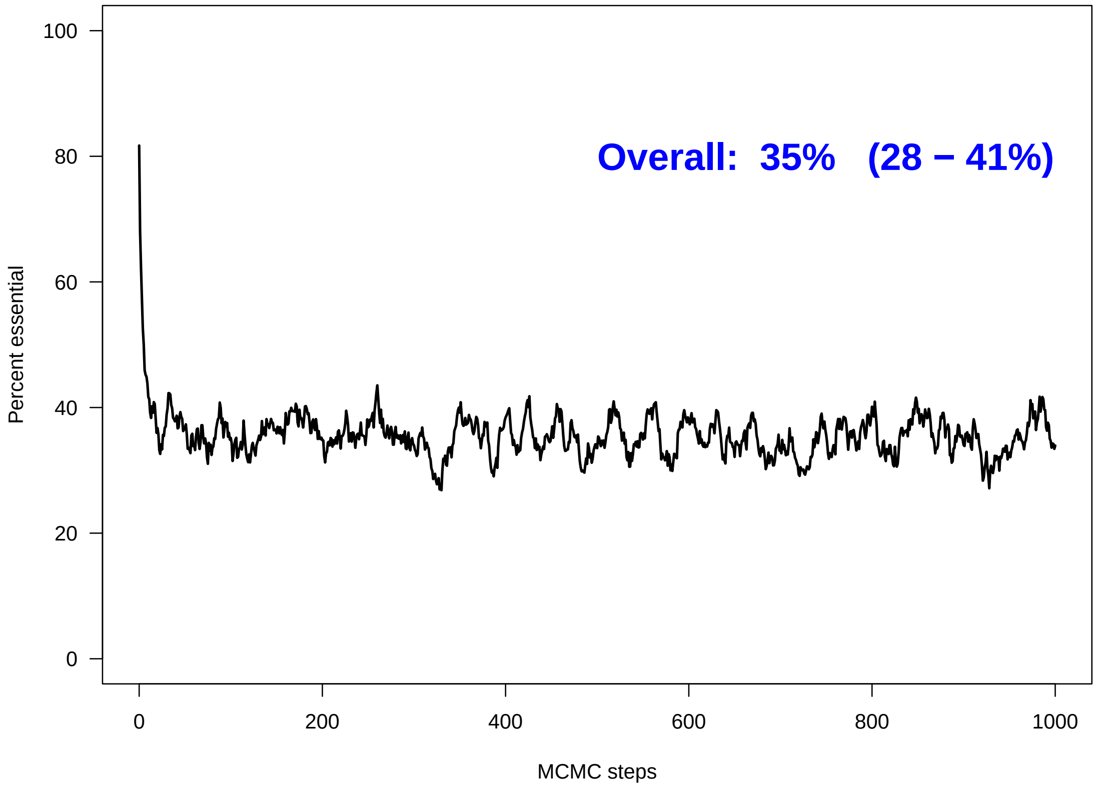


M. tb. mutagenesis data

- 74,403 TA sites total
- 57,934 sites within proximal portion of a gene
- 77 sites shared by two genes
- 4204/4250 genes with at least one such site

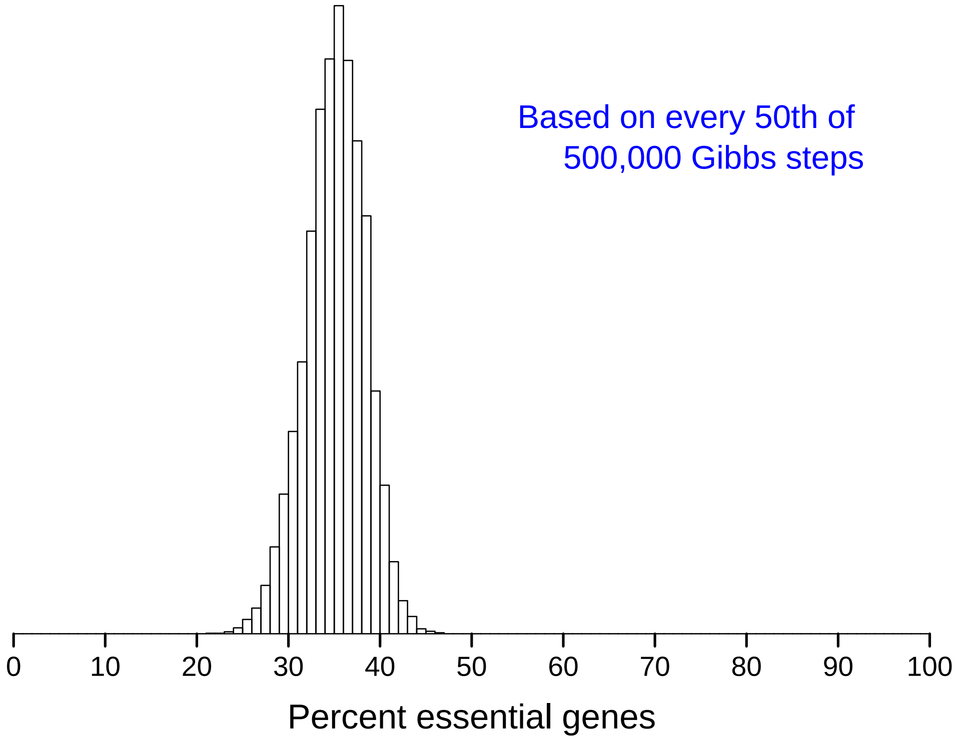
- 1425 insertion mutants
- 1025 within proximal portion of a gene
- 2 mutants for sites shared by two genes
- 770 unique genes hit

Percent essential genes in M. tb.



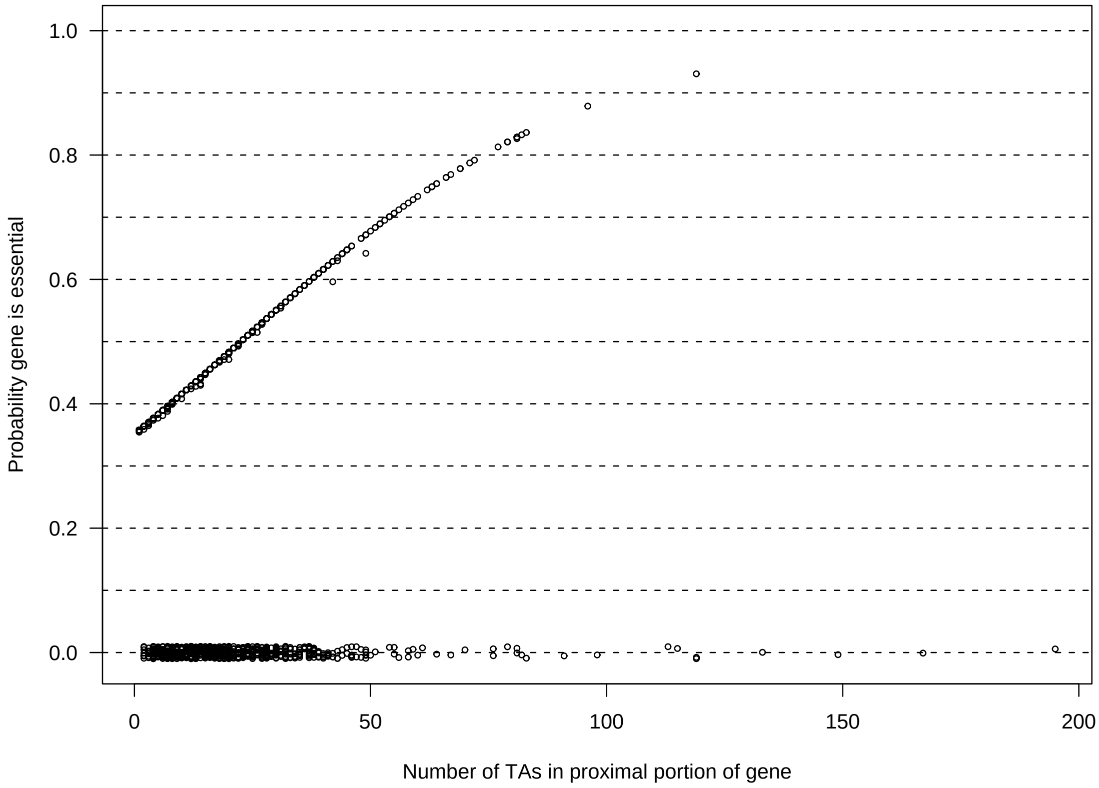
Overall: 35% (28 – 41%)

Percent essential genes in M. tb.



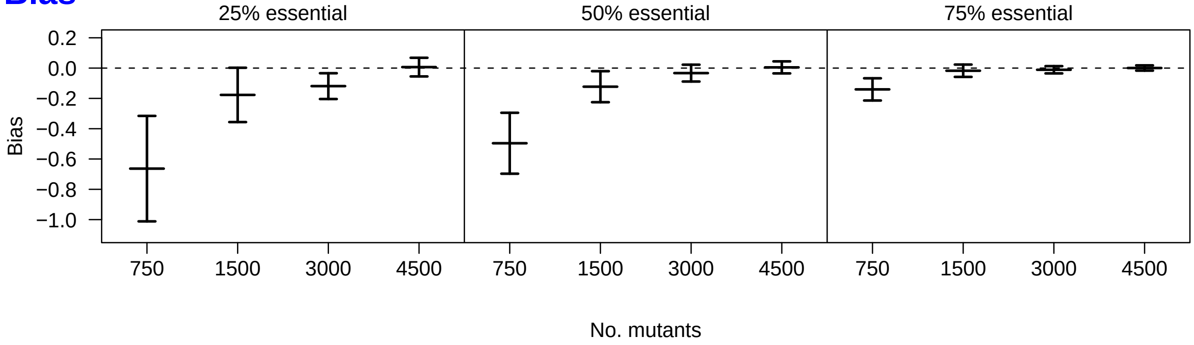
Based on every 50th of
500,000 Gibbs steps

Probability that each gene is essential

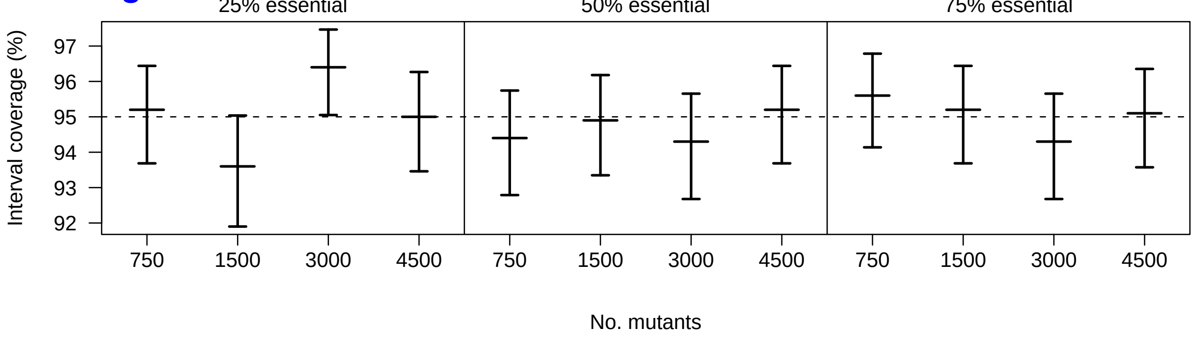


Frequentist properties of $\hat{\theta}_+$

Bias



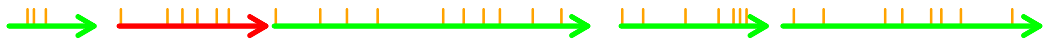
Coverage



Based on 1000 simulations

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.

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.

Acknowledgements

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(and many others)