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: $\sim$ \$15,000
  - Other bacterial pneumonias: $\sim$ \$35
- 4.4 Mbp circular genome, completely sequenced
- 4250 known or inferred genes

Aim

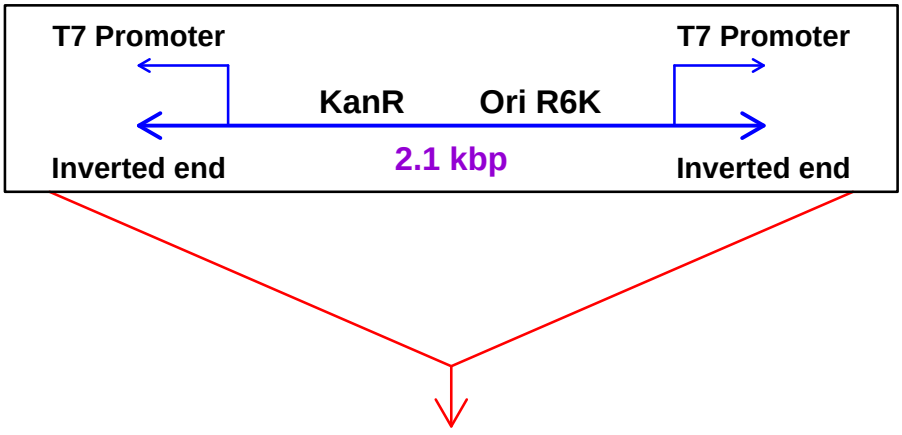
Identify the essential genes

(knock-out $\Longrightarrow$ non-viable mutant)

Method

Random transposon mutagenesis

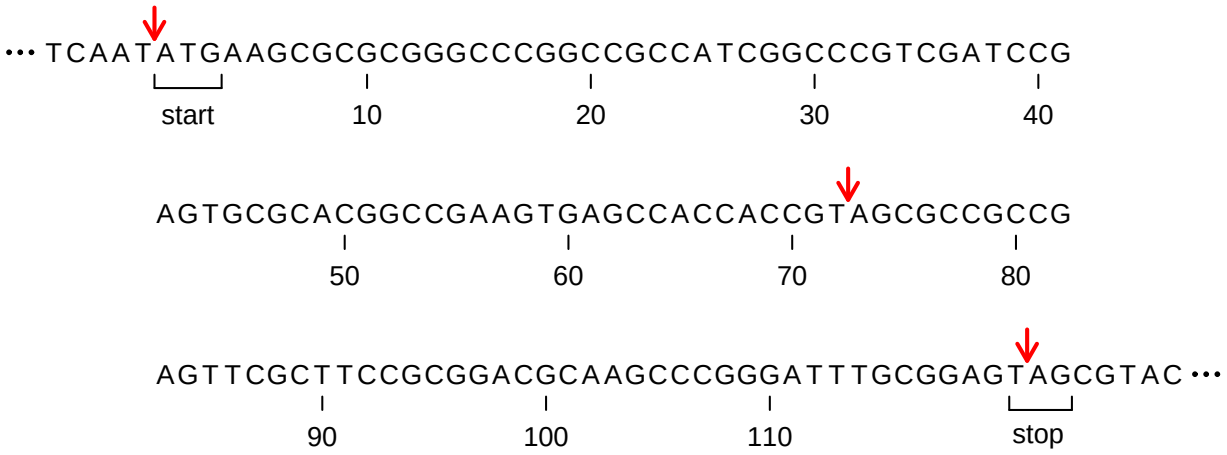
Himar1, a mariner-derived transposon



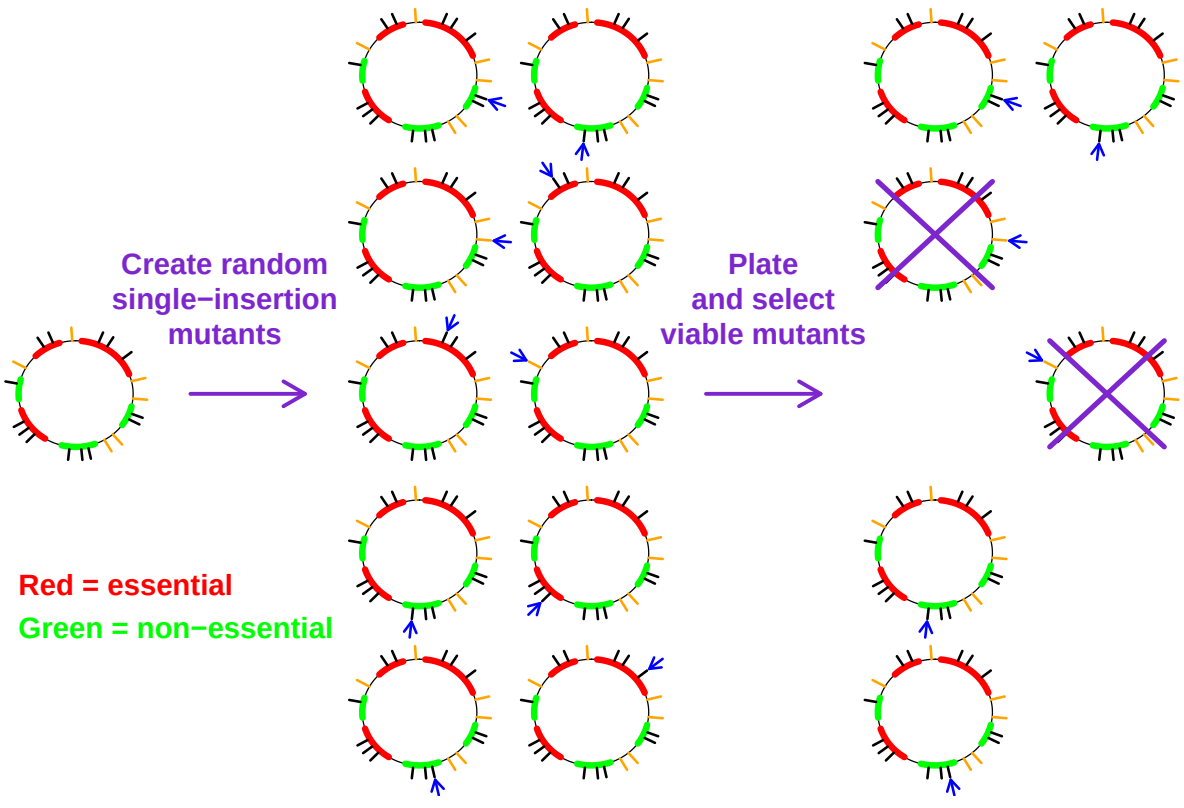
5′-TCGAAGCCTGCGAC**TA**ACGTT**TA**AAGTTTG-3′
3′-AGCTTCGGACGCTG**AT**TGCAA**AT**TTCAAAC-5′

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

Sequence of the gene MT598



Random transposon mutagenesis



Random transposon mutagenesis

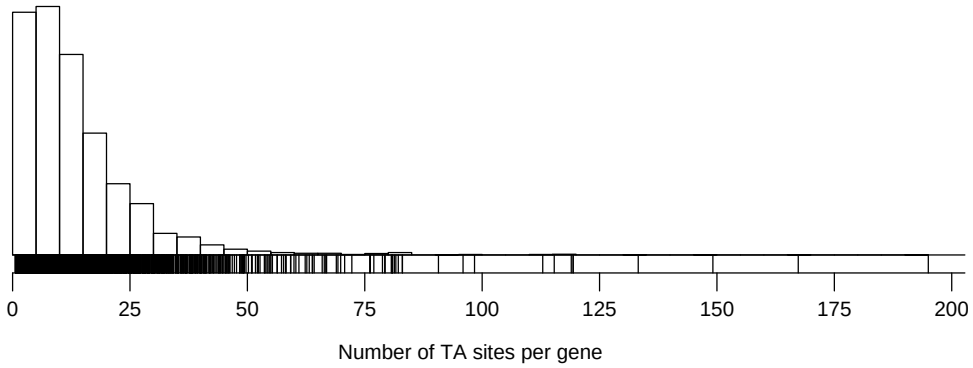
- Location of transposon insertion determined by sequencing across junctions
- Viable insertion within a gene $\Longrightarrow$ gene is non-essential
- Essential genes: we will never see a viable insertion
- Complication: Insertions in the very distal portion of an essential gene may not be sufficently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

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

The data

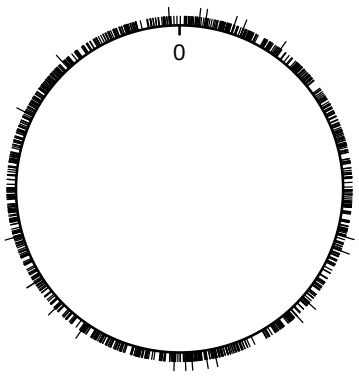
- Number, locations of genes.
- Number of insertion sites in each gene.
- n viable mutants with exactly one transposon insertion.
- Location of the transposon insertion in each mutant.

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?

(i.e., what would we see if we had 10^{100} mutants?)

Statistics, Part 1

- Find a probability model for the process giving rise to the data.
- Parameters in the model correspond to characteristics of the underlying process that we wish to determine.

The model

- Transposon inserts completely at random
  (Each TA site equally likely to be hit)
- Genes are either completely essential or completely non-essential.

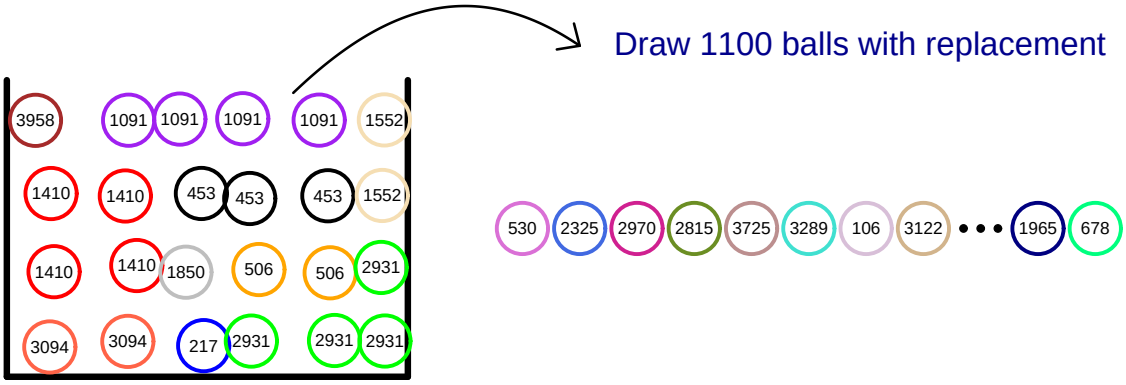
N genes x_i = no. TA sites in gene i

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

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

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

A picture of the model



Urn with balls labelled 1−4204

If essential: 0 balls

If non−essential: no. balls = no. TA sites

Part of the data

gene	no. TA sites	no. mutants
1	31	0
2	29	0
3	34	1
4	3	0
5	39	0
⋮	⋮	⋮
21	11	0
22	49	2
23	20	0
24	1	0
25	12	0
⋮	⋮	⋮
4204	4	0
total	**57934**	**1025**

A related problem

How many species of insects are there in the Amazon?

- Sample n insects at random.
- Classify according to species.
- How many total species exist?

My problem is a lot easier!

- Have a bound on the total number of classes.
- Know the relative proportions (up to a set of 0/1 factors).

Statistics, Part 2

Find an estimate of $\boldsymbol{\theta}$.

We're especially interested in $\theta_+ = \sum_i \theta_i$ and 1 – θ_+/N.

Frequentist approach

- View the parameters $\{\theta_i\}$ as fixed, unknown values.
- Find some estimate (function of the [random] data) that has good properties.
- Think about repeated realizations of the random process.

Bayesian approach

- View the parameters as random.
- Specify their joint prior distribution.
- Do a probability calculation.

The likelihood

$$L(\boldsymbol{\theta} \mid \mathbf{y}) = \Pr(\mathbf{y} \mid \boldsymbol{\theta})$$

$$= \binom{n}{\mathbf{y}} \prod_i (x_i\, \theta_i)^{y_i} \,/\, (\sum_j x_j\, \theta_j)^n$$

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

Note: Depends only on which $y_i > 0$, and not directly on the particular values of y_i.

Frequentist method

Maximum likelihood estimates (MLEs):

Estimate the θ_i by the values for which $L(\boldsymbol{\theta} \mid \mathbf{y})$ achieves its maximum.

$$L(\boldsymbol{\theta} \mid \mathbf{y}) \propto \begin{cases} (\sum_i x_i\, \theta_i)^{-n} & \text{if } \theta_i = 1 \text{ whenever } y_i > 0 \\ 0 & \text{otherwise} \end{cases}$$

And so the MLEs are

$$\hat{\theta}_i = \begin{cases} 1 & \text{if } y_i > 0 \\ 0 & \text{if } y_i = 0 \end{cases}$$

Further, $\hat{\theta}_+ = \sum_i 1\{y_i > 0\}$.

This is a rather stupid estimate!

Bayes: The prior

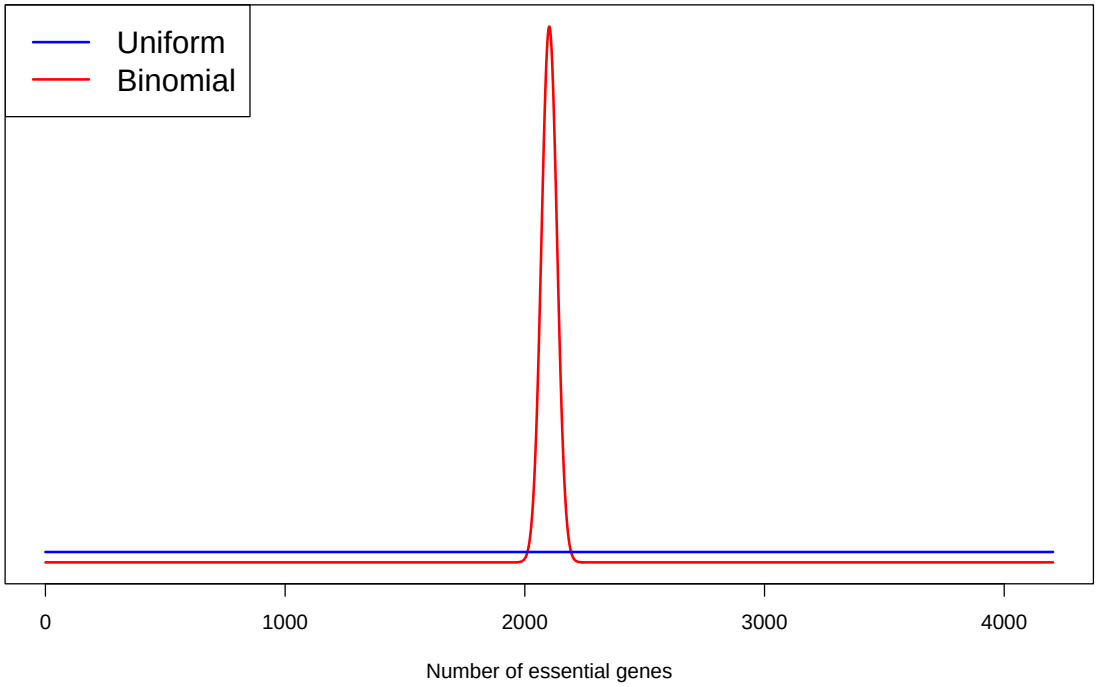
$\theta_+ \sim$ uniform on $\{0, 1, \ldots, n\}$

$\boldsymbol{\theta} \mid \theta_+ \sim$ 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 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).

Uniform vs. Binomial



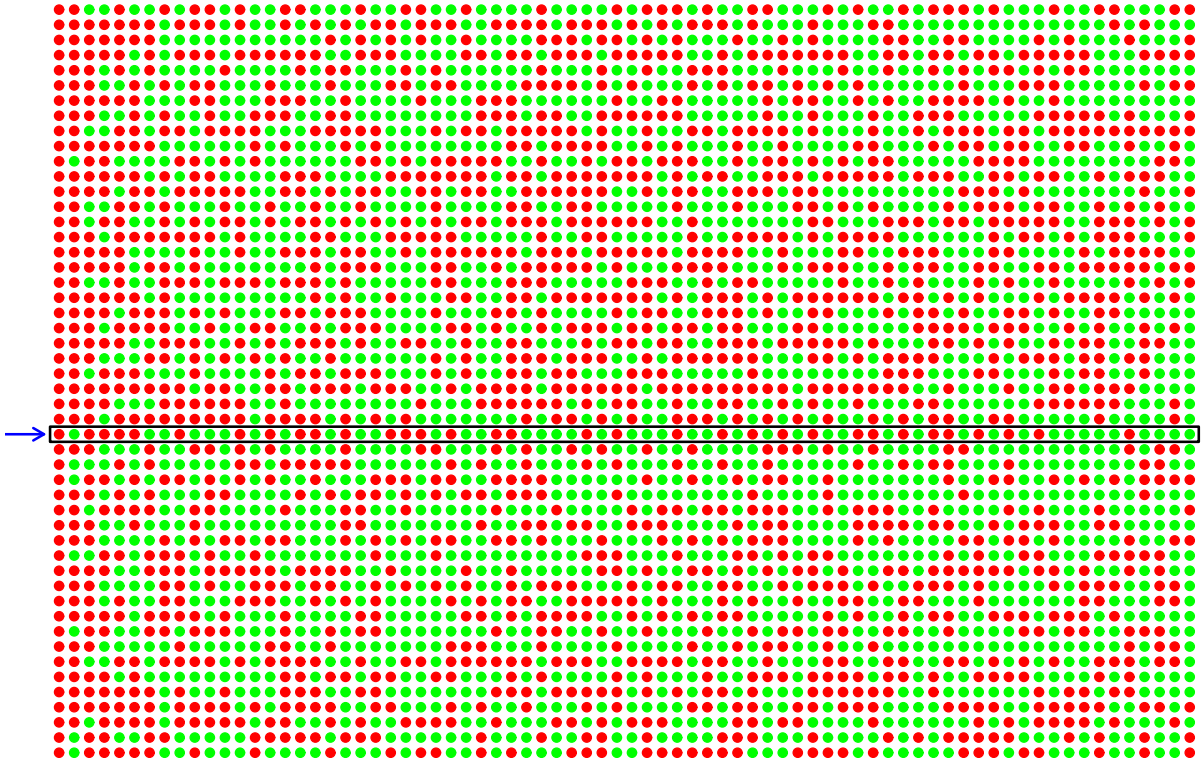
A Gibbs sampler

Goal: Estimate $\Pr(\boldsymbol{\theta}|\mathbf{y}) = \Pr(\mathbf{y} \mid \boldsymbol{\theta}) \Pr(\boldsymbol{\theta}) / \sum_{\boldsymbol{\theta}} \Pr(\mathbf{y} \mid \boldsymbol{\theta}) \Pr(\boldsymbol{\theta})$

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 $\boldsymbol{\theta}_{-i}^{(s)} = (\theta_1^{(s+1)}, \ldots, \theta_{i-1}^{(s+1)}, \theta_{i+1}^{(s)}, \ldots, \theta_n^{(s)})$.
  - 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.

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

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

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

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

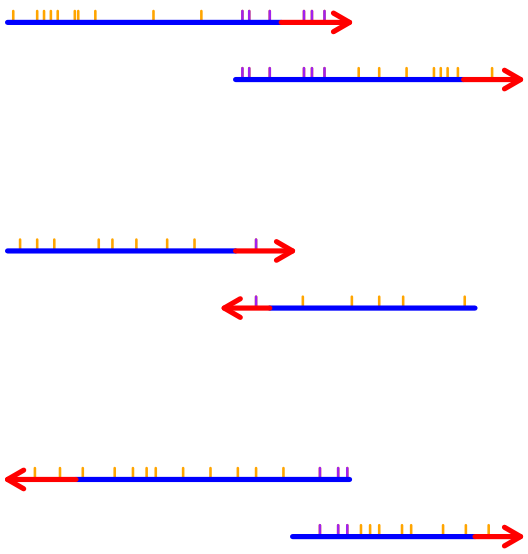
or Rao-Blackwellize:

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

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.

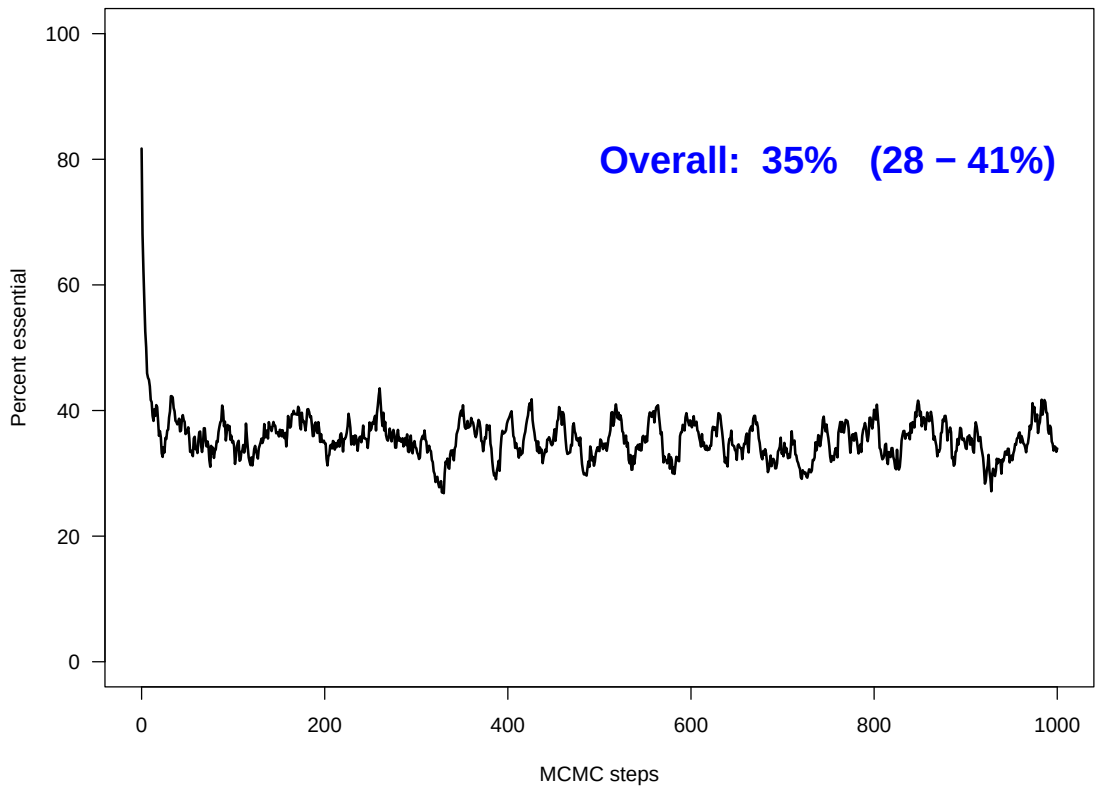


M. tb. mutagenesis data

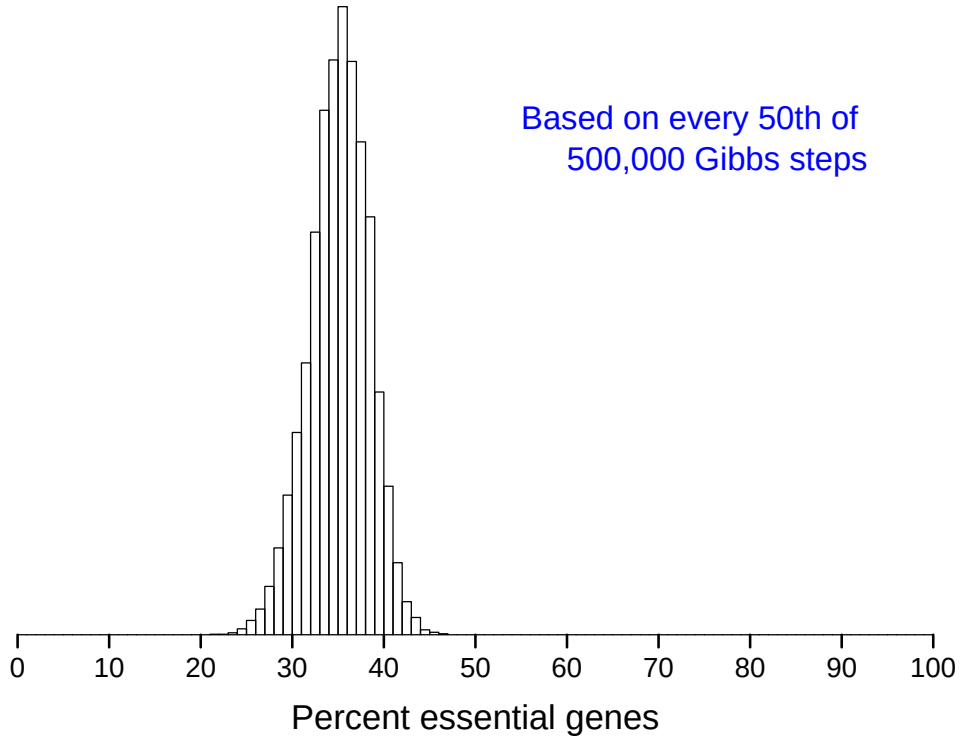
- 74,403 TA sites total
- 57,934 sites within proximal portion of a gene
- 77 sites shared by two genes
- 4204/4250 genes with at least one such site

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

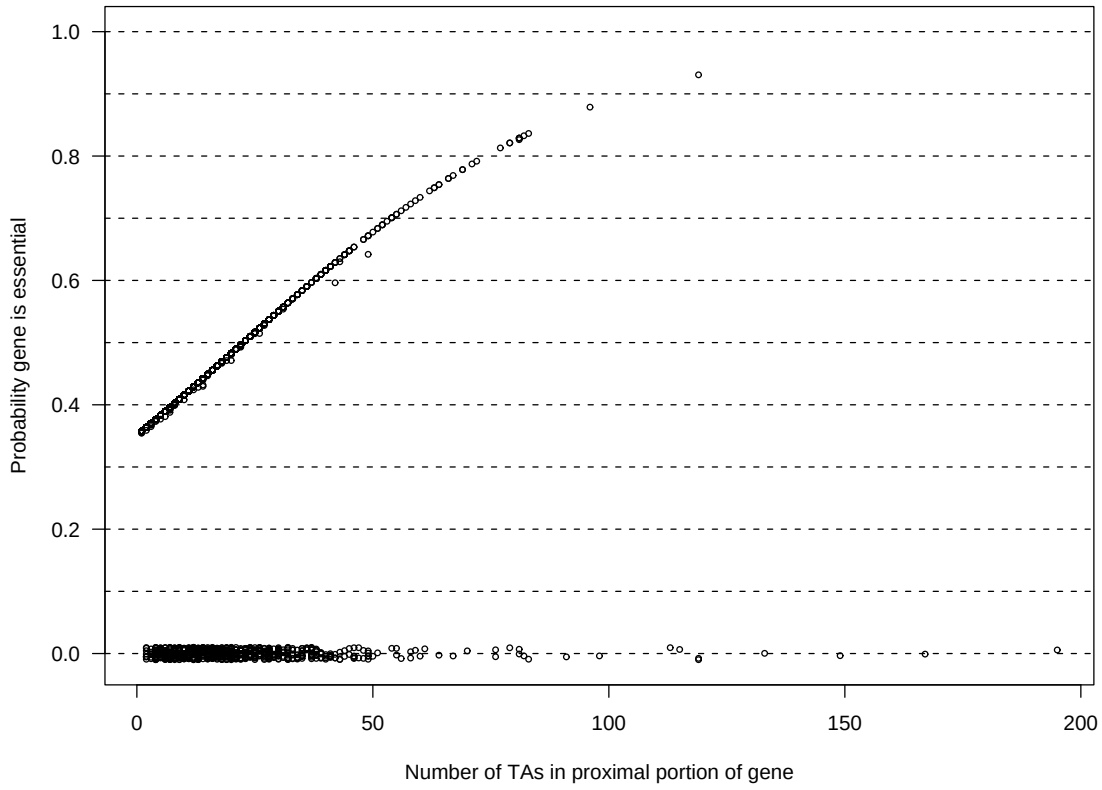
Percent essential genes in M. tb.



Percent essential genes in M. tb.



Probability that 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.

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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