Identification of the essential genes in the *M. tuberculosis* genome by random transposon mutagenesis

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

Typical drug regimens

Tuberculosis

- INH 15g
- RIF 37g
- PZA 141g
- ETB 151g

- ~60 DOT visits

- Cost: > $15,000

Other bacterial pneumonias

- Azithromycin 1.5g

- Self-supervised

- Cost: $35

Mycobacterium tuberculosis genome

- 4.4 Mbp circular genome, completely sequenced
- 4250 known or inferred genes
- 44% of genome has no match to mammals or other bacteria
- $>$250 lipid biosynthesis genes (E. Coli: $\sim$50)
- Mycolic acids: unique, essential
- Cell division time: 24 hr

Bacterial gene products

Essential genes

- Cell division
- DNA replication
- Transcription
- Protein synthesis
- Cell wall formation

Non-essential genes

- Virulence
- Stress response
- DNA modification
- Mobile elements
- Small molecular biosynthesis
- Regulatory genes

Aim

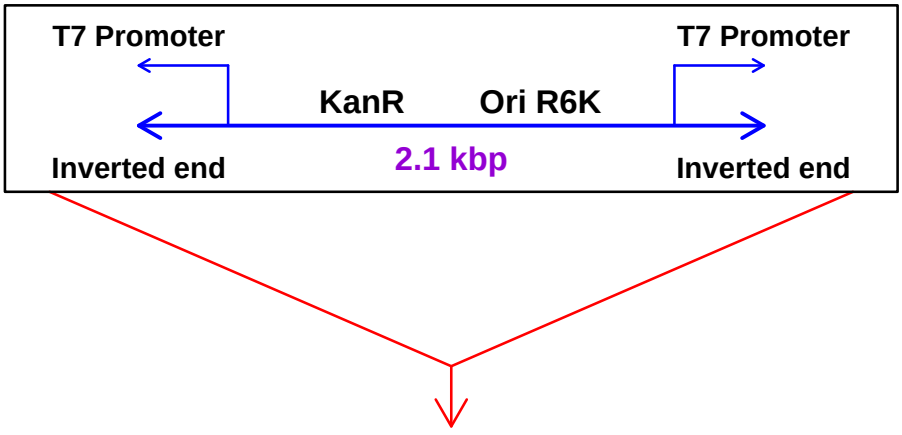
Identify the essential genes

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

Method

Random transposon mutagenesis

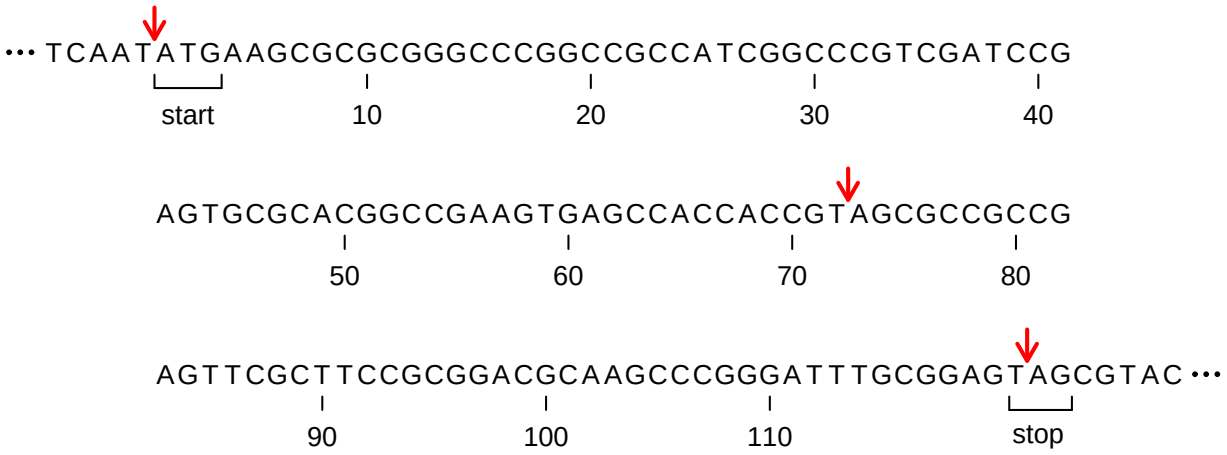
Himar1, a mariner-derived transposon



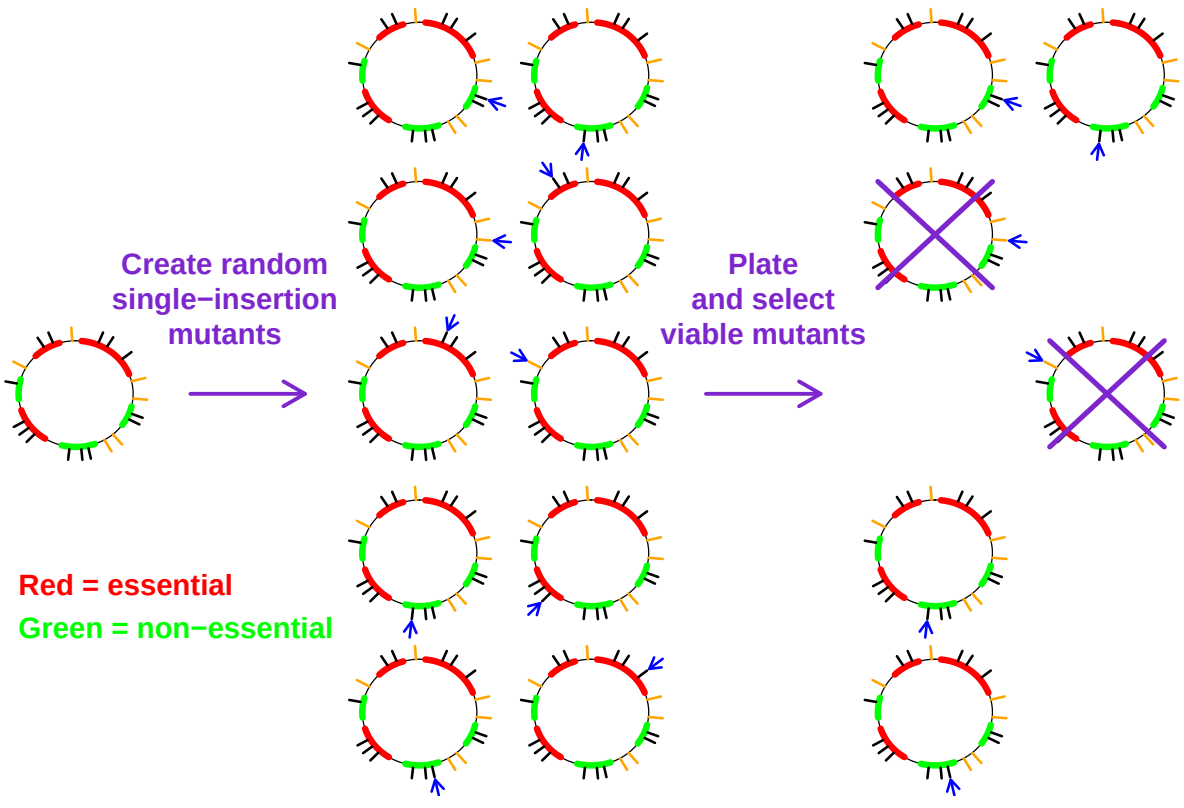
5′-TCGAAGCCTGCGAC**TA**ACGTT**TA**AAGTTTG-3′
3′-AGCTTCGGACGCTG**AT**TGCAA**AT**TTCAAAC-5′

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

Sequence of the gene MT598



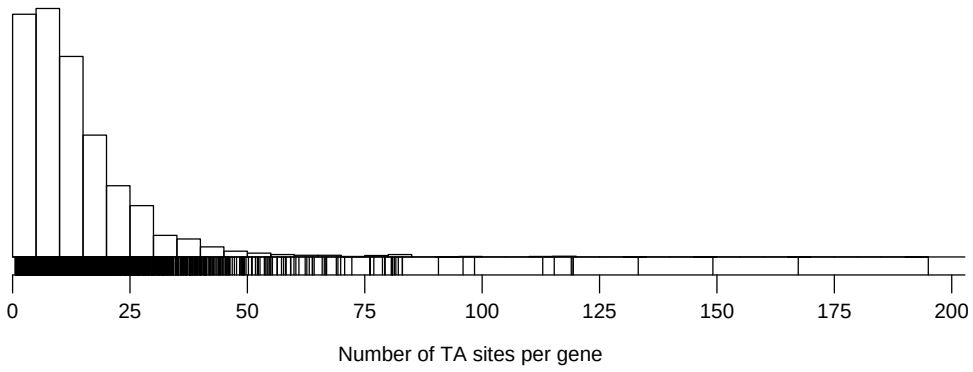
Random transposon mutagenesis



Random transposon mutagenesis

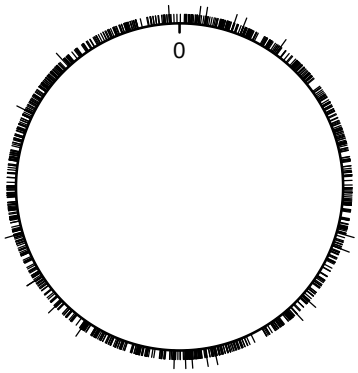
- Location of transposon insertion determined by sequencing across junctions
- Viable insertion within a gene $\Longrightarrow$ gene is non-essential
- Essential genes: we will never see a viable insertion
- Note: We only consider insertion sites within proximal 80% or n–100 basepairs of a gene

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$\{0, 1, \ldots, 4204\}$
- Given no. ess'l genes, each possible subset is equally likely

Bayes with Markov chain Monte Carlo (MCMC):

Approximate calculation of

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

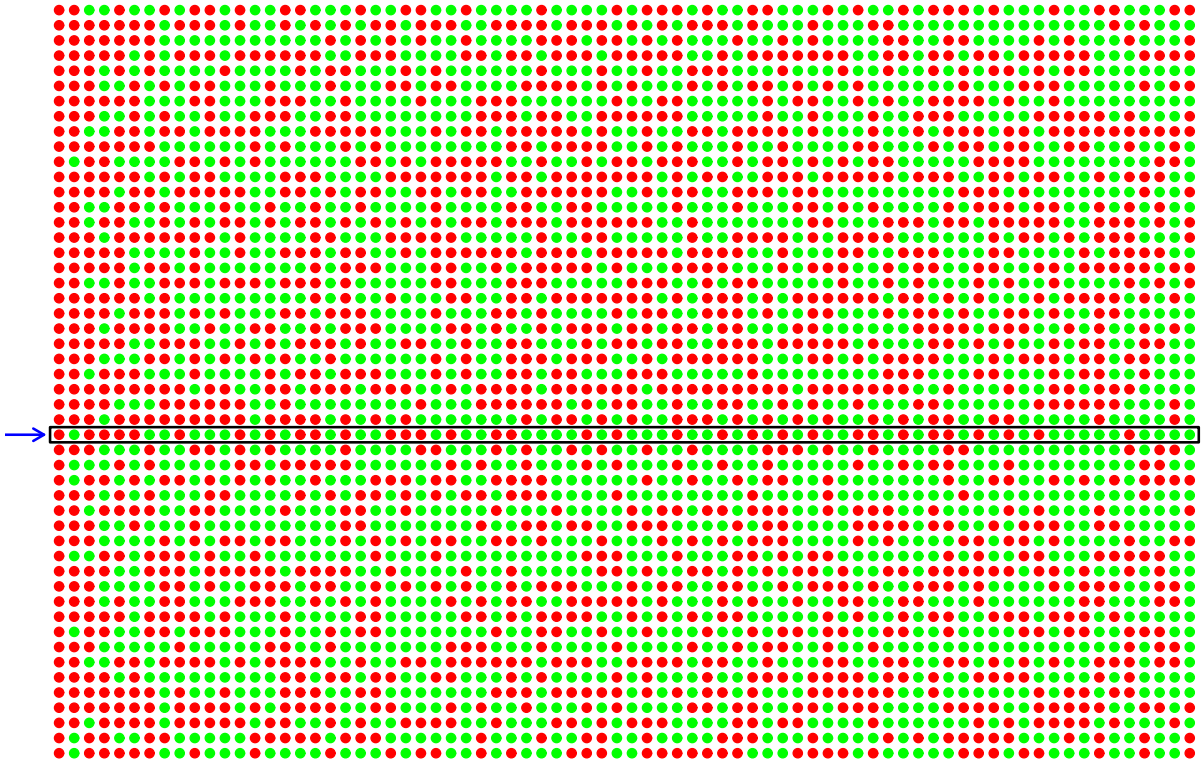
MCMC algorithm

- Begin with initial assignment of essential status of each gene
- Consider each gene, one at a time
  - Calculate

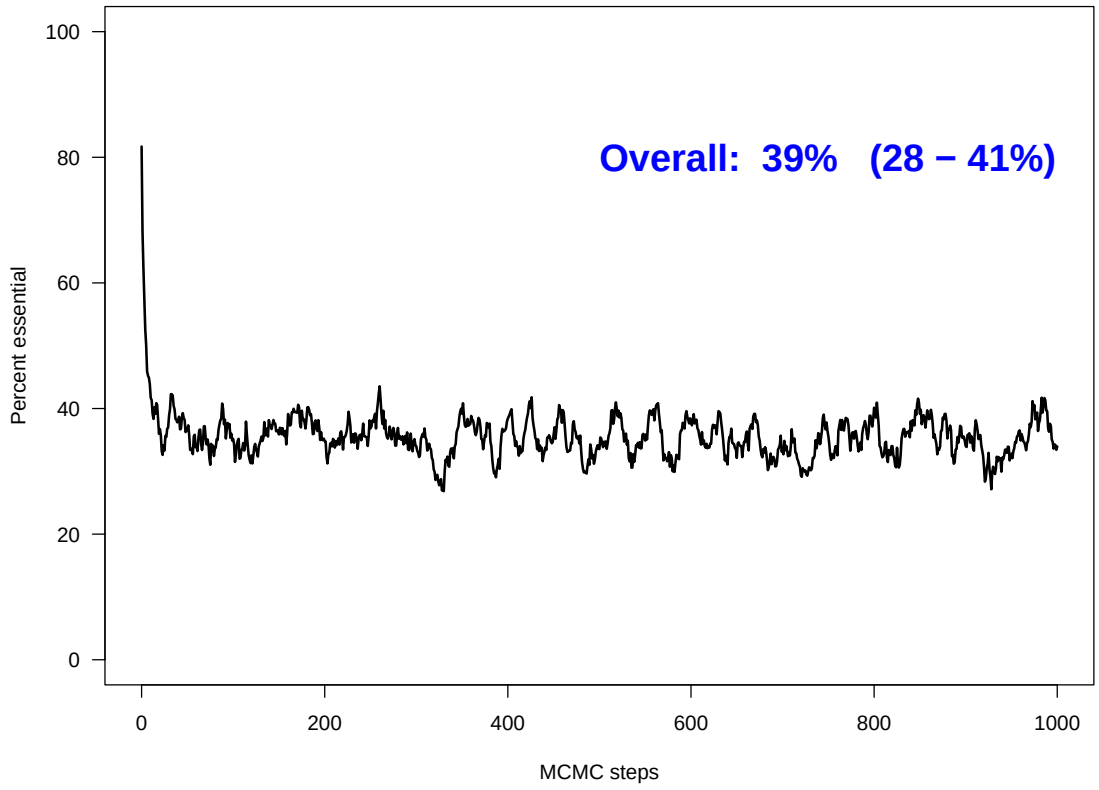
    $\Pr(\text{gene is ess'l} \mid \text{data, status of other genes})$ ⟵ Depends on:

    - No. mutants
    - No. TA sites in gene
    - Total no. viable TA sites
    - No. ess'l genes
  - Randomly assign it to be essential or non-ess'l according to this probability
- Repeat many times
- Summarize results

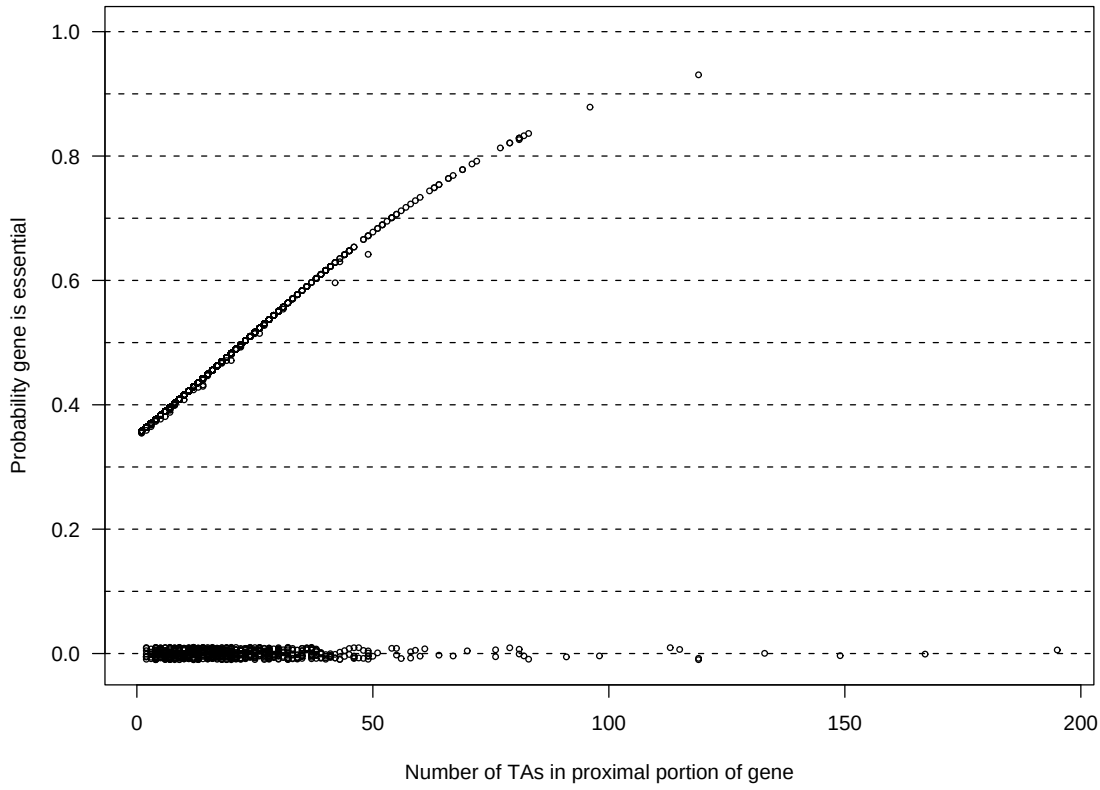
MCMC in action



Percent essential genes in M. tb.



Probability that each gene is essential



Potentially dicey bits

- Insertion sites in regions of gene overlap
- Operons
- The 80% rule
- Relationship between essentiality and number of insertion sites
- Randomness of transposon insertion

Acknowledgements

Bill Bishai

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