Linking genotype to phenotype in *Streptococcus pneumoniae*: Genome-wide approaches for profiling genetic interactions

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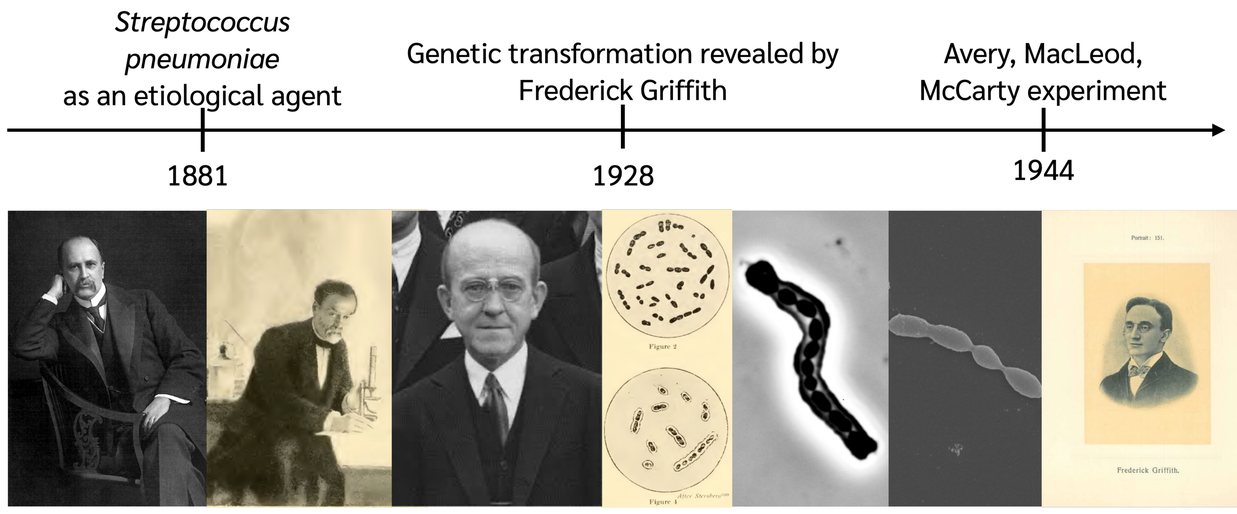
Abstract

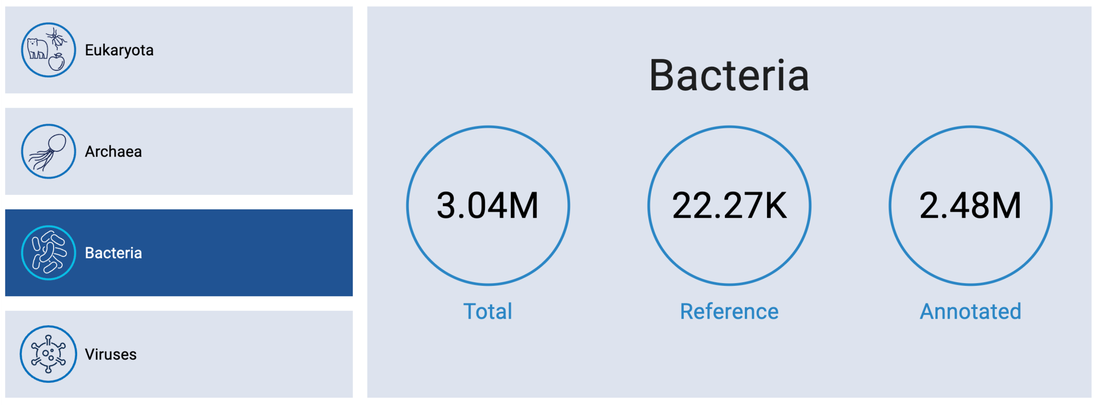
Next-generation DNA sequencing (NGS) has significantly increased the volume of sequencing data available in public databases. However, millions of these sequences remain as genetic "dark matter." For example, about one-third of bacterial genes in the model organism *Escherichia coli* lack known functions, partly due to challenges like gene redundancy. Transposon sequencing (Tn-seq) is a widely used, unbiased approach for studying gene function. This technique tracks changes in the abundance of randomly inserted, gene-inactivating transposons within a saturated mutant pool, helping to identify genes essential for survival under various conditions. However, traditional Tn-seq is not designed to map genetic interaction networks involving more than a few genes. My laboratory recently developed dual Tn-seq and Sup-seq to uncover negative and positive genetic interactions, respectively. These approaches expedite functional genomics, open new research opportunities, and accelerate drug discovery.

Background and the unmet medical needs

Streptococcus pneumoniae

- Kills more than **0.5 million people** worldwide annually.
- A leading cause of lower respiratory infections.
- More than 100 serotypes have been identified. **Licensed vaccines cover at most 23 types**.
- Naturally competent. AMR is on the rise.





Positive genetic interaction

Epistasis / Synthetic viable / suppression

Substrate → **Enzyme A** → **TOXIC** → **Enzyme B (essential)** → Non-essential product

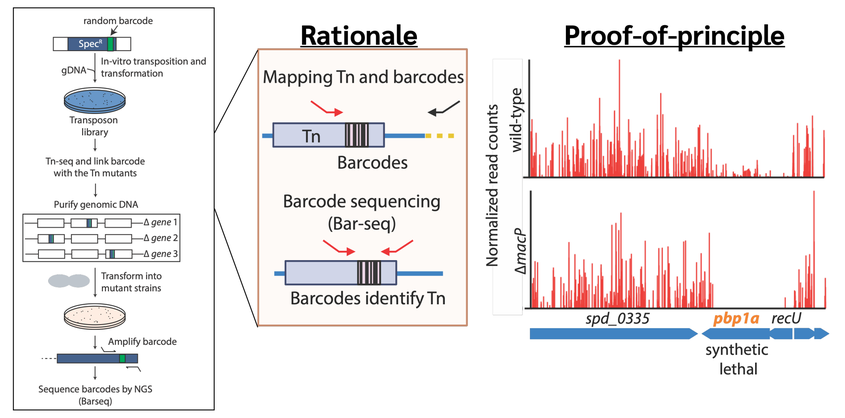
Negative genetic interaction

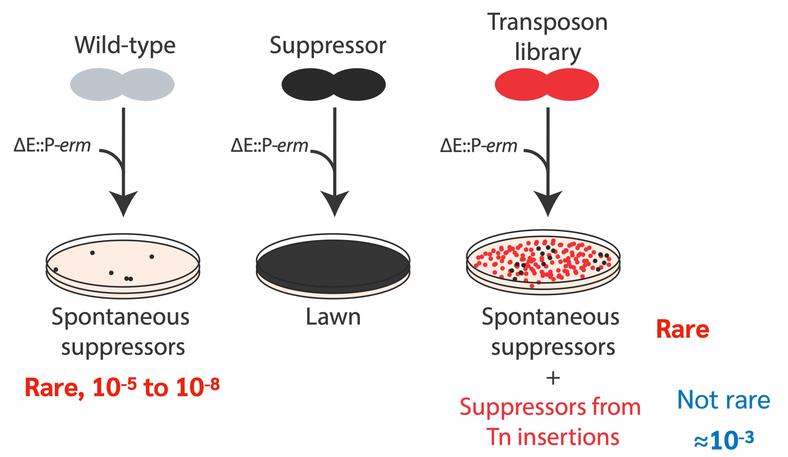
Synthetic lethal / sickness

Substrate → **Enzyme A** + **Enzyme B** → **Product required for survival**

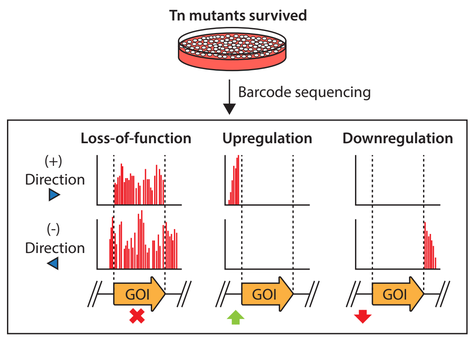
Profiling genetic interactions with Sup-seq

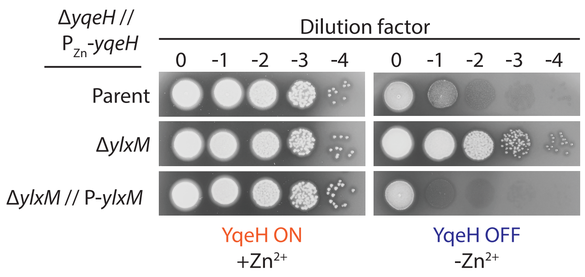
- Complement our recently published **dual Tn-seq** approach (*Science* 2025; PMID: 40997176).
- Randomly barcoded transposon sequencing (RB Tn-seq) enables rapid mapping of transposon mutants.
- Spontaneous suppressor mutants are **rare**. But a specific transposon mutant in a library that disrupts a specific gene is not.
- Transposon insertions that suppress an essential gene function are enriched.
- The process can be iterated, and the Tn mutants are sequenced.

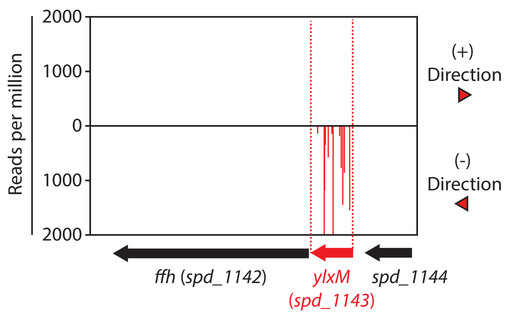


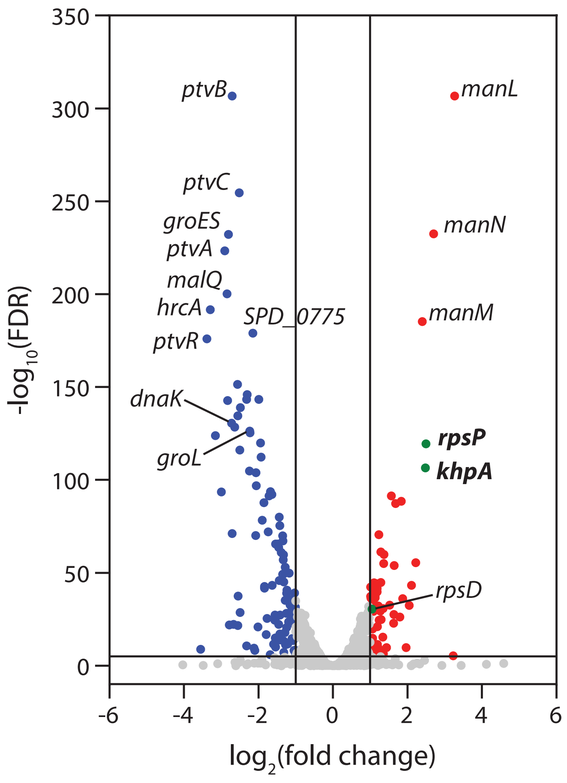


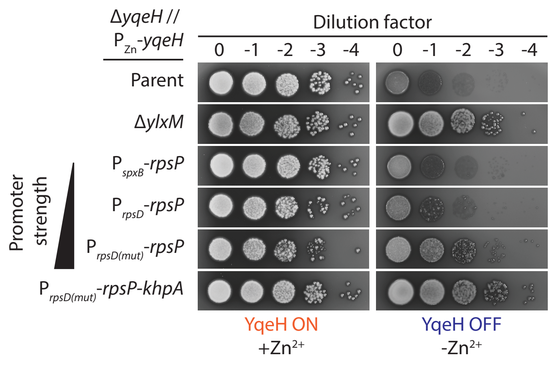
YqeH is suppressed by the loss of YlxM







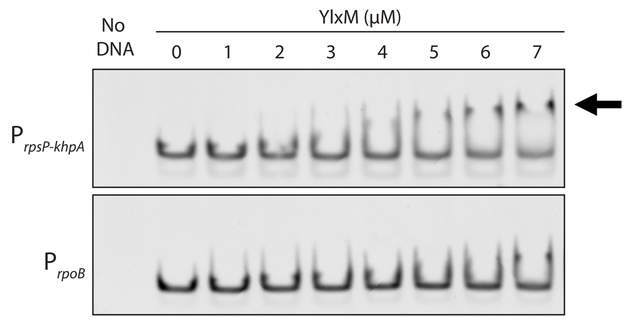


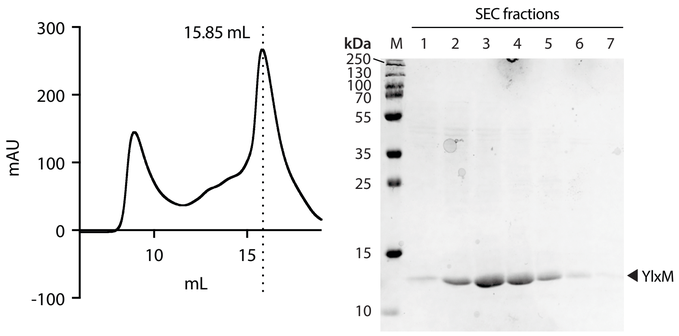


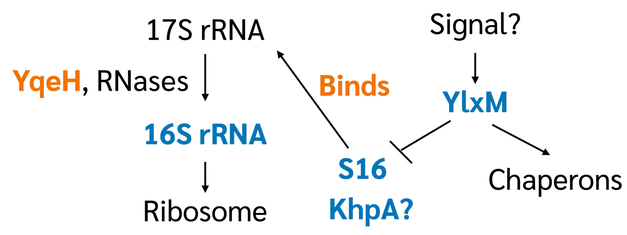
- YqeH is a widely conserved circular permutated GTPase required for **ribosome assembly**.
- Deletion of a small DNA-binding protein, **YlxM**, bypassed YqeH essentiality.
- RNA-sequencing experiments showed that ***rpsP-khpA*** is overexpressed in the Δ*ylxM* mutant, whereas **chaperon genes** are downregulated.
- **Overexpression of *rpsP* is sufficient** to alleviate the phenotype resulting from *yqeH* depletion
- Suppression is enhanced by khpA overexpression.

YlxM is a transcription factor that control translation

- YlxM was purified by Ni-NTA chromatography and size-exclusion chromatography.
- **Purified YlxM binds to the *rpsP-khpA* promoter**, suggesting a direct transcriptional interaction.
- YlxM likely regulates protein folding.







Conclusions

- Identifying genetic interactions is a facile way to understand gene function.
- **Sup-seq is an unbiased approach** that enables genome-wide screens for genetic suppression events.
- As a proof of concept, we screened **7 essential query genes and identified 53 genetic interactions**.
- Functional genomics remains central for uncovering drug and vaccine targets.

7 essential query genes screened

58 candidate suppressors detected

53 confirmed by reconstruction

Acknowledgements

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