BACTERIAL GENOMICS

An ‘i’ for ingenuity

The rapidly dividing bacterium *Vibrio natriegens* holds promise for transforming traditional molecular biology and biotechnology processes. New work demonstrates that CRISPR interference technology is a robust tool for rapid, genome-wide screens in *V. natriegens*, facilitating future bioengineering efforts.

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For decades, *Escherichia coli* has reigned as the de facto workhorse of molecular biology due to its robust, rapid growth and genetic tractability. Recently, a new contender for this role has emerged, quite literally from the depths of the sea. First isolated from a salt marsh, and a cousin of *Vibrio cholerae* (the infectious agent of the global scourge cholera), *Vibrio natriegens* has captured the spotlight with its claim-to-fame being the fastest growing bacterium, with a generation time of <10 min under optimized conditions[1] (Fig. 1a). However, insights into the basis for its prodigious proliferation, and further development as a tool for molecular biology and biotechnology, have been stymied by the lack of a system for high-throughput functional genomics. In this issue of *Nature Microbiology*, Lee et al.[2] perform a comprehensive genomic characterization of this halophile and develop a new genetic screening platform powered by the broad-range clustered regularly interspaced short palindromic repeat interference (CRISPRi) system to begin to unravel the secrets of this bacterium’s fast-paced life.

Dissection of the genetic base of rapid growth has been a long-standing goal for microbiologists. Early efforts to address this issue were very laborious, requiring systematic generation of single deletion strains for every gene. Until recently, the Keio collection in *E. coli* represented the only example of such a monumental effort[3]. The advent of next-generation sequencing coupled with computational statistics approaches enabled harnessing of a traditional genetic mutation technique, transposon mutagenesis, to rapidly identify genes required for growth. Such transposon insertion sequencing (Tn-seq) has been applied broadly, deepening our understanding of growth requirements in diverse bacteria[4]. The field of synthetic biology has drawn on such data and on new genome sequences for genome-scale bioengineering projects, generating reduced genome strains with improved biotechnological properties[5] and new organisms with minimal genomes[6]. However, Tn-seq is unfeasible in certain species or strains, including some more exotic bacteria that have recently garnered increased interest, such as those identified in metagenomic surveys.

Against this backdrop, Lee et al. establish a workflow for functional genomics that sets a new gold standard for comprehensive analyses of exotic prokaryotes. They begin by optimizing in vitro growth media for *V. natriegens* and construct the first closed genome, which consists of two chromosomes, a defining feature of vibrios. Lee et al. also obtained both genomic methylation data and the positions of the origin and terminus of replication for both chromosomes. The latter, determined computationally by exploiting the stereotyped variation in sequencing coverage across the genome of an actively growing bacterium, is a valuable annotation that is often missing in genome publications.

Motivated to engineer the bacterium into a more suitable tool for biotechnology,

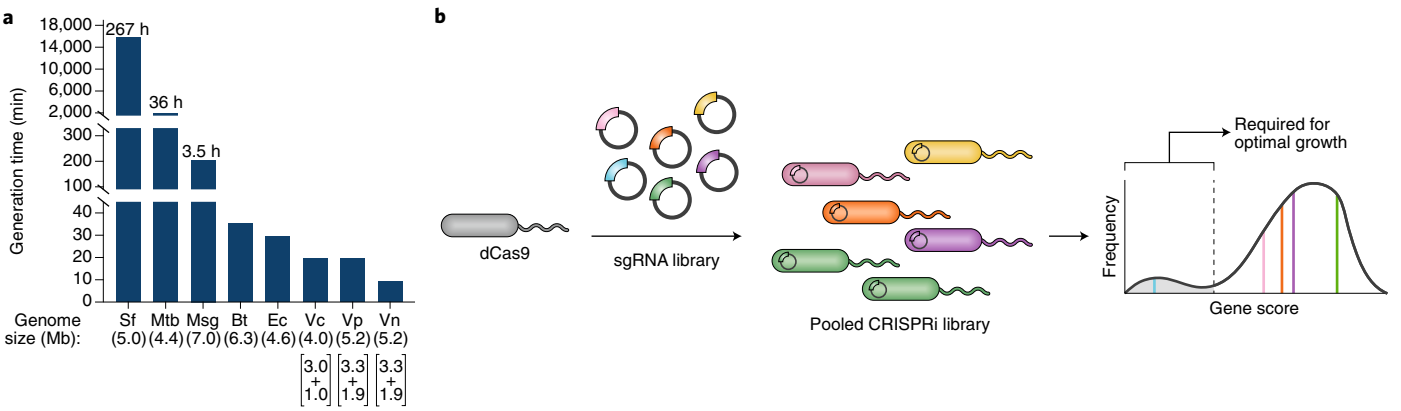

Fig. 1 | *V. natriegens* growth, genome and genome-wide screen. a, Range of in vitro generation times across various bacterial species. Genome size included in parenthesis; chromosome size included in brackets refer to chromosome 1 + chromosome 2. Genome sizes represent that of the reference genome for the species in the NCBI Genome database. Sf, *Syntrophobacter fumaroxidans*[12]; Mtb, *Mycobacterium tuberculosis*[13]; Msg, *Mycobacterium smegmatis*[13]; Bt, *Bacteroides thetaiotaomicron*[14]; Ec, *E. coli*[2]; Vc: *V. cholerae*; Vp, *Vibrio parahaemolyticus*; Vn, *V. natriegens*[1]. **b**, Prokaryotic genome-wide CRISPRi screen described by Lee et al.[2]. Gene score refers to an aggregate measure of the relative abundance of all sgRNAs targeting a gene; frequency refers to the relative number of genes with a given score.

the authors turned to genome-wide methodologies to investigate the genetic requirements for *V. natriegens* growth. Unsuccessful attempts at Tn-seq prompted them to explore the potential of CRISPRi, in which a catalytically inactive version of the Cas9 enzyme (dCas9) is utilized to repress transcription of a targeted gene. Like Cas9, dCas9 is directed to a targeted locus via a single guide RNA (sgRNA). The original dCas9 developed for bacteria[7], though not always the most efficient dCas9 variant[8], functioned well in *V. natriegens*[2].

Lee et al. then designed multiple guide RNAs for all the protein coding genes in the genome, and introduced these sgRNAs into *V. natriegens* with and without dCas9 to generate two pooled genome-wide CRISPRi libraries. Comparison of sgRNA frequencies between these libraries after a few passages, using the library without dCas9 as a control in lieu of non-targeting sgRNAs, identified the genes required for growth in vitro (Fig. 1b). Approximately 600 genes were required for optimal growth; half were likely essential, and half were non-essential but growth-supporting genes, such as those involved in aerobic respiration pathways. The screens also uncovered differences in the relative importance of genes with similar annotations in core metabolic pathways. This gold mine of a dataset provides a rich resource for deeper analysis of specific genes promoting rapid growth plus many starting points for generating enhanced *V. natriegens* strains for biotechnology via genome reduction, for example, by removal of non-essential metabolic pathways and redundant metabolic enzymes. Furthermore, the straightforward screening approach demonstrates the real-world applicability of CRISPRi, as the experiment is the first CRISPRi genome-wide screen performed in a non-model organism, and the second in any bacterium (after previous efforts in *E. coli*)[9].

As a new tool for conducting genome-wide screens in bacteria, CRISPRi is not without its limitations; a key disadvantage compared to Tn-seq is the polar effect of transcriptional repression on genes in multicistronic operons located downstream of the targeted gene, which can lead to an overestimation of genes required for growth. Furthermore, the efficiency of repression[7], dCas9 toxicity[2] and targeting specificity of sgRNAs can vary substantially and potentially lead to off-target effects[9].

Future CRISPRi iterations may rectify these limitations, for example, through improved sgRNA design[9] or dCas9 variants[10]. Furthermore, its inducible and reversible nature permits complex experimental designs that query temporal and/or spatial dimensions, which are largely inaccessible with current Tn-seq approaches. CRISPRi also lends itself to the construction of arrayed or targeted libraries. Finally, as early landmark papers demonstrated[11], CRISPRi is well suited to probe the function of essential genes.

Genome-wide CRISPRi screens represent a breakthrough for functional genomics of the myriad diverse bacteria around and within us, and the findings gleaned from its use in *V. natriegens* are paving the way for the bacterium's emergence as a new molecular biology workhorse for the twenty-first century. ❐

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Competing interests

The authors declare no competing interests.