Forward genetic studies of gene essentiality and sporulation in *Clostridium difficile*

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Clostridium difficile is the most common cause of antibiotic-associated intestinal infections and a significant cause of morbidity and mortality. Therapeutic intervention largely relies on a small number of broad-spectrum antibiotics, which further exacerbate intestinal dysbiosis and leave the patient acutely sensitive to reinfection. *C. difficile* spores are the primary infectious agent and are required for both disease transmission and recurrence, representing an attractive target for intervention. A detailed understanding of the cellular processes that underpin *C. difficile* sporulation and germination could thus have direct implications in infection control and the development of novel therapeutics. To date, this has been hampered by a lack of genetic tools for dissecting the organism.

In order to address these limitations, we developed a new method for rapidly generating large numbers of transposon mutants in clinically important strains of *C. difficile*. We validated our transposon mutagenesis approach in a model strain of *C. difficile* and then generated a comprehensive transposon library in the highly virulent epidemic strain R20291 (027/BI/NAP1) containing over 70,000 unique mutants. Using transposon-directed insertion site sequencing (TraDIS) we have identified a core set of 404 essential genes, required for growth *in vitro*. We then applied this technique to the process of sporulation, an absolute requirement for *C. difficile* transmission and pathogenesis, identifying 798 genes required to produce viable spores. The data generated in this study will form a valuable resource for the *C. difficile* community and inform future research on this important human pathogen.