Assessing Conditional Gene Essentiality in an S-layer Deficient Strain of *C. difficile*

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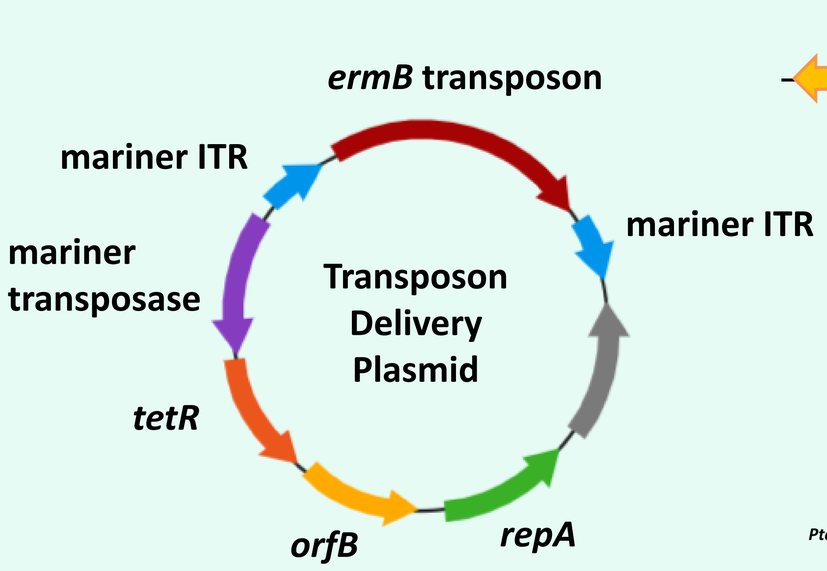
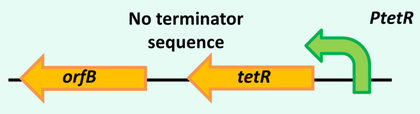
Introduction

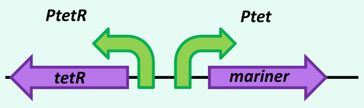
On the *C. difficile* cell surface is a proteinaceous paracrystalline array, known as the S-layer. The S-layer of *C. difficile* is composed of two proteins: the high molecular weight (HMW) and low molecular weight (LMW) S-layer proteins, derived from the pre-protein SlpA (2). Previous efforts to knock out *slpA* have proved unsuccessful. However, by using bacteriocins that specifically target the S-layer, we recently isolated a mutant which had no evident S-layer due to a mutation in the *slpA* gene (3). As the S-layer was previously thought to be essential, it now brings into question which genes become conditionally essential and non-essential in an S-layer deficient strain. Transposon directed insertion site sequencing (TraDIS) combines high-density transposon mutagenesis with high-throughput sequencing, allowing essential genes to be identified (1). TraDIS of an S-layer null strain will allow identification of conditionally essential genes during *in vitro* conditions. Additionally, CRISPR interference (CRISPRi) in *C. difficile* will allow analysis of gene essentiality and also gene function during transient knock-down.

Transposon Directed Insertion Site Sequencing

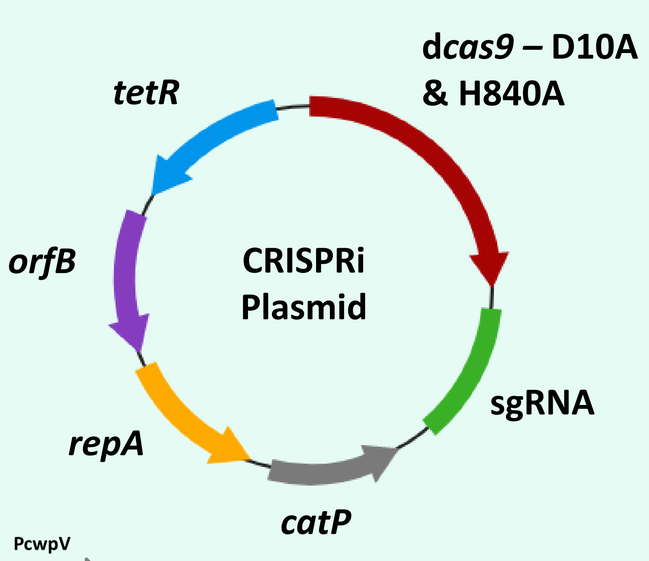


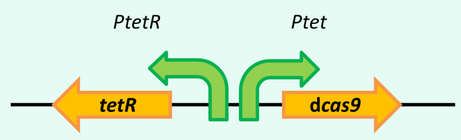


The transposon delivery plasmid consists of a *himar1* transposase under the control of the inducible P_{tet} promoter. An *ermB* transposon lies between two mariner terminal inverted repeats.

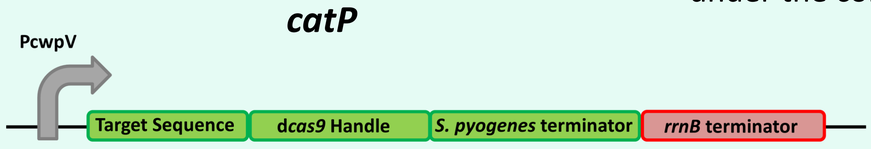


CRISPR Interference

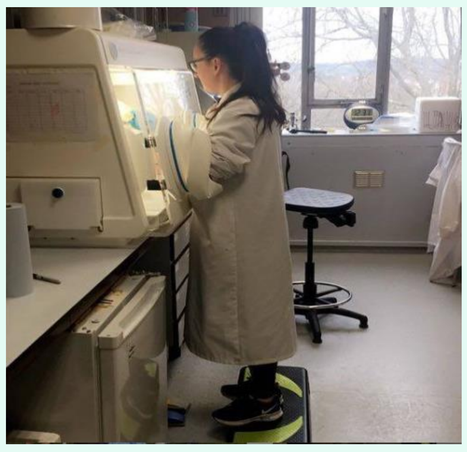




The CRISPRi plasmid for *C. difficile* consists of a deactivated *cas9* (d*cas9*) under the control of an inducible tetracycline promoter. sgRNA is composed of the target sequence, d*cas9*, an *S. pyogenes* terminator and a second terminator, under the control of P_{cwpV}

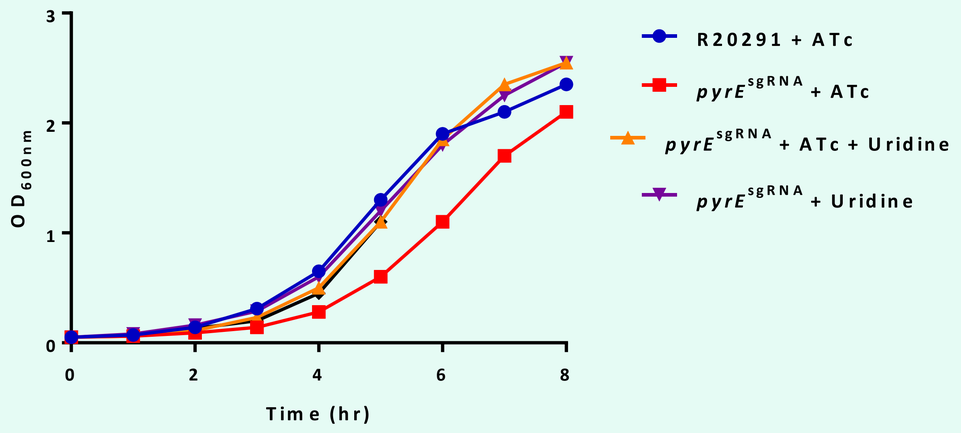


Transposon Libraries



Transposon libraries were made for strains R20291, FM2.5 and FM2.5RW. ~1.5 million colonies were pooled, with each colony representing a unique mutant.

Knock-down of *pyrE* Results in Uracil Auxotrophy



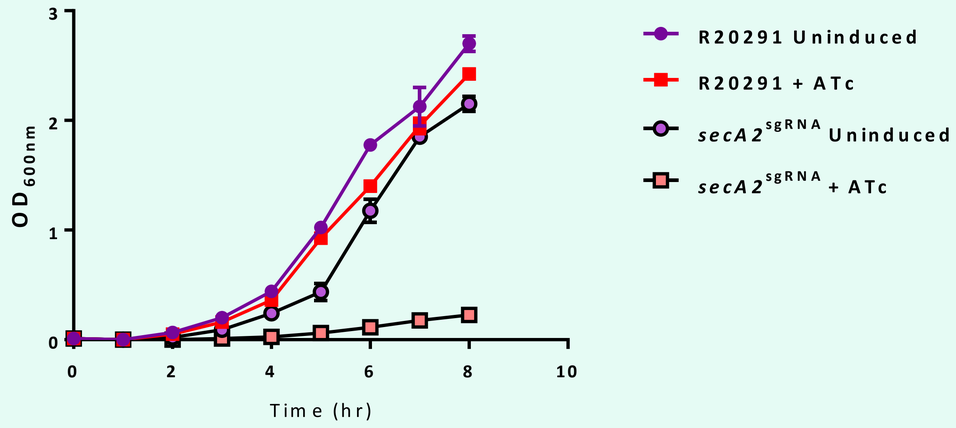
CRISPRi knock-down of *pyrE*. Knock-down of *pyrE* results in uracil auxotrophy, a phenotype restored by uridine (or uracil) supplementation

Optimisation of Transposon Library Production

	% Chromosome Reads	% Plasmid Reads
R20291	0.01%	99.9%
FM2.5	15%	85%

Increasing transposon library density decreases transposon library complexity.

secA2 is essential in *C. difficile* R20291



CRISPRi knock-down of *secA2*. Knock-down of *secA2* results in a ~10-fold viability defect.

References

1. Dembek, M., Barquist, L., Boinett, C.J., Cain, A.K., Mayho, M., Lawley, T.D., Fairweather, N.F., and Fagan, R.P. (2015). High-throughput analysis of gene essentiality and sporulation in Clostridium difficile. MBio *6*, e02383.
2. Kirk, J.A., Banerji, O., and Fagan, R.P. (2017). Characteristics of the Clostridium difficile cell envelope and its importance in therapeutics. Microb Biotechnol *10*, 76-90.
3. Kirk, J.A., Gebhart, D., Buckley, A.M., Lok, S., Scholl, D., Douce, G.R., Govoni, G.R., and Fagan, R.P. (2017). New class of precision antimicrobials redefines role of Clostridium difficile S-layer in virulence and viability. Sci Transl Med *9*.

Future Perspectives

Future work will focus on processing and analysing our agar based biological libraries. Following this, transient knock downs of essential and non-essential genes will be characterised using the CRISPRi system.